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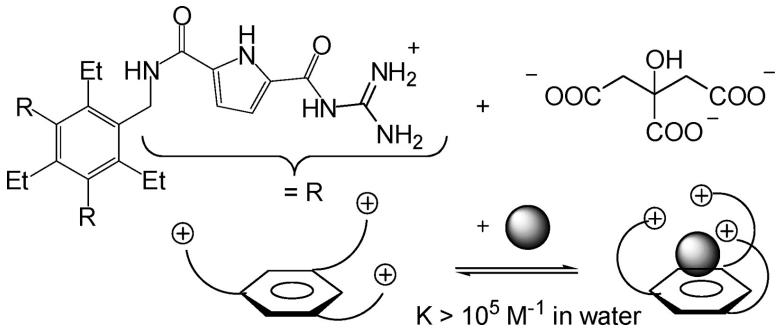

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A Molecular Flytrap for the Selective Binding of Citrate and Other Tricarboxylates in Water

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Abstract: The synthesis and binding properties of a new tricationic guanidiniocarbonyl pyrrole receptor **7** are described. Receptor **7** binds citrate **9** and other tricarboxylates such as trimesic acid tricarboxylate **8** with unprecedented high association constants of $K_{assoc} > 10^5$ M^{-1} in water as determined by UV and fluorescence tritration studies. According to NOESY experiments and molecular modeling calculations, the tricarboxylates are bound within the inner cavity of receptor **7** by ion pairing between the carboxylate groups and the guanidiniocarbonyl pyrrole moieties, favored by the nonpolar microenvironment of the cavity. Hence, receptor **7** can be regarded as a molecular flytrap. In the case of the aromatic tricarboxylate **8**, additional aromatic interactions further strengthen the complex. The complexes with the tricarboxylates are so strong that even the presence of a large excess of competing anions or buffer salts does not significantly affect the association constant. For example, the association constant for citrate changes only from $K_{assoc} = 1.6 \times 10^5$ M^{-1} in pure water to $K_{assoc} = 8.6 \times 10^4$ M^{-1} in the presence of a 170-fold excess of bis-tris buffer and a 1000-fold excess of chloride. This makes **7** one of the most efficient receptors for the binding of citrate in aqueous solvents reported thus far.

Introduction

The detection of a given analyte by a chemosensor requires a receptor unit that selectively interacts with the substrate of choice and a method to read out the binding using a change in a physical signal.[1] In most cases, an additional reporter unit (e.g., a fluorescent chromophore) is covalently attached to the receptor for this purpose. Another possibility is to use a "silent" receptor without an attached read-out device and to employ the indicator displacement method.[2,3] In any case, however, efficient and strong binding of the analyte by the receptor is a necessary prerequisite. For applications in aqueous solvents (e.g., under physiological conditions) this still presents a challenging task.[4] For example, pure hydrogen bond-based receptors work only in organic solvents of low polarity as the strength of hydrogen bonds decreases rapidly with increasing polarity of the solvent.[5] In polar solutions, supramolecular aggregation can even be endothermic[6] and therefore entropy-driven because of the reorganization of the solvent upon complexation.[7] Therefore, new receptor units with improved binding characteristics are needed.[8] We wish to report here the synthesis and binding properties of a new tripodal guanidininocarbonyl pyrrole receptor **7**, which binds citrate and other tricarboxylates with

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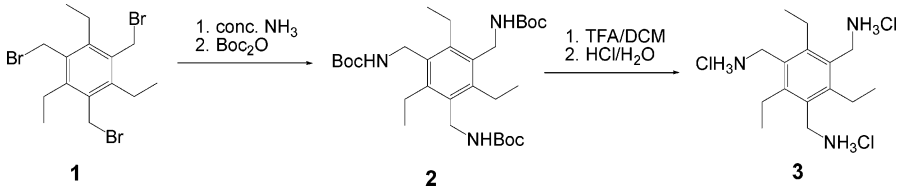
10.1021/ja0433469 CCC: $30.25

Scheme 1. Improved Synthesis of the Triamine Template **3**



1 **2** **3**

unprecedented high association constants of $K_{assoc} \geq 10^5\ M^{-1}$ in water. Because of the built-in pyrrole chromophore, the receptor can be used as an optical chemosensor for citrate[9] without the need for additional reporter units or the use of a competitive indicator displacement assay.

