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Design and Synthesis of Inhibitors of PhzA/B

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Meiner Familie

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1 Introduction

Today as in the past, bacterial infections contribute to a large extent to the morbidity and mortality of human beings. Especially hospitalized patients are prone to several bacterial infections that would not affect a healthy person, such as *Pseudomonas aeruginosa*, which causes 10-15% of all nosocomial infections worldwide.[1]

The prevalent *P. aeruginosa* is furthermore a common cause of hospital-acquired infections and the opportunistic pathogen infects preferentially immunocompromised patients with predisposing factors such as burn victims, patients with neutropenic cancer, bone marrow transplants and AIDS. It has been speculated, that *P. aeruginosa* is a major contributor for chronic lung infections by formation of a biofilm adherent to the human mucins in the lower respiratory tract.[2,3] Biofilm formation in the lungs has a particularly drastic effect on patients having cystic fibrosis (CF), the most common autosomal recessive disorder in Caucasian people. They usually suffer from an airway inflammation caused by a bacterial infection, with the major pathogen *P. aeruginosa*. This chronic lung inflammation leads to airflow obstruction, extensive tissue remodelling and hence destruction which leads to the low life expectancy of CF patients.[4] Treatment is hard to achieve, mainly due to the heavy colonization of the lungs with a biofilm-growing mucoid strain of *P. aeruginosa*. This biofilm is a structured consortium of bacteria which is embedded in a self-produced polymer matrix consisting of proteins, DNA and polysaccharides of which alginates prevail in *P. aeruginosa*. The consequence of this biofilm formation is that the bacteria can resist phagocytosis and other components of the immune system. The immune system is leading an antibody response and thereby the chronic inflammation is initiated, which itself is the main cause for the extensive tissue damage in the lungs.

It is difficult to eradicate these sheltered bacteria, because they feature increased tolerance to antibiotics. This is caused by the increased frequency of mutations of *P. aeruginosa* in the biofilm, the mutation of the antibiotic target molecules in the bacteria, the conventional resistance mechanisms towards antibiotics like the β-lactamases, the up-regulated efflux pumps and generally to a faster adaption to changes in the environment and a swifter development of resistance against antibiotics. *P. aeruginosa* furthermore produces an arsenal of virulence factors, which ensures the bacteria's survival. Those are divided into cell-associated and extracellular virulence factors, of which the last are excreted substances that play their role dissolved in the environmental medium, such as the phenazine "pyocyanin" for *P. aeruginosa*.[2,5]

2 Theoretical background

2.1 Phenazine biosynthesis

2.1.1 Structure and functions of phenazines

In the 1859, Marthurin-Joseph Fordos reported the isolation of the blue pigment “pyocyanin” from purulent wound dressings by chloroform extraction.[6] It took several years until a bacterium, *Bacillus pyocyaneus*, was held responsible for the production of the blue phenazine derivative pyocyanin. The bacterium was later termed as *Pseudomonas aeruginosa* and several other phenazine producing bacteria were discovered. Not only Gram-positive bacteria have the ability to produce this substance class, but also Gram-negative species like *Pseudomonas* and *Streptomyces*. At the present day, more than hundred biological compounds containing the phenazine motif have been isolated[7,8,9,10] and several thousand analogues have been synthesized.[8,11] The condensed tricyclic ring system with the pyrazine moiety in the centre is the characteristic motif of this generally redox-active substance class.[12] (Figure F1)

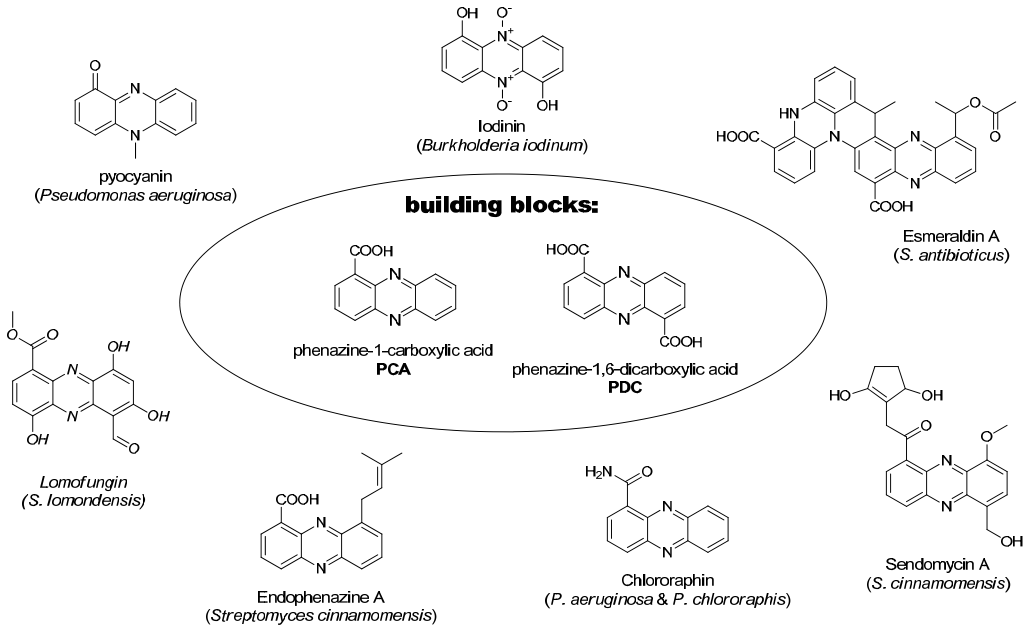


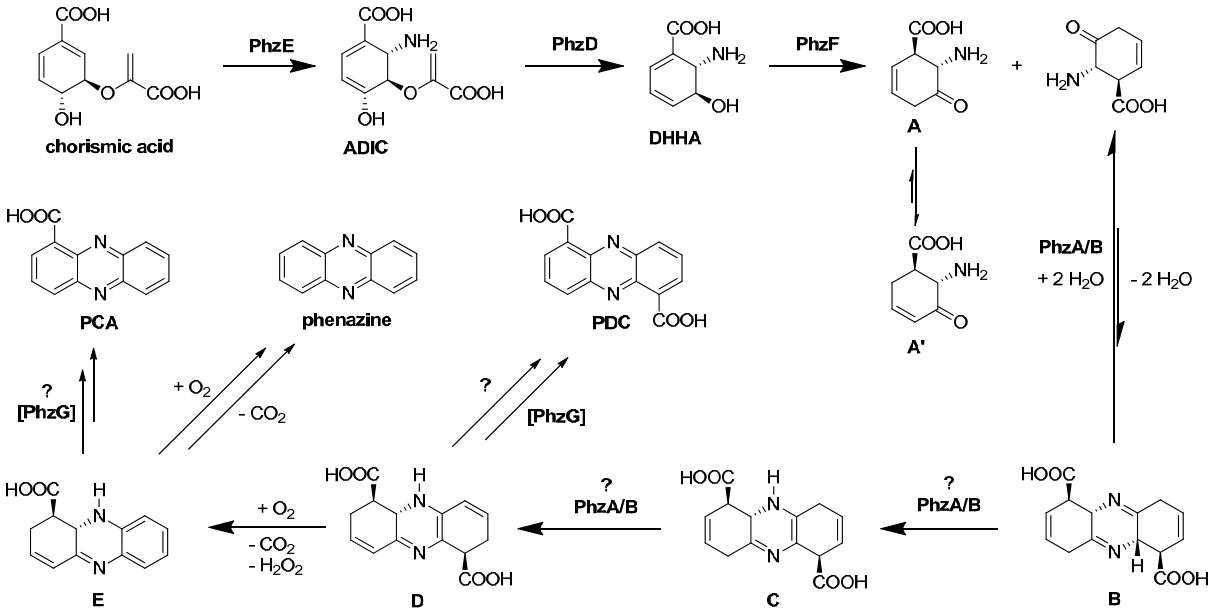
Figure F1: The two core-phenazines **PCA** and **PDC** with naturally occurring derivatives

A general understanding of the secondary metabolites’ roles is about to develop and many functions have already been elucidated.[11,13,14,15,16] Phenazines are redox-active and can reduce molecular oxygen to highly reactive oxygen species, which explains their broad-spectrum antibacterial, antifungal, antiviral and antitumor activity.[7,8] These toxic intermediates trigger also tissue damage in human infectious disease.[17] It was long thought that phenazines mainly act as antibiotics and virulence factors for their producers so as to provide them with a competitive advantage,[18] but new data suggests, that they also play a prominent role in various other biological processes. Phenazine-producing strains residing on roots can protect crops against susceptible bacterial and fungal

pathogens.[19,20,21] In addition, it was shown that phenazines can aid the iron acquisition from the soil by reducing Fe(III) to the more soluble Fe(II).[22] In *P. aeruginosa*, pyocyanin acts as a signalling molecule and controls gene expression by activating the iron-containing transcription factor SoxR.[23] Phenazines also play a prominent role in the primary energy metabolism of the phenazine producer. The secondary metabolites act as respiratory pigments under anaerobic conditions, which are found in the lower layer of a biofilm and reoxidize NADH to sustain glycolysis. The reduced, mobile phenazines diffuse to the surface of the biofilm, where they are reoxidized. Hence an electrochemical gradient is established through the biofilm which ensures the bacteria's survival. As it was anticipated, the deletion of the genes for phenazine biosynthesis and therefore the lack of the signalling molecules and the respiratory pigments, led to a highly decreased capability to form a biofilm in previously phenazine-producing strains.[24,25] The formation of a biofilm complicates the treatment of diseases substantially, since it renders the bacteria in the bulk less accessible for the agents of the immune response and pharmaceuticals. One important phenazine producing bacteria is *P. aeruginosa*, which is a common reason for a large number of nosocomial infections. It is the prime reason for the low life expectancy in patients with cystic fibrosis, whose lungs are chronically colonized by *P. aeruginosa*.[26,27] Strains of *P. aeruginosa* defective in pyocyanin biosynthesis are more susceptible to immune responses in a mouse model of lung infections.[28,29] These pooled observations suggest the assumption, that phenazine biosynthesis may provide a new target for anti-biotic and anti-virulence strategies against infectious disease.[30]

2.1.2 Biosynthesis of Phenazines

Existing knowledge suggests, that all the natural occurring phenazines derive from either phenazine-1-carboxylic acid (**PCA**) or phenazine-1,6-dicarboxylic acid (**PDC**) (Scheme S1).[31,32,33] The enzymes for the biosynthetic pathway are encoded in the "*phz* operon" and it has been proven that five Phz-enzymes are vital for the synthesis of **PCA** and **PDC** from two molecules of chorismic acid.[19] PhzE converts chorismic acid to 2-amino-desoxyisochorismate (**ADIC**)[10] followed by the isochorismatase PhzD, which leads to *trans*-2,3-dihydro-3-hydroxyanthranilate (**DHHA**).[34] This intermediate is the last that can be isolated. In the next step PhzF isomerizes **DHHA** by to 6-amino-5-oxocyclohex-2-ene-1-carboxylic acid (Scheme S1, **A**),[12,35] presumably by initiating a [1,5]-prototropic shift.[35] Two moieties of this reactive ketone condense head-to-tail, followed by isomerization and oxidation to arrive at the aromatic phenazine moiety **B**. In *Pseudomonas*, the core phenazine **PCA** is furnished by an oxidative decarboxylation step, while other bacteria (*Pantoea agglomerans*) produce **PDC** as their core phenazine.[20]



Scheme S1: Biosynthesis of Phenazine-1-carboxylic acid (**PCA**) (Scheme by AHUJA *et al.*[9])

In vitro experiments have shown, that the formation of **PCA** from the ketone **A** (Scheme S1) does not require enzyme catalysis and hence PhzF alone can trigger the conversion to the tricyclic product **D**. The non-catalyzed reaction, however, proceeds with low yield.[12,35] At low substrate concentrations, the bimolecular reaction is very slow due to the exponential decline of the reaction rate with concentration and catalysis by PhzA/B leads to a significant rate acceleration and overall yield, since byproducts are prevented. Phenazine biosynthesis is coupled to the shikimate pathway, which leads low intracellular concentrations of the ketone precursor **A** (Scheme S1) in the bacterial cell. Additionally the unselective conversion of the ketone **A** (Scheme S1), e.g. by oxidation or reaction with free amino groups of proteins, must be avoided and consequently PhzA/B is essential for the catalysis *in vivo*.[19] This has been demonstrated by complete shutdown of phenazine production after deletion of the gene in a *Pantoea agglomerans* strain.[20] The exact roles of the remaining enzymes PhzA/B and PhzG are not clarified yet, but several assumptions have been made. It is believed that the FMN-dependent PhzG is involved in one of the terminal oxidation steps,[36] but the function of PhzA/B is still unclear.[19] Experiments with *E. coli* expressing fragments of the *phz* operon have shown, that although not required, PhzA/B increased the efficiency of the phenazine biosynthesis severalfold.[10,37] PARSONS *et al.* suggested, that the enzyme might be involved in the dimerization step of the two ketones to generate the phenazine tricycle.[12] This could explain, why phenazines are so common in the *Pseudomonads*, which in contrast to other phenazine generating bacteria, possess two adjacent, highly homologous copies of this gene, *phzA* and *phzB* (80% amino acid identity).[19,37,38]

AHUJA et al. have carried out biochemical assays and crystal structure analyses of PhzA/B (from *Burkholderia cepacia* R18194) in order to further enlighten the role of the enzyme in phenazine

biosynthesis.[19] They stated, that phenazine biosynthesis follows the same pathway in both *Burkholderia cepacia* and *Pseudomonas* species, since the same intermediates were observed upon conversion of the substrate. The controversial question whether the condensation of the ketones **A** (Scheme S1) takes place in PhzF was clarified by ^{18}O labelling experiments. It was demonstrated, that the ketone **A** (Scheme S1) is eventually released from the enzyme which further suggests that PhzA/B catalyzes the subsequent condensation reaction. The dimer of PhzA/B possesses a KSI/NTF2-fold and the C-terminus of each chain contributes to the formation of the active centre of the neighbouring monomer. They furthermore hypothesize, that the C-terminus acts as a flexible lid that controls access to the active side. Additionally, the C-terminus contributes to the catalysis and is vital for the activity and stability. Truncation experiments have shown, that shortening the enzyme to 159 residues renders PhzA/B non-functional (Figure F2, A).[19]

The intermediates following the ketone condensation are too unstable to be isolated for crystallographic experiments further elucidating the role of PhzA/B. Instead, substrate analogues and synthetic mimics of the product were synthesized and tested by isothermal titration calorimetry (ITC). Binding of the ketone analogues (mimics to the substrate) to PhzA/B was not observed with ITC, but a range of protein-ligand complexes with product mimics were isolated and investigated by crystallography. The best compounds have affinities between 2 and 25 µM and all of them display a similar binding mode. 2-(Cyclohexylamino)benzoic acid and its binding mode to PhzA/B is displayed below (Figure F2, A).[19]

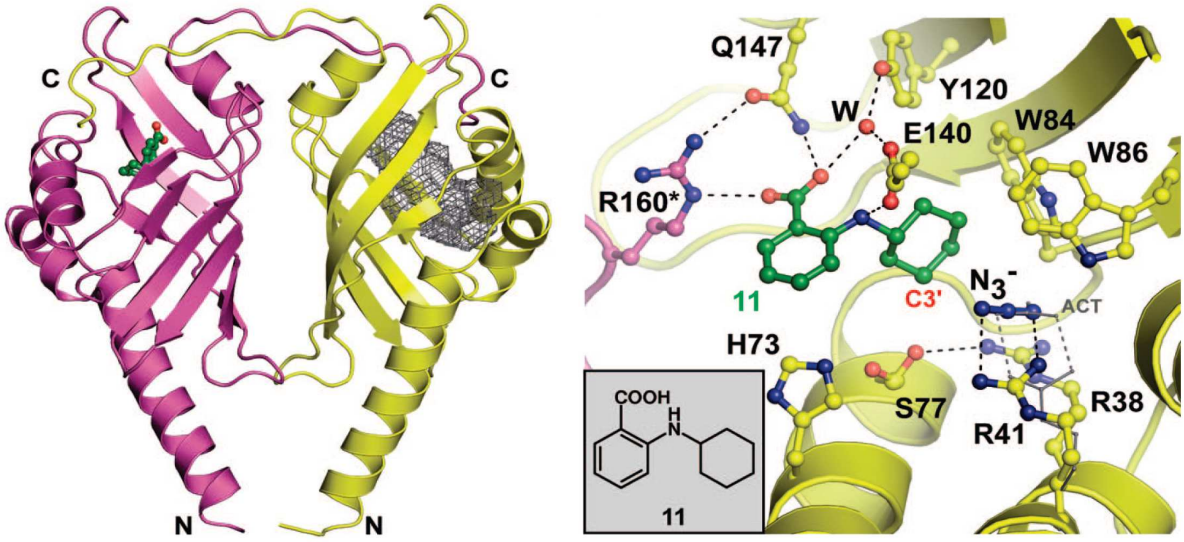


Figure F2: a) Overall crystal structure of *Burkholderia cepacia* PhzA/B with two active sites. N- and C-termini are indicated. b) Binding of 2-(Cyclohexylamino)benzoic acid in PhzA/B (*B. cepacia*). (Pictures taken from AHUJA *et al.*[19])

Because of the requirement of PhzA/B *in vivo*, the class of synthetic probes might act as lead compounds against phenazine biosynthesis in infectious disease.

The development and analysis of further probes with higher affinity based on 2-(Cyclohexylamino)-benzoic acid is ongoing in the research groups of BREINBAUER (Graz University of Technology) and BLANKENFELDT (University of Bayreuth). Matthias Mentel recently synthesized the inhibitor phenazistatin A **(1*R*,3*S*)-1** with an affinity of 51 nM (unpublished result) which was the focus of interest of this thesis.

2.1.3 Mechanism of the PhzA/B catalyzed reaction

AHUJA *et al.* furthermore propose a catalytic mechanism in which PhzA/B enhances the reaction cascade from 6-amino-5-oxocyclohex-2-ene-1-carboxylic acid to the rearranged condensation product (depicted in Figure F3 as **5a**). The catalytic cycle is initiated by binding of the first ketone molecule to R41 and possibly to S77 with its carboxylate group (Figure F3, a). The second substrate molecule binds to H73 and to R160* and this is expected to anchor the flexible C-terminus of the neighbouring monomer so as to shield the active side from the solvent. The nucleophilic attack of the 2-amino group of the latter bound ketone starts the condensation reaction to lead to the first tetrahedral intermediate (Figure F3, b). As a consequence of the first condensation reaction, the 2-amino group of the first ketone comes closer to the carbonyl group of the second, triggering the second condensation and hence the formation of the tricycle (Figure F3, c). This second intramolecular condensation might not require catalysis, but the structural model suggests that H73 and S77 participate in the catalysis by neutralizing the generated negative charge (Figure F3, d). PhzA/B could also participate in the final "maturation" step, where the proton of the double imine is shifted. The model indicates, that the residue E140 would be ideally suited due to its proximity and mobility (Figure F3, e and f).[19]

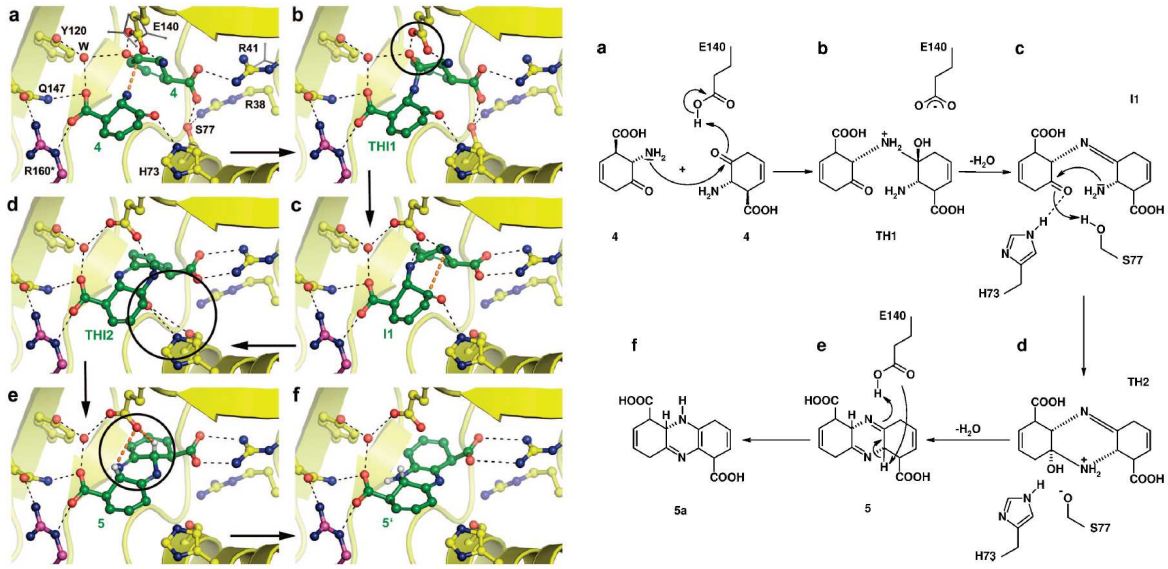


Figure F3: Left: Catalytic proposal for PhzA/B. 6-Amino-5-oxocyclohex-2-ene-1-carboxylic acid is depicted as "**4**". Right: Catalytic proposal for PhzA/B showing the most important proton transfer reactions. (Pictures taken from AHUJA *et al.*[19])

Leading on from the product of the PhzA/B cascade, several oxidative routes to gain aromaticity seem to be possible. This speculation is supported by the detection of phenazine-1,6-dicarboxylic acid (**PDC**) and the unsubstituted phenazine (Scheme S1) as byproducts.

2.2 Bioisosteres

2.2.1 The concept of bioisosterism

The concept of isosterism between simple chemical units was first considered by James Moir in 1909 and was further elaborated by Irving Langmuir, who termed groups of atoms having the same number of atoms and electrons as "isosteres" (e.g. N_2 and CO, N_2O and CO_2).[39,40] This idea was further refined by H. G. Grimm, who postulated the hydride displacement law according to which an atom gets the properties of the atom with the next highest atomic number upon addition of a hydride.[41] Hans Erlenmeyer observed that antibodies could not discriminate between phenyl and thienyl rings or O, NH, and CH_2 when employing artificial antigens derived by reaction of diazonium ions with proteins (Scheme S2).[39,41,42,43]

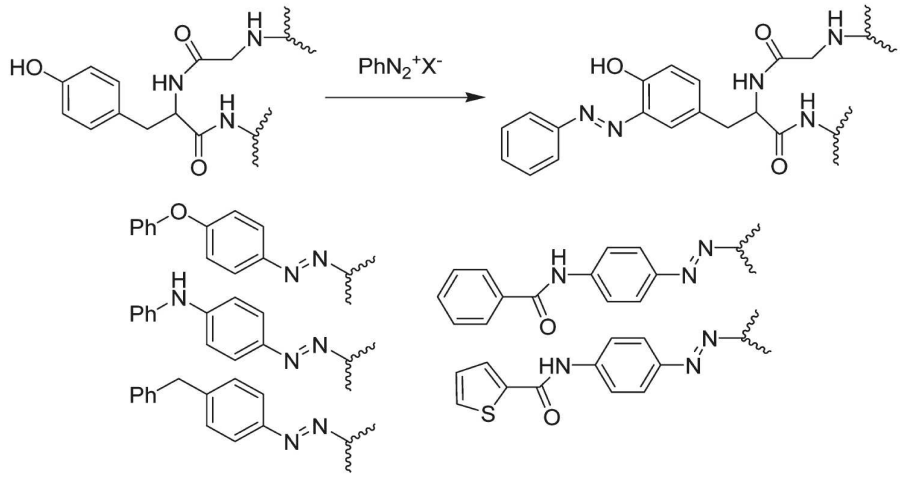


Figure S2: Erlenmeyer's artificial antigens[39,41,42,43] (Figure taken from MEANWELL[52])

The term "bioisostere" was introduced by Harris Friedman in 1950 who defined it as compounds having a similar biological effect and he pointed out, that isosteric compounds are not necessarily bioisosteric.[44] The application of bioisosterism will strongly depend on the context and the understanding of biochemical mimicry will prove more important than of the physicochemical properties. Bioisosteres are typically quite inexact structural mimetics and are often more alike concerning the biological than the physical properties. An effective bioisostere for one application may not translate to another biochemical setting and hence the bioisosteric groups will have to be selected and tailored independently. By means of design of the bioisosteres several attributes can be altered, such as size, shape, electronic distribution, polarizability, dipole, polarity, lipophilicity and pK_a. The development and application of bioisosteres constitute a fundamental strategic approach to

address a number of aspects related with the design and development of drug candidates.[39,41,45,46,47,48,49,50,51]

Manifold applications of bioisosteres are reported, ranging from improving potency, enhancing selectivity, altering physical properties, reducing or redirecting metabolism, eliminating or modifying toxicophores, and acquiring novel intellectual property.[52]

2.2.2 Carboxylic acid isosteres

The isosteres of carboxylic acid have been studied to a great extent, mainly by interest in inhibitors of the arachidonic acid pathway and by the development of angiontensin II receptor antagonists. The typical focus of the studies was increasing potency, decreasing polarity and raising lipophilicity to improve membrane permeability. This measure usually enhances the pharmacokinetic properties for *in vivo* applications and reduces the potential of toxicity.[52,53]

The most common isosteres for carboxylic acid groups are depicted in Figure F4. The use of heterocycles with modifiable substituents, commonly used for alteration of pK_a, widens the repertoire of bioisosteres (Figure F5).[52]

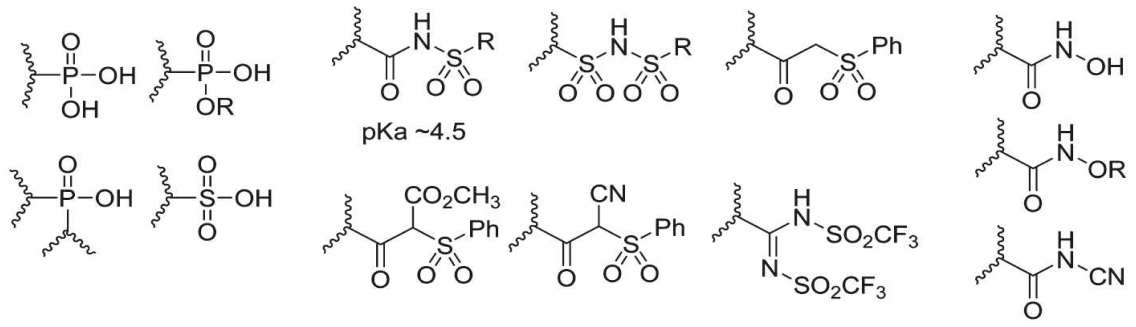


Figure F4: Synopsis of common carboxylic acid isosteres (Picture taken from MEANWELL[52])

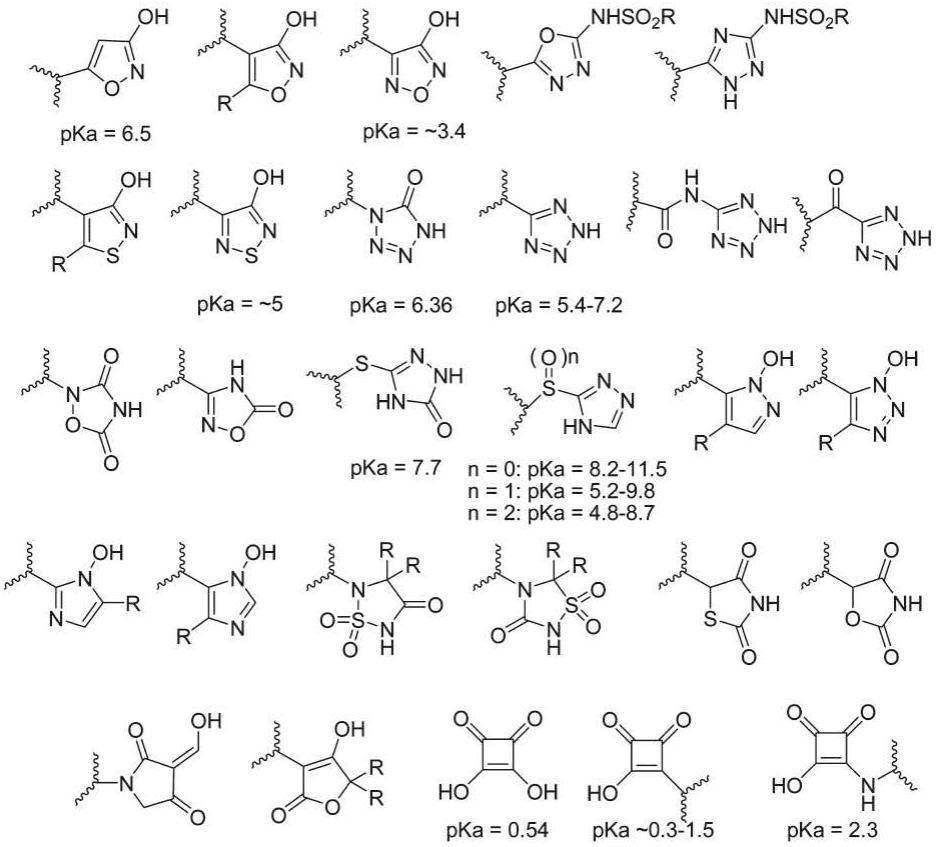


Figure F5: Synopsis of heterocycle-based carboxylic acid isosteres (Picture taken from MEANWELL[52])

2.2.2.1 Angiotensin II receptor antagonists

Angiotensin II receptor antagonists give an informatory insight into the design of carboxylic acid isostere design, since their binding affinity is quite sensitive to the identity of the acidic element. Losartan (Figure F6, **B**) exhibits a 10-fold increase in potency compared to the free carboxylic acid analogue (Figure F6, **A**). This result might be explained through geometrical analysis, which indicates that the acidic N-H proton is in the tetrazole moiety is placed more distant to the aryl ring than the CO_2H. Introducing an acylsulfonamide renders similar geometry and comparable potency to the carboxylic group (Figure F6, **C**). A reverse acylsulfonamide however projects the charge further away from the aryl core, comparable to the distance in the tetrazole. The negative charge would be expected to reside on the carbonyl oxygen (Figure F6, **D**).[52,54,55]

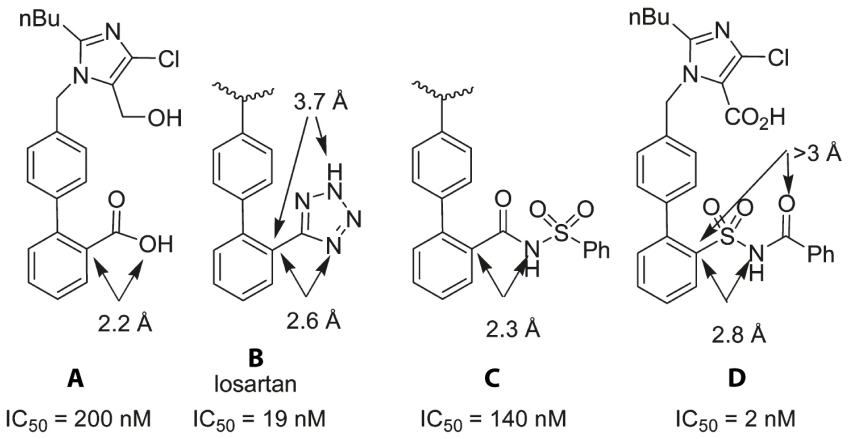


Figure F6: Angiotensin II receptor antagonists[52,54,55] (Picture taken from MEANWELL[52])

A second example from the field of angiotensin antagonists is L-158809, a potent and selective antagonist (IC_{50} = 0.3 nM) to AT_1 which shows prolonged antihypertensive activity in rats over a period of 6 h after dosing. In dogs and rhesus monkey the compound was rapidly cleared from the organism by glucuronidation of the tetrazole moiety. The replacement of the tetrazole with an acylsulfonamide, as represented with L-159282, preserved potency (IC_{50} = 0.2 nM) and extended the antihypertensive effect in rats. With this change, a similarly long effect was observed in the dog and rhesus monkey (Figure F7). The resistance of the acylsulfonamide toward glucuronidation is made responsible for these results.[52,56]

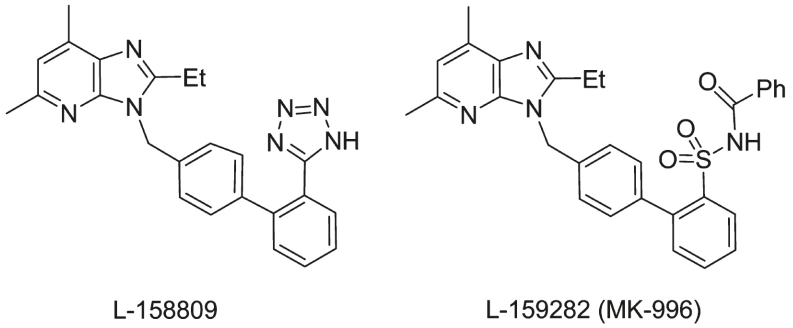


Figure F7: Angiotensin II antagonists[56] Picture taken from MEANWELL[52].

