J|A|C|S
COMMUNICATIONS

Published on Web 07/01/2004

Dipeptide Binding in Water by a de Novo Designed Guanidiniocarbonylpyrrole Receptor

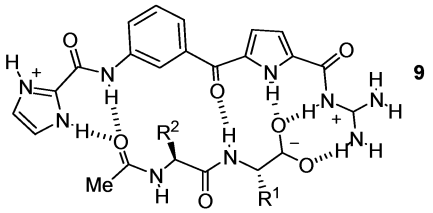
Carsten Schmuck* and Lars Geiger

Institute of Organic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

Received March 11, 2004; E-mail: schmuck@chemie.uni-wuerzburg.de

Today, there are only a few artificial receptors known that allow the complexation of a peptidic substrate in water.[1] One reason for this is that the strength of hydrogen bonds (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.[2,3] We currently explore how additional ionic interactions enhance the binding affinity of hydrogen-bonding motifs.[4] In this context, we present here a new cationic receptor prototype **9** that efficiently binds dipeptides in water with association constants $K_{ass} > 10^4$ M^{-1}.

The synthesis of **9** is described in Scheme 1. A Friedel−Crafts acylation of 2-pyrrole methyl carboxylate **1** with meta nitro benzoyl chloride **2** using $ZnCl_2$ as the catalyst under kinetic control[5] provides the 2,5-disubstitued pyrrole **3** in 26% yield besides 43% of the 2,4-regioisomer. The nitro group in **3** was reduced with hydrazine and Raney nickel to give amine **4** in 92% yield, which was then reacted with imidazole 2-carboxylic acid **5**[6] to provide the corresponding amide **6** (80% yield). The structures of **4** and **6** were confirmed by X-ray analysis. Cleavage of the ester (LiOH, 97%) and subsequent reaction of acid **7** with mono-boc protected guanidine[7] using PyBOP as the coupling reagent (87%) yielded **8**. Deprotection with acid gave the title compound **9**.



Receptor **9** was designed de novo based on theoretical calculations (Macromodel 8.0, Amber*, water solvation)[8] to bind dipeptides with a free carboxylate. The guanidiniocarbonyl pyrrole moiety is expected to form a hydrogen-bonded ion pair with the carboxylate,[9] whereas additional H-bonds between the dipeptide backbone and the receptor further stabilize the complex. The H-bond from the imidazole NH can be either neutral (monocation) or partly ionic (dication), depending on the pH of the solution.

First hints that **9** is indeed capable to bind dipeptides came from ESI MS experiments which show a distinct signal for a 1:1 complex between **9** and Ac-Ala-Ala-OH (**10**) (DMSO/MeOH solution). To probe the complexation properties of **9** in solution we first performed NMR titration experiments in 40% water in DMSO.[10] Upon the addition of **10** (NMe_4^+-salt) to **9** (1 mM, monopicrate salt), significant complexation-induced shift changes can be observed for both the receptor and the dipeptide (Figure 1).[11] For example, the amide NH next to the carboxylate in **10** exhibits a significant downfield shift with increasing equivalents of **9** added, whereas the N-terminal amide NH shows an upfield shift (Figure 1). Furthermore, the coupling constants for the amide NHs increase

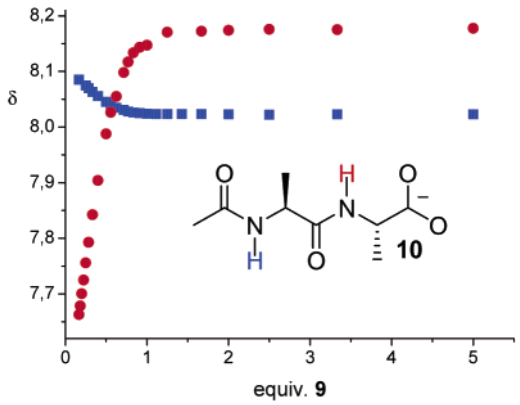
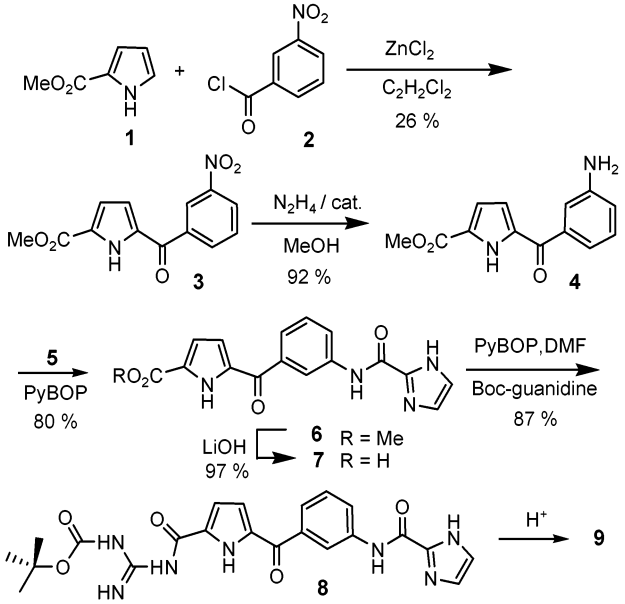


