Carboxylate Binding by 2-(Guanidiniocarbonyl)pyrrole Receptors in Aqueous Solvents: Improving the Binding Properties of Guanidinium Cations through Additional Hydrogen Bonds

Carsten Schmuck[a]

In memory of Professor Wolfgang R. Roth

Abstract: A series of guanidiniocarbonyl pyrrole receptors has been synthesized which bind carboxylates by ion pairing in combination with multiple hydrogen bonds. Their binding properties with various carboxylates have been investigated using NMR titration studies in 40% water/DMSO (*v*/*v*). The best receptor has association constants which are in the order of $K \approx 10^3\ mol^{-1}$ and hence some 30 times larger than with the simple acetyl guanidinium cation. Through a systematic variation of the receptor structure, semiquantitative estimates for the energetic contributions of the individual binding interactions could be derived. These data show that the various hydrogen bonds are not equally important for the binding but differ significantly in their energetic contribution to the overall complexation process. Furthermore, the receptor can be made chiral and shows selectivity upon binding of enantiomeric amino acid carboxylates. Molecular modeling was used to obtain structural information for the various receptor carboxylate complexes and served as a basis to explain the observed differences in binding constants.

Keywords: amino acids · carboxylate receptors · guanidinium cations · molecular recognition · supramolecular chemistry

Introduction

Molecular recognition is based on the interaction of ions or molecules and is thus the chemistry of noncovalent bonds such as hydrogen bonds, electrostatic or van der Waals interactions.[1] Despite all the progress made in this field and the large number of elegant and sophisticated host systems that have been described over the last two decades,[2] noncovalently controlled phenomena are still poorly understood.[3] Only very few receptors exist that work in solvents more polar than chloroform because the strength of hydrogen bonds and electrostatic interactions decreases rapidly as the polarity of the surrounding solvent increases.[4] However, the recognition of substrates such as peptide hormones, neurotransmitters, or carbohydrates under physiological conditions, and therefore necessarily in water is of great importance for the design of biosensors, the targeting of cellular processes or the design of new therapeutics. But the rational design of an artificial receptor which selectively binds a given substrate in such a polar solvent is still a challenging task.[1, 5] One problem is that the qualitative nature of some noncovalent interactions is not yet fully understood and is still under debate (e.g. low barrier hydrogen bonds[6] or cation-π-interactions[7]). Furthermore, we know very little about the quantitative aspects of such interactions.[8] However, without a detailed thermodynamic understanding of hydrogen bonds, electrostatic or hydrophobic interactions the rational design of such artificial receptors is not possible.

Our current approach to this problem is based on substituted 2-(guanidiniocarbonyl)-1*H*-pyrroles such as **1** (Figure 1), which we introduced recently as a new receptor class for the complexation of carboxylates in aqueous solvents.[9] In addition to the ion pairing with the guanidinium unit (well

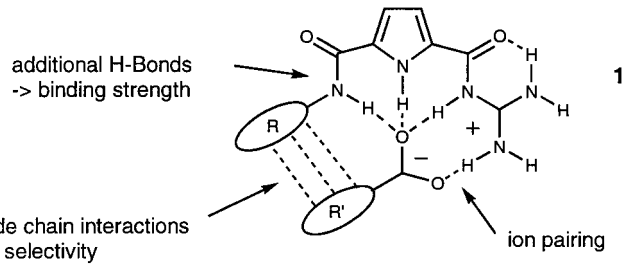

Figure 1. Design of guanidiniocarbonyl pyrrole receptors **1** for the binding of carboxylates; the ion pairing and the hydrogen bonds provide the binding strength whereas additional interactions with the side chain can account for the substrate selectivity.

[a] Dr. C. Schmuck
Institut für Organische Chemie
Universität Köln
Greinstrasse 4, 50939 Köln (Germany)
Fax: (+49) 221-4705102
E-mail: carsten.schmuck@uni-koeln.de

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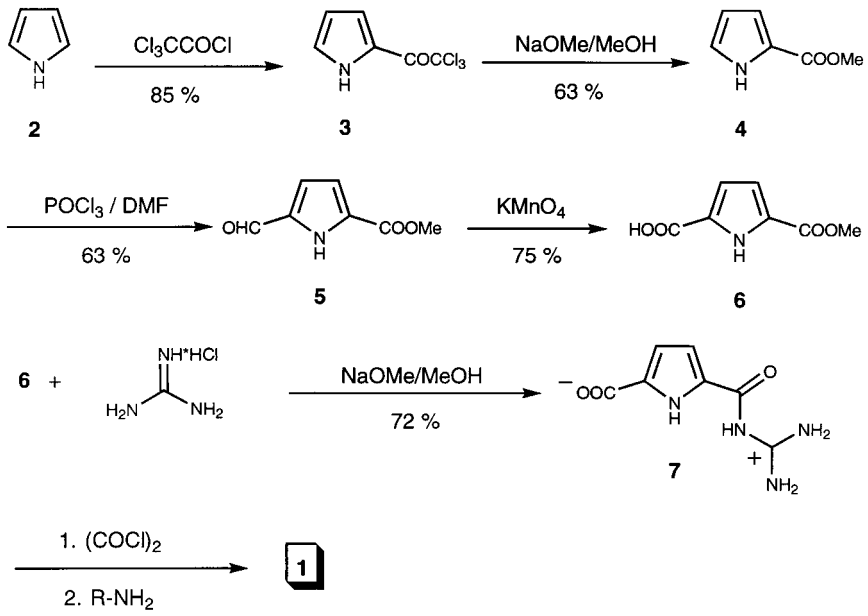
known for the complexation of oxo anions in organic solvents such as chloroform or acetonitrile),[10] the pyrrole NH as well as suitable donor sites in the side chain attached at position 5 of the pyrrole ring can form a hydrogen bond to the bound carboxylate. Therefore, these receptors show stronger binding compared with simple guanidinium cations, thus allowing the effective complexation of carboxylates even in highly polar solvents.[9] Variation of the amine side chain should allow to tune the binding selectivity for different substrates. The receptor can also be made chiral by using amino acids as amine components which means that stereoselective binding of other chiral substrates should also be possible. In this paper the synthesis of such receptors and their binding properties with a variety of carboxylates is described. By comparison of the binding affinities of a series of systematically varied receptors the energetic contributions of the individual binding interactions for the overall association process are estimated and discussed on a structural basis.



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

Results and Discussion

Synthesis: The synthesis of the guanidiniocarbonyl pyrrole receptors is shown in Scheme 1: Pyrrole (**2**) was transformed into 2-methoxycarbonyl pyrrole (**4**) by acylation with trichloroacetyl chloride and subsequent cleavage of the trichloroacetyl group with sodium methoxide.[11] Vilsmeier–Haack-formylation[12] followed by oxidation with permanganate yielded the acid **6**.[13] The guanidinylation of this compound was best achieved by refluxing **6** and an excess of guanidinium chloride with sodium methoxide in methanol.[14] Other attempts, such as reacting the ester with the free guanidine base prepared by ion exchange from the hydrochloride, or reaction of activated acid derivatives with guanidine either failed or gave very low yields.[15] The resulting zwitterion **7** forms highly stable self-assembled dimers and is insoluble in any solvent except DMSO.[16] Therefore, attempts to react **7** directly with an amine in the presence of dicyclohexylcarbodiimide (DCC) or any other reagent commonly used for amino acid coupling failed. However, the zwitterion **7** could be converted into the acyl chloride by reaction with oxalyl chloride in methylene chloride in the presence of catalytic amounts of DMF even though both compound **7** and the resulting acyl chloride are poorly soluble in this solvent. Without further isolation, the crude acyl chloride can then be reacted with an amine in THF to give the desired receptors, which can be isolated after reversed-phase chromatography in form of their chloride or picrate salts in yields of 55–75%.