Results and Discussion

Design and Synthesis of 7. The design of receptor **7** was based on the triamine template **3** advertised by Anslyn in his elegant work on anion sensors.[10] Because of the alternate arrangement of the ethyl and the aminomethylene groups, this template provides a rather rigid scaffold that presents the three amino groups all at the same side of the aromatic ring.[11] When suitable receptor units are attached to these amino groups, a binding cavity with convergent binding sites is formed. In the past, mainly guanidinium cations and/or boronic acid groups were used to achieve complexation of various substrates such as phosphate, citrate, tartrate, or heparine.[4b,11] For example, citrate is bound by a triguanidinium cation based on this template with an association constant of $K_{assoc} = 7 \times 10^3\ M^{-1}$ in pure water. However, in the presence of a buffer the binding affinity dropped by nearly 2 orders of magnitude. Therefore, even stronger binding motifs are needed to allow citrate binding under physiological conditions (= buffered water). We reasoned that the binding affinity for citrate can be improved by using even more efficient carboxylate binding sites than simple guandinium cations.[12] For this purpose, we recently introduced guanidiniocarbonyl pyrroles.[13] Because of the increased acidity of the acylated guanidinium group and additional binding sites in form of the pyrrole amide, their complexes with carboxylates are much stronger than those of simple guanidinium cations.[14] Hence, receptor **7** became a promising candidate to achieve strong complexation of citrate in water even in the presence of other competing anions and buffer salts.

The synthesis of **7** is described in Schemes 1 and 2. Template **3** was synthesized in analogy to the literature procedure starting from the tribromide **1**.[9b] However, to improve the sometimes troublesome isolation and purification of the corresponding triamine **3**, the literature synthesis was modified (Scheme 1). The tribromide **1** was treated with concentrated ammonia in THF/ethanol,[15] and the resulting amine was trapped in situ as the *t*Boc-carbamate by addition of Boc_2O to give **2**, which can be easily isolated by chromatography and stored without problems. Deprotection with trifluoroacetic acid and anion exchange by lyophilization with HCl then provided the desired triamine **3** as the hydrochloride salt in 63% yield starting from **1**.

This triamine **3** was then reacted with the pyrrole dicarboxylic acid monobenzyl ester **4** using PyBOP in DMF as the coupling reagent (Scheme 2). The benzyl ester groups were cleaved off by hydrogenolysis to yield the triacid **5** in 83% overall yield. The guanidine group was introduced in form of the mono-*t*Boc-guanidine **6** again using PyBOP in DMF as the coupling reagent. Acidic cleavage of the *t*Boc-protecting group with TFA gave the desired receptor **7** as the trifluoracetate salt, which was lyophilized several times from aqueous HCl to obtain the chloride salt of the receptor **7** in 72% yield.

NMR Binding Studies. Receptor **7** was designed to bind citrate **9** in water through a combination of ion pairing and hydrogen bonds. To probe the effciency of **7** for anion binding, we first chose trimesic acid tricarboxylate **8** as a substrate, as both substrate and host have the same threefold symmetry giving rise to an optimal geometric complementarity between guest and host binding groups. Upon the addition of trimesic acid tricarboxylate **8** to a solution of **7** (1 mM, chloride salt) in 10% H_2O in DMSO-d_6, significant complexation-induced shift changes of the amide NH and the pyrrole CHs are observed, indicating a strong molecular interaction between **7** and **8** (Figure 1). However, only the protons in the binding arms of the receptor are shifted. The ethyl groups attached to the central benzene ring do not show any shift change, demonstrating that they do not participate in the binding of the substrate. A quantitative analysis of these complexation-induced shift changes shows a linear decrease until a molar ratio of 1:1 is reached (Figure 2),

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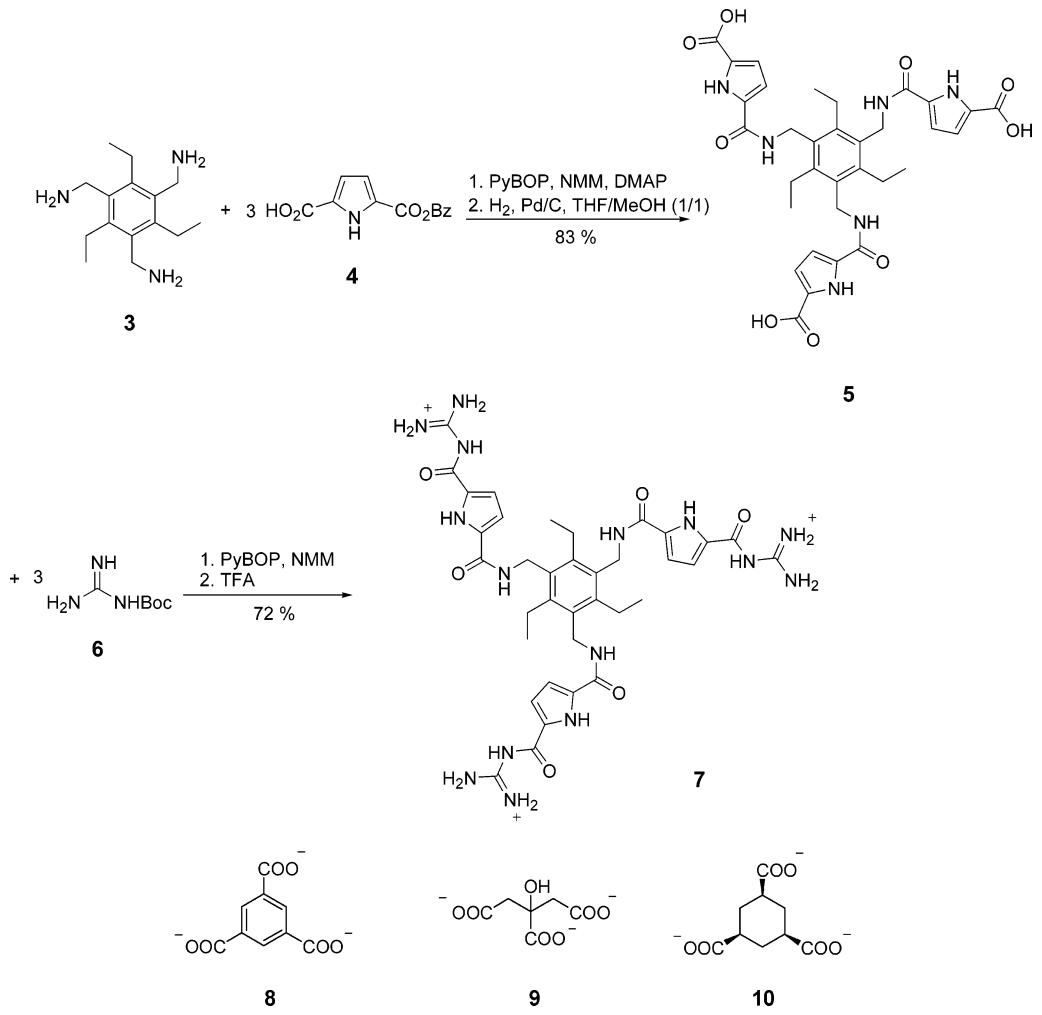
Scheme 2. Synthesis of Receptor **7**