Figure 1. NMR complexation-induced shift changes of the amide NHs of **10** in the presence of **9** (40% H_2O in DMSO-d_6).

Scheme 1. Synthesis of Dipeptide Receptor **9**



from 5 to 6−8 Hz upon binding, indicating a more pronounced β-sheet-like conformation. This is in good agreement with the suggested binding mode depicted above. Corresponding shift changes are also observed for receptor **9** (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 this solvent mixture.

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

10.1021/ja048587v CCC: $27.50

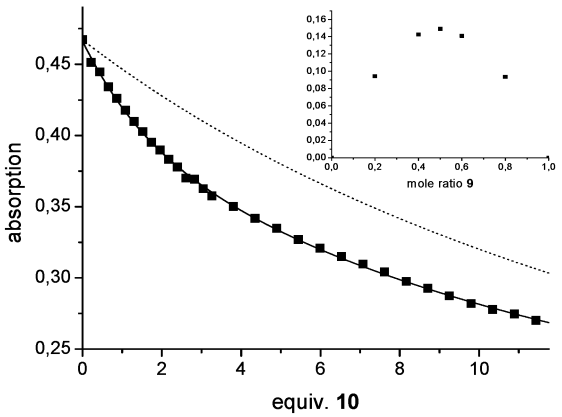


Figure 2. Job plot (inset) and binding isotherm for the complexation of Ac−Ala-Ala-OH (**10**) by receptor **9** in water (dotted line = expected UV change due to simple dilution).

Table 1. Binding Constants of **9** with Various Carboxylates

carboxylate	K_{ass}[a]
Gly-Gly (**11**)	15.900
Ala-Ala (**10**)	30.600
Val-Ala (**12**)	43.800
Val-Val (**13**)	54.300
Ala (**14**)	7.400
Gly (**15**)	5.200

[a] K in M^{-1}, estimated error limit in $K < \pm 25\%$.

was followed by the decrease in the absorption of the pyrrole moiety at $\lambda = 320$ nm (Figure 2) upon the addition of aliquots of the dipeptide to a solution of **9** (0.01586 mM, chloride salt, 0.5 mM bis-tris-buffer at pH = 5.5).[12] A Job plot confirmed the 1:1 binding stoichiometry in water. A nonlinear curve-fitting procedure was used to determine the binding constants (Table 1). The data show that **9** binds dipeptides very efficiently even in water with association constants $K_{ass} > 10^4$ M^{-1}, making **9** one of the most effective dipeptide receptors known so far.[1]

The dipeptides are bound up to 10 times more efficiently than simple amino acids ($K_{ass} \approx (5-7) \times 10^3$ M^{-1}) for which the association constants are similar to those for other guanidiniocarbonyl pyrrole-based carboxylate receptors, therefore representing simple ion pair formation.[1a,4b] Hence, the increase in stability for the dipeptides must be due to the additional binding sites within the complex (the H-bonds between the backbone amides and interactions with the imidazol 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 first glance as there are no specific binding sites for side-chain interactions present in **9**. 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.[13] As the interactions within the complex with **9** 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 H-bonds between the backbone amides from the surrounding solvent (Figure 3) thereby increasing their strength.[14] Hence, all the experimental findings support the binding motif expected from initial receptor design.

