CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200600764

Thickness Recognition of Bolaamphiphiles by α-Cyclodextrin

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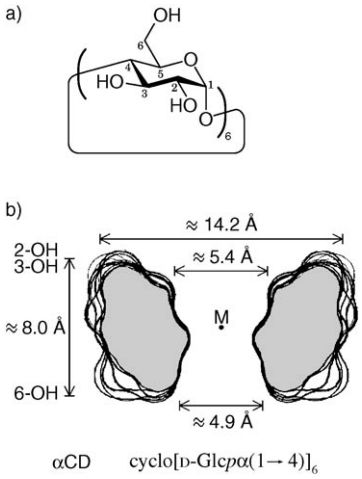
Abstract: The minimum internal diameters of α-, β-, and γ-cyclodextrin were calculated by a space filling algorithm, MolShape, from the electron density maps created by semiempirical AM1 and PM3 calculations using Gaussian03. In addition, the minimum diameters of a series of dicationic bolaamphiphiles were calculated by MolShape as well. The calculated diameters of these hosts and guests allowed prognosis about the stabilities of the corresponding inclusion compounds. The experimental binding data, obtained by isothermal titration calorimetry (ITC), revealed indeed a very pronounced thickness recognition and correlate well with the calculated diameters.

Keywords: calorimetry · cyclodextrins · host–guest systems · molecular recognition · semiempirical calculations

Introduction

Cyclodextrins (CDs) are 1→4 α-linked cyclic oligomers of anhydroglucopyranose.[1–3] Those CDs consisting of six, seven, or eight glucose entities are called α-, β-, or γ-CD, respectively. CDs assume a toroidal shape with the primary hydroxyl groups at the narrow side and the secondary hydroxyl groups at the wide side (Scheme 1). CDs serve as molecular hosts, because they provide a hydrophobic nanoenvironment in aqueous solution, in which a great variety of hydrophobic guest molecules can be incorporated. Formation of so-called CD inclusion compounds can be exploited for many purposes, such as drug delivery,[4,5] creation of hydrogels[6–8] or templated synthesis of rotaxanes and polyrotaxanes.[9,10]

The major driving forces for the formation of CD inclusion compounds are hydrophobic, van der Waals forces, and dipol–dipol interactions, which strongly depend on the space filling of the CD cavity by the guest.[11] Meanwhile, a great number of binding data is available, which allows finding basic rules for inclusion.[12,13] If the guest is too small to fill the CD cavity, binding is weak. For instance, the binding constant of β-CD for benzoic acid $K_S = 20\,M^{-1}$ is much smaller than the one for *tert*-butyl benzoic acid $K_S = 18400\,M^{-1}$.[14] On the other hand, if a guest is too large to fit into the cavity only partial inclusion is observed, which also leads to low binding constants, such as $K_S = 0.6\,M^{-1}$ for D-glucose in β-CD.[15] Consequently, an optimally shaped guest must exist, which fills out the cavity best.



Scheme 1. Schematic drawings of α-cyclodextrin a) molecular formula; b) cross-section along the C_6 axis, according to Lichtenthaler and Immel (Reprinted with permission from ref. [17] Copyright 1994 Elsevier).

The cavities of CDs are about 8.0 Å long in direction of the C_n axis according to X-ray and neutron diffraction studies.[16] The first values of the internal diameters, derived by Saenger in 1980 from CPK models, are listed in Table 1.[1] Since the CD molecules show a conical shape, a distinction has to be drawn between the diameters of the narrow primary side d_{prim} and the wide secondary side d_{sec}. Molecular

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Table 1. Inner widths of cyclodextrins obtained by molecular modeling.

Cyclodextrin Method	n	d_{prim} [Å] CPK[a]	d_{sec} [Å] CPK[a]	$\check{d}_{eq}$ [Å] AM1[b]	$\check{d}_{eq}$ [Å] PM3[b]
α-CD	6	4.7	5.2	4.4	4.4
β-CD	7	6.0	6.4	5.8	6.5
γ-CD	8	7.5	8.3	7.4	8.1

[a] measured from CPK models.[1] [b] Calculated by Gaussian03[18] and MolShape,[19] this work.

modeling calculations of Immel et al. provided more precise pictures. Especially the modeled structure of α-CD showed some constriction at the elevation of carbons C-5 (Scheme 1).[17] Unfortunately, quantitative data of the minimal internal diameters were missing. Therefore, we performed semiempirical quantum mechanical calculations to continue this work.

In this work, we selected a series of bolaamphiphiles as guests for α-CD to investigate the influence of space filling on the binding constant. Bolaamphiphiles are those amphiphiles with two opposite hydrophilic end-groups.[20,21] They were chosen as guests because they allow characterization of the inclusion compounds under homogenous conditions in aqueous solution by highly effective methods such as NMR spectroscopy and isothermal titration calorimetry (ITC).[22–25] Since it is well-known, that the binding constants K_S of bolaamphiphiles and α-CD increase with the lengths of the hydrophobic part of the guest,[2] we focused on the investigation of thickness recognition of the guest while its length was kept constant.

