NMR Detection of Simultaneous Formation of [2]- and [3]Pseudorotaxanes in Aqueous Solution between α-Cyclodextrin and Linear Aliphatic α,ω-Amino acids, an α,ω-Diamine and an α,ω-Diacid of Similar Length, and Comparison with the Solid-State Structures

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The interactions of 11-aminoundecanoic acid (**1**), 12-aminododecanoic acid (**2**), 1,12-diaminododecane (**3**), and 1,13-tridecanoic diacid (**4**) with α-cyclodextrin (αCD) were studied in aqueous solution by NMR spectroscopy. The association modes were established with titration and continuous variation plots, variable temperature NMR spectra, and dipolar interactions as recorded in 2D ROESY spectra. The studies were carried out at pH 7.3 and 13.6. These long, linear bifunctional molecules were found to form simultaneously [2]- and [3]pseudorotaxanes with αCD in the aqueous solution. At the higher pH the 1:1 adducts were present at concentrations higher than at the neutral pH. The longer guests formed complexes enriched in the 2:1 constituent at both pH values. There were clear indications that the [2]pseudorotaxanes are present in two isomeric forms. The presence of isomers also in the [3]pseudorotaxanes was not ruled out. Various exchange rate regimes were observed; clearly in neutral solutions the formation of the 1:1 complexes was fast in the NMR time scale, whereas the threading of a second αCD ring was a slower process. In the solid state, the adduct of αCD/**2** had the structure of a [3]pseudorotaxane, in accordance with previously solved crystal structures of αCD/**3** and *β*CD/**4**. The species in solution, in contrast with those present in the solid state, are therefore of varying nature, and thus the frequently and conveniently assumed 1:1 stoichiometry in similar systems is an oversimplification of the real situation.

The introduction of linear molecules inside cyclodextrins (CDs) has been the subject of investigation in several laboratories.[1−5] Part of the interest arises from the foreseeable possibility of inducing the arrangement of the macrocyclic molecules into larger arrays, by either covalently bonding the guest molecules[1] or by utilizing their known modes of association to achieve further organization into higher order supramolecular systems[2] that could be termed as pseudopolyrotaxanes.[3] Guest molecules with end-functional groups capable of either reacting or associating are therefore desired. Linear long-chain molecules bearing terminal amino or carboxylic functionalities have been popular inserts of mostly αCD,[4] to form the corresponding pseudorotaxanes,[3] whereupon suitable stoppering has afforded various cyclodextrin [2]-rotaxanes, molecules assembled like a wheel and its axle.[2a,b,5] Although solid-state studies have been carried out regarding polycondensation,[1b,c] rotaxane isolation,[5] and crystal structure characterization,[2c] little attention has been paid to the true nature of the species in aqueous solution prior to crystallization, precipitation, or reaction to rotaxane products. In this work we investigate in detail by NMR spectroscopy the interaction of bifunctional aliphatic molecules, 11-aminoundecanoic acid (**1**), 12-aminododecanoic acid (**2**), 1,12-diaminododecane (**3**), and 1,13-tridecanoic diacid (**4**), with αCD (Scheme 1) in aqueous solution. These are all bifunctional molecules, bearing end groups capable of hydrogen bonding, and of length capable of threading in sequence two cyclodextrin rings. Several reports have appeared in the literature. The interaction of **1** with αCD has been studied by microcalorimetry;[6] however, structural information could not be easily extracted from thermodynamic data alone and therefore 1:1 stoichiometry was assumed for the system. Polycondensation of αCD/**1** as the 2:1 complex to form a water-soluble pseudorotaxane polyamide[1b] was an intriguing result, but the work focused on the solid-state process, bypassing the behavior of the species in solution. The threading of diamine **3** into αCD has been a rather popular project.[5a,5c] The corresponding [2]rotaxane, bearing metalloorganic stoppers, was the first cy-

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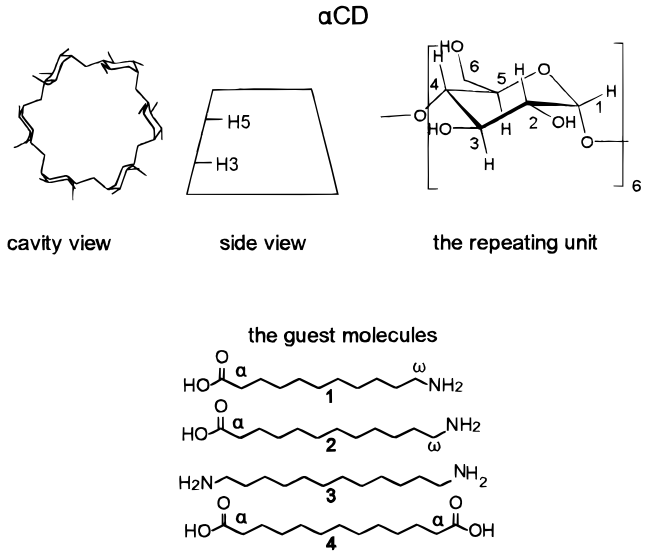
Scheme 1



clodextrin rotaxane to be isolated, and similar preparations with various stoppers have followed.[5a] The corresponding unstoppered adduct, however, crystallized as the [3]-pseudorotaxane, as revealed recently by its X-ray crystal structure.[7] Finally, NMR studies of the interactions of several α,ω-alkanediacarboxylate anions, including the disodium salt of **4**, with αCD[8] were based on the assumption that 1:1 host:guest adducts were formed. In the meantime, the crystal structure of the adduct β*C*D/**4** was solved in our laboratory and turned out also to be the [3]pseudorotaxane.[9] The general picture, therefore, regarding these linear molecules (15−19 Å long when fully extended) is that the [2]rotaxanes are finally produced after stoppering, although the [3]pseudorotaxanes preferentially crystallize from the aqueous solutions of the components. We, therefore, have undertaken a detailed examination of the threading mechanism for guest molecules **1**−**4** into αCD in aqueous solution, prompted by our interest to investigate the nature of the species formed in situ, deduce solution superstructures, and compare them with solid-state results. Further, the choice of two asymmetrically end-substituted guest molecules, **1** and **2**, and of two symmetrical ones, **3** and **4**, was intended to investigate the possibility of forming orientational isomers, previously reported only as distinct rotaxanes with αCD.[5d,e]