2.2.2.2 Acylsulfonamide in Bcl-2 inhibitors

Inhibitors of the antiapoptotic protein Bcl-2 have an application in oncology therapy. NMR screening identified two structural elements, the bipenylcarboxylic acid **A** and the phenol **B** (Figure F8), that bound weakly to the protein. It was hence tried to increase binding affinity by linking those two fragments via the ortho position of the benzoic acid (Figure F8, **C**), but all attempts failed. Significantly higher potency was then achieved by linking the fragments via an acylsulfonamide bridge (Figure F8, **D**), which preserved the acidic functionality of the carboxylic group that interacts with Arg_{139} in the active site of the Bcl-2 protein. The clinical candidate ABT-263 (Figure F8, **E**)

emerged from this work, which is orally bioavailable in spite of an molecular weight of 974 (Figure F8).[52,57,58]

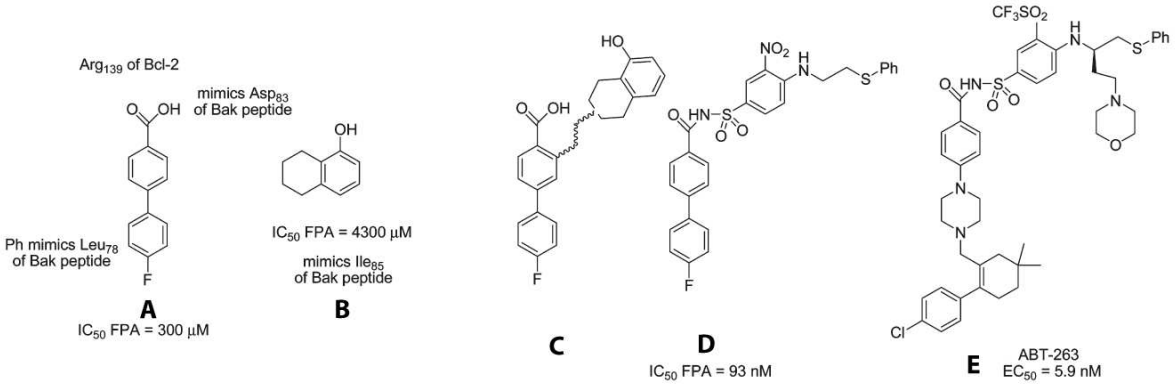


Figure F8: Inhibitors of the antiapoptotic protein Bcl-2[57,58] Picture taken from MEANWELL[52].

2.2.2.3 2,6-Difluorophenol as a CO_2H mimetic

The introduction of fluorine atoms at the positions 2 and 6 of phenol increased its acidity from $pK_a = 9.81$ to a pK_a of 7.12 for 2,6-difluorophenol, which suggested the assumption that this functionality might be used as lipophilic CO_2H mimetic. The concept of this assumption was the combination of the acidity of the OH and the potential of fluorine to imitate the carboxylic acid C=O by acting as a *H*-bond acceptor. 2,6-Difluorophenol derivatives **A** and **B** were synthesized and identified to be competitive inhibitors of GABA amino transferase.[59,52] The 2,6-difluorophenol moiety was also employed as an isostere of the carboxylic acid in aldose reductase inhibitor **C** and yielded **D** with an 6-fold increased potency (Figure F9).[52,60]

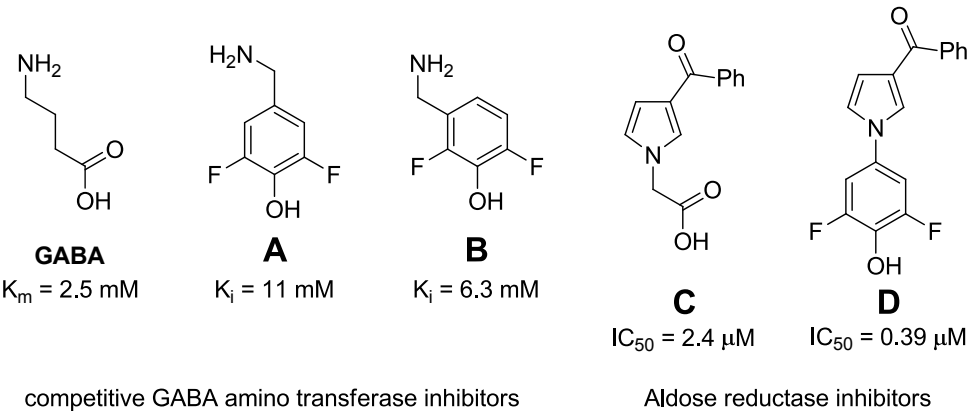


Figure F9: 2,6-Difluorophenol as CO_2H mimetic[52,59,60]

3 Aims of this thesis

In the course of his PhD thesis Matthias Mentelsynthesized a library of Phenazistatin analogues in order to study the binding characteristics towards the protein PhzA/B.[77,94] He was able to synthesize various mimics of the tricyclic product that bound to the protein and protein-ligand complexes were prepared by soaking of the native PhzA/B protein. The structural models obtained by x-ray crystallography revealed the binding mode of the probes and led to an improved understanding of the single events of the molecular catalysis. Isothermal titration calorimetry (ITC) led to the binding affinities (K_D and IC_{50}-values) of the compounds, which ranged from 2 to 25 µM.[19] The ligand **(1*R*,3*S*)-1** (Figure F10) was synthesized eventually and exhibited an affinity of 51 nM, which marked a 40-fold increase towards the earlier lead inhibitor (unpublished result). This lead structure is henceforth referred to as phenazistatin A, or **(1*R*,3*S*)-1**.

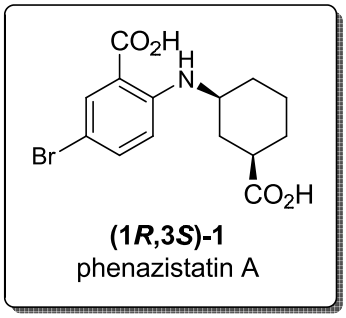


Figure F10: The lead structure penazistatin A

Phenazistatin A bears attractive functional groups for modifications and represents an ideal substructure for future lead structure optimization. For that purpose sufficient supplies of **(1*R*,3*S*)-1** must be provided. This target cannot be achieved with the current synthesis, since phenazistatin A emerged serendipitously as a by-product in the crucial C-N coupling.

The aim of the following thesis was to develop a reliable synthetic route towards Phenazistatin A in order to ensure the supply for starting off with the lead structure optimization. Several expected difficulties will have to be overcome, such as the chiral resolution of the amine building block, the selective introduction at the aryl moiety and the development of selective C-N coupling conditions.

An additional aim was to diversify the class of phenazistatin by replacing the functional groups to modify the physicochemical as well as the biological properties, such as size, shape, polarity, lipophilicity, pK_a, bioavailability, toxicity or potency for *in vitro* and future *in vivo* studies. This could be achieved by converting the free carboxylic acid group into bioisosteric groups like hydroxamic acid, or the more lipophilic 1*H*-tetrazole group. The bromine substituent can be utilized for elongation by Pd-catalyzed cross-couplings such as SUZUKI-, SONOGASHIRA-, HECK-reaction or α-arylation of ketones. Membrane permeability is an important issue for *in vivo* studies and the free carboxylic groups might pose a problem for that. Converting them into more lipophilic ester groups

may lead to an improved permeability and the intracellular hydrolases are expected to cleave carefully chosen ester functionalities to release the active phenazistatin.

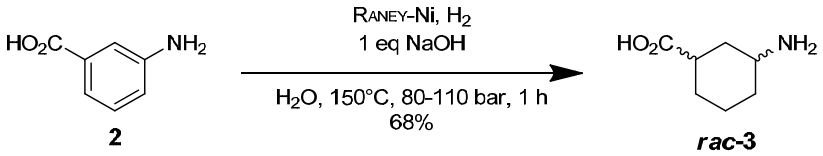
The synthesized phenazistatin will be analyzed for their biological activity and binding mode to PhzA/B and they will be furthermore employed in *in vivo* studies by our cooperation partner Prof. Wulf Blankenfeldt (University of Bayreuth). These experiments may indicate, if inhibition of PhzA/B has a bactericidal effect and if phenazistatines may potentially be employed in future drug discovery.

4 Results and discussion

4.1 Limitations of the current synthesis of phenazistatin A (1*R*,3*S*)-1

The current synthesis of phenazistatin A **(1R,3S)-1** by Matthias Mentel[77] exhibits several drawbacks and is hence not viable to be employed for prospective substrate preparation. A major point mentioned beforehand is the low overall yield of roughly 0.8%, which makes it reasonable to search for a more efficient synthetic pathway. Based on the pros and cons of the current synthesis, a more feasible synthetic strategy needed to be developed.

MENTEL started the synthesis with a catalytic hydrogenation of 3-aminobenzoic acid (**2**) with RANEY-Ni in alkaline solution. In spite of the harsh reaction conditions of 80-110 bar and 150°C, full conversion could not be obtained. A moderate yield of 68% the stereoisomers (37% remaining substrate) was isolated (Scheme S3).



Scheme S3: Catalytic hydrogenation of 3-aminobenzoic acid (**2**) using RANEY-Ni[77]

As a result of the catalytic hydrogenation two chiral centres and four stereoisomers are generated (Figure F11). Catalytic hydrogenation reactions without additives yield an excess of the *cis*-enantiomers, since the all-equatorial conformation is favoured thermodynamically. The extent of this excess usually depends on the conditions and kind of catalyst used in this reaction. BADLAND *et al.* report a *cis*/*trans*-ratio of 5:1 using 5% Rh on charcoal as a catalyst.[61]. The exact ratio of *cis*/*trans* in the current synthesis was not reported, only the emergence of an additional set of NMR-signals was described. For reasons of simplification, these configurations will henceforth be referred to as *cis*- and *trans*-configuration.

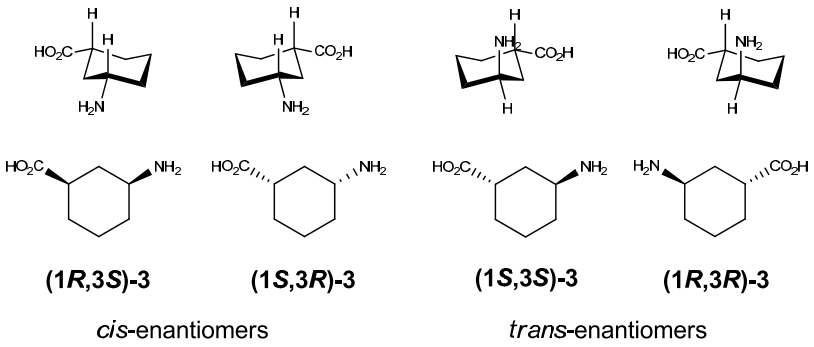
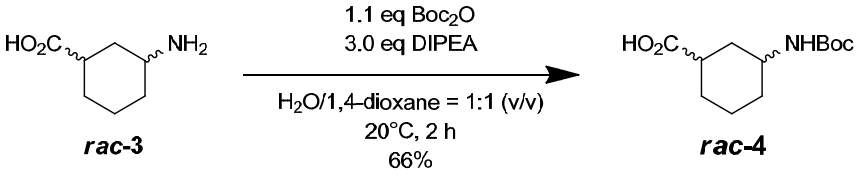


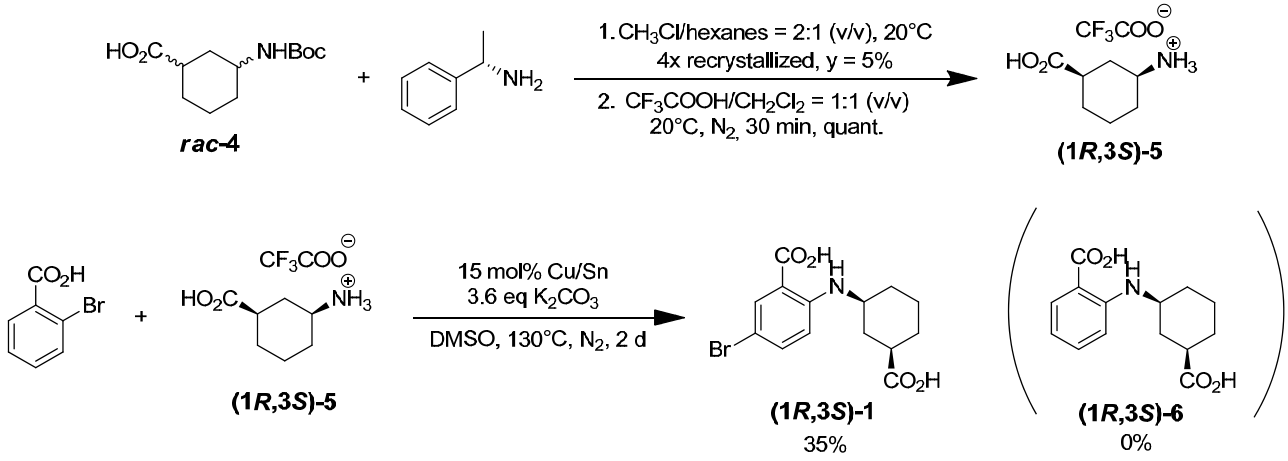
Figure F11: Possible product-stereoisomers of 3-aminocyclohexanecarboxylic acid (**3**)

The amino group of ***rac*-3** was protected smoothly at room temperature, using a Boc_2O/DIPEA protocol in a H_2O/1,4-dioxane mixture and the crude product could be purified by recrystallization affording the product ***rac*-4** in good yield (Scheme S4).



Scheme S4: *N*-protection of 3-aminocyclohexanecarboxylic acid (**3**)

The chiral resolution was performed using 1.0 eq (*S*)-(-)-1-phenylethylamine to generate a diastereomeric salt followed by repeated recrystallization. This resolution outlines a crucial step in the synthesis, since the crude product had to be recrystallized four times from a mixture of chloroform/hexanes (2:1 (v/v)) and yielded only 5% of the product salt **(1*R*,3*S*)-5**. The enantiomerically pure *N*-protected amino acid **(1*R*,3*S*)-4** was isolated quantitatively by acidification and extraction with water. **(1*R*,3*S*)-4** was deprotected with TFA/CH_2Cl_2 = 1:1 (v/v) and was then reacted with 2-bromobenzoic acid using ULLMANN-condensation conditions. Surprisingly, the major product generated (35%) was the brominated **(1*R*,3*S*)-1** and not the expected non-brominated **(1*R*,3*S*)-6** (Scheme S5). MENTEL suggested a bromination by an electrophilic substitution (S_EAr) mechanism during the workup to occurr, that might be mediated by Cu(II) bromide presumably formed during the reaction or workup conditions.[77] The work-up and purification of the ULLMANN-condensation reaction is inconvenient and includes acidification, filtration, centrifugation and semi-preparative RP-HPLC. This expensive means of purification does not allow a future large-scale preparation of the product **(1*R*,3*S*)-1**.



Scheme S5: Chiral resolution of ***rac*-4** and ULLMANN-condensation reaction of **(1*R*,3*S*)-5** with 2-bromobenzoic acid.

This synthesis towards the key intermediate Phenazistatin A **(1*R*,3*S*)-1** needs to be changed considerably. Firstly the catalytic hydrogenation has to be improved so that full conversion and a high *cis*/*trans* – ratio can be obtained. Secondly, the efficiency of the chiral resolution has to be enhanced. In order to build up a decent library of Phenazistatin analogues, gram quantities of the chiral amino acid **(1*R*,3*S*)-4** will be needed. Even though the chiral resolution is placed early in the total synthesis, a tedious recrystallization procedure with a yield of 5% to obtain a sufficient e.e. cannot be tolerated. Beyond that, the hazardous solvents chloroform and hexane should be replaced by safer solvents. Thirdly, the present retrosynthetic strategy with the fortunate introduction of bromine during the fusion of the two building blocks in the ULLMANN-condensation reaction (Scheme S5) has to be reconsidered. The bromine substituent will need to be introduced selectively at the last step or a bifunctional aromatic moiety will have to be employed. The bifunctional aromatic moiety, e.g. a dihaloarene, would need to react stereoselectively during the ULLMANN-condensation reaction. Chemoselectivity and by-product generation will be an issue too, since purifications in a gram-scale cannot be achieved with semi-preparative HPLC.

4.2 A new approach towards Phenazistatin A (1*R*,3*S*)-1

The newly proposed approach to the lead molecule is depicted in Figure F12. It would start off according to the current synthesis with a catalytic hydrogenation, protection and chiral resolution but then lead on proceeds with an ULLMANN-condensation reaction with a bifunctional aromatic moiety to afford Phenazistatin A. The major obstacles to overcome are highlighted in colour, such as the synthesis of the aromatic moiety, the chiral resolution, the optimization of the Ullmann-condensation conditions and subsequently the modification of the Phenazistatin backbone in order to generate analogues.

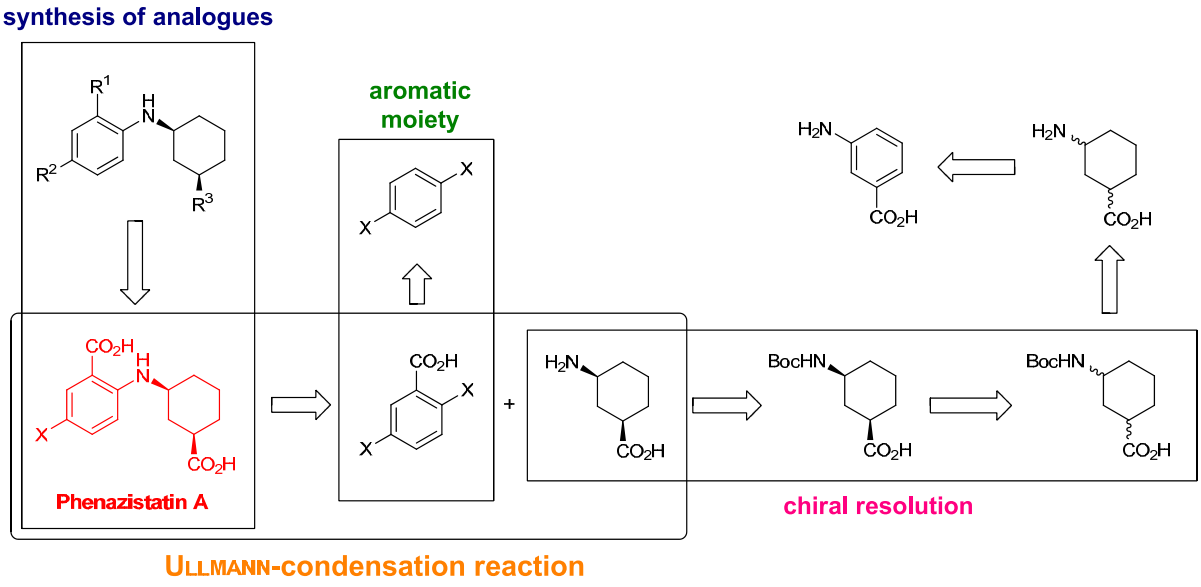
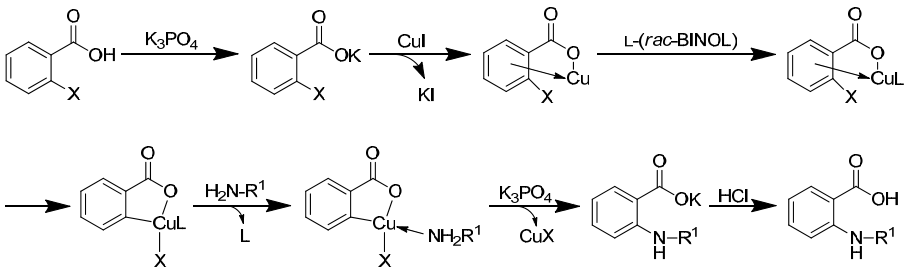


Figure F12: A new retrosynthetic approach towards phenazistatin A (**1**)

The most significant modification in this newly proposed synthetic strategy (Figure F12) is the use of a bifunctional aromatic moiety. The challenge is, how to achieve selective *N*-arylation with just one functional group, while the other one (preferably a bromine) stays idle. One option was proposed by JIANG *et al.*, who report room temperature conditions for a selective mono *N*-arylation of iodobenzenes containing a chlorine- or bromine-substituent. For both substrates, the coupling occurs exclusively at the C-I bond.[62] Hence a 2,5-dihalobenzoic acid holding an iodine- and a bromine-substituent in the respective positions might lead to success. This consideration is additionally supported by the works of ZENG *et al.*, who investigated the *ortho*-substituent effect of the carbonyl group on the ULLMANN-condensation reaction of 2-halobenzoic acids at room temperature. They describe, that the copper-catalyzed aminations of 2-bromo- and 2-iodobenzoic acid derivatives proceed in a regioselective manner only on the carbon-halogen bond adjacent to the carboxyl group at room temperature, yet in presence of –Cl or –Br substituents. They presume, that this is due to the accelerating effect of the carboxyl group and suggested a possible mechanism for the condensation reaction (Scheme S6).[63]



Scheme S6: Suggested accelerating effect of an ortho carboxyl group during an ULLMANN-condensation reaction[63]

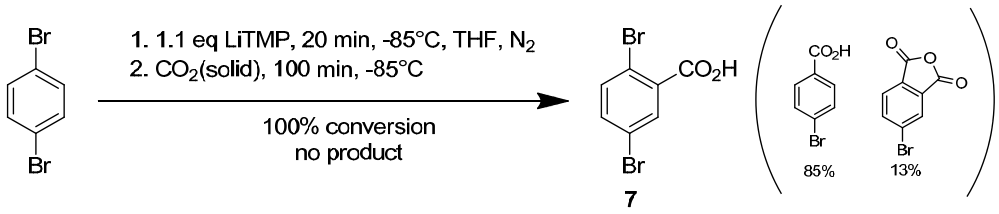
These references suggest two dihalobenzenes, that would be ideal for the selective ULLMANN-condensation reaction: 2,5-dibromobenzoic acid (**7**) and 5-bromo-2-iodobenzoic acid (**8**). Both aromatic moieties would react preferentially in the required position due to the accelerating effect of the carboxyl group in *ortho*-position but 5-bromo-2-iodobenzoic acid (**8**) would have an additional increase of selectivity thanks to the favoured reactivity of iodine over bromine.[62,63] It was hence tried to synthesize both substrates for the verification of this assumption.

4.3 Synthesis of the bifunctional aromatic moiety

The first attempt to synthesize 1,4-dibromobenzoic acid (**7**) was an *ortho*-lithiation reaction at -80°C followed by quenching with CO_2 as described by LULINSKI *et al.*.[64] LiTMP was prepared *in situ* by dropwise addition of *n*-BuLi to a stirred solution of TMP in THF at -85°C and stirring for 10 min. The CO_2 was freshly resublimed from a gas bottle and was stored in a flask with lid to minimize the condensation of water. The reaction mixture was quenched upon completion of the substrate but no product was generated. Instead GC-MS analysis indicated 4-bromobenzoic acid and

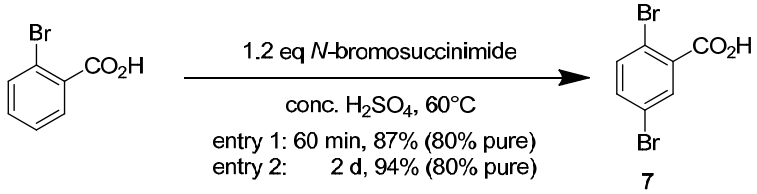
5-bromoisobenzofuran-1,3-dione as the main products (Scheme S7). In both cases a lithiation of the bromine occurred and only in the latter case an *ortho*-lithiation occurred at some point. The reason might be that the sterically hindered base LiTMP had not yet been fully generated during 10 min at -85°C and that the stronger base *n*-BuLi lithiated the C_{Ar}-Br (Scheme S7). Other procedures describe the quick preparation of LiTMP with *n*-BuLi at temperatures up to 0°C, which was tested during the next experiment.[65]

For this try, the *n*-BuLi/TMP mixture was left warming to 0°C within 100 min and was then treated with the 1,4-dibromobenzene solution at -85°C (step 1, Scheme S7). The solution was kept for 60 min at a temperature of -80°C, was cooled to -100°C and dry ice was added. The reaction mixture was left warming to room temperature afterwards. In this reaction, no conversion was observed and GC-MS analysis displayed the pure substrate. It seems like no lithiation occurred at any point of the reaction.



