New Guanidinium Compounds for the Molecular Recognition of Carboxylates and Contributions to the Synthesis of Bivalent NPY Y_1 Receptor Antagonists

Dissertation

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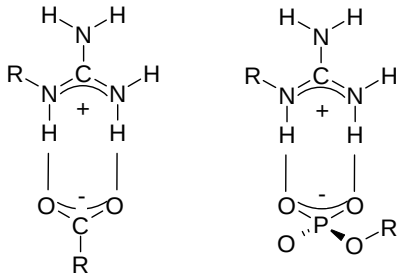
Contents

A. Introduction

Synthesis of guanidines in solution

1. Introduction

Arginine, a naturally occuring amino acid with a guanidinium moiety, is found in numerous enzyme active sites and cell recognition motifs. Horseradish peroxidase,[1] fumarate reductase[2] and creatine kinase[3] are just a few enzymes that have arginine-containing active sites. The tripeptide sequence RGD (Arg-Gly-Asp) is a common cell-recognition motif responsible for the binding of the integrin receptors.[4] This sequence has been used as a lead structure for the development of different integrin antagonists.[5]
Nonpeptide cyclic cyanoguanidines are used as HIV-1 protease inhibitors,[6] while carboxylic guanidino analogs are used as influenza neuroaminidase inhibitors.[7] Guanidinium-based molecules are also extensively used as cardiovascular drugs,[8] antihistaminines,[9] anti-inflammatory agents,[10,11] antidiabetic drugs,[12] antibacterial and antifungal drugs,[13] antiprotozal and other antiparasitic drugs[14] and antiviral drugs.[15] Guanidinium derivatives (Impromidine and related compounds) are also used as histamine H_2-receptor agonists[16] and as NPY Y_1-receptor antagonists.[17]
Guanidinium-containing compounds such as guanidinoacetic acid are used as artificial sweeteners,[18] bicyclic guanidines catalyze the enantioselective Strecker synthesis[19] and modified guanidines are also used as potential chiral superbases.[20,21]
The guanidinium ion and its many derivatives have been widely studied in the context of anion binding.[22,23,24]
The abundant involvement of arginine in the binding of anionic substrates to proteins fostered the suspicion early on that interactions of a guanidinium ion with common oxoanions must hold special virtues. Much later it was concluded from site-directed mutagenesis experiments affecting the active sites of certain enzymes that in the protein enviroment the energetic stabilization of a carboxylate by the guanidinium side chain of arginine outmatches the analogous interaction with the primary ε-ammonium group of lysine by as much –21 kJ/mol.[25,26] The reason for the strong interaction with oxoanions lies in the peculiar binding pattern featuring two strong parallel hydrogen bonds in addition to the electrostatic interaction (Scheme 1).[27]



Scheme 1. Binding pattern of guanidinium groups with oxoanions found in many X-ray structures of the corresponding salts.

The guanidinium moiety is one of the most hydrophilic functional groups known.[28] Solvation by water is so efficient that despite the favorable binding pattern, ion pairing with carboxylates and phosphates in aqueous solution is negligible (K_S < 5 M^{-1}).[29] Bridging by water molecules may even allow the electrostatic repulsion to be overcome and lead to face-to-face dimerization of two guanidinium cations.[30] The extreme basicity of guanidine (13.5), which is conserved or even enhanced by prudent substitution,[31] guarantees a fixed protonation state and opens the entire range of accessible pH values for study.

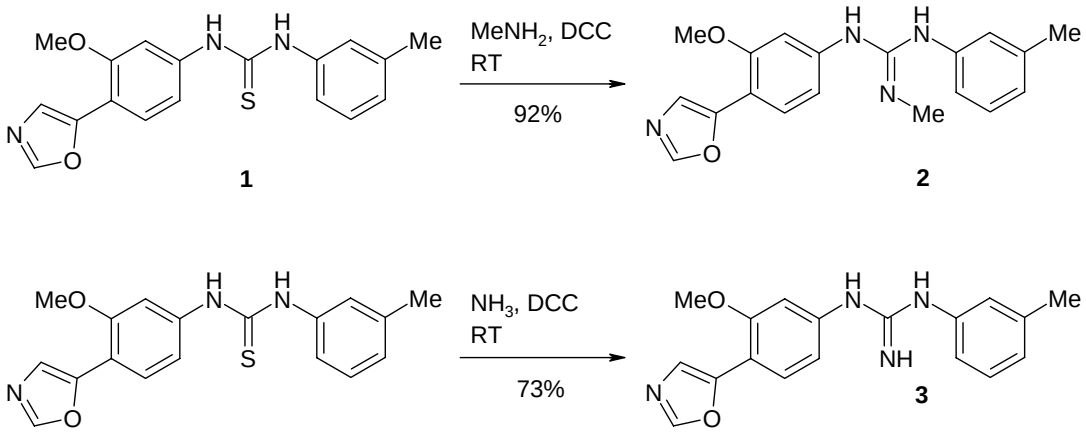
2. Discussion

Guanidines from Thioureas

The thiourea moiety is converted into guanidinium functionalities in the presence of different coupling reagents: *N*,*N*-dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), mercury(II) chloride ($HgCl_2$), mercury(II) oxide (HgO), 2-chloro-1-methylpyridinium iodide (Mukaiyama`s reagent)

DCC Coupling

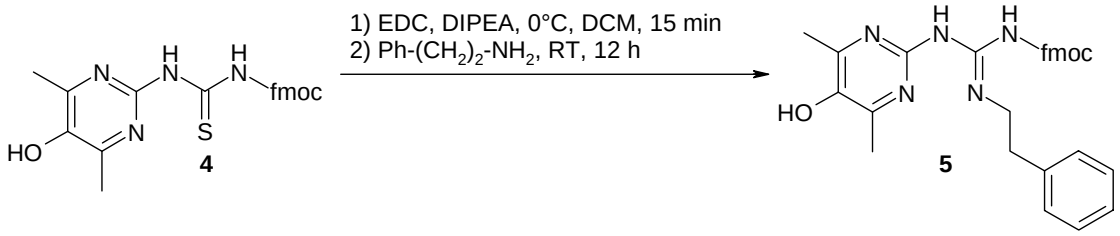
Iwanowicz and co-workers have reported the synthesis of novel guanidine-based inhibitors of inosine monophosphate dehydrogenase through DCC coupling (Scheme 2).[32]



Scheme 2. Synthesis of guanidines using DCC

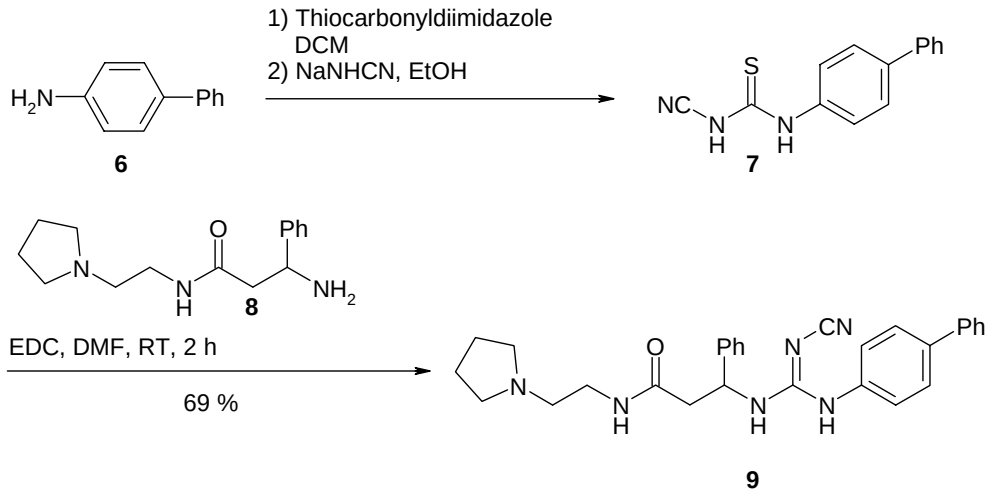
EDC Coupling

Jefferson and co-workers have reported about a structure-activity relationship analysis on a high-throughput small molecule screening lead for HCV-IRES translation inhibition. The study led to the identification of a guanidine-based structure with low μM inhibitory activity.[33] The thiourea, activated with 1-ethyl-3-(3´-dimethylaminopropyl) carbodiimide (EDC), was treated with an arylamine to afford the guanidine derivative (Scheme 3).



Scheme 3. Synthesis of aryl-guanidines using EDC

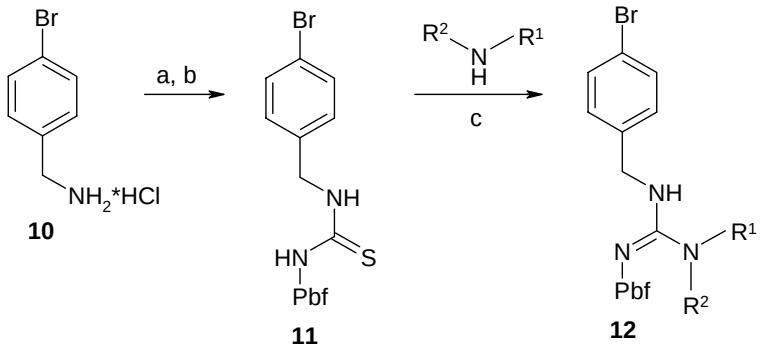
Wollin and co-workers reported on the synthesis of cyanoguanidines (Scheme 4).[34]



Scheme 4. Synthesis of cyanoguanidine β-amino acid derivative

Mukaiyama´s Reagent

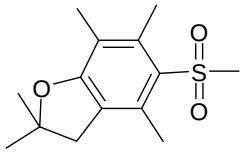
Fan et al. have developed an efficient synthesis of *N*,*N´*-substituted guanidine derivatives via an aromatic sulfonyl-activated thiourea intermediate (Scheme 5).[35] As shown in Scheme 5, a primary amine **10** was first turned into the corresponding pentafluorophenyl thiocarbamate. This allowed for the smooth synthesis of the arylsulfonyl-activated thiourea **11**, using PbfNHK (formed by treating $PbfNH_2$ with potassium *tert*-butoxide) as nucleophile. Compound **11** is an excellent guanidinylating reagent. Treatment of **11** with an amine in the presence of Mukaiyama reagent (2-chloro-1-methylpyridinium iodide) produced the subsequent guanidin derivatives **12a-d** in very good yields at room temperature in 12 – 18 h.



12a R^1 = H, R^2 = cyclohexyl, Yield: 88%
12b R^1 = H, R^2 = *t*-butyl, Yield: 84%
12c R^1 = R^2 = ethyl, Yield: 85%
12d R^1 = R^2 = isopropyl, Yield: 88%

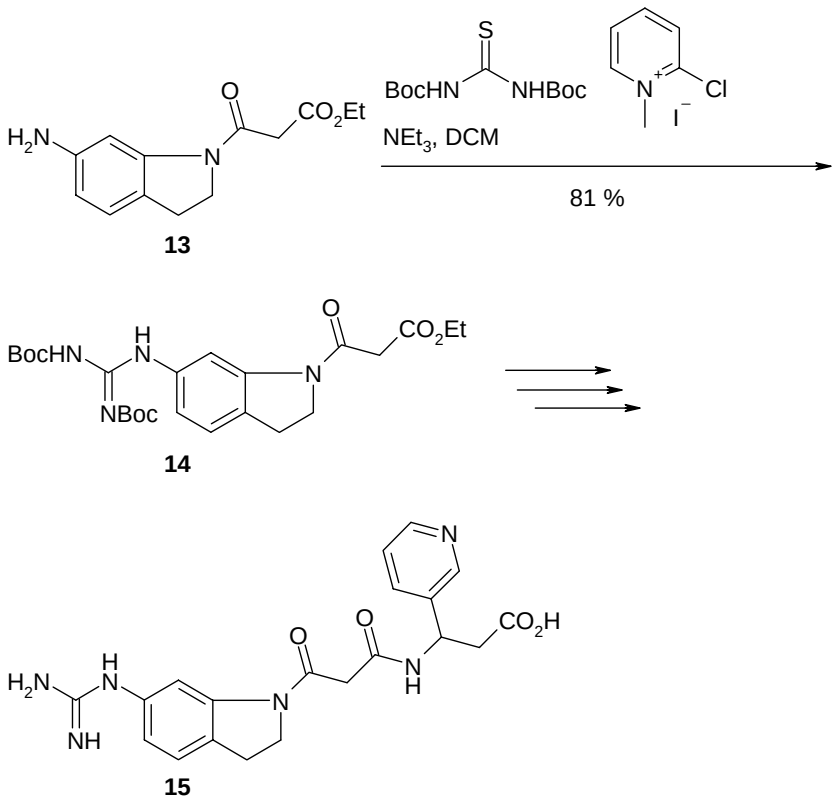
a) pentafluorophenyl chloroformiate, DIPEA, CH_2Cl_2; b) $PbfNH_2$, potassium t-butoxide, DMSO; c) Mukaiyama reagent, DIPEA, THF/DMF

Pbf:



Scheme 5. Arylsulfonylthiourea-assisted synthesis of *N,N´*-substituted guanidines

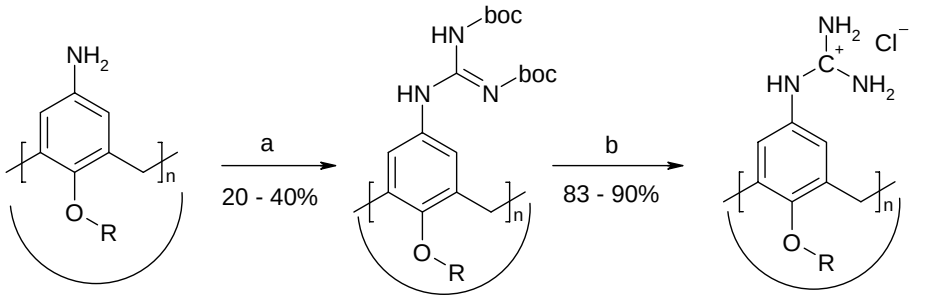
Nagashima and co-workers have reported on the synthesis of malonamide derivatives as $\alpha_v\beta_3$ antagonists. The guanylation of compound **13** with *N,N´*-bis(*tert*-butoxycarbonyl)thiourea in the presence of 2-chloro-1-methylpyridine iodide (Mukaiyama´s reagent) and NEt_3 in dichloromethane afforded the *tert*-butoxycarbonyl (Boc) protected guanidine derivative **14**. Removal of the Boc groups with 4 M HCl – dioxane afforded the desired guanidine compound **15**. (Scheme 6).[36]



Scheme 6. Guanylation with Mukaiyama´s reagent

$HgCl_2$ Coupling

The group of *Ungaro* reported the reaction of O-alkylated p-aminocalix[n]arenes (n = 4, 6, 8) with *N*,*N´*-di(*tert*-butoxycarbonyl)thiourea in the presence of $HgCl_2$. The subsequent removal of the protective groups with hydrochloride acid led to the new water soluble calix[n]guanidinium derivatives (p-guanidiniumcalix[n]arenes) **16** - **19** (Scheme 7).[37]

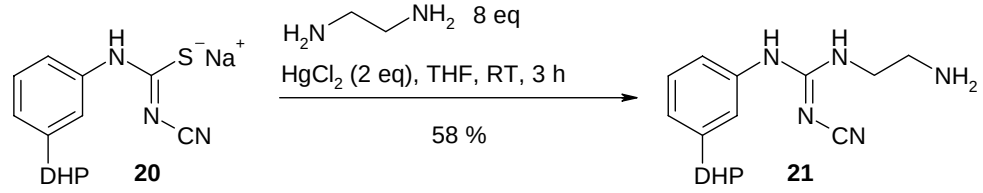


16 n = 4, R = Pr
17 n = 4, R = Oct
18 n = 6, R = Me
19 n = 8, R = Me

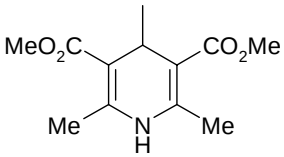
a) $HgCl_2$/Boc-NH-C(S)-NH-Boc/DMF (dry), NEt_3 (dry); b) concd. HCl/1,4-dioxane

Scheme 7. Calix[n]arene guanidinium derivatives

Guanglin and co-workers have reported on the synthesis of a 4-amino-*N*-arylpiperidine moiety in NPY Y_1-receptor antagonists (Scheme 8).[38] Treatment of **20** with excess ethylene diamine provided **21** in good yields and offered none of the bis-functionalized product.



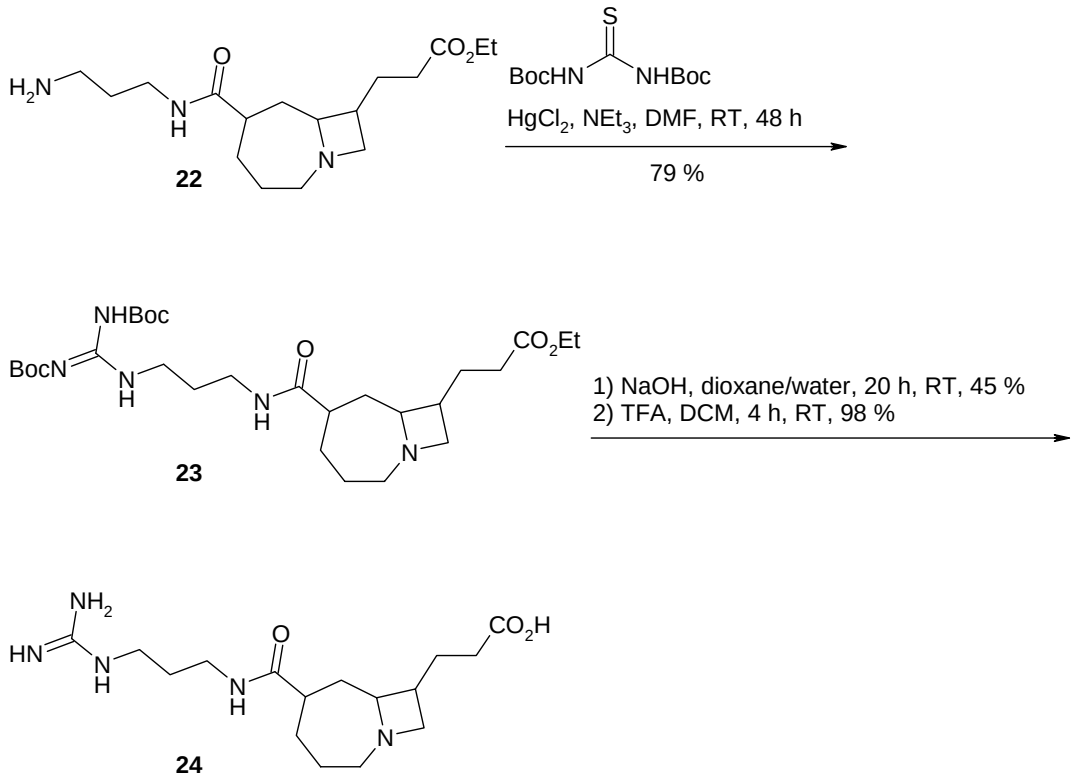
DHP:



Scheme 8. Synthesis of the cyanoguanidine moiety

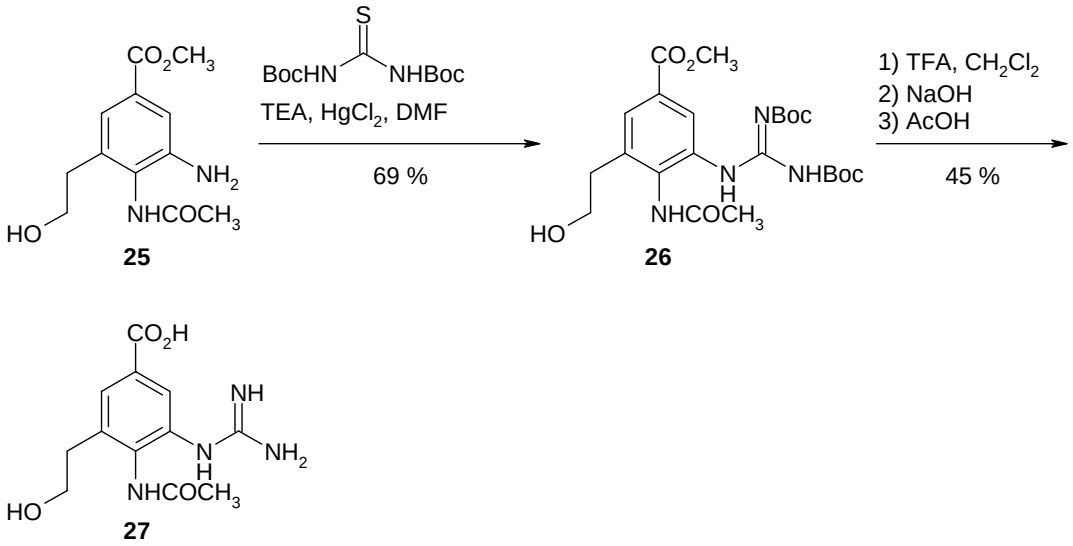
Girard and co-workers have reported on the synthesis of a new bicyclic lactam **22** as a non-peptidic scaffold mimicking the RGD β-turntopology (Scheme 9).[39] The occurrence in these non-peptidic molecules of both guanidinium and carboxylate groups are essential elements for mimicking the Arg and Asp side chains of RGD, respectively. Coupling of **23** in the presence of $HgCl_2$ with *N,N´*-bis-*t*-butoxycarbonylthiourea

resulted in the formation of compound **24** in 79 %. Saponification of the ethyl ester by sodium hydroxide followed deprotection of *N*-*t*-butoxycarbonyl by treatment with TFA gave 5-(guanidinocarboxamido propyl)-1-azabicyclo[5.2.0]nonan-2-one-8-propionic acid **24**.



Scheme 9. Synthesis of non-peptidic RGD antagonists

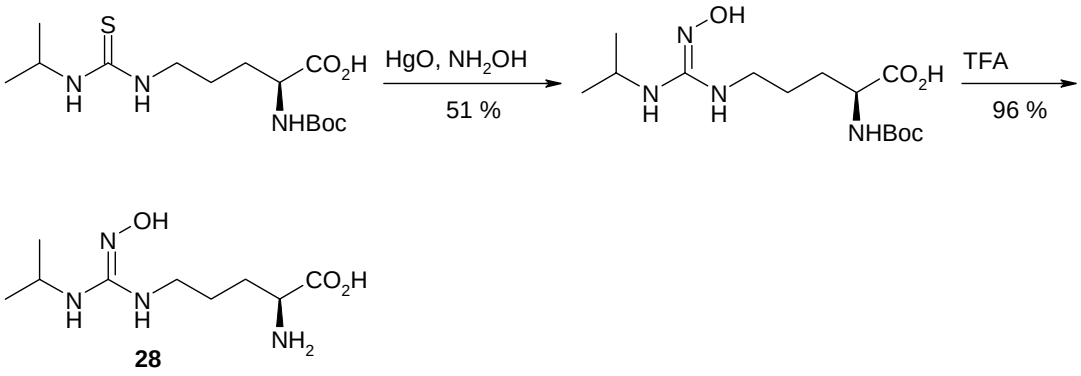
Chand and co-workers have reported on the synthesis and inhibitory activity of benzoic acid and pyridine derivatives on influenza neuraminidase (Scheme 10).[40] The guanidino-group was introduced on the amino group of **25** using *N,N´*-bis(*tert*-butoxycarbonyl)thiourea in the presence of $HgCl_2$ and NEt_3 to furnish **26**. Deprotection of the Boc-groups with trifluoracetic acid, followed by base hydrolysis gave the target compound **27**.



Scheme 10. Synthesis of benzoic acid guanidinium derivative

HgO Coupling

Poulos and co-workers reported on a series of *N*-alkyl-*N´*-hydroxyguanidine **28** compounds, which are non-amino acid substrates for all three nitric oxide synthase (NOS) isoforms (Scheme 11).[41]

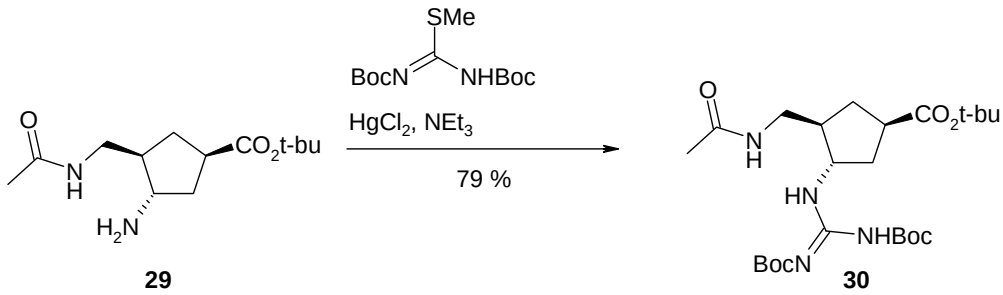


Scheme 11. *N*-alkyl-*N´*-hydroxyguanidine

Guanidines from Isothioureas

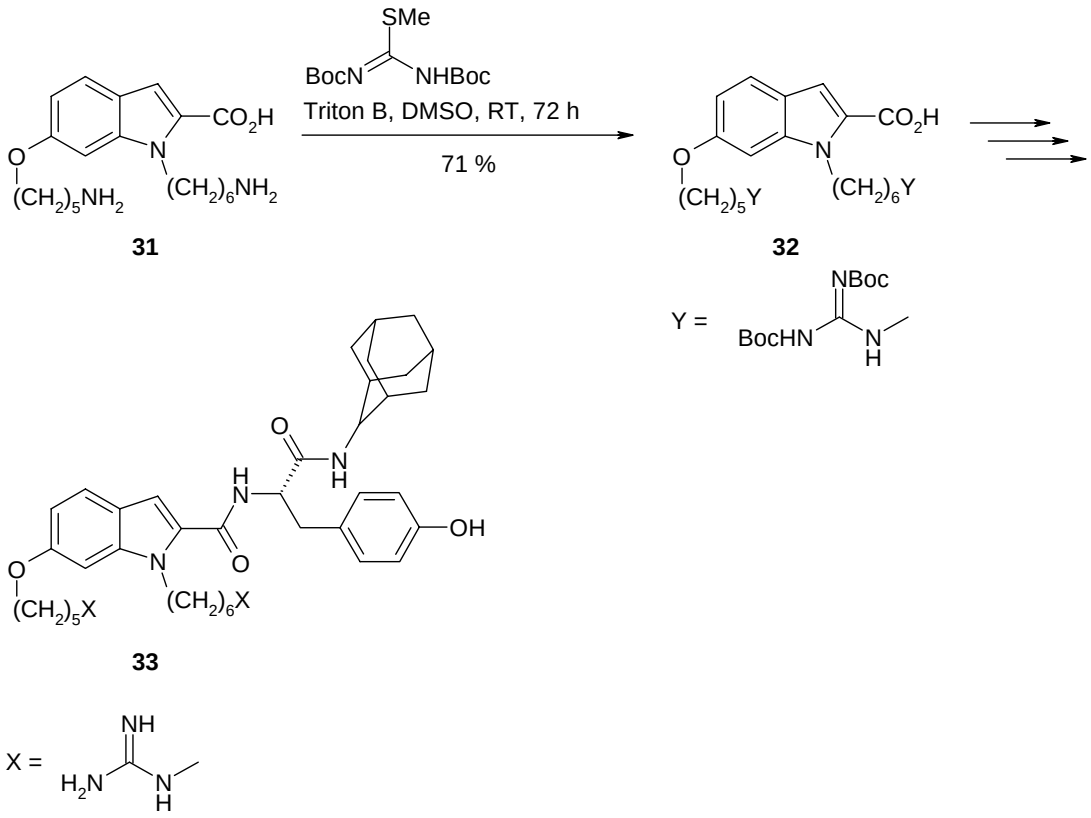
Isothioureas like the *N,N´*-bis-(*tert*-butyloxycarbonyl)-S-methylisothiourea and the *N,N´*-bis-(benzyloxycarbonyl)-S-methylisothiourea are often used as guanylation reagents in the presence of $HgCl_2$.

Chand and co-workers reported on the synthesis of novel multisubstituted cyclopentane derivatives with potent antiinfluenza activity (Scheme 12).[42] The amino compoud **29** was treated with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea in the presence of $HgCl_2$ and NEt_3 to afford compound **30**.



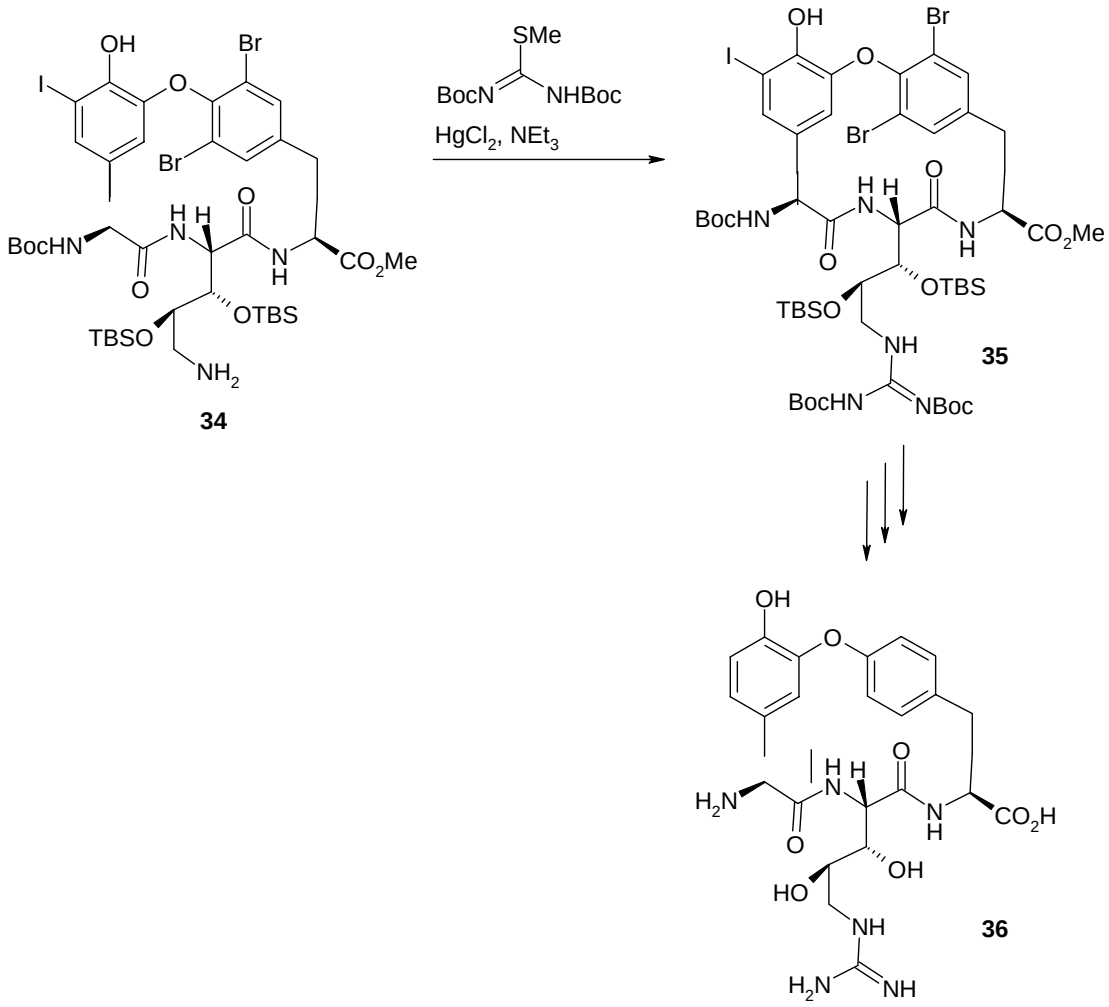
Scheme 12. Synthesis of multisubstituted cyclopentane derivative

Richelson and co-workers have reported the synthesis and binding activity of novel neurotensin (8-13) mimetics. Neurotensin (NT), a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), is widely distributed in the central peripheral nervous systems. It acts as a neuromodulator and is associated with numerous physiological functions. More interestingly, NT has extremely potent antinociceptive activity and may play a major role in the pathogenesis of schizophrenia. It is possible that potent NT analogues could be used as effective therapeutic agents for treating schizophrenia and other diseases (Scheme 13).[43] In the synthesis the guanidinium group was introduced by treatment of compound **31** with *N,N´*-bis-(*tert*-butyloxycarbonyl)-S-methylisothiourea and Triton B in methanolic solution in DMSO.



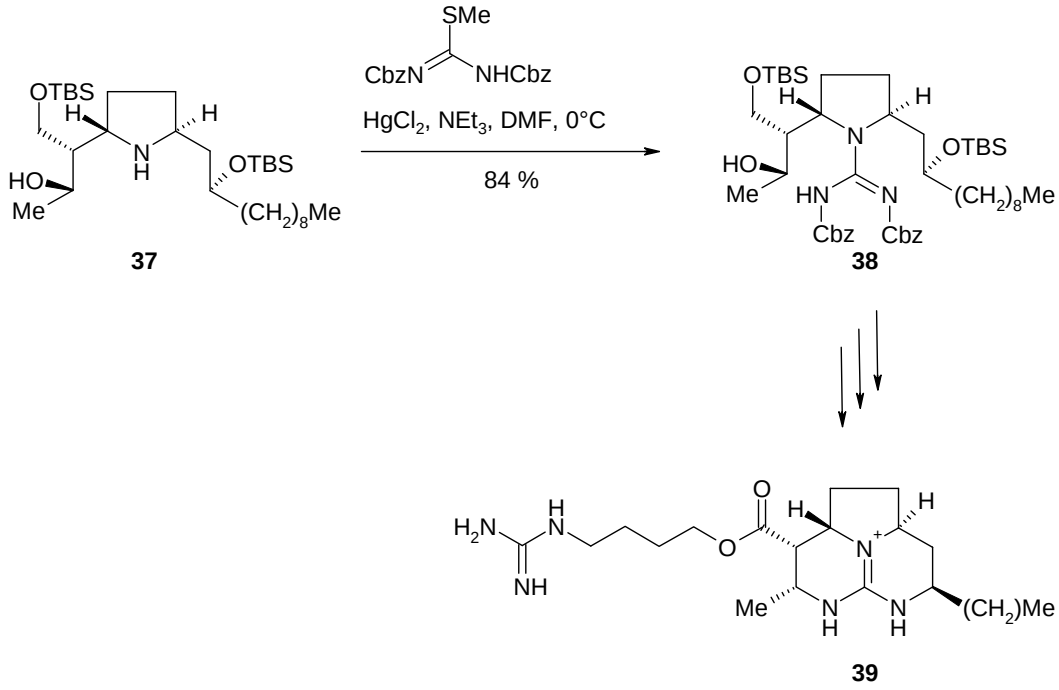
Scheme 13. Synthesis of neurotensin (8-13) mimetic

Nishiyama et al. have reported on a new synthesis of eurypamides, a isodityrosine natural product with a dihydroarginine unit. The isodityrosine are known to exhibit antimicrobial, cytotoxic and enzyme-inhibitory activities. The guanidinium group was introduced by treatment of an amine group with *N,N´*-bis-(*tert*-butyloxycarbonyl)-S-methylisothiourea in the presence of $HgCl_2$ and NEt_3 in DMF with 60 % yield (Scheme 14).[44]



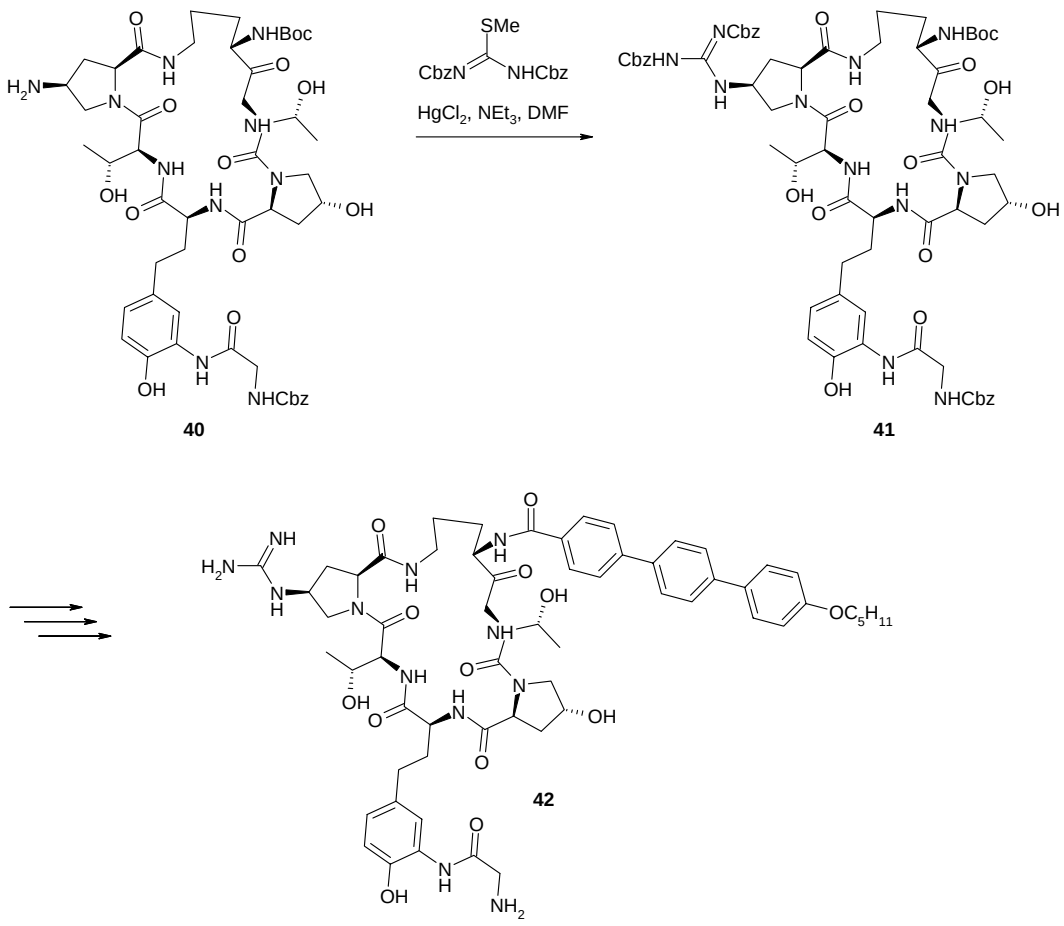
Scheme 14. Synthesis of eurypamides **36**

Batzelladines A – I are members of a new class of polycyclic guanidine alkaloids isolated from Bahamian (batzelladines A – E) and Jamaican sponges (batzelladines F – I) of the genus *Batzella* by a SmithKline Beecham group. Batzelladines A and B inhibit the binding of HIV glycoprotein gp-120 to the human CD4 receptor. *Nagasawa* and co-workers reported on the synthesis of batzellidine D (**39**) (Scheme 15).[45] The 2,5-disubstituted pyrrolidine **37** was reacted with bis-Z-2-methyl-2-isothiourea in the presence of mercury(II) chloride and triethylamine to give **38** in 84 % yield.



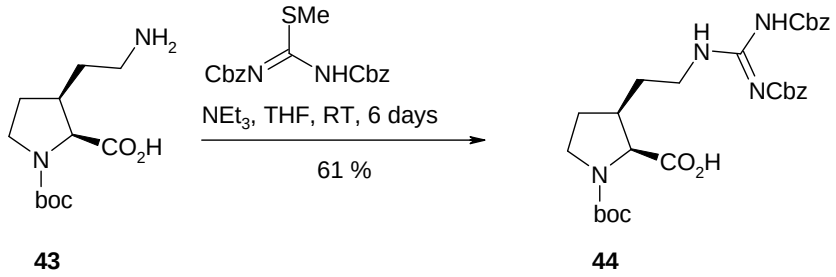
Scheme 15. Synthesis of batzellidine D

Human fungal infections have come to prominence during the past two decades due to an increase in patients with HIV-infection, chemotherapy-induced neutropenia, organ transplantation, hemodialysis or extensive use of broad-spectrum antibiotics and glucocorticosteroids.[46,47,48,49] The echinocandin family of natural hexacyclic-lipopeptides has been of interest in antifungal research over the past three decades due to their fungicidal profiles. Increased interest became apparent during the early-mid eighties after the delineation of their mode of action as β-1,3-glucan synthetase inhibitors. This enzyme is part of a membrane bound protein complex that utilizes UDP-glucose as a substrate to produce oligomeric glucose, which is further processed by a variety of enzymatic steps to mesh the fungal cell wall. Inhibition of such a process induces cell wall breakage, leads to cellular lysis and death.[50] *Bennani* and co-workers have reported the synthesis of A-192411 (**42)**, a structural simplified cyclic hexapeptide with acceptable in vitro and in vivo profiles (Scheme 16).[51] In order to introduce the guanidinium group into compound **40**, they used the bis-Z-2-methyl-2-isothiourea in the presence of mercury(II) chloride and triethylamine.



Scheme 16. Synthesis of A-192411

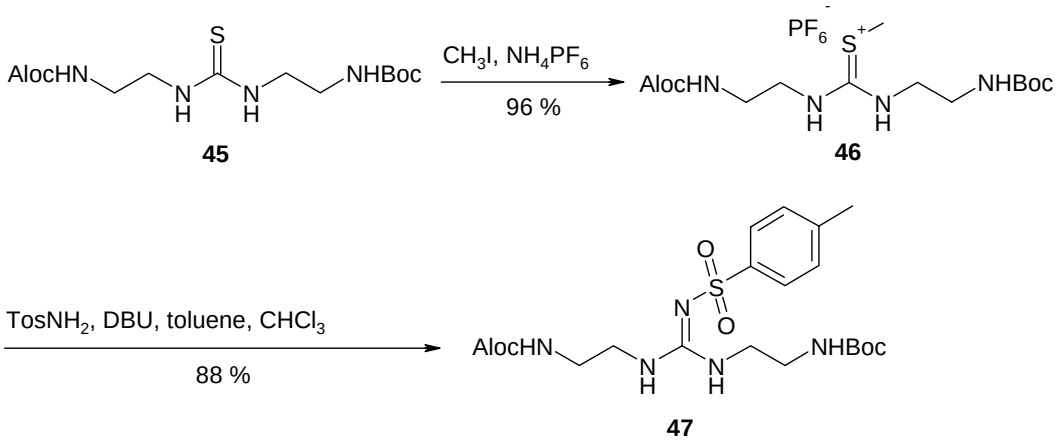
The control of the three-dimensional structure of peptides and proteins by chemical modification of proteinogenic amino acids has been the keystone of peptide research for the past few decades. The aim of these studies is the development of compounds with improved selectivity, bioavailability, stability and permeability.[52,53,54] Among the various approaches (disulfide bridging, lactam cyclization, *N*-methylation, …) described in the de novo design of peptides with a high propensity to fold with predetermined secondary structure, the replacement of a native residue by proline chimeras has been widely used.[55,56,57,58] *Karoyan* and co-workers reported on the synthesis of 3-substituted proline chimeras bearing polar side chains of proteinogenic amino acids (Scheme 17).[59] Guanylation of **43** using *N,N´*-di-Cbz-S-methylisothiourea in THF provided orthogonally protected *cis*-3-prolinoarginine **44** in 61 % yield.



43 **44**

Scheme 17. Synthesis of 3-substituted proline

Kilburn and co-workers have reported on the synthesis of so-called "tweezer" receptors, incorporating a guanidinium "head group" and two peptide derived side arms (Scheme 18).[60] Alkylation of the thiourea **45** with methyl iodide and counterion exchange gave the thiouronium hexafluorophosphate **46**. Treatment with tosyl amine, in the presence of DBU, led to the orthogonally protected guanidine **47**.



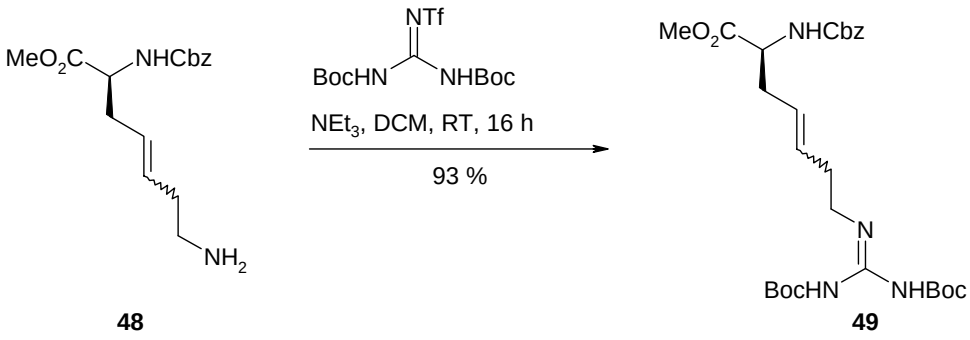
45 **46** **47**

Scheme 18. Synthesis of "tweezer" receptors

Guanidines from Triflylguanidines

An efficient guanidinylation of poorly or sterically hindered amines is obtained with the triflylguanidine derivatives.[61,62]

Pyne and co-workers have reported on the synthesis of (S)-lysine and (S)-arginine homologues (Scheme 19).[63] The amine **48** was treated with *N´´*-triflyl-*N,N´*-di-Boc-protected guanidine and triethylamine to give the arginine homologe **49** in 93 % yield.

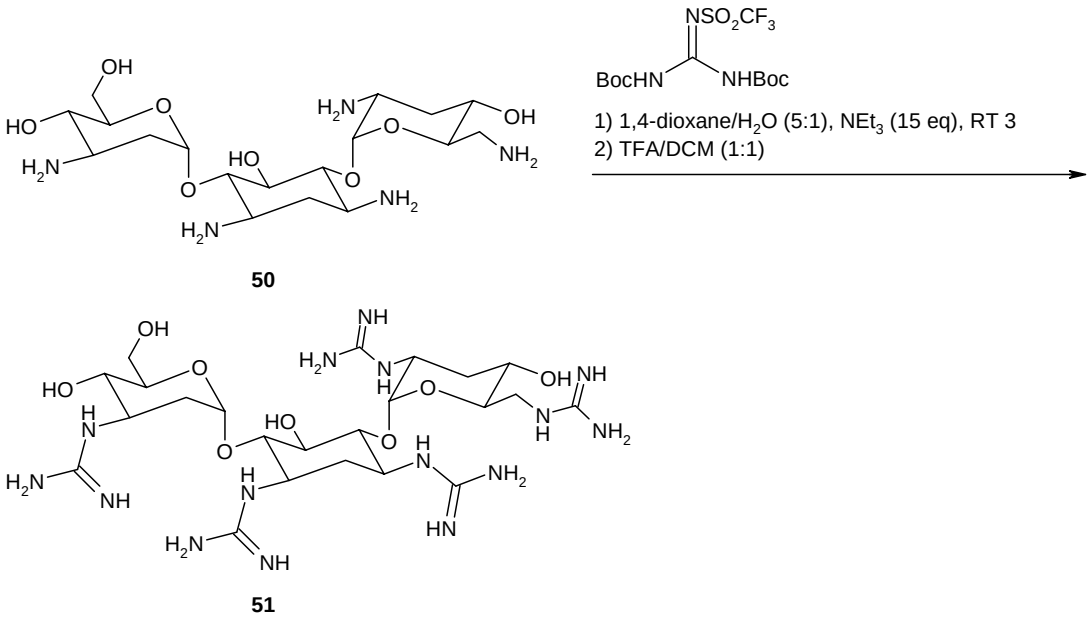


48 **49**

Scheme 19. Synthesis of arginine homologe

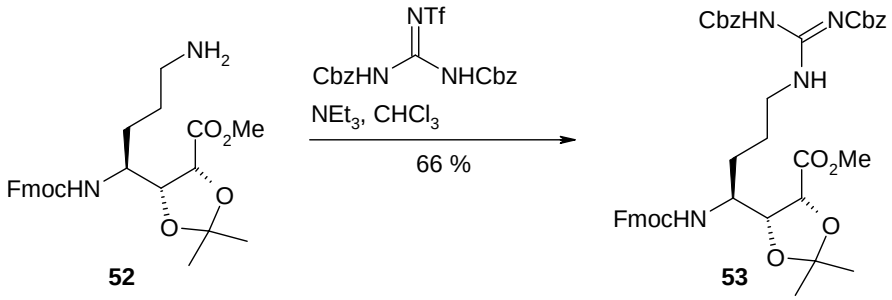
Aminoglycosides are broad-spectrum antibiotics against a variety of clinically important bacteria. Their antibacterial effects are due to binding to bacterial 30S ribosomes and inhibiting protein synthesis. The therapeutic efficacy of aminoglycosides has decreased recently because of increased antibiotic resistance.[64,65] The guanidinium group plays a key role at many RNA – protein binding interfaces, including the complexes formed between transcriptional elongation factors with mRNA, tRNA synthetases with tRNAs, ribosomal proteins with rRNA, and viral regulatory proteins with their cognate RNA binding sites.[66,67]

Seeberger and co-workers and *Tor* and co-workers have reported on the synthesis of guanidinoglycosides using *N,N´*-di-Boc-*N´´*-triflyl-guanidine as a guanidinylating reagent.[68,69] This reagent facilitates the guanidinylation of polyfunctional amines in aqueous media and in high yields. For example, when tobramycin **50** is treated with an excess of *N,N´*-di(Boc)-*N´´*-triflyl-guanidine in a 1,4-dioxane/water mixture, the Boc-protected, fully guanidinylated derivative is obtained. Subsequent deprotection of the Boc–groups affords guanidino-tobramycin **51** (Scheme 20).



