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Design and Synthesis of Biomedically Active Glycomimetics

MASTER'S THESIS

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Meinen Großeltern

Gert und Brigitte

GNŌTHI SEAUTÓN

Preface

The work for this thesis brought me half way around the world. I got to know people, I would never have met and I went to places, I would never have seen, otherwise. I am very grateful for all the experiences I made and everything I learned in this last year.

It took the support of a lot of people to accomplish this thesis. First and foremost I want to thank my supervisor Tanja Wrodnigg for her intellectual support and her continual encouragement throughout my studies. Together with Arnold Stütz she arranged for me to visit New Zealand. I am grateful to you both for this incredible opportunity.

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I also wish to thank all members of the Ferrier Research Institute in Wellington, led by director Richard Furneaux. I owe a special thanks to Peter Tyler, who has been patient beyond limits with me. Keith, Gary, Ralf, Lawrence, Jenny, Doug, Tony, Olga, Karl and Herbert, I'm really grateful for everything I learned from you. Rachael and Chris, thanks for all the organizational aid and taking care of me.

The biggest thanks goes to my parents: Danke, Mama und Papa! Obwohl ihr am Wenigsten mit dieser Arbeit zu tun hattet, steckt von euch wohl die meiste Energie darin.

I want to thank Brett Hirsch and Ross Firestone for carrying out the inhibition studies on my final products. They are members of the Albert Einstein College of Medicine in New York under the direction of Vern Schramm.

Abstract

The bacterial enzyme 5'-methylthioadenosine nucleosidase (MTAN) and the human enzyme 5'-methylthioadenosine phosphorylase (MTAP) both play a key role in thiomethyl-group metabolism. They are part of *S*-adenosylmethionine (SAM) related pathways, such as quorum sensing,[1,2] methylation reactions,[3] purine salvage,[4] polyamine[5] and menaquinone biosynthesis.[1,4,6] Potent and selective inhibitors, designed from experimentally determined transition state structures, have shown to cause anti-bacterial[7,8] and anti-cancer[3,5] effects, respectively. A library of 15 2nd generation MTAN/MTAP inhibitors was designed, synthesized and tested against *Helicobacter pylori* MTAN and human MTAP. Most transition state analogues show slow-onset tight binding inhibition reaching *K*i* values of 25pM. Two effects of enzyme selectivity toward *Hp*MTAN have been observed. In a second project two new reactions were added successfully to the Staudinger/aza-Wittig reaction sequence. A bi-cyclic iminosugar bearing synthetic potential and an iminosugar 1-phophonate, a possible glycosyl phosphate mimetic, were synthesized.

Zusammenfassung

Das bakterielle Enzym 5'-Methylthioadenosinnuklease (MTAN) und das menschliche Enzym 5'-Methylthioadenosinphosphrylase (MTAP) spielen beide eine zentrale Rolle im Metabolismus von Thiomethylverbindungen. Sie stehen in Verbindung mit quorum sensing,[1,2] Methylierungsreaktionen,[3] Purinabbau,[4] Polyamine-[5] und Meaquinonbiosynthese.[1,4,6] Wirksame und selektive Inhibitoren für diese Enzyme haben sich bereits effektiv im Einsatz gegen Bakterien[7,8] bzw. gegen Tumorzellen[3,5] gezeigt. Im Zuge dieser Arbeit wurden 15 MTAN-/MTAP-Inhibitoren der zweiten Generation entworfen, synthetisiert und gegen MTAN aus *Helicobacter pylori* und menschliche MTAP getestet. Die meisten der 15 Inhibitoren bewirken slow-onset Inhibierung, wobei die wirksamsten Verbindungen Dissoziierungskonstanten von 25pM erreichen. Des Weiteren konnten zwei Selektivitätseffekte, wodurch *Hp*MTAN bevorzugt inhibiert wird, festgestellt werden. In einem zweiten Projekt wurden erfolgreich zwei neue Nukleophile in der Staudinger/aza-Wittig-reaktion umgesetzt. Dadurch wurden ein bizyklischer Iminozucker, der mehrfache Möglichkeiten für weitere Synthese aufweist, und ein Iminozucker-1-phosphonat, eine geeignete Glycosylphosphatimitation, synthetisiert.

Contents

1. Introduction

Sugars are the building blocks for carbohydrates. Together with amino acids, lipids and nucleotides they provide the organic foundation for life on our planet. In nature, carbohydrates mainly function as energy storage. Photosynthetic plants consume sunlight to oxidize water to oxygen in order to synthesize adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) in the “light reactions”. In the Calvin Cycle (“dark reactions”) they use ATP and NADPH to reduce carbon dioxide and build up carbohydrates. It is estimated that photosynthesis fixes about 10^{11} tons of carbon annually, which correlates to 10^{18}kJ of energy.[9] Non-photosynthetic organisms, such as human beings, gain this energy by direct or indirect consumption.

A long underestimated function of carbohydrates is their role in cell recognition processes. As part of protein side-chains sugars affect healing, blood clotting, prevention of infection and fertilization.[10] A sperm cell, for example, recognizes and binds the carbohydrate pattern of an egg cell’s surface before it penetrates the cell wall and initiates fertilization.[9]

1.1. Iminosugars

Iminosugars are carbohydrates with the endocyclic oxygen being replaced by nitrogen (Figure 1). In the early 1960s replacement of the ring-oxygen in sugars with heteroatoms such as sulfur, phosphorus and nitrogen was solely an academic exercise.[11] In 1966, when Paulsen published the first synthesis of 1-deoxynjirimycin (DNJ),[12] Inouye *et al.*isolated nojirimycin from *Streptomyces* and proved its antibiotic qualities the same year.[13,14] A decade later, Bayer chemists isolated DNJ from other natural sources and discovered its activity as α-glucosidase inhibitor, drawing interest in iminosugars as therapeutic agents.

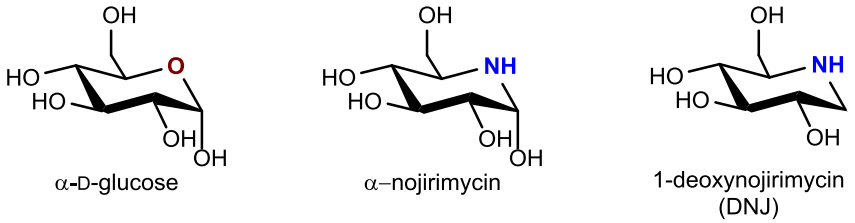


Figure 1: The most common sugar D-glucose and the iminosugar nojirimycin are very similar in structure.

From a stereoelectronical point of view, replacement of the endocyclic oxygen by nitrogen does not change much. Both elements show similar carbon bond lengths and comparable reactivity due to similar carbon bond polarization. However, neutral nitrogen forms three bonds, enabling the introduction of long-chained linkers. More importantly, the ring nitrogen in iminosugars is protonated under physiological conditions, creating a positive charge in the molecule. Enzymes catalyzing the cleavage of glycosidic bonds often stabilize positive charges, according to the reaction mechanisms their substrates undergo. Therefore, iminosugars can be potent inhibitors for such enzymes.

1.2. Enzyme Theory

Enzymes regulate almost all biochemical processes. As catalysts they accelerate reactions by lowering the activation barrier without altering of the chemical equilibrium. Enzymatic rate enhancements between 10^6 and 10^{17} have been found.[15] Therefore, a process catalyzed by an enzyme in one second, would take 10 days to 3 billion years to happen by itself.

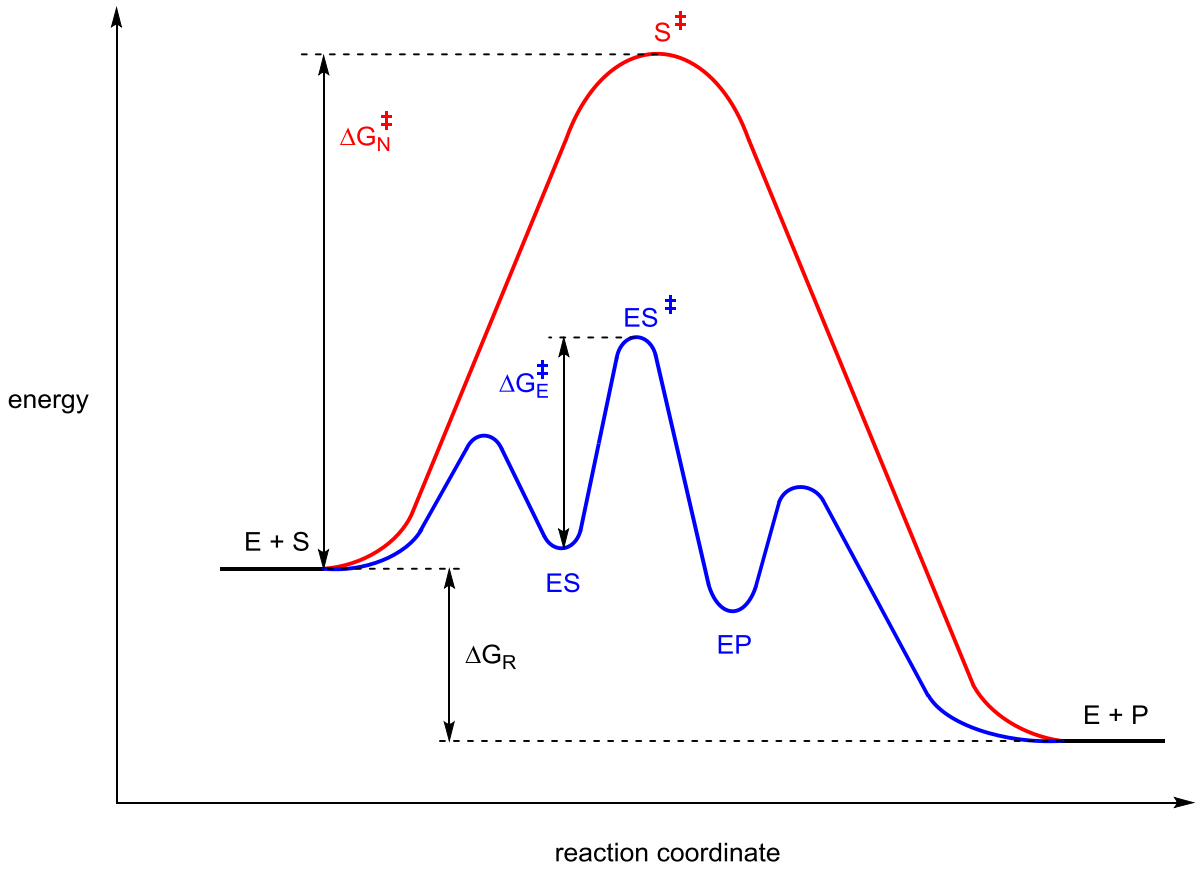


Figure 2: Reaction coordinate diagram for a hypothetical enzyme-catalyzed (blue) and non-catalyzed reaction (red). Transition state stabilization by the enzyme reduces the necessary activation energy $\Delta G_E^{\ddagger}$ compared to $\Delta G_N^{\ddagger}$, significantly increasing the reaction rate. The two smaller dips correlate to the formation of the enzyme-substrate complex (ES) and the dissociation of the enzyme-product complex (EP). For illustration reasons their activation energy is pictured out of proportion in this figure.

As soon as the substrate (S) reaches the active site of an enzyme (E), the substrate-enzyme complex (ES) is formed. Structural rearrangements of the enzyme can affect the solvent restricted active site and causes substrate distortion towards the transition state ($ES^{\ddagger}$) by electrostatic stabilization, substrate-substrate orientation and proton or electron transfer. Enzyme theory, influenced by Pauling and Wolfenden,[16] suggests that the lowered activation energy is a result of tighter binding between the enzyme and the transition state than between the enzyme and the substrate.[17] Successful formation of the product alters the electron distribution again and causes repulsive forces to open the active site and release the product.

1.2.1. Enzyme Inhibition

Apart from controlling the biosynthesis of enzymes their inhibition is nature's way of regulating and interrupting biochemical pathways. If the product of an enzyme accumulates for any reason, it competes with the substrate for the active site and the enzymes activity is decreased by *product inhibition*. In some cases, early enzymes of reaction sequences are inhibited by products of later steps, causing *feedback inhibition*. Some inhibitors are called *uncompetitive*, because they only bind and deactivate the already formed substrate-enzyme complex. An *irreversible inhibitor* links itself covalently to the active site and deactivates the enzyme completely. *Competitive inhibitors* are recognized by the enzyme due to their similarity with the substrate. Most effective competitive inhibitors cannot be converted by the enzyme due to their chemical functionalities. If they mimic the transition state rather than the substrate, they decrease the enzymes activity even more. This is because enzymes stabilize the reaction's transition state better than they bind the substrate (enzyme theory).

Adenosine deaminase converts adenosine to inosine (Figure 3) which has a K_M value of 300mM. Inosine causes moderate product inhibition (K_i=0.3mM) to adenosine deaminase, whereas the *transition state analogue* 1,6-dihydroinosine, which cannot undergo an addition-elimination reaction, inhibits the enzyme with a K_i of 150fM (10^9-fold lower than inosine).[9]

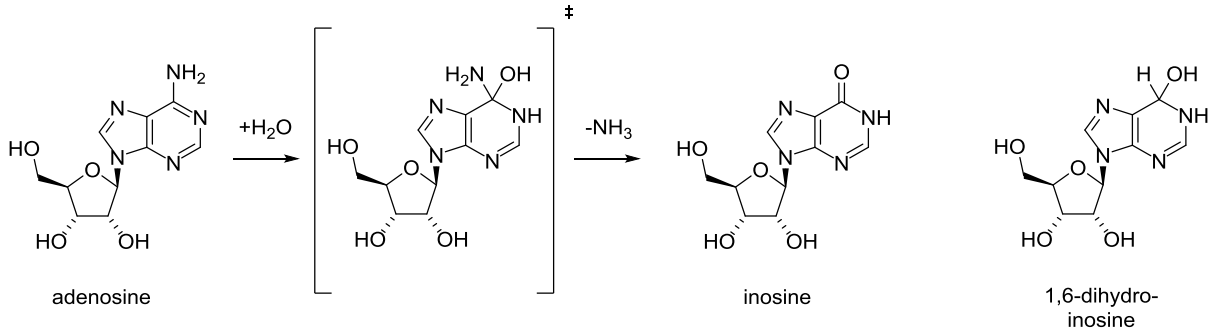


Figure 3: 1,6-Dihydroinosine is a transition state analogue for adenosine deaminase.

Transition state analogues sometimes cause structural changes in the enzyme's periphery to achieve tighter binding. These conformational changes occur in the time scale of standard enzyme assay and are therefore called *slow-onset inhibition*. The formation of a tight bound enzyme-inhibitor complex (EI*) from the enzyme-inhibitor complex (EI) decreases the enzymes activity even further and is generally described by a K_i* value, which is comparable to the K_i value of a normal, competitive inhibitor (cf. 3.3.1. K_i* values).

In drug discovery, enzyme inhibition is a popular tool. It enables the interruption of whole pathways by one specific and effective inhibitor. AIDS, for example, is treated almost exclusively with drugs that inhibit the activities of certain viral enzymes.[9] Slow-onset inhibitors are especially of interest as they show slow dissociation from the drug-target complex.

1.3. Targeting 5'-Methylthioadenosine Enzymes for Drug Development

5'-Methylthioadenosine nucleosidase (MTAN) is a bacterial enzyme catalyzing the irreversible hydrolysis of 5'-methylthioadenosine (MTA, **1a**) and other naturally occurring derivatives (Figure 4). Potent MTAN inhibitors have proven to be effective as anti-bacterial agents.[7,8] The enzyme 5'-methylthioadenosine phosphorylase (MTAP) catalyzes the reversible phosphorolysis of MTA (Figure 5). It is found in mammals and provides a target for cancer treatment.[3,5]

Both MTAN and MTAP are key enzymes in thiomethyl-group metabolism. They are part of *S*-adenosylmethionine (SAM) related pathways, such as quorum sensing,[1,2] methylation reactions,[3] purine salvage,[4] polyamine[5] and menaquinone biosynthesis.[1,4,6]

1.3.1. MTAN Inhibition against Bacteria

Antibiotic resistance and its expansion among bacteria pose a serious problem in today's health system. Thus, there is not only a need for new and selective antibiotics, but also one for antimicrobial agents bacteria do not learn to sustain.

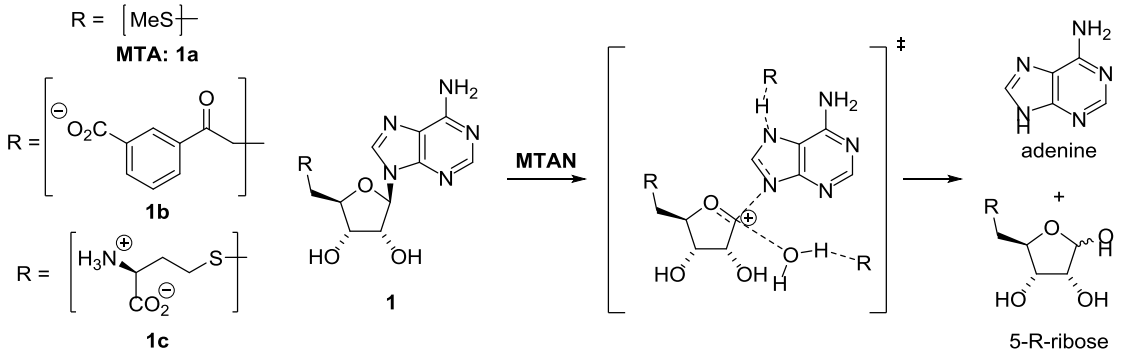
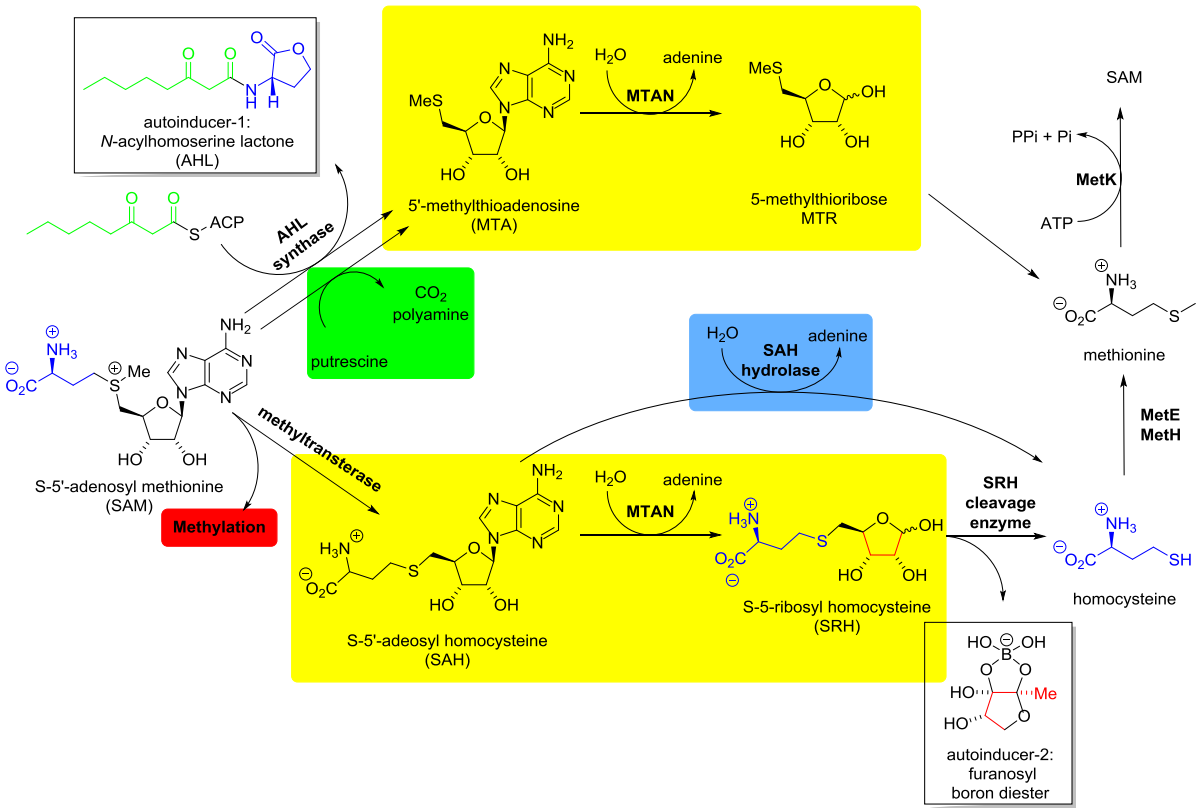


Figure 4: Cleavage of the *N*-glycosidic bond in 5'-methylthioadenosine (MTA, **1a**), 6-amino-6-deoxyfutalosine (**1b**) and *S*-adenosylhomocysteine (SAH, **1c**) to adenine and the respective ribosyl derivate is catalyzed by MTAN.

Many bacteria use MTAN in several processes (Scheme 1 andScheme 2). The MTANs expressed by different bacteria can be distinguished not only in the enzyme's periphery, but also in their active sites. MTANs from *Helicobacter pylori* and *Neisseria meningitides* stabilize early dissociative transition states, whereas MTANs from *E. coli* and *Streptococcus pneumoniae* have late dissociative character.[4] In addition, only *H. pylori and Campylobacter jejuni* are known to convert 6-amino-6-deoxyfutalosine (**1b**) *in vivo* (Figure 4).[18,19] Thus, it should be possible to design selective transition state analogues in order to target certain bacteria without causing side effects.

Quorum Sensing

Decentralized communication among bacteria is called quorum sensing. Depending on their cell density it influences their gene expression. Quorum sensing bacteria synthesize, release and detect signaling molecules called autoinducers (AIs).[2] An autoinducer concentration above a certain threshold can initiate processes like symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation and biofilm formation.[1,20] Thus, quorum sensing is the bacteria's way to save their energy and resources until their numbers are high enough to invest their capital and get active. Therefore, it is legitimately assumed that quorum sensing was an early step in evolution to develop multicellularity.[1]



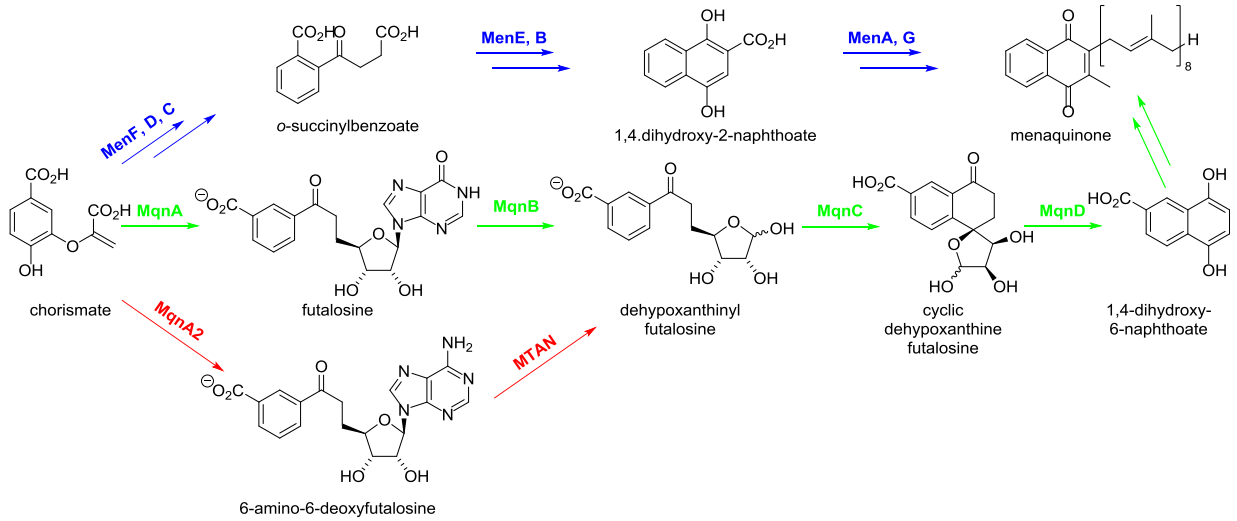
Scheme 1: In bacteria MTAN catalyzed reactions (yellow box) enable SAM recycling from byproducts of methylation reactions (red box), the polyamine pathway (green box) and autoinducer-1 (AHL) biosynthesis. [1,2,20] Autoinducer-2 (shown as furasyl boron diester) forms spontaneously from 4,5-dihydroxy-2,3-pentanedione, a side-product of SRH cleavage enzyme. However, this step can be by-passed by SAH hydrolase (blue box). [20]

MTAN is directly involved in the biosynthesis of *N*-acylhomoserine lactone (**AI-1**) and furanosyl boron diester (**AI-2**) (Scheme 1). Both autoinducers have been connected to quorum sensing pathways effecting biofilm formation, virulence and antibiotic resistance.[22] In Gram-positive and Gram-negative bacteria MTAN cleaves MTA into adenine and 5-methylthioribose (MTR). Adenine is salvaged by adenine phosphoribosyltransferase (purine salvage pathway) and MTR can enter the methionine salvage pathway to recycle to *S*-adenosyl methionine (SAM).[21] Effective MTAN inhibition causes accumulation of MTA. Consequently, the biosynthesis of autoinducer-1 (**AI-1**), used by Gram-negative bacteria, is inhibited due to product inhibition of AHL synthase (50µM MTA causes 67% inhibition[22]). Moreover, MTA is a side product of

spermidine and spermine synthase in the polyamine pathway in prokaryotes as well as eukaryotes (Scheme 3). Both enzymes have been reported to be sensitive to product inhibition by MTA. Less sensitive rat spermidine synthase shows a *K*i value of 50µM[23], but mammalian spermine synthase shows a *K*i value of 0.3µM.[24] MTAN also converts *S*-adenosyl homocysteine (SAH) to *S*-ribosyl homocysteine (SRH), a precursor of autoinducer-2 (**AI-2**) and homocysteine. Homocysteine can enter the methione salvage pathway to recycle SAM. **AI-2** spontaneously forms from SRH- derived 4,5-dihydroxy-2,3-pentanedione.[20] Schramm *et al.* showed that the biosynthesis of AI-2 in enterohemorrhagic *E. coli* O157:H7 (EHEC) is reduced when treated with the MTAN transition state analogue MT-DIA (**3**: IC_{50} value is 600nM).[2] Because SAH is a side product of many methylation reactions, it is reasonable to suggest that MTAN inhibition effects methylation reaction in bacteria via product inhibition, as MTAP inhibition does in mammals. However, SAH hydrolase provides bacteria an alternative way to catabolize SAH without synthesizing AI-2 as side product.[20] Therefore, SAH accumulation and its effect on methylation reaction due to MTAN inhibition is probably insignificant. A big advantage of attacking bacteria via quorum sensing is that cell proliferation is not affected.[2,6] Thus, it is less likely for pathogens to develop resistance against this class of pharmaceuticals.

Menaquinone Biosynthesis

Menaquinone (vitamin K_2, Scheme 2) is a lipid-soluble, essential vitamin that gained Edward Doisy and Henrick Dam a Nobel Prize in 1943 (for the discovery and structure determination, respectively). Bacteria use menaquinone in aerobic and anaerobic respiratory systems to transport electrons within the membrane. Most Gram-negative bacteria (like *E. coli*) use menaquinone only under anaerobic conditions, as they use ubiquinone under aerobic conditions. However, Gram-positive and some Gram-negative bacteria (like *H. pylori*) rely on menaquinone as their sole electron carrier.[25,26]



Scheme 2: Bacterial menaquinone biosynthesis starts from an intermediate in the shikimate pathway, chorismate. The classic pathway (blue) is used by most bacteria and was investigated intensively in *E. coli*.[27,28] Evidence for the futalosine pathway (green) in *S. coelicolor* and *T. thermophiles* as sole menaquinone source was given by Dairi *et al.* in 2008.[18,29] Work from Tanner *et al.* suggests that *H. pylori* and *C. jejuni* use an alternative futalosine pathway (red), involving MTAN rather than MqnB.[19]

As menaquinone (Scheme 2) is essential to most bacteria (including many pathogens), its biosynthetic pathways pose viable targets for antibiotics, by inhibiting its biosynthesis and therefore restricting the spread of pathogens. Whereas, most bacteria use the classic (blue) or the futalosine (green) pathway to synthesize menaquinone (Scheme 2), it has recently been shown that *Helicobacter pylori* (as well as *Campylobacter jejuni)* use an altered futalosine pathway (red).[18,19] MqnA2 of both species converts chorismate to 6-amino-6-deoxyfutalosine rather than futalosine. The intermediates only differ at the C-6 residue, but the amino groug is enough for *Hp*MTAN, an enzyme originally used in methylthio-group metabolism, to recognize 6-amino-6-deoxyfutalosine and cleave the *N*-glycosidic bond to yield dehypoxanthinylfutalosine and adenine. *H. pylori* is a Gram-negative bacterium related to gastric carcinoma,[18] 85% of gastric and 95% of duodenal ulcers.[30] Specific *Hp*MTAN transition state analogues are a viable option for selective, new antibiotics against *H. pylori*, as human beings and commensal intestinal bacteria (like *lactobacilli*) lack this pathway to synthesize menaquinone. However, as MTAN is involved in other bacterial processes (cf. Quorum Sensing), inhibition would probably affect intestinal bacteria, but it has been shown that accumulation of MTA does not affect cell growth[2,6] and therefore should not pose a problem.