Scheme S7: Ortho-lithiation of 1,4-dibromobenzene followed by quenching with CO_2[64]

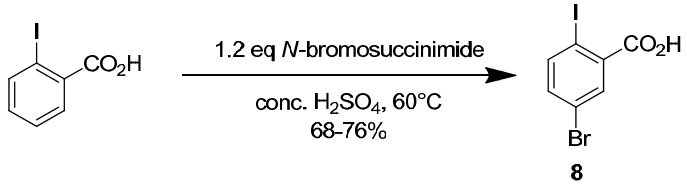
This approach was laid aside and an alternative synthetic path was tested. RAJESH *et al.* reported a convenient bromination of deactivated aromatic systems using *N*-bromosuccinimide (NBS) as a reagent[66] and VALOIS-ESCAMILLA *et al.* used this procedure to prepare 5-bromo-2-iodobenzoic acid (**8**) in good yields.[67] 2-Bromobenzoic acid could be brominated, but the purity of the product did not exceed 80% as determined by ^{1}H-NMR. The yields mentioned below are the crude yields and the purities were determined by ^{1}H-NMR. Recrystallization from H_2O/MeOH = 2:1 (v/v) was attempted, but the purity could not be increased (recrystallization yield = 39%) (Scheme S8).



Scheme S8: Selective bromination of 2-bromobenzoic acid with *N*-bromosuccinimide[66]

Trying exactly the same reaction conditions with 2-iodobenzoic acid afforded the product **8** in good yields. A special feature of this reaction is, that the product starts precipitating as a colourless solid from the reaction mixture after addition of ~1.0 eq NBS (entry 1: 0.98 eq, entry 2: 1.01 eq). The yellowish suspensions could not be stirred and were further heated (entry 1: 2 h, entry 2: 1 h). Isolation of the product was performed by dilution with water, filtration and washing. Traces of

impurities could be detected in ^{1}H-NMR and were removed by recrystallization from H_2O/MeOH = 2:1 (v/v) to give pure 5-bromo-2-iodobenzoic acid (**8**) (Scheme S9).

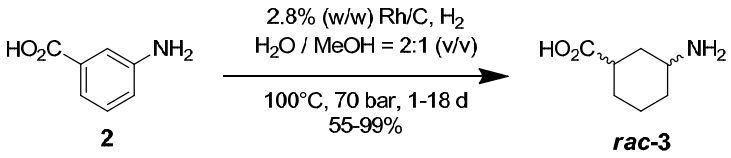


Scheme S9: Selective bromination of 2-iodobenzoic acid with *N*-bromosuccinimide[66]

4.4 Synthesis of 3-((*tert*-butoxycarbonyl)amino)cyclohexane-carboxylic acid (*rac*-4)

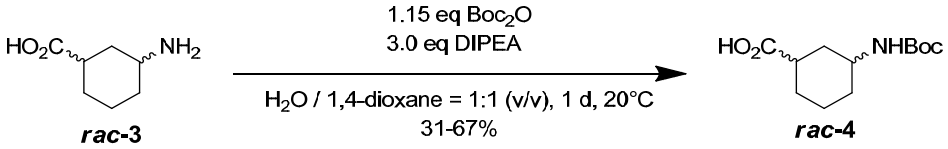
The previously employed catalytic hydrogenation of **2** with RANEY-Ni was put away and a protocol of BADLAND *et al.* was adapted, who used 5% rhodium on charcoal as a catalyst. They could reduce the temperature to 70°C (20 bar H_2) and increase the *cis*/*trans*-ratio up to 5:1. A drawback of the postulated procedure is the high catalyst input of 20% (w/w).[61]

For cost reasons the catalyst loading was considerably reduced to 2.8% (w/w) in all the conducted experiments. This alteration manifested itself in the reaction times, which ranged depending on the scaling from 1 d to 18 d (Scheme S10). As an unfortunate spin-off, the *cis*/*trans*-ratio decreased to 2:1, which was preserved throughout all performed catalytic hydrogenations. The increase of temperature to 100°C might as well be the cause of the drop in the *cis*/*trans*-ratio. It was not attempted to separate the diastereomers at this stage and the crude product from the catalytic hydrogenation was used in the following step (and directly for the ULLMANN-condensation screenings) without further purification.



Scheme S10: Catalytic hydrogenation of 3-aminobenzoic acid (**2**) using Rh/C

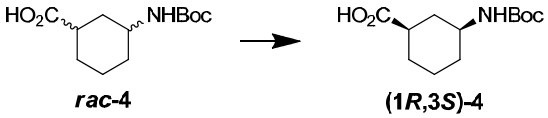
The protection of the amino acid ***rac*-3** was carried out using Boc_2O and the below-mentioned conditions afforded moderate yields (Scheme S11). It was tried to remove the *trans*-enantiomers through recrystallization from hot CH_2Cl_2 followed by precipitation with *n*-pentane as described in the above mentioned publication[61], but the effort miscarried. It might well be, that the low excess of *cis*-enantiomers impedes the clean crystallization of just one pair of enantiomers. As a result, the surplus *trans*-enantiomers were not separated and the crude product (*cis*/*trans* - ratio = 2:1) was subjected to the following chiral resolution.



Scheme S11: *N*-Protection of the amino acid ***rac*-3**

4.5 Chiral resolution of *rac*-3-((*tert*-butoxycarbonyl)amino)-cyclohexanecarboxylic acid (*rac*-4)

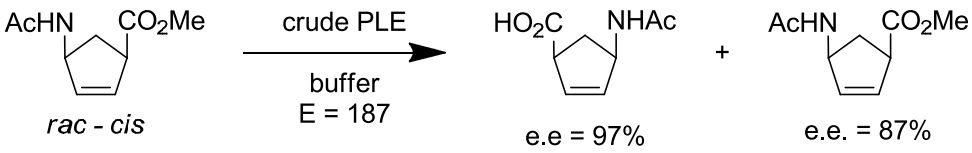
The next hurdle in the synthetic strategy is the chiral resolution of the four stereoisomers of ***rac*-4** to obtain the desired **(1*R*,3*S*)-4** in high enantiomeric purity (Scheme S12). In the course of the laboratory work for this thesis two approaches have been tested. Firstly, the kinetic chiral resolution using porcine liver esterase (PLE) and secondly, the chiral resolution by recrystallization with the aid of a chiral amine have been examined.



Scheme S12: Chiral resolution of *rac*-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (**rac-4)**

4.5.1 Kinetic chiral resolution using PLE

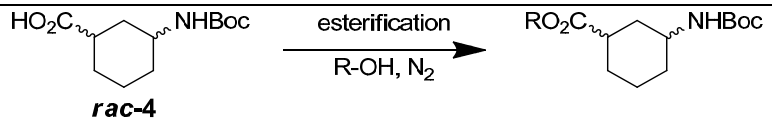
Porcine/pig liver esterase (PLE) has become an important tool in various biotransformations, such as the chiral resolution of racemic esters (Scheme S13). Several features like the general versatility, the wide substrate tolerance and the price contribute to the popularity. For many applications a crude and hence cheap enzyme preparation (a mixture of 6-7 *iso*enzymes) can be used.[68] In the following experiment such a crude enzyme preparation was applied.



Scheme S13: Scheme of a kinetic chiral resolution of *N*-acetylaminocyclopentene carboxylate[68]

As a substrate for the PLE-experiment, both methyl- and ethylester were synthesized using various procedures (Table T1). The methods relying on the *in situ* generation of HCl did not achieve full conversion as was determined by the remaining acidic proton signal of the carboxyl group in ^{1}H-NMR. The reactions were performed under dry conditions, but the generated water seems to drive the reaction to a certain equilibrium state. The reaction might be pushed further by means of water separation (entries 1 and 3). The transesterification reaction using 1,1,1-triethoxyethane and PTSA as a catalyst in EtOH at 20°C yielded 70% of the product ***rac*-9** after column chromatography (entry 4).

The esterification reactions with the coupling reagent *N*,*N*'-diisopropylcarbodiimide proved to be reliable and convenient, and were usually performed as over-night reactions. Most of the generated urea could be removed by filtration and the rest was removed during column chromatography (entries 2 and 5).[69] **(1*R*,3*S*)-9** could later be synthesized with the same conditions in 86% yield by using CH_2Cl_2 as solvent. The *N*,*N*'-diisopropylurea could be removed almost quantitatively by filtration due to the low solubility in CH_2Cl_2 (entry 6).[69]



***rac*-4**

entry	product	reagents	solvent	temperature / °C	duration	% conversion	% yield
1	***rac*-10**	0.1 eq acetyl chloride 0.01 eq TMS-Cl	MeOH	80	6 d	incomplete	traces
2	***rac*-10**	1.05 eq DIC, 1.5 eq MeOH, 0.01 eq 4-DMAP	THF	-78 to 20	17 h	100	42%
3	***rac*-9**	0.1 eq acetyl chloride, 0.01 eq TMS-Cl	EtOH	80	6 d	incomplete	traces
4	***rac*-9**	5 eq 1,1,1-triethoxyethane, 0.01 eq p-tolylsulfonic acid	EtOH	20	17 h	100	70
5 (6)	***rac*-9**	1.05 eq DIC, 1.5 eq EtOH, 0.01 eq 4-DMAP	THF (CH_2Cl_2)	-78 to 20	17 h	100	57 (86)

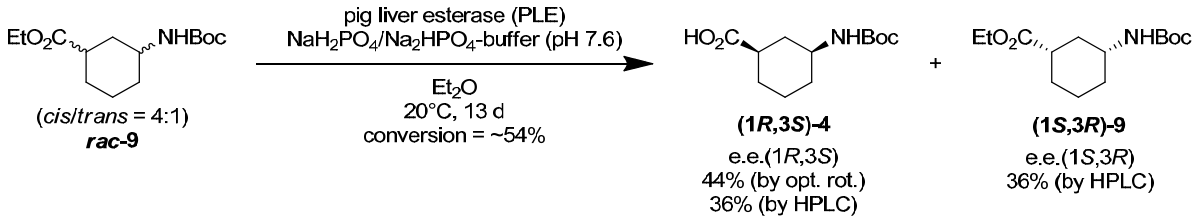
Table T1: Esterification of the *N*-protected ester ***rac*-4**

In the following experiment a precipitate of PLE (a mixture of six to seven *iso*enzymes) in half-saturated $(NH_4)_2SO_4$ was used, which was kindly provided by Prof. Dr. Harald Pichler (IMBT, Graz University of Technology).[70,71,72,73,74] Reaction monitoring was performed by HPLC-UV with a Chiralpak® AD-H column with *n*-heptane/ethanol = 95:5 as an isocratic eluent. The *cis*-enantiomers of the ethylester **9** could be baseline separated using a shorter time program, hence it was decided on using the ethylester ***rac*-9** instead of the methylester ***rac*-10**.

The enantioselectivity (E and k_{rel}) for the substrate ***rac*-9** could not be calculated, since the generated acid ***rac*-4** could not be resolved on chiral HPLC. The calculation requires both enantiomeric excess of the acid **4** (aqueous phase) and the ester **9** (organic phase). Changes of the column (Chiralpak® OD-H), temperature, solvent (MeOH) and the additive (CF_3COOH) had no effect on the chromatographic resolution. As a result of this, it was tried to abort the reaction at a turnover of ~50% (reaction rate estimated by pre-trials) in order to acquire convincing data, whether the kinetic racemic resolution with PLE can be applied in a preparative scale.

This was done by a workup at roughly 50%, and chiral analysis of both the organic phase (remaining substrate ester ***rac*-9** by chiral HPLC) and aqueous phase (acid product ***rac*-4**. indirectly, e.g. by optical

rotation or esterification followed by chiral HPLC). It was assumed, that this procedure would lead to a fair estimate.



Scheme S14: Kinetic chiral resolution of the ester ***rac*-9** with PLE

107 mg ***rac*-9** (*cis*/*trans* = 4:1) were dissolved in a two phase system of Et_2O and 2.0 eq phosphate-buffer (pH 7.6), followed by addition of 400 µL PLE-suspension and stirring (200 rpm) at 20°C (Scheme S14). The turbid reaction mixture was stirred for 13 d until a turnover of 54% was reached, which was determined by the mass of the remaining ester ***rac*-9** in the reaction mixture. Chiral HPLC-UV of the organic phase indicated an e.e.(1*S*/3*R*) of 36% and the excess of the non-desired **(1*S*/3*R*)-9**, and hence the preferred conversion of the desired **(1*R*/3*S*)-9**. The conversion of the desired ester **(1*R*/3*S*)-9** was proven by saponification of the remaining substrate from the organic phase with KOH in THF/H_2O = 1:1 followed by the measurement of the optical rotation. The obtained value of $[\alpha]_D^{20}$ = +4.8° (c = 0.8325, MeOH) suggests a deficiency of **(1*R*/3*S*)-4** (lit.(1*R*,3*S*): -50.5° (c = 1, MeOH))[75]. This measured value is fraught with uncertainty, due to the remaining *trans*-enantiomers and other possible impurities.

The generated **(1*R*/3*S*)-4** was isolated by acidification and extraction of the aqueous phase. Recovery of the substrate and the product for exact data for conversion and yield imposed a problem. Only 61 mol% could be recovered, adding up the extracted ester **9** and the acidified/extracted acid **4**. Discrimination of substrate and product might have happened during the extraction- and filtration processes. The isolated **(1*R*/3*S*)-4** exhibits an e.e.(1*R*,3*S*) of 44% (by optical rotation), but this result also needed to be checked by chiral HPLC-UV. For this purpose **(1*R*/3*S*)-4** was esterified in excellent yield using EDC·HCl, 4-DMAP, EtOH in CH_2Cl_2 at 20°C. Chiral HPLC-UV of the generated **(1*R*/3*S*)-9** gave an e.e.(1*R*, 3*S*) = 36% and hence confirmed the previously obtained value.

The most important parameter for a kinetic chiral resolution using PLE is the enantioselectivity (E or k_{rel}), which describes the reaction rate ratio between the two enantiomers. With low enantioselectivities (k_{rel}) the recovered substrate can usually be prepared in high e.e., just by increasing the conversion at the cost of the isolated total yield. This is not the case for the product, where a high enantioselectivity is required for a high e.e. below 50% conversion (Figure F13).[76] Within the above mentioned experiment low enantiomeric purities have been detected in both the recovered substrate (Et_2O) and the product (buffer) phase, which leads to the assumption that PLE has a very low enantioselectivity for the substrate ***rac*-9**. As described earlier, the recovered

substrate could be isolated in high purities, but unluckily the desired **(1*R*/3*S*)-9** is converted preferentially. These facts lead to the conclusion, that the porcine liver esterase reaction is not the method of choice for the chiral resolution of ***rac*-9**.

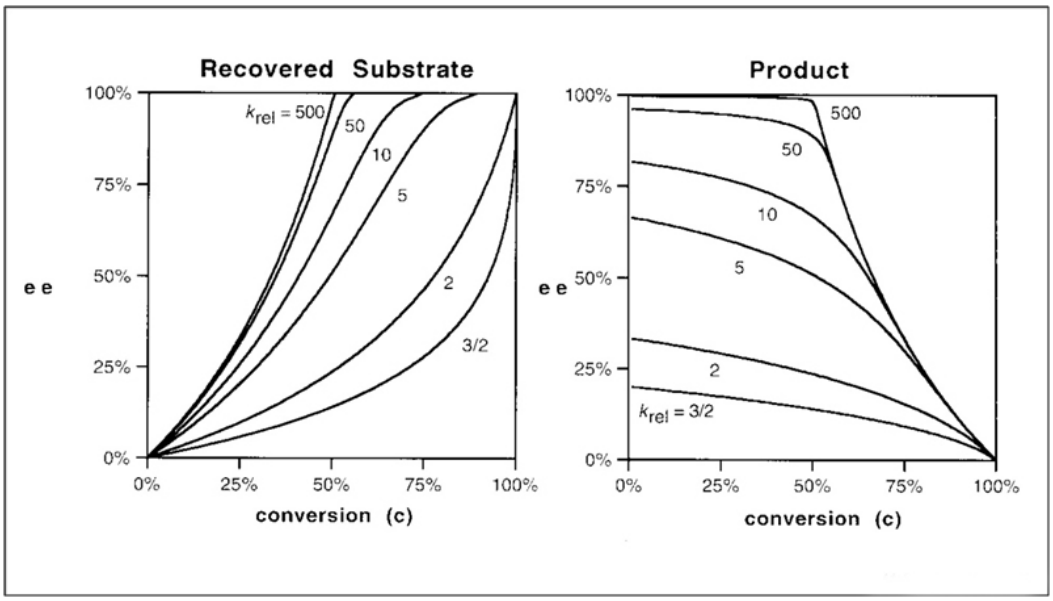
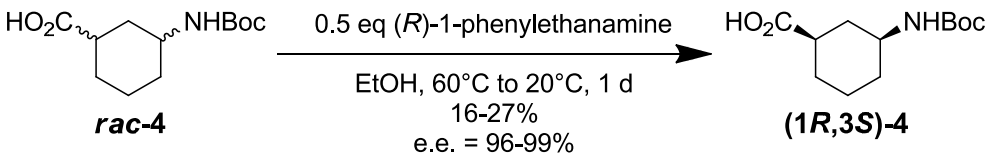


Figure F13: Enantiomeric purities of the recovered substrate and the product, depending on the conversion. Picture taken from KEITH *et al.* [76]

4.5.2 Transformation into diastereomers and recrystallization

Since the chiral resolution with PLE could not provide a viable alternative, the so far low-yielding chiral resolution by recrystallization was tested. Following the procedure of BADLAND *et al.*, the solvent was changed from chloroform/hexanes to the less hazardous EtOH and instead of 1.0 eq (*S*)-1-phenylethanamine just 0.5 eq of (*R*)-1-phenylethanamine was used.[61] With the reduced employment of the chiral amine, **(1*R*,3*S*)-4** could be isolated in moderate yields and excellent enantiomeric purities right after the first crystallization (Scheme S15).



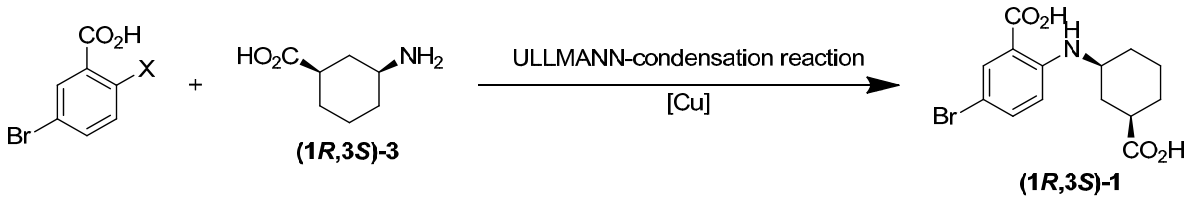
Scheme S15: Chiral resolution of ***rac*-4** using (*R*)-1-phenylethanamine

The enantiomeric purity of diastereomeric salts with lower e.e. (~90%) could not be increased by repeated recrystallization from hot EtOH, not even by seeding with the (*R*)-1-phenylethanamine salt **(1*R*,3*S*)-11** (98% e.e.). It was observed, that seeding a freshly prepared diastereomeric salt of ***rac-4*** with **(1*R*,3*S*)-11** (98% e.e.) at 60°C followed by slow cooling to room temperature and vigorous stirring afforded excellent purities, albeit slightly lower yields. Best results were obtained when ***rac-4*** was dissolved in the lowest possible amount of EtOH at 20°C, followed by addition of 0.5 eq of (*R*)-1-

phenylethanamine. Within 30 min of stirring precipitation occurred and the precipitate was subsequently dissolved at 60°C. The clear solution was removed from the oil bath, seeded with high-e.e. salt and was stirred vigorously for dispersion of the seed crystals for at least 1 d. The yield was improved from 5% (4x recryst.)[77] to 16-27% (1x recryst., e.e. = 96-99%) and this value was seen as sufficient and was hence not further optimized.

4.6 Optimization of the ULLMANN-condensation reaction

The most challenging part of the total synthesis turned out to be the ULLMANN-condensation reaction of the two subunits (Scheme S16). The substrate ***rac*-3** had not been used previously in a Cu-catalyzed cross-coupling, but few procedures for aliphatic, acyclic γ-amino acids have been reported.[78] The effects of an amine with an attached γ-carboxyl group are not well understood yet, but an accelerating effect of α-amino acids has been described[79]. Several methods for β-amino acids and also β-amino esters have been described[80], but on the basis of practicability it was first focussed on the ULLMANN-coupling of the free γ-amino acid ***rac*-3**, since it was directly accessible after chiral resolution and deprotection and any further step would lead to a loss of the enantiomerically pure **(1*R*,3*S*)-3**.



Scheme S16: Scheme of the desired ULLMANN-condensation reaction

For reasons of convenience the screenings were performed using ***rac*-3** with a ratio of *cis*/*trans* = 2:1 and easily available aromatic substrates. Ethyl-2-bromobenzoate and ethyl-2-iodobenzoate were synthesized in good yields using acidic catalysis (3.0 eq conc. H_2SO_4) in EtOH and the other substrates were purchased. The following yields were obtained by analyzing the area % of the corresponding 210 nm HPLC-peaks. This leads to certain inaccuracy, because the reactants feature different UV-absorption spectra and they might be over or underestimated. Since most of the samples were extracted directly from the reaction mixture and analyzed without drying, the solvent peak containing the amino acid ***rac*-3** was not integrated. Conversion was hence roughly determined by the consumption of the aromatic substrate. This procedure was kept the same for the following ULLMANN-condensation screenings.

It has been observed, that the ULLMANN-coupled products share similar properties regarding their properties and consequently the reaction monitoring was simplified. The products are typically yellow-brown solids that are stable at air. Solutions of products dissolved in MeOH, CH_3CN or water

change their colour slowly to pink and later to brown but no significant degradation product could be identified. The substances furthermore feature three definite absorption bands, the highest usually between 330-370 nm. TLC spots at 366 nm exhibit blue fluorescence and can be stained with CAM to develop an intensive red/orange colour.

4.6.1 ULLMANN-Screenings

The first screening was performed to reproduce the reaction conditions used by Matthias Mentel which yielded 35% Phenazistatin A **(1*R*,3*S*)-1** as product.[77] The screening was performed using the same settings, except the workup was carried out differently. Instead of filtering through cotton, eluting with 0.5 M NaOH and acidification with conc. HCl, the solvent was removed in oil pump vacuum and the residue was subjected to column chromatography (Table T2, entries 1-5). Surprisingly, **(1*R*,3*S*)-1** could not be detected in any of the trials and the expected coupling products (non-brominated) were generated as the main product. This result is supported by HPLC analysis of a **(1*R*,3*S*)-1** reference sample. Furthermore, the reaction monitoring by HPLC-MS (an aliquot was diluted, filtrated and subjected to HPLC-MS) indicated no alteration of the product range during the performed workup. The formation of the brominated byproducts in Matthias Mentel's work seems to depend on the alkaline/acidic workup procedure. It was not followed up whether exactly the acidic or alkaline environment was responsible for this side reaction.[77]

In order to elaborate if the yield could be improved by using other high-temperature conditions, $Cu(OAc)_2 \cdot H_2O$ as a copper source and Fe(III) acetyl acetonate as a co-catalyst were tested (Table T2, entries 6-10). This reaction featured similar product yields and trends of reactivity like the previous one, but a noteworthy increase of byproducts was observed. With 2-bromobenzoic acid (entry 6), a by-product (7%) featuring three discrete absorption maxima was spotted, and in (entry 7) saponification of the product occurred. As purification of the generated byproducts by flash-chromatography proved to be difficult, they were not identified.[81]

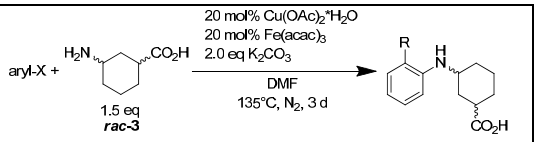
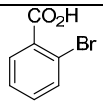
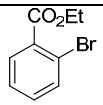
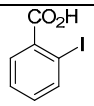
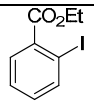
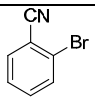
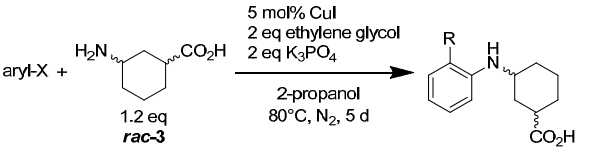
						
aryl-X	entry	% conversion	% yield	entry	% conversion	% yield
	1	100	62 (13[a])	6	100	48
	2	100	32	7	100	7 (3[b])
	3	100	63	8	100	53
	4	100	28 (6[b])	9	N/A	10
	5	12	6	10	21	7

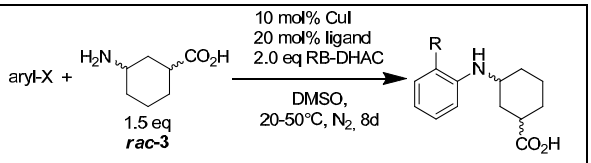
Table T2: ULLMANN-condensation reaction according to GP-U1 (entries 1-5) and GP-U2 (entries 6-10).
a: isolated yield, b: saponified product ***rac*-6**

The high temperature reaction conditions seemed not to provide a high-yielding option for the *N*-arylation , along with the expected selectivity issues when applying two different halogen atoms on a building block. During the last years huge progress in ULLMANN-condensation reactions has been made and with the use of special ligands the reaction temperatures could be lowered significantly. In the first trial ethylene glycol was utilized as a ligand, as described by KWONG *et al.*[82] A lower loading of the amino acid was employed and the reactions were stopped and worked up upon full conversion. The reaction temperature could be lowered to 80°C, but the yield decreased considerably at the cost of mainly one by-product. This product could not be isolated and identified, but it might originate from an *O*-arylation of the ethylene glycol. M. MENTEL described an analogous *O*-arylation competing with the *N*-arylation whilst working with 2-ethoxyethanol as a solvent (Table T3, entries 1-4).[77]

To overcome this difficulty, non-reactive solvents were chosen in the following screenings. For the next attempt DMSO was used as a solvent and the base was altered to a readily available resin-bound organic base (RB-dimethylhydroxyethylammonium carbonate, RB-DHAC) which could be prepared from a commercially available ion exchange resin (Table T3, entries 5-9). HUANG *et al.* have accomplished to couple a wide range of substrates at room temperature, removing the base by filtration and using it again without significant loss of activity.[83] All reactions were started at room temperature and since no progress was observed after 7 d, the temperature was increased to 50°C. This procedure rendered to be very inefficient for all substrates and exclusively the 2-halobenzoic

acids displayed some reactivity. They were converted to byproducts, which could not be identified (Table T3, entries 5 and 8).





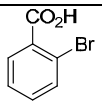
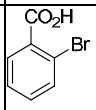
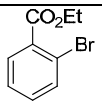
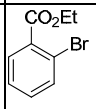
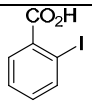
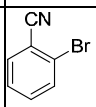
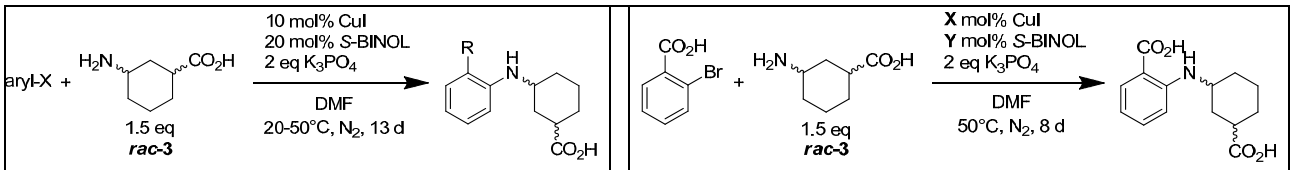
entry	aryl-X	% conversion	% yield[b]	entry	aryl-X	ligand	% conversion	% yield
1		100	17	5		DMEDA	12	0
2		100	5	6		DMEDA	0	-
3		100	21	7		L-proline	0	-
4		100	4	8		L-proline	26[a]	0
				9		L-proline	0[a]	-

Table T3: ULLMANN-condensation reaction according to **GP-U3** (entries 1-4) and **GP-U4a/4b** (entries 5-9). a: L-proline used as a ligand, b: isolated yield.

In order to increase the reactivity towards the product and to lower the reaction temperature a more recent approach employing an easily available ligand, *rac*-BINOL, was chosen.[63] With these conditions (Table T4, entries 1-5) high conversions were monitored for 2-bromobenzoic acid and 2-iodobenzoic acid. In both cases significant amounts of byproducts were generated and the expected product appeared to be a minor-product. Interestingly, both reactions feature the same unidentified by-product in high abundances (Table T4, entries 1 and 3). 2-Bromobenzonitrile led to low conversion but no product was generated. For every reaction the temperature had to be increased to 50°C to enable the conversion.

To test, whether the reactivity could be increased and if side reactions could be suppressed by higher loading of catalyst and ligand, a screening was performed with 2-bromobenzoic acid and varying equivalents of catalyst and ligand (Table T4, entries 6-9). The obtained data suggest, that neither the loading of catalyst nor of the ligand have a significant effect on both conversion and yield. All test reactions stagnated at the same conversion and yield and the product spectrum did not change upon increasing the reaction time. Along with the previous data, this insight prompted to find another approach, since the optimization of the *rac*-BINOL reaction conditions would prove unyielding for a selective route towards the product.