In conclusion, we have shown here that based on a theoretical prediction a new and very efficient dipeptide receptor **9** was successfully realized. The binding properties of **9** are superior to

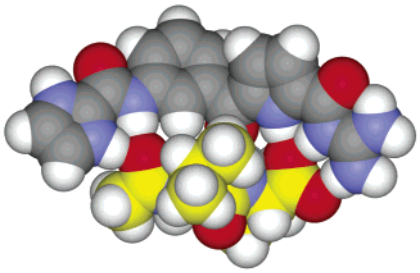


Figure 3. Calculated complex structure for the binding of **13** (yellow) by receptor **9** (gray).

any other dipeptide receptor reported thus far. The general structure of **9** should also allow the development of a second generation of receptors with specifically built in side-chain interactions to further increase the substrate selectivity (for example via an N′-alkylation at the guanidinium moiety)[1b] in the future.

Acknowledgment. This work was supported by the DFG (SCHM 1501/2-2) and the Fonds der Chemischen Industrie.

Supporting Information Available: Experimental details for the synthesis of **9**; binding data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For selected examples, see: (a) Schmuck, C.; Heil, M. *ChemBioChem* **2003**, *4*, 1232−1238. (b) Schmuck, C.; Bickert, V. *Org. Lett.* **2003**, *5*, 4579−4581. (c) Rensing, S.; Schrader, T. *Org. Lett.* **2002**, *4*, 2161−2164. (d) Jensen, K.; Braxmeier, T. M.; Demarcus, M.; Frey, J. G.; Kilburn, J. D. *Chem. Eur. J.* **2002**, *8*, 1300−1309. (e) Xuo, R.; Greiveldinger, G.; Marenus, L. E.; Cooper, A.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 4898−4899. (f) Sirish, M.; Schneider, H.-J. *Chem. Commun.* **1999**, *10*, 907−908. (g) Davies, M.; Bonnat, M.; Guillier, F.; Kilburn, J. D.; Bradley, M. *J. Org. Chem.* **1998**, *63*, 8696−8703. (h) Hossain, Md. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208−11209. (i) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536−3537. (j) Albert, J. S.; Peczuh, M. W.; Hamilton, A. D. *Bioorg. Med. Chem.* **1997**, *5*, 1455−1467.

(2) (a) Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press: New York, 1997. (b) Israelachvili, J. *Intermolecular & Surface Forces*, 2nd ed.; Academic Press: London, 1992.

(3) For examples of solvent effects on the strength of H-bonds in complexes, see: (a) Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072−7080. (b) Ariga, K.; Anslyn, E. V. *J. Org. Chem.* **1992**, *57*, 417−419.

(4) (a) Schmuck, C.; Wienand, W. *J. Am. Chem. Soc.* **2003**, *125*, 452−459. (b) Schmuck, C. *Chem. Eur. J.* **2000**, *6*, 709−718.

(5) Murakami, Y.; Tani, M.; Ariyasu, T., Nshiyama, C.; Watanabe, T.; Yokoyama, Y. *Heterocycles* **1988**, *27*, 1855−1860.

(6) Galeazzi, E.; Guzman, A.; Nava, J. L. *J. Org. Chem.* **1995**, *60*, 1090−1092.

(7) Zapf, C. W.; Creighton, C. J.; Tomioka, M.; Goodman, M. *Org. Lett.* **2001**, *3*, 1133−1136.

(8) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufiled, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(9) For recent reviews on carboxylate binding by artificial receptors including guanidinium-based systems, see: (a) Best, M. D., Tobey, S. L., Anslyn, E. V. *Coord. Chem. Rev.* **2003**, *240*, 3−15. (b) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 191−221. (c) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans 1* **2002**, 841−864. (d) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609−1646.

(10) (a) Wilcox, C. S. In *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H. J., Dürr, H., Eds.; VCH: Weinheim, 1990. (b) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

(11) Dilution studies showed that under these conditions no self-association of **9** occurs.

(12) Anion binding can be regarded as a beginning deprotonation, as the free base shows a significantly smaller absorption, it is therefore not surprising that the UV absorption of the guanidiniocarbonyl pyrrole decreases.

(13) Nowick, J. S.; Insaf, S. *J. Am. Chem. Soc.* **1997**, *119*, 10903−10908.

(14) Chitnumsub, P.; Fiori, W. R.; Lashuel, H. A.; Diaz, H.; Kelly, J. W. *Bioorg. Med. Chem.* **1999**, *7*, *39*−*59*.

JA048587V