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Synthesis of Bacterial Metabolites and the Chemical Control of their Biosynthesis

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Abstract

Phenazines represent an abundant class of bacterial secondary metabolites synthesized mainly by *Pseudomonas* and *Streptomyces* strains. The redox-active, intensively colored bacterial pigments act as virulence factors and provide the producing organism with a competitive advantage. Their ability to reduce molecular oxygen to reactive oxygen species (ROS) not only triggers tissue damage in human infectious disease, but also explains their broad-spectrum antibiotic, antiviral and antifungal activities. Phenazine biosynthesis is linked to the shikimate pathway and the tricyclic phenazine core was found to derive from two chorismic acid molecules. The elucidation of phenazine biosynthesis has received considerable attention and the enzyme PhzA/B was found to catalyze a twofold head-to-tail condensation of two moieties of *trans*-2,3-dihydro-3-hydroxyanthranilate (DHHA). Inhibition of PhzA/B would hence suppress the virulence traits of bacteria and render them more benign so that they could be cleared more readily by the immune system. Furthermore the accumulation of unreacted DHHA is assumed to have a bactericidal effect making the inhibition of PhzA/B an interesting target for studies towards a novel antibiotic for the treatment of *P. aeruginosa* infections.

Synthetic mimics of the instable condensation product of the enzyme reaction were synthesized and tested for their affinity in vitro and the binding mode was extensively studied by X-ray crystallography. A lead compound, Phenazistatin A, was identified exhibiting high affinity towards PhzA/B in vitro. The effects of structural variation of the inhibitors were studied in detail and a library of analogs was synthesized in order to investigate the structure-activity relationships. However, the current structural features of this anthranilate-based inhibitor raise doubts about the efficacy in vivo due to the presence of highly polar carboxylic acid groups that might hamper passive membrane permeability. Therefore various inhibitors bearing bioisosteric replacements of the carboxyl group as well as prodrug esters were generated and evaluated.

Kurzfassung

Phenazine stellen eine häufig vorkommende Klasse von sekundären bakteriellen Metaboliten dar, die hauptsächlich von *Pseudomonas* und *Streptomyces* Stämmen synthetisiert werden. Die redox-aktiven, intensiv gefärbten bakteriellen Pigmente wirken als Virulenzfaktoren und statten den produzierenden Organismus mit einem kompetitiven Vorteil aus. Die Fähigkeit der Phenazine reaktive Sauerstoffverbindungen durch Reduktion von molekularem Sauerstoff herzustellen, führt nicht nur zu Gewebeschäden bei Infektionen von Menschen, sondern erklärt auch das breite Spektrum der antibiotischen, virostatischen und antimykotischen Wirksamkeit. Die Phenazinbiosynthese ist mit dem Shikimisäureweg gekoppelt. Es wurde herausgefunden, dass die trizyklische Kernstruktur der Phenazine aus zwei Molekülen Chorisminsäure gebildet wird. Die Aufklärung der Phenazinbiosynthese wurde intensiv untersucht und das Enzym PhzA/B konnte für die zweifache Kondensation von zwei Molekülen *trans*-2,3-Dihydro-3-hydroxyanthranilat (DHHA) verantwortlich gemacht werden. Inhibition von PhzA/B könnte daher die Virulenzeigenschaften der Bakterien abschwächen und sie somit harmloser machen, sodass sie einfacher durch das Immunsystem bekämpft werden können. Die Anreicherung von unreagiertem DHHA hat darüber hinaus vermutlich eine toxische Wirkung und somit stellt die Inhibition von PhzA/B ein interessantes Gebiet für die Erforschung von neuartigen Antibiotika für die Behandlung von *P. aeruginosa* Infektionen dar.

Synthetische Analoga des Kondensationsprodukts wurden synthetisiert und bezüglich der Affinität und des Bindungsmodus intensiv analysiert. Die Substanz Phenazistatin A zeigte eine vielversprechend hohe in vitro Affinität zu PhzA/B. Die Auswirkungen von Veränderungen der chemischen Struktur der Inhibitoren wurden untersucht und eine große Anzahl von Verbindungen wurde synthetisiert, um Struktur-Aktivitäts-Beziehungen abzuleiten. Die funktionellen Gruppen dieses Inhibitors lassen jedoch an der in vivo Wirksamkeit zweifeln, da die sehr polaren Carbonsäuregruppen bekannt dafür sind, den passiven Membrantransport stark zu behindern. Um diese Eigenschaften zu verbessern wurden Inhibitoren mit bioisosteren Gruppen sowie Prodrug-Ester hergestellt und auf ihre Wirksamkeit überprüft.

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“A General and Direct Reductive Amination of Aldehydes and Ketones with Electron-Deficient Anilines” J. Pletz, B. Berg, R. Breinbauer, *Synthesis* **2016,** *48*, 1301–1317.
DOI: 10.1055/s-0035-1561384

“Enterotoxicity of a nonribosomal peptide causes antibiotic-associated colitis” G. Schneditz, J. Rentner, S. Roier, J. Pletz, K. A. T. Herzog, R. Bücker, H. Troeger, S. Schild, H. Weber, R. Breinbauer, G. Gorkiewicz, C. Högenauer, E. L. Zechner, *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 13181–13186.
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1 Introduction

The serendipitous discovery by penicillin in 1929 by Fleming[1] enabled the control of bacterial infections by Gram-positive pathogens such as *Staphylococcus* and *Streptococcus*. The following discovery of streptomycin in 1943[2] allowed for the control of the tuberculosis agent *Mycobacterium tuberculosis*, which was the first effective treatment of tuberculosis until then. After these discoveries the golden era of antibiotics had started and numerous natural antibacterial products as well as analogs of these pioneering drugs were discovered and chemical modification of existing antibiotics led to a large amount of effective agents. The results were revolutionary and medicine was transformed because infections could be controlled routinely. Invasive surgeries became routine, the immune-system destroying chemotherapy was introduced, deteriorating joints could be radically replaced by artificial ones, organ transplantation led to extended lives, and the quality of life for millions of people was greatly improved. However, the golden era of antibiotics is over and now in the age of antibiotic resistance the management of bacterial infection with safe, cheap and accessible antibiotics cannot longer be taken for granted. The future strategy to treat bacterial infections requires a paradigm shift away from a “best-guess-medicine” using broad-spectrum antibiotics towards an individualized therapy with narrow-spectrum agents that selectively target the pathogen. This personalized medicine would create less off-target effects and reduce the evolutionary pressure to evolve resistance in bacteria. However, the identification of such promising new antibiotics is hard and antibiotic discovery is considered to be target poor, with the drugs limited to interfering with cell-wall synthesis, membrane integrity, translation, transcription, and DNA synthesis. However, this range of activities might simply reflect the targets of the broad-spectrum agents. Narrow-spectrum agents offer a larger and more diverse prospect of targets that are specific to bacteria and pathogenesis. Processes that enable the in vivo growth and virulence are especially promising.[3] The rationale of the antivirulence approach[4] is to suppress the virulence traits of bacteria hence rendering them more benign so that they can be cleared more readily by the immune system. This indirect strategy is expected to reduce the selective pressure for the spread of drug-resistant genes and could lead to therapies that retain a much longer span of efficacy compared to traditional antibiotics.[5,6] In order to devise such narrow-spectrum agents a deep underlying comprehension of bacterial metabolism is paramount and highly-selective modes of action have to be addressed. Phenazines, a natural product class produced efficiently in commonly multi-drug-resistant *P. aeruginosa*, are an attractive example of a bacterial virulence factor that provides its producer with a competitive advantage. The intensively-colored bacterial pigments are produced in copious amounts and lead to tissue damage in human infectious disease, being the prime reason for the low life expectancy of cystic fibrosis patients.[7] In the

last decade the biosynthetic pathway of the phenazines was elucidated and detailed mechanistic insights into the enzymatic catalysis have been utilized to generate inhibitors that are being studied for their potential to strip bacteria off an essential virulence factor. It remains to be explored, whether the intervention in this biosynthetic pathway can lead to a narrow-spectrum antibiotic that selectively targets a single process in bacterial physiology which is unrelated to the established targets of antibiotics.

2 Theoretical Part

2.1 Phenazines

2.1.1 Structure and function of phenazines

Phenazines represent an abundant class of bacterial secondary metabolites and the first member of its class was already isolated in the mid 19th century by Marthurin-Joseph Fordos. He extracted a blue pigment from purulent wound dressings by chloroform extraction which he referred to as "pyocyanine".[8,9] Several years later a bacterium called "*Bacillus pyocyaneus*",[10] today *Pseudomonas aeruginosa*, was identified to be associated with the blue metabolite. Since then several other bacteria classes were identified to produce members of this product class. Gram-positive (e.g. *Streptomyces*) and Gram-negative (e.g. *Pseudomonas*) bacteria were found to be the most proficient producers of phenazines, but also *Methanosarcina* species were identified to produce phenazines. The first isolated members were chlororaphine and iodinine[10,11] and the structural diversity of the phenazines was found to be highly diverse. Until now, over 150 natural products containing the tricyclic phenazine motif have been described[10,12–14] and several thousand analogs have been synthesized.[13,15] The bacterial metabolites show a high structural diversity and substitution at every atom around the aromatic rings has been found (Figure 1). Dimeric structures (Esmeraldin A, *S. antibioticus*), substitution with lipophilic chains (Methanophenazine, *M. sarcina Gö1*) and charge transfer complexes (Iodinine, *B. iodinum*) are just a few examples.

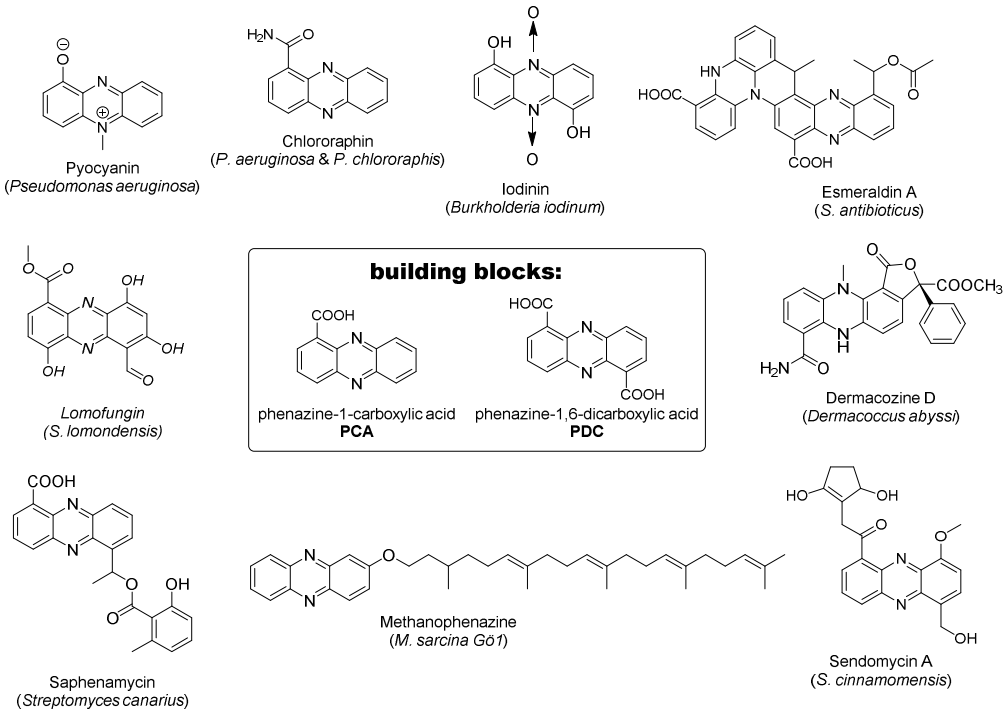


Figure 1: The two core-phenazines **PCA** and **PDC** with phenazine natural products.

The characteristic tricyclic ring system with the central pyrazine moiety leads to a general redox activity of the phenazines.[16] This redox activity is the cornerstone of several effects the phenazines exhibit. Phenazines can directly oxidize molecular oxygen to reactive oxygen species, which trigger tissue damage in human infectious disease,[17] but are also toxic to other microorganisms. The reactive oxygen species explain their broad-spectrum antibacterial, antifungal, antiviral and antitumor activity.[7,12,13] Some strains produce copious amounts of phenazines as high as grams per liter, but the physiological importance of these substances has not yet been fully understood.[18] The phenazines act as virulence factors and equip their producer with a competitive advantage[19] but are also used to fight competitors.[18,20] However, recent studies revealed that phenazines might also play an important role in the physiology of their producer.[10,14] A study by Hernandez et al.[21] describes that excreted phenazines contribute to the iron acquisition process of bacteria by reducing Fe^{3+}-containing minerals to the more soluble Fe^{2+} species, which can be shuttled into the cell by regular siderophores.[14] Phenazine-producing bacteria residing on roots were found to protect crop plants from phenazine-susceptible bacteria and fungi.[7,22,23] In *P. aeruginosa* pyocyanin was found to act as a signaling molecule directly controlling gene expression by activating the iron-containing transcription factor SoxR.[24] Phenazines furthermore play a prominent role in primary energy metabolism of phenazine-producing bacteria. Pyocyanin can directly oxidize NADH, which might be needed to sustain glycolysis under anoxic conditions, such as in lower layers of a bacterial biofilm. Studies with deletion mutants lacking the phenazine biosynthesis genes had a strongly decreased capability for forming a bacterial biofilm.[25–27] The formation of a bacterial biofilm is a strong impediment for antibiotic treatment as well as the human immune response during an infectious disease, because it renders the bacteria less accessible for the agents of the immune response and antibiotics. The abundant human pathogen, phenazine-producing *P. aeruginosa* is an opportunistic pathogen and a leading cause of hospital-acquired infections. It poses a particular threat to cystic fibrosis patients, third-degree burn victims and patients with implanted medical devices.[5,28–30] For patients suffering from cystic fibrosis the chronical colonization of the lungs with *P. aeruginosa* is a leading cause of early mortality.[31,32] Strains of *P. aeruginosa* defective of pyocyanin biosynthesis were found to be more susceptible to immune response in a mouse model of lung infection.[17,33]

2.1.2 Phenazine biosynthesis

The enzymes for the biosynthetic pathway of the phenazines are encoded in the "*phz* operon" and the analysis of sequence data indicates that five enzymes are vital for the synthesis of the tricyclic phenazine precursor molecules phenazine-1-carboxylic acid (**PCA**) and phenazine-1,6-dicarboxylic acid (**PDC**) (Figure 2). Existing knowledge suggests that the core structure of all of the bacterial phenazines derives from either **PCA** or **PDC,**[13–15,34] and that post-tailoring steps give rise to the high structural diversity of these natural products (Figure 1). The molecular mechanism of phenazine biosynthesis was extensively studied and in 1962 it was found that phenazines derive from two molecules of chorismic acid, which was proven by the incorporation of ^{14}C-labelled precursors.[35] PhzE was identified to convert chorismic acid to 2-amino-2-desoxyisochorismate (**ADIC**), which was already postulated in 1982 by Römer and Herbert[36] and later supported by Galbraith et al.[36–38] **ADIC** is further hydrolyzed to *trans*-2,3-dihydro-3-hydroxyanthranilic acid (**DHHA**) and pyruvate by the enzyme PhzD, which was found to be a quite promiscuous enzyme also utilizing isochorismate, chorismate and 4-amino-4-desoxychorismate.[14,39] **DHHA** is a substrate of PhzF, which acts as an isomerase and converts **DHHA** to 6-amino-5-oxocyclohex-2-ene-1-carboxylic acid (Figure 2, **A**). NMR spectroscopy in D_2O revealed that the proton at C3 is not lost during isomerization, but that only a shift from C3 to C1 takes place.[40] This observation suggests that the enzyme PhzF might catalyze a pericyclic reaction. Considerable effort has been put into the elucidation of the reaction mechanism in the groups of Breinbauer and Blankenfeldt.[41] QM/MM studies indicate that the reaction proceeds via an acid-base catalyzed mechanism. Results have been summarized in the PhD thesis of Mario Leypold.[41]

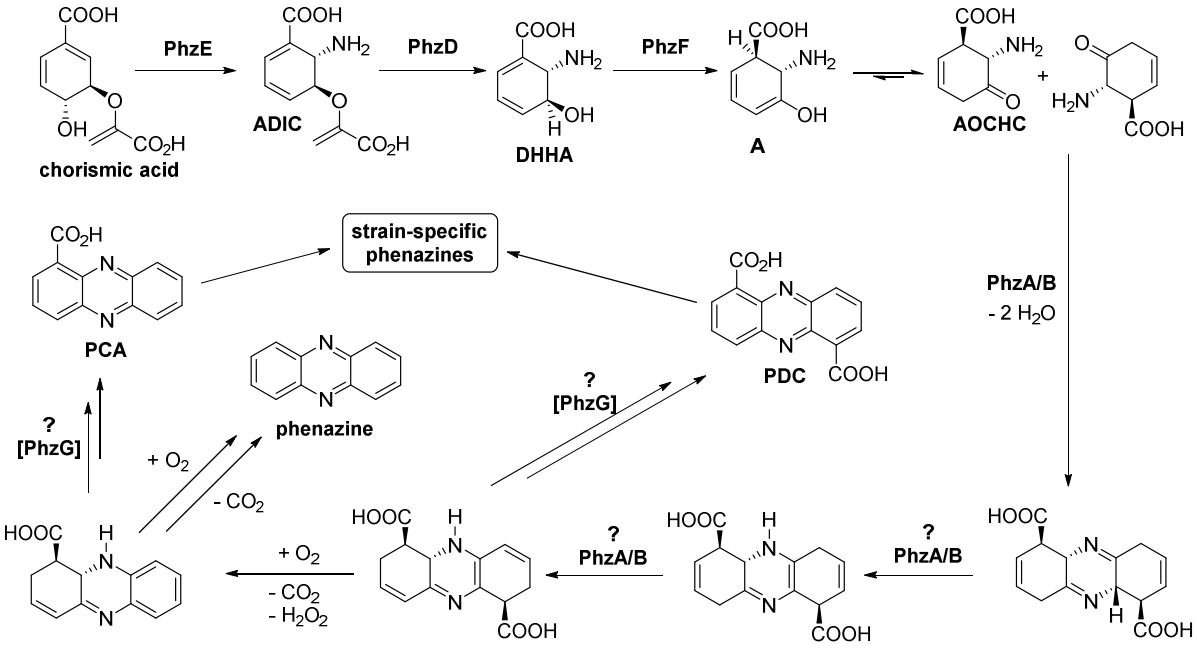


Figure 2: Biosynthesis of the phenazine precursor molecules **PCA** & **PDC.**

The product **A** resulting from the turnover of **DHHA** by PhzF (Figure 2) isomerizes to the ketone, but the ketone **AOCHC** was found to be highly reactive and reacted spontaneously further in a head-to-tail fashion to give a tricyclic phenazine precursor. The diagonal-symmetrical pairing of the head-to-tail condensation as a key step in phenazine biosynthesis was already described in the 1970s, albeit without knowing the structure of the condensation intermediate.[42–44]

Initially it was believed that the homodimeric PhzF, which features two independent active sites facing each other, catalyzed the dimerization reaction at the monomer-monomer interface.[14,40] The likelihood of two molecules meeting each other in the central cavity in the closed conformation would be much higher, if the two active sites of the PhzF dimer release **AOCHC** simultaneously. Since the condensation of two molecules **AOCHC** is a bimolecular reaction it is enhanced when higher concentrations of **DHHA** are present. However, because **AOCHC** probably reacts unspecifically with amines for example in proteins, it is likely toxic to the bacterial cell and its accumulation has to be prevented. It was found that the PhzA/B proteins catalyze the condensation reaction, hence leading to a much faster clearance of these reactive intermediates in the cell.[7] Phenazine production is very prominent in Pseudomonads, which possess two adjacent, highly homologous copies of this gene (*phzA* and *phzB*) with approximately 80% amino acid identity.[45,46] When expressed separately, PhzA and PhzB were found to be homodimers, however heterodimers form when expressed together in which the dimer has only one functional active site.[14] When not referring to one specific enzyme, then generally the term “PhzA/B” is used here. In the active site of PhzA/B two condensation reactions are catalyzed by first placing the bifunctional **AOCHC** molecules in an opposite orientation and then facilitating twofold condensation by acid/base catalysis. The condensation product undergoes a series of isomerizations and oxidations to yield the precursors of the bacterial phenazines **PCA** and **PDC**. The mechanism of the following steps is not well understood, but it is assumed that the enzymes PhzA/B and PhzG play a role in these post-condensation steps. It is believed that the FMN-dependent PhzG is involved in one of the terminal oxidation steps that leads to aromatization,[47] but the role of PhzA/B is not obvious.[7] The steps leading from chorismate to the reduced forms of PCA and PDC are summarized in Figure 3.

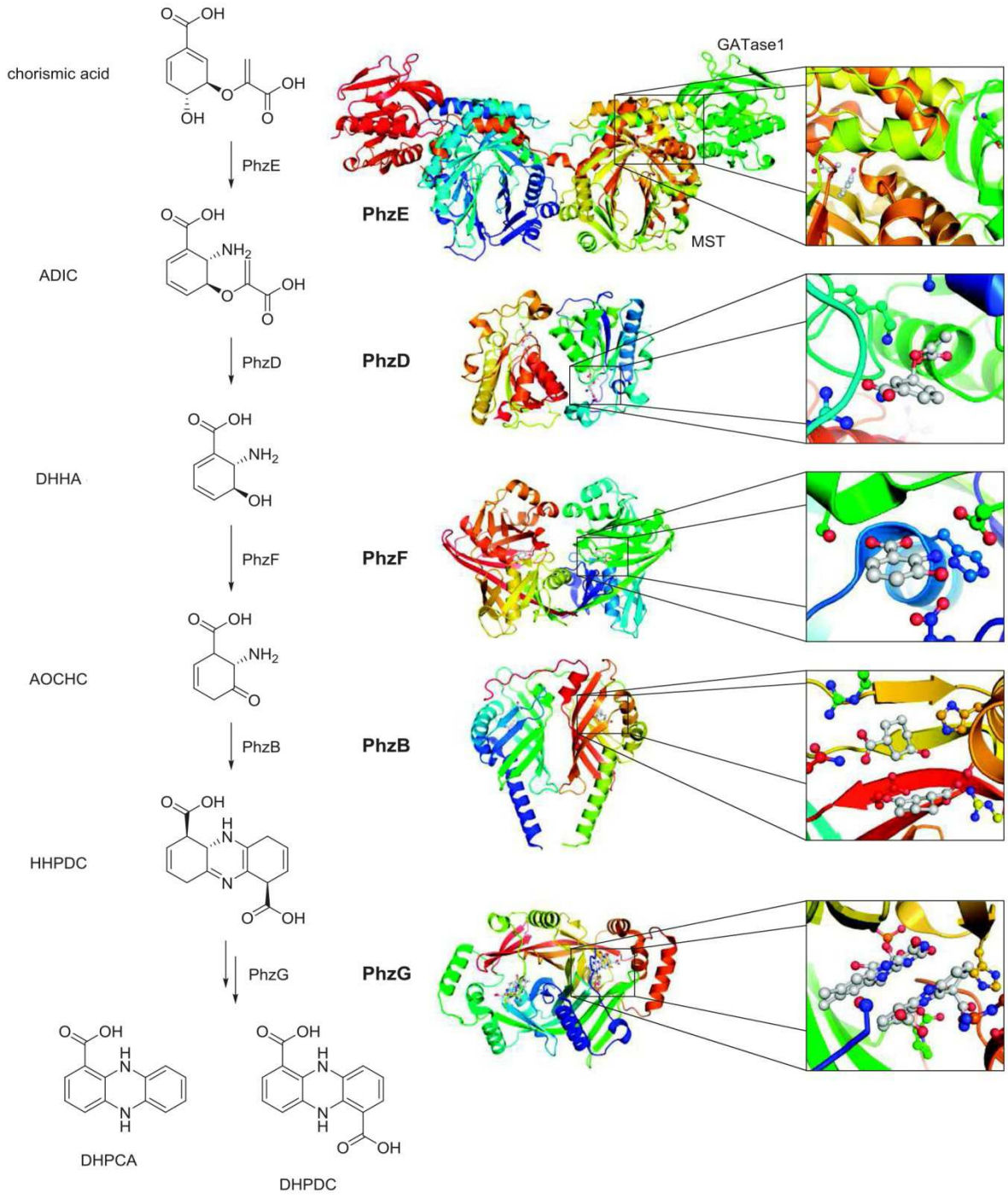


Figure 3: Structural view of the phenazine biosynthesis; the following Protein Data Bank entries[48] have been used: PhzE, 3R75;[49] PhzD, 1NF8;[50] PhzF, 1U1W;[40] PhzB, 3DZL[7] and PhzG, 4HMT;[51] Figures were generated with PyMOL.[10,52] Figure courtesy of Mario Leypold.[41]

Ahuja et al. stated,[7] that phenazine biosynthesis follows the same depicted (Figure 2) pathway in different bacterial species (*B. cepacia*, *Pseudomonas*), which they proved by the observation of the identical intermediates upon conversion of the substrate in bacterial cultures.

2.1.3 Mechanism of the reaction catalyzed by PhzA/B

The heterodimer of PhzA/B has a KSI/NTF2-fold and provides a large binding cavity for **AOCHC** where the *C*-termini of both enzymes contribute to the formation of the active center of the neighboring monomer. As was already described earlier, the heterodimer possesses only one functional binding site where substrate turnover takes place. Ahuja et al. hypothesize,[7] that the *C*-terminus acts as a flexible lid that controls the access of the substrate to the active site. This hypothesis was supported by the crystal structures of inhibitor-bound PhzA/B where the active site is fully shielded from solvent and where all the residues of the neighboring *C*-terminus were present. It was furthermore found that the *C*-terminus is vital for protein function as well as stability. Truncation experiments have shown that truncation at residue 162 leads to impaired activity and ultimately, when the enzyme was shortened to 159 residues, no enzyme function could be observed. This finding was expected, since R160* (Figure 4) participates in substrate binding.

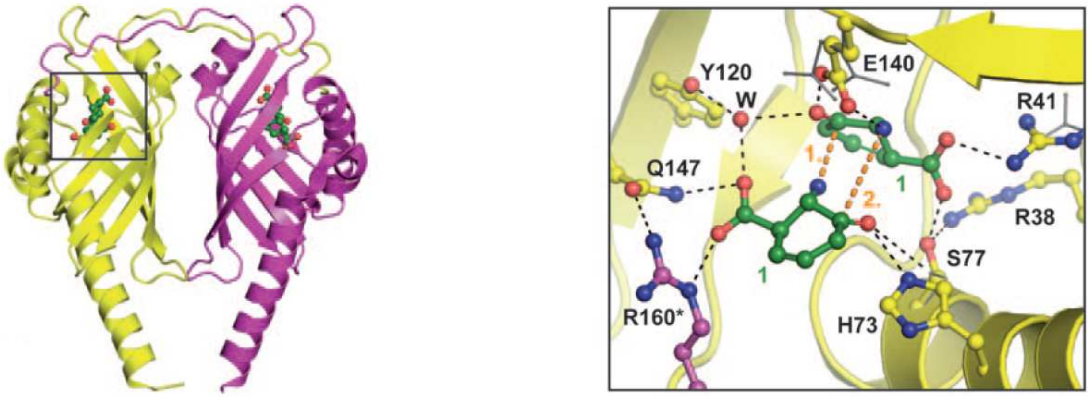


Figure 4: Crystal structure of PhzA/B from *B. cepacia R18194* (PDB ID: 3DZL). The enzyme catalyzes the twofold head-to-tail condensation of **AOCHC** (Picture taken from Mentel et al.[14]).

The catalytic mechanism in which PhzA/B enhances the reaction from AOCHC to the tricyclic condensation product has been described.[7] The catalytic sequence is initiated by binding of the first **AOCHC** molecule to R41 and probably also by H-bonding of the carboxyl group with S77 (Figure 5, a). The second identical substrate molecule binds to H73 via its ketone and to the flexible R160*, which is donated from the neighboring enzyme. This binding is expected to anchor the flexible terminus in an orientation that shields the active site from access of solvent. The amino group of the H73-bound **AOCHC** nucleophilically attacks the ketone of the first bound substrate molecule and forms the first tetrahedral intermediate (Figure 5, b). As a consequence of the first condensation the transient intermediate is contracted and the closer proximity of the second ketone and amine group triggers the second nucleophilic attack furnishing the tricyclic phenazine core (Figure 5, c). The second nucleophilic attack might not require enzyme catalysis, but the structural model suggests that H73 and S77

participate in the condensation by neutralizing the transiently generated negative charge upon $-NH_2$ attack at the ketone (Figure 5, d). It is not clear whether PhzA/B is required for the post-cyclization maturation steps which include the enamine formation. The structural model of the binding site indicates, that the residue E140 would be ideally suited to promote this isomerization due to its proximity and mobility (Figure 5, e & f).

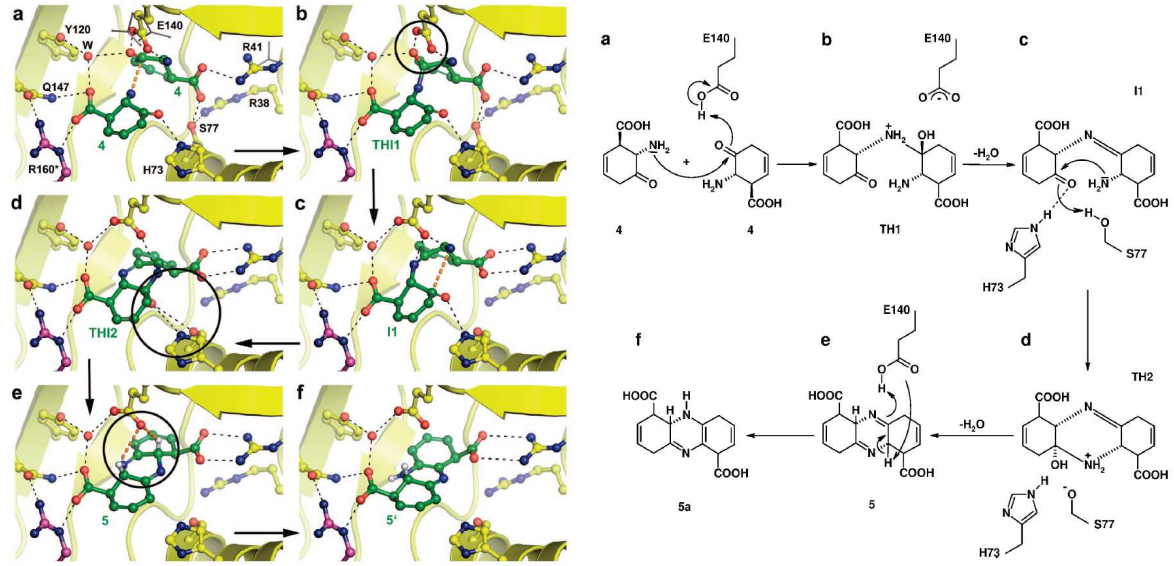


Figure 5: Left: Proposed catalytic sequence for PhzA/B. **AOCHC** is depicted here as **4**. Right: Proposed catalytic sequence for PhzA/B showing the most important proton transfer reactions (Pictures taken from Ahuja et al.[7]).

It would be highly interesting to devise probes that bind to this active site in order to further study the mechanism of PhzA/B and a logical approach would be the synthesis of an analog which is very closely related to the transition states of the condensation steps. Unfortunately the intermediates are highly unstable and could not be synthesized for the biological experiments. Instead, substrate mimics as well as condensation product mimics were synthesized and tested via isothermal titration calorimetry (ITC) and X-ray crystallography.[7] The **AOCHC** mimics synthesized did not bind, but products based on *N*-substituted anthranilic acid showed affinity and crystal structures and ITC values could be obtained. The best compounds synthesized exhibited a binding affinity K_D = 2 to 25 µM and all of them were found to show a similar binding mode as shown in Figure 6. These studies were further conducted in the PhD thesis of Matthias Mentel[53] and inhibitors with nanomolar affinity were found.

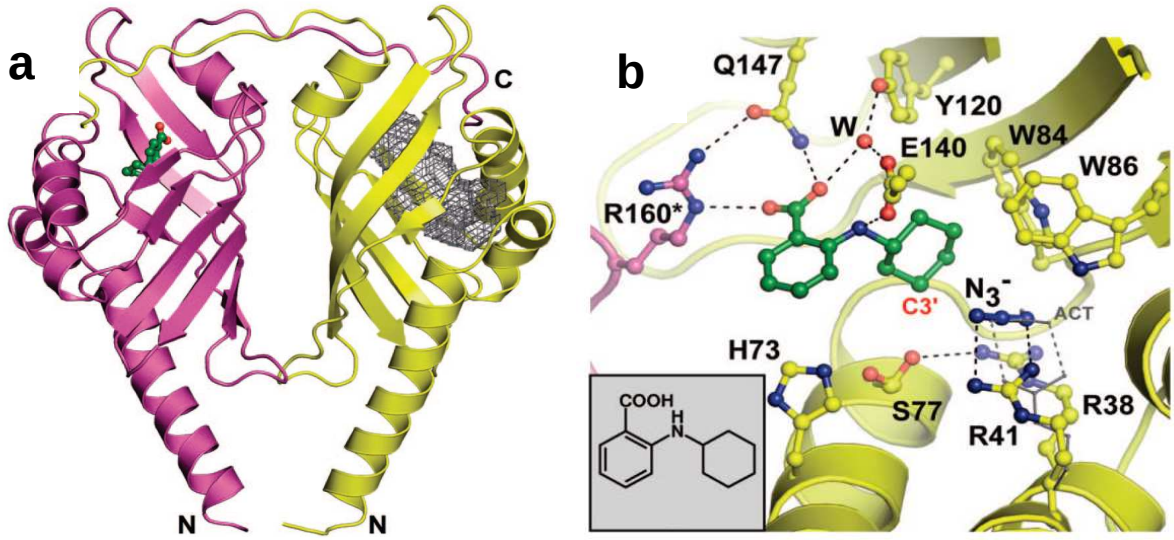


Figure 6: a) Crystal structure of *Burkholderia cepacia* PhzA/B with two active sites. *N*- and *C*-termini are indicated. b) Binding of 2-(cyclohexylamino)benzoic acid in PhzA/B (*B. cepacia*) (pictures taken from AHUJA *et al.*[7]).

2.2 Bioisosteres

The concept of bioisosterism is based on the idea, that single atoms, groups or whole molecules that exhibit similar volume, shape, and/or physicochemical properties can lead to similar biological effects.[54,55] The term “bioisostere” was coined by Harris Friedman in 1950 and he also recognized that compounds might be isosteric, but not necessarily bioisosteric.[56] This notion already suggests that the application of bioisosterism will depend largely on the context, and underlying physicochemical principles will become less important than the actual and observed biochemical mimicry. Hence the bioisosteric replacements are most often rather similar in biological than in physical properties. Thus, an effective bioisosteric replacement for one biochemical application might not translate to a similar setting and the isostere would need to be carefully selected and tailored to suit in the new biological setting. Consequently, the design of bioisosteres frequently necessitates structural changes that might either be beneficial or deleterious, depending on the context. The following parameters were found to play key contributing roles in molecular recognition and mimicry: size, shape, electronic distribution, polarizability, dipole, polarity, lipophilicity and pKa. In current practice of Medicinal Chemistry the use of bioisosteric groups has become a fundamental tactical approach to address a wide number of aspects of drug design and development.[54–62] Bioisosteres are widely used to improve potency, enhance selectivity, alter physical properties, redirect metabolism, eliminate or alter toxicophores or to generate novel intellectual property.[56]

2.2.1 Carboxylic acid bioisosteres

The presence of a carboxylic acid functional group can be essential to a pharmacophore, however, the presence of this moiety in a drug or a drug candidate can lead to several drawbacks such as metabolic instability, toxicity as well as poor passive diffusion across biological membranes. In order to overcome these shortcomings medicinal chemists often investigate the effects of the use of carboxylic acid (bio)isosteres.[63]

The carboxylic acid functional group plays a leading role in the chemistry of living systems as well as in drug design. Several classes of endogenous substances, such as triglycerides, prostanoids and amino acids contain this functional group and it is commonly found in the pharmacophore of therapeutic agents.[64] In fact, more than 450 carboxylic acid-containing drugs have been marketed worldwide including widely used antibiotics, anticoagulants, nonsteroidal anti-inflammatory drugs, cholesterol-lowering statins, and others.[63] The reasons why this functional group often is a key determinant for drug-target interactions is its acidity as well as its ability to form relatively strong electrostatic interactions and hydrogen bonds. However, the carboxylic acid group can also impose problems, for instance a diminished ability to diffuse passively through a biological membrane. This is a significant challenge especially in the field of central nervous system (CNS) drug discovery, where the blood-brain-barrier can be relatively impermeable to negatively charged carboxylate anions.[65] In order to avoid these and possible other shortcomings, the replacement of the carboxylic acid group by a bioisosteric group can represent a suitable and effective strategy. A compilation of the more commonly used carboxylic acid bioisosteres is presented in Figure 7.

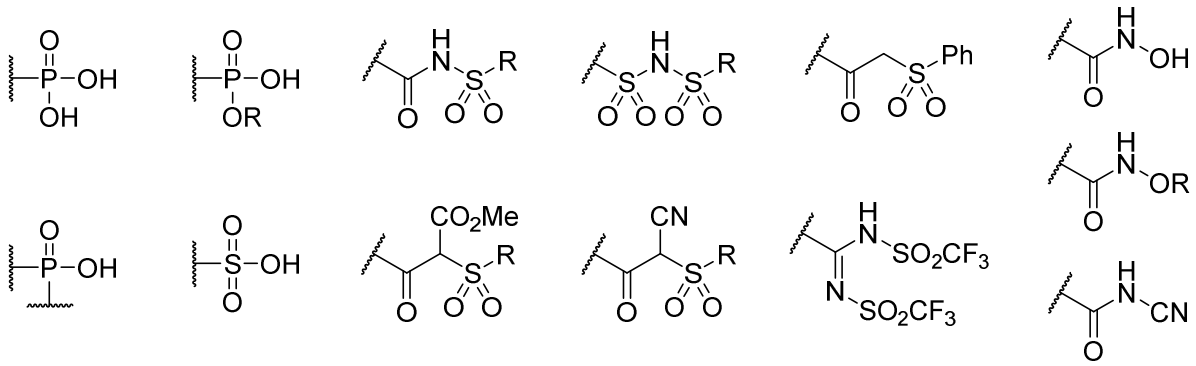


Figure 7: Synopsis of common carboxylic acid isosteres.[56]

The use of heterocycles with intrinsic acidity or with substituents to modulate the pK_a greatly expanded the palette of possible bioisosteres. The large diversity of heterocycles offers additional opportunities by allowing additional structural variation in which the bioisostere could be fine-tuned to enhance complementarity to the target protein. A wide-ranging structural diversity is provided by different patterns of substitution and the potential of charge

delocalization by enolization. A synopsis of acidic heterocycles that have been explored as carboxylic acid bioisosteres is depicted in Figure 8.[56]

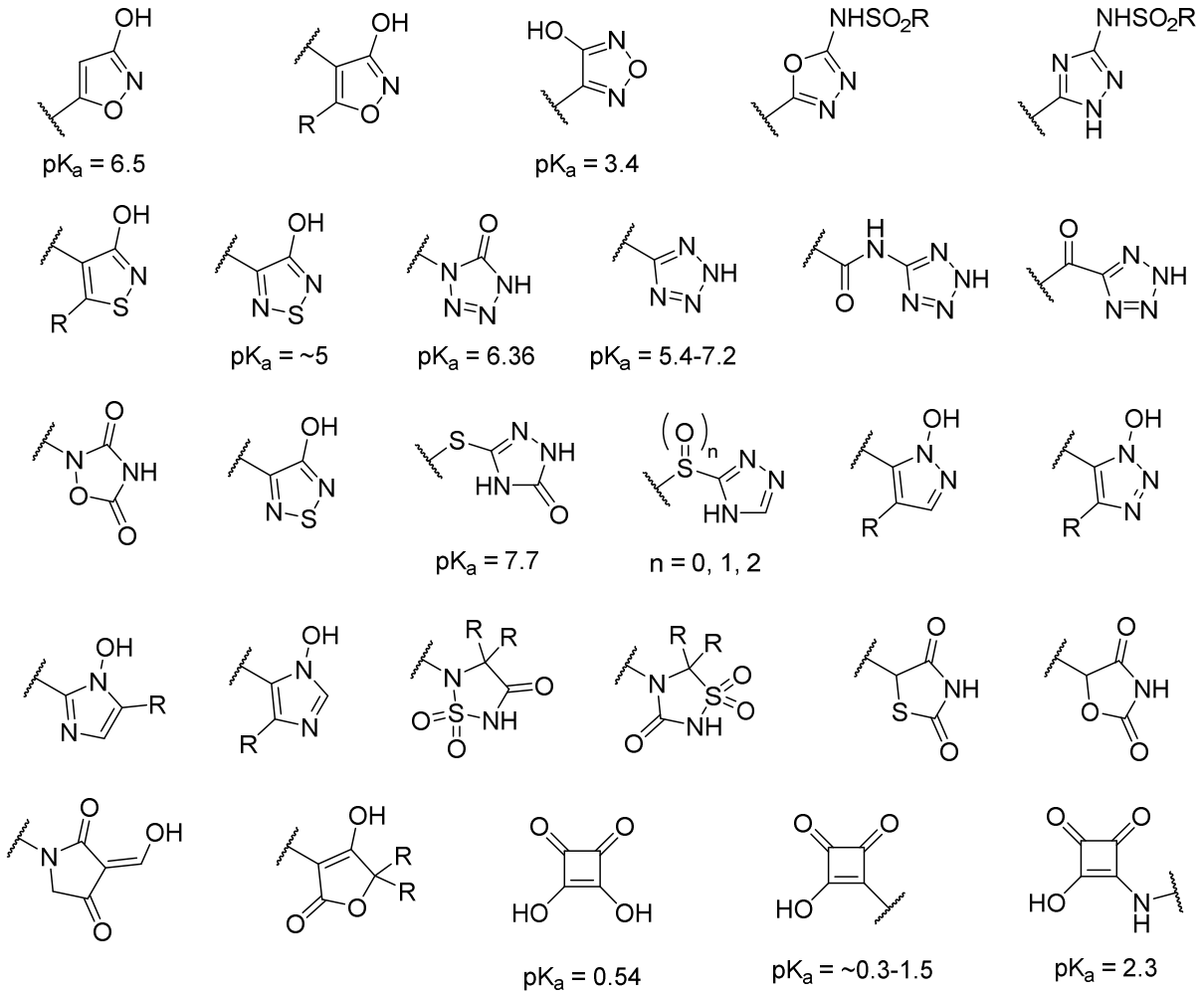


Figure 8: Synopsis of heterocycle-based carboxylic acid isosteres.[56]

2.2.2 Tetrazoles as carboxylic acid isosteres

Tetrazoles are one of the most commonly employed carboxylic acid bioisosteres and their use has been reviewed in recent review articles.[63,66,67] The 5-substituted tetrazoles exist in a tautomeric equilibrium between the 1*H* and the 2*H* tautomers (Figure 9).[63]

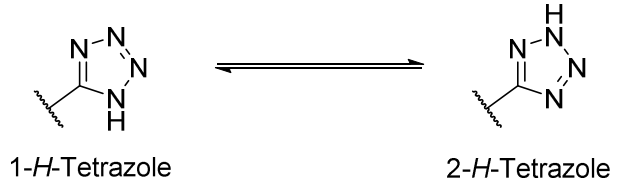


Figure 9: The tautomeric equilibrium between the 1*H* and the 2*H*-tetrazole.

The most important features of the tetrazolic acids are the planar structure and the acidity, which closely resembles the one of carboxylic acids (pK_a = ~4.5-4.9).[68,69] The tetrazoles

require slightly more space than the carboxylic acid equivalents[70,71] and it was found that the hydrogen-bond environment surrounding the tetrazolates extends further than the one of the carboxylates.[71] Tetrazolate anions are more lipophilic than carboxylates,[72] and exhibit a charge distribution due to the delocalization of the negative charge over the heterocyclic ring system.[73] Like carboxylic acids, tetrazoles are capable of forming two-point interactions with amidines, however the stability of the tetrazolate-amidine is lower than the carboxylate-amidine equivalent.[74,75] Tetrazoles can also undergo *N*-glucuronidation,[76] but the resulting glucuronides are less reactive than the carboxylic acid and hydroxamic acid equivalents and have not yet been described to cause toxic effects in humans.

The tetrazole moiety is commonly used in drug development and the most important examples demonstrating the utility of the tetrazole group include several angiotensin II type 1 (AT1) receptor antagonists.[77] The AT1 receptor belongs to the G protein-coupled receptor (GPCR) superfamily which plays an important role in vasoconstriction.[78] The relative importance of these drugs is highlighted by five angiotensin II antagonists amongst the top selling 200 drugs in 2010.[79] Amongst the AT1 receptor antagonists used clinically for the treatment of high blood pressure, five out of six contain a tetrazole moiety.[63,77,80] Typical focus of the drug development studies towards these molecules was placed on increasing potency and increasing lipophilicity in order to improve passive membrane permeability. The detailed studies towards the angiotensin II receptor antagonists gave instructive insight into the design of carboxylic acid bioisosteres, since the binding affinity to the receptor of a series of biphenyl acids (a structural motif of the sartans) was found to be quite sensitive to the nature of the acidic group.[81,82] The use of the tetrazole moiety in Losartan (Figure 10, **B**) led to a 10-fold increase in potency compared to the carboxylic acid analog. When a geometrical analysis was conducted, the tetrazole was found to project the acidic NH 1.5 Å further away from the phenyl ring than the acidic OH in a carboxyl group (**A**). The acyl sulfonamide derivative **C** exhibited a similar geometry to the carboxyl group and the potency was found to be in the same order of magnitude. However, when the reverse acyl sulfonamide group was installed, the acidic NH group was projected further away from the biphenyl core and a largely increased potency compared to the acidic proton in Losartan (**B**) was observed.[81,82]

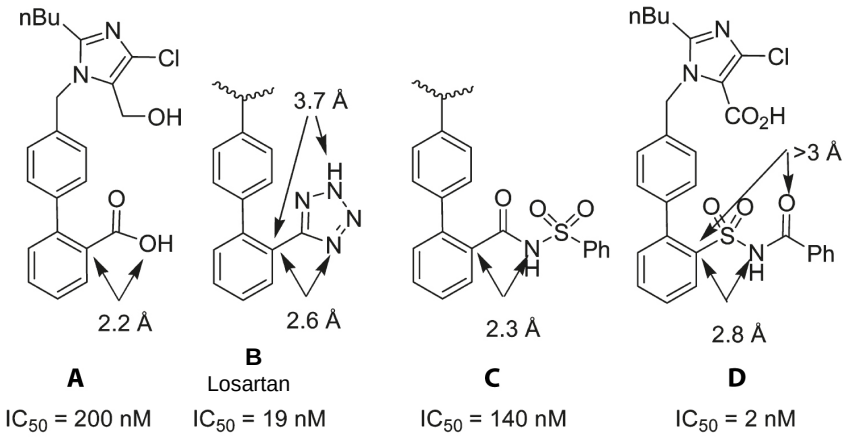


Figure 10: Geometrical arrangements associated with the carboxylic acid moiety and the tetrazole and acyl sulfonamide isosteres in angiotensin II antagonists (Picture taken from Meanwell[56]).

2.3 Prodrugs

Prodrugs are generally inactive, or at least significantly less active, bioreversible derivatives of active drug molecules that have to undergo an enzymatic and/or chemical transformation in vivo in order to release the active parent drug.[83–86] A simplified illustration of the prodrug concept is given in Figure 11. The rationale behind the use of prodrugs is most often to adjust the “drug-like” properties, summarized by the acronym ADMET (absorption, distribution, metabolism, excretion, toxicity), because the parent compound sometimes features unfavorable properties. The prodrug strategy has furthermore been used to increase the selectivity of drugs for their intended target. This would not only improve the efficacy of the drug, but also decrease the possibility for systemic or organ/tissue specific toxicity. The use of a prodrug strategy should be considered in the early stages of preclinical development, because they may favorably alter the tissue distribution, efficacy and sometimes even toxicity of the parent drug. Unfortunately, the use of prodrugs is often still seen as “an act of desperation”[87] and only considered when significant problems are encountered with the original drug candidate. This is somewhat surprising, because approximately 10% of all globally marketed medicines can be counted as prodrugs and in 2008 alone 33% of all approved small-molecular-weight drugs were prodrugs.[84,87]

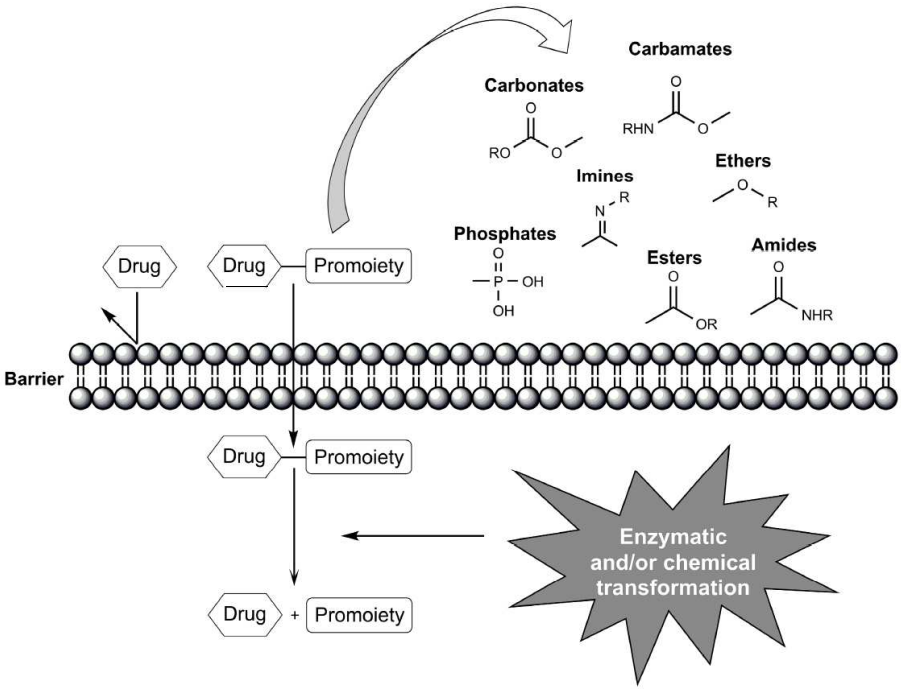


Figure 11: In vivo bioactivation of prodrugs by enzymatic and/or chemical transformations (Picture taken from Jornada et al.[88]).

2.3.1 Clinical considerations of prodrug bioconversion

The prodrug strategy was without doubt a success for a large number of globally used therapeutic agents. However, the prodrug research encounters many different challenges in preclinical and clinical settings, mostly related to understanding the mechanism of bioconversion of the prodrugs. Several enzymes that are involved in the bioactivation have interindividual variabilities in their activities. Several factors can be made responsible for this variability, such as polymorphisms in the genes encoding the relevant bioactivating enzymes as well as interactions caused by other drugs or xenobiotics. In other words, the same prodrug can be insufficiently or excessively converted to the active drug by different patients. Other hurdles for prediction of the prodrug disposition on humans are the interspecies differences in prodrug activation by the enzymes.

The most common class of prodrugs relies on diversity-tolerant enzymes with a wide substrate scope such as the peptidases, phosphatases and especially carboxylesterases.[89] They are ubiquitously distributed and hence the potential for carboxylesterases to become saturated or the substrate to engage in drug-drug interactions is considered to be negligible,[90] although not impossible.[91] Most of the carboxylesterases belong to two isoenzyme families, CES1 and CES2, which are characterized by different substrate specificities, tissue distribution, and gene regulation.[87] The different substrate specificities of the isoenzyme families CES1 and CES2 as well as their different tissue distribution can be

taken into account during prodrug design. It was found, that CES1 prefers the hydrolysis of esters with a large acyl group and small alcohol group (carboxylic acid as the active drug), whereas CES2 hydrolyses preferably esters with small acyl groups and large alcohol groups (alcohol as the active drug) (Figure 12).[92]

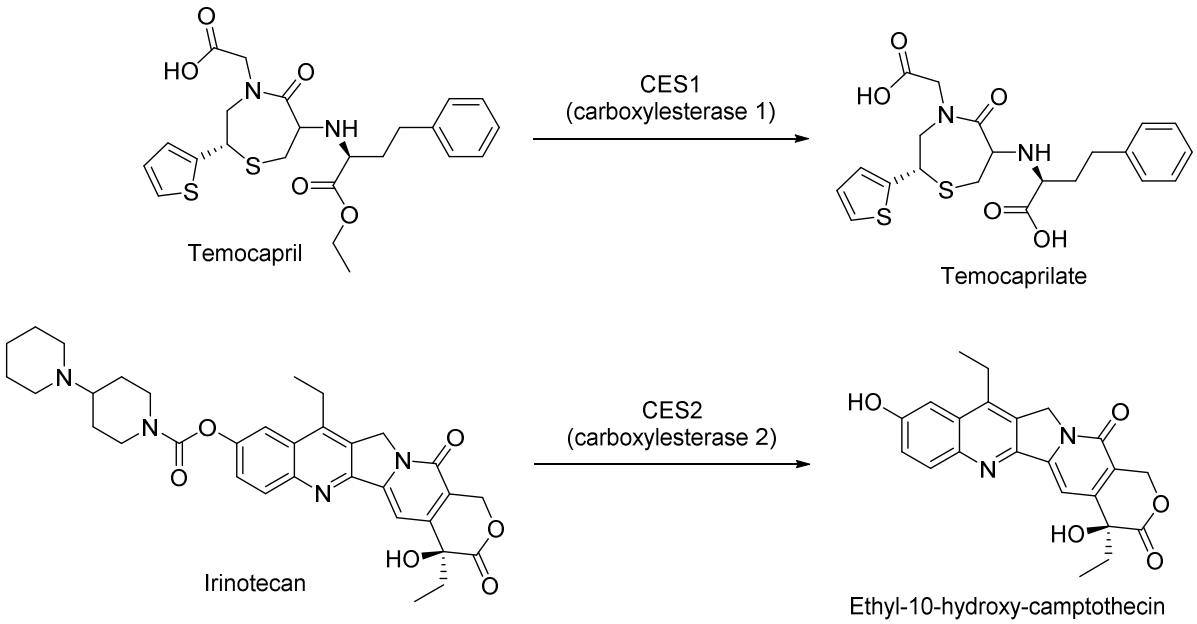


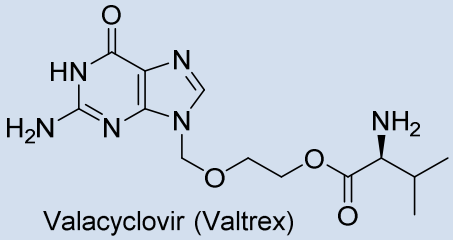
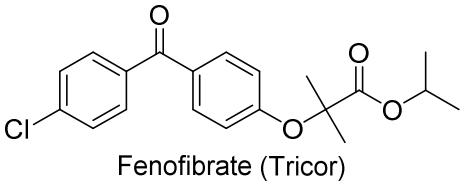
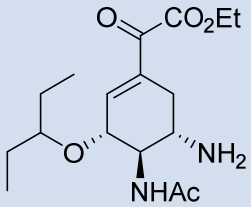
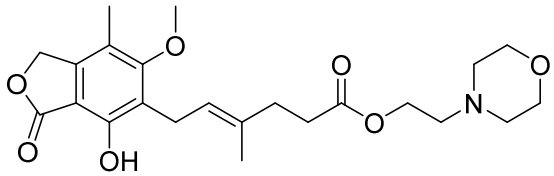
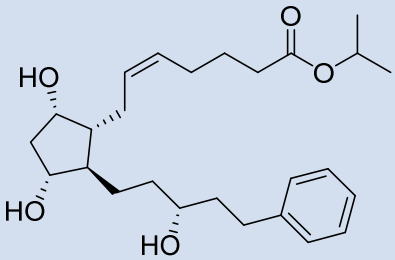
Figure 12: Bioactivation of ester prodrugs temocapril and irinotecan.[87]

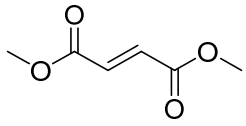
However, although being an attractive target for various prodrugs, the interspecies differences make it very difficult to translate the finding from preclinical studies with animals into humans. As an example, a minimal hydrolase activity in the small intestine of dogs was found, whereas rodent species frequently had higher hydrolase activity than humans.[93] A possible way to evade the interspecies variability would be to use human recombinant enzymes or human tissue extracts, which would help to identify tissue specific hydrolysis in prodrug development.

Another challenge with prodrugs was the less than complete absorption that was observed for several hydrolase-activated prodrugs such as penicillins and cephalosporins. These prodrugs were found to exhibit bioavailabilities of only around 50%, because of their premature hydrolysis in the enterocytes of the intestinal tract during absorption.[89] The hydrolysis within the enterocytes furnishes the active, most often more polar and less membrane permeable drug, which is the more likely to be effluxed into the lumen by passive and carrier-mediated processes rather than to move on into the blood stream, hence limiting the oral bioavailability.

In 2009, 15% of the 100 best-selling small-molecular-weight drugs were prodrugs.[87] Some of the prodrugs are listed in Table 13 with their labile functional group and the prodrug strategy that will lead to the active form.

Table 1: The occurrence of prodrugs among the world's 100 top-selling pharmaceuticals in 2009.[87]

Prodrug Name (Trade Name) and Therapeutic area	Functional Group	Prodrug Strategy
 Valacyclovir (Valtrex) Antiviral agent	L-Valyl ester of acyclovir	Bioconversion by valacyclovir hydrolase (valacyclovirase) Bioavailability improved from 20% (acyclovir) to 54% (valacyclovir)
 Fenofibrate (Tricor) Hypercholesterolemia	Isopropyl ester of fenofibric acid	Lipophilic ester of fenofibric acid
 Oseltamivir (Tamiflu) Influenza	Ethyl ester of oseltamivir carboxylate	Improved bioavailability, allowing oral administration
 Mycophenolate mofetil (CellCept) Immunosuppressant	Morpholinyl ethyl ester of mycophenolic acid	Improved oral bioavailability with less variability
 Latanoprost (Xalatan) Glaucoma	Isopropyl ester of latanoprost acid	Bioconversion by esterases, improved lipophilicity to achieve better ocular absorption and safety

 Dimethyl fumarate (Tecfidera) Relapsing multiple sclerosis	Dimethyl ester of fumaric acid	Bioconversion by esterases

3 Aims of work

Bacteria produce secondary metabolites to fulfill various functions. In this thesis we focused on two classes of metabolites, the phenazines and the pyrrolobenzodiazepines (PBDs). They both play a role in human infectious disease and the accurate understanding of the underlying mechanisms are necessary in order to control these diseases.

The class of phenazistatin derivatives was so far accessed via an ULLMANN-GOLDBERG amination, which often requires harsh conditions lacking selectivity and functional group tolerance. In order to generate libraries of highly functionalized Phenazistatin derivatives a more robust and mild method for the assembly of the phenazine backbone would be highly desirable. One strategy to generate this class of compounds would be the reductive amination of an anthranilic acid derivative with a substituted cyclohexanone (Figure 13). However, the existing protocols for reductive amination were not suitable for these substrates and very low reactivities were observed. The aim of the first part of this thesis was the development of a reliable method for the reductive amination to generate Phenazistatin derivatives.

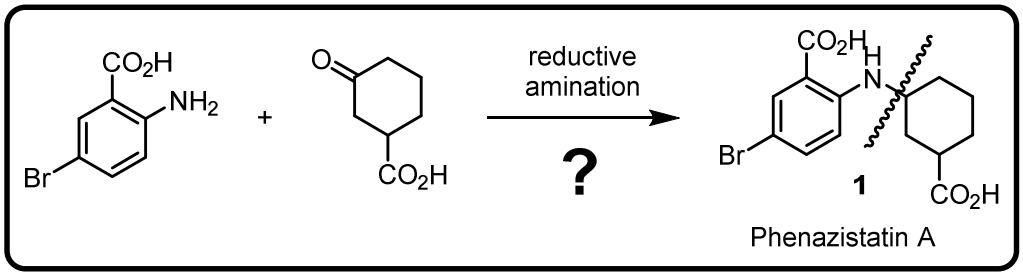


Figure 13: Strategy towards Phenazistatin A (**1**) via reductive amination.

The aim of the second part of this thesis was the generation of different screening sets of Phenazistatin derivatives in order to study the binding to PhzA/B (Figure 14). One part of this effort was the synthesis of inhibitors to increase the affinity to PhzA/B and therefore libraries of Phenazistatin derivatives substituted at the 5-position of the aromatic ring as well as different alicyclic ring sizes should be synthesized. The insights from the binding mode and the affinities to PhzA/B would allow for deducting detailed structure-activity relationships and propel future efforts in order to develop these inhibitors. Furthermore a special emphasis should be placed on the synthesis of Phenazistatin derivatives in order to improve the ADMET properties of the in this respect problematic lead compound. Therefore, inhibitor sets bearing bioisosteric replacements of the carboxyl group as well as prodrug esters of Phenazistatin A (**1**) should be generated. As an additional target the class of Maverick inhibitors should be diversified in order to study the binding behavior to PhzA/B, since the

Br-Maverick **2** exhibited the unprecedented event of simultaneous binding of a racemic mixture into the identical binding pocket of PhzA/B.[94]

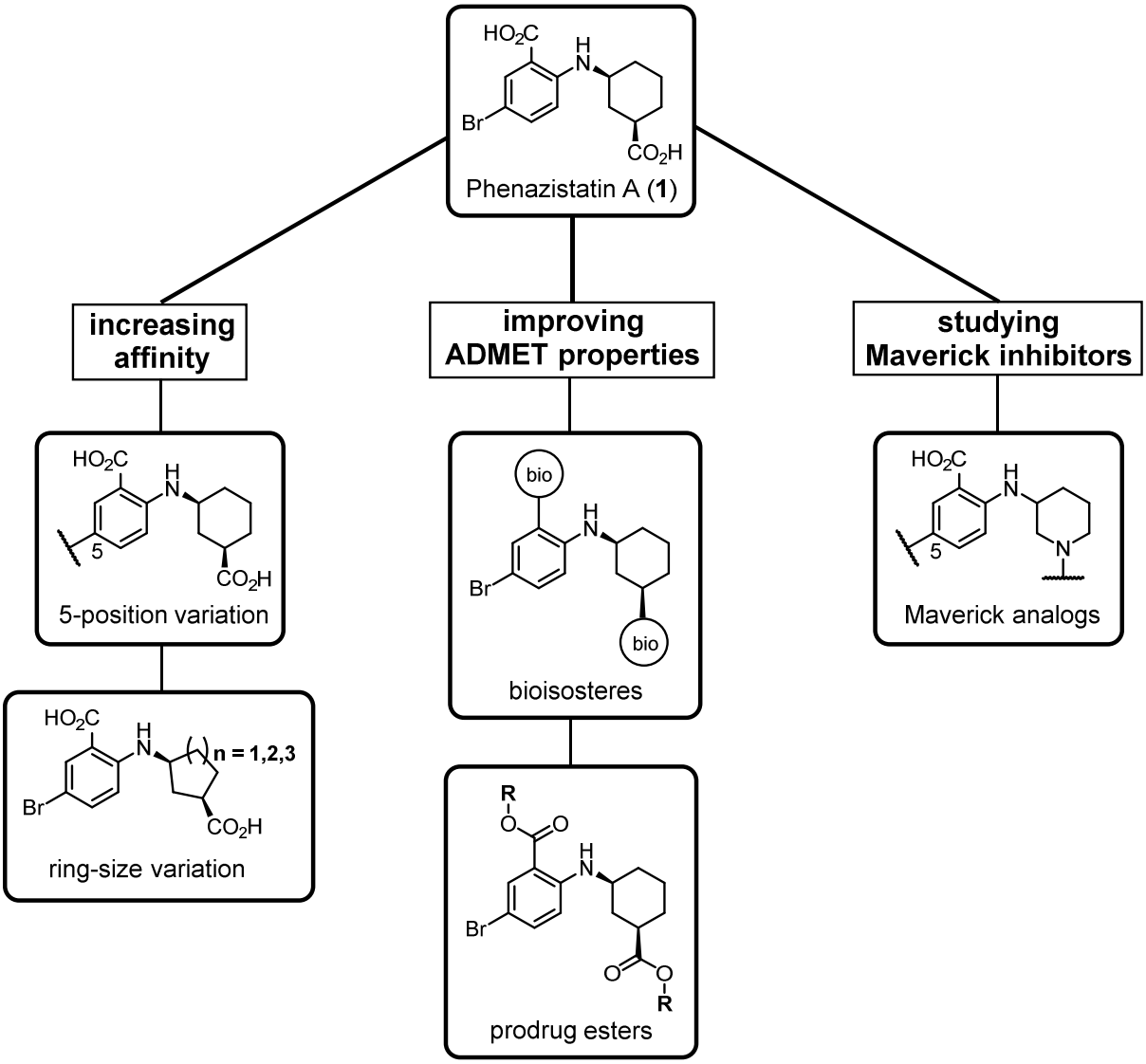


Figure 14: Aims for the diversification of the Phenazistatin and Maverick substance class.

The third part of this thesis was connected to the pyrrolobenzodiazepines, the second class of bacterial metabolites studied. In this case the biosynthetic pathway towards Tilivalline (**3**) and the physiological function of this compound is currently being investigated. Because the bacterial fermentations were low-yielding and not robust, **3** was produced synthetically. The main focus was the generation of sufficient amounts of Tilivalline **3** in order to fuel the biological studies in order to elucidate the physiological target of this PBD (Figure 15). The published total synthesis[95] starts from a quite expensive starting material and hence a cheaper route should be developed to enable the production of larger amounts of Tilivalline, if the need should arise (e.g. animal studies).

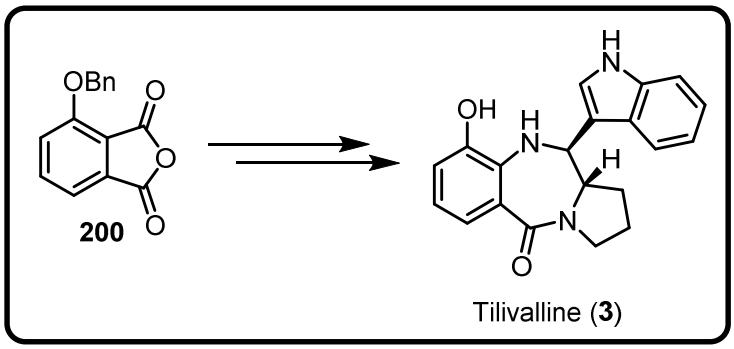


Figure 15: Total synthesis of Tilivalline (**3**).

4 Results and Discussion

4.1 Studies towards the reductive amination of electron-deficient anilines

4.1.1 A general and direct reductive amination of aldehydes and ketones with electron-deficient anilines

This part of the thesis has already been published in:

"A General and Direct Reductive Amination of Aldehydes and Ketones with Electron-Deficient Anilines" J. Pletz, B. Berg, R. Breinbauer, *Synthesis* **2016,** *48*, 1301–1317.
DOI: 10.1055/s-0035-1561384

4.1.1.1 Introduction

In our ongoing effort in preparing tool compounds for investigating and controlling the biosynthesis of phenazines, we recognized the limitations of existing protocols for C-N-bond formation. The ULLMANN-GOLDBERG amination proved to be quite inefficient for the C-N bond formation with these kind of substrates.[96–100] Despite considerable efforts and some success in improving the yields for some substrates by optimizing the reaction conditions, no reliable protocol could be found which was suitable for the diverse and highly functionalized substrates of the desired ligands. A different route towards the Phenazistatin derivatives is the C-N bond formation between the alkyl-C and the aromatic amine (Figure 16). This approach should lead to higher yields and have the additional advantage that the required ketone substrates are better available than the alicyclic amines necessary for the ULLMANN-GOLDBERG-route.

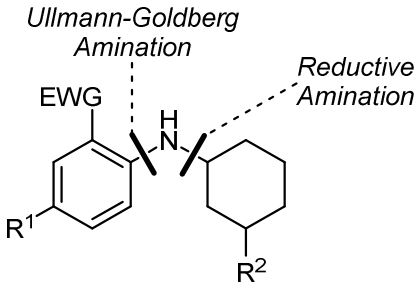


Figure 16: Strategic disconnections for the synthesis of PhzA/B-ligands.

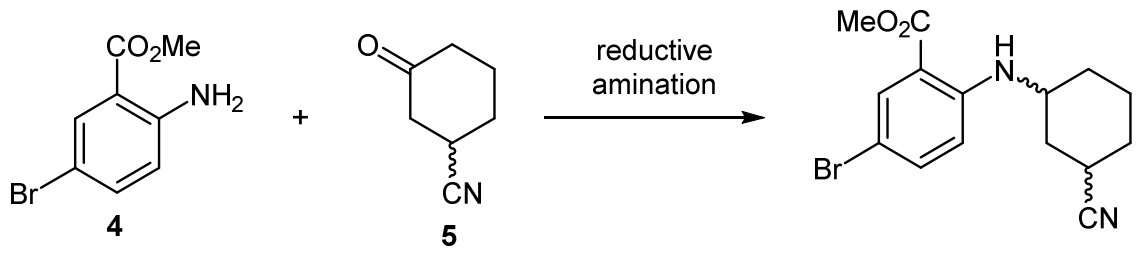
The lack of control of the resulting stereogenic center should not bother us at this early stage of biological testing with PhzA/B, because we have learned from the studies with the Br-Maverick **2** that racemic ligands could offer interesting surprises with this particular protein.[94]

First we had to develop more efficient protocols for reductive aminations as we soon noticed that all established protocols showed to be inefficient for our electron-deficient aniline substrates.

4.1.1.2 Results and discussion

Reductive aminations are one of the most frequently used synthetic reactions for the production of secondary (and tertiary) amines.[101–153] The advantages over other transformations are the ready accessibility of the starting materials and its selectivity avoiding overalkylation. A comprehensive review has been published in 2002,[154] in which the reaction with sterically congested ketone substrates and the reductive amination with electron-deficient amine nucleophiles have been defined as the remaining challenges in this field. The problematic step is often the initial imine formation due to the unreactive nature of either the carbonyl or amine component. Specific examples which have been described as test cases are the reaction of 2,6-disubstituted anilines with aldehydes[155] and aliphatic or alicyclic ketones,[102] of camphor with benzylamine[102] and the reaction of a β-keto ester with 2-fluoroaniline[156] (modest yield). Several methods which perform well specifically for the reductive amination of electron-deficient amines have been published.[102,103,106,107,116,157–159] We tested several of these methods for the reductive amination of methyl 2-amino-5-bromobenzoate (**4**) and 3-oxocyclohexane-1-carbonitrile (**5**) as model substrates (Table 2). Most reactions failed or gave only low conversions of the starting materials due to the unreactive nature of the aromatic amine.

Table 2: Screening of reductive amination methods.



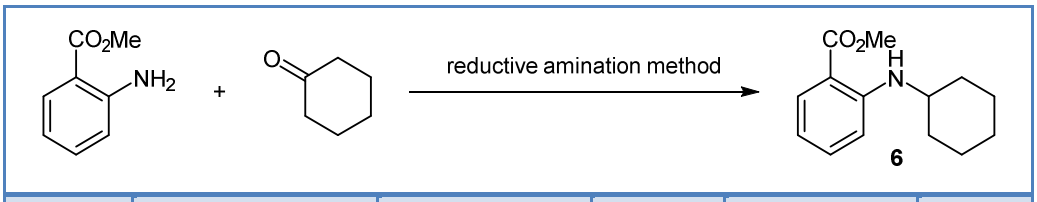
Entry	Conditions	Ref.	Product formation
1	1) toluene, 4 Å MS, reflux, Dean-Stark, 4 d 2) 3 eq $NaBH_4$, MeOH, N_2, RT, 6 h	[160]	--
2	1) PTSA, toluene, reflux, Dean-Stark, 2 d 2) 3 eq $NaBH_4$, MeOH, N_2, RT, 6 h	-	--
3	0.5 eq $TiCl_4$, 2.0 eq Et_3N, CH_2Cl_2, RT, 4 d	[161]	--
4	1) $Ti(O^iPr)_4$, neat, RT, 1 h 2) 0.55 eq $NaCNBH_3$, EtOH, RT, 3 d	[162]	--
5	1) 1.25 eq $Ti(O^iPr)_4$, neat, 40 °C, 16 h 2) 2 eq $NaBH_4$, 0 °C-RT, EtOH, 6 h	[163]	--
6	1) 1.25 eq $Ti(O^iPr)_4$, neat, 40 °C, 16 h 2) 2 eq PMHS, 0 °C-RT, THF, 6 h	[164]	--
9	2 eq $NaBH(OAc)_3$, $HC(OEt)_3/CH_2Cl_2$ = 1:1, RT, 23 h	[165]	--
10	1) 10 eq Na_2SO_4, AcOH, RT, 40 min 2) 3 eq $NaBH(OAc)_3$, RT, 24 h	[166]	0
11	2 eq $NaBH(OAc)_3$, 1 eq AcOH, toluene, RT, 6 d	[167]	0
12	2.8 eq $NaBH(OAc)_3$, 5 eq AcOH, DCE, RT, 30 h[a]	[102]	+
13	1.5 eq Hantzsch ester, 0.1 eq thiourea, 4 Å MS, toluene, 50 °C, 20 h	[123]	+
14	1.5 eq BH_3·THF, CH_2Cl_2/AcOH = 2:1, RT, 3 d	[141]	++

[a]methyl 3-oxocyclohexane-1-carboxylate was used as the ketone; --: no conversion, 0: moderate conversion; +: good conversion; ++: full conversion.

The methods which worked best for the synthesis of the phenazistatin A derivative in these initial screenings (Table 2) were investigated in detail. For further optimization the model

reaction was simplified to methyl anthranilate and cyclohexanone (commercially available) and the reactions were repeated using the same procedures as described in Table 2 (Entries 12, 13, 14). The results are listed in Table 3 (vide infra). The well-established $NaBH(OAc)_3$ (STAB)-method by Abdel-Magid[102] led to incomplete conversion of methyl anthranilate to the product **6** even after 1 week of reaction time (Table 3, Entry 1). The transfer hydrogenation approach by Menche using Hantzsch ester as hydride source and a thiourea organocatalyst[123] resulted in full conversion after 6 d at 50 °C (Entry 2). The third method was the reductive amination using $BH_3 \cdot THF$[141] (or $BH_3 \cdot SMe_2$)[142] in CH_2Cl_2/AcOH at RT, which has been described to enable the reaction of the very electron-deficient 2-nitroaniline with acetone (3 eq) in excellent yields within 16-20 h. However, our attempts to apply the non-optimized method for our model reaction led only to incomplete conversion. We observed that prolonged reaction time and increased carbonyl loading did not improve conversion but increasing the amount of reductant ultimately led to full conversion. In our experience a minimum of 3 eq of $BH_3 \cdot THF$ is needed to ensure full conversion for most substrates. When exploring the scope of this useful transformation with other substrates, optimized Method A (1.5 eq carbonyl substrate, 3 eq $BH_3 \cdot THF$) proved as a reliable and versatile method for the reductive amination of a wide range of electron-deficient amines (Table 3, Entry 3). The detailed substrate scope will be described in the following sections (Table 5, Entries 1A – 11A & Table 6, Entries 1A – 17A).

Table 3: Comparison of the reported reductive amination methods with established protocols.



Entry	Method	Temperature	Time	Conversion	Yield[a]
1	$NaBH(OAc)_3$[b]	RT	7 d	77%[c]	73%
2	Hantzsch ester[d]	50 °C	6 d	full	91%
3	Method A[e]	0 °C	3 h	full	80%
4	Method B[f]	0 °C	15 min	full	97%
5	Method C[g]	0 °C	15 min	full	99%

[a]isolated yield. [b]according to Abdel-Magid et al. 1.0 eq aniline, 2.0 eq ketone, 5 eq AcOH, 2.8 eq $NaBH(OAc)_3$, dichloroethane, RT.[102] [c]*n*-nonane used as an internal standard. [d]according to Menche et al. 1.0 eq aniline, 1.5 eq cyclohexanone, 1.5 eq Hantzsch ester, 0.1 eq thiourea, 5 Å MS, toluene, 50 °C.[123] [e]Method A: 1.00 mmol amine, 1.50 mmol cyclohexanone, 3.0 mmol (3.0 eq) $BH_3 \cdot THF$, CH_2Cl_2/AcOH = 2:1 (v/v), 0 °C to RT. [f]Method B:

300 µmol (1.0 eq) amine, 330 µmol (1.1 eq) cyclohexanone, 750 µmol (2.5 eq) TMSCl, 300 µmol (1.0 eq) BH_3·THF, DMF, 0 °C. [g]Method C: Method C: 300 µmol (1.0 eq) amine, 330 µmol (1.1 eq) cyclohexanone, 750 µmol (2.5 eq) TMSCl, 300 µmol (1.0 eq) $NaBH_4$, DMF, 0 °C

When applying Method A for the synthesis of various Phenazistatin derivatives using substituted aryl amines and cyclohexanones we sometimes obtained lower yields and noticed the formation of two by-products, namely *N*-acetylated and *N*-ethylated aniline substrate (Figure 17). Such by-products have been previously reported for the $NaBH(OAc)_3$-method by Abdel-Magid[102] when AcOH was used as Lewis-acid. In fact protocols for the *N*-ethylation of amines using $NaBH_4$ in neat carboxylic acid have been reported in the literature.[168–170] It is suggested that the product originates from a stepwise process in which acetic acid is reduced to acetaldehyde (or an acetaldehyde equivalent), which reacts with the amine to form an iminium ion. Reduction of this imine results in the ethylamine product.[168] Marchini could isolate the *N*-acetylated product by heating the $NaBH_4$-carboxylic acid mixture before addition of the amine.[170] During the synthesis of Phenazistatin derivative **7** we observed the formation of these by-products ((Figure 17, **2a** & **3a**), which accounted for considerable consumption of the substrate aryl amine.

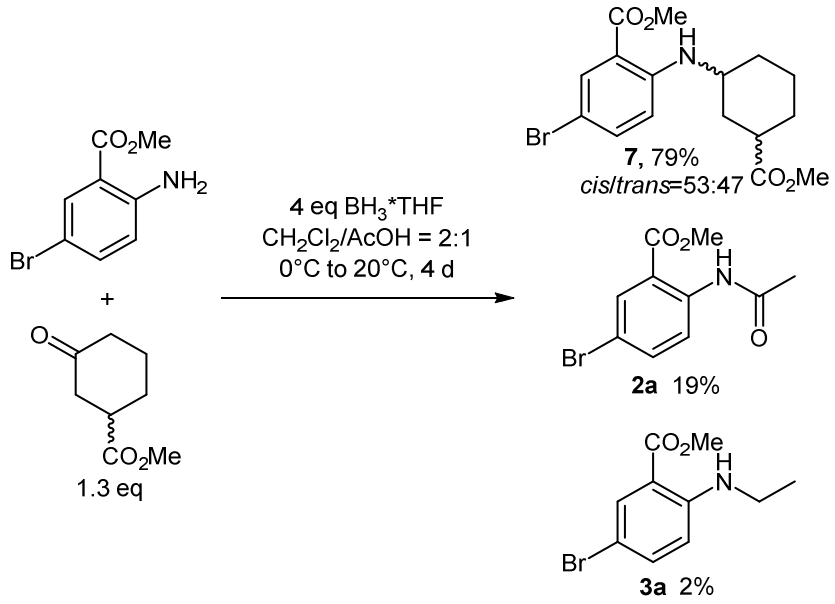


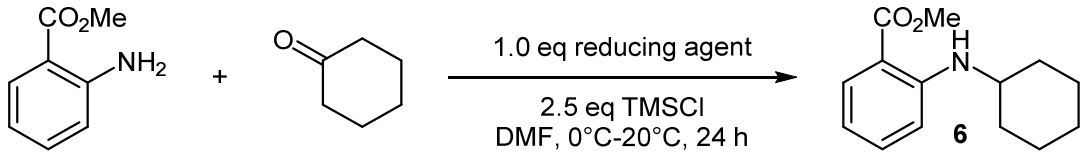
Figure 17: Observed by-product formation in the synthesis of a "Phenazistatin" derivative (percentage given refers to GC-MS area).

We reasoned that by using a different acid additive we might suppress these side reactions and increase the yields. Full conversion of the starting material was obtained with the acids (9 eq each) TFA (12 h), $MeSO_3H$ (3 d), malonic acid (13 h), phenylphosphonic acid (13 h) and the activating agent TMSCl (12 h) for the reaction of 2-aminobenzonitrile and 4-*tert*-butylcyclohexanone in rates similar to AcOH (12 h). Among these reagents TMSCl proved to be the most attractive substitute for AcOH due to its availability, low molecular weight, low

price, and most importantly by its lack of the formation of the common by-products mentioned above for AcOH. TMSCl has been used extensively in combination with boranes for the reduction of carbonyl substrates, along with borohydrides mainly for the in situ generation of borane.[171–177] Blacklock had used TMSCl as an additive for the reductive alkylation of ureas, thioureas and carbamates (with benzaldehydes, $NaBH_4$ in AcOH).[178,179] By screening different solvents we observed that DMF increases the reaction rate significantly and lowers the extent of carbonyl reduction. Optimization of the reaction parameters resulted in optimized Method B (1.1 eq carbonyl substrate, 2.5 eq TMSCl, 1.0 eq $BH_3 \cdot THF$), which gave 97 % in 15 min at 0 °C for the model reaction (Table 3, Entry 4). It is noteworthy, that the reaction proceeds to completion within minutes at 0 °C. An amount of 1.0 eq of $BH_3 \cdot THF$ sufficed for all tested reactions, but full conversion was detected for the reaction of 2-aminobenzonitrile with cyclohexanone even when using only 0.5 eq $BH_3 \cdot THF$. The reductive amination was carried out at high concentrations of the aryl amine in DMF (1.5 M) but was found to perform equally well in higher dilutions (0.5 M). We kept the solvent volume at a minimum to facilitate the removal of DMF during workup.

One parameter which requires special attention is the reducing agent borane-tetrahydrofuran complex. $BH_3 \cdot THF$ is commonly used as a 1.0 M solution in THF (stabilized with $NaBH_4$),[180] which is stable when stored and used at 0 °C, but loses hydride activity when kept at RT, creating a stability and safety concern.[180–184] While $BH_3 \cdot THF$ can be conveniently handled in a research laboratory, it would be desirable to substitute $BH_3 \cdot THF$ by a more inexpensive and inherently safer reducing agent for large-scale applications.[183] We conducted a screening of commonly employed hydride sources in combination with TMSCl as activating agent (Table 4).

Table 4: Test reaction with different hydride sources



Entry	Reducing agent	Conversion[a]	Yield (%)[b]	Prize [€/mol H][c]
1	PMHS	0%	-	14
2	Et_3SiH	0%	-	108
3	$PhSiH_3$	full	84%	218
4	$NaBH_4$	full	88%	6
5	$NaBH(OAc)_3$	92%	66%	207
6	BH_3·THF[d]	full	90%	64
7	pinacolborane	full	91%	350
8	9-BBN[e]	88%	81%	496
9	catecholborane	77%	73%	727

[a]1,2-DME was used as an internal standard. [b]isolated yield. [c]prices from Sigma-Aldrich catalog November 2015. [d]1.0 M solution in THF. [e]0.5 M solution in THF.

PMHS and Et_3SiH did not react using the reaction conditions of Method B (Table 4, Entries 1-2), while $PhSiH_3$ and the boron based reductants resulted in good to high conversions and yields (Entries 3-9). To our delight $NaBH_4$ performed equally well as BH_3·THF in the reductive amination (Entries 4 & 6) allowing us to define the cost efficient Method C (TMSCl, $NaBH_4$, DMF, 0 °C), as the price per hydride equivalent of $NaBH_4$ is significantly lower than for any other active reductant, with BH_3·THF the second least expensive one. In order to explore the substrate scope we conducted a series of experiments using Method C. The results are summarized in the following section. In Table 3 we have compared the performance of the best established procedures in the literature with our new Methods A-C for the same test substrate. Methods A-C deliver product **6** in much shorter reaction time and lower reaction temperatures.

So far all reported reactions have been performed in a dry solvent under inert conditions. To test the sensitivity of Method C towards moisture and air we performed the reaction of methyl anthranilate with cyclohexanone in an open flask using synthesis-grade (99.8%) DMF. We

were pleased to find that the same yield as for the inert conditions was achieved after 15 min reaction time at 0 °C (Table 5, Entry 7C*).

Based on previous studies by Abdel-Magid,[102] Borch,[185] Roth[114] and Schellenberg[186] we have proposed a possible mechanism outlined in Figure 18 suggesting a dual role of TMSCl. First, TMSCl activates the carbonyl substrate for nucleophilic attack by the amine. Second, it shifts the equilibrium to the imine by serving as a dehydrating agent,[177] which also provides the acid required for the formation of the iminium ion, which is the ultimate substrate for hydride attack (Figure 18).

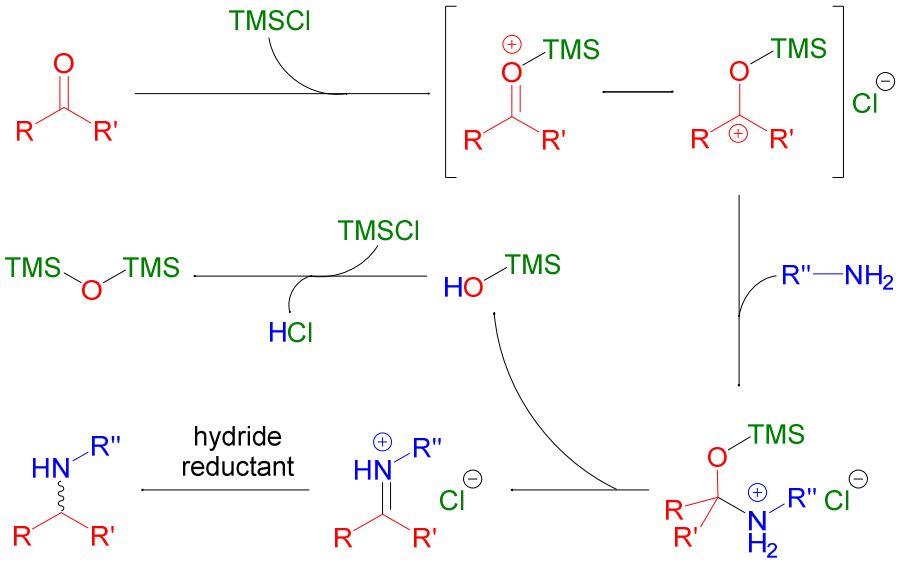


Figure 18: Proposed mechanism of the reductive amination process

4.1.1.2.1 Reductive amination with weakly nucleophilic amines

The described methods are very efficient in reductive amination reactions with weakly basic and nonbasic amines. The results in Table 5 show that a wide range of aryl amines can successfully react in the reductive amination with cyclohexanone. The reductive amination following the BH_3·THF/AcOH/CH_2Cl_2 method (Table 5, Method A) with reaction times from 3-41 h is generally slower than the BH_3·THF/TMSCl/DMF method (Table 5, Method B) and the $NaBH_4$/TMSCl/DMF method (Table 5, Method C), which typically require 10-230 min. It is noteworthy that Method B and Method C proceed at 0 °C within minutes while Method A and other described methods for electron-deficient aromatic amines require room or even higher temperatures and reaction times of hours to days. We found that Method B (BH_3·THF/DMF) and Method C ($NaBH_4$/DMF) yielded comparable results for most of the aromatic amines tested, however, Method B (BH_3·THF/DMF) was chosen for the study of reactive carbonyl groups in the next section (Table 6) as the handling of the liquid BH_3·THF solution was more suitable for parallel operations on a small scale. While there is a significant difference between Method A and Method B/Method C in matters of reactivity and reaction rate, we

observed that the selectivity and tolerance for functional groups seems to be conserved in most cases.

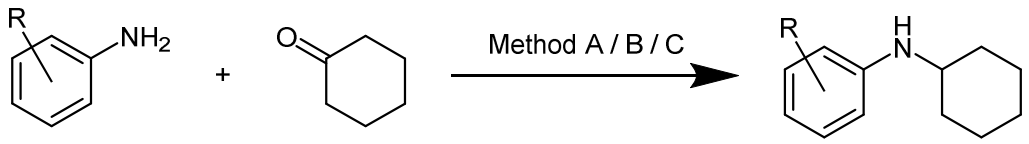
One case in which the selectivity differs between Method A and Method B is the reductive amination of 4-acetylaniline. Method A gives the product **8** in low yields (Table 5, Entry 8A), due to a large amount of self-condensation of the aromatic ketone with the aniline whereas in Method B the aromatic ketone moiety remains untouched and the product **8** is obtained in good yield (Entry 8B).

The described methods give good yields for electron-rich primary anilines and a wide range of electron-deficient primary aromatic amines. The reactions are convenient and simple and show a high degree of tolerance for a variety of functional groups including acetyl, alkoxycarbonyl, carboxy, cyano, diethylphosphonyl, halo, and nitro groups.

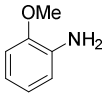
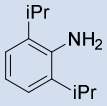
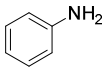
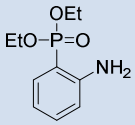
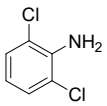
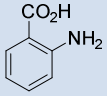
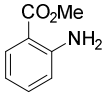
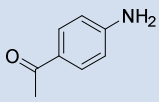
Substitution at the *ortho*-position of the aromatic amines did not seem to have any detrimental effect on the reaction rate and high to excellent yields were obtained (Table 5). As mentioned above the reductive amination of 2,6-disubstituted anilines has been described in the literature as a challenging substrate combination.[102,187] The sterically hindered 2,6-dichloroaniline reacted very slowly using Method A (Entry 5A, 18% conversion after 6 d), but to our delight reacted smoothly with Method B and Method C in 90% resp. 87% yield (Entries 5B & 5C) to give product **9**. The most sterically hindered aniline used, 2,6-diisopropylaniline, failed to undergo reaction with cyclohexanone using both Method B and Method C (Table 5, Entries 2B & 2C). However, the reactivity of Method B could be enhanced by the use of TMSOTf instead of TMSCl (Table 5, Entry 2B*). The reaction proceeded very slowly (230 min) but gave the product **10** in 66% yield.

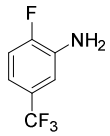
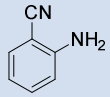
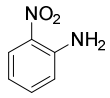
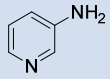
The very electron-deficient 2-nitroaniline gave product **11** in 94% yield after 30 min at 0 °C using Method B (Entry 11B). Abdel-Magid[102] could only obtain 30% conversion to the desired product after 6 d at RT when using the optimized $NaBH(OAc)_3$ conditions. Anthranilic acid reacted nicely with Methods A & B to give product **12** in 91% (Table 5, Entries 6B & 6C). Interestingly, the heterocyclic aryl amine 3-aminopyridine reacted to the reductive amination product **15** in 84% yield using Method B (Entry 12B).

Table 5: Investigation of the aniline substrate scope for the reductive amination according to Methods A, B and C



Method A: amine (1 eq), ketone (1.5 eq), BH_3·THF (3 eq), CH_2Cl_2/AcOH[a]
Method B: amine (1 eq), ketone (1.1 eq), TMSCl (2.5 eq), BH_3·THF (1 eq), DMF[b]
Method C: amine (1 eq), ketone (1.1 eq), TMSCl (2.5 eq), $NaBH_4$ (1 eq), DMF[c]

Amine	Product	Entry	Method	Time	Yield (%)[d]
	**13**	1A 1B 1C	A B C	21 h 15 min 15 min	81 85 90
	**10**	2B 2B* 2C	B B[e,f] C	140 min 230 min 15 min	0 66[g] 0
	**14**	3A 3B 3C	A B C	19 h 15 min 15 min	77 77 70
	**15**	4A 4B	A B	3 h 10 min	89 72
	**9**	5A 5B 5C	A B[f] C	6 d 18 min 15 min	(18[h]) 90 87
	**12**	6B 6C	B C	10 min 15 min	91 91
	**6**	7A 7B 7C 7C*	A B C C[i]	3 h 15 min 15 min 15 min	80 97 99 99
	**8**	8A 8B	A B	21 h 15 min	29 79

	**16**	9A 9B 9C	A B C	3 h 10 min 15 min	72 68 67
	**17**	10A 10B 10C	A B C	15 h 18 min 15 min	94 94 91
	**11**	11A 11B 11C	A B C	21 h 30 min 30 min	74 94 78
	**18**	12B	B	11 min	84

[a]Method A: 1.00 mmol amine, 1.50 mmol cyclohexanone, 3.0 mmol (3.0 eq) BH_3·THF, CH_2Cl_2/AcOH = 2:1 (v/v), 0 °C to RT. [b]Method B: 300 µmol (1.0 eq) amine, 330 µmol (1.1 eq) cyclohexanone, 750 µmol (2.5 eq) TMSCl, 300 µmol (1.0 eq) BH_3·THF, DMF, 0 °C. [c]Method C: 300 µmol (1.0 eq) amine, 330 µmol (1.1 eq) cyclohexanone, 750 µmol (2.5 eq) TMSCl, 300 µmol (1.0 eq) $NaBH_4$, DMF, 0 °C. [d]isolated yield. [e]TMSOTf was used instead of TMSCl. [f]1.5 eq cyclohexanone were used. [g]carbonyl reduction was observed. [h]conversion of the starting material (*n*-nonane used as internal standard). [i]performed in an open flask using synthesis-grade DMF (99.8%).

4.1.1.2.2 Reductive amination of aldehydes and ketones

The results of Table 6 show that the reductive amination of a variety of aromatic aldehydes as well as cyclic and acyclic ketones with test electron-deficient anilines **H**-**I** was successfully accomplished under the standard conditions (Method A and Method B) and furnished the products in moderate to excellent yields. The scope of the reaction includes different aromatic aldehydes (Table 6: Entries 1A, 1B, 2B), cinnamic aldehyde (Entry 3B), alicyclic ketones (Entries 5A, 8A, 9A, 4B–9B), 2-adamantanone (Entries 9A & 9B), saturated acyclic ketones (Entries 10A, 11A, 11B, 12B, 13B*), methyl 3-oxobutanoate (Entry 12B), acetophenone (Entry 16A, 16B*) and 1,1-diethoxycyclohexane (Entry 18B). For the same primary aromatic amine the rate of the reaction was dependent on the steric and electronic factors associated with the carbonyl substrates as well as with the reductive amination protocol used. The substrates reacted consistently more sluggish using the BH_3·THF in CH_2Cl_2/AcOH system (Method A) as compared to the BH_3·THF/TMSCl/DMF system (Method B).

Aldehydes and ketones are known to be reduced by BH_3·THF[185,186] and $NaBH_4$[188] and thus carbonyl reduction could be expected to compete with the reductive amination process.[102] However, the chosen reaction conditions were so selective that the reductive amination with aldehydes and ketones worked efficiently and resulted in clean reactions in most cases.

Cases in which carbonyl reduction was detected involved the sterically hindered 2-(*tert*-butyl)cyclohexan-1-one in both Method A and Method B (Table 6, Entries 14A, 14B, 14B*), 2,6-diisopropylaniline in the modified Method B (Table 5, Entry 2B*), and acetophenone when using the modified Method B (Table 6, Entry 16B*).

Of all the carbonyl substrates used in this study, the alicyclic ketones were the most reactive and gave very good to excellent yields (Table 6, Entries 5A–9A, 4B–9B). Aldehydes gave somewhat lower yields with comparable reaction times (Entries 1A, 1B-3B), but in contrast to established silane based methods we did not observe any over-alkylated product. *trans*-Cinnamaldehyde reacted smoothly with methyl anthranilate providing **19** in 81% yield with no trace of C=C reduction product (Entry 3B), which is a common side reaction with borane reagents. Saturated acyclic ketones reacted more sluggishly and gave lower yields (Entries 10A, 11A, 11B, 13B*). The β-keto ester methyl 3-oxobutanoate (Entry 12B) reacted slowly and gave product **29** in 67% yield. The acid labile ketone 1-Boc-3-piperidone could be reacted nicely with methyl anthranilate using Method A producing **20** (66%) (Entry 8A), whereas Method C yielded a complex mixture. Method A might be the better method for the coupling of more acid-sensitive carbonyl and aryl amine substrates.

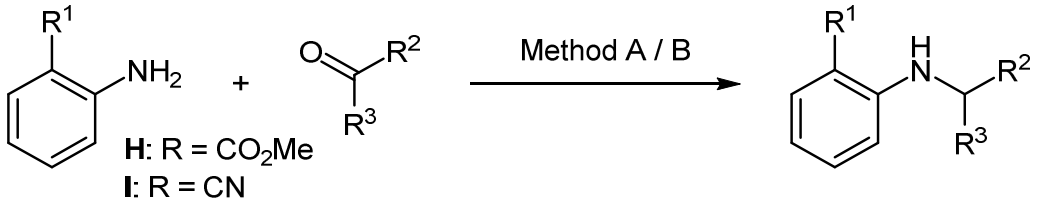
4.1.1.2.3 Reductive amination of aromatic and sterically hindered ketones

The least reactive ketones were aromatic and sterically hindered alicyclic and aliphatic ketones. It is noteworthy, that acetophenone reacted smoothly using Method A to give the desired product **21** in good yields, whereas the unmodified Method B failed (Table 6, Entries 16 A & 16B). For other unreactive carbonyl substrates the aryl amine substrate was quantitatively acetylated to the by-product *N*-(2-cyanophenyl)acetamide, as judged by GC-MS (Entries 14A, 15A, 17A). The formation of small amounts of these by-products was also described for some slow reactions using the established $NaBH(OAc)_3$-protocol.[102] Benzophenone, 2-(*tert*-butyl)cyclohexan-1-one and (+)-camphor failed to react with 2-aminobenzonitrile using Method A (Table 6, Entries 14A, 15A, 17A). These substrates as well as acetophenone and benzophenone could also not be converted in the reaction with methyl anthranilate when using Method B (Table 6, Entries 13B, 14B, 15B, 16B, 17B).

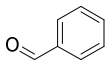
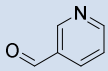
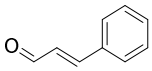
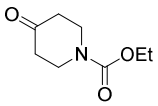
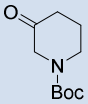
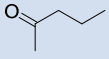
However, the reactivity could be enhanced by using the more reactive TMSOTf instead of TMSCl as demonstrated for the reaction with pinacolone and acetophenone giving the products **22** and **23** in good yields (Entries 13B*, 16B*) after reaction times of 20 h and 23 h, respectively. However, even with the TMSOTf-modified Method B, the other challenging ketones did hardly react with methyl anthranilate and only traces (<5%) of the desired products were detected by GC-MS (Entries 14B*, 15B*, 17B*) after 18-23 h. Interestingly,

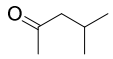
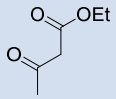
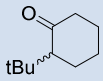
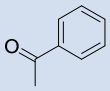
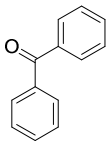
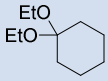
Method B can also be used for the reductive amination with ketals, as exemplified for 1,1-diethoxycyclohexane as a substrate (Entry 18B).

Table 6: Investigation of the carbonyl substrate scope for the reductive amination according to Methods A and B



Method A: amine (1 eq), ketone (1.5 eq), BH_3·THF (3 eq), CH_2Cl_2/AcOH[a]
Method B: amine (1 eq), ketone (1.1 eq), TMSCl (2.5 eq), BH_3·THF (1 eq), DMF[b]

"Carbonyl"	Entry	Amine	Method	Time	Product, Yield (%)[c]
	1A 1B	**I** **H**	A B	15 h 20 min	**24**, 80 **25**, 85
	2B	**H**	B	10 min	**26**, 84
	3B	**H**	B	10 min	**19**, 81
	4B	**H**	B	15 min	**27**, 96
	5A 5B	**I** **H**	A B	15 h 15 min	**17**, 94 **6**, 97
	6B	**H**	B	15 min	**28**, 91
	7B	**H**	B	15 min	**29**, 96
	8A	**H**	A	44 h	**20**, 66
	9A 9B	**I** **H**	A B	41 h 24 min	**30**, 80 **31**, 94
	10A	**I**	A	39 h	**32**, 80

	11A 11B	**I** **H**	A B	39 h 23 h	**33**, 69 **34**, 43
	12B	**H**	B	6 h	**35**, 67
	13B 13B*	**H** **H**	B B[d,e]	17 h 20 h	0 **22**, 50
	14A 14B 14B*	**I** **H** **H**	A B B[d,e]	24 h 17 h 18 h	0[f] 0[f] Traces[g,f]
	15A 15B 15B*	**I** **H** **H**	A B B[d,e]	41 h 23 h 18 h	0 0 Traces[g]
	16A 16B 16B*	**I** **H** **H**	A B B[d,e]	39 h 23 h 23 h	**21**, 70 0 **23**, 87[f]
	17A 17B 17B*	**I** **H** **H**	A B B[d,e]	4 d 17 h 20 h	0 0 Traces[g]
	18B	**H**	B	19 min	**6**, 71[h]

[a]Method A: 1.0 mmol amine, 1.5 mmol cyclohexanone, 3.0 mmol (3.0 eq) BH_3·THF, CH_2Cl_2/AcOH = 2:1 (v/v), 0 °C to RT. [b]Method B: 300 µmol (1.0 eq) amine, 330 µmol (1.1 eq) cyclohexanone, 750 µmol (2.5 eq) TMSCl, 300 µmol (1.0 eq) BH_3·THF, DMF, 0 °C. [c]isolated yield. [d]TMSOTf was used instead of TMSCl. [e]1.5 eq cyclohexanone were used. [f]carbonyl reduction was observed. [g]product mass was identified with GC-MS (<5%). [h]methyl 2-(cyclohexylamino)benzoate was obtained as the product.

In order to demonstrate the scalability of Method B the gram-scale synthesis of the Phenazistatin derivatives **7** and **36** (Figure 19) was performed, which delivered **7** and **36** in 96% and 97% yield, respectively.

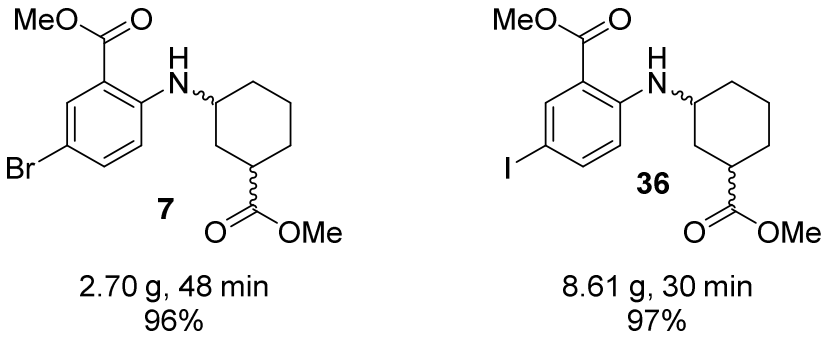


Figure 19: Gram-scale synthesis of Phenazistatin derivatives using Method B

4.1.1.3 Summary and conclusions

In conclusion we have established three new methods which have proven useful for the reductive amination of electron-deficient anilines with ketones (Figure 20). Method A (BH_3·THF/AcOH/CH_2Cl_2) is distinguished by inexpensive reagents and simple workup, but requires longer reaction times and gives rise to acetylated by-products for substrate combinations with slow imine formation. For these substrates, the more reactive activating agent TMSCl is recommended and both Method B (BH_3·THF/TMSCl/DMF) as well as Method C ($NaBH_4$/TMSCl/DMF) offer powerful reagent combinations, which result in full conversions for most substrates within 10-25 min. In case these methods fail, the use of TMSOTf offers additional opportunities to make sterically more congested substrates accessible. Together, these methods have expanded the scope of reductive amination reaction, which will become an even more powerful tool for the organic synthesis of substituted amines.

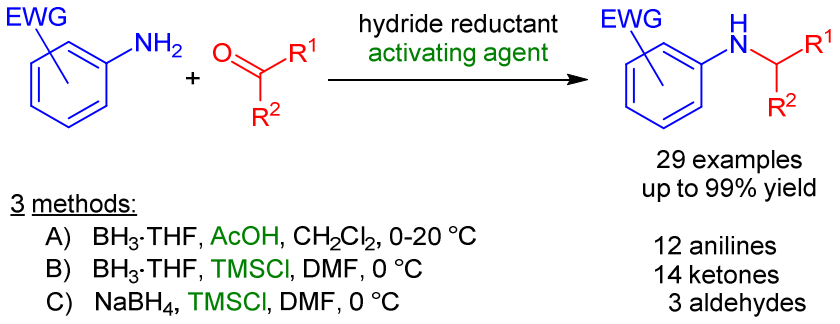


Figure 20: Summary of the developed methods for the reductive amination of electron-deficient amines.

4.1.2 Scope and limitations of the reductive amination methods

The developed reductive amination protocols were found to be very useful for the synthesis of derivatives of Phenazistatin A, which feature an anthranilate motif and a carbocyclic ketone. These procedures were quite generally applicable for the reaction of a variety of electron deficient aromatic amines as well as a broad range of ketones. This chapter is dedicated to point out the limitations of the published protocols, and to discuss limiting parameters as well as highlight some unusual side reactions that might prove useful for future syntheses.

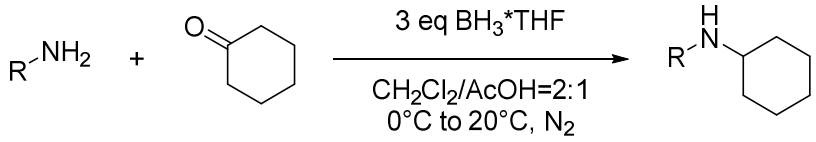
4.1.2.1 Limitations of method A

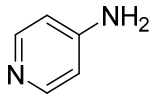
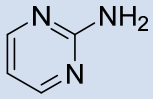
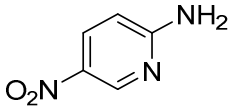
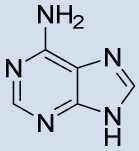
Method A ($BH_3 \cdot THF$, CH_2Cl_2/AcOH) with its simple experimental setup and easily available reagents exhibits a wide substrate scope as was shown in several experiments in the studies of Pletz.[189] However, there are some classes of amine substrates and carbonyl substances, for which the reaction failed or gave inferior results to those already established.

4.1.2.1.1 Failed amine substrates with method A ($BH_3 \cdot THF$, CH_2Cl_2/AcOH)

One of those substrate classes were the heteroaromatic amines depicted in Table 7. When method A was applied to 4-aminopyridine or pyrimidin-2-amine (Entries 1 & 2), full conversion of the starting material was detected by TLC after 3 h and 2 d, respectively. The product could not be identified in the mixture using HPLC-MS. For 4-aminopyridine a colorless precipitate formed throughout the reaction und after treatment with saturated $NaHCO_3$ (standard workup) this precipitate dissolved. However, the reaction monitoring was difficult and TLC and HPLC gave inconclusive results (Entry 1). Pyrimidin-2-amine (Entry 2) seems to have a very low solubility in the reaction solvents and this low solubility hampers also TLC monitoring of the reaction. The reaction was found to be complete by TLC analysis after 2 d, and two spots were generated. The products could not be purified and identified. 5-Nitropyridin-2-amine (Entry 3) showed very little conversion to two less polar products after 2 d, but upon prolonged stirring at RT the reaction mixture darkened and TLC indicated a complex mixture. When adenine (Entry 4) was used as the amine substrate, a mixture of products was obtained after 18 h. HPLC-MS of this mixture showed a complex mixture of products and the corresponding product mass could not be found.

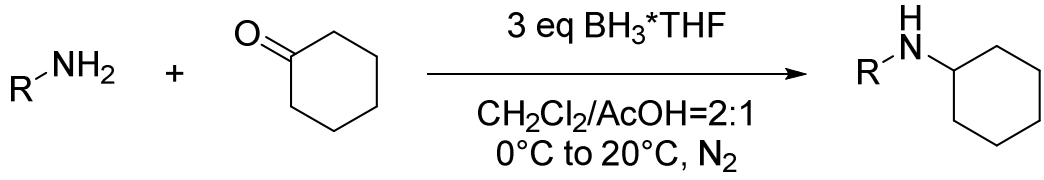
Table 7: Failed aromatic amine substrates for reductive amination with Method A.

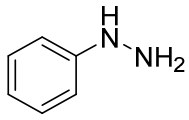
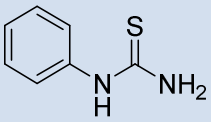
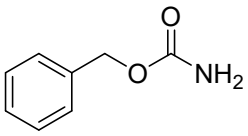
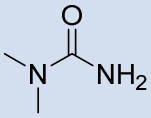
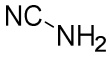
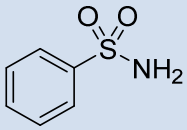


Entry	"amine"	time	conversion [%]	comments
1		3 h	100	inconclusive
2		2 d	100	low solubility inconclusive
3		2 d	>0	very little conversion after 8 d: complex mixture inconclusive
4		18 h	incomplete	complex mixture (HPLC) product not identified

We also tried to extend the scope of the reductive amination to other amine substrates, e.g. the ureas, as it has been shown that these substrates also show certain reactivity towards reductive amination.[102,103,178,179] Therefore, we conducted a screening with cyclohexanone as the carbonyl substrate and the unmodified method A[189] as reaction conditions (Figure 8). Under these conditions phenylhydrazine (Entry 1) was not fully converted after 18 h and a brownish reaction mixture was obtained. TLC indicated a mixture of many products. 1-Phenylthiourea (Entry 2) and benzyl carbamate (Entry 3) showed similar reactivity when subjected to method A. After 8 d low conversion was detected by TLC and numerous by-products had formed, showing that method A is both too unreactive and unselective for these substrate classes. 1,1-Dimethylurea (Entry 4), cyanamide (Entry 5) and benzenesulfonamide (Entry 6) as substrates were partially converted to a less polar product, but the corresponding product masses could not be found in HPLC-MS.

Table 8: Failed “amine” substrates for reductive amination with Method A.

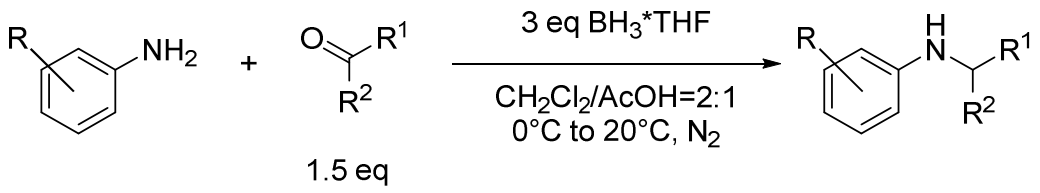


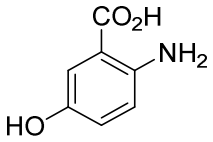
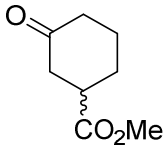
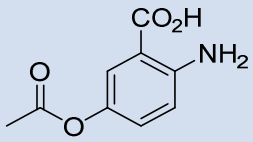
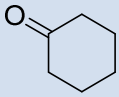
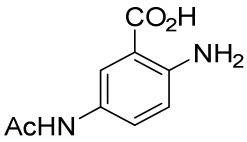
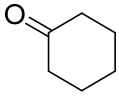
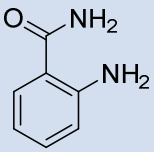
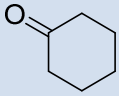
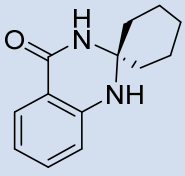
Entry	“amine”	time	conversion of amine [%]	comments
1		18 h	incomplete	complex mixture many spots on TLC
2		8 d	incomplete	low conversion complex mixture
3		8 d	incomplete	low conversion complex mixture
4		2 d	incomplete	less polar product formed mass not found in HPLC-MS
5		2 d	100	less polar product formed mass not found in HPLC-MS
6		2 d	100	less polar product formed mass not found in HPLC-MS,

Method A was found to perform poorly for aromatic amine substrates bearing *N*- or *O*-substituents in 5-position (Table 9, Entries 1-3). 5-Hydroxyanthranilic acid (Entry 1) was partially converted after 3 d at RT to several less polar by-products (all 366 nm UV active) and the product could not be identified as one of those (HPLC-MS). When 5-acetoxy-2-aminobenzoic acid (Entry 2) or 5-acetamido-2-aminobenzoic acid (Entry 3) were used, the substrate was partially converted to only one less polar product. However, also in this case the corresponding product masses could not be found in HPLC-MS. When 2-aminobenzamide (Entry 4) was used as a substrate it was neatly converted to a less polar compound. After workup and purification the ^{1}H- and ^{13}C-APT spectra did not fit with the predicted data and comparison of published spectra led to the conclusion that the spiro-2,3-

dihydroquinazolin-4(1*H*)-one **37** had formed. It was found that 2-aminobenzamide and aldehydes and ketones are known to easily react to the spiro compounds,[190–193] even in the absence of any reagent or catalyst.[194]

Table 9: Failed aromatic amine substrates bearing acidic protons with Method A.



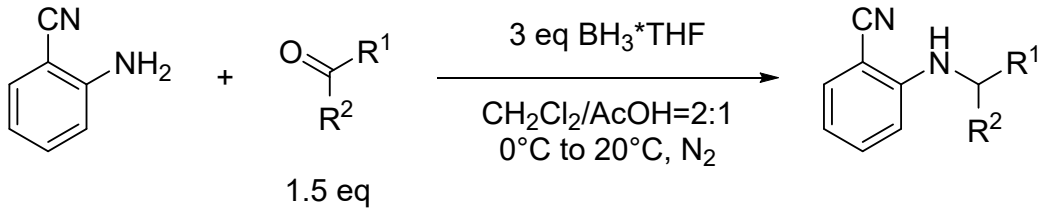
Entry	amine	carbonyl	Time	comments
1			3 d	some conversion several by-products
2			5 d	some conversion traces of less polar product (after 1 d: same TLC)
3			5 d	some conversion traces of less polar product (after 1 d: same TLC)
4			1 d	full conversion only spiro-product formed!  **37** ➔same results as when method B or method C are used!

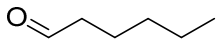
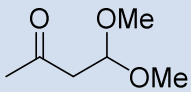
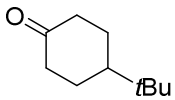
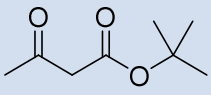
4.1.2.1.2 Failed carbonyl substrates with method A (BH_3·THF, CH_2Cl_2/AcOH)

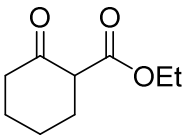
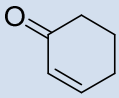
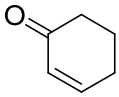
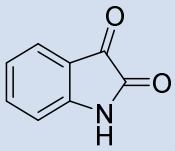
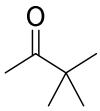
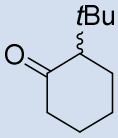
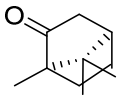
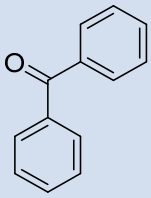
Table 10 shows the carbonyl substrates that have failed to undergo reductive amination with 2-aminobenzonitrile using method A. Full conversion in every case corresponds to 2-aminobenzonitrile, which was the limiting substrate. With hexanal (Entry 1) the amine was fully converted within 14 h, but a complex mixture was formed as judged by TLC. The product could not be identified with GC-MS. With the acetal 4,4-dimethoxybutan-2-one (Entry 2) the amine was fully converted after 44 h (TLC) and only one slightly less polar product was formed, which exhibited an intensive orange staining with CAM. GC-MS shows a quite pure product with a mass of 201.0 but it could not be determined which product had formed. This result indicates that acetals presumably cannot be easily coupled using the developed reductive amination procedures as it was the case with the ketal of cyclohexanone, which reacted smoothly to the corresponding product albeit with slightly lower yield (vide supra). With the sterically well-accessible 4-(*tert*-butyl)cyclohexan-1-one (Entry 3) full conversion of the starting material was obtained after 15 h and 96% of product were isolated after column chromatography. The isomers could not be separated via column chromatography and hence it was not possible to assign the stereochemistry to the two GC-MS peaks with the corresponding product structure (ratio of lower t_R/higher t_R = 67:33). The β-keto ester *tert*-butyl 3-oxobutanoate (Entry 4) reacted equally well (4.5 h) with 2-aminobenzonitrile as the corresponding methyl ester (vide infra, Table 12, Entry 6B), but due to a spillage during workup an isolated yield cannot be stated. The alcohol substituent on the β-keto ester seems to have little impact on the reactivity of the above mentioned methods, since the *tert*-butyl- and methyl-ester react equally fast. However, when using the cyclic β-keto ester ethyl 2-oxocyclohexane-1-carboxylate (Entry 5), the reaction was not complete after 41 h (TLC & GC-MS) and a complex mixture of products was generated. In this case a considerable quantity of acetylated amine substrate was detected in GC-MS. With the α,β-unsaturated cyclohex-2-en-1-one (Entries 6 & 7) full conversion of the amine was detected after 1 d and 15 h, respectively. In the reaction that lasted 1 d (Entry 6), 71% of the total area% of the GC-MS analysis corresponded to the fully reduced methyl 2-(cyclohexylamino)benzoate (**6**), no trace of the desired unsaturated product could be identified. In the reaction which was worked up earlier, already after 15 h (Entry 7), 45% of the fully reduced product **6** could be isolated. Here again no trace of the unsaturated product could be found, which indicates that the reduction process might proceed in the same time scale as the reductive amination process and that control of the saturation/unsaturation will be difficult to achieve by method A. Additionally, in GC-MS a substantial amount of the acetylated substrate amine was detected. The biologically interesting ketone isatin (Entry 8) yielded a complex dark-brown mixture after 41 h and no product could be identified by GC-MS or HPLC-MS. When method A was applied to very bulky carbonyl compounds, the

reaction did not give the desired outcome (Entries 9-12). With pinacolone (Entry 9) several by-products were formed after 41 h (full conversion, TLC) and only 6% of the product was isolated after column chromatography. 2-(*tert*-Butyl)cyclohexan-1-one (Entry 10) did not react to the product and 44% of the acetylated amine substrate (main product) were detected by GC-MS (total area%). In this case the ratio of cyclohexanone-cyclohexanol was 30/12 (area% GC-MS) after 24 h, which once again highlights how slow the reduction of the carbonyl substrate is in this solvent system. When the sterically challenging (+)-camphor was used, the amine substrate was neatly converted to the acetylated amine substrate within 41 h with no trace of the desired product (GC-MS). With the electronically challenging benzophenone (Entry 12) the amine substrate 2-aminobenzonitrile was fully converted after 24 h and was, as with (+)-camphor above, transformed into the acetylated substrate amine. Interestingly no ketone reduction was observed and traces of the desired intermediary imine were detected by GC-MS. The present hydride species seems to have a too low reactivity to reduce benzophenone or the intermediary imine.

Table 10: Failed “carbonyl” substrates for reductive amination with Method A.



Entry	“carbonyl”	time	Conversion of amine [%] [%]	comments
1		14 h	100	complex mixture many by-products generated
2		44 h	100	slightly less polar product intensive orange staining (CAM)
3		15 h	100	96% isolated isomers not separated lower t_R/higher t_R = 67:33 (GC-MS)
4		4.5 h	100	not isolated as fast as methyl ester

5		41 h	incomplete	complex mixture acetylated substrate amine generated
6		1 d	100	71% reduced product **6** (GC-MS)
7		15 h	100	several by-products acetylated substrate amine detected 45% **6** isolated (reduced product)
8		41 h	100	complex mixture acetylated amine substrate detected (GC-MS)
9		41 h	100	several by-products 6% isolated
10		24 h	100	30% ketone, 12% alcohol, 44% acetylated substrate amine (GC-MS) no trace of product
11		41 h	100	only acetylated substrate amine formed no trace of product
12		24 h	100	77% ketone, 21% acetylated substrate amine (GC-MS area) traces desired imine

4.1.2.2 Studies concerning the acid additive of Method A

In our efforts to synthesize Phenazistatin A analogs by means of reductive amination with the racemic ketone we always obtained a mixture of the diastereomers when furnishing the second stereogenic center (Figure 21). Our lead compound for PhzA/B inhibition Phenazistatin A has a *cis*-configuration and so it would be a logical consequence to try to find a method which could give the *cis*- product of any product in excess or, ideally, exclusively.

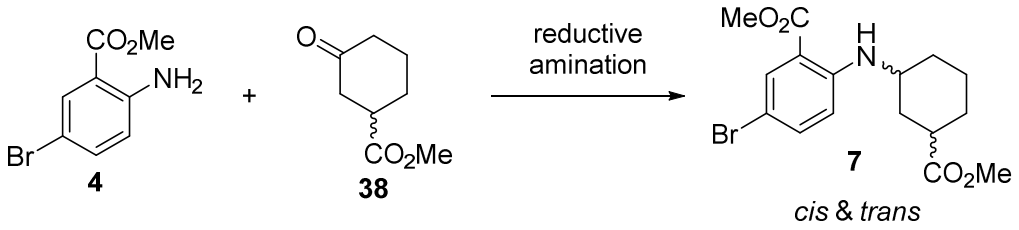


Figure 21: A standard reductive amination leads to a mixture of *cis*- and *trans*-diastereomers.

When we were experimenting with method A ($BH_3 \cdot THF$, CH_2Cl_2/AcOH = 2:1) we observed a slight preference for the formation of the *cis*- over the *trans*-isomer in most cases, usually in the range of 2:1. This reflects well the 1,3-diaxial interaction in the chair conformation of the *trans*-isomer, which renders it thermodynamically less stable compared to the *cis*-isomer (Figure 22).