Results and Discussion

^{1}H NMR Studies at Neutral pH. Evidence for the inclusion of the guests into the αCD cavity was obtained by titration of a buffered D_2O solution of αCD with solid **1**−**4** at pH 7.3. Continuous shielding of the signal of the proton H3, located inside the αCD cavity (Scheme 1), upon increasing guest concentration was observed (Figure 1), a typical indication of guest inclusion into the αCD and fast exchange for the host between free and associated forms in the NMR time scale. Careful examination of the line shape of H3 indicated a slight departure from

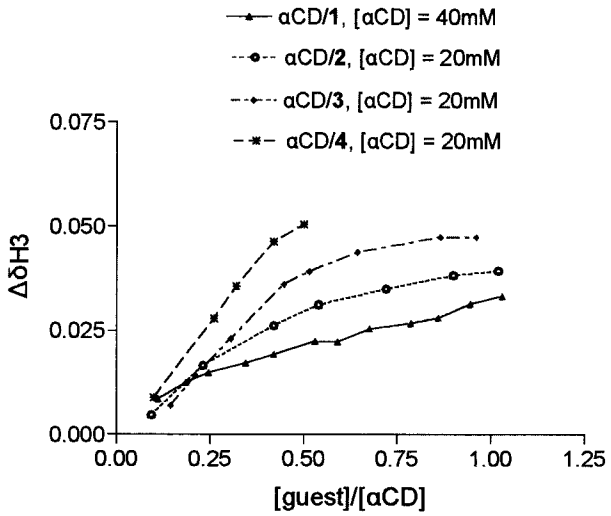


Figure 1. Mole ratio plots at pH 7.3.

this regime, although the corresponding chemical shift could be accurately measured at all times. The mole ratio plots[10] thus constructed (Figure 1) showed a slowly rising shallow curve for **1** that did not reach a clear plateau. The curve for αCD/**2** was steeper, and that for αCD/**3** was even more so, indicating association constants for these guests larger than for **1** and more closely resembling plots of 2:1 adducts.[10b] We note that **2** and **3** are molecules of nearly the same length (∼17 Å), whereas **1** is shorter (∼16 Å) and **4** is longer (∼18 Å).[11] The titrations ended when further addition of the guest resulted in a suspension, and despite heating and sonication more data points, well exceeding the 1:1 mole ratio, could not be collected. The corresponding plot of αCD/**4** (Figure 1), although incomplete for solubility reasons, has the steepest rise of all and a tendency to curve at a [αCD]:[**4**] ratio of 2:1, indicating even larger association constants. We have, therefore, seen that all plots are not typical for 1:1 adducts[10b] and suggest the presence of primarily the 2:1 adducts, especially for the longer guests.

During titration, the signals of the "external" αCD protons experienced both shielding (H1) and deshielding (H2, H4) effects, along with band shape alterations. The signals of **1** and **2** suffered severe broadening, evidently experiencing intermediate rates of exchange (Figure 2a, 298 K). The same was also observed for the signals of **3** up to a [host]:[guest] ratio of 2:1, becoming sharp thereafter. Finally, the signals of **4** were somewhat broadened (Figure 2b, 298 K), but in this case solubility was low and the titration ended at the 2:1 mole ratio. The above results suggest that dynamic phenomena take place at this pH, which arise from exchange of free and complexed guests, from exchange among complexes of different stoichiometries, or both.

^{13}C NMR Studies at Neutral pH. Examination of the room-temperature ^{13}C NMR spectra at several points during the titration at pH 7.3 showed a reduction of the intensity and expansion of the line width of the signals due to αCD C1, C3, and C6, as compared with those of

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(9) Makedonopoulou, S.; Tulinsky, A.; Mavridis, I. M. *Supramol. Chem.*, in press.
(10) (a) Connors, K. A. *Binding Constants. The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987. (b) Chriswell, C. D.; Schilt, A. A. *Anal. Chem.* **1975**, *47*, 1623−1629.
(11) The molecules were created with ChemDraw or Chem3D and minimized with the MM2 routine provided by CS Chem3D Pro (ChemOffice, CambridgeSoft).