Results and Discussion

Calculation of the dimensions of CD cavities: Electron density maps $\rho(x,y,z)$ of α-, β-, and γ-CDs were created in a first step by quantum mechanical calculations using the program Gaussian03.[18] Atom coordinates from both energy minimized quantum mechanical calculations and crystallographic data of α-CD,[26,27] β-CD,[28,29] and γ-CD,[30,31] were used as input structures. An electron density cut-off value of 0.002 au, common in literature,[32–34] was chosen to define the surface of the molecule. The picture of the calculated surface of the cavity of α-CD together with the stick presentation of the molecule is shown in Figure 1. A constriction of the cavity is clearly visible located close to the middle.

The cross-sectional areas $A(z)$ perpendicular to the C_n axis were determined as follows. The electron density has been dissected into small cubes with a base area a_i of 0.04 Å^2, which has been proven to be sufficiently small. Decreasing this area only prolongs the computing time without achieving a better precision. Now, only those cubes $a_i^*(z)$ at a given altitude z, whose electron density fall below a value of 0.002 au, were regarded as being situated inside the cavity and were therefore counted by the program MolShape. The cross-sectional area $A(z)$ was obtained as the sum of a_i^*. The respective diameter $d_{eq}(z)$ of an equivalent

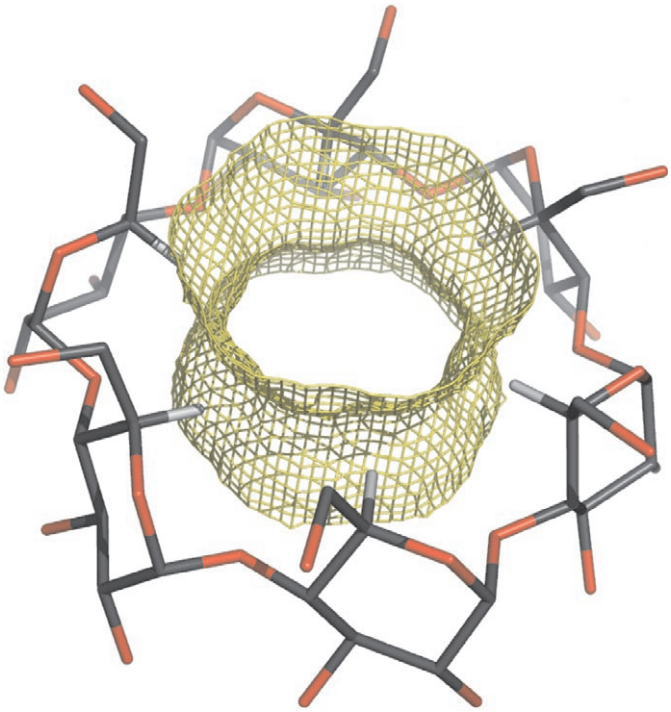


Figure 1. Inner surface of α-CD cavity (yellow) calculated by AM1/Gaussian 03 and stick structure of α-CD, oxygen atoms are in red, hydrogens except H-5, omitted for the benefit of clarity.

circle was calculated according to equation 1, and plotted as a function of z (Figure 2). Again, the constrictions of the CD cavities become evident as a minimum of the internal diameter $\check{d}_{eq}$ at $z \approx 1$ Å, approximately at the altitude of hydrogens H-5.

$$d_{eq}(z) = \sqrt{\frac{4A(z)}{\pi}} = \sqrt{\frac{4\sum a_i^*(z)}{\pi}} \quad (1)$$

In case of α-CD, semiempirical methods, such as AM1, PM3, as well as the density functional theory method B3LYP/6-31G(d) were tested. The resulting minimal diame-

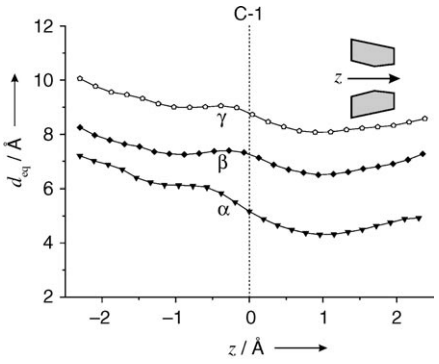


Figure 2. Profiles of the inner widths of α-, β-, and γ-CD along the C_n axis z ($z=0$ at the altitude of atoms C-1), as calculated by MolShape[19] from PM3/Gaussian 03.[18]

ters, $\breve{d}_{eq}$, of α-CD depended only very little on the calculation method of the energy minimized structure and of the electron density map (see Table 2, entries 1–4). In general, calculations were performed forcing C_6 symmetry to save computing time. Very similar results were obtained without this symmetry constraint. Therefore we conclude that this method for determining the minimal internal diameters is quite robust. The PM3 method was selected for further calculations as it contains the best parameterization of hydrogen bonds,[35,36] which are known to internally stabilize the cyclodextrin structure.[37]

Table 2. Minimal cross-sectional areas $\breve{A}$ and equivalent inner widths $\breve{d}_{eq}$ of α-CD, calculated with the program MolShape for various input structures and determination methods of the electron density maps.

