Synthesis of Guanidine Amino Acids and Investigation of their Interactions with Nucleic Acid

Thesis submitted for the degree of Doctor of Philosophy by

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Declaration

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Abbreviations

AAA	2-<u>a</u>mino-2-(2-<u>a</u>minopyrimidin-5-yl)-<u>a</u>cetic acid
AAP	2-<u>a</u>mino-3-(2-<u>a</u>minopyrimidin-5-yl)-<u>p</u>ropanoic acid
Ac	acetyl
AIA	2-<u>a</u>mino-2-(2-<u>i</u>minohexahydro-pyrimidin-5-yl)<u>a</u>cetic acid
AIP	2-<u>a</u>mino-3-(2-<u>i</u>minohexa-hydropyrimidin-5-yl)<u>p</u>ropanoic acid
Ala, A	alanine
Alloc	allyloxycarbonyl
aq.	aqueous
Ar	aromat
Arg, R	arginine
Asn, N	asparagine
Asp, D	aspartic acid
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
Boc	*tert*-butyl carbonate
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
*t*Bu	*tert*-butyl
br	broad
Br$_2$	bromine
Bu$_4$NCl	tetrabutylammonium chloride
Cat.	catalyst
Cys, C	cysteine
cys, c	D-cysteine
CDCl$_3$	deuterated chloroform
CD$_3$OD	deuterated methanol
CO$_2$	carbon dioxide
Cs$_2$CO$_3$	caesium carbonate
DCM	dichloromethane
DIOP	4,5-bis(diphenylphosphinomethyl)-2,2-dimethyl-1,3-dioxolane
DIPAMP	ethylenebis[(2-methoxyphenyl)phenylphosphine]

DMF	dimethylformamide
DNA	deoxyribonucleic acid
Me-DUPHOS	2,2′,5,5′-Tetramethyl-1,1′-(o-phenylene)diphospholane
EI MS	electron ionisation mass spectrometry
eq.	equivalent
ESI MS	electrospray ionisation mass spectrometry
Et_3N	triethylamine
Fe	iron
FDAA	N-(2,4)-dinitro-5-fluorophenyl)-L-alanine amide
Fmoc	fluorenylmethyloxycarbonyl
Gln, Q	glutamine
Glu, E	glutamic acid
Gly, G	glycine
H_2	hydrogen gas
HATU	2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium
HCl	hydrochloric acid
HCN	hydrogen cyanide
His, H	histidine
HIV-1	human immunodeficiency virus 1
HMPA	hexamethylphophoramide
H_2O	water
H_3O^+	hydronium ion
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography or high pressure liquid chromatography
HRMS	high resolution mass spectrometry
Hyp	hydroxyproline
IEP	isoelectric point
Ile, I	isoleucine
IR	infrared
KBr	potassium bromide
KCl	potassium chloride
KH_2PO_4	monopotassium phosphate

K_2HPO_4	dipotassium phosphate
Leu, L	leucine
LiBr	lithium bromide
Lys, K	lysine
M	molar
MALDI-ToF	matrix-assisted laser desorption/ionization - time-of-flight
Me-DUPHOS	1,2-bis(2,5-dimethylpholano)benzene
MeOH	methanol
Met, M	methionine
$MgSO_4$	magnesium sulfate
mM	millimolar
mp	melting point
mRNA	messenger ribonucleic acid
N_2	nitrogen gas
NAG	*N*-acetylglucosamine
NaH	sodium hydride
$NaHCO_3$	sodium hydrogen carbonate or sodium bicarbonate
NAM	*N*-acetylmuramic acid
$NaNO_2$	sodium nitrite
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
Negative-Peptide	$(H_2N-EEEEGGG)_2KGGGX-NH_2$
NH_3	ammonia
NH_4Cl	ammonium chloride
NMR	nuclear magnetic resonance
O_2	oxygen
org.	organic
PAM	Phenylacetamidomethyl
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
$PdCl_2$	palladium chloride
$Pd(OH)_2$	palladium hydroxide
Phe, F	phenylalanine
pm	picometre
Pmc	pentamethylchromansulfonyl

Positive-Peptide	$(H_2N\text{-RKRKGGG})_2\text{KGGGX-NH}_2$
PPh$_3$	triphenylphosphine
PPh$_3$O	triphenylphosphine oxide
ppm	parts per million
i-PrMgCl	isopropylmagnesium chloride
Pro, P	proline
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
Pyl, O	pyrrolysine
Rev	regulator of expression of virion
RNA	ribonucleic acid
rpm	rotation per minute
Rre	Rev response element
sat.	saturated
Sec, U	selenocysteine
Ser, S	serine
SPPS	solid phase peptide synthesis
Tar	trans-activating response element
Tat	trans-activator of transcription
TEAP	triethylammonium phosphate buffer
TFA	trifluoroacetic acid
TGFβ-1	transforming growth factor beta 1
THF	tetrahydrofurane
Thr, T	threonine
TLC	thin layer chromatography
Trp, W	tryptophane
Trt	trityl
Tyr, Y	thyrosine
Val, V	valin
X	Fmoc-Glu(biotinyl-PEG)-OH or *N*-α-Fmoc-*N*-γ-(*N*-biotinyl-3-(2-(2-(3-aminopropyloxy)-ethoxy)-ethoxy)-propyl)-L-glutamine
YB-1	Y-box binding protein-1

Abstract

Certain amino acids have a high propensity to be found at the intermolecular contact regions in protein-nucleic acid complexes. One of them is arginine whose side chain commonly makes contact through electrostatic interactions with phosphates and hydrogen bonding to nucleobases. Rigidified arginine analogues in which the side chain is cyclised would reduce torsional entropy loss occurring on binding. In this project amino acids were synthesised with cyclic guanidine side chains as modular arginine analogue building blocks for preparation of nucleic acid binding peptides.

The synthetic strategies to prepare the amino acids involve Grignard reactions and Heck coupling reactions with iodo-pyrimidines. The Heck coupling is followed by a catalytic asymmetric hydrogenation to generate the stereo centre. An enzymatic kinetic resolution is essential after the Grignard coupling to provide stereoselectivity.

Small molecule interactions with nucleic acids can be used as a tool for modulating processes like transcription and translation. Ultimately peptide binding screens against the mRNA of TGF-β1 may be undertaken to study the role of protein binding in regulation of translation.

Within this thesis a peptide library was prepared via the split and recombine method using macro-beads which provides valuable groundwork for the aim of continuing research. This would be the synthesis of peptides containing rigidified arginine mimetics that interact with mRNA of TGF-β1 or other nucleic acids.

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1. Introduction

1.1 Amino acids

Amino acids are a class of organic compounds that are important as protein building blocks.

Most natural amino acids possess the amino group in the α-position, i.e. bound to the carbon atom adjacent to the carboxylic acid group. This α-carbon atom is a chiral centre in all twenty naturally occurring amino acids that are the base of all proteins (proteinogenic amino acids, Figure 3) with the exception of glycine. Two conventions are in use to describe the stereo chemistry of amino acids: the Fischer-projection and a three-dimensional drawing (Figure 1). In the Fischer-projection all proteinogenic amino acids show the NH$_2$-group on the left side, i.e. they have L-configuration. In accordance with the priorities as defined by Cahn, Ingold and Prelog nearly all amino acids are of *S*-configuration with the exception of cysteine. The cysteine has per definition *R*-configuration because the sulphur in the cysteine side chain has a higher priority than oxygen[1] (Figure 2).

Fischer projection **three-dimensional configuration**
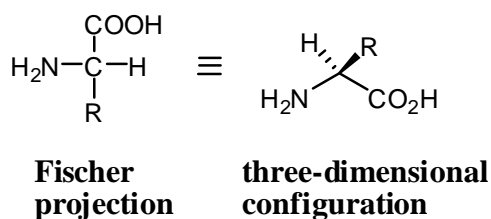
Figure 1: Common conventions to show the stereochemistry of amino acids.
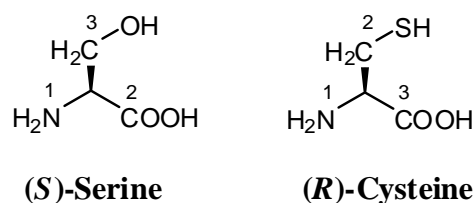
(*S*)-Serine **(*R*)-Cysteine**

Figure 2: Serine and cysteine as examples of naturally occurring *R* and *S* amino acids.

Neutral Amino Acids

Glycine
(Gly, G)

Alanine
(Ala, A)

Valine
(Val, V)

Leucine
(Leu, L)

Isoleucine
(Ile, I)

Cysteine
(Cys, C)

Serine
(Ser, S)

Threonine
(Thr, T)

Asparagine
(Asn, N)

Glutamine
(Gln, Q)

Phenylalanine
(Phe, F)

Tyrosine
(Tyr, Y)

Tryptophan
(Trp, W)

Methionine
(Met, M)

Proline
(Pro, P)

Basic Amino Acids

Lysine
(Lys, K)

Arginine
(Arg, R)

Histidine
(His, H)

Acidic Amino Acids

Aspartic Acid
(Asp, D)

Glutamic Acid
(Glu, E)
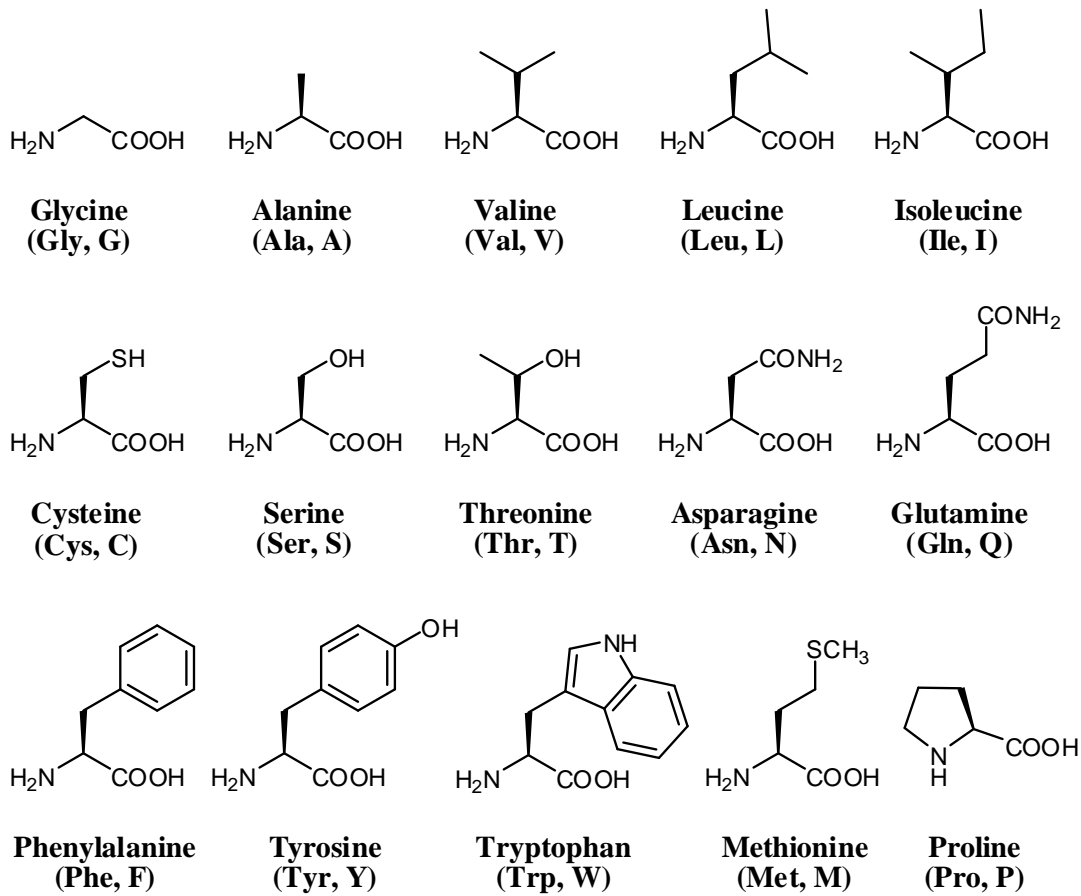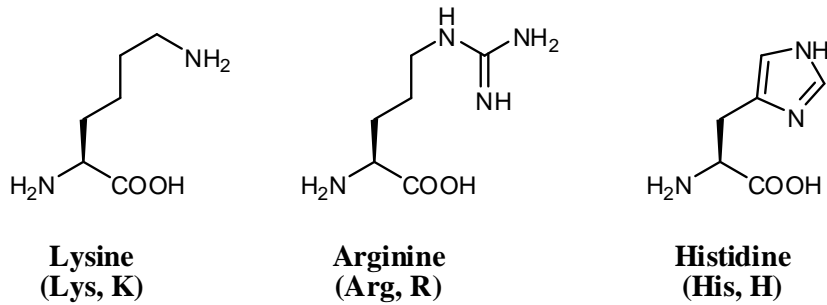
Figure 3: Proteinogenic amino acids distinguished into neutral amino acids, acidic amino acids and basic amino acids.

Three groups of natural amino acids may be distinguished: neutral amino acids (one amino group and one carboxylic acid group), acidic amino acids (one amino and two carboxylic acid groups) and basic amino acids (one carboxylic acid and two basic functionalities). A three-letter-code is often used for simplification to describe amino acids or peptides (e.g. Arg for arginine). The considerably larger proteins can only be described in a practical manner with the one-letter-code (e.g. R for arginine).

The twenty α-amino acids are a necessary requirement of the protein biosynthesis. Only twelve of these can be produced by the human body. The remaining eight are essential amino acids (leucine, isoleucine, valine, threonine, phenylalanine, tryptophan, methionine, lysine) that have to be absorbed from nutrition.[2] In certain proteins further amino acids (Figure 4) do occur as for example hydroxyproline (Hyp, **1**) which is a major component of the protein collagen. Here the hydroxyl group is introduced into the proline on the gamma carbon atom *after* the protein synthesis. This phenomenon is described as "post translational modification".[3] Two further proteinogenic amino acids are selenocysteine (**2**) and pyrrolysine (**3**) which are ribosomally incorporated by transfer RNA with anticodon UCA or CUA, respectively.
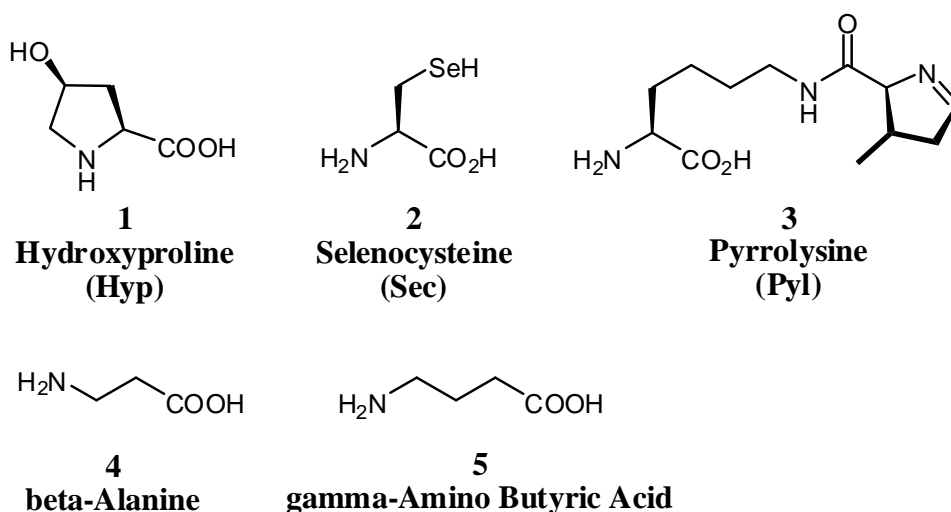
Figure 4: Structures of hydroxyproline, selenocysteine, pyrrolysine, β-alanine and γ-amino butyric acid.

Apart from the α-amino acids there are further biologically important amino acids which are described as β, γ, δ and ε amino acids depending on the position of the amino group. These compounds are not part of proteins but have biological functions as transmitters or

have a part in metabolism. For example β-alanine (**4**) is found in coenzyme A and γ-amino butyric acid (**5**) is an important neurotransmitter in the brain.[3]

Ampholytic character of amino acids

Amino acids present both, basic and acidic properties. Thus an intramolecular neutralisation is possible that leads to zwitterions (Figure 5).
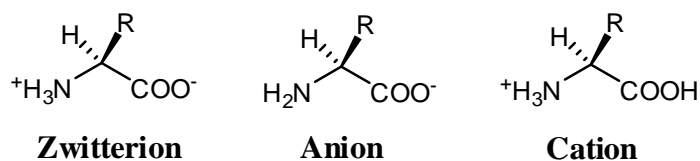
Figure 5: Structure of amino acid depends on pH-value. At isoelectric point (IEP) the amino acid occurs as a zwitterion, at pH-values larger than IEP as anion and at pH-values smaller than IEP as cation.

In aqueous media the amino group can remove the hydrogen ion from the carboxylic group and the NH_3^+ group is then the acidic group of an amino acid. The pK_A is describing the acidity of this group and the pK_B is related to the basic effect of the carboxylate moiety. The isoelectric point (IEP) is the pH-value where the intramolecular neutralisation is complete. A compound occurs as anion if the pH-value is larger than the isoelectric point and as cation if the pH-value is smaller than the isoelectric point.[3]

Isolation and Synthesis of Amino Acids

The proteinogenic amino acids are mainly obtained by isolation from hydrolysed proteins. This procedure cannot be used to gain D-amino acids or unnatural amino acids. Therefore a number of synthetic methods have been developed.

One important method is the Strecker synthesis (Scheme 1). An aldehyde reacts with ammonia and hydrogen cyanide to yield α-aminonitrile. Hydrolysis of the α-aminonitrile gives the final amino acid as racemic mixture.
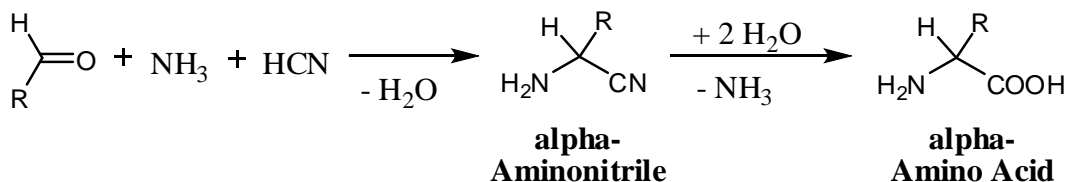
Scheme 1: Strecker synthesis is one of the important methods to synthesise amino acids.

Another important synthetic method is the amination of α-halogen carboxylic acids (Scheme 2). For example α-bromo carboxylic acid can be obtained by Hell-Volhard-Zelinsky halogenation reaction and can then be reacted with an excess of ammonia. The excess is necessary because the amino group of the product amino acid is more nucleophilic than the ammonia and would also react with the α-bromo carboxylic acid.

Hell-Vollhard-Zelinsky Reaction   **Alanine**
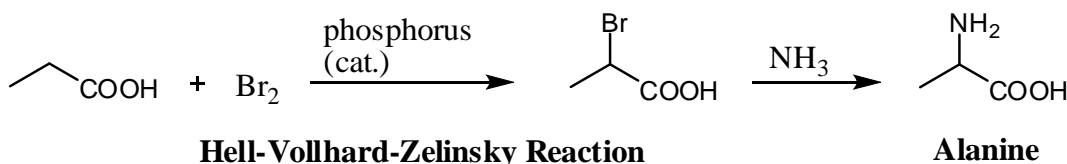
Scheme 2: Alanine synthesis by amination of α-halogen carboxylic acid.

In the Gabriel synthesis this problem is resolved by using potassium phthalimide instead of ammonia (Scheme 3). Potassium phthalimide reacts with for example bromomalonic ester and the product is subsequently alkylated and then hydrolysed. Carbondioxide is set free by heating which yields the desired amino acid.
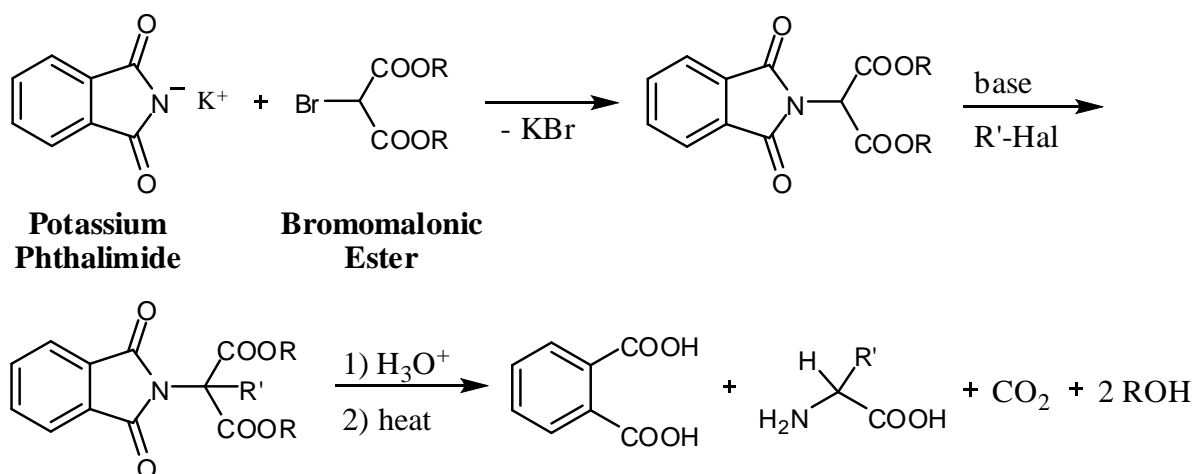
Scheme 3: Gabriel synthesis to prepare amino acids using potassium phthalimide and bromomalonic ester.

The disadvantage of all presented synthesis methods is that the amino acids are obtained as a racemate. In a racemic mixture the amino acid is present as 50/50 mixture of *S*- and *R*-configuration. Separation of the racemate into the enantiomers can take place by certain methods. The three main methods are inoculation of a supersaturated racemic solution with one enantiomer (for racemate which crystallise as conglomerate − pure crystals of each enantiomer), transformation of one enantiomer by enzyme and preparation of diastereomers by reaction with another chiral compound.[1]

Various methods described in the literature for the synthesis of enantiomeric pure compounds include an isomer separation during or at the end of the synthesis. Preparatively this is time consuming and expensive and it is also creates waste for disposal if just one isomer is of significance. Therefore it is of interest to synthetically eliminate unwanted optical isomers as early as possible during the preparation or make both isomers in pure form separately available.

Asymmetric synthesis is the synthesis of optically active compounds from achiral precursors with the use of optically active catalysts or auxiliaries. That offers the possibility to prepare the isomer of interest of amino acids and unnatural amino acids. Unnatural amino acids are of particularly interest for drug development and drug optimisation. For example the generation of peptide derivatives with an increased bioavailability and activity is important for the application of peptide agents. Substitution of *S* amino acids for *R* amino acids results in inhibited proteolysis by proteinase because D amino acids are uncommon in nature. Modifications in the peptide side chains or in the peptide backbone also reduce the degeneration.

1.2 Arginine Derivatives

The 20 naturally occurring amino acids show different propensities to bind with nucleic acids. Protein-DNA and protein-RNA crystal structures show a very high frequency of hydrogen bonding, water mediated hydrogen bonding and van der Waals contacts of arginine to the nucleic acid.[4] The arginine side chain, therefore, is an effective ligand for nucleic acids.

The guanidine group of arginine enables it to perform some specific functions in the organism. Strong ionic binding of the guanidine to carboxylates like aspartic acid ensures specificity at the active site of many target proteins. The concepts and approaches that have been introduced to chemically diversify arginine are numerous and versatile.[5] Therefore only some recent and typical or outstanding examples will be discussed here.

Arginine is an important nitrogen reservoir. As such it participates in the urea cycle and it also is the exclusive precursor of physiological nitrogen oxide (NO). The biochemical functions of NO are manifold and new insights are being gained continuously.

It is known that NO is an important vasodilatator and may provide protection from atherosclerotic events. It is also of importance in blood coagulation. Last but not least it is a potent neurotransmitter that influences functions such as memory and pain regulation. All these physiological processes have motivated researchers in recent years to undertake synthetic efforts in the field of arginine mimetics due to the many potential pharmaceutical applications.

Nitric oxide synthases and arginases are the enzymes that regulate the NO level. A straight forward approach is to identify arginine mimetics that may inhibit selectively arginine sensitive receptors. Foremost the development of thrombin inhibitors has been a field of intensive and extensive work.[6-10]

Already relatively small chemical alterations of arginine as they do occur in natural physiological processes may have significant impact on the binding affinity towards an enzyme. Protein-methyl-Transferases (PRMTs) methylate L-arginine to give N^ω-methylated L-arginines. These compounds are arginase inhibitors. The rate of the biosynthesis of these compounds therefore directly influences the plasma level of arginine and the concentration of NO. Patients that suffer of illnesses such as hypertension, renal disease, high cholesterol or diabetes mellitus do show increased levels of *N*-methylated arginines. An interesting project recently synthesised N^δ-methyl-L-arginine (**6**, Figure 6) and determined its binding properties towards the enzymes that control the NO plasma level. It became apparent that this compound was only a weak inhibitor or in some cases even a poorly binding substrate. Although this compound had also been identified as a natural product previously the authors conclude that their results show that a physiological function in humans is highly unlikely.[8]
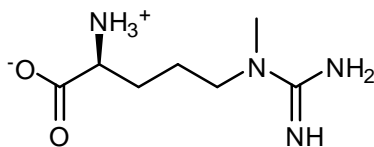
6
N^{δ}**–Methyl-L-Arginine**

Figure 6: Structure of N^{δ}-methyl-L-arginine.

Substrates containing highly basic groups like guanidine are commonly poorly absorbed after oral administration. Hence the bioavailability of arginine mimetics is often far from ideal. A common strategy in the synthesis of arginine mimetics is therefore to reduce the basicity while maintaining or better even improving the binding selectivity. Not long ago an extensive library of arginine based thrombin inhibitors was introduced that showed very good selectivity.[10]

Wang et al. showed that the introduction of a nitro group in the ω-position and alkyl esterification (**7**, Figure 7) improve inhibitory effects on nitric oxide synthase (NOS).[9]
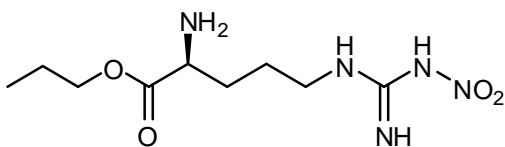
7
N^{ω}**-Nitro-L-Arginine Propyl Ester**

Figure 7: Structure of N^{ω}-nitro-L-arginine propyl ester.

Synthesis strategies to rigidify arginine are of foremost interest. Means to achieve this may be the introduction of double bonds or ring systems. It is generally agreed that a compound promises to be a more effective inhibitor or antagonist for energetic reasons if it possesses fewer degrees of rotational freedom than the natural substrate. Rigidification may also lead to a higher lipophilicity of the molecule and a higher stability towards metabolic enzymes. The latter reasons may both lead to higher bioavailability. It does not come as a surprise that many arginine mimetic synthesis projects appreciate this in their strategies.[6, 10-12]

Enduracididine (**8**, Figure 8) is a rigid arginine derivative and a natural product, too. This compound shows interesting biologic activity. It is a component of the peptide antibiotic enduracidine as well as of the antibiotic minosaminomycin and was also identified in the

cytotoxic fraction of extracts of *Leptoclinides dubius* (a marine ascidian). Recently the stereoselective synthesis was published.[11]

8
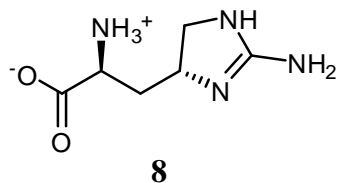
Enduracididine

Figure 8: Structure of enduracididine.

C3a is an important peptide that functions as an anaphylotoxin in the complement system. It promotes inflammatory responses and increased levels of C3a have been related to chronic inflammatory disease such as asthma, rheumatoid arthritis and multiple sclerosis. The inhibition of the C3a receptor (C3aR) therefore seems to be a promising target and Denonne et al. have developed a potent candidate through rigidification of a known inhibitor by incorporating a furan moiety (**9**, Figure 9). However, according to the authors this compound still suffers of the lack absorption when administered orally which is down to the unaltered guanidine moiety.[12]

9
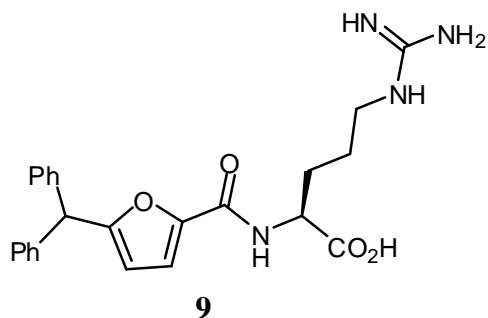
Figure 9: Structure of small molecule ligand of C3a receptor.

1.3 Peptides/Proteins

Amino acids can be polymerised and form chains. This process can be regarded as a condensation reaction (loss of a water molecule). The created CO-NH-linkage is an amide bond but is also commonly described as peptide bond (Figure 10). Depending on the number of linked amino acids these polymers are called di-, tri-, oligo- or polypeptides.
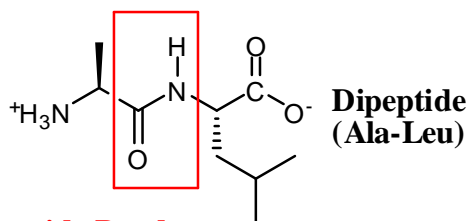
Figure 10: Peptide bond demonstrated as dipeptide Ala-Leu.

Polypeptides are linear polymers and each amino acid takes part in two peptide bonds apart from the ones at the end of the chain. Peptide bonds are stabilised by mesomerism. This mesomerism induces the partial double bond character of the amide group and makes the peptide bond relatively rigid i.e. not freely rotatable (Figure 11). The partial double bond character is confirmed by the measured C-N bond length which is 132 pm in comparison to 147 pm of a normal C-N single bond.[13]
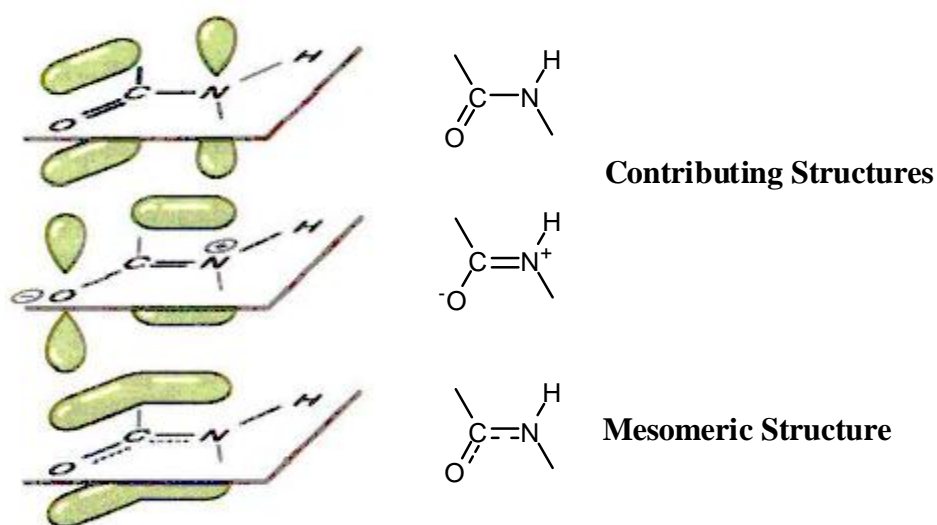
Figure 11: Peptide bonds are stabilised by mesomerism which explains the partial double bond character.[13]

The side chains of the amino acids hinder each other less in the *trans* conformation in which the hydrogen of the substituted amino group is in *trans* position to the oxygen of the carbonyl group (Figure 12). Therefore the *trans* conformation is in general more stable than the *cis* conformation.[13]

trans   **cis**
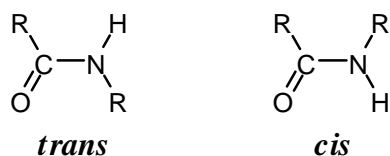
Figure 12: *Trans* and *cis* conformation of the peptide bond.

The peptide backbone is formed by recurring NH-CO-CHR groups with rigid and planar peptide units. The order of the amino acids in the peptide is also called the sequence or primary structure.

Secondary structure accrues by folding from the primary structure and occurs in three forms: α-helix, β-sheet and turns. In the case of L amino acids the α-helix is right-handed and is stabilised by intramolecular hydrogen bonds between every (i +4)th amino acid. The amino acid side chains are directed outwards. β-Sheets are stabilised mainly through hydrogen bonds between neighbouring peptide strands and hydrophobic interactions between side chains on the same face of the sheet. The peptide strands can be organised in parallel or antiparallel alignment and the peptide level resembles a folded sheet. The amino acid side chains point out of the plane.[3] The tertiary structure of a protein describes its overall layout which depends on the configuration of the secondary structure units. Disulfide bonds, salt bridges, hydrogen bonds and hydrophobic interactions are involved in the stabilisation of the tertiary structure. A quatenary structure is formed when several protein subunits form one protein complex.

The proteins are complex structures from folded polypeptide chains which can be several thousand amino acids long. They play an important role in all bioprocesses and have very different applications like as structural proteins (collagen in cartilages, bones and sinews, elastin for elasticity in arteries, keratin in hair and nails), contractile proteins (actin and myosin in muscles), transport proteins (haemoglobin for O_2 transport, transferrin for Fe transport), regulatory proteins (insulin for regulation of blood glucose level, parathormone for regulation of Ca^{2+} and PO_4^{3-} level in the blood), catalytic proteins (enzymes like

oxidoreductase, transferase, hydrolase) and they regulate important functions in gene expression like transcription and translation.[14]

Branched Peptides

Branched peptides are not just a product of synthetic chemistry they are also part of biochemical processes. The protein ubiquitin (76 amino acids) for example binds to other proteins and tags them for degradation. Ubiquitin binds towards other proteins with the amino acid glycine (G76) and forms an amide bond with a lysine side chain of the target protein. It also contains seven lysines which enable the binding of additional ubiquitins.

Another example for naturally occurring branched peptides is peptidoglycan. Peptidoglycan is formed of cross-linked peptide chains of identical peptidoglycan monomers (NAG-NAM-pentapeptide). The monomer consists of two joined amino sugars NAG (*N*-acetylglucosamine) and NAM (*N*-acetylmuramic acid) with a pentapeptide linked to the NAM. The precise structure of the pentapeptide varies with bacterial species and the branching can occur through lysine. The peptidoglycan provides strength in the bacterial cell wall and enables the bacteria to resist osmotic lysis.

It is possible to synthesise branched peptides by reactions on the side chain. Glutamic acid and aspartic acid have a further carboxyl group in the side chain which can react with the amino group of a second amino acid. Lysine and arginine have an amino and guanidine group respectively in the side chain which can react with the carboxyl group of a second amino acid.

Branched peptides have been used in a variety of applications ranging from multiple antigenic peptide systems[15] to gene carriers.[16] Falciani et al.[17] showed for drug-conjugated branched peptides containing the human regulatory peptide neurotensin a retained or increased biological activity by multivalent binding. They also have a considerably higher *in vivo* activity compared to the corresponding peptide monomers due to the fact that they are very resistant towards proteolysis.

1.4 Solid Phase Peptide Synthesis (SPPS)

The solid-phase peptide synthesis (SPPS) has been developed by R. B. Merrifield[18] in 1963. The previous practice of peptide synthesis in solution was very inefficient and the purification has been a major problem. The concept of SPPS involves the covalent binding of the growing amino acid chain on an insoluble resin. That way unreacted soluble reagents can easily be removed by washing and filtration of the resin. Merrifield used a polystyrene resin with CH_2Cl groups on the surface (Merrifield resin). Today a range of resins and linkers are available e.g. Phenylacetamidomethyl (PAM) resin, Rink-Amide resin and Wang resin (Figure 13).
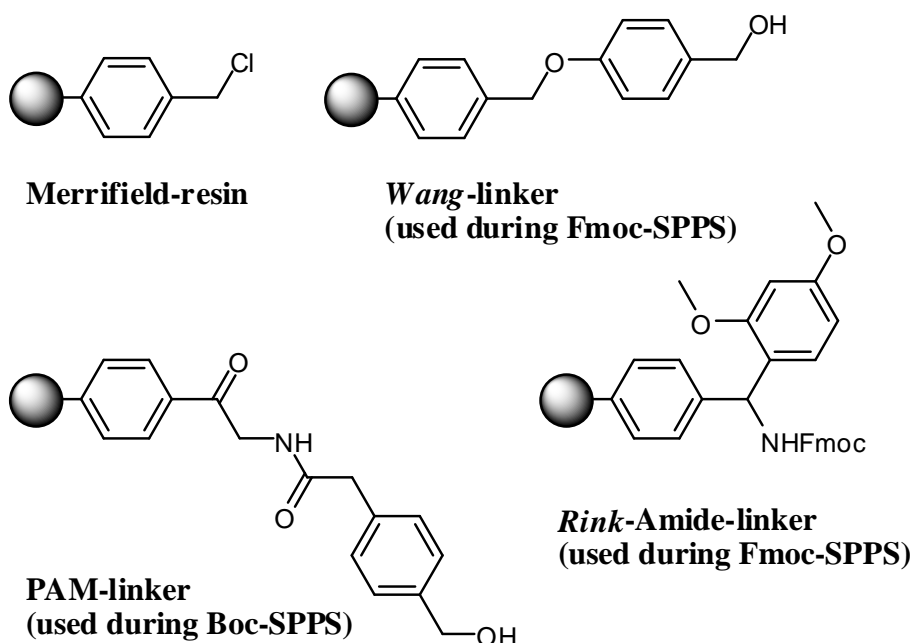
Figure 13: Active group on Merrifield resin surface and examples of linkers for some common resins in SPPS.

