THE VERSATILE USE OF GUANIDINIOCARBONYLPYRROLES: FROM SELF-ASSEMBLY TO PEPTIDE RECOGNITION

DISSERTATION ZUR ERLANGUNG DES
NATURWISSENSCHAFTLICHEN DOKTORGRADES
DER BAYERISCHEN JULIUS-MAXIMILIANS-UNIVERSITÄT
WÜRZBURG

vorgelegt von
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aus
Rösrath

Würzburg 2004

Eingereicht am: _____
bei der Fakultät für Chemie und Pharmazie

1. Gutachter: _____

2. Gutachter: _____

der Dissertation

1. Prüfer: _____

2. Prüfer: _____

3. Prüfer: _____

des öffentlichen Promotionskolloquiums:

Tag des öffentlichen Promotionskolloquiums: _____

Doktorurkunde ausgehändigt am: _____

meiner Familie

Die vorliegende Dissertation wurde unter Anleitung von Prof. Dr. Carsten Schmuck von Oktober 2000 bis März 2002 am Institut für Organische Chemie der Universität zu Köln und im Zeitraum von April 2002 bis April 2004 am Institut für Organische Chemie der Universität Würzburg angefertigt.

Teilergebnisse der vorliegenden Arbeit sind an folgenden Stellen veröffentlicht worden oder zur Veröffentlichung eingereicht:

C. Schmuck, L. Geiger: "Carboxylate Binding by Guanidiniocarbonyl Pyrroles: From Self-Assembly to Peptide Receptors"; *Current Organic Chemistry* **2003**, *7*, 1485-1502.

C. Schmuck, L. Geiger: "Dipeptide Binding in Water by a *de novo* Designed Guanidiniocarbonylpyrrole Rezeptor"; *J. Am. Chem. Soc.*, submitted.

C. Schmuck, L. Geiger, T. H. Rehm: "Chain-length depending Self-Association of flexible Guanidiniocarbonylpyrrole-Carboxylate-Zwitterions in polar solution", *Chem. Comm.*, submitted.

C. Schmuck, L. Geiger: "Dimerization of a guanidiniocarbonyl pyrrole cation in DMSO that can be controlled by the counter anion", *Chem. Comm.*, submitted.

M. Schäfer, C. Schmuck, L. Geiger, H- J. Cooper, C. L. Hendrickson, M. J. Chalmers, A.G. Marshall: „Structurally related non-covalent complexes examined by quadrupole ion trap (QIT) MS^2 and infrared multiphoton dissociation Fourier transform ion cyclotron resonance mass spectrometry IRMPD-FT-ICR MS: evidence for salt-bridge structures in the gas phase"; *Int. J. Mass Spectr.*, submitted.

Danksagung

An dieser Stelle möchte ich allen danken, die es mir ermöglicht haben, diese Arbeit anzufertigen, indem sie mir sowohl bei Problemen und Fragen geholfen, als auch dazu beigetragen haben, die arbeitsreiche Zeit zu verschönen.

Mein Dank gilt an erster Stelle meinem Betreuer Prof. Dr. Carsten Schmuck für die Möglichkeit auf einem faszinierenden Gebiet der Chemie arbeiten zu können. Sein stetes Interesse und die fachliche Unterstützung und Aufmunterungen in Zeiten, in denen es nur langsam vorwärts ging, haben wesentlich zum Gelingen dieser Arbeit beigetragen.

Besonders danken möchte ich ferner meinen Kölner und Würzburger Laborkollegen Wolfgang Wienand, Martin Heil, Michael Schwegmann, Uwe Machon und Thomas Rehm für die gute Zusammenarbeit und die angenehme Arbeitsatmosphäre. Besonders danke ich Wolfgang Wienand für die fachlichen und außerfachlichen Diskussionen und die vielen Ideen die er zu meiner Arbeit beigesteuert hat. Desweiteren möchte ich mich bei den weiteren Arbeitskreismitgliedern in Köln und in Würzburg bedanken.
Auch möchte ich „meinen“ Praktikanten Thomas Rehm, Thomas Kupfer und Michael Merschky herzlich danken, die doch in den letzten Monaten mit ihrem Einsatz manche vielstufige Synthese erst ermöglicht haben.

Für die Durchführung analytischer Messungen und die Unterstützung bei der Auswertung danke ich Dr. Hans Schmickler, Ingrid Hoven, Katrin König, Dr. Mathias Schäfer (Köln) und Dr. Mathias Grüne, Elfriede Ruckdeschel und Dr. Mathias Büchner (Würzburg) ganz herzlich. Hervorzuheben ist hier besonders Dr. Mathias Schäfer ohne dessen ESI-MS IRMPD Studien ein wichtiger Aspekt meiner Arbeit fehlen würde.

Für die kritische Durchsicht und Diskussionen des Manuskripts möchte ich mich bei Iris Krampitz, Uwe Machon und Michael Merschky bedanken.

CONTENTS

1 Introduction

The molecular recognition of biologically relevant substrates (peptides, carbohydrates or hormones) by a specific receptor and the molecular-recognition directed self-assembly are two ubiquitous processes in nature.

In many medicinal processes, the selective interaction of a receptor with its substrate plays an essential role in the function of a biochemical or metabolic pathway, as exemplified by the mode of action of the antibiotic vancomycin or the Ras-protein induced oncogenesis (Fig. 1).[1, 2] The development of artificial receptors for the specific complexation of biologically important substrates such as peptides, hormones, neurotransmitters, or carbohydrates under physiological conditions is thus of great importance for the design of biosensors, the targeting of cellular processes or the discovery of new therapeutics.[3, 4]

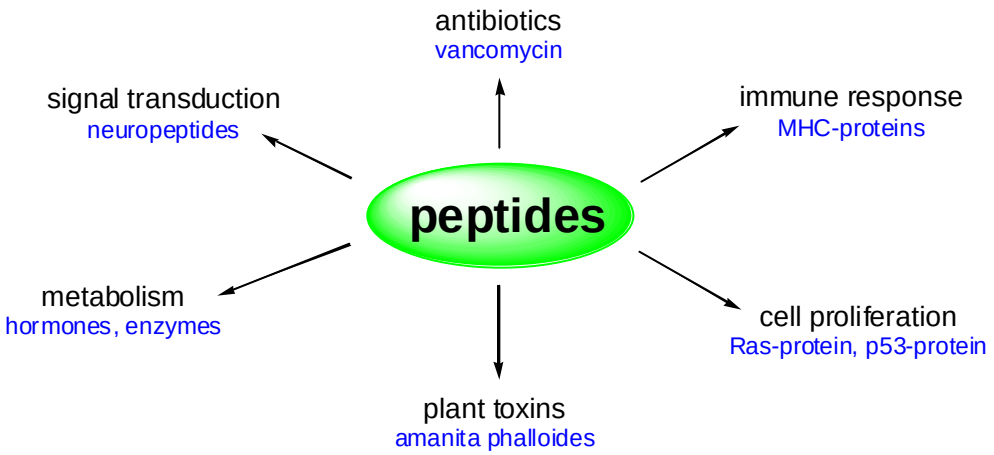


Fig. 1. *The molecular recognition of peptides plays a decisive role in many biologically relevant processes.*

On the other hand, the molecular-recognition directed association, self-assembly and self-organisation of individual molecules results in the formation of highly complex and fascinating structures.[5-11] Examples of self-assembling systems found in nature include the formation of DNA double helices,[12] the formation of insoluble protein plaques in neurodegenerative diseases such as Alzheimer's,[13-17] Scrapie, Creutzfeldt-Jakob and BSE,[18] the molecular architecture of the tobacco virus[19] and the association of microtubuli during mitosis.[20]

The tobacco mosaic virus (TMV) is a fascinating example for the self-organization through self-assembly of relatively simple monomers in nature. The TMV is a rod-shaped virus

300 nm long and 18 nm in diameter. The central right-handed helix is built up by the self-association of 2130 identical protein subunits (M_r = 17500) in the presence of an RNA single strand. This RNA strand carries the genetic information and is placed between these peptide subunits at a distance of 4 nm from the outer diameter (Fig. 2).

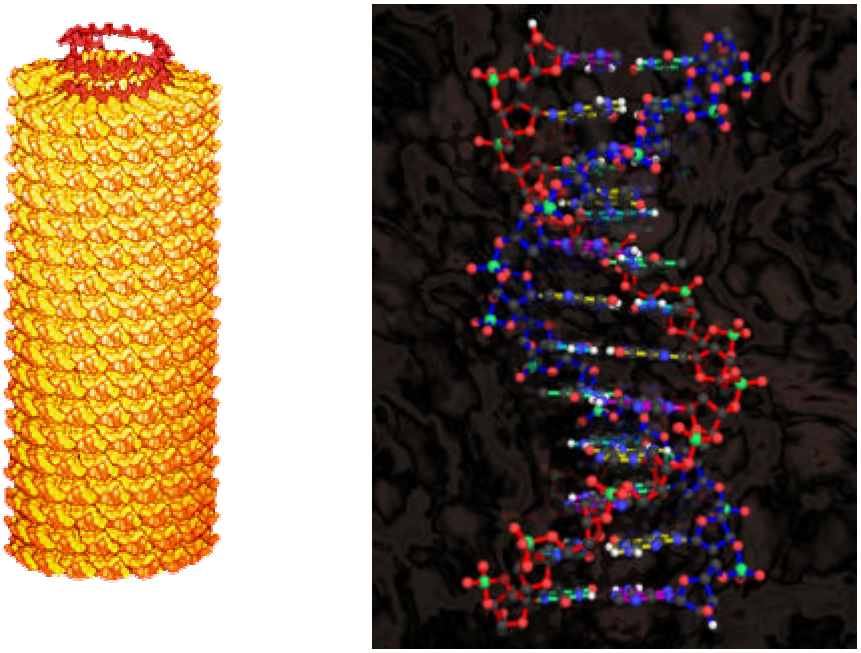


Fig. 2. *Two examples of self-assembly in nature: The tobacco mosaic virus (left) and the DNA-helix (right).*

In recent years, supramolecular research has focused more and more on the development of artificial self-assembling systems which will give access to the controlled formation of well-defined architectures from their molecular components. This could lead to the development of novel materials and nanostructures with tailor-made properties and significant technological potential (e.g. supramolecular polymers, molecular crystals, tailor-made proteins, new catalysts and drugs).

Both of these supramolecular processes -the molecular recognition and self-assembly- are based on intermolecular interactions of single chemical species (ions or molecules) through noncovalent bonds with a substrate (host-guest-chemistry) or with themselves (self-assembly). Thus, supramolecular chemistry is often defined as “chemistry beyond the molecule” which means that it is concerned with intermolecular binding interactions and with entities and assemblies of higher complexity than single molecules themselves.[7]

Over the past three decades, intense research has been directed towards the search for novel building blocks which are capable of self-assembling, the design of artificial receptors for a given substrate and also towards the understanding of the concepts that govern these

processes. Despite the progress over the years, the design of self-assembling molecules and receptor systems is still a challenging task, especially in polar solvents, due to the limited strength of non-covalent interactions in such solvents.[21-24] So far, hydrogen bonds have been mostly used because of their specifity and directionality.[25] However, as the polarity of the surrounding solvent increases, the strength of hydrogen bonds decreases rapidly due to the competitive solvation of donor and acceptor sites by the solvent. Therefore, most systems described to date show complexation only in organic solvents of low polarity. However, it would be desirable to have access to systems that also function in more polar (i.e. more "natural") solvents such as water.[26-30]

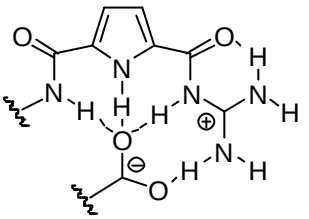
Accordingly, it became evident that there is still a great demand for the design of new artificial binding motifs and also for the design of self-assembling systems that function under physiological (i.e. in water) conditions. The evaluation of the thermodynamics of such noncovalent binding interactions will significantly increase the understanding of the concepts that govern these processes. Additional knowledge of the fundamental principles is not only crucial for the construction of new artificial systems, but it also has a remarkable influence on the understanding of the folding (association) and reactivity (binding process) of biomolecules like DNA, peptides or oligosaccharides.

Additionally, the design of artificial receptors for biological substrates and the design of new supramolecular materials will pave the way for a variety of new technological applications in the near future, like for example the development of biosensors, potential drug candidates, separation of racemic mixtures or the design of new catalysts and supramolecular polymers.

The aim of the present thesis is to explore the use of the guanidiniocarbonyl pyrrole binding motif for the design of new building blocks for supramolecular polymers and bioorganic receptors in aqueous solvents.

2 Project and Concept

In recent years *Schmuck* has introduced guanidiniocarbonyl pyrroles **1** as a new and very efficient artificial receptor class for the complexation of carboxylates and amino acids in polar solution.[31-33] The additional ion pairing, besides the multiple hydrogen bonding, improves the complexation properties as compared to simple guanidinium ions (for more details see chapter 3.1.2). Thus, a complexation of carboxylates in polar media (DMSO or water) was achieved (Fig. 3).



1

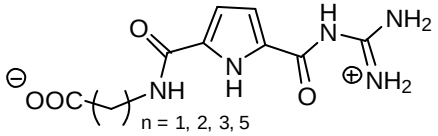
Fig. 3. *Guanidiniocarbonyl pyrroles **1** developed by Schmuck as a binding motif for carboxylates.*

These results provided the starting point for my thesis. With this general binding motif for carboxylates at hand, the goal of my work was to develop new receptor systems for the complexation of amino acids and dipeptides in aqueous media. Additionally, in a supramolecular project new self-assembling zwitterions should be developed. A necessary requirement therefore is that a new, mild and facile synthetic approach towards protected guanidines like **2** was developed which was not yet at hand. (see general Scheme 1 on page 6).

My thesis comprises the following projects:

1. ***Supramolecular Self-Assembly:***

   New flexible self-assembling zwitterions **3-6** should be developed, which aggregate in polar solution and thus pave the way to new interesting supramolecular structures or even supramolecular polymers (Fig. 4)



3-6

Fig. 4. *Flexible zwitterions **3-6** with spacer length n = 1, 2, 3, 5.*

2. Bioorganic receptors for the complexation of amino acids and dipeptides:

a) To synthesize of a series of arginine-analogues **7-10**, which can be utilized in the future as arginine mimetic by incorporation via normal solid-phase synthesis into natural arginine-rich peptides (Fig. 5). This may then have a great influence on the structure and biological properties of such modified peptides

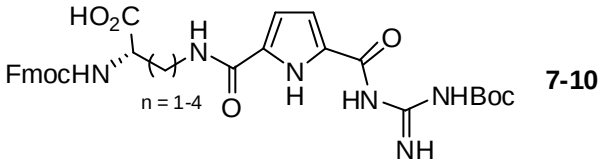


Fig. 5. *Arginine analogues **7-10** with spacer length n = 1-4.*

b) To develop a new tris-cationic receptor **11** for the complexation of amino acids in polar solution (Fig. 6). In course of this particular project the influence of an additional ionic interaction within our guanidiniocarbonyl pyrrole motif should be investigated. Additionally, receptor **11** should be capable to bind amino acids with two carboxylate residues.

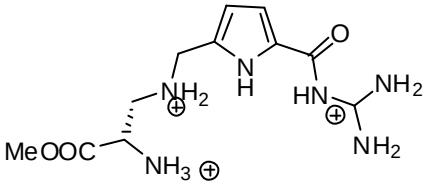


Fig. 6. *Tris-cationic receptor **11** for amino acids.*

c) To synthesize and subsequently evaluate a *de-novo* designed receptor **12** for the complexation of dipeptides in water (Fig. 7)

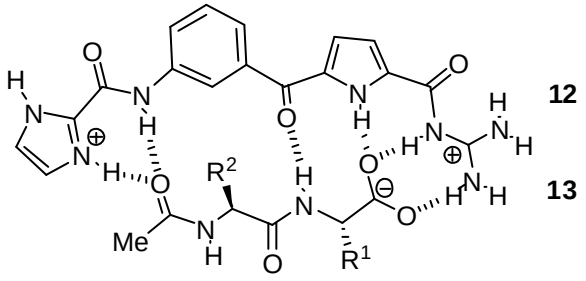


Fig. 7. *Complex of the de-novo designed dipeptide receptor **12** with an acylated dipeptide **13** (left).*

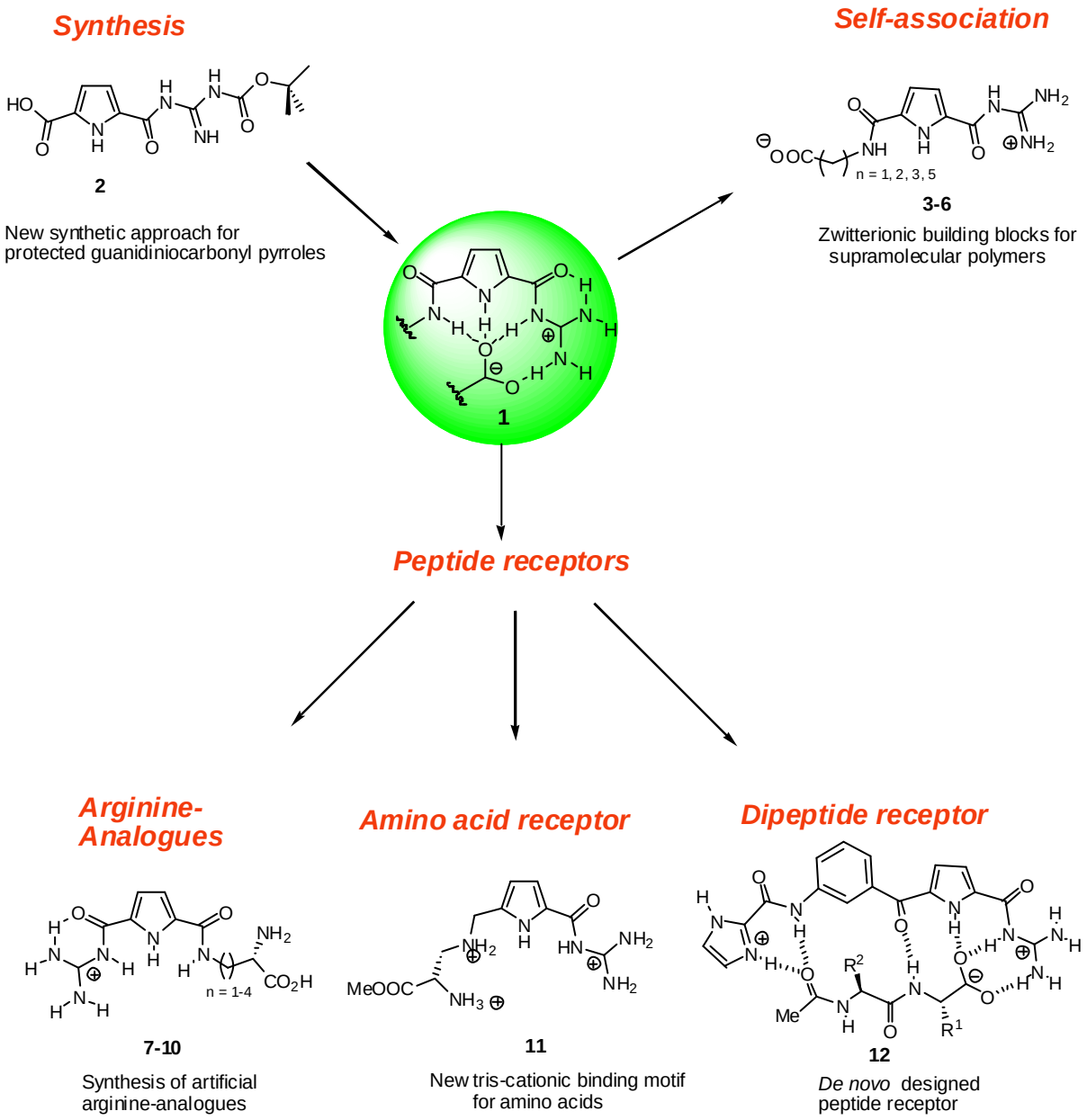
The analysis of the results obtained in these diverse supramolecular and bioorganic projects on basis of the guanidiniocarbonyl pyrrole binding motif should improve our knowledge of the thermodynamic foundations of such self-association or molecular recognition processes. The questions which should be investigated and evaluated in detail within these projects are:

- The *structure* of the intermolecular or intramolecular formed aggregates (zwitterionic project) or the receptor-substrate complexes, respectively.

- The *stability* of the formed aggregates. This means the determination of the binding energy for the receptor-substrate interaction or the intermolecular association in the case of zwitterionic project.

- The *analysis* of these data in terms of structure-stability relationships. By the determination of the stability of the formed aggregates the energetical contribution of individual noncovalent interactions (single hydrogen bonds, additional ionic interactions etc.) should be estimated. Additionally, the influence of these interactions on the structure of the formed aggregates should be studied.

This additional knowledge of the thermodynamic of such noncovalent interactions is crucial for the directed development of new receptors for a given target in the future.
Scheme 1 summarizes the central projects of the present thesis.



Scheme 1. *Schematic representation of the central projects of my thesis.*

Project 1: Flexible Zwitterions

The first objective of my thesis was to create a new class of flexible zwitterions, **3-6**, in which a carboxylate is linked via an alkyl chain to a guanidiniocarbonyl pyrrole cation (Fig. 8).

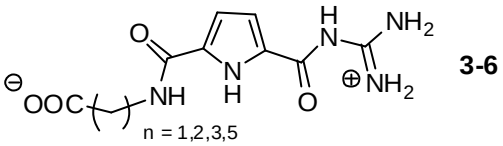


Fig. 8. *Flexible zwitterions **3-6**.*

Due to the fact that guanidiniocarbonyl pyrroles strongly interact with carboxylates in polar solution, *Schmuck* has already synthesized simple rigid zwitterionic compounds which form oligomeric aggregates in DMSO (discussed in detail in chapter 3.2.1).[34]

Based on a newly developed mild and efficient synthesis for such guanidiniocarbonyl pyrroles, it was now possible to synthesize a variety of new zwitterions which could be deliberately modified. The analysis of their self-assembly and therefore the influence of the length and flexibility of the alkyl spacer implemented in these flexible zwitterions **3-6** should be studied within this particular project. These zwitterions should be capable to either *intermolecularly* dimerize and oligomerize or to form *intramolecular* self-folded molecules ("loops") (Fig. 9).

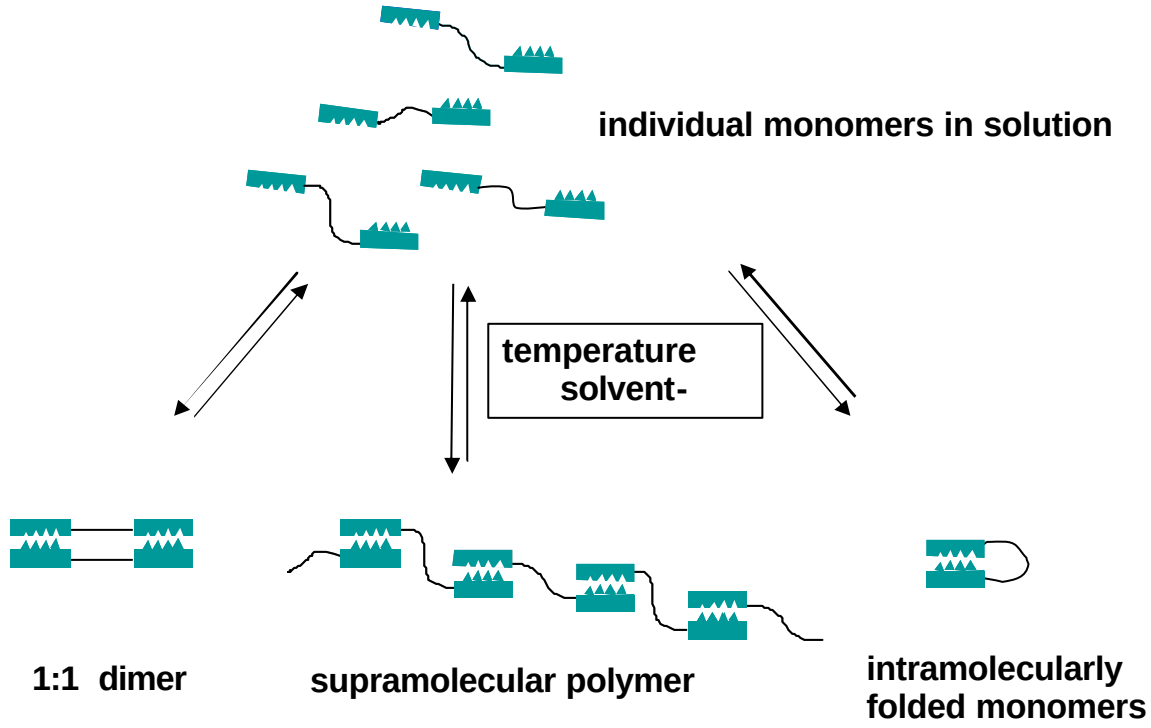


Fig. 9. *Possible association equilibriums of individual monomers.*

The study of such ionic building blocks is useful, since it represent the first steps towards the design of new supramolecular polymers that could function even in polar solution.
Supramolecular polymers are in general reversible assemblies that are formed through noncovalent interactions of individual monomers. These materials combine the many attractive features of conventional polymers such as elasticity or viscosity with reversibility and responsiveness. However, so far the vast majority of artificial self-assembling systems, and thus also supramolecular polymers, rely mainly on hydrogen bonds due to their directionality and versatility. Therefore, most systems function only in unpolar media like hexane or chloroform. In contrast to that, our binding motif incorporates additional ionic interactions which enhance the strength of the hydrogen bonded self-complementary network. This binding motif may therefore allow the design of water-soluble supramolecular polymers in the near future.

Project 2: Bioorganic receptors for the complexation of amino acids and dipeptides

Besides the application in supramolecular research fields, the guanidiniocarbonyl pyrrole binding motif can also be used to develop interesting bioorganic receptors for biologically important substrates.

Project 2a: Arginine Analogues

One other aspect of my research was to synthesize four new analogues **7-10** of the natural amino acid arginine **14** (Fig. 10). Arginine **14** is involved in many physiological and pathophysiological processes usually by stabilization of negatively charged groups (carboxylate, phosphate).[35, 36] Due to the fact that our guanidiniocarbonyl pyrroles bind carboxylates much stronger, than arginine, substitution of **14** by one of our unnatural analogues **7-10** may have a profound influence on the chemical and biological characteristics of arginine rich peptides.

Fig. 10. *The natural amino acid arginine **14** (left) and our arginine analogues **7-10** (right)*

A necessary prerequisite for a facile incorporation of our arginine-analogues **7-10** as a substitute for the natural amino acid arginine **14** into small oligopeptides would be that our protected arginine analogues can be used in a normal solid phase synthesis. Therefore, as a "proof of principle" the arginine analogues **7-10** should be tested in a standard solid-phase synthesis (Fig. 11).

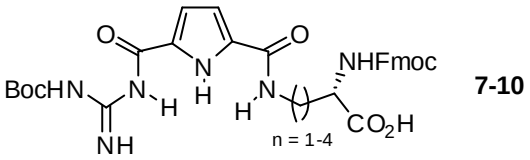


Fig. 11. *The protected arginine analogues **7-10** for the application in solid-phase synthesis.*

Project 2b: Tris-cationic amino acid receptor

In the thermodynamic studies of the binding process of guanidiniocarbonyl pyrroles of type **1** by *Schmuck* it became clear that the amide moiety in 5-position of the guanidiniocarbonyl pyrrole is very important for the binding process. It contributes approximately 25 % to the total binding energy. To study this special energetic contribution in detail, I developed a di-cationic receptor type **15**, in which the amide function was substituted by an ammonium function. With this receptor type the influence of an additional ionic interaction at the 5-position could be determined and compared to the neutral amide functionality. The general design offered also the possibility to synthesize a new tris-cationic receptor of type **11**. This receptor **11** should be capable to strongly complex amino acids with two carboxylate groups (aspartic acid or glutamic acid) in water (Fig. 9).

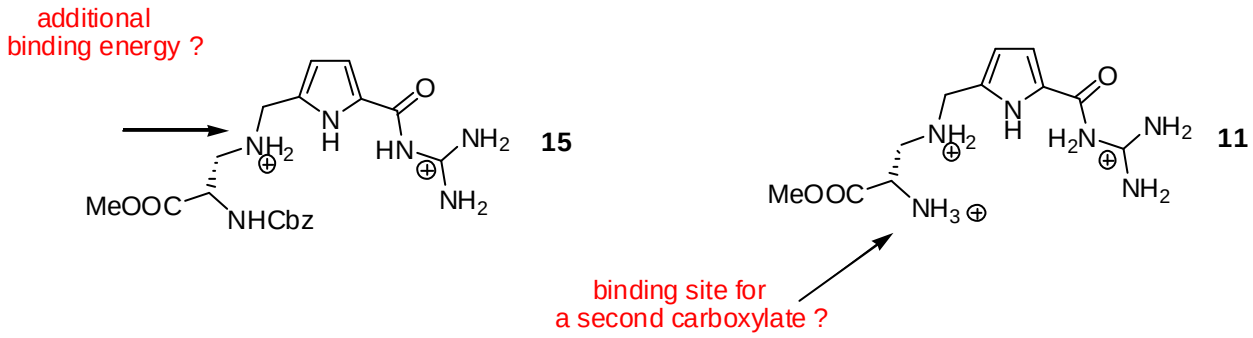


Fig. 12. *Di-cationic receptor **15** (left) and tris-cationic receptor **11** (right) for the complexation of amino acids in water.*

Project 2c: Dipeptide receptor

The receptor **12** was *de-novo* designed based on molecular modelling studies and is therefore predicted to bind C-terminal dipeptides in a β-sheet conformation (Fig. 13).

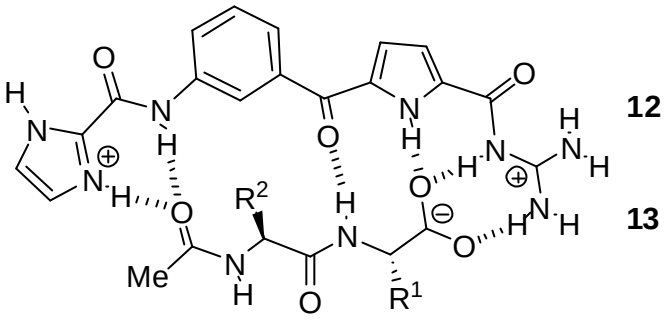


Fig. 13. *Complex of the de-novo designed dipeptide receptor **12** with an acylated dipeptide **13**.*

The guanidiniocarbonyl pyrrole moiety in the receptor is predicted to form a hydrogen bonded ion pair with the carboxylate. Additional hydrogen bonds between the amide group of the peptide backbone **13** and the receptor **12** should further enhance the stability of the complex.

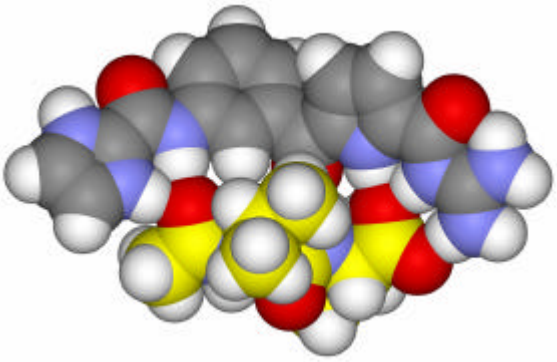


Fig. 14. *Calculated complex structure for the binding of **13** (yellow) by receptor **12** (grey).*

The design of an artificial receptor that efficiently binds to such C-terminal peptides in a β-sheet conformation is interesting, because this will allow to study and induce peptide secondary structures in the future.

3 General Theoretical Section

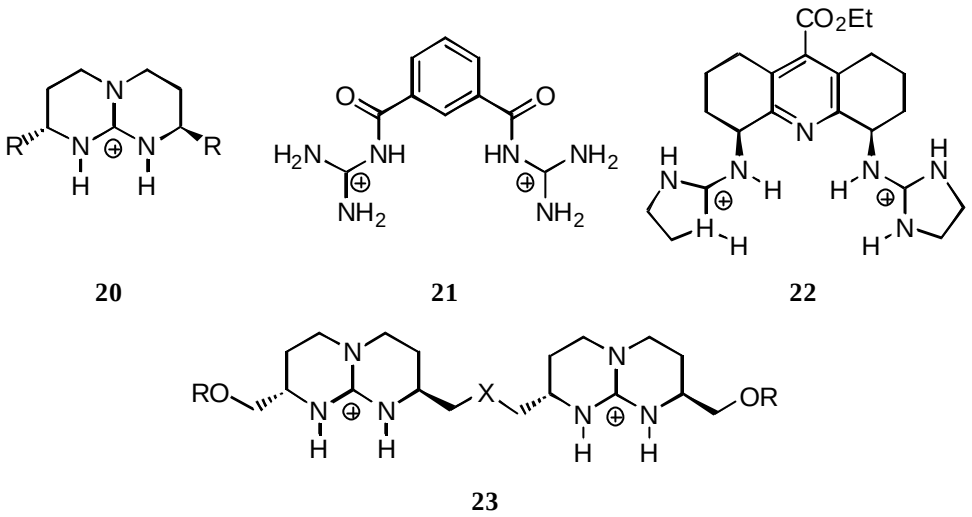
In the following sections I will give a short overview on the current research in fields which are relevant for this thesis. Due to the fact that the guanidiniocarbonyl pyrrole binding motif developed by *Schmuck* is an essential basis for my research, I will at first focus on the design and characterisation of this system. Afterwards, I will describe the general progress and results in the design of self-assembling and self-associating systems over the last years as building blocks for new supramolecular polymers. Finally, I will summarize the relevant work in the field of molecular recognition, i.e. the work dealing with the development of receptors for biological important small molecules, especially peptides.

3.1 Binding motifs for carboxylates

3.1.1 Introduction

Both in nature and supramolecular chemistry guanidinium cations are well-known for the complexation of oxo anions such as phosphates or carboxylates. Arginine **14** for example plays a key role in many biological peptides (e.g. neuropeptide Y, RNA-binding proteins) through binding of carboxylates and is also involved in a large number of metabolic processes.[35, 36]

Lehn was the first to investigate the complexation of carboxylates by different simple bis- and tris-guanidinium salts in the late 70s.[37] In the early 80s, *Schmidtchen* used charged bicyclic guanidinium cations such as **20** for the complexation of anions in chloroform.[38] More recently, *Hamilton* **21**,[39] *Anslyn* **22**,[24] and *Schmidtchen* **23**[40-42] designed poly-guanidinium receptors that function even in more polar solvents such as DMSO, methanol or sometimes water (Fig. 15). Despite the very strong electrostatic interaction of these poly-guanidinium complexes, the binding constants were still moderate ($K = 10^1 - 10^5\ M^{-1}$) and design and synthesis of such receptors remains demanding.[28, 29, 43-48] Theses examples show, that in highly competitive solvents, ion pairing of simple guanidinium cations with carboxylates is normally not strong enough for the formation of stable complexes and that only multiply charged poly-guanidinium receptors achieve reasonably binding constants.



20 **21** **22**

23

Fig. 15. *Mono- and poly-guanidinium receptors **20-23** for the complexation of anions in organic solvents.*

3.1.2 Guanidiniocarbonyl pyrroles as a binding motif for carboxylates

To enhance the complexation properties of simple guanidinium cations, additional binding interactions besides the ion pairing are therefore necessary. Recently, based on theoretical calculations (Macromodel V. 6.5, Amber* force field, GB/SA water solvation treatment)[49] *Schmuck* introduced guanidiniocarbonyl pyrroles, such as **1**, as a new receptor class for carboxylates (Fig. 16). These acyl guanidinium receptors combine several new features compared to simple guanidines, such as **20-23** which improve their binding properties even in competitive solvents.[32]

- The increased acidity of the acyl guanidinium group relative to a guanidinium cation favors the formation of ion pairs via hydrogen bonds and hence increases the binding affinity. Acyl guanidines have pK-values in the order of 6-8 whereas simple guanidiniums have pKs around 13.5.[50, 51]
- Additional hydrogen bonds, from the pyrrole NH or the amide NH, can further enhance the stability of the complex.
- The binding motif is planar and rather rigid and therefore ideally preorganized for the binding of planar anions such as carboxylates.
- Additional secondary interactions between the receptor side chain and the carboxylate can be used to achieve selectivity with respect to the carboxylate.

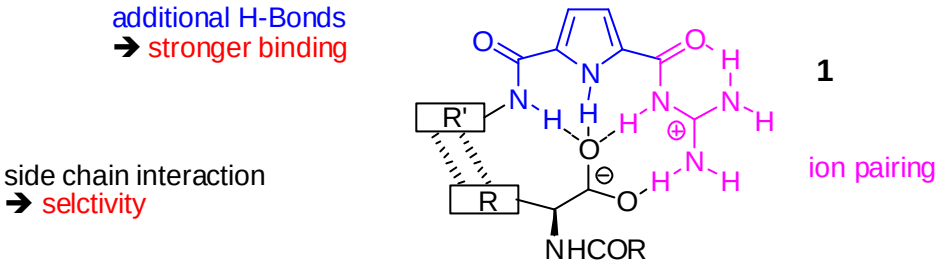


Fig. 16. *Design of guanidiniocarbonyl pyrrole receptors **1** for the binding of carboxylates; the ion pairing and the hydrogen bonds provide the binding strength whereas additional interactions with the side chain can account for the substrate selectivity.*

And indeed guanidiniocarbonyl pyrroles bind carboxylates even in highly polar solvents. Addition of acetate **24** to a solution of the ethylamide-substituted guanidiniocarbonyl pyrrole **25** in DMSO causes significant complexation induced shifts (CIS) of the various protons of **25** in the ^{1}H NMR spectrum (Fig. 17).[52]

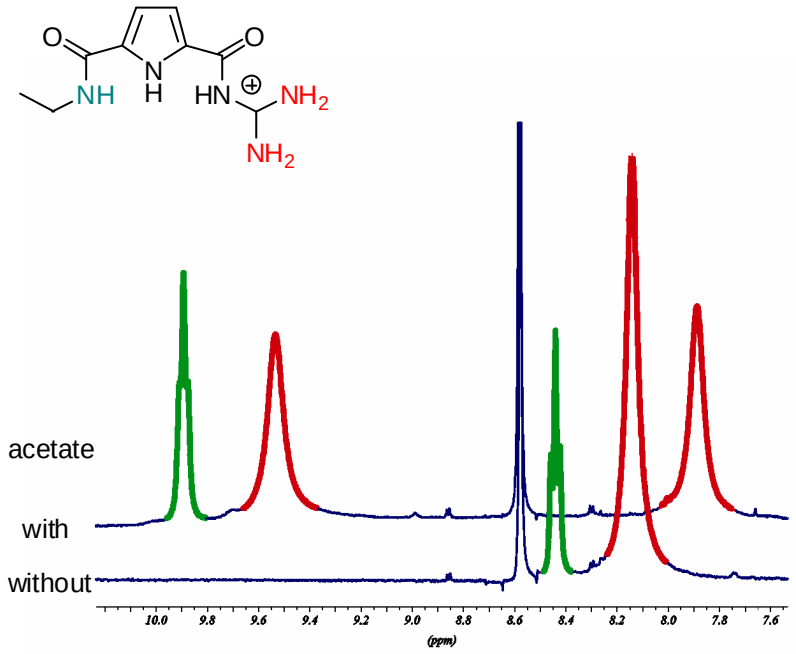


Fig. 17. *^{1}H NMR spectrum of receptor **25** (picrate salt) with (back) and without (front) acetate **24** (NMe_4^+ salt) in DMSO showing the CIS of the guanidinium NH protons and the amide NH.*

The complexation induced shifts clearly indicate the participation of all these NH protons in hydrogen-bonding interactions with the bound carboxylate. The observed shifts changes in the ^{1}H NMR spectrum are consistent with the general binding motif depicted in Fig. 16 and Fig. 18. The guanidinium cation forms an ion pair with the carboxylate which is simultaneously hydrogen bonded by the pyrrole NH and the amide NH.

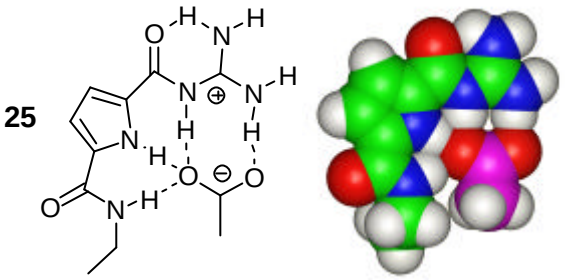


Fig. 17. *Proposed binding motif for the complexation of carboxylates by guanidiniocarbonyl pyrrole receptors like* ***25****.*

In solution the stability of these complexes could be determined via NMR titration experiments, using the dependence of the complexation induced shift changes on the ratio of host to substrate.[53] In DMSO the binding is too strong so that a NMR titration experiment with guanidiniocarbonyl receptor **26** and carboxylate **27** or acetate **24** just showed a linear increase of the shift changes until a molar ratio of 1:1 was reached (Fig. 19).[54] This confirms the expected 1:1 stoichiometry of the complex and reflects an association constant in the order of $K \geq 10^5$-10^6 mol^{-1}. Even in 50 % water/DMSO the association constants are still in the order of $K \geq 10^3$ mol^{-1}. As expected simple guanidinium cations such as the ones *Hamilton*[39] and *Kilburn*[55] have used in their receptors show no complexation under these polar conditions.

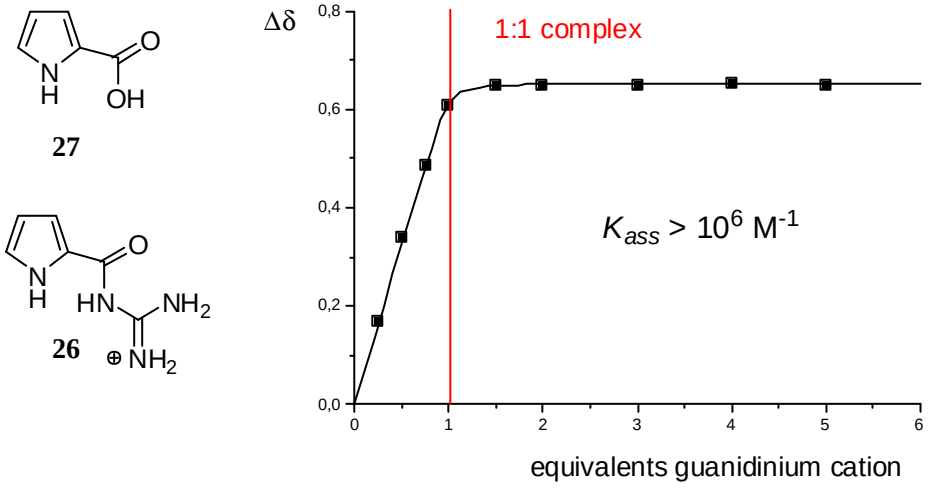
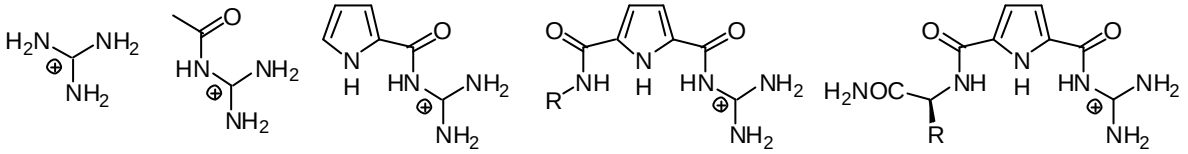


Fig. 19. *NMR titration curve of carboxylate* ***27*** *(1 mM) with guanidinium cation* ***26*** *in DMSO.*

To experimentally determine the individual energetic contribution of an individual bond or type of interaction within (e.g. acyl guanidinium cation, pyrrole-NH, amide-NH, side-chain) this receptor-substrate complex the binding properties of a systematically varied series of

receptors **25**, **26**, **29-30** (Fig. 20) were studied using N-acetyl alanine carboxylate **33** as the substrate. Thus, a quantitative value for the energetical contribution of an individual interaction could be estimated.[52]



28 **29** **26** **25** (R = Et) **31**(R = H)

30 (R = Bu) **32** (R = *i*Pr)

Fig. 20. *Receptors **25**, **26**, **29-32** (picrate salts) used for the binding studies.*

Compared with the parent acyl guanidinium cation **29** the pyrrole derivative **26** contains one more potential hydrogen bond donor (the pyrrole NH), the ethyl and the butyl substituted receptor **25** and **30** two (the pyrrole and the amide NH), and the amino acid derivatives **31** and **32** three (the pyrrole and the amide NH). The binding constants were experimentally determined by NMR titration experiments in 40 % water/DMSO showing the following results:

- The simple guanidinium cation **28** does not bind the carboxylate ($K < 10\ M^{-1}$).
- The more acidic acetyl guanidinium compound **29**[52, 56] shows a weak association constant of $K = 50\ M^{-1}$
- For the guanidinium cations **26**, **25**, **30**-**32** every additionally potential hydrogen bond donor position increases the complexation significantly:
    1. The association constant of the guanidiniocarbonyl pyrrole **26** with its one additional binding site is about three times larger than that of the acetyl guanidinium cation **29** ($K = 130\ M^{-1}$ versus $K = 50\ M^{-1}$).
    2. The additional amide group in the receptors **25** and **30** further increases the binding constant by a factor of five relative to **26** ($K = 770$ and $690\ M^{-1}$).
    3. For the valine substituted receptor **32** the binding constant is again larger by another factor of more than two relative to **25** and **30** ($K = 1610\ M^{-1}$).

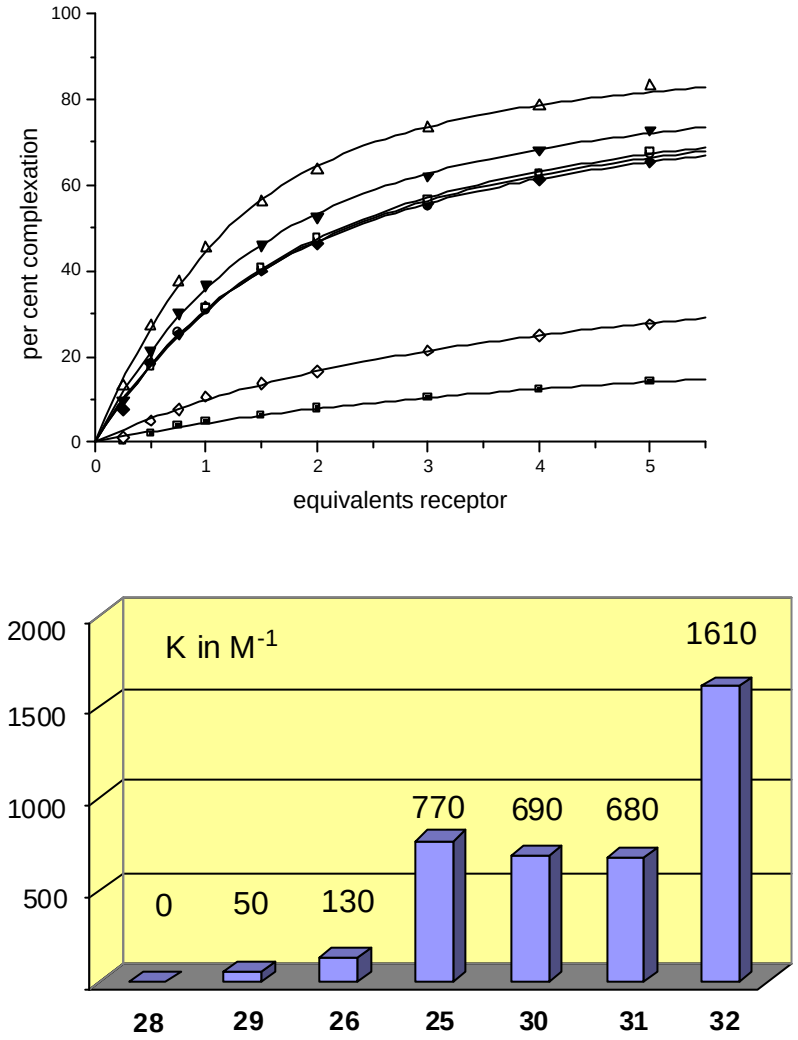


Fig. 21. *NMR titration curves of the various receptors (picrate salts) with alanine carboxylate (NMe_4^+ salt, 1mM) in, 40 % water in DMSO (v/v);* ***32*** *(D),* ***25*** *(▼),* ***30*** *(·),* ***31*** *(›),* ***26*** *(à) and* ***29*** *(■) and below the calculated binding constants (in M^{-1}) are shown.*

Assuming the same complex structures for all receptors (which seems plausible based on molecular modeling findings) estimated semi-quantitative energetical contributions of the various binding interactions for the complexation of carboxylate by guanidiniocarbonyl pyrrole receptors of the general type **1** can be derived, which are shown in Fig. (22).[32, 52]

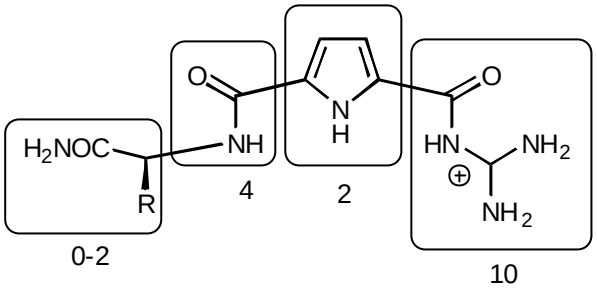


Fig. 22. *Estimated semi-quantitative energetic contributions of the individual binding interactions for the overall complexation of carboxylate by guanidiniocarbonyl pyrroles **1** (DG in kJ·mol^{-1}).*

The data in Fig. (22) show, that the different hydrogen bonds do not contribute equally to the binding process. Besides the ion pairing with the acyl guanidinium moiety, which provides the major binding energy of ΔG = 10 kJ·mol^{-1}, the amide NH next to the pyrrole ring is most important and increases the binding energy by another 4 kJ·mol^{-1}. The pyrrole NH adds only 2 kJ·mol^{-1}, while it is depending on the group R whether the terminal carbamoyl group, because of its rather high flexibility, increases the binding energy at all (maximum up to 2 kJ·mol^{-1}).

The presented thermodynamic findings were further studied within my work. Due to the fact that the amide NH next to the pyrrole contributes nearly 25 % to the total binding energy, I developed a receptor motif in which the amide NH was substituted by an amine.

In general, such guanidiniocarbonyl pyrrole-carboxylate interactions are also interesting for supramolecular research fields. Considering the fact, that this kind of interaction is tremendously strong in polar media, as well interesting *intermolecular* or *intramolecular* supramolecular structures could result. This particular research field will be introduced in the following chapter.

3.2 From self-assembly to supramolecular polymers

As mentioned in the introduction, the self-assembly of individual molecules through noncovalent bonds plays an important role in nature for the formation of highly complex and biological interesting structures.[5-9, 11, 57-60] Besides the study of natural self-association processes, the research in the recent years has focused on artificial self-assembling systems mostly designed as building blocks for supramolecular polymers.

The association of artificial self-complementary binding motifs can thus lead to highly fascinating supramolecular aggregates. Such self-associating building blocks can be used for example in the field of material science to synthesize supramolecular polymers through the self-association of self-complementary monomers (Fig. 23).[61]

The major advantage of such supramolecular polymers, compared to usual covalent polymers, is the reversibility of the noncovalent interactions.

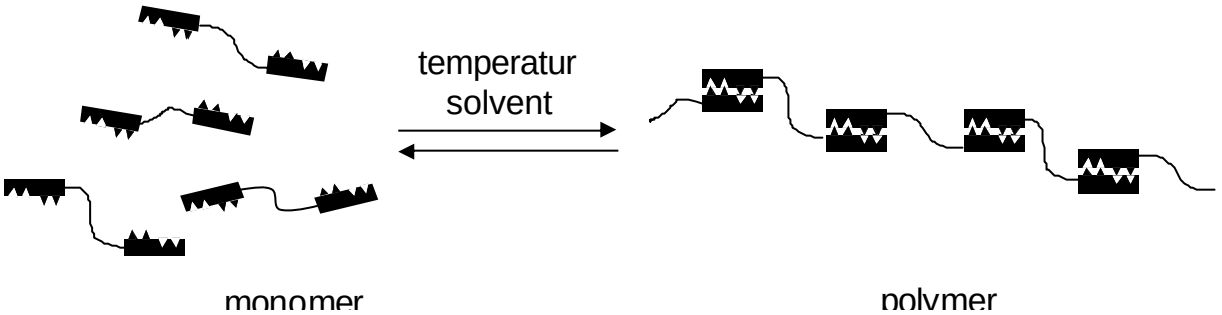


Fig. 23. *Supra-polymerisation of bifunctional monomers.*

The strength of noncovalent interactions depends significantly on the chemical environment (solvent, temperature) and thereby the macroscopic properties of such polymers can be controlled and adjusted by variation of the external parameters. Additionally, the reversibility of the noncovalent interactions leads to thermodynamic controlled polymeric suprastructures. This is of great advantage for the production of polymers with potential for commercial use, since kinetically induced defects like in covalent bonded polymers are avoided.

However, most of the artificial self-assembling systems that were synthesized until now just make use of hydrogen bonds as intermolecular forces as a reason of their directionality and specifity. The main drawback of hydrogen bonds is their limited strength, the more polar the solvent is, the weaker the hydrogen bonds are (Fig. 24).[62]

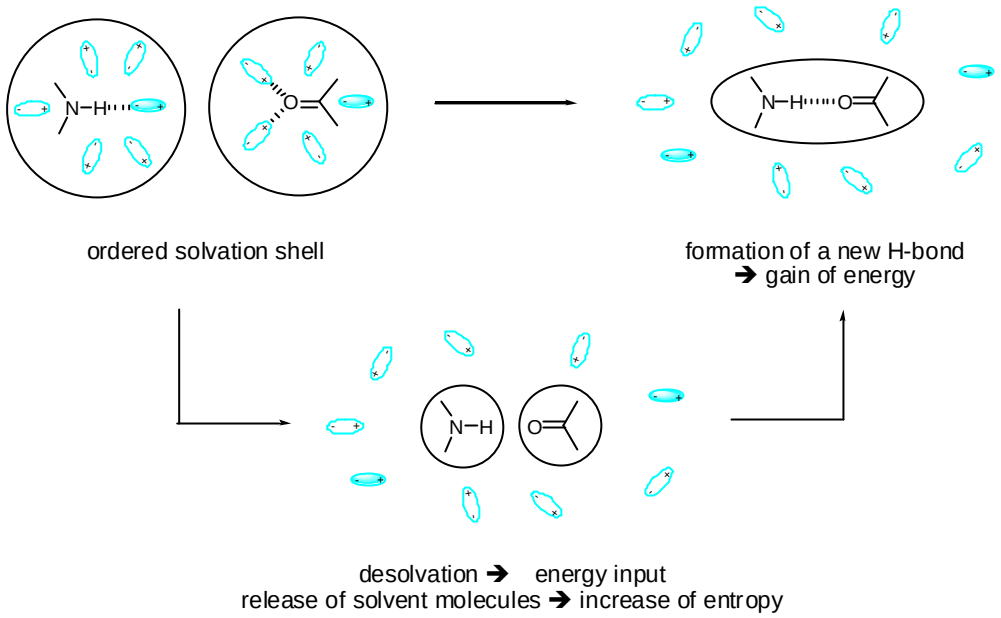
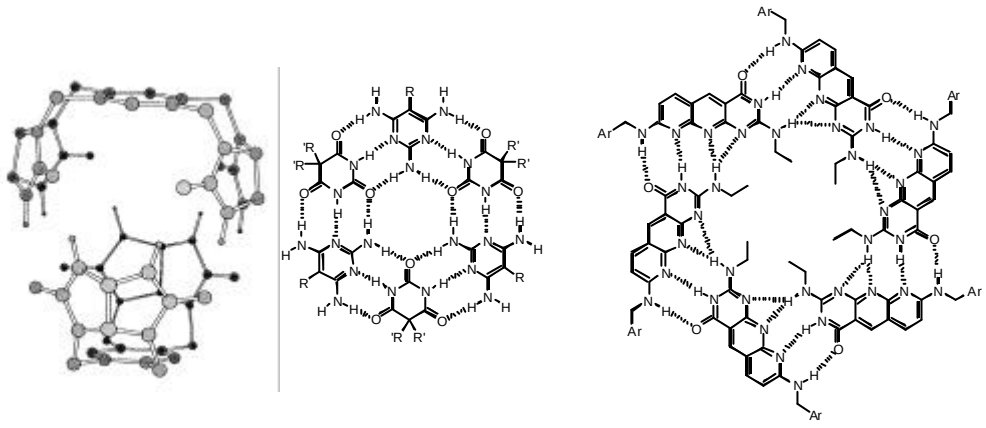


Fig. 24. *Effect of water on the strength of hydrogen bonds.*

Therefore, hydrogen bonds possess a considerable binding energy only in unpolar aprotic solvents ($CHCl_3$, hexane). However, for stable supramolecular assemblies in more polar solvents a combination of several hydrogen bonds with other noncovalent forces, such as ionic or hydrophobic interaction is necessary.

Rebeks supermolecular tennis ball **33**,[63-67] and also *Withesides* hexameric rosettes **34**[68] or *Zimmermans* cyclic self-aggregates **35**[69] are therefore only stable in solvents, such as chloroform and break down upon addition of even smallest amounts of DMSO (Fig. 25).[70]

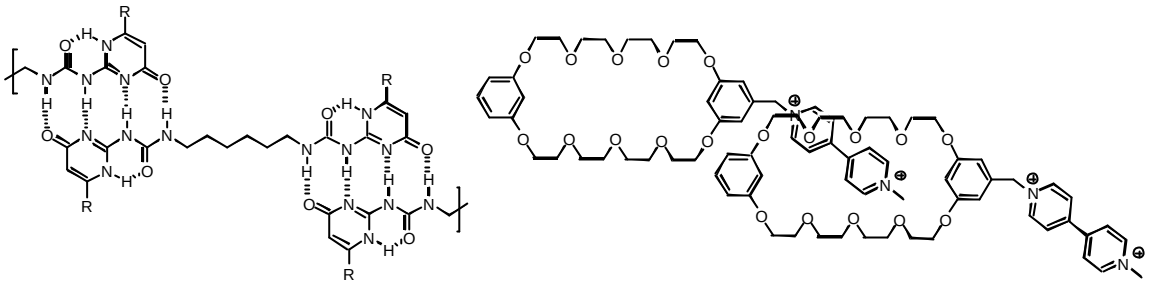


33 **34** **35**

Fig. 25. *Examples for self-assembling systems. All aggregates shown are only stable in chloroform and break down with the addition of polar solvents like DMSO.*

Meijer and co-workers synthesized supramolecular ureidopyrimidone polymers through the self-association of a bifunctional molecule **36**, which is based on four hydrogen bonds in a linear array and accordingly aggregates again only in non-polar solvents.[71-74]

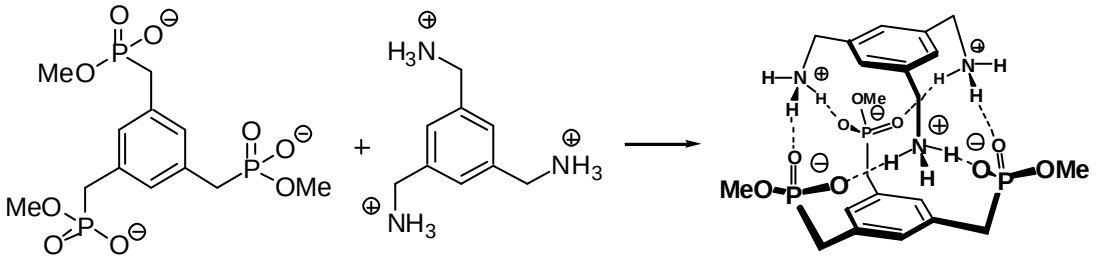
Stoddard[75, 76] and also *Gibson*[77, 78] reported self-associating paraquat-crown ether-monomers, such as **37**, in which in addition to hydrogen bonds π-π-interaction were incorporated, but they also aggregate only in solvents like acetonitril or acetone (Fig. 26).



36 **37**

Fig. 26. *Supramolecular polymers. In nonpolar solvents these compounds aggregate and form linear polymers.*

However, all the above mentioned systems display their unique conformations only in solvents of low polarity such as chloroform; more polar solvents destroy the hydrogen bonds and therefore the self-assembly. Today there exist only a few examples for self-assembly in polar solvents, which do not rely on strong coordinative metal-ligand bonding.[79-81] In these systems, aggregation in water is mostly achieved through the combination of hydrophobic effects or an extensive accumulation of electrostatic interactions.[82] In the case of *Schrader´s* molecular box **38**, the complexation emerges for example from the association of the tris-phosphonate anion **39** with the tris-ammonium cation **40** and therefore possesses a total of six charge interactions (Fig. 27).[83]



38 **39** **40**

Fig 27. S*chrader´s molecular box **40***.

Rheinhoudt´s calix[4]arene-based molecular capsules are also formed by multiple ionic interactions between several sulfonate and amidinium groups.[84, 85] Also the β-peptides, independently disclosed by *Seebach* and *Gellman* in 1996, are an example of molecules, which form defined secondary structures such as helices or β-sheets in water.[86, 87]

These examples show that a self-association in polar media can be achieved by multiple weak noncovalent interactions.

3.3 Self-assembling based on guanidiniocarbonyl pyrrole-carboxylate interaction

Over the past years *Schmuck* has synthesized several very efficient self-assembling systems based on guanidiniocarbonyl pyrrole-carboxylate interaction. The zwitterionic compound **41** for example, forms extremely stable dimers **41•41** in DMSO, which are held together by an array of hydrogen bonds and ion pairing.[33, 54]

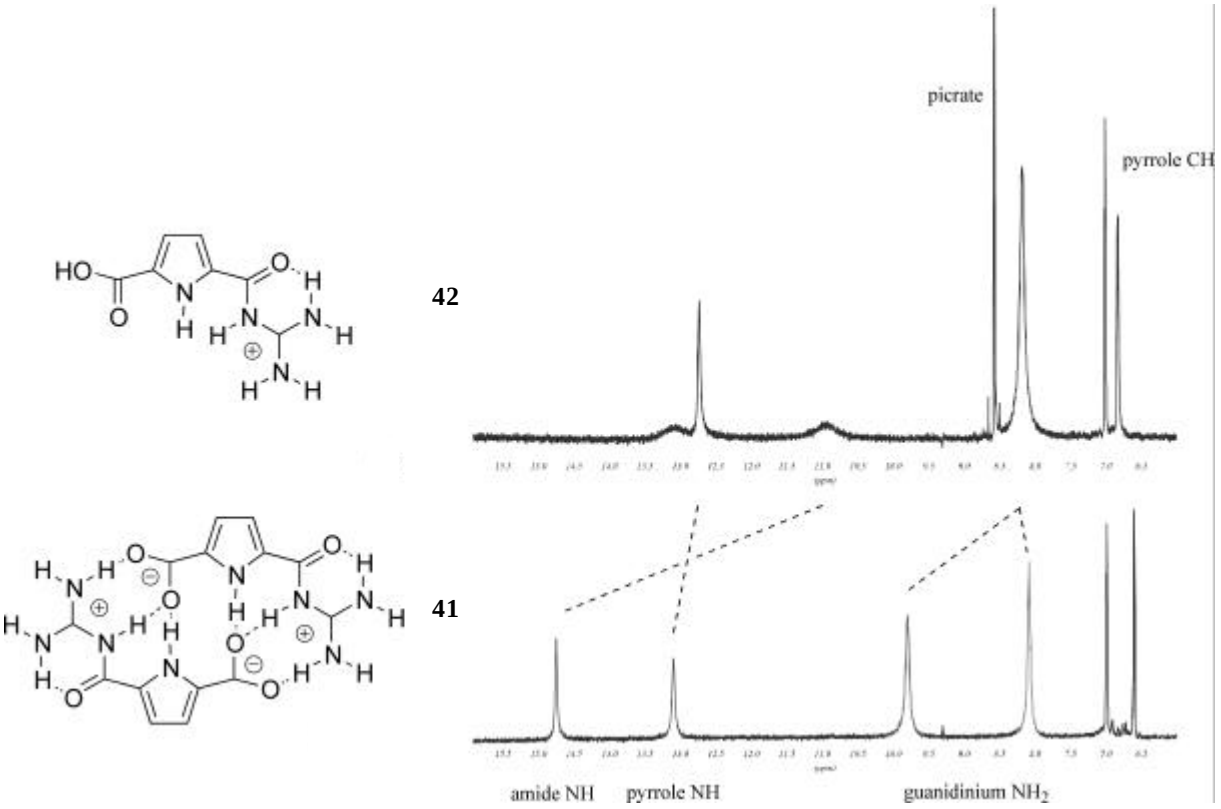
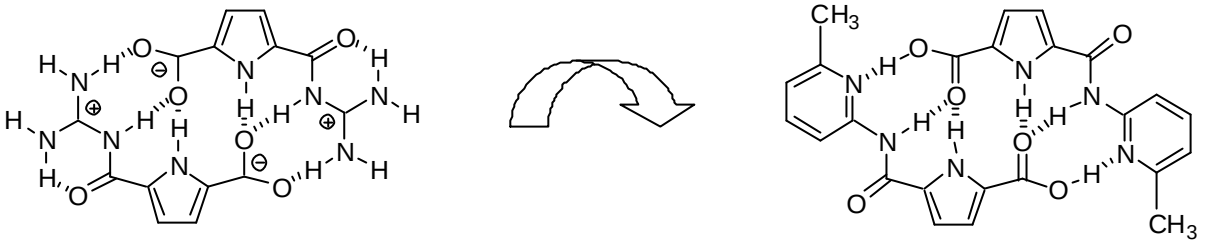


Fig. 38. *^{1}H NMR spectra of the protonated form **42** and the dimeric zwitterion **41·41**.*

Because of the limited solubility of the zwitterion **41** in solvents others than DMSO, the binding motif was modified by *Schmuck* and *Wienand* through the introduction of solubilizing substituents in the 3 and 4 position on the pyrrole moiety. This was accomplished by attaching hydrophilic triethyleneglycol oxymethylgroups. Therefore, the dissociation of the dimer could be studied by NMR dilution experiments in water. In nearly pure water they measured an astonishingly binding constant of K ˜ 170 M^{-1}.[33]

To further investigate the thermodynamic contribution of the ionic interaction on the one hand and the hydrogen bonding on the other to the overall binding energy between the acyl guanidinium and the carboxylate moiety *Schmuck* and *Wienand* synthesized a neutral analogue of zwitterion **41**. In the amidopyridine pyrrole carboxylic acid **43** the ion pairing is switched off, but the same essential hydrogen binding pattern as in **41** is conserved. Hence, any differences in these complex stabilities then must arise from the additional ion pairs, which are not present in the neutral analogue **43** but only in the ionic molecule **41**. Both model systems are given in the following Fig. (29**)** showing the guanidiniocarbonyl pyrrole-carboxylate complex and its translation into the amidopyridine pyrrole-carboxylic acid interaction as a neutral analogue.