3 **4** **5** **6** **7** **8** **9** **10**

proving the formation of a 1:1 complex. For example, upon the addition of 1 equiv of tricarboxylate **8**, the pyrrole H7 shifts from $\delta = 7.24$ to 6.60, whereas the H6 shifts from $\delta = 6.90$ to 6.73, respectively (assignment based on NOESY experiments). The amide NH4 shows a highfield shift from $\delta = 8.41$ to 8.29 followed by a small downfield shift if more than 1 equiv of substrate is added, which could reflect the formation of weak complexes of stoichiometry higher than 1:1.[4d]

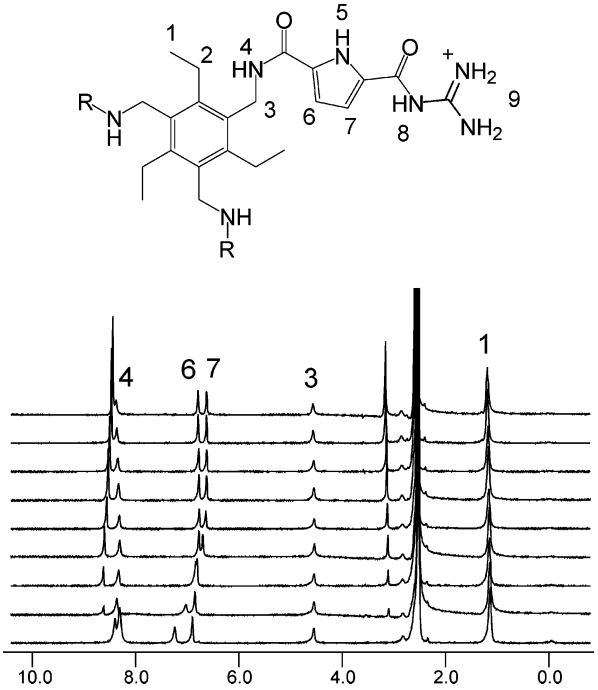


Figure 1. NMR shifts for the titration of **7** with **8** in 90% DMSO/Wasser.

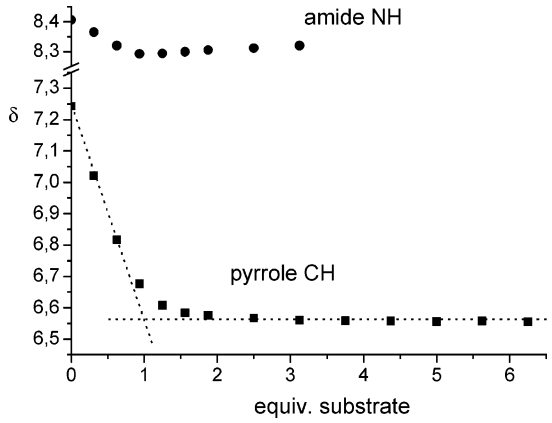


Figure 2. Binding isotherm for the titration of **7** with **8** in 90% DMSO/Wasser.