The peptide synthesis on the resin proceeds from the carboxyl- to the amino terminus which is in reverse order of protein synthesis *in vivo*. First, the *N*α-protected amino acid that is to form the *C*-terminal end of the peptide is bound through a cleavable linker to the resin. A frequently used *N*α-protecting group is the Fmoc (9-fluorenylmethoxycarbonyl)-protecting group which is base labile. This protecting group can be removed by secondary amines like piperidine. During the peptide synthesis it is also important to protect the functional groups of the side chains of the amino acids used. These protecting groups vary

in the different amino acids and need to be base stable and acid labile for Fmoc-SPPS (Figure 14).
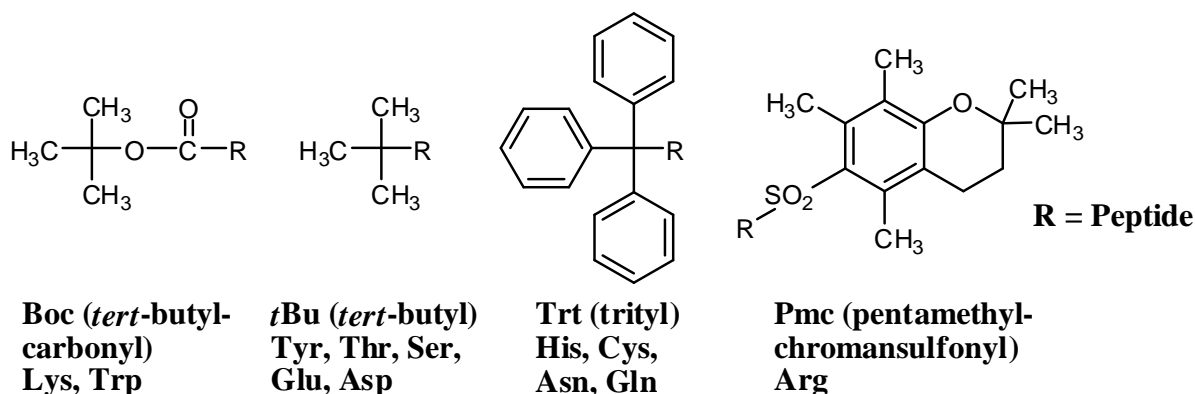
Figure 14: Amino acid side chain protecting groups for Fmoc-Solid Phase Peptide Synthesis.[19] The protecting groups are acid labile and base stable.

The $N\alpha$-protecting group is removed under the appropriate conditions (e.g. piperidine for Fmoc) and the resin is then washed and filtered to remove all soluble reagents. Now the carboxyl group of the amino acid is activated e.g. with BOP or PyBOP to enable the coupling step with the next $N\alpha$-protected amino acid. Under basic conditions BOP or PyBOP and the $N\alpha$-protected amino acid are added at the same time. The free carboxylic acid of the $N\alpha$-protected amino acid reacts with BOP or PyBOP and forms an active ester (Scheme 4 shows activation with BOP). The lone pair of the carboxyl oxygen attacks the positive charged phosphorus of the BOP reagent. A benzotriazololate is split of and attacks then the carboxyl group of the amino acid which creates an ester with a good leaving group. The amino group lone pair of the resin bound peptide attacks the activated carboxyl group and forms a peptide bond under loss of hydroxybenzotriazole (HOBt). The advantage of the PyBOP reagent is that no carcinogen hexamethylphosphoramide (HMPA) is produced during activation.

The amino acid couplings for the peptide growth are characterised by high yields and reproducibility. Often an excess of soluble reagents is used to achieve a full conversion. To remove all soluble reagents after the coupling step the resin is washed and filtered. Now the cycle of $N\alpha$ deprotection and amino acid coupling can be repeated as often as necessary.
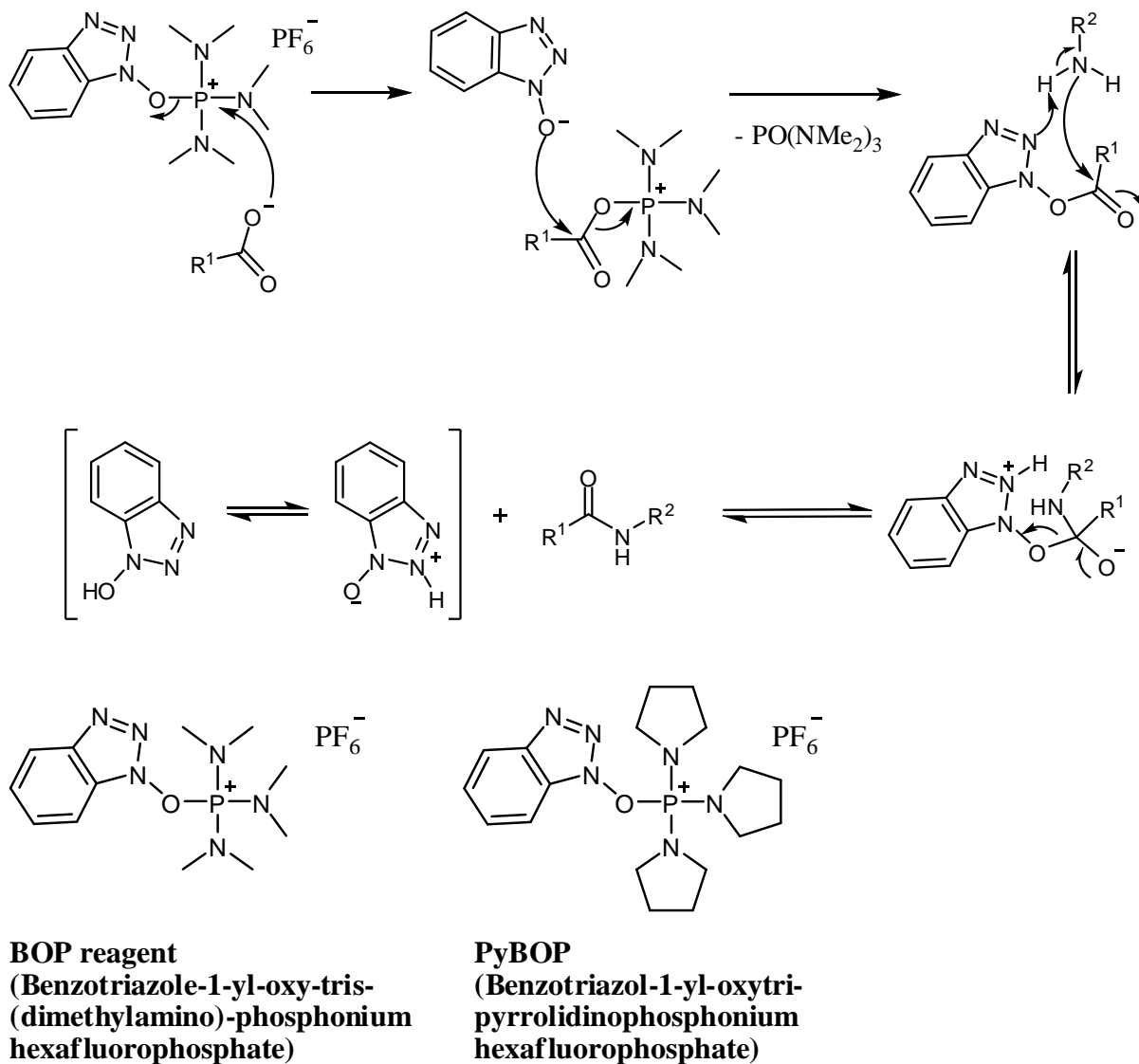
Scheme 4: Mechanism of activation in solid phase peptide synthesis and figures of BOB and PyBOP reagent.

**BOP reagent
(Benzotriazole-1-yl-oxy-tris-
(dimethylamino)-phosphonium
hexafluorophosphate)**

**PyBOP
(Benzotriazol-1-yl-oxytri-
pyrrolidinophosphonium
hexafluorophosphate)**

Repeat of steps
for each amino acid

Deprotection

N-alpha deprotection of resin bound peptide
Washing and filtration of resin to remove excess of soluble reagents

Activation

Activation of the designated amino acid´s carboxylic group and coupling on resin bound peptide
Washing and filtration of resin to remove excess of soluble reagents

Coupling

Cleave peptide from resin and deprotection of side chain protecting groups

Scheme 5: Simplified scheme for solid phase peptide synthesis.

As soon as the desired peptide sequence is synthesised by SPPS, the crude peptide is separated from the resin. This is done under appropriate conditions which remove the protecting groups from the side chains and releases the peptide from the resin. For example a mixture of thioanisol (5.0%), ethanedithiol (2.5%), *m*-cresol (5.0%), water (5.0%) and TFA (82.5%) can be added to the peptide resin and left for some hours at room temperature. Finally, the crude peptide can be purified and analysed.

The SPPS allows the synthesis of defined peptides which can even contain unnatural amino acids. The simplicity of the ever repeating steps in the SPPS also allows the automation of the entire process and offers the possibility to create compound libraries in combinatorial chemistry. A disadvantage of the SPPS is the possible aggregation of the growing peptide chains.[19] The peptide chains are sometimes so intertwined that a further elongation of the peptide strand is no longer possible. The driving force of aggregation is the formation of hydrophobic interactions or hydrogen bonds. Therefore the synthesis of peptides by SPPS with more than 30 amino acids can be difficult.

1.5 Protein/Peptide Binding to Nucleic Acids

One particularly interesting and also challenging problem in molecular biology is to understand the ability of proteins to selectively bind to very specific targets on nucleic acids.

The structural abundance of protein-nucleic acid interactions becomes more and more illustrated with the increasing number of well resolved X-ray structures of complexes described in literature. The very broad spectrum of folding variations of proteins and nucleic acids notably RNA do explain the structural abundance. Consequentially this leads to numerous types of recognition surfaces.[20] To better appreciate interactions between nucleic acids and proteins or potential nucleic-acid-targeted molecules it is crucial to not only determine the structure of nucleic acid complexes but to also clarify their dynamic.[21] The X-ray resolution of complexes is however frequently impaired by crystallisation difficulties or measurement limitations. In such cases studies with simplified model systems may be investigated to afford valuable information. It is possible to determine a complex of the nucleic acid binding domain or a part of the domain and a short

polypeptide in absence of the extensive protein scaffold which recognises the nucleic acid binding domain.[20]

The strength and specificity of peptide-nucleic acid complexes are defined through the orientation of chemical groups on the interface. Typically the attracting forces involved are a combination of hydrophobic, ionic and hydrogen bond interactions. Last but not least also water- or metal-mediated interactions are participating.

Hitherto the general estimation of protein–nucleic acid complexes was based on the view that the task of the protein is to recognise and distinguish specific elements of nucleic acids by applying its tertiary structure. The protein envelopes the nucleic acid and offers certain binding pockets. Moderate changes in the conformation of the protein may take place to optimise the fit. This role allocation was found to be partially reversed in published peptide-RNA complexes.[22] In these cases it was the RNA that wrapped itself around the attached peptide in the complex formation process. Here the tertiary structure of the RNA provided the binding framework and also pockets for specific binding.

Prior to the RNA binding peptides are largely unstructured and develop only upon complex formation mostly α-helix[22, 23] or β-hairpin[24] structure. For example the HIV-1 proteins Tat (trans-activator of transcription, 101 amino acids) and Rev (regulator of expression of virion proteins, 116 amino acids) are unstructured until binding to the mRNA motifs Tar (trans-activating response element) or Rre (Rev response element), respectively. The conclusion is that both RNA and peptide may contribute towards complex optimisation through conformational alterations. Some peptides require a helical structure in the first place to bind specifically to RNA[25] and several α-helix peptides-RNA complexes have been studied.[26] Various peptide-DNA complexes[27] have been investigated in the last decades, too. Conformational alterations of oligopeptides are also known to occur in peptide-DNA complexes. In such complexes the helicity of α-helix peptides increased during binding.[28]

1.6 α-Helix Stabilisation in Oligopeptides

Helices are important recognition motifs and they are the largest class of secondary structure units in proteins. The average length of these units equals two to three helical turns.[29] Separation of the α-helix motif from the tertiary protein structure usually results in peptides with random coil structure which lose much of their ability to specifically bind to the intended target. Different strategies have been developed for α-helix stabilisation in oligopeptides like the introduction of covalent side chain constrains, incorporation of unnatural amino acids and metal complexation.[29]

One complete α-helix turn contains 3.6 amino acids where the side chains in position i, i + 4, i + 7 and i + 11 locate on the same face of the α-helix.[30] Covalent bonds between the i and i + 4 or i + 7 side chain group reduce the conformational flexibility of the peptide and can lock segments of these peptides into helical formation. Common methods are lactam and disulfide bridges (Figure 15). The lactam bridge is formed of amide bonds between the side chains of Lys and Asp/Glu. The side chains are protected with Allyl for Glu and Asp and with Alloc for Lys during peptide synthesis. Treatment with tetrakis (triphenylphosphine) Pd(0) removes these groups without affecting the other protecting groups. The lactam formation can be performed between the carboxyl and the amino function ensuring completion of the reaction by activation with HATU.

Disulfide bonds are formed *via* oxidation (in the air or with iodine) between cysteine side chains in position i and i + 4. The incorporation of D-Cys in position i and L-Cys in position i + 4 is required to realise a helical structure. The L,L-analogue peptides show a highly distorted helical structure and the L,D-analogue results in a random coil or β-sheet peptides.[31] Lactam and disulfide bridges are a powerful tool to stabilise α-helix conformation but they are not always stable in cells.

H-Lys-Glu-Ile-Leu-Arg-Lys-Leu-Leu-Gln-NH$_2$ **Peptide with Lactam-Bridge**
        └──────C(O)-NH──────┘

H-Thr-Trp-Ala-Arg-Asn-cys-Tyr-Asn-Val-Cys-Arg-Leu-Pro-NH$_2$ **Peptide with Disulfide-Bridge**
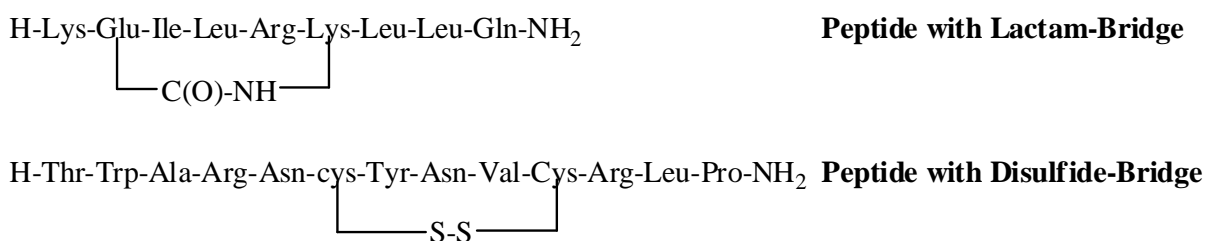   └──────────S-S──────────┘

Figure 15: Examples of peptides with α-helix stabilisation *via* lactam- or D-cys,L-Cys-disulfide-bridge.

This limitation can be overcome by replacing one of the intramolecular hydrogen bonds of the main helix with a hydrocarbon bridge.[30] Unnatural amino acids with olefin side chains are introduced in position i and i + 4 or i + 7. The hydrocarbon bridge is finalised *via* ring closure using Grubbs chemistry (Scheme 6). Introduction of unnatural amino acids have also been reported for α-helix stabilisation *via* disulfide bridges.[32, 33] Jackson et al.[32] for example included the D and L form of *N*-Fmoc-*S*-(acetamidomethyl)-2-amino-6-mercaptohexanoic acid (**10**, Scheme 6) into position i and i + 7 to achieve high helicity in an eight-residue peptide.
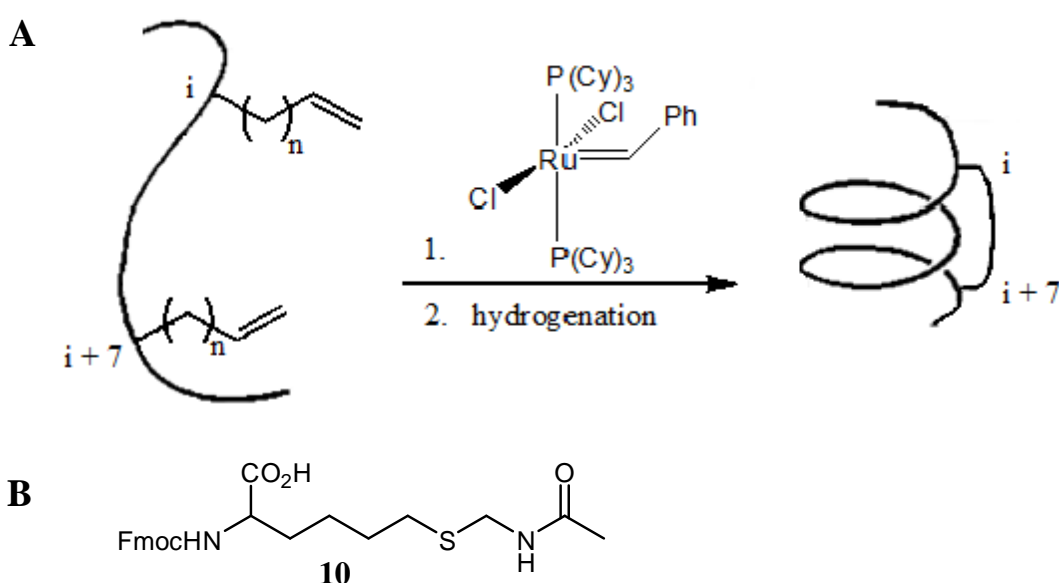
Scheme 6: A: Introduction of unnatural amino acids to stabilise α-helix structure *via* hydrocarbon bridge. B: Unnatural amino acid *N*-Fmoc-*S*-(acetamidomethyl)-2-amino-6-mercaptohexanoic acid (**1**) used for α-helix stabilisation *via* disulfide bridge.

1.7 Transforming Growth Factor beta 1 (TGF-β1)

The TGFB1 gene provides the instructions to produce the protein transforming growth factor beta 1 (TGFβ-1). This protein is a dimer of two identical peptides which are linked by disulfide bridges and consist of 112 amino acids. Each of these peptides is synthesised from a 390 amino acid long protein precursor by proteolytic processes.

The TGFβ-1 protein is found throughout the body and helps to control the growth and division of cells, the differentiation of cells, the cell movement and the apoptosis of cells.[34] The protein also regulates inflammation and wound healing. After the stimulation of a receptor with TGFβ-1 the responds in the cell can be very different depending of the cell type and the state of cell differentiation.

Interestingly the mRNA of TGF-β1 is not translated very well into the protein and protein binding on the GC rich stem loop between nt +77 to +106 of the mRNA (Figure 16) is believed to play an important role for the regulation of translation.[35] The regulation seems to occur by protein complexes where currently only protein YB-1 is identified. YB-1 (Y-box binding protein-1) is a RNA binding protein and functions as a transcription factor. It binds to the GC rich stem loop of the TGF-β1 mRNA and is important for the translation regulation.

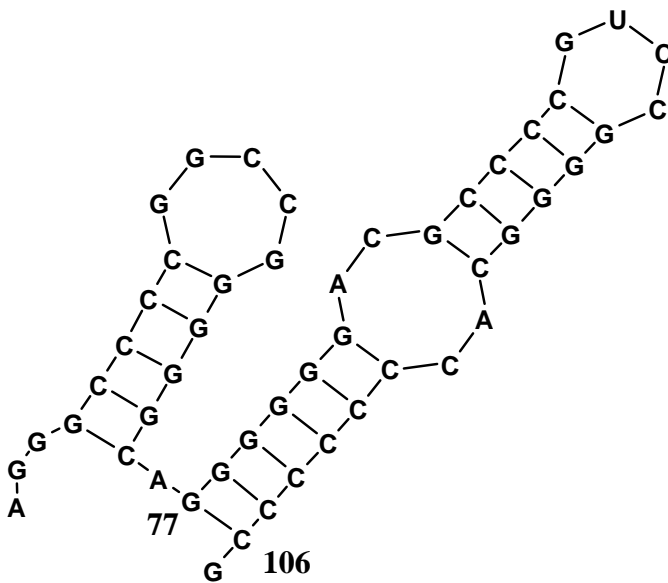**Figure 16:** The GC rich stem loop between nt +77 to +106 of the mRNA of TGF-β1 is believed to play an important role for the regulation of translation.

The GC rich stem loop of the TGF-β1 mRNA is therefore an interesting target to learn more about the protein binding and therefore about the regulation of the translation. Binding test studies with a simplified peptide model system could afford valuable information.

1.8 Scientific Goals

One goal in medical chemistry research is the synthesis of compounds (proteins and small molecules) which interact with nucleic acids to give control over processes like replication of DNA, translation of DNA into RNA and transcription of RNA into proteins. Compounds that interact with nucleic acid have to bind sequence-specifically in order to target solely a definite set of genes or organisms.

This project tries to contribute to this scientific field by gaining new findings in two rather distinct aspects of research.

Firstly the stereoselective synthesis of new constrained unnatural arginine mimetics should be planned and carried out. The planning should incorporate the following determining factors. The syntheses should be short, stereoselective and efficient and where possible also apply strategic concepts that are new to amino acid synthesis.

Secondly the synthesised arginine mimetics should be used to prepare peptide libraries via the split and recombine method using macro-beads. These short peptides should then be used to investigate the binding to TGF-β1 mRNA as this could afford valuable information of the translation of the mRNA.

2. Results and Discussions

2.1 Synthesis of Arginine Mimetics

2.1.1 Introduction

This project investigated hitherto untested synthetic options in the preparation of arginine mimetics. The following considerations influenced the decision on which target molecules were synthetically pursued. The syntheses should be rather short to provide efficient and economically attractive access to the target molecule. It was deemed highly desirable to ensure a high degree of stereoselectivity. The target compounds should be new or only known as racemates. Last but not least two key perceptions of many recent efforts which should also be consulted in this project were the introduction of rigidity and modifications of the guanidine moiety.

2-Amino-5-iodopyrimidine (**11**, Figure 17) was identified to have interesting properties as a starting material that would meet some of the lined out requirements. It is commercially available and as an iodide opens opportunities to use efficient C-C coupling chemistry. It contains a ring system that would provide rigidity and includes the guanidine system. Also it opens the possibility to vary the basicity of any product through hydrogenation of the aromatic ring system.
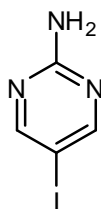
11
2-Amino-5-iodopyrimidine

Figure 17: Structure of commercially available 2-amino-5-iodopyrimidine.

Based on this starting position the molecules **12** and **13** were targeted. They are related to arginine (**14**) but contain a constrained side chain (Figure 18). Similar structures are found

in natural products e.g. capreomycidine antibiotics where the non-proteinogenic amino acid capreomycidine **15** is a constituent. While **12** has not been reported previously **13** has been synthesised but only as a racemate.[36]
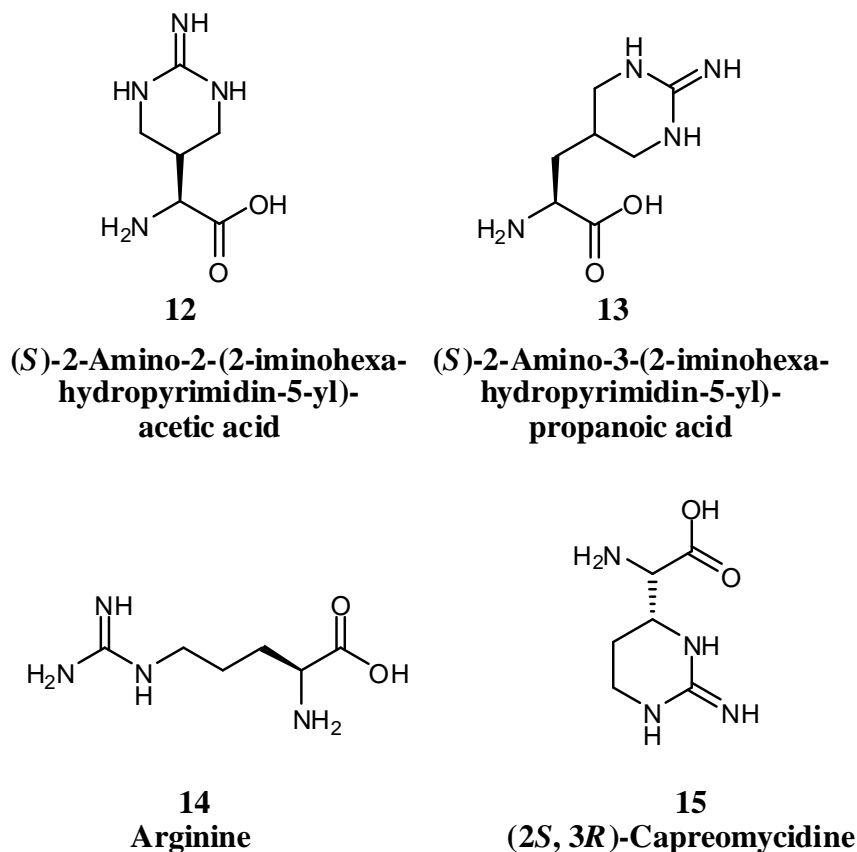

Figure 18: Structure of (*S*)-2-amino-2-(2-iminohexahydropyrimidin-5-yl) acetic acid (**12**), (S)-2-amino-3-(2-iminohexahydropyrimidin-5-yl) propanoic acid (**13**), arginine and (2*S*, 3*R*)-capreomycidine.

The synthetic efforts to prepare these compounds in both enantiomers and in their aromatic or reduced derivatives are described in the following chapter.

2.1.2 Synthesis of (*S*)/(*R*)-AAP and (*S*)/(*R*)-AIP)

(*S*)/(*R*)-AAP (**16**, **17**) and (*S*)/(*R*)-AIP (**13**, **18**) (Figure 19) are four arginine derivatives which were considered to be of interest. These amino acids possess the same number of nitrogen and oxygen atoms and one additional carbon atom as arginine but differ from it by having a constrained side chain. The constrained analogues should show a reduced loss of entropy when binding to a nucleic acid target due to a diminished loss of torsional freedom. The stereocentres of (*S*)/(*R*)-AAP and (*S*)/(*R*)-AIP could be synthesised by the formation of one of the four bonds on the α-centre or a commercially available homochirals could be coupled to a pyrimidine ring.

16	**17**	**13**	**18**
(*S*)-AAP	(*R*)-AAP	(*S*)-AIP	(*R*)-AIP
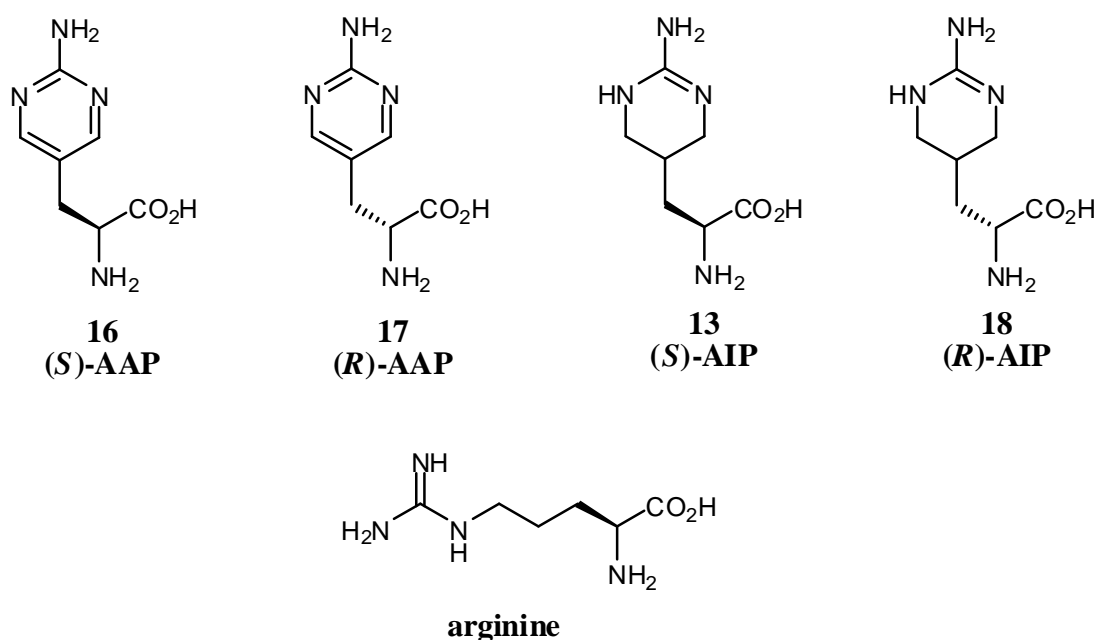
arginine

Figure 19: Structure of (*S*)/(*R*)-2-aminо-3-(2-aminopyrimidin-5-yl)-propanoic acid (**16**, **17**), (*S*)/(*R*)-2-amino-3-(2-iminohexahydropyrimidin-5-yl)propanoic acid (**13**, **18**) and arginine.

The advantage of coupling an amino acid derivative to the pyrimidine ring is that amino acid derivatives are commercially available with very high enantiomerical purity.

2.1.2.1 Synthesis Planning

The group of Richard F. W. Jackson has investigated a palladium catalysed cross-coupling reaction of substituted haloaromatics with organozinc reagents from iodoalanine derivative

19[37, 38] (Scheme 7). Sufficient zinc activation is essential for the formation of the organozinc reagents. The organozinc reagents were prepared using commercial zinc dust which was then activated sequentially with 1,2-dibromoethane and TMSCl in dry DMF. The coupling reaction with haloaromatics were mediated by a palladium catalyst ($Pd(PPh_3)_2Cl_2$ or Pd_2dba_3).[38]
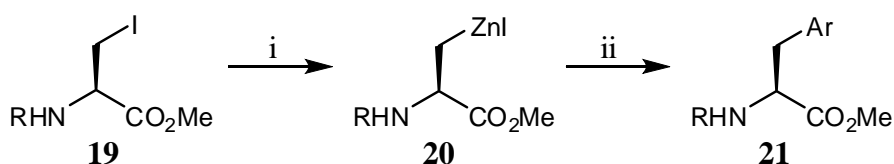
Scheme 7: Reagents and conditions: (i) activated Zn; (ii) $Pd(PPh_3)_2Cl_2$ or Pd_2dba_3, electrophile, DMF, rt.[38]
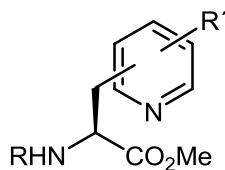
Also a range of pyridyl amino acids (Figure 20) have been synthesised in the group of Richard F. W. Jackson by cross-coupling halopyridine with organozinc reagents based on an iodoalanine derivative **19**.[38]

R = Fmoc or Boc
R′ = NH$_2$ or Br

Figure 20: Pyridyl amino acids.[38]

The results of the Jackson group have shown that theirs is a simple and efficient concept to synthesise unnatural amino acids with high enantiomeric purity. Hence for this project a pathway for the synthesis of the arginine derivatives was chosen (shown in Scheme 8) based on the publications from the Jackson group.[37, 38] The strategy begins with a palladium catalysed Negishi coupling of the commercially available 2-amino-5-iodopyrimidine (**11**) and the enantiomerically pure (*R*) or (*S*) iodoalanine derivative (**22**). In the next step the Boc protecting group would be removed from the coupling product using TFA. A hydrogenolysis of the benzyl protecting group and a reduction of the heteroaromatic ring would be completed using hydrogen gas and a palladium catalyst.
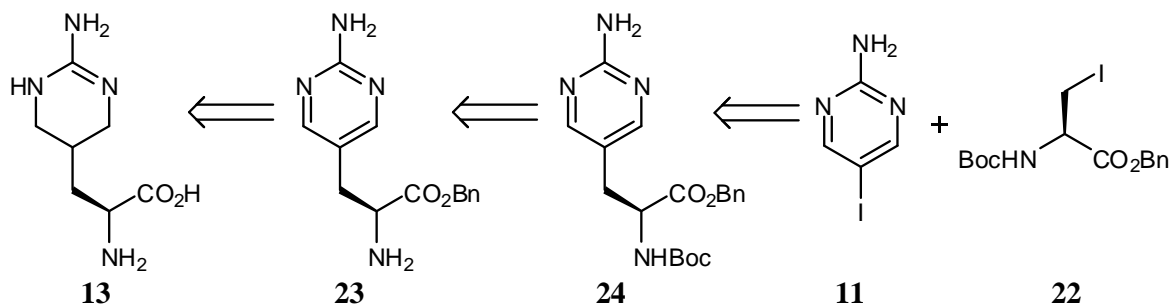
Scheme 8: Retrosynthetic analysis for the synthesis of amino acid **13** based on publications from Richard F. W. Jackson's group.[37, 38]

2.1.2.2 Negishi Coupling

The Negishi coupling was published by Ei-ichi Negishi in 1977.[39] Alkenyl or aryl halide are coupled to an organozinc compound using a nickel or palladium catalyst (Scheme 9). The best results for reaction rate, yield and stereoselectivity are obtained when organozinc compounds are coupled in the presence of Pd(0) catalysts.[39] The active Pd(0) and Ni(0) complexes are relatively unstable but can be generated *in situ* from more stable Pd(II) and Ni(II) complexes. The Negishi coupling has the advantage that many functional groups are tolerated. The reason is that organozinc compounds are less reactive than other organometallic compounds with metals like lithium or magnesium.

R = aryl, alkenyl, alkynyl
R´= aryl, alkenyl, allyl, benzyl
X = Cl, Br, I
L = ligand
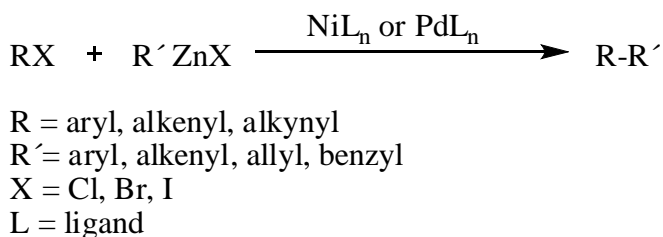
Scheme 9: Principle of Negishi coupling.

For the Negishi coupling of **11** and *N*-(*tert*-butoxycarbonyl)-3-iodo-L-alanine benzyl ester (Boc-beta-iodo-Ala-OBn, **22**) the conditions from the Tabanella[38] publication have been used. First zinc granular was activated in dry DMF with 1,2-dibromoethane and TMSCl. **22** was added and after 6 hours the zinc insertion was complete (TLC). $Pd(PPh_3)_2Cl_2$ and **11** were added and the mixture was stirred for 15 hours at room temperature. Because the TLC monitoring showed still educt **11** the temperature was increased to 40 °C for 4 hours. The work up showed no coupling product and most of the organozinc reagent was

hydrolysed into pyrimidin-2-amine. In further attempts the reaction temperature was first increased to 55 °C and then to 120 °C but no coupling product was found in the work up. The catalyst was changed to $Pd_2(dba)_3$, different solvents like dry THF, dry NMI and dry NMP were tried and as ligands triphenylphosphine or tricyclohexylphosphine were added. The zinc activation was also changed. Zinc dust was used to increase the surface area and the zinc was activated in dry DMF with LiCl, 1,2-dibromoethane and TMSCl (Table 1). The palladium catalysed coupling between **11** and **22** was unsuccessful under these conditions. It was verified that the zinc insertion was performed by working up the mixture of activated zinc and **22** with D_2O after 6 hours. The NMR shoved a full conversion into 5-deuteridepyrimidin-2-amine.

Table 1: Attempts to cross-couple **11** and **22**.

Zinc activation*	Catalyst	Ligand	Solvent (dry)	Temp. [°C]	Time [h]
I	$Pd(PPh_3)_2Cl_2$	-	DMF	rt-40	18
II	$Pd(PPh_3)_2Cl_2$	-	THF	55	24
II	$Pd(PPh_3)_2Cl_2$	-	DMF	120	17
II	$Pd_2(dba)_3$	PPh_3	NMI	120	5
I	$Pd_2(dba)_3$	$PCyp_3$	NMP	100	18

* Zinc activation **I**: zinc, dry DMF and 1,2-dibromoethane heated for 30 min. at 90˚C, TMSCl added at room temperature and stirred for 30 min., **II**: zinc dust plus dry LiCl on vacuum line for 20 min., dry DMF and 1,2-dibromoethane added and heated for 30 min. at 90˚C, TMSCl added at room temperature and stirred for 30 min.

It was considered that the amino group of **11** might hinder the coupling. Normally anilines can be tolerated in coupling reactions with organozinc reagents as shown in the publication of Tabanella[38] but the publication also mentions coupling problems in position 5 of the more acidic 2-amino-5-iodopyrimidine. So the amino group of 2-amino-5-iodopyrimidine was protected with two benzyl protecting groups to investigate the coupling with this compound.