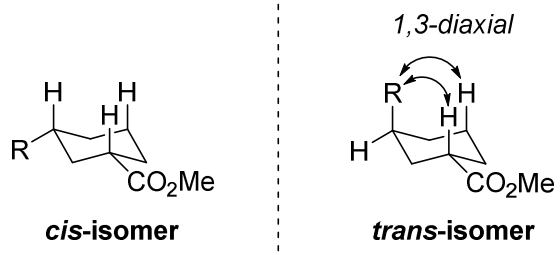


Figure 22: The *cis*-isomer of a 6-ring Phenazistatin derivative is more stable than the *trans*-derivative.

During addition of the $BH_3 \cdot THF$ solution to the substrate solution in CH_2Cl_2/AcOH we observed in every case an intensive bubbling, which discontinued after prolonged stirring and progressing reaction. We therefore assumed that $BH_3 \cdot THF$ is immediately reacting upon addition with the AcOH to form H_2 gas and an acylated borane species (Figure 23). One could think that this process might happen again to form the double substituted borane or even a third time, due to the large excess of AcOH. However, this seems to be not the case since method A works that well and apparently not all the hydride equivalents are consumed upon initial addition. It was reported for $NaBH_4$ that a maximal amount of three AcOH could react with it to give the well-known and stable $NaBH(OAc)_3$ whereas a further reaction could not take place anymore.[102] However, it is not exactly clear, whether this also applies to the free borane in our system.

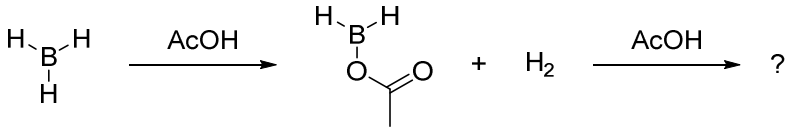


Figure 23: Borane presumably reacts with acetic acid to give a mixture of acylated species.

We speculated that a different acid than AcOH coordinated to the borane during the reduction process of the intermediary prochiral iminium species might have an influence on the stereochemical outcome of the reaction (Figure 24). This might especially be the case for very bulky acids or when two acid equivalents are coordinated at the same time.

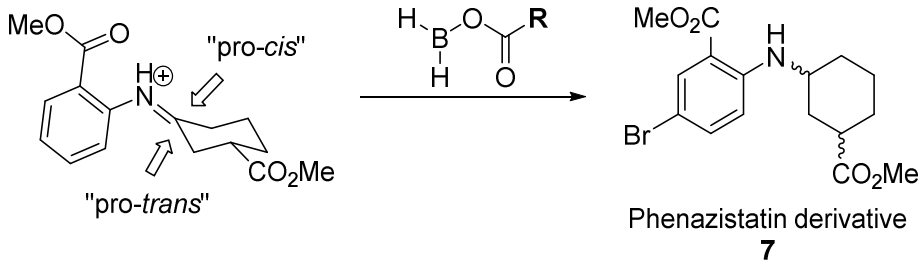


Figure 24: The face from which the reducing agent approaches the pro-chiral iminium ion of a Phenazistatin derivative depends on its electronic and steric properties.

In order to test whether our method could be steered into either direction by the use of different acids we chose the reaction of 2-aminobenzonitrile with 4-(*tert*-butyl)cyclohexan-1-one as ideal for our purposes. The corresponding 3-(*tert*-butyl)cyclohexan-1-one, which would resemble our substrate more, unfortunately was not available. The bulky *tert*-butyl group in 4-position would act as a conformational anchor locking the conformation as shown in Figure 25 so that we could study the influence of different acids on the ratio between the borane approach from the “pro-*cis*” or “pro-*trans*” face.

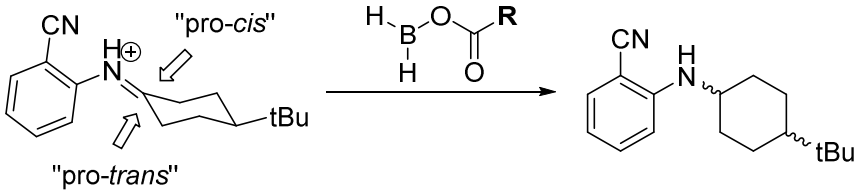


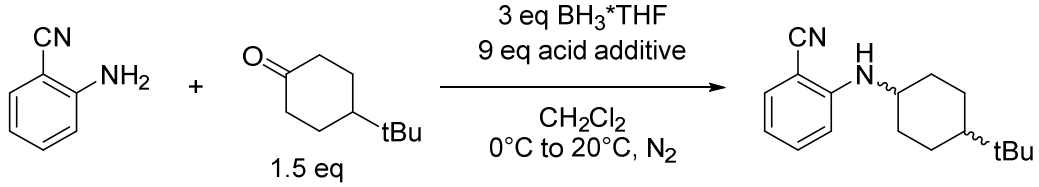
Figure 25: 2-Aminobenzonitrile and 4-tert-butylcyclohexanone were chosen to study the influence of different acidic additives in the reductive amination with BH_3·THF.

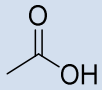
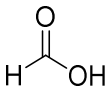
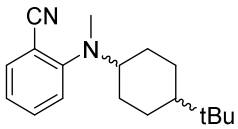
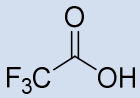
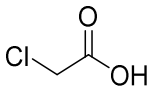
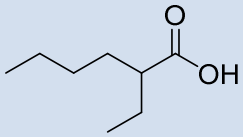
In Table 11 the results from the acid screening are listed. The *cis*- and *trans*-isomer of the product could not be chromatographically separated and structurally assigned, hence they will from now on be referred to as “lower t_R” and “higher t_R”, owing to their different retention time in GC-MS. In order to simulate the large excess of AcOH in method A (~17 eq) we

decided to add 9 eq of every acid to provide enough acid for the (unlikely) triple-substitution of the 1 eq borane added.

When no acid was used as a blank reaction (Figure 11, Entry 1), full conversion of the starting material was detected after 12 h by TLC and GC-MS. The TLC plate indicated the clean formation of a single very polar product (R_f = ~0) which showed a brownish spot after CAM staining. The product was not detected by GC-MS presumably due to low-volatility owing to the high polarity and could not be identified. When the reaction was performed using 9 eq AcOH (Entry 2), which corresponds to the reaction conditions of method A with roughly the half amount of AcOH added, GC-MS indicated a clean reaction without the formation of by-products such as the acetylated substrate amine (as commonly encountered when using methyl anthranilate as substrate). The ratio of the isomers was found to be 67:22 (lower/higher t_R). When using the smaller, more acidic formic acid (Entry 3), clean conversion to a single product was detected by GC-MS, some by-products were detectable on TLC. GC-MS analysis suggested the formation of the methylated product (shown in Entry 3) instead of the expected product. This by-product might have formed in analogy to the ethylated by-product observed by Marchini[170] and Abdel-Magid[102] when using AcOH in reductive aminations, where the acid is reduced to an aldehyde-equivalent which reacts with the corresponding amine to give the imine intermediate. Apparently the generated HCHO is reactive enough to react with the product, a secondary aryl amine. In contrast, the analogous by-product with AcOH was never observed in any other reductive amination performed during this project. This method might be useful in some cases as a domino-reductive amination-methylation method for electron deficient anilines. The highly acidic trifluoroacetic acid (Entry 4) reacted equally fast (12 h) as AcOH (Entry 3) and gave the product in the same isomeric ratio as with AcOH. No by-products were generated according to GC-MS, however some traces of by-products were observed on TLC. When the less-acidic chloroacetic acid (Entry 5) was used, a complex mixture of products was obtained (GC-MS, TLC). The product isomers were detected with an interesting higher/lower t_R-ratio, but the GC-MS spectrum was rather complex so this finding was not followed up any further. When using the sterically demanding and readily available 2-ethylhexanoic acid (Entry 6), the reaction was very slow (4 d, incomplete conversion), but only the product isomers were detected in GC-MS and TLC. The ratio of the isomers was slightly shifted towards the lower t_R-isomer (73:27) as compared to the reaction with AcOH (67:33), but this difference is marginal.

Table 11: Acid additive screening of method A (carboxylic acids).

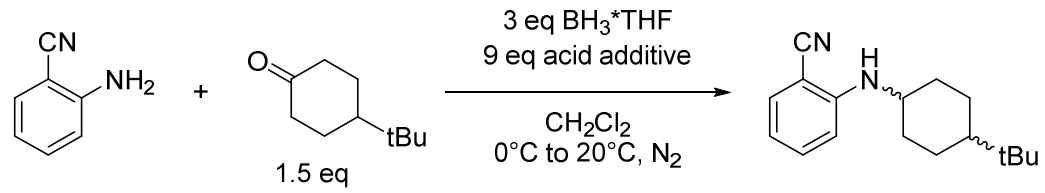


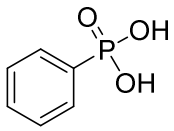
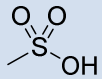
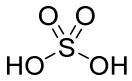
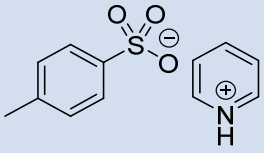
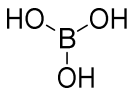
Entry	Acid [9 eq]	Time conversion	ratio (lower t_R/ higher t_R)	comments
1	no acid	12 h 100%	-	polar by-product formed only alcohol detected (GC-MS) product not volatile
2		12 h 100%	67:33	clean (GC-MS)
3		12 h 100%	54:46	methylated product formed 
4		12 h 100%	62:38	clean (GC-MS) traces of by-products (TLC)
5		12 h 100%	92:8	complex mixture (GC-MS, TLC)
6		4 d Incomp.	73:27	clean (GC-MS, TLC)

When phenylphosphonic acid (Table 12, Entry 1) was used as the acid additive, the reaction went to completion within 13 h and no by-products were detected by GC-MS and TLC. There was no improvement in the ratio of the isomers (62:38) when compared to the cheaper and easier to handle (liquid) AcOH. When using the highly acidic $MeSO_3H$ (Entry 2), the reaction proceeded neatly to completion (3 d) and only the expected product was formed. In this reaction a significant shift towards the “lower” isomer was detected (76:24), producing a larger share of the isomer which is already favored in the reaction with AcOH (Entry 2). This bias was weakened when the other *S*-acids H_2SO_4 (Entry 3) or PPTS (Entry 4) were used.

With H_2SO_4 only 57:43 was obtained, which is lower than with AcOH (67:33), and PPTS showed the same result as AcOH (64:36). The reactions were fast (12 h & 13 h respectively), but TLC indicated the formation of many by-products which could not be detected in GC-MS. Boric acid (Entry 5) yielded only traces of the product after 24 h and thick, gel-like mixture was obtained which prevented agitation with the stirring bar. The reaction was found to be inconvenient and was not followed up on any further. The ratio of the small quantity of product isomers formed was 56:44.

Table 12: Acid additive screening of method A (*P*-, *S*- and *B*-acids).

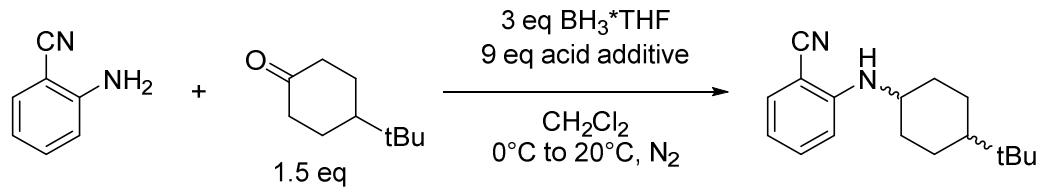


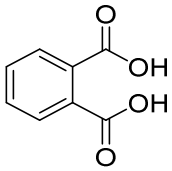
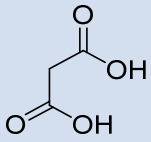
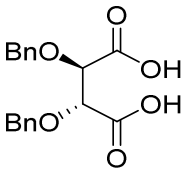
Entry	Acid [9 eq]	Time conversion	ratio (lower t_R/ higher t_R)	comments	versatility
1		13 h 100%	62:38	spot-to-spot (TLC) very clean (GC-MS)	+++
2		3 d 100%	76:24	spot-to-spot (TLC), very clean (GC-MS)	+++
3		12 h 100%	57:43	clean (GC-MS) by-products formed (TLC)	+
4	 PPTS	13 h 100%	64:36	clean (GC) many by-products (TLC)	++
5		24 h Low conv.	56:44	inefficient traces of product	--

When using bifunctional, potentially chelating, carboxylic acids no improvement of isomer ratios could be obtained (Table 13, Entries 1-3). Phthalic acid (Entry 1) and malonic acid (Entry 2) were very reactive and full conversion of the starting material was detected after

13 h. The GC-MS indicated clean production of the product isomers and also TLC showed no formation of by-products. The isomer ratios with 57:43 (phthalic acid) and 61:39 (malonic acid) are close to the standard results obtained with AcOH (67:33). With the more bulky dibenzylether of L-(+)-tartaric acid (Entry 3) no different result could be observed, the reaction performed worse than for the two above stated. An isomer ratio of 66:34 with a strongly reduced reactivity (3 d, incomplete conversion) and many by-products were observed.

Table 13: Acid additive screening of method A (bifunctional carboxylic acids).

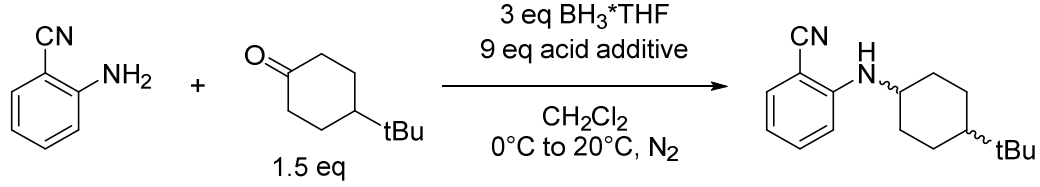


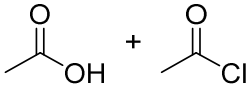
Entry	Acid [9 eq]	Time conversion	ratio (lower t_R/ higher t_R)	comments
1		13 h 100%	57:43	clean (GC-MS) ketone reduction
2		13 h 100%	61:39	spot-to-spot (TLC) very clean (GC-MS)
3		3 d Incomp.	66:34	low conversion traces of product generated many by-products considerable ketone reduction

When using TMSCl (Table 14, Entry 1) in this reaction, a clean reaction towards the two product isomers took place within 12 h. The isomer ratio was found to be 64:36, which is practically identical to the starting point of the screening (AcOH: 67:33). On the positive side, the reaction was exceptionally pure and no trace of a by-product could be detected either by GC-MS or TLC. Considering the low prize and convenient handling TMSCl could be a good replacement of AcOH in this procedure and also in the general reductive amination method A.[189] When AcCl (Entry 2) was tested as a replacement for AcOH, the reaction proceeded sluggishly and after 18 h incomplete conversion of the starting material was

detected by TLC. GC-MS indicated about 50% of the total area to be the acetylated amine and only 33% of the desired product isomers (ratio 66:34). Furthermore a considerable amount of ketone reduction was detected, which makes AcCl a useless acid additive for this reaction. However, when AcCl and AcOH were mixed in equimolar quantities (9 eq each) (Entry 3), the reaction proceeded to completion within 3.5 h. Several by-products were detected by GC-MS and TLC, but interestingly, a bias towards the “earlier” isomer was detected (80:20). In order to test whether the by-products originated from the use of AcCl or AcOH in these selective conditions, AcOH was replaced by TMSCl (Entry 4). To our delight a clean reaction towards the product isomers took place and full conversion of the starting material was detected after 18 h. TLC and GC-MS indicated a spot-to-spot reaction with an isomer ratio of 83:17, which was the best result obtained in this screening. Compared to the result with AcOH (67:33) this represents a rise in selectivity from 2:1 to about 4:1. Interestingly the combination of both “acid” chlorides (TMSCl & AcCl) gives a change in selectivity, while the use of AcCl or TMSCl alone gives no bias. This might be an interesting point for future optimizations of this reaction.

Table 14: Acid additive screening of method A (acid chlorides).



Entry	Acid [9 eq]	Time conversion	ratio (lower t_R/ higher t_R)	comments	versatility
1	TMSCl	12 h 100%	64:36	spot-to-spot (TLC) very clean (GC-MS)	+++
2		18 h Incomp.	66:34	considerable ketone reduction GC-MS: 50% acetylated amine, 33% product	-
3		3.5 h 100%	80:20	by-products generated (GC, TLC)	++
4	TMSCl + 	18 h 100%	83:17	spot-to-spot (TLC), very clean (GC-MS)	+++

So far only the 4-substituted cyclohexanone had been tested as the ketone partner with a generic (not Phenazistatin A-like), but electronically similar aniline. The reaction partners that would lead to the di-ester of Phenazistatin A **7** were tested and only 7.5 eq of a different acid additive were used to investigate the effect of the acid-"bulkiness" onto the stereochemical outcome of the reaction (Figure 26).

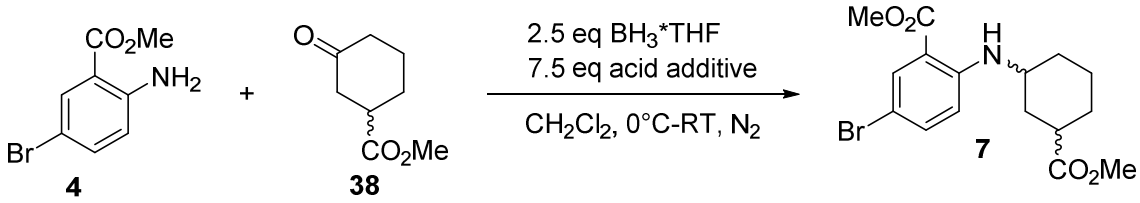
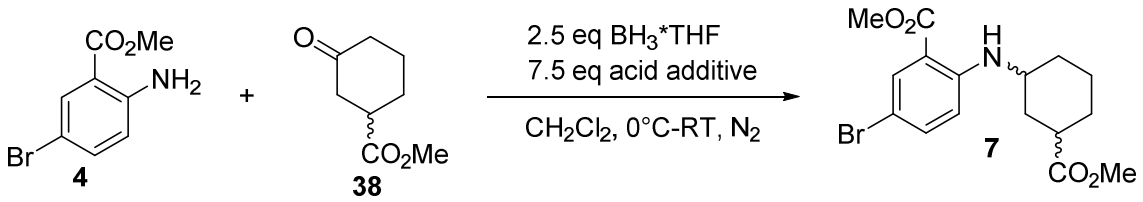


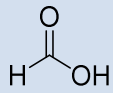
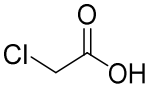
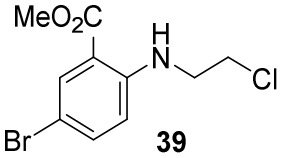
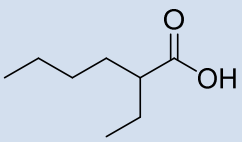
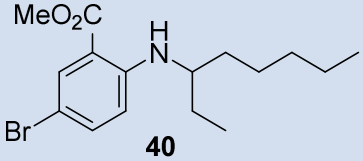
Figure 26: Acid additive screening leading to the di-ester of Phenazistatin A **7**.

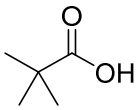
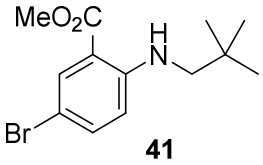
The results of this screening are summarized in Table 15. In the case when no acid was added (Table 15; Entry 1) the same result as for the reaction with the other pair of substrate (2-aminobenzamide & 4-*tert*-butylcyclohexanone) was observed (Table 11, Entry 1). The substrate was neatly converted within 18 h to a very polar product which was stuck on the starting line of the TLC and could not be detected by GC-MS. When the small and more acidic HCO_2H (Table 15, Entry 2) were used, the reaction went to completion within 16 h but a complex mixture of products was obtained as judged by TLC. GC-MS analysis indicated 51% of the desired product (total area %), 29% of the formylated substrate as well as 12% of the methylated substrate. The *cis*/*trans*-ratio was found to be 59:41 which corresponds to a slight bias to the desired direction of stereoselectivity (PhzA/B inhibitors). When an excess of chloroacetic acid (Entry 3) was used in this reaction, full conversion of the starting material was detected after 16 h (TLC). According to GC-MS analysis 22% product were generated with a *cis*/*trans*-ratio of 50:50 along with 55% of the alkylated substrate **39**. When the bulky and inexpensive 2-ethylhexanoic acid (Entry 4) was used, incomplete conversion was detected after 18 h and the reaction did not proceed further upon prolonged reaction time. TLC analysis indicated the formation of by-products and GC-MS confirmed this observation. The product isomers could be detected in 44% abundancy (of total area) in a surprising *cis*/*trans*-ratio of 22:78, which corresponds to a switch in stereoselectivity towards the thermodynamically less favored product isomer. The remaining substrate which had reacted was converted to a broad peak with strong tailing on GC-MS whose mass was identical to the *N*-alkylated substrate amine **40**. NMR analysis of the purified by-product supports this assumption and the CH-group adjacent to the aniline was clearly detectable. This product might again have originated from an acid reduction/imine formation/reduction pathway as

described earlier. This effect was also observed when using pivalic acid (Entry 5) as an additive, just in a more pronounced fashion. Full conversion of the starting material was detected after 18 h and 71% of the alkylated product **41** was detected by GC-MS. Apart from this by-product this reaction was very clean and 29% of the product was detected with a *cis*/*trans*-ratio of 31:69, similar to the results with 2-ethylhexanoic acid (22:78).

Table 15: Acid additives used in Phenazistatin A synthesis.

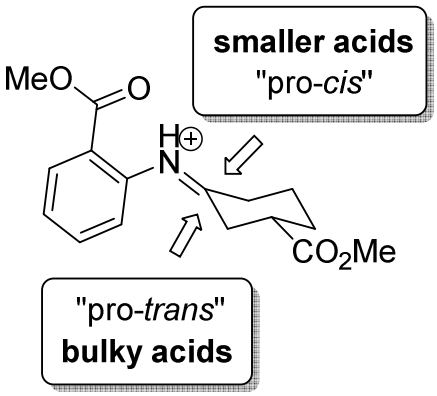


Entry	acid additive	Time / Conversion [%]	comments
1	no acid	18 h 100	polar product generated (TLC) GC-MS: not detectable
2	 (26 eq used)	16 h 100	TLC: complex mixture GC-MS: 29% formylated substrate, 12% methylated substrate, 51% product *cis*/*trans* = 59:41
3	 (15 eq used)	16 h 100	22% product (GC-MS) *cis*/*trans* = 50:50 55% alkylated substrate (GC-MS)  **39**
4		18 h incomplete	TLC: by-products generated *cis*/*trans* = 22:78 44% product, 45% alkylated substrate (GC-MS)  **40**

5		18 h 100	TLC: by-products generated 29% product (GC-MS) *cis*/*trans* = 31:69 71% alkylated product (GC-MS)  **41**

In conclusion, a general trend of the effect of bulkiness of the acid on the stereochemical outcome of the reaction was detected. Smaller acids favor the formation of the *cis*-isomer (reference sample for the di-ester of Phenazistatin A **7** are available), while the more bulky acid additives switch the selectivity towards the *trans*-isomer (Table 16). Due to the high rate of by-product formation these methods cannot yet be considered as viable methods for generating a single isomer selectively, or in very high excess. The main by-product in every case was the alkylated substrate amine. Presumably the generated acyl-borane species is too low in reactivity and/or sterically demanding for the reduction process, so that the rate of reduction of the carboxylic acid becomes completive to the overall reductive amination process. However, the obtained *cis*/*trans*-ratio with 2-ethylhexanoic acid of 22:78 might be interesting for future investigations, also when the formation of the less-interesting (considering PhzA/B inhibitors) was favored.

Table 16: Effect of acid additives in method A on the *cis*/*trans*-ratio of the product isomers.



Entry	Acid	*cis*/*trans*-ratio
1	-	-
2	HCO_2H	59:41
3	chloroacetic acid	50:50
4	2-ethylhexanoic acid	22:78
5	pivalic acid	31:69

4.1.2.3 Limitations of Method B

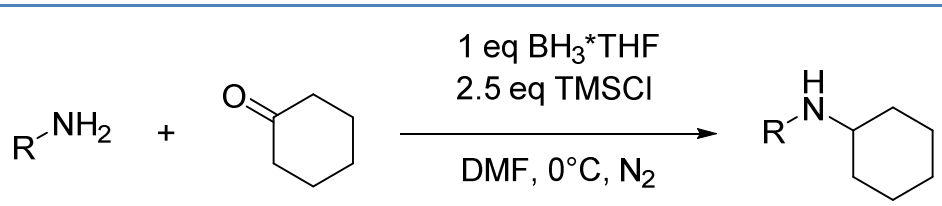
Method B (BH_3·THF, TMSCl, DMF) was found to be the most convenient method for reductive amination of electron-deficient anilines in our hands and enabled us to synthesize a large variety of secondary aryl amines conveniently and reliably. In order to test where the limitations of method B lie, we conducted a series of experiments with challenging substrates. The results from these screenings are summarized in Tables 17-21.

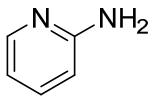
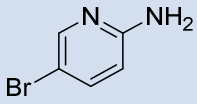
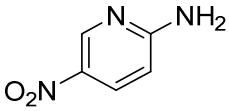
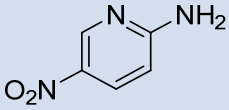
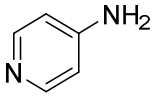
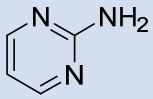
4.1.2.3.1 Failed amine substrates with method B (BH_3·THF, TMSCl, DMF)

One class of very challenging substrates was found to be the heteroaromatic amines with exception of 3-aminopyridine, which was found to react very efficiently with cyclohexanone using the standard conditions (*vide supra*, Table 5, Entry 15). The results from the screenings with heteroaromatic amines are summarized in Table 17. When 2-aminopyridine (Table 17, Entry 1) was used, full conversion of the starting material was detected (TLC) after 15 min and the reaction was worked up. However, GC-MS analysis of the crude product indicated incomplete conversion (33%) with most of the remaining quantity being the starting material (67%). Future reaction controls with 2-aminopyridines were therefore conducted with GC-MS, but these results clearly indicate that 2-aminopyridine can potentially be converted using the standard conditions (15 min is very short, more challenging substrates were usually stirred hours and more) and that the reaction could be optimized if needed. When using the slightly more electron-deficient 5-bromopyridin-2-amine (Entry 2) and taking the lower reactivity and competing ketone reduction into account (1.5 eq cyclohexanone used) 77% conversion was detected after 4 h at 0 °C. The reaction did not go to completion after longer reaction time and after standard workup and column chromatography 20% of the product were isolated. The lower yield could be explained by losses during standard workup, since it was not optimized for the more polar pyridines and the extraction of the aqueous phase after quench was only repeated three times. Unfortunately the purification via column chromatography proved to be challenging due to similar R_f-values and behavior on the column of the product and substrate and hence the isolated product was contaminated. When attempting to couple the very challenging 5-nitropyridin-2-amine (Entry 3) with cyclohexanone, only 15% conversion of the amine could be detected by GC-MS after 2 h. Prolongation of the reaction time had no significant effect and the conversion seemed to stop. When replacing TMSCl with TMSOTf while using the same substrate (Entry 3*) 73% conversion could be detected after 36 min, which is an exceptional increase in reactivity compared to the reaction without TMSOTf (2 h, 15% conversion). The GC-MS spectrum was clean and only substrate and product could be detected. However, the workup and purification with the standard method was again problematic and only 10% of the product

(76% purity, rest is substrate) could be isolated for the same reasons as stated above. When 4-aminopyridine (Entry 4) was used, only ketone reduction was observed by GC-MS after 60 min and no traces of product could be detected. The same outcome was observed for the reaction with pyrimidin-2-amine (Entry 5).

Table 17: Failed heteroaromatic amine substrates with Method B.

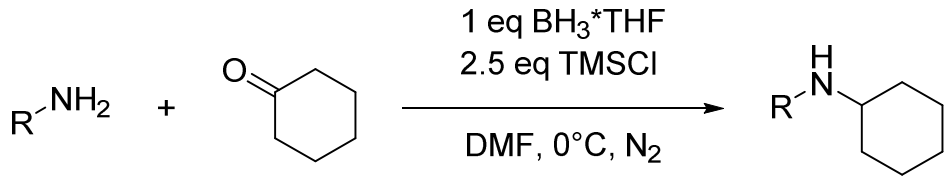


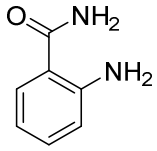
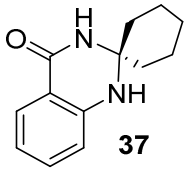
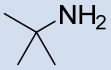
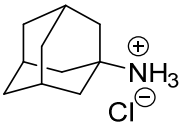
Entry	Amine	Time	Conv. [%]	comments
1		15 min	33	TLC analysis difficult GC-MS(after quench & workup): 67% substrate, 26% product
2		4 h	77	1.5 eq carbonyl used 20% isolated (not NMR pure) difficult to purify (similar R_f-values)
3		2 h	15	TLC analysis difficult conversion stopped after 2 h (GC)
3*		36 min	73	*TMSOTf used instead of TMSCl 10% isolated (76% purity) separation from substrate difficult (column chromatography)
4		60 min	0	only ketone reduction observed complex GC-MS trace no product detected
5		60 min	0	only ketone reduction observed complex GC-MS trace no product detected

4.1.2.3.2 Failed aromatic and aliphatic amine substrates with method B (BH_3·THF, TMSCl, DMF)

When using 2-aminobenzamide (Table 18, Entry 1), clean conversion to a single product was observed after 10 min but no trace of the desired product was detected (GC-MS). HR-MS and NMR analysis indicated that again the spiro product **37** had formed and 82% were isolated after column chromatography. The same reaction product was detected when method A was used (vide supra, Table 9, Entry 4) as well when method C ($NaBH_4$) was used.[190–194] When the very bulky but electron rich amine 2-methylpropan-2-amine (Entry 2) and adamantan-1-amine hydrochloride (Entry 3) were used, full conversion of the amine was detected after 15 min by TLC. Upon addition of H_2O for the workup, an insoluble colorless precipitate formed, which did not dissolve upon prolonged stirring. The solid was isolated via filtration and analyzed by GC-MS, as was the EtOAc extract of the mixture. No traces of the corresponding products or substrates could be detected. The amino-borane complexes that form during a reaction with amines and BH_3 are known to be quite stable towards water (ammonia-borane is more convenient to handle in air than $NaBH_4$),[150] so it can be speculated that either the amine-borane complex of the substrate and/or the product amine had formed which resulted in the precipitation of the colorless solid upon addition of water (reduction of solubility in DMF). A possibility of how to tackle this problem would be to boil the resulting mixture after the H_2O quench with concentrated HCl. This is a known procedure[195] to destroy the amine-borane complexes and to obtain the free amine. However, this theory still needs to be tested and the workup procedures optimized so as to widen the scope of method B (and method C).

Table 18: Failed amine substrates with Method B.

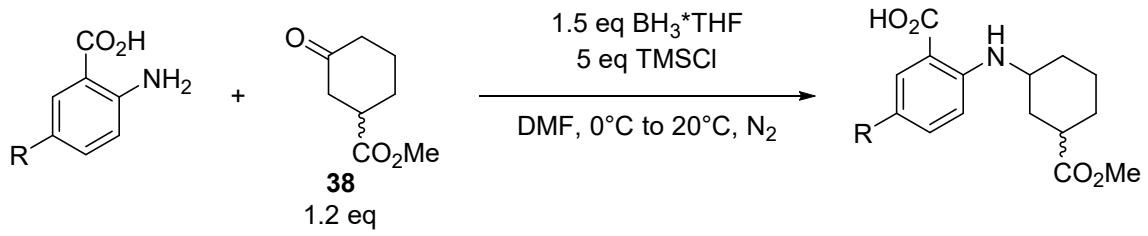


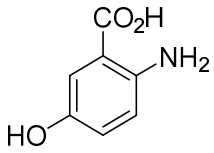
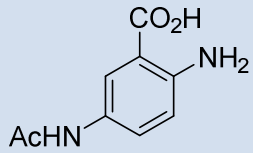
Entry	Amine	Time	Conv. [%]	comments
1		10 min	100	NMR analysis: spiro product formed  **37** 82% isolated (column) ➔same result with method A, B & C.
2		15 min	100	insoluble colorless precipitate formed (upon H_2O addition) amine-borane complex?
3		15 min	100	insoluble colorless precipitate formed (upon H_2O addition) amine-borane complex?

For the synthesis of Phenazistatin A derivatives bearing substituents at the 5-position the direct installation of a –OH or $–NH_2$ group would be especially attractive since it would enable the very convenient production of a wide variety of ester, amide, carbamate, carbonate derivatives. Therefore the reaction of an accordingly decorated anthranilic acid (the free carboxylate group was found to not be interfering with method B) was attempted (Table 19, Entries 1 & 2). Both reactions went to completion within 15 h and TLC indicated a spot-to-spot reaction. During workup it was found that the dissolved crude product darkened and during column chromatography more by-products were formed. The generated spots were isolated by column chromatography (highly enriched fractions were obtained) and subjected to HPLC-MS analysis. However, no peak having the corresponding product mass could be identified. The point of time of the degradation process is uncertain, but it is very likely that the reductive amination proceeded before decomposition started upon air and light exposure, since the substrates are air stable solids which are commercially available. The quinoidic structure of the products might give rise to the decomposition pathways. In conclusion, the reductive amination method B cannot be used for the synthesis of –OH and $–NH_2$ substituted

phenazistatins starting from the readily available anthranilic acid substrates. The general stability of the quinoidic products has to be considered when attempting a different approach towards these structures.

Table 19: Failed amine substrates bearing acidic protons with modified Method B.



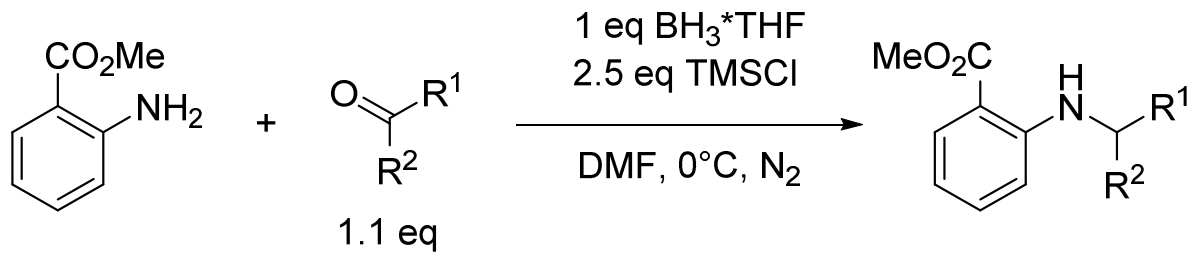
Entry	Amine	Time	Conv. [%]	Comments
1		15 h	100	spot-to-spot reaction on TLC product degradation during workup/column
2		15 h	100	spot-to-spot reaction on TLC product degradation during workup/column

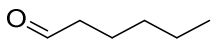
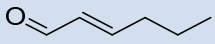
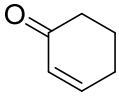
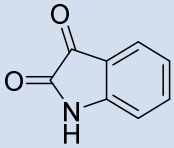
4.1.2.3.3 Failed carbonyl substrates with method B (BH_3·THF, TMSCl, DMF)

The carbonyl substrate scope of method B turned out to be quite broad, but certain limitations were encountered (Table 20). A difficult substrate was the aliphatic aldehyde hexanal (Table 20, Entry 1), which only gave 42% conversion (GC-MS) after 24 h and did not undergo full conversion upon prolonged stirring. In GC-MS no trace of the desired product could be detected, but the substrate was converted to a complex mixture of higher-boiling (than substrate) substances which could not be identified. It can be speculated that again a stable amine-borane complex of the product could have formed and thus hampering the GC-MS analysis. When the α,β-unsaturated aliphatic aldehyde (*E*)-hex-2-enal (Entry 2) was used for the reaction, incomplete conversion was detected after 50 h and a very polar by-product was generated (TLC). The product mass could not be detected on GC-MS, and only traces of a higher-molecular unidentified product were found. Method B seems to have one of its limitations in the reaction with the reaction of aliphatic aldehydes, presumably due to the formation of amine-borane complexes or due to competing aldol condensation. This might be

the case when using α-unbranched aldehydes (Entries 1 & 2), but this theory still has to be tested with further substrates. However, when reacting the α,β-unsaturated ketone cyclohex-2-en-1-one (Entry 3), the reductive amination process was found to proceed and full conversion of the substrate was detected after 60 min. Many by-products were detected by TLC and 40% of the product mixture was isolated. GC-MS indicated the mixture to contain the C=C reduced, as well as the unreduced product in a ratio of 63:37. In a subsequent experiment it was found that an increase in reaction time led to a higher share of the reduced product. Apparently the reaction conditions are not chemoselective for α,β-unsaturated compounds, which however is in stark contrast to the results obtained from the reaction of cinnamic aldehyde with methyl anthranilate (vide supra, Table 6, Entry 3B) which proceeded smoothly in 10 min to give the product in 81% yield without traces of C=C reduction. When using the ketone isatin (Entry 4) a brown complex mixture was obtained (GC-MS) and no traces of product could be detected.

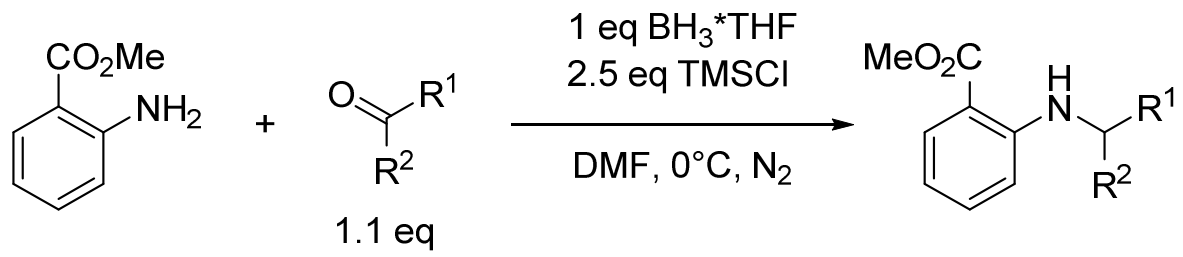
Table 20: Failed carbonyl substrates with Method B.

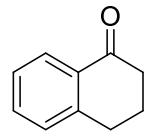
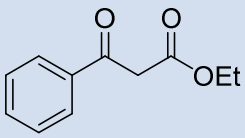
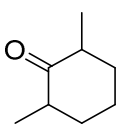


Entry	Carbonyl	Time	Conv. [%]	comments
1		24 h	42 (GC)	complex mixture (GC-MS) no product mass found (GC-MS) amine-borane complex formed?
2		50 h	Incompl.	polar by-product generated (TLC) GC-MS: only anthranilate detected
3		60 min	100	many by-products formed 40% isolated GC-MS: reduced/unreduced=63:37
4		7 d	Incompl.	complex mixture no product detected (GC-MS)

Method B was earlier found to leave aromatic ketones intact and only the use of TMSOTf instead of TMSCl could enhance the reactivity sufficiently so as to make this kind of substrate accessible (vide supra). With these modified reaction conditions α-tetralone was reacted with methyl anthranilate (Table 21, Entry 1*). After 6 d 99% conversion was detected (GC-MS) and the GC-MS spectrum indicated only substrate and product. 66% of the product were isolated via column chromatography, but the product fractions were contaminated with the α-tetralone due to a very similar R_f-value of product and ketone. Hence no analytically pure sample of the product could be obtained. The same strategy was applied for the reaction of the aromatic β-ketoester ethyl 3-oxo-3-phenylpropanoate (Entry 2*), where 92% conversion (GC-MS) were obtained after 6 d by the use of TMSOTf. With this ketone the R_f-value of ketone and product was virtually identical and the product could only be isolated in 88% purity (GC-MS). The problem with very similar R_f-values was the case for all the β-keto esters used in this study since the substrate ("mono-ester") is only transformed to a "di-ester", which gives very similar polarities for both species. When using the sterically very demanding 2,6-dimethylcyclohexan-1-one (Entry 3) very little conversion could be detected after 17 h (TLC, GC-MS) and only traces of the product were detected by GC-MS.

Table 21: Failed carbonyl substrates with Method B.



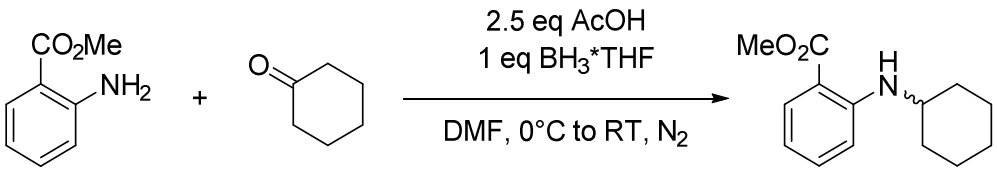
Entry	Carbonyl	Time	Conv. [%]	comments
1*		6 d	99	*TMSOTf used instead of TMSCl 66% isolated (column) not NMR pure
2*		6 d	92	*TMSOTf used instead of TMSCl R_f-value of product and substrate virtually identical not isolated in pure form
3		17 h	>0	GC-MS: traces of product detected

4.1.2.3.4 Variation of method B (BH_3·THF, TMSCl, DMF)

During our studies towards the optimization of the reductive amination methods we conducted a series of screenings, which were not directly connected to the methods or turned out to be dead ends. However, we think they might be of interest to the reader and thereby we have shortly described our findings in the following tables.

When we arrived at the very active method B (BH_3·THF, TMSCl, DMF) starting the optimization from the less-active method A (BH_3·THF, CH_2Cl_2/AcOH), we thought about the different acids that could replace AcOH in method A and were wondering what happened when we tried to replace TMSCl (the new "acid additive") with the "old" AcOH. Therefore we conducted the experiment shown in Table 22. After 7 d only 48% conversion of the amine was detected (GC-MS, area%) and 24% product were observed. In addition 22% of reduced ketone was detected, which indicates a very efficient ketone reduction under these conditions. Using the standard method B, no trace of ketone reduction is observed even after days of stirring, the reduction process somehow seems to be hampered. It was previously described that the use of DMF as the reaction solvent suppressed the aldehyde reduction in reductive amination with $NaCNBH_3$.[110] Interestingly, no acetylated amine substrate was observed in this reaction. To sum up, AcOH cannot replace TMSCl in method B since the overall reaction rate is strongly reduced and the side reaction (ketone reduction) becomes competitive.

Table 22: Replacement of TMSCl by AcOH in method B.

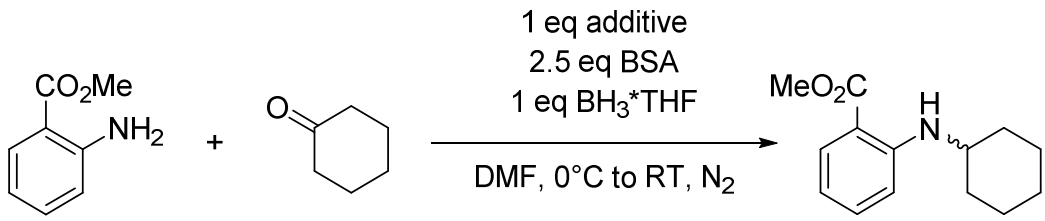


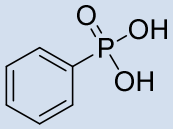
time	Conversion	Comments
7 d	48% (GC)	GC-MS: 26% substrate, 24% product, 22% reduced ketone no acetylated substrate generated

The role of TMSCl as a water scavenger and/or silyl donor for the reductive amination process is highly likely and with that it would be thinkable that TMSCl could be replaced by another water scavenger/silyl-donor. *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) is well known to scavenge water in a non-acidic way liberating only the neutral acetamide upon reaction. We then tried to test the activity of BSA both with and without an acid additive for the model

reaction (Table 23). When no acid additive was used (Entry 1), no conversion could be detected after 3 d. When 1 eq phenylphosphonic acid was used (Entry 2), some clean conversion to the product was detected after 3 d at RT, but according to the intensity on TLC the conversion was rather low. In this case it cannot be deconvoluted whether the rise in activity originates from the combination of BSA & acid or in the acid alone, since the reaction without BSA and the same amount of acid was unfortunately not conducted.

Table 23: Test reactions to replace TMSCl by BSA with and without acid additive.



Entry	Additive	time	conversion	comments
1	-	3 d	0	No conversion detected by TLC
2		3 d	incompl.	low conversion low reactivity of the system

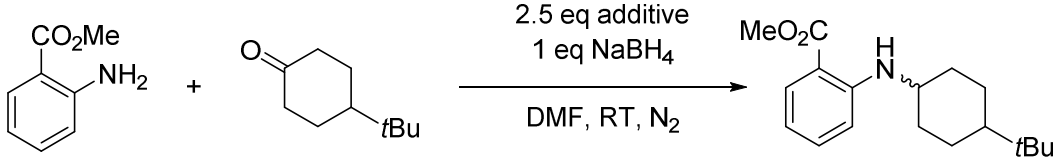
4.1.2.4 Variation of method C ($NaBH_4$, TMSCl, DMF)

The effect of the addition of acid additives on the stereoselectivity of the reaction of 2-aminobenzamide with 4-*tert*-butylcyclohexanone was described above (vide supra, Tables 11-14). Here we would like to report which stereochemical outcome was observed when TMSCl was replaced by a different acid chloride (Table 24). AcCl was chosen since it also showed good reactivity as an acid additive in method A and since it was also described as a water scavenger[178] liberating HCl. The isomer ratio of the product mixture is again referred to as lower & higher t_R, similar to the studies towards the stereochemical outcome when different acid additives were used with method A (vide supra, Tables 11-14) which corresponds to the retention time on GC-MS because it was not possible to separate and assign the isomers by chromatography. When AcCl was used as the acid additive (Entry 1), no formation of product was observed upon full conversion after 15 min at RT. The major product generated was identified to be the acetylated substrate amine (GC-MS) followed by the reduced ketone. Apparently AcCl is an efficient acetylating agent under these conditions, which was for example not the case when it was used in combination with TMSCl (vide

supra, Table 14, Entry 4). When the standard reagent for this method, TMSCl, was used (Entry 2) full conversion of the starting material was detected after 15 min and a clean reaction without the formation of by-products was observed (GC-MS, TLC). The ratio of lower/higher was found to be 68:32. When instead of TMSCl the more bulky TBDMSCl was used (Entry 3), 96% conversion could be detected after 15 min (GC-MS). Upon addition of the TBDMSCl the stirring was hampered and an additional 400% of DMF were necessary to give a stirrable solution. GC-MS indicated the formation of traces of the dehydration product TBDMS-O-TBDMS and 96% of the product (overall area). The ratio of lower/higher t_R with TBDMSCl was found to be 66:34 which is almost identical as the result for TMSCl (68:36).

To summarize, the nature of the silylating agent seems to have no influence on the stereochemical outcome of the reaction. This finding suggests, that the silyl group is not "bound" to the intermediate when the stereo-determining step takes place and is rather necessary to provide HCl and to scavenge the water generated. TMSCl is superior to TBDMS not only when considering the price, but also the removal of the generated siloxane. TMS-O-TMS with a boiling point of 101 °C can be removed under reduced pressure, however the TBDMS-equivalent will require higher vacuum/temperatures or column chromatography.

Table 24: The effect of a TBDMSCl or AcCl on the stereoselectivity (*cis*/*trans*).



Entry	Additive	time	Conversion [%]	Comments
1	AcCl	15 min	100	major product: acetylated substrate (GC-MS) ketone reduction no product formed
2	TMSCl	15 min	100	clean reaction (GC-MS) ratio (lower/higher) = 68:32
3	TBDMSCl	15 min	96 (GC)	+400% DMF necessary to make it stirrable 96% product formed (GC-MS) ratio (lower/higher) = 66:33

4.1.2.5 Attempts towards the reductive amination of (+)-camphor

The most challenging ketone of the ones tested turned out to be (+)-camphor. Here we want to describe our attempts and to give an overview about the reactivity towards reductive amination. When applying method B for the reaction of (+)-camphor with 2-aminobenzonitrile no conversion could be detected after 12 h (TLC), also no side reaction was detected (Figure 27).

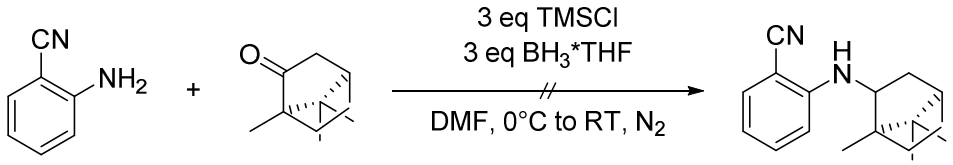


Figure 27: 2-Aminobenzonitrile fails to react with (+)-camphor using method B.

Considering the electron-deficient character of 2-aminobenzonitrile we exchanged the amine to the much more reactive benzylamine and applied the standard conditions of method B (Figure 28).[189] Upon addition of the borane complex the formation of a colorless precipitate was detected and after 4 h at RT the formation of a by-product was observed (TLC), but not of the desired product (confirmed by GC-MS). The reaction was heated to 30 °C for 19 h in order to accelerate the reaction but this led to the formation of more by-products, still no traces of product could be found. The precipitation upon borane addition might be an indication of the formation of an amine-borane complex of benzylamine so that it would not be available as a nucleophile for the reductive amination process.

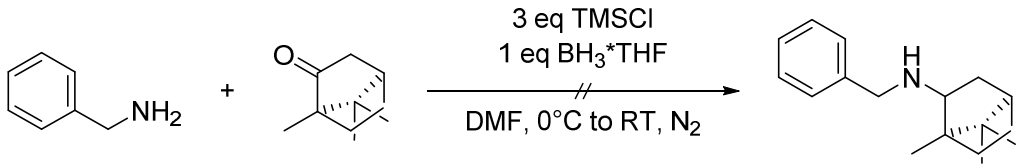


Figure 28: Benzylamine fails to react with (+)-camphor using method B.

Chandrasekhar et al.[116] have reported the facile reductive amination of (+)-camphor with benzylamine using a $Ti(O^iPr)_4$/PMHS system at RT. We tried to reproduce this procedure using the same substrates (Figure 29). Neat stirring with $Ti(O^iPr)_4$ followed by dilution and addition of PMHS at RT and stirring for 3 d at RT resulted in the full conversion of the starting material. TLC indicated the formation of a very apolar spot, which gave an intensive pink staining upon CAM staining. GC-MS analysis indicated 95% of the total area to be the desired intermediary imine. However, no trace of the reduced product was found as they had published. In order to check whether the PMHS (described to be very stable) was inactive, a fresh bottle was bought and tested but the same result was obtained.

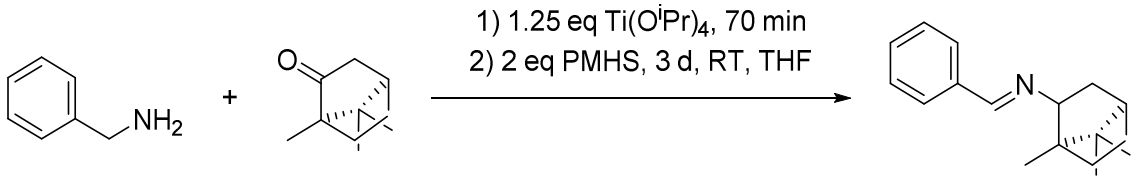
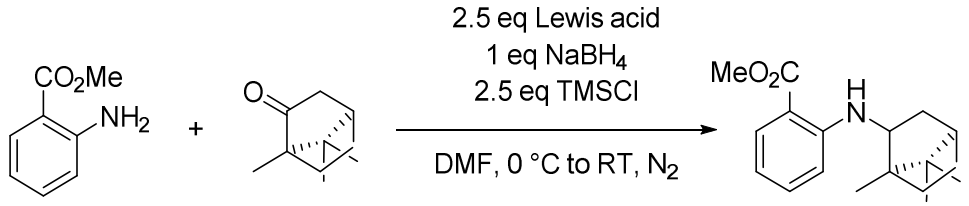


Figure 29: Benzylamine reacts with (+)-camphor neatly to the imine, but fails to undergo reduction with PMHS.

This finding that a Lewis-acid can promote the reactivity prompted us to test the effect of Lewis-acids onto our methods. Therefore we took the standard conditions of method C ($NaBH_4$) and added 2.5 eq Lewis acid (Table 25). When $Ti(O^iPr)_4$ was used (Entry 1), no conversion was detected after 6 h at RT. When $BF_3{\cdot}Et_2O$ (Entry 2) and ZnI_2 (Entry 3) were used as a Lewis acid, the substrate was partially converted to a more polar product which showed the same staining as the substrate with CAM on TLC. The product was not volatile and could not be identified by GC-MS and no trace of the desired product or the intermediary imine was detected. In summary, the addition of a Lewis acid to method C did not enhance the formation of the product.

Table 25: The influence of Lewis acid additives in method C in the reductive amination of camphor.



Entry	additive	time	conversion	comments
1	$Ti(O^iPr)_4$	6 h	0	no product formed, no conversion
2	$BF_3{\cdot}Et_2O$	6 h	incompl.	more polar by-product formed same staining as substrate (CAM: brown) no trace of desired product
3	ZnI_2	6 h	incompl.	more polar by-product formed same staining as substrate (CAM: brown) no trace of desired product

With the use of $BH_3{\cdot}THF$ and also TMSCl/$NaBH_4$ the temperature range which can used for the reactions is somewhat limited, since $BH_3{\cdot}THF$ is known to spontaneously and exothermically decompose over 40 °C. This is a safety concern and we wanted to avoid heating up the reaction mixtures. We found earlier in our studies[189] (vide supra) that $PhSiH_3$

is an efficient reductant and can be used instead of $BH_3 \cdot THF$. An advantage of $PhSiH_3$ is the thermal stability and it can conveniently be heated to 100 °C. In order to test whether the higher reaction with the "same" reaction conditions of method B would have a positive influence on the reaction we conducted the following reaction (Figure 30). Strong gas evolution was observed upon addition of the $PhSiH_3$ which discontinued after some minutes to give a colorless solution. TLC reaction after 12 min showed no conversion and the mixture was heated to 50 °C for 18 h, which led to the formation of a more polar spot (TLC) with the identical staining of the substrate and several other by-products. The product was very similar to the one found with the Lewis acids $BF_3 \cdot Et_2O$ and ZnI_2 above (Table 25, Entries 2 & 3), but could not be detected on GC-MS due to low volatility/high polarity. No traces of the product or the corresponding intermediary imine could be detected by GC-MS. In conclusion, it seems that high temperature promotes the formation of by-products, but does not enable the desired reductive amination to proceed.

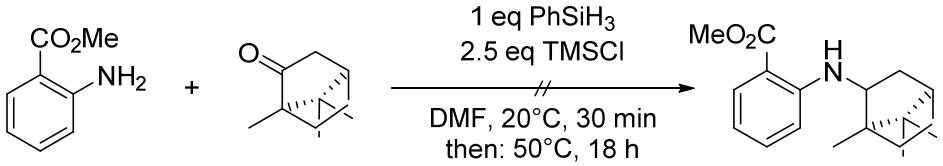


Figure 30: The use of method B with $PhSiH_3$ and elevated temperatures does not promote the reaction between methyl anthranilate and (+)-camphor.

As a common strategy to enhance the reactivity of method B we suggested the use of TMSOTf instead of TMSCl. When this procedure was applied for the reaction of 2-nitroaniline with (+)-camphor no conversion could be detected after 3 d at RT (Figure 31). Neither traces of the product nor by-products were detected by TLC and GC-MS.

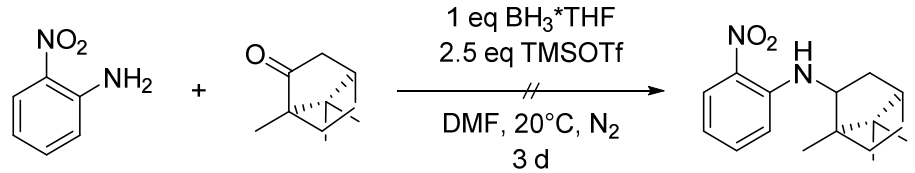


Figure 31: The use of method B with the activity-enhancing TMSOTf fails to promote the reaction between 2-nitroaniline and (+)-camphor.

Taking a step back from the very electron-deficient 2-nitroaniline to the more reactive benzylamine we considered our previous findings for the choice of appropriate reaction conditions. Using method B with $BH_3 \cdot THF$/TMSCl we would get immediate precipitation of a colorless solid, we decided to use method C, which relies on $NaBH_4$ instead of $BH_3 \cdot THF$ and

hence might not lead to this side reaction. In addition, we would boost the reactivity by the use of TMSOTf. We conducted the following reaction and obtained full conversion of the starting material after 170 min and observed the spot-to-spot reaction leading to a slightly less polar product (TLC) showing the same staining as the substrate amine (CAM) (Figure 32). GC-MS analysis of the reaction mixture indicated the exclusive formation of formylated benzylamine and the unreacted (+)-camphor. To sum up, boosting method C with TMSOTf when using electron-rich amines might lead to formylation of the amine and no productive enhancement of reactivity.

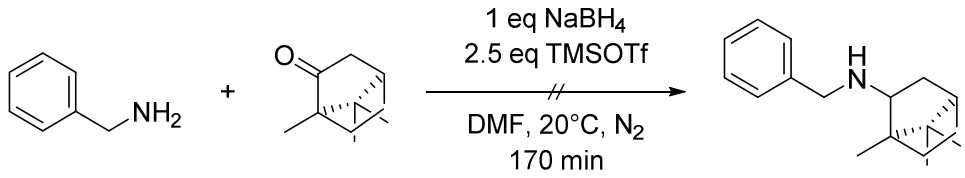


Figure 32: The use of method C with the activity-enhancing TMSOTf fails to promote the reaction between benzylamine and (+)-camphor and instead leads to the formylated benzylamine.

4.2 Synthesis of Phenazistatin A derivatives as Inhibitors of PhzA/B

4.2.1 Phenazistatin derivatives to increase PhzA/B affinity

4.2.1.1 General remarks about the synthesis via ULLMANN-GOLDBERG-aminations

When using the standard ULLMANN-GOLDBERG-aminations described by Mentel et al.[53] for the synthesis of Phenazistatin A derivatives only minute amounts of the desired Phenazistatin products could be obtained (0.8% overall yield of Phenazistatin A from 3-aminobenzoic acid) and further derivatization was limited simply by the low availability. In the MSc thesis of Pletz[196] the reaction conditions for these reaction partners was optimized according to a protocol by Liu et al.[197] and a higher yielding synthesis of Phenazistatin A (**1**) and related compounds was devised. In this optimized synthesis the coupling reaction starts from 2-iodobenzoic acid and an excess of aliphatic aminoester **42** and afforded the crude mono-ester **43** in moderate to low yields and selectivities (Figure 33).

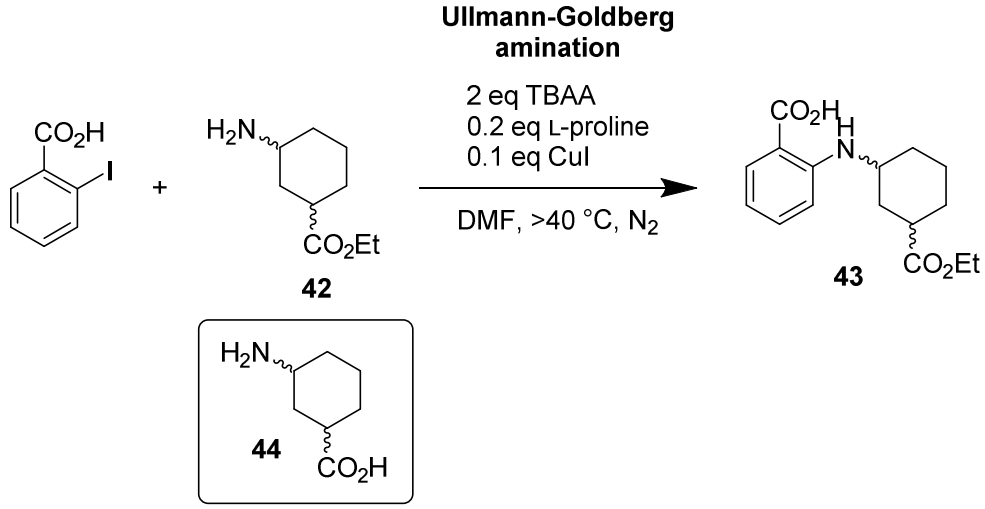


Figure 33: The higher-yielding ULLMANN-GOLDBERG-strategy towards Phenazistatin derivatives.

As has been shown earlier in the MSc thesis of Pletz,[196] the reaction could not be conducted directly with the more easily accessible acid derivative **44** of the aliphatic amine and so additional steps were necessary to produce the substrate ester. The reaction with the free acid derivative failed presumably for reasons of low substrate solubility in the reaction solvents (DMF, DMSO) and so far in our syntheses the use of the ethyl ester proved mandatory to ensure high solubility necessary for reasonable reaction times. In order to produce larger quantities of the 5-substituted products a reliable coupling with a simple and efficient workup would be highly desirable. However, the most active reaction conditions such as the ones by Buchwald et al.[198] using 2-isobutyrylcyclohexan-1-one as a ligand and

Liu et al.[197] using L-proline as a ligand and TBAA as a soluble organic base suffered from considerable sensitivity of the reactivity towards the electronic parameters (the substitution) of the aryl halide. Since we were trying to synthesize a large variety of different products substituted with diverse functional groups this limitation was especially problematic. On top of that, the purification of the reaction products turned out to be cumbersome when unprotected carboxylate groups were present. When employing the optimized coupling conditions with an amino ester and 2-iodobenzoic acid as shown in Figure 33, the reaction product was crude mono-ester **43** contaminated with the remaining substrate (if full conversion was not achieved) and small quantities of unidentified by-products. The logical purification sequence would be to proceed via pathway A (Figure 34), which features a purification at this mono-ester **42** stage followed by a clean saponification with hydroxide to give the desired phenazistatin A derivative **45**. Unfortunately the quite polar carboxylic acids show an intensive tailing on TLC, and the acidic additive AcOH in the eluent has a minor effect on the tailing. Even higher acid contents (up to 5%) and other acidic additives (HCO_2H) had no positive effect on the separation. Hence larger quantities of product generated via ULLMANN-GOLDBERG aminations could not be purified via silica gel chromatography due to by-products with similar R_f-values. In order to achieve NMR-pure samples preparative RP-HPLC using a gradient of MeCN and H_2O/0.01% HCO_2H had to be used, which was not an option on larger scale. The same purification difficulties held true for pathway B (Figure 34), where a global deprotection of the ester functionalities of **43** would afford a di-acid product **45**, which could be purified by careful column chromatography. This pathway was considered as especially tempting, since traces of the di-acid **45** were already detected in most cases where the standard ULLMANN-GOLDBERG amination conditions mentioned beforehand were employed. But as could be expected the double-acid **45** exhibited even more complicated tailing properties than the mono-acid **43**. The separation of the stereoisomers or sometimes the removal of the unreacted aryl halide substrate was generally not possible. The pathway conceived as a consequence of the above stated observations was pathway C (Figure 34), where the crude mono-ester **43** containing traces of the di-acid **45** was converted to a crude di-ester **46** by a global esterification. This esterification was usually conducted with carbodiimide reagents (such as DIC) or by alkylative esterification using Me_2SO_4. Further information will be given when the syntheses of Phenazistatin derivatives are described in detail. As expected, the purification of the di-ester **46** worked well via silica gel column chromatography and by subsequent saponification the desired analytically pure di-acid **45** could be obtained.

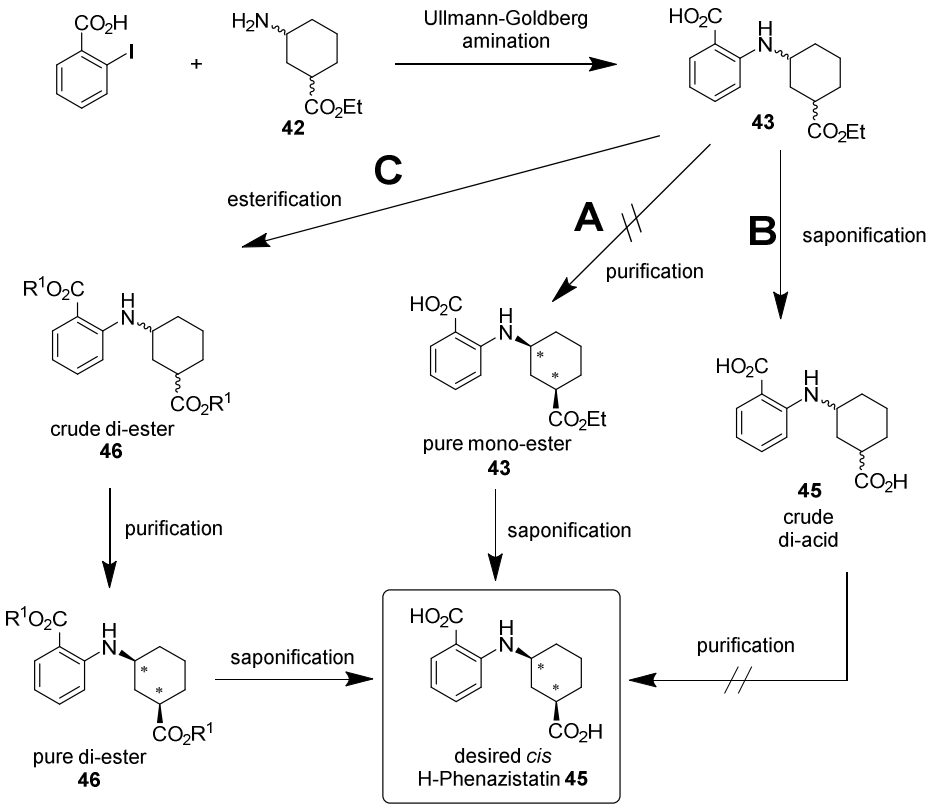


Figure 34: Purification strategies of the ULLMANN-GOLDBERG-amination product.

4.2.1.2 Remarks concerning the building block synthesis and the separation of *cis*/*trans*-isomers

The esterification-saponification strategy via pathway C (Figure 34) made it possible to purify several Phenazistatin A derivatives. Another parameter hampering a reliable upscaling was the synthesis of the amino-ester salt **47** (Figure 35) needed for the amination reaction with the aryl halide. The catalytic hydrogenation in the first step proceeded well on a rather small 2.00 g scale producing the aliphatic amino acid **44** in excellent yield as a mixture of *cis*- & *trans*-isomers (2:1). This reaction worked well for this small scale, but it was found that due to the limited volume (200 mL) of the autoclave an increase in substrate input had a deleterious effect on the overall performance of the reaction. An increase in substrate loading while keeping the reaction volume (and thus the gas volume) constant resulted as expected in longer overall reaction times. However, it was found that in many cases the turnover stopped at a certain degree of conversion and raising the temperature or the H_2-pressure did not lead to further turnover. Further additions of portions of Rh/C were necessary to ensure full conversion, with overall catalyst loadings reaching ~10 wt% Rh/C. This unacceptably high catalyst loading was especially problematic for batches of 15 g, which was the maximum substrate load that could be dissolved in the maximum volume of 100-150 mL reaction solvent H_2O/MeOH = 2:1 (v/v) in the reaction heat (~1 - 1.5 g Rh/C required). Attempts to

stop the catalytic hydrogenation at a certain point and to separate the fully hydrogenated product **44** from the unconverted 3-aminobenzoic acid by recrystallization failed due to poor crystallization behavior. Attempts to recycle the Rh/C catalyst for other rounds of catalytic hydrogenation (via filtration, acid washing with acid and water) were not successful and an inactive catalyst was obtained. Unfortunately, no commercial vendor could be found selling the hydrogenation product **44** of 3-aminobenzoic acid, presumably since the hydrogenation reactions yield an ill-defined mixture of varying content of the *cis*- & *trans*-isomer.

Another problematic step in the synthesis was the separation of the *cis*- and *trans*-isomers by recrystallization after *N*-Boc protection of **44** (Figure 35). Badland et al.[199] have stated in their synthesis of the same substrate a *cis*/*trans*-ratio of the catalytic hydrogenation of 4:1, corresponding to a high loading of the desired *cis*-isomer. However, we were not able to reproduce these results and only ratios between 3:1 and 2:1 were obtained. These findings indicate a much lower loading of the desired *cis*-product **44** in the fully hydrogenated mixture than published. The isomers of **44** could not be separated by recrystallization at this stage (the aliphatic acid does not crystallize well, usually oils are obtained after evaporation) and only installation of a Boc-group led to sufficient crystallizability enabling recrystallization. The separation of the Boc-protected isomers of **48** as published[200] by dissolving in the minimum amount of CH_2Cl_2 and precipitation with *n*-pentane worked to a certain extent and analytically pure samples could be obtained in moderate yields. Recrystallization of larger quantities was not successful and only small fractions of the pure *cis*-product could be isolated with the majority of the desired isomer being buried in a mixed fraction. For those larger-scale recrystallizations a less-careful recrystallization (= a higher excess of *n*-pentane was added to the saturated CH_2Cl_2 solution to drive precipitation) was conducted to give a highly enriched *cis*-product (~5-10% of the *trans*-isomer). It was reasoned that this enrichment would suffice to obtain sufficient amounts of a clean Phenazistatin derivative when carrying out a column chromatography at the di-ester stage.

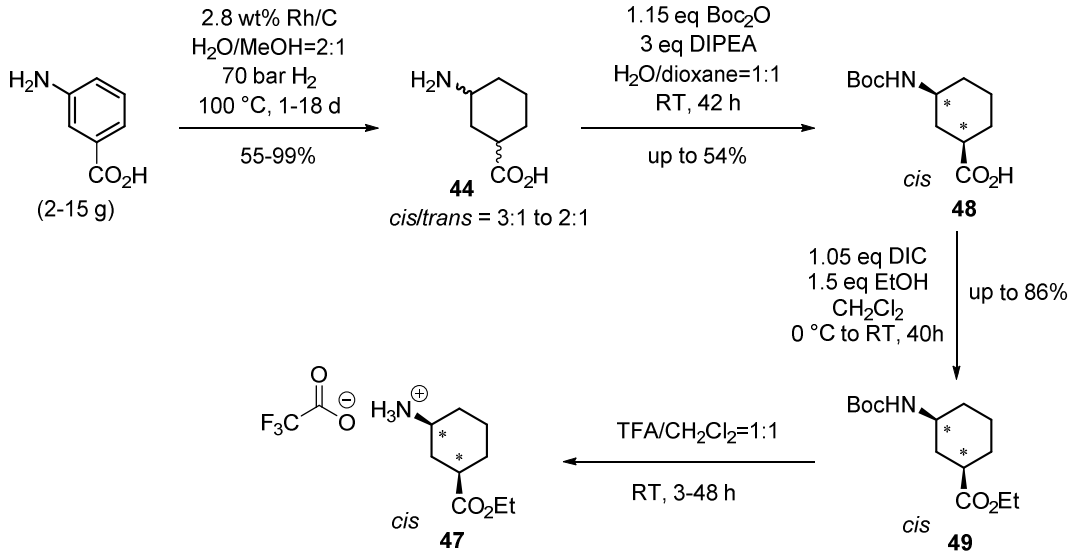


Figure 35: Synthesis of pure *cis*-isomer via catalytic hydrogenation.

4.2.1.3 Synthesis of inhibitors substituted at the 5-position of the aromatic ring

4.2.1.3.1 Synthesis of H-Phenazistatin

The synthesis of a phenazistatin A derivative bearing a proton instead of bromine **45** was initially attempted via the RT ULLMANN-GOLDBERG-amination published by Liu.[197] 5-Bromo-2-iodobenzoic was found to react smoothly with **42** at elevated temperatures to the corresponding 5-bromo derivative **50** during the synthesis of enantiomerically pure Phenazistatin A (**1**).[196] However, when the standard reaction conditions where applied for 2-iodobenzoic acid (with higher temperature to accelerate the reaction rate) incomplete conversion of the starting material was obtained after 11 d and a mixture of products was generated (Figure 36). When this mixture was worked up and telescoped to the saponification, a complex mixture was obtained where the product **45** could not be isolated.

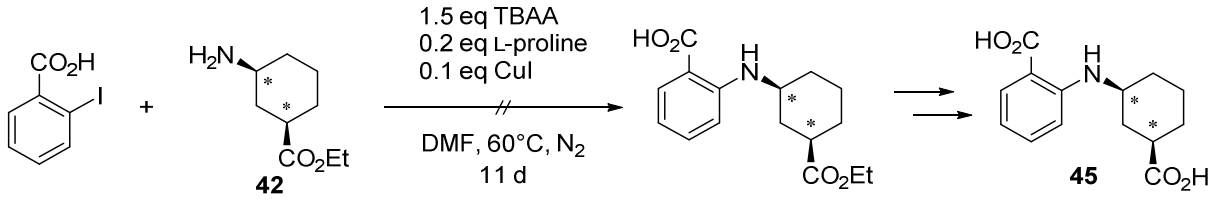


Figure 36: Synthesis of H-Phenazistatin **45** with the conditions by Yang et al..[197]

Due to these limitations the reaction conditions of the developed method B[189] for the reductive amination were applied to the corresponding substrates. The reaction went smoothly to the di-ester product **51** within 27 min at 0 °C and the expected stereoisomers were the only obtained products detectable with TLC and GC-MS (Figure 37). Column

chromatography allowed for the partial separation of the *cis*- and *trans*-isomers and product **51** was isolated in 67% total yield. Standard hydrolysis yielded the clean isomers of H-Phenazistatin **45** in moderate yield (only the cleanest fractions were pooled and sent to biological evaluation).

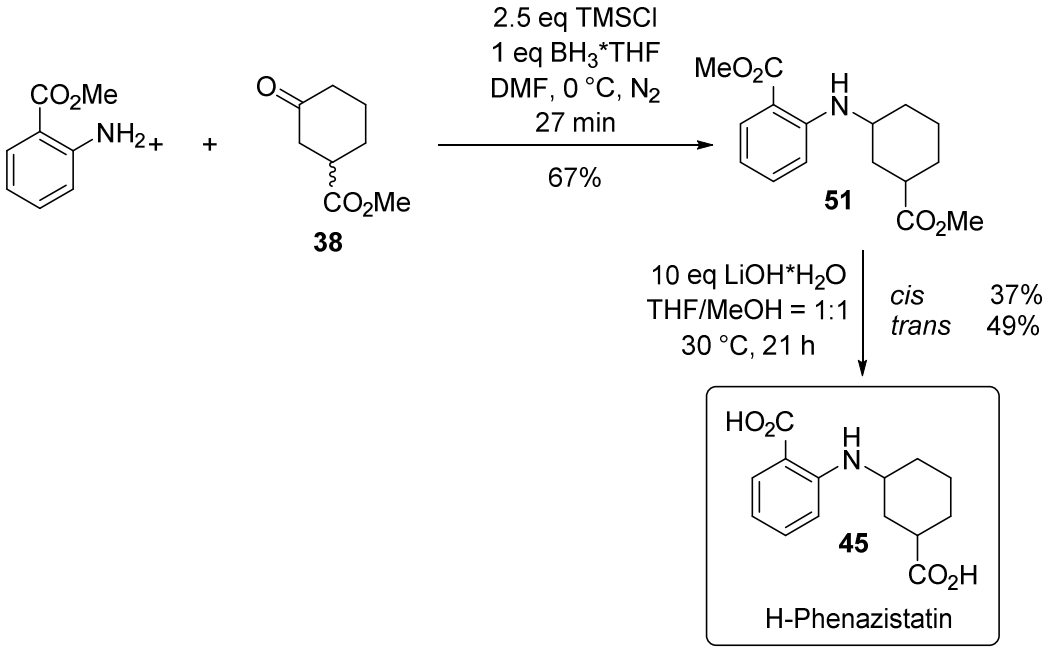


Figure 37: Synthesis of the H-Phenazistatin **45** via the reductive amination by Pletz et al..[189]

4.2.1.3.2 Synthesis of F-Phenazistatin

During our attempts to synthesize F-Phenazistatin **52**, we could not find a commercial source for an ULLMANN-GOLDBERG-amination- (aryl iodide/bromide) or reductive amination-precursor (aniline) bearing the fluorine in the desired 5-position selling it at a reasonable price. We therefore decided to synthesize the anthranilic ester derivative **53** via an iodination/Pd-catalyzed cyanation/Pinner reaction-sequence, which would additionally yield us the valuable nitrile **54**, which is a surrogate for the easily introduced tetrazole (Figure 38).

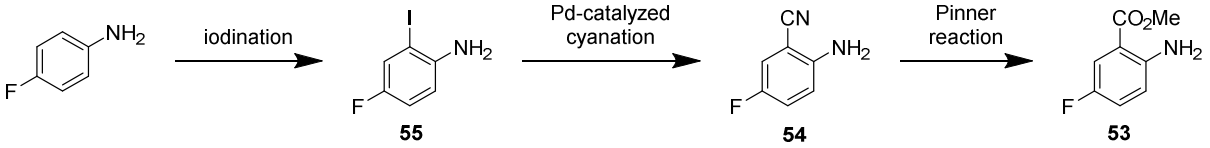


Figure 38: Synthetic strategy towards the precursor **53**.

During our test reaction for the iodination[201] of 4-fluoroaniline we realized, that the product **55** seems to be highly sensitive when concentrated and exposed to air and light. TLC control of the reaction mixture only indicated the product (buffered dilute solution) but when the

mixture was filtrated through silica gel, concentrated and was then left neatly on either RT or -18 °C, a large variety of spots appeared on TLC and the redish-brown liquid would change its color to a dark brown-black. We therefore decided to telescope the crude reaction mixture of a larger batch directly and without delay to the following Pd-catalyzed cyanation reaction.[202] The iodination of the larger batch 4-fluoroaniline went to completion within 60 min and a spot-to-spot reaction was observed via TLC (Figure 39). The product was worked up (73% isolated) and was immediately used in the cyanation reaction without further purification. Upon the cyanation conditions by Quan et al.[202] full conversion of the aryl iodide **55** was detected after 20 h at 90 °C and TLC indicated a clean reaction, apart from the many minor degradation product spots of the substrate (as judged by comparison of a "degraded" sample of the substrate with the reaction mixture). Column chromatography yielded 37% of the desired nitrile product **54**, which was found to be stable at RT.

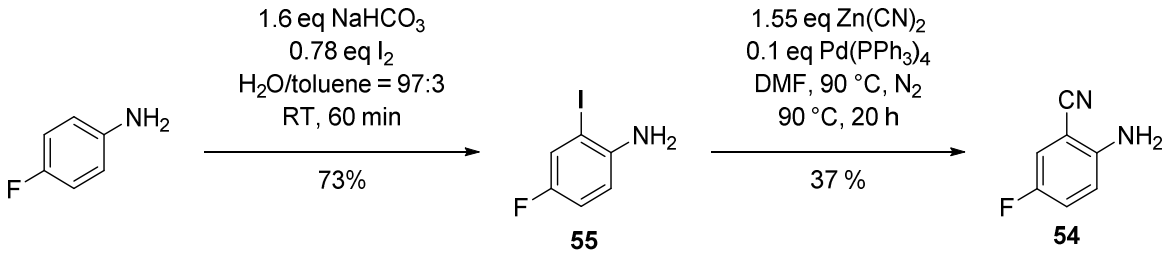


Figure 39: Iodination/Pd-catalyzed cyanation-sequence towards the building block **54**.

It was then tried to convert the obtained nitrile **54** directly towards the ester **56** via a Pinner reaction with in-situ generated HCl in anhydrous MeOH (Figure 40).[203] After 3 d at RT the reaction mixture was quenched with water and the mixture was analyzed by GC-MS. No conversion could be detected and the substrate **54** was recovered unchanged from the reaction mixture. The applied PINNER-reaction conditions seem not to be reactive enough for the conversion of benzonitriles. In order to test whether the reaction would work once the full Phenazistatin core is assembled, the benzonitrile **54** was reacted with the ketone **38** to give the Phenazistatin derivative **57** in 86% using standard conditions.[189] Unfortunately the PINNER-reaction was as well not reactive enough to convert this substrate **57** to the di-ester **58**. Heating the reaction mixture under reflux might provide a boost in reactivity, but this experiment was not tested.

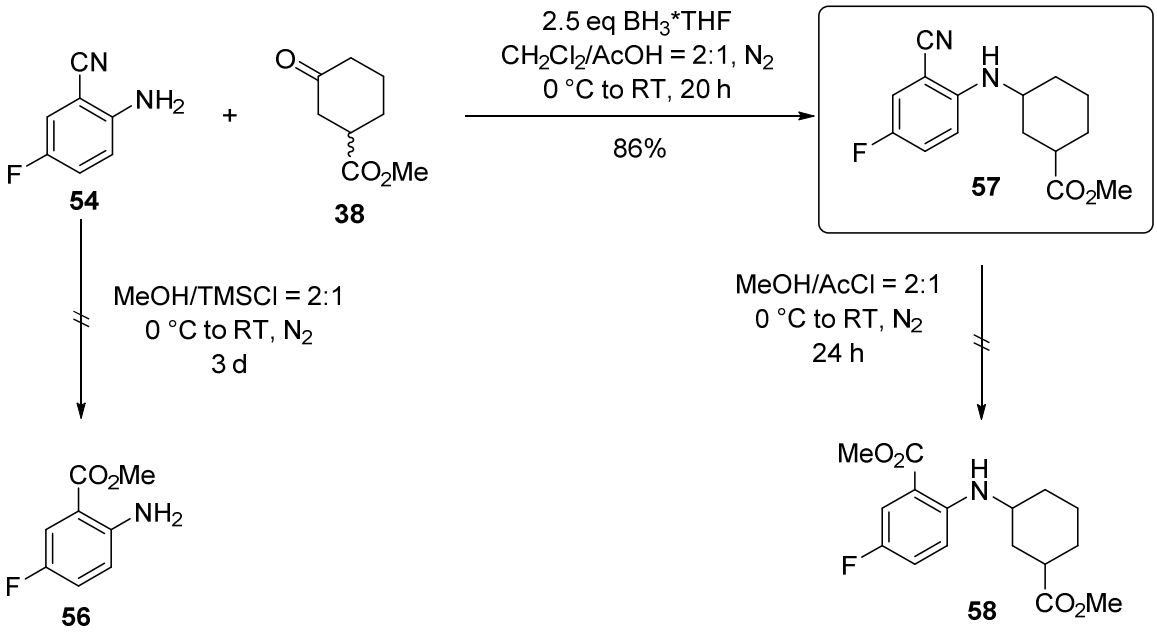


Figure 40: Attempts to convert the fluorinated benzonitriles **54** & **57** via PINNER-reaction into the esters **56** & **58**.

At this point we made the decision to purchase the desired methyl ester **56** from a commercial vendor and used it for the reductive amination using the developed standard conditions.[189] The reaction did not proceed to completion within 70 min and another addition of ketone **38** (0.2 eq) as well as $BH_3 \cdot THF$ (0.2 eq) proved necessary to ensure full conversion of the starting aniline **56** (Figure 41). The product isomers of **58** could be partially separated via column chromatography and **58** was isolated in 46% yield. Saponification furnished the desired F-Phenazistatin **52**. However, the reactions proceeded very sluggishly and additional portions of $LiOH \cdot H_2O$ (*cis*: 15 eq, *trans*: 3 x 15 eq) were required to hydrolyze both ester functionalities.

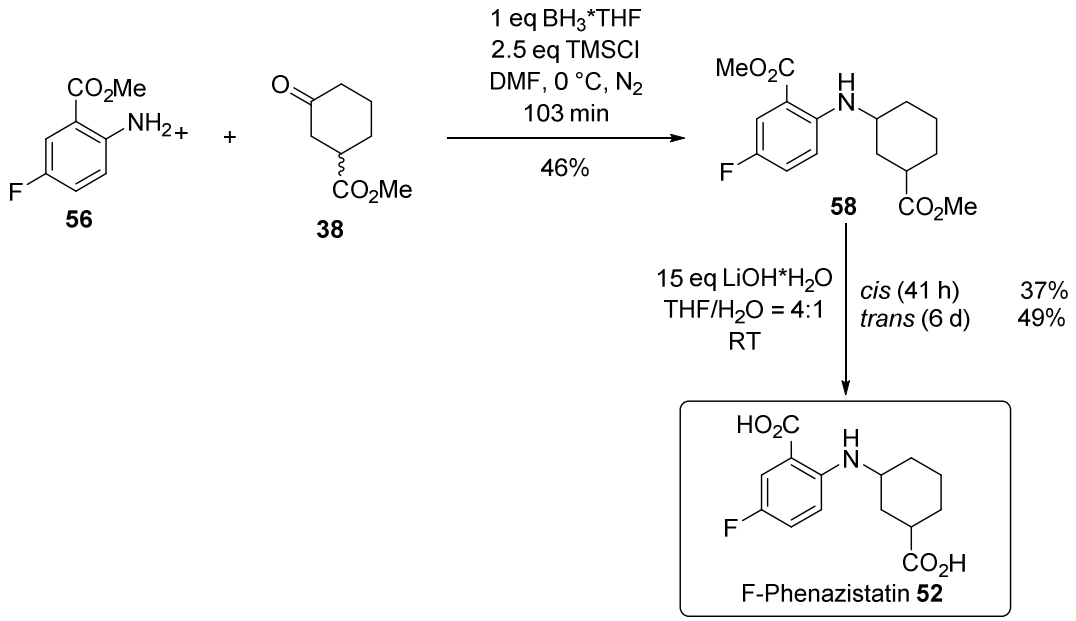


Figure 41: Synthesis of the F-Phenazistatin **52** via reductive amination[189] and hydroxide saponification.

4.2.1.3.3 Synthesis of Cl-Phenazistatin

Cl-Phenazistatin **59** was synthesized using an ULLMANN-GOLDBERG-amination while the sufficiently active reductive amination methods had not yet been developed.

The required 5-chloro-2-iodobenzoic acid (**60**) had to be synthesized and a chlorination of 2-iodobenzoic acid was attempted. The method by Valois-Escamilla et al.[204] for the NBS bromination of inactivated aromatic substrates in concentrated H_2SO_4 was attempted with NCS, but no conversion of the starting material could be observed by ^{1}H-NMR after 16 h (Figure 42). The same results were obtained when the dipolar aprotic solvent DMF was used.

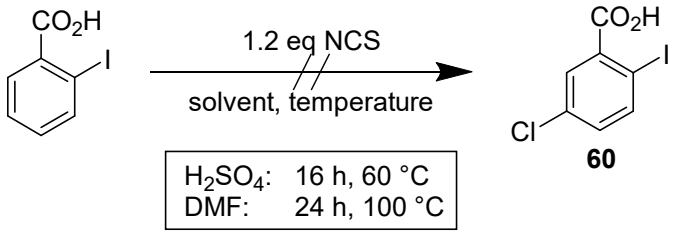


Figure 42: Chlorination attempts of 2-iodobenzoic acid using conditions by Valois-Escamilla.[204]

We hypothesized, that chlorination of a more electron-rich anthranilic acid derivative could be possible using the procedures described above followed by a SANDMEYER reaction to give the desired aryl iodide **60**. Therefore, anthranilic acid was treated with 1 eq NCS in DMF and the mixture was heated to 100 °C until full conversion of the starting material was detected by TLC (Figure 43). Standard workup yielded 54% of a solid which was identified with ^{1}H-NMR analysis as a mixture of the *para*- and *ortho*-chlorinated anthranilic acid. Attempts to separate this mixture by recrystallization failed and hence a different strategy to synthesize the product had to be employed.

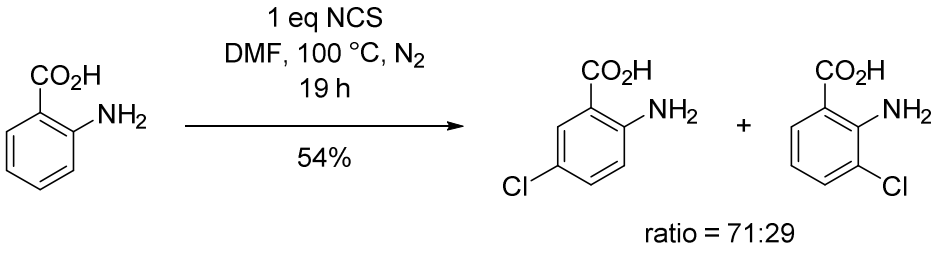


Figure 43: Chlorination attempts of anthranilic acid using NCS.

In order to alter the selectivity inherent to the reaction we first tried to change the substrate to the readily available methyl anthranilate which can be transformed to the desired chlorinated anthranilic acid **61** according to Sun et al.[205] using a hypochlorite chlorination followed by a hydroxide saponification. In the first step the chlorination reaction was left stirring in the

thawing ice bath for 2 h (TLC: inconclusive) followed by an extraction, a $Na_2S_2O_3$-quench and concentration (Figure 44). ^{1}H-NMR analysis indicated the presence of two species, presumably the desired intermediary ester and parts of the already hydrolyzed anthranilic acid derivative **61**. The saponification was conducted with 3 eq KOH in MeOH, but was found to have a very low reaction rate. In total 3 h at RT and 3 d stirring at elevated temperature (60 °C) were necessary to furnish the desired product **61** in 80% crude yield. This product could be telescoped to the next step, a purity upgrade by recrystallization was facilitated after the following step. The SANDMEYER reaction was performed according to a standard procedure[206] and yielded 54% of the desired aryl iodide **60** as a single product after recrystallization from MeOH/H_2O.

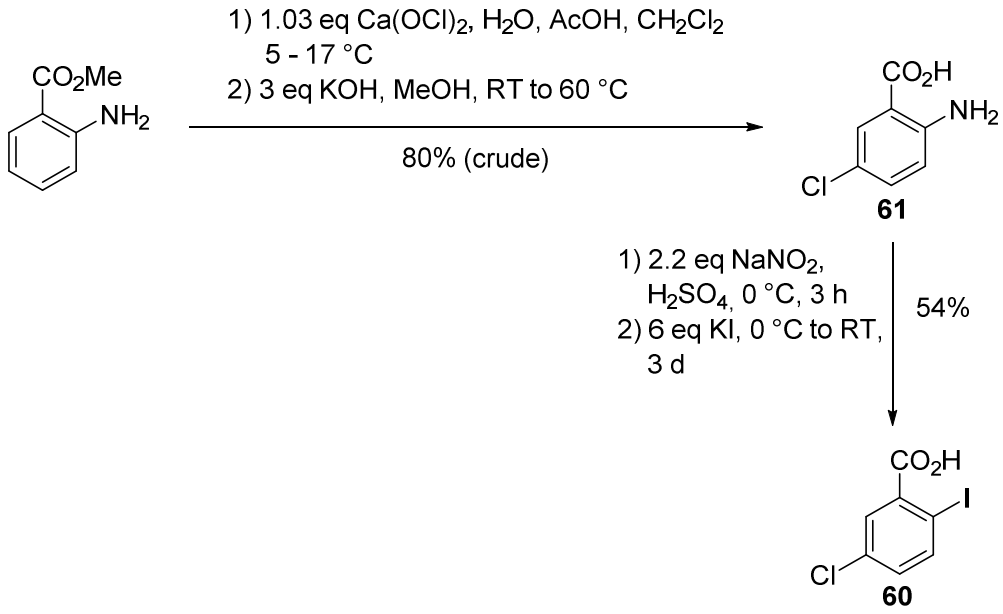


Figure 44: Synthesis of 5-chloro-2-iodobenzoic acid **60** from methyl anthranilate.

The final Cl-Phenazistatin **59** was produced by the ULLMANN-GOLDBERG-amination method published by Liu.[197] After 6 d reaction time only traces of substrate **60** were detectable by TLC and the reaction mixture was worked up and telescoped without purification to the consecutive saponification step (Figure 45). The saponification proceeded very slowly and only after 13 d full conversion of the starting material could be detected. The product was purified via column chromatography and a portion of clean *cis*-stereoisomer of **59** could be isolated for biological testing.

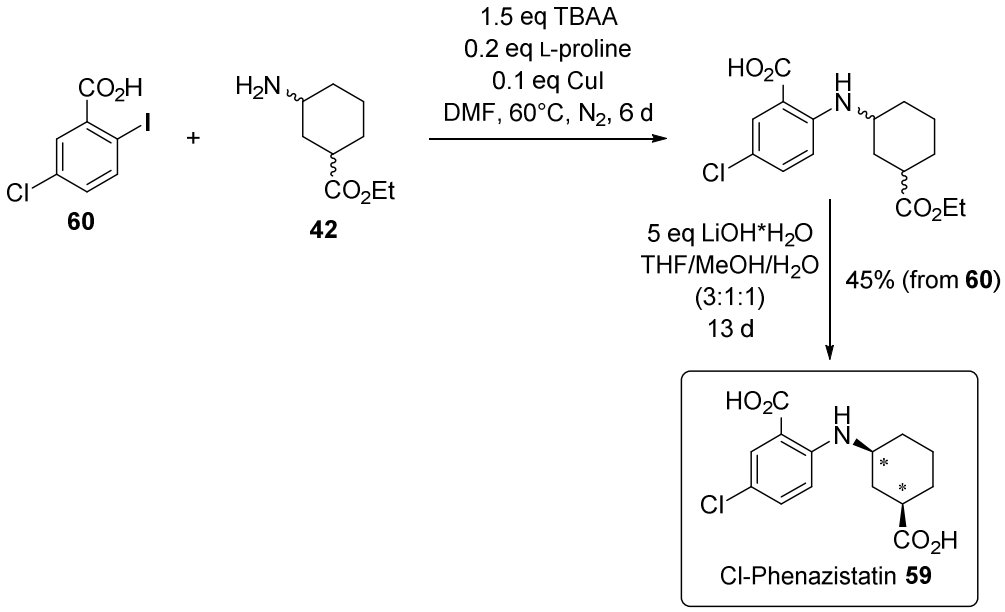


Figure 45: Synthesis of Cl-Phenazistatin **59** via an ULLMANN-GOLDBERG-amination/saponification approach.

4.2.1.3.4 Synthesis of Br-Phenazistatin (Phenazistatin A)

Phenazistatin A **1** was in general prepared according to the procedure by Liu et al.[197] and this optimized synthesis was already disclosed in the Master thesis of Pletz.[196] However, there was another method tested to synthesize the product and the mono-ester **50** of Phenazistatin A **1** was synthesized, which shall be stated here.

When 5-bromo-2-iodobenzoic acid[204] and the corresponding amine **42** to give Phenazistatin A **1** were reacted according to Buchwald et al.,[198] incomplete conversion of the starting material (5-bromo-2-iodobenzoic acid) was observed after 2 d. HPLC-MS analysis of the mixture indicated a complex mixture with 20% product **50** and 47% undesired homocoupling-product **62**, which corresponds to unproductive turnover of the substrate halide (Figure 46). The reaction was considered as too unselective to be developed further.

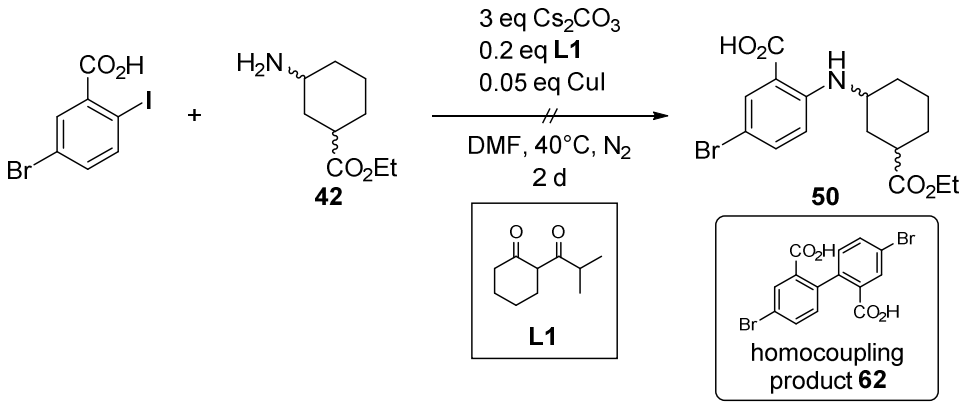


Figure 46: Attempted synthesis of the Br-Phenazistatin precursor **50** via a procedure by Buchwald.[198]

When the methods developed in our laboratory[189] for the reductive amination of electron-deficient anilines were applied for the synthesis of the di-ester of Phenazistatin A **7**, good yields were obtained in short reaction times (Figure 47). These reactions will be described in detail in the following section. We tested whether the free 2-amino-5-bromobenzoic acid could be reacted using the same conditions and found that the reaction proceeds to completion within 46 min at 0 °C and 74% yield were isolated via column chromatography. Our attempts to separate the stereoisomers via column chromatography failed due to tailing effects attributed to the free carboxylic acid.

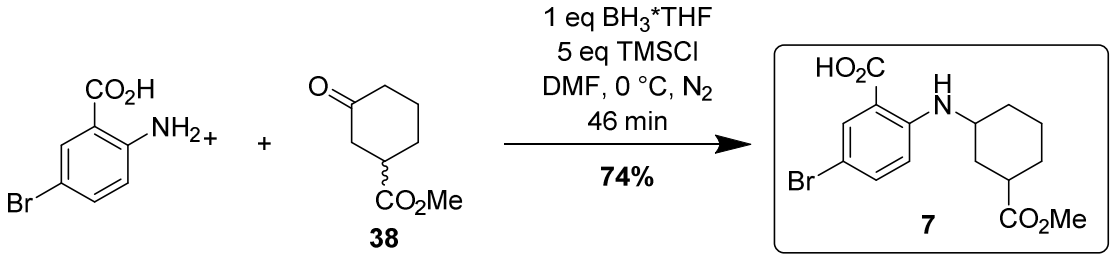


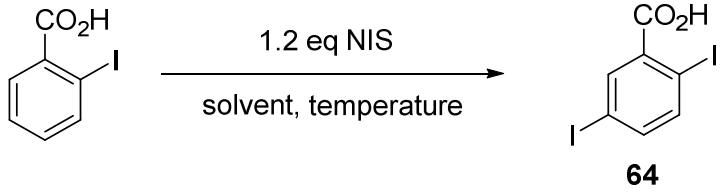
Figure 47: Synthesis of the di-ester of Phenazistatin A **7** via reductive amination.[189]

4.2.1.3.5 Synthesis of I-Phenazistatin

The I-Phenazistatin **63** was initially attempted to be synthesized using an ULLMANN-GOLDBERG-amination method[197] and later by reductive amination.[189]

In order to produce the 2,5-diiodobenzoic acid (**64**) required for testing the ULLMANN-GOLDBERG-amination, the synthesis of the aryl iodide was attempted by iodination with NIS in AcOH (Table 26, Entry 1), but no conversion of the substrate was detected. However, when the bromination agent NBS was replaced by NIS in the method for the bromination of electron-deficient aryl substrates by Valois-Escamilla et al.,[204] full conversion of the starting material was detected after 5 h and 48% of the desired 2,5-diiodobenzoic acid (**64**) were isolated after recrystallization (Entry 2).

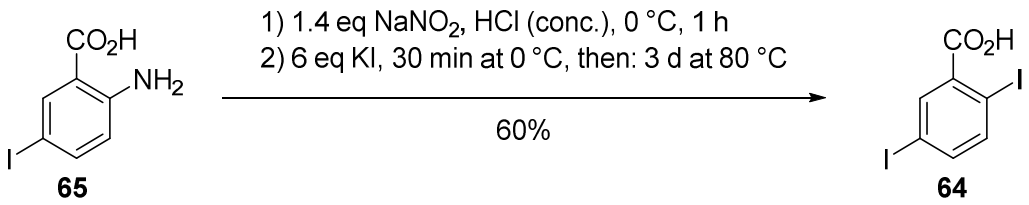
Table 26: Synthesis of 2,5-diiodobenzoic acid (**64**) by iodination with NIS.



64

Entry	solvent	temperature	time	comments
1	AcOH	20 to 50 °C	5 d	no conversion
2	H_2SO_4 (conc.)	60 °C	5 h	full conversion 48% isolated

During later stages of the project larger quantities of the aryl di-halide **64** were required and an alternative method for the synthesis was sought, considering the high price of NIS. The SANDMEYER reaction had already been successfully employed for the synthesis of the chloro-derivative **60** (vide supra) and was hence tried (Figure 48) with the readily available anthranilic acid derivative **65** (prepared from anthranilic acid according to a procedure by Kini et al.[207]) and 60% of the product were isolated after standard workup and recrystallization (H_2O/MeOH).



65 **64**

Figure 48: Synthesis of 2,5-diiodobenzoic acid (**64**) via a SANDMEYER-reaction.

The ULLMANN-GOLDBERG-amination method by Liu et al.[197] with 2,5-diiodobenzoic (**64**) was performed and after 6 d incomplete conversion of the starting material **64** was detected (TLC) (Figure 49). No further conversion could be detected upon prolonged reaction time and so the mixture was worked up and telescoped into the consecutive step. The hydrolysis at RT proceeded very slowly and only after 13 d full conversion of the mono-ester **66** could be detected. Workup and separation of the stereoisomers via column chromatography yielded a fraction of 21% of the pure *cis*-I-Phenazistatin **63**.

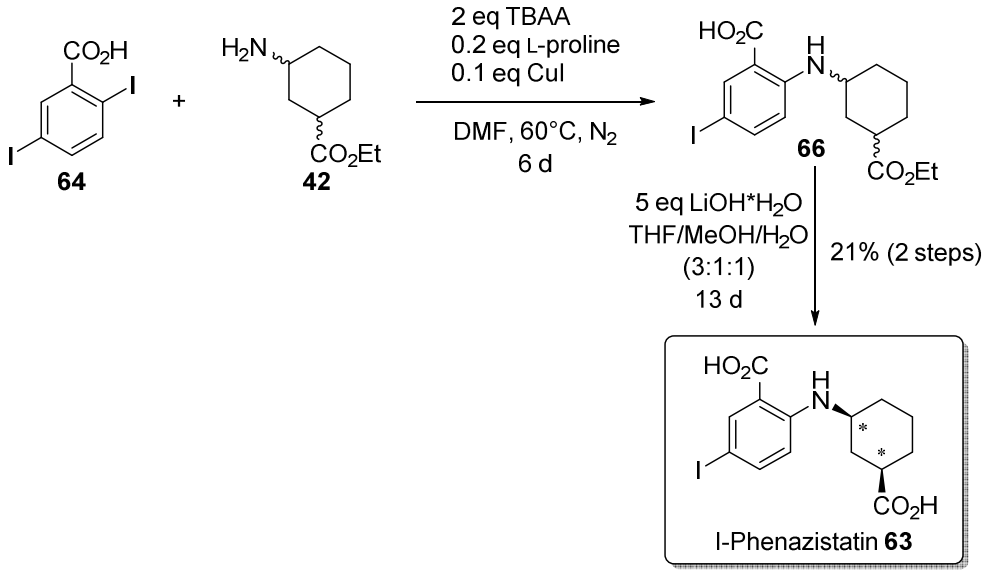


Figure 49: Synthesis of I-Phenazistatin **63** via conditions by Liu.[197]

The ULLMANN-GOLDBERG-amination described above delivered the desired I-Phenazistatin **63**, albeit in low yield, because during the amination reaction many by-products had formed diminishing the overall yield. We then tried to apply the developed reductive amination method[189] for the synthesis of the I-Phenazistatin **63**.

2-Amino-5-iodobenzoic acid hydrochloride was conveniently synthesized according to a procedure by Pal et al.[208] on a 100 mmol (15 g) scale (Figure 50). The reaction proceeded smoothly to full conversion of the starting material (3 d, TLC). During the reaction a colorless precipitate had formed which was found to be the product hydrochloride **67**. The solid was collected via filtration, repeatedly rinsed with H_2O, and was found to be NMR pure after drying. Recrystallization of this solid to improve the morphology or to facilitate drying failed due to the low-solubility of the hydrochloride **67**. For future preparations the reaction should be conducted in the presence of an equimolar amount of base (NaOAc, $NaHCO_3$, etc.) in order to neutralize the HCl generated and to yield the free aniline, which could be more easily recrystallized.

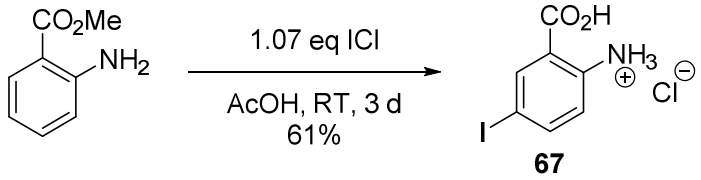


Figure 50: Synthesis of **67** via iodination with ICl.[208]

The reductive amination of the hydrochloride **67** proceeded nicely to the desired product and a total amount of 8.61 g product (97%) were isolated after column chromatography (Figure 51). The use of the hydrochloride had no influence on the reactivity and the only

change made in the reaction setup was the use of +200% DMF because the hydrochloride **67** did not fully dissolve using the standard amount. When using the free aniline, generally a clear solution was obtained with the standard conditions of method B.[189] A careful second column chromatography was conducted with 1.00 g isomer mixture in order to separate the *cis*- and *trans*-isomers of **36** for analysis. The separation was on the one hand very time- and solvent intensive (38 cm x 2.4 cm), but on the other hand 551 mg *cis*- and 405 mg *trans*-isomer of **36** could be isolated in high purity. For the synthesis of Phenazistatin derivatives this di-ester stage of the iodide **36** seems to be the best point to separate the isomers, especially when the di-ester aryl iodide **36** is being used as a starting material for further reactions (e.g. cross-coupling reactions). Standard saponification and workup of the pure isomer fractions (acidification, solvent removal, preparative RP-HPLC) yielded the pure stereoisomers of I-Phenazistatin **63** in good isolated yield.

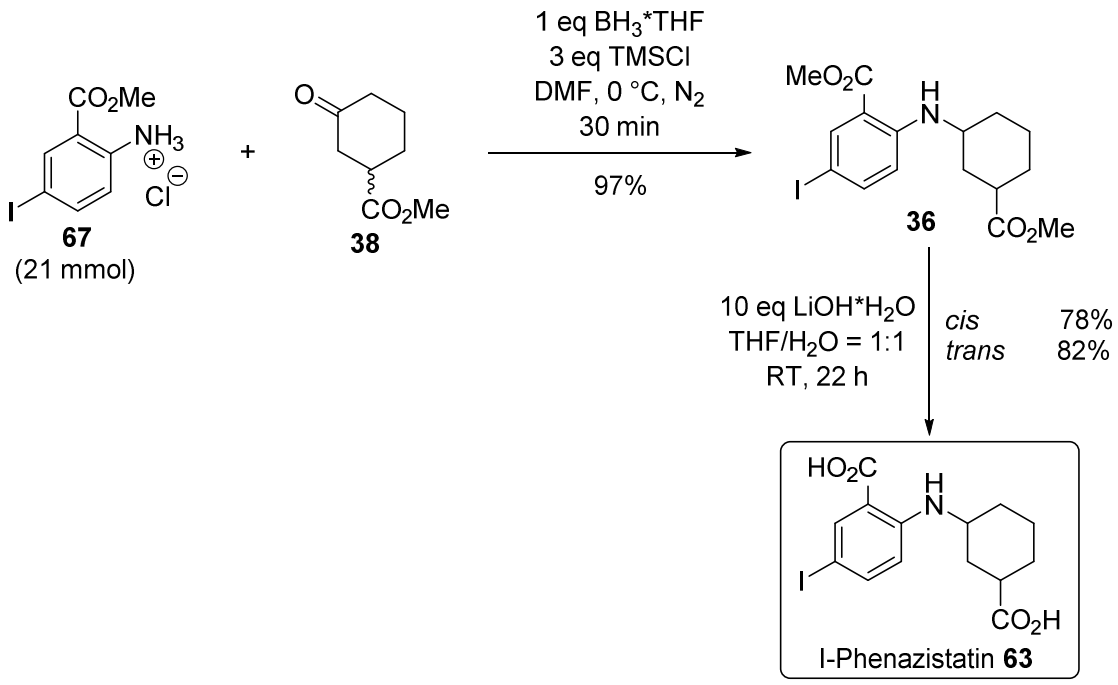


Figure 51: Synthesis of I-Phenazistatin **63** via reductive amination[189] and saponification.

4.2.1.3.6 Synthesis of Me-Phenazistatin

For the synthesis of Me-Phenazistatin **68** the same synthetic strategy was applied as for Cl-Phenazistatin **59**: a SANDMEYER reaction in order to produce the 2-iodo-5-methylbenzoic acid (**69**) and the ULLMANN-GOLDBERG-amination with the pure *cis*-amine **42**. Later in the project some quantities of the product were also successfully synthesized according to a reductive-amination/methylation-sequence.

The aryl iodide precursor **59** was synthesized according to a standard SANDMEYER procedure by Kini et al.[207] and 86% of the desired product were obtained after standard workup and recrystallization (MeOH/H_2O) (Figure 52).

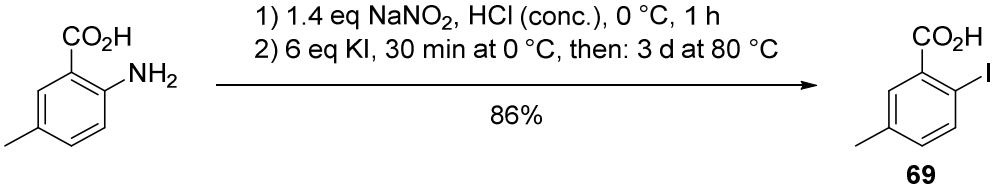


Figure 52: Synthesis of 2-iodo-5-methylbenzoic acid (**69)** via a SANDMEYER-reaction.[207]

The synthesis of Me-Phenazistatin **68** via the ULLMANN-GOLDBERG-amination proceeded very sluggishly and after 11 d reaction time still traces of substrate **69** could be detected by TLC (Figure 53). The complex reaction mixture was worked up and the crude reaction mixture was telescoped into the subsequent saponification step. The saponification step was started with 2 eq $LiOH \cdot H_2O$ and after 28 h incomplete conversion was detected and additional portions of hydroxide were needed to complete the reaction (+2 eq and 23 h, +2 eq and 24 h). A minor quantity of the desired *cis*-Me-Phenazistatin **68** could be isolated via column chromatography.

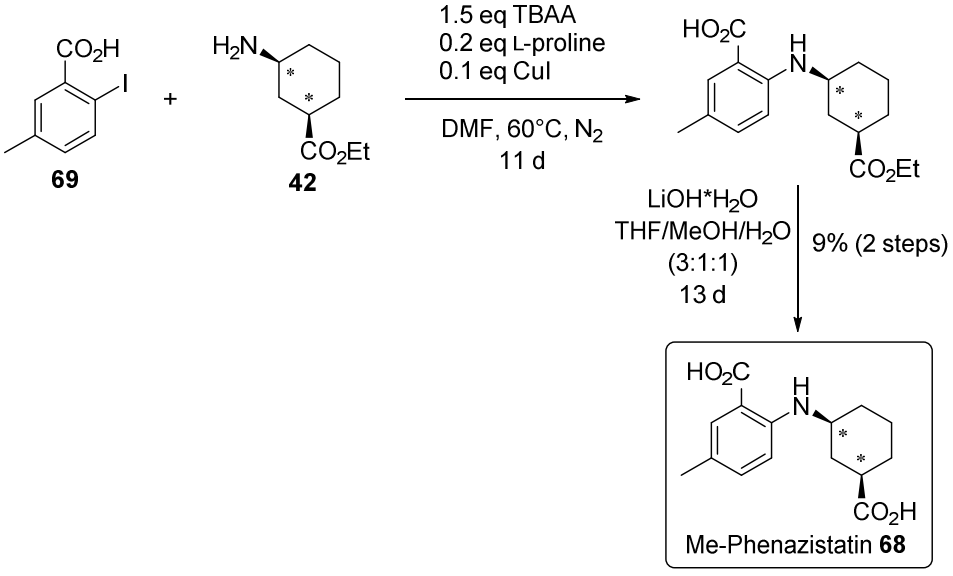


Figure 53: Synthesis of Me-Phenazistatin (**68)** via reductive amination/saponification.[189]

Also for this substrate the ULLMANN-GOLDBERG-amination reaction was found to be quite unselective and several by-products could be detected. The reductive amination approach was tested, but it was first tried to convert the substrate acid **70** to the ester **71** in order to directly obtain easily separable stereoisomers (di-esters) after the reductive amination. We

tested an alkylative esterification with Me_2SO_4 in the presence of equimolar quantities of Na_2CO_3 (Figure 54).[209] Full conversion of the starting material was detected after 3 d. However, the reaction mixture was found to contain a complex mixture of 52% mono-methylated, 43% dimethylated and 6% trimethylated product (GC-MS), which could hardly be resolved (and hence separated) by TLC. The alkylative esterification with Me_2SO_4 seems to be too unselective for this substrate. The Fischer-esterification (boiling the substrate in MeOH in the presence of 3 eq H_2SO_4 (conc.)) was not found to be a viable alternative, since several by-products with similar polarity were generated and probably a column chromatography would be necessary to obtain a clean product.

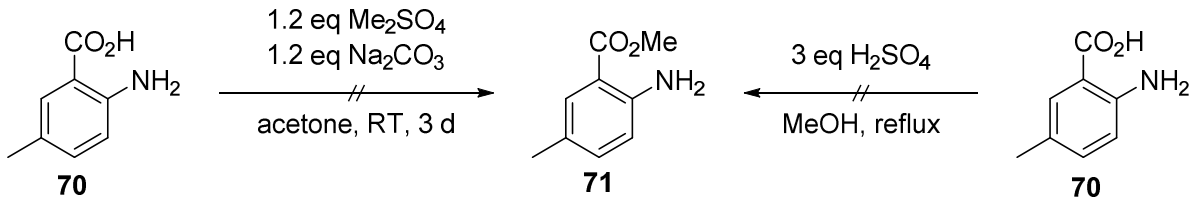


Figure 54: Attempted alkylative esterifications with **70** as substrate.

We reasoned, that we might suppress over-methylation on the aniline **70** by introducing a sterically bulky substituents on the nitrogen. The logical protecting step would be to first conduct the reductive amination (an inherent protecting group would be installed) followed by the methylation step. However, there are some problems connected to this strategy when considering previous findings concerning the reductive amination. The stereochemical outcome of the reductive amination cannot be controlled and a mixture of *cis*- and *trans*-isomers of **72** would be obtained. In previous experiments we found that we cannot separate a mono-ester product (which is obtained directly from the reductive amination) via column chromatography, due to intensive tailing effects. We hence had to proceed via the previously discussed pathway C (vide supra, Figure 34) of the general purification strategies, which includes a detour via a reductive amination/esterification/purification/saponification to yield the final product **68**.

The reductive amination was conducted using method B[189] and the reaction mixture was stirred until full conversion of the starting material **70** was detected after 3 d (Figure 55). The mixture was worked up according to the standard procedure and the crude product was treated with a slight excess of Me_2SO_4 in the presence of base. The methylation reaction proceeded very slowly and after 10 d incomplete conversion of the starting material was detected by TLC and another portion (0.6 eq) of the reagents was added. Full conversion was obtained after another 24 h and the mixture was worked up and purified via column chromatography. We were very pleased to find that no over-methylated product was formed

in the reaction, in spite of the excess of Me_2SO_4 (GC-MS). Clean fractions of the *cis*- and *trans*-isomer of **73** could be isolated, which were telescoped to the subsequent hydrolysis step. The saponification proceeded smoothly to give the pure isomers of Me-Phenazistatin **68** for biological testing.

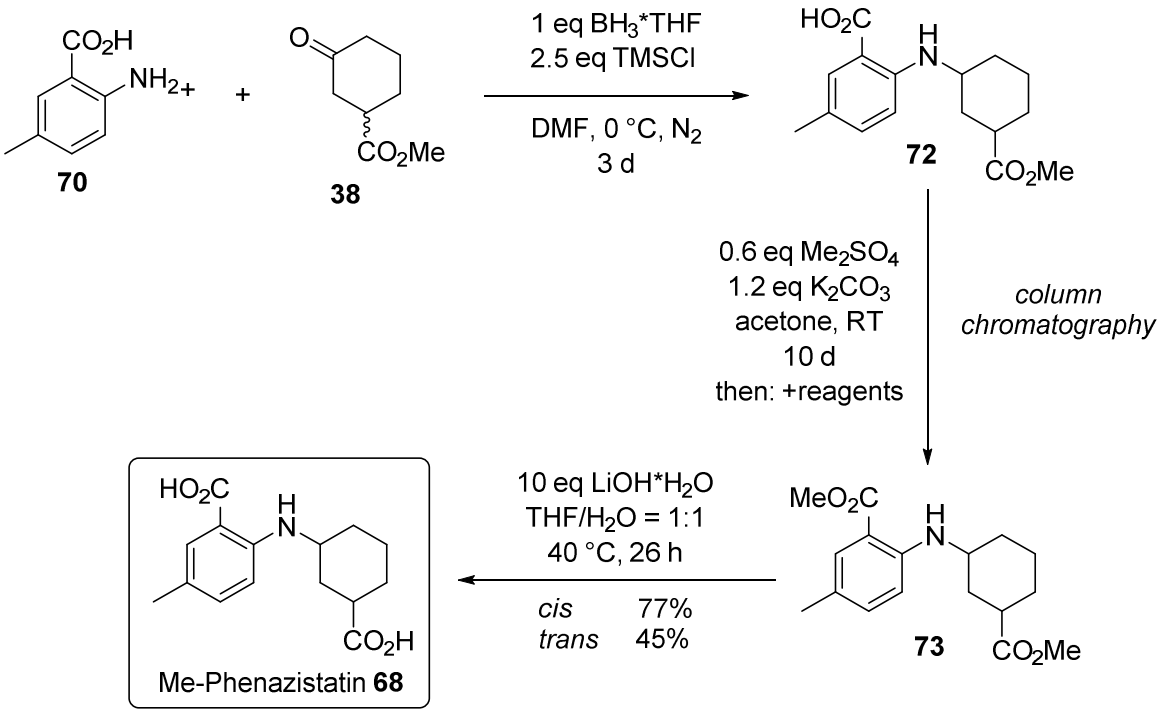


Figure 55: Synthesis of Me-Phenazistatin **68** via reductive amination/saponification.[189]

4.2.1.3.7 Synthesis of MeO-Phenazistatin

The synthesis of MeO-Phenazistatin **74** was initially conducted via an ULLMANN-GOLDBERG-amination starting from the readily available 2-bromo-5-methoxybenzoic acid (**75**),[210] which was synthesized from 3-methoxybenzoic acid and purified by recrystallization (Figure 56).

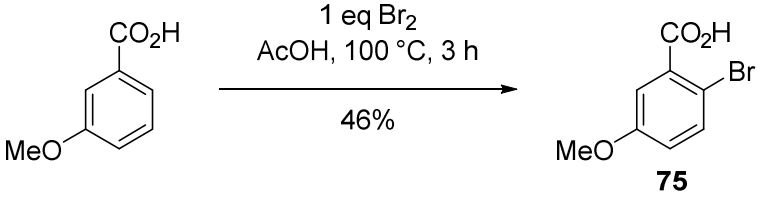


Figure 56: Synthesis of 2-bromo-5-methoxybenzoic acid (**75)** via bromination.[210]

The ULLMANN-GOLDBERG-amination[197] with 2-bromo-5-methoxybenzoic acid (**75**)[210] as a substrate proceeded to completion within 22 h and the reaction mixture was in this case not worked up, but was treated directly with an equal volume of H_2O followed by 10 eq $LiOH \cdot H_2O$ (Figure 57). The mixture was stirred 22 h at 40 °C, but TLC indicated incomplete conversion of mono-ester **76** so an additional portion of 10 eq $LiOH \cdot H_2O$ was added and full conversion

was detected after an additional 31 h at 40 °C. The product could be purified via repeated preparative RP-HPLC and a glassy, amorphous solid was obtained in >100% yield (insufficiently dried, repeated azeotropic drying with various solvents had no effect). However, ^{1}H-NMR analysis indicated the presence of the clean desired *cis*-isomer of MeO-Phenazistatin **74**. We decided to apply a reductive amination approach in order to generate a sufficiently clean sample for biological testing.

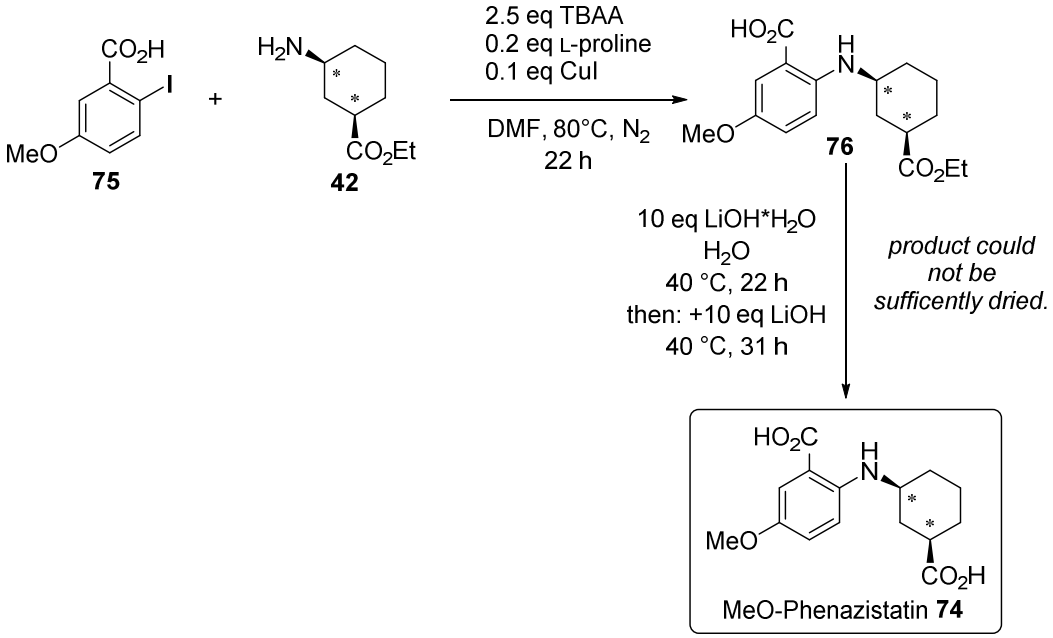


Figure 57: Synthesis of MeO-Phenazistatin**74** via an ULLMANN-GOLDBERG-amination/saponification approach.[197]

When we wanted to apply the reductive amination strategy the commercially available 2-amino-5-methoxybenzoic acid (**77**), we had to cope with the identical issues like it was the case when using the Me-anthranilic acid **70** in the synthesis of Me-Phenazistatin **68** (vide infra) (Figure 58). In addition to the separation problems for the reductive amination product **78** (mono-acid) we also could not synthesize the corresponding methyl ester of **79** by standard alkylative- or Fischer-esterification protocols. When 1 eq of the 2-amino-5-hydroxybenzoic acid was treated with only a slight excess of 2.4 eq methyl-equivalents (= 1.2 eq Me_2SO_4) a mixture of three distinct peaks was detected on GC-MS, which presumably correspond to the mono-, bis- and tris-methylated products according to their mass signals. The distribution of methyl groups is somewhat counterintuitive, since 2.4 methyl equivalents would suggest a stronger preference for the bis- and the tris-methylated product. Anyway, the products could not be cleanly separated on TLC and hence it was not attempted to separate them by column chromatography. We therefore decided to employ the same strategy as it was used previously to synthesize Me-Phenazistatin **68** (vide supra) to circumvent this problem, namely the reductive amination/methylation/purification/ saponification-strategy.

