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Amino acid binding in water by a new guanidiniocarbonyl pyrrole dication: the effect of the experimental conditions on complex stability and stoichiometry

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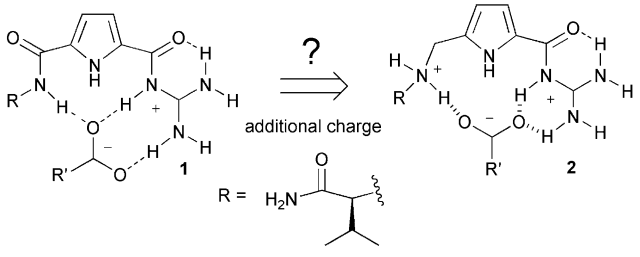
Abstract—The synthesis and binding properties of a new guanidiniocarbonyl pyrrole dication **2** are reported, which efficiently binds alanine carboxylate with $\log K_{ass} = 3.9$ in buffered water. Due to the increased charge density in this dication, the binding constant is five times larger than for the parent guanidiniocarbonyl pyrrole monocation **1** ($\log K = 3.2$). However, the experimental conditions for determining the binding constant significantly influence both complex stability and stoichiometry. With increasing amount of substrate added during the titration, the overall complex stability decreases due to the increasing ionic strength of the solution. Furthermore, the formation of 1:2 complexes between **2** and **7** becomes increasingly important. Therefore, for the comparison of binding data it has to be assured that exactly the same experimental conditions are used for their determination.

1. Introduction

Today, there are only a few artificial receptors known, that allow the complexation of an amino acid or a small peptidic substrate in water.[1,2] One reason for this is that the strength of H-bonds, often successfully used for molecular recognition in organic solvents, decreases rapidly with increasing polarity of the solvent making the design of receptors for aqueous media challenging. Therefore most systems reported so far either use hydrophobic cavities for substrate binding or rely on the much stronger metal–ligand binding interaction, for example. We currently explore how additional ionic interactions enhance the binding affinity of simple hydrogen bonding motifs.[3] In this context, we have introduced a new and highly efficient receptor for the complexation of carboxylates and amino acid carboxylates, the guanidiniocarbonyl pyrroles.[4] Due to the increased acidity of the acyl guanidinium moiety and the additional H-bonds, these complexes are much stronger than those of simple guanidinium cations, which only form stable ion pairs in organic solvents of low polarity such as chloroform or acetonitrile.[5] Therefore, our guanidiniocarbonyl pyrroles also allow the complexation of carboxylates even in aqueous solvents.[1a–c,5,6] Hence, this new recognition motif has already found versatile use in various fields of supramolecular[3] and bioorganic chemistry.[1a–c]

In an earlier investigation we had studied the origin of these highly efficient complexation properties of the guanidiniocarbonyl pyrroles by comparing complex stabilities within a series of systematically varied receptors.[5] This study revealed that the ion pairing is the main attractive interaction providing roughly 50% of the binding energy. The H-bond between the amide NH attached to position 5 of the pyrrole ring and the bound carboxylate is responsible for another 25% of the total binding energy whereas the pyrrole NH seems to be of only minor importance. As charged hydrogen bonds



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are normally stronger than neutral ones,[7] we were interested to see whether a second positive charge at the position of the amide NH would further increase the complex stability. For that reason, we chose receptor **2**, a bis-cation, as a new receptor prototype to test this hypothesis.

2. Results

Receptor **2** was synthesized according to Scheme 1. Starting from the pyrrole aldehyde **3**,[8] the methyl ester functionality was cleaved with LiOH and the resulting carboxylic acid **4** was subsequently coupled with N-Boc-guanidine.[9] A reductive amination of **5** with H-Val-NH_2 using $NaBH_3CN$ provided after deprotection with HCl the desired receptor **2**, isolated as the bis-chloride salt.

The complexation properties of **2** were first probed qualitatively by NMR studies in 40% H_2O in DMSO, the same solvent mixture in which the monocation **1** was previously studied.[5] Upon the addition of N-Ac-L-Ala-O^- (NMe_4^+-salt) **7** to a solution of **2** significant complexation induced shift changes for protons of both substrate and receptor are observed indicating a molecular interaction between anionic guest and cationic receptor in this solvent mixture. A quantitative analysis of these shift changes was however not possible as the data could neither be fitted adequately with a simple 1:1 or 1:2 (**2**:**7**) binding model nor a combination of both.