1.3.2. MTAP Inhibition as Cancer Treatment

MTAP is the mammalian pendant to MTAN in terms of MTA conversion. As humans do not synthesize menaquinone or autoinducers, MTAP's sole function in humans is to recycle MTA to SAM via the methionine salvage pathway (Scheme 3).[31]

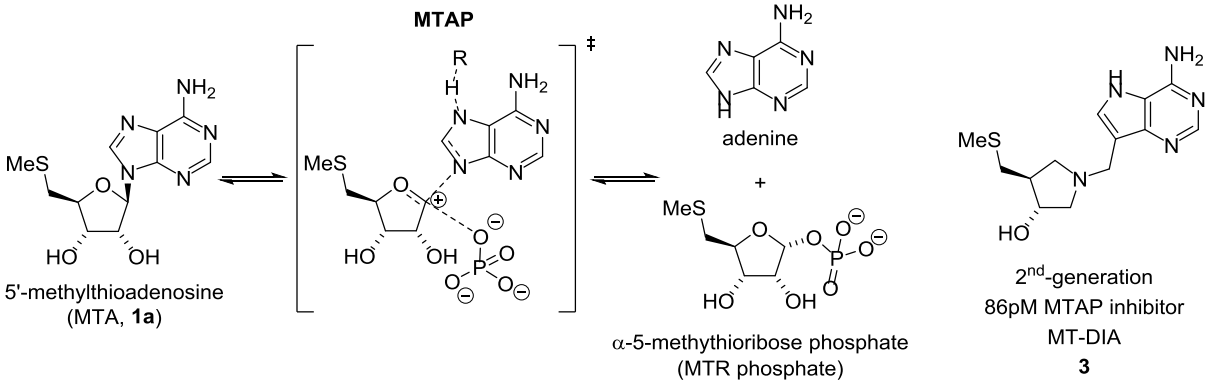
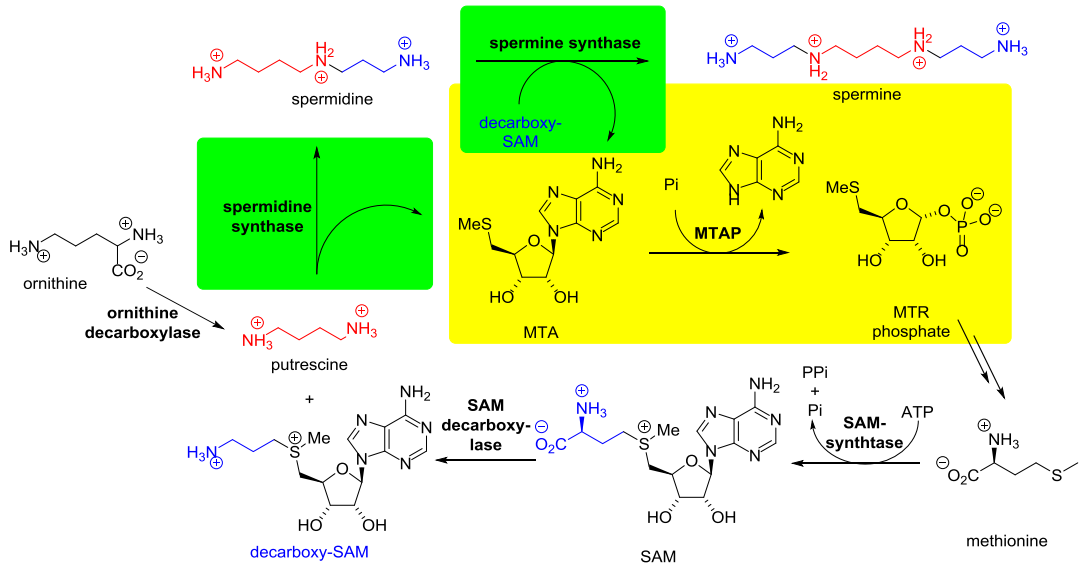


Figure 5: In mammals MTA is converted reversibly by 5'-methylthioadenosine (MTAP).

Spermidine and spermine synthase are enzymes in the polyamine pathway (Scheme 3). Both produce MTA as side product and are sensitive to MTA product inhibition[23,24] (cf. Quorum Sensing). Polyamine biosynthesis has been associated with cellular proliferation[32] and validated as anti-cancer target.[33,34,35] Schramm *et al.* already showed that treatment of head and neck cancer cell lines with the 86pM MTAP inhibitor MT-DIA (**3**) in combination with MTA induced apoptosis, whereas the same treatment did not affect human fibroblast cell lines or even breast cancer cell lines with MTAP gene deletion.[31] Treatment of all cell lines with the inhibitor MT-DIA (**3**) alone did not induce apoptosis, implicating MTA as active agent.



Scheme 3: Spermidine and spermine synthase (green) are two consecutive enzymes in the polyamine pathway. They consume decarboxy-SAM to extend the linear polyamine chain of ornithine derived putrescine to yield spermine. MTA is produced as side product in both steps. In mammals it is catabolized my MTAP and further on recycled as SAM.

Accumulation of MTA caused by MTAP inhibition also prevents SAM recycling via the purine salvage and methionine salvage pathways (Scheme 3). As the pattern of SAM mediated DNA methylation is changed in tumor cells,[31,36] it is likely that disrupted SAM recycling reduces cancer cell proliferation. Although MTA has already been proven to interfere with methylation reactions in the early 1980s,[37,38] the effect has yet to be compared with polyamine biosynthesis interruption. Most likely both effects add up, making MTAP inhibitors (in combination with MTA) potential anti-cancer agents.

1.3.3. Design of MTAN and MTAP Transition State Analogues

Understanding an enzyme's transition state is necessary for rational inhibitor design. Unfortunately, transition states are very unstable and short-lived species (approximately 10^{-13}s).[16] They cannot be analyzed by direct physical observation like NMR, MS or crystallography, which are typically used for structure determination of stable compounds. Schramm *et al.*[4,39] used kinetic isotope effect (KIE) measurements in combination with *in silico* modeling to approximate the transition states of human MTAP and several bacterial MTANs (Figure 6).

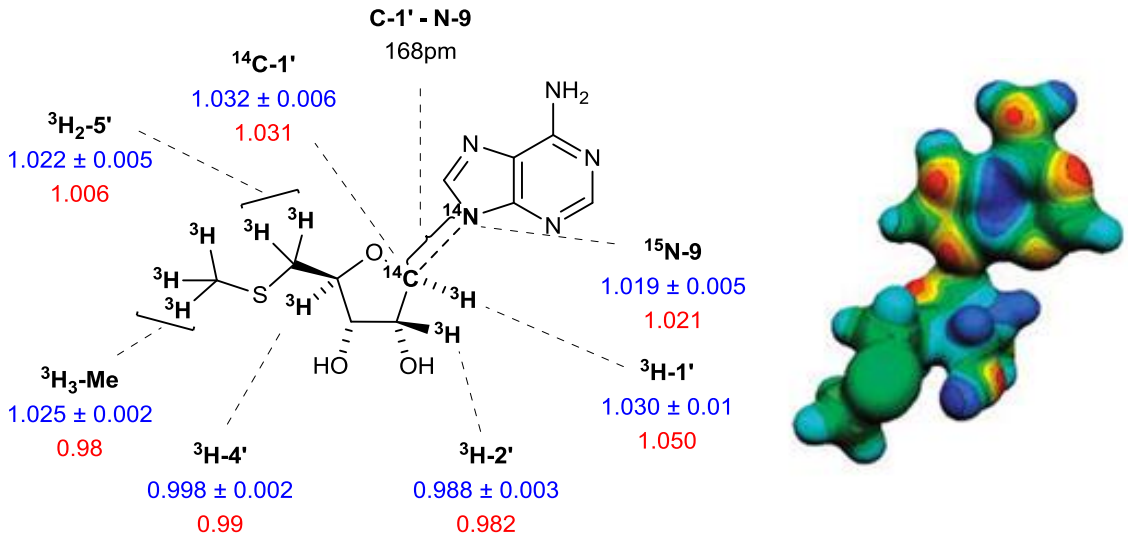


Figure 6: Intrinsic (blue) and calculated (red) KIEs for the conversion of isotope labeled MTA with *Neisseria meningitis* MTAN (left). The MEP model of the transition state was calculated at HF/STO3G (Gaussian 98/cube) and gave an *N*-glycosidic bond length of 168pm.[40]

The KIE is a popular tool in mechanism determination. It is based on the fact that different isotopes show different bond vibration frequencies due to their unequal masses. If the respective atom is close to a formed or broken bond, the reaction rate is altered for isotope enriched substrates. KIE values are calculated as the ratio of a non-labeled substrate divided by a labeled substrate and are close to one. Schramm *et al.* used MTA (labeled on different positions) and measured the conversion by different MTANs (Table 1).

Table 1: Intrinsic KIE values and calculated C-N bond length suggest an early transition state for *N. meningitides* and *H. pylory*. *K. pneumoniae*, *S. aureus*, *E. coli* and *S. pneumoniae* undergo a late transition state.

MTANs	Transition state character	^{14}C-1' KIE	^{3}H-1' KIE	C-N bond length [Å]
N. meningitides[40]	Early D_N*A_N	1.032 ± 0.006	1.030 ± 0.010	1.68
H. pylori[4]	Early D_N*A_N	1.007 ± 0.004	1.036 ± 0.004	NA
K. pneumoniae[4]	S_N1	1.000 ± 0.001	1.094 ± 0.005	>3
S. aureus[4]	S_N1	1.001 ± 0.004	1.122 ± 0.005	>3
E. coli[41]	S_N1	1.004 ± 0.003	1.160 ± 0.004	3.00
S. pneumoniae[42,43]	S_N1	1.000 ± 0.005	1.235 ± 0.002	>3.2

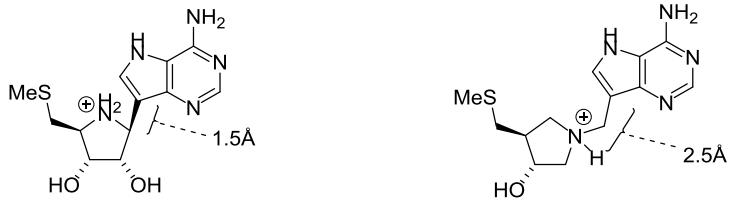
Short C-N bond lengths, high ^{14}C-1' KIE values and low ^{3}H-1' KIE values for *Nm*MTAN and *Hp*MTAN consist with an early D_N*A_N (addition-elimination) mechanism. The partial C-1' – N-9 bond, suggested by the calculated bond length, sterically restricts out of plane bending and therefore reduces the ^{3}H-1' KIE value.[40] The ^{14}C-1' KIE value is enhanced, as a new bond to the anomeric carbon is formed in the rate-determining step.

MTANs from *K. pneumoniae*, *S. aureus*, *E. coli* and *S. pneumoniae* catalyze a S_N1-like mechanism with no significant order towards the attacking water or the leaving adenine. A oxacarbenium ion with partial double bond character between the oxygen and the anomeric carbon is formed. α-Primary ^{14}C and α-secondary ^{3}H KIEs are useful do determine between S_N1 and S_N2. Fully dissociated S_N1 transition states show ^{14}C KIEs close to unity (Table 1), whereas KIE values for associative S_N2 typically range from 1.08 to 1.13.[41] Enhanced ^{3}H-1' KIE are due to more steric freedom enabling more out-of-plane bending at the transition state.

KIE suggests that in contrast to *Ec*MTAN adenine is not fully protonated in *S. pneumoniae* prior to the rate determining step, creating an unflavored anionic leaving group. This is a reasonable explanation why MTA hydrolysis at full saturation is 100 times faster in *Ec*MTAN ($k_{cat}=20s^{-1}$)[41] than in *Sp*MTAN ($k_{cat}=0.2s^{-1}$)[42].

1.3.4. MTAN and MTAP 1st and 2nd Generation Inhibitors

KIE values provide boundary conditions for computational modeling to estimate an enzyme's transition state structure, which can be used as a blueprint to synthesize transition state analogues. Due to given bond lengths and angles, it is only possible to synthesize stable imitations of the transition state.



MT-ImmA
(1st-generation)
2

MT-DIA
(2nd-generation)
3

Figure 7: 1st- and 2nd-generation inhibitors MT-ImmA and MT-DIA developed by the Ferrier Research Institute are *tight binding* transition state analogues *(slow on-set* inhibitors) for MTAN and MTAP.

The protonated crystal of the 1st-generation inhibitor MT-ImmA has a 1.5Å distance between C-1' and C-9, mimicking an early transition state (*N*-glycosidic bond in *Nm*MTAN transition state has 1.68Å). The longer distance of 2.5Å in 2nd-generation inhibitor MT-DIA (**3**), caused by the inserted methylene group, makes the molecule resemble a late transition state (Table 1). The K_i* values shown in Table 2 support this model. Generally MT-DIA (**3**) is a more powerful MTAN inhibitor than MT-ImmA (**3**), but its dissociation constants are only about half for the early transition state MTANs from *N. meningitides* and *H. pylori* and ~45 times lower for the late transition state MTANs.

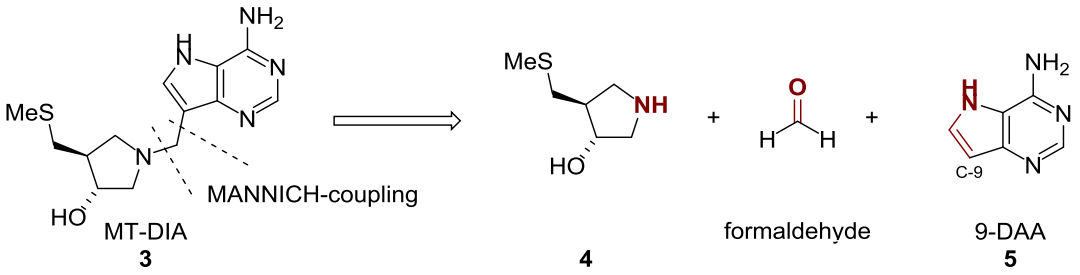
Table 2: Dissociation constants of MT-ImmA and MT-DIA for different MTANs and human MTAP.[4]

Enzymes	K_i* (MT-ImmA)	K_i* (MT-DIA)	$\frac{K_i^*(\text{MT-ImmA})}{K_i^*(\text{MT-DIA})}$
*Nm*MTAN	360	140	2.6
*Hp*MTAN	$1.2 \cdot 10^3$	571	2.1
*Kp*MTAN	$45 \cdot 10^3$	784	57
*Sa*MTAN	$77 \cdot 10^3$	$1.4 \cdot 10^3$	55
*Ec*MTAN	77	2	38
*Sp*MTAN	$1.0 \cdot 10^6$	$24 \cdot 10^3$	42
Human MTAP	$1.0 \cdot 10^3$	86	12

Human MTAP shows a K_i*-ratio of 12 placing itself between early and late transition state MTANs. However, KIE values suggest that *h*MTAP adopts a late transition state.[4]

1.4. Synthesis of MT-DIA (3)

The library of 2nd-generation inhibitors was designed by scientists of the Albert Einstein College of Medicine, directed by Vern Schramm, and chemists of the Ferrier Research Institute, directed by Richard Furneaux. The below-demonstrated synthesis of MT-DIA (**3**) has already been adapted for large scale and is patented by the Ferrier Research institute.[44]

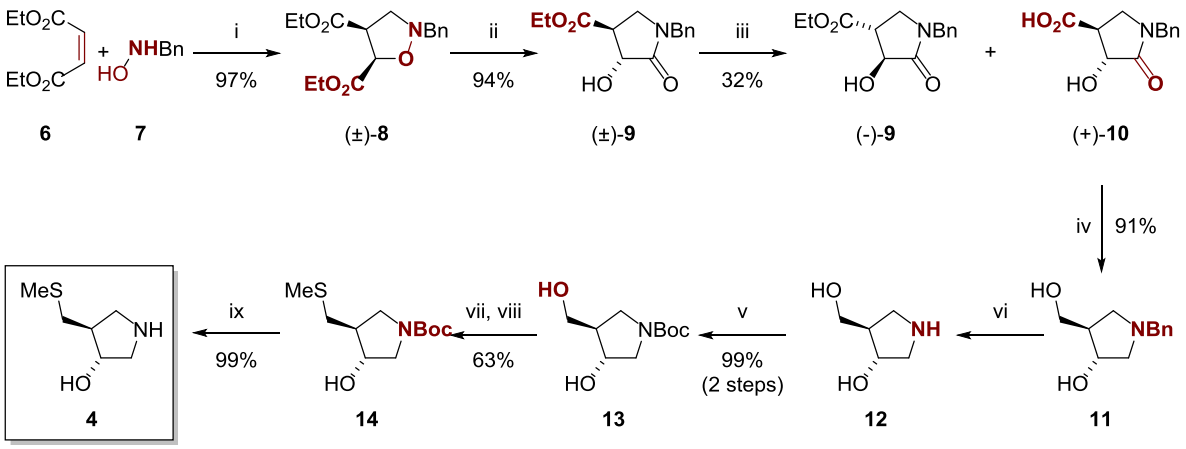


Scheme 4: Retrosynthetic analysis of MT-DIA (**3**).

The Mannich-coupling as first step in the retrosynthetic analysis cleaves the target molecule into two almost equivalent halves and makes the introduction of an aldehyde at the C-9 position of 9-DAA (**5**) for reductive amination unnecessary. Although the nitrogen in pyrrolidine **4** is by far the most basic one involved, the two other nitrogens bearing protons in 9-DAA (**5**) can undergo the same reaction mechanism, creating side-products with similar polarity in the process. Therefore, the reaction was optimized to limit those side-products to a minimum and a procedure to crystalize the final product as phosphate salt was developed. The reactants are stirred with formaldehyde in an ethanol/water mixture at room temperature. As soon as limiting reactant **4** is used up after approximately 24h, phosphoric acid and more ethanol is added to precipitate the product as the phosphate salt of **3** with 88% yield.

1.4.1. (3*R*,4*R*)-4-Hydroxymethylpyrrolidin-3-ol (4)

Free pyrrolinol **12** has already been synthesized from D-glucose[45] and D-xylose.[46] Other published synthetic routes use chiral auxiliaries,[47] diastereomer separation[48] and opening of a Sharpless epoxide with cyanide.[49] Scheme 5 shows a synthetic route designed for kilogram scale with only one chromatographic purification required.



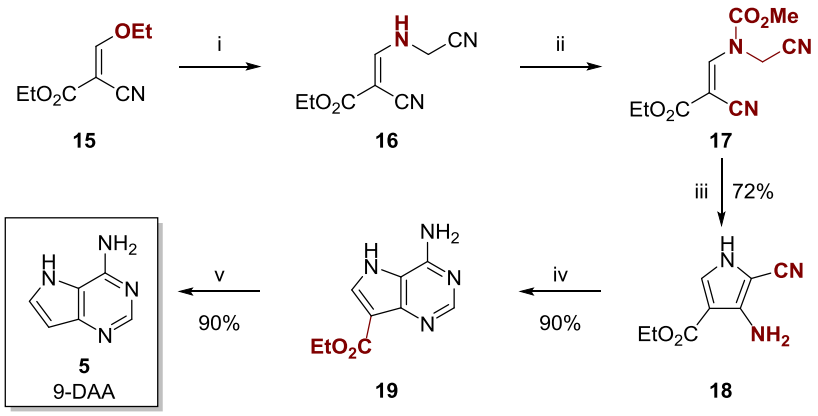
Scheme 5: Synthesis of pyrrolindine half **4**: (i) formaldehyde, EtOH, reflux, 2.5h; (ii) Zn, AcOH, 1h; (iii) Novozym 435, acetone, water, pH=7.5, 27°C, 5.7h; (iv) $BF_3.OEt_2$, $NaBH_4$, THF, 72h; (v) H_2, Pd/C; (vi) Boc anhydride, DMAP; (vii) MsCl, 2,6-lutidine, acetone, 2d; (viii) NaSMe, acetone, 50°C, 2h; (ix) TFA, toluene.[44,50]

Racemic *cis*-isoxazolidine (±)-**8** is obtained from a 1,3-dipolar cycloaddition between diethyl maleate (**6**) and the nitrone (formed by treatment of *N*-benzylhydroxylamine (**7**) with formaldehyde) in 97% yield. Cleavage of the N-O bond employing zinc in acetic acid causes spontaneous formation of lactam (±)-**9** (94% yield). Hydrolytic resolution of the racemic ester (±)-**9** with commercially available Novozym 435 gives enantiomer (-)-**9** in acceptable yield (32%) and high enantiomeric purity (95%ee) after isolation by simple extraction. Reduction with borane gave *N*-benzyl porrolinol **11** in 91% yield. As sulfur is introduced in a later step a protecting group change from *N*-benzyl (**11**) to *N*-boc (**13**) is necessary at this point. No purification by chromatography to obtain >90% pure intermediates was required so far. Selective mesylation of the primary alcohol **13** allow the introduction of the methylthio group in moderate yield (63%) after chromatography. Treatment with trifluoroacetic acid gives pyrrolinol **4** in quantitative yield after crystallization from ethanol with oxalic acid.[44,50]

1.4.2. 9-Deaza-adenine (5)

The synthesis of the 9-DAA (**5**) half has also been designed and carried out on large scale. Acrylate ester **15** undergoes Michael addition-elimination with aminoacetonitrile to give amine **16** in quantitative yield after aqueous work up. Treatment with methyl chloroformate and triethylamine gives carbamate **17** in quantitative yield after aqueous work up. Deprotonation with DBU initiates ring closure. Subsequent treatment with methanol, ammonium acetate and water gives pyrrole **18** in 72% yield with 80% purity. Ring formation is achieved by treatment

with foramidine acetate at 90°C for 20 hours. Pure pyrimidine **19** is obtained by recrystallization from water in a high yield (90%). Treatment with potassium hydroxide at 120°C for 6h causes saponification and decarboxylation to give adequate pure 9-DAA (**5**) after recrystallization from hot water in 90% yield.[44]

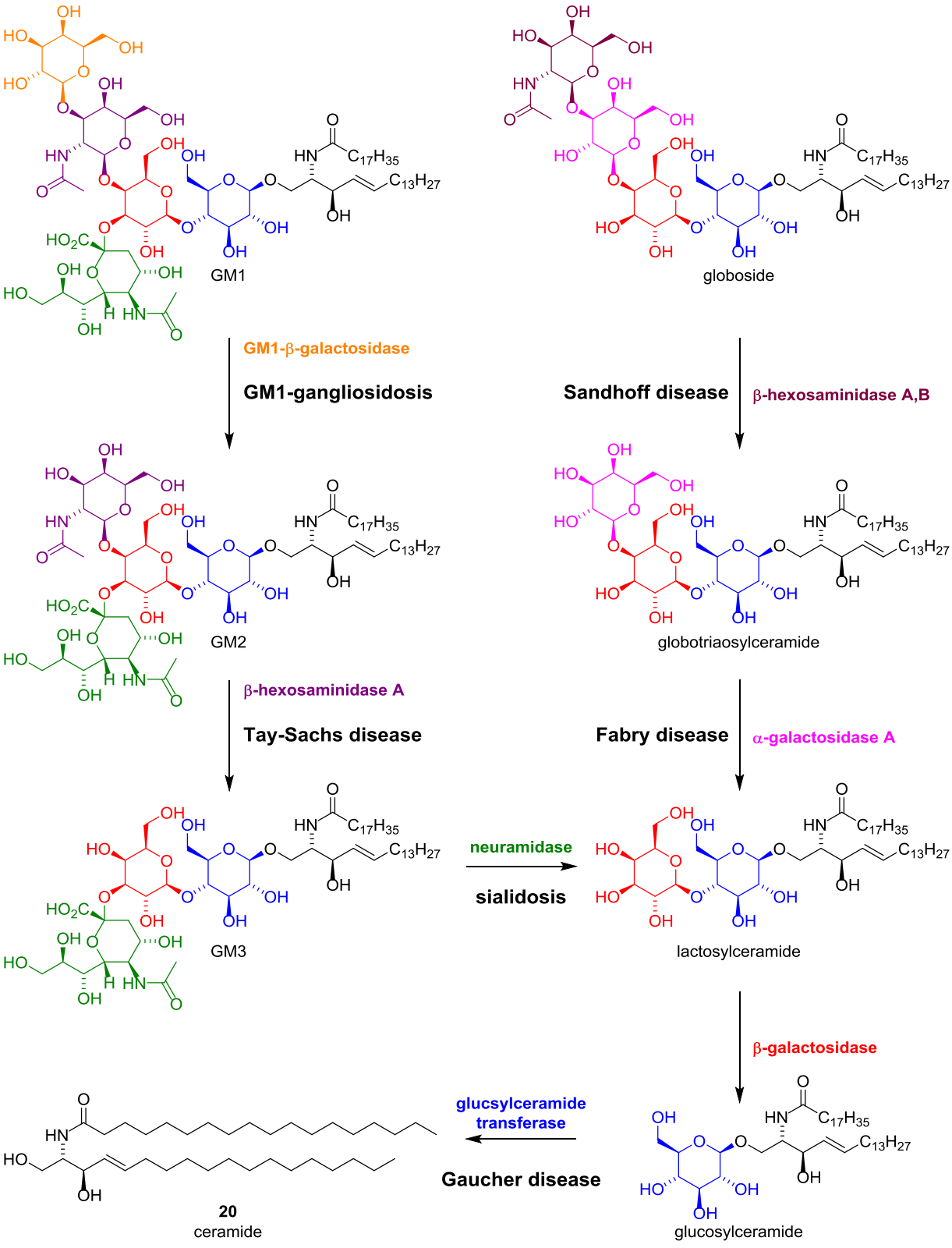


Scheme 6: Synthesis of 9-deaza-adenine (9-DAA); i: aminoacetonitrile bisulfate, Et_3N, methanol, 2h; ii: methyl chloroformate, Et_3N, ethyl acetate, 5°C, 10min; iii: DBU, CH_2Cl_2 then methanol then ammonium acetate; iv: formamidine acetate, formamide, 90°C, 20h; v: KOH, water, reflux, 6h.[44]

1.5. Glycosidase Inhibitors against Lysosomal Storage Disease

Lysosomal storage disease (LSD) describes a group of metabolic diseases with interrupted catabolism of glycosphingolipids, proteins, lipids, glycogen or glycoprotein. Glycoside hydrolases, originally responsible for the acidic degradation of macromolecules in the lysosome, of the affected organisms are malfunctioning. The concluding accumulation of those macromolecules causes disruption of intra cellular processes to the point of apoptosis.[51] Scheme 7 shows the degradation of two glycosphingolipids to ceramide. The stated diseases are caused by malfunction of the respective enzyme. Alone 50 known forms of LSD are connected only with the degradation of glycosphingolipids.

One of 4000 to 6000 newborn is affected by some form of LSD.[52] Symptoms depend on the specific disease and the age of the patient. Delayed development, deafness, blindness, movement disorder, abnormal bone growth, organ enlargement as well as neurological pathology are described.[53] LSD is incurable, partially successful treatment has been achieved by *enzyme replacement* (ERT) and *substrate reduction therapy* (SRT). Recombinant enzymes have been approved for the treatment of Gaucher's,[54] Fabry's,[55] Pompe's[56] and mucopolysaccharidoses[57,58,59] to replace the respective mutant enzyme. Substrate reduction therapy focuses on the inhibition of anabolic enzyme activity in order to minimize the substrate of the malfunctioning enzyme. Its main advantage over ERT is that SRT agents such as *N*-butyl-1-DNJ with ability to cross the blood-brain barrier have been approved to treat neurological symptoms of Gaucher's.[60] Furthermore, *gene therapy* and *chaperone mediated therapy* are promising methods of treatment for future.



Scheme 7: Catabolic pathways for the degradation of two glycosphingolipids to ceramide (**20**) with glycosidases for each step in color and the diseases in black, if the respective enzyme is malfunctioning.[53]

1.5.1. Chaperone Mediated Therapy (CMT)

Mutated enzymes, unable to fold in their native conformation, cause lysosomal storage diseases. Such proteins are recognized and decomposed by the endoplasmic reticulum-associated degradation (ERAD) pathway (Figure 8). Active site specific chaperones (ASCCs) are originally inhibitors of the respective enzyme, but when dosed in subinhibitory concentrations they can function as folding templates by stabilizing the native conformation.[61] The enzyme-chaperone complex can pass through the Golgi apparatus into the lysosome, where it dissociates due to substrate excess and the lowered pH-value, providing the organism a working enzyme.

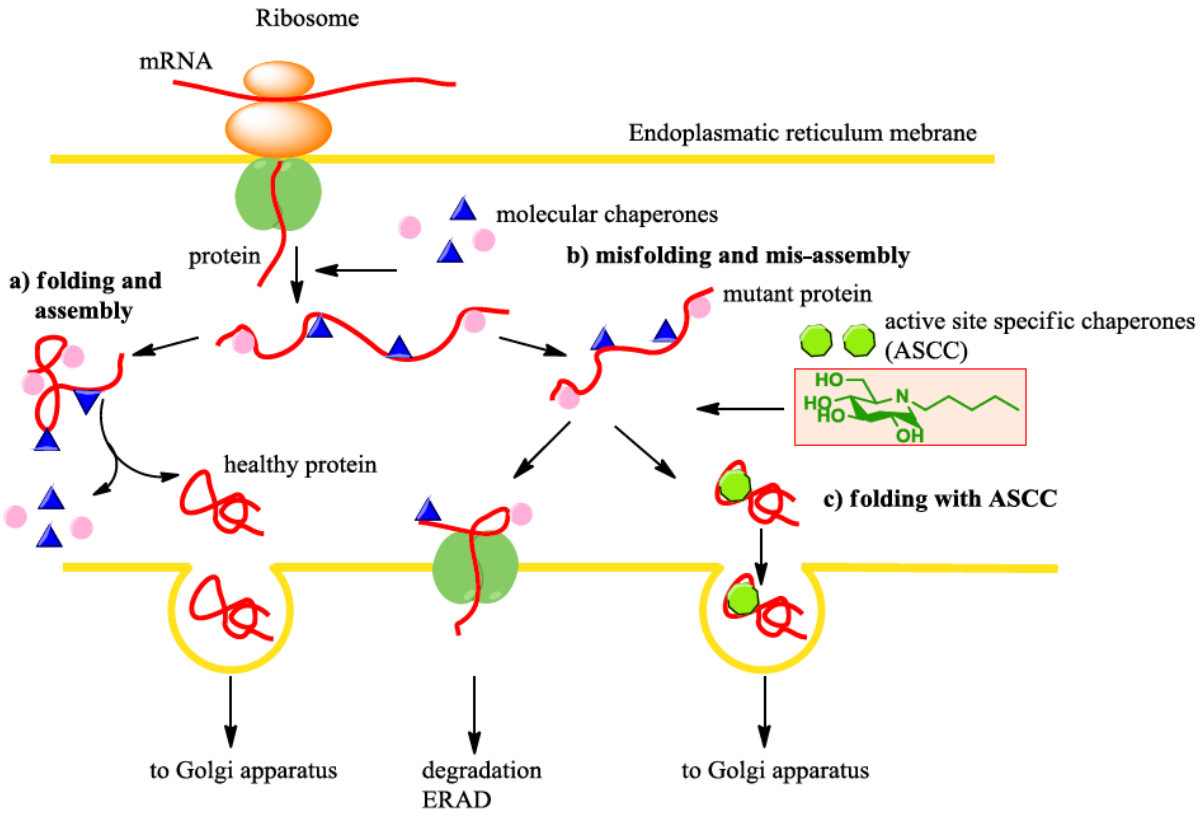


Figure 8: Enzymes are synthesized in the endoplasmic reticulum. In healthy organisms chaperone mediated folding provides proteins in their native conformations **(a)**. Healthy enzymes are passed on into the Golgi apparatus to enter the organism. Some mutated proteins undergo misfolding and are therefore degradated by ERAD **(b)**. Active site specific chaperones (ASCCs) can function as folding templates for such proteins, allowing them to enter the Golgi apparatus **(c)**. [62]

Advantages of treating LSDs with iminosugar based pharmacological chaperones are that it is possible to synthesize small active molecules with cell permeability and the ability to cross the blood-brain barrier at relatively low cost of production, compared to ERT, allowing the treatment of neuropathological forms of LSD.