These NMR shift changes not only prove the formation of a 1:1 complex between the tripodal receptor **7** and tricarboxylate **8**, but the linearity of the binding isotherm also shows that under

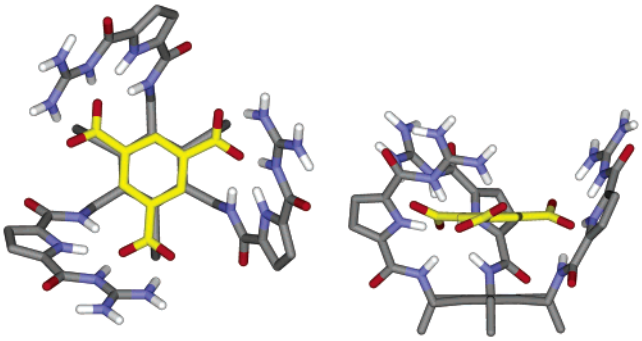


Figure 3. Calculated energy-minimized structure of the complex between receptor **7** (gray) and tricarboxylate **8** (yellow). Nonpolar hydrogens are omitted for clarity.

these conditions (10% water in DMSO) the association is too strong to be measured by NMR techniques, suggesting a binding constant $K_{assoc} > 10^5$ M^{-1}.[16] Unfortunately, at millimolar concentrations as needed for NMR studies, increasing the water content to >40% led to a precipitation of the complex during the titration, preventing NMR studies in more polar solvent mixtures.

Complex Structure. Complex formation could also be proven by NOESY experiments (DMSO, room temperature), which show cross-peaks between the CH of trimesic acid **8** and both the pyrrole NH and the amide NH of the receptor **7**. Hence, these cross-peaks not only confirm that the substrate is bound but also confirm that the guanidiniocarbonyl pyrrole moiety in the binding arms of receptor **7** adopts a syn conformation with regard to these NHs. This conformation is not the energetical favored ground state of receptor **7** (even though in water the differences in energy between the various conformers are expected to be rather small according to modeling studies)[13d,17] but the one needed for anion binding by a guanidiniocarbonyl pyrrole. To obtain more information about the most likely complex structure, we performed molecular modeling calculations (Macromodel V 8.0, Amber* force field, GB/SA water solvation treatment).[18] A Monte Carlo conformational search was performed (10 000 steps), and the obtained energy-minimized complex structure was further subjected to a MD simulation at 300 K_{assoc} (10-ps simulation time, 1.5-fs time steps). The resulting complex structure is shown in Figure 3. The substrate (yellow) lies atop the benzene ring of the receptor (gray) within van der Waals distance, probably allowing for an attractive π-stacking between the electron-rich benzene ring of the receptor **7** and the electron-poor trimesic acid tricarboxylate **8**.[19,20] Each carboxylate group is bound by one of the guanidiniocarbonyl pyrrole arms by ion pair formation with the guanidinium cation and additional hydrogen bonds from the pyrrole NH and the amide NH (highlighted in Figure 4). This binding motif is the same as that we previously observed for the binding of monocarboxylates by simple monocationic guanidiniocarbonyl pyrrole receptors.[13] This overall binding scheme is furthermore in good agreement with the observed NOE signals and shift changes in the NMR.

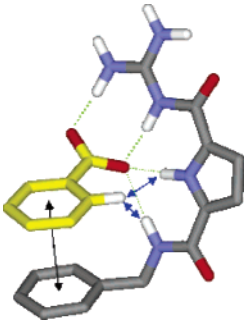


Figure 4. Main binding interactions within the complex between **7** and **8**. The carboxylate interacts with the guanidiniocarbonyl pyrrole cation (dashed green lines), whereas the two aromatic rings π-stack (black arrow). Observed NOE signals are shown in blue.

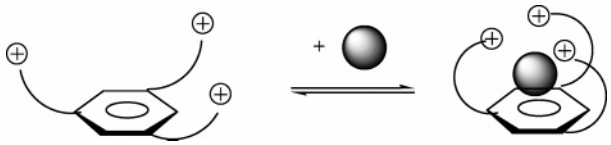


Figure 5. Guest binding by receptor **7** resembles a "molecular flytrap".

According to this calculated structure, receptor **7** can be seen as a "molecular flytrap" (Figure 5). In the absence of a substrate, the three arms are pointing away from each other because of their mutual electrostatic repulsion, providing a more open form of the receptor. Upon the binding of the substrate, the three arms close up and completely surround the guest, locking it in place.

This binding mode also explains the high stability of this complex, which most likely stems from three factors. First, the binding properties of the guanidiniocarbonyl pyrrole cation relative to simple guanidinium or ammonium cations are superior.[14] Second, π-stacking interactions between the two aromatic rings, one electron-rich and the other electron-poor, further stabilize the complex. Third, the three binding arms of **7** provide an extensive hydrophobic shielding for the substrate once it is bound within the inner cavity. Hence, the microenvironment around the binding sites is more hydrophobic than the bulk solvent, favoring both ion pair interactions and hydrogen bonds. Most parts of the bound guest molecule, and therefore also its binding sites, are not accessible by the solvent anymore (Figure 6), as can be seen also from the calculated solvent accessible surface.