The dibenzyl protection turned out to be difficult probably due to the delocalization of the amine lone pair of **11** into the aromatic ring system. Parameters like the nature of the base ($NaOH$, K_2CO_3, DBU), reaction time (0.5 h (MW) to 16 h), reaction temperature and

solvent system were investigated but just led to the monoprotected amine (Table 2). C. B. Reese[40] and T. Saitoh[41] published the dibenzyl protection of aromatic amines using sodium hydride as base. Using this strong base finally resulted in the dibenzyl protected amine **17** in good yields (Table 2, Scheme 10).

Table 2: Investigated parameters for dibenzyl protection of **11**.

BnBr [eq.]	Base (eq.)	Solvent	Time [h]	Temp. [°C]	Monoprotected [%]	Diprotected [%]
2.0	NaOH (2.2)	water	0.5 (MW)	80	31	-
2.9	K₂CO₃ (3.2)	dry DMF	16	80	33	-
2.9	K₂CO₃ (3.2)	dry THF	16	80	34	-
2.9	K₂CO₃ (3.2)	dry MeCN	16	80	40	-
5.9	K₂CO₃ (3.2)	-	15	100	50	-
2.9	DBU (2.9)	dry DMF	16	80	39	-
2.9	DBU (2.9)	dry THF	16	80	39	-
2.9	DBU (2.9)	dry MeCN	16	80	39	-
5.9	DBU (9.9)	-	15	100	34	-
2.5	NaH (3.2)	dry THF	19	rt	14	78
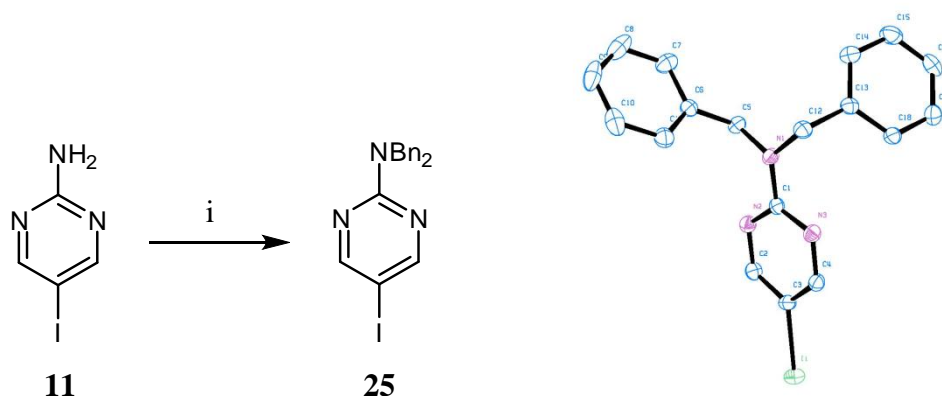
Scheme 10: Reagents and conditions: (i) NaH, BnBr, THF, 0 °C – rt, 19 h at rt, 78%; Crystal structure of **25**.

The palladium catalysed coupling with zinc activation was then investigated between the dibenzylprotected pyrimidine **25** and the (*S*) iodoalanine derivative **22**. Different catalyst

and ligand were investigated (Table 3) but it seems that position 5 of **25** is too unreactive for the Negishi coupling and no desired product was found in the work up.

Table 3: Attempts to cross-couple **25** and **22**.

Zinc activation*	Catalyst	Ligand	Solvent (dry)	Temp. [°C]	Time [h]
II	Pd(PPh$_3$)$_2$Cl$_2$	-	DMF	100	15
II	Pd(PPh$_3$)$_2$Cl$_2$	-	DMF	100	21
II	Pd(PPh$_2$)$_2$(Cp)$_2$FeCl$_2$	AsPh$_3$	DMF	100	24

* Zinc activation **II**: zinc dust plus dry LiCl on vacuum line for 20 min., DMF and 1,2-dibromoethane added and heated for 30 min. at 90°C, TMSCl added at room temperature and stirred for 30 min.

2.1.2.3. Heck Reaction

A new synthesis strategy was devised, where the pyrimidine compound would be coupled to a 2-aminoacrylic acid derivative **26** *via* Heck coupling followed by stereoselective reduction of the double bond. The amino protecting group would be removed by an acid and the ester hydrolysed with a base (Scheme 11).
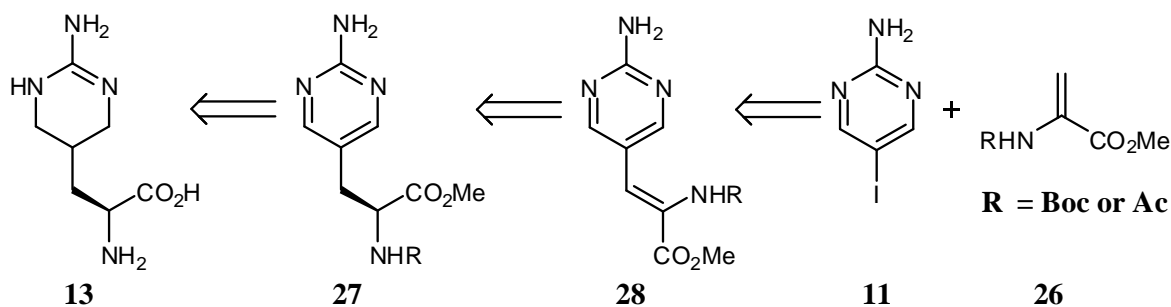
Scheme 11: New synthesis pathway for the synthesis of amino acid **13** based on Heck coupling and stereoselective reduction of the double bond.

Heck reactions are palladium catalysed C-C couplings where a vinyl hydrogen is substituted with a moiety like aryl, vinyl or benzyl. A stoichiometric amount of base is required and the double bond is maintained in this process.

C. Chan et al. [42] reported a Heck coupling with Pd(OAc)$_2$ and tri-*o*-tolylphosphine where the double bond of the terminal alkene moiety is created *in situ* from the commercially available compound **30** with four equivalents of triethylamine (Scheme 12).
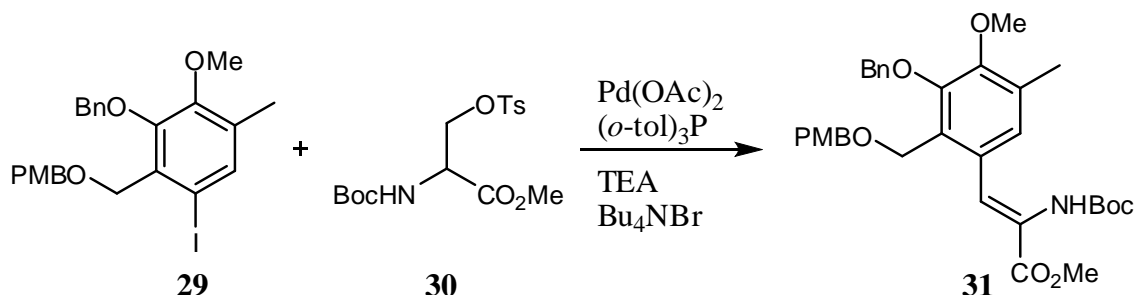

Scheme 12: Synthesis of **31** by *in situ* formation of the double bond from compound **30** followed by Heck coupling to compound **29**.[42]

Hence the Heck coupling was investigated with compound **11** under these conditions but no coupling product was observed. The reaction temperature was then increased from 80 °C to 120 °C and the solvent changed from acetonitrile to DMF but still no coupling product was discovered (Scheme 13).
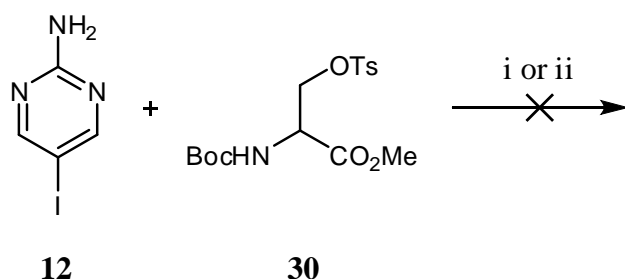
Scheme 13: Heck coupling reaction based on publication from C. Chan [42] did not work for compound **11** with (i) Pd(OAc)$_2$, Bu$_4$NCl, Et$_3$N, CH$_3$CN, 80 °C, 72 h or (ii) Pd(OAc)$_2$, PPh$_3$, Et$_3$N, DMF, 120 °C, 88 h.

The amino group of **11** was then protected with two Boc-protecting groups to exclude the free amino group as a possible inhibitor of the Heck coupling. Pyrimidine derivative **11** was refluxed in THF with di-*tert*-butyldicarbonate (2.4 eq.) and 4-dimethyl-aminopyridine (0.26 eq.) to give the di-Boc-protected compound **32** with 97% yield (Scheme 14).
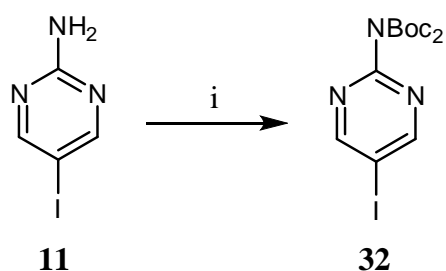
Scheme 14: Reagents and conditions: (i) Boc₂O, DMAP, THF, reflux at 80 °C, 24 h, 97%.

The Heck coupling with compound **30** and di-Boc-protected compound **32** was investigated and parameters like the type of the base, catalyst, reaction temperature and solvent system have been tested (Table 4) but no desired product was observed under the tested conditions.

Table 4: Heck coupling attempts between **26** and **24**.

Base	Catalyst	Ligand	Solvent	Temp. [°C]	Time [h]
Et₃N	Pd(OAc)₂	PPh₃	CH₃CN	80	24
Et₃N	Pd(OAc)₂	PPh₃	DMF	110	18.5*
NaHCO₃+ TBACl	Pd(PPh₃)₂Cl₂	-	NMP	130	18*
CaCO₃ + TBACl	Pd(PPh₃)₂Cl₂	-	NMP	130	18*

* Compound **26** was added after two hours of waiting time.

M. J. Reno[43] reported Heck coupling reactions also with 2-amino-5-iodopyrimidine as substrate which yielded 26% and 36% coupling product (Scheme 15). Therefore it was surprising not to find a coupling product at all even not with a small yield under the numerous applied conditions.
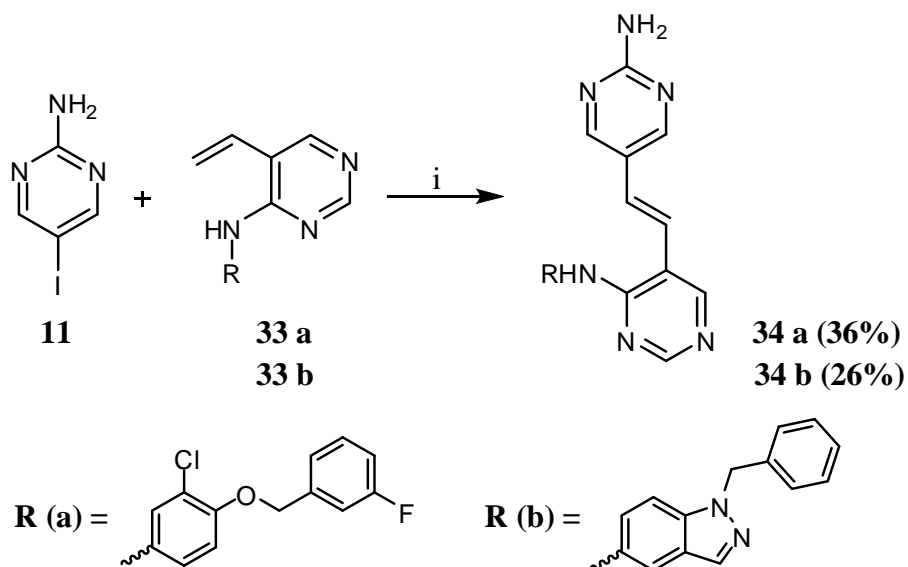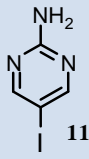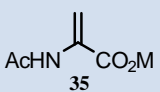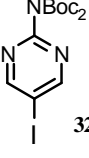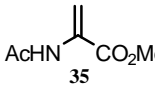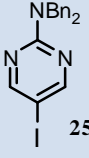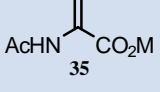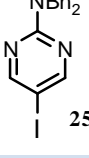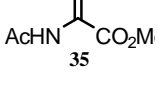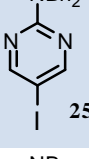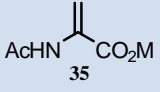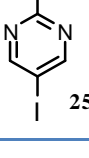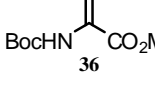
Scheme 15: Heck coupling using 2-amino-5-iodopyrimidine (**11**) published by M. J. Reno.[43] Reagents and conditions: (i) Pd(OAc)$_2$, (o-tol)$_3$P, Et$_3$N, DMF, 100 °C, 5 h.

It was thought that maybe the creation of the double bond *in situ* from **30** was the difficulty as the alkene was not found in the work-up. Therefore methyl-2-acetamidoacrylate (**35**) was used instead of **30**.

Using Pd(OAc)$_2$, triethylamine and tri-o-tolylphosphine in DMF at 100 °C resulted in no coupling product for **11** and **35** (Table 5, Entry 1) and **32** and **35** (Table 5, Entry 2). Traces of product have been found with the dibenzyl protected derivative **25** (Entry 3). Changing the base to NaHCO$_3$ and adding Bu$_4$NCl gave again just traces of product with derivative **25** (Entry 4) but changing the catalyst to PdCl$_2$ and working under dry conditions with dry DMF finally resulted in 80% of coupling product **37** (Entry 5).

Table 5: Heck coupling attempts between pyrimidine derivatives and **35**.

Pyrimidine Deriv.	Ene Deriv.	Base	Catalyst	Ligand	Solv.	Temp. [°C]	Prod.
NH_2 pyrimidine, I, **11**	AcHN–CO$_2$M **35**	Et$_3$N	Pd(OAc)$_2$	(*o*-tol)$_3$P	DMF	100	-
$NBoc_2$ pyrimidine, I, **32**	AcHN–CO$_2$M **35**	Et$_3$N	Pd(OAc)$_2$	(*o*-tol)$_3$P	DMF	100	-
NBn_2 pyrimidine, I, **25**	AcHN–CO$_2$M **35**	Et$_3$N	Pd(OAc)$_2$	(*o*-tol)$_3$P	DMF	100	traces
NBn_2 pyrimidine, I, **25**	AcHN–CO$_2$M **35**	NaHCO$_3$ Bu$_4$NCl	Pd(OAc)$_2$	(*o*-tol)$_3$P	DMF	100	traces
NBn_2 pyrimidine, I, **25**	AcHN–CO$_2$M **35**	NaHCO$_3$ Bu$_4$NCl	**PdCl$_2$**	(*o*-tol)$_3$P	**dry DMF**	100	**80%**
NBn_2 pyrimidine, I, **25**	BocHN–CO$_2$M **36**	NaHCO$_3$ Bu$_4$NCl	**PdCl$_2$**	(*o*-tol)$_3$P	**dry DMF**	70	**70%**

For the coupling of **25** to methyl-2-(tert-butoxycarbonylamino)acrylate (**36**) the temperature had to be dropped to 70 °C otherwise the Boc protecting group was removed. Because of this lower temperature the reaction time had to be increased to 19 hours to give coupling product **38** in 70% yield (Entry 6 and Scheme 16).
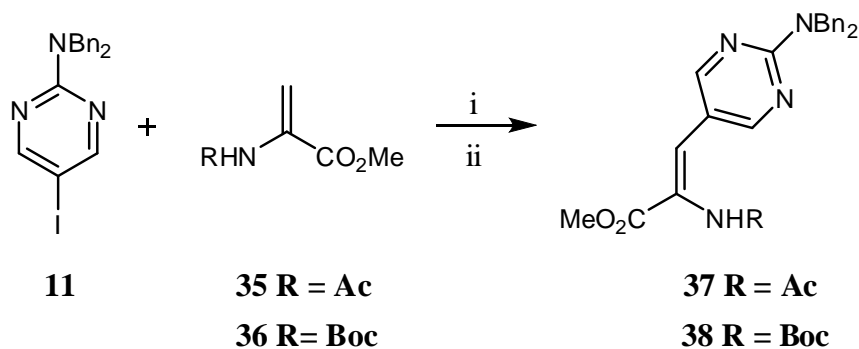
Scheme 16: Reagents and conditions: (i) for R = Ac: PdCl$_2$ (15 mol%), (*o*-tol)$_3$P (15 mol%) , NaHCO$_3$, Bu$_4$NCl, dry DMF, 100 °C, 4 h, 80% (ii) for R = Boc: PdCl$_2$ (20 mol%), (*o*-tol)$_3$P (15 mol%), NaHCO$_3$, Bu$_4$NCl, dry DMF, 70 °C, 19 h, 70%.

It was assumed that the cleavage of the benzyl protecting groups would require comparatively harsh conditions so the Heck coupling was also undertaken with the di-Boc-protected pyrimidine **32** and compound **36** under dry conditions. This yielded in 30% coupling product **39** and 20% of the 5,5´-bipyridine **40** (Scheme 17).
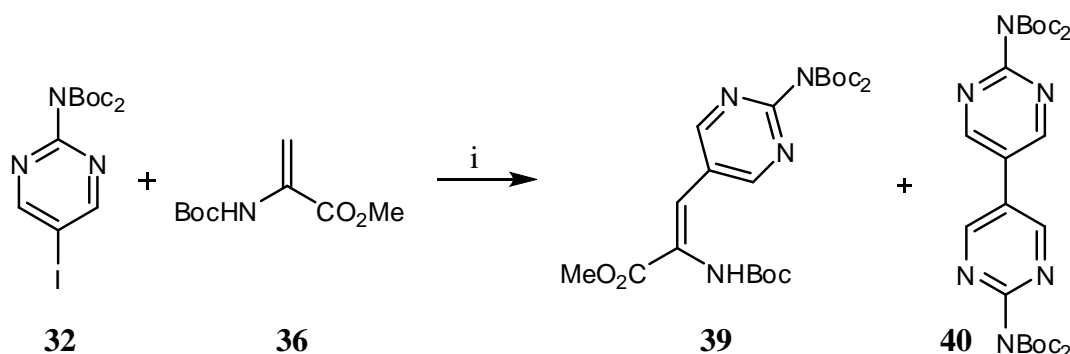
Scheme 17: Reagents and conditions: (i) PdCl$_2$ (20 mol%), (*o*-tol)$_3$P (20 mol%) , NaHCO$_3$, Bu$_4$NCl, dry DMF, 70 °C, 19 h, 30%.

One major issue is, that the reaction is very slow at 70˚C and after 19 hours reaction time both starting materials are still visible on the TLC. But at higher temperatures the Boc-protecting groups get removed and the reaction ends in a complex mixture. Another issue is, that the di-Boc-protected pyrimidine **32** tends to couple with itself to afford a 5,5´-bipyrimidine. This was also reported from J. R. Medina[44] who published the synthesis of 2-substituted-5-hydroxypyrimidines (Scheme 18). 5-Bromopyrimidines were subjected to a Miyaura-type aryl boronate synthesis. The di-Boc-protected 5-bromo-pyrimidine **41** showed a significant amount of the symmetric biaryl in the reaction mixture, demonstrating the propensity to undergo Suzuki coupling under these mild conditions. The

biaryl formation was than minimized by using three equivalent of bis(pinacolato)diboron relative to the starting material.[44]
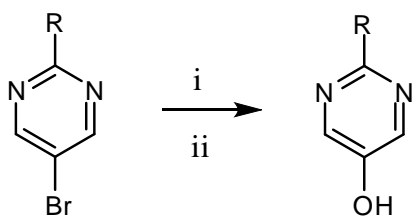
41 R = NBoc$_2$ **42** R = NBoc$_2$

Scheme 18: Reagents and conditions: (i) Pd(OAc)$_2$, KOAc, bis-(pinacolato)diboron (3 eq.), DMF, 85 °C, overnight; (ii) NaBO$_3 \cdot$ 4 H$_2$O, THF/H$_2$O (1:1), rt, overnight, 96%. [44]

During the Heck reaction the substitution is favoured on the sterically less hindered carbon of the olefin. The Heck product normally is preferentially generated as the thermodynamically more stable E-configured olefin. However exceptions are known in the literature. In cases of substrates with amino and carbonyl moieties (Z)-stereochemistry is strongly preferred.[42, 43, 45, 46] So it was investigated if the (Z)- or the (E)-geometry was achieved in the Heck coupling of the pyrimidines. The NOESY of compound **31** and **32** was measured and showed a cross peak between H-1 and H-2 (Figure 21) which defines the compounds as (Z)-coupling products.
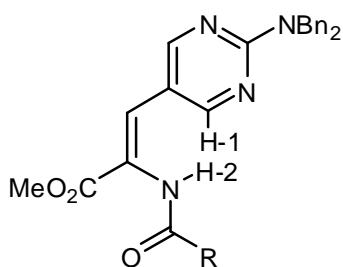
Figure 21: In a Heck-(Z)-coupling product H-1 and H-2 are close enough to show a cross peak in a NOESY measurement.

Aside from the NOESY measurements it was tried to investigate the stereochemistry by a proton coupled ^{13}C NMR experiment. Prasad[45] determined the geometry of the double bond by proton coupled ^{13}C NMR experiments. They measured the H-C three-bond coupling constant between the vinyl proton and the carbonyl carbon of the methyl ester (Figure 22) and compared the data to the known $^3J_{COOR, H}$ coupling constants (cis = 8 Hz, trans = 12.6 Hz).[47]
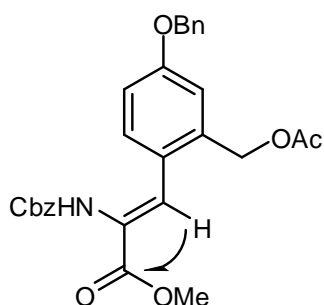
Figure 22: Stereochemistry determined by Prasad[45] by measuring the H-C three-bond coupling constant between the vinyl proton and the carbonyl carbon in a proton coupled ^{13}C NMR experiment.

To verify the structure with the same method, a proton coupled ^{13}C NMR was measured of compound **38**. But also with over 4000 scans it was not possible to obtain a definite multiplet to determine the coupling constants (Figure 23 A). Ester **38** was than hydrolyzed with three eq. LiOH · H$_2$O (Scheme 19) to eliminate the coupling of the carbonyl carbon with the protons of the methyl group. The proton coupled ^{13}C NMR was than measured with compound **43** and resulted in an unresolved multiplet looking like a broad singlet for the carbonyl carbon of interest (Figure 23 B).
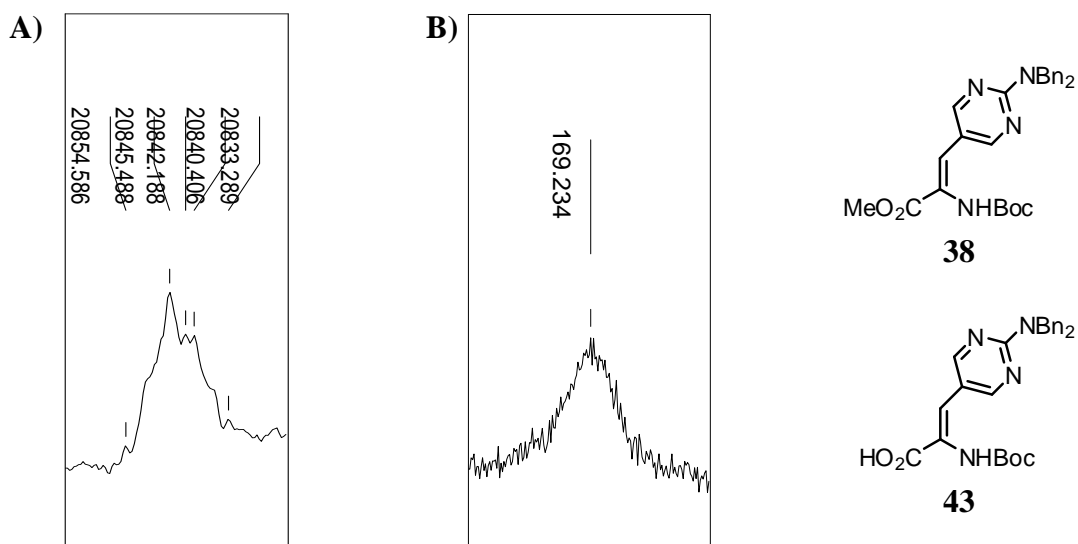
Figure 23: Proton coupled ^{13}C NMRs were measured with over 4000 scans to investigate the stereochemistry A) compound **38** showed an undefined multiplet for the carbonyl carbon of the methyl ester; B) compound **43** showed a broad singulet for the carbonyl carbon of the carboxylic acid.
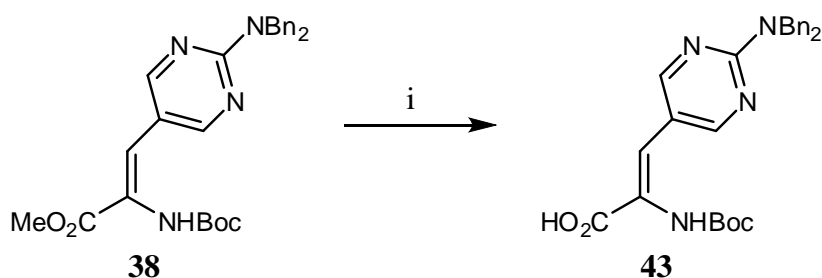
Scheme 19: Reagents and conditions: (i) Hydrolysis of ester **38** with lithium hydroxide monohydrate (3 eq.), MeOH/THF/water (8:4:1), rt, 27 h, 95%.

The proton coupled ^{13}C NMR experiments have not provided the defined multiplet to determine the coupling constants. The problem might be that the carbonyl signal is not very strong in the ^{13}C NMR anyway and H-C three-bond couplings are weak leading to a noisy poorly resolved signal from which the coupling constant cannot be determined. Fortunately, the stereochemistry was also resolved by crystal structure of the Heck coupling compound **37** (Figure 24).

Crystals were grown using the Vapour Diffusion Method. Some material is dissolved in a solvent (S1) where it is good soluble in and placed in a closed vial with a small hole in the lid. This vial is placed in a larger closed vial containing a second solvent (S2) in which the material is less soluble in. Slow diffusion of S2 into the smaller vial and S1 out of that vial causes crystals to form.

Various solvents were tested for S1 and S2 to crystallise **37** and **38** but resulted always in thin needles. Finally a crystallisation attempt with compound **37**, chloroform (S1) and hexane (S2) gave large enough needles to determine the crystal structure. The structure shows a Z configuration with a disordered OCH$_3$ group. The OCH$_3$ group points in two orientations in the crystal.
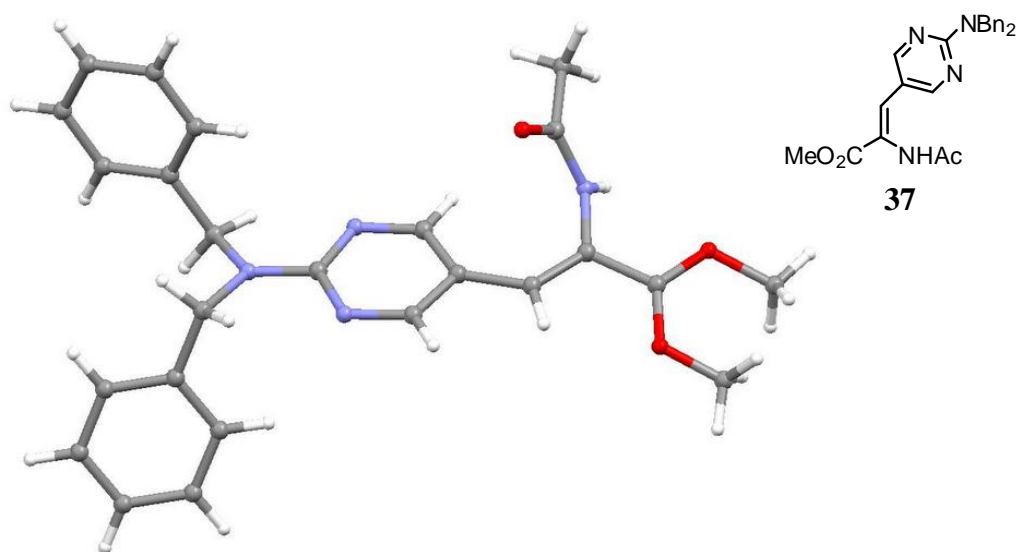
Figure 24: Crystal structure of Heck coupling compound **37** with a disordered OCH₃ group. The compound crystallises in thin needles where two orientations are present for the OCH₃ group.

In order to introduce chirality into the Heck coupling compounds the asymmetric hydrogenation was chosen. It uses inexpensive hydrogen gas and a very small amount of a chiral catalyst. This provides a powerful way to produce enantio-enriched compounds in large quantity without forming waste.

2.1.2.4 Asymmetric Hydrogenation

In the mid-sixties Geoffrey Wilkinson discovered the properties of chlorotris-(triphenylphosphine)rhodium (RhCl(PPh₃)₃, Wilkinson catalyst) as soluble hydrogenation catalyst for unhindered olefins.[48] By replacing the triphenylphosphine with a chiral phosphine an asymmetric hydrogenation takes place. Since the two enantiomers of a chiral drug molecule often have different effects on cells, it is important to be able to produce each of the two forms in high purity. Various chiral bidentate bisphosphine ligands have been developed for this purpose, some of the more popular being DIOP, BINAP, DUPHOS and DIPAMP (Figure 25).
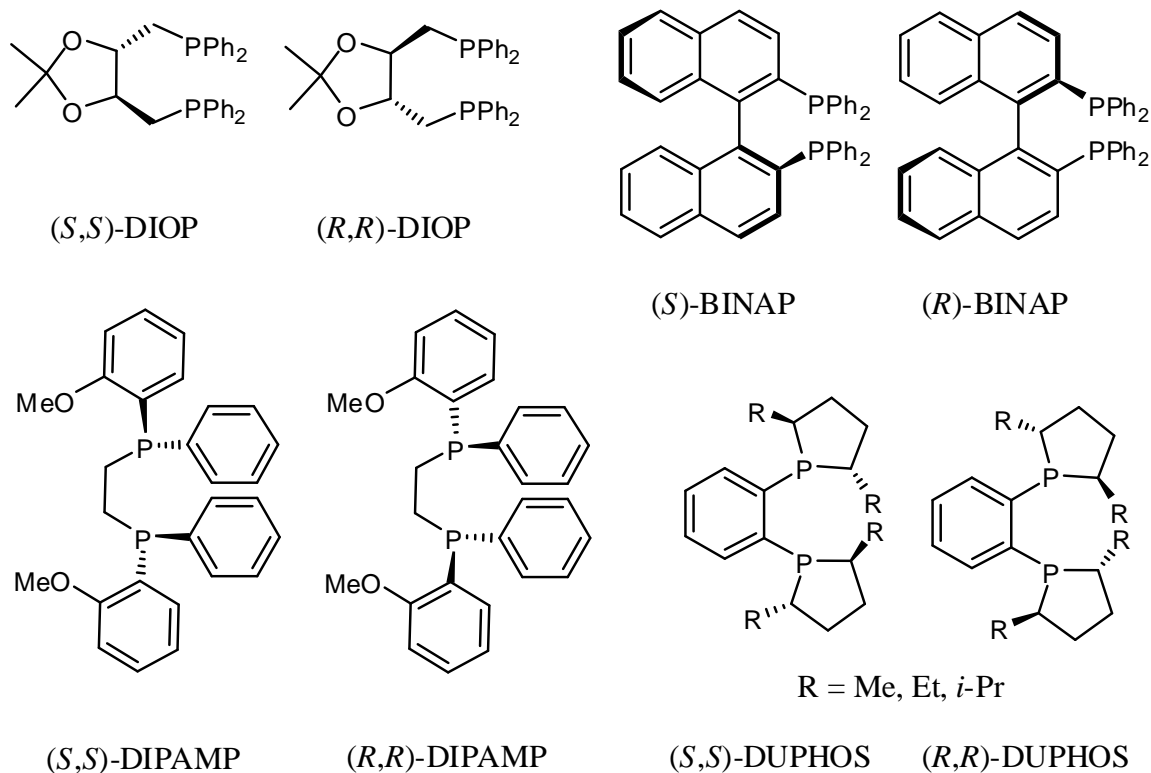
Figure 25: Examples of chiral bisphosphine ligands for the asymmetric hydrogenation.

The well known and inexpensive bisphosphine ligand BINAP was chosen to test how the asymmetric hydrogenation would work out. Rh(COD)$_2$BF$_4$ (5 mol%) and (*R*)-BINAP (10 mol%) were added to compound **37** in a stainless steel high pressure reactor. The mixture was stirred in degassed methanol at room temperature with 5 bar hydrogen pressure. The reaction progress was checked by TLC but after 20 hours there was still no product produced. So the hydrogen pressure was increased to 7 bar which is the maximum pressure the hydrogen generator can produce. After a further 21 hours the reaction solution was worked up and 10% hydrogenation product was isolated by column chromatography (Table 6, Entry 1). The asymmetric hydrogenation was repeated with compound **38** at 40°C and with 7 bar of hydrogen from the reaction onset. The reaction was TLC monitored and showed a slower reactivity in comparison to compound **37**. A very small product spot was visible on an especially long TLC (starting material and product have very similar R$_f$ values) but the product could not be isolated by column chromatography (Table 6, Entry 2).

W. S. Knowles discussed the use of different phosphine ligands for olefins with a free carboxylic acid or an ester. Seven-membered bis(phosphines) like DIOP or BINAP are often inefficient when the free carboxylic acid is changed to a derivative such as an ester. In comparison five-membered bis(phosphines) like DIPAMP usually work quite well.[48]

Therefore the hydrogenation was carried out with compound **38**, 5 mol% Rh(COD)$_2$BF$_4$, 10 mol% (*S,S*)-DIPAMP and 20 bar of hydrogen gas (using a hydrogen gas cylinder) in degassed methanol at room temperature. After 16 hours the TLC just showed a little product spot. So the hydrogen gas pressure was increased again to 35 bar and the reaction mixture was stirred for a further two days. Even with this high pressure the TLC showed still starting material after the two days. Because the starting material and the product have very similar R$_f$ values the conversion was measured by a crude proton NMR (Figure 26). The NMR data demonstrate that 63% of the starting material was left and that 37% had been converted to the reduced product (Table 6, Entry 3).
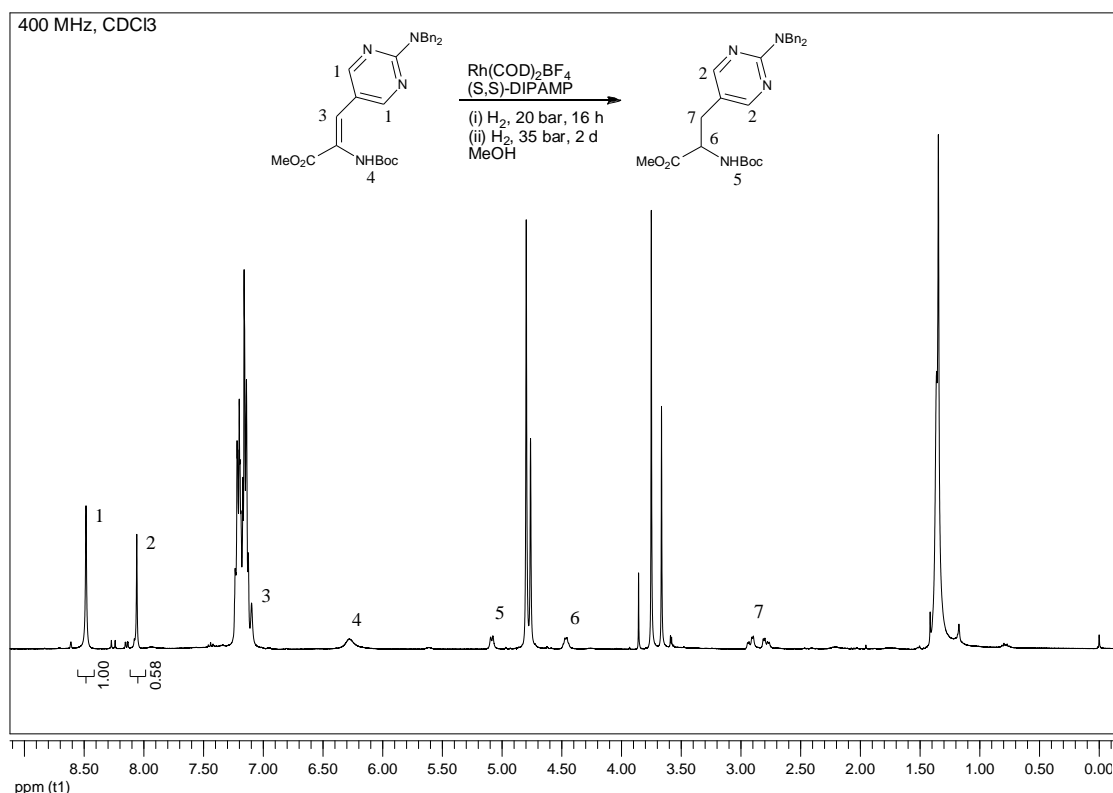
Figure 26: Crude proton NMR shows a conversion of 37% into the reduced product by hydrogenation with 5 mol% Rh(COD)$_2$BF$_4$ and 10 mol% (*S,S*)-DIPAMP at 20 bar for 16 hours and two days at 35 bar.