In order to avoid the use of the insoluble zwitterion **7**, we tried to introduce the guanidinium group in the last step of the synthesis. The acid **6** can be easily coupled with amines in the presence of DCC to give the corresponding amides (in the case of valine in 55% yield). However, no method was found to achieve effective guanidinylation of these amides under mild conditions. Refluxing with guanidinium chloride in sodium methoxide gave the desired products only in small amounts, caused decomposition and the racemization of the stereocenter in amino acids.

A systematically varied series of receptors **8**–**14** (Scheme 2) was synthesized according to the general route in Scheme 1 by reacting the acyl chloride of **7** with butyl amine, glycine or valine amide. *N*-Acetyl guanidinium (**8**),

Abstract in German: *Guanidiniocarbonylpyrrol-Rezeptoren bilden mit Carboxylaten Ionenpaare, die zusätzlich durch mehrere Wasserstoffbrückenbindungen stabilisiert werden. Eine Reihe solcher Rezeptoren wurde synthetisiert und ihre Bindungseigenschaften mit verschiedenen Carboxylaten durch NMR Titrationen in 40% Wasser/DMSO (v/v) untersucht. Der beste Rezeptor weist Assoziationskonstanten in der Größenordnung von $K \approx 10^3\ mol^{-1}$ auf, die somit ca. 30 mal größer sind als mit der Stammverbindung, dem Acetylguanidinium-Kation. Durch systematische Variation der Rezeptorstruktur konnten die energetischen Beiträge der einzelnen Wechselwirkungen zur Gesamtkomplexbildung abgeschätzt werden. Hierbei zeigte sich, daß die verschiedenen Wasserstoffbrückenbindungen nicht alle gleich wichtig sind, sondern sich erheblich in ihrer Stärke unterscheiden. Einer der hier vorgestellten Rezeptoren ist chiral und bindet enantiomere Aminosäurecarboxylate stereoselektiv. Die Diskussion der beobachteten Unterschiede im Komplexierungsverhalten der verschiedenen Rezeptoren erfolgt auf der Basis der mit Hilfe von Kraftfeldrechnungen ermittelten Strukturen der Komplexe.*

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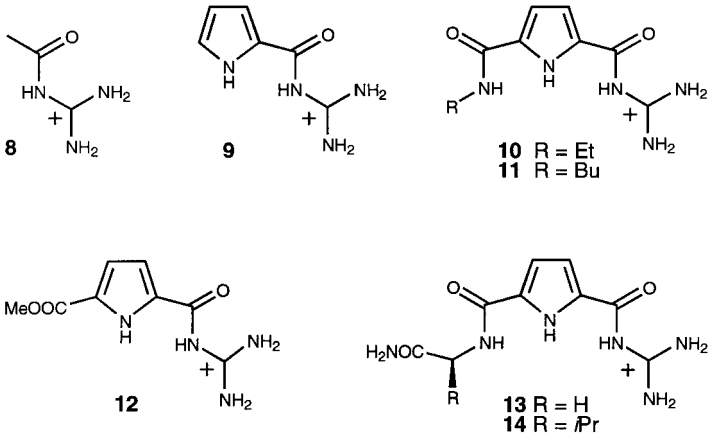


Scheme 2. Receptors **8**–**14** (picrate salts) used for the binding studies.

guanidiniocarbonyl pyrrole (**9**), the ethyl derivative **10** and the methyl ester **12** were synthesized as reported elsewhere.[9, 16] Compared with the parent acylguanidinium cation **8**, the pyrrole derivative **9** contains one more potential hydrogen bond donor (the pyrrole NH), the ethyl and the butyl substituted receptors **10** and **11** two (the pyrrole and the amide NHs), and the amino acid derivatives **13** and **14** three (the pyrrole NH and two amide NHs). The ester **12** has one more potential hydrogen bond donor and one acceptor (the ester carboxyl group) relative to **8**. For all the following studies the receptors were used in form of their picrate salts and all carboxylates in form of their NMe_4^+ salts (unless otherwise stated in the text).

Structure of the complexes: The 2-(guanidiniocarbonyl)-1*H*-pyrroles were designed to bind carboxylates through a combination of ion pairing and multiple hydrogen bonds. A mixture of the ethyl substituted receptor **10** and 2-pyrrole carboxylate (**15**) in [D_6]DMSO showed indeed significant complexation induced shifts (CIS) of the various protons of both substrate and host in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of the receptor shows the "normal" signals expected for an acylguanidinium cation (see Figure 2):[17] A broad signal at $\delta = 8.1$ for the four guanidinium NH protons, a triplet at $\delta = 8.4$ for the ethyl amide NH, a singlet for the pyrrole NH at $\delta = 12.7$, and a broad signal at $\delta = 10.9$ for the guanidinium amide NH. The unsplit signal for the four guanidinium NH protons indicates that in DMSO no intramolecular hydrogen bonding between these protons and the adjacent carbonyl group occurs or that the corresponding conformational exchange is fast on the NMR time scale. Upon addition of the carboxylate, large downfield shifts are observed (Figure 2): The signal for the guanidinium NH protons splits into two signals (two protons each) at $\delta = 7.9$ and 9.5. The ethyl amide NH gives a signal at $\delta = 9.9$ which is a downfield shift of 1.5 ppm. The signals for the guanidinium amide NH and the pyrrole NH protons are too broad and cannot be detected (probably due to traces of water in the sample and fast exchange with the solvent or an unsuitable complexation kinetics).

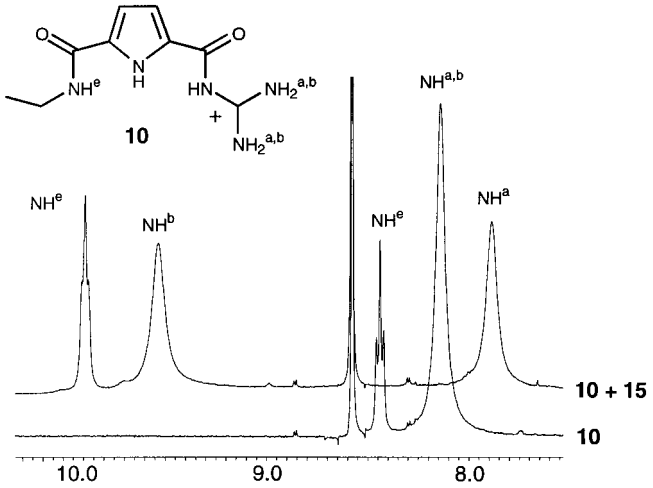


Figure 2. ^{1}H NMR spectrum of receptor **10** (picrate salt) with (back) and without (front) carboxylate **15** (NMe_4^+ salt) in [D_6]DMSO showing the CIS of the guanidinium NH protons and the amide NH.

In the case of the valine substituted receptor **14** the same CIS as described above can be observed upon addition of acetate **16** (see Figure 3). The signal for the guanidinium protons shows the same splitting into two signals as for **10**, and the valine α-NH amide and one proton of the terminal carbamoyl NH_2 group show downfield shifts of 1.7 and 0.7 ppm to $\delta = 10.1$ (formerly $\delta = 8.4$) and $\delta = 8.2$ (formerly $\delta = 7.5$). The signal of the second carbamoyl NH_2 proton shifts slightly upfield from $\delta = 7.1$ to $\delta = 6.9$. This indicates that even the terminal carbamoyl NH_2 group is involved in the complexation of the bound carboxylate.