Scheme 20. Synthesis of guanidino-tobramycin **51**

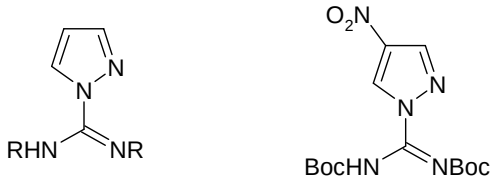
The *N,N´*-di-*Z*-*N´´*-trifluormethanesulfonyl-guanidine was used by *Lipton* and co-workers to introduce the guanidinium group in an ornithine derivative (Scheme 21).[70]



Scheme 21. Synthesis of an guanylated ornithine derivative **53**

Pyrazole-based guanidinylation reagents

Another useful guanidinylation reagents are the 1*H*-pyrazole-1-[*N,N´*-bis-(*tert*-butoxycarbonyl)]carboxamidine **54a**, the 1*H*-pyrazole-1-[*N,N´*-bis-(benzyloxycarbonyl)]carboxamidine **54b** and the more electrophilic 4- nitro-1*H*-pyrazole-1-[*N,N´*-bis-(*tert*-butoxycarbonyl)]carboxamidine **55** (Figure 1).[71,72]

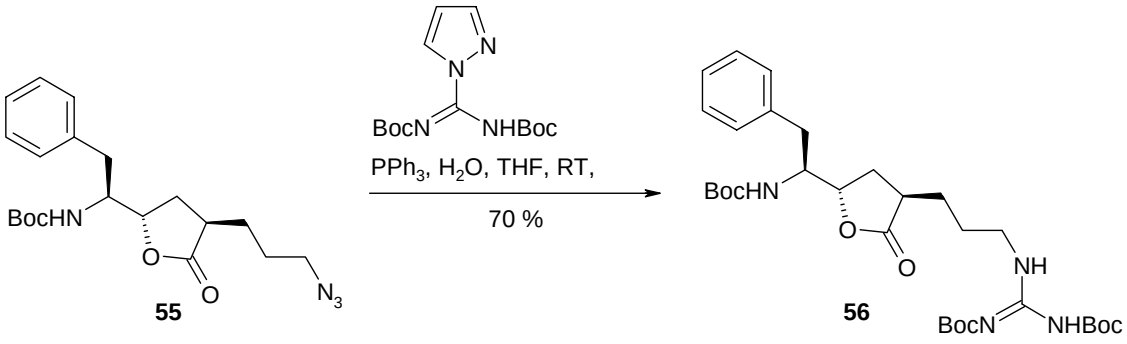


54a R = Boc
54b R = Cbz

55

Figure 1. Pyrazole-based guanidinylation reagents **54** and **55**

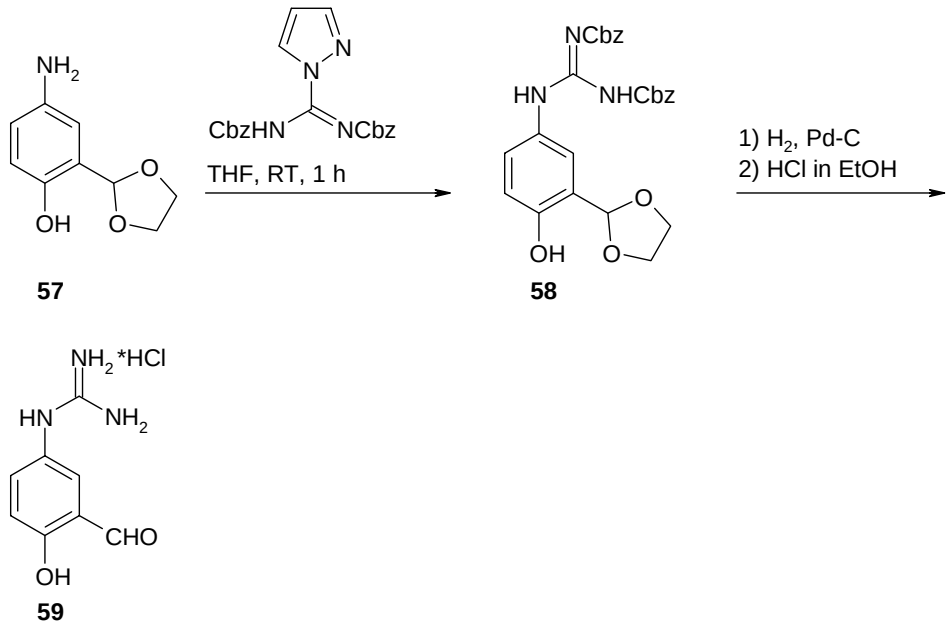
The Staudinger reaction is a mild conversion of azides to phosphazenes via treatment with phosphines. While phosphazenes display a wide range of reactivities, they are readily hydrolyzed to the corresponding primary amine and phosphine oxide in the presence of water.[73,74] *Rich* and co-workers have reported on the synthesis of a protected hydroxyethylene dipeptide isostere of Phe-Arg as component of potential peptidase inhibitors (Scheme 22).[75]



55 **56**

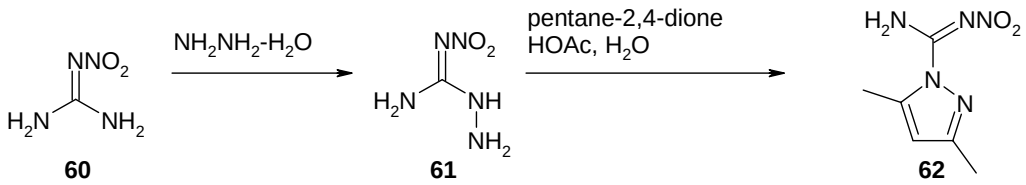
Scheme 22. Staudinger reaction

Tanizawa and co-workers have reported on the synthesis and evaluation of guanidine-containing Schiff Base copper(II), zinc (II) and iron (II) chelates as trypsin inhibitors (Scheme 23).[76] 3-Formyl-4-hydroxyphenylguanidine hydrochloride **59**, a key component in this study, was prepared from 2-hydroxy-5-nitrobenzaldehyde. Compound **57** was reacted with the guanylation reagent 1-[*N*,*N*´-bis(benzyloxycarbonyl)amidino]pyrazole in THF at room temperature for 1 h to give the guanidinium compound **58** in 90 % yield.



Scheme 23. Synthesis of the 3-Formyl-4-hydroxyphenylguanidine hydrochloride **59**

Castillo-Meléndez and co-workers have reported on the synthesis of 3,5-dimethyl-*N*-nitro-1-pyrazole-1-carboxamidine (DMNPC) **62** (Scheme 24).[77]



Scheme 24. Synthesis of DMNPC **62**

Several nitroguanidine derivatives were prepared to test the versatility of DMNPC (**62**) as a guanidinylating reagent. On reaction with DMNPC in MeOH at room temperature for 12 hours, a variety of amines (aliphatic, benzylic and aromatic) gave excellent yields (70 – 100 %) of *N*-alkyl-*N´*-nitroguanidines **63 –72** (Figure 2).

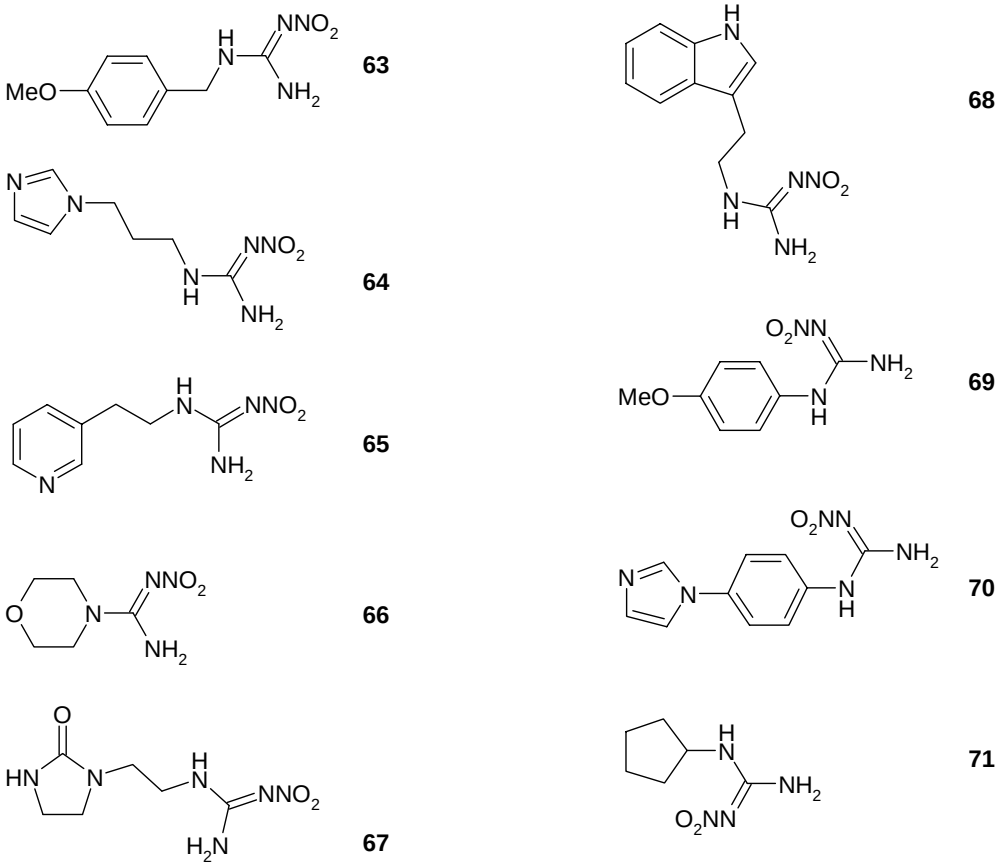
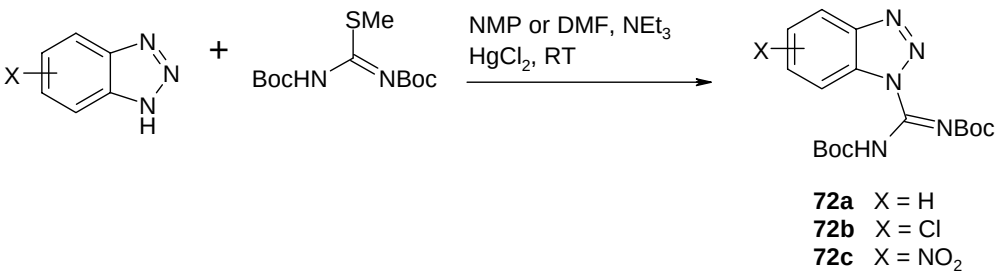


Figure 2. Examples of nitroguanidines prepared by nitroguanidinylation of amines by the DMNPC method

Moroder et al. reported on the synthesis of *N,N´*-di-*tert*-butoxycarbonyl-1-carboxamidine derivatives. By enhancing the leaving group character of benzotriazole via electron-withdrawing substituents such as 5-chloro or 6-nitro derivatives, highly efficient reagents are obtained for the conversion of primary and secondary amines in solution and in solid phase to diprotected guanidines (Scheme 25).[78]

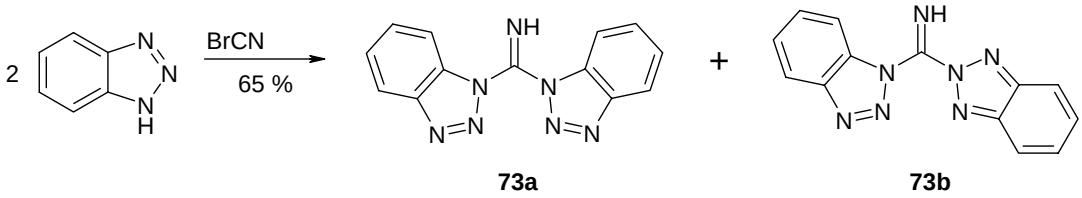


Scheme 25. Synthesis of *N,N´*-di-*tert*-butoxycarbonyl-1-carboxamidine derivatives **72a –c**

As expected from the electron-withdrawing substituents, guanidinylation of aniline as a model amine of poor nucleophilicity by the benzotriazole-based reagents occurs at rates in the rank order **72a** < **72b** < **72c**. However, all three reagents are more efficient amidine donors than the *N,N´*-di-*tert*-butoxycarbonyl-1H-pyrazole-1-carboxamidine (**54a**) and the triflylguanidine derivatives.

Di(benzotriazole-1-yl)methanimine

The group of *Katritzky* have reported on Di(benzotriazole-1-yl)methanimine (**73a-b**), a new reagent for the synthesis of tri- and tetrasubstituted guanidines (Scheme 26).[79]



73a **73b**

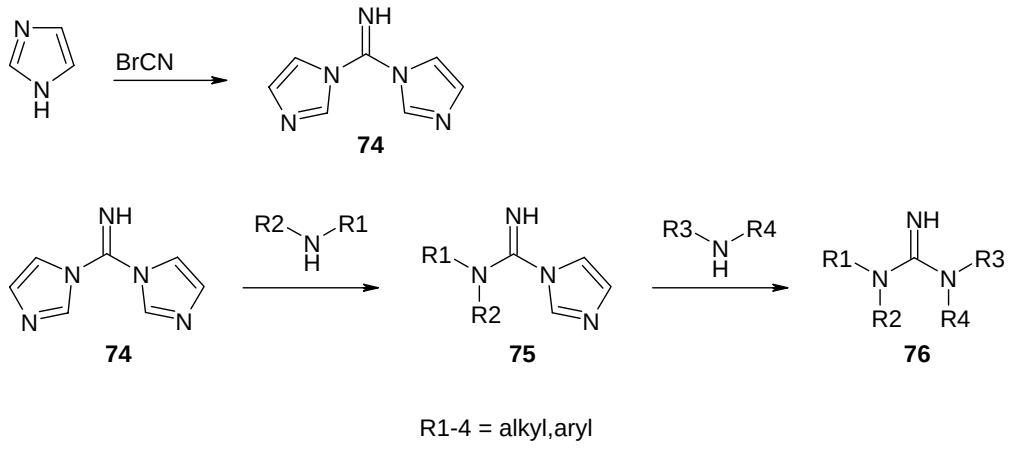
Scheme 26. Synthesis of Di(benzotriazole-1-yl)methanimine **(73a-b)**

The guanidinylation reagent **73** was obtained from the reaction of benzotriazole with cyanogen bromide as a mixture of di(1*H*- benzotriazole-1-yl)methanimine (**73a**) and 1*H*-benzotriazole-1-yl(2*H*-benzotriazole-2-yl)methanimine (**73b**) in overall 60 – 65 % yield. The displacement of the first benzotriazole moiety was effected by the addition of an amine to a solution of the mixture of isomers **73a** + **73b** in THF. A diverse range of aromatic and aliphatic primary and secondary amines in refluxing THF successfully displaced the remaining benzotriazole group and gave the *N,N,N´*-trisubstituted or the *N,N,N´,N´*-tetrasubstituted guanidines in good yields.

Di(imidazole-1-yl)methanimine

Another interesting guanidinylation reagent was developed by the group of *Wu*. The di(imidazole-1-yl)methanimine was synthesized by treating cyanogen bromide with imidazole in good yields (Scheme 27).[80] The sequential condensation of **74** with two amines leads to the substituted guanidines directly. It was found that both primary and secondary alkyl and arylamines were effective for the first displacement of imidazole,

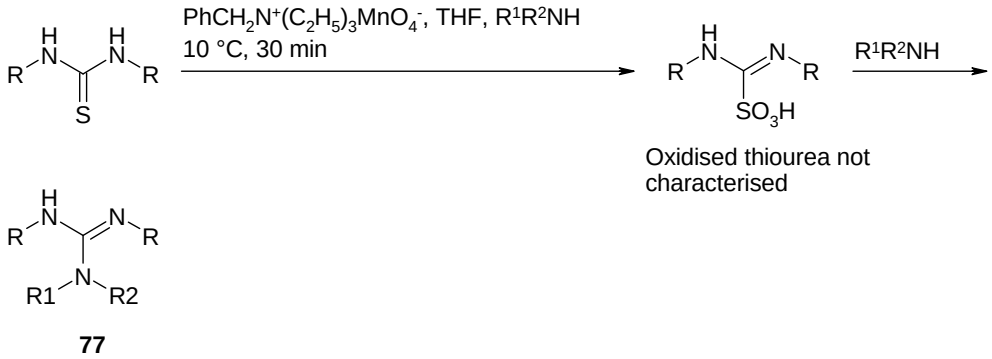
affording compound **75**. The second imidazole displacement by amines for the formation of guanidine **76** could only take place at reaction temperatures above 60 °C in THF.



Scheme 27. Synthesis of Di(imidazole-1-yl)methanimine (**74)**

Quaternaryammonium permanganates

Ramadas and co-workers have reported the use of quaternaryammonium permanganate to transform 1,3-diarylthioureas in the presence of an amine to the respective trisubstituted guanidines **77** in excellent yields (Scheme 28).[81]

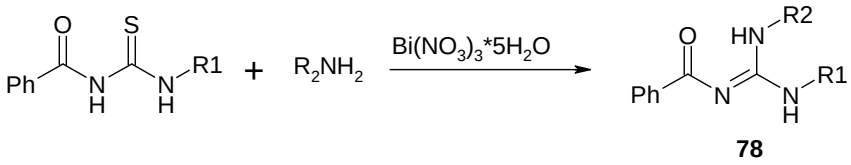


Scheme 28. Synthesis of trisubstituted guanidines via oxidation of thioureas

In a comparative study they found that the benzyltriethylammonium permanganate (BTEAP) gave the best results.

Bismuth nitrate pentahydrate

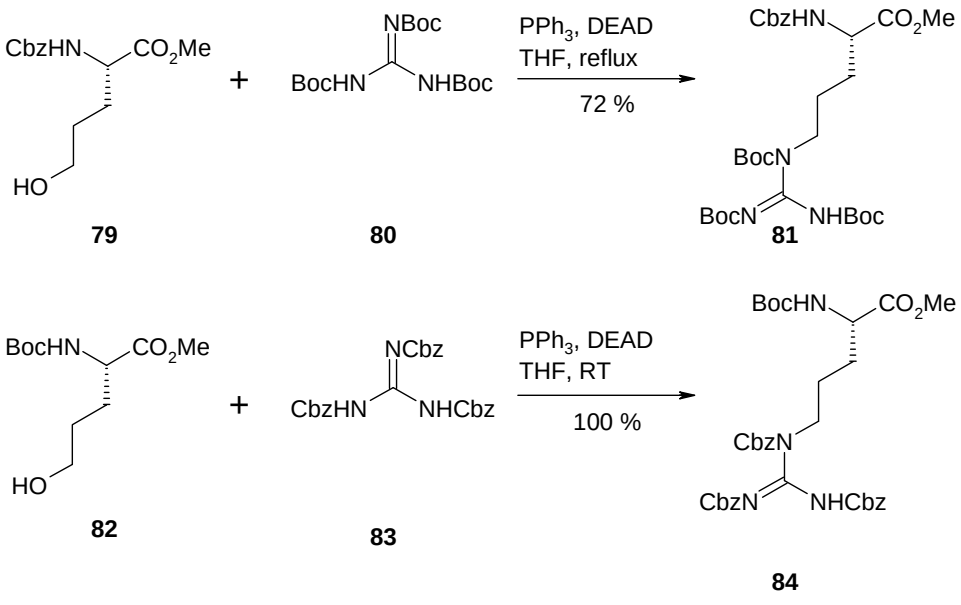
Cunha and co-workers reported on the synthesis of *N*-benzoyl-guanidines using bismuth nitrate as a guanidinylation reagent as an alternative to the quite often used mercury(II) chloride (Scheme 29).[82]



Scheme 29. Synthesis of *N*-benzoyl-guanidines **78**

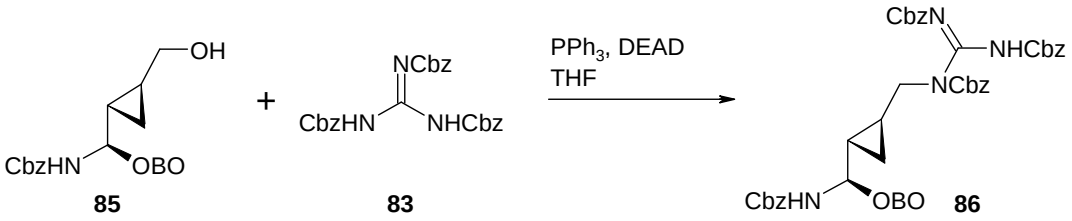
Mitsunobu Reaction

Goodman and co-workers have reported on the synthesis of protected alkylated guanidines via condensation of a primary or secondary alcohol with the guanidinylation reagents *N*,*N´*,*N´´*-tri-Boc-guanidine or *N*,*N´*,*N´´*-tri-Cbz-guanidine (Scheme 30).[83]



Scheme 29. Mitsunobu reaction

Lajoie and co-workers have reported on the synthesis of *trans*-cyclopropyl arginine via Mitsunobu reaction (Scheme 30).[84]



Scheme 30. Synthesis of *trans*-cyclopropyl arginine (**86**)

3. Conclusion

In the past couple of decades, the interest of guanidinium groups in biological, pharmaceutical and supramolecular applications has been ignited. They are valuable precursors of numerous medicinally important molecules and are assets in biological research. The chemical properties of the guanidinium group as well as its ability to form H-bonds, charge pairing and cation-π interactions opens up a large number of possibilities in molecular recognition. In summary, we report herein the most important methods for the synthesis of guanidines in solution. We focus on methods, which are used to synthesis more complex molecules in the field of natural product synthesis and pharmaceutical research. The use of thiourea and isothiourea as precursors is of clear importance. These precursors can be utilized in the presence of diverse reagents and coupling reagents.

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B. Main Part

1. Synthesis of Ethoxycarbonyl Guanidinium Amino Acids

1.1 Introduction

Arginine residues and their function as anion binding sites are ubiquitous in nature. They are found in the binding region of a large number of enzymes and signaling proteins. These proteins employ arginine residues, more specifically, guanidine moieties, to interact with negatively charged anionic[1,2] or π-electron-rich aromatic moieties[3] of substrates or cofactors.

The reason for the strong interaction with oxoanions lies in the peculiar binding pattern featuring two parallel hydrogen bonds in addition to the electrostatic interaction (Figure 1).[4]

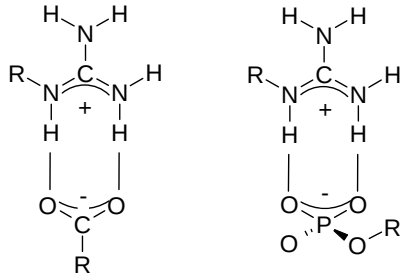


Figure 1. Binding pattern of guanidinium groups with oxoanions found in many X-ray structures of the corresponding salts.

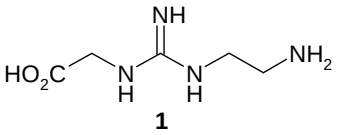
The extremely high basicity of guanidine (pK_a = 13.5) guarantees protonation over a wide pH range. This biological motif has inspired chemists to make use of the guanidinium group as an attractive anchor subunit in various artificial receptors.[2,4,5]

Lehn was among the first to investigate the use of guanidinium salts in the complexation of carboxylates by synthesising a series of structurally different guanidinium salts and measuring their association constants with carboxylate salts in a 10% H_2O – MeOH mixture, using pH-metric titration experiments.[6] The binding of a simple guanidinium salt to a carboxylate can be enhanced by incorporation of additional hydrogen bonding functionality. *Schmuck* has recently described guanidino-carbonyl pyrrole receptors

which bound carboxylates by ion pairing in combination with multiple hydrogen bonds.[7] *Schmidtchen* first reported the use of a bicyclic guanidinium salt for the formation of host-guest complexes with simple carboxylates.[8]

1.2 Results and discussion

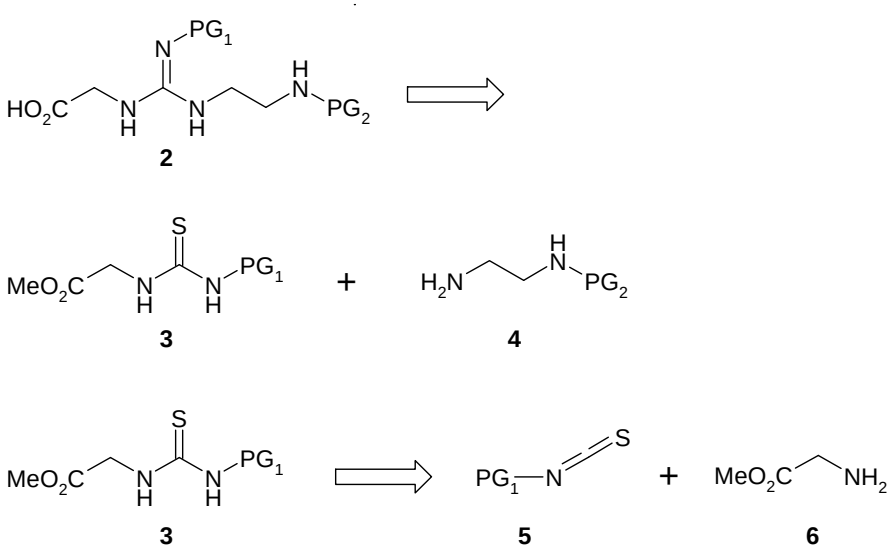
Kilburn and co-workers reported on the synthesis of "tweezer" receptors, incorporating a guanidinium "head group" in the backbone of an diamine and two peptide derived side arms.[9] *Vaillancourt* and co-workers reported on the synthesis and biological activity of aminoguanidine and diaminoguanidine analogues of the antidiabetic/antiobesity agent 3-Guanidinopropionic acid.[10,11] One of these compounds was an artificial guanidine with an amino acid structure **1** (Scheme 1). Protecting groups are not used in the synthesis.



1

Scheme 1. Artificial Guanidine Amino Acid (GuAA) **1**

Our approach is also to bring the guanidinium group from the side chain of arginine into the backbone of an artificial Guanidine Amino Acid (GuAA) **1** in order to get a more rigid receptor compared to arginine. Guanidine Amino Acids (GuAA´s) like **2** contain a guanidine group, which has to be protected orthogonally (PG_1) to the primary amine function (PG_2) in order to avoid side reactions on the guanidine group during the coupling steps (Scheme 2).

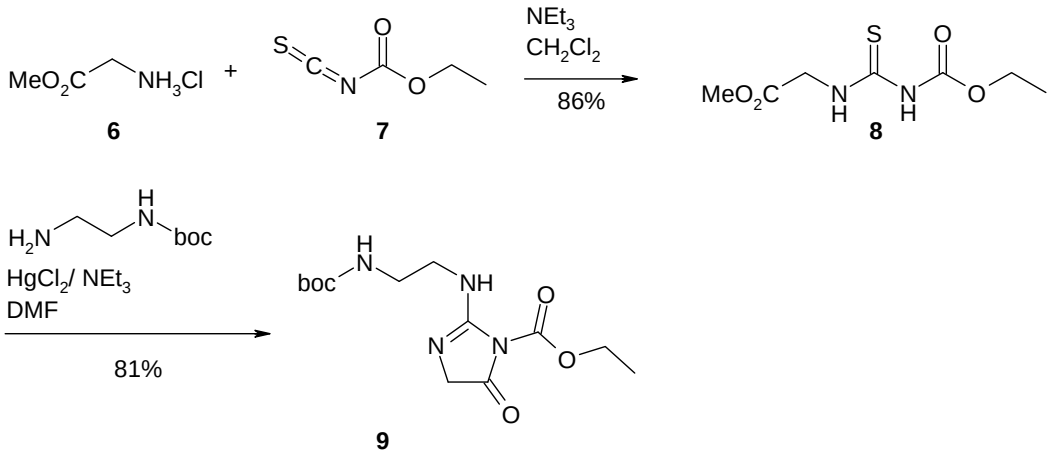


Scheme 2. General approach for the synthesis of GuAA **2** (PG_1/PG_2 = different protecting groups)

Various methods exist for the synthesis of guanidine derivatives from different starting materials and reagents.[12] One of the well-known methods is the conversion of thioureas, protected with electron withdrawing groups like Boc[13] or Cbz,[14] into guanidinium moities in the presence of a coupling reagent.[15] Most thioureas that contain these activating groups can be only coupled with primary amines. Furthermore, the efficiency of guanylation depends on the group attached to the primary amine. Thioureas mostly react with primary amines attached to primary carbons due to the bulkiness of the protecting groups. The efficiency of guanylation also depends upon whether the group that is attached to the amine is electron withdrawing or electron donating. Amines attached to electron withdrawing groups tend to give lower yields in guanylation.

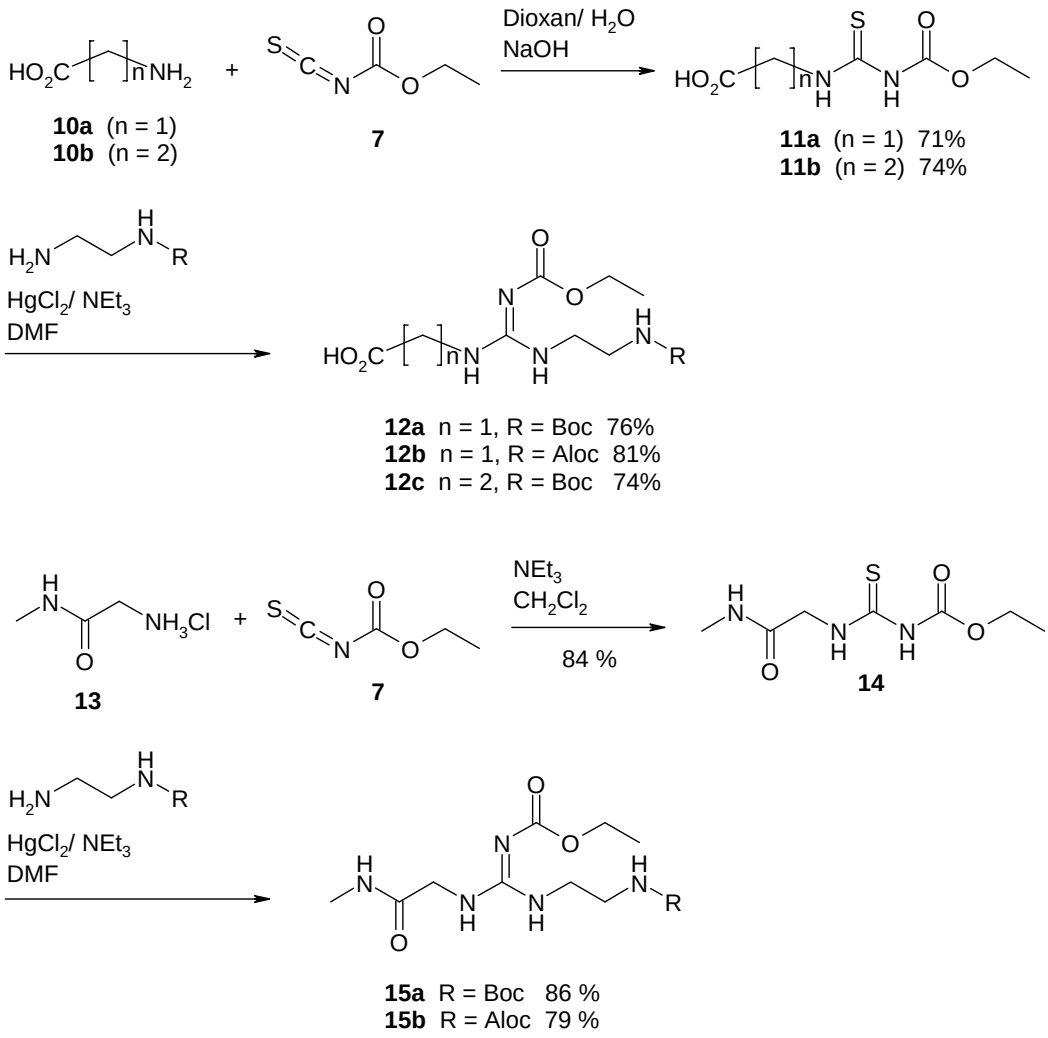
Ansyln and co-workers described a highly efficient synthesis of guanidinium derivatives using the ethyl carbamate protecting group and EDCl as a coupling reagent.[16] The advantages of the ethyl carbamate protecting group are on the one hand the commercial availability of the starting material, on the other hand the rapid formation of the protected thiourea.

We have started our approach with the formation of the thiourea derivative **8** obtained in very good yields. Transformation of the thiourea derivative **8** with mono-Boc-protected *N,N`*-ethylenediamine[17] in the presence of $HgCl_2$ gave not the acyclic guanidinium amino acid but the cyclic guanidinium derivative **9** in good yields (Scheme 3).



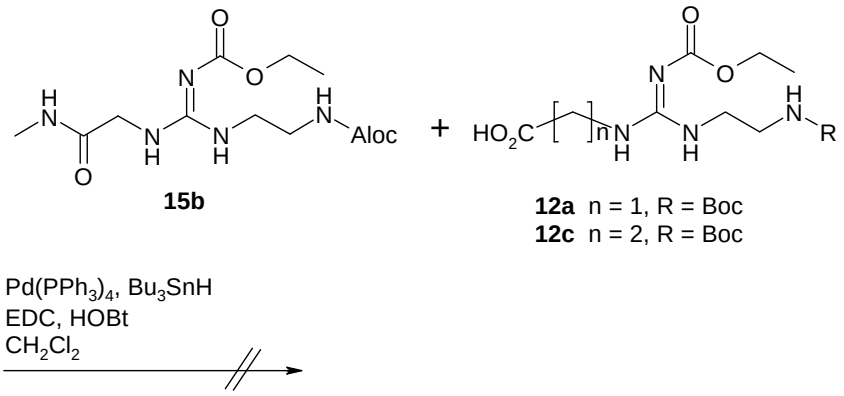
Scheme 3. Synthesis of the cyclic guanidine derivative **9**

In order to avoid such cyclisation reactions, it is necessary to replace the ester functionality by the unprotected carboxylic acid or by an amide functionality. Both variations were used for the synthesis of the ethoxycarbonyl-protected acyclic guanidinium amino acids (Scheme 4).



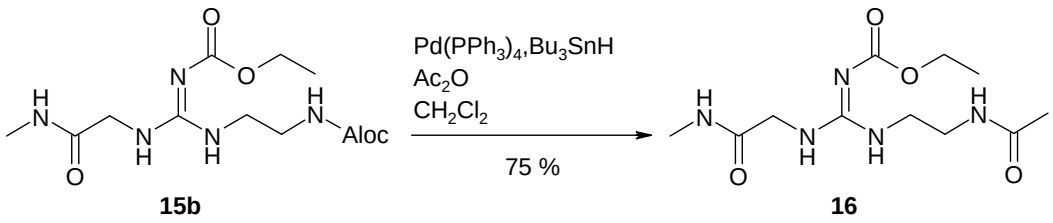
Scheme 4. Synthesis of different acyclic guanidinium amino acids (GuAA´s)

We have synthesised different acyclic guanidinium amino acids both with an Aloc-protecting and with a Boc-protecting group on the primary amine. When the palladium-catalyzed deprotection is carried out by using tributyltin hydride as nucleophile in the presence of an active acylating agent a new acyl group is introduced on nitrogen. Successful acylating agents include carboxylic acid anhydrides, acid chlorides and activated esters.[18] Unfortunately, the synthesis of a dimeric compound, using an activated GuAA as the acylating agent failed in all attempts (Scheme 5).



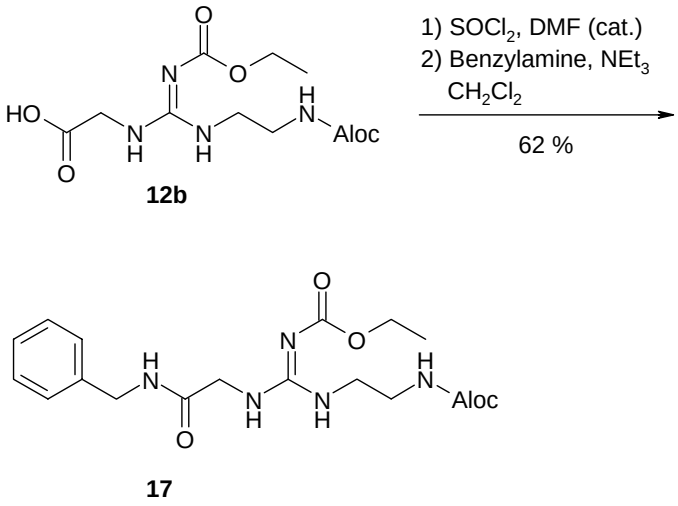
Scheme 5. Attempted synthesis of a dimeric compound

Compound **15b** was transformed into the corresponding acylated compound **16** in good yields (Scheme 6).



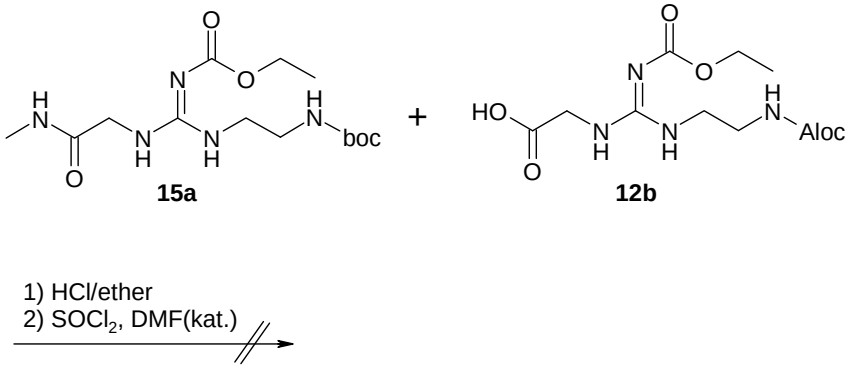
Scheme 6. Acylation of compound **15b**

This reaction shows that in principle a coupling on the amino function is possible. Next we have examined the coupling conditions on the carboxylic function. Peptide coupling via standard active esters methods (e. g. EDC/HOBT, HBTU/HOBt, HATU/HOAt) failed with all guanidinium amino acids. The activation of the carboxylic acid with $SOCl_2$ and catalytic amounts of DMF and following coupling with benzylamine led to compound **17** in 62 % yield (Scheme 7). The reason for this result could be that the two NH-groups of the guanidinium moiety from strong hydrogen bonds to the carboxylic group, so that an activation with the large phosphonium and uronium activation reagents is not possible. $SOCl_2$ is smaller in size and much more reactive than the phosphonium or uronium reagents.



Scheme 7. Synthesis of **17** via activation of **12b** with $SOCl_2$

The Boc-protected guanidine **15a** was first deprotected with HCl saturated ether and the resulting ammonium chloride was then added to the activated carboxylic acid **12b**. Unfortunately, the synthesis of a dimer via activation with $SOCl_2$ failed also as the synthesis via standard peptide coupling methods as described above (Scheme 8).

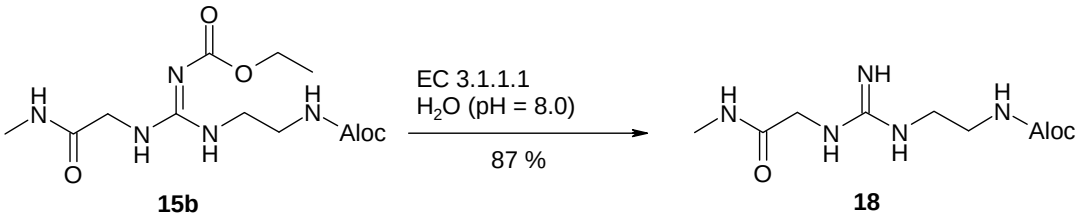


Scheme 8. Attempted synthesis of a dimer

Anslyn et al. described the removal of the ethyl carbamate group using Me_3SiBr under reflux in DMF followed by protonation with methanol to give the unprotected guanidinium compounds in yields of 95 %.[13] Experiments with our compounds failed.
Hutzler has described the enzymatic hydrolysis of an *N,N´*-Bis(ethoxycarbonyl) substituted guanidine with pig liver esterase.[i] Pig liver esterase (PLE, E.C. 3.1.1.1) is

[i] Hutzler, C. PhD Thesis, **2001**, University of Regensburg, Germany.

one of the most useful hydrolases for the enantiotopos-differentiating hydrolysis of prochiral cyclic dicarboxylic diesters.[19] The treatment of **15b** with the pig liver esterase EC 3.1.1.1 showed that a selective enzymatical cleavage of the ethoxycarbonyl-group is possible (Scheme 9). The Aloc-group is stable under these conditions.



Scheme 9. Selective cleavage of the Ethoxycarbonyl-group with EC 3.1.1.1

We have also studied the binding properties of such guanidinium amino acids. In the case of this trisubstituted guanidines the binding motif is in DMSO similar to the binding motif of thioureas ot ureas. The binding affinities of the compound **15** to acetate was determined by ^{1}H-NMR spectra titrations in DMSO-d_6. The binding constant was determined by non-linear fitting of chemical induced shift (CIS) of the NH proton of the guanidinium group.

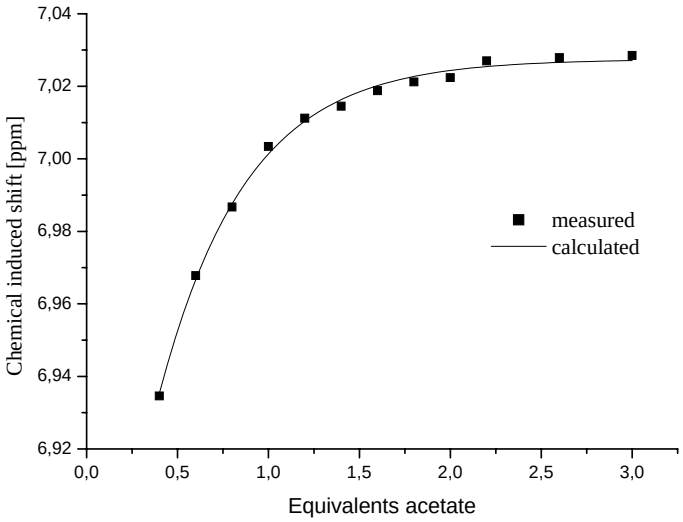


Figure 2. NMR-titration curve of a 2 mM of **15** with Bu_4NOAc in DMSO-d_6, monitoring the CIS of the NH proton of the guanidine.

The titration shows a binding constant of $K_a = (1.98 \pm 0.38)*10^3$ in DMSO. The value is similar to the binding constants of thioureas to carboxylates.[20]

1.3 Conclusion

Artificial Ethoxycarbonyl Guanidine Amino Acids were prepared efficiently by the reaction of glycine derivatives and commercially available ethoxycarbonylisothiocyanat **7** in good yields.

Carboxy – and amino-groups can be selectively deprotected, but peptide coupling reactions are limited to simple amines and acids.

The Ethoxycarbonyl-group can be selectively cleaved by the pig liver esterase EC 3.1.1.1

The binding ability of the Ethoxycarbonyl-protected guanidine to carboxylates in DMSO was investigated by NMR-titrations. The value is comparable with the binding constant of thioureas in DMSO.

1.4 Experimental Section

Melting points (mp) were determined with a Büchi SMP 20 and are uncorrectd.

IR-spectra were recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

NMR : Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K), Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [pp] relative external standards (solvent residual peak). The spectra were analysed by first order, the coupling constants are in Hertz [Hz]. Characterisation of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet. Integration is determined as the relative number of atoms. Error of reported values: 0.01 ppm for ^{1}H-NM, 0.1 ppm for ^{13}C-NMR; coupling constants: 0.1 Hz. The used solvent is reported for each spectrum.

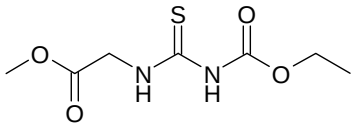
MS-Spectra: Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

Elemental Analysis: Microanalytical Laboratory of the University of Regensburg.

Thin Layer Chromatography (TLC) was performed on aluminia plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 nm). Visualisation was accomplished byUV-light (λ = 254 nm) and ninhydrine in MeOH.

Column Chromatography was performed on silica gel (70-230 mesh) from Merck.

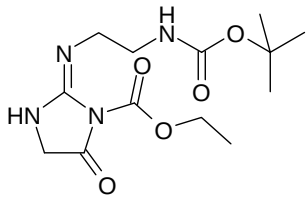
Synthesis



***N*-(Methoxycarbonylmethyl) –*N´*-(ethoxycarbonyl)thiourea (8).**

Ethoxycarbonylisothiocyanat **7** (2.32 g, 17.7 mmol) was added to a solution of glycine-methylester **6** (3.33 g, 26.5 mmol) and NEt_3 (3.05 g, 4.10 mL, 30.1 mmol) in dichloromethane (40 mL) and stirred at room temperature for 5 h. After addition of water (30 mL) the phases were separated and the organic phase was washed twice with water (30 mL). The organic phase was dried (anhydrous Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **8** (3.34 g, 15.2 mmol, 86 %) as a pale yellow solid. R_f = 0.6 (EtOAc)

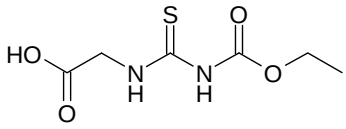
mp: 81 °C. - IR (KBr) [cm^{-1}]: 2950, 2910, 1719, 1537, 1384, 1147. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.29 (t, J = 7.1 Hz, 3 H, CH_2-C*H*$_3$), 3.78 (s, 3 H, Methoxy), 4.23 (q, J = 7.1 Hz, 2 H, C*H*$_2$-CH_3), 4.41 (d, J = 4.9 Hz, 2 H, C*H*$_2$-NH), 8.46 (bs, 1 H, N*H*), 10.13 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.2 (+), 46.9 (-), 52.6 (+), 62.9 (-), 152.7 (C_{quat}), 168.9 (C_{quat}), 179.8 (C_{quat}). - MS (EI, 70 eV): m/z (%) = 220.1 (100) [M^+], 188.0 (50) [M – CH_3OH], 160.0 (33) [M – CH_3OH – C_2H_4], 132.0 (17) [M – $CH_3CH_2CO_2NH$]. – Elemental analysis calcd. (%) for $C_7H_{12}N_2O_4S$ (220.24): C 38.17, H 5.50, N 12.73; found C 38.11, H 5.43, N 12.78.



[2-(1-Ethoxycarbonyl-5-oxo-4,5-dihydro-1H-imidazol-2-ylamino)-ethyl]-carbamic acid *tert*-butyl ester (9).

Thiourea **8** (88 mg, 0.39 mmol) was added to a solution of 1-(*tert*-Butyloxycarbonyl)ethylene diamine[15] (92 mg, 0.58 mmol) and NEt_3 (79 mg, 0.11 mL, 0.78 mmol) in DMF (20 mL). Mercury(II)-chloride (125 mg, 0.46 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **9** (99 mg, 0.32 mmol, 81 %) as a white solid. R_f = 0.5 (EtOAc)

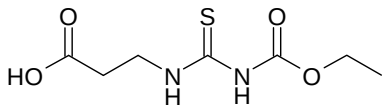
mp: 81 °C. - IR (KBr) [cm^{-1}]: 3389, 3325, 2976, 2924, 1750, 1688, 1533, 1474, 1251. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.19 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 1.35 (s, 9 H, Boc), 3.07 – 3.17 (m, 2 H, -C*H*$_2$-NH-Boc), 3.50 (t, J = 5.8 Hz, 2 H, -C*H*$_2$-CH_2), 3.96 – 4.08 (m, 4 H, 2 x C*H*$_2$), 6.87 (bs, 1 H, N*H*-Boc), 8.92 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.3 (+), 28.1 (+), 37.5 (-), 38.9 (-), 47.4 (-), 60.1 (-), 77.5 (C_{quat}), 161.1 (C_{quat}), 162.3 (C_{quat}), 171.9 (C_{quat}). - MS (EI, 70 eV): m/z (%) = 314.1 (9) [M^+], 269.1 (4) [M^+ - C_2H_5O], 241.0 (14) [M^+ - C_4H_9O], 213.0 (11) [M^+ - $C_4H_9CO_2$]. - Elemental analysis calcd. (%) for $C_{13}H_{22}N_4O_5$ (314.16): C 49.66, H 7.06, N 17.83; found C 49.59, H 7.09, N 17.96.



(3-Ethoxycarbonyl-thiourea)-acetic acid (11a).

Ethoxycarbonylisothiocyanat **7** (1.16 g, 1.00 mL, 8.87 mmol) in dioxane (20 mL) was added to a solution of glycine **10a** (1.00 g, 13.3 mmol) in 10 mL H_2O and 5 mL 1N NaOH. The mixture was stirred at room temperature for 12 h. the organic solvent was removed under reduced pressure. The water phase was acidified with 5 % $KHSO_4$ solution to pH = 2. After addition of EtOAc (25 mL) the phases were separated and the and the water phase was extracted twice with EtOAc (40 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **11a** (1.29g, 6.29 mmol, 71 %) as white solid. R_f (EtOAc) = 0.35

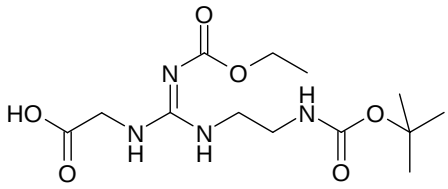
mp: 78 °C. - IR (KBr) [cm^{-1}]: 3180, 2970, 2908, 1720, 1545, 1448, 1414, 1255, 1205, 1146. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.23 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 4.17 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 4.27 (d, J = 5.2 Hz, 2 H, HO_2C-C*H*$_2$-), 10.15 (bs, 1 H,CH_2-N*H*-), 11.14 (s, 1 H, N*H*), 13.0 (bs, 1 H, CO_2*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.1 (+), 46.4 (-), 61.7 (-), 153.3 (C_{quat}), 169.8 (C_{quat}), 179.9 (C_{quat}). - MS (EI, 70 eV): m/z (%) = 206.1 (100) [M^+], 188.0 (16) [M^+ - H_2O], 160.0 (23) [M^+ - C_2H_5OH]. – $C_6H_{10}N_2O_4S$ (206.04).



(3-Ethoxycarbonyl-thiourea)-propionic acid (11b).

Ethoxycarbonylisothiocyanat **7** (981 mg, 0.845 mL, 7.48 mmol) in dioxane (20 mL) was added to solution of β-Ala-OH **10b** (1.00 g, 11.22 mmol) in 10 mL H_2O and 5 mL 1N NaOH. The mixture was stirred at room temperature for 12 h. the organic solvent was removed under reduced pressure. The water phase was acidified with 5 % $KHSO_4$ solution to pH = 2. After addition of EtOAc (25 mL) the phases were separated and the and the water phase was extracted twice with EtOAc (40 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **11b** (1.22g, 5.53 mmol, 74 %) as a white solid. R_f (EtOAc) = 0.45

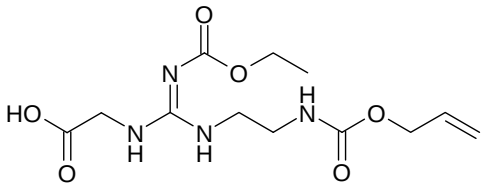
mp: 78 °C. - IR (KBr) [cm^{-1}]: 3183, 2973, 2915, 1720, 1548, 1416, 1259, 1151. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.21 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 2.58 (t, J = 6.6 Hz, 2 H, HO_2C-C*H*$_2$-), 3.71 – 3.78 (m, 2 H, -C*H*$_2$-NH-), 4.14 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 9.92 – 10.08 (m, 1 H, N*H*), 10.95 (bs, 1 H, N*H*), 12.37 (bs, 1 H, CO_2*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.0 (+), 32.3 (-), 40.2 (-), 61.6 (-), 153.3 (C_{quat}), 172.8 (C_{quat}), 179.4 (C_{quat}). - MS (CI - MS, NH_3): m/z (%) = 220.9 (100) [MH^+]. - Elemental analysis calcd. (%) for $C_7H_{12}N_2O_4S$ (220.24): C 38.17, H5.49, N 12.72; found C 38.15, H5.21, N 12.73.



[*N´*-(2-*tert*-Butoxycarbonylamino-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-acetic acid (12a).

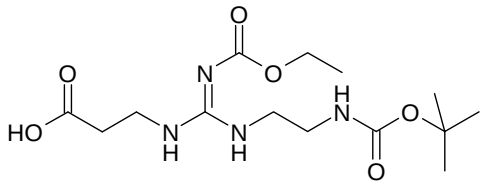
Thiourea **11a** (300 mg, 1.45 mmol) was added to a solution of 1-(*tert*-Butyloxycarbonyl)ethylene diamine[15] (348 mg, 2.17 mmol) and NEt_3 (323 mg, 0.442 mL, 3.19 mmol) in DMF (20 mL). Mercury(II)-chloride (472 mg, 1.74 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O (25 mL) and acidified with 5 % $KHSO_4$ to pH = 2-3. The aqueous layer was extracted three times with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **12a** (366 mg, 1.10 mmol, 76 %) as a white solid. R_f = 0.2 (EtOAc)

mp: 93 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.21 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 1.38 (s, 9 H, Boc), 2.92 –2.98 (m, 2 H, C*H*$_2$), 3.06 – 3.12 (m, 2 H, C*H*$_2$), 3.75 (d, J = 5.2 Hz, 2 H, HO_2C-C*H*$_2$-), 4.12 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 6.82 (bs, 1 H, -N*H*-Boc), 7.95 - 8.15 (m, 2 H, N*H*), 10.05 (s, 1 H, CO_2*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.1 (+), 28.1 (+), 38.7 (-), 39.7 (-), 42.4 (-), 61.1 (-), 77.6 (C_{quat}), 152.8 (C_{quat}), 154.3 (C_{quat}), 155.5 (C_{quat}), 168.5 (C_{quat}). - MS (EI, 70 eV): m/z (%) = 332.1 (5) [M^+], 276.0 (10) [M^+ - C_4H_8], 259.0 (20) [M^+ - C_4H_8O], 231.1 (5) [M^+ - $C_4H_8CO_2$], 203.0 (40) [M^+ - Boc – C_2H_4]. – $C_{13}H_{24}N_4O_6$ (332.36).



[*N´*-(2-Allyloxycarbonyl-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-acetic acid (12b).