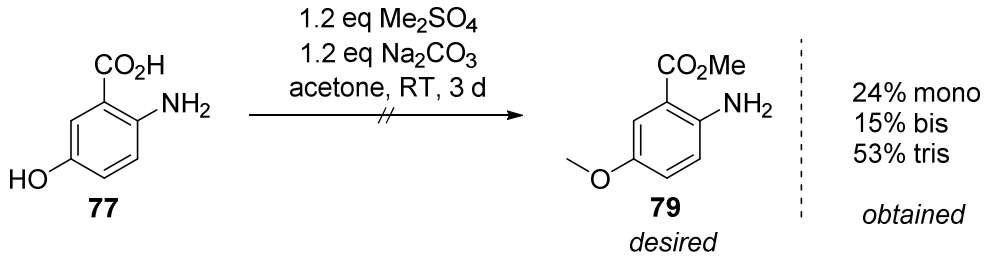


Figure 58: Synthesis of **79** via alkylative esterification of 2-amino-5-hydroxybenzoic acid.

The reductive amination proceeded smoothly to a single spot on TLC, which did not show any separation of the TLC as was expected for the mono-acid **78** (Figure 59). The mixture was worked up according to the standard procedure[189] and telescoped to the alkylative esterification step, which proceeded sluggishly and full conversion could only be detected after 8 d. The reaction mixture was worked up and the stereoisomers of the di-ester **80** could be partially separated via column chromatography. The clean fractions were telescoped to the saponification step, but 15 eq hydroxide were not enough to saponify both esters and three additional portions of base (a total of 60 eq) over a period of 6 d were necessary to ensure full hydrolysis. In order to obtain analytically pure samples of the isomers (some impurities, presumably the other isomers) they were repeatedly purified via preparative RP-HPLC, which resulted in low yields of the desired isomers of MeO-Phenazistatin **74**.

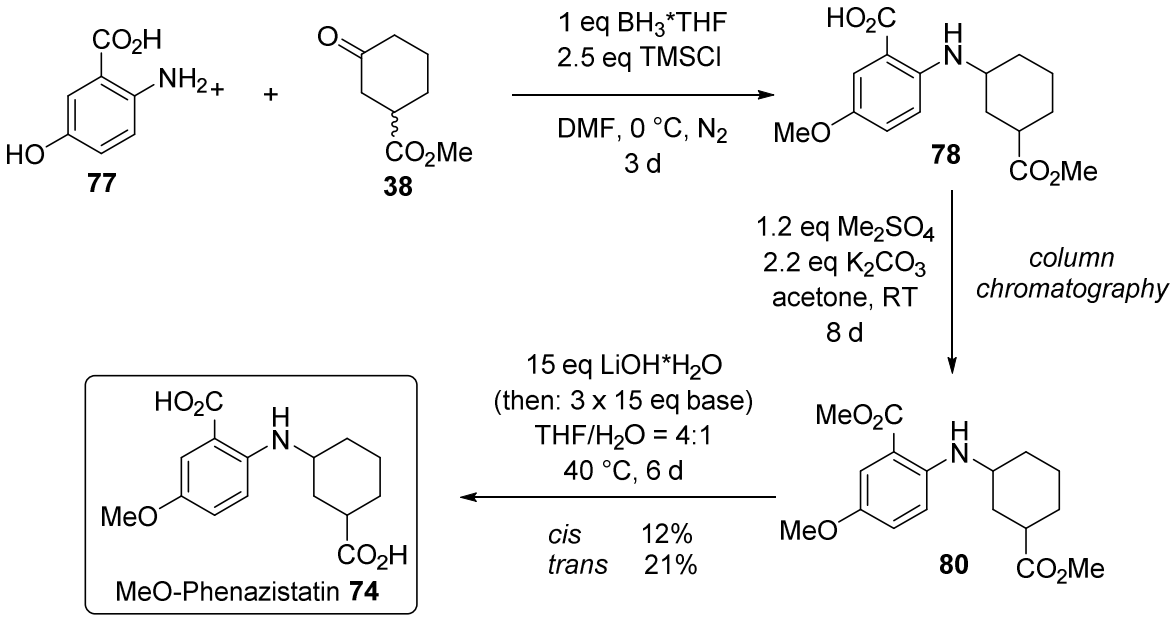


Figure 59: Synthesis of Me-Phenazistatin **74** via reductive amination/alkylative esterification/saponification.

4.2.1.3.8 Synthesis of NO_2-Phenazistatin

In order to prepare the corresponding ULLMANN-GOLDBERG-amination precursor **81** for the synthesis of NO_2-phenazistatin **82**, 2-bromobenzoic acid was nitrated using a standard procedure (Figure 60). This procedure worked smoothly on small scale and 66% of the product **81** could be isolated after recrystallization. When conducting this reaction on a larger scale special care should be taken with large amounts of nitrous gases generated (neutralization of the gases in aqueous base solution with security washing bottles is recommended).

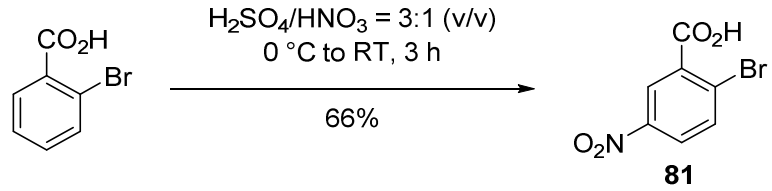
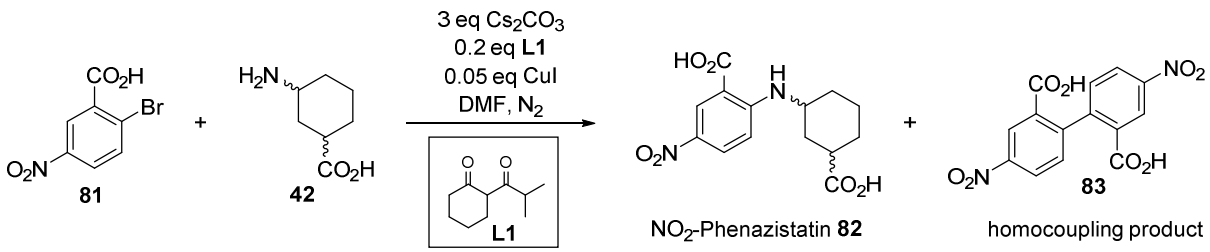


Figure 60: Synthesis of 2-bromo-5-nitrobenzoic acid (**81**) via nitration of 2-bromobenzoic acid.

With this substrate the effect on the halide/amine loading and the temperature on the product distribution of the ULLMANN-GOLDBERG-amination were studied. The well-established and very active conditions by Buchwald et al.[198] were used for this screening with 2-bromo-5-nitrobenzoic acid and 3-aminocyclohexane-1-carboxylic acid as substrates.

It could be shown, that significant amounts of the undesired homocoupling-product **83** were produced in each case (Table 27, Entries 1-3) and that the loading of the amine had a direct influence on the amount of **83** produced. As expected, the more amine was present, the higher the product **82** share was (Entries 1-3). When the temperature was increased, a higher share of product **82** and less homocoupling product **83** was observed (Entry 2 and Entry 4). Only when using 80 °C for this reaction the reaction could be brought to completion within 1 d, but when using 40 °C after 8 d all the other reactions remained incomplete. These findings suggests, that the used substrate combination is very unreactive, since Buchwald et al.[198] had developed this method for the RT C-N coupling of aryl halides. Unfortunately, these observations also indicate, that these conditions cannot be employed for the selective synthesis of Phenazistatin derivatives.

Table 27: Halide- / amine-loading and temperature screening of the ULLMANN-GOLDBERG-amination by Buchwald et al.[198] (ratios given according to HPLC-MS total area%).



Entry	Halide [eq]	Amine [eq]	Temperature [°C]	Ratio: homocoupling/product	
				1 d (conv.)	8 d (conv.)
1	1	1.5	40	40:60	37:63 (incompl.)
2	1	1	40	41:59	38:62 (incompl.)
3	1.5	1	40	58:42	50:50 (incompl.)
4	1	1	80	30:70 (100%)	-

The first quantity of NO_2-Phenazistatin **82** was synthesized by an ULLMANN-GOLDBERG-amination according to the procedure by Liu et al.[197] starting from 2-iodo-5-nitrobenzoic acid (**84**) (Figure 61). The amination reaction went to completion within 29 h at 80 °C and the mixture was cooled down and used as such for the consecutive step. Therefore an equal volume in respect to the reaction solvent was added followed by a large excess of 20 eq $LiOH \cdot H_2O$. The mixture was heated to 80 °C, because for the Me-Phenazistatin **68** it was found that the hydrolysis proceeds very slowly in the DMF/H_2O mixture. Full conversion of the starting material was detected after 3 d and 51% NO_2-Phenazistatin were isolated after preparative RP-HPLC.

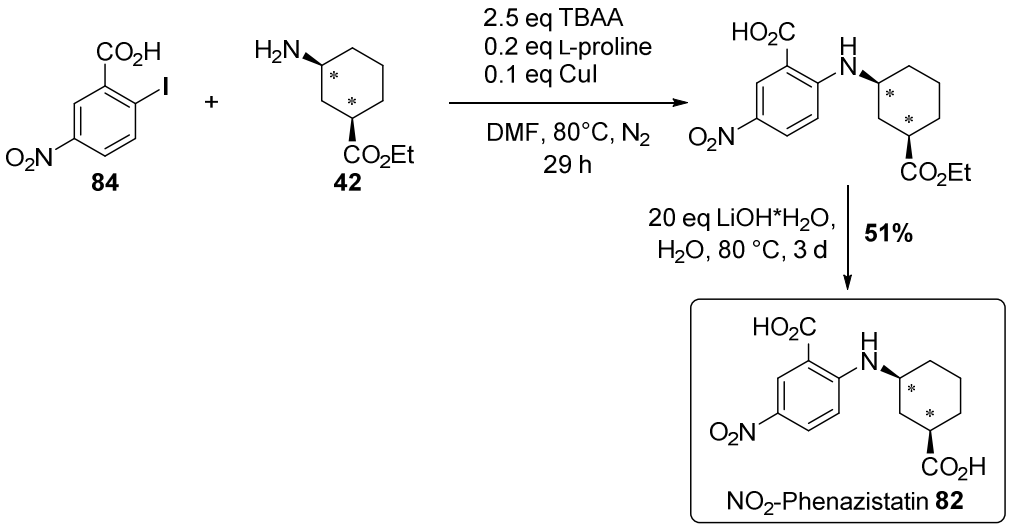


Figure 61: Synthesis of NO_2-Phenazistatin **82** via an ULLMANN-GOLDBERG-amination/saponification approach.[197]

We then tried to produce NO_2-Phenazistatin **82** via reductive amination using our developed method (Figure 62). Unfortunately, again only the free anthranilic acid derivative 2-amino-5-nitrobenzoic acid (**85**) was commercially available and hence the same approach containing a detour as for the previous Phenazistatin derivatives (Me-, MeO-Phenazistatin) had to be applied (reductive amination/esterification/purification/saponification-sequence). In the reductive amination incomplete conversion of the starting material was detected after 3 d, but the reaction was worked up and telescoped to the alkylative esterification step. After 8 d at RT incomplete conversion of the mono-acid **86** was detected, but no additional alkylating agent was added in order to prevent overalkylation (on the secondary amine) and the mixture was worked up. The isomers of the di-ester **87** of NO_2-Phenazistatin could be partially separated via column chromatography and a total of 26% were isolated. The clean isomers of the di-ester **87** were hydrolyzed using much milder conditions than the previous Phenazistatin derivatives, which needed additional quantities of base or heating to ensure full conversion.

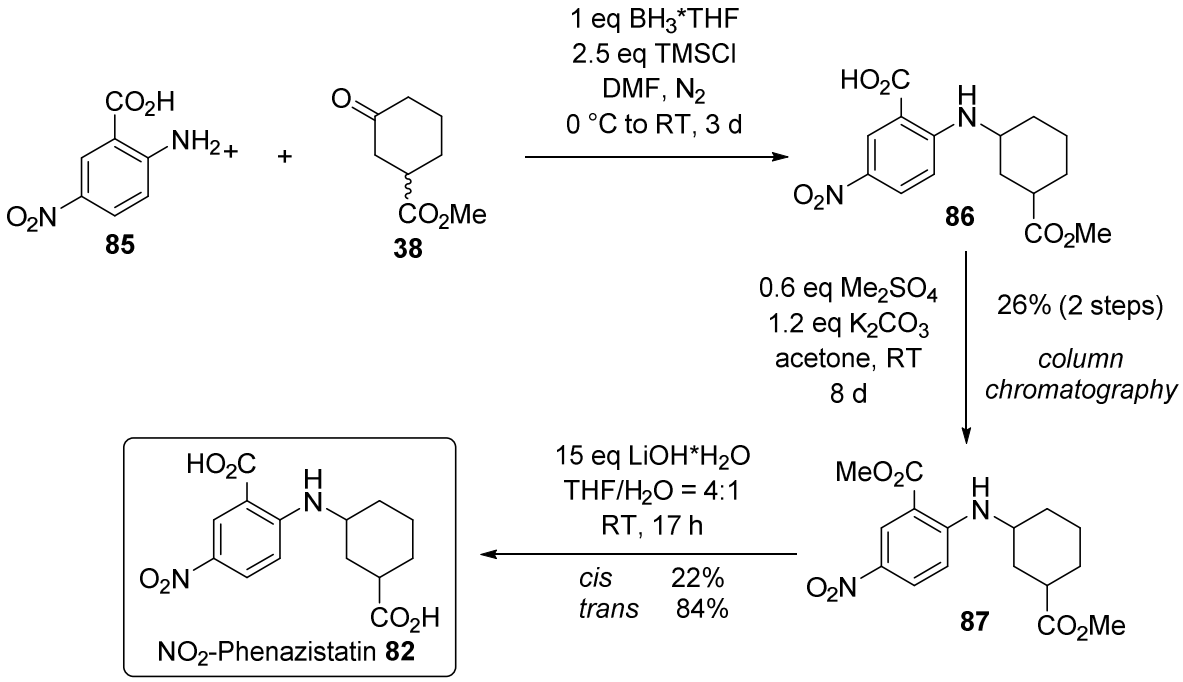


Figure 62: Synthesis of NO_2-Phenazistatin **82** via reductive amination/alkylative esterification/saponification.

The synthesis of the NO_2-derivative was carried out in parallel to the other syntheses where the readily available anthranilic acid derivative could not be successfully esterified by standard conditions (vide supra, Me- & MeO-Phenazistatin). We expected a similar behavior of the NO_2 derivative during alkylation, so in order to test this hypothesis we conducted a similar alkylative esterification experiment as for the Me- & OH-derivative, where this reaction yielded a wild mixture of products (Figure 63). When an excess of 3.2 alkylation equivalents (1.6 eq Me_2SO_4) were applied onto 2-amino-5-nitrobenzoic acid (**85**), full conversion of the starting material was detected after 22 h and both GC-MS and TLC indicated the formation of

a mono-alkylated product **88**. Apart from some minor impurities the product **88** was pure. For future efforts to produce the NO_2-Phenazistatin **82**, the already protected ester building block **88** should be used, in order to facilitate the purification steps and to increase the overall yield.

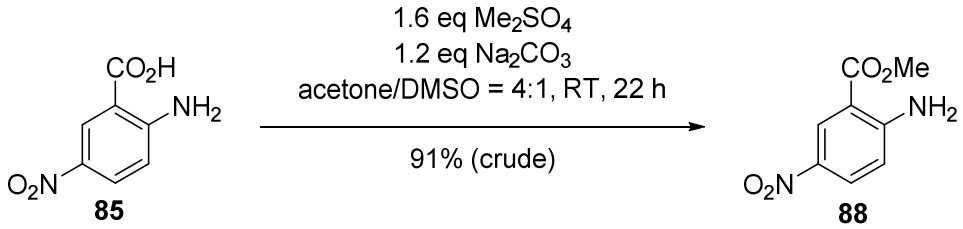


Figure 63: Synthesis of **88** via alkylative esterification with Me_2SO_4.

4.2.1.3.9 Synthesis of NH_2-Phenazistatin

As was described earlier (vide supra, Table 19), the direct synthesis of NH_2-Phenazistatin was not possible using the reductive amination approach due to instability of the product or an intermediary product.

We tried whether the NH_2-Phenazistatin **89** could be synthesized directly from the NO_2-Phenazistatin **82**, hence circumventing the reductive amination procedure which might have caused degradation by itself (Figure 64). When standard catalytic hydrogenation conditions were applied, full conversion of the starting material was detected after 3 d (TLC). When the reaction mixture was applied to the silica gel TLC plate, the spot immediately turned to an intensive violet-black color and showed an intensive tailing when developed in the chamber (same effect was found when an acid additive was used). HPLC-MS and ^{1}H-NMR analysis was inconclusive and showed a complex mixture and hence we assume that the product might have degraded presumably by oxidation of the hydroiminoquinone.

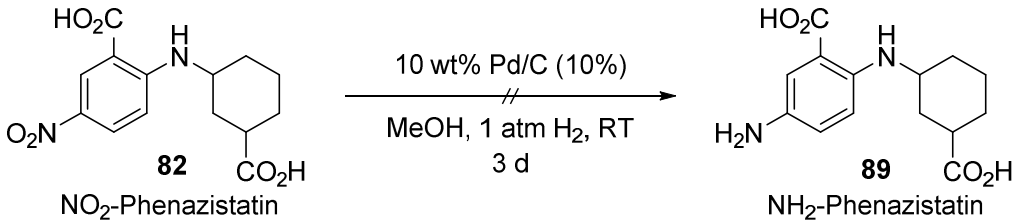


Figure 64: Synthesis of NH_2-Phenazistatin **89** via catalytic hydrogenation.

4.2.1.4 Synthesis of inhibitors via cross-coupling reactions at the 5-position of the aromatic ring

The newly developed methods[189] for the reductive amination of electron-deficient anilines made the di-ester of the I-Phenazistatin **36** available in large quantities, which allowed the diversification using cross-coupling reactions. It would be interesting what effect a longer substituent at the 5-position would have on the binding affinity to PhzA/B. The classes of conducted cross-couplings are summarized in Figure 65.

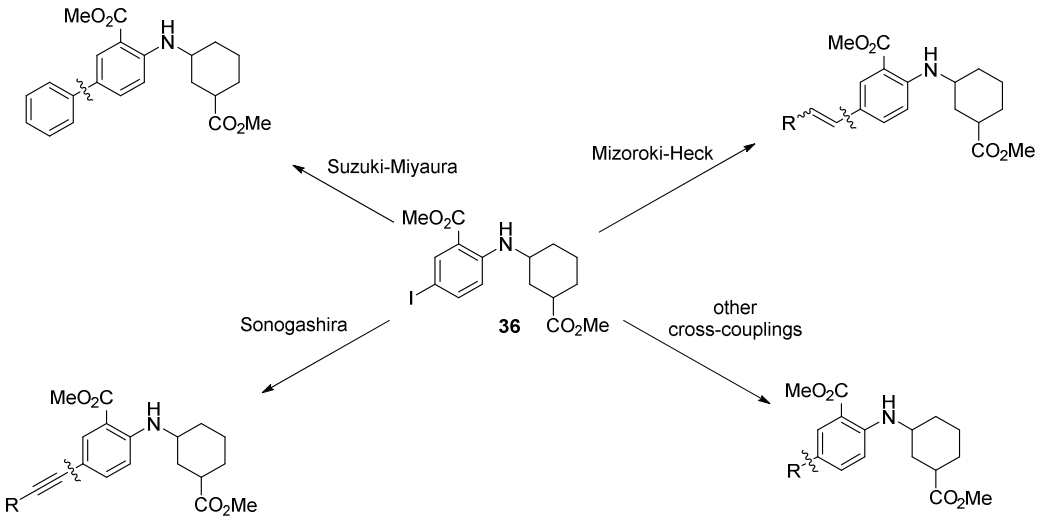
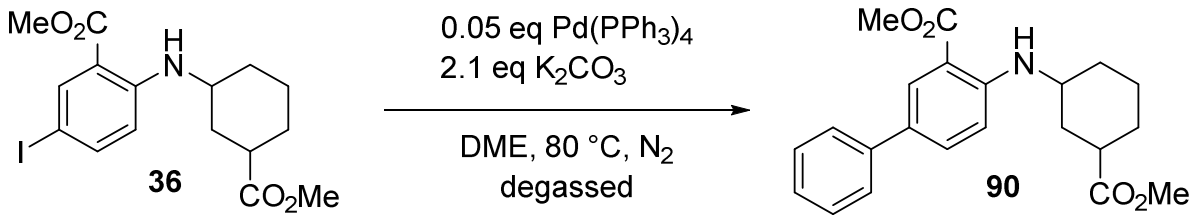


Figure 65: Cross-coupling reactions carried out with the di-ester of I-Phenazistatin **36**.

4.2.1.4.1 SUZUKI-MIYAURA cross-coupling

For the synthesis of the phenyl-derivative **90** two different reaction conditions were tested. When applying the standard reactions for the SUZUKI-MIYAURA-reaction (Table 28, Entry 1) to substrate **36** incomplete conversion was detected after 15 h, but the product **90** was the only spot generated. This test reaction was taken as a proof-of-concept that the substrate is reactive for SUZUKI-MIYAURA couplings and the preparative scale was performed using the well-established reaction conditions established by Melanie Trobe (Entry 2).[211] The reaction was heated to 80 °C and full conversion of the starting material was detected after 5 d (intensive red CAM staining of the product on TLC) and after workup 66% of product **90** was isolated (not optimized). Fractions of the pure *cis*- and *trans*-isomers of **90** were isolated via column chromatography for further reactions towards the elongated phenazistatin derivatives. GC-MS analysis failed with this and the following SUZUKI-MIYAURA products, since they were not volatile enough and were detected in the respective following run.

Table 28: Synthesis of **90** via SUZUKI-MIYAURA-reaction from **36** using different conditions.



Entry	Catalyst / Base	Solvent	T [°C]	Time / Conversion [%]	Comments
1	0.05 eq $Pd(PPh_3)_4$ 2.1 eq K_2CO_3	DME	80	15 h incompl.	incomplete
2[211]	0.05 eq $PdCl_2(dppf)_2$ 2.1 eq CsF	DME	80	5 d 100	66% isolated *cis*/*trans* separated

The hydrolysis of **90** towards the phenyl-Phenazistatin **91** was performed using standard saponification in THF/H_2O at RT (Figure 66). The reaction proceeded sluggishly and complete conversion was detected only after 4 d. The isolation of the products was low yielding, since impurities with a similar R_f-value were present and many contaminated fractions were obtained (only highly pure fractions were pooled for the biological screenings). It is very likely that those impurities are the respective other isomer, which has a very similar R_f-value. An intensive tailing of the di-acid **91** makes it extremely difficult to separate an isomeric mixture at this stage via column chromatography, therefore a separation at the di-ester **90** stage is mandatory.

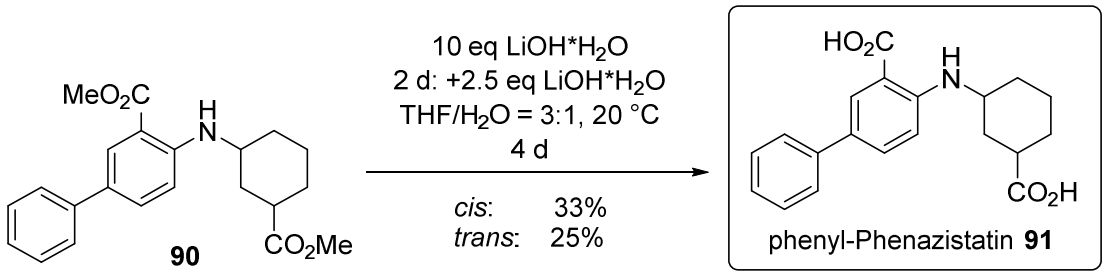


Figure 66: Synthesis of phenyl-Phenazistatin**91**via saponification of **90**.

The same SUZUKI-MIYAURA conditions were applied to synthesize the 4-nitrophenyl-phenazistatin **92** and the cross-coupling proceeded in 56% yield to give the isomer mixture of the product **92** as a yellow solid (Figure 67). 5% of the homo coupling product of the boronic acid could be detected with GC-MS. Unfortunately only minute amounts of the *cis*-isomer but no clean *trans*-fraction of the di-ester **92** could be obtained via column chromatography and the fractions were telescoped to the subsequent hydrolysis step. In the hydrolysis step full

conversion of the starting material was detected after 21 h but due to very similar polarities and the intensive tailing not even traces of the pure isomers of **93** could be obtained in this micro-scale reaction. In the case where the pure *cis*-isomer of **92** was used (14.4 mg) not enough material could be isolated for complete characterization and biological testing.

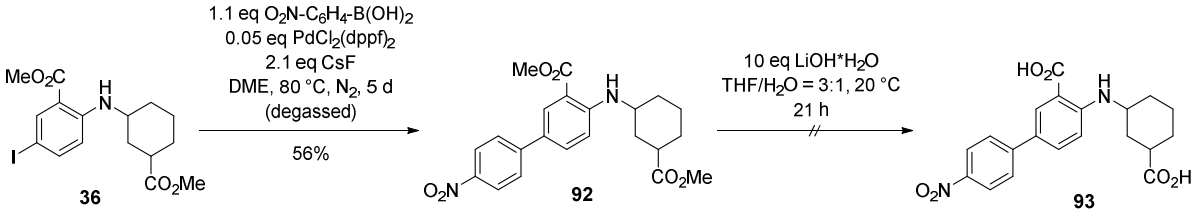


Figure 67: Synthesis of 4-nitro-phenyl-Phenazistatin **93** via SUZUKI-MIYAURA-reaction/saponification.

When reacting (3-(ethoxycarbonyl)-phenyl)boronic acid **94** with the aryl iodide **36** full conversion of the starting material was detected after 5 d (TLC) and product **95** was isolated via column chromatography (Figure 68). Analytically pure *cis*- and *trans*-fractions of **95** could be obtained. The subsequent hydrolysis did yield a mixture of different mono- and di-esters and prolonging the reaction time did not have an effect on conversion. Since the reaction was conducted on a test micro-scale it was not possible to isolate the desired product **96** amount for full characterization and biological testing.

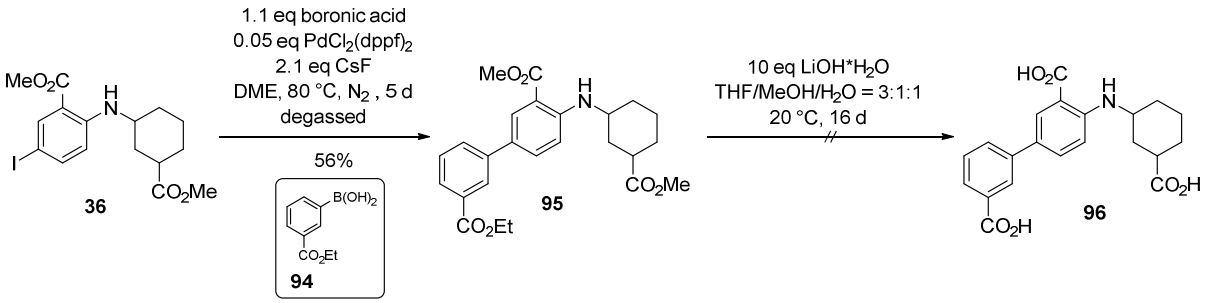


Figure 68: Synthesis of 3-ethoxycarbonyl-phenyl-Phenazistatin **96** via SUZUKI-MIYAURA-reaction/saponification.

4.2.1.4.2 MIZOROKI-HECK reaction

The MIZOROKI-HECK reaction with aryl halide **36** and methyl acrylate proceeded smoothly using standard conditions[212] and 83% of product **97** was isolated via column chromatography (Figure 69). The *cis*- & *trans*-isomers of **97** could not be isolated purely and only a total purity of 94% and 95% could be obtained, respectively. According to GC-MS minor amounts of the respective *Z*-isomer had formed during the MIZOROKI-HECK reaction contaminating the enriched fractions. It was hence decided to simplify the overall separation

by telescoping the enriched fractions to a catalytic hydrogenation. The hydrogenation proceeded sluggishly and full conversion of the starting material **97** was detected after 10 d. The product isomers of **97** were separated via column chromatography and were subjected to saponification. Moderate to good yields of the clean isomers of carboxyethyl-Phenazistatin **98** were obtained after column chromatography.

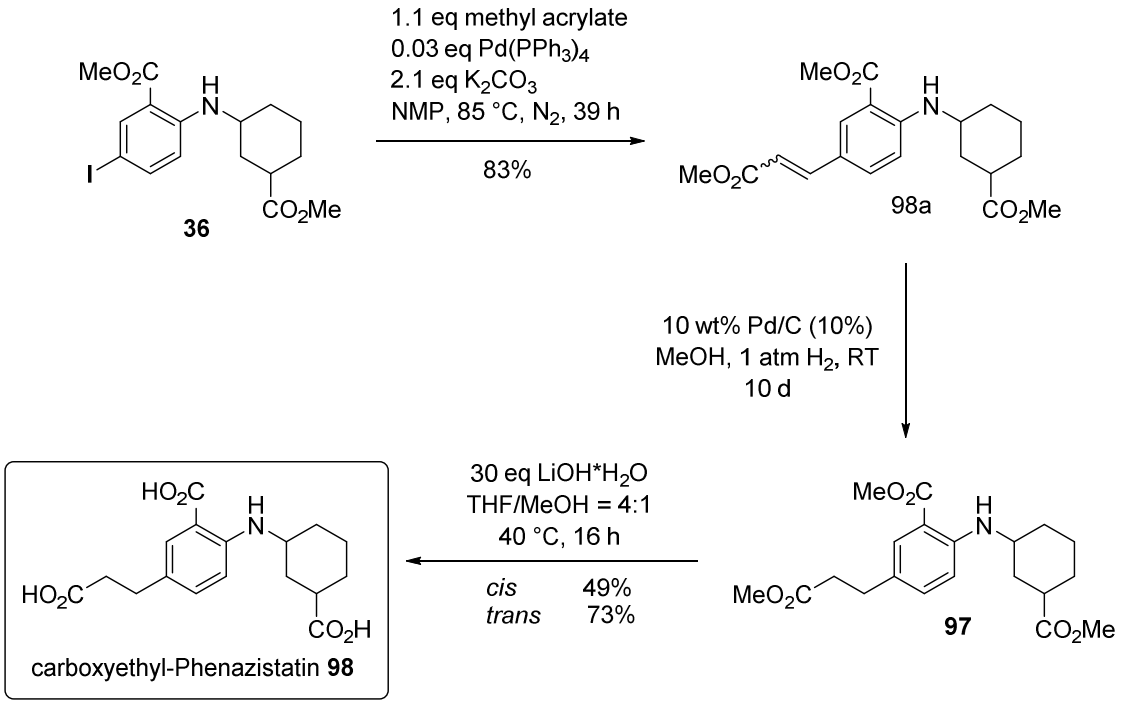


Figure 69: Synthesis of carboxyethyl-Phenazistatin **98** via MIZOROKI-HECK reaction/catalytic hydrogenation/saponification.

When the same synthetic strategy was used for cyanoethyl-Phenazistatin derivative **99**, several problems concerning the regioselectivity of the MIZOROKI-HECK reaction were encountered (Figure 70). The cross-coupling proceeded smoothly to full conversion within 38 h and 80% of the product isomers **100** could be isolated via column chromatography. Clean isomer fractions could not be isolated. GC-MS analysis indicated more than two peaks, which was expected due to the formation of the *E*- and *Z*-isomers. However, after catalytic hydrogenation of the double bonds GC-MS still indicated the presence of two peaks, which can only be explained by the lack of regioselectivity in the MIZOROKI-HECK reaction step and the generation of the two regioisomers depicted below. The polarities of the products were very similar and it was not possible to separate them via column chromatography.

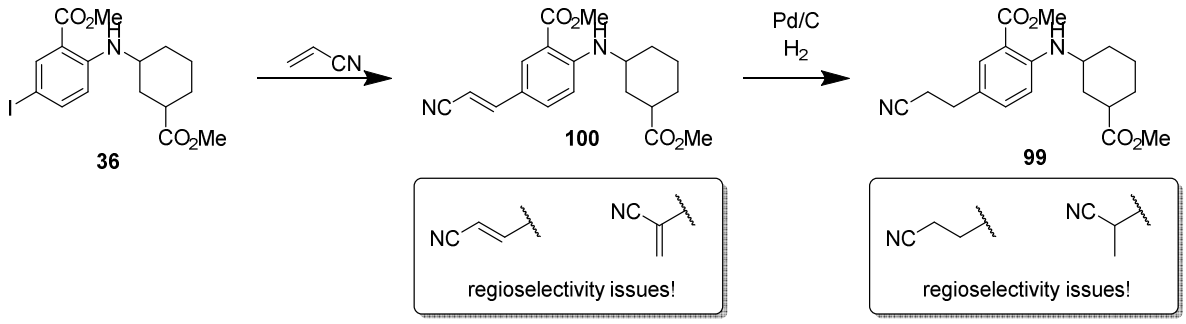


Figure 70: Synthesis of cyanoethyl-Phenazistatin **99** via MIZOROKI-HECK reaction/catalytic hydrogenation.

When acrylic acid amide was used for the same sequence, a more polar product was obtained after the MIZOROKI-HECK reaction (Figure 71). GC-MS indicated a mixture of products, which could not be separated by repeated attempts of column chromatography. In our experience the polarities do not change much upon catalytic hydrogenation and separation of diastereomers grows more complicated once the esters are hydrolyzed and therefore this route was not further pursued.

Prop-1-en-2-yl acetate (**101**) would be an interesting substrate for the MIZOROKI-HECK reaction, since upon acidic workup the obtained product **102** would be converted to the ketone. However, when prop-1-en-2-yl acetate (**101**) was used as a substrate several products appeared on TLC and the most intensive spot had the same R_f–value as the substrate and was just distinguished by a different CAM staining (Figure 71). GC-MS indicated a complex mixture and hence the purification of the reaction was not attempted.

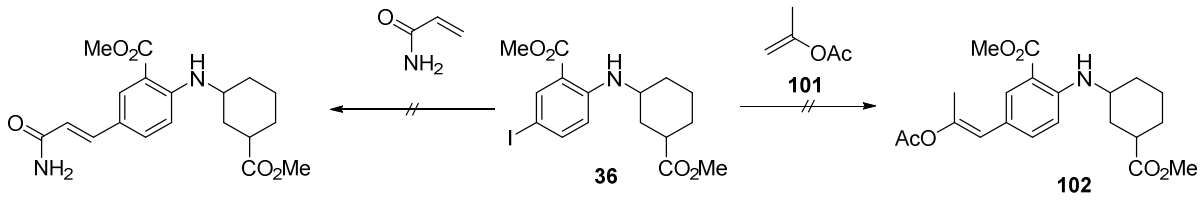


Figure 71: Synthesis of different Phenazistatin derivatives via MIZOROKI-HECK reaction using acrylic acid amide and prop-1-en-2-yl acetate (**101**).

4.2.1.4.3 Sonogashira reaction

The SONOGASHIRA reaction with the aryl halide **36** and a slight excess phenylacetylene proceeded smoothly in 28 h to full conversion and 64% **103** were isolated after column chromatography (Figure 72). Pure isomer fractions of **103** could be obtained via column chromatography. The corresponding *trans*-phenylacetylene-Phenazistatin derivative **104** was obtained after standard saponification. Not enough *cis*-isomer **104** could be prepared to allow for full characterization.

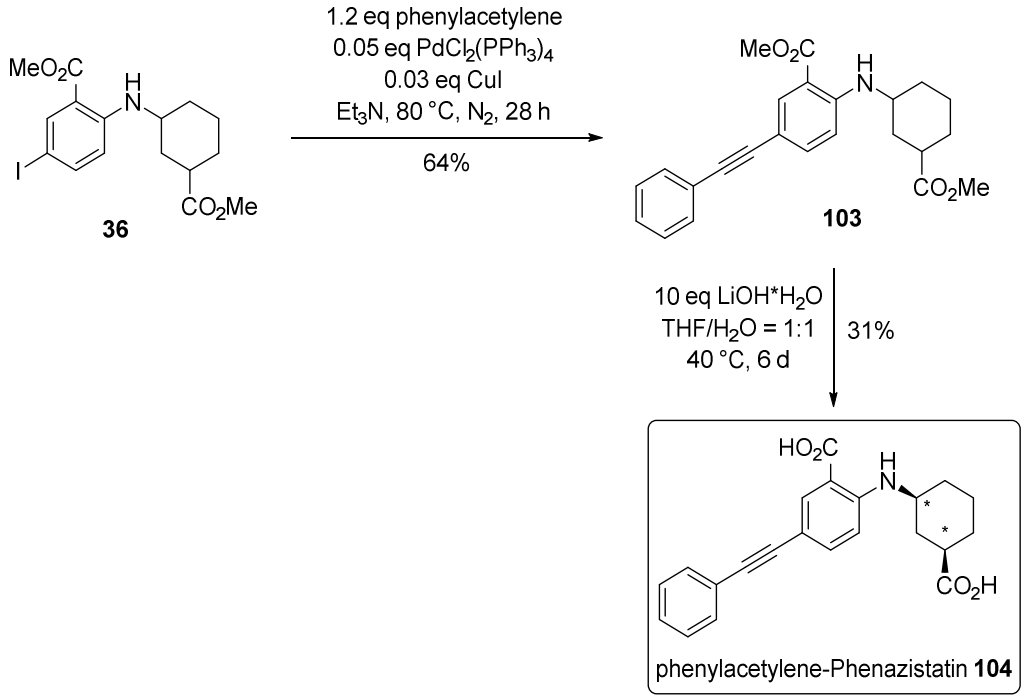


Figure 72: Synthesis of phenylacetylene-Phenazistatin **104** via SONOGASHIRA reaction/saponification.

A fraction of the produced di-ester of phenylacetylene-Phenazistatin **103** was subjected to catalytic hydrogenation and full conversion was detected by GC-MS after 3 d (Figure 73). Product and substrate had the same R_f–value on TLC, but differed in their CAM staining (substrate: violet, product: yellow). GC-MS analysis was necessary to confirm full conversion. Partial separation of the product **105** isomers was achieved via column chromatography and the pure *cis*- & *trans*-fractions were used for consecutive saponification. Accidentally MeOH was used instead of H_2O for the saponification and hence the reaction was much slower and not complete after 7 d, presumably due to the low solubility of $LiOH \cdot H_2O$ in the solvent mixture but after another 3 d at 40 °C full conversion of the starting material **105** was detected. The product isomers of the phenethyl-Phenazistatin **106** were isolated in good yields by acidification, solvent evaporation and column chromatography.

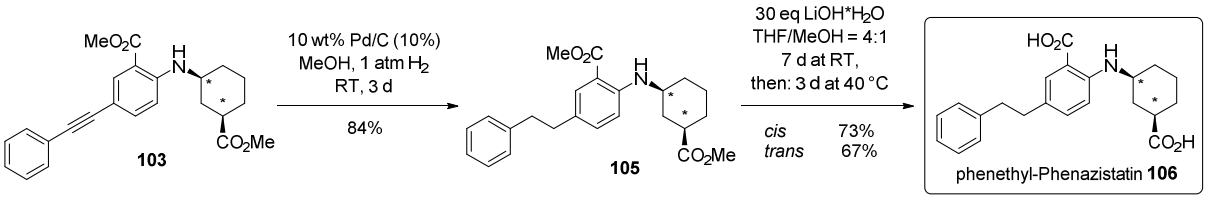


Figure 73: Synthesis of phenethyl-Phenazistatin **106** via catalytic hydrogenation/saponification.

When 1-pentyne was used for the SONOGASHIRA reaction with the diastereomeric mixture of aryl halide **36**, the product **107** isomers could not be fully separated via column chromatography and only minor quantities of the *cis*-isomer were isolated (Figure 74). In

order to produce sufficient amounts of the PhzA/B inhibitor **108** the reaction was conducted with pure *cis*-aryl halide **36** to evade these problems (shown in depiction below). Using the pure isomer **36** the synthesis and isolation was simple and sufficient quantities of the pentyne-phenazistatin **108** were produced.

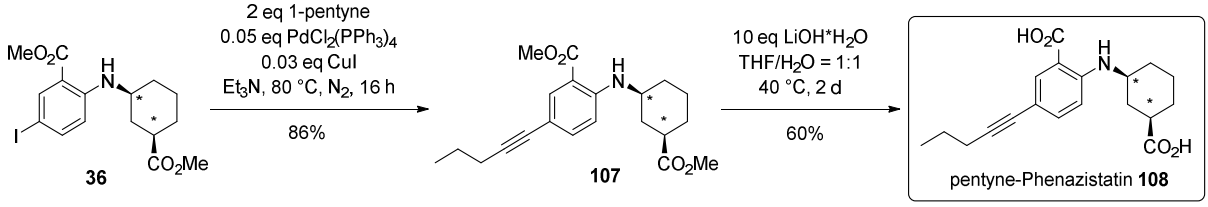


Figure 74: Synthesis of pentyne-Phenazistatin **108** via SONOGASHIRA reaction/saponification.

When alkynes bearing hydroxy functionalities, such as 2-methylbut-3-yn-2-ol (**109**) and but-3-yn-1-ol (**110**) were used, the reaction with the racemic aryl halide **36** proceeded nicely in 2 d to the expected SONOGASHIRA products (Figure 75). The products showed a much higher polarity on TLC than the substrate and unfortunately also exhibited some tailing. Hence the product isomers of **111** & **112** could not be isolated via column chromatography. Because of the low availability of the pure *cis*-aryl iodide **36** (only ~300 mg could once be cleanly isolated from 1 g isomer mixture using a solvent- and time-intensive (~3 L solvent, 1 day) column chromatography) the reactions were not repeated.

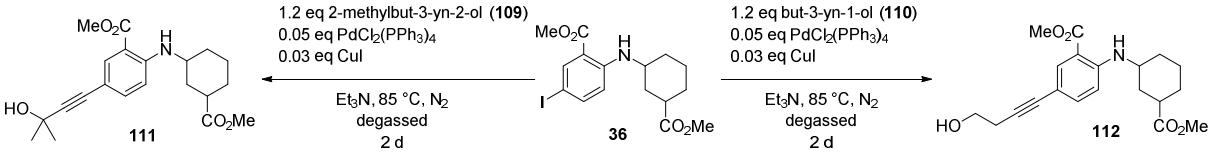


Figure 75: Synthesis of products **111** & **112** bearing hydroxy functionalities via SONOGASHIRA reaction.

To summarize, the SONOGASHIRA reaction works perfectly for aryl iodide **36** and so far any alkyne could be efficiently coupled. However, the separation of the *cis*- and *trans*-isomers represents the bottleneck in these syntheses. The optimal approach would be to use the desired pure aryl iodide **36** isomer which makes any desired SONOGASHIRA product easily available.

4.2.1.4.4 Miscellaneous cross-coupling reactions

The established cross-coupling reactions worked nicely with aryl halide **36**, so it was tested whether cyanide or amine nucleophiles could as well be incorporated by alternative cross-coupling reactions to give novel Phenazistatin derivatives (Figure 76).

When the conditions by Quan et al.[202] for the Pd-catalyzed cyanation of aryl halides was tested on our racemic aryl iodide **36** incomplete conversion of the starting material **36** was detected after 60 h at 90 °C (Figure 76). Two 366 nm UV-active products had formed (TLC) which had almost identical R_f–values. ^{1}H-NMR analysis after workup indicated the presence of several species.

When the well-established copper-catalyzed amination of aryl halides conditions by Buchwald et al.[198] were tested with **36** and the very reactive $BnNH_2$, many products were formed and after 4 d at 70 °C the starting material **36** had not been fully consumed. The corresponding product mass could not be detected in the mixture using HPLC-MS or GC-MS.

When the conditions by Buchwald et al.[213] for the copper-catalyzed amidation of aryl-halides was attempted using racemic aryl iodide **36** and pyrrolidin-2-one, clean conversion to a single more polar product (TLC) could be detected. The isomers of the mixture could not be separated due to very similar polarities and the HPLC-MS and GC-MS gave inconclusive results so it can only be speculated that the desired product had formed.

Earlier in this thesis the difficulties during the synthesis of the NH_2-Phenazistatin using the reductive amination methods were described (vide supra). The syntheses were presumably hampered by the instability of the products toward air and/or light. A method by Taillefer et al.[214] described the simple installation of an amino group by a Cu-catalyzed direct amination without the use of a masked amine which would need to be deprotected subsequently (e.g. $BnNH_2$,…). It was tested, whether the di-ester of the NH_2-Phenazistatin **113** could be synthesized directly from the aryl-halide **36** by these conditions and if the instability issues in the previous attempts were connected to the reductive amination reaction conditions. When the conditions by Taillefer et al.[214] were applied, incomplete conversion of the starting material was detected after 3 d (TLC, GC-MS). A complex mixture with various products was obtained (TLC) while the starting material was still the most prominent spot. The corresponding product mass of **113** could not be identified by HPLC-MS or GC-MS.

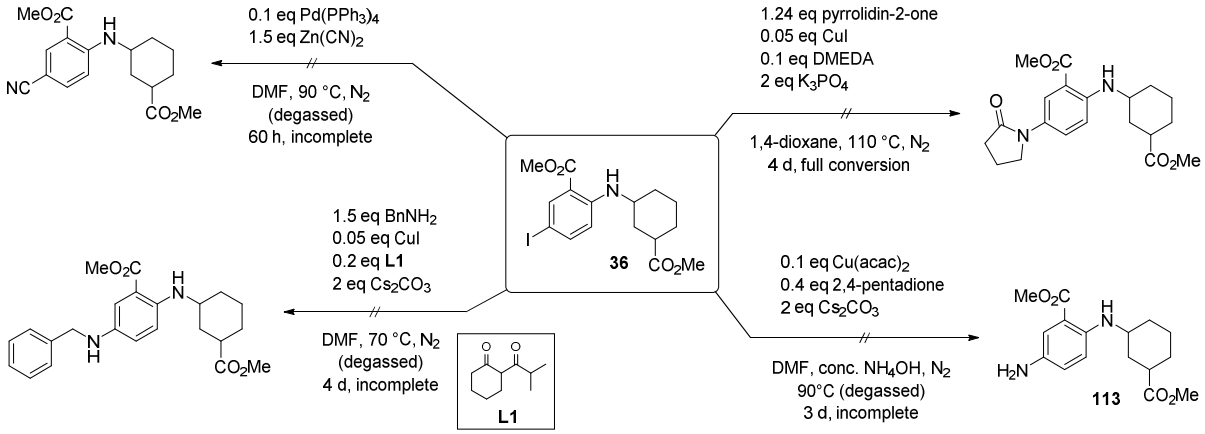


Figure 76: Synthesis of Phenazistatin derivatives via alternative cross-coupling reactions.

To sum up, only the reaction conditions for the copper-catalyzed amidation of aryl halides by Buchwald et al.[213] were found suitable for the synthesis of Phenazistatin derivatives. The remaining tested conditions suffer from poor selectivities or low conversion rates, possibly related to the inherent reactivity of the aryl iodide substrate **36**. In order to produce -N or -CN substituted Phenazistatin derivatives using these cross-coupling reactions, these reactions will have to be studied in more detail.

4.2.1.5 Synthesis of inhibitors with varied alicyclic ring-size

4.2.1.5.1 Synthesis of the 6-ring phenazistatin A

The synthesis of pure *cis*- or *trans*-Phenazistatin A (**1**) (bearing a 6-membered carbocyclic) proved to be straightforward employing the previously reported methods.[189] The substrate ketone **38** was easily accessed by a MICHAEL-[215] and PINNER-reaction[203] sequence (Figure 77). The MICHAEL-reaction was conducted in batch sizes of up to 500 mmol. TLC was used to monitor the reaction and even after longer reaction times traces of substrate were still detected, but we found that prolonged reaction times promoted the formation of by-products. However, those impurities generated were just minor constituents as judged by GC-MS even if they stained intensively with CAM. The cyanide addition was performed taking highest safety precautions and two HCN detectors were used at all times positioned between the round-bottom reaction flask and the operator. Upon full conversion (3 d in the case of the 500 mmol scale) solid Na_2CO_3 was added and the pH was checked to be ~10.8 (pK_a(HCN)= 9.21) and H_2O was added to dissolve the precipitates. The mixture was exhaustively extracted with $CHCl_3$ (significant product losses were made when just extracted three times), followed by standard workup procedure. For safety reasons the aqueous phase containing the remaining KCN was basified with KOH (pH = 14), the mixture was stirred and cooled in an ice bath and an excess of 30% H_2O_2 was added to oxidize remaining cyanide. The mixtures were stirred overnight or longer, until the excess of H_2O_2 had decomposed (KI paper stripes). The crude product was purified by vacuum distillation and the main impurity in the fractions was found to be unreacted 2-cyclohexen-1-one, which could be removed by repeated vacuum distillation.

The isolated 3-oxocyclohexane-1-carbonitrile (**5**) was subjected to a PINNER-reaction, where MeOH and AcCl were mixed at 0 °C to generate HCl in situ followed by the addition of the aliphatic nitrile **5** and warming up to RT. The sequence of addition was found to be not important and as good results were obtained when AcCl was added to a cooled substrate **5** solution in MeOH. Upon completion of the reaction (aqueous mini-workup, then GC-MS) a colorless precipitate had formed. The generated PINNER-salt and the excess AcCl were carefully hydrolyzed by addition of H_2O to the cooled suspension and the mixture was stirred until all solids had dissolved. Standard workup yielded the product ester **38** in very high GC-MS and NMR purity and the PINNER-reaction generally proceeded in exceptional selectivity and gave a clean product. It was found, that either the nitrile **5** or the ester **38** could be purified by distillation or column chromatography, and that the PINNER-reaction could be conducted with the crude product from the MICHAEL-reaction. The purification of either nitrile **5** or ester **38** is equally laborious (vacuum distillation) and gives comparable overall yields. Column chromatography gave much better recoveries of the product than distillation (even

on larger scale), but was not conducted generally due to cost reasons and the good accessibility of the starting material.

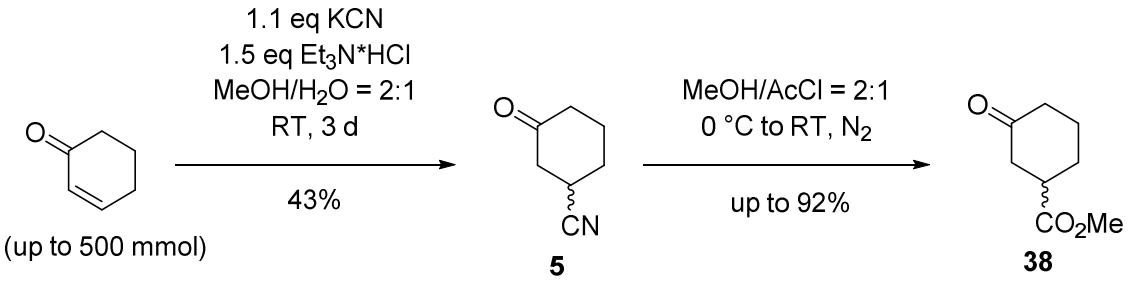


Figure 77: Synthesis of the building block methyl 3-oxocyclohexane-1-carboxylate (**38**).

The di-ester of Phenazistatin A **7** can be synthesized in excellent yields using our described methods,[189] but here we want to report a slightly altered reaction. Instead of using the free aniline for the reaction we used the hydrobromide **114** of the aniline, since we reasoned that the overall acidic conditions would not be influenced by an extra acid equivalent (Figure 78). The hydrobromide **114** precipitated directly in high purity from an unbuffered (no base like KOAc used) bromination of methyl anthranilate with Br_2 in AcOH and could be conveniently produced in larger quantities. With these alterations 2.37 g **7**, corresponding to a yield of 96%, were isolated after column chromatography. The reaction time did not differ from the reported smaller scale reactions and full conversion of **114** could be detected after 48 min (TLC). This time point corresponds to the first TLC reaction control and it was not checked if the reaction was complete even after a shorter reaction time.

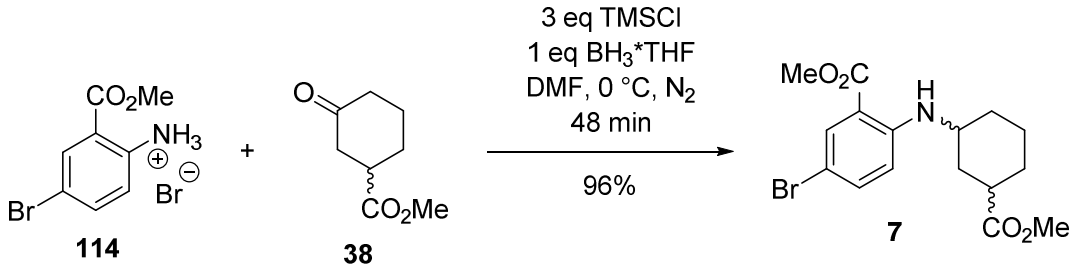


Figure 78: Synthesis of di-ester of Phenazistatin A **7** using method B.[189]

GC-MS of the isolated product indicated an isomer ratio of *cis*/*trans* = 42:58. This finding was slightly unexpected, since the *trans*-isomer corresponds to the thermodynamically less stable isomer with one substituent, e.g. the carboxymethyl group, in axial position. Clean fractions of the isomers could be separated by very slow (high back pressure, up to 40 cm) column chromatography. However, this was not carried out for the whole portion of the product since significant amounts of solvents and silica were needed. For the production of derivatives we usually first made the reaction and then purified the products via column chromatography. In cases where the R_f-values of the product isomers were even closer than the ones of the

substrate isomers, clean fractions of the starting material had to be used under appropriate reaction conditions.

During our studies and syntheses generating Phenazistatin derivatives, we observed that the isolated isomers could be clearly assigned using NMR spectroscopy. An HSQC spectrum shows the representative “fingerprint” of the carbocyclic core of both *cis*- and *trans*-, which seems to be highly conserved even when altering substituents on the aromatic core as well as exchanging the aliphatic carboxy group to bioisosteric replacements or prodrug esters. Figure 79 shows the much more pronounced difference of the ^{1}H-shift of the diastereotopic carbocyclic CH_2 protons in the *cis*-**7** species, whereas in the *trans*-**7** species this effect is not that prominent.

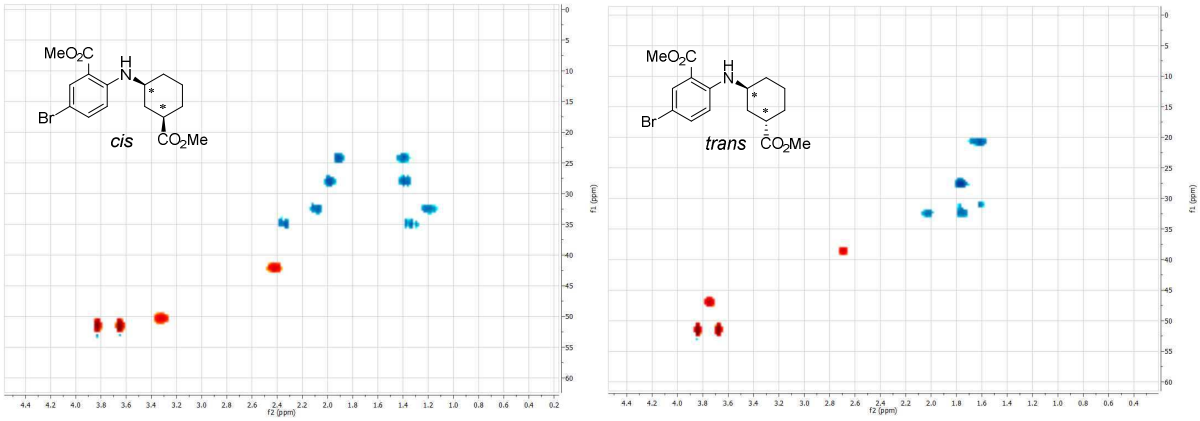


Figure 79: HSQC-NMR spectrum of the aliphatic region of the di-ester isomers 6-ring-Phenazistatin derivative **7**.

In addition to this difference in the HSQC-fingerprint the *cis*- & *trans*-isomers of the Phenazistatin derivatives generally exhibited some other similarities. The *trans*-isomer was in every case less polar on the TLC than the *cis*-isomer and in GC-MS (if applicable) the *cis*-isomer had generally a higher retention time than the *trans*-isomer, indicating a higher boiling point or stronger interactions with the stationary phase.

The pure isomers of Phenazistatin A **1** were synthesized by standard saponification of **7** and were purified via preparative RP-HPLC (Figure 80). The yields are not optimized and the main focus was placed on product purity for enzyme studies with PhzA/B.

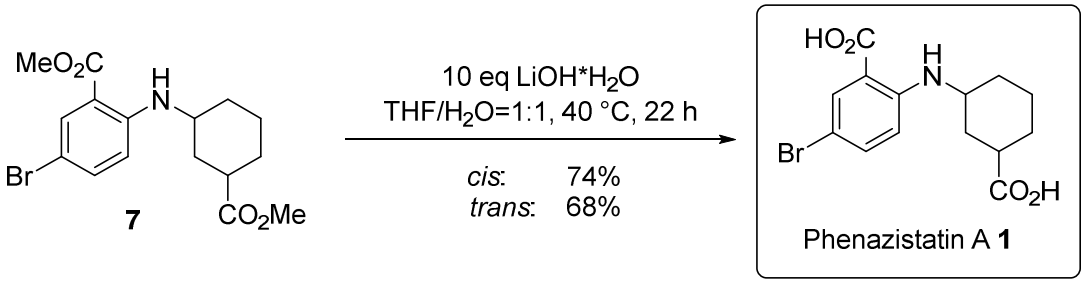


Figure 80: Synthesis of Phenazistatin A (**1**) via saponification from **7**.

4.2.1.5.2 Synthesis of the 5-ring Phenazistatin A

The 5-ring analog of Phenazistatin A **115** was synthesized in analogy to the aforementioned 6-ring analog **1** (Figure 81). MICHAEL-reaction with potassium cyanide[215] at the readily available 2-cyclopenten-1-one followed by PINNER-reaction[203] and purification via column chromatography gave the product **116** in good yield.

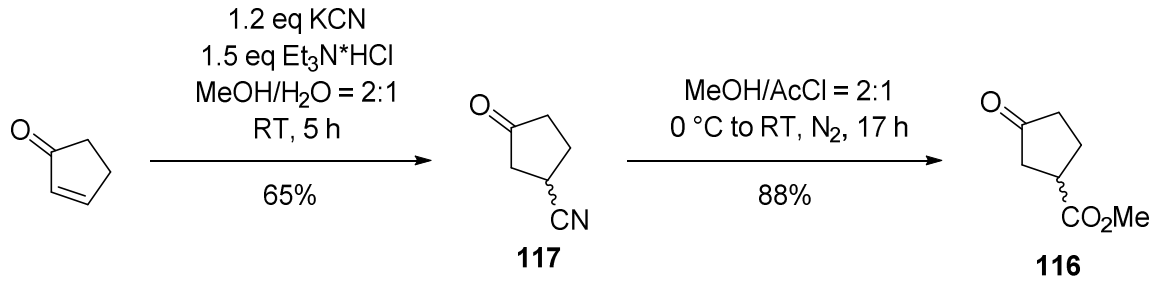


Figure 81: Synthesis of the building block methyl 3-oxocyclopentane-1-carboxylate (**116**).

Method A[189] was used for the reductive amination of methyl 3-oxocyclopentane-1-carboxylate (**4**) with methyl 2-amino-5-bromobenzoate (**4**) to give the product **118** in moderate yield with 22 h reaction time (Figure 82). The more active method B had not been developed at that time but might give higher yields if applied to these substrates owing to the lack of the previously described by-product formation. In this reaction, GC-MS showed the formation of the acetylated substrate to an extent of 25% and the ratio of *cis*/*trans* was found to be 84:16. The isomers of the product **118** could be separated via column chromatography and were used separately for following reactions.

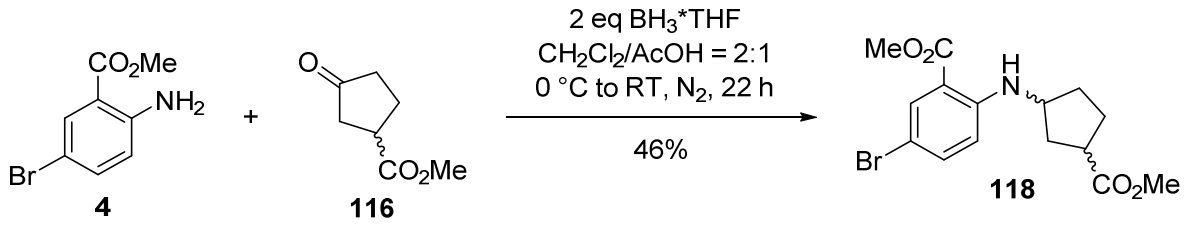


Figure 82: Synthesis of di-ester of the 5-ring-Phenazistatin **118** using method A.[189]

Similar to the 6-ring Phenazistatins, the HSQC-NMR spectra of the pure 5-ring Phenazistatin isomers showed a distinct fingerprint for either isomer (Figure 83).

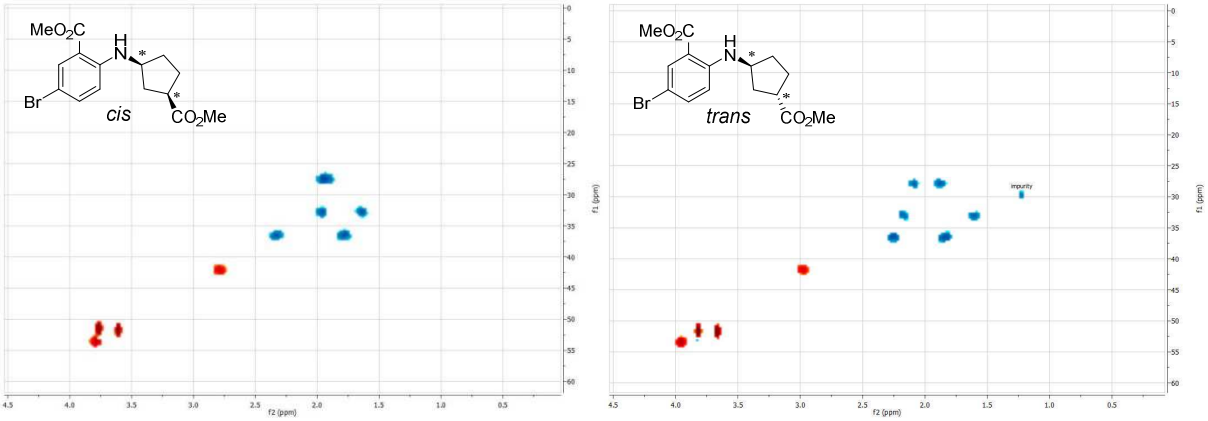


Figure 83: HSQC-NMR spectrum of the aliphatic region of the di-ester of cyclopentyl-Phenazistatin derivative **118**.

The pure isomers of the 5-ring Phenazistatin **115** were synthesized by standard saponification and were purified via column chromatography (Figure 84). The yields are not optimized and the main focus was placed on product purity for enzyme studies with PhzA/B.

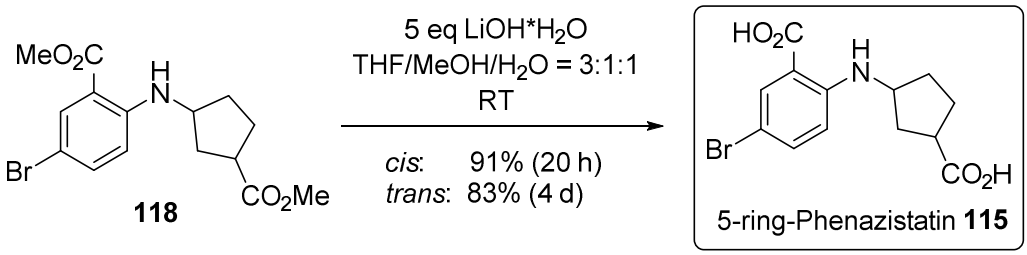


Figure 84: Synthesis of the 5-ring-Phenazistatin **115** via saponification from the di-ester **118**.

4.2.1.5.3 Synthesis of the 7-ring Phenazistatin A

For the 7-ring derivative of Phenazistatin A **119** the same approach as for the successful syntheses of the 5-ring and 6-ring derivatives was used (Figure 85). However, to our dismay we observed the formation of a plethora of by-products during the standard MICHAEL-reaction[215] with potassium cyanide. Unfortunately, the starting material cyclohept-2-en-1-one is quite expensive (1 g: ~110€, abcr product catalog, July 2016). The product **120** was isolated via column chromatography as a single spot on TLC and the combined fractions were analyzed by GC-MS. Apart from the product **120** peak in 85% abundancy a second peak was found (15%) which showed the mass of the ester product **121**. This product might originate from a nitrile hydrolysis followed by an esterification, but formally it corresponds to the desired PINNER-reaction product **121**. Separation of these two spots was not possible due to the identical R_f-value of the two products.

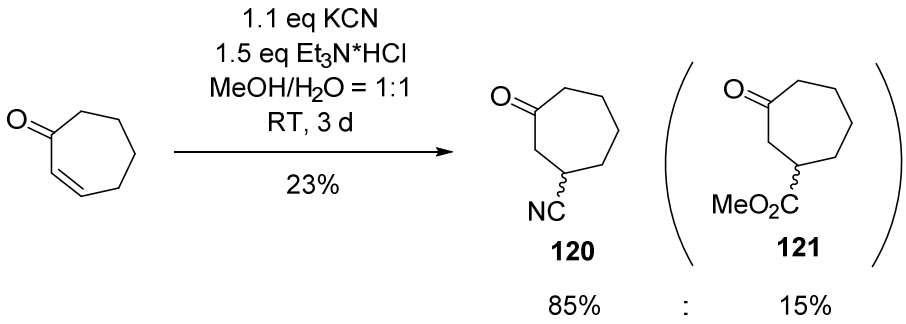


Figure 85: Synthesis of 3-oxocycloheptane-1-carbonitrile (**120**) via MICHAEL-addition.[215]

A logical approach for tackling this problem was to telescope the reaction mixture into the next reaction step to converge the mixture to the desired PINNER-reaction product **120** (Figure 86).

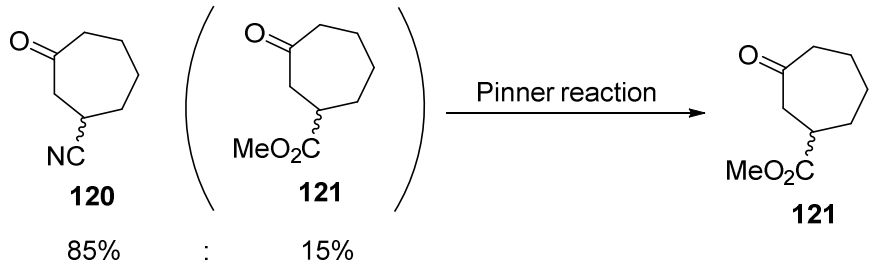


Figure 86: Synthesis of methyl 3-oxocycloheptane-1-carboxylate (**120**) via PINNER-reaction.

The next step was the reductive amination, which was expected to perform excellently for this class of substrates. However, we were somehow reluctant to subject the total quantity of 221 mg of the valuable nitrile/ester directly to the PINNER-reaction. This was mainly for two reasons. Firstly, the reductive amination was found to perform better when using a larger scale and secondly, the average isolated yield of the PINNER-reaction varied from 61 to 92% with good yields obtained in scales >1 g substrate. We reasoned that a PINNER-reaction at this stage would diminish our total product quantity, rendering the subsequent reductive amination step less efficient. We therefore envisaged that a reversal of the steps would give a higher overall yield and, more importantly, a safer route to sufficient quantities of the desired 7-ring analog **119** which would require a subsequent hydrolysis step (Figure 87).

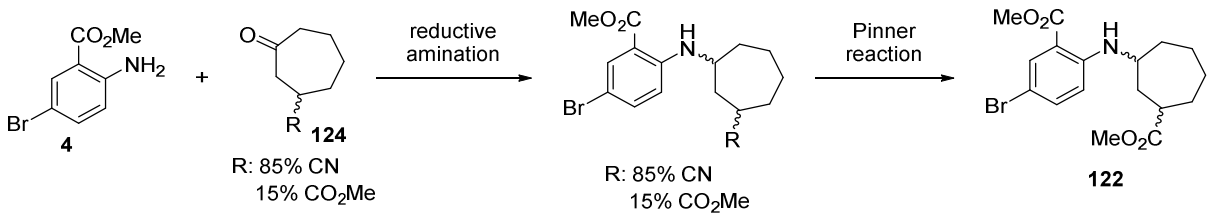


Figure 87: Approach to the di-ester of the 7-ring-Phenazistatin **122** via reductive amination/PINNER-reaction.

In order to test whether this proposed sequence could be performed, it had to be tested whether the PINNER-reaction performed equally well on a C-N coupled Phenazistatin substrate. We therefore synthesized the 6-ring analog **7** and tested the standard conditions for this substrate (Figure 88). We were very pleased to find that all the starting material **123** had been consumed after 3 d and GC-MS and TLC indicated a clean reaction without any by-product formation. This confirmed the feasibility of our anticipated reaction sequence and encouraged us in testing it on the valuable 7-ring substrate **124**.

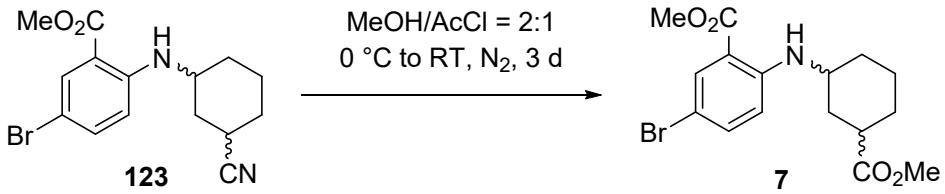


Figure 88: A test reaction as a proof of concept that the PINNER-reaction is amenable to the aliphatic nitrile **123**.

The reductive amination via method B[189] (BH_3·THF, TMSCl, DMF) worked well and full conversion of the limiting component (carbonyl compound **124**) could be detected after 101 min by GC-MS (Figure 89). TLC reaction control gave inconclusive results, since several products were formed during the reaction which exhibit quite similar R_f-values. The reaction mixture was worked up and was dried in oil pump vacuum and was telescoped to the PINNER-reaction step. After 20 h at RT full conversion of the nitrile **125** could be detected by TLC and the product **122** was detected to have formed in a major/minor-ratio of 4:1 (GC-MS). Curiously excess aniline substrate from the reductive amination was not acetylated under these conditions. After workup and very careful column chromatography (30 cm, R_f = 0.20) 47% of the pure major-diastereomer **122** could be isolated. The diastereomers did not show any difference in R_f-value and a very careful column chromatography with GC-MS fraction analysis was indispensable for the purification.

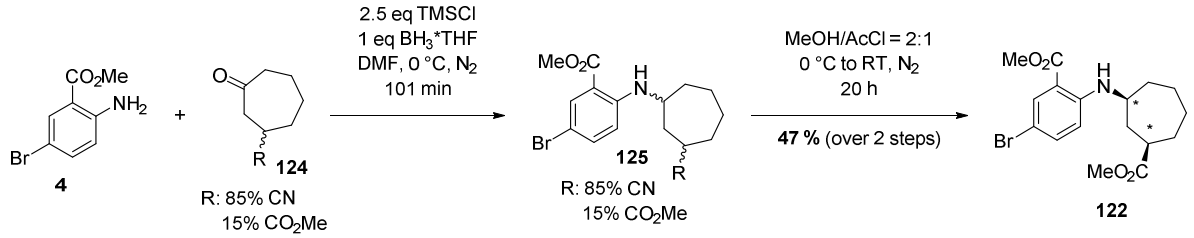


Figure 89: Assembly of the di-ester **122** of the 7-ring Phenazistatin.

The HSQC-NMR fingerprint of the 7-ring Phenazistatin derivative **122** is shown in Figure 90. The difference in the chemical shift of the diastereotopic protons is not as high as in the *cis*-isomer of Phenazistatin A **1** (6-ring), but higher than the *trans*-isomer and a little smaller compared to both isomers of the 5-ring derivatives.

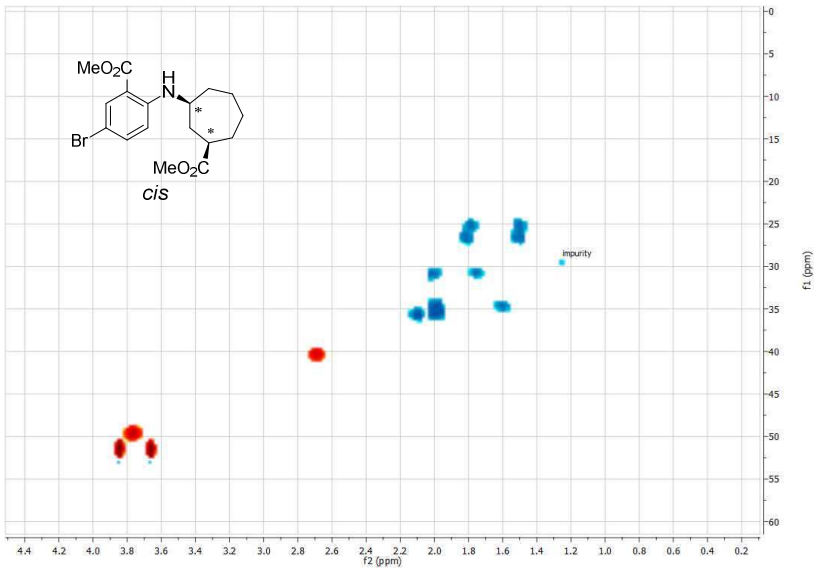


Figure 90: HSQC-NMR spectrum of the aliphatic region of the di-ester of cycloheptyl-Phenazistatin derivative **122**.

The pure 7-ring-Phenazistatin **119** was synthesized from the major product of **122** by standard saponification and was purified via preparative RP-HPLC (Figure 91).

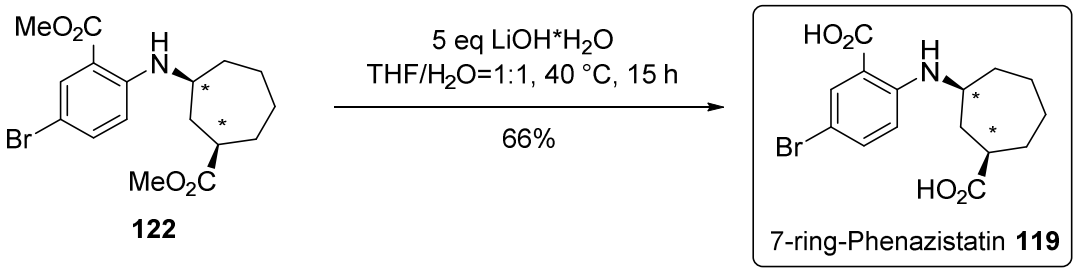


Figure 91: Synthesis of the 7-ring-Phenazistatin **119** via saponification.

The stereochemistry of the racemic product was assigned in collaboration with Prof. Hansjörg Weber (Graz University of Technology) by NOESY-NMR studies and was found to be the *cis*-diastereomer (Figure 92).

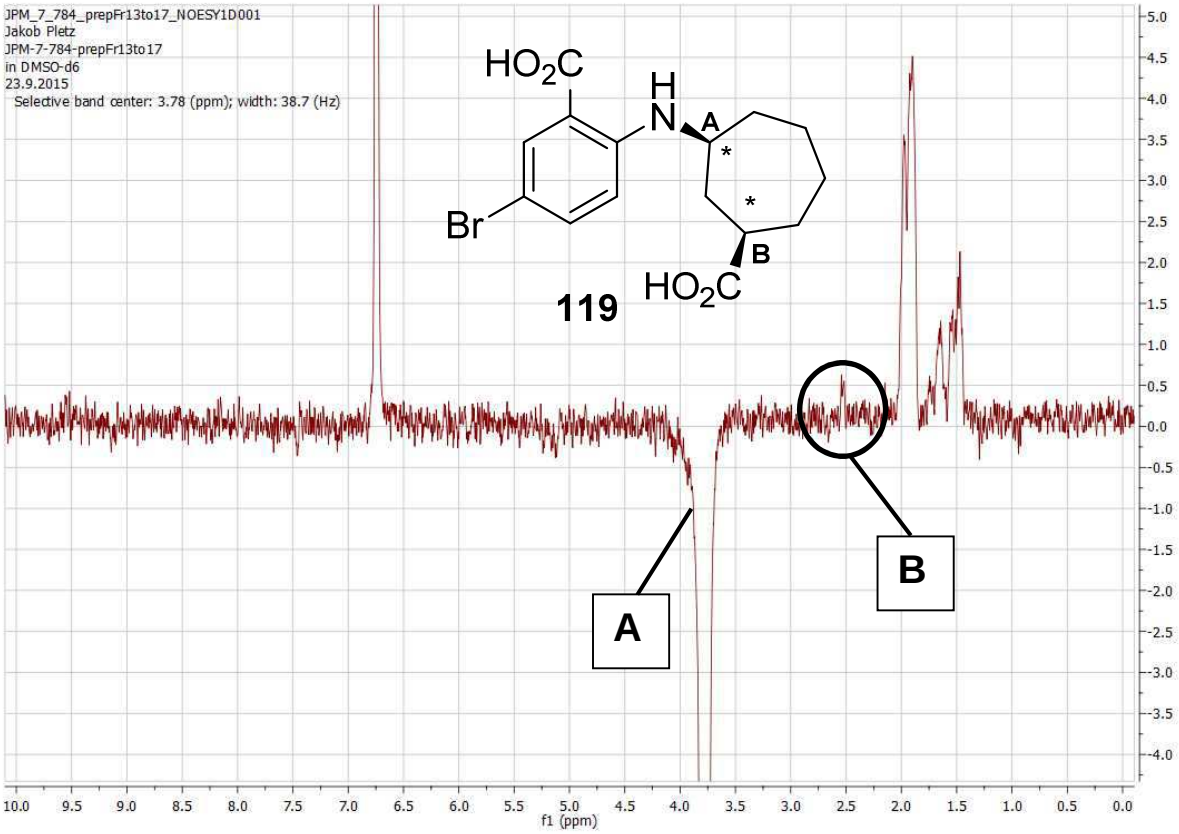


Figure 92: NOESY-NMR spectrum of the 7-ring-Phenazistatin **119.**

However, there are doubts about the stereochemical assignment of the 7-ring Phenazistatin since protein crystal structures with PhzA/B indicate an electron density that could also fit for the *trans*-diastereomer of **119**. In the reductive amination generating the second stereogenic center (Figure 89) a 4:1 ratio of the product diastereomers was observed by GC-MS. This indicates a quite large bias towards one of the two configurations (with the 6-ring analog usually only about 2:1 or lower was obtained).

Mario Leypold (Graz University of Technology) conducted quantum mechanical calculations in order to determine the relative energy difference between the *cis*- and the *trans*-diastereomer (Figure 93). He found the *trans*-form to be 3.25 $kJ \cdot mol^{-1}$ less stable than the *cis*-form, which results in a thermodynamic *cis*/*trans*-ratio of ~3.8:1. This ratio is very similar to the ratio of products from the reductive amination, however it might be a coincidence because we have no information whether the generation of the second stereogenic center was thermodynamically or kinetically controlled. The energetically most stable conformers of **119** are shown in Figure 93.

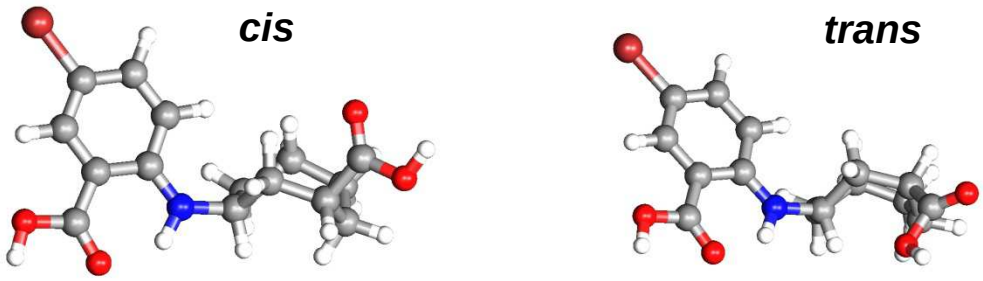


Figure 93: Energetically most stable conformers of the *cis*- and *trans*-diastereomer of the 7-ring Phenazistatin **119**. Free enthalpies (ΔG) calculated with PCM (THF) B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d).[216–220]

In summary, the stereochemical assignment of the isolated 7-ring Phenazistatin **119** could not be unequivocally made. NOESY-NMR as well as the quantum mechanical calculation favor the formation of the cis-diastereomers, however the result from the protein-ligand crystal structure is still ambiguous.

4.2.2 Phenazistatin derivatives to optimize ADMET-properties

4.2.2.1 Synthesis of derivatives bearing bioisosteric replacements of the carboxyl group

4.2.2.1.1 Synthesis of 1*H*-tetrazole bioisosteres

1*H*-tetrazoles were selected as a first class of bioisosteres, which represent a very convenient bioisosteric group to the carboxyl group, since it can often be introduced directly during the last step from a nitrile or it can be easily protected and deprotected (e.g. trityl) if needed. We therefore aimed for the synthesis of the appropriate nitrile precursors in order to synthesize various Phenazistatin-tetrazole precursors. As a building block 2-amino-5-bromobenzonitrile was synthesized according to a standard procedure[221] by NBS bromination from 2-aminobenzonitrile and could be isolated directly via filtration from the reaction mixture (Figure 94).

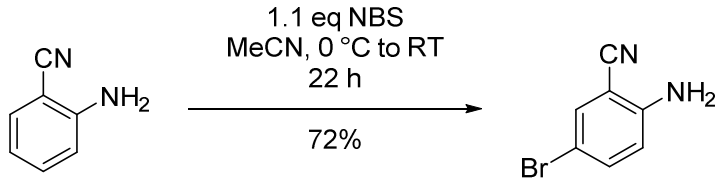
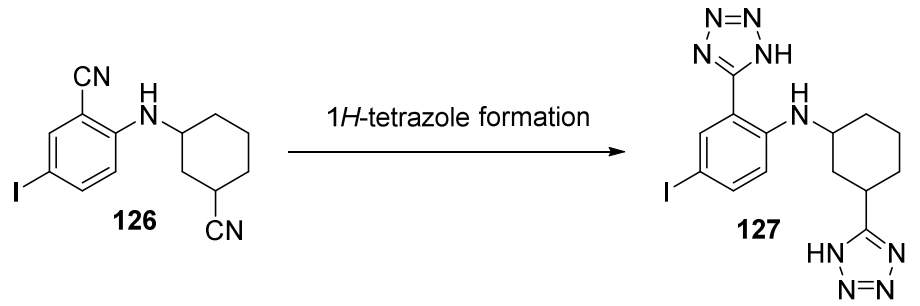


Figure 94: Synthesis of 2-amino-5-benzonitrile from 2-aminobenzonitrile by NBS-bromination.

In order to test which method of 1*H*-tetrazole formation worked best various methods were tested on 2-((3-cyanocyclohexyl)amino)-5-iodobenzonitrile (**126**) (Table 29), which was easily prepared by a procedure by Pletz.[189] For the introduction of the 1*H*-tetrazole we decided to rely on the more safe and solid NaN_3 instead of the liquid and highly toxic organic azide reagents. When the methods by Das et al.[222] (catalytic iodine) and Aridoss et al.[223] (Zn/Cu couple) were used, incomplete conversion of the starting material **126** was detected after 4 d. The same result was obtained after 3 d when the method by Jaitak et al.[224] ($ZnCl_2$) was tested. When the method by Cullen et al.[225] with $Et_3N{\cdot}HCl$ as an additive was used in combination with an excess of 4 eq NaN_3, full conversion of the starting material and conversion to a single more polar product spot was detected. The product tetrazole **127** was found to exhibit an intensive crimson-red staining upon contact with the cold CAM staining solution, which was in general a good indicator for the presence of an alkyl- as well as aryl-1*H*-tetrazole group. Separation of the product **127** isomers was not observed on TLC and acid additives had no effect on the separation. The acidic nature of the product **127** led to tailing on TLC as well as during column chromatography comparable to the parent compounds, the Phenazistatins with the carboxylic acid groups.

Table 29: Screening for the reaction conditions to produce the bis-1*H*-tetrazole **127**.



Entry	Reagents	Solvent / Temperature	Time	Conversion
1[226]	3 eq NaN_3 0.12 eq I_2	DMF 100 °C	4 d	incomplete
2[223]	2.8 eq NaN_3 10 wt% Zn/Cu	DMF 120 °C	4 d	incomplete
3[224]	2.5 eq NaN_3 1.25 eq $ZnCl_2$	DMF 100 °C	3 d	incomplete
4[225]	4 eq NaN_3 4 eq $Et_3N{\cdot}HCl$	toluene 120 °C	4 d	complete

With this method for the formation of 1*H*-tetrazoles at hand, we tried to synthesize the corresponding bis-tetrazole derivative of Phenazistatin A **128**[225] from the easily available 5-bromo-2-((3-cyanocyclohexyl)amino)benzonitrile **129** (prepared by method A[189]) (Figure 95). The reaction proceeded smoothly to full conversion after 45 min and the reaction progress could be visually detected by the formation of a brown oil at the bottom of the Schlenk flask with the clear toluene phase above. The mixture was carefully (fume hood!) acidified with 1 M HCl and further processed according to standard procedures. Unfortunately, all attempts to separate the mixture of *cis*- and *trans*-stereoisomers of the product **128** failed for all methods tested (TLC, careful column chromatography, analytical RP-HPLC, preparative RP-HPLC, recrystallization, salt formation and recrystallization with amines). As we failed to separate the isomers at this stage, we went one step back and tried to separate the isomers of the di-nitrile substrate **129**, but we found it equally difficult to separate them. The influence of the small –CN groups on the overall polarity upon *cis*- or *trans*-substitution seems to be very small. Sometimes when dealing with very similar R_f-values on the TLC we found that the substances could be separated by a very careful column chromatography by selecting a column with >40 cm length and adjusting the R_f-value to about 0.1 or smaller with fraction

analysis by NMR, GC-MS or HPLC-MS. When this strategy was applied to the di-nitrile **129** (45 cm length, R_f = 0.10) only a slight enrichment of the *cis*- and *trans*-isomers of **129** could be detected by GC-MS in the first and last fraction (60:40 and 40:60, respectively). It was hence not possible to produce a pure fraction of either isomer of the bis-1*H*-tetrazole **128** for biological testing by this strategy.

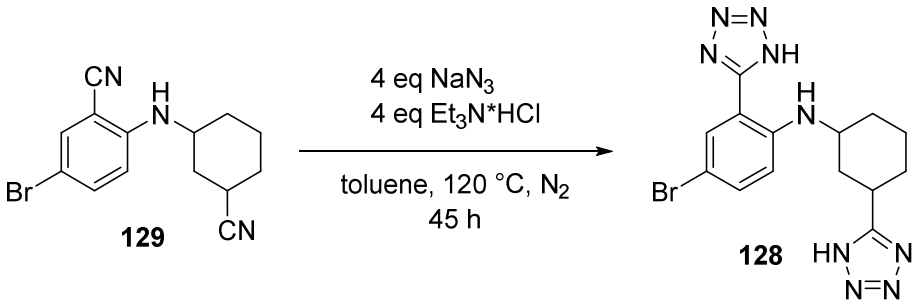


Figure 95: Synthesis of the bis-1*H*-tetrazole analog **128** of Phenazistatin A.[225]

Earlier on we observed that the di-ester **7** of Phenazistatin A could be separated via column chromatography, albeit with considerable effort. Since we could not separate the isomers of the di-nitrile **129**, we decided to produce the corresponding mono-nitrile **130** since we expected a larger influence of the one remaining ester group on the polarity, and hence the separability of the isomers (Figure 96). The required mono-nitrile **130** was prepared according to reductive amination method A[189] in moderate yield and was purified via column chromatography. Clean fractions of the isomers of **130** could be obtained and the pure isomers were used in the 1*H*-tetrazole formation reaction, since again poor separability of the products was expected.

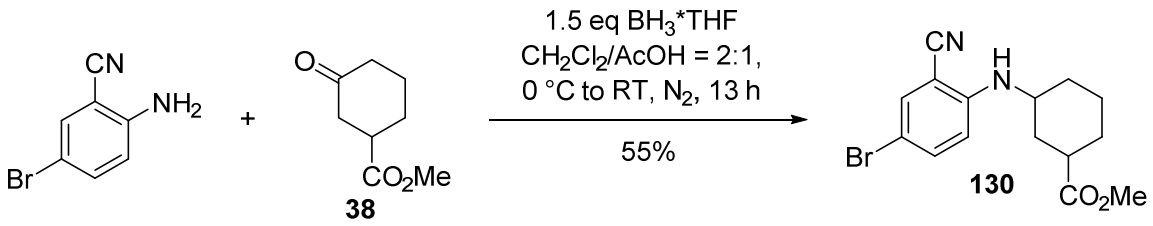


Figure 96: Synthesis of the mono-nitrile **130** analog of Phenazistatin A via reductive amination method A.[189]

The corresponding mono-1*H*-tetrazole **131** was produced by a telescoped process, since the separability of the intermediary tetrazole-ester **132** was equally poor as a free carboxylate group (Figure 97). The formation of the 1*H*-tetrazole worked nicely with a twofold excess of NaN_3 and full conversion of the starting material was detected after 18 h in boiling toluene. After acidification, extraction and concentration the crude product was used as such for the hydrolysis step. Full conversion of the starting material was detected by TLC after 18 h and standard workup yielded the pure isomers of the desired mono-1*H*-tetrazole **131** in sufficient

purity. The separation of the generated minor impurities during the reactions resulted in low overall yield.

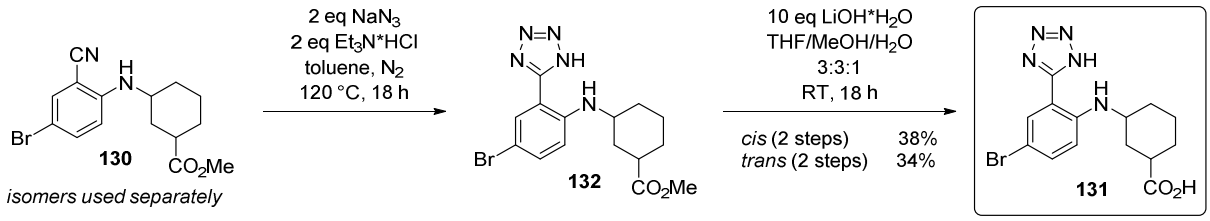


Figure 97: Synthesis of mono-1*H*-tetrazole **131** via tetrazole formation/saponification.[225]

It was also tried to apply this purification strategy to the easily available 5-ring analogs of Phenazistatin A. The corresponding di-nitrile **132** was synthesized according to method A[189] (BH_3·THF, CH_2Cl_2/AcOH) and 61% of the product **132** could be isolated after 25 h (Figure 98). The stereoisomers of the product **132** could be partially separated via column chromatography. The tetrazole formation was conducted with the pure *cis*-isomer of di-nitrile **132** with 2 eq of both NaN_3 and Et_3N·HCl but incomplete conversion was detected after 4 d. Additional 4 eq of both reagents and another 31 h of heating led to full conversion of the starting material **133**. The very polar bis-tetrazole product **134** could be isolated via column chromatography.

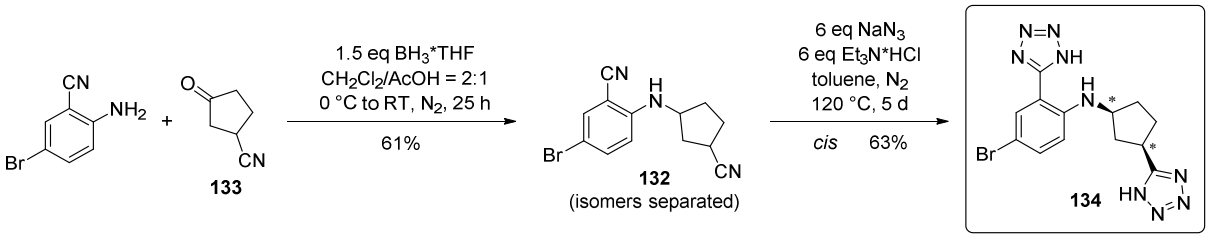


Figure 98: Synthesis of bis-1*H*-tetrazole analog **134** of the 5-ring-Phenazistatin **115** via reductive amination/tetrazole formation/saponification.[189,225]

The synthesis of one of the 5-ring analogs of the mono-1*H*-tetrazoles **135** was tried using the same strategy (Figure 99). Method A[189] gave mono-nitrile **136** in good yield and the stereoisomers were separated via column chromatography. The pure *cis*-isomer of **136** was telescoped into the tetrazole formation reaction and full conversion of the starting material **136** was detected after 23 h at 120 °C. The mono-tetrazole product **135** was purified via column chromatography, but the hydrolysis could not be conducted due to the low overall quantity produced.

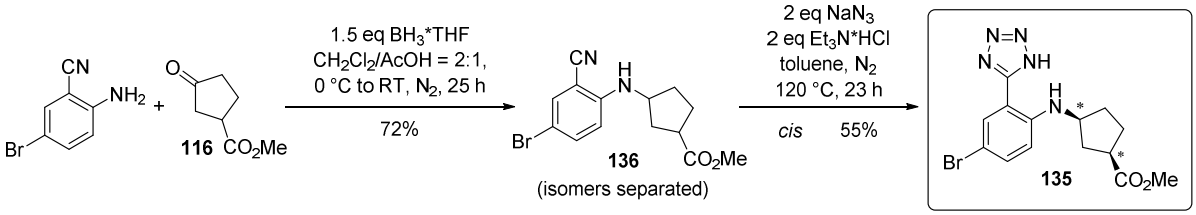


Figure 99: Synthesis of mono-1*H*-tetrazole analog **135** via reductive amination/tetrazole formation/saponification.[189,225]

The synthesis of the other 5-ring mono-1*H*-tetrazole **137** with the mirrored ester/nitrile substitution was conducted according to the same strategy as the previous one (Figure 100). During the reductive amination with method A[189] only a clean fraction of the *cis*-isomer of **138** could be isolated in low yield and the remaining product fractions (*trans*- and remaining *cis*-) were contaminated with by-products and were discarded. The tetrazole formation with *cis*-**138** to give **137** proceeded slowly and after 4 d still traces of the substrate **138** were detectable on TLC.[225] The reaction was not very selective and three new, more polar spots had appeared on TLC, which exhibited a similar CAM staining as the substrate nitrile **138**. The most prominent polar spot was isolated and identified to be the correct product **137** by ^{1}H-NMR, ^{13}C-NMR and HR-MS.

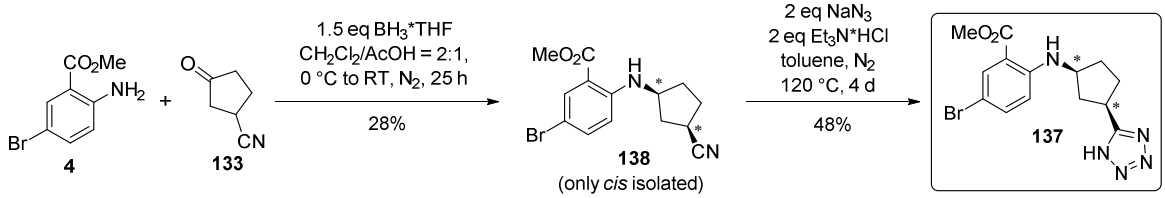


Figure 100: Synthesis of mono-1*H*-tetrazole analog **137** via reductive amination/tetrazole formation/saponification.[189,225]

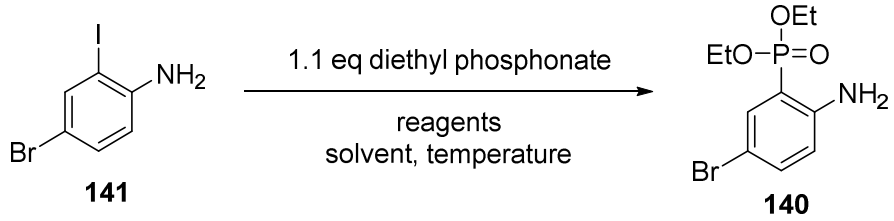
4.2.2.1.2 Synthesis of phosphonic acid bioisosteres

Next the synthesis of the very polar phosphonic acid derivative **139** of Phenazistatin A was attempted. According to literature[227] the phosphonic ester group can be easily introduced by Pd-catalyzed cross-coupling reaction from the corresponding aryl bromide or iodide and hence it was decided to synthesize Phenazistatin A derivative **139**, bearing the phosphoric acid on the aromatic ring.

First it was tried to assemble the respective phosphonic ester building block **140** via a selective mono-functionalization of 4-bromo-2-iodoaniline (**141**). Therefore the substrate was iodinated according to a literature procedure[228] with ICl in AcOH in moderate yield. Many by-products were formed during the iodination reaction and column chromatography was necessary to obtain sufficiently clean product. When the Cu-catalyzed selective introduction of just one diethyl phosphonate was attempted using a procedure by Gelman et al.,[229]

incomplete conversion of the starting material was detected after 17 h and two more polar products were generated which exhibited the same intensities (TLC, CAM staining) (Table 30). When the Pd-catalyzed procedure by Brown et al.[230] was used, the same two products were generated, but the intensities on TLC indicated a much higher abundance of the more polar spot (Entry 2). In order to identify this product a preparative batch using the same conditions was made (Entry 3). To our surprise the reaction was not complete after 42 h and many by-products had formed. The isolation of the putative product was attempted, but the by-product could not be separated via column chromatography. This strategy was then abandoned and a more convenient strategy was searched for which avoids these selectivity issues.

Table 30: Screening of reaction conditions for the synthesis of **140**.



Entry	Reagents	Solvent / Temperature	Time	Comments
1[229]	0.05 eq CuI 2 eq Cs_2CO_3 0.3 eq DMEDA	toluene 110 °C	17 h	incomplete conversion two products generated
2[230]	0.1 eq $Pd(PPh_3)_4$ 3.5 eq Et_3N	toluene 110 °C	17 h	100% conversion two products generated
3	0.1 eq $Pd(PPh_3)_4$ 3.5 eq Et_3N	toluene 110 °C	42 h	preparative batch: incomplete conversion many by-products generated

We then decided to construct the building block by a sequential introduction of the functional groups, starting from the readily available 2-bromoaniline. When the Hirao-reaction was performed following a procedure by Gooßen et al.[231] full conversion of the starting material was detected by TLC after 6 d and the product **142** was isolated via column chromatography (Figure 101). Standard bromination with NBS yielded the desired building block **140**. The reductive amination using method A[189] was rather slow and a large amount of the acetylated by-product was formed (24%) as judged by GC-MS. The *trans*-isomer could not be separated from the by-products and only 25% of the pure *cis*-product could be isolated via

column chromatography. The *cis*/*trans*-ratio in this reaction was found to be 58:42. When the pure *cis*-product **143** was subjected to standard hydrolysis conditions for the phosphonic ester, a complex mixture was obtained after 6 d at 80 °C and the product **144** could not be identified in this mixture. The final steps towards the product will need to be further optimized, eventually by the use of TMSI as simultaneous deprotecting agent for both the phosphonic ester and the carboxylic ester as published by Olah.[232,233]

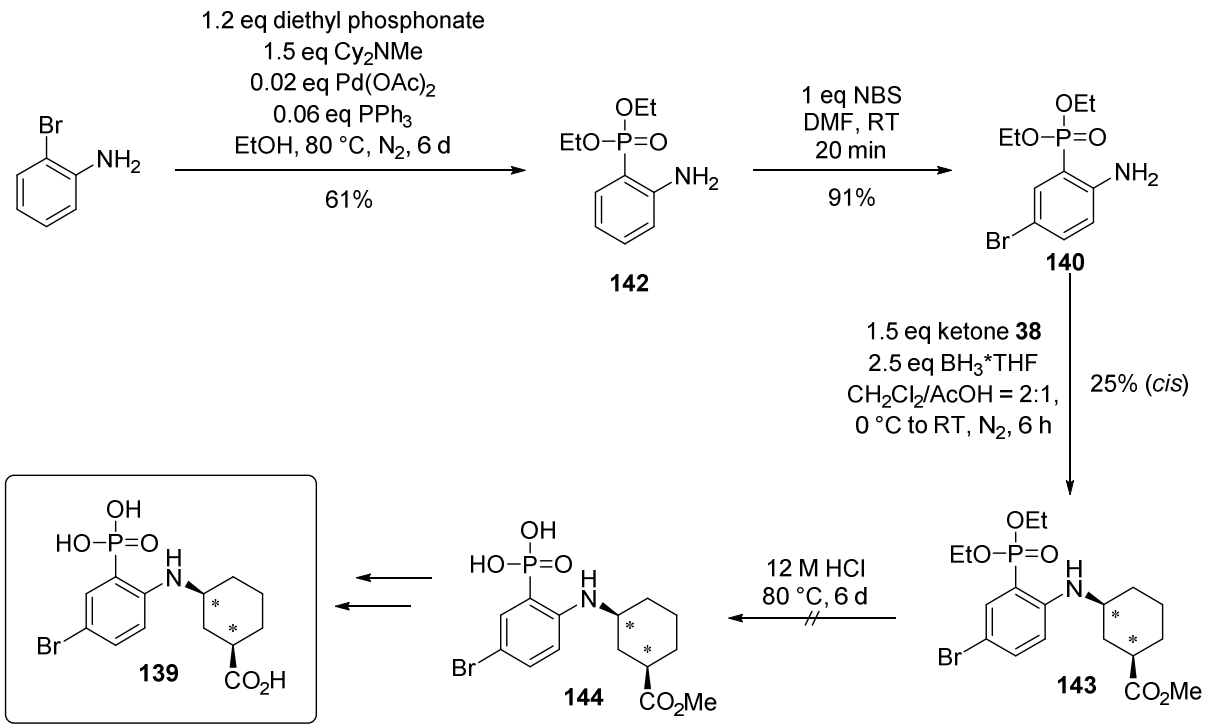


Figure 101: Synthesis of the mono-phosphoric acid analog **139** of Phenazistatin A **1** via Hirao-reaction/bromination/reductive amination/saponification.[189]

4.2.2.1.3 Synthesis of acyl sulfonamide- and acyl cyanamide bioisosteres

First the synthesis of the acyl sulfonamide derivative **145** was tried with the Boc-protected carboxylic acid **146** in order generate a pre-installed building block for the acyl sulfonamide derivative synthesis via reductive amination (Figure 102). When standard peptide coupling conditions with EDC·HCl in a mixture of CH_2Cl_2 and THF were used[234] (the methanesulfonamide had low solubility in CH_2Cl_2 and hence little THF was added to facilitate the solubility), incomplete conversion of the starting material **146** was detected after 19 h (Figure 102, A). The TLC analysis was not conclusive and in HPLC-MS no mass corresponding to the product **145** could be found. Instead, the main product formed in this reaction was the EDC-adduct **147** of the starting material, which was found to be quite stable. When the reaction mixture was heated to 50 °C for 5 d the EDC-adduct **147** was still the main product, but several other by-products had formed. Maybe the steric bulk of the

adjacent Boc-group hinders the attack of the weakly nucleophilic methanesulfonamide at the stage of the EDC-adduct **147**. When DCC was used instead[235] and the reaction was heated under reflux for 3 d, full conversion of the starting material was detected (Figure 102, B). However, no product was formed according to HPLC-MS analysis and this time the generated main products could not be identified.

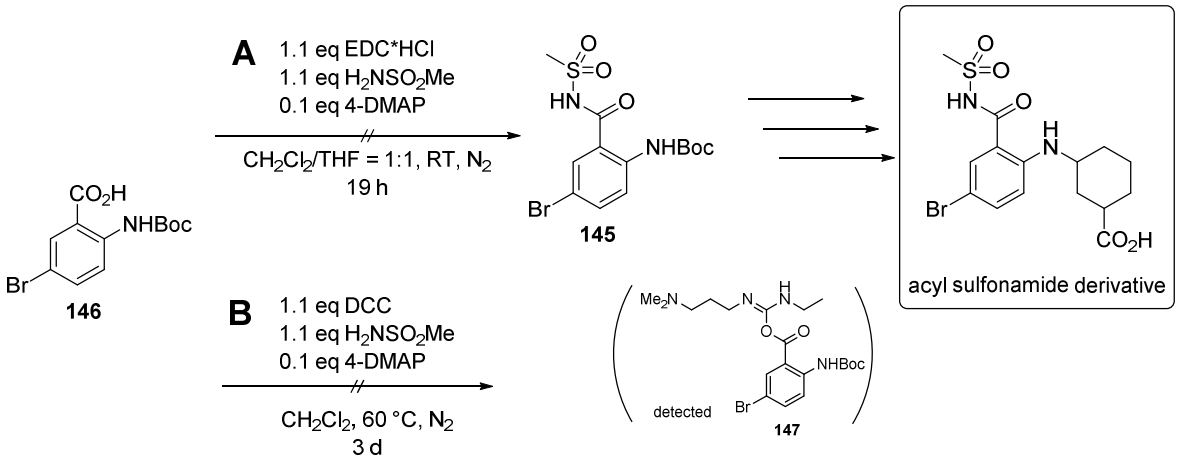


Figure 102: Synthesis of acyl sulfonamide Phenazistatin derivatives using carbodiimide reagents.

We observed the same behavior during the coupling reaction of equally weakly nucleophilic cyanamide **148** with the Boc-protected acid **149** (Figure 103) following the procedure by Choong.[234] After 19 h the main product formed was the stable EDC-substrate-adduct and further heating to 50 °C did not lead to any conversion, only the occurrence of more by-products was detected.

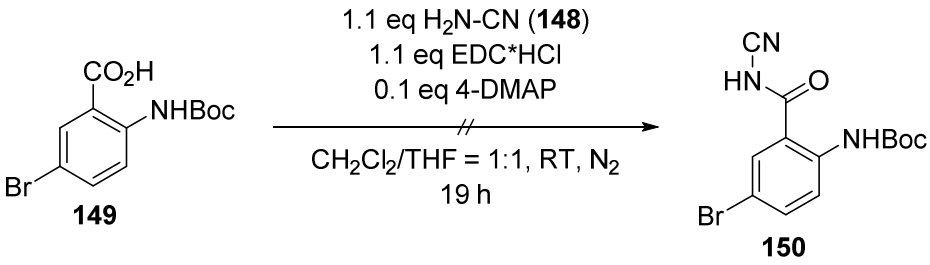


Figure 103: Synthesis of *N*-cyanobenzamide derivative **150** using EDC-coupling conditions.[234]

Since the steric bulk of the Boc group on the quite small building block **149** might prevent the desired reaction, we reasoned that it might be of advantage when the acyl sulfonamide group is installed directly on the fully assembled Phenazistatin derivative (Figure 104). Required selectivity between the acid groups could be achieved by using an arbitrary carboxylic acid precursor, where only one carboxylate is protected by an ester. These building blocks have proven to be easily synthesized using the developed methods for reductive amination and hence we tried to use the mono-acid **151** with a more reactive system of installing the acyl sulfonamide system. When the mono-acid **151** was reacted with 4-methylbenzenesulfonyl

isocyanate in the presence of base according to a procedure by Stoltz,[236] a very complex mixture of products was detected by HPLC after 6 d at 60 °C. The product **152** could be identified as a minor constituent of the mixture (11% total area%), but isolation was not attempted due to the little quantities of **152** generated in this test reaction.

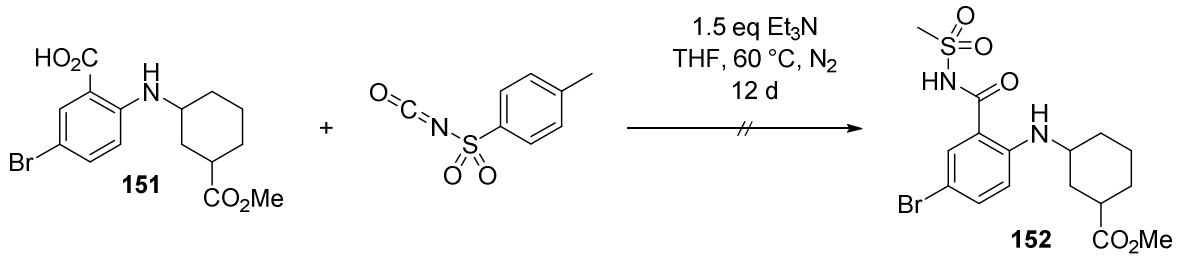


Figure 104: Synthesis of mono-acyl sulfonamide derivative **152.**

We then decided, that it would be anyway more interesting to synthesize the bis-acyl sulfonamide **153** of Phenazistatin A **1**, which would be the direct replacement of both carboxylic acid groups by a more lipophilic bioisostere (Figure 105). We used racemic Phenazistatin A **1** in procedure by Choong[234] with EDC·HCl and methanesulfonamide. Full conversion of the starting material **1** was detected after 25 h and the product **153** isomers were generated as the major products of the reaction. Repeated column chromatographies followed via preparative RP-HPLC facilitated the isolation of 3% of the *cis*-isomer of the desired bis-acyl sulfonamide **153** for biological testing. In this reaction it proved very difficult to separate the isomers of **153** and only clean fractions of the *cis*-isomer could be obtained. The fractions containing the *trans*-isomer of **153** remained contaminated with the other isomer throughout every purification step.

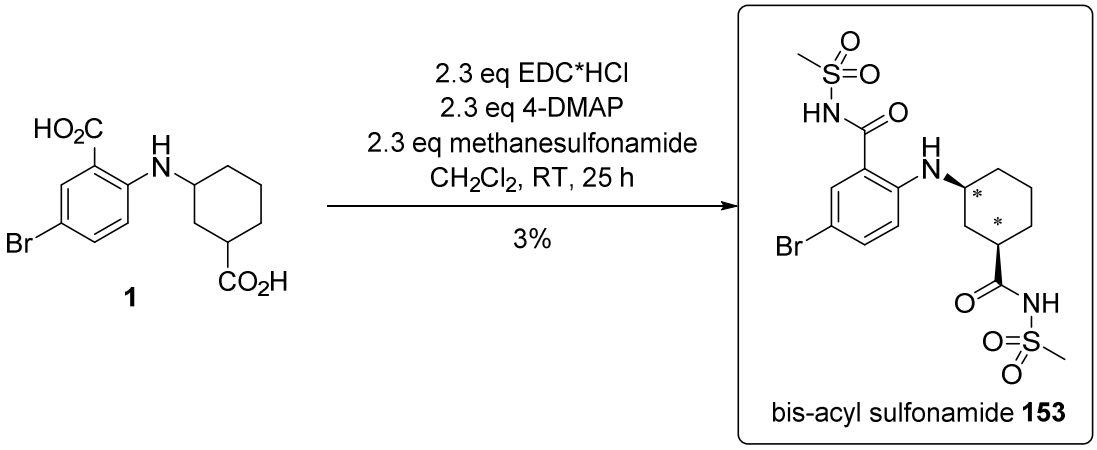


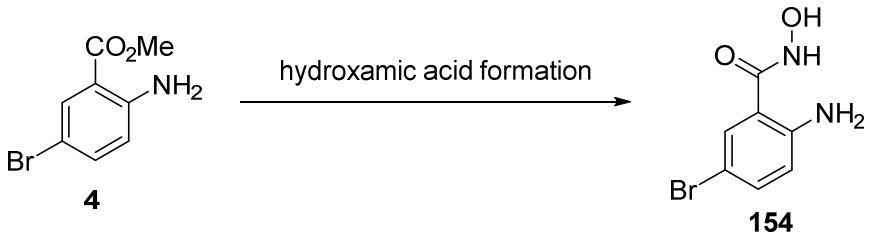
Figure 105: Synthesis of bis-acyl sulfonamide analog **153** of Phenazistatin A (**1**).

4.2.2.1.4 Synthesis of hydroxamic acid bioisosteres

For the formation of the hydroxamic bioisostere we again tried to assemble the corresponding building block so we could generate any desired hydroxamic acid Phenazistatin analog by reductive amination from the respective starting materials.

When ester **4** was used in the procedure described by Hauser et al.[237] employing hydroxylamine hydrochloride with KOH (Table 31, Entry 1), full conversion of the starting material **4** was detected after 2 d reflux. A very polar product was detected on TLC, which showed intensive tailing and no TLC conditions could be found where the dilute and/or neutralized reaction mixture showed a normal behavior on TLC. The polar product was isolated by silica filtration and analyzed by ^{1}H-NMR, but the analysis was inconclusive due to a large variety of peaks formed. When the procedure with NaOMe was used described by Stolberg (Table 31, Entry 2),[238] full conversion of the starting material **4** was detected after 4 d heating under reflux. Upon reaction a colorless solid precipitated from the reaction and was isolated via filtration. The small amount of precipitate (not weighed) was analyzed by ^{1}H-NMR in D_2O and was found to be a clean product just showing three aromatic peaks, which suggests to be the sodium salt of the product **154**. A second fraction of product was isolated from the mother liquor by precipitation with HCl in an aqueous solution. However, this precipitate had an inferior quality and HPLC-MS analysis showed three peaks with roughly 33% (total area) each and one showing the right product mass. TLC analysis indicated a strongly tailing mixture of products.

Table 31: Synthesis of the hydroxamic acid derivative **154**.



4 **154**

Entry	Reagents	Solvent / Temperature	Time / Conversion	Comments
1[237]	2 eq KOH 1 eq $H_2NOH \cdot HCl$	MeOH 80 °C	2 d 100%	very polar product isolation difficult
2[238]	3 eq NaOMe 1 eq $H_2NOH \cdot HCl$	MeOH 70 °C	4 d 100%	mixture of products product mass found

With this hydroxamic acid building block in hands, we were ready to try the reductive amination using the developed methods. However, initial attempts to react the building block **154** with the reductive amination method B (TMSCl, $BH_3 \cdot THF$) failed and gave several product spots which showed unacceptably strong tailing effects on TLC. We therefore reasoned that we would not be able to achieve any separation of the stereoisomers at this stage. This intensive tailing (on both TLC and column) of the hydroxamic is probably due to its excellent hydrogen bond donor/acceptor capabilities paired with its acidic proton. In order to obtain a suitable hydroxamic acid building block which could be coupled, we tried to protect the acidic group with 2,2-diethoxypropane and CSA by a procedure by Couturier.[239] The reaction was described to proceed at RT within hours, but for our hydroxamic acid **154** we could not detect any conversion by either TLC or HPLC after 26 h (Figure 106).

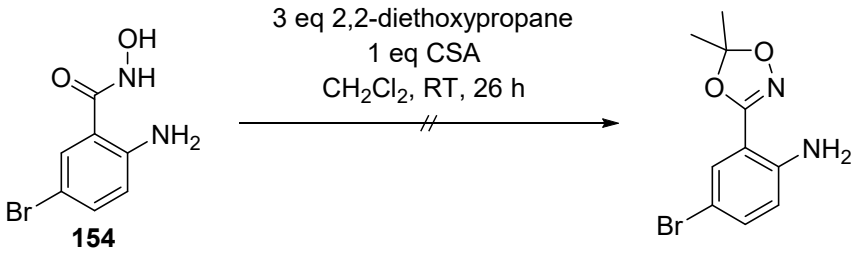


Figure 106: Attempt to protect the hydroxamic acid functionality in **154**.

In order to avoid the many side reactions and the dirty reductive amination with the hydroxamic acid building block **154**, we then decided to introduce the hydroxamic acid functionality onto the fully assembled Phenazistatin backbone (Figure 107). This could again be achieved by coupling reagents using the free carboxylic acids. We therefore tried to couple Phenazistatin A (**1**) with two equivalents of hydroxylamine using conditions by Dallavalle.[240] Full conversion of the starting material **1** was detected after 3 d and a more polar spot (TLC), which showed intensive red CAM staining. The strongly tailing spot showed a strong violet staining with the $FeCl_3$ stain, which is characteristic for hydroxamic acids and phenols. The reaction was worked up according to the described standard procedure and in order to remove the by-products and to obtain an NMR of the product isomer mixture silica gel column chromatography was conducted. Unfortunately, the product **155** could not be isolated via column chromatography, which might be connected with its high polarity and strong tailing properties or because it was lost during the workup.

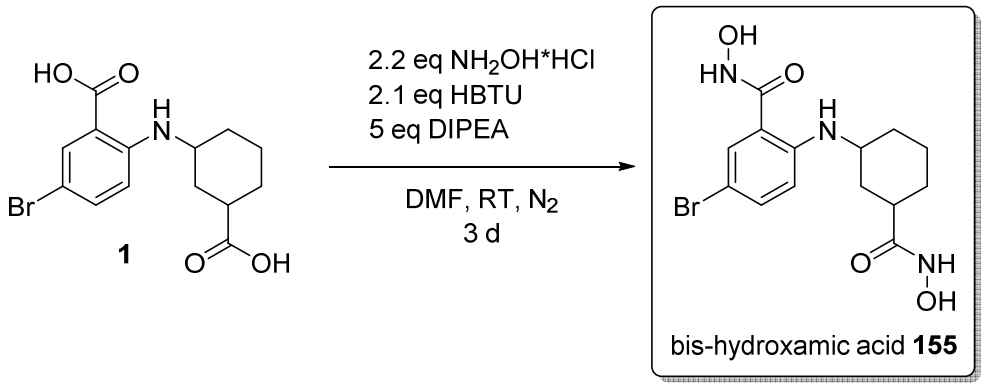


Figure 107: Synthesis of the di-hydroxamic acid **155** from Phenazistatin A **1**.

We were confident that the coupling reaction had worked, but again the isolation of the highly polar product **155** would be an issue. Due to the described rather low yields for this reaction we did not want to invest the valuable pure isomers of Phenazistatin A (**1**) as a starting material, but we were searching for a method where we could again separate the isomers of a protected hydroxamic acid after column chromatography, followed by deprotection at the end of the total synthesis.

We were pleased to find, that the *O*-benzyl-protected hydroxylamine had been previously employed in coupling reactions with carboxylic acids followed by Bn-deprotection. We therefore tried the carbodiimide coupling reaction conditions by Hao et al. with the readily available *O*-benzylhydroxylamine (Figure 108).[241] TLC monitoring turned out to be quite problematic for this reaction and HPLC-MS had to be used instead. After a very long period of 20 d incomplete conversion of the starting material **151** was detected (HPLC-MS: 25% substrate **151** of the total area% remained). In HPLC-MS 27% of the desired product **156** (correct mass signals) was detected, along with 22% of an unidentified by-product (double-peak with same mass signals, usually indication of stereoisomers). TLC analysis however showed a single spot, and therefore it was impossible to separate the products via this method. Optimization of the analytical HPLC method for the separation of the product stereoisomers **156** could not be achieved either. In summary, in order to synthesize the hydroxamic acid bioisosteres of Phenazistatin A, the coupling reactions will need to be optimized and the use of pure stereoisomers of the Phenazistatin precursors will be necessary. As a method for purification preparative RP-HPLC should be used, TLC and column chromatography seem to be highly unreliable since the intensive tailing of the hydroxamic acids could not be controlled.

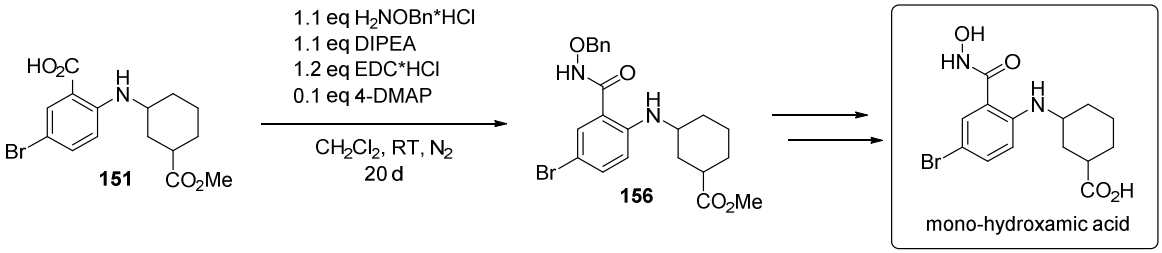


Figure 108: Synthesis of the mono-*O*-protected hydroxamic acid derivative **156**.[241]

4.2.2.1.5 Synthesis of oxadiazolone bioisosters

The oxadiazolone derivatives of Phenazistatin A were accessed in a linear fashion starting from 2-amino-5-bromobenzonitrile (Figure 109). Therefore, the aromatic building block **157**, carrying the fully assembled bioisosteric replacement of the carboxyl group was synthesized using a procedure by Valgeirsson.[242] The first reaction step worked smoothly and after 3 d and standard workup 76% of the intermediary amidoxime **157a** was obtained. Cyclization to give the oxadiazolone **157** proceeded nicely in boiling EtOH within 17 h to give the product **157** in 68% yield. The product **157** was isolated by trituration and was NMR pure as such.