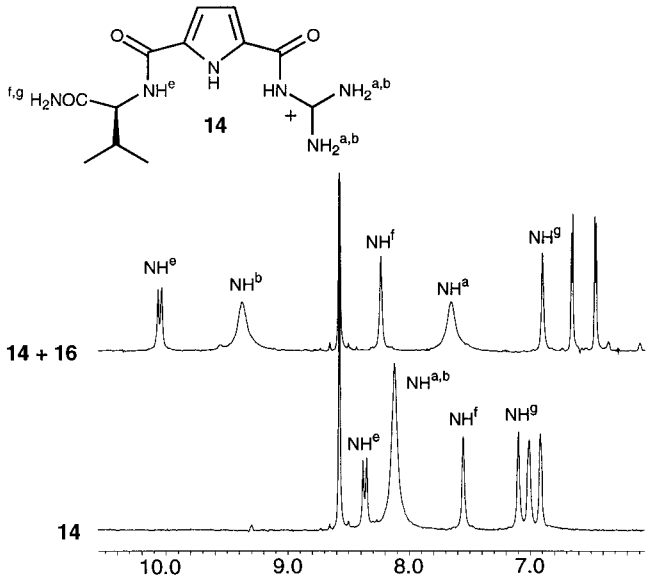


Figure 3. ^{1}H NMR spectrum of receptor **14** (picrate salt) with (top) and without (bottom) acetate (**16**) (NMe_4^+ salt) in [D_6]DMSO showing the CIS of the guanidinium NH protons and the amide NHs.

Again, both the pyrrole and the guanidinium amide NHs cannot be detected. From other studies, however, we know that upon complexation with a carboxylate these protons also show significant downfield shifts of 0.4 and 3.8 ppm to $\delta = 13.1$ and $\delta = 14.7$.[16] The large downfield shift of the guanidinium amide NH is especially noteworthy and clearly indicates the

participation of this proton in hydrogen-bonding interactions with the bound carboxylate.

These observed shift changes in the ^{1}H NMR spectrum are consistent with the general binding motif given in Figure 4 for the valine substituted receptor **14**. The guanidinium cation forms an ion pair with the carboxylate which is simultaneously

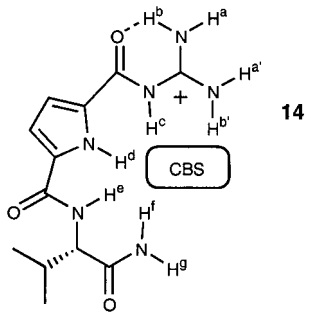
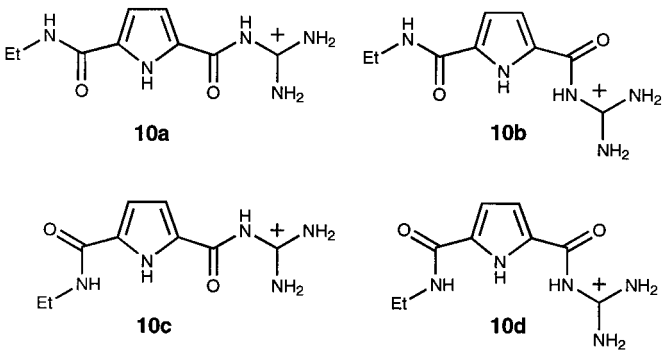


Figure 4. Proposed binding motif for the complexation of carboxylates by guanidiniocarbonyl pyrrole receptors such as **14** (CBS = carboxylate binding site).

hydrogen bonded by the pyrrole NH and the various amide NHs. The resonances for the two guanidinium protons H^a and the amide proton H^g, which do not form hydrogen bonds to the carboxylate, shift slightly upfield, whereas the other two guanidinium protons H^b, the amide NHs c, e and f, and the pyrrole NH^d, all of which take part in the complexation process, show significant downfield shifts indicating hydrogen-bonding interactions.

This binding mode requires a receptor conformation in which all the partially positively charged NHs point towards each other, forming the carboxylate binding site (CBS). This might seem unfavorable as a result of the mutual repulsion between these NHs. This eletrostatic repulsion is indeed important in chloroform or nonpolar organic solvents and strongly disfavors this conformation in such solvents. According to a molecular dynamics calculation of the ethyl substituted receptor **10** in chloroform (10 ns simulation at 300 K with the AMBER force field and the GB/SA water solvation treatment as implemented in MacroModel V6.0),[18] there is an energy difference of more than 13 kJ mol^{-1} between conformation **10d** and the lowest energy conformation **10b** (Scheme 3). But with increasing polarity of the solvent, the repulsion becomes more and more negligable. Finally, in pure water, all receptor conformations **10a–d** fall within an energy range of 2 kJ mol^{-1}. Therefore, it is not surprising that under the conditions used in these studies the receptor can easily adopt conformation **10d** with all NHs pointing inwards as required for binding.

In order to further visualize the structure of the complex, molecular mechanics calculations were carried out on the complex between the valine substituted receptor **14** and acetate **16**. A Monte Carlo simulation produced a number of conformationally different structures of the complex, reflecting the rather high flexibility of the side chain in **14**. However, all structures within 10 kJ mol^{-1} of the lowest energy conformation had the same general feature: The simultaneous binding of the carboxylate by the guanidinium cation moiety,



solvent	10a	10b	10c	10d
$CHCl_3$	1	0	8	13
water	1	0	0	2

Scheme 3. Calculated relative energies of the various conformations of receptor **10** in water and chloroform (in kJ mol^{-1}).

the pyrrole NH, and the two amide NHs. The various conformations differ only in the position and orientation of the terminal carbamoyl group, which—though hydrogen bonded to the carboxylate—is rather flexible and does not seem to have a clear preference for a single binding geometry. The lowest energy conformation obtained for this complex is shown in Figure 5.

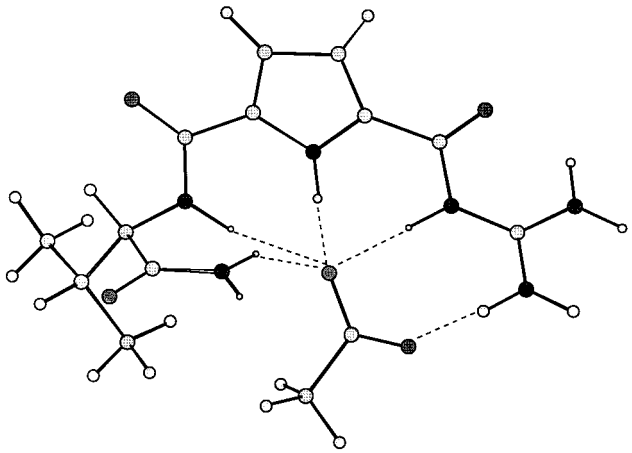


Figure 5. Lowest energy conformation of the complex between receptor **14** and acetate **16**.