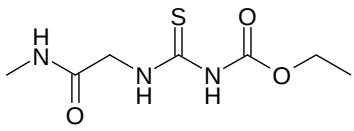
Thiourea **4a** (513 mg, 2.48 mmol) was added to a solution of 1-Allyloxycarbonylethylene diamine[16] (672 mg, 3.72 mmol) and NEt_3 (1.00 g, 1.37 mL, 9.92 mmol) in DMF (25 mL). Mercury(II)-chloride (809 mg, 2.98 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O (25 mL) and acidified with 5 % $KHSO_4$ to pH = 2-3. The aqueous layer was extracted three times with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **12b** (635 mg, 2.01 mmol, 81 %) as a white solid. R_f = 0.1 (EtOAc)
mp: 104 °C. - IR (KBr) [cm^{-1}]: 3325, 3085, 2984, 2943, 1698, 1655, 1552, 1249, 1152, 1101, 1017, 912, 875, 763, 687. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.21 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 2.98 – 3.18 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.75 (d, J = 5.2 Hz, 2 H, HO_2C-C*H*$_2$), 4.12 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 4.46 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH_2O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH_2O), 5.83 – 5.97 (m, 1 H, CH_2=C*H*CH_2O), 7.23 (bs, 1 H, -N*H*-Aloc), 8.00 - 8.17 (m, 2 H, 2 x N*H*), 10.06 (bs, 1 H, CO_2*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.1 (+), 38.5 (-), 39.6 (-), 42.4 (-), 61.1 (-), 64.2 (-), 116.9 (-), 133.6 (+), 152.8 (C_{quat}), 154.3 (C_{quat}), 155.9 (C_{quat}), 168.6 (C_{quat}). - MS (CI - MS, NH_3): m/z (%) = 334.1 (10) [MNH_4^+], 317.1 (100) [MH^+]. - Elemental analysis calcd. (%) for $C_{12}H_{20}N_4O_6$ (316.14): C 45.57, H 6.37, N 17.71; found C 45.24, H 6.35, N 17.76.



[*N´*-(2-*tert*-Butoxycarbonylamino-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-propionic acid (12c).

Thiourea **11b** (300 mg, 1.36 mmol) was added to a solution of 1-(*tert*-Butyloxycarbonyl)ethylene diamine[15] (327 mg, 2.04 mmol) and NEt_3 (303 mg, 0.414 mL, 2.99 mmol) in DMF (20 mL). Mercury(II)-chloride (442 mg, 1.63 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O (25 mL) and acidified with 5 % $KHSO_4$ to pH = 2-3. The aqueous layer was extracted three times with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **12c** (350 mg, 1.01 mmol, 74 %) as a white solid. R_f = 0.25 (EtOAc)

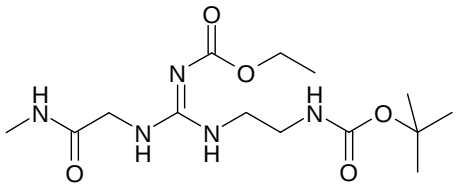
mp: 95 °C. - IR (KBr) [cm^{-1}]: 3325, 2980, 1690, 1644, 1536, 1367, 1243, 1173, 1018, 979, 769. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.18 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 1.37 (s, 9 H, Boc), 2.27 (t, J = 6.9 Hz, 2 H, HO_2C-C*H*$_2$-), 2.94 – 3.08 (m, 4 H, 2 x C*H*$_2$), 3.29 – 3.38 (m, 2 H, C*H*$_2$), 4.09 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 6.78 (bs, 1 H, -N*H*-Boc), 7.92 – 7.99 (m, 2 H, 2 x N*H*), 9.92 (s, 1 H CO_2*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.1 (+), 28.1 (+), 35.0 (-), 35.5 (-), 38.6 (-), 39.4 (-), 61.0 (-), 77.5 (C_{quat}), 152.6 (C_{quat}), 154.3 (C_{quat}), 155.5 (C_{quat}), 170.5 (C_{quat}). - MS (CI - MS, NH_3): m/z (%) = 364.0 (3) [MNH_4^+], 347.0 (100) [MH^+], 290.9 (26) [MH^+ - C_4H_8], 247.0 (5) [MH^+ - Boc]. – $C_{14}H_{26}N_4O_6$ (346.19).



***N*-(Methylacetamide) –*N´*-(ethoxycarbonyl)thiourea (14).**

Ethoxycarbonylisothiocyanat **7** (2.32 g, 17.7 mmol) was added to a solution of glycine-methylamide **13** (3.30 g, 26.5 mmol) and NEt_3 (3.05 g, 4.10 mL, 30.1 mmol) in dichloromethane (40 mL) and stirred at room temperature for 5 h. After addition of water (30 mL) the phases were separated and the organic phase was washed twice with water (30 mL). The organic phase was dried (anhydrous Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **14** (3.27 g, 14.9 mmol, 84 %) as a white solid. R_f = 0.45 (EtOAc)

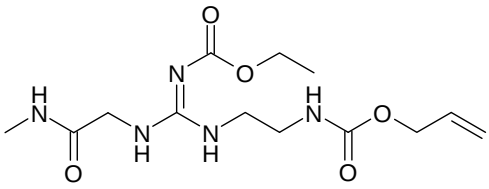
mp: 101 °C. - IR (KBr) [cm^{-1}]: 3307, 3177, 3034, 2935, 1720, 1657, 1546, 1414, 1247, 1205, 1043, 963, 739, 615. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.21 (t, J = 7.0 Hz, 3 H, -C*H*$_3$), 2.60 (d, J = 5.2 Hz, 3 H, -NH-C*H*$_3$), 4.17 (q, J = 7.0 Hz, 2 H, -C*H*$_2$-CH_3), 4.27 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-NH), 8.04 (bs, 1 H, N*H*), 9.76 (bs, 1 H, N*H*), 10.16 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.0 (+), 25.4 (+), 47.6 (-), 61.6 (-), 153.3 (C_{quat}), 167.3 (C_{quat}), 179.2 (C_{quat}). - MS (EI, 70 eV): m/z (%) = 219.3 (100) [M^+], 173.2 (10) [M – C_2H_5OH]. – Elemental analysis calcd. (%) for $C_7H_{13}N_3O_3S$ (219.26): C 38.35, H 5.98, N 19.16; found C 38.15, H 6.48, N 19.23.



[*N´*-(2-*tert*-Butyloxycarbonylamino-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-methyl acetamide (15a).

Thiourea **14** (41 mg, 0.186 mmol) was added to a solution of 1-Boc-ethylene diamine[15] (44 mg, 0.279 mmol) and NEt_3 (37 mg, 0.050 mL, 0.372 mmol) in DMF (10 mL). Mercury(II)-chloride (55 mg, 0.205 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O (15 mL) and acidified with 5 % $KHSO_4$ to pH = 2-3. The aqueous layer was extracted three times with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **15a** (57 mg, 0.166 mmol, 86 %) as a white solid. R_f = 0.15 (EtOAc)

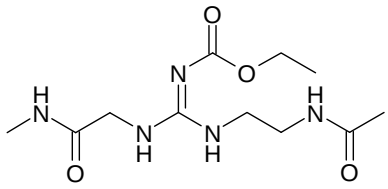
mp: 112 °C. - IR (KBr) [cm^{-1}]: 3335, 2979, 2935, 1642, 1605, 1535, 1395, 1369, 1286, 1170, 1089, 972, 864, 798, 654. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.13 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 1.37 (s, 9 H, Boc), 2.61 (d, J = 5.2 Hz, 3 H, -NH-C*H*$_3$), 2.97 – 3.24 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.76 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-NH), 3.91 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 6.91 (bs, 1 H, -N*H*-Boc), 7.80 (bs, 1 H, N*H*), 8.95 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 346.5 (100) [MH^+], 290.4 (35) [MH^+ - C_4H_8], 246.5 (25) [MH^+ - Boc]. – $C_{14}H_{27}N_5O_5$ (345.40).



[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-methyl acetamide (15b).

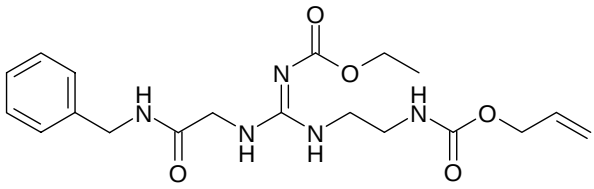
Thiourea **14** (360 mg, 1.64 mmol) was added to a solution of 1-(Allyloxycarbonyl)ethylene diamine[15] (580 mg, 2.37 mmol) and NEt_3 (830 mg, 1.14 mL, 8.20 mmol) in DMF (20 mL). Mercury(II)-chloride (535 mg, 1.97 mmol) was added in one portion and the reaction mixture was stirred for 15 h at room temperature. The mixture was filtered over Celite and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O (25 mL) and acidified with 5 % $KHSO_4$ to pH = 2-3. The aqueous layer was extracted three times with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **15b** (428 mg, 1.30 mmol, 79 %) as a white solid. R_f = 0.10 (EtOAc)

mp: 102 °C. - IR (KBr) [cm^{-1}]: 3323, 3085, 2982, 2948, 1721, 1655, 1456, 1251, 1151, 1108, 1027, 914, 874, 769, 688. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.21 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 2.60 (d, J = 5.2 Hz, 3 H, -NH-C*H*$_3$), 2.98 – 3.18 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.75 (d, J = 5.2 Hz, 2 H, C*H*$_2$-NH), 4.12 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 4.46 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH_2O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH_2O), 5.83 – 5.97 (m, 1 H, CH_2=C*H*CH_2O), 7.23 (bs, 1 H, -N*H*-Aloc), 8.03 (bs, 1 H, N*H*), 8.67 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.2 (+), 25.3 (+), 38.5 (-), 39.7 (-), 42.6 (-), 61.0 (-), 64.3 (-), 117.0 (-), 133.5 (+), 152.8 (C_{quat}), 154.3 (C_{quat}), 155.9 (C_{quat}), 168.6 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 330.1 (100) [MH^+], 299.0 (5) [MH^+ - CH_3NH_2], 284.0 (25) [MH^+ - EtOH]. – $C_{13}H_{23}N_5O_5$ (329.36).



[*N´*-(2-Acetylamino-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-methyl acetamide (16).
Compound **15b** (77 mg, 0.23 mmol) and Ac_2O (60 mg, 0.050 mL, 0.58 mmol) were dissolved in 10 mL DCM. $Pd(PPh_3)_4$ (13 mg, 5 mol%) and immediately Bu_3SnH (81 mg, 0.074 mL, 0.28 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **16** (49 mg, 0.17 mmol, 75 %) as a colourless oil. R_f (EtOAc) = 0.25

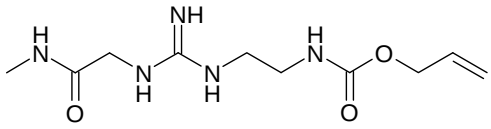
IR (KBr) [cm^{-1}]: 3312, 3071, 2972, 2939, 1719, 1641, 1367, 1134, 1108, 1007, 923, 856, 774. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.17 (t, J = 7.1 Hz, 3 H, -C*H*$_3$), 1.81 (s, 3 H, C*H*$_3$-CO), 2.61 (d, J = 5.2 Hz, 3 H, -NH-C*H*$_3$), 2.98 – 3.18 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.75 (d, J = 5.2 Hz, 2 H, C*H*$_2$-NH), 3.91 (q, J = 7.1 Hz, 2 H, -C*H*$_2$-CH_3), 7.04 (bs, 1 H, N*H*), 7.89 (bs, 1 H, N*H*), 8.07 (bs, 1 H, N*H*), 9.23 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 310.0 (10) [MNa^+], 288.0 (100) [MH^+], 241.8 (55) [MH^+ - EtOH]. – $C_{11}H_{21}N_5O_4$ (287.32).



[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´´*-ethoxycarbonyl-guanidino]-methyl benzylamide (17).

Compound **12b** (220 mg, 0.70 mmol) was dissolved in 8 mL DCM, $SOCl_2$ (833 mg, 0.51 mL, 7.0 mmol) and 2 drops DMF were added and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and the product was dried under high vacuum for 1 h. The acid chloride was dissolved in 10 mL DCM, benzylamine (75 mg, 0.080 mL, 0.70 mmol) and NEt_3 (71 mg, 0.100 mL, 0.70 mmol) were added and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **17** (174 mg, 0.43 mmol, 62 %) as a light brown oil.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.18 (t, J = 7.0 Hz, 3 H, -C*H*$_3$), 2.98 – 3.18 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.73 (d, J = 5.1 Hz, 2 H, C*H*$_2$-NH), 4.12 (q, J = 7.0 Hz, 2 H, -C*H*$_2$-CH$_3$), 4.46 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 4.58 (s, 2 H, Ph-C*H*$_2$), 5.18 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.29 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.82 – 5.96 (m, 1 H, CH$_2$=C*H*CH$_2$O), 7.13 – 7.31 (m, 6 H, Ph/N*H*-Aloc), 8.03 (bs, 2 H, N*H*), 8.67 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 406.1 (100) [MH^+]. – $C_{19}H_{27}N_5O_5$ (405.20).



[2-(*N´*-Methylcarbamoylmethyl-guanidino)-ethyl]-carbamic acid allyl ester (18).

Compound **15b** (77 mg, 0.23 mmol) was dissolved in 10 mL H_2O (Tris-buffer, pH = 8.0) and 10 mg EC 3.1.1.1 was added. The reaction mixture was stirred at room temperature for 20 h. The pH value was adjusted to pH = 10 with 1 N NaOH. The organic layer was extracted with DCM (2 x 25 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (EtOAc/MeOH = 4/1) to obtain **18** as a colourless oil. R_f (EtOAc/MeOH = 4/1) = 0.35

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.61 (d, J = 5.1 Hz, 3 H, -NH-C*H*$_3$), 3.06 – 3.34 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.78 –3.87 (m, 2 H, C*H*$_2$-NH), 4.47 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.80 – 5.95 (m, 1 H, CH$_2$=C*H*CH$_2$O), 7.33 (bs, 1 H, -N*H*-Aloc), 7.56 – 7.73 (m, 2 H , 2 x N*H*), 7.89 (bs, 1 H, N*H*), 8.23 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 25.5 (+), 43.6 (-), 45.2 (-), 59.7 (-), 64.4 (-), 117.0 (-), 133.5 (+), 156.0 (C_{quat}), 156.8 (C_{quat}), 167.6 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 258.8 (100) [MH^+]. – $C_{10}H_{19}N_5O_3$ (257.29).

1.5 Appendix

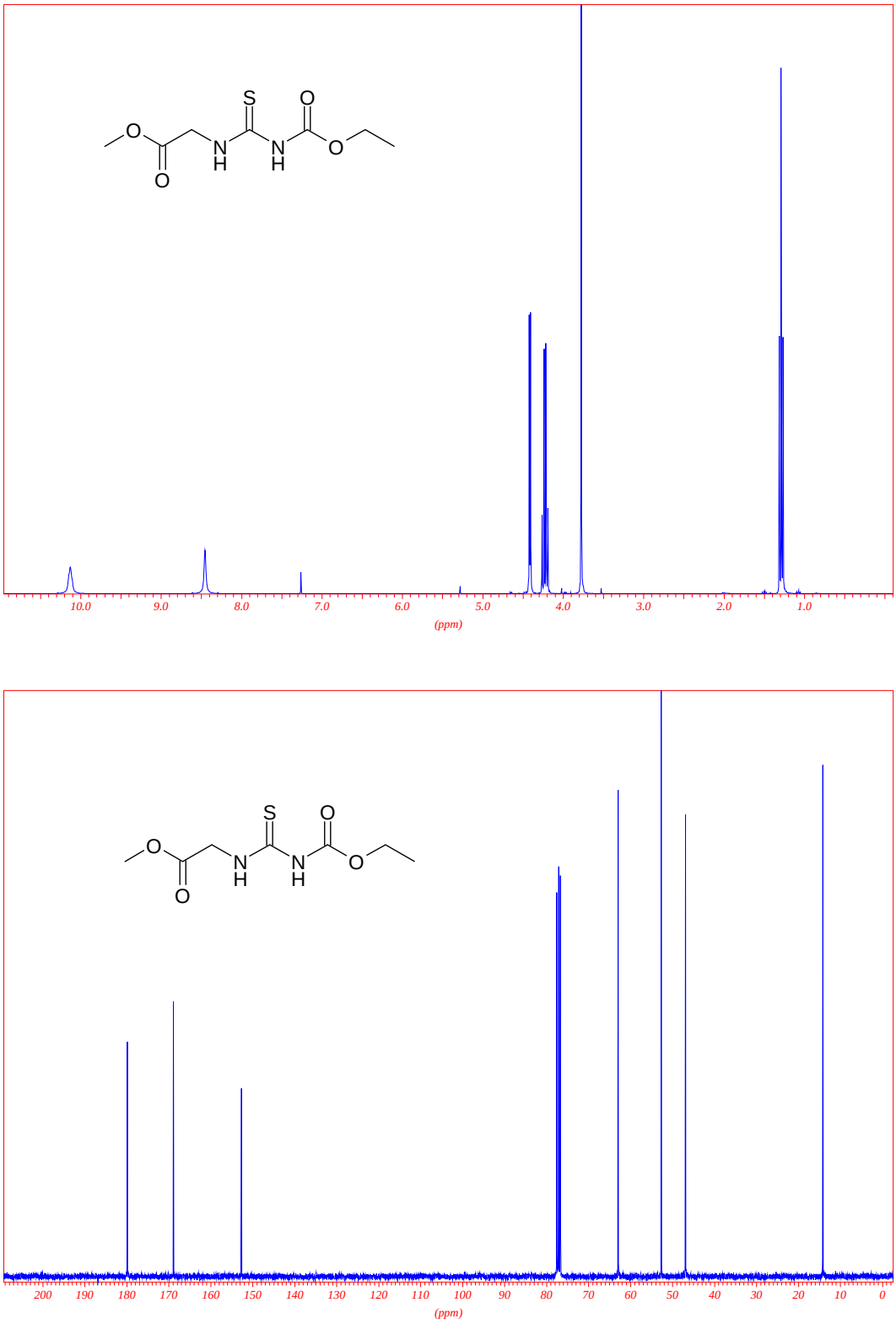


Figure 3. ^{1}H-NMR and ^{13}C-NMR of compound **8**

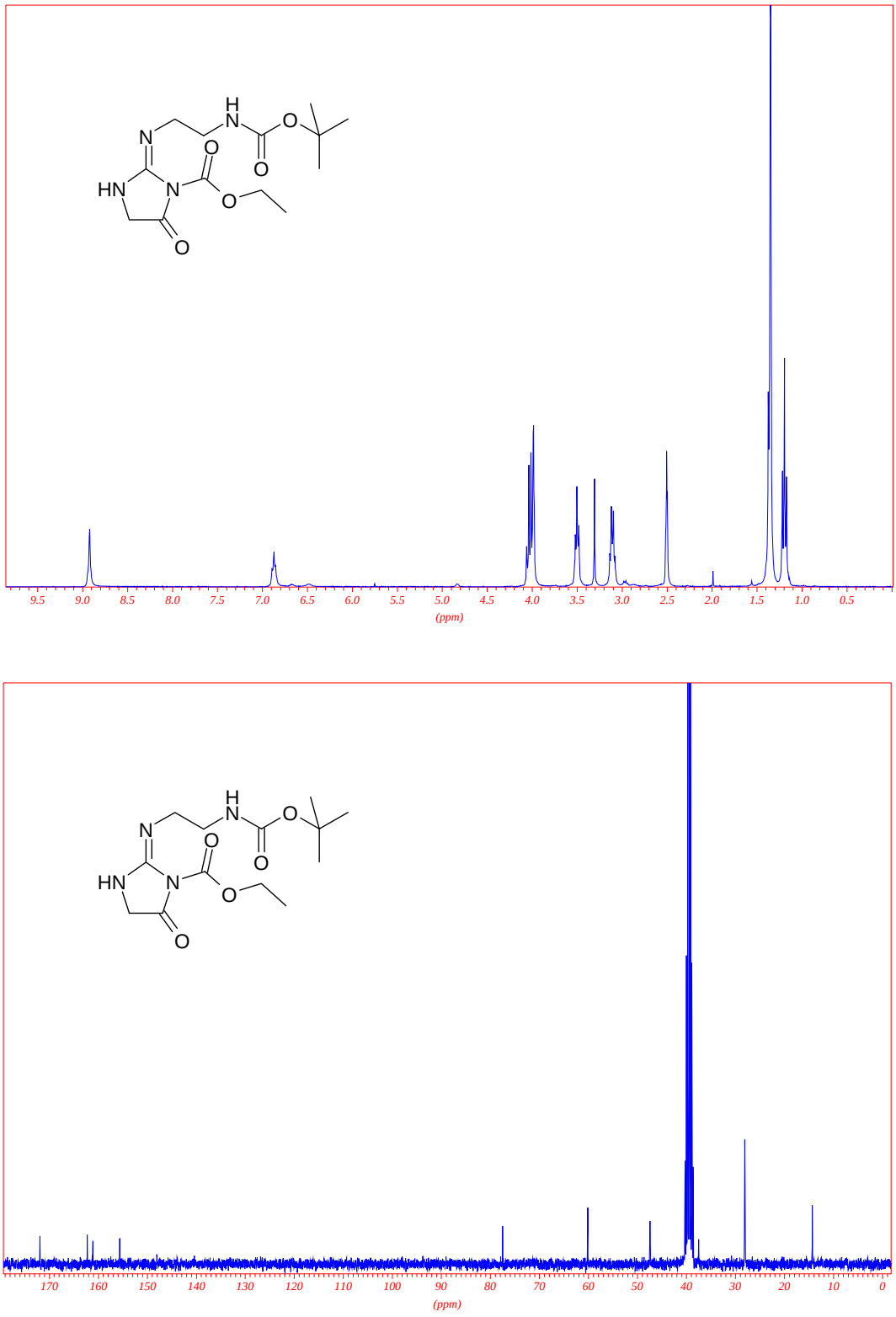
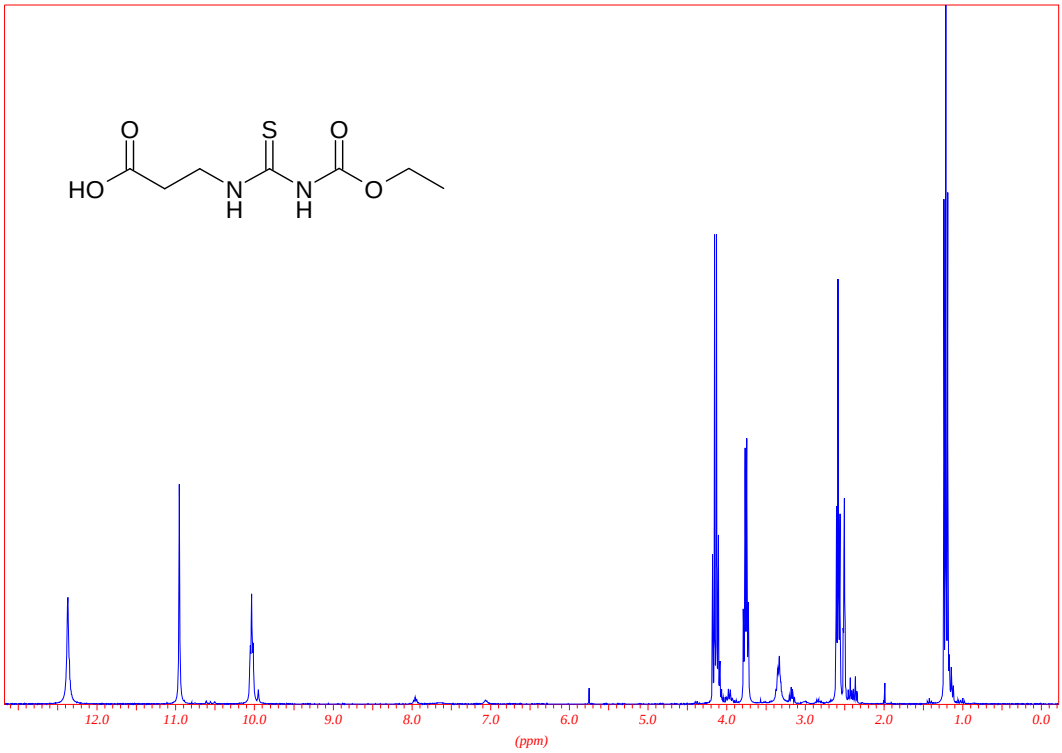


Figure 4. ^{1}H-NMR and ^{13}C-NMR of compound **9**



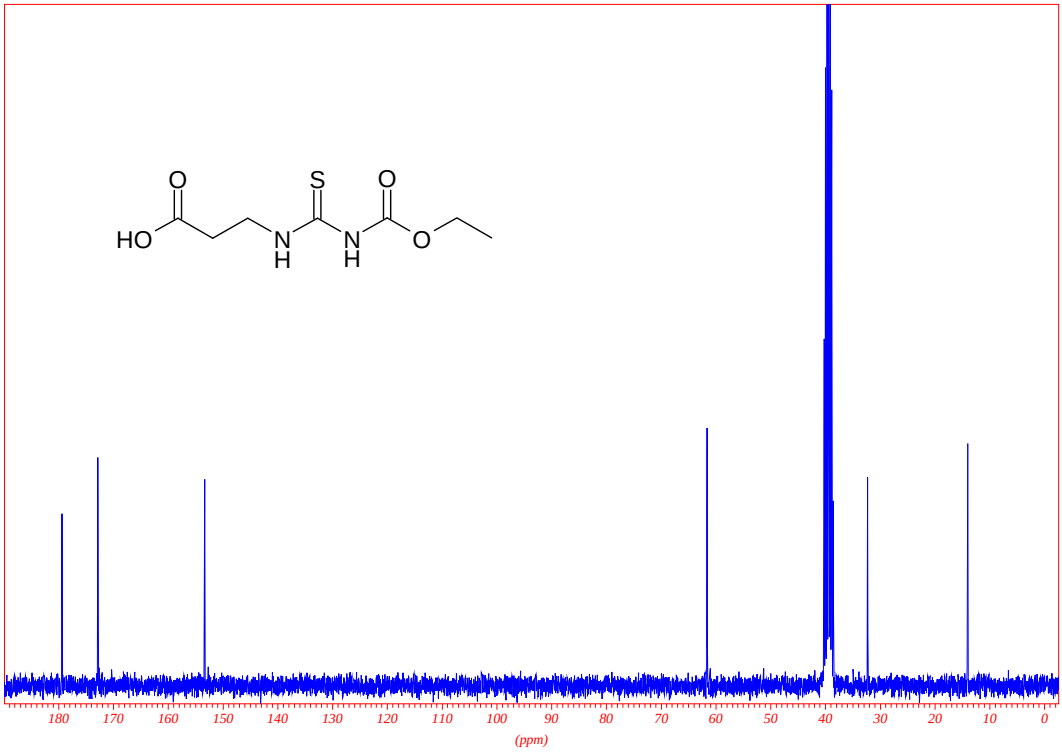
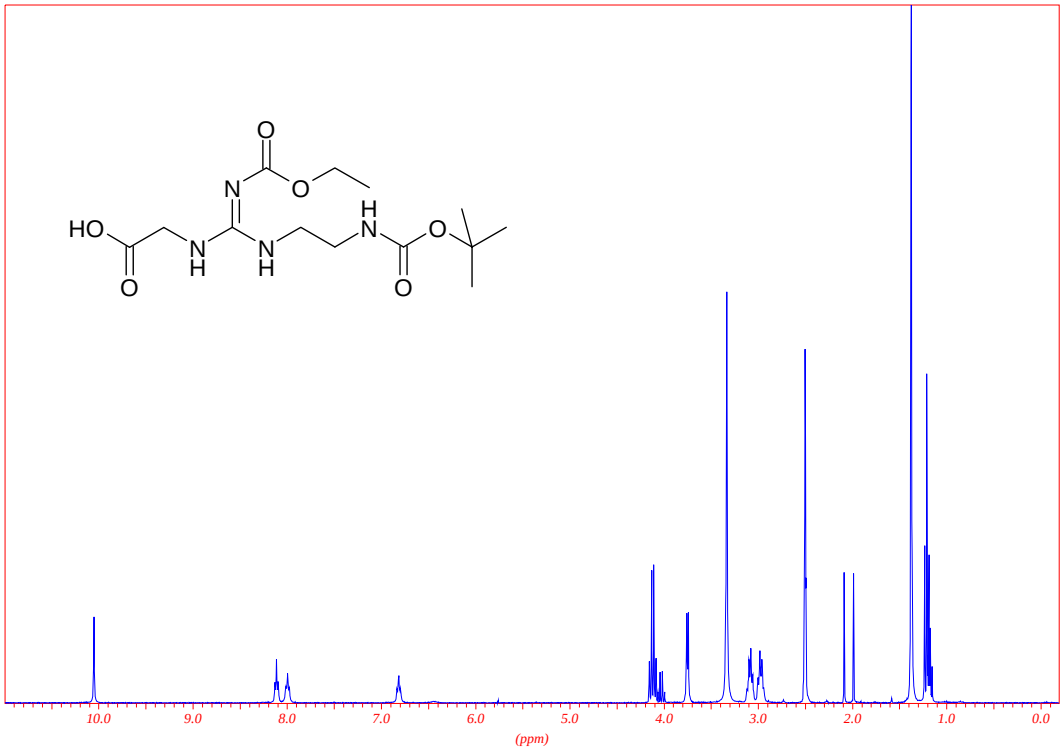


Figure 5. ^{1}H-NMR and ^{13}C-NMR of compound **11b**



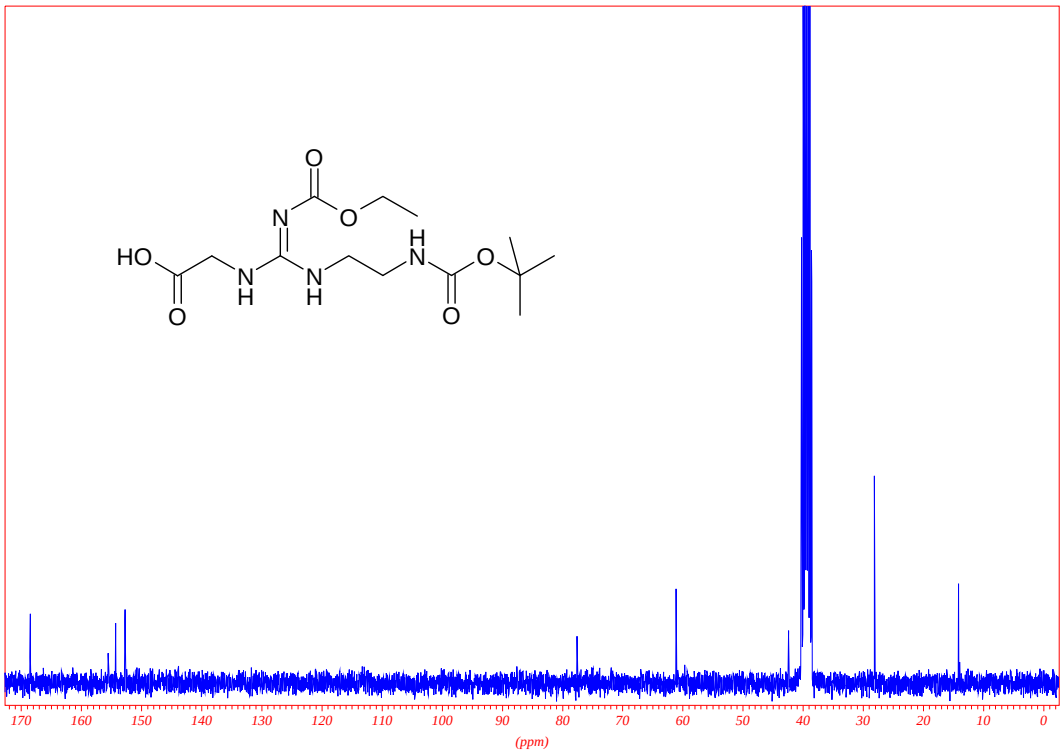


Figure 6. ^{1}H-NMR and ^{13}C-NMR of compound **12a**

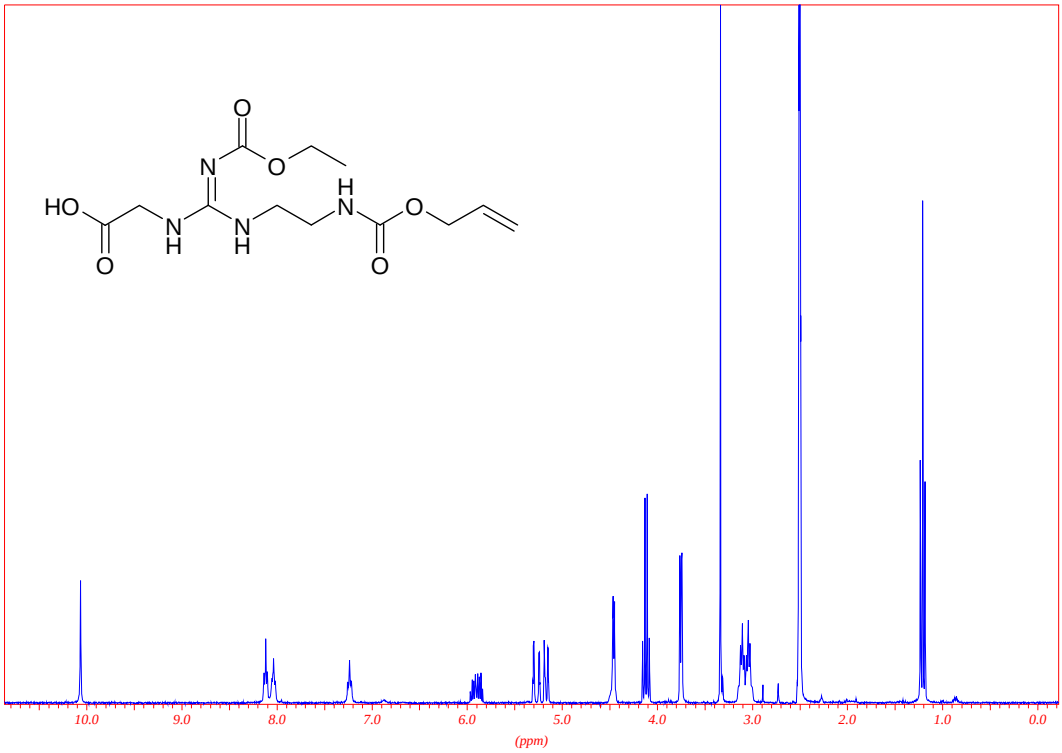


Figure 7. ^{1}H-NMR of compound **12b**

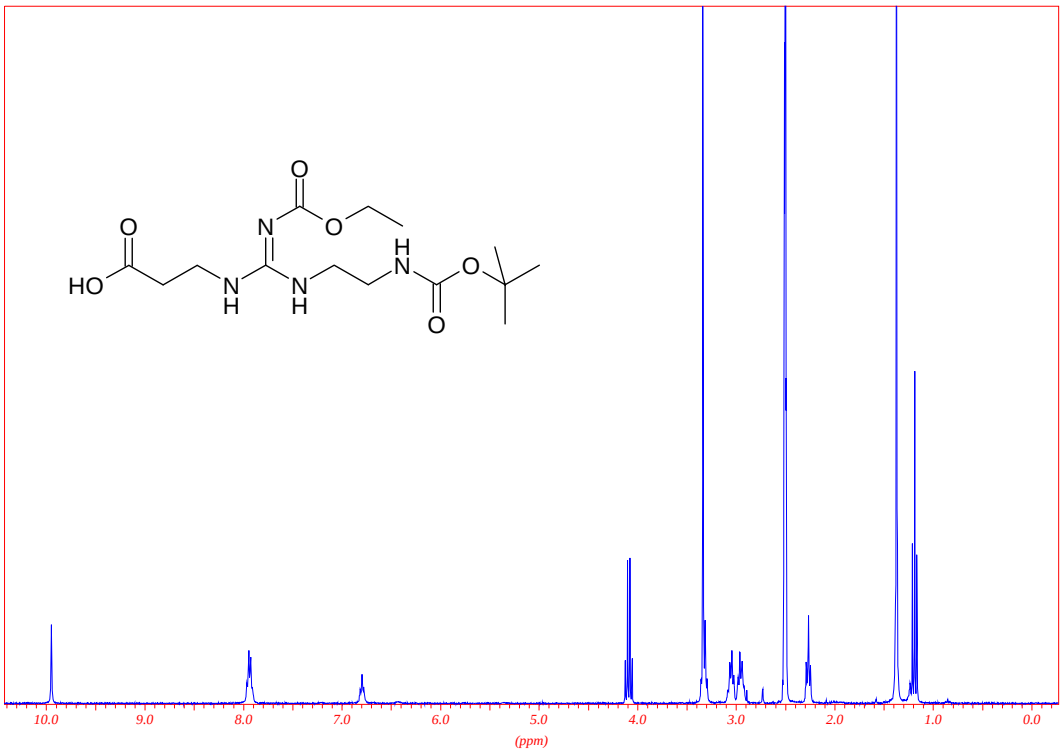


Figure 8. ^{1}H-NMR of compound **12c**

1.6 References and notes

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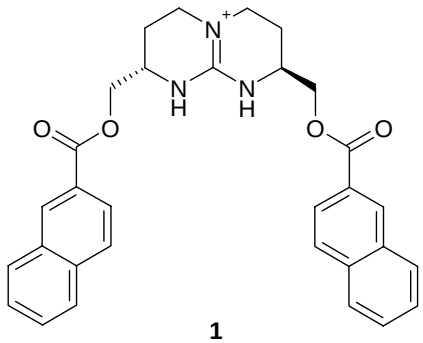
2. Synthesis of Fluorescent Guanidinium Amino Acids

2.1 Introduction

Since the early days of anion-recognition chemistry, guanidinium-based anion recognition has attracted attention because of their special ability to form both hydrogen bonds and electrostatic interactions. They remain protonated over a wide pH-range (pK_a = 13.5)[1] and provide two protons that point in roughly the same direction and which can stibilize two parallel hydrogen bonds, as seen from the X-ray crystal structures of many guanidinium salts.[2] As a consequence, guanidinium-based receptors often display high affinities for oxoanion substrates, even in polar solvents and aqueous environments. In 1978, *Lehn* and co-workers reported the synthesis and anion-binding-properties of some guanidinium-based macrocycles.[3] These macrocycles bind trianionic phosphate (PO_4^{3-}) in a 1:1 ratio and with affinity constants of log K_a = 3.1 – 4.3 in methanol-water (9:1) solution at 20 °C. *Lehn* and *de Mendoza* prepared the first chiral bicyclic guanidinium receptor **1** (Figure 1).[4]

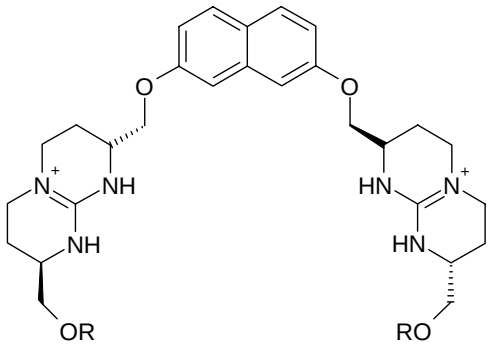


1

Figure 1. Chiral bicyclic guanidinium receptor

It was found that this receptor is capable of extracting *p*-nitrobenzoate anions from aqueous media into organic solvents, confirming that it can bind oxyanions. Quantitative analysis of the binding constants by use of standard ^{1}H-NMR titration methods gave a K_a of 1609 M^{-1} for the binding of *p*-nitrobenzoate (studied as the corresponding triethylammonium salt) in $CDCl_3$. Interestingly, the asymmetric nature of **1**-SS enabled enantioselective recognition of chiral carboxylate anions such as the sodium salts of (S)-mandelate and (S)-naproxenate, as judged from extraction

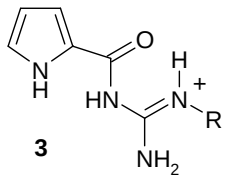
experiments. Similarly, receptors **1**-SS and **1**-RR could be used to extract L- and D-*N*-acetyltryptophan from a racemic mixture of the L and D enantiomers. In 1993 *Schmidtchen* reported the bisguanidinium receptor **2** (Figure 2), in which bicyclic guanidiniums are linked via ether linkages, and showed that such systems could be used to effect dicarboxylate anion recognition.[5]



2 R = $Si(C_6H_5)_2C(CH_3)_3$

Figure 2. Bisguanidinium receptor **2**

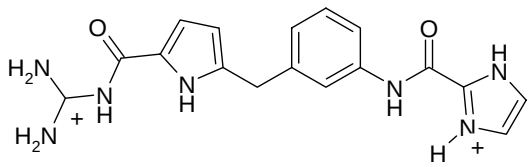
Schmuck and co-workers have reported on the synthesis of *N´*-substituted guanidinocarbonyl pyrrols **3** (Figure 3).[6] These guanidinium cations are efficient receptors for the complexation of amino acid carboxylates even in water ($K_{assoc} > 10\ M^{-1}$).



3

Figure 3. *N´*-substituted guanidinocarbonyl pyrrol **3**

A de Novo designed guanidinocarbonyl receptor **4** for the dipeptide binding in water was described by the same working group (Figure 4).[7] The complexation properties of **4** were studied by UV titration in water (with 10 % DMSO added for solubility reasons) with various dipeptides and amino acids as substrates.

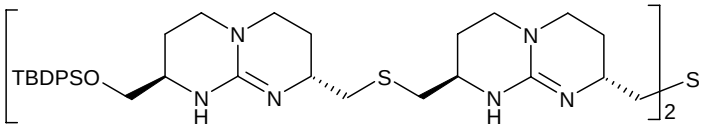


4

Figure 4. Guanidinocarbonyl receptor **4** for dipeptide binding in water

The binding was followed by the decrease in the absorption of the pyrrole moiety at λ = 320 nm upon the addition of aliquots of the dipeptide to a solution of **4** (0.01586 mM, chloride salt, 0.5 mM bis-tris-buffer at pH = 5.5). A Job´s plot confirmed a 1:1 binding stoichiometry in water. **4** binds dipeptides very efficiently even in water with association constants $K_{ass} > 10^4$ M^{-1}. The dipeptides are bound up to 10 times more efficiently than simple amino acids ($K_{ass} \approx$ (5-7) x 10^3 M^{-1}) for which the association constants are similar to those for other guanidiniocarbonyl pyrrole-based receptors.[8,9]

Hamilton and *de Mendoza* have extended the bicyclic guanidinium motif to create synthetic receptors that similarly stabilise the α-helical conformation of glutamate and aspartate rich peptides.[10,11] They have shown the association between tetraguanidinium receptor **5** and a peptide with an *i*, *i* +3, *i* + 6, *i* + 9 arrangement of aspartate residues in 10 % aqueous methanol.



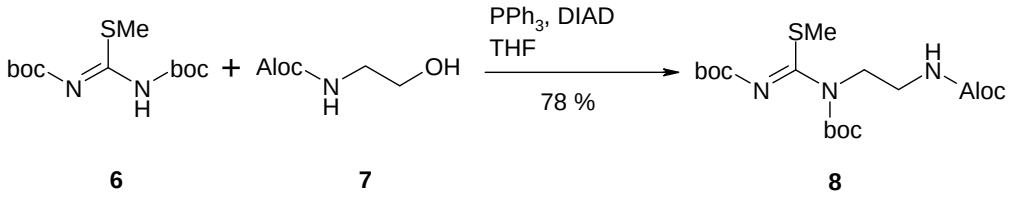
5

Figure 5. Bicyclic guanidinium tetramer **5**

The synthesis of such bicyclic guanidinium moieties is difficult and the yields are small. The binding constants are determined via NMR-titrations or in the case of the tetramer 5 via CD-titrations. We report here about the synthesis of fluorescent acyclic guanidine amino acids (GuAA`s) and their binding properties.

2.2 Synthesis of Fluorescent Guanidines

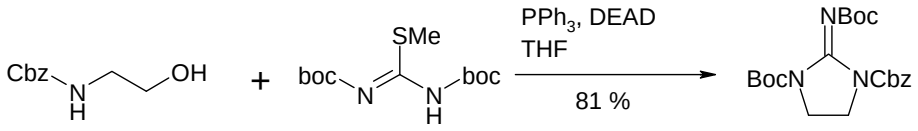
The synthesis started with the conversion of *N*-Aloc-protected ethanolamine (**7)**[12] and 1,3-di-Boc-2-methyl-isothiourea (**6**)[13] under standard Mitsunobu reaction conditions to provide the alkylated isothiourea **8** in good yields (Scheme 1).



6 **7** **8**

Scheme 1. Synthesis of the isothiourea derivative **8**

Kim and co-workers described the synthesis of the cyclic guanidine **10** using mono-Cbz protected ethanolamine **9** and 1,3-di-Boc-2-methyl-isothiourea (**7**) (Scheme 2).[14]

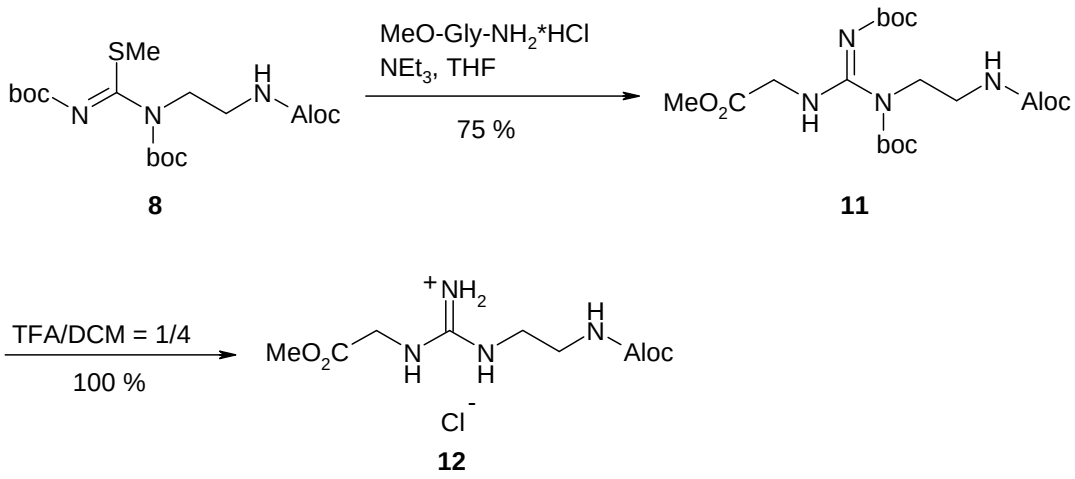


9 **7** **10**

Scheme 2. Synthesis of the cyclic product **10**

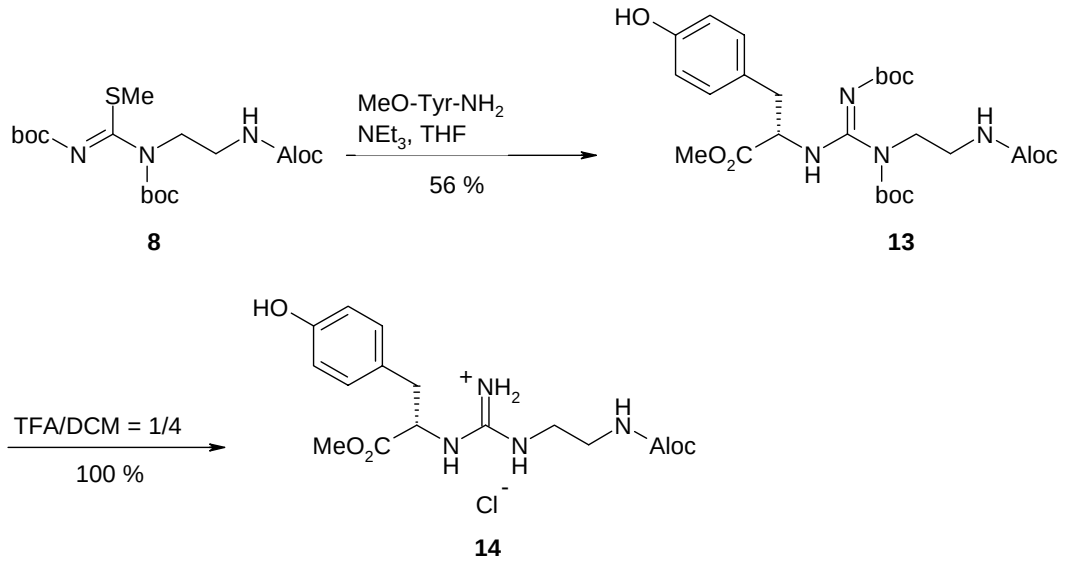
Using the Aloc-group (Scheme 1), a cyclisation is avoided.

Conversion of the isothiourea **8** with glycine-methyl ester in refluxing THF over night gave the expected guanidine **11** in 75 % yield (Scheme 3). Using $HgCl_2$ as an "activation reagent" for the isothiourea, the yields go down to 45 %. Cleavage of the two Boc-groups with TFA:DCM = 1:4 and anion exchange against chloride yielded in the guanidinium cation **12**.



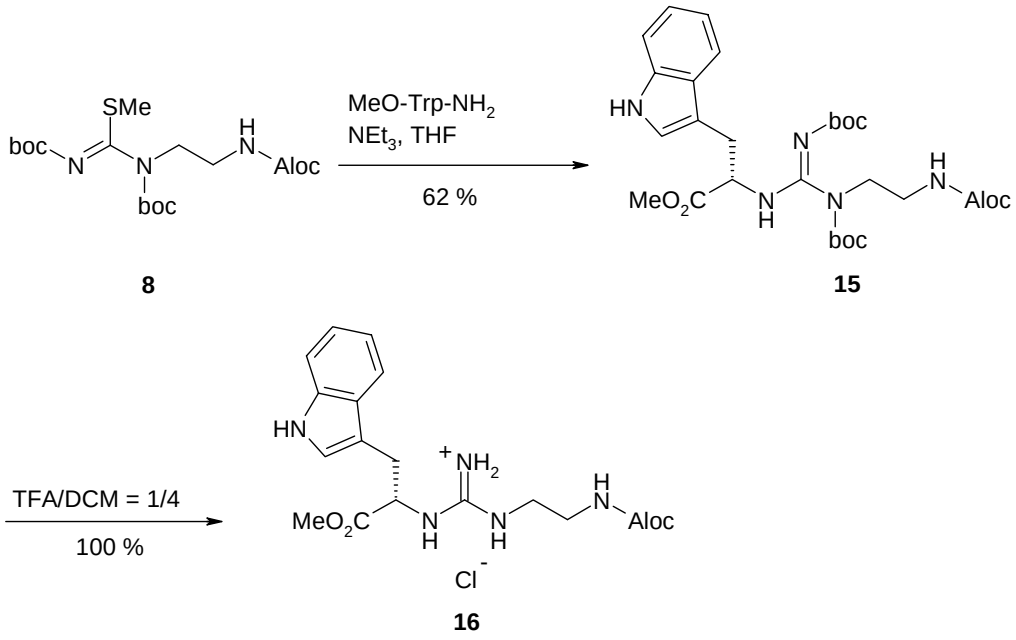
Scheme 3. Synthesis of the guanidinium cation **12**

The natural amino acids Tyr and Trp show emission around 303 nm (excitation 274 nm) and 350 nm (280 nm). The conversion of the isothiourea with these amino acids should lead to simple fluorescent guanidinium compounds. The preparation of the tyrosine-guanidinium compound **13** follows the same reaction procedure shown in Scheme 3. The MeO-Tyr-NH_2 gave lower yields than the MeO-Gly-NH_2*HCl, 56 % compared to 75 % with glycine (Scheme 4).



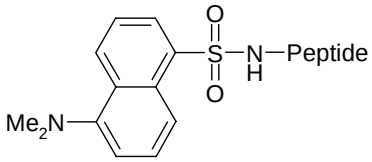
Scheme 4. Synthesis of the tyrosine-guanidinium compound **14**

As a second fluorescent guanidinium compound we have synthesized the tryptophan-guanidinium **16** (Scheme 5).