Spectroscopic Titration Studies in Water. Because of high complex stability in aqueous DMSO further binding studies were performed in water using UV-titration studies. At the much lower concentrations needed for UV studies (~0.01 mM), the solubility of both the receptor and the complex was sufficient even in water. Aliquots of a stock solution of the carboxylate **8** (0.2 mM) were added to a solution of the receptor **7** (0.012 mM). The complexation was monitored by the decrease of the UV absorbance of the pyrrole moiety at $\lambda = 300$ nm. The corresponding binding isotherm (Figure 7) was then analyzed

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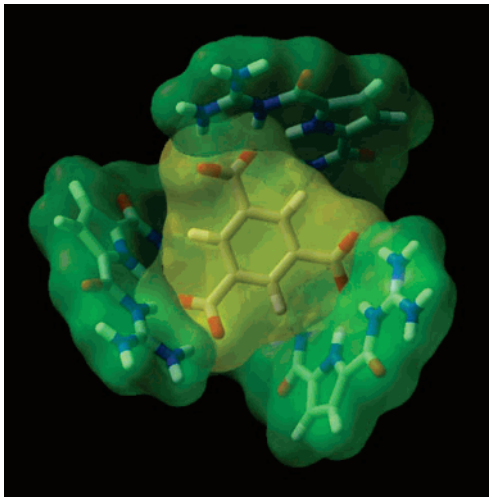

Figure 6. Solvent-accessible surfaces of receptor (green) and substrate (yellow) within the complex. The substrate's carboxylates are completely shielded from the solvent, allowing for a strong interaction with the guanidiniocarbonyl pyrrole moieties even in water.

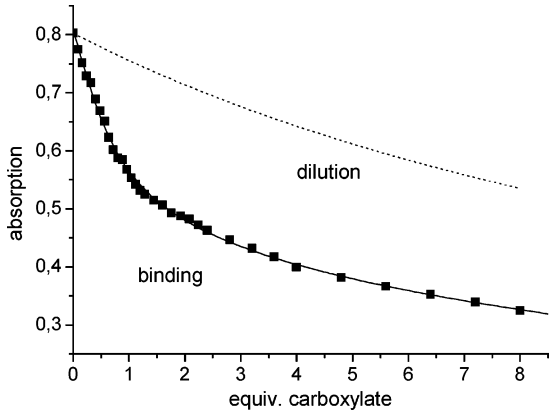

Figure 7. Binding isotherm at $\lambda = 300$ nm for the titration of **7** (1.2×10^{-5} M) with trimesic acid tricarboxylate **8** (2×10^{-4} M) in water. The solid line represents the calculated curve fit for the experimental data (■), whereas the dotted line indicates the expected change in absorption due to simple dilution of the sample during the titration.

using a nonlinear curve fitting with a 1:1 association model.[16,21] This 1:1-complex stoichiometry was confirmed by a Job plot (Figure 8).[22]

According to this UV titration, in water at pH = 6.3 trimesic acid tricarboxylate **8** is bound by receptor **7** with an association constant of $K_{assoc} = 3.4 \times 10^5$ M^{-1}! This makes **7** one of the most efficient carboxylate receptors for aqueous solvents reported thus far. This surprisingly high binding constant was independently confirmed by a fluorescence titration study, in which the decrease of the fluorescence signal at $\lambda = 335$ nm was monitored. The fluorescence titration provided an association constant of $K_{assoc} = 4.4 \times 10^5$ M^{-1}, which is in excellent agreement with the value obtained from the UV titration. A clean 1:1 binding stoichiometry was again confirmed by a job plot.

Next we tested citrate **9**, a more flexible and less symmetric tricarboxylate, as a substrate, which is however bound nearly

(21) (a) Connors, K. A. *Binding Constants*; Wiley: New York, 1987. (b) Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.
(22) (a) MacCarthy, P. *Anal. Chem.* **1978**, *50*, 2165. (b) Job, P. *C. R. Acad. Sci.* **1925**, *180*, 928; *Ann. Chim. (Paris) (Serie 10)* **1928**, *9*, 113−203; *(Serie 11)* **1936**, *6*, 97−144.

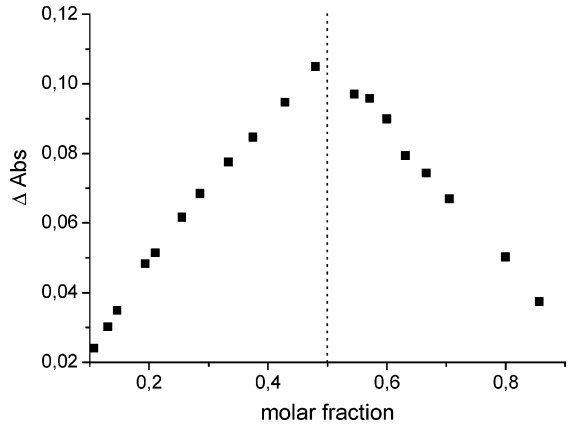

Figure 8. Job plot extracted from the UV titration indicating the 1:1 complex stoichiometry.