Association constants: The dependence of the complexation induced shift changes on the ratio of host to substrate can be used to calculate the binding constant. However, at millimolar concentrations in [D_6]DMSO, the binding is so strong that a NMR titration experiment just showed a linear increase of the shift changes until a molar ratio of 1:1 was reached. This corresponds to an association constant $K > 10^6$ mol^{-1} (much too high for exact binding studies) and also clearly proves the 1:1 binding stoichiometry.[19]

Even in 40% water/DMSO (v/v) the association constants are still in the order of $K \approx 10^3$ mol^{-1} and hence suitable for NMR titration experiments at millimolar substrate concentrations.[20] If lower water contents are used, the binding is too

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strong, whereas in solvents with higher water contents, the receptors are not soluble enough. Also, with increasing water content it becomes more and more difficult to follow the shift changes accurately as a result of signal overlap. Furthermore, the use of water requires presaturation of the water signal during the NMR experiment so that protons which show fast exchange with the solvent, such as the receptor NHs, cannot be detected. Therefore, for the binding studies aliquots of the receptor were titrated to a solution of the carboxylate (in form of its NMe_4^+ salt) in 40% water/[D_6]DMSO (*v*/*v*). The binding constants were then calculated from the shift changes of the protons of the carboxylates by using nonlinear least-squares fitting with a 1:1 association model.[20] In all cases there was a good fit of the measured data with the theoretical model. Where the binding constants could be calculated following the shift changes of more than one proton the results were consistent and within the margin of the experimental error.

According to the general binding motif in Figure 4 the association constants for the complexation of a given carboxylate by these guanidiniocarbonyl pyrrole receptors **9**–**14** should be larger than with the parent acylguanidinium cation **8** itself due to the additional hydrogen bonds by the pyrrole NH and the amide NHs. To learn more about the individual energetic contributions of these different noncovalent interactions, the binding properties of the systematically varied receptors **9**–**14** were studied in 40% water/DMSO (*v*/*v*) with *N*-acetyl alanyl carboxylate (**17**) as the substrate (Figure 6). The calculated association constants for these receptors are summarized in Table 1.

Addition of guanidinium chloride to a 10 mM solution of the carboxylate **17** does not result in any shift changes in the NMR spectrum; this clearly shows that in this highly polar solvent no complexes are formed. Hence, the association constant must be $K < 10$ mol^{-1}.[20] The parent acetyl guanidinium cation **8** binds **17** with $K = 50$ mol^{-1}, a binding constant that is smaller by a factor of 32 compared with $K = 1610$ mol^{-1} for the binding of **17** by the valine substituted receptor **14**. The binding constants for the other receptors **9**–**13** fall in between these two values. For the guanidiniocarbonyl pyrrole **9** with its one more binding site (the pyrrole NH) the binding constant is

Table 1. Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for Ac-L-Ala-O$^-$ **17** (NMe_4^+ salt, 1 mM) with receptors **8**–**14** (picrate salts) in 40% water/[D_6]DMSO (*v*/*v*) at 25 °C.

Receptor	K_{ass} [mol^{-1}][a]	$-\Delta G_{ass}$ [kJ mol^{-1}] [kJ mol^{-1}]
Guanidinium chloride	< 10	< 5.7
8	50	9.7
9	130	12.1
10	770	16.5
11	690	16.2
12	940	17.0
13	680	16.2
14	1610	18.3

[a] error limits in *K* were estimated to be < ±10%.

about three times larger than with **8** ($K = 130$ mol^{-1} versus $K = 50$ mol^{-1}). The additional amide group in the receptors **10** and **11** further increases the binding constant by a factor of five ($K = 770$ and 690 mol^{-1}, respectively) relative to **9**. As the glycine derivative **13** has more or less the same binding constant ($K = 680$ mol^{-1}) as the ethyl and the butyl derivatives **10** and **11** the terminal carbamoyl group in **13** is obviously not involved in the complexation process in this case. For the valine substituted receptor **14**, however, the binding constant ($K = 1610$ mol^{-1}) is again larger by another factor of two relative to the binding by **10** or **13**. This is in accordance with the observed shift changes in the NMR spectrum. In the glycine substituted receptor **13** no significant shift change of the terminal carbamoyl NH could be observed, whereas in the case of the valine derivative **14** this proton resonance shifts downfield indicating hydrogen-bonding interactions (at least in DMSO). The isopropyl group in **14** probably favors receptor conformations in which the terminal carbamoyl group can take part in the binding process (valine is known for inducing β-sheet formation).[21] In the glycine derivative **13** the side chain is too flexible and any possible enthalpy gain from the additional hydrogen bond is more or less counterbalanced by the loss of entropy upon binding. Figure 7 shows a superposition of 25 structures of the complex between **13** and acetate **16** generated by a molecular dynamics calculation (100 ns simulation with one structure every 4 ns at 300 K in

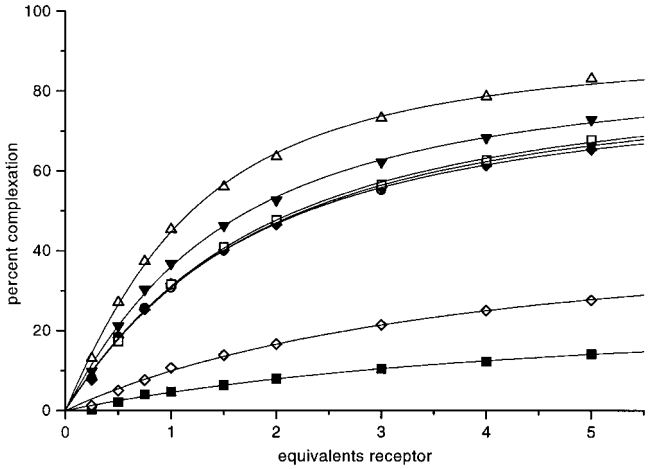


Figure 6. NMR titration curves of the various receptors (picrate salts) with carboxylate **17** (NMe_4^+ salt, 1 mM) in 40% water/[D_6]DMSO (*v*/*v*); **14** (△), **12** (▼), **10** (□), **11** (○), **13** (◆), **9** (◇), and **8** (■).

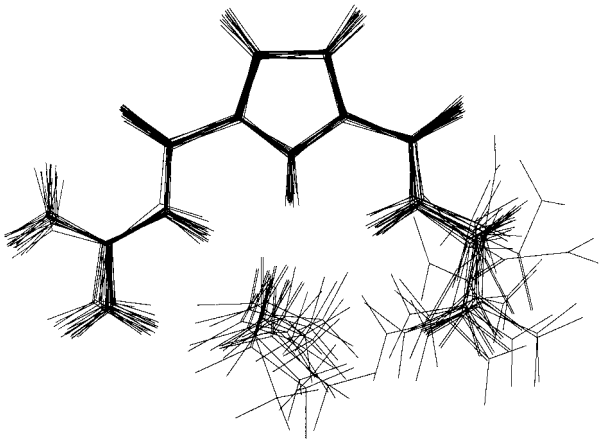
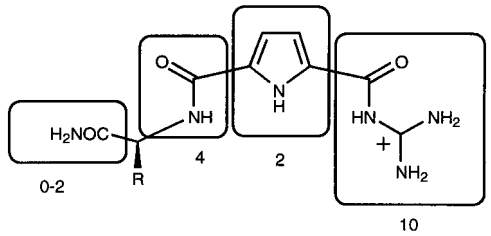


Figure 7. Superposition of 25 structures of the complex between the glycine derivative **13** and acetate **16** generated by a molecular dynamics calculation showing the high flexibility of the terminal carbamoyl group (shown on the right side).

water). It can be seen clearly that the terminal carbamoyl group has a high flexibility in contrast to the rest of the receptor. There are only very few structures in which the terminal carbamoyl group is involved in the binding of the carboxylate, whereas all other hydrogen bonds seem to be stable, at least for the time period of the simulation. It is therefore not surprising that this group has no pronounced effect on the association constant of **13**.