41•41 **43•43**

Fig. 29. *Concept of a neutral analogue to the guanidiniocarbonyl pyrrole carboxylic zwitterion **41**: the amidopyridine pyrrole-carboxylic acid **43**.*

Both systems were crystallized and investigated by X-ray diffraction. As it can be seen in the superposition of the resulting structures in Fig. (30), their hydrogen bond network are indeed essentially the same.

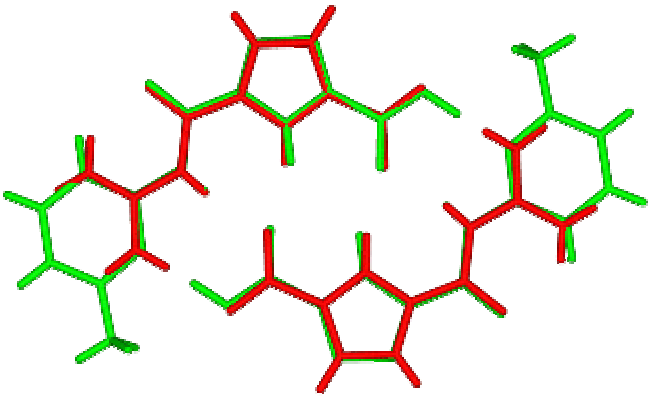
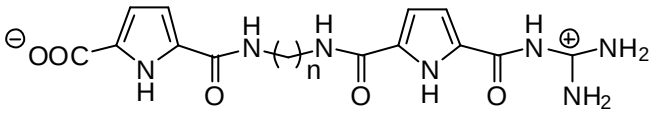


Fig. 30. Sup*erposition of the dimeric solid state structures of the zwitterionic guanidiniocarbonyl pyrrole carboxylate **41•41** and the amidopyridine pyrrole carboxylic acid **43•43**.*

It was shown by NMR dilution experiments, that the amidopyridine pyrrole carboxylic acid forms highly stable dimers in pure chloroform with binding constants well above 10^4 M^{-1}. However, upon addition of only minimal amounts of dimethyl sulfoxide (1 % in chloroform) the binding constant dramatically decreases by two orders of magnitude (K_{dim} ~ 100 M^{-1}). This indicates a very weak and finally completely suppressed dimerization of the monomeric amidopyridine pyrrole carboxylic acid **43** under polar conditions with high amounts of dimethyl sulfoxide. In contrast to this, is zwitterion **41** fully dimerized under such conditions and only with ratios of 10 % DMSO and below in water the dissociation of the zwitterion was observed.[33]

These comparative NMR studies have shown that despite of the identical hydrogen bond network the ionic binding interaction is crucial for the binding in high polar solvents. Moreover, the combination of both types of weak interaction, ion pairing and hydrogen bonding leads to very stable supramolecular complexes even under most challenging conditions, i.e. water. It can also be stated that the described zwitterionic binding motif **41•41** is one of the first supramolecular systems at all to form stable associates even in pure water solely relying on self-complementary electrostatic interactions.

In contrast to rigid zwitterions like **41**, the self-aggregation properties of more flexible zwitterions should differ significantly. To investigate this, *Schmuck* synthesized zwitterions **44** in which the pyrrole carboxylate moiety was linked to a guanidiniocarbonyl pyrrole receptor unit using a flexible spacer (Fig. 31).[52]



44 n = 2,4

Fig. 31. Flexible zwitterions **44**.

Through ROESY and H/D-solvent exchange NMR-studies a self-folding loop structure could be verified for the C_4 spacer zwitterionen.

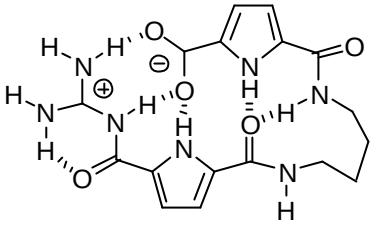


Fig. 32. Self-folding of the zwitterion **44b**.

In contrast to that, the C_2 spacer zwitterionen **44a** is according to molecular modelling studies too short to allow a folding into a well-defined loop. And indeed the C_2-zwitterion **44a** forms only oligomeric structures in solution.

This study provides the starting point for my investigation of the association properties of flexible zwitterion. The question which should be investigated within this particular project is whether the intermolecular association can be controlled and adjusted by the length and thereby flexibility of the spacer. A systematic variation of length and flexibility of an alkyl chain between carboxylate and guanidinium group and their influence on the structure and stability of the formed zwitterionic aggregates in polar solution should be studied. These zwitterionic compounds should either be capable to form intramolecular self-folded loop structure like described for **44a** or intermolecular oligomeric or even polymeric associates.

3.4 Artificial receptors for amino acids and peptides in water

As mentioned before, the molecular recognition of C-terminal oligopeptides plays a tremendous role in a variety of biological processes like for example in the mode of action of the antibiotic vancomycin[1, 88-91] or in the formation of protein plaques in neurodegenerative diseases.[13-17] Due to the high conformational flexibility of the peptides and the weak binding energy of noncovalent bonds in water, the development of artificial receptors for peptides in polar solvents is still a challenging task. Therefore, there are only a few examples that allow the complexation of peptidic substrates in water so far (Fig. 33).[55, 92-95] In most of these cases a complexation is achieved through:

(1) Multiple H-bond interactions with a peptide backbone (β-sheet-tweezer-rezeptors of type **45** of *Kelly* and *Kilburn*)[55, 96]

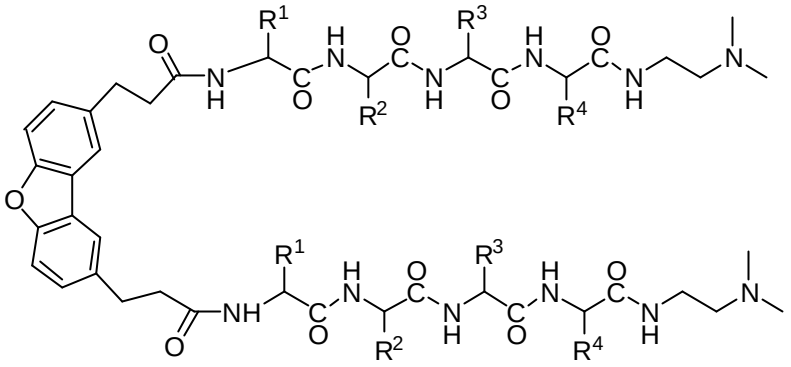
(2) Binding of hydrophobic side chains (Cyclodextrin-dimer-receptors like **46** of *Breslow*),[26, 97]

(3) Additional ionic interaction with ionic amino acid side chains (*Hamilton´s* bis-guanidine-receptors like **47**) for the recognition of aspartic acid on protein epitopes[27, 98, 99]

(4) Interaction with a free carboxy-terminus of the peptide (tripeptidrezeptor **48** of *Schneider*)[94]

or

(5) Interaction of metalloreceptors like **49** (*Mizutani*) with histidine containing peptides.[100]



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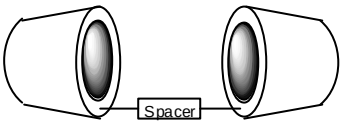

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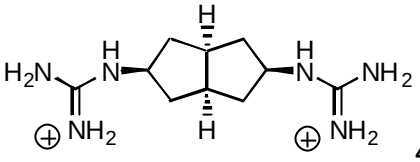

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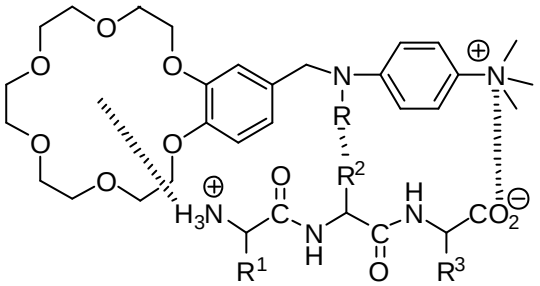

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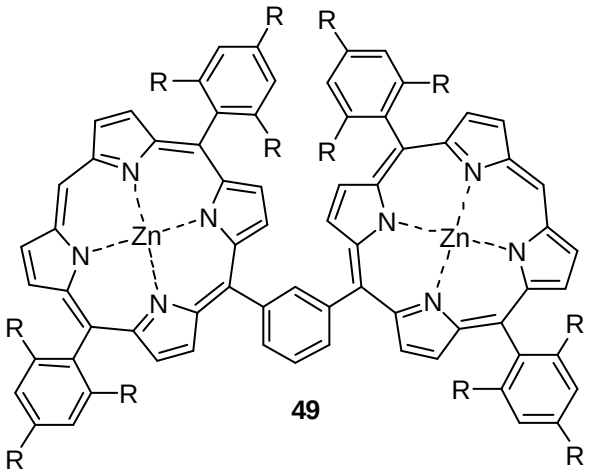
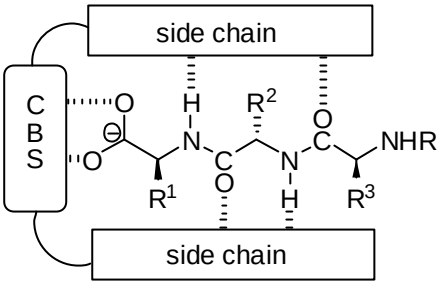


49

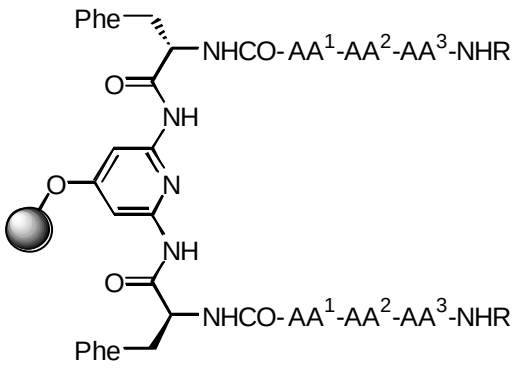
Fig. 33. *Synthetic receptors **45-49** for the binding of peptides in polar solution.*

The importance of additional binding interactions for an effective complexation, compared to hydrogen-bonded receptor/ligand interaction is demonstrated for instance in the case of *Kilburns* tweezer receptors shown in Fig. 34, which are able to recognize C-terminal tripeptides in polar solution.



50

CBS = carboxylate-binding side (ionic or neutral)



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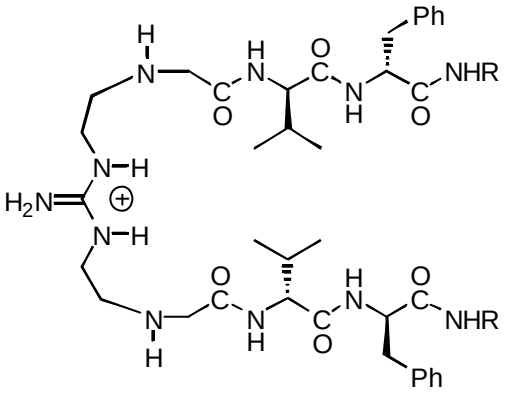

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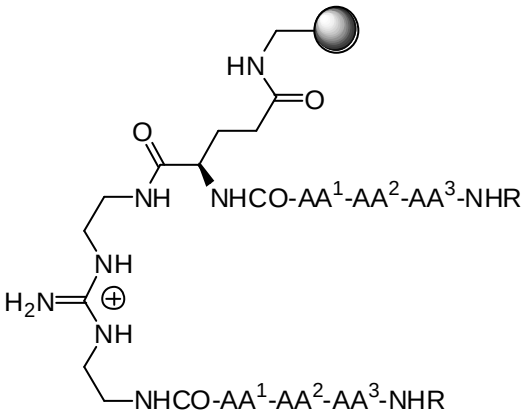


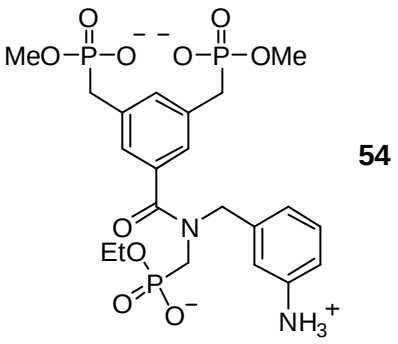
53

Fig. 34. *Peptide receptors **50-53** of Kilburn.*

A receptor library with a neutral carboxylate binding head group (diaminopyridine) **51**[101, 102] and also a receptor with a cationic guanidinium group **52**[55, 103] were synthesized and tested for the binding properties against tripeptides. Due to the fact that no ionic interaction is present in the receptor motif **51**, a strong complexation is only achieved in chloroform or acetonitril. The addition of just 10 % DMSO leads to a complete loss of any binding properties. Related sulfonyl peptide tweezer receptors, that show nearly the same decrease in binding properties by addition of a polar co-solvent, were previously introduced by *Gennari* and *Nestler*.[104, 105]

The ionic receptor **52** binds tripeptides in contrast to the non-ionic receptor **51** in water/DMSO-mixtures (8/2) with binding constants of 10^4 M^{-1}. This underlines again the relevance of ionic interactions for a strong complexation in polar solvents.

In 2002, *Schrader et al.* introduced a receptor **54** for the RGD-tripeptide sequence (Arg-Gly-Asp) in water.[106]



54

Fig. 35. *RGD-receptor **54** by Schrader.*

This specific tripeptide was chosen as a substrate because it plays a vital role in cell-cell and cell-matrix adhesion processes. The interaction of integrines (heterodimeric glycoproteins) anchored inside the cell membrane with RGD containing proteins control essential body functions such as embryogenesis, cell differentiation, angiogenesis, heamostasis or immune response. The association constant for the tripeptide Arg-Gly-Asp is $K \cong 1300\ M^{-1}$ in water as determined by NMR titration.

With the guanidiniocarbonyl pyrroles of type **1**, *Schmuck* was not only able to complex simple carboxylates but also amino acids. Even the simple guanidiniocarbonyl pyrroles show specific secondary interactions between receptor and carboxylate residue. As depicted in Fig. (35), the binding strength for the complexation of various N-acetyl amino acid carboxylates by the ethylamide-substituted receptor **25** significantly depends on the type of amino acid. The association constant for the binding of *D*/*L*-phenylalanine ($K = 1700\ M^{-1}$) is more than

two times stronger than for *D*/*L*-alanine (K = 770 M^{-1}); whereas the association constant for *D*/*L*-lysine (K = 360 M^{-1}) is about two times weaker (Fig. 35).[31]

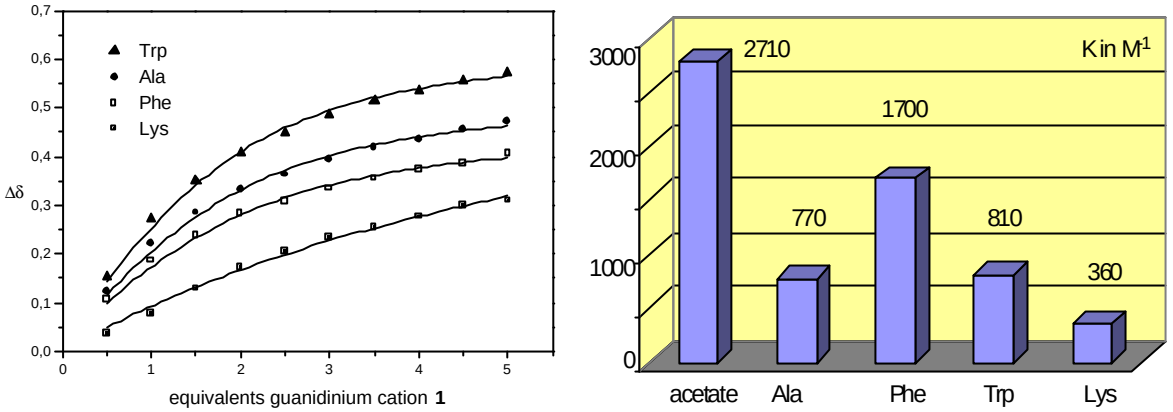


Fig. 35. *^{1}H NMR titration curves of **25** with various amino acid carboxylates in 40 % water/DMSO (left: titration curves; right: binding constants).*

This side chain selectivity could be explained with the help of theoretical calculations (Macromodel V. 6.5, Amber* force field, GB/SA water solvation treatment). In contrast to simple carboxylates, the complexes formed by receptor **25** with N-acetylated amino acids are not any longer planar. This results from an unfavourable steric interaction of the N-acetyl group of the amino acids with the receptor, which explains the lower complexation constant for all amino acids compared to acetate (K = 2790 M^1). In the case of phenylalanine the aromatic ring π-stacks with the acyl guanidinium unit of **25** (Fig. 36). This cation-π interaction further stabilizes the complex.[107, 108] Hence, the association constant for the binding of phenylalanine is more than two times larger than for the binding of alanine. The positively charged ammonium group in lysine decreases the binding affinity relative to alanine due to unfavourable electrostatic interactions with the positively charged guanidinium group.

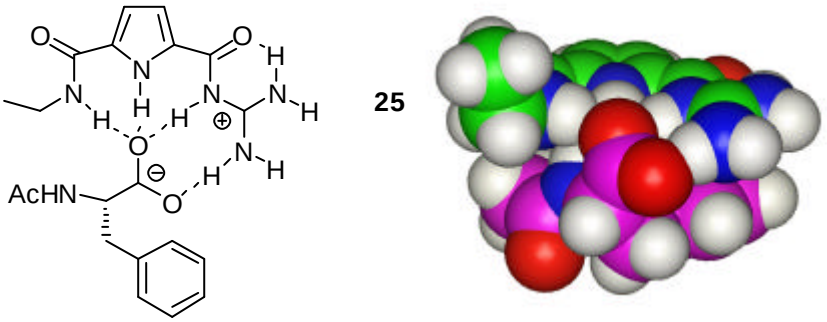
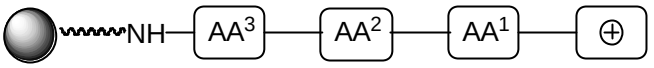


Fig. 36. *Complexation of phenylalanine by **25**. Attractive cation-p interaction favour the binding of phenylalanine by the guanidiniocarbonyl pyrrole receptor **25** (left: schematic representation; right: calculated structure).*

As a further approach to efficient and selective bioorganic receptors not only for single amino acids, but also for small peptides, our group recently synthesized a combinatorial library of 512 receptor structures. This receptor library **55** was screened in an on bead fluorescence assay for binding of the tetrapeptide Val-Val-Ile-Ala, a model for the *C*-terminal end (39-42) of amyloid *b*-peptide (A*b*) (Fig. 37) which is one of the two domains of A*b* being mainly responsible for A*b* self-aggregation in Alzheimer disease.[92, 108]



variable building block: AA^x = amino acid; ⊕ = guanidiniocarbonyl pyrrole cation

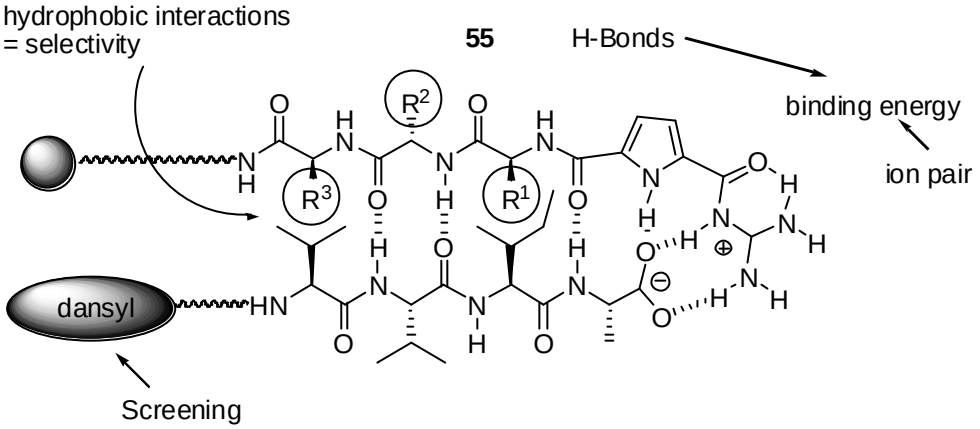


Fig. 37. *A tripeptide-based library of cationic guanidiniocarbonyl pyrrole receptors **55** of the general structure resin-PEG-AA^1-AA^2-AA^3-Å (AA = amino acid, Å = guanidiniocarbonyl pyrrole cation) designed for the binding of Val-Val-Ile-Ala, a tetrapeptide representing the C-terminus of Ab (above: general structure; below: schematic presentation).*

In these receptors (Fig. 38), a tripeptide unit was chosen to provide the necessary binding sites for the formation of a hydrogen bonded antiparallel *b*-sheet with the backbone of the tetrapeptide substrate. A combinatorial variation of the three amino acids in the receptor side chain was then used to identify structures in which additional hydrophobic and steric interactions between these side chains and the substrate further enhance the binding within the *b*-sheet and also render the recognition event selective for this specific tetrapeptide. With this receptor library design *Schmuck* and *Heil* were able to identify several receptors which selectively complex this specific tetrapeptide with association constants up to $K \sim 9000\ M^{-1}$ relative to formate in water. In order to check whether the one-armed receptors of type **55** were also able to bind tetrapeptides in free solution and not only when attached to the solid

support, the receptors **56** and **57** were resynthesized (Fig. 38). The association constants for these were determined in a UV titration to 1770 M^{-1} and 2660 M^{-1} in water.[92]

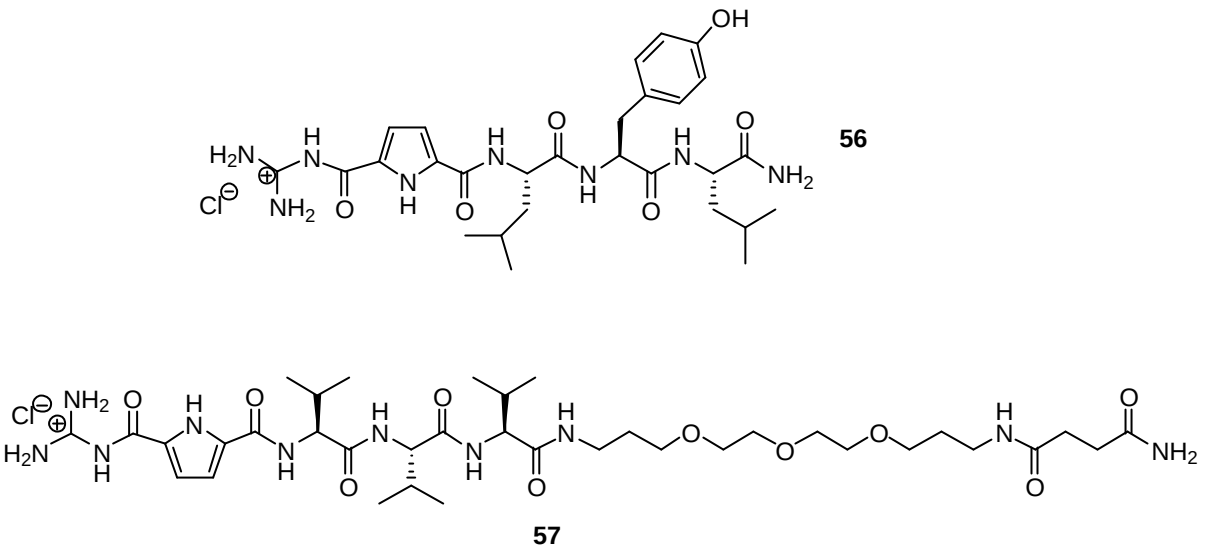


Fig. 38. *Receptors **56** and **57** that were synthesized and tested in solution*

The examples presented show that there are only a few artificial receptor systems known until now that provide a binding of amino acids or peptides in water. And compared to strength of receptor/ligand interaction found in nature with association constants of up to 10^9-10^{13} (biotin-streptavidin), the binding energies of around 10^2-10^3 for single amino acids and approximately 10^3 to 10^4 for peptidic substrates (tri- or tetrapeptides) are still very moderate. Despite all the progress made in this field and the large number of elegant host systems that have been described over the decades, a rational design of a receptor for a given substrate could be realized only in minor cases. Due to the fact that the basic principles of noncovalent interaction are still poorly understood, combinatorial approaches were mainly chosen to identify high-affinity receptors. Hence, there is still a great demand for the design of new efficient receptor systems for polar media and also for understanding the concepts which govern these processes to reach a level, at which a rational receptor design for a given biological target becomes possible.

4 Results and Discussion

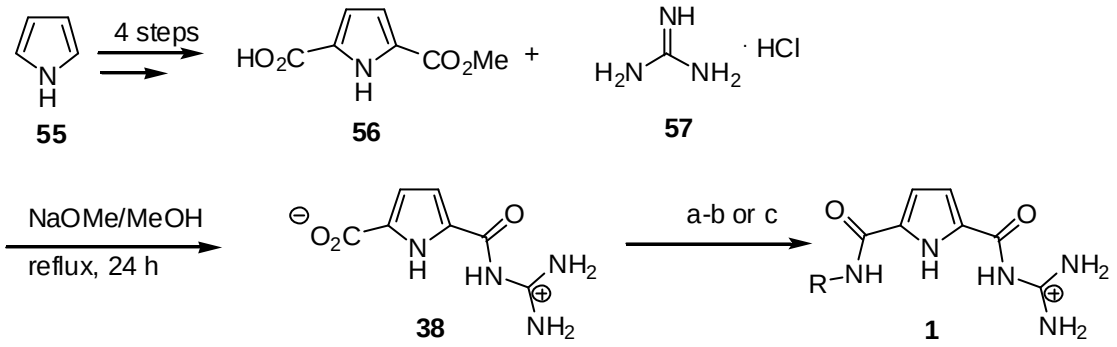
In the following sections, I will first describe the synthetic work that formed the basis for both the bioorganic and the supramolecular research projects, which were introduced in chapter two. Afterwards, I will discuss the physical-organic characterization and the evaluation of the resulting binding or aggregation processes, respectively. This particular section is divided in five parts:

(1) Development of a new mild synthetic method for acyl guanidines
(2) Flexible zwitterions which aggregate in polar solution
(3) Synthesis of four new arginine-analogues
(4) New tris-cationic binding motif for amino acids
(5) *De-novo* designed dipeptide receptor

A necessary requirement for the realization of these projects was the development of an efficient synthetic approach towards guanidiniocarbonyl pyrroles that will be described in the following section.

4.1 A new method for the synthesis of acyl guanidines

In previous projects of our group, it became obvious that a totally new synthetic approach for the central guanidinylation step towards guanidiniocarbonyl pyrroles of type **1** was necessary. The original method used in our group for the synthesis of simple acyl guanidines started from the 2,5-disubstituted pyrrole compound **56**, which is readily available in a known four step synthesis from pyrrole **55**. The central guanidinylation step was then achieved by a S_N2_t-reaction with guanidinium chloride **57** in sodium methoxide to yield the zwitterion **38** (Scheme. 2).[54]



a) $(COCl)_2$; b) RNH_2, between 60-70% over both steps; c) PyBOP, RNH_2, 75-95 %.

Scheme 2. *Synthesis of guanidiniocarbonyl pyrrole receptors of type **1**.*

This zwitterion can be converted to the acyl chloride using oxalyl chloride in DMF and can subsequently be coupled with a variety of amines to yield the desired receptor compounds of type **1**.[32] Alternatively, the carboxylic acid group can be directly reacted with amines after activation by the coupling reagent PyBOP in DMF.

In previous synthetic work it became clear that this guanidinylation method using sodium methoxide in refluxing methanol is rather harsh and turned out to be not compatible with a wide range of functional groups or stereogenic centers in more complex molecules than **1**. Additionally, in synthetic practice, acyl guanidines like the zwitterion **38** or the target molecules of type **1** are difficult to handle (work-up, purification) due to their high polarity and also very low solubility in typical organic solvents. Especially the zwitterion **38** is, as a result of its strong self-association properties (discussed in section 3.2.1), an extremely insoluble compound.[54] The purification of such polar guanidiniocarbonyl pyrroles of type **1** by recrystallization or chromatographically methods failed in most cases or gave only poor yields. Therefore, an alternative synthetic approach for the central guanidinylation step towards protected acyl guanidines was needed to enable the synthesis of larger, more sophisticated systems.

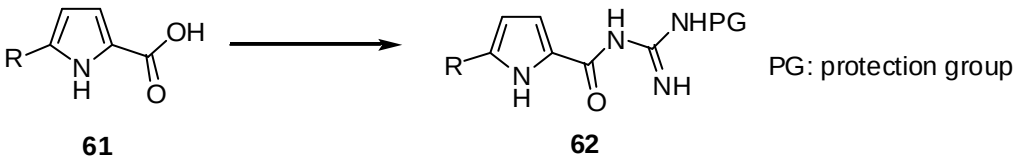
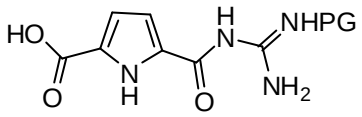


Fig. 40. *Synthetic step from a pyrrole carboxylate **58** to a protected acyl guanidines **59**.*

Such a new synthetic route should combine several advantages compared to the original sequence. The method should generate the pyrrole acyl guanidine moiety under mild conditions and the acyl guanidinium function should be protected. Through the protection of the guanidinium function, a less polar compound of type **59** would be obtained which should be much easier to isolate than the polar and cationic guanidinium compounds like **1**.
In particular, a protected version of the synthetic key intermediate the zwitterion **38** should be developed (Fig. 41).



60

Fig. 41. *Protected 2,5-substituted guanidiniocarbonyl pyrrole carboxylic acid **60**.*

Direct protection-route:

At the beginning of my thesis, many guanidinylation protocols for the synthesis of guanidines from amines had been published, but there existed only a few methods for the generation of acyl guanidines from carboxylic acids.
A possible, straight approach to generate a protected guanidinocarbonyl pyrrole **60** would be to make use of a protected guanidine in the central guanidinylation step. Thus, a nucleophilic substitution on a pyrrole carboxylic acid **64** with a protected guanidine as a nucleophile was tested. Since di-protected guanidines are common compounds, I tested a di-carboxybenzoylcarbonyl (Cbz) protected guanidine **61** for the nucleophilic substitution at the pyrrole carboxylic acid **64**.[109]

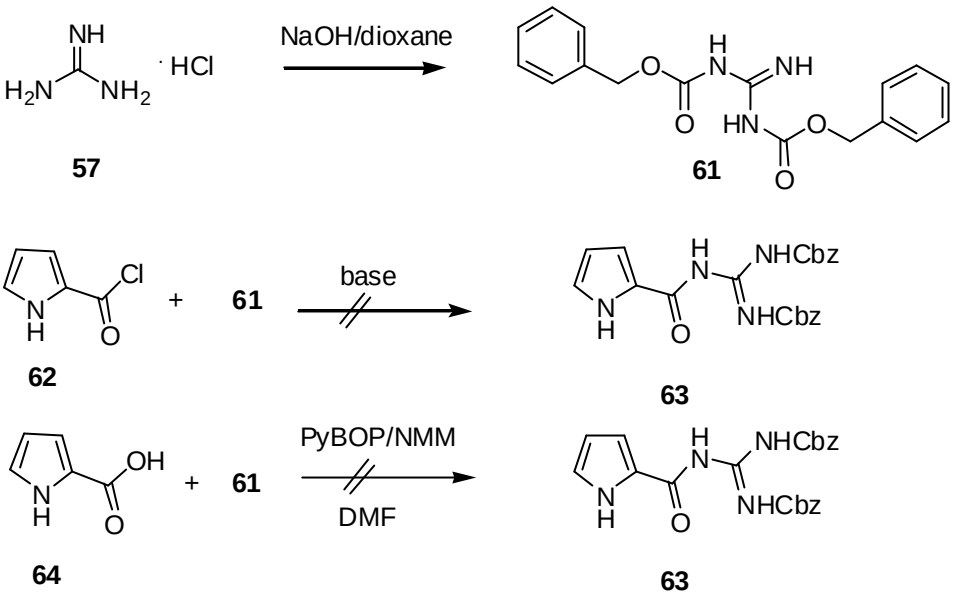
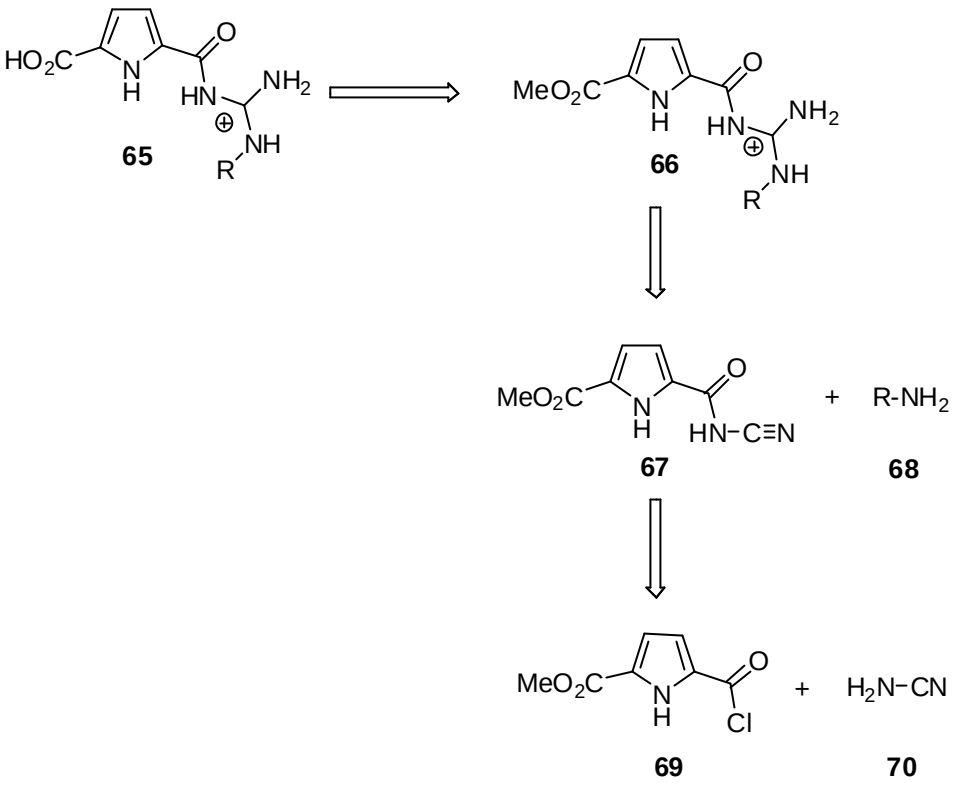


Fig. 42. *Reaction of a di-Cbz protected guanidine **61** with a pyrrole acyl chloride **62** or with the pyrrole carboxylic acid **64**.*

But neither the deprotonation of the Cbz-protected guanidine **61** and subsequently reaction with the acyl chloride **62,** nor the reaction with the pyrrole carboxylic acid **64** activated by the coupling reagent PyBOP under basic conditions yielded, to the desired protected guanidinocarbonyl pyrrole (Fig. 42). This might be a result of the low nucleophilicity of the di-Cbz-protected guanidine and the reduced activity of the pyrrole acyl chloride.

Cyanamide route:

As all of these attempts to directly synthesize a protected version of our guanidinocarbonyl pyrroles **1** failed, the next idea was to build an acyl guanidine from simple precursor compounds. A well known and often applied method for the synthesis of acyl guanidines, in general, is the reaction of an acyl cyanamide with an amine yielding the acyl guanidine moiety.[110] As a consequence, I was interested to use cyanamides for the generation of our desired guanidiniocarbonyl pyrroles **1**, according to the retrosynthetic Scheme 3.



Scheme 3. *Retrosynthesis of guanidiniocarbonyl pyrrole **57** via a pyrrole cyanamide **67**.*

The realization of this approach for the preparation of guanidiniocarbonyl pyrroles like **65** via a pyrrole cyanamide offers also the possibility to introduce a second residue on the guanidinio moiety. Depending on the amine used in the second reaction step, a variety of hitherto unknown N,N´-substituted guanidiniocarbonyl pyrroles **71** should be obtained. The synthesis of such N,N´-substituted guanidiniocarbonyl pyrroles **71** would be highly interesting, as it paves the way to a new tweezer like receptor class (Fig. 43).

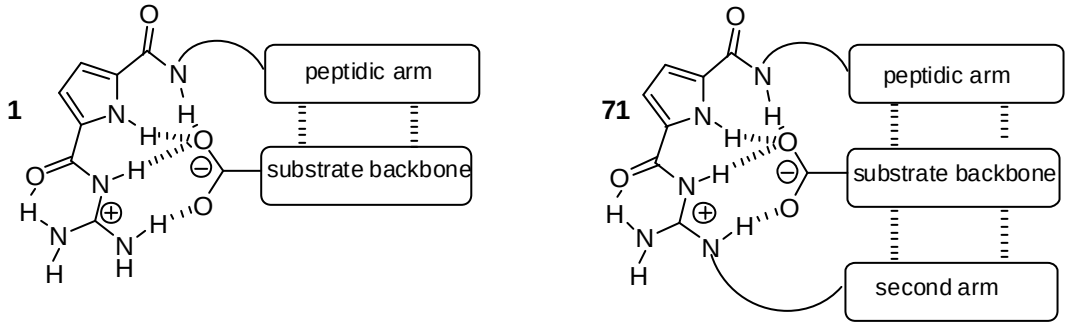


Fig. 43. *Complex of a tweezer receptor **71** with a carboxylate (right). Additional binding interactions in complexes between N,N´-substituted guanidiniocarbonyl pyrroles **71** and carboxylate should alter the stability and selectivity compared to **1**.*

These kinds of receptors may be more efficient in the complexation of peptidic substrates, because these tweezer-receptors **71** could contribute additional binding interactions with the backside of the substrate. So far, only one side of the carboxylate is used for binding interactions with the receptor. The other side of the substrate is still completely free and exposed only to the solvent. Thus, the cyanamide route seemed at first sight to be the ideal method for the synthesis of guanidiniocarbonyl pyrroles in general.

As a first test to determine the properties of such cyanamides, I carried out a well known literature reaction between benzoylchloride **72** and calcium cyanamide **73**.[111] The acyl chloride **72** and the calcium cyanamide **73** were reacted in an aqueous sodium hydroxide solution at room temperature yielding the desired benzoylcyanamide **74** corresponding to the literature in a yield of 80 %. Subsequently, the isolated benzoylcyanamide compound **74** was reacted with aniline **75** to the substituted acyl guanidine **76** in 70 % (Fig. 44).

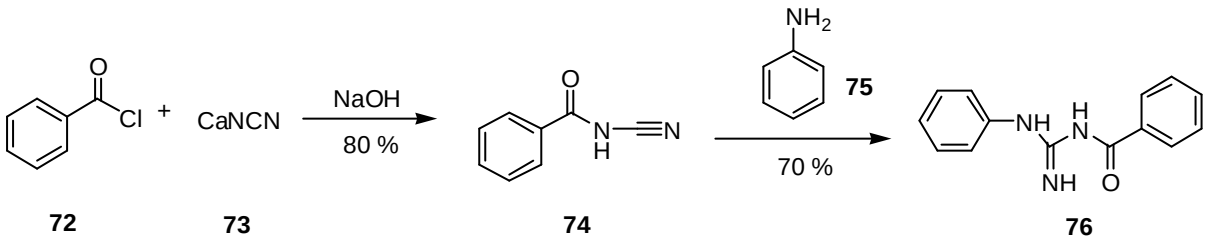


Fig. 44. *Reaction of benzoylchloride **72** with calcium cyanamide **73** and subsequently addition of aniline **75** yielding the acyl guanidine **76**.*

This proves that cyanamides are, in general, perfectly suited intermediates for the preparation of acyl guanidine. Unfortunately, our pyrrole acyl chloride **69** does not react with calcium cyanamide under the same reaction conditions. After reaction times varying from 30 min to 12 h at room temperature and/or elevated temperatures only the hydrolysis product of the acyl chloride **69**, the pyrrole dicarboxylic acid **77**, could be detected by TLC and NMR analysis (Fig. 45).

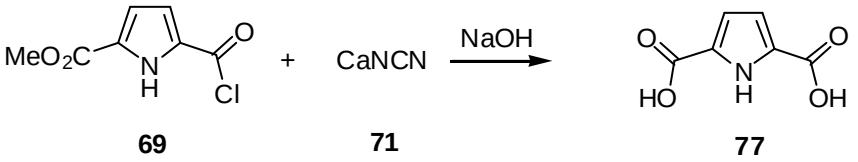
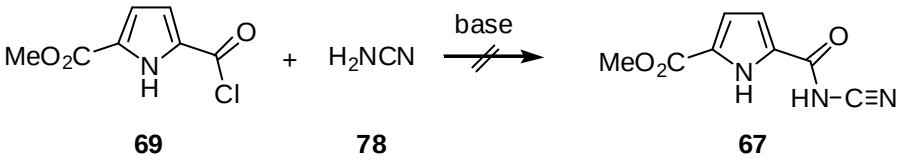


Fig. 45. *Hydrolysis of the acyl chloride **69** by a reaction with calcium cyanamide **73** in a sodium hydroxide solution.*

Consequently, non-aqueous reaction conditions were also tested. Due to the fact that calcium cyanamide is nearly insoluble in any organic solvent, the free cyanamide was used instead.



69 **78** **67**

Fig. 46. *Attempt to synthesize the pyrrole cyanamide **67** with free cyanamide **78**.*

Some representative reaction conditions, that were tested, are summarized in Table 1. In most of these reactions aprotic polar solvents (DMF or acetone) were used, which should facilitate such S_N2_t-reactions. As a base for the deprotonation of the cyanamide **78** both sodium hydride as a strong base or potassium carbonate as a weaker base was used.

Table. 1: Representative reaction conditions for the synthesis of **69**.

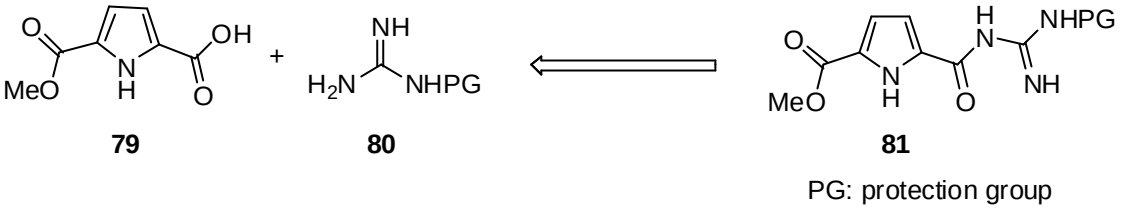
solvent	base	additive	temperature
THF	NaH	/	room temperature
acetone	K_2CO_3	/	room temperature
DMF	K_2CO_3	/	room temperature
DMF	NMM	PyBOP	room temperature
DMF	K_2CO_3	NH_4Cl	room temperature
pyridine	/	/	room temperature
DMF	K_2CO_3	/	reflux, 6 h
dichloromethane	K_2CO_3	DMAP	reflux, 12 h

However, under the tested reaction conditions no pyrrole cyanamide **67** could be detected or isolated in significant yield. Additionally, the trapping of a cyanamide compound **67**, possibly formed *in-situ* during the reaction by adding of ammonium chloride to the reaction mixture failed (Tab. 1).

These experiments have shown that the cyanamide route was not successful with our pyrrole derivatives.

Mono-protected guanidine route:

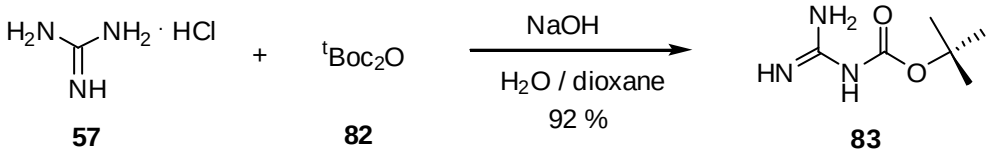
Finally, as all of the previously reported attempts failed, I tried to generate the acyl guanidine moiety by a reaction of a mono-protected guanidine **81** with the pyrrole carboxylic acid **79** (Scheme 3).



79 **80** **81**

Scheme. 3. *Retrosynthetic approach towards protected guanidiniocarbonyl pyrrole **81** via a nucleophilic substitution with mono protected guanidine **80**.*

This attempt differs from the above described di-Cbz-protection experiments only in the nature of the applied nucleophile. A mono-protected guanidine **80** should be more nucleophilic, compared to a guanidine which possesses two electron withdrawing urethane-protection groups like **61**. Therefore, I modified a literature synthesis for di-^{t}Boc protected guanidines to prepare mono ^{t}Boc guanidine **83** (Fig. 47).[109]



57 **82** **83**

Fig. 47. *Synthesis of mono ^{t}Boc-protected guanidine **83**.*

Instead of an equimolar mixture of guanidine hydrochloride **57** and ^{t}Boc$_2$O **82** a fivefold excess of the guanidinium hydrochloride **57** was used. To ensure only mono ^{t}Boc-protection, the addition time of ^{t}Boc$_2$O **82** to a solution of guanidine **57** in mixture of aqueous sodium hydroxide and dioxane was extended to eight hours. A comparable reaction protocol for the synthesis of mono ^{t}Boc-guanidine **83** was published shortly after my work by *Goodman et al.* in 2001.*[112]*

Now this ^{t}Boc-guanidine **83** was reacted with our pyrrole carboxylic acid **79** in DMF using overnight activation with the amide coupling reagent PyBOP. Fortunately, the desired ^{t}Boc-protected methylester **84** was isolated after chromatographic purification with an excellent yield of 95 % (Fig. 48).

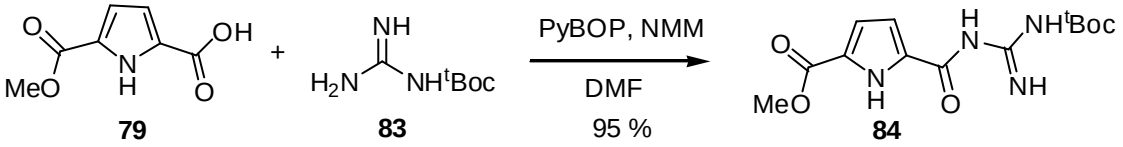


Fig. 48. *Coupling of the mono ^{t}Boc-protected guanidine **83** with the pyrrole carboxylic acid **79**.*

It must be emphasized that only phosphonium coupling reagents like PyBOP efficiently activate the carboxylic acid group in compound **79**, and therefore enable the reaction. Other coupling reagents like DCC, EDCI did not react or gave only poor yields like for instance the Mukajama reagent (2-Chlor-N-methyl-pyridiniumjodid) (30-40 %).

In conclusion, this newly developed synthetic approach provides the possibility to prepare a protected acyl guanidine directly from carboxylic acid. Moreover, this reaction works also in the case of our deactivated pyrrole carboxylic acids. To finally arrive at our desired compound the ^{t}Boc-protected guanidiniocarbonyl pyrrole carboxylic acid **85**, it was only necessary to cleave the methylester group in **84**. Hence, I tried to hydrolyse the methylester moiety by a standard reaction protocol with a fivefold excess of lithium hydroxide in a mixture of tetrahydrofuran/water (4:1).

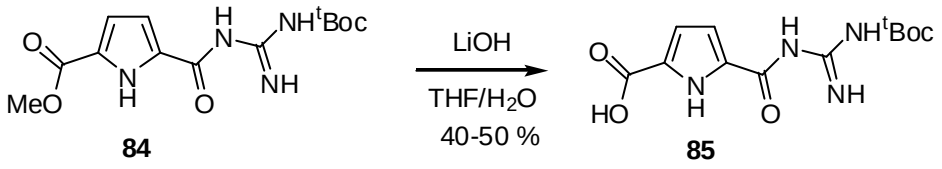


Fig. 49. *Hydrolysis of the methylester compound **84** with LiOH in THF/water.*

Due to the reduced reactivity of pyrrole methylesters the reaction was still incomplete after a reaction time of two days. In addition, the isolation and purification of the desired compound **85** proved to be extremely difficult, as the carboxylic acid **85** could not be precipitated at a pH value around 4, which is the absolute minimum to ensure that the ^{t}Boc-protection group is not cleaved at the same time. A chromatographic isolation of the carboxylic acid was also problematic and gave only low yields between 40 % to 50 %. One explanation for the slow reaction and the moderate isolated yields could be that the methylester **84** is not very soluble in the THF/water mixture. The resulting two phase reaction might explain the observed long reaction time. Therefore, I performed the reaction in a methanol/water mixture. The examination of the reaction by thin layer chromatography showed a complete conversion of

the starting material after already one hour. Unfortunately, the product which could be isolated in nearly quantitative yield was not the desired ^{t}Boc-protected guanidiniocarbonyl pyrrole **63**, but the pyrrole dicarboxylic acid **86** (Fig. 50).

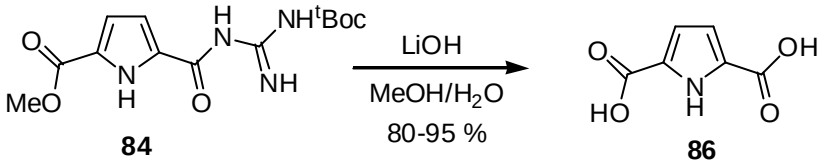


Fig. 50. *Complete hydrolysis of the methylester compound **84** with LiOH in methanol/water.*

Apparently, the reaction in methanol/water is so fast, that besides the hydrolysis of the methylester, also an attack on the less reactive guanidinylated carbonyl takes place (Fig. 51).

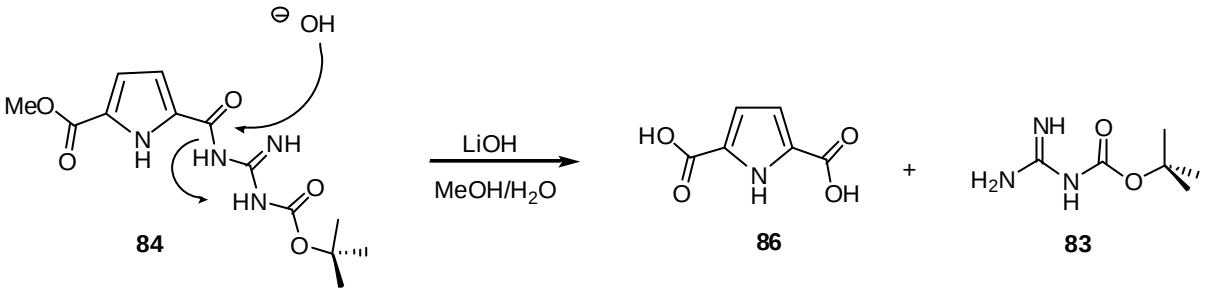


Fig. 51. *Attack of a hydroxide anion on the guanidinylated carbonyl under nucleophilic conditions.*

Obviously, the electron withdrawing capacity of the urethane protection group (^{t}Boc) makes the ^{t}Boc-guanidine to good a leaving group. This possible side reaction also explains the moderate yields which were isolated during the hydrolysis in THF/water mixtures. The ability of the ^{t}Boc-guanidine **83** to function as a leaving group under nucleophilic reaction conditions, has also been proven within another project in our group realized by *Wienand*.[113] In an amidation reaction of his guanidiniocarbonyl pyrrole receptor **87** with ammonia in methanol, he observed besides the desired amidation of the methylester, also an amidation of the guanidinocarbonyl pyrrole moiety (Fig. 52).

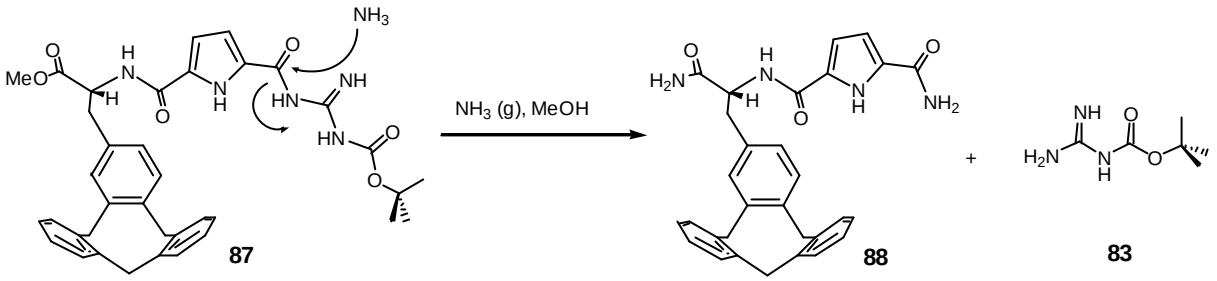
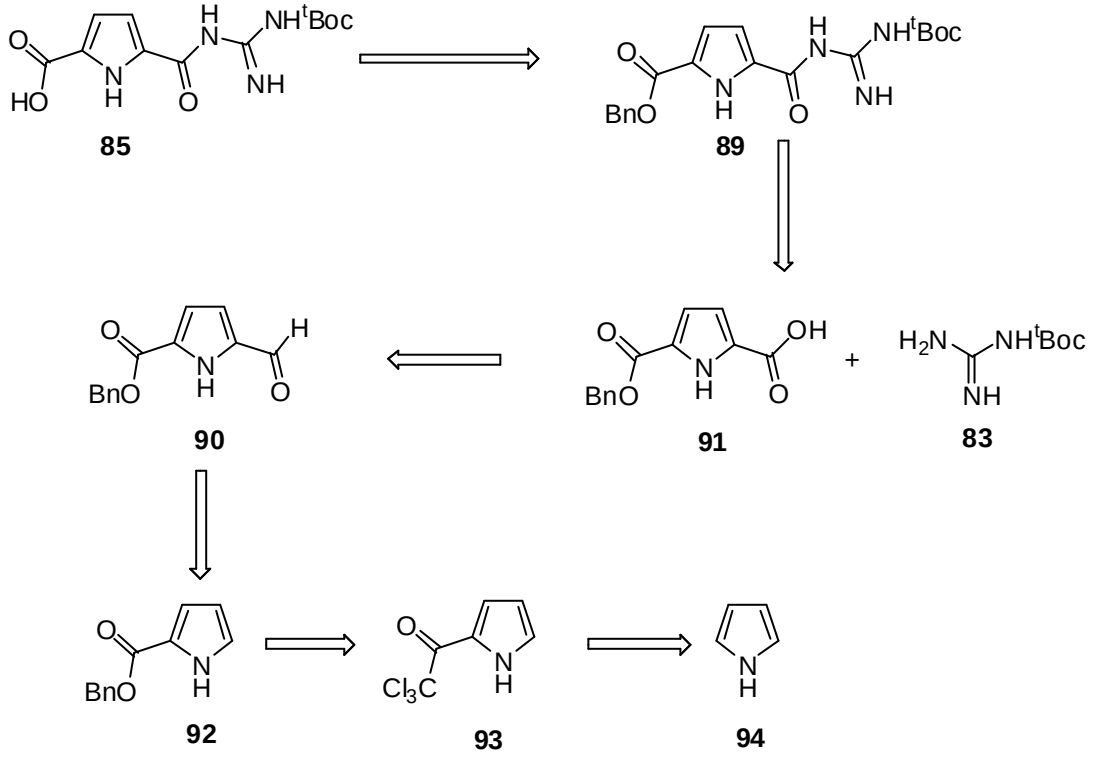


Fig. 52. *Amidation of the guanidinylated carbonyl under nucleophilic conditions.*

As a result of the foregoing, a new ester functionality has to be prepared in the 5-position of the guanidiniocarbonyl pyrrole which can be cleaved off under non-nucleophilic and non-acidic conditions. Thus, I prepared the hitherto unknown pyrrole benzylesters **91** and **89**, according to the retrosynthetic Scheme 4, through a modification of the synthetic route for the methylester compound **79** described above.



Scheme 4. *Retrosynthesis of the guanidinocarbonyl pyrrole **85** via the benzylester **89**.*

Pyrrole **94** was transformed into benzyl 2-pyrrole carboxylate **92** by acylation with trichloroacetyl chloride **95** (yield: 95 %) and subsequent cleavage of the trichloroacetyl group with sodium benzylate with a yield of 95 % yield (Fig. 53).[114]

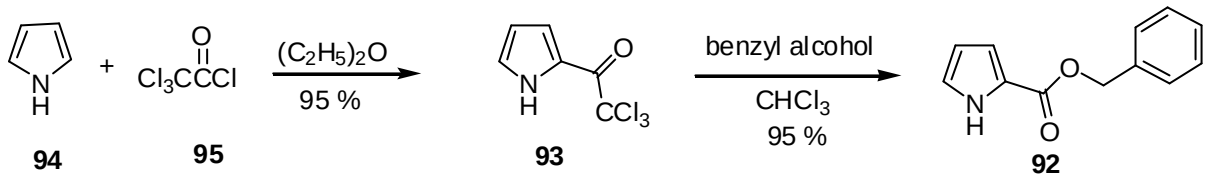


Fig. 53. *Synthesis of benzyl 2-pyrrole carboxylate **92** from pyrrole **94**.*

Vilsmeier-Haack formylation of **92** gave the aldehyde **90** in 62 % yield beside 33 % of the 2,4-substituted isomer **96** (Fig. 54).[115]

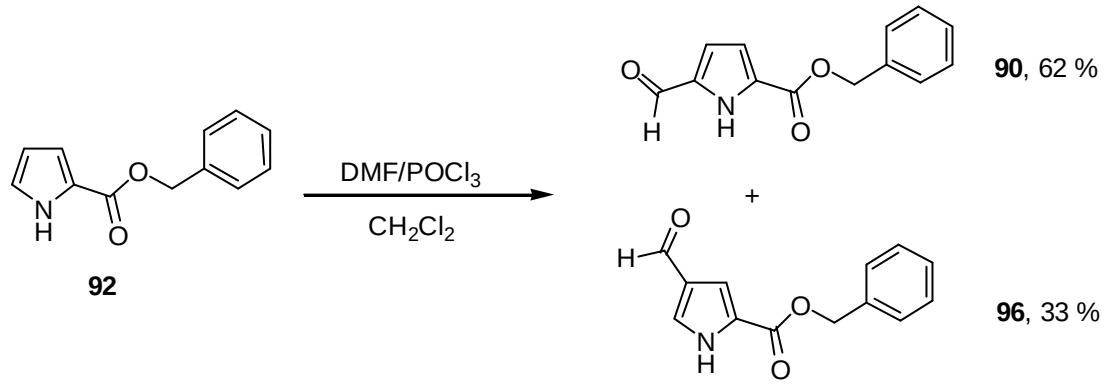


Fig. 54. *Vilsmeier-Haack formylation of benzyl 2-pyrrole carboxylate **92**.*

Oxidation of the aldehyde **90** gave the desired carboxylic acid **91** with a good yield of 67 %.

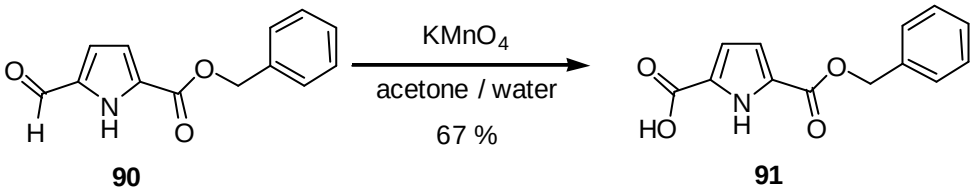


Fig. 55. *Oxidation of aldehyde **90** with $KMnO_4$.*

Subsequently, the carboxylic acid **91** was reacted with mono ^{t}Boc-guanidine **83** by activation with PyBOP to yield the ^{t}Boc-protected pyrrole **89** in an excellent yield of 89 % (Fig. 56).

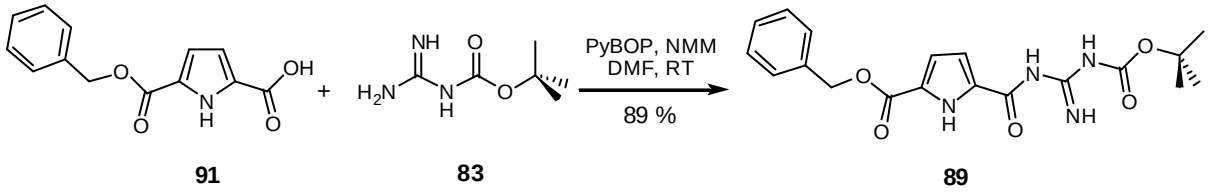


Fig. 56. *Coupling of the carboxylic acid **91** with ^{t}Boc-guanidine **83**.*

The ester function was then cleaved by hydrogenolysis of the benzyl ester **89** in an excellent yield of 95 % to give the desired protected zwitterion **84**.

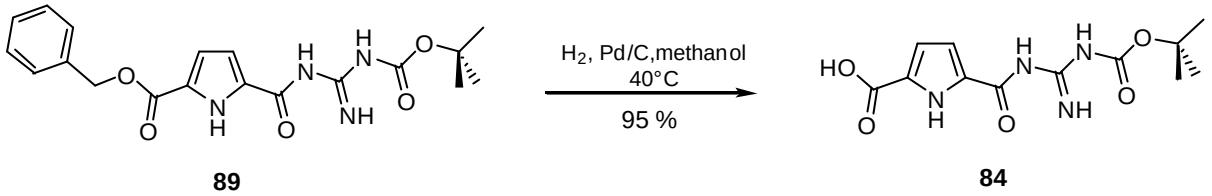


Fig. 57. *Hydrogenolysis of benzylester **89**.*

The ^{t}Boc-protected guanidiniocarbonyl pyrrole **84** is, in contrast to the zwitterion **38**, soluble in organic solvents and thus can easily react in good yields (normally 70-95 %) with amines (Fig. 58). The striking new feature of this method is that the resulting protected amides **97** can easily be isolated by normal column chromatography. The solubility of **97** in organic solvents facilitates any further reaction and allows therefore the synthesis of larger and more sophisticated systems.

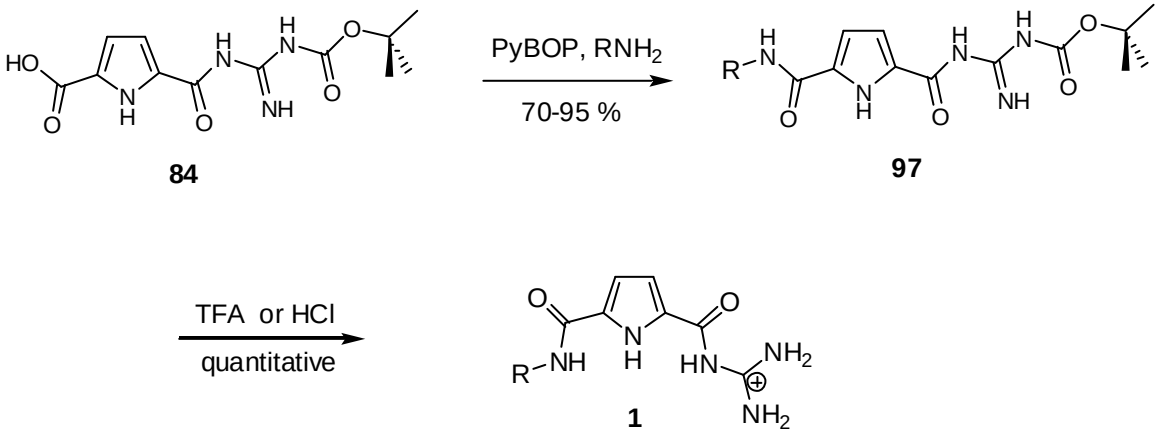
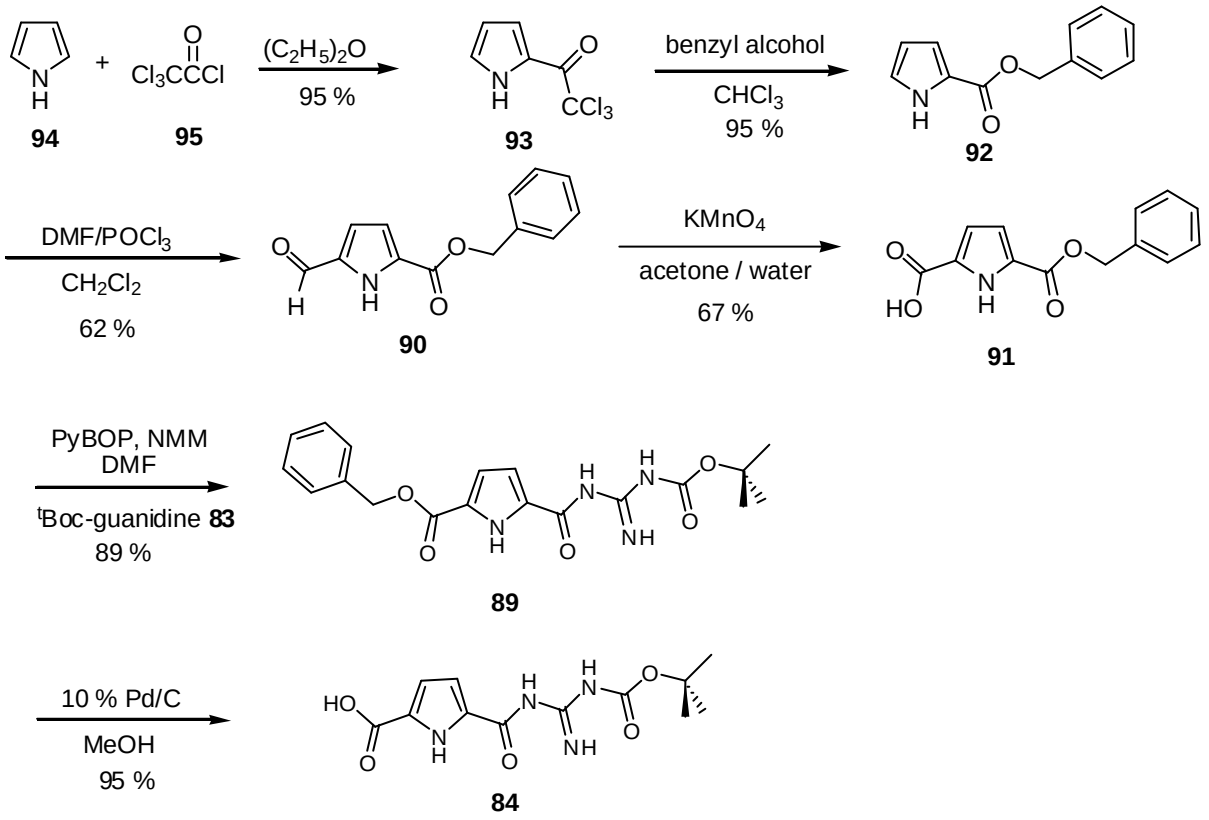


Fig. 58. *Coupling of **84** with amines and subsequently deprotection of the ^{t}Boc-group yielding to the receptors **1**.*

Finally, the deprotection of ^{t}Boc protection group using aqueous hydrochloride acid or TFA gave the desired cationic compounds of type **1** in quantitative yield.

Conclusion:

The newly developed synthesis for the ^{t}Boc-protected guanidiniocarbonyl pyrrole **84** by the route summarized in Scheme 5 is a tremendous improvement for the facile and high yield preparation of guanidiniocarbonyl pyrroles.



