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A New Receptor Molecule for Lysine and Histidine in Water: Strong Binding of Basic Amino Acid Esters by a Macrocyclic Host

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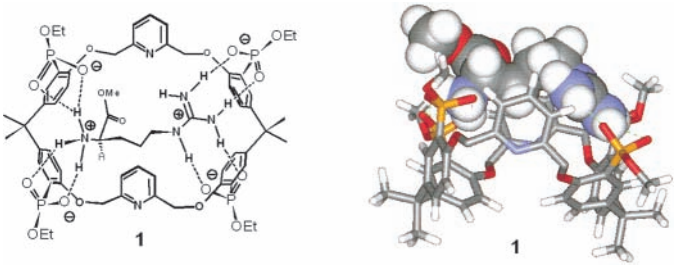
ABSTRACT



We present the new host molecule 1 which binds basic amino acid esters in water. It recognizes both positively charged groups of the amino acid esters by electrostatic and hydrogen bond interactions with its four strategically placed phosphonate anions. Selectivity for lysine is achieved by the correct distance between both bisphosphonate pairs. By contrast, the smaller amino acid esters arginine, ornithine, and histidine form 2:1 complexes with 1. In methanol, a double chelate assembly enforced by π-cation interactions with the imidazolium cation leads to a very high association constant for the 1:histidine complex of 3×10^4 M^{-1}.

The effective and selective molecular recognition of amino acids in water is still a challenge in supramolecular chemistry. Because of the frequent use of the side chains of basic amino acids (Lys, Arg, His) for biological processes, the molecular recognition of these amino acids by synthetic receptor molecules is of special interest.[1] Recently we presented a new macrocyclic receptor molecule (**2**), which binds arginine and lysine in a stereoselective fashion (Figure 1).[2] The mechanism of enantioselective recognition relies on two

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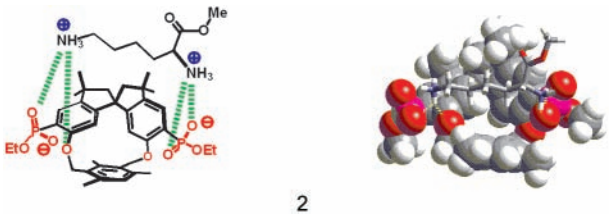

Figure 1. Chiral recognition of lysine methyl ester by macrocyclic bisphosphonate receptor molecule **2** in DMSO.[2]

simultaneous cation−phosphonate interactions, i.e., binding of a specific cationic group of the amino acid to a specific phosphonate moiety in the receptor molecule. After docking, the amino acid comes into van der Waals contact with the chiral surface of the chiral bridging unit in **2** and one enantiomer is bound preferentially.

However, because of the relatively weak binding energy from the classical ammonium phosphonate interaction, the overall binding constants are in the range of 10^4 M^{-1} only in DMSO. We asked ourselves if strong binding in more polar solvents (preferably in water) could be achieved would two chelating bisphosphonate moieties instead of two single phosphonates be incorporated in a macrocycle, so that each cationic functionalitiy of the amino acid would be involved in a highly effective chelate complex.[3] This led to the design of macrocycle **1** which according to X-ray analysis and molecular modeling of its octamethyl ester precursor adopts a favorable open conformation.[4] The respective tetraanion should be able to perform an induced fit on approach of a dication with the correct spacer. Thus, a highly stable 1:1 complex could be formed, possibly even in water (Figure 2).

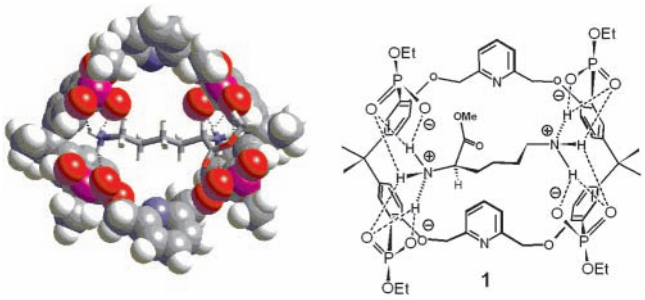

Figure 2. (a) Tetrakisphosphonate receptor molecule **1** and its complex with lysine methyl ester dication (proposed binding mode): molecular mechanics calculations (cylinders, arginine; CPK, host **1**). (b) Tetrakisphosphonate receptor molecule **1** and its complex with lysine methyl ester dication (proposed binding mode): Lewis structures.

