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A Chiral Sensor for Arginine and Lysine

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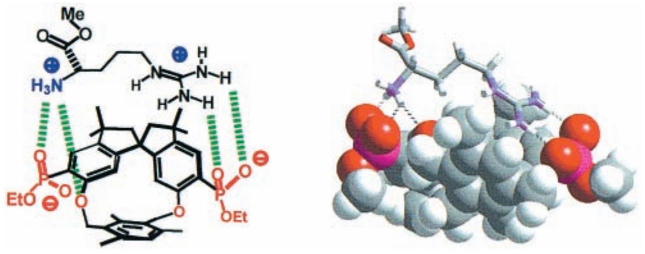
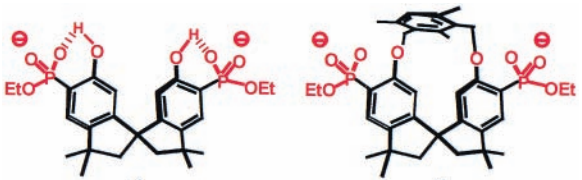
ABSTRACT



We provide access to a new class of C_1- or C_2-symmetrical host molecules 1 and 2 based on a spirobisindane skeleton. Whereas 1 is selective for short, rigid diamines, 2 prefers longer α,ω-dications. Of all the amino acid methyl esters, only those of lysine and arginine with the correct distance between their cationic groups form strong 1:1 complexes in DMSO with 2. NMR titrations reveal high association constants as well as discrimination between the enantiomers of lysine and arginine.

We recently introduced the xylylene bisphosphonate moiety as a new general binding motif for amino alcohols[1,2] and used it for the construction of biomimetic adrenaline hosts[3] as well as for the anomerselective recognition of amino sugars.[4] In this paper we present a new class of cleftlike receptor molecules based on bisphosphonates and fully equipped for multipoint binding of their dicationic substrate (Figure 1).



1 2

Figure 1. Two new host molecules: the open-chain **1** has C_2- whereas macrocycle **2** has C_1-symmetry.

The new host molecules were prepared by careful monodealkylation of the respective cleftlike bisphosphonic acid tetraalkyl esters[5] with LiBr: Refluxing a 40 mM solution of the tetraalkyl ester with 1 equiv of dry lithium bromide in 2-hexanone or acetonitrile afforded after 5−100 h a precipitate of the respective lithium salt, which was in many cases analytically pure.[6] The spirobisindane skeleton in **1** and **2** guarantees a high degree of rigidity, which should lead to a pronounced preorganization of the different binding sites. The mesitylene spacer in **2** was deliberately chosen, because in former conformational analyses it was found that only such sterically demanding moieties prevent rotation of the bridge.[5] A series of NMR binding experiments revealed a remarkably different behavior of the two new host molecules with respect to their chemoselectivity as well as their enantioselectivity.

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(1) Schrader, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2649−2651.
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(5) (a) Consiglio, G. A.; Failla, S.; Finocchiaro, P. *Phosphorus, Sulfur Silicon* **1998**, *413*, 134−139. (b) Consiglio, G. A.; Finocchiaro, P.; Failla, S.; Hardcastle, K. I.; Ross, C.; Caccamese, S.; Giudice, G. *Eur. J. Org. Chem.* **1999**, 2799−2806.
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Job plots[7] with **1**, **2**, and several α,ω-diammonium compounds (Figure 2) proved that **1** forms 1:1 complexes in all cases, whereas **2** binds two guest molecules as long as they are shorter than lysine or arginine (Figure 3). This divergent behavior could be explained with the different sterical accessibility of the cleft in **1** and **2**: In **1** there is ample room for both cationic groups to approach the phosphonate anions from within the cleft, with the effect that small α,ω-diammonium compounds should lead to the highest binding enthalpy, governed mainly by optimal electrostatic interactions (Figure 4a). In **2**, on the contrary, the cleft is closed by the rigid mesitylene bridge, so that only long α,ω-dicationic guests can reach from one phosphonate group to the other; at the same time their carbon chain comes into close contact with the mesitylene bridge, so that molecular recognition can be expected to become more sensitive (Figure 4b).