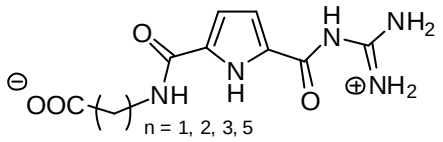
Scheme 5. *Synthesis of the key compound, the ^{t}Boc-protected guanidinocarbonyl pyrrole **63**.*

The key step, the coupling of a ^{t}Boc-protected guanidine **83** with pyrrole carboxylic acids by the amide coupling reagent PyBOP proved to be a very versatile and mild procedure for the preparation of acyl guanidines. The ^{t}Boc-protected guanidiniocarbonyl pyrrole carboxylic acid **84** is an extremely important intermediate in the synthesis of more complex guanidiniocarbonyl pyrroles. It is, in contrast to the zwitterion **38**, soluble in organic solvents like DMF and thus the carboxylic acid in 5-position of the pyrrole can easily be reacted in excellent yield with a variety of amines. The resulting amide compounds **97** can be isolated by extraction and/or by normal silica gel chromatography in high yields (normally between

75 % to 95 %) and can subsequently be converted into the desired cations by cleavage of the ^{t}Boc-group with TFA. This mild guanidinylation route and its application for synthesis of our guanidinocarbonyl pyrroles is now the standard protocol in our group for the solution and also solid-phase synthesis of guanidinocarbonyl pyrroles.

4.2 Flexible Zwitterions

To study the self-association properties of a series of flexible zwitterions, four guanidiniocarbonyl-carboxylate zwitterions **3-6** with varying spacer length were synthesized and evaluated.



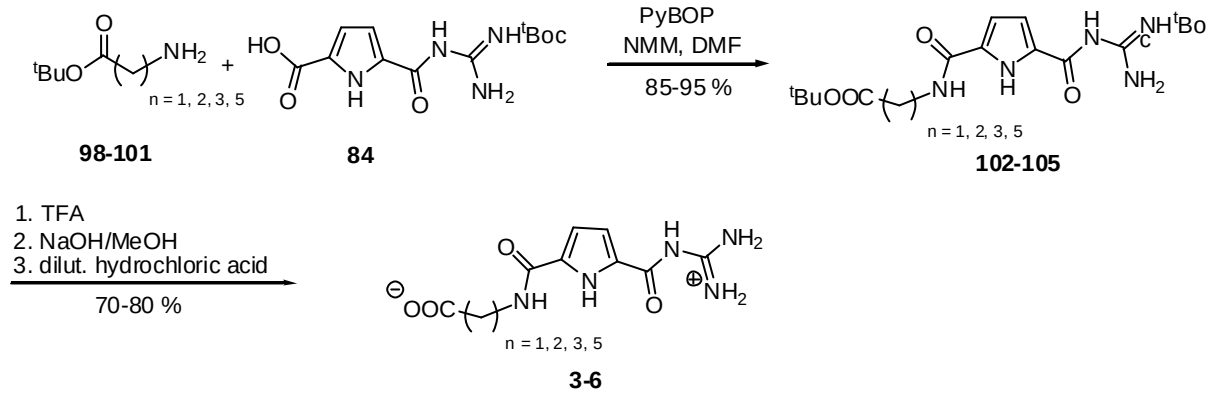
3-6

Fig. 59. *Flexible zwitterions **3-6** with spacer length n = 1, 2, 3, 5.*

Within this particular project, the influence of the length and flexibility of a spacer between guanidiniocarbonyl pyrrole and carboxylate on the stability and structure of the formed aggregates should be evaluated. Thereby, more information of the thermodynamic basis of such supramolecular aggregation processes could be obtained. This may lead in the future to a more directed design of self-assembling and self-association building blocks.

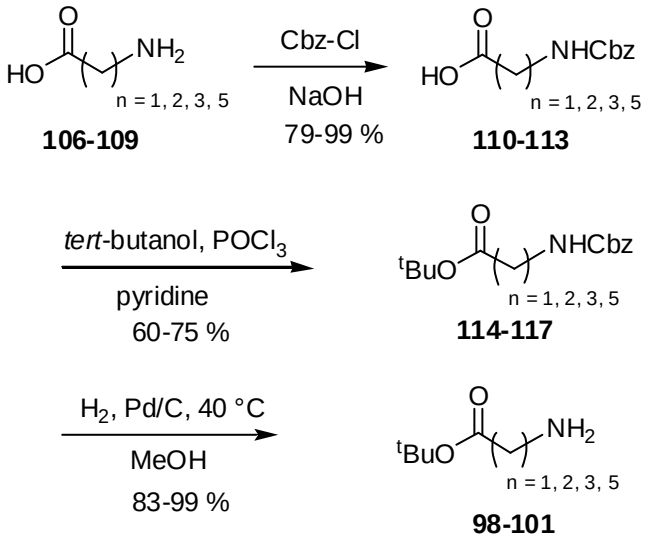
4.2.1 Synthesis

Based on the synthesis of the key intermediate described above, the ^{t}Boc-protected guanidiniocarbonyl pyrrole carboxylic acid **84**, a synthesis for the zwitterions **3-6** was developed which is outlined in Scheme 6. The ^{t}Boc-protected guanidiniocarbonyl pyrrole **84** was coupled with the *tert*-butylester **98-101** of the corresponding ω-amino acids to yield to the diprotected zwitterions **102-105**.



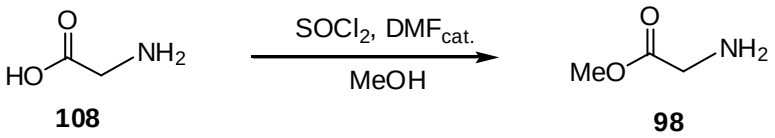
Scheme 6. *Synthesis of the zwitterions **3-6**.*

The necessary *tert*-butylesters **98-101** were prepared, in analogy to literature procedures, in a three step synthesis from the corresponding commercially available ω-amino acids **106-109** (Scheme 7).[116] The amino function of the ω-amino acid **106-109** was first protected with a Cbz-protection group, subsequently the Cbz-protected *tert*-butyl esters **110-113** were prepared and removal of the Cbz-function finally led to the desired *tert*-butyl esters **100-103**.



Scheme 7. *Synthesis of the tert-butyl esters **98-101**.*

The direct one-step preparation of the *tert*-butyl esters **98-101** from the amino acids **106-109** through an activation of the carboxylic acid with thionylchloride in *tert*-butanol gave only crude product mixtures, which could not be further purified. This might seem surprisingly at first sight, because in the case of the preparation of methylesters (glycine, β-alanine) from the corresponding amino acids **106-107** this is the standard procedure (Fig. 60).[117]

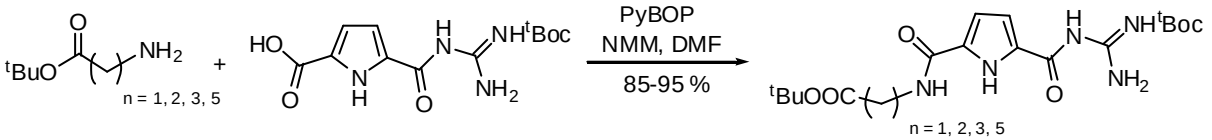


108 **98**

Fig. 60. *Synthesis of glycine methylester* ***98*** *via activation of the carboxylic group with thionylchloride in methanol.*

However, for the preparation of the β-alanine *tert*-butyl ester **99** the indirect method via Cbz-protection of the amino function is the recommended and always applied procedure according to literature.[116] The adaptation of this method for the preparation of the other *tert*-butylesters **98, 100-101** proved that this route is, though being slightly longer, preferable. With 47 % up to 62 % the overall yields for the three step synthesis are good and the purity of the obtained *tert*-butyl esters **98-101** is very good in contrast to the direct method.

The next step in synthesis, the coupling of the ^{t}Boc-protected guanidiniocarbonyl pyrrole **84** with the *tert*-butylesters **98-101** was achieved through standard amide coupling using PyBOP as a coupling reagent. The desired di-protected compounds **102-103** were obtained after chromatographic purification in good yields of 85-95 % (Fig. 61).



98 **84** **102-105**

Fig. 61. *Synthesis of the di-protected zwitterions* ***102-105****.*

Simultaneous deprotection of the *tert*-butyl esters and the ^{t}Boc-protection group with concentrated trifluoroacetic acid gave the triflate salts of the compounds **3-6**. The triflate salts were then dissolved in a mixture of aqueous sodium hydroxide and methanol and adjusted to a pH of 5 with dilute hydrochloric acid yielding the desired zwitterions **3-6**. The precipitation procedure of the zwitterionic compounds was repeated several times to remove all remaining trifluoroacetate and to ensure a defined zwitterionic protonation state, that was confirmed in the NMR spectra.

4.2.2 Results

The self-association behaviour of **3-6** was studied using both NMR dilution experiments in DMSO-*d6* which will be discussed in the following part, and also ESI MS studies which will be presented afterwards.

Studies in solution:[118]

Depending on the length and therefore flexibility of the alkyl spacer, one could expect that these zwitterions can, on the one hand either *intermolecularly* dimerize, oligomerize or polymerize or on the other hand form *intramolecular* self-folded molecules ("loops") (Fig. 64). The concentration dependency of NMR shift changes can be used to analyze such aggregation processes.[53, 119] In general, shift changes result from the differences between the free and aggregated state of two species. The interaction of two molecules through noncovalent bonds causes a different chemical surrounding of individual atoms and therefore also a chemical shift change of the observed atoms. In this particular case the concentration dependent shifts of the various NHs clearly indicate an *intermolecular* interaction of the carboxylate anion of one molecule with the guanidiniocarbonyl pyrrole cation of another. For example, for **3** (n = 1) the signal for the guanidinium amide NH shifts from δ = 10.7 in a highly diluted solution (0.25 mM) to δ = 11.25 at already 20 mM concentration (Fig. 61). In contrast to the situation described, an *intramolecular* self-folding would be concentration independent, whereas with increasing dilution the formation of intermolecular self-associated oligomers or polymers become less and less favorable. Hence, a rather strong self-aggregation for **3** (n = 1) occurs even in DMSO and an intramolecular formation of a "loop" structure can be excluded.

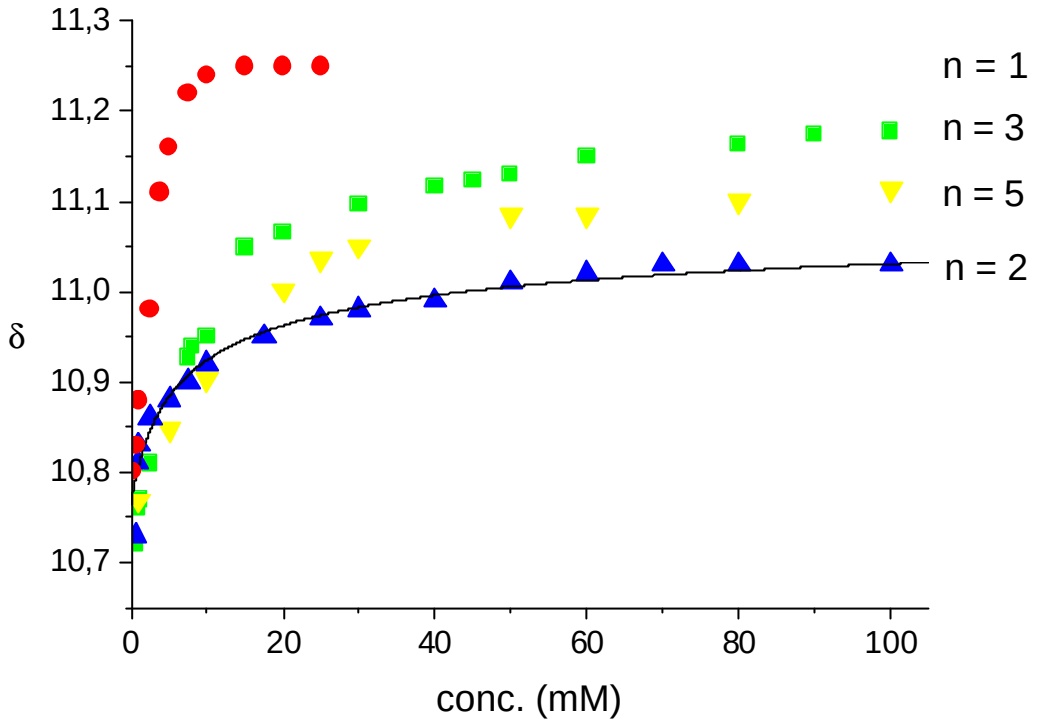


Fig. 62. *NMR dilution data of the guanidinium amide NH for **3**-**6**. The solid line for n = 2 shows the curve fitting for a 1:1-dimerization model.*

For the the more flexible zwitterions with n = 2, 3 or 5, the aggregation is weaker as implied by the data in Figure 62: The plateau value corresponding to the limiting shift of the amide NH within the aggregate is reached only at much higher concentrations (> 100 mM), compared to n = 1. Therefore, the aggregates of **3** (n = 1) are remarkably more stable than for the other zwitterions. This is also reflected by the much lower solubility of zwitterion **3** compared to its higher analogues. Whereas the zwitterion **3** (n = 1) is only soluble in DMSO up to 20 mM, the other more flexible zwitterions can be dissolved up to 100 mM. Even the introduction of only one additional CH_2-group in **3** to give **4** obviously significantly reduces the stability of the aggregates probably due to the higher flexibility of the monomers which entropically disfavors the self-association or also allows a competing intramolecular charge interaction for longer spacers.

The extremely strong tendency of **3** (n = 1) to self-associate is further supported by ESI-MS experiments from DMSO solution (discussed in detail in the next part). In addition to the monomer, signals of nearly similar intensity for higher oligomers can be observed (Fig. 63). For the other zwitterions, ESI-MS experiments also show the formation of oligomers in solution but with weaker intensity relative to the monomer. Unfortunately, due to the complex oligomerization equilibrium present in solution, a quantitative analysis of the NMR dilution data is not possible for this zwitterion. However, the efficient self-association of **3** (n = 1) is in good agreement with data from amino acid recognition studies, which show that simple

carboxylates can be bound by guanidiniocarbonyl pyrroles with association constants $K > 10^3\ M^{-1}$ in DMSO.

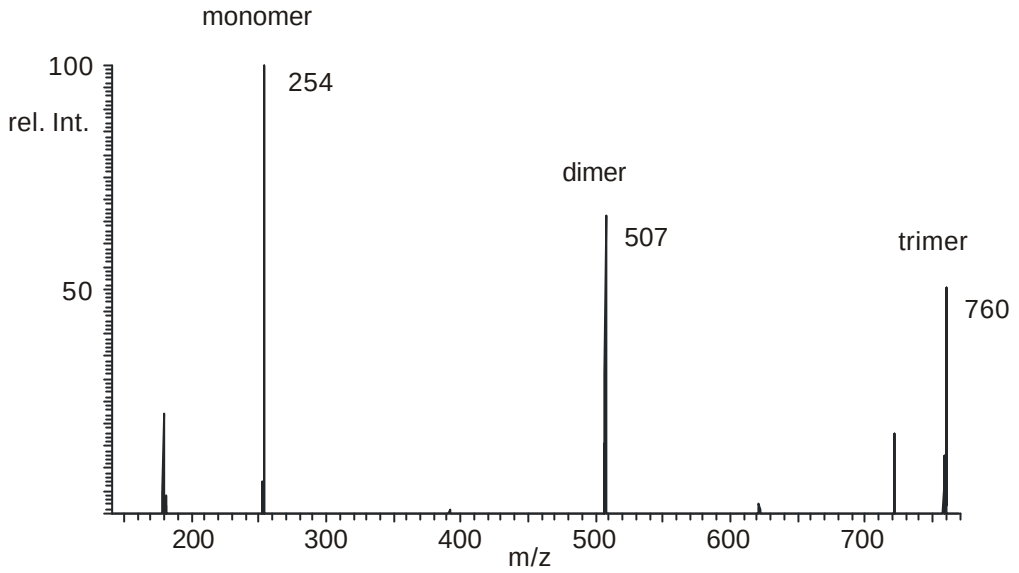


Fig. 63. *ESI MS spectrum of **3**. In addition to the M^+-signal at m/z = 254 the signals at m/z = 507 and 760 indicate the formation of dimers and trimers in solution (DMSO).*

The formation of oligomers for zwitterion **3** (n = 1) is also supported by dynamic light scattering experiments. Preliminary studies indicate the formation of larger aggregates of 10 – 100 nm size in solution. However, due to solubility problems at higher concentrations a detailed analysis was not yet possible.

All these data indicate that zwitterion **3** (n = 1) forms stable oligomers of significant size in DMSO solution even at relative low concentrations (< 20 mM). However, these experiments do not reveal the structure of such aggregates. For this purpose a MD simulations (Macromodel 8.0, Amber*, water solvation)[49] was performed, which suggested that **3** forms a helical oligomer (shown in Figure 64) held together both by ion pairing within the plane and π-stacking interactions along the helix axis. The helix has a repeat of about five molecules. The formation of such helical stacks would also explain the limited solubility of **3**, compared to the other zwitterions.

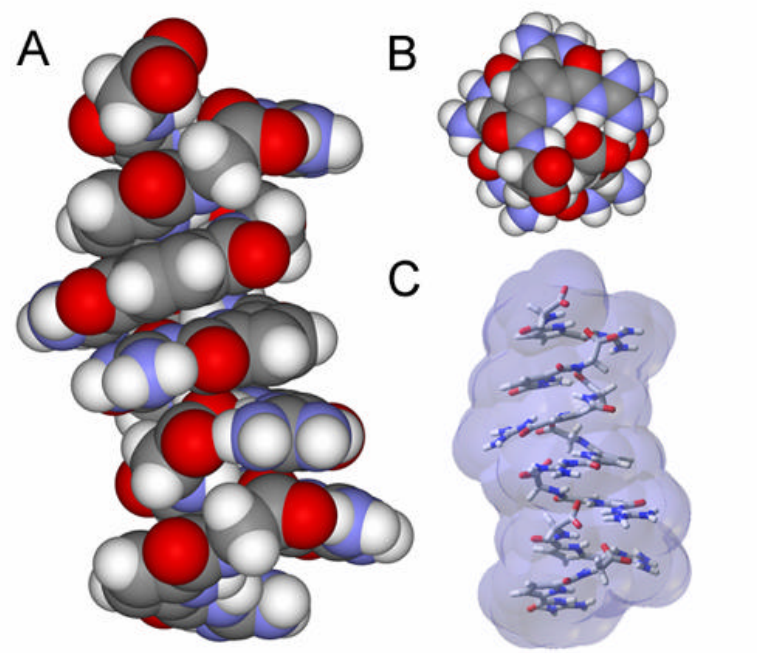


Fig. 64. *Side (**A**) and top (**B**) view of the calculated structure for the helical oligomer of zwitterion **3** (n = 1) and the solvent accessible surface (**C**).*

For the more flexible zwitterions with n = 2, 3 or 5, a similar tight and stable stacking is not possible. Accordingly, the NMR dilution data indicate a much weaker intermolecular association in the order of n = 1 >> 3 > 5 > 2, reflecting the increasing flexibility of the monomers with the exception of zwitterion **4** with n = 2. Even though the molecule is less flexible than **5** and **6**, its aggregation seems to be weaker. However, the extrapolated shift for the amide NH within the aggregate for n = 2 (δ = 11.05) is different from the other three zwitterions which all approach a limiting shift of δ ~ 11.25. This indicates the formation of a different kind of aggregate for n = 2 compared to n = 1, 3 or 5. And indeed the dilution data for **4** (n = 2) can be perfectly well fitted with a 1:1-dimerization model (Figure 62) (explained in detail in section 4.4.2) according to the following equation:

$$d_{beo} = d_C + \frac{\Delta d}{4 \cdot [C_0] \cdot K_{\dim}} \cdot \left\{ 1 + 4 \cdot [C_0] \cdot K_{\dim} - \sqrt{1 + 8 \cdot [C_0] \cdot K_{\dim}} \right\}$$

Eq. 1

$$mit \quad \Delta d = d_{\dim} - d_C \qquad und \qquad K_{\dim} = \frac{1}{K_{diss}}$$

The dimerization constant for **4** (n = 2) was calculated to K = 190 M^1 at 298 K. For zwitterions **5** and **6** the deviation of the data from a 1:1 dimerization model is significant, indicating more complex oligomerization equilibria in solution.

However, if one uses the limiting shift of δ = 11.25 from the aggregation of **3** (n = 1) for these two zwitterions, one can estimate the average number of aggregated monomers. As the complexation is fast on the NMR time scale, the observed chemical shift δ_{obs} is the weighted average of the shifts for the complexed (δ_{oligo}) and the uncomplexed molecule (δ_{free}):

$$\delta_{obs} = \delta_{oligo}\, p + \delta_{free}\, (1\text{-}p) \qquad \textbf{Eq. 2}$$

The parameter p is the fraction of complexed molecules at each concentration. From this value, the number n of aggregated monomers can be estimated according to equation **3**:

$$n = 1 / (1\text{-}p) \qquad \textbf{Eq. 3}$$

Therefore, one can estimate that at a concentration of 100 mM an average of 7 (n = 3) or 4 (n = 5) monomers are aggregated, respectively. In contrast to these oligomeric aggregation of **5** and **6**, zwitterion **4** (n = 2) forms only dimers.

Theoretical calculations confirm that for **4** (n = 2), the formation of a 1:1-head to tail dimer is energetically most favorable in this series. The calculated structure for this dimer is shown in Fig. 65.

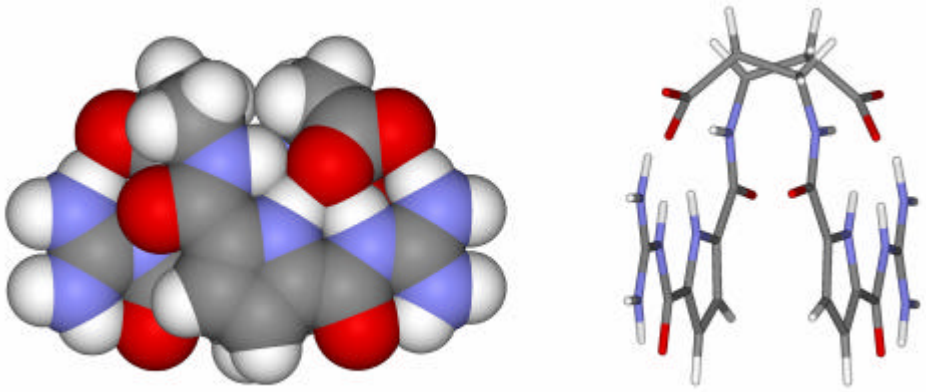


Fig. 65. *Calculated structure for the 1:1-dimer formed by zwitterion **4** (n = 2).*

Each carboxylate forms a tight ion pair with the guanidiniocarbonyl pyrrole moiety of the second molecule whereas the pyrrole rings are π-stacked. For **3** (n = 1) a similar dimer can not form due to steric strain as the alkyl spacer is not yet long enough to allow a simultaneous ion pairing and π-stacking. For the more flexible zwitterions probably either entropic reasons or

the competing now more and more possible *intramolecular* charge interaction disfavors dimerization.

In conclusion, both experimental data as well as calculations suggest that the flexible zwitterions with n = 1, 3 and 5 form linear oligomers in DMSO solution. For n = 1, highly stable helical aggregates with nanometer sized dimensions are formed. For the more flexible systems with n = 3 and 5, the aggregation is much weaker. In contrast to this, the zwitterion with n = 2 forms discrete head to tail dimers instead of linear oligomers in the concentration range studied.

Gas phase studies:[120]

To learn more about the association equilibria of our zwitterionic compounds **3-6** and the intermolecular forces that govern these processes, we also investigated the properties of these aggregates by electrospray ionization mass spectrometry (ESI-MS) studies. ESI is an exceptionally soft ionization technique, in which even weakly bound noncovalent complexes can be transferred intact into the gas phase.[121-123]

In this context, we investigated the stability and the fragmentation kinetic of our series of structurally related zwitterions **3-6** upon infrared laser activation by infrared multiphoton dissociation Fourier transform ion cyclotron resonance mass spectrometry (IRMPD-FT-ICR MS).[124-126] IRMPD-FT-ICR MS is an extremely valuable technique for this purpose, as the noncovalent complexes can be transferred into the gas phase by ESI, trapped by an ion cyclotron resonance mass spectrometer (ICR) and subsequently the dissociation kinetic could be measured upon IR activation with a CO_2 laser. Additionally, IRMPD-FT-ICR MS is perfectly suited to study weakly, noncovalent complexes, as it offers the possibility to measure quantitatively the energetics of such complexes.[127-130] Thus, the character and the strength of noncovalent interaction can be probed and quantified in the gas phase. Because gas phase measurements necessarily take place in the absence of solvent, comparison to data obtained in solution can elucidate the influence of solvent molecules on the stability and structure of supramolecular complexes.

As the protonated and especially the sodiated dimeric complexes $[2M_n + Na]^+$ can easily be generated and examined in the gas phase by electrospray MS, we selected the dimeric complexes $[2M_n + Na]^+$ of the zwitterions n = 2, 3 and 5 as precursor ions for IRMPD tandem MS experiments in a FT ICR MS. Unfortunately, the zwitterion **3** with n = 1 could not

be examined within these studies due to the fact, that it could not be transferred into the gas phase under the conditions necessary for this study. The selected noncovalent complexes dissociate exclusively into the respective monomeric sodium adduct ion $[M_n + Na]^+$ which is shown for example in Fig. 66 for the zwitterion with n = 5.

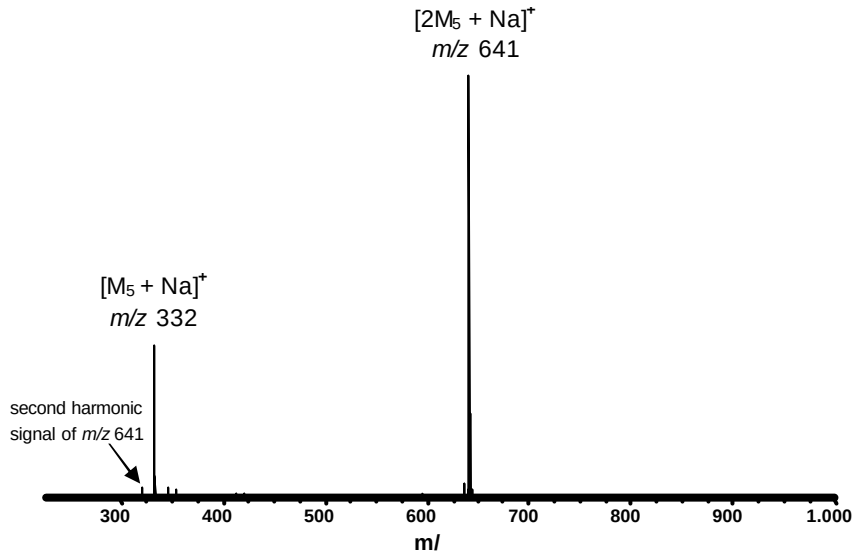


Fig. 66. *IRMPD of the zwitterion* ***6*** *(n = 5) with 8.9 Wcm^{-2} CO_2 laser power density and an irradiation time period of 500 ms.*

The analogous IRMPD behavior of the structurally close related complexes allows to establish relative ordering of gas phase dissociation energy barriers E_a^{laser} for the disruption of the respective noncovalent interaction. The necessary prerequisites for the application of the IRMPD measurements for the determination of relative stability ordering are fulfilled when closely related ions of moderate size (≥ 550 Da) are activated and analogous fragmentation reactions are observed governed by energy exchange of comparable oscillators. These conditions are met in this case, so that the determination of the relative energy barriers E_a^{laser} for the disruption of the noncovalent complexes could be conducted.

To identify activation energies E_a^{laser} of first order gas phase dissociation reactions by IRMPD, the corresponding rate constants of the fragmentation reactions had to be determined. The rate constants of the unimolecular dissociation of $[2M_n + Na]^+$ precursor ions were therefore measured for five laser power densities in the range between 4.5 to 10.3 Wcm^{-2} (Fig. 67 and Fig. 68). The abundance of $[2M_n + Na]^+$ relative to the product ion $[M_n + Na]^+$ was calculated after each of 4-6 irradiation periods ranging from 150 ms to 60 s.

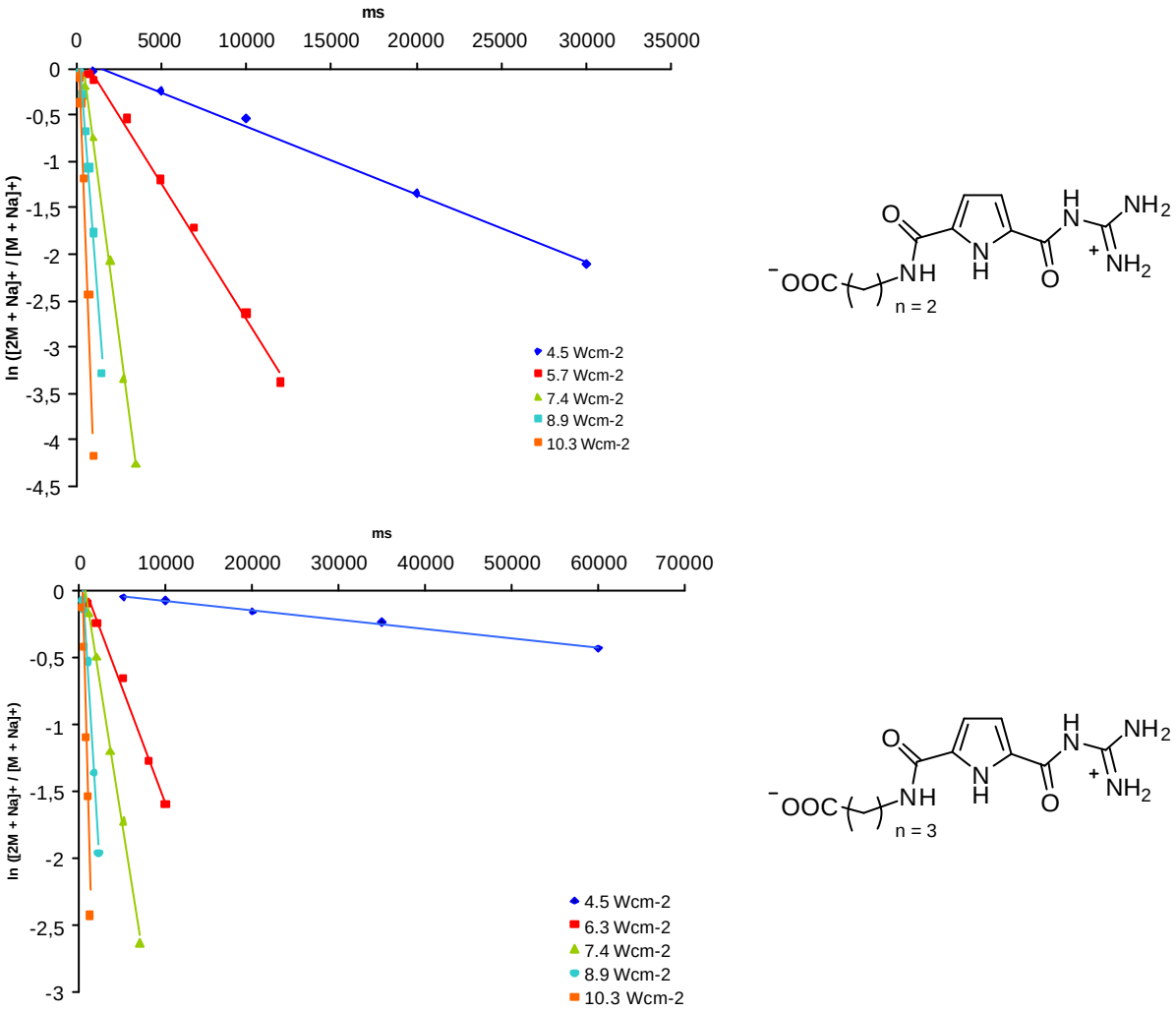


Fig. 67. *Natural logarithm of the abundance of the precursor ion* $[2M_2 + Na]^+$ *(m/z 557) relative to the abundance of the primary fragment* $[M_2 + Na]^+$ *(m/z 290)(above) and the precursor ion* $[2M_3 + Na]^+$ *(m/z 585) relative to the abundance of the primary fragment* $[M_3 + Na]^+$ *(m/z 304) (below), vs. laser irradiation time period, for each of five indicated laser power densities.*

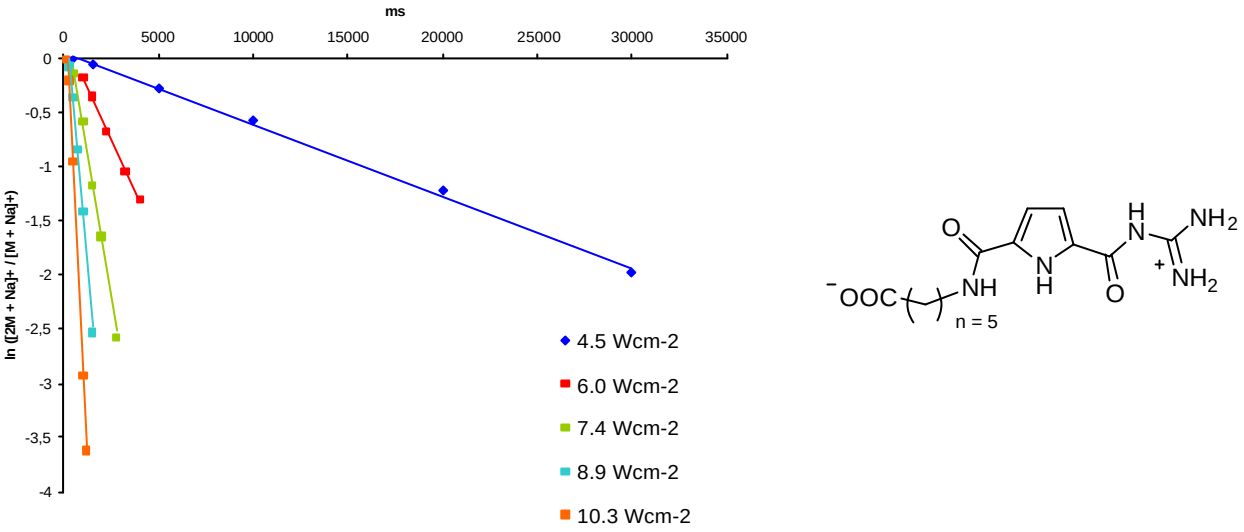


Fig. 68. *Natural logarithm of the abundance of the precursor ion $[2M_5 + Na]^+$ (m/z 641) relative to the abundance of the primary fragment $[M_5 + Na]^+$ (m/z 332) vs. laser irradiation time period, for each of five indicated laser power densities.*

To calculate the activation energies E_a^{laser} of first order gas phase dissociation reactions from the measured data, the rate constants (k_{diss}) and laser power densities (P_{laser}) are applied to **Eq. 4** as proposed by Dunbar.[131]

$$E_a^{laser} = qh\nu \frac{d \ln k_{diss}}{d \ln P_{laser}} \qquad \textbf{Eq. 4}$$

In **Eq. 4**, E_a^{laser}, is the activation energy (in eV) of the gas phase dissociation reaction, q is the partition function for the vibrational mode that absorbs the incoming radiation, h is Planck's constant, ν is the laser frequency, k_{diss} is the first-order dissociation rate constant, P_{laser} is the laser power density (Wcm^{-2}). Because q varies slightly with temperatures between 1.01 and 1.1, an average value of 1.05 was chosen for the maximum expected range of the internal temperature (280-580 K). It should be noted, that this factor q used to describe the number and character of oscillators involved, can only be estimated.[127, 131, 132] Hence, only relative activation energies E_a^{laser} can be determined with **Eq. 4**.

Furthermore, to obtain the activation energies E_a^{laser} from these data with **Eq. 4**, a correction factor has to be introduced, because the size of the complexes had to be considered.[133-135] The complex of M_5 has more atoms n_a and therefore has more degrees of freedom N to distribute energy, compared to the complexes of M_3 and of M_2. Therefore, it requires more energy to break the noncovalent bond in the former complex than the equivalent noncovalent

bond in the latter complexes, within the same time frame. This factor is compensated by correcting the relative activation energy barriers for the number of degrees of freedom ($N = 3n_a - 6$) in the complexes (Tab. 2).

Table 2. *$[2M_n + Na]^+$ precursor ions for the IRMPD study; n_a number of atoms in the respective complex ion $[2M_n + Na]^+$.*

n	$[2M_n + Na]^+$ *m/z*	Number of Oscillators *N* ($N = 3n_a - 6$)
2	557	189
3	585	207
5	641	243

The number of degrees of freedom of the complex $[2M_3 + Na]^+$ was chosen as $N_{reference}$ for the scaling of the other two complexes which results in the following equation.

$$\left(E_a^{laser}\right)_{corrected} = E_a^{laser} * \left(\frac{N_{reference}}{N_{complex}}\right) \qquad \textbf{Eq. 5}$$

With this equation **Eq. 5**, at hand the first-order dissociation rate constant k_{diss} [s^{-1}] can be calculated from the slope of the lines in Fig. 68, which shows the plot of the natural logarithm of the dissociation rate constants k_{diss} for each laser power density versus the natural logarithm of the laser power density (Fig. 69).

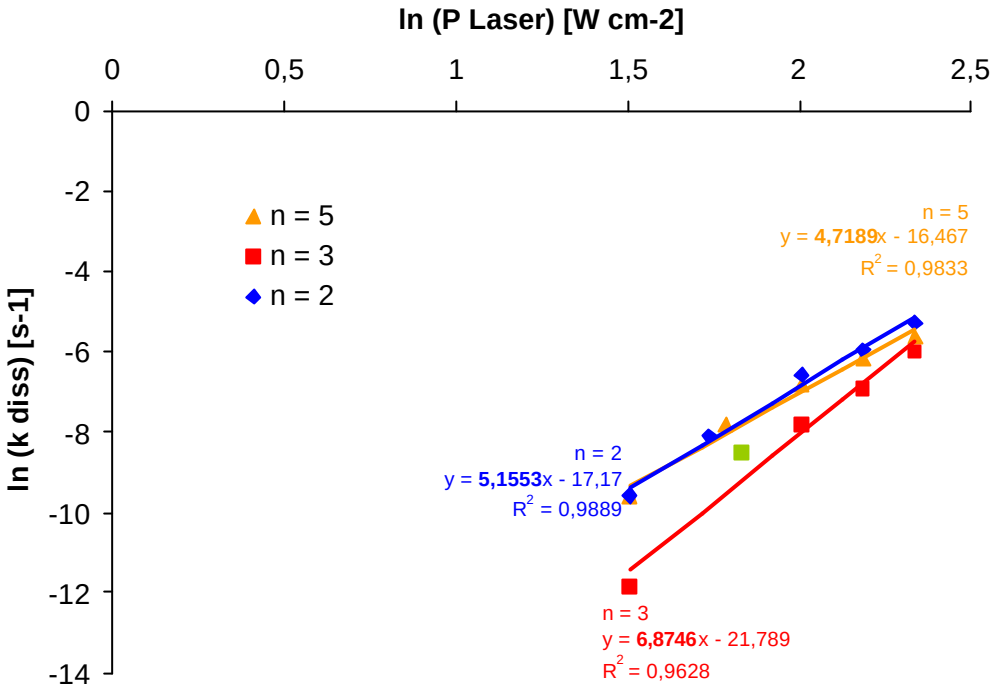


Fig. 69. *Plot of the natural log of the dissociation rate constants k_{diss} for each respective laser power density vs. the natural log of the laser power density in watts per area.*

The $(E_a^{laser})_{corrected}$ for the dissociation of the $[2M_n + Na]^+$ complexes, calculated with **Eq. 5**, were 0.69eV (67 kJmol^{-1}) for M_2, 0.84eV (81 kJmol^{-1}) for M_3 and 0.49eV (47 kJmol^{-1}) for M_5.

Thus, the dimeric complex ion $[2M_3 + Na]^+$ is more stable than the respective complexes of M_2 and M_5, which also can be seen very clearly in Fig. 70, that shows the dependence of the logarithm of the abundance of the precursor ion $[2M_n + Na]^+$ (n = 2, 3, 5) relative to the abundance of the respective primary fragment $[M_n + Na]^+$, upon an irradiation period of the laser at 4.5 Wcm^{-2} power density.

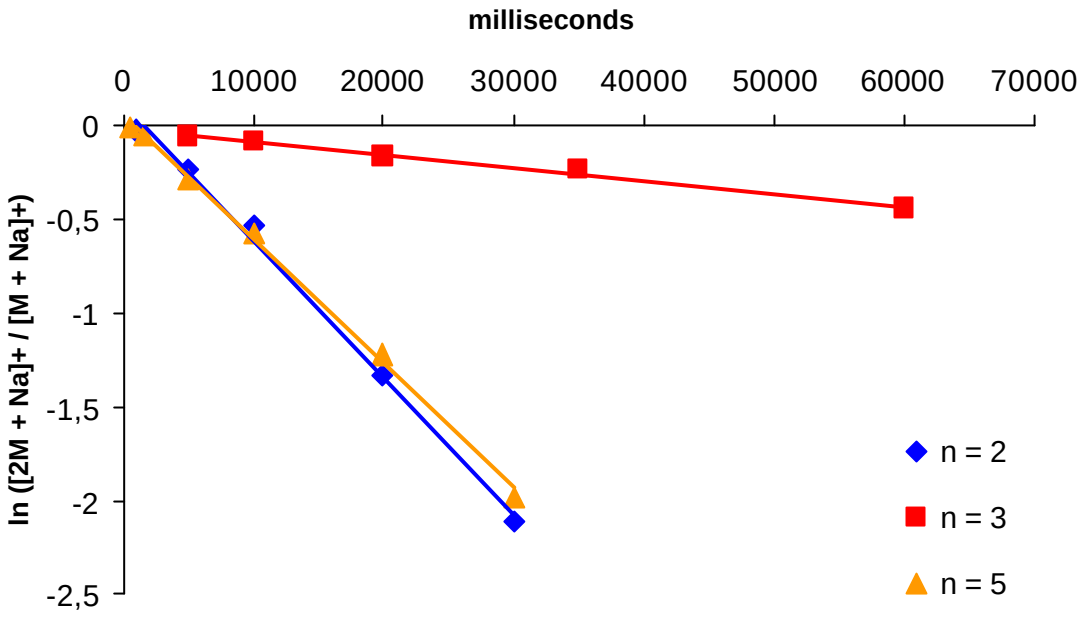


Fig. 70. *Natural logarithm of the abundance of the precursor ion $[2M_n + Na]^+$ (n = 2, 3, 5) relative to the abundance of the respective primary fragment $[M_n + Na]^+$, vs. irradiation time period of the laser at 4.5 Wcm^{-2} power density.*

Obviously, $[2M_3 + Na]^+$ dissociates slower and is therefore substantially more stable than the complexes of M_2 and M_5, respectively. The molecules M_2, M_3 and M_5 have very closely related structures, so that the basicity of the guanidiniocarbonyl pyrrole functionality is expected to be very similar. Anticipated was that the stability of the complexes is limited or determined by the flexibility and the length of the spacer. With increasing flexibility of the spacer the resulting complexes should be less stable due to entropic factor of the longer spacer. Apparently, the relative difference in activation energy observed for the dissociation of the cationized dimeric complexes is not related to their flexibility alone.

The maximum stability of the three examined complexes was found for the complex of M_3. Therefore, geometrical reasons are probably responsible for the unexpected high stability of the $[2M_3 + Na]^+$ complex. Thus, a conformational analysis by molecular dynamics calculations of gas phase $[2M_n + Na]^+$ ions with subsequent energy minimization of the theoretically proposed structures is currently in progress.

The free energies of the aggregates in the gas phase are much higher ($\Delta G \approx$ 50-80 $kJmol^{-1}$) than compared to the solution data ($\Delta G \approx$ 13 $kJmol^{-1}$). However, a direct comparison of these values is not possible, because in the gas phase the sodiated dimeric complexes and in solution oligomeric zwitterions were studied. As a result, the influence of this cation on the structure and stability is not yet clear and has to be further investigated. In addition, in solution the solvation of the formed oligomeric complexes influences their stability.

Outlook:

Within this work, the first general synthetic approach towards a series of flexible zwitterions with different spacer length between guanidiniocarbonyl moiety and carboxylate could be established. It could be shown that in DMSO dimeric or even higher aggregates are formed. Zwitterion with n = 1 even forms highly stable helical aggregates with nanometer sized dimensions. Within gas phase studies of sodiated dimeric complexes it was possible to measure significant differences in the stability of the formed complexes depending on the spacer length. Our group is currently, based on these findings, exploring the possibility to use such ion pair interactions to obtain water-stable supramolecular polymers.

One idea to arrive to a strong association even in aqueous solution, is to accumulate multiple guanidiniocarbonyl pyrrole-carboxylate interaction in a monomeric building block like in the case of **118** (Fig. 71). Through this general design it could be possible to get a three dimensional network which possibly polymerizes in DMSO or water.

Additionally, the general binding forces could be enhanced through other noncovalent interactions like π-stacking or hydrophobic interactions. Therefore, our guanidiniocarbonyl pyrrole binding motif could for example be attached to an aromatic core molecule like perylene or phenylenevinylene. Such a design should provide an assembly by intermolecular π-stacking and/or hydrophobic interaction of the aromatic core units which thereby should tremendously increase the binding energy especially in water.

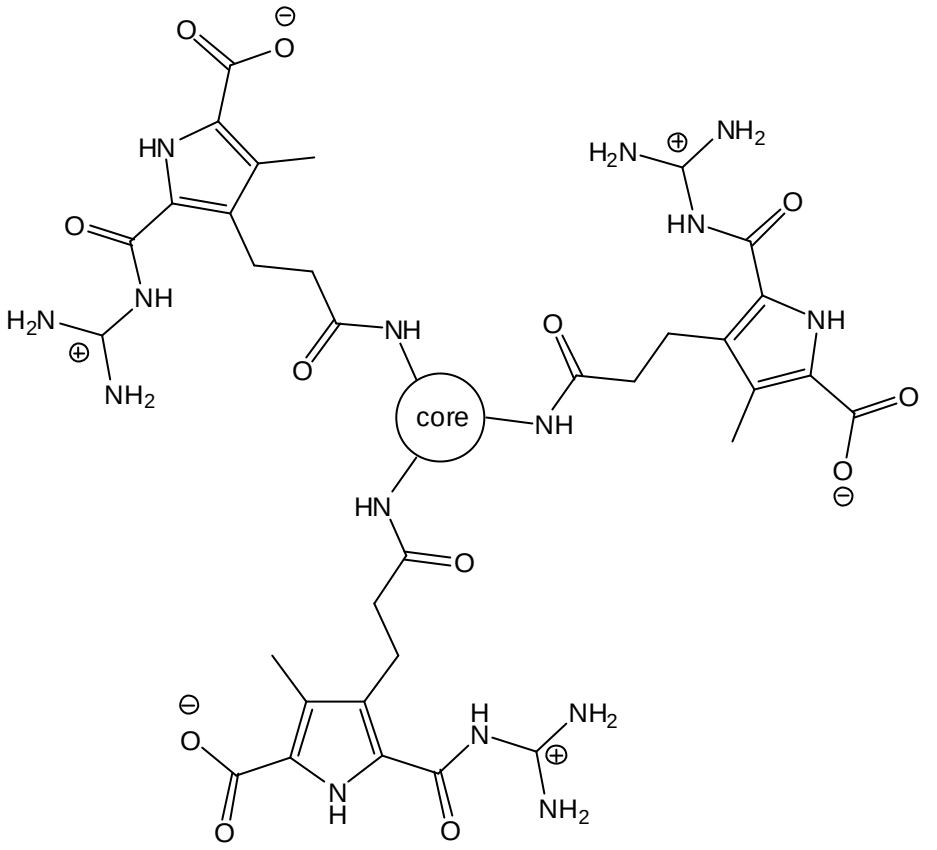


Fig. 71. *Possible structure of a guanidiniocarbonyl pyrrole monomer* ***118*** *with an aromatic core unit, which could polymerize into a three dimensional network.*

4.3 Arginine analogues

The development of peptidomimetics has found wide application to discover potent mimetics of naturally occurring biologically active peptides.[136] The natural amino acid *L*-arginine is involved in many important physiological and metabolic processes and many enzymes display a preference for the arginine residue that is found in a lot of substrates and inhibitors.
Due to the fact that the guanidiniocarbonyl pyrroles bind carboxylates much stronger than guanidine, it could have a tremendous effect on the chemical and biological properties of arginine rich peptides, if the arginine analogues **7-10** are implemented as a substitute for the natural amino acid (Fig. 72).

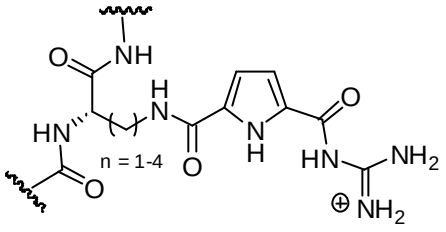


Fig. 72. *Schematic representation of the arginine-analogues **7-10** implemented in a peptidic strand*

Oligopeptides which possess cumulative arginines in the active site are for example RNA-binding peptides or the neuropeptide Y (NPY).[137] The NYP is the most abundant peptide in the mammalian brain and it consists of 36 amino acids. Moreover, NYP has been shown to be vital in a variety of physiological situations, including cardiovascular regulation and control of food intake. The understanding of the function of NYP and their associated pathways could therefore provide the basis for the development of novel therapeutic approaches.
Another field of application of our newly designed arginine-analogues could be the stabilization of helical secondary structures in small peptides. In order to study the role that an isolated helix might play in a biological system, it is necessary to design peptides or peptidomimetics that will adopt a stable helical conformation in water. Therefore, in the recent years much effort has been expended on the study of helical propensities of amino acids using small peptides. *Marqusee and Baldwin* for example designed a 17-residue peptide which adopted a stable helical structure in water. The helical stabilisation in this case arises from ionic interactions between Glu and Lys residues (Fig. 73).[138]

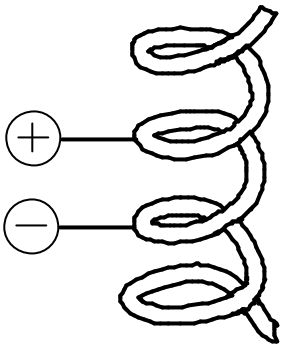


Fig. 73. *Stabilising of an a-helical structure by ion pair interaction.*

As ion pair and hydrogen bond interactions between amino acid side chains stabilize a helical structure and on the other hand the guanidiniocarbonyl pyrrole-carboxylate interaction is extremely strong, our arginine analogues should be an ideal candidate to be tested for their α-helical stabilisation propensities.

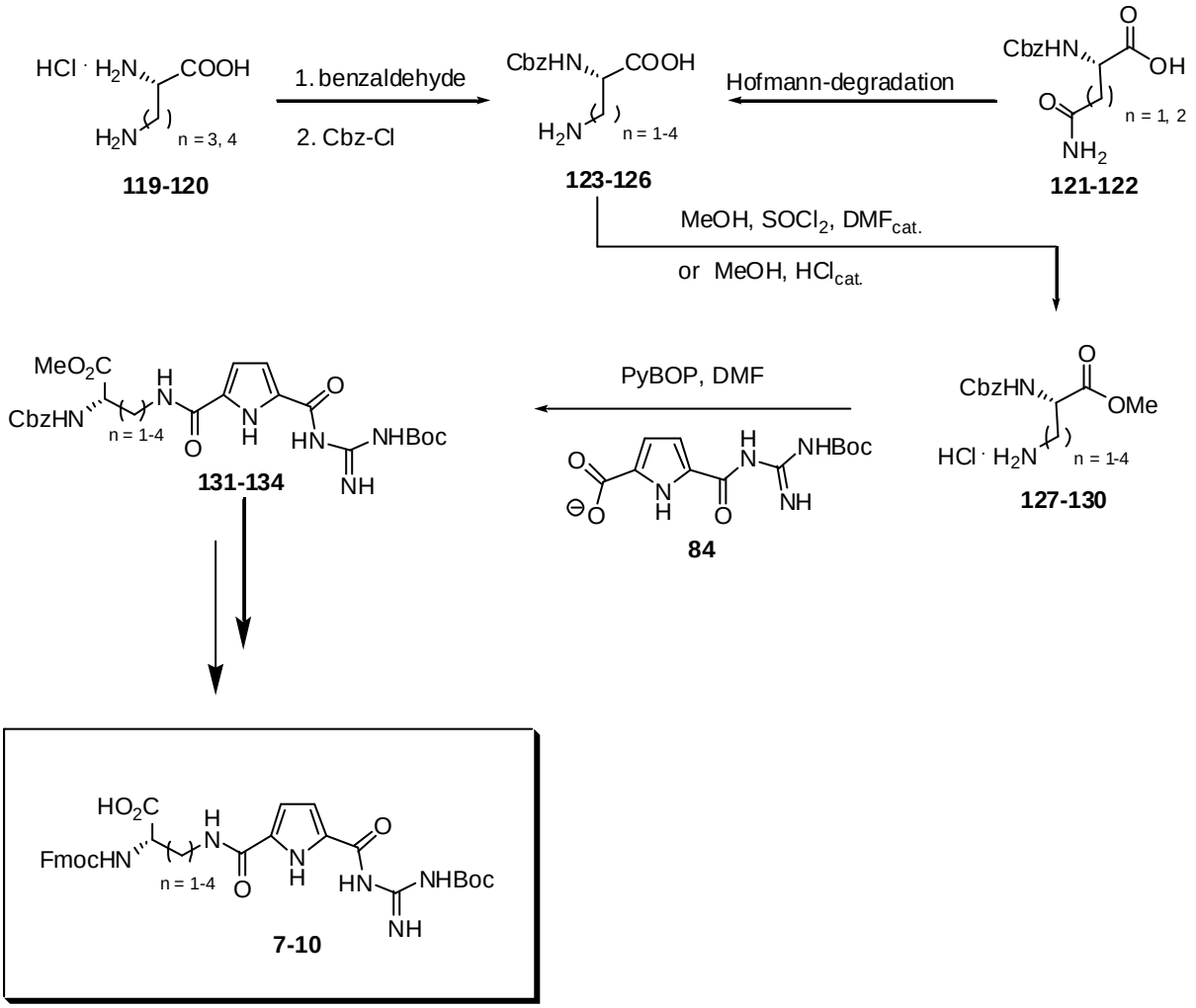
A third potential application of our arginine mimetics is their use for the design of molecular transporter systems. In the recent years, several classes of peptide molecules have been identified that are water-soluble and undergo facilitated uptake into cells and tissues. These peptides include the TAT protein from HIV, the antennapedia protein from Drosophila, and arginine rich peptides. The common feature of these peptides is their highly cationic nature. Extensive structure-function studies of the HIV-tat transporter sequence (RKKRRQRRR) have led to the finding that short oligomers of arginine (R7-9) and, more specifically, guanidinium-based oligomers often exhibit superior membrane translocation activity. However, the mechanism of the protein translocation is still unclear.[139]

This obviously offers the potential to study the cellular uptake ability of short peptide transporters which are based on our arginine analogues. The comparison with normal arginine peptide sequence of the same length would allow to study the influence the guanidinium group in such receptors systems and thus help to understand the mechanism of protein translocation.

These examples demonstrate that there are a lot of research fields, in which arginine-analogues could be utilized and therefore could help to gain more understanding of such natural processes. For these diverse potential applications in near future, I developed a synthetic route towards four new Fmoc-protected arginine analogues **7-10** with a spacer length from one to four carbon atoms. Their synthesis and test in a solid-phase synthesis as a "proof of principal" will be presented in the next chapter.

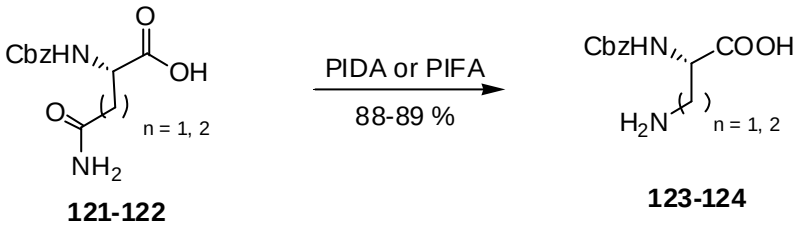
4.3.1 Synthesis

The four Fmoc-protected arginine analogues **7-10** were synthesized according to Scheme 8:



Scheme. 8 *Synthesis of the Fmoc-protected arginine analogues **7-10**.*

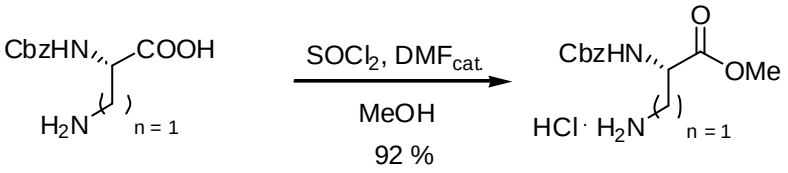
The Cbz-protected amino acids **123-126** were synthesized by two different routes: The amino acids with spacer length n = 1-2 were prepared through a Hofmann-degradation from the commercially available Cbz-protected asparagine **121** (n = 1) and Cbz-protected glutamine **122** (n = 2) (Fig. 74). The amino acids with spacer length n = 3-4 were prepared via selective mono-protection of the α-amino function of natural amino acids ornithine **119** and lysine **120**. The Hofmann degradation of Cbz-protected asparagine **121** and Cbz-protected glutamine **122** was performed according to literature procedures with the hypervalent iodine compounds bis(acetoxyiodo)benzene (PIDA) or the analogous bis(trifluoroacetoxyiodo)benzene (PIFA) with yields of 88 % (n = 1) and 89 % (n = 2) (Fig. 74).[140]



121-122 **123-124**

Fig. 74. *Hofmann-degradation of Cbz-protected asparagine **121** and Cbz-protected glutamine **122**.*

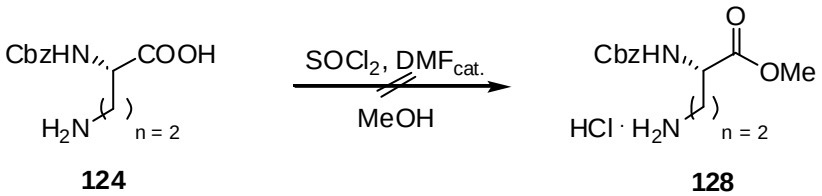
The methylester of the Cbz-protected diamino carboxylic acid **127** (n = 1) was then easily prepared in a good yield of 92 % through a reaction with thionylchloride and catalytic amounts of dimethylformamide in methanol (Fig. 75).[141]



123 **127**

Fig. 75. *Synthesis of the methylester of n =1.*

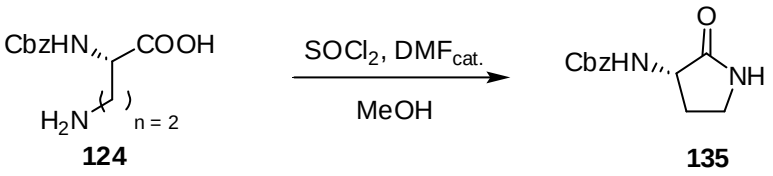
In the case of the Cbz-protected diamino carboxylic acid **124** with n = 2 the analogous reaction conditions did not yield to the corresponding methylester **128**. However, the investigation by thin layer chromatography showed a complete conversion of the starting material **127** after a reaction time of 12 h. The NMR spectra revealed that the isolated product did not possess any methyl group (Fig. 76).



124 **128**

Fig. 76. *Attempt to synthesize the methylester **128** of n =2.*

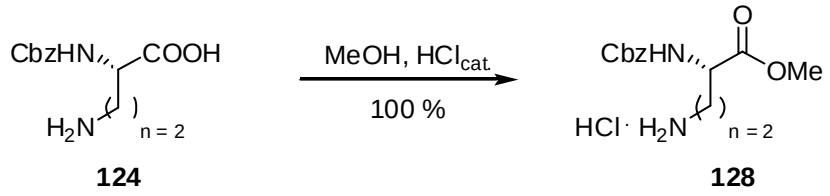
Thus, an intramolecular lactonization to a five membered ring was supposed, because this would be according to the Baldwin rules a favored 5-exo-trig reaction (Fig. 77). Indeed, *Cox et al.* had described this lactonization under this particular reaction conditions.[142]



124 **135**

Fig. 77. *Lactonization reaction of the Cbz-protected amino acid **124** by a reaction with $SOCl_2$ in methanol.*

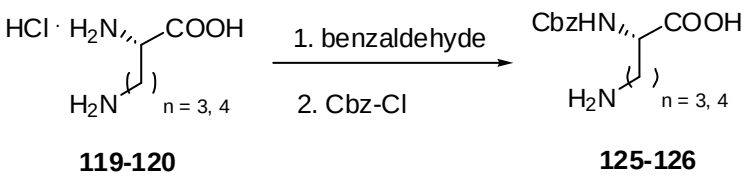
However, they also reported that the preparation of the methyle ster of compound **124** could be achieved under milder reaction conditions by a reaction catalyzed by hydrochloric acid in methanol. Under this particular reaction conditions, the amine group is protonated and consequently only a weak nucleophile. As a result, the intramolecular reaction is suppressed by such catalytic protonation conditions, therefore just producing the methylester



124 **128**

Fig. 78. *Synthesis of the methylester **128** (n = 2) in methanol with hydrochloric acid.*

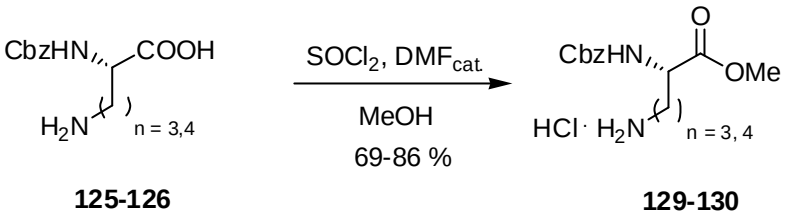
In contrast to the preparation of the Cbz-protected amino acids **123-124** (n = 1-2), the selective Cbz-protection of the α-amino group of ornithine and lysine was realized according to *Zervas et al.* by a two step synthesis.[143] At first, the ω-amino function was selectively protected as an imine, in this specific case as a benzylidene. This could be achieved by a reaction with benzaldehyd in sodium hydroxide, since the more reactive ω-amino moiety reacts preferentially compared to the α-amino function. In the next step simultaneously the α-amino moiety was Cbz-protected and the imine was hydrolyzed yielding the desired Cbz-protected amino acids in 49 % and 56 %, respectively (Fig. 79).



119-120 **125-126**

Fig.79. *Selective protection of the N_a-amino function of ornithine **119** and lysine **120**.*

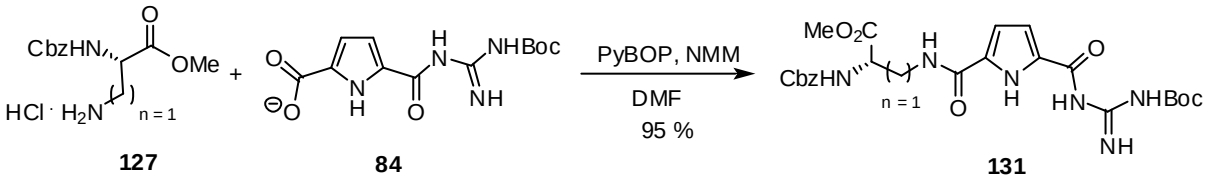
The methylesters **129-130** of the Cbz-protected amino acids **125-126** (n = 3, 4) were then prepared by a reaction with thionylchloride in methanol catalyzed by DMF yielding the desired compounds in yields of 69-86 %.[144]



125-126 **129-130**

Fig. 80. *Synthesis of the methylester **129-130** of n = 3, 4 by a reaction with thionylchloride in methanol.*

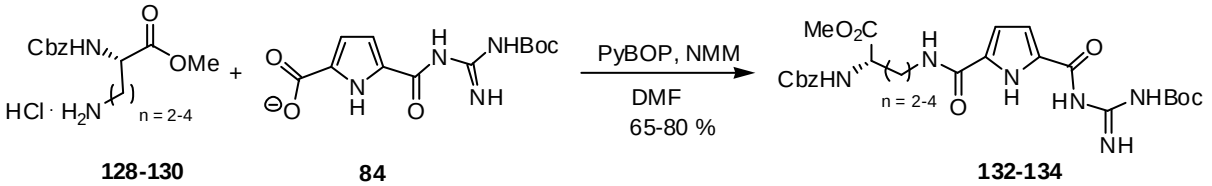
The key step for the synthesis of the arginine analogues **7-10**, the coupling of the amino acid methylesters **127-130** with the ^{t}Boc-protected guanidiniocarbonyl pyrrole carboxylic acid **84** was achieved in DMF by activation with PyBOP. In the case of the amino acid **127** (n = 1) the coupling reaction yielded the amide **131** in a very good yield of 95 %.



127 **84** **131**

Fig. 81. *Coupling of the amino acid **127** (n = 1) with the ^{t}Boc-protected guanidiniocarbonyl pyrrole **84**.*

The other amide coupling products **132-134** were also obtained in good yields of 65 % in the case of the amino acid **128** (n = 2) and 79 % to 80 % for the amino acids **129-130** (n = 3-4) (Fig. 82).



128-130 **84** **132-134**

Fig. 82. *Coupling of the amino acids **128-130** (n = 2-4) with the ^{t}Boc-protected guanidinocarbonyl pyrrole **84**.*

It must be emphasized again, that the application of the ^{t}Boc-protected guanidinocarbonyl pyrrole **84** in this coupling step is inevitable for a successful realization of this and the

following synthetic steps. Previous to the development of this central compound, I have reacted the known zwitterion **38** by activation with PyBOP with the corresponding amino acids (Fig. 83).

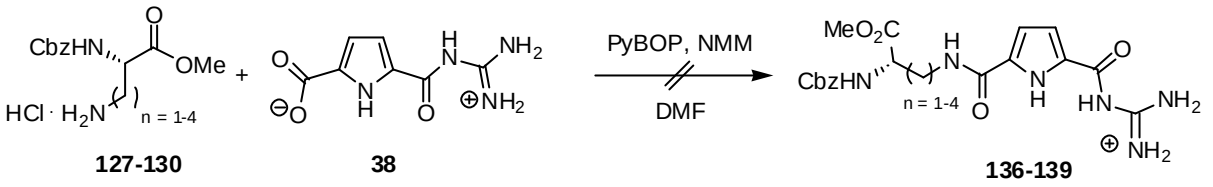


Fig. 83. *Coupling of the amino acids **127-130** (n = 1-4) with the zwitterion **38** led only to impure product mixtures.*

However, the isolation of the resulting cationic guanidiniocarbonyl pyrroles **136-139** by chromatography or by precipitation as the picrate salt led only to impure product mixtures. In addition to this, the isolated picrate salts were only poorly soluble in organic solvents and therefore the following reactions and their work-up were highly problematic or even impossible. This underlines again the importance of the newly developed synthesis introduced in section 4.1.