The syntheses of the neutral macrocycle corresponding to **1** and some related structures have recently been described by the Finocchiaro group.[4] In a very efficient one-pot synthesis, 2,6-bis(bromomethyl)pyridine is directly treated with phosphonate-modified bisphenol A in a dipolar aprotic solvent with a mild base. The neutral macrocycle is obtained in 70% yield by formation of four C−O bonds in one step. For mild and selective monodealkylation of all four diethylphosphonates, we modified a procedure originally described by Karaman et al.[5] Heating of a 2-hexanone solution of the octaethyl ester precursor with 4 equiv of dry lithium bromide for 1 week afforded, after recrystallization and dialysis, the receptor molecule **1** as the tetralithium salt (Figure 3). This compound is very soluble in polar solvents

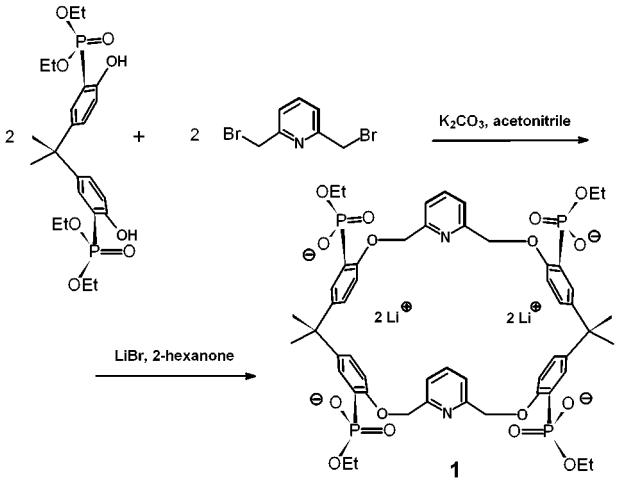

Figure 3. Synthesis of macrocyclic tetraphosphonate receptor molecule **1**.

such as methanol and water but insoluble in DMSO and acetonitrile.

In a preliminary ^{1}H NMR spectroscopic experiment, a 1:1 mixture of **1** and lysine methyl ester dihydrochloride produced significant complexation-induced shifts of CH protons both in the amino acid and in the receptor molecule (Figure 4). To check the complex stoichiometries with lysine, arginine, ornithine, and histidine, Job plots were taken for each of the amino acid complexes with **1**.[6] The result was remarkable: while the smaller amino acid esters histidine, ornithine, and arginine produced a clear 2:1 stoichiometry, only lysine was bound by **1** in a clean 1:1 complex.

This is a good indication for the postulated binding mode: Only in lysine are the two cationic groups able to span the distance from one bisphosphonate moiety to the

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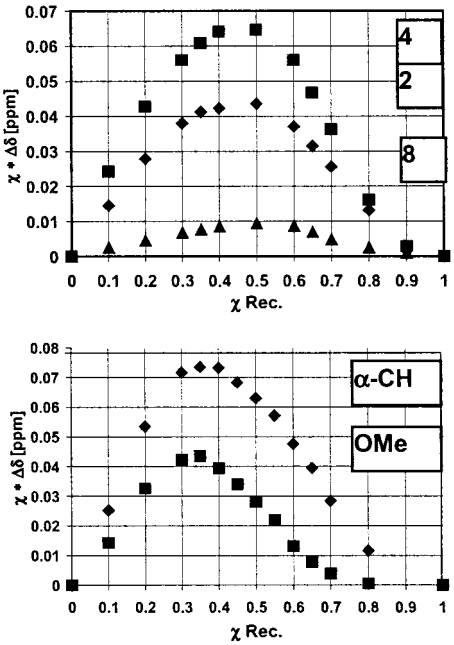

Figure 4. (a) Job plot for the complex between **1** and lysine methyl ester (^{1}H NMR experiments). (b) Job plot for the complex between **1** and histidine methyl ester; for the proton numbering scheme of **1**, see Supporting Information.