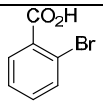
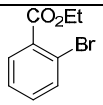
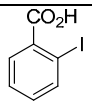
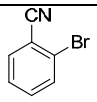
entry	aryl-X	% conversion	% yield	entry	CuI/*S*-BINOL	% conversion	% yield
1		87	21 (26[a])	6	10/20	63	22
2		0	0	7	10/40	60	17
3		85	21 (38[a])	8	20/20	64	20
4		0	0	9	20/40	62	17
5		6	0				

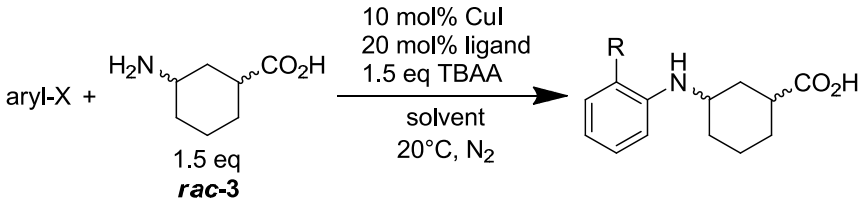
Table T4: ULLMANN-condensation reaction according to **GP-U5** (entries 1-5) and **GP-U6** (entries 6-9). a: unidentified by-product with characteristic UV bands 227 nm, 283 nm, 332 nm.

To condense the results of the executed experiments, some important trends of reactivity can be stated. The aromatic substrates with a carboxyl group exhibited higher reactivity than those bearing an ester functionality and 2-bromobenzonitrile exhibited the lowest reactivity in all screenings. Aryl iodides react faster than aryl bromides and an increase in temperature leads to an increase of both conversion and side reactions. Saponification was observed in high temperature ULLMANN-condensation reactions (Table T2, entries 4 and 7) but not using lower temperatures and ligands (Table T3, entries 2 and 4). Classical room temperature conditions did not work with the free amino acid ***rac*-3** as a substrate and the temperature needed to be increased to at least 50°C. Only aryl halides with a free carboxyl group could be coupled with the amino acid (Table T4, entries 1 and 3). Full conversion of the substrate (except 2-bromobenzonitrile) was only obtained with the high-temperature conditions (Table T2 and Table T3, entries 1-4) and using room temperature conditions the conversion could be gradually increased with the reaction temperature. The low solubility of the amino acid ***rac*-3** at room temperature may be responsible for this behaviour.

A recent publication by YANG *et al*. describes the use of a soluble organic base instead of the previously employed inorganic bases. They report significantly increased yields with the well-soluble organic bases tetrabutylphosphonium malonate and tetrabutylammonium adipate with simple ligands such as L-proline and L-(*N,N*-dimethylglycine).[84] The organic bases were easily prepared by neutralizing the aqueous solution of 1.0 eq tetrabutylammonium hydroxide with 0.5 eq adipic acid

and removal of the water by lyophilization and drying in oil pump vacuum. Traces of water did not impose problems in the following coupling studies.

Remarkably, 2-iodobenzoic acid was almost fully converted (conversion = 85%) at room temperature and afforded 30% product (Table T5, entry 2). Additionally, no formation of C-N coupling byproducts was observed. The corresponding ethyl-2-iodobenzoate was not converted (entry 3) and 2-bromobenzoic acid gave poor yields, while using L-(*N,N*-dimethylglycine) as a ligand (entry 1).



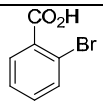
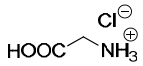
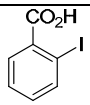
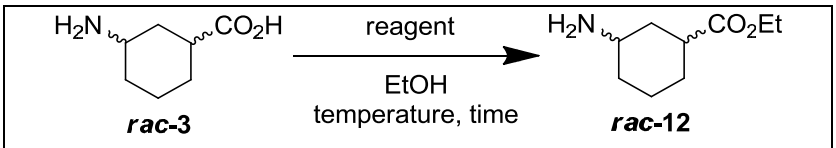
entry	aryl-X	ligand	solvent	time	% conversion	% yield
1			DMSO	6 d	58	6
2		L-proline	DMF	16 d	85	30
3		L-proline	DMF	16 d	0	0

Table T5: ULLMANN-condensation reaction according to **GP-U7**

These first observed room temperature conditions for the amino acid ***rac*-3** were promising, but the yield of 30% and the relatively low conversion still needed improvement. This huge increase in reactivity compared to the previously employed ULLMANN-condensation conditions might be due to the better soluble organic base TBAA. Since the loading of catalyst and ligand seems to have little impact on the reactivity (Table T4, entries 6-9) and since the base/ligand system has been optimized thoroughly by YANG *et al.*[84] another parameter had to be changed. The only one left was the amino acid ***rac*-3** and the simplest transformation seemed to be the protection of the carboxyl group by esterification. MA *et al.* have described β-amino esters as good substrates in ULLMANN-condensation reactions[80] and assuming γ-amino esters feature a similar performance, ethyl 3-aminocyclohexane-carboxylate (***rac*-12**) was synthesized.

The ethyl ester ***rac*-12** was prepared using H^+-catalyzed methods in EtOH from the free amino acid ***rac*-3** and was produced in poor yields (Table T6). The reason for that might be the adjustment of the equilibrium depending on the amount of generated water. An increase in yield might have been achieved by proper removal of water and even the use of dry solvents (Table T6, entry 2 and 3) did not lead to any improvement of yields.[85] Surprisingly, the dark-yellow amino ester ***rac*-12** features a strong and pleasant smell like the candy Fizzers™.

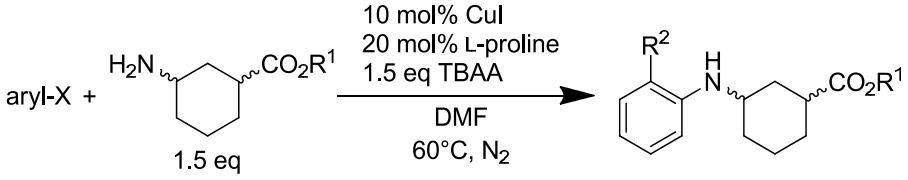


entry	reagent	T / °C	time / d	% yield
1	conc.H_2SO_4 (3 eq)	100	4	38
2	acetyl chloride (2 eq)	0 to 20	1	27
3	TMS-Cl (2.5 eq)	0 to 90	3	18

Table T6: Esterification of amino acid ***rac*-3**

The new concept was put to the test in the following three reactions, which proved to be the key experiment of this thesis (Table T7). An aryl-iodide was reacted with either the amino acid ***rac*-3** or the amino ester ***rac*-12** using the organic base conditions. To achieve rate enhancement, the reactions were carried out at 60°C where still low by-product formation and a higher solubility of the reactants was expected. The alteration from the amino acid ***rac*-3** to the amino ester ***rac*-12** led to higher conversions (51% to 88%) and a dramatic increase in yield, from 25% to 67% (Table T7, entries 1 and 2).

Using the amino ester ***rac*-12**, only a single unidentified by-product was generated (entry 2) while the free amino acid ***rac*-3** led to three others (entry 1). Additionally, no saponified product was observed in the reaction with the γ-amino ester ***rac*-12**, whereas MA *et al.* describe the saponification of the β-amino ester to occur prior the catalytic cycle using their ULLMANN-condensation conditions (10 mol% CuI, H_2O (cat.), DMF, 90-100°C).[80] The just employed reaction conditions seem to be considerably more selective than all the previous approaches.



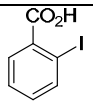
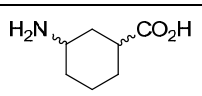
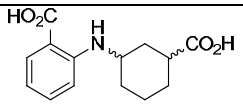
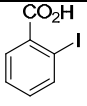
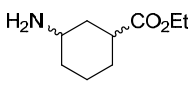
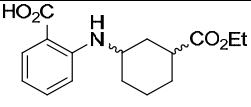
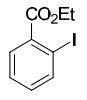
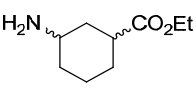
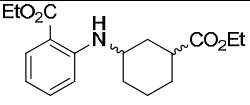
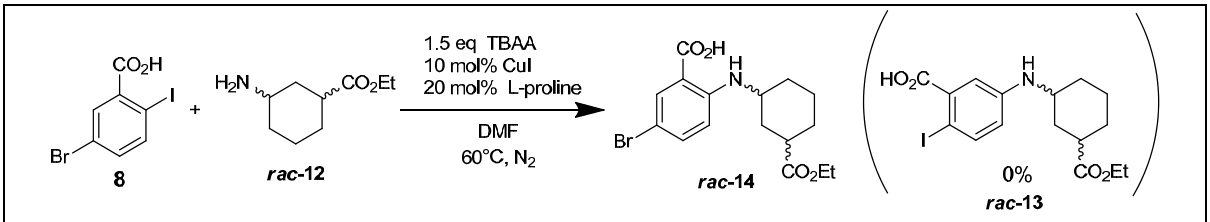
entry	aryl-X	amine	product	time / d	% conversion	% yield
1				21	51	25[a]
2				25	88	67[b]
3				29	11	0[b]

Table T7: Comparison of the reactivity of amino acid ***rac*-3** and amino ester ***rac*-12** with 2-iodobenzoic acid. (a: GP-U8a, b: GP-U8b)

The proof of concept needed to be made, that the doubly functionalized 5-bromo-2-iodobenzoic acid (**8**) can be reacted selectively on the iodine using the above developed reaction conditions. Furthermore, the effect of lowering the amino ester ***rac*-12** loading was examined in order to determine the smallest possible amount for the reaction to work, since the ester will be employed enantiomerically pure (Table T8). Hence **8** was reacted with an excess of ethyl 3-aminocyclohexane-carboxylate (***rac*-12**) using the previously employed ULLMANN-condensation conditions. As luck would have it, 5-bromo-2-iodobenzoic acid (**8**) was coupled in high yields compared to 2-iodobenzoic acid. Outstandingly, only the carbon-iodine bond adjacent to the carboxyl group reacted and no trace of the unselectively coupled by-product ***rac*-13** was detected (Table T8). Another important conclusion of this experiment was the effect of the amino ester ***rac*-12** loading. With lower input the same conversions and yields could be obtained (Table T8, entries 1 and 2), which might lead to a possible change of the limiting substrate towards the soon-to-be enantiomerically pure amino ester **(1*R*,3*S*)-12**. The product ***rac*-14** could not be purified completely, since its R_f-value is very similar to 5-bromo-2-iodobenzoic acid (**8**). Hence the crude product was not subjected to flash chromatography at this stage.



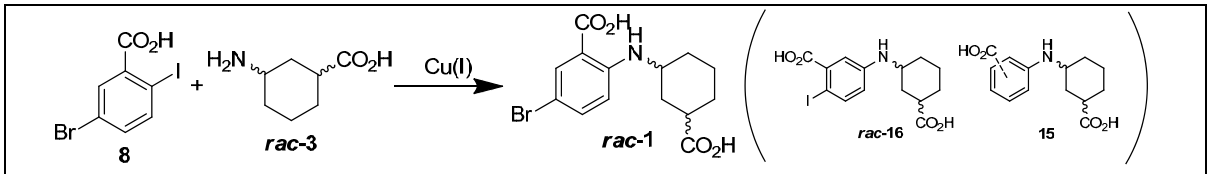
entry	eq ***rac*-12**	time / d	conversion / %	isolated yield / %
1	1.5	2	90	62
2	1.3	3	87	67

Table T8: "Proof of Concept" Coupling between 5-bromo-2-iodobenzoic acid (**8**) and amino ester ***rac*-12**

4.6.2 Concerning chemoselectivity of the ULLMANN-condensation conditions

The evidence was provided, that the bifunctional aromatic moiety can be coupled in good yields and selectively on the iodine substituent using the TBAA/L-proline-system. In order to find out the highest-yielding, still chemoselective ULLMANN-condensation conditions, the following screenings were performed with the help of Christina Kofler (Table T9).

Firstly, the previously employed coupling conditions were tested with 5-bromo-2-iodobenzoic acid (**8**) and the free amino acid ***rac*-3**. As expected, the selectivity decreased with an increase in temperature and the TBAA/L-proline protocol represented the most selective conditions as exclusively the product ***rac*-14** was generated. Additionally, full conversion was only achieved for the high temperature approaches (Table T9, entries 1-3). But the screening revealed some surprising characteristics of the Cu-catalyzed coupling with dihalobenzoic acids. The previously assumed preferential reactivity of the iodine over the bromine substituent, also because of the postulated accelerating effect of the carboxyl group, seemed to be reversed at high temperatures and a significant excess of the inverse product ***rac*-13** was detected (entries 1-3). During the reaction with the copper bronze, a good deal of the generated product was dehalogenated to yield **15** and aside from that, only the inverse product ***rac*-13** was observed (entry 1). Lowering the reaction temperature to the boiling point of 2-propanol, the product ***rac*-1** and the inverse product ***rac*-13** were generated in roughly the same abundances (entry 4). Below this temperature and with the use of more sophisticated ligands, exclusively the desired product ***rac*-14** was generated (entries 5-7). Apparently the published reactivity trends for 2-halobenzoic acids (*vide supra*) are only valid for 5-bromo-2-iodobenzoic acid (**8**) at low temperature and with the use of ligands.



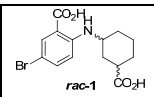
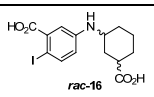
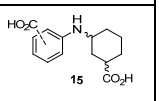
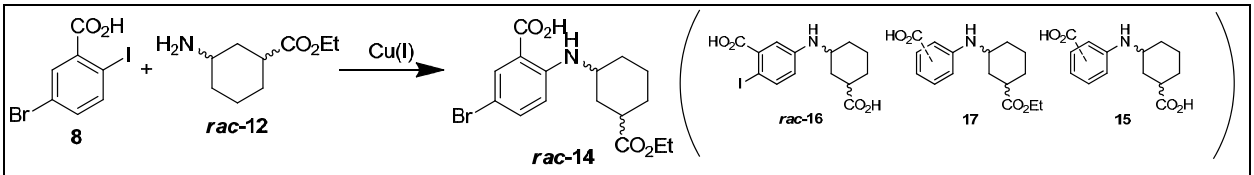
entry	conditions	solvent °C / time	eq ***rac*-3**	% conversion			
1	15 mol% Cu/Sn 3.6 eq K_2CO_3	DMSO 135 / 2 d	1.5	100	-	19	39
2	20 mol% $Cu(OAc)_2 \cdot H_2O$ 20 mol% $Fe(acac)_3$ 2 eq K_2CO_3	DMF 135 / 2 d	1.5	100	27	67	-
3	10 mol% CuI 5 mol% H_2O 3 eq K_2CO_3	DMF 135 / 2 d	0.83	100	20	75	-
4	5 mol% CuI 2 eq ethylene glycol 2 eq K_3PO_4	2-propanol 120 / 5 d	1.2	87	13	19	-
5	10 mol% CuI 20 mol% *S*-BINOL 2 eq K_3PO_4	DMF 50 / 5 d	1.5	63	8	-	-
6	10 mol% CuI 20 mol% DMG·HCl 1.5 eq TBAA	DMF 50 / 5 d	1.5	91	7	-	-
7	10 mol% CuI 20 mol% L-proline 1.5 eq TBAA	DMF 50 / 5 d	1.5	50	12	-	-

Table T9: Selectivity screening, Ullmann-condensation of amino acid ***rac*-3** with 5-bromo-2-iodobenzoic acid (**8**). The general procedures for the Ullmann-coupling were applied. DMG·HCl: L-(*N*,*N*-dimethylglycine)·hydrochloride

As was shown in previous experiments, the amino ester ***rac*-12** featured higher reactivity towards the product ***rac*-14**. It was hence tested, whether the ULLMANN-condensation reaction with the amino ester ***rac*-12** has higher selectivity than with the corresponding amino acid ***rac*-3**. Hence *rac*-ethyl-3-aminocyclohexanecarboxylate (***rac*-12**) was reacted with 5-bromo-2-iodobenzoic acid (**8**) and the products were monitored with HPLC-MS (Table T10).

In contrast to the reaction with the amino acid ***rac*-3**, only the high-temperature reaction with Cu/Sn yielded an inverse product ***rac*-16**, which besides was saponified. In accordance with the previous discoveries, exclusively the Cu/Sn-procedure yielded saponified products, even a both dehalogenated and saponified product **15** (entry 1). The other high-temperature procedures generated substantial amounts of the dehalogenated product **17** and low yields of the product ***rac*-14** were obtained (entries 2 and 3). The coupling with the ligand ethylene glycol led to very low yields and to a range of

byproducts. This again suggests the assumption, that *O*-arylation products might have been generated (*vide supra*, entry 4). The low-temperature approaches on the contrary, yielded the product ***rac*-14** in fair yields and in the case of the TBAA reactions, no formation of other *N*-arylation products (three characteristic UV absorption bands) was observed (entries 5-7).



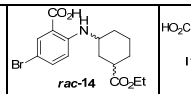
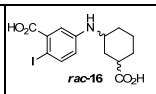
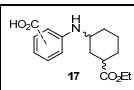
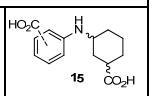
entry	conditions	solvent °C / time	eq ***rac*-12**	% conversion				
1	15 mol% Cu/Sn 3.6 eq K_2CO_3	DMSO 135 / 2 d	1.5	100	-	35	33	3
2	20 mol% $Cu(OAc)_2 \cdot H_2O$ 20 mol% $Fe(acac)_3$ 2 eq K_2CO_3	DMF 135 / 2 d	1.5	100	14	-	15	-
3	10 mol% CuI 5 mol% H_2O 3 eq K_2CO_3	DMF 135 / 2 d	0.83	100	1	-	11	-
4	5 mol% CuI 2 eq ethylene glycol 2 eq K_3PO_4	2-propanol 120 / 5 d	1.2	86	8	-	-	-
5	10 mol% CuI 20 mol% *S*-BINOL 2 eq K_3PO_4	DMF 50 / 5 d	1.5	87	46	-	-	-
6	10 mol% CuI 20 mol% DMG·HCl 1.5 eq TBAA	DMF 50 / 5 d	1.5	67	25	-	-	-
7	10 mol% CuI 20 mol% L-proline 1.5 eq TBAA	DMF 50 / 5 d	1.5	100	54	-	-	-

Table T10: Selectivity screening, Ullmann-condensation of amino ester ***rac*-12** with 5-bromo-2-iodobenzoic acid (**8**). The general procedures for the Ullmann-coupling were applied. DMG·HCl: L-(*N*,*N*-dimethylglycine)·hydrochloride

These findings lead to the conclusion, that the CuI/TBAA/L-proline procedure is superior to all other employed ULLMANN-condensation conditions for the substrates *rac*-ethyl-3-aminocyclohexane-carboxylate (***rac*-12**) and 5-bromo-2-iodobenzoic acid (**8**). There seems to be no difference in selectivity depending on the substrate used (amino acid ***rac*-3** or amino ester ***rac*-12**). The high-temperature conditions fail, because they promote the formation of the inverse product ***rac*-16** and lead to various side-reactions, especially while using the amino acid ***rac*-3**. The generation of the

inverse product ***rac*-16** appears prominently in the amino acid screening (Table T9), but in the amino ester screening only the Cu/Sn-reaction features this by-product ***rac*-16** (Table T10, entry 1).

4.7 Transformation of the *N*-protected amino acid *rac*-4 to the amino ester *rac*-12

Another obstacle in the total synthesis of Phenazistatin A **1** was the transformation of the *N*-protected amino acid ***rac*-4** to the amino ester ***rac*-12** which exhibits such improved coupling properties over the free amino acid ***rac*-4**. In order to do so, three possible routes would be possible (Figure F14).

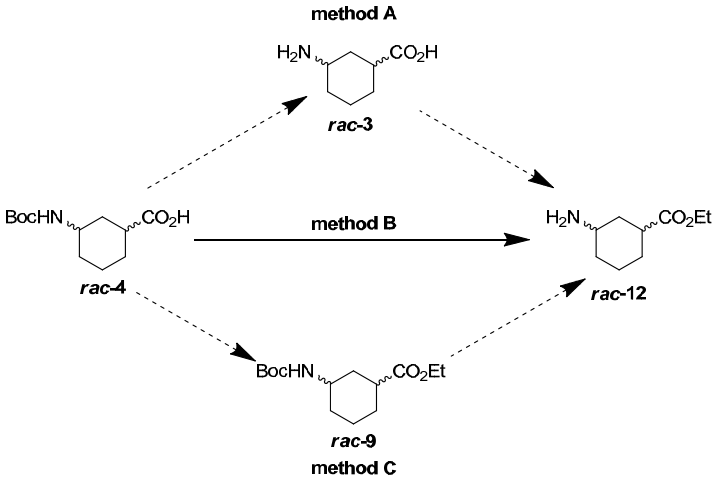


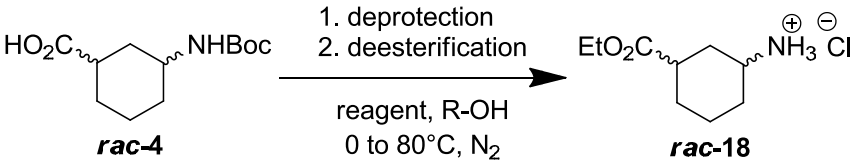
Figure F14: Possible routes for the transformation of ***rac*-4** into the amino ester ***rac*-12**.

Method A appeared unattractive, since the esterification of the free amino acid ***rac*-3** proceeds in such low yields (*vide supra*, Table T6).

Method B would include simultaneous deprotection and esterification. This could be realized by combining two separate procedures using the reagent acetyl chloride in ethanol, since both a mild deprotection in MeOH[86] and esterification of α-amino acids in MeOH at reflux[87] have been reported. Method B was tested using 3.0 eq acetyl chloride at 80°C and with 3.5 eq TMS-Cl at 80°C[85] (Table T11, entries 1-3). All reactions did not reach full conversion, as was determined by the remaining acidic proton signal of the carboxyl group in the ^{1}H-NMR spectrum. The protecting group was successfully cleaved, but the esterification was incomplete and could not be improved by longer reaction times.

Method C includes an esterification followed by a deprotection. Conditions for this conversion have already been tested during the PLE-substrate preparation (Table T1) and the DIC/EtOH/4-DMAP system (Table T1, entries 5 and 6) was selected for the following total synthesis of **(1*R*,3*S*)-1**.[69] Deprotection of the *N*-protected amino acid ***rac*-4** was realized by reacting the dissolved substrate in a mixture of TFA/CH_2Cl_2 = 1:1 (v/v) followed by evaporation of the solvent upon completion.

Evolution of gas bubbles was observed upon addition of the trifluoroacetic acid and quantitative conversion was attained after 30 min. The crude product could be used as such in the following ULLMANN-condensation reaction without any detected change of reactivity or selectivity.



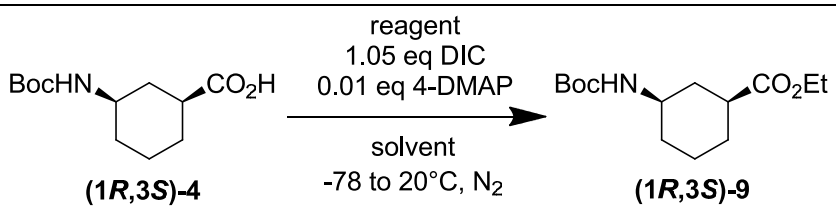
entry	R	reagents	duration	conversion
1	Me	3.5 eq TMS-Cl	4 d	incomplete
2	Et	3.5 eq TMS-Cl	4 d	incomplete
3	Et	3.0 eq acetyl chloride	18 h	incomplete

Table T11: Method B: Approaches for the direct conversion of ***rac*-4** to ***rac*-18**. Entries 1 and 2[85], entry 3:[86,87]

4.8 Total synthesis of (1*R*,3*S*)-1

Since the single steps of the total synthesis have been tested and optimized in the previous section, the respective reaction conditions were applied to the enantiomerically pure *N*-protected amino acid **(1*R*,3*S*)-4**.

The esterification of **(1*R*,3*S*)-4** was performed using the coupling reagent DIC (Table T12) in different solvents. In one case EtOH was used as a solvent and in the other as a reagent. Both reactions seemed to proceed with the same reaction rate and gave high yields of the ester **(1*R*,3*S*)-9**, even though 4-DMAP is said to be more active in an apolar and aprotic solvent like CH_2Cl_2. The most noticeable difference between the two reactions conditions is the solubility of the generated *N*,*N*'-diisopropylurea in the applied solvent. Most of the urea precipitates in CH_2Cl_2 and much less in EtOH and can hence be removed by filtration. In each case the product could be easily purified by flash chromatography and the separation of urea did not impose problems. For future attempts, the product might be purified by a silica filtration since no byproducts are formed. Pre-trials could furthermore show that cooling to -78°C during the addition of the coupling reagent is not necessary for the selective esterification.



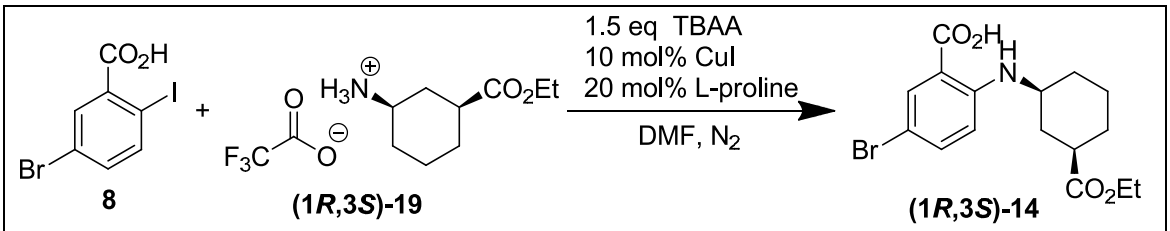
entry	scale / mg	eq reagent	solvent	time / d	yield / %
1	200	1.5 eq EtOH	CH_2Cl_2	2	86
2	300	-	EtOH	2	82

Table T12: Esterification of **(1*R*,3*S*)-4**[69]

The deprotection was carried out with TFA/CH_2Cl_2 = 1:1 (v/v) and the crude TFA-salt **(1*R*,3*S*)-19** was used for the ULLMANN-condensation reaction without further purification in the same Schlenk tube (Table T13).

The ULLMANN-condensation reaction was performed using the same stoichiometry and conditions as in the screening with the racemic substrate.[84] As a difference, all the reactants in the enantiomerically pure experiment were dissolved at 50-60°C generating an ink-blue solution, while the racemic reactions gave a dark-blue suspension. The product **(1*R*,3*S*)-14** was obtained as the main product in both performed reactions, but full conversion was not reached. Higher loading of TFA-salt **(1*R*,3*S*)-19** and the increase of temperature to 60°C led to slightly higher conversion, but in return caused more byproducts (entry 2). It could be shown, that the reaction is not very sensitive to air and traces of water. Upon addition of all reactants in entry 2 the reaction mixture turned yellow and not to ink-blue like normally and no conversion was observed after 6 d. The pH value of the utilized, freshly prepared TBAA was measured in water and indicated slightly acidic conditions (pH 5-6). Assuming the TBAA was not active, the reaction mixture was reduced to the half, neutralized with sat. $NaHCO_3$ solution, extracted with EtOAc, dried over Na_2SO_4 and the solvent was removed in vacuum. The residue was transferred to a Schlenk tube without extensive drying and TBAA (of the old batch) and the remaining reactants were added. This time the reaction mixture turned dark-blue again and was worked up after 6 d at a conversion of 90%. In this case the reaction mixture was acidified, extracted, washed and not subjected to column chromatography (Table T13, entry 2), but directly to saponification in the following step (see Table T14, entry 2).

The problem with the TBAA might originate from the preparation, by a wrong stoichiometry from tetrabutylammonium hydroxide (TBA-OH) and adipic acid. The content of the commercially obtained solution of TBA-OH was declared as 40% and maybe overestimated. Thus the excess of adipic acid would explain the acidity of the "organic base". For future experiments the real content of TBA-OH will have to be specified by titration.

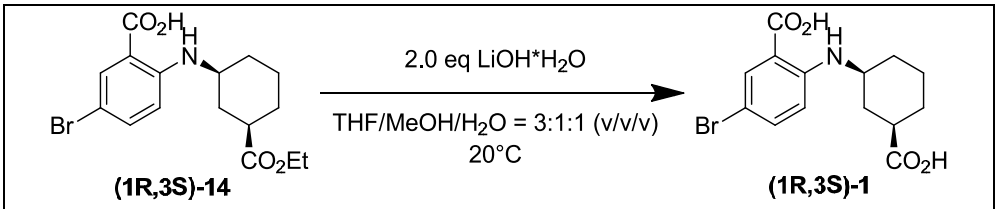


entry	scale / mg	**(1*R*,3*S*)-19**	temperature / °C	time / d	% conversion	% yield
1	160	1.3 eq	50	3	82	84
2	240	1.5 eq	60	6	90	N/A

Table T13: ULLMANN-condensation reaction of the pure **(1*R*,3*S*)-19** with 5-bromo-2-iodobenzoic acid (**8**) [84]

The last remaining step in the total synthesis of **(1*R*,3*S*)-1** is the saponification of **(1*R*,3*S*)-14**. Pre-trials have shown, that the ULLMANN-product ***rac*-14** is readily saponified using 2 eq $LiOH \cdot H_2O$ in the solvent mixture THF/MeOH/H_2O = 3:1:1 (v/v/v). The product could be purified at this stage, not only the unconverted 5-bromo-2-iodobenzoic acid (**8**) was separated but also the *cis*- and *trans*-enantiomers were resolved.