In order to be able to implement these arginine analogues **7-10** easily into oligopeptides, it is inevitable that these compounds can be used in a normal solid phase synthesis. Therefore, the methylester must be hydrolyzed and the Cbz-functionality must be transformed into an Fmoc-protection group. Thus, the Cbz-moiety was cleaved off by a catalytic hydrogenation reaction in methanol affording the corresponding amines **140-143** in yields of 90-95 % (Fig. 84).

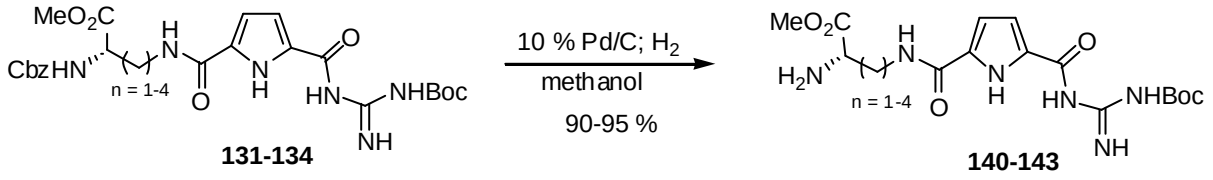


Fig. 84. *Catalytic hydrogenation of the Cbz-protection group.*

Finally, the methylesters **140-143** were hydrolyzed with lithium hydroxide in a THF/water mixture and subsequently Fmoc-protected by addition of Fmoc-chloride in a dioxane/water mixture.[145]

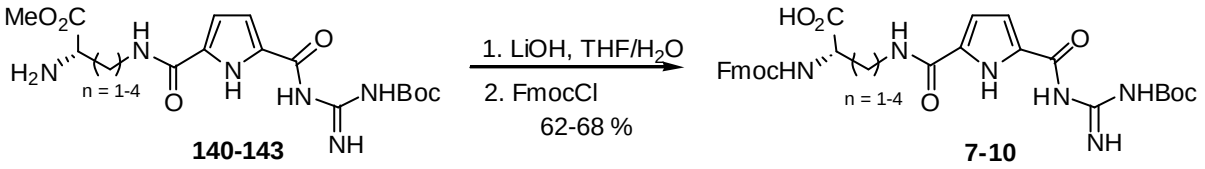


Fig. 85. *Hydrolysis of the methylester and subsequently Fmoc-protection of the amine moiety yielding the desired arginine analogues **7-10**.*

The hydrolysis and the work-up must be performed under special precautions. Due to the fact that the ^{t}Boc-guanidino group can also act as a very good leaving group under nucleophilic conditions (discussed in section 4.1) only a slight excess of lithium hydroxide (1.5 eq.) was used in the reaction. Additionally, the reaction time was kept on a minimum. Therefore, the reaction was intensively controlled by thin layer chromatography and immediately stopped when the hydrolysis was completed. The reaction mixture was then neutralized and directly lyophilized. This work-up procedure ensures that the reaction is stopped after complete hydrolysis of the methylesters. The carboxylic acids of the corresponding methylesters **140-143** were not isolated, but the lithium salts directly reacted with Fmoc-chloride in a mixture of dioxane and aqueous sodium carbonate for 90 minutes. The resulting reaction mixture was hydrolyzed again and the desired Fmoc-protected arginine analogues **7-10** isolated by column chromatography in good yields of 62-68 %.

With this multi-step synthesis I was able to prepare these four, new arginine analogues with the implemented guanidiniocarbonyl pyrrole binding motif in total yields from 25 % to 45 %.

4.3.2 Results

A necessary prerequisite for the successful application of our Fmoc-protected arginine analogues in further projects is that **7-10** can be implemented into oligopeptides by a normal solid phase synthesis. Hence, I tested the application of **7-10** in a normal Fmoc-protocol peptide synthesis. As a test reaction I synthesized a tripeptide consisting of alanine, the arginine analogue **7** with n = 1 and valine. If this test reaction works properly, this would be an evidence for the possibility and reliability of amide couplings on solid support with our arginine analogues **7-10**. The synthesis of the tripeptide Ala-AA_1-Val (AA_1: arginine analogous n = 1) was performed on Rink amide MBHA resin by a standard protocol. After every coupling step the completeness of the reaction was controlled by a Kaiser-test. A positive result (i.e. colored beads) in this ninhydrin-type test is a sign for the existence of free amino groups. Since the Kaiser-test did not show an undesired positive result in any coupling step, this is proof that our arginine analogue can be coupled on solid support like normal amino acids. The tripeptide was subsequently cleaved from the solid support by shaking the resin with CH_2Cl_2/TFA mixture (5:95) yielding after isolation the desired triflate salt of the peptide.

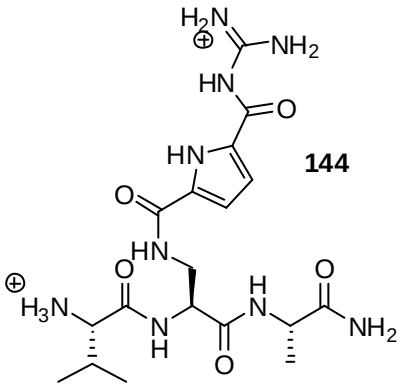


Fig. 86. Structure of the tripeptide Ala-AA_1-Val (AA: arginine analogue) synthesized on Rink amide solid support.

The ^{1}H NMR, the ^{13}C NMR and the high resolution ESI-MS spectra of the isolated product are consistent with the depicted tripeptide structure **144** in Fig. 86. The ^{1}H NMR spectrum in the range from 3.3 to 4.7 ppm shows the expected multipletts of the three α protons of the tripeptide (Fig. 87).

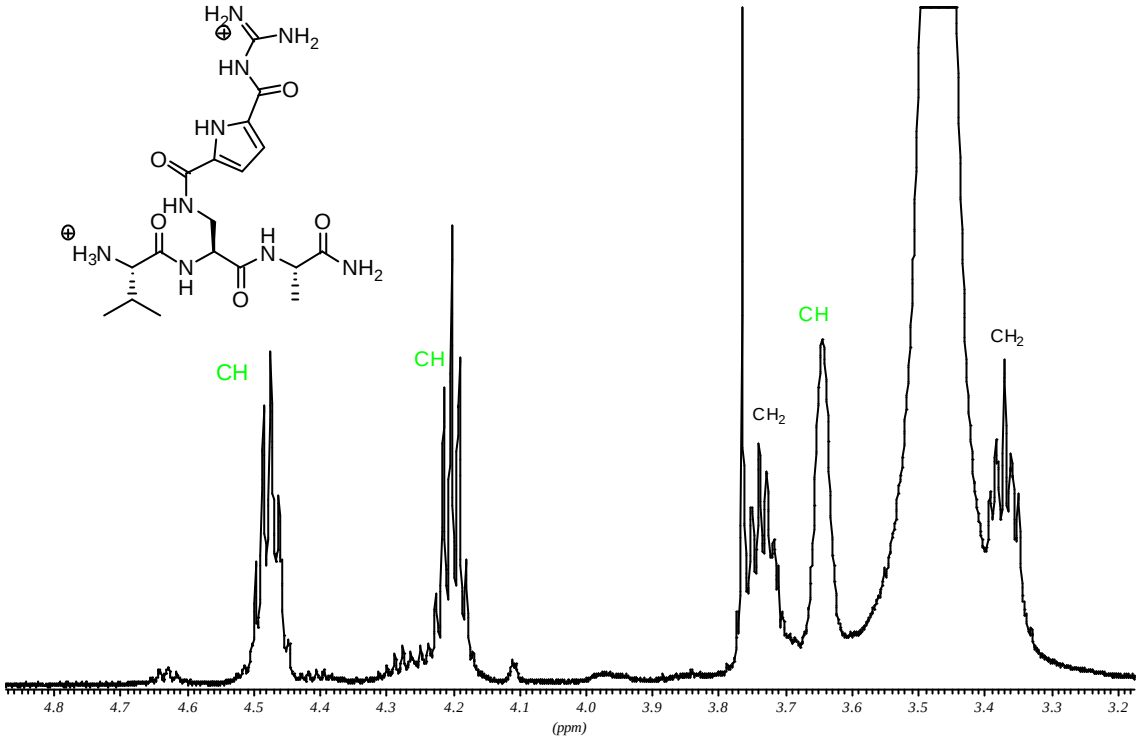


Fig. 87. *Part of the ^{1}H NMR spectrum of the tripeptide **144** Ala-AA$_1$-Val (AA: arginine analogue) showing the expected CH-protons.*

Additionally, the ^{13}C NMR spectrum shows besides the signal of the single methylene group and the CH-carbon atoms (48-57 ppm) also the characteristic pyrrole signals for the two CH-carbons at 113 and 116 ppm and the quaternary carbons at around 130 ppm (Fig. 88). Thus, these spectra prove that the desired tripeptide **144** consisting of our arginine analogue with n = 1 was successfully synthesized on the solid support.

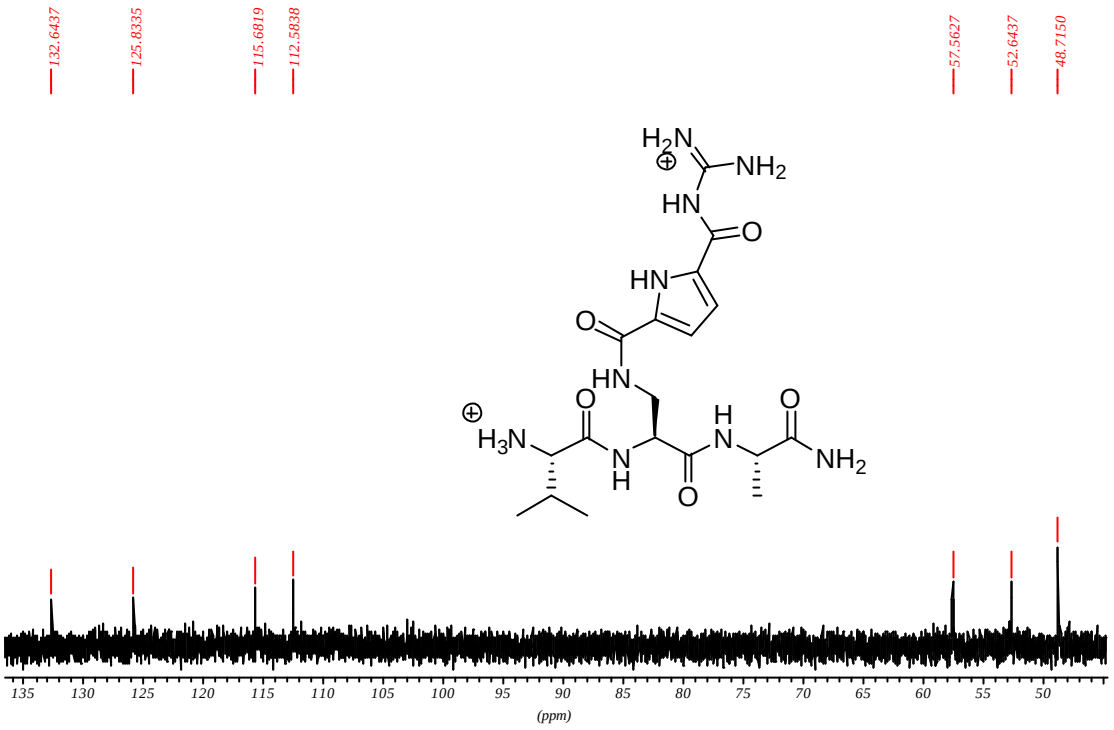


Fig. 88. *Part of the ^{13}C NMR spectrum of the tripeptide Ala-AA$_1$-Val (AA: arginine analogue) showing the expected pyrrole carbon atoms.*

In summary, I have shown here that the synthesized arginine analogues can be applied in a standard solid phase peptide synthesis. This is a necessary requirement for the use of these arginine analogues **7-10** in new projects in the future.

Outlook:

With these interesting arginine mimetics at hand a variety of interesting projects can be developed in the future. Besides the implementation of our arginine analogues **7-10** as a substitute for arginine in biologically important peptides, a tremendously interesting project would be to study the α-helical stabilizing propensities of our unnatural amino acids. Among the 22 proteinogenic amino acids, alanine (Ala) has been used mostly for designing helices due to its high helix propensity.[138] Stabilized by Ala residues, isolated peptides need to be typically at least 20 residues long. In the recent years, many research groups have tried to stabilize short alanine based helical structures by electrostatic or hydrogen-bond interactions

between amino acid side chains. Most of these peptidic models contain Glu-Lys, Gln-Glu, His-Asp, Gln-Asn, Glu-Lys or Asp-Arg interactions. Within these studies the effect of i(i+3) and i(i+4) spacing and orientation between these residues were determined. Since our guanidiniocarbonyl pyrrole-carboxylate interaction is extremely strong also in competitive solvents like water, it would be highly interesting to implement such interactions in a short peptide strand (Fig. 89).

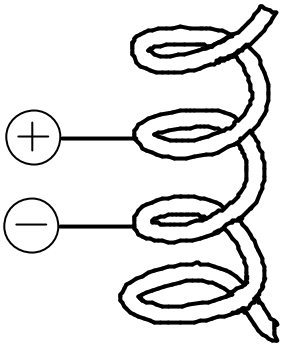


Fig. 89. *Stabilising of an a-helical structure by ion pair interaction.*

In order to study the ability of our guanidiniocarbonyl pyrrole-carboxylate interaction to stabilize peptide helices, it would be necessary to implement one or several arginine-analogues together with aspartic or glutamic acid into a short peptide. Within these studies also the effect of the length and therefore flexibility of our arginine arginine **7-10** can be evaluated.

4.4 Tris-cationic receptor

In his thermodynamic studies of the interaction of guanidiniocarbonyl pyrroles with carboxylates, *Schmuck* established a semi-quantitative supramolecular structure-binding relationship (SSBR) for the complexation of carboxylates by guanidiniocarbonyl pyrroles. In this context he determined the quantitative value for the energetic contribution of individual interactions within this binding motif by a comparative study of a series of systematically varied receptors (discussed in detail in section 3.1.2) (Fig. 90).[32]

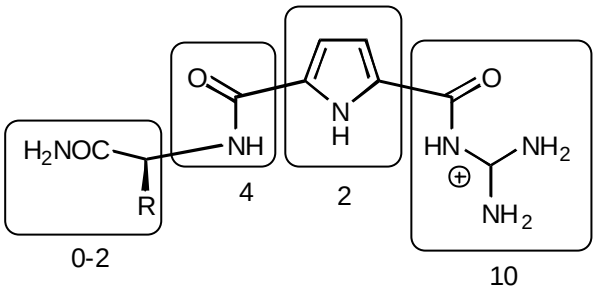
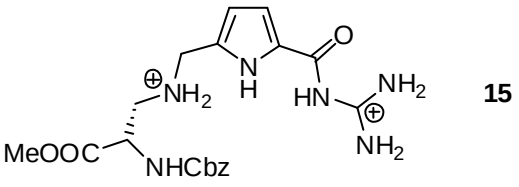


Fig. 90. *Estimated semi-quantitative energetic contributions of the individual binding interactions for the overall complexation of carboxylate by guanidiniocarbonyl pyrroles **1** (DG in kJ·mol^{-1}).*

Besides the acyl guanidinium moiety, which provides the major binding energy of $\Delta G = 10$ kJ·mol^{-1}, the amide NH next to the pyrrole ring is most important and increases the binding energy by another 4 kJ·mol^{-1}. In order to study this specific energetic contribution in more detail, I designed a di-cationic receptor **15** in which the amide functionality is substituted by an ammonium group.



15

Fig. 91. *Di-cationic receptor design as a new binding motif for carboxylates.*

With this general receptor type at hand, the influence of additional ionic interactions at the other side of the pyrrole could be evaluated and compared with the neutral amide function. Possibly, the additional ionic interaction between this guanidiniocarbonyl pyrrole binding motif and a carboxylate further enhances the binding strength (Fig. 92). The influence of this

additional ionic interaction for the stability of the modified guanidiniocarbonyl pyrrole-carboxylate interaction was therefore evaluated.

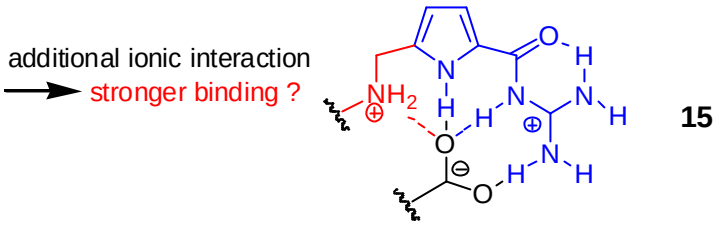


Fig. 92. *Di-cationic receptor design as a new binding motif for carboxylates.*

The general design of receptor **15** also offers the possibility to synthesize a tris-cationic receptor of type **11**. This receptor possesses, besides the guanidiniocarbonyl pyrrole binding site, also a second cationic ammonium group (Fig. 93).

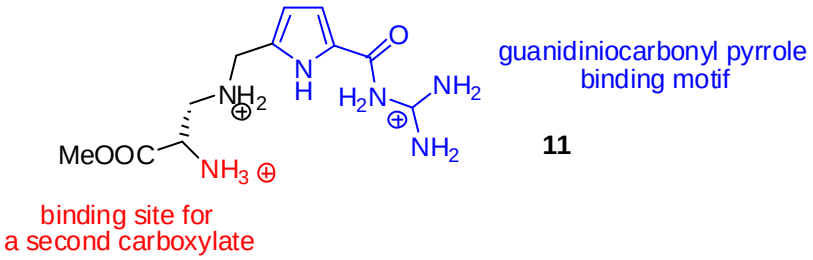


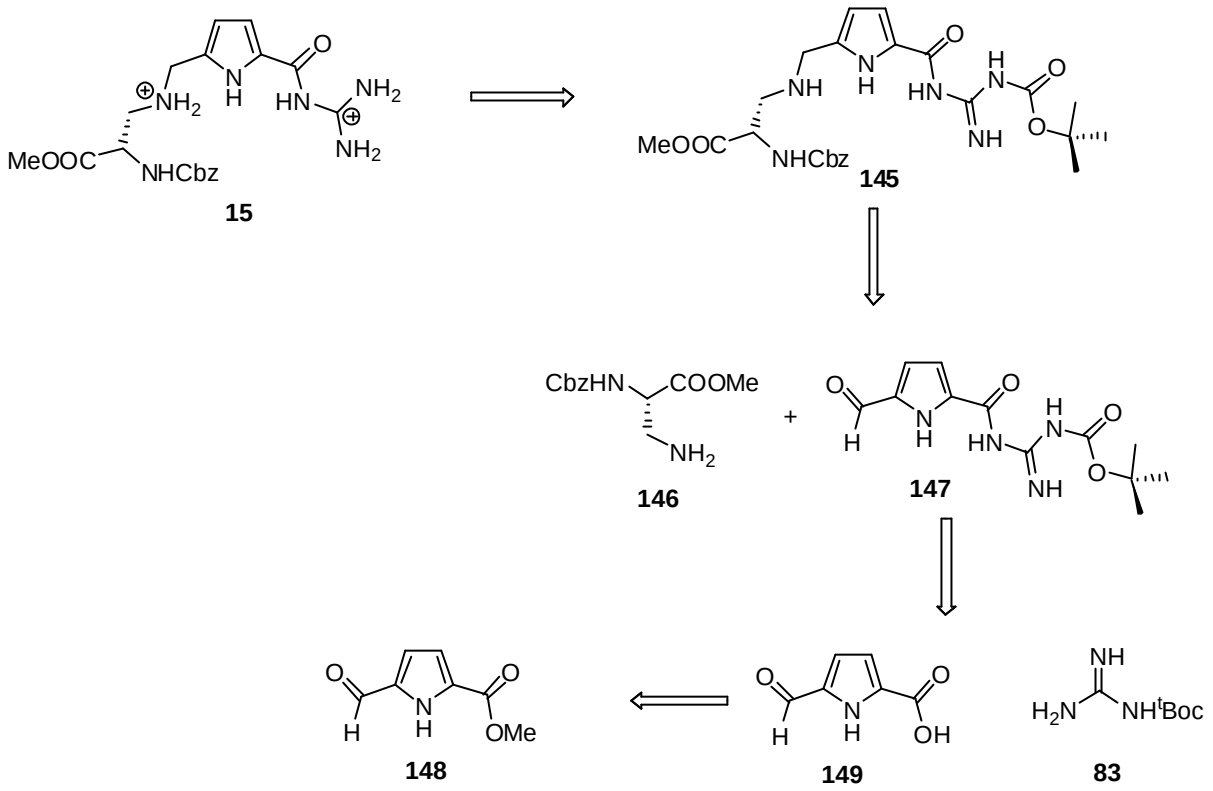
Fig. 93. *Tris-cationic receptor design as a new binding motif for amino acids with two carboxylates.*

Since ammonium cations are often used to complex carboxylates, this receptor should be able to bind substrates with two carboxylates.[7] One carboxylate can be bound by the guanidiniocarbonyl pyrrole and the other anionic group should interact with the ammonium group. Hence, this general receptor structure should be ideally preorganized to strongly complex amino acids with two carboxylates like for example aspartic or glutamic acid.

I developed a synthesis for these two target molecules which will be presented in the following. The detailed evaluation of the association constants towards a variety of acylated amino acids will be discussed in afterwards.

4.4.1 Synthesis

The retrosynthesis of the di-cationic receptor is outlined in Scheme. 9.



Scheme 9. *Retrosynthesis of the di-cationic receptor **15**.*

According to the Scheme **9** the receptor **11** is synthesized starting from the known and readily available 2,5-substituted pyrrole methylester **148**.[32] The methylester **148** was first hydrolyzed in adaptation of a literature procedure by refluxing in a water/ethanol-mixture with added potassium hydroxide.[146] The carboxylic acid could be isolated in nearly quantitative yield.

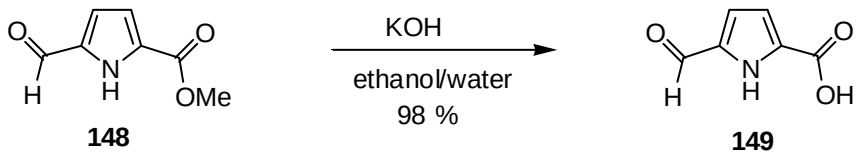


Fig. 94. *Hydrolysis of the methylester**148** .*

In the following step the guanidino moiety was introduced by coupling of the carboxylic acid **149** with mono ^{t}Boc-protected guanidine **83** using PyBOP as the coupling reagent to yield the ^{t}Boc-protected compound **147** in 90 %.

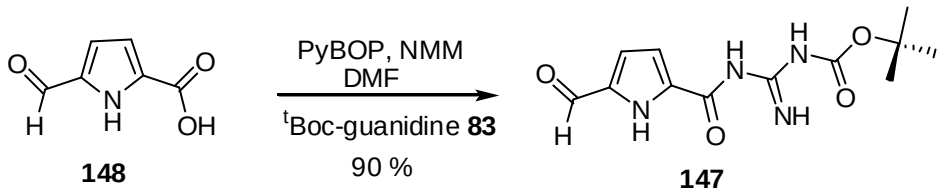


Fig. 95. *Coupling of the carboxylic acid **148** with tBoc-guanidine **83** by activation with PyBOP.*

The Cbz-protected amino acid **123**,[140] which was synthesized from Cbz-protected asparagine **121** via a Hofmann-degradation (described in section **4.3.1**), was now reacted with the ^{t}Boc-protected aldehyde **147** in methanol under addition of sodium cyanoborohydride. In this reductive amination reaction, the formed imine from the condensation of the aldehyde **147** and the amine **123** is reduced *in-situ* by sodium cyanoborohydride yielding the amine **145** in a yield of 65 % (Fig. 96). Comparable yields were achieved in similar reactions described in the literature.

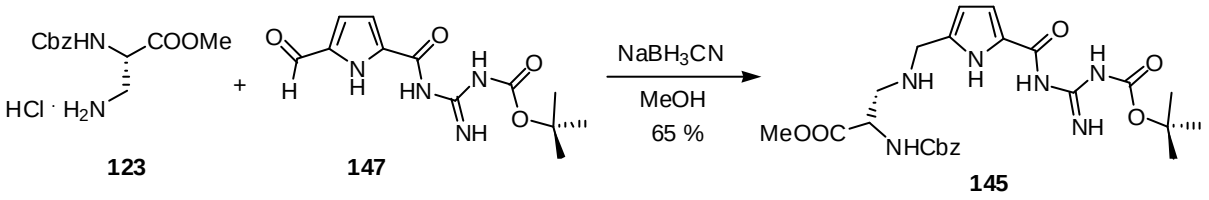


Fig. 96. *Reductive amination of the aldehyde **147** with the amino acid **123** by addition of cyanoborohydride.*

The di-cationic receptor is subsequently prepared by cleavage of the ^{t}Boc-group with TFA and precipitation of the picrate salt in methanol in quantitative yield.

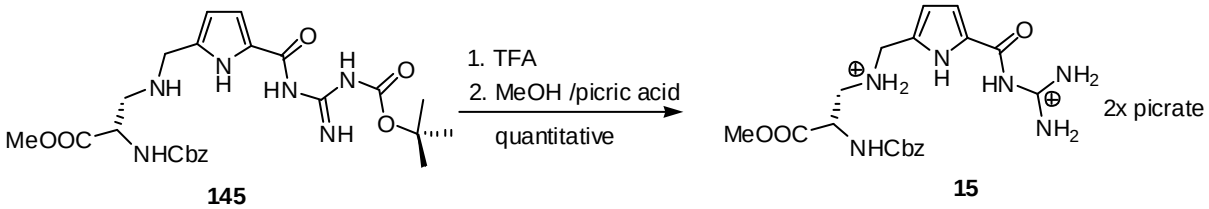
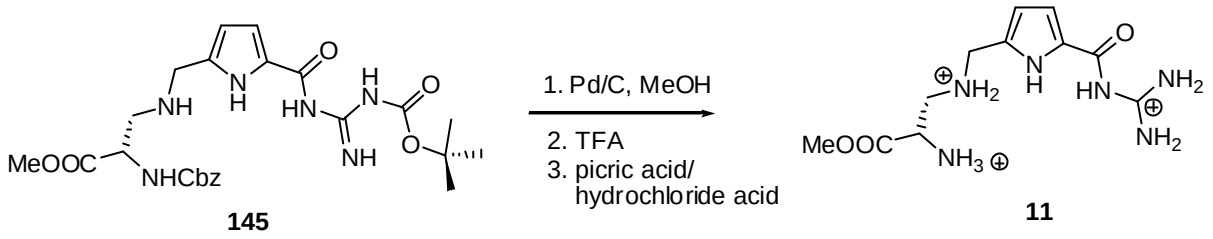


Fig. 97. *Synthesis of the picrate salt of the di-cationic receptor **15**.*

The tris-cationic receptor **11** was synthesized on the nearly the same route, except that the Cbz-protection group was cleaved off by hydrogenation prior to the cleavage of the ^{t}Boc-group. The tris-cationic receptor **11** was thus obtained in quantitative yield (Fig 96).



145 **11**

Fig. 98. *Synthesis of the tris-cationic receptor **11** by hydrogenolysis of Cbz-group and subsequent deprotection with TFA.*

The binding studies of these two, newly developed receptor systems will be discussed in the following section.

4.4.2 Binding studies

For the determination of the association constants of receptor-ligand interactions there are several methods known. Frequently used physical-organic methods are the UV- and fluorescence-spectroscopy or especially the NMR-spectroscopy.[53, 147, 148] A major advantage of the NMR method over other optical techniques is that the results are not greatly affected by the presence of minor impurities and valuable structural information can be obtained.

The determination of association constants by NMR-titration experiments is based on the chemical shift changes between free and bound state of the ligand caused by differences in the chemical surrounding of individual atoms. This can be caused by interaction through hydrogen bonds or other weak noncovalent interactions in the complex. The difference $\Delta\delta$ between the chemical shift in the complex and in the free molecule δ_{free} is called *complexation-induced shift* (CIS). For simplification herein only a 1:1 complex formation equilibrium between the receptor R, the substrate S and the heterodimeric complex RS will be concerned:

$$R + S \rightleftharpoons RS$$

The equilibrium constant for the association K_{ass} or the dissociation K_{diss} results from the law of mass action (**Eq. 6**). The activity coefficients are generally unknown and are therefore disregarded in order to simplify the discussion.

$$K_{ass} = \frac{[RS]}{[R]\cdot[S]} = \frac{1}{K_{diss}} \qquad \textbf{Eq. 6}$$

The equilibrium constants of receptor and substrate can be expressed by the known initial concentration $[R_0]$ and $[S_0]$.

$$[R] = [R_0] - [RS] \qquad or \qquad [S] = [S_0] - [RS] \qquad \textbf{Eq. 7}$$

Due to the fact that association processes are dynamic equilibria, the exchange rate of the free compounds R, S and the complex RS are critical for the analysis. If the receptor-ligand complexation equilibrium has a very slow exchange rate compared to the NMR time scale, there will be both peaks for the free compounds and also one for the complex. The determination of the complex constant is achieved through a simple integration of the signals.
If the receptor-ligand complexation has a very fast exchange compared to the NMR time scale, a single coalescence absorption signal will be detected. The observed shift δ_{obs} can be expressed with:

$$d_{obs} = \frac{[R_0] - [RS]}{[R_0]} \cdot d_R + \frac{[RS]}{[R_0]} \cdot d_{RS} \qquad or \qquad d_{obs} = d_R + \frac{[RS]}{[R_0]} \cdot \Delta d$$

$$with \qquad \Delta d = d_{RS} - d_R \qquad \textbf{Eq. 8}$$

Thus, the NMR observable δ_{obs} is a function of the initial receptor concentration $[R_0]$, the chemical shift of the free receptor δ_R, the chemical shift of the complex δ_{RS} and of the equilibrium concentration of the complex [RS].
Combining the equations for the dissociation constants (**Eq. 6**) and the equilibrium concentration (**Eq. 7**) and afterwards solving the resulting quadratic equation gives an equation for the complex concentration:

$$[RS] = \frac{1}{2} \cdot \left\{ K_{diss} + [R_0] + [S_0] - \sqrt{(K_{diss} + [R_0] + [S_0])^2 - 4 \cdot [R_0] \cdot [S_0]} \right\} \qquad \textbf{Eq. 9}$$

Substitution of the complex concentration [RS] in the equation (**Eq. 8**) through (**Eq. 9**) gives an equation, in which the observed complexation shift δ_{obs} is expressed as a function of the known initial concentration of the receptor $[R_0]$, the substrate $[S_0]$, the chemical shift of the free receptor δ_R, the chemical shift of the complex δ_{RS} and of the dissociation constants K_{diss} which is the wanted parameter:

$$d_{obs} = d_R + \frac{\Delta d}{2 \cdot [R_0]} \cdot \left\{ K_{diss} + [R_0] + [S_0] - \sqrt{(K_{diss} + [R_0] + [S_0])^2 - 4 \cdot [R_0] \cdot [S_0]} \right\} \qquad \textbf{Eq. 10}$$

Equation **10** describes a hypersurface, which is determined by the parameters $[R_0]$, $[S_0]$ and δ_{obs}. For simplification one parameter in an NMR experiment is normally held constant, for instance $[R_0]$ and the other component is added. The resulting titration curve is therefore a two-dimensional cut through this hypersurface. For the analysis the equation (**Eq. 10**) is iteratively fitted by a nonlinear regression to the experimentally obtained NMR data. Whereas, the initial concentrations $[R_0]$, $[S_0]$ and the observed chemical shift δ_{obs} are known, the dissociation constants K_{diss} results from the curve fitting.

The real strength of a receptor-ligand interaction is not given by the absolute values of the observed chemical shift changes, it is determined by the curvature of the titration graph. However, for experimental reason in the majority of cases atoms are observed which possess a large chemical shift, because this results in a reduced error margin of the calculated parameters.

For weak complexes *Pearson and Deranleau* could show, that the “best” data for the association constant could be obtained by analysing a certain range of the titration curve. This range of the titration curve is called “probability of binding” and is defined as the ratio of concentration of complex to maximum possible concentration of complex. The minimum error in the measurement of K_{ass} occurs at $p = 0.5$, and the best data are obtained from the range $0.2 \leq p \leq 0.8$. In other words, K_{ass} is best defined by titration data that curve measurably and approach a limiting shift. The problem with measuring small K_{ass} is that there is a large

error associated with the extrapolation to $\Delta\delta_{max}$. The problem with measuring large K_{ass} ($\geq 10^5$-10^6 M^{-1}) is that there is no curvature in the titration graph. In order to observe curvature in the titration plot the solution would need to be diluted by several orders of magnitude (µmol range). However, NMR is a time intensive technique and thus experiments are routinely performed in the mmol range.

It is obvious, that before equation (**Eq. 10**) can be applied for the analysis of experimental NMR data, the stoichiometry of the formed complex must be known. There are today different methods to determine the stoichiometry of such complexes. In most cases the Continuous Variation Method, also called "JOB-plot" is used. The experimental procedure consists of preparing a series of solutions of receptor and substrate under the condition that the sum of the total receptor and ligand concentration is constant. The experimentally observed parameter is sensitive to the receptor/substrate complex formation and an extremum in the absorbance changes or chemical shift change in the case of an NMR experiment is related to the molar ratio of receptor to substrate. Some other approaches to get further "indirect" information of the complex structures in solution are mass spectrometry or x-ray structure analysis.

The discussion until now describes only the association of two different molecules to a heterodimeric complex (receptor-substrate complex) in general. For the special case of a homodimer 1:1 interaction, i.e. the self-association of molecules the variables $[R_0]$ and $[S_0]$ have to be substituted with the variable $[C_0]$ (i.e. $[R_0] = [S_0] = [C_0]$). $[C_0]$ is the total concentration of the self-complementary compound C. The equation for the NMR titration (**Eq. 10**) changes in the case of a self-association process to (see section 4.2):

$$d_{beo} = d_C + \frac{\Delta d}{4 \cdot [C_0] \cdot K_{\dim}} \cdot \left\{ 1 + 4 \cdot [C_0] \cdot K_{\dim} - \sqrt{1 + 8 \cdot [C_0] \cdot K_{\dim}} \right\}$$

Eq. 1

$$mit \quad \Delta d = d_{\dim} - d_C \qquad und \qquad K_{\dim} = \frac{1}{K_{diss}}$$

Experimentally, the measurement of a self-association constant is not a titration experiment, it is rather a dilution study. Hence, the dependency of the chemical shift from the substrate concentration $[C_0]$ is observed during dilution. The degree of dimerization is determined by the equilibria constant K_{dim} and the substrate concentration which changes during the dilution experiment.

The equations above derived for the NMR-titration were used to determine the binding constants. Due to the fact that the binding constants for the original guanidiniocarbonyl pyrrole binding motif had been determined by NMR-titration experiments in 40 % water/60 % DMSO,[32] I used the same conditions for my newly developed binding motifs **15, 11**. Therefore, I am able to compare my results with the previously obtained binding data. At first, I performed a NMR titration with the picrate salt of the di-cationic receptor **15** in 40 % water in order to study the influence of an additional implemented positive charge on the other side of the guanidiniocarbonyl pyrrole moiety. As anticipated, addition of **15** to a solution of N-acetyl-*L*-alanine carboxylate **29** in 40 % water/60 % DMSO caused significant complexation induced shifts of the various protons of **29** in the ^{1}H NMR spectrum (Fig. 99).

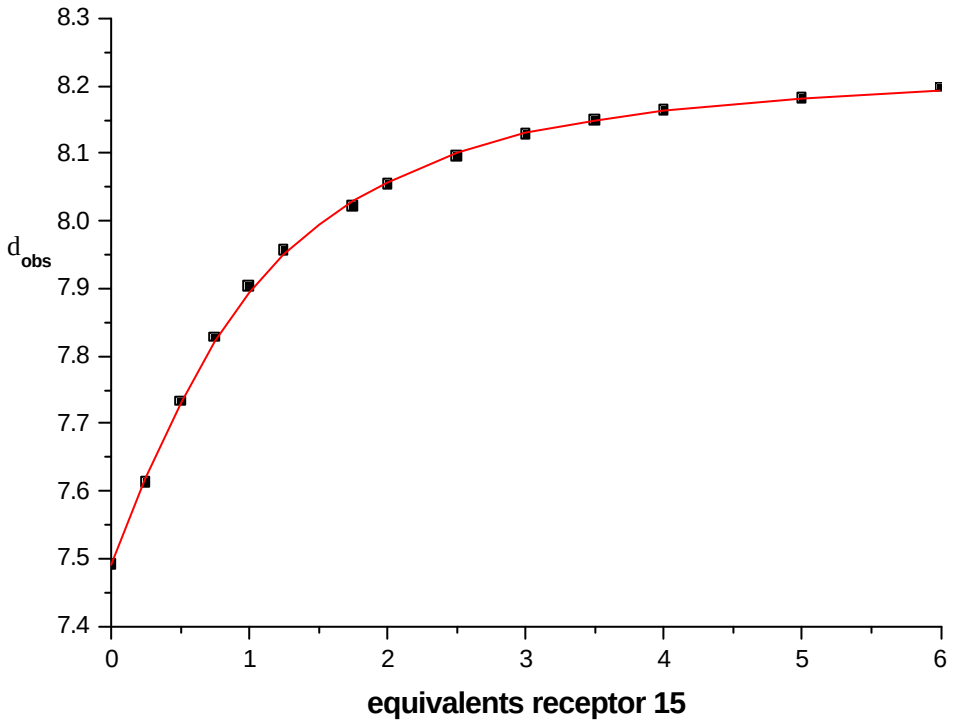


Fig. 99. *NMR titration curve of N-acetyl-L-alanine **29** with the di-cationic receptor **15** in 40 % water/60 % DMSO.*

The binding constant was calculated from the observed shift changes of the amide-NH of alanine **29** using nonlinear least squares fitting with a 1:1 association model. The di-cationic receptor **15** binds alanine **29** with K_{ass} = 1642 mol^{-1}. The similar glycine substituted receptor **27** developed by *Schmuck* possesses a binding constant of K_{ass} = 680.[32] In conclusion, the ionic interaction within this newly developed receptor **15** led to a stronger binding compared to previous receptors like **27**. The binding constant is larger by a factor of two. Apparently,

the loss of the amide-carboxylate hydrogen bond is counterbalanced by the additional ionic interactions at this position.

To compare the association constant of the tris-cationic receptor **11** with these results, a NMR titration of receptor **11** against alanine carboxylate **29** was performed under the same conditions (40 % water in DMSO) (Fig. 100). However, the titration graph differs entirely from the titration curve obtained for **15**. There is only a linear increase in the chemical shift change until a molar ratio of 1:1 is reached (Fig. 100), clearly indicating the 1:1 stoichiometry of the binding interaction. Since the titration graph possesses no measurable curvature a binding constant can only be estimated to be $K \geq 10^6$ mol^{-1} (Fig. 100).

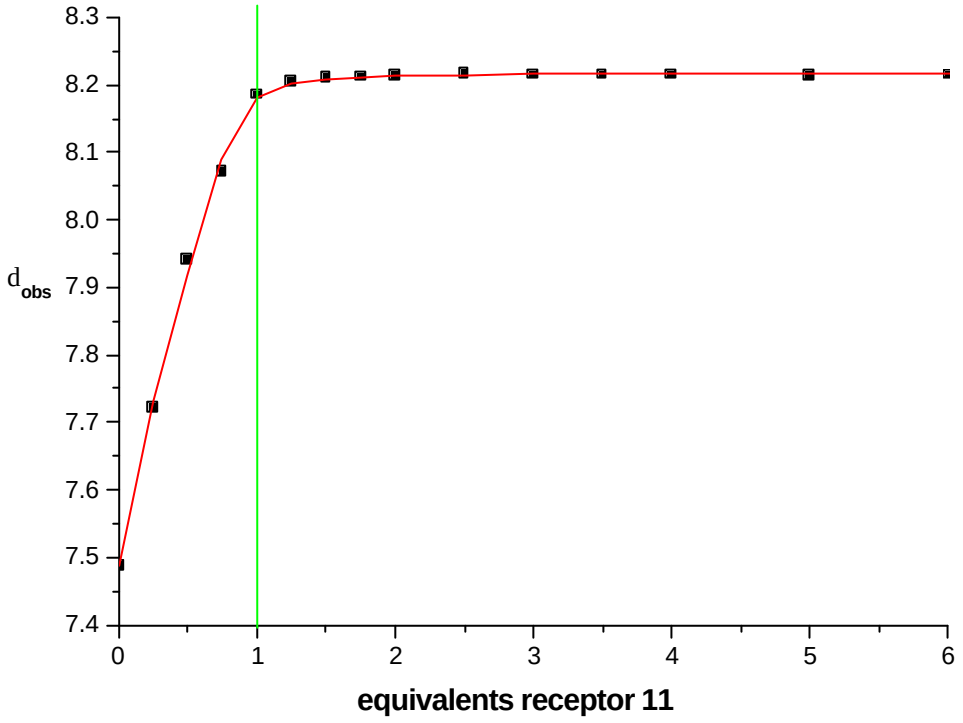


Fig. 100. *NMR titration curve of N-acetyl-L-alanine **29** with the tris-cationic receptor **11** in 40 % water/60 % DMSO.*

According to the data obtained the tris-cationic receptor **11** binds alanine carboxylate **29** tremendously stronger than the di-cationic receptor **15**. The binding constant differs by a factor of approximately 10^2. Based on these findings, I also performed NMR titrations with the dicarboxylates of the amino acid aspartic acid and glutamic acid in 40 % water. Since the tris-cationic receptor possess two potential binding sites, the guanidiniocarbonyl pyrrole and the terminal ammonium group, the resulting complexes could have in principal 1:1 and also

1:2 stoichiometry. First of all, I made a NMR titration with glutamic acid in 40 % water (Fig. 101).

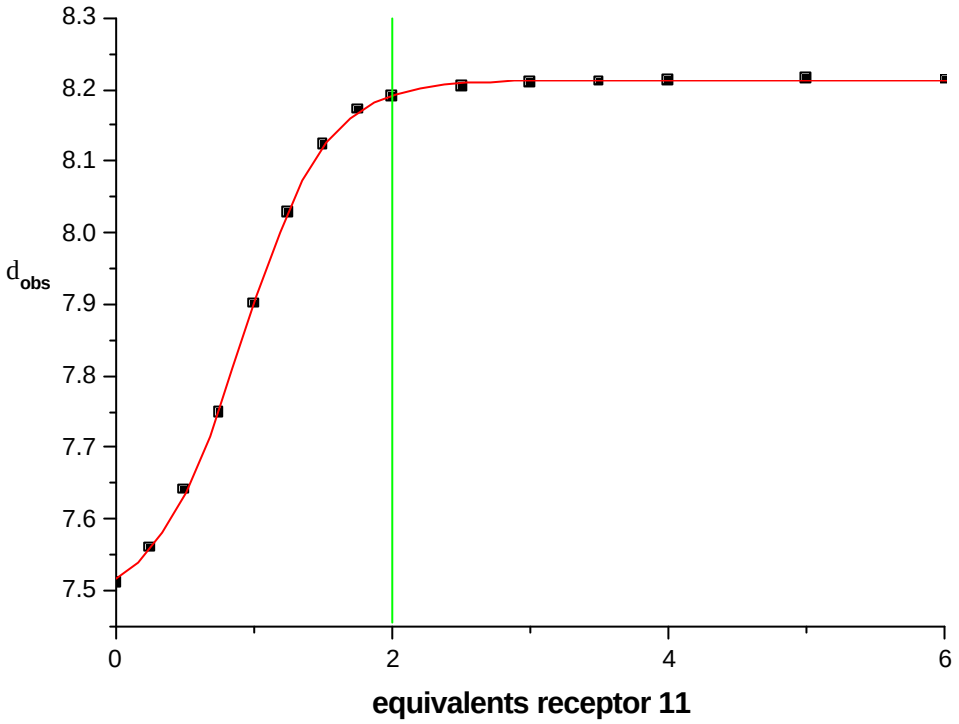


Fig. 101. *NMR titration curve of glutamic acid (NMe_4-salt)* ***150*** *with the tris-cationic receptor* ***11*** *in 40 % water/60 % DMSO.*

The obtained NMR titration curve shows a sigmoid binding curve which is a sign for a cooperative effect. There is also a nearly linear increase in the chemical shift change, but in contrast to alanine the maximum is reached at a 1:2 molar ratio of substrate to receptor. Therefore, this titration graph clearly proves that two receptors **11** bind one glutamic acid molecule **150**. The binding constant is again too strong to be calculated from these data.

In contrast to this, the titration curve in the case of aspartic acid **151** with the tris-cationic receptor **11** indicated no distinct stoichiometry. The maximum was not achieved at a molar ratio of neither 1:1 nor 1:2. This could be either a result of experimental mistakes in the preparation of the samples or it could result from the simultaneous existence of 1:1 and 1:2 complexes during the titration. Therefore, the exact stoichiometry has to be determined in further experiments, like for example a JOB-plot, to be able to calculate the binding constants from the obtained titration data.

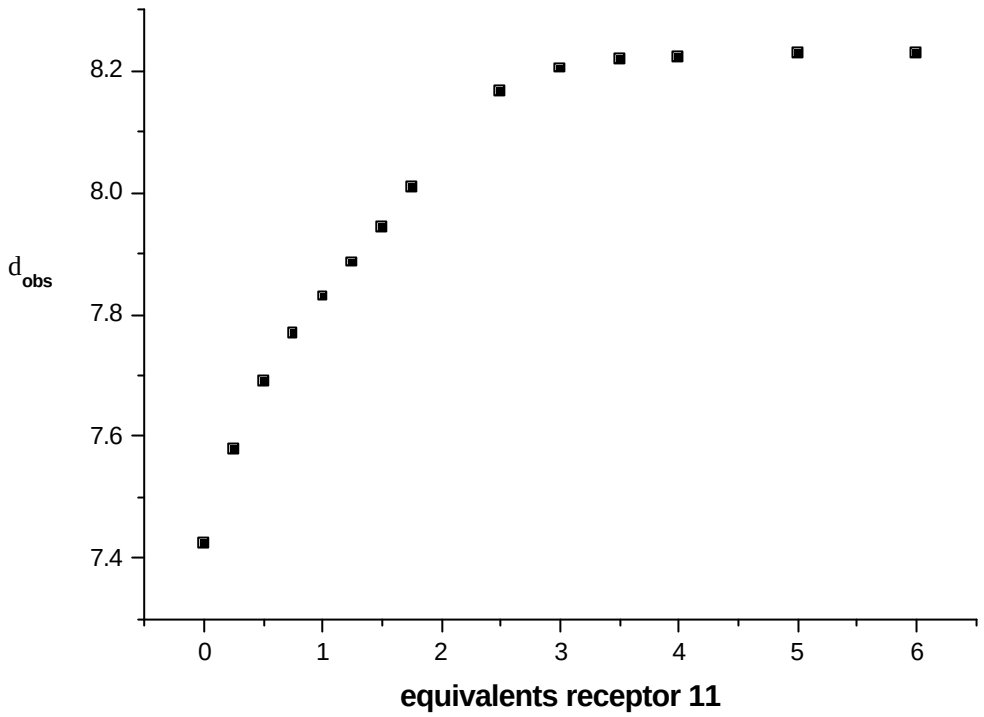


Fig. 102. *NMR titration curve of aspartic acid (NMe_4-salt)* ***151*** *with the tris-cationic receptor* ***11*** *in 40 % water/60 % DMSO.*

Since even in 40 % water in DMSO the association constants are too strong ($K \geq 10^{-6}$ mol^{-1}) to be exactly determined, a more competitive solvent (i.e. more water) was used in further experiments. Due to the low solubility of the tris-picrate of the receptor **11** in solvents with higher water contents, the tris-chloride salt of the receptor **11** was used for the following binding studies. The tris-chloride receptor **11** was therefore dissolved in 90 % water by addition of 10 % DMSO for solubility reasons. The titration curve of alanine **29** with the tris-chloride salt of receptor **11** is shown in Fig. 103. The binding constant was calculated using a 1:1 association model to K_{ass} = 1988 M^{-1} in 90 % water. In comparison to the di-cationic receptor **15** with an association constant of K_{ass} = 1642 M^{-1} in 40 % water in DMSO this is a tremendous increase in the binding constant.

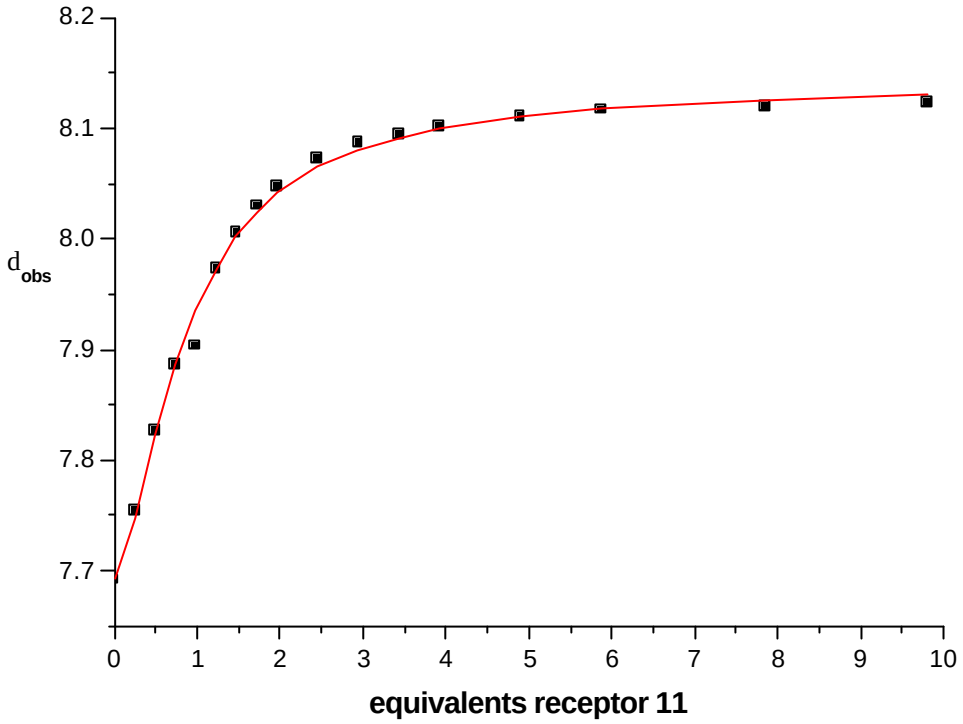


Fig. 103. *NMR titration curve of N-acetyl-L-alanine **29** with the tris-cationic receptor **11** in 90 % water/10 % DMSO.*

The titration curve for of glutamic acid **150** with the tris-chloride salt of receptor **11** in 90 % water is shown in Fig. 104. The sigmoidic binding curve results from the cooperative 2:1 binding process which prevented the calculation of a binding constant in this case.

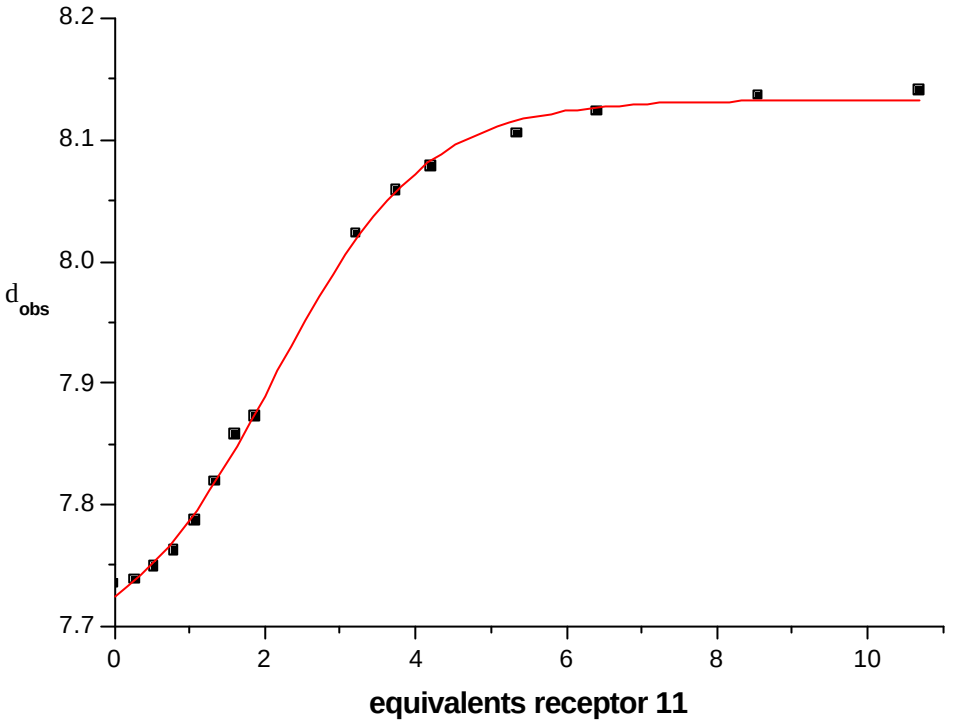


Fig. 104. *NMR titration curve of glutamic acid **150** with the tris-cationic receptor11 in 90 % water/10 % DMSO.*

Before any determination of the association constant could be performed in the case of the complexation of aspartic acid **151** by the tris-chloride of receptor **11**, it is essential to determine the exact stoichiometry of the complex. This is most readily achieved by means of the method of continuous variations (JOB-plot). Thus, I measured a JOB plot for the determination of the aspartic acid/receptor **11** complexation which is shown in Fig. 105.

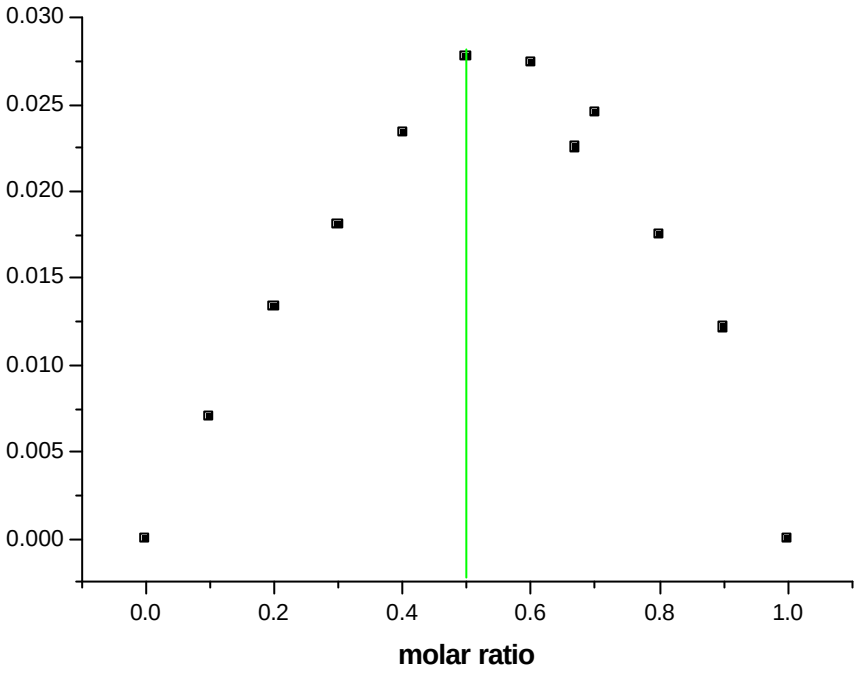


Fig. 105. *JOB-plot of aspartic acid **151** with the tris-cationic receptor **11** in 90 % water/10 % DMSO.*

The x-coordinate at the maximum in the curve of the JOB-plot of the receptor **11** with aspartic acid **151** is 0.5. This proves that the stoichiometry of the complex is 1:1 under those conditions and thus the 1:1 association model was used to calculate the binding constant.

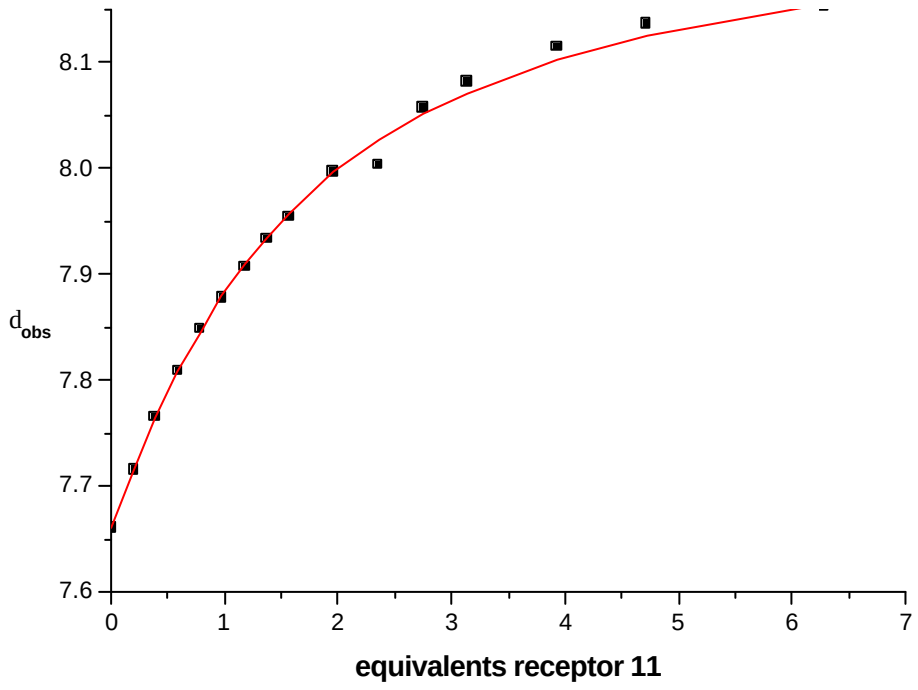


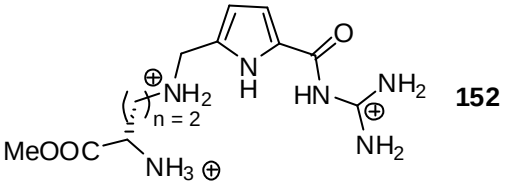
Fig. 106. *NMR titration curve of aspartic acid **151** with the tris-cationic receptor **11** in 90 % water/10 % DMSO.*

This proves that the stoichiometry of the complex is 1:1 under those conditions and thus the 1:1 association model was used to calculate the binding constant. The binding constant is about five times smaller (K = 388 M^{-1}) than for alanine. According to molecular modelling studies, one carboxylate is bound by our guanidiniocarbonyl pyrrole, while the other carboxylate is complexed by the ammonium group. Possibly due to entropic reasons (solvation) is the 2:1 complex less stabile than the 1:1 complex.

In conclusion, I have demonstrated that by the additional ionic interaction in the receptor **15** the association constant was improved by a factor of two compared to similar receptors developed by *Schmuck*. In contrast to **15** the tris-cationic receptor **11** possess in 40 % water in DMSO binding constants in the range of K $\approx 10^6$ M^{-1}. In nearly pure water (10 % DMSO), the association constant for the complexation of alanine is still 1988 M^{-1}. This shows that this new binding motif is the best amino acid receptor known in our group so far.

Outlook:

In the future this very interesting new binding motif for amino acids can be evaluated in more detail. Therefore, NMR titration studies against a variety of different amino acid substrates should be investigated to get more information about the exact binding conformation. In addition, molecular modelling studies may underline the experimental data. Also a new tris-cationic receptor **152** can be developed with a spacer n = 2. This receptor might be preorganized to complex glutamic acid with a 1:1 stoichiometry.



152

Fig. 107. *Design of a tris-cationic receptor **152** that possibly binds glutamic acid in a 1:1 stoichiometry.*

4.5 *De-novo* designed dipeptide receptor

The receptor **12** was *de-novo* designed based on theoretical calculations (Macromodel 8.0, Amber*, water solvation)[49] to bind dipeptides with a free carboxylate. The receptor possesses several functional groups which should enable him to strongly complex C-terminal dipeptides in a β-sheet conformation:[149]

- The guanidiniocarbonyl pyrrole moiety as a complementary binding site for the free carboxylate.
- The aromatic rigid spacer, which should enable a binding of larger substrates like dipeptides.
- Additional hydrogen bonds between the dipeptide backbone and the receptor (keto-function, amide and imidazolium NH) further stabilize the complex.

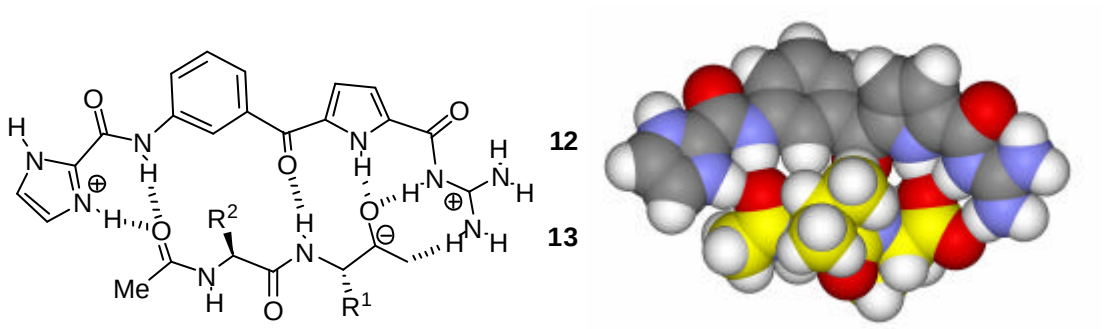
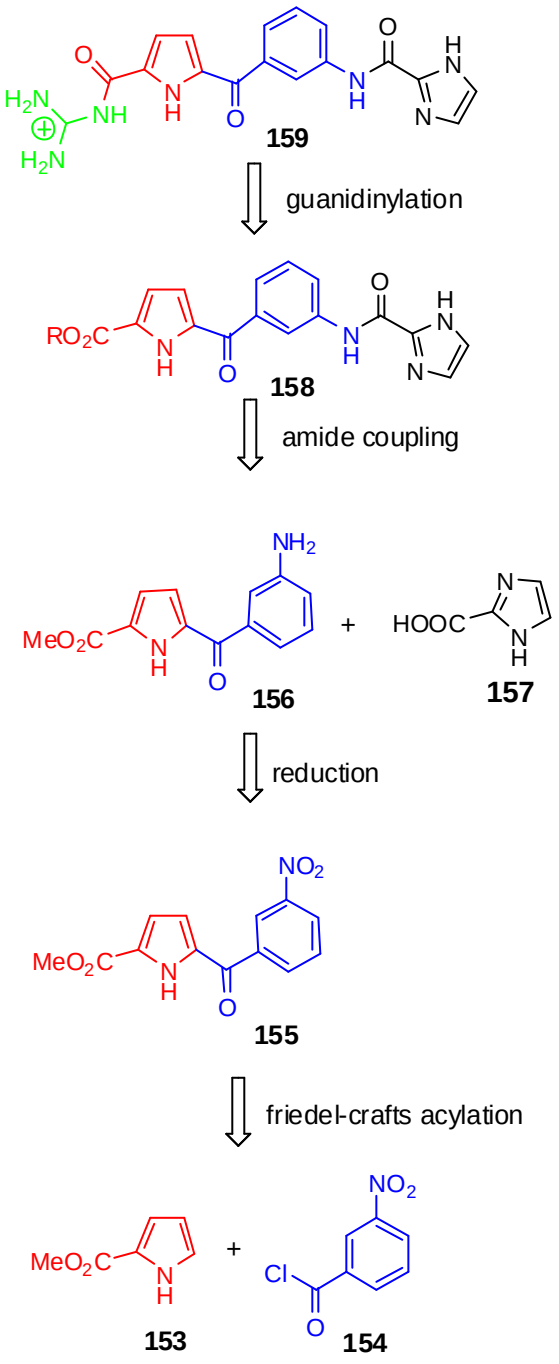


Fig. 108. *Complex of the de-novo designed dipeptide receptor* ***12*** *with an acylated dipeptide* ***13*** *(left) and calculated complex structure (right).*

4.5.1 Synthesis

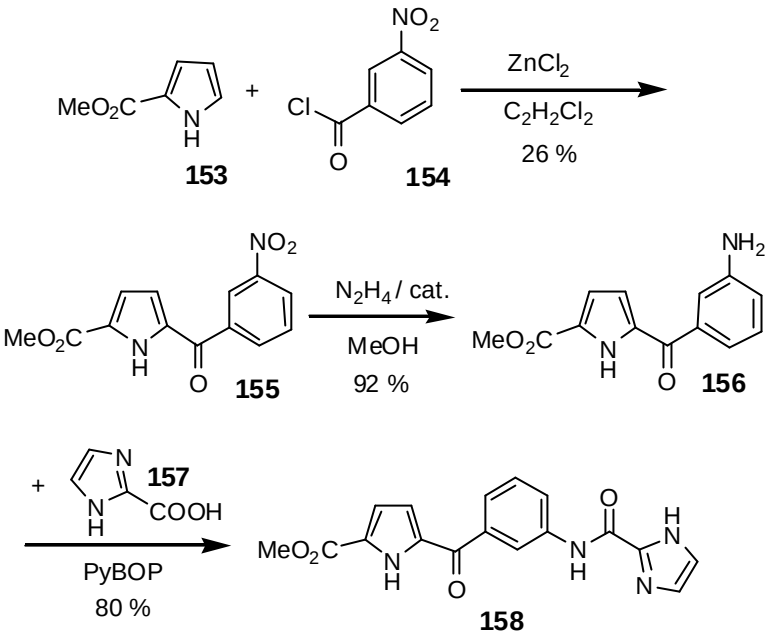
The retrosynthetic approach is outlined in Scheme 10.



Scheme 10. *Retrosynthetic scheme of the receptor **12**.*

The first three reactions steps of this project had been already successfully realized during my diploma thesis.[150] A Friedel-Crafts-acylation of 2-pyrrole methyl carboxylate **153** with *meta* nitro benzoyl chloride **154** using $ZnCl_2$ as the catalyst under kinetic control provides the 2,5-

disubstitued pyrrole **155** in 26 % yield besides 43 % of the 2,4-regioisomer.[151] The nitro group in **155** was reduced with hydrazine and Raney-nickel to give amine **156** in 92 % yield,[152] which was then reacted with imidazole 2-carboxylic acid **157**[153] to provide the corresponding amide **158** (80 % yield) (Scheme 11).



Scheme 11. *Synthesis of pyrrole compound **158**.*

However, the final reaction step, the introduction of the guanidinium functionality in **158** failed. At that time only the guanidinylation reaction with an excess of guanidinium chloride in refluxing sodium methoxide was established in our group (see discussion in section X).