No.	Input structure	Method	$\breve{A}$ [Å^2]	$\breve{d}_{eq}$ [Å]
1	AM1/C_6 symmetry/EM[a]	AM1	14.77	4.34
2	PM3/C_6 symmetry/EM	PM3	14.82	4.35
3	AM1/C_6 symmetry/EM	B3LYP/6-31G(d)	14.59	4.32
4	PM3/C_6 symmetry/EM	B3LYP/6-31G(d)	14.77	4.34
5	X-ray structure[26]	PM3	18.71	4.88
6	X-ray structure[39]	PM3	17.90	4.77
7	X-ray structure[40]	PM3	17.72	4.75
8	neutron structure[27]	PM3	16.80	4.62

[a] EM: energy minimization.

Those diameters $\breve{d}_{eq}$ based on X-ray structures (see Table 2, entries 5–7) were ≈0.4 Å larger than those from semiempirical input structures. The value of $\breve{d}_{eq}$ for the neutron structure (see Table 2, entry 8) is also somewhat smaller than the ones of the X-ray structures. Crystal structures significantly differ from solution structures, because of stronger intermolecular interactions in the solid state. We assumed that the energy minimized calculated structures are better representations of the solution structure than any crystal structure. Differences between X-ray structures and calculated solution structures of α-CD are already known.[38] Therefore the value $\breve{d}_{eq}$=4.4 Å based on the semiempirical input structures was taken as a measure of the inner width of α-CD. This calculated inner width of α-CD is somewhat smaller than the one published by Saenger,[1] because it takes better into account the constriction of the cavity.

Also the inner widths $\breve{d}_{eq}$ for β-, and γ-CDs (see Table 1) were obtained accordingly. The values of $\breve{d}_{eq}$ from PM3 calculations were ≈0.7 Å larger than those from AM1. The β-, and γ-CD structures seem to collapse to a funnel-shaped conformation for the AM1 parameterization which is not the case for the PM3 calculations. Therefore, the PM3 values in Table 1 are considered as the most reliable ones. The usefulness of these calculated internal diameters has already been demonstrated recently explaining the stoichiometries of polymeric channel inclusion compounds.[10]

Design of an optimal guest molecule for α-CD: The optimal guest should exhibit a waisted molecular shape which should fit well within the constricted cavity of α-CD. Therefore we thought, stilbene derivatives might fit well because of their narrow part in the middle of the molecule. The bolaamphiphile (*E*)-4,4′-bis(aminomethyl)stilbene (**2**) was chosen because of its sufficient solubility in water. Again, the program MolShape could be used to calculate the thickness of this waisted guest. In case of a guest the area increments $\Delta a_i^*(z)$ were defined as those elements of the cross-section at the coordinate z, which exceed an electron density of 0.002 au. The sum of Δa_i^* yielded the cross-sectional area $A(z)$. The equivalent thickness $d_{eq}(z)$, calculated according to Equation (1), is plotted in Figure 3 as the function of the

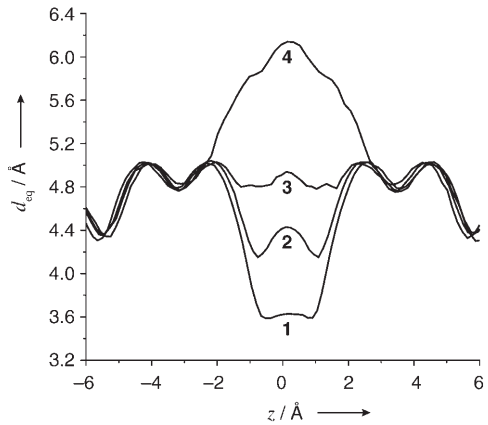
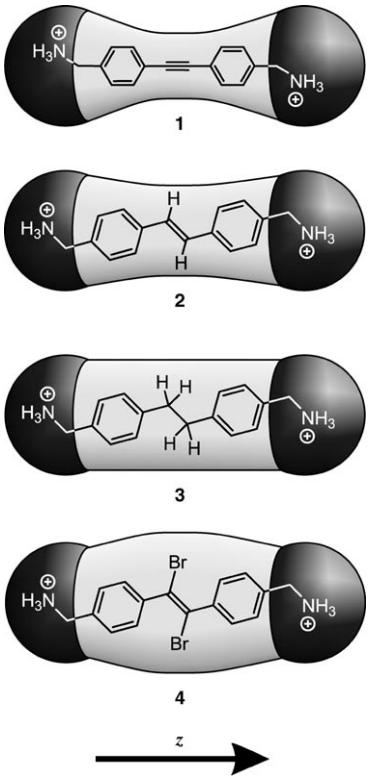