Scheme 5. Synthesis of the tryptophan-guanidinium compound **16**

The dansyl (5-dimethylaminonaphthalene-sulfonyl) group **9** is often used in peptide chemistry as a fluorescence-active group (fluorophore) (Figure 3).[15]

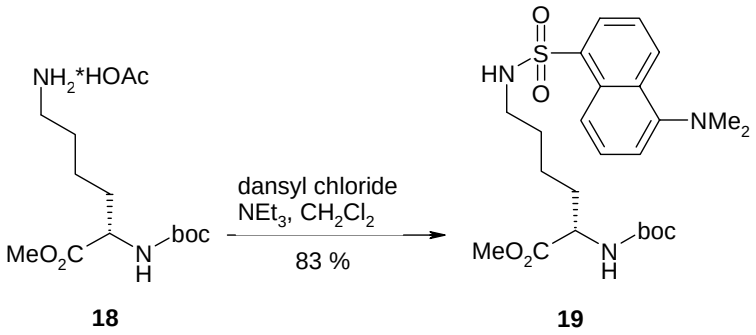


17

absorption: 330 nm
emission: 518 nm

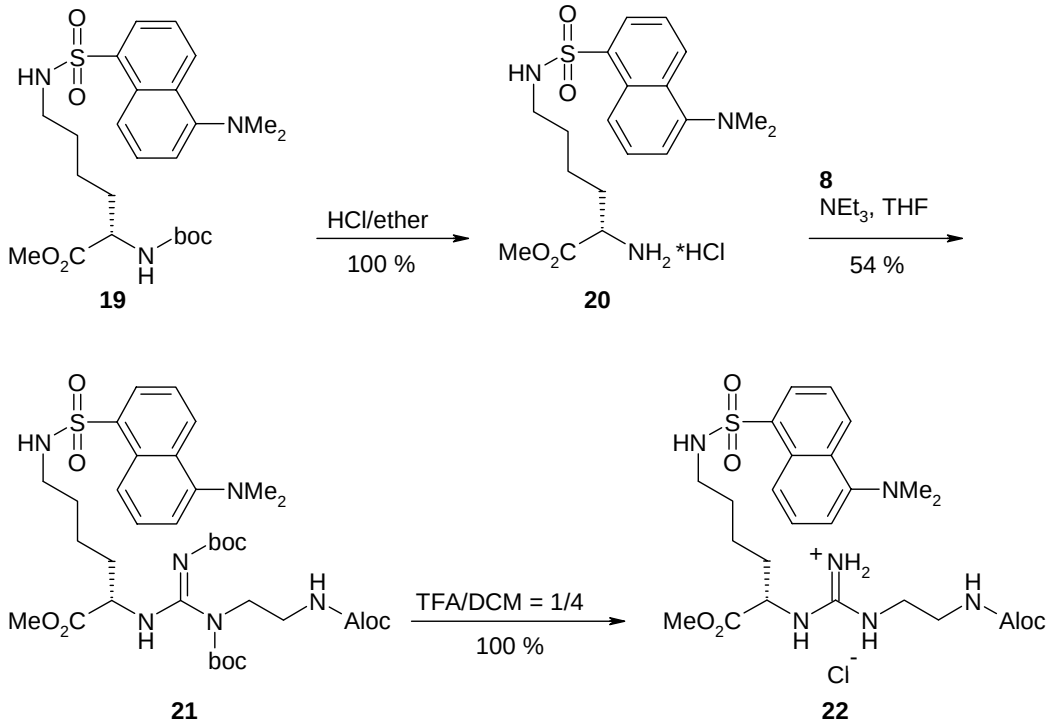
Figure 6. Dansyl group at the amino-end of a peptide **17**

The synthesis of fluorescent guanidinium compound started with the introduction of the dansyl group to the amino side chain of lysine (Scheme 6).



Scheme 6. Introduction of the dansyl group to get the lysine derivative **19**

In the next step the Boc-group was deprotected in HCl saturated ether in quantitative yields to obtain the ammonium chloride **20**, which was then transformed into the guanidinium compound **22** in good yields (Scheme 7).



Scheme 7. Synthesis of the dansyl guanidinium compound **22**

2.3 Spectroscopic Investigation of Fluorescent Guanidines

The emission quantum yields of the fluorescent guanidines were measured in different solvents with quinine bisulfate as reference. The effect of solvent on emission is similar for all compounds showing decreasing quantum yields with higher polarity. Table 1 summarizes the results.

Entry	DMSO	MeOH	H_2O
14	13	13	10
16	18	14	13
22	15	14	7

Table 1. Relative quantum yields of guanidines in %. Quinine bisulfate was used as standard; concentration: $5*10^{-5}$

The relative quantum yields of the fluorescent guanidines are in all cases lower compared to the quantum yields of the amino acids.[16] Figure 7 shows the emission spectrum of **14** in H_2O with an emission maximum at 304 nm (Excitation 274 nm).

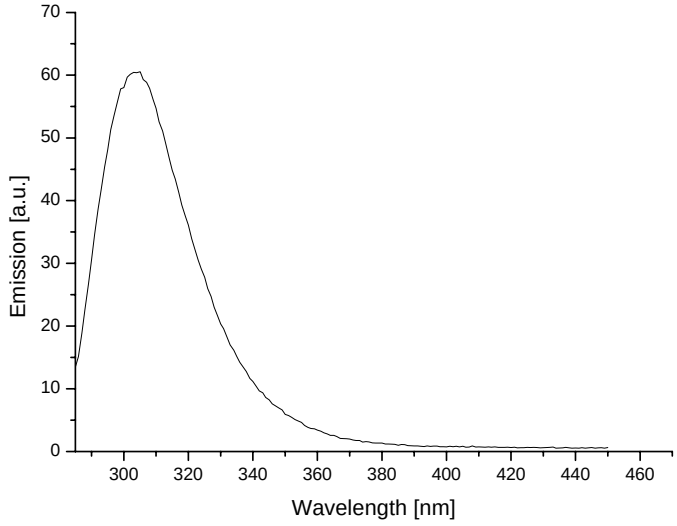


Figure 7. Emission spectra of **14** in a $1*10^{-4}$ M solution in H_20

Figure 8 shows the emission spectrum of **16** in MeOH with an emission maximum at 344 nm (Excitation 280 nm).

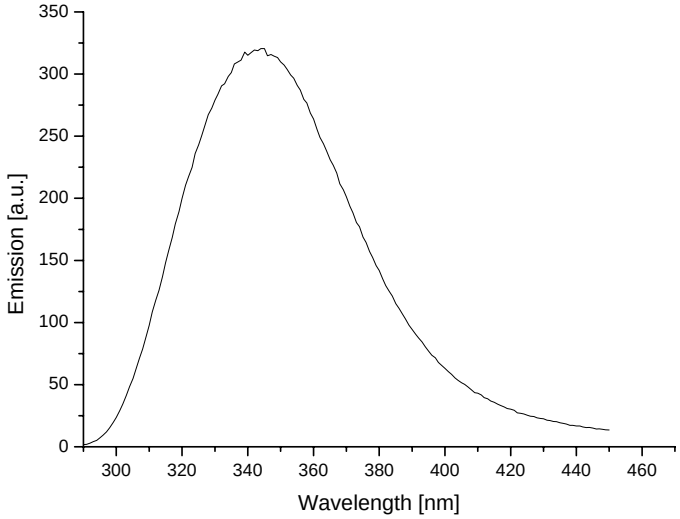


Figure 8. Emission spectra of **16** in a $5*10^{-5}$ M solution in MeOH

Figure 9 shows the emission spectrum of **22** in MeOH with an emission maximum at 518 nm (Excitation 330 nm).

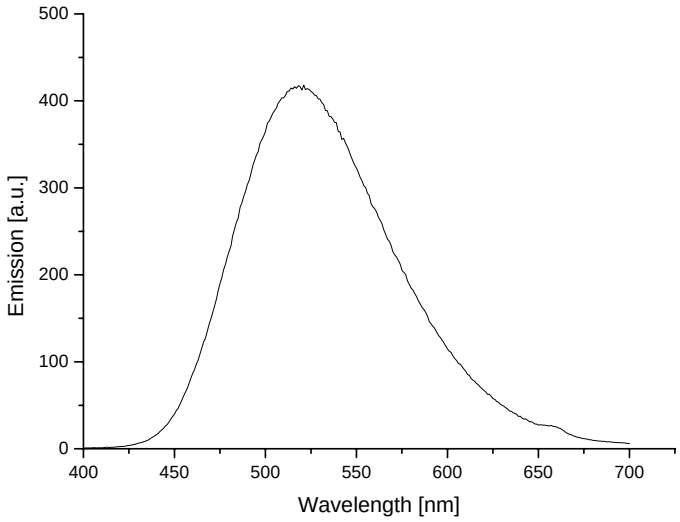


Figure 9. Emission spectra of **22** in a $5*10^{-5}$ M solution in MeOH

The binding affinities of the fluorescent guanidines **14**, **16** and **22** to carboxylates were determined by fluorescence titrations in DMSO, MeOH and H_2O. Binding constants were determined by non-linear fitting of the decrease of emission intensity. The binding constants of the tyrosine guanidine **14** and the tryptophan guanidine **16** are identical. This means that the fluorophore have no influence on the binding process. Interestingly,

the binding constants of the dansyl guanidine **22** are a little lower compared to the binding constants of **14** and **16**. This could be an indication that the dansyl group is involved in the binding process. An exact answer can not be given within the scope of this work.

Entry	Guanidine	Guest	Solvent	K_a [L/mol][a]
1	**14**	Bu_4NOAc	DMSO	$2.45*10^4$
2	**14**	Bu_4NOAc	MeOH	$5.25*10^5$
3	**14**	NaOAc	H_2O (Tris, pH 7.0	$7.94*10^3$
4	**14**	Boc-Gly-OH	H_2O (Tris, pH 7.0	$7.88*10^3$
5	**14**	Boc-Ala-OH	H_2O (Tris, pH 7.0	$7.91*10^3$
6	**14**	Boc-Val-OH	H_2O (Tris, PpH 7.0	$7.91*10^3$
7	**16**	Bu_4NOAc	DMSO	$2.42*10^4$
8	**16**	Bu_4NOAc	MeOH	$5.36*10^5$
9	**16**	NaOAc	H_2O (Tris, pH 7.0	$7.90*10^3$
10	**22**	Bu_4NOAc	DMSO	$2.19*10^4$
11	**22**	Bu_4NOAc	MeOH	$5.02*10^5$
12	**22**	NaOAc	H_2O (Tris, pH 7.0	$7.76*10^3$

[a] All binding constants have errors of approx. ± 6 %

Table 2. Binding constants of different luminescent guanidines. The binding constants were determined by adaption to a model of a 1:1 complex. The buffer concentration is 50 mM.

Figure 10 shows the fluorescence titration of a $2.02*10^{-4}$ M solution of guanidinium compounds **14, 16** and **22** with Bu_4NOAc in DMSO (Entry 1,7 and 9 in Table 2).

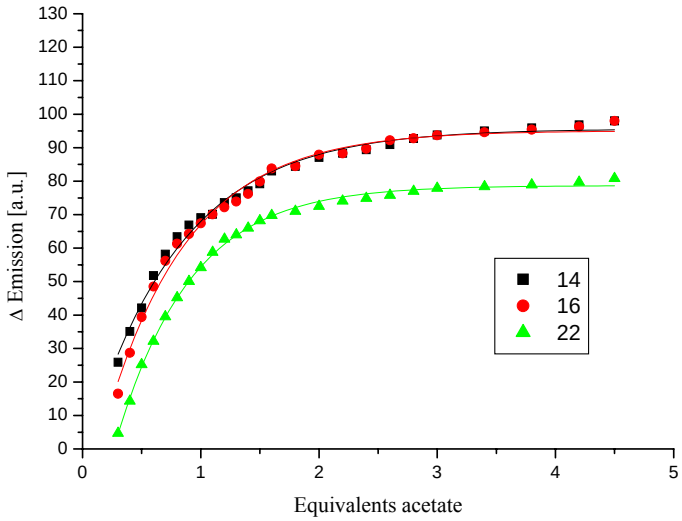


Figure 10. Fluorescence titrations of a $2.02*10^{-4}$ M solution of guanidinium compounds **14** (black), **16 (**red) and **22** (green) with Bu_4NOAc in DMSO

The titration curves show saturation after the addition of 2.0 equivalents of acetate. The Job´s plot analysis of the binding process shows a maximum at x = 0.5, confirming a 1:1 binding motif of the guanidinium compound **14** in DMSO (Figure 11).

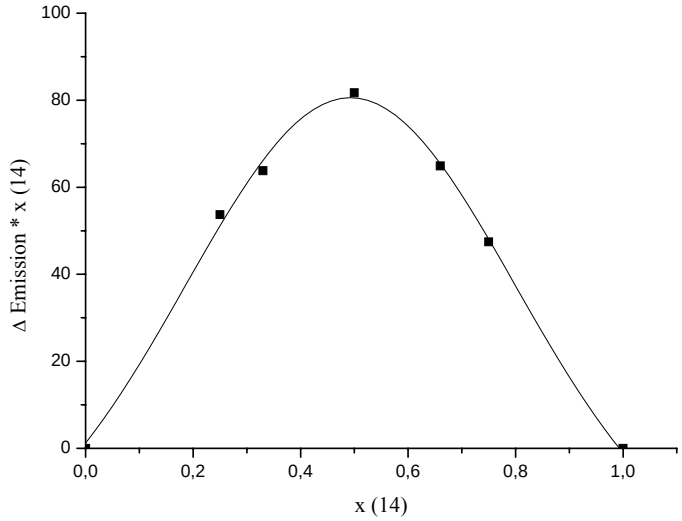


Figure 11. Job´s plot of **14** and Bu_4NOAc in DMSO

Figure 12 shows the fluorescence titration of a $2.45*10^{-4}$ M solution of guanidinium compounds **14, 16** and **22** with Bu_4NOAc in MeOH (Entry 2,8 and 10 in Table 2).

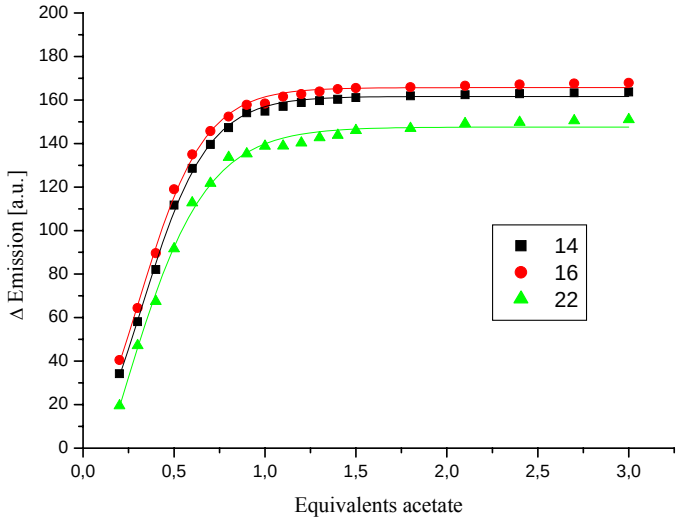


Figure 12. Fluorescence titrations of a $2.45*10^{-4}$ M solution of guanidinium compounds **14 (**black), **16** (red) and **22** (green) with Bu_4NOAc in MeOH

The titration curves show a saturation a fter the addition of 1.2 equivalents of acetate. The Job´s plot analysis shows a maximum at x = 0.5, confirming a 1:1 binding motif of the guanidinium compound **14** in MeOH (Figure 13).

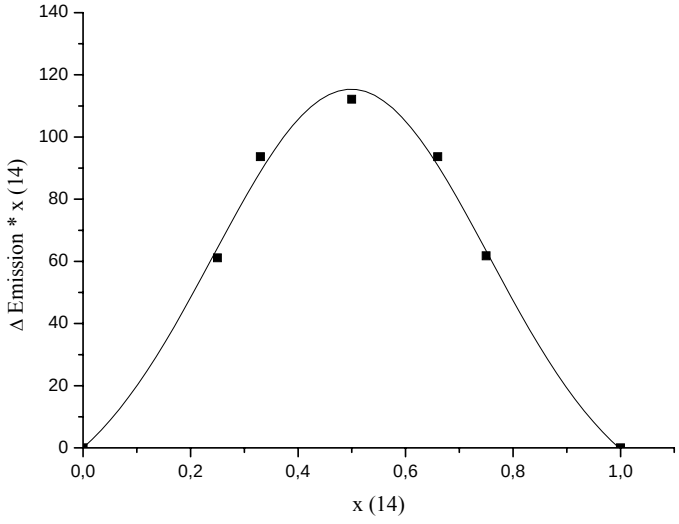


Figure 13. Job´s plot of **14** and Bu_4NOAc in MeOH

Figure 14 shows the fluorescence titration of a $2.57*10^{-4}$ M solution of guanidinium **14** with different carboxylates in H_2O (Tris, pH = 7.0, 50 mM).

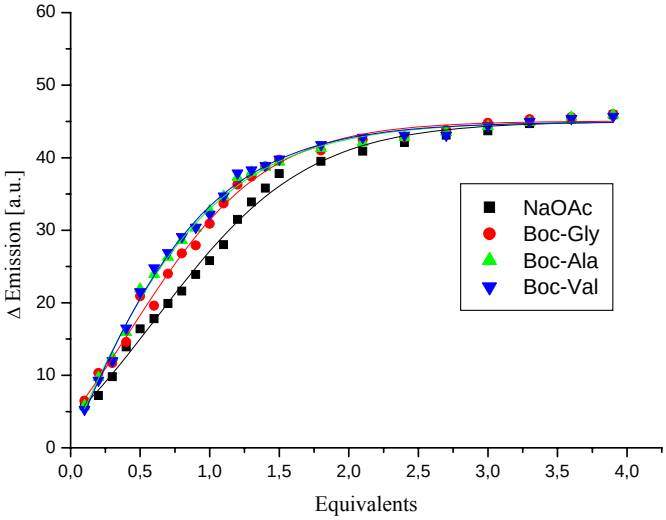


Figure 14. Fluorescence titrations of a $2.57*10^{-4}$ M solution of guanidinium **14** with different carboxylates

Figure 15 shows the fluorescence titration of a $2.57*10^{-4}$ M solution of guanidinium compounds **14, 16** and **22** with NaOAc in H_2O (Tris, pH = 7.0, 50 mM).

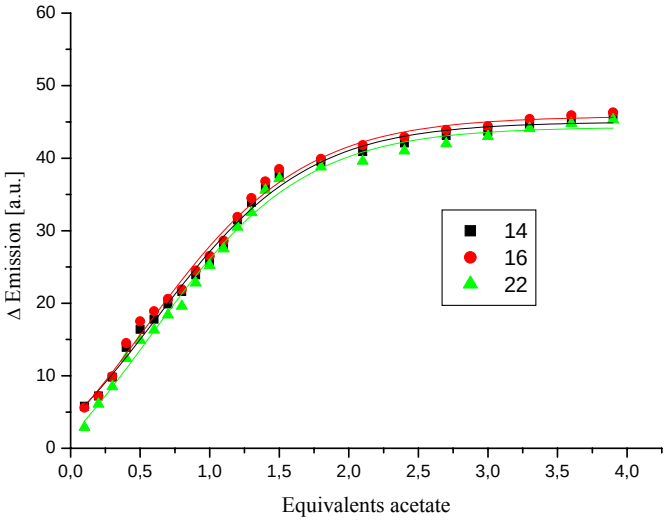


Figure 15. Fluorescence titrations of a $2.57*10^{-4}$ M solution of guanidinium compounds **14 (**black), **16** (red) and **22** (green) with NaOAc in H_2O

The Job´s plot analysis of the binding process shows a maximum at x = 0.5, confirming a 1:1 binding motif of the guanidinium compound **14** in H_2O (Tris, pH = 7.0, 50 mM) (Figure 16).

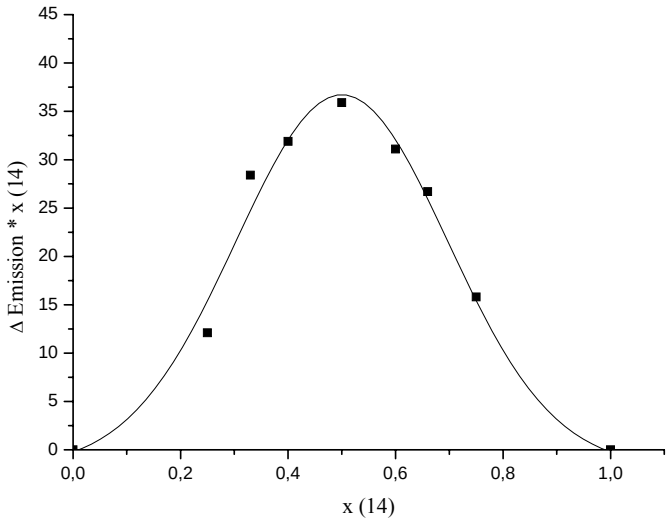
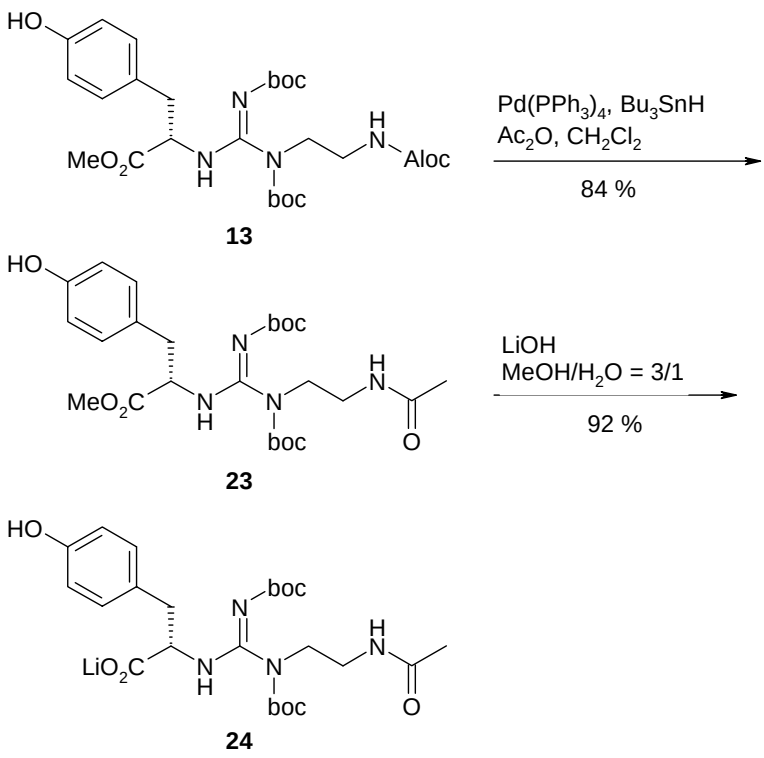


Figure 16. Job´s plot of **14** and NaOAc in H_2O (Tris, pH = 7.0, 50 mM).

2.4 Synthesis of Dimers

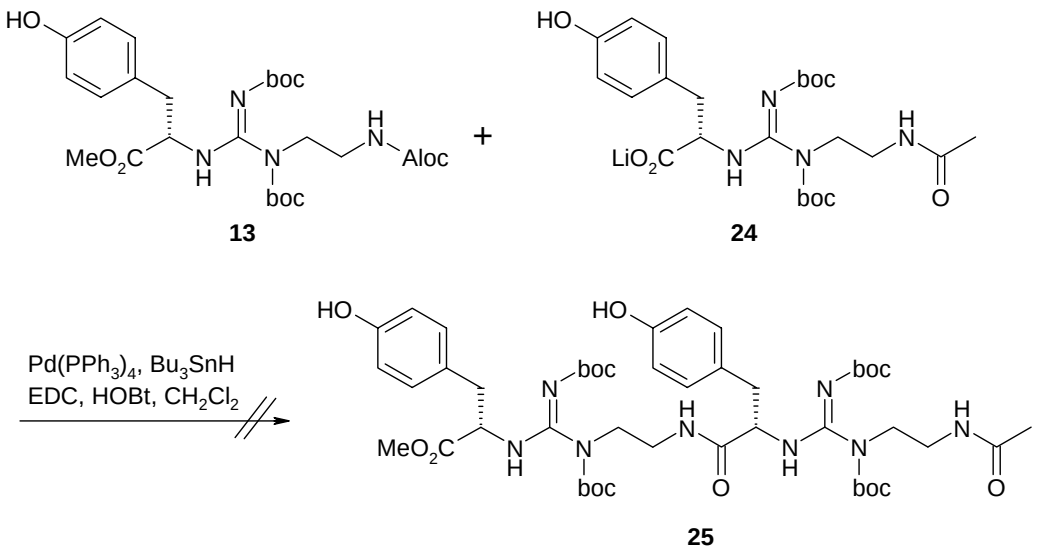
We have developed fluorescent guanidinium amino acids with a simple synthesis in two steps and good binding properties in DMSO, MeOH and water. By connection of two of these guanidines with peptide coupling methods, it should be possible to create systems, which can bind to aspartate or glutamate residues in the side chain of peptides. The binding properties could be examined by fluorescence spectroscopy.

We started our synthesis with the transformation of the tyrosine guanidine **13** to the acylated guanidine **23** (Scheme 8). The acylated compound was obtained from Aloc-protected guanidine **13** by direct transacylation, without necessitating the removal of the amine-protective group in an initial, separate step. Compound **7** was reacted with a catalytic amount (5 mol-%) of $Pd(PPh_3)_4$ and 1.1 eq of Bu_3SnH in the presence of 2.5 eq of acetic anhydride, which furnished after 30 min *N*-acetyl-guanidine **23** in 84 % yields. The product was purified by colomn chromatography.



Scheme 8. Synthesis of the Li-carboxylate **24**

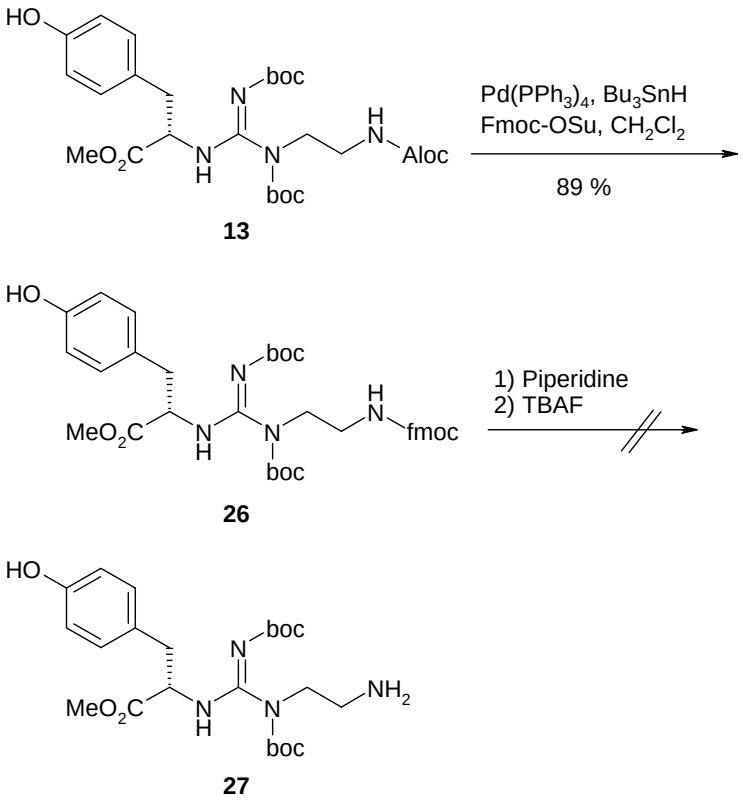
The Bu_3SnH acts as a very fast hydride donor in this process. By reaction of the initially formed π-allylpalladium complex with the tin hydride a tin carbamate and an intermediate palladium hydride species is formed. The hydride is then transformed via the metal to the allyl cation to release propene. In the next step we tried to couple the Li-salt **24** with the Aloc-protected guanidine **13** via a transprotection reaction (Scheme 9). Generally speaking, the coupling of two peptide fragments requires three separate reaction steps, namely: i) deprotection of the amine nitrogen of the first fragment; ii) activation of the carboxylic moiety of the second fragment and iii) coupling of the two fragments that result from these operations. In some cases, the second and third step can be performed in one pot by activating the carboxylic moiety *in situ* using coupling reagents such as DCC or other carbodiimides. Using the Aloc-group as a protection group for nitrogen, the three peptide bond formation reaction steps may be combined into a single.



Scheme 9. Attempted synthesis of the dimeric guanidine **25**

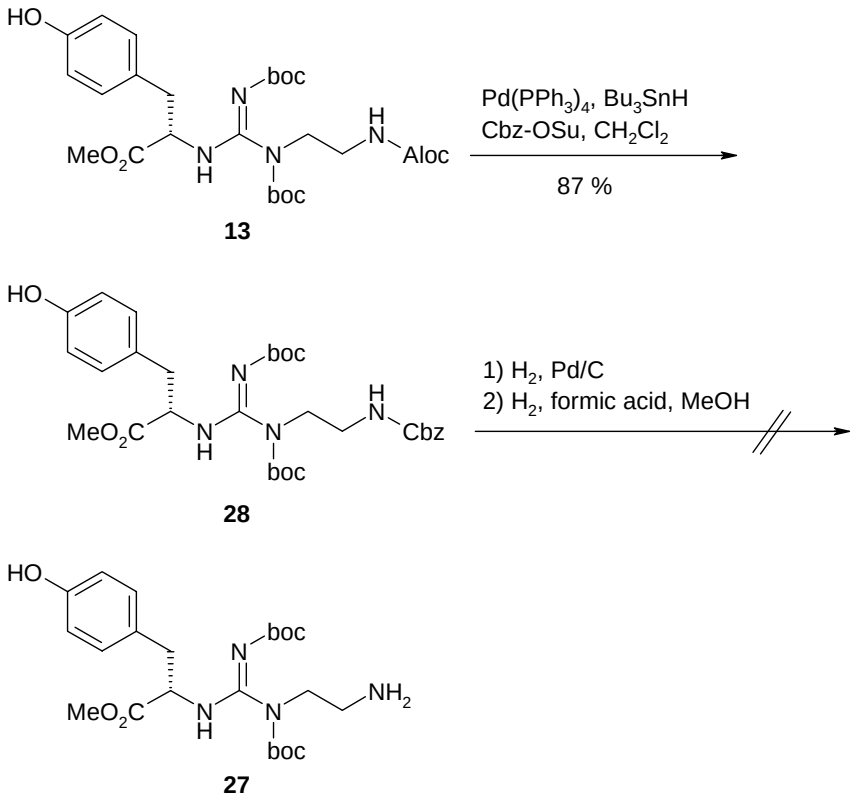
Unfortunately, we were not able to synthesis the dimeric guanidine **25**. The reaction mixture contained neither the starting material **13** nor the activated carboxylic guanidine **24**. One by-product shows in the NMR spectrum the typical AA´BB`-spinsystem of the aromatic ring of the tyrosine and the methyl ester, but characterisation of the compound was not possible.

Variations of the reaction conditions, adding DIPEA as base or using DCC and DIC instead of the EDC, gave also no product formation. After several unsuccessful attempts we changed the strategy for the synthesis of the dimeric guanidine **25**. First we replaced the Aloc-group of the tyrosine guanidine **13** by a Fmoc-protection group. This reaction was done under the same reaction conditions as described above (Scheme 10).



Scheme 10. Synthesis of the Fmoc-protected guanidine **26** and attempted cleavage of the Fmoc-group

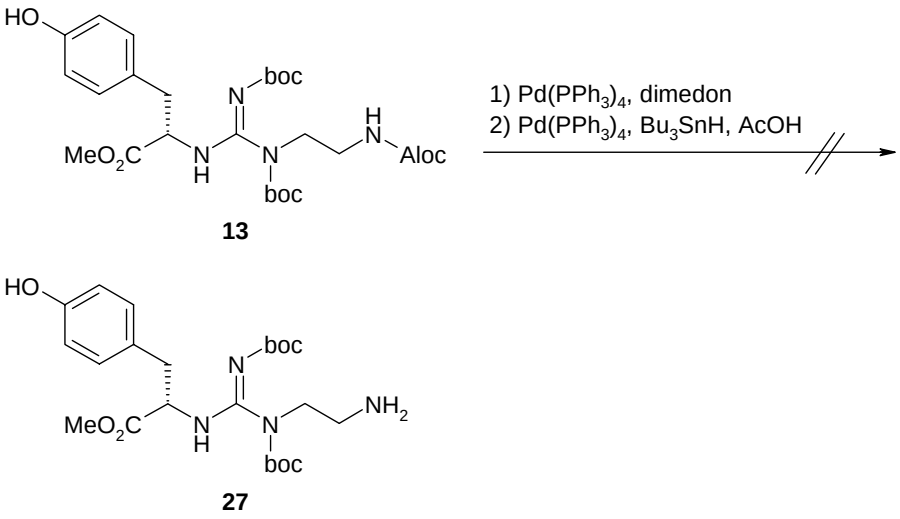
The Fmoc-group of **26** should be cleaved with a solution of 20 % piperidine in DMF or TBAF to give the free amine **27**. Peptide coupling of compound **27** with the Li-salt **24** should then give the dimeric guanidine **25**. Unfortunately, the cleavage of the Fmoc-group was not successful, neither with piperidine in DMF nor with TBAF. The starting material **26** was isolated from the reaction mixture in yields between 70 % and 85 %. Variations in the reaction conditions (longer reaction times, higher temperatures) produced also no product. After these results we tried the replacement of the Aloc-group by a Cbz-group (Scheme 11).



Scheme 11. Synthesis of the Cbz-protected guanidine **28**

Hydrogenolytic cleavage with palladium on charcoal failed. After the addition of a few drops of AcOH, the starting material **28** was isolated in nearly quantitative yields. The addition of 1 eq AcOH led to decomposition of **28**. As a third method we tried the cleavage with palladium on charcoal in MeOH in the presence of formic acid (1 eq). In this case the starting material **28** was isolated from the reaction mixture in nearly quantitative yields.

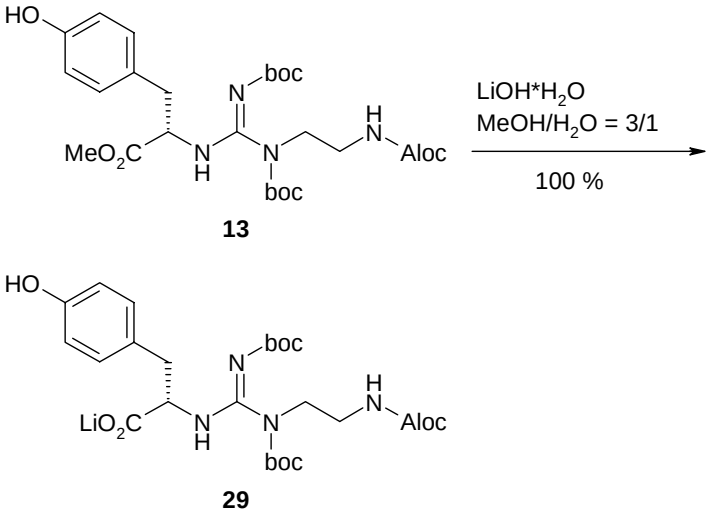
Kunz and co-workers have reported about the Aloc deprotection from a late step in the *Kunz-Unverzagt* synthesis of a fucosyl-chitobiose glycopeptide which constitutes a partial sequence of a viral envelope protein.[17] By simple treating of the complex trisaccharide-asparagine conjugate in THF at r.t. with 8 equivalents of dimedone (5,5-dimethyl-1,3-cyclohexanedione) in the presence of 10 mol% of a Pd(0) catalyst, the amino group in the asparagine moiety was liberated in 92 % yield without detriment to the labile *O*-glycosidic link. *Guibe* and co-workers have reported about the cleavage of the Aloc-group using Bu_3SnH, Pd(0) and AcOH.[18]



Scheme 12. Attempted cleavage of the Aloc-group

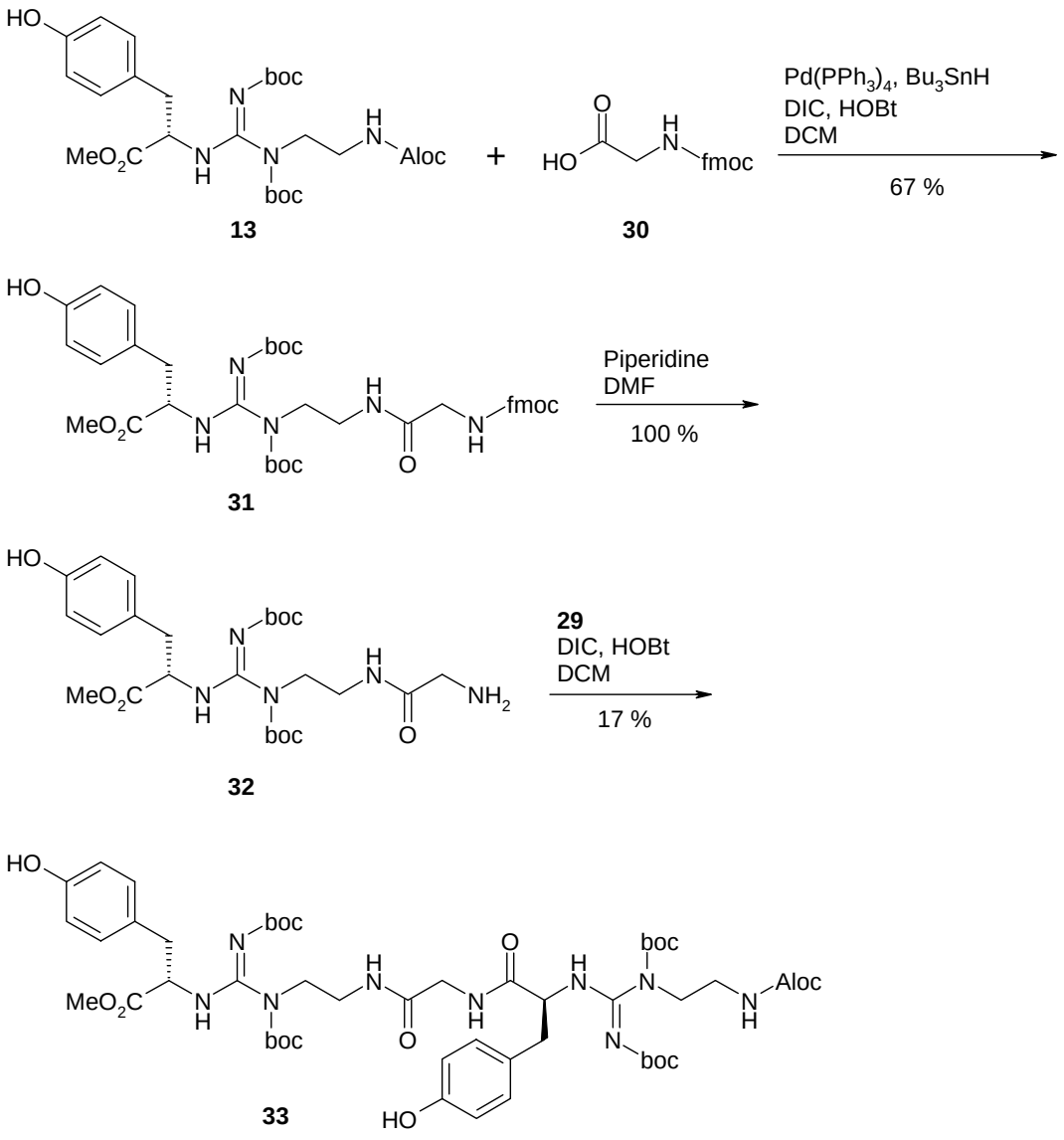
Careful analysis showed of the mass spectrum showed a compound with the molecular mass of 285. A possible explanation of this molecular ion is a cyclisation of the free amino group to give a 5-membered cyclic guanidine.

Next the synthesis of a glycine-bridged bis-guanidinium compound was attempted. First the methyl ester of compound **13** was cleaved in quantitative yields (Scheme 13).



Scheme 13. Cleavage of the methyl ester

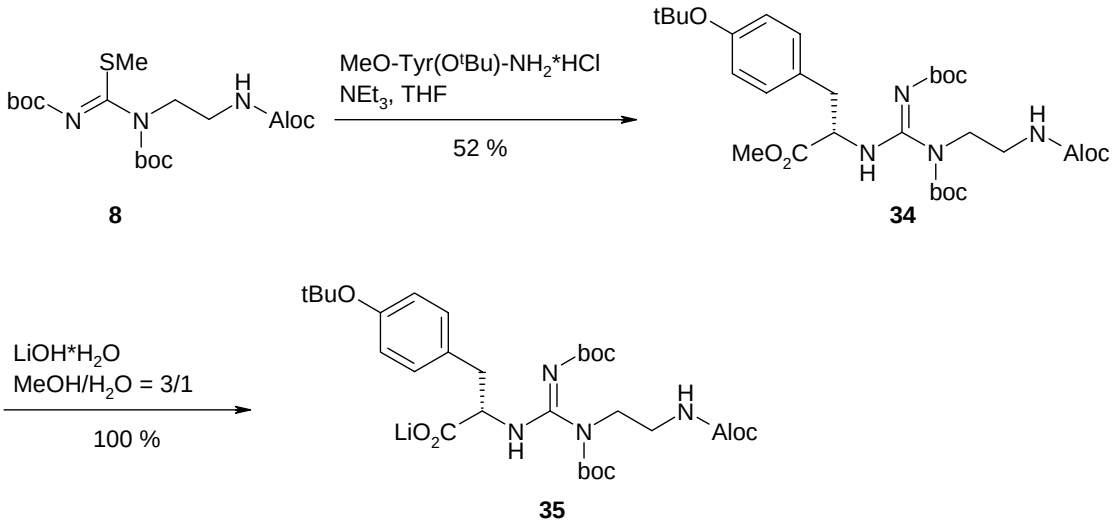
The guanidine **13** was coupled with Fmoc-glycine **30** in 67 % yield. The resulted guanidine **31** was treated with 20 % piperidine in DMF to cleave the Fmoc-group. The reaction progress was monitored by TLC (EtOAc). Compound **32** was used without further purification and gave the resulting dimer **33** in 17 % yield (Scheme 14).



Scheme 14. Synthesis of the dimer **33**

2.5 Solid Phase Synthesis

The first step in preparation for a SPPS protocol was the synthesis of a full protected dipeptide **35** (Scheme 15).

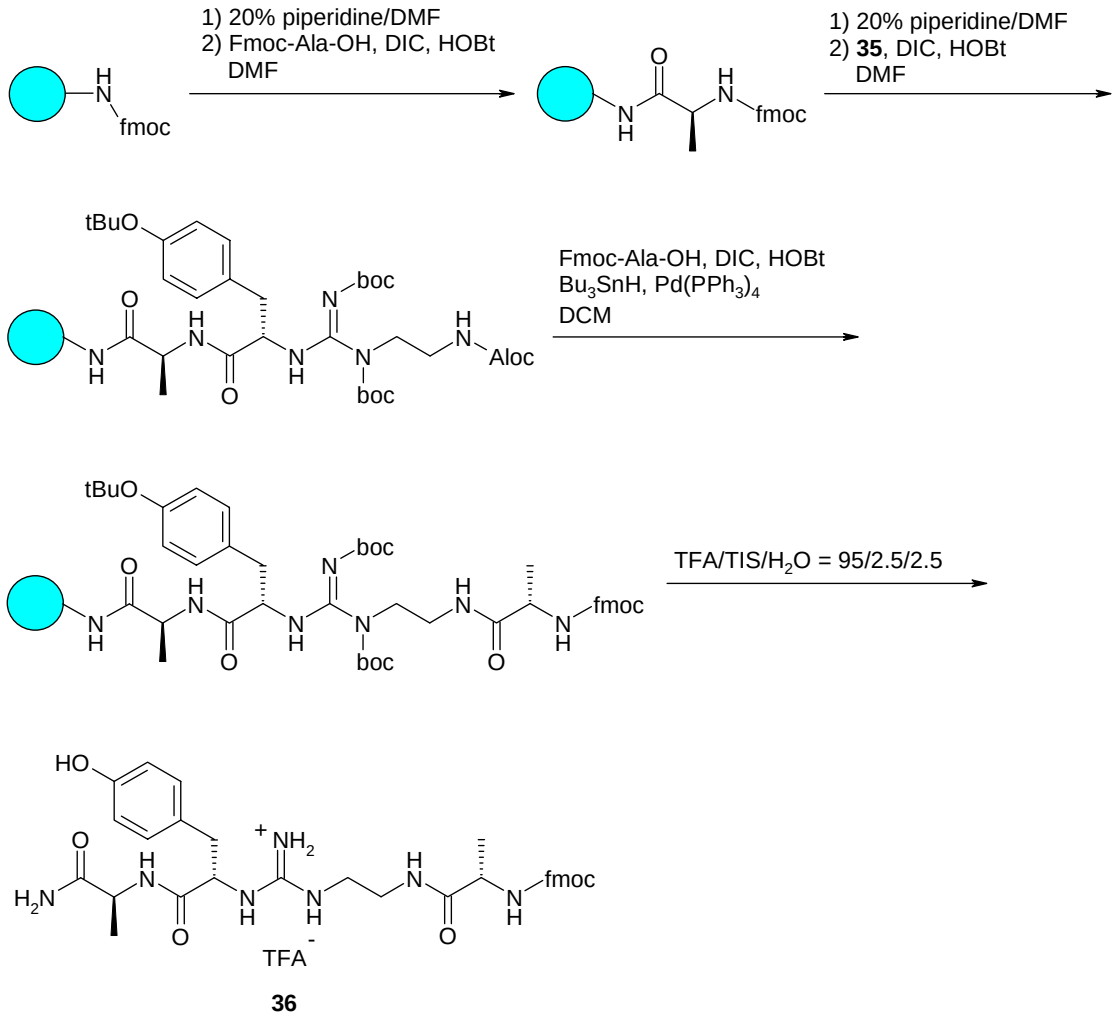


Scheme 15. Synthesis of the Li-carboxylate **35**

The procedure for the solid phase synthesis is shown in Scheme 16.

The Fmoc-protected Rink amide MBHA resin was first treated with 20 % piperidine/DMF to cleavage the Fmoc-group. In the first coupling step Fmoc-Ala-OH was activated with DIC/HOBt and added to the resin. In the second step the Fmoc-group was cleaved under basic conditions and the activated guanidine **35** was added to the resin. In the third step Fmoc-Ala-OH, DIC and HOBt were dissolved in DCM, Bu_3SnH and Pd(0) were added and the solution was given to the resin. In the final step the peptide was cleaved from the resin.

The exact procedure is described in the experimental part.



Scheme 16. Synthesis of the peptide **36** on the solid phase

The careful analysis of the product showed as the main product the dipeptide H_2N-Ala-Ala-Fmoc. The desired peptide **36** could be detected in the mass spectrum. Other fragments could not be found in the mass spectrum. The solid phase synthesis did not work well and gave the desired peptide **36** only in very low yields. An explanation could be that the coupling of the Li-carboxylate **35** did not work very well.

2.6 Conclusion

We have described the synthesis of luminescent guanidino amino acids (GuAA´s) **14**, **16** and **22** as new synthetic receptor building blocks for molecular recognition of carboxylates.

The binding constants were determined by fluorescence titrations in different solvents.
The binding constants are in MeOH in the range of $5*10^5$ M^{-1}, in DMSO $2*10^4$ M^{-1} and in H_2O (pH = 7.0) in the range of $7*10^3$ M^{-1}.
The stoichiometry of all binding processes is 1:1 and was determined by Job`s plot analysis.

The synthesis of a tripeptide **33** containing two GuAA molecules gave the target compound in 17 % yield.

A first attempt to prepare a short GuAA containing peptide on solid phase was not successful.

2.7 Experimental Section

Melting points (mp) were determined with a Büchi SMP 20 and are uncorrectd.

IR-spectra were recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

NMR : Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K), Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [pp] relative external standards (solvent residual peak). The spectra were analysed by first order, the coupling constants are in Hertz [Hz]. Characterisation of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet. Integration is determined as the relative number of atoms. Error of reported values: 0.01 ppm for ^{1}H-NM, 0.1 ppm for ^{13}C-NMR; coupling constants: 0.1 Hz. The used solvent is reported for each spectrum.

MS-Spectra: Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

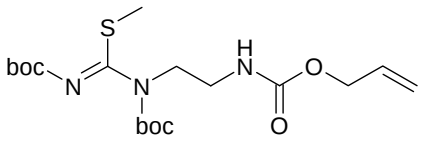
Elemental Analysis: Microanalytical Laboratory of the University of Regensburg.

Thin Layer Chromatography (TLC) was performed on aluminia plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 nm). Visualisation was accomplished byUV-light (λ = 254 nm) and ninhydrine in MeOH.

Column Chromatography was performed on silica gel (70-230 mesh) from Merck.

Optical rotation was measured on a Perkin Elmer Polarimeter 241 with sodium lamp at 589 nm in a specified solvent.

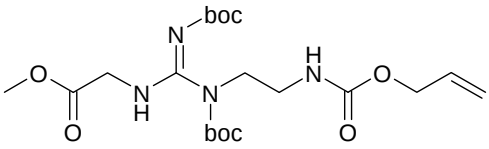
Synthesis



[2-(1,3-Di-(*tert*-butoxycarbonyl)-2-methyl-isothioureido)-ethyl]-carbamic acid allyl ester (8).

Compound **6** (250 mg, 0.222 mL, 1.72 mmol), compound **7** (500 mg, 1.72 mmol) and PPh_3 (677 mg, 2.58 mmol) were dissolved in 25 mL THF and DIAD (522 mg, 0.500 mL, 2.58 mmol) was added. The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to obtain **8** (552 mg, 1.32 mmol, 78 %) as a colourless oil. R_f (EtOAc) = 0.35

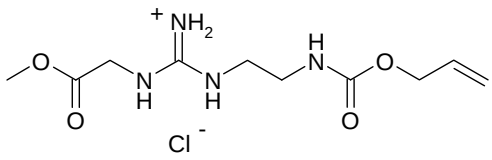
IR (KBr) [cm^{-1}]: 3256, 3178, 2979, 2912, 1721, 1656, 1511, 1455, 1378, 1166, 987, 845, 756. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.48 (s, 9 H, Boc), 2.38 (s, 3 H, SMe), 3.30 – 3.39 (m, 2 H, -C*H*$_2$-NH), 3.78 – 3.86 (m, 2 H, NBoc-C*H*$_2$), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.83 – 5.97 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.22 (bs, 1 H, -N*H*-Aloc). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.5 (+), 14.2 (+), 28.0 (+), 28.1 (+), 28.2 (+), 28.4 (+), 42.4 (-), 42.8 (-), 60.4 (C_{quat}), 67.3 (-), 68.3 (-), 80.1 (C_{quat}), 83.4 (C_{quat}), 131.4 (C_{quat}), 165.2 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 418.2 (100) [MH^+], 318.1 (15) [MH^+ - Boc], 218.0 (20) [MH^+ - 2 Boc]. - Elemental analysis calcd. (%) for $C_{18}H_{31}N_3O_6S$ (417.19): C 51.78, H 7.48, N 10.06; found C 51.69, H 7.68, N 9.80.



[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´*,*N´´*-di-(*tert*-butoxycarbonyl)-guanidino]-acetic acid methyl ester (11).

Compound **8** (504 mg, 1.21 mmol) was dissolved in 20 mL THF. After addition of Gly-OMe (456 mg, 3.63 mmol) and NEt_3 (367 mg, 0.503 mL, 3.63 mmol) the reaction mixture was refluxed for 15 h. The solid was filtered off and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **11** (403 mg, 0.91 mmol, 75 %) as a white solid. R_f (EtOAc) = 0.65

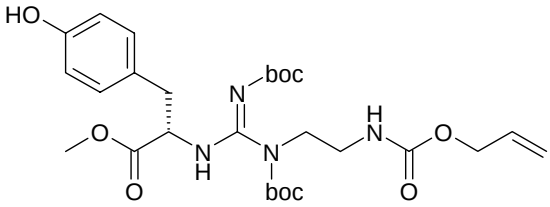
mp: 95 °C. - IR (KBr) [cm^{-1}]: 3245, 3009, 2079, 2921, 2856, 1718, 1645, 1434, 1367, 1123, 956. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 9 H, Boc), 1.48 (s, 9 H, Boc), 3.21 – 3.59 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.78 (s, 3 H, CO_2Me), 4.06 (d, J = 5.2 Hz, 2 H, CO-C*H*$_2$-NH), 4.54 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.19 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.30 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.83 – 5.97 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.87 (bs, 1 H, -N*H*-Aloc), 8.58 (bs, 1 H, N*H*). - ^{13}C- NMR (75 MHz, $CDCl_3$): δ = 28.0 (+), 28.1 (+), 28.2 (+), 28.4 (+), 40.6 (-), 44.9 (-), 47.5 (-), 52.6 (+), 65.4 (-), 79.9 (C_{quat}), 83.4 (C_{quat}), 117.4 (-), 117.8 (C_{quat}), 133.1 (-), 156.7 (C_{quat}), 169.3 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 481.3 (15) [MNa^+], 459.3 (100) [MH^+], 403.2 (5) [MH^+ - C_4H_8], 359.2 (10) [MH^+ - Boc]. – $C_{20}H_{34}N_4O_8$ (458.24).