In the Glyco Group a variety of iminosugars with glycosidase inhibiting properties have been developed over the last years. Figure 9 shows a selection of compounds with high potential to be used as pharmaceutical chaperones.[63]

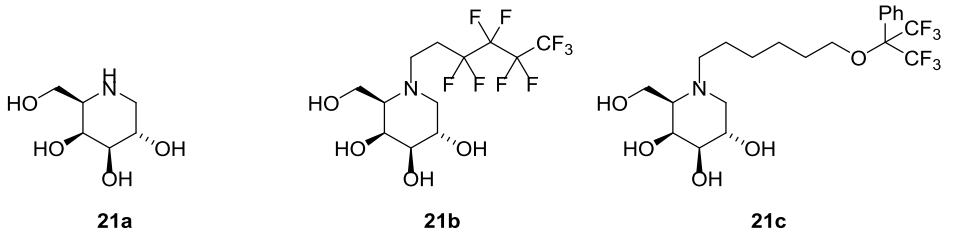


Figure 9: Pharmaceutical Chaperones inhibiting galactosidases: **21a** (K_i=13nM for α-galactosidase of green coffee beans), **21b** (K_i=0.36µM for human lysosomal β-galactosidase) and **21c** (K_i=0,10µM for human lysosomal β-galactosidase).[63]

Synthesizing Iminosugars via intermolecular Staudinger/aza-Wittig

The Staudinger/aza-Wittig (SAW) reaction sequence is a robust tool for the synthesis of 5-, 6-, 7- and 8- membered heterocycles, such as iminosugars.[64] It is a well-established method in the Glyco Group to synthesize iminosugars like **22a**-**c**.[65] By this approach C-glycosyl type iminosugar derivatives are accessible, which are believed to be versatile building blocks for the synthesis of pharmacological chaperones.

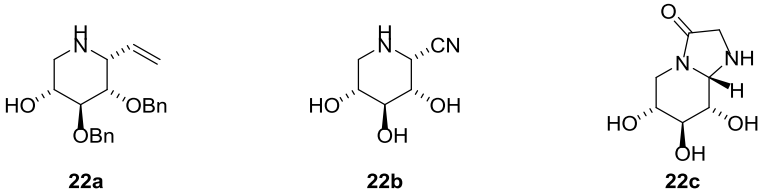
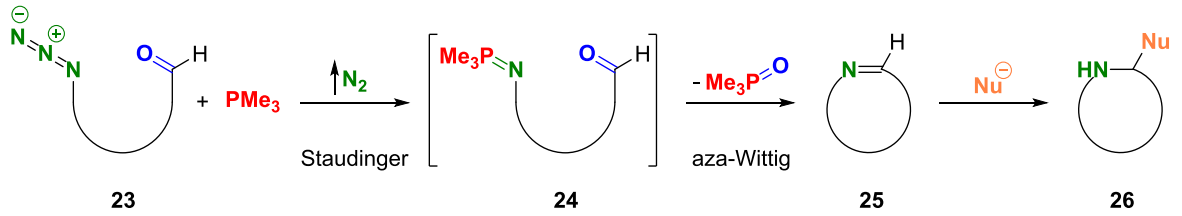


Figure 10: Successful examples of the Staudinger/aza-Wittig reaction. The formed imine was converted via Strecker (24), Grignard (25) and with an amino acid methyl ester, prepared by chemists of the Glyco Group.[65,66]

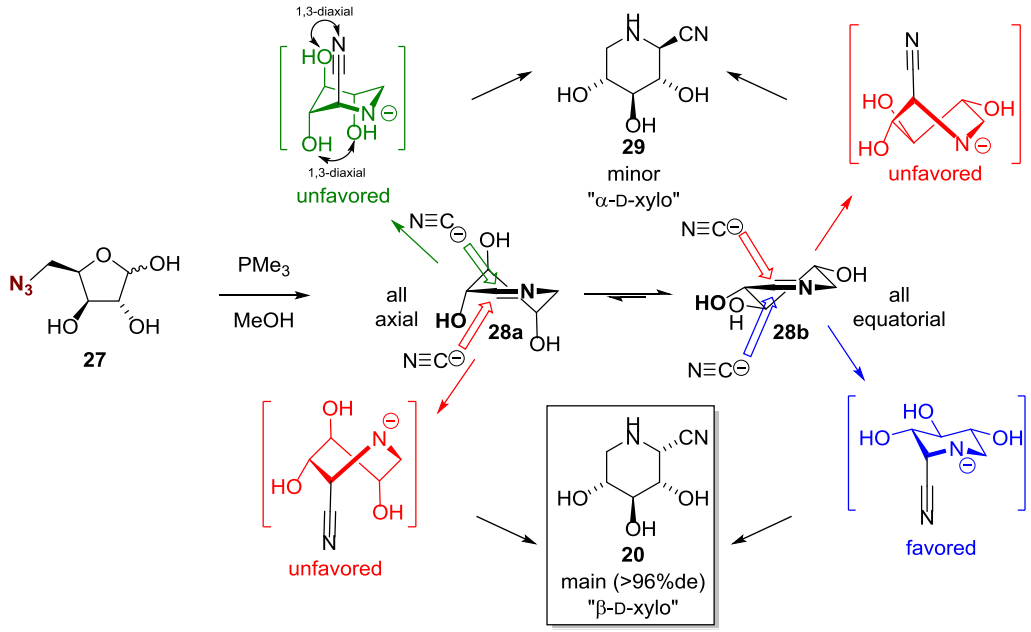
Substrates bearing an azide and an aldehyde (or hemi-acetal) form in an intramolecular fashion cyclic imines (**25**) spontaneously when treated with phosphines (Scheme 8). In the first mechanistic step the nucleophilic phosphorus attacks the nitrogen at the terminal end of the azide. The formed phophazide undergoes a cyclic 4-membered transition state and molecular nitrogen emerges when iminophosphorane or aza-ylide **24** is formed. In a simple Staudinger reduction this iminophosphorane would be quenched with water to give the free amine. The very nucleophilic nitrogen in aza-ylide **24** attacks the aldehyde to form a bicyclic oxazaphosphetane, which spontaneously dissociates into cyclic imine **25** and phosphine oxide (aza-Wittig).



Scheme 8: Intermolecular Staudinger/aza-Wittig Reactions are applicable for substrates bearing azido and aldehyde functionality in convenient distance for ring closure. Molecular nitrogen and phosphine oxide are formed in the process. The respective, cyclized imine is a reactive electrophile and can be attacked nucleophilically.

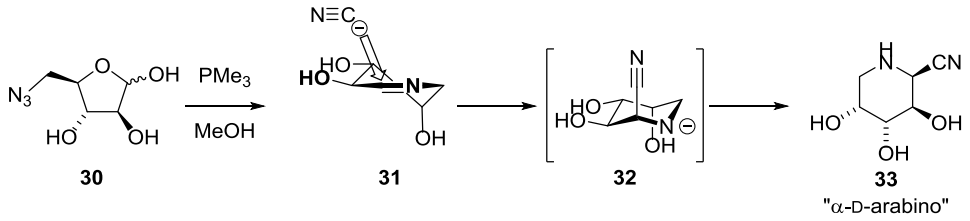
The nucleophile to give product **26** can be present while the SAW sequence is started or added after conformation of full substrate conversion, making it a one-pot multicomponent or tandem reaction, respectively. If Grignard or organolithium reagents are used as nucleophiles, best yields were obtained when the SAW reaction was performed in methanol and the solvent was changed to tetrahydrofuran before addition of the C-nucleophile.[66]

Figure 10 indicates that the nucleophilic attack is sterically controlled by the substrate's chirality. In fact, the stereoselectivity is so high that main diastereoisomer **20** was obtained in a 98% yield, whereas minor diastereoisomer **29** could not be isolated at all when SAW-Strecker reaction was carried out on 5-azido-5-deoxy-D-xylose (**27**).[65] This phenomenon can be rationalized by a model developed by K. A. Woerpel which focuses on the 6-membered ring conformations and their stability of the intermediates formed (Scheme 9).[67]



Scheme 9: SAW of **27** gives cyclic imine **28**. The endocyclic double bond forces the intermediate into a half-chair conformation with all residues in axial (**28a**) or more stable equatorial (**28b**) position. A nucleophilic attack of cyanide along the pseudo-equatorial trajectory (red) is unflavored, because it leads to intermediates in high-energy twist-boat conformation. The attack along the pseudo-axial trajectory (green and blue) allows maximal orbital overlap and results directly into stable chair conformations. However, **28a** is expected to be the less populated than **28b** and its reaction with cyanide results in two highly unfavored 1,3-diaxial interactions (green). Therefore, the blue pathway is expected to be the dominant one.

Scheme 9 illustrates that **28b** is preferred over **28a** and its reaction with cyanide along the pseudo-axial trajectory (blue) gives the most stable intermediate. Thus, product **20** was obtained with >96%de in β-D-xylo configuration. It has to be mentioned that the positioning of residues at heteroatom containing rings and therefore the equilibrium in Scheme 9 are not as straight-forward as it seems. Woerpel showed that cyclic oxocarbenium ions (similar to **28**) in half-chair conformation often prefer residues containing electronegative atoms in axial position. This is especially true for residues bearing the electronegative atom within close distance to the ring-heteroatom (C-4 in sugar nomenclature) and if the heteroatom is positively charged. Scheme 10 shows that SAW-Strecker on 5-azido-5-deoxy-D-arabinose (**30**) leads to the α-D-arabino product **33** as the only product isolated. Imine **31** shows the hydroxyl group attached to C-4 in axial position in the most stable conformation. The nucleophilic attack of cyanide along the pseudo-axial trajectory gives the more stable intermediate **32**.



Scheme 10: SAW-Strecker on 5-azido-5-deoxy-D-arabinose **(30)** leads solely to the α-D-arabino product **33**.

Although it is extremely useful, Woerpel's model does not take the size of the nucleophile into consideration. It is representative for small nucleophiles such as cyanide, but steric hindrance might overrule any of the above mentioned effects for large nucleophiles.

2. Problem Statement

The practical work for this master's thesis has been mainly focused on the synthesis of organic compounds, including the execution of reactions, isolation and characterization of intermediate and final products as well as the interpretation of the inhibition study results.

2.1. MTAN and MTAP Inhibitors

Deriving from the benchmark inhibitor MT-DIA (**3**) all MTAN/MTAP related targets can be divided into two groups. The first one includes products with modifications regarding the adenine structure (**34**). Whereas MT-DIA (**3**) consists of 9-deaza-adenine, these compounds have a changed nitrogen pattern. The other group differs in the nature of the thio ether group attached at the 5' position of compound **35**. Lipophilic chains and nitrogen containing heterocycles are to be introduced here.

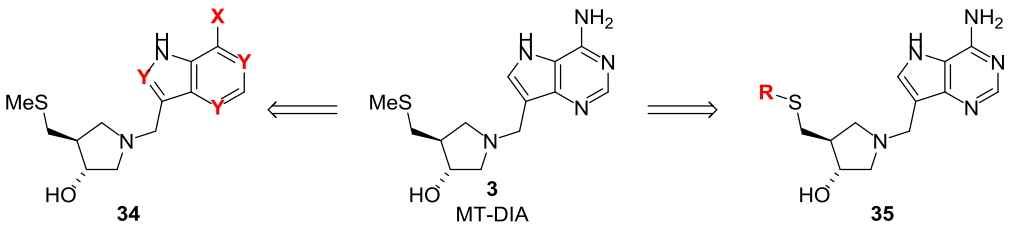


Figure 11: Two classes of inhibitors derived from MT-DIA. **34** shows different nitrogen patterns in the aromatic half (**X** = C or N; **Y** = OH or N) and **35** shows variable thio ether residues at 5'-position.

The task has been to synthesize a small library of compounds at a scale large enough to characterize the products properly by ^{13}C-NMR (>10mg). The main objective has been to produce a reasonable number of compounds in order to test their ability as inhibitors of bacterial MTAN and human MTAP. Optimization of reaction conditions, minimization of synthetic steps and the use of large scale friendly methods were of secondary focus.

2.2. Staudinger/aza-Wittig Reactions

The objective of this project has been to synthesize substrate **36**, expand the SAW reaction employing novel nucleophiles (**37**) and convert the formed cyclic imine with suitable dienes via a Diels-Alder reaction (**38**). Conditions for possible reactions should be optimized to maximize the yield. The stereoselectivity of the reaction should be investigated by suitable product characterization methods.

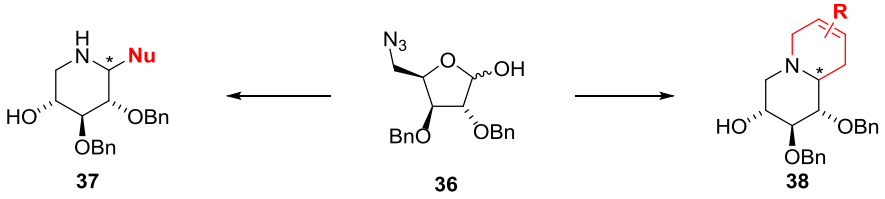


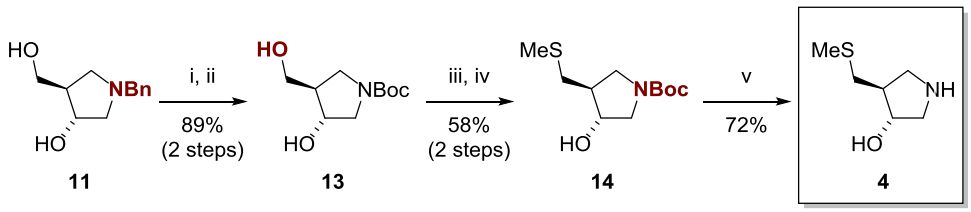
Figure 12: Expected product of the SAW reaction with a nucleophile (**37**) and general structure of a SAW-Diels-Alder product (**38**).

3. Results and Discussion

In the course of this thesis 15 potential MTAN/MTAP-inhibitors were successfully synthesized at a milligram scale and tested against MTAN and *h*MTAP. Some results were achieved on the synthetic route to 1-deaza-MT-DIA (**66**), although the target molecule could not be synthesized. Furthermore, two new nucleophiles were introduced to the Stauding/aza-Wittig reaction, yielding iminosugar 1-phosphonate **109** and the bi-cyclic iminosugar **111**.

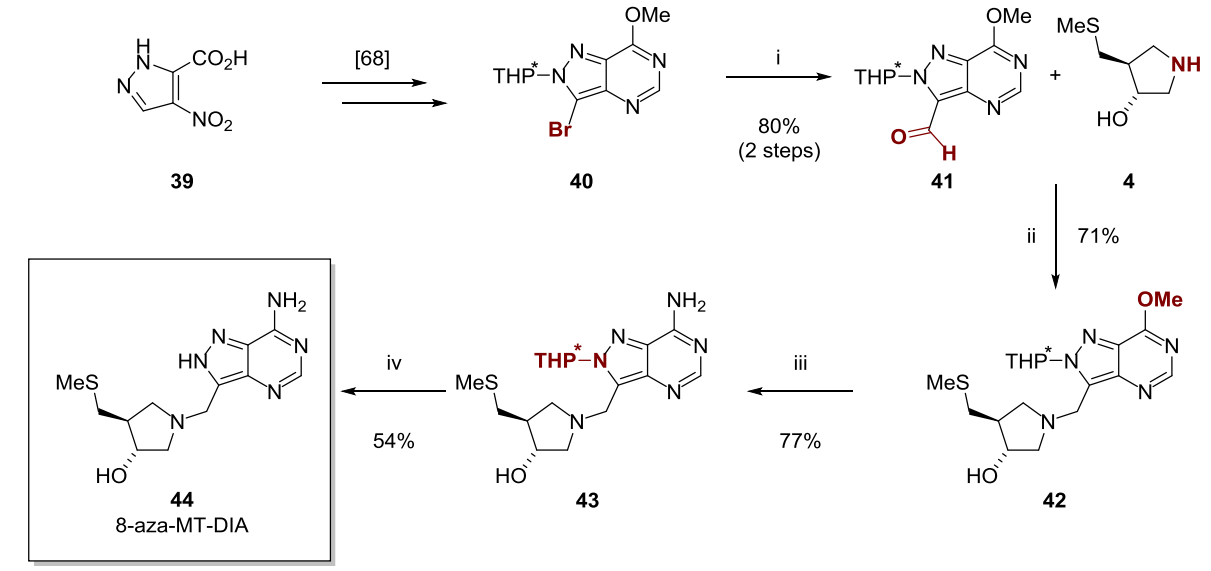
3.1. Synthesis of MT-DIA Adenine Derivatives

Known methylthio-pyrrolidine **4** was synthesized from provided diol **11** following large-scale procedures described above (1.4. Synthesis of MT-DIA (3)). The protecting group change from *N*-benzyl **11** to *N*-Boc **13** in methanol is straight forward, although the hydrogenolysis with Pearlman's catalyst took six days and five charges of catalyst. Replacement of the primary alcohol with sodium thiomethoxide in the presence of a secondary alcohol was achieved by selective mesylation with 2.1 equivalents of 2,6-lutidine and 1.1 equivalents of mesyl chloride in acetone. In this step most of the lost material was due to bis-mesylation and consequently introduction of two methylthio ethers. For the introduction of a thioacetate ester (**69**) iodization with imidazole (Garegg reaction, 3.2. Synthesis of 5'-S-MT-DIA Derivatives) at 0°C followed by its displacement was chosen with similar yields.



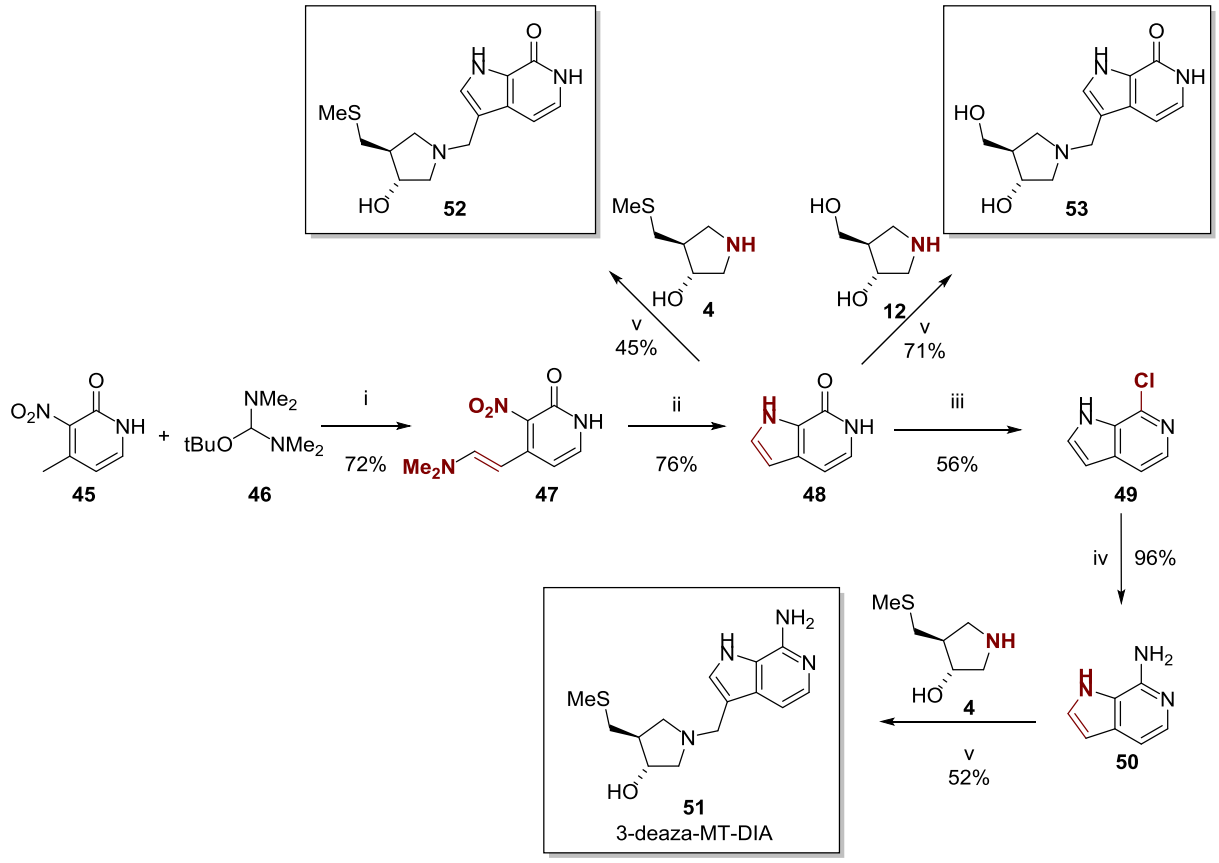
Scheme 11: (i) H_2, Pd/C, MeOH, 7 days; (ii) Boc2O, MeOH, 10min; (iii) 2,6-lutidine, MsCl, acetone, 3days; (iv) NaSMe, acetone, 3h; (v) TFA, BnH, 4h.

8-Aza-MT-DIA (**44**) was synthesized from prepared bromide **40**[68] in 5 steps with an overall yield of 24% (Scheme 12). The lithium-halogen exchange on **40** was quenched with DMF at -78°C and gave aldehyde **41** in high yield. Racemate **41** was reacted with chiral pyrrolidine **4** in a reductive amination with picoline borane to give **42** as a mixture of diastereomers. Exchange of the methoxy group with methanolic ammonia at 120°C in a pressure vessel gave amine **43**. Both intermediates **42** and **43** gave the expected peaks by HRMS and NMR, but due to the mixture of diastereomers, it was not possible to fully characterize them by NMR. Only when the chiral THP protecting group was removed under acidic conditions to give final product **44** full characterization by NMR was possible.



Scheme 12: (i) *n*BuLi, THF at -78°C, 30min then DMF; (ii) pic-BH_3, MeOH, 8h; (iii) NH_3 in MeOH (7N), 120°C, over night; (iv) MeOH/HCl(conc.) 3/1, 1h.

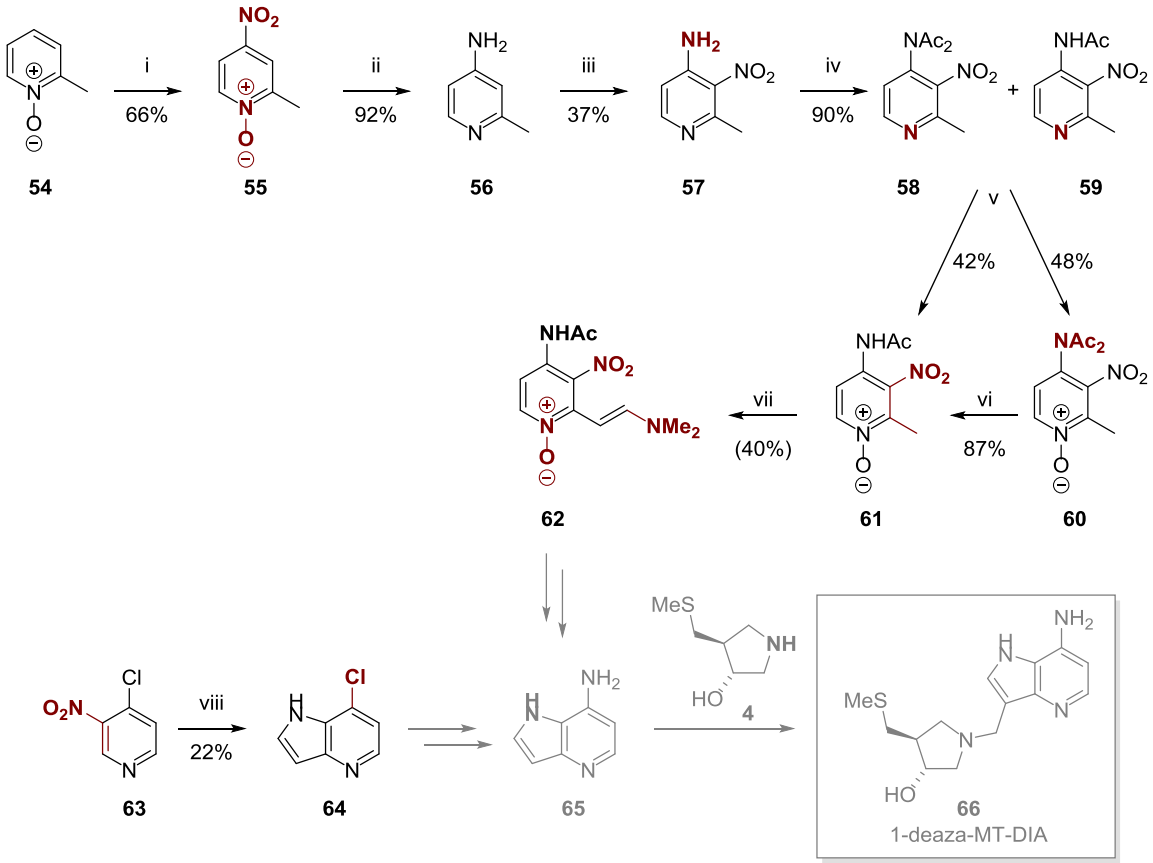
3-Deaza-MT-DIA (**51**) was synthesized from commercial available 2-hydroxy-4-methyl-3-nitropyridine (**45**) in five steps with an overall yield of 15% (Scheme 13). Final products **52** and **53** were synthesized via Mannich coupling as enough of intermediate **48** was left over. They are potential inhibitors for PNP, a human enzyme similar to the here discussed MTAP.[69]



Scheme 13: (i) DMF, 100°C, over night; (ii) Zn (dust), AcOH, 1.5h; (iii) $OPCl_3$, 100°C, 2h; (iv) NH_3(aq), CuCl, 120°C, over night; (v) formaldehyde, EtOH/water 1/1, 80-100°C, 2-4h.

Electronpoor aromatic rings bearing a methyl in ortho position to a nitro group are potential substrates for indole synthesis with the Bredereck's reagent (**46**).[70] At high temperatures the reagent is basic enough to deprotonate the activated methyl group, which forms a C-C double bond after nucleophilic attack and elimination. Reduction of nitro compound **47** to the respective amine causes spontaneous ring-closure to give aza-indole **48** in moderate yields. Introduction of the amine required activation with neat phosphoryl chloride at high temperatures to give chloride **49** in an reasonable yield. Replacement of chloride **49** with aqueous ammonia and catalytic copper(I) chloride gave amine **50** in an excellent yield. However, reaction with methanolic ammonia and sodium azide did not work. Mannich coupling with pyrrolidine **4** gave final product **51** in an acceptable yield.

In the first attempt to synthesize 1-deaza-MT-DIA (**66**) we chose a partially known route,[70] starting from commercial 2-picoline *N*-oxide (**54**). However, the synthesis turned out to be more step intensive and low yielding than expected. The Bartoli-indole synthesis[71] from commercial available 4-chloro-3-nitropyridine (**63**) would provide a more elegant alternative route, but unfortunately, due to time problems, time towards the end of the project this synthesis could not be finished.



Scheme 14: (i) H_2SO_4, HNO_3, 160°C; (ii) Fe, AcOH, 100°C; (iii) H_2SO_4, HNO_3, 60°C; (iv) Ac_2O, BnH; (v) mCPBA, $CHCl_3$; (vi) MeONa, MeOH, 5min; (vii) DMF dimethyl acetale, DMF; (viii) vinylMgBr, THF, -50° to -20°C, over night.