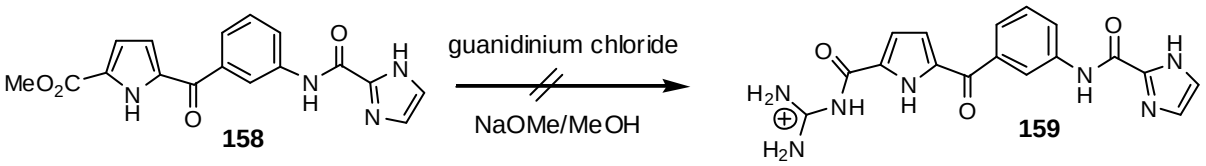


Fig. 109. *Guanidinylation of the methylester **109** with guanidinium chloride in sodium methoxide.*

In this case these guanidinylation conditions led to a destruction of the methylester compound **158**. After HPLC chromatographic purification of the reaction mixture only fragments of the starting materials and product could be isolated. Thereafter, I developed the method to generate acyl guanidines via nucleophilic reactions of pyrrole carboxylates with ^{t}Boc-protected guanidines (discussed in section 4.1). With this method at hand, I was finally able to complete the synthesis of the dipeptide receptor **12**. Cleavage of the methylester **158** (LiOH,

97 %) and subsequent reaction of acid **160** with mono ^{t}Boc protected guanidine **83**[112] using PyBOP in DMF as the coupling reagent (87 %) afforded the protected receptor **161**. Deprotection with trifluoroacetic acid and precipitation with picric acid finally gave the title compound **9** isolated as the mono-picrate salt.

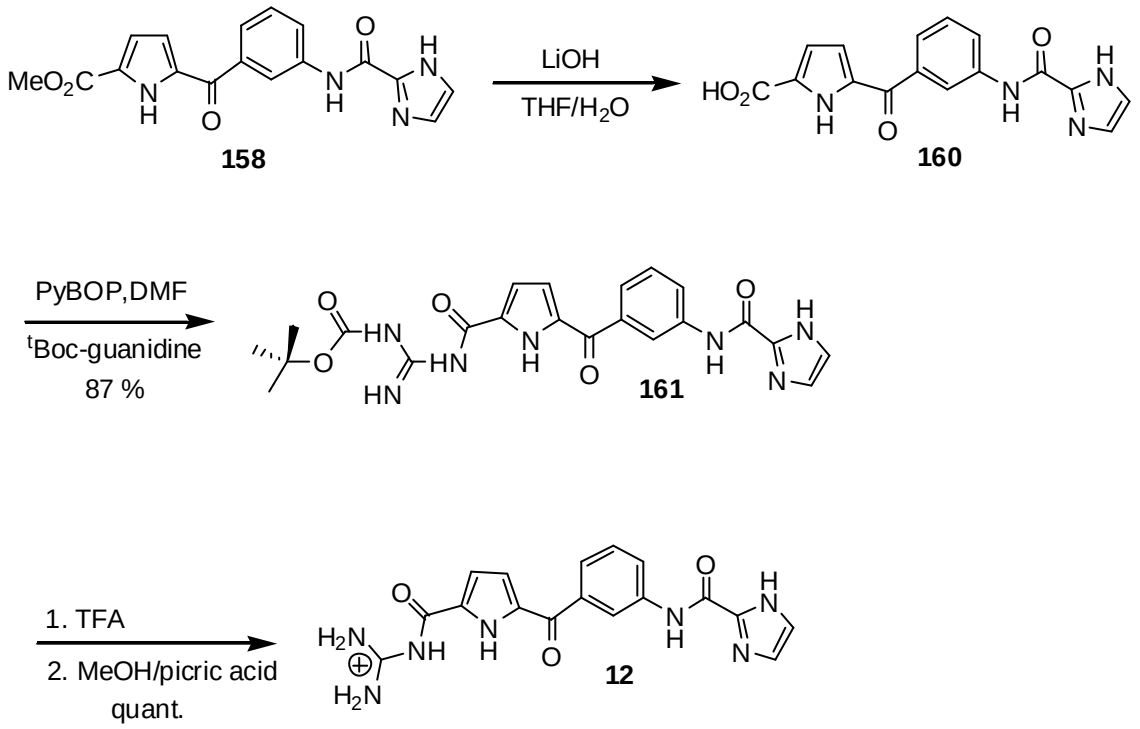


Fig. 110. *Synthesis of the dipeptide receptor **12** from the methylester compound **158**.*

4.5.2 Results and binding studies

A first unexpected result was that the deprotection of the corresponding ^{t}Boc protected guanidine as either the picrate or the chloride salt only yielded to the mono-cationic species.

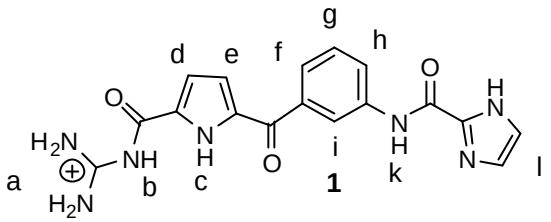


Fig. 111. *Mono cationic receptor compound **12**.*

The ^{1}H NMR spectrum only showed protonation of the guanidinium group which can be clearly seen by the characteristic signal around δ = 8.3 for the four NH_2 protons (Fig. 112).

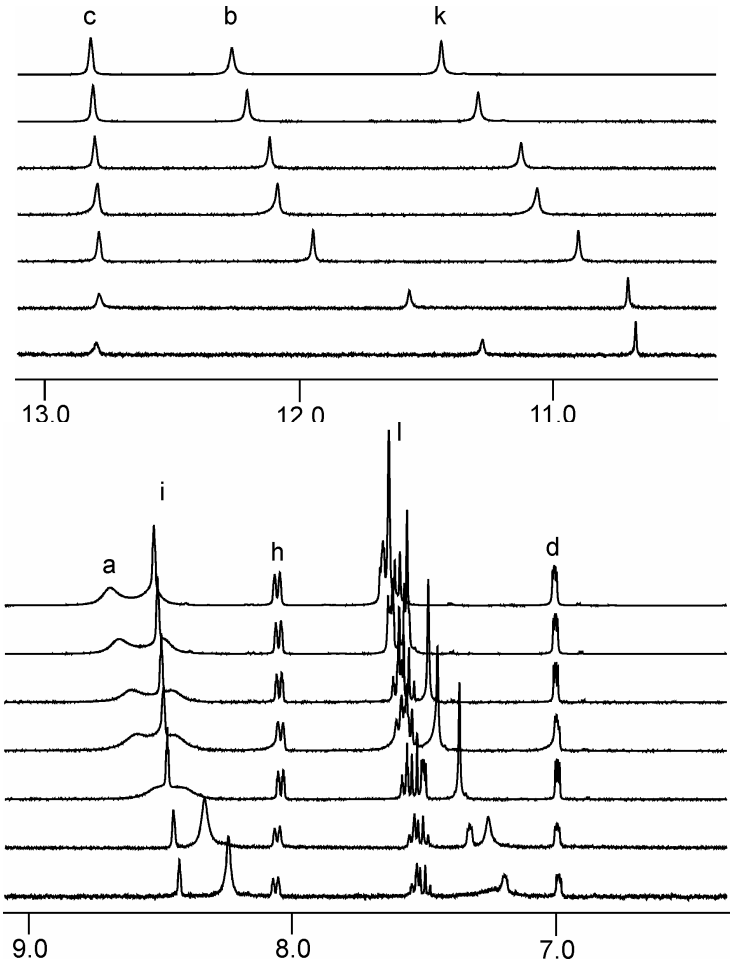


Fig. 112. *Parts of the ^{1}H NMR spectrum (300 MHz, 303 K) of **12** in [D6]DMSO showing the dimerization induced shift changes (concentrations from bottom to top: 1, 5, 10, 12.5, 15, 25, 50 mM).*

Furthermore, a closer look at the NMR spectrum of the chloride salt revealed that the mono cation self-aggregates: the NMR shifts are concentration dependent (Fig. 112, chloride salt).[154] The ^{1}H NMR spectrum of **12** at submillimolar concentrations in [D6]DMSO (0.1 mM at 300 K) is consistent with a non interacting species: The signal at δ = 8.25 for the four guanidinium NH_2 protons, a signal for the guanidinium amide NH at δ = 10.75 and for the imidazolium amide NH at δ = 10.71 and a singlet at δ = 12.86 for the pyrrole NH. However, these shifts are concentration dependent. In a 50 mM solution in [D6]DMSO the signal for the guanidinium NH_2 protons has split into two signals and shifted to δ = 8.7 and 8.5. The signal for the imidazolium amide NH hast shifted to δ = 11.4, and the signal for the guanidinium amide NH to δ = 12.3, respectively. These concentration dependant shift changes are indicative of an *intermolecular* interaction that is more pronounced in more concentrated solutions. As the addition of NaCl to a diluted solution of **12** (0.5 mM) does not cause similar shift changes, interactions of the cation with the chloride anion can not be the reason. Hence, self-association of the cation **12** by itself must be the reason.

To determine the binding constant for the self-association of cation **12** quantitatively, I studied the concentration dependence of the ^{1}H NMR spectrum of **12** in the concentration range from 1 to 50 mM. A plot of the observed chemical shift versus concentration gives an isothermic binding curve (Figure 113).

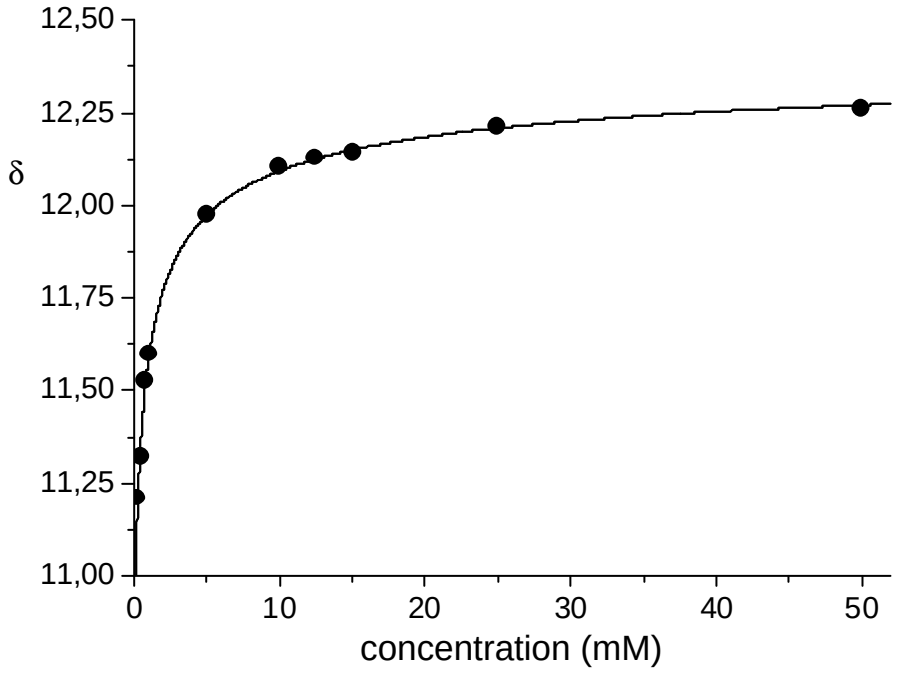


Fig. 113. *Binding isotherms for the guanidinium amide NH of 12. The solid line shows the curve fitting for a 1:1-dimerization.*

The association constant for the dimerization of cation **12** is calculated to be K_{dim} = 1080 M^{-1} at 298 K using both the shift changes of the polar guanidinium amide NH or the guanidinium NH_2 as well as the benzene CHs (see equation in chapter 4.2.2).[53] The value of 1080 M^{-1} for the dimerization of **12** is surprisingly high for a cationic species in such a highly polar solvent as DMSO and suggests that the binding within the dimer is probably not just only due to hydrogen bonding. The dimer is most likely further stabilized by additional π-stacking interactions between the aromatic rings. To gain further insight into the binding interactions, we performed molecular modelling calculations. A Monte Carlo simulation (Macromodel 8.0,[5] Amber*, water solvation)[49] revealed that the dimer is held together by a combination of hydrogen bonds and π-stacking interactions (Fig. 114).

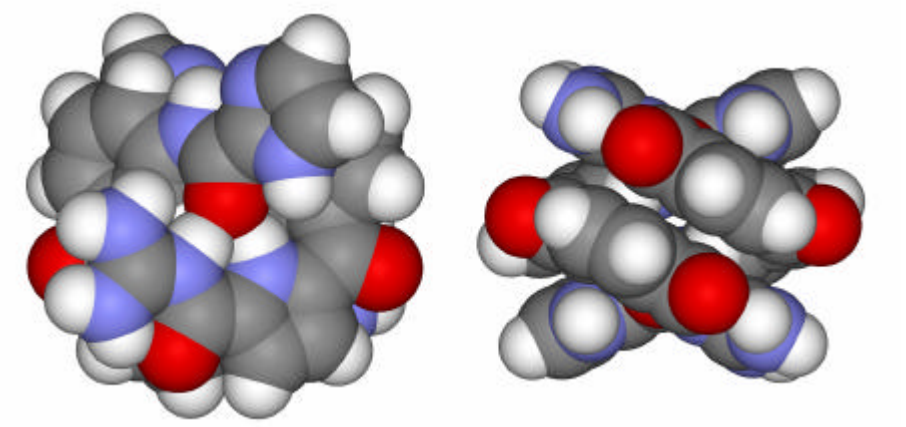


Fig. 114. *Calculated structure of the dimer.*

In contrast to the situation found in DMSO, in more polar media this self-association process is suppressed. For example at the concentration of below 2 x 10^{-5} M^{-1} in 90 % water in DMSO which were applied in the following UV titration experiments the self-association is not relevant anymore. This could be proved by a UV dilution experiment, which showed a linear decrease according to the Lambert-Beer law.

To find out whether the guanidiniocarbonyl pyrrole **12** is an efficient receptor for the complexation of C-terminal dipeptides, I performed at first NMR titration experiments in 40 % water in DMSO. These conditions were chosen, because the simple guanidiniocarbonyl pyrrole receptors by *Schmuck* bind amino acids with association constants of approximate 10^2-10^3 M^{-1} under these conditions.

First hints that **12** is indeed capable to bind dipeptides came from ESI MS experiments which show a distinct signal for a 1:1 complex between **12** and Ac-Ala-Ala-OH (**162**) (DMSO/MeOH solution) (Fig. 115).

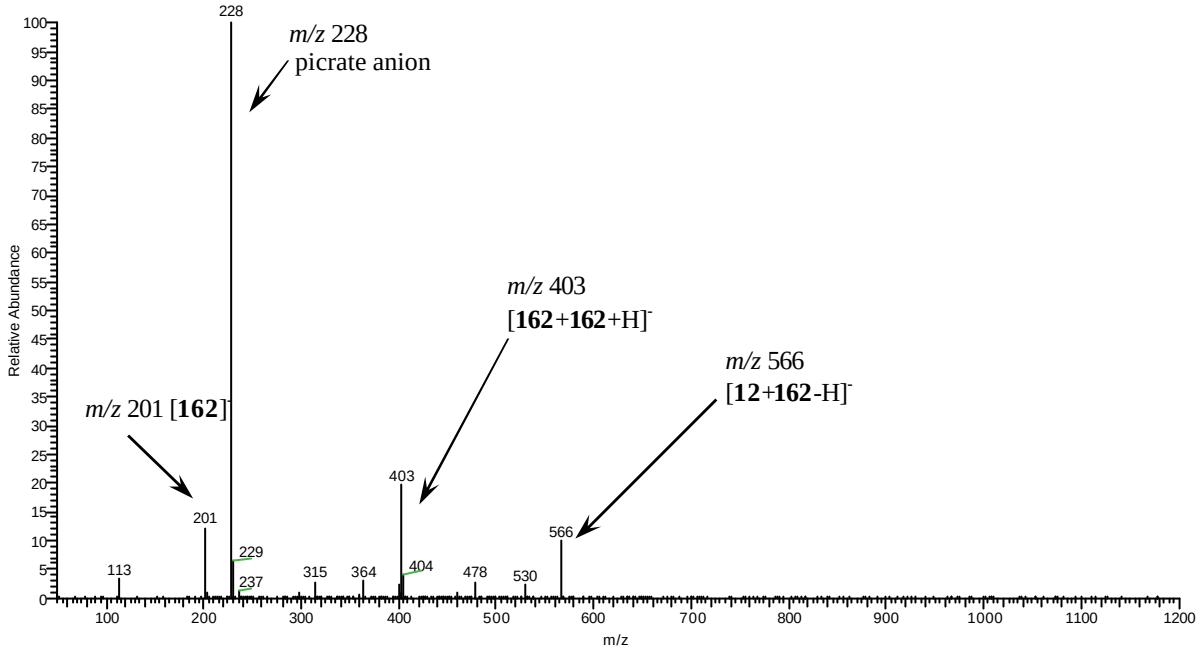


Fig. 115. *Positive ESI MS experiment with the picrate of the receptor **12** and Ac-Ala-Ala-OH **162**.*

Therefore, I probed the complexation properties of **12** in solution by NMR titration experiments. Upon the addition of **162** (NMe_4^+-salt) to **12** (1 mM, picrate salt) in 40 % water in DMSO, significant complexation induced shift changes can be observed for both the receptor and the dipeptide (Figure 116). For example, the amide NH next to the carboxylate in 10 exhibits a significant downfield shift with increasing equivalents of **12** added, whereas the N-terminal amide NH shows an upfield shift (Figure 116).

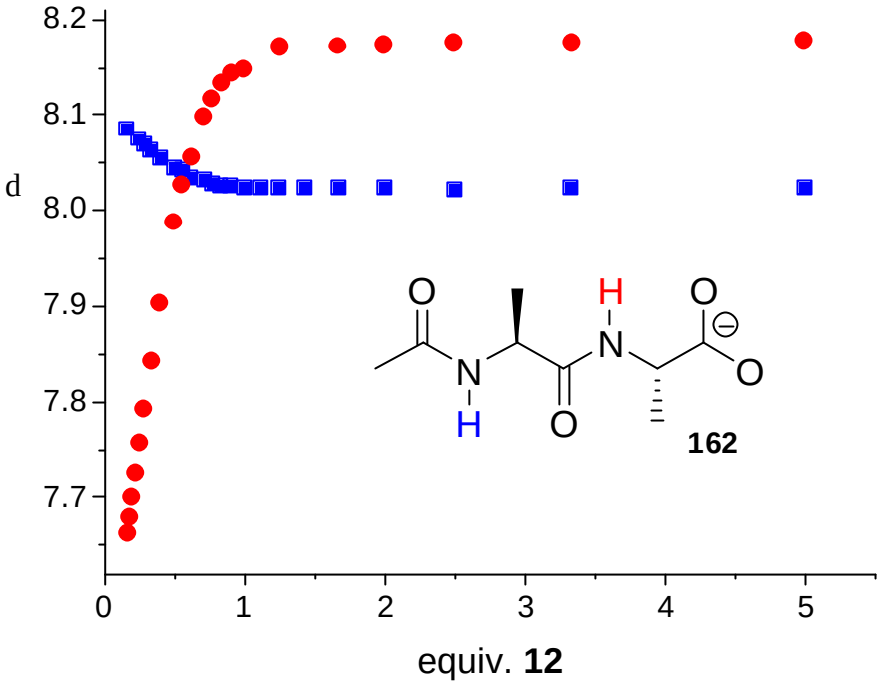


Fig. 116. *NMR complexation induced shift changes of the amide NHs of **162** in the presence of **12** (40 % H_2O in DMSO-d6).*

This is in total agreement with the suggested binding mode depicted in Fig. 108. Corresponding shift changes are also observed for receptor **12** (e.g. for the imidazolium CHs). The linearity of the shift changes not only proves the 1:1-complex stoichiometry but also shows that even in aqueous DMSO complex formation is too strong to measure by NMR. The association constant for the binding of 10 is therefore estimated to be $K_{ass} > 10^6$ M^{-1} in 40 % water in DMSO. To determine the binding constant in further NMR experiments a more competitive solvent mixture has to be used (e.g. more water). Due to the fact that the receptor **12** was not soluble in a mmolar concentration in solvent mixtures containing more water, a quantitative measurement of the association constant by NMR titration experiments was not possible. To experimentally determine the association constant nevertheless, a method must be used which is routinely carried out under more diluted conditions, so that the receptor X can be dissolved in a more competitive solvent. Optical methods like UV or fluorescence spectroscopy are herefore perfectly suited, because they are performed in a concentration range between 10^{-5} to 10^{-4} M^{-1}.

The complexation properties of **9** were therefore studied by UV titration in water (with 10 % DMSO added for solubility reasons) with various dipeptides and amino acids as substrates. The binding was followed by the decrease in the absorption of the pyrrole moiety at

λ = 320 nm (Figure 117) upon the addition of aliquots of the dipeptide to a buffered solution of **12** (0.01586 mM, chloride salt, 0.5 mM bis-tris-buffer at pH = 5.5). Changes in the UV spectrum of the receptor at λ = 320 were recorded and used to determine the binding constants as the absorbance of the pyrrole moiety decreases upon complex formation (Fig. 117).

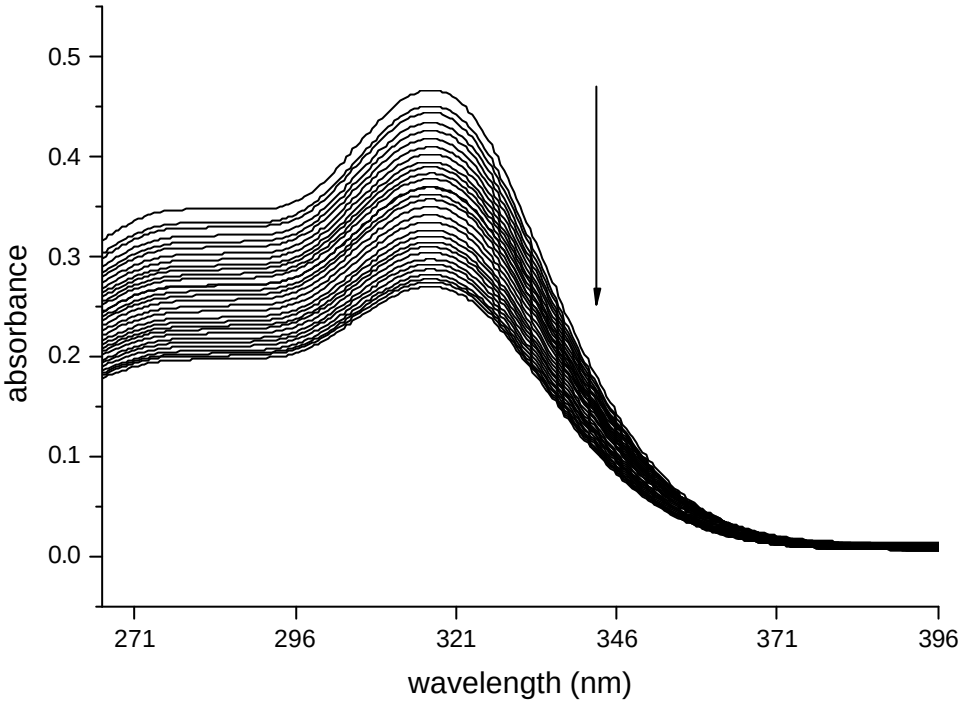


Fig. 117. *Binding isotherm for the complexation of Ac-Ala-Ala-OH (**162**) by receptor **12** in water (pH = 5.5, bis-tris-buffer).*

To confirm the stoichiometry of the complex under these conditions, a JOB plot was measured (Fig. 118).

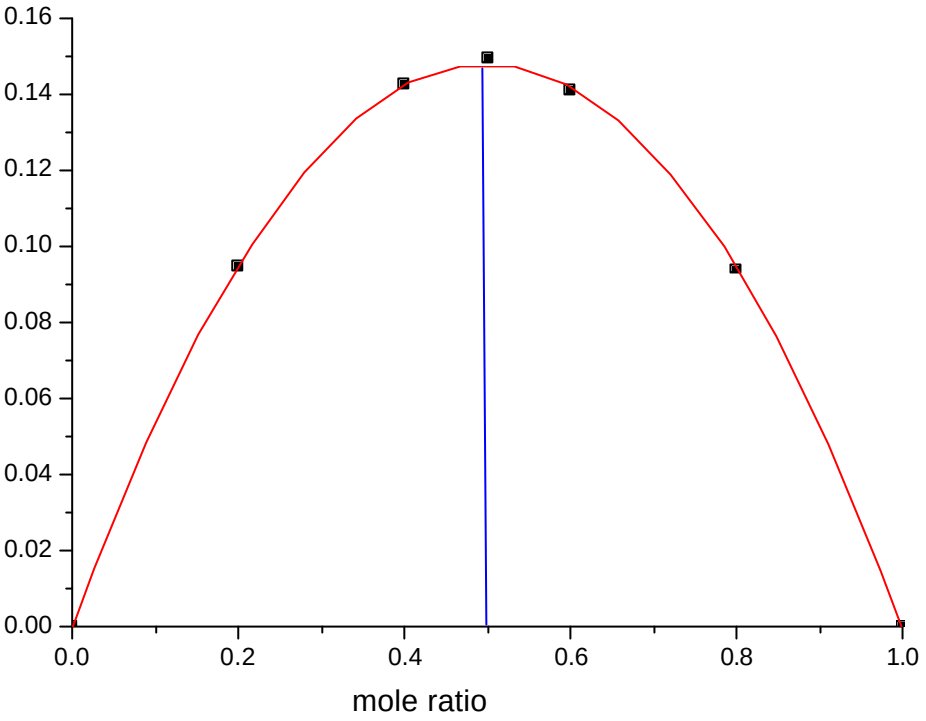


Fig. 118. *JOB-Plot of receptor **12** with Ac-Ala-Ala **162**.*

The x-coordinate at the maximum in the curve of the JOB-plot of the receptor **12** with Ac-Ala-Ala **162** is 0.5. Thus, this JOB plot confirms the 1:1 binding stoichiometry in water. This is consistent with the stoichiometry determined by the NMR titration experiments in 40 % water in DMSO that was indicated by the linear increase in chemical shift changes until a molar ratio of 1:1. Therefore, a non linear curve fitting procedure for a 1:1 association model was used to determine the binding constants. In Figure 119 the binding isotherm for the complexation of Ac-Ala-Ala-OH **162** by receptor **12** in water (pH = 5.5, bis-tris-buffer) is shown. The dotted line shows the expected change in absorption due to simple dilution of the sample.

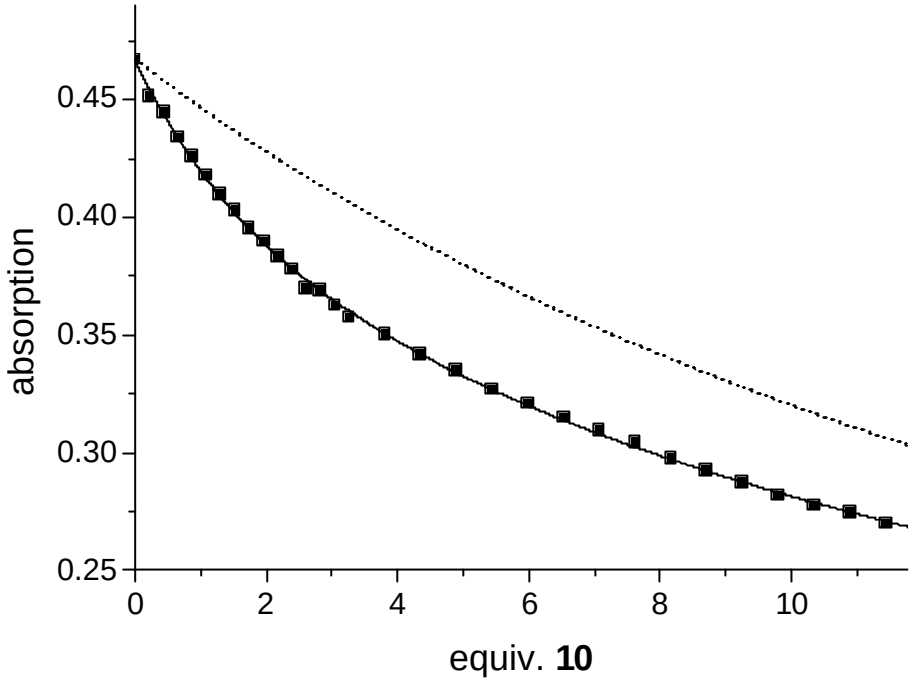


Fig. 119. *Binding isotherm for the complexation of Ac-Ala-Ala-OH (**162**) by receptor **12** in water (pH = 5.5, bis-tris-buffer). The dotted line shows the expected change in absorption due to simple dilution of the sample.*

As further substrates two amino acids (Gly-OH, Ala-OH) and three dipeptides were studied. If the predicted receptor-substrate complex shown in Fig. 108 would be correct, then amino acid must be bound worse than dipeptidic substrates, because only with larger substrates the complex could benefit from additional hydrogen-bonds. In addition, to analyze whether the hydrophobicity of the dipeptide influences the binding affinity a series of hydrophobic dipeptides from Ala-Ala-OH to Val-Val-OH was chosen.

As shown in Table 3 the dipeptides (K_{ass} ~ 2-5•10^3 M^{-1}) are bound up to ten times more efficiently than simple amino acids (K_{ass} ~ 5-7•10^4 M^{-1}) for which the association constants are similar to other guanidiniocarbonyl pyrrole based carboxylate receptors therefore representing simple ion pair formation.

Table 3: Binding constants of 12 with various carboxylates

carboxylate	K_{ass} [a]
Gly-Gly	15.900
Ala-Ala	30.600
Val-Ala	43.800
Val-Val	54.300
Ala	7.400
Gly	5.200

a K in M^{-1}, estimated error limit in K < ± 25 %.

Hence, the increase in stability for the dipeptides must be due to the additional binding sites within the complex (the hydrogen bonds between the backbone amides and interactions with the imidazolium group). Within the series of dipeptides studied the complex stability increases depending on the side chains present in the order Gly < Ala < Val. This might be surprising at the first glance as there are no specific binding sites for side chain interactions present in **12**. However, the increase in stability in this order is in good agreement with both the decreasing flexibility of the peptide and the increasing hydrophobicity of the side chains. For example, valine is known to induce peptide conformations that favor the formation of β-sheets. As the interactions within the complex with **12** are similar to those found in a β-sheet, it is not surprising that Val-Val is bound better than Ala-Ala or Gly-Gly, respectively. Furthermore, within the complex the isopropyl side chains effectively shield the hydrogen bonds between the backbone amides from the surrounding solvent (Figure 120) thereby increasing their strength.

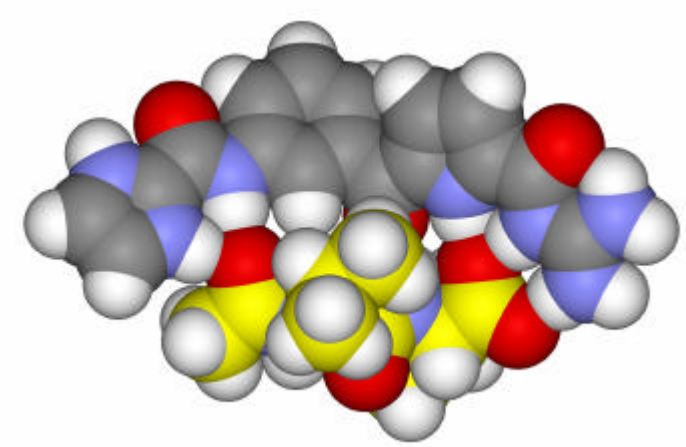


Fig. 120 . *Calculated complex structure for the binding of **162** (yellow) by receptor **12** (grey).*

In conclusion, I have shown here that based on a theoretical prediction a new and very efficient dipeptide receptor **12** was successfully realized. The binding properties of **12** are superior to any other dipeptide receptor reported so far. The association constants achieved in our group for the complexation of amino acids and tetrapeptides were always in the range of 10^2 to 10^3 M^{-1} so far. Therefore, this rather small receptor displays an enormous binding constant for dipeptides in water. This proves that the basic receptor design, namely the idea to improve the binding strength of guanidiniocarbonyl pyrroles by introduction of additional binding interactions to the peptide backbone in addition to the ion pairing with the guanidinium cation, was successful. NMR and UV binding studies with a systematically varied series of hydrophobic substrates showed that the receptor-substrate stability is increased depending on the flexibility and hydrophobicity of the dipeptide. This again underlines our general receptor design depicted in Fig. 108.

Outlook:

Taking into account that the receptor **12** is still rather small, this is a rather promising starting point for the design of receptors of even more pronounced stability and selectivity. However, for a more effective and facile approach to this general receptor class, the first step in the synthesis should be altered. Due to the fact that the selective Friedel-Crafts-acylation of 2-pyrrole methyl carboxylate **153** with *meta* nitro benzoyl chloride **154** under kinetic control was the limiting step in this synthetic approach, this step should be avoided.

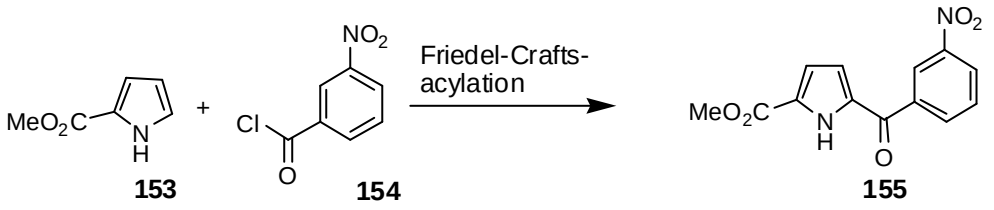


Fig. 121. The limiting step in the synthesis of receptor: The kinetic controlled Friedel-Crafts acylation.

Therefore, I tried within this work also a Friedel-Crafts acylation on a 3,4-dimethyl pyrrole **168**. Due to the fact that this compound possesses only one free position on the pyrrole, the Friedel-Crafts acylation can be carried out under thermodynamic control with a more reactive catalyst like for example aluminium chloride. The necessary 3,4-dimethyl pyrrole **168** was prepared according to literature procedures on large scale with a yield of 17 % (Fig. 122)[155]

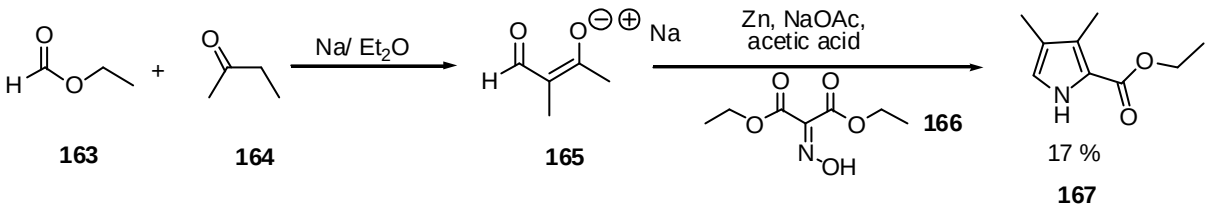


Fig. 122. S*ynthesis of 3,4-dimethyl pyrrole **168**.*

Subsequently, the Friedel-Crafts acylation on the 3,4-dimethyl pyrrole **168** gave the corresponding compound **170** in very good yield of 70 %.

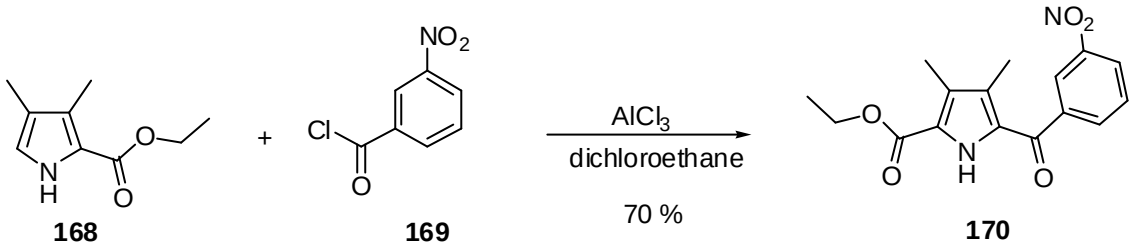


Fig. 123. *Friedel-Crafts acylation on the 3,4-dimethyl pyrrole **168**.*

This approach may in the future facilitate the large scale synthesis of others receptors of type **12**.

The general structure of **12** should also allow to develop a second generation of receptors with specifically built in side chain interactions to further increase the substrate selectivity. Therefore, the binding motif can be enlarged for example by attaching further functional groups on the imidazolium moiety. Another promising concept could be to N'-alkylate the guanidinium moiety and thus arrive at tweezer like receptors. The side chains attached to the N' of the guanidiniocarbonyl pyrrole could therefore help to further increase the complex stability and also to increase the selectivity of the recognition event. In summary, this receptor class paves the way to a variety of promising new receptor systems in the near future.

5 Summary

The present thesis encompasses two parts. The first supramolecular part focuses on the development of new flexible self-assembling zwitterions as building blocks for supramolecular polymers. In the second part, the aim was to develop bioorganic receptors for amino acids and dipeptides in aqueous media. Both research projects are based on the guanidiniocarbonyl pyrrole **1** as a new efficient binding motif for the complexation of carboxylates in polar solution.

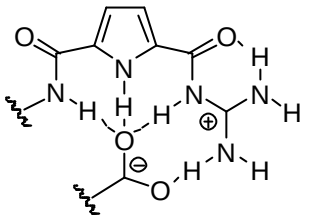


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Fig. 118. *Guanidiniocarbonyl pyrroles **1** developed by Schmuck as a binding motif for carboxylates.*

A necessary requirement for the realization of these research projects was to develop an efficient and mild synthetic approach for the cationic guanidiniocarbonyl pyrroles in general. The harsh reaction conditions of the previously used method and the problematic purification of the cationic guanidinocarbonyl pyrroles so far prevented a more extensive exploration in bioorganic and supramolecular research. In the course of this work I successfully developed a new synthesis starting with mono ^{t}Boc-protected guanidine **83** that was coupled with a benzyl protected pyrrole carboxylic acid **91**. After deprotection of the benzyl group, a key intermediate in the newly developed synthesis, the ^{t}Boc-protected guanidinocarbonyl pyrrole acid **84**, was obtained (Fig. 119).

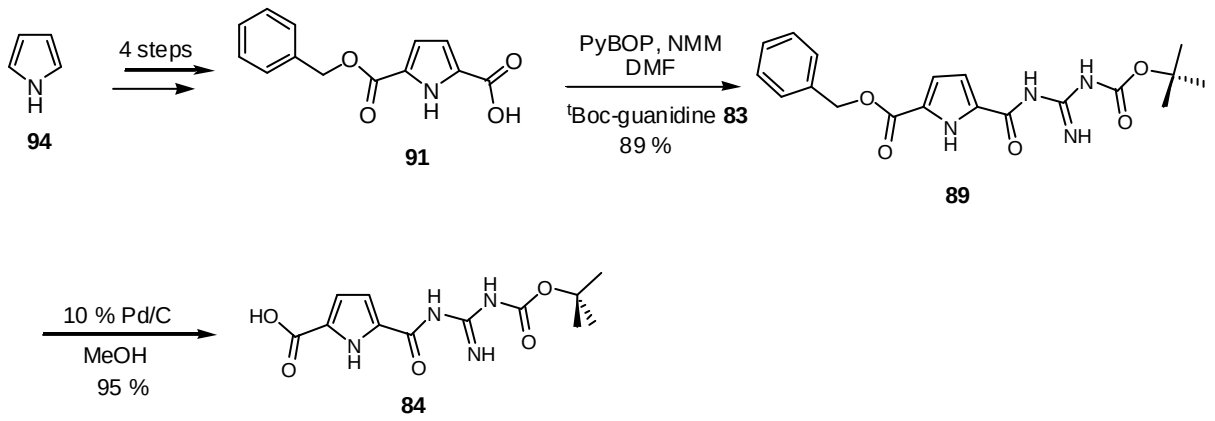
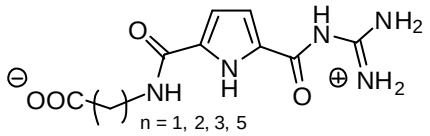


Fig. 119. *Synthesis of the key intermediate, the tBoc-protected guanidiniocarbonyl pyrrole acid **84**.*

In contrast to cationic guanidiniocarbonyl pyrroles this compound is soluble in organic solvents. Hence, this protected guanidiniocarbonyl pyrrole can easily be coupled with amines under mild conditions and can subsequently be purified and isolated with high yields and purity (80-90 %) by extraction or column chromatography. This new, mild and extremely efficient synthetic approach for the introduction of acyl guanidines is now the standard procedure in our group for the preparation of both solution and solid-phase guanidiniocarbonyl pyrroles.

With this facile method at hand, a new class of flexible zwitterions **3-6**, in which a carboxylate is linked via an alkyl chain to a guanidiniocarbonyl pyrrole cation was synthesized.



3-6

Fig. 120. *Flexible zwitterions **3-6** with spacer length n = 1, 2, 3, 5.*

The self-aggregation and the influence of the length and therefore flexibility of the alkyl spacer on the structure and stability of the formed aggregates were studied in solution and gas phase. In solution the aggregation was studied by NMR-dilution experiments in DMSO which suggest that flexible zwitterions with n = 1, 3 and 5 form oligomers (Fig. 121).

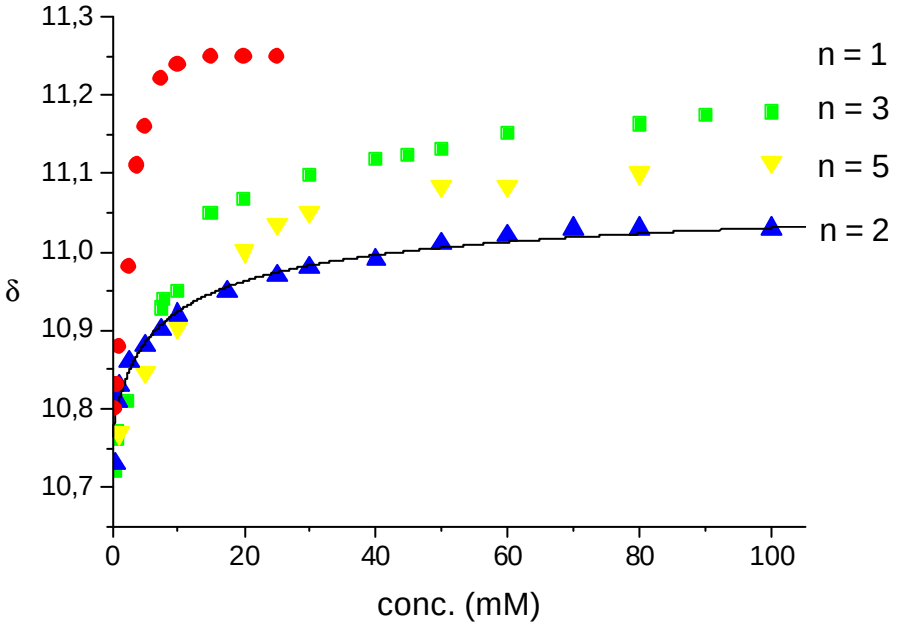
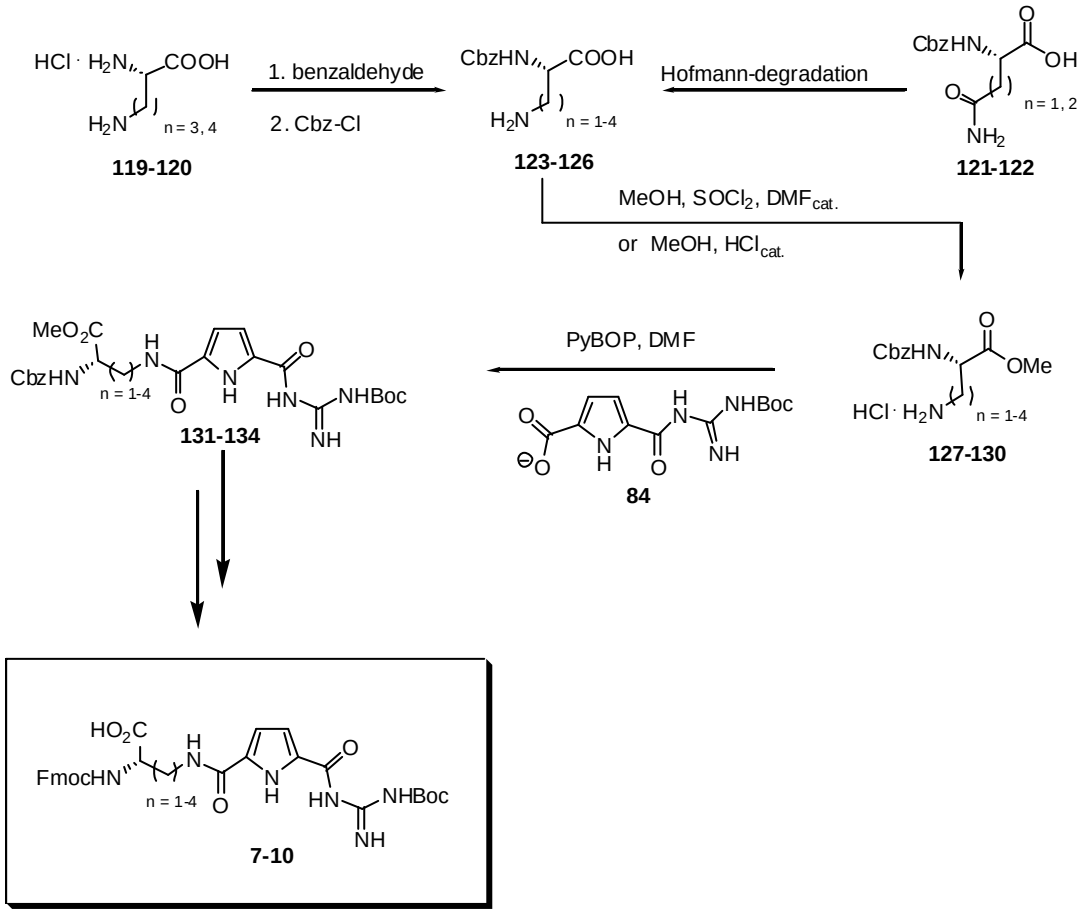


Fig. 121. *NMR dilution data of the guanidinium amide NH for **3-6**. The solid line for n = 2 shows the curve fitting for a 1:1-dimerization model.*

For n = 1, highly stable helical aggregates with nanometer size are formed. In contrast to this, the zwitterion with n = 2 forms discrete head to tail dimers instead of oligomers.

In the gas phase studies the stability and the fragmentation kinetics of a series of sodiated dimeric zwitterions with n = 2, 3 and 5 were investigated. This was done by infrared multiphoton dissociation Fourier transform ion cyclotron resonance mass spectrometry (IRMPD-FT-ICR-MS). The maximum stability of the three examined complexes was found for the complex with n = 3. Therefore the stability of the complexes is not limited or determined by the flexibility or length of the spacer but obviously by the structure of the sodiated complexes. These kinds of studies can be used in the future for a more directed design of supramolecular building blocks

The bioorganic research part comprises three different projects. In a first project I synthesized four new arginine analogues **7-10** which can be implemented in peptides as a substitute for arginine. Therefore, I developed the new multi-step synthesis shown below for these arginine analogues. As a test for their application in normal solid phase synthesis, I successfully prepared a tripeptide sequence Ala-AA_1-Val (AA: arginine analogue) which shows that these compounds can be easily coupled on the solid support.



Scheme 12. *Synthesis of the Fmoc-protected arginine analogue **7-10**.*

In a second project I studied the influence of additional ionic interactions within our binding motif. I synthesized a di-cationic **15** and a tris-cationic receptor **11** and evaluated the binding properties via NMR titration experiments against a variety of amino acids (Fig. 122). Especially, the tris-cationic receptor was capable to strongly complex amino acids. The association constants were about a factor of 10^2 higher than those for the guanidiniocarbonyl pyrroles known so far. Even in 90 %water/10 % DMSO the association constants determined by NMR titration were extremely high with values around $K_{ass} \approx 2000\ M^{-1}$.

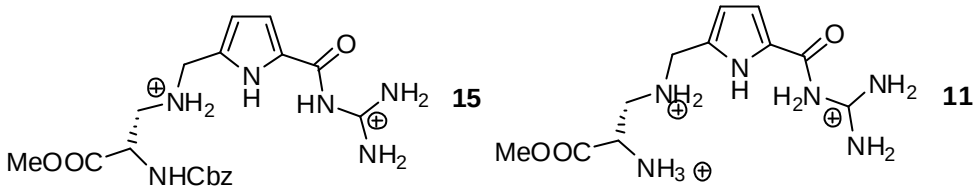


Fig. 122. *Di-cationic receptor **15**(left) and the tris-cationic receptor **11** (right).*

In the third project I developed a *de-novo* designed receptor for C-terminal dipeptides in a β-sheet conformation based on molecular calculations.

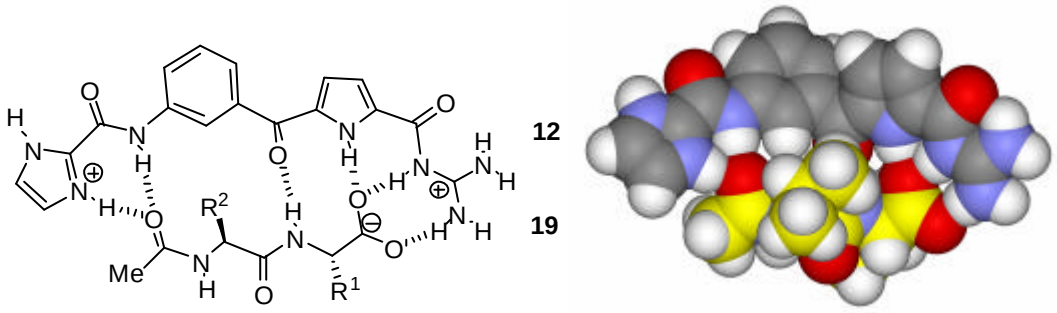


Fig. 123. *Complex of the de-novo designed dipeptide receptor **12** with an acylated dipeptide **19** (left) and calculated complex structure (right).*

This receptor was studied in NMR and also UV titration experiments. In 40 % water/ 60 % DMSO the association constants were too strong to be measured by NMR titration experiments. Therefore, the complexation properties of **12** were studied by UV titration in water (with 10 % DMSO added for solubility reasons) with various dipeptides and amino acids as substrates. The data show that **12** binds dipeptides very efficiently even in water with association constants $K_{ass} > 10^4\ M^{-1}$, making **12** one of the most effective dipeptide receptors known so far. In contrast to that, simple amino acids are bound up to ten times less efficiently ($K_{ass} \approx 10^3\ M^{-1}$) than dipeptides (Table 4).

Table 4: Binding constants of **12** with various carboxy lates

carboxylate	K_{ass}[a]
Gly-Gly	15.900
Ala-Ala	30.600
Val-Ala	43.800
Val-Val	54.300
Ala	7.400
Gly	5.200

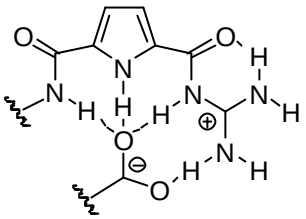
a K in M^{-1}, estimated error limit in K < ± 25 %.

In the series of dipeptides studied the complex stability increases depending on the side chains present in the order Gly < Ala < Val which is a result of the decreasing flexibility of the peptide and the increasing hydrophobicity of the side chains. The binding properties of this receptor are superior to any other dipeptide receptor reported so far.

Within my thesis I have not only developed an essential, mild and efficient synthetic approach for guanidiniocarbonyl pyrroles in general, but also a new binding motif for the complexation of amino acids **15, 11** and in addition a dipeptide receptor **12** that is superior to all dipeptides receptors known so far.

6 Zusammenfassung

Die vorliegende Arbeit gliedert sich in zwei Themenschwerpunkte. Ein supramolekulares Projekt beinhaltete die Entwicklung von neuen flexiblen, selbst-aggregierenden Zwitterionen als Bausteine für supramolekulare Polymere. In einem zweiten bioorganischem Teil bestand das Ziel darin, Rezeptoren für Aminosäuren und Dipeptide in Wasser zu entwickeln. Beide Projekte basieren auf dem Guanidiniocarbonylpyrrol als effizientes Bindungsmotiv für die Komplexierung von Carboxylaten in wässrigen Lösungen



1

Fig. 124. *Das Guanidiniocarbonylpyrrol-Bindungsmotiv **1** entwickelt von Schmuck zur effizienten Komplexierung von Carboxylaten in polarer Lösung.*

Eine notwendige Voraussetzung für die Realisierung dieser Projekte war jedoch zunächst die Entwicklung einer allgemeinen, effizienten und milden Synthese für Guanidiniocarbonylpyrrole. Die bei der zuvor verwendeten Methode aggressiven Reaktionsbedingungen und die problematische Aufreinigung der entstandenen kationischen Guanidinocarbonylpyrrole verhinderten eine größere Anwendung dieses Bindungsmotivs in bioorganischen und supramolekularen Projekten. Im Rahmen dieser Arbeit gelang es mir erfolgreich eine neue Syntheseroute zu entwickeln. Hierbei wurde mono-^{t}Boc-Guanidine **83** mit dem Benzylester **91** mittels PyBOP gekuppelt und nach Entschützung der Benzylschutzgruppe wurde die zentrale Zwischenstufe für die weiteren Synthesen, die ^{t}Boc-geschützte Guanidinocarbonylpyrrol-Säure **84** erhalten (Fig. 125).

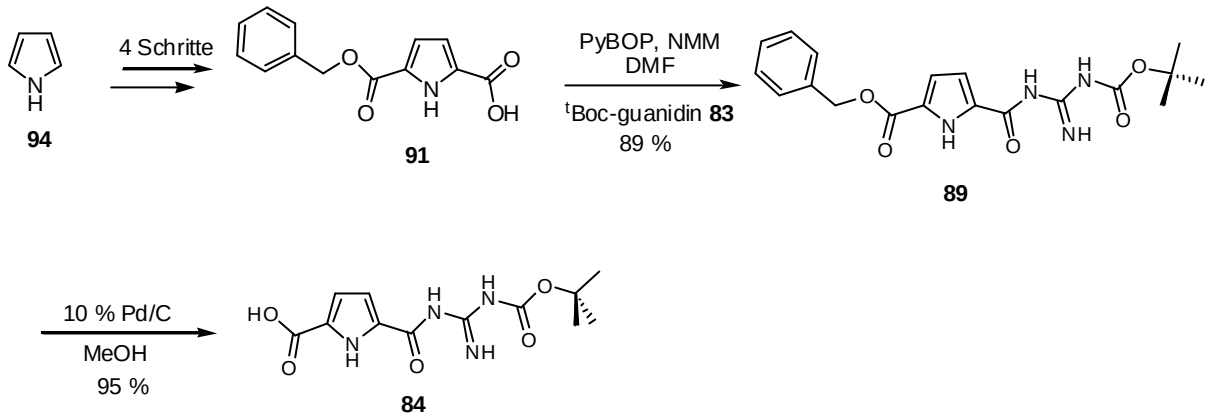
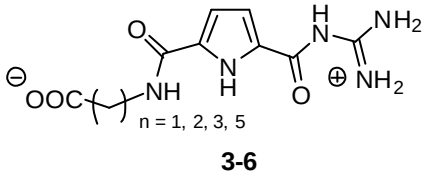


Fig. 125. *Synthese der zentralen Zwischenstufe, die* ^{t}*Boc-geschützte Guanidinocarbonylpyrrole-Säure* ***84***.

Diese Verbindung ist im Gegensatz zu den geladenen Guanidiniocarbonylpyrrolen leicht löslich in organischen Lösemitteln und kann dadurch sehr einfach und unter milden Bedingungen mit Aminen gekuppelt werden. Die Reinigung der entstehenden Verbindungen gelingt mit hoher Reinheit und exzellenter Ausbeute (80-90 %). Dieser gleichermaßen effiziente und milde synthetische Zugang zu Acylguanidinen kann sowohl zur Synthese von Guanidinocarbonylpyrrolen in Lösung wie auch an fester Phase eingesetzt werden.

Durch diese neuartige Synthese war es möglich, eine Reihe von flexiblen Zwitterionen **3-6** herzustellen und deren Selbst-Aggregation und den Einfluß der Kettenlänge und somit Flexibilität der Alkylkette auf Struktur und Stabilität der gebildeten Aggregate in Lösung sowie auch in der Gasphase zu untersuchen.



3-6

Fig. 126. *Flexible Zwitterionen* ***3-6*** *mit Alkylketten von n = 1, 2, 3, 5.*

In DMSO deuten NMR-Verdünnungsreihen darauf hin, dass die flexiblen Zwitterionen mit n = 1, 3 und 5 oligomere Strukturen ausbilden (Fig. 127). Im Falle von n = 1 werden hoch stabile helicale und Nanometer große Aggregate in der gebildet. Im Gegensatz dazu bildet das Zwitterion mit n = 2 diskrete „Kopf-Schwanz“-Dimere.

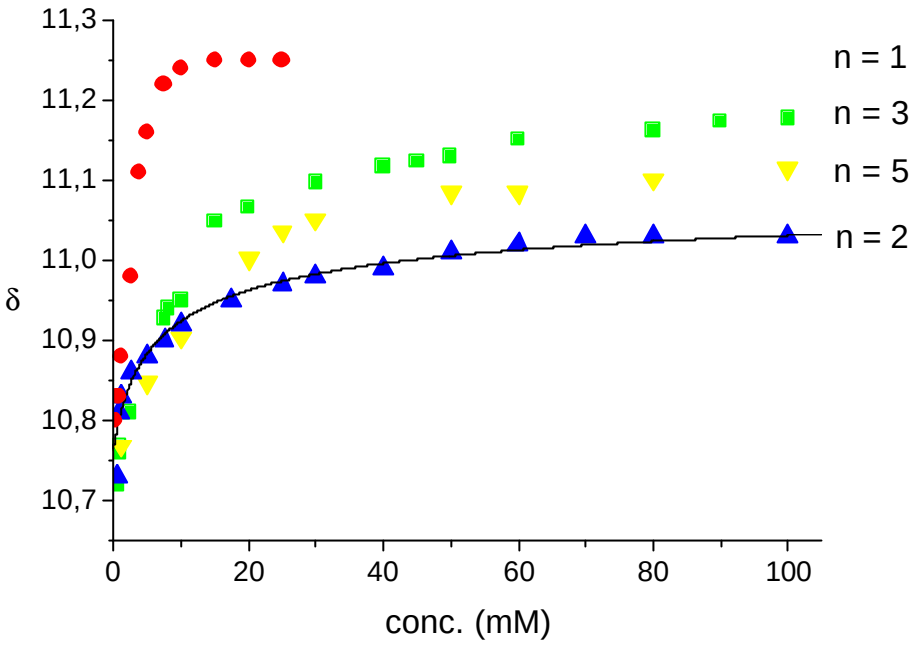
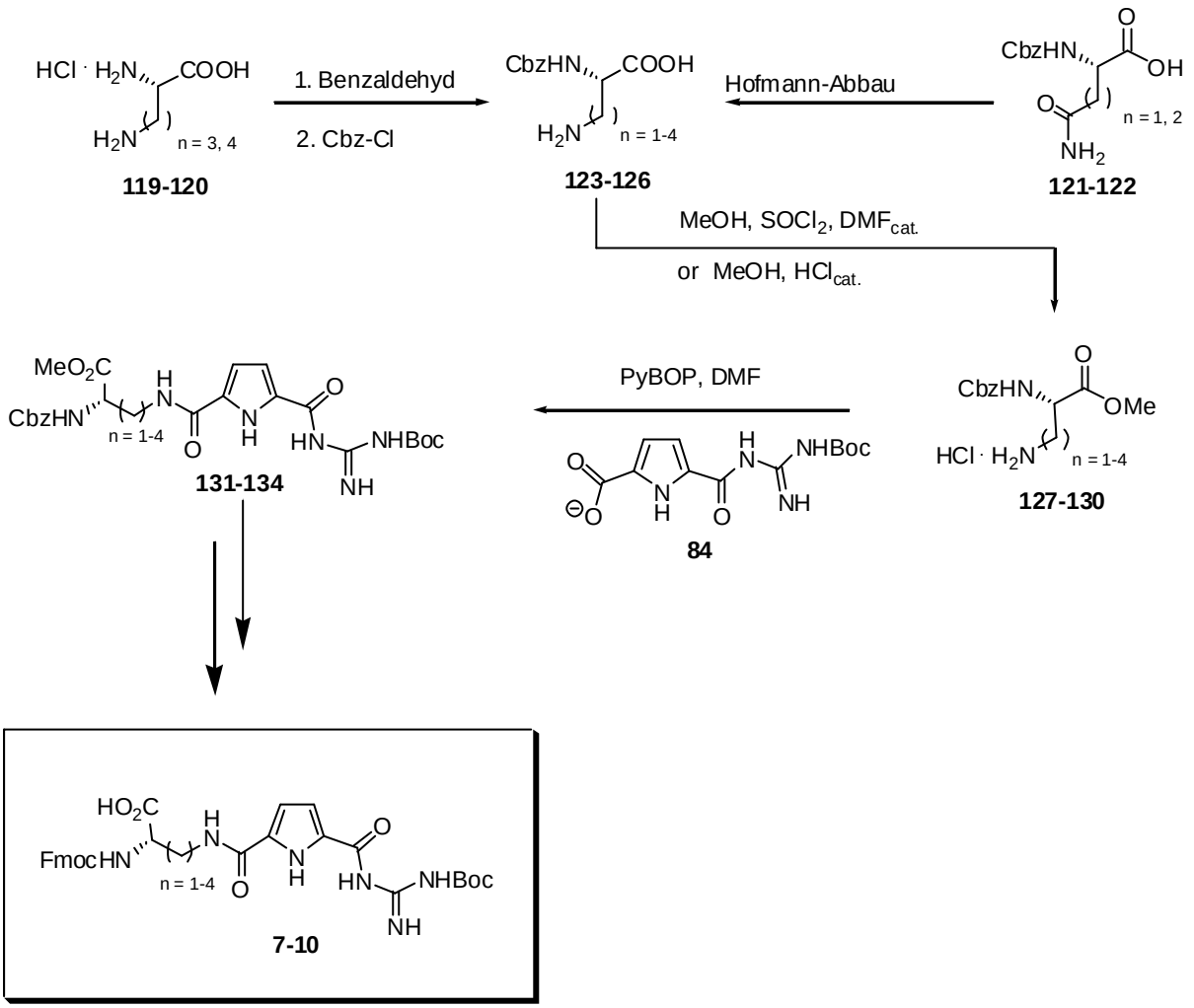


Fig. 127. *NMR-Verdünnungsdaten des Guanidiniumamid NHs für **3-6**. Die durchgezogene Linie für n = 2 zeigt die basierend auf einem 1:1-Dimerisierungsmodel angeglichene Kurve.*

In den Gasphasen-Studien wurde die Stabilität und Zerfallskinetik einer Reihe von Natriumaddukten der Dimere von n = 2, 3 und 5 untersucht. Dieses gelang durch die Methode der „infrared multiphoton dissociation Fourier transform ion cyclotron resonance mass spectrometry“ (IRMPD-FT-ICR MS). Das Maximum der Stabilität der drei untersuchten Komplexe wurde für n = 3 gemessen. Somit hängt die Stabilität der Komplexe nicht nur von der Länge und damit Flexibilität der Alkylkette ab, sondern offensichtlich auch von der Struktur der gebildeten Dimere. Solche Studien ermöglichen möglicherweise in Zukunft das gezielte Design von supramolekularen Bausteinen.

Der bioorganische Teil meiner Arbeit setzte sich aus drei Einzelprojekten zusammen. So synthetisierte ich durch eine fünfstufige Synthesesequenz vier neue Arginin-Analoga **7-10**, die in Zukunft als Ersatz für Arginin in Peptide eingebaut werden können (Fig. 128). Als Testreaktion für die Eignung dieser Verbindungen in einer Festphasenpeptidsynthese, wurde ein Tripetid Ala-AA_1-Val (AA: Arginin-Analogon) mit einem eingebauten Arginin-Analogon erfolgreich hergestellt. Dies zeigte, dass diese Verbindungen sehr einfach an der Festphase gekuppelt werden können.



Schema 13. *Synthese der Fmoc-geschützten Arginin-Analoga **7-10**.*

In einem zweiten Projekt habe ich den Einfluß einer zusätzlichen ionischen Wechselwirkung in unserem Bindungsmotiv untersucht. Dazu wurde ein zweifach-kationischer Rezeptor **15** und der dreifach-geladenen Rezeptor **11** synthetisiert und physikalisch-organisch ihre Bindungseigenschaften mit Hilfe von NMR-Titrationsexperimenten gegen eine Reihe von Aminosäuren untersucht. Der dreifach-kationische Rezeptor **11** zeigte hierbei herausragende Bindungseigenschaften und war um ca. den Faktor 10^2 besser als für die bisher bekannten Guanidiniocarbonylpyrrole. Die Assoziationskonstanten waren auch fast reinem Wasser (90 % Wasser mit 10 % DMSO) mit bis zu $K_{ass} \approx 2000$ noch bemerkenswert hoch.

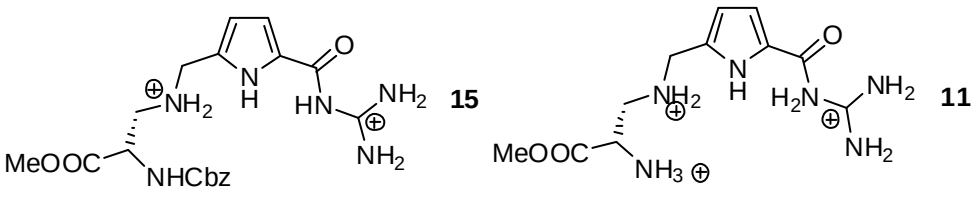


Fig. 128. *Di-kationische Rezeptor **15**(links) und der tris-kationische Rezeptor **11** (rechts).*

Im dritten Projekt habe ich einen *de-novo* entwickelten Rezeptor für C-terminale Dipeptide in einer β-Faltblatt Struktur basierend auf Molecular Modelling Berechnungen entwickelt.

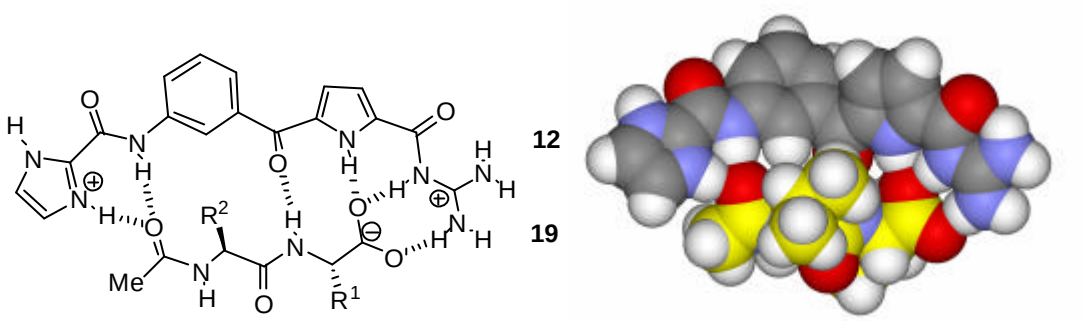


Fig. 129. *Komplex des de-novo entwickelten Dipeptid-Rezeptors **12** mit einem acylierten Dipeptid **19** und die daraus berechnete Struktur.*

Dieser Rezeptor wurde mittels NMR and UV-Titrationen untersucht. In 40 % Wasser/ 60 % DMSO waren die Bindungskonstanten zu hoch um überhaupt quantifiziert zu werden. Deshalb wurden die Bindungseigenschaften des Rezeptors **12** mittels UV Titrationen in einer Mischung aus 90 % Wasser mit 10 % DMSO gegen eine Reihe von Dipeptiden und Aminosäuren getestet. Die Bindungsdaten zeigen, dass Rezeptor **12** Dipeptide mit ausgezeichneten Bindungskonstanten ($K_{ass} > 10^4\ M^{-1}$) komplexiert. Im Gegensatz dazu bindet der Rezeptor **12** Aminosäuren um den Faktor zehn schlechter ($K_{ass} \approx 10^3\ M^{-1}$).