The crude product of **(1*R*,3*S*)-14** was subjected to saponification without any purification. The surplus reaction components were expected not to have a negative effect on the separability afterwards, especially not to generate a by-product with similar polarity to the product. This was realized using 2.0 eq $LiOH \cdot H_2O$ as a base and THF/MeOH/H_2O = 3:1:1 (v/v/v).[88] Pure **(1*R*,3*S*)-14**, as obtained in a pre-trial, was converted in excellent yields (Table T14, entry 1) but the unpurified crude product from the ULLMANN-condensation reaction posed a problem. No conversion took place using 2.0 eq of $LiOH \cdot H_2O$ and after additional 2.0 eq base slow turnover was monitored. A verdigris-coloured solid precipitated and the reaction mixture was filtrated through a pad of silica gel in order to remove copper salts and other polar components. The recovered substrate was deployed to identical saponification conditions and was purified by flash chromatography. Phenazistatin A **(1*R*,3*S*)-1** was obtained in high purity (>93%) and excellent yield (80%) after two steps, starting from 5-bromo-2-iodobenzoic acid (**8**) (Table T14, entry 2).



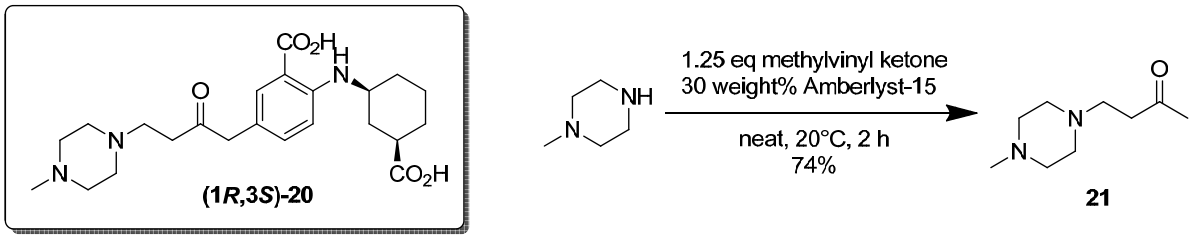
entry	scale / mg	time / d	yield / %
1	40	1	94
2	325	5	80[a]

Table T14: Final step in the sythesis of Phenazistatin A **(1*R*,3*S*)-1**. Saponification of **(1*R*,3*S*)-14**.

a: after two steps (starting from 5-bromo-2-iodobenzoic acid **8**)[88]

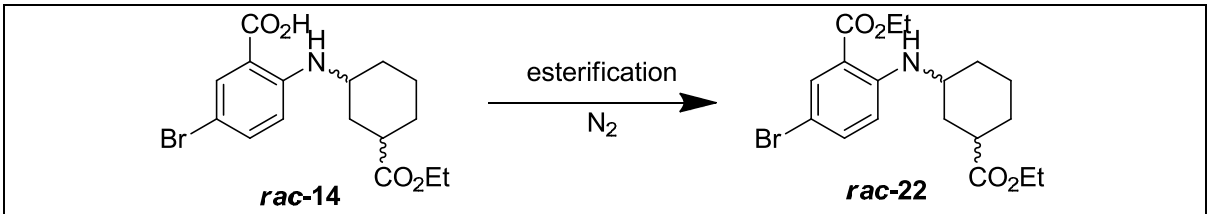
4.9 Post-ULLMANN modifications

The protein-ligand crystal structure of Phenazistatin A **(1*R*,3*S*)-1** by BLANKENFELDT *et al.* revealed, that the bromine substituent directs into an unexploited cavity of the binding pocket and that a well-chosen group might lead to an increase in affinity to PhzB. Estimations and calculations of novel ligand structures led to the best *in silico* compound **(1*R*,3*S*)-20** (Figure F15). Hence the bromine substituent was tried to be replaced by an alkanone containing piperazine moiety using procedures for Pd-catalyzed α-arylation of ketones. The ketone substrate **21** for that reaction was easily prepared by a neat MICHAEL-addition reaction of *N*-methylpiperazine to an excess of methylvinyl ketone in presence of 30% (w/w) anion exchange resin Amberlyst-15™ at room temperature.[89] The reaction was finished after 2 h and a wide variety of byproducts was generated. Nonetheless, the ketone **21** could be isolated in good yields and high purity through flash chromatography (Scheme F15).



Scheme F15: the best *in silico* compound **(1*R*,3*S*)-20** and synthesis of the ketone **21**[89]

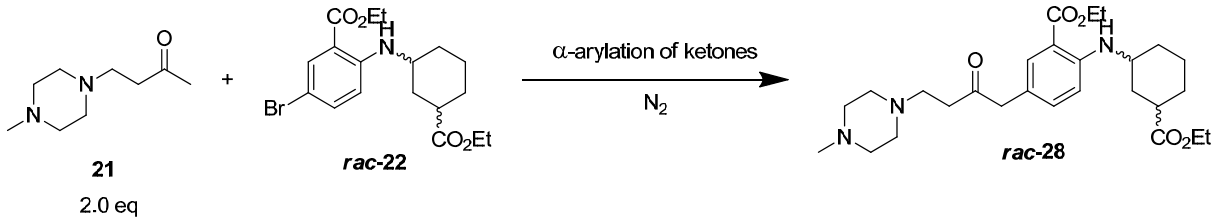
The Pd-catalyzed cross-coupling was expected to be sensitive to the two carboxyl groups of Phenazistatin A, which would need to be protected by esterification. This was realized by already tested reaction conditions starting from the ULLMANN-product ***rac*-14** before saponification. The DIC/4-DMAP/EtOH-protocol gave the product in excellent yields and the other procedures featured the same limitations as in the above mentioned esterifications of amino acid ***rac*-3** and *N*-protected amino acid ***rac*-4**. Full conversion could only be obtained using the DIC/4-DMAP/EtOH-protocol (Table T15).



entry	scale / mg	reagents	solvent / T	time / d	% conversion	% yield
1	30	4 eq TMS-Cl	EtOH / 90°C	3	incomplete	-
2	100	3 eq conc. H_2SO_4	EtOH[a] / 90°C	4	incomplete	-
3	30	1.05 eq DIC, 1.5 eq EtOH, 0.01 eq 4-DMAP	CH_2Cl_2 / 20°C	1	100	95

Table T15: Preparation of the diester ***rac*-22**. (entry 1[85], entry 3[69]) a: No inert conditions

Following reaction conditions for the α-arylation of ketones have been applied, but no product formation could be observed (Table T16). Using the first procedure,[90] conversion of the ester substrate ***rac*-22** was determined by GC-MS, but no additional product spot on TLC could be found or isolated by flash chromatography. Product discrimination might have occurred during the work-up, especially during the filtration through silica gel and Celite® to remove the catalyst. The low concentration of the GC-MS sample might have also led to the wrong assumption of full conversion. Neither the structures nor the type of the proceeded reactions could be illuminated, due to the bad ionization properties of both substrates and generated products in ESI (HPLC-MS) (entry 1). Adopting the more recent method with KHMDS as a base, no conversion was achieved either (entry 2).[91] The approach of extending Phenazistatin A by α-arylation of the ketone **21** will need considerable attention.



entry	scale	catalyst / ligand /base	base / solvent	temperature / °C	time / d	% conversion	yield
1	23 mg	1.5 mol% $Pd_2(dba)_3$ 3.6 mol% *rac*-BINAP 1.3 eq NaO*t*-Bu	THF	70 to 50°C	9	100	-
2	30 mg	30 mol% $Pd(OAc)_2$ 30 mol% *t*-Bu_3P 2.6 eq KHMDS	toluene	20 to 120°C	2	0	-

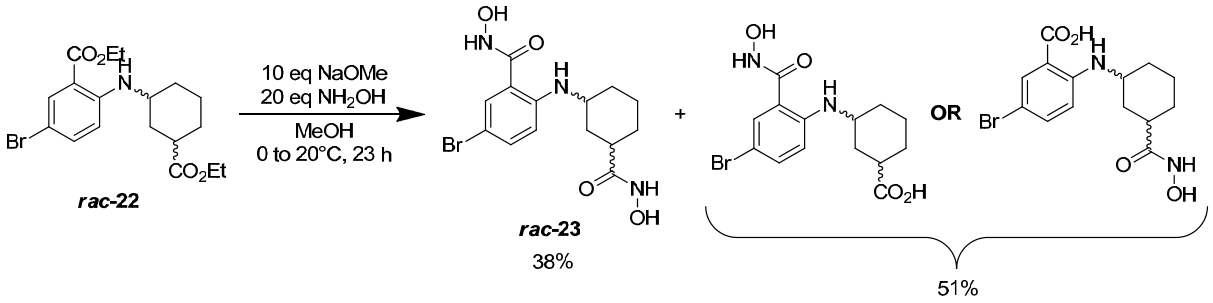
Table T16: Attempts for the α-arylation of the ketone **21** (entry 1[90], entry 2[91])

In order to design high affinity inhibitors of PhzB as tool compounds against phenazine biosynthesis *in vitro* and *in vivo*, not only the affinity towards PhzB needs to be increased, but also the bioavailability needs to be improved. The carboxylic groups state the biggest problem, since the target bacteria for these studies (*Pseudomonads*) possess highly efficient efflux pumps for polar xenobiotics. The membrane permeability might impose an additional difficulty. These problems could be overcome either by converting the free carboxylic acids into prodrug esters, which will be converted by intracellular hydrolases, or by replacement by bioisosteric groups, that feature higher lipophilicity like the well-known 1*H*-tetrazole.

In the course of the α-arylation attempts above, the diethyl ester ***rac*-22** was already generated as the first prodrug ester for further studies (Table T15).

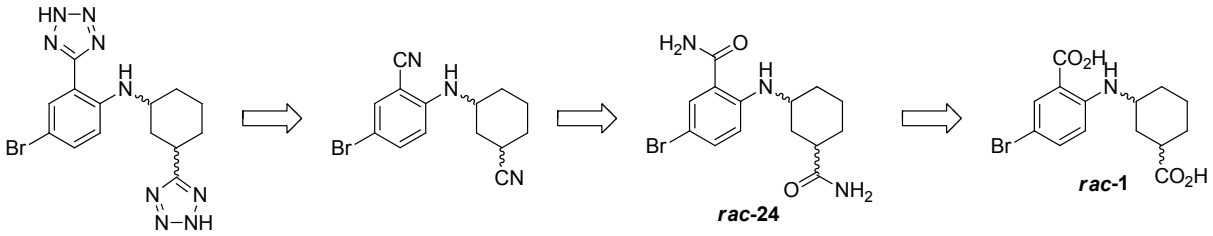
As the first attempt to directly synthesize an analogue with bioisosteric replacements of the carboxyl group, the hydroxamic acid group was chosen. This functional group is readily available using the

same conditions with hydroxylamine from either Phenazistatin A **(1*R*,3*S*)-1** or the diethyl ester ***rac*-22**. The diethyl ester ***rac*-22** was chosen, because of availability issues that time. The reactants were mixed at 0°C and the reaction process was monitored with HPLC-MS. Full conversion of ***rac*-22** was reached after 4 h but a wide diversity of intermediates could be observed. Among which some were identified as the stochastic variations of ethylcarboxylate-, carboxylate- and hydroxamic acid groups on the Phenazistatin-backbone. The reaction was left stirring and the diversity vanished overnight and only two main products with a share of 38:51 (percent of the total products) were observed. Those were identified as product ***rac*-22** and one of the depicted byproducts (Scheme S17). Attempts to isolate either of the main products failed on account of instability on silica gel during TLC-analysis and flash-chromatography in the chosen solvents. In consequence of the small scale (20 mg) further attempts of purification could not be made. The effect of rising the temperature or the reaction time will have to be examined to further drive the reaction to completion and a single product.



Scheme S17: Transformation of the diester ***rac*-22** into the carboxamide ***rac*-23**[93]

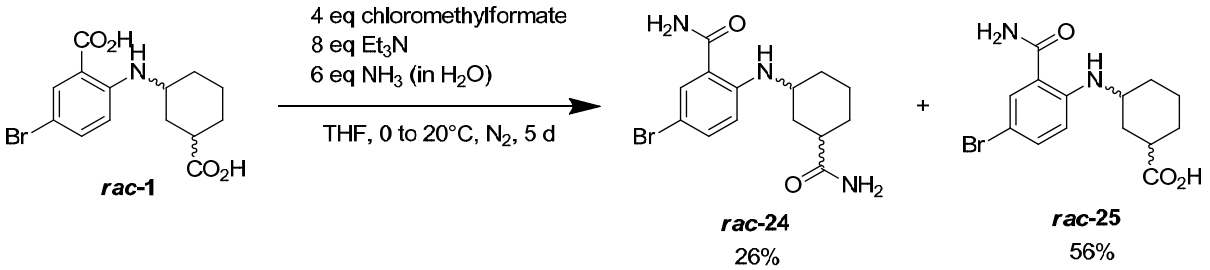
In order to replace the carboxyl group by the more lipophilic tetrazole group, several transformations need to be made. In the first step, the carboxylic group would need to be replaced by a carboxamide, which would then be dehydrated to the corresponding nitrile. The nitrile would then be converted to the 1*H*-tetrazole, using an azide reagent (Scheme S18). In the course of this work only the first step was achieved.



Scheme S18: Retrosynthetic approach to replace the carboxyl groups in ***rac*-1** by 1*H*-tetrazole groups.

The first transformation to the carboxamide groups attached on the phenazine backbone was tested employing a procedure by CAO *et al.*.[92] ***Rac*-1** was deprotonated with Et_3N and activated with chloromethyl formate and at 0°C. After 90 min the generated anhydride was quenched with conc. NH_4OH in water. The substrate was fully converted after 5 d and two main products were obtained

with a 26:56-share (% of the total products). The products were separated by flash chromatography and were identified (Scheme S19) by NMR and HPLC-MS. The correct assignment of the carboxamide-group in the by-product ***rac*-25** was achieved by ^{13}C-NMR. The reason for the incomplete double-substitution might be the incomplete activation with the chloromethyl formate in the first place, or the competitive attack of the less nucleophilic hydroxide after the activation. The second possibility could be excluded in future experiments by using ammonia in 1,4-dioxane instead of water. The isolated products are currently being investigated by Wulf Blankenfeldt for their protein-ligand crystal structures and the K_D-values. The crystals have already been diffracted at the synchrotron and suggest binding of the ligand to the protein.



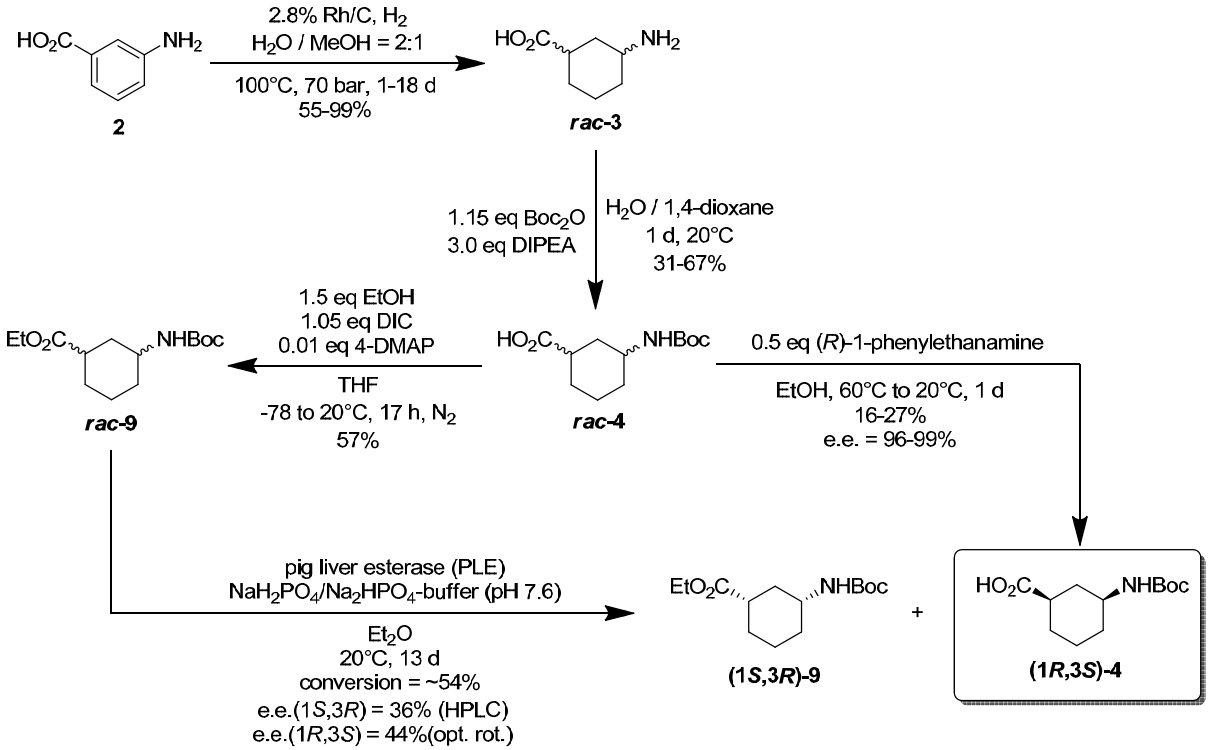
Scheme S19: Transformation of Phenazistatin A **(1*R*,3*S*)-1** into the carboxamides[92]

5 Summary and future work

5.1 Summary

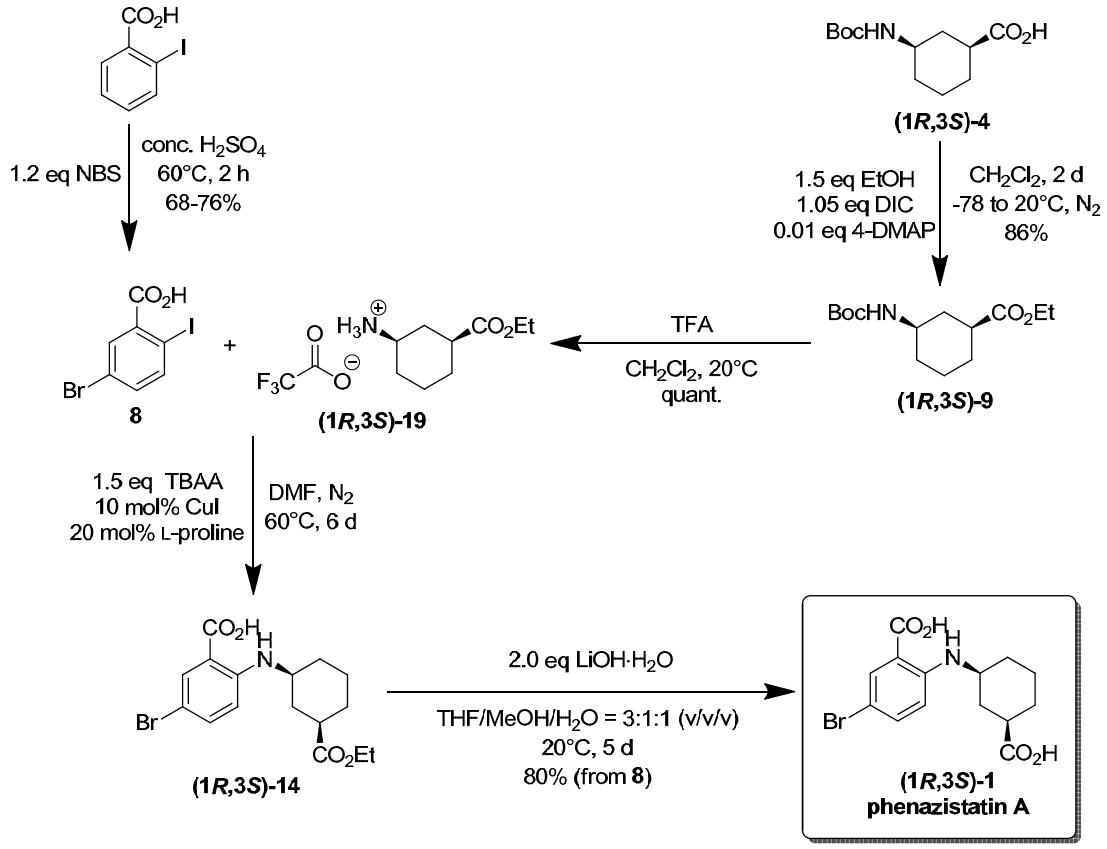
The aim of this thesis was to develop a synthetic route towards penazistatin A, which is easy, reliable and applicable for the synthesis of larger quantities of product. Starting from this substructure the lead structure optimization should be initiated to increase the potency and to alter the other physiochemical and biological properties.

In the course of this Master thesis both tasks were dealt with and a new synthetic route towards phenazistatin A **(1*R*,3*S*)-1** was optimized. For the synthesis of the *N*-protected racemic amino acid ***rac*-4** the conditions were adapted from the previous work of Matthias Mentel.[77] The chiral resolution of this substrate was tested using different approaches, at first the kinetic chiral resolution of the racemic ester ***rac*-9** using the selective saponification with pig liver esterase (PLE) and secondly the more classic method of chiral resolution of the *N*-protected amino acid ***rac*-4** using a chiral amine. It has been shown that the PLE reaction is not the method of choice chiral resolution, since the enzyme mixture exhibits just a low stereoselectivity for the conversion of the desired enantiomer. On the other hand, a versatile and simple method for chiral resolution has been developed, by employing a chiral amine. Moderate yields, but excellent enantiomeric purities of **(1*R*,3*S*)-4** could be obtained without chromatographic purification, starting from 3-aminobenzoic acid (**2**) (Scheme S20)



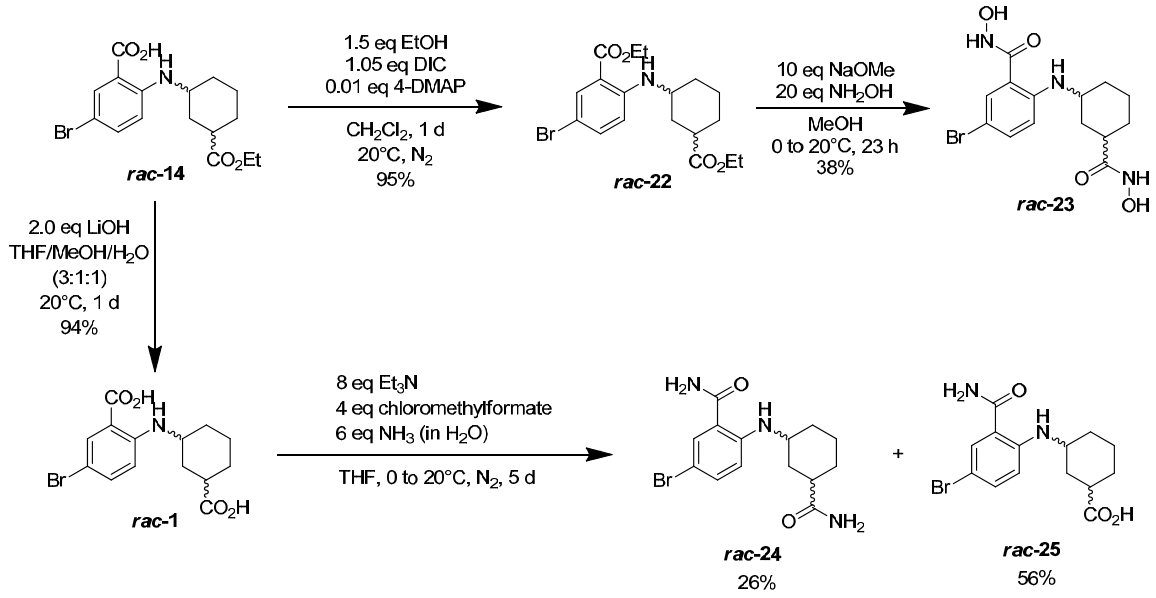
Scheme S20: Optimization of the chiral resolution of **(1*R*,3*S*)-4**

It was found that the ULLMANN-condensation represents the biggest problem within the total synthesis of phenazistatin A and that only the reaction of the amino ester **(1*R*,3*S*)-12** (or its TFA-salt **(1*R*,3*S*)-19**) with the bifunctional moiety **8** leads to high yields (Scheme S21). The preparation of the amino ester **(1*R*,3*S*)-12** requires an additional step and hence loss of substrate, but the use of the free amino acid **3** is not sustainable for the C-N coupling, since it features very low reactivity. Several other ULLMANN-condensation conditions have been tested using **8** and both the amino acid ***rac*-3** and the amino ester ***rac*-12**, and as assumed, a decrease in chemoselectivity was monitored. Intriguingly, a reversion of chemoselectivity was observed by rising the temperature, rendering the carbon-bromine bond more reactive and leading solely to the bromine-coupled by-product. These findings complement the publications, that propose a rate enhancing effect of the ortho carboxylic group and an increased reactivity of iodine over bromine.[62,63]



Scheme S21: Finishing the total synthesis of phenazistatin A **(1*R*,3*S*)-1**

As a part of the aim to generate a library of analogues of phenazistatin A, the diethylester ***rac*-22**, the dihydroxamic acid ***rac*-23** and the mono-/dicarboxamide ***rac*-24 / *rac*-25** have been synthesized (Scheme S22). They are currently being tested for their biological properties and their affinity towards PhzA/B by the group of Prof. Blankenfeldt (University of Bayreuth). First results from X-ray diffraction at the synchrotron indicate ligand binding.

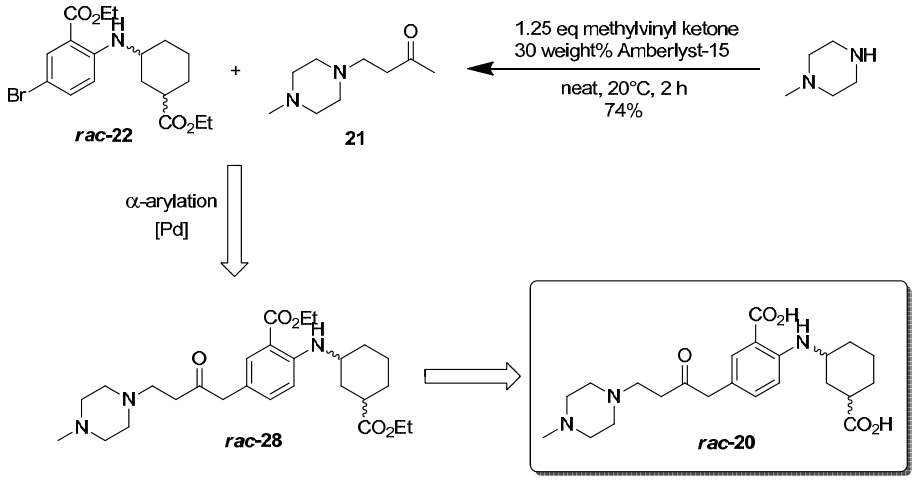


Scheme S22: Post-ULLMANN modifications of **(1*R*,3*S*)-14**

To summarize the work done, a reliable synthetic route towards phenazistatin A has been developed which so far requires two chromatographic steps and proceeds in an overall yield of 12%, which represents a 15-fold increase towards the previously employed strategy. It has been shown that the two carboxylic groups are easily replaced by various substituents, which will facilitate the generation of phenazistatin A analogues in future.

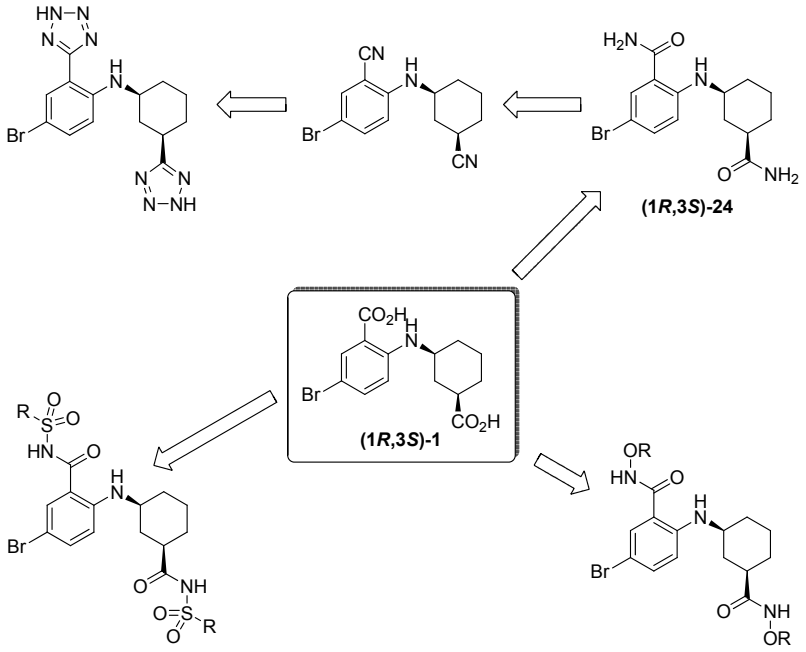
5.2 Future work

As calculations by Matthias Mentel have shown, the ligand ***rac*-20** might feature increased affinity and hypothetically potency, due to the increased hydrophobic interaction area and the hydrophilic piperazine moiety. First synthetic efforts have been difficult, but the promising *in silico* data encourage for future efforts (Scheme S23).