[*N´*-(2-Allyloxycarbonylamino-ethyl)-guanidino]-acetic acid methyl ester hydrochloride (12).

Compound **11** (403 mg, 0.91 mmol) was dissolved in 15 mL DCM/TFA = 4/1 and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 10 mL 1 N HCl. After freeze drying compound **12** (0.91 mmol) was obtained as a white solid.

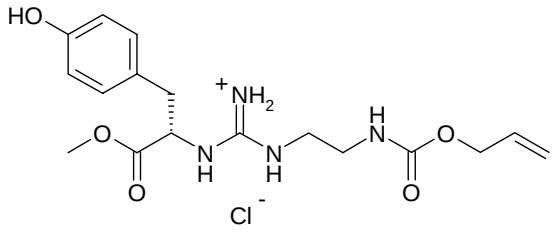
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.39 – 3.68 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 3.87 (s, 3 H, CO_2Me), 4.11 (d, J = 5.0 Hz, 2 H, CO-C*H*$_2$-NH), 4.54 (d, J = 5.2 Hz, 2 H -C*H*$_2$-CH), 5.21 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.32 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.83 – 5.97 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.88 (bs, 1 H, -N*H*-Aloc), 8.98 (bs, 2 H, 2 x N*H*), 9.34 (bs, 1 H, N*H*), 10.67 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 259.3 (100) [MH^+]. – $C_{10}H_{19}N_4O_4$ (259.14).



(S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester (13).

Compound **8** (820 mg, 1.96 mmol) and MeO-Tyr-NH_2 (574 mg, 2.94 mmol) were dissolved in 20 mL THF and the reaction mixture was refluxed for 15 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to obtain **13** (621 mg, 1.10 mmol, 56 %) as a pale yellow solid. R_f (EtOAc/hexane = 1/1) = 0.40

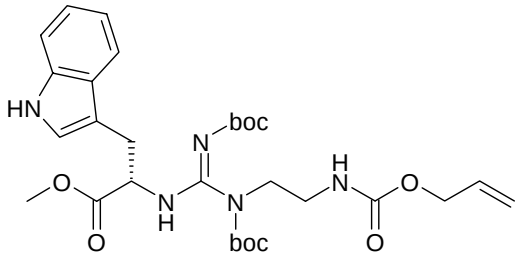
mp: 112 °C. – $[\alpha]^{20}_D$ = - 13.5 (c = 0.6 in MeOH). - IR (KBr) [cm^{-1}]: 3374, 3076, 2979, 1720, 1615, 1517, 1448, 1368, 1251, 1147, 1069, 994, 929, 840, 773. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.45 (s, 9 H, Boc), 2.81 – 3.50 (m, 6 H, 3 x C*H*$_2$), 3.78 (s, 3 H, CO_2Me), 4.05 – 4.22 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH_2O), 5.28 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH_2O), 5.83 – 5.97 (m, 1 H, CH_2=C*H*CH_2O), 6.78 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.87 (bs, 1 H, -N*H*-Aloc), 6.99 (d, J = 8.0 Hz, 2 H, Ar-*H*), 10.12 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.0 (+), 28.1 (+), 40.2 (-), 52.6 (+), 65.5 (-), 80.2 (C_{quat}), 116.0 (C_{quat}), 117.5 (-), 127.1 (C_{quat}), 130.4 (+), 133.0 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 565.4 (100) [MH^+], 509.3 (20) [MH^+ - C_4H_8], 465.4 (25) [MH^+ - Boc]. – HRMS ($C_{27}H_{41}N_4O_9$): calcd. 565.2874, found 565.2879 ± 0.02 ppm.



(S)-2-[*N´*-(2-allyloxycarbonylamino-ethyl)-guanidino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester hydrochloride (14).

Compound **13** (621 mg, 1.10 mmol) was dissolved in 15 mL DCM/TFA = 4/1 and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 10 mL 1 N HCl. After freeze drying compound **14** (440 mg, 1.10 mmol, 100 %) was obtained as a yellow solid.

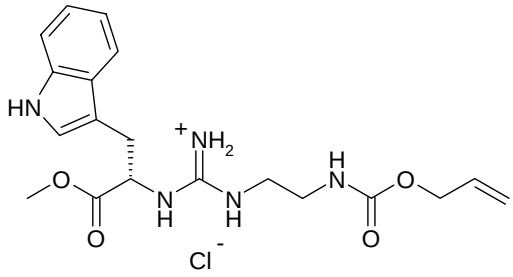
IR (KBr) [cm^{-1}]: 3340, 3197, 1696, 1636, 1516, 1444, 1352, 1265, 1170, 1107, 933, 833. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.76 – 3.21 (m, 6 H, 3 x C*H*$_2$), 3.78 (s, 3 H, CO_2Me), 4.05 – 4.22 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.28 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.83 – 5.97 (m, 1 H, CH$_2$=C*H*CH$_2$O),), 6.75 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.99 (d, J = 8.0 Hz, 2 H, Ar-*H*), 7.23 (bs, 1 H, -N*H*-Aloc), 7.61 –7.98 (m, 3 H, 3 x N*H*), 9.39 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 31.2 (C_{quat}), 36.4 (-), 39.2 (-), 39.6 (+), 40.7 (-), 48.5 (+), 52.3 (+), 55.1 (C_{quat}), 64.4 (-), 115.1 (+), 117.0 (-), 125.7 (C_{quat}), 130.1 (+), 133.5 (+), 155.5 (C_{quat}), 170.6 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 365.1 (100) [MH^+]. – $C_{17}H_{25}N_4O_5Cl$ (400.15).



(S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´,N´´*-di-(*tert*-butoxycarbonyl)-guanidino]-3-(1H-indol-3-yl)-propionic acid methyl ester (15).

Compound **8** (1.44 g, 3.45 mmol) and MeO-Trp-NH_2 (1.32 g, 5.18 mmol) were dissolved in 20 mL THF and the reaction mixture was refluxed for 15 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to obtain **15** (1.26 g, 2.14 mmol, 62 %) as a orange oil. R_f (EtOAc/hexane = 1/1) = 0.45

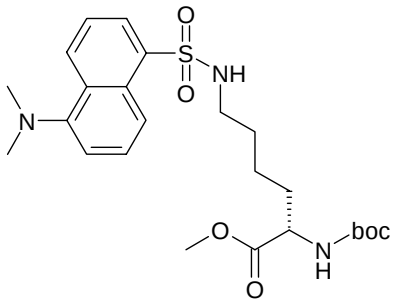
$[\alpha]^{20}_D$ = - 24.7 (c = 0.6 in MeOH). - IR (KBr) [cm^{-1}]: 3388, 3059, 2978, 1716, 1618, 1533, 1437, 1368, 1249, 1147, 1064, 1011, 928, 743. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.46 (s, 9 H, Boc), 2.81 – 3.50 (m, 6 H, 3 x C*H*$_2$), 3.78 (s, 3 H, CO_2Me), 4.30 – 4.48 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.18 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH_2O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH_2O), 5.81 – 5.94 (m, 1 H, CH_2=C*H*CH_2O), 6.97 – 7.21 (m, 4 H, Ar-*H*/N*H*), 7.33 (d, J = 8.0 Hz, 1 H, Ar-*H*), 7.55 (d, J = 8.0 Hz, 1 H, Ar-*H*), 8.36 (bs, 1 H, N*H*), 10.17 (bs, 1 H, N*H*). - ^{13}C- NMR (75 MHz, $CDCl_3$): δ = 28.0 (+), 28.1 (+), 28.2 (+), 28.4 (+), 40.1 (-), 52.4 (C_{quat}), 52.6 (-), 65.3 (-), 82.9 (C_{quat}), 109.6 (C_{quat}), 111.5 (+), 117.3 (-), 119.5 (+) 122.4 (+), 122.9 (+), 123.4 (+), 133.3 (C_{quat}), 136.2 (C_{quart}), 152.7 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 588.4 (100) [MH^+], 532.3 (10) [MH^+ - C_4H_8], 488.3 (15) [MH^+ - Boc]. $C_{29}H_{41}N_5O_8$ (587.29).



(S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)guanidino]-3-(1H-indol-3-yl)-propionic acid methyl ester hydrochloride (16).

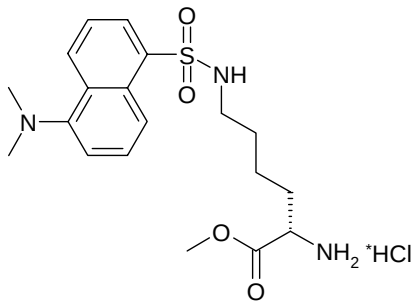
Compound **15** (1.26 g, 2.14 mmol) was dissolved in 15 mL DCM/TFA = 4/1 and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 10 mL 1 N HCl. After freeze drying compound **16** (906 mg, 2.14 mmol, 100 %) was obtained as a orange solid.

IR (KBr) [cm^{-1}]: 3327, 1697, 1635, 1527, 1341, 1261, 1151, 932, 745. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.91 – 3.34 (m, 6 H, 3 x C*H*$_2$), 3.78 (s, 3 H, CO_2Me), 4.30 – 4.48 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.18 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.81 – 5.94 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.97 – 7.21 (m, 4 H, Ar-*H*/N*H*), 7.33 (d, J = 8.0 Hz, 1 H, Ar-*H*), 7.55 (d, J = 8.0 Hz, 1 H, Ar-*H*), 7.59 – 7.79 (m, 3 H, 3 x N*H*), 7.93 (bs, 1 H, N*H*), 9.19 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 388.2 (100) [MH^+]. – $C_{19}H_{26}N_5O_4Cl$ (423.17).



(S)-6-(5-Dimethylamino-naphthalene-1-sulfonylamino)-2-(*tert*-butoxycarbonyl)amino-hexanoic acid methyl ester (19).

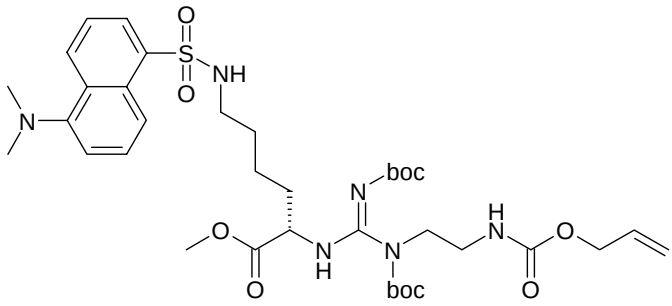
Compound **18** (400 mg, 1.54 mmol) was dissolved in 14 mL DCM. After addition of dansyl chloride (415 mg, 1.54 mmol) and NEt_3 (467 mg, 0.640 mL, 4.62 mmol), the solution was stirred at room temperature for 15 h. After filtration the filtrate was evaporated. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **19** (631 mg, 1.28 mmol, 83 %) as a yellow solid. R_f (EtOAc) = 0.55 mp: 108 °C. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.31 – 1.73 (m, 15 H, Boc/3 x C*H*$_2$), 2.83 – 2.98 (m, 8 H, 2 x Me/C*H*$_2$), 3.70 (s, 3 H, CO_2Me), 4.10 –4.23 (m, 1 H, C*H*), 4.79 (bs, 1 H, N*H*), 4.95 (d, J = 8.0 Hz, 1 H, N*H*), 7.22 (d, J = 7.7 Hz, 1 H, Ar-*H*), 7.51 – 7.61 (m, 2 H, Ar-*H*), 8.21 – 8.34 (m, 2 H, Ar-*H*), 8.58 (d, J = 7.8 Hz, 1 H, Ar-*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.2 (-), 28.3 (+), 29.0 (-), 32.2 (-), 42.9 (-), 45.4 (+), 52.3 (+), 53.0 (+), 115.2 (+), 118.8 (+), 123.2 (+), 128.4 (+), 129.7 (+), 130.4 (+), 134.7 (C_{quat}), 152.0 (C_{quat}), 155.6 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 494.4 (100) [MH^+], 438.3 (20) [MH^+ - C_4H_8], 394.3 (5) [MH^+ - Boc]. - Elemental analysis calcd. (%) for $C_{24}H_{35}N_3O_6S$ (493.22): C 58.39, H 7.15, N 8.52; found C 58.26, H 7.29, N 8.46.



(S)-6-(5-Dimethylamino-naphthalene-1-sulfonylamino)-2-amino-hexanoic acid methyl ester hydrochloride (20).

Compound **19** (631 mg, 1.28 mmol) was dissolved in 8 mL DCM and 15 mL HCl saturated ether was added. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dried under high vacuum. Compound **20** (906 mg, 1.28 mmol, 100 %) was obtained as a yellow solid.

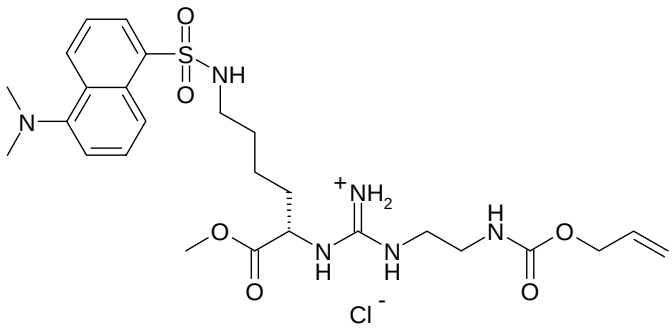
IR (KBr) [cm^{-1}]: 3406, 3192, 2951, 1701, 1637, 1516, 1449, 1437, 1322, 1259, 1143, 794, 586. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.30 – 1.52 (m, 4 H, 2 x C*H*$_2$), 1.61 – 1.72 (m, 2 H, C*H*$_2$), 2.73 – 2.91 (m, 8 H, 2 x Me/C*H*$_2$), 3.72 (s, 3 H, CO_2Me), 3.81 – 3.99 (m, 2 H, C*H*/N*H*) , 7.20 (d, J = 8.0 Hz, 1 H, Ar-*H*), 7.51 – 7.61 (m, 2 H, Ar-*H*), 8.21 – 8.34 (m, 2 H, Ar-*H*), 8.59 (d, J = 8.0 Hz, 1 H, Ar-*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 787.6 (8) [$2MH^+$], 394.3 (100) [MH^+]. – $C_{19}H_{28}N_3O_4SCl$ (429.15).



(S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´*,*N´´*-di-(*tert*-butoxycarbonyl)-guanidino]-6-(5-dimethylamino-naphthalene-1-sulfonylamino)-hexanoic acid methyl ester (21).

Compound **20** (397 mg, 1.00 mmol) and compound **8** (626 mg, 1.5 mmol) were dissolved in 20 mL THF. NEt_3 (121 mg, 0.166 mL, 1.20 mmol) was added and the reaction mixture was refluxed for 18 h. After filtration the solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to obtain **21** (412 mg, 0.54 mmol, 54 %) as a yellow solid. R_f (EtOAc/hexane) = 0.30

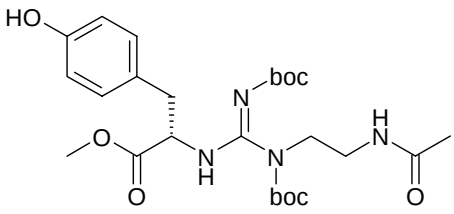
mp: 103 °C. - $[\alpha]^{20}_D$ = + 31.0 (c = 0.6 in MeOH). - IR (KBr) [cm^{-1}]: 3256, 3112, 2978, 2934, 1722, 1645, 1523, 1411, 1367, 1287, 1188, 1002, 956, 845. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.31 – 1.65 (m, 20 H, 2 x Boc/C*H*$_2$), 1.71 – 1.83 (m, 2 H, C*H*$_2$), 2.83 – 2.93 (m, 8 H, 2 x Me/C*H*$_2$), 3.21 – 3.37 (m, 2 H, C*H*$_2$), 3.70 (s, 3 H, CO_2Me), 4.10 –4.21 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.28 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.52 (bs, 1 H, N*H*), 5.83 – 5.97 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.87 (bs, 1 H, -N*H*-Aloc), 7.13 (d, J = 7.8 Hz, 1 H, Ar-*H*), 7.41 – 7.53 (m, 2 H, Ar-*H*), 8.11 – 8.22 (m, 2 H, Ar-*H*), 8.34 (d, J = 7.8 Hz, Ar-*H*), 9.82 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.2 (+), 27.8 (-), 28.1 (+), 28.2 (+), 28.4 (+), 40.4 (C_{quat}), 42.7 (-), 45.5 (+), 52.5 (+), 65.5 (-), 68.6 (-), 115.3 (+), 117.4 (C_{quat}), 123.3 (+), 128.3 (+), 129.4 (+), 129.8 (C_{quat}), 130.2 (+), 152.3 (C_{quat}), 157.5 (C_{quat}), 171.4 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 763.5 (100) [MH^+], 663.4 (15) [MH^+ - Boc]. – $C_{36}H_{54}N_6O_{10}S$ (762.36).



(S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-guanidino]-6-(5-dimethylamino-naphthalene-1-sulfonylamino)-hexanoic acid methyl ester hydrochloride (22).

Compound **21** (412 mg, 0.54 mmol) was dissolved in 15 mL DCM/TFA = 4/1 and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the product was dissolved in 10 mL 1 N HCl. After freeze drying compound **22** (306 mg, 0.54 mmol, 100 %) was obtained as a pale yellow solid.

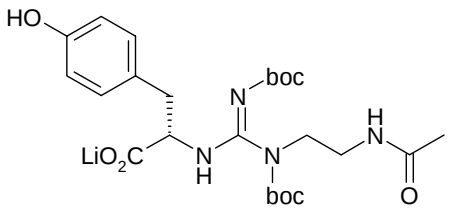
mp: 104 °C. – IR (KBr) [cm^{-1}]: 3385, 2945, 2868, 2787, 1710, 1575, 1508, 1456, 1363, 1319, 1161, 1085, 791, 625, 571. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.34 – 1.75 (m, 4 H, 2 x C*H*$_2$), 2.83 – 2.93 (m, 8 H, 2 x Me/C*H*$_2$), 3.21 – 3.37 (m, 2 H, C*H*$_2$), 3.72 (s, 3 H, CO_2Me), 4.10 –4.20 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH_2O), 5.28 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH_2O), 5.52 (bs, 1 H, N*H*), 5.83 – 5.97 (m, 1 H, CH_2=C*H*CH_2O), 6.89 (bs, 1 H, -N*H*-Aloc), 7.13 (d, J = 7.8 Hz, 1 H, Ar-*H*), 7.41 – 7.53 (m, 4 H, Ar-*H*/2 x N*H*), 8.11 – 8.22 (m, 3 H, Ar-*H*/N*H*), 8.34 (d, J = 7.8 Hz, 1 H, Ar-*H*), 8.56 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 563.4 (100) [MH^+]. – $C_{26}H_{39}N_6O_6SCl$ (566.26).



(S)-2-[*N´*-(2-Acetylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester (23).

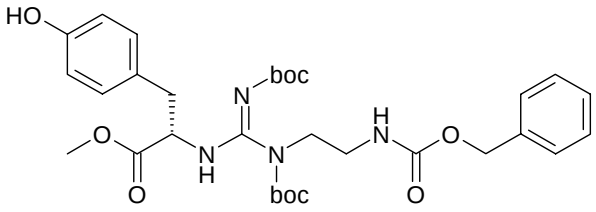
Compound **13** (345 mg, 0.61 mmol) and Ac_2O (156 mg, 0.144 mL, 1.53 mmol) were dissolved in 15 mL DCM. $Pd(PPh_3)_4$ (35 mg, 5 mol%) and Bu_3SnH (195 mg, 0.177 mL, 0.67 mmol) were added and the reaction mixture was stirred ar room temperature for 45 min. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **23** (266 mg, 0.51 mmol, 84 %) as a colourless oil. R_f (EtOAc) = 0.50

IR (KBr) [cm^{-1}]: 3256, 3008, 2988, 2923, 1722, 1623, 1455, 1367, 1278, 1189, 1007, 984, 845, 734. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 9 H, Boc), 1.45 (s, 9 H, Boc), 1.87 (s, 3 H, CO-C*H*$_3$), 2.80 – 3.19 (m, 6 H, 3 x C*H*$_2$), 3.71 (s, 3 H, CO_2Me), 4.05 – 4.22 (m, 1 H, C*H*), 6.81 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.93 (bs, 1 H, -N*H*-$COCH_3$), 7.06 (d, J = 8.0 Hz, 2 H, Ar-*H*), 10.01 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 523.3 (100) [MH^+], 467.2 (10) [MH^+ - C_4H_8], 423.3 (10) [MH^+ - Boc]. – $C_{25}H_{38}N_4O_8$ (522.27).



Lithium (S)-2-[*N´*-(2-Acetylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-hydroxy-phenyl)-propionic acid (24).

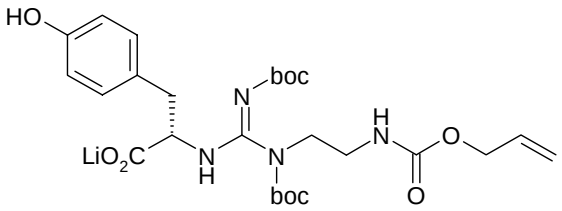
Compound **23** (266 mg, 0.51 mmol) was dissolved in 10 mL MeOH/H_2O = 3/1 and LiOH*H_2O (21 mg, 0.51 mmol) was added. The reaction mixture was stirred at room temperature for 18 h. The solvents were removed and the product was dried under high vacuum. Compound **24** (242 mg, 0.47 mmol, 92 %) was obtained as a pale yellow solid. mp: 123 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.41 (s, 9 H, Boc), 1.45 (s, 9 H, Boc), 1.89 (s, 3 H, CO-C*H*$_3$), 2.84 – 3.29 (m, 6 H, 3 x C*H*$_2$), 4.05 – 4.14 (m, 1 H, C*H*), 6.81 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.91 (bs, 1 H, -N*H*-$COCH_3$), 7.06 (d, J = 8.0 Hz, 2 H, Ar-*H*), 10.45 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 507.6 (100) [M-H$^+$]. – $C_{24}H_{35}N_4O_8Li$ (514.26).



(S)-2-[*N´*-(2-Benzyloxycarbonylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-hydroxy-phenyl)-propionic acid methyl ester (28).

Compound **13** (323 mg, 0.57 mmol) and Cbz-OSu (157 mg, 0.63 mmol) were dissolved in 15 mL DCM. $Pd(PPh_3)_4$ (33 mg, 6 mol%) and Bu_3SnH (183 mg, 0.166 mL, 0.63 mmol) were added and the reaction mixture was stirred ar room temperature for 45 min. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **28** (305 mg, 0.50 mmol, 87 %) as a colourless oil. R_f (EtOAc) = 0.65

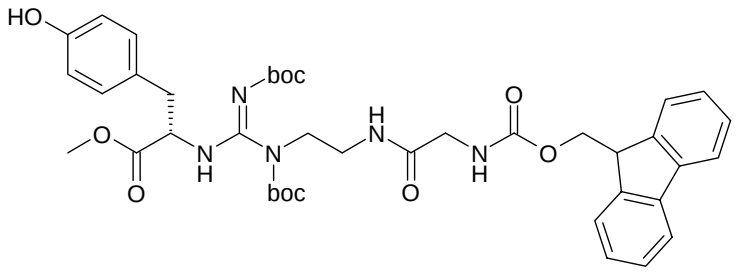
IR (KBr) [cm^{-1}]: 3267, 2989, 2923, 1722, 1685, 1477, 1365, 1298, 1123, 945, 834, 756, 689. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.47 (s, 9 H, Boc), 2.83 – 3.25 (m, 6 H, 3 x C*H*$_2$), 3.76 (s, 3 H, CO_2Me), 4.08 – 4.21 (m, 1 H, C*H*), 5.04 (s, 2 H, C*H*$_2$-Ph), 6.45 (bs, 1 H, N*H*-Cbz), 6.79 (d, J = 8.2 Hz, 2 H, Ar-*H*), 7.04 (d, J = 8.2 Hz, 2 H, Ar-*H*), 7.19 – 7.36 (m, 5 H, Ph), 10.08 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 10.0 (-), 13.7 (+), 25.5 (-), 27.5 (-), 28.0 (+), 28.1 (+), 30.6 (-), 40.2 (-), 52.5 (+), 66.6 (-), 72.8 (-), 80.0 (C_{quat}), 115.8 (C_{quat}), 127.2 (C_{quat}), 128.0 (+), 128.4 (+), 128.7 (+), 128.8 (+), 130.4 (+), 133.2 (C_{quat}), 168.7 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 615.6 (100) [MH^+]. – $C_{31}H_{42}N_4O_9$ (614.30).



Lithium (S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-hydroxy-phenyl)-propionic acid (29).

Compound **13** (190 mg, 0.34 mmol) was dissolved in 10 mL MeOH/H_2O = 3/1 and LiOH*H_2O (14 mg, 0.34 mmol) was added. The reaction mixture was stirred at room temperature for 15 h. The solvents were removed and the product was dried under high vacuum. Compound **29** (189 mg, 0.34 mmol, 100 %) was obtained as a pale red solid.

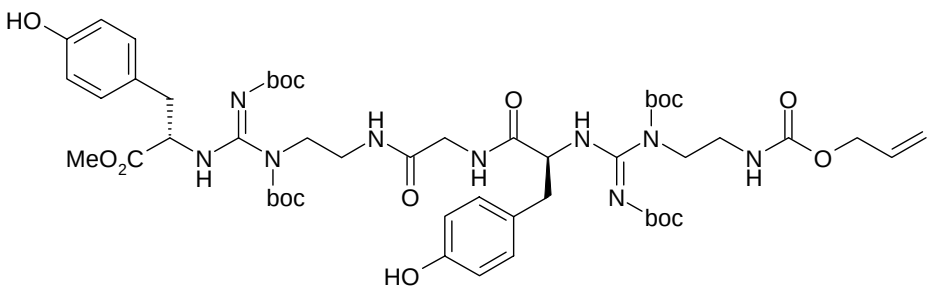
mp: 89 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.39 (s, 9 H, Boc), 1.44 (s, 9 H, Boc), 2.81 – 3.34 (m, 6 H, 3 x C*H*$_2$), 3.89 – 4.03 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.28 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.83 – 5.97 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.78 – 6.91 (m, 3 H, Ar-*H*/N*H*), 6.99 – 7.06 (m, 2 H, Ar-*H*), 9.81 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 549.26 (100) [M – H^+]. – $C_{26}H_{37}N_4O_9Li$ (556.27).



(S)-2-(*N´,N´´*-Di-(*tert*-butoxycarbonyl)-*N´*-{2-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-acetylamino]-ethyl}-guanidino)-3-(4-hydroxy-phenyl)-prppionic acid methyl ester (31).

Compound **30** (110 mg, 0.37 mmol), DIC (47 mg, 0.057 mL, 0.37 mmol) and HOBt (50 mg, 0.37 mmol) were dissolved in 15 mL DCM and the reaction mixture was stirred at room temperature for 30 min. Compound **13** (207 mg, 0.37 mmol), $Pd(PPh_3)_4$ (29 mg, 7 mol%) and Bu_3SnH (119 mg, 0.108 mL, 0.41 mmol) were added and the solution was stirred at room temperature for 12 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **31** (182 mg, 0.25 mmol, 67 %) as a colourless oil. R_f (EtOAc) = 0.45

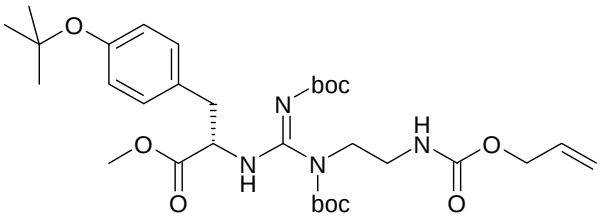
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.47 (s, 9 H, Boc), 2.81 – 3.17 (m, 6 H, 3 x C*H*$_2$), 3.81 (s, 3 H, CO_2Me), 4.08 – 4.21 (m, 2 H, 2 x C*H*), 4.31 – 4.43 (m, 4 H, 2 x C*H*$_2$), 6.02 (bs, 1 H, N*H*), 6.77 (d, J = 8.2 Hz, 2 H, Ar-*H*), 7.01 (d, J = 8.2 Hz, 2 H, Ar-*H*), 7.11 –7.20 (m, 4 H, Ar-*H*), 7.43 – 7.50 (m, 2 H, Ar-*H*), 7.56 – 7.63 (m, 2 H, Ar-*H*), 9.01 (bs, 1 H, N*H*), 10.12 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 760.4 (100) [MH^+]. – $C_{40}H_{49}N_5O_{10}$ (759.35).



GuAA-Gly-GuAA (33).

Compound **31** (164 mg, 0.21 mmol) was dissolved in 10 mL 20 % piperidine/DMF and the reaction mixture was stirred at room temperature for 1 h. The reaction process was monitored by TLC. The solvent was evaporated and dried under high vacuum. The amine was used without further purification. The amine was dissolved in 10 mL DCM, compound **32** (117 mg, 0.21 mmol), DIC (27 mg, 0.033 mL, 0.21 mmol) and HOBt (28 mg, 0.21 mmol) were added and the reaction was stirred at room temperature for 18 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **33** (38 mg, 0.036 mmol, 17 %) as a yellow solid.

mp: 93 °C. - IR (KBr) [cm^{-1}]: 3245, 3006, 2988, 2923, 1723, 1634, 1412, 1367, 1234, 1145, 1098, 934, 823. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.35 (s, 9 H, Boc), 1.39 (s, 9 H, Boc), 1.43 (s, 18 H, 2 x Boc), 2.79 – 3.43 (m, 12 H, 6 x C*H*$_2$), 3.78 (s, 3 H, CO_2Me), 3.85 – 3.97 (m, 1 H, C*H*), 4.11 – 4.19 (m, 1 H, C*H*), 4.53 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 4.79 (m, 2 H, C*H*$_2$), 5.17 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.28 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.83 – 5.97 (m, 1 H, CH_2=C*H*CH$_2$O), 6.73 – 7.08 (m, 7 H, Ar-*H*/N*H*), 7.13 – 7.19 (m, 2 H, Ar-*H*), 8.23 (bs, 1 H, N*H*), 8.49 (bs, 1 H, N*H*) 9.12 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.0 (+), 21.8 (+), 23.2 (+), 24.4 (-), 25.2 (-), 25.7 (C_{quat}) 26.2 (-), 27.4 (+), 27.6 (+), 27.7 (+), 27.8 (+), 28.1 (+), 33.2 (+), 40.2 (C_{quat}), 40.6 (-), 47.4 (+), 51.8 (+), 54.2 (+), 59.6 (C_{quat}), 64.1 (-), 67.7 (-), 109.6 (-), 115.0 (+), 116.8 (-), 119.8 (C_{quat}), 119.9 (+), 121.3 (+), 125.3 (C_{quat}), 126.6 (+), 127.1 (+), 129.8 (+), 133.5 (C_{quat}), 133.6 (C_{quat}), 155.8 (C_{quat}), 156.1 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 1070.6 (100) [MH^+], 970.6 (10) [MH^+ - Boc]. – $C_{51}H_{75}N_9O_{16}$ (1069.53).

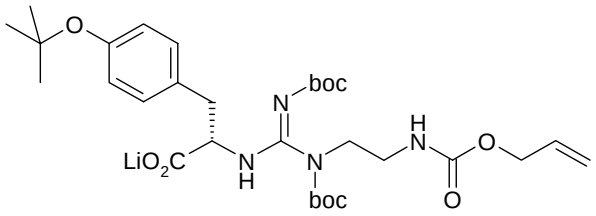


(S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-*tert*-butoxy-phenyl)-propionic acid methyl ester (34).

Compound **8** (1.23 g, 2.94 mmol), MeO-Tyr(OtBu)-NH_2*HCl (1.15 g, 3.99 mmol) and NEt_3 (404 mg, 0.550 mL, 3.99 mmol) were dissolved in 25 mL THF and the reaction mixture was refluxed for 18 h. The solid was filtered off and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (EtOAc/hexane = 1/1) to obtain **34** (943 mg, 1.53 mmol, 52 %) as a pale yellow solid.

R_f (EtOAc/hexane = 1/1) = 0.35

mp: 100 °C. - IR (KBr) [cm^{-1}]: 3312, 2977, 2912, 1712, 1645, 1567, 1423, 1267, 1123, 1006, 987, 856, 723. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.36 (s, 9 H, tBu), 1.40 (s, 9 H, Boc), 1.45 (s, 9 H, Boc), 2.85 – 3.33 (m, 6 H, 3 x C*H*$_2$), 3.69 (s, 3 H, CO_2Me), 4.05 – 4.22 (m, 1 H, C*H*), 4.51 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.19 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.24 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.81 – 5.94 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.62 (bs, 1 H, -N*H*-Aloc), 6.86 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.99 (d, J = 8.0 Hz, 2 H, Ar-*H*), 9.87 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.4 (+), 22.0 (+), 23.9 (-), 25.9 (-), 27.9 (+), 28.0 (+), 28.2 (+), 28.6 (+), 29.2 (-), 39.3 (C_{quat}), 40.1 (-), 42.3 (-), 47.4 (-), 52.5 (+), 59.2 (C_{quat}), 65.3 (-), 67.6 (-), 68.5 (-), 69.9 (+), 79.9 (C_{quat}), 81.0 (+), 98.3 (C_{quat}), 107.8 (+), 117.2 (-), 124.4 (+), 129.8 (+), 133.4 (+), 133.5 (C_{quat}), 154.6 (C_{quat}), 156.5 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 621.4 (100) [MH^+]. – $C_{31}H_{48}N_4O_9$ (620.34).



Lithium (S)-2-[*N´*-(2-Allyloxycarbonylamino-ethyl)-*N´,N´´*-di-(-(*tert*-butoxycarbonyl)-guanidino]-3-(4-*tert*-butoxy-phenyl)-propionic acid (35).

Compound **34** (943 mg, 1.53 mmol) was dissolved in 10 mL MeOH/H_2O = 3/1 and LiOH*H_2O (64 mg, 0.51 mmol) was added. The reaction mixture was stirred at room temperature for 18 h. The solvents were removed and the product was dried under high vacuum. Compound **35** (291 mg, 0.51 mmol, 100 %) was obtained as a pale yellow solid.

mp: 110 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9 H, tBu), 1.40 (s, 9 H, Boc), 1.45 (s, 9 H, Boc), 2.85 – 3.33 (m, 6 H, 3 x C*H*$_2$), 4.05 – 4.22 (m, 1 H, C*H*), 4.51 (d, J = 5.2 Hz, 2 H, -C*H*$_2$-CH-), 5.19 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.24 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.81 – 5.94 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.61 (bs, 1 H, -N*H*-Aloc), 6.86 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.99 (d, J = 8.0 Hz, 2 H, Ar-*H*), 10.01 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 563.5 [M – H^+]. – $C_{28}H_{43}N_4O_8Li$ (570.32).

Solid Phase Protocol

Fmoc-Deprotection

The resin (0.1 mmol) is preswollen in DCM for 1 h. The resin is treated with 2 mL 20 % piperidine/DMF for 5 min. The deprotection step was repeated for two times. After completion the resin was washed several times with DMF.

Coupling of Fmoc-AA

The Fmoc-AA (155 mg, 0.5 mmol), HOBt (67 mg, 0.5 mmol) and DIC (0.077 mL, 0.5 mmol) were dissolved in 2 mL DMF. The coupling mixture was added to the resin (preswollen in DMF). The resin was filtered off after 4 h. The completion of the coupling step was monitored using the *Kaiser* test. The resin was washed thoroughly with DMF.

Fmoc-Deprotection

The resin is treated with 2 mL 20 % piperidine/DMF for 5 min. The deprotection step was repeated for two times. After completion the resin was washed several times with DMF

Coupling of GuAA

The GuAA (131 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol) and DIC (0.031 mL, 0.2 mmol) were dissolved in 2 mL DMF. The coupling mixture was added to the resin (preswollen in DMF). The resin was filtered off after 6 h and the coupling step was repeated two times. The completion of the coupling step was monitored using the *Kaiser* test. The resin was washed thoroughly with DMF.

Deprotection of the Aloc-group and coupling with Fmoc-AA

The Fmoc-AA (155 mg, 0.5 mmol), HOBt (67 mg, 0.5 mmol) and DIC (0.077 mL, 0.5 mmol) were dissolved in 2 mL DCM. $Pd(PPh_3)_4$ (11 mg, 10 mol%) and Bu_3SnH (0.080 mL, 0.3 mmol) were added and the mixture was added to the resin. The resin was filtered off after 6 h and the deprotection/coupling step was repeated two times. The completion of the coupling step was monitored using the *Kaiser* test. The resin was washed thoroughly with DCM and DMF.

Cleavage

The resin was washed with DMF (3 x), DCM (3 x) and methanol. The resin was dried under high vacuum for 3 h and then placed in a flask. A solution of 95% TFA/2.5% H_2O/2.5% TIS (4 mL) was added and leave to stand at room temperature for 2 h. The resin is filtered off and washed twice with TFA. Cold ether is added to the filtrate in a 10 fold volume. The precipitate is filtered off and lyophilized.

2.8 Appendix

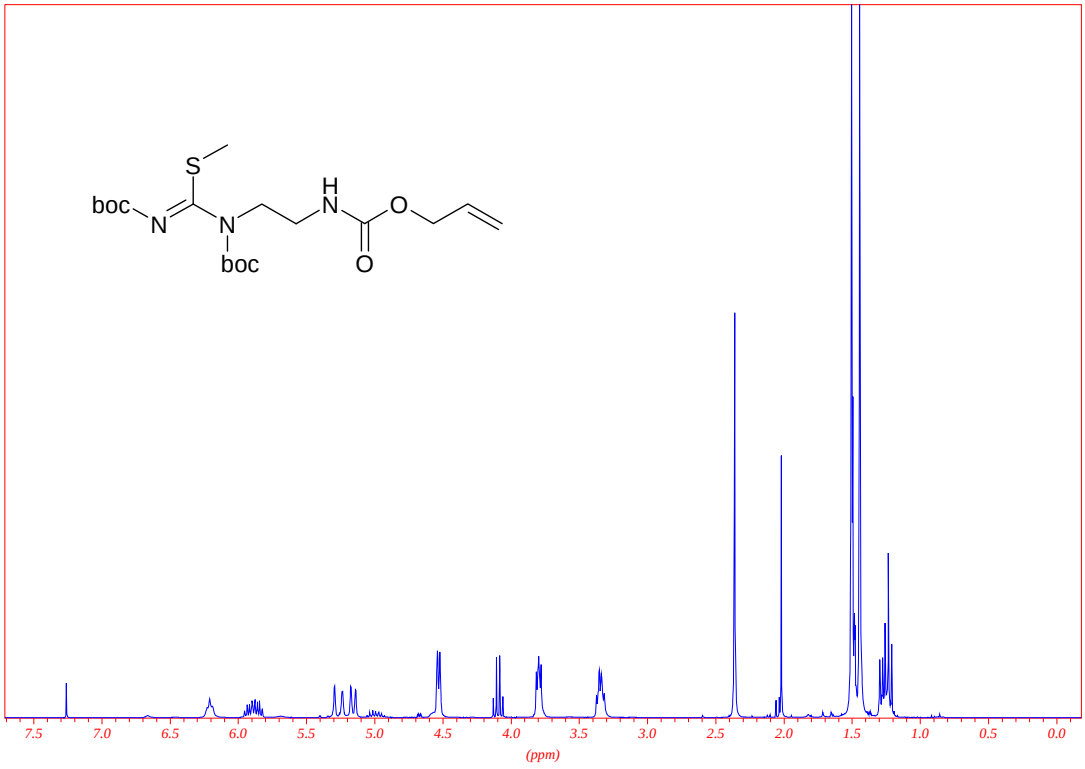
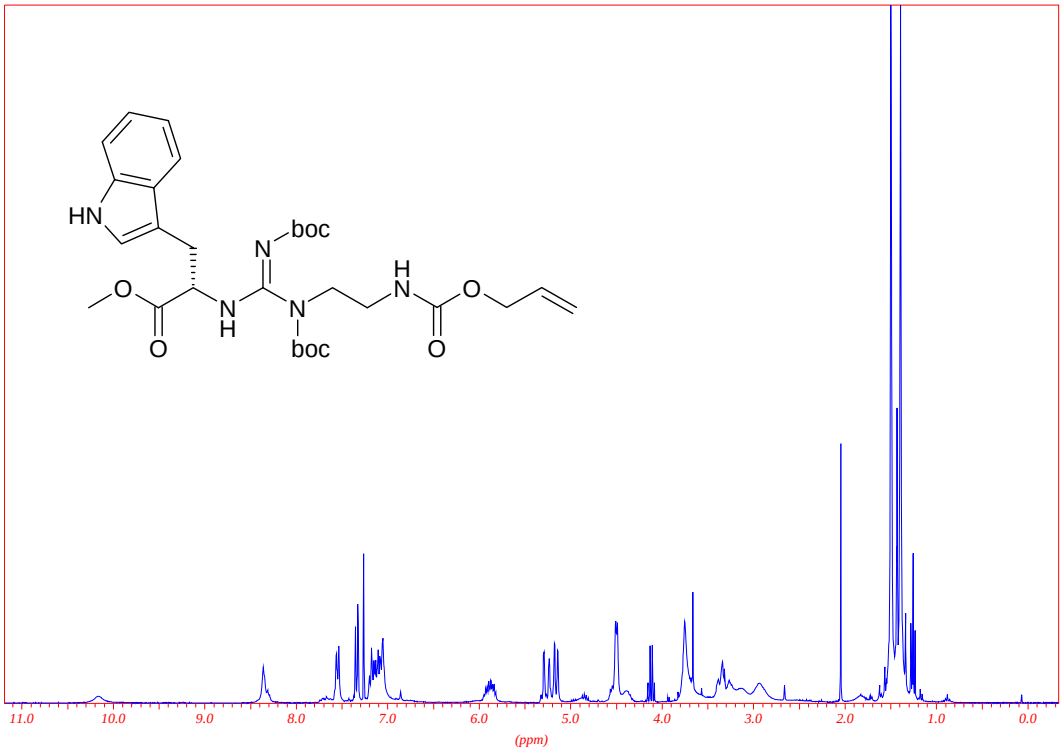


Figure 17. ^{1}H-NMR of compound **8**



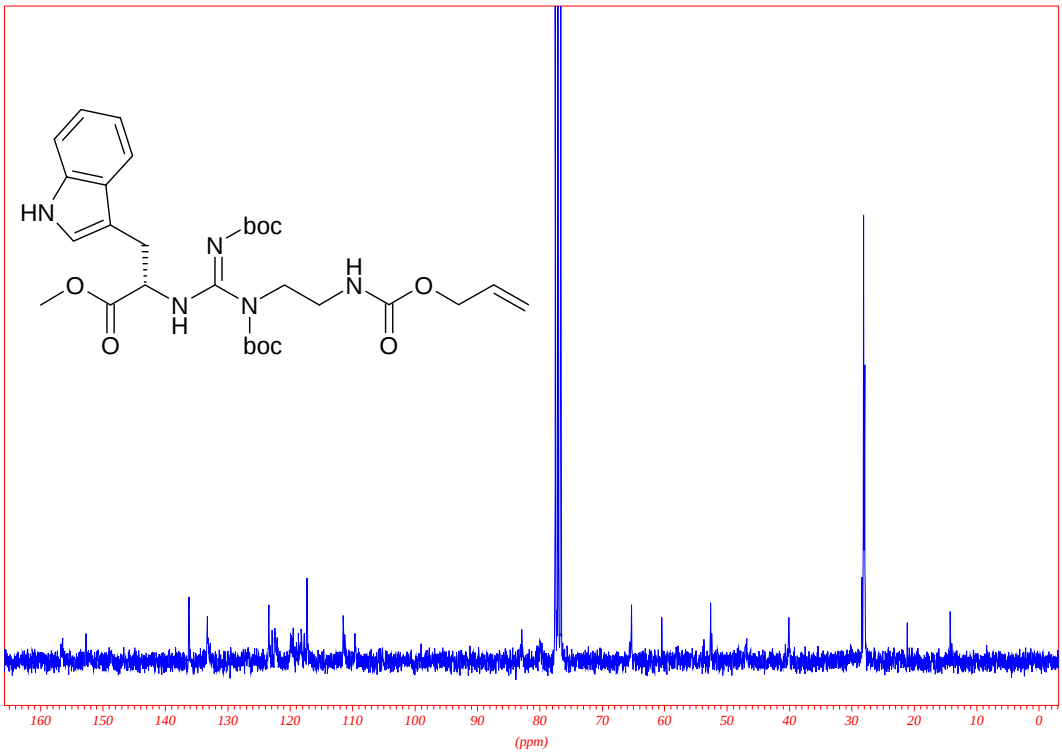


Figure 18. ^{1}H-NMR and ^{13}C-NMR of compound **15**

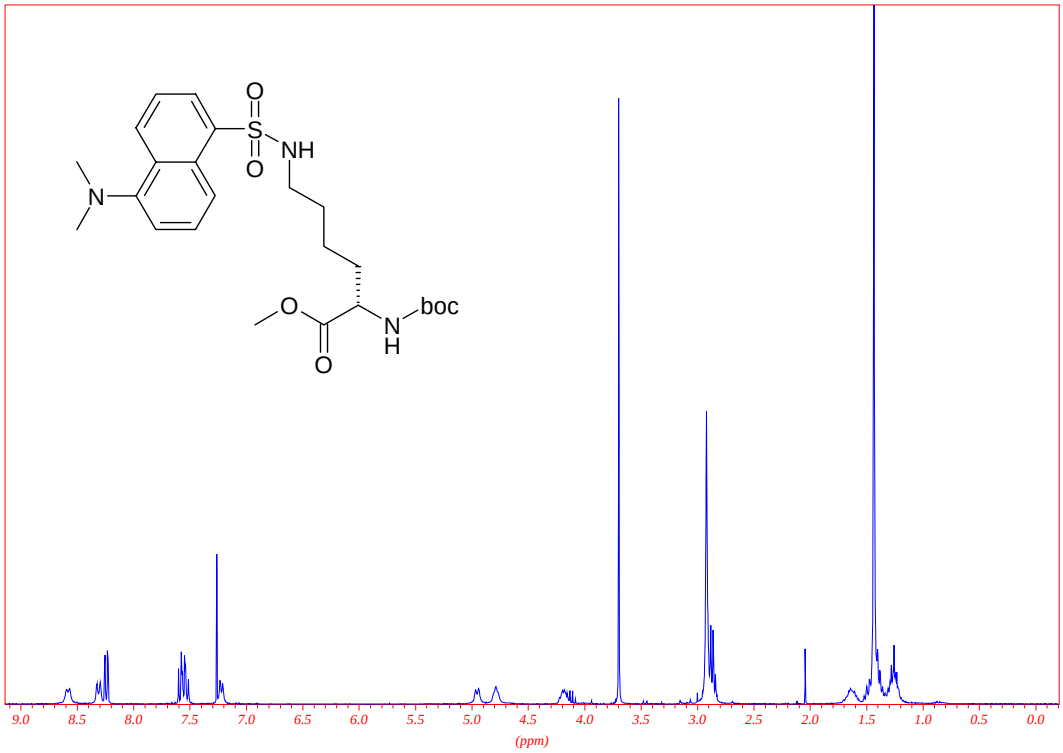


Figure 19. ^{1}H-NMR of compound **19**

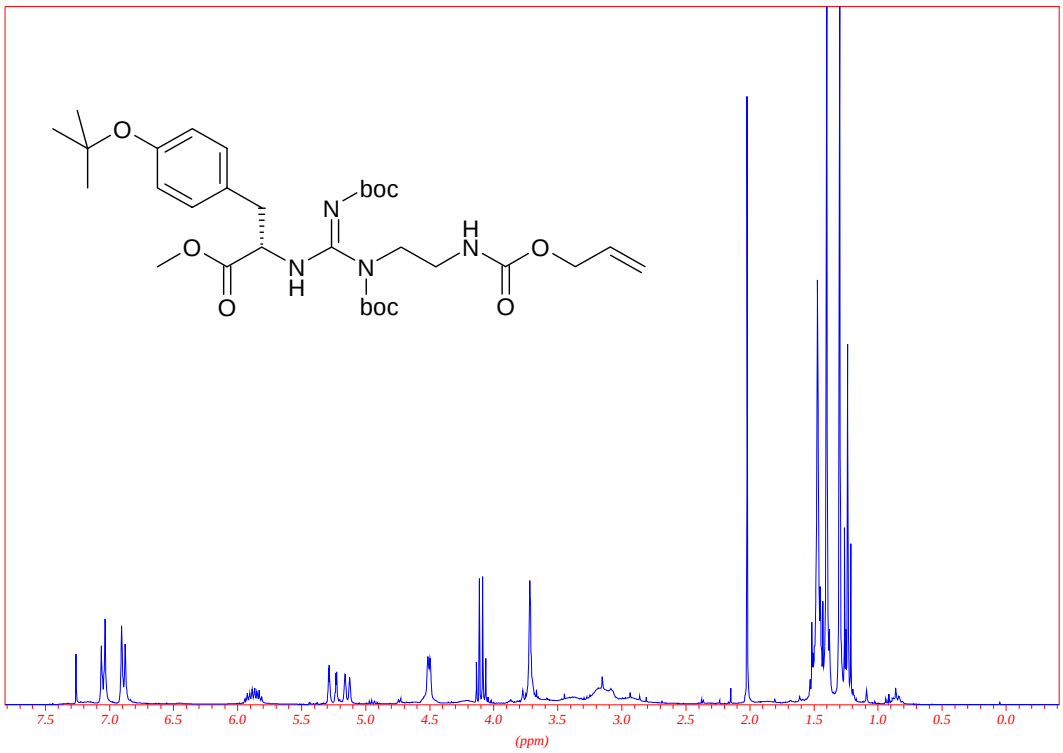


Figure 20. ^{1}H-NMR of compound **34**

2.9 References

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3. Bivalent NPY Y_1 Receptor Antagonists

3.1 Introduction

3.1.1 Neuropeptide Y (NPY)

Isolated in 1982 from porcine brain,[1] neuropeptide Y (NPY) is a 36-residue C-terminally amidated peptide that has raised an incredibly high number of studies in the last 20 years. On the basis of structural and evolutionary criteria,[2] NPY is believed to belong to a family of polypeptides, also called the NPY family, whose other two members are pancreatic polypeptide (PP) isolated from chicken pancreas in 1975[3] and peptide YY (PYY) isolated from porcine intestine.[4,5] Common structural features of this family are the 36 amino acids of the sequence, the amidated C-terminus, the presence of several tyrosine residues, and the considerable amino acis homology (Figure 1).[6]

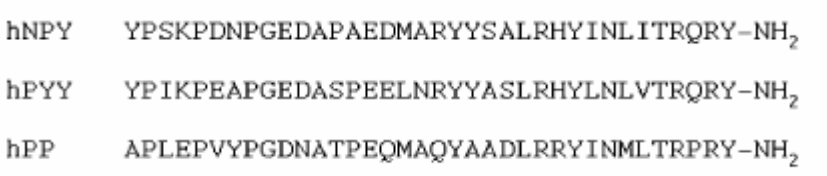


Figure 1. Amino Acid sequences of human NPY (hNPY), human peptide Y (hPYY) and human pancreatic polypeptide (hPP), the three members of the so-called NPY family

NPY acts mainly as neurotransmitter, while PYY and PP act as hormones.[7] Expressed by neurons in the central and peripheral nervous system, NPY is one the most abundant peptides in the nervous system of mammalians.[8] The largest group of NPY neurons are centrally localized in the hypothalamous and the amigdala. PYY is expressed in endocrine cells in the intestine,[9] and in certain species, in nerves innervating the stomach walls.[10] PP is expressed in the pancreas[11] and in gastrointestinal endocrine cells.[12] NPY stimulates the secretion in insulin and luteinizing hormone (LH).[13] Central administration of a low dose of NPY reduces the secretion of corticotropic-releasing hormone (CRH), adrenocorticotrophic releasing hormone (ACTH), and cortisol.[14] An important effect of NPY is the regulation of blood pressure, which is exerted through a vasoconstriction effect in muscles,[15] heart,[16] kidney,[17] eyes and brain.[18] NPY is able to influence the body energy balance also regulating thermogenesis.[19] The circadian or

daily cycle generated by mammalian brain to create an internal rhythm for the body is also influenced by NPY.[20]

The first and so far only member of the NPY family for which a high-resolution X-ray crystallographic structure is available is the avian (turkey) pancreatic polypeptide (aPP).[21] On the basis of the high homology, molecular modeling studies undertaken on pancreatic polypeptides of different species and supported by CD results suggested that the PP fold is adopted by all the members of the pancreatic polypeptide family. Based on analogous considerations, different investigations have extended the PP-fold model also to NPY. Figure 2 shows the structure of NPY bound to micelles.[22]

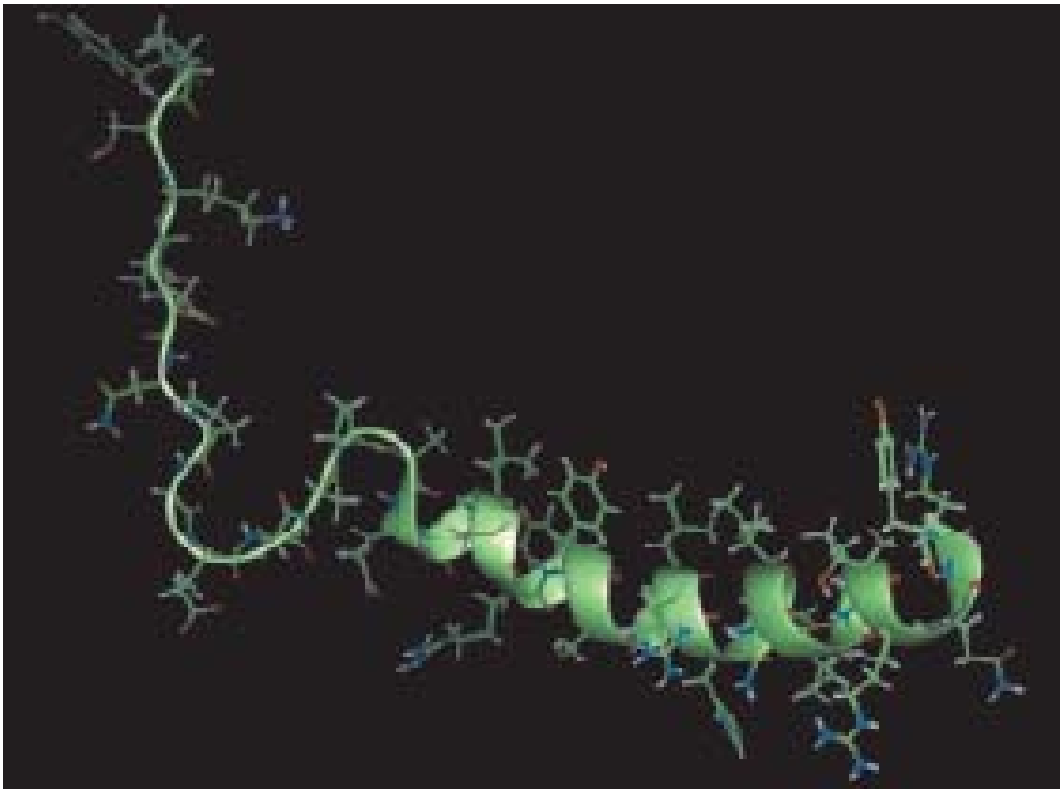


Figure 2. The structure of NPY bound to micelles. The N-terminal part is flexible and completely mobile, while the C-terminal α-helix interacts with the membrane surface.