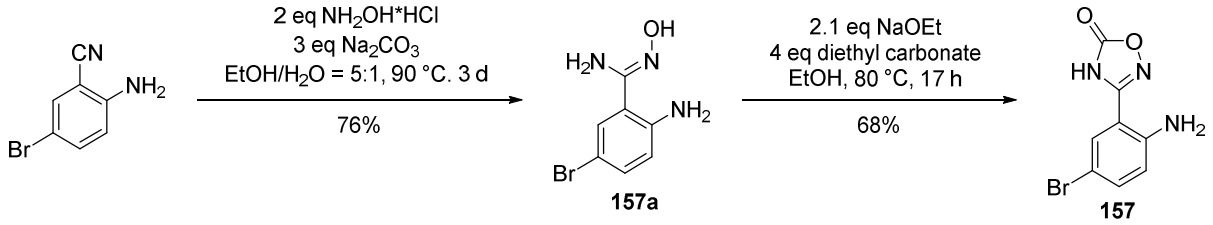


Figure 109: Synthesis of the oxadiazolone building block **157**.[242]

The Phenazistatin skeleton was assembled using reductive amination[189] and product **158** was isolated in low yield (Figure 110). Some unidentified by-products were formed during this reaction, but sufficient amounts of **158** were obtained to carry on the synthesis. The isomers of **158** could be partially separated via column chromatography and the subsequent saponification was carried out with the pure fractions of **158** (When we tried to telescope the isomeric mixture of **158** to the saponification step, then we could not isolate the oxadiazolone-Phenazistatin **159** isomers afterwards). When the saponification was carried out with the pure fractions, then the desired product isomers of the oxadiazolone-Phenazistatin **159** could be isolated via preparative RP-HPLC in moderate yields. Here again purity was paramount and contaminated product fractions were discarded, hence the low yields. It was not possible to obtain HRMS data for these compounds using DI-EI since M^+ was apparently not stable under the ionization conditions, but the ESI-MS (coupled to the

HPLC system) gave clearly the desired masses of $[M+H]^+$ with the correct isotope pattern for mono-bromine substitution.

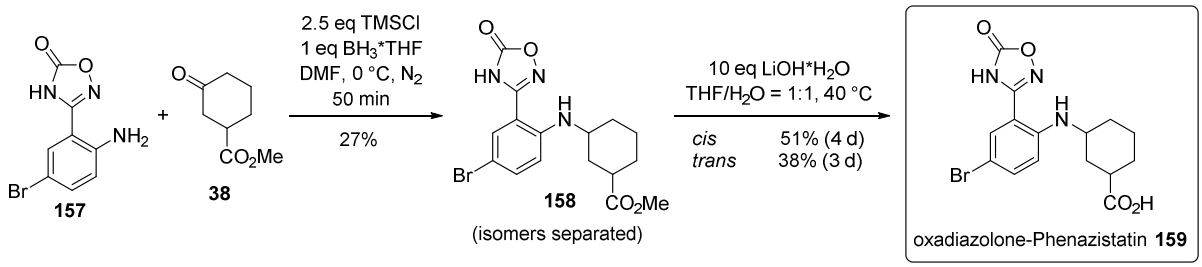


Figure 110: Synthesis of mono-oxadiazolone-Phenazistatin **159** via reductive amination/saponification.[189]

As the synthesis for the mono-oxadiazolone **159** worked well, we wanted to try whether we could synthesize the bis-oxadiazolone Phenazistatin **160** from di-nitrile **129** (Figure 111). In this molecule both potentially troublesome carboxyl groups would be replaced by the bioisosteric groups, occupying slightly more space in the binding pocket. When the reaction was carried out, full conversion of the starting material was obtained after 17 h and a very polar product was generated. HPLC-MS analysis indicated the formation of two peaks with the corresponding product **160** masses (potentially isomers). This finding encourages future attempts to the bis-oxadiazolone Phenazistatin **161**, which would be an interesting surrogate for the parent compound Phenazistatin A (**1**).

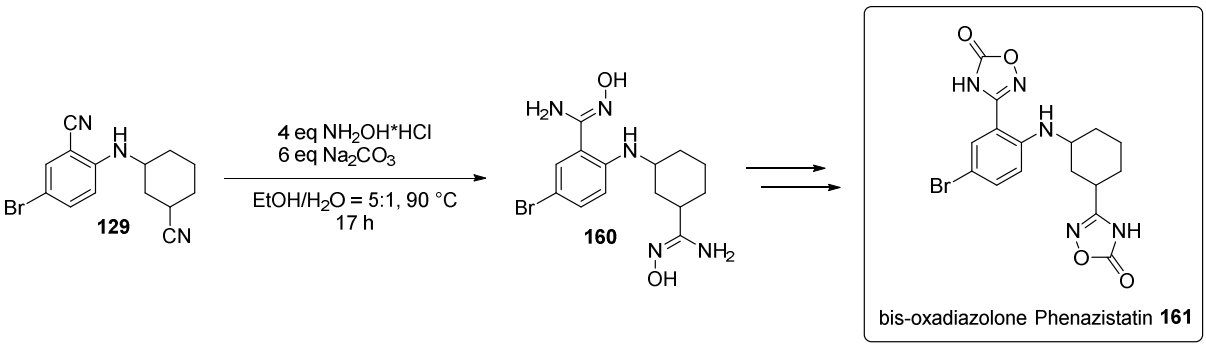


Figure 111: Synthesis of the bis-oxadiazolone-Phenazistatin **161**.

These results prompted to ask whether the reaction could also be conducted with a Phenazistatin derivative containing an ester as well as a nitrile group (Figure 112). This reaction would be a complementary approach towards the linear sequence where a pre-decorated building block would be used (vide supra). When the ester-nitrile substrate **130** was reacted with a slight excess of the reagents, a complex mixture (TLC & HPLC-MS) was obtained after 2 h under reflux. Many peaks in similar intensities were generated from a clean starting material **130** and the desired product mass **162** was not detected. Presumably the ester functionality is not inert against the nucleophilic attack by the excellent nucleophile hydroxylamine and hence the side reactions were observed.

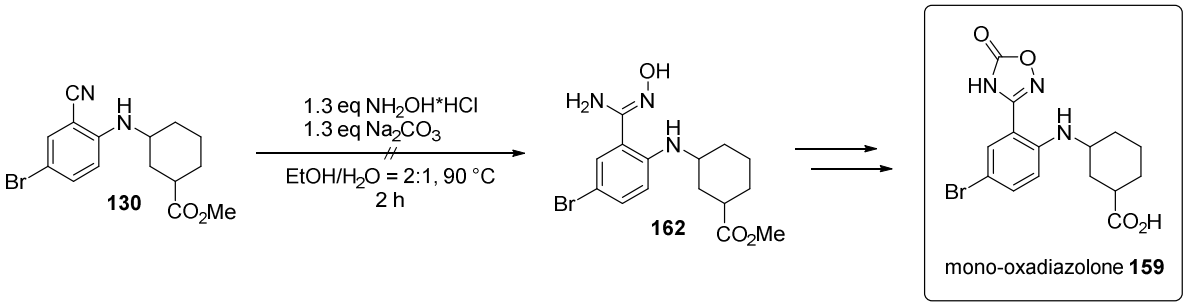


Figure 112: Synthesis of the mono-oxadiazolone-Phenazistatin **159** starting from benzonitrile **130**.

4.2.2.1.6 Synthesis of hydrazine-related heterocyclic bioisosteres

There are several heterocyclic bioisosteres of the carboxylic acid that originate from the reaction of a hydrazine moiety with a carbonyl compound. We wanted to attempt how well they are accessible starting from the synthesized Phenazistatin building blocks.

At first we tried to synthesize the 1,3,4-oxadiazole-2(3*H*)-thione precursor **163** for the synthesis of the corresponding Phenazistatin derivatives (Figure 113). When ester **4** was heated under reflux with an excess of hydrazine hydrate,[243] full conversion of the starting material **4** was detected after 10 h and product **164** could be isolated as colorless needles in 61% after recrystallization.

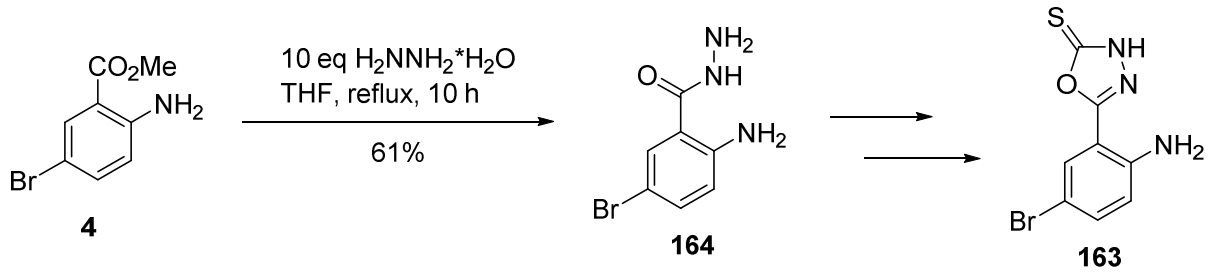


Figure 113: Synthesis of 1,3,4-oxadiazole-2(3*H*)-thione precursor **163**.

When product **164** from the previous step was used for the formation of the 1,3,4-oxadiazole-2(3*H*)-thione **163** following a procedure by Tagad et al.,[243] full conversion of the starting material **164** was obtained after 20 h and two more polar spots were generated with roughly the same intensities on TLC (Figure 114). When the TLC plate was treated with CAM staining solution, the spots did not stain. In our experience the free $-NH_2$ group usually leads to a staining with CAM, which might indicate that the free amino group has participated in the reaction mechanism that led to the two observed products. Furthermore HPLC-MS analysis of the crude product was inconclusive and the mass of the desired product **163** could not be detected in the mixture. When the reaction was repeated on a larger scale, a total of three more polar products were observed. The solvent of the reaction mixture was removed, the

residue was dissolved in the minimum amount of H_2O and upon acidification with 1 M HCl a precipitate formed, which was collected via filtration. This precipitate formation is an indicator for the presence of an acidic proton in the pK_a-range where our product would be expected. The crude product was purified via column chromatography, but HPLC-MS analysis of the isolated fractions was inconclusive and the corresponding product mass of **163** was not found. We hypothesized, that the free amino group in *ortho*-position to the benzohydrazide might have participated in the reaction and led to different reaction products.

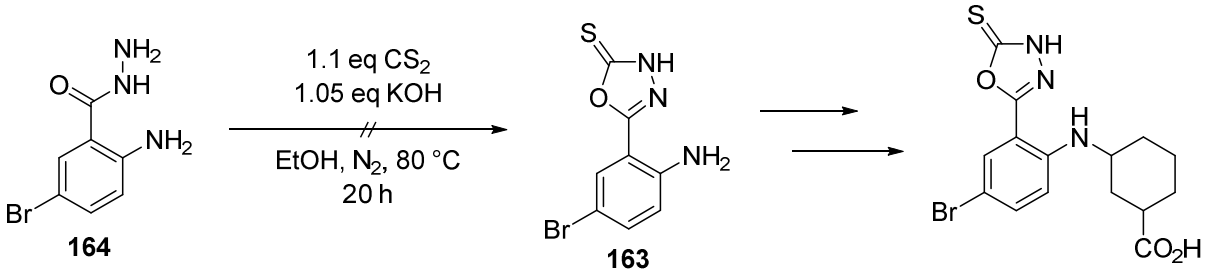
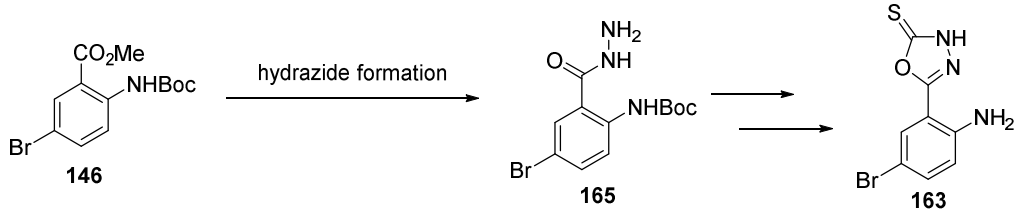


Figure 114: Synthesis of the 1,3,4-oxadiazole-2(3*H*)-thione precursor **163**.

We reasoned that we might suppress these by-product formations by the protection of the free amino group in proximity to the reactive side. Therefore we chose the easily introduced Boc-protecting group (Table 32). When the hyrazide formation was conducted with the Boc-protected ester **146** using conditions by Tagad et al.,[243] full conversion of **146** was detected after 3 d and the putative product was isolated by recrystallization as beautiful colorless needles (Table 32, Entry 1). However, ^{1}H-NMR analysis indicated that the Boc-group had been cleaved under this conditions and the deprotected product **164** was obtained. We decided to use milder reaction conditions with lower hydrazine loadings and RT to limit the rate of Boc-deprotection. When the conditions by Voronkov et al.[244] were tried in MeOH/DMF (Entries 2 & 3) only partial Boc-deprotection was detected after 20 h at RT. Additionally a slightly more polar product (in respect to the substrate) had formed which stained intensively red with CAM solution. This spot might correspond to the desired product **165**, however the correct product mass could not be detected by HPLC-MS from the mixture. Due to the small scale and the variety of products generated the mixture was not worked up and purified. Summarizing, the Boc-group appears to be not sufficiently stable for the hydrazide formation and a more stable *N*-protecting group should be applied in the future.

Table 32: Hydrazide formation from the Boc-protected ester **165**.



Entry	Conditions	Solvent / Temperature	Time	Comments
1[243]	10 eq $NH_2NH_2 \cdot H_2O$	THF 80 °C	3 d	putative product isolated ^{1}H-NMR: deprotected substrate
2[244]	7.5 eq $NH_2NH_2 \cdot H_2O$	MeOH RT	20 h	partially deprotected different product generated
3[244]	7.5 eq $NH_2NH_2 \cdot H_2O$	DMF RT	20 h	partially deprotected different product generated

In order to avoid this issue with the additional reaction steps of protection & deprotection we decided to attempt the formation of the di-hydrazide **166** from the di-ester **7** of Phenazistatin A, and hence taking advantage of the inherent "alkyl"-protection of the aromatic $-NH_2$ group (Figure 115). After 7 d heating under reflux in THF[243] incomplete conversion (HPLC: 72%) of the starting material was detected. HPLC-MS indicated the formation of two more polar sets of products with the masses corresponding to the product. Unfortunately, the product **166** could not be assigned to either peak, because the masses of all possible products are too similar (see Figure 115). The small difference in the second digit behind the comma would require a higher resolution of mass spectrometry system. However, it can be assumed that the most polar product (10% total area%) corresponds to the product and the more polar product (30%) to a mono-hydrazone. These results indicate that a harsher method would be necessary in order to convert all the reactive ester groups into the corresponding hydrazides.

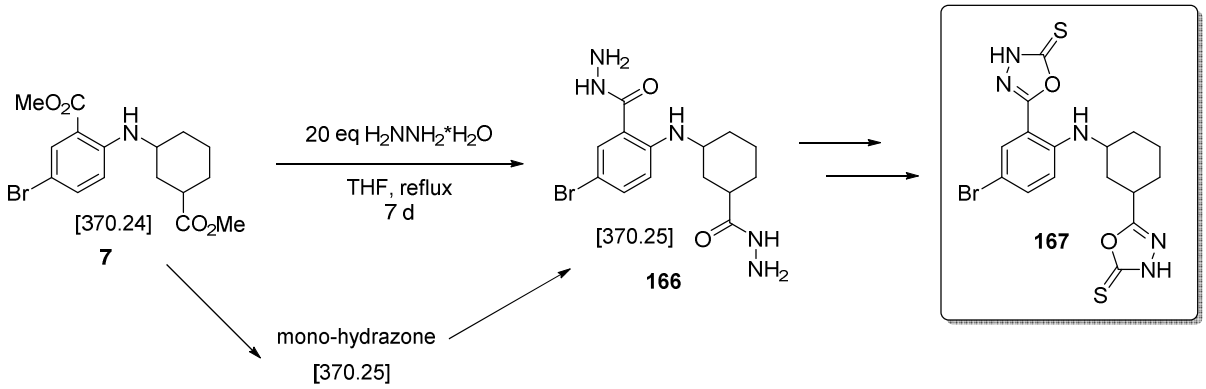


Figure 115: Synthesis of the di-1,3,4-oxadiazole-2(3*H*)-thione-Phenazistatin **167**.

4.2.2.1.7 Synthesis of miscellaneous heterocyclic bioisosteres

From the amidoxime precursor **158** which was obtained in the synthesis of the oxadiazolone bioisosteres (vide supra) a variety of other heterocyclic carboxylic acid bioisosteres can be synthesized. We attempted to synthesize the aniline precursor of the 1,2,4-thiadiazol-5(4*H*)-one bioisoster **168** and the 1,2,4-oxadiazole-5(4*H*)-thione bioisoster **169** according to a procedure by Tagad et al. (Figure 116).[243]

When we used the reaction conditions[243] to produce the 1,2,4-thiadiazol-5(4*H*)-one product **168** (Figure 116, A), we observed full conversion of the starting material **158** after 90 min at RT (TLC). The TLC analysis indicated a messy reaction, but the crude mixture was worked up according to the procedure and telescoped to the next step without any purification. For the second step full conversion to the intermediary product was detected after 4 h at RT and after workup a single product was isolated. The orange solid was found not to be the desired product **168** (HPLC-MS, NMR), but it could not be determined which product had actually formed during the reaction. The reaction was repeated in order to reproduce this finding, but the same outcome was obtained.

The synthesis of the precursor of the 1,2,4-oxadiazole-5(4*H*)-thione **169** bioisoster was attempted by a one-pot procedure[243] from precursor **158** (Figure 116, B). Full conversion of the starting material was detected after 1 h but TLC indicated the formation of many by-products which all were more polar than the starting material **158**. HPLC-MS analysis as well indicated a very complex mixture and hence this reaction was not further pursued.

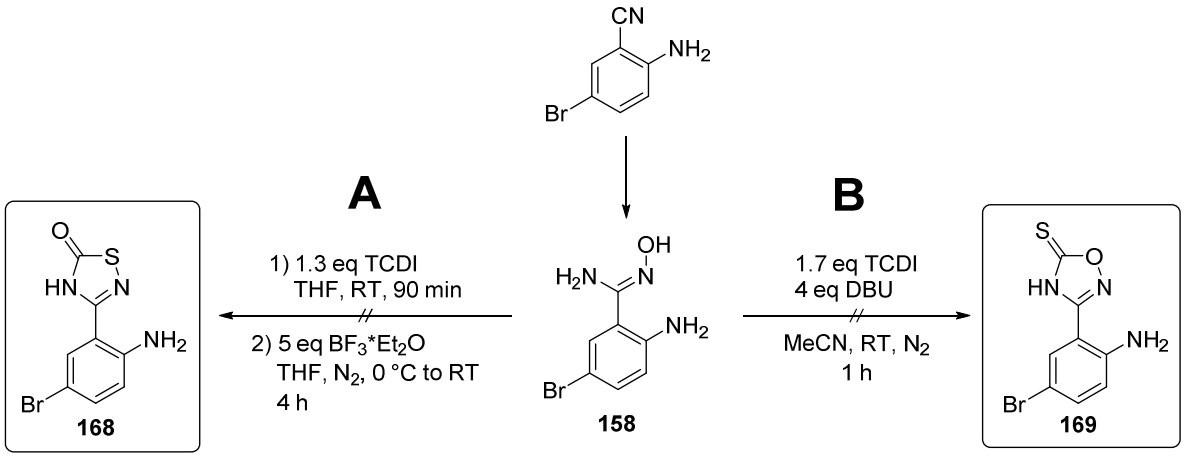


Figure 116: Synthesis of the precursors of the 1,2,4-thiadiazol-5(4*H*)-one bioisoster **168** and the 1,2,4-oxadiazole-5(4*H*)-thione bioisoster **169**.

In summary, for both reactions the desired product could not be detected in the reaction mixture. In the first case (Figure 116, A) a single product was isolated and in the second case (Figure 116, B) a complex mixture was obtained. It can be hypothesized, that cross-reactions with the free $-NH_2$ group have taken place, as was already speculated with the hydrazides previously (vide supra). For a selective reaction to take place, we hypothesized that the neighboring amino group will need to be properly protected in order to exclude it from the reaction. In order to check this hypothesis we synthesized the acetyl protected precursor and conducted the identical reactions, but to our dismay the reactions were equally messy as for the unprotected substrate. The acetyl group was probably a way too reactive protective group for these transformations. A protective group more inert to nucleophilic attack might prevent cross-reactivity here.

4.2.2.2 Synthesis of produg esters of Phenazistatin A

In literature a large variety of prodrug esters have been published, which all aim for capping the polar carboxylate group rendering the prodrug more lipophilic so its membrane permeability is modified. Phenazistatin A was the basic structure for modification, since it exhibits the highest affinity towards PhzA/B and because the two carboxylate groups might largely reduce the ability to permeate a bacterial cell wall. The easily introduced and well-established pivaloyloxymethyl prodrug ester[245] was chosen as the ester (Figure 117).

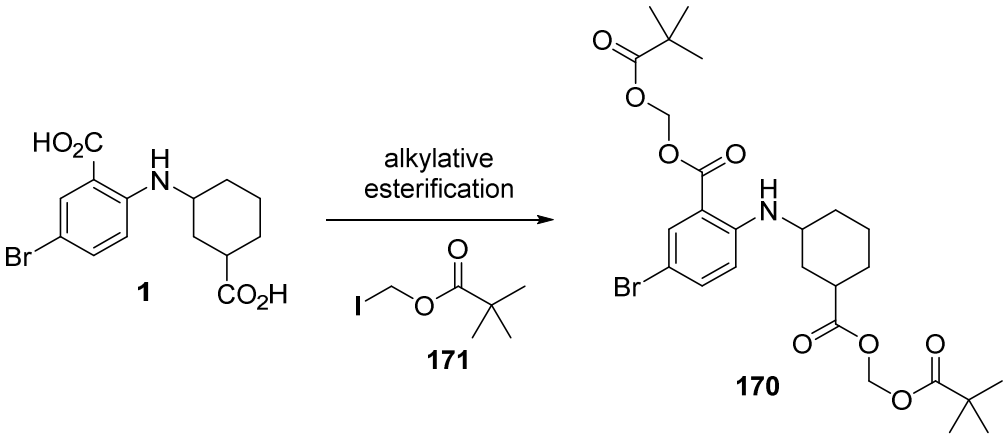


Figure 117: The strategy towards the pivaloyloxymethyl prodrug ester **170**.

The synthesis of the prodrug ester **170** is relatively simple and the alkylating agent iodomethyl pivalate (**171**) was accessed in a one-step procedure from the corresponding chloromethyl pivalate (**172**) (Figure 118).[245] The halogen exchange reaction in MeCN proceeded smoothly at 30 °C within 15 h and the reaction mixture was worked up by a standard workup procedure. A single product was detectable in GC-MS and it was surprising when only 25% were isolated after evaporation and drying in oil pump vacuum. It turned out that the product was highly volatile (the intensive pleasantly fruity odor might have suggested that already) and that large amounts were condensed into the cooling trap. When the reaction was repeated the product was only dried 60 min in vacuum (50 mbar, 40 °C bath) to give 89% of the desired product **171**. Traces of MeCN would not interfere in the subsequent alkylative esterification and so the product was used in the following step without further purification.

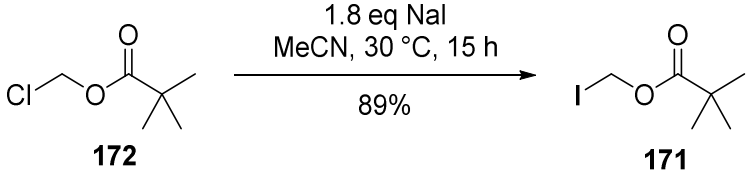


Figure 118: Synthesis of iodomethylpivalate (**171**) from chloromethylpivalate (**172**).[245]

The alkylative esterification was conducted by a standard procedure[245] and an excess of iodomethyl pivalate (3.2 eq) was added in the beginning at 0 °C (Figure 119). After 2.5 h incomplete conversion was detected by TLC and additional 1.6 eq of **171** were added but after another 30 min no change could be detected by TLC. The reaction mixture was warmed to RT to accelerate the reaction and was left stirring overnight. No change in the TLC trace was detected and so the reaction was worked up. To our dismay the product diastereomers (we started from a *cis*-/*trans*-mixture of Phenazistatin A (**1**) had an identical R_f–value on TLC and changes in the eluent had no effect on the separation. It was therefore decided to carry out a very slow column chromatography (30 x 1.1 cm, R_f–value = 0.13) and to analyze the product fractions by a different method. GC-MS failed to detect the products presumably since they are not volatile or stable enough, but ^{1}H-NMR fraction analysis gave the relieving result that clean fractions of the pure *cis*- and *trans*-isomers of **170** had been obtained. The low overall yield corresponds to the isolated clean isomer fractions of **170** and the mixed fractions were not collected.

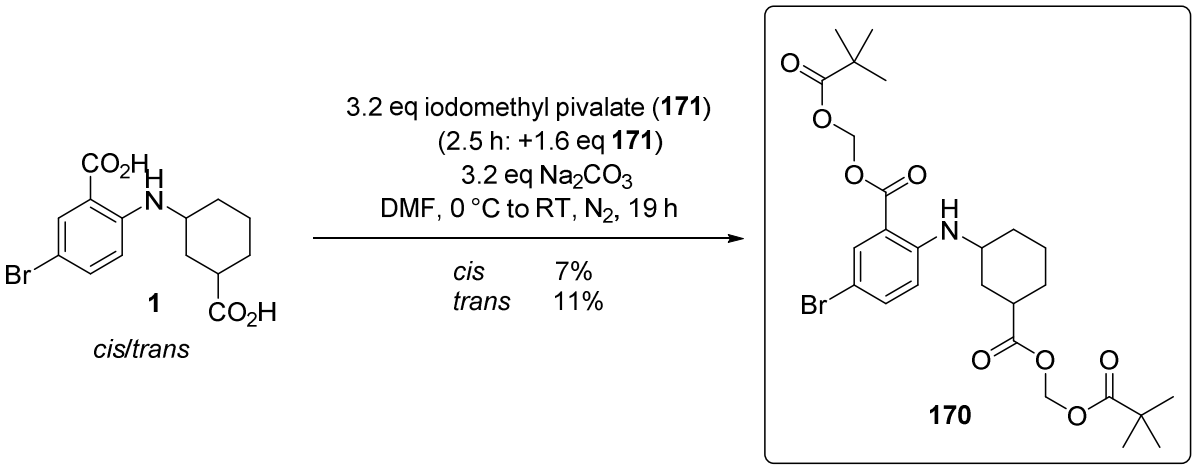


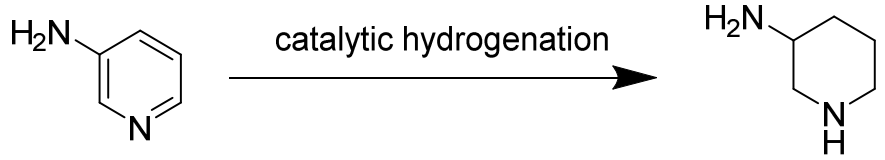
Figure 119: Synthesis of the pivaloyloxymethyl prodrug ester **170**.[245]

4.2.3 Synthesis of Maverick derivatives as PhzA/B inhibitors

4.2.3.1 Synthesis of Maverick building blocks

The building blocks required for the synthesis of the Maverick derivatives via the ULLMANN-GOLDBERG amination are all derived from readily available 3-aminopyridine. The most challenging and time-consuming part was the catalytic hydrogenation of 3-aminopyridine to 3-aminopiperidine. When the reaction conditions from the PhD thesis of Matthias Mentel[53] were used, full conversion of 3-aminopyridine was obtained after 14 d and 86% of 3-aminopiperidine was isolated after filtration and evaporation (Table 33, Entry 1). However, a high catalyst loading of 50 wt% should not be upscaled and hence a cheaper alternative was sought for. When the reaction was conducted with 1 g starting material and 5 wt% Pd/C in the autoclave using 70 bar pressure and an elevated temperature, full conversion could be detected after 2 d (Entry 2). NMR analysis of the product mixture indicated the formation of several by-products, probably acetylated species due to the presence of CH_3-signals in the respective region after azeotropic drying to remove the AcOH. When the solvent was exchanged to EtOH and the reaction was conducted on a 10 g scale with a higher catalyst loading 60% conversion of 3-aminopyridine was detected by ^{1}H-NMR. The absence of an acid apparently has a retarding effect on the reaction rate, but in the stainless steel autoclave no acid additive can be added because of the risk of corrosion. The addition of AcOH might again lead to acetylated by-products hence this measure was avoided. When another portion of 3 wt% Pd/C was added to the reaction mixture (60% conversion) no further conversion could be detected after another 8 d of heating. The separation of the sticky and very polar product from the substrate was only achieved via column chromatography, but this cost intensive method was sought to be avoided on larger scale.

Table 33: Screening for the catalytic hydrogenation of 3-aminopyridine.



Entry	Conditions	Solvent / Temperature	Time / Conversion	Comments
1	50 wt% Rh/C 1 atm H_2 (Matthias PhD)	EtOH, HCl 60 °C	14 d 100%	86% isolated
2	5 wt% Pd/C (10%) 70 bar H_2	AcOH, 80 °C	2 d 100%	complex mixture
3	10 wt% Pd/C (10%) 70 bar H_2	EtOH, 80 °C	18 d 60%	additional portion Pd/C added after 18 d 26 d: no further conversion

The electron-donating amino group at the pyridine ring seems to slow down the catalytic hydrogenation towards the saturated piperidine. In patent literature[246] we found an example where the amino group of 3-aminopyridine was first acetylated to facilitate hydrogenation followed by an acidic deacetylation to give the desired product **173** as a dihydrochloride (Figure 120). This reaction worked very well and the desired dihydrochloride **173** was prepared in an overall 73% yield. The catalytic hydrogenation needed only 2.5 wt% Pd/C and 10 d were necessary to ensure full conversion. Although this overall process needs quite long, it could be performed on 15 g scale and did not need any special attention since the product **173** was obtained in the last step by simple evaporation of the solvent.

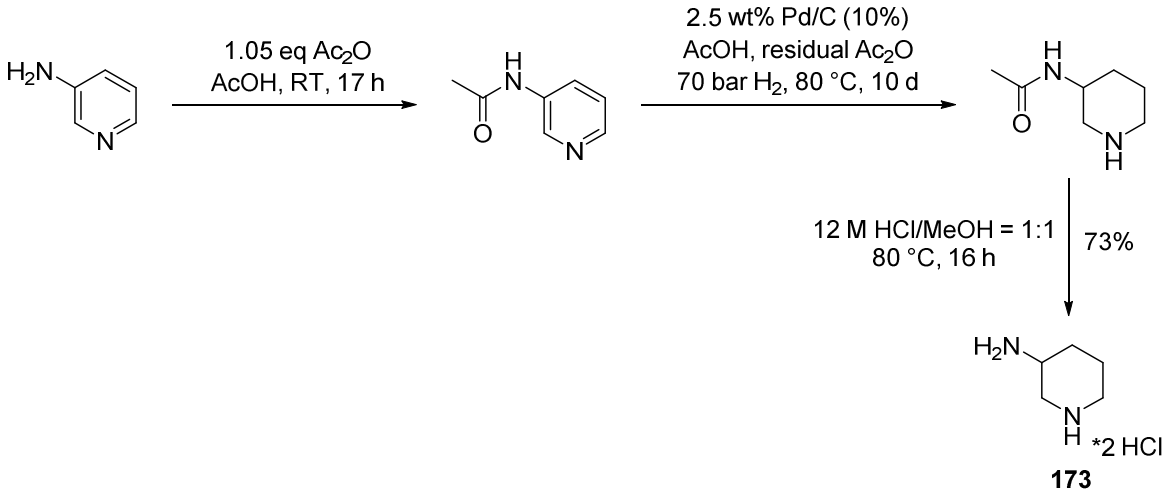


Figure 120: Synthesis of the dihydrochloride of 3-aminopiperidine **173**.[246]

We then tried to synthesize the Maverick derivatives by an ULLMANN-GOLDBERG amination approach. After several attempts using the di-hydrochloride **173** for selective ULLMANN-GOLDBERG aminations we concluded that we needed to protect the secondary amine in order to avoid extensive by-product formation. We prepared the precursor with the Boc-protected secondary amine **174** according to a convenient procedure by Laduron (Figure 121).[247] The dihydrochloride **173** was suspended in MIBK and treated with Na_2CO_3 under DEAN-STARK conditions and the water was azeotropically removed to furnish the imine **175** (MIBK boils at 116 °C and forms an azeotrope with water (bp = 88 °C)). The secondary amine was then in the same pot Boc-protected (alkylation would also be possible) by addition of 1 eq of Boc_2O and upon completion the mixture was washed with water (MIBK is insoluble in water), and then heated to 50 °C in a water/IPA mixture to hydrolyze the Boc-protected imine **176**. The free desired product amine **174** was isolated in excellent 91% yield over three steps.

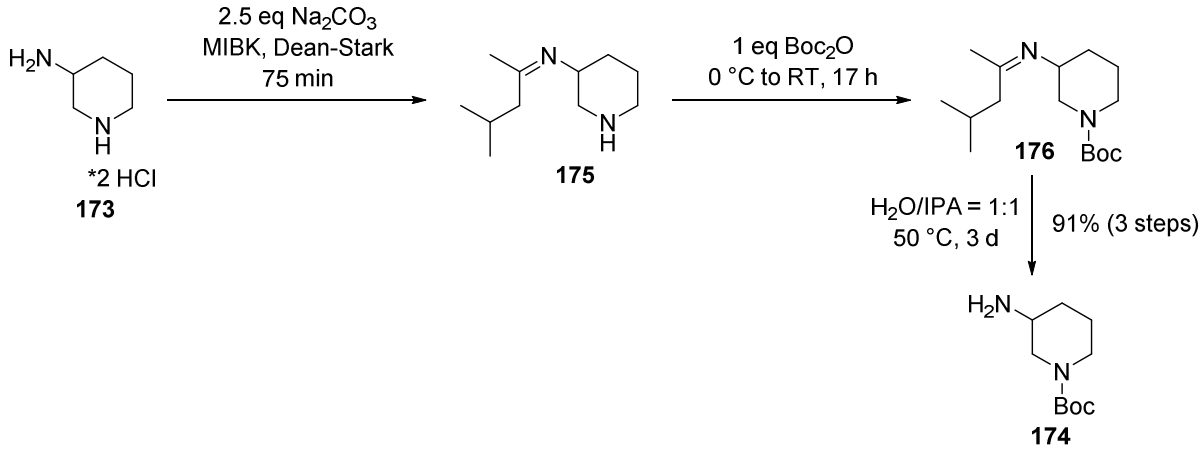


Figure 121: Synthesis of *N*-Boc protected 3-aminopiperidine **174**.[247]

4.2.3.2 Synthesis of H-Maverick

The H-Maverick **177** was synthesized via an ULLMANN-GOLDBERG amination using a procedure by Buchwald et al.[198] from the protected piperidine precursor **174** and 2-iodobenzoic acid (Figure 122). The amination proceeded smoothly to full conversion after 3 h at 70 °C and the product **178** was purified by silica filtration in order to remove the polar components of the reaction. The enriched fractions (a by-product with similar polarity as the product remained in the mixture) were telescoped into the next step, where a Boc-deprotection in the presence of anisole was conducted. The starting material was converted to a very polar product, which was purified via preparative RP-HPLC to give pure H-Maverick **177**.

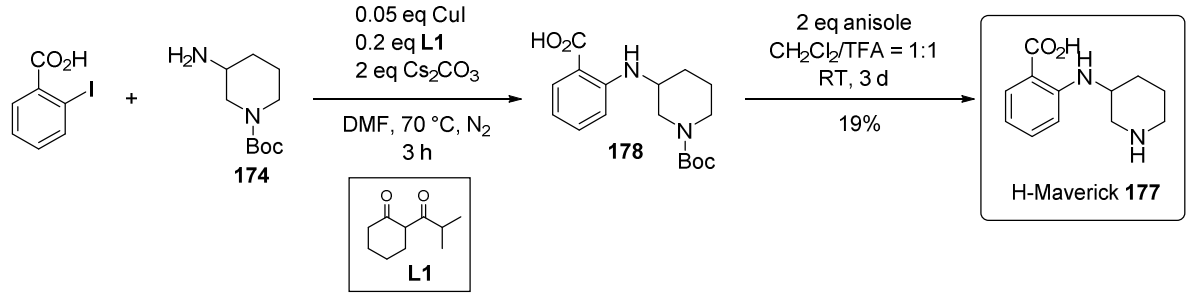


Figure 122: Synthesis of H-Maverick **177** via an ULLMANN-GOLDBERG amination/deprotection approach.[198]

Alternatively, the C-N bond formation for the generation of the H-Maverick **177** could be performed by reductive amination using method A (Figure 123).[189] Full conversion of methyl anthranilate was detected after 44 h (substrate and product had very similar R_f-values, only the CAM staining was different) and standard workup and column chromatography yielded 66% of H-Maverick precursor **178**.

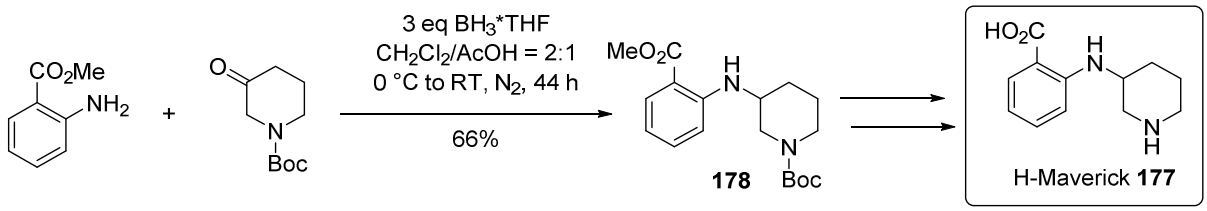


Figure 123: Synthesis of H-Maverick precursor **178** via reductive amination from methyl anthranilate.[189]

The method mentioned above failed with both method B and method C,[189] presumably due to the instability of the Boc-group towards the in situ generated HCl. The reliable formation of the fully protected H-Maverick **178** opens up the door for the synthesis of practically any other Maverick derivative, provided the aniline precursor is available. However, it would be nice if the Maverick core could be constructed using a free acid, hence saving one deprotection step (Figure 124). We therefore performed the experiment above again using anthranilic acid instead of methyl anthranilate. The mixture was left stirring for 1 d and was worked up for reaction control. TLC analysis was not useful due to strong tailing and similar R_f-values. HPLC-MS indicated very low conversion and the formation of <5% of the product **179**. To sum up, the H-Maverick core structure is best assembled with the fully protected building blocks since the reaction does not proceed with the free anthranilic acid. The use of the unprotected piperidine is not an option, due to the high instability of the corresponding amino ketone.

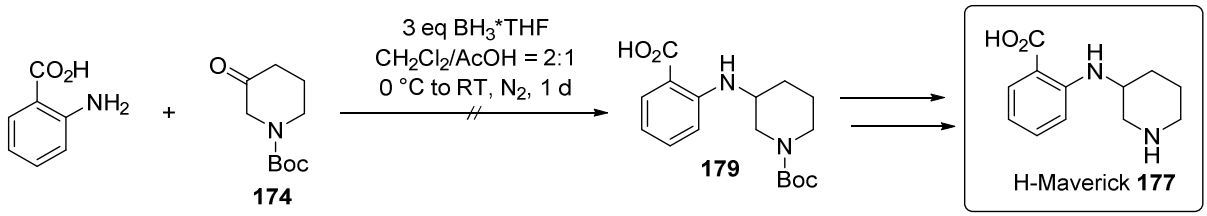


Figure 124: Synthesis of H-Maverick precursor **179** via reductive amination from anthranilic acid.[189]

4.2.3.3 Synthesis of the F-Maverick

With the methyl 2-amino-5-fluorobenzoate commercially available, the F-Maverick **180** was synthesized by a reductive amination sequence using method A (Figure 125).[189] The first reaction was worked up after 6 d although TLC gave no useful information (R_f-values of starting material and product very similar) and was telescoped as a crude product directly to the next step. Standard saponification followed by Boc-deprotection[248] and preparative RP-HPLC yielded the desired F-Maverick **180** in 41% over three steps.

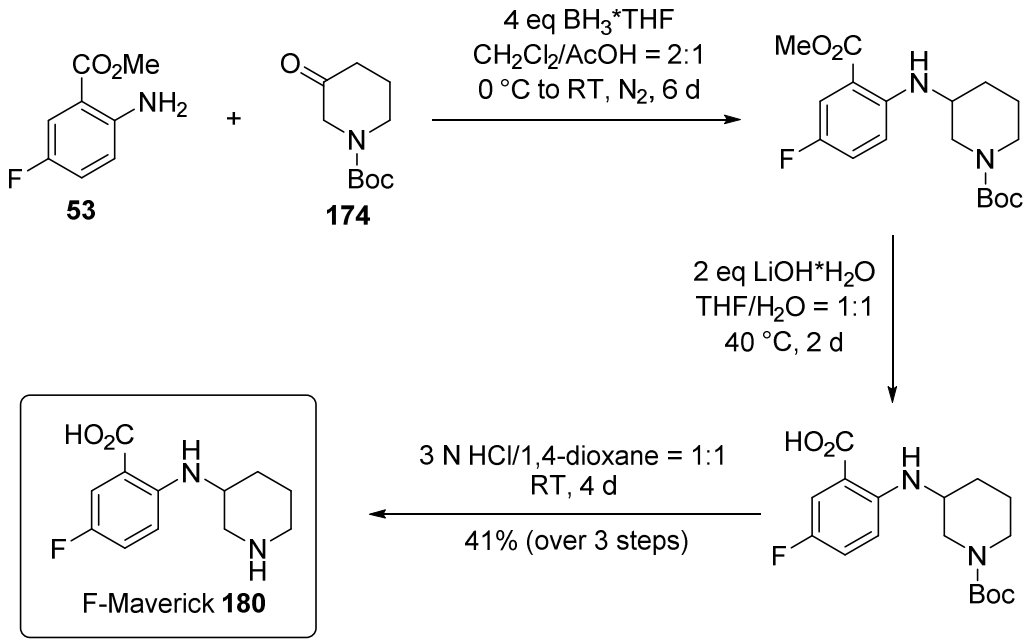


Figure 125: Synthesis of F-Maverick **180** via reductive amination/saponification/Boc-deprotection.[189,248]

4.2.3.4 Synthesis of Cl-Maverick

The synthesis of Cl-Maverick **181** was attempted by an ULLMANN-GOLDBERG amination sequence since 5-chloro-2-iodobenzoic acid (**60**) was available from the campaign towards the Cl-Phenazistatin **59** (Figure 126). When the selective ULLMANN-GOLDBERG amination was attempted on the unprotected 3-aminopiperidine using the conditions by Liu et al.,[197] the desired Cl-Maverick **181** could not be isolated. Instead product **182** resulting from a double

arylation of 3-aminopiperidine was isolated in good yield. This highlights again the necessity of the protecting group on the secondary amine, since it readily participates in the Cu-catalyzed C-N bond formations by these conditions. The double-Cl-Maverick **182** was fully characterized and sent for biological testing.

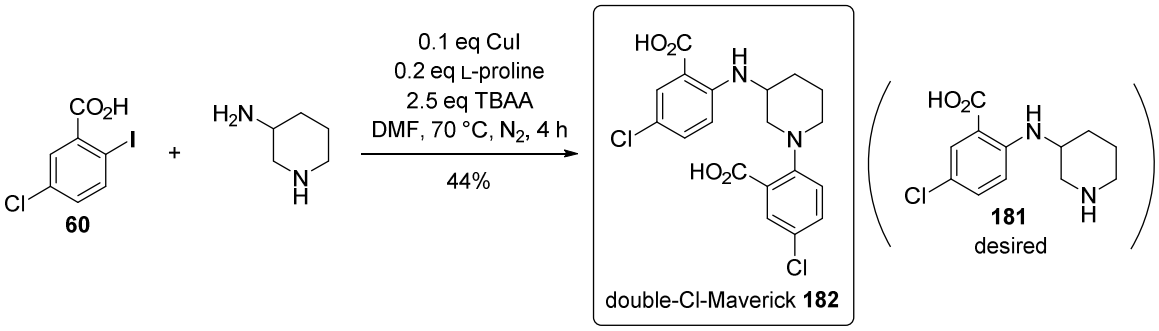


Figure 126: Attempted synthesis of Cl-Maverick **181** via ULLMANN-GOLDBERG amination using 3-aminopiperidine.[197]

When the Cl-Maverick **181** was attempted to synthesize by the conditions of Buchwald[198] only very little conversion of the starting material **60** could be detected after 3 d at 70 °C (Figure 127). The products generated exhibited very similar R_f-values so the separation of the product **183** was not accomplished by TLC. The Cl-Maverick **181** could not be synthesized in pure form via the ULLMANN-GOLDBERG amination, however it should be easily accessible via the reductive amination approach when the necessary anthranilic ester is available.

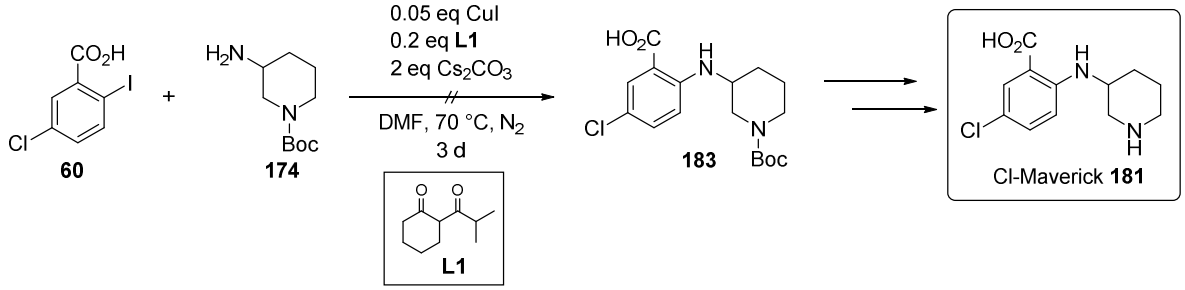


Figure 127: Attempted synthesis of the Cl-Maverick **181** via ULLMANN-GOLDBERG amination using *N*-Boc-protected 3-aminopiperidine **174**.[197]

4.2.3.5 Synthesis of Br-Maverick

The synthesis of Br-Maverick **2**, which was found to exhibit the unprecedented simultaneous binding of a racemic mixture into a binding pocket,[94] was conducted with the methods at hand. The ULLMANN-GOLDBERG amination[198] proceeded smoothly and after 3 d at 70 °C full conversion to the product **184** was detected (Figure 128). The mixture was worked up and telescoped to the Boc-deprotection step. To our surprise several by-products appeared to be

formed during this step and the product **2** was tried to be purified via column chromatography and repeated preparative RP-HPLC. Unfortunately, no analytically pure fraction of the Br-Maverick **2** could be obtained from this reaction.

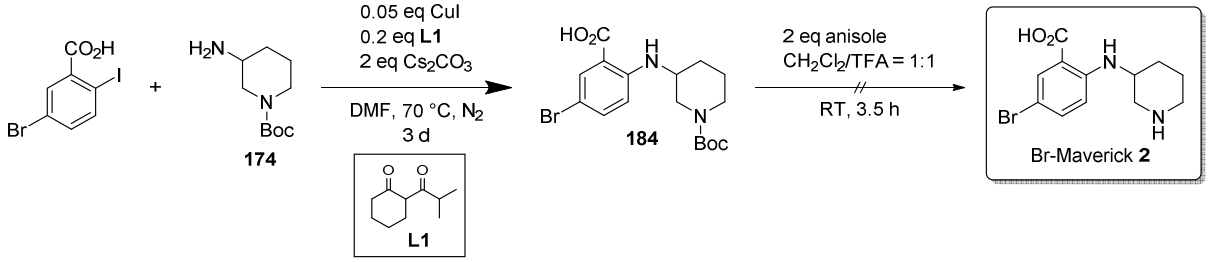


Figure 128: Synthesis of Br-Maverick (**2**) via ULLMANN-GOLDBERG amination/saponification.[198]

To our delight the reductive amination approach using method A[189] worked nicely (Figure 129) and 47% of the desired Br-Maverick **2** was obtained via preparative RP-HPLC using the same standard procedure[189,248] as for the F-Maverick **180** above (vide supra).

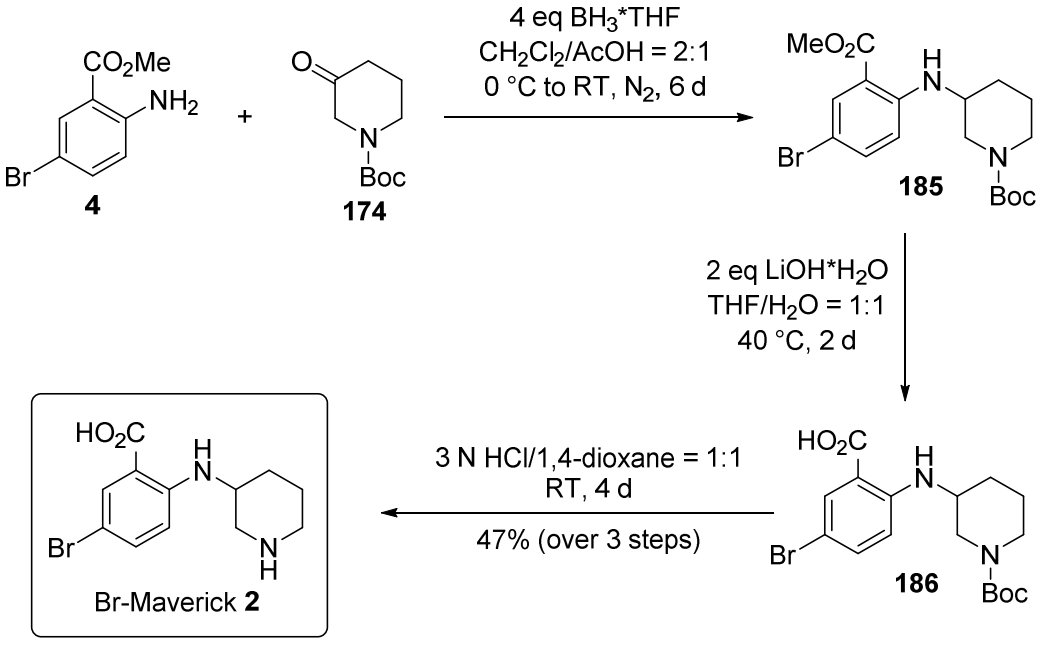


Figure 129: Synthesis of Br-Maverick **2** via reductive amination/saponification/Boc-deprotection.[189,248]

In order to obtain the bromo derivative **187** of the interesting double arylated product that was obtained during the synthesis of the Cl-Maverick (**181**, vide supra), the reaction was repeated using the more active ULLMANN-GOLDBERG amination method by Buchwald (Figure 130).[198] Full conversion of the aryl iodide substrate was detected after 3 d and product **187** was isolated by repeated preparative RP-HPLC in minor quantities. The reaction

suffered from intensive by-product formation (e.g. homocoupling) and the purification of the polar product (intensive tailing) turned out to be very difficult.

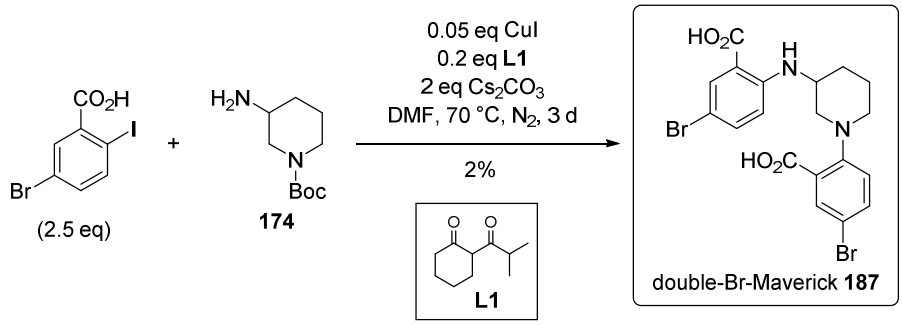


Figure 130: Synthesis of double-Br-Maverick **187** via ULLMANN-GOLDBERG amination.[198]

4.2.3.6 Synthesis of I-Maverick

The synthesis of I-Maverick **188** was done according to an ULLMANN-GOLDBERG amination[198]/deprotection sequence (Figure 131) as it was tried for the H-Maverick **177** and the Br-Maverick **2**. In the amination reaction a single product **189** was generated (TLC) and after a standard workup the crude product was telescoped to the Boc-deprotection reaction. To our surprise several by-products were generated here which complicated the subsequent purification considerably. After column chromatography and two preparative RP-HPLC purifications the product still contained amounts of an unknown by-product which could not be separated. There might be a problem adherent to the deprotection of the Maverick precursor **189** with the procedure using CH_2Cl_2/TFA with anisole as additive by Pan et al.[249] and in the future the procedure by Waal et al.[248] (3 N HCl/1,4-dioxane) was used.

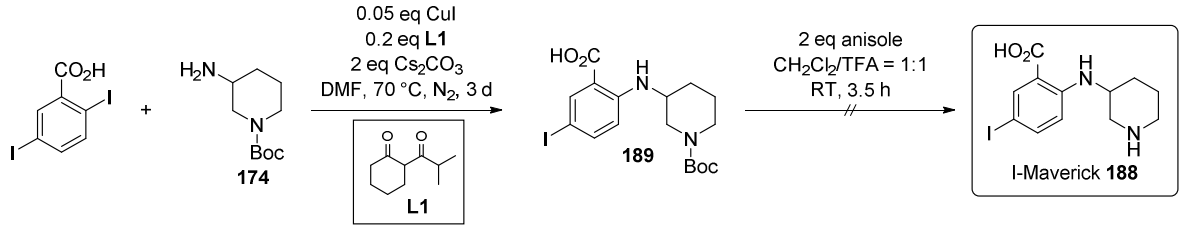


Figure 131: Synthesis of I-Maverick **188** via ULLMANN-GOLDBERG amination/Boc-deprotection.[198]

In order to produce analytically pure I-Maverick **188** for biological testing again the reductive amination/saponification/deprotection approach was chosen, since it gave the most reliable results for the synthesis of Maverick derivatives of all methods attempted so far (Figure 132). This time only 20% of the pure I-Maverick **188** could be isolated over three steps since some similarly polar impurities complicated purification.

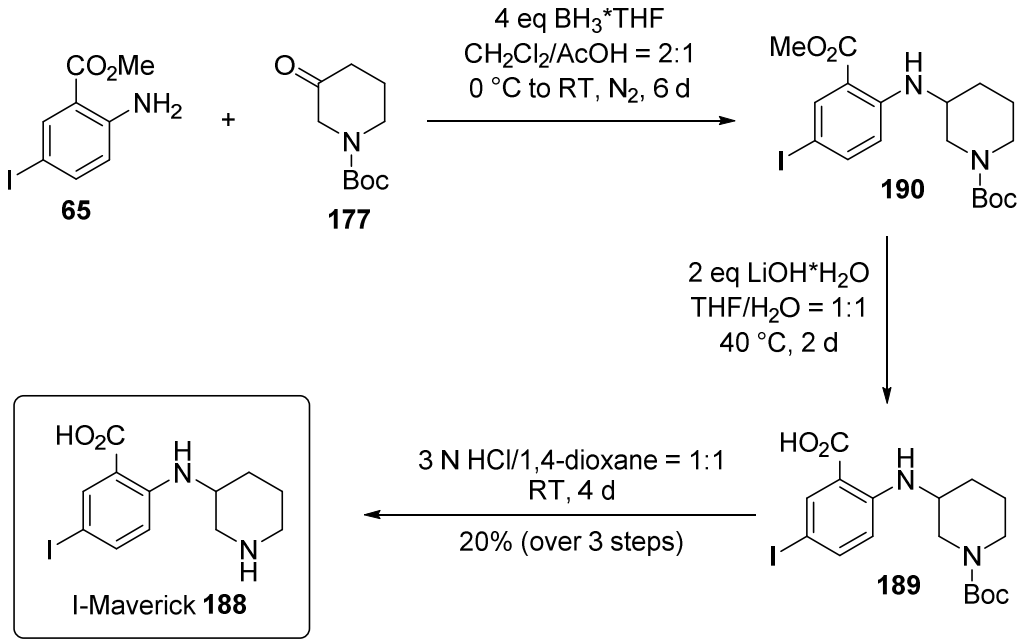


Figure 132: Synthesis of I-Maverick **189** via reductive amination/saponification/Boc-deprotection.[189,248]

4.2.3.7 Synthesis of Me-Maverick

When the usually productive amination conditions by Liu et al.[197] were used for the synthesis of Me-Maverick **191** from 2-iodo-5-methylbenzoic acid (**69**), incomplete conversion and a complex mixture was obtained after 6 d at 60 °C (Figure 133). The desired product **191** could not be isolated by chromatography from this mixture.

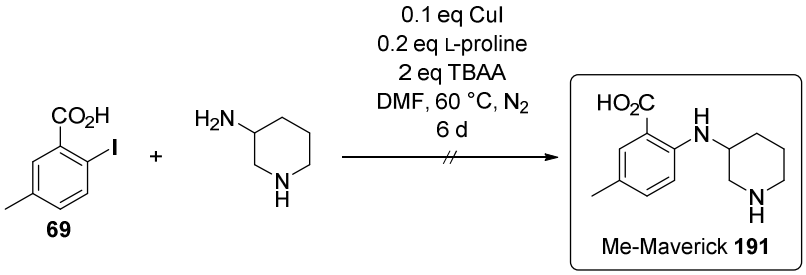


Figure 133: Synthesis of the Me-Maverick **191** via ULLMANN-GOLDBERG amination.[197]

When the same ULLMANN-GOLDBERG amination[198]/deprotection sequence as for the H-, Br- and I-Maverick was used, the corresponding Me-Maverick **191** could not be isolated (Figure 134). Similar difficulties as compared to the I-Maverick **188** were encountered (vide supra). After a clean amination reaction[198] the crude product was telescoped to the deprotection step, which was this time performed using the conditions described by Waal et al.[248] (3 N HCl/1,4-dioxane) instead of the more harsh conditions by Pan.[249] Previously we hypothesized that the very acidic TFA environment might have caused the side reactions during the I-Maverick **188** synthesis, but in this case the same problems were encountered during the purification and the pure Me-Maverick **191** could not be isolated via preparative RP-HPLC.

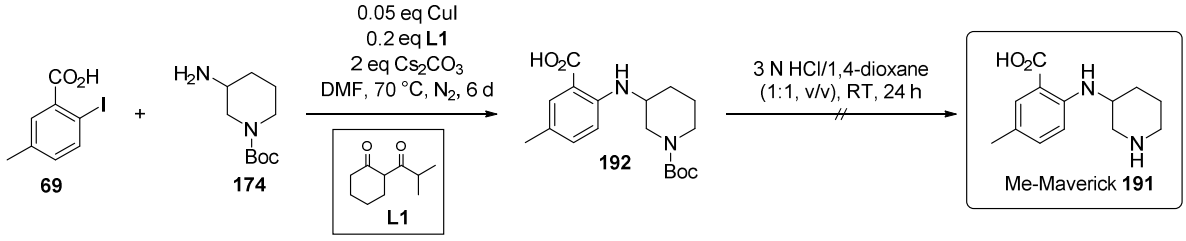


Figure 134: Synthesis of the Me-Maverick **191** via ULLMANN-GOLDBERG amination/Boc-deprotection.[198,248]

4.2.3.8 Synthesis of MeO-Maverick

The MeO-Maverick was synthesized according to ULLMANN-GOLDBERG amination conditions by Liu[197] and a standard Boc-deprotection procedure (Figure 135). 19% of the MeO-Maverick **193** could be isolated via preparative RP-HPLC after two steps. When the amination conditions by Buchwald et al.[198] and the deprotection by Pan[249] (TFA/CH_2Cl_2)

were used, the purification of the product failed similarly to several above mentioned procedures.

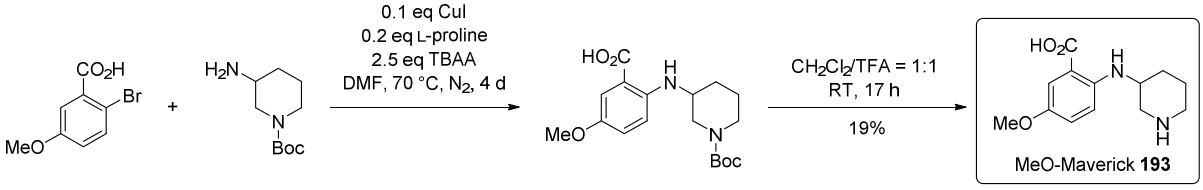


Figure 135: Synthesis of the MeO-Maverick **193** via ULLMANN-GOLDBERG amination/Boc-deprotection.[197]

4.2.3.9 Synthesis of NO_2-Maverick

The NO_2-Maverick **194** was synthesized using ULLMANN-GOLDBERG amination conditions by Buchwald et al.[198] followed by a Boc-deprotection by Waal (Figure 136).[248] In this synthesis the intermediary amination product **195** had to be purified via column chromatography before it was subjected to the Boc-deprotection step. Without the purification at this stage the NO_2-Maverick **194** could not have been isolated in a pure form by chromatography. With this substrate the Boc-deprotection proceeded smoothly and 58% of the desired NO_2-Maverick **194** could be isolated.

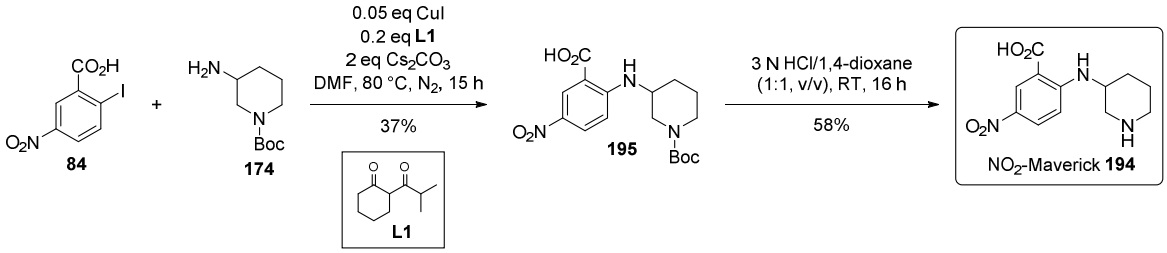


Figure 136: Synthesis of the NO_2-Maverick **194** via ULLMANN-GOLDBERG amination/Boc-deprotection.[198,248]

With the NO_2-Maverick **194** as well as the Boc-protected precursor **195** in hand it was tried whether the NH_2-Maverick **196** could be synthesized directly from the parent NO_2 compound **195** (Figure 137). Severe problems have been encountered when the NH_2-Phenazistatin **89** or the related precursors were attempted, presumably due to product instability (vide supra). When the Boc-protected NO_2-Maverick **195** was catalytically hydrogenated with Pd/C and H_2, full conversion of starting material **195** was obtained after 24 h and a more polar product was detected by TLC. The putative product could not be isolated in this small scale and when all the fractions were combined after the column and analyzed with HPLC-MS, the corresponding product mass of product **197** was not found. The spot on TLC as well as the product spectrum of the reaction appeared to be stable towards the column chromatography and no detectable degradation (darkening, etc) was observed. When a one-pot, reduction/deprotection was attempted using Zn/HCl (Figure 137) full conversion of the starting material **195** was detected (TLC) after 17 h. HPLC indicated the formation of a very

polar product which eluted in the solvent cut-off. In comparison DMSO was less polar on the HPLC column than the main product of the reaction. When the method was changed the corresponding product mass could not be found. The reaction mixture was worked up and the extremely polar product was purified via column chromatography. When the combined fractions (dark-yellow residue) were dissolved in MeOH and left standing at ambient conditions, a darkening of the yellow solution towards a dark-yellow/brown tone was observed within minutes. Judging from these observations, the isolated product degraded to a certain extent when it was brought in contact with air in methanolic solution. Unfortunately the structure of the isolated product could not be identified to make a clear statement about the stability of the NH_2-Maverick **196** (HPLC-MS, NMR).

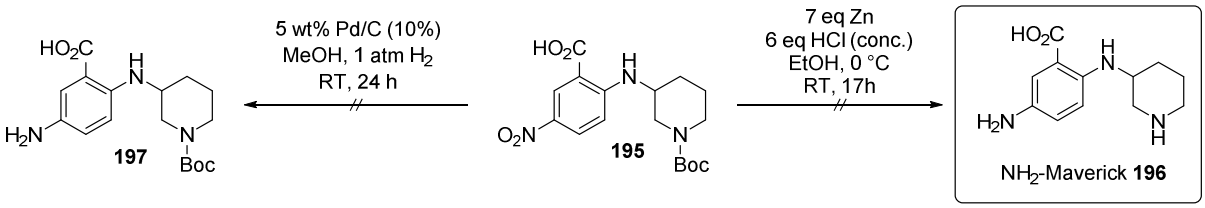


Figure 137: Attempted syntheses towards NH_2-Maverick precursor **197** and NH_2-Maverick **196**.

4.2.3.10 Synthesis of hydroxyethyl-Maverick

In order to explore how the binding characteristics of Br-Maverick (**2**) are affected by substitution of the secondary piperidine amine, we decided to synthesize the polar hydroxyethyl-Maverick **198** (Figure 138). The synthesis started from Br-Maverick (**2**) which was accessed by a reductive amination sequence.[189] Alkylation of the secondary amine was conducted using standard conditions and 17% of the desired hydroxyethyl-Maverick **198** was isolated after preparative RP-HPLC. It would have been wise to carry out the alkylation while the ester functionality of the Maverick backbone was still intact, but at that time this intermediary substrate was not available. For future syntheses the highly polar Br-Maverick (**2**) could be selectively modified at the secondary amine by reductive amination with readily available aldehydes.

The hydroxyethyl-Maverick **198** was also synthesized by a different sequence (Figure 138), starting with an ULLMANN-GOLDBERG amination using the fully decorated amino piperidine **199** (prepared according to a procedure by Laduron et al.[247]). However, this approach was inferior to the previously described approach mainly due to the unselective ULLMANN-GOLDBERG amination where a mixture of mono- and di-arylated as well as a large fraction of homocoupling product was formed.

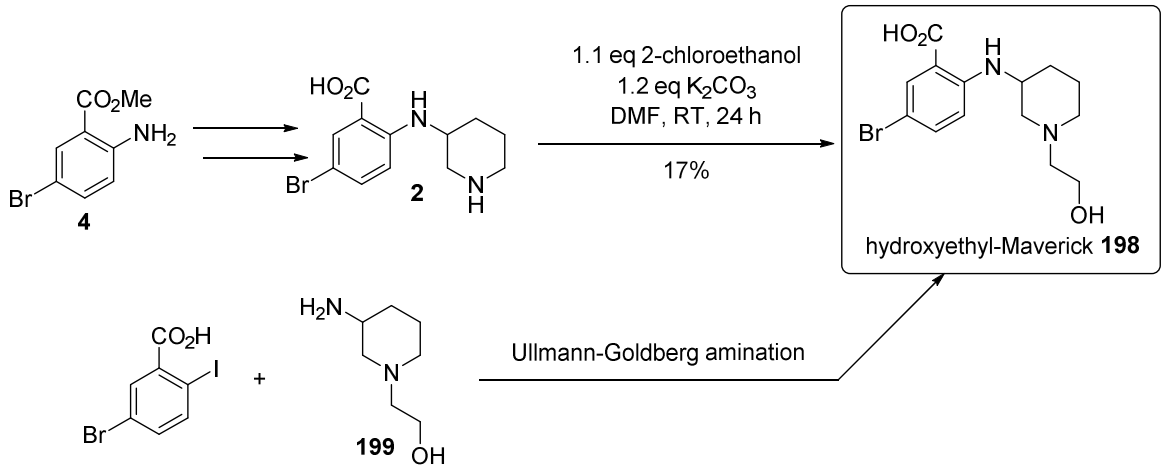


Figure 138: Synthesis of hydroxyethyl-Maverick **198**.

4.2.4 Biological evaluation of the inhibitors

The extensive biological evaluation of the inhibitors and the X-ray crystallographic experiments were carried out by Christina Diederich at the Helmholtz-Centre for Infection Research in Braunschweig under the supervision of Prof. Wulf Blankenfeldt.

In order to evaluate the inhibitors towards their capability to reversibly inhibit the enzyme PhzA/B, two different techniques were used. Firstly, an in vitro inhibition assay was conducted which reported the residual activities in % of the heterodimeric enzyme PhzA/B isolated from *Burkholderia cepacia*. This assay could be performed in parallel and most of the inhibitors could be analyzed. The experiments were conducted with a concentration of 1 mM DHHA (enzyme substrate) and the data were generated for titrations with stock solutions of different concentrations (10 mM and 100 mM) of the inhibitors. Secondly, Isothermal Titration Calorimetry (ITC) measurements were conducted in order to obtain thermodynamic binding data for the inhibitors. ITC measurements were only conducted for the most promising inhibitors from the in vitro assay, due to the limited availability of the ITC instrument and the large number of compounds synthesized. For some ITC curves the K_D-value was additionally calculated using a different method and the values are given in italics below the one calculated using the standard method. For these values a statistical lower and upper limit is given in parentheses.

Many enzyme-inhibitor structures could be obtained by crystallography of protein crystals soaked with ligands and some of which are shown here. The remaining structures are discussed in more detail in the PhD thesis of Christina Diederich.

4.2.4.1 Inhibitors substituted at the 5-position of the aromatic ring

At first the Phenazistatin derivatives which bear a short group at the 5-position of the aromatic ring were tested and the results are shown in Figure 139 (residual activities) and Table 34 (ITC). Phenazistatin A (**1**), with a Br-substituent, was so far the best inhibitor with an ITC K_D of 64 nM (Table 34, Entry 6) and was used as a reference here to compare the other synthesized inhibitors. Phenazistatin A (**1**) gives a residual activity below 10% for both concentrations tested. The *trans*-isomer of Phenazistatin-H **45** derivative gives a slightly higher residual activity at the 10 µM experiment than Phenazistatin A (**1**), hence it can be assumed to be less active in ITC. However, this assumption could not be confirmed by ITC and it was found that the *cis*-isomer of **45** (K_D = 3.6 µM, Table 34, Entry 1) bound weaker than the *trans*-isomer of **45** (K_D = 120 nM, Table 34, Entry 2). This observation was somewhat unexpected, since we know from previous studies[53] that the *cis*-isomer of Phenazistatin A (**1**) binds much stronger than the *trans*-isomer. When F-Phenazistatin **52** was tested, the residual activities indicated the same trend as for Phenazistatin H **45** and the *cis*-isomer of F-Phenazistatin **52** (Figure 139) showed a much lower residual activity than the *trans*-isomer. ITC analysis indicated an upside-down result and showed that *cis*-F-Phenazistatin **52** is a much weaker binder (K_D = 1.0 µM, Table 34, Entry 3) than *trans*-F-Phenazistatin **52** (K_D = 47 nM, Table 34; Entry 4). Interestingly, *trans*-F-Phenazistatin **52** was found to exhibit a higher affinity in the conducted ITC measurements than the lead compound Phenazistatin A (**1**) (K_D = 64 nM, Table 34, Entry 6). Additionally, X-ray structures of the enzyme-inhibitor complex could be obtained for both isomers of **52** showing that they exhibit the same binding mode as Phenazistatin A (**1**) (vide supra, Figures 140 & 141). The Phenazistatin derivatives bearing the halogens chlorine **59** and iodine **63** were found to give a similar result in the residual activity assay, however ITC only gave a moderate binding affinity for the *cis*-Cl-Phenazistatin **59** (9.4 µM, Table 34; Entry 5) and the *cis*-I-Phenazistatin **63** (K_D = 5.8 µM, Table 34, Entry 7). When the bromine was replaced by a methyl group, the residual activity was found to be higher in both concentrations measured and ITC gave a mediocre binding affinity for the *cis*-Me-Phenazistatin **68** (K_D = 1.7 µM, Table 34, Entry 8). The *trans*-MeO-Phenazistatin **74** on the other hand gave a lower residual activity again and the K_D-value was found to be a magnitude lower (K_D = 130 nM (Table 34, Entry 9), which is comparable to the results from *trans*-H-Phenazistatin **45** (K_D = 120 nM, Table 34, Entry 2). Both isomers of NO_2-Phenazistatin **82** did not seem to inhibit Phz(A/B) at either concentration and a residual activities around 90% were measured at 100 µM (Figure 139).

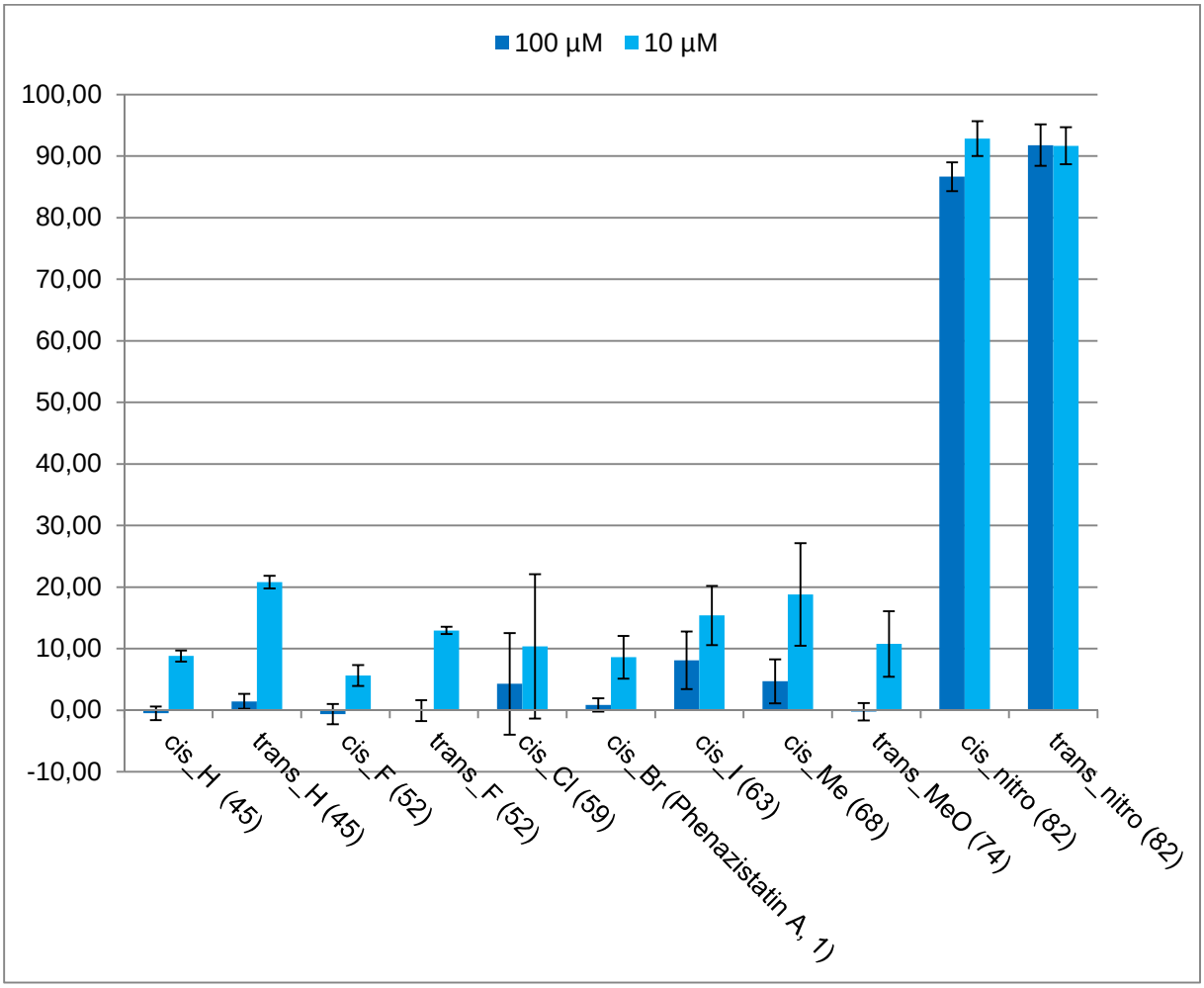
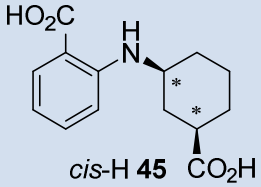
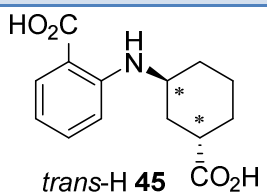
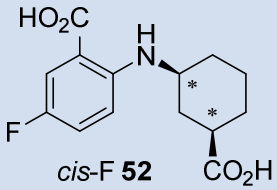
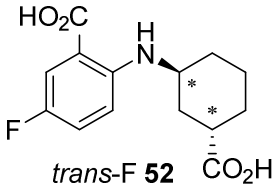
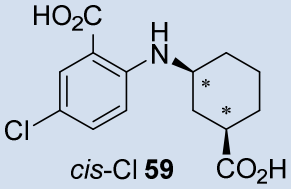
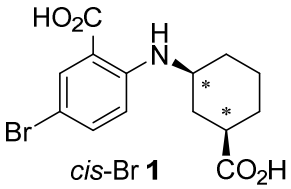
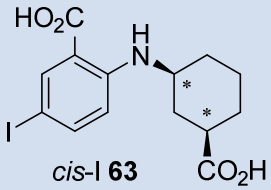
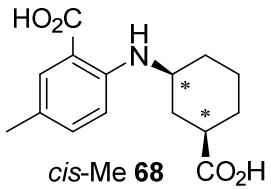
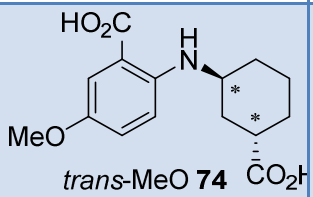
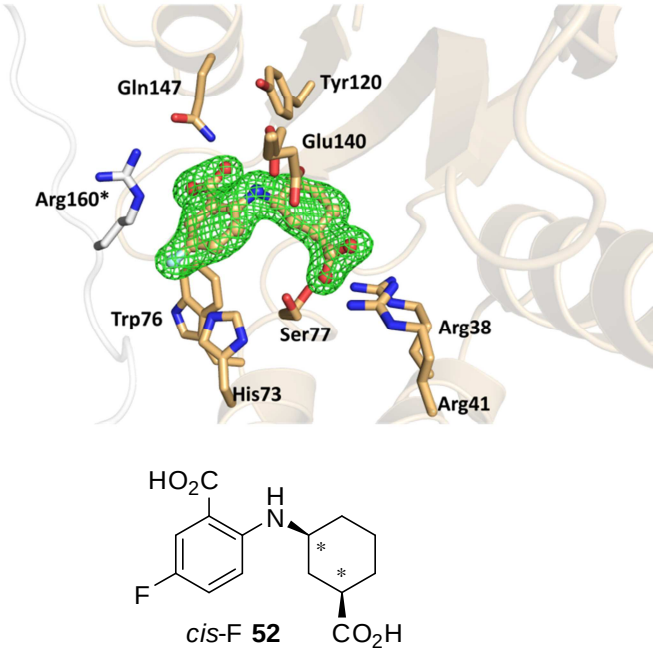


Figure 139: Residual activity of BcPhzAB (1 mM DHHA) with inhibitors substituted at the 5-position of the aromatic ring.

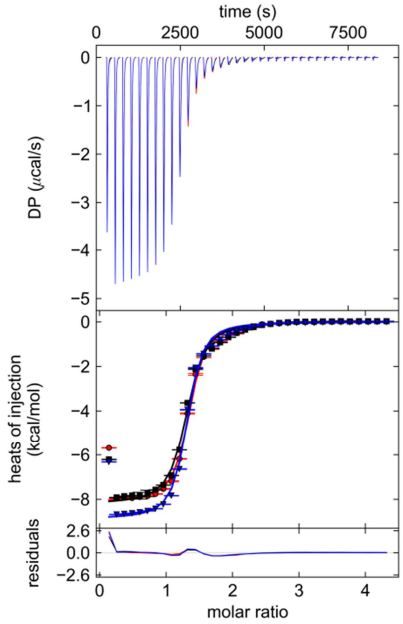
Table 34: ITC data for inhibitors substituted at the 5-position of the aromatic ring.

Entry	Ligand	K_D (µM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	-T*ΔS (kcal mol^{-1})	*N*	c
1	 *cis*-H **45**	3.6 ± 0.4 *4.5 (3.4, 5.8)*	-7.4 ± 0.1 *-7.3*	-8.4 ± 0.3 *-9.0*	1.0 ± 0.3 *1.7*	1.3 ± 0.1 *1.3*	28.5 ± 3.4 *22.3*
2	 *trans*-H **45**	1.2 ± 0.3 • 10^{-1}	-9.4 ± 0.1	-3.2 ± 0.0	-6.3 ± 0.1	1.2 ± 0.0	855.8 ± 176.9

3	 *cis*-F **52**	1.0 ± 0.2	-8.2 ± 0.1	-10.8 ± 0.4	2.6 ± 0.3	1.3 ± 0.0	103.7 ± 16.2
4	 *trans*-F **52**	4.7 ± 0.1 • 10^{-2}	-10.0 ± 0.0	-5.0 ± 0.3	-5.0 ± 0.3	1.1 ± 0.1	2126.1 ± 41.9
5	 *cis*-Cl **59**	9.4 ± 1.5 *7.3 (4.8, 11.5)*	-6.9 ± 0.1 *-7.0*	-10.2 ± 0.5 *-8.4*	3.4 ± 0.6 *-1.4*	2.4 ± 0.0 *2.3*	10.9 ± 1.8 *13.7*
6	 *cis*-Br **1**	6.4 ± 1.1 • 10^{-2}	-9.8 ± 0.1	-10.3 ± 0.2	0.5 ± 0.2	1.8 ± 0.1	1610.6 ± 242.7
7	 *cis*-I **63**	5.8 ± 0.2	-7.1 ± 0.0	-9.4 ± 0.2	2.3 ± 0.2	2.8 ± 0.0	17.3 ± 0.7
8	 *cis*-Me **68**	1.7 ± 0.2	-7.9 ± 0.1	-8.7 ± 0.4	0.8 ± 0.4	1.9 ± 0.0	58.4 ± 5.8
9	 *trans*-MeO **74**	1.3 ± 0.2 • 10^{-1}	-9.4 ± 0.1	-4.5 ± 0.1	-4.9 ± 0.1	1.1 ± 0.0	793.6 ± 151.9

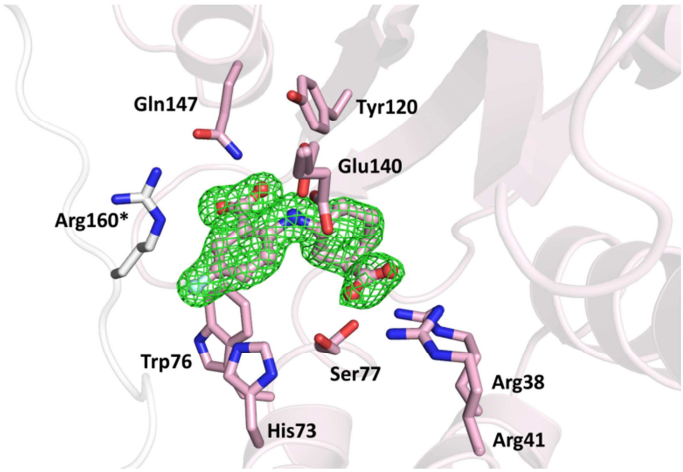


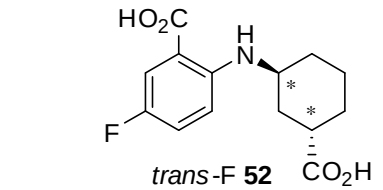
cis-F **52**



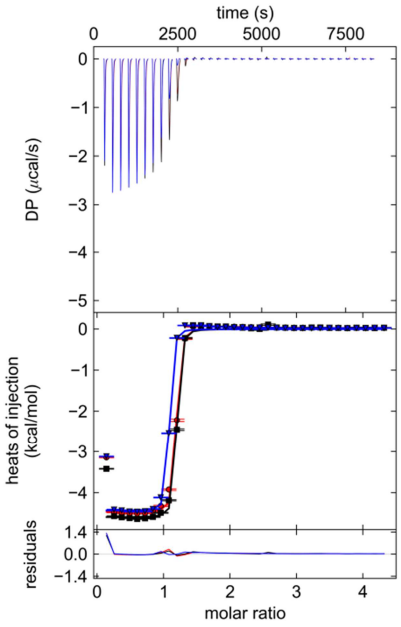
K_D = 1.0 ± 0.2 µM

Figure 140: Crystal structure of *cis*-F-Phenazistatin **52** and its ITC curve.





trans-F **52**



K_D = 47 ± 1 nM

Figure 141: Crystal structure of *trans*-F-Phenazistatin **52** and its ITC curve.

4.2.4.2 Inhibitors via cross-coupling reactions at the 5-position of the aromatic ring

With the above mentioned inhibitors the effect of electronic, H-bonding and steric effects onto the binding affinity has been tested. In order to test how sensitive the binding affinity is to a substitution by larger substituents at the aromatic ring the corresponding Phenazistatin derivatives were tested. The results from the residual activity assay are summarized in Figure 142 and the ITC data in Table 35.

The isomers of phenyl-Phenazistatin **91** showed a strong effect on the residual affinity of PhzA/B (Figure 142). The *cis*-isomer of **91** exhibited a stronger effect than the *trans*-isomer (41%) and a residual activity of 7% was already obtained at 10 µM (Figure 142). With the *trans*-isomer 100 µM of the inhibitor were needed to obtain the same effect (2%). ITC of the *cis*-isomer of **91** gave a K_D = 3.4 µM (Table 35, Entry 1), which is a quite low affinity when compared to the Phenazistatin A (**1**) lead (K_D = 64 nM). It is however in the same magnitude as the inhibitors bearing short substituents at the aromatic ring (vide supra, Table 34). An apolar, aromatic substituent at this position apparently does not increase binding affinity, however no detrimental effect was observed either.

The isomers of carboxyethyl-Phenazistatin **98**, which were synthesized via a MIZOROKI-HECK reaction followed by a catalytic hydrogenation, showed only minor activity in the residual activity assay. 100 µM of the *cis*-isomer of **98** were needed so that a significant suppression (47%) of the enzyme reaction could be achieved (Figure 142). The *trans*-isomer of **98** seems to have a very low affinity and even at 100 µM only 88% residual activity were detected (Figure 142). This might be an indication that a short, very polar group is not well-tolerated by the enzyme.

When the bromine atom was replaced to give the linear, apolar phenylacetylene-Phenazistatin **104**, activity in the residual activity assay was detected (Figure 142). The *cis*-isomer of **104** was more active than the *trans*-isomer and 9% residual activity was already detected at 10 µM, whereas the *trans*-isomer required 100 µM to give a similar effect (12%). With this long and rigid stick-like appendage on the inhibitor **104** a pronounced difference between both isomers was detected at 10 µM (*cis*: 9%, *trans*: 72% residual activity). For most of the above mentioned inhibitors with the short substituents at the aromatic ring (Table 34) the difference between the isomers is very low, at least when looking at the results from the residual activity. It appears that the longer and the more rigid the substituent gets, the higher the difference in energy of binding of the two isomers becomes. This observation is supported by the results of the rigid, but shorter phenyl-Phenazistatin **91** (Figure 142), where the difference at 10 µM was found to be *cis*: 7%, *trans*: 41%. To sum up, the *cis*- and *trans*-orientation of the inhibitor becomes more and more energetically different the longer and more rigid the substituent at the aromatic ring gets.

This theory is further supported by the results from phenethyl-Phenazistatin **106**, where the rigid structure of the phenylacetylene-Phenazistatin **104** was transformed into a flexible side chain by catalytic hydrogenation (Figure 142). According to the theory mentioned earlier, the activity would need to increase greatly and the difference in activity between the *cis*- and the *trans*-product would shrink. We were very pleased to observe the expected result and both isomers of the phenethyl-Phenazistatin **106** showed a remarkably low residual activity (*cis*: 2%, *trans*: 1%) already at 10 µM. The flexibility of the side chain apparently allows both isomers of **106** to access the binding pocket of PhzA/B equally well. ITC of the *cis*-phenethyl-Phenazistatin **106** however only indicated a mediocre K_D-value of 5.6 µM (Table 35, Entry 2).

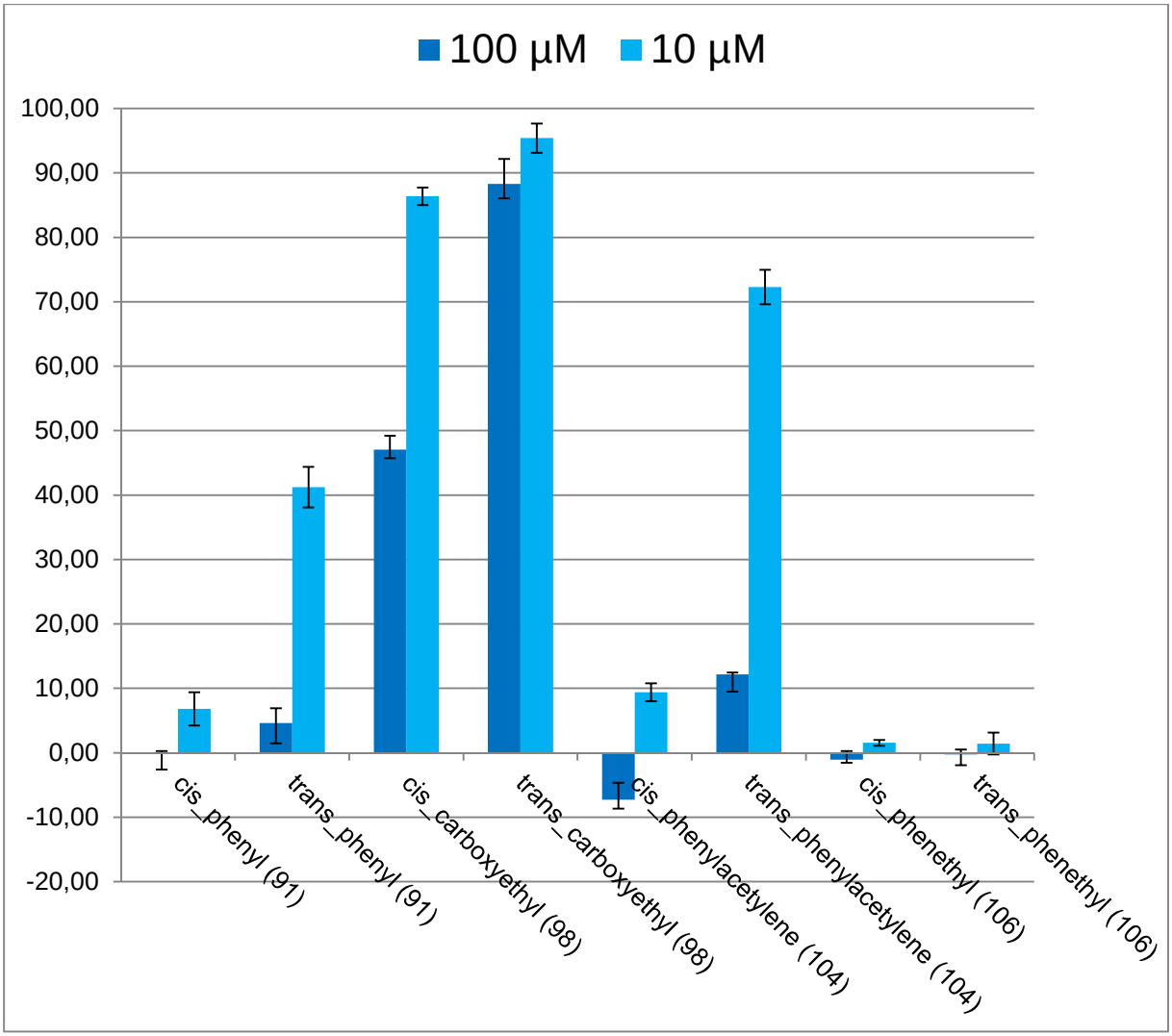
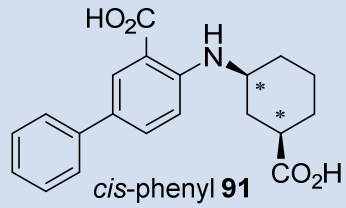
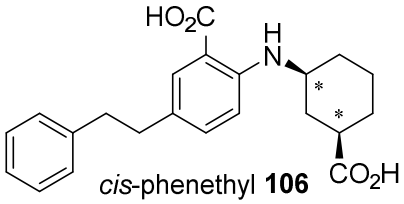


Figure 142: Residual activity of BcPhzAB (1 mM DHHA) with inhibitors substituted at the 5-position of the aromatic ring (synthesized via cross-coupling reactions).

Table 35: ITC data for inhibitors substituted at the 5-position of the aromatic ring (synthesized via cross-coupling reactions).

Ligand	K_D (µM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	-T*ΔS (kcal mol^{-1})	*N*	c
 *cis*-phenyl **91**	3.4 ± 0.2	-7.5 ± 0.0	-7.6 ± 0.0	0.2 ± 0.1	1.8 ± 0.0	29.7 ± 1.6
 *cis*-phenethyl **106**	5.6 ± 0.4 *5.6 (4.3, 7.3)*	-7.2 ± 0.0 *-7.2*	-8.0 ± 0.4 *-8.0*	0.9 ± 0.4 *0.8*	1.7 ± 0.0 *1.7*	17.9 ± 1.2 *17.8*

4.2.4.3 Biological evaluation of the alicyclic ring-size altered inhibitors

Next the inhibitors bearing different ring sizes of the alicyclic ring were tested. It was found that the 5-ring stereoisomers of **115** did not have a very large inhibitory effect on the residual activity of BcPhzAB (Figure 143). With 10 µM inhibitor 76-80% residual activity was measured and with 100 µM still 34-38%. The 6-ring- (**1**) and the 7-ring analogs (**119**) were much more active in the assay and already with 10 µM inhibitor concentration <10% residual activity was detected.

The ITC analysis of the inhibitors (Table 36) indicated, that the *cis*-6-ring Phenazistatin A (**1**) has the highest affinity with a K_D = 64 nM, and both the *cis*-5-ring Phenazistatin **115** (K_D = 470 nM) as well as the *cis*-7-ring Phenazistatin **119** (K_D = 450 nM) showed affinities one magnitude smaller but still in the nanomolar range.

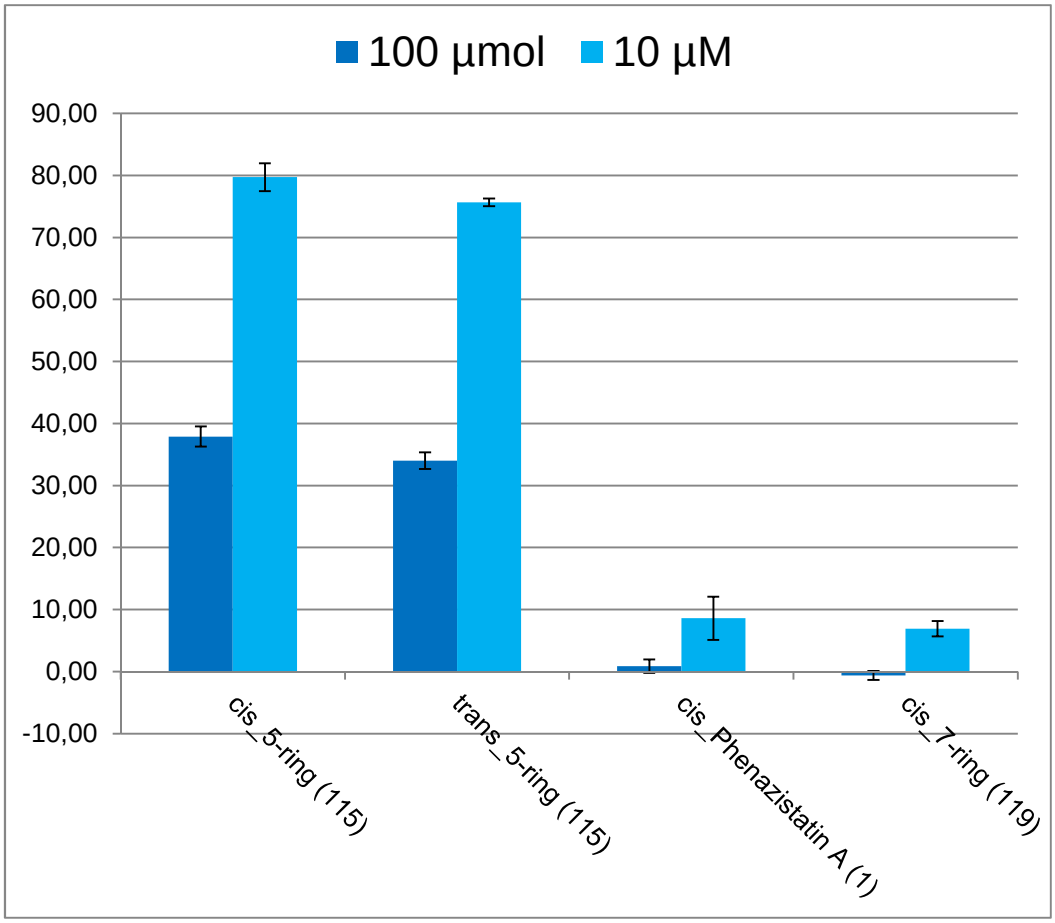
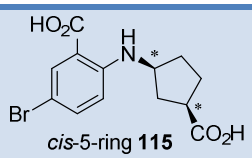
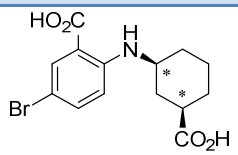
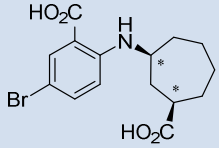
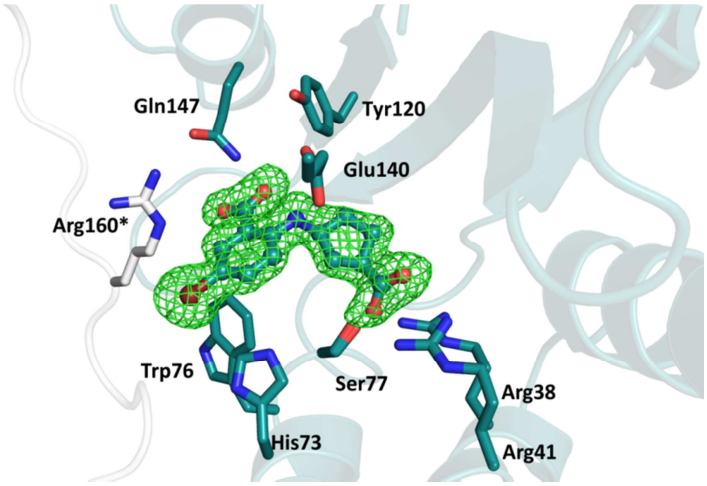


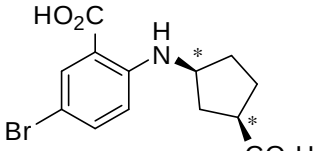
Figure 143: Residual activity of BcPhzAB (1 mM DHHA) with alicyclic ring-size altered inhibitors.

Table 36: ITC data for alicyclic ring-size altered inhibitors.

Ligand	K_D (nM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	-T*ΔS (kcal mol^{-1})	*N*	c
 *cis*-5-ring **115**	470 ± 30	-8.6 ± 0.0	-1.3 ± 0.0	-7.3 ± 0.1	3.0 ± 0.1	212.2 ± 14.1
 *cis*-Phenazistatin A **1**	64 ± 11	-9.8 ± 0.1	-10.3 ± 0.2	0.5 ± 0.2	1.8 ± 0.1	1610.6 ± 242.7
 *cis*-7-ring **119**	450 ± 30	-8.7 ± 0.0	-7.3 ± 0.1	-1.3 ± 0.1	0.9 ± 0.0	222.3 ± 16.0

Christina Diederich could obtain protein-inhibitor complexes and X-ray crystal structures for the new inhibitors (5-ring Phenazistatin **115** & 7-ring Phenazistatin **119**). The analogs exhibit the same binding mode as the parent molecule Phenazistatin A (**1**) (6-ring analog) and the structures as well as the ITC curves are shown in Figure 144 (5-ring) & Figure 145 (7-ring).





cis-5-ring **115**

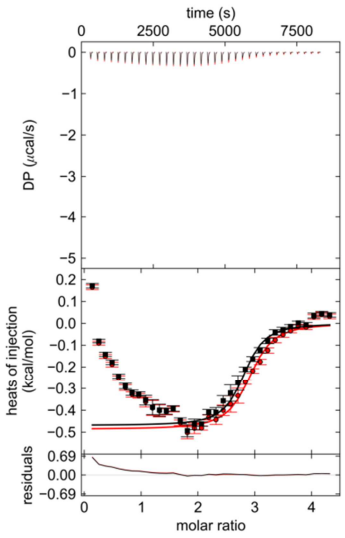
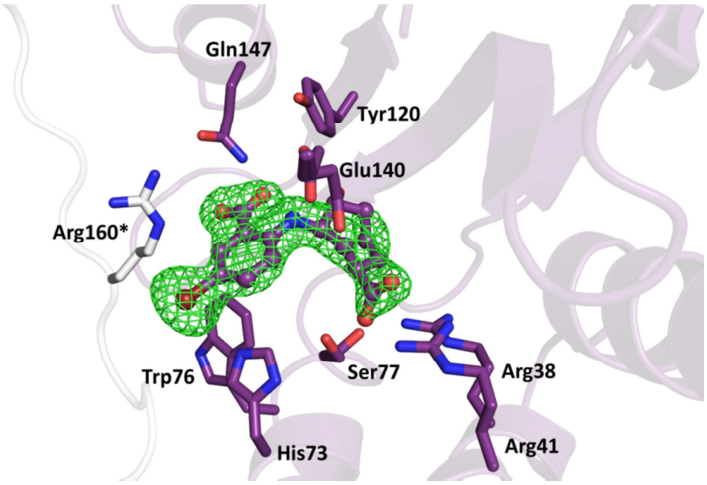
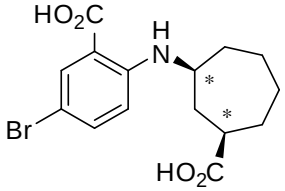


Figure 144: Crystal structure of *cis*-5-ring-Phenazstatin **115** and its ITC curve.





cis-7-ring **119**

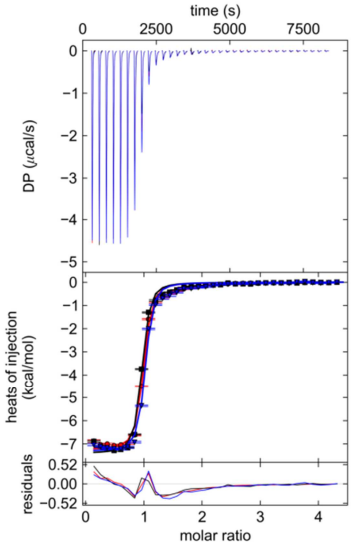


Figure 145: Crystal structure of *cis*-7-ring-Phenazstatin **119** and its ITC curve.

4.2.4.4 Biological evaluation of derivatives bearing bioisosteric replacements of the carboxyl group

As a next step the Phenazistatin derivatives bearing bioisosteric replacements of the carboxyl group were tested in the residual activity assay and ITC. The results are summarized in Figure 146 (residual activity) and Table 37 (ITC).

The 6-ring Phenazistatin derivatives bearing a 1*H*-tetrazole at the aromatic ring (*cis*-**132**, *cis*-**131**, *trans*-**131**) were the most promising amongst the ones tested. When both isomers of **131** were applied in a 10 µM concentration, residual activities of BcPhzAB of <40% were detected with an improvement to 6-7% at 100 µM (Figure 146). ITC measurement (Table 37) indicated a good affinity for the *cis*-isomer **131** (K_D = 400 nM) and in contrast a considerably weaker one for the *trans*-isomer **131** (K_D = 5.1 µM). It could be shown how important the presence of a second free carboxyl (or bioisosteric) group is for the inhibitory effect, since the (protected) methylester precursor **132** of the inhibitor **131** showed a much lower residual activity than the fully deprotected derivative **132** (Figure 146). The ITC measurement confirmed this observation (K_D = 17.4 µM).

The methyl ester protected 5-ring derivatives (**135** & **137**) bearing 1*H*-tetrazole replacements of the carboxyl groups were found to be inactive in the in vitro assay (Figure 146). This is not surprising considering the methyl-protection (prodrug) and the low activity of the parent 5-ring-Phenazistatins *cis*-**115** & *trans*-**115** (vide supra, Figure 143, 30-40% residual activity at 100 µM). When both carboxyl groups of the *cis*-5-ring-Phenazistatin **115** were replaced by 1*H*-tetrazoles, the bis-1*H*-tetrazole **134** was inactive towards the inhibition of BcPhzAB (~95% residual activity at 10 µM & 100 µM). In this case the replacement by two 1*H*-tetrazole groups rendered the substance less active when compared to the parent compound **115** (vide supra, Figure 143).

The prodrug of the phosphonic acid derivative **143** was found to be inactive in the in vitro assay (Figure 146).

When both carboxy groups of Phenazistatin A **1** were replaced by the sterically demanding bioisosteric methylsulfonamides to give the di-methylsulfonamide derivative **153**, no inhibitory effect was detected in the residual activity assay (~97%, Figure 146).

When the aromatic carboxyl group was replaced to give the oxadiazolone derivative **159**, both *cis*- and *trans*-isomer showed a moderate activity in the in vitro assay (Figure 146) and residual activities of 46% and 52% at 100 µM were measured, respectively. This weak binding of the isomers of **159** was confirmed in the ITC measurement (*cis*: K_D = 16.9 µM, *trans*: K_D = 13.0 µM). For the *trans*-isomer **159** a crystal structure could be obtained which

indicated the general binding mode of Phenazistatin A (**1**) with the considerably bigger bioisosteric replacement of the carboxyl group (Figure 147).

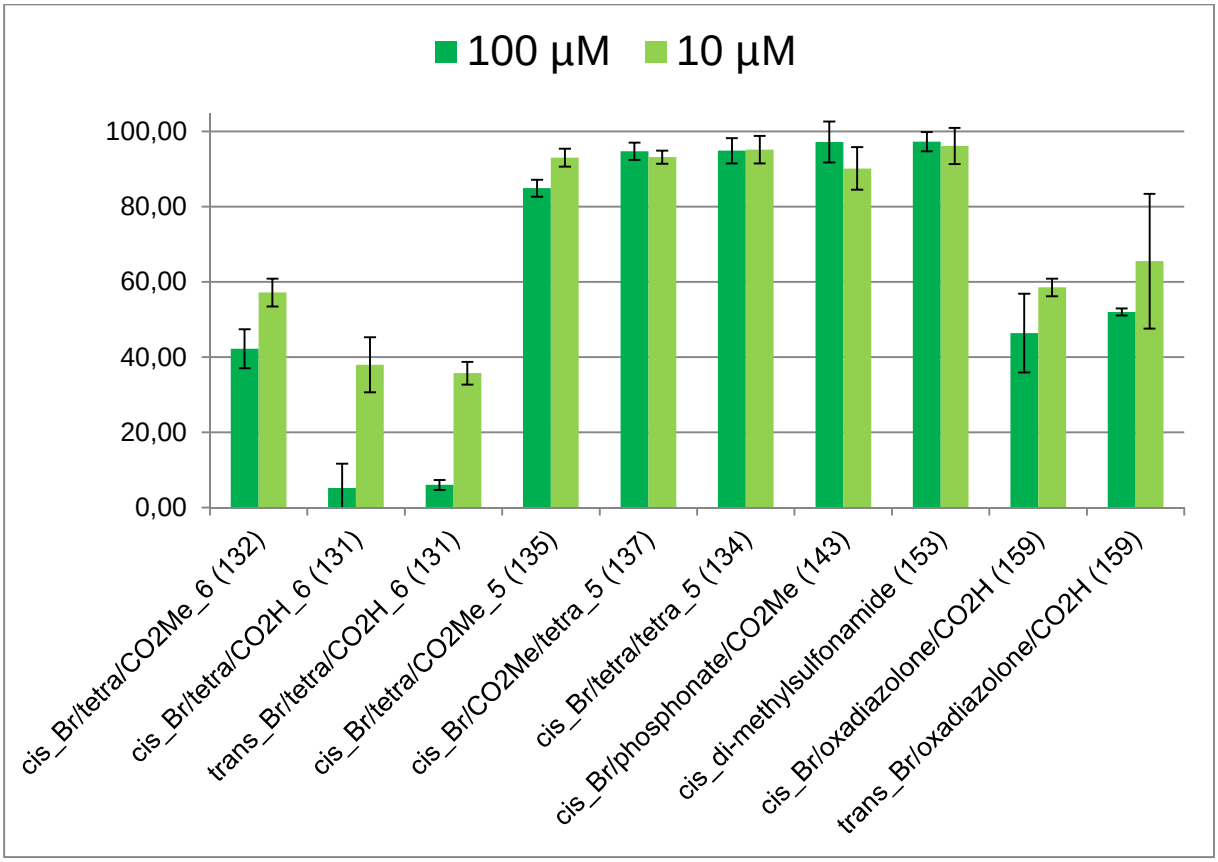
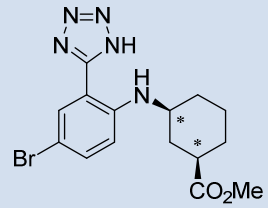
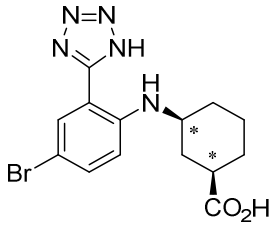
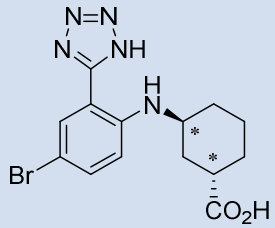
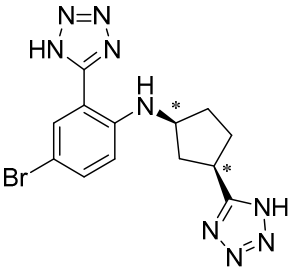
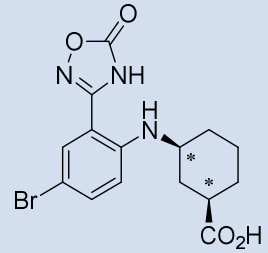
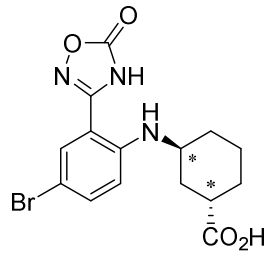


Figure 146: Residual activity of BcPhzAB (1 mM DHHA) with inhibitors bearing bioisosteric replacements of the carboxyl group.

Table 37: ITC data for inhibitors bearing bioisosteric replacements of the carboxyl group.

Ligand	K_D (µM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	-T*ΔS (kcal mol^{-1})	*N*	c
 *cis*-Br/tetra/CO_2Me **132**	17.4 ± 1.0	-6.5 ± 0.0	-4.1 ± 0.1	-2.4 ± 0.0	1.2 ± 0.1	5.8 ± 0.3
 *cis*-Br/tetra/CO_2H **131**	4.0 ± 0.6 • 10^{-1}	-8.7 ± 0.1	1.3 ± 0.0	-10.0 ± 0.1	1.0 ± 0.0	255.8 ± 39.0
 *trans*-Br/tetra/CO_2H **131**	5.1 ± 0.4	-7.2 ± 0.0	4.3 ± 0.1	-11.5 ± 0.1	1.3 ± 0.0	19.7 ± 1.5
 *cis*_Br/tetra/tetra_5 **134**	81.2 ± 12.8	-5.6 ± 0.1	2.6 ± 0.2	-8.2 ± 0.1	1.6 ± 0 .1	1.3 ± 0.2
 *cis*-oxadiazolone **159**	16.9 ± 1.8	-6.5 ± 0.1	3.3 ± 0.2	-9.8 ± 0.3	2.4 ± 0.1	6.0 ± 0.7
 *trans*-oxadiazolone **159**	13.0 ± 0.1	-6.7 ± 0.0	5.0 ± 0.0	-11.7 ± 0.0	1.9 ± 0 .2	7.7 ± 0.1

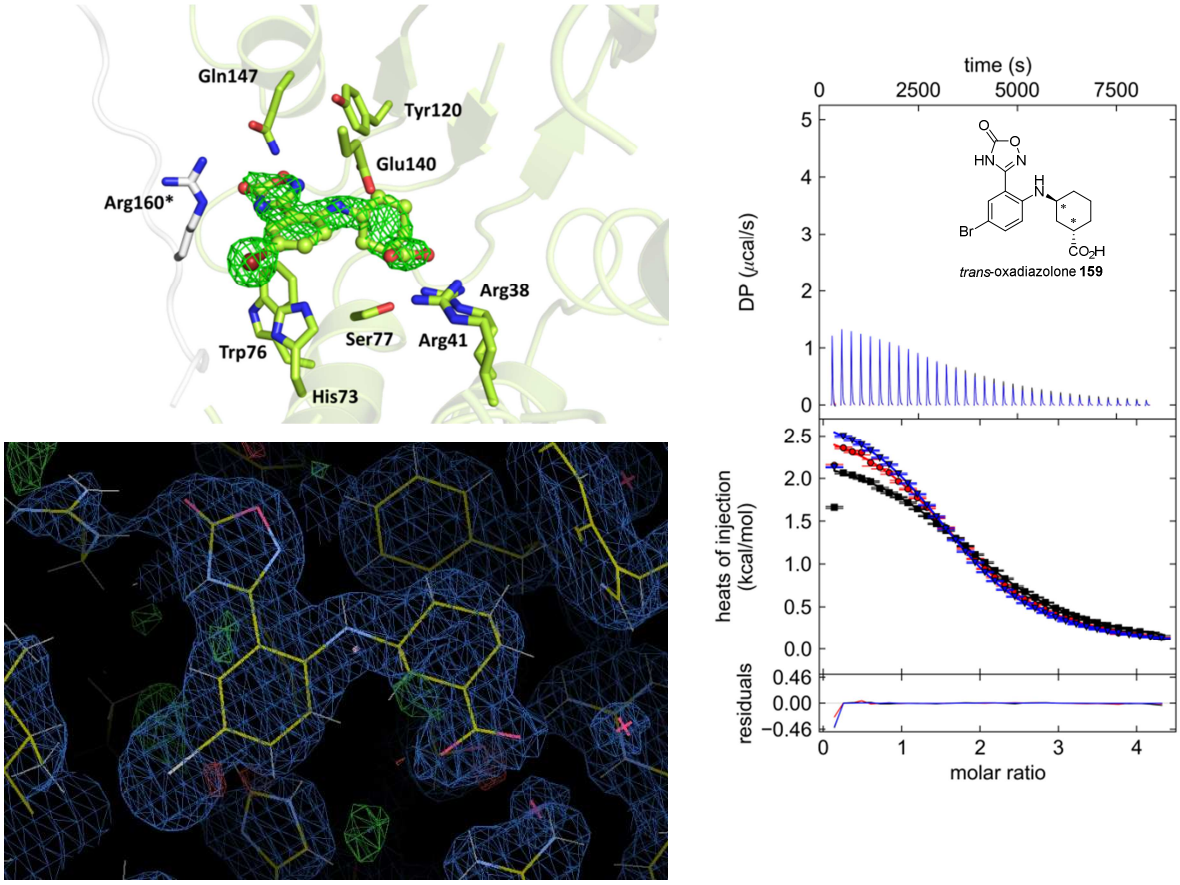


Figure 147: Crystal structure of *trans*-oxadiazolone-Phenazistatin **159** and its ITC curve.

4.2.4.5 Biological evaluation of produg esters of Phenazistatin A

The prodrug esters are currently being investigated for their capability of permeating artificial bacterial membranes. These studies are being conducted by Florian Gräf under the supervision of Prof. Claus-Michael Lehr at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) in Germany.

In order to test the activity of the prodrugs as such on BcPhzAB we carried out the in vitro assay as for the above mentioned compounds (Figure 148). We did not find any activity of the tested prodrug esters of the isomers of the 5-ring Phenazistatin **118**, the 6-ring Phenazistatin **7** or the pivaloyloxy di-esters **170**. These results are not surprising and future studies will show if the prodrug ester functionalities can enable the active Phenazistatin derivatives (free carboxylic acid groups) to enter the bacterial cells.

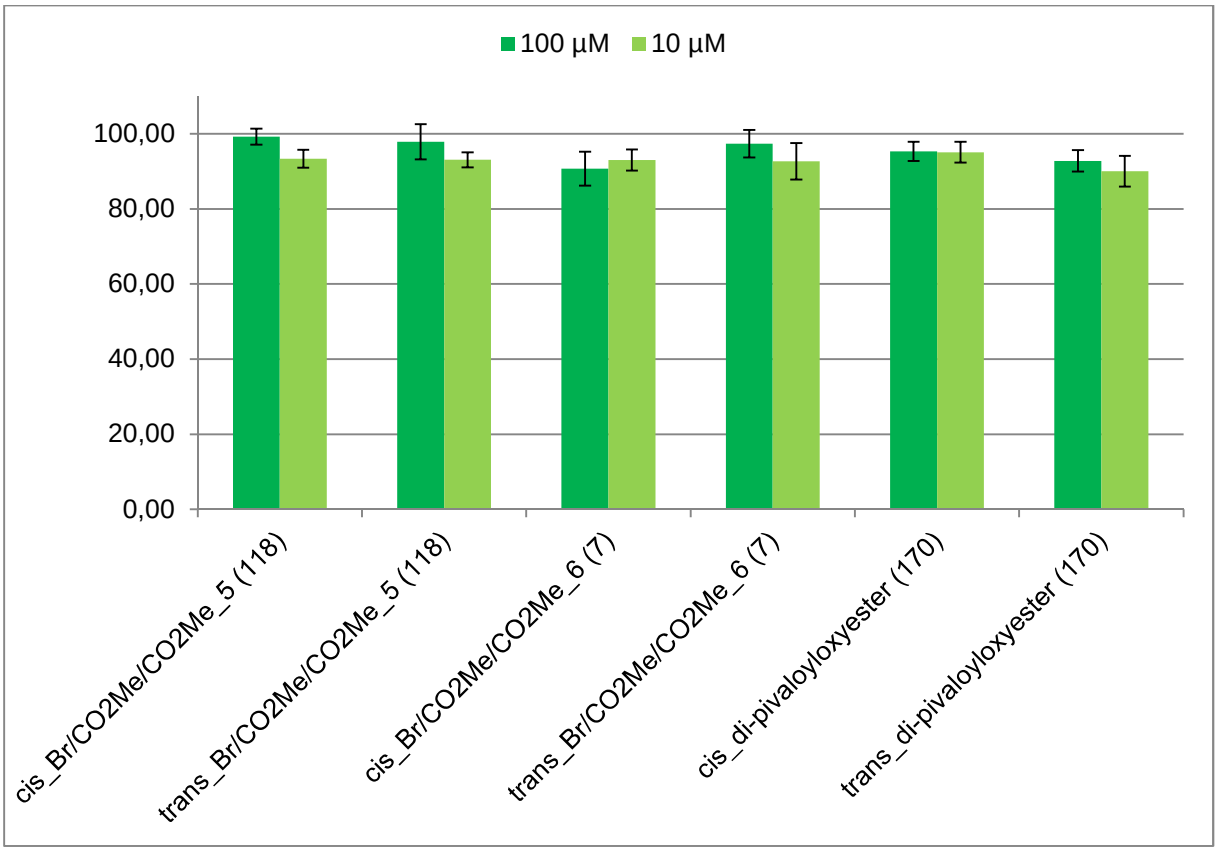


Figure 148: Residual activity of BcPhzAB (1 mM DHHA) with prodrug Phenazistatin derivatives.

We furthermore tested all the other prodrug compounds and intermediates that originated from the synthesis routes whether they showed activity in the residual activity assay (Figure 149). Most of the compounds tested were di-esters followed by nitrile intermediates. None of the compounds tested showed a significant activity in the in vitro assay. However, these samples are expected to show no effect with in vitro tests (activity assay, ITC) because they require enzymatic or chemical activation. The prodrugs are currently being investigated for their in vivo activity by Prof. Rolf W. Hartmann at the HIPS in Saarbrücken.