We therefore performed UV titration studies in buffered water. Aliquots of the amino acid carboxylate **7** (NMe_4^+-salt) were added to a solution of receptor **2** (chloride salt) in water (bis–tris-buffer, 4.7 mM, pH = 6.0, $[\mathbf{2}]_o = 4.8 \times 10^{-5}$ M). Changes in the UV spectrum of the receptor were recorded and used to determine the binding constants as the absorbance of the pyrrole moiety at $\lambda = 294$ nm decreases upon complex formation (Fig. 1). This decrease in absorbance is in good agreement with the pH-dependance of the UV spectrum of receptor **2**. In basic solution (pH > 9) the absorption of the guanidiniocarbonyl pyrroles decreases significantly due to the deprotonation of the guanidinium moiety. As hydrogen bonding to an anion can be regarded as a beginning deprotonation, it is not surprising that the UV absorption of the guanidiniocarbonyl pyrrole decreases upon carboxylate binding. A quantitative analysis of these changes in the UV spectra was performed using a nonlinear least-squares fitting with a 1:1 association model[10] using the Specfit/32 software program from Spectrum Software Associates. The 1:1 complex stoichiometry under these experimental conditions was confirmed by a modified Job plot analysis (Fig. 2).[11]

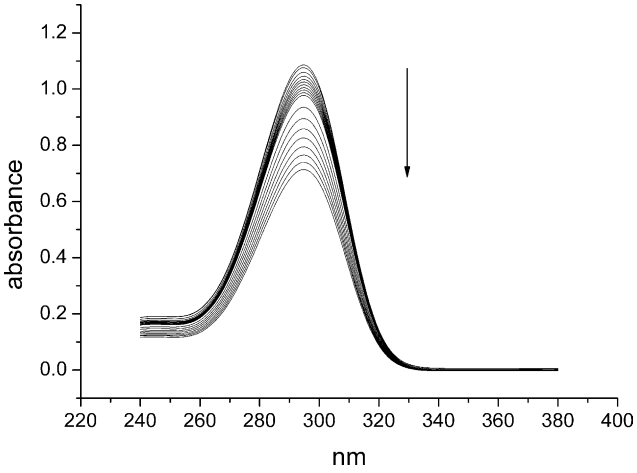


Figure 1. Changes in the UV spectra for the complexation of Ac-L-Ala-O^- (**7**) by receptor **2** in water at pH = 6.

According to this UV titration, the new bis-cationic receptor **2** binds N-acetyl alanine carboxylate **2** with an association constant of $K_{ass} = 7.95 \times 10^3\ M^{-1}$ ($\log K = 3.9$) in water. Under exactly the same conditions the mono-cationic receptor **1** binds **7** with only $K_{ass} = 1.60 \times 10^3\ M^{-1}$ ($\log K = 3.2$). Hence, the additional positive charge in bis-cation **2** favors the binding of the negatively charged substrate relative to the mono-cation **1** by a factor of five.[7] A charged H-bond at position 5 of the pyrrole is indeed superior to a neutral amide NH at the same position.

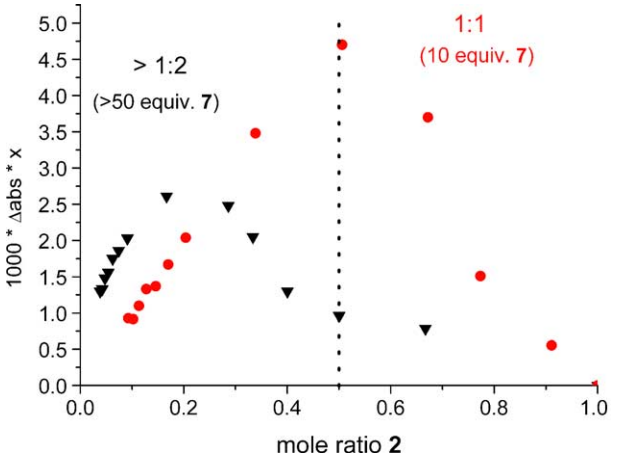
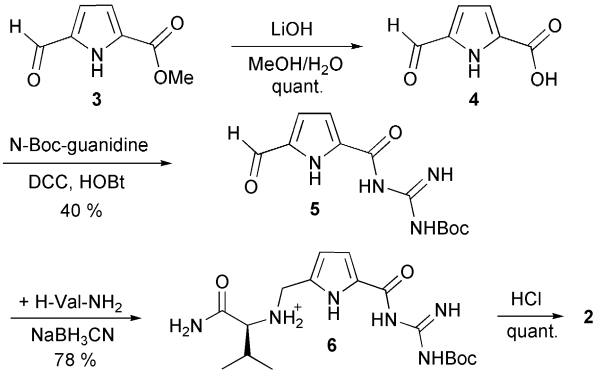


Figure 2. Modified Job plot extracted from the titration data indicating the change of complex stoichiometry with increasing amounts of added substrate.



Scheme 1. Synthesis of the bis-cationic receptor **2** via reductive amination of aldehyde **5** with H-Val-NH_2.