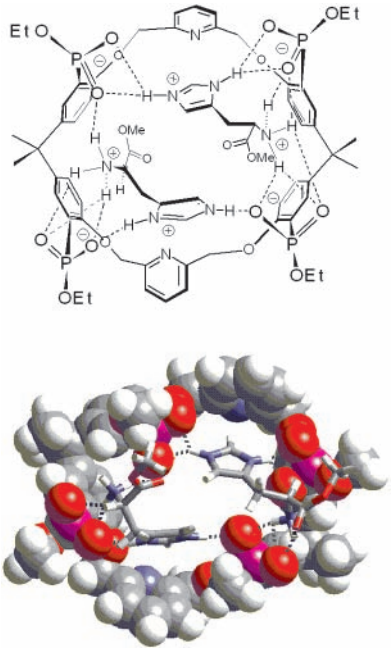

Figure 5. Postulated binding mode of histidine methyl ester in its 2:1 complex with tetraphosphonate **1** (energy minimization).

other.[2] By contrast, the smaller amino acid esters arginine, ornithine, and histidine can only reach one bisphophonate moiety at a time, so that two amino acid molecules can be bound by receptor molecule **1**. However, even in this case a relatively stable assembly may be formed, characterized by an array of alternating positive and negative charges according to energy minimizations.[7] Histidine is bound remarkably well in this 2:1 complex. The reason for this may lie in the special hydrogen bond donor arrangement of the imidazolium ion: according to force-field calculations, two histidine molecules can each be involved in a double chelate assembly with three phosphonate groups. While the ammonium functionality is complexed as usual by the bisphosphonate, the imidazolium ring acts as a hydrogen bond donor bridge to one of the phosphonate moieties at the opposite end of the receptor molecule (Figures 5 and 6).[8]

We then performed NMR titrations for each of the four complexes in methanol. The resulting binding curves were analyzed by nonlinear regression; the respective association constants are listed in Table 1.[9]

In methanol, all amino acid esters are bound strongly by **1**. The four-point interaction in the lysine complex with **1** is more powerful than the two-point interaction in the related assemblies with ornithine and arginine (Figure 7). However, despite its 2:1 stoichiometry, histidine is even superior to lysine. This is again in good agreement with the above-discussed double chelate binding mode suggested by molecular modeling. In addition to the enhanced electrostatic attraction, histidine is able to form two strong hydrogen

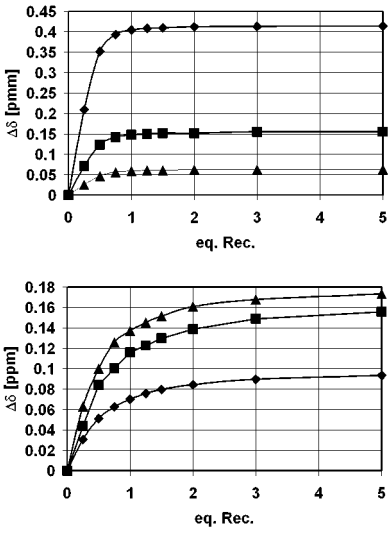

Figure 6. (a) NMR titration curves for the complexation of histidine methyl ester by **1**. (b) NMR titration curves for the complexation of lysine methyl ester by **1**.

(7) We found this array in numerous 1:1complexes of bisphosphonates and short diammonium cations (unpublished results); Molecular Modeling Program CERIUS[2] from Molecular Simulations Inc.; Force field: Dreiding 2.21.

(8) Strong highfield shifts of both the imidazole and the pyridine ring protons indicate π,π-interactions, supporting the binding mode of Figure 5.

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Table 1. Association Constants (K_a) [M^{-1}] and Stoichiometries for the Complexes of Basic Amino Acid Esters with Receptor Molecule **1** from NMR Titrations in Methanol and Water at 20 °C[a]

amino acid × HCl	K_a [M^{-1}] (methanol)[b]	(amino acid vs **3**)[c]	K_a [M^{-1}] (water)[b]	(amino acid vs **3**)[b]
histidine	29000 ± 11%	2:1	650 ± 18%	2:1
ornithine	9500 ± 9%	2:1	221 ± 33%	2:1
arginine	8800 ± 14%	2:1	165 ± 38%	2:1
lysine	21000 ± 35%	1:1	1200 ± 25%	1:1

[a] Because of the strongly hygroscopic character of both titration partners, the d_4-methanol solution contained ~0.1% of water. Errors in K_a are standard deviations and were calculated to be ±15−38%. [b] For the 2:1 complexes, the association constants for each complexation step were assumed to be equal. [c] From Job plots.

bonds between the imidazolium moiety and the bisphosphonate ions. This may contribute to its superior binding constant.

From methanol to water, the stoichiometry of all complexes is retained, but a 20−50-fold drop is observed in the association constants of the four investigated amino acid esters. This demonstrates the powerful competition of water molecules and is in accord with the results reported by other groups.