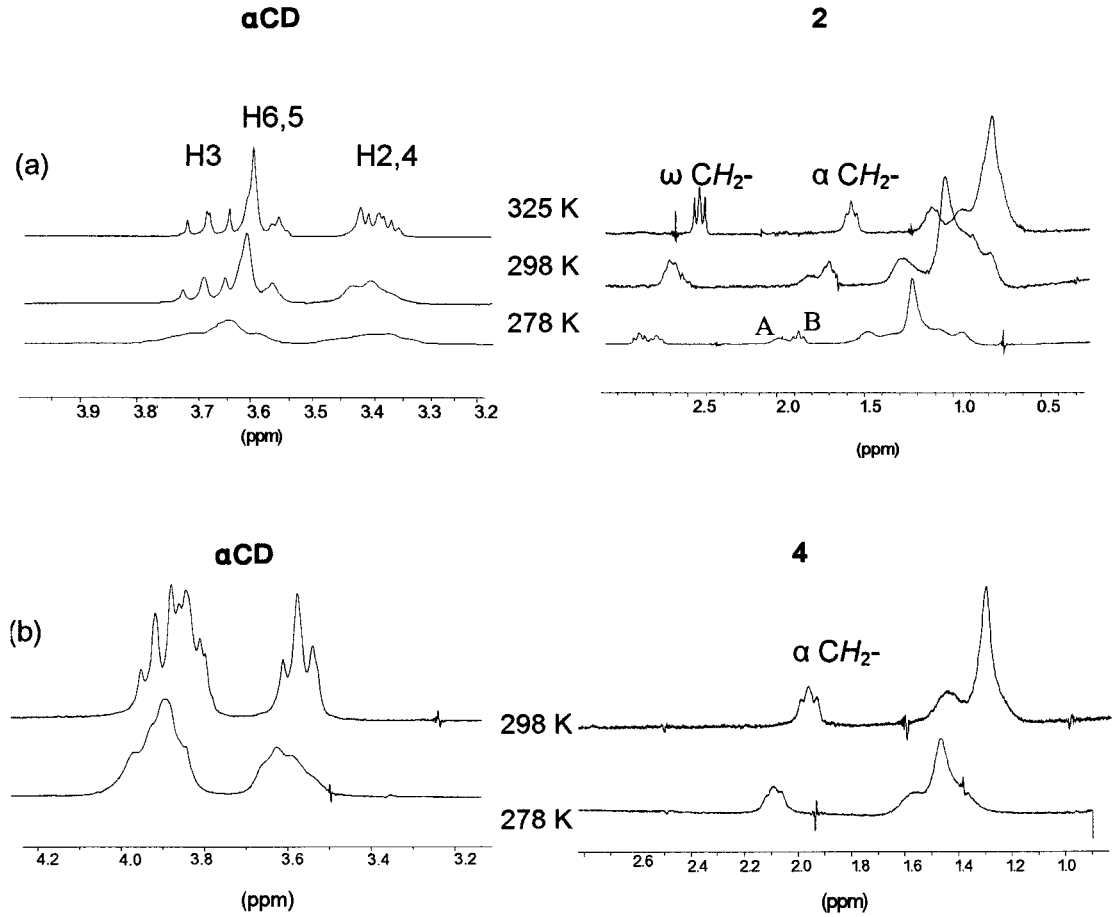


Figure 2. Representative variable-temperature ^{1}H NMR spectra (250 MHz, D_2O) at pH = 7.3: (a) αCD/**2**, [αCD] = 20 mM, (b) αCD/**4**, [αCD] = 20 mM. The anomeric proton H1 is not shown.

C2, C4, and C5, up to a [host]:[guest] ratio of 2:1 (Figure 3a,b, traces at 298 K). Broadening followed by severe reduction in intensity was similarly observed for the guests' signals. Introduction of larger relaxation delays (up to 2 s) before spectral acquisition did not alter the intensities of the affected peaks, and therefore unusually long relaxation times for C1 and C3 could be ruled out. All signals became sharp when the [host]:[guest] ratio nearly reached 1:1 (for example, Figure 3b, top trace at 298 K). Possible changes in macrocyclic conformation, usually invoked to account for such observations, would seem unlikely for the relatively rigid αCD structure and the "slim" guest molecules. Intermediate rates of exchange, on the other hand, among mixed pseudorotaxanes in the solution would be a more reasonable explanation for the observed line shapes. Furthermore, the dependence of the line shapes on the [host]:[guest] ratio could be rationalized by considering that when αCD is in excess, the association of the guest is nearly complete and a slow-to-intermediate exchange of the pseudorotaxanes is observed. When the concentration of the guest increases, the fast exchange between the free guest and free host dominates the line shapes. This situation was also encountered in the ^{1}H NMR spectra of αCD/**3**. Acquisition of both ^{1}H and ^{13}C NMR spectra at low temperatures was deemed to be necessary.

Variable-Temperature Studies. All ^{1}H NMR spectra were examined at 278 K in D_2O at pH 7.3 at [host]:[guest] ratios of 1:1, 2:1 and 4:1 (for **1**, **2**, and **4**) and 1:1, 4:1, and 8:1 (for **3**). At this temperature, signal broadening was observed for the signals of αCD, whereas those of the guests resolved into triplets (Figure 2). Specifically, the broad signals of **1**, **2**, and **3** at 2.3−1.8 ppm (298 K, $-CH_2-$COOH) and at 3.0−2.5 ppm (298 K, H_2N-CH_2-) separated into two apparent triplets at 278 K which coalesced on heating (Figure 2, top traces).[12] The spectrum of αCD/**2** was also examined at 400 MHz at 278 K (Figure 4a), whereupon apart from the two very well resolved triplets of H_2N-CH_2- (peaks A and B) another triplet at higher frequency appeared (peak C), assigned to the free amino acid **2** (vide infra) having the same chemical shift as that of pure **2** in a very dilute solution of D_2O. Similar observations were made for αCD/**3** (Figure 4b), whose α-methylene groups were resolved into two triplets (peaks A and B) at 278 K, but no extra peak was observed. Interestingly, the signals of αCD in the presence of **3**, somewhat split at 250 MHz, had clearly reverted to the slow exchange regime at 400 MHz and 278 K, and even the signal of H1 of αCD had been resolved into two doublets. Finally, the corresponding α-methylene peak of the diacid **4** became only broader with the temperature decrease and did not resolve at 278 K and 250 MHz (Figure 2b). We were, therefore, observ-

(12) $\Delta\nu$ = 46 Hz, T_c = 310 K for αCD/**1**; $\Delta\nu$ = 27 Hz and T_c = 312 K for αCD/**2**; and $\Delta\nu$ = 22.4 Hz, T_c = 297 K for αCD/**3**, where T_c is the approximate coalescence temperature. The corresponding values for the energies of activation are $\Delta G^{\ddagger}$ =15.3 kcal/mol, $\Delta G^{\ddagger}$ =15.0 kcal/mol, and $\Delta G^{\ddagger}$ = 15.1 kcal/mol, respectively, calculated using the approximate Eyring equation (Günther, H. *NMR Spectroscopy−An Introduction*; J. Wiley: New York, 1987; Chapter VIII) for the exchanging species.