To improve the conversion an extensive literature research was undertaken which revealed that DUPHOS is frequently used as ligand especially for asymmetric hydrogenations with a Boc-protected α-enamides.[42, 49] Thus the reaction was carried out with compound **38**, 5 mol% Rh(COD)$_2$BF$_4$ and 10 mol% (*S*,*S*)-Me-DUPHOS at 35 bar of hydrogen pressure. After 20 hours a sample was taken and the crude proton NMR showed conversion of 80%. The mixture was left for further two days at 35 bar hydrogen pressure and the crude proton NMR showed now a conversion of 67% (Table 6, Entry 4). Because the yield had decreased already the reaction mixture was worked up. Purification with a very long column chromatography with CH$_2$Cl$_2$/MeOH (200:1) failed because of the too similar R$_f$ values of product and starting material. A preparative TLC separation was then undertaken with CH$_2$Cl$_2$/MeOH (200:1) but the compounds absorbed very strongly to the silica which hindered most of the recovery. In the end just 7% product was recovered. A hydrogenation with compound **38**, 5 mol% Rh(COD)$_2$BF$_4$ and 10 mol% (*R*,*R*)-Me-DUPHOS at 35 bar of hydrogen pressure showed a conversion of 70% after 48 hours (Table 6, Entry 5) and gave 8% yield after the preparative TLC separation. The hydrogenation was then performed with compound **37**, 5 mol% Rh(COD)$_2$BF$_4$ and 10 mol% (*S*,*S*)-Me-DUPHOS at 35 bar of hydrogen pressure for 24 hours. This yielded 92% product (Table 6, Entry 6) after column purification with hexane/ethyl acetate (1:2). Because the reaction works very well with compound **37** under these conditions the amount of used catalyst was reduced. Using 1 mol% Rh(COD)$_2$BF$_4$ and 2 mol% (*S*,*S*)-Me-DUPHOS at 35 bar of hydrogen pressure gave a yield of 67% after column chromatography purification (Table 6, Entry 9). The amount of catalyst was increased again to 2 mol% which yielded 93% product using 4 mol% (*S*,*S*)-Me-DUPHOS or 4 mol% (*R*,*R*)-Me-DUPHOS (Table 6, Entry 7 and 8).

Table 6: Asymmetric hydrogenation on compounds **37** and **38** with Rh(COD)$_2$BF$_4$ and different ligands.
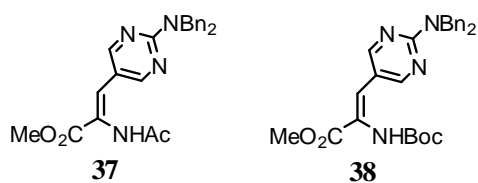
	Compound	Rh cat.	Ligand	Time [h]	H$_2$ [bar]	Temp. [°C]	Conv. [%]
1	37	5 mol%	(R)-BINAP (10 mol%)	20 21	5 7	rt	10
2	38	5 mol%	(R)-BINAP (10 mol%)	20	7	40	traces*
3	38	5 mol%	(S,S)-DIPAMP (10 mol%)	16 48	20 35	rt	37**
4	38	5 mol%	(S,S)-Me-DUPHOS (10 mol%)	48	35	rt	67**
5	38	5 mol%	(R,R)-Me-DUPHOS (10 mol%)	24	35	rt	70**
6	37	5 mol%	(S,S)-Me-DUPHOS (10 mol%)	24	35	rt	92
7	37	2 mol%	(S,S)-Me-DUPHOS (4 mol%)	24	35	rt	93
8	37	2 mol%	(R,R)-Me-DUPHOS (4 mol%)	24	35	rt	93
9	37	1 mol%	(S,S)-Me-DUPHOS (2 mol%)	24	35	rt	67

*determined by TLC; ** calculated by crude proton NMR

All attempts to grow crystals from the hydrogenation product using the Vapour Diffusion Method resulted in needles which were too thin to determine a crystal structure. Hence the stereocentre was investigated with the Marfey's reagent (FDAA).

2.1.2.5 Investigation of Stereocentre

Marfey was the first to synthesise 1-fluoro-2,4-dinitropheyl-5-L-alanineamide (FDAA) and use it as a reagent for derivatisation of a D- and L-amino acid mixture.[50] FDAA was prepared by substitution of one of the two fluorine atoms in 1,5-difluoro-2,4-dinitrobenzene (DFDNB) by L-alanine amide (Scheme 20). FDAA then reacts with the amino group of L- and D-amino acids to yield diastereomers which can be separated by HPLC using a reverse phase column. The diastereomers may be detected at 340 nm with nanomolar sensitivity.[50]
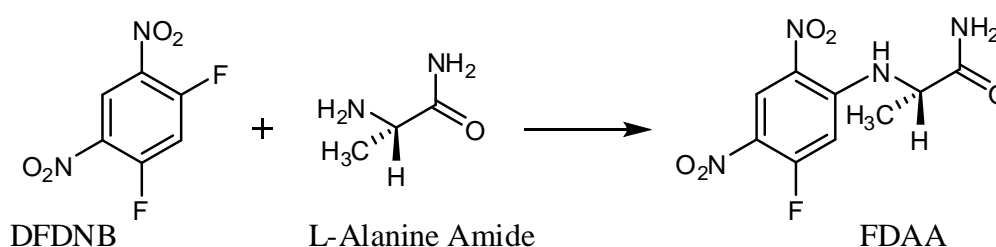
Scheme 20: Synthesis of 1-fluoro-2,4-dinitropheyl-5-L-alanineamide (FDAA) by Marfey[50] using L-Ala-NH$_2$·HCl, 1 M NaOH, acetone, anhydrous MgSO$_4$ and 1,5-difluoro-2,4-dinitrobenzene (DFDNB).

To synthesise the diastereomers it is necessary to have a free amine group on the amino acid. So the hydrogenation products **44** and **45** prepared with ligand (*S*,*S*)-Me-DUPHOS and ligand (*R*,*R*)-Me-DUPHOS were refluxed with 6 M HCl. This removed the acetyl protecting group and hydrolysed the ester to give HCl salts **46** and **47** in 98% yield (Scheme 21).
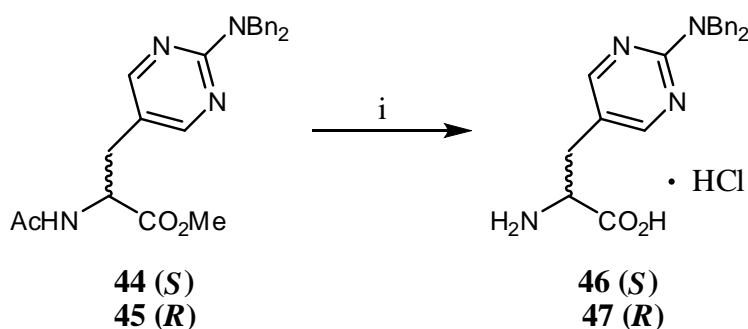
Scheme 21: To synthesise diastereomers using the Marfey´s reagent it was necessary to remove the acetyl protecting group. Reagents and conditions: (i) 6 M HCl, 110 °C, 6 h, 98%.

The diastereomers were prepared by stirring a mixture of FDAA (1.1 eq.), Cs_2CO_3 (20 eq.) and **46** respectively **47** in acetone/water (1:1) at 40 °C for two hours (Scheme 22). The solvent was removed under reduced pressure and the resulting orange solids were used crude for the HPLC experiments (Figures 27 and 28).
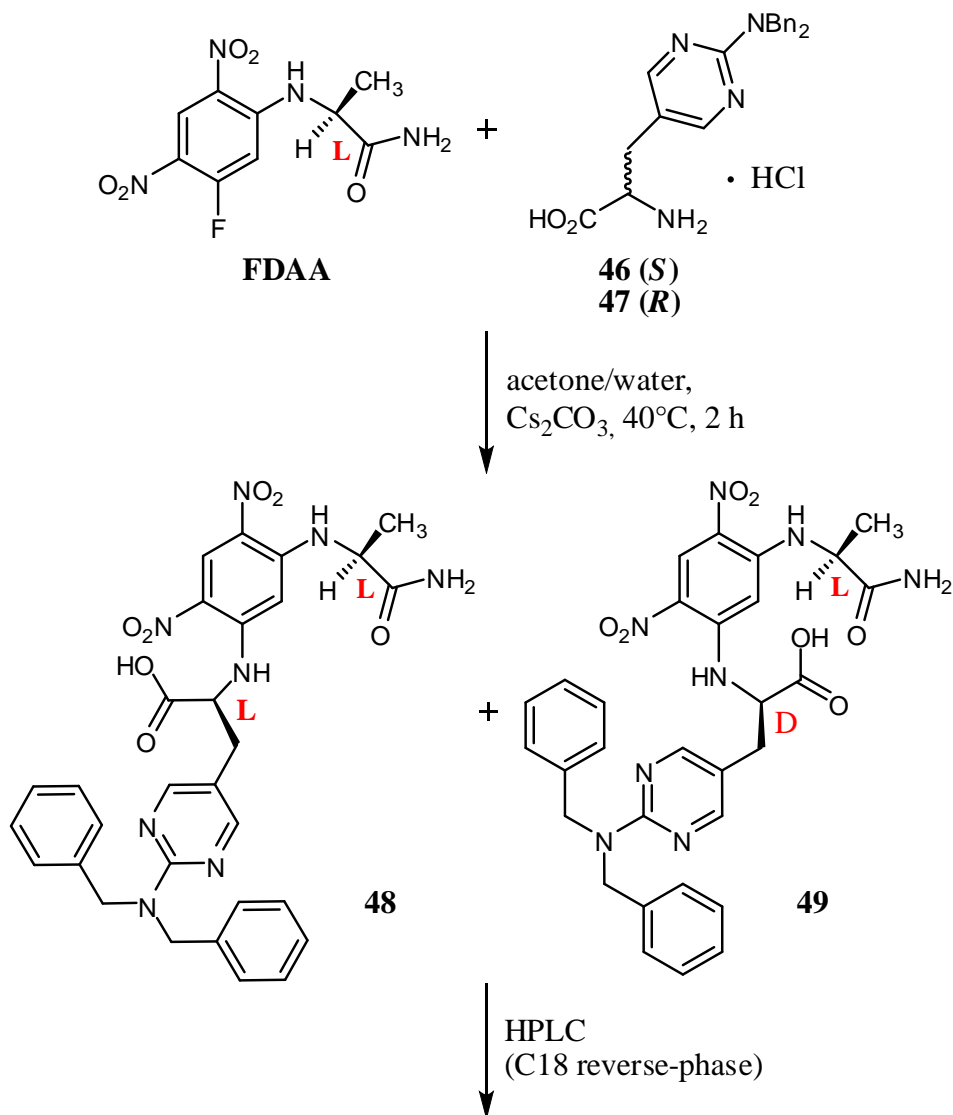
L,L-diastereomers are eluted from column before D,L-diasteromers
Stronger intramolecular H-bonding in D,L- than in L,L-diastereomers

Scheme 22: Synthesis of diastereomers **41** and **42** using Marfey´s reagent FDAA. The diastereomers elute differently from the HPLC (C18 reverse-phase). The L,L-diastereomer elutes first because of stronger intramolecular H-bonding in the D,L- than in the L,L-diastereomer.

The D,L- and the L,L-diastereomer were analysed by HPLC (Dionex Ultimate 3000, C18 reverse phase column, 5 μm particle size, 15 cm × 6.0 mm). A linear gradient of 100% TEAP buffer (50 mM, pH 3.0) to 100% MeCN over 50 min. was used (Figure 27 and 28).
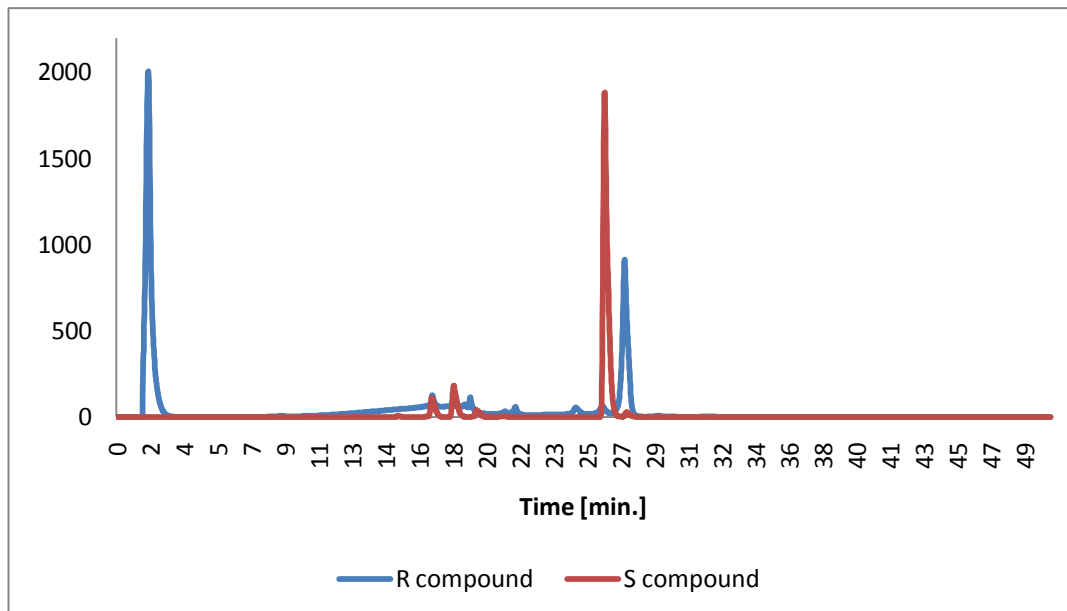
Figure 27: HPLC (C18 reverse-phase column) run of diastereomers **48** (red) and **49** (blue) using a linear gradient of 100% TEAP buffer (50 mM, pH 3.0) to 100% MeCN over 50 min.
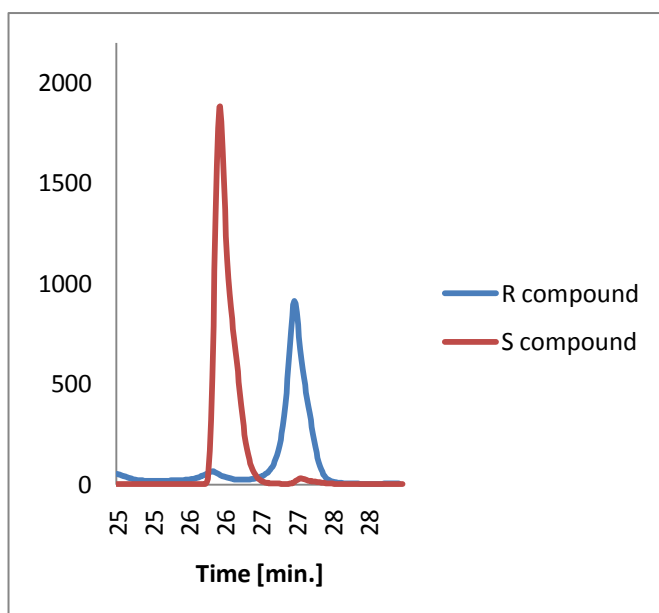
Figure 28: Expanded region of Figure 9 showing elution peaks for compound **48** (red) and **49** (blue). Red: high peak at 26.13 min. and small peak at 27.30 min., calculated ee = 98; Blue: high peak at 27.20 min. and small peak at 26.02 min., calculated ee = 96.

The red HPLC run shows that compound **48** synthesised with the (*S*,*S*)-Me-DUPHOS ligand gave a major peak at 26.1 min. and a minor peak at 27.3 min. The enantiomeric excess calculated from the peak areas is 98. The blue HPLC run shows that compound **49** synthesised with the (*R*,*R*)-Me-DUPHOS ligand gave a high peak at 27.20 min. and a small peak at 26.02 min. The enantiomeric excess calculated from the peaks areas is 96. The L,D-diastereomer has stronger intramolecular hydrogen bonding and elutes therefore after the L,L-diastereomer from a reverse phase column. That confirms that the (*S*,*S*)-Me-DUPHOS ligand gave the product **44** with a *S*-stereocentre and (*R*,*R*)-Me-DUPHOS ligand gave the product **45** with a *R*-stereocentre.

The enantiomeric excess of the asymmetric hydrogenation with catalyst $Rh(COD)_2^+$ and ligand Me-DUPHOS was measured by HPLC (Shimadzu LC10A VP, ODH-column, hexane/isopropanol (20:80), 25°C). Compound **44** was produced with a *S*-stereocentre in an enantiomeric excess of 98 and compound **45** was produced with a *R*-stereocentre in an enantiomeric excess of 99 (Table 7).

Table 7: Asymmetric hydrogenation with catalyst $Rh(COD)_2$ and ligand Me-DUPHOS provided products in high yields and high enantiomeric excess.

44 (*S*)-enantiomer   **45** (*R*)-enantiomer
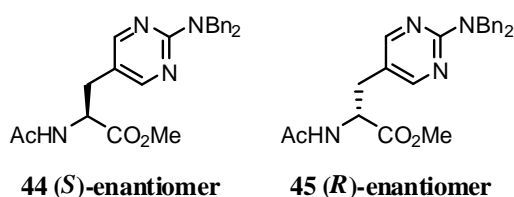
Rh(COD)$_2$	Me-DUPHOS	Product	Yield	ee
5 mol%	(*S*,*S*) 10 mol%	37	92%	98
2 mol%	(*S*,*S*) 4 mol%	37	93%	98
2 mol%	(*R*,*R*) 4 mol%	38	93%	99

2.1.2.6 Cleavage Attempts of Benzyl Protection

In the literature several examples are known for *N*-debenzylation from an aromatic amine by hydrogenolysis using palladium on carbon[51] as catalyst. Also palladium hydroxide is widely used for reductive *N*-debenzylation[52] and was chosen to reduce the pyrimidine ring and to remove the benzyl protecting groups in one step.

46 was treated with palladium hydroxide in methanol under hydrogen gas at room temperature but no reaction occurred and all starting material was recovered (Scheme 23). The hydrogen pressure was increased and at 15 bar the pyrimidine ring was reduced but both benzyl protecting groups remained. No debenzylation occurred even after increasing the hydrogen pressure to 30 bar (Scheme 23).
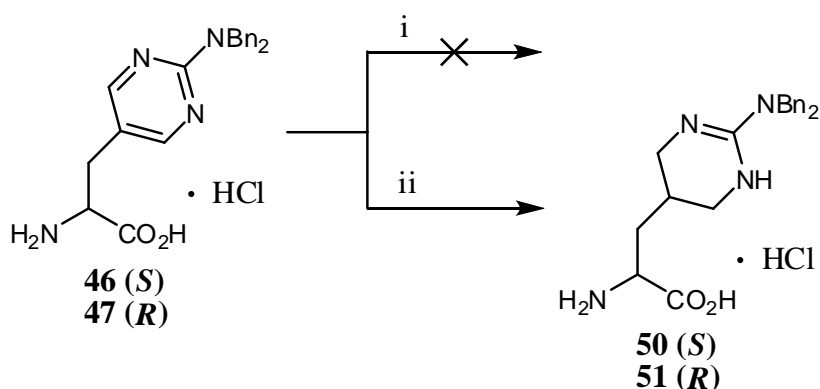
Scheme 23: Reagents and conditions: (i) **46** (*S*), Pd(OH)$_2$, H$_2$, 1 bar, degassed MeOH, rt, 24 h or **39** (*S*), Pd(OH)$_2$, H$_2$, 7 bar, degassed MeOH, rt, 24 h; (ii) **46** (*S*), Pd(OH)$_2$, H$_2$, 15 bar, degassed MeOH, rt, 24 h, 99% or **47** (*R*), Pd(OH)$_2$, H$_2$, 30 bar, degassed MeOH, rt, 24 h, 99%

Another method for *N*-debenzylation involves transfer hydrogenation catalysed by palladium on carbon with ammonium formate.[53] This method was also successful in case of debenzylation for aromatic amines.[54]

A mixture of compound **46**, ammonium formate (13 eq.) and palladium on carbon (10 w%) was refluxed in methanol (Scheme 24). The reaction was monitored by TLC but showed no reaction even after 24 hours and the starting material was recovered in the work up.
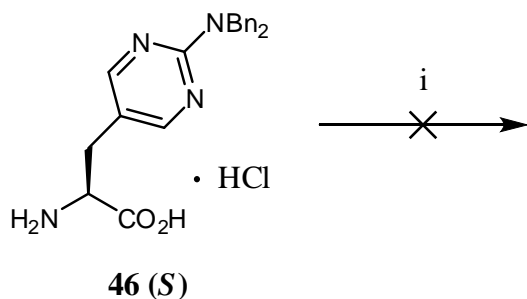
Scheme 24: Reagents and conditions: (i) **46**, Pd/C (10 w%), NH$_4$HCO$_2$ (13 eq.), MeOH, 70 °C (reflux), up to 24 h.

Literature also illustrates the *N*-debenzylation with sodium in liquid ammonia.[55] The sodium metal is added to ammonia at −78 °C and dissolves. Each dissolving sodium atom provides an electron into the liquid ammonia which solvates the sodium cation and the electron. The appearing blue colour is characteristic of a solution containing solvated electrons. This method was also applied in case of debenzylation for aromatic amines.[56, 57] The yields of the debenzylation for aromatic amines have been low (20% for Schwarz et al.[57] and 40% crude for Young et al.[56]) but it was worth considering the method.
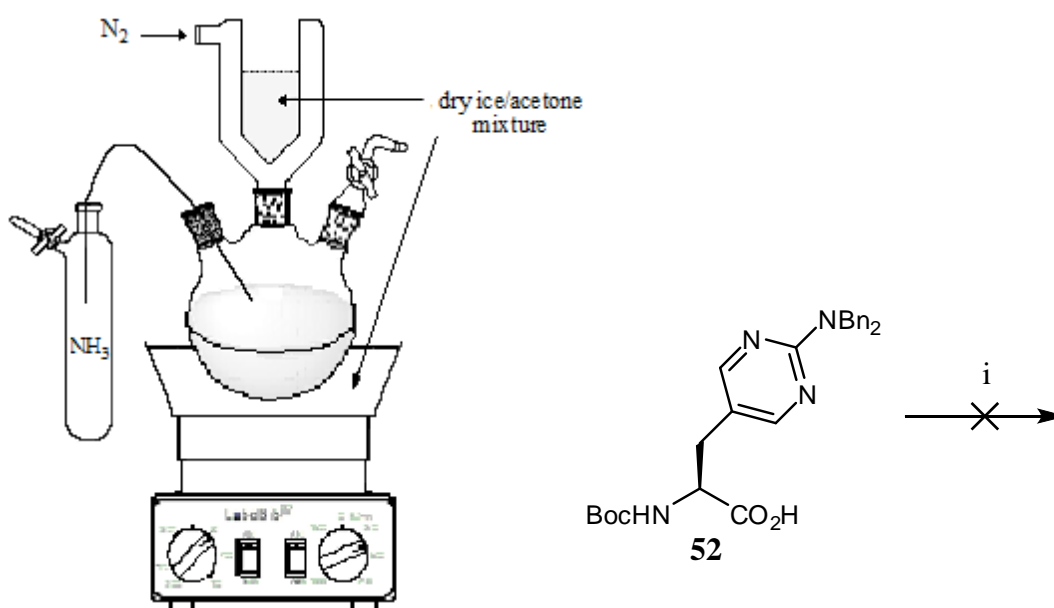
Scheme 25: Schematic construction of attempted reduction with reagents and conditions: (i) compound **52**, Na, liquid ammonia, dry THF, −78 °C to −30 °C, 1.5 hours at −30 °C.

Reductive *N*-debenzylation with sodium and liquid ammonia was attempted with 2-amino pyrimidine compound **52** (Scheme 25). Sodium was added to liquid ammonia at −78 °C. The cooling bath underneath the flask was then removed and the mixture stirred for 1.5 hours at −30 °C. According to TLC a reaction occurred. The substrate was transformed to several products of which none could be isolated or identified. The crude NMR did not indicate the presence of the desired product.

In an extensive literature research a publication of C. B. Reese[58] was found. Ammonium cerium(IV)nitrate (CAN) was used for oxidative di-*N*-debenzylation of 5-bromo-2-(dibenzylamino)pyridine (**53**). Further publications[59] reported that CAN is effective for *N*-debenzylation of tertiary amines but not of secondary amines. Reese states in the article that they were pleasantly surprised to find that the treatment of **53** with an excess of CAN

was effective[60] (Scheme 26). Both benzyl protecting groups have been removed and the reaction was quenched with saturated aqueous sodium hydrogen carbonate. After filtration the product was transferred into the organic layer by washing with ethyl acetate.
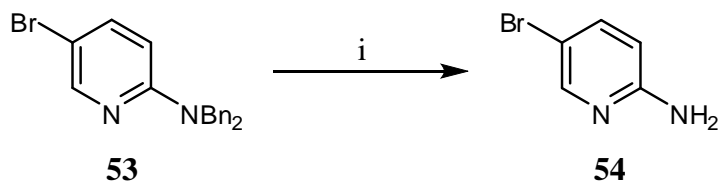
Scheme 26: Reagents and conditions: (i) CAN (5 eq.), MeCN/H$_2$O (9:1), rt, 30 min.[58]

The oxidative di-*N*-debenzylation of **46** was attempted under the same conditions as described but the reaction was left for three hours instead of 30 minutes (Scheme 27). The work up was problematic because some starting material, the side product of *N*-debenzylation and main product of di-*N*-debenzylation stayed together with the CAN in the water layer. The product was precipitated by changing the pH number of the water layer from 1 to 6 with 3 M NaOH which yielded in a yellow solid. The NMR showed the product but with impurities (Figure 29).
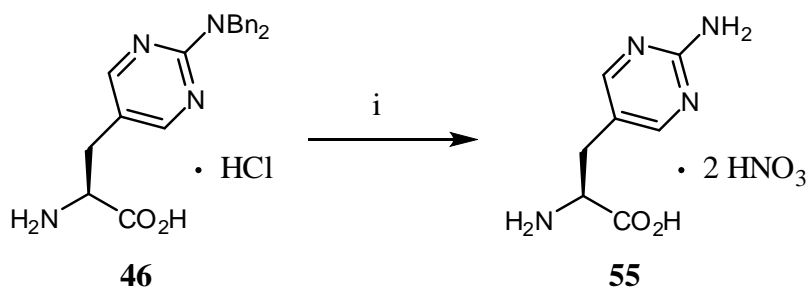
Scheme 27: Reagents and conditions: (i) **46**, CAN (5 eq.), MeCN/H$_2$O (9:1), rt, 3 h.
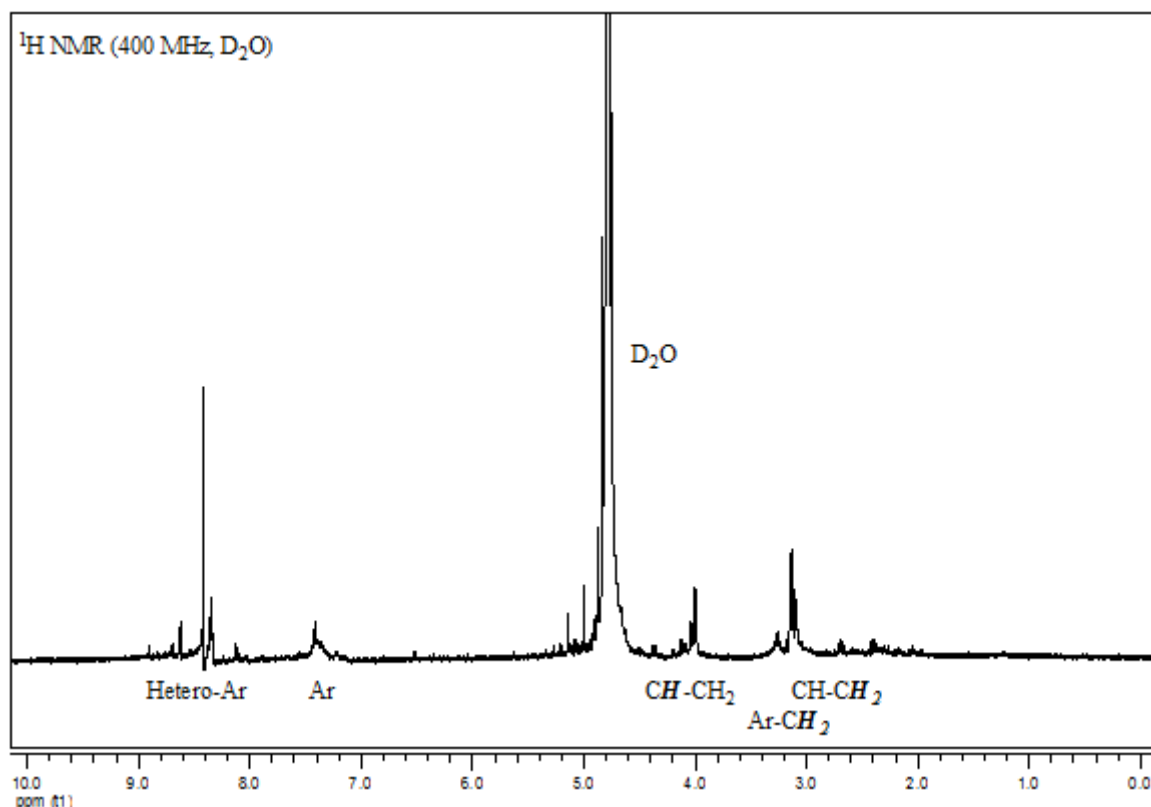
Figure 29: ^1H NMR in D_2O of compound **48** with impurities.

To increase the solubility of the product in organic solvents the reaction was repeated with compound **44** which contains an acetyl and an ester protecting group. Because the reaction was not completed with compound **46** the reaction time was increased to 6 hours and the amount of CAN was increased to 6 eq. (Scheme 28).
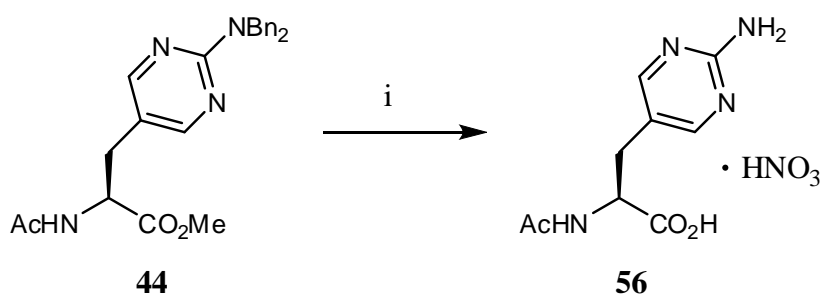
Scheme 28: Reagents and conditions: (i) **44**, CAN (6 eq.), MeCN/H_2O (9:1), rt, 6 h, 92%.

During the work up it became apparent that the product was again in the water layer. This time the CAN was precipitated as brown solid by changing the pH number of the water layer from 1 to 12 with 3 M NaOH. The solvent from the water layer was removed under reduced pressure and the resulting light yellow solid was dried. Methanol was added to the

solid and the sodium hydroxide was filtered off from the resulting yellow solution. After removing the solvent again the NMR confirmed the synthesis of compound **56** (Figure 30).
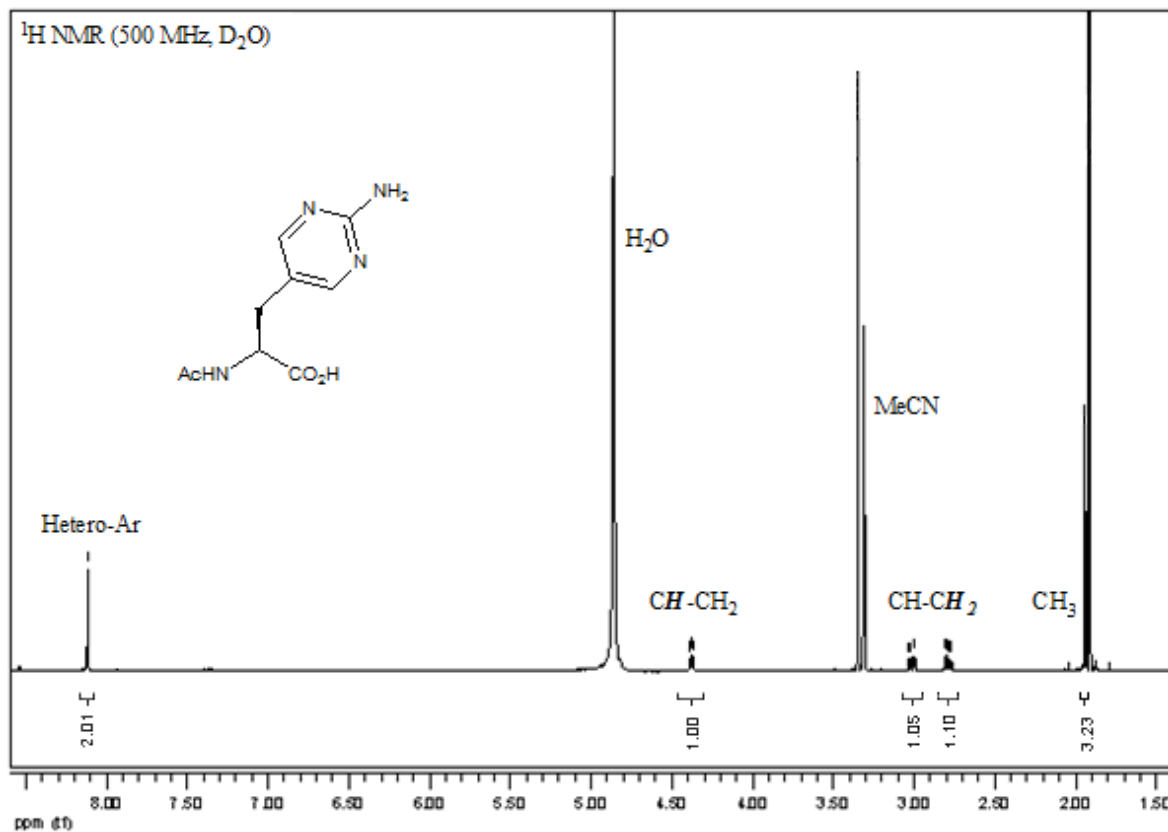
Figure 30: ^{1}H NMR in D_2O of compound **56**.

The IR showed broad NO_3^- peaks at 1567 cm^{-1} (N-O asymmetric) and at 1385 cm^{-1} (N-O symmetric). It was not possible to get a mass spectrum of the product. It could be that not all cerium was precipitated by changing the pH number from 1 to 12 and the cerium caused problems by the measurement of the mass spectrum. It could also be that the cerium ion coordinates to the guanidine part of the product. Several compounds have been published where metal ions coordinate to the guanidine part of pyrimidin-2-amine derivatives (Figure 31).

51
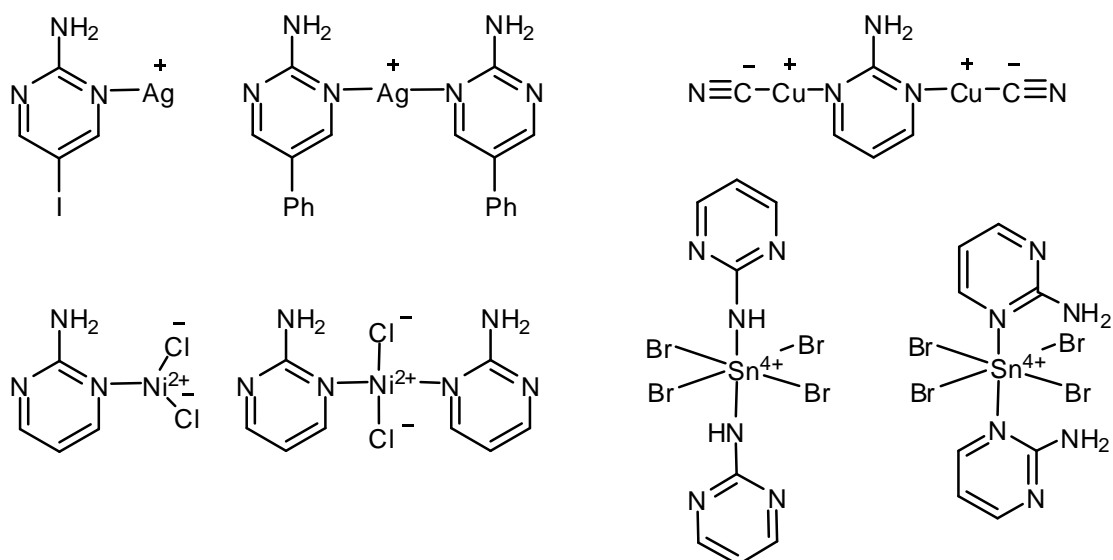
Figure 31: Examples of pyrimidin-2-amine derivatives with coordinated metal ions on the guanidine part.

An acetyl deprotection was attempted with compound **56**. The solid was refluxed in 3 M hydrogen chloride solution for six hours. The crude NMR did indicate that the compound decomposed and the aromatic ring hydrolysed. So the reaction time was decreased by up to 3 hours but the compound still hydrolysed.