Table 1: Bindungskonstanten für **12** gegen eine Reihe von Aminosäuren

carboxylate	K_{ass} [a]
Gly-Gly	15.900
Ala-Ala	30.600
Val-Ala	43.800
Val-Val	54.300
Ala	7.400
Gly	5.200

a K in M^{-1}, abgeschätzter Fehler in K < ± 25 %.

Die Komplexstabilität nimmt hierbei in Abhängigkeit von der Seitenkette des Dipeptids in der Reihe Gly < Ala < Val zu, was sich mit der abnehmenden Flexibilität und zunehmenden Hydrophobizität der Seitenkette erklären lässt. Diese Eigenschaften machen den Rezeptor **12** zu dem besten bisher bekannten Dipeptidrezeptor in wässrigen Lösungen.

Innerhalb meiner Arbeit gelang es mir somit, nicht nur eine essentiell wichtige, milde und effiziente Synthese für Guanidinocarbonylpyrrole zu entwickeln, sondern es gelang mir ebenso ein neues Bindungsmotiv für die Komplexierung von Aminosäuren in Wasser zu

entwickeln. Zusätzlich konnte noch der Dipeptidrezeptor **12** erfolgreich synthetisiert und untersucht werden. Mit Bindungskonstanten für von $K_{ass} \approx 10^4\ M^{-1}$ ist er der derzeit beste Dipeptidrezeptor in wässrigen Lösung

7 Experimental Section

7.1 General experimental methods

Solvents and Chemicals

All solvents were dried according to literature procedures.[156] Dichloromethane, *N*,*N*-dimethylformamide, acetonitril, benzene, toluene and all amines were dried by distillation from calcium hydride. Hexane and chloroform from phosphorus pentoxide. Diethylether and tetrahydrofuran were distilled from sodium-benzophenone ketyl. Methanol was distilled from magnesium. All other commercial reagents were purchased and used as received unless otherwise specified.

Inert gas

Reactions with air-sensitive compounds were carried out under technical argon (4.6 or 4.8) of the company *Linde* (99.966 % or 99.998 %), which was dried with phosphorpentoxid and freed from oxygen with a BTS-catalyst of the company *BASF*.

Vacuum pumps

Equipment: *Vacubrand* membrane pumps of different types and implementations
Leybold oil pumps of different types and implementations

7.2 Analytic methods

Thin Layer Chromatography (TLC)

Material: *Machery-Nagel* POLYGRAM SIL G/UV_{254} (40 × 80 mm plates, 0.25 mm)

Reactions were monitored by TLC on silica gel 60 F254 precoated plates. Visualization of the spots was carried out by fluorescence quenching with 254-nm UV light and/or with iodine or ninhydrin vapours. The TLC-elution mixtures are reported in volumes except otherwise stated.

Flash Chromatography

Material: *Macherey-Nagel* MN silica gel 60 (0.04-0.063 mm)
ICN Biomedicals GmbH silica gel 60 (0.032-0.063 mm)

Flash chromatography was performed on silica gel with the indicated solvent system and on columns of different diameter and length. Solvent mixtures used for flash chromatography are reported in volume. Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure, unless otherwise stated. Purified compounds were further dried for 12-72 h under high vacuum (0.01-0.05 Torr). Yields refer to chromatographically purified and spectroscopically pure compounds, unless otherwise stated.

Nuclear magnetic resonance (NMR)

Equipment *Bruker* AC 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz)
Bruker DPX 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz)
Bruker DRX 500 (^{1}H: 500 MHz, ^{13}C: 125 MHz)
Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz)
Bruker Avance 600 (^{1}H: 600 MHz, ^{13}C: 150 MHz)

The ^{1}H NMR spectra were recorded either at 300, 400, 500 or 600 MHz, and the ^{13}C NMR spectra were recorded at 75, 100, 125 or 150 MHz at room temperature, employing DMSO-d_6, or $CDCl_3$ as solvent. The chemical shifts are reported in ppm from TMS (δ scale) but were measured against the solvent signals and the apparent coupling constants are given in Hertz. The description of the fine structure means: s = singulett, br.s = broad singulett, d = dublett, t = triplett, q = quartett, m = multiplett, br. = broad signal. All assignments have been performed according to literature.[157]

Infrared spectra (IR)

Equipment *Perkin-Elmer* FT-IR 1600

All IR spectra were measured as KBr-pellets on a *PerkinElmer* FT-IR 1600 spectrophotometer. The maxima are classified in three intensities: s (strong), m (middle), w (weak) and are reported in cm^{-1}.

Mass spectra (MS)[158]

Equipment Electron ionization (EI): *Finnigan* MAT Incos 500 or MAT 90
Electrospray ionization (ESI): *Finnigan* MAT 900 S

Lyophilisation

Equipment *Christ* Alpha 1-4

UV spectra

Equipment Jasco V530 spectrometer

All UV spectra were measured at room temperature in 10 mm rectangular cells of the company *Hellma.*

Fluorescence spectra

Equipment *Jasco* FP-6500 spectofluorometer

All fluorescence spectra were measured at room temperature in 10 mm rectangular cells of the company *Hellma.*

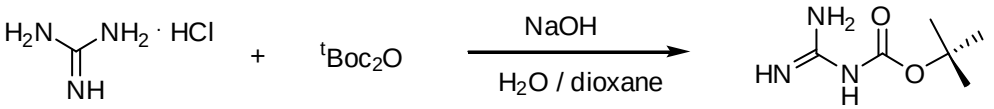
Melting points

Equipment Büchi apparatus according to *Dr. Tottoli*

Melting points were measured in open end glass capillary tubes and are uncorrected.

7.3 Synthesis of the protected guanidinocarbonyl pyrrole compounds

N-^{t}Boc-guanidine [112]



CH_6ClN_3	$C_{10}H_{18}O_5$		$C_6H_{13}N_3O_2$
95.53	218.25		159.19
57	**82**		**83**

A solution of $^{t}Boc_2O$ **82** (12.0 g, 55.0 mmol, 1eq.) in dioxane (100 ml) was added very slowly at 0°C (ice bath) under vigorous stirring to a mixture of guanidinium chloride **57** (26.3 g, 275 mmol, 5 eq.) in an aqueous sodium hydroxide solution (12.0 g, 0.3 mol NaOH in 50 ml water). The resulting suspension was stirred at room temperature for additional 20 h and then extracted with ethyl acetate (3 times with 100 ml). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting white crystals were dried *in vacuo* to yield 8.02 g (92 %) of analytically pure **83**.

$C_6H_{13}N_3O_2$ Mw = 159.19

yield 8.02 g (92 %)

R_f 0.25 (SiO_2, dichlormethane/methanol/trietylamine = 5/1 + 1 ml triethylamine per 100 ml)

mp. 165 °C (decomposition)

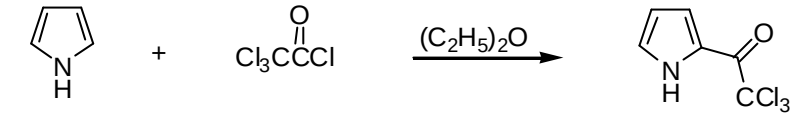
FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3408 [s] , 1650 [s], 1540 [s], 1450 [m], 1311 [s], 1253 [m], 1142 [s], 1066 [s], 950 [w], 806 [m]

HR-MS (EI) m/z = 159.101 ± 0.005 (calculated for $^{12}C_6H_{13}N_3O_2$: 159.101)

^{1}H-NMR (300MHz, [D_6]DMSO) δ = 1.33 (s, 9H, C*H*$_3$), 6.79 (br.s, 3H, guanidinium-N*H*)

^{13}C-NMR (75MHz, [D_6]DMSO) δ = 28.4 (*C*H_3), 75.8 (*C*$(CH_3)_3$), 162.8 (*Cq* or *C*=O), 163.4 (*Cq* or *C*=O)

Trichloroacetyl pyrrole[114]



C_4H_5N	C_2Cl_4O	$C_6H_4Cl_3NO$
67.09	181.83	212.46
95	**95**	**93**

To a solution of trichloroacetyl chloride **95** (225 g, 1.23 mol, 1.1 eq.) in dry diethyl ether (220 ml) freshly distilled pyrrole **94** (77.0 g, 1.15 mol, 1 eq.) was added over 3 h. The mixture was stirred for an additional hour at room temperature and afterwards neutralized with an aqueous potassium carbonate solution (100 g, 0.72 mol K_2CO_3 in 300 ml water). The organic phase was separated, dried with $MgSO_4$, activated charcoal was added to the solution and then filtered through a Celite pad. Concentration under reduced pressure and recrystallization from *n*-hexane (225 ml) gave a crystalline, gray-metallic solid of 219 g (95 %).

$C_6H_4Cl_3NO$ Mw = 212.46

yield 219 g (95 %)

R_f 0.49 (SiO_2, dichloromethane)

mp. 75-75.5 °C (Lit: 73.5-74 °C)

FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3323 [br.s], 2921 [w], 1656 [s], 1536 [w], 1425 [w], 1387 [m], 1137 [m], 1112 [m], 1064 [m], 1036 [m], 843 [w], 756 [m], 740 [m]

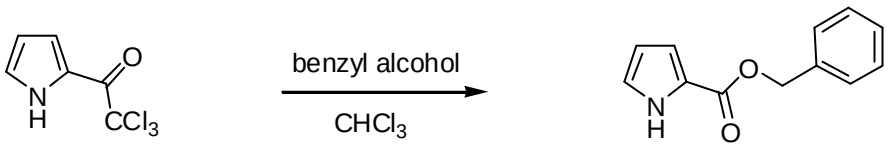
MS (EI): *m/z* = 211 $[M]^+$ (5), 148 (5), 94 (100), 66 (33), 39 (24)

HR-MS (EI) m/z = 210.9358 ± 0.0001 (calculated for $^{12}C_6H_4Cl_3NO$: 210.9361)

^{1}H-NMR (400MHz, $CDCl_3$) δ = 6.35-6.39 (m, 1H, pyrrole-C*H*), 7.15-7.24 (m, 1H, pyrrole-C*H*), 7.36-7.37 (m, 1H, pyrrole-C*H*), 9.60 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, $CDCl_3$) δ = 94.9 (*C*Cl_3), 111.9 (pyrrole-*C*H), 121.2 (pyrrole-*C*H), 123.0 (pyrrole-*Cq*), 127.2 (pyrrole-*C*H), 173.2 (*C*=O)

Benzyl 2-pyrrole carboxylate[114]



$C_6H_4Cl_3NO$
212.46

93

$C_{12}H_{11}NO_2$
201.22

92

A sodium benzylate solution [prepared from Na (550 mg, 23.9 mmol) in benzylalcohol (26.7 ml, 258.83 mmol)] was added to a solution of trichloroacetyl pyrrole **93** (50.0 g, 235 mmol, 1 eq.) in chloroform (50 ml). The resulting dark mixture was stirred for an additional hour at room temperature and the solvent was evaporated under reduced pressure yielding a black oil, which crystallizes after a few days. This raw product was pure enough to be used in the further reaction steps or could be recrystallized for analytical purpose from dichloromethane/*n*-hexane resulting in off-white needles (45.0 g, 95 %).

$C_{12}H_{11}NO_2$ Mw = 201.22

yield 45.0 g (95 %)

R_f 0.83 (SiO_2, hexane/ethyl acetate = 3/2)

0.55 (SiO_2, hexane/ethyl acetate/dichloromethane = 70/15/15)

mp. 55 °C (Lit: 54-55 °C)

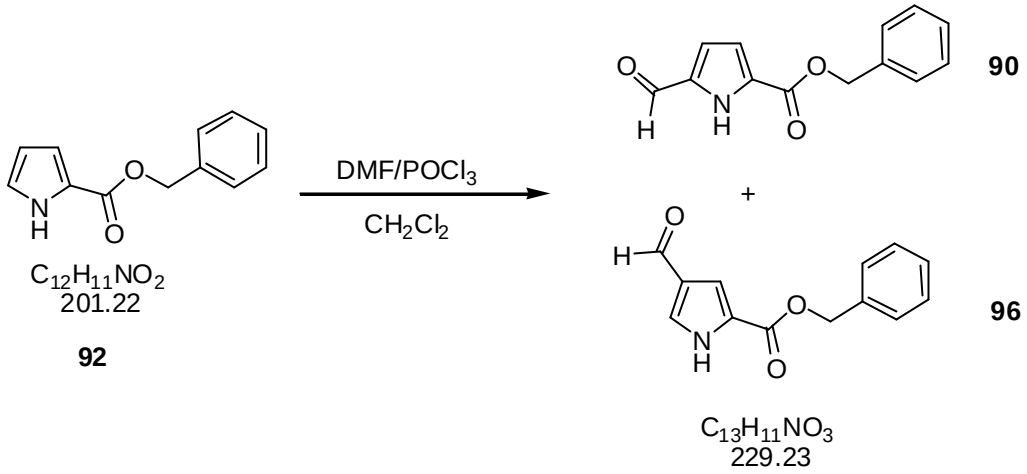
FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3310 [s], 3190 [s], 2325 [m], 2320 [m], 1696 [s], 1420 [s], 1320 [s], 1190 [s], 1130 [s], 735 [m], 745 [m], 765 [m]

HR-MS (EI) m/z = 201.0793 ± 0.0001 (calculated for $^{12}C_{12}H_{11}NO_2$: 201.0790)

^{1}H-NMR (400MHz, $CDCl_3$) δ = 5.31 (s, 2H, C*H*$_2$), 6.24-6.27 (m, 1H, pyrrole-C*H*), 6.92-6.94 (m, 1H, pyrrole-C*H*), 6.95-6.98 (m, 1H, pyrrole-C*H*), 7.32-7.44 (m, 5H, aryl-C*H*), 9.38 (br.s, 1H, N*H*)

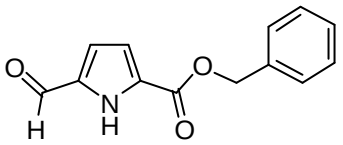
^{13}C-NMR (100MHz, $CDCl_3$) δ = 66.0 (benzyl-*C*H$_2$), 110.5 (pyrrole-*C*H), 115.6 (pyrrole-*C*H), 122.5 (pyrrole-*Cq*), 123.1 (pyrrole-*C*H), 128.1 (aryl-*C*H), 128.2 (aryl-*C*H), 128.5 (aryl-*C*H), 136.1 (aryl-*Cq*), 160.0 (*C*=O)

Benzyl 5-Formylpyrrole-2-carboxylate[115]



Phosphorylchloride (11.2 ml, 0.12 mol, 2 eq.) was added dropwise to dimethylformamide (23.0 ml, 0.3 mol, 5 eq.) at 5-10 °C. Stirring for 30 min produced the Vilsmeier reagent. A portion of **92** (12.1 g, 0.06 mol, 1 eq.) was dissolved in 250 ml of dichloromethane, the solution was cooled to -20 °C, and the Vilsmeier reagent was added dropwise over 12-15 min. The reaction mixture was stirred at 0°C for 3 h and then at room temperature for 24 h. Aqueous sodium bicarbonate (150 ml) was added, and the mixture was refluxed for 15 min. The organic layer was separated, washed twice with brine, dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to give a yellow oil. Flash chromatography on silica gel yielded in the white 2,5-substituted product **90** (8.45 g, 62 %) and in the red 2,4-substituted product **96** (4.50 g, 33 %).

$C_{13}H_{11}NO_3$ Mw = 229.23



yield 8.45 (62 %)

R_f 0.73 (SiO_2, cyclohexane/ethyl acetate = 3/2)

0.55 (SiO_2, cyclohexane/ethyl acetate/dichlormethane = 70/15/15)

mp. 105 °C

FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3280 [s], 2320 [m], 2350 [m], 1690 [s], 1700 [s], 1550 [s], 1390 [s], 1340 [s], 1220 [s], 780 [m], 770 [m], 700 [m]

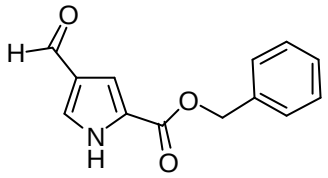
MS (EI) m/z = 229 $[M]^+$ (12), 123 (21), 122 (11), 91 (100), 65 (17), 39 (13)

HR-MS (EI) m/z = 229.0739 ± 0.0001 (calculated for $^{12}C_{13}H_{11}NO_3$: 229.0741)

^{1}H-NMR (400MHz, $CDCl_3$) δ = 5.33 (s, 2H, benzyl-C*H*$_2$), 6.90-6.97 (m, 2H, pyrrole-C*H*), 7.33-7.42 (m, 5H, aryl-C*H*), 9.63 (s, 1H, aldehyde-C*H*), 10.02 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, $CDCl_3$) δ = 67.0 (aryl-*C*H$_2$), 116.0 (pyrrole-*C*H), 119.7 (pyrrole-*C*H), 128.5 (aryl-*C*H), 128.6 (aryl-*C*H), 128.7 (aryl-*C*H), 134.5 (*Cq*), 135.2 (*Cq*), 160.1 (*C*=O), 180.4 (aldehyde-*Cq*)

$C_{13}H_{11}NO_3$ Mw =229.23



yield 4.50 g (33 %)

R_f 0.4 (SiO_2, cyclohexane/ethyl acetate = 3/2)

0.13 (SiO_2, cyclohexane/ethyl acetate/dichlormethane = 70/15/15)

mp. 99 °C

FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3138 [m], 2990 [m], 1707 [s], 1636 [s], 1567 [m], 1502 [m], 1452 [m], 1416 [m], 1383 [m], 1344 [m], 1268 [m], 1202 [s], 1152 [m], 1113 [m], 953 [w], 909 [w], 810 [w], 788 [w], 759 [m], 693 [m], 615 [w],

584 [w], 536 [w]

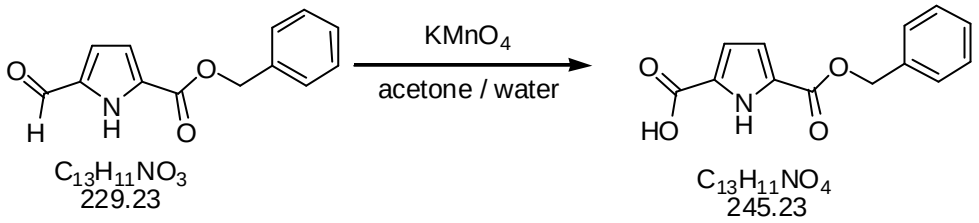
MS (EI) m/z = 229 $[M]^+$ (23), 122 (23), 91 (100), 71 (10), 65 (12), 57 (16)

HR-MS (EI) m/z = 229.0739 0.0002 (calculated for $^{12}C_{13}H_{11}NO_3$: 229.0741)

^{1}H-NMR (400MHz, $CDCl_3$) δ = 5.3 (s, 2H, benzyl-C*H*$_2$), 7.32-7.41 (m, 6H, aryl-C*H* and pyrrole-C*H*), 7.52-7.54 (m, 1H, pyrrole-C*H*), 9.80 (s, 1H, aldehyde-C*H*), 10.20 (pyrrole-N*H*)

^{13}C-NMR (100MHz, $CDCl_3$) δ = 66.7 (benzyl-*C*H$_2$), 114.7 (pyrrole-*C*H), 127.5 (pyrrole-*C*H), 128.2 (aryl-*C*H), 128.5 (aryl-*C*H), 128.6 (aryl-*C*H), 128.7 (*Cq*), 135.4 (aryl-*Cq*), 160.7 (*C*=O), 185.7 (aldehyde-*Cq*)

1*H*-Pyrrole-2,5-dicarboxylic acid monobenzylester



90 **91**

To a solution of the aldehyde **90** (15.0 g, 74.55 mmol, 1 eq.) in acetone (100 ml) a suspension of $KMnO_4$ (23.6 g, 149.1 mmol, 2 eq.) in acetone/water (1:1, 300 ml) was dropwise added over 1 h. The solution was heated over 1 h to 40 °C and stirred at room temperature for an additional hour. A few grams (1-2 g) of sodium dithionite were added, the mixture filtered through a Celite pad, thoroughly washed with an aqueous sodium hydroxide solution (150 ml, 5 %) and the combined filtrates acidified with hydrochloric acid (5 %). The colorless precipitate was filtered off, washed with hydrochloric acid (5 %) and dried over phosphorus pentoxide affording the carboxylic acid **91** as a colorless powder (12.2 g, 67 %).

$C_{13}H_{11}NO_4$ Mw = 245.23

yield 12.2 g (67 %)

R_f 0.59 (SiO_2, dichloromethane/ethyl acetate = (5/5 + 1ml acetic acid per 100 ml)

mp. 234 °C (decomposition)

FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3287 [s], 2360 [m], 2350 [m], 1731 [s], 1730 [s], 1684 [m], 1557 [m], 1277 [s], 770,4 [m], 770,3 [m]

HRMS m/z = 245.0688 ± 0.0002 (calculated for $^{12}C_{13}H_{11}NO_4$: 245.0687)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 5.28 (s, 2H, benzyl-C*H*$_2$), 6.75-6.77 (m, 1H, pyrrole-C*H*), 6.82-6.85 (m, 1H, pyrrole-C*H*), 7.30-7.47 (m, 5H, aryl-C*H*), 12.54 (s, 1H, N*H*), 12.82 (br.s, 1H, COO*H*)

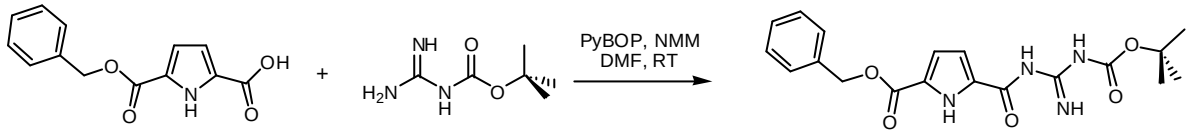
^{13}C-NMR (100MHz, [D_6]DMSO) δ = 65.7 (benzyl-*C*H$_2$), 115.3 (pyrrole-*C*H), 115.8 (pyrrole-*C*H), 126.1 (pyrrole-*Cq*), 126.6 (pyrrole-*Cq*), 128.1 (aryl-*C*H), 128.2 (aryl-*C*H), 128.6 (aryl-*C*H), 136.4 (aryl-*Cq*), 159.8 (*C*=O), 161.4 (*C*=O)

EA $C_{13}H_{11}NO_4$; Mw = 245.23 g/mol

calc.: C: 63.67 % H: 4.52 % N: 5.71 %

found: C: 63.43 % H: 4.58 % N: 5.67 %

H-Pyrrole-5-*N*-^{t}Boc-guanidiniocarbonyl-2-carboxylic acid benzylester



$C_{13}H_{11}NO_4$
245.23

91

$C_6H_{13}N_3O_2$
159.19

83

$C_{19}H_{22}N_4O_5$
386.4

89

A mixture of the benzylester **91** (1.0 g, 4.07 mmol, 1 eq), PyBOP (2.12 g, 4.07 mmol, 1 eq.) and N-methyl morpholine (1 ml, 9.09 mmol) was stirred in DMF (20 ml) at room temperature for 30 min. ^{t}Boc-guanidine **83** (649 mg, 4.07 mmol, 1 eq) was added and the resulting solution stirred over night. The red solution was hydrolyzed with water (40 ml), the slightly yellow precipitate filtered and dried over phosphorus pentoxide. Then the product was purified by column chromatography (SiO_2, hexane/ethyl acetate = 3/2 + 1 ml triethylamine per 100 ml) yielding a colorless crystalline powder (1.35 g, 89 %).

$C_{19}H_{22}N_4O_5$ Mw = 386.41

yield 1.35 g (89 %)

R_f 0.51 (SiO_2, hexane/ethyl acetate = 3/2 + 1 ml triethylamine per 100 ml eluent)

mp. 88°C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3393 [br. m], 3256 [br. m], 2360 [s], 2340 [m], 1719 [s], 1717 [s], 1635 [s], 1540 [s], 1286 [s], 1149 [s], 842 [w]

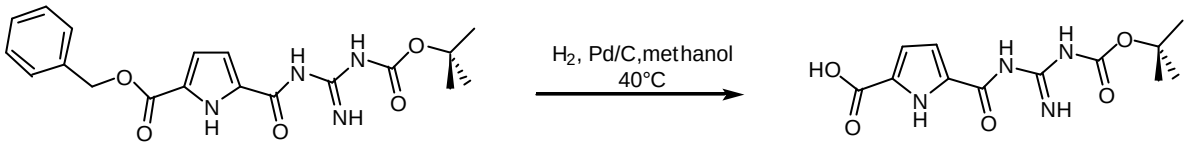
HR-MS (ESI) m/z = 409.149 ± 0.005 (calculated for $^{12}C_{13}H_{18}N_4O_5Na$: 409.146)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, ^{t}Boc-C*H*$_3$), 5.30 (s, 2H, benzyl-C*H*$_2$), 6.83 (m, 2H, pyrrole-C*H*), 7.30-7.46 (m, 5H, aryl-C*H*), 8.56 (br.s, 1H,

N*H*), 9.30 (br.s, 1H, N*H*), 10.73 (br.s, 1H, N*H*), 11.61 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 27.9 (^{t}Boc-*C*H_3), 65.7 (*C*H_2), 116.0 (pyrrole-*C*H), 128.1 (*C*H), 128.2(*C*H), 128.6 (*C*H), 136.4 (*C*H); 158.5 (*C*=O), 160.0 (*C*=O)

1*H*-Pyrrole-5-*N*-^{t}Boc-guanidiniocarbonyl-2-carboxylic acid



$C_{19}H_{22}N_4O_5$
386.4

89

$C_{12}H_{16}N_4O_5$
296.28

84

A mixture of the benzylester **89** (1.00 g, 2.68 mmol) and 10 % Pd/C (100 mg) in methanol was vigorously stirred at 40 °C for 5 h under hydrogen atmosphere. The resulting suspension was evaporated and the residue was washed several times with hot DMF until all product was dissolved. The DMF solution was filtered through a G4-frit and if necessary also through a Celite pad. Water (10 ml) was added to the DMF solution and the resulting precipitate was filtered and dried in the desiccator over phosphorus pentoxide yielding a colorless powder (754 mg, 95 %).

$C_{12}H_{16}N_4O_5$ Mw = 296.28

yield 754 mg (95 %)

R_f 0.64 (SiO_2, dichloromethane/methanol = 8/2 + 1 ml triethylamine per 100 ml)

mp. >300°C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3393 [br. m], 2958 [w], 1650 [s], 1542 [s], 1319 [s];

HR-MS (neg.ESI) m/z = 295 $[M-H^+]^-$

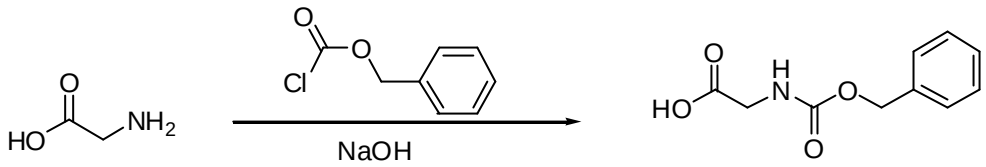
^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.4 (s, 9H, ^{t}Boc-C*H*$_3$), 6.73 (s, 1H, pyrrole-C*H*), 6.80 (s, 1H, pyrrole-C*H*), 8.56 (br.s, 1H, N*H*), 9.29 (br.s, 1H, N*H*); 11.29 (br.s, 1H, pyrrole-N*H*), 11.67 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 28.0 (^{t}Boc-*C*H$_3$), 81.4 (*Cq*), 114.0 (pyrrole-*C*H), 114.7 (pyrrole-*C*H), 127.7 (*Cq*), 132.2 (*Cq*), 156.0 (*Cq*), 158.5 (*Cq*), 162.5 (*Cq*), 167.9 (*Cq*)

7.4 Synthesis of the zwitterionic building blocks

7.4.1 Synthesis of the protected n = 1 zwitterion

N-Cbz-glycine[116]



$C_2H_5NO_2$
75.07

106

$C_{10}H_{11}NO_4$
209.20

110

A solution of glycine **106** (7.50 g, 99.91 mmol, 1.0 eq.) in a 2 *N* aqueous sodium hydroxide solution was cooled to 0 °C. Under vigorous stirring benzylchloroformiate (17.0 g, 99.9 mmol, 1.0 eq.) and a 4 *N* aqueous sodium hydroxide solution (25 ml) were added simultaneously. The reaction mixture was stirred for 30 min at room temperature, extracted with diethylether (2 x 50 ml) and acidified under cooling with an ice-water bath to pH 2 with 1 *N* hydrochloric acid. The white precipitate was filtered off, washed with cooled water and dried to yield the title compound **110** as a white solid (16.4 g, 79 %).

$C_{10}H_{11}NO_4$ Mw = 209.20

yield 16.4 g (79 %)

mp. 119-120 °C

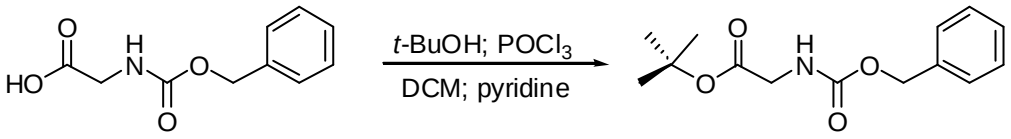
R_f 0.60 (SiO_2, chloroform/methanol = 9:1 + 1 ml of acetic acid per 100 ml eluent)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3330 [s], 2939 [w], 2566 [w], 1729 [s], 1680 [s], 1538 [s], 1453 [w], 1433 [w], 1413 [w], 1322 [m], 1290 [m], 1253 [s], 1170 [w], 1055 [w], 980 [s], 912 [w], 792 [m], 764 [m], 731 [m], 702 [m], 599 [m], 499 [w], 428 [w]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.67 (s, 2H, glycine-CH_2), 5.04 (s, 2H, benzyl-CH_2), 7.35 (m, 5H, aryl-C*H*), 7.53 (m, 1H, amide-N*H*), 12.55 (br.s, 1H, COO*H*)

^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.4 (glycine-*C*H_2), 65.7 (benzyl-*C*H_2), 127.9 (aryl-*C*H), 128.0 (aryl-*C*H), 128.6 (aryl-*C*H), 137.2 (aryl-*Cq*), 156.7 (*C*=O), 171.8 (*C*OOH)

N-Cbz-glycine-*tert*-butylester[116]



$C_{10}H_{11}NO_4$
209.20

110

$C_{14}H_{19}NO_4$
265.31

114

To a stirred solution of N-Cbz-glycine **110** (10.0 g, 47.9 mmol, 1.0 eq.) in *tert*-butanol (19.3 g, 5.1 eq.), dry dichloromethane (160 ml) and pyridine (19.3 g, 2.0 eq.) phosphorylchloride (8.95 ml, 2.0 eq.) was added at -10 °C. Upon stirring at room temperature for 18 h, the reaction mixture was diluted with chloroform (300 ml), washed three times with water (3 x 200 ml), then with a 3% sodium hydrogencarbonate solution (3 x 200 ml) and again with

water (3 x 200 ml). The organic phase was dried with Na_2SO_4 and concentrated *in vacuo* to afford the title compound114 as a colorless oil (8.05 g, 63 %).

$C_{14}H_{19}NO_4$ Mw = 265.31

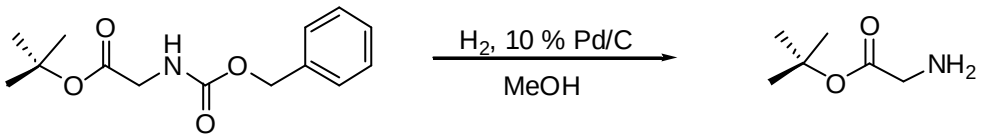
yield 8.05 g (63 %)

FT-IR $\tilde{n}$ (neat) [cm^{-1}] = 3352 [m], 3039 [w], 2979 [s], 1724 [s], 1523 [s], 1455 [m], 1367 [m], 1362 [w], 1223 [m], 1156 [s], 1054 [m], 993 [w], 848 [m], 752 [m], 699 [s]

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.45 (s, 9H, *tert*-butyl-C*H*$_3$), 3.85 (s, 2H, glycine-C*H*$_2$), 5.10 (s, 2H, benzyl-C*H*$_2$), 5.26 (br.s, 1H, amide-N*H*), 7.33 (m, 5H, aryl-C*H*)

^{13}C-NMR ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 28.0 (*tert*-butyl-*C*H$_3$), 43.4 (glycine-*C*H$_2$), 66.9 (benzyl-*C*H$_2$), 82.2 (*tert*-butyl-*Cq*), 128.1-128.7 (aryl-*C*H), 137.4 (aryl-*Cq*), 156.2 (*C*=O), 169.0 (*C*OOH)

Glycine-*tert*-butylester[116]



$C_{14}H_{19}NO_4$
265.31

114

$C_6H_{13}NO_2$
131.17

98

A mixture of N-Cbz-glycine-*tert*-butylester **114** (7.66 g, 28.9 mmol) and 10 % Pd/C (0.76 g) was vigorously stirred for 5 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the *tert*-butylester compound **98** as a colorless oil, which crystallizes after a few days in the refrigerator (3.58 g, 95 %).

$C_6H_{13}NO_2$ Mw = 131.17

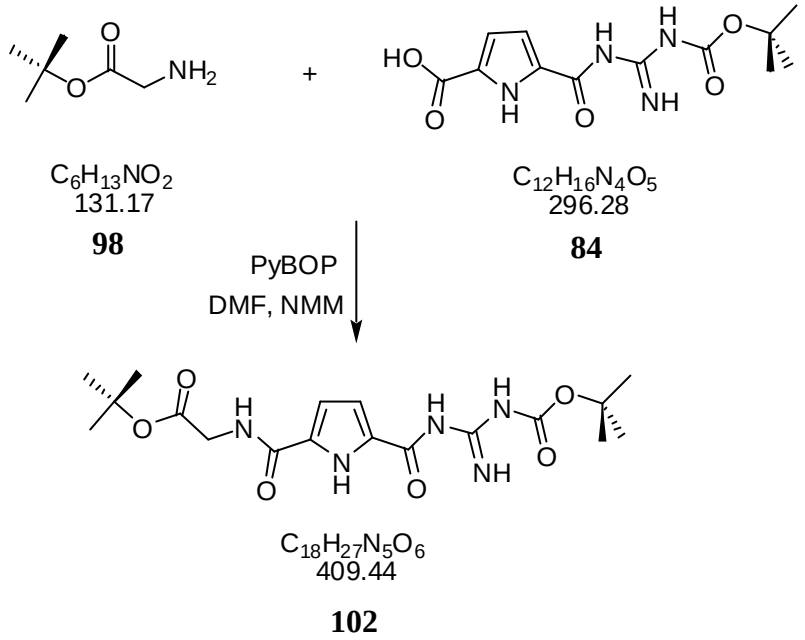
yield 3.58 g (95 %)

FT-IR $\tilde{n}$ (neat) [cm^{-1}] = 3350 [m], 2979 [w], 1741 [s], 1374 [m], 1253 [s] 1160 [s], 1038 [m], 843 [m]

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.44 (s, 9H, *tert*-butyl-C*H*$_3$), 3.78 (br.s, 2H, glycine-C*H*$_2$), 7.10 (br.s, 2H, amine-N*H*$_2$)

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 27.1 (*tert*-butyl-*C*H$_3$), 40.1 (glycin-*C*H$_2$), 82.6 (*tert*-butyl-*Cq*), 165.9 (*C*=O)

Protected guanidiniocarbonyl pyrrole zwitterion with n = 1



A mixture of the ^{t}Boc-protected guanidinocarbonyl pyrrole compound **84** (800 mg, 2.70 mmol, 1 eq.), PyBOP (1.40 g, 2.70 mmol, 1 eq.) and N-methyl morpholine (1 ml) was stirred in DMF (20 ml) at room temperature for 15 min. Glycine *tert*-butylester **98** (470 mg, 3.2 mmol, 1.2 eq.) was added to the solution and then stirred for 12 h at room temperature. The resulting dark solution was hydrolyzed with water (50 ml), the precipitate was filtered and dried in the desiccator over phosphorus pentoxide. The resulting solid was purified by column chromatography (SiO_2, cyclohexane/acetone = 3/2) yielding the title compound **102** as a colorless crystalline powder (1.05 g, 95 %).

$C_{18}H_{27}N_5O_6$ Mw = 409.44

yield 1.05 g (95 %)

R_f 0.25 (SiO_2, cyclohexane/acetone = 3/2)

mp. 157 °C

FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3401 [m], 3295 [m], 2978 [w], 2927 [w], 1722 [s], 1637 [s], 1544 [m], 1471 [m], 1369 [m], 1288 [s], 1236 [s], 1151 [s], 843 [w]

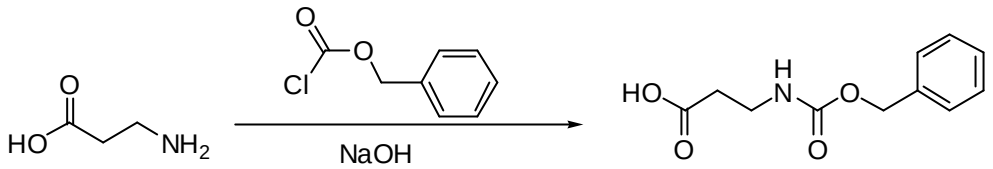
HR-MS (ESI) m/z = 432.185 ± 0.005 (calculated for $^{12}C_{18}H_{27}N_5O_6Na$: 432.186)

^{1}H-NMR ^{1}H-NMR (400 MHz, [D_6]DMSO) δ = 1.41 (s, 9H, C*H*$_3$), 1.45 (s, 9H, C*H*$_3$), 3.87 (d, J = 5.8 Hz, 2H, C*H*$_2$), 6.80 (br.s, 2H, pyrrole-C*H*), 8.55 (br.s, 1H, N*H*), 8.71 (t, J = 5.8 Hz, 1H, N*H*), 9.31 (br.s, 1H, N*H*), 10.82 (br.s, 1H, N*H*), 11.21 (br.s, 1H, N*H*)

^{13}C-NMR (100 MHz, [D_6]DMSO) δ = 27.9 (C*H*$_3$), 27.9 (C*H*$_3$), 41.5 (C*H*$_2$), 80.1 (*Cq*), 112.2 (pyrrole-C*H*), 126.0 (pyrrole-C*H*), 132 (*Cq*), 160.1 (*C*=O), 169.2 (*C*=O)

7.4.2 Synthesis of the protected n = 2 zwitterion

***N*-Cbz-*b*-Alanine**[116]



107 **111**

A mixture of *b*-alanine **107** (3.00 g, 33.7 mmol, 1 eq.) in an 2 *N* aqueous sodium hydroxide solution (17.0 ml) was chilled with an ice bath to 0°C. Under vigorous stirring benzylchloroformiate (5.22 ml, 37.1 mmol, 1.1 eq.) and a 2*N* aqueous sodium hydroxide solution (19.0 ml) were simultanously added over 2 minutes. The mixture was stirred for 20 minutes at room temperature, extracted with diethylether (4 x 40 ml), the aqueous layer was seperated and acidified with conc. hydrochloric acid to pH 2. The resulting emulsion was extracted with ethyl acetate (3 x 30 ml), the organic phases were combined, washed with brine and dried (Na_2SO_4). Concentration *in vacuo* gave the title compound **111** as white needles, which were dried *in vacuo* (7.51 g, 99 %).

$C_{11}H_{13}NO_4$ Mw = 223.07

yield 7.51 g (99 %)

R_f 0.28 (SiO_2, dichloromethane/ethyl acetate = 9/1)

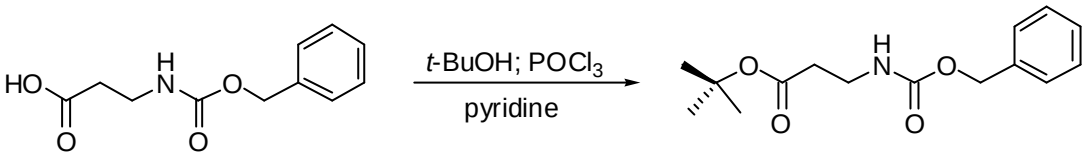
mp. 103 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3334 [s], 3070 [w], 2950 [w], 1685 [s], 1537 [s], 1220 [m], 730 [m], 697 [m]

^{1}H-NMR ^{1}H-NMR (400MHz, $CDCl_3$) δ [ppm] = 2.56 (br.s, 2H, C*H*$_2$), 3.44 (br.s, 2H, C*H*$_2$), 5.08 (s, 2H, benzyl-C*H*$_2$), 5.33 (br.s, 1H, N*H*), 7.26-7.34 (m, 5H, aryl-C*H*), 8.51 (br.s, 1H, COO*H*)

^{13}C-NMR (100MHz, $CDCl_3$) δ [ppm] = 34.6 (*C*H$_2$), 36.7 (*C*H$_2$), 67.3 (benzyl-*C*H$_2$), 128.5 (aryl-*C*H), 128.6 (aryl-*C*H), 128.9 (aryl-*C*H), 136.8 (aryl-*Cq*), 156.8 (*C*=O), 171.7 (*C*OOH)

***N*-Cbz-*b*-Alanine-*tert*-butylester**[116]



$C_{11}H_{13}NO_4$
223.2252

111

$C_{15}H_{21}NO_4$
279.3315

115

A mixture of *N*-Cbz-*b*-alanine **111** (4.00 g, 17.9 mmol, 1 eq.), pyridine (27.0 ml) and *tert*-butanol (45.0 ml) was chilled to -10 °C. Under stirring phosphorylchloride (1.81 ml, 19.7 mmol, 1.1 eq.) was added and the solution was stirred at -10 °C for 30 min. The clear, yellow mixture was stirred at room temperature over night, concentrated *in vacuo* and diluted with water (20.0 ml). The mixture was extracted with ethyl acetate (3 x 5.0 ml), the organic layers were combined, washed with water (3 x 55.0 ml), saturated aqueous sodium sulfate solution (3 x 55.0 ml), again water (3 x 55.0 ml), 2 *N* aqueous potassium hydrogen sulfate solution (3 x 55.o ml), brine (2 x 30.0 ml) and dried over Na_2SO_4. The solvent was evaporated yielding an orange oil, which was dried *in vacuo* (3.75 g, 75 %).

$C_{15}H_{21}NO_4$ Mw = 279.11

yield 3.75 g (75 %)

R_f 0.67 (SiO_2, dichloromethane/ethyl acetate = 9/1)

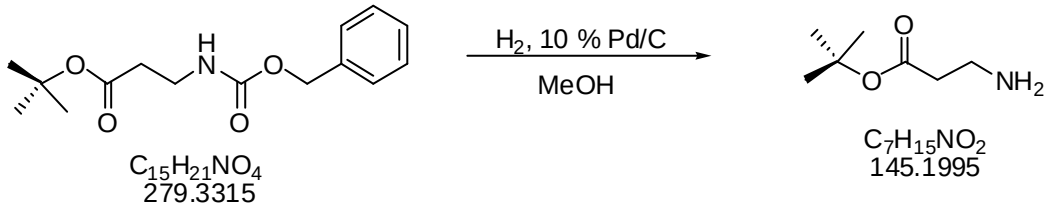
FT-IR $\tilde{n}$ (neat) [cm^{-1}] = 3350 [m], 3070 [m], 2979 [m], 1725 [s], 1522 [s], 1367 [m], 740 [m], 699 [m]

HR-MS (ESI) 302.1368 ± 0.005 (calculated for $C_{15}H_{21}NO_4Na$: 302.1368)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.39 (s, 9H, *tert*-butyl-C*H*$_3$), 2.40 (t, 2H, J = 6.7 Hz, C*H*$_2$), 2.70 (t, 2H, J =6.6 Hz, C*H*$_2$), 5.02 (benzyl-C*H*$_2$), 5.33 (br.s, 1H, N*H*), 7.25-7.33 (m, 5H, aryl-C*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 27.9 (*tert*-butyl-*C*H$_3$), 37.2 (*C*H$_2$), 38.7 (*C*H$_2$), 67.2 (benzyl-*C*H$_2$), 79.7 (*C*(CH$_3$)$_3$), 128.5 (aryl-*C*H), 128.6 (aryl-*C*H), 128.9 (aryl-*C*H), 136.8 (aryl-*Cq*), 156.8 (*C*=O), 171.6 (*C*=0)

***b*-Alanine-*tert*-butylester**[116]



$C_{15}H_{21}NO_4$
279.3315

115

$C_7H_{15}NO_2$
145.1995

99

A mixture of *N*-Cbz-*b*-alanine-*tert*-butylester **115** (3.41 g, 12.2 mmol) and 10 % Pd/C (0.34 g) in methanol (70 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with ethyl acetate. The filtrate and washings were combined and evaporated to give the *tert*-butylester **99** as a orange oil, which was dried *in vacuo* (1.47 g, 83 %).

$C_7H_{15}NO_2$ Mw = 145.05

yield 1.47 g (83 %)

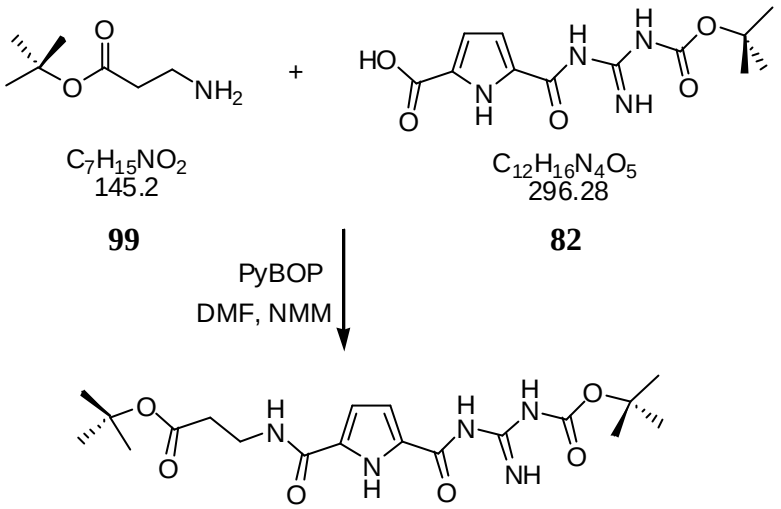
R_f 0.66 (SiO_2, dichloromethane/ethanol = 7/3)

FT-IR $\tilde{n}$ (neat) [cm^{-1}] = 3379 [m], 2977 [w], 1725 [s], 1367 [s], 1247 [s], 845 [m]

^{1}H-NMR (400MHz, [D_6]DMSO) δ [ppm] = 1.39 (s, 9H, *tert*-butyl-C*H*$_3$), 2.28 (t, 2H, J = 6.7 Hz, C*H*$_2$), 2.70 (t, 2H, J = 6.6, C*H*$_2$), 3.10 (br.s, 2H, N*H*$_2$)

^{13}C-NMR (100MHz, [D_6]DMSO) δ [ppm] = 27.9 (*tert*-butyl-*C*H_3), 37.7 (*C*H_2), 38.7 (*C*H_2), 79.7 (*C*(CH_3)$_3$), 171.6 (*C*=O)

Protected guanidiniocarbonyl pyrrole zwitterion with n = 2



A mixture of the ^{t}Boc-protected guanidinocarbonylpyrrole compound **84** (800 mg, 2.7 mmol, 1 eq.), PyBOP (1.4 g, 2.7 mmol, 1 eq.) and N-methyl morpholine (1 ml) was stirred in DMF (20 ml) at room temperature for 15 min. β-Alanine-*tert*-butylester **99** (424 mg, 3.2 mmol, 1.2 eq.) was added to the solution and then stirred for 12 h at room temperature. The resulting dark solution was hydrolyzed with water (50 ml), the precipitate was filtered and dried in the desiccator over phosphorus pentoxide. The resulting solid was purified by column chromatography (SiO_2, cyclohexane/acetone = 3/2) yielding the title compound **103** as a colorless crystalline powder (970 mg, 85 %).

$C_{19}H_{29}N_5O_6$ Mw = 423.47

yield 970 mg (85 %)

R_f 0.43 (SiO_2, cyclohexane/acetone = 3/2)

mp. 98 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3387 [w], 2921 [w], 1727 [m], 1634 [s], 1554 [s], 1301 [s], 1243 [s], 844 [m]

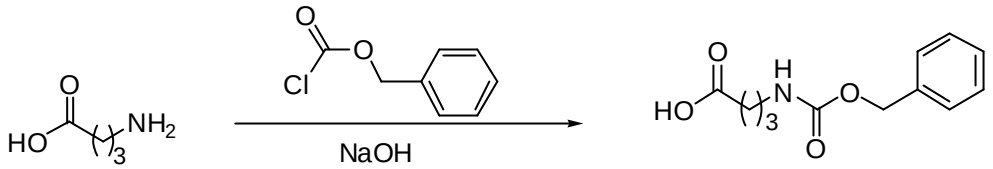
HR-MS (ESI) m/z = 446.201 ± 0.005 (calculated for $C_{19}H_{29}N_5O_6Na$: 446.202)

^{1}H-NMR (400MHz, [D_6]DMSO) δ [ppm] = 1.38 (s, 9H, C*H*$_3$), 1.45 (s, 9H, C*H*$_3$), 2.45 (t, 2H, J = 6.8 Hz, C*H*$_2$), 3.38-3.43 (m, 2H, C*H*$_2$), 6.73-6.84 (m, 2H, pyrrole-C*H*), 8.39 (t, 1H, J = 5.5 Hz, N*H*), 8.55 (br.s, 1H, N*H*), 9.30 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*), 11.23 (s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ [ppm] = 27.9 (*tert*-butyl-*C*H$_3$), 35.1 (*C*H$_2$), 35.2 (*CH*$_2$), 80.1 (*C*(CH$_3$)$_3$), 111.9 (pyrrole-*C*H), 159.7 (*C*=O), 170.7 (*C*=O)

7.4.3 Synthesis of the protected n = 3 zwitterion

***N*-Cbz-4-aminobutyric acid**



$C_4H_9NO_2$
103.06

108

$C_{12}H_{15}NO_4$
237.10

112

A mixture of 4-aminobutyric acid **108** (2.00 g, 19.4 mmol, 1 eq.) and a 2 *N* aqueous sodium hydroxide solution (9.50 ml) was chilled in an ice bath to 0 °C. Under vigorous stirring both benzylchloroformiate (3.00 ml, 21.3 mmol, 1.1 eq.) and an aqueous 2 *N* sodium hydroxide solution were simultanously added over 2 min. After stirring for 20 min at room temperature the mixture was extracted four times with diethylether (4 x 20 ml). The aqueous layer was separated and acidified with conc. hydrochloric acid to pH 2. The suspension was extracted with ethyl acetate (15 ml). The organic phases were combined, washed with brine, dried (Na_2SO_4) and evaporated to give a slightly yellow oil. Through addition of *n*-hexane a white solid crystallizes, which was filtered and dried *in vacuo* (4.03 g, 87 %).

$C_{12}H_{15}NO_4$ Mw = 237.08

yield 4.03 g (87 %)

R_f 0.20 (SiO_2, dichloromethane/ethyl acetate = 9/1)

mp. 59 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3332 [s], 3070 [w], 2933 [w], 1687 [s], 1549 [s],

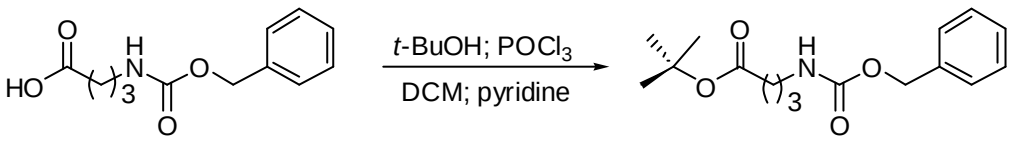
1273 [s], 1208 [s], 694 [m]

MS (neg.ESI) m/z = 236.14 $[M-H^+]^-$

^{1}H-NMR (400MHz, $CDCl_3$) δ [ppm] = 1.77-1.84 (m, 2H, C*H*$_2$), 2.36 (t, 2H, J = 7.2 Hz, C*H*$_2$), 3.18-3.26 (m, 2H, C*H*$_2$), 5.00 (br.s, 1H, N*H*), 5.08 (s, 2H, benzyl-C*H*$_2$), 7.27-7.36 (m, 5H, aryl-C*H*), 9.86 (br.s, 1H, COO*H*)

^{13}C-NMR (100MHz, $CDCl_3$) δ [ppm] = 24.9 (*C*H$_2$), 31.1 (*C*H$_2$), 40.2 (*C*H$_2$), 66.8 (benzyl-*C*H$_2$), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.5 (aryl-*C*H), 136.4 (aryl-*Cq*), 156.6 (*C*=O), 178.2 (*C*OOH)

***N*-Cbz-4-aminobutyric acid-*tert*-butylester**



$C_{12}H_{15}NO_4$
237.10

112

$C_{16}H_{23}NO_4$
293.16

116

A mixture of *N*-Cbz-4-aminobutyric acid **112** (4.00 g, 17.9 mmol, 1 eq.), pyridine (25.0 ml) and *tert*-butanol (42.0 ml) was chilled to -10 °C. Under vigorous stirring phosphorylchloride (1.68 ml, 18.4 mmol, 1.1 eq.) was added and the solution was stirred at -10 °C for 30 min. The clear yellow mixture was stirred at room temperature over night, concentrated *in vacuo* and diluted with water (20.0 ml). The mixture was extracted with ethyl acetate (3 x 40 ml), the organic layers were combined, washed with water (3 x 50.0 ml), saturated aqueous sodium sulfate solution (3 x 50.0 ml), again with water (3 x 50.0 ml), 2 *N* aqueous potassium hydrogensulfate solution (3 x 55. ml), brine (2 x 30.0 ml) and dried over Na_2SO_4. The solvent was evaporated yielding a slightly yellow oil, which was dried *in vacuo* (3.75 g, 62 %).

$C_{21}H_{23}NO_4$ Mw = 353.17

yield 3.75 g (62 %)

R_f 0.66 (SiO_2, dichloromethane/ethyl acetate = 9/1)

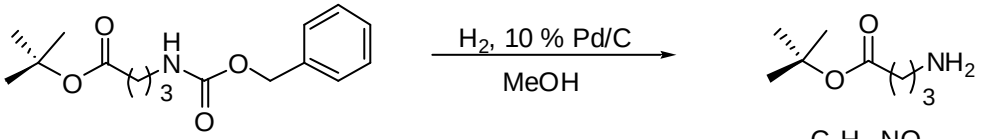
FT-IR $\tilde{n}$ (neat) [cm^{-1}] = 3350 [m], 3070 [w], 2976 [m], 1727 [s], 1537 [s], 698 [m]

HR-MS (ESI) m/z = 316.153 ± 0.005 (calculated for $^{12}C_{21}H_{23}NO_4Na$: 316.153)

^{1}H-NMR (400MHz, $CDCl_3$) δ [ppm] = 1.43 (s, 9H, *tert*-butyl-C*H*$_3$), 1.75-1.82 (m, 2H, C*H*$_2$), 2.30 (t, 2H, J = 7.1 Hz, C*H*$_2$), 3.18-3.26 (m, 2H, C*H*$_2$), 4.90 (br.s., 1H, N*H*), 5.09 (s, 2H, C*H*$_2$), 7.30-7.36 (m, 5H, aryl-C*H*)

^{13}C-NMR (100MHz, $CDCl_3$,) δ [ppm] = 25.2 (*C*H$_2$), 28.0 (*C*H$_3$), 32.8 (*C*H$_2$), 40.5 (*C*H$_2$), 66.6 (benzyl-*C*H$_2$), 80.5 (*C*(CH$_3$)$_3$), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.5 (aryl-*CH*), 136.6 (C*q*), 156.4 (C=O), 172.5 (*C*=O)

4-Aminobutyric acid-*tert*-butylester



$C_{16}H_{23}NO_4$
293.16

116

$C_8H_{17}NO_2$
159.13

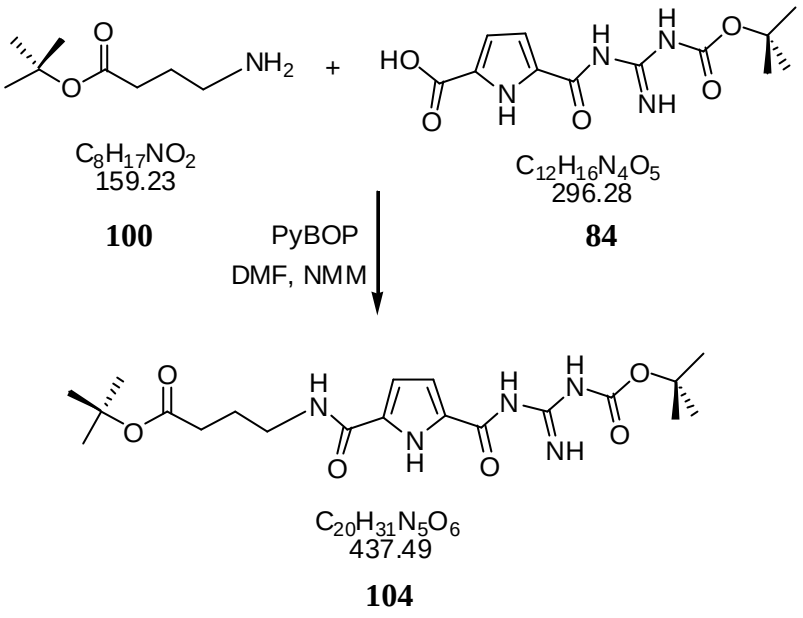
100

A mixture of *N*-Cbz-aminobutyric acid-*tert*-butylester **116**(7.50 g, 25.5 mmol) and 10 % Pd/C (0.75 g) in tetrahydrofuran (150 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with ethylester.

The filtrate and washings were combined and evaporated to give the *tert*-butylester **100** as an slightly yellow oil, which was dried *in vacuo* (3.77 g, 94 %).

$C_8H_{17}NO_2$	Mw = 159.06
yield	3.77 g (94 %)
R_f	0.66 (SiO_2, dichloromethane/ethanol = 7/3)
FT-IR	$\tilde{n}$ (neat) [cm^{-1}] = 3372 [m], 2976 [m], 1728 [s], 1455 [m], 1367 [m], 1255 [m], 847 [m]
^{1}H-NMR	(400MHz, $CDCl_3$) δ = 1.39 (s, 9H, *tert*-butyl-C*H*$_3$), 1.66-1.71 (m, 2H, C*H*$_2$), 1.77 (br.s, 2H, N*H*$_2$), 2.22 (t, 2H, J = 7.2 Hz, C*H*$_2$), 2.69 (t, 2H, J = 6.69 Hz, C*H*$_2$)
^{13}C-NMR	(100MHz, [D_6]DMSO) δ = 28.0 (*tert*-butyl-*C*H_3), 28.8 (*C*H_2), 32.9 (*C*H_2), 41.4 (*C*H_2), 80.1 (*Cq*), 172.8 (*C*=O)

Protected guanidiniocarbonyl pyrrole zwitterion with n = 3



A mixture of the ^{t}Boc-protected guanidinocarbonyl pyrrole compound **84** (2.00 g, 6.75 mmol, 1 eq.), PyBOP (3.51 g, 6.75 mmol, 1 eq.) and N-methyl morpholine (2 ml) in DMF (40 ml) was stirred at room temperature for 15 min. The *tert*-butylester **100** (1.07 g, 6.75 mmol, 1 eq.) was added to the solution and then stirred for 12 h at room temperature. The resulting red solution was hydrolyzed with water (100 ml), the resulting suspension was extracted with diethyl ether (3 x 100 ml), the organic phases were combined, dried (Na_2SO_4) and evaporated. The resulting orange oil was purified by column chromatography (SiO_2, hexane/ethyl acetate= 3/2) yielding compound **104** as a slightly yellow, crystalline powder (2.05 g, 85 %).

$C_{20}H_{31}N_5O_6$ Mw = 437.49

yield 2.50 g (85 %)

R_f 0.57 (SiO_2, hexane/ethyl acetate = 3/2)

mp. 100-103 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3385 [m], 3320 [br. m.], 2979 [m], 1728 [s], 1556 [s],1469 [s], 1393[s], 1369 [m], 1300 [s], 1243 [s], 1152 [s], 1050 [w], 843.7 [m], 789 [w], 757 [w], 602 [w]

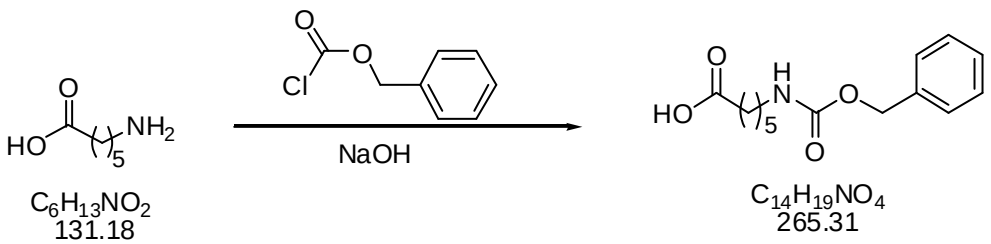
HR-MS (ESI) m/z = 460.217 ± 0.005 (calculated for $^{12}C_{20}H_{31}N_5O_6Na$: 406.217)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.33 (s, 9H, C*H*$_3$), 1.34 (s, 9H, C*H*$_3$), 1.62-1.69 (m, 2H, C*H*$_2$), 2.19 (t, 2H, J = 7.5 Hz, C*H*$_2$), 3.15-3.20 (m, 2H, C*H*$_2$), 6.71 (s, 1H, pyrrole-C*H*), 6.75 (s, 1H, pyrrole-C*H*), 8.25 (t, 1H, N*H*, J = 5.5 Hz), 8.50 (br.s, 1H, N*H*), 9.25 (br.s, 1H, N*H*), 10.87 (br.s, 1H, N*H*), 11.22 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 25.1 (*C*H$_2$), 28.1 (*C*H$_3$), 28.1 (*C*H$_3$), 32.7 (*C*H$_2$), 38.4 (*C*H$_2$), 79.9 (*Cq*), 112 (pyrrole-*C*H), 158.8 (*Cq*), 160.0 (*C*=O), 172.3 (*C*=O)

7.4.4 Synthesis of the protected n = 5 zwitterion

***N*-Cbz-6-aminocaproic acid**



$C_6H_{13}NO_2$
131.18

109

$C_{14}H_{19}NO_4$
265.31

113

A mixture of 6-aminocaproic acid **109** (5.00 g, 38.1 mmol, 1 eq.) and an aqueous 2 *N* sodium hydroxide solution (19.0 ml) was chilled in an ice bath to 0°C. Under vigorous stirring

simultanously both benzylchloroformiate (5.9 ml, 41.9 mmol, 1.1 eq.) and an aqueous 2 *N* sodium hydroxide solution (21.0 ml) were added over 2 min. After stirring for 20 min at room temperature the mixture was extracted four times with diethylether (4 x 50 ml). The aqueous layer was separated and acidified with conc. hydrogen chloride to pH 2. The suspension was extracted with ethyl acetate (35 ml). The organic phases were combined, washed with brine, dried (Na_2SO_4) and evaporated to give a white solid which was dried *in vacuo* (9.67 g, 99 %)

$C_{14}H_{19}NO_4$ Mw = 256.31

yield 9.67 g (99 %)

R_f 0.41 (SiO_2, dichloromethane/ethyl acetate = 9/1)

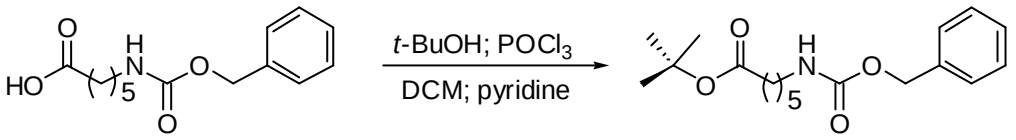
mp. 56 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3344 [s], 3050 [w], 2920 [s], 2852 [s], 1685 [s], 1528 [s], 1273 [m], 1236 [s], 696 [w];

^{1}H-NMR (400MHz, $CDCl_3$) δ [ppm] = 1.35-1.42 (m, 2H, C*H*$_2$), 1.50-1.58 (m, 2H, C*H*$_2$), 1.63-1.70 (m, 2H, C*H*$_2$), 2.36 (t, 2H, J = 7.4 Hz, C*H*$_2$), 3.21 (t, 2H, J = 6.3 Hz, C*H*$_2$), 4.87 (br.s., 1H, N*H*), 5.13 (s, 2H, benzyl-C*H*$_2$), 7.33-7.39 (m, 5H, aryl-C*H*), 9.36 (br.s., 1H, COO*H*)

^{13}C-NMR (100MHz, $CDCl_3$) δ [ppm] = 24.2 (*C*H$_2$), 26.1 (*C*H$_2$), 29.5 (*C*H$_2$), 33.8 (*C*H$_2$), 40.8 (*C*H$_2$), 66.7 (benzyl-CH$_2$), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.5 (aryl-*C*H), 136.5 (aryl-*Cq*), 156.5 (*C*=O), 179.0 (*C*=O)

N-Cbz-6-Aminocaproic acid *tert*-butylester



$C_{14}H_{19}NO_4$
256.31

113

$C_{18}H_{27}NO_4$
321.42

117

A mixture of *N*-Cbz-6-aminocaproic acid **113** (9.57 g, 36.1 mmol, 1 eq.), pyridine (54.0 ml) and *tert*-butanol (54.0 ml) was chilled to -10 °C. Under vigorous stirring phosphorylchloride (3.70 ml, 39.7 mmol, 1.1 eq.) was added and the solution was stirred at -10 °C for 30 min. The clear yellow mixture was stirred at room temperature over night, concentrated *in vacuo* and diluted with water (20.0 ml). The mixture was extracted with ethyl acetate (3 x 40 ml), the organic layers were combined, washed with water (3 x 50.0 ml), saturated aqueous sodium sulfate solution (3 x 50.0 ml), again with water (3 x 50.0 ml), 2 *N* aqueous potassium hydrogensulfate solution (3 x 55.0 ml), brine (2 x 30.0 ml) and dried with Na_2SO_4. The solvent was evaporated yielding a slightly yellow oil, which was dried *in vacuo* (3.75 g, 62 %).