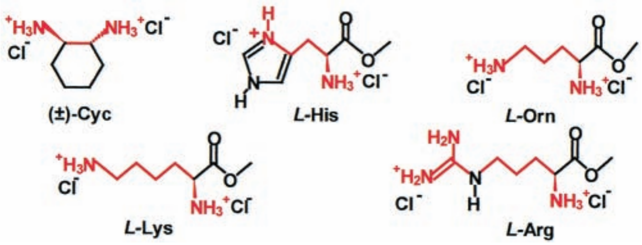

Figure 2. Investigated chiral guest molecules: the distance between the charged nitrogen atoms is successively increased by one carbon or nitrogen atom.

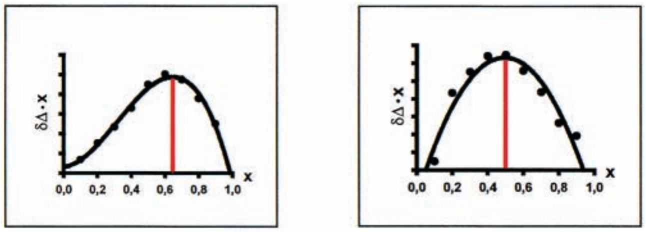

Figure 3. Job plots with **2** show 2:1 complexes for short guest molecules, but 1:1 complexes for lysine and arginine. Left: complexation of **2** with diaminocyclohexane (Cyc). Right: **2** with arginine methyl ester (Arg).

We performed NMR titrations[8] of various dicationic guests with a successively increasing distance between their positively charged nitrogen atoms (Figure 2).

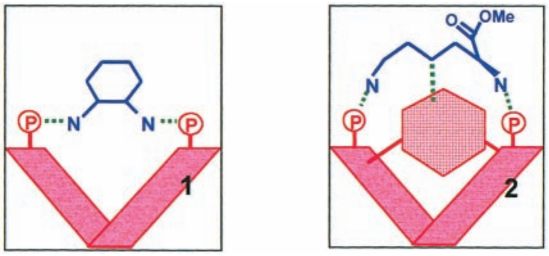

Figure 4. (a) The open host **1** allows short guest molecules to penetrate deeply into the cleft. (b) The mesitylene bridge in **2**, however, provides a third hydrophobic binding site. This leads to strong and chiral recognition of longer α,ω-diamines in DMSO.

Table 1. Association Constants K_a [M^{-1}] from NMR Titrations for Complexes of Hosts **1** and **2** with Dicationic Guests of Varying N,N-Distance in DMSO at 20 °C[a]

guest	N^+,N^+-distance	$K_{a(1:1)}$ [M^{-1}] open-chain **1**[b,c]	$K_{a(1:1)}$ [M^{-1}] macrocycle **2**[b,d]	complex	chiral recogntn
Cyc	2 atoms	$8.4 \times 10^3 \pm 32\%$[a]	$2.0 \times 10^3 \pm 20\%$	2:1	no
His	3 atoms	$2.4 \times 10^3 \pm 11\%$	$1.0 \times 10^3 \pm 32\%$	2:1	no
Orn	4 atoms	$2.4 \times 10^3 \pm 28\%$	$3.1 \times 10^2 \pm 38\%$	n.d.	no
Lys	5 atoms	$2.5 \times 10^3 \pm 14\%$	$2.1 \times 10^4 \pm 26\%$	1:1	yes
Arg	6 atoms	$9.0 \times 10^2 \pm 43\%$	$9.4 \times 10^3 \pm 42\%$	1:1	yes

[a] Because of the strongly hygroscopic character of the bisphosphonate salts, the DMSO solution contained ~0.1% of water. [b] Errors are standard deviations of the nonlinear regressions. [c] With **1** a 1:1 complex is formed in all cases. [d] In all 2:1 complexes the phosphonates were calculated as independent binding sites.