However, this time, lysine is complexed 5−7 times more strongly than ornithine and arginine and even twice as strongly as histidine. If hydrophobic forces are weak as in our case, the contribution of hydrogen bonds in water is negligible, while electrostatic interactions represent the major attractive force. It is known that in this respect the hard ammonium ion with its high charge density is superior to the softer guanidinium and also the imidazolium ion, where the positive charge is delocalized across several atoms.[10] In our case, the electrostatic attraction exerted by the second ammonium functionality of lysine is obviously much stronger than that of arginine's guanidinium ion and even histidine's imidazolium ion. Thus, receptor molecule **1** is moderateley selective for lysine in water.

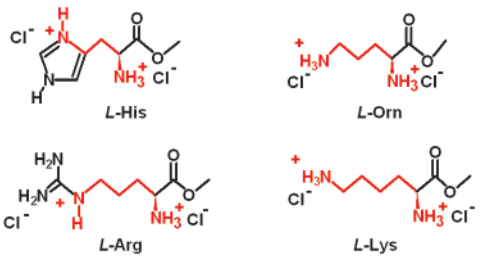

Figure 7. Investigated dicationic guest molecules: the distance between the charged nitrogen atoms is successively increased by one carbon or nitrogen atom.

Recently, Bell[11] and Dougherty[12] published new binding motifs for arginine, which both operate very efficiently in water. While Bell's rigid halfmoon-shaped receptor molecule is highly preorganized (K_a = 900 M^{-1}), Dougherty makes use of the π-cation interaction between electron-rich benzene rings and the guanidinium moiety, enforced by multiple electrostatic interactions with additional carboxylates in the periphery of the receptor molecule (K_a = 5000 M^{-1}). However, both hosts are large molecules, accessible only through a multistep synthesis. In this respect our modular synthesis from two building blocks in one step may offer the advantage of a fast entry into a whole family of related tetraphosphonate hosts.

In the future, we intend to incorporate chiral building blocks in our receptor molecules (preferably fom natural sources such as amino acids) as well as implement more rigid elements for a high degree of preorientation to achieve enantioselective *and* strong recognition of basic amino acids in water.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support.

Supporting Information Available: Experimental procedures and full characterization for compound **1**, NMR titration curves, and Job plots in various solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) **17,17,40,40-Tetramethyl-1,10,24,33-tetraoxy[2](2,6)pyridino[2.1]paracyclo[2](2,6)-pyridino[2.1]paracyclophane-12,20,35,43-tetraphosphonic acid tetraethylester tetra lithium salt 1:** 146.7 mg (0.1215 mmol) of the macrocycle[4] and 46.3 mg (4.1 equiv) of lithium bromide were dissolved in 7 mL of dry 2-hexanone under an argon atmosphere and heated for 120 h at 100 °C. The residue was filtrated and washed with 2-hexanone. The salt was purified via dialysis: afterward it contained only minute amounts of lithium bromide. Yield: 116 mg (85%). ^{1}H NMR (500 MHz, CD_3OD): δ = 1.13 (t, 12 H, J = 7.3 Hz, CH_3CH_2O), 1.64 (s, 12 H, CH_3C), 3.81 (q, 8 H, J = 7.0 Hz, CH_3CH_2O), 5.26 (s, 8 H, CH_2O), 6.76 und 6.90 (m, 2 × 4 $H_{arom.}$), 7.69 (d, 4 H_{py}, J = 6.9 Hz), 7.76 (t, 2 H_{py}, J = 6.9 Hz), 7.86 (m, 4 $H_{arom.}$). ^{13}C NMR (125 MHz, CD_3OD) δ = 17.44 (s), 31.87 (s), 39.00 (s), 43.37 (s), 61.74 (d, 5.0 Hz), 113.75 (d, 8.8 Hz), 122.36 (s), 123.92 (s), 125.31 (s), 129.36 (d, 82.3 Hz), 132.39 (s), 133.58 (d, 6.8 Hz), 139.68 (s), 144.36 (d, 12.5 Hz), 159.01 (d, 42,3 Hz). ^{31}P NMR (200 MHz, CD_3OD) δ = 12.85. Anal. Cacld for $C_{52}H_{58}N_2O_{16}P_4Li_4$: C 55.83; H 5.23; N 2.50. Found: C 54.71; H 5.45; N 2.08. MS (FAB, glycine matrix, Xe): m/z 1125 (M + Li^+, 55%).