Figure 3. Profiles of the guests **1**–**4** $d(z)$ along the main axis z of the molecules calculated by MolShape from B3LYP/6-31G(d)//B3LYP/6-31G(d) calculations with Gaussian03.[18] Origin of z at the center of inversion of the guest.

z coordinate. The waist is clearly visible, the minimal diameter $\breve{d}_{eq}$=4.4 Å in the middle (z=0) is nearly identical to the inner width of α-CD. The two small side minima that are lower than $d_{eq}(0)$ were not taken into account, as they are too narrow to be recognized by the α-CD constriction. Three further bolaamphiphiles **1**, **3**, and **4** with nearly the same lengths were considered for the exclusive investigation of the influence of the minimal diameter $\breve{d}_{eq}$ on the binding constant K_S. The calculated profiles of $d_{eq}(z)$ are given in Figure 3, and the minimal diameters $\breve{d}_{eq}$ are listed in Table 3. The value of $\breve{d}_{eq}$ for the tolane derivative **1** was smaller, while the one for the bibenzyl derivative **3** was larger than the internal diameter $\breve{d}_{eq}$=4.4 Å of α-CD. The dibromo derivative **4** did not show any waist at all, it is an "overweight" guest, and should not be complexed at all (Scheme 2).

Table 3. Minimal cross-sectional areas and thicknesses of bolaamphiphilic guests **1**–**4** as calculated by Gaussian03/MolShape. Origin of z at the center of inversion of the guest.

Guest	$\breve{A}$ [Å^2]	$\breve{d}_{eq}$ [Å]
1	10.2	3.6
2	15.2	4.4
3	18.9	4.9
4	29.2	6.1



Scheme 2. Schematic drawings of bolaamphiphilic guests, dark gray: hydrophilic, light gray: hydrophobic, z: main axis of the molecule.

Synthesis of bolaamphiphilic guest molecules 1–4: 4,4′-Bis(aminomethyl)tolane (**1**), (*E*)-4,4′-bis(aminomethyl)stilbene (**2**), and (*E*)-4,4′-bis(aminomethyl)-α,α′-dibromostilbene (**4**), were synthesized by Delépine reaction of urotropine with 4,4′-bis(bromomethyl)tolane,[41] (*E*)-4,4′-bis(bromomethyl)-stilbene,[42] and (*E*)-4,4′-bis(bromomethyl)-α,α′-dibromostilbene (**5**), respectively. 4,4′-Bis(aminomethyl)-bibenzyl (**3**) was obtained by catalytic hydrogenation of the stilbene derivative **2**.

Determination of the binding data by ITC: Bolaamphiphiles **1–4** were sufficiently water-soluble to allow isothermal microcalorimetric titration with α-CD (ITC). The titration curve was fitted by nonlinear regression, assuming a 1:1 stoichiometry of the inclusion compound. The binding constant K_S and the molar binding enthalpy $\Delta H°$ were obtained as fitting parameters, from which the binding free energy $\Delta G°$ and binding entropy $\Delta S°$ were derived (Table 4). In addition, these binding data are plotted in Figure 4 as the function of the thickness $\check{d}_{eq}$ of the guest. The tremendous influence of $\check{d}_{eq}$ on the stability of the α-CD inclusion compounds becomes evident. The tolane derivative **1** with a somewhat loose fit showed surprisingly the highest binding free energy while the dibromo compound **4** was not included at all. This finding shows that some mobility of the guest inside the CD cavity is essential for reaching a high binding constant. Otherwise, the loss of entropy becomes too unfavorable, as demonstrated by the data of the bibenzyl derivative **3**. A similar influence of the mobility of the guest on the binding free energy $\Delta G°$ was already found for the inclusion of naphthalene derivatives in α-, β- and γ-CD by Schneider et al. 15 years ago.[43] On the other hand, for the inclusion of alicyclic, alibicyclic and alitricyclic amphiphilic guests in β-CD no upper limit for the size of the guest was found, but the binding free energy increased linearly with the size of the guest as well.[44] The steric constraints for normal amphiphilic guests might be less pronounced than for bolaamphiphilic ones. A normal amphiphile can still move along the C_n axis to avoid steric hindrance, while the location of a bolaamphiphile is locked in the CD because of both hydrophilic head groups persisting to stay out of the cavity. Therefore bolaamphiphiles are ideal guests for studying thickness recognition in detail.

Table 4. Thermodynamic data of the inclusion of bolaamphiphilic guests **1–4** in α-CD in 50 mM phosphate buffer pH 3, measured by isothermal titration microcalorimetry.