The multiplicity of physiological processes in which NPY is involved is owing to its binding to at least 6 G-protein coupled receptors (GPCRs). They are termed Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 because the members of the NPY family of ligands contain several tyrosine residues, which are identified by the letter Y in the single-letter amino acid code.[2] G-protein coupled receptors are a superfamily of integral membrane proteins[23] characterized by seven α-helical fragments (7 TM) that span the cellular membrane.[6]

80 % of all bioactive molecules act through the interaction with GPCRs.[24] Typical ligands for this family of receptors are not only peptides but also monoamine messengers and proteins. The binding of a ligand to the GPCR induces intracellular binding of a heterotrimeric G-protein. The activated α-subunit of the G-protein exchanges its bound GDP and GTP and dissociates from the $G_{\beta\gamma}$ complex. At this point, the activated G_{α} interacts with an effector protein that can be, for instance, an enzyme (Figure 3).[6]

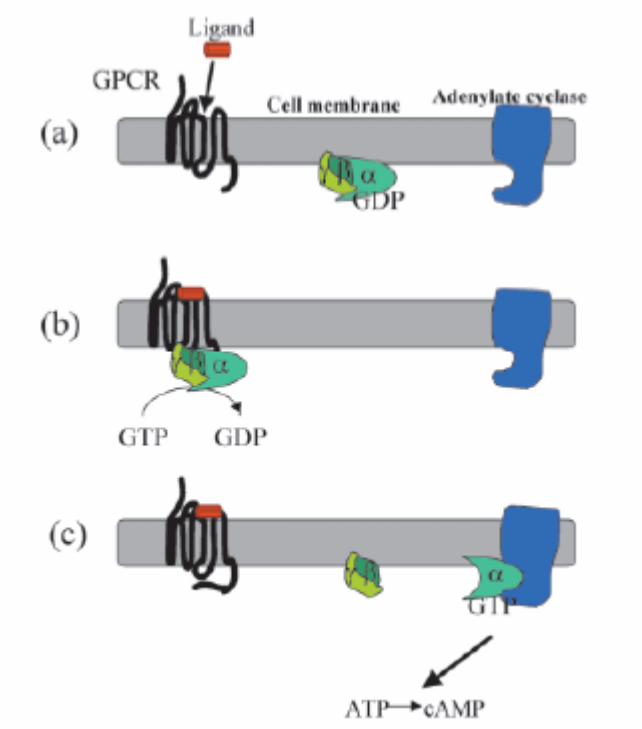


Figure 3. Signal transduction by G-protein coupled receptors. The binding of the ligand (a) determines the interaction of the receptor with the heterotrimeric G-protein whose α-subunit exchanges bound GDP for GTP (b). The G_{α}-GTP complex dissociates from the $G_{\beta\gamma}$ dimer and interacts with an effector protein, which in this case is the enzyme adenylate cyclase (c)

Five of the six Y receptors, namely Y_1, Y_2, Y_4, Y_5 and Y_6, have been cloned so far, while the Y_3 receptor has been described only pharmacologically and might be also derived from posttranslational modifications of one of the cloned receptors.[25] The Y_1, Y_2 and Y_5 receptors bind preferentially to NPY and PYY, while Y_4 is selective for PP. Together with Y_5, the Y_1 receptor is involved in regulation of food intake.[26] The Y_2 receptor subtype is responsible for the presynaptic inhibition of neurotransmitters release[27] and is expressed in various parts of the central nervous system and in

peripheral tissues.[28] Expressed mainly in peripheral tissues like colon, small intestine, prostate and pancreas,[29] the Y_4 receptor is involved in the regulation of the gastrointestinal activity.[30]

A rational mimetic strategy based on the structure of NPY led to the synthesis of the first highly active and Y_1 selective nonpeptidic antagonist, BIBP 3226 (**1**) (Figure 4) at Boehringer Ingelheim Pharma.[31,32,33]

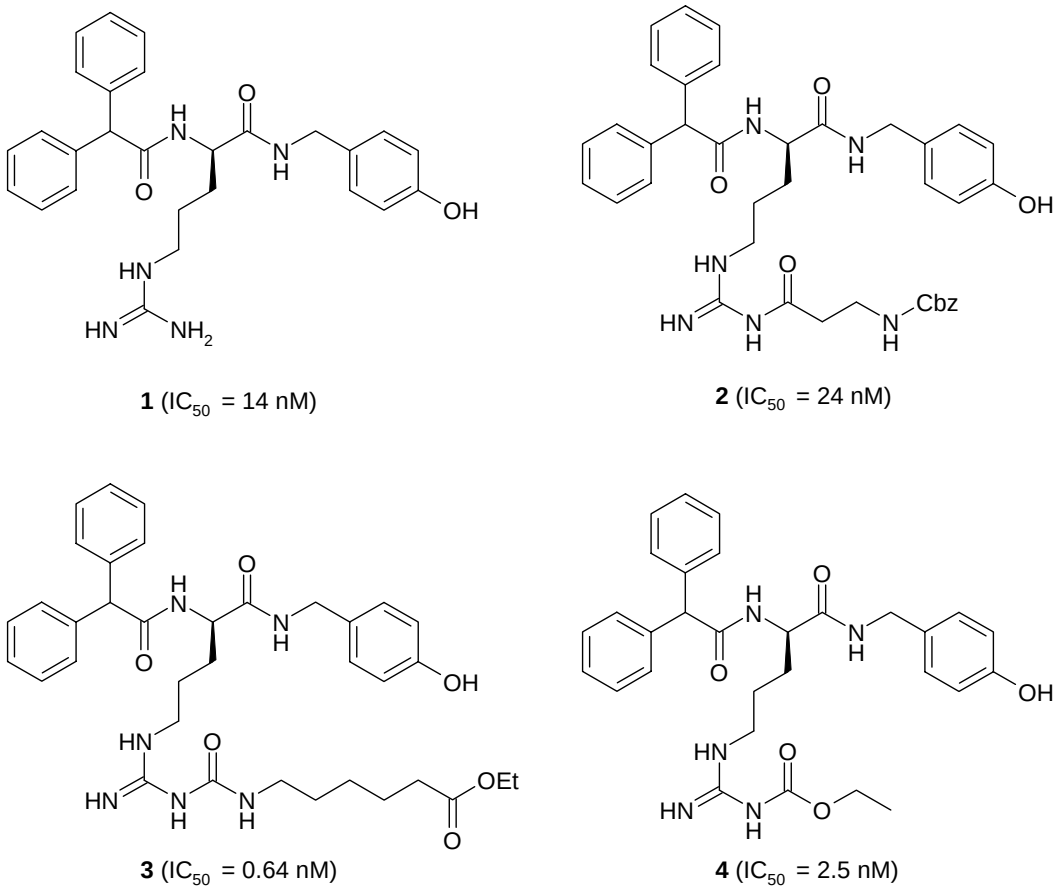


Figure 4. Structure of the Y_1 receptor antagonist BIBP 3226 (**1**) and N^G-acylated BIBP 3226 derivatives.[i] Inhibition of NPY (10 nM) stimulated Ca^{2+} mobilization in HEL cells

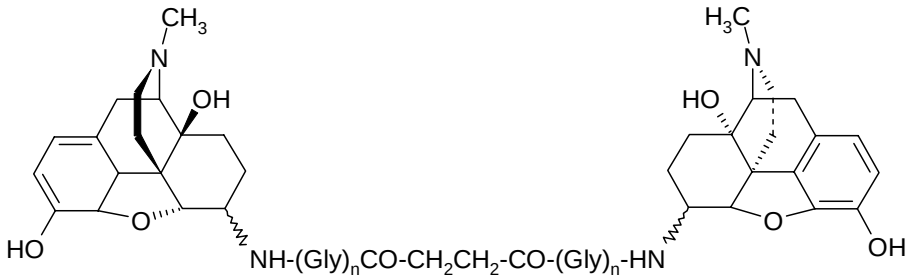
The complete alanine scan of NPY revealed that the C-terminal tetrapeptide, in particular Arg^{35} and Tyr^{36}, is most important for Y_1 receptor binding.[34] Recently, the Y_1 receptor binding models of BIBP 3226[35] were used to suggest that appropriate N^G-substituentes at the D-arginine side chain will retain or even increase antagonistic activity (Figure 5, compounds **2**, **3** and **4**).[i,36] With the intension to reduce the basicity of

[i] Hutzler, C. PhD Thesis, **2001**, University of Regensburg, Germany.

the guanidino group and, by this, to increase the hydrophobicity of the ligands for better blood-brain passage, electron-withdrawing substituentes were introduced. Some of these compounds are up to 20 times more active in the functional test and show more than 30 times higher Y_1 selectivity than BIBP 3226. Y_1 selectivity is even increased in most cases. The basicity of the guanidinium group is reduced to pK_a values of about 8, indicating that considerable amounts of the N^G-acylated argininamides are uncharged under physiological conditions. Probably, the ionic interaction of BIBP 3226 with Asp[287] can be replaced by a charge-assisted hydrogen bond.[37]

3.1.2 The Concept of Bivalent Ligands

The term "bivalent ligand" was defined as a molecule that contains two pharmacophores linked through a spacer. *Portoghese* and co-workers have reported first on the synthesis of opioid agonist and antagonist bivalent ligands as receptor probes (Figure 5).[38,3940]



5

Figure 5. Example of an bivalent ligand **5**

The authors envisaged that a bivalent ligand with a spacer of optimal length would exhibit a potency that is greater than the sum of its two monovalent pharmacophores. Such synergy was based on the assumption that a bivalent ligand should first undergo univalent binding, followed by binding of the second pharmacophore to a recognition site on a neighboring receptor (Figure 6).[41] When the bivalent ligand is in the univalently bound state, the pathway to bivalent binding should be favored over univalent binding of a second ligand because of the small containment volume of the tethered, unbound pharmacophore that is in the region of the unoccupied neighboring

receptor site. If the neighboring receptors are allosterically coupled, any affinity change due to occupation of a neighboring site by a single ligand (bridging) may also be a function of the type of cooperativity that is involved.

The spacer length is the critical factor with respect to the ability of the bivalent ligand to bridge neighboring receptors. A spacer of insufficient length would not permit bridging, and an excessively long spacer would tend to reduce bridging by increasing the confinement volume of the free pharmacophore so that it would spend less time in the vicinity of the unoccupied, neighboring recognition site.

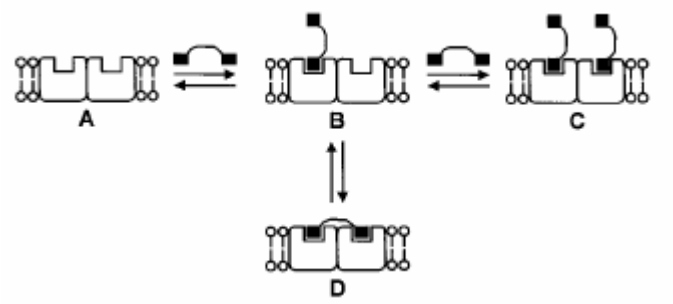


Figure 6. An schematic illustration of the bivalent ligand concept. The unoccupied dimer (A) undergoes univalet binding that leads to state B. The unoccupied site in B can be “bridged” to give D, or be bound by a second ligand to give the dimer with both sites occupied (C)

3.2 Results and Discussion

In this project bivalent NPY Y_1 receptor antagonists should be synthesized (Figure 7).

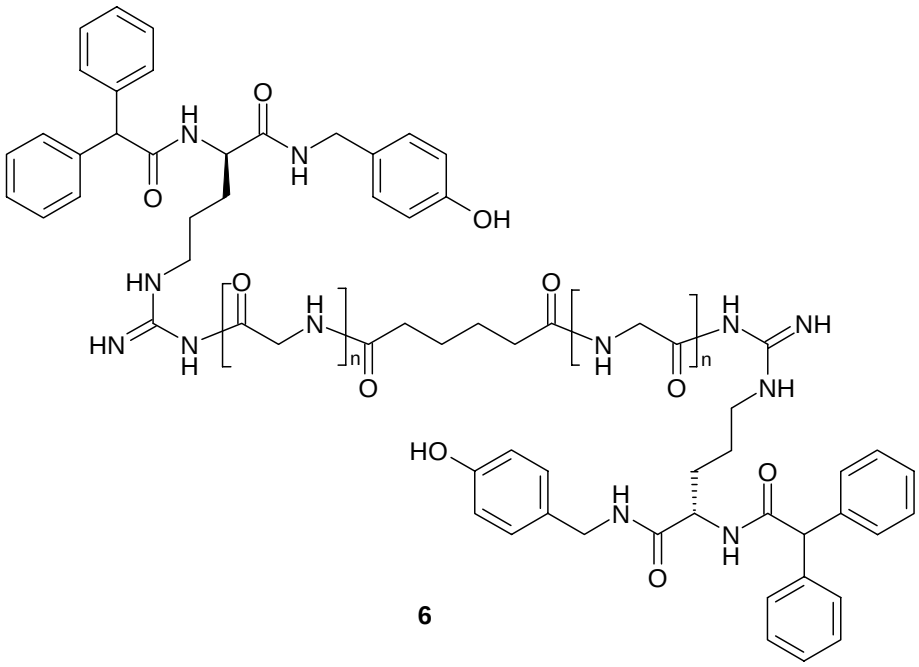
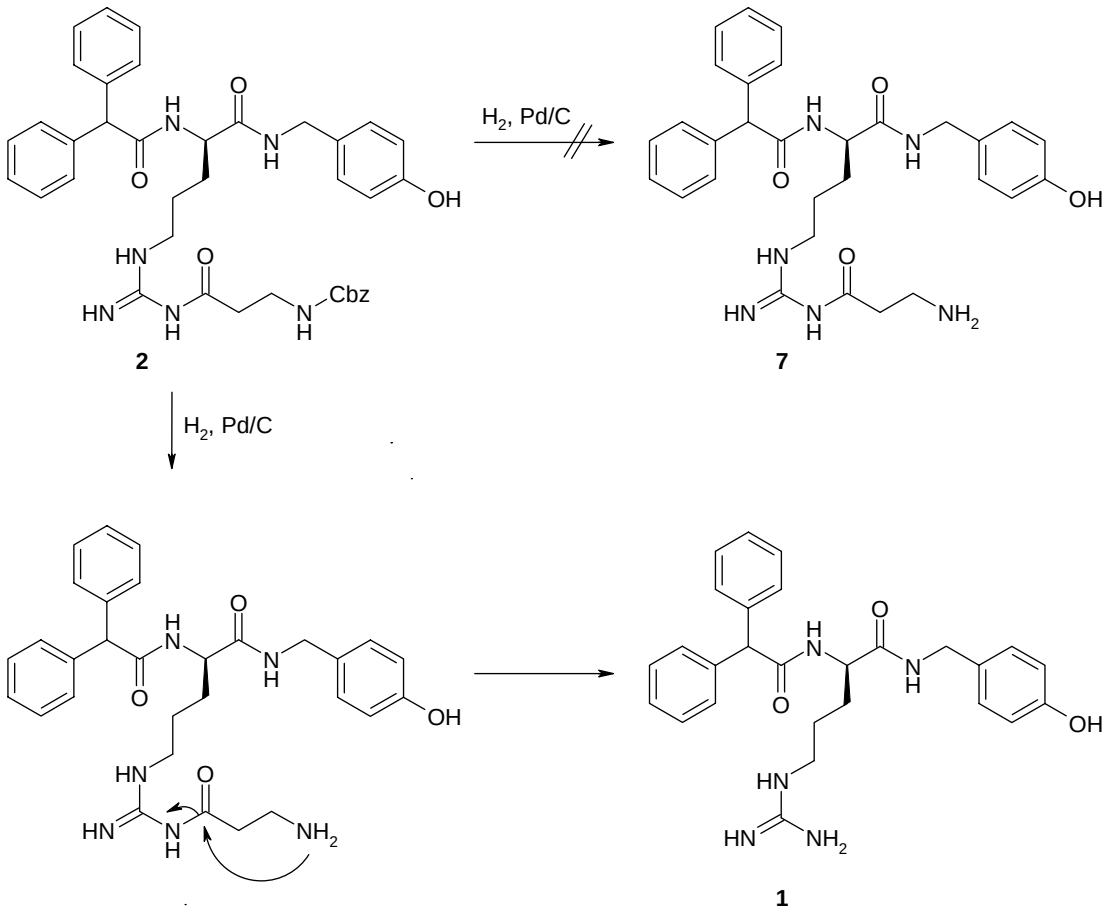


Figure 7. Example of a bivalent NPY Y_1 receptor antagonist **6**

The two pharmacophores are be connected via the guanidinium group, because *Hutzler*[i] has shown in his PhD thesis that N^G-acylated argininamides have in most cases higher binding affinities than the BIBP 3226. The connection via a peptide spacer is useful because of his easy synthetic access. The use of glycyl units avoided cumulative incremental increases in hydrophobicity that would occur upon homologation by an alkyl chain. Symmetry was introduced into the oligoglycine spacers by the inclusion of a central adipinyl moiety.

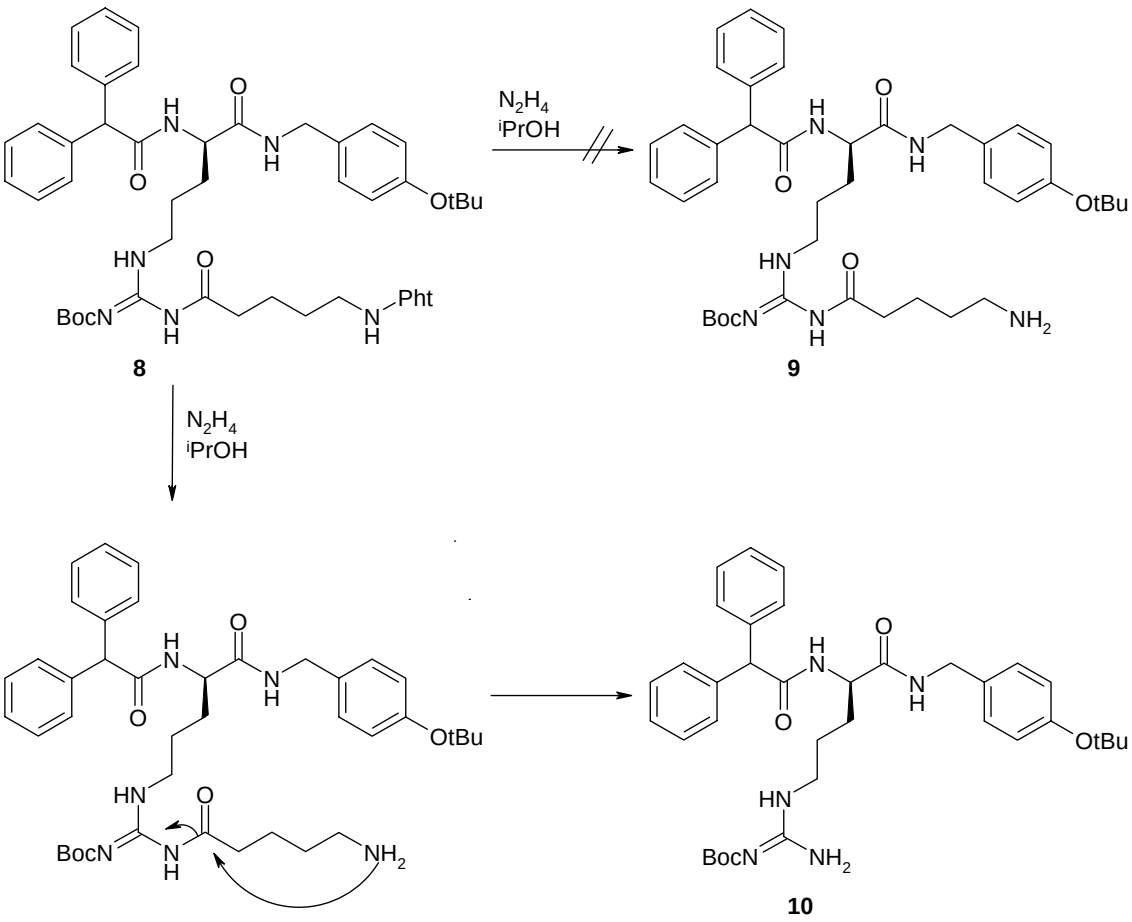
The direct approach to get a bivalent ligand by cleaving the Cbz-group of compounds like **2** and following coupling of the free amine **7** with an activated carbonic diacid is not possible. Fragmentation of the free amine yields BIBP 3226 (**1**) exclusivly (Scheme 1).[i]



Scheme 1. Cyclisation of **2** to give BIBP 3226 (**1**)

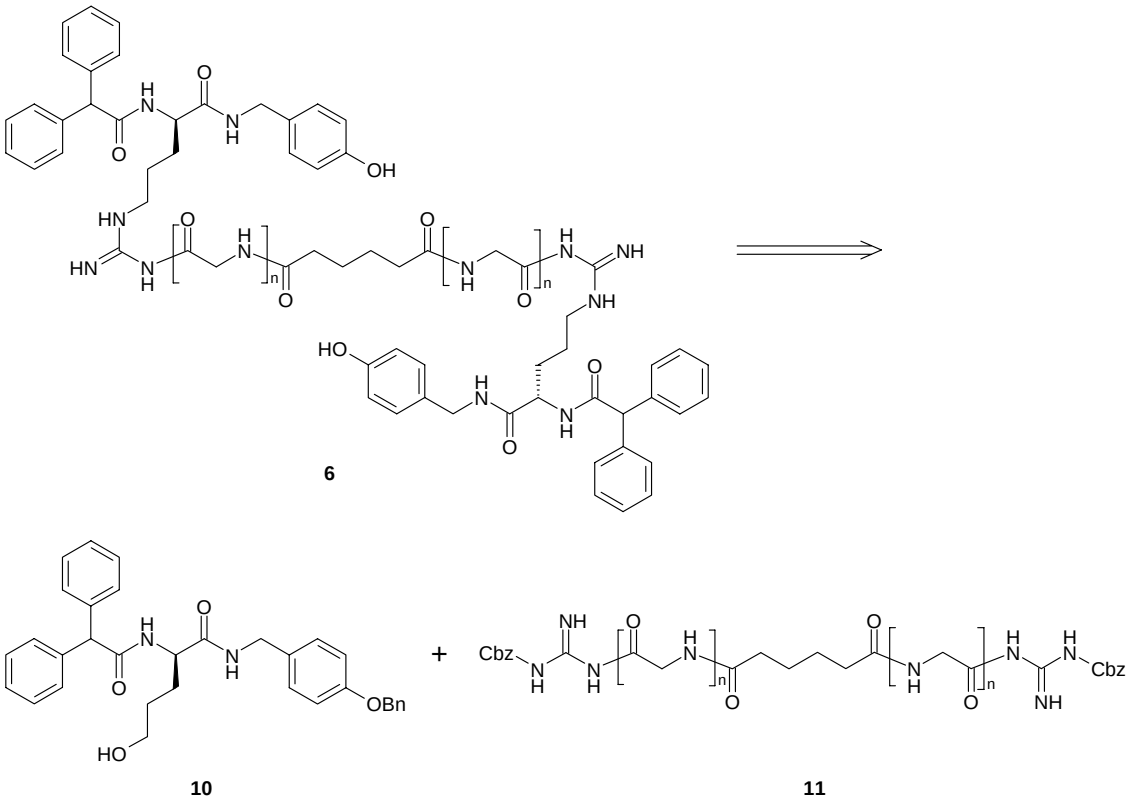
Freund has reported in his Diploma Thesis on the synthesis of the argininamide **8** (Scheme 2).[ii] All attempts to cleave the phthaloyl group with hydrazine in isopropanol failed. In all cases the 5-aminopentanoyl group is cleaved to obtain the arginineamide **10**.

[ii] Freund, M. Diploma Thesis, **2004**, University of Regensburg, Germany



Scheme 2. Cyclisation of **8**

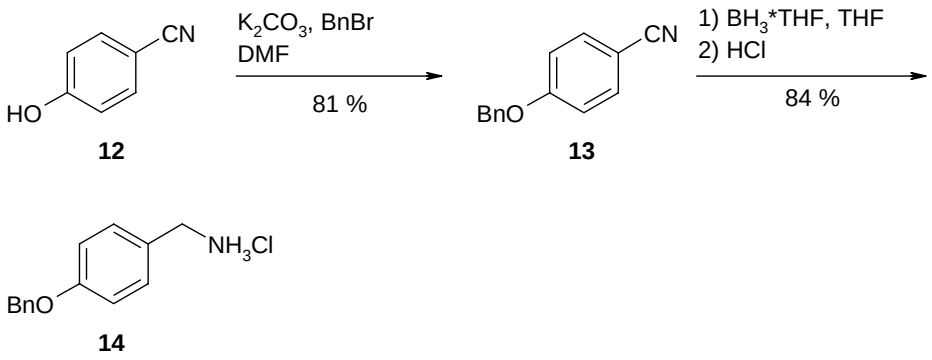
For this reasons a new synthetic route is necessary to prepare bivalent NPY Y_1 receptor antagonists like **6**. Scheme 3 shows the retrosynthetic analysis for a possible synthetic access to bivalent NPY Y_1 antagonists.



Scheme 3. Retrosynthetic analysis of the target molecule **6**

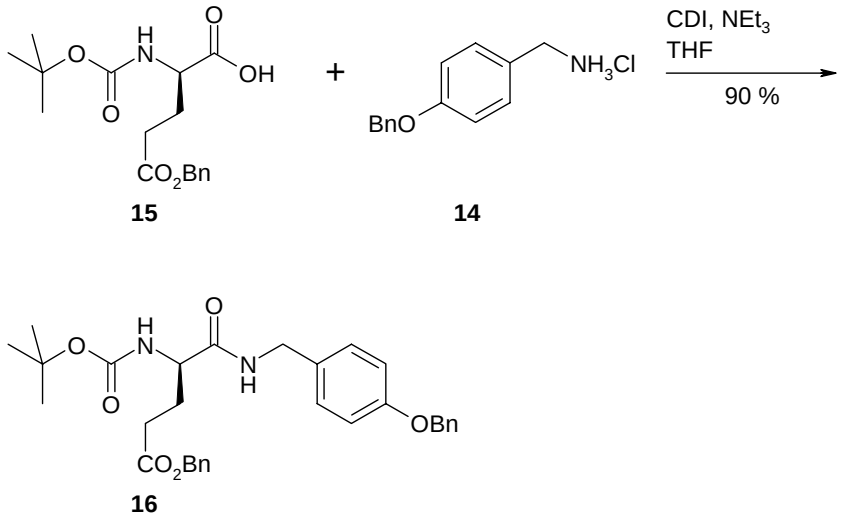
The Mitsunobu reaction is an efficient method to build up complex guanidine-containing compounds.[42] Two eqivalents of the alcohol **10** should react with one equivalent of the bis-guanidines **11** in a double Mitsunobu reaction to give the bivalent ligands **6** with different spacer lengths. For the synthesis it is necessary that all protection groups can be cleaved in one step. The Cbz-groups on the guanidinium moiety and the benzyl-group can easily be removed via hydrogenolytic cleavage.

The 4-benzyloxy-benzylamine hydrochloride **14** was prepared according to a literature known procedure (Scheme 4),[43] starting from commercially available 4-hydroxybenzonitrile (**12)** and benzylbromide to obtain the 4-benzyloxybenzonitrile (**13**) in good yields. The reduction of the nitrile group to the primary amine with boran-THF-complex gives the target compound **14** in very good yields.



Scheme 4. Synthesis of the benzyloxy-benzylamine hydrochloride **14**

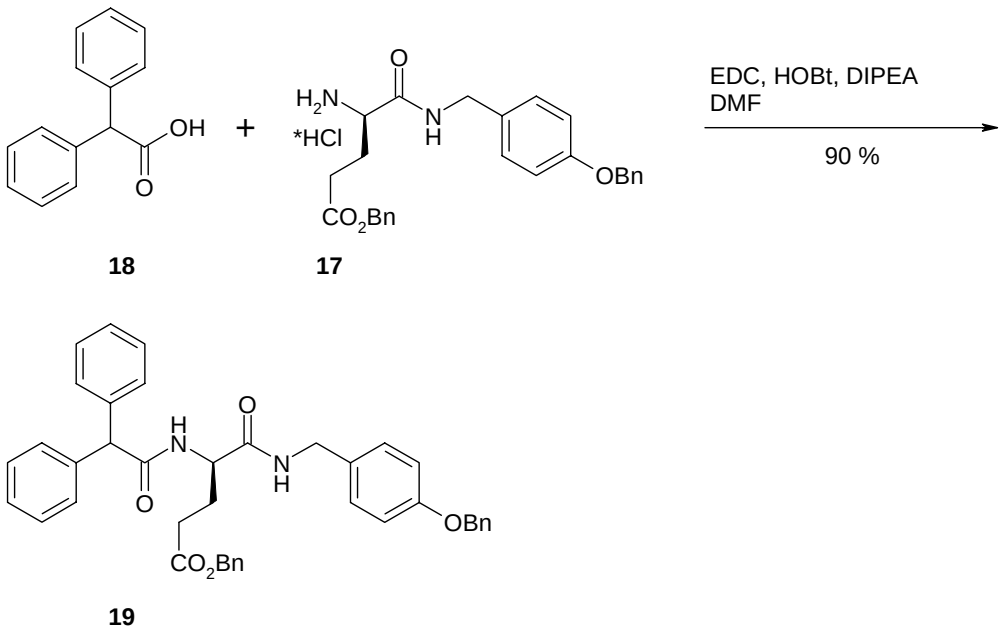
The benzylamine derivative **14** was then coupled with the commercially available (D)-Boc-Glu(OBn)-OH (**15**) in very good yields using CDI as an activation reagent for the glutamic acid (Scheme 5).



Scheme 5. Synthesis of the glutamic acid derivative **16**

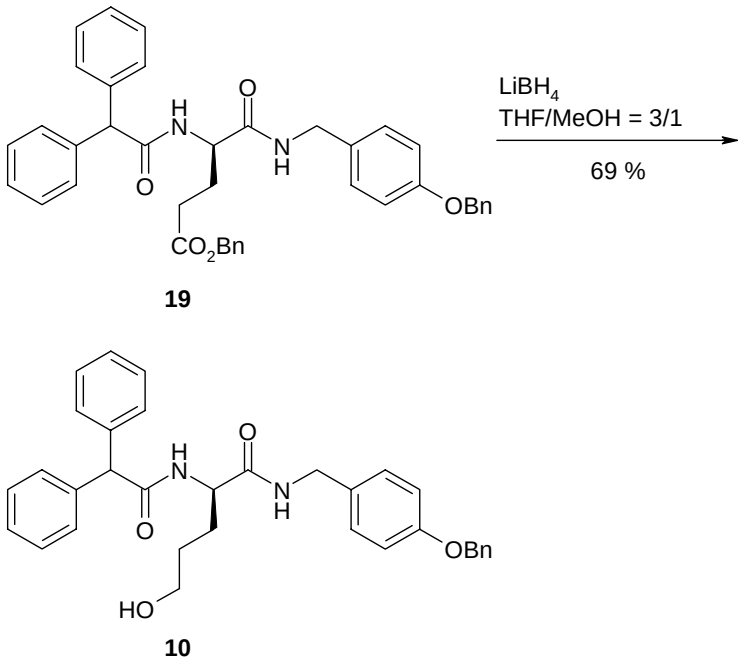
The resulting glutamic acid derivative **16** was purified by column chromatography on silica gel. Reaction conditions using EDC and HOBt to create the active ester of **15** gave nearly the same yields. The following cleavage of the Boc-group in HCl saturated ether yielded the ammonium chloride **17** quantitatively.

Peptide coupling with 1,1-diphenylacetic acid (**18**) resulted in **19** (Scheme 6). Using EDC/HOBt to create the active ester of **18** the yields are 90 %, while CDI gave only 60 %.



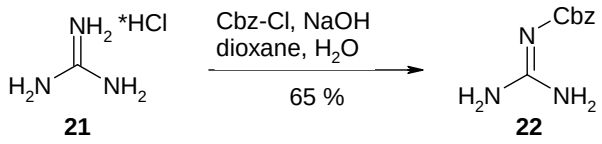
Scheme 6. Synthesis of the glutamic acid derivative **19**

In the last step the benzylester in the side chain of **19** was reduced to the primary alcohol with $LiBH_4$ in a mixture of THF/MeOH = 3/1 at room temperature in good yields (Scheme 7).



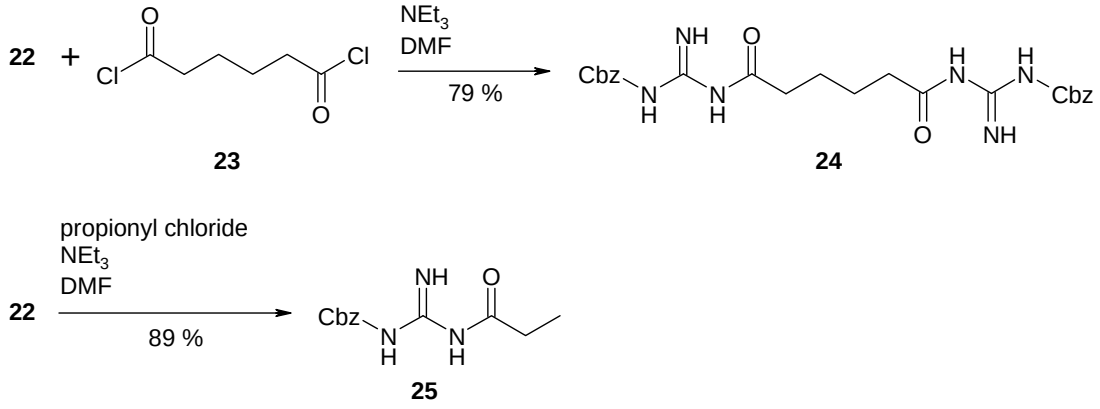
Scheme 7. Reduction of **19** to obtain the primary alcohol **10**

The synthesis of the bis-guanidines started from mono-Cbz-protected guanidine, which was prepared from guanidinium hydrochloride (**21**) and Cbz-Cl by a literature known procedure (Scheme 8).[44]



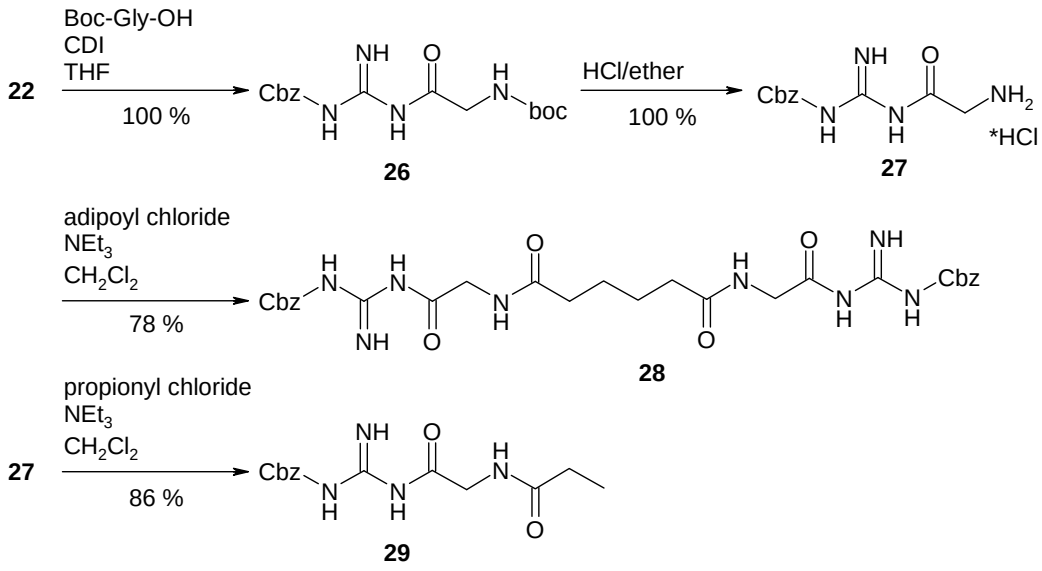
Scheme 8. Synthesis of mono-Cbz-protected guanidine **22**

The next steps followed well-known peptide coupling procedures. The guanidinium **22** was coupled with adipinic acid dichloride (**23**) to obtain the bis-guanidine **24**. The corresponding mono-guanidine **25** was synthesized via coupling of the mono-Cbz-protecting guanidine **22** with propionyl chloride in very good yields (Scheme 9).



Scheme 9. Synthesis of mono-Cbz-protected guanidine **22**

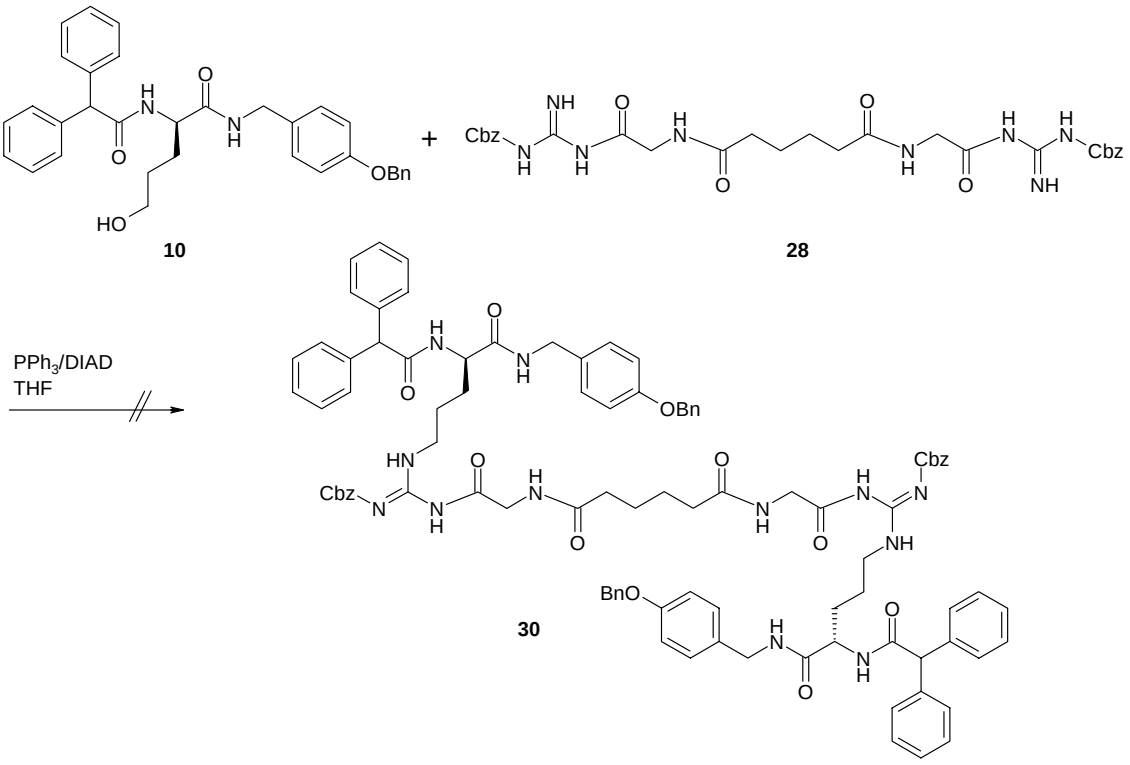
The synthesis of bis-guanidines with longer spacers followed the same synthetic procedure (Scheme 10).



Scheme 10. Synthesis of bis-guanidine **28**

Guanidinium **22** was coupled with Boc-Gly-OH, which was activated by CDI, to obtain the acylated guanidine **26** in quantitative yields. Cleavage of the Boc-group with HCl saturated ether led to ammonium chloride **27**, which was coupled with adipinic acid dichloride to the bis-guanidine **28**. Coupling of the ammonium chloride **27** with propionyl chloride gave the corresponding mono-guanidine **29** in good yields. In order to obtain longer spacers the dipeptide Boc-Gly-Gly-OH or the tripeptide Boc-Gly-Gly-Gly-OH may be used instead of Boc-Gly-OH.

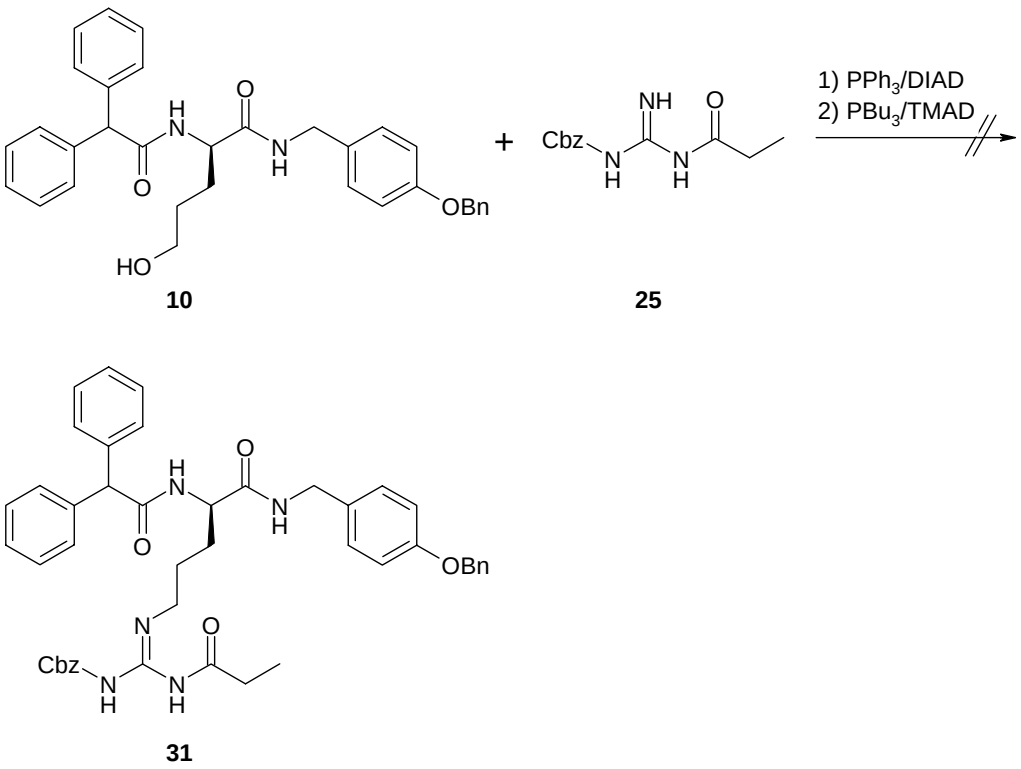
In the next step the alcohol **20** and the bis-guanidine **11** should be converted into the bivalent compound **6** under standard Mitsunobu reaction conditions (Scheme 11).



Scheme 11. Mitsunobu reaction to obtain the bivalent compound **30**

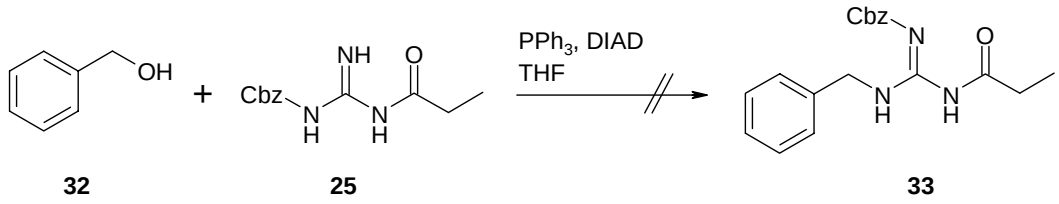
Unfortunately, the synthesis of the bivalent compound **30** via a double Mitsunobu reaction failed in all attempts. Variations of the solvent and variations of the temperature showed no conversion. The use of DEAD instead of DIAD and PBu_3 instead of PPh_3 showed also no conversion. *Harusawa* and co-workers have reported on the synthesis of (+)-α-Skytanthine, using the *N*,*N*,*N´*,*N´*-tetramethylazodicarboxamide (TMAD) in combination with tributylphosphine as a powerful azodicarboxylate in the Mitsunobu reaction. [45] In this case the alcohol **10** was isolated nearly quantitatively.

The Mitsunobu reactions of the alcohol **10** with the mono-guanidine **25** to obtain the monovalent compound failed (Scheme 12).



Scheme 12. Attempted Mitsunobu reaction to obtain the monovalent compound **31**

To investigate the reason for the failure of the Mitsunobu reaction, simple model reactions were carried out. In the first series the reactivity of the guanidines was investigated. The monoguanidine **25** was treated with benzylalcohol under standard Mitsunobu reaction conditions (Scheme 13).



Scheme 13. Mitsunobu test reaction to obtain the monovalent compound **33**

A formation of the product **33** was not observed. The monoguanidine **25** was isolated from the reaction mixture in nearly quantitative yields. The repetition of the same reaction with MeOH instead of the benzylalcohol **32** as alcohol component led to the same negative result. Attempts with monoguanidine **29** showed also no formation of the expected products. In the next trials the reactivity of the alcohol compound **10** was

investigated. *Goodman* and co-workers have shown that the *N,N´,N´´*-tri-Boc-guanidine (**34**) and the *N,N´,N´´*-tri-Cbz-guanidine (**35**) are useful guanidinylation reagents for the facile conversion of alcohols to substituted guanidines (Figure 8).[42]

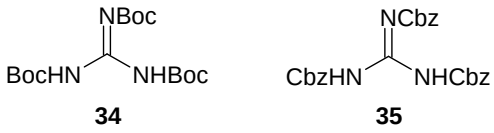
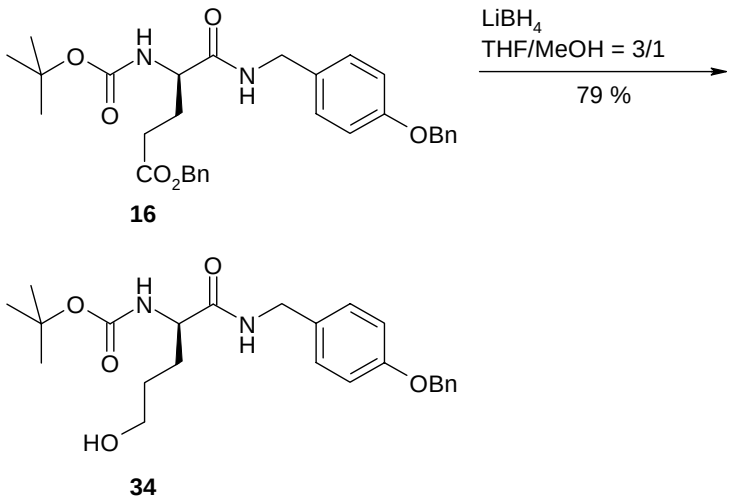


Figure 8. Guanidinylation reagents **34** and **35**

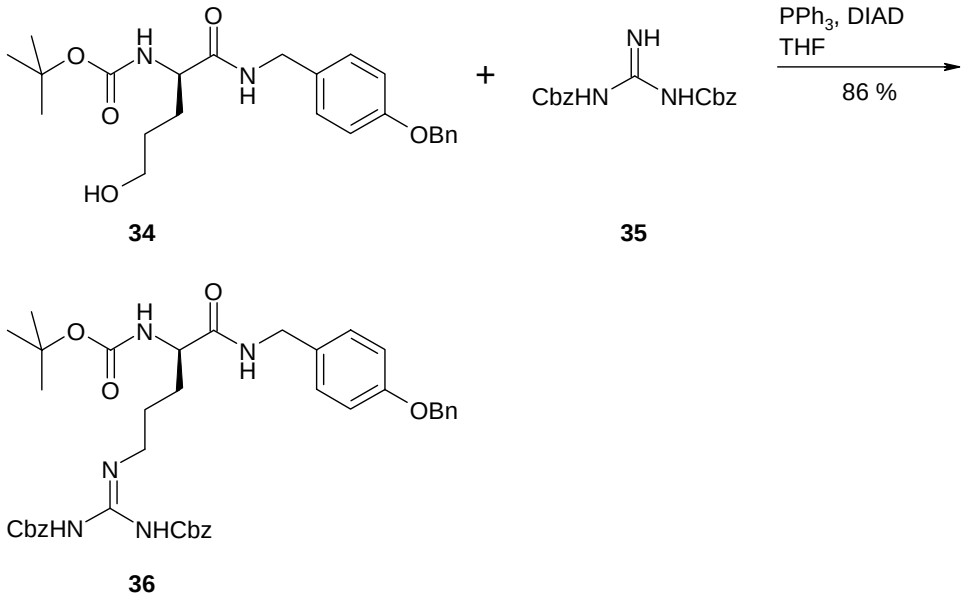
They synthesised a series of arginine analogues via condensation of a primary or secondary alcohol with the guanidinylation reagents **34** and **35** under Mitsunobu conditions to produce protected alkylated guanidines. The alcohol compound **10** was therefore treated both with *N,N´,N´´*-tri-Boc-guanidine (**34**) and with the *N,N´,N´´*-tri-Cbz-guanidine (**35**). Unfortunately, we observed no formation of the expected products in both cases. The alcohol **10** and the guanidinylation reagents **34** and **35** were isolated nearly quantitatively. An explanation for these results could not be provided within the scope of this PhD thesis.