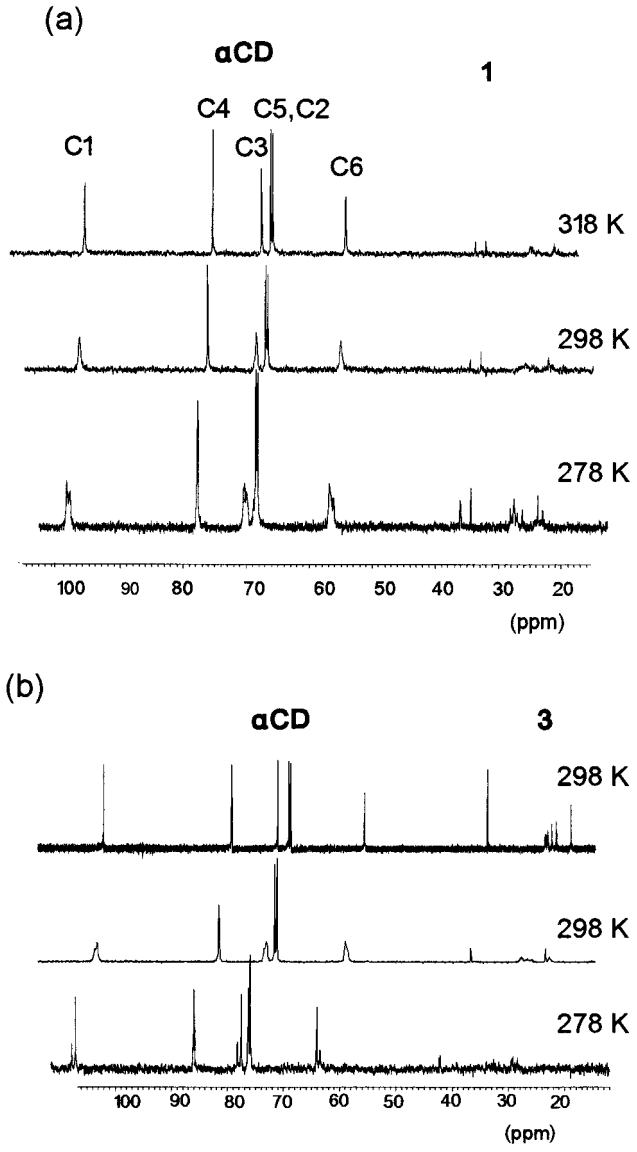


Figure 3. Variable-temperature ^{13}C NMR spectra at a [host]:[guest] ratio of 2:1: (a) αCD/**1**; (b) αCD/**3**; the upper trace is at a [host]:[guest] ratio of 1:1.

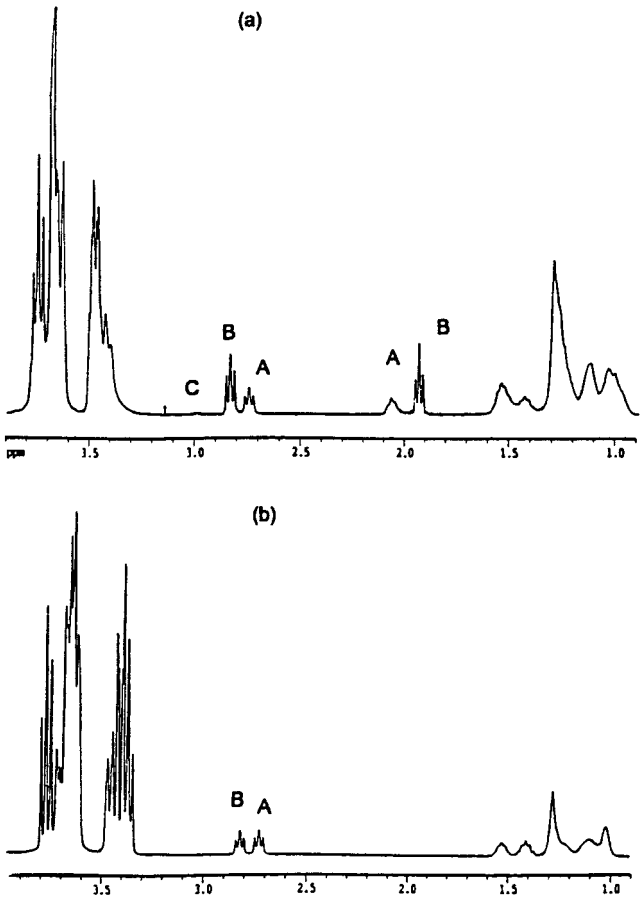


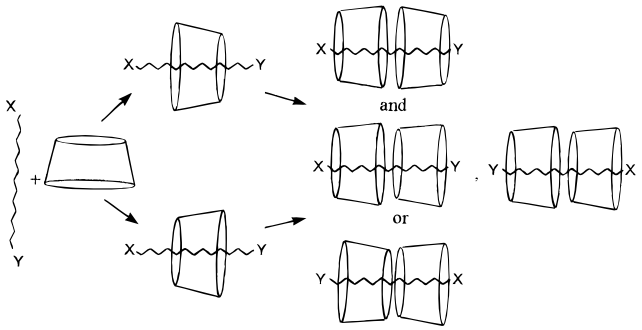
Figure 4. Partial ^1H NMR spectra at 400 MHz, 278 K, pH 7.3 of (a) αCD/**2**; (b) αCD/**3**.