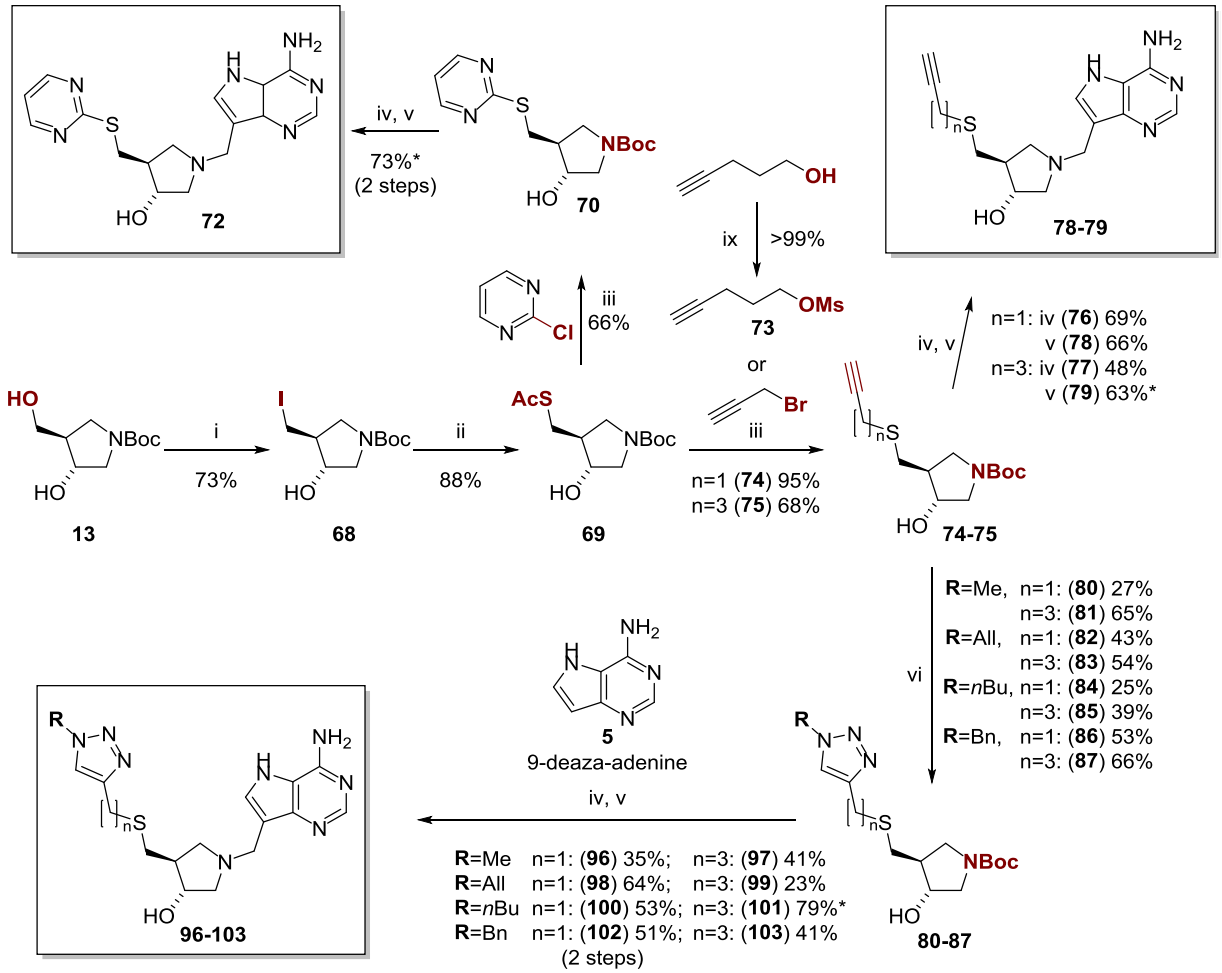
2-Picoline *N*-oxide (**54**) was nitrated to give nitro-compound **55** in moderate yields. Reduction to pyridine **56** turned out to be tricky, as molecular hydrogen with palladium on carbon and zinc dust in acetic acid both gave the coupled hydrazine product as main product. Reduction with iron dust gave the desired product, but separation of the formed iron species was only achieved, when appropriate extraction conditions were developed. Nitration to 4-amino-3-nitro-2-picoline (**57**) gave high amounts of the unusable 5-nitro- and 3,5-di-nitro-by-products even when nitric acid was used only stoichiometrically. Treatment of amine **57** with the Bredereck's reagent did not give any desired product and therefore the free amine was protected as acetamide. Selective formation of mono-acetamide **58** was impossible and separation of bis-acetamide **59** was also hardly possible. Thus, oxidation with *m*CPBA was carried out on the mixture to give the separable *N*-oxides **60** and **61**. At this stage bis-acetamide **60** was transformed into mono-acetamide **61** with sodium methoxide. Treatment of *N*-oxide **61** with DMF dimethylacetale gave a mixture of multiple compounds. HRMS and NMR suggest that compound **62** was formed, but the desired product could not be isolated due to the small amount of material left.

The Bartoli reaction on 4-chloro-3-nitropyridine (**63**) was performed as described by Wang *et al.* with vinylmagnesium bromide (3 equivalents).[71] However, azide replacement of the chloride in DMF at high temperatures could not be observed.

3.2. Synthesis of 5'-S-MT-DIA Derivatives

A small library of 11 compounds, with the same backbone as MT-DIA (**3**), but different thio-ether substituents at the 5'-position, was synthesized from diol **13** (Scheme 15). Final compounds **72, 78** and **79** were synthesized in seven steps with an overall yield of 28%, 25% and 12%, respectively. Triazoles **96**-**103** were synthesized in eight steps with an overall yield of 4% to 15%.

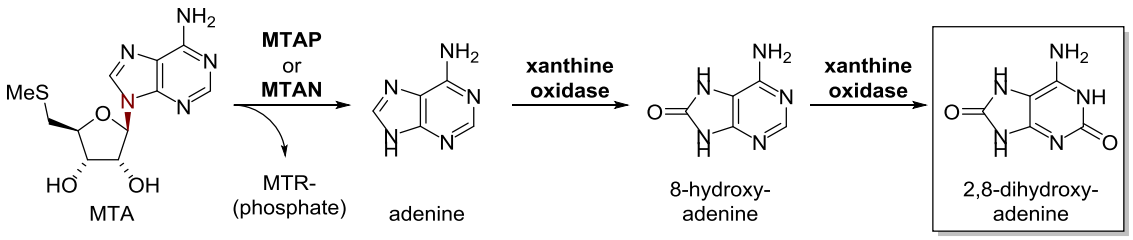
The primary alcohol of diol **13** was activated following Garegg's procedure[72] with triphenylphosphine, imidazole and iodine at 0°C. Thioacetate ester **69** was obtained after treatment of **69** with potassium thioacetate in good yields. Formation of thioethers **70**, **74** and **75** was performed employing the respective halide after the thioacetate ester was cleaved with sodium methoxide to give the free thiol. Copper(I) catalyzed one-pot multicomponent 1,3-dipolar cycloaddition was performed on alkynes **74** and **75** with sodium azide employing different halides such as methyl iodine, allyl bromide, *n*-butyl bromide and benzyl bromide. The last 2 steps for all 11 compounds shown in Scheme 15 are Boc deprotection with hydrochloric acid and Mannich coupling with 9-deaza-adenine and formaldehyde.



Scheme 15: (i) PPh_3, I_2, imidazole, CH_2Cl_2, 0°, over night; (ii) AcSK, DMF, 0° to rt, 30min; (iii) MeONa, MeOH, 30min then 3-propargyl bromide, 2-chloropyrimidine or **73**, over night; (iv) MeOH/HCl(conc.) 3/1, 1h-over night; (v) 9-deaza-adenine (**5**), formaldehyde, EtOH/water 1/1, 70-100°C (microwave), 2-6h; (vi) RX (MeI, allyl bromide, *n*BuBr or BnBr), NaN_3, CuI, MeOH. *: purity only partially above 95%.

3.3. Inhibition Studies

The conversion of MTA by MTAN or MTAP in the presence of the respective inhibitor was measured by an indirect enzymatic assay. Activity was monitored by the increase in absorbance at 305nm. The coupled reactions catalyzed by xanthine oxidase are irreversible and allow to determine the formation of 2,8-dihydroxy-adenine from adenine.

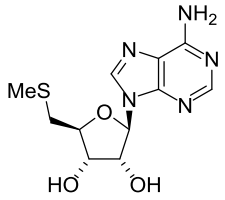
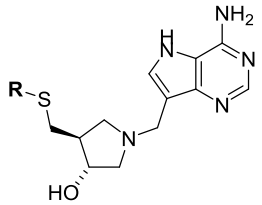
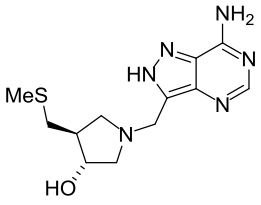


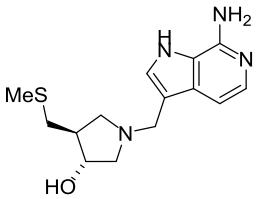
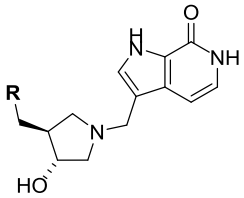
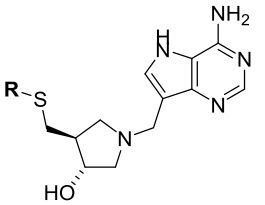
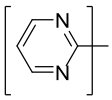
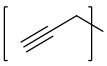
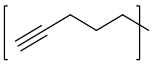
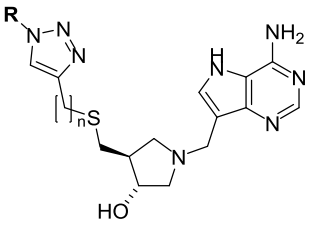
Scheme 16: Xanthine oxidase converts formed adenine quantitatively and irreversibly to 2,8-dihydroxy-adenine, which was quantified via its absorbance at 305nm.

3.3.1. K_i* values

Slow-onset tight binding inhibition of MTAN and MTAP by transition state analogue inhibitors normally involves a two-step process. First the enzyme undergoes reversible competitive binding with the inhibitor to form an enzyme-inhibitor-complex: E + I ↔ EI. The equilibrium of this process is described with a K_i value as dissociation constant. In the second step the slow-onset inhibitor causes a conformational change in the enzyme's periphery to achieve tighter binding of the enzyme-inhibitor-complex: EI ↔ E*I. This second step happens at a relatively lower rate and causes stronger inhibition of the enzyme. The overall equilibrium of the process: E + I ↔ E*I is described with the K_i* value as dissociation constant, which can be compared with K_i values of conventional competitive inhibitors. Table 3 shows K_i* values only.

Table 3: Intrinsic K_i* values for all synthesized products. First section shows the known and the measured K_M values of MTA (**1a**) for the respective enzyme. Benchmark inhibitors MT-DIA and BT-DIA were measured as control inhibitors. Both values vary from known values, but they stay in the same magnitude and are therefore acceptable. For determination of the *Hp*MTAN-K_i* values a mixture of HEPES buffer (0.1M, pH=7.2), NaCl (0.1M), dithiothreitol (1mM), xanthine oxidase (1U/mL), MTA (1mM) and the respective inhibitor (0-2µM) in water (1mL total reaction volume) was treated with *Hp*MTAN (0.6nM) for 80min at 25°C. For *h*MTAP a mixture of potassium phosphate (0.1M), dithiothreitol (1mM), xanthine oxidase (1U/mL), MTA (800µM) and the respective inhibitor in water (1mL total reaction volume) was treated with *h*MTAP (2nM) for 120min at room temperature. For both enzymes the absorption at 305nm (2,8-dihydroxy-adenine, ε_i=15.5mM^{-1}cm^{-1}) was measured with 6 data points.

	Inhibitor	K_i* (*Hp*MTAN)[73]	K_i* (*h*MTAP)[74]
1a		- (lit.: K_M = 0.6 µM[6])	K_M = 1.74 µM (lit.: K_M = 1.8 µM[75])
MT-DIA (**3**)	 **R** = Me	- (lit.: K_i* = 571 pM[6])	K_i* = 191 pM (lit.: K_i* = 86 pM[4])
BT-DIA (**67**)	**R** = *n*Bu	K_i* = 22 pM (lit.: K_i* = 36 pM[6])	- (lit.: K_i* = 110 pM[4])
44		235 ± 64 pM	18.9 ± 1.5 nM

51		>5 µM	52.0 ± 3.3 nM
			
52	**R** = MeS	3.04 ± 25 nM	8.18 ± 0.81 nM
53	**R** = HO	>5 µM	>50 µM
			
72	**R** = 	58 ± 6 pM	12.7 ± 4.8 nM
78	**R** = 	55 ± 25 pM	626 ± 81 pM
79	**R** = 	**26 ± 6 pM**	937 ± 118 pM
			
96	n=1	**R** = Me	274 ± 96 pM	66.1 ± 6.6 nM
97	n=3	**R** = Me	**25 ± 2.1 pM**	2.98 ± 0.17 nM
98	n=1	**R** = All	151 ± 99 pM	70.0 ± 3.2 nM
99	n=3	**R** = All	**26 ± 4.8 pM**	2.42 ± 0.25 nM
100	n=1	**R** = *n*Bu	373 ± 135 pM	37.1 ± 6.9 nM
101	n=3	**R** = *n*Bu	**28 ± 10 pM**	1.25 ± 0.05 nM
102	n=1	**R** = Bn	73 ± 21 pM	13.1 ±1.4 nM
103	n=3	**R** = Bn	36 ± 10 pM	1.37 ± 0.09 nM

The structure activity relationship study with the compounds depicted in Table 3 shows that compounds **79**, **97**, **99** and **101** are the most potent inhibitors for *Hp*MTAN. With inhibition constants down to 25pM they are among the strongest known inhibitors for *Hp*MTAN. With K_M/K_i^* values of up to $24 \cdot 10^3$ they bind to the enzyme 24,000-times tighter than its substrate MTA. Inhibitors **78** and **79** are the most powerful inhibitors among the library for *h*MTAP. K_M/K_i^* values up to $2.8 \cdot 10^3$ make them potent inhibitors, although they are 60-fold weaker than other known inhibitors for *h*MTAP.

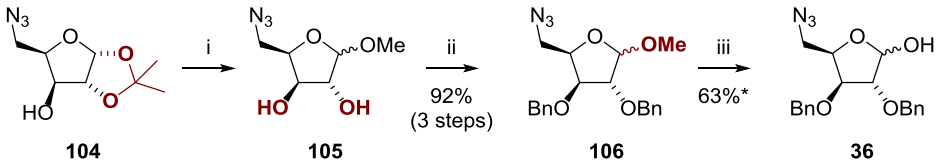
A thorough structural analysis shows that the introduced modifications at position 5' of MT-DIA (3) are highly selective for *Hp*MTAN over *h*MTAP. This is due to the fact that *Hp*MTAN accepts three different substrates (MTA, 6-amino-6-deoxyfutalosine and SAH), which are 5'-derivatives of each other. Therefore, *Hp*MTAN provides a lipophilic pocket next to the active site.[4] *h*MTAP on the other hand, only converts MTA and hence lacks a spacious, lipophilic pocket. This circumstance can be used to develop tight-binding inhibitors for *Hp*MTAN with lowered activity towards *h*MTAP. Such compounds can be used as antibiotics without affecting the human metabolism. Compound **98** shows the highest $K_i^*_{(h\text{MTAP})}/K_i^*_{(Hp\text{MTAN})}$ value of 464, which is more than 150-times higher than the same value of BT-DIA (**67**).

Compared with MT-DIA (**3**) the introduced nitrogen at position 8 in 8-aza-MT-DIA (**44**) increases its inhibition properties for *Hp*MTAN by 2-fold, but decreases the inhibition for *h*MTAP by a factor of 10. Combination of the above described effect of bulky 5'-residues and an additional nitrogen in the adenine backbone will probably create an even more specific *Hp*MTAN inhibitor. Other changes to the nitrogen pattern of 9-deaza-adenine did not increase the inhibition. However, it is dubious why compound **52** shows lower K_i^* values than **51**, as all natural substrates of MTANs and MTAPs bear adenine- and not hypoxanthine-residues.

One aspect K_i values do not address is bioavailability. Although compounds like MT-DIA (**3**) are usually perfectly soluble in water, the introduction of large lipophilic 5'-residues might cause micelle formation or membrane binding. Table 3 shows that *Hp*MTAN accepts transition state analogous like **72** and **96**-**103**, bearing nitrogen containing, aromatic heterocycles at the 5'-terminus, perfectly well. For the triazole compounds it seems to be crucial that the aromatic residue is pointing far enough into the enzymes lipophilic pocket, as compounds **96**, **98** and **100** show decreased inhibition properties. This could be explained by the long-chain triazole from compounds **97**, **99**, **101** and **103** actually poking through the lipophilic pocket and interacting with the polar surface of the protein. However, this theoretically possible scenario has to be proven yet.

3.4. Synthesis of 5-Azido-5-deoxy-2,3,-di-*O*-benzyl-α,β-D-xylose (36)

Azide **36** was synthesized from provided substrate **104** in four steps with an overall yield of 58% following a straight forward protecting group procedure.[76] The cleavage of acetal **106** was performed in equal amounts of water and acetonitrile with hydrochloric acid at reflux. The reaction was stopped before full conversion was achieved, as one epimer was reacting significantly faster and decomposition of the product was observed at an earlier attempt.



104 **105** **106** **36**

Scheme 17: (i) AcCl, MeOH, 0°C to rt, over night; (ii) NaH, DMF, 0°C then BnBr, rt, 10min; (iii) HCl (conc), MeCN, reflux. *: includes 10% substrate recovery.

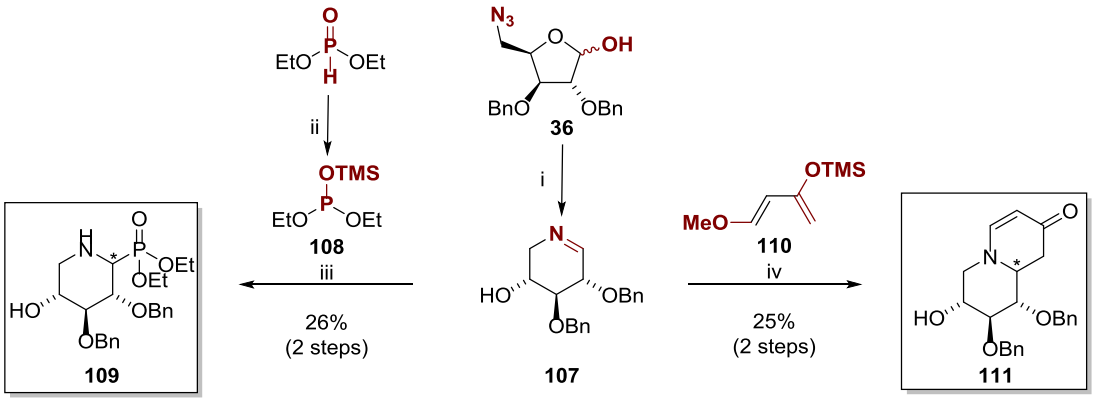
3.5. Novel nucleophiles for Staudinger/aza-Wittig Reaction Sequence

Although cyclic imine **107** can be transferred into other solvents, it cannot be isolated or stored and therefore was synthesized freshly for every reaction. The cyclic imine formation was performed in methanol with trimethylphosphine, after full conversion the reaction was concentrated and the next step was performed straight away.

The synthesis of iminosugar 1-phosphonate **109** required a second reagent with limited stability, which had to be prepared directly before conversion. Diethyl trimethylsilyl phosphite (**108**) was prepared from diethyl phosphite, triethylamine and trimethylsilyl chloride with 26% yield.[77]

Bi-cyclic iminosugar **111** was isolated after cyclic imine **107** was treated with dried zinc(II) chloride and Danishefsky's diene (**110**). Attempts to increase the yield of 25% by the choice of the Lewis acid, the work up conditions or the reaction temperature failed so far. Literature suggests that Lewis acid catalyzed reactions of imines with Danishefsky's diene are rather stepwise Mukaiyama-Aldol/Mannich-Michael reactions than concerted Diels-Alder reactions with enolether cleavage and elimination.[78,79]

^{1}H-NMR coupling constants of both products **109** and **110** suggest that their formation followed Woerpel's model and that they have β-configuration (sugar nomenclature). However, attempts to crystalize the products have failed so far, however x-ray structure analysis will be crucial to determine the products' stereochemistry.



Scheme 18: (i) PMe_3, MeOH, 30min; (ii) TMSCl, Et_3N, CH_2Cl_2, 0°C, 10min; (iii) **108** added to **109**, CH_2Cl_2, 1h; (iv) $ZnCl_2$ (dry), THF then Danishefsky's diene (**111**).

4. Conclusions and Outlook

A small library of 15 MTAN- and MTAP- inhibitors has been successfully synthesized and tested against *Helicobacter pylori* MTAN and human MTAP. Various 2nd-generation DIA-inhibitors bearing long, linear 5'-thio ethers show K_M/K_i* values up to $24 \cdot 10^3$. If the 5'-thio ethers contain bulky, aromatic, nitrogen-heterocycles, like pyrimidines and triazoles, a specific inhibition affinity toward bacterial MTAN can be achieved, with less affinity towards mammalian MTAP.

A similar effect was achieved when a carbon in the aromatic adenine residue of MT-DIA (**3**) was replaced by nitrogen. 8-Aza-MT-DIA (**44**) showed higher affinity to *Hp*MTAN and less toward *h*MTAP, compared to MT-DIA (**3**). For further investigations it would be interesting to synthesize 5'-derivatives of 8-aza-MT-DIA to see, if these two effects multiply each other and result in an highly specific MTAN- inhibitor.

Other changes to the nitrogen-pattern on MT-DIA (**51**-**53**) did not lead to an increase of inhibition affinity or enzyme selectivity. The alternative route to synthesize 1-deaza-MT-DA (**66**) starting from 4-chloro-3-nitropyridine (**63**) with a Bartoli reaction is promising, as it is at least 6 synthetic steps shorter than the originally chosen route.

For the Staudinger/aza-Wittig reaction sequence the partially protected substrate **36** was synthesized in gram quantities following a protecting group protocol and two new nucleophiles were successfully converted with the intermediate cyclic imine **107**. Reaction with Danishefsky's diene (**110**) provided the bi-cyclic iminosugar **111**, with various possibilities of follow-up chemistry. Conversion of imine **107** via Arbuzov reaction with prepared phosphite **108** gives iminosugar 1-phosphonate **109**. The stereochemistry of both products **109** and **111** is estimated from NMR coupling constants and Woerpel's model. It has not been confirmed by x-ray crystallography yet, which is the next important thing to do.

Compounds, like **109**, with a phosphonate directly bound to the pseudo anomeric carbon are suitable glycosyl phosphate mimetics, as they are reasonable stable and have polarities similar to those of natural sugar 1-phosphates.[80] Therefore, an approach including 1'-phosphonates might be an efficient way to design highly selective MTAP- inhibitors.

5. Experimental

Synthetic work for this thesis was carried out in two different laboratories. The synthesis of MTAN/MTAP inhibitor synthesis has been performed in Lower Hutt, Wellington under supervision of Peter C. Tyler in the facilities of the Ferrier Research Institute (Victoria University of Wellington). Data regarding work, reactions and analysis performed in Wellington are labeled with a section sign ([§]). Synthesis for the Staudinger-aza-Wittig reaction has been performed done in Graz under supervision of Tanja M. Wrodnigg and Manuel Zoidl in the facilities of the Glyco Group (Graz University of Technology). Data regarding work, reactions and analysis performed in Graz are labeled with a double dagger ([‡]). The inhibition studies of compounds **44**, **51**-**53**, **72**, **78**-**79** and **96**-**103** were carried out by Brett Hirsch (*Hp*MTAN) and Ross Firestone (*h*MTAP) at the Albert Einstein College of Medicine (Yeshiva University, New York).

5.1. General Synthetic Procedures

All reactions were performed under an argon[§] or nitrogen[‡] atmosphere, unless water was used as solvent or the reaction mixture was heated above 100°C. Organic solutions were dried over anhydrous magnesium sulfate[§] or sodium sulfate[‡] and the volatiles were evaporated under reduced pressure at 40°C. Anhydrous and chromatography solvents were obtained commercially and used without any further purification. Ion exchange resins Amberlyst 21 and 26 were rinsed with water and methanol before used. Thin layer chromatography (TLC) was performed on aluminum sheets coated with 60 F254 silica gel. Organic compounds were visualized under UV light[§,‡] or a dip of di-methylaminebenzaldehyde (1g, Ehrlich's reagent), sulfuric acid (conc., aq., 25mL), methanol (150mL)[§,‡] or cerium(IV) sulfate (8g, CAM), ammonium molybdate (100g), sulfuric acid (conc., aq., 108mL), water (975mL)[‡] or potassium permanganate (2g), potassium carbonate (13g), sodium hydroxide (aq, 1M, 3.5mL, water (200mL)[§]. Chromatography (flash column[§,‡] or an automated system with continuous gradient facility[§]) was performed on silica gel (40-63µm[§] and 35-70µm[‡]). Solvent mixtures are states as percentage of the polar solvent respective to total volume. All final compounds gave satisfactory purity (≥95%) by HPLC[§] and NMR.[§,‡] ^{1}H NMR spectra were measured in $CDCl_3$ (δ=7.26), CD_3OD (center line, δ=3.31), DMSO-d6 (δ=2.50), APT and ^{13}C NMR spectra in $CDCl_3$ (center line, δ=77.16), CD_3OD (center line, δ=49.0) or DMSO-d6 (center line δ=39.52) at 500MHz[§] and 300MHz[‡]. Assignments of ^{1}H and ^{13}C resonances were based on 2D (1H-1H DQF-COSY, 1H-13C HSQC), DEPT[§] and APT[‡] experiments. Abbreviations used: s, singlet; d, doublet; t, triplet; q, quartet; pent, pentet; sext, sextet; dt, doublet of triplets, ddt, doublet of doublets of triplets, etc. High resolution electrospray mass spectra (ESI-HRMS) were recorded on a Q-TOF Tandem Mass Spectrometer[§].

5.1.1. Thioether Formation from Thioacetate ester 69[§]

A solution of sodium methoxide (1.1eq, 3%) in methanol was degased with argon and treated with an inert prepared solution of thioacetate ester **69** in methanol. The respective halide (1.2eq) was added after 30min. After an additional hour reaction time the volatiles were removed under reduced pressure and the residue was dissolved in chloroform. Aqueous work-up gave crude products in quantitative yields. Purification (column chromatography: gradient from petrol ether to ethyl acetate) was only necessary, if excess amounts of non-volatile halides were used as reactants.

5.1.2. Multicomponent 1,3-Dipolar Cycloaddition[§]

The respective alkyne was dissolved in methanol under argon atmosphere and treated with sodium azide (2eq), the respective halide (1.1eq) and a catalytic amount of copper(I) iodide (ca. 0.05eq). After the reaction mixture was stirred at room temperature overnight it was directly adsorbed onto silica and the desired product was obtained by chromatography (petrol ether/ethyl acetate/methanol or toluene/acetone).

5.1.3. *N*-Boc Deprotection[§]

The respective Boc protected pyrrolidine was dissolved in methanol and treated with hydrochloric acid (conc., aq.) to give a volume ratio methanol/hydrochloric acid of 3/1. After full conversion of the staring material was determined by tlc (1-10h), isopropyl alcohol and toluene were added and the volatiles were removed under reduced pressure. The residue was redissolved in methanol and neutralized with ion exchanger Amberlyst 21 to pH=7-9. Filtration and evaporation gave crude product often with sufficient purity. If necessary, the crude product was purified by chromatography (absorbed on silica, gradient from dichloromethane to 20% methanolic ammonia (7M) in dichloromethane).

5.1.4. MANNICH Coupling[§]

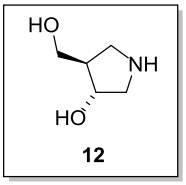
The respective pyrrolidine (1-1.2eq) and the respective aza-aminoindole (1-1.2eq) were dissolved in water and ethanol (volume ratio: 1/1). The mixture was treated with formaldehyde (1.2eq of a 37%aq. solution) and heated to 70-100°C in a sealed vessel conventionally or with radiation (microwave). The limiting reactant was occasionally changed due to better separation of product and excess starting material. After complete consumtion of the starting material (2-6h) the volatiles were removed under reduced pressure and the product isolated by column chromatography (absorbed on silica, gradient from dichloromethane to 30% methanolic ammonia (7M) in dichloromethane). If purification with methanolic ammonia did not succeed, aqueous ammonia with isopropyl alcohol or chloroform and methanol was used due to unequal shifts of R_f values.

5.2. MTAN/MTAP Inhibitors

5.2.1. Synthesis of Methylthio Pyrrolidine 4

(3*R*,4*R*)-4-(Hydroxymethyl)pyrrolidin-3-ol (12)[§]

Boc deprotection of synthesized substrate **13** (1.29g, 5.94mmol) was carried out as described above (General Synthetic Procedures). Crude product **12** was obtained as brown oil in quantitative yield (782mg, 6.68mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



12

MW: 117.15g/mol
TLC-solvent: $CHCl_3$/MeOH: 50%
TLC-detection: Ehrlich's
CC-solvent: -
yield: >99%

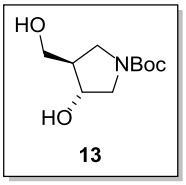
^{1}H NMR (CD_3OD center line 3.31): δ = 4.33 (ddd, *J*=2.6, 5.0, 2.6Hz, 1H), 3.63 (dd, *J*=11.2, 5.8Hz, 1H), 3.60 (dd, *J*=11.2, 5.6Hz, 1H), 3.51 (dd, *J*=11.8, 8.0Hz, 1H), 3.35 (dd, *J*=12.2, 5.0Hz, 1H), 3.17-3.12 (m, 2H), 2.43-2.37 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 73.7 (CH), 62.3 (CH_2), 53.9 (CH_2), 50.1 (CH), 47.8 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_5H_{12}NO_2$ (MH^+) calcd 118.0868, found 118.0863.

tert-Butyl-(3*R*,4*R*)-3-hydroxy-4-(hydroxymethyl)pyrrolidine-1-carboxylate (13)[§]

Provided compound **11** (50.0g, 217mmol, ~90% pure) in methanol (500mL) was treated with Pearlman's catalyst (5g, $Pd(OH)_2$ on carbon, 20 wt.%), degased and placed under hydrogen atmosphere. Additional charges of catalyst (5g) were added after 21h, 28h and 71h. The mixture was filtrated through celite after 6 days. Boc anhydride (64g, 290mmol) and DMAP (502mg, 4.07mmol) was added to the red filtrate. After 10min reaction time all volatiles were evaporated to give the crude product (50.4g) as brown oil as. The material was purified by column chromatography to afford product **13** (42.1g, 194mmol) as pale syrup.



13

MW: 217.27g/mol
TLC-solvent: $CHCl_3$/MeOH: 50%
TLC-detection: Ehrlich's
CC-solvent: EA/MeOH: 10%
yield: 89% (2 steps)

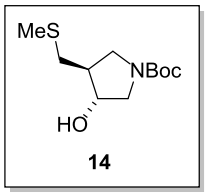
^{1}H NMR (CD_3OD center line 3.31): δ = 4.17-4.12 (m, 1H), 3.59-3.50 (m, 3H), 3.49-3.42 (m, 1H), 3.25-3.18 (m, 2H), 2.29-2.20 (m, 1H), 1.46 (s, 9H).

^{13}C NMR (CD_3OD center line 49.0): δ = 156.6 (C), 80.9 (C), 72.7, 72.0 (CH), 62.6 (CH_2), 54.0, 53.6 (CH_2), 50.0, 49.3 (CH), 48.1, 47.6 (CH_2), 28.7 (3xCH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{10}H_{19}NO_4Na$ (MNa^+) calcd 240.1212, found 240.1207.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-((methylthio)methyl)pyrrolidine-1-carboxylate (14)[§]

Diol **13** (1.99g, 9.16mmol) in acetone (20mL) was treated with 2,6-lutidine (2.2mL, 19mmol) and methansulfonyl chloride (0.80 mL, 10 mmol). After 3 days the white precipitate (lutidine hydrochloride) was filtered off, the filtrate was treated with sodium thiomethoxide (aq., 3M, 4mL, 12mmol) and heated to 50°C. After 3.5h all volatiles were evaporated, the residue dissolved in diethyl ether (80mL) and washed with hydrochloric acid (aq., 2M, 30mL) and sodium bicarbonate (sat. aq., 30mL). The organic layer was dried and concentrated to give the crude product (1.98g) as yellow oil. The material was purified by column chromatography to afford product **14** (1.31g, 5.30mmol) as pale syrup.



