JOC Article

Oxoanion Binding by Flexible Guanidiniocarbonyl Pyrrole−Ammonium Bis-Cations in Water

Carsten Schmuck* and Volker Bickert

Universität Würzburg, Institut für Organische Chemie, Am Hubland, 97074 Würzburg, Germany

schmuck@chemie.uni-wuerzburg.de

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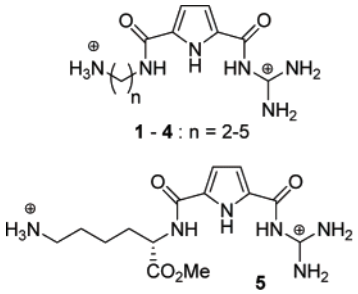

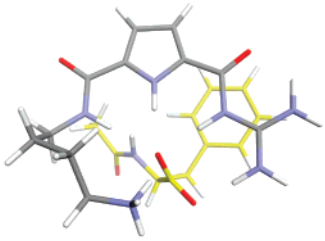

The syntheses of several bis-cations **1**−**5** with a simple primary ammonium cation attached via flexible linkers of varying length to a guanidiniocarbonyl pyrrole oxo anion binding site are reported. In UV-binding studies in aqueous buffer solution these bis-cations showed efficient binding of various *N*-acetyl amino acid carboxylates. However, complex affinity is significantly depending on both the anion and the length of the linker in the bis-cation. With increasing linker length, complex stability first increases until an optimum is reached for bis-cation **3** with a C4-linker. Then the complex stability decreases again. The best binding substrate in this series is *N*-acetyl phenyl alanine, most likely due to additional cation−π-interactions between the aromatic ring and the guanidiniocarbonyl pyrrole cation. The formation of the complex between bis-cation **3** and *N*-acetyl phenyl alanine carboxylate was investigated further by fluorescence titrations and NOE studies, as well as molecular mechanics calculations.

Introduction

The vast majority of supramolecular anion complexation[1] has been and still is based on directed electrostatic interactions, namely H-bonds[2] and ion pair formation,[3] respectively. This, however, often limits such host−guest systems to organic solvents of low polarity as due to the competitive solvation of donor and acceptor sites H-bonds and ion pairs are significantly weakened in more polar solvents.[4] To achieve strong complexation also in aqueous solvents, metal ligand interactions are often used as their strength can approach that of covalent bonds.[5] However, the use of metals can have other disadvantages such as unfavorable complexation kinetics or bioincompatibility if toxic metals are used. Another possibility is to use hydrophobic contacts[6] or aromatic interactions[7] which are especially strong

* Address correspondence to this author. Fax: +49 931 8884625.

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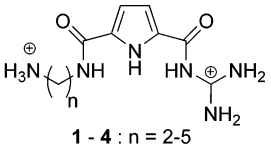
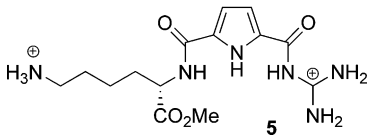
in aqueous solvents. They are, however, difficult to use deliberately as they are in general less directional and less specific than, e.g., H-bonds, respectively. Therefore the selective complexation of a given substrate solely based on solvophobic contacts in aqueous solvents is rather difficult. Furthermore, substrates which allow for extensive hydrophobic interactions often have only limited solubility in aqueous solvents. With the development of more efficient binding motifs, it was demonstrated in recent years that also electrostatic interactions can be used for substrate binding in water.[8] In general, several such weak electrostatic interactions have to be combined to achieve a strong and selective substrate binding under these competitive conditions ("Gulliver-Effect").[2a]

In this context, we are exploring how a clustering of ion pairs can be used for oxoanion binding in aqueous solvents.[9] For example, we recently showed that tetrapeptides with a free carboxylate terminus can be efficiently recognized in water by multi-cationic receptors solely based on electrostatic interactions.[10] In one of our works we have quantitatively screened a combinatorial receptor library derived from a tripeptide with an appended tailor-made guanidiniocarbonyl pyrrole carboxylate binding site[11,12] for the binding of the polar tetrapeptide N-Ac-D-Glu-Lys-D-Ala-D-Ala.[13] From these screenings, it appears that the guanidiniocarbonyl pyrrole anion binding site could be further improved by an additional second positive charge, which can simultaneously interact with the bound carboxylate thereby strengthing the complex. Whenever a lysine was directly next to the guanidiniocarbonyl pyrrole cation, complex stability significantly increased compared to that of other, non-cationic amino acids in this position.[14] We have therefore now systematically investigated the influence of a second charge on oxoanion binding by guanidiniocarbonyl pyrrole cations. For this purpose, we have synthesized a series of flexible guanidiniocarbonyl pyrrole−ammonium bis-cations **1**−**5** in which the distance between the ammonium cation and the guanidiniocarbonyl pyrrole cation varies from two to five carbons. These bis-cations were then tested for their binding affinity toward amino acid carboxylates and other oxoanions by using UV titrations in buffered water. We find that complex stability significantly depends on both the length of the linker and the amino acid side chain with a pronounced optimum for the binding of phenylalanine by the longer bis-cation **3** with a C4 spacer.



1 - **4** : n = 2-5



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Results and Discussion

Synthesis of Receptors 1−4. The general structure of bis-cations **1**−**4** is shown in Figure 1: a guanidiniocarbonyl pyrrole cation is attached to a diamino alkane with a chain length varying from two to five carbons. The synthesis of these cations is described in Scheme 1: Pyrrole dicarboxylic acid monobenzyl ester **6** was reacted with *t*Boc-guanidine **7** with PyBOP as the coupling reagent (93% yield). The benzyl ester in **8** was quantitatively cleaved off by hydrogenation with a catalytic amount of palladium on activated charcoal. The resulting carboxylic acid **9** was then reacted with the mono-*t*Boc protected diamines **10** with PyBOB as the coupling reagent. The yields varied from 52% to 74% yield for the different diamines. Finally, the remaining protecting groups were cleaved off by acidic treatment with concentrated hydrochloric acid in methanol. After lyophilization the desired bis-cationic receptors **1**−**4** were obtained as their hydrochloride salts.