ing at least two species in solution for all systems under consideration, which as suggested by the mole ratio plots could be the 1:1 and 2:1 pseudorotaxanes. Integration of peaks A and B (278 K, 250 MHz) showed that the ratio [A]/[B] increases upon increasing the [host]:[guest] ratio (1:1 to 4:1), suggesting that peak A is the 2:1 species and peak B is the 1:1 species. Moreover, peak A was more abundant in the longer amino acid **2**, further strengthening this assignment. However, because the α-methylene signal of free **1** or **2** (in the *absence* of αCD in D_2O at 298 K) appears at a higher frequency as compared with the peaks in question and was observed only in the spectrum of αCD/**2** at 400 MHz and 278 K (Figure 4a, peak C), the presence of the free guest under one of peaks A or B seemed unlikely. The α-methylene signals of either **3** or **4** appear at the same frequency either free or in the presence of αCD, so that the existence of unthreaded guests under the broad peaks in these complexes could not be ruled out. Surprisingly, the relative abundance of peaks A and B in αCD/**3** was not measurably altered at different [host]:[guest] ratios (8- and 4-fold excess of αCD). Finally, the appearance of the strongly coupled protons of the aliphatic chain around 1.3 ppm also varied with the temperature and the [host]:[guest] ratio variations (Figures 2 and 4). Introduction of larger relaxation delays (up to 2 s) did not result in any alteration of the line shapes, and a rise in temperature led to coalescence of these methylene signals, too (Figure 2). The above indicate that the broad peaks arising from the backbone of the guests are due to exchange phenomena rather than to a change in the relaxation rates or to a dispersion of the chemical shifts arising from inclusion of the linear guests into the asymmetric αCD cavity.[1a]

Simultaneously, the broad ^{13}C NMR peaks of αCD (C1, C3, C6) at 298 K mentioned previously, split into two signals at 278 K, and typical spectra are shown in Figure 3 (bottom traces). The broad peaks of the guest became even broader at 278 K. These experiments therefore suggested that we presumably observe the 2:1 adducts in exchange with the 1:1 adducts and that the threading of the second αCD ring to form [3]pseudorotaxanes is an intermediate-to-slow rate process in the NMR time scale at ambient temperature, as already deduced from the ^1H NMR data. A further complication is that each of the asymmetric guests **1** and **2** could be threaded into the first αCD ring in two modes (Scheme 2), a possibility that has not been ruled out so far.

^1H NMR Studies at Basic pH. The limited aqueous solubility at neutral pH of the systems under examination prevented construction of continuous variation (Job) plots[10a] necessary to resolve the question of stoichiometry in solution. At pH 13.6 guests **1**, **2**, and **4** were soluble

Scheme 2



but diamine **3** was not. At this pH all dissolved guests and the αCD should be largely ionized.[13] Direct comparison with results obtained at pH 7.3 would, therefore, not be wise, but the qualitative features of the spectra could provide more information about the systems. Continuous variation curves were obtained by monitoring the change of chemical shift of H3 of the host. The plots obtained (Figure 5a1−5c1) do not reach a maximum at 0.5 (exclusively 1:1 adducts) or 0.66 (exclusively 2:1 adducts)[10a] on the abscissa axis. Simultaneously, the curves due to the α-CH_2 signals of **1** and **2** (Figure 5a2−5c2) were not in agreement (break at mole fraction 0.5) with their counterparts, indicating that competing processes occur rather than a single equilibrium,[14] as we also observed at pH 7.3. Further, because the deflection points were close to the theoretically anticipated value of 2:1 (Figures 5a−c), one might infer that there is more [3]pseudorotaxane present in αCD/**2** and αCD/**4** than in αCD/**1**, as was observed at neutral pH. Therefore, we have now verified that at pH 13.6 there exist both binary and ternary complexes in solution.

The observed rates of exchange were modified at the alkaline pH; the signals of the methylene peaks in the solutions of both αCD/**1** and αCD/**2** had become sharp at ambient temperature, indicating fast exchange between the various species (Figure 6a). The exchange rate, however, had been retarded for αCD/**4**, as previously observed for the corresponding dicarboxylate anions[8] at this basic pH (Figure 6b). Separate triplet peaks due to the α-methylene emerged as the mixing of the solutions proceeded (see the Experimental Section) resulting in two triplets and a broad peak, labeled as A, B, and C at an [αCD]:[**4**] ratio of about 1:1. There was no continuous variation in the frequency of α-CH_2 (peak A, concentration-independent chemical shift), but there was a variation in its intensity. Plotting the area of peak A as the fraction of the total area of peaks A, B, and C yielded a curve with a deflection point at a mole fraction of 0.5 (Figure 5c2), suggesting that peak A is due to the 1:1 adduct. It follows that peaks B and C would most likely arise from the 2:1 adduct, as well as the free diacid. We have therefore verified the existence of both [2]- and [3]-pseudorotaxanes in the basic solution for αCD/**4**.