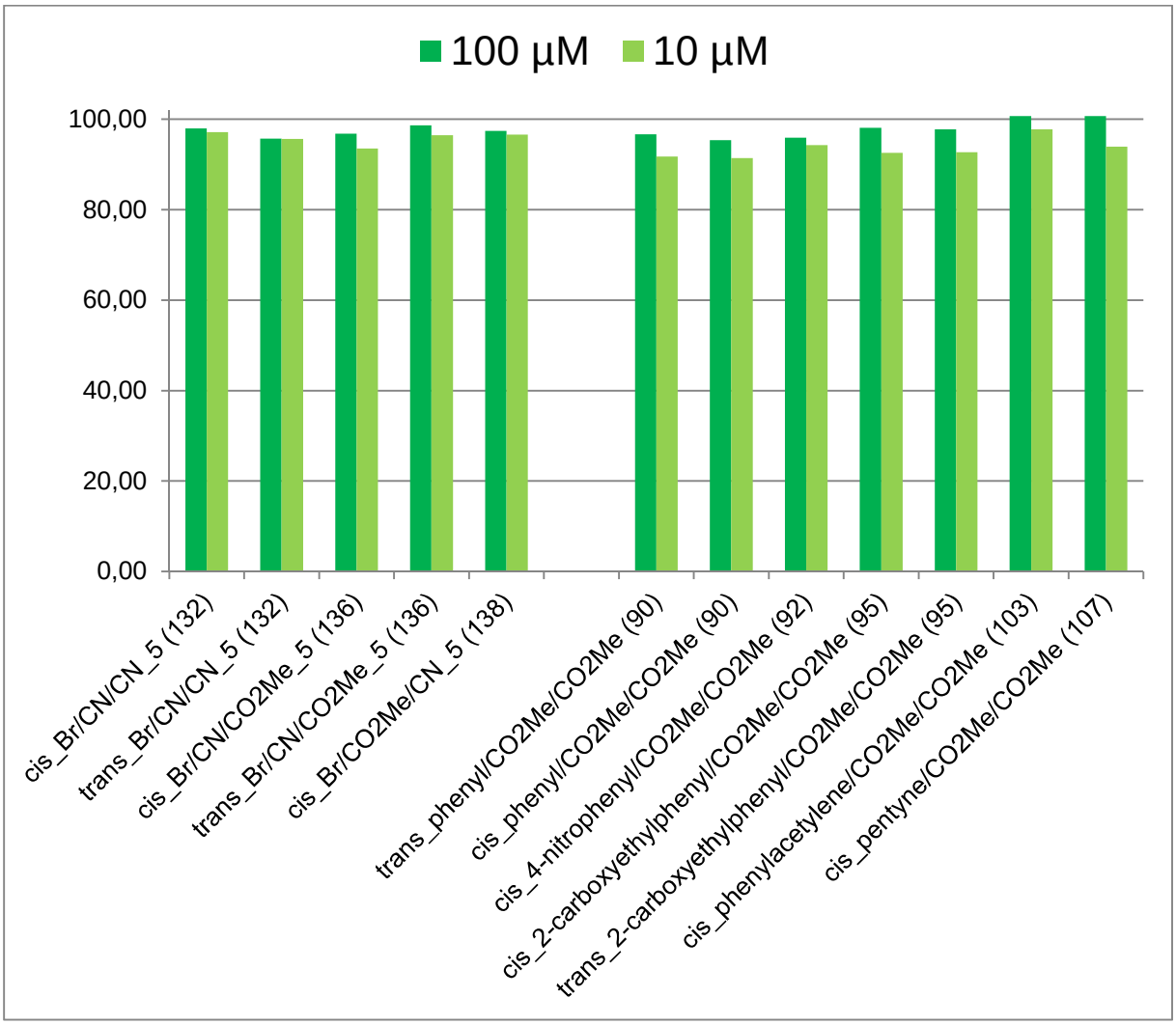


Figure 149: Residual activity of BcPhzAB (1 mM DHHA) with other prodrugs and intermediates of Phenazistatin synthesis.

4.2.4.6 Biological evaluation of Maverick derivatives as PhzA/B inhibitors

The Maverick derivatives were synthesized mainly in order to evaluate their binding mode and to investigate whether the simultaneous binding of two enantiomers[94] of the Br-Maverick (**2**) was a unicorn, or if there were other closely related structures which show the same fascinating binding behavior. We already knew from our previous studies[94] that the Maverick inhibitors are much weaker inhibitors than the corresponding Phenazistatin derivatives, however they were still tested by the in vitro assay and ITC.

Figure 151 shows the obtained residual activities for the Maverick inhibitors that were synthesized. It could be shown that the H-Maverick **177**, the Cl-Maverick **181** as well as the different enantiomers of the Br-Maverick (**2**) show very little to no activity in the residual activity assay. For the H-Maverick **177** an ITC measurement was conducted (K_D = 22.9 µM) corresponding to a very weak inhibitor (Table 38).

The Me-Maverick **191** showed some activity and at 100 µM the residual activity of BcPhzAB was around 84%. ITC revealed higher affinity than the H-Maverick **177** with K_D = 11.3 µM. A nice crystal structure could be obtained with the Me-Maverick **191** (Figure 152). The MeO-Maverick was found to give a similar level of residual activity, but ITC (Table 38) indicated a much weaker binding than the other two Maverick derivatives (K_D = 36.4 µM).

When the structure of the Br-Maverick (**2**) was changed so that the secondary amine in the piperidine ring was substituted with a hydroxyethyl group to give the hydroxyethyl-Maverick **198**, the affinity was found to increase (K_D = 2.4 µM, Table 38) while the residual activity of BcPhzAB remained around 80% for both 10 µM and 100 µM (Figure 151).

When the by-product from the synthesis of the Cl-Maverick **181**, the double-Cl-Maverick **182** was tested similar residual activities as for the previously described Maverick derivatives was found (Figure 151) and the ITC indicated a weak affinity (K_D = 27.7 µM, Table 38). However, when the Cl-substituent at the aromatic ring was exchanged against bromine and the double-Br-Maverick **187** was obtained, the K_D-value improved from 27.7 µM to 6.1 µM (Table 38). The residual activity was found to be the lowest of all Maverick derivatives tested with a value of 58% (100 µM, Figure 151). It is interesting, that also for the double-halogen-Maverick products the introduction of a bromine substituent (in double-Br-Maverick **187**) increased the affinity, as it was the case for Phenazistatin A (**1**) where the Br-derivative was long time the most active one.

Further information about the binding characteristics of all the Maverick derivatives can be found in the PhD thesis of Christina Diederich.

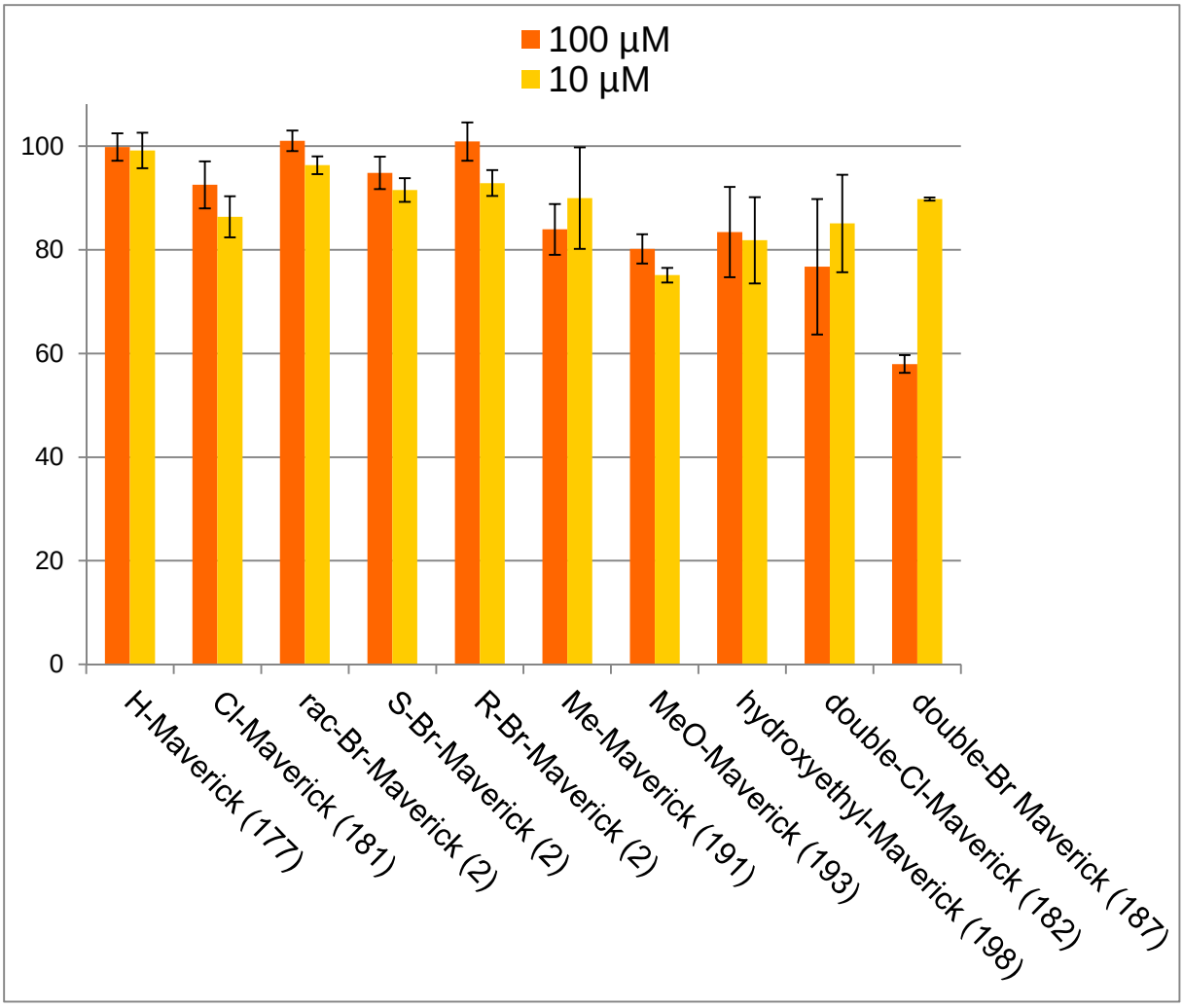
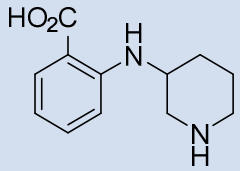
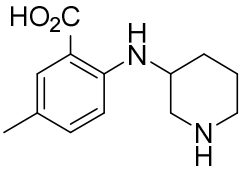
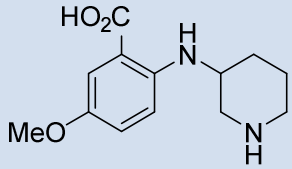
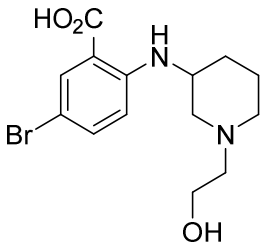
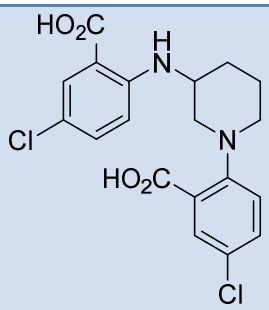
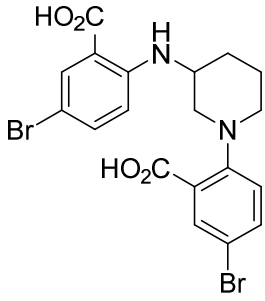
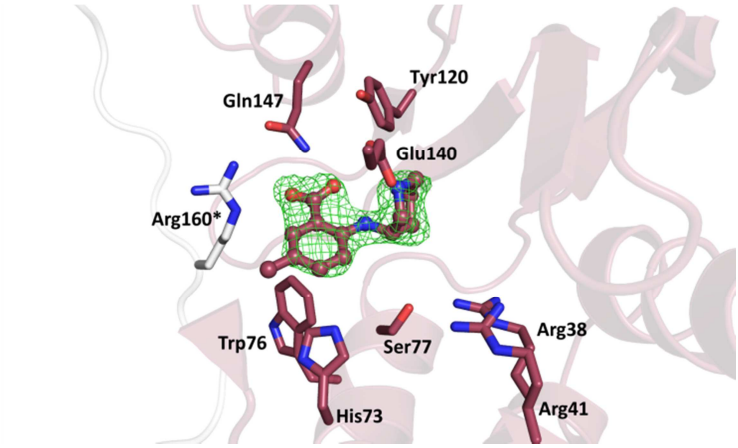
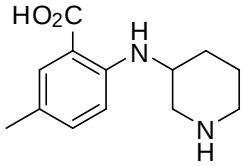


Figure 151: Residual activities of BcPhzAB with 100 µM and 10 µM Maverick inhibitors.

Table 38: ITC data for Maverick derivatives as PhzA/B inhibitors.

Ligand	K_D (μM)	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	-T*ΔS (kcal mol^{-1})	*N*	c
 H-Maverick **177**	22.9 ± 0.5	-6.3 ± 0.0	-8.9 ± 0.1	2.6 ± 0.2	3.3 ± 0.1	4.4 ± 0.1
 Me-Maverick **191**	11.3 ± 0.1	-6.7 ± 0.0	-10.8 ± 0.1	4.0 ± 0.1	2.6 ± 0 .1	8.8 ± 0.1
 MeO-Maverick **193**	36.4 ± 9.4	-6.1 ± 0.1	-9.5 ± 0.6	3.5 ± 0.8	3.7 ± 0.9	2.9 ± 0.7
 hydroxyethyl-Maverick **198**	2.4 ± 0.5	-7.7 ± 0.1	-6.7 ± 0.9	-1.0 ± 1.0	4.2 ± 0. 5	43.1 ± 8.0
 double-Cl-Maverick **182**	27.7 ± 0.7	-6.2 ± 0.0	8.1 ± 0.2	-14.3 ± 0.2	1.8 ± 0.0	3.6 ± 0.1
 double-Br-Maverick **187**	6.1 ± 0.2	-7.1 ± 0.0	4.7 ± 0.1	-11.9 ± 0.1	1.7 ± 0. 1	16.5 ± 0.5





Me-Maverick **191**

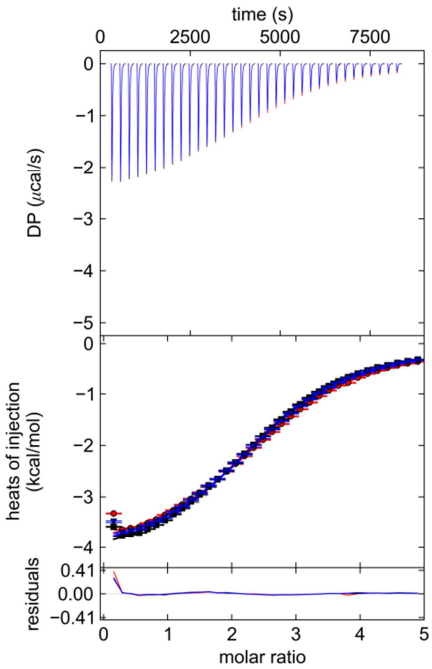


Figure 152: Crystal structure of Me-Maverick **191** and its ITC curve.

K_D = 11.3 ± 0.1 µM

4.3 Total synthesis of Tilivalline

4.3.1 Introduction and relevance of Tilivalline

Recently, the group of Ellen Zechner at the Institute of Molecular Biosciences at the University of Graz identified a biosynthetic gene cluster in *Klebsiella oxytoca* which was found responsible for the production of a cytotoxin.[250] This low-molecular weight cytotoxin was identified by Jana Rentner in the group of Rolf Breinbauer at the Institute of Organic Chemistry at Graz University of Technology to be Tilivalline (**3**), belonging to the class of pyrrolobenzodiazepines (PBDs).[251] Tilivalline **3** is associated with antibiotic-associated hemorrhagic colitis (AAHC), a disease associated with enterobacterial overgrowth, which is triggered by antibiotic treatment. This bacterial overgrowth is dominated by *Klebsiella oxytoca*, which is a resident of the gut (2-10%) of healthy individuals.[252–254] Tilivalline is the only PBD produced by gram-negative bacteria (*Klebsiella*)[255] and usually gram-positive Actinomycetales produce this class of cytostatics.[256,257]

In order to supply the studies of the group of Ellen Zechner with sufficient amounts of Tilivalline (**3**), bacterial cultures were grown and the cytotoxin was isolated by *n*-butanol extraction followed by steps of preparative RP-HPLC. The amounts of Tilivalline (**3**) produced were found to vary strongly between different batches and in the best case 41 mg **3** were isolated from 1 L bacterial supernatant. When the fermentation was repeated, only minute amounts of **3** could be isolated, endangering the supply for the ongoing studies. We therefore set out to synthesize Tilivalline (**3**) according to a procedure by Nagasaka et al.,[95] which had to be optimized first because it was found to be not easily reproduced.

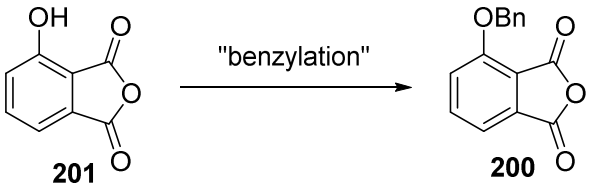
4.3.2 Total synthesis of Tilivalline

4.3.2.1 Synthesis of the 4-(benzyloxy)isobenzofuran-1,3-dione

The building block 4-(benzyloxy)isobenzofuran-1,3-dione (**200**) was the central intermediate towards Tilivalline (**3**). In the original total synthesis by Nagasaka et al.[95] the building block **200** was prepared from commercially available 4-hydroxyisobenzofuran-1,3-dione (**201**) by benzylation with BnCl in water.[258] We decided to purchase 1 g starting material **201** (226 €, Sigma-Aldrich catalog, July 2016) for initial studies, however the price was not very encouraging to make this the starting point of our synthesis. When we repeated the synthesis of the benzyl protected anhydride **200** as described in the paper[95,259] (Table 39, Entry 1), we obtained full conversion of the starting material in the first step after 17 h and telescoped the crude product to the next step without further purification. Neat heating to 200 °C for 85 min, recrystallization and ^{1}H-NMR however indicated that no benzyl group was attached to the

obtained product. Presumably the anhydride was hydrolyzed and the di-acid was obtained. We then tried the slightly modified conditions by Nakamura et al.[259] (Entry 2), which contained NaI in order to facilitate the nucleophilic attack. After 3 d at 110 °C in the closed Schlenk tube we detected full conversion of the starting material (^{1}H-NMR) and the mixture was acidified and worked up according to a standard procedure. The crude material was dissolved in CH_2Cl_2 and 5 eq Ac_2O were added as a dehydrating agent to enable the anhydride formation. After 41 h full conversion was detected by TLC, the mixture was filtrated through a pad of silica gel and the solvents were removed in vacuum to give the pure product **200** as a light-yellow solid in 52% yield.

Table 39: Synthesis of the 4-(benzyloxy)isobenzofuran-1,3-dione (**200**) building block.



201 **200**

Entry	Conditions	Comments
1[95,259]	1) 1.1 eq BnCl, 5 eq KOH, H_2O, 120 °C, 17 h 2) neat, 200 °C, 85 min	no product isolated
2[259]	1) 1.23 BnCl, 5 eq KOH, 0.1 eq NaI, H_2O, 110 °C, 3 d 2) 5 eq Ac_2O, CH_2Cl_2, N_2, 60 °C, 41 h	52% isolated (recrystallization)

The low 52% yield of **200** from the expensive commercially available starting material in the first step of the total synthesis motivated us to find a procedure with which we could produce a large quantity of the desired 4-(benzyloxy)isobenzofuran-1,3-dione (**200**) cheaply. Literature search led us to conclude that the formation of the desired backbone would be most facile via a Diels-Alder reaction from diethyl acetylenedicarboxylate and furan furnishing the bicycle **202**. Acid catalyzed ring-opening/aromatization would lead to phenol **203,** which could be simply converted to the desired anhydride **200** using standard chemistry (Figure 153).

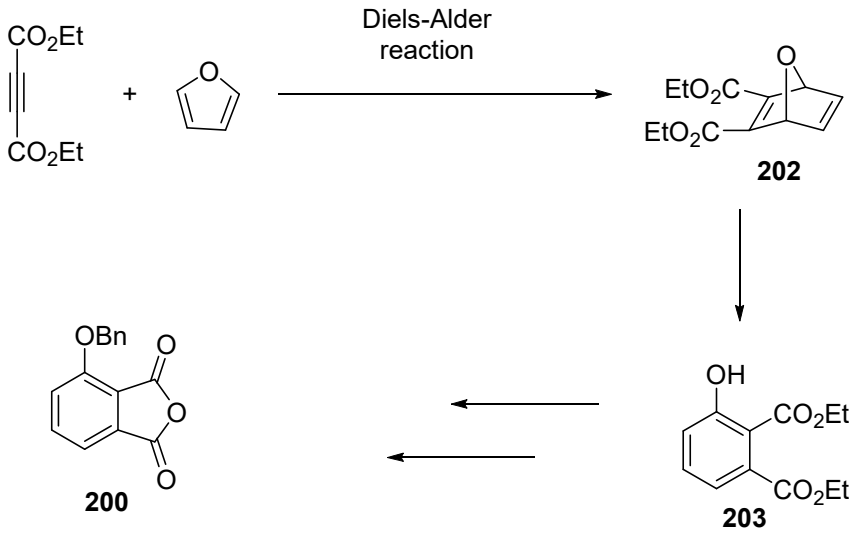
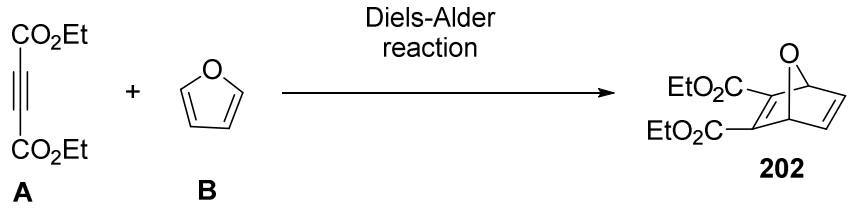


Figure 153: Strategy towards the central building block **200**.

We then tried several literature protocols for the formation of the desired product **202** (Table 40) and found that the DIELS-ALDER reaction proceeded best when the conditions by Soret et al.[260] were used, where the diethyl acetylenedicarboxylate and furan were heated in toluene to 80 °C (Table 40, Entry 2). The product **202** could be isolated by simple solvent evaporation and was NMR pure without further purification. In the other conditions the reaction either did not proceed to completion or by-products were formed.

Table 40: Screening of the DIELS-ALDER reaction to produce the bicycle **202**.



Entry	Reagents / ratio: **A** / **B**	Solvent / Temperature	Time / conversion	Comments
1[261]	0.3 eq $AlCl_3$ 1 eq / 1 eq	CH_2Cl_2 60 °C	5 d incomplete	traces of product
2[260]	- 1 eq /1.2 eq	Toluene 80 °C	5 d 100%	clean
3[262]	- 1 eq / 1.2 eq	Et_2O 60 °C	5 d incomplete	clean
4[263]	- 1.2 eq /1 eq	1,4-dioxane 80 °C	5 d incomplete	by-products

While performing the reaction on a preparative scale it was found that a slight excess of furan was beneficial for the reaction to proceed until completion and remaining furan was easily removed in vacuum from the mixture. When the reaction was run on a 5 g scale, full conversion of diethyl acetylenedicarboxylate was detected after 6 d at 80 °C and product **202** was purified via column chromatography (to remove minor impurities) to give **202** in 65% yield (Figure 154). In a similar 15 g batch the product **202** was isolated in 83% yield after column chromatography (3 d reaction time, 80 °C).

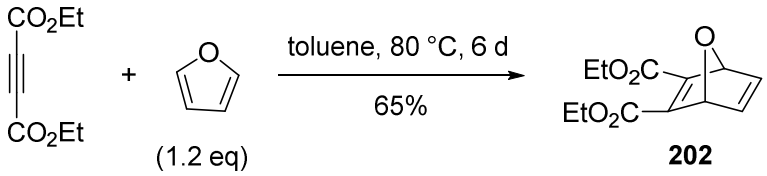


Figure 154: Synthesis of bicycle **202** via a DIELS-ALDER-reaction.

The LEWIS-acid catalyzed ring-opening was performed following a procedure by Ram et al.,[264] where the starting material **202** was treated with pure $BF_3 \cdot Et_2O$ and heated to 60 °C until full conversion of **202** was detected after 6 d (Figure 155). The ^{1}H-NMR of the crude product **203** after workup indicated high purity. The product **203** was isolated in 81% yield. It was decided to telescope the brown liquid to the next step and to perform the purification at a subsequent step.

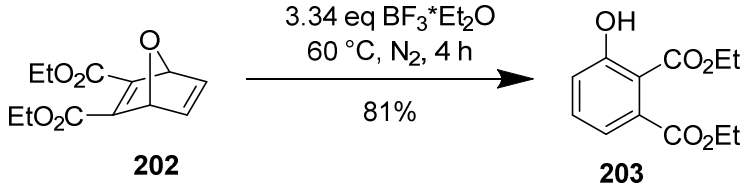


Figure 155: Synthesis of phenol **203** acid-catalyzed ring-opening/aromatization.

In a series of test reactions it was found that the benzylation was best carried out with phenol **203**, since the least amount of alkylating agent was necessary and unproductive alkylative esterification was prevented (in case the free di-acid was used) (Figure 156). The *O*-alkylation proceeded smoothly to full conversion of the starting material **203** after 22 h at 75 °C, the reaction was worked up and a brown liquid was obtained. Considering the solvent volume necessary to purify the product via column chromatography at this batch size, we explored the subsequent reaction steps and found that the desired anhydride **200** was crystalline, suggesting it could be purified via recrystallization at that stage. Hence we decided to telescope the crude product **204** to the next step. Saponification with 10 eq $LiOH \cdot H_2O$ at RT only led to selective mono-hydrolysis of one ester moiety (which one was not elucidated) and another portion of 10 eq KOH followed by heating to 50 °C for 15 h was necessary to obtain full hydrolysis. The mixture was acidified, extracted and product **205** was

attempted to be recrystallized at this stage. However, it turned out that the di-acid **205** showed poor crystallization behavior and only sticky solids or oils were obtained when recrystallizations were attempted. The crude di-acid **205** was telescoped as such to the dehydratization step. The di-acid **205** was suspended in a small amount of dichloromethane with 5 eq of Ac_2O and heated to 65 °C. It was observed that the brown precipitate dissolved after 15 min heating and a brown-black solution was obtained. After 19 h at 65 °C full conversion of the starting material **205** was detected by ^{1}H-NMR and the mixture was allowed to slowly cool to RT. To our delight a large fraction of product **200** precipitated directly from the reaction mixture in the shape of beige crystals and a second fraction of **200** could be isolated by dissolving in the minimum amount of EtOAc and precipitation with *n*-pentane. A total of 10.2 g (72%) of the product **200** could be isolated from this single batch starting from 25 g diethyl acetylenedicarboxylate, which was bought for 46 € (TCI Chemicals, July 2016). We were very pleased that we have found a cheap alternative to buying the 4-hydroxyisobenzofuran-1,3-dione (**201**) (1 g costs 226 € at Sigma-Aldrich catalog, July 2016) which would still need a low-yielding Bn-protection to give the desired Bn-protected anhydride **200**.

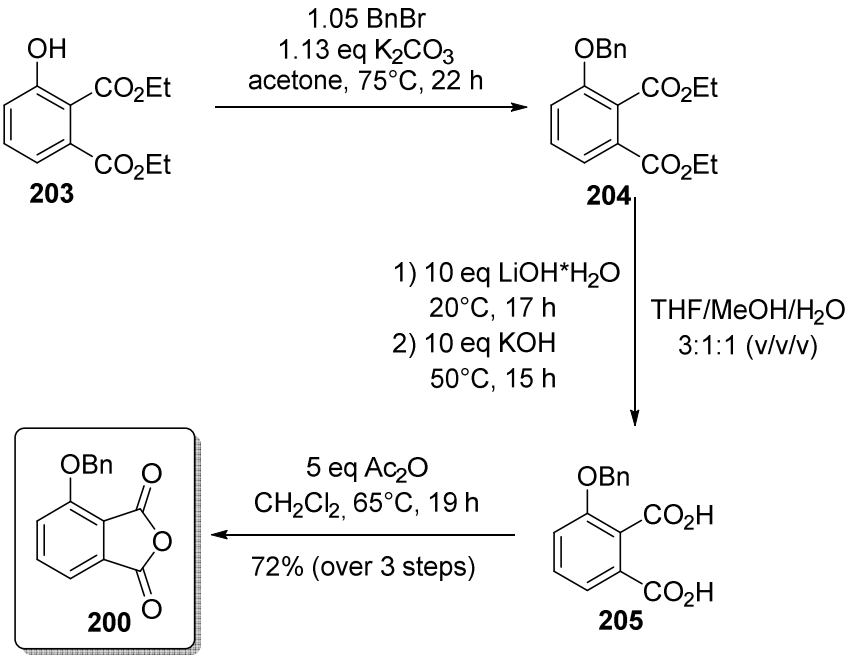
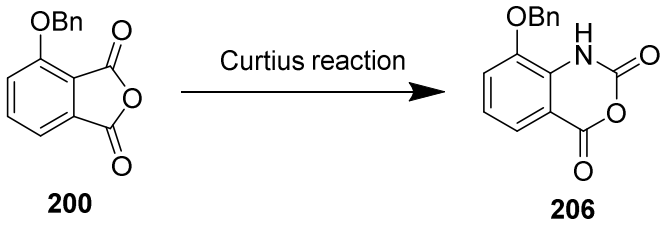


Figure 156: Synthesis of the central building block **200** from phenol **203** via benzylation/saponification/anhydride formation.

4.3.2.2 CURTIUS-reaction with 4-(benzyloxy)isobenzofuran-1,3-dione

The key step in the overall Tilivalline synthesis is the CURTIUS-reaction from the Bn-protected anhydride **200** to introduce selectively the nitrogen in the position adjacent to the protected phenol (Figure 157).

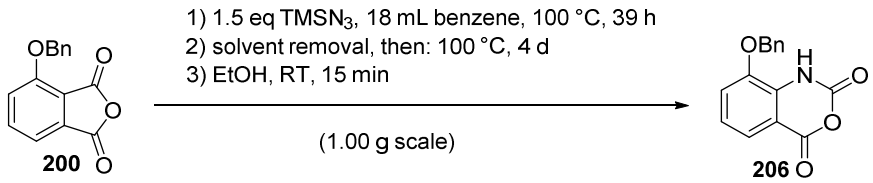


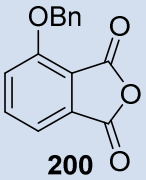
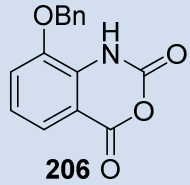
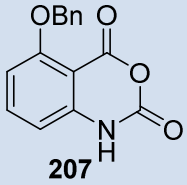
200 **206**

Figure 157: Synthesis of isatoic anhydride **206** from anhydride **200** via CURTIUS-reaction.

It turned out that this reaction was not very reproducible and the published yields of the isatoic anhydride **206** using the described reaction times and temperatures could not be repeated. For safety reasons we only performed this reaction on a 1 g scale of **200** in a 100 mL Schlenk flask behind a blast shield. In order to scale this reaction up, parallel reactions were set up and treated with the identical portions of reagents in the same heating bath. Substrate **200** was charged into the Schlenk flask, dried in vacuum, the solvent and $TMSN_3$ were added and the reaction was sealed and placed into a preheated (100 °C) oil bath. After 39 h (no ^{1}H-NMR control was done here, the reaction proceeds only to completion in the next step) the excess of $TMSN_3$ and benzene was removed with a cooling trap at RT until a dark-yellow oil remained and this oil was subjected to further neat heating to 100 °C (bath) for 4 d. Cooling to RT and stirring the reaction mixture with EtOH until all the solid parts dissolved gave the product **206**, which was analyzed by ^{1}H-NMR. The benzylic CH_2-shift was a good indicator which product had formed. To our dismay even when performing identical parallel reaction completely different outcomes were observed, in respect to conversion as well as selectivity. The results from one scale-up campaign with five identical parallel reactions are shown in Table 41. The conversions between those flasks ranged between 24% and 69%, and up to 20% of the undesired regioisomer **207** was formed. The cause for the different outcome in these reactions is still unclear.

Table 41: Scale-up of the CURTIUS-reaction with 4-(benzyloxy)isobenzofuran-1,3-dione (**200**).

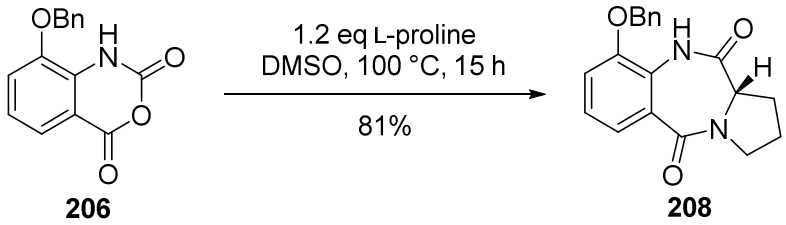


Parallel reaction vessel	 **200** [%]	 **206** [%]	 **207** [%]
Schlenk 1	31	49	20
Schlenk 2	40	45	15
Schlenk 3	69	31	-
Schlenk 4	72	28	-
Schlenk 5	76	24	-

Luckily crude products that still contained substrate could be subjected to an additional CURTIUS-reaction without significant product decomposition observed via ^{1}H-NMR. In any case, the by-product **207** had to be removed by repeated recrystallization (mainly from EtOAc). The regioisomers could be easily distinguished by the ^{1}H-NMR shift of the benzylic CH_2-group. Unfortunately, no exact yield of product **206** for this reaction can be given here, but in the best case 31% of product **206** was isolated from four parallel 1 g reactions of starting material **200**. It was found that heating the neat crude mixture after the partial removal of the benzene to 110 °C (bath temperature) was beneficial to the reaction rate and product selectivity as compared to the reaction at 100 °C.

4.3.2.3 Final synthetic steps towards Tilivalline

The subsequent step of the insertion of the L-proline into the isatoic anhydride **206** was performed as described by Nagasaka.[95] Full conversion of the starting material **206** was observed after 15 h at 100 °C (TLC), the reaction mixture was poured into H_2O, the solid was collected via filtration and washed to remove the DMSO (Figure 158). We found that a hot filtration (dissolving in hot toluene and passing through a sintered glass frit) gave the product **208** in excellent purity upon solvent evaporation. This hot filtration allowed for the use of slightly impure isatoic anhydride **206**, where the by-product was not soluble in hot toluene and could be removed. It was however not tested, whether the undesired regioisomer (resulting from the wrong CURTIUS-product) might be separated at this stage. This question remains to be answered but if recrystallization conditions could be found that efficiently separate the regioisomers, a much more efficient (or at least convenient) process could be devised. Recrystallization attempts from toluene alone failed because the product **208** showed poor crystallization behavior.



206 **208**

Figure 158: Synthesis of **208** from isatoic anhydride **206**.

The next steps were performed according to the paper by Nagasaka (Figure 159).[95] The Cbz-protection of the amide **208** was conducted at RT since the proton seemed to be quite hard to abstract. In this case freshly bought LiHMDS solution seemed to be essential, since older solutions with a lower titer did somehow not result in full conversion of **208,** even when a larger excess was added. The completion of deprotection could be visibly determined by the formation of a thick, colorless suspension, which immediately turned clear again upon addition of the CbzCl. Standard workup yielded the Cbz-protected product **209** in 92% yield. The $NaBH_4$ reduction of **209** proceeded smoothly to completion within 1 h at 0 °C and solvent removal with a cooling trap (at 0 °C) followed via column chromatography gave the desired alcohol **210** in 82% yield. In one batch the reaction mixture was instead filtrated through a wet pad (THF) of silica gel to remove the borohydride followed by rotary evaporation at 40 °C bath temperature. However when the concentrated mixture was analyzed, an additional by-product had appeared, which greatly lowered the overall yield of **210** (54%). This degradation might have been caused by traces of $NaBH_4$ remaining in solution and have been triggered by the elevated temperature on the rotary evaporator. The installation of the indole on the

alcohol product **210** was as well performed by the published procedure.[95] When the procedure was used, the product **211** could be nicely isolated via column chromatography in 78% yield. It was beneficial to degas the reaction mixtures properly prior to the heating. If not performed, the color or the solution will darken to a brownish tone and lower yields might be obtained. When the reaction was degassed for 30 min by N_2-bubbling in an ultrasonic bath a light-yellow solution was obtained and good overall yields of **211** were isolated. There were some doubts about the product stability since some NMR samples of the product **211** turned pink in $CDCl_3$ solution, but no degradation products could be detected by NMR analysis and after several days at RT the same spectra were recorded. Interestingly this class of compounds, especially the latter ones, gave the best resolution and spot shape on TLC by using the quite uncommon mixture of CH_2Cl_2/cyclohexane/acetone = 2:1:1 and all other tested commonly employed eluents gave inferior results.

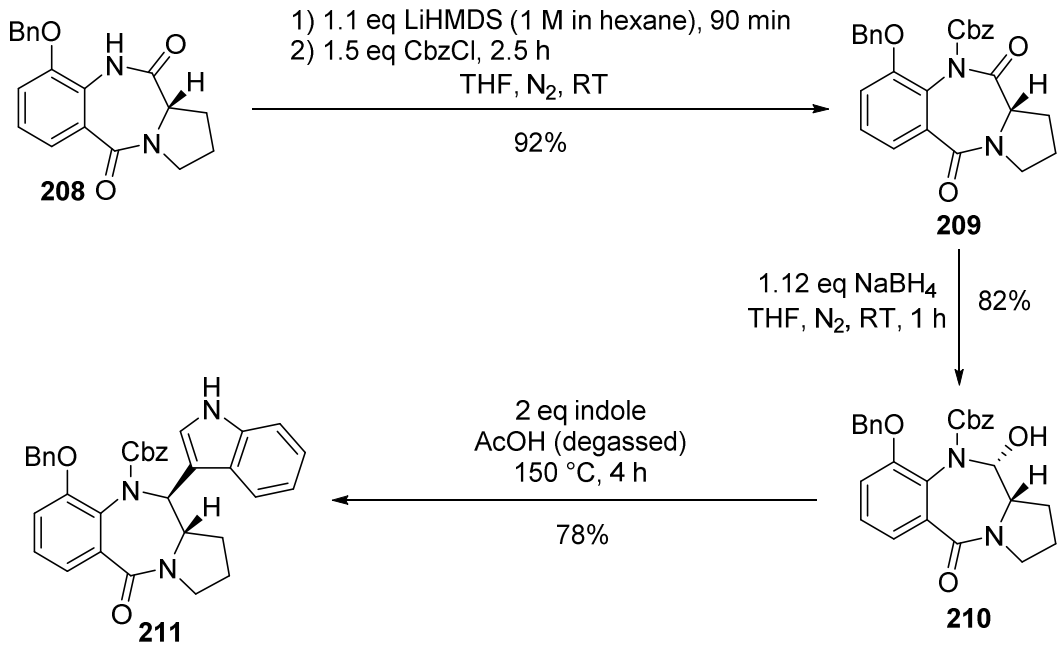


Figure 159: Synthesis of the Tilivalline precursor **211** from unprotected amide **208**.

The final global deprotection of the protected precursor **211** towards the desired Tilivalline **3** was conducted using catalytic hydrogenation with Pd/C and 1 atm H_2 (Figure 160). Full conversion of the starting material **211** was observed after 15 h and the highly polar product **3** was isolated via filtration, solvent removal and preparative RP-HPLC. On larger scale the loading of 5 wt% was not sufficient to ensure full conversion and up to 20 wt% had to be added to obtain full conversion within days. The low yield is due to the formation of some unidentified by-products. Care needs to be taken that the hydrogenation is run to completion so as not to lose product by mono-deprotection of **211** (TLC: the spot between product **3** and substrate **211**). However, there seems to be another by-product with very similar R_f-value to

Tilivalline (**3**) that can only be separated by very careful column chromatography (or RP-HPLC). If all precautionary measures are taken, then product **3** can be isolated in good yields as high as 72% (167 mg).

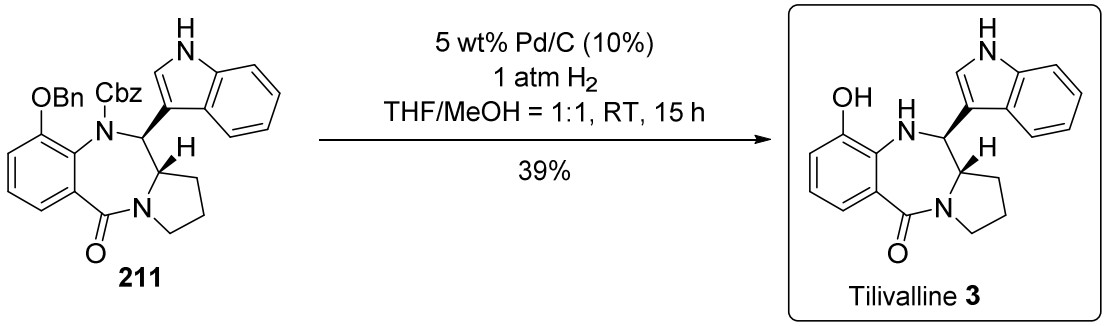


Figure 160: Synthesis of Tilivalline (**3**) via catalytic hydrogenation of the protected precursor **211**.

5 Summary and Outlook

5.1 Studies towards the reductive amination of electron-deficient anilines

In the first part of this thesis three new methods for the reductive amination of electron-deficient amines with aldehydes and ketones were developed (Figure 161).[189] The optimized reaction conditions made a series of Phenazistatin derivatives accessible which could not have been synthesized otherwise due to the low selectivity and rate of the established approach via ULLMANN-GOLDBERG amination. The new methods exhibit a much higher reactivity for electron-deficient amines than published reductive amination methods and full conversions were usually obtained within minutes to hours at temperatures as low as 0 °C. Established methods required elevated temperatures, long reaction times and/or an excess of reagents and were shown to be inferior to the new methods.

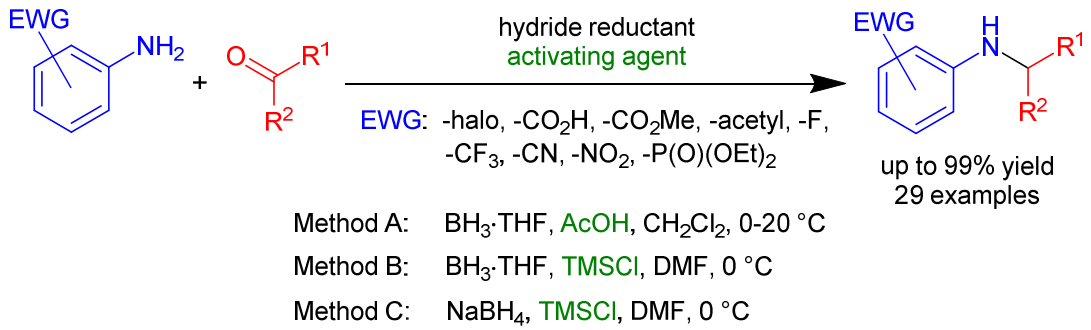


Figure 161: Reductive amination methods for the conversion of electron-deficient anilines and the substrate scope of which.[189]

The amine substrate scope of the reductive amination methods was found to be broad and a large number of functional groups were tolerated that normally would cause side reactions or that would prevent the reaction for steric reasons (Figure 162). Some of the tolerated amine substrates with method B (which makes use of the novel reagent combination BH_3·THF/TMSCl/DMF) are shown below. The high yields for the 2,6-dichloroaniline and the 2,6-diisopropylaniline are especially intriguing, because established methods give extremely low rates of reaction for these substrates. The described methods show minor differences concerning their reactivity and functional group tolerance.

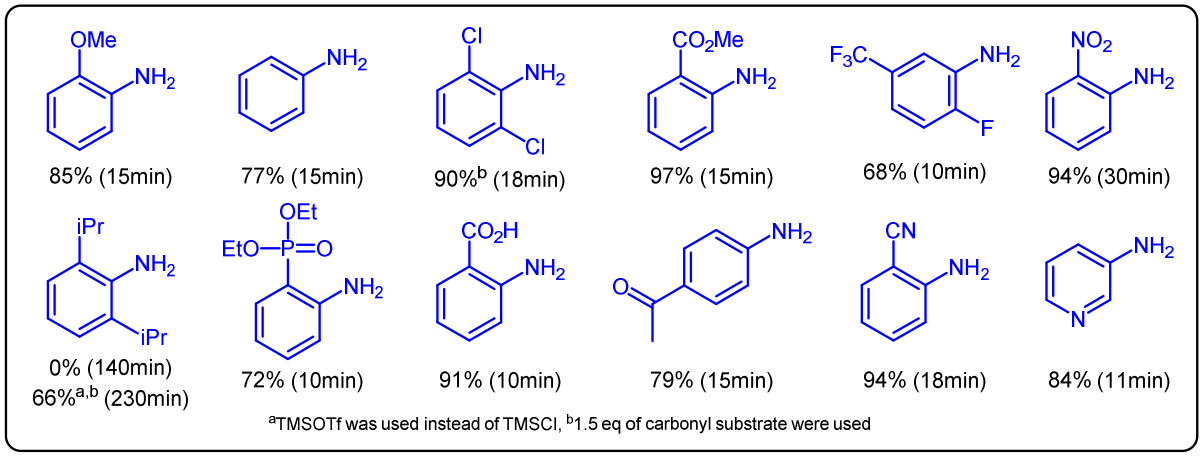


Figure 162: Amine substrate scope of method B.[189]

The carbonyl substrate scope for the methods was as well explored and a broad range of ketones and aldehydes could be reacted with different aromatic amines (Figure 163). It was found, that carbonyl substrates with low reactivity could be made accessible when TMSOTf was used instead of TMSCl. The three described methods had their limitations with sterically very bulky carbonyl substrates as well as aliphatic aldehydes. Acid sensitive groups (Boc) are not tolerated in method B & method C, where TMSCl is used as an additive, but method A employing AcOH gives good yields for the respective Boc-protected substrates.

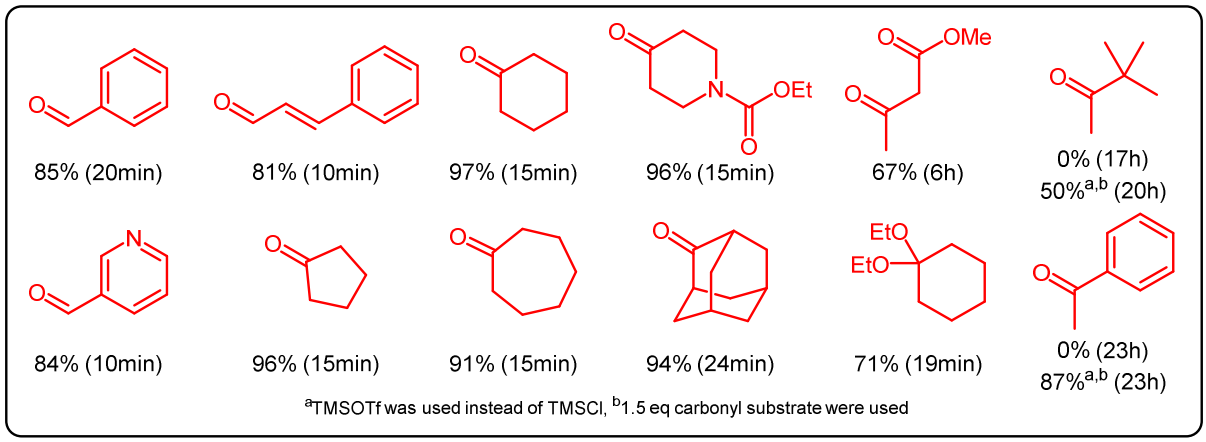


Figure 163: Carbonyl substrate scope of method B.[189]

During the development of the reductive amination methods we encountered several difficulties and identified a variety of substrate classes that cannot yet be reacted using the standard conditions. One of these challenges is the following reaction between an electron-deficient aniline and an enolizable, aliphatic aldehyde (Figure 164). The reaction looks very simple on paper, but when standard conditions are applied generally no reaction is observed. One reason for this might be that the iminium-ion formation is hindered due to the low nucleophilicity of the aniline. When alkylating reaction conditions or silane-mediated

alkylations are used over-alkylation might be problematic. When aliphatic aldehydes were used with the BH_3·THF/TMSCl/DMF or the other published methods, then either incomplete conversion with by-products formed or full conversion with an insoluble precipitate (formed upon workup) was observed. It would be highly interesting to investigate these substrates a little further so that the scope of the reductive amination methods could be extended and that the already powerful methodology might become even more useful to the synthetic community as a selective mono-alkylation method for electron-deficient anilines.

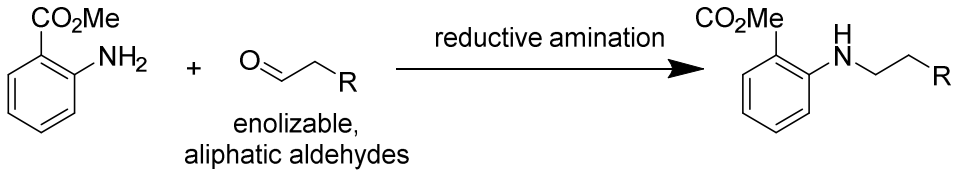


Figure 164: Enolizable, aliphatic aldehydes are challenging substrates for the developed methods.[189]

Yet another class of challenging substrates would be (+)-camphor which so far in any case failed to react with an electron-deficient aniline. Further optimization will be necessary to enhance the reactivity of the reductive amination enough in order to make this substrate accessible.

5.2 Synthesis of Phenazistatin A derivatives as Inhibitors of PhzA/B

In the second part of the thesis novel inhibitors of PhzA/B were synthesized and biologically evaluated in collaboration with the group of Wulf Blankenfeldt at the Helmholtz-Centre for Infection Research in Braunschweig.

As a first class of inhibitors Phenazistatin A derivatives with substitution at the 5-position of the aromatic ring were investigated. The Phenazistatin derivatives bearing H-, halogens F-, Cl-, Br-, I- as well as Me-, MeO- and NO_2- were synthesized by different methods (Figure 165). We observed that different approaches were necessary to produce these inhibitors, since the ULLMANN-GOLDBERG amination protocols did not always work as planned and the substituents on the aromatic ring were found to have a quite big influence on the reaction rate, by-product generation and selectivity. With our new method for the reductive amination[189] most attempts to synthesize the target compounds were successful. However, the separation of the generated diastereomers was difficult and only separations of the fully protected (methyl esters) molecules was successful. In case the substituted methyl anthranilate (with the protected carboxylic acid) was used, the preparations were simple and led reliably to high yields of the product.

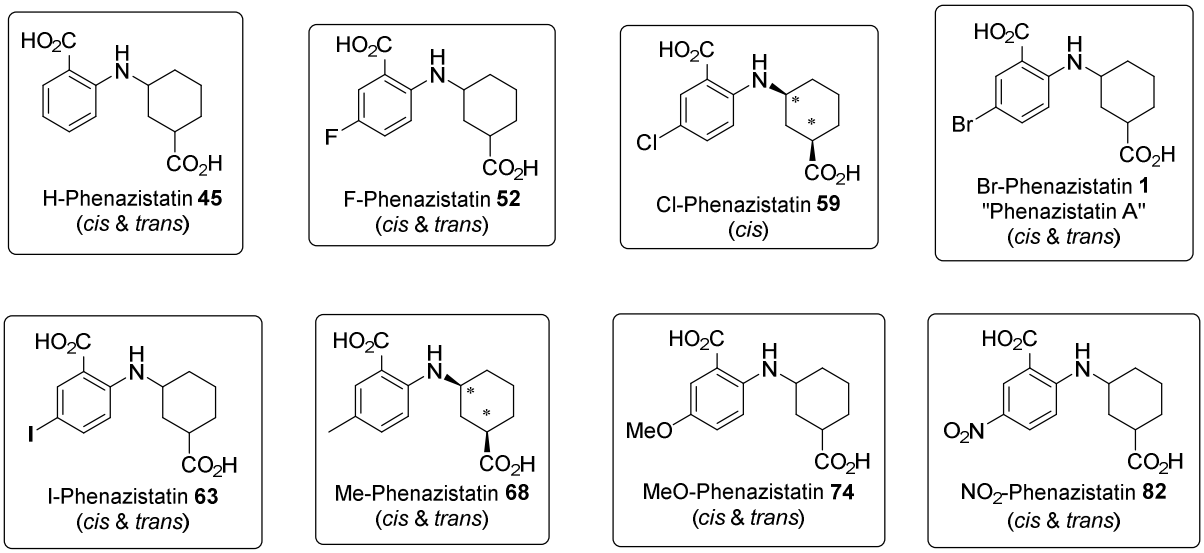


Figure 165: Phenazistatin derivatives synthesized bearing a short substituent at the 5-position of the aromatic ring.

The use of different cross-coupling reactions made it possible to install longer substituents at the 5-position of the Phenazistatin core structure and hence the effect on the inhibitor binding could be investigated (Figure 166). The synthesis of these probes was straightforward and started from the di-ester of I-Phenazistatin **36**.

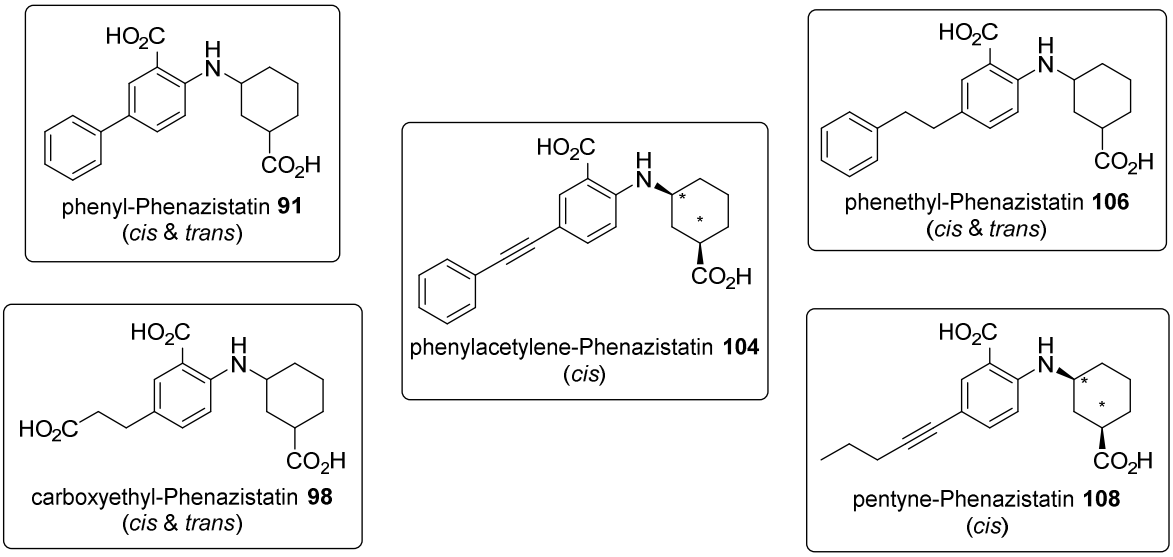


Figure 166: Phenazistatin derivatives synthesized bearing a long substituent at the 5-position of the aromatic ring which was introduced via cross-coupling reactions.

The biological evaluation gave insight into the binding modes as well as the capability to inhibit the enzyme reaction of PhzA/B. It was found that different substituents at the 5-position of the aromatic ring are well-tolerated and most of the compounds showed high activity in the residual activity assay. However, when these compounds were analyzed by ITC, only the *trans*-F-Phenazistatin **52** (K_D = 47 nM) was found to have a higher affinity

towards PhzA/B than Phenazistatin A (**1**) (K_D = 64 nM), the hit compound where the optimization started from.

We were also successful in synthesizing Phenazistatin A derivatives where the ring size of the alicyclic ring was varied (Figure 167). The syntheses for all these compounds relied on a MICHAEL-addition/PINNER-reaction/reductive amination sequence. The utilization of the developed reductive amination protocols[189] made the syntheses very smooth and effortless. When the inhibitors were biologically evaluated the 6-ring Phenazistatin **1** was found to be the most active one (K_D = 64 nM) with the 5-ring analogs **115** (K_D = 470 nM) and the 7-ring analog **119** (K_D = 450 nM) being one order of magnitude weaker binders as judged by ITC. The crystal structures of the inhibitor-enzyme complexes of **115** & **119** revealed the same binding mode as for the 6-ring derivative **1**.

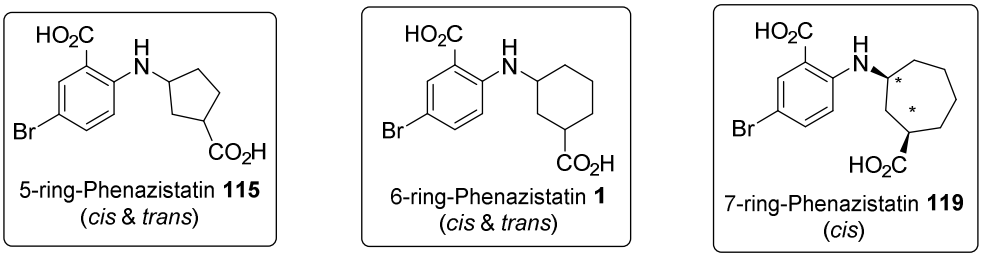


Figure 167: Phenazistatin derivatives synthesized with varied alicyclic ring size.

Several Phenazistatin derivatives bearing bioisosteric replacements of the carboxyl group could be synthesized using different strategies (Figure 168). However, biological evaluation indicated a lower activity than the parent compounds in the residual activity assay as well as via ITC. The 6-ring 1*H*-tetrazole derivatives **131** were found to be the most active ones (K_D(*cis*) = 400 nM, K_D(*trans*) = 5.1 µM) followed by the oxadiazolone derivatives **159** & **158**. The bis-acyl sulfonamide 153 as well as the 5-ring-1*H*-tetrazole derivatives (**137**, **135**, **134**) were found to have a very low affinity.

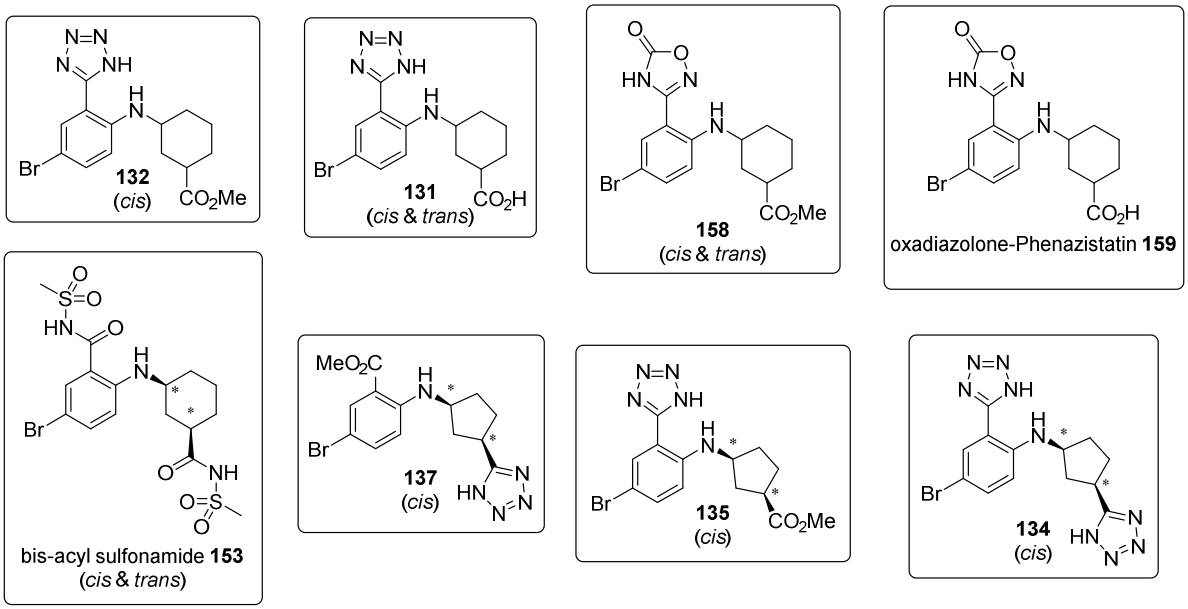


Figure 168: Phenazistatin derivatives synthesized bearing bioisosteric replacements for the carboxylic acid group.

A large number of prodrug esters of Phenazistatin A (**1**) and of other Phenazistatin derivatives could be generated (Figures 169-171). The biological evaluation of these compounds is ongoing in the group of Claus-Michael Lehr at the Department for Drug Delivery at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). As expected, the substances were found to have no inhibitory effect on the enzyme PhzA/B in the residual activity assay, but are expected to give promising in vivo results as the enzymatic activation of the substances by hydrolases is possible then.

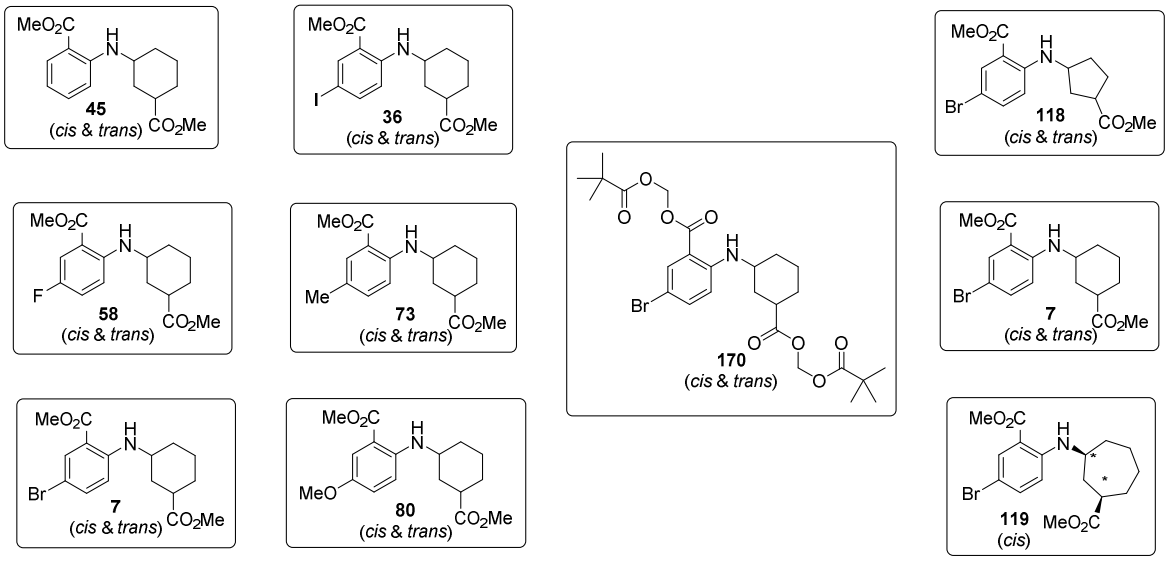


Figure 169: Prodrug esters of Phenazistatin A (**1**) and derivatives.

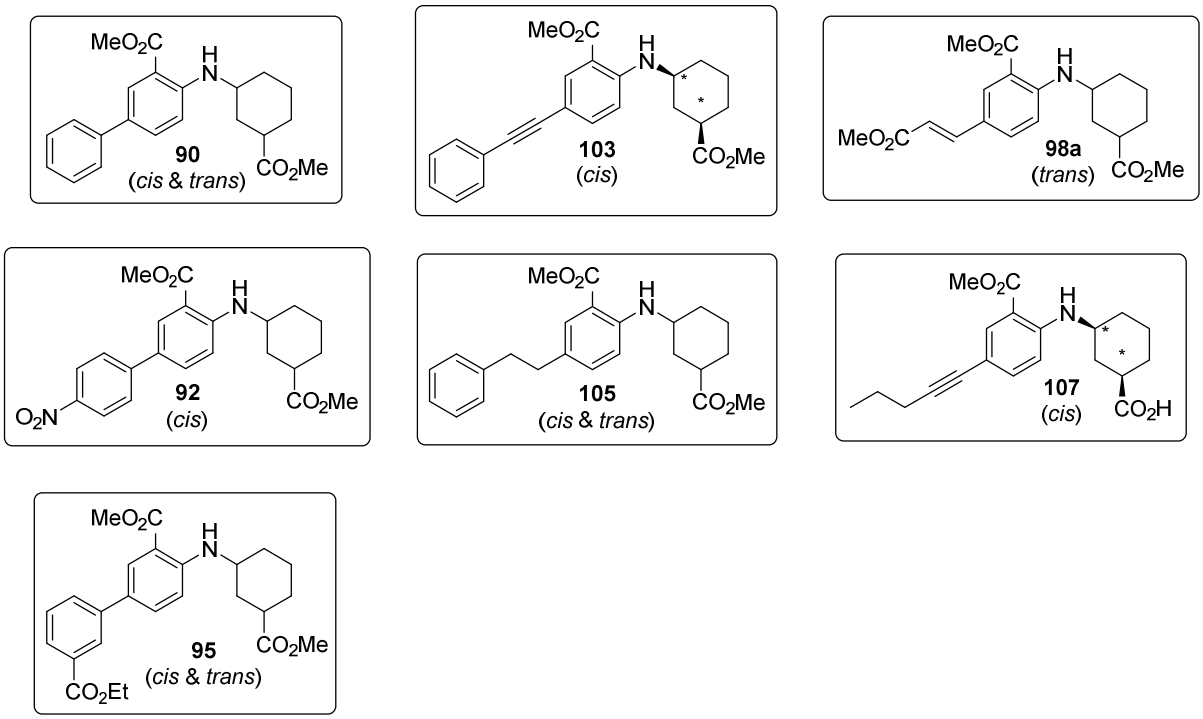


Figure 170: Prodrug esters synthesized via cross-coupling reactions from aryl iodide **36**.

With the developed reductive amination methods, the class of Phenazistatin inhibitors can now be easily accessed from readily available anthranilic acid derivatives. During the synthesis of the derivatives bearing bioisosteric replacements of the carboxylate group, several problems were encountered. In some syntheses the reactivity of the free (or protected) aniline which is placed in vicinity to the reactive center might be responsible for the cross-reactivity. With further optimization and a smart selection of appropriate protecting groups these problems might be overcome.

Our results showed that a large number of inhibitors substituted at the 5-position could bind to Phz A/B, but the activity could not be significantly enhanced. The major problem of these compounds will be rather the bioavailability and the activity in vivo with live cells. At this point the biological studies with bacterial cultures will have to be conducted in order to ascertain whether the compounds show a biological effect or not. *Pseudomonas aeruginosa* is known to possess a large number of adaptations that renders it less accessible to antibiotics, such as an outer membrane of low permeability, a multitude of efflux pumps and several degradative enzymes that disable antibiotics. Preliminary data concerning membrane permeability from the group of Claus-Michael Lehr (HIPS Saarbrücken) indicate that Phenazistatin A (**1**) can permeate through an outer membrane model in a transwell system, which might suggest that the inhibitors could reach the interior of bacterial cells. However, the results still need to be confirmed and it needs to be determined whether Phenazistatin A (**1**) is actively effluxed by *P. aeruginosa*.

5.3 Total synthesis of Tilivalline

In the third part of this thesis a new synthesis of the anhydride **200** was developed, from which the total synthesis of Tilivalline by Nagasaka et al.[95] can start (Figure 172). The five step procedure to produce anhydride **200** made it possible to generate larger amounts of the intermediate from cheap starting materials via reliable and simple synthetic steps. The synthesis by Nagasaka et al.[95] was revisited and it was found that the CURTIUS-rearrangement of the anhydride **200** was the bottleneck of the total synthesis. A varying degree of by-product formation and chemoselectivity were observed when parallel reactions on same scale were performed. The purification of the desired regioisomer of the isatoic anhydride **206** was laborious and low-yielding. However, since the starting material **200** is easily available in gram quantities by the devised route a sufficient amount of the desired Tilivalline (**3**) could be synthesized for ongoing biological studies in the group of Ellen Zechner at the University of Graz (Figure 172).

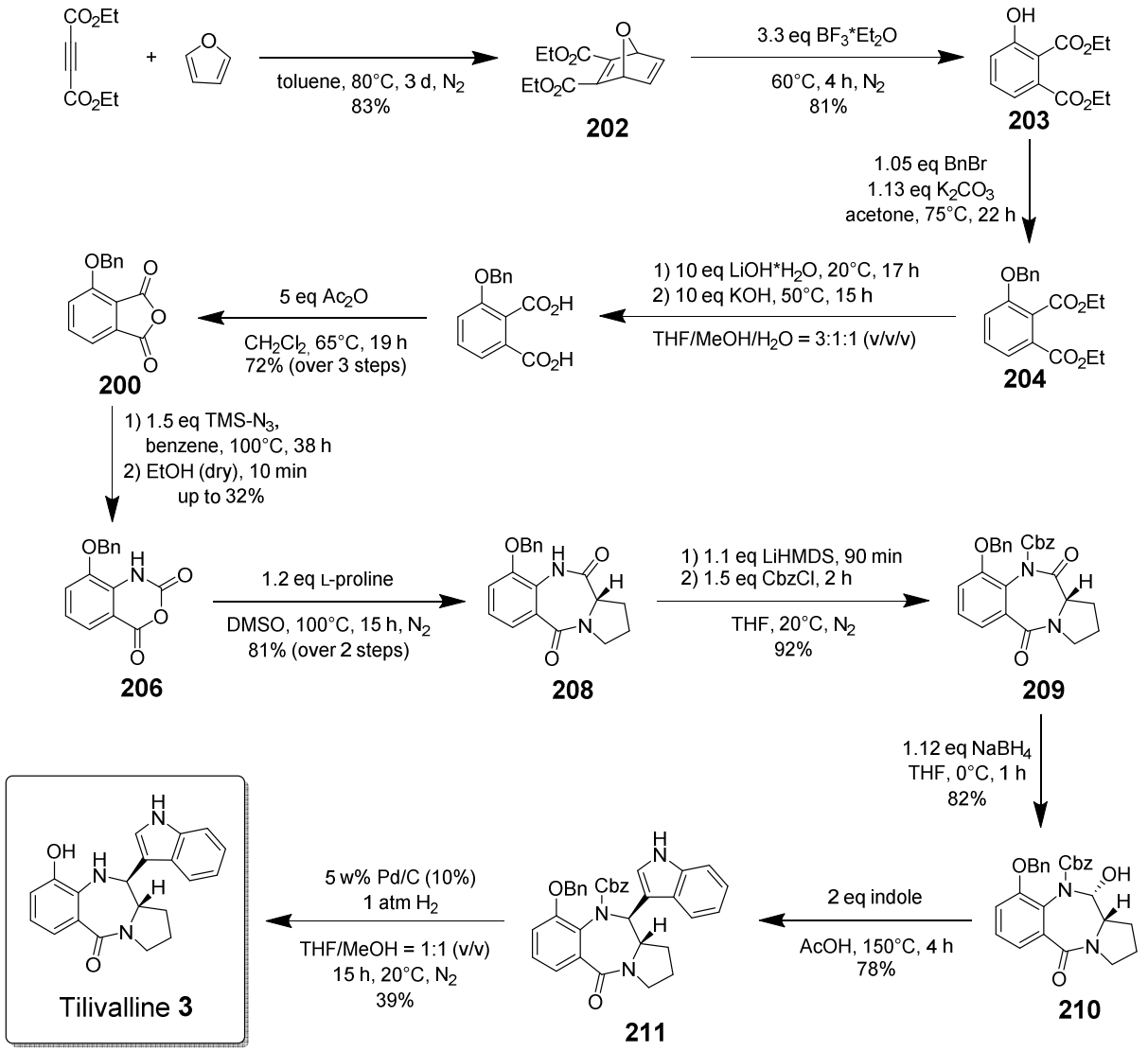


Figure 172: Total synthesis of Tilivalline (**3**) starting from diethyl acetylenedicarboxylate and furan.

There are still limitations to the existing route towards Tilivalline (**3**), especially when it comes to the CURTIUS-rearrangement, which proceeds unreliably with varying selectivities and unreproducible yields. If the need for gram-quantities of Tilivalline (**3**) should arise in future (e.g. animal studies as food additive), then this step should be revisited and the selectivity and safety optimized. The use of different solvents might offer additional possibilities and probably the less volatile and safer diphenyl phosphoryl azide (DPPA, 25 g approximately 77€, abcr product catalog, July 2016) should be used instead of the more hazardous $TMSN_3$ (bp = 95-99 °C). The following steps are high yielding and operationally simple and large quantities of Tilivalline (**3**) could thus be conveniently generated. The molecule **3** could be most easily ^{13}C- or ^{14}C- labelled if a labelled building block (L-proline, indole) was directly introduced at the step of the L-proline insertion (Figure 172) or the indole introduction. The introduction of the label at the indole might be the cheapest, safest, cleanest (if radioactive material is used), and highest-yielding alternative (only one subsequent step), however there are doubts about the metabolic stability of the C-C bond that connects the indole with the Tilivalline-backbone. This might be an issue especially if degradation products shall be studied. Introduction of the label in the L-proline moiety might prove superior for that purpose. However, four additional steps and at least two column chromatographies would be necessary to provide clean material via this strategy. Derivatives of Tilivalline (**3**) with different substituents on the aromatic ring might be introduced either by using an appropriately decorated starting material or via electrophilic aromatic substitution at the fully protected amide **209** (before $NaBH_4$ reduction). Several pyrrolobenzodiazepines are described that bear substituents at the L-proline moiety[256,257] and this substance class would be most conveniently introduced using a substituted proline derivative.

6 Experimental Section

6.1 General Experimental Aspects

All commercially available reagents and solvents were purchased from Acros Organics, Alfa, Aesar, ABCR, EGA-Chemie, Fisher Scientific, Fluka, Fluorochem, Heraeus, LOBA-Chemie, Merck, Roth, Sigma-Aldrich, TCI Chemicals, Ueticon and VWR and were of reagent grade or better and were used without further purification except otherwise stated. When it was required, e.g. with Et_2O, THF, 1,4-dioxane and $CHCl_3$, non-dry solvents were distilled before use.

If reactions were performed under inert conditions, e.g. exclusion of water, oxygen or both, all experiments were carried out using established Schenk techniques. Herein solvents were dried and/or degassed with common methods and afterwards stored under inert gas atmosphere (argon or N_2) over molecular sieves. In some cases, when explicitly mentioned, dry solvents were received from the mentioned suppliers. In general, when high vacuum was declared in experimental procedures, typically a vacuum of 10^{-2}-10^{-3} mbar was applied. All reactions were stirred with Teflon-coated magnetic stirring bars unless otherwise stated and Glindemann® PTFE sealing rings were used for the glass stoppers.

Degassing of solvents was performed by applying two different procedures. For small amounts of solvents (10 mL or less) vacuum was subjected to an appropriate reaction vessel, the solvent was frozen in liquid N_2, warmed to RT until the solvent or reaction mixture started to boil and was afterwards purged with an inert gas. This procedure was repeated at least for three times, depending also on the solvent volume. Larger amounts of solvents were degassed by bubbling argon from a balloon via cannula through the solvent during ultrasonification for about 20 min.

Molecular sieves (Sigma-Aldrich, beads with 8-12 mesh) were activated in a round-bottom flask with a gas inlet adapter by heating them carefully in a heating mantle at level 1 for two days under high vacuum until a constant vacuum was obtained. These activated molecular sieves were stored at RT under argon atmosphere.

In general, temperatures were measured externally if not otherwise stated. When working at a temperature of 0 °C, an ice-water bath served as the cooling medium. Lower temperatures were achieved by either using an acetone/dry ice cooling bath or a cryostatic temperature regulator. Reactions, which were carried out at higher temperatures than RT, were heated in a silicon oil bath on a heating plate (RCT basic IKAMAG® safety control, 0-1500 rpm) equipped with an external temperature controller.

When catalytic hydrogenation reactions were performed special care was taken. The hydrogenation reactor was purged with argon after the hydrogenation reaction and the catalyst was separated by filtration in an inert frit (argon!). The catalyst was kept wet at all time and was then disposed of appropriately (phlegmatized with an excess of water in a container).

6.2 Solvents

Solvents and chemicals listed below were prepared according to the following procedures:

Acetonitrile (MeCN): Anhydrous MeCN stored over 3 Å MS was purchased from Acros Organics. It was transferred into an amber 1000 mL Schenk bottle and stored over activated 3 Å MS under argon atmosphere (water content according to specification: <100 ppm).

Chloroform ($CHCl_3$): Anhydrous $CHCl_3$ stored over 4 Å MS (stabilized with amylene) was purchased from Acros Organics. It was transferred into an amber 1000 mL Schenk bottle and stored over activated 4 Å MS under argon atmosphere (water content according to specification: <50 ppm). Non-inert $CHCl_3$ was distilled before use to remove the stabilizer and was stored in an amber glass bottle.

Dichloromethane (CH_2Cl_2): Anhydrous CH_2Cl_2 was produced by pre-drying EtOH stabilized CH_2Cl_2 over P_4O_{10} and afterwards heating it under reflux over CaH_2 for 24 h under argon atmosphere. It was distilled in an amber 1000 mL Schlenk bottle over activated 4 Å MS and under argon atmosphere.

Diethylether (Et_2O): Anhydrous Et_2O was produced by heating it over Na under reflux for 24 h under argon atmosphere until benzophenone indicated its dryness by turning into deep blue color. It was distilled over a 20 cm Vigreux column and immediately used afterwards.

***N*,*N*-Dimethylformamide (DMF):** Anhydrous DMF stored over 3 Å MS was purchased from Acros Organics. It was transferred into an amber 1000 mL Schenk bottle and stored over activated 3 Å MS under argon atmosphere (water content according to specification: <50 ppm).

Tetrahydrofuran (THF): Anhydrous THF was produced by heating it over Na under reflux for 48 h under argon atmosphere until benzophenone indicated its dryness by turning into deep blue color. It was distilled into an amber 1000 mL Schlenk bottle and stored over 4 Å MS and under argon atmosphere.

The following solvents were used in reactions as well as workup processes, which were directly performed under atmospheric conditions: cyclohexane, dichloromethane (CH_2Cl_2) ethyl acetate (EtOAc) and methanol (MeOH) purchased from VWR or Fisher Scientific,

diethylether (Et_2O), ethanol (EtOH) and tetrahydrofuran (THF) purchased from Roth as well as acetonitrile (MeCN) purchased from Riedel-de Haën. All solvent were used without further purification except Et_2O and THF. These two were distilled before use and stored over solid KOH in amber glass bottles.

Saturated NaCl solution (brine): Solid NaCl was dissolved in H_2O until remaining solid was left.

Saturated $NaHCO_3$ solution: Solid $NaHCO_3$ was dissolved in H_2O until remaining solid was left.

Half saturated $NaHCO_3$ solution: Saturated $NaHCO_3$ solution was diluted with an equal volume of H_2O.

Saturated Na_2CO_3 solution: Solid Na_2CO_3 was dissolved in H_2O until remaining solid was left.

6.3 Reagents

Chlorotrimethylsilane (98%) and $BH_3 \cdot THF$ (1.0 M solution in THF) were purchased from Sigma Aldrich.

TBAA: 2 eq (n-Bu)$_4$NOH (40% solution in H_2O) were mixed with 1 eq adipic acid and the mixture was stirred at RT for 1 d. The product was dried by lyophilization and was dried in oil pump vacuum for at least 2 d to give a free flowing, colorless product. The dry TBAA was very hygroscopic and was stored under N_2.[197]

6.4 Analytical Methods

6.4.1 Thin layer chromatography (TLC)

Analytical thin layer chromatography (TLC) was carried out on Merck TLC silica gel aluminum sheets (silica gel 60, F_{254}, 20 x 20 cm) and spots were visualized by UV light (λ = 254 nm and/or λ = 366 nm) and by the listed staining reagents and developed by heating with a heat gun. If heating was not necessary to visualize the spots after staining, it is noted before the respective staining reagent, e.g. “cold CAM”. Eluents, R_f-values, staining reagents and colors of the stained spots are stated in the experimental descriptions.

Iodine (I_2 adsorbed on silica gel): Powdered iodine was mixed in a TLC chamber with silica gel.

Cerium ammonium molybdate (CAM): 50 g $(NH_4)_6Mo_7O_{24}$ were dissolved in 400 mL H_2O and afterwards 50 mL conc. H_2SO_4 as well as 2.0 g $Ce(SO_4)_2$ were added, respectively.

Potassium permanganate ($KMnO_4$): 0.3 g $KMnO_4$ as well as 20 g K_2CO_3 were dissolved in 300 mL H_2O and afterwards 5.0 mL 5 % aqueous NaOH were added.

Ninhydrin: 1.5 g ninhydrin were dissolved in 100 mL *n*-butanol and then 3.0 mL AcOH were added.

Bromocresol green: 0.04 g bromocresol green were dissolved in 100 mL EtOH and a 0.1 M solution of aqueous NaOH was added dropwise until the solution turned dark blue.

Vanillin: 15 g vanillin was dissolved in 250 mL EtOH and 2.5 mL conc. H_2SO_4.

$FeCl_3$: 5 g $FeCl_3$*$6H_2O$ was dissolved in 100 mL 0.1 M HCl.

6.4.2 Column chromatography

Column chromatography was performed on silica gel 60 from Acros Organics with particle sizes 35 - 70 µm. A 30- to 100-fold excess of silica gel was used with respect to the mass of dry crude product, depending on the separation problem. The crude material was either dissolved in the minimal amount of eluent or in the case of an insoluble or sticky sample, it was dissolved in an appropriate solvent (EtOAc, CH_2Cl_2 or MeOH) and subsequently adsorbed on the 2.5-fold excess of Celite™. Afterwards the solvent was removed in vacuum and the adsorbed crude material was dried in oil pump vacuum. The dimension of the column was adjusted to the required amount of silica gel and formed a pad between 10 cm and 40 cm. In general, the silica gel was mixed with the eluent and charged into the column before equilibration. For smaller column dimensions the dry silica gel was filled into the column and was equilibrated by forcing an appropriate amount of eluent through by over-pressure. Subsequently, the dissolved or adsorbed crude material was loaded onto the top of the silica gel and the mobile phase was forced through the column by pressure exerted by a rubber bulb pump. The volume of each collected fraction was adjusted between 20 % and 40 % of the silica gel volume, according to the separation problem.

6.4.3 Gas chromatography with mass selective detection (GC-MS)

GC-MS analyses were performed on an Agilent Technologies 7890A GC system equipped with a 5975C mass selective detector (inert MSD with Triple Axis Detector system) by electron-impact ionization (EI) with a potential of E = 70 eV. Herein, the samples were separated depending on their boiling point and polarity. The desired crude materials or pure

compounds were dissolved either in CH_2Cl_2, EtOAc or MeOH and the solutions were injected by employing the autosampler 7683B in a split mode 1/20 (inlet temperature: 280 °C; injection volume: max. 1.0 µL). Separations were carried out on an Agilent Technologies J&W GC HP-5MS capillary column ((5 %-phenyl)methylpolysiloxane, 30 m x 0.2 mm x 0.25 µm) with a constant helium flow rate (He 5.0 (Air Liquide), 1.085 $mL.min^{-1}$, average velocity: 41.6 $cm.s^{-1}$). A general temperature-gradient method was used (initial temperature: 50 °C for 1 min, linear increase to 300 °C (40 $°C.min^{-1}$), hold for 5 min, 1 min post-run at 300 °C, detecting range: 50.0-550.0 amu, solvent delay of 2.80 min).

GC-FID measurements were performed employing an Agilent Technologies 6890N GC system with an Agilent Technologies J&W GC-column DB-1701 ((14%-cyanopropylphenyl)-methylpolysiloxane; length: 30 m; inner-diameter: 0.250 mm; film: 0.25 µm). The injection was executed by an Agilent Technologies 7683 Series autosampler in split mode. Nitrogen 5.0 was used as carrier gas and for detection a flame ionization detector (FID) with Hydrogen 5.0 and air as detector gases were used. A general temperature-gradient method was used (80 °C 1 min, ramp: 30 °C·min-1 linear to 280 °C, 280 °C 3 min).

When GC was used for reaction monitoring, the samples were prepared using a microscale workup. Therefore an aliquot was taken from the reaction mixture, quenched with ~1 mL aqueous solution (in some cases saturated $NaHCO_3$ or 1.0 M HCl solution and extracted with ~1 mL EtOAc. After proper mixing and phase separation, the organic layer was collected, and dried over $MgSO_4$. Samples from reaction mixtures containing transition metals were prepared by filtering through a short pad of silica gel (~1 cm) (eluted with EtOAc or MeOH).

6.4.4 High performance liquid chromatography (HPLC)

Analytical HPLC-MS measurements were performed on a Shimadzu Nexera LCMS-2020 system (CBM-20A Prominence system controller, Nexera SIL-30AC autosampler, DGU-20A3 and DGU-20A5 on-line degassers, Nexera LC-30AD binary pump, FCV-20AH2 valve unit, CTO-20AC Prominence column oven, SPD-M20A Prominence photodiode array (PDA) detector (deuterium lamp, tungsten lamp, 190-800 nm)) equipped with single quadrupole ultra-fast LC/MS detector “LCMS-2020” or an Agilent Technologies 1200 Series system (G1379 Degasser, G1312 Binary Pump, G1367C HiP ALS SL Autosampler, G1330B FC/ALS Thermostat, G1316B TCC SL column compartment, G1365C MWD SL multiple wavelength detector (deuterium lamp, 190-400 nm)) equipped with a single quadrupole LCMS detector “6120 LC/MS” using electrospray ionization source (ESI in positive and negative mode). All separations were carried out on a reversed phase Agilent Poroshell 120 SB-C18 (100 x 3.0 mm, 2.7 µm) column equipped with a Merck LiChroCART® 4-4 pre-column. Samples

were either dissolved in MeCN, MeOH or DMSO and in the case of undissolved particles the suspension was filtered through PTFE syringe filters (VWR International, w/0.2 µm PTFE membrane). Generally 0.01% HCO_2H were added to the H_2O eluent. A general solvent gradient method was used (0-2.00 min: MeCN:H_2O = 10:90 (v/v), 2.00-10.00 min: linear increase to MeCN:H_2O = 95:5 (v/v), 10.00-16.00 min: holding of MeCN:H_2O = 95:5 (v/v), oven temperature: 40 °C, solvent flow: 0.700 mL/min).

Reversed phase preparative HPLC purifications were run on a Thermo Scientific UltiMate 3000 system (Dionex UltiMate Pump 3000, Dionex UltiMate Autosampler, Dionex UltiMate Column Compartment, Dionex UltiMate Diode Array Detector and Dionex UltiMate Automated Fraction Collector). The separations were carried out on a Macherey-Nagel 125/21 Nucleodur® 100-5 C18ec (125 x 21 mm, 5.0 µm) column. Two methods were used:

Method_PREPHPLC (gradient program: 0-2.00 min: linear increase from MeCN/0.01% HCOOH = 3:97 (v/v) to MeCN/0.01% HCOOH = 10:90 (v/v), 2.00-7.00 min MeCN/0.01% HCOOH = 10:90 (v/v), 7.00-16.5 min linear increase to MeCN/0.01% HCOOH = 80:20 (v/v), 16.5-17.00 min linear increase to MeCN/0.01% HCOOH = 100:0 (v/v), 17.00-21.00 min MeCN/0.01% HCOOH = 100:0 (v/v), oven temperature: 30 °C; solvent flow: 14.0 mL/min).

Method_TILIVALLINE (gradient program: 0-60 min: linear increase from MeCN/0.01% HCOOH = 5:95 (v/v) to MeCN/0.01% HCOOH = 10:90 (v/v), oven temperature: 30 °C; solvent flow: 14.0 mL/min).

6.4.5 Nuclear magnetic resonance spectroscopy (NMR)

In general ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III 300 spectrometer (^{1}H: 300.36 MHz; ^{13}C: 75.53 MHz) with autosampler or on a Varian Unity Inova 500 spectrometer (^{1}H: 499.88 MHz; ^{13}C: 125.69 MHz, ^{19}F: 470.35 MHz, ^{31}P: 202.35 MHz). Some ^{19}F (282.47 MHz) and ^{31}P (121.58 MHz) NMR spectra were recorded on a Varian INOVA 300 spectrometer. Chemical shifts were either referenced to tetramethylsilane as internal standard or to the residual proton and carbon signal of the deuterated solvent ($CDCl_3$: δ = 7.26 ppm (^{1}H), 77.16 ppm (^{13}C); DMSO-d_6: δ = 2.50 ppm (^{1}H), 39.52 ppm (^{13}C); MeOH-d_4: δ = 3.31 (^{1}H), 49.00 ppm (^{13}C); D_2O: d_4: δ = 4.79 (^{1}H)). Chemical shifts δ are given in ppm (parts per million) and coupling constants *J* in Hz (Hertz). If necessary, 1D spectra (APT and NOESY) as well as 2D spetra (HH-COSY, HSQC and HMBC) were recorded for the identification and confirmation of the structure. Signal multiplicities are abbreviated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of doublet), td (triplet of doublet), t (triplet), dt (doublet of triplet), q (quadruplet), dq (doublet of quadruplet), p (pentet), h (hexet) and m (multiplet). Additionally, quarternary carbon atoms

are designated as C_q and aromatic carbon atoms bearing a hydrogen as CH_{arom}. Deuterated solvents for nuclear resonance spectroscopy were purchased from euriso-top®. For acid sensitive substrates $CDCl_3$ was neutralized by filtering it through basic Alox (aluminum oxide activated, basic type 5016A, 58 Å, particle size: 150 mesh, Brockmann Grade I) from Acros Organics.

6.4.6 High resolution mass spectrometry (HRMS)

HRMS spectra were recorded in the research group of Prof. Robert Saf (ICTM, TU Graz) on a "Waters Micromass GCT Premier" system. Ionization was realized by an electron impact source (EI ionization) at a constant potential of 70 eV. Herein, individual samples were either inserted directly (direct inlet electron impact ionization; DI-EI) or prior to this gas chromatographically separated on a "Hewlett Packard BC 7890A" system equipped with an Agilent Technologies J&W GC-column DB-5MS (length: 30 m; inner-diameter: 0.250 mm; film: 0.25 µm) at a constant helium flow. Molecule ions were analyzed by a time-of-flight (TOF) mass analyzer in the positive mode (TOF MS EI^+). Besides molecular formulas, calculated as well as determined *m/z* ratios of each molecule peak are denoted.

6.4.7 Determination of melting points

Melting points were determined on a Mel-Temp® melting point apparatus from Electrothermal with an integrated microscopical support. They were measured in open capillary tubes with a mercury-in-glass thermometer and were not corrected.

6.4.8 Attenuated total reflection infrared spectroscopy (ATR-IR)

ATR-IR spectra were recorded using an ALPHA FT-IR spectrometer (Bruker; Billerica, MA, USA). For the measurement an attenuated total reflection (ATR) attachment was used with 48 scans at a resolution of 4 cm^{-1} and a scan range between 4000 and 400 cm^{-1}. The data were analyzed with OPUS 7.5 software.

6.4.9 Specific optical rotation

The specific optical rotation was determined on a Perkin Elmer Polarimeter 341 with an integrated sodium vapor lamp. All samples were measured at the D-line of the sodium light (λ = 589 nm) under non-tempered conditions between 25 °C and 34 °C. Concentrations

between 5.0 $g.L^{-1}$ (c = 0.50) and 10.0 $g.L^{-1}$ (c = 1.00) depending on the solubility of the sample were chosen, whereas MeCN, $CHCl_3$, H_2O, MeOH and DMSO were used as solvents. All solvents were either purchased from Sigma-Aldrich or Fluka and had HPLC quality or higher.

6.5 Biological Assays

6.5.1 Isothermal titration calorimetry

The dimeric enzyme PhzA/B from *Burkholderia cepacia* R18194 was produced recombinantly in *E. coli* and used for microcalorimetric analysis. Affinity titrations with different potential ligands were carried out at 25 °C using a MicroCal™ VP-ITC System (GE Healthcare, now Malvern Instruments), while the sample cell was filled with the protein to a final concentration of 100 µM in 20 mM TRIS/HCl pH 8.0, 150 mM NaCl. After an initial delay of 60 s, the protein was titrated with 2 mM solutions of different ligands. These solutions were prepared from aqueous (10 mM in 200 mM TRIS/HCl pH 9.0) or from DMSO (200 mM) stock solutions (the samples were stored dissolved in the above mentioned solvents). If residual DMSO (up to 1 %) was present in the syringe, the same amount was added to the sample cell to ensure homogeneity of the buffer. The ligands were injected at a rate of 0.5 µL s^{-1} and a stirring rate of 307 rpm with 240 s gaps between individual injections using 10 µcal s^{-1} reference power in high feedback mode. The data were analyzed and plotted using the programs NITPIC [265,266] SEDPHAT (Houtman et al.[267]; Zhao et al.[268]) and GUSSI (Brautigam[269]; and for review Brautigam et al.[270]).

6.5.2 *In vitro* inhibition assay

The inhibitory effect of different ligands on PhzB activity was analyzed using a coupled photometric condensation assay. Standard solutions containing 1 µM of the target enzyme *Bc*PhzA/B as well as the coupled enzyme PhzF in 50 mM sodium phosphate buffer at pH 7.5 were used for titration experiments. A serial ten times dilution (from aqueous or DMSO stock solutions) of the respective compound, *i.e.* 100, 10, 1 and 0.1 µM, was titrated against 125 µM and 1 mM DHHA respectively. All experiments were carried out continuously in UV-Star® 96-Well microplates (Greiner Bio-One) at 25 °C in an Infinite® M200 microplate reader (Tecan Group Ltd.). A final reaction volume of 150 µL in reaction buffer supplemented with 2 % (v/v) dimethyl sulfoxide (DMSO) was prepared by the addition of 75 µL of the pre-mixed enzyme solution to an equal volume of each substrate/inhibitor dilution. Data acquisition was carried out over a 20 min time course in 40 s intervals while the increasing absorption of a tricyclic phenazine intermediate, tetrahydro-phenazine-1-carboxylic acid (THPCA), was monitored at 330 nm. Reaction mixture lacking inhibitor were used as reference. The background reaction was evaluated using reaction mixtures lacking *Bc*PhzA/B and the potential inhibitor. After normalization to zero, reaction rates were determined from the slopes of the linear phase of each curve. After background correction, the percentage

residual enzyme activity was calculated in relation to an inhibitor free sample. All samples were analyzed in triplicate using Microsoft Excel 2010 (Microsoft Corporation).

6.5.3 X-ray structure determination

The crystallization experiments in order to obtain the X-ray structures of the Phenazistatin derivatives were performed by Christina Diederich at the Helmholtz-Centre for Infection Research in Braunschweig, under supervision of Prof. Wulf Blankenfeldt.

6.6 Experimental procedures

6.6.1 Studies towards the reductive amination of electron-deficient anilines

6.6.1.1 Test reaction with different hydride sources

A dry 20 mL Schlenk flask with magnetic stirring bar was charged consecutively with 39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 39.2 µL 1,2-DME (internal standard), 200 µL dry DMF and 96.8 µL (750 µmol, 2.5 eq) TMSCl at RT in a N_2 counter-stream. The reaction mixture was cooled to 0 °C and 300 µmol (1.0 eq) of the reducing agent were added in one portion (the reductant was added over a period of 1 min if employed as a solution (9-BBN, BH_3·THF)). The flask was sealed with a glass stopper and the mixture was kept stirring in the thawing ice bath. A reaction aliquot was sampled after 24 h and worked up for GC-FID analysis (same workup procedure as stated below). The reaction was stopped after 24 h by the addition of 3 mL distilled water. The mixtures were left stirring at RT for 18 h and 5 mL EtOAc was added followed by 2 mL saturated Na_2CO_3 solution. The mixtures were kept stirring for 60 min until gas evolution had ceased. The phases were separated and the aqueous layer was extracted with EtOAc (3 x 8 mL). The combined organic phases were reduced in vacuum and the product was purified via column chromatography (cyclohexane/EtOAc = 150:1 (v/v)), size: 8.0 x 0.8 cm).

6.6.1.2 General procedure A: Reductive amination of aryl amines and aldehydes/ketones with BH_3·THF in CH_2Cl_2/AcOH

A dry 20 mL Schlenk flask with magnetic stirring bar was charged consecutively with 1.0 mmol (1.0 eq) aryl amine, 1.5 mmol (1.5 eq) carbonyl substrate, 2.0 mL dry CH_2Cl_2 and 1.0 mL glacial AcOH in a N_2 counter-stream. The reaction mixture was cooled to 0 °C, the glass stopper was replaced by a rubber septum and 3.0 mL (3.0 mmol, 3 eq) BH_3·THF (1.0 M solution in THF) were added slowly via syringe over a period of 10 - 20 min. The flask was sealed with a glass stopper and the mixture was kept stirring in the thawing ice bath until full conversion of the starting material was detected by TLC. The vigorously stirred mixture was cooled to 0 °C again and 5 mL saturated $NaHCO_3$ were added carefully (CO_2 evolution) followed by 10 mL EtOAc. The biphasic mixture was kept stirring until the gas evolution had ceased (typically 20 - 60 min). The phases were separated and the aqueous layer was extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The crude product (adsorbed on Celite™) was purified via column chromatography.

6.6.1.3 General procedure B: Reductive amination of aryl amines and aldehydes/ketones with BH_3·THF/TMSCl in DMF

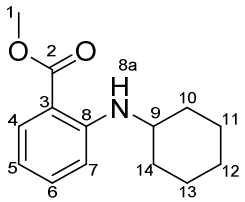
A dry 20 mL Schlenk flask with magnetic stirring bar was charged consecutively with 300 µmol (1.0 eq) aryl amine, 330 µmol (1.1 eq) carbonyl substrate, 200 µL dry DMF and 96.8 µL (750 µmol, 2.5 eq) TMSCl in a N_2 counter-stream. The reaction mixture was cooled to 0 °C, the glass stopper was replaced by a rubber septum and 300 µL (300 µmol, 1.0 eq) BH_3·THF (1.0 M solution in THF) were added slowly via syringe over a period of 10 - 20 min. The flask was sealed with a glass stopper and the reaction mixture was kept stirring at 0 °C until full conversion of the starting material was detected by TLC. The vigorously stirred reaction mixture was cooled to 0 °C again and 3 mL H_2O were added and the mixture was stirred for 20 min. 5 mL EtOAc were added followed by 2.5 mL saturated Na_2CO_3 solution (CO_2 evolution). The biphasic mixture was kept stirring until the gas evolution had ceased (typically 20-60 min). In case the aqueous phase was turbid, small amounts of H_2O were added to provide a clear aqueous phase. The phases were separated and the aqueous layer was extracted with EtOAc (5 x 8 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The residue was dissolved in a small portion of EtOAc or MeOH and a 2.5-fold amount of Celite™ (in respect to the mass of the crude product) was added. The solvents were removed in vacuum and the crude product (adsorbed onto Celite™) was purified via column chromatography.

6.6.1.4 General procedure C: Reductive amination of aryl amines and aldehydes/ketones with $NaBH_4$/TMSCl in DMF

A dry 20 mL Schlenk flask with magnetic stirring bar was charged consecutively with 1.0 mmol (1.0 eq) aryl amine, 1.1 mmol (1.1 eq) carbonyl substrate, 670 µL dry DMF and 323 µL (2.5 mmol, 2.5 eq) TMSCl in a N_2 counter-stream. The reaction mixture was cooled to 0 °C and 39.0 mg (1.0 µmol, 1.0 eq) $NaBH_4$ were added in one portion. The flask was sealed with a glass stopper and the reaction mixture was kept stirring at 0 °C until full conversion of the starting material was detected by TLC. The vigorously stirred reaction mixture was cooled to 0 °C again and 5 mL saturated $NaHCO_3$ solution were added carefully (CO_2 evolution) followed by 5 mL EtOAc. The biphasic mixture was kept stirring until the gas evolution had ceased (typically 20-60 min). In case the aqueous phase was turbid, small amounts of H_2O were added to provide a clear aqueous phase. The phases were separated and the aqueous layer was extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The residue

was dissolved in a small portion of EtOAc or MeOH and the 2.5-fold amount of Celite™ (in respect to the mass of the crude product) was added. The solvents were removed in vacuum and the crude product (adsorbed onto Celite™) was purified via column chromatography.

6.6.1.5 Methyl 2-(cyclohexylamino)benzoate (6)



according to GP-A:

131 µL (1.0 mmol, 1.0 eq) methyl anthranilate, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 3 h reaction time, column chromatography (cyclohexane/EtOAc = 150:1 (v/v), size: 11 x 2.8 cm, 22 g silica gel).

yield: 186 mg (797 µmol, 80%) light-yellow, viscous liquid

according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 200:1, 150:1 (v/v), size: 7.5 x 2.2 cm, 10 g SiO_2).

yield: 68.2 mg (292 µmol, 97%) light-yellow, viscous liquid.

according to GP-B: (starting from 1,1-diethoxycyclohexane)

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 64.5 µL (330 µmol, 1.1 eq) 1,1-diethoxycyclohexane, 19 min reaction time, column chromatography (cyclohexane/EtOAc = 150:1 (v/v), size: 8 x 0.8 cm).

yield: 49.4 mg (212 µmol, 71%) light-yellow, viscous liquid.

according to GP-C:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 200:1, 150:1 (v/v), size: 7.5 x 2.2 cm, 10 g silica gel).

yield: 69.1 mg (296 µmol, 99%) light-yellow, viscous liquid.

$C_{14}H_{19}NO_2$ [233.31]

R_f = 0.67 (cyclohexane/EtOAc = 8:1 (v/v)) (254 nm, 366 nm, cold CAM: orange).

IR (ATR): 3346, 2928, 2852, 1680, 1606, 1579, 1516, 1436, 1328, 1249, 1223, 1187, 1161, 1137, 1107, 1075, 1045, 745, 702, 565, 525 cm^{-1}.

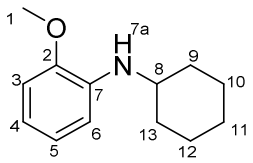
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.93-7.84 (m, 1H, H-4), 7.82-7.69 (m, 1H, H-8a), 7.36-7.21 (m, 1H, H-6), 6.74-6.63 (m, 1H, H-7), 6.56-6.45 (m, 1H, H-5), 3.84 (s, 3H, H-1), 3.47-3.30 (m, 1H, H-9), 2.10-1.94 (m, 2H, CH_2), 1.84-1.69 (m, 2H, CH_2), 1.67-1.53 (m, 1H, CH_2), 1.49-1.20 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.3 (C_q, C-2), 150.5 (C_q, C-8), 134.6 (CH_{arom}, C-6), 132.0 (CH_{arom}, C-4), 114.0 (CH_{arom}, C-5), 111.8 (CH_{arom}, C-7), 109.6 (C_q, C-3), 51.5 (CH_3, C-1), 50.6 (CH, C-9), 33.0 (CH_2, C-10, C-14), 26.0 (CH_2, C-12), 24.8 (CH_2, C-11, C-13).

GC-MS: t_R = 6.909 min, *m/z* (%) = 233.2 (M^+, 56), 207.1 (32), 190.1 (86), 158.1 (100), 145.1 (24).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{19}NO_2$: 233.1416; found: 233.1424

6.6.1.6 *N*-Cyclohexyl-2-methoxyaniline (13)



according to GP-A:

114 µL (1.00 mmol, 1.0 eq) 2-methoxyaniline, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 21 h reaction time, column chromatography (cyclohexane/EtOAc = 40:1 (v/v), size: 11 x 2.8 cm).

yield: 166 mg (810 µmol, 81%) amorphous, colorless solid.

according to GP-B:

34.2 µL (300 µmol, 1.0 eq) 2-methoxyaniline, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 100:1 (v/v), size: 14 x 0.8 cm).

yield: 52.6 mg (256 µmol, 85%) colorless solid.

according to GP-C:

114 µL (1.00 mmol, 1.0 eq) 2-methoxyaniline, 115 µL (1.1 mmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexanone/EtOAc = 100:1, 50:1 (v/v), size: 20 x 1.4 cm).

yield: 185 mg (902 µmol, 90%) amorphous, colorless solid.

$C_{13}H_{19}NO$ [205.30]

R_f = 0.31 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: purple).

mp: 32 °C

IR (ATR): 3422, 2919, 2849, 1600, 1507, 1449, 1252, 1234, 1219, 1125, 1026, 738 cm^{-1}.

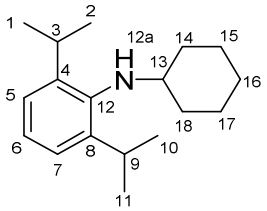
^{1}H NMR (300.36 MHz, $CDCl_3$): δ = 6.87 (dt, $^{3}J_{HH}$ = 7.8 Hz, $^{4}J_{HH}$ = 1.3 Hz, 1H, ArH), 6.78 (dd, $^{3}J_{HH}$ = 8.1 Hz, $^{4}J_{HH}$ = 1.0 Hz, 1H, ArH), 6.70-6.58 (m, 2H, 2 x ArH), 4.16 (s, 1H, NH), 3.86 (s, 3H, CH_3), 3.35-3.20 (m, 1H, CH), 2.17-2.00 (m, 2H, CH_2), 1.87-1.60 (m, 3H, CH_2), 1.50-1.12 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 146.8 (C_q, C-2), 137.4 (C_q, C-7), 121.4 (C-5), 115.9 (C-4), 110.3 (C-6), 109.7 (C-3), 55.5 (C-8), 51.5 (C-1), 33.6 (C-9, C-13), 26.1 (C-11), 25.2 (C-10, C-12).

GC-MS: t_R = 6.436 min, *m/z* (%) = 205.2 (M^+, 45), 162.1 (100), 134.1 (15), 108.1 (7).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{19}NO$: 205.1467; found: 205.1471.

6.6.1.7 *N*-Cyclohexyl-2,6-diisopropylaniline (10)



according to GP-B (modified):
62.9 µL (300 µmol, 1.0 eq) 2,6-diisopropylaniline, 47.2 µL (450 µmol, 1.5 eq) cyclohexanone, 139 µL (750 µmol, 2.5 eq) TMSOTf (instead of TMSCl), 230 min reaction time, column chromatography (cyclohexane/EtOAc = 100:1 (v/v), size: 8 x 0.8 cm).
yield: 51.6 mg (199 µmol, 66%) colorless, viscous liquid.

$C_{18}H_{29}N$ [259.44]

R_f = 0.76 (cyclohexane/EtOAc = 9:1 (v/v)) (254 nm, $KMnO_4$: yellow).

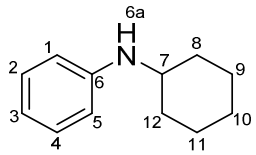
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.15–6.97 (m, 3H, H-5, H-6, H-7), 3.39–3.18 (m, 2H, H-3, H-9), 3.03–2.68 (m, 2H, H-12a, H-13), 2.12–1.92 (m, 2H, H-14a, H-18a), 1.86–1.54 (m, 3H, H-15a, H-16a, H-17a), 1.37–1.06 (m, 17H, H-1, H-2, H-10, H-11, H-14b, H-15b, H-16b, H-17b, H-18b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 142.0 (C_q, C-12), 141.8 (C_q, C-4, C-8), 123.5 (C-5, C-7), 123.0 (C-6), 59.6 (C-13), 34.7 (C-14, C-18), 28.0 (C-3, C-9), 26.2 (C-16), 26.1 (C-15, C-16), 24.3 (C-1, C-2, C-10, C-11).

GC-MS: t_R = 6.474 min, *m/z* (%) = 259.2 (M^+, 44), 188.1 (60), 176.1 (100), 146.1 (70).

The spectra were in accordance with the previously reported data.[271]

6.6.1.8 *N*-Cyclohexylaniline (14)



according to GP-A:

92.0 µL (1.00 mmol, 1.0 eq) aniline, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 19 h reaction time, column chromatography (cyclohexane/EtOAc = 45:1 (v/v), size: 11 x 2.8 cm).
yield: 134 mg (765 µmol, 77%) light-yellow, viscous liquid.

according to GP-B:

27.7 µL (300 µmol, 1.0 eq) aniline, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 150:1, 80:1 (v/v), size: 14 x 0.8 cm).
yield: 40.4 mg (230 µmol, 77%) colorless liquid.

according to GP-C:

92.0 µL (1.00 mmol, 1.0 eq) aniline, 115 µL (1.1 mmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 45:1 (v/v), size: 15.5 x 3.5 cm, 25 g silica gel).
yield: 123 mg (702 µmol, 70%) yellow, viscous liquid.

$C_{12}H_{17}N$ [175.28]

R_f = 0.79 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, cold CAM: flesh-colored).

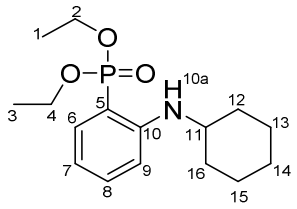
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.23-7.11 (m, 2H, H-2, H-4), 6.74-6.54 (m, 3H, H-1, H-3, H-5), 3.35-3.19 (m, 1H, H-7), 2.15-2.00 (m, 2H, CH_2), 1.85-1.60 (m, 3H, CH_2), 1.49-1.06 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 147.5 (C_q, C-6), 129.4 (CH_{arom}, C-2, C-4), 116.9 (C_q, C-6), 113.3 (CH_{arom}, C-1, C-5), 51.8 (CH, C-7), 33.6 (CH_2, C-8, C-12), 26.1 (CH_2, C-10), 25.2 (CH_2, C-9, C-11).

GC-MS: t_R = 5.809 min, *m/z* (%) = 175.1 (M^+, 34), 132.1 (100), 118.1 (20), 93.0 (12), 77.0 (10).

The spectra were in accordance with the previously reported data.[272]

6.6.1.9 Diethyl (2-(cyclohexylamino)phenyl)phosphonate (15)



according to GP-A:

229 mg (1.0 mmol, 1.0 eq) diethyl (2-aminophenyl)phosphonate,[273] 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 3 h reaction time, column chromatography (cyclohexane/EtOAc = 5:1 (v/v), size: 11 x 2.8 cm, 22 g silica gel).

yield: 276 mg (886 µmol, 89%) light-yellow, very viscous liquid.

according to GP-B:

68.8 mg (300 µmol, 1.0 eq) diethyl (2-aminophenyl)phosphonate,[231] 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 10 min reaction time, column chromatography (cyclohexane/EtOAc = 5:1 (v/v), size: 15 x 0.8 cm).

yield: 67.6 mg (217 µmol, 72%) light-yellow, very viscous liquid.

$C_{16}H_{26}NO_3P$ [311.36]

R_f = 0.24 (cyclohexane/EtOAc = 5:1 (v/v)) (254 nm, 366 nm).

IR (ATR): 3314, 2981, 2853, 1598, 1582, 1523, 1456, 1325, 1220, 1046, 1017, 955, 747, 559, 532, 513 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.51-7.38 (m, 1H, H-6), 7.36-7.24 (m, 1H, H-8), 6.69-6.44 (m, 3H, H-7, H-9, H-10a), 4.20-3.94 (m, 4H, H-2, H-4), 3.40-3.25 (m, 1H, H-11), 2.05-1.92 (m, 2H, CH_2), 1.84-1.68 (m, 2H, CH_2), 1.65-1.53 (m, 1H, CH_2), 1.47-1.19 (m, 11H, CH_2, H-1, H-3).

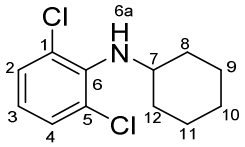
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 151.2 (d, $^2J_{CP}$ = 9.0 Hz, C_q, C-10), 134.2 (d, $^4J_{CP}$ = 2.1 Hz, CH_{arom}, C-8), 134.0 (d, $^2J_{CP}$ = 7.3 Hz, CH_{arom}, C-6), 114.8 (d, $^3J_{CP}$ = 14.2 Hz, CH_{arom}, C-7), 111.5 (d, $^3J_{CP}$ = 12.2 Hz, CH_{arom}, C-9), 107.5 (d, $^1J_{CP}$ = 182.6 Hz, C_q, C-5), 62.0 (d, $^2J_{CP}$ = 4.9 Hz, CH_2, C-2, C-4), 50.8 (s, CH, C-11), 32.8 (s, CH_2, C-12, C-16), 26.0 (s, CH_2, C-14), 24.7 (s, CH_2, C-13, C-15), 16.4 (d, $^3J_{CP}$ = 6.7 Hz, CH_3, C-1, C-3).

^{31}P-NMR (121.58 MHz, $CDCl_3$): δ = 22.06 (s).

GC-MS: t_R = 7.326 min, *m/z* (%) = 311.1 (M^+, 40), 268.1 (100), 194.0 (17), 156.0 (16), 98.1 (16).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{26}NO_3P$: 311.1650; found: 311.1669.

6.6.1.10 2,6-Dichloro-*N*-cyclohexylaniline (9)



according to GP-B:

49.6 mg (300 µmol, 1.0 eq) 2,6-dichloroaniline, 47.2 µL (450 µmol, 1.5 eq) cyclohexanone, 18 min reaction time, column chromatography (cyclohexane/EtOAc = 400:1 (v/v), size: 14 x 0.8 cm).

yield: 66.0 mg (270 µmol, 90%) colorless, viscous liquid

according to GP-C:

165 mg (1.00 mmol, 2.0 eq) 2,6-dichloroaniline, 116 µL (1.1 mmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 300:1, 150:1 (v/v), size: 8 x 2.7 cm).

yield: 213 mg (872 µmol, 87%) light-yellow, viscous liquid.

$C_{12}H_{15}Cl_2N$ [244.16]

R_f = 0.84 (cyclohexane/EtOAc = 9:1% (v/v)) (254 nm).

IR (ATR): 3355, 2926, 2851, 1580, 1493, 1447, 1421, 1249, 1139, 1081, 889, 836, 805, 762, 723, 623, 528 cm^{-1}.

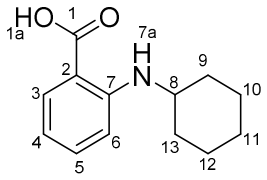
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.22 (d, $^3J_{HH}$ = 8.0 Hz, 2H, H-2, H-4), 6.75 (t, $^3J_{HH}$ = 8.0 Hz, 1H, H-3), 3.98–3.37 (m, 2H, H-6a, H-7), 2.03–1.88 (m, 2H, CH_2), 1.82–1.68 (m, 2H, CH_2), 1.67–1.53 (m, 1H, CH_2), 1.40–1.08 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 142.1 (C_q, C-6), 128.8 (C-2, C-4), 126.7 (C_q, C-1, C-5), 121.5 (C-3), 55.4 (C-7), 34.5 (C-8, C-12), 25.9 (CH_2), 25.2 (CH_2).

GC-MS: t_R = 6.481 min, *m/z* (%) = 243.1 (M^+, 31), 200.0 (100), 161.0 (43), 117.0 (16).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{12}H_{15}Cl_2N$: 243.0582; found: 243.0582.

6.6.1.11 2-(Cyclohexylamino)benzoic acid (12)



according to GP-B:

42.0 mg (300 µmol, 1.0 eq) anthranilic acid, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 10 min reaction time, column chromatography (cyclohexane/EtOAc/AcOH = 10:1:1% (v/v/v), size: 15 x 0.8 cm).

yield: 59.9 mg (273 µmol, 91%) light-yellow solid.

according to GP-C:

140 mg (1.00 mmol, 1.0 eq) anthranilic acid, 115 µL (1.1 mmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc/AcOH = 10:1:1% (v/v/v), size: 15 x 3.5 cm).

yield: 200 mg (912 µmol, 91%) light-yellow solid.

$C_{13}H_{17}NO_2$ [219.28]

R_f = 0.18 (cyclohexane/EtOAc/AcOH = 10:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: orange-green).

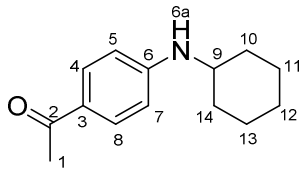
mp: 116 °C

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.42 (bs, 1H, H-1a), 8.20–7.64 (m, 2H, H-7a, CH_{arom}), 7.31 (t, $^3J_{HH}$ = 7.1 Hz, 1H, CH_{arom}), 6.74 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.50 (t, $^3J_{HH}$ = 7.4 Hz, 1H, CH_{arom}), 3.50–3.32 (m, 1H, C-8), 2.01–1.81 (m, 2H, CH_2), 1.76–1.48 (m, 3H, CH_2), 1.48-1.09 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 170.1 (C-1, C_q), 150.0 (C-7, C_q), 134.4 (CH_{arom}), 131.9 (CH_{arom}), 113.7 (CH_{arom}), 111.6 (CH_{arom}), 109.7 (C-2, C_q), 49.4 (C-8), 32.3 (C-9, C-13), 25.4 (C-11), 24.0 (C-10, C-12).

The spectra were in accordance with the previously reported data.[274]

6.6.1.12 1-(4-(Cyclohexylamino)phenyl)ethan-1-one (8)



according to GP-A:

138 mg (1.00 mmol, 1.0 eq) 4-aminoacetophenone, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 21 h reaction time, column chromatography (cyclohexane/EtOAc = 40:1 (v/v), size: 13 x 2.8 cm).

yield: 62.5 mg (288 µmol, 29%) light-orange, sticky solid.

according to GP-B:

41.4 mg (300 µmol, 1.0 eq) 4-aminoacetophenone, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 9:1 (v/v), size: 15 x 0.8 cm).

yield: 51.5 mg (237 µmol, 79%) light-yellow solid.

$C_{14}H_{19}NO$ [217.31]

R_f = 0.43 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm).

mp: 115-117 °C

IR (ATR): 3324, 2924, 2914, 2852, 1648, 1584, 1569, 1451, 1431, 1359, 1339, 1279, 1257, 1170, 1147, 1106, 1069, 953, 823, 611, 568, 498, 468 cm^{-1}.

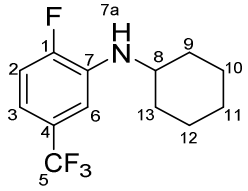
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.79 (d, $^3J_{HH}$ = 8.7 Hz, 2H, H-4, H-8), 6.52 (d, $^3J_{HH}$ = 8.7 Hz, 2H, C-5, H-7), 4.47–3.85 (m, 1H, H-6a), 3.42–3.24 (m,1H, H-9), 2.48 (s, 3H, H-1), 2.12–1.96 (m, 2H, CH_2), 1.86–1.58 (m, 3H, CH_2), 1.49–1.07 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 196.3 (C_q, C-2), 151.5 (C_q, C-6), 131.0 (C-4, C-6), 126.3 (C_q, C-3), 111.6 (C-5, C-7), 51.4 (C-9), 33.2 (CH_2), 26.0 (C-1), 25.8 (CH_2), 24.9 (CH_2).

GC-MS: t_R = 7.312 min, *m/z* (%) = 217.1 (M^+, 53), 174.1 (100), 146.1 (23), 120.0 (22).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{19}NO$: 217.1467; found: 217.1470.

6.6.1.13 *N*-Cyclohexyl-2-fluoro-5-(trifluoromethyl)aniline (16)



according to GP-A:

134 µL (1.0 mmol, 1.0 eq) 2-fluoro-5-(trifluoromethyl)aniline, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 3 h reaction time, column chromatography (cyclohexane, size: 9.5 x 3.6 cm).
yield: 187 mg (717 µmol, 72%) colorless, viscous liquid.

according to GP-B:

40.2 µL (300 µmol, 1.0 eq) 2-fluoro-5-(trifluoromethyl)aniline, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 10 min reaction time, column chromatography (cyclohexane/EtOAc = 300:1 (v/v), size: 15 x 0.8 cm).
yield: 53.2 mg (204 µmol, 68%) colorless, viscous liquid.

according to GP-C:

134 µL (1.0 mmol, 1.0 eq) 2-fluoro-5-(trifluoromethyl)aniline, 116 µL (1.1 mmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane, size: 15.5 x 3.0 cm, 25 g silica gel).
yield: 174 mg (666 µmol, 67%) light-yellow, viscous liquid.

$C_{13}H_{15}F_4N$ [261.26]

R_f = 0.85 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, $KMnO_4$: yellow).

IR (ATR): 3435, 2932, 2857, 1624, 1534, 1444, 1351, 1322, 1302, 1283, 1259, 1240, 1195, 1161, 1091, 1066, 934, 853, 805, 658, 625, 610 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.07-6.94 (m, 1H, C-2), 6.91-6.78 (m, 2H, C-3, C-6), 3.95 (bs, 1H, H-7a), 3.41-3.19 (m, 1H, H-8), 2.13-1.93 (m, 2H, CH_2), 1.87-1.57 (m, 3H, CH_2), 1.51-1.10 (m, 6H, CH_2).

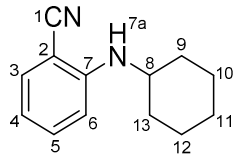
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 153.0 (d, $^1J_{CF}$ = 243.1 Hz, C_q, C-1), 136.4 (d, $^2J_{CF}$ = 12.3 Hz, C_q, C-7), 127.3 (dd, $^2J_{CF}$ = 32.1 Hz, $^4J_{CF}$ = 3.2 Hz, C_q, C-4), 124.4 (d, $^1J_{CF}$ = 271.9 Hz, CF_3), 114.7 (d, $^2J_{CF}$ = 20.3 Hz, CH_{arom}, C-2), 113.3-112.7 (m, CH_{arom}, C-3), 109.0-108.7 (m, CH_{arom}, C-6), 51.4 (s, CH, C-8), 33.3 (s, CH_2, C-9, C-13), 25.9 (s, CH_2, C-11), 25.0 (s, CH_2, C-10, C-12).

^{19}F-NMR (282.47 MHz, $CDCl_3$): δ = -62.07, -132.19.

GC-MS: t_R = 5.518 min, *m/z* (%) = 261.1 (M^+, 28), 218.0 (100), 179.0 (23), 55.1 (12).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{15}F_4N$: 261.1141; found: 261.1145.

6.6.1.14 2-(Cyclohexylamino)benzonitrile (17)



according to GP-A:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 15 h reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 11 x 2.6 cm).

yield: 188 mg (938 µmol, 94%) colorless solid.

according to GP-B:

36.2 mg (300 µmol, 1.0 eq) 2-aminobenzonitrile, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 18 min reaction time, column chromatography (cyclohexane/EtOAc = 100:1 (v/v), size: 16 x 0.8 cm).

yield: 56.2 mg (281 µmol, 94%) colorless solid.

according to GP-C:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 116 µL (1.10 mmol, 1.1 eq) cyclohexanone, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 70:1 (v/v), size: 7 x 2.7 cm).

yield: 182 mg (910 µmol, 91%) light-yellow solid.

$C_{13}H_{16}N_2$ [200.29]

R_f = 0.32 (cyclohexane/EtOAc = 30:1 (v/v)) (254 nm, 366 nm, cold CAM: orange).

mp: 61-62 °C

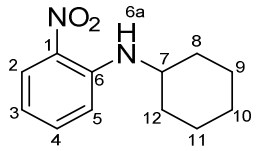
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.43-7.28 (m, 2H, H-3, H-5), 6.72-6.55 (m, 2H, H-4, H-6), 4.53-4.35 (m, 1H, H-7a), 3.43-3.26 (m, 1H, H-8), 2.11-1.96 (m, 2H, H-9a, H-13a), 1.87-1.73 (m, 2H, H-10a, H-12a), 1.72-1.56 (m, 1H, H-11a), 1.48-1.15 (m, 5H, H-9b, H-10b, H-11b, H-12b, H-13b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 149.6 (C_q, C-7), 134.2/133.0 (C-3/C-5), 118.2 (C_q, C-2), 116.0/111.1 (C-4/C-6), 95.6 (C_q, C-1), 51.5 (CH, C-8), 33.1 (CH_2, C-9, C-13), 25.8 (CH_2, C-11), 24.9 (CH_2, C-10, C-12).