The benzylester function in the side chain of the glutamic acid derivative **16** can easily be reduced to the primary alcohol **36** in good yields, using $LiBH_4$ as the reducing reagent (Scheme 14).



Scheme 14. Reduction of the glutamic acid derivative **16** to the primary alcohol **34**

The primary alcohol **34** was treated in the following reaction with *N,N´*-di-Cbz-Guanidine (**35**) under Mitsunobu conditions and the expected product **36** was isolated from the reaction mixture by column chromatographie in 86 % yield (Scheme 15).



Scheme 15. Mitsunobu reaction of the alcohol **34** with *N,N´*-Di-Cbz-guanidine (**35**)

N,N´-di-Cbz-guanidine (**35**) was prepared according to a literature known procedure.[42] The outcome of this reaction has shown that the diphenyl-part in the primary alcohol **10** has an influence on the reaction mechanism of the Mitsunobu reaction. One explanation

could be the steric shielding of the OH-function by the two phenyl rings. Based on the results of the Mitsunobu reaction shown in Scheme 15, a new strategy to build up bivalent NPY Y_1 receptor antagonists was developed. In this new strategy the two pharmacophores should be connected via a peptide spacer, but linked on the guanidine function by an alkyl chain (Figure 10).

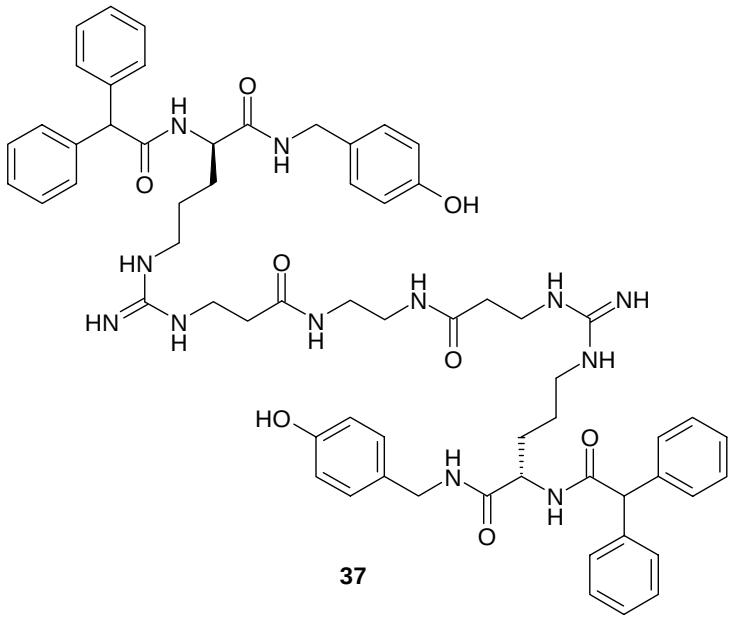
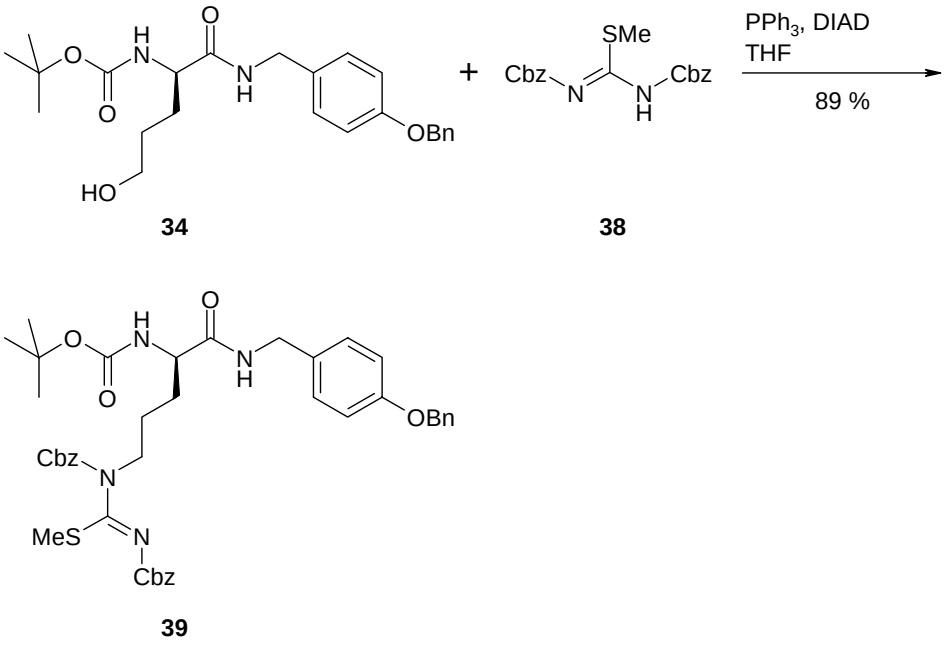


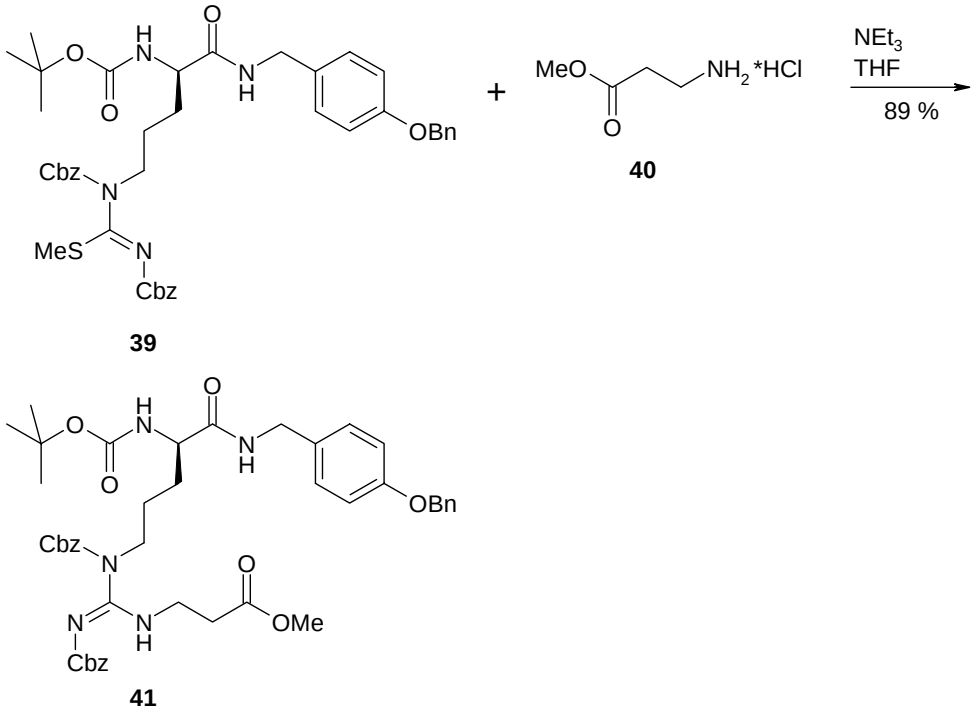
Figure 10. Alkylated bivalent ligand **37**

The synthesis starts with the condensation of the primary alcohol **34** and the 1,3-di-Cbz-2-methyl-isothiourea (**38**), which is commercially available, under Mitsunobu conditions (Scheme 16). The isothiourea derivative **39** was isolated from the reaction mixture by column chromatographie in 89 % yield.



Scheme 16. Mitsunobu reaction of the alcohol **34** with 1,3-di-Cbz-2-methyl-isothiourea (**38**)

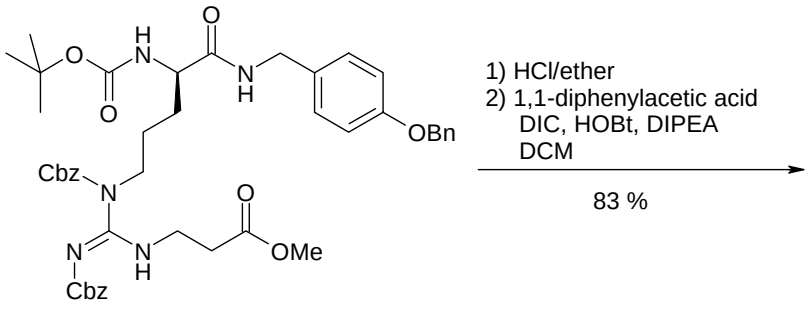
In the next step the isothiourea compound **39** was treated with β-Ala-OMe (**40**) to give the corresponding guanidinium derivative **41** (Scheme 17).



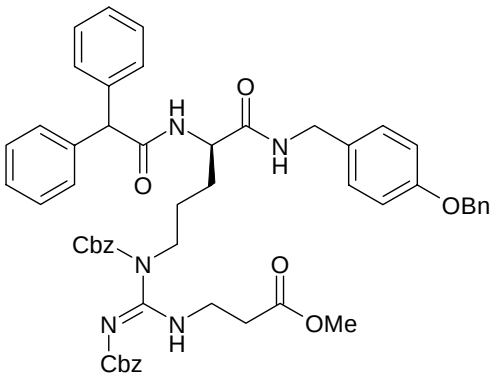
Scheme 17. Transformation of the isothiourea **39** to the guanidine **41**

For the transformation of isothioureas to guanidines mercury(II)-chloride ($HgCl_2$) is often used. In this case the use of $HgCl_2$ gave guanidine **41** in yields between 25 % and 35 %. The TLC indicates the formation of many side products. This and the lower yields compared to the reaction in refluxing THF indicates that by the use of $HgCl_2$ side reactions and decomposition are induced.

The Boc-group was then cleaved with HCl saturated ether in very good yields to obtain the ammonium chloride, which was coupled next with 1,1-diphenylacetic acid in good yields. The product **42** was isolated from the reaction mixture by column chromatography.



41

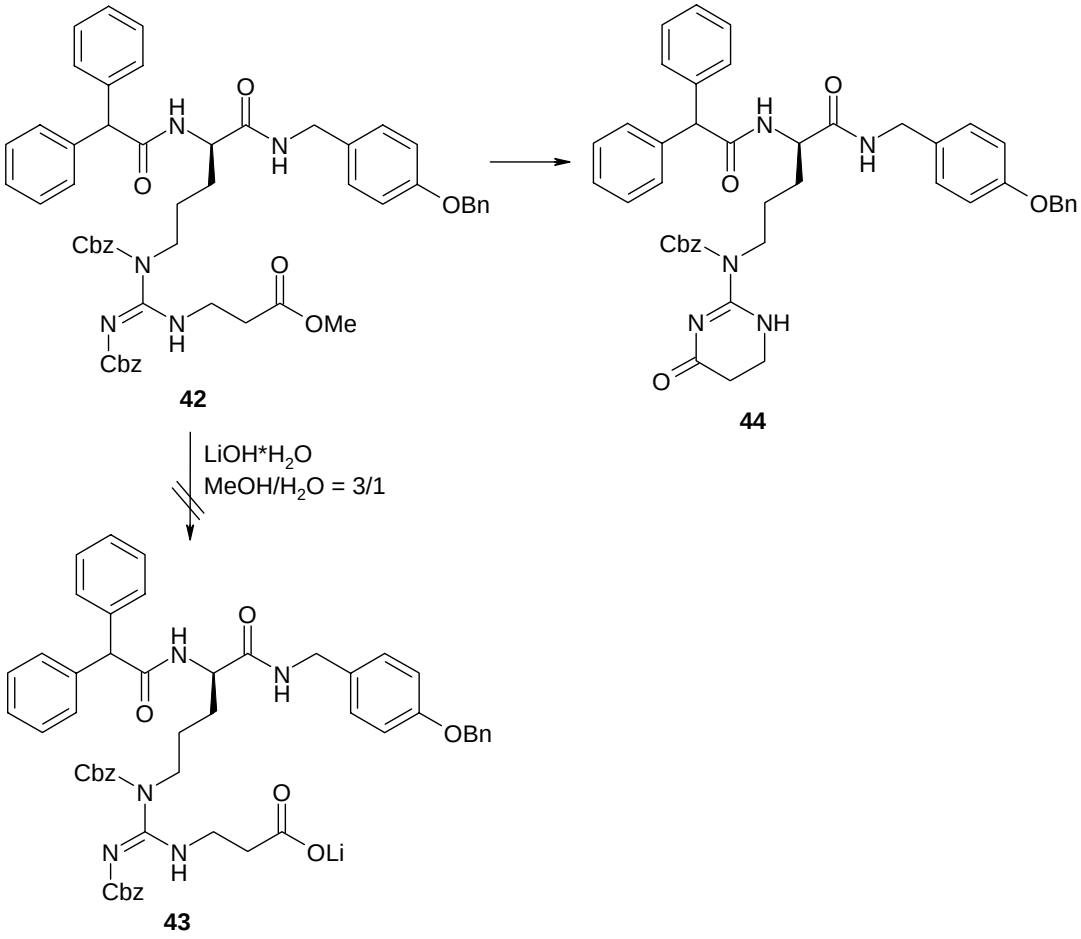


42

Scheme 18. Synthesis of the protected alkylated BIBP 3226 derivative **42**

Saponification of the methyl ester with LiOH and the following peptide coupling with ethylene diamine should gave the alkylated bivalent ligand **37**. For the cleavage of the methylester the guanidinium methylester **43** was dissolved in a mixture of MeOH/H_2O = 3/1 and 1 equivalent of LiOH*H_2O was added to the reaction mixture. After 15 min a solid began to precipitate from the reaction mixture. After 12 h the solid was filtered off

and the analysis revealed the formation of a single cyclic product **45** in pure form. The carboxylate **44** could not be identified in the filtrate (Scheme 19).



Scheme 19. Saponification of the methylester **42**

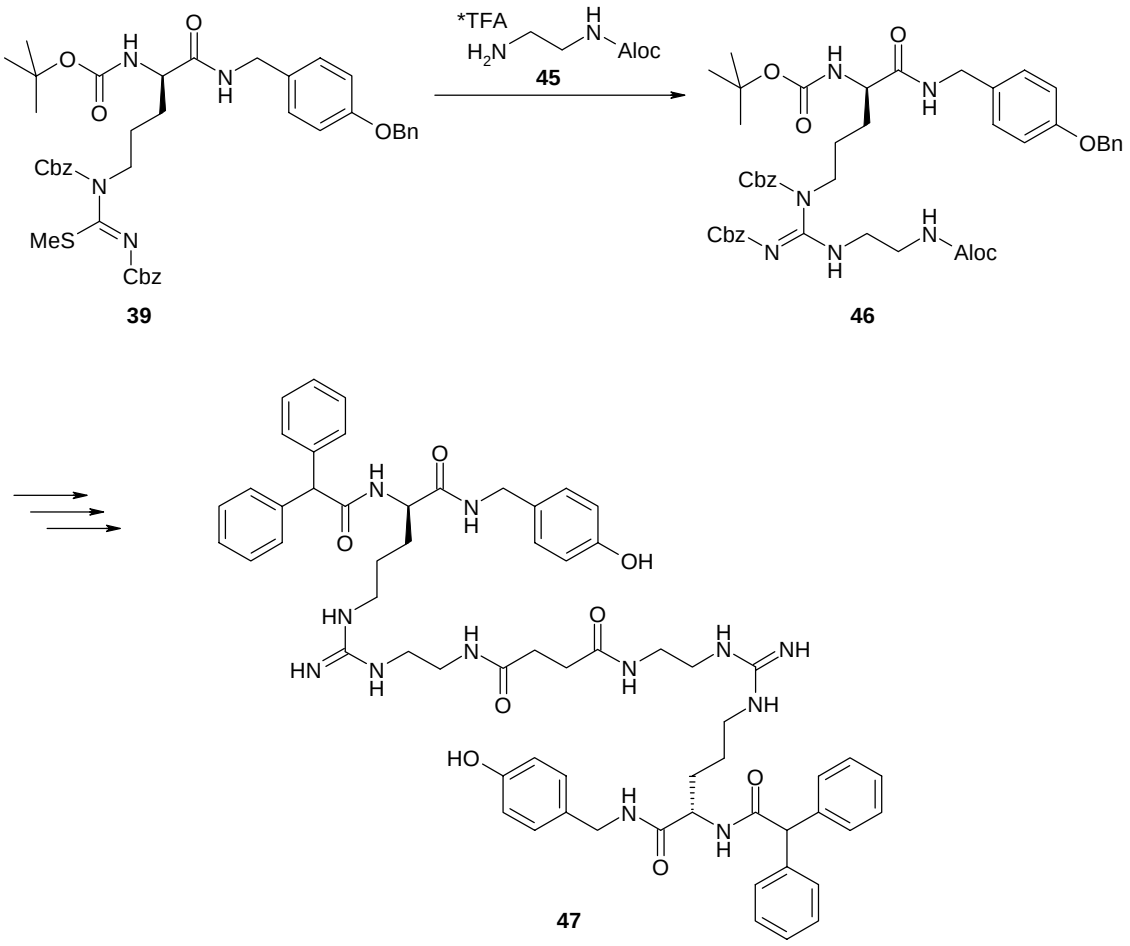
The explanation for this result is that the LiOH has first cleaved the Cbz-group and the free amino-group attacked the methylester to form the lactame. Attempts to cleave the ester under acid conditions or enzymatically failed also. A nucleophilic cleavage of the methyl ester with NaI was not successful. The careful analysis of the reaction mixture showed complete decomposition of the starting material.

Although we were not able to synthesis the bivalent compound **37**, we found an effective access to alkylated argininamide derivatives like **43**. Instead of the β-Ala-OMe it should be possible to use other amines or amino acids for the reaction shown in Scheme 17.

Dangles et al. have reported on the selective cleavage of an Aloc-group in the presence of a Cbz-group.[46] They used Aloc-Lys(Cbz)-OH in their studies and the selective

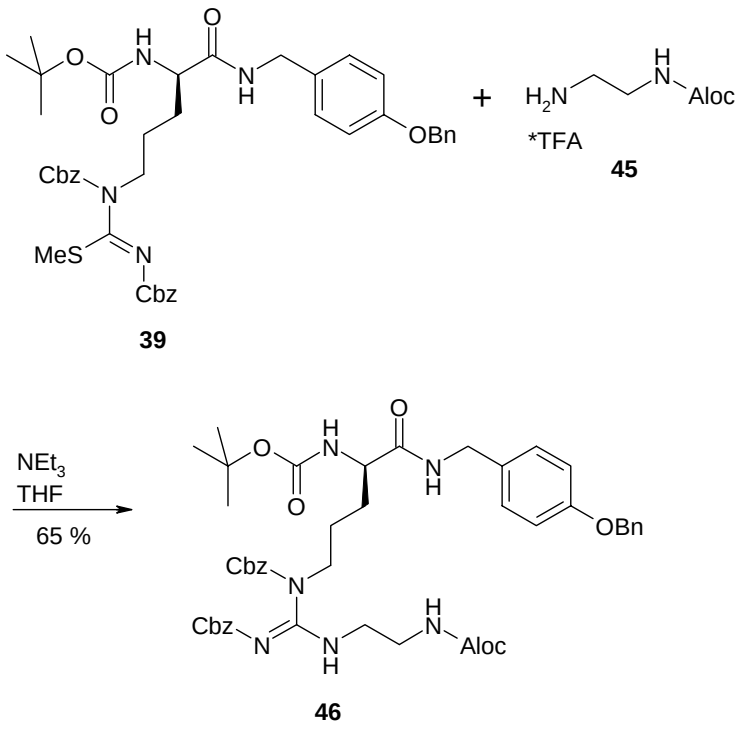
cleavage of the Aloc-group with $Pd(PPh_3)_4$ and Bu_3SnH obtained H-Lys(Cbz)-OH in yields between 95 % and 100 %.

The treatment of the isothiourea derivative **39** with mono-Aloc-protected ethylene diamine (**45**)[47], selective cleavage of the Aloc-group of compound **46** and peptide coupling with succinyl chloride should lead to the bivalent compound **47** (Scheme 20).



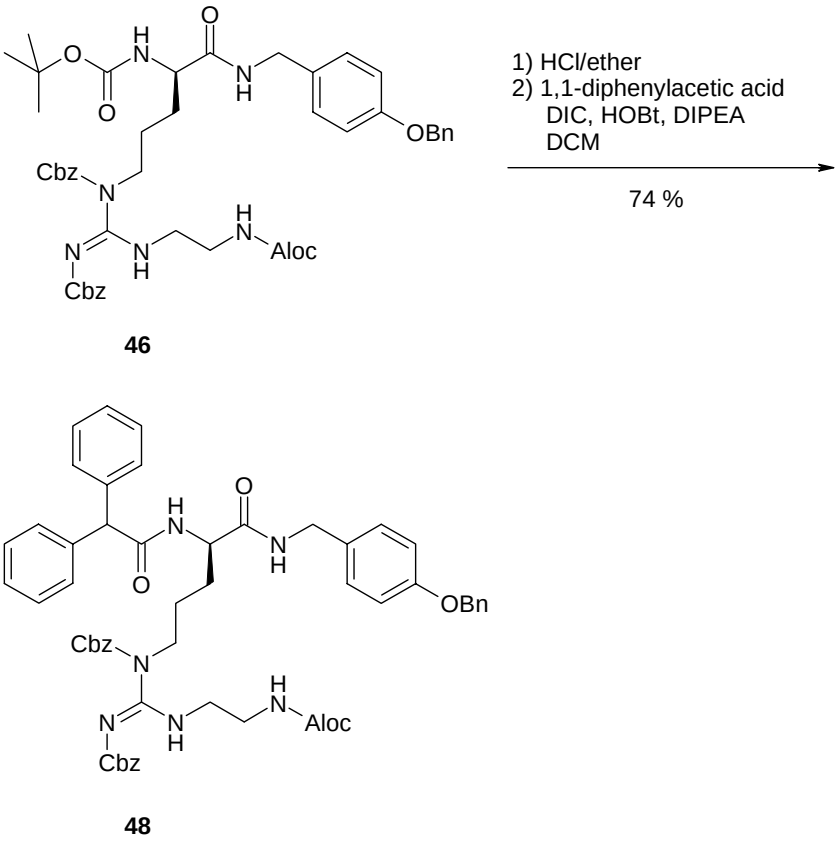
Scheme 20. New synthetic route via succinyl moiety

The synthesis starts with the treatment of the isothiourea with mono-Aloc-protected ethylene diamine (**45**) in refluxing THF to obtain the guanidine **46** in 65 % (Scheme 21).



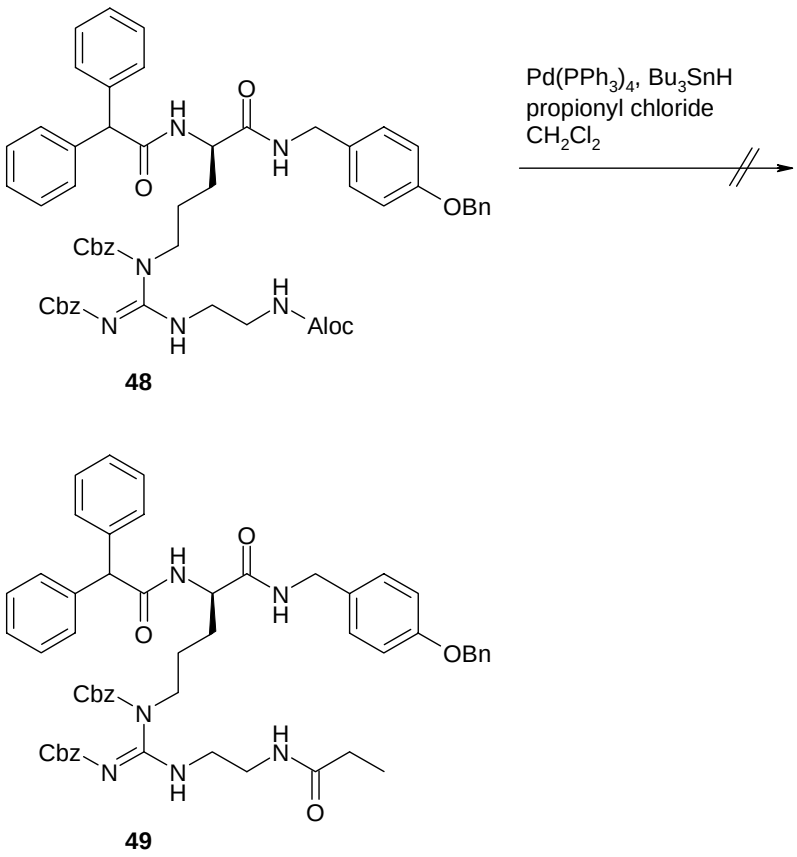
Scheme 21. Synthesis of the guanidine **46**

In the following step the Boc-group was cleaved with HCl saturated ether, the corresponding ammonium salt was treated with 1,1-diphenylacetic acid under standard peptide coupling conditions to give compound **48** in good yields (Scheme 22).



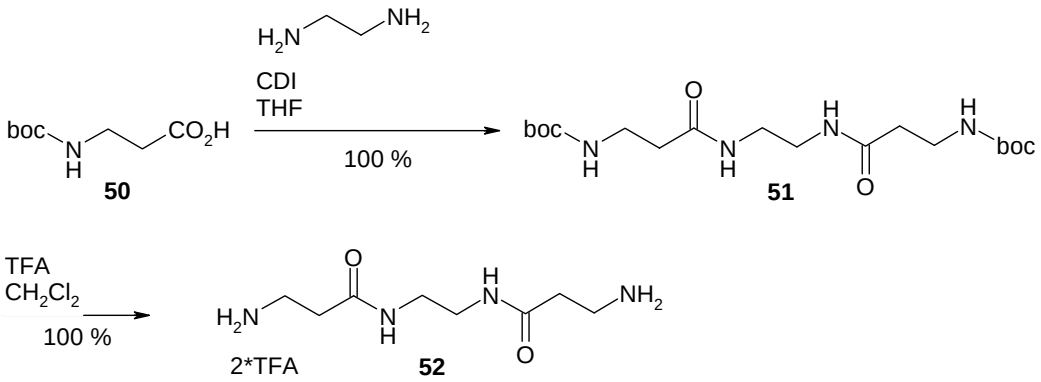
Scheme 22. Synthesis of the protected alkylated BIBP 3226 derivative **48**

Unfortunately, the selective cleavage of the Aloc-group and coupling with propionyl chloride to obtain the monovalent compound **49** failed (Scheme 23). Reaction monitoring by TLC showed complete conversion of the starting material. In the mass spectral analysis of the reaction mixture the peak of the product can not be found. Instead of this several peaks with lower masses are found. This is an indication that a decomposition of the starting material took place. A possible explanation is that the Cbz-groups are also cleaved under this conditions. Attempts with succinyl dichloride to obtain the corresponding bivalent compound failed.



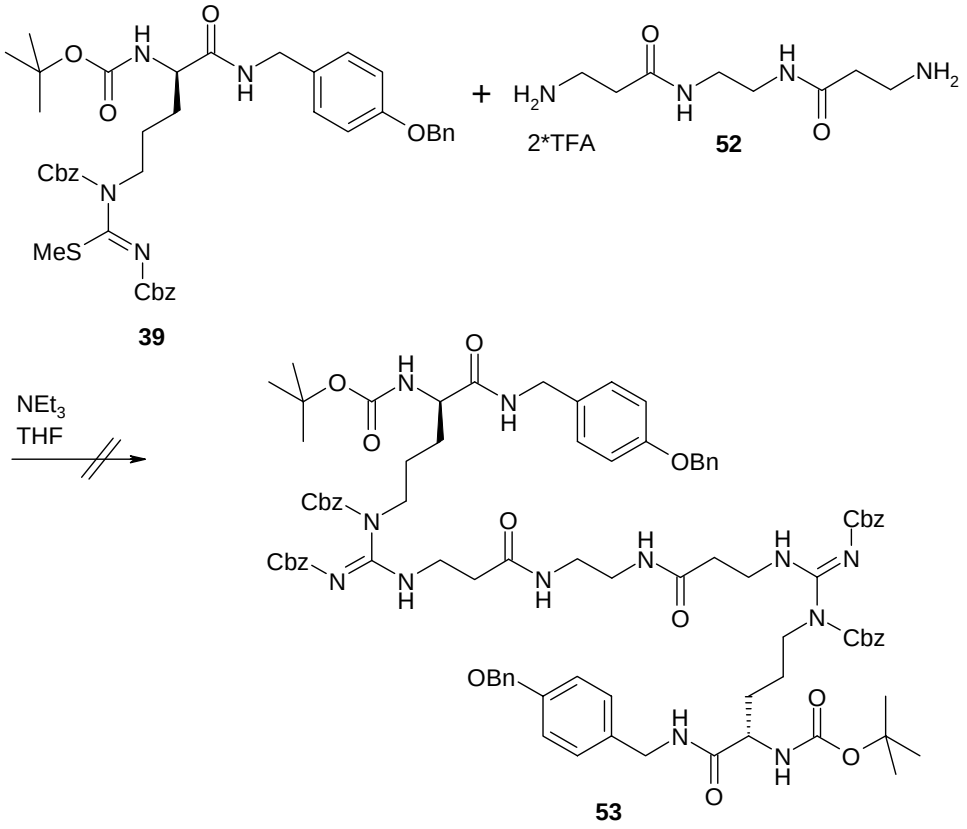
Scheme 23. Failed attempt to cleave the Aloc-group of the compound **48**

Based on these results we have tried another variant. The isothiourea **39** should be treated with a diamine to give the corresponding bivalent compound. The following cleavage of the two Boc-groups and coupling with the 1,1-diphenylacetic acid and final cleavage of the Cbz-groups and the benzyl-group should lead to the bivalent NPY Y_1 receptor antagonist **37**. We have first synthesized diamine **52**, starting from Boc-protected β-alanine and ethylene diamine in quantitative yields (Scheme 24).



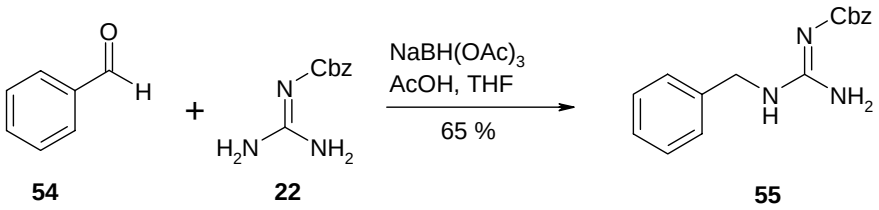
Scheme 24. Synthesis of the diamine **52**

Unfortunately, the attempted synthesis of the bivalent compound **53** failed (Scheme 25). Careful analysis of the reaction mixture revealed a complete decomposition of the starting material and no formation of the bivalent ligand **53**. Attempts with longer alkyl diamines like 1,6-diaminohexane and 1,8 diaminooctane did not improve the outcome of the reaction.



Scheme 25. Attempt to synthesis the bivalent compound **53**

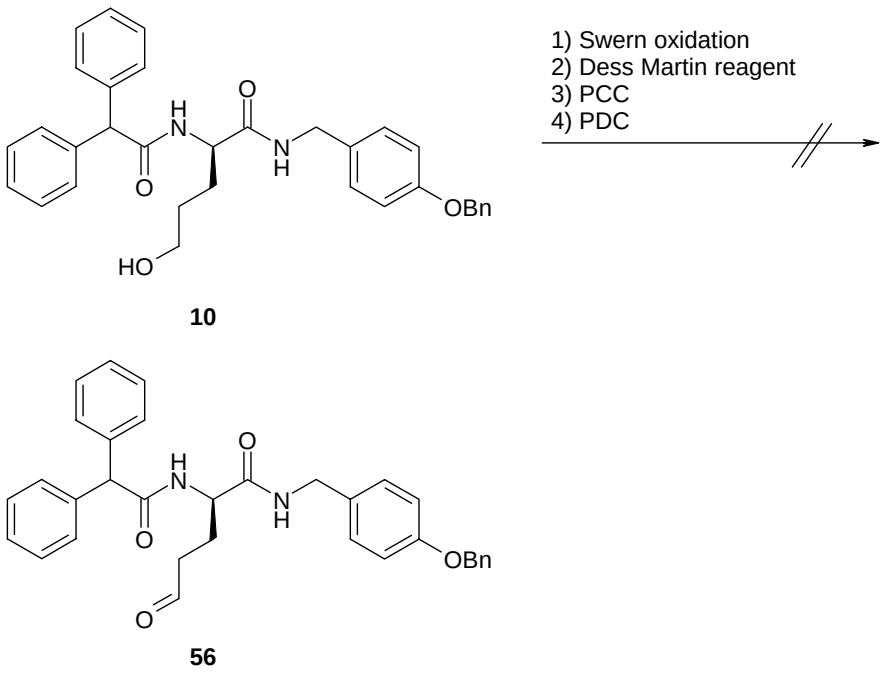
Reductive amination is another method for the synthesis of alkylated guanidines. The reductive amination reaction is the direct reaction of carbonyl compounds and amines with a proper reducing agent without prior isolation of the intermediate imine or iminium salt. One of the most common methods uses sodium cyanoborohydride ($NaBH_3CN$) for reduction.[48] Another reagent is the sodium triacetoxyborohydride [$NaBH(OAc)_3$].[49] We used this reagent for the synthesis of alkylated guanidines via reductive aminaion of an aldehyde with a guanidine (Scheme 26).



54 **22** **55**

Scheme 26. Reductive amination of benzaldehyde (**53**) with Cbz-guanidine **22**

Based on this result, we intended to synthesise bivalent and the corresponding monovalent compounds via reductive amination. Therefore the first step in this synthetic route was the preparation of the aldehyde **56** (Scheme 27).



Scheme 27. Attempted oxidation of the alcohol **10** to the aldehyde **56**

The synthesis of the aldehyde **56** failed in all four attempts. The analysis of the reaction mixture showed in the mass spectrum as the main product a compound with the molecular mass of 502. This corresponds to a difference of 18 to the aldehyde **56**. A possible explanation is that during the reaction water is eliminated and a double bond is formed. An exact analysis by means of the NMR-spectrum was not possible.

3.3 Conclusion

In summary, we have reported the synthesis of bis-guanidines **24** and **28**. The alcohol **10** could be obtained in good yields starting from commercially available Boc-D-Glu(OBn)-OH **15**. The double Mitsunobu reaction of alcohol **10** with bis-guanidine **24** to form the bivalent ligand **30** failed. To investigate the reason for the failure of the Mitsunobu reaction, simple model reactions were carried out. As a result we found that the diphenyl-part in the primary alcohol **10** has an influence on the reaction mechanism of the Mitsunobu reaction. One explanation could be the steric shielding of the OH-function by the two phenyl rings.

Furthermore the synthesis of alkylated BIBP 3226 derivatives could be achieved by simple Mitsunobu reaction of the alcohol **34** with 1,3-di-Cbz-2-methyl-isothiourea **38** to get the isothiourea **39**. The compound is useful as a precursor for the synthesis of alkylated guanidines by treatment with different amines.

Reductive amination is another method for the synthesis of alkylated guanidines. The reductive amination of benzaldehyde (**53**) with Cbz-guanidine **22** yielded the alkylated guanidine **55** in 65 %. However, a synthesis of an aldehyde corresponding to alcohol **10** was not possible.

3.4 Experimental Section

Melting points (mp) were determined with a Büchi SMP 20 and are uncorrectd.

IR-spectra were recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

NMR: Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz, T = 300 K), Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 300 K), Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [pp] relative external standards (solvent residual peak). The spectra were analysed by first order, the coupling constants are in Hertz [Hz]. Characterisation of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet. Integration is determined as the relative number of atoms. Error of reported values: 0.01 ppm for ^{1}H-NM, 0.1 ppm for ^{13}C-NMR; coupling constants: 0.1 Hz. The used solvent is reported for each spectrum.

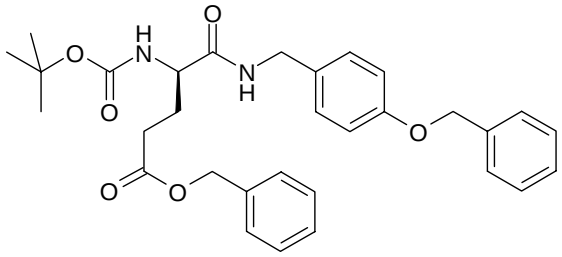
MS-Spectra: Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

Elemental Analysis: Microanalytical Laboratory of the University of Regensburg.

Thin layer chromatography (TLC) was performed on aluminia plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 nm). Visualisation was accomplished byUV-light (λ = 254 nm) and ninhydrine in MeOH.

Column Chromatography was performed on silica gel (70-230 mesh) from Merck.

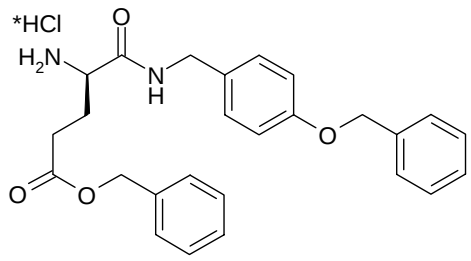
Synthesis



(R)-4-(Benzyloxy-benzylcarbamoyl)-4-*tert*-butoxycarbamoylamino-butyric acid benzylester (16).

Boc-D-Glu(OBn)-OH (782 mg, 2.32 mmol) **(15)** was dissolved in 25 mL THF and CDI (376 mg, 2.32 mmol) was added. The reaction mixture was stirred at room temperature for 15 min. Next the hydrochloride **14** (579 mg, 2.32 mmol) and NEt_3 (352 mg, 0.482 mL, 3.48 mmol) were added and the solution was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **16** (1.10 g, 2.07 mmol, 90 %) as a white solid. R_f = 0.85 (EtOAc)

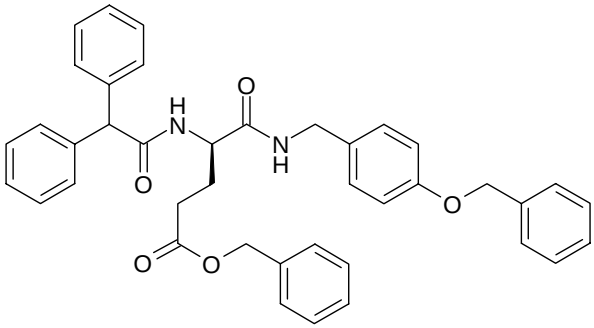
mp: 73 °C. - IR (KBr) [cm^{-1}]: 3389, 3326, 2977, 2925, 1750, 1689, 1655, 1535, 1474, 1363, 1251, 1172. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.89 – 2.59 (m, 4 H, -C*H*$_2$-C*H*$_2$-), 4.15 – 4.26 (m, 1 H, NH-C*H*-CO), 4.34 (d, J = 5.6 Hz, 2 H, NH-C*H*$_2$-), 5.03 (s, 2 H, -C*H*$_2$-Ph), 5.10 (s, 2 H, -C*H*$_2$-Ph), 5.43 (d, J = 7.8 Hz, 1 H, Boc-N*H*-), 6.70 (bs, -N*H*-CH_2-), 6.87 – 6.94 (m, 2 H, AA´-part of a AA´BB´-system), 7.13 – 7.19 (m, 2 H, BB´-part of a AA´BB´-system), 7.30 – 7.45 (m, 10 H, 2 x Ph). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.0 (+), 28.3 (-), 30.5 (-), 43.0 (-), 53.8 (+), 66.6 (-), 70.0 (-), 80.1 (C_{quat}), 115.0 (+), 127.5 (+), 128.0 (+), 128.3 (+), 128.4 (+), 128.6 (+), 129.0 (+), 130.3 (C_{quat}), 135.7 (C_{quat}), 136.9 (C_{quat}), 155.8 (C_{quat}), 158.2 (C_{quat}), 171.4 (C_{quat}), 173.1 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 533.3 (100) [MH^+], 477.2 (66) [$MH^+ - C_4H_8$], 433.2 (10) [MH^+ – Boc]. – Elemental analysis calcd. (%) for $C_{31}H_{36}N_2O_6$ (532.28): C 69.89, H 6.82, N 5.26; found C 69.84, H 6.67, N 5.01.



(R)-4-(Benzyloxy-benzylcarbamoyl)-4-amino-butyric acid benzylester hydrochloride (17).

Compound **16** (1.10 g, 2.07 mmol) was dissolved in 5 mL dichloromethane and 15 mL HCl saturated ether was added. The reaction mixture was stirred at room temperature for 2 h. The solvent is evaporated and the product dried under high vacuum. **17** (969 mg, 2.07 mmol, 100 %) is a white solid.

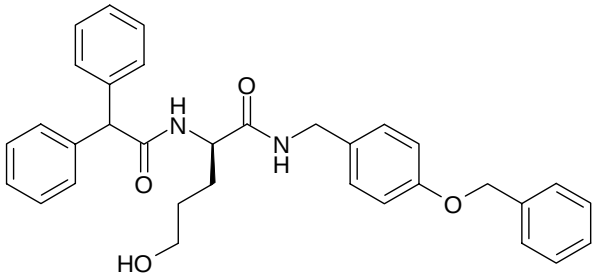
mp: 97 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.98 – 2.10 (m, 2 H, -CH-C*H*$_2$-), 2.41 – 2.47 (m, 2 H, -C*H*$_2$-CO_2Bn), 4.18 – 4.30 (m, 2 H, -NH-C*H*$_2$-), 5.02 (s, 2 H, -C*H*$_2$-Ph), 5.08 (s, 2 H, -C*H*$_2$-Ph), 6.92 – 7.00 (m, 2 H, AA´-part of a AA´BB´-system), 7.18 – 7.25 (m, 2 H, BB´-part of a AA´BB´-system), 7.30 – 7.48 (m, 10 H, 2 x Ph), 8.39 (bs, 2 H, N*H*$_2$). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 26.1 (-), 29.0 (-), 39.6 (+), 41.7 (-), 51.5 (+), 65.6 (-), 69.0 (-), 114.6 (+), 127.5 (+), 127.7 (+), 127.9 (+), 128.0 (+), 128.3 (+), 128.4 (+), 128.7 (+), 135.9 (C_{quat}), 137.0 (C_{quat}), 157.3 (C_{quat}) 167.7 (C_{quat}), 171.4 (C_{quat}). - MS (ESI, AcN/H_2O): m/z (%) = 865.8 (22) [2 MH^+], 433.4 (100) [MH^+]. – $C_{26}H_{29}N_2O_4Cl$ (468.18).



(R)-4-(Benzyloxy-benzylcarbamoyl)-4-diphenylacetylamino-butyric acid benzylester (19).

Compound **17** (1.07 g, 2.29 mmol), Diphenylacetic acid **18** (486 mg, 2.29 mmol) and HOBt (309 mg, 2.29 mmol) were dissolved in 20 mL DMF. After addition of EDC (356 mg, 0. 405 mL, 2.29 mmol) and DIPEA (296 mg, 0.392 mL, 2.29 mmol) the reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **19** (1.29 g, 2.06 mmol, 90 %) as a white solid. R_f = 0.80 (EtOAc)

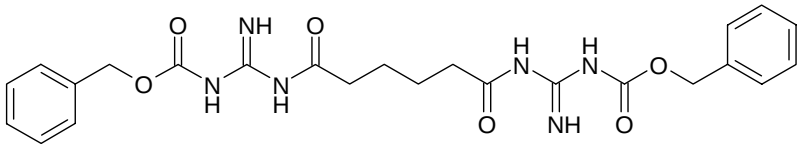
mp: 93 °C. - IR (KBr) [cm^{-1}]: 3278, 3061, 3008, 2911, 2889, 1728, 1645, 1539, 1513, 1452, 1248, 1174, 1032, 742, 697. – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.83 – 2.60 (m, 4 H, C*H*$_2$-C*H*$_2$), 4.28 (d, J = 6.3 Hz, NH-C*H*$_2$-), 4.45 – 4.56 (m, 1 H, C*H*), 4.83 – 4.91 (m, 1 H, C*H*), 5.03 (s, 2 H, -C*H*$_2$-Ph), 5.08 (s, 2 H, -C*H*$_2$-Ph), 6.52 – 6.65 (m, 2 H, 2 x N*H*), 6.92 – 7.00 (m, 2 H, AA´-part of a AA´BB´-system), 7.18 – 7.25 (m, 2 H, BB´-part of a AA´BB´-system), 7.30 – 7.48 (m, 20 H, 4 x Ph). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 27.5 (-), 30.4 (-), 43.0 (-), 52.7 (+), 58.7 (+), 66.6 (-), 70.0 (-), 115.0 (+), 127.4 (+), 127.5 (+), 128.0 (+), 128.3 (+), 128.4 (+), 128.6 (+), 128.8 (+), 128.8 (+), 128.9 (+), 129.0 (+), 130.2 (C_{quat}), 135.7 (C_{quat}), 137.0 (C_{quat}), 139.0 (C_{quat}), 158.2 (C_{quat}), 170.7 (C_{quat}), 172.5 (C_{quat}), 173.2 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 1253.6 (8) [2M + H^+], 644.3 (10) [M + NH_4^+], 627.3 (100) [MH^+]. - Elemental analysis calcd. (%) for $C_{40}H_{38}N_2O_5$ (626.28): C 76.64, H 6.12, N 4.47; found C 76.73, H 6.19, N 4.54.



(R)-2-Diphenylacetylamino-5-hydroxy-pentanoic acid 4-benzyloxy-benzylamide (10).

Compound 19 (888 mg, 1.41 mmol) was dissolved in 20 mL THF/MeOH = 3/1 and $LiBH_4$ (92 mg, 4.23 mmol) was added in small portions. After 15 h a saturated solution of $NaHCO_3$ (25 mL) was added and the organic solvent was evaporated. The water phase was extracted with DCM (2 x 30 mL). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain 10 (506 mg, 0.97 mmol, 69 %) as a white solid. R_f = 0.40 (EtOAc).

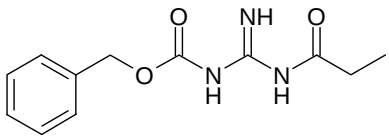
mp: 81 °C. - IR (KBr) [cm^{-1}]: 3278, 3061, 3008, 2911, 2889, 1728, 1645, 1539, 1513, 1452, 1248, 1174, 1032, 742, 697. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.65 – 1.93 (m, 4 H, -C*H*$_2$-C*H*$_2$), 3.48 – 3.62 (m, 2 H, -C*H*$_2$-OH), 4.28 (d, J = 6.2 Hz, 2 H, -NH-C*H*$_2$-), 4.90 (s, 1 H, Ph-C*H*-Ph), 5.03 (s, 2 H, -C*H*$_2$-Ph), 6.75 – 6.83 (m, 2 H, AA´-part of a AA´BB´-system), 7.08 – 7.12 (m, 2 H, BB´-part of a AA´BB´-system), 7.19 – 7.44 (m, 15 H, 3 x Ph). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 27.8 (-), 29.6 (-), 43.0 (-), 53.0 (+), 58.7 (+), 62.2 (-), 70.0 (-), 115.0 (+), 127.4 (+), 127.5 (+), 128.0 (+), 128.6 (+), 128.8 (+), 128.9 (+), 129.0 (+), 130.2 (C_{quat}), 136.9 (C_{quat}), 139.0 (C_{quat}), 158.1 (C_{quat}), 171.3 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 523.3 (100) [MH^+]. - Elemental analysis calcd. (%) for $C_{33}H_{34}N_2O_4$ (522.25): C 75.83, H 6.56, N 5.36; found C 75.60, H 6.60, N 5.04.



_N_-(Benzyloxycarbonyl)-_N´_-{6-[_N´_-(benzyloxycarbonyl)-guanidino]-6-oxy-hexanoyl}-guanidine (24).

Guanidine 22 (694 mg, 3.59 mmol) was dissolved in 15 mL DMF, NEt_3 (363 mg, 0.498 mL, 3.59 mmol) and adipoyl chloride 23 (329 mg, 0.262 mL, 1.79 mmol) were added. The reaction mixture was stirred at 60 °C for 18 h. The solution was allowed to cool at 0 °C and treated with water. The precipitate was filtered off and washed thoroughly with ice-water. The product was dried under high vacuum. 24 (699 mg, 1.41 mmol, 79 %) is a white solid.

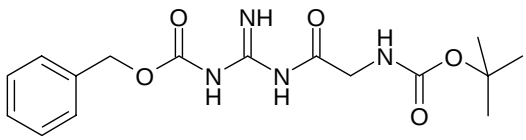
mp: 109 °C. - IR (KBr) [cm^{-1}]: 3363, 3250, 2995, 2944, 1694, 1627, 1542, 1269, 1164, 814, 744, 694. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.47 – 1.56 (m, 4 H, 2 x CH_2), 2.30 – 2.40 (m, 4 H, 2 x CH_2), 5.05 (s, 4 H, 2 x CH_2-Ph), 7.26 – 7.45 (m, 10 H, 2 x Ph), 8.87 (bs, 2 H, 2 x N*H*), 9.14 (bs, 2 H, 2 x N*H*), 11.00 (bs, 2 H, 2 x N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 23.5 (+), 35.6 (-), 39.4 (+), 39.7 (+), 39.9 (+), 65.4 (-), 127.2 (+), 127.5 (+), 127.6 (+), 128.2 (+), 128.3 (+), 137.3 (C_{quat})159.0 (C_{quat}), 163.1 (C_{quat}), 176.3 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 497.2 (100) [MH^+]. - Elemental analysis calcd. (%) for $C_{24}H_{28}N_6O_6$ (496.21): C 58.04, H 5.69, N 16.93; found C 57.52, H 5.65, N 16.54.



***N*-(Benzyloxycarbonyl)-*N´*-propionyl-guanidine (25).**

Guanidine **22** (416 mg, 2.15 mmol) was dissolved in 10 mL DMF, NEt_3 (217 mg, 0.298 mL, 2.15 mmol) and propionyl chloride (198 mg, 0.188 mL, 2.15 mmol) were added. The reaction mixture was stirred at room temperature for 12 h. The solution was allowed to cool at 0 °C and treated with water. The precipitate was filtered off and washed thoroughly with ice-water. The product was dried under high vacuum. **25** (475 mg, 1.91 mmol, 89 %) is obtained as a white solid.

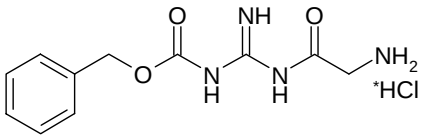
mp: 118 °C. - IR (KBr) [cm^{-1}]: 3388, 3317, 3268, 3011, 2967, 2879, 1713, 1652, 1615, 1545, 1449, 1386, 1286, 1180, 1102, 809, 752, 700. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.01 (t, J = 7.0 Hz, 3 H, -C*H*$_3$), 2.36 (q, J = 7.0 Hz, 2 H, -C*H*$_2$-CH$_3$), 5.06 (s, 2 H, -C*H*$_2$-Ph), 7.26 – 7.40 (m, 5 H, Ph), 8.87 (bs, 1 H, N*H*), 9.04 (bs, 1 H, N*H*), 10.92 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 8.5 (+), 29.3 (-), 65.3 (-), 127.2 (+), 127.5 (+), 128.2 (+), 137.3 (C_{quat}), 159.9 (C_{quat}). - MS (CI-MS, NH_3): m/z (%) = 250.0 (100) [MH^+]. – Elemental analysis calcd. (%) for $C_{12}H_{15}N_3O_3$ (249.11): C 57.82, H 6.07, N 16.86; found C 57.72, H 6.22, N 16.93.