Mole ratio plots were also constructed at pH 13.6 and are shown in Figure 7. We observed chemical shift variations for all protons of the host macrocycle, including the outer protons. This could be a result of the ionization of αCD and the breaking of the intramolecular H-bonds of the secondary hydroxyl groups, thus allowing the cyclic oligosaccharide to gain flexibility. The plots were obtained by recording the chemical shift difference of the cavity proton H3 from the "outer" proton H1. In addition, the signals were well-defined, indicating fast exchange, which was further verified by observing concentration-dependent host *and* guest chemical shifts. The manifestation of mixed stoichiometries of complexed species owing to competing equilibrium processes was more striking in these diagrams, because the unusually curved plots (presence of a maximum in the binding isotherms)[10a] for αCD/**1** and αCD/**2** suggest that there exist various 1:1 and 2:1 pseudorotaxanes in the solution, having intrinsic chemical shifts $\Delta\delta^{\circ}_{11}$ and $\Delta\delta^{\circ}_{12}$ such that $\Delta\delta^{\circ}_{11} \neq \Delta\delta^{\circ}_{12}$, the subscripts denoting the 1:1 and the 2:1 adducts, respecively. The shape of the curve for αCD/**1** suggested that the alkaline solution is enriched in the [2]pseudorotaxane, as compared to the neutral pH. Although the plot of αCD/**2** is similar, the smaller curvature may indicate that there is less [2]pseudorotaxane in αCD/**2** than in αCD/**1**. This result could probably be anticipated, since **2** is longer by one methylene unit. The adduct of diamine **3** was insoluble at this pH. However, αCD/**4** gave a curve (Figure 7) that rises as expected and, compared with the corresponding curve at neutral pH (Figure 2), shows a relative prevalence of the 1:1 adduct. The latter is most probably due to the stronger repulsions between the ionized carboxylic ends of **4** with the negatively charged hydroxyls of αCD in the basic solution. Nevertheless, the 2:1 adduct is present, as shown in Figure 6b. We therefore conclude that at the basic pH the solutions are uniformly enriched with the 1:1 adducts and the binding of the diacid **4** is stronger than any of the amino acids, as compared with the corresponding situation at the neutral pH.

Dipolar Interactions. ROESY spectra of αCD/**1** and of αCD/**2** at 298 K at both pH 7.3 and 13.5 revealed interactions between CD H3,5,6 and the entire amino acid chain, which were stronger with the primary side protons H5, H6,6′ (αCD/**1**, Figure 8), indicating inclusion. There were also exchange cross-peaks (in phase with the diagonal) between peaks A and B arising from the α-methylene and, less intensely, the *ω*-methylene groups at 298 K in αCD/**1** and αCD/**2**. Each of the mutually exchanging α-methylenes showed dipolar interactions with the protons of the αCD cavity. Signal B, attributed to the 1:1 adduct as shown above, showed dipolar interactions with both the secondary (H3) and the primary (H5,6,6′) sides of the host. This observation suggests that there are two isomeric [2]pseudorotaxanes as a result of the amino acid's insertion into the macrocyclic ring in two different orientations, with the carboxyl group exiting either the primary or the secondary side of the αCD ring (Scheme 2). To our knowledge, this is the first example of isomeric [2]pseudorotaxanes detected in solution, although isolation of related orientational isomeric [2]rotaxanes has been reported.[5d,e] Signal A, on the other hand, which is attributed to the 2:1 adduct, also had dipolar interactions with the protons on both sides of the hosts' cavity. The threading of the second αCD ring (Scheme 2) could be head to head, as it is usually observed in the solid-state structures (see below), to benefit from the dimerization of αCD through H-bonding in the secondary side. However, the presence of isomeric [3]pseudorotaxanes in tail to tail or even head to tail

(13) pK_a of αCD is 12.332 (Szejtli, J. *Chem. Rev.* **1998**, 1743−1753).
(14) Djedaïni, F.; Perly, B. *Nuclear Magnetic Resonance of Cyclodextrins, Derivatives, and Inclusion Compounds*. In *New trends in Cyclodextrins and Derivatives*; Duchêne, D., Ed.; Editions de Santé: Paris, France, 1991.