The lysine derivative **5** was synthesized accordingly (Scheme 2). The pyrrole carboxylic acid **9** was reacted with H-Lys(Cbz)-OMe **12** with PyBOP as the coupling reagent in a mixture of DCM and DMF (yield 86%). Removal of the Cbz-protecting group with H_2/Pd/C provided the desired receptor **5** as the chloride salt after lyophilization from aqueous HCl in methanol. The moderate yield (60%) of the cleavage reaction is due to the fact that under these conditions also the methyl ester is cleaved to some extent, giving the free carboxylic acid as the byproduct.

Binding Studies. The binding properties of the five bis-cations **1**−**5** were investigated by using UV-titration studies[15] in buffered water with 10% DMSO added to increase the solubility of the *N*-acetyl amino acid carboxylate used as substrate. The bis-cations ($c = 7 \times 10^{-5}$) were dissolved in bis-tris buffer ($c = 5 \times 10^{-3}$) and the pH was adjusted to 6.0 to make sure that the receptor is fully protonated and the substrates are deprotonated. Control studies have shown that the pK_a of the guanidiniocarbonyl pyrrole cation is around 6.5 whereas the ammonium group has a pK_a of 9.2−9.7. These values were determined with use of the Henderson−Hasselbalch equation by measuring the pH at the half equivalence point of each host in water. As substrates a variety of oxo-anions ($c = 1.5 \times 10^{-3}$) such as several *N*-acetyl amino acid carboxylates as well as sulfate and acetate were used. Aliquots of a stock solution of the corresponding anion were addedd to the solution of the bis-cation and the changes in the UV spectra were

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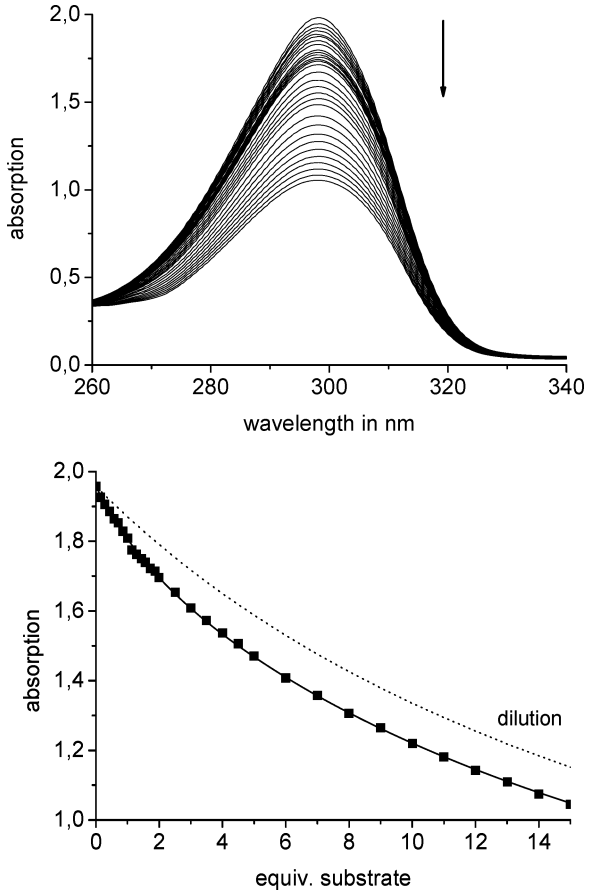
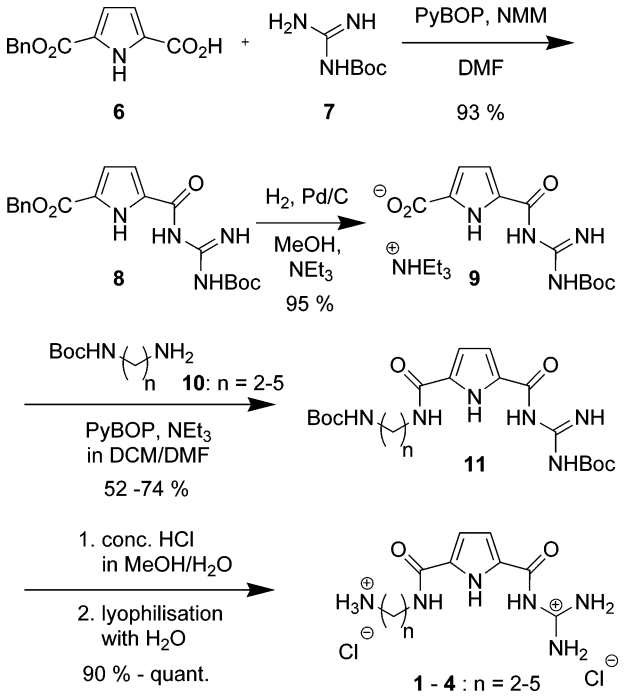


FIGURE 1. Top: Change of the UV absorption in the course of the titration of receptor **3** with *N*-acetyl phenyl alanine carboxylate. Bottom: Resulting binding isotherm at $\lambda = 300$ nm used to calculate the complex stability based on a nonlinear curve-fitting procedure. The dashed line represents the expected change in absorbance if only a dilution of the sample had taken place.