14

MW: 247.35g/mol
TLC-solvent: EA
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 20%→40%
yield: 58% (2 steps)

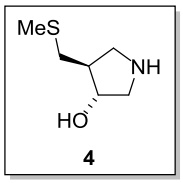
^{1}H NMR ($CDCl_3$ center line 7.26): δ = 4.16 (bs, 1H), 3.71-3.62 (bm, 2H), 3.28-3.17 (bm, 1H), 3.13 (dd, *J*=11.1, 6.6Hz, 1H), 2.75-2.66 (bd, *J*=14.4, 1H), 2.61-2.44 (m, 2H), 2.35-2.24 (bm, 1H), 2.13 (s, 3H), 1.44 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.7 (C), 79.7 (C), 75.0, 74.2 (CH), 52.6, 52.4 (CH_2), 49.3, 49.0 (CH_2), 45.3, 44.5 (CH), 35.9 (CH_2), 28.6 (3xCH_3), 16.0 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{11}H_{21}NO_3SNa$ (MNa^+) calcd 270.1140, found 270.1144.

(3*R*,4*S*)-4-((Methylthio)methyl)pyrrolidin-3-ol (4)[§]

Substrate **14** (1.31g, 5.30mmol) in toluene (40mL) was cooled to 0° and treated with Trifluoroacetic acid (4.5mL, 58mmol). After 4h isopropyl alcohol was added and the reaction mixture was evaporated. The residue was redissolved in methanol and basified with ion exchanger Amberlyst 26 to pH=7-9. Filtration and evaporation gave the crude product as dark oil. The material was purified by column chromatography to afford product **4** (558mg, 3.79mmol) as yellow sticky solid.



4

MW: 147.24g/mol
TLC-solvent: EA
TLC-detection: Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→20%
yield: 72%

^{1}H NMR (CD_3OD center line 3.31): δ = 4.13 (ddd, *J*=3.4, 5.4, 3.4Hz, 1H), 3.33 (dd, *J*=11.6, 7.5Hz, 1H), 3.12 (dd, *J*=12.1, 5.3Hz, 1H), 2.89 (dd, *J*=12.1, 3.3Hz, 1H), 2.79 (dd, *J*=11.8, 5.6Hz, 1H) , 2.65 (dd, *J*=13.1, 6.6Hz, 1H) , 2.45 (dd, *J*=13.1, 8.8Hz, 1H) , 2.31-2.24 (m, 1H), 2.12 (s, 3H).

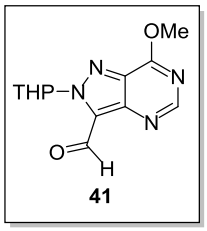
^{13}C NMR (CD_3OD center line 49.0): δ = 76.9 (CH), 54.2 (CH_2), 50.8 (CH_2), 48.3 (CH), 36.9 (CH_2), 15.6 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_6H_{14}NOS$ (MH^+) calcd 148.0796, found 148.0790.

5.2.2. Synthesis of 8-aza-MT-DIA (44)

7-Methoxy-2-(tetrahydro-2*H*-pyran-2-yl)-2*H*-pyrazolo[4,3-d]pyrimidine-3-carbaldehyde (41)[§]

Provided bromide **40** (492mg, 1.57mmol) in THF (25mL) was cooled to -78°C under argon atmosphere. *n*-Butyllithium (1.9M in hexane, 1.7mL, 3.3mmol) was added dropwise without warming the reaction mixture above -70°C. DMF (0.87mL, 11mmol) was added after 25min. After additional 30min the reaction was quenched with ammonium chloride (sat. aq., 10mL), warmed to room temperature, diluted with water (40mL) and extracted with toluene (2x100mL). Combined organic layers were dried and concentrated to give the crude product (419mg) as yellow solid. The material was purified by column chromatography to afford product **41** (328mg, 1.25mmol) as yellow solid.



41

MW: 262.27g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: UV, Ehrlich's
CC-solvent: PE/EA: 20%
yield: 80%

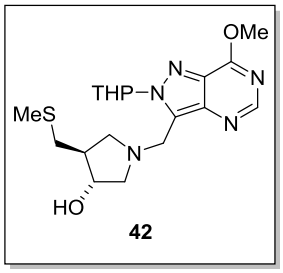
^{1}H NMR ($CDCl_3$ center line 7.26): δ = 10.41 (s, 1H), 8.69 (s, 1H), 6.54 (dd, *J*=9.7, 2.6Hz, 1H), 4.23 (s, 3H), 4.12-4.07 (m, 1H), 3.82 (td, *J*=11.2, 2.6Hz, 1H), 2.54-2.44 (m, 1H), 2.18-2.11 (m, 1H), 2.11-2.05 (m, 1H), 1.84-1.70 (m, 2H), 1.67-1.61 (m, 1H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 178.2 (CH), 162.7 (C), 155.6 (CH), 145.1 (C), 131.3 (C), 130.0 (C), 88.1 (CH), 68.5 (CH_2), 54.6 (CH_3), 29.8 (CH_2), 24.9 (CH_2), 22.3 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{12}H_{15}N_4O$ (MH^+) calcd 263.1144, found 263.1140.

(3*R*,4*S*)-1-((7-Methoxy-2-(tetrahydro-2*H*-pyran-2-yl)-*2H*-pyrazolo[4,3-d]pyrimidin-3-yl)methyl)-4-((methylthio)methyl)pyrrolidin-3-ol (42)[§]

Pyrrolidine **4** (185mg, 1.26mmol) and aldehyde **41** (328mg, 1.25mmol) were added to a prepared solution of acetyl chloride (60µL, 0.824mmol) and methanol (2mL). 2-Picoline-borane complex (175mg, 1.63mmol) was added when the mixture turned homogeneous at 40°C (pH=5). All volatiles were evaporated after 8h to give the crude product (587mg) as yellow foam. The material was purified by column chromatography to afford a diastereomeric mixture of **42** (353mg, 0.897mmol) as yellow solid.



42

MW: 393.51g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 5%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→10%
yield: 71%

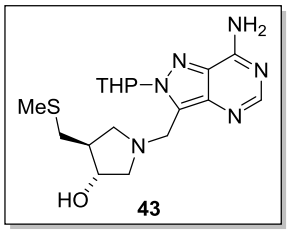
^{1}H NMR ($CDCl_3$ center line 7.26) and ^{13}C NMR ($CDCl_3$ center line 77.2) indicate a diastereomeric mixture of the desired product free of other impurities.

MS: ESI-HRMS (TOF) *m/z* for $C_{18}H_{28}N_5O_3S$ (MH^+) calcd 394.1913, found 394.1905.

$[\alpha]_D^{20}$: not measured

(3*R*,4*S*)-1-((7-Amino-2-(tetrahydro-2*H*-pyran-2-yl)-2*H*-pyrazolo[4,3-d]pyrimidin-3-yl)methyl)-4-((methylthio)methyl)pyrrolidin-3-ol (43)[§]

The diastereomeric mixture of **42** (328mg, 0.834mmol) was dissolved in methanolic ammonia (7M, 5mL) and heated to 120°C in a sealed pressure vessel for 16h. Evaporation of all volatiles gave the crude product (386mg) as brown foam. The material was purified by column chromatography to afford a diastereomeric mixture of **43** (243mg, 0.642mmol) as yellow foam.



43

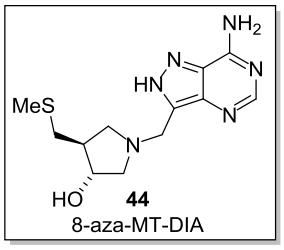
MW: 378.50g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→10%
yield: 77%

^{1}H NMR (CD_3OD center line 3.31 and ^{13}C NMR (CD_3OD center line 49.0) indicate a diastereomeric mixture of the desired product free of other impurities.

MS: ESI-HRMS (TOF) *m/z* for $C_{17}H_{27}N_6O_2S$ (MH^+) calcd 379.1916, found 379.1912.

(3*R*,4*S*)-1-((7-Amino-2*H*-pyrazolo[4,3-d]pyrimidin-3-yl)methyl)-4-((methylthio) methyl)pyrrolidin-3-ol (8-aza-MT-DIA, 44)[§]

The diastereomeric mixture of **43** (231mg, 0.610mmol) was dissolved in methanol (10mL) and treated with hydrochloric acid (conc., 0.78mL). The reaction mixture was concentrated from toluene and isopropyl alcohol after 3h. The residue was dissolved in methanol and neutralized with Amberlyst 21. The ion exchange resin was filtered off and the filtrate was concentrated to give the crude product (204mg) as brown oil. The material was purified by column chromatography to afford product **44** (98mg, 0.33mmol) as white solid.



MW: 294.38g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 20%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 5%→20%
yield: 54%

^{1}H NMR (CD_3OD center line 3.31): δ = 8.21 (s, 1H), 4.03 (d, *J*=13.8Hz, 1H), 4.00 (d, *J*=13.8Hz, 1H), 3.96 (ddd, *J*=4.1, 6.3, 4.1Hz, 1H), 3.09 (dd, *J*=7.9, 9.6Hz, 1H), 2.90 (dd, *J*=10.2, 6.3Hz, 1H), 2.70 (dd, *J*=10.2, 4.0Hz, 1H), 2.66 (dd, *J*=12.9, 6.4Hz, 1H), 2.47 (dd, *J*=12.9, 8.9Hz, 1H), 2.41 (dd, *J*=9.7, 6.8Hz, 1H), 2.25-2.17 (m, 1H), 2.06 (s, 3H).

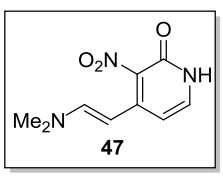
^{13}C NMR (CD_3OD center line 49.0): δ = 154.1 (C), 152.9 (CH), 140.5 (C), 140.0 (C), 125.6 (C), 76.9 (CH), 62.6 (CH_2), 59.1 (CH_2), 49.6 (CH_2), 48.2 (CH), 38.0 (CH_2), 15.5 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{12}H_{19}N_6OS$ (MH^+) calcd 295.1341, found 295.1336.

5.2.3. Synthesis of 3-deaza-MT-DIA (51)

(*E*)-4-(2-(Dimethylamino)vinyl)-3-nitropyridin-2(1*H*)-one (47)[§]

Commercial available 4-methyl-3-nitro-pyridin-2-ol (**45**, 1.64g, 10.6mmol) in DMF (60mL) was treated with Bredereck's reagent (**46**, 6.25mL, 773mmol) and heated to 100°C for 20h. The product was precipitated by the addition of ice and water (150mL) and filtered off. The crude compound **47** (1.61g, 72%) was obtained as orange solid, which showed sufficient purity by NMR and was used in the next step without further purification.



MW: 209.21g/mol
TLC-solvent: EA/MeOH: 10%
TLC-detection: UV
CC-solvent:-
yield: 72%

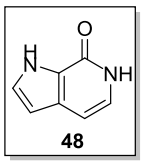
^{1}H NMR ($(CD_3)_2SO$ at 2.50): δ = 11.32 (bs, 1H), 7.66 (d, *J*=13.0Hz, 1H), 7.16 (d, *J*=7.3Hz, 1H), 6.47 (d, *J*=7.4Hz, 1H), 4.77 (d, *J*=13.0Hz, 1H), 2.94 (bs, 6H).

^{13}C NMR ($(CD_3)_2SO$ center line 39.5): δ = 155.4 (C), 150.0 (CH), 144.5 (C), 134.0 (CH), 133.1 (C), 99.1 (CH), 85.0 (CH).

MS: ESI-HRMS (TOF) *m/z* for $C_9H_{11}N_3O_3Na$ (MNa^+) calcd 232.0698, found 232.0692.

1,6-Dihydro-7*H*-pyrrolo[2,3-c]pyridin-7-one (48)[§]

Substrate **47** (1.57g, 7.50mmol) in acetic acid (100mL) was treated with zinc dust (2.48g, 37.2mmol). The reaction mixture was filtered and the filtrate concentrated after 1.5h to give the crude product (2.8g) as green residue. The material was purified by column chromatography to afford product **48** (769mg, 5.73mmol) as powder.



48

MW: 134.14g/mol
TLC-solvent: EA/MeOH: 10%
TLC-detection: UV, Ehrlich's (red spot)
CC-solvent: EA/MeOH: 5%
yield: 76%

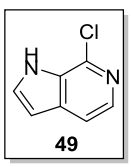
^{1}H NMR ($(CD_3)_2SO$ at 2.50): δ = 11.93 (bs, 1H), 10.88 (bs, 1H), 7.27 (d, *J*=2.6Hz, 1H), 6.86 (d, *J*=7.0Hz, 1H), 6.45 (d, *J*=6.9Hz, 1H), 6.30 (d, *J*=2.7Hz, 1H).

^{13}C NMR ($(CD_3)_2SO$ center line 39.5): δ = 155.0 (C), 130.3 (C), 126.4 (CH), 124.3, (CH), 123.9 (C), 102.7 (CH), 100.6 (CH).

MS: ESI-HRMS (TOF) *m/z* for $C_7H_7N_2O$ (MH^+) calcd 135.0558, found 135.0556.

7-Chloro-1*H*-pyrrolo[2,3-c]pyridine (49)[§]

Compound **48** (41mg, 0.31mmol) was dissolved in phosphoryl chloride (1.00mL, 10.7mmol) and heated to 100°C. All volatiles were evaporated after 2.5h and the white, solid residue was partitioned between sodium hydroxide (aq., 0.2M, 11mL) and chloroform (3x10mL). Combined organic layers were dried and concentrated to the give crude product (35mg) as white solid. The material was subject to silica filtration to afford product **49** (26mg, 0.17mmol) as powder.



49

MW: 152.58g/mol
TLC-solvent: EA
TLC-detection: UV
CC-solvent: EA
yield: 56%

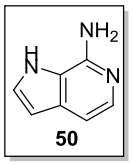
^{1}H NMR (CD_3OD center line 3.31): δ = 7.85 (d, *J*=5.5Hz, 1H), 7.56 (d, *J*=3.2Hz, 1H), 7.55 (d, *J*=5.4Hz, 1H), 6.63 (d, *J*=3.2Hz, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 137.7 (CH), 136.6 (C), 135.3 (C), 131.6 (CH), 131.3 (C), 116.2 (CH), 103.5 (CH).

MS: ESI-HRMS (TOF) *m/z* for $C_7H_6N_2{}^{35}Cl$ (MH^+) calcd 153.0220, found 153.0224.

1*H*-Pyrrolo[2,3-c]pyridin-7-amine (50)[§]

Chloride **49** (19mg, 0.12mmol) was dissolved in ammonia (aq., 2.8mL, 25mass%) and heated with copper(I) chloride (22mg, 0.22mmol) and copper dust (6.4mg, 0.10mmol) at 120°C for 17h in a sealed pressure vessel. The reaction mixture was filtrated through sinter, washed with water (10mL), the filtrate extracted with ethyl acetate (3x25mL) and the combined organic layers were dried and concentrated to give the crude product (21mg). The material was purified by column chromatography to afford product **50** (16mg, 0.12mmol) as grey powder.



50

MW: 133.15g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: UV, (Ehrlich's)
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
yield: 96%

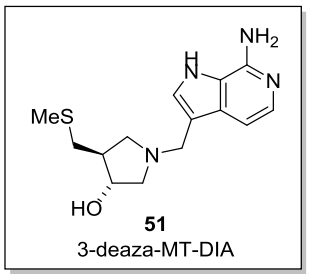
^{1}H NMR (CD_3OD center line 3.31): δ = 7.45 (d, *J*=5.8Hz, 1H), 7.33 (d, *J*=3.0Hz, 1H), 6.90 (d, *J*=5.8Hz, 1H), 6.40 (d, *J*=3.0Hz, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 147.4 (C), 135.8 (CH), 134.4 (C), 127.9 (CH), 121.7 (C), 108.0 (CH), 102.9 (CH).

MS: ESI-HRMS (TOF) *m/z* for $C_7H_8N_3$ (MH^+) calcd 134.0718, found 134.0716.

(3*R*,4*S*)-1-((7-Amino-1*H*-pyrrolo[2,3-c]pyridin-3-yl)methyl)-4-((methylthio)methyl)pyrrolidin-3-ol (51)[§]

The Mannich coupling of pyrrolidine **4** (27.3 mg, 0.185 mmol) and enamine **50** (21.9mg, 0.164mmol) was carried out as described above (General Synthetic Procedures) in water (0.5mL) and ethanol (0.5mL) with formaldehyde (37%aq. solution, 15mg, mmol) at 80°C for 6h. The crude product was purified by column chromatography twice to afford product **51** (25mg, 85µmol) as colorless oil.



51
3-deaza-MT-DIA

MW: 292.40g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%
$iPrOH/NH_3$(aq., 25mass%): 0%→2.5%
yield: 52%

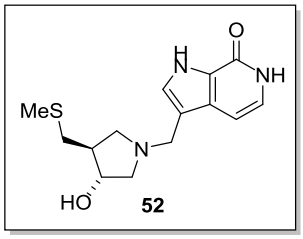
^{1}H NMR (CD_3OD center line 3.31): δ = 7.48 (d, *J*=6.1Hz, 1H), 7. 46 (s, 1H), 7.07 (d, *J*=6.1Hz, 1H), 4.00 (ddd, *J*=4.3, 6.4, 4.3Hz, 1H), 3.85 (d, *J*=13.5Hz, 1H), 3.79 (d, *J*=13.5Hz, 1H), 3.04 (dd, *J*=10.0, 7.9Hz, 1H), 2.89 (dd, *J*=10.4, 6.5Hz, 1H), 2.72-2.65 (m, 2H), 2.48 (dd, *J*=12.9, 9.1Hz, 1H), 2.42 (dd, *J*=10.0, 7.0Hz, 1H), 2.28-2.20 (m, 1H), 2.07 (s, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 146.8 (C), 134.1 (C), 133.5 (CH), 129.6 (CH), 121.0 (C), 113.0 (C), 106.6 (CH), 76.5 (CH), 62.2 (CH_2), 58.9 (CH_2), 50.4 (CH_2), 47.8 (CH), 37.8 (CH_2), 15.5 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{14}H_{21}N_4OS$ (MH^+) calcd 293.1436, found 293.1430.

3-(((3*R*,4*S*)-3-Hydroxy-4-((methylthio)methyl)pyrrolidin-1-yl)methyl)-1,6-dihydro-7*H*-pyrrolo[2,3-c]pyridin-7-one (52)[§]

The Mannich coupling of pyrrolidine **4** (101 mg, 0.686 mmol) and enamine **48** (77mg, 0.574mmol) was carried out as described above (General Synthetic Procedures) in water (2mL) and ethanol (2mL) with formaldehyde (37%aq. solution, 65mg, 0.80mmol) at 80°C for 3h. The crude product was purified by column chromatography to afford product **52** (77mg, 0.26mmol).



52

MW: 293.39g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 20%
TLC-detection: UV, Ehrlich's
CC-solvent: iPrOH/NH_3(aq., 25mass%): 0%→5%
yield: 45%

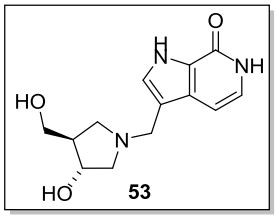
^{1}H NMR (CD_3OD center line 3.31): δ = 7.34 (s, 1H), 6.99 (d, *J*=6.9Hz, 1H), 6.78 (d, *J*=6.9Hz, 1H), 3.98 (ddd, *J*=4.1, 6.3, 4.1Hz, 1H), 3.75 (d, *J*=13.4Hz, 1H), 3.70 (d, *J*=13.4Hz, 1H), 3.00 (dd, *J*=9.6, 8.3Hz, 1H), 2.82 (dd, *J*=10.2, 6.4Hz, 1H), 2.68 (dd, *J*=12.8, 6.3Hz, 1H), 2.62 (dd, *J*=10.2, 4.1, 1H), 2.48 (dd, *J*=12.9, 9.0, 1H), 2.35 (dd, 9.8, 7.0, 1H), 2.26-2.18 (m, 1H), 2.06 (s, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 157.3 (C), 133.4 (C), 128.9 (CH), 125.0 (CH), 124.8 (C), 114.6 (C), 102.8 (CH), 76.8 (CH), 62.6 (CH_2), 59.1 (CH_2), 50.8 (CH_2), 48.1 (CH), 38.1 (CH_2), 15.6 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{14}H_{20}N_3O_2S$ (MH^+) calcd 294.1276, found 294.1271.

3-(((3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidin-1-yl)methyl)-1,6-dihydro-7*H*-pyrrolo[2,3-c]pyridin-7-one (53)[§]

The Mannich coupling of pyrrolidine **12** (119 mg, 1.02 mmol) and enamine **48** (164mg, 1.22mmol) was carried out as described above (General Synthetic Procedures) in water (3mL) and ethanol (3mL) with formaldehyde (37%aq. solution, 108mg, 1.33mmol) in the microwave at 100°C for 2h. Product **53** (190mg, 0.710mmol) was obtained after purification by column chromatography.



53

MW: 263.30g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%→30%
yield: 71%

^{1}H NMR (CD_3OD center line 3.31): δ = 7.55 (s, 1H), 7.06 (d, *J*=7.1Hz, 1H), 6.84 (d, *J*=7.0Hz, 1H), 4.30 (d, *J*=13.7Hz, 1H), 4.25 (d, *J*=13.8Hz, 1H) overlaps with 4.24 (ddd, *J*=3.2, 5.8, 3.2Hz, 1H), 3.63 (dd, *J*=10.1, 5.4Hz, 1H) overlaps with 3.59 (dd, *J*=10.9, 6.1Hz, 1H), 3.45 (dd, *J*=11.1, 8.3Hz, 1H), 3.25 (dd, *J*=11.4, 5.7Hz, 1H), 3.08 (dd, *J*=11.2, 2.8Hz, 1H), 2.97 (dd, *J*=11.1, 6.8Hz, 1H), 2.41-2.34 (m, 1H).

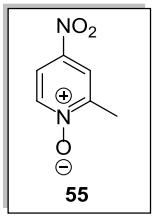
^{13}C NMR (CD_3OD center line 49.0): δ = 157.2 (C), 133.3 (C), 130.6 (CH), 126.1 (CH), 125.2 (C), 109.8 (C), 102.0 (CH), 73.2 (CH), 62.6 (CH_2), 61.6 (CH_2), 55.9 (CH_2), 50.5 (CH_2), 50.3 (CH).

MS: ESI-HRMS (TOF) *m/z* for $C_{13}H_{18}N_3O_3$ (MH^+) calcd 264.1348, found 264.1344.

5.2.4. Synthesis of 1-deaza-MT-DIA (66)

2-Methyl-4-nitropyridine 1-oxide (55)[§]

Commercial picoline *N*-oxide (**54**, 498mg, 4.47mmol) in sulfuric acid (95mass%, 4mL) and nitric acid (69mass%, 4mL) was heated to 160°C. After 2h the reaction mixture was poured into ice water (15mL) and extracted with chloroform (3x10mL). Combined organic layers were dried and concentrated to give the crude product (502mg) as yellow solid. The material was purified by column chromatography to afford product **55** (458mg, 2.97mmol) as yellow solid.



55

MW: 154.13g/mol
TLC-solvent: $CHCl_3$/MeOH: 10%
TLC-detection: UV
CC-solvent: $CHCl_3$/MeOH: 0.5%
yield: 66%

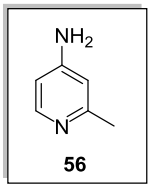
^{1}H NMR ($CDCl_3$ at 7.26): δ = 8.29 (d, *J*=7.2Hz, 1H), 8.12 (d, *J*=3.1Hz, 1H), 7.97 (dd, *J*=7.2, 3.2Hz, 1H), 2.55 (s, 3H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 150.7 (C), 141.8 (C), 140.1 (CH), 120.7 (CH), 118.2 (CH), 18.1 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_6H_7N_2O_3$ (MH^+) calcd 155.0457, found 155.0460.

2-Methylpyridin-4-amine (56)[§]

4-Nitro-2-picoline *N*-oxide (**55**, 1.74g, 11.3mmol) in acetic acid (100mL) was treated with iron dust (6.18g, 110mmol) and heated to 100°C for 3h. The reaction mixture was filtrated, washed with ethyl acetate and the filtrate was concentrated. The residue was partitioned between sodium hydroxide (aq. 2M, 50% sat. with sodium chloride, 100mL) and chloroform (5x200mL). The combined organic layers were dried and concentrated to give the crude product as white solid. The material was purified by column chromatography to afford product **56** (1.13g, 10.5mmol) as white crystals.



56

MW: 108.14g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%
TLC-detection: UV
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→10%
yield: 93%

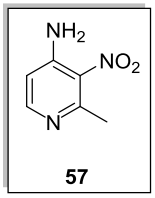
^{1}H NMR (CD_3OD center line 3.31): δ = 7.83 (d, *J*=5.8Hz, 1H), 6.42 (d, *J*=2.2Hz, 1H), 6.38 (dd, *J*=5.9, 2.3Hz, 1H), 2.29 (s, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 158.7 (C), 157.2 (C), 149.1 (CH), 109.3 (CH), 108.0 (CH), 23.5 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_6H_9N_2O$ (MH^+) calcd 109.0766, found 109.0759.

2-Methyl-3-nitropyridin-4-amine (57)[§]

4-Amino-2-picoline (**56**, 1.96g, 18.6mmol) in sulfuric acid (20mL, 95mass%) and nitric acid (1.47mL, 22.9mmol, 69mass%) was heated to 65°C. After exactly 3h the reaction mixture was diluted with ice water (50mL), neutralized with sodium hydroxide (0.9g) and extracted with chloroform (3x500mL). Combined organic layers dried and concentrated to give the crude product (2g) as yellow solid. The material was purified by multiple column chromatographies and recrystallization from boiling toluene to afford product **57** (1.05g, 6.88mmol) as yellow crystals.



57

MW: 153.14g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: UV
CC-solvent: PE/EA: 15%→100%
yield: 37%

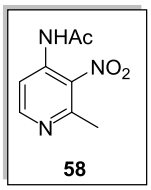
^{1}H NMR (CD_3OD center line 3.31): δ = 7.89 (d, *J*=6.1Hz, 1H), 6.74 (d, 6.1Hz, 1H), 2.56 (s, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 156.1 (C), 151.0 (C), 149.6 (CH), 133.7 (C), 112.0 (CH), 23.5 (CH_3).

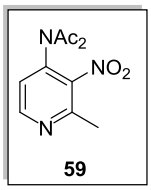
MS: ESI-HRMS (TOF) *m/z* for $C_6H_8N_3O_2$ (MH^+) calcd 154.0617, found 154.0614.

Mixture of *N*-(2-Methyl-3-nitropyridin-4-yl)acetamide (58) and *N*-acetyl-N-(2-methyl-3-nitropyridin-4-yl)acetamide (59)[§]

Amine **57** (47mg, 0.307mmol) in toluene (1mL) was refluxed with acetic anhydride (0.28mL, 2.96mmol) over night. The reaction mixture was concentrated and the residue partitioned between sodium carbonate (sat. aq., 10mL) and chloroform (3x25mL). The combined organic layers were dried and concentrated to give a mixture of the desired mono- (**58**) and the bis- (**59**) acetamide in a ratio: 10/7 by NMR as brown oil (59mg, **58**: 0.16mmol, **59**: 0.11mmol). The compounds could not be separated by chromatography and therefore were used as mixture for the next step.



58



59

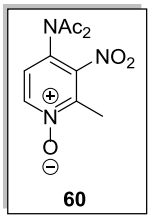
MW **58**: 195.18g/mol; **59**: 237.22g/mol
TLC-solvent: EA
TLC-detection: UV
CC-solvent:-
yield **58**: 53%; **59**: 37% (calc. from NMR-ratio)

^{1}H NMR ($CDCl_3$ at 7.26) and ^{13}C NMR ($CDCl_3$ center line 77.2) indicate a mixture of mono- (**58**) and the bis- (**59**) acetamide free of other impurities.

MS: ESI-HRMS (TOF) *m/z* for $C_8H_{10}N_3O_3$ (mono, MH^+) calcd 196.0722, found 196.0723. ESI-HRMS (TOF) *m/z* for $C_{10}H_{11}N_3O_4Na$ (bis, MNa^+) calcd 260.0647, found 260.0650.

4-(*N*-Acetylacetamido)-2-methyl-3-nitropyridine 1-oxide (60)[§]

A mixture (266mg) of compounds **58** (0.920mmol) and **59** (0.365mmol) in chloroform (2mL) was treated with mCPBA (927mg, 3.23mmol) over night. The reaction mixture was quenched with sodium bicarbonate (sat. aq., 15mL) and extracted with chloroform (5x70mL). The combined organic layers were dried and concentrated to give the crude product mixture (370mg) of **60** and **61** as orange oil. The material was purified by column chromatography to afford products **60** (44mg, 0.17mmol) and **61** (81mg, 0.38mmol) both as yellow solids.

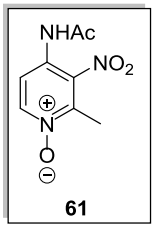


MW: 253.21g/mol
TLC-solvent: $CHCl_3$/MeOH: 10%
TLC-detection: UV
CC-solvent: PE/acetone: 20%→100%
yield: 48%

^{1}H NMR ($CDCl_3$ at 7.26): δ = 8.42 (d, *J*=7.0Hz, 1H), 7.12 (d, *J*=7.0Hz, 1H), 2.52 (s, 3H), 2.34 (s, 6H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 171.2 (2xC), 147.5 (C), 145.8 (C), 141.8 (CH), 127.2 (C), 124.7 (CH), 26.3 (2xCH_3), 14.1 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{10}H_{12}N_3O_5$ (MH^+) calcd 254.0777, found 254.0776.



MW: 211.18g/mol
TLC-solvent: PE
TLC-detection: UV, Ehrlich's
CC-solvent: PE/acetone: 20%→100%
yield: 42%

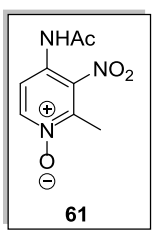
^{1}H NMR (CD_3OD center line 3.31): δ = 8.31 (d, *J*=7.3Hz, 1H), 8.05 (d, 7.8Hz, 1H), 2.51 (s, 3H), 2.17 (s, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 171.0 (C), 145.2 (C), 141.4 (CH), 141.0 (C), 130.6 (C), 118.2 (C), 23.8 (CH_3), 14.2 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_8H_{10}N_3O_4$ (MH^+) calcd 212.0671, found 212.0665.