Because the synthesis came to a standstill there it was tried to improve the promising looking di-*N*-debenzylation of compound **46** (Scheme 20). The resulting product lacks the acetyl protecting group but the work up was difficult and the resulting yield small. Because all the reaction products stay in the water layer and cannot be separated it was attempted to reach full conversion. Parameters like the amount of CAN, solvent ratio, reaction time and reaction temperature were investigated (Table 8).

Table 8: Investigation of different parameters for di-*N*-debenzylation.
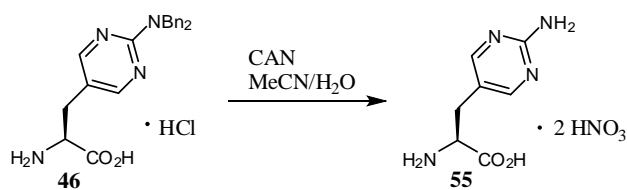
Entry	CAN [eq.]	MeCN/H₂O	Temp.	Time [h]
1	6.0	6:1	rt	7 ½
2	8.0	8:1	30 °C	24
3	8.2	4:1	rt	24
4	6.0	2:1	rt	5
5	6.0	4:1	rt	7 ½

First the amount of CAN was increased to six equivalents, the reaction time was increased to 7 ½ hours and the solvent ratio was changed to MeCN/H₂O 6:1 (Table 8, Entry 1). The proton NMR looked promising but it also showed a still uncompleted debenzylation. Therefore the amount of CAN was increased to eight equivalents, the reaction time was increased to 24 hours and the temperature was increased to 30 °C (Table 8, Entry 2). The proton NMR still showed a reaction mixture. Because the NMR of the reaction with the solvent ratio of MeCN/H₂O 6:1 looked more promising, the solvent ratio was changed to MeCN/H₂O 4:1 (Table 8, Entry 3). The proton NMR showed an increased conversion but the debenzylation was not complete. Therefore the solvent ratio was changed to MeCN/H₂O 2:1 (Table 8, Entry 4) which resulted in a mixture of mono and di deprotected products. The conversion of the reaction was decreased compared to entry 3. The solvent ratio was changed back to MeCN/H₂O 4:1 and the reaction time was decreased to 7 ½ hours to minimise possible decomposing (Table 8, Entry 5). The proton NMR showed a product mixture with uncompleted debenzylation. That is very problematic because the different products stay in the water layer and cannot be separated by column chromatography or recrystallisation.

The oxidative di-*N*-debenzylation appeared to be more promising than the reductive di-*N*-debenzylation. But it also seemed that the cerium metal ion coordinated to the guanidine part of the deprotected product causing difficulties in the work up and characterisation. Therefore a different oxidation method without metal ions was undertaken. In the literature the debenzylation of di-protected amines with *N*-iodosuccinimide (NIS)[61] gave

high yields for the removal of one benzyl group with three equivalents NIS and yields of 50 to 72% for the di-*N*-debenzylation with ten equivalent NIS.

Compound **44** and ten equivalents NIS were stirred in dry dichloromethane at 25 °C under dry conditions while the flask was protected from ambient light. According to TLC no reaction occurred even after leaving the reaction for 23 hours. This was a significant problem because without the removal of the benzyl protecting groups the guanidine moity of the amino acid cannot bind.

2.1.2.7 Altered Strategy with PMB Protection

A synthesis pathway with 4-methoxybenzyl as protecting group instead of the benzyl protecting group was pursued while working on the di-*N*-debenzylation. The advantage of the 4-methoxybenzyl protecting group (PMB) is that it can be removed with trifluoroacetic acid.[62]

The synthesis starts with di-protection of **11** with 4-methoxybenzyl chloride. The protected pyrimidine compound would be coupled to a 2-aminoacrylic acid derivative *via* Heck coupling followed by stereoselective reduction of the double bond. All protecting groups would be removed under acidic conditions and the aromatic ring of compound **16** reduced with hydrogen (Scheme 29).
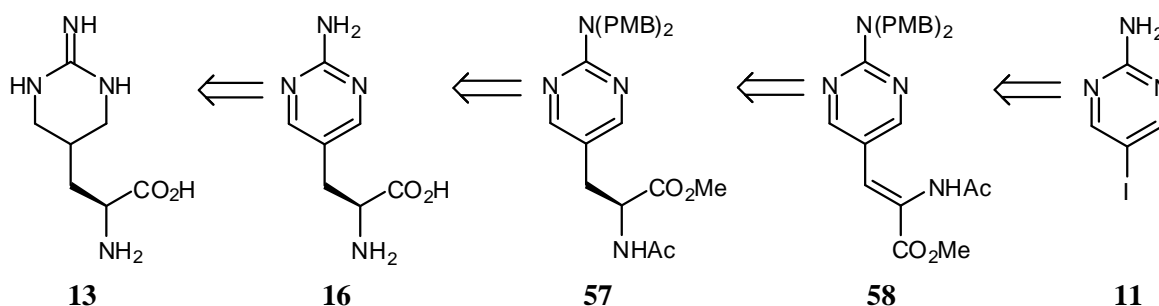
Scheme 29: Retrosynthetic analysis for the synthesis of amino acid **13**.

The benzyl protecting group and the 4-methoxybenzyl protecting group have a similar structure. Therefore the reaction conditions which have been successful for the di-benzylation have been applied for the reaction. Compound **11** was reacted with three equivalent sodium hydride and five equivalent 4-methoxybenzyl chloride (**59**). at room temperature under dry conditions. The reaction was monitored by TLC but after 21 hours

reaction time at room temperature most of the starting material was still unreacted. The temperature was increased to 30 °C and the mixture was stirred for further 23 hours until most of the starting material was converted. Because the reaction at room temperature was too slow it was decided to perform the reaction at 30 °C which gave a yield of 91% (Scheme 30).
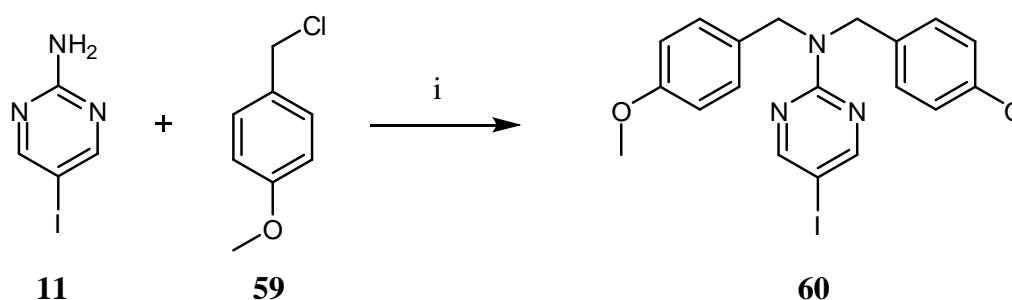
Scheme 30: Reagents and conditions: (i) **11**, sodium hydride (3 eq.), 4-methoxybenzyl chloride (5 eq.), dry THF, 0 °C to 30 °C, 23 hours at 30 °C, 91%.

The most successful condition for the Heck coupling of di-benzyl protected **25** (Table 5, Entry 5) has been used to attempt the Heck coupling of **60**. This condition, using alkene **35**, PdCl$_2$, NaHCO$_3$, Bu$_4$NCl and tri-*o*-tolylphosphine in dry DMF at 100 °C resulted in no coupling product (Table 9, Entry 1). To investigate if the product decomposed under this condition the reaction temperature was decreased to 60 °C but no reaction occurred and both staring materials have been recovered. The same outcome was reached at 90 °C. Therefore the amounts of catalyst and ligand were increased to 20 mol% and 40 mol%, respectively. This resulted in a 43% yield of coupling product **61** at 110 °C (Table 9, Entry 4). The highest yield (62%) for the Heck coupling between **60** and **35** was achieved with 30 mol% catalyst and 30 mol% ligand at 100 °C (Table 9, Entry 5).

Table 9: Investigation of different parameters for Heck coupling between compound **35** and **60**.

Entry	PdCl$_2$ [mol%]	(o-tol)$_3$P [mol%]	NaHCO$_3$ [eq.]	Bu$_4$NCl [eq.]	Temp. [°C]	Time [h]	Yield [%]
1	15	15	2.0	1.2	100	23	-
2	15	15	2.0	1.2	60	2.5	-
3	15	15	2.0	1.2	90	3	-
4	20	40	2.0	1.2	110	24	43
5	30	30	2.0	1.2	100	22	62

The NOESY of compound **61** showed a cross peak between H-1 and H-2 (Figure 32) which defines the compound as (Z)-coupling product.
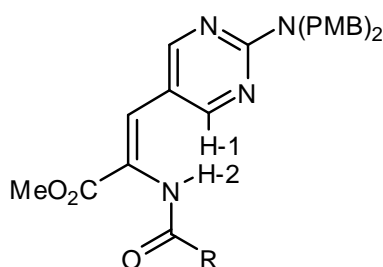
Figure 32: In a Heck-(Z)-coupling product H-1 and H-2 are close enough to couple and show a cross peak in a NOESY measurement.

For introducing chirality into the Heck coupling compound **61** the asymmetric hydrogenation was chosen which has been very successful with the di-benzyl protected compound **37**. The most successful condition for the asymmetric hydration of di-benzyl protected **25** (Table 6, Entry 7 and 8) has been used to attempt the asymmetric hydration of **61**. The reaction was carried out with compound **61**, 2 mol% Rh(COD)$_2$BF$_4$, 4 mol% (*S,S*)-Me-DUPHOS and 35 bar of hydrogen gas (using a hydrogen gas cylinder) in degassed methanol at room temperature and yielded in 7% product. To increase the yield the amount of catalyst and ligand has been doubled and the hydrogen gas pressure was

increased to 38 bar. This lead to a yield of 90% for the reaction with (*S*,*S*)-Me-DUPHOS and 91% for the reaction with (*R*,*R*)-Me-DUPHOS (Table 10).

Table 10: Asymmetric hydrogenation with catalyst Rh(COD)$_2$ and ligand Me-DUPHOS provided products in high yields and high enantiomeric excess.

62 (*S*)-enantiomer   **63** (*R*)-enantiomer
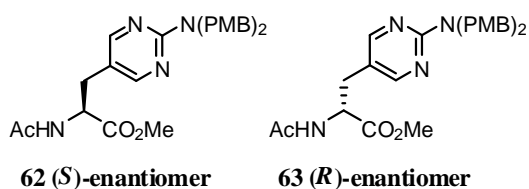
Rh(COD)$_2$	Me-DUPHOS	Product	Yield	ee
4 mol%	(*S*,*S*) 8 mol%	57	90%	99
4 mol%	(*R*,*R*) 8 mol%	58	91%	99

The enantiomeric excess of the asymmetric hydrogenation compounds **62** and **63** was measured by HPLC (Shimadzu LC10A VP, ODH-column, hexane/isopropanol (20:80), 25°C).

Crystallisation of compound **62** and **63** in different solvent systems just resulted in long thin needles. The X-ray data are not good enough to determine the absolute configuration of the stereocentre. There have been similar problems with the crystallisation of the dibenzyl protected asymmetric compounds **44** and **45**. The configuration of them was then determined by preparing diastereomers with FDAA (Scheme 15). The L,D-diastereomer has stronger intramolecular hydrogen bonding and elutes therefore after the L,L-diastereomer from a C 18 reverse phase column. The results showed (Figure 9 and 10) that the asymmetric hydrogenation of compound **37** with the (*S*,*S*)-Me-DUPHOS ligand resulted in the product with a *S*-stereocentre and the reaction with the (*R*,*R*)-Me-DUPHOS ligand resulted in the product with a *R*-stereocentre. Because of the similarity between compounds **37** and **61** (Figure 33) it was assumed that the achieved results also apply for compound **61**.
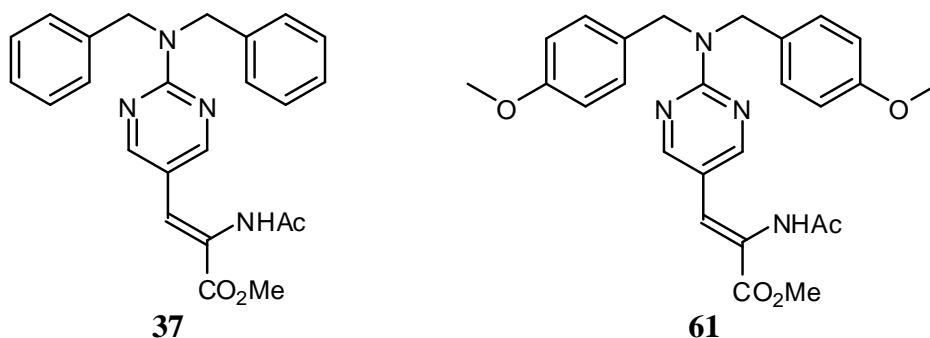
Figure 33: Figure shows the similarity between the structures of compounds **37** and **61**.

It is known that the 4-methoxybenzyl protecting group can be removed by trifluoroacetic acid.[62] But there are also publications where the 4-methoxybenzyl protecting group was removed by hydrochloric acid[63] or hydrobromic acid[64], respectively (Scheme 31).
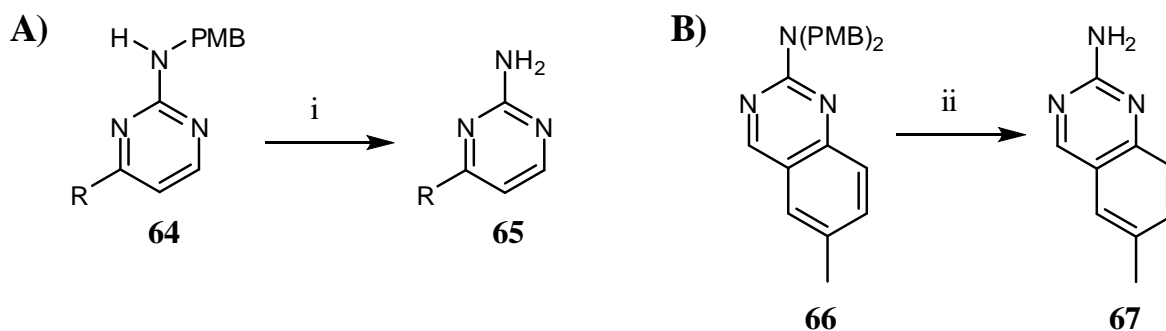
Scheme 31: Reagents and conditions: A) (i) **64**, 3 M hydrochloric acid, 110 °C, 30 h, 59%;[63] B) (ii) **66**, 5.7 M hydrobromic acid in toluene, reflux, 8 h, 66%.[64]

It was attempted to remove the PMB and acetyl protecting groups as well as cleave the ester in one step. Compound **62** was refluxed in 3 M hydrochloric acid for 3.5 hours. After the work up the proton NMR showed that the reaction resulted in a mixture of deprotected **64** and still di-PMB protected **63** (Scheme 32, i).Therefore the reaction time was increased to six hours what yielded in a product mixture of deprotected **64** and mono-PMB protected **65** (Scheme 32, ii).
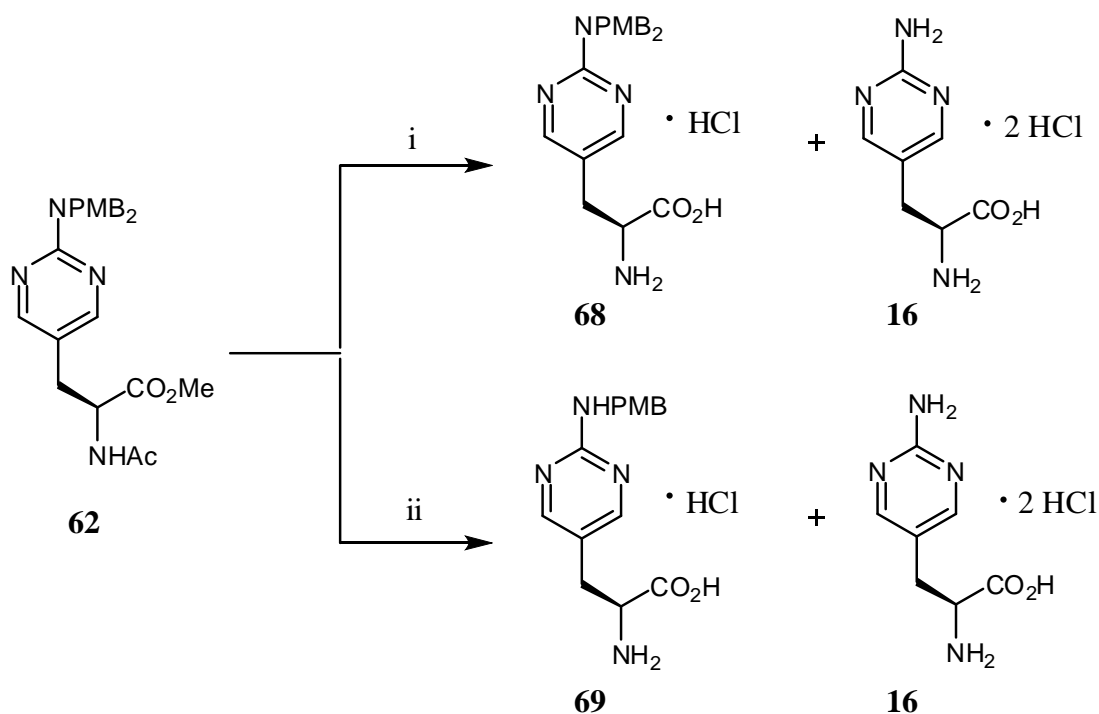
Scheme 32: Reagents and conditions: (i) **62**, 3 M HCl, 110 °C, 3 ½ h; (ii) **57**, 3 M HCl, 110 °C, 6 h.

The desired fully deprotected product and the partly deprotected products are very polar and cannot be separated easily by column chromatography. As a result it is important to achieve a conversion of 100% for the reaction. This was realised by a two step reaction (Scheme 33). First compound **62/63** was heated for four hours in TFA to remove the 4-methoxybenzyl protecting groups. The TFA was removed under reduced pressure and the crude product was refluxed in 3 M hydrochloric acid for four hours. That gave the fully deprotected arginine derivatives **16** and **17**, respectively in quantitative yield. By reducing the aromatic ring of **16** and **17** *via* hydrogenation on Pd(OH)$_2$ two further arginine derivatives have been synthesised (Scheme 33).
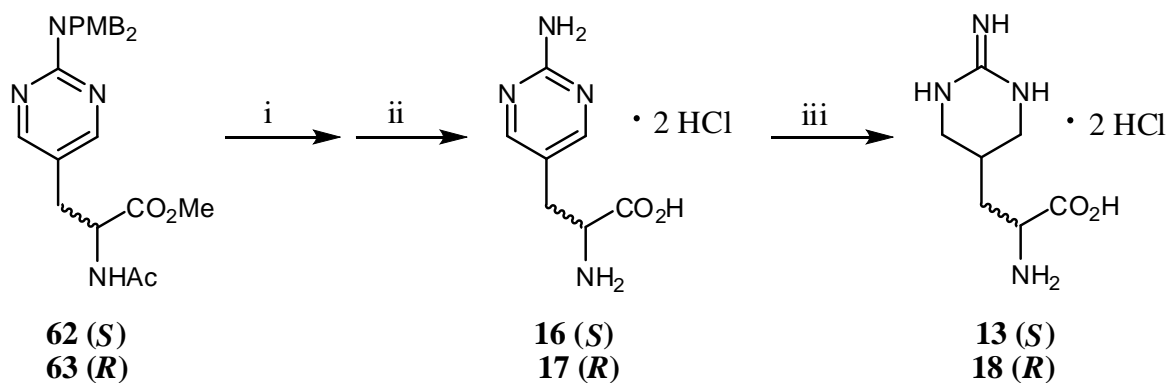
Scheme 33: Reagents and conditions: (i) TFA, 50 °C, 4 h; (ii) 3 M HCl, 110 °C, 4 h, quant. ; (iii) Pd(OH)$_2$, H$_2$, H$_2$O, 15 bar, rt, 24 hours, quant.

2.1.2.8 Summary

These interesting arginine mimetics were all synthesised with very high stereoselectivity in both enantiomers (Figure 34). They could show intriguing binding properties towards nucleic acid when build into peptides. In contrast to arginine the guanidine side chain is conformationally constrained. This should result in a favourable entropic component when the peptide binds toward a nucleic acid target. The constrained analogues should show a reduced loss of entropy when binding due to a diminished loss of torsional freedom. This could also improve the selectivity for a target relative to peptides containing arginine with its highly flexible side chain instead.
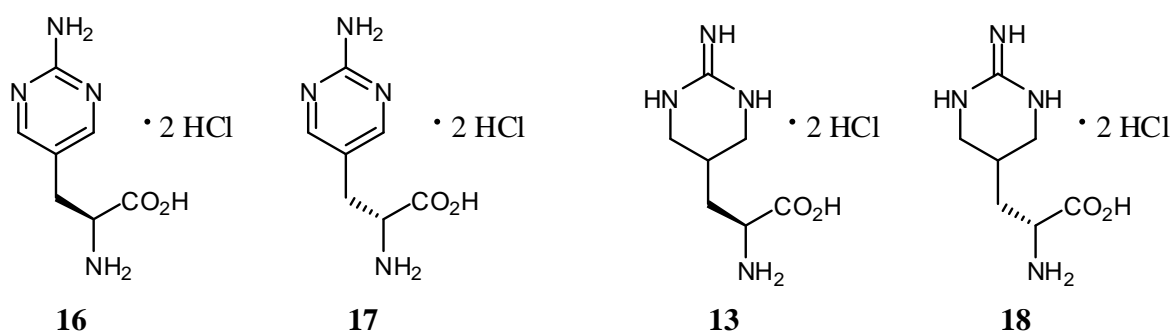
Figure 34: Structure of the stereoselective synthesised arginine mimetics.

All four arginine mimetics build into the peptide can bind towards the base and the backbone of the nucleic acid target. The aromatic and partly planar character of the arginine derivatives **16** and **17** may open additional binding opportunities by intercalation between base pairs of DNA.

2.1.3 Synthetic Efforts towards (*S*)/(*R*)-AAA and (*S*)/(*R*)-AIA

(*S*)/(*R*)-AAA (**70**, **71**) and (*S*)/(*R*)-AIA (**12**, **72**) (Figure 35) are two possible arginine mimetics which were considered to be of interest. These amino acids possess the same number of carbons, nitrogens and oxygens as arginine but have a constrained side chain. As mentioned before the constrained analogues should show a reduced entropy loss by binding to a nucleic acid. To realise the synthesis of the arginine mimetics it was necessary to connect the stereocentre directly to the amino tetrahydropyrimidine ring.

70
(*S*)-AAA

71
(*R*)-AAA

12
(*S*)-AIA

72
(*R*)-AIA
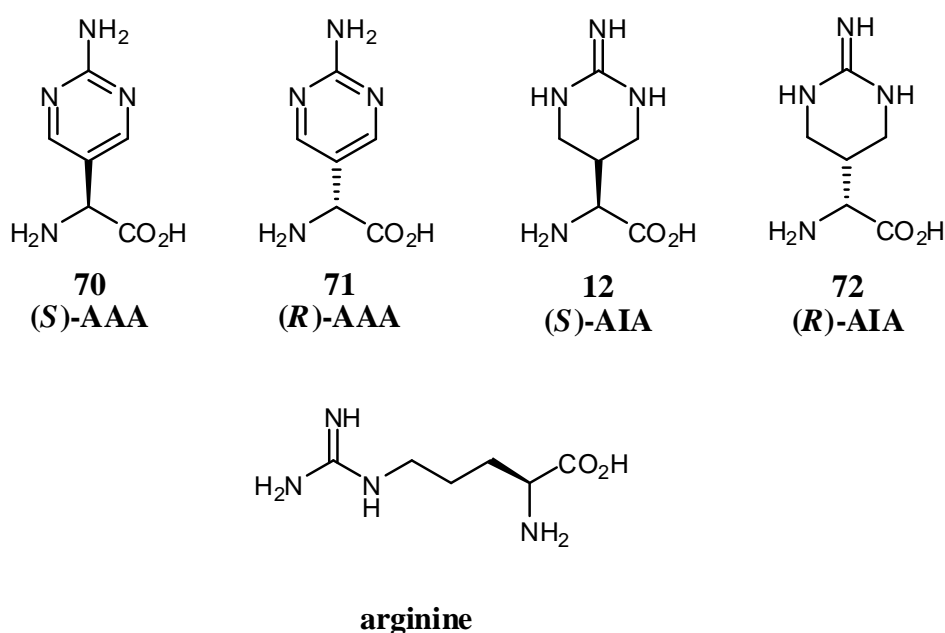
arginine

Figure 35: Structure of (*S*)-2-amino-2-(2-aminopyrimidin-5-yl)acetic acid (**70**), (*R*)-2-amino-2-(2-aminopyrimidin-5-yl)acetic acid (**71**) (*S*)-2-amino-2-(2-iminohexahydropyrimidin-5-yl)acetic acid (**12**), (*R*)-2-amino-2-(2-iminohexahydropyrimidin-5-yl)acetic acid (**72**) and arginine.

2.1.3.1 Synthesis Planning

The chosen pathway is based on a publication of P. Cali and M. Begtrup.[65] They synthesised 1-hydroxypyrazole α-amino acids by introducing the glycine functionality with diethyl *N*-Boc iminolate (**73**) *via* Grignard reaction. The racemic amino acid was realised by reaction with lithium hydroxide at room temperature and hydrochloric acid in Et$_2$O at 60 °C (Scheme 34).
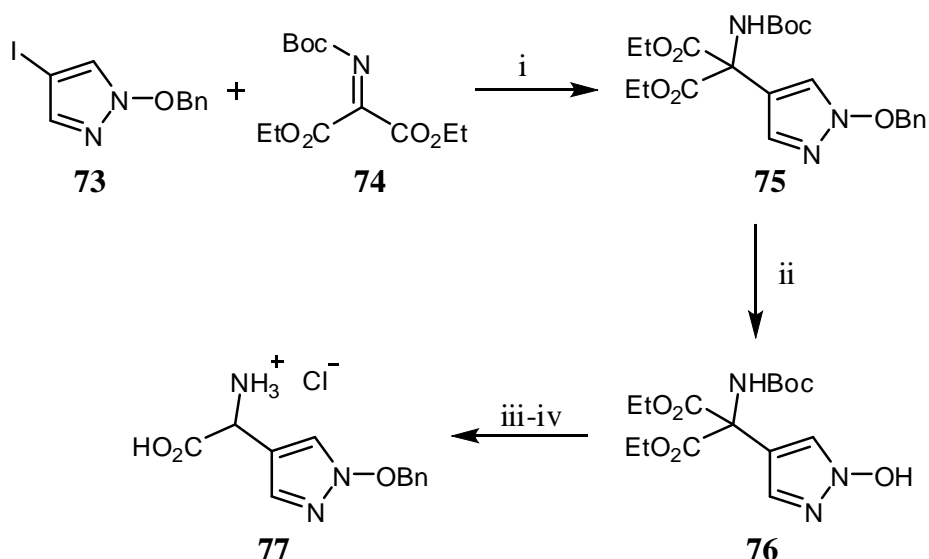
Scheme 34: Reagents and conditions: (i) *i*-PrMgCl, THF, 0 °C, 30 min., then cooling to −78 °C, 3 h; (ii) H_2 (1 atm), 10% Pd/C, MeOH, 0 °C, 30 min.; (iii) LiOH, rt, 4 h; (iv) 2 M HCl in Et_2O, 60 °C, 30 min.[65]

The synthesis plan for the amino acids starts with the commercially available 2-amino-5-iodopyrimidine. The iminolate **74** and the di-benzyl protected pyrimidine **25** would be coupled by a Grignard reaction. After formation of the stereocentre as racemate the Boc protecting group needs to be changed to an acetyl protecting group. This opens the opportunity to separate the enantiomers enzymatic using Aminoacylase. The heteroaromatic ring would be reduced by hydrogenation (Scheme 35).
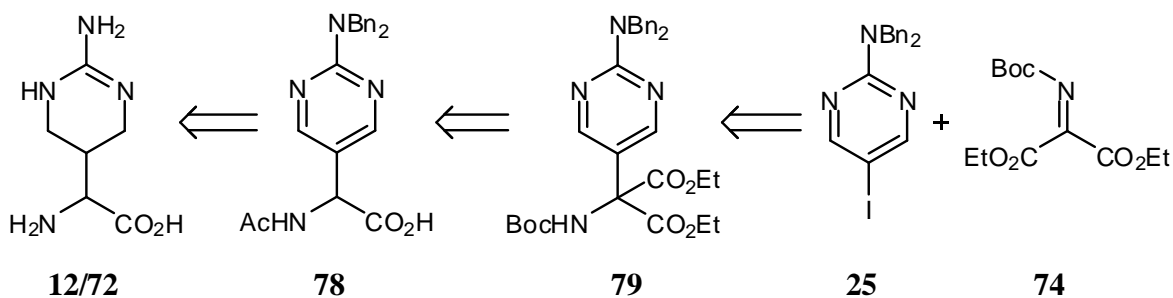
Scheme 35: Retrosynthetic analysis for the unnatural arginine mimetics based on publication of P. Cali.[65]

2.1.3.2 Grignard Reaction

To synthesise iminolate **74**, *tert*-butylcarbazate (**80**) was stirred with sodiumnitrite at 0 °C. The produced azide was then extracted with diethylether and used directly for the second step. Triphenylphosphine was added to this solution and the precipitate *N*-Boc-triphenyliminophosphorane (**24**) was obtained after filtering. **24** and diethylmesoxalate were refluxed in dry THF and after filtering of the side product Ph₃PO the crude product was purified by kugelrohr distillation to give **74** as colourless oil (Scheme 36).
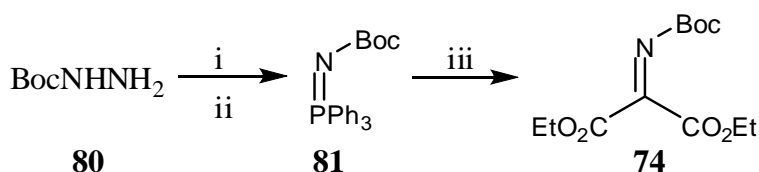
Scheme 36: Reagents and conditions: (i) NaNO₂, AcOH/H₂O, 0 °C; (ii) PPh₃, 0 °C – rt, 1.5 h, 83%; **(iii)** CO(CO₂Et)₂, THF, reflux, 16 h, 50%.

The di-benzyl protected iodo pyrimidine **25** was synthesized using benzyl bromide and the strong base sodium hydride. Compound **25** and iminolate **74** were then coupled through a Grignard reaction with *i*-PrMgCl to yield **79** as yellow oil in 30% yield (Scheme 37).
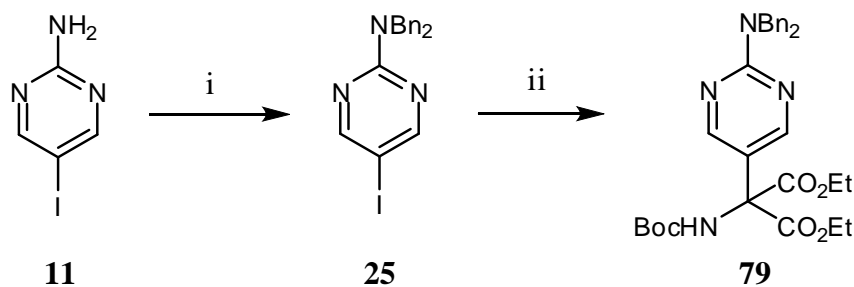
Scheme 37: Reagents and conditions: (i) NaH, BnBr, dry THF, 0 °C – rt, 19 h at rt, 78%; (ii) *i*-PrMgCl, dry THF, rt, 5 h, then iminolate **74**, rt, 16 h 30%.

The advantage of this reaction is that the prochiral centre can be connected directly to the aromatic ring system. The disadvantage is that the Grignard might also attack the nitrogen and the carbonyl groups of **74** which might have led to the low yield.

2.1.3.3 Synthesis of Racemate

The plan was to remove the Boc protecting group of compound **79** with 2 M HCl in Et$_2$O but because of solubility problems the Boc-group was not eliminated. With 10% TFA in DCM the Boc protecting group could be removed at room temperature with a yield of 96% (Scheme 38).
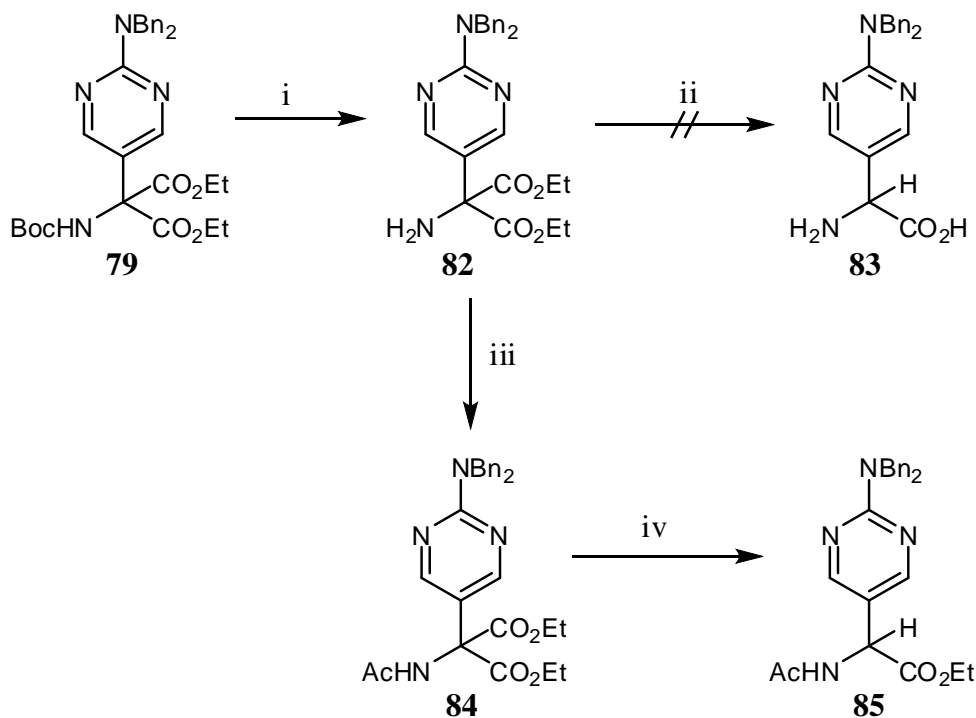
Scheme 38: Reagents and conditions: (i) 10% TFA in DCM, rt, 4 h, 96%; (ii) 6 M HCl/MeCN (1:1), 100 °C, 5 h; (iii) Ac$_2$O, Et$_3$N, rt, 4 h, quant.; (iv) LiBr, DMF, H$_2$O, 130 °C, 19 h, 56%.

The experiment to hydrolyse the esters and decarboxylate compound **82** with 6 M HCl/MeCN (1:1) at 100 °C failed and destroyed the compound. So instead the amino group of compound **82** was first protected with an acetyl protecting group using acetic anhydride and triethylamine. The acetyl protecting group is an advantage for the enzymatic separation of the enantiomers of a racemic mixture. The enzyme Aminoacylase just removes the acetyl protecting group from an amino acid with the *S* stereocentre and therefore generates two separable compounds.

To avoid the protecting group exchange from Boc-protected amine **79** to acetyl-protected amine **84** diethyl 2-(acetylimino)malonate (**87**) was synthesised (Scheme 39).
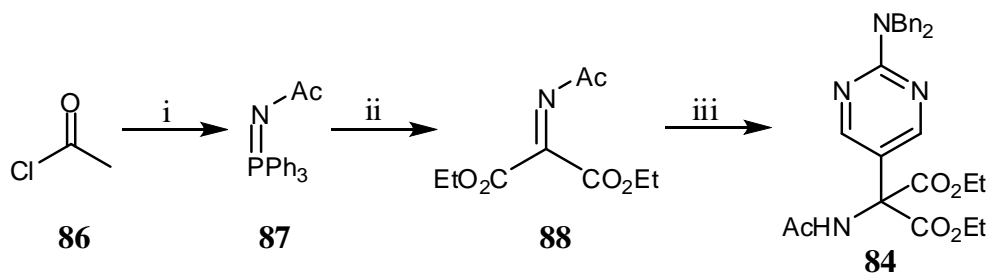
Scheme 39: Reagents and conditions: (i) Me$_2$CO, 0 °C, NaN$_3$, PPh$_3$, rt, 40 min., 76%; (ii) dry THF, CO(CO$_2$Et)$_2$, 80 °C, 17 h, 51% (iii) dry THF, **25**, *i*-PrMgCl, 0 °C, 3 h, **88**, rt, 19 h, 7%.

Acetylchloride (**86**) was converted with sodium azide followed by triphenylphosphine to give **87** in 76% yield. **87** and diethylmesoxalate were then refluxed in dry THF for 17 hours and after filtering off the side product Ph$_3$PO the crude product was purified by column chromatography to give **88** in 51% yield. Di-benzyl protected pyrimidine derivative **25** and malonate **88** were then coupled through a Grignard reaction with *i*-PrMgCl to yield **84** in just 7% yield (Scheme 39). This is a surprising result and an increased yield was expected from this Grignard reaction compared to the Grignard coupling with malonate **74**. Less side reactions have been expected because the carbon of the carbonyl group of the Acetyl protecting group should be less electrophilic than the carbon of the carbonyl group of the Boc protecting group. Compound **84** was then treated with lithium bromide in a H$_2$O/DMF mixture which successfully removed one of the two ester groups. This resulted in amino acid **85** as a racemic mixture (Scheme 38).