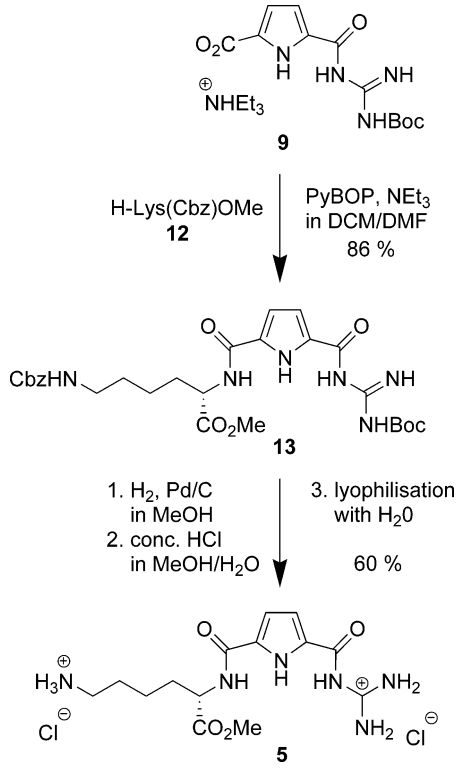
recorded after each addition. Interaction of the anion with the guanidiniocarbonyl pyrrole cation causes a distinct decrease in the molar absorbance at 300 nm, which can be used to follow the complexation quantitatively. From this decrease in UV absorbance the binding constants were calculated by using a nonlinear curve-fitting procedure for a 1:1 complexation taking into account the dilution of the sample during titration. All measurements were repeated twice, and the results were identical within the experimental error of the method. A representative binding isotherm is shown in Figure 1. The complex stoichiometry was independently confirmed by a Job plot[16] (Figure 2) as well as ESI-MS experiments. The resulting binding constants are summarized in Table 1 and in Figure 3.

Data Interpretation. As the data show, the binding of oxoanions by these bis-cations depends quite significantly on the length of the linker. One might expect a steady decrease of complex stability with increasing length and flexibility of the linker as has been observed in other cases. It is generally assumed that freezing of an internal torsion upon guest complexation reduces complex stability. For example, Schneider has studied the influence of alkyl spacers on the binding between flexible bis-ammonium cations and bis-anions in water. He indeed found a steady decrease of complex stability with

SCHEME 1. Synthesis of the Bis-Cationic Receptors 1−4



SCHEME 2. Synthesis of the Lysine Derivative 5



increasing linker length.[17] However, in this case for all anions complex stability first increases until a pronounced maximum in complex stability is reached for bis-cation **3** with a linker length of four methylene groups (Figure 3). Only afterward does the complex stability drop again when the linker becomes even longer. In general, the smaller bis-cations **1** and **2** do not bind anions very efficiently under these experimental conditions (*K*

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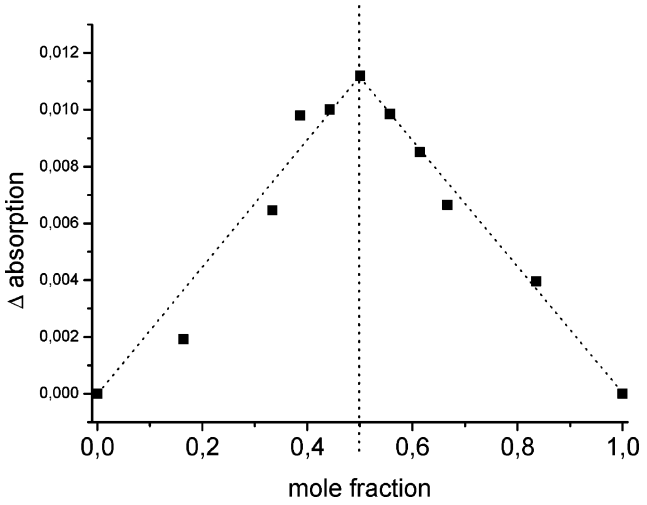

FIGURE 2. Job plot for the complex formation between receptor **3** and *N*-acetyl phenyl alanine carboxylate confirming a 1:1 complex stoichiometry.

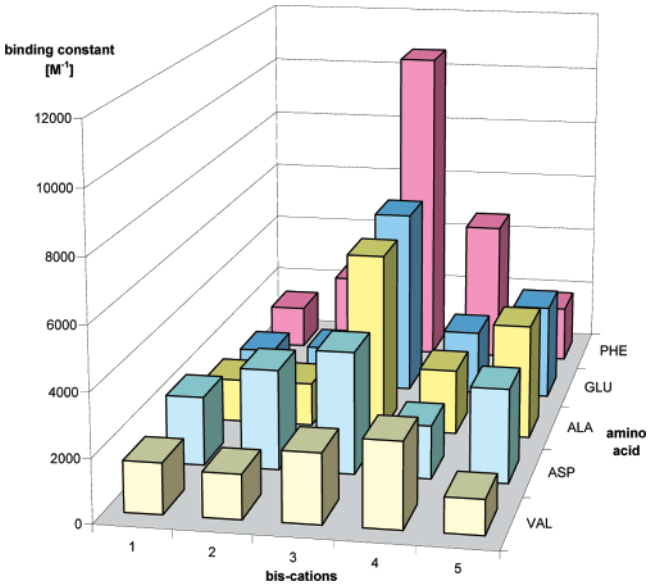

FIGURE 3. Binding constants for bis-cationic receptors **1**−**5** and various *N*-acetyl amino acid carboxylates calculated from UV-titration experiments in buffered water (K_{ass} in M^{-1}).

TABLE 1. Binding Constants (K_{ass} in M^{-1}) for Complex Formation between the Bis-Cationic Hosts 1−5 and Several Oxo-Anions As Determined from UV-Titration Experiments in Buffered Water

host	ALA	PHE	VAL	GLU	ASP	NaOAc	Na_2SO_4
1	1400	1400	1600	1100	2200	1500	1300
2	1400	2600	1400	1300	3200	<1000	<1000
3	5800	10700	2200	6100	3900	3600	1800
4	2100	4700	2700	2100	1700	3300	2600
5	3700	1850	1100	3100	3000	3300	2400

< 1500 M^{-1}). Complex stability is similar to what is expected for anion binding by a simple guanidiniocarbonyl pyrrole monocation.[9] Most likely, the linker is too short to allow a significant additional interaction of the ammonium group with the bound carboxylate. With four carbon atoms in the linker as in **3**, the linker is now long enough to allow a strain-free charge interaction of the NH_3 cation with the anions. If the linker becomes even longer (bis-cation **4** and the lysine derivative **5**, both with five atoms in the linker) complex stability decreases again. Perhaps now the entropic costs required to orientate the longer and more flexible linker compensate to some extent the additional attractive charge interaction. These entropic costs should be larger in **4** and **5** than in **3**.[18]

Among the different anions, *N*-acetyl phenyl alanine carboxylate is bound much more efficiently than the other amino acid anions by all bis-cations (except the lysine derivative **5**). Again, the maximum complex stability is seen with bis-cation **3** (Figure 3) with an association constant of $K = 10.700\ M^{-1}$, which is surprisingly large for amino acid binding in aqueous buffer. This binding constant is nearly twice as large as that with alanine ($K = 5.800\ M^{-1}$), for example, and more than five times larger as that for the binding of valine ($K = 2.200\ M^{-1}$), respectively. Valine in general is bound only rather weakly by these bis-cations. The higher affinity for phenylalanine most likely reflects additional cation−π-interactions of the aromatic phenyl ring with the guanidinium cation or aromatic stacking interactions with the pyrrole (vide infra).