4-Acetamido-2-methyl-3-nitropyridine 1-oxide (61) from 60[§]

Bis-acetamide **60** (112mg, 0.442mmol) in methanol (10mL) was treated with sodium methoxide (30mass% in methanol, 39mg, 0.22mmol). The reaction was quenched with brine (10mL) and extracted with chloroform (3x90mL) after 5min. The combined organic layers were dried and concentrated to give the crude product as orange oil. The material was purified by column chromatography to afford product **61** (81mg, 0.38mmol) as orange powder.



MW: 211.18g/mol
TLC-solvent: acetone
TLC-detection: UV
CC-solvent: PE/acetone: 20%→100%
yield: 87%

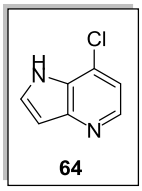
^{1}H NMR (CD_3OD center line 3.31): δ = 8.31 (d, *J*=7.3Hz, 1H), 8.05 (d, 7.8Hz, 1H), 2.51 (s, 3H), 2.17 (s, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 171.0 (C), 145.2 (C), 141.4 (CH), 141.0 (C), 130.6 (C), 118.2 (C), 23.8 (CH_3), 14.2 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_8H_{10}N_3O_4$ (MH^+) calcd 212.0671, found 212.0667.

7-Chloro-1*H*-pyrrolo[3,2-b]pyridine (64)‡

Commercial 4-chloro-3-nitro-pyridine (**63**, 554mg, 3.14mmol) in THF (20mL) was cooled to -50°C, treated with vinylmagnesium bromide (0.7M in THF, 14.5mL, 10.1mmol) and warmed to -20°C over night. The reaction was quenched with ammonium chloride (aq., 20mass%, 15mL) and extracted with ethyl acetate (3x30mL). The combined organic layers were dried and concentrated to give the crude product (0.7g) as red oil. The material was purified by column chromatography to afford product **64** (110mg, 721µmol) as red powder.



64

MW: 152.58g/mol
TLC-solvent: C/EA: 50%
TLC-detection: UV, (Ehrlich's)
CC-solvent: C/EA: 25%
yield: 23%

^{1}H NMR (CD_3OD center line 3.31): δ = 8.21 (d, *J*=5.2Hz, 1H), 7.63 (d, *J*=3.2Hz, 1H), 7.21 (d, *J*=5.2Hz, 1H), 6.66 (d, *J*=3.2Hz, 1H).

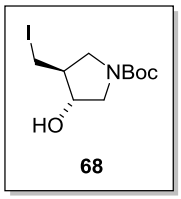
^{13}C NMR (CD_3OD center line 49.0): δ = 148.4 (C), 143.5 (CH), 131.4 (CH), 128.0 (C), 117.4 (CH), 114.8 (C), 103.3 (CH).

MS: GC-EIMS *m/z* for $C_7H_5ClN_2$ (M^+) calcd 152.01, found 152.01.

5.2.5. Synthesis of MT-DIA 5'-S Derivatives

***tert*-Butyl-(3*R*,4*S*)-3-hydroxy-4-(iodomethyl)pyrrolidine-1-carboxylate (68)§**

Diol **13** (26.2g, 121mmol) in dichloromethane (200mL) was treated with triphenylphosphine (44.7g, 169mmol) and imidazole (11.6g, 169mmol) at 0°C. Iodine (36.8g, 145mmol) was added in portions. The reaction was quenched with sodium metabisulphite (aq., 2.4M, 200mL) after 3 days at 0°C. The organic layer was separated, washed with water (2x150mL), dried and concentrated to give the crude product (107g) as a solid. The material was purified by column chromatography to afford product **68** (29.0g, 88.6mol) as yellow oil.



68

MW: 327.16g/mol
TLC-solvent: $CHCl_3$/MeOH: 10%
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 25%
yield: 73%

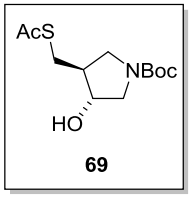
^{1}H NMR ($CDCl_3$ at 7.26): δ = 4.21-4.03 (bm, 1H), 3.78-3.58 (bm, 2H), 3.34-3.21 (bm, 2H), 3.21-3.09 (bm, 2H), 2.74, 2.65 (bs, 1H), 2.42-2.27 (bm, 1H), 1.46 (s,9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.6 (C), 80.0 (C), 75.3, 74.4 (CH), 52.8, 52.5 (CH_2), 50.6, 50.2 (CH_2), 48.6, 47.9 (CH), 28.6 (CH_3), 5.4 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{10}H_{18}INO_3Na$ (MNa^+) calcd 350.0229, found 350.0229.

tert-Butyl-(3*S*,4*R*)-3-((acetylthio)methyl)-4-hydroxypyrrolidine-1-carboxylate (69)[§]

Iodine **68** (258mg, 749µmol) in DMF (5mL) was treated with potassium thioacetate (257mg, 2.25mmol) at 0°C. After reaction at room temperature over night the mixture was diluted with toluene (45mL) and extracted with water (5x5mL) and brine (5mL). The combined organic layers were dried and concentrated to give the crude product (219mg) as brown oil. The material was purified by column chromatography to afford product **69** (180mg, 654µmol) as pale syrup.



69

MW: 275.36g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 10%→75%
yield: 88%

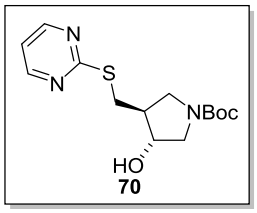
^{1}H NMR ($CDCl_3$ at 7.26): δ = 4.06-3.98 (bm, 1H), 3.72-3.53 (bm, 2H), 3.28-3.17 (bm, 1H), 3.14-3.04 (bm, 1H), 3.04-2.97 (bm, 1H), 2.97-2.88 (bm, 1H), 2.54-2.46 (bm, 1H), 2.36 (s, 3H), 2.34-2.25 (bm, 1H), 1.45 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 196.3 (C), 154.6 (C), 79.8 (C), 73.5, 73.0 (CH), 52.1, 51.9 (CH_2), 48.7, 48.2 (CH_2), 46.2, 45.6 (CH), 30.7 (CH_3), 29.4, 29.3 (CH_2), 28.6 (3xCH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{12}H_{21}NO_4SNa$ (MNa^+) calcd 298.1089, found 298.1087.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-((pyrimidin-2-ylthio)methyl)pyrrolidine-1-carboxylate (70)[§]

The thioether formation from thioacetate ester **69** (190mg, 655µmol) with sodium methoxide (0.750mmol) and 2-chloropyrimidine (104mg, 863µmol) was carried out as described above (General Synthetic Procedures) in methanol (5mL). Product **70** (135mg, 434µmol) was obtained as pale syrup after purification by column chromatography.



70

MW: 311.40g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: UV, Ehrlich's
CC-solvent: PE/EA: 20%→100%
yield: 66%

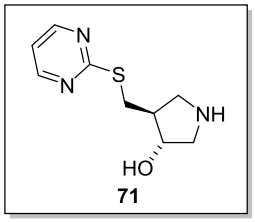
^{1}H NMR ($CDCl_3$ at 7.26): δ = 8.51 (d, *J*=4.9Hz, 2H), 7.00 (t, *J*=4.81Hz, 1H), 4.22-4.12 (bm, 1H), 3.75-3.58 (bm, 2H), 3.42-3.11 (bm, 5H), 2.54-2.42 (bm, 1H), 1.43 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 172.3 (C), 157.5 (2xCH), 154.7 (C), 116.9 (CH), 79.6 (C), 73.2, 72.7 (CH), 51.9, 51.7 (CH_2), 48.9, 48.3 (CH_2), 46, 45.5 (CH), 31.0, 30.8 (CH_2), 28.6 ($3xCH_3$).

MS: ESI-HRMS (TOF) *m/z* for $C_{14}H_{21}N_3O_3NaS$ (MNa^+) calcd 334.1201, found 334.1199.

(3*R*,4*S*)-4-((Pyrimidin-2-ylthio)methyl)pyrrolidin-3-ol (71)[§]

Boc deprotection of substrate **70** (130mg, 0.418mmol) was carried out as described above (General Synthetic Procedures). Crude product **71** was obtained as pale oil in quantitative yield (93mg, 0.44mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



71

MW: 211.28g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: UV, Ehrlich's
CC-solvent: -
yield: >99%

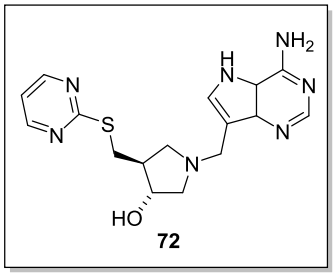
^{1}H NMR (CD_3OD center line 3.31): δ = 8.59 (d, *J*=4.8Hz, 2H), 7.18 (t, *J*=4.9Hz, 1H), 4.37 (ddd, *J*=2.9, 5.4, 2.9Hz, 1H), 3.60 (dd, 12.0, 7.5Hz, 1H), 3.51 (dd, *J*=12.3, 4.7Hz, 1H), 3.39 (dd, *J*=14.3, 7.3Hz, 1H), 3.26-3.20 (m, 2H), 3.17 (dd, *J*=14.1, 8.1Hz, 1H), 2.74-2.66 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 172.4 (C), 159.0 (2xCH), 118.5 (CH), 74.6 (CH), 52.7 (CH_2), 49.4 (CH_2), 47.8 (CH), 31.9 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_9H_{14}N_3OS$ (MH^+) calcd 212.0858, found 212.0858.

(3*R*,4*S*)-1-((4-Amino-4a,7a-dihydro-5H-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((pyrimidin-2-ylthio)methyl)pyrrolidin-3-ol (72)[§]

The Mannich coupling of pyrrolidine **71** (85mg, 0.38mmol) and 9-deaza-adenine (**5**, 65mg, 0.49mmol) was carried out as described above (General Synthetic Procedures) in water (2mL) and ethanol (2mL) with formaldehyde (37%aq. solution, 37mg, 0.46mmol) in the microwave at 70°C for 2h. Product **72** (74mg, 0.21mmol, >95% purity and 32mg, 91µmol, ~60% purity) was obtained after purification by column chromatography.



72

MW: 359.45g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%
yield: 55% (+14%, ~60 pure by ^{1}H-NMR)

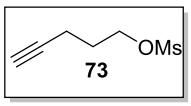
^{1}H NMR (CD_3OD center line 3.31): δ = 8.51 (d, *J*=5.0Hz, 2H), 8.17 (s, 1H), 7.68 (s, 1H), 7.11 (t, *J*=5.0Hz, 1H), 4.31 (bm, 2H), 4.24 (ddd, *J*=3.5, 5.8, 3.5Hz, 1H), 3.55 (dd, *J*=11.4, 7.9Hz, 1H), 3.41-3.34 (m, 2H), 3.19-3.10 (m, 2H), 3.01 (dd, *J*=11.4, 6.9Hz, 1H), 2.65-2.57 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 172.4 (C), 158.7 (2xCH), 152.4 (C), 151.6 (CH), 146.6 (C), 131.8 (CH), 118.3 (CH), 115.4 (C), 107.3 (C), 75.0 (CH), 60.7 (CH_2), 57.3 (CH_2), 49.2 (CH_2), 47.8 (CH), 32.6 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{16}H_{20}N_7OS$ (MH^+) calcd 358.1450, found 358.1443.

Pent-4-yn-1-yl methanesulfonate (73)[§]

Commercial 4-pentyn-1-ol (909mg, 10.5mmol) and triethylamine (2.2mL, 16mmol) were combined with dichloromethane (20mL) and cooled to 0°C. Methanesulfonyl chloride (1mL, 12.7mmol) was added and after 10min the reaction mixture was warmed to room temperature. After 2 days the reaction mixture was quenched by addition of water (20mL) and extracted with dichloromethane (20mL). Combined organic layers washed with sodium bicarbonate (sat. aq., 20mL), dried and concentrated to afford crude product **73** (1.72g, 10.6mmol) in quantitative yield as yellow liquid. The material showed sufficient purity by NMR and was used in the next step without further purification.



73

MW: 162.20g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: $KMnO_4$
CC-solvent: -
yield: >99%

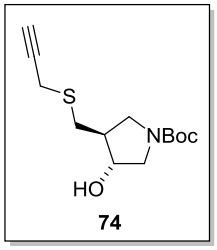
^{1}H NMR ($CDCl_3$ at 7.26): δ = 4.35 (t, *J*=6.1Hz, 2H), 3.02 (s, 3H), 2.36 (td, *J*=6.9, 2.7Hz, 2H), 2.00 (t, *J*=2.7Hz, 1H), 1.96 (qunit, *J*=6.58Hz, 2H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 82.2 (C), 69.9 (CH), 68.4 (CH_2), 37.4 (CH_3), 28.0 (CH_2), 14.8 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_6H_{10}O_3NaS$ (MNa^+) calcd 185.0248, found 185.0251.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-((prop-2-yn-1-ylthio)methyl)pyrrolidine-1-carboxylate (74)[§]

The thioether formation from thioacetate ester **69** (5.01mg, 17.3mmol) with sodium methoxide (19mmol) and propargyl bromide (80mass% in toluene, 2.3mL, 21mmol) was carried out as described above (General Synthetic Procedures) in methanol (25mL). Product **74** (4.44g, 16.4mmol) was obtained as pale syrup after purification by column chromatography.



74

MW: 271.38g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: Ehrlich's, $KMnO_4$
CC-solvent: PE/EA: 5%→75%
yield: 95%

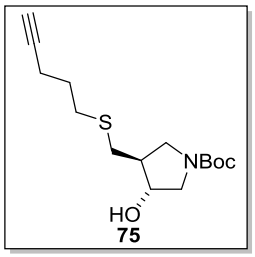
^{1}H NMR ($CDCl_3$ at 7.26): δ = 4.25-4.16 (bs, 1H), 3.74-3.60 (bm, 2H), 3.33-3.23 (m, 3H), 3.21-3.15 (bm, 1H), 2.81-2.65 (bm, 2H), 2.40-2.29 (bm, 2H), 2.27 (t, *J*=2.6Hz, 1H), 1.45 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.7 (C), 79.8 (C), 79.6 (C), 75.0, 74.3 (CH), 71.8 (CH), 52.7, 52.4 (CH_2), 49.3, 49.0 (CH_2), 45.5, 44.8 (CH), 33.1 (CH_2), 28.6 (3xCH_3), 19.8 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{13}H_{21}NO_3NaS$ (MNa^+) calcd 294.1140, found 294.1134.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-((pent-4-yn-1-ylthio)methyl)pyrrolidine-1-carboxylate (75)[§]

The thioether formation from thioacetate ester **69** (1.88mg, 6.48mmol) with sodium methoxide (6.89mmol) and mesylate **73** (1.41mg, 7.60mmol) was carried out as described above (General Synthetic Procedures) in methanol (10mL). Product **75** (1.23g, 4.42mmol) was obtained as pale syrup after purification by column chromatography.



75

MW: 299.43g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: Ehrlich's, $KMnO_4$
CC-solvent: PE/EA: 10%→30%
yield: 68%

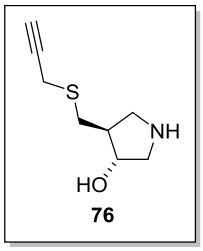
^{1}H NMR ($CDCl_3$ at 7.26): δ = 4.17 (bs, 1H), 3.72-3.59 (bm, 2H), 3.29-3.18 (bm, 1H), 3.13 (dd, *J*=11.2, 6.5Hz, 1H), 2.66 (t, *J*=7.3Hz, 2H), 2.63-2.48 (bm, 3H), 2.32 (td, *J*=6.9, 2.6Hz, 2H), 2.28 (bs, 1H), 1.97 (t, *J*=2.7Hz, 1H), 1.80 (pent, *J*=7.0Hz, 2H), 1.44 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.7 (C), 83.4 (C), 79.7 (C), 75.1, 74.3 (CH), 69.3 (CH), 52.6, 52.4 (CH_2), 49.4, 49.1 (CH_2), 45.9, 45.1 (CH), 33.7 (CH_2), 31.3 (CH_2), 28.6 (3xCH_3), 28.3 (CH_2), 17.6 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{15}H_{25}NO_3NaS$ (MNa^+) calcd 322.1453, found 322.1444.

(3*R*,4*S*)-4-((Prop-2-yn-1-ylthio)methyl)pyrrolidin-3-ol (76)[§]

Boc deprotection of substrate **74** (145mg, 508µmol) was carried out as described above (General Synthetic Procedures). The crude material was purified by column chromatography to afford product **76** (60mg, 129µmol) as yellow oil.



76

MW: 271.26g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 20%
TLC-detection: Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%→20%
yield: 69%

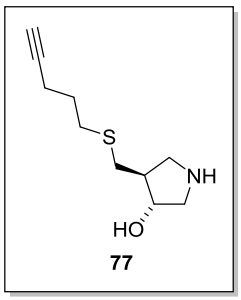
^{1}H NMR (CD_3OD center line 3.31): δ = 4.22 (ddd, *J*=3.2, 5.3, 3.2Hz, 1H), 3.47 (dd, *J*=11.8, 7.5Hz, 1H), 3.34 (d, *J*=2.3Hz, 2H), 3.27 (dd, *J*=12.2, 5.2Hz, 1H), 3.04 (dd, *J*=12.2, 2.9Hz, 1H), 3.00 (dd, *J*=11.8, 5.6Hz, 1H), 2.86 (dd, *J*=13.3, 6.7Hz, 1H), 2.63 (dd, *J*=13.3, 8.8Hz, 1H) overlaps with 2.62 (t, *J*=2.5Hz, 1H), 2.45-2.38 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 80.8 (C), 75.8 (CH), 72.6 (CH), 53.3 (CH_2), 50.0 (CH_2), 47.6 (CH), 33.7 (CH_2), 19.8 (CH_2).

MS: ESI-MS (TOF) *m/z* for $C_8H_{14}NO_3S$ (MH^+) calcd 172.08, found 172.1.

(3*R*,4*S*)-4-((Pent-4-yn-1-ylthio)methyl)pyrrolidin-3-ol (77)[§]

Boc deprotection of substrate **75** (108mg, 361µmol) was carried out as described above (General Synthetic Procedures). The crude material was purified by column chromatography to afford product **77** (35mg, 176µmol) as pale solid.



77

MW: 199.31g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 20%
TLC-detection: Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→20%
yield: 48%

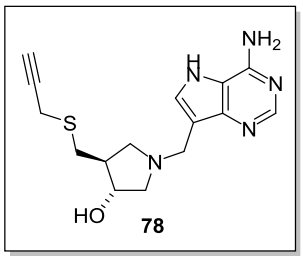
^{1}H NMR (CD_3OD center line 3.31): δ = 4.06 (ddd, *J*=3.4, 6.9, 3.4Hz, 1H), 3.29-3.23 (bm, 1H), 3.08-3.01 (bm, 1H), 2.84-2.78 (bm, 1H), 2.72-2.64 (m, 4H), 2.47 (dd, *J*=12.9, 8.8Hz, 1H), 2.31 (td, *J*=7.0, 2.7Hz, 2H), 2.23 (t, *J*=2.7Hz, 1H), 2.22-2.14 (m, 1H), 1.78 (pent, *J*=7.1Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 84.2 (C), 77.6 (CH), 70.1 (CH), 54.6 (CH_2), 51.3 (CH_2), 49.1 (CH), 34.9 (CH_2), 31.9 (CH_2), 29.6 (CH_2), 18.1 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{10}H_{18}NOS$ (MH^+) calcd 200.1109, found 200.1112.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((prop-2-yn-1-ylthio)methyl)pyrrolidin-3-ol (78)[§]

The Mannich coupling of pyrrolidine **76** (60mg, 0.33mmol) and 9-deaza-adenine (**5**, 42.7mg, 0.318mmol) was carried out as described above (General Synthetic Procedures) in water (2mL) and ethanol (2mL) with formaldehyde (37%aq. solution, 34mg, 0.42mmol) at 80°C for 2h. Product **78** (25mg, 0.079mmol, >95% purity and 45mg, 0.14mmol, ~60% purity by ^{1}H-NMR) was obtained after purification by column chromatography.



78

MW: 317.41g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%
yield: 24% (+42%, ~60% pure by ^{1}H-NMR)

^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.50 (s, 1H), 3.99 (ddd, *J*=4.2, 6.4, 4.2, 1H), 3.87 (d, 13.4Hz, 1H), 3.82 (d, 13.4Hz, 1H), 3.27 (d, *J*=2.6, 2H), 3.07 (dd, 9.8, 8.0Hz, 1H), 2.90-2.85 (m, 2H), 2.70-2.63 (m, 2H), 2.54 (t, *J*=2.6Hz, 1H), 2.41 (dd, *J*=10.0, 7.0Hz, 1H), 2.29-2.19 (m, 1H).

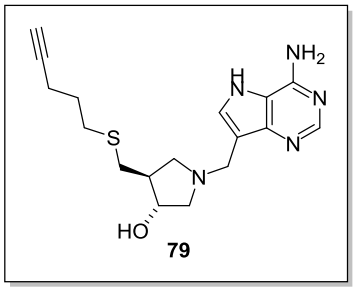
^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.0 (CH), 147.0 (C), 130.1 (CH), 115.15 (C), 112.3 (C), 81.0 (CH), 76.8 (CH), 72.2 (C), 62.3 (CH_2), 58.8 (CH_2), 49.0 (CH_2), 48.0 (CH), 35.3 (CH_2), 19.8 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{15}H_{20}N_5OS$ (MH^+) calcd 318.1389, found 318.1391.

$[\alpha]_D^{20}$: not measured

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((pent-4-yn-1-ylthio)methyl)pyrrolidin-3-ol (79)[§]

The Mannich coupling of pyrrolidine **77** (35mg, 0.18mmol) and 9-deaza-adenine (**5**, 29mg, 0.22mmol) was carried out as described above (General Synthetic Procedures) in water (1mL) and ethanol (1mL) with formaldehyde (37%aq. solution, 25mg, 0.31mmol) in the microwave at 70°C for 2h. Product **79** (38mg, 0.11mmol) was obtained after purification by column chromatography.



79

MW: 345.47g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→20%

yield: 63%

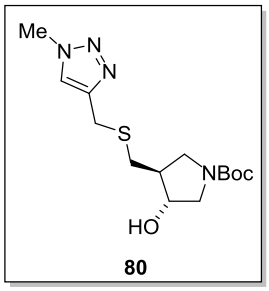
^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.49 (s, 1H), 3.96 (ddd, *J*=4.2, 8.4, 4.2Hz, 1H), 3.86 (d, *J*=13.5Hz, 1H), 3.81 (d, *J*=13.5Hz, 1H), 3.07 (dd, *J*=9.9, 8.1Hz, 1H), 2.86 (dd, *J*=10.3, 6.4Hz, 1H), 2.73 (dd, *J*=12.7, 6.1Hz, 1H), 2.67 (dd, *J*=10.4, 4.2Hz, 1H), 2.60 (t, *J*=7.2Hz, 2H), 2.49 (dd, *J*=12.8, 9.2Hz, 1H), 2.40 (dd, *J*=9.9, 7.1Hz, 1H), 2.27 (td, *J*=7.0, 2.7Hz, 2H), 2.22-2.14 (m, 1H) overlaps with 2.20 (t, *J*=2.6Hz, 1H), 1.73 (pent, *J*=7.1Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.0 (CH), 147.0 (C), 130.1 (CH), 115.2 (C), 112.4 (C), 84.2 (C), 76.8 (CH), 70.0 (C), 62.3 (CH_2), 58.8 (CH_2), 49.0 (CH_2), 48.6 (CH), 35.7 (CH_2), 31.9 (CH_2), 29.6 (CH_2), 18.0 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{17}H_{24}N_5OS$ (MH^+) calcd 346.1702, found 346.1698.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-((((1-methyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidine-1-carboxylate (80)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **74** (198mg, 730µmol), methyl iodide (117mg, 816µmol) and sodium azide (101mg, 1.54mmol) was carried out as described above (General Synthetic Procedures) in methanol (2.5mL). Product **80** (64mg, 0.19mmol) was obtained after purification by column chromatography.



80

MW: 328.43g/mol
TLC-solvent: EA or PE/acetone: 50%
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 50%→EA→acetone
yield: 27%

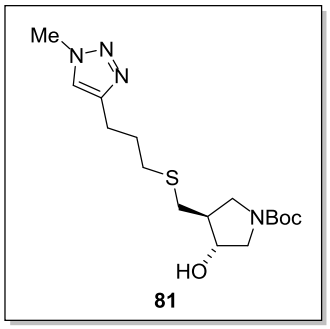
^{1}H NMR (CD_3OD center line 3.31): δ = 7.88 (s, 1H), 4.11-4.04 (bm, 1H) overlaps with 4.09 (s, 3H), 3.88 (s, 2H), 3.63-3.51 (bm, 2H), 3.22-3.14 (bm, 2H), 2.68 (dd, *J*=13.0, 5.5Hz, 1H), 2.46 (dd, *J*=12.6, 8.8Hz, 1H), 2.33-2.24 (bm, 1H), 1.46 (s, 9H).

^{13}C NMR (CD_3OD center line 49.0): δ = 156.8 (C), 146.3 (C), 125.4 (CH), 81.0 (C), 74.9, 74.2 (CH), 53.6, 53.2 (CH_2), 50.2, 49.9 (CH_2), 46.9, 46.3 (CH), 37.2 (CH_3), 34.2 (CH_2), 28.8 (3xCH_3), 27.1 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{14}H_{24}N_4O_3NaS$ (MNa^+) calcd 351.1467, found 351.1460.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-(((3-(1-methyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidine-1-carboxylate (81)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **75** (197mg, 658µmol), methyl iodide (100mg, 698µmol) and sodium azide (89mg, 1.4mmol) was carried out as described above (General Synthetic Procedures) in methanol (2.5mL). Product **81** (153mg, 429µmol) was obtained as syrup after purification by column chromatography.



81

MW: 356.49g/mol
TLC-solvent: EA
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 50%→EA→EA/acetone: 20%
yield: 65%

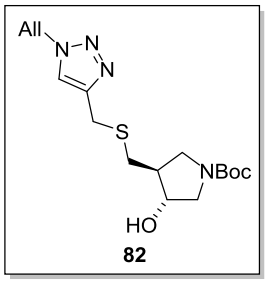
^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.71 (s, 1H), 4.09 (q, *J*=7.4Hz, 1H), 3.59 (dd, *J*=11.2, 7.4Hz, 1H), 3.58-3.52 (m, 1H), 3.24-3.15 (m, 2H), 2.81 (t, *J*=7.4Hz, 2H), 2.69 (dd, *J*=13.0, 6.0Hz, 1H), 2.59 (td, *J*=7.2, 1.0Hz, 2H), 2.43 (dd, *J*=13.0, 9.0Hz, 1H), 2.28-2.20 (m, 1H), 1.95 (pent, *J*=7.3Hz, 2H), 1.45 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 156.6 (C), 148.5 (C), 124.4 (CH), 81.0 (C), 74.9, 74.2 (CH), 53.7, 53.3 (CH_2), 50.3, 49.8 (CH_2), 47.4, 46.8 (CH), 37.0 (CH_3), 34.1 (CH_2), 32.5 (CH_2), 30.3 (CH_2), 28.8 (3xCH_3), 25.1 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{16}H_{28}N_4O_3NaS$ (MNa^+) calcd 379.1780, found 379.1782.

tert-Butyl-(3*R*,4*S*)-3-hydroxy-4-((((1-((*Z*)-prop-1-en-1-yl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidine-1-carboxylate (82)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **74** (43mg, 0.16mmol), allyl bromide (24mg, 0.20mmol) and sodium azide (17mg, 0.26mmol) was carried out as described above (General Synthetic Procedures) in methanol (1.5mL). Product **82** (43mg, 0.12mmol) was obtained as pale syrup after purification by column chromatography.



82

MW: 354.47g/mol
TLC-solvent: PE/EA: 50%
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 50%→100%
yield: 77%

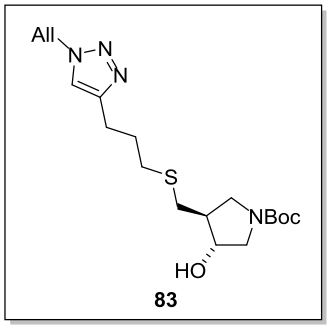
^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.49 (s, 1H), 6.01 (ddt, *J*=16.5, 10.2, 6.3Hz, 1H), 5.36 (dq, *J*=10.3, 1.0Hz, 1H), 5.31 (dq, *J*=17.0, 1.1Hz, 1H), 4.95 (dt, *J*=6.3, 1.2Hz, 2H), 4.18-4.11 (bm, 1H), 3.82 (s, 2H), 3.69-3.57 (bm, 2H), 3.43 (bs, 1H), 3.29-3.17 (bm, 1H), 3.15-3.08 (bm, 1H), 2.67-2.59 (bm, 1H), 2.57-2.49 (bm, 1H), 2.39-2.27 (bm, 1H), 1.44 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.5 (C), 145.6 (C), 131.1 (CH), 121.6 (CH), 120.4 (CH_2), 79.5 (C), 74.3, 73.6 (CH), 52.9 (CH_2), 52.4, 52.1 (CH_2), 49.2, 48.7 (CH_2), 45.6, 45.1 (CH), 32.7 (CH_2), 28.5 (3xCH_3), 26.2(CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{16}H_{27}N_4O_3S$ (MH^+) calcd 355.1804, found 355.1804.

tert-Butyl-(3_R_,4_S_)-3-hydroxy-4-(((3-(1-((_Z_)-prop-1-en-1-yl)-1_H_-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidine-1-carboxylate (83)[§]

Alkyne **75** (208mg, 695µmol) in methanol (2.5mL) under argon atmosphere was treated with sodium azide (57mg, 0.87mmol), allyl bromide (107mg, 876µmol) and a catalytic amount of copper(I) iodide. Additional charges of sodium azide (91mg, 1.4mmol), allyl bromide (0.12mL, 1.4mmol) and a copper(I) iodide were added after 21h and 48h. After 4 days the reaction mixture was heated to 50°C for 4h, adsorbed onto silica and purified by column chromatography. NMR- and MS-analysis was performed on one fraction, but the purity of the obtained product **83** (149mg, 390µmol) was high enough to perform the next step.