{2-[*N*-(Benzyloxycarbonyl)-guanidino]-2-oxo-ethyl}-carbamic acid *tert*-butyl ester (26).

Guanidine **22** (1.03 g, 5.32 mmol) was dissolved in 15 mL THF and CDI (863 mg, 5.32 mmol) was added. The solution was stirred at room temperature for 15 min. Boc-Gly-OH (932 mg, 5.32 mmol) was added. After 15 h at room temperature the solvent was evaporated and the crude product was purified by column chromatography on R_f = 0.2 (EtOAc)

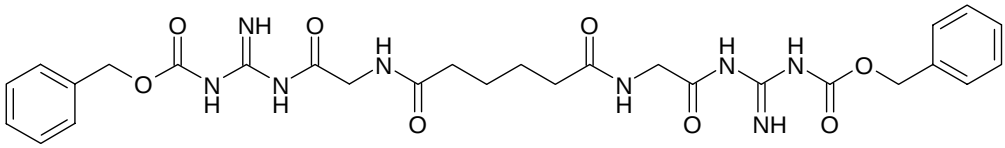
mp: 89 °C. - IR (KBr) [cm^{-1}]: 3386, 3287, 3065, 3013, 2976, 2933, 1728, 1624, 1522, 1437, 1375, 1292, 1167, 1053, 1028, 922, 817, 703. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.38 (s, 9 H, Boc), 3.71 (d, J = 8.4 Hz, 2 H, -C*H*$_2$-NH), 5.08 (s, 2 H, Ph-C*H*$_2$), 7.18 (t, J = 8.4 Hz, 1 H, Boc-N*H*), 7.27 – 7.40 (m, 5 H, Ph), 8.90 (bs, 2 H, 2 x N*H*), 11.10 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 27.8 (+), 28.0 (+), 39.7 (+), 39.9 (+), 44.0 (-), 54.8 (C_{quat}), 65.5 (-), 78.2 (C_{quat}), 127.2 (+), 127.5 (+), 128.2 (+), 137.1 (C_{quat}), 155.8 (C_{quat}), 158.5 (C_{quat}), 162.5 (C_{quat}), 173.8 (C_{quat}). - MS (CI-MS, NH_3): m/z (%) = 351.1 (100) [MH^+], 294.9 (4) [MH^+ - C_4H_8], 251.0 (3) [MH^+ - Boc]. – Elemental analysis calcd. (%) for $C_{16}H_{22}N_4O_5$ (350.16): C 54.83, H 6.33, N 16.00; found C 54.53, H 6.29, N 15.90.



***N*-(2-Amino-acetyl)-*N´*-(benzyloxycarbonyl)-guanidine hydrochloride (27).**

Compound **26** (1.86 g, 5.32 mmol) was dissolved in 5 mL DCM and 15 mL HCl saturated ether was added. The reaction mixture was stirred at room temperature for 45 min and monitored by TLC. The solvent was evaporated and the product was dried under high vacuum. **27** (1.52 g, 5.32 mmol, 100 %) is a white solid.

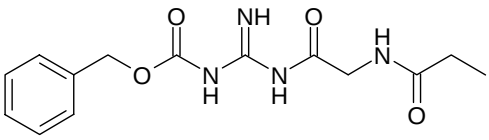
mp: 89 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.81 – 3.89 (m, 2 H, -C*H*$_2$-NH), 5.10 (s, 2 H, Ph-C*H*$_2$), 7.29 – 7.41 (m, 5 H, Ph), 7.89 (bs, 2 H, N*H*$_2$), 8.99 (bs, 2 H, 2 x N*H*), 11.10 (bs, 1 H, N*H*). - MS (CI-MS, NH_3): m/z (%) = 251.0 (100) [MH^+]. – $C_{11}H_{15}N_4O_3Cl$ (286.08).



Hexanedioic acid bis-{[2-(*N´*-(benzyloxycarbonyl)-guanidino)-2-oxo-ethyl]-amide} (28).

Guanidine **27** (1.02 g, 3.59 mmol) was dissolved in 15 mL DMF, NEt_3 (363 mg, 0.498 mL, 3.59 mmol) and adipoyl chloride (329 mg, 0.262 mL, 1.79 mmol) were added. The reaction mixture was stirred at 60 °C for 15 h. The solution was allowed to cool at 0 °C and treated with water. The precipitate was filtered off and washed thoroughly with ice-water. The product was dried under high vacuum. **28** (848 mg, 1.39 mmol, 78 %) is a white solid. R_f (EtOAc) = 0.25

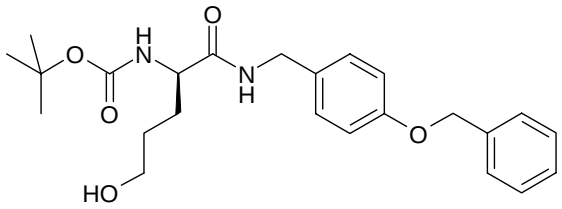
mp: 119 °C. - IR (KBr) [cm^{-1}]: 3289, 3212, 2989, 2923, 2893, 1723, 1634, 1513, 1455, 1367, 1298, 1188, 1009, 956, 854, 793, 696. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.37 – 1.48 (m, 4 H, 2 x C*H*$_2$), 1.97 – 2.08 (m, 4 H, 2 x C*H*$_2$), 5.05 (s, 4 H, 2 x C*H*$_2$-Ph), 7.26 – 7.43 (m, 10 H, 2 x Ph), 7.85 (bs, 2 H, 2 x CH_2-N*H*), 8.87 (bs, 2 H, 2 x N*H*), 9.05 (bs, 2 H, 2 x N*H*), 10.95 (bs, 2 H, 2 x N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 611.3 (100) [MH^+]. – $C_{28}H_{34}N_8O_8$ (610.25).



***N*-{2-[*N´*-(Benzyloxycarbonyl)-guanidino]-2-oxo-ethyl}-propionamide (29).**

Guanidine **27** (326 mg, 1.14 mmol) was dissolved in 10 mL DMF, NEt_3 (115 mg, 0.158 mL, 1.14 mmol) and propionyl chloride (105 mg, 0.099 mL, 1.14 mmol) were added. The reaction mixture was stirred at room temperature for 18 h. The solution was allowed to cool at 0 °C and treated with water. The precipitate was filtered off and washed thoroughly with ice-water. The product was dried under high vacuum. **29** (300 mg, 0.98 mmol, 86 %) is obtained as a white solid.

mp: 105 °C. - IR (KBr) [cm^{-1}]: 3312, 3199, 2989, 2934, 1734, 1711, 1656, 1478, 1299, 1034, 965, 829, 756. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.05 (t, J = 7.5 Hz, 3 H, -C*H*$_3$), 2.04 (q, J = 7.6 Hz, 2 H, -C*H*$_2$-CH$_3$), 3.78 (d, J = 8.2 Hz, 2 H, -C*H*$_2$-NH), 5.10 (s, 2 H, Ph-C*H*$_2$), 7.28 – 7.41 (m, 5 H, Ph), 7.85 (bs, 1 H, N*H*), 8.81 (bs, 1 H, N*H*), 9.05 (bs, 1 H, N*H*), 11.05 (bs, 1 H, N*H*). - MS (CI-MS, NH_3): m/z (%) = 324.1 (15) [MNH_4^+], 307.1 (100) [MH^+]. – $C_{14}H_{18}N_4O_4$ (306.13).

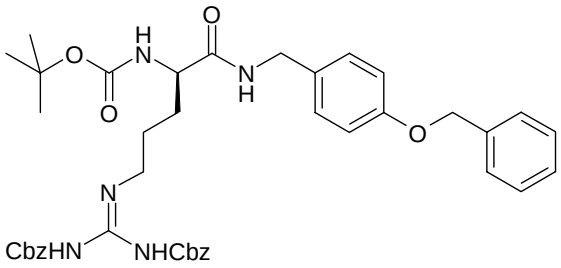


[(R)-1-(4-Benzyloxy-benzylcarbamoyl)-4-hydroxy-butyl]-carbamic acid *tert*-butyl ester (34).

Compound **16** (2.97 g, 5.58 mmol) was dissolved in 20 mL THF/MeOH = 3/1, $LiBH_4$ (30 mg, 13.95 mmol) was added and the reaction mixture was stirred at room temperature for 15 h. A saturated solution of $NaHCO_3$ (25 mL) was added and the organic solvent was evaporated. The water phase was extracted with DCM (2 x 30 mL). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc) to obtain **34** (506 mg, 0.97 mmol, 79 %) as a colourless oil. R_f = 0.40 (EtOAc).

IR (KBr) [cm^{-1}]: 3386, 3031, 2978, 1728, 1649, 1610, 1450, 1379, 1099, 1013, 810, 746, 693. – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.56 – 1.98 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 3.67 (t, J = 6.0 Hz, 2 H, HO-C*H*$_2$-), 4.15 – 4.25 (m, 1 H, C*H*), 4.35 (d, J = 5.5 Hz, 2 H, -NH-C*H*$_2$), 5.04 (s, 2 H, -C*H*$_2$-Ph), 5.31 (d, J = 7.7 Hz, 1 H, Boc-N*H*), 6.71 (bs, 1 H, N*H*), 6.88 – 6.95 (m, 2 H, AA´-part of a AA´BB´-system), 7.16 – 7.21 (m, 3 H, BB´-part of a AA´BB´-system/N*H*), 7.28 – 7.45 (m, 5 H, Ph). – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 28.2 (+), 28.3 (-), 29.8 (-), 43.0 (-), 54.1 (C_{quat}), 62.3 (-), 70.0 (-), 80.2 (C_{quat}), 115.0 (+), 127.5 (+), 128.0 (+), 128.6 (+), 129.1 (+), 130.2 (C_{quat}), 136.9 (C_{quat}), 158.2 (C_{quat}), 171.9 (C_{quat}). - MS (CI-MS, NH_3): m/z (%) = 446.2 (20) [MNH_4^+],

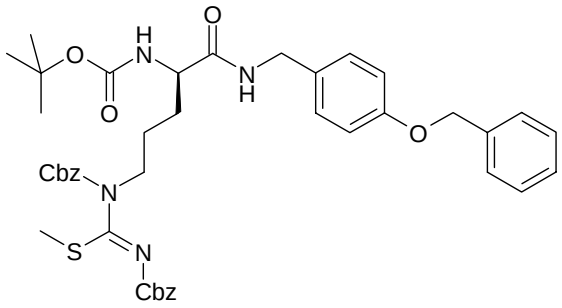
429.2 (100) [MH^+]. - Elemental analysis calcd. (%) for $C_{24}H_{32}N_2O_5$ (428.23): C 67.25, H 7.53, N 6.54; found C 67. 15, H 7.42, N 6.45.



[(R)-1-(4-benzyloxy-benzylcarbamoyl)-4-(*N´,N´´*-bis-Cbz-guanidino)-butyl]-carbamic acid *tert*-butyl ester (36).

Compound **34** (415 mg, 0.97 mmol), Di-Cbz-guanidine **35** (639 mg, 1.94 mmol) and PPh_3 (383 mg, 1.46 mmol) were dissolved in 15 mL THF and DIAD (295 mg, 0.283 mL, 1.46 mmol) was added dropwise. The mixture was stirred at room temperature for 12 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **36** (615 mg, 0.83 mmol, 86 %) as a white solid. R_f = 0.70 (EtOAc).

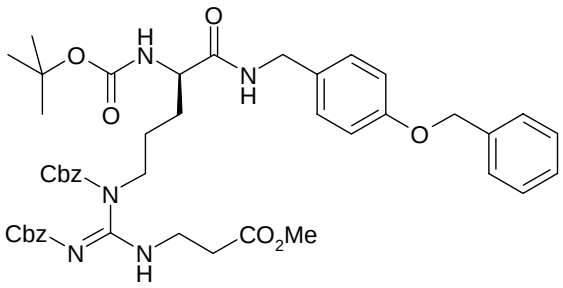
mp: 88 °C. - IR (KBr) [cm^{-1}]: 3396, 3325, 3033, 2976, 1722, 1655, 1612, 1512, 1450, 1383, 1255, 1172, 1099, 1009, 809, 746, 696. – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.55 – 1.80 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 3.73 – 3.86 (m, 1 H, C*H*), 3.98 – 4.15 (m, 2 H, Cbz-N-C*H*$_2$-), 4.30 (d, J = 5.5 Hz, 2 H, -NH-C*H*$_2$), 5.01 (s, 2 H, -C*H*$_2$-Ph), 5.13 (s, 2 H, -C*H*$_2$-Ph), 5.23 (s, 2 H, -C*H*$_2$-Ph), 5.78 (d, J = 7.7 Hz, 1 H, Boc-N*H*), 6.80 – 6.86 (m, 2 H, AA´-part of a AA´BB´-system), 6.90 – 7.03 (m, 3 H, BB´-part of a AA´BB´-system/N*H*), 7.27 – 7.45 (m, 15 H, 3 x Ph), 9.28 (bs, 1 H, N*H*), 9.40 (bs, 1 H, N*H*). – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 24.6 (-), 28.4 (+), 28.8 (-), 42.7 (-), 44.0 (-), 67.0 (-), 67.7 (-), 69.0 (-), 70.0 (-), 114.8 (+), 127.5 (+), 127.9 (+), 128.0 (+), 128.1 (+), 128.2 (+), 128.4 (+), 128.4 (+), 128.5 (+), 128.6 (+), 128.7 (+), 128.9 (+), 129.0 (+), 130.5 (C_{quat}), 134.6 (C_{quat}), 136.4 (C_{quat}), 137.0 (C_{quat}), 155.8 (C_{quat}), 158.0 (C_{quat}), 160.8 (C_{quat}), 163.4 (C_{quat}), 171.8 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 760.4 (5) [MNa^+], 738.5 (100) [MH^+]. – $C_{41}H_{47}N_5O_8$ (737.34).



[()-1-(4-Benzyloxy-benzylcarbamoyl)-4-(1,3-di-Cbz-2-methyl-isothioureido)-butyl]-carbamic acid *tert*-butyl ester (39).

Compound **34** (231 mg, 0.53 mmol), **38** (286 mg, 0.80 mmol) and PPh_3 (209 mg, 0.80 mmol) were dissolved in 15 mL THF and DIAD (161 mg, 0.155 mL, 0.80 mmol) was added dropwise. The mixture was stirred at room temperature for 12 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (hexane/EtOAc = 1/1) to obtain **39** (362 mg, 0.47 mmol, 89 %) as a colourless oil. R_f = 0.20 (hexane/EtOAc = 1/1)

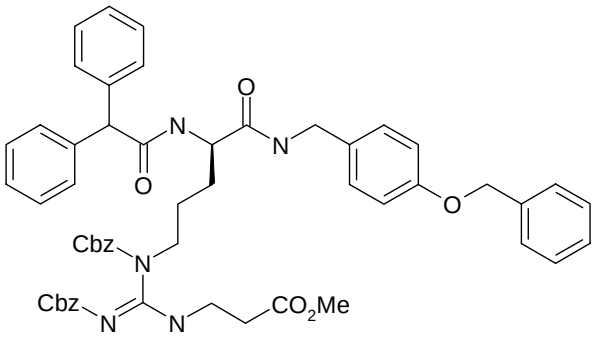
IR (KBr) [cm^{-1}]: 3335, 3029, 2993, 2978, 2867, 1745, 1648, 1523, 1451, 1383, 1053, 814, 741. – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 9 H, Boc), 1.53 – 1.90 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 2.34 (s, 3 H, SC*H*$_3$), 3.59 – 3.88 (m, 1 H, C*H*), 4.10 – 4.18 (m, 2 H, Cbz-N-C*H*$_2$-), 4.34 (d, J = 5.5 Hz, 2 H, -NH-C*H*$_2$), 4.94 (s, 2 H, -C*H*$_2$-Ph), 4.96 (s, 2 H, -C*H*$_2$-Ph), 5.00 (s, 2 H, -C*H*$_2$-Ph), 5.30 (bs, 1 H, Boc-N*H*), 6.65 (bs, 1 H, N*H*), 6.88 – 6.93 (m, 2 H, AA´-part of a AA´BB´-system), 7.15 – 7.21 (m, 2 H, BB´-part of a AA´BB´-system), 7.28 – 7.42 (m, 15 H, 3 x Ph). – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.2 (C_{quart}), 15.6 (+), 25.2 (C_{quart}), 28.3 (+), 29.9 (-), 43.0 (-), 48.3 (-), 68.4 (-), 68.6 (-), 70.0 (-), 115.0 (+), 127.4 (+), 128.0 (+), 128.1 (+), 128.4 (+), 128.5 (+), 128.6 (+), 128.6 (+), 128.8 (+), 128.9 (+), 129.1 (+), 130.4 (C_{quat}), 135.2 (C_{quat}), 135.3 (C_{quat}), 136.9 (C_{quat}), 153.1 (C_{quat}), 158.2 (C_{quat}), 158.8 (C_{quat}), 165.1 (C_{quat}), 171.8 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 769.4 (100) [MH^+], 713.3 (10) [MH^+ - C_4H_8], 669.3 (8) [MH^+ - Boc]. - Elemental analysis calcd. (%) for $C_{42}H_{48}N_4O_8S$ (768.32): C 65.60, H 6.30, N 7.29; found C 65.54, H 6.21, N 7.35.



3-{*N´*-[(R)-4-(4-Benzyloxy-benzylcarbamoyl)-4-*tert*-butoxycarbonylamino-butyl]-*N´*,*N´´*-di-Cbz-guanidino}-propionic acid methyl ester (41).

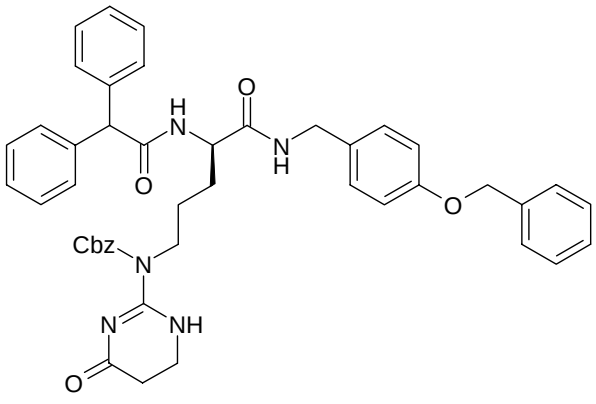
Compound **39** (647 mg, 0.84 mmol), **40** (176 mg, 1.26 mmol) and NEt_3 (127 mg, 0.175 mL, 1.26 mmol) were dissolved in 15 mL THF and the reaction mixture was refluxed for 15 h. The finished reaction was filtered and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (hexane/EtOAc = 1/1) to obtain **41** (617 mg, 0.75 mmol, 89 %) as a colourless oil. R_f = 0.15 (hexane/EtOAc = 1/1)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 9 H, Boc), 1.52 – 1.72 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 2.41 – 2.49 (m, 2 H, -C*H*$_2$-CO_2Me), 3.26 – 3.38 (m, 2 H, -C*H*$_2$-CH_2-CO_2Me), 3.65 (s, 3 H, CO_2Me), 3.79 – 3.90 (m, 2 H, Cbz-N-C*H*$_2$-), 4.01 – 4.09 (m, 1 H, C*H*), 4.19 (d, J = 5.6 Hz, 2 H, -NH-C*H*$_2$), 5.01 (s, 2 H, -C*H*$_2$-Ph), 5.05 (s, 2 H, -C*H*$_2$-Ph), 5.12 (s, 2 H, -C*H*$_2$-Ph), 5.37 (d, J = 7.8 Hz, 1 H, Boc-N*H*), 6.65 (bs, 1 H, N*H*), 6.84 – 6.90 (m, 2 H, AA´-part of a AA´BB´-system), 7.08 – 7.14 (m, 2 H, BB´-part of a AA´BB´-system), 7.28 – 7.42 (m, 15 H, 3 x Ph), 9.82 (bs, 1 H, N*H*). – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 23.4 (-), 24.6 (C_{quat}), 28.3 (+), 33.3 (-), 42.8 (-), 52.1 (+), 67.4 (-), 67.5 (-), 68.5 (-), 70.0 (-), 98.5 (+), 114.9 (+), 127.5 (+), 128.0 (+), 128.1 (+), 128.3 (+), 128.4 (+), 128.6 (+), 128.7 (+), 129.0 (+), 130.6 (C_{quat}), 136.9 (C_{quat}), 158.0 (C_{quat}), 171.8 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 846.4 (5) [MNa^+] 824.5 (100) [MH^+]. – $C_{45}H_{53}N_5O_{10}$ (823.38).



3-{*N´*-[(R)-4-(4-Benzyloxy-benzylcarbamoyl)-4-diphenylacetylamino-butyl]-*N´,N´´*-di-Cbz-guanidino}-propionic acid methyl ester (42).

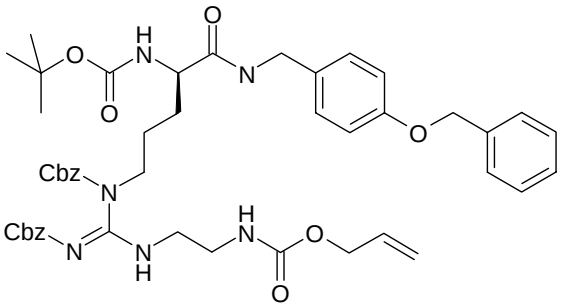
Compound 41 (91 mg, 0.11 mmol) was dissolved in 5 mL DCM and 10 mL HCl saturated ether was added. The mixture was stirred at room temperature for 45 min. The solvent was evaporated and the white solid was dried under high vacuum. After 1h the solid was dissolved in 10 mL DCM, diphenylacetic acid (23 mg, 0.11 mmol), HOBt (15 mg, 0.11 mmol), DIC (14 mg, 0.017 mL, 0.11 mmol) and DIPEA (14 mg, 0.019 mmol, 0.11 mmol) was added. After stirring 12 h at room temperature the solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain 42 (84 mg, 0.091 mmol, 83 %) as a colourless oil. R_f = 0.75 (EtOAc) IR (KBr) [cm^{-1}]: 3256, 3221, 3013, 2978, 2956, 1744, 1634, 1567, 1411, 1377, 1101, 1089, 845, 734. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.53 – 1.91 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 2.55 (t, J = 6.1 Hz, 2 H, -C*H*$_2$-CO_2Me), 3.28 – 3.38 (m, 2 H, -C*H*$_2$-CH_2-CO_2Me), 3.65 (s, 3 H, CO_2Me), 3.81 – 3.89 (m, 2 H, Cbz-N-C*H*$_2$-), 4.00 – 4.10 (m, 2 H, 2 x C*H*), 4.26 (d, J = 5.6 Hz, 2 H, -NH-C*H*$_2$), 5.00 (s, 2 H, -C*H*$_2$-Ph), 5.03 (s, 2 H, -C*H*$_2$-Ph), 5.09 (s, 2 H, -C*H*$_2$-Ph), 6.31 (bs, 1 H, N*H*), 6.83 – 6.89 (m, 2 H, AA´-part of a AA´BB´-system), 7.03 – 7.10 (m, 2 H, BB´-part of a AA´BB´-system), 7.18 – 7.48 (m, 26 H, 5 x Ph/N*H*), 9.78 (bs, 1 H, N*H*). - ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 42.7 (C_{quat}), 70.0 (-), 114.9 (+), 124.9 (C_{quat}), 127.2 (+), 127.5 (+), 128.0 (+), 128.4 (+), 128.6 (+), 128.6 (+), 128.7 (+), 128.7 (+), 128.8 (+), 128.9 (+), 129.2 (+), 130.5 (C_{quat}), 136.2 (C_{quat}), 137.0 (C_{quat}), 158.0 (C_{quat}), 171.2 (C_{quat}). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 918.5 (100) [MH^+]. – $C_{54}H_{55}N_5O_9$ (917.40).



(R)-2-Diphenylacetylamino-5-[benzyloxycarbonyl-(4-oxo-1,4,5,6-tetrahydro-pyrimidin-2-yl)-amino]-pentanoic acid 4-benzyloxy-benzylamide (44).

Compound **42** (183 mg, 0.20 mmol) and LiOH*H_2O (8.4 mg, 0.20 mmol) were dissolved in 10 mL MeOH/H_2O = 3/1 and the reaction mixture was stirred at room temperature for 12 h. The precipitated solid was filtered off and washed thoroughly with cold MeOH. The solid was dried under high vacuum to obtain **44** (132 mg, 0.18 mmol, 89 %). R_f (EtOAc) = 0.35

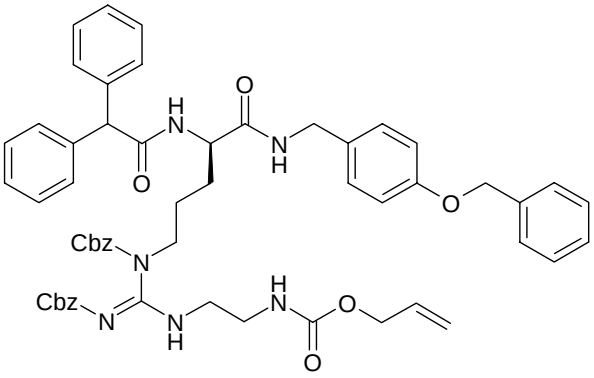
mp: 93 °C. - IR (KBr) [cm^{-1}]: 3289, 3178, 2993, 2921, 1745, 1712, 1634, 1423, 1178, 978, 866, 791, 694. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.52 – 1.95 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 2.63 (bs, 2 H, CO-C*H*$_2$-CH$_2$), 3.42 (bs, 2 H, -CH$_2$-C*H*$_2$-NH), 3.81 – 3.89 (m, 2 H, Cbz-N-C*H*$_2$-), 4.00 – 4.10 (m, 2 H, 2 x C*H*), 4.24 (d, J = 5.6 Hz, 2 H, -NH-C*H*$_2$), 5.00 (s, 2 H, -C*H*$_2$-Ph), 5.09 (s, 2 H, -C*H*$_2$-Ph), 6.38 (bs, 1 H, N*H*), 6.83 – 6.89 (m, 2 H, AA´-part of a AA´BB´-system), 7.03 – 7.10 (m, 2 H, BB´-part of a AA´BB´-system), 7.18 – 7.48 (m, 21 H, 4 x Ph/N*H*), 9.77 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 752.4 (100) [MH^+]. – $C_{45}H_{45}N_5O_6$ (751.34).



[(R)-4-[*N´*-(2-allyloxycarbonylamino-ethyl)-*N,N´´*-bis-(benzyloxycarbonyl)-guanidino]-1-(4-benzyloxy-benzylcarbamoyl)-butyl]-carbamic acid *tert*-butyl ester (46).

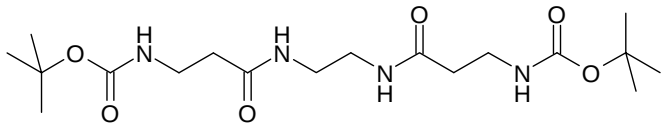
Compound **39** (120 mg, 0.15 mmol), **45** (155 mg, 0.60 mmol) and NEt_3 were dissolved in 15 mL THF and the reaction mixture was refluxed for 15 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to give **46** (90 mg, 0.10 mmol, 65 %) as a colourless oil. R_f (EtOAc) = 0.65

IR (KBr) [cm^{-1}]: 3278, 3134, 3004, 2978, 2945, 1751, 1687, 1523, 1474, 1312, 1056, 943, 856, 723. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 9 H, Boc), 1.50 – 1.69 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 2.98 – 3.18 (m, 4 H, -C*H*$_2$-C*H*$_2$-NH-Aloc), 3.78 – 3.88 (m, 2 H, Cbz-N-C*H*$_2$-), 4.01 – 4.08 (m, 1 H, C*H*), 4.16 (d, J = 5.6 Hz, 2 H, -NH-C*H*$_2$-Ar), 4.46 (d, J = 5.2 Hz, 2 H, O-C*H*$_2$-CH), 5.01 (s, 2 H, -C*H*$_2$-Ph), 5.04 (s, 2 H, -C*H*$_2$-Ph), 5.12 (s, 2 H, -C*H*$_2$-Ph), 5.19 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.43 (bs, 1 H, Boc-N*H*), 5.82 – 5.94 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.65 (bs, 1 H, N*H*), 6.84 – 6.90 (m, 2 H, AA´-part of a AA´BB´-system), 7.08 – 7.14 (m, 2 H, BB´-part of a AA´BB´-system), 7.28 – 7.42 (m, 16 H, 3 x Ph/N*H*), 9.81 (bs, 1 H, N*H*). – MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 865.4 (100) [MH^+]. – $C_{47}H_{56}N_6O_{10}$ (864.41).



(2-{*N´*-[(R)-4-(4-Benzyloxy-benzylcarbamoyl)-4-diphenylacetylamino-butyl]-*N´,N´´*-bis-(benzyloxycarbonyl)-guanidino}-ethyl)-carbamic acid allyl ester

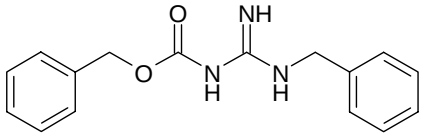
Compound **46** (90 mg, 0.10 mmol) was dissolved in 5 mL DCM and 10 mL HCl saturated ether was added. The mixture was stirred at room temperature for 45 min. The solvent was evaporated and the white solid was dried under high vacuum. After 1 h the solid was dissolved in 10 mL DCM, diphenylacetic acid (27 mg, 0.13 mmol), HOBt (18 mg, 0.13 mmol), DIC (16 mg, 0.020 mL, 0.13 mmol) and DIPEA (17 mg, 0.022 mmol, 0.13 mmol) was added. After stirring 16 h at room temperature the solvent was evaporated and the crude product was purified by column chromatography on silica gel (EtOAc) to obtain **48** (71 mg, 0.074 mmol, 74 %) as a colourless oil. R_f = 0.60 (EtOAc) IR (KBr) [cm^{-1}]: 3181, 2970, 2908, 1721, 1634, 1449, 1256, 1206, 1167, 1078, 966, 842, 753. - ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.52 – 1.90 (m, 4 H, -CH-C*H*$_2$-C*H*$_2$), 2.98 – 3.18 (m, 4 H, -C*H*$_2$-C*H*$_2$-NH-Aloc), 3.81 – 3.89 (m, 2 H, Cbz-N-C*H*$_2$-), 4.00 – 4.10 (m, 2 H, 2 x C*H*), 4.23 (d, J = 5.4 Hz, 2 H, -NH-C*H*$_2$), 4.48 (d, J = 5.2 Hz, 2 H, O-C*H*$_2$-CH), 5.01 (s, 2 H, -C*H*$_2$-Ph), 5.03 (s, 2 H, -C*H*$_2$-Ph), 5.09 (s, 2 H, -C*H*$_2$-Ph), 5.19 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H, C*H*H=CHCH$_2$O), 5.27 (dd, J = 1.7 Hz, J = 16.2 Hz, 1 H, CH*H*=CHCH$_2$O), 5.82 – 5.94 (m, 1 H, CH$_2$=C*H*CH$_2$O), 6.31 (bs, 1 H, N*H*), 6.83 – 6.89 (m, 2 H, AA´-part of a AA´BB´-system), 7.03 – 7.10 (m, 2 H, BB´-part of a AA´BB´-system), 7.18 – 7.48 (m, 27 H, 5 x Ph/2 x N*H*), 9.89 (bs, 1 H, N*H*). - MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): m/z (%) = 959.6 (100) [MH^+]. – $C_{56}H_{58}N_6O_9$ (958.43).



{2-[2-(3-*tert*-Butoxycarbonylamino-propionylamino)-ethylcarbamoyl]-ethyl}-carbamic acid *tert*-butyl ester (51).

Compound **50** (4.0 g, 21.1 mmol) was dissolved in 30 mL DCM and CDI (3.42 g, 21.1 mmol) was added slowly. After stirring at room temperature for 15 min ethylene diamine (424 mg, 0.476 mL, 7.05 mmol) was added and the reaction mixture was stirred at room temperature for additionally 18 h. A white solid precipitated during this time. The solid was filtered off and washed thoroughly with DCM. After drying under high vacuum **51** (2.83 g, 7.04 mmol, 100 %) was obtained as a white solid.

mp: 98 °C. - ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 18 H, 2 x Boc), 2.17 – 2.27 (m, 4 H, -C*H*$_2$-CO-), 3.01 – 3.16 (m, 8 H, 4 x C*H*$_2$), 6.75 (bs, 2 H, 2 x Boc-N*H*), 7.88 (bs, 2 H, 2 x CH$_2$-N*H*). - MS (CI-MS, NH_3): m/z (%) = 403.2 (100) [MH^+], 347.0 (5) [MH^+ - C_4H_8], 302.9 (10) [MH^+ - Boc]. – $C_{18}H_{34}N_4O_6$ (402.25).



***N*-(Benzyloxycarbonyl)-*N´*-benzyl-guanidine (55).**

Benzaldehyde **54** (164 mg, 0.156 mL, 1.55 mmol) and compound **22** (300 mg, 1.55 mmol) were dissolved in 15 mL THF and $NaBH(OAc)_3$ (491 mg, 2.32 mmol) was added. After addition of acetic acid (93 mg, 0.088 mL, 1.55 mmol) the suspension was stirred at room temperature for 12 h. 1N NaOH (20 mL) was added and the water phase was extracted with EtOAc (2 x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography on silica gel (EtOAc) to give compound **55** (285 mg, 1.01 mmol, 65 %) as a white solid. R_f (EtOAc) = 0.3

mp: 102 °C. - IR (KBr) [cm^{-1}]: 3473, 3286, 3030, 1591, 1427, 1285, 1134, 1060, 1027, 802, 696. – ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.32 (s, 2 H, Ph-C*H*$_2$-NH), 5.05 (s, 2 H, Ph-CH_2), 7.22 – 7.42 (m, 10 H, 2 x Ph), 8.60 (bs, 2 H, 2 x N*H*), 10.55 (bs, 1 H, N*H*). – ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 43.2 (C_{quat}), 65.2 (-), 127.0 (+), 127.3 (+), 128.3 (+), 137.9 (C_{quat}), 161.3 (C_{quat}), 163.0 (C_{quat}). - MS (CI-MS, NH_3): m/z (%) = 284.1 (100) [MH^+]. – $C_{16}H_{17}N_3O_2$ (283.13).

3.5 Appendix

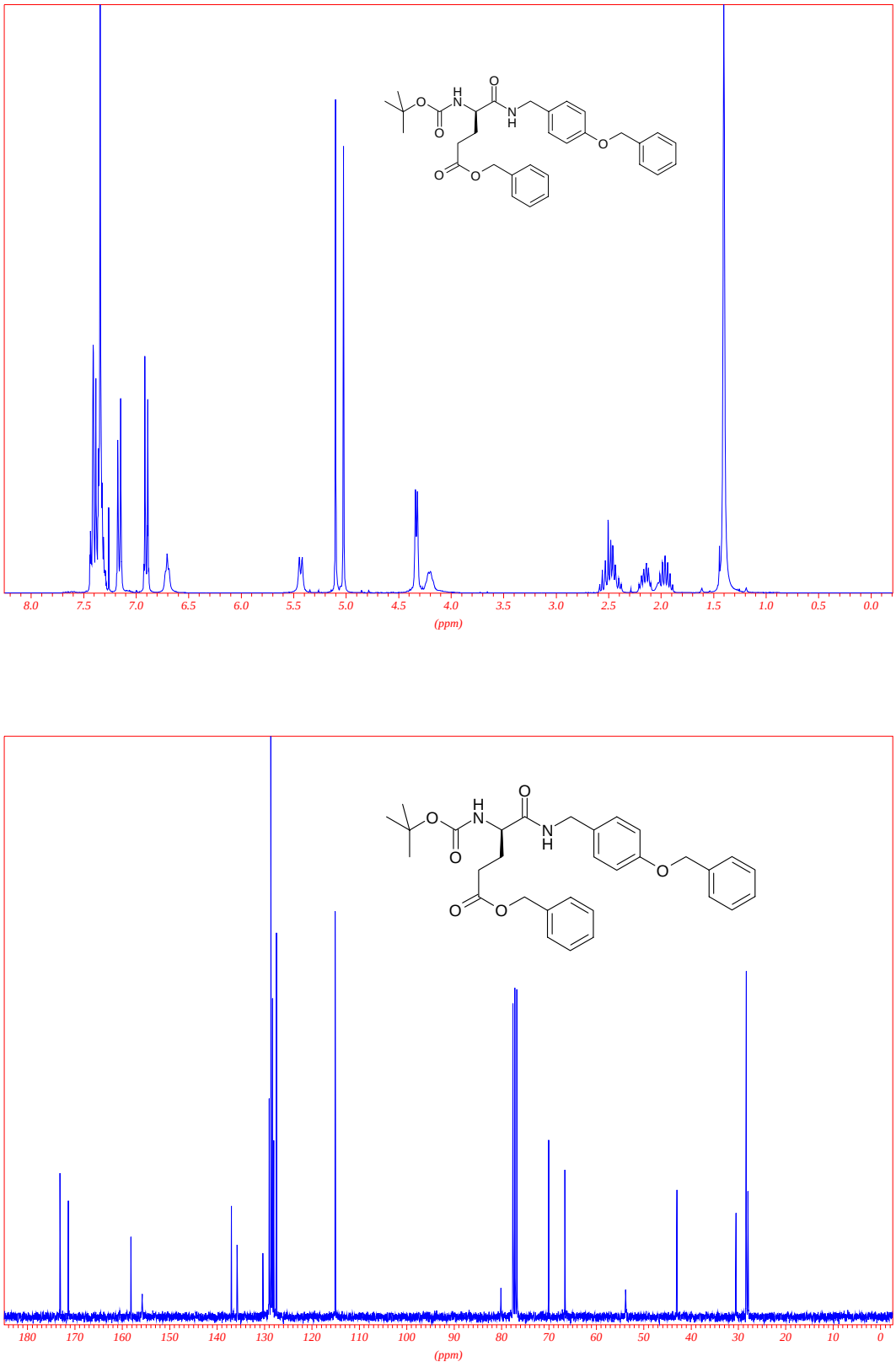
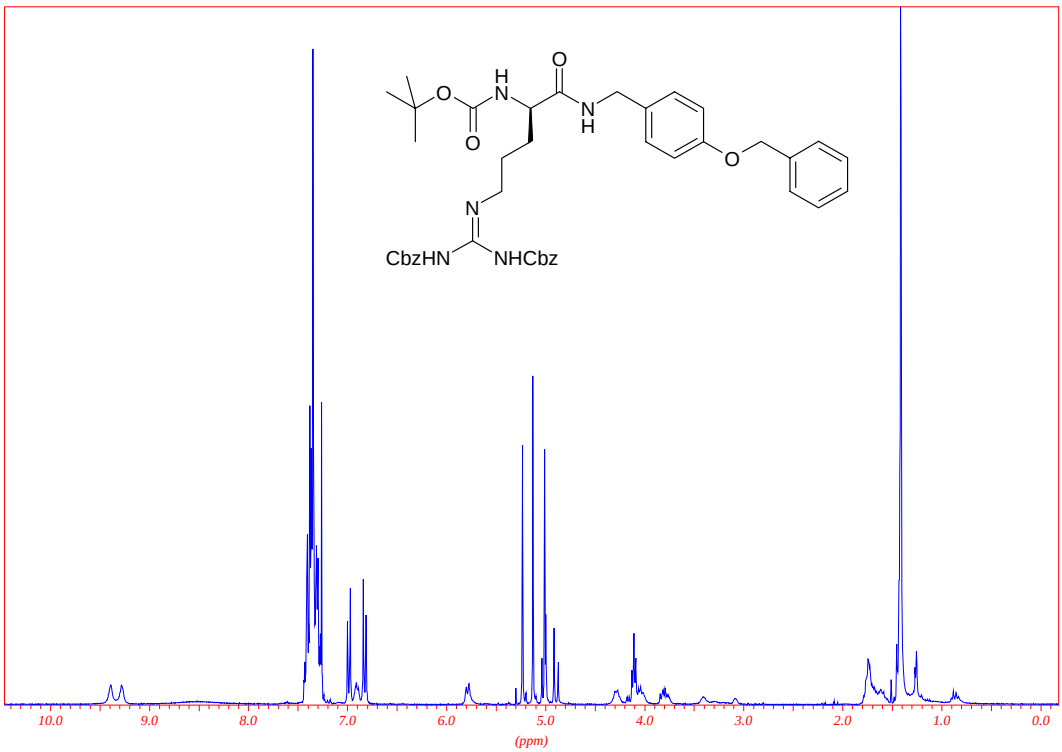


Figure 9. ^{1}H-NMR and ^{13}C-NMR of compound **16**



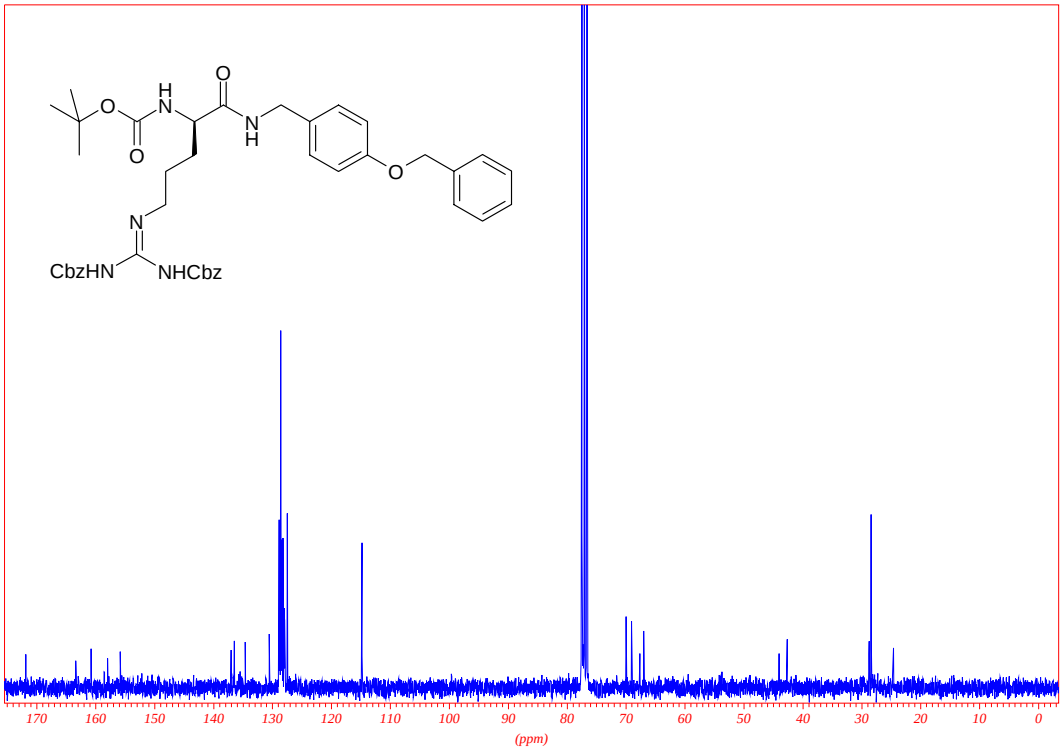


Figure 10. ^{1}H-NMR and ^{13}C-NMR of compound **36**

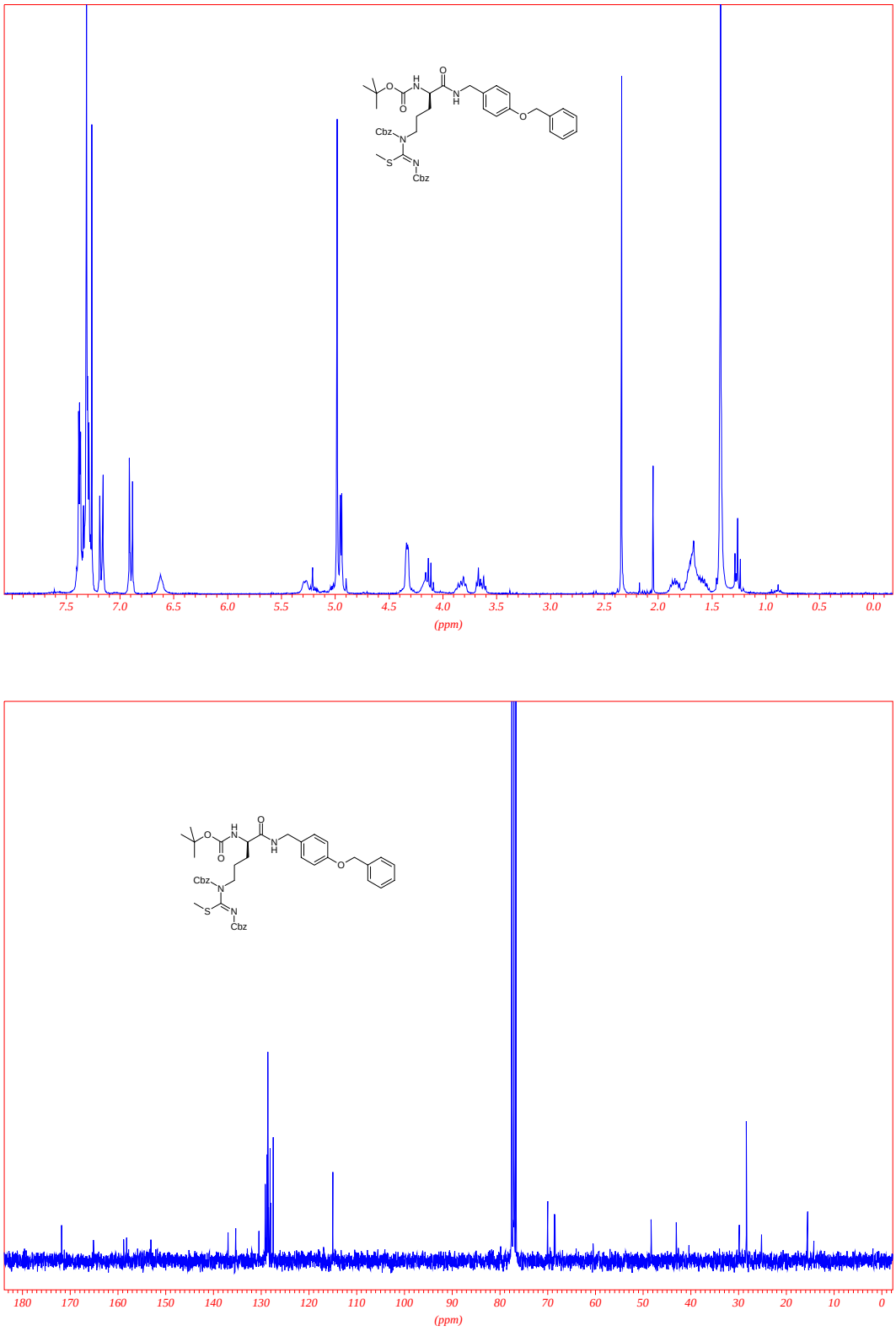


Figure 11. ^{1}H-NMR and ^{13}C-NMR of compound **39**

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C. Summary

The chemical properties of the guanidinium group as well as its ability to form H-bonds, charge pairing and cation-π interactions opens up a large number of possibilities in molecular recognition and pharmaceutical chemistry.

In the first chapter, the synthesis of Artificial Ethoxycarbonyl Guanidinium Amino Acids is reported. Carboxy– and amino-groups can be selectively deprotected, but peptide coupling reactions are limited to simple amines and acids. The Ethoxycarbonyl-group can be selectively cleaved by the pig liver esterase EC 3.1.1.1. The binding ability of the Ethoxycarbonyl-protected guanidine to carboxylates in DMSO was investigated by NMR-titrations. The value is comparable with the binding constant of thioureas in DMSO.

In further studies, luminescent Guanidinium Amino Acids (GuAA´s) as new synthetic receptor building blocks for molecular recognition of carboxylates were prepared. The binding constants were determined by fluorescence titrations in different solvents. The binding constants are in MeOH in the range of $5*10^5$ M^{-1}, in DMSO $2*10^4$ M^{-1} and in H_2O (pH = 7.0) in the range of $7*10^3$ M^{-1}. The stoichiometry of all binding processes is 1:1 and was determined by Job`s plot analysis. The synthesis of a tripeptide **33** containing two GuAA molecules gave the target compound in 17 % yield.

Guanidinium compounds play a major role in pharmaceutical chemistry. The Y_1 receptor antagonist BIBP 3226 and N^G-acylated BIBP 3226 derivatives are highly active compounds. We have reported the synthesis of bis-guanidines **24** and **28**. The alcohol **10** could be obtained in good yields starting from commercially available Boc-D-Glu(OBn)-OH **15**. The double Mitsunobu reaction of alcohol **10** with bis-guanidine **24** to form the bivalent ligand **30** failed. To investigate the reason for the failure of the Mitsunobu reaction, simple model reactions were carried out. As a result we found that the diphenyl-part in the primary alcohol **10** has an influence on the reaction mechanism of the Mitsunobu reaction. One explanation could be the steric shielding of the OH-function by the two phenyl rings. Furthermore the synthesis of alkylated BIBP 3226 derivatives could be achieved by simple Mitsunobu reaction of the alcohol **34** with 1,3-

di-Cbz-2-methyl-isothiourea **38** to get the isothiourea **39**. The compound is useful as a precursor for the synthesis of alkylated guanidines by treatment with different amines.

D. Abbreviations

Ala	Alanine
Ar	Aryl
Arg	Arginine
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
c	Concentration
Cbz	Benzyloxycarbonyl
CDI	*N*,*N´*-Carbonyldiimidazole
d	days
DIC	Diisopropylcarbodiimide
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropyl ethyl amine (Huenig´s base)
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EtOAc	Ethylacetate
EI	Electronic Ionisation
eq	Equivalents
ESI	Electronic spray ionisation
FAB	Fast-Atom Bombardment
Glu	Glutamic Acid
Gly	Glycine
GPCR	G-protein coupled receptor
h	hours
HOBt	1-Hydroxybenzotriazole
HRMS	High resolution mass spectroscopy
IR	Infrared spectroscopy
J	Coupling Constant
MeOH	methanol
Mg_2SO_4	Magnesium sulfate

min	minutes
mp	melting point
MS	Mass spectroscopy
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
NEt_3	Triethyl amine
NMR	Nuclear Magnetic Resonance
NPY	Neuropeptide Y
Ph	Phenyl
quant.	quantitative
quat	quaternary
R_f	Retention Factor
rt	room temperature
TFA	Trifluoracetic Acid
THF	tetrahydrofuran
TIS	Triisopropyl silane
TLC	Thin Layer Chromatography
x	Mole Fraction

E. Appendix

Poster Presentations

- *Summerschool of Medicinal Chemistry* (09/2002), Regensburg, Germany
- *1st World Congress on Synthetic Receptors* (10/2003), Lisboa, Portugal
- *2nd Summerschool of Medicinal Chemistry* (10/2004), Regensburg, Germany
- *Workshop of the Graduate College Medicinal Chemistry* (12/2005), Windberg, Germany

Oral Presentations

- *Acyclic Guanidinium Oligomers – Recognition of carboxylic side chains in peptides* (05/2003), Workshop of the Graduate College Medicinal Chemistry, Regen, Germany
- *Bivalent NPY Y_1 Receptor Antagonists* (12/2005), Workshop of the Graduate College Medicinal Chemistry, Windberg, Germany

Conferences

- Conference *Oberflächenanalytik* (11/1999), Karlsruhe, Germany
- Symposium *Molecular Recognition* (06/2003), Prague, Czech Republic
- Seminar of Peptide Synthesis with Microwaves (11/2004), Munich, Germany

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G. Curriculum Vitae

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03/99 – 04/99	Infineon Technology, Regensburg (Project Management)
08/99 – 10/99	Infineon Technology, Munich (Research Department)
08/00 – 11/00	Infineon Technology, Regensburg (Chemistry Department)