2.1.3.4 Cleavage Attempts of Benzyl Protection

The next step should have been the reduction of the aromatic ring of **85** with simultaneous removal of the benzyl protecting groups. Pd/C and Pd(OH)$_2$ were tried as catalyst with a H$_2$-balloon (1.0 bar) in methanol, but no reaction could be observed (Scheme 40).
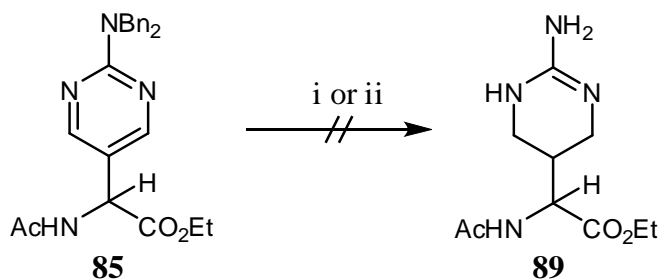
Scheme 40: Reagents and conditions: (i) Pd/C, H_2-balloon (1 bar), MeOH, rt; (ii) Pd(OH)$_2$, H_2-balloon (1 bar), MeOH, rt.

The reaction was repeated with Pd(OH)$_2$ and the hydrogen pressure was increased to 7 bar. No reaction occurred and all starting material was recovered.

2.1.3.5 Summary

It is reasonable to assume that the difficulty to remove the benzyl protecting groups from **85** will be comparably challenging as already described in the case of **44** although this cleavage was not investigated as thoroughly as for **44**. Ultimately this synthesis was not quite finished due to lack of time but it would be straightforward to attempt an alternative synthetic strategy using PMB protection analogue to the successful synthesis of **16** and **17**.

2.2 Synthesis of Peptides

2.2.1 Introduction

The development of combinatorial chemistry facilitated the search for new drugs. The principle of combinatorial chemistry is the synthesis of a variety of different compounds under identical condition at once. The generated compound collection is called a library which can be screened for the desired quality.

Split-and-Recombine-Method

A very elegant and efficient method to synthesise a combinatorial library is the Split-and-Recombine-Method also called One-Bead-One-Compound-Synthesis. The method is based on the simultaneous synthesis of a large number of molecules on a solid phase (Scheme 41).
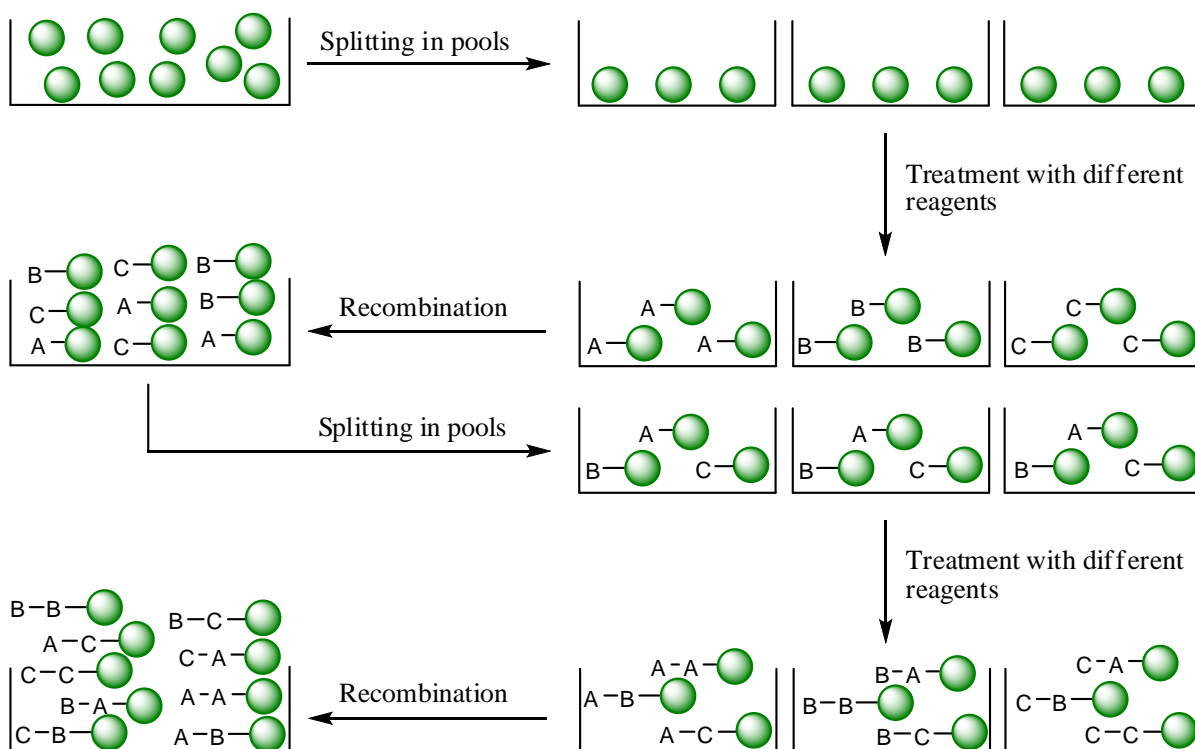
Scheme 41: Principle of the Split-and-Recombine-Method.

Macro beads are convenient to use and are widely applied. The macro beads are equally divided into a series of pools. Each pool is treated with a different reagent. After the reaction is finished the beads are washed and excess of reagent is filtered off. The beads are recombined and mixed to uniformity. Then the next cycle starts and the beads are again split in pools. The number of different possible compounds depends on the number of cycles and the number of used coupling reagents. The advantage of the method is that each bead contains one compound and the beads can be easily separated.

2.2.2 Synthesis of Peptide Library

The aim was to synthesise peptides with a partly stabilised α-helix structure. Oligopeptides are normally unfolded in aqueous solution but some peptides require a helical structure in the first place to bind specifically to the nucleic acid target. Different strategies have been developed for α-helix stabilisation in oligopeptides for example the introduction of covalent side chain constrains with disulfide bridges.

One complete α-helix turn contains 3.6 amino acids where the side chains in position i and i + 4 locate on the same face of the α-helix. Disulfide bonds are formed *via* oxidation (in the air or with iodine) between the cysteine side chains in position i and i + 4. It is required to incorporation D-Cys in position i and L-Cys in position i + 4. That way the two side chains point towards each other and the obtained disulfide constrain has the required length to realise a helical loop. The full helical structure is often realised during the binding (Scheme 42).
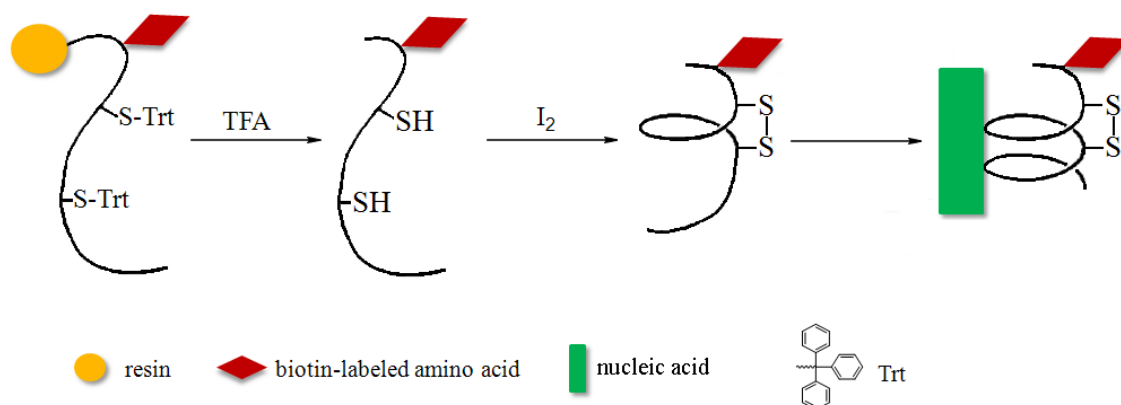
Scheme 42: Principle of obtaining α-helix loop stabilisation via disulfide bridging and realising the full helical structure during binding.

The amino acids in position i + 2, i + 3, i + 6 and i + 7 of the α-helix are the one of interest for binding towards a nucleic acid target. The amino acids in these positions have been varied to create the peptide library (Figure 36). The chosen amino acids have a high propensity for binding nucleic acids (Table 11). It was also very important to create a library where the synthesised peptide can be identified by MALDI-ToF. The number of amino acids used and therefore the number of compounds finally synthesised in the library has been limited to achieve that. Keeping the beads in the different pools separated after adding the last amino acid (so the last amino acid of the peptide is known) and measuring

the exact mass by MALDI-ToF provides all information needed to identify each synthesised peptide. In the α-helix structure the amino acids in position i + 5 and i + 8 are not important for the binding towards the nucleic acid target and therefore the amino acid alanine as strong helix former has been chosen for both positions.
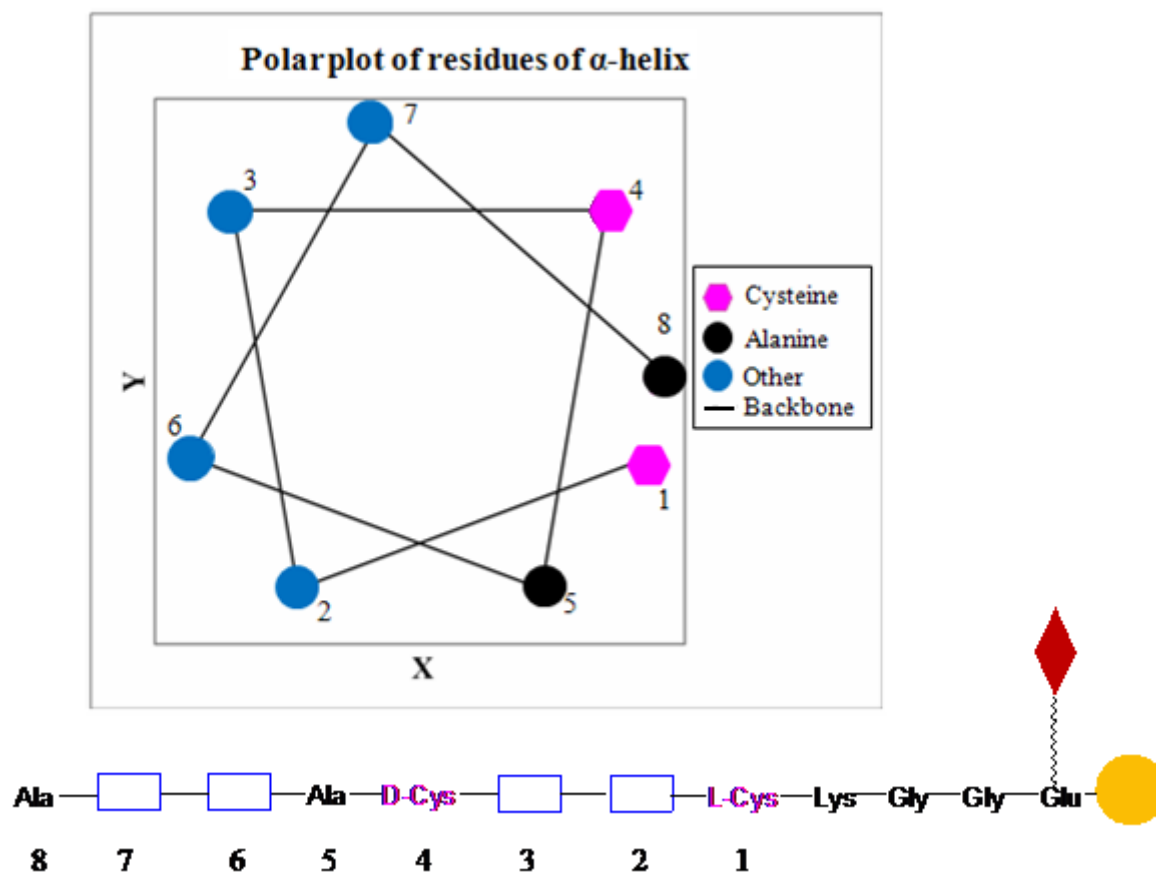
Figure 36: Plot of an eight residue α-helix showing the positions of the varied amino acids in the peptide library in blue. Linear presentation of the peptide library peptides.

To become familiar with the SPPS procedure it was decided to initially perform the peptide synthesis with arginine and not with the arginine mimetics (Table 11 positions 3 and 6). This also opens the possibility to test the binding of these peptides towards the mRNA of TGF-β1 first and determine which peptides are strong binders. These strong binders can then selectively be synthesised including the arginine mimics.

Table 11: Shows the varying amino acids of the synthesised peptide library in blue.

Amino Acid Position	Library
1	**L-Cys**
2	Phe, Ile, Thr, Tyr
3	Lys, **Arg**
4	**D-Cys**
5	Ala
6	**Arg**, Ser
7	Leu, Asn, Val, Tyr
8	Ala

The peptide library was synthesised by Fmoc peptide synthesis using the Mix-and-Recombine-Method in plastic syringes with a polypropylene sinter base. Polystyrene AM RAM macro beads (0.50-0.56 mm, 62 nmol per bead) were swollen in NMP. For the Fmoc deprotection a piperidine/NMP (1:1) mixture was used for two times 10 min. The amino acids were coupled with PyBOP (4 eq.) and DIEA (4 eq.) for two times 60 min. After the penultimate amino acid the four pools kept divided to alleviate the identification by MALDI-ToF. 96 well-plates were used to cleave the peptide from the resin and one bead was added to each well. A mixture of TFA (95%), H_2O (2%), m-cresol (1%) and 1, 2-ethanedithiol (1%) was added to the wells and left for one hour.

Figure 37: Three examples of peptides identified by MALDI-ToF.

Six samples from the library have been analysed by MALDI-ToF mass analysis to confirm if the peptide library synthesis was successful and to see if the peptides can be explicitly identified with this method. Three samples and their measured monoisotopic masses results are shown in Figure 37.

All MALDI-ToF spectra of the samples showed a definite peak which could be identified as peptides from the target peptide library.

2.2.3 Synthesis of Branched Peptides

Before the products could undergo systematic ELISA screening of their binding potential towards the mRNA of TGF-β1 the ELISA conditions have to be tested. Therefore a peptide with many positive charges which will bind very well towards nucleic acid and a peptide with many negative charges which should not bind at all towards nucleic acid were synthesised by standard Fmoc methodology (Figure 38) with Rink Amide MBHA resin (100-200 µm, 0.34 mmol/g, 0.06 g).
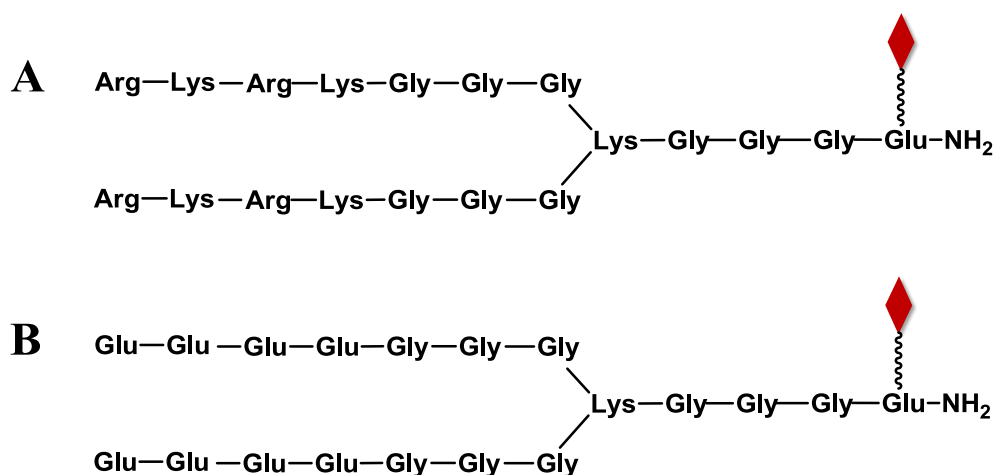
Figure 38: A: Synthesised peptide with many positive charges. B: Synthesised peptide with many negative charges.

For the Fmoc deprotection a piperidine/NMP (1:1) mixture was used for two times 10 min. The amino acids up to the two times Fmoc protected Lys (to creating the branch) were

added with one coupling cycle of 45 min. with PyBOP (4 eq.) and DIEA (4 eq.). The following amino acids were added with 8 eq. of Fmoc protected amino acid, PyBOP and DIEA. The identity of the two peptides was confirmed by MALDI-ToF mass spectrometry.

The prerequisites for a systematic ELISA screening have been created with the synthesis of these peptides and the peptide library.

3. Conclusions and Perspectives

The synthesis of unnatural arginine mimetics as well as their application as building block in peptides to investigate binding properties towards nucleic acids are modern and exiting concepts in medicinal chemistry and were the subject of this thesis.

Two different synthetic pathways towards new arginine analogues were planned and investigated.

The first synthesis although overall successful was a good example for a crucial aspect of amino acid synthesis, the protecting group strategy. Due to the cleavage problems with the benzyl group on the last step the synthesis had to be restarted all over at a rather late stage of the project. Also noticeable is the rather surprising failure of the Negishi chemistry. However this was well recovered by shifting to the Heck reaction in combination with a successful high yielding and very stereoselective asymmetric hydrogenation.

The second synthesis strategy required an enzymatic racemate separation as no straightforward conceptual alternatives had been identified while planning the synthesis. This synthesis shared the same problem with regard to the removal of the benzyl groups on the last step as already mentioned above. For timing reasons it was not possible to re-perform the whole synthesis analogue to the first synthesis. However, it is likely that by applying the same slight alterations to the protection group strategy an equally satisfactory result can be achieved.

Over all important synthetic concepts regarding the preparation of pyrimidine based arginine mimetics have been established, namely regarding stereoselectivity, C-C coupling chemistry and protection group considerations. With these procedures established it should be possible to create a larger amount of similar unnatural amino acids in relative short time.

The biochemical part of the research focused on the synthesis of peptides which could be used to investigate the translation of mRNA of TGF-β1 into the protein by binding towards the mRNA. To become familiar with the SPPS procedure it was decided to initially perform the peptide synthesis with arginine and not with the arginine mimetics. The peptide library was successfully prepared by Fmoc peptide synthesis using the Mix-and-Recombine-Method with macro beads (0.50-0.56 mm, 62 nmol per bead). Before the peptides could undergo systematic ELISA screening of their binding potential the ELISA

conditions have to be tested. Therefore a peptide as positive standard and a peptide as negative standard was synthesised by standard Fmoc methodology with Rink Amide MBHA resin.

Thus the preconditions to perform the ELISA have been created which will facilitate ELISA work in future research efforts. For methodical screening peptides that show a positive ELISA binding response will be synthesised with a constrained arginine mimetic instead of the arginine.

4. Experimental Part

4.1 Materials and Methods

4.1.1 Chemicals

Solvents

All solvents were used as purchased from commercial suppliers. Anhydrous solvents were distilled from an appropriate drying agent and degassed methanol was prepared on the vacuum line under argon.

Chemicals

Chemicals were purchased from Acros, Aldrich, Fluka, Strem, Alfa Aesar and Novabiochem and were used without further purification.

Phosphate Buffer (50 mM with 100 mM KCl)

K_2HPO_4 (13.97 g), KH_2PO_4 (2.69 g) and KCl (14.91 g) were dissolved in two litre of distillated water. The pH was then adjusted to 7.4 with 0.1 M HCl.

Triethylammonium phosphate Buffer (50 mM)

Triethylamine (2.53 g) was added to 300 mL of distillated water. The pH was adjusted to 3.0 with 0.1 M phosphoric acid and the volume was made up to 500 mL with distilled water.

4.1.2 Analytical Methods

Thin Layer Chromatography/ Column Chromatography

Thin Layer Chromatography (TLC) analysis was performed on Merck aluminium sheets with silica 60 (fluorescence marker (F_{254}). Detection was carried out by UV (wavelength 254 nm and 366 nm) and by staining solutions (potassium permanganate, cerium/molybdenium, ninhydrin).

Preparative column chromatography was performed on Merck silica gel 60 (230 – 400 mesh).

Melting Points

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.

NMR Spectroscopy

^{1}H and ^{13}C spectra were recorded on a Bruker DPX 400 or on a Bruker Avance 500. $CDCl_3$, D_2O, CD_3OD, $(CD_3)_2CO$ and DMSO-d_6 were used as deuterated solvents. Chemical shifts are given in parts per million using the residual solvent peak as a reference.

Mass Spectroscopy

Mass spectroscopy was performed by EPSRC Mass Service Centre, Swansea University or by Dr. R. Jenkins, R. Hicks or D. Walker at Cardiff University.

MALDI-ToF measurements were recorded on a Waters Micromass MALDI micro MX^{TM}.

IR Spectroscopy

Infrared spectra were recorded on a FT/IR – 660 plus spectrometer operating from 4000 – 500 cm^{-1} as thin film or as KBr disk.

Optical Rotation

Optical rotations were measured on a AA-1000 polarimeter at room temperature with sodium D line (589 nm), c = 0.1 and L = 0.5 dm and are uncorrected.

X-ray Crystallography

Crystals were grown using the Vapour Diffusion Method and measured by Dr. B. Kariuki at Cardiff University.

HPLC

HPLC analyses were performed on Dionex Ultimate 3000, Merck-Hitachi LaChrom L 7000 series or Shimadzu LC10A VP.

4.2 Procedures

(*S*)-2-Amino-3-(2-iminohexahydropyrimidin-5-yl)propanoic acid (13)

(*S*)-2-Amino-3-(2-aminopyrimidin-5-yl)propanoic acid (**16**) (0.03 g, 0.12 mmol) and Pd(OH)$_2$ (0.03 g) were added in a steel autoclave. After purging with H$_2$ water (30 mL) was inserted and the reaction mixture was degassed for six times. The reaction mixture was stirred at 15 bar of H$_2$ at room temperature for 24 h. After releasing the H$_2$ pressure the mixture was filtrated and the solvent was removed under reduced pressure to give (*S*)-2-amino-3-(2-iminohexahydropyrimidin-5-yl)propanoic acid **13** (0.03 g, quantitative) as yellow solid. (m.p.: 68-70 °C, hygroscopic).

13
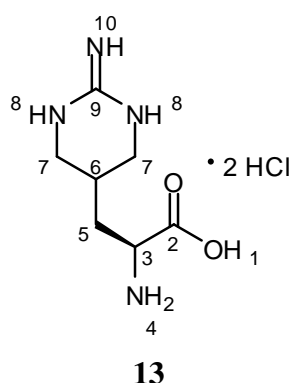
^{1}H NMR (400 MHz, D$_2$O): δ = 1.56 (1H, m, H-5), 1.71 (1H, m, H-5), 1.97 (1H, m, H-6), 2.75 (2H, m, H-7), 3.16 (2H, m, H-7), 3.71 (1H, m, H-3) ppm; ^{13}C NMR (125 MHz, D$_2$O): δ = 26.1 (C-6), 30.7 (C-5), 42.1 (C-7), 50.3 (C-3), 153.5 (C-9), 1651.9 (C-2) ppm;

ESI MS (*m/z*): 187 [M+H]$^+$, 163, 130; HRMS (ESI): calc. for C$_7$H$_{15}$O$_2$N$_4^+$ ([M+H]$^+$): 187.1190, found: 187.1188; IR (KBr disk, cm^{-1}): 3368 (br), 1662, 1639, 1405, 1355, 1329, 1300, 1174; Optical rotation: [α]$_D$ = + 26° (c = 0.1, dest. H$_2$O).

(*S*)-2-Amino-3-(2-aminopyrimidin-5-yl)propanoic acid (16)

(*S*)-Methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)propanoate (**62**) (0.06 g, 0.12 mmol, 1.0 eq.) was dissolved in trifluoroacetic acid (4 mL) and stirred for 4 h at 50 °C. The solution was washed with chloroform (2 × 4 mL) and the solvent was removed under reduced pressure from the water layer to give a solid. The solid was then refluxed in 3 M HCl (4 mL) at 110 °C for 4 h. The solvent was removed under reduced pressure to give (*S*)-2-amino-3-(2-aminopyrimidin-5-yl)propanoic acid **16** as yellow solid (0.03 g, 100%). (m.p.: ~ 180 °C decomp.).
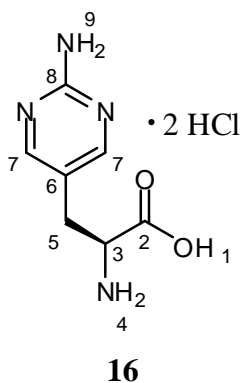
16

^{1}H NMR (400 MHz, D$_2$O): δ = 3.02-3.15 (2H, m, H-5), 3.96-4.98 (1H, m, H-3), 8.49 (2H, s, H-7) ppm; ^{13}C NMR (125 MHz, D$_2$O): δ = 29.2 (C-5), 53.3 (C-3), 117.7 (C-6), 155.0 (C-8), 157.6 (C-7), 171.0 (C-2) ppm; ESI MS (*m/z*): 365 [2×M+H]$^+$, 279, 217, 183 [M+H]$^+$, 169, 163, 149; HRMS (ESI): calc. for C$_7$H$_{11}$O$_2$N$_4^+$ ([M+H]$^+$): 183.0877, found: 183.0870; IR (KBr disk, cm^{-1}): 3415 (br), 3124, 3007, 2917, 2849, 1740, 1670, 1633, 1551, 1492, 1437, 1403, 1370, 1291, 1213, 1143, 1110, 1057, 1012; Optical rotation: [α]$_D$ = + 48° (c = 0.1, dest. H$_2$O).

(*R*)-2-Amino-3-(2-aminopyrimidin-5-yl)propanoic acid (17)

(*R*)-Methyl-2-acetamido-3-(2-(bis (4-methoxybenzyl) amino) pyrimidin-5-yl) propanoate (**63**) (0.06 g, 0.12 mmol, 1.0 eq.) was dissolved in trifluoroacetic acid (4 mL) and stirred for 4 h at 50 °C. The solution was washed with chloroform (2 × 4 mL) and the solvent was removed under reduced pressure from the water layer to give a solid. The solid was then refluxed in 3 M HCl (4 mL) at 110 °C for 4 h. The solvent was removed under reduced pressure to give (*S*)-2-amino-3-(2-aminopyrimidin-5-yl)propanoic acid **17** as yellow solid (0.03 g, 100%). (m.p.: ~ 180 °C decomp.).
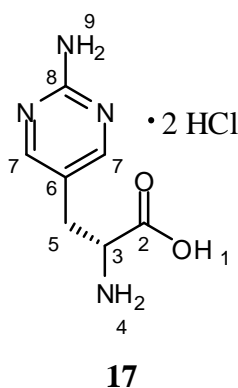
17

^{1}H NMR (400 MHz, D$_2$O): δ = 3.02-3.15 (2H, m, H-5), 3.96-4.98 (1H, m, H-3), 8.49 (2H, s, H-7) ppm; ^{13}C NMR (125 MHz, D$_2$O): δ = 29.2 (C-5), 53.3 (C-3), 117.7 (C-6), 155.0 (C-8), 157.6 (C-7), 171.0 (C-2) ppm; ESI MS (*m/z*): 365 [2×M+H]$^+$, 279, 217, 183 [M+H]$^+$, 169, 163, 149; HRMS (ESI): calc. for C$_7$H$_{11}$O$_2$N$_4$$^+$ ([M+H]$^+$): 183.0877, found: 183.0870; IR (KBr disk, cm^{-1}): 3416 (br), 3127, 2917, 2849, 1740, 1670, 1633, 1551, 1492, 1437, 1403, 1370, 1291, 1213, 1143, 1110, 1056, 1012; Optical rotation: [α]$_D$ = − 48° (c = 0.1, dest. H$_2$O).

(*R*)-2-Amino-3-(2-iminohexahydropyrimidin-5-yl)propanoic acid (18)

(*R*)-2-Amino-3-(2-aminopyrimidin-5-yl)propanoic acid (**17**) (0.03 g, 0.12 mmol) and Pd(OH)$_2$ (0.03 g) were added in a steel autoclave. After purging with H$_2$ water (30 mL) was inserted and the reaction mixture was degassed for six times. The reaction mixture was stirred at 15 bar of H$_2$ at room temperature for 24 h. After releasing the H$_2$ pressure

the mixture was filtrated and the solvent was removed under reduced pressure to give (*R*)-2-amino-3-(2-iminohexahydropyrimidin-5-yl)propanoic acid **18** (0.03 g, quantitative) as yellow solid. (m.p.: 68-70 °C, hygroscopic).


18

^{1}H NMR (400 MHz, D$_2$O): δ = 1.56 (1H, m, H-5), 1.71 (1H, m, H-5), 1.96 (1H, m, H-6), 2.75 (2H, m, H-7), 3.16 (2H, m, H-7), 3.70 (1H, m, H-3) ppm; ^{13}C NMR (125 MHz, D$_2$O): δ = 26.2 (C-6), 30.7 (C-5), 42.2 (C-7), 50.3 (C-3), 153.5 (C-9), 1651.9 (C-2) ppm; ESI MS (*m/z*): 187 [M+H]$^+$, 163, 130; HRMS (ESI): calc. for C$_7$H$_{15}$O$_2$N$_4$$^+$ ([M+H]$^+$): 187.1190, found: 187.1188; IR (KBr disk, cm^{-1}): 3368 (br), 1662, 1639, 1405, 1355, 1329, 1300, 1174; Optical rotation: [α]$_D$ = − 26° (c = 0.1, dest. H$_2$O).
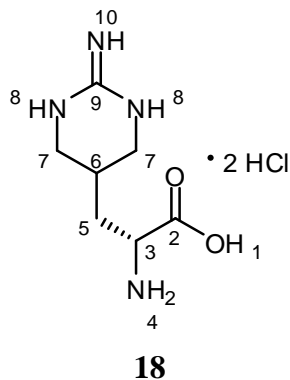
N, N-Dibenzyl-5-iodopyrimidin-2-amine (25)

Under nitrogen atmosphere sodium hydride (0.03 g, 0.73 mmol, 3.2 eq., 60% NaH in oil) was added to a solution of 2-amino-5-iodopyrimidine (0.05 g, 0.23 mmol, 1.0 eq.) in dry THF (1 mL) at 0 °C. Benzyl bromide (0.07 mL, 0.58 mmol, 2.5 eq.) was added at 0 °C after 30 min. and the reaction mixture was then allowed to warm up to room temperature. After 19 h water (3 mL) and ethyl acetate (2 mL) were added and the water phase was washed with ethyl acetate (3 mL). The combined organic phase was washed with brine (5 mL), dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane to hexane/ethyl acetate, 10:1, R_f = 0.57) to give N, N-dibenzyl-5-iodopyrimidin-2-amine **25** (0.07 g, 78%) as a colourless solid. (m.p.: 117 °C).
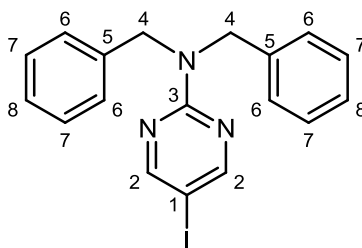
25

^{1}H NMR (500 MHz, CDCl$_3$): δ = 4.74 (4H, s, H-4), 7.24-7.13 (10H, m, H-Ar), 8.35 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 48.5 (C-4), 75.8 (C-1), 127.7 (C-8), 127.9 (C-6), 128.7 (C-7), 137.9 (C-5), 160.7 (C-3), 162.8 (C-2) ppm; EI MS (*m/z*): 401 [M]$^+$, 311, 232, 204, 184, 91; HRMS (EI): calc. for C$_{18}$H$_{16}$IN$_3$$^{\cdot +}$ ([M]$^+$): 401.0383, found: 401.0376; IR (thin film, cm^{-1}): 1570, 1512, 1426, 1393, 1362, 930.

N, N-Di-*tert*-butoxycarbonyl-5-iodopyrimidin-2-amine (32)

2-Amino-5-iodopyrimidine (1.0 g, 4.52 mmol, 1.0 eq.), di-*tert*-butyldicarbonate (2.39 g, 10.9 mmol, 2.4 eq.) and 4-dimethylaminopyridine (0.14 g, 1.15 mmol, 0.26 eq.) were dissolved in THF (50 mL) and then refluxed at 80 °C for 24 h. The solvent was removed under reduced pressure and the brown solid was purified *via* column chromatography (hexane/ethyl acetate, 3:1, R_f = 0.60) to give *N*, *N*-di-*tert*-butoxycarbonyl-5-iodopyrimidin-2-amine **32** (1.85 g, 97%) as a colourless solid. (m.p.: 147 °C).

32

^{1}H NMR (500 MHz, CDCl$_3$): δ = 0.47 (18H, s, H-6), 8.90 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 27.8 (C-6), 83.9 (C-5), 89.7 (C-1), 150.4 (C-4), 157.5 (C-3), 164.0 (C-2) ppm; EI MS (*m/z*): 421 [M]$^+$, 321, 277, 247, 221, 57; HRMS (ESI): calc. for

$C_{14}H_{21}O_4N_3I_1^+$ ([M+H]$^+$): 422.0571, found: 422.0569; IR (thin film, cm^{-1}): 3425 (br, O-H), 2983 (C-H), 1797, 1761 (C=O), 1541, 1422, 1370, 1291.

(Z)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate (37)

N, *N*-Dibenzyl-5-iodopyrimidin-2-amine (**25**) (0.080 g, 0.20 mmol, 1.0 eq.), methyl-2-acet-amidoacrylate (0.037 g, 0.26 mmol, 1.3 eq.), NaHCO$_3$ (0.034 g, 0.40 mmol, 2.0 eq.), Bu$_4$NCl (0.068 g, 0.24 mmol, 1.2 eq.), PdCl$_2$ (0.005 g, 0.03 mmol, 15 mol%) and tri-*o*-tolylphosphine (0.009 g, 0.03 mmol, 15 mol%) were mixed with DMF (anhydrous, 2 mL) and stirred for 4 h at 100 °C under nitrogen. The reaction was quenched with brine (15 mL) and ethyl acetate (15 mL) was added. The org. layer was separated, washed with brine (2 × 15 mL), dried over MgSO$_4$ and filtered. The solvent was removed under reduced pressure and the brown solid was purified *via* column chromatography (hexane/ethyl acetate, 1:1, R_f = 0.18) to give (Z)-methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate **37** (0.065 g, 80%) as a colourless solid. (m.p.: 160 °C).

37

^{1}H NMR (500 MHz, CD$_3$OD): δ = 2.12 (3H, s, H-6), 3.81 (3H, s, H-1), 4.89 (4H, s, H-11), 7.18-7.34 (10H, m, H-13, H-14, H-15), 7.35 (1H, s, H-7), 8.61 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 23.5 (C-6), 49.1 (C-11), 52.6 (C-1), 116.8 (C-8), 120.8 (C-3), 127.2 (C-15), 127.5 (C-13), 128.1 (C-7), 128.5 (C-14), 137.4 (C-12), 159.6 (C-9), 161.4 (C-10), 165.7 (C-2), 168.8 (C-5) ppm; ESI MS (*m/z*): 417 [M+H]$^+$, 375, 325, 283, 144, 129; HRMS (ESI): calc. for $C_{24}H_{25}O_3N_4^+$ ([M+H]$^+$): 417.1921, found: 417.1921; IR

(KBr disk, cm^{-1}): 3274, 3027, 2952, 2901, 2845, 1720, 1670, 1598, 1523, 1453, 1427, 1402, 1358, 1239, 1211, 1189, 1147, 1112, 1029.

(Z)-Methyl-2-(*tert*-butoxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate (38)

N, *N*-Dibenzyl-5-iodopyrimidin-2-amine (**25**) (0.50 g, 1.25 mmol, 1.0 eq.), methyl-2-(*tert*-butoxycarbonylamino)acrylate (0.33 g, 1.62 mmol, 1.3 eq.), NaHCO$_3$ (0.21 g, 2.50 mmol, 2.0 eq.), Bu$_4$NCl (0.45 g, 1.62 mmol, 1.3 eq.), PdCl$_2$ (0.06 g, 0.25 mmol, 20 mol%) and tri-*o*-tolylphosphine (0.08 g, 0.25 mmol, 20 mol%) were mixed with DMF (anhydrous, 4 mL) and stirred for 19 h at 70 °C under nitrogen. The reaction was quenched with sat. aq. NaHCO$_3$-solution (20 mL) and ethyl acetate (20 mL) was added. The org. layer was separated, washed with water (2 × 20 mL), dried over MgSO$_4$ and filtered. The solvent was removed under reduced pressure and the brown solid was purified *via* column chromatography (hexane/ethyl acetate, 5:1, R_f = 0.26) to give (Z)-methyl-2-(*tert*-butoxy-carbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate **38** (0.415 g, 70%) as a light yellow solid. (m.p.: 122 °C).