The resulting binding curves were analyzed by nonlinear regression methods and furnished the association constants found in Table 1. It is obvious that the dependence of the order of total binding energies from the guest structure differs enormously between host **1** and **2**: the open chain host **1** is selective for short rigid α,ω-diamines (K_a up to 8×10^3 M^{-1}); macrocycle **2**, however, prefers longer dications (K_a up to 1.2×10^4 M^{-1}). This is in perfect agreement with the above-postulated binding mode.

No enantiodiscrimination is found for the open chain host **1**, and even **2** can only distinguish between enantiomeric guests with an $N^+ \cdots N^+$ distance of more than five bonds. Force field calculations[9] suggest that only lysine and arginine are long enough to span the bridged cleft of **2**, so that two salt bridges can be formed between their cationic groups and the respective phosphonates. This indicates once more that for an efficient chiral recognition the two binding sites within the C_2-symmetrical open chain host molecule **1** are not sufficient. Only the additional third binding site offered in C_1-symmetrical macrocycle **2** by the chiral surface of the

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[7] (a) Job, P. *Compt. Rend.* **1925**, *180*, 928. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626.

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[9] Molecular Modeling Program: CERIUS^2 from Molecular Simulations Inc., Force field: Dreiding 2.21.

mesitylene bridge leads to a detectable enantiomeric discrimination. Although the ee's are not very high (**2**:L-Arg, 17%; **2**:L-Lys, 33%), well-resolved NMR signals are produced during the NMR titration, so that optically pure **2** may be used as a shift reagent for the quantitative determination of the enantiomeric purity of arginine and lysine derivatives (Figure 5).

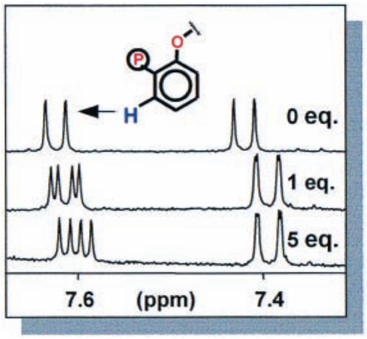

Figure 5. Formation of the diastereomeric complexes can be monitored as a typical signal splitting of distinct host NMR signals. Different upfield shifts are observed for the two nonequivalent aromatic receptor protons *ortho* to the phosphonate moiety in **2**, on complex formation with lysine methyl ester.

Additional structural information from a careful analysis of the NMR data provides a more detailed picture of the molecular recognition of long basic amino acids by **2**: The signal splitting of an aromatic host proton observed in the complex between **2** and arginine occurs at the opposite end of **2** compared to its complex with lysine. Since this effect must originate from an interaction of that host proton with the chiral environment of the α-C atom, the amino acids obviously prefer an opposite orientation in the complex. Only one of the nonequivalent O-CH_2 methylene groups in **2** shows a strong downfield shift during the titration (used for K_a calculation). This correlates well with an additional $N{-}H^{\delta+}\cdots O^{\delta-}$ hydrogen bond observed in the energy minimization process (Figure 6). Only an ammonium group is able to undergo this typical hydrogen bond pattern. Whereas in arginine this must be the α-NH_3^+ group, lysine probably prefers the flexible and sterically mobile ϵ-amino group. Now three binding sites evolve in the amino acid molecules, with each exhibiting a different binding strength. This is the prerequisite for chemoselective and also enantioselective recognition, met only by arginine and lysine in our experiments.

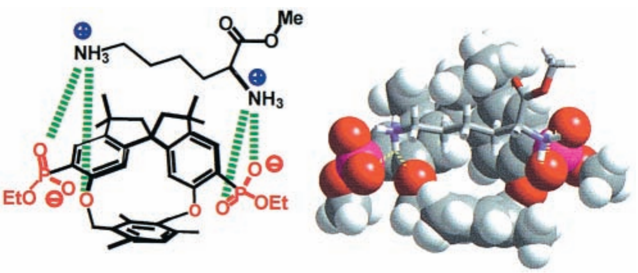

Figure 6. Energy-minimized structure of the proposed complex geometry for Lys-**2**. Note the additional H-bond to the only accessible ether oxygen on the left, which determines the relational orientation of both partners.