For the two bis-carboxylates, *N*-acetyl aspartate and *N*-acetyl glutamate, also 1:1 complex stoichiometries were found according to Job plots calculated from the titration data. These two polar amino acids are also bound most efficiently by bis-cation **3**. For example, the complex stability for glutamate by the shorter bis-cation **2** is about five times smaller than that with **3** (K = 1300 vs 6100 M^{-1}, respectively). For the longer bis-cations **4** and **5** complex stability drops again as seen with the mono-carboxylates. An interesting aspect is that the shorter bis-cations **1** and **2** interact more strongly with aspartate than glutamate whereas **3**−**5** bind glutamate more strongly than aspartate (e.g., for **3** $K = 6100$ vs $3900\ M^{-1}$, respectively). This change in the relative binding affinities is not unreasonable. The shorter bis-cations (**1** and **2**) prefer the shorter bis-anion (Asp) whereas the longer bis-cations (**3**−**5**) prefer the longer bis-anion (Glu). Molecular modeling studies (Macromodel 8.0, Amber* force field, GB/SA water solvation)[19] suggest that the distance between the two charges in **1** or **2** is not sufficient to allow a simultaneous strain free interaction with both carboxylates in glutamate whereas the longer linker in **3** just has the right length (Figure 4). This could explain the significant increase in complex stability for glutamate going from bis-cation **2** to **3**. For aspartate with one methylene group less between the two carboxylates even in bis-cation **1** both positively charged groups can interact with both carboxylates of the substrate. However, at least according to the modeling studies the ion pair between the guanidiniocarbonyl pyrrole cation and the α-carboxylate is somewhat distorted. The geometry is not the usual planar bidentate ion pair indicating some strain introduced by the additional interaction with the second positive charge in the side chain.

The two inorganic anions, acetate and sulfate, were also studied. They are not efficiently bound by the two shorter bis-cations **1** and **2** (K < 1500 M^{-1}). The binding affinity significantly increases for bis-cation **3**. However, complex stability does not vary much for the longer bis-cations **4** and **5**.

(18) However, it might also be that the increased flexibility in the host allows for more favorable intramolecular interactions within the host, which also weakens intermoelcular substrate binding. See discussion and references in: Mardis, K. L. *J. Phys. Chem. B* **2006**, *110*, 971.

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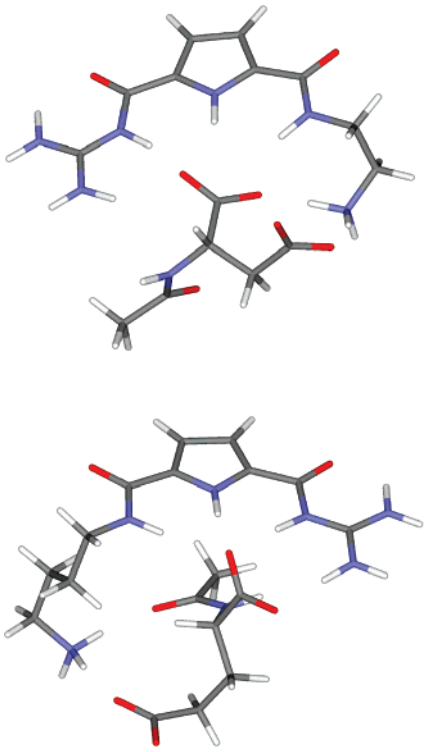

FIGURE 4. Calculated energy minimized structure of the complexes between receptor **1** and *N*-acetyl aspartate (top) and between receptor **3** and *N*-acetyl glutamate (bottom) as obtained from a Monte Carlo conformational search.

For example, acetate is bound by **3** with $K = 3600\ M^{-1}$ and by **4** and **5** with $K = 3300\ M^{-1}$, respectively. The binding of acetate is stronger than that of sulfate, which most likely reflects the increased basicity of the former or differences in the solvation properties of the anions.

Further Studies of Complex 3@Phe. As the complex between bis-cation **3** and *N*-acetyl phenyl alanine carboxylate is significantly more stable than any other complex in this series, this complex was investigated further. Unfortunately, we were not able to determine any binding constants using isothermal microcalorimetry (ITC)[20,21] as only very small heat changes were observed (see the Supporting Information). No reliable data interpretation was possible based on these small effects. At best the data hinted to a slightly exothermic and hence mildly enthalpic and entropic driven association, which would not be surprising for the combination of both ion pair formation and cation−π-interactions in aqueous solvents.

We also examined complex formation between **3** and *N*-acetyl phenyl alanine using a fluorescence titration experiment measuring light emission from 250 to 400 nm with a synchronous excitation of $\Delta\lambda = 20$ nm (aqueous bis-tris buffer, 10 mM, pH 6.0 with 10% DMSO added; $[\mathbf{3}]_0 = 7\ \mu M$; $[\text{guest}]_0 = 0.15$ mM). Upon the addition of the guest an intense band at $\lambda = 285$ nm due to the fluorescence of the phenylalanine occurs, whereas