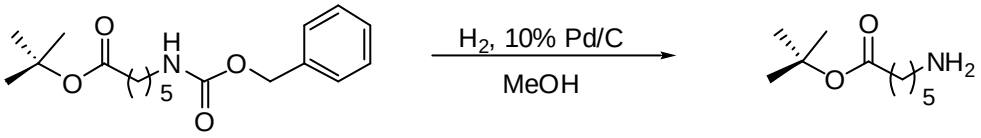
$C_{21}H_{23}NO_4$ Mw = 353.42

yield 6.99 g (60 %)

^{1}H-NMR (400MHz, [D_6]DMSO) δ [ppm] = 1.20-1.28 (m, 2H, C*H*$_2$), 1.38 (s, 9H, *tert*-butyl-C*H*$_3$), 1.45-1.47 (m, 2H, C*H*$_2$), 2.13-2.17 (m, 2H, C*H*$_2$), 2.34 (t, 2H, J = 7.4 Hz, C*H*$_2$), 2.96-2.97 (m, 2H, C*H*$_2$), 4.99 (s, 2H, benzyl-C*H*$_2$), 7.21 (br.s, 1H, N*H*), 7.30-7.37 (m, 5H, aryl-C*H*)

^{13}C-NMR (100MHz, DMSO) δ [ppm] = 24.2 (*C*H$_2$), 26.1 (*C*H$_3$), 29.5 (*C*H$_2$), 33.9 (*C*H$_2$), 35.5 (CH$_2$), 40.5 (*C*H$_2$), 66.6 (*C*H$_2$), 80.5 (*C*(CH$_3$)$_3$), 128.0 (aryl-*C*H), 128.5 (aryl-*CH*), 136.6 (C*q*), 156.4 (C=O), 172.5 (*C*=O)

6-Aminocaproic acid-*tert*-butylester



$C_{18}H_{27}NO_4$
321.42

117

$C_{10}H_{21}NO_2$
187.28

101

A mixture of *N*-Cbz-aminocaproic acid-*tert*-butylester **117** (6.99 g, 21.8 mmol) and 10 %-Pd/C (0.70 g) in tetrahydrofuran (140 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with ethyl acetate. The filtrate and washings were combined and evaporated to give the *tert*-butylester **X** as an orange oil, which was dried *in vacuo* (4.04 g, 21.60 mmol, 99 %).

$C_{10}H_{21}NO_2$ Mw = 187.28

yield 4.04 g (99 %)

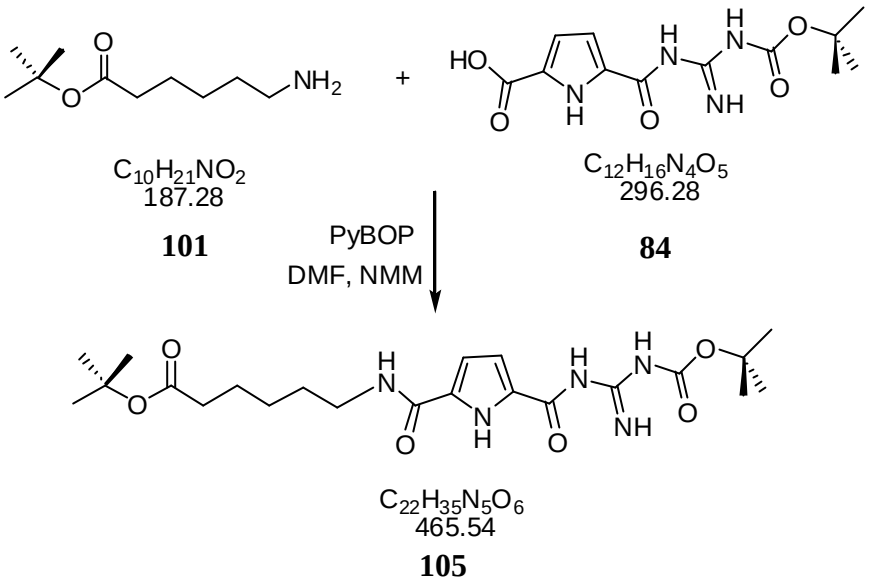
R_f 0.66 (SiO_2, dichloromethane/ethanol = 7/3)

FT-IR $\tilde{n}$ (neat) [cm^{-1}] = 3378 [m], 2931 [w], 1729 [s], 1457 [s], 1367 [s], 848 [m]

^{1}H-NMR (400MHz, $CDCl_3$) δ [ppm] = 1.23-1.28 (m, 2H, C*H*$_2$), 1.35 (s, 9H, *tert*-butyl-CH_3), 1.37-1.41 (m, 2H, C*H*$_2$), 1.47-1.55 (m, 2H, C*H*$_2$), 2.12 (t, 2H, J = 7.44 Hz, C*H*$_2$), 2.60 (t, 2H, J = 6.69 Hz, C*H*$_2$)

^{13}C-NMR (100MHz, $CDCl_3$) δ [ppm] = 24.7 (*C*H_2), 26.2 (*C*H_2), 27.9 (*tert*-butyl-CH_3), 33.2 (*C*H_2), 35.4 (*C*H_2), 41.8 (*C*H_2), 79.8 (*Cq*), 171.9 (C=O)

Protected guanidiniocarbonyl pyrrole zwitterion with n = 5



A mixture of the ^{t}Boc-protected guanidinocarbonyl pyrrole compound **84** (2.00 g, 6.75 mmol, 1 eq.), PyBOP (3.51 g, 6.75 mmol, 1 eq.) and N-methyl morpholine (2 ml) was stirred in DMF (40 ml) at room temperature for 15 min. The *tert*-butylester **101** (1.51 g, 8.1 mmol, 1.2 eq.) was added to the solution and then stirred for 12 h at room temperature. The resulting red solution was hydrolyzed with water (100 ml), the suspension extracted with diethylether (3 x 100 ml) and the organic phases were combined, dried (Na_2SO_4) and evaporated. The resulting orange oil was purified by column chromatography (SiO_2, hexane/ethyl acetate= 3/2) yielding the title compound **105** as a slightly yellow, crystalline powder (2.83 g, 90 %).

$C_{20}H_{31}N_5O_6$ Mw = 437.49

yield 2.83 g (90 %)

R_f 0.57 (SiO_2, hexane/ethyl acetate = 3/2)

mp. 100-103°C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3400 [m], 3320 [br. m.], 2980 [m], 1730 [s], 1550 [s],1469 [s], 1393[s], 1300 [s], 1243 [s], 1152 [s], 1050 [w], 843.7 [m], 789 [w], 760 [w], 602 [w]

HR-MS (ESI) m/z = 460.217 ± 0.005 (calculated for $^{12}C_{20}H_{31}N_5O_6Na$: 406.217)

^{1}H-NMR (400MHz, [D_6]DMSO) δ [ppm] = δ = 1.21-1.34 (m, 2H, C*H*$_2$).1.36 (s, 9H, C*H*$_3$), 1.45 (s, 9H, C*H*$_3$), 1.45-1.54 (m, 4H, C*H*$_2$), 2.17 (t, 2H, C*H*$_2$, J = 7.3 Hz), 3.15-3.20 (m, 2H, C*H*$_2$), 6.75 (s, 1H, pyrrole-C*H*), 6.80 (s, 1H, pyrrole-C*H*), 8.27 (t, 1H, N*H*, J = 5.4 Hz), 8.55 (br.s, 1H, N*H*), 9. 30 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*), 11.26 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ [ppm] = 24.5 (*C*H$_2$), 26.0 (*C*H$_2$), 27.9 (*C*H$_3$), 27.9 (*C*H$_3$), 28.9 (*C*H$_2$), 34.8 (*C*H$_2$), 38.6 (*C*H$_2$), 79.9 (*Cq*), 111.7 (pyrrole-*C*H), 113.9 (pyrrole-*C*H), 124.3 (*Cq*), 129.9 (*Cq*), 158.6 (*Cq*), 159.6 (*Cq*), 172.3 (*Cq*)

7.4.5 Zwitterionic compounds with n = 1, 2, 3, 5

General Method for the diprotection of the ^{t}Boc and *tert*-butyl group

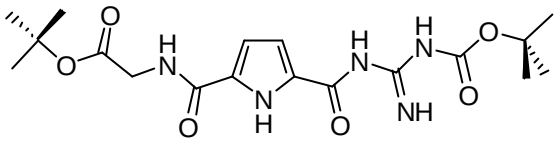
A solution of the corresponding protected guanidiniocarbonyl pyrrole compound (100 mg) in trifluoroacetic acid (5 ml) was stirred for 30 min at room temperature. The trifluoroacetic acid was evaporated *in vacuo* yielding a slightly yellow oil. Addition of a few drops of diethylether produced the white trifluoroacetate salt, which was filtered and dried *in vacuo*.

The picrate salts of the guanidiniocarbonyl pyrrole compounds were produced for analytic purpose by solving the trifluoroacetate salt in methanol (10 ml) and addition of aqueous picric acid to afford the yellow picrate salt, which was filtered and dried over phosphorus pentoxide in the desiccator (yield: quantitative)

The guanidiniocarbonyl pyrrole carboxylate zwitterions were produced via solving the trifluoroacetate salts in 1*N* aqueous sodium hydroxide solution and adjusting the pH to a

value of 5 with 1 *N* aqueous hydrochloride acid. The white precipitate was filtered (yield = 70-80 %) and dried over phosphorus pentoxide in the desiccator. The recrystallization of the zwitterions were repeated four times to ensure an analytically pure zwitterionic product.

Picrate of the zwitterion n = 1

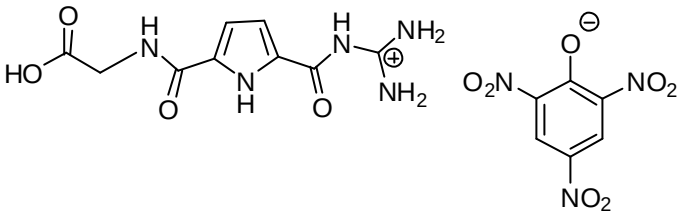


$C_{18}H_{27}N_5O_6$
409.44

102

1.TFA

2. MeOH, picric acid



$C_{15}H_{14}N_8O_{11}$
482.32

3

$C_{15}H_{14}N_8O_{11}$ Mw = 483.32

mp. 243-244 °C (decomposition)

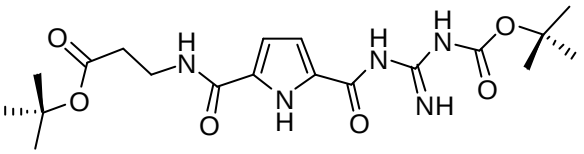
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3404 [m], 3325 [m], 3168 [m], 1703 [s], 1611 [s], 1557 [m], 1427 [w], 1365 [m], 1337 [s], 1268 [s], 1082 [w], 911[w], 816 [w], 763 [w]

HR-MS (ESI) m/z = 254.089 ± 0.005 (calculated for $^{12}C_9H_{12}N_5O_4$: 254.089)

^{1}H-NMR (400 MHz, [D$_6$]DMSO) δ = 3.94 (d, J = 5.8 Hz, 2H, C*H*$_2$), 6.91 (m, 1H, pyrrole-C*H*), 7.03 (m, 1H, pyrrole-C*H*), 8.16 (br.s, 4H, guanidium-N*H*$_2$), 8.58 (s, 2H, picrate-C*H*), 8.81 (t, J = 5.8 Hz, 1H, N*H*), 10.94 (br.s, 1H, guanidinium-N*H)*, 12.46 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100 MHz, [D$_6$]DMSO) δ = 40.9 (*C*H$_2$), 112.8 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 124.4 (picrate-*C*H), 125.38 (*Cq*), 125.9 (*Cq*), 132.4 (*Cq*), 142.0 (*Cq*), 155.0 (*Cq*), 159.6 (*Cq*), 159.6 (*Cq*), 161.0 (*Cq*), 171.3 (*Cq*)

Picrate of the zwitterion n = 2

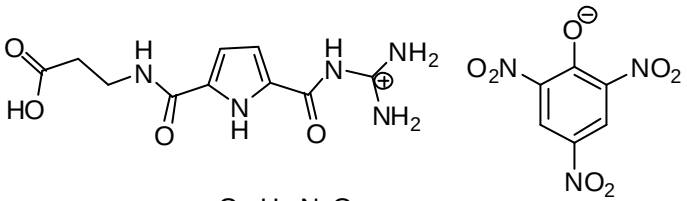


$C_{19}H_{29}N_5O_6$
423.46

103

1.TFA

2. MeOH, picric acid



$C_{16}H_{16}N_8O_{11}$
496.35

4

$C_{16}H_{16}N_8O_{11}$ Mw = 496.35

mp. 250-251 °C (decomposition)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3400 [m], 3325 [m], 3168 [m], 1705 [s], 1611 [s], 1557 [m], 1427 [w], 1370 [m], 1335 [s], 1268 [s], 1082 [w], 814 [w], 763

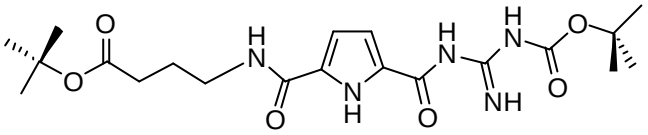
HR-MS (ESI) m/z = 268.105 ± 0.005 (calculated for $^{12}C_{10}H_{14}N_5O_4$: 268.105)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 3.43 (q, J = 6.3 Hz, 2H, C*H*$_2$), 6.85-6.87 (m, 1H, pyrrole-C*H*), 7.00-7.02 (m, 1H, pyrrole-C*H*), 8.16 (br.s, 4H, guanidinium-N*H*$_2$), 8.54 (t, J = 11.6, 1H, N*H*), 8.58 (s, 2H, picrate-C*H*), 10.94 (br.s, 1H, guanidinium-N*H*), 12.42 (br.s, 1H, pyrrole-N*H*)

one C*H*$_2$ peak is superposed by the solvent signal

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 33.9 (*C*H$_2$), 35.3 (*C*H$_2$), 112.7 (pyrrole-*C*H), 115.6 (pyrrole-*C*H), 125.4 (picrate-*C*H), 125.6 (*Cq*), 132.8 (*Cq*), 142.0 (*Cq*), 155.0 (*Cq*), 159.3 (*Cq*), 159.6 (*Cq*), 161.0 (*Cq*), 173.0 (*C*OOH)

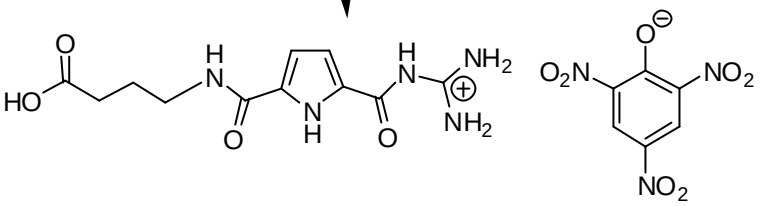
Picrate of the zwitterion n = 3



$C_{20}H_{31}N_5O_6$
437.49

104

1. TFA
2. MeOH, picric acid



$C_{17}H_{18}N_8O_{11}$
510.37

5

$C_{17}H_{18}N_8O_{11}$ Mw = 510.37

mp. 248 °C (decomposition)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3546 [w], 3388 [s], 3132 [m], 2924 [w], 1714 [s], 1634 [s], 1567 [s], 1476 [m], 1433 [m], 1287 [s], 1204 [m], 1080 [w], 616 [w]

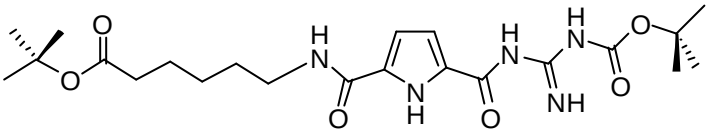
HR-MS (ESI) m/z 282.121 ± 0.005 (calculated for $^{12}C_{11}H_{16}N_5O_4$: 282.120);

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.69-1.77 (m, 2H, C*H*$_2$), 2.27 (t, 2H, C*H*$_2$, J = 7.45 Hz), 3.23-3.28 (m, 2H, C*H*$_2$), 6.85-6.87 (m, 1H, pyrrole-N*H*), 7.01-7.03 (m, 1H, pyrrole-N*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.42 (t, 1H, J = 5.43 Hz, N*H*), 8.57 (s, 2H, picrate-C*H*), 10.93 (br.s, 1H, N*H*), 12.33

(br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D$_6$]DMSO) δ = 24.6 (C*H*$_2$), 31.2 (C*H*$_2$), 38.4 (C*H*$_2$), 112.4 (pyrrole-C*H*), 115.7 (pyrrole-C*H*), 124.4 (*Cq*), 125.4 (C*H*), 133.0 (*Cq*), 142.0 (*Cq*), 155.0 (*Cq*), 159.2 (*Cq*), 159.6 (*Cq*), 161.0 (*Cq*), 174.3 (*Cq*)

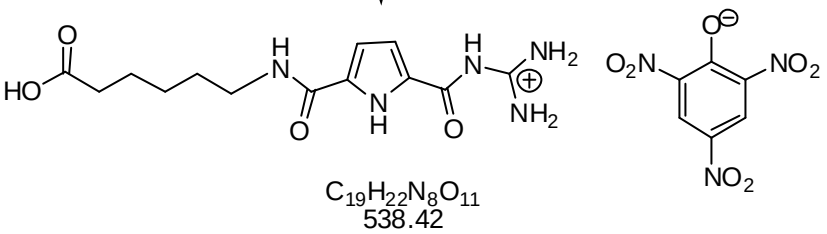
Picrate of the zwitterion n = 5



$C_{22}H_{35}N_5O_6$
465.54

105

1. TFA
2. MeOH, picric acid



$C_{19}H_{22}N_8O_{11}$
538.42

6

$C_{19}H_{22}N_8O_{11}$ Mw = 538.43

mp. 250 °C (decomposition)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3541 [w], 3388 [s], 2930 [w], 1711 [s], 1634 [s], 1570 [s], 1476 [m], 1430 [m], 1287 [s], 1200 [m], 1084 [w]

HR-MS (ESI) m/z 310.152 ± 0.005 (calculated for $^{12}C_{13}H_{20}N_5O_4$: 310.152)

^{1}H-NMR (400MHz, [D$_6$]DMSO) δ = 1.27-1.32 (m, 2H, C*H$_2$*), 1.48-1.54 (m, 2H, C*H$_2$*), 2.20 (t, 2H, C*H$_2$*, J = 7.2 Hz), 3.21-3.23 (m, 2H, C*H$_2$*), 6.86 (s, 1H, pyrrole-C*H*), 7.01 (s, 1H, pyrrole-C*H*), 8.15 (br.s, 4H, guanidinium-N*H$_2$*), 8.39 (t, 1H, N*H*, J = 5.0 Hz), 8.57 (s, 2H, picrate-C*H*), 10.92 (br.s, 1H, N*H*), 12.33 (br.s, 1H, pyrrole-N*H*)

one C*H$_2$* peak is superposed by the solvent signal

^{13}C-NMR (100MHz, [D$_6$]DMSO) δ = 24.4 (*C*H$_2$), 26.2 (*C*H$_2$), 29.0 (*C*H$_2$), 33.8 (*C*H$_2$), 38.8 (*C*H$_2$), 112.4 (pyrrole-*CH*), 115.7 (pyrrole-*C*H), 124.4 (*Cq*), 125.4 (picrate-*C*H), 133.1 (*Cq*), 142.0 (*Cq*), 155.0 (*Cq*), 159.2 (*Cq*), 159.6 (*Cq*), 161.0 (*Cq*)

7.4.6 NMR dilution data

NMR dilution experiments:

Solutions of zwitterions **3-6** with varying concentrations were obtained by diluting aliquots of concentrated stock solutions in the DMSO to a total volume of 500 µl. The chemical shifts were recorded (Bruker Avance 400 spectrometer) for each sample relative to the deuterated solvent.

Tab.4: *Data of the NMR dilution experiments of the zwitterions **3-4**.*

C1-Zwitterion

c [mM]	δ (guanidinium-NH) [ppm]
25	11.25
20	11.25
15	11.25
10	11.24
7.5	11.22
5	11.16
3.75	11.11
2.5	10.98
1	10.88
0.75	10.83
0.5	10.83
0.25	10.8

C2-Zwitterion

c [mM]	δ (guanidinium-NH) [ppm]
100	11.03
80	11.03
70	11.03
60	11.02
50	11.01
40	10.99
30	10.98
25	10.97
25	10.97
17.5	10.95
10	10.92
7.5	10.9
5	10.88
2.5	10.86
1	10.83
0.75	10.81
0.5	10.73

Tab.5: *Data of the NMR dilution experiments of the zwitterions **5-6**.*

C3-Zwitterion

c [mM]	δ (guanidinium-NH) [ppm]
100	11.1773
90	11.1735
80	11.1625
60	11.1501
50	11.13
45	11.1236
40	11.117
30	11.097
20	11.0662
15	11.049
10	10.95
8	10.94
7.5	10.9273
2.5	10.8099
1	10.7694
0.75	10.76
0.5	10.72

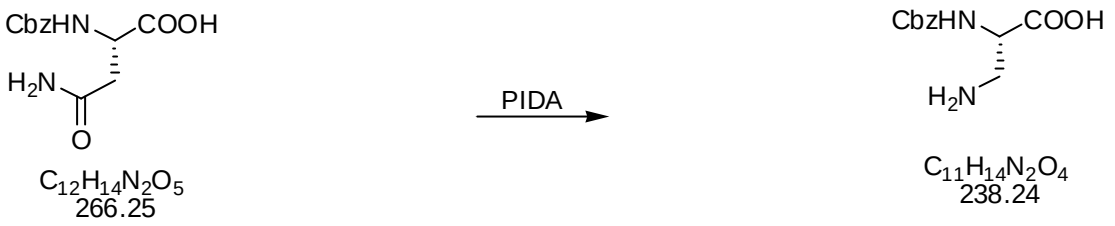
C5-Zwitterion

c [mM]	δ (guanidinium-NH) [ppm]
100	11.1142
80	11.1009
60	11.0845
50	11.0842
30	11.0507
25	11.0365
20	11.0018
10	10.9033
5	10.8474
1	10.7691

7.5 Synthesis of the arginine analogues

7.5.1 Synthesis of the arginine analogue with n = 1

N_a-Cbz-b-amino-*L*-alanine[140]



$C_{12}H_{14}N_2O_5$
266.25

121

$C_{11}H_{14}N_2O_4$
238.24

123

A slurry of N_α-Cbz-*b*-amino-L-asparagine **121** (5.00 g, 18.8 mmol), ethyl acetate (24.0 ml), acetonitrile (24.0 ml), water (12.0 ml), and bis(acetoxy)iodobenzene (PIDA) (7.26 g, 22.5 mmol) was cooled and stirred at 16 °C for 30 min. The temperature was allowed to reach room temperature, and the reaction was stirred until completion (4 h). The mixture was cooled to 5 °C, and the product was filtered, washed with diethyl ether (10 mL), and dried *in vacuo* at 50 °C to give **123** (3.92 g, 88%).

$C_{11}H_{14}N_2O_4$ Mw = 238.24

yield 3.92 g (88 %)

R_f 0.73 (SiO_2, ethyl acetate/*n*-butanol/acetic acid/water = 2/1//1)

0.69 (SiO_2, dichloromethane/ methanol/ethyl acetate = 5/3/1)

mp. 237 °C (decomposition)

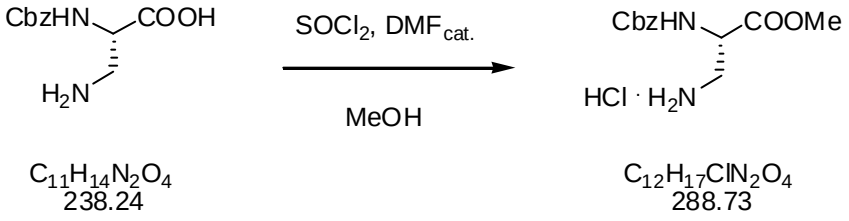
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3326 [m], 3028 [m], 1725 [m], 1696 [s]

HR-MS (ESI) m/z = 239.103 ± 0.005 (calculated for $^{12}C_{12}H_{16}N_2O_4+H^+$: 239.102)

^{1}H-NMR (400MHz, [D_6]DMSO/TFA) δ = 2.93-3.10 (m, 1H, C*H*$_2$), 3.14-3.29 (m, 1H, C*H*$_2$), 4.39-4.27 (m, 1H, C*H*), 5.05 (s, 2H, C*H*$_2$-O), 7.25-7.42 (m, 5H, aryl-C*H*), 7.77 (d, 1H, J = 8.5 Hz, N*H*), 8.21 (br.s, 3H, N*H*$_3^+$)

^{13}C-NMR (100MHz, [D_6]DMSO/TFA) δ = 39.4 (*C*H$_2$-NH$_2$), 52.1 (*C*H), 66.1 (*C*H$_2$), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.6 (aryl-*C*H), 137.0 (*Cq*), 156.4 (*C*=O), 171.1 (*C*OOH)

N$_a$-Cbz-b-amino-*L*-alaninemethylester hydrochloride[141]



$C_{11}H_{14}N_2O_4$
238.24

122

$C_{12}H_{17}ClN_2O_4$
288.73

127

To a chilled (ice bath) suspension of the Cbz-protected amino acid **123** (2.00 g, 8.40 mmol, 1 eq.) in abs. methanol (20 ml) was added dropwise thionyl chloride (670 µl, 9.24 mmol, 1.1 eq.) and catalytic amounts of dimethylformamide. The resulting solution was stirred over night at 50 °C, the solvent evaporated and the residue recrystallized from methanol/diethyl ether to yield colorless crystals of the methylester **127**.

$C_{12}H_{17}ClN_2O_4$ Mw = 288.73

yield 2.23 g (92 %)

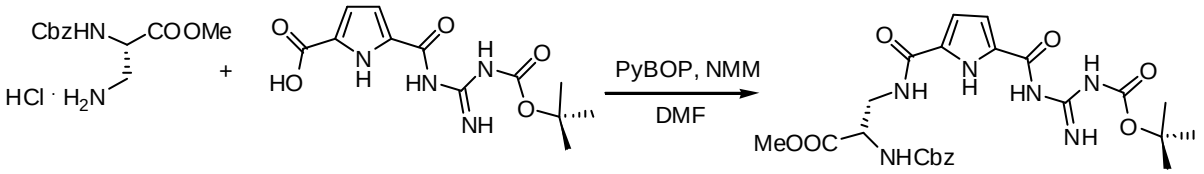
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3316 [m], 2873 [m], 2363 [w], 2021 [w], 1737 [s], 1692 [s], 1598 [w], 1542 [s], 1443 [w], 1309 [m], 1265 [m], 1228 [m], 1060 [w], 1015 [m], 753 [w], 664 [w]

mp. 165 °C

^{1}H-NMR (400MHz, [D_6]DMSO/TFA) δ = 2.99-3.11 (m, 1H, C*H*$_2$), 3.14-3.26 (m, 1H, C*H*$_2$), 3.66 (s, 3H, C*H*$_3$), 4.41-4.48 (m, 1H, C*H*), 5.05 (s, 2H, C*H*$_2$-O), 7.29-7.39 (m, 5H, aryl-C*H*); 7.96 (d, 1H, J = 8.5 Hz, N*H*), 8.38 (br. s, 3H, NH_3^+)

^{13}C-NMR (100MHz, [D_6]DMSO/TFA) δ = 39.2 (*C*H$_2$-NH$_2$), 52.0 (*C*H), 52.7 (*C*H$_3$) 66.1 (*C*H$_2$), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.6 (aryl-*C*H), 136.9 (C*q*), 156.3 (*C*=O), 170.0 (*C*OOH)

Cbz-protected methylester of the arginine analogue n = 1



$C_{12}H_{17}ClN_2O_4$ 288.73 — **127**

$C_{12}H_{16}N_4O_5$ 296.28 — **84**

$C_{24}H_{30}N_6O_8$ 530.53 — **131**

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **84** (2.00 g, 6.76 mmol, 1 eq.), PyBOP (3.52 g, 6.76 mmol, 1 eq.) and N-methyl morpholine (2 ml, 18.18 mmol) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **127** (2.15 g, 7.43 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with diethyl ether (3 x 100 ml). The combined

organic phases were washed with water (100 ml), brine (100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 7/3) to yield a colorless solid (3.40 g, 95 %).

$C_{24}H_{30}N_6O_8$ Mw = 530.53

yield 3.40 g (95 %)

R_f 0.64 (SiO_2, dichloromethane/acetone = 7/3)

mp. 112 °C

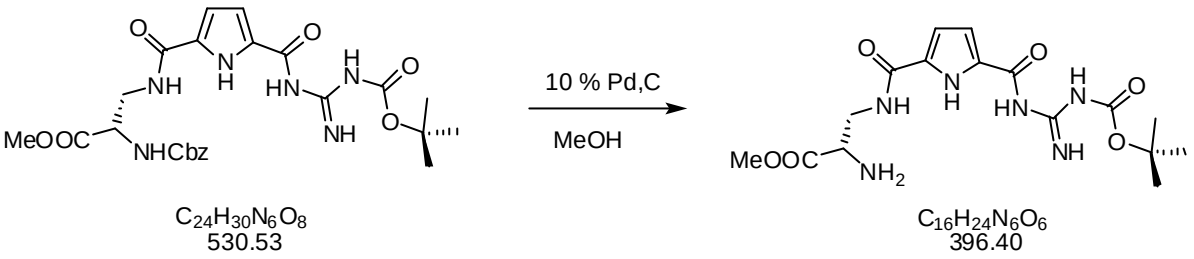
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3386 [m], 2978 [w], 1726 [s], 1636 [s], 1557 [s], 1470 [m], 1301 [s], 1241 [s], 1148 [s], 1051 [m], 842 [w], 780 [w], 755 [w], 697 [w]

HR-MS (ESI) m/z = 553.202 ± 0.005 (calculated for $^{12}C_{24}H_{30}N_6O_8$ + Na: 553.201)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, C*H*$_3$), 3.49-3.57 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 4.29 (q, 1H, C*H*), 6.74-6.76 (m, 1H, pyrrole-C*H*), 6.81 (br.s, 1H, pyrrole-C*H*) 7.33 (br.s, 5H, benzyl-C*H*), 7.73 (d, J = 7.93 Hz, 1H, N*H*), 8.46-8.51 (m, 1H, N*H*), 8.55 (br.s, 1H, N*H*), 9.32 (br.s, 1H, N*H*), 10.84 (br.s, 1H, N*H*), 11.32 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 27.9 (Boc-*C*H$_3$), 45.8 (*C*H$_2$), 52.2 (*C*H$_3$), 54.0 (*C*H), 65.8 (*C*H$_2$), 81.1 (*Cq*), 112.3 (pyrrole-*C*H), 113.8 (pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.5 (aryl-*C*H), 136.9 (aryl-*Cq*), 156.1 (*Cq*), 158.6 (*Cq*), 160.3 (*Cq*), 160.6 (*Cq*), 171.2 (*Cq*)

Methylester of the arginine analogue n = 1



$C_{24}H_{30}N_6O_8$
530.53

131

$C_{16}H_{24}N_6O_6$
396.40

140

A mixture of Cbz-protected amine **131** (1.88 g, 3.55 mmol) and 10 % Pd/C (180 mg) in methanol (140 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **140** as a colorless solid (1.33 g, 95%). The picrate salt was synthesized for analytic purpose.

$C_{16}H_{24}N_6O_6$ Mw = 396.40

yield 1.33 g (95 %)

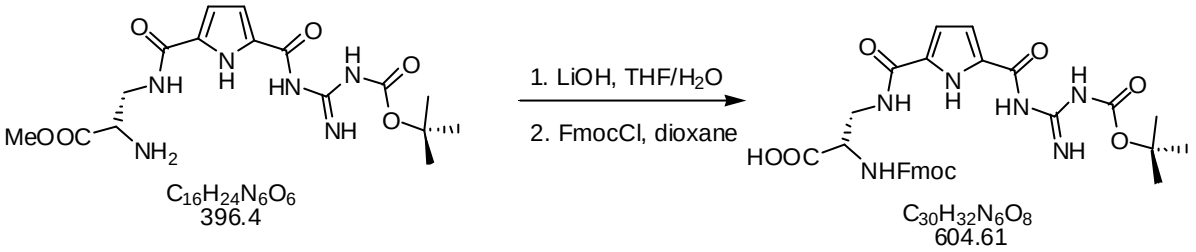
mp. 129 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] (picrate-salt) = 3324 [br.m], 3196 [br.m], 1705 [s], 1612 [s], 1516 [s], 1268 [s], 1078 [m], 911 [w], 745 [m], 710 [m]

^{1}H-NMR (400MHz, [D_6]DMSO, picrate-salt) δ = 3.70-3.83 (m, 2H, CH_2), 3.75 (s, 3H, CH_3), 4.19 (t, J = 5.3, 1H, C*H*), 6.86-6.89 (m, 1H, pyrrole-C*H*), 7.03-7.05 (m, 1H, pyrrole-C*H*), 8.17 (br.s, 4H, guanidinium-NH_2), 8.40 (br.s, 3H, NH_3^+), 8.58 (s, 4H, picrate-C*H*), 8.65 (t, J = 5.8, 1H, N*H*), 10.97 (br.s, 1H, N*H*), 12.41 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO, picrate-salt) δ = 39.8 (*C*H_2), 53.3 (*C*H_3), 53.5 (*C*H), 113.1 (pyrrole-*C*H), 115.5 (pyrrole-*C*H), 124.4 (*Cq*), 125.4 (picrate-*C*H), 125.9 (*Cq*), 132.1 (*Cq*), 142.0 (*Cq*), 155.0 (*Cq*), 159.6 (*Cq*), 160.4 (*Cq*), 161.0 (*Cq*), 168.5 (*C*OOMe)

Arginine analogue with n = 1



140 **7**

A mixture of the methylester **140** (500 mg, 1.26 mmol, 1 eq.) and lithium hydroxide monohydrate (79.0 mg, 1.5 eq.) in THF/water (10 ml, 4/1) was stirred for 90 min until hydrolysis was complete (tlc). The solution was neutralized with hydochloric acid (5 %) and lyophylised. The residue was suspended in an aqueous Na_2SO_4 solution (10 ml, 10 %) and a solution of Fmoc-Cl (326 mg) in dioxane (10 ml) was added at 0 °C. The solution was stirred for additional 90 min at room temperature, the solution lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (472 mg, 62 %).

$C_{30}H_{32}N_6O_8$ Mw = 604.61

yield 472 mg (62 %)

R_f 0.40 (SiO_2, dichlormethane/methanol = 9/1)

mp. 154 °C (picrate salt)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] (picrate salt) = 3375 [br.m], 3202 [br.m], 1701 [s], 1632 [s], 1605 [s], 1550 [s], 1330 [m], 1273 [s], 742 [w]

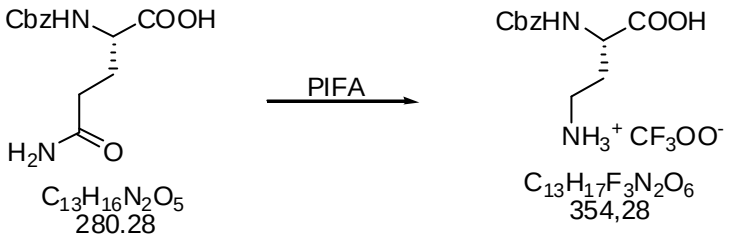
HR-MS (ESI) m/z = 505.181 ± 0.005 (calculated for $^{12}C_{25}H_{25}N_6O_6$: 505.184) (picrate salt)

^{1}H-NMR (400MHz, [D_6]DMSO, picrate salt) δ = 3.45-3.52 (m, 1H, C*H*$_2$), 3.64-3.75 (m, 1H, C*H*$_2$), 4.13-4.34 (m, 4H, fluorenyl-C*H*$_2$ und 2C*H*), 6.84-6.89 (m, 1H, pyrrole-C*H*), 7.00-7.05 (m, 1H, pyrrole-C*H*), 7.26-7.34 (m, 2H, fluorenyl-C*H*), 7.38-7.41 (m, 2H, fluorenyl-C*H*), 7.64-7.70 (m, 3H, 2 fluorenyl-C*H´*s und 1 N*H*), 7.87-7.88 (m, 2H, fluorenyl-C*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.58 (s, 3H, picrate-C*H* und N*H*), 10.93 (br.s, 1H, N*H*), 12.43 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO, picrate-salt) δ = 40.4 (*C*H$_2$), 46.8 (fluorenyl-*C*H), 53.8 (amino acid-*C*H), 65.9 (fluorenyl-*C*H$_2$), 112.9 (pyrrole-*C*H), 115.5 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.4 (pyrrole-*Cq*), 125.3 (picrate-*C*H), 125.7 (fluorenyl-*Cq*), 127.2 (fluorenyl-*C*H), 127.8 (fluorenyl-*C*H), 132.6 (picrate-*Cq*), 140.9 (fluorenyl-*Cq*), 142.0 (picrate-*Cq*), 143.9 (fluorenyl-*Cq*), 154.9 (*Cq*), 156.2 (*Cq*), 159.6 (*Cq*), 159.7 (*Cq*), 160.9 (*Cq*), 172.1 (*C*OOH)

7.5.2 Synthesis of the arginine analogue with n = 2

N_a-Cbz-*L*-2,4-diaminobutyric acid



122 **124**

A slurry of N_α-Cbz-*b*-amino-*L*-glutamine **122** (5.00 g, 17.8 mmol) in ethyl acetate (24 ml), acetonitrile (24 ml), water (12 ml), and bis(trifluoroacetoxy)iodobenzene (PIFA) (7.65 g, 17.8 mmol) was cooled and stirred at 16 °C for 30 min. The mixture was stirred at 40 °C for 4 h and subsequently heated to 70 °C for 10 min. The solvent was evaporated *in vacuo*, the oily residue was dissolved in 100 ml water and extracted with diethyl ether (2 x 30 ml). The aqueous phase was evaporated and the colorless solid (5.80 g, 89 %) was dried *in vacuo*.

$C_{13}H_{17}F_3N_2O_6$ Mw = 274.27

yield 5.80 g (89 %)

mp. 96-98 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3318 [br.s], 1684 [br.s], 1520[s], 1210 [s], 1188 [s], 725 [m]

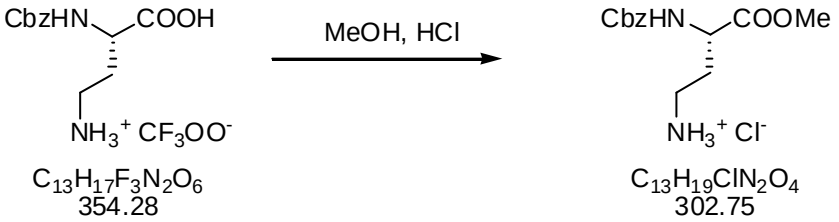
HR-MS (ESI) m/z = 253.119 ± 0.005 (calculated for $^{12}C_{12}H_{17}N_2O_4$: 253.119)

^{1}H-NMR (400MHz, [D_6]DMSO/TFA) δ = 1.84-1.94 (m, 1H, CH_2), 1.99-2.07 (m, 1H,

C*H*$_2$), 2.78-2.93 (m, 2H, C*H*$_2$), 4.07-4.13 (m, 1H, C*H*), 5.03 (s, 2H, C*H*$_2$-O), 7.28-7.35 (m, 5H, aryl-C*H*); 7.69 (d, 1H, J = 8.0 Hz, N*H*), 7.85 (br.s, 3H, N*H*$_3^+$)

^{13}C-NMR (100MHz, [D$_6$]DMSO/TFA) δ = 29.2 (CH_2), 39.4 (*C*H$_2$), 52.9 (*C*H), 66.0 (*C*H$_2$), 128.1 (aryl-*C*H), 128.2 (aryl-*C*H), 128.7 (aryl-*C*H), 137.3 (*Cq*), 156.6 (*C*=O), 173.3 (*C*OOH)

Methyl-N$_a$-Cbz-*L*-2,4-diaminobutyrate hydrochloride[142]



122 **128**

To a suspension of the Cbz-protected amino acid **122** (5.80 g, 16.4 mmol, 1 eq.) in abs. methanol (500 ml) was added drop wise conc. hydrochloric acid (5 ml). The resulting solution was stirred for 4 h at room temperature, the solvent evaporated and the oily residue was digerated with diethyl ether in a ultrasound bath. The diethyl ether was decanted, the residue was dissolved in water (30 ml) and lyophilized yielding a colorless powder (4.98 g, 100 %)

$C_{13}H_{29}ClN_2O_4$ Mw = 302.75

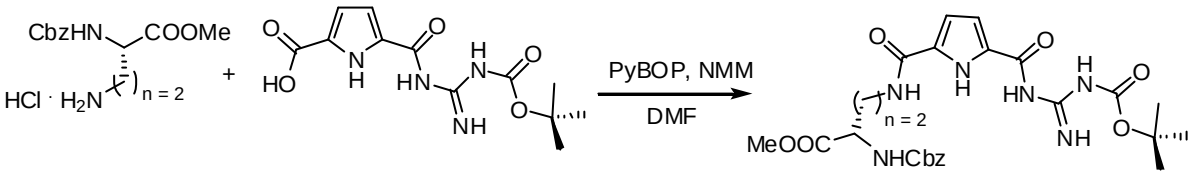
yield 4.98 g (100 %)

^{1}H-NMR (400MHz, [D$_6$]DMSO) δ = 1.86-1.99 (m, 1H, C*H*$_2$), 2.01-2.10 (m, 1H, C*H*$_2$), 2.81 (br.s, 2H, C*H*$_2$), 3.63 (s, 3H, C*H*$_3$), 4.16-4.22 (m, 1H, C*H*),

5.03 (s, 2H, C*H*$_2$-O), 7.27-7.38 (m, 5H, aryl-C*H*), 7.90 (d, 1H, J = 7.7 Hz, N*H*), 8.25 (br. s, 3H, NH_3^+)

^{13}C-NMR (100MHz, [D$_6$]DMSO) δ = 28.7 (C*H*$_2$), 36.1 (*C*H$_2$), 51.7 (*C*H), 52.3 (*C*H$_3$) 65.8 (*C*H$_2$), 127.9 (aryl-*C*H), 128.0 (aryl-*C*H), 128.6 (aryl-*C*H), 137.0 (C*q*), 156.3 (*C*=O), 172.1 (*C*OOH)

Cbz-protected methylester of the arginine analogue n = 2



$C_{13}H_{19}ClN_2O_4$
302.75

$C_{12}H_{16}N_4O_5$
296.28

$C_{25}H_{32}N_6O_8$
544.56

128 **84** **132**

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **84** (1.50 g, 5.06 mmol, 1 eq.), PyBOP (2.63 g, 5.57 mmol, 1.1 eq.) and N-methyl morpholine (2 ml) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **128** (1.69 g, 5.57 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with ethyl acetate (3 x 60 ml). The combined organic phases were washed with brine (100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 7/3) to yield a colorless solid (1.09 g, 2.00 mmol).

$C_{25}H_{32}N_6O_8$ Mw = 544.56

yield 1.09 g (40 %)

R_f 0.64 (SiO_2, dichlormethane/acetone = 7/3)

mp. 112°C

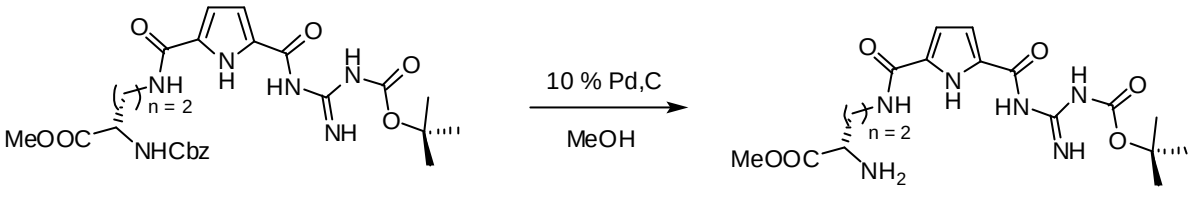
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3380 [br.m], 3280 [br.m], 2928 [m], 1730 [s], 1635 [s], 1556 [s], 1468 [m], 1240 [s], 1149 [s], 842 [w], 755 [w]

HR-MS (ESI) m/z = 567.218 ± 0.005 (calculated for $^{12}C_{25}H_{32}N_6O_8 + Na^+$: 567.218)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, C*H*$_3$), 1.77-1.87 (m, 1H, C*H*$_2$), 1.95-2.03 (m, 1H, C*H*$_2$), 3.25-3.32 (m, 2H, C*H*$_2$), 3.61 (s, 3H, C*H*$_3$), 4.09-4.15 (m, 1H, C*H*), 5.03 (d, J = 4.2 Hz, 2H, C*H*$_2$), 6.74-6.75 (m, 1H, pyrrole-C*H*), 6.76 (br.s, 1H, pyrrole-C*H*), 7.32-7.38 (m, 5H, benzyl-C*H*), 7.78 (d, J = 7.7 Hz, 1H, N*H*), 8.35 (t, J = 4.8 Hz, 1H, N*H*), 8.52 (br.s, 1H, N*H*), 9.30 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*), 11.14 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 27.9 (Boc-*C*H$_3$), 30.6 (C*H*$_2$), 35.8 (*C*H$_2$), 51.9 (*C*H), 52.1 (*C*H$_3$), 65.7 (*C*H$_2$), 79.3 (Boc-*Cq*), 112.0 (pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.5 (aryl-*C*H), 137.0 (aryl-*Cq*), 156.3 (*Cq*), 159.8 (*Cq*), 171.9 (*Cq*)

Methylester of the arginine analogue n = 2



$C_{25}H_{32}N_6O_8$
544.56
132

$C_{17}H_{26}N_6O_6$
410.42
141

A mixture of Cbz-protected amine **132** (1.00 g, 1.84 mmol) and 10 % Pd/C (100 mg) in methanol (100 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **141** as a colorless solid (680 mg, 90 %).

$C_{17}H_{26}N_6O_6$ Mw = 438.48

yield 680 mg (90 %)

mp. 80 °C

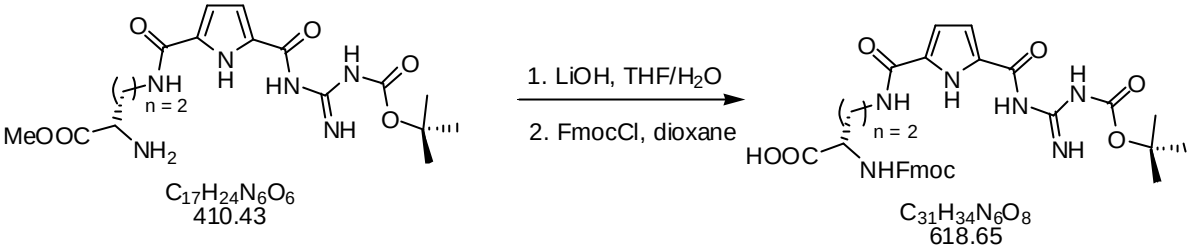
FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3385 [br.m], 3296 [m], 2978 [w], 1732 [s], 1635 [s], 1556 [s], 1468 [m], 1370 [m], 1298 [s], 1241 [s], 1150 [s], 842 [w], 755 [w]

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, Boc-C*H*$_3$), 1.56-1.65 (m, 1H, C*H*$_2$),1.82-1.96 (m, 1H, C*H*$_2$), 3.26-3.38 (m, 4H, C*H*$_2$ + N*H*$_2$), 3.59 (s, 3H, C*H*$_3$), 4.06 (br.s, 1H, C*H*), 6.74-6.75 (m, 1H, pyrrole-C*H*), 6.80-6.81 (m, 1H, pyrrole-C*H*), 8.37 (t, J = 5.4, 1H, N*H*), 8.55 (br.s, 1 H, N*H*), 9.30 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 28.1 (*C*H$_3$), 34.4 (*C*H$_2$), 36.0 (*C*H$_2$), 51.5 (*C*H$_3$), 52.2 (*C*H), 80.9 (*Cq*), 112.0 (pyrrole-*C*H), 114.1 (pyrrole-*C*H),

129.9 (pyrrole-*Cq*), 131.0 (pyrrole-*Cq*), 158.6 (*Cq*), 159.6 (*Cq*), 176.1 (*Cq*)

Arginine analogue with n = 2



141 **8**

A mixture of the methylester **141** (560 mg, 1.36 mmol, 1 eq.) and lithium hydroxide monohydrate (86.0 mg, 1.5 eq.) in THF/water (10 ml, 4/1) was stirred for 90 min until completion. The solution was neutralized with hydochloric acid (5 %) and lyophylized. The residue was suspended in an aqueous Na_2SO_4 solution (15 ml, 10 %) and at 0 °C a solution of Fmoc-Cl (353 mg) in dioxane (15 ml) was added. The solution was stirred for additional 90 min at room temperature, the solution was lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder.

$C_{31}H_{34}N_6O_8$ Mw = 618.65

yield 563 mg (67 %)

R_f 0.47 (SiO_2, dichloromethane/methanol = 9/1)

mp. 200 °C (decomposition)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] (picrate salt) = 3360 [br.m], 3200 [br.m], 1701 [s], 1634 [s], 1600 [s], 1530 [s], 1330 [m], 1273 [s],

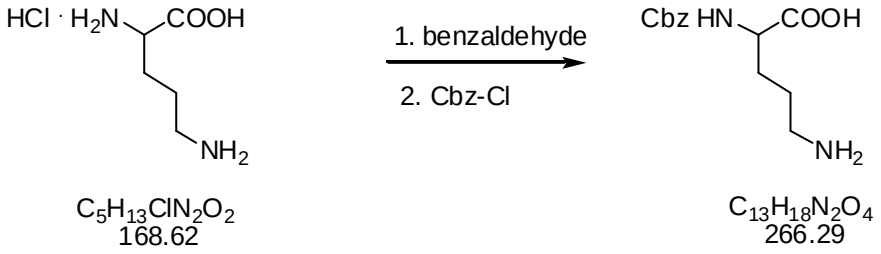
HR-MS (ESI) m/z = 519.198 ± 0.005 (calculated for $^{12}C_{26}H_{27}N_6O_6$: 519.199) (picrate salt)

^{1}H-NMR (400MHz, [D$_6$]DMSO, picrate salt) δ = 1.78-1.89 (m, 1H, C*H*$_2$), 1.98-2.07 (m, 1H, C*H*$_2$), 3.27-3.37 (m, 2H, C*H*$_2$), 4.00-4.06 (m, 1H, CH), 4.18-4.34 (m, 3H, fluorenyl-C*H*$_2$ and C*H*), 6.84-6.89 (m, 1H, pyrrole-C*H*), 6.99-7.03 (m, 1H, pyrrole-C*H*), 7.29-7.35 (m, 2H, fluorenyl-C*H*), 7.37-7.44 (m, 2H, fluorenyl-C*H*), 7.67-7.77 (m, 2H, fluorenyl-C*H*), 7.86-7.88 (m, 2H, fluorenyl-C*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.45 (br.s, 1H, C*H*), 8.58 (s, 3H, picrate-C*H*), 10.93 (br.s, 1H, N*H*), 12.36 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D$_6$]DMSO) δ = 30.6 (*C*H$_2$), 36.3 (*C*H$_2$), 46.9 (fluorenyl-*C*H), 51.9 (amino acid-*C*H), 65.9 (fluorenyl-*C*H$_2$), 112.6 (pyrrole-*C*H), 115.6 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.7 (pyrrole-*Cq*), 125.4 (picrate-*C*H), 125.5 (pyrrole-*Cq*), 127.3 (fluorenyl-*C*H), 127.6 (fluorenyl-*C*H), 132.9 (picrate-*Cq*), 140.9 (fluorenyl-*Cq*), 142.0 (picrate-*Cq*), 144.0 (fluorenyl-*Cq*), 154.9 (*Cq*), 156.4 (*Cq*), 159.4 (*Cq*), 159.6 (*Cq*), 160.8 (*Cq*), 173.9 (*C*OOH)

7.5.3 Synthesis of the arginine analogue with n = 3

N$_a$-Cbz-*L*-ornithine[143]



$C_5H_{13}ClN_2O_2$
168.62

119

$C_{13}H_{18}N_2O_4$
266.29

125

The Nε-benzylidene-*L*-ornithine was obtained in a first step by addition of benzaldehyde (24.6 g, 231 mmol) to a chilled solution of *L*-ornithine monohydrochloride **119** (25.3 g, 0.15 mmol) in dilute lithium hydroxide (75 ml, 2 *N*). After shaking for a very short time, the benzaldehyd dissapeared and the benzylidene precipitated out. The precipitate was filtered off, thoroughly washed with water and ethanol and dried in the desiccator affording the Nε-benzylidene-*L*-ornithine as a colorless solid (28.5 g, 81 %).

Then a chilled (0 °C) solution of sodium hydroxide (1 *N*, 150 ml) and benzyl chloroformate (17 ml) were added in two portions within a brief period (5 min) to a cold, vigorously stirred solution of Nε-benzylidene-*L*-ornithine (23.4 g, 0.10 mole) in sodium hydroxide (1 *N*, 100 ml). Stirring was continued for a short interval (0 °C, 10 min; room temperature, 10 min), after which concentrated hydrochloric acid (25 ml) was added, and the mixture was heated (50 °C, 5 min) and extracted with two portions of diethyl ether (100 ml). The water layer was adjusted to pH 6.2 and filtered to remove Nε-benzyloxycarbonyl-*L*-ornithine. The remaining aqueous solution was concentrated *in vacuo* (100 ml) and cooled in the refrigerator over night. The precipitate was filtered off and recrystallized from water affording a colorless powder (16.0 g, 60 %)

$C_{13}H_{18}N_2O_4$ Mw = 266.29

yield 16.0 g (60 %)

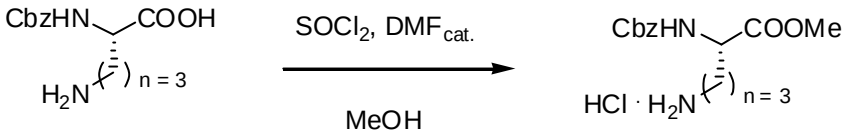
mp. 225-228°C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3343 [m], 2058 [s], 1736 [s], 1689 [s], 1533 [m], 1469 [m], 1249 [m], 1045 [w], 1015 [w], 754 [w]

^{1}H-NMR (400MHz, [D_6]DMSO/TFA) δ = 1.57-1.81 (m, 4H, CH_2), 2.72-2.78 (m, 2H, CH_2), 3.91-3.95 (m, 1H, C*H*), 5.02 (s, 2H, CH_2-O), 7.27-7.37 (m, 5H, aryl-C*H*), 7.60 (d, 1H, J = 8.5 Hz, N*H*), 7.94 (br.s, 3H, NH_3^+)

^{13}C-NMR (100MHz, [D_6]DMSO/TFA) δ = 24.2 (*C*H_2), 28.1 (*C*H_2), 38.7 (*C*H_2-NH_2), 53.7 (*C*H), 65.8 (*C*H_2), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.7 (aryl-*C*H), 137.3 (*Cq*), 156.5 (*C*=O), 173.9 (*C*OOH)

Na **-Cbz-*L*-ornithine methylester**[144]



$C_{13}H_{18}N_2O_4$
266.30
125

$C_{14}H_{21}ClN_2O_4$
316.79
129

To a chilled (ice bath) suspension of the Cbz-protected amino acid **125** (2.00 g, 7.5 mmol, 1 eq.) in abs. methanol (20 ml) was added drop wise thionyl chloride (570 µl, 8.26 mmol, 1.1 eq.) and catalytic amounts of dimethylformamide . The resulting solution was stirred over night at 50 °C, the solvent evaporated and the residue digerated with diethyl ether to yield a colorless solid of the methylester **129**.

$C_{14}H_{21}ClN_2O_4$ Mw = 316.79

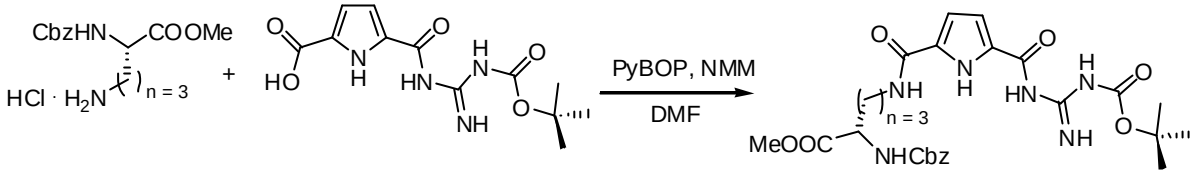
yield 1.63 g (69 %)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.51-1.57 (m, 4H, C*H*$_2$), 2.71-2.73 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 3.97-4.03 (m, 1H, C*H*), 5.02 (s, 2H, C*H*$_2$-O), 7.27-7.39 (m, 5H, aryl-C*H*), 7.73 (d, 1H, J = 7.7 Hz, N*H*), 8.13 (br. s, 3H, NH_3^+)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 22.5 (*C*H_2), 24.5 (*C*H_2), 38.5 (*C*H_2) 52.0 (*C*H),

53.9 (*C*H$_3$) 65.7 (*C*H$_2$), 127.9 (aryl-*C*H), 128.0 (aryl-*C*H), 128.5 (aryl-*C*H), 137.0 (C*q*), 156.3 (*C*=O), 173.0 (*C*OOH)

Cbz-protected methylester of the arginine analogue n = 3



$C_{14}H_{21}ClN_2O_4$ 316.79 — **129**

$C_{12}H_{16}N_4O_5$ 296.28 — **84**

$C_{26}H_{34}N_6O_8$ 558.59 — **133**

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **84** (2.00 g, 6.76 mmol, 1 eq.), PyBOP (3.52 g, 6.76 mmol, 1 eq.) and N-methyl morpholine (2 ml, 18.18 mmol) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **129** (2.46 g, 7.43 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic phases were washed with water (100 ml), brine (2 x 100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 1/1) to yield a colorless solid (2.98 g, 79 %).

$C_{26}H_{34}N_6O_8$ Mw = 558.59

yield 2.98 g (79 %)

R_f 0.65 (SiO_2, dichloromethane/acetone = 1/1)

mp. 95-97 °C

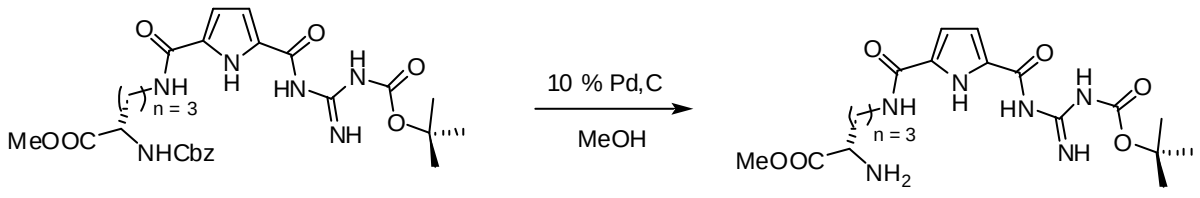
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3375 [br.m], 3268 [br.m], 2928 [m], 1727 [s], 1635 [s], 1555 [s], 1470 [m], 1241 [s], 1150 [s], 842 [w], 754 [w],

HR-MS (ESI) m/z = 581.233 ± 0.005 (calculated for $^{12}C_{26}H_{35}N_6O_8 + Na^+$: 581.234)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, C*H*$_3$), 1.57-1.67 (m, 4H, C*H*$_2$), 3.18-3.25 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 4.03-4.09 (m, 1H, C*H*), 5.02 (s, 2H, C*H*$_2$), 6.74-6.76 (m, 1H, pyrrole-C*H*), 6.81 (br.s, 1H, pyrrole-C*H*), 7.28-7.37 (m, 5H, benzyl-C*H*), 7.75 (d, J = 7.8 Hz, 1H, N*H*), 8.30 (t, J = 5.4 Hz, 1H, N*H*), 8.54 (br.s, 1H, N*H*), 9.30 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 27.9 (^{t}Boc-*C*H$_3$), 29.7 (*C*H$_2$), 32.3 (*C*H$_2$), 38.2 (*C*H$_2$), 52.0 (*C*H$_3$), 53.9 (*C*H), 65.7 (*C*H$_2$), 81.1 (*Cq*), 111.7 (pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.5 (aryl-*C*H), 137.1 (aryl-*Cq*), 156.3 (*Cq*), 158.5 (*Cq*), 159.7 (*Cq*), 173.0 (*Cq*)

Methylester of the arginine analogue n = 3



$C_{26}H_{34}N_6O_8$
558.58
138

$C_{18}H_{28}N_6O_6$
422.45
142

A mixture of Cbz-protected amine **133** (600 mg, 1.07 mmol) and 10 %-Pd/C (60 mg) in methanol (100 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **142** as a pale green solid (407 mg, 90 %).

$C_{18}H_{28}N_6O_6$ Mw = 422.45

yield 407 mg (90 %)

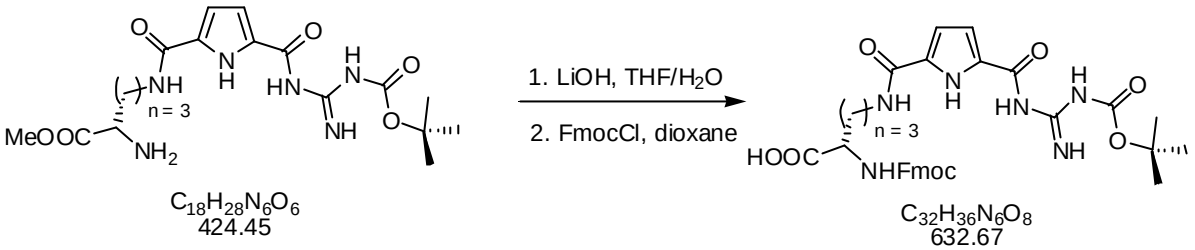
mp. 74 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3390 [br.m], 2955 [br.m], 1732 [s], 1635 [s], 1530 [s], 1470 [m], 1241 [s], 1155 [s], 842 [w]

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 3H, ^{t}Boc-C*H*$_3$), 1.49-1.64 (m, 4H, C*H*$_2$), 3.14-3.25 (m, 2H, C*H*$_2$), 3.60 (s, 3H, C*H*$_3$), 4.06 (m, 1H, C*H*), 6.75-6.76 (m, 1H, pyrrole-C*H*), 6.80 (br.s, 1H, pyrrole-C*H*), 8.35 (t, J = 5.4, 1H, N*H*), 8.55 (br.s, 1 H, N*H*), 9.30 (br.s, 1H, N*H*), 10.84 (br.s, 1H, N*H*), 11.40 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 26.2 (*C*H$_2$), 26.8 (*C*H$_2$), 28.1 (*C*H$_3$), 38.7 (*C*H$_2$), 38.7 (*C*H$_2$), 50.6 (*C*H$_3$), 56.0 (*C*H), 112.0 (pyrrole-*C*H), 114.0 (pyrrole-*C*H), 158.6 (*Cq*), 159.6 (*Cq*), 176.4 (*Cq*)

Arginine analogue with n = 3



$C_{18}H_{28}N_6O_6$
424.45

142

$C_{32}H_{36}N_6O_8$
632.67

9

A mixture of the methylester **142** (500 mg, 1.18 mmol, 1 eq.) and lithium hydroxide monohydrate (74.0 mg, 1.5 eq.) in a THF/water mixture (10 ml, 4/1) was stirred for 90 min until complete hydrolysis (tlc). The solution was neutralized with hydochloric acid (5 %) and lyophylized. The residue was suspended in an aqueous Na_2SO_4 solution (10 ml, 10 %) and at 0 °C a solution of Fmoc-Cl (305 mg, 1.18 mmol, 1 eq.) in dioxane (10 ml) was added. The solution was stirred for additional 90 min at room temperature, the mixture was lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (507 mg, 68 %).

$C_{32}H_{36}N_6O_8$ Mw = 632.67

yield 507 mg (68 %)

R_f 0.50 (SiO_2, dichlormethane/methanol = 9/1)

mp. 210-220 °C (decomposition) (picrate salt)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3375 [br.m], 2965 [br.m], 1720 [s], 1620 [s], 1541 [s], 1271 [s], 742 [w]

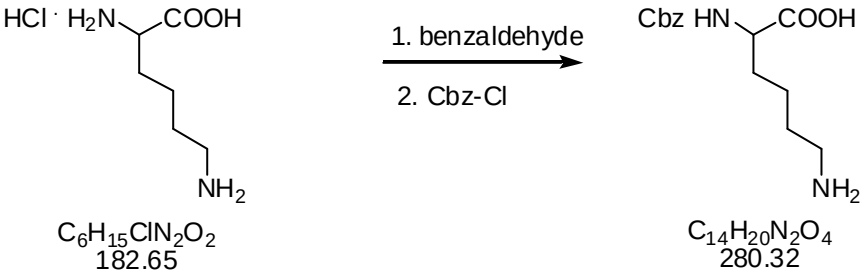
HR-MS (ESI) m/z = 533.216 ± 0.005 (calculated for $^{12}C_{27}H_{29}N_6O_6$: 533.215) (picrate)

^{1}H-NMR (400MHz, [D_6]DMSO, picrate salt) δ = 1.49-1.82 (m, 4H, C*H*$_2$), 3.23-3.28 (m, 2H, C*H*$_2$), 3.94-4.00 (m, 1H, C*H*), 4.19-4.24 (m, 1H, C*H*) 4.27-4.28 (C*H*$_2$), 6.86-4.88 (m, 1H, pyrrole-C*H*), 7.02-7.03 (m, 1H, pyrrole-C*H*), 7.29-7.33 (m, 2H, fluorenyl-C*H*), 7.37-7.42 (m, 2H, fluorenyl-C*H*), 7.60-7.70 (m, 1H, fluorenyl-C*H*), 7.86-7.88 (m, 2H, fluorenyl-C*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.42 (t, J = 5.18 Hz, 1H, N*H*), 8.58 (s, 2H, picrat-C*H*), 10.93 (br.s, 1H, NH), 12.34 (br.s, 1H, NH)

^{13}C-NMR (100MHz, [D_6]DMSO, picrate salt) δ = 26.1 (*C*H$_2$), 28.5 (*C*H$_2$), 38.5 (*C*H$_2$), 46.8 (fluorenyl-*C*H), 53.8 (amino acid-*C*H), 65.8 (fluorenyl-*C*H$_2$), 112.4 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.4 (pyrrole-*Cq*), 125.4 (picrate-*C*H), 125.4 (pyrrole-*Cq*), 127.2 (fluorenyl-*C*H), 127.8 (fluorenyl-*C*H), 133.0 (picrate-*Cq*), 140.9 (fluorenyl-*Cq*), 140.9 (fluorenyl-*Cq*), 142.0 (picrate-*Cq*), 144.0 (fluorenyl-*Cq*), 144.0 (fluorenyl-*Cq*), 155.0 (*Cq*), 156.3 (*Cq*), 159.2 (*Cq*), 159.6 (*Cq*), 161.0 (*Cq*), 174.0 (*C*OOH)

7.5.4 Synthesis of the arginine analogue with n = 4

N$_a$-Cbz-*L*-lysine[143]



$C_6H_{15}ClN_2O_2$ 182.65 — **120**

$C_{14}H_{20}N_2O_4$ 280.32 — **126**

The Nε-benzylidene-*L*-lysine was obtained in a first step by addition of benzaldehyde (24.6 g, 231 mmol) to a chilled solution of *L*-lysine monohydrochloride **120** (27.3 g, 0.15 mmol) in dilute lithium hydroxide (75 ml, 2 *N*). After shaking for a very short time, the benzaldehyd

dissapeared and the benzylidene precipitated out. The precipitate was filtered off, thoroughly washed with water and ethanol and dried in the desiccator affording the Nε-benzylidene-*L*-lysine as a colorless solid (28.1 g, 80 %).