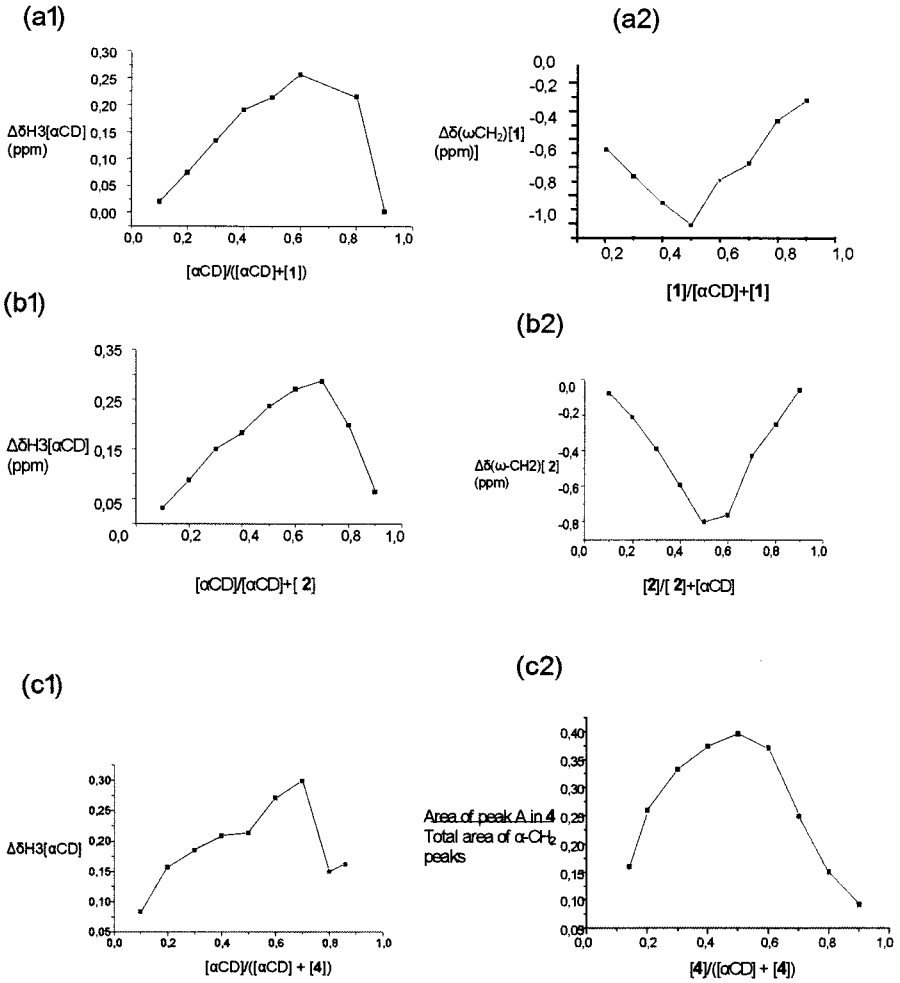


Figure 5. Continuous-variation plots at pH 13.6 for αCD/**1** (a1, a2), αCD/**2** (b1, b2), and αCD/**4** (c1, c2).

orientation cannot be ruled out[15] in the solution, because these structures would also yield the same dipolar interactions with the host. A recent report[16] on X-ray studies of hexa(ethylene glycol) threaded into αCD also involves a head to head and a tail to tail assembly of the macrocyclic rings to maintain a channel superstructure.

ROESY spectra of αCD/**2** at 278 K and at 400 MHz revealed similar correlations; however, the small triplet at 3.0 ppm (see Figure 4, peak C), clearly observed along the diagonal, did not give any correlation with the αCD protons. This observation allowed as to assign the triplet with certainty to the unthreaded amino acid **2**. ROESY spectra of the αCD/**3** (pH = 7.3, 400 MHz, 278 K) also gave a similar picture. There was no free diamine detectable, and both α-methylene triplets (Figure 4) showed dipolar interactions with the cyclodextrin protons. It therefore follows that one is due to the [2]- and the other to the [3]pseudorotaxane, but their exact correspondence with either one of the triplets is not known. In addition, the signals of the free diamine **3** could be overlapping with one of the triplets, because the corresponding resonance of the free diamine is located at their chemical shift range. Finally, the ROESY spectra of αCD/**4** were obtained only at pH 13.6 because at neutral pH resolution of the diacid's α-methylene signals was not possible. At the basic pH and at low temperature (Figure 9), we observed two clearly resolved triplets A and B and a broad peak C (see also Figure 6b, bottom trace) in the α-methylene region. There were dipolar interactions of both triplets with the α-cyclodextrin cavity, but there was also a very weak correlation due to the broad peak, which could arise from exchange transfer. Cross-peaks observed between αCD H2 and H4 and the guest are not genuine but due to dipolar transfer via their *J* coupling to H3 and H5, as previously observed in such systems.[17] The dipolar correlations therefore provide support to the previous results: a uniform picture has emerged, according to which all guests under consideration thread into αCD fast to form [2]pseudorotaxanes, in two modes, detectable only in the spectra of asymmetric molecules **1** and **2**. The threading of the second αCD ring is a slower process in the NMR time scale, and the formed [3]pseudorotaxanes are more abundant in the case of the longer molecules. At the basic pH, the equilibrium shifts toward the [2]pseudorotaxanes, evidently as a result of increased electrostatic repulsions between the fully or partly ionized guests and the hydroxy anions of the ionized host αCD. It is anticipated

(15) Examination of the αCD ^{1}H NMR spectra in H_2O (without deuterium lock) with and without the amino acid guests present revealed an alteration of its very broad hydroxyl peaks, indicating that hydrogen bonding of the host plays a role in the assembly of the pseudorotaxanes. More details, however, could not be extracted from these spectra.

(16) Harada, A.; Li, J.; Kamachi, M.; Kitagawa, Y.; Katsube, Y. *Carbohydr. Res.* **1998**, *305*, 127−129.

(17) Botsi, A.; Yannakopoulou, K.; Perly, B.; Hadjoudis, E. *J. Org. Chem.* **1995**, 60, 4017−4023.