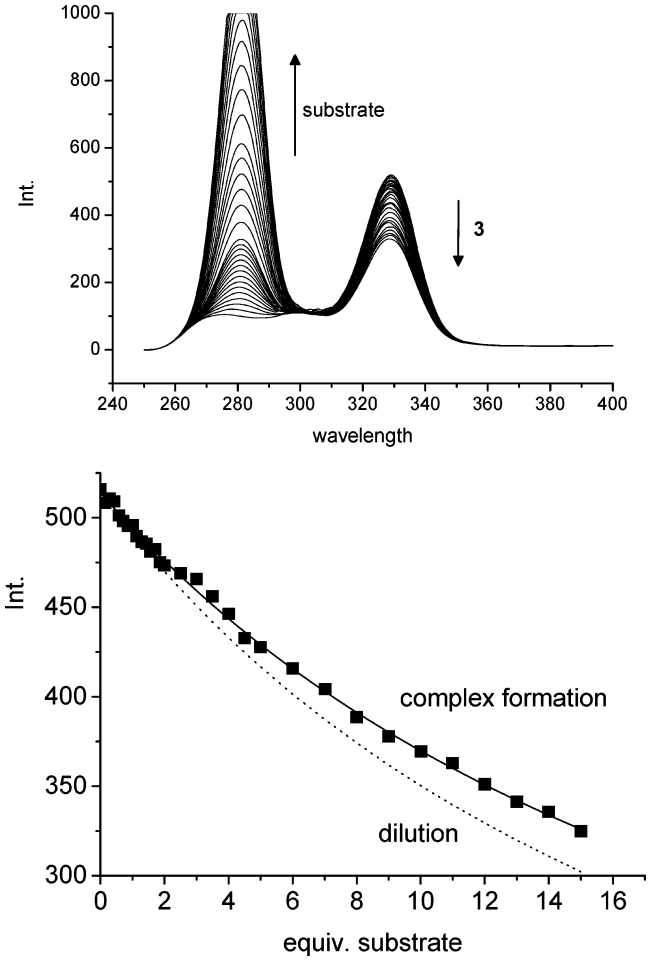

FIGURE 5. Top: Change of the fluorescence in the course of the titration of receptor **3** with *N*-acetyl phenyl alanine carboxylate by a synchronous excitation of $\Delta\lambda = 20$ nm. Bottom: Resulting binding isotherm at $\lambda = 285$ nm.

the fluorescence band of the pyrrole moiety of host **3** at $\lambda = 335$ nm decreases. After subtracting the changes due to simple dilution of the sample, one sees that this emission band actually slightly increases upon complex formation (Figure 5). On the basis of this binding isotherm the association constant K_{ass} was calculated by using a nonlinear curve-fitting procedure. With $K_{ass} = 4600\ M^{-1}$ the fluorescence titration also indicates the formation of a rather strong complex between host **3** and *N*-acetyl phenyl alanine carboxylate. The somewhat smaller value compared to the UV titrations might reflect the differences in the experimental conditions (different concentration of host and guest as well as the buffer).

We then turned to NMR studies. Upon addition of the carboxylate to a solution of **3** in water (with 10% DMSO-d_6), shift changes are observed for various protons of **3**. For example, the pyrrole CH protons show distinct high-field shifts as observed in other cases of oxoanion binding by guanidiniocarbonyl pyrrole cations. However, the shift changes are too small too allow a reliable calculation of a binding constant. Signals which would be expected to show more significant shift changes (such as the amide or guanidinium NHs) are not detectable under these protic conditions due to fast exchange with the solvent. However, the shift changes again indicate complex formation.

In line with this, a NOESY spectrum (d_3-MeOH, 213 K, 300 ms mixing time) revealed distinct cross-peaks between the phenyl-

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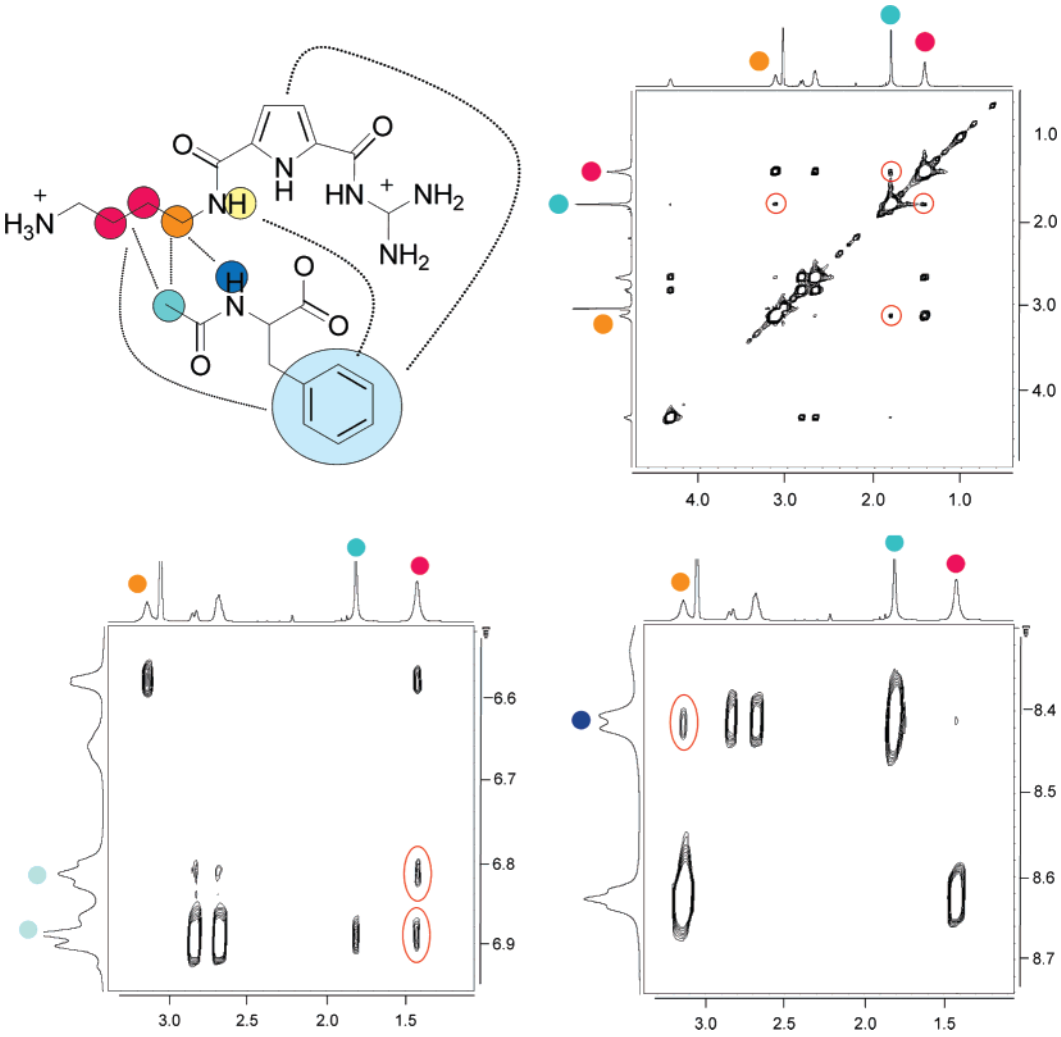