83

MW: 382.52g/mol

TLC-solvent: PE/EA: 75%

TLC-detection: Ehrlich's

CC-solvent: PE/acetone: 15%→100%

yield: 56%

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.31 (s, 1H), 6.00 (ddt, *J*=16.5, 10.2, 6.2Hz, 1H), 5.33 (dq, *J*=10.3, 1.0Hz, 1H), 5.28 (dq, *J*=17.1, 1.1Hz, 1H), 4.93 (dt, *J*=6.2, 1.4Hz, 2H), 4.25-4.15 (bm, 1H), 3.70-3.58 (bm, 2H), 3.40 (bs, 1H), 3.27-3.18 (bm, 1H), 3.14-3.08 (bm, 1H), 2.89-2.75 (m, 2H), 2.66-2.47 (bm, 2H) overlaps with 2.58 (t, *J*=7.4Hz, 2H), 2.32-2.22 (bm, 1H), 1.97 (pent, *J*=7.2Hz, 2H), 1.44 (s, 9H).

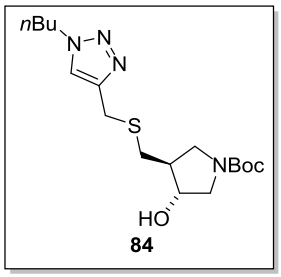
^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.6 (C), 147.3 (C), 131.4 (CH), 120.8 (CH), 120.0 (CH_2), 79.5 (C), 74.4, 73.7 (CH), 52.7 (CH_2), 52.5, 52.2 (CH_2), 49.2, 48.8 (CH_2), 45.7, 45.1 (CH), 33.2 (CH_2), 31.5 (CH_2), 29.0 (CH_2), 28.5 (3xCH_3), 24.3 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{18}H_{30}N_4O_3S$ (MH^+) calcd 383.2117, found 383.2122.

$[\alpha]_D^{20}$: not measured

tert-Butyl-(3_S_,4_R_)-3-((((1-butyl-1_H_-1,2,3-triazol-4-yl)methyl)thio)methyl)-4-hydroxypyrrolidine-1-carboxylate (84)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **74** (352mg, 1.30mmol), 1-bromobutane (0.28mL, 2.6mmol) and sodium azide (186mg, 2.83mmol) was carried out as described above (General Synthetic Procedures) in methanol (6mL). Product **84** (133mg, 359µmol) was obtained as colorless oil after purification by column chromatography.



84

MW: 370.51g/mol

TLC-solvent: PE/EA: 50%

TLC-detection: Ehrlich's

CC-solvent: PE/EA: 50%→100%

yield: 28%

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.46 (s, 1H), 4.33 (t, *J*=7.3Hz, 2H), 4.18-4.12 (bm, 1H), 3.83 (s, 2H), 3.72-3.57 (bm, 2H), 3.27-3.16 (bm, 1H), 3.16-3.08 (bm, 1H), 2.67-2.59 (bm, 1H), 2.59-2.50 (bm, 1H), 2.39-2.28 (bm, 1H), 1.88 (quint, *J*=7.5Hz, 2H), 1.44 (s, 9H), 1.36 (sext, *J*=7.5Hz, 2H), 0.95 (t, *J*=7.4Hz, 3H).

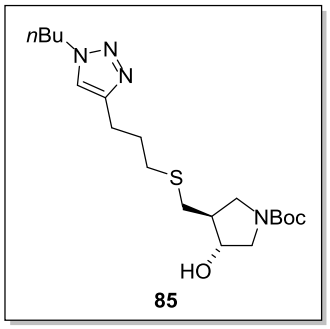
^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.5 (C), 145.3 (C), 121.6 (CH), 79.5 (C), 74.3, 73.7 (CH), 52.4, 52.0 (CH_2), 50.2 (CH_2), 49.2, 48.7 (CH_2), 45.7, 45.1 (CH), 32.6 (CH_2), 32.2 (CH_2), 28.5 (3xCH_3), 26.2 (CH_2), 19.7 (CH_2), 14.1 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{17}H_{30}N_4O_3NaS$ (MNa^+) calcd 393.1936, found 393.1937.

$[\alpha]_D^{20}$: not measured

tert-Butyl-(3*S*,4*R*)-3-(((3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)-4-hydroxypyrrolidine-1-carboxylate (85)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **75** (186mg, 621µmol), 1-bromobutane (0.14mL, 1.3mmol) and sodium azide (105mg, 1.60mmol) was carried out as described above (General Synthetic Procedures) in methanol (2.5mL). Product **85** (95mg, 0.24mmol) was obtained as colorless oil after purification by column chromatography.



85

MW: 398.57g/mol

TLC-solvent: PE/acetone: 50%

TLC-detection: Ehrlich's

CC-solvent: PE/acetone: 20%%→50%

yield: 39%

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.28 (s, 1H), 4.30 (t, *J*=7.2Hz, 2H), 4.24-4.16 (bm, 1H), 3.74-3.57 (bm, 2H), 3.47-3.33 (bm, 1H), 3.29-3.17 (bm, 1H), 3.17-3.07 (bm, 1H), 2.89-2.75 (m, 2H), 2.67-2.47 (bm, 2H) overlaps with 2.59 (t, *J*=7.4Hz, 2H), 2.33-2.22 (bm, 1H), 1.98 (pent, *J*=7.1Hz, 2H), 1.86 (pent, *J*=7.4Hz, 2H), 1.44 (s, 9H), 1.34 (sext, *J*=7.5Hz, 2H), 0.94 (t, *J*=7.4Hz, 3H).

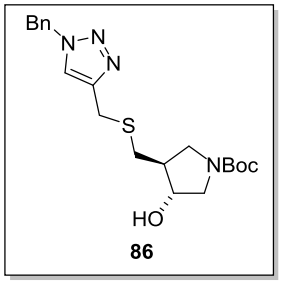
^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.7 (C), 147.0 (C), 120.9 (CH), 79.6 (C), 74.5, 73.8 (CH), 52.6, 52.3 (CH_2), 50.1 (CH_2), 49.3, 49.0 (CH_2), 45.8, 45.2 (CH), 33.4 (CH_2), 32.4 (CH_2), 31.7 (CH_2), 29.1 (CH_2), 28.6 (3xCH_3), 24.4 (CH_2), 19.8 (CH_2), 13.6 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{19}H_{34}N_4O_3NaS$ (MNa^+) calcd 421.2249, found 421.2254.

$[\alpha]_D^{20}$: not measured

tert-Butyl-(3*S*,4*R*)-3-((((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)-4-hydroxypyrrolidine-1-carboxylate (86)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **74** (112mg, 413µmol), benzyl bromide (60µg, 0.49mmol) and sodium azide (33mg, 0.50mmol) was carried out as described above (General Synthetic Procedures) in methanol (1.5mL). Product **86** (88mg, 0.22mmol) was obtained as colorless oil after purification by column chromatography twice.



86

MW: 404.53g/mol
TLC-solvent: $CHCl_3$/MeOH: 10%
TLC-detection: Ehrlich's
CC-solvent: $CHCl_3$/MeOH: 0%→10%
$CHCl_3$/EA(1/2)/MeOH: 0%→10%
yield: 53%

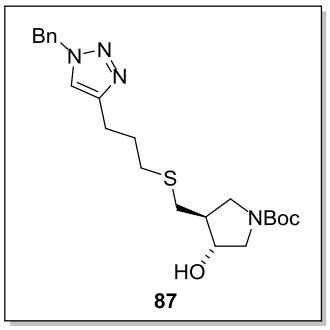
^{1}H NMR (TMS at 0.00): δ = 7.41 (s, 1H), 7.40-7.35 (m, 3H), 7.28-7.24 (m, 2H), 5.50 (s, 2H), 4.16-4.08 (bm, 1H), 3.79 (s, 2H), 3.69-3.56 (bm, 3H), 3.27-3.17 (bm, 1H), 3.14-3.07 (bm, 1H), 2.67-2.59 (bm, 1H), 2.54-2.46 (bm, 1H), 2.37-2.26 (bm, 1H), 1.45 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.6 (C), 145.8 (C), 134.5 (C), 129.2 (2xCH), 128.9 (CH), 128.2 (2xCH), 121.9 (CH), 79.6 (C), 74.3, 73.6 (CH), 54.4 (CH_2), 52.5, 52.2 (CH_2), 49.3, 48.8 (CH_2), 45.7, 45.2 (CH), 32.8 (CH_2), 28.6 (3xCH_3), 26.3 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{20}H_{29}N_4O_3S$ (MH^+) calcd 405.1960, found 405.1957.

tert-Butyl-(3*S*,4*R*)-3-(((3-(1-benzyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)-4-hydroxypyrrolidine-1-carboxylate (87)[§]

The copper(I) catalyzed 1,3-dipolar cycloaddition of alkyne **75** (226mg, 755µmol), benzyl bromide (153mg, 877µmol) and sodium azide (62mg, 0.94mmol) was carried out as described above (General Synthetic Procedures) in methanol (2.5mL). Product **87** (215mg, 197µmol) was obtained as yellow oil after purification by column chromatography.



87

MW: 432.58g/mol
TLC-solvent: PE/EA: 75%
TLC-detection: Ehrlich's
CC-solvent: PE/EA: 25%→100%
yield: 66%

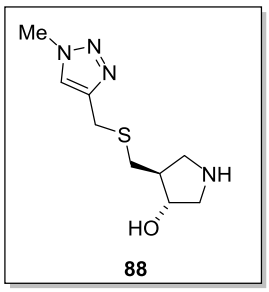
^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.40-7.33 (m, 3H), 7.28-7.21 (m, 3H), 5.48 (s, 2H), 4.19 (bm, 1H), 3.72-3.57 (bm, 2H), 3.31-3.18 (bm, 2H), 3.15-3.07 (bm, 1H), 2.88-2.73 (m, 2H), 2.64-2.47 (m, 2H) overlaps with 2.58 (t, *J*=7.1Hz, 2H), 2.32-2.21 (m, 1H), 1.96 (pent, *J*=7.3Hz, 2H), 1.45 (s, 9H).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 154.7 (C), 147.6 (C), 134.9 (C), 129.2 (2xCH), 128.8 (CH), 128.2 (2xCH), 121.0 (CH), 79.6 (C), 74.6, 73.9 (CH), 54.2 (CH_2), 52.6, 52.3 (CH_2), 49.3, 49.0 (CH_2), 45.8, 45.12 (CH), 33.4 (CH_2), 31.7 (CH_2), 29.0 (CH_2), 28.6 (3xCH_3), 24.4 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{22}H_{33}N_4O_3S$ (MH^+) calcd 433.2273, found 433.2274.

(3*R*,4*S*)-4-((((1-Methyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (88)[§]

Boc deprotection of substrate **80** (64mg, 195µmol) was carried out as described above (General Synthetic Procedures). The crude material was purified by column chromatography to afford product **88** (25mg, 110µmol).



88

MW: 228.31g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%
yield: 56%

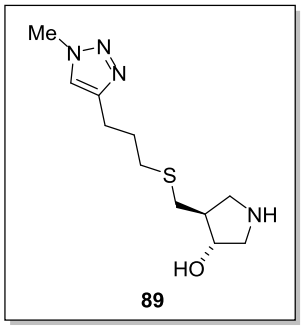
^{1}H NMR (CD_3OD center line 3.31): δ = 7.84 (s, 1H), 4.09-4.06 (m, 4H), 3.82 (s, 2H), 3.28 (dd, *J*=10.9, 7.9Hz, 1H) overlaps with solvent, 3.11-3.02 (m, 1H), 2.88-2.81 (m, 1H), 2.75-2.69 (m, 1H), 2.66 (dd, *J*=13.0, 6.8Hz, 1H), 2.47 (dd, *J*=13.0, 8.6Hz, 1H), 2.27-2.19 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 146.8 (C), 125.1 (CH), 77.1 (CH), 54.4 (CH_2), 51.1 (CH_2), 48.5 (CH), 37.1 (CH_3), 34.5 (CH_2), 26.6(CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_9H_{17}N_4OS$ (MH^+) calcd 229.1123, found 229.1123.

(3*R*,4*S*)-4-(((3-(1-Methyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (89)[§]

Boc deprotection of substrate **81** (116mg, 0.325mmol) was carried out as described above (General Synthetic Procedures). Crude product **89** was obtained as pale oil in quantitative yield (88mg, 0.343mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



89

MW: 256.37g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: -
yield: >99%

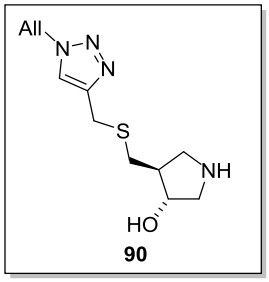
^{1}H NMR (CD_3OD center line 3.31): δ = 7.75 (s, 1H), 4.35-4.29 (bm, 1H), 4.07 (s, 3H), 3.63-3.56 (bm, 1H), 3.46-3.39 (bm, 1H), 3.25-3.17 (bm, 2H), 2.81 (t, *J*=7.5Hz, 2H), 2.74 (dd, *J*=12.9, 6.2Hz, 1H), 2.62 (t, *J*=7.1Hz, 2H), 2.52 (dd, *J*=12.8, 8.9Hz, 1H), 2.49-2.42 (bm, 1H), 1.96 (pent, *J*=7.3Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 148.8 (C), 124.7 (CH), 74.5 (CH), 52.6 (CH_2), 49.4 (CH_2), 47.5 (CH), 37.0 (CH_3), 33.4 (CH_2), 32.3 (CH_2), 30.3 (CH_2), 25.1 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{11}H_{21}N_4OS$ (MH^+) calcd 257.1436, found 257.1437.

(3*R*,4*S*)-4-((((1-((*Z*)-Prop-1-en-1-yl)-1H-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (90)[§]

Boc deprotection of substrate **82** (64mg, 181µmol) was carried out as described above (General Synthetic Procedures). Crude product **90** was obtained as pale oil in quantitative yield. The material showed sufficient purity by NMR and was used in the next step without further purification.



90

MW: 254.35g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: -
yield: >99%

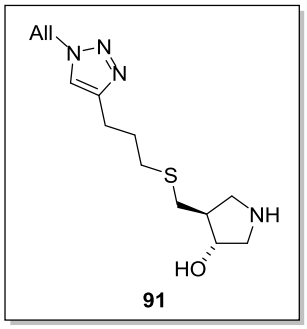
^{1}H NMR (CD_3OD center line 3.31): δ = 7.90 (s, 1H), 6.08 (ddt, *J*=16.4, 10.3, 6.0Hz, 1H), 5.32 (dq, *J*=10.4, 1.3Hz, 1H), 5.26 (dq, *J*=17.0, 1.4Hz, 1H), 5.02 (dt, *J*=6.0, 1.5Hz, 2H), 4.22 (ddd, *J*=3.0, 5.3, 3.0Hz, 1H), 3.86 (s, 2H), 3.49 (dd, *J*=11.8, 7.4Hz, 1H), 3.29 (dd, *J*=12.4, 5.0Hz, 1H), 3.07 (dd, *J*=9.5, 2.8Hz, 1H), 3.02 (dd, *J*=12.0, 5.4Hz, 1H), 2.69 (dd, *J*=13.3, 6.7Hz, 1H), 2.51 (dd, *J*=13.2, 8.6Hz, 1H), 2.45-2.38 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 146.7 (C), 133.2 (CH), 124.2 (CH), 119.9 (CH_2), 75.4 (CH), 53.7 (CH_2), 53.2 (CH_2), 49.9 (CH_2), 47.6 (CH), 33.7 (CH_2), 26.6 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{11}H_{19}N_4OS$ (MH^+) calcd 255.1280, found 255.1281.

(3*R*,4*S*)-4-(((3-(1-((*Z*)-Prop-1-en-1-yl)-1H-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (91)[§]

Boc deprotection of substrate **83** (144mg, 0.377mmol) was carried out as described above (General Synthetic Procedures). Crude product **91** was obtained as pale oil in quantitative yield (101mg, 0.358mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



91

MW: 282.41g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: -
yield: 95%

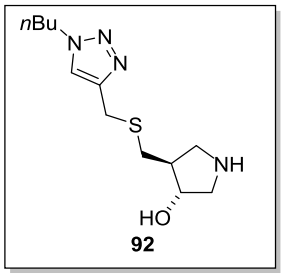
^{1}H NMR (CD_3OD center line 3.31): δ = 7.74 (s, 1H), 6.07 (ddt, *J*=16.4, 10.2, 6.0Hz, 1H), 5.31 (dq, *J*=10.2, 1.2Hz, 1H), 5.25 (dq, *J*=17.0, 1.4Hz, 1H), 4.99 (dt, *J*=6.1, 1.4Hz, 2H), 4.24 (ddd, *J*=3.1, 5.3, 3.1Hz, 1H), 3.49 (dd, *J*=11.8, 7.6Hz, 1H), 3.32-3.28 (m, 1H), 3.07 (dd, *J*=12.3, 2.8Hz, 1H), 3.03 (dd, *J*=11.9, 5.5Hz, 1H), 2.82 (t, *J*=7.6Hz, 2H), 2.71 (dd, *J*=13.2, 6.6Hz, 1H), 2.61 (t, *J*=7.1Hz, 2H), 2.49 (dd, *J*=13.1, 8.8Hz, 1H), 2.40-2.33 (m, 1H), 196 (pent, *J*=7.4Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 148.5 (C), 133.3 (CH), 123.4 (CH), 119.7 (CH_2), 75.4 (CH), 53.5 (CH_2), 53.2 (CH_2), 50.0 (CH_2), 48.0 (CH), 33.8 (CH_2), 32.2 (CH_2), 30.3 (CH_2), 25.1 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{13}H_{23}N_4OS$ (MH^+) calcd 283.1593, found 283.1594.

(3*R*,4*S*)-4-((((1-Butyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (92)[§]

Boc deprotection of substrate **84** (133mg, 0.346mmol) was carried out as described above (General Synthetic Procedures). Crude product **92** was obtained as pale oil in quantitative yield (101mg, 0.356mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



92

MW: 270.40g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: -
yield: >99%

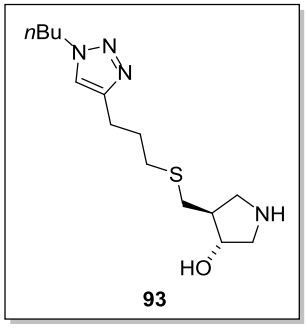
^{1}H NMR (CD_3OD center line 3.31): δ = 7.93 (s, 1H), 4.39 (t, *J*=7.1Hz, 2H), 4.26 (ddd, *J*=3.0, 5.4, 3.0Hz, 1H), 3.68 (s, 2H), 3.56 (dd, *J*=11.9, 7.0Hz, 1H), 3.37 (dd, *J*=12.4, 4.8Hz, 1H), 3.15 (dd, *J*=12.5, 2.9Hz, 1H) overlaps with 3.12 (dd, *J*=11.8, 4.7Hz, 1H), 2.70 (dd, *J*=12.8, 6.3Hz, 1H), 2.52 (dd, *J*=13.0, 8.8Hz, 1H) overlaps with 2.50-2.43 (m, 1H), 1.88 (pent, *J*=7.3Hz, 2H), 1.34 (sext, *J*=7.5Hz, 2H), 0.96 (t, *J*=7.4Hz, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 146.3 (C), 124.2 (CH), 74.8 (CH), 52.8 (CH_2), 51.2 (CH_2), 49.6 (CH_2), 47.3 (CH), 33.4 (CH_2), 33.3 (CH_2), 26.5 (CH_2), 20.7 (CH_2), 13.8 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{12}H_{22}NONaS$ (MNa^+) calcd 293.1412, found 293.1407.

(3*R*,4*S*)-4-(((3-(1-Butyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (93)[§]

Boc deprotection of substrate **85** (95mg, 0.238mmol) was carried out as described above (General Synthetic Procedures). Crude product **93** was obtained as pale oil in quantitative yield (69mg, 0.231mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



93

MW: 298.45g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: -
yield: 97%

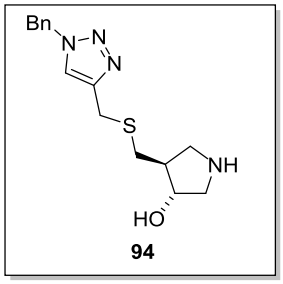
^{1}H NMR (CD_3OD center line 3.31): δ = 7.75 (s, 1H), 4.36 (t, *J*=7.1Hz, 2H), 4.12 (ddd, *J*=3.3, 5.4, 3.3Hz, 1H), 3.33 (dd, *J*=10.5, 2.9Hz, 1H) overlaps with solvent, 3.12 (dd, *J*=12.1, 5.3Hz, 1H), 2.88 (dd, *J*=12.2, 3.2Hz, 1H), 2.83-2.77 (m, 3H), 2.69 (dd, *J*=12.9, 6.7Hz, 1H), 2.59 (t, *J*=7.2Hz, 2H), 2.47 (dd, *J*=13.0, 8.7Hz, 1H), 2.27-2.19 (m, 1H), 1.96 (pent, *J*=7.4Hz, 2H), 1.87 (pent, *J*=7.3Hz, 2H), 0.96 (t, *J*=7.4Hz, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 148.3 (C), 123.3 (CH), 76.8 (CH), 54.2 (CH_2), 51.0 (CH_2), 50.9 (CH_2), 48.7 (CH), 34.5 (CH_2), 33.3 (CH_2), 32.3 (CH_2), 30.3 (CH_2), 25.2 (CH_2), 20.6 (CH_2), 13.7 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{14}H_{27}N_4OS$ (MH^+) calcd 299.1906, found 299.1897.

(3*R*,4*S*)-4-((((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (94)[§]

Boc deprotection of substrate **86** (90mg, 0.223mmol) was carried out as described above (General Synthetic Procedures). Crude product **94** was obtained as pale oil in quantitative yield (65mg, 0.214mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



94

MW: 304.41g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%
TLC-detection: Ehrlich's
CC-solvent: -
yield: 96%

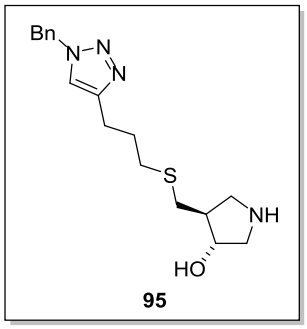
^{1}H NMR (CD_3OD center line 3.31): δ = 7.91 (s, 1H), 7.40-7.29 (m, 5H), 5.58 (s, 2H), 4.26-4.23 (m, 1H), 3.84 (s, 2H), 3.53 (dd, *J*=12.2, 7.4Hz, 1H), 3.34 (dd, *J*=12.4, 2.0Hz, 1H), 3.14 (dd, *J*=12.4, 2.0Hz, 1H), 3.10 (dd, *J*=12.2, 5.0Hz, 1H), 2.67 (dd, *J*=13.0, 6.4Hz, 1H), 2.53-2.41 (m, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 146.8 (C), 136.8 (C), 130.1 (2xCH), 129.6 (CH), 129.2 (2xCH), 124.3 (CH), 74.8 (CH), 55.0 (CH_2), 52.8 (CH_2), 49.5 (CH_2), 47.3 (CH), 33.3 (CH_2), 26.5 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{15}H_{21}N_4OS$ (MH^+) calcd 305.1436, found 305.1443.

(3*R*,4*S*)-4-(((3-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (95)[§]

Boc deprotection of substrate **87** (193mg, 0.446mmol) was carried out as described above (General Synthetic Procedures). Crude product **95** was obtained as pale oil in quantitative yield (145mg, 0.436mmol). The material showed sufficient purity by NMR and was used in the next step without further purification.



95

MW: 332.47g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 10%

TLC-detection: Ehrlich's

CC-solvent: -

yield: 98%

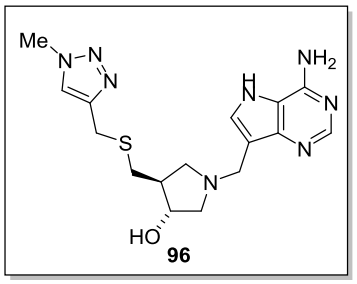
^{1}H NMR (CD_3OD center line 3.31): δ = 7.76 (s, 1H), 7.39-7.30 (m, 5H), 5.55 (s, 2H), 4.30 (ddd, *J*=2.9, 5.4, 2.9Hz, 1H), 3.58 (dd, *J*=12.0, 7.1Hz, 1H), 3.40 (dd, *J*=12.3, 4.8Hz, 1H), 3.21-3.15 (m, 2H), 2.80 (t, *J*=7.5Hz, 2H), 2.71 (dd, *J*=12.7, 6.2Hz, 1H), 2.59 (t, *J*=7.2Hz, 2H), 2.49 (dd, *J*=12.8, 8.8Hz, 1H), 2.46-2.40 (m, 1H), 1.94 (pent, *J*=7.4Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 148.7 (C), 136.9 (C), 130.0 (2xCH), 129.6 (CH), 129.1 (2xCH), 123.5 (CH), 74.6 (CH), 54.9 (CH_2), 52.7 (CH_2), 49.4 (CH_2), 47.5 (CH), 33.4 (CH_2), 32.2 (CH_2), 30.3 (CH_2), 25.2 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{17}H_{25}N_4OS$ (MH^+) calcd 333.1749, found 33.1755.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((((1-methyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (96)[§]

The Mannich coupling of pyrrolidine **88** (25mg, 0.11mmol) and 9-deaza-adenine (**5**, 18mg, 0.13mmol) was carried out as described above (General Synthetic Procedures) in water (0.5mL) and ethanol (0.5mL) with formaldehyde (37%aq. solution, 17mg, 0.21mmol) in the microwave at 70°C for 2h. Product **96** (26mg, 69µmol) was obtained after purification by column chromatography.



96

MW: 374.47g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%

yield: 63%

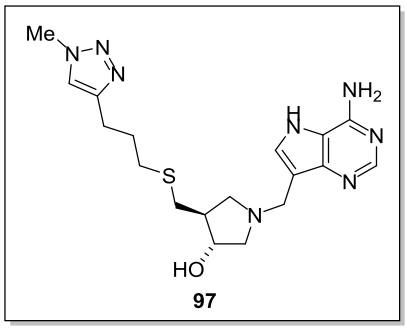
^{1}H NMR (CD_3OD center line 3.31): δ = 8.17 (s, 1H), 7.80 (s, 1H), 7.53 (s, 1H), 4.06 (s, 3H), 3.99 (ddd, *J*=4.1, 6.2, 4.1Hz, 1H), 3.94 (d, *J*=13.6, 1H), 3.90 (d, *J*=13.5Hz, 1H), 3.77 (s, 2H), 3.14 (dd, *J*=10.0, 8.0Hz, 1H), 2.94 (dd, *J*=10.5, 6.3Hz, 1H), 2.76 (dd, *J*=10.6, 3.9Hz, 1H), 2.70 (dd, *J*=12.8, 6.4Hz, 1H), 2.52-2.45 (m, 2H), 2.27-2.19 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.2 (C), 151.1 (CH), 146.9 (C), 146.7 (C), 130.4 (CH), 125.1 (CH), 115.2 (C), 111.3 (C), 76.4 (CH), 62.0 (CH_2), 58.5 (CH_2), 49.9 (CH_2), 48.0 (CH), 37.0 (CH_3), 35.3 (CH_2), 26.6 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{16}H_{22}N_8ONaS$ (MNa^+) calcd 397.1535, found 397.1530.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-(((3-(1-methyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (97)[§]

The Mannich coupling of pyrrolidine **89** (83mg, 0.32mmol) and 9-deaza-adenine (**5**, 43mg, 0.32mmol) was carried out as described above (General Synthetic Procedures) in water (2mL) and ethanol (2mL) with formaldehyde (37%aq. solution, 27mg, 0.33mmol) in the microwave at 70°C for 2h. Product **97** (54mg, 0.13mmol) was obtained after purification by column chromatography.



97

MW: 402.52g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%

yield: 41%

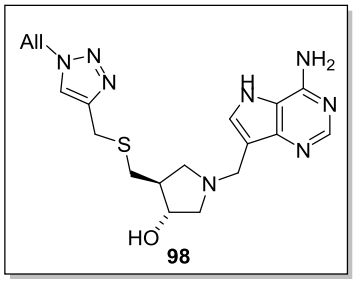
^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.67 (s, 1H), 7.50 (s, 1H), 4.05 (s, 3H), 3.97 (ddd, *J*=4.1, 6.3, 4.1Hz, 1H), 3.87 (d, *J*=13.4Hz, 1H), 3.83 (d, *J*=13.4Hz, 1H), 3.07 (dd, *J*=9.9, 8.1Hz, 1H), 2.87 (dd, *J*=10.4, 6.3Hz, 1H), 2.76 (t, *J*=7.4Hz, 2H), 2.72 (dd, *J*=12.7, 6.2Hz, 1H), 2.68 (dd, *J*=10.5, 4.3Hz, 1H), 2.53 (td, *J*=7.2, 1.9Hz, 2H) overlaps with 2.49 (dd, *J*=12.8, 9.3Hz, 1H), 2.40 (dd, *J*=9.9, 7.1Hz, 1H), 2.21-2.13 (m, 1H), 1.90 (pent, *J*=7.3Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 148.5 (CH), 147.0 (C), 130.1 (C), 124.3 (CH), 115.2 (CH), 115.2 (C), 112.2 (C), 76.7 (CH), 62.2 (CH_2), 58.7 (CH_2), 48.9 (CH_2), 48.5 (CH), 36.9 (CH_3), 35.6 (CH_2), 32.2 (CH_2), 30.3 (CH_2), 25.1 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{18}H_{27}N_8OS$ (MH^+) calcd 403.2029, found 403.2032.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((((1-((*Z*)-prop-1-en-1-yl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (98)[§]

The Mannich coupling of pyrrolidine **90** (45mg, 0.18mmol) and 9-deaza-adenine (**5**, 28mg, 0.21mmol) was carried out as described above (General Synthetic Procedures) in water (1.5mL) and ethanol (1.5mL) with formaldehyde (37%aq. solution, 24mg, 0.30mmol) in the microwave at 70°C for 2h. Product **98** (43mg, 0.11mmol) was obtained after purification by column chromatography.



