Communication

Efficient Complexation of *N*-Acetyl Amino Acid Carboxylates in Water by an Artificial Receptor: Unexpected Cooperativity in the Binding of Glutamate but Not Aspartate

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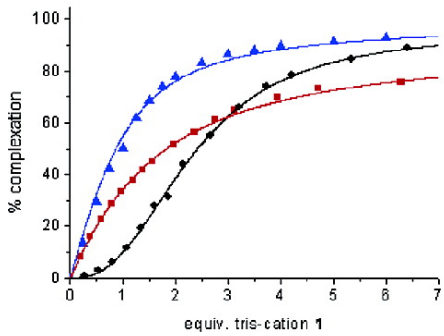

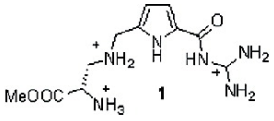

Ala **7**: K_1 = 2100 M^{-1}
Asp **8**: K_1 = 480 M^{-1}
Glu **9**: K_1 = 460 M^{-1}
K_2 = 3300 M^{-1}

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Efficient Complexation of *N*-Acetyl Amino Acid Carboxylates in Water by an Artificial Receptor: Unexpected Cooperativity in the Binding of Glutamate but Not Aspartate

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The design of artificial receptors, which efficiently bind amino acids under physiological conditions, still remains a challenging task.[1] Most amino acid receptors reported in the literature so far need either hydrophobic[1a−c] and/or strong metal−ligand interactions[1f,g,2] to achieve substrate binding in water. We now present for the first time a new tris-cationic receptor **1** which binds amino acid carboxylates efficiently with $K_{ass} \geq 10^3$ M^{-1} in water solely based on electrostatic interactions. Furthermore, **1** shows an unexpected cooperative 2:1 complex formation with *N*-acetyl glutamate—but not aspartate.

The receptor design is based on our guanidiniocarbonyl pyrroles, which we introduced for the binding of carboxylates in polar solvents.[3] Their H-bond-enforced ion pairs with amino acid carboxylate are much stronger than those of other organic cations (e.g., the parent guanidinium cation),[4] but still not strong enough to allow efficient binding in water at millimolar salt concentrations. We reasoned that the additional positive charges in **1** should further stabilize the complex. The synthesis of tris-cation **1** is shown in Scheme 1. The pyrrole acid **2** is first coupled with tBoc−guanidine **3** to give **4**. A reductive amination with amino acid **5** provided the protected receptor **6**. After deprotection and ion-exchange with HCl, the water-soluble tris-cation **1** was obtained as the chloride salt.

The binding properties of this new tris-cation **1** were studied by NMR titration experiments (for more details on the titrations and the data analysis, see Supporting Information).[5] In 40% water in DMSO (v/v), the tris-cation **1** binds *N*-acetyl-L-alanine carboxylate **7** so effectively that the binding isotherm shows only a linear increase up to a ratio of 1:1, indicating an association constant of $K \geq 10^5$ M^{-1}. Hence, the complex is at least 2 orders of magnitude more stable than with corresponding monocationic guanidiniocarbonyl pyrroles ($K \leq 10^3$ M^{-1} in 40% water in DMSO).[3c,d] Even in 90% water (10% DMSO was added for solubility reasons), **1** binds alanine carboxylate **7** with a surprisingly high association constant of $K = 2100$ M^{-1}, as obtained from a nonlinear curve fitting of the binding isotherm (Figure 1). To the best of our knowledge, tris-cation **1** is hence the first simple receptor that allows the efficient complexation of an amino acid carboxylate in water solely based on electrostatic interactions with $K > 10^3$ M^{-1} at millimolar salt concentrations and, therefore, medium ionic strength.

This surprisingly efficient binding probably results from the clustering of electrostatic interactions in this binding motif. However, the three charges do not contribute equally to the complex stability, as can be seen by comparison of tris-cation **1** with the parent guanidiniocarbonyl pyrrole monocations[3c,d] and a previously reported dication.[3a] The significantly improved affinity of **1** must, therefore, come from the terminal α-ammonium group.[6] According to the calculated energy-minimized structure[7] for the complex between tris-cation **1** and **7**, this ammonium group can form an additional chelate interaction with the two carboxylate oxygens (Figure 2). On the basis of this complex structure, no stereoselective

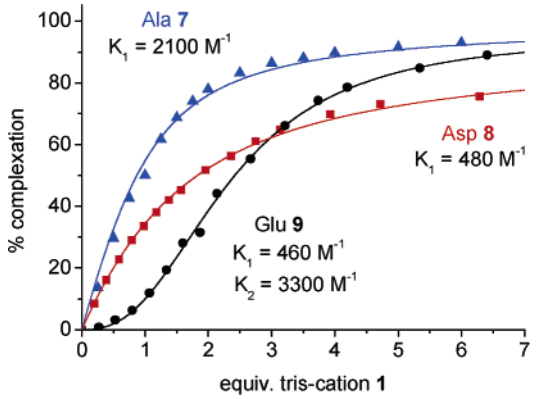


Figure 1. Binding isotherms for the amide NH of the amino acid carboxylates **7**−**9** (c_0 = 1.5 mM, NMe_4 salts) upon the addition of tris-cation **1** (chloride salt) in 90% water/DMSO. The solid lines represent the curve fitting.