Guest	K_S [M^{-1}]	$-\Delta G°$ [kJ mol^{-1}]	$-\Delta H°$ [kJ mol^{-1}]	$-\Delta S°$ [J mol^{-1} K^{-1}]
1	8880 ± 329	22.54 ± 0.38	25.87 ± 0.09	11.2 ± 1.3
2	708 ± 7	16.28 ± 0.10	21.85 ± 0.03	18.7 ± 0.3
3	59 ± 2	10.13 ± 0.29	19.31 ± 0.50	30.8 ± 1.9
4	no binding	–	–	–

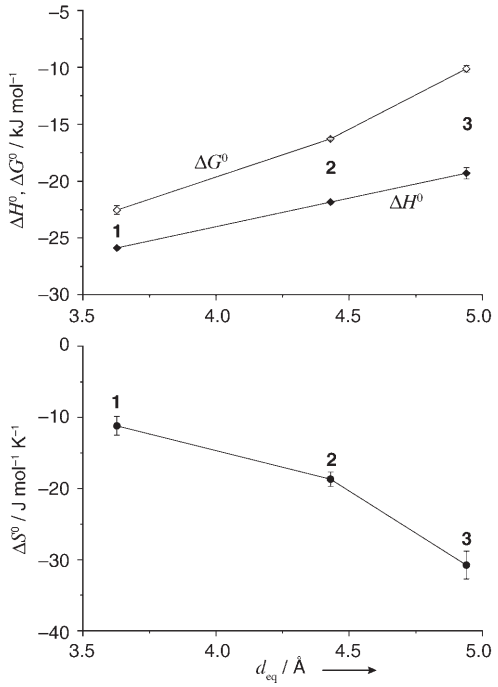


Figure 4. ITC binding enthalpies $\Delta H°$ and binding free energies $\Delta G°$ (top), and binding entropies $\Delta S°$ (bottom) as the function of the thickness $\check{d}_{eq}$ of the guest.

Conclusion

Cross-sectional diameters of host and guests, derived from electron density maps by the program MolShape, are strongly correlated with the stability of host guest complexes. For entropic reasons bolaamphiphilic guests should be about 0.8 Å thinner than the inner width of the CD host to reach high binding constants.

Experimental Section

Measurements: NMR spectra were recorded on a Bruker Avance 500 spectrometer (^{1}H: 500.00 MHz, ^{13}C: 125.71 MHz). The following internal standards were used for ^{1}H NMR: for D_2O HOD 4.75 ppm, for $CDCl_3$ $CHCl_3$ 7.25 ppm; for ^{13}C NMR: for D_2O CH_3CN 1.30 ppm, for $CDCl_3$ $CDCl_3$ 77.0 ppm. The following abbreviations were used: s singlet, d doublet, t triplet, m multiplet, br broad signal.

Mass spectra were recorded with an ESI single quadrupole mass spectrometer, micromass ZQ 4000 (Waters) from solutions in methanol. The following standard setting was used: capillary voltage 3.80 kV, cone voltage 20 V, extractor voltage 5 V.

The microcalorimetric titrations were performed at $T = 25.0$ °C with an AutoITC isothermal titration calorimeter (MicroCal, Northampton, USA) using 1.4144 mL sample and reference cells. The reference cell was filled with distilled water. The sample cell was filled with a 5 mM solution of the respective guest in 50 mM phosphate buffer pH 3.00 and constantly stirred with 450 rpm. The 71 mM solution of α-CD in the same buffer was added automatically by a syringe within 25 portions of 10 μL. The resulting 25 heat signals were integrated to yield the mixing heats, which were corrected by the corresponding dilution enthalpies of α-CD. The resulting differential inclusion heats were plotted versus the molar ratio of α-CD and guest and fitted by nonlinear regression using the program Origin for ITC 7.0 as shown in Figure 5.

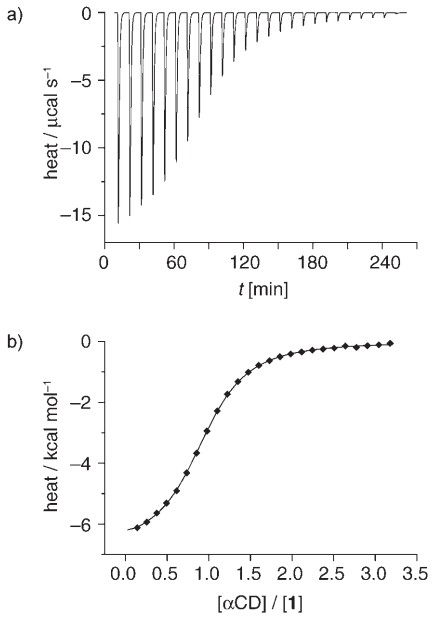


Figure 5. Differential inclusion heats of 5.00 mM tolane derivative **1** with 70.72 mM α-CD (in 0.050 M phosphate buffer pH 3.00) plotted versus the molar ratio of α-CD and guest and fitted by nonlinear regression using the program Origin for ITC 7.0.