FIGURE 6. Parts of the NOE spectrum for a 1:1 mixture of receptor **3** and *N*-acetyl phenyl alanine carboxylate in d_3-MeOH with selected intermolecular NOEs (red circles) indicating complex formation.

alanine and bis-cation **3** confirming the formation of a defined complex (Figure 6). For example, cross-peaks between the *N*-acetyl group of the anion and the methylene groups of the linker in **3** are observed. These methylene groups of the linker also show cross-peaks with the phenyl alanine amide NH as well as the phenyl CH protons. The aromatic ring gives cross-peaks with the linker amide NH of **3** as well as one of the pyrrole CH protons. Hence, these intermolecular cross-peaks, which are schematically summarized in Figure 6, are a clear indication for the formation of a complex with a defined structure.

A possible complex structure was elucidated by using molecular mechanics calculations (Macromodel 8.0, Amber* force field, GB/SA water solvation). The energy minimum structure obtained from a MC conformational search with 100.000 steps is shown in Figure 7. At least according to these calculations, the aromatic ring of the phenyl alanine indeed stacks with the guanidiniocarbonyl pyrrole moiety. A similar effect has already been observed for the binding of *N*-acetyl amino acid carboxylates by a simple guanidiniocarbonyl pyrrole mono-cation.[11]

All the observed intermolecular NOEs (Figure 6) are in good agreement with the calculated complex structure shown above in Figure 7. For example, in this structure, the *N*-acetyl group of the amino acid is orientated next to the linker, as indicated by the corresponding NOE signals and the stacking of the phenyl ring below the guanidiniocarbonyl pyrrole moiety brings it into close proximity of the pyrrole CH as well as the amide NH and the methylene groups of the linker. The benzylic methylene protons, however, point away from the bis-cation and accordingly no intramolecular NOE signals were observed.

Conclusion

It is generally assumed that flexible single bonds in anion hosts should be avoided as the increasing flexibility should cause a decrease in complex stability. However, we show here that within the series of bis-cations **1**−**5** complex stability first significantly increases with increasing linker length and flexibility. Whereas the two shortest bis-cations **1** and **2** show only very weak binding of *N*-acetyl amino acid carboxylates in aqueous buffer solution, for the longer bis-cation **3** a significant increase in complex stability is observed. The complex stability then drops again for the longer bis-cations **4** and **5**. Hence, a minimum linker length of four carbons is necessary to allow efficient anion binding by both positive charges. This underlines that not just unspecific Coulomb interactions are responsible for anion binding in this case but rather the formation of directed ion pairs which require a certain relative geometry and arrange-

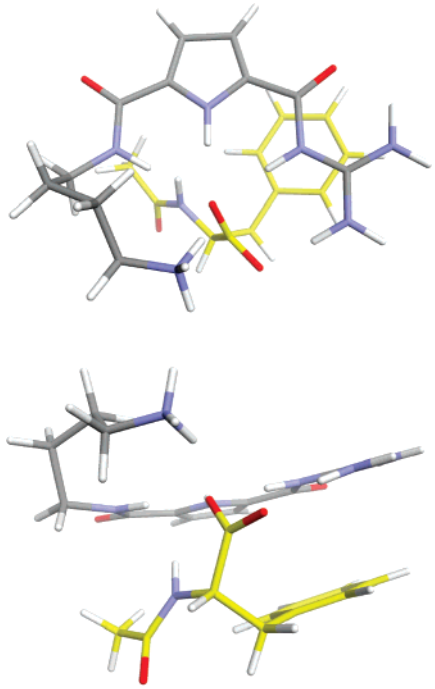

FIGURE 7. Calculated energy minimized structure of the complex between receptor **3** and *N*-acetyl phenyl alanine carboxylate as obtained from a Monte Carlo conformational search. Whereas the carboxylate is interacting with both cationic groups, the aromatic phenyl ring π-stacks with the planar guanidiniocarbonyl pyrrole moiety.

ment between the charged groups in the host and guest. Hence, increasing flexibility in the design of anion hosts is not always detrimental.

Experimental Section

Synthesis of 5-((*tert*-Butyloxycarbonyl)guanidiniocarbonyl)-1*H*-pyrrole-2-carboxylic Acid Benzylester 8. A mixture of the 1*H*-pyrrole-2,5-dicarboxylic acid monobenzyl ester **6** (2.45 g, 10.0 mmol, 1 equiv), PyBOP (5.72 g, 11 mmol, 1.1 equiv), and *N*-methyl morpholine (2.45 mL, 22 mmol, 2.2 equiv) was stirred in DMF (50 mL) at room temperature for 30 min then *tert*-butyloxycarbonylguanidine **7** (3.19 g, 20.0 mmol, 2 equiv) was added and the resulting solution was stirred over night. The yellow solution was slowly poured into vigorously stirred water (150 mL) causing the formation of a white solid that was dissolved in diethyl ether (150 mL). After phase separation and twice extracting the water/DMF phase with diethyl ether (150 mL) the solvent was evaporated and the crude product was further purified by flash chromatography yielding the colorless powder **8** (3.59 g, 93%).