38

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.38 (9H, s, H-7), 3.77 (3H, s, H-1), 4.81 (4H, s, H-12), 7.11 (1H, s, H-3), 7.14-7.26 (10H, m, H-14, H-15, H-16), 8.49 (2H, s, H-10) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 21.2 (C-7), 49.5 (C-12), 52.8 (C-1), 81.4 (C-6), 117.5 (C-9), 122.1 (C-3), 126.7 (C-8), 127.3 (C-16), 127.9 (C-14), 128.7 (C-15), 137.9 (C-13), 153.0 (C-5), 159.6 (C-10), 161.6 (C-11), 166.1 (C-2) ppm; ESI MS (*m/z*): 949 [2×M+H]$^+$, 748,

497, 475 $[M+H]^+$, 419; HRMS (ESI): calc. for $C_{27}H_{31}O_4N_4^+$ ($[M+H]^+$): 475.2340, found: 475.2332; IR (KBr disk, cm^{-1}): 3314, 3027, 2977, 2947, 1720, 1696, 1637, 1598, 1519, 1492, 1454, 1438, 1392, 1360, 1303, 1230, 1161.

(Z)-Methyl-3-(2-(bis(*tert*-butoxycarbonyl)-amino)pyrimidin-5-yl)-2-(*tert*-butoxycarbonylamino)acrylate (39)

N, *N*-Dibenzyl-5-iodopyrimidin-2-amine (**25**) (0.53 g, 1.25 mmol, 1.0 eq.), methyl-2-(*tert*-butoxycarbonylamino)acrylate (0.36 g, 1.80 mmol, 1.4 eq.), NaHCO₃ (0.21 g, 2.50 mmol; 2.0 eq.), Bu₄NCl (0.45 g, 1.62 mmol, 1.3 eq.), PdCl₂ (0.06 g, 0.25 mmol, 20 mol%) and tri-*o*-tolylphosphine (0.08 g, 0.25 mmol, 20 mol%) were mixed with DMF (anhydrous, 6 mL) and stirred for 19 h at 70 °C under nitrogen. The reaction was quenched with sat. aq. NaHCO₃-solution (20 mL) and ethyl acetate (20 mL) was added. The org. layer was separated, washed with water (2×20 mL), dried over MgSO₄ and filtered. The solvent was removed under reduced pressure and the brown solid was purified *via* column chromatography (hexane/ethyl acetate, 3:1, R_f = 0.26) to give (Z)-methyl-3-(2-(bis(*tert*-butoxycarbonyl)-amino)pyrimidin-5-yl)-2-(*tert*-butoxycarbonylamino)acrylate **39** (0.19 g, 30%) as a colourless solid. (m.p.: 143 °C).

39

^{1}H NMR (500 MHz, CDCl₃): δ = 1.44 (27H, s, H-7, H-14), 3.89 (3H, s, H-1), 6.74 (1H, s, H-4), 7.14 (1H, s, H-8) ppm; ^{13}C NMR (125 MHz, CDCl₃): δ = 27.8 (C-14), 28.1 (C-7), 53.1 (C-1), 81.9 (C-6), 83.5 (C-13), 120.2, 126.7, 126.9, 150.3 (C-12), 152.0 (C-5), 157.2

(C-11), 158.4 (C-10), 165.0 (C-2) ppm; ESI MS (*m/z*): 989 [2×M+H]$^+$, 495 [M+H]$^+$, 395, 321, 265; HRMS (ESI): calc. for $C_{23}H_{35}O_8N_4^+$ ([M+H]$^+$): 495.2449, found:. 495.2454; IR (KBr disk, cm^{-1}): 3355, 2980, 2932, 1799, 1719, 1651, 1578, 1550, 1504, 1432, 1368, 1281, 1162, 1106.

(Z)-2-(*tert*-Butoxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylic acid (43)

(Z)-Methyl-2-(*tert*-butoxy-carbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)acryl-ate (**38**) (0.15 g, 0.32 mmol, 1.0 eq.) and lithium hydroxide monohydrate (0.07 g, 0.95 mmol, 3.0 eq.) were dissolved in a mixture of MeOH (8 mL), THF (4 mL) and water (1 mL). The solution was stirred for 27 h at room temperature, diluted with ethyl acetate (40 mL) and washed with 1 M HCl (40 mL). The org. phase was separated, dried with MgSO$_4$ and filtered. The solvent was removed under reduced pressure to give a light yellow solid of (Z)-2-(*tert*-butoxycarbonylamino)-3-(2-(dibenzyl-amino)pyrimidin-5-yl)acrylic acid **43** (0.14 g, 95%). (m.p.: 143-146 °C).

43

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.37 (3H, s, H-7), 4.83 (4H, s, H-12), 6.41 (1H, s, H-4), 7.11-7.33 (10H, m, H-14, H-15, H-16), 8.55 (2H, s, H-10), 9.40 (1H, s, H-1) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 28.0 (C-7), 49.4 (C-12), 81.2 (C-6), 117.3 (C-9), 122.2 (C-3), 126.6 (C-8), 127.2 (C-14), 127.5 (C-16), 128.5 (C-15), 137.0 (C-13), 153.0 (C-5), 159.2 (C-10), 160.4 (C-11), 169.0 (C-2) ppm; ESI MS (*m/z*): 921 [2×M+H]$^+$, 461 [M+H]$^+$, 405; HRMS (ESI): calc. for $C_{26}H_{29}O_4N_4^+$ ([M+H]$^+$): 461.2183, found: 461.2176; IR (KBr

disk, cm^{-1}): 3322, 3061, 3028, 2979, 2929, 2627 (br), 1702, 1636, 1596, 1519, 1453, 1423, 1393, 1358, 1235, 1159.

(*S*)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate (44)

(*Z*)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate (**37**) (3.01 g, 7.23 mmol, 1.0 eq.), bis(1,5-cyclooctadiene)rhodium(I)tetrafluoroborate (0.06 g, 0.15 mmol, 2 mol%) and (*S,S*)-Me-DUPHOS (0.09 g, 0.29 mmol, 4 mol%) were added to a steel autoclave. After purging two times with H$_2$, degassed MeOH (30 mL) was inserted and the reaction mixture was degassed again three times. The reaction was stirred at room temperature with 35 bar of H$_2$ for 24 h and stopped *via* release of H$_2$ pressure. After filtration through Celite the crude oil was purified *via* column chromatography (hexane/ethyl acetate, 1:2, R_f = 0.33) to give (*S*)-methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate **44** (2.82 g, 93%) as a colourless solid. (m.p.: 123 °C).

44

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.95 (3H, s, H-6), 2.85-2.96 (2H, m, H-7), 3.68 (3H, s, H-1), 4.70-4.78 (5H, m, H-3, H-11), 6.09 (1H, d, $^3J_{HH}$ = 7.2 Hz, H-4), 7.09-7.26 (10H, m, H-13, H-14, H-15), 8.03 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 23.2 (C-6), 31.9 (C-7), 49.1 (C-11), 52.6 (C-1), 53.1 (C-3), 116.9 (C-8), 127.1 (C-15), 127.6 (C-13), 128.1 (C-14), 138.1 (C-12), 158.4 (C-9), 161.7 (C-10), 169.7 (C-5), 171.8 (C-2) ppm; ESI MS (*m/z*): 419 [M+H]$^+$, 417, 276, 217, 129, 102, 74; HRMS (ESI): calc. for C$_{24}$H$_{27}$O$_3$N$_4$$^+$ ([M+H]$^+$): 419.2078, found: 419.2077; IR (KBr disk, cm^{-1}): 3297, 3061, 3026, 2921,
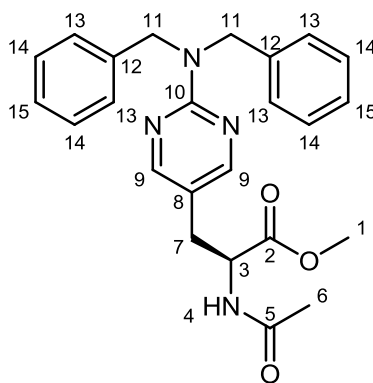
2851, 1948, 1746, 1654, 1609, 1541, 1509, 1452, 1429, 1403, 1359, 1259, 1228, 1202, 1169, 1120, 1028; Enantiomeric excess (ee): 98 % [HPLC: ODH column, 25 °C, hexane/*i*-PrOH: 20/80, 1 mL/min., ret. times: 10.1 min. (minor), 19.3 min. (major)]; Optical rotation: $[\alpha]_D = +80°$(c = 0.1, CH_2Cl_2).

(*R*)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate (45)

(Z)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate (**37**) (0.30 g, 0.720 mmol, 1.0 eq.), bis(1,5-cyclooctadiene)rhodium(I)tetrafluoroborate (0.006 g, 0.015 mmol, 2 mol%) and (*R,R*)-Me-DUPHOS (0.009 g, 0.029 mmol, 4 mol%) were added to a steel autoclave. After purging two times with H_2, degassed MeOH (30 mL) was inserted and the reaction mixture was degassed again three times. The reaction was stirred at room temperature with 35 bar of H_2 for 24 h and stopped *via* release of H_2 pressure. After filtration through Celite the crude oil was purified *via* column chromatography (hexane/ethyl acetate, 1:2, R_f = 0.33) to give (*R*)-methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate **45** (0.28 g, 93%) as a colourless solid. (m.p.: 123 °C).
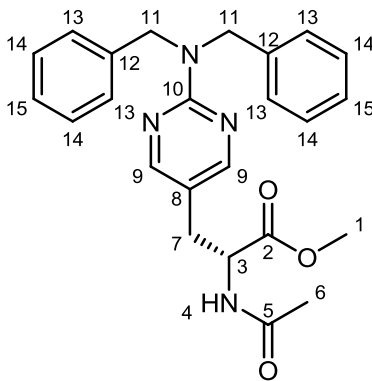
45

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.97 (3H, s, H-6), 2.86-2.97 (2H, m, H-7), 3.70 (3H, s, H-1), 4.74-4.78 (5H, m, H-3, H-11), 6.06 (1H, d, $^3J_{HH}$ = 7.3 Hz, H-4), 7.12-7.26 (10H, m, H-13, H-14, H-15), 8.02 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 23.2 (C-6), 31.8 (C-7), 49.1 (C-11), 52.6 (C-1), 53.0 (C-3), 116.8 (C-8), 127.1 (C-15), 127.5 (C-13), 128.5 (C-14), 138.1 (C-12), 158.4 (C-9), 161.8 (C-10), 169.7 (C-5), 171.8 (C-2) ppm; ESI

MS (m/z): 419 [M+H]$^+$, 441; HRMS (ESI): calc. for $C_{24}H_{27}O_3N_4^+$ ([M+H]$^+$): 419.2078, found: 419.2076.IR (KBr disk, cm^{-1}): 3296, 3064, 3025, 2916, 2849, 1938, 1746, 1653, 1610, 1541, 1510, 1452, 1430, 1404, 1360, 1261, 1230, 1201, 1168, 1117, 1029; Enantiomeric excess (ee): 99 % [HPLC: ODH column, 25 °C, hexane/i-PrOH: 20/80, 1 mL/min., ret. times: 10.1 min. (major), 19.3 min. (minor)]; Optical rotation: $[\alpha]_D = -80°$ (c = 0.1, CH_2Cl_2).

(*S*)-1-Carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride (46)

3 M HCl (20 mL) was added to a solution of (*S*)-methyl 2-acetamido-3-(2-(dibenzyl-amino)pyrimidin-5-yl)propanoate (**44**) (0.235 g, 0.56 mmol) in acetone (0.5 mL). The solution was refluxed at 110 °C for 6 h. After room temperature was reached the solvent was removed under reduced pressure to yield (*S*)-1-carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride **46** (0.22 g, 98%) as light yellow solid. (R_f = 0.56 (ethanol/ acetic acid, 80:20); m.p.: 143 − 146 °C)

46
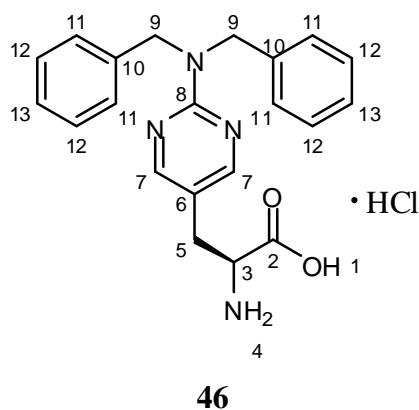
^{1}H NMR (500 MHz, MeOD): δ = 3.20-3.41 (2H, m, H-5), 4.41 (1H, t, $^3J_{HH}$ = 7.3 Hz, H-3), 5.00 (4H, s, H-9), 7.23-7.40 (10H, m, H-11, H-12, H-13), 8.63 (2H, s, H-7) ppm; ^{13}C NMR (125 MHz, MeOD): δ = 29.0 (C-5), 55.4 (C-9), 52.5 (C-3), 117.2 (C-6), 127.2 (C-11), 127.5 (C-13), 128.3 (C-12), 134.8 (C-10), 155.3 (C-7), 157.6 (C-8), 169.0 (C-2) ppm; ESI MS (m/z): 725 [2×M-Cl]$^+$, 421, 363 [M-Cl]$^+$, 290; HRMS (ESI): calc. for $C_{21}H_{23}O_2N_4^+$ ([M-Cl]$^+$): 363.1816, found: 363.1819; IR (KBr disk, cm^{-1}): 3397 (br), 2928 (br), 1963 (br), 1735, 1647, 1613, 1584, 1545, 1496, 1453, 1410, 1352, 1207, 1079, 1027.

(*R*)-1-Carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride (47)

3 M HCl (20 mL) was added to a solution of (**45**) (*R*)-methyl-2-acetamido-3-(2-(dibenzylamino)-pyrimidin-5-yl)propanoate (0.2 g, 0.49 mmol) in acetone (1 mL). The solution was refluxed at 110 °C for 6 h. After room temperature was reached the solvent was removed under reduced pressure to yield (*R*)-1-carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride **47** (0.2 g, 102%) as light yellow solid. (R_f = 0.56 (ethanol/ acetic acid, 80:20); m.p.: 143 − 146 °C)

47
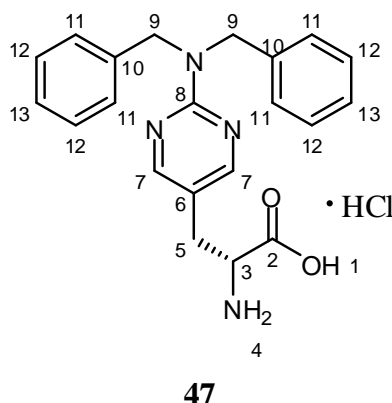
^{1}H NMR (500 MHz, MeOD): δ = 3.20-3.42 (2H, m, H-5), 4.40 (1H, t, $^3J_{HH}$ = 7.3 Hz, H-3), 5.01 (4H, s, H-9), 7.21-7.40 (10H, m, H-11, H-12, H-13), 8.63 (2H, s, H-7) ppm; ^{13}C NMR (125 MHz, MeOD): δ = 29.0 (C-5), 55.3 (C-9), 52.5 (C-3), 117.1 (C-6), 127.2 (C-11), 127.5 (C-13), 128.2 (C-12), 134.8 (C-10), 155.3 (C-7), 157.5 (C-8), 169.0 (C-2) ppm; ESI MS (*m/z*): 725 [2×M-Cl]$^+$, 421, 363 [M-Cl]$^+$, 290; HRMS (ESI): calc. for $C_{21}H_{23}O_2N_4^+$ ([M-Cl]$^+$): 363.1816, found: 363.1819; IR (KBr disk, cm^{-1}): 3400 (br), 2925 (br), 2349, 1964 (br), 1740, 1646, 1612, 1543, 1496, 1453, 1137, 1080, 1027.

(*S*)-2-(5-((*S*)-1-Amino-1-oxopropan-2-ylamino)-2,4-dinitrophenylamino)-3-(2-(di-benzylamino)pyrimidin-5-yl)propanoic acid (48)

(*S*)-1-Carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride **46** (0.06 g, 0.16 mmol, 1.0 eq.), *N*-(2,4)-dinitro-5-fluorophenyl)-L-alanine amide (FDAA) (0.05 g, 0.17 mmol, 1.1 eq.) and Cs$_2$CO$_3$ (0.99 g, 3.04 mmol, 20 eq.) were dissolved in a 1:1 mixture of acetone and water (2 mL). The reaction was protected with aluminium foil and

stirred at 40 °C for two hours. The solvent was removed under reduced pressure and the resulting orange solid was used crude for the HPLC experiments.
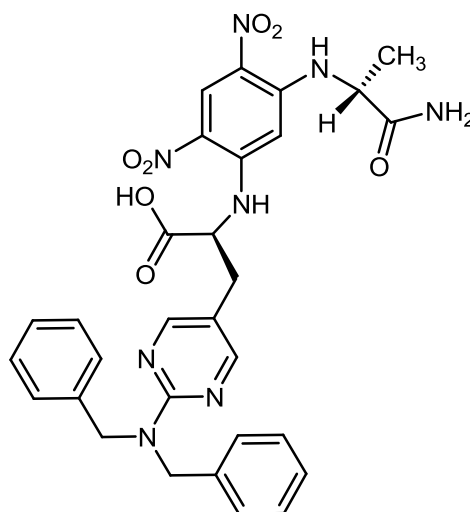
48

Enantiomeric excess (ee): 96 % [HPLC: C18 reverse phase column, 100% TEAP-buffer to 100% MeCN, 1 mL/min., ret. times: 26.1 min. (major), 27.3 min. (minor)].

(*R*)-2-(5-((*S*)-1-Amino-1-oxopropan-2-ylamino)-2,4-dinitrophenylamino)-3-(2-(di-benzylamino)pyrimidin-5-yl)propanoic acid (49)

(*R*)-1-Carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride **47** (0.01 g, 0.025 mmol, 1.0 eq.), *N*-(2,4-dinitro-5-fluorophenyl)-L-alanine amide (FDAA) (0.007 g, 0.027 mmol, 1.1 eq.) and Cs$_2$CO$_3$ (0.164 g, 0.50 mmol, 20 eq.) were dissolved in a 1:1 mixture of acetone and water (2 mL). The flask was wrapped in aluminium foil to exclude light and stirred at 40 °C for two hours. The solvent was removed under reduced pressure and the resulting orange solid was used crude for the HPLC experiments.

49

Enantiomeric excess (ee): 98 % [HPLC: C18 reverse phase column, 100% TEAP-buffer to 100% MeCN, 1 mL/min., ret. times: 26.1 min. (minor), 27.3 min. (major)].

(1*S*)-1-Carboxy-2-(2-(dibenzylamino)-1,4,5,6-tetrahydropyrimidin-5-yl)ethanaminium chloride (50)

(*S*)-1-Carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride (**46**) (0.06 g, 0.15 mmol) and Pd(OH)$_2$ (0.04 g) were added in a steel autoclave. After purging with H$_2$ degassed MeOH (30 mL) was inserted and the reaction mixture was degassed again for three times. The reaction mixture was stirred at 15 bar of H$_2$ at room temperature for 24 h. After releasing the H$_2$ pressure the mixture was filtrated through Celite and the solvent was removed under reduced pressure to give (1*S*)-1-carboxy-2-(2-(dibenzylamino)-1,4,5,6-tetrahydropyrimidine-5-yl)ethanaminium chloride **50** (0.06 g, 99%) as yellow solid. (R_f = 0.24 (ethanol/ acetic acid, 80:20); m.p.: 123 - 125 °C).

50

^{1}H NMR (400 MHz, D$_2$O): δ = 1.70-1.92 (2H, m, H-5), 2.07-2.23 (1H, m, H-6), 3.00-3.49 (4H, s, H-7), 3.74 (1H, t, $^3J_{HH}$ = 7.3 Hz, H-3), 4.53 (4H, s, H-10), 7.14-7.40 (10H, m, H-12, H-13, H-14), 8.07 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, MeOD): δ = 26.0 (C-6), 31.0 (C-5), 43.1 (C-7), 51.3 (C-10), 52.1 (C-3), 126.7 (C-12), 128.1 (C-14), 129.1 (C-13), 135.0 (C-11), 144.3 (C-9), 164.7 (C-2) ppm.ESI MS (*m/z*): 733 [2×M-Cl]$^+$, 423, 367 [M-Cl]$^+$, 294; HRMS (ESI): calc. for C$_{21}$H$_{27}$O$_2$N$_4^+$ ([M-Cl]$^+$): 367.2129, found: 367.2128; IR (KBr disk, cm^{-1}): 3382 (br), 3237 (br), 3031, 2922, 1623, 1496, 1453, 1422, 1079, 1028.

(1*S*)-1-Carboxy-2-(2-(dibenzylamino)-1,4,5,6-tetrahydropyrimidin-5-yl)ethanaminium chloride (51)

(*S*)-1-Carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethanaminium chloride (**47**) (0.06 g, 0.15 mmol) and Pd(OH)$_2$ (0.04 g) were added in a steel autoclave. After purging with H$_2$ degassed MeOH (30 mL) was inserted and the reaction mixture was degassed again for three times. The reaction mixture was stirred at 30 bar of H$_2$ at room temperature for 24 h. After releasing the H$_2$ pressure the mixture was filtrated through Celite and the solvent was removed under reduced pressure to give (1*S*)-1-carboxy-2-(2-(dibenzylamino)-1,4,5,6-tetrahydropyrimidine-5-yl)ethanaminium chloride **51** (0.06 g, 99%) as yellow solid. (R_f = 0.24 (ethanol/ acetic acid, 80:20); m.p.: 123 - 125 °C).

51
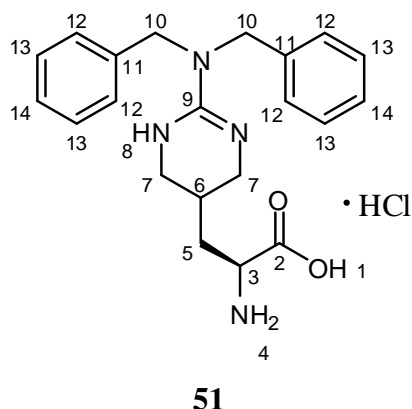
^{1}H NMR (400 MHz, D$_2$O): δ = 1.71-1.94 (2H, m, H-5), 2.06-2.23 (1H, m, H-6), 3.02-3.48 (4H, s, H-7), 3.75 (1H, t, $^3J_{HH}$ = 7.3 Hz, H-3), 4.53 (4H, s, H-10), 7.14-7.41 (10H, m, H-12, H-13, H-14), 8.07 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, MeOD): δ = 26.1 (C-6), 31.0 (C-5), 43.1 (C-7), 51.2 (C-10), 52.2 (C-3), 126.7 (C-12), 128.0 (C-14), 129.1 (C-13), 135.0 (C-11), 144.3 (C-9), 164.7 (C-2) ppm.ESI MS (*m/z*): 733 [2×M-Cl]$^+$, 423, 367 [M-Cl]$^+$, 294; HRMS (ESI): calc. for C$_{21}$H$_{27}$O$_2$N$_4$$^+$ ([M-Cl]$^+$): 367.2129, found: 367.2128; IR (KBr disk, cm^{-1}): 3382 (br), 3237 (br), 3033, 2921, 1623, 1496, 1454, 1422, 1078, 1028.

(*S*)-5-(2-Acetamido-2-carboxyethyl)-2-aminopyrimidin-1-ium nitrate (56)

(*S*)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate **44** (0.11 g, 0.26 mmol, 1.0 eq.) and ammoniumcerium(IV)nitrate (0.83 g, 1.51 mmol, 6.0 eq.) were dissolved in a 9:1 mixture of acetonitrile and water (10 mL) and stirred for 6 ½ h at room temperature. The acetonitrile was removed under reduced pressure and the cerium salt was precipitated from the water by changing the pH from one to twelve with 3 M NaOH. The solid was filtered off and washed with ethyl acetate (10 mL). The water layer was separated and the solvent was removed under reduced pressure to give a light yellow solid. MeOH (15 mL) was added to the solid and the solvent was removed from the filtrate to yield (*S*)-5-(2-acetamido-2-carboxyethyl)-2-aminopyrimidin-1-ium nitrate **56** (0.07 g, 92%) as yellow solid. (m.p.: ~ 150 °C decomp.)

56

^{1}H NMR (500 MHz, MeOD): δ = 1.94 (3H, s, H-5), 2.75-2.83 (1H, dd, $^3J_{HH}$ = 4.8 Hz, 9.0 Hz, H-6), 2.98-3.05 (1H, dd, $^3J_{HH}$ = 4.8 Hz, 9.0 Hz, H-6), 4.36-4.39 (1H, dd, $^3J_{HH}$ = 4.8 Hz, 2.2 Hz, H-2), 8.12 (2H, s, H-8) ppm; ^{13}C NMR (125 MHz, MeOD): δ = 23.7 (C-5), 33.3 (C-6), 57.0 (C-2) 120.7 (C-7), 158.5 (C-8), 171.1 (C-9), 176.0 (C-4), 187.2 (C-1) ppm; IR (KBr disk, cm^{-1}): 3442 (br), 2963, 2931, 2857, 2426 (br), 1789, 1715 (C=O), 1642 (C=O), 1567 (N-O asym), 1385 (N-O sym), 1208, 1145, 1112, 1049.

5-Iodo-*N, N*-bis(4-methoxybenzyl)pyrimidin-2-amine (60)

2-Amino-5-iodopyrimidine (1.00 g, 4.5 mmol, 1.0 eq.) and sodium hydride (0.54 g, 13.8 mmol, 3.0 eq., 60% NaH in oil) were mixed with dry THF (40 mL). 4-Methoxybenzyl chloride (3.1 mL, 22.8 mmol, 5.0 eq.) was added at 0 °C and the reaction mixture was then stirred at 30 °C for 23 h. The reaction was quenched with water (20 mL) and ethyl acetate (20 mL). The water layer was washed with ethyl acetate (2 × 10 mL) and the combined org. layer was dried over MgSO$_4$ and filtered. The crude product was purified *via* column chromatography (hexane/ethyl acetate, 20:1 to 10:1, R_f = 0.33 for 10:1) to give 5-iodo-*N, N*-bis(4-methoxybenzyl)pyrimidin-2-amine **60** (1.90 g, 91%) as a colourless solid. (m.p.: 80-81 °C).

60

^{1}H NMR (400 MHz, CDCl$_3$): δ = 3.80 (6H, s, H-9), 4.72 (4H, s, H-4), 6.84 (4H, d, $^3J_{HH}$ = 8.7 Hz, H-7), 7.15 (4H, d, $^3J_{HH}$ = 8.7 Hz, H-6), 8.43 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 48.4 (C-4), 55.3 (C-9), 75.4 (C-1), 113.9 (C-7), 128.9 (C-6), 129.8 (C-5), 158.8 (C-8), 160.4 (C-3), 162.6 (C-2) ppm; ESI MS (*m/z*): 500 [M+NH$_4$]$^+$, 462 [M+H]$^+$, 419, 391, 276, 199, 137; HRMS (ESI): calc. for C$_{20}$H$_{21}$O$_2$IN$_3$$^+$ ([M+H]$^+$): 462.0673, found: 462.0671; IR (thin film, cm^{-1}): 3008 (m) , 2954 (w), 2934 (w), 2910 (w), 2835 (m), 1612 (m), 1569 (s), 1512 (s), 1464 (w), 1418 (w), 1392 (m), 1359 (m), 1303 (m), 1248 (s), 1174 (m), 1109 (w), 1036 (m).
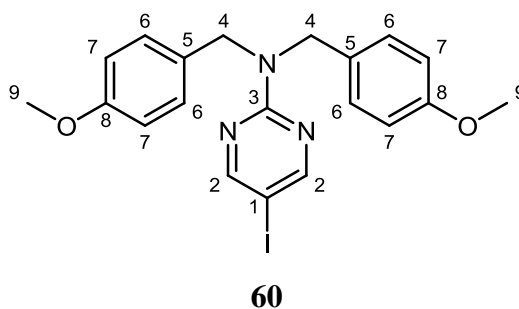
(Z)-Methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)acrylate (61)

5-Iodo-*N, N*-bis(4-methoxybenzyl)pyrimidin-2-amine (**60**) (0.289 g, 0.63 mmol, 1.0 eq.), methyl-2-acet-amidoacrylate (0.100 g, 0.70 mmol, 1.1 eq.), NaHCO$_3$ (0.106 g, 1.26 mmol, 2.0 eq.), Bu$_4$NCl (0.211 g, 0.76 mmol, 1.2 eq.), PdCl$_2$ (0.023 g, 0.13 mmol, 20 mol%) and tri-*o*-tolylphosphine (0.077 g, 0.25 mmol, 40 mol%) were mixed with DMF (anhydrous, 12 mL) and stirred for 24 h at 110 °C under nitrogen. The reaction was quenched with ethyl acetate (12 mL) and washed with water (3 × 15 mL). The combined water layer was washed with ethyl acetate (12 mL). The combined org. layer was dried over MgSO$_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified *via* column chromatography (hexane/ethyl acetate, 1:2, R_f = 0.47) to give (Z)-methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)-pyrimidin-5-yl)acrylate **61** (0.130 g, 43%) as a colourless solid. (m.p.: 172 - 173 °C).
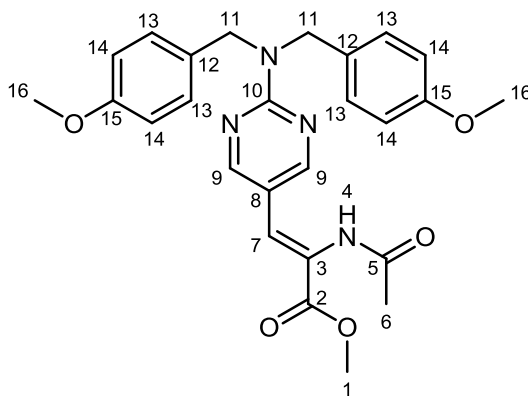
61

^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.17 (3H, s, H-6), 3.79 (4H, s, H-16), 3.83 (3H, s, H-1), 4.78 (4H, s, H-11), 6.83 (4H, d, J = 8.6 Hz, H-14), 7.14 (4H, d, J = 8.6 Hz, H-13), 7.27 (1H, s, H-4), 7.30 (1H, s, H-7), 8.46 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 23.6 (C-7), 48.2 (C-11), 52.6 (C-1), 55.2 (C-16), 113.9 (C-14), 116.7 (C-8), 120.4 (C-3), 128.1 (C-7), 128.9 (C-13), 129.5 (C-12), 158.9 (C-15), 159.6 (C-9), 161.3 (C-10), 165.8 (C-2), 168.6 (C-5) ppm; ESI MS (m/z): 975 [2×M+Na]$^+$, 953 [2×M+H]$^+$, 499 [M+Na]$^+$,477 [M+H]$^+$,462, 279, 163; HRMS (ESI): calc. for C$_{26}$H$_{29}$O$_5$N$_4$$^+$ ([M+H]$^+$): 477.2132, found: 477.2124; IR (thin film, cm^{-1}): 3371 (br), 2957, 2920, 1715, 1665, 1595, 1511, 1435, 1359, 1246, 1174, 1111, 1036.

(*S*)-Methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)propanoate (62)

(Z)-Methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)acrylate (**61**) (0.50 g, 1.05 mmol, 1.0 eq.), bis(1,5-cyclooctadiene)rhodium(I)tetrafluoroborate (0.018 g, 0.044 mmol, 4 mol%) and (*S*,*S*)-Me-DUPHOS (0.085 g, 0.085 mmol, 8 mol%) were added in a steel autoclave. After purging with H$_2$ two times degassed MeOH (30 mL) was inserted and the reaction mixture was degassed again for three times. The reaction was stirred at room temperature with 38 bar of H$_2$ for 24 h and stopped *via* release of H$_2$ pressure. After filtration through Celite the crude oil was purified *via* column chromatography (hexane/ethyl acetate, 1:2, R_f = 0.29) to give (*S*)-methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)propanoate **62** (0.45 g, 90%) as a colourless solid. (m.p.: 66-68 °C).

62

^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.04 (3H, s, H-6), 2.90-3.08 (2H, m, H-7), 3.78 (3H, s, H-1), 3.79 (6H, s, H-16), 4.74 (4H, s, H-11), 4.81-4.87 (1H, m, H-3), 6.09 (1H, d, J = 7.4 Hz, H-4), 6.83 (4H, d, $^3J_{HH}$ = 8.7 Hz, H-14), 7.14 (4H, d, $^3J_{HH}$ = 8.7 Hz, H-13), 8.09 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 23.2 (C-6), 31.8 (C-7), 48.1 (C-11), 52.6 (C-1), 53.0 (C-16), 55.3 (C-3), 113.9 (C-14), 116.8 (C-8), 128.9 (C-13), 130.2 (C-12), 158.4 (C-9), 158.8 (C-15), 161.8 (C-10), 169.7 (C-2), 171.7 (C-5) ppm; ESI MS (*m/z*): 957 [2×M+H]$^+$, 501, 479 [M+H]$^+$, 391, 234, 199, 163; HRMS (ESI): calc. for C$_{26}$H$_{31}$O$_5$N$_4$$^+$ ([M+H]$^+$): 479.2289, found: 479.2284; IR (thin film, cm^{-1}): 3417 (br), 3006, 2954, 2836, 1743, 1658, 1607, 1539, 1511, 1436, 1418, 1361, 1110, 1034; Enantiomeric excess (ee): 99 % [HPLC: ODH column, 25 °C, hexane/*i*-PrOH: 20/80, 1 mL/min., ret. times: 13.2 min. (minor), 24.2 min. (major)]; Optical rotation: [α]$_D$ = +120° (c = 0.1, CH$_2$Cl$_2$).

(*R*)-Methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)propanoate (63)

(Z)-Methyl-2-acetamido-3-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)acrylate   (**61**) (0.15 g, 0.32 mmol, 1.0 eq.), bis(1,5-cyclooctadiene)rhodium(I)tetrafluoroborate (0.005 g, 0.013 mmol, 4 mol%) and (*R*,*R*)-Me-DUPHOS (0.008 g, 0.0025 mmol, 8 mol%) were added in a steel autoclave. After purging with H$_2$ two times degassed MeOH (20 mL) was inserted and the reaction mixture was degassed again for three times. The reaction was stirred at room temperature with 38 bar of H$_2$ for 24 h and stopped *via* release of H$_2$ pressure. After filtration through Celite the crude oil was purified *via* column chromatography (hexane/ethyl acetate, 1:2, R_f = 0.29) to give (*S*)-methyl-2-acetamido-3-

(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)propanoate **63** (0.14 g, 91%) as a colourless solid. (m.p.: 66 - 68 °C).
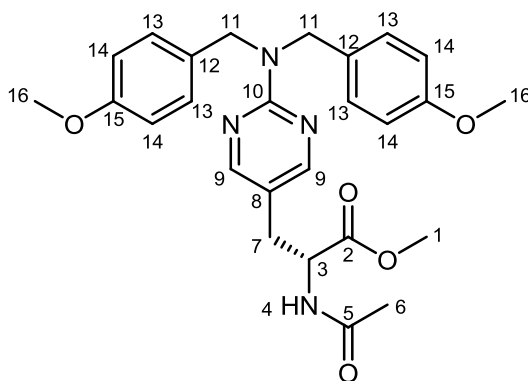

63

^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.04 (3H, s, H-6), 2.90-3.08 (2H, m, H-7), 3.78 (3H, s, H-1), 3.79 (6H, s, H-16), 4.74 (4H, s, H-11), 4.81-4.87 (1H, m, H-3), 6.09 (1H, d, J = 7.4 Hz, H-4), 6.83 (4H, d, $^3J_{HH}$ = 8.7 Hz, H-14), 7.14 (4H, d, $^3J_{HH}$ = 8.7 Hz, H-13), 8.09 (2H, s, H-9) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 23.2 (C-6), 31.8 (C-7), 48.1 (C-11), 52.6 (C-1), 53.0 (C-16), 55.3 (C-3), 113.9 (C-14), 116.8 (C-8), 128.9 (C-13), 130.2 (C-12), 158.4 (C-9), 158.8 (C-15), 161.8 (C-10), 169.7 (C-2), 171.7 (C-5) ppm; ESI MS (*m/z*): 957 [2×M+H]$^+$, 501, 479 [M+H]$^+$, 391, 234, 199, 163; HRMS (ESI): calc. for C$_{26}$H$_{31}$O$_5$N$_4$$^+$ ([M+H]$^+$): 479.2289, found: 479.2283; IR (thin film, cm^{-1}): 3417 (br), 3006, 2954, 2836, 1743, 1658, 1607, 1539, 1511, 1436, 1418, 1361, 1110, 1034; Enantiomeric excess (ee): 99 % [HPLC: ODH column, 25 °C, hexane/*i*-PrOH: 20/80, 1 mL/min., ret. times: 13.3 min. (major), 24.2 min. (minor)]; Optical rotation: [α]$_D$ = -120° (c = 0.1, CH$_2$Cl$_2$).

Diethyl-2-(*tert*-butoxycarbonylimino)malonate (74)

Under nitrogen atmosphere *N*-Boc-triphenyliminophophorane **81** (11.84 g, 31.38 mmol, 1.1 eq.) was dissolved in dry THF (60 mL) and diethylmesoxalate (4.94 g, 28.36 mmol, 1.0 eq.) was added at room temperature. The THF was removed under reduced pressure after 16 h at 80 °C and a colourless solid was obtained. Cooled dry diethyl ether (20 mL) was added and the solid (PPh$_3$O) was filtered of by washing with cooled dry diethyl ether. The solvent was removed under reduced pressure and the light yellow oil was purified by

kugelrohr distillation to give diethyl-2-(*tert*-butoxycarbonylimino)malonate **74** (9.05 g, 50%) as a colourless oil.