GC-MS: t_R = 6.480 min, *m/z* (%) = 200.2 (27, M^+), 157.1 (100), 118.1 (20).

The spectra were in accordance with the previously reported data.[275]

6.6.1.15 *N*-Cyclohexyl-2-nitroaniline (11)



according to GP-A:

141 mg (1.00 mmol, 1.0 eq) 2-nitroaniline, 157 µL (1.5 mmol, 1.5 eq) cyclohexanone, 21 h reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 11 x 2.8 cm).
yield: 162 mg (735 µmol, 74%) bright-orange solid.

according to GP-B:

42.3 mg (300 µmol, 1.0 eq) 2-nitroaniline, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 30 min reaction time, column chromatography (cyclohexane/EtOAc = 80:1 (v/v), size: 7.5 x 2.2 cm, 10 g silica gel).
yield: 62.1 mg (282 µmol, 94%) bright orange solid.

according to GP-C:

42.3 mg (300 µmol, 1.0 eq) 2-nitroaniline, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 30 min reaction time, column chromatography (cyclohexane/EtOAc = 80:1 (v/v), size: 7.5 x 2.2 cm, 10 g silica gel).
yield: 51.5 mg (234 µmol, 78%) bright-orange solid.

$C_{12}H_{16}N_2O_2$ [220.27]

R_f = 0.70 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, VIS: orange).

mp: 101-103 °C

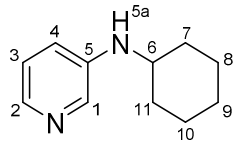
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.23–8.04 (m, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 1.4 Hz, 2H, H-2, H-6a), 7.45-7.32 (m, 1H, H-4), 6.86 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-5), 6.64-6.51 (m, 1H, H-3), 3.59-3.40 (m, 1H, H-7), 2.14-1.96 (m, 2H, CH_2), 1.90-1.72 (m, 2H, CH_2), 1.72-1.57 (m, 1H, CH_2), 1.52-1.21 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 144.9 (C_q, C-6), 136.1 (CH_{arom}, C-4), 131.7 (C_q, C-1), 127.2 (CH_{arom}, C-2), 114.9 (CH_{arom}, C-3), 114.3 (CH_{arom}, C-5), 51.1 (CH, C-7), 32.9 (CH_2, C-8, C-12), 25.7 (CH_2, C-10), 24.7 (CH_2, C-9, C-11).

GC-MS: t_R = 7.116 min, *m/z* (%) = 220.1 (M^+, 39), 177.1 (100), 130.1 (35), 106.0 (36).

The spectra were in accordance with the previously reported data.[276]

6.6.1.16 *N*-Cyclohexylpyridin-3-amine (18)



according to GP-B:

28.5 mg (300 µmol, 1.0 eq) 3-aminopyridine, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 11 min reaction time, column chromatography (CH_2Cl_2/MeOH = 80:1, size: 13 x 1 cm).
yield: 44.2 mg (251 µmol, 84%) light-beige solid.

$C_{11}H_{16}N_2$ [176.26]

R_f = 0.20 (CH_2Cl_2/MeOH = 80:1 (v/v)) (254 nm, 366 nm, $KMnO_4$: yellow).

mp: 86-89 °C

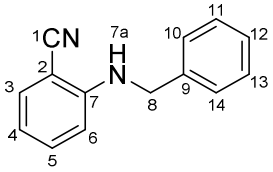
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.98 (d, $^4J_{HH}$ = 1.5 Hz, 1H, H-1), 7.89 (d, $^3J_{HH}$ = 3.9 Hz, 1H, H-2), 7.09–6.97 (m, 1H, H-3), 6.89–6.77 (m, 1H, H-4), 3.58 (bs, 1H, H-5a), 3.32–3.15 (m, 1H, H-6), 2.10–1.96 (m, 2H, H-7a, H-11a), 1.84–1.57 (m, 3H, H-8a, H-9a, H-10a), 1.46–1.05 (m, 5H, H-7b, H-8b, H-9b, H-10b, H-11b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 143.5 (C_q, C-5), 138.3 (C-2), 136.5 (C-1), 123.8 (C-3), 118.8 (C-4), 51.6 (C-6), 33.3 (C-7, C-11), 25.9 (C-9), 25.0 (C-8, C-10).

GC-MS: t_R = 6.198 min, *m/z* (%) = 176.1 (37, M^+), 133.1 (100), 119.0 (15), 94.0 (14).

The spectra were in accordance with the previously reported data.[198]

6.6.1.17 2-(Benzylamino)benzonitrile (24)



according to GP-A:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 155 µL (1.5 mmol, 1.5 eq) benzaldehyde, 15 h reaction time, column chromatography (cyclohexane/EtOAc = 30:1 (v/v), size: 11 x 2.6 cm).
yield: 167 mg (803 µmol, 80%) colorless solid.

$C_{14}H_{12}N_2$ [208.26]

R_f = 0.15 (cyclohexane/EtOAc = 30:1 (v/v)) (254 nm, 366 nm, cold CAM: violet-grey).

mp: 110-112 °C

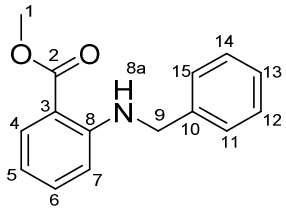
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.47-7.28 (m, 7H, H-3, H-5, H-10, H-11, H-12, H-13, H-14), 6.75-6.58 (m, 2H, H-4, H-6), 5.03 (bs, 1H, H-7a), 4.44 (bs, 2H, H-8).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 150.2 (C_q, C-7), 137.8 (C_q, C-9), 134.4 (CH_{arom}), 132.9 (CH_{arom}), 129.0 (CH_{arom}, C-11, C-13), 127.8 (CH_{arom}), 127.3 (CH_{arom}, C-10, C-14), 118.0 (C_q, C-1), 117.0 (CH_{arom}, C-4), 111.2 (CH_{arom}, C-6), 96.1 (C_q, C-2), 47.6 (CH_2, C-8).

GC-MS: t_R = 7.045 min, *m/z* (%) = 208.2 (M^+, 49), 207.1 (35), 91.0 (100).

The spectra were in accordance with the previously reported data.[277]

6.6.1.18 Methyl 2-(benzylamino)benzoate (25)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 33.9 µL (330 µmol, 1.1 eq) benzaldehyde, 20 min reaction time, column chromatography (cyclohexane/EtOAc = 100:1, 70:1 (v/v), size: 12 x 2.4 cm, 10 g silica gel).
yield: 61.6 mg (255 µmol, 85%) light-yellow, viscous liquid.

$C_{15}H_{15}NO_2$ [241.29]

R_f = 0.72 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm).

IR (ATR): 3380, 2945, 1672, 1601, 1576, 1516, 1494, 1453, 1434, 1323, 1287, 1225, 1187, 1166, 1147, 1102, 1077, 753, 728, 703, 693, 595, 566, 527, 458 cm^{-1}.

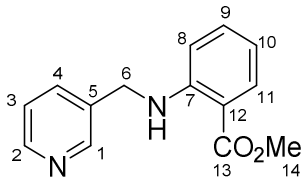
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.09 (bs, 1H, H-8a), 7.84 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{4}J_{HH}$ = 1.3 Hz, 1H, CH_{arom}), 7.36–7.12 (m, 6H, CH_{arom}), 6.63–6.45 (m, 2H, CH_{arom}), 4.37 (s, 2H, H-9), 3.78 (s, 3H, H-1).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.2 (C_q, C-2), 151.1 (C_q, C-8), 139.0 (C_q, C-10), 134.7 (CH_{arom}), 131.8 (CH_{arom}), 128.8 (CH_{arom}), 127.3 (CH_{arom}), 127.2 (CH_{arom}), 115.0 (CH_{arom}), 111.8 (CH_{arom}), 110.4 (C_q, C-3), 51.6 (C-1), 47.2 (C-9).

GC-MS: t_R = 7.134 min, *m/z* (%) = 241.1 (M^+, 74), 208.1 (100), 180.1 (48), 132.0 (25), 106.1 (45), 91.1 (99).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{15}NO_2$: 241.1103; found: 241.1108.

6.6.1.19 Methyl 2-((pyridin-3-ylmethyl)amino)benzoate (26)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 31.6 µL (330 µmol, 1.1 eq) 3-pyridinecarboxaldehyde, 10 min reaction time, column chromatography (cyclohexane/EtOAc = 3:1, 2:1 (v/v), size: 15 x 0.8 cm).
yield: 61.1 mg (252 µmol, 84%) light-yellow, viscous liquid.

$C_{14}H_{14}N_2O_2$ [242.28]

R_f = 0.16 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, 366 nm).

IR (ATR): 3473, 3365, 2950, 2846, 1680, 1607, 1586, 1516, 1487, 1452, 1297, 1238, 1188, 1161, 1094, 1024, 962, 748, 699, 665, 526, 499 cm^{-1}.

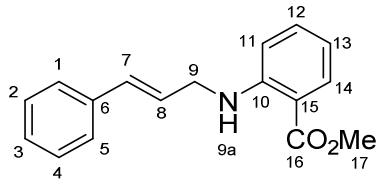
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.70–8.62 (m, 1H, CH_{arom}), 8.55 (d, $^3J_{HH}$ = 3.9 Hz, 1H, CH_{arom}), 8.23 (bs, 1H, H-6a), 7.96 (dd, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, CH_{arom}), 7.70 (d, $^3J_{HH}$ = 7.8 Hz, 1H, CH_{arom}), 7.39–7.23 (m, 2H, CH_{arom}), 6.71–6.58 (m, 2H, CH_{arom}), 4.50 (d, $^3J_{HH}$ = 5.5 Hz, 2H, H-6), 3.89 (s, 3H, C-14).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.2 (C_q, C-13), 150.6 (C_q, C-7), 149.1 (CH_{arom}), 148.8 (CH_{arom}), 134.9 (CH_{arom}), 134.8 (CH_{arom}), 134.5 (C_q, C-5), 131.9 (CH_{arom}), 123.7 (CH_{arom}), 115.5 (CH_{arom}), 111.6 (CH_{arom}), 110.71 (C_q, C-12), 51.7 (C-14), 44.7 (C-6).

GC-MS: t_R = 7.374 min, *m/z* (%) = 242.1 (M^+, 100), 209.1 (72), 181.1 (65), 132.0 (37), 107.1 (44), 92.1 (55), 65.1 (38).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{14}N_2O_2$: 242.1055; found: 242.1058.

6.6.1.20 Methyl 2-(cinnamylamino)benzoate (19)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 42.4 mg (330 µmol, 1.1 eq) cinnamic aldehyde, 10 min reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 15 x 0.8 cm).

yield: 65.0 mg (243 µmol, 81%) yellow-orange, amorphous solid.

$C_{17}H_{17}NO_2$ [267.33]

mp: 33-35 °C

R_f = 0.88 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

IR (ATR): 3386, 3362, 3023, 3003, 2947, 2844, 1675, 1605, 1575, 1513, 1436, 1318, 1278, 1254, 1219, 1191, 1163, 1149, 1096, 1081, 1048, 963, 743, 730, 691, 664, 528 cm^{-1}.

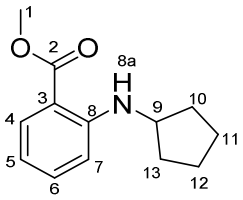
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.10-7.75 (m, 2H, NH, ArH), 7.41-7.14 (m, 6H, ArH), 6.71 (d, $^3J_{HH}$ = 8.5 Hz, 1H), 6.66-6.53 (m, 2H), 6.37-6.22 (m, 1H), 4.05 (d, $^3J_{HH}$ = 11.5 Hz, 2H, CH_2), 3.85 (s, 3H, CH_3).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.2 (C-16, C_q), 151.1 (C-10, C_q), 137.0 (C-6, C_q), 134.7, 131.8, 131.6, 128.7 (ArH), 127.6, 126.51 (ArH), 126.47, 115.0 (ArH), 111.8 (ArH), 110.4 (C-15, C_q), 51.6 (C-17), 45.1 (C-9).

GC-MS: t_R = 7.888 min, *m/z* (%) = 267.1 (60, M^+), 234.1 (30), 132.1 (28), 117.1 (100), 91.1 (56).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{17}NO_2$: 267.1259; found: 267.1273.

6.6.1.21 Methyl 2-(cyclopentylamino)benzoate (27)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 29.5 µL (330 µmol, 1.1 eq) cyclopentanone, 15 min reaction time, column chromatography (cyclohexane/EtAOc = 250:1 (v/v), size: 8.0 x 0.8 cm).

yield: 63.0 mg (287 µmol, 96%) colorless liquid.

$C_{13}H_{17}NO_2$ [219.28]

R_f = 0.20 (cyclohexane/EtOAc = 200:1 (v/v)) (254 nm, 366 nm, cold CAM: orange-green).

IR (ATR): 3353, 2950, 2869, 1680, 1606, 1579, 1514, 1457, 1435, 1331, 1256, 1231, 1184, 1161, 1138, 1086, 1077, 1046, 745, 702, 526 cm^{-1}.

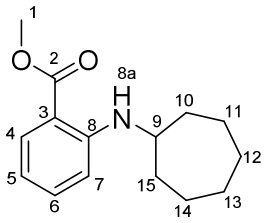
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.98–7.65 (m, 2H, H-8a, CH_{arom}), 7.40–7.28 (m, 1H, CH_{arom}), 6.72 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.55 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.95–3.75 (m, 4H, H-1, H-9), 2.14–1.93 (m, 2H, CH_2), 1.88–1.49 (m, 6H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.3 (C_q, C-2), 150.9 (C_q, C-8), 134.6 (CH_{arom}), 131.8 (CH_{arom}), 114.2 (CH_{arom}), 112.2 (CH_{arom}), 109.8 (C_q, C-3), 53.8 (C-9), 51.5 (C-1), 33.6 (CH_2), 24.2 (CH_2).

GC-MS: t_R = 6.489 min, *m/z* (%) = 219.1 (M^+, 57), 204.1 (32), 190.1 (55), 186.1 (39), 158.0 (100), 130.0 (35), 77.0 (23).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{17}NO_2$: 219.1259; found: 219.1260.

6.6.1.22 Methyl 2-(cycloheptylamino)benzoate (28)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 39.3 µL (330 µmol, 1.1 eq) cycloheptanone, 15 min reaction time, column chromatography (cyclohexane/EtAOc = 200:1 (v/v), size: 8.0 x 0.8 cm).

yield: 67.5 mg (273 µmol, 91%) colorless, very viscous liquid.

$C_{15}H_{21}NO_2$ [247.34]

R_f = 0.23 (cyclohexane/EtOAc = 200:1 (v/v)) (254 nm, 366 nm, cold CAM: orange-green).

IR (ATR): 3349, 2923, 2853, 1681, 1606, 1579, 1515, 1457, 1436, 1328, 1227, 1187, 1161, 1142, 1087, 1046, 841, 745, 702, 569, 525 cm^{-1}.

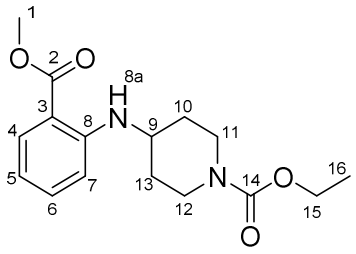
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.12–7.67 (m, 2H, H-8a, CH_{arom}), 7.39–7.28 (m, 1H, CH_{arom}), 6.73–6.46 (m, 2H, CH_{arom}), 3.85 (s, 3H, H-1), 3.66–3.47 (m, 1H, H-9), 2.09–1.89 (m, 2H, CH_2), 1.78–1.43 (m, 10H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.3 (C_q, C-2), 150.2 (C_q, C-8), 134.6 (CH_{arom}), 132.0 (CH_{arom}), 114.1 (CH_{arom}), 112.1 (CH_{arom}), 109.9 (C_q, C-3), 52.9 (C-9), 51.5 (C-1), 34.7 (CH_2), 28.4 (CH_2), 24.4 (CH_2).

GC-MS: t_R = 7.133 min, *m/z* (%) = 247.1 (M^+, 39), 190.1 (74), 158.0 (100).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{21}NO_2$: 247.1572; found: 247.1589.

6.6.1.23 Ethyl 4-((2-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate (29)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 50.7 µL (330 µmol, 1.1 eq) ethyl 4-oxopiperidine-1-carboxylate, 15 min reaction time, column chromatography (cyclohexane/EtOAc = 7:1, 6:1 (v/v), size: 6 x 2.2 cm).
yield: 88.3 mg (288 µmol, 96%) light-yellow, viscous liquid.

$C_{16}H_{22}N_2O_4$ [306.36]

R_f = 0.30 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: orange-green).

IR (ATR): 3342, 2981, 2948, 2856, 1680, 1605, 1581, 1517, 1455, 1431, 1379, 1330, 1238, 1220, 1192, 1155, 1128, 1096, 1075, 1032, 974, 848, 747, 703, 559 cm^{-1}.

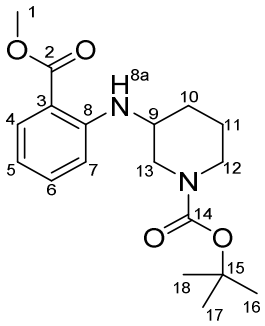
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99–7.69 (m, 2H, H-4, H-8a), 7.31 (t, $^3J_{HH}$ = 7.8 Hz, 1H, H-6), 6.67 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.56 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 4.12 (q, $^3J_{HH}$ = 7.0 Hz, 2H, H-15), 4.04-3.87 (m, 2H, H-11a, H-12a), 3.82 (s, 3H, H-1), 3.65–3.49 (m, 1H, H-9), 3.22–3.00 (m, 2H, H-11b, H-12b), 2.10–1.89 (m, 2H, H-10a, H-13a), 1.62–1.39 (m, 2H, H-10b, H-13b), 1.25 (t, $^3J_{HH}$ = 7.0 Hz, 3H, H-16).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.1 (C_q, C-2), 155.6 (C_q, C-14), 149.9 (C_q, C-8), 134.6 (CH_{arom}), 132.0 (CH_{arom}), 114.7 (CH_{arom}), 111.5 (CH_{arom}), 110.1 (C_q, C-3), 61.4 (C-15), 51.5 (C-1), 48.5 (C-9), 42.2 (C-11, C-12), 31.7 (C-10, C-13), 14.7 (C-16).

GC-MS: t_R = 8.135 min, *m/z* (%) = 306.1 (M^+, 50), 158.1 (45), 156.1 (45), 155.1 (100), 151.1 (41), 146.1 (32), 126.0 (43), 82.1 (33), 56.0 (37).

HR-MS: *m/z* $[M]^+$ calcd for $C_{16}H_{22}N_2O_4$: 306.1580; found: 306.1595.

6.6.1.24 *tert*-Butyl 3-((2-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate (20)



according to GP-A:

131 µL (1.00 mmol, 1.0 eq) methyl anthranilate, 305 mg (1.50 mmol, 1.5 eq) 1-Boc-3-piperidone, 44 h reaction time, column chromatography (cyclohexane/EtOAc = 20:1 (v/v), size: 20 x 1.2 cm).

yield: 220 mg (657 µmol, 66%) light-yellow, very viscous liquid.

$C_{18}H_{26}N_2O_4$ [334.42]

R_f = 0.49 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: crimson).

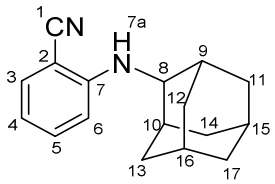
IR (ATR): 3342, 2974, 2935, 2857, 1681, 1606, 1581, 1517, 1457, 1421, 1365, 1331, 1255, 1237, 1141, 1076, 973, 867, 747, 704, 526 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.97-7.70 (m, 2H, H-8a, CH_{arom}), 7.34 (dd, $^3J_{HH}$ = 11.3 Hz, $^4J_{HH}$ = 4.2 Hz, 1H, CH_{arom}), 6.80 (d, $^3J_{HH}$ = 8.2 Hz, 1H, CH_{arom}), 6.59 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 4.41–3.61 (m, 5H, H-1, CH_2), 3.54–3.37 (m, 1H, H-9), 3.26–2.56 (m, 2H, CH_2), 2.18-1.95 (m, 1H, CH_2), 1.89–1.27 (m, 12H, CH_2, H-16, H-17, H-18).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.2 (C_q, C-2), 154.8 (C_q, C-14), 150. (C_q, C-8), 134.8 (CH_{arom}), 131.9 (CH_{arom}), 115.0 (CH_{arom}), 111.8 (CH_{arom}), 110.3 (C_q, C-3), 79.7 (C_q, C-15), 51.6 (CH_3), 48.8 (CH_2), 48.4 (CH_3), 44.1 (CH_2), 31.0 (CH_2), 28.5 (C-16, C-17, C-18), 23.8 (CH_2).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{18}H_{26}N_2O_4$: 334.1893; found 334.1894.

6.6.1.25 2-((-Adamantan-2-yl)amino)benzonitrile (30)



according to GP-A:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 228 mg (1.50 mmol, 1.5 eq) 2-adamantanone, 41 h reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 12 x 2.7 cm).

yield: 203 mg (803 µmol, 80%) colorless solid.

$C_{17}H_{20}N_2$ [252.36]

R_f = 0.30 (cyclohexane/EtOAc = 50:1 (v/v)) (254 nm, 366 nm, cold CAM: light violet).

mp: 96-100 °C

IR (ATR): 3379, 3368, 3074, 2901, 2888, 2845, 2215, 1603, 1573, 1513, 1463, 1449, 1322, 1285, 1164, 1129, 1064, 1026, 810, 799, 740, 503 cm^{-1}.

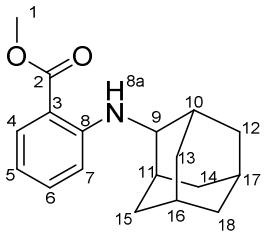
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.42–7.27 (m, 2H, H-3, H-5), 6.71–6.49 (m, 2H, H-4, H-6), 5.02–4.79 (m, 1H, H-7a), 3.69–3.54 (m, 1H, H-8), 2.09–1.55 (m, 14H, CH, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 149.5 (C_q, C-7), 134.2 (CH_{arom}), 132.9 (CH_{arom}), 118.2 (C_q, C-1), 116.0 (CH_{arom}), 111.2 (CH_{arom}), 95.8 (C_q, C-2), 56.5 (CH, C-8), 37.6 (CH_2, C-17), 37.3 (CH_2, C-11, C-12),31.6 (CH_2, C-13, C-14), 31.5 (CH, C-9, C-10), 27.4/27.2 (CH, C-15/C-16).

GC-MS: t_R = 7.817 min, *m/z* (%) = 252.2 (100, M^+), 135.1 (75), 118.1 (29), 79.0 (25).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{20}N_2$: 252.1626; found: 252.1627.

6.6.1.26 Methyl 2-((adamantan-2-yl)amino)benzoate (31)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 50.1 mg (330 µmol, 1.1 eq) 2-adamantanone, 24 min reaction time, column chromatography (cyclohexane/EtOAc = 150:1, 120:1 (v/v), size: 4 x 2.2 cm).

yield: 80.8 mg (283 µmol, 94%) light-yellow, waxy solid.

$C_{18}H_{23}NO_2$ [285.39]

R_f = 0.73 (cyclohexane/EtOAc = 9:1 (v/v)) (254 nm, 366 nm, cold CAM: crimson).

mp: 98-100 °C

IR (ATR): 3360, 2961, 2905, 2853, 1681, 1604, 1579, 1516, 1456, 1433, 1328, 1254, 1227, 1162, 1076, 1062, 796, 746, 701, 583, 551, 526, 497 cm^{-1}.

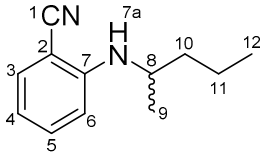
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.21 (bs, 1H, H-8a), 7.83 (d, $^3J_{HH}$ = 8.0 Hz, 1H, H-4), 7.23 (t, $^3J_{HH}$ = 7.8 Hz, 1H, H-6), 6.59 (d, $^3J_{HH}$ = 8.6 Hz, 1H, CH_{arom}), 6.44 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.79 (s, 3H, H-1), 3.66–3.52 (m,1H, H-9), 2.06–1.45 (m, 14H, CH_2, CH).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 168.3 (C_q, C-2), 149.3 (C_q, C-8), 133.5 (CH_{arom}), 130.9 (CH_{arom}), 112.8 (CH_{arom}), 110.6 (CH_{arom}), 108.5 (C_q, C-3), 55.0 (C-9), 50.4 (C-1), 36.7 (CH_2), 36.3 (CH), 30.8 (CH_2), 30.6 (CH), 26.4 (CH), 26.3 (CH).

GC-MS: t_R = 7.976 min, *m/z* (%) = 285.2 (M^+, 84), 270.1 (100), 252.1 (71), 132.0 (48), 119.0 (29), 77.0 (28).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{18}H_{23}NO_2$: 285.1729; found: 285.1740.

6.6.1.27 2-(Pentan-2-ylamino)benzonitrile (32)



according to GP-A:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 164 µL (1.5 mmol, 1.5 eq) 2-pentanone, 39 h reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 11 x 2.6 cm).

yield: 150 mg (795 µmol, 80%) light-yellow, sticky solid.

$C_{12}H_{16}N_2$ [188.27]

R_f = 0.31 (cyclohexane/EtOAc = 30:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

IR (ATR): 3407, 3363, 2960, 2931, 2872, 2210, 1604, 1575, 1512, 1461, 1380, 1324, 1289, 1167, 1042, 744, 568, 495 cm^{-1}.

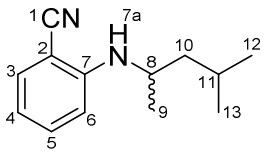
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.41-7.29 (m, 2H, H-3, H-5), 6.70-6.55 (m, 2H, H-4, H-6), 4.34 (bs, 1H, H-7a), 3.64-3.47 (m, 1H, H-8), 1.67-1.31 (m, 4H, H-10, H-11), 1.22 (d, $^3J_{HH}$ = 6.3 Hz, 3H, H-9), 0.94 (t, $^3J_{HH}$ = 7.1 Hz, 3H, H-12).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 149.9 (C_q, C-7), 134.3/133.0 (CH_{arom}, C-3/C-5), 118.2 (C_q, C-1), 116.0 (CH_{arom}, C-4), 111.0 (CH_{arom}, C-6), 95.7 (C_q, C-2), 48.3 (CH, C-8), 39.2 (CH_2, C-10), 20.7 (CH_3, C-9), 19.4 (CH_2, C-11), 14.1 (CH_3, C-12).

GC-MS: t_R = 5.877 *m/z* (%) = 188.2 (M^+, 9), 145.1 (100), 118.1 (11), 77.0 (6).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{12}H_{16}N_2$: 188.1313; found: 188.1315.

6.6.1.28 2-((4-Methylpentan-2-yl)amino)benzonitrile (33)



according to GP-A:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 191 µL (1.5 mmol, 1.5 eq) 4-methylpentan-2-one, 39 h reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 11 x 2.6 cm).

yield: 139 mg (685 µmol, 69%) colorless, amorphous solid.

$C_{13}H_{18}N_2$ [202.30]

R_f = 0.34 (cyclohexane/EtOAc = 30:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

mp: 30-32 °C

IR (ATR): 3356, 2959, 2918, 2863, 2839, 2214, 1604, 1574, 1516, 1464, 1432, 1366, 1324, 1292, 1268, 1165, 1035, 742, 567, 523, 489, 430 cm^{-1}.

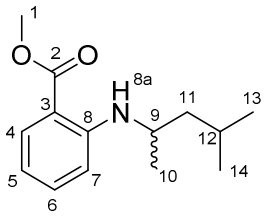
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.43-7.28 (m, 2H, H-3, H-5), 6.72-6.54 (2H, H-4, H-6), 4.30 (bs, 1H, H-7a), 3.70-3.50 (m, 1H, H-8), 1.84-1.66 (m, 1H, H-11), 1.58-1.41 (m, 1H, H-10a), 1.40-1.27 (m, 1H, H-10b), 1.19 (d, $^3J_{HH}$ = 6.9 Hz, 3H, H-9), 1.02-0.80 (m, 6H, H-12,H-13).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 149.9 (C_q, C-7), 134.3/133.3 (CH_{arom}, C-3/C-5), 118.2 (C_q, C-1), 116.0 (CH_{arom}, C-4), 110.9 (CH_{arom}, C-6), 95.7 (C_q, C-2), 46.6/46.5 (C-8/C-10), 25.1 (CH, C-11), 22.8/22.7 (C-12/C-13), 21.0 (CH_3, C-9).

GC-MS: t_R = 6.004 min, *m/z* (%) = 202.2 (M^+, 9), 145.1 (100), 118.0 (12), 77.0 (5).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{18}N_2$: 202.1470; found: 202.1484.

6.6.1.29 Methyl 2-((4-methylpentan-2-yl)amino)benzoate (34)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 42.2 µL (330 µmol, 1.1 eq) 4-methylpentan-2-one, 23 h reaction time, column chromatography (cyclohexane/EtOAc = 150:1, 120:1 (v/v), size: 12 x 2.4 cm, 10 g silica gel).
yield: 30.6 mg (130 µmol, 43%) light-yellow, viscous liquid.

$C_{14}H_{21}NO_2$ [235.33]

R_f = 0.77 (cyclohexane/EtOAc = 9:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

IR (ATR): 3346, 2955, 2927, 2870, 1682, 1606, 1579, 1516, 1458, 1435, 1325, 1249, 1224, 1189, 1163, 1132, 1083, 1047, 746, 703, 527 cm^{-1}.

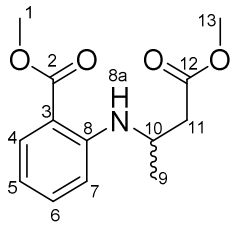
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.89 (d, $^3J_{HH}$ = 8.0 Hz, 1H, H-4), 7.66 (bs, 1H, H-8a), 7.33 (t, $^3J_{HH}$ = 7.8 Hz, 1H, H-6), 6.70 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.53 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.85 (s, 3H, H-1), 3.71–3.55 (m, 1H, H-9), 1.87–1.70 (m, 1H, H-12), 1.65–1.50 (m, 1H, H-11a), 1.44–1.15 (m, 4H, H-10, H-11b), 1.03–0.80 (m, 6H, H-13, H-14).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.3 (C_q, C-2), 150.8 (C_q, C-8), 134.7 (CH_{arom}), 132.0 (CH_{arom}), 114.0 (CH_{arom}), 111.6 (CH_{arom}), 109.7 (C_q, C-3), 51.5 (C-1), 46.7 (C-11), 45.8 (C-9), 25.2 (C-12), 22.9 (C-13, C-14), 21.1 (C-10).

GC-MS: t_R = 5.992 min, *m/z* (%) = 202.2 (M^+, 8), 145.1 (100), 118.1 (12), 77.0 (5).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{21}NO_2$: 235.1572; found: 235.1585.

6.6.1.30 Methyl 2-((4-methoxy-4-oxobutan-2-yl)amino)benzoate (35)



according to GP-B:

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 36.0 µL (330 µmol, 1.1 eq) methyl acetoacetate, 6 h reaction time, column chromatography (cyclohexane/EtOAc = 17:1 (v/v), size: 8 x 0.8 cm).

yield: 50.8 mg (202 µmol, 67%) colorless, viscous liquid.

$C_{13}H_{17}NO_4$ [251.28]

R_f = 0.55 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: orange-green).

IR (ATR): 3341, 2952, 1735, 1682, 1605, 1581, 1516, 1457, 1435, 1323, 1299, 1239, 1190, 1162, 1130, 1077, 1047, 1005, 748, 703, 527 cm^{-1}.

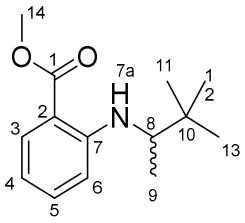
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.00–7.65 (m, 2H, H-8a, CH_{arom}), 7.41–7.30 (m, 1H, CH_{arom}), 6.75 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.59 (t, $^3J_{HH}$ = 7.3 Hz, 1H, CH_{arom}), 4.16–3.99 (m, 1H. C-10), 3.85 (s, 3H, CH_3), 3.69 (s, 3H, CH_3), 2.73 (dd, $^2J_{HH}$ = 15.1 Hz, $^3J_{HH}$ = 5.0 Hz, 1H, C-11a), 2.43 (dd, $^2J_{HH}$ = 15.1 Hz, $^3J_{HH}$ = 7.8 Hz, 1H, C-11b), 1.33 (d, $^3J_{HH}$ = 6.4 Hz, 3H, C-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 172.1 (C_q, C-12), 169.1 (C_q, C-2), 149.8 (C_q, C-8), 134.8 (CH_{arom}), 132.0 (CH_{arom}), 115.0 (CH_{arom}), 111.8 (CH_{arom}), 110.4 (C_q, C-3), 51.8 (CH_3), 51.6 (CH_3), 45.1 (C-10), 41.2 (C-11), 20.8 (C-9).

GC-MS: t_R = 6.570 min, *m/z* (%) = 251.1 (M^+, 13), 204.0 (7), 178.1 (49), 147.0 (10), 146.0 (100), 77.0 (11).

HR-MS: *m/z* $[M]^+$ calcd for $C_{13}H_{17}NO_4$: 251.1158; found: 251.1172.

6.6.1.31 Methyl 2-((3,3-dimethylbutan-2-yl)amino)benzoate (22)



according to GP-B (modified):

57.4 µL (450 µmol, 1.5 eq) pinacolone, 39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 450 µL (450 µmol, 1.5 eq) $BH_3 \cdot THF$ (1.0 M in THF), 139 µL (750 µmol, 2.5 eq) TMSOTf (used instead of TMSCl), left stirring in the thawing ice bath, 20 h reaction time, column chromatography (cyclohexane/EtOAc = 1:0, 120:1 (v/v), size: 14 x 2.5 cm, 15 g silica gel).
yield: 35.2 mg (150 µmol, 50%) yellow, viscous liquid.

$C_{14}H_{21}NO_2$ [235.33]

R_f = 0.23 (cyclohexane/EtOAc = 120:1 (v/v)) (254 nm, 366 nm, cold CAM: orange-yellow).

IR (ATR): 3345, 2955, 2871, 1680, 1606, 1580, 1518, 1457, 1435, 1370, 1350, 1321, 1254, 1232, 1189, 1161, 1119, 1080, 1046, 745, 702, 520 cm^{-1}.

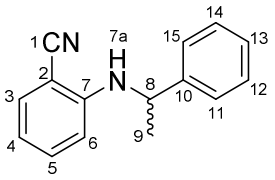
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.17–7.81 (m, 2H, CH_{arom}, H-7a), 7.37–7.27 (m, 1H, CH_{arom}), 6.73 (d, $^3J_{HH}$ = 8.6 Hz, 1H, CH_{arom}), 6.51 (t, $^3J_{HH}$ = 7.4 Hz, 1H, CH_{arom}), 3.85 (s, 3H, H-14), 3.45–3.30 (m, 1H, H-8), 1.14 (d, $^3J_{HH}$ = 6.5 Hz, 3H, H-9), 1.02 (s, 9H, H-11, H-12, H-13).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.5 (C_q, C-1), 151.3 (C_q, C-7), 134.7 (CH_{arom}), 132.0 (CH_{arom}), 113.8 (CH_{arom}), 111.6 (CH_{arom}), 109.5 (C_q, C-2), 56.6 (C-8), 51.6 (C-14), 35.0 (C_q, C-10), 26.7 (C-11, C-12, C-13), 15.7 (C-9).

GC-MS: t_R = 6.121 min, *m/z* (%) = 235.1 (M^+, 5), 178.1 (47), 146.1 (100), 77.0 (8).

HR-MS: *m/z* $[M]^+$ calcd for $C_{14}H_{21}NO_2$: 235.1572; found: 235.1602.

6.6.1.32 2-((1-Phenylethyl)amino)benzonitrile (21)



according to GP-A:

121 mg (1.00 mmol, 1.0 eq) 2-aminobenzonitrile, 178 µL (1.5 mmol, 1.5 eq) acetophenone, 39 h reaction time, column chromatography (cyclohexane/EtOAc = 50:1 (v/v), size: 11 x 2.6 cm).

yield: 156 mg (701 µmol, 70%) colorless, crystalline solid.

$C_{15}H_{14}N_2$ [222.29]

R_f = 0.27 (cyclohexane/EtOAc = 30:1 (v/v)) (254 nm, 366 nm, cold CAM: red-grey).

mp: 84 °C

IR (ATR): 3361, 3024, 2973, 2930, 2866, 2213, 1603, 1577, 1517, 1491, 1470, 1448, 1342, 1330, 1316, 1291, 1261, 1167, 1147, 1095, 1023, 947, 759, 741, 700, 608, 565, 541, 484, 445 cm^{-1}.

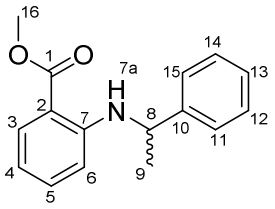
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.41-7.14 (m, 7H, H-3, H-5, H-11, H-12, H-13), 6.60 (t, $^3J_{HH}$ = 7.5 Hz, 1H, H-4), 6.41 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H-6), 4.89 (bs, 1H, H-7a), 4.62-4.47 (m, 1H, H-8), 1.57 (d, $^3J_{HH}$ = 6.7 Hz, 1H, H-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 149.4 (C_q, C-7), 143.8 (C_q, C-10), 134.2 (CH_{arom}), 132.7 (CH_{arom}), 129.0 (CH_{arom}), 127.4 (CH_{arom}), 125.7 (CH_{arom}), 118.1 (C_q, C-1), 116.8 (CH_{arom}), 112.1 (CH_{arom}), 96.0 (C_q, C-2), 53.4 (C-8), 25.0 (C-9).

GC-MS: t_R = 6.879 min, *m/z* (%) = 222.2 (M^+, 23), 207.1 (52), 129.1 (15), 118.1 (30), 105.1 (100), 77.0 (30), 51.0 (12).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{14}N_2$: 222.1157; found: 222.1167.

Methyl 2-((1-phenylethyl)amino)benzoate (23)



according to GP-B (modified):

39.2 µL (300 µmol, 1.0 eq) methyl anthranilate, 53.5 µL (450 µmol, 1.5 eq) acetophenone, 139 µL (750 µmol, 2.5 eq) TMSOTf, 300 µL (300 µmol, 1.0 eq) BH_3·THF, left stirring in the thawing ice bath, 23 h reaction time, column chromatography (cyclohexane/EtAOc = 80:1 (v/v), size: 8 x 0.8 cm).

yield: 66.3 mg (260 µmol, 87%) colorless, viscous liquid.

$C_{16}H_{17}NO_2$ [255.32]

R_f = 0.14 (cyclohexane/EtOAc = 120:1 (v/v)) (254 nm, 366 nm, CAM: orange-red).

IR (ATR): 3352, 3026, 2965, 2950, 2869, 1681, 1605, 1580, 1514, 1451, 1436, 1324, 1241, 1202, 1163, 1124, 1080, 1046, 1017, 747, 698, 557, 522 cm^{-1}.

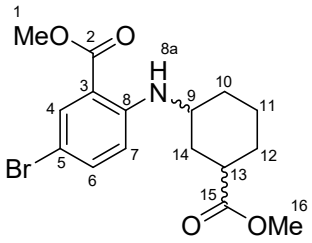
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.13 (bs, 1H, H-7a), 7.82 (dd, $^3J_{HH}$ = 8.0 Hz, $^4J_{HH}$ = 1.2 Hz, 1H, CH_{arom}), 7.33–7.03 (m, 6H, CH_{arom}), 6.45 (t, $^3J_{HH}$ = 7.6 Hz, 1H, CH_{arom}), 6.36 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 4.57–4.41 (m, 1H, H-8), 3.81 (s, 3H, H-16), 1.51 (d, $^3J_{HH}$ = 6.7 Hz, 3H, H-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.4 (C_q, C-1), 150.3 (C_q, C-7), 145.0 (C_q, C-10), 134.6 (CH_{arom}), 131.6 (CH_{arom}), 128.8 (CH_{arom}), 127.0 (CH_{arom}), 125.9 (CH_{arom}), 114.9 (CH_{arom}), 112.9 (CH_{arom}), 110.2 (C_q, C-2), 53.0 (C-8), 51.6 (C-16), 25.2 (C-9).

GC-MS: t_R = 7.037 min, *m/z* (%) = 255.1 (M^+, 32), 240.1 (78), 208.1 (100), 119.0 (33), 105.1 (77), 77.1 (39).

HR-MS (GC-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{17}NO_2$: 255.1259; found: 255.1267.

6.6.1.33 Methyl 5-bromo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (7)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 2.37 g (7.62 mmol, 1.0 eq) methyl 2-amino-5-bromobenzoate hydrobromide and the solid was dried in oil pump vacuum for 10 min. To the Schlenk flask were added 5 mL dry DMF, 1.25 g (8.00 mmol, 1.05 eq) methyl 3-oxocyclohexane-1-carboxylate[215,278] and 2.95 mL (22.9 mmol, 3.0 eq) TMSCl at RT. The glass stopper was replaced by a rubber septum. The yellow biphasic mixture was cooled to 0 °C and 7.60 mL (7.60 mol, 1.0 eq) BH_3·THF were added via syringe over a period of 42 min. The flask with the colorless solution with colorless precipitate was sealed and was left stirring for 48 min at 0 °C until full conversion of the starting material was detected by TLC. 10 mL H_2O were added carefully, followed by neutralization with 25% NH_4OH. The solvents were removed in vacuum, the residue was adsorbed on Celite™ (7 g, suspending in MeOH) and was purified via column chromatography (cyclohexane/EtOAc = 20:1, 15:1, 12:1 (v/v), 80 g silica gel, size: 18 x 3.5 cm).

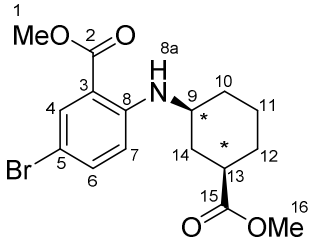
yield: 2.70 g (7.30 µmol, 96%) light-yellow, very viscous liquid.

ratio (*trans*/*cis*) = 57:43

$C_{16}H_{20}BrNO_4$ [370.24]

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}BrNO_4^+$: 369.0576; found: 369.0580

6.6.1.34 Methyl 5-bromo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (7)



R_f = 0.40 (cyclohexane/EtOAc = 5:1 (v/v)) (254 nm, 366 nm, CAM: orange).

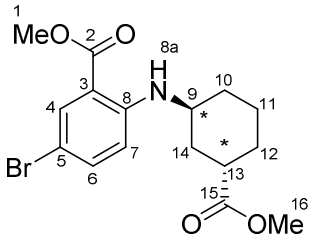
IR (ATR): 3339, 2946, 2859, 1733, 1685, 1598, 1574, 1502, 1435, 1407, 1308, 1245, 1201, 1127, 1108, 1074, 1015, 966, 898, 807, 787, 706, 644, 569, 519 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^{4}J_{HH}$ = 2.3 Hz, 1H, H-4), 7.71 (d, $^{3}J_{HH}$ = 7.2 Hz, 1H, H-8a), 7.37 (dd, $^{3}J_{HH}$ = 9.0 Hz, $^{4}J_{HH}$ = 2.2 Hz, 1H, H-6), 6.58 (d, $^{3}J_{HH}$ = 9.1 Hz, 1H, H-7), 3.84 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 3.44-3.25 (m, 1H, H-9), 2.51-2.30 (m, 2H, H-13, CH_2), 2.19-1.84 (m, 3H, CH_2), 1.52-1.12 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-15), 168.1 (C_q, C-2), 149.1 (C_q, C-8), 137.2 (C-6), 134.2 (C-4), 113.6 (C-7), 111.4 (C_q, C-5), 105.6 (C_q, C-3), 51.84 (CH_3), 51.81 (CH_3), 50.7 (C-9), 42.5 (C-13), 35.3 (C-14), 32.6 (C-10), 28.5 (C-12), 24.4 (C-11).

GC-MS: t_R = 8.819 min, *m/z* (%) = 371.1/369.1 (100, BP), 312.1/310.1 (56), 270.0/268.0 (53), 238.0/236.0 (53).

6.6.1.35 Methyl 5-bromo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (7)



R_f = 0.47 (cyclohexane/EtOAc = 5:1 (v/v)) (254 nm, 366 nm, CAM: orange).

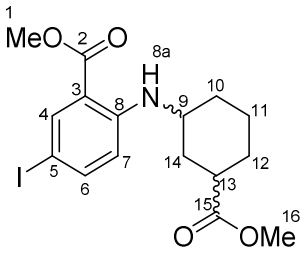
IR (ATR): 3344, 2936, 2860, 1730, 1683, 1575, 1502, 1435, 1314, 1215, 1188, 1113, 1079, 1042, 967, 883, 808, 788, 706 644, 564, 521 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^{4}J_{HH}$ = 2.3 Hz, 1H, H-4), 7.90 (d, $^{3}J_{HH}$ = 6.8 Hz, 1H, H-8a), 7.39 (dd, $^{4}J_{HH}$ = 9.0, $^{3}J_{HH}$ = 2.2 Hz, 1H, H-6), 6.67 (d, $^{3}J_{HH}$ = 9.1 Hz, 1H, H-7), 3.92-3.62 (m, 7H, C-9, 2 x CH_3), 2.77-2.63 (m, 1H, H-13), 2.14-1.96 (m, 1H, CH_2), 1.89-1.49 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.9 (C_q, C-15), 168.3 (C_q, C-2), 149.2 (C_q, C-8), 137.4 (C-6), 134.1 (C-4), 113.7 (C-7), 111.3 (C_q, C-5), 105.6 (C_q, C-3), 51.8 (2 x CH_3), 47.0 (C-9), 38.7 (C-13), 32.6 (C-14), 31.1 (C-10), 28.1 (C-12), 21.2 (C-11).

GC-MS: t_R = 8.647 min, *m/z* (%) = 371.1/369.1 (100, BP), 312.1/310.1 (57), 270.0/268.0 (55), 238.0/236.0 (57).

6.6.1.36 Methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (36)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 6.69 g (21.3 mmol, 1.0 eq) methyl 2-amino-5-iodobenzoate hydrochloride and the solid was dried in oil pump vacuum for 10 min. To the Schlenk flask were added 40 mL dry DMF followed by 3.50 g (22.4 mmol, 1.05 eq) methyl 3-oxocyclohexane-1-carboxylate[203,215] and 8.30 mL (64.0 mmol, 3.0 eq) TMSCl. The glass stopper was replaced by a rubber septum, the flesh-colored, biphasic mixture was cooled in an ice bath and 23.0 mL (23.0 mmol, 1.08 eq) BH_3·THF were added over a period of 60 min. The flask was sealed and the mixture was left stirring at 0 °C until full conversion of the starting material was detected by TLC (30 min). The light-yellow solution was carefully treated with 20 mL H_2O, followed by neutralization with 25% NH_4OH. The solvents were removed in vacuum, the residue was adsorbed on Celite™ (24 g, suspended in MeOH) and was purified via column chromatography (cyclohexane/EtOAc = 15:1, 10:1, 8:1, 6:1 (v/v), 300 g silica gel, size: 13 x 7.5 cm).

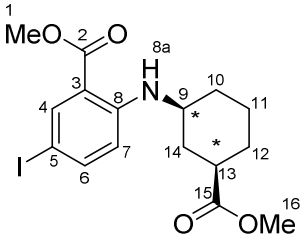
$C_{16}H_{20}INO_4$ [417.24]

yield: 8.61 g (20.6 mmol, 97%) yellow, very viscous liquid.

ratio (*trans*/*cis*) = 57:43

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}INO_4^+$: 417.0437; found: 417.0443.

6.6.1.37 Methyl 5-iodo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (36)



R_f = 0.46 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

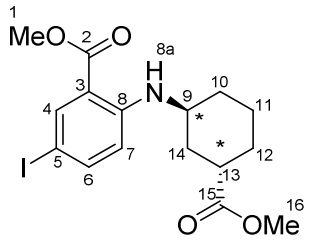
IR (ATR): 3338, 2937, 2859, 1732, 1682, 1591, 1571, 1500, 1435, 1403, 1320, 1308, 1247, 1201, 1127, 1106, 1075, 1015, 965, 899, 806, 788, 758, 697, 637, 572, 518 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.15 (d, $^{4}J_{HH}$ = 2.1 Hz, 1H, H-4), 7.73 (d, $^{3}J_{HH}$ = 7.2 Hz, 1H, NH), 7.51 (dd, $^{3}J_{HH}$ = 8.9 Hz, $^{4}J_{HH}$ = 1.9 Hz, 1H, H-6), 6.48 (d, $^{3}J_{HH}$ = 9.0 Hz, 1H, H-7), 3.83 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 3.41–3.25 (m, 1H, H-9), 2.52–2.28 (m, 2H, H-13, CH_2), 2.17–1.82 (m, 3H, CH_2), 1.52–1.11 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-15), 168.0 (C_q, C-2), 149.5 (C_q, C-8), 142.7 (CH_{arom}), 140.2 (CH_{arom}), 114.1 (CH_{arom}), 112.2 (C_q, C-3), 73.9 (C_q, C-5), 51.84 (CH_3), 51.79 (CH_2), 50.6 (C-9), 42.4 (C-13), 35.3 (CH_2), 32.6 (CH_2), 28.4 (CH_2), 24.4 (CH_2).

GC-MS: t_R = 9.333 min, *m/z* (%) = 417.1 (M^+, 100, BP), 358.1 (33), 316.0 (31), 284.0 (30), 207.1 (23).

6.6.1.38 Methyl 5-iodo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (36)



R_f = 0.51 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

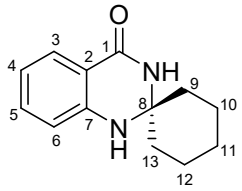
IR (ATR): 3342, 2946, 2859, 1730, 1681, 1591, 1571, 1499, 1435, 1404, 1314, 1215, 1188, 1140, 1111, 1081, 1042, 807, 788, 697, 644, 562, 520 cm^{-1}.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.16 (d, $^{4}J_{HH}$ = 2.0 Hz, 1H, H-4), 7.93 (d, $^{3}J_{HH}$ = 7.0 Hz, 1H, NH), 7.53 (dd, $^{3}J_{HH}$ = 8.9 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1H, C-6), 6.57 (d, $^{3}J_{HH}$ = 9.0 Hz, 1H, H-7), 3.94–3.62 (m, 7H, CH_3, C-9), 2.78–2.62 (m, 1H, H-13), 2.13–1.92 (m, 1H, CH_2), 1.90–1.51 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.9 (C_q, C-15), 168.2 (C_q, C-2), 149.6 (C_q, C-8), 142.8 (CH_{arom}), 140.1 (CH_{arom}), 114.3 (CH_{arom}), 112.1 (C_q, C-3), 73.9 (C_q, C-5), 51.8 (2 x CH_3), 46.9 (C-9), 38.7 (C-13), 32.5 (CH_2), 31.0 (CH_2), 28.1 (CH_2), 21.2 (CH_2).

GC-MS: t_R = 9.118 min, *m/z* (%) = 417.1 (M^+, 100, BP), 358.1 (30), 316.0 (43), 284.0 (30).

6.6.1.39 1'*H*-Spiro[cyclohexane-1,2'-quinazolin]-4'(3'*H*)-one (37)



according to GP-B:

41.7 mg (300 µmol, 1.0 eq) 2-aminobenzamide, 34.6 µL (330 µmol, 1.1 eq) cyclohexanone, 10 min reaction time, column chromatography (cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v), size: 14 x 0.8 cm).

yield: 53.4 mg (247 µmol, 82%) colorless solid.

according to GP-C:

139 mg (1.0 mmol, 1.0 eq) 2-aminobenzamide, 115 µL (1.1 mmol, 1.1 eq) cyclohexanone, 60 min reaction time, column chromatography (cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v), size: 15 x 3.5 cm).

yield: 199 mg (920 µmol, 92%) light-yellow solid.

$C_{13}H_{16}N_2O$ [216.28]

R_f = 0.36 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: crimson).

mp: 222-223 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.91 (bs, 1H, NH), 7.56 (d, $^3J_{HH}$ = 7.4 Hz, 1H, CH_{arom}), 7.21 (t, $^3J_{HH}$ = 7.2 Hz, 1H, CH_{arom}), 6.80 (d, $^3J_{HH}$ = 8.1 Hz, 1H, CH_{arom}), 6.69–6.52 (m, 2H, CH_{arom}, NH), 1.86–1.10 (m, 10H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 163.2 (C_q, C-1), 146.7 (C_q, C-7), 133.1 (CH_{arom}), 127.1 (CH_{arom}), 116.5 (CH_{arom}), 114.6 (CH_{arom}), 114.4 (C_q, C-2), 67.8 (C-8), 37.1 (C-9, C-13), 24.6 (C-11), 20.9 (C-10, C-12).

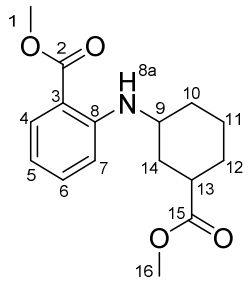
HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{18}N_2O$: 216.1263 ; found: 216.1253.

6.6.2 Synthesis of Phenazistatin A derivatives as Inhibitors of PhzA/B

6.6.2.1 Phenazistatin derivatives to increase PhzA/B affinity

6.6.2.1.1 H-Phenazistatin

6.6.2.1.1.1 Methyl 2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (51)

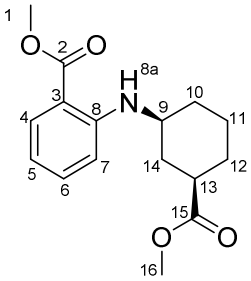


A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 69.8 µL (81.5 µmol, 1.0 eq) methyl anthranilate, 350 µL dry DMF, 100 mg (640 µmol, 1.2 eq) methyl 3-oxocyclohexane-1-carboxylate[203,215] and 172 µL (1.33 µmol, 2.5 eq) TMSCl. The mixture was cooled to 0 °C and 534 µL (534 µmol, 1.0 eq) BH_3·THF were added over a period of 3 min. The mixture was left stirring in the thawing ice bath for 3 d until full conversion of the starting material was detected by TLC. 3 mL H_2O were added and the mixture was stirred at RT for 23 h. The mixture was extracted with EtOAc (5 x 8 mL), the combined organic layers were dried over Na_2SO_4, filtrated through a Pore 3 sintered-glass frit and the solvent was removed in vacuum. The residue was adsorbed on 370 mg Celite™ (EtOAc) and the product was purified via column chromatography (size: 27 x 0.8 cm, cyclohexane/EtOAc = 25:1). Pure fractions of the isomers were obtained.[189]

$C_{16}H_{21}NO_4$ [291.35]

yield: 99.0 mg (340 µmol, 64%) colorless, viscous liquid

6.6.2.1.1.2 Methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (51)



R_f = 0.28 (cyclohexane/EtOAc = 9:1 (v/v)) (254 nm, 366 nm).

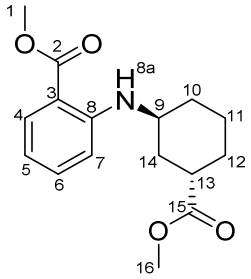
GC-MS: t_R = 7.721 min, *m/z* (%) = 291.1 (M^+, BP, 100), 232.1 (70), 190.1 (74), 158.0 (95).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.89 (dd, $^{3}J_{HH}$ = 8.0 Hz, $^{4}J_{HH}$ = 1.3 Hz, 1H, H-4), 7.73 (bs, 1H, H-8a), 7.39–7.28 (m, 1H, H-6), 6.70 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.55 (t, $^{3}J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.84 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 3.47–3.29 (m, 1H, H-9), 2.54–2.34 (m, 2H, H-13, H-14a), 2.20–2.08 (m, 1H, H-10a), 2.08–1.84 (m, 2H, H-11a, H-12a), 1.54–1.12 (m, 4H, H-10b, H-11b, H-12b, H-14b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.5 (C_q, C-15), 169.2 (C_q, C-2), 150.1 (C_q, C-8), 134.7 (C-6), 132.0 (C-4), 114.5 (CH_{arom}), 111.8 (CH_{arom}), 110.0 (C_q, C-3), 51.8 (CH_3), 51.6 (CH_3), 50.7 (C-9), 42.6 (C-13), 35.5 (C-14), 32.8 (C-10), 28.5 (C-12), 24.5 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{21}NO_4^+$: 291.1471; found: 291.1469.

6.6.2.1.1.3 Methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (51)



R_f = 0.33 (cyclohexane/EtOAc = 9:1 (v/v)) (254 nm, 366 nm).

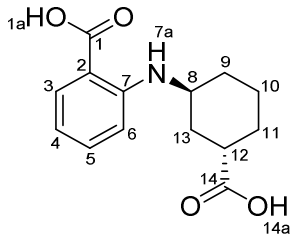
GC-MS: t_R = 7.605 min, *m/z* (%) = 291.1 (M^+, BP, 100), 232.1 (65), 207.0 (44), 190.0 (65), 158.0 (90).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.10–7.79 (m, 2H, H-4, H-8a), 7.41–7.28 (m, 1H, H-6), 6.77 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.56 (t, $^{3}J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.92–3.74 (m, 4H, H-9, CH_3), 3.68 (s, 3H, CH_3), 2.80–2.63 (m, 1H, H-13), 2.13–1.94 (m, 1H, H-14a), 1.91–1.55 (m, 7H, H-10, H-11, H-12, H-14b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.1 (C_q, C-15), 169.4 (C_q, C-2), 150.2 (C_q, C-8), 134.8 (C-6), 131.9 (C-4), 114.4 (CH_{arom}), 111.9 (CH_{arom}), 109.9 (C_q, C-3), 51.8 (CH_3), 51.6 (CH_3), 46.9 (C-9), 38.7 (C-13), 32.6 (C-14), 31.0 (C-10), 28.2 (C-12), 21.2 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{21}NO_4^+$: 291.1471; found: 291.1464.

6.6.2.1.1.4 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)benzoic acid (45)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 35.9 mg (123 µmol, 1.0 eq) methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**51**), 3 mL THF/H_2O = 2:1 (v/v) and 52.2 mg (1.23 mmol, 10 eq) LiOH·H_2O. An air cooler was fitted on the flask and the mixture was heated to 30 °C for 21 h until full conversion of the starting material was detected by TLC. The reaction mixture was acidified with 6 N HCl. The solvent was removed in vacuum and the residue was dried in oil pump vacuum. The product was purified via column chromatography (size: 7 x 0.8 cm, cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v), 5 mL fractions).

$C_{14}H_{17}NO_4$ [263.29]

yield: 15.9 mg (60.4 µmol, 49%) colorless solid.

R_f = 0.35 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: green-orange.

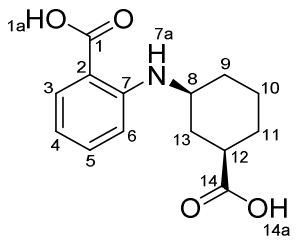
mp: 203-205 °C (turns yellow upon melting).

^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 7.88 (d, $^3J_{HH}$ = 8.0 Hz, 1H, CH_{arom}), 7.38–7.26 (m, 1H, CH_{arom}), 6.81 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.53 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.87–3.74 (m, 1H, H-8), 2.73–2.60 (m, 1H, H-12), 2.13–1.96 (m, 1H, H-13a), 1.88–1.52 (m, 7H, H-9, H-10, H-11, H-13b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 179.2 (C_q, C-14), 172.3 (C_q, C-1), 151.7 (C_q, C-7), 135.6 (CH_{arom}), 133.4 (CH_{arom}), 115.3 (CH_{arom}), 112.9 (CH_{arom}), 111.4 (C_q, C-2), 48.1 (C-8), 39.9 (C-12), 34.0 (C-13), 32.0 (C-9), 29.1 (C-11), 22.1 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{17}NO_4^+$: 263.1158; found: 263.1159.

6.6.2.1.1.5 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)benzoic acid (45)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 17.9 mg (61.4 µmol, 1.0 eq) methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**51**), 1.5 mL THF/H_2O = 2:1 (v/v) and 26.0 mg (614 µmol, 10 eq) LiOH·H_2O. An air cooler was fitted on the flask and the mixture was heated to 30 °C for 21 h until full conversion of the starting material was detected by TLC. The reaction mixture was acidified with 6 N HCl, the solvent was removed in vacuum and the residue was dried in oil pump vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{14}H_{17}NO_4$ [263.29]

yield: 6.0 mg (22.8 µmol, 37%) colorless, amorphous solid.

mp: 100-105 °C.

R_f = 0.32 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: green-orange).

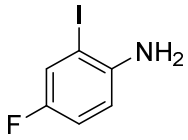
^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 7.87 (d, $^3J_{HH}$ = 7.9 Hz, 1H, CH_{arom}), 7.32 (t, $^3J_{HH}$ = 7.1 Hz, 1H, CH_{arom}), 6.78 (d, $^3J_{HH}$ = 8.5 Hz, 1H, CH_{arom}), 6.53 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.53–3.38 (m, 1H, H-8), 2.55–2.29 (m, 2H, H-12, H-13a), 2.19–1.84 (m, 3H, H-9a, H-10a, H-11a), 1.61–1.09 (m, 4H, H-9b, H-10b, H-11b, H-13b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 179.1 (C_q, C-14), 172.2 (C_q, C-1), 151.6 (C_q, C-7), 135.5 (CH_{arom}), 133.4 (CH_{arom}), 115.4 (CH_{arom}), 112.9 (CH_{arom}), 111.6 (C_q, C-2), 51.6 (C-8), 43.6 (C-12), 36.8 (C-13), 33.9 (C-9), 29.8 (C-11), 25.4 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{17}NO_4^+$: 263.1158; found: 263.1159.

6.6.2.1.2 F-Phenazistatin

6.6.2.1.2.1 4-Fluoro-2-iodoaniline (55)



A 500 mL round-bottom flask with magnetic stirring bar was charged with 100 mL distilled H_2O, 3 mL toluene, 6.05 g (71.3 mmol, 1.6 eq) $NaHCO_3$ and 4.27 mL (44.5 mmol, 1.0 eq) 4-fluoroaniline. The yellow solution was stirred vigorously and 8.91 g (34.7 mmol, 0.78 eq) I_2 were added in small portions over a period of 30 min. The mixture was stirred at RT for 60 min until full conversion of the starting material was detected by TLC. The mixture was treated with 100 mL H_2O and was extracted with Et_2O (2 x 100 mL). The combined organic layers were washed with 5% aqueous $Na_2S_2O_3$ solution (100 mL), brine (100 mL) and then dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The crude product was purified via silica gel filtration (cyclohexane/EtOAc = 3:1 (v/v)). The product was used immediately in the following step as it degraded rapidly at RT as well as when stored at -18 °C.[279]

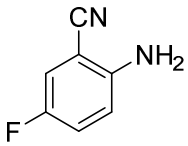
C_6H_5FIN [237.02]

yield: 7.73 g (32.6 mmol, 73%) red-brown liquid.

R_f = 0.70 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm).

GC-MS: t_R = 5.200 min, *m/z* (%) = 237.0 (M^+, 100, BP), 126.9 (17), 110.0 (33), 83.0 (36).

6.6.2.1.2.2 2-Amino-5-fluorobenzonitrile (54)



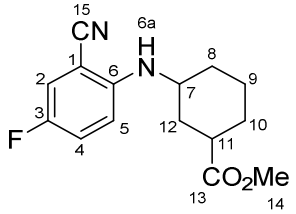
A dry 100 mL Schlenk tube was charged with 3.00 g (12.7 mmol, 1 eq) 4-fluoro-2-iodoaniline (**55**), 2.30 g (19.6 mmol, 1.55 eq) $Zn(CN)_2$, 292 mg (253 µmol, 0.02 eq) $Pd[PPh_3]_4$ and 50 mL dry DMF. The brown mixture was degassed (15 min N_2 bubbling in ultrasonic bath) and was heated to 90 °C for 20 h until full conversion was detected by TLC. The mixture was cooled to RT, 50 mL 5% aqueous NH_4OH was added and the mixture was extracted with EtOAc (150 mL). No phase separation was observed and the solvent was removed on the rotary evaporator. The residue was treated with EtOAc (100 mL) and the mixture was washed with H_2O (70 mL). The organic layer was dried over Na_2SO_4, filtrated and the solvent was

removed in vacuum. The product was purified via column chromatography (100 g silica gel, size: 10 x 3 cm, cyclohexane/EtOAc = 4:1 (v/v)). The product was not analytically pure (^{1}H-NMR showed minor impurities) but the product was used in the following steps without further purification.[202]

yield: 630 mg (4.63 mmol, 37%) red-orange, crystalline solid.

R_f = 0.46 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm).

6.6.2.1.2.3 Methyl 3-((2-cyano-4-fluorophenyl)amino)cyclohexane-1-carboxylate (57)

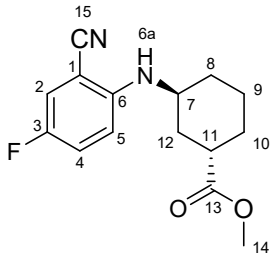


A dry 50 mL Schlenk flask with magnetic stirring bar was charged with 459 mg (3.37 mmol, 1.0 eq) 2-amino-5-fluorobenzonitrile (**54**), 4 mL dry CH_2Cl_2, 2 mL dry AcOH and 685 mg (4.38 mmol, 1.3 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**)[203,215] in a N_2 counter-stream. The deep-red solution was cooled to 0 °C and 8.43 mL (8.43 mmol, 2.5 eq) BH_3·THF (1.0 M solution in THF) were slowly added to the stirred solution over a period of 20 min. The brown mixture was left stirring in the thawing ice bath until full conversion of the starting material was detected after 20 h (GC-MS). The reaction mixture was treated with 30 mL H_2O and 10 mL brine. The crude product was extracted with EtOAc (3 x 30 mL), the combined organic layers were washed with $NaHCO_3$ (2 x 20 mL), dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The crude product (brown solid) was dried in oil pump vacuum ($yield_{crude}$ = 1.05 g). 200 mg (19%) of the crude product were purified via column chromatography and clean isomer fractions could be obtained (15 g silica gel, size: 8 x 2.5 cm, eluent: cyclohexane/Et_2O = 8:1 (v/v), 5 mL fractions).[141,189]

$C_{15}H_{17}FN_2O_2$ [276.31]

yield (total) : 152 mg (549 µmol, 86%) viscous, brown liquid.

6.6.2.1.2.4 Methyl (1*S**,3*S**)-3-((2-cyano-4-fluorophenyl)amino)cyclohexane-1-carboxylate (57)



yield: 63.7 mg (230 µmol, 36%) viscous, brown liquid.

R_f = 0.66 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, CAM: pink).

GC-MS: t_R = 7.332 min, *m/z* (%) = 276.2 (M^+, 100), 245.1 (25), 136.1 (65).

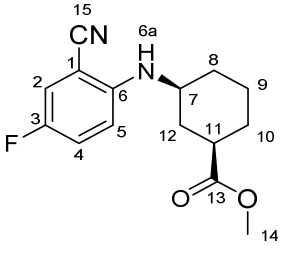
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.20-7.02 (m, 2H, H-2, H-4), 6.77 (dd, $^3J_{HH}$ = 9.2 Hz, $^4J_{HF}$ = 4.2 Hz, 1H, H-5), 4.33 (d, $^3J_{HH}$ = 6.3 Hz, 1H, H-6a), 3.71 (s, 3H, H-14), 3.67 (d, $^3J_{HH}$ = 4.7 Hz, 1H, H-7), 2.81-2.70 (m, 1H, H-11), 2.29-2.16 (m, 1H, H-12a), 1.90 (s, 2H, H-8a, H-10a), 1.77-1.63 (m, 2H, H-8b, H-9a), 1.61-1.34 (m, 3H, H-9b, H-10b, H-12b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.6 (C-13), 153.6 (d, $^1J_{CF}$ = 237.0 Hz, C-3), 146.5 (C-6), 122.4 (d, $^2J_{CF}$ = 22.6 Hz, C-4), 118.3 (d, $^2J_{CF}$ = 24.9 Hz, C-2), 117.1 (C-15), 112.7 (d, $^3J_{CF}$ = 7.4 Hz, C-5), 95.5 (d, $^3J_{CF}$ = 9.0 Hz, C-1), 51.9 (C-14), 48.2 (C-7), 38.9 (C-11), 32.9 (C-12), 31.8 (C-8), 27.7 (C-10), 21.5 (C-9).

^{19}F-NMR (470.35 MHz, $CDCl_3$): δ = -127.81 (td, *J* = 7.9 Hz, 4.3 Hz).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{17}FN_2O_2^+$: 276.1274; found: 276.1263.

6.6.2.1.2.5 Methyl (1*R**,3*S**)-3-((2-cyano-4-fluorophenyl)amino)cyclohexane-1-carboxylate (57)



yield: 88.0 mg (318 µmol, 50%) viscous, brown liquid.

R_f = 0.60 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, CAM: pink).

GC-MS: t_R = 7.463 min, *m/z* (%) = 276.2 (M^+, 100), 245.1 (25), 136.1 (65).

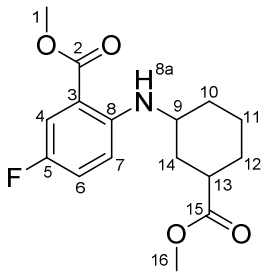
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.17-7.02 (m, 2H, H-2, H-4), 6.61 (dd, $^{3}J_{HH}$ = 9.1 Hz, $^{4}J_{HF}$ = 4.2 Hz, 1H, H-5), 4.29 (d, $^{3}J_{HH}$ = 7.1 Hz, 1H, H-6a), 3.67 (s, 3H, H-14), 3.31 (s, 1H, H-7), 2.56-2.26 (m, 2H, H-11, H-12a), 2.13-1.85 (m, 3H,H-8a, H-9a, H-10a), 1.46-1.09 (m, 4H, H-8b, H-9b, H-10b, H-12b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.1 (C-13), 153.6 (d, $^{1}J_{CF}$ = 237.3 Hz, C-3), 146.3 (C-6), 122.2 (d, $^{2}J_{CF}$ = 22.7 Hz, C-4), 118.6 (d, $^{2}J_{CF}$ = 24.9 Hz, C-2), 116.9 (d, $^{4}J_{CF}$ = 2.7 Hz, C-15), 112.6 (d, $^{3}J_{CF}$ = 7.4 Hz, C-5), 95.8 (d, $^{3}J_{CF}$ = 8.9 Hz, C-1), 51.9 (C-14), 51.7 (C-7), 42.3 (C-11), 35.3 (C-12), 32.6 (C-8), 28.4 (C-10), 24.7 (C-9).

^{19}F-NMR (470.35 MHz, $CDCl_3$): δ = -127.56 (td, *J* = 8.0 Hz, 4.4 Hz).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{17}FN_2O_2^+$: 276.1274; found: 276.1264.

6.6.2.1.2.6 Methyl 5-fluoro-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (58)

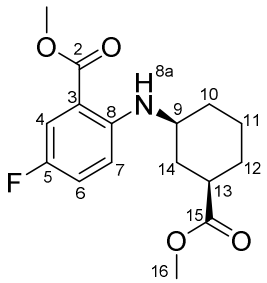


A dry 15 mL Schlenk flask with magnetic stirring bar was charged with 208 µL (1.52 mmol, 1.0 eq) methyl 2-amino-5-fluorobenzoate, 250 mg (1.60 mmol, 1.05 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**),[203,215] 1.00 mL dry DMF and 492 µL (3.81 mmol, 2.5 eq) TMSCl. The orange-yellow, biphasic mixture was cooled to 0 °C and 1.52 mL (1.52 mmol, 1.0 eq) BH_3·THF (1.0 M in THF) were added over a period of 12 min. The yellow solution was stirred at 0 °C for 90 min (TLC: incomplete conversion of the aniline) and 59.0 mg (380 µmol, 0.25 eq) methyl 3-oxocyclohexane-1-carboxylate[203,215] and 300 µL (300 µmol, 0.2 eq) TMSCl were added to the stirred solution. Full conversion of the aromatic amine was detected after additional 15 min at 0 °C (TLC: cyclohexane/EtOAc = 3:1 (v/v)). The reaction mixture was treated with 5 mL H_2O and 5 mL EtOAc and was stirred at RT for 80 min. The phases were separated and the aqueous phase was extracted with EtOAc (4 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in oil pump vacuum. The residue was adsorbed on 1.20 g Celite™ (EtOAc) and was purified via column chromatography. Pure fractions of the isomers could be obtained (size: 25 x 2.2 cm, cyclohexane/EtOAc = 25:1, 20 mL fractions).[189]

$C_{16}H_{20}FNO_4$ [309.34]

yield (total): 215 mg (695 µmol, 46%) faint-yellow, viscous liquid.

6.6.2.1.2.7 Methyl 5-fluoro-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-benzoate (58)



yield: 41.2 mg (133 µmol, 9%) faint-yellow, viscous liquid.

R_f = 0.27 (cyclohexane/EtOAc = 10:1 (v/v)) (254 nm, 366 nm, cold CAM: carnate).

GC-MS: t_R = 7.669 min, *m/z* (%) = 309.1 (100, M^+), 250.1 (76), 208.1 (79), 176.0 (85).

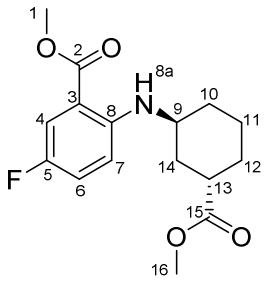
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.63–7.41 (m, 2H, H-4, H-8a), 7.15–7.02 (m, 1H, H-6), 6.68–6.58 (m, 1H, H-7), 3.84 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 3.40–3.24 (m, 1H, H-9), 2.51–2.30 (m, 2H, H-13, H-14a), 2.17–2.06 (m, 1H, H-10a), 2.04–1.83 (m, 2H, H-11a, H-12a), 1.51–1.12 (m, 4H, H-10b, H-11b, H-12b, H-14b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.4 (C_q, C-15), 168.3 (d, $^4J_{CF}$ = 2.8 Hz, C-2), 152.9 (d, $^1J_{CF}$ = 233.3 Hz, C-5), 147.0 (C_q, C-8), 122.4 (d, $^2J_{CF}$ = 23.0 Hz, C-6), 117.1 (d, $^2J_{CF}$ = 23.2 Hz, C-4), 113.0 (d, $^3J_{CF}$ = 6.9 Hz, C-7), 109.9 (C_q, d, $^3J_{CF}$ = 6.7 Hz, C-3), 51.8 (C-1, C-16), 51.1 (C-9), 42.5 (C-13), 35.5 (C-14), 32.8 (C-10), 28.5 (C-12), 24.4 (C-11).

^{19}F-NMR (470.35 MHz, $CDCl_3$): δ = -130.66 (m).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}FNO_4^+$: 309.1376; found: 309.1378.

6.6.2.1.2.8 Methyl 5-fluoro-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-benzoate (58)



yield: 104.9 mg (339 µmol, 21%) faint-yellow, viscous liquid.

R_f = 0.33 (cyclohexane/EtOAc = 10:1 (v/v)) (254 nm, 366 nm, cold CAM: carnate).

GC-MS: t_R = 7.556 min, *m/z* (%) = 309.1 (100, M^+), 250.1 (72), 208.1 (75), 176.1 (83).

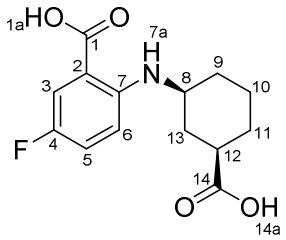
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.73 (bs, 1H, H-8a), 7.63–7.49 (m, 1H, H-4), 7.17–7.03 (m, 1H, H-6), 6.79–6.66 (m, 1H, H-7), 3.85 (s, 3H, CH_3), 3.80–3.70 (m, 1H, H-9), 3.68 (s, 3H, CH_3), 2.78–2.64 (m, 1H, H-13), 2.12–1.97 (m, 1H, H-14a), 1.89–1.50 (m, 7H, H-10, H-11, H-12, H-14b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.9 (C_q, C-15), 168.4 (d, $^{4}J_{CF}$ = 3.0 Hz, C-2), 152.8 (C_q, d, $^{1}J_{CF}$ = 233.1 Hz, C-5), 146.9 (C_q, C-8), 122.5 (d, $^{2}J_{CF}$ = 23.0 Hz, C-6), 116.8 (d, $^{2}J_{CF}$ = 23.2 Hz, C-4), 113.0 (d, $^{3}J_{CF}$ = 6.9 Hz, C-7), 109.6 (d, $^{3}J_{CF}$ = 6.8 Hz, C-3), 51.70/51.68 (C-1/C-16), 47.2 (C-9), 38.6 (C-13), 32.5 (C-14), 31.0 (C-10), 28.0 (C-12), 21.1 (C-11).

^{19}F-NMR (470.35 MHz, $CDCl_3$): δ = -130.84 (m).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}FNO_4^+$: 309.1376; found: 309.1367.

6.6.2.1.2.9 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-fluorobenzoic acid (52)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 41.2 mg (133 µmol, 1.0 eq) methyl 5-fluoro-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**58**), 2 mL THF/MeOH = 4:1 (v/v) and 84.7 mg (2.00 mmol, 15 eq) $LiOH \cdot H_2O$. The light-carnate colored mixture was stirred for 17 h at RT (TLC. incomplete conversion) and 84.7 mg (2.00 mmol, 15 eq) $LiOH \cdot H_2O$ were added. The mixture was left stirring at RT and full conversion of the starting material was detected by TLC after another 23 h. The mixture was acidified with 6 N HCl (2.5 mL), diluted with H_2O (5 mL) and extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on 103 mg Celite™ (MeOH) and the product was purified via column chromatography (size: 15 x 0.8 cm, cyclohexane/EtOAc/AcOH = 4:1:1%, 3 mL fractions).

$C_{14}H_{16}FNO_4$ [281.28]

yield: 20.9 mg (74.3 µmol, 56%) light-brown solid.

R_f = 0.20 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: black).

mp: 174-175 °C.

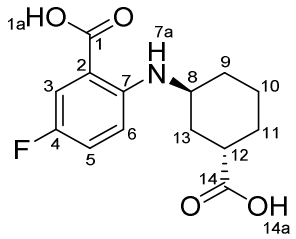
^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 7.64–7.49 (m, 1H, C-3), 7.21–7.06 (m, 1H, C-5), 6.88–6.71 (m, 1H, C-6), 3.52–3.33 (m, 1H, H-8), 2.58–2.24 (m, 2H, H-12, H-13a), 2.18–1.81 (m, 3H, H-9a, H-10a, H-11a), 1.61–1.01 (m, 4H, H-9b, H-10b, H-11b, H-13b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 178.9 (C_q, C-14), 171.0 (C_q, d, $^{4}J_{CF}$ = 2.6 Hz, C-1), 154.2 (C_q, d, $^{1}J_{CF}$ = 232.1 Hz, C-4), 148.4 (C_q, C-7), 123.0 (d, $^{2}J_{CF}$ = 23.2 Hz, C-5), 118.1 (d, $^{2}J_{CF}$ = 23.1 Hz, C-3), 114.4 (d, $^{3}J_{CF}$ = 7.0 Hz, C-6), 111.6 (C_q, d, $^{3}J_{CF}$ = 6.4 Hz, C-2), 52.0 (C-8), 43.4 (C-12), 36.7 (C-13), 33.9 (C-9), 29.7 (C-11), 25.4 (C-10).

^{19}F-NMR (470.35 MHz, DMSO-d_6): δ = -130.77 (m).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}FNO_4^+$: 281.1063; found: 281.1059.

6.6.2.1.2.10 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-fluorobenzoic acid (52)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 28.0 mg (90.5 µmol, 1.0 eq) methyl 5-fluoro-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**58**), 1 mL THF/MeOH = 4:1 (v/v) and 57.5 mg (1.36 mmol, 15 eq) LiOH·H_2O. The light-carnate colored mixture was stirred for 17 h at RT (TLC: incomplete conversion) and 57.5 mg (1.36 mmol, 15 eq) LiOH·H_2O were added. The mixture was stirred for another 24 h at RT (TLC: incomplete conversion) and 76.2 mg (1.36 mmol, 15 eq) KOH and 750 µL H_2O were added. The mixture was left stirring for 23 h (TLC: incomplete conversion) and 57.5 mg (1.36 mmol, 15 eq) LiOH·H_2O were added. Full conversion of the starting material was detected by TLC after another 3 d of stirring. The mixture was acidified with 6 N HCl (2.5 mL), diluted with H_2O (5 mL) and extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on 64 mg Celite™ (MeOH) and the product was purified via column chromatography (15 x 0.8 cm, 3 mL fractions, cyclohexane/EtOAc/AcOH = 4:1:1%).

$C_{14}H_{16}FNO_4$ [281.28]

yield: 18.1 mg (64.3 µmol, 71%) light-yellow solid.

R_f = 0.26 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: black).

mp: 220-223 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.51 (bs, 2H, H-1a, H-14a), 7.57–7.42 (m, 1H, H-3), 7.32–7.17 (m, 1H, H-5), 6.88–6.71 (m, 1H, H-6), 3.79–3.63 (m, 1H, H-8), 2.52–2.48 (m, 1H, H-12), 2.01–1.84 (m, 1H, H-13a), 1.80–1.39 (m, 7H, H-9, H-10, H-11, H-13b).

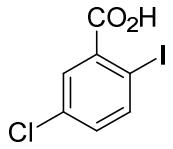
^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.2 (C_q, C-14), 169.3 (C_q, d, $^{4}J_{CF}$ = 2.6 Hz, C-1), 151.9 (C_q, d, $^{1}J_{CF}$ = 230.8 Hz, C-4), 146.9 (C_q, C-7), 122.1 (d, $^{2}J_{CF}$ = 22.8 Hz, C-5), 116.6 (d, $^{2}J_{CF}$ = 22.7 Hz, C-3), 113.1 (d, $^{3}J_{CF}$ = 7.0 Hz, C-6), 110.0 (C_q, d, $^{3}J_{CF}$ = 6.2 Hz, C-2), 46.5 (C-8), 38.0 (C-12), 32.3 (C-13), 30.4 (C-9), 27.4 (C-11), 20.7 (C-10).

^{19}F-NMR (470.35 MHz, DMSO-d_6): δ = -130.67 (m).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}FNO_4^+$: 281.1063; found: 281.1059.

6.6.2.1.3 Cl-Phenazistatin

6.6.2.1.3.1 5-Chloro-2-iodobenzoic acid (60)



Step 1: A 100 mL two-neck round-bottom flask, equipped with magnetic stirring bar and an internal thermometer, was charged with 50 mL CH_2Cl_2, 2.5 mL AcOH and 5.00 g (33.1 mmol, 1.0 eq) methyl anthranilate, respectively. The mixture was cooled in an ice bath and 25 mL H_2O were added, followed by the quick addition of 7.50 g (34.1 mmol, 1.03 eq) $Ca(OCl)_2$. The temperature increased to 11 °C but dropped to 3 °C after 15 min resulting in a colour change from orange to brown. Stirring was prolonged for 1 h at RT. The mixture was washed with 50 mL H_2O. The organic layer was dried over Na_2SO_4, filtrated, and the solvent was removed in vacuum. The brown residue was treated with 40 mL *n*-pentane, agitated in the ultrasonic bath for 5 min and stored in the refrigerator overnight. The chocolate brown precipitate was collected by filtration and washed with *n*-pentane (2 x 10 mL). A solution of 1 N KOH (5.57 g, 99.2 mmol, 3.00 eq) in 20 mL MeOH was added to the crude methyl ester and was heated under reflux overnight at 60 °C. After cooling to RT, 27 mL AcOH were added. The mixture was extracted with EtOAc (140 mL) and the dark orange organic layers were dried over Na_2SO_4, filtrated, and the solvent was removed in vacuum. The crude product (4.53 g, 80%) was used in the following step without further purification.[205]

Step 2: A 250 mL round-bottom flask equipped with a magnetic stirring bar was charged with 3.00 g (17.5 mmol, 1 eq) crude 5-chloroanthranilic acid and 12.5 mL conc. H_2SO_4. The thick, brown suspension was stirred vigorously while being cooled in an ice bath. 2.40 g (38.5 mmol, 2.2 eq) $NaNO_2$ were added in small portions over a period of 10 min. The

suspension was stirred for a further 3 h followed by the quick addition of 100 mL ice. The reaction mixture was added dropwise over a period of 10 min to an ice cold solution of 15.7 g (105 mmol, 6 eq) potassium iodide in 80 mL H_2O. Stirring at RT was continued for 60 h. Addition of 25 mL $Na_2S_2O_3$ resulted in a colour change from brown to yellow. The light brown precipitate was collected by filtration, washed with H_2O (3 x 10 mL) and dried in vacuum. The crude product was suspended in 40 mL MeOH, the solid parts were removed by filtration through a pad of Celite® (1 cm, elution with MeOH) and the solvent was removed in vacuum. The product was purified via column chromatography (75 g silica gel, cyclohexane/EtOAc/AcOH= 7:1:1%, 5:1:1% (v/v/v)). The isolated product fractions (some impurities were present) were recrystallized from H_2O/MeOH (3:1 (v/v), 125 mL). The resulting pale yellow crystals were collected by filtration and dried in high vacuum.[206]

$C_7H_4O_2ICl$ [282.48]

yield: 1.18 g (4.19 mmol, 24%), light-yellow needles.

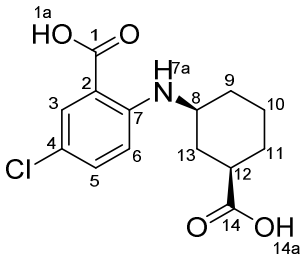
R_f = 0.10 (cyclohexane/EtOAc/AcOH = 5:1:1% (v/v/v)) (254 nm,CAM: blue).

mp: 155 -160 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.00 (d, $^4J_{HH}$ = 2.6 Hz, 1H), 7.98 (d, $^3J_{HH}$ = 8.5 Hz, 1H), 7.20 (dd, $^3J_{HH}$ = 8.48 Hz, $^3J_{HH}$ = 2.6 Hz, 1H).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.7, 143.2, 134.9, 134.6, 133.8, 132.2, 92.0.

6.6.2.1.3.2 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-chlorobenzoic acid (59)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 61.7 mg (227 µmol, 1.3 eq) ethyl (1*R**,3*S**)-3-((*tert*-butoxycarbonyl)amino)cyclohexane-1-carboxylate (**42**), 500 µL dry CH_2Cl_2 and 500 µL TFA. The mixture was stirred at RT for 16 h and the solvent was removed in oil pump vacuum. To the carnate oil were added 30.0 mg (175 µmol, 1.0 eq) 5-chloro-2-iodobenzoic acid (**60**), 4.00 mg (35.0 µmol, 0.2 eq) L-proline, 3.40 mg (17.5 µmol, 0.1 eq) CuI, 275 mg (437 µmol, 2.5 eq) TBAA and 2.0 mL dry DMF. The green-blue solution was heated to 70 °C for 3 d until full conversion of the starting material was detected by TLC. The solvent was removed in vacuum, the residue was treated with H_2O (10 mL) and EtOAc (10 mL) and was acidified with 6 N HCl (pH = 1). The phases were separated and the

aqueous layer was extracted with EtOAc (3 x 10 mL), the combined organic layers were reduced in vacuum and the residue was adsorbed on 130 mg Celite™ (MeOH). The intermediary product was purified via column chromatography (size: 13 x 0.8 cm, cyclohexane/EtOAc/AcOH = 6:1:1%) and the isolated fractions were transferred to a 10 mL round-bottom flask with magnetic stirring bar. 2 mL THF/H_2O (1:1, v/v) and 74.2 mg (1.75 mmol, 10 eq) LiOH·H_2O were added. The light-yellow, biphasic mixture was heated to 40 °C for 3 d until full conversion of the starting material was detected by TLC. The mixture was acidified with 6 N HCl (pH = 1) and the solvent was removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).[197]

$C_{14}H_{16}ClNO_4$ [297.73]

yield: 13.8 mg (46.3 µmol, 26%) light yellow solid.

mp: 200-205 °C (decomposition).

R_f = 0.50 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: red-brown).

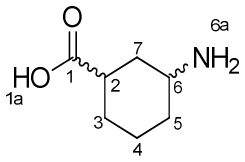
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.54 (bs, 2H, CO_2H), 7.98–7.59 (m, 2H, H-3, NH), 7.36 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-5), 6.85 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 3.53–3.36 (m, 1H, H-8), 2.48-2.31 (m, 1H, H-12), 2.28–2.12 (m, 1H, CH_2), 2.05–1.70 (m, 3H, CH_2), 1.55–0.99 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C_q), 169.0 (C_q), 148.6 (C_q, C-7), 134.0 (C-5), 130.6 (C-3), 117.2 (C_q), 113.8 (C-6), 110.9 (C_q), 49.6 (C-8), 41.3 (C-12), 35.0 (C-13), 32.1 (C-9), 28.1 (C-11), 23.7 (C-10).

HR-MS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{14}H_{17}ClNO_4$: 298.0841; found: 298.0849.

6.6.2.1.4 Br-Phenazistatin

6.6.2.1.4.1 3-Aminocyclohexane-1-carboxylic acid (44)



A 200 mL Teflon® vessel with magnetic stirring bar was charged with 2.00 g (14.6 mmol) 3-aminobenzoic acid, 30 mL H_2O/MeOH (2:1, v/v) and 56.0 mg (2.8 wt%) Rh/C (5%). The container was put into the autoclave ("Berghof RHS 295", with a thermostat "Berghof Bar 945"), the flange and the valves were closed and the autoclave was pressurized to 70 bar with H_2 and was purged twice. A pressure of 70 bar H_2 was applied, the stirring was activated and the autoclave mantle was heated to 100 °C. After 5 d the autoclave was cooled to RT (^{1}H-NMR after mini-workup indicated full conversion of the starting material), the catalyst was removed via filtration through a bed of wet (H_2O) Celite™ (3 cm) and was washed with H_2O and MeOH (each 3 x with the double volume of the Celite™ pad). The solvent was removed in vacuum and drying in oil pump vacuum afforded the title compound as an off-white solid.[200]

$C_7H_{13}NO_2$ [143.18]

yield: 2.08 g (14.6 mmol, 99%), off-white solid.

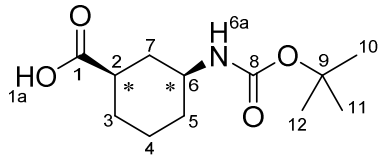
R_f = 0.26 (MeOH/CH_2Cl_2 = 5:1 (v/v), ninhydrin: violet).

mp = >230 °C (decomp.).

^{1}H-NMR (300.36 MHz, D_2O): δ = 3.62-3.46 (m, 0.3H, H-6^{trans}), 3.35-3.16 (m, 0.7H, H-6^{cis}), 2.70-2.58 (H-2^{trans}), 2.39-2.11 (m, 1.7H, H-2^{cis}, H-7a), 2.10-1.89 (m, 3H, H-3a, H-4a, H-5a), 1.89-1.14 (m, 4H, H-3b, H-4b, H-5b, H-7b).

^{13}C-NMR (75.53 MHz, D_2O) δ = 183.9/183.2^{trans} (C-1), 49.9/47.7^{trans} (C-6), 45.0/40.7^{trans} (C-2), 33.5/31.3^{trans} (C-7), 29.9/29.1^{trans} (C-5), 28.4/27.5^{trans} (C-3), 23.7^{trans}/23.2 (C-4).

6.6.2.1.4.2 (1*R**,3*S**)-3-((*tert*-Butoxycarbonyl)amino)cyclohexane-1-carboxylic acid (48)



A 500 mL round-bottom flask with magnetic stirring bar was charged with 12.0 g (83.8 mmol, 1.0 eq) crude 3-aminocyclohexane-1-carboxylic acid (**44**) and 240 mL H_2O/1,4-dioxane (1:1, (v/v)). To the brown suspension 43.8 mL (251 mmol, 3.0 eq) DIPEA were added and the mixture was stirred for 30 min at RT. 21.5 g (96.4 mmol, 1.15 eq) Boc_2O were added in one portion and the brown solution was stirred for 42 h at RT. The reaction mixture was acidified (pH = 1) with 12 M HCl and was extracted with CH_2Cl_2 (4 x 150 mL). The combined organic extracts were dried over $MgSO_4$, filtrated and the solvent was removed in vacuum. The sticky, brown-carnate crude product was dissolved in 70 mL MeOH, stirred until fully dissolved and was treated with 150 mL *n*-pentane at RT. Precipitation of a colorless solid occurred after 15 min stirring and the generated very turbid suspension was stirred for 17 h at RT. The product was isolated via filtration through a sintered glass frit (Pore 3), washed with *n*-pentane (2 x 20 mL) and the colorless solid was dried in oil pump vacuum.[200]

$C_{12}H_{21}NO_4$ [243.30]

yield: 11.0 g (45.1 mmol, 54%), colorless solid.

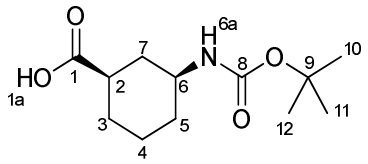
R_f = 0.77 (CH_2Cl_2/MeOH = 5:1 (v/v), CAM: blue).

mp = 135-140 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.69-4.28 (m, 1H, H-6a), 3.90-3.70 (m, 0.3H, H-6^{trans}), 3.56-3.29 (m, 0.7H, H-6^{cis}), 2.63 (bs, 0.3H, H-2^{trans}), 2.49-2.33 (m, 0.7H, H-2^{cis}), 2.27 (d, $^3J_{HH}$ = 11.8 Hz, 1H, H-7a), 1.97 (d, $^3J_{HH}$ = 10.1 Hz, 2H, H-3a, H-5a), 1.90-1.80 (m, 1H, H-4a), 1.63-1.51 (m, 1H, H-3b), 1.44 (s, 9H, H-10, H-11, H-12), 1.38-1.14 (m, 2H, H-4b, H-7b), 1.13-0.93 (m, 1H, H-5b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 180.5 (C-1), 155.3 (C-8), 79.5 (C-9), 49.0 (C-6), 42.2 (C-2), 35.5 (C-7), 32.8 (C-5), 28.5 (C-10, C-11, C-12), 28.2 (C-3), 24.3 (C-4).

6.6.2.1.4.3 (1*R*,3*S*)-3-((*tert*-Butoxycarbonyl)amino)cyclohexanecarboxylic acid (48)



A 25 mL round-bottom flask with magnetic stirring bar was charged with 2.00 g (8.22 mmol, 1.0 eq) (1*R**,3*S**)-3-((tert-butoxycarbonyl)amino)cyclohexane-1-carboxylic acid (**48)** and 8.0 mL EtOH. The colorless solution was stirred and 535 µL (4.12 mmol, 0.5 eq) D-(+)-alpha-methylbenzylamine were added. The turbid, colorless suspension was heated to 60 °C until a clear solution was obtained (30 min) and was kept stirring vigorously for 4 d at RT during which time precipitation occurred. The colorless precipitate was isolated via filtration through a sintered glass frit (Pore 3), washed with *n*-pentane (3 x 4 mL) and dried in vacuum. As a test for the obtained enantiomeric excess, 46.6 mg (128 µmol) of the colorless solid was transferred into a separation funnel, suspended in 10 mL EtOAc and was washed with 1 M HCl (10 mL, 5 mL). The organic layer was dried over $MgSO_4$, filtrated, the solvent was removed in vacuum and the colorless solid was dried in oil pump vacuum. The obtained 31.0 mg (127 µmol, yield of salt extraction: 99%) title compound were dissolved in 2.00 mL MeOH and the optical rotation was determined. The remaining diastereomeric salt was transferred to a 250 mL separation funnel, suspended in 150 mL EtOAc and washed with 1 M HCl (3 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed in vacuum and in oil pump vacuum.[200]

$C_{12}H_{21}NO_4$ [243.30]

yield: 404 mg (1.66 mmol, 20%) snow-white light solid.

R_f = 0.77 (CH_2Cl_2/MeOH = 5:1 (v/v), CAM).

mp = 140 °C.

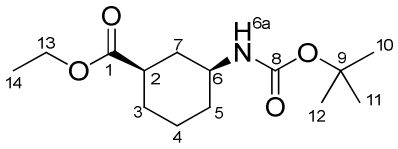
$[\alpha]_D^{20}$ = -48.4° (c = 1.55, MeOH).

$[\alpha]_D^{20}$ = -50.5 °(c = 1, MeOH, lit.).[280]

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.47 (d, $^4J_{HH}$ = 5.7 Hz, 1H, H-6a), 3.58-3.36 (m, 1H, H-6), 2.53-2.33 (m, 1H, H-2), 2.26 (d, $^3J_{HH}$ = 11.4 Hz, 1H, H-7a), 1.96 (d, $^3J_{HH}$ = 10.9 Hz, 2H, H-3a, H-5a), 1.90-1.74 (m, 1H, H-4a), 1.43 (s, 10H, H-3b, H-10/11/12), 1.37-1.29 (m, 1H, H-4b), 1.29-1.13 (m, 1H, H-7b), 1.13-0.92 (m, 1H, H-5b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 180.5 (C-1), 155.3 (C-8), 79.5 (C-9), 49.0 (C-6), 42.2 (C-

6.6.2.1.4.4 (1*R*,3*S*)-Ethyl 3-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylate (49)



A dry 5 mL Schlenk flask with magnetic stirring bar was charged with 201 mg (822 µmol, 1.0 eq) (1*R*,3*S*)-3-((tert-butoxycarbonyl)amino)cyclohexane-1-carboxylic acid (**48**) and 2.00 mg (16.4 µmol, 0.02 eq) 4-DMAP in a N_2 counter-stream. The flask was evacuated and refilled with N_2 for three times. To the flask were added 72.0 µL (1.23 mmol, 1.5 eq) dry EtOH and 2.00 mL dry CH_2Cl_2 were added and the colorless solution was cooled to -78 °C and 136 µL (863 µmol, 1.05 eq) *N,N'*-diisopropylcarbodiimide were added over a period of 3 min. The slightly-yellow solution was allowed to warm to RT overnight and was stirred over the weekend. The colorless suspension was filtered through a sintered glass frit (Pore 4), the filter cake was washed with Et_2O (2 x 3 mL) and the filtrate was concentrated in vacuum and was dried in oil pump vacuum to afford a colorless crude product (254 mg). The crude product was dissolved in MeOH, adsorbed on 630 mg Celite™, the solvent was removed in vacuum and the product was purified via column chromatography (15 g silica gel, size: 10 x 2 cm, cyclohexane/EtOAc = 20:1, fraction size: 10 mL).[281]

$C_{14}H_{25}NO_4$ [271.35]

yield: 191 mg (704 µmol, 86%) off-white solid.

mp: 54 °C.

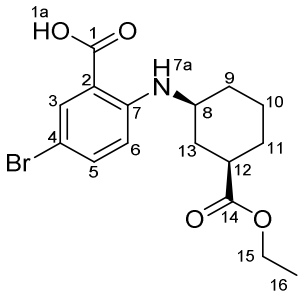
$[\alpha]_D^{20}$ = -29.9° (c = 1.31, MeOH).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.42 (s, 1H, H-6a), 4.11 (q, $^3J_{HH}$ = 7.1 Hz, 2H, H-13), 3.46 (s, 1H, H-6), 2.37 (t, $^3J_{HH}$ = 11.9 Hz, 1H, H-2), 2.23 (d, $^3J_{HH}$ = 12.1 Hz, 1H, H-7a), 2.03-1.76 (m, 3H, H-3a, H-4a, H-5a), 1.42 (s, 9H, H-10, H-11, H-12), 1.37-1.10 (m, 6H, H-3b, H-4b, H-7b, H-14), 1.09-0.94 (m, 1H, H-5b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.2 (C-1), 155.2 (C-8), 79.4 (C_q, C-9), 60.5 (C-13), 49.1 (C-6), 42.5 (C-2), 35.8 (C-7), 33.0 (C-5), 28.5 (C-10, C-11, C-12), 28.3 (C-3), 24.3 (C-4), 14.3 (C-14).

GC-MS: t_R = 6.50 min ; *m/z* (%) = 201 (17), 184 (11), 156 (30), 141 (15), 126 (19), 98 (32), 81 (52), 70 (24), 56 (100).

6.6.2.1.4.5 5-Bromo-2-(((1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (50)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 30.0 mg (111 µmol, 1.3 eq) (1*R*,3*S*)-ethyl 3-((*tert*-butoxycarbonyl)amino)cyclohexane-1-carboxylate (**47**), 500 µL dry CH_2Cl_2 and 500 µL TFA. The mixture was stirred at RT for 16 h and the solvents were removed in oil pump vacuum. To the carnate oily residue were added 27.8 mg (85.0 µmol, 1.0 eq) 5-bromo-2-iodobenzoic acid, 134 mg (213 µmol, 2.5 eq) TBAA, 2.00 mg (17.0 µmol, 0.2 eq) L-proline and 1.70 mg (8.50 µmol, 0.1 eq) CuI followed by 2 mL dry DMF. The flask was sealed and the mixture was heated to 60 °C for 23 h until full conversion of the starting material was detected by TLC. The solvent was removed in oil pump vacuum and the product was purified via preparative RP-HPLC (Method_PREPHPLC).[197]

$C_{16}H_{20}BrNO_4$ [370.24]

yield: 13.9 mg (37.5 µmol, 44%) yellow, amorphous solid.

R_f = 0.70 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: yellow).

$[\alpha]_D^{20}$: -16.5 (c = 0.645, DMSO).

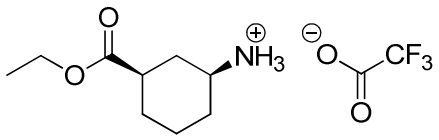
mp: 46-50 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.97 (bs, 1H, H-1a), 7.95–7.65 (m, 2H, H-3, NH), 7.46 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-5), 6.81 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 4.03 (q, $^3J_{HH}$ = 7.1 Hz, 2H, H-15), 3.57–3.39 (m, 1H, H-8), 2.59–2.42 (m, 1H, H-12, DMSO-overlap), 2.24–2.12 (m, 1H, CH_2), 2.03–1.71 (m, 3H, CH_2), 1.52–1.08 (m, 7H, H-16, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 174.2 (C_q), 168.8 (C_q), 148.8 (C_q, C-7), 136.7 (C-5), 133.5 (C-3), 114.2 (C-6), 111.5 (C_q), 104.3 (C_q), 59.8 (C-15), 49.3 (C-8), 41.1 (C-12), 34.7 (C-13), 32.0 (C-9), 28.0 (C-11), 23.5 (C-10), 14.1 (C-16).

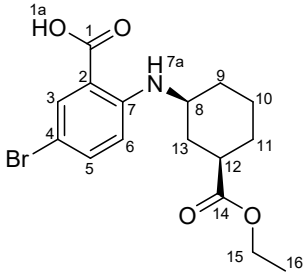
HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}BrNO_4^+$: 369.0576; found: 369.0568.

6.6.2.1.4.6 (1*S*,3*R*)-3-(Ethoxycarbonyl)cyclohexan-1-aminium 2,2,2-trifluoroacetate (47)



A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 250 mg (876 µmol) (1*R*,3*S*)-ethyl 3-((*tert*-butoxycarbonyl)amino)cyclohexane-1-carboxylate (**49**) and was subsequently evacuated and refilled with N_2. 1.25 mL dry CH_2Cl_2 and 1.25 mL TFA were added in a N_2 counter-stream. The tube was sealed after the gas evolution had ceased and the flesh-colored solution was stirred at RT for 4 h. The solvent was removed in oil pump vacuum to afford a flesh-colored viscous liquid which was used in the following step without further purification.

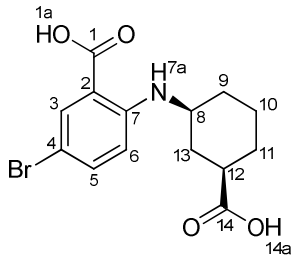
6.6.2.1.4.7 5-Bromo-2-(((1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (50)



To the Schlenk flask containing the crude (1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexan-1-aminium 2,2,2-trifluoroacetate (**47**) from the previous step (876 µmol, 1.5 eq) were added 191 mg (584 µmol, 1.0 eq) 5-bromo-2-iodobenzoic acid,[204] 551 mg (876 µmol, 1.5 eq) TBAA, 13.6 mg (117 µmol, 0.2 eq) L-proline and 11.4 mg (58.0 µmol, 0.1 eq) CuI in a N_2 counter-stream. The tube was evacuated and refilled with N_2, 3.5 mL dry DMF were added and the yellow suspension was heated to 60 °C. After 18 h (TLC: incomplete conversion) additional 11.4 mg (0.1 eq) CuI were added but no further conversion could be obtained upon prolonged stirring. The solvent was removed in vacuum, the orange/brown residue was treated with saturated $NaHCO_3$-solution (3 mL) and the greenish-yellow mixture was extracted with EtOAc (4 x 5 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum and the residue was dried in oil pump vacuum. The emerald-green viscous liquid was transferred to a dry 10 mL Schlenk flask and 191 mg (584 µmol, 1.0 eq) 5-bromo-2-iodobenzoic acid,[204] 551 mg (876 µmol, 1.5 eq) TBAA, 13.6 mg (117 µmol, 0.2 eq) L-proline, 11.4 mg (58 µmol, 0.1 eq) CuI and 3.5 mL dry DMF were added in a N_2 counter-stream. The bright-blue suspension was heated to 60 °C and the obtained dark-blue solution was kept at this temperature for 6 d (TLC: full conversion

of the starting material). The dark-green reaction mixture was transferred to a 50 mL round-bottom flask and the solvent was removed in vacuum. The green residue was treated with 20 mL EtOAc and was washed with 1 M HCl (20 mL), H_2O (25 mL) and brine (2 x 25 mL). The yellow organic layer was dried over $MgSO_4$, filtrated and the solvent was removed in vacuum and the residue was dired in oil pump vacuum to yield an iodine-brown viscous liquid (752 mg) which was used in the following step without further purification.[197]

6.6.2.1.4.8 5-Bromo-2-(((1*S*,3*R*)-3-carboxycyclohexyl)amino)benzoic acid (1)



The crude 5-bromo-2-(((1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (**50**) from the previous step (max. 876 µmol, 1.0 eq) was transferred into a 25 mL round-bottom flask with magnetic stirring bar and was dissolved in 3.0 mL THF, 1.0 mL MeOH and 1.0 mL H_2O. To the orange solution were added 49.0 mg (1.17 mmol, 2.0 eq) $LiOH \cdot H_2O$ and the greenish reaction mixture was stirred for 20 h at RT (TLC: incomplete conversion of the starting material). Additional 57.2 mg (1.36 mmol, 1.6 eq) $LiOH \cdot H_2O$ were added and the deep grass-green reaction mixture was stirred for 3 d at RT (TLC: incomplete conversion). The reaction mixture was filtered through a wet pad of silica gel (3 cm) to remove the verdigris-colored precipitate and the components were eluted using cyclohexane/EtOAc = 1:1, EtOAc and MeOH. The eluted fractions were combined and the solvent was removed in vacuum. The viscous, orange liquid was dissolved in 3.0 mL THF, 1.0 mL MeOH and 1.0 mL H_2O, 49.0 mg (2.0 eq) $LiOH \cdot H_2O$ was added and the olive-green reaction mixture was stirred at RT for 22 h until full conversion of the starting material was detected by TLC. The green suspension was acidified with 1 M HCl (5 mL), extracted with Et_2O (5 x 10 mL), the combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed in vacuum. The orange-yellow crude product (465 mg) was dissolved in 20 mL CH_2Cl_2, adsorbed on 1.4 g Celite™ (MeOH) and the solvent was removed in vacuum. The product was purified via column chromatography (20 g silica gel, size: 230 x 15 mm, cyclohexane/EtOAc/AcOH = 600:100:1, 500:100:1, 300:100:1, fraction size: 20 mL).[282]

$C_{14}H_{16}BrNO_4$ [342.19]

yield: 160 mg (469 µmol, 80%) canary-yellow solid.

R_f = 0.38 (cyclohexane/EtOAc/AcOH = 1:1:1%).

mp = >190 °C (decomp.).

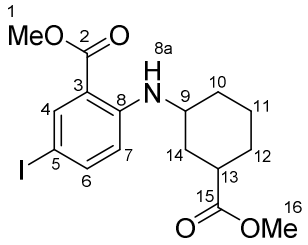
$[\alpha]_D^{20}$= +18° (c = 0.5 (5 g/L), MeOH).[77]

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.47 (bs, 2H, H-1a, H-14a), 7.84 (ds, $^4J_{HH}$ = 2.4 Hz, 1H, H-3), 7.45 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-5), 6.80 (d, $^3J_{HH}$ = 9.2 Hz, 1H, H-6), 3.43 (m, 1H, H-8), 2.41 (t, $^3J_{HH}$ = 12.0 Hz,1H, H-12), 2.19 (d, $^3J_{HH}$ = 11.8 Hz, 1H, H-13a), 1.98 (d, $^3J_{HH}$ = 11.3 Hz, 1H, H-9a), 1.89 (d, $^3J_{HH}$ = 12.3 Hz, 1H, H-11a), 1.78 (d, $^3J_{HH}$ = 13.3 Hz, 1H, H-10a), 1.42 (m, 1H, H-10b), 1.31-1.01 (m, 3H, H-11b, H-13b, H-9b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C-14), 168.9 (C-1), 148.9 (C-7), 136.6 (C-5), 133.5 (C-3), 114.2 (C-6), 111.6 (C-4), 104.2 (C-2), 49.5 (C-8), 41.3 (C-12), 35.0 (C-13), 32.1 (C-9), 28.1 (C-11), 23.7 (C-10).

6.6.2.1.5 I-Phenazistatin

6.6.2.1.5.1 Methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (36)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 6.69 g (21.3 mmol, 1.0 eq) methyl 2-amino-5-iodobenzoate hydrochloride (**67**) and the solid was dried in oil pump vacuum for 10 min. To the Schlenk flask were added 40 mL dry DMF followed by 3.50 g (22.4 mmol, 1.05 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**)[203,215] and 8.30 mL (64.0 mmol, 3.0 eq) TMSCl. The flesh-colored, biphasic mixture was cooled in an ice bath and 23 mL (23.0 mmol, 1.08 eq) BH_3·THF were added over a period of 60 min. The flask was sealed and the mixture was left stirring for 30 min at 0 °C until full conversion of the starting material was detected by TLC. The light-yellow solution was carefully treated with 20 mL H_2O, followed by neutralization with 25% NH_4OH. The solvents were removed in vacuum, the residue was adsorbed on Celite™ (24 g, dissolved in MeOH) and was purified via column chromatography (300 g silica gel, size: 13 x 7.5 cm). Clean fractions of the *cis*- and *trans*-stereoisomers could be separated by a very solvent-intensive column chromatography.[189]

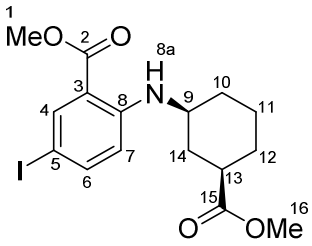
$C_{16}H_{20}INO_4$ [417.24]

yield: 8.61 g (20.6 mmol, 97%) yellow, very viscous liquid.

ratio (*trans*/*cis*) = 57:43.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}INO_4^+$: 417.0437; found: 417.0443.

6.6.2.1.5.2 Methyl 5-iodo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (36)



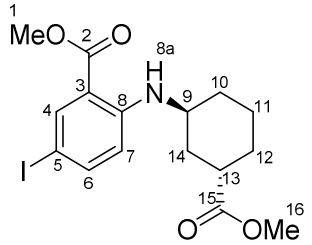
R_f = 0.46 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

GC-MS: t_R = 9.333 min, *m/z* (%) = 417.1 (M^+, 100), 358.1 (33), 316.0 (31), 284.0 (30), 207.1 (23).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.15 (d, $^4J_{HH}$ = 2.1 Hz, 1H, H-4), 7.73 (d, $^3J_{HH}$ = 7.2 Hz, 1H, NH), 7.51 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 1.9 Hz, 1H, H-6), 6.48 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-7), 3.83 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 3.41–3.25 (m, 1H, H-9), 2.52–2.28 (m, 2H, H-13, CH_2), 2.17–1.82 (m, 3H, CH_2), 1.52–1.11 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-15), 168.0 (C_q, C-2), 149.5 (C_q, C-8), 142.7 (CH_{arom}), 140.2 (CH_{arom}), 114.1 (CH_{arom}), 112.2 (C_q, C-3), 73.9 (C_q, C-5), 51.84 (CH_3), 51.79 (CH_2), 50.6 (C-9), 42.4 (C-13), 35.3 (CH_2), 32.6 (CH_2), 28.4 (CH_2), 24.4 (CH_2).

6.6.2.1.5.3 Methyl 5-iodo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (36)



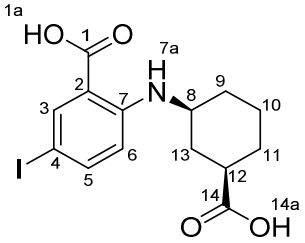
R_f = 0.51 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

GC-MS: t_R = 9.118 min, *m/z* (%) = 417.1 (M^+, 100), 358.1 (30), 316.0 (43), 284.0 (30).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.16 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-4), 7.93 (d, $^3J_{HH}$ = 7.0 Hz, 1H, NH), 7.53 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, C-6), 6.57 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-7), 3.94–3.62 (m, 7H, CH_3, C-9), 2.78–2.62 (m, 1H, H-13), 2.13–1.92 (m, 1H, CH_2), 1.90–1.51 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.9 (C_q, C-15), 168.2 (C_q, C-2), 149.6 (C_q, C-8), 142.8 (CH_{arom}), 140.1 (CH_{arom}), 114.3 (CH_{arom}), 112.1 (C_q, C-3), 73.9 (C_q, C-5), 51.8 (CH_3), 46.9 (C-9), 38.7 (C-13), 32.5 (CH_2), 31.0 (CH_2), 28.1 (CH_{arom}), 21.2 (CH_{arom}).

6.6.2.1.5.4 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-iodobenzoic acid (63)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 46.2 mg (111 µmol, 1.0 eq) methyl 5-iodo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**), 1.10 mL THF/H_2O (1:1, v/v) and 46.9 mg (1.11 mmol, 10 eq) LiOH·H_2O. The mixture was placed into a preheated (40 °C) oil bath and was left stirring for 22 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 1 M HCl and the solvents were removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{14}H_{16}INO_4$ [389.19]

yield: 33.5 mg (86.1 µmol, 78%) yellow, amorphous solid.

R_f = 0.12 (cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: green-orange).

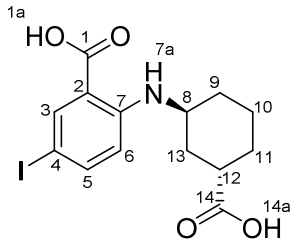
mp: 195 °C (decomp.)

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.44 (bs, 2H, H-1a, H-14a), 8.08–7.66 (m, 2H, NH, ArH), 7.56 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, ArH), 6.68 (d, $^3J_{HH}$ = 9.0 Hz, 1H, ArH), 3.51–3.33 (m, 1H, H-8), 2.48–2.30 (m, 1H, H-12), 2.25–2.12 (m, 1H, CH_2), 2.04–1.68 (m, 3H, CH_2), 1.51–0.97 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C_q, C-14), 168.8 (C_q, C-1), 149.2 (C_q, C-7), 142.0 (C-5), 139.5 (C-3), 114.7 (C-6), 112.5 (C_q, C-2), 73.8 (C_q, C-4), 49.4 (C-8), 41.3 (C-12), 35.0 (C-13), 32.1 (C-9), 28.1 (C-11), 23.7 (C-10).

HR-MS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{14}H_{17}INO_4^+$: 390.0197; found: 390.0210.

6.6.2.1.5.5 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-iodobenzoic acid (63)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 35.2 mg (84.4 µmol, 1.0 eq) methyl 5-iodo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**), 840 µL THF/H_20 (1:1, v/v) and 35.8 mg (744 µmol, 10 eq) $LiOH \cdot H_2O$. The mixture was placed into a preheated (40 °C) oil bath and was left stirring for 22 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 1 M HCl and the solvents were removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{14}H_{16}INO_4$ [389.19]

yield: 26.9 mg (69.1 µmol, 82%) yellow, amorphous solid.

R_f = 0.17 (cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: green-orange).

mp: 190 °C (decomp., turns dark brown).

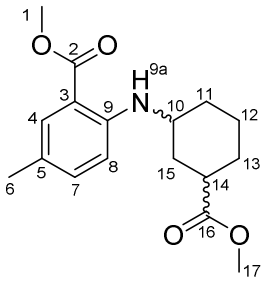
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.42 (bs, 2H, 2 x CO_2H), 8.65–7.85 (m, 2H, NH, ArH), 7.54 (d, $^3J_{HH}$ = 8.4 Hz, 1H, ArH), 6.63 (d, $^3J_{HH}$ = 8.7 Hz, 1H, ArH), 3.84–3.57 (m, 1H, H-8), 2.64–2.38 (m, 1H, H-12, DMSO overlap), 2.05–1.33 (m, 8H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.2 (C_q, C-14), 169.1 (C_q, C-1), 149.1 (C_q, C-7), 141.7 (CH_{arom}), 139.6 (CH_{arom}), 114.4 (CH_{arom}), 113.6 (C_q, C-2), 73.8 (C_q, C-4), 46.2 (C-8), 38.0 (C-12), 32.2 (C-13), 30.2 (C-9), 27.4 (C-11), 20.6 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}INO_4^+$: 389.0124; found: 389.0149.

6.6.2.1.6 Me-Phenazistatin

6.6.2.1.6.1 2-((3-Carboxycyclohexyl)amino)-5-methylbenzoic acid (73)

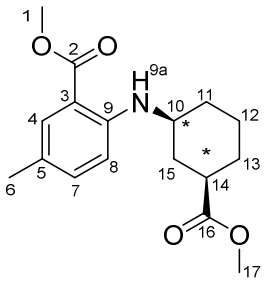


A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 156 mg (1.00 mmol, 1.0 eq) 2-amino-5-methylbenzoic acid, 600 µL dry DMF, 172 mg (1.10 mmol, 1.1 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**)[203,215] and 323 µL (2.50 mmol, 2.5 eq) TMSCl at RT. The yellow solution was cooled to 0 °C, the glass stopper was replaced by a rubber septum and 1.00 mL (1.00 mmol, 1.0 eq) BH_3·THF were slowly added over a period of 10 min via syringe. The mixture was left stirring in the thawing ice bath for 3 d (TLC: full conversion of the starting material), 5 mL H_2O were added and the reaction mixture was extracted with EtOAc (3 x 8 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The residue was transferred into a 30 mL screw-cap vial with magnetic stirring bar and was dissolved in 3 mL distilled acetone. 168 mg (1.2 mmol, 1.2 eq) K_2CO_3 and 57.3 µL (600 µL, 0.6 eq) Me_2SO_4 were added at RT to the stirred solution. The reaction mixture was stirred for 10 d (TLC: incomplete conversion) and another portion of 84.0 mg (600 µmol, 0.6 eq) K_2CO_3 and 28.7 µL (300 µL, 0.3 eq) Me_2SO_4 was added. Full conversion of the starting material was detected after another 24 h. The solids were removed via filtration through a Pore 3 sintered glass frit, the filter cake was repeatedly rinsed with distilled acetone and the solvent was removed in vacuum. The residue was dissolved in 50 mL EtOAc and was washed with H_2O (5 x 20 mL). The solvent was removed in vacuum and the product was adsorbed on 725 mg Celite™ (EtOAc) and was purified via column chromatography (size: 24 x 1.4 cm, cyclohexane/EtOAc = 25:1 (v/v)). Pure fractions of the isomers could be isolated.[189]

$C_{17}H_{23}NO_4$ [305.37]

yield (*cis*&*trans*): 93.3 mg (306 µmol, 31%) light-yellow, viscous liquid.

6.6.2.1.6.2 Methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-methylbenzoate (73)



R_f = 0.55 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: carnate).

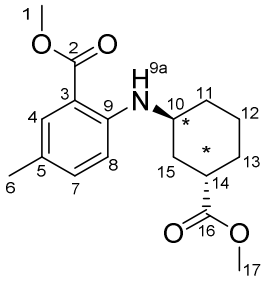
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.70 (s, 1H, H-4), 7.60–7.45 (m, 1H, NH), 7.15 (d, $^3J_{HH}$ = 7.4 Hz, 1H, H-7), 6.62 (d, $^3J_{HH}$ = 8.6 Hz, 1H, H-8), 3.83 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.43–3.26 (m, 1H, H-10), 2.53–2.33 (m, 2H, H-14, CH_2), 2.21 (s, 3H, C-6), 2.17–2.06 (m, 1H, CH_2), 2.03–1.86 (m, 2H, CH_2), 1.53–1.11 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.5 (C_q, C-16), 169.2 (C_q, C-2), 148.3 (C_q, C-9), 135.7 (C-7), 131.7 (C-4), 123.4 (C_q), 111.9 (C-8), 109.8 (C_q), 51.8 (CH_3), 51.5 (CH_3), 50.7 (C-10), 42.6 (C-14), 35.6 (C-15), 32.8 (C-11), 28.5 (C-13), 24.5 (C-12), 20.2 (C-6).

GC-MS: t_R = 7.953 min, *m/z* (%) = 305.1 (M^+, 100), 246.1 (45), 214.1 (24), 204.1 (40), 172.0 (52).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{23}NO_4^+$: 305.1627; found: 305.1630.

6.6.2.1.6.3 Methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-methylbenzoate (73)



R_f = 0.60 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: carnate).