Mp 88 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (s, 9 H, CH_3), 5.31 (s, 2 H, CH_2), 6.85 (m, 2 H, CH), 7.30−7.46 (m, 5 H, CH), 8.58 (br s, 1 H, NH), 9.31 (br s, 1 H, NH), 10.74 (br s, 1 H, NH), 11.62 (br s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.7 (CH_3), 65.5 (CH_2), 81.2 (quat C), 113.8, 115.8 (both CH), 124.5 (quat C), 127.8, 128.0, 128.4 (all CH), 134.0, 136.2, 155.6, 158.3, 159.8, 168.0 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3393 (m), 3256 (m), 2360 (s), 2340 (m), 1719 (s), 1717 (s), 1635 (s), 1540 (s), 1286 (s), 1149 (s), 842 (w); HR-MS (positive ESI) *m/e* calcd for $C_{19}H_{22}N_4NaO_5^+$ (M + Na^+) 409.146, found 409.149.

Synthesis of 5-((*tert*-Butyloxycarbonyl)guanidiniocarbonyl)-1*H*-pyrrole-2-carboxylate 9. A mixture of the benzyl ester **8** (1.94 g, 5.00 mmol, 1 equiv), a catalytic amount of Pd/C (~200 mg), and triethylamine (1.05 mL, 7.50 mmol, 1.5 equiv) in methanol (30 mL) was vigorously stirred at 40 °C for 5 h under hydrogen atmosphere. The resulting solution was filtered through a celite pad, which was washed several times with methanol with 2% triethylamine. The solvent was evaporated under reduced pressure. After adding water (10 mL) to the resulting oil, the solution was lyophilized giving the product **9** as a white solid (1.89 g, 95%).

Mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.08 (t, 9 H, ^3J(H,H) = 7.20 Hz, CH_3), 1.45 (s, 9 H, CH_3), 2.79 (q, 6 H, ^3J(H,H) = 7.20 Hz, CH_2), 6.47 (d, ^3J(H,H) = 3.64 Hz, 1 H, CH),6.77 (d, ^3J(H,H) = 3.68 Hz, 1 H, CH), 8.58 (br s, 1 H, NH), 9.31 (br s, 1 H, NH), 10.84 (br s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 9.7 (CH_3), 27.8 (CH_3), 45.2 (CH_2), 80.2 (quat C), 112.1, 114.1 (both CH), 128.8, 133.0, 158.5, 160.6, 163.9, 167.2 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3393 (m), 2958 (w), 1650 (s), 1542 (s), 1319 (s); MS (negative ESI) *m/e* calcd for $C_{12}H_{15}N_4O_5^-$ (M − H^+) 295.11, found 295.1.

General Procedure of the Synthesis of the *t*Boc-Protected Receptors 11 and 13. 5-(*t*Boc-Guanidiniocarbonyl)-1*H*-pyrrole-2-carboxylate **9** (397 mg, 1 mmol, 1 equiv) was added to a solution of PyBOP (780 mg, 1.5 mmol, 1.5 equiv) and triethylamine (278 μL, 2 mmol, 2 equiv) in dry dichloromethane (30 mL) with dry DMF (2 mL) and the solution was stirred for 15 min. This slightly yellow suspension was then added dropwise to a solution of an amine **10**/**12** (2 mmol, 2 equiv) and triethylamine (278 μL, 2 mmol, 2 equiv) in dichloromethane (30 mL) with dry DMF (2 mL) and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated and the residue taken up in water (60 mL) and ethyl ether (90 mL). For better solubility ultrasound was used. The water layer was extracted with ethyl ether (five times with 90 mL) and then the combined organic layers were dried with magnesium sulfate and afterward the solvent was evaporated. Further purification by filtration column (silica gel, 3:2 dichloromethane/acetone with 1% triethylamine) yielded white to slightly yellow solids (52% to 86%).

General Procedure of the Synthesis of the Receptors 1−4. The cleavage of the N-protecting group was done twice with the *t*Boc-protected receptors **11** (1 mmol, 1 equiv) in 2 mL of concd hydrochloric acid in 10 mL of methanol. These solutions were each stirred at room temperature for 2 h. After repeated lyophilization with 20 mL of water the receptors were obtained as white chloride salts (95% to quant.).

Bis-Cation 1. White solid (52%). Mp 210 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.98 (m, 2 H, CH_2), 3.50 (q, ^3J(H,H) = 5.68 Hz, 2 H, CH_2), 6.92 (d, ^3J(H,H) = 2.28 Hz, 1 H, CH), 7.06 (s, 1 H, NH), 7.56 (s, 1 H, CH), 8.06 (br s, 3 H, NH_3), 8.48 (br s, 2 H, NH_2), 8.67 (br s, 2 H, NH_2), 8.83 (t, ^3J(H,H) = 5.04 Hz), 1 H, NH), 12.10 (s, 1 H, NH), 12.35 (s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.6, 38.5 (all CH_2), 112.7, 115.9 (both CH), 125.5, 132.5, 155.6, 159.3, 159.7 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3336 (s), 3191 (s), 3005 (m), 1697 (s), 1656 (s), 1558 (s), 1478 (m), 1295 (s), 1257 (w), 1201 (w), 1067 (w), 812 (w), 759 (m), 665 (w); HR-MS (positive ESI) *m/e* calcd for $C_9H_{15}N_6O_2^+$ (M + H^+) 239.125, found 239.122; HR-MS (positive ESI) *m/e* calcd for $C_9H_{14}N_6NaO_2^+$ (M + Na^+) 261.107, found 261.107.