Then a chilled (0 °C) solution of sodium hydroxide (1 *N*, 150 ml) and benzyl chloroformate (17 ml) were added in two portions within a brief period (5 min) to a cold, vigorously stirred solution of Nε-benzylidene-*L*-lysine (23.4 g, 0.10 mole) in sodium hydroxide (1 *N*, 100 ml). Stirring was continued for a short interval (0 °C, 10 min; room temperature, 10 min), after which concentrated hydrochloric acid (25 ml) was added, and the mixture was heated (50 °C, 5 min) and extracted with two portions of ether (100 ml). The water layer was adjusted to pH 6.2 and filtered to remove Nε-benzyloxycarbonyl-*L*-lysine. The remaining aqueous solution was concentrated *in vacuo* to a volume of 100 ml and cooled in the refrigerator over night. The precipitate was filtered off and recrystallized from water affording a colorless powder (19.6 g, 70 %)

$C_{14}H_{20}N_2O_4$ Mw = 280.32

yield 14.0 g (50 %)

mp. 229-233 °C

MS (neg.ESI) 279 [M^+-H]

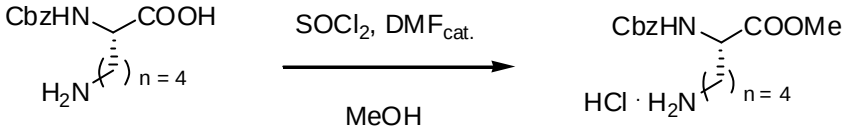
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3328 [m], 2947 [m], 1721 [s], 1559 [s], 1518 [s], 1403 [m], 1242 [m], 1047 [m], 1019 [m], 756 [w]

^{1}H-NMR (400MHz, [D_6]DMSO/TFA) δ = 1.29-1.64 (m, 6H, C*H*$_2$), 2.70-2.80 (m, 2H, C*H*$_2$), 3.87-3.96 (m, 1H, C*H*), 5.02 (s, 2H, C*H*$_2$-O), 7.26-7.37 (m, 5H, aryl-C*H*), 7.56 (d, 1H, J = 8.1 Hz, N*H*), 7.82 (br.s, 3H, N*H*$_3^+$)

^{13}C-NMR (100MHz, [D_6]DMSO/TFA) δ = 22.7 (CH2), 26.7 (CH2), 30.9 (CH2), 38.7

(CH_2-NH_2), 53.9 (*C*H), 65.6 (*C*H_2), 128.0 (aryl-*C*H), 128.1 (aryl-*C*H), 128.6 (aryl-*C*H), 137.2 (*Cq*), 156.4 (*C*=O), 171.1 (*C*OOH)

Na **-Cbz-*L*-lysine methyl ester**



$C_{14}H_{20}N_2O_4$
280.32
126

$C_{15}H_{23}ClN_2O_4$
330.82
130

To a chilled (ice bath) suspension of the Cbz-protected amino acid **126** (2.00 g, 7.13 mmol, 1 eq.) in abs. methanol (20 ml) was added drop wise thionyl chloride (570 µl, 7.85 mmol, 1.1 eq.) and catalytic amounts of dimethylformamide. The resulting solution was stirred over night at 50 °C, the solvent evaporated and the residue digerated with diethyl ether to yield a colorless solid of the methylester **130**.

$C_{15}H_{23}ClN_2O_4$ Mw = 330.82

yield 2.02 g (86 %)

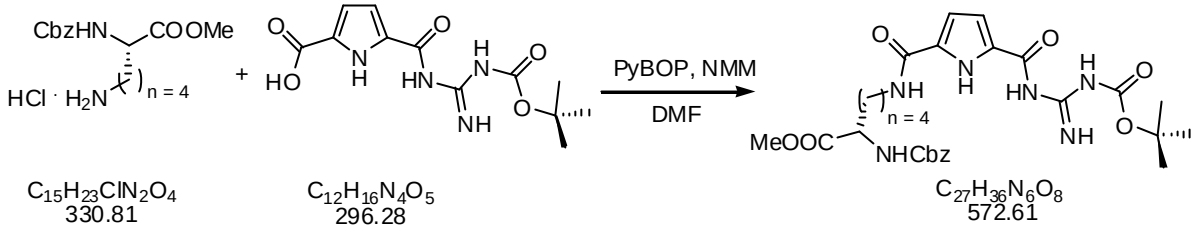
mp. 75 °C

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.27-1.36 (m, 2H, C*H*$_2$), 1.43.1.71 (m, 4H, C*H*$_2$), 2.65-2.77 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 3.97-4.03 (m, 1H, C*H*), 5.02 (s, 2H, C*H*$_2$-O), 7.27-7.39 (m, 5H, aryl-C*H*), 7.73 (d, 1H, J = 7.7 Hz, N*H*), 8.01 (br. s, 3H, NH_3^+)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 22.5 (*C*H_2), 26.5 (*C*H_2), 30.2 (*C*H2), 38.5 (*C*H_2) 52.0 (*C*H), 53.9 (*C*H_3) 65.7 (*C*H_2), 127.9 (aryl-*C*H), 128.0 (aryl-

*C*H), 128.5 (aryl-*C*H), 137.0 (C*q*), 156.3 (*C*=O), 173.0 (*C*OOH)

Cbz-protected methylester of the arginine analogue n = 4



130 **84** **134**

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **84** (2.00 g, 6.76 mmol, 1 eq.), PyBOP (3.52 g, 6.76 mmol, 1 eq.) and N-methyl morpholine (2 ml, 18.18 mmol) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **130** (2.46 g, 7.43 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic phases were washed with water (100 ml), brine (2 x 100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 1/1) to yield a colorless solid (3.10 g, 80 %).

$C_{27}H_{36}N_6O_8$ Mw = 572.61

yield 3.10 g (80 %)

R_f 0.65 (SiO_2, dichloromethane/acetone = 1/1)

mp. 78 °C

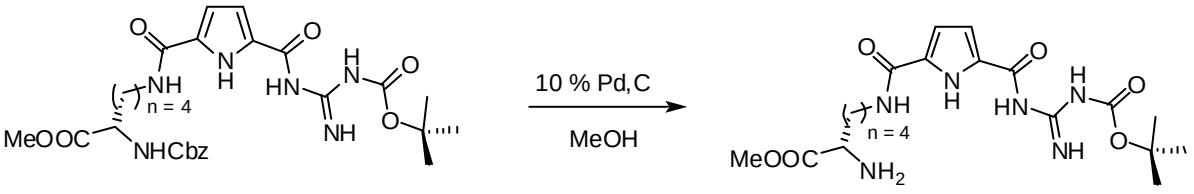
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3384 [br.m], 3268 [br.m], 2928 [m], 1727 [s], 1635 [s], 1556 [s], 1467 [m], 1241 [s], 1149 [s], 842 [w], 754 [w], 697 [w]

HR-MS (ESI) m/z = 573.267 ± 0.005 (calculated for $^{12}C_{27}H_{36}N_6O_8 + H^+$: 573.267)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.30-1.75 (m, 6H, C*H*$_2$), 1.44 (s, 9H, CH_3), 3.17-3.22 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 3.98-4.05 (m, 1H, C*H*),5.02 (s, 2H, C*H*$_2$), 6.74-6.76 (m, 1H, pyrrol-N*H*), 6.81 (br.s, 1H, pyrrol-C*H*) 7.29-7.36 (m, 5H, benzyl-C*H*), 7.70 (d, J = 7.83 Hz, 1H, N*H*), 8.29 (t, J = 5.43 Hz, 1H, N*H*), 8.55 (br.s, 1H, N*H*), 9.31 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*), 11.16 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 23.1 (CH_2), 27.9 (^{t}Boc-*C*H_3), 28.8 (*C*H_2), 30.5 (*C*H_2), 38.5 (*C*H_2), 51.9 (*C*H_3), 54.0 (*C*H), 65.6 (*C*H_2), 81.1 (*Cq*), 111.7 (pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.5 (aryl-*C*H), 137.1 (aryl-*Cq*), 156.1 (*Cq*), 158.6 (*Cq*), 159.7 (*Cq*), 173.1 (*Cq*)

Methylester of the arginine analogue n = 4



$C_{27}H_{36}N_6O_8$
572.61

134

$C_{19}H_{30}N_6O_6$
438.48

143

A mixture of Cbz-protected amine **134** (2.00 g, 3.49 mmol) and 10 %-Pd/C (200 mg) in methanol (160 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and

washings were combined and evaporated to give the amine compound **143** as a pale green solid (1.33 g, 95%).

$C_{19}H_{30}N_6O_6$ Mw = 438.48

yield 1.45 g (95 %)

mp. 77 °C

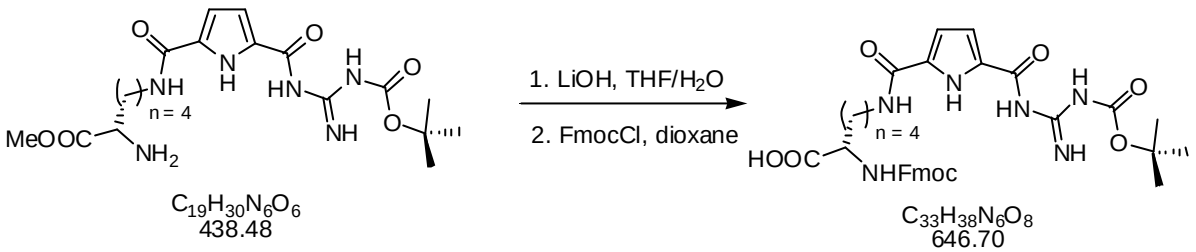
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3384 [br.m], 2952 [br.m], 1732 [s], 1633 [s], 1544 [s], 1467 [m], 1241 [s], 1150 [s], 842 [w]

HR-MS (ESI) m/z = 439.231 ± 0.005 (calculated for $^{12}C_{19}H_{30}N_6O_6 + H^+$: 439.231)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.27-1.38 (m, 2H, C*H*$_2$), 1.45 (s, 3H, ^{t}Boc-C*H*$_3$), 1.47-1.62 (m, 2H, C*H*$_2$), 3.18-3.22 (m, 2H, C*H*$_2$), 3.26-3.32 (m, 2H, C*H*$_2$), 3.59 (s, 3H, C*H*$_3$), 4.06 (m, 1H, C*H*), 6.74-6.75 (m, 1H, pyrrole-C*H*), 6.80-6.81 (m, 1H, pyrrole-C*H*), 8.28 (t, J = 5.4, 1H, N*H*), 8.55 (br.s, 1 H, N*H*), 9.30 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 22.9 (*C*H$_2$), 27.9 (*C*H$_3$), 29.1 (*C*H$_2$), 34.4 (*C*H$_2$), 38.7 (*C*H$_2$), 51.5 (*C*H$_3$), 54.0 (*C*H), 80.9 (*Cq*), 111.7 (pyrrole-*C*H), 113.9 (pyrrole-*C*H), 129.9 (pyrrole-*Cq*), 131.0 (pyrrole-*Cq*), 158.6 (*Cq*), 159.6 (*Cq*), 176.4 (*Cq*)

Arginine analogue with n = 4



$C_{19}H_{30}N_6O_6$
438.48

143

$C_{33}H_{38}N_6O_8$
646.70

10

A mixture of the methylester **143** (500 mg, 1.14 mmol, 1 eq.) and lithium hydroxide monohydrate (71.0 mg, 1.5 eq.) in a THF/water mixture (10 ml, 4/1) was stirred for 90 min until complete hydrolysis (tlc). The solution was neutralized with hydochloric acid (5 %) and lyophylized. The residue was suspended in an aqueous Na_2SO_4 solution (10 ml, 10 %) and at 0 °C a solution of Fmoc-Cl (295 mg, 1.14 mmol, 1 eq.) in dioxane (10 ml) was added. The solution was stirred for additional 90 min at room temperature, the mixture was lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (486 mg, 66 %).

$C_{33}H_{38}N_6O_8$ Mw = 646.70

yield 486 mg (66 %)

R_f 0.47 (SiO_2, dichlormethane/methanol = 9/1)

mp. 220-230 °C (decomposition) (picrate salt)

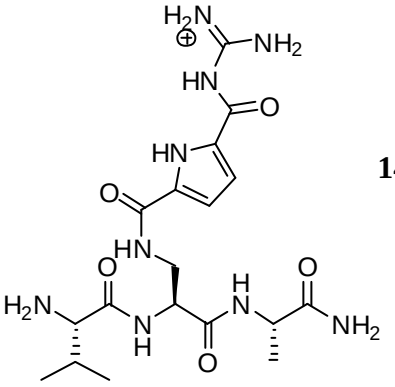
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3385 [br.m], 2965 [br.m], 1700 [s], 1623 [s], 1541 [s], 1271 [s], 742 [w]

HR-MS (ESI) m/z = 647.283 ± 0.005 (calculated for $^{12}C_{33}H_{38}N_6O_8 + H^+$: 647.283)

^{1}H-NMR (400MHz, [D_6]DMSO, picrate salt) δ = 1.28-1.78 (m, 6H, CH_2), 3.23-3.24 (m, 2H, CH_2), 3.89-3.96 (m, 1H, C*H*), 4.17-4.28 (m, 3H, C*H* and CH_2), 6.84-4.86 (m, 1H, pyrrole-C*H*), 7.01-7.02 (m, 1H, pyrrole-C*H*), 7.28-7.32 (m, 2H, fluorenyl-C*H*), 7.38-7.41 (m, 2H, fluorenyl-C*H*), 7.60-7.62 (m, 1H, fluorenyl-C*H*), 7.69-7.71 (m, 1H, fluorenyl-C*H*), 7.86-7.87 (m, 2H, fluorenyl-C*H*), 8.18 (br.s, 4H, guanidinium-NH_2), 8.42 (t, J = 5.18 Hz, 1H, N*H*), 8.57 (s, 2H, picrat-C*H*), 10.96 (br.s, 1H, NH), 12.32 (br.s, 1H, NH)

^{13}C-NMR (100MHz, [D_6]DMSO, picrate salt) δ = 23.4 (*C*H_2), 28.8 (*C*H_2), 30.7 (*C*H_2), 38.8 (*C*H_2), 46.9 (fluorenyl-*C*H), 54.0 (amino acid-*C*H), 65.8 (fluorenyl-*C*H_2), 112.5 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.5 (pyrrole-*Cq*), 125.4 (picrate-*C*H), 125.5 (pyrrole-*Cq*), 127.3 (fluorenyl-*C*H), 127.9 (fluorenyl-*C*H), 133.1 (picrate-*Cq*), 140.9 (fluorenyl-*Cq*), 140.9 (fluorenyl-*Cq*), 142.1 (picrate-*Cq*), 144.0 (fluorenyl-*Cq*), 144.1 (fluorenyl-*Cq*), 155.1 (*Cq*), 156.4 (*Cq*), 159.3 (*Cq*), 159.7 (*Cq*), 161.0 (*Cq*), 174.2 (*C*OOH)

7.5.5 Solid-Phase Synthesis of the tripeptide Ala-AA_1-Val



144

The synthesis of the tripeptide Ala-AA_1-Val **144** (AA1: arginine analogous n = 1) was synthesized on Rink amide MBHA resin by a standard protocol: Rink amide MBHA resin (300 mg, 0.67 mmol/g) was swollen in DMF for 1.5 h. The Fmoc protection group was

removed by twice agitation with piperidine in DMF (20 %) for 20 min. The peptide was synthesized with the resulting free amine. Coupling conditions for the amino acids: 2.5 eq. of Fmoc amino acid, 2.5 eq. PyBOP, DMF containing NMM 3% (10 ml). The mixture was shaken for 3.5 h to ensure quantitative coupling. The attachment of the unnatural amino acid AA_1 was performed under related conditions: 2.5 eq. PyBOP, DMF containing 5 % NMM. After every coupling step the completion of the reaction was checked by a Kaiser-test. The product was cleaved from the solid support by shaking the resin with CH_2Cl_2/TFA mixture (5:95). The solvent was evaporated up to a volume of 2 ml, and the remaining oil was treated with dry diethyl ether. The white precipitate was centrifuged and dried *in vacuo* yielding the desired tripeptide as a colorless solid (72.0 mg, 53 %)

yield 72.0 mg (53 %)

mp. 186 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3328 [br.s, m], 1672 [br.s, s], 1558 [s], 1286 [m], 1202 [s], 1138 [m], 839 [w], 759 [w], 723 [w]

HR-MS (ESI) m/z = 452.237 ± 0.005 (calculated for $^{12}C_{18}H_{29}N_9O_5 + H^+$: 452.237)

^{1}H-NMR (600MHz, [D_6]DMSO) δ = 0.90 (d, 3H, J = 6.9 Hz, C*H*$_3$), 0.92 (d, 3H, J = 6.9 Hz, C*H*$_3$), 1.22 (d, 3H, J = 7.1 Hz, C*H*$_3$), 2.03-2.08 (m, 1H, C*H*), 3.35- 3.39 (m, 1H, C*H*$_2$), 3.65 (br.s, 1H, C*H*-NH_3^+), 3.71-3.76 (m, 1H, C*H*$_2$), 4.18-4.23 (m, 1H, C*H*), 4.46-4.50 (m, 1H, C*H*), 6.89-6.90 (m, 1H, pyrrole-C*H*), 7.14-7.16 (m, 1H, pyrrole-C*H*), 7.19 (br.s, 1H, N*H*$_2$), 7.54 (br.s, 1H, N*H*$_2$), 8.27 (d, 1H, J = 6.8 Hz, N*H*), 8.12 (br.s, 3H, NH_3^+), 8.40-8.80 (br.s, 4H, guanidinium-N*H*$_2$),8.60 (t, 1H, J = 5.5 Hz, N*H*), 8.71 (t, 1H, J = 7.6 Hz, N*H*), 11.51 (br.s, 1H, N*H*), 12.27 (br.s, 1H, pyrrole-N*H*)

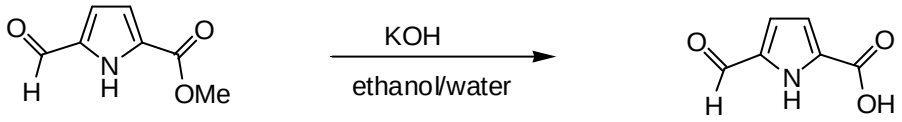
^{13}C-NMR (150MHz, [D_6]DMSO) δ = 17.8 (*C*H$_3$), 18.1 (*C*H$_3$), 18.5 (*C*H$_3$), 30.0 (*C*H), 40.5 (*C*H$_2$),48.7 (*C*H), 52.6 (*C*H), 57.6 (*C*H), 112.6 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 125.8 (pyrrole-*Cq*), 132.6 (pyrrole-*Cq*), 158.7 (*Cq*), 159.0

(*Cq*), 159.7 (*Cq*), 159.9 (*Cq*), 168.0 (*Cq*), 169.9 (*Cq*)

7.6 Synthesis of the di-cationic and tris-cationic receptors

7.6.1 Synthesis of the di-cationic receptor

5-Formyl-1*H*-pyrrole-2-carboxylic acid[146]



$C_7H_7NO_3$
153.14

148

$C_6H_5NO_3$
139.11

149

A solution of the methylester **148** (6.50 g, 42.5 mmol, 1 eq.) and potassium hydroxide (2.62 g, 46.7 mmol, 1.1 eq.) in ethanol/water (30 ml EtOH + 7 ml water) was refluxed for 3 h. The solvent was evaporated, the residue dissolved in water (50 ml) and acidified with conc. hydrochloride acid. The slightly red precipitate was filtered, washed with water and dried *in vacuo* affording the carboxylic acid **149** (5.8 g, 98 %).

$C_6H_5NO_3$ Mw = 139.11

yield 5.8 g (98 %)

R_f 0.69 (SiO_2, hexane/ethyl acetate/acetic acid = 1/1 + 1ml acetic acid per 100 ml)

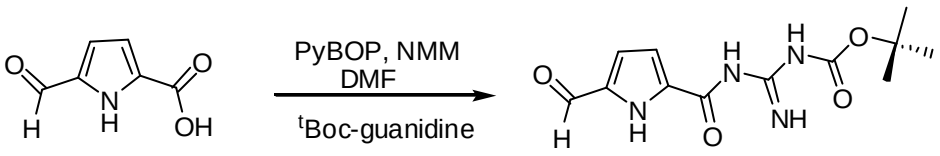
mp. 198 °C (decomposition)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3134 [m], 1687 [s], 1651 [s], 1549 [m], 1504 [m], 1430 [m], 1405 [m], 1325 [m], 1243 [s], 1145 [m], 1045 [w], 1002 [w], 916 [m], 826 [m], 777 [m], 547 [m]

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 6.81-6.83 (m, 1H, pyrrole-C*H*), 6.91-6.93 (m, 1H, pyrrole-C*H*), 9.67 (s, 1H, aldehyde-C*H*), 12.83 (br.s, 1H, pyrrole-N*H*), 13.04 (br.s, 1H, COO*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 115.6 (pyrrole-*C*H), 116.6 (pyrrole-*C*H), 129.3 (pyrrole-*Cq*), 135.5 (pyrrole-*Cq*), 161.7 (*C*OOH), 181.5 (*C*H=O),

5-Formyl-2-guanidiniocarbonyl-*1H*-pyrrole



$C_6H_5NO_3$
139.11

149

$C_{12}H_{16}N_4O_4$
280.28

147

A mixture of the carboxylic acid **149** (3.00 g, 21.7 mmol, 1 eq.), PyBOP (12.4 g, 23.9 mmol, 1.1 eq.) and N-methyl morpholine (1.50 ml) in abs. DMF (40 ml) was stirred for 60 min at room temperature. ^{t}Boc-guanidine **83** (6.98 g, 43.4 mmol, 2 eq.) was added and the resulting solution stirred over night. The solution was hydrolyzed with water (120 ml) and extracted with ethyl acetate (4 times with 100 ml). The combined organic phases were washed with water (150 ml), brine (150 ml), dried ($MgSO_4$) and evaporated. The resulting solid was purified by column chromatography (SiO_2, dichloromethane/ethyl acetate = 7/3) yielding a pink solid (5.49 g, 90 %)

$C_{12}H_{16}N_4O_4$ Mw = 280.28

yield 5.49 g (90 %)

R_f 0.4 (SiO_2, dichloromethane/ethyl acetate = 1/1)

mp. 145 °C (decomposition)

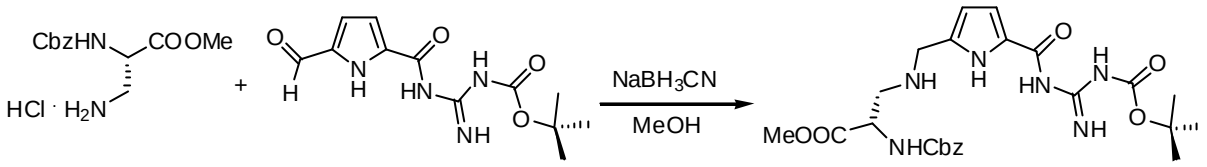
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3393 [br.s], 3240 [br.s], 2980 [m], 1724 [s], 1667 [s], 1531 [s], 1240 [s], 1144 [s], 827 [s]

MS (EI) 179 (100) [M^+-Boc]

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.46 (^{t}Boc-C*H*$_3$), 6.83-6.88 (m, 1H, pyrrole-C*H*), 6.92-6.94 (m, 1H, pyrrole-C*H*), 8.57 (br.s, 1H, N*H*), 9.35 (br.s, 1H, N*H*), 9.65 (s, 1H, aldehyde-C*H*), 10.74 (br.s, 1H, N*H*), 12.12 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 27.9 (Boc-*C*H$_3$), 81.9 (Boc-*Cq*), 114.2 (pyrrole-*C*H), 117.5 (pyrrole-CH), 134.5 (pyrrole-*Cq*), 158.0 (*Cq*), 158.7 (*Cq*), 181.3 (H*C*=O)

Protected di-cationic receptor



$C_{12}H_{17}ClN_2O_4$ 288.73 — **123**

$C_{12}H_{16}N_4O_4$ 280.28 — **147**

$C_{24}H_{32}N_6O_7$ 516.55 — **145**

To a mixture of the ^{t}Boc-protected aldehyde **147** (500 mg, 1.78 mmol, 1 eq.) and the amine hydrochloride salt **123** (1.28 g, 5.35 mmol, 3 eq.) in abs. methanol (10ml) was added at 0 °C triethylamine (179 µl, 1.28 mmol, 0.72 eq.) and $NaBH_3CN$ (112 mg, 1.78 mmol, 1 eq.). The solution was stirred at room temperature for 24 h, then the mixture was poured into ice-water (50 ml) and the off-white precipitate was filtered off. The solid was purified by column chromatography (SiO_2, dichloromethane/acetone = 1/1) affording the amine **145** as a colorless powder (600 mg, 65 %).

$C_{24}H_{32}N_6O_7$ Mw = 516.55

yield 600 mg (65 %)

R_f 0.57 (SiO_2 dichloromethane/acetone = 1/1)

mp. 79-81 °C

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3384 [br.m], 1725 [s], 1628 [s], 1542 [m], 1481 [m], 1332 [m], 1240 [s], 1150 [s], 842 [w]

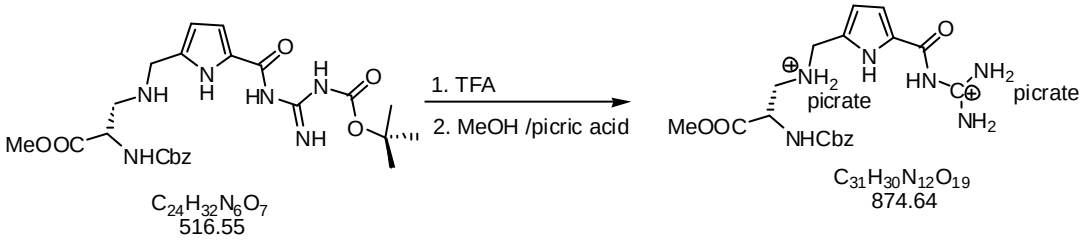
HR-MS (ESI) 517.242 ± 0.005 (calculated for $C_{24}H_{32}N_6O_7 + H^+$: 517.241)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.42 (s, 9H, C*H*$_3$), 2.72-2.79 (m, 2H, C*H*$_2$), 3.61 (s, 3H, C*H*$_3$), 3.62 (br.s, 2H, C*H*$_2$), 4.18 (q, J = 7.7 Hz, 1H, C*H*), 5.03 (s,

2H, benzyl-CH_2), 5.98 (br.s, 1H, pyrrole-C*H*); 6.97 (br.s, 1H, pyrrole-C*H*) 7.32-7.38 (m, 5H, benzyl-C*H*), 7.61 (d, J = 7.7 Hz, 1H, N*H*), 8.56 (br.s, 1H, N*H*), 9.10 (br.s, 1H, N*H*), 10.8 (br.s, 1H, N*H*), 11.38 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 28.0 (Boc-*C*H_3), 45.1 (*C*H_2), 49.1 (*C*H_2), 52.0 (*C*H_3), 54.4 (*C*H), 65.7 (benzyl-*C*H_2), 108.5 (pyrrole-*C*H), 114.8 (pyrrole-*C*H), 128.0 (benzyl-*C*H), 128.0 (benzyl-*C*H), 128.5 (benzyl-*C*H), 137.0 (benzyl-*Cq*), 156.2 (*Cq*), 158.9 (*Cq*), 172.1 (*Cq*)

Di-cationic receptor



145 **15**

The ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **145** (100 mg, 1.94 mmol) was dissolved in trifluoracetic acid and stirred for 15 min. The trifloroacetic acid (3 ml) was evaporated, the residue dissolved in methanol (10 ml) and picric acid was dropwise added. The yellow precipitate was filtered and dried *in vacuo*.

$C_{31}H_{30}N_{12}O_{19}$ MW = 874.639

yield 169 mg (quantitative)

mp. 118-120 °C (decomposition)

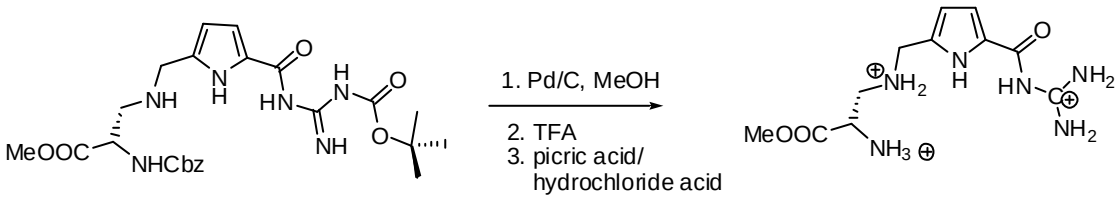
FT-IR $\tilde{n}$ (KBr) [cm^{-1}] = 3360 [br.s], 1702 [s], 1608 [s], 1612 [s], 1566 [m], 1319 [s], 1163 [m], 1080 [w], 912 [w], 789 [w], 744 [w], 710 [w]

HR-MS (ESI) 417.189 ± 0.005 (calculated for $C_{19}H_{25}N_6O_5^+$: 417.189)

^{1}H-NMR (400MHz, [D$_6$]DMSO) δ = 3.18 (br.s, 1H, C*H*$_2$), 3.35 (br.s, 1H, C*H*$_2$), 3.67 (s, 3H, C*H*$_3$), 4.23 (br.s, 2H, C*H*$_2$), 4.47-4.52 (m, 1H, C*H*), 5.07 (s, 2H, benzyl-C*H*$_2$), 6.43-6.45 (m, 1H, pyrrole-C*H*), 7.03-7.04 (m, 1H, pyrrole-C*H*), 7.32-7.38 (m, 5H, benzyl-C*H*), 7.89 (d, J= 8.5 Hz, 1H, N*H*), 8.14 (br.s, 4H, guanidinium-N*H*$_2$), 8.57 (s, 4H, picrate-C*H*), 8.98 (br.s, 2H, N*H*$_2^+$), 10.85 (br.s, 1H, N*H*), 12.20 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D$_6$]DMSO) δ = 43.0 (*C*H$_2$), 46.4 (*C*H$_2$), 51.0 (*C*H), 52.9 (*C*H$_3$), 66.3 (benzyl-*C*H$_2$), 112.8 (pyrrole-*C*H), 115.2 (pyrrole-*C*H), 124.3 (pyrrole-*Cq*), 124.3 (pyrrole-*Cq*), 125.3 (picrate-*C*H), 128.0 (benzyl-*C*H), 128.2 (benzyl-*C*H), 128.6 (benzyl-*C*H), 130.1 (*Cq*), 136.6 (benzyl-*Cq*), 142.0 (*Cq*), 155.1 (*Cq*), 156.3 (*Cq*), 159.9 (*Cq*), 161.0 (*Cq*), 169.6 (*C*OOMe)

7.6.2 Synthesis of the tris-cationic receptor



145 **11**

The Cbz-protected compound **145** (300 mg) and 10 % Pd/C (30.0 mg) was dissolved in methanol and stirred for 4 h under hydrogen atmosphere until reaction was complete (tlc). The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound as colorless powder. To obtain the trispicrate salt, the amine **145** was dissolved in trifluoroacetic acid (5 ml) and

stirred for 20 min at room temperature. The trifluoroacetic acid was evaporated *in vacuo*, the residue dissolved in methanol (8 ml) and picric acid was added (20 ml). The yellow picrate precipitate was filtered off and dried *in vacuo* yielding the picrate salt of the receptor **11** in quantitative yield.

To obtain the chloride salt of the receptor **11**, the amine **145** was dissolved in trifluoroacetic acid (5 ml) and stirred for 20 min at room temperature. The trifluoroacetic acid was evaporated *in vacuo*, addition of a few drops of diethyl ether afforded the triflate salt as a colorless solid. The triflate salt was filtered, dissolved in diluted hydrochloric acid (5 %) and lyophilized. This procedere was repeated three times yielding the tris-chloride as a slightly brown powder.

mp. 119-121 °C (decomposition) (picrate-salt)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3369 [br.m], 1756 [w], 1701 [m], 1612 [s], 1537 [s], 1318 [s], 1257 [s], 1171 [w], 1070 [w], 709 [w]

HR-MS (ESI) 283.152 ± 0.005 (calculated for $C_{11}H_{19}N_6O_3$: 283.152)

^{1}H-NMR (400MHz, [D_6]DMSO) (picrate-salt) δ = 3.20-3.26 (m, 1H, C*H*), 3.39-3.44 (m, 1H, CH_2), 3.78 (s, 3H, CH_3), 4.27-4.32 (m, 2H, CH_2), 4.35 (t, J = 5.94 Hz, 1H, C*H*), 6.48 (s, 1H, pyrrole-C*H*), 7.03 (s, 1H, pyrrole-C*H*), 8.13 (br.s, 4H, guanidinium-NH_2), 8.30-8.55 (br.s, 2H, NH_2^+), 8.58 (s, 6H, picrate-C*H*), 10.83 (br.s, 1H, N*H*), 12.24 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) (picrate-salt) δ = 43.6 (CH_2), 45.4 (CH_2), 49.5 (*C*H), 53.8 (CH_3), 112.4 (pyrrole-*C*H), 115.3 (pyrrole-*C*H), 124.2 (*Cq*), 124.5 (*Cq*), 125.4 (picrate-*C*H), 142.0 (*Cq*), 155.1 (*Cq*), 159.7 (*Cq*), 161.0 (*Cq*), 167.1 (*Cq*)

7.6.3 Data from the binding studies

NMR-Binding studies

All NMR titrations were carried out by adding aliquots of a stock solution of the the receptor (concentration given in each table) to a solution of the acylated amino acid (NMe_4^+-salt) The chemical signals were recorded with presaturation of the water signal (Bruker Avance 400 spectrometer) after each addition.

Table 6: Titration of alanine against the di-picrate salt of receptor **15** in 40 % water in DMSO.

equivalents	NH	alanine-CH_3	acetyl-CH_3
0	7.491	1.1199	1.803
0.25	7.6117	1.1379	1.8054
0.5	7.7326	1.1549	1.8076
0.75	7.8274	1.1684	1.8098
1	7.9022	1.1785	1.8108
1.25	7.956	1.1865	1.8119
1.5	/	1.1916	1.8127
1.75	8.021	1.1963	1.8134
2	8.0533	1.2	1.1837
2.5	8.0954	1.2061	1.1841
3	8.1277	1.2101	1.8148
3.5	8.1495	1.2134	1.8156
4	8.1641	1.2152	1.8159
5	8.1819	1.2181	1.8163
6	8.1978	1.2196	1.8163

c(alanine) = 0.001579699 mol/l; c(rezeptor) = 0.015825957 mol /l

Table 7: Titration of alanine against the di-picrate salt of receptor **11** in 40 % water in DMSO.

equivalents	NH	alanine-CH_3	acetyl-CH_3
0.00	7.4888	1.1202	1.8033
0.25	7.7214	1.1535	1.8077
0.50	7.9410	1.1925	1.8113
0.75	8.0710	1.2053	1.8146
1.00	8.1853	1.2185	1.8164
1.25	8.2058	1.2218	1.8168
1.50	8.2106	1.2221	1.8172
1.75	8.2127	1.2225	1.8172
2.00	8.2142	1.2225	1.8172
2.50	8.2157	1.2229	1.8172
3.00	8.2149	1.2225	1.8168
3.50	8.2153	1.2225	1.8168
4.00	8.2153	1.2225	1.8168
5.00	8.2146	1.2225	1.8168
6.00	8.2153	1.2221	1.8168
8.00	8.2168	1.2218	1.8164
10.00	8.2153	1.2218	1.8164

c(alanine) = 0.0015 mol/l; c(rezeptor) = 0.015 mol /l

Table 8: Titration of glutamic acid against the di-picrate salt of receptor **11** in 40 % water in DMSO.

equivalents	NH	CH_2	acetyl-CH_3
0.00	7.5103	1.9304	1.8130
0.25	7.5600	2.0791	1.8212
0.50	7.6415	2.1561	1.8234
0.75	7.7488	2.1919	1.8259
1.00	7.9008	2.2244	1.8285
1.25	8.0290	2.2511	1.8310
1.50	8.1229	2.2668	1.8329
1.75	8.1726	2.2751	1.8336
2.00	8.1908	2.2789	1.8340
2.50	8.2047	2.2811	1.8340
3.00	8.2095	2.2818	1.8340
3.50	8.2116	2.2829	1.8340
4.00	8.2124	2.2829	1.8340
5.00	8.2149	2.2829	1.8343
6.00	8.2142	2.2822	1.8343
8.00	8.2142	2.2822	1.8340
10.00	8.2142	2.2822	1.8340

c(glutamic acid) = 0.0015 mol/l; c(rezeptor) = 0.015 mol /l

Table 9: Titration of aspartic acid against the di-picrate salt of receptor **11** in 40 % water in DMSO.

equivalents	NH	CH_2	acetyl-CH_3
0.00	7.4230	2.3801	1.7956
0.25	7.5779	2.4473	1.8055
0.50	7.6904	2.5590	1.8128
0.75	7.7693	2.6054	1.8172
1.00	7.8296	2.6310	1.8197
1.25	7.8851	2.6463	1.8215
1.50	7.9424	2.6595	1.8230
1.75	8.0078	2.6708	1.8248
2.00	/	2.6810	1.8256
2.50	8.1664	2.6978	1.8285
3.00	8.2043	2.7041	1.8296
3.50	8.2182	2.7059	1.8296
4.00	8.2222	2.7072	1.8303
5.00	8.2285	2.7088	1.8300
6.00	8.2292	2.7082	1.8296

c(aspartic acid) = 0.0015 mol/l; c(rezeptor) = 0.015 mol /l

Table 10: Titration of alanine against the tris-chloride salt of receptor **11** in 90 % water in DMSO.

equivalents	NH	alanine-CH_3	acetyl-CH_3
0.00	7.6922	1.1323	1.8091
0.25	7.754	1.1454	1.8113
0.50	7.8277	1.1604	1.8142
0.75	7.887	1.1721	1.8164
1.00	7.9041	1.1827	1.8183
1.25	7.9742	1.1918	1.8197
1.50	8.0064	1.1984	1.8212
1.75	8.0305	1.2093	1.8223
2.00	8.0484	1.2075	1.823
2.50	8.0725	1.213	1.8241
3.00	8.0871	1.2156	1.8245
3.50	8.0948	1.2178	1.8248
4.00	8.1021	1.2188	1.8248
5.00	8.1105	1.2203	1.8252
6.00	8.1174	1.2218	1.8256
8.00	8.12	1.2225	1.8256
10.00	8.1236	1.229	1.8256

c(alanine) = 0.00152952 mol/l; c(rezeptor) = 0.015 mol /l

Table 11: Titration of glutamic acid against the tris-chloride salt of receptor **11** in 90 % water in DMSO.

equivalents	NH	CH_2	acetyl-CH_3
0.00	7.735	2.0119	1.8285
0.27	7.7383	2.0761	1.8285
0.53	7.7485	2.1225	1.8289
0.80	7.762	2.158	1.8296
1.07	7.7865	2.1868	1.8303
1.34	7.819	2.208	1.831
1.60	7.8573	2.2248	1.8325
1.87	7.8723	2.2354	1.8336
2.14	/	2.246	1.8347
2.67	/	/	1.8365
3.21	8.023	2.271	1.838
3.74	8.059	2.2795	1.8391
4.20	8.0776	2.284	1.8398
5.35	8.105	2.2902	1.8413
6.41	8.1233	2.2928	1.8413
8.55	8.1364	2.296	1.842
10.69	8.1404	2.2975	1.842

c(glutamic acid) = 0.00152952 mol/l; c(rezeptor) = 0.015 mol /l

Table 12: Titration of aspartic acid acid against the tris-chloride salt of receptor **11** in 90 % water in DMSO.

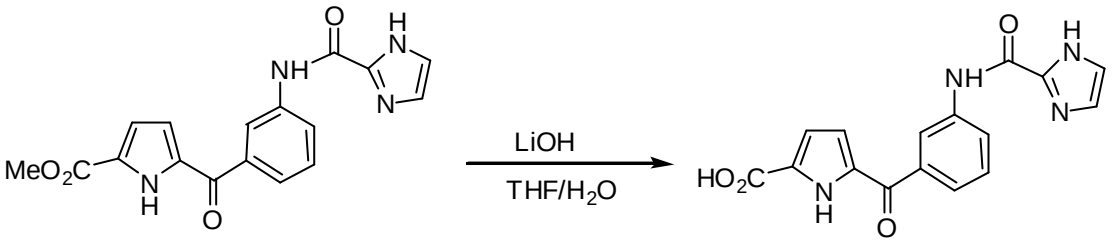
equivalents	NH	acetyl-CH_3
0.00	7.6608	1.8146
0.20	7.7152	1.8186
0.39	7.7653	1.8219
0.59	7.8088	1.8252
0.79	7.8486	1.8270
0.98	7.8782	1.8289
1.18	7.9074	1.8307
1.38	7.9337	1.8318
1.57	7.9545	1.8325
1.96	7.9967	1.8351
2.36	8.0034	1.8362
2.75	8.0575	1.8369
3.14	8.0823	1.8380
3.93	8.1147	1.8391
4.72	8.1364	1.8394
6.29	8.1525	1.8409
7.86	8.1623	1.8416

c(aspartic acid) = 0.00152952 mol/l; c(rezeptor) = 0.015 mol /l

7.7 Dipeptide receptor

7.7.1 Synthesis of the dipeptide receptor compounds

Carboxylic acid 166



$C_{17}H_{14}N_4O_4$
338.3175

158

$C_{16}H_{12}N_4O_4$
324.29

166

A mixture of the methylester **158** (1.00 g, 2.96 mmol, 1 eq.) and lithium hydroxide monohydrate (620 mg, 14.8 mmol, 5 eq.) was suspended in a tetrahydrofuran/water mixture (100 ml, 4:1) and stirred at room temperature for two days until hydrolysis was complete (tlc). The mixture was evaporated to a volume of 15 ml and acidified with aqueous hydrochloric acid (5 %) to pH 3. The colorless precipitate was filtered off, washed with water, dried over phosphorus pentoxide in the desiccator affording the carboxylic acid **160** as the hydrochloride salt (1.03 g, 97 %).

$C_{16}H_{12}N_4O_4$	Mw = 324.29
yield	1.03 g (97 %)
R_f	0.39 (SiO_2, dichloromethane/methanol = 9/1 + 1 ml triethylamine per 100 ml)
mp.	286-287 °C

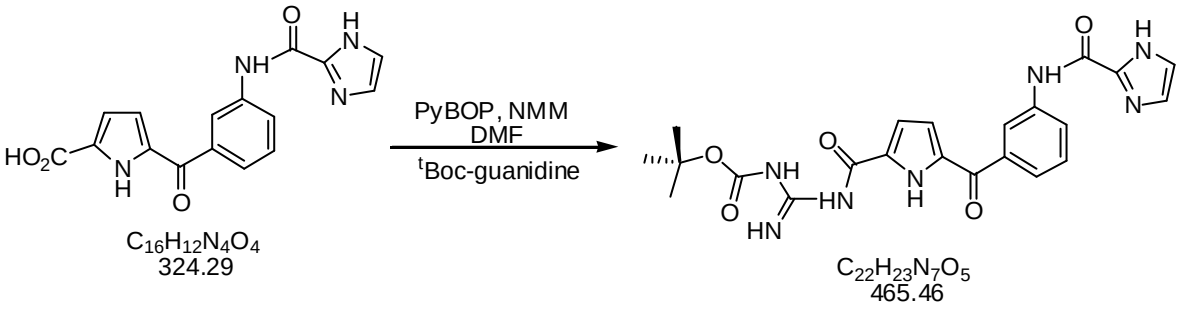
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3421 [br.m], 3233 [br.s], 1688 [s], 1666 [s], 1441 [m], 1298 [s], 1153 [w], 772 [w], 751 [w]

HR-MS (EI) m/z = 324.0858 ± 0.0002 (calculated for $^{12}C_{16}H_{12}N_4O_4$: 324.0858)

^{1}H-NMR (250MHz, [D_6]DMSO) δ = 6.83-6.87 (m, 2H, pyrrole-C*H*), 7.54-7.64 (m, 2H, C*H*), 7.74 (s, 2H, aryl-C*H*), 8.07 (dd, J = 7.02, J = 2.14, 1H, aryl-C*H*), 8.45 (t, ^{5}J = 1.64 Hz, 1H, aryl-C*H*); 11.83 (br.s, 1H, N*H*), 12.44 (br.s, 1H, pyrrole-N*H*);

^{13}C-NMR (65MHz, [D_6]DMSO) δ = 115.4 (pyrrole-*CH*), 119.1 (pyrrole-*C*H), 121.0 (*C*H), 124.2 (*C*H), 124.7 (*C*H), 129.4 (*Cq*), 129.4 (*C*H), 133.5 (*Cq*), 138.3 (*Cq*), 138.6 (*Cq*), 140.0 (*Cq*), 155.7 (*Cq*), 161.7 (*Cq*), 184.3 (*Cq*)

^{t}Boc-protected dipeptide receptor



160 **161**

A mixture of the carboxylic acid hydrochloride salt **160** (500 mg, 1.38 mmol, 1 eq.), PyBOP (795 mg, 1.53 mmol, 1.1 eq.), N-methyl morpholine (1 ml) in DMF (20 ml) was stirred for 15 min at room temperature. ^{t}Boc-guanidine **83** (244 mg, 1.53 mmol, 1.1 eq.) was added and the mixture stirred over night. The solution was hydrolyzed with water (50 ml) and the colorless precipitate was filtered off, washed with water and dried *in vacuo* yielding the protected guanidiniocarbonyl pyrrole receptor **161** as a colorless powder (558 mg, 87 %).

$C_{22}H_{23}N_7O_5$ Mw = 465.46

yield 558 mg (87 %)

mp. 279 °C

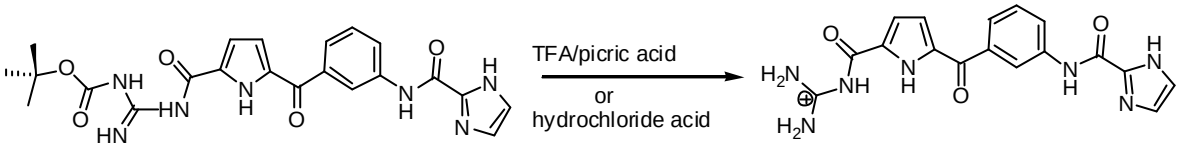
FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3399 [m], 3286 [m], 2927 [w], 1634 [s], 1527 [s], 1438 [m], 1304 [s], 1248 [m], 1151 [s], 839 [w], 753 [w]

HR-MS (ESI) m/z = 488.165 ± 0.005 (calculated for $^{12}C_{16}H_{12}N_4O_4$ + Na: 488.166)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 1.47 (Boc-C*H*$_3$), 6.86 (br.s, 1H, pyrrole-C*H*), 6.96-6.97 (m, 1H, pyrrole-C*H*), 7.16 (s, 1H, imidazole-C*H*), 7.39 (s, 1H, imidazole-C*H*), 7.49-7.57 (m, 2H, aryl-C*H*), 8.06-8.10 (m, 1H, aryl-C*H*), 8.46-8.47 (t, 1H, aryl-C*H*), 8.58 (br.s, 1H, N*H*), 9.40 (br.s, 1H, N*H*), 10.70 (br.s, 1H, N*H*), 11.16 (br.s, 1H, N*H*), 13.26 (br.s, 1H, N*H*);

^{13}C-NMR (100MHz, [D_6]DMSO) δ = δ = 27.9 (Boc-C*H*$_3$), 80.1 (*Cq*), 119.1 (pyrrole-*CH*), 119.3 (pyrrole-*C*H), 120.8 (*C*H), 120.9 (*C*H), 124.1 (*C*H), 129.4 (*Cq*), 129.4 (*C*H), 138.0 (*Cq*), 138.7 (*Cq*), 140.0 (*Cq*), 155.7 (*Cq*), 161.7 (*Cq*), 184.0 (*Cq*)

Dipeptide receptor



$C_{22}H_{23}N_7O_5$
465.46

161 **12**

To obtain the picrate salt of the receptor **12** the protected pyrrole receptor **161** (100 mg) was dissolved in trifluoroacetic acid (5 ml) and stirred for 20 min at room temperature. The trifluoroacetic acid was evaporated *in vacuo*, the residue dissolved in methanol (8 ml) and picric acid was added (20 ml). The yellow picrate precipitate was filterd off and dried in vacuo yielding the picrate salt of the receptor **12** in quantitative yield.

To obtain the chloride salt of the receptor **12**, the protected pyrrole receptor **161** (100 mg) was disssolved in conc. hydrochloric acid and stirred for 45 min. The solvent was evaporated and dried *in vacuo* to yield the chloride salt of **12** in quantitative yield.

yield quantitative

mp. 120 °C (decomposition) (picrate salt)

FT-IR $\tilde{n}$ (KBr-pellet) (picrate salt) [cm^{-1}] = 3386 [br.s], 1701 [m], 1637 [m], 1321 [m], 1264 [s], 1080 [s], 804 [w], 465 [w]

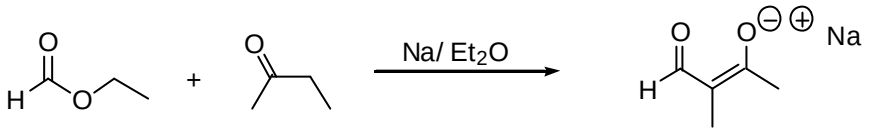
HR-MS (EI) m/z = 366.132 ± 0.005 (calculated for $^{12}C_{17}H_{16}N_7O_3$: 366.131)

^{1}H-NMR (400MHz, [D_6]DMSO) δ = 6.98-6.99 (m, 1H, pyrrole-C*H*), 7.50-7.51 (m, 1H, pyrrole-C*H*), 7.59-7.68 (m, 2H, aryl-C*H*), 7.74 (s, 2H, imidazole-C*H*), 8.03-8.05 (m, 1H, aryl-C*H*), 8.45-8.47 (m, 1H, aryl-C*H*), 8.41-8.56 (br.s, 4H, guanidinium-NH_2), 8.58 (s, 2H, picrate-C*H*), 11.46 (br.s, 1H, N*H*),

11.92 (br.s, 1H, N*H*), 12.85 (br.s, 1H, N*H*)

^{13}C-NMR (100MHz, [D_6]DMSO) δ = 116.2 (pyrrole-*CH*), 119.0 (pyrrole-*C*H), 121.2 (*C*H), 122.9 (*C*H), 122.9 (*C*H), 124.5 (*CH*), 124.6 (*Cq*), 125.5 (CH), 125.5 (*Cq*), 129.0 (*C*H), 129.9 (*Cq*), 135.7 (*Cq*), 138.0 (*Cq*), 138.2 (*Cq*), 138.7 (*Cq*), 142.2 (*Cq*), 153.5 (*Cq*), 155.5 (*Cq*), 159.9 (*Cq*), 161.7 (*Cq*), 184.4 (*Cq*)

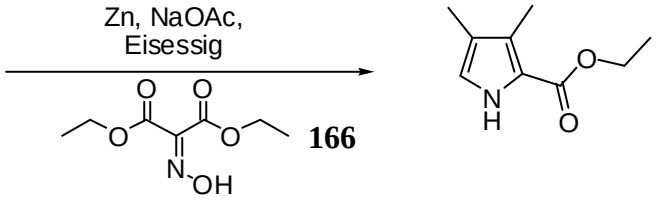
3,4-Dimethyl-1*H*-pyrrole-2-ethylester[155]



$C_3H_6O_2$ 74.08 — **163**

C_4H_8O 72.11 — **164**

$C_5H_7O_2Na$ 122.10 — **165**



166

$C_7H_{11}NO_5$ 189.17

$C_9H_{13}NO_2$ 167.21 — **167**

To a chilled (ice-bath) mixture of 2-butanon **164** (30.0 g, 1.4 eq.), ethyl formate **163** (30.0 g, 1.3 eq.) in dry diethyl ether (400 ml) was added over 6 h freshly cut sodium (9.20 g, 0.40 mol). The mixture was stirred for additional 14 h and the solvent evaporated affording a

brown solid. After addition of glacial acid (174 ml), sodium acetate (48.0 g) and diethyl aminomalonate **166** (57.0 g, 0.30 mol, 1 eq.) was the mixture heated to 90 °C until all compounds were dissolved. Then zinc (62.0 g) was added carefully and the temperature was kept between 95-100 °C. The mixture was heated to reflux for 2 h and poured onto ice-water (400 ml). The solution was extracted with dichloromethane (3 x 150 ml), the combined organic phases dried (Na_2SO_4) and the residue purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate = 8/2) affording the compound **167** as a yellow solid (8.44 g, 17 %).

$C_9H_{13}NO_2$ Mw = 167.21

yield 8.44 g (17 %)

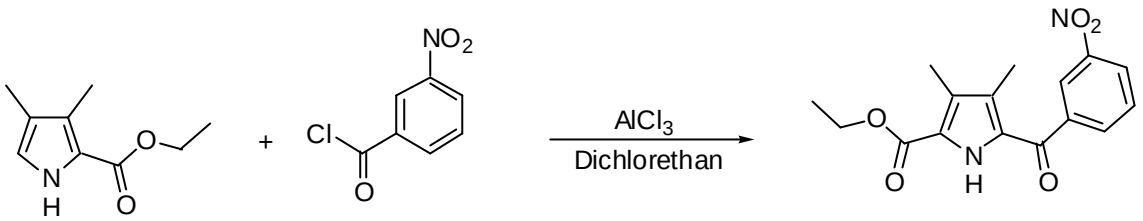
R_f 0.47 (SiO_2, *n*-hexane/ethyl acetate = 8/2)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3328 [s], 2990]w], 2918 [w], 1659 [s], 1463 [m], 1411 [s], 1382 [m], 1345 [w], 1281 [s], 1207 [w], 1152 [s], 1100 [m], 1027 [m], 922 [m], 777 [m], 599 [m]

^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.26 (t, 3H, J = 7.5 Hz, ethyl ester-C*H*$_3$), δ = 1.91 (s, 3H, pyrrole-C*H*$_3$), δ = 2.16 (s, 3H, pyrrole-C*H*$_3$), δ = 4.19 (m, 2H, ethyl ester-C*H*$_2$), δ = 6.69 (s, 1H, pyrrole-C*H*), δ = 11.11 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 9.9 (pyrrole-*C*H$_3$), 10.3 (pyrrole-*C*H$_3$), 14.6 (ethyl ester-*C*H$_3$), 59.1 (*C*H$_2$), 118.4 (pyrrole-CH), 118.9 (pyrrole-*Cq*), 121.4 (pyrrole-*Cq*), 125.5 (pyrrole-*Cq*), 161.1 (*C*=O)

4-Dimethyl-5-(3-nitrobenzoyl)-1*H*-pyrrole-2-ethyl ester



$C_9H_{13}NO_2$
167.21

$C_7H_4ClNO_3$
185.56

$C_{16}H_{16}N_2O_5$
316.31

168 **169** **170**

A mixture of 3,4-dimethyl-1*H*-pyrrole-2-ethylester **168** (1.78 g, 10.65 mmol, 1.0 eq.), 3-nitrobenzoyl chloride **169** (2.20 g, 1.1 eq.) and aluminium chloride (4.26 g, 3.0 eq.) in 1,2-dichloroethane (40 ml) was stirred at room temperature for 4 h. The dark brown mixture was hydrolyzed with water (150 ml) and extracted with dichoromethane (3 x 100 ml). The combined organic phases were washed with aqueous sodium hydrogen carbonate solution (5 %, 100 ml), with water (3 x 100 ml) and evaporated *in vacuo*. The product was purified by column chromatography (SiO_2, dichloromethane/ethyl acetate = 9/1 + 1 ml triethylamine per 100 ml) yielding a yellow solid **170** (2.36 g, 70 %).

$C_{16}H_{16}N_2O_5$ Mw = 316.31

yield 2.36 g (70 %)

R_f 0.81 (SiO_2, dichloromethane/ethyl acetate = 9/1 + 1 ml triethylamine per 100 ml)

FT-IR $\tilde{n}$ (KBr-pellet) [cm^{-1}] = 3221 [br.w], 2923 [w], 1670 [s], 1645 [s], 1530 [s], 1352 [w], 1296 [m], 1265 [s], 721 [w]

MS (EI) *m/z* = 316 $[M]^+$ (100), 269 (74), 253 (39), 148 (45), 65 (19), 39 (6)

HR-MS (EI) 316.1054 ± 0.0002 (calculated for $C_{16}H_{16}N_2O_5$: 316.1059)

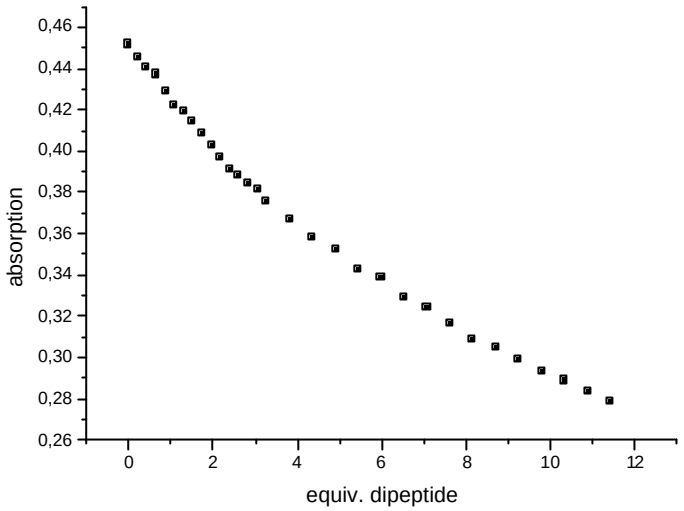
^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.37 (t, 3H, J = 7.1 Hz, ethyl ester-C*H*$_3$), 1.95 (s, 3H, pyrrole-C*H*$_3$), 2.26 (s, 3H, pyrrole-C*H*$_3$), 4.35 (q, 2H, J = 7.1 Hz, C*H*$_2$), 7.67 (t, 1H, J = 8.1 Hz, aryl-C*H*), 8.00 (dt, 1H, ^{3}J = 7.8 Hz, ^{4}J = 1.2 Hz, aryl-C*H*), 8.39 (m, 1H, aryl-C*H*), 8.1 (t, 1H, ^{4}J = 1.2 Hz, aryl-C*H*), 9.51 (br.s, 1H, pyrrole-N*H*)

^{13}C-NMR (100 MHz, $CDCl_3$) δ = 11.0 (pyrrole-*C*H_3), 11.4 (pyrrole-*C*H_3), 14.3 (ethyl ester-*C*H_3), 60.9 (ethyl ester-*C*H_2), 123.8 (aryl-*C*H), 124.1 (*Cq*), 126.4 (aryl-*C*H), 127.7 (*Cq*), 127.9 (*Cq*), 128.9 (*Cq*), 129.8 (aryl-*C*H), 134.2 (aryl-*C*H), 140.2 (pyrrole-*Cq*), 148.1 (aryl-*Cq*-NO_2), 160.9 (C=O), 184.5 (C=O)

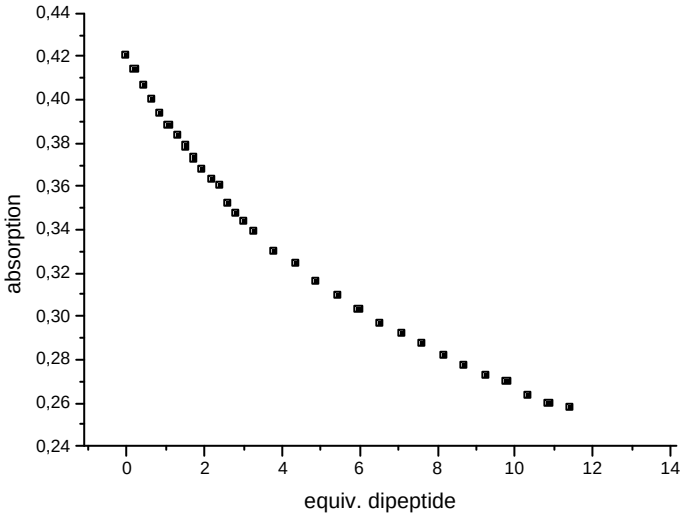
7.7.2 UV-titration data

The complexation properties of **12** were studied by UV titration in water (with 10 % DMSO added for solubility reasons) with various dipeptides and amino acids as substrates. The initial receptor concentration was 0.01586 mM in 0.5 mM bis-tris-buffer at pH = 5.5. Aliquots of the dipeptide in the same solvent mixture (concentration given below) were added to the receptor solution and the UV spectra were recorded in the range from 280 – 360 nm. The binding was then analyzed with a 1:1-complexation model using the decrease in the absorption of the pyrrole moiety at λ = 320 nm (binding isotherms shown below). All measurements were repeated twice. Sample dilution and background absorption of the added substrate were taken into account during data analysis.

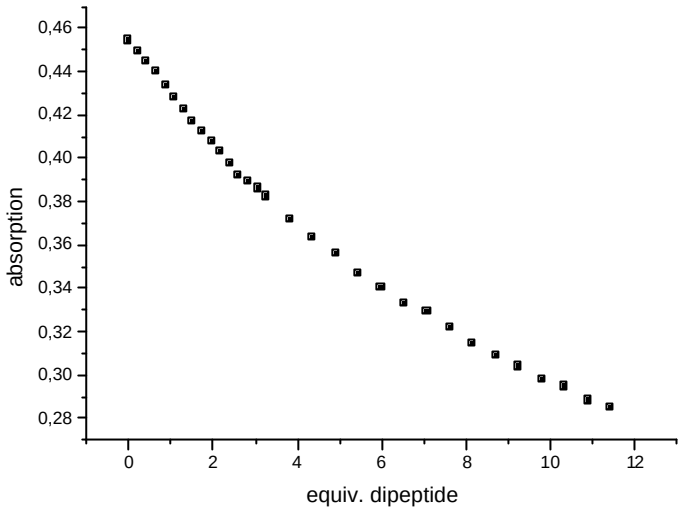
Ac-Gly-OH ; c = 0,321947 mM



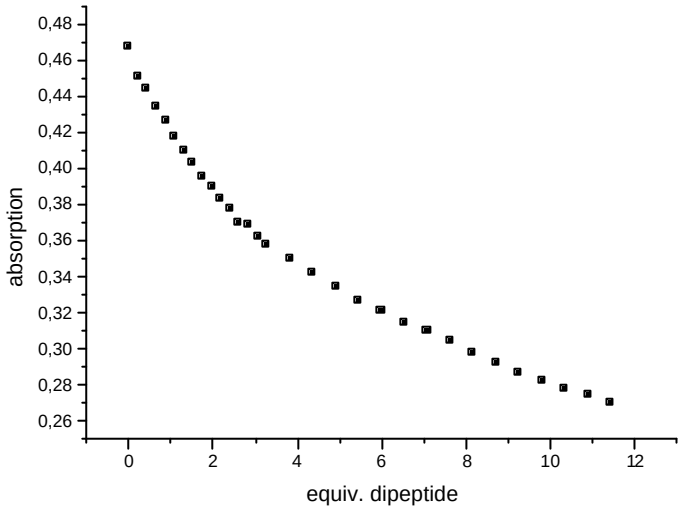
Ac-Ala-OH ; c = 0,321947 mM



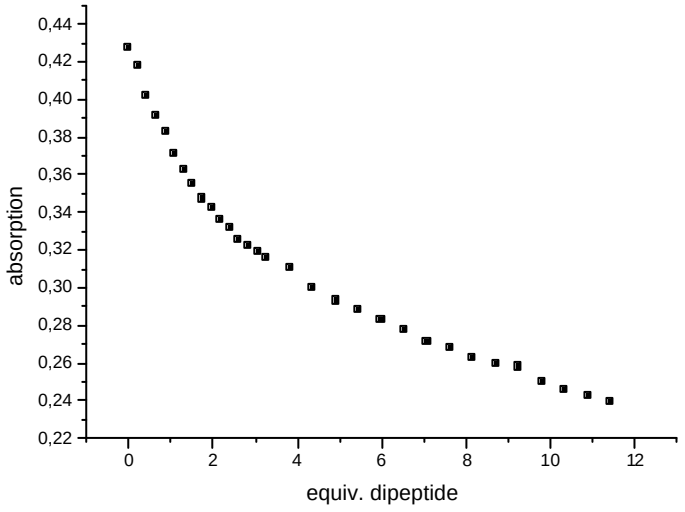
Ac-Gly-Gly-OH ; c = 0,3941 mM



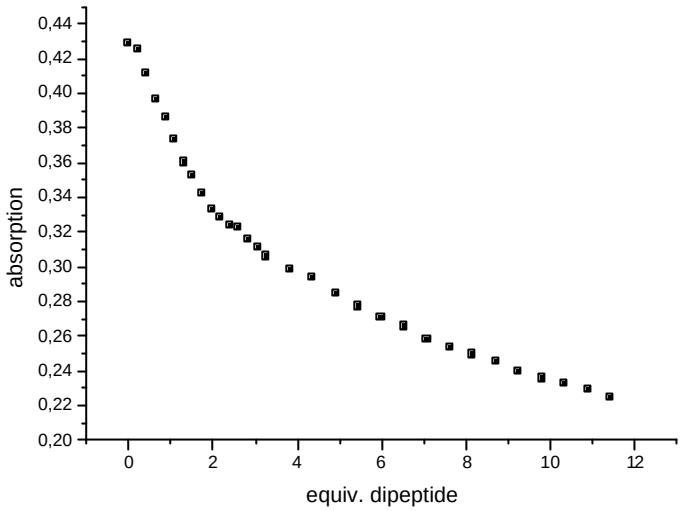
Ac-Ala-Ala-OH ; c = 0,34425 mM



Ac-Val-Ala-OH ; c = 0,3669 mM



Ac-Val-Val-OH ; c = 0,3766 mM



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9 Appendix

Abbreviations

Å	Ångstrøm
Ac	acetyl
Ala	alanine
abs.	absolute
aq.	aqueous
Bn	benzyl
^{t}Boc	*tert*-butyloxycarbonyl
c	concentration
°C	grad Celsius
Cbz	carboxybenzoyl
CBS	carboxylate-binding side
cm	centimeter
d	day(s)
DMSO	dimethylsulfoxide
δ	chemical shift
EA	elementary analysis
ESI	electrospray ionization
Et	ethyl
Fig.	figure
Fmoc	9-Fluorenylmethyloxycarbonyl
g	gram
HOAc	acetic acid
K_{ass}/K	assoziation constant
K_{dim}	dimerization constant
K_{diss}	dissoziation constant
l	liter
Me	methyl
MHz	megahertz
µl	microliter
mm	millimeter
mmol	millimole
mp	melting point
MS	mass spectrometry
m/z	mass per charge
Mw	molecular weight
N	normale
neg.	negative
NEt_3	triethylamine
NMM	*N*-methyl morpholine
R_f	retention factor
Si/SiO_2	silicagel
TFA	trifluoracetic acid
tert-Bu/^{t}Bu	*tert*-butyl
THF	tetrahydrofurane

Lebenslauf

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