Calculations: The quantum mechanical calculations were performed with the program Gaussian03.[18] The resulting electron density function was transformed into a discret cubic electron density $\rho(x,y,z)$ by the program Cubegen (from Gaussian). The cross-sectional area $A(z)$ was calculated from $\rho(x,y,z)$ by the program MolShape.[19]

Synthesis: ***4,4′-Bis(aminomethyl)tolane dihydrochloride (1)***: 4,4′-Bis-(bromomethyl)-tolane (0.112 g, 308 μmol, synthesized according to Houk et al.[41]) was added to a solution of urotropine (0.098 g, 699 μmol) in $CHCl_3$ (3 mL) stirred and heated under reflux for 1 h. The resulting precipitate was separated by filtration, washed with $CHCl_3$ and suspended in methanol (5 mL). After addition of HCl (6 M, 1 mL) the mixture was heated under reflux for further 1.5 h. The white precipitate was filtrated off, washed with methanol and acetone and dried in vacuo (59 mg, 191 μmol, 62 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, TMS): $\delta = 4.04$ (s, 4H; CH_2), 7.56, 7.59 (2 × d, ^3J(H,H) = 8.2 Hz, 8H; phenyl), 8.58 ppm (br, 6H; NH_3); ^{13}C{^{1}H} NMR (125.71 MHz, [D$_6$]DMSO, TMS): $\delta = 41.77$ (s, CH_2), 89.39 (s, C), 122.05, 129.31, 131.44, 134.79 ppm (all s, phenyl); IR (ATR): $\tilde{\nu} = 832$ (C-H, phenyl), 855, 876, 975, 1066 (CH_2-NH_2), 1107, 1212 (CH_2-NH_2), 1380, 1417, 1456, 1517 (NH_3^+), 1570 (NH_3^+), 1917, 2871 (N-H), 3097 (N-H), 3313 cm^{-1} (N-H); MS (3.80 kV, ESI, methanol): m/z (%): 220.17 (100) $[M-HCl-Cl-NH_3]^+$, 237.17 (30) $[M-HCl-Cl]^+$; elemental analysis calcd (%) for $C_{16}H_{16}N_2 \cdot 2HCl$ (309.23): C 62.14, H 5.87, N 9.06; found: C 61.97, H 5.68, N 9.21.

(E)-4,4′-Bis(aminomethyl)stilbene dihydrochloride (2): (*E*)-4,4′-bis(bromomethyl)stilbene (1.00 g, 2.73 mmol, synthesized according to Drefahl et al.[42]), was added to a solution of urotropine (0.84 g, 5.99 mmol) in $CHCl_3$ (8 mL) and heated under reflux for 3 h. The precipitate separated by filtration, washed with $CHCl_3$ and suspended in methanol (50 mL). After addition of HCl (6 M, 3 mL) the mixture was heated under reflux for 16 h. The white precipitate was filtrated off, washed with diethyl ether and dried in vacuo (0.72 g, 2.32 mmol, 85 %). ^{1}H NMR (500 MHz, D_2O): $\delta = 4.16$ (s, 4H; CH_2), 7.23 (s, 2H; CH), 7.44 (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl), 7.63 ppm (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl); ^{13}C{^{1}H} NMR (125.71 MHz, D_2O): $\delta = 43.51$ (s, CH_2), 127.78 (s, CH=CH), 129.44, 129.82, 132.69, 138.43 ppm (all s, phenyl); IR (ATR): $\tilde{\nu} = 827$ (CH=CH), 844, 877, 945, 958, 1075 (CH_2-NH_2), 1117, 1212 (CH_2-NH_2), 1381, 1463, 1477 (NH_3^+), 1515 (NH_3^+), 2878 (N-H), 2954 (N-H), 3425 cm^{-1} (N-H); MS (3.80 kV, ESI, methanol): m/z (%): 222.00 (100) $[M-NH_3-HCl-Cl]^+$; UV/vis (50 mM phosphate buffer pH 3.0): $\lambda_{max} = 312$ nm, $\varepsilon = (29\,930 \pm 43)$ cm^2 mmol^{-1}; elemental analysis calcd (%) for $C_{16}H_{18}N_2 \cdot 2HCl$ (311.25): C 61.74, H 6.38, N 9.00; found: C 61.67, H 6.33, N 9.22.

4,4′-Bis(aminomethyl)bibenzyl dihydrochloride (3): A solution of **2** (210 mg, 675 μmol) in water (40 mL) was filled in a stainless steel autoclave together with 10 % Pd on charcoal (70 mg). The suspension was exposed to a hydrogen pressure of 5 bar and stirred for 28 h at 25 °C. After lyophilization of the filtrate the product was obtained as a white powder (193.2 mg, 617 μmol, 91 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, TMS): $\delta =$ 2.86 (s, 4H; CH_2), 3.90 (s, 4H; CH_2), 7.23 (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl), 7.37 ppm (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl); ^{13}C{^{1}H} NMR (125.71 MHz, [D$_6$]DMSO, TMS): $\delta = 36.34$ (s, CH_2), 42.07 (s, CH_2), 128.45, 128.77, 132.28, 141.43 ppm (all s, phenyl); IR (ATR): $\tilde{\nu} = 829$ (C-H), 876, 967 (C-H), 1069, 1217 (CH_2-NH_2), 1380, 1403, 1465 (N-H), 1481, 1514 (NH_3^+), 1592 (NH_3^+), 2886 (N-H), 2970 (N-H); MS (3.80 kV, ESI, methanol): m/z (%): 112.59 (100) $[M-NH_3-2Cl]^{2+}$, 241.29 (32) $[M-HCl-Cl]$, 121.08 (12) $[M-2Cl]^{2+}$; elemental analysis calcd (%) for $C_{16}H_{20}N_2 \cdot 2HCl$ (313.3): C 61.34, H 7.08, N 8.94; found: C 61.17, H 6.91, N 9.10.