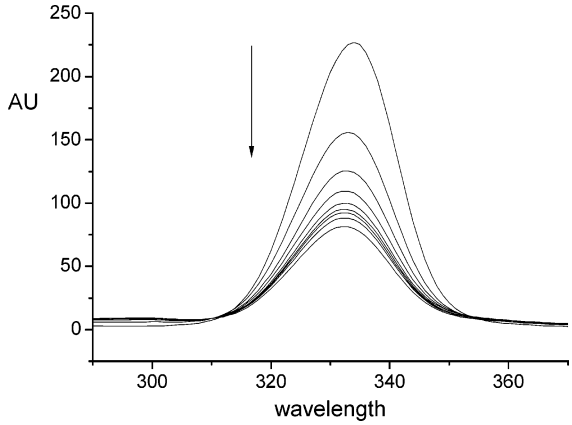

Figure 9. Fluorescence titration of **7** (1.2×10^{-5} M) with citrate **9** (2×10^{-4} M) in water.

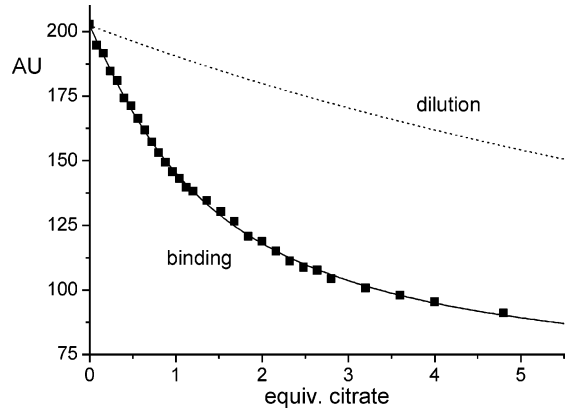

Figure 10. Binding isotherm at $\lambda = 300$ nm for the titration of **7** (1.2×10^{-5} M) with citrate **9** (2×10^{-4} M) in water for the fluorescence titration. The solid line represents the calculated curve fit for the experimental data (■), whereas the dotted line indicates the expected change in absorption due to simple dilution of the sample during the titration.

as well. The UV titration provided an association constant of $K_{assoc} = 1.6 \times 10^5$ M^{-1}, whereas the fluorescence titration (Figures 9−11) gave $K_{assoc} = 2.3 \times 10^5$ M^{-1}, respectively. To the best of our knowledge, **7** is hence the most efficient receptor for the binding of citrate in water reported thus far. With a binding constant of $K_{assoc} \geq 10^5$ M^{-1} in water, the complex is nearly 2 orders of magnitude more stable than Anslyns' citrate receptor ($K_{assoc} = 7 \times 10^3$ M^{-1}). Even in the presence of a large excess of bis-tris buffer and chloride anions, the binding

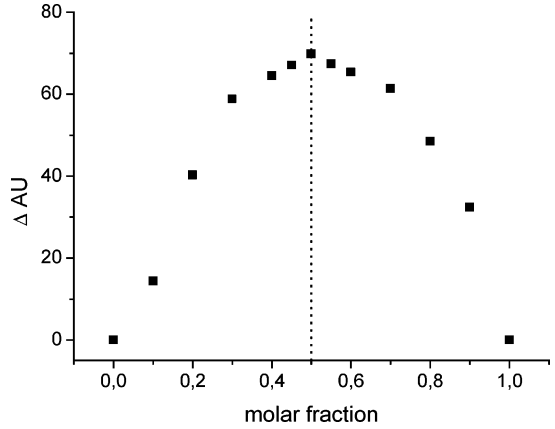

Figure 11. Job plot for the complex formation between receptor **7** and citrate **9** in pure water as obtained from the changes in fluorescence, confirming the 1:1 binding stoichiometry.

constant for citrate is still $K_{assoc} = 8.6 \times 10^4\ M^{-1}$ (see below), whereas for example Anslyn's receptor shows only a rather weak binding of citrate in buffer solution.

To probe whether the better binding of the aromatic tricarboxylate **8** relative to citrate **9** is due to the additional aromatic π-stacking or the rigidity and threefold symmetry of **8**, which matches the C_{3v} symmetry of the receptor, we also tested Kemps triacid **10**. Kemps triacid is also a rigid tricarboxylate with C_{3v} symmetry, but it is not aromatic. If rigidity and symmetry are important, **10** should be bound as well as **8**. Kemps triacid **10** is, however, bound even less efficiently than citrate **9**. In aqueous buffer, the association constant for **10** is $K_{assoc} = 5.1 \times 10^4\ M^{-1}$ compared to $K_{assoc} = 8.6 \times 10^4\ M^{-1}$ for citrate. The better binding of citrate in this case despite its larger flexibility might be due to the α-OH group. It was already demonstrated for other guanidinium hosts that α-OH carboxylates can be even better guests than a carboxylate.[10g]