However, in comparing binding data for individual receptors one has to be very cautious. Depending on the conditions under which the binding constants were determined significant deviations can occur, which might lead to misinterpretations. If the binding is primarily electrostatic in nature as in these cases, the overall solvent composition significantly influences the complexation. Especially the total ionic strength of the solution plays a major role. However, differences in the ionic strength cannot only be due to different buffer concentrations[14a] but also can have their origin in the titration itself. Depending on how many equivalents of the ionic substrate are added to the receptor, the calculated binding constants can vary. The more substrate is added, the smaller the overall association constant is due to the increasing 'salt effect' caused by the substrate itself. For example, for receptor **2**, the binding constant varies from $K = 7950\ M^{-1}$ with 5 equiv of **7** added to $K = 5900\ M^{-1}$ at 20 equiv of **7** even though Job plots indicate the predominant formation of 1:1 complexes in both cases. If larger amounts of substrate are added the apparent association constant further decreases but now complexes of higher stoichiometry also form in significant amounts (vide infra; Fig. 2). However, also the addition of large amounts of NaCl causes a similar decrease in the observed association constant. If 10 mM NaCl is added, no association constant can be determined using UV titration anymore indicative of an association constant $K \leqslant 1000\ M^{-1}$.[10] But with too little substrate added the data set is not significant enough to allow a calculation of the binding constant as the extent of complexation is then not sufficiently large.[10] One has to assure therefore, that only binding constants obtained under exactly the same experimental conditions, as done here, are used for comparisons. Otherwise any data interpretation especially of small effects has only limited value.

Furthermore, also the complex stoichiometry can vary upon the addition of larger amounts of substrate. Whereas for the bis-cationic receptor **2** in water upon the addition of 10 equiv of carboxylate **7**, a Job plot from the titration data[11] clearly demonstrates the 1:1 complex stoichiometry, 1:2 (**2**:**7**) and probably even higher complexes are formed with increasing equivalents of substrate (Fig. 2).

If one does not consider these two effects (decrease of K due to the increasing salt effect and changes in stoichiometry during titration, respectively) upon setting up an experiment, the calculated binding constant can be misleading. We found that for our systems the addition of 10 equiv of substrate is a good compromise to assure a significant amount of clean 1:1 complexation as needed for data analysis with a still neglectable salt effect.

To get an idea about the structure of the complex between **2** and **7**, we performed molecular mechanics calculations. According to a MD simulation (Macromodel V 8.0, Amber* force field, GB/SA water solvation treatment, 100 ps simulation time, 1.5 fs time steps at 300 K)[12] one of the carboxylate oxygens is bound by both of the cationic guanidinium NHs forming bifurcated H-bonds whereas the other is primarily bound by the ammonium cation with assistance by the terminal carboxamide NH (Fig. 3). The binding motif in **2** is hence different from the one found for the mono-cationic guanidiniocarbonyl pyrrole **1**,[5] where the guanidinium moiety forms bidentate hydrogen bonds to both carboxylate oxygens. The higher positive charge of the ammonium group in **2** compared to the neutral amide NH in **1** causes the shifting of the carboxylate toward this H-bond donor.

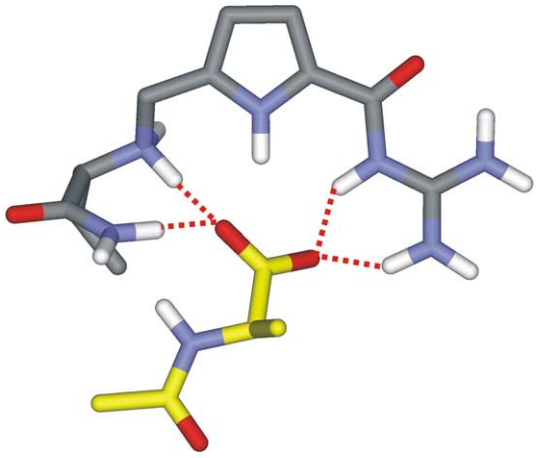


Figure 3. Calculated energy minimized structure for the complex between **7** (yellow) and **2** (gray). Hydrogen bonds are shown in red. Nonpolar hydrogens are omitted for clarity.

The five times higher binding affinity of receptor **2** relative to **1** is in good agreement with its higher positive charge density.[7,13] Though this electrostatic effect is probably somewhat counterbalanced by the higher flexibility of **2** making complexation entropically less favorable causing in total only to an increase by approximately a factor of five in complex stability. Also the bis-cationic receptor **2** is likely much more solvated in water as is mono-cation **1**.[13] Therefore, the desolvation costs are probably also larger especially in water. This desolvation, which is necessary before complexation can take place, sometimes even causes ion pair interactions to be endothermic and hence entropy driven in polar solutions.[14] In this respect an increase in complex stability by a factor of five in water is a significant improvement demonstrating how a clustering of electrostatic interactions, as weak as they individually are in water, can lead to an overall stronger complexation.

Furthermore, this also underlines that suitable binding sites at position 5 of our guanidiniocarbonyl pyrrole motif can have a significant effect on the complex stability opening the door for further improvements of the receptor design.

3. Conclusion

We have presented here the synthesis and evaluation of the binding properties of a new prototype of a

bis-cationic guanidiniocarbonyl pyrrole receptor **2**. Due to the additional positive charge **2** has superior binding properties compared to an analogous mono-cationic receptor **1** underlining the importance of electrostatic interactions for guest binding in water.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.12.120. Details of the synthesis of receptor **2**.

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