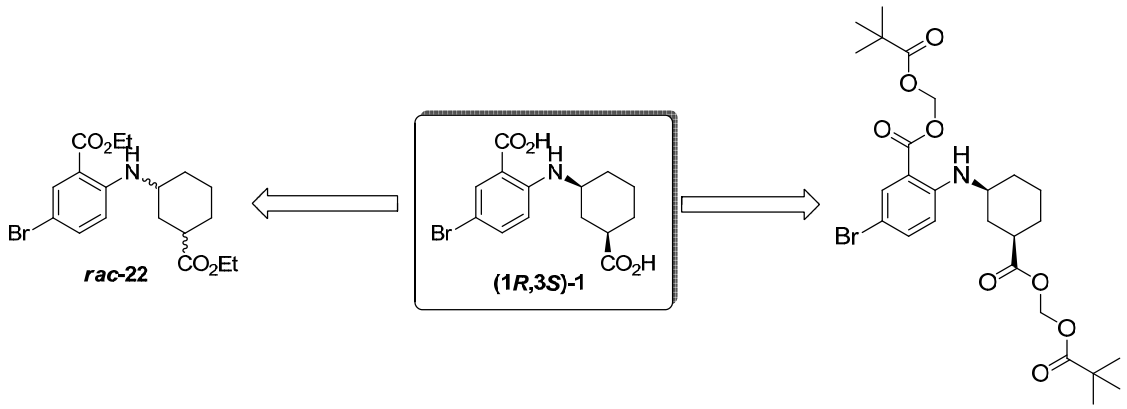
Scheme S23: Elongation of ***rac*-22** by α-arylation of the ketone ***rac*-21**

In order to tune the properties of the PhzA/B inhibitors without changing the biological properties significantly, several replacements by bioisosteric groups can be made. The polar carboxylic groups might pose a problem for membrane permeability and bioavailability, since *Pseudomonads* possess highly effective efflux pumps for xenobiotics. Acylsulfonamides and hydroxamic acid derivatives are easily accessible and feasible alternatives in order to render the compound more hydrophobic. The replacement by a 1*H*-tetrazole is a common tool to increase lipophilicity in drug design.[95,96] The first transformation towards the carboxamide has already been made in the course of this thesis (Scheme S24).



Scheme S24: Transforming phenazistatin A **(1*R*,3*S*)-1** into analogues with bioisosteric groups

Another approach to cope with the membrane permeability issues is the transformation into a prodrug ester, which is expected to be converted by intracellular hydrolases and to release the active drug without the formation of toxic cleavage products. The diethyl ester ***rac*-22** has already been synthesized and the pivoloxymethyl ester would be another easily accessible compound (Scheme S25).



Scheme S25: Transforming phenazistatin A **(1*R*,3*S*)-1** into prodrug esters

6 Experimental section

6.1 General aspects, materials and methods

6.1.1 General

The reactions were carried out on air, unless noted otherwise. Inert reactions were carried out employing standard Schlenk techniques and only dry solvents were used. Dry solvents were prepared by the below-mentioned procedures and were stored under argon. Before use the apparatuses were dried in oil pump vacuum by heating with a heat-gun, cooled to room temperature and flushed with the inert gas, nitrogen or argon. Reagents and solvents were always added under an inert gas counter-stream. Before the addition of the solvents, the solid components were dried by 3-fold evacuation and refilling with inert gas (if possible). Sticky substrates were transferred to the dry apparatuses dissolved in an appropriate solvent (dichloromethane, diethyl ether or methanol) followed by evaporation of the solvent and drying in oil pump vacuum. For reasons of safety, the catalyst of hydrogenation reactions was removed by filtration through a wet bed of Celite® in an inverse filter funnel under nitrogen. The stated temperatures generally refer to the oil bath or cooling bath temperature.

All chemicals and reagents used were purchased from the companies ABCR, ACROS Organics, Aldrich, Fluka, LOBA-Chemie, Merck, Riedel-de Haën, Sigma-Aldrich, Strem Chemicals and VWR and were used without further purification unless otherwise stated.

6.1.2 Solvents

Acetonitrile: Dry acetonitrile was purchased from ACROS Organics in 99.9% purity without stabilizer stored over 3 Å molecular sieves and was transferred into a brown 1 L Schlenk bottle with activated 3 Å molecular sieves and was stored under an argon atmosphere.

Dichloromethane: Dichloromethane was treated with phosphorus pentoxide, distilled, then heated to reflux for two days over calcium hydride and then distilled under argon atmosphere into a dry brown 1 L Schlenk bottle with activated 4 Å molecular sieves.

Dimethylsulfoxide: Dry dimethylsulfoxide was purchased from ACROS Organics and was stored over activated 4 Å molecular sieves in a brown 1 L Schlenk bottle under argon atmosphere.

Ethanol: Ethanol was treated with sodium and diethyl phthalate in an inert distillation apparatus and was heated slowly until hydrogen an intensive gas generation was observed. The mixture was heated to reflux for 2 h and was distilled and stored over activated 3 Å molecular sieves in a brown 1 L Schlenk bottle under argon atmosphere. Ethanol used for non-inert conditions was purchased from Merck (stabilized with 1% methylethyl ketone) and was used without further purification.

Methanol: Methanol was heated to reflux over magnesium turnings and was then distilled under argon atmosphere into a dry brown 1 L Schlenk bottle with activated 3 Å molecular sieves.

***N,N*-Dimethylformamide:** *N,N*-Dimethylformamide was purchased from ACROS Organics as extra dry solvent (<50 ppm water, over 3 Å molecular sieves, AcroSeal®) and was transferred to a dry brown 1 L Schlenk bottle with activated 3 Å molecular sieves and stored under argon atmosphere.

Tetrahydrofuran: Dry tetrahydrofuran was purchased from Sigma-Aldrich, transferred to a dry brown 1 L Schlenk bottle with activated 3 Å molecular sieves and stored under argon atmosphere. Tetrahydrofuran used for non-inert reactions was purchased from Roth and the stabilizer was removed by distillation using a rotary evaporator. The solvent was stored above KOH pellets in a brown-glass bottle.

Toluene: Toluene was purchased from Sigma-Aldrich (99.7%) and was dried using an aluminium oxide column (Pure Solv® by Innovative Technology) and was stored over activated 4 Å molecular sieves in a brown 1 L Schlenk bottle under argon atmosphere.

1,4-Dioxane: 1,4-Dioxane was purchased from Merck and the stabilizer was removed by distillation using a rotary evaporator. The solvent was stored above KOH pellets in a brown-glass bottle.

Diethyl ether: Diethyl ether was purchased from Roth and the stabilizer was removed by distillation using a rotary evaporator. The solvent was stored above KOH pellets in a brown-glass bottle.

Cyclohexane, dichloromethane, ethyl acetate and **methanol** were purchased from Fisher Scientific as analytical grade (99.99%) and were used as obtained.

***n*-Pentane:** *n*-Pentane was distilled and stored in a brown-glass bottle.

6.1.3 Reagents

***S*-BINOL:** *S*-BINOL was synthesized in the organic chemistry laboratory course by Matthias Pickl under supervision of Dr. Jana Rentner.

CO_2: Dry ice was used right after preparation by resublimation of liquid CO_2.

CuI: CuI was purchased from ACROS Organics and was purified by Soxhlet-extraction with THF and was stored under argon atmosphere at 4°C.

***N*-Bromosuccinimide:** NBS was purchased from Merck and was recrystallized from water (20 g NBS/175 mL water).

***n*-Butyllithium:** n-Butyllithium was purchased from ACROS Organics and Aldrich as a 2.5 M solution and the actual concentration was determined by titration according to the method by W. G. KOFRON and L.M.BACLAWSK.[97] A dry 8 mL Schlenk tube with magnetic stirring bar was charged with 250.0 mg diphenylacetic acid and 10 mL dry THF. To the stirred colourless solution the *n*-BuLi solution was added via a 1 mL syringe until the colour of the suspension changed to canary yellow. The added amount of *n*-BuLi corresponds to the weighted amount of diphenylacetic acid. The concentration was determined as the average value of three repetitions.

Tetrabutylammonium adipate: TBAA was prepared using a procedure by YANG *et al.*.[84] Tetrabutylammonium hydroxide was purchased as a 40% solution in water from Fluka and adipic acid from LOBA Chemie. A round bottom flask with magnetic stirring bar was charged with 2 eq *n*-Bu_4NOH solution and 1 eq adipic acid and the colourless solution was stirred for 24 h. The water was removed by lyophilization and the colourless solid was dried in oil pump vacuum.

Pig liver esterase-precipitate (PLE): The PLE-precipitate (purchased by Fluka) in half-saturated $(NH_4)_2SO_4$-solution was gratefully received from Prof. Dr. Harald Pichler (IMBT, Graz University of Technology) and was used after careful whirling up the sedimented part.

NaH_2PO_4/Na_2HPO_4-buffer (pH 7.6): The NaH_2PO_4/Na_2HPO_4-buffer was prepared by dissolving 12.0 g NaH_2PO_4 (purchased from Fluka) in 1.0 L deionised water followed by dropwise addition of NaOH solution (3.0 M) to the stirred solution until a pH of 7.6 was reached.

Resin-bound dimethylhydroxyethylammonium carbonate (RB-DHAC): Amberlite "IRA-400" ion-exchange resin (chloride form) was purchased from Aldrich, charged into a column with sintered glass frit and was rinsed with 200 mL 1 M HCl, 500 mL H_2O, 400 mL 1 M Na_2CO_3 and 400 mL H_2O. The light-yellow polymer beads were dried in oil pump vacuum to afford orange-brown polymer beads. The resin-bound organic base was stored under a nitrogen atmosphere.[83]

6.1.4 Analytical Methods

6.1.4.1 Thin-layer chromatography

Analytical thin layer chromatography was performed using TLC-plates purchased from Merck (TLC aluminium foil, silica gel 60 F_{254}). The TLC plates were generally screened using a UV lamp with λ = 254 nm (fluorescence quenching) and λ = 366 nm (immanent fluorescence of the analytes). Alternatively, a stain reagent was applied and the plates were developed using a stream of hot air.

CAM-solution: 2.0 g Cer(IV)-sulfate, 50.0 g ammonium molybdate and 50 mL conc. H_2SO_4 in 400 mL water.

Ninhydrin: 250.0 mg Ninhydrin, 100 mL EtOH

The developing solvents and the R_f-values are denoted in the experimental procedures.

6.1.4.2 Flash chromatography

Preparative column chromatography was performed using silica gel 60 from ACROS Organics (particle size 35-70 µm). Depending on the separation problem, the mass of silica gel used was the 30- to 100-fold (w/w) amount of the dry crude product. Sticky and other non-transferable crude products were dissolved in an appropriate amount of solvent and were adsorbed on the twofold amount of Celite® (w/w). Column dimensions were chosen in order to reach a separation length between 15-30 cm and the actual sizes along with the eluents used are stated in the experimental procedures.

6.1.4.3 Gas chromatography

Analytical gas chromatography was performed employing an „Agilent Technologies 7890A GC system“ with a polar HP-5MS column (30 m x 250 µm x 0.25 µm); injection was executed by an “Agilent Technologies 7683 Series Autosampler“ in split mode; carrier gas: helium 5.0; electron impact (EI) ionisation source with a potential of E = 70 eV; mass selective detector “Agilent Technologies 5975C inert MSD with Triple Axis Detector”.

JPM_50_S: 50°C 1 min, ramp 40°C/min linear to 300°C, 5 min

For quantitative statements concerning yield and conversion, the area percent of the peaks was used. Those values are not exact, since no internal standard was employed. Along with the retention times t_R of the main products, the intensities of the molecular fragments normalized by the basis peak are given.

6.1.4.4 High performance liquid chromatography

Analytical HPLC measurements were performed on three different instruments. Most of the qualitative and quantitative data were acquired on a “Shimadzu Nexera Liquid Chromatograph” with thermostatically controlled column oven. The separation of the analytes was carried out using a “C-18 reversed-phase” column of the type „Poroshell® 120 SB-C18, 3.0 x 100 mm, 2.7 µm“ by Agilent Technologies. Detection of the substances was accomplished with a „Shimadzu SPD-M20A Prominence Diode Array Detector“ at a wavelength of λ = 210 nm and with the mass selective detector „Shimadzu LCMS-2020 Liquid Chromatograph Mass Spectrometer” in the modes “ESI positive” and “ESI negative”. As eluents acetonitrile and water with 0.01% HCOOH as an additive were used.

Method_A1: 0.0-0.1 min 98% water/HCOOH and 2% CH_3CN, 0.1-15.0 min linear to 60% CH_3CN, 16.0-16.5 min linear to 100% CH_3CN, 16.5-18.5 min 100% CH_3CN, 18.5-19.0 min linear to 2% CH_3CN, 19.5-20.0 min 98% water/HCOOH and 2% CH_3CN; 1.00 mL/min, 30°C

-15.0 min 70% water/HCOOH and 30% CH_3CN,

Method_A2: 0.0-1.5 min 3% CH_3CN and 97% water/HCOOH, 1.5-4.5min linear to 80% CH_3CN, 4.5-5.0 min 80% CH_3CN, 5.0-5.05 min linear to 100% CH_3CN, 5.05-5.9 min 100% CH_3CN, 5.9-7.0 min linear to 3% CH_3CN; 0.70 mL/min, 40°C

Method_A3: 0.0-1.5 min 3% CH_3CN and 97% water/HCOOH, 1.5-4.5 min linear to 80% CH_3CN, 4.5-5.0 min 80% CH_3CN, 5.0-5.05 min linear to 100% CH_3CN, 5.05-5.9 min 100% CH_3CN, 5.9-7.0 min linear to 3% CH_3CN

The analysis of chiral substances was performed on an „Agilent 1100 Series“ instrument with thermostatically controlled column oven. The chiral column „Chiralpak® AD-H, 4.6 x 250 mm, 5.0 µm“ by „Daicel Chemical Industries“ was used. The signal was acquired with an „Agilent Technologies 1200 Series MWD SL“ UV-detector at λ = 210 nm.

Method_A4: isocratic with *n*-heptane / ethanol = 95:5 (v/v), 1.1 mL/min, 15°C, 60 bar

6.1.4.5 Nuclear magnetic resonance spectroscopy

The described nuclear resonance spectra were acquired with the following instrument:
Bruker AVANCE III with Autosampler: 300.36 MHz-^{1}H-NMR, 75.53 MHz-^{13}C-NMR
Chemical shifts δ are referenced to the residual protonated solvent signals as internal standard. ^{13}C-NMR and APT spectra were acquired proton-decoupled. Signal multiplicities *J* are abbreviated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplet), td (triplet of doublet), q (quadruplet), pd (pentet of doublet) and m (multiplet). For the correct assignment of the signals APT, HSQC and HH-COSY were recorded if necessary. In the case of mixtures of diastereomers, correctly assigned ^{1}H- or ^{13}C-signals were indicated by the superscript ^{cis} or ^{trans}, e.g. H-1^{trans}. Furthermore, the deuterated solvent, the chemical shifts δ in ppm (parts per million), the coupling constants *J* in Hertz (Hz) and the integral and assignment of the respective signal are given. Quaternary atoms are denoted as C_q (^{13}C-data) and aromatic protons as CH_{Ar} (^{1}H-data).
Deuterated solvents for nuclear resonance spectroscopy were purchased from euriso top® (DMSO-d_6, $CDCl_3$, MeOH-d_4) and Deutero® (D_2O).

6.1.4.6 Determination of the melting point

Melting points are uncorrected and were determined using a “Mel-Temp®” apparatus with integrated microscopical support by the company Electrothermal. The temperature was measured using a mercury thermometer.

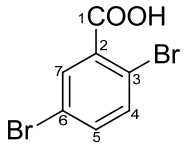
6.1.4.7 Determination of the optical rotation

The optical rotation was obtained using a “Polarimeter 341” by the company Perkin Elmer with integrated sodium lamp. All measurements were conducted at 20°C using the D-band of the sodium spectrum (λ = 589 nm). Methanol was used as the solvent (“Chromasolv® for HPLC“ by Sigma-Aldrich) and concentrations of 0.3 - 1.6 (3 g/L-16 g/L) were measured.

6.2 Experimental procedures and analytical data

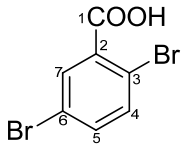
6.2.1 Synthesis of the bifunctional aromatic moiety

6.2.1.1 2,5-Dibromobenzoic acid (7)



A dry 40 mL Schlenk tube with magnetic stirring bar was charged with 0.81 mL (4.66 mmol, 1.1 eq) TMP and 7 mL dry THF in a N_2 counter-stream. The colourless reaction mixture was cooled to -85°C (cooling bath) and 3.3 mL (4.66 mmol, 1.1 eq) *n*-BuLi solution in hexane (1.19 M) was added dropwise and stirred for 10 min. 1.00 g (4.24 mmol, 1.0 eq) 1,4-dibromobenzene in 5 mL dry THF was added at -85°C and the yellow solution was stirred for 80 min not exceeding - 60°C. The dirty yellow solution was cooled to -80°C and solid CO_2 was added during 20 min. The orange/brown reaction mixture was warmed to 20°C over a period of 1 h. The bright-orange, clear solution was quenched by addition of 1.5 M H_2SO_4 (26 mL), the layers were separated and the aqueous layer was extracted with Et_2O (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed by rotary evaporation. The bright-yellow solid was transferred into a funnel with a sintered glass frit and was washed with H_2O (3x20 mL), toluene (2x10 mL, yellow eluate) and n-pentane (1x20 mL). The light-yellow solid was dried in oil pump vacuum and analyzed with GC-MS.[83]

6.2.1.2 2,5-Dibromobenzoic acid (7)



A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 500 mg (2.41 mmol, 1.0 eq) 2-bromobenzoic acid and 4.0 mL conc. H_2SO_4. The light-yellow solution was heated to 60°C and 515 mg (2.90 mmol, 1.2 eq) *N*-bromosuccinimide was added in small portions over a period of 3 h and was left stirring for 1 h. The reaction mixture was poured on 30 g ice and was filtered through a sintered glass frit (Pore 3). The colourless solid was washed with H_2O (2 x 5 mL), transferred to a separating funnel and dissolved in EtOAc (25 mL). The organic phase was washed with H_2O (20 mL), brine (20 mL) and was dried over $MgSO_4$. The drying agent was removed by filtration and the solvent was removed by rotary evaporation and the crude product was dried in oil pump vacuum.[67]

yield: 588 g (20% by-product) colourless solid

The crude product was recrystallized from 5 mL H_2O/MeOH = 2:1 (v/v), filtrated, washed with H_2O (2x5 mL) and dried in vacuum.

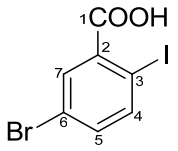
yield: 228.4 mg (20% by-product remaining) colourless solid

$C_7H_4Br_2O_2$ [279.91]

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.13 (d, $^4J_{HH}$ = 2.3 Hz, 1H, H-7), 7.58 (d, $^3J_{HH}$ = 8.5 Hz, 1H, H-4), 7.51 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-5).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 169.6 (C-1), 136.7/136.4/135.4 (C-4, C-5, C-7), 131.9 (C-2), 121.5/121.3 (C-3, C-6).

6.2.1.3 5-Bromo-2-iodobenzoic acid (8)



A 250 mL two-necked round-bottom flask with magnetic stirring bar was charged with 10.00 g (39.5 mmol, 1.0 eq) 2-iodobenzoic acid and 80 mL conc. H_2SO_4. The light-yellow solution was heated to 60°C and 8.44 g (47.4 mmol, 1.2 eq) *N*-bromosuccinimide was added in small portions over a period of 90 min. The reaction mixture became very turbid after the addition of ~7.11 g (1.01 eq) *N*-bromosuccinimide, no further stirring was possible. The reaction mixture was poured onto ice (~600 mL) and the colourless precipitate was isolated by filtration through a sintered glass frit (Pore 3). The colourless solid was washed with H_2O (3 x 30 mL), dissolved in EtOAc (250 mL) and transferred into a separating funnel. The organic phase was washed with H_2O (2 x 150 mL) and brine (150 mL) and was dried over $MgSO_4$. The drying agent was removed by filtration and the solvent was removed by rotary evaporation. The crude product was purified by recrystallization ($MeOH/H_2O$ = 13:10 (v/v), 230 mL) and was dried in oil pump vacuum.[67]

$C_7H_4BrIO_2$ [326.91]

yield: 9.822 g (30 mmol, 76%) colourless, fluffy solid

R_f = 0.58 (CH_2Cl_2/MeOH/AcOH = 50:1:1) (254 nm)

mp: 155-156°C

HPLC-MS (Method_A1): t_R = 13.32 min

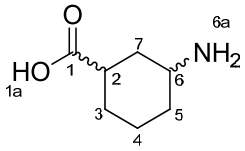
HPLC-MS (Method_A2): t_R = 4.78 min

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.93-7.83 (m, 2H, H-4, H-7), 7.34 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-5).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 168.5 (C-1), 144.0 (C-4), 139.6 (C-2), 136.4 (C-5), 134.3 (C-7), 123.1 (C-6), 92.6 (C-3).

6.2.2 Chiral resolution of *rac*-4

6.2.2.1 *rac*-3-Aminocyclohexanecarboxylic acid (rac-3)



A 200 mL Teflon® container with magnetic stirring bar was charged with 2.0 g (14.6 mmol) 3-aminobenzoic acid (**2**), 30 mL H_2O/MeOH (2:1, (v/v)) and 56.0 mg (2.8% (w/w)) 5% Rh/C. 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 room temperature, the catalyst was removed by filtration through a bed of Celite® (3 cm) and was washed with H_2O and MeOH (each 3 x double volume of the Celite®-bed). The solvent was removed by rotary evaporation and drying in oil pump vacuum afforded the title compound as an off-white solid.[61]

$C_7H_{13}NO_2$ [143.18]

yield: 2.08 g (14.6 mmol, 99%), off-white solid

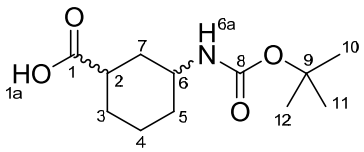
R_f = 0.26 (MeOH/CH_2Cl_2 = 5:1 (v/v), ninhydrin)

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).

6.2.2.2 *rac*-3-((*tert*-Butoxycarbonyl)amino)cyclohexanecarboxylic acid (*rac*-4)



A 500 mL round-bottom flask with magnetic stirring bar was charged with 12.00 g (83.8 mmol, 1.0 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**) 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 stirred for 30 min. 21.5 g (96.4 mmol, 1.15 eq) Boc_2O were added and the brown solution was stirred for 42 h. The reaction mixture was acidified (pH = 1) with conc. HCl, extracted with CH_2Cl_2 (4 x 150 mL). The combined organic extracts were dried over $MgSO_4$, the drying agent was removed by filtration and the solvent

was removed by rotary evaporation. The gluey, brown-flesh-coloured crude product was dissolved in 70 mL MeOH, stirred thoroughly and treated with 150 mL *n*-pentane. Precipitation of a colourless solid occurred after 15 min and the generated very turbid suspension was stirred for 17 h at 20°C. The product was isolated by filtration through a sintered glass frit (Pore 3), washed with *n*-pentane (2 x 20 mL) and the colourless solid was dried in oil pump vacuum.[61]

$C_{12}H_{21}NO_4$ [243.30]

yield: 10.9795 g (45.1 mmol, 54%), colourless solid

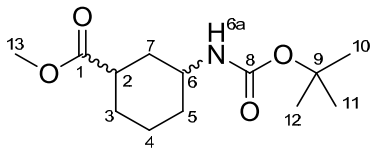
R_f = 0.77 (CH_2Cl_2/MeOH = 5:1 (v/v), CAM)

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).

6.2.2.3 *rac*-Methyl-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylate (*rac*-10)



A dry Schlenk-tube with magnetic stirring bar was charged with 1.00 g (4.11 mmol, 1.00 eq) *rac*-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (**rac-4**), 5.1 mg (41.1 µmol, 0.01 eq) 4-DMAP, 250 µL (61.7 mmol, 1.5 eq) dry MeOH and 10 mL dry THF in a N_2 counter-stream. The dirty-yellow reaction mixture was cooled to -78°C and 680 µL (4.32 mmol, 1.05 eq) *N,N*'-diisopropylcarbodiimide were added dropwise. The reaction mixture was left stirring at room temperature overnight. Full conversion was observed after 18 h (TLC). The light-yellow suspension was filtered and the filtrate was concentrated in vacuum to give a viscous, honey-brown liquid. The product was purified by flash-chromatography (40 g silica gel, 27 x 2 cm, cyclohexane/EtOAc = 10:1, fraction size: 20 mL, fractions 16 to 26 pooled).[69]

$C_{13}H_{23}NO_4$ [257.33]

yield: 441.7 mg (1.72 mmol, 42%), colourless viscous liquid

R_f = 0.51 (cyclohexane/EtOAc = 3:1 (v/v), CAM)

mp = 48-54°C

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.65-4.20 (m, 1H, H-6a), 3.80 (bs, 0.3H, H-6^{trans}), 3.70-3.62 (m, 3H, H-13), 3.41 (bs, 0.7H, H-6^{cis}), 2.65-2.49 (m, 0.3H, H-2^{trans}), 2.48-2.30 (m, 0.7H, H-2^{cis}), 2.23 (d,

$^{3}J_{HH}$ = 12.4 Hz, 1H, H-7a), 2.06-1.79 (m, 3H, H-3a, H-4a, H-5a), 1.48-1.37 (m, 9H, H-10, H-11, H-12), 1.36-1.12 (m, 3H, H-3b, H-4b, H-7b), 1.12-0.93 (m, 1H, H-5b).

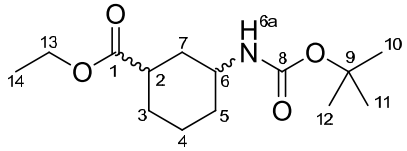
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.6 (C-1), 155.3 (C-8), 79.4 (C_q, C-9), 51.8 (C-13), 49.1 (C-6), 42.4 (C-2), 35.8 (C-7), 32.9 (C-5), 28.5 (C-10, C-11, C-12), 28.4 (C-3), 24.3 (C-4).

GC-MS (JPM_50_S): ratio (*cis*/*trans*) = 4 :1 (area/area)

t_R = 6.44^{trans} min; *m/z* = 201 (27%), 184 (17%), 156 (63%), 141 (32%), 96 (48%), 81 (44%), 56 (100%, BP).

t_R = 6.50^{cis} min ; *m/z* = 201 (17%), 184 (11%), 156 (30%), 141 (15%), 126 (19%), 98 (32%), 81 (52%), 70 (24%), 56 (100%, BP).

6.2.2.4 *rac*-Ethyl-3-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylate (*rac*-9)



A dry Schlenk tube with magnetic stirring bar was charged with 50.0 mg (206 µmol, 1.00 eq) *rac*-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (**rac-4**) and 2.0 mg (2.06 µmol, 0.01 eq) p-tolylsulfonic acid in a N_2 counter-stream. The Schlenk tube was evacuated for 30 min, refilled with N_2 and was charged with 170 µL (1.03 mmol, 5.0 eq) 1,1,1-triethoxyethane and 800 µL dry EtOH. The light-yellow solution was stirred for 24h at 20°C and additional 0.1 eq p-tolylsulfonic acid were added. After further 4 d (still incomplete conversion) the reaction mixture was concentrated by rotary evaporation. The product was purified by flash-chromatography (2.0 g silica gel, cyclohexane/EtOAc = 10:1)

$C_{14}H_{25}NO_4$ [271.35]

yield: 39.1 mg (144 µmol, 70%), colourless oil

R_f = 0.57 (cyclohexane/EtOAc = 3:1 (v/v), CAM)

mp = 54°C

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.67-4.22 (m, 1H, H-6a), 4.20-4.02 (m, 2H, H-13), 3.82 (bs, 0.3H, H-6^{trans}), 3.45 (bs, 0.7H, H-6^{cis}), 2.61-2.45 (m, 0.3H, H-2^{trans}), 2.45-2.29 (m, 0.7H, H-2^{cis}), 2.23 (d, $^{3}J_{HH}$ = 12.5 Hz, 1H, H-7a), 2.01-1.77 (m, 3H, H-3a, H-4a, H-5a), 1.55-1.37 (m, 9H, H-10, H-11, H-12), 1.36-1.12 (m, 6H, H-3b, H-4b, H-7b, H-14), 1.11-0.93 (m, 1H, H-5b).