98

MW: 400.51g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%
yield: 61%

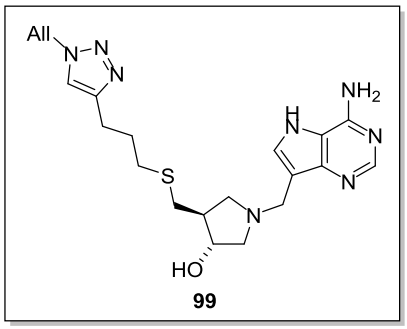
^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.83 (s, 1H), 7.50 (s, 1H), 6.05 (ddt, *J*=16.8, 10.5, 6.1Hz, 1H), 5.30 (dq, *J*=10.3, 1.3Hz, 1H), 5.23 (dq, *J*=17.0, 1.2Hz, 1H), 4.99 (dt, *J*=6.0, 1.4Hz, 2H), 3.96 (ddd, *J*=4.0, 6.1, 4.0Hz, 1H), 3.88 (d, *J*=13.6Hz, 1H), 3.84 (d, *J*=13.6Hz, 1H), 3.79 (s, 2H), 3.07 (dd, *J*=9.8, 8.3Hz, 1H), 2.88 (dd, *J*=10.4, 6.3Hz, 1H), 2.73-2.67 (m, 2H), 2.50 (dd, *J*=12.9, 8.8Hz, 1H), 2.40 (dd, *J*=9.8, 7.0Hz, 1H), 2.25-2.17 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.1 (CH), 147.0 (C), 146.9 (C), 133.2 (CH), 130.3 (CH), 124.2 (CH), 119.9 (CH_2), 115.2 (C), 112.0 (C), 76.6 (CH), 62.2 (CH_2), 58.7 (CH_2), 53.6 (CH_2), 49.0 (CH_2), 48.1 (CH), 35.5 (CH_2), 26.7 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{18}H_{25}N_8OS$ (MH^+) calcd 401.1872, found 401.1874.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-(((3-(1-((*Z*)-prop-1-en-1-yl)-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (99)[§]

The Mannich coupling of pyrrolidine **91** (101mg, 358µmol) and 9-deaza-adenine (**5**, 60mg, 0.45mmol) was carried out as described above (General Synthetic Procedures) in water (3mL) and ethanol (3mL) with formaldehyde (37%aq. solution, 34mg, 0.42mmol) in the microwave at 70°C for 2h. Product **99** (37mg, 86µmol) was obtained after purification by column chromatography.



99

MW: 428.56g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%

yield: 24%

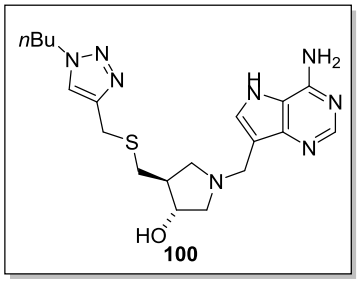
^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.70 (s, 1H), 7.50 (s, 1H), 6.05 (ddt, *J*=16.7, 10.6, 6.0Hz, 1H), 5.29 (dq, *J*=10.4, 1.2Hz, 1H), 5.22 (dq, *J*=17.0, 1.4Hz, 1H), 4.97 (dt, *J*=6.0, 1.4Hz, 2H), 3.97 (ddd, *J*=4.3, 6.2, 4.3Hz, 1H), 3.88 (d, *J*=13.4Hz, 1H), 3.83 (d, *J*=13.5Hz, 1H), 3.07 (dd, *J*=9.7, 7.9Hz, 1H), 2.88 (dd, *J*=10.5, 6.4Hz, 1H), 2.78 (t, *J*=7.5Hz, 2H), 2.75-2.67 (m, 2H), 2.53 (td, *J*=7.3, 1.9Hz, 2H), 2.49 (dd, *J*=12.7, 9.0Hz, 1H), 2.41 (dd, *J*=10.0, 7.0Hz, 1H), 2.21-2.14 (m, 1H), 1.91 (pent, *J*=7.3Hz, 2H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.1 (CH), 148.6 (C), 147.0 (C), 133.3 (CH), 130.2 (CH), 123.3 (CH), 119.7 (CH_2), 115.2 (C), 112.1 (C), 76.7 (CH), 62.2 (CH_2), 58.8 (CH_2), 53.5 (CH_2), 49.0 (CH_2), 48.5 (CH), 35.6 (CH_2), 32.3 (CH_2), 30.3 (CH_2), 25.2 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{20}H_{29}N_8OS$ (MH^+) calcd 429.2185, found 429.2189.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((((1-butyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (100)[§]

The Mannich coupling of pyrrolidine **92** (101mg, 374µmol) and 9-deaza-adenine (**5**, 66mg, 0.49mmol) was carried out as described above (General Synthetic Procedures) in water (3mL) and ethanol (3mL) with formaldehyde (37%aq. solution, 38mg, 0.47mmol) in the microwave at 70°C for 2h. Product **100** (81mg, 0.19mmol) was obtained after purification by column chromatography.



100

MW: 416.55g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%

yield: 52%

^{1}H NMR (CD_3OD center line 3.31): δ = 8.19 (s, 1H), 7.87 (s, 1H), 7.51 (s, 1H), 4.36 (t, *J*=7.1Hz, 2H), 4.02-3.83 (bm, 3H), 3.78 (s, 2H), 3.15-3.06 (bm, 1H), 2.96-2.86 (bm, 1H), 2.76-

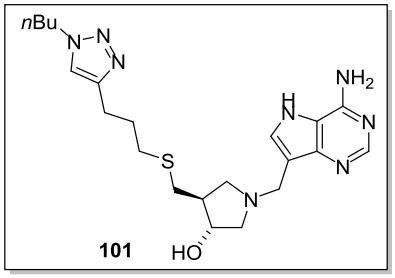
2.66 (bm, 2H), 2.55-2.40 (bm, 2H), 2.26-2.17 (bm, 1H), 1.86 (pent, *J*=7.3Hz, 2H), 1.31 (sext, *J*=7.5Hz, 2H), 0.94 (t, *J*=7.4Hz, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.1 (CH), 147.0 (C), 146.6 (C), 130.3 (CH), 124.2 (CH), 115.4 (C), 111.8 (C), 76.6 (CH), 62.1 (CH_2), 58.7 (CH_2), 51.1 (CH_2), 49.0 (CH_2), 48.1 (CH), 35.4 (CH_2), 33.3 (CH_2), 26.7 (CH_2), 20.6 (CH_2), 13.7 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{19}H_{29}N_8OS$ (MH^+) calcd 417.2185, found 417.2179.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-(((3-(1-butyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (101)[§]

The Mannich coupling of pyrrolidine **93** (51mg, 0.17mmol) and 9-deaza-adenine (**5**, 29mg, 0.22mmol) was carried out as described above (General Synthetic Procedures) in water (2mL) and ethanol (2mL) with formaldehyde (37%aq. solution, 18mg, 0.22mmol) in the microwave at 70°C for 2h. Product **101** (25mg, 56µmol, >95% purity and 37mg, 84µmol ~70% purity by ^{1}H-NMR) was obtained after purification by column chromatography.



101

MW: 444.60g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%

yield: 33% (+ 49%, ~70% purity by ^{1}H-NMR)

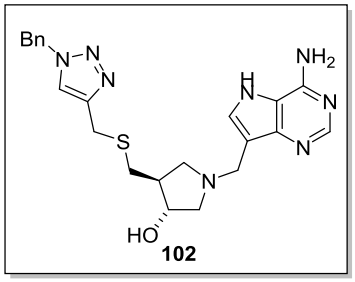
^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.72 (s, 1H), 7.50 (s, 1H), 4.34 (t, *J*=7.1Hz, 2H), 3.96 (ddd, *J*=4.2, 6.4, 4.2Hz, 1H), 3.86 (d, *J*=13.5Hz, 1H), 3.81 (d, *J*=13.5Hz, 1H), 3.05 (dd, *J*=9.9, 8.0Hz, 1H), 2.86 (dd, *J*=10.3, 4.2Hz, 1H), 2.78 (t, *J*=7.4Hz, 2H), 2.72 (dd, *J*=12.8, 6.2Hz, 1H), 2.67 (dd, *J*=10.3, 4.2Hz, 1H), 2.53 (td, *J*=7.2, 2.1Hz, 2H), 2.49 (dd, *J*=12.7, 9.2Hz, 1H), 2.39 (dd, *J*=10.0, 7.0Hz, 1H), 2.21-2.13 (m, 1H), 1.92 (pent, *J*=7.4Hz, 2H), 1.85 (pent, *J*=7.5Hz, 2H), 1.31 (sext, *J*=7.5Hz, 2H), 0.94 (t, *J*=7.4Hz, 3H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.0 (CH), 148.3 (C), 147.0 (C), 130.1 (CH), 123.3 (CH), 115.1 (C), 112.4 (C), 76.8 (CH), 62.3 (CH_2), 58.8 (CH_2), 51.0 (CH_2), 48.9 (CH_2), 48.6 (CH), 35.7 (CH_2), 33.3 (CH_2), 32.3 (CH_2), 30.3 (CH_2), 25.2 (CH_2), 20.6 (CH_2), 13.7 (CH_3).

MS: ESI-HRMS (TOF) *m/z* for $C_{21}H_{32}N_8ONaS$ (MNa^+) calcd 467.2317, found 467.2312.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-((((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)thio)methyl)pyrrolidin-3-ol (102)[§]

The Mannich coupling of pyrrolidine **94** (61mg, 0.20mmol) and 9-deaza-adenine (**5**, 33mg, 0.25mmol) was carried out as described above (General Synthetic Procedures) in water (1.5mL) and ethanol (1.5mL) with formaldehyde (37%aq. solution, 22mg, 0.27mmol) in the microwave at 70°C for 2h. Product **102** (48mg, 0.11mmol) was obtained after purification by column chromatography.



102

MW: 450.57g/mol

TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 20%

TLC-detection: UV, Ehrlich's

CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→20%

yield: 53%

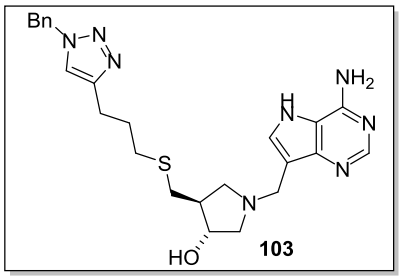
^{1}H NMR (CD_3OD center line 3.31): δ = 8.16 (s, 1H), 7.83 (s, 1H), 7.49 (s, 1H), 7.38-7.26 (m, 5H), 5.54 (s, 2H), 3.94 (ddd, *J*=4.2, 8.1, 4.2Hz, 1H), 3.86 (d, *J*=13.5Hz, 1H), 3.82 (d, *J*=13.5Hz, 1H), 3.76 (s, 2H), 3.04 (dd, *J*=9.8, 8.1Hz, 1H), 2.85 (dd, *J*=10.5, 6.4Hz, 1H), 2.70-2.64 (m, 2H), 2.47 (dd, *J*=12.8, 8.8Hz, 1H), 2.36 (dd, *J*=10.0, 7.1Hz, 1H), 2.22-2.14 (m, 1H).

^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.1 (CH), 147.1 (C), 147.0 (C), 136.8 (C), 130.2 (CH), 130.0 (2xCH), 129.6 (CH), 129.1 (2xCH), 124.2 (CH), 115.2 (C), 112.0 (C), 76.6 (CH), 62.2 (CH_2), 58.7 (CH_2), 55.0 (CH_2), 49.0 (CH_2), 48.1 (CH), 35.5 (CH_2), 26.6 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{22}H_{27}N_8OS$ (MH^+) calcd 451.2029, found 451.2030.

(3*R*,4*S*)-1-((4-Amino-5*H*-pyrrolo[3,2-d]pyrimidin-7-yl)methyl)-4-(((3-(1-benzyl-1*H*-1,2,3-triazol-4-yl)propyl)thio)methyl)pyrrolidin-3-ol (103)[§]

The Mannich coupling of pyrrolidine **95** (140mg, 421µmol) and 9-deaza-adenine (**5**, 68mg, 0.51mmol) was carried out as described above (General Synthetic Procedures) in water (3mL) and ethanol (3mL) with formaldehyde (37%aq. solution, 35mg, 0.43mmol) in the microwave at 70°C for 2h. Product **103** (85mg, 0.18mmol) was obtained after purification by column chromatography.



103

MW: 478.62g/mol
TLC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 15%
TLC-detection: UV, Ehrlich's
CC-solvent: CH_2Cl_2/NH_3(7M in MeOH): 0%→30%
yield: 42%

^{1}H NMR (CD_3OD center line 3.31): δ = 8.15 (s, 1H), 7.70 (s, 1H), 7.49 (s, 1H), 7.37-7.26 (m, 5H), 5.53 (s, 2H), 3.95 (ddd, *J*=4.1, 6.3, 4.1Hz, 1H), 3.86 (d, *J*=13.5Hz, 1H), 3.82 (d, *J*=13.5Hz, 1H), 3.05 (dd, *J*=9.8, 8.0Hz, 1H), 2.86 (dd, *J*=10.3, 6.4Hz, 1H), 2.76 (t, *J*=7.5Hz, 2H), 2.70 (dd, *J*=13.1, 6.2Hz, 1H) overlaps with 2.67 (dd, *J*=9.4, 4.2Hz, 1H), 2.51 (td, *J*=7.1, 1.4Hz, 2H) overlaps with 2.47 (dd, *J*=12.8, 9.1Hz, 1H), 2.39 (dd, *J*=9.8, 7.1Hz, 1H), 2.20-2.12 (m, 1H), 1.89 (pent, *J*=7.3Hz, 2H).

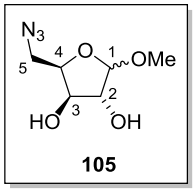
^{13}C NMR (CD_3OD center line 49.0): δ = 152.1 (C), 151.1 (CH), 148.8 (C), 147.0 (C), 136.9 (C), 130.2 (CH), 130.0 (2xCH), 129.5 (CH), 129.0 (2xCH), 123.4 (CH), 115.2 (C), 112.3 (C), 76.7 (CH), 62.2 (CH_2), 58.8 (CH_2), 54.9 (CH_2), 49.0 (CH_2), 48.5 (CH), 35.6 (CH_2), 32.3 (CH_2), 30.2 (CH_2), 25.2 (CH_2).

MS: ESI-HRMS (TOF) *m/z* for $C_{24}H_{30}N_8ONaS$ (MNa^+) calcd 501.2161, found 501.2157.

5.3. SAW-Reactions

(2*R*,3*R*,4*R*)-2-(Azidomethyl)-5-methoxytetrahydrofuran-3,4-diol (105)‡

Acetyl chloride (1.65mL, 23mmol) in methanol (100mL) was cooled to 0°C for 20min. Provided substrate **104** (10.0g, 46.5mmol) was added as a solution in methanol (50mL). After 20min the mixture was warmed to room temperature and after 21h the pH value was adjusted to 6.5 by the addition of sodium carbonate. The mixture was filtrated and the filtrate concentrated. The residue was partitioned between water (50mL) and ethyl acetate (3x150mL). The combined organic layers were dried and concentrated to give an epimeric mixture of **105** (9.8g, 52mmol) as brown oil. The material showed sufficient purity by NMR and was used in the next step without further purification.



105

MW: 189.17g/mol
TLC-solvent: C/EA: 50%
TLC-detection: CAM
CC-solvent:-
yield: >99%

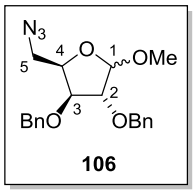
Epimeric mixture (ratio: 0.7/0.3):

^{1}H NMR (CD_3OD, HOD at 4.87): δ = 4.89 (s, 0.3H, H-1), 4.78 (s, 0.7H, H-1), 4.34 (ddd, *J*=5.0, 8.3, 5.0Hz, 0.7H, H-4), 4.28-4.16 (m, 0.6H, H-4, H-3), 4.07 (dd, *J*=5.1, 2.1Hz, 0.7H, H-3), 4.05-3.99 (bm, 1H, H-2), 3.54-3.35 (m, 5H, H-5, H-Me).

^{13}C-APT NMR (CD_3OD center line 49.0): δ = 111.0 (C-1), 103.7 (C-1), 82.7 (C-4), 82.2 (C-2), 79.0, 78.6, 77.3, 76.7 (C-2, 2xC-3, C-4), 55.9 (C-Me), 55.7 (C-Me), 52.9 (C-5), 52.2 (C-5).

(2*R*,3*S*,4*R*)-2-(Azidomethyl)-3,4-bis(benzyloxy)-5-methoxytetrahydrofuran (106)‡

The epimeric mixture of diol **105** (9.8g, 52mmol) in DMF (150mL) was cooled to 0°C and pretreated sodium hydride (8.3g, 0.21mol, washed with cyclohexane) was added. After 30min the mixture was treated with benzyl bromide (15mL, 0.12mol) and warmed to room temperature. The reaction was quenched with methanol (50mL) after 10min, diluted with toluene (2L) and washed with water (5x250mL). The organic layer was dried and concentrated to give the crude product (20g) as pale, yellow liquid. The material was purified by silica filtration to afford product **106** (16g, 43mmol) as pale, yellow oil. A small amount was used to separate the epimers for NMR-characterization.



106

MW: 369.42g/mol
TLC-solvent: C/EA: 33%
TLC-detection: UV, Ehrlich's
CC-solvent: C then EA
yield: 92% (2 steps)

106α ($J_{H\text{-}1\rightarrow H\text{-}2}$=1.2Hz):

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.42-7.24 (m, 10H, H-Ph), 4.93 (d, *J*=1.2Hz, 1H, H-1), 4.64-4.44 (m, 4H, H-Bn), 4.38 (ddd, *J*=6.3, 8.4, 4.7Hz, 1H, H-4), 4.10 (dd, *J*=6.2, 3.2Hz, 1H, H-3), 4.06-4.01 (bm, 1H, H-2), 3.57 (dd, *J*=13.0, 8.5Hz, 1H, H-5a), 3.43 (s, 3H, H-Me) overlaps with 3.39 (dd, *J*=13.1, 4.5Hz, 1H, H-5b).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 137.6 (C-Ph), 137.5 (C-Ph), 128.6-127.9 (10xC-Ph), 108.4 (C-1), 86.9 (C-4), 82.0 (C-3), 80.0 (C-2), 72.5 (**CH_2**-Ph), 72.3 (**CH_2**-Ph), 55.9 (C-Me), 52.1 (C-5).

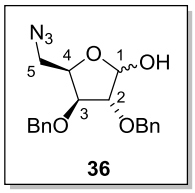
106β ($J_{H\text{-}1\rightarrow H\text{-}2}$=4.1Hz):

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.42-7.24 (m, 10H, H-Ph), 4.82 (d, *J*=4.1Hz, 1H, H-1), 4.72-4.51 (m, 4H, H-Bn), 4.35-4.27 (m, 2H, H-3, H-4), 4.03 (bt, *J*=4.5Hz, 1H, H-2), 3.49-3.38 (m, 5H, H-5, H-Me).

^{13}C NMR ($CDCl_3$ center line 77.2): δ = 137.9 (C-Ph), 137.6 (C-Ph), 128.6-127.9 (10xC-Ph), 100.7 (C-1), 84.1 (C-2), 81.3, 75.7 (C-3, C-4), 72.9 (**CH_2**-Ph), 72.6 (**CH_2**-Ph), 55.4 (C-Me), 51.3 (C-5).

(3*R*,4*S*,5*R*)-5-(Azidomethyl)-3,4-bis(benzyloxy)tetrahydrofuran-2-ol (36)‡

The epimeric mixture of acetal **106** (16g, 43mmol) in acetonitrile (160mL) and water (160mL) was treated with hydrochloric acid (conc., 10mL) and heated to reflux for 2h. The mixture was concentrated and partitioned between sodium bicarbonate (sat. aq., 30mL) and dichloromethane (5x50mL). The combined organic layers were dried and concentrated to give the crude product (12.6g) as black oil. The material was purified by column chromatography to afford product **36** (8.12g, 23mmol) as pale oil.



36

MW: 355.39g/mol
TLC-solvent: EA/MeOH: 10%
TLC-detection: UV, Ehrlich's
CC-solvent: C/EA: 5%→20%
yield: 53% (+ 10% substrate recovery)

epimeric mixture (ratio: 4/6)

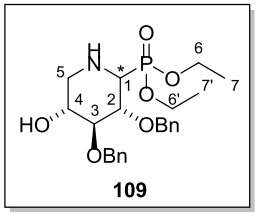
^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.44-7.22 (m, 10H, H-Ph), 5.78 (dd, *J*=9.4, 4.0Hz, 0.4H, H-1), 5.30 (d, *J*=10.7Hz, 0.6H, H-1), 4.73-4.41 (m, 4H, H-Bn), 4.40-4.27 (m, 1H, H-4), 4.04-3.98 (m, 1.6H, H-3, H-2), 3.96 (dd, *J*=4.0, 2.4Hz, 0.4H, H-2), 3.83 (bd, *J*=9.4Hz, 0.4H, H-OH), 3.68-3.43 (m, 2H, H-5), 3.39 (bd, *J*=10.7Hz, 0.6H, H-OH).

^{13}C NMR ($CDCl_3$ center line 77.2): δ =137.4-136.7 (4xC-Ph), 128.9-127.9 (20xC-Ph), 101.6, 96.4 (2xC-1), 84.7, 81.3 (2xC-2), 81.0, 81.7 (2xC-3), 80.2, 77.1 (2xC-4), 73.4, 72.8, 72.4, 72.2 (4x**CH_2**-Ph), 50.9, 50.3 (2xC-5).

Diethyl ((3*R*,4*S*,5*R*)-3,4-bis(benzyloxy)-5-hydroxypiperidin-2-yl)phosphonate (109)‡

Compound **36** (198mg, 557µmol) in methanol (2mL) was treated with trimethylphosphine (1M in toluene, 1.0mL, 1.0mmol). After 45min toluene was added and the solvents were removed under reduced pressure, dissolved in dichloromethane (2mL) and stored at 0°C. Simultaneously, diethyl phosphite (0.11mL, 0.85mmol) in dichloromethane (3mL) was cooled to 0° and treated with triethylamine (0.12mL, 0.87mmol) and trimethylsilyl chloride (0.11mL, 0.86mmol) in a separate flask. The mixture was warmed to room temperature after 10min and combined with the first reaction mixture (imine) after 15min. The reaction was quenched with sodium hydroxide (aq., 2M, 5mL) and extracted with dichloromethane (2x10mL) after 3h. The combined organic layers were dried and concentrated to give the crude product (238mg) as colorless oil. The material was dissolved in methanol (3mL) and treated with hydrochloric acid

(conc., 1mL). After 10min isopropyl alcohol and toluene were added and the volatiles were removed under reduced pressure. The residue was dissolved in methanol and the pH was adjusted to 7-9 by addition of ion exchange resin (Amberlyst 21). Filtration and evaporation gave the crude product as a colorless oil. The crude material was purified by column chromatography to afford product **109** (76mg, 0.17mmol) as pale syrup.



109

MW: 449.48g/mol

TLC-solvent: PE

TLC-detection: UV, Ehrlich's

CC-solvent: C/E: 50% then EA then EA/MeOH: 10%

yield: 26%

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.37-7.16 (m, 10H, H-Ph), 4.67 (d, *J*=10.8Hz, 1H, H-Ph), 4.53 (d, *J*=10.8Hz, 1H, H-Bn), 4.46 (bs, 2H, H-Bn), 4.17-3.91 (m, 4H, H-6, H-6'), 3.83 (bs, 1H, H-2), 3.62 (bs, 1H, H-3), 3.56 (bs, 1H, H-4), 3.45 (d, $^{2}J_{H,P}$=21.3Hz, 1H, H-1), 3.03 (bd, *J*=14.0Hz, 1H, H-5a), 2.89 (bd, *J*=14.0Hz, 1H, H-5b), 1.28 (bt, *J*=6.8Hz, 3H, H-7), 1.18 (bt, *J*=6.9Hz, 3H, H-7').

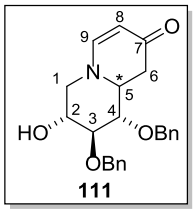
^{13}C NMR ($CDCl_3$ center line 77.2): δ = 137.8 (C-Ph), 137.0 (C-Ph), 128.7-127.7 (10xC-Ph), 74.3 (d, $^{2}J_{C,P}$=2.6Hz, C-2), 73.9 (**CH_2**-Ph), 72.5 (d, $^{3}J_{C,P}$=8.5Hz, C-3), 72.3 (**CH_2**-Ph), 66.5 (C-4), 62.6 (d, $^{2}J_{C,P}$=6.6Hz, C-6), 62.2 (d, $^{2}J_{C,P}$=6.8Hz, C-6'), 53.2 (d, $^{1}J_{C,P}$=161.2Hz, C-1), 48.1 (d, $^{3}J_{C,P}$=14.9Hz, C-5), 16.5, (d, $^{3}J_{C,P}$=16.4Hz, C-7) overlaps with 16.4 (d, $^{2}J_{C,P}$=16.5Hz, C-7').

MS: to be measured

$[\alpha]_D^{20}$: to be measured

(7*R*,8*S*,9*S*)-8,9-bis(Benzyloxy)-7-hydroxy-1,6,7,8,9,9a-hexahydro-2*H*-quinolizin-2-one (111)‡

Compound **36** (198mg, 557µmol) in methanol (2mL) was treated with trimethylphosphine (1M in toluene, 1.0mL, 1.0mmol). After 45min toluene was added and the reaction mixture was concentrated. The residue was dissolved in THF (5mL), treated with Danishefsky's diene (**110**, 247mg, 1.43mmol) and dry zinc(II) chloride (~0.1g, ~0.7mmol). The reaction was quenched with hydrochloric acid (aq., 2M, 5mL) after additional 2h and extracted with dichloromethane (3x20mL). The combined organic layers were washed with sodium bicarbonate (sat. aq., 5mL) and re-extracted with dichloromethane (3x20mL). All organic layers were combined, dried and concentrated to give the crude product (214mg) as red syrup. The material was purified by column chromatography to afford product **111** (54mg, 0.14mmol) as orange syrup.



111

MW: 379.46g/mol

TLC-solvent: C/EA: 33%; EA/MeOH: 10%

TLC-detection: UV, CAM, Ehrlich's (intense red spot)

CC-solvent: C/EA: 20%→100%

yield: 25%

^{1}H NMR ($CDCl_3$ at 7.26): δ = 7.35-7.13 (m, 10H, H-Ph), 6.81 (d, *J*=7.7Hz, 1H, H-9), 4.90 (d, *J*=7.7Hz, 1H, H-8), 4.58-4.36 (m, 4H, H-Bn), 3.86-3.70 (m, 3H, H-1, H-2, H-3), 3.45 (d, *J*=13.2Hz, 1H, H-1a), 3.36 (s, 1H, H-2), 3.23 (d, *J*=13.4Hz, 1H, H-1b), 2.70 (dd, *J*=16.6, 11.5Hz, 1H, H-6a), 2.31 (dd, *J*=16.6, 7.0Hz, 1H, H-6b).

^{13}C NMR ($CDCl_3$ center line 77.2): δ =192.2 (C-7), 155.6 (C-9), 137.4 (C-Ph), 136.1 (C-Ph), 128.9-127.8 (10xC-Ph), 99.5 (C-8), 77.3 (C-2), 73.7 (**CH_2**-Ph), 72.4 (**CH_2**-Ph), 71.0, 67.3 (C-3, C-4), 55.4 (C-5), 55.1 (C-1), 37.7 (C-6).

MS: to be measured

$[\alpha]_D^{20}$: -79.95 ($CHCl_3$)

6. Appendix

6.1. Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources or resources and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

German version:

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die vorliegende Arbeit selbstständig verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Quellen wörtlich und inhaltlich entnommene Stellen als solche kenntlich gemacht habe.

Graz, am _____ _____

(Oskar Hoff)

6.2. Literature

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6.3. List of Abbreviations

9-DAA	9-deaza-adenine (chemical compound)
$[\alpha]_D^{20}$	specific rotation
Ac	acetyl (functional group)
All	allyl (functional group)
ATP	adenosine triphosphate (chemical compound)
AI	autoinducer
AIDS	acquired Immune Deficiency Syndrome (disease)
Boc	tert-butyloxycarbonyl (protecting group)
Bn	benzyl (protecting or functional group)
C	cyclohexane (solvent)
CC	column chromatography
Cj	*Campylobacter jejuni* (bacterium)
CMT	chaperone mediated therapy
conc.	concentrated
DBU	1,8-diazabicycloundec-7-ene (chemical compound)
DNJ	1-deoxynojirimycin (chemical compound)
DIPEA	*N,N*-diisopropylethylamine (Hünig's base, chemical compound)
DMAP	4-dimethylaminopyridine (chemical compound)
DMF	dimethylformamide (solvent)
EA	ethyl acetate (solvent)
Ec	*Escherichia coli* (bacterium)
ee	enantiomeric excess
ERAD	endoplasmic rediculum-associated degradation
ERT	enzyme replacement therapy
ESI-HRMS	high resolution electrospray mass spectrometry (analytic method)
GC-EIMS	gas chromatography electron impact mass spectrometry (analytic method)
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (chemical compound)
*h*MTAP	*human* MTAP (→ MTAP, enzyme)
Hp	*Helicobacter pylori* (bacterium)
K_i	inhibition constant
K_i^*	inhibition constant for slow-onset inhibitors
KIE	kinetic isotopic effect
Kp	*Klebsiella pneumoniae* (bacterium)
LSD	lysosomal storage disease
M	mol/l
mCPBA	*meta*-chloroperbenzoic acid (chemical compound)
Me	methyl (functional group)

MT-DIA	2nd generation inhibitor for MTAN and MTAP (chemical compound)
MTA	5'-methylthioadenosine (chemical compound)
MTAN	5'-Methylthioadenosine nucleosidase (enzyme)
MTAP	5'-methylthioadenosine phosphorylase (enzyme)
MT-ImmA	1st generation inhibitor for MTAN and MTAP (chemical compound)
MS	mass spectrometry (analytic method)
MW	molecular weight
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate (chemical compound)
*n*Bu	n-butyl (functional group)
Nm	*Neisseria meningitides* (bacterium)
NMR	nuclear magnetic resonance (spectroscopy, analytic method)
PE	petrol ether (solvent)
PNP	purine nucleoside phosphorylase (enzyme)
R_f	retention factor
rt	room temperature
Sa	*Staphylococcus aureus* (bacterium)
SAM	*S*-adenosylmethionine (chemical compound)
SAW	Staudinger/aza-Wittig (named reaction)
Sp	*Streptococcus pneumoniae* (bacterium)
SRT	substrate reduction therapy
THF	tetrahydrofuran (chemical compound)
TLC	thin layer chromatography (analytic method)
v/v	volume/volume ratio