As the pK_a values of the *N*-acetyl guanidinium cation **8** (pK_a 7.6) and the guanidiniocarbonyl pyrrole receptors (pK_a 6.9–7.9) are essentially the same,[22] the increase in binding strength cannot only be caused by different acidities of the guanidinium moieties but rather reflects the additional hydrogen bonds formed in the complexes. This clearly shows that the binding affinity of guanidinium cations for carboxylates can indeed be significantly improved (in the case of receptor **14** by a factor of 32) by the introduction of further suitable binding sites. The data in Table 1 also show that the different hydrogen bonds do not contribute equally to the binding process (Scheme 4). Assuming the same complex structures for all receptors (which seems plausible based on



Scheme 4. Estimated semiquantitative energetic contributions of the individual binding interactions for the overall complexation of carboxylate **17** by guanidiniocarbonyl receptors of the general type **1** (ΔG in kJ mol^{-1}).

molecular modeling findings) at least some semiquantitative estimates for the individual energetic contributions of the various binding interactions can be derived from the data in Table 1. Besides the ion pairing with the acylguanidinium moiety, which has a 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}. 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}).[23]

The methyl ester **12** shows rather strong binding (the binding constant for carboxylate **17** is larger by a factor of 7 compared with **9**). As it lacks the amide group next to the pyrrole ring, we expected that the binding constant should be the same as with the unsubstituted guanidiniocarbonyl pyrrole **9**. However, according to a molecular mechanics calculation, the complex structure is somewhat different from the other receptors as there is an additional hydrogen-bonding interaction between the amide NH of the *N*-acetyl alanyl carboxylate (**17**) and the receptor ester oxygen (see Figure 8). Thus the total number of hydrogen bonds in the complex of **17** with **12** or **10** is the same and the binding energy therefore more or less also the same ($\Delta G = 16.5$ and 17.0 kJ mol^{-1}, respectively).

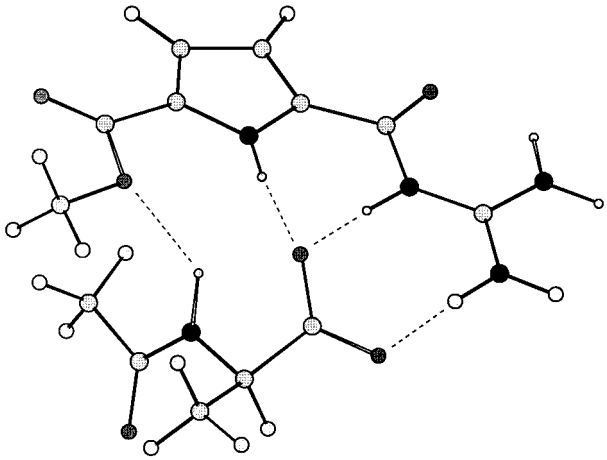
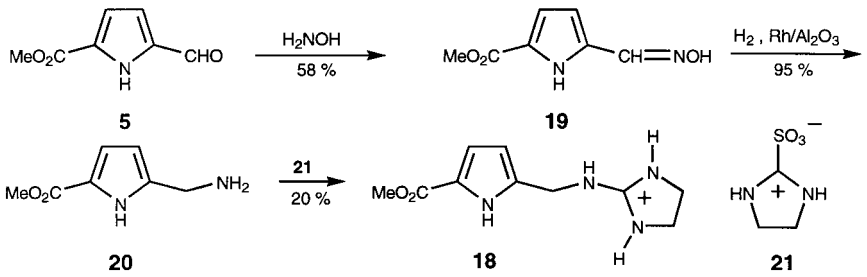


Figure 8. Lowest energy conformation of the complex between receptor **12** and alanyl carboxylate **17**.

To investigate the influence of the basicity on the binding strength for a receptor analogous to **12**, the more basic guanidine **18** was synthesized according to the following Scheme 5: Starting from the pyrrole carbaldehyde **5** the corresponding oxime **19** was catalytically reduced to the amine **20** with hydrogen in the presence of rhodium on alumina.[24] The amine then reacted with the imidazolium salt **21**[25] to give the desired guanidine **18** in 11% overall yield.

As with the parent guanidinium chloride no indication for the complexation of a carboxylate by **18** was observed in 40% water/DMSO (*v*/*v*) (see Figure 9). In pure DMSO, however, **18** binds carboxylate **17** with an association constant of $K = 5900$ mol^{-1}, which is smaller by a factor of 10^3 relative to the binding by the guanidiniocarbonyl pyrrole receptor **12** ($K \approx 10^6$ mol^{-1}). This decrease in binding strength is probably due to three factors: First, the much lower acidity of the guanidinium cation compared with an acylguanidinium cation disfavors the formation of hydrogen bonds. Guanidines have pK_a values around 13 whereas the guanidiniocarbonyl pyrroles have pK_a values of 7–8.[22] As the propensity to form hydrogen bonds increases with increasing acidity of the guanidinium moiety, a stronger binding for the acylguanidines is therefore expected. Second, the pyrrole NH of **18** is less



Scheme 5. Synthesis of guanidinium cation **18**.

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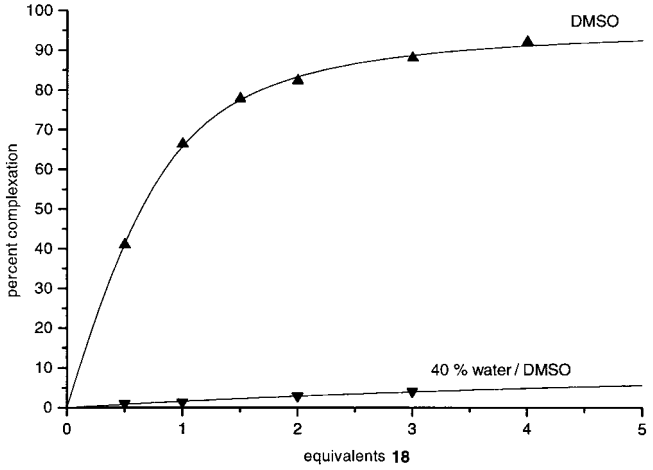

Figure 9. NMR titration curves of receptor **18** (picrate salt) with alanyl carboxylate **17** (NMe_4^+ salt, 1 mM).

acidic than that of **12** and therefore also less available for the formation of hydrogen bonds. Third, the molecule is not planar in contrast to the receptors **9**–**14**. According to molecular mechanics, the pyrrole ring in **18** is tilted relative to the plane of the guanidinium cation. Therefore, upon complexation of the carboxylate the receptor has to undergo unfavorable conformational changes.

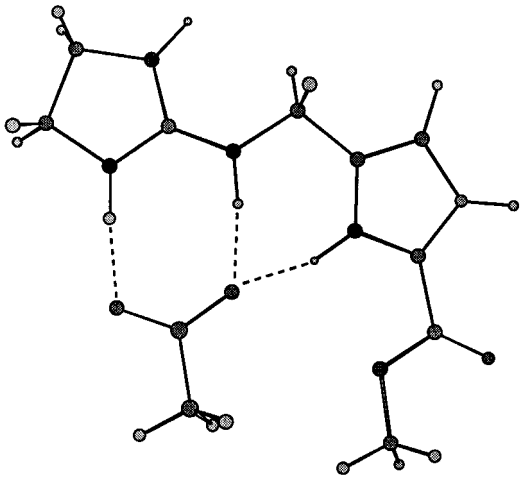

Figure 10. Lowest energy conformation of the complex between **18** and acetate.

Substrate selectivity: Receptor **14** not only shows the strongest binding in the studied series but is also chiral. Thus enantioselective recognition of amino acid carboxylates could be possible.[26] Therefore, the binding of the carboxylates of alanine, phenylalanine, and tryptophan by the valine derived receptor **14** was investigated (Figure 11). The results are summarized in Table 2.