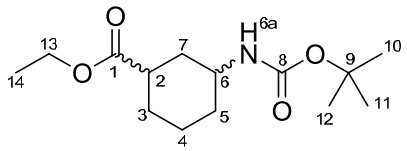
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.2 (C-1), 155.3 (C-8), 79.4 (C_q, C-9), 60.5 (C-13), 49.1 (C-6), 42.5 (C-2), 35.8 (C-7), 33.0 (C-5), 28.6 (C-10, C-11, C-12), 28.4 (C-3), 24.4 (C-4), 14.3 (C-14).

GC-MS (JPM_50_S): ratio (*cis*/*trans*) = 4:1 (area/area)

t_R = 6.59^{trans} min; *m/z* = 215 (29%), 198 (9%), 170 (100%, BP), 154 (6%), 141 (25%), 126 (4%), 109 (6%), 96 (49%), 81 (37%), 70 (19%), 56 (85%).

t_R = 6.67^{cis} min ; *m/z* = 215 (20%), 198 (11%), 170 (49%), 152 (18%), 142 (21%), 126 (30), 109 (13%), 98 (37%), 81 (52%), 70 (15%), 57 (100%, BP).

6.2.2.5 *rac*-Ethyl-3-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylate (*rac*-9)



A dry Schlenk tube with magnetic stirring bar was charged with 1.00 g (4.11 mmol, 1.00 eq) 3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (**rac-4**), 5.1 mg (41.1 µmol, 0.01 eq) 4-DMAP, 360 µL (61.7 mmol, 1.5 eq) dry EtOH and 10 mL dry THF in a N_2 counter-stream. The dirty-yellow reaction mixture was cooled to -78°C and 680 µL (4.32 mmol, 1.05 eq) *N,N*'-diisopropylcarbodiimide was added dropwise. The yellow solution was left stirring at room temperature overnight. Full conversion was observed after 18 h (TLC). The light-yellow suspension was filtered and the filtrate was concentrated in oil pump vacuum to give a viscous, honey-brown liquid. The product was purified by flash-chromatography (40 g silica gel, 27 x 2 cm, cyclohexane/EtOAc = 10:1, fraction size: 20 mL, fractions 12 to 22 pooled).[69]

$C_{14}H_{25}NO_4$ [271.35]

yield: 640.0 mg (2.36 mmol, 57%), colourless solid

R_f = 0.55 (cyclohexane/EtOAc = 3:1 (v/v), CAM)

mp = 54°C

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.67-4.22 (m, 1H, H-6a), 4.20-4.02 (m, 2H, H-13), 3.82 (bs, 0.3H, H-6^{trans}), 3.45 (bs, 0.7H, H-6^{cis}), 2.61-2.45 (m, 0.3H, H-2^{trans}), 2.45-2.29 (m, 0.7H, H-2^{cis}), 2.23 (d, $^3J_{HH}$ = 12.5 Hz, 1H, H-7a), 2.01-1.77 (m, 3H, H-3a, H-4a, H-5a), 1.55-1.37 (m, 9H, H-10, H-11, H-12), 1.36-1.12 (m, 6H, H-3b, H-4b, H-7b, H-14), 1.11-0.93 (m, 1H, H-5b).

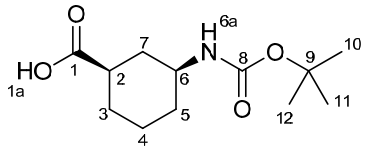
^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.2 (C-1), 155.3 (C-8), 79.4 (C_q, C-9), 60.5 (C-13), 49.1 (C-6), 42.5 (C-2), 35.8 (C-7), 33.0 (C-5), 28.6 (C-10, C-11, C-12), 28.4 (C-3), 24.4 (C-4), 14.3 (C-14).

GC-MS (JPM_50_S): ratio (*cis*/*trans*) = 4:1

t_R = 6.59^{trans} min; *m/z* = 215 (29%), 198 (9%), 170 (100%, BP), 154 (6%), 141 (25%), 126 (4%), 109 (6%), 96 (49%), 81 (37%), 70 (19%), 56 (85%).

t_R = 6.67^{cis} min ; *m/z* = 215 (20%), 198 (11%), 170 (49%), 152 (18%), 142 (21%), 126 (30), 109 (13%), 98 (37%), 81 (52%), 70 (15%), 57 (100%, BP).

6.2.2.6 (1*R*,3*S*)-3-((*tert*-Butoxycarbonyl)amino)cyclohexanecarboxylic acid ((1*R*,3*S*)-4)



A 25 mL round-bottom flask with magnetic stirring bar was charged with 106.5 mg (392 µmol, 1.0 eq) *rac*-ethyl-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylate (***rac*-9**) and 4.0 mL Et_2O. To the colourless solution were added 7.7 mL 0.1 M NaH_2PO_4/Na_2HPO_4-buffer (pH 7.6) and 400 µL PLE-precipitate (suspension in half-saturated $(NH_4)_2SO_4$). The colourless two-phase system was stirred (100 rpm) for 13 d at 20°C. The organic layer was removed with a Pasteur pipette, dried by filtering through a pipette with $MgSO_4$ and the solvent was removed in vacuum.

yield: 49.4 mg (18.2 µmol, 46% of the substrate ester ***rac*-9**) colourless oil

A 5 mL round-bottom flask with magnetic stirring bar was charged with the crude product (18.2 µmol, 1.0 eq) and 500 µL THF and 550 µL (275 µmol, 15 eq) 0.5 M KOH were added. The colourless solution was stirred for 18 h at 20 h and the solvent was removed using the rotary evaporator. The residue was treated with 3 mL H_2O and was acidified (pH = 1.5) with conc. HCl (3 drops). The colourless suspension was extracted with CH_2Cl_2 (3 x 3 mL) and the combined organic layers were dried by filtration through a Pasteur pipette with cotton and $MgSO_4$. The solvent was removed by rotary evaporation and the product was dried using oil pump vacuum.[98]

yield: 33.3 mg (137 µmol, 35%) colourless foamy solid

$[\alpha]_D^{20}$ = +4.8°(c = 0.8325, MeOH)

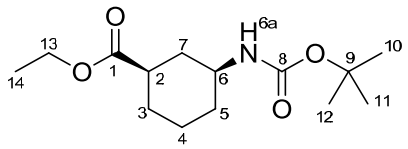
The aqueous layer of the PLE-experiment was acidified (pH 2) with 1 M HCl (6 mL) and was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtrated through a sintered glass frit (Pore 3), the solvent was removed by rotary evaporation and was dried using oil pump vacuum.

yield: 13.7 mg (56.3 µmol, 14%) colourless oil

$[\alpha]_D^{20}$ = -22.3° (c = 0.3425, MeOH)

$[\alpha]_D^{20}$ = -50.5° (c = 1, MeOH, lit.)[75]

6.2.2.7 (1*R*,3*S*)-Ethyl 3-((*tert*-butoxycarbonyl)amino)cyclohexanecarboxylate ((1*R*,3*S*)-9)



The crude (1*R*,3*S*)-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid **(1*R*,3*S*)-4** (55.5 µmol, 1.0 eq) was transferred to a dry 8 mL Schlenk tube with magnetic stirring bar and was dried in oil pump vacuum. The colourless foamy solid was dissolved in 400 µL dry CH_2Cl_2 and 8.3 µL (141 µmol, 2.5 eq) EtOH and 13.0 mg (106 µmol, 1.9 eq) 4-DMAP were added in a N_2 counter-stream. The colourless solution was cooled to 0°C and 27.5 mg (141 µmol, 2.5 eq) EDC·HCl was added. The cooling was removed after 5 min and the reaction mixture was stirred for 30 h at 20°C. The colourless reaction mixture was treated with CH_2Cl_2 (5 mL) and was washed with H_2O (2 x 3 mL). The turbid organic phase was dried by filtering through a pipette with cotton and $MgSO_4$ and the solvent was removed by rotary evaporation. The crude product (greenish / yellow solid) was dissolved in 30 mL EtOAc, adsorbed on 75 mg Celite and the solvent was removed by rotary evaporation and oil pump vacuum. The product was purified by flash chromatography (3 g SiO_2, 130 x 8 mm, cyclohexane/EtOAc = 10:1 (v/v), fraction size: ~2 mL).

$C_{14}H_{25}NO_4$ [271.35]

yield: 11.9 mg (43.9 µmol, 79%) colourless solid in viscous liquid

$[\alpha]_D^{20}$ = -3.7°(c = 0.595, MeOH)

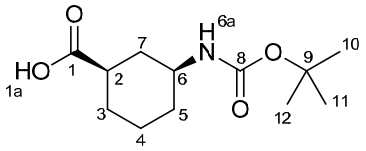
chiral-HPLC-UV (Method_A4): t_R = 5.25^{minor}/5.96 (*trans*-enantiomers), 7.48^{minor}/8.78 (*cis*-enantiomers).

^{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 (JPM_50_S): 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%, BP).

6.2.2.8 (1*R*,3*S*)-3-((*tert*-Butoxycarbonyl)amino)cyclohexanecarboxylic acid ((1*R*,3*S*)-4)



A 25 mL round-bottom flask with magnetic stirring bar was charged with 2.00 g (8.22 mmol, 1.0 eq) *rac*-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (**rac-4)** and 8.0 mL EtOH. The colourless solution was stirred and 535 µL (4.12 mmol, 0.5 eq) D-(+)-alpha-methylbenzylamine were added. The turbid, colourless suspension was heated to 60°C until a clear solution was obtained (30 min) and was kept stirring vigorously for 4 d at 20°C. The colourless salt was isolated by 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 colourless 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 $MgSO_4$, filtered, the solvent was removed by rotary evaporation and the colourless 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 by rotary evaporation and in oil pump vacuum.[61]

$C_{12}H_{21}NO_4$ [243.30]

yield: 404.0 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

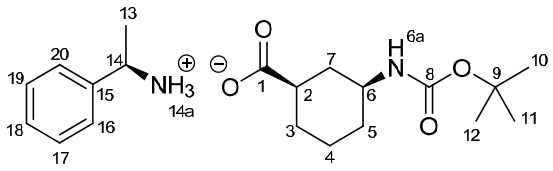
$[\alpha]_D^{20}$ = -48.4° (c = 1.55, MeOH)

$[\alpha]_D^{20}$ = -50.5 °(c = 1, MeOH, lit.)[75]

^{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-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).

Diastereomeric salt **(1*R*,3*S*)-11**



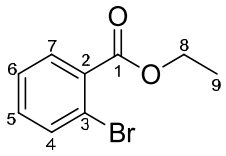
$C_{20}H_{32}N_2O_4$ [364.48]

^{1}H-NMR (300.36 MHz, MeOH-d_4): δ = 7.44-7.29 (m, 5H, CH_{Ar}), 4.36 (q, $^3J_{HH}$ = 6.9 Hz, 1H, H-14), 3.26 (m, 1H, H-6), 2.14 (t, $^3J_{HH}$ = 11.8Hz, 1H, H-2), 2.00 (d, $^3J_{HH}$ = 12.3 Hz, 1H, H-7a), 1.92-1.65 (m, 3H, H-3a, H-4a, 5a), 1.56 (d, $^3J_{HH}$ = 6.9 Hz, 3H, H-13), 1.38 (s, 9H, H-10, H-11, H-12), 1.30-0.94 (m, 4H, H-3b, H-4b, H-5b, H-7b).

^{13}C-NMR (75.53 MHz, MeOH-d_4): δ = 183.6 (C-1), 157.7 (C-8), 130.2 (C-16, C-17, C-19, C-20), 129.9 (C-15), 127.6 (C-18), 79.7 (C-9), 52.3 (C-6), 50.7 (C-14), 47.2 (C-2), 37.6 (C-7), 34.0 (C-5), 30.5 (C-3), 28.8 (C-10, C-11, C-12), 25.8 (C-4), 21.3 (C-13).

6.2.3 Optimization of the Ullmann-condensation reaction

6.2.3.1 Ethyl 2-bromobenzoate



A 10 mL Schlenk tube with magnetic stirring bar was charged with 500 mg (2.41 mmol, 1.0 eq) 2-bromobenzoic acid, 2.0 mL EtOH and 390 µL (7.23 mmol, 3.0 eq) conc. H_2SO_4. The reaction mixture was heated to 85°C for 20 h and then concentrated by rotary evaporation. The yellow residue was neutralized with sat. $NaHCO_3$-solution and was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed by rotary evaporation and in oil pump vacuum. The crude product was ^{1}H-NMR pure and was used without further purification in the following ULLMANN-condensation reaction screenings.

$C_9H_9BrO_2$ [229.07]

yield: 527.3mg (2.30 mmol, 95%) light-yellow liquid, pleasant smell

R_f = 0.69 (cyclohexane/EtOAc = 5:1 (v/v), UV 254 nm)

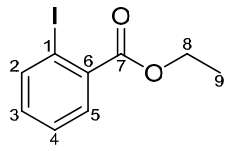
HPLC-MS (Method A1): t_R = 16.99 min

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.78 (dd, $^3J_{HH}$ = 7.4 Hz, $^4J_{HH}$ = 2.0 Hz, 1H, H-7), 7.65 (dd, $^3J_{HH}$ = 7.6 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, H-4), 7.34 (pd, $^3J_{HH}$ = 7.4 Hz, $^4J_{HH}$ = 1.7 Hz, 2H, H-5, H-6), 4.40 (q, $^3J_{HH}$ = 7.1 Hz, 2H, H-8), 1.41 (t, $^3J_{HH}$ = 7.1 Hz, 3H, H-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 166.3 (C-1), 134.2 (C-4), 132.7 (C-2), 132.5 (C-5/C-6), 131.2 (C-7), 127.2 (C-5/C-6), 121.6 (C-3), 61.7 (C-8), 14.3 (C-9).

GC-MS (JPM_50_S): t_R = 5.47 min; *m/z* = 230/228 (18%, M^+), 201/199 (36%), 185/183 (100%, BP), 157/155 (34%), 76 (35%).

6.2.3.2 Ethyl 2-iodobenzoate



A 10 mL Schlenk tube with magnetic stirring bar was charged with 611 mg (2.41 mmol, 1.0 eq) 2-iodobenzoic acid, 2.0 mL EtOH and 390 µL (7.23 mmol, 3.0 eq) conc. H_2SO_4. The reaction mixture was heated to 85°C for 20 h and was concentrated by rotary evaporation. The yellow residue was neutralized with sat. $NaHCO_3$-solution and was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtrated and the solvent was removed by rotary evaporation and in oil pump vacuum. The crude product was ^{1}H-NMR pure and was used without further purification in the following ULLMANN-condensation reaction screenings.

$C_9H_9IO_2$ [276.07]

yield: 572.2 mg (2.07 mmol, 86%) light-yellow liquid, pleasant smell

R_f = 0.96 (CH_2Cl_2/MeOH = 5:1 (v/v), UV 254 nm and CAM)

HPLC-MS (Method_A2): t_R = 5.34 min

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 7.99 (dd, $^3J_{HH}$ = 7.9 Hz, $^4J_{HH}$ = 0.9 Hz, 1H, H-5), 7.79 (dd, $^3J_{HH}$ = 7.8 Hz, , $^4J_{HH}$ = 1.6 Hz, 1H, H-2), 7.40 (td, $^3J_{HH}$ = 7.7 Hz, $^4J_{HH}$ = 1.1 Hz, 1H, H-3), 7.14 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, H-4), 4.40 (q, $^3J_{HH}$ = 7.1 Hz, 2H, H-8), 1.41 (t, $^3J_{HH}$ = 7.1 Hz, 3H, H-9).

^{13}C-NMR (75.53 MHz, $CDCl_3$):): δ = 166.8 (C-7), 141.4 (C-5), 135.7 (C-6), 132.6 (C-4), 131.0 (C-2), 128.0 (C-3), 94.1 (C-1), 61.9 (C-8), 14.4 (C-9).

GC-MS (JPM_50_S): t_R = 5.83 min; *m/z* = 276 (60%, M^+), 248 (33%), 231 (100%, BP), 203 (33%), 76 (33%).

6.2.3.3 General procedures ULLMANN-condensation reaction

GP-U1:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (349 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 233 µmol (1.0 eq) aryl-halide (if solid), 117 mg (838 µmol, 3.6 eq) K_2CO_3 and 24.0 mg (34.9 µmol, 0.15 eq) Cu/Sn (10:1 (w/w)) in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times and 233 µmol (1.5 eq) aryl-halide (if liquid) and 1.0 mL dry DMSO was added. The suspensions were heated to 120°C for 3 d, the solvent was removed by rotary evaporation and the product was purified by column chromatography (CH_2Cl_2/MeOH/AcOH = 200:1:1 (v/v/v)). Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[77]

GP-U2:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (349 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 233 µmol (1.0 eq) aryl-halide (if solid), 65.0 mg (466 µmol, 2.0 eq) K_2CO_3, 17.0 mg (46.6 µmol, 0.2 eq) $Fe(acac)_3$ and 8.6 mg (46.6 µmol, 0.2 eq) $Cu(OAc)_2$ in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times and 233 µmol (1.5 eq) aryl-halide (if liquid) and 1.0 mL dry DMF was added. The suspensions were heated to 135°C for 3 d. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[81]

GP-U3:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 43.3 mg (298 µmol, 1.2 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 248 µmol (1.0 eq) aryl-halide (if solid), 106.3 mg (497 µmol, 2.0 eq) K_3PO_4 and 2.4 mg (12 µmol, 0.05 eq) CuI in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 248 µmol (1.2 eq) Aryl-halide (if liquid), 34.0 µL (497 µmol, 2.0 eq) ethylene glycol and 1.0 mL 2-propanol were added in a N_2 counter-stream. The suspension was heated to 80°C for 5 d. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[82]

GP-U4a:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (350 µmol, 1.0 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 524 µmol (1.5 eq) aryl-bromide (if solid), 150 mg RB-DHAC, 6.7 mg (34.8 µmol, 0.1 eq) CuI and 8.8 µL (69.8 µmol, 0.2 eq) DMEDA in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 524 µmol (1.5 eq) aryl-bromide (if liquid) and 1.0 mL dry DMSO was added via a syringe. The suspension was first stirred at 20°C for 6 d, then heated to 100°C for 1 d. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[83]

GP-U4b:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (350 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 233 µmol (1.0 eq) aryl-iodide (if solid), 150 mg RB-DHAC, 4.4 mg (23.3 µmol, 0.1 eq) CuI and 5.4 mg (46.6 µmol, 0.2 eq) L-proline in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 524 µmol (1.5 eq) Aryl-iodide (if liquid) and 1.0 mL dry DMSO was added via a syringe. The suspension was first stirred at 20°C for 6 d, then heated to 100°C for 1 d. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[83]

GP-U5:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (349 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 233 µmol (1.0 eq) aryl-halide (if solid), 13.3 mg (46.6 µmol, 0.2 eq) *S*-BINOL and 100.9 mg (466 µmol, 2.0 eq) K_3PO_4 in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 233 µmol (1.5 eq) Aryl-halide (if liquid), 4.5 mg (23.3 µmol, 0.1 eq) CuI and 1.0 mL dry DMF was added. The suspension was first stirred at 20°C for 5 d, then heated to 100°C for 1 d. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[63]

GP-U6:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (349 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 48.2 mg (233 µmol, 1.0 eq) 2-bromobenzoic acid, 100.9 mg (466 µmol, 2.0 eq) K_3PO_4 and variable amounts of *S*-BINOL and CuI in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times, 1.0 mL dry DMF was added and the turquoise suspension was heated to 50°C for 8 d. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[63]

GP-U7:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (349 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 233 µmol (1.0 eq) aryl-halide (if solid), 330 mg (524 µmol, 1.5 eq) TBAA and 4.6 µmol (0.2 eq) ligand in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 233 µmol (1.5 eq) Aryl-halide (if liquid) and 1.0 mL dry solvent were added. The blue/green-turquoise suspensions were stirred at 20°C for the denoted time and were diluted with 2 mL EtOAc and concentrated by rotary evaporation. HPLC-MS samples were prepared by dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[84]

GP-U8a:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 50.0 mg (349 µmol, 1.5 eq) *rac*-3-aminocyclohexanecarboxylate (***rac*-12**), 58.9 mg (233 µmol, 1.0 eq) 2-iodobenzoic acid, 220 mg (349 µmol, 1.5 eq) TBAA, 5.4 mg (46.6 µmol, 0.2 eq) L-proline and 4.4 mg (23.3 µmol, 0.1 eq) CuI in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times and 1.0 mL dry DMF was added in a N_2 counter-stream. The sky-blue suspension was stirred 20 d at 20°C and was heated to 60°C for 1 d, diluted with EtOAc and concentrated by rotary evaporation. HPLC-MS samples were prepared by dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[84]

GP-U8b:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 25.0 mg (146 µmol, 1.5 eq) *rac*-ethyl 3-aminocyclohexanecarboxylate (***rac*-12**), 97.3 µmol (1.0 eq) aryl-iodide (if solid), 91.8 mg (146 µmol, 1.5 eq) TBAA, 2.3 mg (19.5 µmol, 0.2 eq) L-proline and 1.9 mg (9.7 µmol, 0.1 eq) CuI in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 97.3 µmol (1.5 eq) Aryl-iodide (if liquid) and 500 µL dry DMF were added. The suspensions were stirred 20 d at 20°C and then heated for 5 d to 60°C, diluted with EtOAc and concentrated by rotary evaporation. HPLC-MS samples were prepared by dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[84]

GP-U9:

A dry 10 mL Schlenk tube with magnetic stirring bar was charged with 30.0 mg (210 µmol, 1.0 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**), 248 µmol (1.2 eq) aryl-halide (if solid), 90.0 mg (630 µmol, 3.0 eq) K_2CO_3 and 4.0 mg (21 µmol, 0.1 eq) CuI in a N_2 counter-stream. The Schlenk tube was evacuated and refilled with N_2 for two times. 248 µmol (1.2 eq) Aryl-halide (if liquid), 5.0 µL (278 nmol, 1.3 meq) H_2O and 1.0 mL dry DMF were added via a syringe. The coloured suspension was heated to 100°C and kept stirring. Work-up for HPLC-MS reaction control was performed by extraction of an aliquot, dissolution in H_2O/CH_3CN = 1:1 (v/v) and filtration through cotton/micro filter.[99]

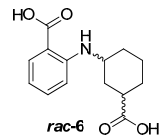
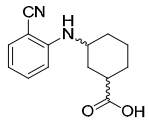
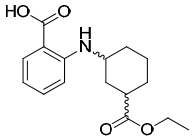
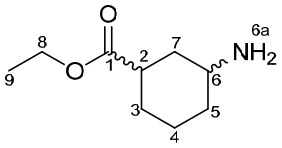
ULLMANN-product	retention time (t_R) (Method_A1)	UV absorption bands / nm
 *rac*-6	12.07 min	221 258 353
	13.77 min	219 256 338
	16.61 min	225 259 355

Table T17: Analytical data of the ULLMANN-condensation products

6.2.3.4 *rac*-Ethyl 3-aminocyclohexanecarboxylate (*rac*-12)



A 50 mL Schlenk tube with magnetic stirring bar was charged with 1.00 g (6.98 mmol, 1.0 eq) *rac*-3-aminocyclohexanecarboxylic acid (***rac*-3**) and 15 mL EtOH. 1.09 mL (20.8 mmol, 3.0 eq) conc. H_2SO_4 was added and the colourless suspension was heated to 90°C for 4 d. The yellow reaction mixture was concentrated by rotary evaporation, neutralized with sat. $NaHCO_3$-solution (40 mL) and was extracted with EtOAc (4 x 30 mL). The combined organic layers were dried over $MgSO_4$, the drying agent was removed by filtration and the solvent was removed by rotary evaporation and drying in oil pump vacuum.

$C_9H_{17}NO_2$ [171.24]

yield: 453.4 mg (2.65 mmol, 38%) light-yellow liquid, strong Fizzers™-smell

R_f = 0.50 (CH_2Cl_2/MeOH = 5:1) (CAM)

GC-MS (JPM_50_S): ratio (*cis*/*trans*) = 1:1 (area/area). Peaks not assigned to the stereoisomers.

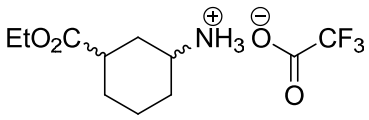
t_R = 7.84 min; *m/z* = 325 (15%), 282 (18%), 252 (100%, BP), 210 (64%), 170 (15%), 81 (11%).

t_R = 7.96 min; *m/z* = 325 (13%), 282 (19%), 252 (100%, BP), 234 (25%), 210 (46%), 81 (11%).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 4.19-4.03 (m, 2H, H-8), 3.13-2.99 (m, 0.3H, H-6^{trans}), 2.78-2.57 (m, 1H, H-6^{cis}, H-2^{trans}), 2.41-2.21 (m, 0.7H, H-2^{cis}), 2.09 (d, $^3J_{HH}$ = 12.5 Hz, 1H, H-7a), 2.03-1.41 (m, 3H, H-3a, H-4a, H-5a), 1.39-1.12 (m, 6H, H-3b, H-4b, H-7b, H-9), 1.13-0.90 (m, 1H, H-5b).

^{13}C-NMR (75.53 MHz, $CDCl_3$): (only *cis* assigned) δ = 175.2 (C-1), 60.5 (C-8), 50.1 (C-6), 42.5 (C-2), 37.4 (C-7), 34.5 (C-5), 28.2 (C-3), 24.2 (C-4), 14.3 (C-9).

6.2.3.5 *rac*-3-(Ethoxycarbonyl)cyclohexanaminium 2,2,2-trifluoroacetate (*rac*-5)

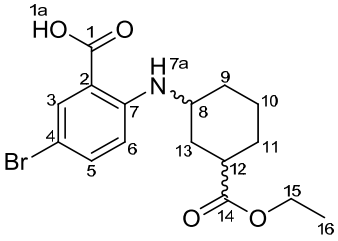


A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 54.2 mg (200 µmol) *rac*-ethyl 3-aminocyclohexanecarboxylate (***rac*-9**) and 1.5 mL dry CH_2Cl_2 in a N_2 counter-stream. 1.5 mL TFA were added, whereby gas evolution occurred. The slightly pink solution was stirred for 5 h at 20°C and the solvent was removed in oil pump vacuum. The crude product can be used in the ULLMANN-condensation reaction instead of *rac*-ethyl-3-aminocyclohexanecarboxylate (***rac*-12**).[77]

$C_{11}H_{18}F_3NO_4$ [285.26]

yield: 62.3 mg, light-yellow viscous liquid

6.2.3.6 *rac*-5-Bromo-2-((3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (*rac*-14)



A dry 10 ml Schlenk flask with magnetic stirring bar was charged with 293.7 mg (898 µmol, 1.0 eq) 5-bromo-2-iodobenzoic acid (**8**), 847.7 mg (1.35 mmol, 1.5 eq) TBAA, 20.9 mg (180 µmol, 0.2 eq) L-proline and 17.5 mg (89.8 µmol, 0.1 eq) CuI, the tube was evacuated and refilled with N_2 for two times. 200 mg (1.17 mmol, 1.3 eq) *Rac*-ethyl-3-aminocyclohexanecarboxylate (***rac*-12**) were added via a pipette which was subsequently rinsed with 4.0 mL dry DMF. The blue suspension was heated to 60°C for 65 h, the solvent was removed in vacuum and the emerald-green, viscous liquid was diluted with MeOH, adsorbed on 1.4 g Celite and the solvent was removed in vacuum. The crude product was purified via flash-chromatography (30 g SiO_2, 180 x 22 mm, eluent: CH_2Cl_2/MeOH/AcOH = 400:1:1, fraction size: 20 mL). Residues of AcOH were removed by repeated co-evaporation with cyclohexane (mixing in ultrasonic bath) by rotary evaporation and drying in oil pump vacuum.[84]

$C_{16}H_{20}BrNO_4$ [370.24]

yield: 224.6 mg (606 µmol, 67%, HPLC-purity 77%) light-brown, sticky solid

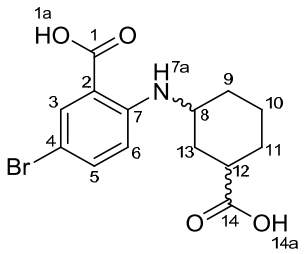
R_f = 0.40 (CH_2Cl_2/MeOH/AcOH = 50:1:1) (UV 254+366 nm, CAM: red)

HPLC-MS (Method_A1): t_R = 17.77 min

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 10.43 (bs, 1H, H-1a), 8.13^{trans}/8.08 (d, $^{4}J_{HH}$ = 2.3/2.3 Hz, 1H, H-3), 7.43/7.32^{trans} (dd, $^{3}J_{HH}$ = 9.1/8.4 Hz, $^{4}J_{HH}$ = 2.5/2.4 Hz, 1H, H-5), 6.75^{trans}/6.65 (d, $^{3}J_{HH}$ = 9.2/9.1 Hz, 1H, H-6), 4.14 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, H-15), 3.76^{trans}/3.37 (m, 1H, H-8), 2.72^{trans}/2.45 (m, 1H, H-12), 2.36 (d, $^{3}J_{HH}$ = 15.6 Hz, 1H, H-9a), 2.13 (d, $^{3}J_{HH}$ = 11.6 Hz, 1H, H-13a), 2.07-1.98 (m, 1H, H-11a), 1.98-1.90 (m, 1H, H-10a), 1.53-1.35 (m, 3H, H-9b, H-10b, H-13b), 1.30-1.21 (m, 4H, H-11b, H-16).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.4^{trans}/175.1 (C-14), 172.7^{trans}/172.5 (C-1), 149.5^{trans}/149.2 (C-7), 138.4^{trans}/138.3 (C-5), 135.1^{trans}/135.0 (C-3), 114.4^{trans}/114.3 (C-6), 110.5/110.1^{trans} (C-4), 106.4/106.2^{trans} (C-2), 60.8/60.7^{trans} (C-15), 51.2/47.6^{trans} (C-8), 42.6/39.0^{trans} (C-12), 35.1 (C-9), 32.5 (C-13), 28.4 (C-11), 24.3 (C-10), 14.4^{trans}/14.3 (C-16).