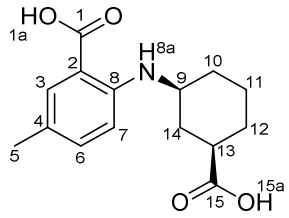
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.82–7.66 (m, 2H, H-4, NH), 7.17 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, H-7), 6.69 (d, $^3J_{HH}$ = 8.6 Hz, 1H, H-8), 3.89–3.73 (m, 2H, H-10, OCH_3), 3.68 (s, 3H, OCH_3), 2.78–2.64 (m, 1H, H-14), 2.22 (s, 3H, C-6), 2.09–1.94 (m, 1H, CH_2), 1.90–1.53 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.1 (C_q, C-16), 169.3 (C_q, C-2), 148.4 (C_q, C-9), 135.9 (C-7), 131.6 (C-4), 123.2 (C_q), 112.0 (C-8), 109.6 (C_q), 51.7 (OCH_3), 51.5 (OCH_3), 46.9 (C-10), 38.6 (C-14), 32.7 (C-15), 31.0 (C-11), 28.2 (C-13), 21.1 (C-12), 20.2 (C-6).

GC-MS: t_R = 7.826 min, *m/z* (%) = 305.1 (M^+, 100), 246.1 (40), 214.1 (22), 204.1 (40), 172.1 (48).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{23}NO_4^+$: 305.1627; found: 305.1633.

6.6.2.1.6.4 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-methylbenzoic acid (68)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 40.4 mg (132 µmol, 1.0 eq) methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-methylbenzoate (**73**), 1.10 mL THF/H_2O (1:1, v/v) and 55.9 mg (1.32 mmol, 10 eq) LiOH·H_2O. The mixture was placed into a preheated (40 °C) oil bath and was left stirring for 26 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 1 M HCl and the solvents were removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{15}H_{19}NO_4$ [277.32]

yield: 28.3 mg (102 µmol, 77%) light-yellow, amorphous solid.

R_f = 0.21 (cyclohexane/EtOAc/AcOH = 60:40:1 (v/v/v)) (254 nm, 366 nm, cold CAM: carnate).

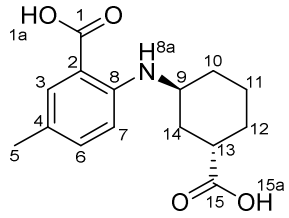
mp: 198-199 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.23 (bs, 2H, H-1a, H-15a), 7.59 (s, 1H, H-3), 7.17 (d, $^3J_{HH}$ = 8.3 Hz, 1H, H-6), 6.71 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H-7), 3.49–3.32 (m, 1H, H-9), 2.48–2.31 (m, 1H, H-13), 2.28–2.07 (m, 4H, H-5, CH_2), 2.07–1.71 (m, 3H, CH_2), 1.52–0.95 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.0 (C_q, C-1), 170.1 (C_q, C-15), 148.0 (C_q, C-8), 135.3 (CH_{arom}), 131.6 (CH_{arom}), 122.3 (C_q, C-4), 112.0 (CH_{arom}), 109.8 (C_q, C-2), 49.6 (C-9), 41.5 (C-13), 35.4 (C-14), 32.4 (C-10), 28.2 (C-12), 23.9 (C-11), 19.8 (C-5).

HR-MS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{15}H_{20}NO_4^+$: 278.1387; found: 278.1386.

6.6.2.1.6.5 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-methylbenzoic acid (68)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 52.9 mg (173 µmol, 1.0 eq) methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-methylbenzoate (**73**), 1.50 mL THF/H_2O (1:1, v/v) and 73.3 mg (1.73 mmol, 10 eq) LiOH·H_2O. The mixture was placed into a preheated (40 °C) oil bath and was left stirring for 26 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 1 M HCl and the solvents were removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{15}H_{19}NO_4$ [277.32]

yield: 21.7 mg (78.2 µmol, 45%) light-yellow, amorphous solid.

R_f = 0.25 (cyclohexane/EtOAc/AcOH = 60:40:1 (v/v/v)) (254 nm, 366 nm, cold CAM: carnate).

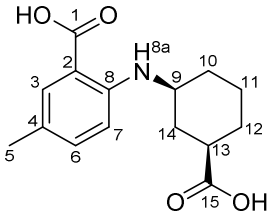
mp: 227-228 °C.

^{1}H-NMR (300.36 MHz, MeOH-d_4): δ = 7.70 (s, 1H, H-3), 7.17 (d, $^3J_{HH}$ = 8.6 Hz, 1H, H-6), 6.74 (d, $^3J_{HH}$ = 8.6 Hz, 1H, H-7), 3.84–3.72 (m, 1H, H-9), 2.71–2.59 (m, 1H, H-13), 2.20 (s, 3H, H-5), 2.10–1.96 (m, 1H, CH_2), 1.87–1.50 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, MeOH-d_4): δ = 179.2 (C_q, C-1), 172.3 (C_q, C-15), 149.6 (C_q, C-8), 136.6 (CH_{arom}), 133.1 (CH_{arom}), 124.6 (C_q, C-4), 113.4 (CH_{arom}), 111.5 (C_q, C-2), 48.3 (C-9), 39.8 (C-13), 34.0 (C-14), 32.0 (C-10), 29.1 (C-12), 22.1 (C-11), 20.3 (C-5).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{19}NO_4^+$: 277.1314; found: 277.1303.

6.6.2.1.6.6 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-methylbenzoic acid (68)



A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 148.0 mg (544 µmol, 1.5 eq) (1*S**,3*R**)-ethyl 3-aminocyclohexane-1-carboxylate (**42**), 1.0 mL dry CH_2Cl_2 and 500 µL TFA. The light-yellow solution was stirred at RT for 21 h and the solvents were removed in vacuum. To the yellow oil were added 7.0 mg (36.3 µmol, 0.1 eq) CuI, 8.4 mg (72.5 µmol, 0.2 eq) L-proline, 342.0 mg (544 µmol, 1.5 eq) TBAA, 100 mg (363 µmol, 1.0 eq) 2-iodo-5-methylbenzoic acid (**69**) and 2.0 mL dry DMF in N_2 counter-stream. To the yellow solution were added 76.0 mg (544 µmol, 1.5 eq) K_2CO_3 and the turquoise suspension was heated to 60 °C for 11 d (TLC: full conversion of the starting material). The reaction mixture was cooled to RT, acidified with 1 N HCl (2 mL) and the mixture was extracted with EtOAc (3 x 3 mL). The combined organic layers were concentrated in vacuum. To the residue were added 5 mL THF/MeOH/H_2O (3:1:1 (v/v/v)) and 30.5 mg (726 µmol, 2.0 eq) $LiOH \cdot H_2O$ and the brown solution was stirred at RT for 2 d (further 4.0 eq $LiOH \cdot H_2O$ were necessary for full conversion of the starting material). The green suspension was acidified with 1 N HCl (3 mL) and the orange solution was extracted with Et_2O (5 x 4 mL). The solvent was removed in vacuum and the residue was adsorbed on 100 mg Celite™ (MeOH). The product was purified via column chromatography (13 g silica gel, size: 120 x 15 mm, cyclohexane/EtOAc/AcOH = 5000:1000:1, 10 mL fractions).[197]

$C_{15}H_{19}NO_4$ [277.32]

yield: 9.0 mg (32.5 µmol, 9%), brown solid.

R_f = 0.51 (cyclohexane/EtOAc/AcOH = 1000:1000:1) (UV 254 nm & 366 nm, CAM: orange)

HPLC (Method_3to80): t_R = 4.53 min, λ_{max}= 227 nm, 260 nm, 364 nm

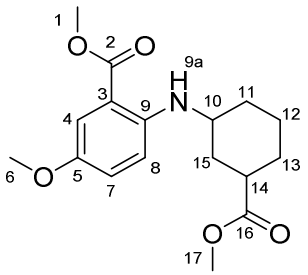
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.39 (bs, 2H, H-1, H-15), 7.58 (d, $^4J_{HH}$ = 1.7 Hz, 1H, H-3), 7.17 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, H-6), 6.72 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-7), 3.56-3.19 (m, 1H, H-9), 2.47-2.33 (m, 1H, H-13), 2.26-2.09 (m, 4H, H-5, H-14a), 1.99 (d, $^3J_{HH}$ = 12.5 Hz, 1H, H-10a), 1.89 (d, $^3J_{HH}$ = 12.8 Hz, 1H, H-12a), 1.78 (d, $^3J_{HH}$ = 13.1 Hz, 1H, H-11a), 1.57-1.32 (m, 1H, H-11b), 1.31-0.96 (m, 3H, H-10b, H-12b, H-14b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C-15), 170.0 (C-1), 148.0 (C-8), 135.3 (C-6), 131.5 (C-3), 122.3 (C-4), 112.0 (C-7), 109.6 (C-2), 49.5 (C-9), 41.4 (C-13), 35.3 (C-14), 32.4 (C-10), 28.2 (C-12), 23.8 (C-11), 19.8 (C-5).

HR-MS (ESI-MS): *m/z* $[M+H]^+$ calcd for $C_{15}H_{20}NO_4^+$: 278.1387; found: 278.1386.

6.6.2.1.7 MeO-Phenazistatin

6.6.2.1.7.1 Methyl 5-methoxy-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (80)

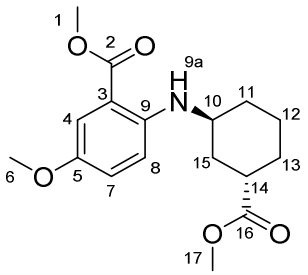


A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 375 mg (2.40 mmol, 1.0 eq) 5-hydroxyanthranilic acid, 2.3 mL dry DMF, 412 mg (2.64 mmol, 1.1 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**)[203,215] and 930 µL (7.2 mmol, 3.0 eq) TMSCl at RT in a N_2 counter-stream. The mixture was cooled to 0 °C and 3.6 mL (3.6 mmol, 1.5 eq) $BH_3 \cdot THF$ (1.0 M solution in THF) was added slowly over a period of 10 min. The light-yellow suspension was left to warm to RT in the ice bath and full conversion of the starting material was detected with TLC (EtOAc/cyclohexane/AcOH = 2:1:1%) after 3 d. The reaction mixture was carefully treated with 5 mL H_2O and the mixture was extracted with EtOAc (3 x 8 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The violet-brown liquid residue was transferred to a 20 mL screw-cap vial with a magnetic stirring bar and the crude product was dissolved in 7 mL distilled acetone. 737 mg (5.28 mmol, 2.2 eq) K_2CO_3 were added, the mixture was stirred and 275 µL (2.88 mmol, 1.2 eq) Me_2SO_4 were added in one portion at RT. Full conversion of the starting material was detected by TLC after 3 h and the mixture was filtrated through a Pore 3 glass frit, the filter cake was rinsed with acetone and the filtrate was concentrated in vacuum. The orange, oily residue was adsorbed on 237 mg Celite™ (acetone) and the crude product was purified via column chromatography (size: 14 x 1 cm, cyclohexane/EtOAc = 15:1, 4 mL fractions). Clean fractions of the stereoisomers could be obtained.[189]

$C_{17}H_{23}NO_5$ [321.37]

yield (*cis* & *trans*): 84.0 mg (261 µmol, 11%) yellow, viscous liquid.

6.6.2.1.7.2 Methyl 5-methoxy-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-benzoate (80)



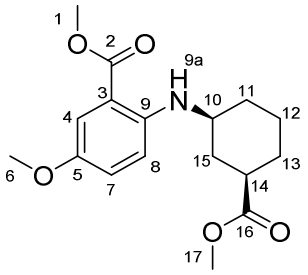
yield: 41.0 mg (127 µmol, 5%) yellow, viscous liquid.

R_f = 0.26 (cyclohexane/EtOAc = 10:1 (v/v)) (254 nm, 366 nm, air: carnate, CAM: redish).

GC-MS: t_R = 8.179 min, *m/z* (%) = 321.2 (100, M^+), 262.1 (28), 220.1 (34), 188.1 (54), 160.0 (19), 81.1 (17).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.42 (d, $^3J_{HH}$ = 2.8 Hz, 1H, H-4), 7.04 (dd, $^3J_{HH}$ = 9.1 Hz, $^4J_{HH}$ = 3.0 Hz, 1H, H-7), 6.90–6.71 (m, 1H, H-8), 3.86 (s, 3H, CH_3), 3.75 (s, 4H, CH_3, H-10), 3.67 (s, 3H, CH_3), 2.78–2.64 (m, 1H, H-14), 2.11–1.94 (m, 1H, H-15a), 1.90–1.49 (m, 7H, H-11, H-12, H-13, H-15b).

6.6.2.1.7.3 Methyl 5-methoxy-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-benzoate (80)



yield: 15.9 mg (49.4 µmol, 2%) yellow/orange solid.

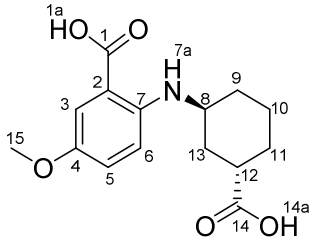
R_f = 0.21 (cyclohexane/EtOAc = 10:1 (v/v)) (254 nm, 366 nm, air: carnate, CAM: redish).

mp: 80-83 °C.

GC-MS: t_R = 8.317 min, *m/z* (%) = 321.2 (100, M^+), 262.1 (30), 220.1 (31), 188.1 (54), 160.1 (17), 81.1 (17).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.43 (d, $^4J_{HH}$ = 2.7 Hz, 1H, H-4), 7.04 (dd, $^3J_{HH}$ = 9.1 Hz, $^4J_{HH}$ = 2.7 Hz, 1H, H-7), 6.93–6.70 (m, 1H, H-8), 3.86 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 3.42–3.26 (m, 1H, H-10), 2.50–2.31 (m, 2H, H-14, H-15a), 2.21–2.08 (m, 1H, H-11a), 2.05–1.88 (m, 2H, H-12a, H-13a), 1.51–1.16 (m, 4H, H-11b, H-12b, H-13b, H-15b).

6.6.2.1.7.4 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-methoxybenzoic acid (74)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 41.0 mg (128 µmol, 1.0 eq) methyl 5-methoxy-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**80**), 2 mL THF/MeOH = 4:1 (v/v) and 81.1 mg (1.91 mmol, 15 eq) $LiOH \cdot H_2O$. The dark-yellow, brownish mixture was stirred for 18 h at RT (TLC: incomplete conversion of the starting material) and 81.1 mg (1.91 mmol, 15 eq) $LiOH \cdot H_2O$ were added. The mixture was stirred for 23 h (TLC: incomplete) and 108 mg (1.91 mmol, 15 eq) KOH were added and the mixture was stirred for 25 h when another portion of 81.1 mg (1.91 mmol, 15 eq) $LiOH \cdot H_2O$ was added. Full conversion of the starting material was detected after another 3 d. The mixture was acidified with 6 N HCl (pH~1) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (93 mg, MeOH) and purified via column chromatography (size: 17 x 0.8 cm, cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v), 3 mL fractions). The product was further purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{15}H_{19}NO_5$ [293.32]

yield: 7.7 mg (26.3 µmol, 21%) yellow solid.

R_f = 0.10 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: yellow).

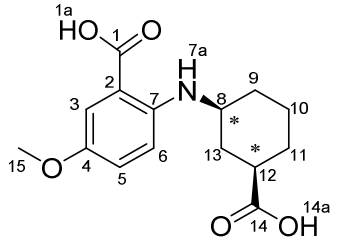
mp: 200-203 °C.

^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 7.47 (d, $^4J_{HH}$ = 3.0 Hz, 1H, H-3), 7.04 (dd, $^3J_{HH}$ = 9.1 Hz, $^4J_{HH}$ = 3.0 Hz, 1H, H-5), 6.86 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 3.83–3.69 (m, 4H, H-8, H-15), 2.74–2.59 (m, 1H, H-12), 2.12–1.95 (m, 1H, H-13a), 1.87–1.49 (m, 7H, H-9, H-10, H-11, H-13b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 179.2 (C_q, C-14), 172.0 (C_q, C-1), 151.7 (C_q), 145.3 (C_q), 123.6 (CH_{arom}), 116.3 (CH_{arom}), 115.8 (CH_{arom}), 113.9 (C_q, C-2), 56.3 (C-15), 49.5 (C-8), 39.9 (C-12), 33.9 (C-13), 31.9 (C-9), 29.1 (C-11), 22.1 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{19}NO_5^+$: 293.1263; found: 293.1264.

6.6.2.1.7.5 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-methoxybenzoic acid (74)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 15.9 mg (49.5 µmol, 1.0 eq) methyl 5-methoxy-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**80**), 1 mL THF/MeOH = 4:1 (v/v) and 31.5 mg (742 µmol, 15 eq) $LiOH \cdot H_2O$. The dark-yellow, brownish mixture was stirred for 18 h at RT and 31.5 mg (742 µmol, 15 eq) $LiOH \cdot H_2O$ were added. The mixture was stirred and 41.7 mg (1.91 mmol, 15 eq) KOH were added after 23 h followed by 31.5 mg (742 µmol, 15 eq) $LiOH \cdot H_2O$ after another 25 h. Full conversion of the starting material was detected after 3 d, the mixture was acidified with 6 N HCl (pH~1) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (36 mg, MeOH) and purified via column chromatography (size: 17 x 0.8 cm, cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v), 3 mL fractions). The product was further purified via preparative RP-HPLC (Method_PREPHPLC). Not enough product could be synthesized for full NMR characterization.

$C_{15}H_{19}NO_5$ [293.32]

yield: 1.7 mg (5.80 µmol, 12%) yellow solid.

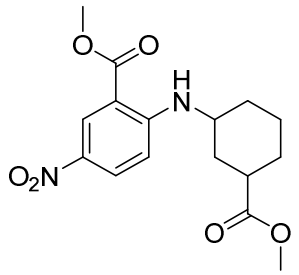
R_f = 0.07 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: yellow).

mp: 86-87 °C.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{19}NO_5^+$: 293.1263; found: 293.1266.

6.6.2.1.8 NO_2-Phenazistatin

6.6.2.1.8.1 Methyl 2-((3-(methoxycarbonyl)cyclohexyl)amino)-5-nitrobenzoate (87)



A dry 20 mL Schlenk flask with a magnetic stirring bar was charged with 409 mg (2.20 mmol, 1.0 eq) 2-amino-5-nitrobenzoic acid, 1.4 mL dry DMF, 378 mg (2.42 mmol, 1.1 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**)[203,215] and 710 µL (5.50 mmol, 2.5 eq) TMSCl. The orange, turbid suspension was cooled to 0 °C and 2.2 mL (2.20 mmol, 1.0 eq) BH_3·THF (1.0 M solution in THF) were added over a period of 10 min. The reaction was allowed to warm to RT and was left stirring for 3 d (TLC: full conversion of the starting material). The mixture was treated with 5 mL H_2O and then extracted with EtOAc (3 x 8 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was transferred with 7 mL distilled acetone into a 30 mL screw-cap vial with magnetic stirring bar and was treated with 369 mg (2.64 mmol, 1.2) K_2CO_3. The mixture was stirred and 126 µL (1.32 mmol, 0.6 eq) Me_2SO_4 were added in one portion. The reaction mixture was stirred for 8 d at RT and was filtrated through a Pore 4 sintered glass frit, rinsed with acetone. The solvent was removed in vacuum and the residue was adsorbed on Celite™ (1.84 g, acetone). The product was purified via column chromatography (75 g silica gel, size: 17 x 2.8 cm, cyclohexane/EtOAc = 10:1, 10 mL fractions). The diastereomers were not fully separated and the fractions with varying content were used in the following saponification step without further purification.[189]

$C_{16}H_{20}N_2O_6$ [336.34]

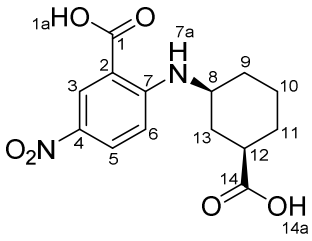
yield: 194 mg (576 µmol, 26%) yellow, very viscous liquid.

R_f = 0.42 (*trans*), 0.38 (*cis*) (cyclohexane/EtOAc = 3:1% (v/v/v)) (254 nm, VIS: yellow).

GC-MS (*trans*): t_R = 9.480 min, *m/z* (%) = 336.1 (M^+, 67), 321.1 (35), 277.1 (65), 235.1 (55), 207.0 (100), 203.0 (54), 73.0 (35).

GC-MS (*cis*): t_R = 9.723 min , *m/z* (%) = 336.1 (M^+, 96), 321.1 (54), 277.1 (100), 235.0 (76), 207.1 (99), 203.0 (85), 81.0 (35).

6.6.2.1.8.2 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-nitrobenzoic acid (82)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 126 mg (374 µmol, 1.0 eq) methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-nitrobenzoate (**87**, ~80% *cis*), 8 mL distilled THF/H_2O (4:1 (v/v)) and 238 mg (5.61 mmol, 15 eq) LiOH·H_2O. The light-yellow suspension was stirred at RT for 17 h until full conversion of the starting material was detected by TLC. The reaction mixture was acidified with 6 N HCl (3 mL), diluted with 5 mL H_2O and extracted with EtOAc (3 x 10 mL). The combined organic layers were concentrated in vacuum and the residue was dried in oil pump vacuum. The yellow solid was adsorbed on Celite™ (288 mg, MeOH) and was purified via column chromatography (size: 17 x 1 cm, cyclohexane/EtOAc/AcOH = 4:1:1%, 5 mL fractions).

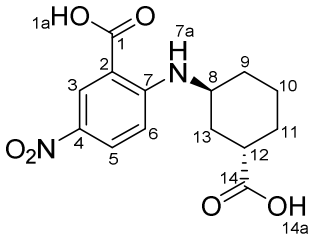
$C_{14}H_{16}N_2O_6$ [308.29]

yield: 24.8 mg (80.4 µmol, 22%) intensive-yellow solid (purity (NMR) = 85%).

R_f = 0.09 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, VIS: yellow).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}N_2O_6^+$: 308.1008; found: 308.1004.

6.6.2.1.8.3 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-nitrobenzoic acid (82)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 67.9 mg (202 µmol, 1.0 eq) methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-nitrobenzoate (**87**, 98% *trans*), 4 mL distilled THF/H_2O (4:1 (v/v)) and 128 mg (3.03 mmol, 15 eq) LiOH·H_2O. The light-yellow suspension was stirred at RT for 17 h until full conversion of the starting material was detected by TLC. The reaction mixture was acidified with 6 N HCl (3 mL), diluted with 5 mL H_2O and extracted with EtOAc (3 x 10 mL). The combined organic layers were concentrated in vacuum and the residue was dried in oil pump vacuum. The yellow

solid was adsorbed on Celite™ (156 mg, MeOH) and the product was purified via column chromatography (size: 14 x 1 cm, cyclohexane/EtOAc/AcOH = 2:1:1%, 4 mL fractions).

$C_{14}H_{16}N_2O_6$ [308.29]

yield: 52.2 mg (169 µmol, 84%) intensive-yellow solid.

R_f = 0.15 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, VIS: yellow).

mp: 259-260 °C (decomp., turns black).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.76 (bs, 2H, H-1a, H-14a), 8.98 (d, $^3J_{HH}$ = 7.3 Hz, 1H, C-7a), 8.65 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-3), 8.17 (dd, $^3J_{HH}$ = 9.3 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, H-5), 6.94 (d, $^3J_{HH}$ = 9.6 Hz, 1H, H-6), 4.00–3.82 (m, 1H, H-8), 2.63–2.52 (m, 1H, H-12), 2.05–1.47 (m, 8H, H-9, H-10, H-11, H-13).

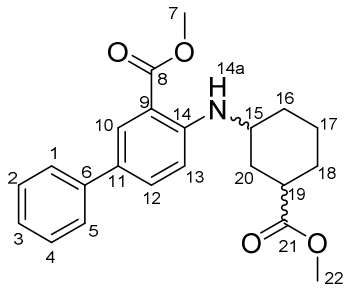
^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.0 (C_q, C-14), 169.0 (C_q, C-1), 153.7 (C_q, C-7), 134.6 (C_q, C-4), 129.5 (C-5), 128.8 (C-3), 111.7 (C-6), 109.3 (C_q, C-2), 47.1 (C-8), 38.0 (C-12), 32.0 (C-13), 30.1 (C-9), 27.2 (C-11), 20.6 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}N_2O_6^+$: 308.1008; found: 308.1001.

6.6.2.1.9 Miscellaneous Phenazistatin derivatives

6.6.2.1.10 Suzuki-Miyaura reaction

6.6.2.1.10.1 Methyl 4-((3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3-carboxylate (90)



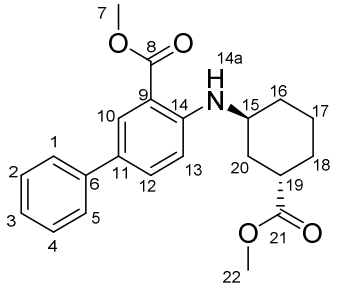
A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 5 mL dry DME and 200 mg (479 µmol, 1.0 eq) methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**) and the mixture was degassed for 15 min (N_2-bubbling in ultrasonic bath). Another 20 mL Schlenk flask with magnetic stirring bar was charged with 154 mg (1.01 µmol, 2.1 eq) CsF, 60.3 mg (479 µmol, 1.0 eq) phenylboronic acid, 19.3 mg (24.0 µmol, 0.05 eq) $PdCl_2(dppf)_2 \cdot Et_2O$ and the flask was evacuated in oil pump vacuum (10 min). The degassed aryl iodide solution was added to the solids, the Schlenk flask was sealed and placed into a pre-heated oil bath (85 °C). The red-brown suspension was kept at this temperature for 5 d

until full conversion of the starting material was detected by TLC (cyclohexane/EtOAc = 4:1 (v/v)). The reaction mixture was filtrated through a 3 cm pad of silica gel (wettened with EtOAc) and the pad was repeatedly rinsed with EtOAc (5 x 20 mL). The solvents were removed in vacuum and the light-brown, oily residue was dried in oil pump vacuum (173 mg). The crude product was adsorbed on Celite™ (440 mg, Et_2O) and purified via column chromatography (size: 14 x 2.2 cm, cyclohexane/EtOAc = 20:1 (v/v), 10 mL fractions). Pure fractions of the stereoisomers could be isolated.[211]

$C_{22}H_{25}NO_4$ [367.45]

yield (total): 117 mg (318 µmol, 66%) yellow, viscous liquid.

6.6.2.1.10.2 Methyl 4-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3-carboxylate (90)



yield: 30.4 mg (82.7 µmol, 17%) yellow, very viscous liquid.

R_f = 0.52 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: crimson).

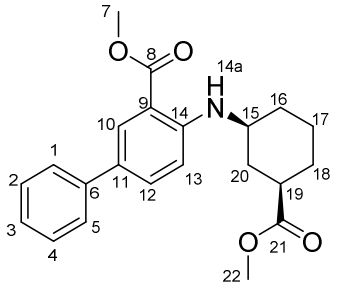
GC-MS: t_R = 10.773 min, *m/z* (%) = 368.1 (20), 367.1 (M^+, 100), 308.1 (20), 276.1 (18), 266.1 (20), 234.1 (52), 221.1 (20), 167.1 (19), 152.1 (26), 81.1 (18).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.15 (d, $^4J_{HH}$ = 2.2 Hz, 1H, H-10), 8.02-7.93 (m, 1H, H-14a), 7.60 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, H-12), 7.51(d, $^3J_{HH}$ = 7.4 Hz, 2H, CH_{arom}), 7.37 (t, $^3J_{HH}$ = 7.6 Hz, 2H, CH_{arom}), 7.28-7.19 (m, 1H, CH_{arom}), 6.84 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-13), 3.89-3.78 (m, 4H, H-7, H-15), 3.67 (s, 3H, H-22), 2.77-2.65 (m, 1H, H-19), 2.11-2.03 (m, 1H, H-20a), 1.91-1.51 (m, 7H, H-16, H-17, H-18, H-20b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.0 (C_q, C-21), 169.4 (C_q, C-8), 149.5 (C_q, C-14), 140.7 (C_q, C-6), 133.5 (C-12), 130.2 (C-10), 128.8 (CH_{arom}), 127.4 (C_q, C-11), 126.4 (CH_{arom}), 126.2 (CH_{arom}), 112.4 (C-13), 110.1 (C_q, C-9), 51.8/51.7 (CH_3, C-7, C-22), 47.1 (C-15), 38.7 (C-19), 32.7 (C-20), 31.1 (C-16), 28.2 (C-18), 21.2 (C-17).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{22}H_{25}NO_4^+$: 367.1783; found: 367.1792.

6.6.2.1.10.3 Methyl 4-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3-carboxylate (90)



yield: 12.0 mg (32.7 µmol, 7%) faint-yellow, very viscous liquid.

R_f = 0.47 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: crimson).

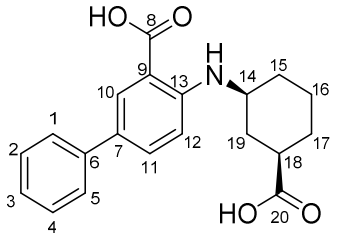
GC-MS: t_R = 11.136 min, *m/z* (%) = 368.1 (25), 367.1 (M^+, 100), 308.1 (22), 276.1 (19), 266.1 (20), 234.1 (53), 221.1 (19), 207.0 (16), 167.0 (19), 152.1 (20), 81.0 (17).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.14 (d, $^3J_{HH}$ = 2.2 Hz, 1H, H-10), 7.83-7.73 (m, 1H, H-14a), 7.58 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-12), 7.51 (d, $^3J_{HH}$ = 7.2 Hz, 2H, CH_{arom}), 7.37 (t, $^3J_{HH}$ = 7.6 Hz, 2H, CH_{arom}), 7.25-7.19 (m, 1H, CH_{arom}), 6.77 (d, $^3J_{HH}$ = 8.8 Hz, 1H, H-13), 3.84 (s, 3H, H-7), 3.64 (s, 3H, H-22), 3.48-3.31 (m, 1H, H-15), 2.52-2.34 (m, 2H, H-19, H-20a), 2.14 (d, $^3J_{HH}$ = 12.9 Hz, 1H, H-16a), 2.02-1.86 (m, 2H, H-17a, H-18a), 1.47-1.16 (m, 4H, H-16b, H-17b, H-18b, H-20b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.4 (C_q, C-21), 169.2 (C_q, C-8), 149.3 (C_q, C-14), 140.6 (C_q, C-6), 133.3 (C-12), 130.3 (C-10), 128.9 (CH_{arom}), 127.7 (C_q, C-11), 126.5 (CH_{arom}), 126.3 (CH_{arom}), 112.4 (C-13), 110.4 (C_q, C-9), 51.9/51.7 (CH_3, C-7, C-22), 50.9 (C-15), 42.6 (C-19), 35.5 (C-20), 32.8 (C-16), 28.5 (C-18), 24.5 (C-17).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{22}H_{25}NO_4^+$: 367.1783; found: 367.1782.

6.6.2.1.10.4 4-(((1*S**,3*R**)-3-(Methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3-carboxylic acid (91)



A 3 mL screw-cap vial with magnetic stirring bar was charged with 22.5 mg (61.2 µmol, 1.0 eq) methyl 4-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3-

carboxylate (**36**), 620 µL THF/MeOH=1:1 (v/v) and 27.2 mg (642 µmol, 10.5 eq) $LiOH \cdot H_2O$. The dark-brown solution was stirred at RT for 43 h (TLC: incomplete conversion) and 7.2 mg (170 µmol, 2.8 eq) $LiOH \cdot H_2O$ were added. Full conversion of the starting material was detected after 48 h (TLC) and the mixture was diluted with 3 mL THF and acidified with 6 N HCl (2 mL). The yellow mixture was extracted with EtOAc (3 x 5 mL), the organic phases were pooled and the solvent was removed in vacuum. The crude product was adsorbed on Celite™ (52 mg, MeOH) and purified via column chromatography (size: 9 x 1 cm, cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v), 2 mL fractions).

$C_{20}H_{21}NO_4$ [339.39]

yield: 6.9 mg (20.3 µmol, 33%) yellow solid.

R_f = 0.21 (cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: dark red).

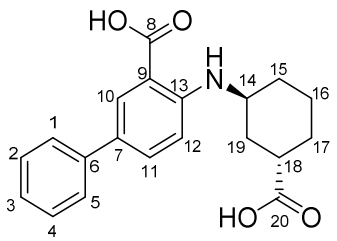
mp: 168-170 °C (decomp., turns brown).

^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 8.17 (d, $^4J_{HH}$ = 2.1 Hz, 1H, H-10), 7.63 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.1 Hz, 1H, CH_{arom}), 7.54 (d, $^3J_{HH}$ = 7.4 Hz, 2H, CH_{arom}), 7.37 (t, $^3J_{HH}$ = 7.6 Hz, 2H, CH_{arom}), 7.22 (t, $^3J_{HH}$ = 7.3 Hz, 1H, CH_{arom}), 6.88 (d, $^3J_{HH}$ = 8.9 Hz, 1H, CH_{arom}), 3.57–3.42 (m, 1H, H-14), 2.55–2.33 (m, 2H, H-18, H-19a), 2.20–2.09 (m, 1H, H-15a), 2.06–1.86 (m, 2H, H-16a, H-17a), 1.61–1.13 (m, 4H, H-15b, H-16b, H-17b, H-19b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 179.6 (C_q, C-20), 176.0 (C_q, C-8), 150.8 (C_q, C-13), 142.0 (C_q, C-6), 133.6 (CH_{arom}), 131.4 (C-10), 129.8 (CH_{arom}), 128.4 (C_q, C-7), 127.1 (CH_{arom}), 126.9 (CH_{arom}), 113.5 (C-12), 112.9 (C_q, C-9), 51.8 (C-14), 44.0 (C-18), 37.0 (C-19), 34.0 (C-15), 29.9 (C-17), 25.5 (C-16).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{20}H_{21}NO_4^+$: 339.1471; found: 339.1470.

6.6.2.1.10.5 4-(((1*S**,3*S**)-3-carboxycyclohexyl)amino)-[1,1'-biphenyl]-3-carboxylic acid (91)



A 3 mL screw-cap vial with magnetic stirring bar was charged with 30.0 mg (81.6 µmol, 1.0 eq) methyl 4-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3-carboxylate (**36**), 408 µL THF/MeOH=1:1 (v/v) and 35.5 mg (837 µmol, 10.3 eq) $LiOH \cdot H_2O$.

The light-yellow solution was stirred at RT for 43 h (TLC: incomplete conversion) and 8.6 mg (204 µmol, 2.5 eq) $LiOH \cdot H_2O$ were added. Full conversion of the starting material was detected after 48 h (TLC) and the mixture was diluted with 3 mL THF and acidified with 6 N HCl (2 mL). The yellow mixture was extracted with EtOAc (3 x 5 mL), the organic phases were pooled and the solvent was removed in vacuum. The crude product was adsorbed on Celite™ (70 mg, MeOH) and purified via column chromatography (size: 9 x 1 cm, cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v), 2 mL fractions).

$C_{20}H_{21}NO_4$ [339.39]

yield: 6.9 mg (20.3 µmol, 25%) yellow solid.

R_f = 0.24 (cyclohexane/EtOAc/AcOH = 3:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: dark red).

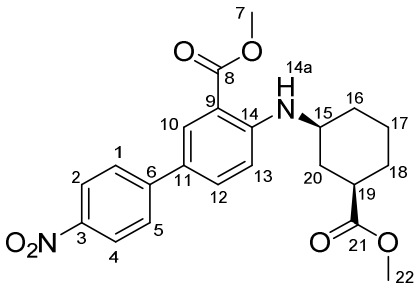
mp: 204-205 °C (decomp., turns brown, gas evolution).

^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 8.18 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-10), 7.69-7.59 (m, 1H, CH_{arom}), 7.54 (d, $^3J_{HH}$ = 7.5 Hz, CH_{arom}), 7.38 (t, $^3J_{HH}$ = 7.6 Hz, 2H, CH_{arom}), 7.23 (t, $^3J_{HH}$ = 7.2 Hz, 1H, CH_{arom}), 6.93 (d, $^3J_{HH}$ = 8.8 Hz, 1H, CH_{arom}), 3.93-3.81 (m, 1H, H-14), 2.76-2.61 (m, 1H, H-18), 2.18-2.01 (m, 1H, H-19a), 1.92-1.55 (m, 7H, H-15, H-16, H-17, H-19b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 179.3 (C_q, C-20), 172.4 (C_q, C-8), 151.0 (C_q, C-13), 141.9 (C_q, C-6), 133.9 (CH_{arom}), 131.4 (C-10), 129.8 (CH_{arom}), 128.4 (C_q, C-7), 127.2 (CH_{arom}), 126.9 (CH_{arom}), 113.5 (C-12), 112.0 (C_q, C-9),48.3 (C-14), 40.0 (C-18), 34.1 (C-19), 32.1 (C-15), 29.1 (C-17), 22.2 (C-16).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{20}H_{21}NO_4^+$: 339.1471; found: 339.1469.

6.6.2.1.10.6 Methyl 4-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-4'-nitro-[1,1'-biphenyl]-3-carboxylate (92)



A dry 20 mL Schlenk flask was charged with 5 mL dry 1,2-DME and 200 mg (479 µmol, 1.0 eq) methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**). The light-yellow solution was degassed for 15 min (N_2 bubbling, ultrasonic bath). A separate dry 20 mL Schlenk flask with magnetic stirring bar was charged with 154 mg (1.01 mmol, 2.1 eq) CsF,

80.8 mg (479 µmol, 1.0 eq) 4-nitrobenzeneboronic acid, 19.3 mg (24.0 µmol, 0.05 eq) $PdCl_2(dppf)\cdot Et_2O$ and the flask was evacuated and backfilled with N_2 two times. The degassed aryl iodide solution was added to the flask and the flask was sealed and placed into a preheated (85 °C) oil bath for 34 h until full conversion of the starting material was detected with TLC. The dark green-brown suspension was filtrated through a pad of silica gel (3 cm, EtOAc wettened) and the pad was repeatedly rinsed with EtOAc. The solvent was removed in vacuum and the residues were dried in oil pump vacuum (209 mg, red-orange viscous liquid). The crude product was adsorbed on Celite™ (530 mg, Et_2O) and was purified via column chromatography (40 g silica gel, cyclohexane/EtOAc = 8:1 (v/v), 10 mL fractions). A quantity of pure *cis*-isomer could be isolated.[211]

$C_{22}H_{24}N_2O_6$ [412.44]

yield (*cis&trans*): 110 mg (267 µmol, 56%) intensive orange/yellow viscous oil/foam.

yield (*cis*): 14.4 mg (34.9 µmol, 7%) intensive yellow, oily foam.

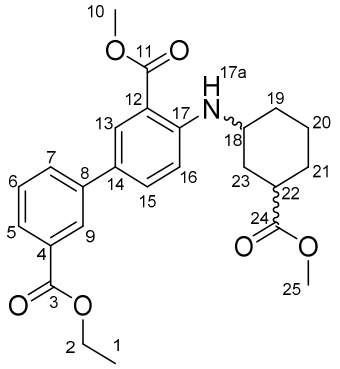
R_f = 0.36 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366nm: intensive yellow fluorescence, yellow without staining, cold CAM: green).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.30–8.20 (m, 3H, H-2, H-4, H-10), 7.99 (d, $^3J_{HH}$ = 6.9 Hz, 1H, H-14a), 7.75-7.58 (m, 3H, H-1, H-5, H-12), 6.82 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-13), 3.90 (s, 3H, H-7), 3.68 (s, 3H, H-22), 3.53–3.37 (m, 1H, H-15), 2.57–2.36 (m, 2H, H-19, H-20a), 2.23–2.11 (m, 1H, H-16a), 2.09–1.90 (m, 2H, H-17a, H-18a), 1.56–1.14 (m, 4H, H-16b, H-17b, H-18b, H-20b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-21), 168.8 (C_q, C-8), 150.4 (C_q), 147.0 (C_q), 146.2 (C_q), 133.2 (C-12), 131.0 (C-10), 126.3 (C-1/C-5), 124.5 (C_q, C-11), 124.4 (C-2, C-4), 112.6 (C-13), 110.5 (C_q, C-9), 51.9 (C-7/C-22), 50.9 (C-15), 42.5 (C-19), 35.4 (C-20), 32.7 (C-16), 28.5 (C-18), 24.4 (C-17).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{22}H_{24}N_2O_6^+$: 412.1634; found: 412.1638.

6.6.2.1.10.7 3'-Ethyl 3-methyl 4-((3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3,3'-dicarboxylate (95)

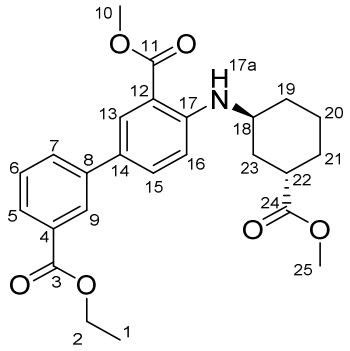


A dry 20 mL Schlenk flask was charged with 5 mL dry 1,2-DME and 200 mg (479 µmol, 1.0 eq) methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**). The light-yellow solution was degassed for 15 min (N_2 bubbling, ultrasonic bath). A separate dry 20 mL Schlenk flask with magnetic stirring bar was charged with 154 mg (1.01 mmol, 2.1 eq) CsF, 93.9 mg (479 µmol, 1.0 eq) 3-ethoxycarbonylphenylboronic acid, 19.3 mg (24.0 µmol, 0.05 eq) $PdCl_2(dppf)\cdot Et_2O$ and the flask was evacuated and backfilled with N_2 two times. The degassed aryl iodide solution was added to the flask and the flask was sealed and placed into a preheated (85 °C) oil bath for 34 h until full conversion of the starting material was detected with TLC. The dark brown suspension was filtrated through a pad of silica gel (3 cm, EtOAc wettened) and the pad was repeatedly rinsed with EtOAc. The solvent was removed in vacuum and the residue was dried in oil pump vacuum (211 mg, light-brown viscous liquid). The crude product was adsorbed on Celite™ (530 mg, Et_2O) and was purified via column chromatography (40 g silica gel, cyclohexane/EtOAc = 8:1 (v/v), 10 mL fractions). Analytically pure *cis*- and *trans*-product could be isolated.[211]

$C_{25}H_{29}NO_6$ [439.51]

yield: 117 mg (267 µmol, 56%) pale-yellow, turbid viscous liquid.

6.6.2.1.10.8 3'-Ethyl 3-methyl 4-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3,3'-dicarboxylate (95)



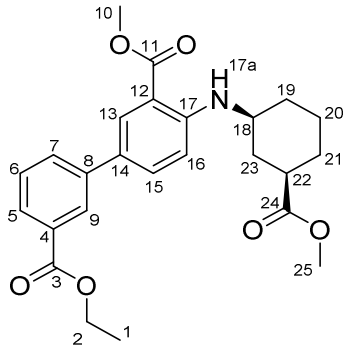
R_f = 0.34 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: blue).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.28–8.17 (m, 2H, H-9, H-13), 7.94 (d, $^3J_{HH}$ = 7.7 Hz, 1H, H-5), 7.79–7.60 (m, 2H, H-7, H-15), 7.46 (t, $^3J_{HH}$ = 7.7 Hz, 1H, H-6), 6.90 (d, $^3J_{HH}$ = 8.8 Hz, 1H, H-16), 4.41 (q, $^3J_{HH}$ = 7.1 Hz, 2H, H-2), 3.90 (s, 4H, H-10, H-18), 3.70 (s, 3H, H-25), 2.82–2.67 (m, 1H, H-22), 2.20–2.02 (m, 1H, H-23a), 1.93–1.53 (m, 7H, H-19, H-20, H-21, H-23b), 1.42 (t, $^3J_{HH}$ = 7.1 Hz, 3H, H-1).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.0 (C_q, C-24), 169.3/166.9 (C_q, C-3/C-11), 149.6 (C_q, C-17), 140.9 (C_q, C-8), 133.4 (CH_{arom}), 131.1 (C_q, C-4), 130.5 (CH_{arom}), 130.3 (CH_{arom}), 128.88 (CH_{arom}), 127.42 (CH_{arom}), 127.29 (CH_{arom}), 126.5 (C_q, C-14), 112.75 (C-16), 110.4 (C_q, C-12), 61.2 (C-2), 51.83/51.79 (C-10/C-25), 47.3 (C-18), 38.8 (C-22), 32.7 (C-23), 31.1 (C-19), 28.2 (C-21), 21.2 (C-20), 14.5 (C-1).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{25}H_{29}NO_6^+$: 439.1995; found: 439.1996.

6.6.2.1.10.9 3'-Ethyl 3-methyl 4-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-[1,1'-biphenyl]-3,3'-dicarboxylate (95)



R_f = 0.30 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: blue).

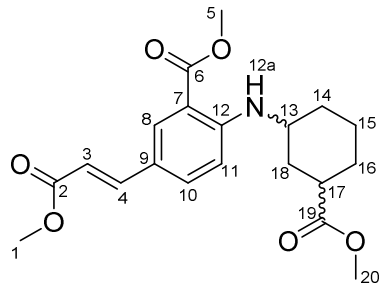
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.25-8.16 (m, 2H, H-9, H-13), 7.98–7.79 (m, 2H, H-5, H-17a), 7.72 (d, $^{3}J_{HH}$ = 7.8 Hz, 1H, H-7), 7.64 (dd, $^{3}J_{HH}$ = 8.8, $^{4}J_{HH}$ = 2.3 Hz, 1H, H-15), 7.46 (t, $^{3}J_{HH}$ = 7.7 Hz, 1H, H-6), 6.83 (d, $^{3}J_{HH}$ = 8.9 Hz, 1H, H-16), 4.41 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, H-2), 3.89 (s, 3H, H-10), 3.67 (s, 3H, H-25), 3.52-3.35 (m, 1H, H-18), 2.56–2.35 (m, 2H, H-22, H-23a), 2.17 (d, $^{3}J_{HH}$ = 12.0 Hz, 1H, H-19a), 2.07–1.91 (m, 2H, H-20a, H-21a), 1.54–1.15 (m, 7H, H-1, H-19b, H-20b, H-21b, H-23b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.4 (C_q, C-24), 169.1/166.9 (C_q, C-3/C-11), 149.4 (C_q, C-17), 140.8 (C_q, C-8), 133.3 (CH_{arom}), 131.2 (C_q, C-4), 130.6 (CH_{arom}), 130.4 (CH_{arom}), 128.9 (CH_{arom}), 127.5 (CH_{arom}), 127.3 (CH_{arom}), 126.7 (C_q, C-14), 112.7 (C-16), 110.6 (C_q, C-12), 61.2 (C-2), 51.9/51.8 (C-10/C-25), 51.0 (C-18), 42.6 (C-22), 35.4 (C-23), 32.7 (C-19), 28.5 (C-21), 24.5 (C-20), 14.5 (C-1).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{25}H_{29}NO_6^+$: 439.1995; found: 439.1986.

6.6.2.1.11 Mizoroki-Heck reaction

6.6.2.1.11.1 Methyl (*E*)-5-(3-methoxy-3-oxoprop-1-en-1-yl)-2-((3-(methoxycarbonyl)-cyclohexyl)amino)benzoate (98a)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 16.6 mg (14.4 µmol, 0.03 eq) $Pd[PPh_3]_4$ and 144 mg (1.03 mmol, 2.15 eq) K_2CO_3. The flask was evacuated and backfilled with N_2. 1.00 mL degassed NMP (10 min, N_2-bubbling in ultrasonic bath) were added followed by 200 mg (479 µmol, 1.0 eq) methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**) and 48.3 µL (527 µmol, 1.1 eq) methyl acrylate. The light brown suspension was heated to 85 °C for 38 h. The solvent was removed in oil pump vacuum (0.01 mbar, 85 °C) and the black/brown residue was treated with H_2O (4 mL) and the mixture was extracted with Et_2O (5 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum (m=198 mg, ochre, viscous liquid). The crude product was adsorbed on Celite™ (500 mg, Et_2O) and was purified via column chromatography (40 g silica gel, cyclohexane/EtOAc = 8:1 (v/v), 10 mL fractions). In order to obtain an analytically pure *trans* product a second column

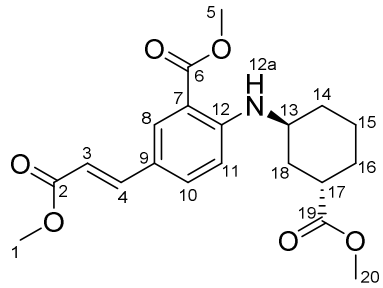
chromatography was conducted (2 g silica gel, cyclohexane/EtOAc = 10:1 (v/v), 1 mL fractions).[283]

$C_{20}H_{25}NO_6$ [375.42]

yield (*cis* & *trans*) : 150 mg (400 µmol, 83%) yellow, turbid viscous liquid.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{20}H_{25}NO_6^+$: 375.1682; found: 375.1676.

6.6.2.1.11.2 Methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (98a)



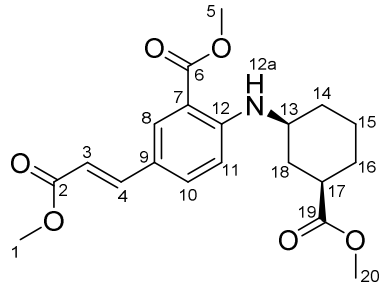
R_f = 0.22 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, CAM: carnate).

GC-MS: t_R = 10.818 min (*trans*), *m/z* (%) = 375.1 (M^+, 100), 344.1 (25), 316.1 (28), 274.1 (26), 242.1 (51), 81.1 (30).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.28 (d, $^3J_{HH}$ = 6.2 Hz, 1H, H-12a), 8.09 (d, $^4J_{HH}$ = 1.9 Hz, 1H, H-8), 7.64–7.49 (m, 2H, H-4, H-10), 6.80 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-11), 6.23 (d, $^3J_{HH}$ = 15.9 Hz, 1H, H-3), 3.91–3.85 (m, 4H, H-5, H-13), 3.78 (s, 3H, H-1), 3.70 (s, 3H, H-20), 2.79–2.65 (m, 1H, H-17), 2.15–2.01 (m, 1H, H-18a), 1.89–1.53 (m, 7H, H-14, H-15, H-16, H-18b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.8 (C_q, C-19), 169.0 (C_q), 168.2 (C_q), 151.5 (C_q, C-12), 144.8 (C-4), 133.8 (C-10), 133.4 (C-8), 120.9 (C_q, C-9), 113.1 (C-3), 112.3 (C-11), 109.9 (C_q, C-7), 51.9/51.6 (CH_3, C-1/C-5/C-20), 47.2 (C-13), 38.7 (C-17), 32.7 (C-18), 31.1 (C-14), 28.1 (C-16), 21.2 (C-15).

6.6.2.1.11.3 Methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (98a)



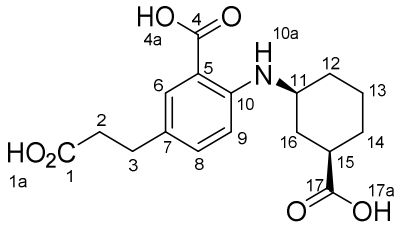
R_f = 0.17 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, CAM: carnate).

GC-MS: t_R = 11.185 min (*cis*), *m/z* (%) = 375.1 (M^+, 100), 344.1 (20), 316.1 (30), 274.1 (25), 242.1 (52), 81.1 (26).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.15–7.96 (m, 2H, CH_{arom}, H-12a), 7.63–7.44 (m, 2H, CH_{arom}, CH_{olefin}), 6.68 (d, $^3J_{HH}$ = 9.0 Hz, 1H, CH_{arom}), 6.21 (d, $^3J_{HH}$ = 15.9 Hz, 1H, CH_{olefin}), 3.84 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 3.49–3.31 (m, 1H, H-13), 2.52–2.29 (m, 2H, H-17, H-18a), 2.18–1.88 (m, 3H, H-14a, H-15a, H-16a), 1.53–1.12 (m, 4H, H-14b, H-15b, H-16b, H-18b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.2 (C_q, C-19), 168.7/168.1 (C_q, C-2/C-6), 151.3 (C_q, C-12), 144.6 (C-4), 133.6 (CH_{arom}), 133.4 (CH_{arom}), 120.9 (C_q, C-9), 113.0/112.1 (C_{olefin}/CH_{arom}), 109.9 (C_q, C-7), 51.8 (CH_3), 51.8 (CH_3), 51.5 (CH_3), 50.7 (C-13), 42.4 (C-17), 35.3 (C-18), 32.6 (C-14), 28.4 (C-16), 24.3 (C-15).

6.6.2.1.11.4 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-(2-carboxyethyl)benzoic acid (98)



A dry 10 mL round-bottom flask with two nitrogen inlets was charged with 49.2 mg (131 µmol, 1.0 eq) methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**97**), 4.9 mg (10 wt%) Pd on activated charcoal (10%) and 4 mL dry MeOH. The flask was degassed three times (cycles of evacuating and backfilling with N_2) and was refilled with H_2 the last time. The black suspension was stirred vigorously at RT for 10 d (GC-MS: incomplete conversion of the

starting material), 10 mg (20 wt%) of Pd/C suspended in little dry MeOH were added and the mixture was stirred for further 5 h (GC-MS: full conversion). The reaction mixture was filtered through a pad of silica gel (3 cm) and the pad was repeatedly rinsed with MeOH (5 x 4 mL). The solvent was removed in vacuum, the residue was dissolved in 4 mL THF/MeOH = 4:1 (v/v) and 167 mg (3.93 mmol, 30 eq) $LiOH \cdot H_2O$ were added at RT. The mixture was heated to 40 °C for 18 h until full conversion of the starting material was detected by TLC. The reation mixture was acidified with 6 N HCl (2.5 mL) and extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (110 mg, MeOH) and the product was purified via column chromatography (size: 14 x 0.8 cm, cyclohexane/EtOAc/AcOH = 2:1:1%, 1:1:1%, 1:2:1% (v/v/v), 3 mL fractions).

$C_{17}H_{21}NO_6$ [335.36]

yield: 21.4 mg (63.8 µmol, 49%) beige solid.

R_f = 0.18 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm).

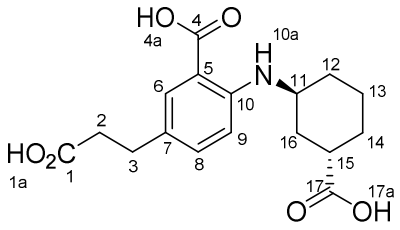
mp: 218-220 °C (decomp., turns brown upon melting, gas evolution).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.62 (s, 1H, H-6), 7.20 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H-8), 6.72 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-9), 3.49–3.30 (m, 1H, H-11), 2.76–2.60 (m, 2H, CH_2), 2.56–2.31 (m, 3H, CH_2, H-15), 2.29–2.11 (m, 1H, H-16a), 2.06–1.71 (m, 3H, H-12a, H-13a, H-14a), 1.50–0.96 (m, 4H, H-12b, H-13b, H-14b, H-16b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.0 (C_q, CO_2H), 173.8 (C_q, CO_2H), 170.2 (C_q, CO_2H), 148.4 (C_q, C-10), 134.4 (C-8), 131.1 (C-6), 126.0 (C_q, C-7), 111.9 (C-9), 110.2 (C_q, C-5), 49.6 (C-11), 41.5 (C-15), 35.7 (CH_2), 35.4 (C-16), 32.4 (C-12), 29.4 (CH_2), 28.2 (C-14), 23.9 (C-13).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{21}NO_6^+$: 335.1369; found: 335.1366.

6.6.2.1.11.5 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-(2-carboxyethyl)benzoic acid (98)



A dry 10 mL round-bottom flask with two nitrogen inlets was charged with 65.0 mg (173 µmol, 1.0 eq) methyl 5-((*E*)-3-methoxy-3-oxoprop-1-en-1-yl)-2-(((1*S**,3*S**)-3-

(methoxycarbonyl)cyclohexyl)amino)benzoate (**97**), 6.5 mg (10 wt%) Pd on activated charcoal (10%) and 6 mL dry MeOH. The flask was degassed three times (cycles of evacuating and backfilling with N_2) and was refilled with H_2 the last time. The black suspension was stirred vigorously at RT for 10 d until full conversion of the starting material was detected by GC-MS. The reaction mixture was filtrated through a pad of silica gel (3 cm) and the pad was repeatedly rinsed with MeOH (5 x 4 mL). The solvent was removed in vacuum, the residue was dissolved in 4 mL THF/MeOH = 4:1 (v/v) and 220 mg (5.19 mmol, 30 eq) $LiOH \cdot H_2O$ were added. The mixture was heated to 40 °C for 18 h until full conversion of the starting material was detected by TLC. The reation mixture was acidified with 6 N HCl (2.5 mL) and extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (145 mg, MeOH) and the product was purified via column chromatography (size: 14 x 0.8 cm, cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v), 4 mL fractions).

$C_{17}H_{21}NO_6$ [335.36]

yield: 42.1 mg (126 µmol, 73%) light-yellow solid.

R_f = 0.22 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm).

mp: 188-190 °C (decomp., turns black).

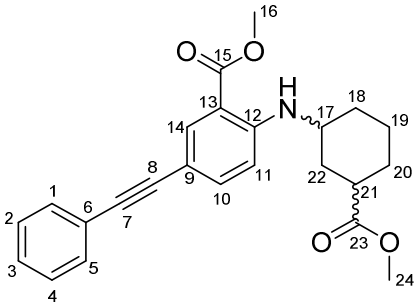
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.26 (bs, 3H, H-1a, H-4a, H-17a), 8.22–7.58 (m, 2H, H6, H-10a), 7.22 (d, $^3J_{HH}$ = 7.2 Hz, 1H, H-8), 6.72 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-9), 3.78–3.63 (m, 1H, H-11), 2.76–2.59 (m, 2H, CH_2), 2.51–2.33 (m, 3H, H-15, CH_2), 2.00–1.80 (m, 1H, H-16a), 1.80–1.37 (m, 7H, H-12, H-13, H-14, H-16b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.3 (C_q, CO_2H), 173.9 (C_q, CO_2H), 170.2 (C_q, CO_2H), 148.5 (C_q, C-10), 134.8 (C-8), 131.2 (C-6), 126.1 (C_q, C-7), 111.8 (C-9), 109.8 (C_q, C-5), 46.3 (C-11), 38.1 (C-15), 35.6 (CH_2), 32.5 (C-16), 30.5 (C-12), 29.4 (CH_2), 27.5 (C-14), 20.7 (C-13).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{21}NO_6^+$: 335.1369; found: 335.1370.

6.6.2.1.12 Sonogashira reaction

6.6.2.1.12.1 Methyl 2-((3-(methoxycarbonyl)cyclohexyl)amino)-5-(phenylethynyl)benzoate (103)



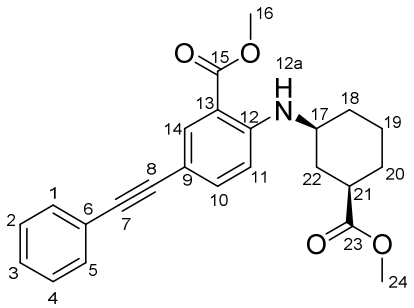
A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 17.2 mg (24.0 µmol, 0.05 eq) $Pd[PPh_3]_4$, 2.8 mg (14.4 µmol, 0.03 eq) CuI and 1.0 mL degassed Et_3N (10 min N_2-bubbling in ultrasonic bath) in a N_2 counter-stream. To the yellow solution were added 200 mg (479 µmol, 1.0 eq) methyl 5-iodo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**) followed by 64.5 µL (575 µmol, 1.2 eq) phenylacetylene. The dark, mud-green suspension was heated to 85 °C for 28 h until full conversion of the starting material was detected by TLC. The solvent was removed in oil pump vacuum and the residue was treated with 4 mL distilled H_2O. The mixture was extracted with Et_2O (5 x 4 mL), the combined organic layers were dried over Na_2SO_4, filtrated, and the solvent was removed in vacuum. The crude product (230 mg, yellow, viscous liquid) was adsorbed on Celite™ (570 mg, Et_2O) and purified via column chromatography (20 g silica gel, cyclohexane/EtOAc = 15:1 (v/v), 10 mL fractions). To obtain an analytically pure *cis*-product a further column chromatography was necessary (2 g silica gel, cyclohexane/EtOAc = 18:1).[284]

$C_{24}H_{25}NO_4$ [391.47]

yield (*cis* & *trans*): 119.1 mg (304 µmol, 64%) dark orange, viscous liquid.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{24}H_{25}NO_4^+$: 391.1783; found: 391.1788.

6.6.2.1.12.2 Methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-(phenylethynyl)benzoate (103)



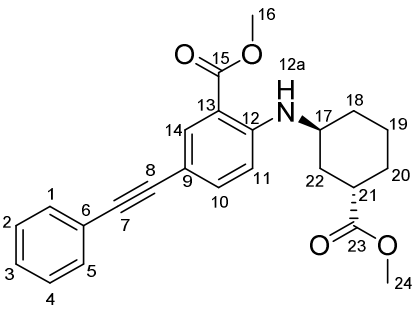
R_f = 0.37 (cyclohexane/EtOAc = = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: redish-violet).

GC-MS: t_R = 7.941 min, *m/z* (%) = 391.2 (M^+, 100), 258.1 (37), 191.1 (19), 81.1 (18).

^{1}H-NMR (300.36 MHz, $CDCl_3$) δ = 8.12 (d, $^4J_{HH}$ = 1.9 Hz, 1H, H-14), 7.95 (d, $^3J_{HH}$ = 7.4 Hz, 1H, H-12a), 7.54–7.42 (m, 3H, H-1, H-5, H-10), 7.39–7.27 (m, 3H, H-2, H-3, H-4), 6.67 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-11), 3.86 (s, 3H, H-16), 3.67 (s, 3H, H-24), 3.50–3.33 (m, 1H, H-17), 2.54–2.31 (m, 2H, H-21, H-22a), 2.14 (d, $^3J_{HH}$ = 12.3 Hz, 1H, H-18a), 2.07–1.85 (m, 2H, H-19a, H-20a), 1.51–1.16 (m, 4H, H-18b, H-19b, H-20b, H-22b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-23), 168.6 (C_q, C-15), 149.8 (C_q, C-12), 137.5 (CH_{arom}), 135.8 (CH_{arom}, C-14), 131.5 (CH_{arom}), 128.4 (CH_{arom}), 127.8 (CH_{arom}), 124.0 (C_q, C-6), 111.8 (C-11), 109.9 (C_q), 108.8 (C_q), 89.8 (C_q), 87.2 (C_q), 51.9/51.8 (C-16/C-24), 50.7 (C-17), 42.5 (C-21), 35.4 (C-22), 32.7 (C-18), 28.5 (C-20), 24.4 (C-19).

6.6.2.1.12.3 Methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-(phenylethynyl)benzoate (103)



R_f = 0.42 (cyclohexane/EtOAc = = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: redish-violet).

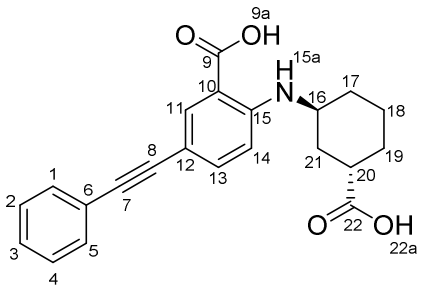
GC-MS: t_R = 7.228 min, *m/z* (%) = 391.2 (M^+, 100), 258.1 (35), 191.1 (17), 81.1 (18).

^{1}H-NMR (300.36 MHz, $CDCl_3$) δ = 8.14–8.02 (m, 2H, H-12a, CH_{arom}), 7.46–7.36 (m, 3H, CH_{arom}), 7.31-7.16 (m, 3H, CH_{arom}), 6.68 (d, $^3J_{HH}$ = 8.9 Hz, 1H, CH_{arom}), 3.85–3.69 (m, 4H,

H-17, CH_3), 3.62 (s, 3H, CH_3), 2.72–2.58 (m, 1H, H-21), 2.07–1.93 (m, 1H, CH_2), 1.83–1.45 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.9 (C_q, C-23), 168.8 (C_q, C-15), 149.9 (C_q, C-12), 137.7 (CH_{arom}), 135.8 (CH_{arom}), 131.5 (CH_{arom}), 128.4 (CH_{arom}), 127.8 (CH_{arom}), 124.0 (C_q, C-6), 111.9 (CH_{arom}), 109.8 (C_q), 108.7 (C_q), 89.8 (C_q, C_{alkyne}), 87.2 (C_q, C_{alkyne}), 51.83/51.78 (C-16/C-24), 47.0 (C-17), 38.7 (C-21), 32.7 (C-22), 31.1 (C-18), 28.1 (C-20), 21.2 (C-19).

6.6.2.1.12.4 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-(phenylethynyl)benzoic acid (104)



A 3 mL screw-cap vial with magnetic stirring bar was charged with 30.0 mg (76.6 µmol, 1.0 eq) methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-(phenylethynyl)benzoate (**103**), 770 µL THF/MeOH = 3:1 (v/v) and 16.2 mg (383 µmol, 5.0 eq) LiOH·H_2O. The dark-brown mixture was stirred RT and full conversion of the starting material was detected with TLC after 6 d. The reaction mixture was acidified with 6 N HCl (2.7 mL) and extracted with EtOAc (4 x 2 mL). The combined organic phases were dried over $MgSO_4$, filtrated, and the solvents were removed in vacuum. The residue was adsorbed on Celite™ (68 mg, MeOH) and the product was purified via column chromatography (size: 10 x 1 cm, cyclohexane/EtOAc/AcOH = 7:3:1% (v/v/v), 2 mL fractions).

$C_{22}H_{21}NO_4$ [363.41]

yield: 8.6 mg (23.7 µmol, 31%) yellow solid.

R_f = 0.27 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: dark orange-brown).

mp: 200-202 °C (decomp., turns black).

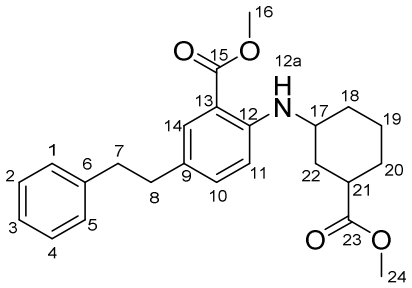
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.49 (bs, 2H, H-9a, H-22a), 8.48–8.30 (m, 1H, H-15a), 7.96 (d, $^3J_{HH}$ = 1.7 Hz, 1H, H-11), 7.55–7.29 (m, 6H, CH_{arom}), 6.83 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-14), 3.91–3.71 (m, 1H, H-16), 2.58–2.51 (m, 1H, H-20), 2.04–1.85 (m, 1H, H-21a), 1.85–1.41 (m, 7H, H-17, H-18, H-19, H-21b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.1 (C_q, C-22), 169.5 (C_q, C-9), 149.7 (C_q, C-15), 136.8 (CH_{arom}), 135.3 (C-11), 131.0 (CH_{arom}), 128.6 (CH_{arom}), 128.0 (CH_{arom}), 123.0 (C_q, C-6), 112.0 (C-14), 110.5 (C_q, C-12), 107.1 (C_q, C-10), 89.9 (C_{alkyne}), 86.9 (C_{alkyne}), 46.4 (C-16), 38.0 (C-20), 32.3 (C-21), 30.3 (C-17), 27.4 (C-19), 20.6 (C-18).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}NO_4^+$: 364.42; found: 364.2.

6.6.2.1.12.5 Methyl 2-((3-(methoxycarbonyl)cyclohexyl)amino)-5-phenethylbenzoate (105)



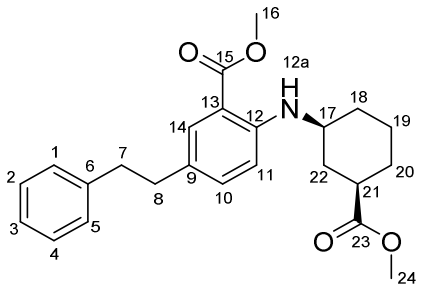
A dry 25 mL round-bottom flask with two nitrogen-inlets and a magnetic stirring bar was charged with 152 mg (388 µmol, 1.0 eq) methyl 2-((3-(methoxycarbonyl)cyclohexyl)amino)-5-(phenylethynyl)benzoate. 18 mg Pd on activated charcoal (10 wt%) and 10 mL dry MeOH. The flask was evacuated and backfilled with N_2 for three times and the last time the flask was flushed with H_2. The black/green colored reaction mixture was stirred vigorously at RT and after 3 d full conversion of the starting material was detected by TLC. The catalyst was removed via filtration (small plug of Celite, eluent: MeOH) and the solvent was removed in vacuum. The crude product was purified via column chromatography (size: 23 x 1.4 cm, cyclohexane/EtOAc = 25:1, 10 mL fractions).

$C_{24}H_{29}NO_4$ [395.50]

yield (*cis&trans*): 129 mg (325 µmol, 83%)

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{24}H_{29}NO_4^+$: 395.2097; found: 395.2117.

6.6.2.1.12.6 Methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-phenethylbenzoate (105)



yield: 62.4 mg (157 µmol, 40%) orange solid.

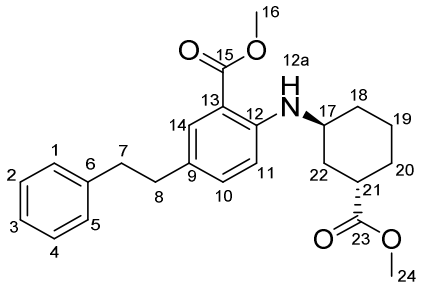
R_f = 0.12 (cyclohexane/EtOAc = 25:1 (v/v), 254 nm, 366 nm, CAM).

mp = 97-98°C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.65 (d, $^4J_{HH}$ = 1.6 Hz, 1H, H-10), 7.52 (s, 1H, H-14), 7.26-7.01 (m, 7H, CH_{arom}), 6.56 (d, $^3J_{HH}$ = 8.6 Hz, 1H, H-11), 3.76 (s, 3H, H-16), 3.58 (s, 3H, H-24), 3.28 (s, 1H, H-17), 2.85-2.65 (m, 4H, H-7, H-8), 2.35 (dd, $^2J_{HH}$ = 19.5 Hz, $^3J_{HH}$ = 10.3 Hz, 2H, H-22), 2.12-1.75 (m, 4H, H-17, H-18, H-19a), 1.44-1.05 (m, 5H, H-19b, H-20, H-21).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.5 (C_q, C-23), 169.2 (C_q, C-15), 148.5 (C_q, C-12), 141.9 (C-5), 135.1 (CH_{arom}), 131.3 (CH_{arom}), 128.6 (CH_{arom}), 128.4 (CH_{arom}), 127.7 (C_q, C-9), 125.9 (CH_{arom}), 111.9 (C-11), 109.9 (C_q, C-13), 51.7/51.5 (CH_3, C-16, C-24), 50.8 (C-17), 42.6 (C-21), 38.3 (CH_2), 36.9 (CH_2), 35.5 (C-22), 32.8 (C-18), 28.5 (C-20), 24.5 (C-19).

6.6.2.1.12.7 Methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-phenethylbenzoate (105)



yield: 10.7 mg (27.0 µmol, 7%) yellow viscous liquid.

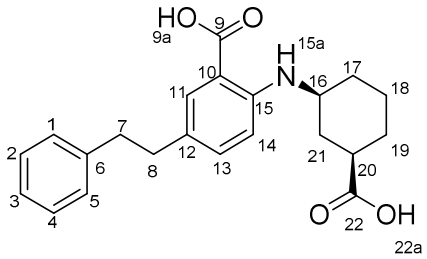
R_f = 0.18 (cyclohexane/EtOAc = 25:1 (v/v), 254 nm, 366 nm, CAM).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.98-7.55 (m, 2H, CH_{arom}), 7.28-7.17 (m, 2H, CH_{arom}), 7.11 (dd, $^3J_{HH}$ = 6.7 Hz, $^4J_{HH}$ = 3.5 Hz, 4H, CH_{arom}), 6.66 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-11), 3.79 (s, 3H,

H-16), 3.61 (s, 3H, H-24), 2.91-2.55 (m, 5H, H-7, H-8, H-17), 2.07-1.87 (m, 1H, H-22a), 1.85-1.39 (m, 8H, H-18, H-19, H-20, H-21, H-22b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.1 (C_q, C-23), 169.3 (C_q, C-15), 147.5 (C_q, C-12, low intensity), 142.0 (C-6), 135.3 (CH_{arom}), 131.2 (CH_{arom}), 128.6 (CH_{arom}), 128.5 (CH_{arom}), 126.0 (CH_{arom}), 112.4 (C-11), 51.8 (C-16), 51.6 (C-24), 47.3 (C-17), 38.7 (C-21), 38.5 (CH_2), 37.0 (CH_2), 32.6 (CH_2), 31.0 (CH_2), 28.2 (CH_2), 21.2 (CH_2).

6.6.2.1.12.8 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-phenethylbenzoic acid (106)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 31.8 mg (80.4 µmol, 1.0 eq) methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-phenethylbenzoate (**105**), 4 mL THF/H_2O = 4:1 (v/v) and 102 mg (2.41 mmol, 30 eq) LiOH·H_2O. The yellow mixture was stirred at RT for 3 d (TLC: incomplete conversion of the starting material) and 102 mg (2.41 mmol, 30 eq) LiOH·H_2O were added. After 30 h stirring (TLC: incomplete) another portion of 102 mg (2.41 mmol, 30 eq) LiOH·H_2O was added followed by 3 d of stirring at RT (TLC: incomplete). The mixture was heated to 40 °C for 3 d and full conversion of the starting material was detected by TLC. The mixture was acidified with 6 N HCl and extracted with EtOAc (5 x 8 mL). The combined organic layers were dried over Na_2SO_4, filtrated, and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (74 mg, MeOH) and the product was purified via column chromatography (size: 13 x 0.8 cm, cyclohexane/EtOAc/AcOH = 4:1:1%, 3:1:1% (v/v/v), 4 mL fractions).

$C_{22}H_{25}NO_4$ [367.45]

yield: 21.7 mg (59.1 µmol, 73%) light-yellow solid.

R_f = 0.48 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm).

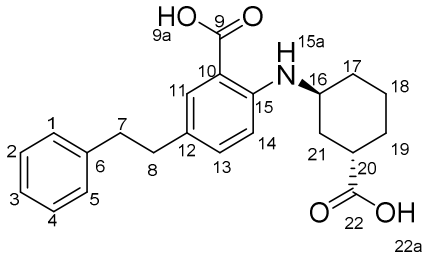
mp: 221-223 °C (decomp., turns brown).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.28 (bs, 2H, H-9a, H-22a), 7.67–7.58 (m, 1H, H-11), 7.33–7.08 (m, 6H, CH_{arom}), 6.72 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-14), 3.47–3.33 (m, 1H, H-16), 2.86–2.65 (m, 4H, H-7, H-8), 2.48–2.30 (m, 1H, H-20), 2.30–2.12 (m, 1H, H-21a), 2.05–1.69 (m, 3H, H-17a, H-18a, H-19a), 1.55–0.94 (m, 4H, H-17b, H-18b, H-19b, H-21b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.0 (C_q, C-22), 170.1 (C_q, C-9), 148.3 (C_q, C-15), 141.6 (C_q, C-6), 134.8 (CH_{arom}), 131.2 (CH_{arom}), 128.4 (CH_{arom}), 128.2 (CH_{arom}), 126.7 (C_q, C-12), 125.7 (CH_{arom}), 111.9 (C-14), 109.7 (C_q, C-10), 49.5 (C-16), 41.4 (C-20), 37.4 (CH_2), 36.0 (CH_2), 35.4 (C-21), 32.4 (C-17), 28.2 (C-19), 23.8 (C-18).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{22}H_{25}NO_4^+$: 367.1783; found: 367.1792.

6.6.2.1.12.9 2-(((1*S**,3*S**)-3-Carboxycyclohexyl)amino)-5-phenethylbenzoic acid (106)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 10.7 mg (27.1 µmol, 1.0 eq) methyl 2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-(phenylethynyl)benzoate (**105**), 1.5 mL THF/H_2O = 4:1 (v/v) and 34.4 mg (812 µmol, 30 eq) LiOH·H_2O. The yellow mixture was stirred at RT for 3 d (TLC: incomplete conversion of the starting material) and 34.4 mg (812 µmol, 30 eq) LiOH·H_2O were added. After 30 h stirring (TLC: incomplete) another portion of 34.4 mg (812 µmol, 30 eq) LiOH·H_2O was added followed by 3 d of stirring at RT (TLC: incomplete). The mixture was heated to 40 °C for 3 d and full conversion of the starting material was detected by TLC. The mixture was acidified with 6 N HCl and extracted with EtOAc (5 x 8 mL). The combined organic layers were dried over Na_2SO_4, filtrated, and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (25 mg, MeOH) and the product was purified via column chromatography (size: 13 x 0.8 cm, cyclohexane/EtOAc/AcOH = 4:1:1%, 3:1:1% (v/v/v), 4 mL fractions). The product was not analytically pure and was further purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{22}H_{25}NO_4$ [367.45]

yield: 3.9 mg (10.6 µmol, 39%) colorless solid.

mp: 210-212 °C.

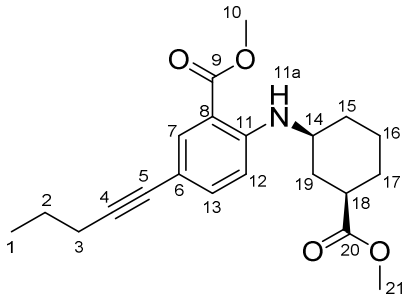
R_f = 0.52 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.30 (bs, 2H, H-9a, H-22a), 7.93 (bs, 1H, H-15a), 7.64 (s, 1H, H-11), 7.33–6.99 (m, 6H, CH_{arom}), 6.71 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-14), 3.78–3.62 (m, 1H, H-16), 2.87–2.65 (m, 4H, H-7, H-8), 2.58–2.50 (m, 1H, H-20), 2.00–1.81 (m, 1H, H-21a), 1.81–1.41 (m, 7H, H-17, H-18, H-19, H-21b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.2 (C_q, C-22), 170.2 (C_q, C-9), 148.3 (C_q, C-15), 141.6 (C_q, C-6), 134.7 (CH_{arom}), 131.18 (CH_{arom}), 128.38 (CH_{arom}), 128.17 (CH_{arom}), 126.71 (C_q, C-12), 125.7 (CH_{arom}), 111.7 (C-14), 110.0 (C_q, C-10), 46.2 (C-16), 38.0 (C-20), 37.4 (CH_2), 36.1 (CH_2), 32.4 (CH_2), 30.5 (CH_2), 27.5 (CH_2), 20.7 (CH_2).

HR-MS (EI-EI): *m/z* $[M]^+$ calcd for $C_{22}H_{25}NO_4^+$: 367.1783; found: 367.1788.

6.6.2.1.12.10 Methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-(pent-1-yn-1-yl)benzoate (107)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 1.00 mL Et_3N followed by 50.0 mg (120 µmol, 1.0 eq) methyl 5-iodo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**36**). The light-yellow solution was degassed for 5 min (N_2-bubbling, ultrasonic bath) and 23.9 µL (240 µL, 2.0 eq) 1-pentyne followed by 4.3 mg (5.99 µmol, 0.05 eq) $PdCl_2(PPh_3)_2$ and 0.7 mg (3.60 µmol, 0.03 eq) CuI were added. The yellow suspension was stirred 12 min at RT and was placed into a preheated (75 °C) oil bath. The appearance changed to a brown suspension within 5 min. Full conversion of the starting material was detected after 3 d and the solvent was removed in vacuum. The brown residue was adsorbed on Celite™ (110 mg, EtOAc) and the product was purified via column chromatography (size: 15 x 0.8 cm, cyclohexane/EtOAc = 18:1).[285]

$C_{21}H_{27}NO_4$ [357.45]

yield: 37.0 mg (104 µmol, 86%) dirty-yellow viscous liquid.

R_f = 0.46 (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, 366 nm, cold CAM: turquoise).

GC-MS: t_R =9.841 min (*cis*) *m/z* (%) = 357.2 (M^+, 100), 298.1 (20), 256.1 (17), 224.1 (42), 156.0 (20), 81.1 (18).

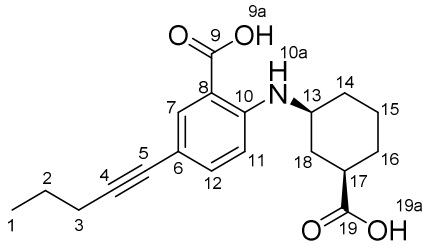
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.97 (d, $^4J_{HH}$ = 1.9 Hz, 1H, H-7), 7.82 (d, $^3J_{HH}$ = 7.3 Hz, 1H, H-11a), 7.34 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 1.9 Hz, 1H, H-13), 6.60 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-12), 3.83 (s, 3H, H-10), 3.66 (s, 3H, H-21), 3.45–3.28 (m, 1H, H-14), 2.52–2.29 (m, 4H, H-3, H-18, H-19a), 2.18–2.06 (m, 1H, H-15a), 2.06–1.88 (m, 2H, H-16a, H-17a), 1.69–1.51

(m, 2H, H-2), 1.49–1.14 (m, 4H, H-15b, H-16b, H-17b, H-19b), 1.03 (t, $^{3}J_{HH}$ = 7.3 Hz, 3H, H-1).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.4 (C_q, C-20), 168.7 (C_q, C-9), 149.3 (C_q, C-11), 137.5 (C-13), 135.5 (C-7), 111.6 (C-12), 109.8 (C_q, C-6), 87.5 (C_q), 80.5 (C_q), one C_q missing!(low intensity), 51.8/51.7 (C-10/C-21), 50.6 (C-14), 42.5 (C-18), 35.4 (C-19), 32.7 (C-15), 28.5 (C-17), 24.4 (C-16), 22.5 (C-2), 21.6 (C-3), 13.7 (C-1).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{21}H_{27}NO_4^+$: 357.1940; found: 357.1940.

6.6.2.1.12.11 2-(((1*S**,3*R**)-3-Carboxycyclohexyl)amino)-5-(pent-1-yn-1-yl)benzoic acid (108)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 33.4 mg (93.4 µmol, 1.0 eq) methyl 2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-5-(pent-1-yn-1-yl)benzoate (**107**), 2 mL THF/H_2O (1:1 (v/v)) and 39.6 mg (934 µmol, 10 eq) $LiOH \cdot H_2O$. The dirty-yellow, biphasic mixture was heated to 40 °C for 2 d until full conversion of the starting material was detected by TLC. The mixture was acidified with 6 N HCl (pH = 1) and the solvent was removed in vacuum. The residue was dissolved in 1.0 mL DMSO and was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{19}H_{23}NO_4$ [329.40]

yield: 18.6 mg (56.5 µmol, 60%) yellow solid.

R_f = 0.49 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: carnate-orange).

mp: 195-200 °C (decomp.).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.45 (bs, 2H, H-9a, H-19a), 7.97 (bs, 1H, NH), 7.77 (s, 1H, H-7), 7.32 (d, $^{3}J_{HH}$ = 8.6 Hz, 1H, ArH), 6.77 (d, $^{3}J_{HH}$ = 8.7 Hz, 1H, ArH), 3.55–3.37 (m, 1H, H-13), 2.47–2.27 (m, 3H, H-3, H-17), 2.26-2.12 (m, 1H, CH_2), 2.06–1.70 (m, 3H, CH_2), 1.62–0.90 (m, 9H, H-1, H-2, CH_2).

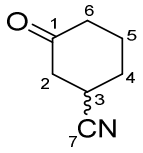
^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C_q, CO_2H), 169.5 (C_q, CO_2H), 149.1 (C_q, C-10), 136.8 (ArH), 134.9 (ArH), 112.0 (ArH), 110.0 (C_q), 108.4 (C_q), 87.2 (C_q), 80.6 (C_q), 49.5

(C-13), 41.3 (C-17), 35.1 (CH_2), 32.2 (CH_2), 28.1 (CH_2), 23.8 (CH_2), 21.9 (H-2), 20.6 (H-3), 13.4 (H-1).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{19}H_{23}NO_4^+$: 329.1627; found: 329.1640.

6.6.2.1.13 Synthesis of the 6-ring Phenazistatin A

6.6.2.1.13.1 3-Oxocyclohexane-1-carbonitrile (5)



(Safety warning: HCN detectors were used at all times throughout the reaction!!!) A 1 L round-bottom flask with magnetic stirring bar was charged with 106 g (765 mmol, 1.5 eq) $Et_3N{\cdot}HCl$, 200 mL MeOH, 100 mL H_2O and 36.9 g (561 mmol, 1.1 eq) KCN. 50.0 mL (510 mmol, 1 eq) cyclohex-2-en-1-one were added over a period of 1 h to the stirred mixture. The milky, light-yellow suspension was stirred at RT for 3 d (TLC: full conversion). 20 g Na_2CO_3 were added and the brown mixture was transferred to a separation funnel. 300 mL distilled H_2O were added to dissolve the precipitate and the mixture was exhaustively extracted with $CHCl_3$ (6 x 200 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum to give a brown viscous liquid. The product was purified by vacuum distillation (20 cm Vigreux column, 1 cm diameter, vacuum insulated).[215]

C_7H_9NO [123.16]

yield: 23.2 g (188 mmol, 37%) colorless liquid.

R_f = 0.20 (cyclohexane/EtOAc = 2:1 (v/v)) (CAM: blue).

bp: 98-100 °C (0.46 mbar).

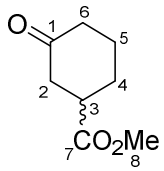
GC-MS: t_R = 4.755 min, *m/z* (%) = 123.1 (46, M^+), 94.1 (14), 55.0 (100).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 3.10-2.95 (m, 1H, H-3), 2.72-2.50 (m, 2H, H-2), 2.46-2.29 (m, 2H, CH_2), 2.22-1.74 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 205.6 (C_q, C-1), 120.3 (C_q, C-7), 43.3 (CH_2), 40.8 (CH_2), 28.7 (CH, C-3), 28.2 (CH_2), 23.8 (CH_2).

The spectra were in accordance with previously reported data.[215]

6.6.2.1.13.2 Methyl 3-oxocyclohexane-1-carboxylate (38)



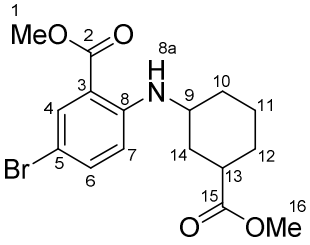
A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 20 mL dry MeOH and cooled in an ice bath. 10 mL (140 mmol, 2.8 eq) AcCl were slowly added to the Schlenk flask in a N_2 counter-stream (gas evolution). 6.02 g (48.9 mmol, 1.0 eq) 3-oxocyclohexane-1-carbonitrile (**5**) was added to the cooled solution and the color changed to bright-yellow. The reaction mixture was stirred overnight at RT and full conversion of the starting material was detected after 24 h (GC-MS). The yellow suspension containing colorless precipitate was cooled to 0 °C and 20 mL H_2O were added slowly. Most of the MeOH was removed with a rotary evaporator, 20 mL H_2O were added and the mixture was extracted with EtOAc (5 x 50 mL). The solvent was removed in vacuum and the yellow/orange, viscous liquid was dried in oil pump vacuum. The product was used in the following steps without further purification.[203]

$C_8H_{12}O_3$ [156.18]

yield: 6.38 g (40.8 mmol, 83%), yellow-orange, viscous liquid.

GC-MS: t_R = 4.91 min, *m/z* (%) = 156 (M^+), 124 (10), 97 (100), 55 (56).

6.6.2.1.13.3 Methyl 5-bromo-2-((3-(methoxycarbonyl)cyclohexyl)amino)benzoate (7)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 2.37 g (7.62 mmol, 1.0 eq) methyl 2-amino-5-bromobenzoate hydrobromide (**114**) and the solid was dried in oil pump vacuum for 10 min. To the Schlenk flask were added 5 mL dry DMF, 1.25 g (8.00 mmol, 1.05 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**) and 2.95 mL (22.9 mmol, 3.0 eq) TMSCl. The yellow biphasic mixture was cooled to 0 °C and 7.6 mL (7.60 mol, 1.0 eq) BH_3·THF were added over a period of 42 min. The colorless solution with colorless precipitate was left stirring for 48 min at 0 °C (TLC: full conversion of the starting material). The mixture was cooled again to 0 °C and 10 mL H_2O were added carefully, followed by

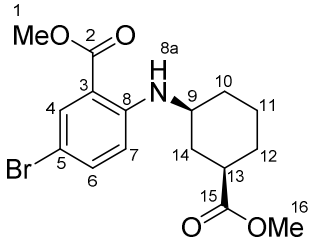
neutralization with 25% NH_4OH. The solvents were removed in vacuum, the residue was adsorbed on Celite™ (7 g, MeOH) and was purified via column chromatography (80 g silica gel, size: 18 x 3.5 cm, 20 mL fractions). Pure fractions of the stereoisomers could be obtained on careful column chromatography.[189]

yield: 2.70 g (7.30 µmol, 96%) light-yellow, very viscous liquid.

$C_{16}H_{20}BrNO_4$ [370.24]

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{16}H_{20}BrNO_4^+$: 369.0576; found: 369.0580

6.6.2.1.13.4 Methyl 5-bromo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)-benzoate (7)



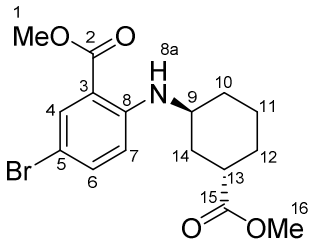
R_f = 0.40 (cyclohexane/EtOAc = 5:1 (v/v)) (254 nm, 366 nm, CAM: orange).

GC-MS: t_R = 8.819 min, *m/z* (%) = 371.1/369.1 (100), 312.1/310.1 (56), 270.0/268.0 (53), 238.0/236.0 (53).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^4J_{HH}$ = 2.3 Hz, 1H, H-4), 7.71 (d, $^3J_{HH}$ = 7.2 Hz, 1H, H-8a), 7.37 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, H-6), 6.58 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-7), 3.84 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 3.44-3.25 (m, 1H, H-9), 2.51-2.30 (m, 2H, H-13, CH_2), 2.19-1.84 (m, 3H, CH_2), 1.52-1.12 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-15), 168.1 (C_q, C-2), 149.1 (C_q, C-8), 137.2 (C-6), 134.2 (C-4), 113.6 (C-7), 111.4 (C_q, C-5), 105.6 (C_q, C-3), 51.84 (CH_3), 51.81 (CH_3), 50.7 (C-9), 42.5 (C-13), 35.3 (C-14), 32.6 (C-10), 28.5 (C-12), 24.4 (C-11).

6.6.2.1.13.5 Methyl 5-bromo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)-benzoate (7)



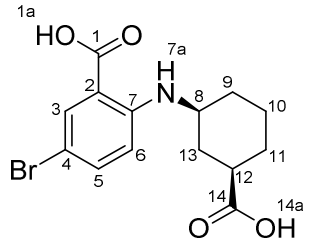
R_f = 0.47 (cyclohexane/EtOAc = 5:1 (v/v)) (254 nm, 366 nm, CAM: orange).

GC-MS: t_R = 8.647 min, *m/z* (%) = 371.1/369.1 (100), 312.1/310.1 (57), 270.0/268.0 (55), 238.0/236.0 (57).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^4J_{HH}$ = 2.3 Hz, 1H, H-4), 7.90 (d, $^3J_{HH}$ = 6.8 Hz, 1H, H-8a), 7.39 (dd, $^4J_{HH}$ = 9.0, $^3J_{HH}$ = 2.2 Hz, 1H, H-6), 6.67 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-7), 3.92-3.62 (m, 7H, C-9, 2 x CH_3), 2.77-2.63 (m, 1H, H-13), 2.14-1.96 (m, 1H, CH_2), 1.89-1.49 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.9 (C_q, C-15), 168.3 (C_q, C-2), 149.2 (C_q, C-8), 137.4 (C-6), 134.1 (C-4), 113.7 (C-7), 111.3 (C_q, C-5), 105.6 (C_q, C-3), 51.8 (2 x CH_3), 47.0 (C-9), 38.7 (C-13), 32.6 (C-14), 31.1 (C-10), 28.1 (C-12), 21.2 (C-11).

6.6.2.1.13.6 5-Bromo-2-(((1*S**,3*R**)-3-carboxycyclohexyl)amino)benzoic acid (1)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 25.7 mg (69.4 µmol, 1.0 eq) methyl 5-bromo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**7**), 700 µL THF/H_2O (1:1, v/v) and 29.4 mg (694 µmol, 10 eq) LiOH·H_2O. The mixture was placed into a preheated (40 °C) oil bath and was left stirring for 22 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 1 M HCl and the solvents were removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{14}H_{16}BrNO_4$ [342.19]

yield: 17.6 mg (51.4 µmol, 74%) yellow, amorphous solid.

R_f = 0.16 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: green-orange).

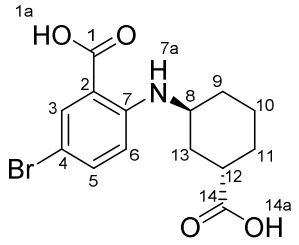
mp: 226 °C (decomp.).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 8.20–7.72 (m, 2H, H-3, NH), 7.43 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, H-5), 6.78 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 3.50–3.33 (m, 1H, H-8), 2.47–2.31 (m, 1H, H-12), 2.27–2.12 (m, 1H, CH_2), 2.07–1.71 (m, 3H, CH_2), 1.53–0.99 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C_q, C-14), 169.2 (C_q, C-1), 148.8 (C_q, C-7), 136.3 (C-5), 133.6 (C-3), 114.1 (C-6), 112.5 (C_q, C-4), 104.2 (C_q, C-2), 49.5 (C-8), 41.4 (C-12), 35.1 (C-13), 32.1 (C-9), 28.1 (C-11), 23.8 (C-10).

HRMS data were not recorded, since they were already disclosed by Mentel.[53]

6.6.2.1.13.7 5-Bromo-2-(((1*S**,3*S**)-3-carboxycyclohexyl)amino)benzoic acid (1)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 21.7 mg (58.6 µmol, 1.0 eq) methyl 5-bromo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclohexyl)amino)benzoate (**7**), 600 µL THF/$H_2$0 (1:1, v/v) and 24.8 mg (586 µmol, 10 eq) LiOH·H_2O. The mixture was placed into a preheated (40 °C) oil bath and was left stirring for 22 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 1 M HCl and the solvents were removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{14}H_{16}BrNO_4$ [342.19]

yield: 13.7 mg (40.0 µmol, 68%) light-yellow solid.

R_f = 0.23 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: green-orange).

mp: 228-230 °C

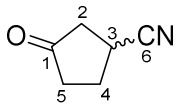
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.48 (bs, 2H, H-1a, H-14a), 8.21–7.94 (m, 1H, NH), 7.85 (d, $^4J_{HH}$ = 2.2 Hz, 1H, H-3), 7.46 (dd, $^3J_{HH}$ = 9.0 Hz, $^3J_{HH}$ = 2.2 Hz, 1H, H-5), 6.77 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 3.81–3.66 (m, 1H, H-8), 2.57–2.44 (m, 1H, H-12, DMSO overlap), 1.99–1.81 (m, 1H, CH_2), 1.81–1.41 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.1 (C_q, C-14), 169.0 (C_q, C-1), 148.9 (C_q, C-7), 136.7 (C-5), 133.6 (C-3), 114.0 (C-6), 111.9 (C_q, C-4), 104.3 (C_q, C-2), 46.4 (C-8), 38.0 (C-12), 32.2 (C-13), 30.3 (C-9), 27.4 (C-11), 20.6 (C-10).

HRMS data were not recorded, since they were already disclosed by Mentel.[53]

6.6.2.1.14 Synthesis of the 5-ring Phenazistatin A

6.6.2.1.14.1 3-Oxocyclopentane-1-carbonitrile (133)



(Safety warning: HCN detectors were used at all times throughout the reaction!!!) A 50 mL round-bottom flask with magnetic stirring bar was charged with 4.97 g (35.8 mmol, 1.5 eq) $Et_3N{\cdot}HCl$, 8 mL distilled H_2O, 8 mL MeOH and 1.87 g (28.6 mmol, 1.2 eq) KCN. 2.04 mL (23.9 mmol, 1 eq) 2-cyclopenten-1-one were added dropwise to the stirred suspension over a period of 15 min and the light-brown suspension was stirred for 5 h at RT until full conversion of the starting material was detected by TLC. Two spatulas of Na_2CO_3 were added followed by 15 mL H_2O and the mixture was extracted with $CHCl_3$ (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (100 g silica gel, size: 11 x 4.5 cm, cyclohexane/EtOAc = 2:1, 50 mL fractions).[215]

C_6H_7NO [109.13]

yield: 1.69 g (15.5 mmol, 65%) faint-yellow liquid.

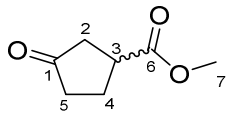
R_f = 0.32 (cyclohexane/EtOAc = 2:1 (v/v)) (CAM: blue).

GC-MS: t_R = 4.254 min, *m/z* (%) = 109.1 (76, M^+), 81.0 (73), 53.0 (100).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 3.26-3.07 (m, 1H, H-3), 2.68-2.14 (m, 6H, H-2, H-4, H-5).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 212.8 (C_q, C-1), 120.9 (C_q, C-6), 41.5 (C-2), 36.7 (C-5), 27.4 (C-4), 25.7 (C-3).

6.6.2.1.14.2 Methyl 3-oxocyclopentane-1-carboxylate (116)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 2 mL dry MeOH and was cooled in an ice bath. To the cooled flask 1 mL AcCl was slowly added, the mixture was stirred for 3 min and 1.00 g (9.16 mmol) 3-oxocyclopentane-1-carbonitrile (**133**) was added with a pipette. The flask was sealed and the light-yellow mixture was kept stirring in the thawing ice bath until full conversion of the starting material was detected after 16 h (GC-MS). The yellow suspension (colorless precipitate) was cooled again to 0 °C and 10 mL H_2O were slowly added. The mixture was left stirring until the precipitate dissolved (~30 min), neutralized with saturated $NaHCO_3$ solution and was extracted with EtOAc (5 x 30 mL). The combined organic layers were dried over Na_2SO_4, filtrated, the solvent was removed in vacuum and the crude product was dried in oil pump vacuum. The product was GC-pure (>99%) and was used in the following steps without further purification.[203]

$C_7H_{10}O_3$ [142.15]

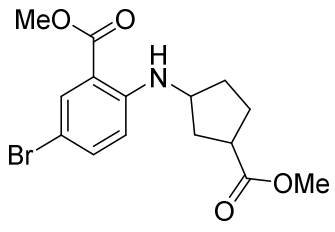
yield: 1.14 mg (8.03 mmol, 88%) dark-yellow liquid.

GC-MS: t_R = 4.449 min, *m/z* (%) = 142.1 (M^+, 15), 114.0 (72), 83.0 (48), 55.0 (100).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 3.70 (s, 3H, H-7), 3.18-3.03 (m, 1H, H-3), 2.56-2.03 (m, 6H, H-4, H-6, H-7).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 216.6 (C_q, C-1), 174.8 (C_q, C-2), 52.2 (C-7), 41.2 (CH_2), 40.9 (C-3), 37.5 (CH_2), 26.7 (CH_2).

6.6.2.1.14.3 Methyl 5-bromo-2-((3-(methoxycarbonyl)cyclopentyl)amino)benzoate (118)



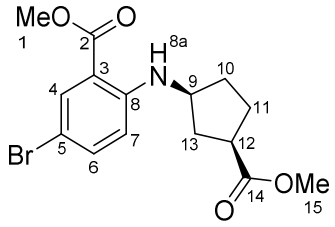
A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 562 mg (2.34 mmol, 1.0 eq) methyl 2-amino-5-bromobenzoate (**4**), 3.0 mL dry CH_2Cl_2, 1.5 mL dry AcOH and 400 mg (2.81 mmol, 1.2 eq) methyl 3-oxocyclopentane-1-carboxylate (**116**). The dark-yellow solution was cooled to 0 °C and 4.7 mL (4.7 mmol, 2.0 eq) BH_3·THF (1.0 M solution in THF)

were added over a period of 15 min. The turbid mixture was left stirring in the thawing ice bath until full conversion of the starting material was detected after 25 h (TLC). The light-yellow suspension was neutralized with saturated aqueous Na_2CO_3 solution (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was dried in oil pump vacuum and purified via column chromatography (85 g silica gel, size: 10 x 4.5 cm, cyclohexane/EtOAc = 10:1), 20 mL fractions). Pure fractions of the stereoisomers could be obtained.[141,189]

$C_{15}H_{18}BrNO_4$ [356.22]

yield (*cis* & *trans*): 387 mg (1.09 mmol, 46%) yellow, viscous liquid.

6.6.2.1.14.4 Methyl 5-bromo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclopentyl)amino)-benzoate (118)



yield: 72.6 mg (204 µmol, 9%) light-yellow, viscous liquid.

R_f = 0.57 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, CAM: yellow).

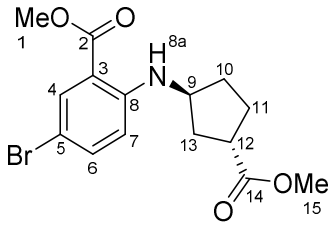
GC-MS: t_R = 8.445 min, *m/z* (%) = 357.1/355.1 (M^+, 100), 340.1/342.1 (39), 324.1 (31), 296.1 (35), 268.0 (44), 238.0/236.0 (56).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-4), 7.85 (bs, 1H, H-8a), 7.38 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-6), 6.56 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-7), 3.92-3.80 (m, 4H, H-9, CH_3), 3.69 (s, 3H, CH_3), 2.96-2.80 (m, 1H, H-12), 2.49-2.34 (m, 1H, H-13a), 2.13-1.94 (m, 3H, H-10a, H-11), 1.93-1.80 (m, 1H, H-13b), 1.79-1.64 (m, 1H, H-10b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.1 (C_q, C-14), 168.0 (C-2), 149.4 (C_q, C-8), 137.1 (C-6), 134.0 (C-4), 113.8 (C-7), 111.6 (C_q, C-5), 105.9 (C_q, C-3), 53.7 (C-9), 52.0 (CH_3), 51.8 (CH_3), 42.5 (C-12), 36.8 (C-13), 33.0 (C-10), 28.0 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{18}BrNO_4^+$:355.0419; found: 355.0416.

6.6.2.1.14.5 Methyl 5-bromo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclopentyl)amino)-benzoate (118)



yield: 33.0 mg (92.6 µmol, 4%) light-yellow, viscous liquid.

R_f = 0.62 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, CAM: yellow).

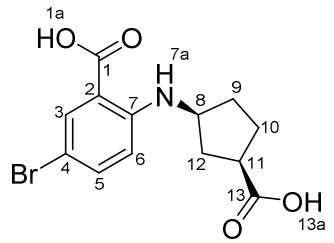
GC-MS: t_R = 8.403 min, *m/z* (%) = 357.1/355.1 (M^+, 100), 340.1/342.1 (42), 324.1 (48), 296.1 (37), 268.0 (48), 238.0/236.0 (61).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.98 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-4), 7.76 (d, $^3J_{HH}$ = 4.9 Hz, 1H, H-8a), 7.39 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-6), 6.58 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-7), 4.04-3.91 (m, 1H, H-9), 3.84 (s, 3H, CH_3), 3.69 (s, 3H, CH_3), 3.07-2.93 (m, 1H, H-12), 2.36-2.03 (m, 3H, H-10a, H-11a, H-13a), 2.00-1.80 (m, 2H, H-11b, H-13b), 1.72-1.55 (m, 1H, H-10b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.6 (C_q, C-14), 168.2 (C_q, C-2), 149.4 (C_q, C-8), 137.2 (C-6), 134.0 (C-4), 113.9 (C-7), 111.4 (C_q, C-5), 106.0 (C_q, C-3), 53.5 (C-9), 52.0 (CH_3), 51.8 (CH_3), 42.1 (C-12), 36.8 (C-13), 33.1 (C-10), 28.2 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{18}BrNO_4^+$:355.0419; found: 355.0417.

6.6.2.1.14.6 5-Bromo-2-(((1S*,3*R**)-3-carboxycyclopentyl)amino)benzoic acid (115)



A 5 mL round-bottom flask with magnetic stirring bar was charged with 35.0 mg (98.3 µmol, 1 eq) methyl 5-bromo-2-(((1*S**,3*R**)-3-(methoxycarbonyl)cyclopentyl)amino)benzoate (**118**), 1.0 mL THF/MeOH/H_2O (3:3:1 (v/v/v)) and 20.8 mg (491 µmol, 5 eq) LiOH·H_2O. The yellow solution was stirred at RT until full conversion of the starting material was detected by TLC (20 h). The mixture was acidified with 1 M HCl (2 mL) and extracted with EtOAc (4 x 4 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was removed in vacuum. The crude product was dissolved in the minimum amount of

cyclohexane/EtOAc/HCO_2H (1:1:1% (v/v/v)) and was filtrated through a pad of silica gel (4 cm) eluting with the identical solvent. The solvent was removed in vacuum to give the pure product.

$C_{13}H_{14}BrNO_4$ [328.16]

yield: 29.5 mg (89.9 µmol, 91%) light-yellow solid.

R_f = 0.51 (cyclohexane/EtOAc/HCO_2H = 1:1:1% (v/v/v)) (254 nm, 366 nm, CAM: yellow).

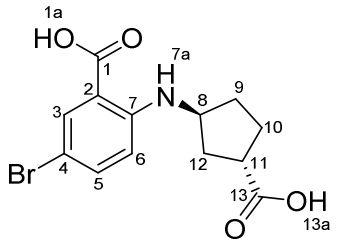
mp: 198-200 °C (decomp.).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.84 (bs, 1H, H-3), 7.45 (d, $^3J_{HH}$ = 7.1 Hz, 1H, H-5), 6.73 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-6), 3.95–3.80 (m, 1H, H-8), 2.93–2.69 (m, 1H, H-11), 2.43–2.25 (m, 1H, H-12a), 2.14–1.76 (m, 3H, H-9a, H-10), 1.73–1.38 (m, 2H, H-9b, H-12b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.5 (C_q, C-13), 168.9 (C_q, C-1), 149.2 (C_q, C-7), 136.5 (C-5), 133.4 (C-3), 114.3 (C-6), 112.1 (C_q, C-4), 104.4 (C_q, C-2), 52.8 (C-8), 41.7 (C-11), 36.1 (C-12), 32.4 (C-9), 27.4 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{14}BrNO_4^+$: 327.0106; found: 327.0107.

6.6.2.1.14.7 5-Bromo-2-(((1*S**,3*S**)-3-carboxycyclopentyl)amino)benzoic acid (115)



A 5 mL round-bottom flask with magnetic stirring bar was charged with 21.0 mg (59.0 µmol, 1 eq) methyl 5-bromo-2-(((1*S**,3*S**)-3-(methoxycarbonyl)cyclopentyl)amino)benzoate (**118**), 600 µL THF/MeOH/H_2O (3:3:1 (v/v/v)) and 12.5 mg (298 µmol, 5 eq) $LiOH \cdot H_2O$. The yellow solution was stirred at RT until full conversion of the starting material was detected by TLC (4 d). The mixture was acidified with 1 M HCl and extracted with EtOAc (4 x 4 mL). The combined organic phases were dried over $MgSO_4$, filtrated and the solvent was removed in vacuum. The crude product was dissolved in the minimum amount of cyclohexane/EtOAc/HCO_2H (1:1:1% (v/v/v)) and was filtrated through a pad of silica gel (4 cm, eluting with the identical solvent). The solvent was removed in vacuum to give the pure product.

$C_{13}H_{14}BrNO_4$ [328.16]

yield: 16.1 mg (49.1 µmol, 83%) light-yellow, greyish solid.

R_f = 0.53 (cyclohexane/EtOAc/HCO_2H = 1:1:1% (v/v/v)) (254 nm, 366 nm, CAM: yellow).

mp: 175-180 °C.

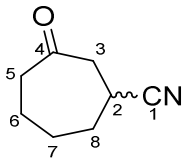
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.84 (d, $^4J_{HH}$ = 2.3 Hz, 1H, H-3), 7.45 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-5), 6.69 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-6), 3.99–3.85 (m, 1H, H-8), 2.96–2.79 (m, 1H, H-11), 2.25–1.91 (m,3H, H-9a, H-10a, H-12a), 1.85–1.62 (m, 2H, H-10b, H-12b), 1.57–1.37 (m, 1H, H-9b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.7 (C_q, C-13), 169.1 (C_q, C-1), 149.1 (C_q, C-7), 136.2 (C-5), 133.5 (C-3), 114.1 (C-6), 113.0 (C_q, C-4), 104.5 (C_q, C-2), 52.7 (C-8), 41.6 (C-11), 36.0 (C-12), 32.5 (C-9), 27.6 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{14}BrNO_4^+$: 327.0106; found: 327.0101.

6.6.2.1.15 Synthesis of the 7-ring Phenazistatin A

6.6.2.1.15.1 3-Oxocycloheptane-1-carbonitrile (124)



(Safety warning: HCN detectors were used at all times throughout the reaction!!!) A 50 mL round-bottom flask with magnetic stirring bar was charged with 1.45 g (10.5 mmol, 1.5 eq) $Et_3N{\cdot}HCl$, 8 mL H_2O and 8 mL MeOH. The colorless solution was cooled in an ice bath and 481 mg (7.32 mmol, 1.05 eq) KCN were added. To the vigorously stirred solution were added 800 mg (6.97 mmol, 1.0 eq) 2-cyclohepten-1-one over a period of 16 min. The light-yellow suspension was left stirring in the thawing ice bath and was stirred for 16 h until GC-MS indicated full conversion of the starting material. The yellow solution was treated with 10 mL saturated Na_2CO_3 solution and the mixture was extracted with $CHCl_3$ (5 x 30 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The yellow, oily residue was adsorbed on 1.3 g Celite™ (CH_2Cl_2) and was purified via column chromatography (size: 20 x 3.5 cm, cyclohexane/EtOAc = 2:1, 20 mL fractions). The product could not be purified higher than a purity of ~85% by conventional column chromatography and was used in the following step without further purification.[215]

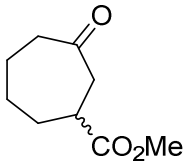
$C_8H_{11}NO$ [137.18]

yield: 221 mg (85% GC purity, rest is ester product!) yellow, viscous liquid.

R_f = 0.16 (cyclohexane/EtOAc = 3:1 (v/v)) ($KMnO_4$: yellow).

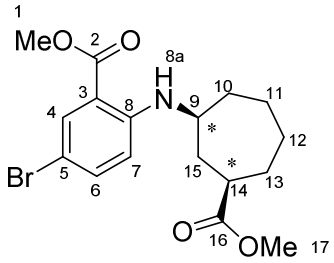
GC-MS: t_R = 4.994 min, *m/z* (%) = 137.1 (M^+, 10), 82.1 (17), 55.0 (100).

by-product:



GC-MS: t_R = 5.168 min, *m/z* (%) = 170.1 (M^+, 20), 138.0 (18), 127.0 (40), 111.0 (48), 110.0 (23), 88.0 (22), 69.0 (20), 59.0 (25), 55.0 (100).

6.6.2.1.15.2 Methyl (1*R**,3*S**)-3-((4-bromo-2-(methoxycarbonyl)phenyl)amino)cycloheptane-1-carboxylate (122)



Step 1: A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 221 mg (max. 1.61 mmol, 1.1 eq) of the crude product from the previous step, 351 mg (1.47 µmol, 1.0 eq) methyl 2-amino-5-bromobenzoate (**4**) and 1.0 mL dry DMF. To the yellow solution were added 473 µL (3.66 mmol, 2.5 eq) TMSCl in one portion at RT. The yellow solution was cooled to 0 °C and 1.47 mL (1.47 mmol, 1.0 eq) BH_3 THF (1.0 M solution in THF) were slowly added over a period of 9 min. The yellow solution was stirred at 0 °C for 110 min until full conversion of the starting material was detected by GC-MS. The yellow mixture was treated with 5 mL H_2O at 0 °C and the mixture was stirred for 30 min at RT. 5 mL EtOAc were added and the mixture was treated with 5 mL saturated Na_2CO_3 solution. The phases were separated, the aqueous layer was extracted with EtOAc (5 x 10 mL), the combined organic layers were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The yellow, oily residue was dried in oil pump vacuum. The crude product was used in the following step without further purification.[189]

Step 2: The crude product from the previous step was transferred (CH_2Cl_2) into a 100 mL Schlenk flask with magnetic stirring bar and the solvents were removed in oil pump vacuum. 5 mL dry MeOH were added to the yellow residue and the mixture was cooled to 0 °C. 2.5 mL AcCl were added dropwise to the vigorously stirred solution over a period of 3 min in a N_2 counter-stream, the Schlenk flask was sealed and the mixture was left stirring in the ice bath. The mixture was left stirring in the thawing ice bath until full conversion of the starting material was detected after 20 h (GC-MS). The mixture was again cooled to 0 °C and 10 mL

H_2O were added carefully. The light-yellow suspension was kept stirring at 0 °C for 80 min and was extracted with EtOAc (5 x 15 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvents were removed in vacuum. The residue was dried in oil pump vacuum and was adsorbed on Celite™ (1.40 g, EtOAc). The product diastereomers exhibited no observable R_f-value difference and hence a very careful column chromatography was performed (size: 30 x 1.8 cm, cyclohexane/EtOAc = 25:1, R_f = 0.20, 20 mL fractions). The fractions were checked with GC-MS and the (1*R**,3*S**)-product could be isolated as a single diastereomer.[203]

$C_{17}H_{22}BrNO_4$ [384.27]

yield (*cis*&*trans*): 268 mg (697 µmol, 47%) light-yellow, very viscous liquid.

R_f (*cis*&*trans*) = 0.20 (cyclohexane/EtOAc = 25:1 (v/v)) (254 nm, 366 nm, cold CAM: yellow).

yield (*cis*): 129 mg (335 µmol, 23%) light-yellow, very viscous liquid.

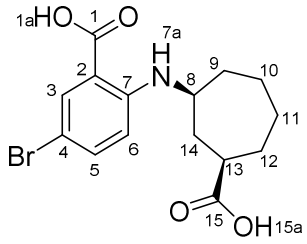
GC-MS: t_R = 8.878 min, *m/z* (%) = 385.1 (M^+, 96), 383.0 (M^+, 98), 326.0 (80), 324.0 (63), 269.9 (96), 267.9 (100), 237.9 (89), 235.9 (90), 228.9 (46), 207.0 (61), 196.9 (43), 95.0 (70), 67.0 (48), 55.0 (50).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-4), 7.82 (d, $^3J_{HH}$ = 7.1 Hz, 1H, H-8a), 7.38 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-6), 6.61 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-7), 3.93–3.69 (m, 4H, H-9, CH_3), 3.66 (s, 3H, CH_3), 2.75–2.61 (m, 1H, C-14), 2.18–1.90 (m, 4H, CH_2), 1.90–1.39 (m, 6H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 177.0 (C_q, C-16), 168.3 (C_q, C-2), 149.0 (C_q, C-8), 137.3 (C-6), 134.1 (C-4), 113.9 (C-7), 111.3 (C_q), 105.6 (C_q), 51.9 (CH_3), 51.8 (CH_3), 50.0 (C-9), 40.6 (C-14), 36.1 (CH_2), 35.1 (CH_2), 30.8 (CH_2), 26.9 (CH_2), 25.3 (CH_2).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{17}H_{22}BrNO_4^+$: 383.0732; found: 383.0739.

6.6.2.1.15.3 (1*R**,3*S**)-3-((4-Bromo-2-carboxyphenyl)amino)cycloheptane-1-carboxylic acid (119)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 50.0 mg (130 µmol, 1.0 eq) methyl (1*R**,3*S**)-3-((4-bromo-2-(methoxycarbonyl)phenyl)amino)cycloheptane-1-carboxylate (**122**), 1.5 mL distilled THF and 1.5 mL H_2O. To the faint-yellow solution were added 27.5 mg (651 µmol, 5.0 eq) $LiOH \cdot H_2O$ and the mixture was heated to 30 °C for 15 h until full conversion of the starting material was detected by TLC. The reaction mixture was acidified with 6 N HCl (200 µL, pH~1) and the solvent was removed in vacuum. The residue was dried in oil pump vacuum and was dissolved in 1.0 mL DMSO for purification with preparative RP-HPLC (Method_PREPHPLC). The isolated product was dried by azeotropic distillation with benzene in order to remove traces of HCO_2H.

$C_{15}H_{18}BrNO_4$ [356.22]

yield: 30.4 mg (85.3 µmol, 66%%) light-yellow solid.

R_f = 0.19 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: orange).

mp: 215-217 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 8.01 (bs, 1H, H-7a), 7.85 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-3), 7.44 (dd, $^3J_{HH}$ = 8.9 Hz, 2.0 Hz, 1H, H-5), 6.73 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 3.83–3.68 (m, 1H, H-8), 2.60–2.50 (m, 1H, H-13, DMSO overlap!), 2.05–1.80 (m, 4H, CH_2), 1.80–1.36 (m, 6H, CH_2).

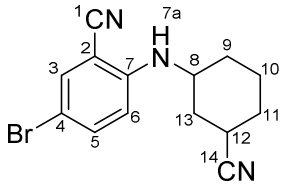
^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 177.3 (C_q, C-15), 169.1 (C_q, C-1), 148.7 (C_q, C-7), 136.6 (C-5), 133.6 (C-3), 114.3 (C-6), 111.9 (C_q,), 104.3 (C_q,), 49.2 (C-8), 39.8 (C-13), 35.5 (CH_2), 34.1 (CH_2), 30.2 (CH_2), 26.5 (CH_2), 24.5 (CH_2).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{18}BrNO_4^+$: 355.0419; found: 355.0418.

6.6.2.2 Phenazistatin derivatives to optimize ADMET-properties

6.6.2.2.1 Bioisosteres

6.6.2.2.1.1 5-Bromo-2-((3-cyanocyclohexyl)amino)benzonitrile (129)



A dry 200 mL Schlenk flask with magnetic stirring bar was charged with 2.00 g (10.2 mmol, 1.0 eq) 2-amino-5-bromobenzonitrile, 14 mL dry CH_2Cl_2, 7 mL glacial AcOH and 1.50 g (12.2 mmol, 1.2 eq) methyl 3-oxocyclohexane-1-carboxylate (**5**). The light-yellow solution was cooled to 0 °C and 20.5 mL (20.5 mmol, 2.0 eq) $BH_3 \cdot THF$ were added over a period of 42 min. The mixture was left stirring in the thawing ice bath and full conversion of the starting material was detected by GC-MS after 46 h. The light-yellow solution was slowly treated with 10 mL H_2O and neutralized with saturated Na_2CO_3 solution. The mixture was extracted with EtOAc (3 x 75 mL), the combined organic layers were dried over Na_2SO_4, filtrated through a Pore 3 sintered-glass frit and the solvent was removed in vacuum. The residue was adsorbed on the 2.5-fold amount of Celite™ (EtOAc) and was purified via column chromatography (225 g silica gel, size: 16 x 6 cm, cyclohexane/EtOAc = 5:1, 4:1, 100 mL fractions). The diastereomers could not be separated via column chromatography.[141,189]

$C_{14}H_{14}BrN_3$ [304.19]

yield: 2.28 g (7.50 mmol, 73%) colorless, amorphous solid.

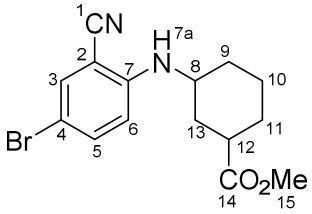
R_f = 0.21 (*cis*&*trans*) (cyclohexane/EtOAc = 4:1 (v/v)) (254 nm, cold CAM: light-yellow).

mp: 80-90 °C.

GC-MS: t_R = 4.453 min (*trans*), 4.715 min (*cis*); ratio(*trans*/*cis*) = 29:71.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{14}BrN_3^+$: 303.0371; found: 303.0381.

6.6.2.2.1.2 Methyl 3-((4-bromo-2-cyanophenyl)amino)cyclohexane-1-carboxylate (130)



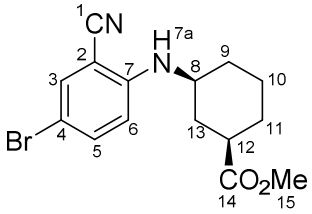
A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 1.97 mg (10.0 mmol, 1.0 eq) 2-amino-5-bromobenzonitrile, 10 mL dry CH_2Cl_2, 5 mL glacial AcOH and 1.87 mg (12.0 mmol, 1.2 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**). The light-yellow solution was cooled to 0 °C, the glass stopper was replaced by a rubber septum and 15 mL (15.0 mmol, 1.5 eq) BH_3·THF (1.0 M solution in THF) were added dropwise via syringe over a period of 30 min. The flask was sealed and the mixture was stirred in the thawing ice bath for 13 h until full conversion of the starting material was detected (TLC). The reaction mixture was cooled to 0 °C and neutralized with saturated $NaHCO_3$ (15 mL) and solid Na_2CO_3. The phases were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL), the combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The crude product was purified via column chromatography (150 g silica gel, cyclohexane/EtOAc = 16:1 (v/v)).[141,189]

$C_{15}H_{17}BrN_2O_2$ [337.22]

yield: 1.84 g (5.46 mmol, 55%) light-yellow, very viscous liquid.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{15}H_{17}BrN_2O_2^+$: 336.0472; found: 336.0476.

6.6.2.2.1.3 Methyl (1*R**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclohexane-1-carboxylate (130)



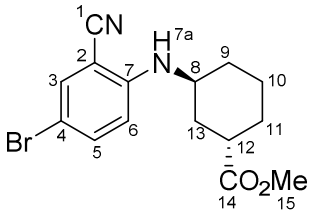
R_f = 0.60 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, cold CAM: orange).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.50–7.37 (m, 2H, ArH), 6.56 (d, $^3J_{HH}$ = 9.0 Hz, 1H, ArH), 4.46 (bs, 1H, NH), 3.67 (s, 3H, H-15), 3.41–3.25 (m, 1H, H-8), 2.51–2.27 (m, 2H, H-12, CH_2), 2.15–1.83 (m, 3H, CH_2), 1.51–1.12 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.0 (C_q, C-14), 148.2 (C_q, C-7), 137.2 (CH_{arom}), 134.9 (CH_{arom}), 116.6 (C_q), 112.8 (CH_{arom}), 107.1 (C_q), 97.6 (C_q), 52.0/51.4 (C-8/C-15), 42.3 (C-12), 35.1 (C-13), 32.5 (C-9), 28.3 (C-11), 24.2 (C-10).