As can be seen from the data in Table 2, receptor **14** strongly binds carboxylates with association constants ranging from $K = 350$ up to $K = 5275\ mol^{-1}$ in 40% water/DMSO (v/v). It is expected that the association constants vary with the basicity of the carboxylate. The more basic the carboxylate, the higher its propensity to form hydrogen bonds and the stronger should the complex be formed with **14**. However, the data in Table 2 reveal that this difference in acidity cannot

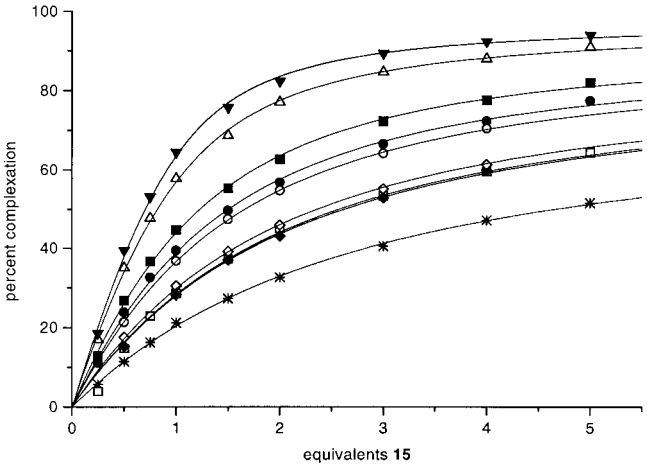

Figure 11. NMR titration curves of carboxylates (NMe_4^+ salt, 1 mM) with receptor **14** (picrate salt) in 40% water/[D_6]DMSO (v/v); **15** (▼), **16** (△), **17** (■), **26** (●), **27** (○), **25** (■), **24** (◆), **23** (■), and **22** (∗).

Table 2. Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for various carboxylate substrates (NMe_4^+ salt, 1 mM) with receptor **14** (picrate salt) in 40% water/[D_6]DMSO (v/v) at 25 °C.

Carboxylate	K_{ass} [mol^{-1}][a]	$-\Delta G_{ass}$ [$kJ\,mol^{-1}$]
AcO^- (**16**)	3380	20.1
$HCOO^-$ (**22**)	350	14.5
2-pyrrole-COO^- (**15**)	5275	21.2
Ac-L-Ala-O^- (**17**)	1610	18.3
Ac-D-Ala-O^- (**23**)	930	16.9
Ac-L-Phe-O^- (**24**)	585	15.8
Ac-D-Phe-O^- (**25**)	680	16.2
Ac-L-Trp-O^- (**26**)	1145	17.4
Ac-D-Trp-O^- (**27**)	1005	17.1

[a] error limits in K were estimated to be $< \pm 10\%$.

be the only reason for the differences in the association constants. Formic acid has the same pK_a as Ac-L-Ala-OH ($pK_a = 3.8$),[27] but its binding constant is five times lower. Acetic acid is even slightly less acidic (pK_a 4.8) than 2-pyrrole carboxylic acid ($pK_a = 4.6$),[28] but its association constant is lower. It could already been shown that in the case of the ethyl substituted receptor **10** differences in the binding constants with amino acid carboxylates stem mainly from additional interactions of the carboxylate substituents with the receptor.[9] Hence, the differences in the association constants in Table 2 should be understandable on a structural basis.

The complexes between **14** and acetate **16**, formate **22** and the 2-pyrrole carboxylate **15** all have essentially the same structure (see Figure 5) according to a molecular mechanics calculation. The differences in the binding constants are not quite clear at present but probably result from differences in the solvation/desolvation behavior of the anions. The smaller, highly solvated formate is bound less strongly than **15** or **16**. In the case of the amino acid carboxylates, the steric bulk of the *N*-acetyl group decreases the binding affinity relative to **15** or **16** (see Figure 12). However, as the data in Table 2 show, the binding is slighty stereoselective: The association constants for the enantiomers differ by a factor of 1.2 for phenylalanine and tryptophan and 1.6 for alanine, respectively.[29] In the case of alanine and tryptophan the L-enantiomer is bound better

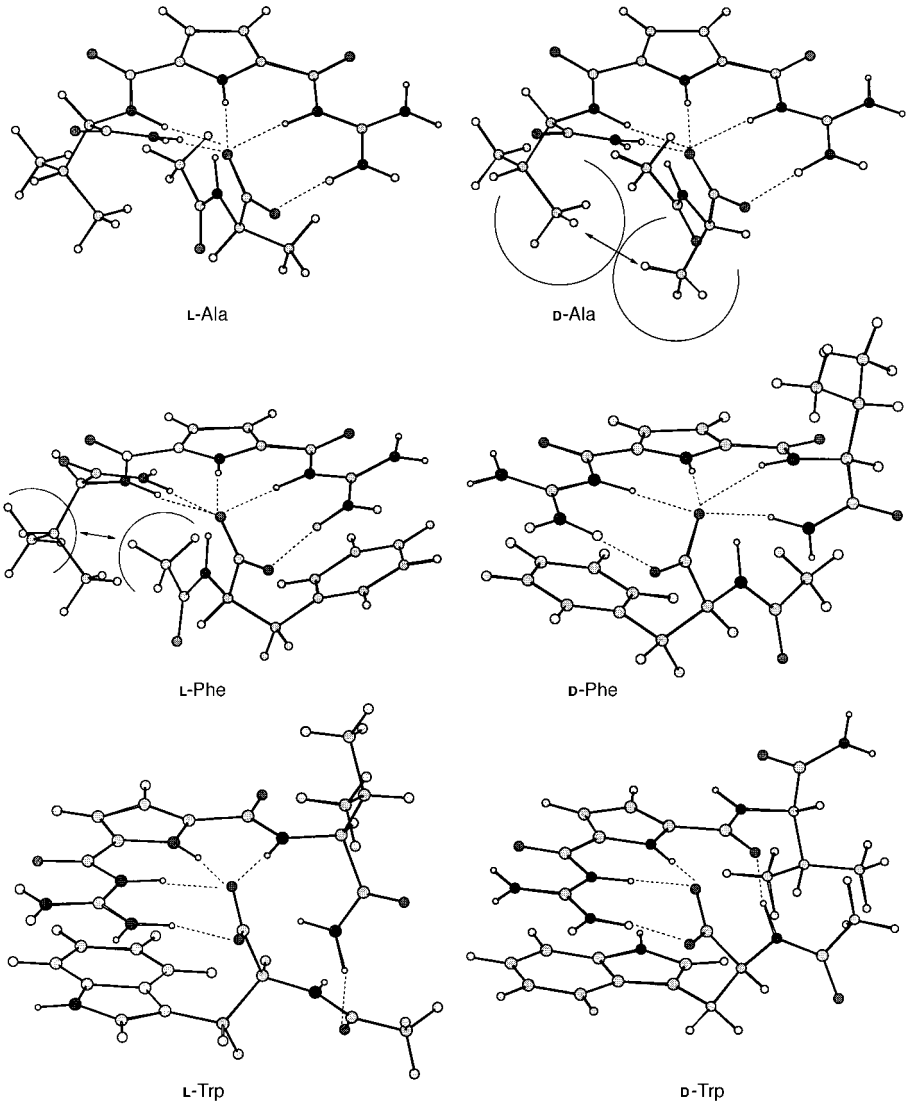

Figure 12. Lowest energy conformations of the complexes between receptor **14** and the amino acid carboxylates of alanine (top), phenylalanine (middle) and tryptophan (bottom); left: L-carboxylate, right: D-carboxylate.