6.2.3.7 *rac*-5-Bromo-2-((3-carboxycyclohexyl)amino)benzoic acid (*rac*-1)

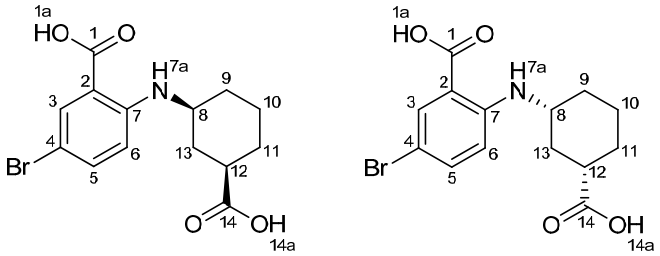


A 10 mL Schlenk tube with magnetic stirring bar was charged with 200.1 mg (540 µmol, 1.0 eq) *rac*-5-bromo-2-((3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (***rac*-14**) and 5 mL of a THF / MeOH / H_2O-mixture (3:1:1 (v/v/v)). 46.6 mg (1.08 mmol, 2.0 eq) $LiOH{\cdot}H_2O$ were added and the yellow solution was kept stirring at 20°C for 29 h. The reaction mixture was treated with 1M HCl (3 mL), extracted with Et_2O (3 x 10 mL), the combined organic layers were dried over $MgSO_4$, the drying agent was removed by filtration and the solvent was removed by rotary evaporation. The yellow crude product (181.8 mg) was dissolved in MeOH (5 mL), adsorbed on 400 mg Celite® and the solvent was removed in vacuum. The product was purified by flash chromatography (20 g SiO_2, 200 x 15 mm, eluent: cyclohexane/EtOAc/AcOH = 500:1:1, fraction size: 15 mL).[88]

$C_{14}H_{16}BrNO_4$ [342.19]

yield (*cis*&*trans*): 113.8 mg (333 µmol, 62%) light-yellow solid

cis-enantiomers: **(1*R*,3*S*)-3** & **(1*S*,3*R*)-3** (fractions 21-38):



yield: 44.6 mg (130 µmol, 24%) light-yellow solid

R_f = 0.16 (cyclohexane/EtOAc/AcOH = 2:1:1) (UV 366 nm, CAM: red)

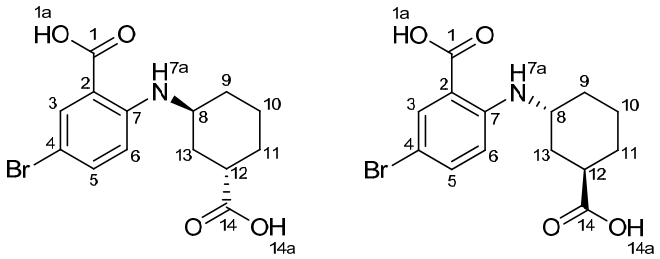
mp = 220°C

HPLC-MS (Method_A3): t_R = 4.93 min

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.56 (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).

trans-enantiomers: **(1*S*,3*S*)-3** & **(1*R*,3*R*)-3** (fractions 12-14):



yield: 21.9 mg (64.0 µmol, 12%) yellow solid

R_f = 0.23 (cyclohexane/EtOAc/AcOH = 2:1:1) (UV 366 nm, CAM: red)

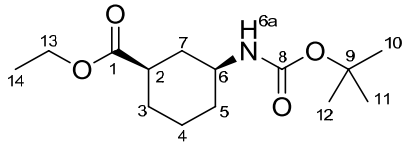
HPLC-MS (Method_A3): t_R = 4.91 min

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.63 (bs, 2H, H-1a, H-14a), 7.84 (d, $^4J_{HH}$ = 2.5 Hz, 1H, H-3), 7.45 (dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 2.5 Hz, 1H, H-5), 6.78 (d, $^3J_{HH}$ = 9.2 Hz, 1H, H-6), 3.72 (s, 1H, H-8), 2.48 (s, 1H, H-12), 2.00-1.82 (m, 1H, H-13a), 1.81-1.59 (m, 4H, H-9a, H-11a, H-11b, H-13b), 1.59-1.40 (m, 3H, H-9b, H-10a, H-10b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.1 (C-14), 169.0 (C-1), 148.9 (C-7), 136.8 (C-5), 133.6 (C-3), 114.1 (C-6), 111.5 (C-4), 104.3 (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).

6.2.4 Total synthesis of Phenazistatin A (1*R*,3*S*)-1

6.2.4.1 (1*R*,3*S*)-Ethyl 3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylate ((1*R*,3*S*)-9)



A dry 5 mL Schlenk flask with magnetic stirring bar was charged with 201.0 mg (822 µmol, 1.0 eq) (1*R*,3*S*)-3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylic acid (**(1*R*,3*S*)-4**) and 2.0 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. 72 µL dry EtOH and 2.0 mL dry CH_2Cl_2 were added, the colourless solution was cooled to -78°C and 136 µL (863µL, 1.05 eq) *N,N'*-diisopropylcarbodiimide were added during 3 min. The slightly-yellow solution was allowed to warm to 20°C overnight and was stirred for overall 40 h. The colourless 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 by rotary evaporation and was dried in oil pump vacuum to afford a colourless crude product (254.9 mg). The crude product was dissolved in MeOH, adsorbed on 630 mg Celite®, the solvent was removed in vacuum and the colourless powder was subjected to flash-chromatography (15 g SiO_2, 10 x 2 cm, eluent: cyclohexane/EtOAc = 20:1, fraction size: 10 mL).[69]

$C_{14}H_{25}NO_4$ [271.35]

yield: 191.0 mg (704 µmol, 86%) off-white solid

mp: 54°C

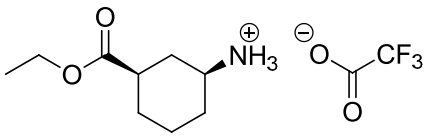
$[\alpha]_D^{20}$ = -29.9° (c = 1.31, MeOH)

^{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).

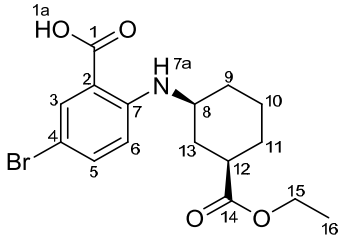
GC-MS (JPM_50_S): t_R = 6.50 min ; *m/z* = 201 (17%), 184 (11%), 156 (30%), 141 (15%), 126 (19%), 98 (32%), 81 (52%), 70 (24%), 56 (100%, BP).

6.2.4.2 (1*S*,3*R*)-3-(Ethoxycarbonyl)cyclohexanaminium 2,2,2-trifluoroacetate ((1*R*,3*S*)-19)



A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 250.0 mg (876 µmol) (1*R*,3*S*)-ethyl 3-((tert-butoxycarbonyl)amino)cyclohexanecarboxylate (**(1*R*,3*S*)-9**) 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-coloured solution was stirred at 20°C for 4 h. The solvent was removed in oil pump vacuum to afford a flesh-coloured viscous liquid which was used in the following step without further purification.[100]

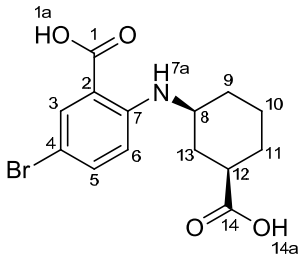
5-Bromo-2-(((1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid ((1*R*,3*S*)-14)



To the Schlenk tube containing the crude (1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexanaminium 2,2,2-trifluoroacetate (**(1*R*,3*S*)-19**) (876µmol, 1.5 eq) were added 191.0 mg (584 µmol, 1.0 eq) 5-bromo-2-iodobenzoic acid (**8**), 551.3 mg (876 µmol, 1.5 eq) TBAA, 13.6 mg (117 µmol, 0.2 eq) L-proline and 11.4 mg (58 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 was added and the yellow suspension was heated to 60°C. After 18 h additional 11.4 mg (0.1 eq) CuI were added but no conversion could be obtained. The solvent was removed in vacuum, the orange/brown residue was treated with saturated $NaHCO_3$-solution (3 mL) and the greenish-yellow solution was extracted with EtOAc (4 x 5 mL). The combined organic layers were dried over Na_2SO_4, the drying agent was removed by filtration and the solvent was removed by rotary evaporation and oil pump vacuum. The emerald-green viscous liquid was transferred to a dry 10 mL Schlenk flask and 191.0 mg (584 µmol, 1.0 eq) 5-bromo-2-iodobenzoic acid (**8**), 551.3 mg (876 µmol, 1.5 eq) TBAA (old batch!), 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. 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 above $MgSO_4$, the drying agent was removed by filtration and the solvent was removed by rotary evaporation and oil pump vacuum to yield an

iodine-brown viscous liquid (751.9 mg) which was used in the following step without further purification.[84]

6.2.4.4 5-Bromo-2-(((1*S*,3*R*)-3-carboxycyclohexyl)amino)benzoic acid ((1*R*,3*S*)-1)



The crude 5-bromo-2-(((1*S*,3*R*)-3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (**(1*R*,3*S*)-14**) ("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·H_2O and the greenish reaction mixture was stirred for 20 h at room temperature. Additional 57.2 mg (1.36 mmol, 1.6 eq) LiOH·H_2O was added and the deep grass-green reaction mixture was stirred for 3 d at room temperature. The reaction mixture was filtered through a wet pad of silica gel (3 cm) to remove the verdigris-coloured precipitate and the components were eluted using cyclohexane/EtOAc = 1:1, EtOAc and MeOH. The eluted fractions were combined and the solvent was removed by rotary evaporation. 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·H_2O was added and the olive-green reaction mixture was stirred at 20°C for 22 h until complete conversion. The green suspension was treated with 1 M HCl (5 mL), extracted with Et_2O (5 x 10 mL), the combined organic layers were dried over $MgSO_4$, the drying agent was removed by filtration and the solvent was removed using the rotary evaporator. The orange-yellow crude product (465 mg) was dissolved in 20 mL CH_2Cl_2, adsorbed on 1.4 g Celite and the solvent was removed in vacuum. The product was purified by flash chromatography (20 g SiO_2, 230 x 15 mm, eluent: cyclohexane/EtOAc/AcOH = 600:100:1 then 500:100:1 then 300:100:1, fraction size: 20 mL, fractions 30 to 63 were pooled).[88]

$C_{14}H_{16}BrNO_4$ [342.19]

yield: 160.1 mg (469 µmol, 80%) canary-yellow solid

R_f = 0.38 (cyclohexane/EtOAc/AcOH = 1:1:drop)

mp = >190°C (decomp.)

$[\alpha]_D^{20}$= +18° (c = 0.5 (5 g/L), MeOH)[77]

HPLC-MS (Method_A3): t_R = 4.94 min

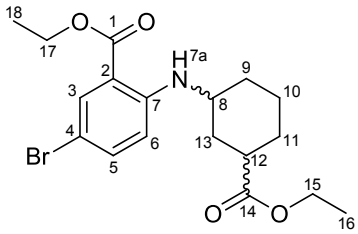
^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 12.56 (bs, 2H, H-1a, H-14a), 7.84 (ds, $^4J_{HH}$ = 2.4 Hz, 1H, H-3), 7.45 (dd, $^3J_{HH}$ = 9.0 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-5), 6.80 (d, $^3J_{HH}$ = 9.2 Hz, 1H, H-6), 3.43 (m, 1H, H-8),

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).

6.2.5 Post-ULLMANN modifications

6.2.5.1 *rac*-Ethyl 5-bromo-2-((3-(ethoxycarbonyl)cyclohexyl)amino)benzoate (*rac*-22)



A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 30.0 mg (81.0 µmol, 1.0 eq) *rac*-5-bromo-2-((3-(ethoxycarbonyl)cyclohexyl)amino)benzoic acid (***rac*-14**), 1.8 mg (14.7 µmol, 0.18 eq) 4-DMAP, 7.1 µL dry EtOH and 500 µL dry CH_2Cl_2. To the stirred yellow solution were added 13.3 µL *N,N*'-diisopropylcarbodiimide at 20°C and the mixture was stirred for 17 h until completion. The reaction mixture was filtrated to remove the generated *N,N*'-diisopropylurea. The filter cake was washed with CH_2Cl_2 and the filtrate was concentrated by rotary evaporation. The residue was dissolved in MeOH and the crude product was adsorbed on 130 mg Celite. The solvent was removed in vacuum and the crude product was purified by flash chromatography (4.3 g SiO_2, 120 x 10 mm, cyclohexane/EtOAc = 20:1, fraction size: 3 mL).[69]

$C_{18}H_{24}BrNO_4$ [398.29]

yield: 23.7 mg (59.5 µmol, 73%) red solid in viscous liquid

R_f = 0.67 (cyclohexane/EtOAc = 3:1 (v/v)), (254 nm, 366 nm, CAM: slowly red)

GC-MS (JPM_50_S, *cis*): t_R = 9.29 min; *m/z* = 399/397 (100%, M^+), 368/370 (58%), 326 (69%), 324 (75%), 309 (36%), 306 (29%), 284 (50%), 282 (56%), 238 (67%), 236 (72%), 199 (27%), 197 (31%), 172 (19%), 170 (36%), 81 (73%), 79 (33%), 55 (33%).

GC-MS (JPM_50_S, *trans*): t_R = 9.08 min; *m/z* = 399/397 (89%, M^+), 370/368 (55%), 326 (66%), 324 (70%), 284 (53%), 282 (56%), 238 (66%), 236 (71%), 207 (38%), 170 (34%), 109 (39%), 81 (100%, BP), 79 (44%), 55 (35%).

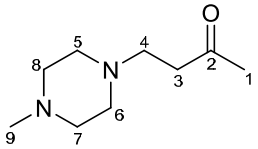
HPLC-MS (Method_A2): t_R = 6.51/6.57^{trans} min

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 8.02 (d, $^4J_{HH}$ = 2.4 Hz, 1H, H-3), 7.41 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 2.4 Hz, 1H, H-5), 6.74^{trans}/6.68 (d, $^3J_{HH}$ = 9.1 Hz, 1H, H-6), 4.31 (q, $^3J_{HH}$ = 7.1 Hz, 2H, H-17), 4.11 (q,

$^{3}J_{HH}$ = 7.1 Hz, 2H, H-15), 3.74^{trans}/3.34 (m, 1H, H-8), 2.70 (m, 0.3H, H-12^{trans}), 2.50-2.29 (m, 1.7H, H-9a, H-12), 2.12 (d, $^{3}J_{HH}$ = 12.0 Hz, 1H, H-13a), 2.07-1.96 (m, 1H, H-11a), 1.96-1.87 (m, 1H, H-10a), 1.49-1.32 (m, 6H, H-9b, H-10b, H-13b, H-18), 1.32-1.11 (m, 4H, H-11b, H-16).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 175.4^{trans}/174.9 (C-14), 167.9^{trans}/167.7 (C-1), 149.0^{trans}/148.6 (C-7), 137.2^{trans}/137.1 (C-5), 134.2/134.1^{trans} (C-3), 114.0 (C-6), 112.1/111.8^{trans} (C-4), 106.2/105.8^{trans} (C-2), 60.9 (C-15), 60.6 (C-17), 51.1 (C-8^{cis}), 47.4 (C-8^{trans}), 42.6 (C-12^{cis}), 38.9 (C-12^{trans}), 35.2 (C-9), 32.5 (C-13), 28.5 (C-11), 24.4 (C-10), 14.5/14.4 (C-16, C-18).

6.2.5.2 4-(4-Methylpiperazin-1-yl)butan-2-one (21)



A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 150 mg Amberlyst-15 ion-exchange resin, was cooled to 0°C and 506 µL (6.24 mmol, 1.25 eq) methylvinyl ketone were added. 554 µL (4.99 mmol, 1.0 eq) 1-methylpiperazine were added dropwise within 20 min at 0°C. GC-MS reaction control indicated full conversion after 60 min and the reaction mixture was dissolved in 20 mL CH_2Cl_2, filtrated and the solvent was removed in vacuum. The very polar product was purified by flash chromatography (34 g SiO_2, eluent: EtOAc/cyclohexane/triethylamine = 50:10:1, then MeOH!)[89]

$C_9H_{18}N_2O$ [170.25]

yield: 630.3 mg (3.70 mmol, 74%) yellow-brown liquid

R_f = 0.85 (MeOH/CH_2Cl_2 = 5:1 (v/v), CAM: blue)

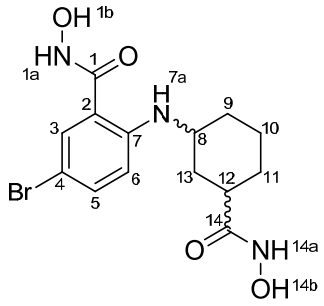
GC-MS (JPM_50_S): t_R = 5.05 min, *m/z* = 170 (20%, M^+), 127 (15%), 113 (26%), 100 (26%), 70 (78%), 55 (BP).

HPLC-MS (Method_A3): t_R = 4.09 min

^{1}H-NMR (300.36 MHz, $CDCl_3$):): δ = 2.70-2.56 (m, 4H, H-3, H-4), 2.46 (bs, 8H, H-5, H-6, H-7, H-8), 2.27 (s, 3H, H-9), 2.15 (s, 3H, H-1).

^{13}C-NMR (75.53 MHz, $CDCl_3$): δ = 207.9 (C-2), 55.1/53.1 (C-5, C-6, C-7, C-8), 52.8 (C-4), 46.1 (C-9), 41.3 (C-3), 30.3 (C-1).

6.2.5.3 *rac*-5-Bromo-*N*-hydroxy-2-((3-(hydroxycarbamoyl)cyclohexyl)amino)benzamide (*rac*-23)



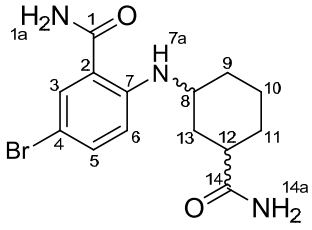
A 10 mL Schlenk flask with magnetic stirring bar was charged with 20.1 mg (50.5 µmol, 1 eq) *rac*-ethyl 5-bromo-2-((3-(ethoxycarbonyl)cyclohexyl)amino)benzoate (***rac*-22**) and 500 µL MeOH. The yellow solution was cooled to 0°C and 29.0 mg (521 µmol, 10.3 eq) NaOMe and 59.2 µL (100 µmol, 20 eq) 50% NH_2OH solution in H_2O were added. The reaction mixture was stirred at 0°C for 3h and 1 h at 20°C until TLC indicated complete conversion. The yellow solution was acidified with 1 M HCl (1 mL) to pH = 6 and the solvent was removed in vacuum. The crude product could not be purified by flash chromatography due to instability on silica gel.[93]

$C_{14}H_{18}BrN_3O_4$ [372.21]

HPLC-MS (Method_A2): t_R = 3.99 min^{cis} (4.07 min^{trans})

HPLC-MS (Method_A2, by-product): t_R = 4.40 min (4.47 min^{trans})

6.2.5.4 *rac*-5-Bromo-2-((3-carbamoylcyclohexyl)amino)benzamide (*rac*-24)



A dry 10 mL Schlenk flask with magnetic stirring bar was charged with 47.3 mg (138 µmol, 1.0 eq) *rac*-5-bromo-2-((3-carboxycyclohexyl)amino)benzoic acid (***rac*-1**) and 1.0 mL dry THF. The dark-yellow solution was cooled to 0°C and 43.0 µL (304 µmol, 2.2 eq) dry Et_3N and 30.1 µL (304 µmol, 2.2 eq) chloroethyl formate were added. The yellow-green suspension was stirred at 0°C for 90 min and 440 µL (2.76 mmol, 20 eq) conc. NH_4OH (25% in water) were added within 10 min at 0°C. The yellow/orange reaction mixture was allowed to warm to 20°C within 6 h until full conversion of the substrate was obtained and was stirred for additional 5 d. The solvent was removed in vacuum, the brown-greyish residue was dissolved in MeOH (5 mL), adsorbed on Celite (250 mg) and the solvent was removed in vacuum. The product was purified by flash chromatography (10 g SiO_2, 100 x 15 mm, eluent: EtOAc/MeOH = 20:1 then 10:1 then 5:1 then 3:2, fraction size: 10 mL).[92]

$C_{14}H_{18}BrN_3O_2$ [340.22]

yield: 14.9 mg (43.8 µmol, 32%), light yellow solid

R_f = 0.81 (EtOAc/MeOH = 2 :1 (v/v), UV 254/366nm and CAM)

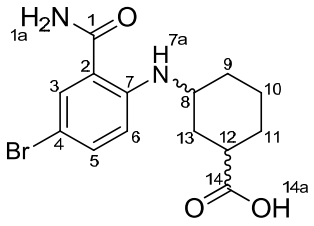
mp: >230°C (decomp.)

HPLC-MS (Method_A2): t_R = 4.26 min^{cis} (4.33 min^{trans})

^{1}H-NMR (300.36 MHz, DMSO-d_6): δ = 8.01-7.84 (bs, 2H, H-1a), 7.79-7.68 (m, 1H, H-3), 7.33 (d, $^3J_{HH}$ = 8.9 Hz, 1H, H-5), 7.29-7.08 (m, 2H, H-14a), 6.75-6.59 (m, 1H, H-6), 3.28 (m, 1H, H-8), 2.38-2.09 (m, 1H, H-12), 2.09-1.83 (m, 2H, H-9a, H-13a), 1.83-1.61 (m, 2H, C-10a, C-11a), 1.60-1.40 (m, 1H, C-10b), 1.39-0.88 (m, 3H, H-9b, H-11b, H-13b).

^{13}C-NMR (75.53 MHz, DMSO-d_6): δ = 176.7^{trans}/176.4 (C-14), 170.5^{trans}/170.4 (C-1), 148.0^{trans}/147.9 (C-7), 135.0^{trans}/134.8 (C-5), 131.3 (C-3), 115.3/115.2^{trans} (C-2), 113.9/113.8^{trans} (C-6), 104.3/104.1^{trans} (C-4), 49.8/46.0^{trans} (C-8), 42.7/38.4^{trans} (C-12), 35.6 (C-13), 32.3 (C-9), 28.6 (C-11), 24.0 (C-10).

Byproduct: *rac*-3-((4-bromo-2-carbamoylphenyl)amino)cyclohexanecarboxylic acid (***rac*-25**)



$C_{14}H_{17}BrN_2O_3$ [341.20]

yield: 47.2 mg (138 µmol, “99%”)

R_f = 0.63 (EtOAc/MeOH = 2 :1 (v/v), UV 254/366nm and CAM)

mp: 170-172°C

HPLC-MS (Method_A2): t_R = 4.53 min (4.59 min^{trans})

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8 Abbreviations

Analytical methods:

^{13}C-NMR	carbon NMR
^{1}H-NMR	proton NMR
APT	Attached Proton Test
bs	broad singlet
CH_{Ar}	aromatic proton
C_q	quarternary carbon
d	doublet
dd	doublet of doublet
decomp.	decomposition
dt	doublet of triplet
e.e.	enantiomeric excess
ESI	electrospray ionization
eV	electron volt
GC-MS	gas chromatography
GC-MS	gas chromatography mass spectroscopy
HH-COSY	proton-proton correlation spectroscopy
HPLC	high performance liquid chromatography
HPLC-MS	high performance liquid chromatography mass spectroscopy
HPLC-UV	high performance liquid chromatography ultraviolet detection
HSQC	heteronuclear single quantum coherence
Hz	Hertz
ITC	isothermal titration calorimetry
J	signal multiplicity
m	multiplet
m/z	mass/charge-ratio
M^+	molecule peak
MHz	megahertz
mp	melting point
NMR	nuclear magnetic resonance
opt. rot.	optical rotation
pd	pentet of doublet
ppm	parts per million

q	quadruplet
R_f	retention factor
RP-HPLC	reversed phase-HPLC
s	singlet
t	triplet
td	triplet of doublet
TLC	thin layer chromatography
t_R	retention time
UV	ultraviolet
δ	chemical shift

Chemical abbreviations:

4-DMAP	4-(dimethylamino)-pyridine
Ac	acetyl
acac	acetyl acetonate
AcOH	acetic acid
ADIC	2-amino-2-desoxyisochorismic acid
BINOL	1,1'-bi-2-naphthol
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
CAM	cerium ammonium molybdate
$CDCl_3$	deuterated chloroform
D_2O	deuterium oxide
dba	dibenzylideneacetone
DHHA	*trans*-2,3-dihydro-3-hydroxyanthranilic acid
DIC	*N,N*'-diisopropylcarbodiimid
DIPEA	*N*,*N*-diisopropylethylamine
DMEDA	*N*,*N*'-dimethylethylenediamine
DMF	*N,N*-dimethylformamide
DMG·HCl	L-(*N*,*N*-dimethylglycine) hydrochloride
DMSO	dimethylsulfoxide
DMSO-d_6	deuterated dimethylsulfoxide
EDC·HCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimid hydrochloride
Et	ethyl
Et_2O	diethyl ether

Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
KHMDS	potassium bis(trimethylsilyl)amide
LiTMP	lithium 2,2,6,6-tetramethylpiperidin-1-ide
Me	methyl
MeOD-d_4	deuterated methanol
MeOH	methanol
NaOMe	sodium methoxide
NBS	*N*-bromosuccinimide
n-Bu_4NOH	tetrabutylammonium hydroxide
n-BuLi	*n*-butyllithium
PCA	phenazine-1-carboxylic acid
PDC	phenazine-1,6-dicarboxylic acid
PLE	porcine/pig liver esterase
PTSA	*p*-toluenesulfonic acid
rac-BINAP	*rac*- 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
RB-DHAC	resin-bound dimethylhydroxyethylammonium carbonate
SiO_2	silica gel
TBAA	tetrabutylammonium adipate
TBA-OH	tetrabutylammonium hydroxide
t-Bu	tertiary butyl
t-Bu_3P	tri(tert-butyl)phosphine
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TMP	2,2,6,6-tetramethylpiperidine
TMS-Cl	trimethylsilyl chloride

Biolological abbreviations:

AIDS	Acquired Immune Deficiency Syndrome
AT_1	angiotensin II receptor subtype 1
Bcl-2	protein
CF	cystic fibrosis
DNA	desoxyribonucleic acid
E140	glutamic acid 140

FMN	flavine mononucleotide
GABA	γ-aminobutyric acid
H73	histidine 73
IC_{50}	half maximal inhibitory concentration
K_D	dissociation constant
KSI/NTF2	Δ5-3-ketosteroid isomerase / nuclear transport factor 2
NADH	nicotinamide adenine dinucleotide
P.	*Pseudomonas*
R160	arginine 160
R41	arginine 41
S.	*Streptomyces*
S77	serine 77

Others:

(v/v)	volume/volume
(v/v/v)	volume/volume/volume
(w/w)	weight/weight
°C	Celsius
Å	Ångström
C_{Ar}-Br	aromatic carbon-bromine bond
cat.	catalytic
cm	centimeter
conc.	concentrated
d	day/-s
E	potential / enantioselectivity
e. g.	exempli gratia (lat.: for example)
EI	electron impact
eq	equivalents
et. al.	*et alii* (lat.: and co-workers)
g	gram
h	hour/-s
H^+	acidic
IMBT	Institute for Molecular Biotechnology (Graz University of Technology)
K_D	dissoziation constant
L	litre

lit.	literature
m	meter
M	molar (mol/L)
min	minute/-s
mL	milliliter
mm	millimeter
nm	nanometer
nM	nanomolar
pK_a	negative logarithmic acid dissociation constant
ppm	parts per million
quant.	quantitative
rac	racemic
recryst.	recrystallized
rpm	rounds per minute
sat.	saturated
S_EAr	electrophilic aromatic substitution
λ	wavelength
λ_{max}	absorption maximum
µL	microliter
µm	micrometer
µM	micromolar

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