Bis-Cation 2. White solid (63%). Mp >250 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.81 (quin, ^3J(H,H) = 6.84 Hz, ^3J(H,H) = 7.38 Hz, 2 H, CH_2), 2.84 (quin, ^3J(H,H) = 5.56 Hz, ^3J(H,H) = 6.68 Hz, 2 H, CH_2), 3.31 (t, ^3J(H,H) = 6.60 Hz, 2 H, CH_2), 6.88 (dd, ^3J(H,H) = 4.04 Hz, ^4J(H,H) = 2.28 Hz, 1 H, CH), 7.54 (dd, ^3J(H,H) = 4.04 Hz, ^4J(H,H) = 2.40 Hz, 1 H, CH), 7.94 (br s, 3 H, NH_3), 8.48 (br s, 2 H, NH_2), 8.66 (br s, 2 H, NH_2), 8.73 (t, ^3J(H,H) = 5.62 Hz), 1 H, NH), 12.08 (s, 1 H, NH), 12.36 (s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.3, 35.9, 36.8 (all CH_2), 112.5, 116.0 (both CH), 125.5, 132.7, 155.5, 159.3, 159.6 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3343 (s), 3164 (s), 2995 (m), 1698 (s), 1646 (s), 1560 (s), 1476 (m), 1287 (s), 1262 (w), 1199 (w), 1059 (w), 808 (w), 761 (m); HR-MS (positive ESI) *m/e* calcd for $C_{10}H_{17}N_6O_2^+$ (M + H^+) 253.141, found 253.140; HR-MS (positive ESI) *m/e* calcd for $C_{10}H_{16}N_6NaO_2^+$ (M + Na^+) 275.123, found 275.123.

Bis-Cation 3. White solid (71%). Mp >250 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.58 (m, 2 H, CH_2), 2.79 (m, 2 H, CH_2), 3.31 (q, ^3J(H,H) = 5.92 Hz, 2 H, CH_2), 6.86 (dd, ^3J(H,H) = 4.04 Hz, ^4J(H,H) = 2.40 Hz, 1 H, CH), 7.56 (dd, ^3J(H,H) = 4.04 Hz, ^4J(H,H) = 2.40 Hz, 1 H, CH), 7.92 (br s, 3 H, NH_3), 8.50 (br s, 2 H, NH_2), 8.62 (t, ^3J(H,H) = 5.62 Hz), 1 H, NH), 8.69 (br s, 2 H, NH_2), 12.12 (s, 1 H, NH), 12.34 (s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.5, 26.0, 38.0, 38.5 (all CH_2), 112.4, 116.0 (both CH), 125.3, 132.9, 155.6, 159.1, 159.6 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3327 (s), 3098 (s), 2931 (m), 1698 (s), 1655 (s), 1558 (s), 1476 (m), 1286 (s), 1256 (w), 1194 (w), 1063 (w), 853 (w), 751 (m), 605 (w); HR-MS (positive ESI) *m*/*e* calcd for $C_{11}H_{19}N_6O_2^+$ (M + H$^+$) 267.156, found 267.157; HR-MS (positive ESI) *m*/*e* calcd for $C_{11}H_{18}N_6NaO_2^+$ (M + Na$^+$) 289.138, found 289.137.

Bis-Cation 4. White solid (74%). Mp 205 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (m, 2 H, CH_2), 1.51−1.60 (m, 4 H, 2 CH_2), 2.76 (m, 2 H, CH_2), 3.24 (q, ^3J(H,H) = 6.68 Hz, 2 H, CH_2), 6.85 (dd, ^3J(H,H) = 4.04 Hz, ^4J(H,H) = 2.40 Hz, 1 H, CH), 7.51 (dd, ^3J(H,H) = 4.04 Hz, ^4J(H,H) = 2.40 Hz, 1 H, CH), 7.83 (br s, 3 H, NH_3), 8.44 (br s, 2 H, NH_2), 8.50 (t, ^3J(H,H) = 5.50 Hz), 1 H, NH), 8.63 (br s, 2 H, NH_2), 12.01 (s, 1 H, NH), 12.31 (s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.2, 26.6, 28.4, 38.4, 38.6 (all CH_2), 112.4, 115.9 (both CH), 125.3, 132.9, 155.5, 159.0, 159.6 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3363 (s), 3047 (s), 2931 (m), 1697 (s), 1626 (s), 1561 (s), 1475 (m), 1286 (s), 1286 (s), 1199 (m), 1070 (w), 853 (w), 758 (m), 598 (m); HR-MS (positive ESI) *m*/*e* calcd for $C_{12}H_{21}N_6O_2^+$ (M + H$^+$) 281.172, found 281.173.

Synthesis of the Receptor 5. For the cleavage of the CBz-protecting group by hydrogenation the benzyl ester **13** (115 mg, 0.2 mmol) was solved in methanol (20 mL) and Pd/C (~10 mg) was added. This mixture was vigorously stirred at 40 °C for 5 h under hydrogen atmosphere. The resulting solution was filtered through a suction filter (Celite 545), which was washed several times with methanol. After that further *t*Boc-cleavage was done in this solution as described above (49 mg, 60%). White solid (52%). Mp 190 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (m, 2 H, CH_2), 1.59 (m, 2 H, CH_2), 1.79 (m, 2 H, CH_2), 2,77 (q, ^3J(H,H) = 5.96 Hz), 2 H, CH_2), 3.66 (s, 3 H, CH_3), 4.41 (m, 1 H, CH), 6.93 (dd, ^3J(H,H) = 3.80 Hz, ^4J(H,H) = 2.24 Hz, 1 H, CH), 7.58 (dd, ^3J(H,H) = 3.68 Hz, ^4J(H,H) = 2.16 Hz, 1 H, CH), 7.93 (br s, 3 H, NH_3), 8.53 (br s, 2 H, NH_2), 8.71 (br s, 2 H, NH_2), 8.86 (d, ^3J(H,H) = 7.32 Hz), 1 H, NH), 12.15 (s, 1 H, NH), 12.49 (s, 1 H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.5, 26.4, 30.1, 38.4 (all CH_2), 52.0 (CH_3), 52.1 (CH), 113.5, 115.8 (both CH), 125.8, 131.9, 155.5, 159.2, 159.7, 172.4 (all quat C); FT-IR (KBr disk) $\tilde{\nu}$ [cm^{-1}] 3327 (s), 3147 (s), 2957 (m), 1699 (s), 1654 (s), 1556 (s), 1475 (m), 1292 (s), 1248 (s), 1225 (s), 1155 (w), 814 (w), 754 (m), 602 (m); HR-MS (positive ESI) *m*/*e* calcd for $C_{14}H_{23}N_6O_4^+$ 339.178, found 339.177.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**5**, **8**, and **9** and ITC data for complex formation between **3** and *N*-acetyl phenyl alanine carboxylate. This material is available free of charge via the Internet at http://pubs.acs.org.

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