Few host molecules with enantioselective recognition of arginine and lysine have been published to date: Jung et al. developed monolayers of cyclohexapeptides immobilized on gold whose interactions with aqueous amino acid solutions were transduced by changes of the resonance frequency of a quartz crystal microbalance.[10] Enantiomeric arginine and lysine recognition has also been achieved by podand and crown-type amide derivatives of the naturally occurring monensin ionophore.[11] Polyclonal antibodies can be used in an enantioselective enzyme-linked immunosorbent assay for dinitrophenylamino acids.[12] Finally, Famulok et al. used an in vitro selection process to produce an L-citrullin binding RNA aptamer, which was subjected to further evolution into an L-arginine binder.[13] All the above-mentioned methods,

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(14) **Dilithium bis(5,5′ethylphosphonato)-6,6′-dihydroxy-3,3,3′,3′-tetramethyl-1,1′-spiro-bisindane 1:** To a solution of **1** (0.3 mmol) in dry methylbutyl ketone (15 mL) was added lithium bromide (0.6 mmol), and the mixture was heated to reflux for 1 h. The white precipitate was filtered, suspended in diethyl ether, and sonicated for 0.5 h. The solid was filtered again and dried under reduced pressure at 130 °C over phosphorus pentoxide: yield 95%; ^{1}H NMR (500 MHz, [D$_6$]DMSO) $\delta = 0.79$ (t, 6H, $^{3}J_{HH} = 14.5$ Hz), 1.01 (s, 6H), 1.07 (s, 6H), 1.87 (d, 2H, $^{2}J_{HH} = 13.2$ Hz), 1.99 (d, 2H, $^{2}J_{HH} = 13.2$ Hz), 3.38 (m, 4H), 5.69 (d, 2H$_{arom}$, $^{4}J_{HP} = 4.4$ Hz), 6.94 (d, 2H$_{arom}$, $^{3}J_{HP} = 12.6$ Hz); ^{13}C NMR (126 MHz, [D$_6$]DMSO) $\delta = 14.13$, 16.86 (d, $^{3}J_{CP} = 7.3$ Hz), 22.03, 25.70, 30.03, 30.84, 32.10, 42.69, 57.34, 59.13, 59.26 (d, $^{1}J_{CP} = 42.5$ Hz), 110.17 (d, $^{3}J_{CP} = 10.9$ Hz), 125.43 (d, $^{2}J_{CP} = 6.07$ Hz), 141.34 (d, $^{3}J_{CP} = 12.1$ Hz), 154.21, 160.15 (d, $^{2}J_{CP} = 7.28$ Hz); ^{31}P{^{1}H} NMR (202 MHz, [D$_6$]DMSO) $\delta = 13.85$ ppm; FAB-MS $m/z = 537$ (100%, M$^+$); IR (cm^{-1}) 2956, 2903, 1508, 1474, 1418, 1363, 1184, 1125, 1041, 946, 780. To get an analytically pure sample the lithium salt was protonated with HCl and filtered and the resulting bisphosphonic acid dried under reduced pressure; calcd C 57.21, H 6.53; found C 57.90, H 7.00. **Dilithium bis(5,5′-ethylphosphonato)-6,6′-(2,4,5-trimethyl-1,3-benzyloxy)-3,3,3′,3′-tetramethyl-1,1′-spiro-bisindane 2**: To a solution of **2** (0.3 mmol) in dry acetonitrile (15 mL) was added lithium bromide (0.6 mmol), and the mixture was heated to reflux for 100 h. The white precipitate was filtered, suspended in diethyl ether, and sonicated for 0.5 h. The solid was filtered again and dried under reduced pressure at 130 °C over phosphorus