(E)-4,4′-Bis(aminomethyl)-α,α′-dibromostilbene dihydrochloride (4): A mixture of 4,4′-dimethyltolane (1.15 g, 5.57 mmol, synthesized according to Drefahl et al.[45]), *N*-bromosuccinimide (4.07 g, 22.9 mmol), and AIBN (16 mg, 100 μmol) in CCl_4 (30 mL, distilled and dried over sieves) was heated under reflux for 4 d under N_2. Additional portions of AIBN (3 × 8 mg, 50 μmol) were added daily. The reaction mixture was cooled to 25 °C and the precipitate was filtrated off and washed with CCl_4 (60 mL). The combined filtrates were washed with water (100 mL). The aqueous phase was separated and extracted with $CHCl_3$ (40 mL). The combined filtrates together with the $CHCl_3$ phase were tried over $MgSO_4$ and con-

centrated in vacuo. The product was obtained after recrystallization of the residue from petroleum ether (10 mL, b.p. 100–140 °C) as a white solid (0.23 g, 0.44 mmol, 8%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 4.51 (s, 4H; CH), 7.44 (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl), 7.49 (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl). The crude product (82 mg, 157 μmol) was dissolved in $CHCl_3$ (4 mL) and heated under reflux with urotropine (95 mg, 678 μmol) for 3.5 h. The reaction mixture was cooled to 25 °C and the precipitate was filtrated off and washed with $CHCl_3$ (10 mL). The colorless residue was suspended in a mixture of methanol (6 mL) and HCl (6 M, 1.5 mL) and heated under reflux for 3.5 h. The colorless solid precipitate was isolated by filtration, and washed with methanol (2 mL) and acetone (1 mL) and dried in vacuo (26 mg, 55 μmol, 35%). ^{1}H NMR (500 MHz, D_2O, HOD): δ = 4.24 (s, 4H; CH_2), 7.55 (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl), 7.67 ppm (d, ^3J(H,H) = 8.2 Hz, 4H; phenyl); ^{13}C{^{1}H} NMR (125.71 MHz, D_2O): δ = 42.73 (s, CH_2), 117.47 (s, =CBr), 129.12, 129.71, 133.67, 141.28 ppm (all s, phenyl); IR (ATR): $\tilde{\nu}$ = 832 (C-H, phenyl), 970 (C-H), 1215 (CH_2-NH_2), 1391, 1469 (N-H), 1509 (NH_3^+), 1604 (NH_3^+), 2618, 2901 (N-H), 2971 (N-H); MS (3.80 kV, ESI, methanol): *m*/*z* (%): 190.54/191.51 (100) $[M-2\,Cl-NH_3]^{2+}$, 378.10/380.09/382.09 (30) $[M-HCl-Cl-NH_3]^+$, 395.07/397.13/399.12 (18) $[M-HCl-Cl]^+$, 198.13/199.03/200.12 (13) $[M-2Cl]^{2+}$; elemental analysis calcd (%) for $C_{16}H_{16}Br_2N_2\cdot 2\,HCl$ (469.04): C 40.97, H 3.87, N 5.97; found: C 40.11, H 3.67, N 6.16.