Substrate Selectivity. Receptor **7** binds citrate **10** with an unprecedented high affinity. Whereas the two tricarboxylates **8** and **10** are of no relevance in terms of natural occurrence, other anions such as tatrate, maleate, acetate, or chloride could interfere with citrate binding. And indeed, chloride anions also interact with the receptor as shown by the decrease of the UV absorption of **7** upon addition of NaCl to a solution of the receptor. However, even in the presence of a 1000-fold excess of chloride anions, citrate still forms an extremely stable complex with **7**. For example, in 2 mM bis tris buffer (pH = 6.3) with 10 mM sodium chloride receptor **7** (12 μM, trifluoracetate salt) binds citrate **9** with $K_{assoc} = 8.6 \times 10^4\ M^{-1}$ *relative to all other anions present in the buffer mixture*. Trimesic acid tricarboxylate **8** is bound with $K_{assoc} = 1.5 \times 10^5\ M^{-1}$ under these conditions. Obviously, the tricarboxylate complexes are so strong even in water, that despite the large excess chloride binding cannot compete with their formation. This is a major improvement compared to other citrate receptors for which the complex stability decreases up to 2 orders of magnitude in the presence of buffer or other competing anions. Even dicarboxylates such as tartrate ($K_{assoc} = 7.0 \times 10^3\ M^{-1}$) or maleate ($K_{assoc} = 1.1 \times 10^4\ M^{-1}$) are bound much less efficiently than citrate as determined by UV titration. Therefore, receptor **7** allows the complexation of citrate **9** also in water in the presence of a large excess of other competing anions.

Conclusion

We have presented here a new tripodal receptor **7** that binds citrate **9** and two tricarboxylates **8** and **10** with unprecedented high association constants of $K_{assoc} > 10^5\ M^{-1}$ in water as could be shown by UV and fluorescence titration studies. In all cases a clean 1:1 complex stoichiometry was found. The binding interactions within the complex are mainly electrostatic, favored by the hydrophobic environment formed by the cavity of the receptor. Therefore, structure and flexibility of the carboxylate substrate play only a minor role in determining complex stability. More important is the charge complementarity. Therefore, the binding of citrate is enormously selective relative to anions such as trifluoroacetate or chloride. Even complexes with tartrate or maleate are around 1 order of magnitude weaker. A further variation of the sidearms of **7** by introduction of additional binding sites should lead to more selective receptors in the future.

Experimental Section

General Remarks. Reaction solvents were dried and distilled under argon before use. All other reagents were used as obtained from either Aldrich or Fluka. ^1H and ^{13}C NMR shifts are reported relative to the deuterated solvents. Peak assignments are based on either DEPT, 2D NMR studies, and/or comparison with literature data. IR spectra were recorded using samples prepared as tablets (NaBr). Melting points are not corrected.

1,3,5-Tris(methylammonium Chloride)-2,4,6-triethylbenzene (3). To a solution of the tribromide **1** (1.00 g, 2.3 mmol) in THF/ethanol (80 mL, 1/1), concentrated ammonia (40 mL) was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the resulting solid was suspended with NaOH (0.40 g, 10.0 mmol) in dioxan/H_2O (40 mL, 1/1). The suspension was treated with Boc_2O (2.18 g, 10 mmol) in dioxane at 0 °C for 1 h and then stirred at room temperature for 3 h. The reaction mixture was extracted with DCM (3 × 30 mL), and the solvent was removed under reduced pressure. The resulting solid was purified by column chromatography (SiO_2, hexane/ethyl acetate/TEA = 7/3/0.1), yielding a white solid. This was dissolved in DCM (20 mL), trifluoroacetic (4 mL) acid was added, and the solution was stirred overnight. After evaporation to dryness the resulting oil was dissolved in HCl (40 mL, 1 M) and lyophilized. This procedure was repeated twice to get the chloride salt **3** as a white solid. (0.50 g, 1.4 mmol, 63%); for spectroscopic data see the literature.[9b]

1,3,5-Tris(5-ethylcarbamoyl-1*H*-pyrrole 2-carboxylic acid)-2,4,6-triethylbenzene (5). To a solution of compound **3** (0.03 g, 0.09 mmol) and NaOH (0.01 g, 0.25 mmol) in DMF/water (10 mL; 5/1), carboxylic acid **4** (0.20 g, 0.77 mmol), PyBOP (0.4 g, 0.77 mmol), DMAP (0.04 g, 0.3 mmol), and *n*-methyl morpholine (0.5 mL) were added, and the reaction mixture was stirred at room temperature for 3 days under nitrogen. The brown solution was hydrolyzed with water (20 mL), and the resulting solid was filtered off. The solution was extracted with DCM (3 × 20 mL) and combined with the filtered solid, and the solvent was removed under reduced pressure. The resulting brown oily residue was purified by column chromatography (SiO_2, DCM/methanol = 20/1), yielding the product as a white solid.