pentoxide: yield 90%; ^{1}H NMR (500 MHz, [D$_4$]-methanol) $\delta = 1.20$ (s, 3H), 1.32 (t, 3H, $^{3}J_{HH} = 14.5$ Hz), 1.35 (t, 3H, $^{3}J_{HH} = 14.5$ Hz), 1.36 (s, 6H), 1.37 (s, 3H), 1.63 (s, 3H), 1.81 (d, 1H, $^{2}J_{HH} = 12.6$ Hz), 1.92 (d, 1H, $^{2}J_{HH} = 12.7$ Hz), 2.30 (d, 1H, $^{2}J_{HH} = 12.6$ Hz), 2.40 (d, 1H, $^{2}J_{HH} = 12.0$ Hz), 2.41 (s, 3H), 2.57 (s, 3H), 3.84 (m, 1H), 3.93 (m, 1H), 3.98 (m, 2H), 5.28 (dd, 2H, $^{2}J_{HH} = 95.2$ Hz), 5.29 (d, 2H, $^{2}J_{HH} = 22.05$ Hz), 5.53 (d, 1H$_{arom}$, $^{4}J_{HP} = 5.1$ Hz), 6.04 (d, 1H$_{arom}$, $^{4}J_{HP} = 5.1$ Hz), 6.83 (s, 1H$_{arom}$); 7.54 (d, 1H$_{arom}$, $^{3}J_{HP} = 13.9$ Hz), 7.78 (d, 1H$_{arom}$, $^{3}J_{HP} = 13.3$ Hz); ^{13}C NMR (126 MHz, [D$_4$]methanol) $\delta = 14.88$, 17.11 (m), 19.84, 21.80, 31.02, 31.39, 31.70, 43.93, 44.00, 58.91, 59.12, 59.71, 61.08 (d, $^{2}J_{CP} = 6.06$ Hz), 61.26 (d, $^{2}J_{CP} = 6.06$ Hz), 65.35, 72.24, 109.46 (d, $^{3}J_{CP} = 9.72$ Hz), 121.64 (d, $^{3}J_{CP} = 10.9$ Hz), 123.63 (d, $^{1}J_{CP} = 175.93$ Hz), 129.38 (d, $^{2}J_{CP} = 6.07$ Hz), 129.61 (d, $^{2}J_{CP} = 6.07$ Hz), 130.31, 132.23, 132.32, 132.35, 138.01, 139.63, 141.07, 143.61 (d, $^{3}J_{CP} = 13.3$ Hz), 149.70 (d, $^{3}J_{CP} = 13.36$ Hz), 153.77 (d, $^{4}J_{CP} = 2.43$ Hz), 154.49, 158.76 (d, $^{2}J_{CP} = 2.42$ Hz), 160.90 (d, $^{2}J_{CP} = 2.42$ Hz); ^{31}P{^{1}H} NMR (202 MHz, [D$_4$]-methanol) $\delta = 12.90/14.09$; FAB-MS $m/z = 681$ (0.6%, M$^+$); IR (cm^{-1}) 2956, 2870, 2900, 1508, 1474, 1396, 1362, 1200, 1185, 1125, 1046, 700, 781; (**2** + H_2O) calcd C 61.53, H 6.93; found C 61.94, H 6.73.

however, either use complex synthetic structures or require elaborated microbiological protocols.

In summary, we have presented a new class of rigid receptor molecules for organic dicationic guests. Depending on the accessibility of their cleft, these hosts are selective for short or long α,ω-diammonium and -guanidinium compounds. Because of their inherent chirality, we could examine their potential for chiral discrimination and found that only those host−guest combinations which allow a three-point interaction were effective. Thus, the bridged macrocyle **2** represents a chiral shift reagent for arginine and lysine derivatives. In the future we intend to develop new sensors from **2** which selectively recognize *N*-terminal arginine and lysine in peptides and proteins.

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Supporting Information Available: Experimental procedures and full characterization for compounds **1** and **2**, NMR titration curves, Job plots, and force field calculations of complex geometries for selected complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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