[1] W. Saenger, *Angew. Chem.* **1980**, *92*, 343–361; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344–362.
[2] G. Wenz, *Angew. Chem.* **1994**, *106*, 851–870; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 803–822.
[3] J. Szejtli, *Chem. Rev.* **1998**, *98*, 1743–1753.
[4] K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* **1998**, *98*, 2045–2076.
[5] G. Wenz, *Clin. Drug Invest.* **2000**, *19*, *Suppl. 2*, 21–25.
[6] K. M. Huh, T. Ooya, W. K. Lee, S. Sasaki, I. C. Kwon, S. Y. Jeong, N. Yui, *Macromolecules* **2001**, *34*, 8657–8662.
[7] J. Li, X. Li, Z. H. Zhou, X. P. Ni, K. W. Leong, *Macromolecules* **2001**, *34*, 7236–7237.
[8] M. Weickenmeier, G. Wenz, J. Huff, *Macromol. Rapid Commun.* **1997**, *18*, 1117–1123.
[9] F. M. Raymo, J. F. Stoddart, *Chem. Rev.* **1999**, *99*, 1643–1663.
[10] G. Wenz, B.-H. Han, A. Müller, *Chem. Rev.* **2006**, *106*, 782–817.
[11] L. Liu, Q.-X. Guo, *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *42*, 1–14.
[12] K. A. Connors, *Chem. Rev.* **1997**, *97*, 1325–1357.
[13] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875–1917.
[14] T. Höfler, G. Wenz, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *25*, 81–84.
[15] F. Hacket, J. M. Coteron, H. J. Schneider, V. P. Kazachenko, *Can. J. Chem.* **1997**, *75*, 52–55.
[16] W. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S. M. Smith, T. Takaha, *Chem. Rev.* **1998**, *98*, 1787–1802.
[17] F. W. Lichtenthaler, S. Immel, *Tetrahedron: Asymmetry* **1994**, *5*, 2045–2060.
[18] Gaussian 03 (Revision B.03), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian Inc., Pittsburgh, PA (USA), **2003**.
[19] A. Müller, G. Wenz, written as a Perl script. The script and a windows GUI-version plus manual can be downloaded from http://www.uni-saarland.de/fak8/wenz/molshape, Saarbrücken (Germany), **2006**.
[20] R. M. Fuoss, D. Edelson, *J. Am. Chem. Soc.* **1951**, *73*, 269–273.
[21] J. H. Fuhrhop, C. Boettcher, D. Spiroski, *J. Am. Chem. Soc.* **1993**, *115*, 1600–1601.
[22] W. Herrmann, S. Wehrle, G. Wenz, *Chem. Commun.* **1997**, 1709–1710.
[23] W. Herrmann, B. Keller, G. Wenz, *Macromolecules* **1997**, *30*, 4966–4972.
[24] L. Avram, Y. Cohen, *J. Org. Chem.* **2002**, *67*, 2639–2644.
[25] K. Yannakopoulou, I. M. Mavridis, *Curr. Org. Chem.* **2004**, *8*, 25–34.
[26] R. Puliti, C. A. Mattia, L. Paduano, *Carbohydr. Res.* **1998**, *310*, 1–8.
[27] K. Lindner, W. Saenger, *Acta Crystallogr. Sect. B* **1982**, *38*, 203–210.
[28] K. Lindner, W. Saenger, *Carbohydr. Res.* **1982**, *99*, 103–115.
[29] I. Nicolis, A. W. Coleman, P. Charpin, C. deRango, *Acta Crystallogr. Sect. B* **1996**, *52*, 122–130.
[30] J. P. Ding, T. Steiner, W. Saenger, *Acta Crystallogr. Sect. B* **1991**, *47*, 731–738.
[31] T. Steiner, W. Saenger, *Acta Crystallogr. Sect. B* **1998**, *54*, 450–455.
[32] R. F. W. Bader, W. H. Henneker, P. E. Cade, *J. Chem. Phys.* **1967**, *46*, 3341–3363.
[33] T. Kihara, K. Sakai, *Acta Crystallogr. A* **1978**, *34*, 326–329.
[34] J. Bentley, *J. Phys. Chem. A* **1998**, *102*, 6043–6051.
[35] Y.-J. Zheng, J. Kenneth M. Merz Jr., *J. Comp. Chem.* **1992**, *13*, 1151–1169.
[36] L. Liu, X.-S. Li, K.-S. Song, Q.-X. Guo, *J. Mol. Struct.* **2000**, *531*, 127–134.
[37] B. Hingerty, B. Klar, G. L. Hardgrove, C. Betzel, W. Saenger, *J. Biomol. Struct. Dyn.* **1984**, *2*, 249–260.
[38] J. E. H. Koehler, W. Saenger, W. F. v. Gunsteren, *J. Mol. Biol.* **1988**, *203*, 241–250.
[39] H. Onagi, B. Carrozzini, G. L. Cascarano, C. J. Easton, A. J. Edwards, S. F. Lincoln, A. D. Rae, *Chem. Eur. J.* **2003**, *9*, 5971–5977.
[40] J. Terao, A. Tang, J. J. Michels, A. Krivokapic, H. L. Anderson, *Chem. Commun.* **2004**, 56–57.
[41] K. N. Houk, S. Menzer, S. P. Newton, F. M. Raymo, J. F. Stoddart, D. J. Williams, *J. Am. Chem. Soc.* **1999**, *121*, 1479–1487.
[42] G. Drefahl, G. Plötner, *Chem. Ber.* **1958**, *91*, 1274–1277.
[43] H. J. Schneider, T. Blatter, S. Simova, *J. Am. Chem. Soc.* **1991**, *113*, 1996–2000.
[44] M. R. Eftink, M. L. Andy, K. Bystrom, H. D. Perlmutter, D. S. Kristol, *J. Am. Chem. Soc.* **1989**, *111*, 6765–6772.
[45] G. Drefahl, G. Plötner, *Chem. Ber.* **1958**, *91*, 1280–1285.

Received: May 31, 2006
Published online: December 13, 2006