This was dissolved in a mixture of methanol/THF (20 mL, 1/1), 10% Pd/C (0.01 g) was added, and the suspension was stirred at 40 °C for 3 h under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate was evaporated to give the carboxylic acid **5** as a white solid (0.05 g, 0.07 mmol, 82%): mp 209−210 °C dec; ^1H NMR (400 MHz, [D6]DMSO) δ =1.12 (t, 9H, ^3J = 7.4 Hz, CH_2-CH_3), 2.79−2.81 (m, 6H, CH_2-CH_3), 4.52−4.53 (m, 6H, CH_2-NH), 6.69−6.70 (m, 3H, pyrrole-C*H*), 6.76−6.78 (m, 3H, pyrrole-C*H*), 8.22 (s, 3H, amide-N*H*), 12.07 (s, 3H, pyrrole-

N*H*); ^{13}C NMR (100 MHz, [D6]DMSO) δ = 16.2 (CH_2-CH_3), 22.7 (CH_2-CH_3), 37.2 (CH_2-NH), 113.6 (pyrrole-*C*H), 114.5 (pyrrole-*C*H), 125.6 (pyrrole-*C*q), 130.1 (pyrrole-*C*q), 132.4 (aryl-*C*q), 143.8 (aryl-*C*q), 158.8, 161.6 (2 carbonyl *C*O); MS (pos. ESI): *m*/*z* = 683 [M + Na^+]$^+$; HR-MS (pos. ESI): *m*/*z* = 683.244 (calcd. for $C_{33}H_{36}N_6O_9$ + Na^+: 683.2441); IR (KBr-pellet) $\tilde{\nu}$ = 3258 (m, br), 2971 (m, br), 1678 (s), 1627 (m), 1559 (s), 1475 (m), 1357 (w), 1270 (s), 1196 (m), 1046 (w), 817 (w), 756 (m) [cm^{-1}].

1,3,5-Tris(5-ethylcarbamoyl-1*H*-pyrrole-2-carbonylguanidinium Chloride)-2,4,6-triethylbenzene (7). To a mixture of the tricarboxylic acid **5** (0.05 g, 0.07 mmol) and *n*-methyl morpholine (0.5 mL) in DMF (10 mL), PyBOP (0.33 g, 0.63 mmol) and *t*Boc-guanidine **6** (0.1 g, 0.63 mmol) were added, and the solution was stirred overnight. The brown reaction mixture was hydrolyzed with water (20 mL), and the resulting brown solid was filtered off. The solution was extracted with DCM (3 × 20 mL) and combined with the solid, and the suspension was evaporated in vacuo. The resulting brown oily residue was purified by column chromatography (SiO_2, DCM/methanol/TEA = 30/1/0.3), yielding the protected receptor as a white solid.

This was dissolved in DCM (10 mL), and trifluoroacetic acid (2 mL) was added to the suspension. The resulting solution was stirred at room temperature overnight and then evaporated in vacuo. The pale yellow oil was dissolved in HCl (20 mL, 1 M) and lyophilized to remove any trifluoroacetic acid. This procedure was repeated twice to obtain the chloride salt **7** as a white solid (0.05 mg, 0.05 mmol, 73%): mp 248−249 °C dec; ^{1}H NMR (400 MHz, [D6]DMSO) δ = 1.14 (t, 9H, ^3J = 7.4 Hz, CH_2-CH_3), 2.82−2.84 (m, 6H, CH_2-CH_3), 4.56 (s, 6H, CH_2-NH), 6.91 (s, 3H, pyrrole-C*H*), 7.48 (s, 3H, pyrrole-C*H*), 8.42 (bs, 12H, guanidinium-NH_2), 8.57 (s, 3H, amide-N*H*), 11.92 (s, 3H, guanidinium-N*H*), 12.47 (s, 3H, pyrrole-N*H*); ^{13}C NMR (100 MHz, [D6]DMSO) δ = 16.2 (CH_2-CH_3), 22.7 (CH_2-CH_3), 37.2 (CH_2-NH), 113.6 (pyrrole-*C*H), 115.6 (pyrrole-*C*H), 125.5 (pyrrole-*C*q), 131.9 (aryl-*C*q), 132.4 (pyrrole-Cq), 143.8 (aryl-*C*q), 155.2, 158.5, 159.7 (2 carbonyl *C*O, guanidinium CN); MS (pos. ESI): *m*/*z* = 784 [M − 2H$^+$]$^+$, 393 [M − H$^+$]$^{2+}$, 262 [M]$^{3+}$; HR-MS (pos. ESI): *m*/*z* = 392.690 (calcd. for $C_{36}H_{45}N_{15}O_6$ + 2H$^+$: 392.6917); IR (KBr-pellet) $\tilde{\nu}$ = 3258 (s, br), 2966 (w), 1699 (s), 1636 (m), 1558 (m), 1473 (m), 1274 (s), 1195 (m), 1092 (s), 755 (w), 615 (w), 482 (m) [cm^{-1}].

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