74

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.49 (6H, m, H-7), 1.70 (9H, s, H-1), 4.52 (4H, m, H-6) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 14.6, 28.6, 63.8, 64.0, 85.3, 152.3, 158.7, 158.9 ppm.

tert-Butyl di-(ethoxycarbonyl)(2-(dibenzylamino)pyrimidin-5-yl)methylcarbamate (79)

Under nitrogen atmosphere **25** (0.8 g, 2.0 mmol, 1.0 eq.) was dissolved in THF (10 mL) and *i*-PrMgCl (1.1 mL, 2.2 mmol, 1.1 eq., 2M in THF) was added via syringe at room temperature. A solution of **74** (0.44 g, 2.0 mmol, 1.0 eq.) in THF (1 mL) was added after 5 h and the reaction solution was stirred further for the next 16 hours. Sat. aq. NH$_4$Cl (4 mL) was added and the water phase was washed with ethyl acetate (2x5 mL). The combined organic phase was washed with brine (10 mL), dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (first column hexane/ethyl acetate, 10:1, R_f = 0.12; second column dichloromethane, R_f = 0.16) to give *tert*-butyl di-(ethoxycarbonyl)(2-(dibenzylamino)-pyrimidin-5-yl)methylcarbamate **79** (0.22g, 20%) as a yellow oil.

79

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.27 (6H, t, $^3J_{HH}$ = 7.1 Hz, H-12), 3.57 (9H, s, H-16), 4.16-4.39 (4H, m, H-11), 4.66 (4H, s, H-4), 6.29 (1H, s, H-13), 7.17-7.35 (10H, m, H-6, H-7, H-8), 8.62 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 14.0 (C-12), 28.3 (C-16), 49.0 (C-4), 63.2 (C-11), 65.9 (C-9), 81.0 (C-15), 116.1 (C-1), 127.1 (C-8), 127.7 (C-6), 128.6 (C-7), 138.0 (C-5), 154.0 (C14), 157.6 (C-2), 162.0 (C-3), 167.1 (C-10) ppm; ESI MS (*m/z*): 549 [M+H]$^+$, 477, 475, 457, 434, 401, 375, 362, 276, 270, 184, 176, 106, 91, 78, 58, 44; HRMS (ESI): calc. for C$_{30}$H$_{37}$O$_6$N$_4^+$ ([M+H]$^+$): 549.2708, found: 549.2709.IR (KBr disk, cm^{-1}): 3684, 3420, 2983, 1741, 1715, 1570, 1515, 1485, 1454, 1426, 1394, 1368, 1257, 1161, 1025.

N-Boc-triphenyliminophosphorane (81) (Armstrong, Alan; Org. Lett.)

A solution of *tert*-butylcarbazate (**80**) (5.00 g, 37.8 mmol, 1.0 eq.) in acetic acid (10 mL) and water (20 mL) was cooled to 0 °C. NaNO$_2$ (2.87 g, 41.6 mmol, 1.1 eq.) was added in small portions and the reaction mixture was then stirred for 25 min. at 0 °C. The produced azide was extracted with diethylether (2x30 mL) and the combined organic phase was washed with water (60 mL), sat. aq. NaHCO$_3$ (40 mL, quickly) and brine (40 mL). This ether solution was used directly for the second step. PPh$_3$ (9.92 g, 37.8 mmol, 1.0 eq.) was added in small portions (strong N$_2$ evolution) at 0 °C. After 1.5 h at room temperature the precipitated product was filtered and dried. *N*-Boc-triphenyliminophophorane **81** (11.8 g, 83%) was obtained as colourless solid.

81

^{1}H NMR (250 MHz, CDCl$_3$): δ = 3.39 (9H, s, H-1), 7.47-7.68 (8H, m, H-5, H-7), 7.72-7.75 (6H, m, H-6) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 28.3 (C-1), 77.8 (C-2), 128.5 (C-6, $^{3}J_{CP}$ = 12 Hz), 128.6 (C-4, $^{1}J_{CP}$ = 101 Hz), 132.1 (C-7, $^{4}J_{CP}$ = 3 Hz), 133.1 (C-5, $^{2}J_{CP}$ = 10 Hz), 161.2 (C-3) ppm.

Diethyl-2-amino-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate (82)

Diethyl-2-(*tert*-butoxycarbonylamino)-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate (**79**) (0.15 g, 0.27 mmol) was dissolved in 10% TFA in DCM (10 mL) at room temperature for 4 h. The yellow solution was then washed with sat. aq. NaHCO$_3$ (2 × 20 mL) to remove the TFA. The solvent was removed under reduced pressure to give diethyl-2-amino-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate **82** (1.12 g, 97%) as a yellow oil. R_f = 0.41 (dichloromethane plus 1% TEA).

82

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.23 (6H, t, $^{3}J_{HH}$ = 7 Hz, H-12), 4.21 (4H, m, H-11), 4.80 (4H, s, H-4), 7.09 – 7.32 (10H, m, H-6, H-7, H-8), 8.49 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, acetone-d$_6$): δ = 15.3 (C-12), 50.9 (C-4), 63.8 (C-11), 68.0 (C-9), 119.0 (C-1), 121.8 (C-1), 128.8 (C-8), 129.4 (C-6), 130.2 (C-7), 140.4 (C-5), 159.2 (C-2), 163.7 (C-3), 172.2 (C-10) ppm; EI MS (*m/z*): 448 [M]$^{+}$, 375, 357, 284, 209, 91; HRMS (ESI): calc. for C$_{25}$H$_{29}$O$_4$N$_4$$^{+}$ ([M+H]$^{+}$): 449.2183, found: 449.2188; IR (thin film, cm^{-1}): 1737, 1602, 1515, 1453, 1426, 1364, 1028.
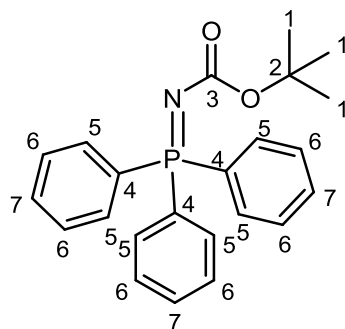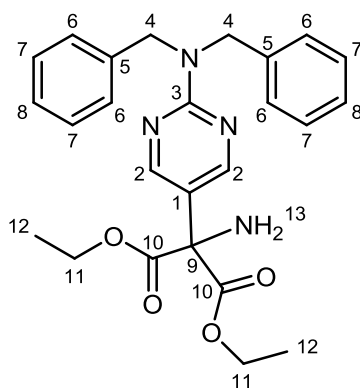
Diethyl-2-acetamido-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate (84)

Triethylamine (0.13 mL, 0.93 mmol, 4.0 eq.) was added into a solution of diethyl-2-amino-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate **82** (0.10 g, 0.22 mmol, 1.0 eq.) in acetic anhydride (5 mL). The yellow solution was stirred at room temperature for 4 h until the conversion was complete (TLC control). The reaction was quenched with water (20 mL). Ethyl acetate (15 mL) was added and the org. phase was washed with water (2 × 10 mL), dried over Na$_2$SO$_4$ and filtered. The solvent was removed under reduced pressure to yield diethyl-2-acetamido-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate **84** (0.12 g, 99%) as a brown oil. R_f = 0.20 (dichloromethane).
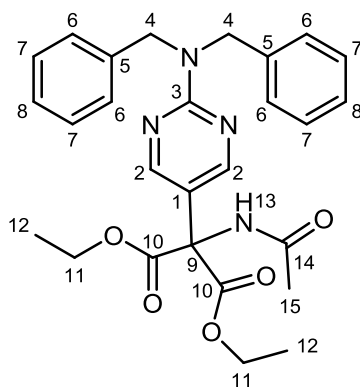
84

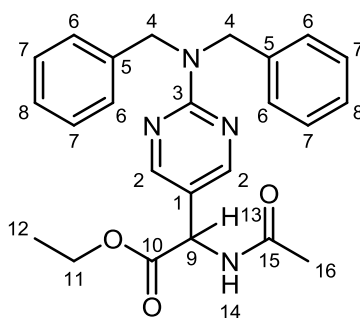^{1}H NMR (400 MHz, acetone-d$_6$): δ = 1.08 (6H, t, $^{3}J_{HH}$ = 7 Hz, H-12), 1.98 (3H, s, H-15), 4.22 (4H, m, H-11), 4.85 (4H, s, H-4), 7.03 – 7.26 (10H, m, H-6, H-7, H-8), 7.87 (1-H, s, H-13), 8.41 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, acetone-d$_6$): δ = 15.2 (C-12), 23.5 (C-15), 51.0 (C-4), 64.4 (C-11), 67.8 (C-9), 119.0 (C-1), 128.8 (C-8), 129.4 (C-6), 130.3 (C-

7), 140.3 (C-5), 159.4 (C-2), 163.6 (C-3), 168.6 (C-14), 171.0 (C-10) ppm; CI MS (m/z): 491 [M+H]$^+$, 434, 419, 362, 276, 106, 77; HRMS (ESI): calc. for $C_{27}H_{31}O_5N_4^+$ ([M+H]$^+$): 491.2289, found: 491.2287; IR (thin film, cm^{-1}): 3680, 3428, 2982, 1740, 1608, 1515, 1454, 1426, 1368, 1255, 1165, 1027.

Ethyl-2-acetamido-2-(2-(dibenzylamino)pyrimidin-5-yl)propanoate (85)

Diethyl-2-acetamido-2-(2-(dibenzylamino)pyrimidin-5-yl)malonate **84** (0.13 g, 0.26 mmol, 1.0 eq.) and LiBr (0.045 g, 0.52 mmol, 2.0 eq.) were dissolved in DMF (10 mL) and one drop of water. After stirring at 130 °C for 19 h the reaction was quenched with water (20 mL) and ethyl acetate (15 mL) was added. The organic layer was washed with brine (15 mL) and the combined water layer was then washed with ethyl acetate (15 mL). The combined organic phase was dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. The crude solid was purified *via* column chromatography (hexane/ethyl acetate, 1:1, R_f = 0.35) to give ethyl-2-acetamido-2-(2-(dibenzylamino)-pyrimidin-5-yl)propanoate **85** (0.06 g, 56%) as a light yellow oil.

85

^{1}H NMR (500 MHz, acetone-d$_6$): δ = 1.21 (3H, t, $^3J_{HH}$ = 7 Hz, H-12), 1.96 (3H, s, H-16), 4.18 (2H, m, H-11), 4.86 (4H, 2 × d, J_{HH} = 15 Hz, 4 Hz, H-4), 5.39 (1-H, d, $^3J_{HH}$ = 7 Hz, H-13), 7.22 − 7.34 (10H, m, H-6, H-7, H-8), 7.86 (1-H, bd, H-14), 8.41 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, acetone-d$_6$): δ = 15.4 (C-12), 23.5 (C-16), 51.1 (C-4), 54.3 (C-11), 63.1 (C-9), 121.1 (C-1), 128.9 (C-8), 129.4 (C-6), 130.3 (C-7), 140.4 (C-5), 159.2 (C-2), 163.9 (C-3), 170.9 (C-10), 172.1 (C-15) ppm; EI MS (m/z): 418 [M]$^+$, 345, 372, 303, 281, 253, 211, 91; HRMS (ESI): calc. for $C_{24}H_{27}O_3N_4^+$ ([M+H]$^+$): 419.2078, found: 419.2078;

IR (thin film, cm^{-1}): 3682, 3428, 2980, 1741, 1608, 1514, 1455, 1426, 1367, 1254, 1165, 1025.

N-Acetyl-triphenyliminophophorane (87)

A mixture of sodium azide (0.85 g, 13.0 mmol, 1.3 eq.) in acetone (50 mL) was cooled to 0 °C. Acetylchloride (0.71 mL, 10.0 mmol, 1.0 eq.) was added *via* syringe followed by triphenylphosphine (2.62 g, 10.0 mmol, 1.0 eq.) which was added in small portions. The reaction was stirred at room temperature for 40 min. Acetone was removed under reduced pressure and dichloromethane (20 mL) was added to the colourless solid. The salts were filtered off and the product was precipitated by adding diethyl ether (six drops) to the solution. The colourless solid was filtered off and dried to give *N*-acetyl-triphenyliminophophorane **87** (2.41 g, 76%).
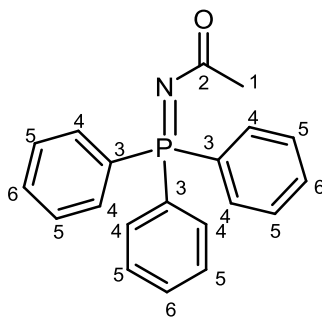
87

^{1}H NMR (500 MHz, CDCl$_3$): δ = 2.26 (3H, d, $^{3}J_{HH}$ = 2.9 Hz, H-1), 7.43-7.49 (6H, m, H-5), 7.52-7.58 (3H, m, H-6), 7.70-7.77 (6H, m, H-4) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 27.5 and 27.6 (*J* = 19.9 Hz, C-1), 128.3 and 128.4 (*J* = 7.0 Hz, C-3), 128.6 and 128.7 (*J* = 12.3 Hz, C-5), 132.2 (C-6), 133.0 and 133.1 (*J* = 9.9 Hz, C-4), 182.8 (C-2) ppm.

Diethyl-2-(acetylimino)malonate (88)

Under nitrogen atmosphere *N*-acetyl-triphenyliminophophorane **87** (3.80 g, 11.9 mmol, 1.1 eq.) was dissolved in dry THF (40 mL) and diethylmesoxalate (1.63 mL, 10.7 mmol, 1.0 eq.) was added at room temperature. The THF was removed under reduced pressure after refluxing for 17 h at 80 °C and a yellow solid was obtained. Cooled dry diethyl ether (6 mL) was added and the solid (PPh$_3$O) was filtered of by washing with cooled dry diethyl ether. The solvent was removed under reduced pressure and the light yellow oil was purified by kugelrohr distillation to give diethyl-2-(acetylimino)malonate **88** (1.18 g, 51%) as a colourless oil.

88
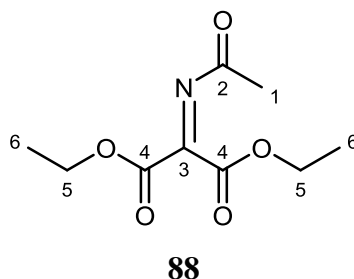
^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.13-1.32 (6H, m, H-6), 2.03 (3H, s, H-1), 4.12-4.33 (4H, m, H-5) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 13.2 (C-6), 22.3 (C-1), 62.1 (C-5), 162.6 (C-3), 165.0 (C-4), 169.3 (C-2) ppm.

2-(5-Iodopyrimidin-2-yl)isoindoline-1,3-dione (90)

The reaction was done in a microwave reactor without solvent. 2-Amino-5-iodopyrimidine (0.028 g, 0.13 mmol, 1.0 eq.) and phthalic anhydride (0.029 g, 0.20 mmol, 1.5 eq.) were added to the microwave tube and the tube was sealed. The reaction was carried out at 300 watts, 220 °C for 5 minutes. Chloroform (5 mL) and sat. aq. NaHCO$_3$ solution (5 mL) were added to the tube and the org. layer was separated and dried over MgSO$_4$. The crude product was purified *via* column chromatography (DCM/ethyl acetate, 50:1, R_f = 0.50) to give 2-(5-iodopyrimidin-2-yl)isoindoline-1,3-dione (**90**) (0.028 g, 62%) as colourless solid. (m.p.: 134-136 °C).

90
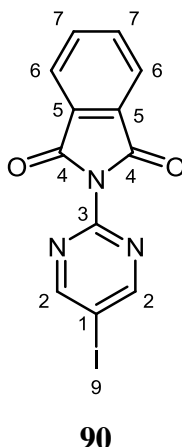
^{1}H NMR (500 MHz, CDCl$_3$): δ = 7.84 (2H, m, H-6), 8.00 (2H, m, H-7), 9.09 (2H, s, H-2) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 76.2 (C-1), 124.3 (C-6), 131.6 (C-5), 134.9 (C-7), 163.2 (C-3), 164.5 (C-2), 165.4 (C-4) ppm; ESI MS (*m/z*): 703 [2×M+H]$^+$, 374, 369, 352 [M+H]$^+$, 282, 217, 163, 150; HRMS (ESI): calc. for C$_{12}$H$_6$IN$_3$O$_2$$^+$ ([M+H]$^+$): 351.9577, found: 351.9580; IR (thin film, cm^{-1}): 3033, 2954, 2834, 1784, 1756, 1720, 1537, 1465, 1418, 1378, 1363, 1260, 1098, 1082, 1008.

N, *N*-Di-*tert*-butoxycarbonyl-2-aminopyrimidin-5-ol (91)

N, *N*-Di-*tert*-butoxycarbonyl-5-iodopyrimidin-2-amine **32** (0.10 g, 0.24 mmol, 1.0 eq.), bis(pinacolatodiboron) (0.18 g, 0.71 mmol, 3.0 eq.), potassium acetate (0.07 g, 0.71 mmol, 3.0 eq.) and palladium(II)acetate (0.003 g, 0.013 mmol, 5.6 mol%) were mixed with DMF (2 mL) and heated up to 85 °C. After 6 ½ h the reaction was not finished and bis(pinacolatodiboron) (0.11 g, 0.43 mmol, 1.8 eq.) and palladium(II)acetate (0.002 g, 0.009 mmol, 3.8 mol%) were added. The reaction was quenched after 24 hours with water (2 mL) and extracted with ethyl acetate (3 × 3 mL). The combined org. layer was washed with brine (2 mL) and water (2 mL). The org. layer was concentrated and dried *in vacuo* and THF (2 mL) and water (2 mL) were added to the yellow oil. Sodium perborate tetrahydrate (0.11 g, 0.73 mmol, 3.0 eq.) was added and the mixture was stirred for 24 h at room temperature. Sat. aq. NH$_4$Cl solution (5 mL) was added and the solution extracted with ethyl acetate (6 mL). The org. layer was dried over MgSO$_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified *via* column chromatography (ethyl acetate/chloroform, 30:70, R_f = 0.16) to give *N*, *N*-di-*tert*-

butoxycarbonyl-2-amino-pyrimidin-5-ol **91** (0.05 g, 67%) as a light yellow solid. (m.p.: 133 - 135 °C).

91

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.41 (18H, s, H-7), 8.32 (2H, s, H-3), ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 27.8 (C-7), 83.9 (C-6), 146.0 (C-3), 150.0 (C-2), 150.5 (C-4), 151.2 (C-5) ppm; ESI MS (*m/z*): 645 [2×M+Na]$^+$, 623 [2×M+H]$^+$, 487, 433, 380, 334 [M+Na]$^+$, 312 [M+H]$^+$, 212, 176; HRMS (ESI): calc. for C$_{14}$H$_{21}$O$_5$N$_3$Na$^+$ ([M+Na]$^+$): 334.1373, found: 334.1377; IR (KBr disk, cm^{-1}): 2981, 2851 (br), 2704 (br), 2587 (br), 1757, 1735, 1723, 1568, 1439, 1397, 1375, 1292, 1260, 1149, 1107, 1061, 1030.

(*S*)-Methyl-2-(tert-butoxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)propan-oate (92)

(*Z*)-Methyl-2-(*tert*-butoxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)-acrylate (**38**) (0.02 g, 0.04 mmol, 1.0 eq.), bis(1,5-cyclooctadiene)rhodium(I)tetra-fluoroborate (0.008 g, 0.02 mmol, 4.5 mol%) and (*S*,*S*)-Me-DUPHOS (0.005 g, 0.015 mmol, 3.5 mol%) were added to a steel autoclave. After purging three times with H$_2$, degassed MeOH (20 mL) was inserted and the reaction mixture was degassed again three times. The reaction was stirred at room temperature with 35 bar of H$_2$ for three days and stopped *via* release of H$_2$ pressure. After filtration through Celite the proton NMR of the crude oil showed 67% product and 33% starting material. Because the compound mixture is not separable *via* column chromatography the crude oil was purified by preparative TLC (DCM/methanol, 200:1, R_f = 0.25) to give (*S*)-methyl-2-(*tert*-butoxy-carbonylamino)-3-(2-(dibenzyl-amino)pyrimi-din-5-yl)propanoate **92** (0.0012 g, 7%) as a colourless solid.

92

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.25 (9H, s, H-7), 2.77-2.95 (2H, m, H-8), 3.69 (3H, s, H-1), 4.49 (1H, m, H3), 4.78 (4H, s, H-12), 5.03 (1H, d, $^3J_{HH}$ = 7.3 Hz, H-4), 7.13-7.26 (10H, m, H-14, H-15, H-16), 8.07 (2H, s, H-10) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 28.3 (C-7), 32.1 (C-8), 49.1 (C-12), 53.1 (C-1), 55.0 (C-3), 116.8 (C-9), 127.0 (C-16), 127.6 (C-14), 128.5 (C-15), 138.1 (C-13), 158.8 (C-10), 162.5 (C-11), 166.5 (C-5), 171.7 (C-2) ppm; ESI MS (*m/z*): 478 [M+H]$^+$, 387, 377, 197; IR (thin film, cm^{-1}): 3374, 3086, 3062, 3026, 2979, 2930, 1744, 1714, 1606, 1541, 1509, 1453, 1435, 1403, 1365, 1253, 1227, 1165, 1057, 1028.

(*R*)-Methyl-2-(tert-butoxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)-propanoate (93)

(*Z*)-Methyl-2-(*tert*-butoxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)acrylate (**38**) (0.02 g, 0.04 mmol, 1.0 eq.), bis(1,5-cyclooctadiene)rhodium(I)tetra-fluoroborate (0.008 g, 0.02 mmol, 4.5 mol%) and (*R,R*)-Me-DUPHOS (0.005 g, 0.015 mmol, 3.5 mol%) were added to a steel autoclave. After purging three times with H$_2$, degassed MeOH (20 mL) was inserted and the reaction mixture was degassed again three times. The reaction was stirred at room temperature with 35 bar of H$_2$ for two days and stopped *via* release of H$_2$ pressure. After filtration through Celite the proton NMR of the crude oil showed 70% product and 30% starting material. Because the compound mixture is not separable *via* column chromatography the crude oil was purified by preparative TLC

(DCM/methanol, 200:1, R_f = 0.25) to give (*R*)-methyl-2-(*tert*-butoxy-carbonylamino)-3-(2-(dibenzyl-amino)pyrimi-din-5-yl)propanoate **93** (0.0014 g, 8%) as a colourless solid.
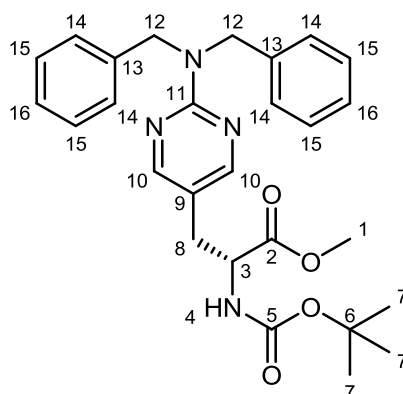

93

^{1}H NMR (500 MHz, CDCl$_3$): δ = 1.25 (9H, s, H-7), 2.77-2.95 (2H, m, H-8), 3.69 (3H, s, H-1), 4.49 (1H, m, H3), 4.78 (4H, s, H-12), 5.03 (1H, d, $^3J_{HH}$ = 7.3 Hz, H-4), 7.13-7.26 (10H, m, H-14, H-15, H-16), 8.07 (2H, s, H-10) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 28.3 (C-7), 32.1 (C-8), 49.1 (C-12), 53.1 (C-1), 55.0 (C-3), 116.8 (C-9), 127.0 (C-16), 127.6 (C-14), 128.5 (C-15), 138.1 (C-13), 158.8 (C-10), 162.5 (C-11), 166.5 (C-5), 171.7 (C-2) ppm; ESI MS (*m/z*): 478 [M+H]$^+$, 387, 377, 197; IR (thin film, cm^{-1}): 3374, 3086, 3062, 3026, 2979, 2930, 1744, 1714, 1606, 1541, 1509, 1453, 1435, 1403, 1365, 1253, 1227, 1165, 1057, 1028.

(*S*)-Methyl-2-amino-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate (94)

(*S*)-Methyl-2-acetamido-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate (0.18 g, 0.43 mmol, 1.0 eq.) was dissolved in methanol (4 mL). Thionyl chloride (0.15 mL, 2.07 mmol, 4.8 eq.) was slowly added at 0 °C and the solution was then refluxed at 80 °C for 18 h. Methanol was removed under reduced pressure and ethyl acetate (20 mL) and sat. aq. NaHCO$_3$ solution (20 mL) were added. The water layer was washed with ethyl acetate (2 × 15 mL) and the combined org. layer was then dried over MgSO$_4$ and filtered. The crude product was purified by column chromatography (hexane/ethyl acetate, 1:8 with 0.1% TEA, R_f = 0.26) to give (*S*)-methyl-2-amino-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate **94** (0.12 g, 76%) as light yellow oil.

94

^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.63-2.86 (2H, m, H-5), 3.56-3.62 (1H, m, H-3), 3.67 (3H, s, H-1), 4.71-4.85 (4H, dd, J = 15.7 Hz, 17.6 Hz, H-9), 7.10-7.27 (10H, m, H-11, H-12, H-13), 8.15 (2H, s, H-7) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 34.7 (C-5), 49.0 (C-9), 52.2 (C-1), 55.3 (C-3), 117.9 (C-6), 127.0 (C-13), 127.5 (C-11), 128.4 (C-12), 138.2 (C-10), 158.5 (C-7), 161.7 (C-8), 175.2 (C-2) ppm; ESI MS (*m/z*): 399 [M+Na]$^+$, 377 [M+H]$^+$, 360, 279; HRMS (ESI): calc. for C$_{22}$H$_{25}$O$_2$N$_4$$^+$ ([M+H]$^+$): 377.1972, found: 377.1971; IR (thin film, cm^{-1}): 3383 (br), 3086, 3062, 3027, 2951, 2922, 1739, 1674, 1605, 1540, 1510, 1453, 1424, 1402, 1362, 1258, 1229, 1175, 1028.
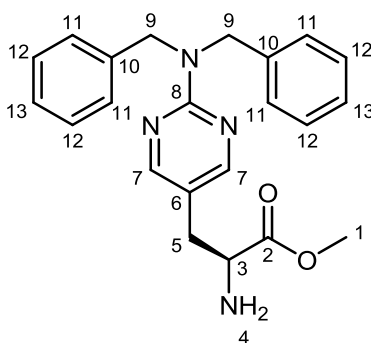
(*S*)-2-(Benzyloxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoic acid (95)

Under nitrogen atmosphere (*S*)-1-carboxy-2-(2-(dibenzylamino)pyrimidin-5-yl)ethan-aminium chloride (**46**) (0.18 g, 0.45 mmol, 1.0 eq.), *N*-hydroxybenzotriazol (0.07 g, 0.52 mmol, 1.1 eq.) and benzyl chloroformate (0.09 ml, 0.63 mmol, 1.4 eq.) were mixed with dry DCM (6 ml). Triethylamine (0.44 ml, 3.17 mmol, 7.0 eq.) was added and the light yellow solution stirred at room temperature for 4 h. 1 M HCl (6 ml) was added and the org. layer dried over MgSO$_4$. The crude product was purified *via* column chromatography (hexane/ethyl acetate plus 0.1% acetic acid, 1:1, R_f = 0.26) to give (*S*)-2-(benzyloxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoic acid **95** (0.16 g, 72%) as colourless solid. (m.p.: 164 – 165 °C).
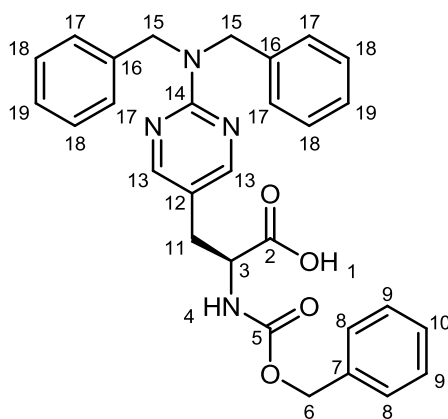
95

^{1}H NMR (500 MHz, DMSO-d$_6$, 80 °C): δ = 2.69-2.98 (2H, m, H-11), 4.15-4.22 (1H, m, H-3), 4.81 (4H, s, H-15), 5.00 (2H, s, H-6), 7.17-7.35 (15H, m, H-Ar), 7.42 (1H, br s, H-4), 8.27 (2H, s, H-13) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 31.5 (C-11), 49.8 (C-15), 54.4 (C-3), 67.1 (C-6), 117.3 (C-12), 2×127.2 (C-Ar), 128.1 (C-Ar), 128.3 (C-Ar), 128.6 (C-Ar), 136.2 (C-7), 137.2 (C-16), 155.6 (C-5), 158.5 (C-13), 160.4 (C-14), 172.5 (C-2) ppm; ESI MS (*m/z*): 519 [M+Na]$^+$, 497 [M+H]$^+$, 453, 391, 199; HRMS (ESI): calc. for C$_{29}$H$_{29}$O$_4$N$_4^+$ ([M+H]$^+$): 497.2183, found: 497.2176; IR (thin film, cm^{-1}): 3419 (br), 3029, 2925, 1713, 1607, 1542, 1506, 1453, 1423, 1362, 1260, 1218, 1057, 1028.

(*S*)-Methyl-2-(benzyloxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoate (96)

Iodomethane (0.024 mL, 0.39 mmol, 1.5 eq.) was added drop wise to a mixture of (*S*)-2-(benzyloxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)propanoic acid (**95**) (0.128 g, 0.26 mmol, 1.0 eq.) and potassium carbonate (0.072 g, 0.52 mmol, 2.0 eq.) in DMF (5 mL). After stirring for 18 h at room temperature the reaction mixture was washed with water (2 × 8 mL). The org. layer was washed with sat. aq. NaHCO$_3$ solution (2 × 8 mL), dried over MgSO$_4$, filtered and concentrated in vacuo. The crude product was purified *via* column chromatography (hexane/ethyl acetate, 2:1, R_f = 0.42) to give (*S*)-methyl 2-(benzyloxycarbonylamino)-3-(2-(dibenzylamino)pyrimidin-5-yl)propan-oate **96** (0.082 g, 62%) as colourless oil.
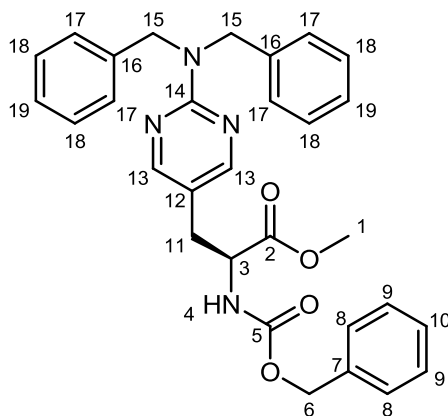
96

^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.76-2.96 (2H, m, H-11), 3.64 (3H, s, H-1), 4.46-4.56 (1H, m, H-3), 4.75 (4H, dd, J = 16 Hz, 2 Hz, H-15), 5.00 (2H, dd, J = 12 Hz, 6 Hz, H-6), 5.39 (1H, d, J = 7.7 Hz, H-4), 7.00-7.31 (15H, m, H-Ar), 8.02 (2H, s, H-13) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 32.1 (C-11), 49.0 (C-15), 52.4 (C-1), 54.5 (C-3), 67.0 (C-6), 116.7 (C-12), 126.9 (C-Ar), 127.4 (C-Ar), 128.0 (C-Ar), 128.1 (C-Ar), 128.4 (C-Ar), 128.5 (C-Ar), 136.0 (C-7), 138.0 (C-16), 155.5 (C-5), 158.3 (C-13), 161.7 (C-14), 171.4 (C-2) ppm; ESI MS (*m/z*): 533 [M+Na]$^+$, 511 [M+H]$^+$, 419, 391, 327; HRMS (ESI): calc. for C$_{30}$H$_{31}$O$_4$N$_4$$^+$ ([M+H]$^+$): 511.2340, found: 511.2335; IR (thin film, cm^{-1}): 3341 (br), 3087, 3063, 3029, 2952, 1951, 1722, 1606, 1540, 1506, 1453, 1435, 1403, 1362, 1258, 1217, 1059, 1028.

General procedure for Fmoc-peptide synthesis

Resin preparation:
The resin was added to a plastic syringe with a glass sinter base. The outlet of the syringe was connected to a membrane pump *via* a collecting tank. NMP was added in order to swell the resin and left for at least 2 h before the solvent was removed by vacuum filtration.

Fmoc-Deprotecting / Coupling cycle:
For Fmoc removal, a 50% piperidine/NMP (v/v) solution was added and stirred for 10 min. at room temperature. The solvent was removed by vacuum filtration and this step was repeated. The resin was washed four times with NMP. A solution of Fmoc protected

amino acid (4 eq.; 2 eq. for amino acid with biotinyl tag), PyBOP (4 eq.) and DIEA (4 eq.) in NMP was added and stirred for 45 min. at room temperature. The solvent was removed by vacuum filtration and the coupling step was repeated. The resin was then washed four times with NMP.

The deprotecting / coupling cycle was repeated for each of the subsequent amino acids.

Removal of the *N*-terminal Fmoc group and drying the peptide resin:

A 50% piperidine/NMP (v/v) solution was added and stirred for 10 min. at room temperature. The solvent was removed by vacuum filtration and this step was repeated. The resin was washed three times with NMP, three times with chloroform, three times with methanol and three times with diethyl ether.

Cleavage of the peptide from resin and deprotection of side chains:

Mixture of thioanisol (5.0%), ethanedithiol (2.5%), m-cresol (5.0%), water (5.0%) and TFA (82.5%) was added to the peptide resin and left for 6 h at room temperature. The filtrate was collected and the resin washed with TFA. Diethyl ether (40 mL) was added to a tube containing the combined filtrate and washing to precipitate the peptide. The tube was centrifuged (5 min., 4 °C, spin 2800 rpm) and the solution decanted from the peptide pellet. The peptide pellet was then washed with diethyl ether (40 mL) and centrifuged for three more times. The peptide was dried under nitrogen flow.

Positive-Peptide ((H$_2$N-RKRKGGG)$_2$KGGGX-NH$_2$)

General procedure was used with Rink Amide MBHA resin (100-200 μm, 0.34 mmol/g, 0.06 g). The amino acids up to the two times Fmoc protected Lys (to creating the branch) were added with just one coupling cycle of 45 min. The following amino acids were added with 8 eq. of Fmoc protected amino acid, PyBOP and DIEA. Peptide identity was confirmed by MALDI-ToF mass spectrometry.

MALDI-ToF: calculated M+H: 2354; found M+H: 2354.

Negative-Peptide ((H$_2$N-EEEEGGG)$_2$KGGGX-NH$_2$)

General procedure was used with Rink Amide MBHA resin (100-200 μm, 0.34 mmol/g, 0.06 g). The amino acids up to the two times Fmoc protected Lys (to creating the branch) were added with just one coupling cycle of 45 min. The following amino acids were added with 8 eq. of Fmoc protected amino acid, PyBOP and DIEA. Peptide identity was confirmed by MALDI-ToF mass spectrometry.

MALDI-ToF: calculated M+K: 2288, M+Na: 2271; found major peak M+K: 2288, minor peak M+Na: 2271.

Peptide Library Fragment (Fmoc-CKGGX-resin)

General procedure was used with Polystyrene AMRAM resin (500-560 μm, 0.53 mmol/g, 0.0468 g). The peptide was cleaved off four test beads to confirm the identity of the peptide by MALDI-ToF mass spectrometry.

MALDI-ToF: calculated M+H: 920; found M+H: 920.

Peptide Library

Half of the amount of the prior prepared Peptide Library Fragment was used. The library was synthesised using the general procedure in combination with the split and recombine strategy. The beads were split in four pools and Fmoc protected Phe, Ile, Thr or Tyr was coupled to the peptide. The beads were then all recombined for the Fmoc-deprotection. The beads were split in two pools to couple Fmoc protected Lys or Arg and recombined for the Fmoc-deprotection. The next two Fmoc protected amino acids D-Cys and Ala were added to all beads in one pool. After splitting the beads in two pools Fmoc protected Arg or Ser was coupled to the peptide and the beads were recombined for the deprotecting step. The beads were split again in four pools and Fmoc protected Leu, Asn, Val or Tyr was coupled on. This time the beads were not recombined for the Fmoc-deprotection and the addition of the last Fmoc protected amino acid Ala. 96 well plates were used for the cleavage of the peptides from the resin. One bead was placed per well because each bead contained a different peptide. The cleavage mixture was evaporated under a nitrogen flow after the cleavage. Peptide identity can be confirmed by MALDI-ToF mass spectrometry as demonstrated on six examples.

MALDI-ToF:

H_2N-ANSAC´KTCKGGX-NH_2+H: 1596.1614 and H_2N-ANSAC´KTCKGGX-NH_2+Na: 1618.1984.

H_2N-AVSAC´KYCKGGX-NH_2+H: 1643.2166 and H_2N-AVSAC´KYCKGGX-NH_2+Na: 1665.2657.

H_2N-ALSAC´KYCKGGX-NH_2+H: 1658.0498 and H_2N-ALRAC´RYCKGGX-NH_2+Na: 1679.0645.

H_2N-ALRAC´RYCKGGX-NH_2+H: 1754.1547.

H_2N-ALRAC´RTCKGGX-NH_2+H: 1692.1808.

H_2N-ANSAC´RFCKGGX-NH_2+H: 1670.1793.

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