than the D-enantiomer, whereas with phenylalanine the D-enantiomer is preferred. Experimental structural information not being at hand, molecular modeling was used to help rationalize the observed binding selectivities. According to these calculations with D-alanine there is an unfavorable steric repulsion between the methyl group of the amino acid and the isopropyl side chain of the receptor which is not present in the complex with the L-enantiomer (see Figure 12). In accordance with this, the binding constant for the D-enantiomer is even slightly lower than with the ethyl substituted receptor **10** ($K = 770\ \mathrm{mol}^{-1}$)[9] which lacks the sterically demanding isopropyl group. In the complex with the L-enantiomer there is no steric repulsion as the methyl group points away from the isopropyl group. Accordingly, the binding constant is larger relative to the binding by **10** due to the additional hydrogen bond. With phenylalanine and tryptophan the aromatic system and the guanidiniocarbonyl pyrrole moiety of the receptor are π-stacked probably because of an attractive cation-π-interaction. However, the much bulkier side chains compared with alanine cause unfavorable steric interactions with the isopropyl group of the receptor and decrease the binding energy for both enantiomers relative to the binding of alanine. In the case of phenylalanine the binding constants are again lower than with the ethyl substituted receptor **10** ($K = 1700\ \mathrm{mol}^{-1}$).[9] The steric repulsion is more severe in the complex with the L-enantiomer whereas the D-enantiomer can adopt a complex conformation which orientates the isopropyl group farther away from the *N*-acetyl group. In the complexes with tryptophan the binding constants are slightly larger than with **10** ($K = 810\ \mathrm{mol}^{-1}$) probably because of the more extensive hydrophobic interactions or π-stacking, which is much better in the complex with the L-enantiomer than with the D-enantiomer (which also lacks one hydrogen bond).

Conclusion

In conclusion, we demonstrated that guanidiniocarbonyl pyrrole receptors can be used to effectively bind carboxylates in aqueous media with binding constants which are much larger than with simple guanidinium cations. This proves that the basic receptor design, namely the idea to improve the binding strength by introduction of additional binding sites in addition to the ion pairing with the guanidinium cation moiety, was successful. Binding studies with a systematically varied series of receptors show that the amide NH next to the pyrrole ring is mainly responsible for the stronger binding whereas the pyrrole NH and the terminal carbamoyl group (as in **13** or **14**) contribute less to the overall binding. In the case of amino acid carboxylates the chiral receptor **14** shows a slight binding enantioselectivity which may not be as pronounced as described with other more complex systems. However, taking into account that the receptor is still rather flexible and contains only one chiral center, this is a promising starting point for the design of receptors of more pronounced stereoselectivity.

Experimental Section

General remarks: Solvents were dried and distilled under argon before use. All other reagents were used as obtained from either Aldrich or Fluka. All

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experiments were run in oven-dried glassware under argon unless otherwise stated. Products were dried in high vacuum (10^{-3} mbar) over phosphorus pentoxide at room temperature overnight. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM300 spectrometer. Shifts are reported relative to the deuterated solvents. Elemental analysis was carried out with an Elementar Vario EL.

5-(Guanidiniocarbonyl)-1*H*-pyrrole-2-carboxylate (7): Ester **6**[13] (2.0 g, 12 mmol) and guanidinium hydrochloride (5.7 g, 60 mmol) were added to a solution of sodium methoxide (prepared from 1.3 g, 56 mmol sodium in 50 mL methanol). The reaction mixture was refluxed for 12 hours and the solvent evaporated. The oily residue was dissolved in water (50 mL). Upon acidification with concentrated hydrochloric acid the crude product precipitated. It was filtered off and washed thoroughly with methanol to provide a white solid (1.7 g, 72%). The picrate salt was obtained by dissolving **7** in an aqueous picric acid solution, and recrystallized from the hot, filtered solution as yellow needles. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 6.84$ (not resolved, 1H, pyrrole CH), 7.01 (not resolved, 1H, pyrrole CH), 8.16 (brs, 4H, guanidinium NH$_2$), 8.58 (s, 2H, picrate), 11.07 (brs, 1H, amide NH), 12.74 (s, 1H, pyrrole NH), 13.16 (brs, 1H, acidic NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): $\delta = 115.26$ (CH), 115.84 (CH), 124.36 (quat. C), 125.39 (CH), 127.05, 132.5, 142.06, 155.05, 159.53, 160.99 (all quat. C); $C_{13}H_{11}N_7O_{10} \times 1\,H_2O$ (443.08): calcd C 35.02, H 2.93, N 22.12; found C 35.37, H 3.03, N 22.40.

Guanidiniocarbonyl pyrrole receptors 1 (general procedure): Compound **7** (644 mg, 3.3 mmol) was suspended in dry CH_2Cl_2 (20 mL), dry DMF (three drops), and oxalyl chloride (501 mg, 4.0 mmol, 1.2 equiv) were added and the reaction mixture was refluxed for four hours. The slightly yellow suspension was poured into a suspension of the corresponding amine or ammonium salt (6.6 mmol, 2 equiv) and triethylamine (13.2 mmol, 4 equiv) in THF and stirred at room temperature overnight. The solvent was evaporated and the residue taken up in water, purified by reversed-phase chromatography (RP 18, solvent water/methanol mixtures) and precipitated as the picrate salt which was recrystallized from water/methanol.

[5-(Butylcarbamoyl)-1*H*-pyrrole-2-carbonyl]guanidinium picrate (11): Obtained in 65% yield as yellow needles. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.89$ (t, ^3J(H,H) = 6.9 Hz, 3H, butyl CH$_3$), 1.31 (m, 2H, butyl CH$_2$), 1.48 (m, 2H, butyl CH$_2$), 3.23 (m, 2H, butyl CH$_2$), 6.86 (dd, J(H,H) = 4.0, 2.4 Hz, 1H, pyrrole CH), 7.01 (dd, J(H,H) = 4.0, 2.4 Hz, 1H, pyrrole CH), 8.15 (brs, 4H, guanidinium NH$_2$), 8.38 (t, ^3J(H,H) = 5.5 Hz, 1H, butyl amide NH), 8.58 (s, 2H, picrate), 10.92 (brs, 1H, guanidinium amide NH), 12.35 (s, 1H, pyrrole NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): $\delta = 13.85$ (CH$_3$), 19.77 (CH$_2$), 31.31 (CH$_2$), 38.60 (CH$_2$), 112.37 (CH), 115.66 (CH), 125.38 (CH), 125.44 (quat. C), 133.09, 142.02, 154.97, 159.15 (all quat. C); $C_{17}H_{20}N_8O_9 \times 1\,H_2O$ (498.16): calcd C 40.97, H 4.45, N 22.48; found C 40.83, H 4.58, N 22.59.

5-[(Carbamoylmethyl)carbamoyl]-1*H*-pyrrole-2-carbonylguanidinium picrate (13): Obtained in 55% yield as dark orange needles. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.81$ (d, ^3J(H,H) = 5.9 Hz, 1H, α-CH$_2$), 6.86 (d, ^3J(H,H) = 2.5 Hz, 1H, pyrrole CH), 7.03 (d, ^3J(H,H) = 2.5 Hz, 1H, pyrrole CH), 7.08 (s, 1H, carbamoyl NH), 7.43 (s, 1H, carbamoyl NH), 8.15 (brs, 4H, guanidinium NH$_2$), 8.58 (s, 2H, picrate), 8.83 (t, ^3J(H,H) = 5.9 Hz, 1H, amide NH), 10.97 (brs, 1H, guanidinium amide NH), 12.42 (s, 1H, pyrrole NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): $\delta = 42.03$ (CH$_2$), 112.90 (CH), 115.46 (CH), 124.41 (quat. C), 125.44 (CH), 132.59, 142.06, 155.27, 159.59, 161.03, 170.88, 177.20 (all quat. C); $C_{15}H_{15}N_9O_{10} \times 1\,H_2O$ (499.11): calcd C 36.06, H 3.43, N 25.25; found C 36.36, H 3.52, N 25.17.