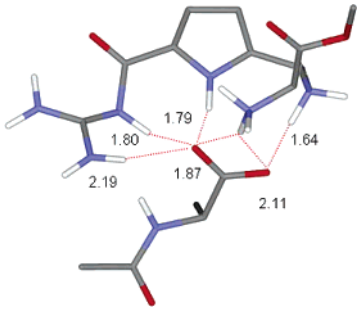
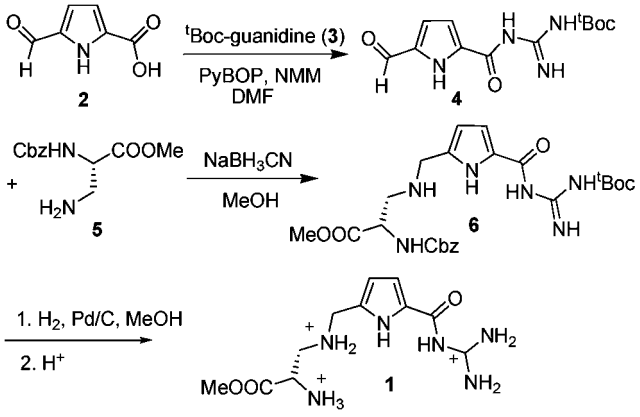


Figure 2. Calculated structure for the complex between **1** and **7** [nonpolar hydrogens omitted for clarity, H-bond distances in Å].

Scheme 1. Synthesis of Tris-Cation **1**



substrate binding is expected, even though tris-cation **1** is chiral, and indeed, *N*-acetyl-D-alanine carboxylate is bound with the same affinity as its enantiomer ($K = 2040$ M^{-1}).

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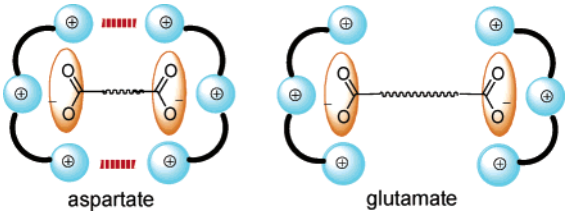

Figure 3. Steric and/or electrostatic interactions (red) prevent the formation of a 2:1 complex for aspartate but not glutamate.

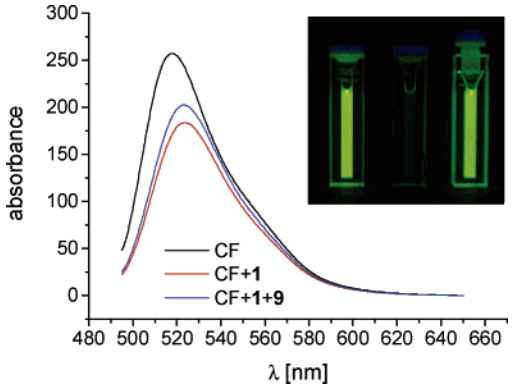

Figure 4. Indicator-displacement assay in water ([CF] = 10 μM, [**1**] = 1 mM, [**9**] = 0.5 mM in 2 mM bis-tris buffer, pH = 6.3). Inset: Naked-eye detection of glutamate in aqueous DMSO.

Tris-cation **1** should also be prone for the binding of multianionic substrates. We, therefore, tested its affinity for *N*-acetyl-L-aspartate **8** and *N*-acetyl-L-glutamate **9**. In 90% water/DMSO (v/v), aspartate **8** forms a 1:1 complex with tris-cation **1** with an association constant of 480 M^{-1} (Figure 1).[8] A Job plot confirmed the formation of a 1:1 complex. Surprisingly, the binding of *N*-acetyl glutamate **9**, which compared to aspartate **8** has only one additional methylene group in the side chain, is completely different. Receptor **1** forms a 2:1 complex with glutamate **9** (receptor:glutamate), and the sigmoidal curvature of the binding isotherm indicates a positive cooperativity effect in the formation of this 2:1 complex (Figure 1).[9] The tris-cation **1** is hence capable of differentiating between glutamate and aspartate, which is remarkable regarding their structural similarity and flexibility. A possible explanation is suggested in Figure 3. The smaller distance between the two carboxylates in alanine compared to glutamate might prevent the formation of 2:1 complexes due to unfavorable steric/electrostatic interactions.

The positive cooperativity in the binding of **1** to glutamate is also evident from the binding constants. A nonlinear curve fitting of the titration data with a 2:1 association model provided K_1 = 460 M^{-1} and K_2 = 3300 M^{-1}. The binding constant for the second association step is larger by a factor of 7 than the one for the first step ($K_1 < K_2$), leading to the observed positive cooperativity. Such allosteric binding processes are tremendously important in many biological systems,[9] but still difficult to achieve in small artificial receptors.[10]

The binding affinity of tris-cation **1** for amino acid carboxylates is large enough to allow their naked-eye detection using an indicator displacement assay,[11] as shown for glutamate as an example in Figure 4. The fluorescence of carboxyfluorescein CF is quenched in the presence of tris-cation **1** due to complex formation and increases again upon the addition of glutamate, a much better binding substrate. The simple tris-cation **1** is, of course, not yet selective enough for different amino acid carboxylates to allow their distinction, but the introduction of additional binding sites, for example, using the ester group in **1** should allow the design of modified versions with improved selectivity for individual amino acids. Such work is currently in progress.

In conclusion, we show here that a clustering of electrostatic interactions as in tris-cation **1** allows the efficient complexation of amino acid carboxylates in water. Additional hydrophobic or metal−ligand interactions are not needed. Furthermore, even small and flexible artificial receptors can show remarkable cooperativity, thereby discriminating between structurally closely related guests.

Acknowledgment. This work was supported by the DFG and the Fonds der Chemischen Industrie.

Supporting Information Available: Experimental details for the synthesis of **1**; binding data. This material is available free of charge via the Internet at http://pubs.acs.org.

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