GC-MS: t_R = 8.464 min, *m/z* (%)= 338.1 (M^+, 90), 336.1 (M^+, 91), 279.0 (77), 277.0 (98), 249.0 (47), 237.0 (71), 235.0 (79), 225.0 (48), 222.9 (63), 208.9 (48), 198.0 (72), 195.9 (68), 155.1 (98), 142.1 (37), 116.1 (49), 81.1 (100, BP), 59.0 (75), 55.1 (49).

6.6.2.2.1.4 Methyl (1*S**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclohexane-1-carboxylate (130)



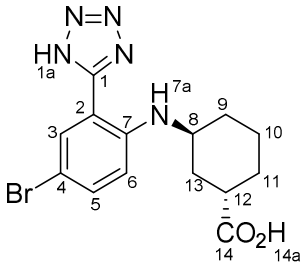
R_f = 0.69 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, 366 nm, CAM: orange).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.50–7.40 (m, *2*H, ArH), 6.72 (d, $^3J_{HH}$ = 9.7 Hz, 1H, ArH), 4.57–4.39 (m, 1H, NH), 3.79–3.61 (m, 4H, H-8, H-15), 2.82–2.69 (m, 1H, H-12), 2.30–2.16 (m, 1H, CH_2), 2.01–1.80 (m, 2H, CH_2), 1.80–1.36 (m, 5H, CH_2).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.50 (s), 148.39 (s), 137.38 (s), 134.65 (s), 116.76 (s), 113.07 (s), 107.04 (s), 97.33 (s), 51.95 (s), 48.06 (s), 38.89 (s), 32.77 (s), 31.68 (s), 27.62 (s), 21.53 (s).

GC-MS: t_R = 8.286 min, *m/z* (%)= 338.1 (M^+, 82), 336.0 (M^+, 82), 279.0 (71), 277.0 (86), 249.0 (44), 237.0 (56), 234.9 (62), 225.0 (65), 223.0 (72), 209.0 (40), 198.0 (59), 195.9 (55), 155.1 (86), 116.1 (47), 81.1 (100), 59.1 (58), 55.1 (46).

6.6.2.2.1.6 (1*S**,3*S**)-3-((4-Bromo-2-(1*H*-tetrazol-5-yl)phenyl)amino)cyclohexane-1-carboxylic acid (131)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 100 mg (297 µmol, 1.0 eq) methyl (1*S**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclohexane-1-carboxylate (**130**), 39.0 mg (594 µmol, 2.0 eq) NaN_3, 82.8 mg (594 µmol, 2.0 eq) $Et_3N{\cdot}HCl$ and 2 mL dry toluene. The flask was sealed, and the light-yellow suspension was heated to 120 °C for 18 h until full conversion of the starting material was detected by TLC. The flask was cooled to RT, 2.5 mL 1 M HCl were added and the mixture was stirred at RT for 5 h. The mixture was extracted with EtOAc (3 x 5 mL), the combined organic phases were dried over $MgSO_4$, filtrated and the solvent was removed in vacuum. The yellow residue was dissolved in 5 mL THF/MeOH/H_2O = 3:3:1 (v/v/v) and 126 mg (2.97 mmol, 10 eq) $LiOH{\cdot}H_2O$ were added. The light-yellow suspension was stirred at RT for 18 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 6 M HCl and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (2 g silica gel, cyclohexane/EtOAc/HCO_2H = 5:1:1%).[225]

$C_{14}H_{16}BrN_5O_2$ [366.22]

yield: 36.8 mg (100 µmol, 34%) greyish solid.

R_f = 0.64 (cyclohexane/EtOAc/HCO_2H = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: intensive red).

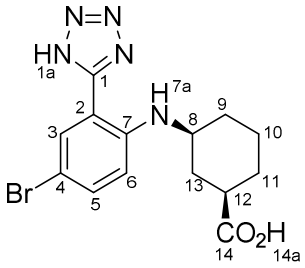
mp: 210-215 °C (decomp.).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.31 (bs, 1H), 8.09–7.58 (m, 2H, H-3, NH), 7.48 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, H-5), 6.89 (d, $^3J_{HH}$ = 9.2 Hz, 1H, H-6), 3.94–3.74 (m, 1H, H-8), 2.64–2.49 (m, 1H, H-12), 2.04–1.49 (m, 8H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.2 (C_q, C-14), 154.1 (C_q, C-1), 144.7 (C-7), 134.6 (CH_{arom}), 130.5 (CH_{arom}), 114.1 (CH_{arom}), 107.3 (C_q), 105.5 (C_q), 46.8 (C-8), 38.0 (C-12), 32.1 (C-13), 30.2 (C-9), 27.5 (C-11), 20.6 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}BrN_5O_2^+$: 365.0487; found: 365.0486.

6.6.2.2.1.7 (1*R**,3*S**)-3-((4-Bromo-2-(1*H*-tetrazol-5-yl)phenyl)amino)cyclohexane-1-carboxylic acid (131)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 100 mg (297 µmol, 1.0 eq) methyl (1*R**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclohexane-1-carboxylate (**130**), 39.0 mg (594 µmol, 2.0 eq) NaN_3, 82.8 mg (594 µmol, 2.0 eq) $Et_3N{\cdot}HCl$ and 2 mL dry toluene. The flask was sealed, and the yellow suspension was heated to 120 °C for 18 h until full conversion of the starting material was detected by TLC. The flask was cooled to RT, 2.5 mL 1 M HCl were added and the mixture was stirred at RT for 5 h. The mixture was extracted with EtOAc (3 x 5 mL), the combined organic phases were dried over $MgSO_4$, filtrated and the solvent was removed in vacuum. The yellow residue was dissolved in 5 mL THF/MeOH/H_2O = 3:3:1 (v/v/v) and 126 mg (2.97 mmol, 10 eq) $LiOH{\cdot}H_2O$ were added. The light-yellow suspension was stirred at RT for 18 h until full conversion of the starting material was detected by TLC. The mixture was acidified with 6 M HCl and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (2 g silica gel, cyclohexane/EtOAc/HCO_2H = 5:1:1%).[225]

$C_{14}H_{16}BrN_5O_2$ [366.22]

yield: 41.6 mg (113 µmol, 38%) greyish solid.

R_f = 0.54 (cyclohexane/EtOAc/HCO_2H = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: intensive red).

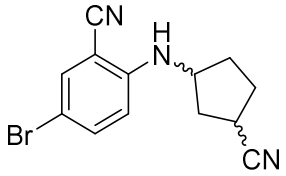
mp: 225-230 °C (decomp.).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.16 (bs, 1H, H_{acidic}), 8.00 (d, $^4J_{HH}$ = 2.1 Hz, 1H, H-3), 7.45 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.1 Hz, 1H, H-5), 6.91 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 3.60–3.42 (m, 1H, H-8), 2.51–2.34 (m, 1H, H-12, DMSO overlap), 2.26 (d, *J* = 11.2 Hz, 1H, CH_2), 2.05 (d, *J* = 10.8 Hz, 1H, CH_2), 1.99–1.72 (m, 2H, CH_2), 1.55–1.07 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C_q, C-14), 154.4 (C_q, C-1), 144.7 (C_q, C-7), 134.2 (C-5), 130.4 (C-3), 114.2 (C-6), 107.7 (C_q), 105.5 (C_q), 50.0 (C-8), 41.4 (C-12), 35.1 (C-13), 32.2 (C-9), 28.1 (C-11), 23.8 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}BrN_5O_2^+$: 365.0487; found: 365.0487.

6.6.2.2.1.8 5-Bromo-2-((3-cyanocyclopentyl)amino)benzonitrile (132)

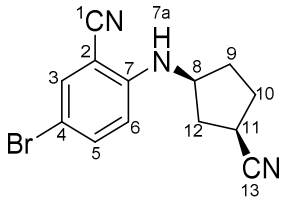


A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 451 mg (2.29 mmol, 1.0 eq) 2-amino-5-bromobenzonitrile, 3.0 mL dry CH_2Cl_2, 1.5 mL dry AcOH and 300 mg (2.75 mmol, 1.2 eq) 3-oxocyclopentane-1-carbonitrile (**133**) in a N_2 counter-stream. The faint-yellow solution was cooled to 0 °C and 4.6 mL (4.6 mmol, 2.0 eq) $BH_3 \cdot THF$ (1.0 M solution in THF) were added over a period of 15 min. The turbid mixture was left stirring in the thawing ice bath until full conversion of the starting material was detected after 25 h. The mixture was cooled to 0 °C, neutralized with saturated Na_2CO_3 solution (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (65 g silica gel, size: 12 x 3.5 cm, cyclohexane/EtOAc = 6:1, 15 mL fractions).[141,189]

$C_{13}H_{12}BrN_3$ [290.16]

yield (*cis* & *trans*): 402 mg (1.39 mmol, 61%) red-orange, waxy solid.

6.6.2.2.1.9 5-Bromo-2-(((1*S**,3*R**)-3-cyanocyclopentyl)amino)benzonitrile (132)



yield: 230 mg (795 µmol, 35%) red/orange, waxy solid.

R_f = 0.08 (cyclohexane/EtOAc = 6:1 (v/v)) (254 nm, CAM: yellow).

mp: 90-96 °C.

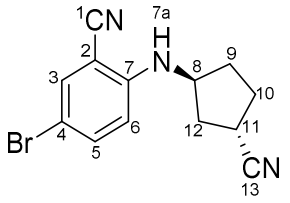
GC-MS: t_R = 8.232 min, *m/z* (%) = 291.1/289.1 (M^+, 100), 262.0/260.0 (70), 237.0/235.0 (83), 209.0/207.0 (54), 155.1 (85).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.54-7.40 (m, 2H, H-3, H-5), 6.52 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-6), 4.65 (d, $^3J_{HH}$ = 5.3 Hz, 1H, H-7a), 3.99-3.81 (m, 1H, H-8), 2.98-2.80 (m, 1H, H-11), 2.65-2.48 (m, 1H, H-12a), 2.27-2.08 (m, 3H, H-9a, H-10), 1.97-1.71 (m, 2H, H-9b, H-12b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 148.2 (C_q, C-7), 137.3 (CH_{arom}), 135.0 (CH_{arom}), 122.1 (C_q, CN), 116.3 (C_q, CN), 112.9 (C-6), 108.1 (C_q, C-4), 98.1 (C_q, C-2), 53.7 (C-8), 37.8 (C-12), 32.5 (C-9), 29.2 (C-10), 26.7 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{12}BrN_3^+$: 289.0215; found: 289.0222.

6.6.2.2.1.10 5-Bromo-2-(((1*S**,3*S**)-3-cyanocyclopentyl)amino)benzonitrile (132)



yield: 172 mg (592 µmol, 26%) red/orange, waxy solid.

R_f = 0.16 (cyclohexane/EtOAc = 6:1 (v/v)) (254 nm, CAM: yellow).

mp: 82-86 °C.

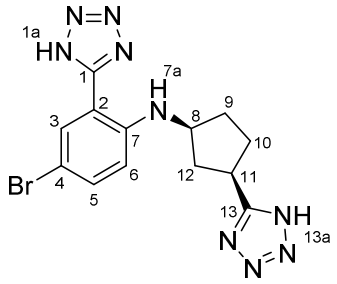
GC-MS: t_R = 8.062 min, *m/z* (%) = 291.1/289.1 (M^+, 100), 262.0/260.0 (67), 237.0/235.0 (80), 207.0 (86), 155.1 (83).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.55-7.44 (m, 2H, H-3, H-5), 6.56 (d, $^3J_{HH}$ = 9.7 Hz, 1H, H-6), 4.50 (d, $^3J_{HH}$ = 3.4 Hz, 1H, H-7a), 4.14-3.97 (m, 1H, H-8), 3.11-2.95 (m, 1H, H-11), 2.45-2.18 (m, 3H, H-9a, H-10a, H-12a), 2.12-1.91 (m, 2H, H-10b, H-12b), 1.75-1.56 (m, 1H, H-9b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 148.2 (C_q, C-7), 137.4 (CH_{arom}), 134.9 (CH_{arom}), 122.3 (C_q, CN), 116.4 (C_q, CN), 113.0 (C-6), 108.2 (C_q, C-4), 98.0 (C_q, C-2), 53.4 (C-8), 37.8 (C-12), 32.6 (C-9), 29.4 (C-10), 26.7 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{12}BrN_3^+$: 289.0215; found: 289.0218.

6.6.2.2.1.12 *N*-((1*S**,3*R**)-3-(1*H*-Tetrazol-5-yl)cyclopentyl)-4-bromo-2-(1*H*-tetrazol-5-yl)aniline (134)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 35.0 mg (121 µmol, 1 eq) 5-bromo-2-(((1*S**,3*R**)-3-cyanocyclopentyl)amino)benzonitrile (**132**), 31.4 mg (482 µmol, 4 eq) NaN_3, 66.9 mg (482 µmol, 4 eq) $Et_3N{\cdot}HCl$ and 1.2 mL dry toluene in a N_2 counter-stream. The suspension was heated to 120 °C for 4 d (TLC: incomplete conversion of the starting material) and additional NaN_3 (31.4 mg (482 µmol, 4 eq)) were added. The mixture was heated to 120 °C and full conversion (TLC) was detected after 1 d. 5 mL 1 M HCl were added followed by 5 mL EtOAc. The mixture was vigorously stirred until all solid parts had dissolved. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was removed in vacuum. The product was purified via column chromatography (3 g silica gel, size: 8 x 1 cm, EtOAc/cyclohexane/HCO_2H = 2:1:1% (v/v/v), 3 mL fractions).[225]

$C_{13}H_{14}BrN_9$ [376.22]

yield: 28.5 mg (75.8 µmol, 63%) light-yellow, collapsed foam.

R_f = 0.48 (EtOAc/cyclohexane/HCO_2H = 2:1:1% (v/v/v)) (254 nm, CAM: crimson).

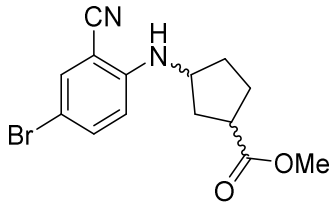
mp: >65 °C (decomp.).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 8.00 (d, $^4J_{HH}$ = 2.1 Hz, 1H, H-3), 7.49 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.1 Hz, 1H, H-5), 6.89 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 4.18–4.05 (m, 1H, H-8), 3.62–3.46 (m, 1H, H-11), 2.79-2.65 (m, 1H, H-12a), 2.36–2.12 (m, 2H, H-9a, H-10a), 2.07–1.90 (m, 1H, H-10b), 1.90–1.63 (m, 2H, H-9b, H-12b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 159.0 (C_q, C-13), 154.2 (C_q, C-1), 145.2 (C_q, C-7), 134.4 (C-5), 130.3 (C-3), 114.4 (C-6), 107.7 (C_q, C-4), 105.8 (C_q, C-2), 53.1 (C-8), 38.5 (C-12), 32.4 (C-11), 32.2 (C-9), 29.6 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{14}BrN_9^+$: 375.0555; found: 375.0573.

6.6.2.2.1.14 Methyl 3-((4-bromo-2-cyanophenyl)amino)cyclopentane-1-carboxylate (136)

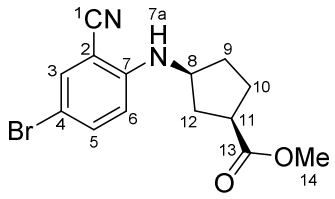


A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 462 mg (2.34 mmol, 1.0 eq) 2-amino-5-bromobenzonitrile, 3.0 mL dry CH_2Cl_2, 1.5 mL dry AcOH and 400 mg (2.81 mmol, 1.2 eq) methyl 3-oxocyclopentane-1-carboxylate (**116**). The dark-yellow solution was cooled to 0 °C and 4.7 mL (4.7 mmol, 2.0 eq) $BH_3 \cdot THF$ (1.0 M solution in THF) were added over a period of 15 min. The turbid mixture was left stirring in the thawing ice bath until full conversion of the starting material was detected after 25 h (TLC). The light-yellow suspension was neutralized with saturated Na_2CO_3 solution (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was dried in oil pump vacuum and purified via column chromatography (100 g silica gel, size: 11 x 4.5 cm, cyclohexane/EtOAc = 8:1), 20 mL fractions).[141,189]

$C_{14}H_{15}BrN_2O_2$ [323.19]

yield (*cis* & *trans*): 544 mg (1.68 mmol, 72%) light-yellow, viscous liquid.

6.6.2.2.1.15 Methyl (1*R**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclopentane-1-carboxylate (136)



yield: 150 mg (464 µmol, 20%) light-yellow, viscous liquid.

R_f = 0.51 (cyclohexane/EtOAc = 1:1 (v/v)) (254 nm, CAM: carnate).

GC-MS: t_R = 8.066 min, *m/z* (%) = 324.1/322.1 (M^+, 84), 263.1 (76), 237.0/235.0 (85), 225.0/223.0 (100), 155.1 (91), 67.0 (67).

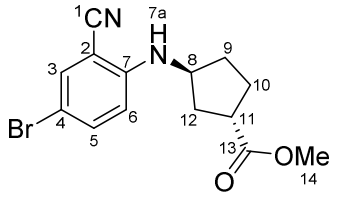
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.50-7.36 (m, 2H, H-3, H-5), 6.55 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-6), 5.08 (d, $^3J_{HH}$ = 6.0 Hz, 1H, H-7a), 4.00-3.85 (m, 1H, H-8), 3.73 (s, 3H, H-14), 3.01-2.86

(m, 1H, H-11), 2.41-2.23 (m, 1H, H-12a), 2.11-1.86 (m, 4H, H-9a, H-10, H-12b), 1.83-1.69 (m, 1H, H-9b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.5 (C_q, C-13), 148.7 (C_q, C-7), 137.1 (CH_{arom}), 134.9 (CH_{arom}), 116.6 (C_q, C-1), 112.9 (C-6), 107.0 (C_q, C-4), 97.6 (C_q, C-2), 54.4 (C-8), 52.3 (C-14), 42.2 (C-11), 36.4 (C-12), 32.8 (C-9), 28.2 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{15}BrN_2O_2^+$: 322.0317; found: 322.0317.

6.6.2.2.1.16 Methyl (1*S**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclopentane-1-carboxylate (136)



yield: 35.8 mg (111 µmol, 5%) light-yellow, viscous liquid

R_f = 0.56 (cyclohexane/EtOAc = 1:1 (v/v)) (254 nm, CAM: carnate)

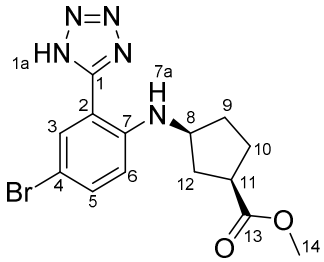
GC-MS: t_R = 8.019 min, *m/z* (%) = 324.1/322.1 (M^+, 75), 263.1 (72), 237.0/235.0 (78), 225.0/223.0 (100), 155.1 (77), 67.0 (57).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.50-7.39 (m, 2H, H-3, H-5), 6.58 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-6), 4.58-4.43 (m, 1H, H-7a), 4.03-3.88 (m, 1H, H-8), 3.70 (s, 3H, H-14), 3.07-2.93 (m, 1H, H-11), 2.40-2.07 (m, 3H, H-9a, H-10a, H-12a), 1.99-1.73 (m, 2H, H-10b, H-12b), 1.66-1.50 (m, 1H, H-9b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 176.3 (C_q, C-13), 148.7 (C_q, C-7), 137.3 (CH_{arom}), 134.7 (CH_{arom}), 116.6 (C_q, C-1), 113.1 (C-6), 107.4 (C_q, C-4), 97.6 (C_q, C-2), 54.1 (C-8), 52.1 (C-14), 41.9 (C-11), 36.5 (C-12), 33.1 (C-9), 28.1 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{15}BrN_2O_2^+$: 322.0317; found: 322.0329.

6.6.2.2.1.17 Methyl (1*R**,3*S**)-3-((4-bromo-2-(1*H*-tetrazol-5-yl)phenyl)amino)cyclopentane-1-carboxylate (135)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 50.0 mg (155 µmol, 1 eq) methyl (1*R**,3*S**)-3-((4-bromo-2-cyanophenyl)amino)cyclopentane-1-carboxylate (**136**), 20.3 mg (309 µmol, 2 eq) NaN_3, 42.5 mg (309 µmol, 2 eq) $Et_3N{\cdot}HCl$ and 1.5 mL dry toluene in a N_2 counter stream. The faint-yellow suspension was heated to 120 °C for 22 h until full conversion of the starting material was detected by TLC. The mixture was cooled to RT and treated with 2.5 mL 2 M HCl and 4 mL EtOAc. The suspension was stirred vigorously until all solid parts had dissolved. The phases were separated and the aqueous phase was extracted

with EtOAc (3 x 4 mL). The combined organic phases were dried over $MgSO_4$ and the solvent was removed in vacuum. The product was purified via column chromatography (4 g silica gel, size: 10 x 1 cm, cyclohexane/EtOAc/HCO_2H = 5:1:1% (v/v/v), 2 mL fractions).[225]

$C_{14}H_{16}BrN_5O_2$ [366.22]

yield: 31.5 mg (86.0 µmol, 55%) dirty-yellow solid.

R_f = 0.25 (cyclohexane/EtOAc/HCO_2H = 2:1:1% (v/v/v)) (254 nm, 366 nm, CAM: crimson).

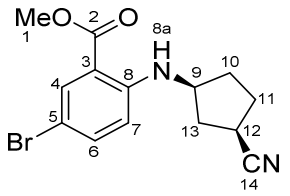
mp: 117-118 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.99 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-3), 7.48 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.0 Hz, 1H, H-5), 6.86 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 4.08–3.91 (m, 1H, H-8), 3.58 (s, 3H, H-14), 3.01–2.83 (m, 1H, H-11), 2.48–2.34 (m, 1H, H-12a), 2.18–2.01 (m, 1H, H-9a), 2.01–1.86 (m, 2H, H-10), 1.81–1.49 (m, 2H, H-9b, H-12b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.3 (C_q, C-13), 154.0 (C_q, C-1) 145.2 (C_q, C-7), 134.5 (C-5), 130.3 (C-3), 114.4 (C-6), 107.3 (C_q, C-4), 105.8 (C_q, C-2), 53.2 (C-8), 51.6 (C-14), 41.5 (C-11), 36.1 (C-12), 32.30 (C-9), 27.33 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}BrN_5O_2^+$: 365.0487; found: 365.0430.

6.6.2.2.1.18 Methyl 5-bromo-2-(((1*S**,3*R**)-3-cyanocyclopentyl)amino)benzoate (138)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 527 mg (2.29 mmol, 1.0 eq) methyl 2-amino-5-bromobenzoate (**4**), 3.0 mL dry CH_2Cl_2, 1.5 mL dry AcOH and 300 mg (2.75 mmol, 1.2 eq) 3-oxocyclopentane-1-carbonitrile (**133**) in a N_2 counter-stream. The faint-yellow solution was cooled to 0 °C and 4.6 mL (4.6 mmol, 2.0 eq) BH_3·THF (1.0 M solution in THF) were added over a period of 15 min. The turbid mixture was left stirring at 0 °C until full conversion of the starting material was detected after 25 h. The mixture was neutralized with saturated Na_2CO_3 solution (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography and only a clean fraction of the *cis*-isomer could be isolated via column chromatography (100 g silica gel, size: 10 x 4.5 cm, cyclohexane/EtOAc = 8:1, 7:1, 5:1, 20 mL fractions).[141,189]

$C_{14}H_{15}BrN_2O_2$ [323.19]

yield: 210 mg (650 µmol, 28%) yellow, amorphous solid.

R_f = 0.47 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, 366 nm, CAM: yellow).

mp: 100-105 °C.

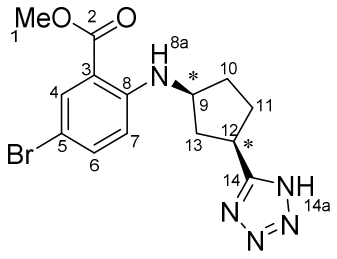
GC-MS: t_R = 8.477 min, *m/z* (%) = 324.1/322.1 (M^+, 100), 291.0/289.1 (53), 263.1 (64), 236.0 (58), 210.0 (38), 155.0 (35).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.01 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-4), 7.89 (d, $^3J_{HH}$ = 5.2 Hz, 1H, H-8a), 7.40 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-6), 6.50 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-7), 3.99–3.79 (m, 4H, H-1, H-9), 2.95–2.78 (m, 1H, H-12), 2.63–2.47 (m, 1H, H-13a), 2.21–2.05 (m, 3H, H-10a, H-11), 1.96–1.75 (m, 2H, H-10b, H-13b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 168.0 (C_q, C-2), 149.0 (C_q, C-8), 137.2 (C-6), 134.2 (C-4), 122.3 (C_q, C-14), 113.6 (C-7), 112.0 (C_q, C-5), 106.5 (C_q, C-3), 53.0 (C-9), 52.0 (C-1), 38.0 (C-13), 32.7 (C-10), 29.4 (C-11), 26.7 (C-14).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{15}BrN_2O_2^+$: 322.0317; found: 322.0312.

6.6.2.2.1.19 Methyl 2-(((1*S**,3*R**)-3-(1*H*-tetrazol-5-yl)cyclopentyl)amino)-5-bromobenzoate (137)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 50.0 mg (155 µmol, 1 eq) methyl 5-bromo-2-(((1*S**,3*R**)-3-cyanocyclopentyl)amino)benzoate (**138**), 20.3 mg (309 µmol, 2 eq) NaN_3, 42.5 mg (309 µmol, 2 eq) $Et_3N{\cdot}HCl$ and 1.5 mL dry toluene in a N_2 counter stream. The faint-yellow suspension was heated to 120 °C for 4 d (traces of substrate remain). The mixture was cooled to RT and treated with 2.5 mL 2 M HCl and 4 mL EtOAc, and stirred vigorously until all solid parts had dissolved. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 4 mL). The combined organic phases were dried over $MgSO_4$, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (4 g silica gel, size: 10 x 1 cm, cyclohexane/EtOAc/HCO_2H = 2:1:1% (v/v/v), 2 mL fractions).[225]

$C_{14}H_{16}BrN_5O_2$ [366.22]

yield: 27.5 mg (75.1 µmol, 48%) light-mauve solid.

R_f = 0.10 (cyclohexane/EtOAc/HCO_2H = 2:1:1% (v/v/v)) (254 nm, 366 nm, CAM: orange).

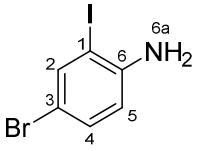
mp: 180-185 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.85 (d, $^4J_{HH}$ = 2.3 Hz, 1H, H-4), 7.73 (d, $^3J_{HH}$ = 6.6 Hz, 1H, H-8a), 7.52 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-6), 6.82 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-7), 4.15–3.98 (m, 1H, H-9), 3.79 (s, 3H, H-1), 3.59–3.44 (m, 1H, H-12), 2.74–2.59 (m, 1H, H-13a), 2.26–2.10 (m, 2H, H-10a, H-11a), 2.03–1.85 (m, 1H, H-11b), 1.83-1.54 (m, 2H, H-10b, H-13b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 167.0 (C_q, C-2), 159.1 (C_q, C-14), 149.0 (C_q, C-8), 137.1 (C-6), 132.9 (C-4), 114.7 (C-7), 110.8 (C_q, C-5), 104.9 (C-1), 52.7 (C-9), 51.9 (C-1), 38.4 (C-13), 32.4 (C-12), 32.2 (C-10), 29.6 (C-11).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{16}BrN_5O_2^+$: 365.0487; found: 365.0496.

6.6.2.2.1.20 4-Bromo-2-iodoaniline (141)



A 100 mL round-bottom flask with magnetic stirring bar was charged with 2.04 g (11.8 mmol, 1.0 eq) 4-bromoaniline and 30 mL glacial AcOH. The bright orange-yellow solution was stirred and 2.07 g (12.7 mmol, 1.05 eq) ICl in 10 mL glacial AcOH were added dropwise over a period of 25 min at RT. The reaction mixture was stirred at RT for 20 h until full conversion of the starting material was detected by TLC. The reaction mixture was poured on 100 mL H_2O and 6 mL aqueous $Na_2S_2O_3$ solution (50%). The reaction mixture was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic layers were dried over Na_2SO_4, filtrated, and the solvent was removed in vacuum. The product was purified via column chromatography (150 g silica gel, size: 27 x 6 cm, cyclohexane/Et_2O = 6:1 (v/v), fraction size: 20 mL).[286]

C_6H_5BrIN [297.92]

yield: 1.85 g (6.02 mmol, 52%) dark purple solid.

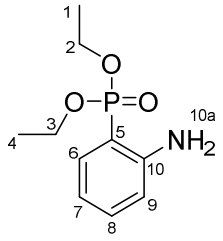
R_f = 0.59 (cyclohexane/EtOAc = 3:1) (UV 254 nm, CAM: purple).

mp = 64 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.73 (d, $^4J_{HH}$ = 2.1 Hz, 1H, H-6), 7.22 (dd, $^3J_{HH}$ = 8.5 Hz, 2.2 Hz, 1H, H-4), 6.61 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H-3), 4.10 (s, 2H, H-2a).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 146.1 (C_q, C-2), 140.5 (C-6), 132.2 (C-4), 115.7 (C-3), 110.0 (C_q, C-5), 84.2 (C_q, C-1).

6.6.2.2.1.21 Diethyl (2-aminophenyl)phosphonate (142)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 1.15 mL (10.0 mmol, 1.0 eq) 2-bromoaniline, 1.56 mL (12.0 mmol, 1.2 eq) diethylphosphite, 40 mL dry EtOH, 3.31 mL (15.0 mmol, 1.5 eq) Cy_2NMe, 159 mg (600 µmol, 0.06 eq) PPh_3, 44.9 mg (200 µmol, 0.02 eq) $Pd(OAc)_2$ and the orange solution was degassed for 20 min (N_2-bubbling, ultrasonic bath). The mixture was heated under reflux (oil bath 90 °C) until full conversion of the starting material was detected after 6 d. The reaction mixture was cooled to Rt, diluted with 300 mL EtOAc, washed with 1 M HCl (100 mL, 50 mL) and saturated aqueous $NaHCO_3$ solution (2 x 50 mL). The phases were separated and the organic phase was dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (250 g silica gel, size: 9 x 6 cm, cyclohexane/EtOAc = 1:1 (v/v), 20 mL fractions).[231]

$C_{10}H_{16}NO_3P$ [229.22]

yield: 1.41 g (6.14 mmol, 61%) orange-yellow liquid.

R_f = 0.29 (cyclohexane/EtOAc = 1:1 (v/v)) (254 nm, CAM: light-blue).

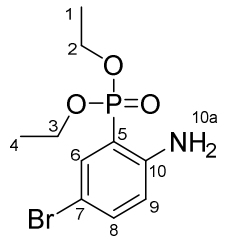
GC-MS: t_R = 6.178 min, *m/z* (%) = 229.1 (M^+, 52), 201.1 (27), 173.0 (43), 156.0 (24), 155.0 (100), 120.1 (19), 93.1 (18), 65.0 (20).

^{1}H-NMR (499.89 MHz, $CDCl_3$): δ = 7.49–7.40 (m, 1H, ArH), 7.30–7.22 (m, 1H, ArH), 6.74–6.62 (m, 2H, ArH), 5.16 (bs, 2H, NH_2), 4.20–3.98 (m, 4H, H-2, H-3), 1.38–1.28 (m, 6H, H-1, H-4).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 151.3 (d, $^2J_{CP}$ = 8.4 Hz, C-10), 134.0 (d, J = 2.3 Hz, CH_{arom}), 133.3 (d, J = 7.3 Hz, CH_{arom}), 117.0 (d, J = 13.9 Hz, CH_{arom}), 116.4 (d, J = 12.7 Hz, CH_{arom}), 108.2 (d, $^1J_{CP}$ = 183.6 Hz, C-5), 62.1 (d, $^2J_{CP}$ = 4.9 Hz, C-2, C-3), 16.4 (d, $^3J_{CP}$ = 6.6 Hz, C-1, C-4).

^{31}P-NMR (202.35 MHz, $CDCl_3$): δ = 21.23 (s).

6.6.2.2.1.22 Diethyl (2-amino-5-bromophenyl)phosphonate (140)



A 50 mL round-bottom flask with $CaCl_2$ drying tube was charged with 1.00 g (4.36 mmol, 1.0 eq) diethyl (2-aminophenyl)phosphonate (**142**) and 20 mL DMF. The yellow solution was stirred and 789 mg (4.36 mmol, 1.0 eq) NBS were added in small portions over a period of 25 min. Full conversion of the starting material was detected after 20 min (TLC) and the mixture was treated with 10 mL H_2O and 1 mL half-saturated $Na_2S_2O_3$ solution. The solvent was removed in vacuum, the residue was diluted with 50 mL H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The amber-brown, viscous liquid crude product was purified via column chromatography (100 mL silica gel, cyclohexane/EtOAc = 1:1 (v/v), 20 mL fractions). (Succinimide could not be fully separated via column chromatography, R_f-value seems to be identical).

$C_{10}H_{15}BrNO_3P$ [308.11]

yield: 1.23 g (3.99 mmol, 91%) light-yellow solid. (Succinimide impurity)

R_f = 0.42 (cyclohexane/EtOAc = 1:1 (v/v)) (254 nm, cold CAM: dark red).

mp: 65-68 °C.

GC-MS: t_R = 6.928 min, *m/z* (%) = 309.0/307.0 (M^+, 70), 281.0/279.1 (38), 252.9/250.9 (50), 234.9/232.9 (100), 207.0 (35).

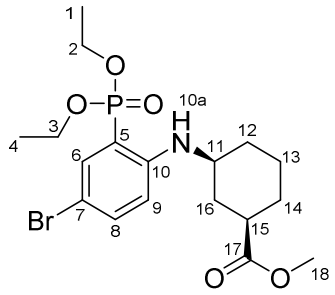
^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.51 (dd, $^3J_{HP}$ = 14.6 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, H-6), 7.31 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 1.9 Hz, 1H, H-8), 6.53 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HP}$ = 7.4 Hz, 1H, H-9), 5.20 (bs, 2H, NH), 4.23–3.97 (m, 4H, 2 x CH_2), 1.33 (t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 x CH_3).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 150.2 (d, $^2J_{CP}$ = 8.2 Hz, C-10), 136.6 (d, $^4J_{CP}$ = 2.2 Hz, C-8), 135.2 (d, $^2J_{CP}$ = 7.9 Hz, C-6), 118.2 (d, $^3J_{CP}$ = 13.6 Hz, C-9), 110.4 (d, $^1J_{CP}$ = 183.6 Hz, C-5), 108.3 (d, $^3J_{CP}$ = 18.2 Hz, C-7), 62.48, 62.42, 16.42, 16.33.

^{31}P-NMR (202.35 MHz, $CDCl_3$): δ = 18.76 (s).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{10}H_{15}BrNO_3P^+$: 306.9973; found: 306.9984.

6.6.2.2.1.23 Methyl (1*R**,3*S**)-3-((4-bromo-2-(diethoxyphosphoryl)phenyl)amino)cyclohexane-1-carboxylate (143)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 500 mg (1.62 mmol, 1.0 eq) diethyl (2-amino-5-bromophenyl)phosphonate (**140**), 2.8 mL dry CH_2Cl_2, 1.4 mL dry AcOH and 380 mg (2.43 mmol, 1.5 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**). The light-yellow solution was cooled to 0 °C and 4.1 mL (4.1 mmol, 2.5 eq) $BH_3 \cdot THF$ solution (1.0 M in THF) were added slowly over a period of 20 min. The reaction mixture was gradually warmed to RT and stirred for 6 h until GC-MS reaction control indicated full conversion of the starting material. The mixture was treated with distilled H_2O (5 mL) followed by saturated Na_2CO_3 solution (10 mL). The turbid, biphasic mixture was stirred for 15 h at RT. The phases were separated and the aqueous phase was extracted with EtOAc (5 x 5 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The yellowish, viscous crude product was purified via column chromatography (11 g silica gel, size: 20 x 1.3 cm, cyclohexane/EtOAc = 5:1 (v/v), 10 mL fractions). A clean fraction of the *cis*-isomer could be isolated.[141,189]

$C_{18}H_{27}BrNO_5P$ [448.29]

yield: 179 mg (399 µmol, 25%) colorless, viscous liquid.

R_f = 0.44 (cyclohexane/EtOAc = 2:1 (v/v)) (254 nm, 366 nm, CAM: yellow/orange).

GC-MS: t_R = 9.440 min, *m/z* (%) = 449.2/447.2 (100, M^+), 390.1/388.1 (73), 348.1/346.1 (75), 235.9 (33), 156.1 (35).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.50 (dd, *J* = 15.1 Hz, *J* = 2.2 Hz, 1H, ArH), 7.35 (dd, *J* = 8.9 Hz, *J* = 2.0 Hz, 1H, ArH), 6.66–6.47 (m, 2H, ArH, NH), 4.21–3.94 (m, 4H, H-2, H-3), 3.65 (s, 3H, H-18), 3.34–3.15 (m, 1H, H-11), 2.49–2.28 (m, 2H, H-15, CH_2), 2.17–1.84 (m, 3H, CH_2), 1.47–1.08 (m, 10H, H-1, H-4, CH_2).

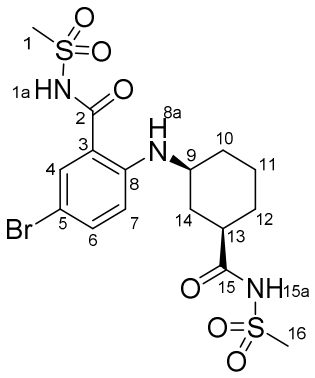
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.3 (C_q, C-17), 149.8 (d, $^2J_{CP}$ = 8.7 Hz, C_q, C-10), 136.8 (d, $^4J_{CP}$ = 2.1 Hz, C-8), 135.9 (d, $^2J_{CP}$ = 7.8 Hz, C-6), 113.4 (d, $^3J_{CP}$ = 13.2 Hz, C_q, C-9), 110.2 (d, $^1J_{CP}$ = 182.3 Hz, C_q, C-5), 106.6 (d, $^3J_{CP}$ = 18.5 Hz, C_q, C-7), 62.4 (d, $^2J_{CP}$ = 5.0 Hz, C-2,

C-3), 51.8 (C-18), 51.1 (C-11), 42.5 (C-15), 35.2 (C-16), 32.5 (C-12), 28.4 (C-14), 24.4 (C-13), 16.4 (d, $^{3}J_{CP}$ = 6.5 Hz, C-1, C-4).

^{31}P-NMR (202.35 MHz, $CDCl_3$): δ = 19.36 (s).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{18}H_{27}BrNO_5P^+$: 449.0792; found: 449.0800.

6.6.2.2.1.24 5-Bromo-*N*-(methylsulfonyl)-2-(((1*S**,3*R**)-3-((methylsulfonyl)carbamoyl)cyclohexyl)amino)benzamide (153)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 2.5 mL CH_2Cl_2 and 318 mg (929 µmol, 1.0 eq) 5-bromo-2-(((1*S**,3*R**)-3-carboxycyclohexyl)amino)benzoic acid (**1**). 247 mg (2.02 mmol, 2.3 eq) 4-DMAP and 196 mg (2.06 mmol, 2.3 eq) methanesulfonamide were added to the yellow, turbid mixture at RT. 389 mg (2.03 mmol, 2.3 eq) EDC·HCl were added and the mixture turned to an orange-yellow solution. The solution was stirred at RT and full conversion of the starting material was detected by TLC after 25 h. The reaction mixture was concentrated in vacuum and the crude product (brownish, viscous residue) was purified via column chromatography (22 x 1.8 cm, cyclohexane/EtOAc/HCOOH = 2:1:1% (v/v/v), fraction size: 20 mL). In order to obtain an analytically pure sample the product was purified via preparative RP-HPLC (Method_PREPHPLC).[234]

$C_{16}H_{22}BrN_3O_6S_2$ [496.39]

yield: 12.5 mg (25.2 µmol, 3%) dark-yellow oil.

R_f = 0.27 (cyclohexane/EtOAc/HCO_2H = 1:1:1% (v/v/v)) (254 nm, cold CAM: orange).

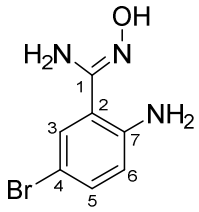
^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 7.77 (d, $^{4}J_{HH}$ = 2.1 Hz, 1H, H-4), 7.44 (dd, $^{3}J_{HH}$ = 9.0 Hz, $^{4}J_{HH}$ = 2.2 Hz, 1H, H-6), 6.79 (d, $^{3}J_{HH}$ = 9.1 Hz, 1H, H-7), 3.51–3.38 (m, 1H, H-9), 3.33 (s, 3H, CH_3), 3.22 (s, 3H, CH_3), 2.53–2.36 (m, 1H, H-13), 2.31–2.17 (m, 1H, H-14a), 2.15–2.03 (m, 1H, H-10a), 2.00–1.61 (m, 2H, H-11a, H-12a), 1.61–1.13 (m, 4H, H-10b, H-11b, H-12b, H-14b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 176.5 (C_q, C-15), 170.2 (C_q, C-2), 150.1 (C_q, C-8), 138.4 (CH_{arom}), 133.5 (CH_{arom}), 115.4 (CH_{arom}), 114.4 (C_q, C-3), 106.6 (C_q, C-5), 51.6 (C-9), 45.0 (C-13), 41.8 (CH_3), 41.3 (CH_3), 35.9 (C-14), 33.4 (C-10), 29.3 (C-12), 25.1 (C-11).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{16}H_{23}BrN_3O_6S_2^+$: 496.02; found: 496.0.

6.6.2.2.1.25 2-Amino-5-bromo-*N*'-hydroxybenzimidamide (157a)



A 150 mL Schlenk flask with magnetic stirring bar was charged with 2.00 g (10.2 mmol, 1 eq) 2-amino-5-bromobenzonitrile and 50 mL EtOH. A mixture of 3.26 g (30.5 mmol, 3 eq) Na_2CO_3, 12 mL H_2O and 1.42 g (20.3 mmol, 2 eq) $NH_2OH \cdot HCl$ were added to the light yellow suspension. The suspension was heated to 100 °C (bath) for 3 d (colorless suspension thickened considerably) until full conversion of the starting material was detected by TLC. The solvent was removed in vacuum and the colorless residue was suspended in distilled H_2O (60 mL). The mixture was stirred vigorously for 15 min and was filtrated through a Pore 4 sintered glass frit. The colorless solid was azeotropically dried with toluene (30 mL) and the product was dried in oil pump vacuum.[242]

$C_7H_8BrN_3O$ [230.07]

yield: 1.78 g (7.75 mmol, 76%) off-white solid.

R_f = 0.42 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, CAM: blue).

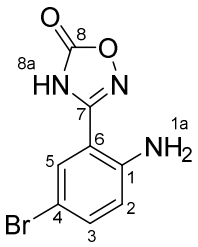
mp: 140-145 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 9.72 (s, 1H, OH), 7.50 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-3), 7.14 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 2.1 Hz, 1H, H-5), 6.63 (d, $^3J_{HH}$ = 8.7 Hz, 1H, H-6), 6.42 (s, 2H, NH_2), 5.82 (s, 2H, NH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 151.9 (C_q), 146.1 (C_q), 131.3 (C-5), 129.3 (C-3), 117.4 (C-6), 115.8 (C_q), 105.4 (C_q).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_7H_8BrN_3O^+$: 228.9851; found: 228.9845.

6.6.2.2.1.26 3-(2-Amino-5-bromophenyl)-1,2,4-oxadiazol-5(4*H*)-one (157)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 15 mL dry EtOH and 230 mg (10 mmol, 2.3 eq) Na (cyclohexane washed, cut into ~3 x 3 mm pieces) were slowly added. The mixture was stirred for 15 min until a clear solution was obtained. 1.00 g (4.35 mmol, 1 eq) 2-amino-5-bromo-*N*'-hydroxybenzimidamide (**157a**) was added followed by 2.12 mL (17.4 mmol, 4 eq) diethyl carbonate. The light-yellow solution was heated to 100 °C for 17 h until full conversion of the starting material was detected by TLC. The dirty-yellow solution with cloudy precipitate was transferred to a 100 mL round-bottom flask and the solvent was removed in vacuum. The light-red, solid residue was triturated with 0.5 N HCl (30 mL) and the beige solid was isolated via filtration and triturated with toluene (40 mL). The product was isolated via filtration and dried in oil pump vacuum (50 °C).[242]

$C_8H_6BrN_3O_2$ [256.06]

yield: 760 mg (2.97 mmol, 68%) light-beige, fluffy solid.

R_f = 0.66 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (254 nm, CAM: brown).

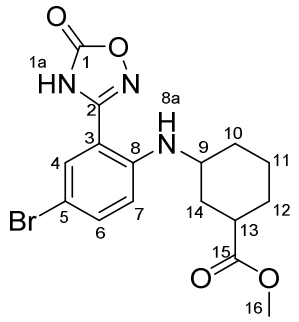
mp: 170-172 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.68 (d, $^4J_{HH}$ = 1.6 Hz, 1H, H-5), 7.35 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, H-3), 6.82 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 159.7/158.0 (C_q, C-7/C-8), 146.8 (C_q, C-1), 134.5 (C-3), 129.8 (C-5), 118.3 (C-2), 106.0/105.6 (C_q, C-4/C-6).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_8H_6BrN_3O_2^+$: 254.9643; found: 254.9644.

6.6.2.2.1.27 Methyl 3-((4-bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylate (158)

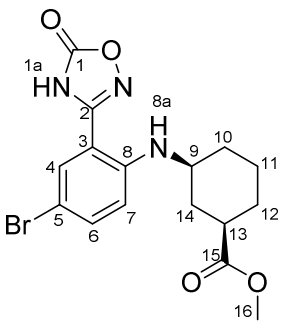


A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 300 mg (1.17 mmol, 1.0 eq) 3-(2-amino-5-bromophenyl)-1,2,4-oxadiazol-5(4*H*)-one (**157**), 800 µL dry DMF, 201 mg (1.29 mmol, 1.1 eq) methyl 3-oxocyclohexane-1-carboxylate (**38**) and 378 µL (2.93 mmol, 2.5 eq) TMSCl at RT. The dark-red, biphasic mixture was cooled to 0 °C and 1.17 mL (1.17 mmol, 1.0 eq) $BH_3 \cdot THF$ were added over a period of 5 min. The mixture was left stirring at 0 °C and full conversion of the starting material was detected after 50 min (TLC). The mixture was treated with 3 mL H_2O and 5 mL EtOAc and was left stirring at RT for 18 h. The mixture was neutralized with saturated aqueous Na_2CO_3 solution (3 mL) and extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over Na_SSO_4, filtrated and the solvents were removed in vacuum. The residue was adsorbed on Celite™ (1.25 g, MeOH) and the product was purified via column chromatography (size: 27 x 2 cm, cyclohexane/EtOAc/AcOH = 7:1:1%, 5:1:1% (v/v/v)). Pure isomer fractions could be obtained and were azeotropically dried by co-evaporation with benzene.[189]

$C_{16}H_{18}BrN_3O_4$ [396.24]

yield: 126 mg (319 µmol, 27%) light-yellow solid.

6.6.2.2.1.28 Methyl (1*R**,3*S**)-3-((4-bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylate (158)



R_f = 0.47 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, cold CAM: rust brown).

mp: 147-150 °C.

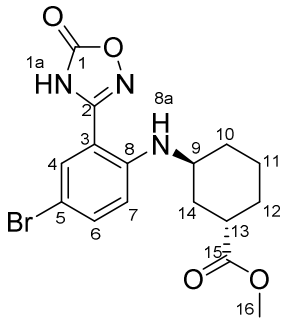
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.74 (d, $^{4}J_{HH}$ = 2.1 Hz, 1H, C-4), 7.48 (dd, $^{3}J_{HH}$ = 9.0 Hz, $^{4}J_{HH}$ = 2.0 Hz, 1H, C-6), 6.91 (d, $^{3}J_{HH}$ = 9.2 Hz, 1H, C-7), 6.60–6.45 (m, 1H, NH), 3.65–3.46 (m, 4H, H-9, H-16), 2.56–2.47 (m, 1H, H-13, DMSO overlap), 2.28–2.14 (m, 1H, CH_2), 2.05–1.73 (m, 3H, CH_2), 1.48–1.05 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 174.6 (C_q, C-15), 158.8 (C_q), 157.5 (C_q), 145.0 (C_q, C-8), 135.1 (C-6), 130.5 (C-4), 114.2 (C-7), 106.3 (C_q), 105.6 (C_q), 51.4 (C-16), 49.9 (C-9), 41.0 (C-13), 34.6 (C-14), 31.8 (C-10), 27.9 (C-12), 23.6 (C-11).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{16}H_{19}BrN_3O_4^+$: 396.06; found: 396.1.

6.6.2.2.1.29 Methyl (1*S**,3*S**)-3-((4-bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylate (158)



R_f = 0.50 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, cold CAM: rust brown).

mp: 85-90 °C.

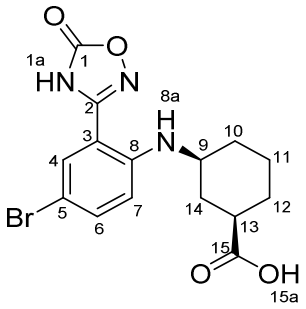
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.76 (s, 1H, ArH), 7.49 (d, $^{3}J_{HH}$ = 8.6 Hz, 1H, ArH), 6.93–6.64 (m, 2H, ArH, NH), 3.89–3.71 (m, 1H, H-9), 3.61 (s, 3H, H-16), 2.68–2.54 (m, 1H, H-13), 2.01–1.84 (m, 1H, CH_2), 1.84–1.40 (m, 7H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 174.9 (C_q, C-15), 159.2 (C_q), 158.1 (C_q), 144.9 (C_q, C-8), 135.1 (CH_{arom}), 130.5 (CH_{arom}), 113.9 (CH_{arom}), 106.7 (C_q), 105.6 (C_q), 51.5 (C-16), 46.9 (C-9), 38.0 (C-13), 31.9 (C-14), 30.1 (C-10), 27.3 (C-12), 20.6 (C-11).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{16}H_{19}BrN_3O_4^+$: 396.06; found: 396.0.

6.6.2.2.1.30 (1*R**,3*S**)-3-((4-Bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylic acid (159)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 46.8 mg (118 µmol, 1.0 eq) methyl (1*R**,3*S**)-3-((4-bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylate (**158**), 1.20 mL THF/H_2O (1:1, v/v) and 50.1 mg (1.18 mmol, 10 eq) LiOH·H_2O. The mixture was heated to 40 °C for 4 d until full conversion of the starting material was detected by TLC. The mixture was acidified with 6 N HCl and the solvent was removed in vacuum. The residue was dissolved in 1 mL DMSO and the product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{15}H_{16}BrN_3O_4$ [382.21]

yield: 23.1 mg (60.4 µmol, 51%) light-carnate, fluffy solid.

R_f = 0.30 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, cold CAM: brown).

mp: 138-140 °C.

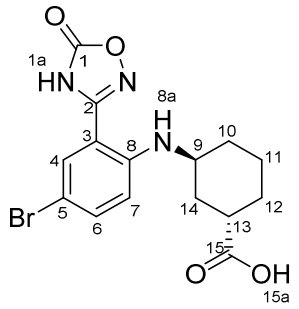
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.26 (bs, 2H, H_{acidic}), 7.74 (s, 1H, H-4), 7.46 (d, $^3J_{HH}$ = 8.2 Hz, 1H, H-6), 6.88 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-7), 6.61 (bs, 1H, NH), 3.59–3.39 (m, 1H, H-9), 2.48–2.31 (m, 1H, H-13), 2.30–2.12 (m, 1H, CH_2), 2.07–1.70 (m, 3H, CH_2), 1.55–1.01 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 175.9 (C_q, C-15), 159.7 (C_q), 158.3 (C_q), 145.0 (C_q), 134.9 (C-6), 130.5 (C-4), 114.1 (C-7), 106.9 (C_q), 105.5 (C_q), 50.1 (C-9), 41.3 (C-13), 34.9 (CH_2), 32.0 (CH_2), 28.1 (CH_2), 23.7 (CH_2).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}BrN_3O_4^+$: 382.04; found: 382.0.

6.6.2.2.1.31 (1*S**,3*S**)-3-((4-Bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylic acid (159)



A 10 mL round-bottom flask with magnetic stirring bar was charged with 32.4 mg (81.8 µmol, 1.0 eq) methyl (1*S**,3*S**)-3-((4-bromo-2-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)amino)cyclohexane-1-carboxylate (**158**), 900 µL THF/H_2O (1:1, v/v) and 34.7 mg (818 µmol, 10 eq) LiOH·H_2O. The mixture was heated to 40 °C for 3 d until full conversion of the starting material was detected by TLC. The mixture was acidified with 6 N HCl and the solvent was removed in vacuum. The residue was dissolved in 1 mL DMSO and the product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{15}H_{16}BrN_3O_4$ [382.21]

yield: 11.8 mg (30.9 µmol, 38%) colorless, amorphous solid.

R_f = 0.33 (cyclohexane/EtOAc/AcOH = 2:1:1% (v/v/v)) (254 nm, cold CAM: brown).

mp: 155-157 °C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.30 (bs, 2H, H_{acidic}), 7.78 (s, 1H, H-4), 7.45 (d, $^3J_{HH}$ = 8.9 Hz, 1H, ArH), 7.00–6.77 (m, 2H, ArH, NH), 3.90-3.68 (m, 1H, H-9), 2.57–2.44 (m, 1H, H-13, DMSO overlap), 2.03–1.40 (m, 8H, CH_2).

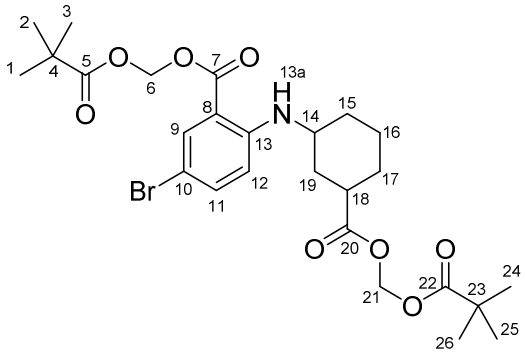
^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.2 (C_q, C-15), 161.2 (C_q), 159.6 (C_q), 144.9 (C_q), 134.6 (C-6), 130.5 (C-4), 113.7 (C-7), 108.0 (C_q), 105.4 (C_q), 46.9 (C-9), 38.0 (C-13), 32.0 (CH_2), 30.2 (CH_2), 27.4 (CH_2), 20.6 (CH_2).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}BrN_3O_4^+$: 382.04; found: 382.0.

6.6.2.2.2 Prodrug esters

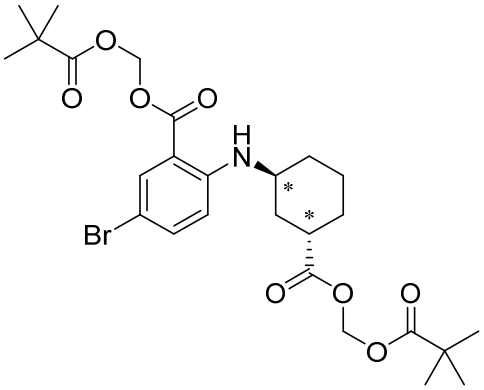
6.6.2.2.2.1 (Pivaloyloxy)methyl 5-bromo-2-((3-(((pivaloyloxy)methoxy)carbonyl)-cyclohexyl)amino)benzoate (170)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 150 mg (438 µmol, 1.0 eq) 5-bromo-2-((3-carboxycyclohexyl)amino)benzoic acid (**1**), 150 mg (1.40 mmol, 3.2 eq) Na_2CO_3 and 4 mL dry DMF. The dirty yellow suspension was cooled to 0 °C and 340 mg (1.40 mmol, 3.2 eq) iodomethyl pivalate (synthesized according to a literature procedure[245]) were added over a period of 2 min. The mixture was stirred at 0 °C for 150 min (TLC: incomplete conversion of the starting material) and further 170 mg (700 µmol, 1.6 eq) were added. The mixture was allowed to warm to RT and stirred for 17 h (TLC: incomplete). To the mixture were added another 74 mg (306 µmol, 0.70 eq) iodomethyl pivalate and the mixture was stirred for 3 h (TLC: traces of starting material remain). The reaction mixture was transferred to a vigorously stirred mixture of 30 mL EtOAc, 30 mL H_2O and 8 mL saturated aqueous Na_2SO_3 solution. The yellow biphasic mixture was stirred for 15 min, the phases were separated and the aqueous layer was extracted with EtOAc (4 x 20 mL), the combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The residue was adsorbed on Celite™ (420 mg, EtOAc) and the product was purified via column chromatography (size: 30 x 1.1 cm, cyclohexane/EtOAc = 20:1 (v/v), 8 mL fractions).[245]

$C_{26}H_{36}BrNO_8$ [570.48]

6.6.2.2.2.2 (Pivaloyloxy)methyl 5-bromo-2-(((1*S**,3*S**)-3-(((pivaloyloxy)methoxy)carbonyl)cyclohexyl)amino)benzoate (170)



yield: 26.9 mg (47.2 µmol, 11%) yellow, very viscous liquid.

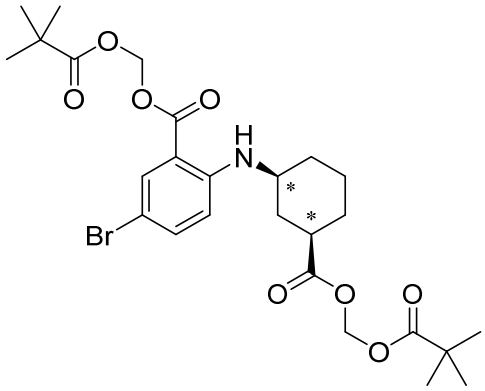
R_f = 0.44 (cyclohexane/EtOAc = 6:1 (v/v)) (254 nm, 366 nm, cold CAM: dark red).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (d, $^4J_{HH}$ = 2.2 Hz, 1H, H-9), 7.76 (d, $^3J_{HH}$ = 6.1 Hz, 1H, H-13a), 7.43 (dd, $^3J_{HH}$ = 9.1 Hz, $^5J_{HH}$ = 2.1 Hz, 1H, H-11), 6.70 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-12), 5.94 (s, 2H, CH_2), 5.78 (dd, $^3J_{HH}$ = 13.1 Hz, $^3J_{HH}$ = 5.4 Hz, 2H, CH_2), 3.82–3.65 (m, 1H, H-14), 2.83–2.67 (m, 1H, H-18), 2.20–2.06 (m, 1H, H-19a), 1.93–1.48 (m, 7H, H-15, H-16, H-17, H-19b), 1.30–1.11 (m, 18H, H-1/2/3, H-24/25/26).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 177.4 (C_q), 177.3 (C_q), 174.0 (C_q, C-20), 166.3 (C_q, C-7), 149.6 (C_q, C-13), 138.2 (C-11), 134.2 (C-9), 114.0 (C-12), 110.0 (C_q), 105.9 (C_q), 79.6 (CH_2), 79.5 (CH_2), 47.2 (C-14), 39.0 (C_q), 38.9 (C_q), 38.8 (C-18), 32.3 (C-19), 31.3 (C-15), 27.8 (C-17), 27.04 (CH_3), 27.00 (CH_3), 21.2 (C-16).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{26}H_{36}BrNO_8^+$: 571.1608; found: 571.1652.

6.6.2.2.2.3 (Pivaloyloxy)methyl 5-bromo-2-(((1*S**,3*R**)-3-(((pivaloyloxy)methoxy)carbonyl)cyclohexyl)amino)benzoate (170)



yield: 17.9 mg (31.4 µmol, 7%) yellow, very viscous liquid.

R_f = 0.44 (cyclohexane/EtOAc = 6:1 (v/v)) (254 nm, 366 nm, cold CAM: dark red).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.93 (d, $^4J_{HH}$ = 2.1 Hz, 1H, H-9), 7.57 (d, $^3J_{HH}$ = 6.3 Hz, 1H, H-13a), 7.33 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.0 Hz, 1H, H-11), 6.52 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-12), 5.86 (s, 2H, H-6), 5.68 (s, 2H, H-21), 3.38–3.19 (m, 1H, H-14), 2.50–2.34 (m, 1H, H-18), 2.34–2.21 (m, 1H, H-19a), 2.11–1.99 (m, 1H, H-15a), 1.99–1.80 (m, 2H, H-16a, H-17a), 1.43–0.97 (m, 22H, H-1/2/3, H-24/25/26, H-15b, H-16b, H-17b, H-19b).

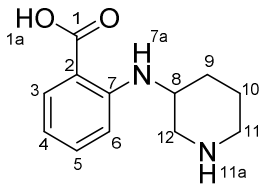
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 177.4 (C_q), 177.3 (C_q), 173.4 (C_q, C-20), 166.2 (C_q, C-7), 149.4 (C_q, C-13), 138.0 (C-11), 134.4 (C-9), 113.7 (C-12), 110.2 (C_q), 106.0 (C_q), 79.6 (CH_2), 79.5 (CH_{arom}), 50.7 (C-14), 42.3 (C-18), 39.0 (C_q), 38.9 (C_q), 34.8 (C-19), 32.5 (C-15), 28.2 (C-17), 27.04 (CH_3), 26.99 (CH_3), 24.3 (C-16).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{26}H_{36}BrNO_8^+$: 571.1608; found: 571.1605.

6.6.2.3 Synthesis of Maverick derivatives as PhzA/B inhibitors

6.6.2.3.1 H-Maverick

6.6.2.3.1.1 2-(Piperidin-3-ylamino)benzoic acid (177)



Step 1: A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 50.0 mg (200 µmol, 1.0 eq) 2-iodobenzoic acid, 2.0 mg (10 µmol, 0.05 eq) CuI and 130 mg (400 µmol, 2.0 eq) Cs_2CO_3. The flask was evacuated and backfilled with N_2 (this procedure was repeated three times). To the solids were added 500 µL dry DMF followed by 52.1 mg (260 µmol, 1.3 eq) *tert*-butyl 3-aminopiperidine-1-carboxylate and 6.7 mg (10 µmol, 0.2 eq) 2-isobutyrylcyclohexan-1-one. The dark-blue solution was heated to 70 °C for 3 h until full conversion of the starting material was detected by TLC. The green solution was cooled to RT, diluted with EtOAc (3 mL) and the colorless precipitate was removed by centrifugation. The solvent was removed with a rotary evaporator and the residue was dried in oil pump vacuum. The intermediary product was purified via column chromatography (10 g silica gel, size: 12 x 1 cm, cyclohexane/EtOAc/AcOH = 300:100:1 (v/v/v)). The combined fractions (not NMR pure) were subjected to the following step without further purification.

Step 2: A 10 mL round-bottom flask with magnetic stirring bar was charged with the product from the previous step and 1.2 mL CH_2Cl_2 and 30 µL anisole were added followed by 300 µL TFA (the solution turned light orange) at RT and the mixture was stirred for 3 d until full conversion of the starting material was detected by TLC. The solution was basified with 5 mL saturated aqueous Na_2CO_3 and 5 mL half-saturated KOH and extracted with EtOAc followed by CH_2Cl_2. The product could not be extracted from the aqueous phase. The solvents were removed in vacuum and the residue was slurried in 20 mL MeOH and the solids were removed via filtration through a pad of silica gel (3 cm). The solvent was removed in vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).[198,249]

$C_{12}H_{16}N_2O_2$ [220.27]

yield: 8.5 mg (38.6 µmol, 19% over 2 steps) colorless sticky solid.

R_f = 0.14 (CH_2Cl_2/MeOH/Et_3N = 6:1:1% (v/v/v)) (265 nm, CAM: red).

^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 8.38 (bs, 1H, NH), 7.92 (d, $^3J_{HH}$ = 7.8 Hz, 1H, CH_{arom}), 7.35 (t, $^3J_{HH}$ = 7.8 Hz, 1H, CH_{arom}), 6.79 (d, $^3J_{HH}$ = 8.3 Hz, 1H, CH_{arom}), 6.65 (t, $^3J_{HH}$ = 7.5 Hz, 1H, CH_{arom}), 3.93–3.77 (m, 1H, H-8), 3.53–3.41 (m, 1H, H-12a), 3.37–3.29 (m, 1H, H-11a),

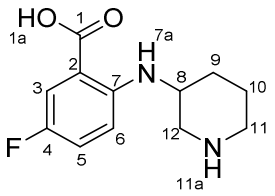
3.15–2.99 (m, 1H, H-11b), 2.99–2.84 (m, 1H, H-12b), 2.27–2.01 (m, 2H, H-9a, H-10a), 2.01–1.80 (m, 1H, H-10b), 1.80–1.60 (m, 1H, H-9b).

^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 173.2 (C_q, C-1), 150.4 (C_q, C-7), 135.2 (CH_{arom}), 133.5 (CH_{arom}), 116.8 (CH_{arom}), 114.7 (C_q, C-2), 112.3 (CH_{arom}), 48.3 (C-12), 47.3 (C-8), 45.0 (C-11), 30.0 (C-9), 21.9 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{12}H_{16}N_2O_2^+$: 220.1212; found: 220.1207.

6.6.2.3.2 F-Maverick

6.6.2.3.2.1 5-Fluoro-2-(piperidin-3-ylamino)benzoic acid (180)



Step 1: A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 85.0 mg (500 µmol, 1 eq) methyl-2-amino 5-fluorobenzoate, 2 mL CH_2Cl_2, 1 mL AcOH and 199 mg (1.00 mmol, 2 eq) *tert*-butyl 3-oxopiperidine-1-carboxylate in a N_2 counter-stream. The stirred solution was cooled to 0 °C and 2.00 mL (2.00 mmol; 4 eq) BH_3·THF solution (1.0 M in THF) was added dropwise over a period of 10 min. The solution was left stirring in the thawing ice bath and full conversion of the starting material was detected after 144 h (GC-MS, TLC showed very similar R_f-values of substrate and product). The reaction mixture was neutralized with 5 mL saturated aqueous $NaHCO_3$ solution and 10 mL EtOAc were added. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The crude product was used in the following step without further purification.[141,189]

Step 2: A 50 mL round-bottom flask with magnetic stirring bar was charged with the crude *tert*-butyl 3-((4-fluoro-2-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate from the previous step, 2 mL distilled THF, 2 mL distilled H_2O and 42.0 mg (1.00 mmol, 2 eq) LiOH·H_2O. The reaction mixture was heated to 40 °C and stirred for 48 h until full conversion of the starting material was detected by HPLC-MS. The solvent was removed in vacuum and the crude product was used in the next step.

Step 3: A 50 mL round-bottom flask with magnetic stirring bar was charged with the crude 5-fluoro-2-((1-(*tert*-butoxycarbonyl)piperidin-3-yl)amino)benzoic acid from the previous step, 3 mL 1,4-dioxane and 3 mL 3 N HCl. The reaction mixture was stirred for 96 h at RT until full

conversion of the starting material was detected by TLC. The solvent was removed in vacuum and the light-brown residue was dissolved in 1.0 mL warm DMSO. The dissolved product was purified via preparative RP-HPLC (Method_PREPHPLC).[248]

$C_{12}H_{15}FN_2O_2$ [238.11]

yield: 49 mg (206 µmol, 41% over 3 steps) light brown solid.

R_f = 0.20 (MeOH/EtOAc = 3:1% (v/v)) (265 nm, CAM: light red).

mp: 172°C.

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.64–7.48 (m, 1H, ArH), 7.08–6.94 (m, 1H, ArH), 6.78–6.64 (m, 1H, ArH), 3.77–3.59 (m, 1H, H-8), 3.43–3.28 (m, 1H, CH_2), 3.28–3.12 (m, 1H, CH_2), 2.90–2.75 (m, 1H, CH_2), 2.69–2.55 (m, 1H, CH_2), 2.10–1.95 (m, 1H, CH_2), 1.93–1.65 (m, 2H, CH_2), 1.57–1.33 (m, 1H, CH_2).

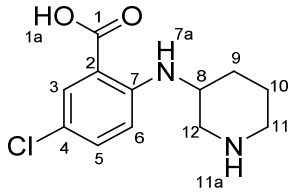
^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 170.9 (C_q, C-1), 152.7 (d, $^1J_{CF}$ = 230.5 Hz, C-4), 145.5 (C_q, C-7), 119.0 (C_q, d, $^3J_{CF}$ = 4.3 Hz, C-2), 118.3 (d, $^2J_{CF}$ = 22.2 Hz, CH_{arom}), 117.3 (d, $^2J_{CF}$ = 21.6 Hz, CH_{arom}), 111.8 (d, $^1J_{CF}$ = 6.6 Hz, CH_{arom}), 47.0 (CH_2), 46.4 (CH), 42.7 (CH_2), 29.5 (CH_2), 21.5 (CH_2).

^{19}F-NMR (470.35 MHz, DMSO-d_6): δ = -130.48 (m).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{12}H_{15}FN_2O_2^+$: 238.1118; found: 238.1114.

6.6.2.3.3 Cl-Maverick

6.6.2.3.3.1 5-Chloro-2-(piperidin-3-ylamino)benzoic acid (181)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 212 mg (750 µmol, 1.0 eq) 5-chloro-2-iodobenzoic acid (**60**), 150 mg (750 mg, 1.0 eq) *tert*-butyl 3-aminopiperidine-1-carboxylate, 7.2 mg (38.0 µmol, 0.05 eq) CuI, 25.4 mg (150 µmol, 0.2 eq) 2-isobutyrylcyclohexan-1-one, 237 mg (2.50 mmol, 2.0 eq) *tert*-butyl acetoacetate and 1.00 mL dry DMF. The flask was sealed and the ink-blue reaction mixture (solution) was heated to 70 °C for 4 d. The solvent was removed in vacuum, the residue was treated with 1 M HCl (3 mL) and extracted with EtOAc (3 x 3 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The brown residue was dissolved in 2 mL MeOH and was treated with 6 M HCl (5 mL). The suspension was kept

stirring at RT for 20 h until full conversion of the starting material was detected by TLC. The solvents were removed in vacuum, the residue was adsorbed on Celite™ (500 mg, MeOH) and purified via column chromatography (30 g silica gel, EtOAc/MeOH/Et_3N = 7:1:1% to 1:3:1% (v/v/v)).

$C_{12}H_{15}ClN_2O_2$ [254.71]

yield: 28.6 mg (112 µmol, 15%) light-brown, amorphous sticky solid.

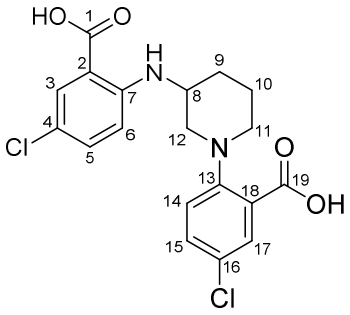
R_f = 0.13 (EtOAc/MeOH/Et_3N = 1:1:1% (v/v/v)) (254 nm).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 9.09 (bs, 1H, NH), 7.77 (d, $^4J_{HH}$ = 2.6 Hz, 1H, H-3), 7.13 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.6 Hz, 1H, H-5), 6.68 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-6), 3.70–3.54 (m, 1H, H-8), 3.36–3.23 (m, 1H, CH_2), 3.20–3.07 (m, 1H, CH_2), 2.88–2.70 (m, 1H, CH_2), 2.68–2.55 (m, 1H, CH_2), 2.07–1.93 (m, 1H, CH_2), 1.92–1.59 (m, 2H, CH_2), 1.55–1.34 (m, 1H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 170.4 (C_q, C-1), 147.5 (C_q, C-7), 131.1 (CH_{arom}), 130.6 (CH_{arom}), 120.5 (C_q), 117.4 (CH_{arom}), 112.2 (C_q), 47.4, 46.4, 43.1, 29.5, 21.8.

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{12}H_{15}ClN_2O_2^+$: 254.0822; found: no mass could be detected with DI-EI.

6.6.2.3.3.2 2-(3-((2-Carboxy-4-chlorophenyl)amino)piperidin-1-yl)-5-chlorobenzoic acid (182)



A dry 15 mL Schlenk flask with magnetic stirring bar was charged with 37.7 mg (220 µmol, 2.2 eq) 5-chloro-2-iodobenzoic acid (**60**), 157 mg (250 µmol, 2.5 eq) TBAA, 2.3 mg (20.0 µmol, 0.2 eq) L-proline, 1.9 mg (10.0 µmol, 0.1 eq) CuI, 2.0 mL dry DMF followed by 10.0 mg (100 µmol, 1.0 eq) 3-aminopiperidine. The blue-turquoise solution was heated to 70 °C for 4 d until full conversion of the starting material was detected by TLC. The DMF was removed in oil pump vacuum, the residue was treated with EtOAc (10 mL) and H_2O (10 mL) and the mixture was acidified with 6 N HCl (pH = 1). The phases were separated, the aqueous layer was extracted with EtOAc (3 x 10 mL), the combined organic layers were

concentrated in vacuum and the residue was further dried in oil pump vacuum. The product was purified via preparative RP-HPLC (Method_PREPHPLC).[197]

$C_{19}H_{18}Cl_2N_2O_4$ [409.26]

yield: 18.0 mg (44.0 µmol, 44%) dirty-yellow solid.

mp: 111-114 °C (decomp.).

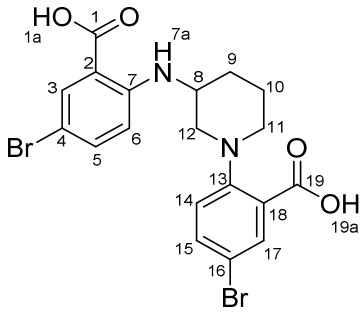
R_f = 0.44 (EtOAc/MeOH/AcOH = 200:100:1) (UV 254 nm & 366 nm, CAM: red).

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 15.70 (bs, 1H), 13.06 (bs, 1H), 12.10 (bs, 2H, H-1a, H-19a), 7.98 (d, $^3J_{HH}$ = 6.6 Hz, 1H), 7.81 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-17), 7.72 (d, $^4J_{HH}$ = 2.6 Hz, 1H, H-3), 7.63 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 2.5 Hz, 1H, H-15), 7.54 (d, $^3J_{HH}$ = 8.8 Hz, 1H, H-14), 7.36 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.6 Hz, 1H, H-5), 6.89 (d, $^3J_{HH}$ = 9.2 Hz, 1H, H-6), 3.86-3.68 (m, 1H, CH_2N), 3.51-3.19 (m, 2H, CH_2N, CHN), 3.17-3.03 (m, 1H, CH_2N), 3.02-2.74 (m, 1H, CH_2N), 2.12-1.67 (m, 4H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 169.0 (C-1), 166.2 (C-19), 149.8 (C-13), 148.4 (C-7), 134.1 (C-5), 133.0 (C-15), 130.7 (C-3), 130.1 (C-17), 129.1 (C-16), 126.6 (C-18), 124.3 (C-14), 117.8 (C-4), 113.7 (C-6), 111.6 (C-2), 56.7 (C-12), 52.8 (C-11), 48.1 (C-8), 29.3 (C-9), 23.4 (C-10).

6.6.2.3.4 Br-Maverick

6.6.2.3.4.1 5-Bromo-2-(3-((4-bromo-2-carboxyphenyl)amino)piperidin-1-yl)benzoic acid (187)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 531 mg (1.63 mmol 2.5 eq) 5-bromo-2-iodobenzoic acid, 5.0 mg (25 µmol, 0.04 eq) CuI, 326 mg (1.00 mmol, 1.5 eq) Cs_2CO_3 and 17.0 mg (100 µmol, 0.15 eq) 2-isobutyrylcyclohexan-1-one in a N_2 counter-stream. 100 mg (650 µmol, 1.0 eq) piperidin-3-amine and 1.00 mL DMF were added via syringe. The dark-blue reaction mixture was stirred at 70 °C for 72 h until full conversion of the starting material was detected by TLC. The black mixture was diluted with 5 mL EtOAc (colorless precipitate) and filtered through a pad of Celite™ (3 cm). The solvent was removed

in vacuum. The black, viscous liquid crude product was diluted with 0.9 mL DMSO and purified via preparative RP-HPLC (Method_PREPHPLC).[198]

$C_{19}H_{18}Br_2N_2O_4$ [498.17]

yield: 5.6 mg (11 µmol, 2%) colorless solid.

R_f = 0.28 (cyclohexane/EtOAc/AcOH = 1:1:1% (v/v/v)) (265 nm, CAM: red).

mp: 161-163°C.

^{1}H-NMR (300.36 MHz, MeOD-d_4): δ = 8.20 (d, $^4J_{HH}$ = 2.2 Hz, 1H, CH_{arom}), 7.96 (d, $^4J_{HH}$ = 2.3 Hz, 1H, CH_{arom}), 7.77 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, CH_{arom}), 7.55 (d, $^3J_{HH}$ = 8.6 Hz, 1H, CH_{arom}), 7.41 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, CH_{arom}), 6.76 (d, $^3J_{HH}$ = 9.1 Hz, 1H, CH_{arom}), 3.96–3.80 (m, 1H, H-8), 3.47–3.36 (m, 1H, H-12a), 3.28–3.05 (m, 2H, H-11), 3.05–2.90 (m, 1H, H-12b), 2.29–1.85 (m, 3H, H-9a, H-10), 1.77–1.56 (m, 1H, H-9b).

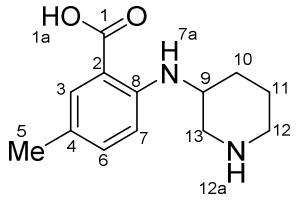
^{13}C-NMR (75.53 MHz, MeOD-d_4): δ = 170.9/168.9 (C_q, C-1/C-19), 150.1/149.9 (C_q, C-7/C-13), 138.1 (CH_{arom}), 137.8 (CH_{arom}), 135.5 (CH_{arom}), 135.3 (CH_{arom}), 128.1 (C_q), 125.7 (CH_{arom}), 121.6 (C_q), 114.6 (CH_{arom}), 114.1 (C_q), 107.2 (C_q), 58.5 (C-12), 55.1 (C-11), 49.8 (C-8), 30.5 (C-9), 24.7 (C-10).

HR-MS (DI-EI): no product mass could be detected.

ESI-MS (coupled with HPLC): *m/z* $[M+H]^+$ calcd for $C_{19}H_{19}Br_2N_2O_4^+$: 499.18; found: 499.0.

6.6.2.3.5 Me-Maverick

6.6.2.3.5.1 5-Methyl-2-(piperidin-3-ylamino)benzoic acid (191)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 157 mg (600 µmol, 1.0 eq) 2-iodo-5-methylbenzoic acid, 7.2 mg (38.0 µmol, 0.05 eq) CuI, 1.00 mL dry DMF, 25.4 mg (150 µmol, 0.2 eq) 2-isobutyrylcyclohexan-1-one, 237 mg (2.50 mmol, 2.0 eq) *tert*-butyl acetoacetate and 150 mg (750 mg, 1.0 eq) *tert*-butyl 3-aminopiperidine-1-carboxylate. The flask was sealed and the dark-blue/green reaction mixture (solution) was heated to 70 °C for 4 d (full conversion of the starting material). The solvent was removed in vacuum, the residue treated with 1 M HCl (3 mL) and extracted with EtOAc (3 x 3 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum.

The oily residue was dissolved in 2 mL MeOH and 5 mL 6 M HCl were added. The mixture was stirred at RT for 8 d. The solvent was removed in vacuum and the residue was adsorbed on 500 mg Celite™ (MeOH). The product was purified via column chromatography (30 g silica gel, EtOAc/MeOH/Et_3N = 7:1:1% to 1:3:1% (v/v/v)).

$C_{13}H_{18}N_2O_2$ [234.30]

yield: 17.3 mg (73.8 µmol, 12%) light-brown, amorphous solid/oil.

R_f = 0.08 (EtOAc/MeOH/Et_3N = 5:1:1% (v/v/v)) (254 nm).

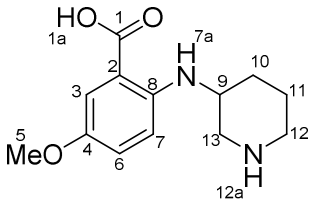
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 8.38 (bs, 1H, NH), 7.64 (s, 1H, H-3), 7.05 (d, $^3J_{HH}$ = 7.3 Hz, 1H, H-6), 6.65 (d, $^3J_{HH}$ = 8.4 Hz, 1H, H-7), 3.66–3.51 (m, 1H, H-9), 3.33–3.20 (m, 1H, CH_2), 3.12–2.98 (m, 1H, CH_2), 2.85–2.63 (m, 1H, CH_2), 2.60-2.43 (m, DMSO-overlap, CH_2), 2.16 (s, 1H, H-5), 2.06–1.91 (m, 1H, CH_2), 1.89–1.56 (m, 2H, CH_2), 1.56–1.33 (m, 1H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 171.2 (C_q, C-1), 147.1 (C_q, C-8), 133.1 (CH_{arom}), 132.0 (CH_{arom}), 122.3, 111.2, 48.6, 47.0, 43.8, 29.9 (CH_2), 22.6 (CH_2), 19.9 (C-5).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{18}N_2O_2^+$: 234.1368; found: 234.1373.

6.6.2.3.6 MeO-Maverick

6.6.2.3.6.1 5-Methoxy-2-(piperidin-3-ylamino)benzoic acid (193)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 50.0 mg (216 µmol, 1.0 eq) 2-bromo-5-methoxybenzoic acid, 340 mg (541 µmol, 2.5 eq) tetrabutylammonium adipate, 5.0 mg (43.3 µmol, 0.2 eq) L-proline, 4.2 mg (21.6 µmol, 0.1 eq) CuI and 4.0 mL dry DMF followed by 65.0 mg (325 µmol, 1.5 eq) *tert*-butyl 3-aminopiperidine-1-carboxylate. The dark-turquoise, blue solution was heated to 70 °C (bath) for 4 d until full conversion of the starting material was detected by TLC. The solvent was removed in oil pump vacuum and the residue was treated with H_2O (10 mL) and EtOAc (10 mL). The brown mixture was acidified to pH = 1 with 6 N HCl, extracted with EtOAc (3 x 10 mL) and the combined organic phases were concentrated in vacuum. The residue was transferred into a 10 mL round-bottom flask with magnetic stirring bar, dissolved in 2 mL CH_2Cl_2 and treated with 2 mL TFA. The color of the solution changed to a deep red-brown and the mixture was left stirring at RT

for 17 h until full conversion of the starting material was detected by TLC. The liquid constituents were removed in oil pump vacuum and the residue was purified via preparative RP-HPLC (Method_PREPHPLC).[197]

$C_{13}H_{18}N_2O_3$ [250.30]

yield: 10.2 mg (40.8 µmol, 19%) light-yellow, amorphous sticky solid.

R_f = 0.12 (EtOAc/MeOH/Et_3N = 1:1:1% (v/v/v)) (254 nm, 366 nm, cold CAM: carnate).

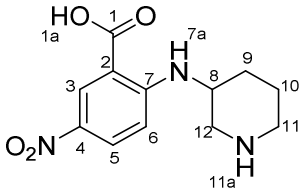
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.36 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-3), 7.03 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, H-6), 6.78 (d, $^3J_{HH}$ = 9.0 Hz, 1H, H-7), 3.78–3.62 (m, 4H, H-5, H-9), 3.41–3.15 (m, 2H, CH_2), 2.91–2.62 (m, 2H, CH_2), 2.10–1.96 (m, 1H, CH_2), 1.96–1.62 (m, 2H, CH_2), 1.62–1.40 (m, 1H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 170.2 (C_q, C-1), 149.2 (C_q), 144.0 (C_q), 121.7 (CH_{arom}), 115.1 (CH_{arom}), 112.9 (CH_{arom}), 55.4, 46.8 (CH_2), 46.2, 42.9 (CH_2), 29.1 (CH_2), 21.2 (CH_2).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{13}H_{18}N_2O_3^+$: 250.1317; found: 250.1320.

6.6.2.3.7 NO_2-Maverick

6.6.2.3.7.1 5-Nitro-2-(piperidin-3-ylamino)benzoic acid (194)



A 50 mL round-bottom flask with magnetic stirring bar was charged with 100 mg (274 □mol) 2-((1-(*tert*-butoxycarbonyl)piperidin-3-yl)amino)-5-nitrobenzoic acid (prepared according to Buchwald et al. [198] from 2-iodo-5-nitrobenzoic acid and used without further purification) and 2 mL 3 M HCl/1,4-dioxane (1:1 (v/v)) were added in one portion. The yellow solution was stirred at RT and the formation of a colorless precipitate was observed. Full conversion of the starting material was observed after 16 h and the solvents were removed in vacuum. The residue was dissolved in DMSO and the product was purified via preparative RP-HPLC (Method_PREPHPLC).

$C_{12}H_{15}N_3O_4$ [265.27]

yield: 57.7 mg (188 µmol, 58%) yellow solid.

R_f = 0.47 14 (CH_2Cl_2/MeOH/Et_3N = 4:1:1% (v/v/v)) (VIS: yellow).

mp: 265°C (decomp.).

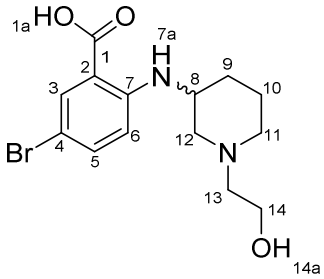
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 9.40 (bs, 2H, H-11a), 8.71 (d, $^{3}J_{HH}$ = 7.8 Hz, 1H, H-3), 8.62 (d, $^{4}J_{HH}$ = 2.7 Hz, 1H, H-7a), 8.19 (dd, $^{3}J_{HH}$ = 9.4 Hz, $^{4}J_{HH}$ = 2.5 Hz, 1H, H-5), 7.06 (d, $^{3}J_{HH}$ = 9.5 Hz, 1H, H-6), 4.25–4.03 (m, 1H, H-8), 3.40–3.15 (m, 2H, CH_2), 2.96–2.74 (m, 2H, CH_2), 2.14–1.99 (m, 1H, CH_2), 1.95–1.80 (m, 2H, CH_2), 1.75–1.57 (m, 1H, CH_2).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 168.6 (C_q, C-1), 153.5 (C_q, C-7), 135.4 (C_q, C-4), 129.5 (C-5), 128.5 (C-3), 111.9 (C-6), 109.8 (C_q, C-2), 45.9 (C-8), 45.5 (CH_2), 42.5 (CH_2), 28.2 (CH_2), 20.7 (CH_2).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{12}H_{15}N_3O_4^+$: 265.1063; found: 265.1064.

6.6.2.3.8 Hydroxyethyl-Maverick

6.6.2.3.8.1 5-Bromo-2-((1-(2-hydroxyethyl)piperidin-3-yl)amino)benzoic acid (198)



Step 1: A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 115 mg (500 µmol, 1 eq) methyl-2-amino 5-bromobenzoate, 2 mL CH_2Cl_2, 1 mL AcOH and 199 mg (1.00 mmol, 2 eq) *tert*-butyl 3-oxopiperidine-1-carboxylate in a N_2 counter-stream. The stirred solution was cooled to 0 °C and 2.00 mL (2.00 mmol; 4 eq) BH_3·THF solution (1.0 M in THF) were added dropwise over a period of 10 min to the cooled solution. The stirred solution was left stirring in the thawing ice bath until full conversion of the starting material was detected by GC-MS after 144 h. The reaction mixture was neutralized with 5 mL saturated $NaHCO_3$ solution and 10 mL EtOAc were added. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The crude product was used in the following step without further purification.[141,189]

Step 2:

A 50 mL round-bottom flask with magnetic stirring bar was charged with the crude *tert*-butyl 3-((4-bromo-2-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate from the previous step and 2 mL THF, 2 mL H_2O and 42.0 mg (1.00 mmol, 2 eq) of LiOH·H_2O were added. The reaction mixture was heated to 40 °C and stirred for 48 h until full conversion of the starting

material was detected by HPLC-MS. The solvent was removed in vacuum and the crude product was used in the following step without further purification.

Step 3:

A 50 mL round-bottom flask with magnetic stirring bar was charged with the crude 5-bromo-2-((1-(*tert*-butoxycarbonyl)piperidin-3-yl)amino)benzoic acid from the previous step, 3 mL 1,4-dioxane and 3 mL 3 N HCl. The reaction mixture was stirred for 96 h at RT until full conversion of the starting material was detected by TLC. The solvent was removed in vacuum and the light-brown residue was dissolved in 1.0 mL warm DMSO. The product was purified via preparative RP-HPLC. 40.0 mg of the 71.0 mg (237 µmol, 47% over 3 steps) obtained 5-bromo-2-(piperidin-3-ylamino)benzoic acid were used in the following step.[248]

Step 4:

A 10 mL round-bottom flask with magnetic stirring bar was charged with 40.0 mg (134 µmol 1 eq) 5-bromo-2-(piperidin-3-ylamino)benzoic acid, 22.1 mg (160 µmol, 1.2 eq) K_2CO_3, 9.90 µL (147 µmol, 1.1 eq) 2-chloroethan-1-ol and 500 µL DMF. The light-red suspension was stirred for 24 h at RT until full conversion of the starting material was detected by TLC. The solvent was removed in vacuum and the light-red residue was dissolved in 1.0 mL hot DMSO. The dissolved crude product was purified via preparative RP-HPLC.

$C_{14}H_{19}BrN_2O_3$ [343.22]

yield: 7.7 mg (22.4 µmol, 8% over 4 steps), colorless solid.

R_f = 0.23 (MeOH/EtOAc = 3:1% (v/v)) (265 nm, CAM: light red).

mp: 186°C.

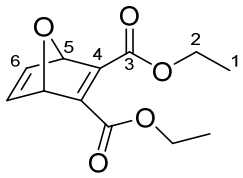
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 7.90 (d, $^4J_{HH}$ = 2.3 Hz, 1H, H-3), 7.28 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 2.3 Hz, 1H, H-5), 6.69 (d, 8.9 Hz, 1H, H-6), 4.26-4.1 (m, 1H, H-8), 3.70-3.64 (m, 2H, H-14), 3.55-3.47 (m, 2H, H-11a,H-12a), 3.38-3.27 (m, 2H, H-13), 3.23-3.13 (m, 2H, H-11b, H-12b), 2.91-2.58 (m, 3H), 2.56-2.43 (m, 11H), 2.11-1.93 (m, 3H), 1.91-1.59 (m, 2H, H-10), 1.59-1.38 (m, 1H, H-9b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 170.2 (C-1), 147.9 (C-7), 134.0 (C-5), 133.9 (C-3), 112.9 (C-6), 112.3 (C-4), 105.0 (C-2), 59.2 (bs, C-13), 58.2 (C-11/C-12), 46.8 (bs, C-14), 46.1 (C-11/C-12), 42.8 (bs, C-8), 29.2 (C-9), 21.5 (C-10).

HR-MS (DI-EI): *m/z* $[M]^+$ calcd for $C_{14}H_{19}BrN_2O_3^+$: 342.0579; found: 342.0587.

6.6.3 Total Synthesis of Tilivalline

6.6.3.1 Diethyl 7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (202)



A dry 150 mL Schlenk flask with magnetic stirring bar was charged with 50 mL dry toluene, 14.1 mL (15.0 g, 83.7 mmol, 1.0 eq) diethyl acetylenedicarboxylate and 7.3 mL (6.84 g, 100 mmol, 1.2 eq) furan in an N_2 counter-stream. The light-yellow reaction mixture was heated to 80 °C for 3 d until full conversion of the starting material was observed by GC-MS. The solvent was removed in vacuum and the crude product was purified via column chromatography (300 g silica gel, size: 20 x 6 cm, cyclohexane/EtOAc = 3:1 (v/v), 200 mL fractions).[260]

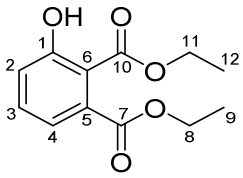
yield: 16.5 g (69.4 mmol, 83%) light-yellow liquid.

R_f = 0.23 (cyclohexane/EtOAc = 1:1 (v/v) (CAM: dark-blue).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.22 (s, 2H, H-6), 5.67 (s, 2H, H-5), 4.27 (q, $^3J_{HH}$ = 7.1 Hz, 4H, H-2), 1.32 (t, $^3J_{HH}$ = 7.1 Hz, 6H).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 163.2 (C-3), 152.8 (C-4), 143.3 (C-6), 85.2 (C-5), 61.5 (C-2), 14.2 (C-6).

6.6.3.2 Diethyl 3-hydroxyphthalate (203)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 16.5 g (69.4 mmol, 1.0 eq) diethyl 7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (**202**) and 30 mL (32.8 g, 232 mmol, 3.34 eq) $BF_3 \cdot Et_2O$ in an N_2 counter-stream. The dark-brown solution was heated to 60 °C for 4 h until full conversion of the starting material was detected by ^{1}H-NMR (pressure equilibration!). The reaction mixture was poured on 200 mL cold saturated $NaHCO_3$ solution and solid Na_2CO_3 was added until neutral. The dark-brown suspension was extracted with Et_2O (3 x 100 mL, 1 x 150 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The brown liquid was dried in oil pump vacuum and was used in the following steps without further purification.[264]

$C_{12}H_{14}O_5$ [238.24]

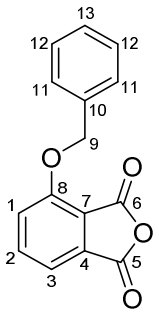
yield: 13.4 g (56.1 mmol, 81%).

R_f = 0.48 (cyclohexane/EtOAc = 3:1 (v/v)) (254 nm, CAM: brown).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.44 (t, $^3J_{HH}$ = 7.9 Hz, 1H, H-3), 7.07 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, H_{arom}), 6.94 (dd, $^3J_{HH}$ = 7.4 Hz, $^4J_{HH}$ = 1.0 Hz, 1H, H_{arom}), 4.45-4.28 (m, 4H, H-8, H-11), 1.37 (t, $^3J_{HH}$ = 7.1 Hz, 6H, H-9, H-12).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.2 (C_q), 169.0 (C_q), 136.0 (C_q), 134.6 (C-3), 119.7 (CH_{arom}), 119.1 (CH_{arom}), 62.3 (CH_2), 61.7 (CH_2), 14.2 (CH_3), 14.0 (CH_3).

6.6.3.3 4-(Benzyloxy)isobenzofuran-1,3-dione (200)[95]



Step 1: A 250 mL round-bottom flask with magnetic stirring bar was charged with 13.3 g (55.9 mmol, 1.0 eq) diethyl 3-hydroxyphthalate (**203**), 8.82 g (63.2 mmol, 1.13 eq) K_2CO_3 and 100 mL distilled acetone. To the stirred suspension were added 7.16 mL (10.3 g, 58.7 mmol, 1.05 eq) benzyl bromide and the brown suspension was heated to 75 °C for 22 h until full conversion of the starting material was detected (TLC). The mixture was cooled to RT and the solvent was removed in vacuum. The brown residue was dissolved in 150 mL THF/MeOH/H_2O (3:1:1 (v/v/v)) and was treated with 41.9 g (988 mmol, 17 eq) $LiOH \cdot H_2O$. The mixture was stirred at RT for 17 h (^{1}H-NMR: full mono-saponification) and was treated with 34.9 g (559 mmol, 10 eq) KOH. The mixture was heated to 50 °C for 15 h until complete saponification was detected (^{1}H-NMR). The red-brown suspension was cooled to 0 °C and acidified with 12 M HCl (130 mL). The mixture was extracted with EtOAc (3 x 200 mL), the combined organic layers were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The brown solid was used in the following step without further purification.[287]

Step 2: A 200 mL Schlenk flask with magnetic stirring bar was charged with the crude 3-(benzyloxy)phthalic acid, 26.5 mL (28.8 g, 279 mmol, 5 eq) Ac_2O and 75 mL CH_2Cl_2. The brown suspension was heated to 65 °C for 19 h and the brown solution was cooled to RT and was left standing for 2 h. The product was isolated via filtration through a sintered glass frit, washed with *n*-pentane (3 x 40 mL) and dried in oil pump vacuum (first fraction, 8.48 g,

60%). The filtrate was concentrated in vacuum and the brown/black solid was dissolved in 50 mL EtOAc. The mixture was treated with 40 mL *n*-pentane and the cloudy precipitate was collected via filtration through a sintered glass frit. The beige crystals (second fraction) were washed with *n*-pentane (2 x 20 mL) and were dried in oil pump vacuum (second fraction, 1.68 g, 12%).

$C_{15}H_{10}O_4$ [254.24]

yield: 10.2 g (40.0 mmol, 72%) light-yellow crystals.

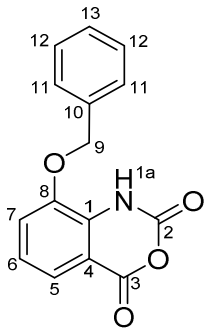
R_f = 0.36 (cyclohexane/EtOAc = 2:1 (v/v)) (UV: 254 nm, 366 nm, CAM: dark blue).

mp: 140 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.76 (t, $^3J_{HH}$ = 7.9 Hz, 1H, H-2), 7.56 (d, $^3J_{HH}$ = 7.3 Hz, 1H, H_{arom}), 7.51-7.44 (m, 2H, H-11), 7.43-7.28 (m, 4H, H_{arom}), 5.38 (s, 2H, H-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 163.0 (C_q), 160.6 (C_q), 157.4 (C-8, C_q), 138.3 (C-2, C_q), 135.1 (C_q), 133.5 (C_q), 129.0 (C-12), 128.6 (C-13), 127.0 (C-11), 120.5 (C_q), 117.8 (C_q), 71.3 (C-9).

6.6.3.4 8-(Benzyloxy)-2*H*-benzo[*d*][1,3]oxazine-2,4(1*H*)-dione (206)



A dry 50 mL Schlenk flask with magentic stirring bar was charged with 854 mg (3.36 mmol, 1.0 eq) 4-(benzyloxy)isobenzofuran-1,3-dione (**200**) and the solid was dried in oil pump vacuum for 10 min. 13 mL dry benzene were added under N_2 counter-stream followed by 740 µL (618 mg, 5.04 mmol, 1.5 eq) TMS-N_3. The yellow suspension was heated to 100 °C for 16 h. The dirty-yellow solution was cooled to RT and most of the solvent was removed using a cooling trap (~1 mL yellow viscous liquid left). The residue was heated to 100 °C for 22 h, cooled to RT and 6 mL dry EtOH were added. The light brown suspension was stirred for 5 min, the solvent was removed with a cooling trap and the product was dried in oil pump vacuum to yield a light brown solid. The crude product (891 mg, brown solid) was used in the following step without further purification. The yield of the by-product 5-(benzyloxy)-2*H*-benzo[*d*][1,3]oxazine-2,4(1*H*)-dione was determined as <1% (^{1}H-NMR) and typically ranges

from 0-28% in analogous experiments. The by-product can be removed by recrystallization from EtOAc.[288]

$C_{15}H_{11}NO_4$ [269.26]

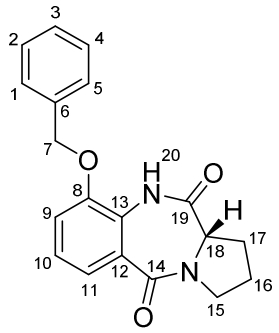
R_f = 0.53 (cyclohexane/EtOAc = 1:1 (v/v)) (UV: 254 nm, CAM: blue).

mp: 194-195 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.10 (bs, 1H, H-1a), 7.67 (d, $^3J_{HH}$ = 7.8 Hz, 1H, H_{arom}), 7.50-7.34 (m, 5H, H_{arom}), 7.23-7.12 (m, 2H, H_{arom}), 5.18 (s, 2H, H-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 159.8 (C-3, C_q), 146.9 (C-8, C_q), 145.0 (C-2, C_q), 136.1 (C-10, C_q), 131.9 (C-1, C_q), 128.3 (C_{arom}), 128.0 (C_{arom}), 127.9 (C_{arom}), 123.3 (C_{arom}), 120.0 (C_{arom}), 119.0 (C_{arom}), 111.1 (C-4, C_q), 70.1 (C-9).

6.6.3.5 (*S*)-9-(Benzyloxy)-1,2,3,11a-tetrahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*)-dione (208)



A dry 20 mL Schlenk flask with magnetic stirring bar was charged with 1.30 g (4.81 mmol, 1.0 eq) crude 8-(benzyloxy)-2*H*-benzo[*d*][1,3]oxazine-2,4(1*H*)-dione (**206**) from the previous step, 672 mg (5.78 mmol, 1.2 eq) L-proline and 6 mL dry DMSO in a N_2 counter-stream. The beige suspension was heated to 100 °C for 15 h until full conversion of the starting material was detected by TLC. The dark-yellow solution was poured into distilled H_2O (30 mL) and the colorless suspension was stirred for 2 h at RT. The light-yellow solid was isolated via filtration, washed with H_2O (2 x 10 mL) and dried in oil pump vacuum. The solid was dissolved in boiling toluene (50 mL) and was filtrated through a (heated) sintered glass frit. The solvent was removed in vacuum and the resulting solid was dried in oil pump vacuum.[95]

$C_{19}H_{18}N_2O_3$ [322.36]

yield: 1.25 g (3.89 mmol, 81%) colorless, amorphous solid.

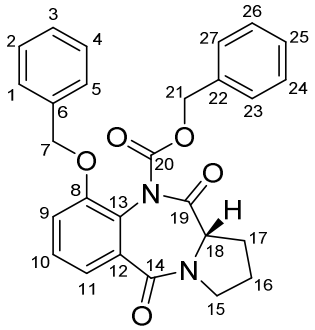
R_f = 0.42 (cyclohexane/CH_2Cl_2/acetone = 2:2:1 (v/v/v)) (UV: 254 nm, CAM: blue).

mp: 177-178 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.98 (s, 1H, H-20), 7.58 (d, $^{3}J_{HH}$ = 7.5 Hz, 1H, H-11), 7.47-7.30 (m, 5H, H_{arom}), 7.17 (t, $^{3}J_{HH}$ = 7.8 Hz, 1H, H-10), 7.09 (d, $^{3}J_{HH}$ = 7.6 Hz, 1H, H-9), 5.12 (s, 2H, H-7), 4.02 (d, $^{3}J_{HH}$ = 5.1 Hz, 1H, H-18), 3.89-3.71 (m, 1H, H-15a), 3.68-3.50 (m, 1H, H-15b), 2.86-2.65 (m, 1H, H-17a), 2.16-1.89 (m, 3H, H-16, H-17b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 170.5 (C-19, C_q), 165.2 (C-14, C_q), 147.8 (C-8, C_q), 135.6 (C-6, C_q), 129.0 (C_{arom}), 128.7 (C-3), 127.9 (C_{arom}), 127.6 (C-13, C_q), 125.7 (C-12, C_q), 124.8 (C-10), 122.9 (C-11), 114.6 (C-9), 71.5 (C-7), 57.0 (C-18), 47.5 (C-15), 26.4 (C-16), 23.5 (C-17).

6.6.3.6 Benzyl (*S*)-9-(benzyloxy)-5,11-dioxo-2,3,11,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate (209)



A dry 200 mL Schlenk flask with magnetic stirring bar was charged with 1.20 g (3.72 mmol, 1.0 eq) (*S*)-9-(benzyloxy)-1,2,3,11a-tetrahydro-5*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*)-dione (**208**) and 72 mL dry THF in a N_2 counter-stream. To the colorless solution were added 4.1 mL (4.09 mmol, 1.1 eq) LiHMDS (1.0 M in hexanes) over a period of 5 min at RT. After 90 min a thick colorless suspension has formed and 821 µL (982 mg, 5.58 mmol, 1.5 eq) CbzCl were added in one portion. The light-yellow solution was stirred at RT for 150 min until full conversion of the starting material was detected (TLC). 2 mL saturated NH_4Cl solution were added and the mixture was stirred for 5 min. The mixture was diluted with CH_2Cl_2 (50 mL) and the solid was removed via filtration through a sintered glass frit and was rinsed with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtrated and the solvent was removed in vacuum. The product was purified via column chromatography (60 g silica gel, size: 20 x 3 cm, cyclohexane/CH_2Cl_2/acetone = 10:10:1 (v/v/v), 20 mL fractions).[95]

$C_{27}H_{24}N_2O_5$ [456.50]

yield: 1.56 g (3.42 mmol, 92%) faint-yellow foam.

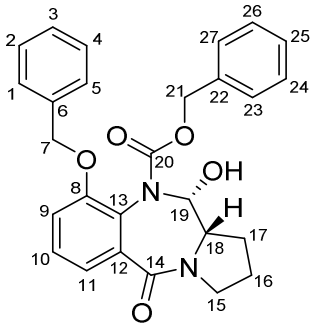
R_f = 0.52 (cyclohexane/CH_2Cl_2/acetone = 2:2:1 (v/v/v)) (UV: 254 nm, CAM: blue).

mp: 50-52 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.49 (dd, $^{3}J_{HH}$ = 7.8 Hz, $^{4}J_{HH}$ = 1.0 Hz, 1H, H_{arom}), 7.40-7.31 (m, 2H, H_{arom}), 7.30-7.25 (m, 2H, H_{arom}), 7.24-7.15 (m, 5H, H_{arom}), 7.14-7.03 (m, 3H, H_{arom}), 5.10 (d, $^{3}J_{HH}$ = 8.0 Hz, 1H, H-7a), 4.90-4.88 (m, 2H, H-7b, H21a), 4.78 (d, $^{3}J_{HH}$ = 12.0 Hz, 1H, H-21b), 3.92 (d, $^{3}J_{HH}$ = 6.7 Hz, 1H, H-18), 3.86-3.71 (m, 1H, H-15a), 3.58-3.41 (m, 1H, H-15b), 2.73-2.59 (m, 1H, H-17a), 2.20-1.83 (m, 3H, H-16, H-17b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.0 (C-19, C_q), 164.7 (C-14, C_q), 152.4 (C_q), 151.8 (C_q), 135.7 (C_q), 134.8 (C_q), 133.1 (C_q), 129.0, 128.7, 128.5, 128.3, 128.2, 128.1, 127.3, 125.8 (C_q), 121.9 (C-11), 116.2 (C-9), 71.6 (C-21), 68.8 (C-7), 59.3 (C-18), 46.7 (C-15), 26.5 (C-17), 23.7 (C-16).

6.6.3.7 Benzyl (11*R*,11a*S*)-9-(benzyloxy)-11-hydroxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate (210)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 390 mg (854 µmol, 1.0 eq) benzyl (*S*)-9-(benzyloxy)-5,11-dioxo-2,3,11,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate (**209**) and 14 mL dry THF in a N_2 counter-stream. The colorless solution was cooled to 0 °C and 34.6 mg (957 µmol, 1.12 eq) $NaBH_4$ were added in one portion. The mixture was stirred at 0 °C until full conversion of the starting material was detected after 1 h (TLC). The solvent was carefully removed in vacuum (max. 35 °C) and the polar residue was removed via silica gel filtration (3 g silica gel, 100 mL cyclohexane/CH_2Cl_2/acetone = 2:2:1). The solvent was removed in vacuum and the product was dried in oil pump vacuum to obtain pure product.[95]

$C_{27}H_{26}N_2O_5$ [458.51]

yield: 321 mg (700 µmol, 82%) colorless, amorphous solid.

R_f = 0.32 (cyclohexane/CH_2Cl_2/acetone = 2:2:1 (v/v/v)) (UV: 254 nm, CAM: blue).

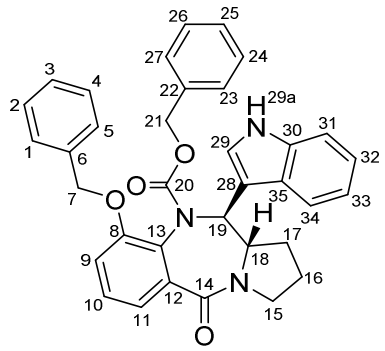
mp: 170-174 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.41-7.16 (m, 11H, H_{arom}), 7.09-6.98 (m, 2H, H_{arom}), 5.63 (d, $^{3}J_{HH}$ = 9.7 Hz, 1H, H-19), 5.09 (d, $^{3}J_{HH}$ = 11.2 Hz, 1H, H-7a), 5.04-4.89 (m, 2H, H- 7b, H-

21a), 4.79 (d, $^{3}J_{HH}$ = 12.0 Hz, 1H, H-21b), 3.82-3.69 (m, 1H, H-15a), 3.62-3.39 (m, 2H, H-15b, H-18), 2.20-2.10 (m, 2H, H-17), 2.08-1.94 (m, 2H, H-16).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 166.9 (C_q, C-14), 156.8 (C_q), 154.6 (C_q), 136.5 (C_q), 135.9 (C_q), 135.2 (C_q), 128.9, 128.7, 128.4, 128.0, 127.9, 127.6, 126.7, 124.9 (C_q), 120.9 (C-11), 115.2 (C-9), 86.3 (C-19), 70.6/67.9 ($CH_{2,\ benzyl}$), 59.9 (C-18), 46.4 (C-15), 28.8 (C-17), 23.2 (C-16).

6.6.3.8 Benzyl (11*S*,11a*S*)-9-(benzyloxy)-11-(1*H*-indol-3-yl)-5-oxo-2,3,11,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate (211)



A dry 4 mL pressure tube with magnetic stirring bar was charged with 49.9 mg (109 µmol, 1.0 eq) benzyl (11*R*,11a*S*)-9-(benzyloxy)-11-hydroxy-5-oxo-2,3,11,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate (**210**), 25.5 mg (218 µmol, 2.0 eq) indole and 1.5 mL AcOH. The light-yellow solution was degassed by bubbling with N_2 in an ultrasonic bath (10 min). The pressure tube was sealed and placed into a preheated oil bath (150 °C) and was stirred for 4 h until full conversion of the starting material was observed (TLC). The mixture was cooled to RT, light-brown solution was concentrated in vacuum and the redish-brown residue was purified via column chromatography (4 g silica gel, cyclohexane/CH_2Cl_2/acetone = 2:2:1, 4 mL fractions).[95]

$C_{35}H_{31}N_3O_4$ [557.65]

yield: 47.5 mg (85.2 µmol, 78%) light-red solid.

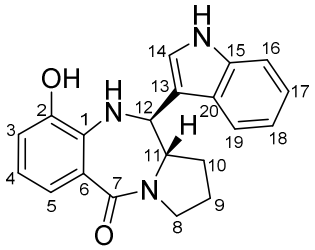
R_f = 0.38 (cyclohexane/CH_2Cl_2/acetone = 2:2:1) (UV: 254 nm, CAM: blue).

mp: 250-254 °C.

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.23 (s, 1H, H-29a), 7.51-6.86 (m, 18H, H_{arom}), 6.34 (d, $^{3}J_{HH}$ = 7.3 Hz, 1H), 5.69 (d, , $^{3}J_{HH}$ = 11.6 Hz, 1H, H-19), 4.91 (AB_q, $^{2}J_{HH}$ = 12.9 Hz, 2H, H-7), 4.44 (d, $^{2}J_{HH}$ = 11.9 Hz, 1H, H-21a), 4.36-4.22 (m, 2H, H-18, H-21b), 3.95-3.80 (m, 1H, H-15a), 3.76-3.56 (m, 1H, H-15b), 2.24-2.06 (m, 1H, H-16a), 2.06-1.88 (m, 2H, H-16b, H-17a), 1.84-1.73 (m, 1H, H-17b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 167.6 (C-14), 155.6 (C_q), 155.4 (C_q), 136.6 (C_q), 136.5 (C_q), 136.4 (C_q), 136.2 (C_q), 129.2 (C_{arom}), 128.2 (C_{arom}), 128.1 (C_{arom}), 127.5 (C_{arom}), 127.2 (C_{arom}), 127.1 (C_{arom}), 126.5 (C_q), 126.0 (C_{arom}), 123.8 (C_{arom}), 122.3 (C_{arom}), 120.7 (C_{arom}), 120.6 (C_{arom}), 119.7 (C_{arom}), 114.9 (C_{arom}), 113.8 (C_q), 111.3 (C_{arom}), 69.7 (C-21), 67.2 (C-7), 63.0 (C-19), 59.7 (C-18), 46.7 (C-15), 29.5 (C-17), 23.3 (C-16).

6.6.3.9 Tilivalline (3)



A dry 100 mL Schlenk flask with magnetic stirring bar was charged with 150 mg (269 µmol) benzyl (11*S*,11a*S*)-9-(benzyloxy)-11-(1*H*-indol-3-yl)-5-oxo-2,3,11,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-10(5*H*)-carboxylate (**211**), 4 mL dry THF, 4 mL dry MeOH and 7.5 mg Pd/C (10% on activated charcoal) in a N_2 counter-stream. The flask was degassed by repeated vacuum/N_2-cycles (3 x) and was filled with H_2 (1 atm) the last time. The suspension was stirred at RT for 15 h until full conversion of the starting material was detected by TLC. The catalyst was removed via filtration through a pad of Celite™ (2 cm, wetted with MeOH) and the pad was rinsed with MeOH (3 x 25 mL). The solvent was removed in vacuum and the product was purified via preparative RP-HPLC (Method_TILIVALLINE).[95]

$C_{20}H_{19}N_3O_2$ [333.39]

yield: 33.8 mg (101 µmol, 38%) light-carnate solid.

R_f = 0.09 (cyclohexane/CH_2Cl_2/acetone = 2:2:1) (UV: 255/366 nm, CAM: orange).

mp = 179-182 °C.

^{1}H-NMR (499.89 MHz, DMSO-d_6): δ = 11.10 (bs, 1H, NH), 7.44-7.36 (m, 3H, H-14, H-16, H-19), 7.16 (d, $^3J_{HH}$ = 7.4 Hz, 1H, H-5), 7.09 (t, $^3J_{HH}$ = 7.3 Hz, 1H, H-17), 6.94 (t, $^3J_{HH}$ = 7.3 Hz, 1H, H-18), 6.77 (d, $^3J_{HH}$ = 7.1 Hz, 1H, H-3), 6.53 (t, $^3J_{HH}$ = 7.8 Hz, 1H, H-4), 5.09 (bs, 1H, OH), 4.73 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-12), 4.21-4.13 (m, 1H, H-11), 3.73-3.66 (m, 1H, H-8a), 3.60-3.51 (m, 1H, H-8b), 1.94-1.84 (m, 1H, H-9a), 1.75-1.65 (m, 2H, H-9b, H-10a), 1.58-1.49 (m, 1H, H-10b).

^{13}C-NMR (125.70 MHz, DMSO-d_6): δ = 166.4 (C-7, C_q), 145.2 (C-2, C_q), 136.5 (C-15, C_q), 134.7 (C-1, C_q), 125.1 (C-20, C_q), 123.7 (C-14, C_{arom}),121.6 (C-17, C_{arom}), 121.3 (C-5, C_{arom}),

120.1 (C-18, C_{arom}), 118.90 (C-19, C_{arom}), 118.87 (C-13, C_q), 116.4 (C-4, C_{arom}), 116.2 (C-6, C_q), 114.9 (C-3, C_{arom}), 111.8 (C-16, C_{arom}), 60.9 (CH, C-12), 59.5 (CH, C-11), 47.5 (CH_2, C-8), 30.2 (CH_2, C-10), 22.0 (CH_2, C-9).

$[\alpha]_D^{20}$= +178 (c = 0.29 in MeOH).

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8 Abbreviations

Analytical Methods

APT	attached proton test
COSY	correlation spectroscopy
DI-EI	direct inlet electron impact
EI	electron impact
ESI	electrospray ionization
ESI-MS	electrospray ionization – mass spectrometry
GC	gas chromatography
GC-FID	gas chromatography flame ionization detector
GC-MS	gas chromatography mass spectrometry
HMBC	heteronuclear multiple-bond correlation spectroscopy
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography mass spectrometry
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
ITC	isothermal titration calorimetry
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
RP-HPLC	reversed-phase high performance liquid chromatography
TOF	time of flight
bs	broad singlet
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
dq	doublet of quadruplet
dt	doublet of triplet
h	hexet
m	multiplet
p	pentet
q	quadruplet
s	singlet
t	triplet
td	triplet of doublet
$[\alpha]_D^{20}$	specific optical rotation at 20 °C
BP	basis peak

δ	chemical shift in ppm (parts per million)
Hz	Hertz
J	coupling constant
λ	wavelength
MHz	Megahertz
min	minute/minutes
M^+	molecule peak
$[M+H]^+$	molecule peak with proton
m/z	mass to charge ratio
nm	nanometer
NOE	nuclear Overhauser effect
ppm	parts per million
R_f	retention factor
TLC	thin layer chromatography
t_R	retention time
UV	ultraviolett
v/v	volume per volume
v/v/v	volume per volume per volume
wt%	weight percent
w/w	mass per mass

Chemical Abbreviations

Ac	acetyl
AcCl	acetyl chloride
Ac_2O	acetic anhydride
AcOH	acetic acid
ADIC	(5*S*,6*S*)-6-amino-5-hydroxy-1,3-cyclohexadiene-1-carboxylic acid
Al_2O_3	aluminum oxide
AOCHC	6-amino-5-oxocyclohex-2-ene-1-carboxylic acid
AT1	angiotensin II receptor type 1
9-BBN	9-borabicyclo[3.3.1]nonane
BcPhzAB	*Burkholderia cepacia* PhzA/B
BnBr	benzyl bromide
BnCl	benzyl chloride
$BnNH_2$	benzylamine
Boc	*tertiary*-butyloxycarbonyl

Boc_2O	di-*tertiary*-butyl dicarbonate
brine	saturated aqueous sodium chloride solution
BSA	*N,O*-bis(trimethylsilyl)acetamide
CAM	cerium ammonium molybdate
CbzCl	benzyl chloroformate
$CDCl_3$	chloroform-*d*
CES1	carboxylesterase 1
CES2	carboxylesterase 2
CO_2	carbon dioxide
conv.	conversion
CSA	camphorsulfonic acid
$Cu(acac)_2$	copper(II) acetylacetonate
Cy_2NMe	*N,N*-dicyclohexylmethylamine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIC	diisopropylcarbodiimide
DCC	dicyclohexylcarbodiimide
DCE	dichloroethane
DHHA	*trans*-2,3-dihydro-3-hydroxyanthranilic acid
DHHB	*trans*-2,3-dihydro-3-hydroxybenzoic acid
DHHS	*trans*-2,3-dihydro-3-hydroxysalicylic acid
DHPCA	dihydrophenazine-1-carboxylic acid
DHPDC	dihydrophenazine-1,6-dicarboxylic acid
DIPEA	*N*,*N*-diisopropylethylamine
4-DMAP	(4-dimethylamino)pyridine
DMEDA	*N*,*N*′-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	dimethyl sulfoxide-d_6
DNA	deoxyribonucleic acid
D_2O	deuterated water
DPPA	diphenyl phosphoryl azide
EDC.HCl	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
Et	ethyl
Et_3B	triethylborane
Et-DHHA	ethyl *trans*-2,3-dihydro-3-hydroxyanthranilate
Et_3N	triethylamine
$Et_3N \cdot HCl$	trimethylamine hydrochloride

Et_2O	diethyl ether
EtOAc	ethylacetate
EtOH	ethanol
FMN	flavin mononucleotide
HBTU	(2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)
HHPDC	hexahydrophenazine-1,6-dicarboxylic acid
IPA	isopropanol
KSI/NTF2	ketosteroid isomerase/nuclear transport factor 2
LHMDS	lithium bis(trimethylsilyl)amide
$LiOH.H_2O$	lithium hydroxide monohydrate
Me	methyl
MeCN	acetonitrile
MeOH	methanol
Me_2SO_4	dimethyl sulfate
$MeSO_3H$	methanesulfonic acid
B. iodinum	*Burkholderia iodinum*
MIBK	4-methyl-2-pentanone
MsCl	methanesulfonyl chloride
*n*Pr	*n*-propyl
*n*BuLi	*n*-butyllithium
NADH	nicotinamide adenine dinucleotide
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NIS	*N*-iodosuccinimide
OAc	acetate
OTf	trifluoromethanesulfonate
PCA	phenazine-1-carboxylic acid
PBD	pyrrolobenzodiazepine
PDC	phenazine-1,6-dicarboxylic acid
PPh_3	triphenylphosphine
PMHS	polymethylhydrosiloxane
PPTS	pyridinium p-toluenesulfonate
PTFE	polytetrafluoroethylene
PTSA	*p*-toluenesulfonic acid
STAB	sodium triacetoxyborohydride
TBAA	tetrabutylammonium adipate

TBDMS	*tert*-butyldimethylsilyl ether
TBDMSCl	*tert*-butyldimethylsilyl chloride
TCDI	1,1′-thiocarbonyldiimidazole
TMSCl	chlorotrimethyl silane
TMSI	trimethylsilyl iodide
$TMSN_3$	azidotrimethylsilane
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TRIS	tris(hydroxymethyl)aminomethan
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	tetramethylsilane

Others

Å	Angstrom
AAHC	antibiotic-associated hemorrhagic colitis
ADMET	absorption, distribution, metabolism, excretion, toxicity
aq	aqueous
b_p	boiling point
c	concentration
cm	centimeter
CNS	central nervous system
d	day
decomp.	decomposition
°	degree
°C	degree Celsius
ΔG	free enthalpy
e.g.	exempli gratia
eq	equivalent/equivalents
et al.	et alii
eV	electron volts
g	gram
h	hour/hours
K_d	dissociation constant
kJ	Kilojoule
L	litre
M	Molar
max	maximum

mbar	millibar
mg	milligram
min	minimum
mL	Milliliter
mM	Millimolar
mmol	millimol
µL	Microliter
µm	Micrometer
µmol	micromol
mp	melting point
MS	molecular sieves
nM	Nanomolar
%	percent
pH	pH value
pK_a	logarithm of acid dissociation constant
ppm	parts per million
QM/MM	quantum mechanics/molecular mechanics
rac	racemic
ROS	reactive oxygen species
RT	room temperature
s	second/seconds
tert	*tertiary*
V	volume