5-[((*S*)-1-Carbamoyl-2-methylpropyl)carbamoyl]-1*H*-pyrrole-2-carbonyl guanidinium picrate (14): Obtained in 73% yield as orange needles. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.88$ (d, ^3J(H,H) = 6.6 Hz, 3H, isopropyl CH$_3$), 0.90 (d, ^3J(H,H) = 6.6 Hz, 3H, isopropyl CH$_3$), 2.05 (m, ^3J(H,H) = 6.6 Hz, 1H, isopropyl CH), 4.35 (dd, ^3J(H,H) = 8.9 Hz and 6.6, 1H, α-CH), 6.92 (dd, J(H,H) = 4.0, 2.5 Hz, 1H, pyrrole CH), 7.02 (dd, J(H,H) = 4.0, 2.5 Hz, 1H, pyrrole CH), 7.09 (s, 1H, carbamoyl NH), 7.55 (s, 1H, carbamoyl NH), 8.15 (brs, 4H, guanidinium NH$_2$), 8.36 (d, ^3J(H,H) = 8.9 Hz, 1H, amide NH), 8.58 (s, 2H, picrate), 10.94 (brs, 1H, guanidinium amide NH), 12.75 (s, 1H, pyrrole NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): $\delta = 18.43$, 19.56 (both CH$_3$), 30.63 (CH) 57.86 (CH), 114.11 (CH), 115.11 (CH), 124.39 (quat. C), 125.41 (CH), 132.69, 142.04, 155.11, 158.94, 161.01, 172.99 (all quat. C); $C_{18}H_{21}N_9O_{10} \times 1\,H_2O$ (541.16): calcd C 39.93, H 4.28, N 23.28; found C 39.79, H 4.28, N 23.51.

Methyl 5-[(hydroxyimino)methyl]-1*H*-pyrrole-2-carboxylate (19): The carbaldehyde **5**[13] (10.0 g, 65 mmol) was dissolved in water at 65 °C, a solution of hydroxylamine hydrochloride (6.7 g, 96 mmol) and potassium carbonate (5.5 g, 40 mmol) in water (30 mL) was added dropwise. Upon cooling a white precipitate formed which was filtered and recrystallized from water/ethyl acetate (3:1) to give oxime **19** in form of a 3:5 mixture of the two diastereomers (6.4 g, 38 mmol, 58% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.80/3.85$ (s, 3H, CH$_3$), 6.41/6.83 (d, ^3J(H,H) = 3.5 Hz, 1H, pyrrole CH), 6.80/6.95 (d, ^3J(H,H) = 3.5 Hz, 1H, pyrrole CH), 8.05/7.52 (s, 1H, oxime CH), 11.18/11.63 (s, 1H, pyrrole NH), 12.21/12.05 (s, oxime NOH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): $\delta = 51.44/51.65$ (CH$_3$), 108.44/115.71 (CH) 115.80/116.39 (CH), 128.23/131.15 (quat. C), 136.56/140.60 (CH), 160.74/160.87 (quat. C); $C_7H_8N_2O_3$ (168.05): calcd C 49.98, H 4.80, N 16.67; found C 49.66, H 4.76, N 16.22.

Methyl 5-(aminomethyl)-1*H*-pyrrole-2-carboxylate (20): Oxime **19** (2.0 g, 12 mmol) was dissolved in methanol (40 mL), saturated with ammonia, and hydrogenated in the presence of rhodium on alumina (200 mg), in a stainless steel autoclave at 120 bar hydrogen pressure for 12 hours. The solution was filtered, the solvent evaporated, and the residue recrystallized from ethyl acetate/hexane to give amine **20** in form of a white solid (1.76 g, 11.4 mmol, 95% yield). ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 1.9$ (brs, NH$_2$), 3.83 (s, 3H, CH$_3$), 3.95 (s, 2H, CH$_2$), 6.05 (d, ^3J(H,H) = 3.0 Hz, 1H, pyrrole CH), 6.83 (d, ^3J(H,H) = 3.0 Hz, 1H, pyrrole CH), 10.30 (brs, 1H, pyrrole NH); ^{13}C NMR (75.5 MHz, CDCl$_3$): $\delta = 38.96$ (CH$_2$), 51.31 (CH$_3$), 107.41 (CH) 115.97 (CH), 121.59, 138.92, 161.87 (all quat. C); $C_7H_{10}N_2O_2$ (154.17): calcd C 54.54, H 6.54, N 18.17; found C 54.32, H 6.54, N 17.87.

2-({[5-Methoxycarbonyl)-1*H*-pyrrol-2-yl]methyl}amino)-4,5-dihydro-1*H*-imidazolium picrate (18): Amine **20** (308 mg, 2 mmol) and imidazolium sulfate **21** (330 mg, 2.2 mmol) were stirred in a mixture of methanol, water, and triethylamine (6 mL, 1:1:1) overnight at room temperature. The solvent was evaporated, the residue taken up in water and acidified with hydrochloric acid. The solution was again evaporated and the remaining solid dissolved in methanol. Picric acid was added and the precipitating salt was recrystallized from methanol to give orange needles (185 mg, 20% yield). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.60$ (s, 4H, CH$_2$), 3.75 (s, 3H, CH$_3$), 4.32 (d, ^3J(H,H) = 6.1 Hz, 2H, CH$_2$), 6.11 (d, ^3J(H,H) = 3.0 Hz, 1H, pyrrole CH), 6.73 (d, ^3J(H,H) = 3.0 Hz, 1H, pyrrole CH), 7.5–8.5 (brs, 2H, guanidinium NH), 8.43 (t, ^3J(H,H) = 6.1 Hz, 1H, NH), 8.58 (s, 2H, picrate), 11.90 (brs, 1H, pyrrole NH); ^{13}C NMR (75.5 MHz, [D$_6$]DMSO): $\delta = 39.05$ (CH$_2$), 42.76 (CH$_2$), 51.33 (CH$_3$), 109.09 (CH) 115.48 (CH), 121.97, 124.36, 125.41, 133.36, 142.04, 159.35, 160.92, 161.01 (all quat. C); $C_{16}H_{17}N_7O_9$ (451.35): calcd C 42.58, H 3.80, N 21.72; found C 42.51, H 3.84, N 21.62.

NMR titrations: All NMR titrations were carried out by adding aliquots of a 10 mM solution of the guanidinium cation (picrate salt) to a 1 mM solution of the carboxylate (NMe_4^+ salt) and recording the chemical shifts after each addition. Dilution was taken into account when analyzing the data. Each titration was performed with 6–8 measurements. Where possible, different NMR signals of the carboxylate were used to calculate the binding constants. For the titrations in water, presaturation of the water signal was used.

Molecular modeling: All calculations described in this paper were performed on a SGI O_2 work station with the software package Macromodel 6.0. Conformational searches were done with at least 1000 steps until the minimum structure was found several times. Molecular dynamics calculations were done by using a constant temperature bath at 300 K with 1.5 fs time steps and 100 ps total time. The AMBER force field and the GB/SA water solvation model implemented in Macromodel were used in all studies.

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