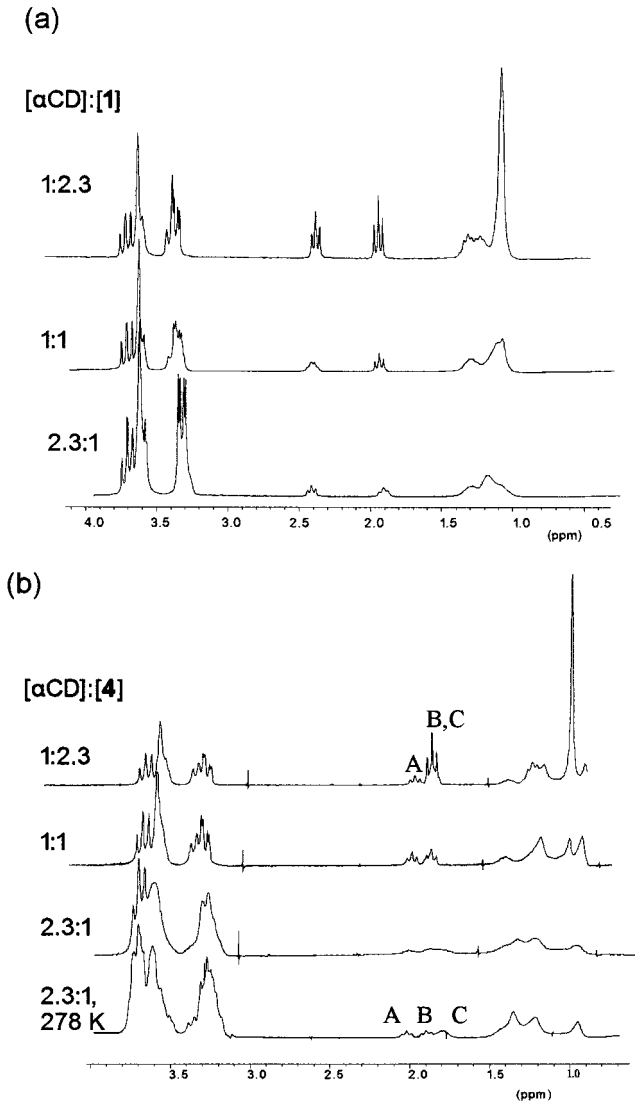


Figure 6. Partrial ^{1}H NMR spectra obtained during continuous variation of the [host]:[guest] ratio at pH = 13.6 and 298 K unless indicated otherwise: (a) αCD/**1** and (b) αCD/**4**.

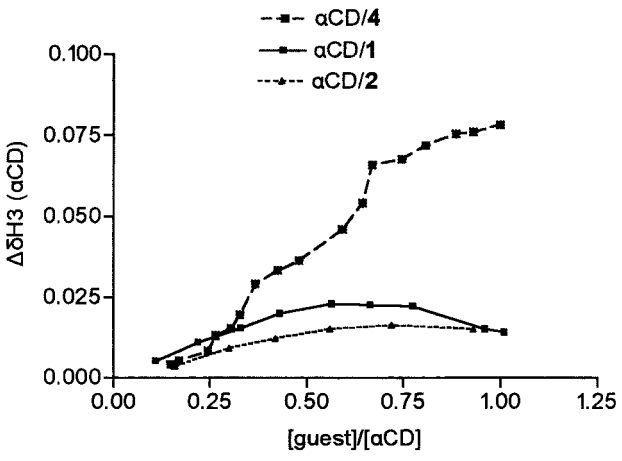


Figure 7. Mole ratio plots at pH 13.6.

that in the [2]pseudorotaxanes there must be some coiling of the aliphatic chain of the guests inside the cavity to reduce unfavorable interactions with the aqueous environment and maximize favorable contacts with the cavity interior. Figure 10 shows such a model, created[11] from

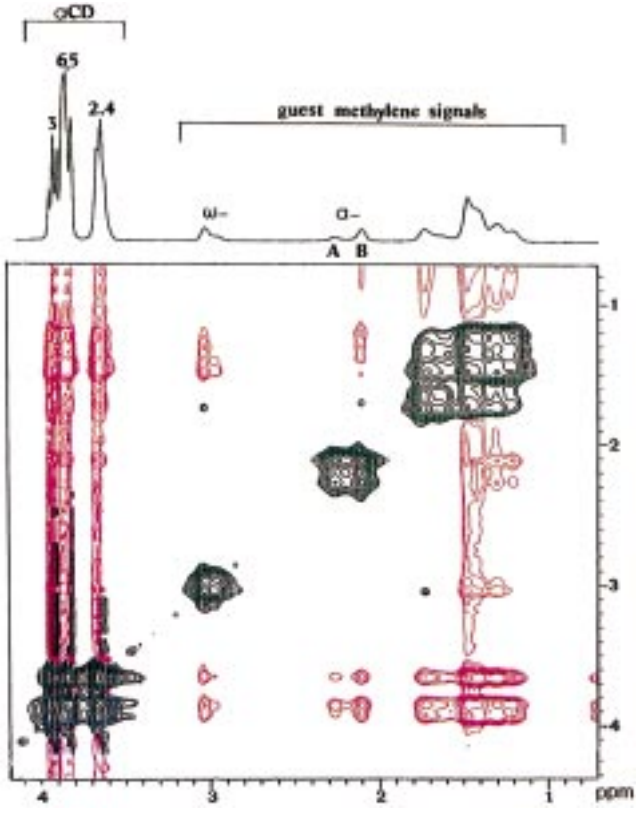


Figure 8. Partial ROESY correlations in αCD/**1** at 400 MHz, 298 K, pH 7.3.

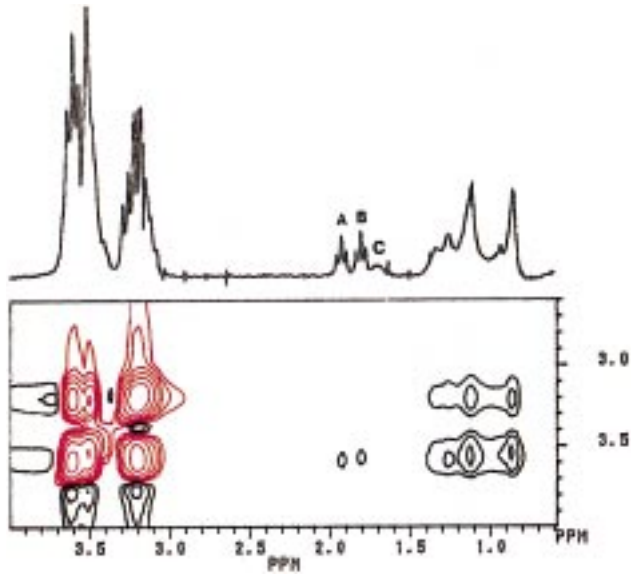


Figure 9. Partial ROESY correlations in αCD/**4** at 250 MHz, 278 K, pH 13.6.

the crystallographic coordinates of αCD, where the calculated van der Waals surfaces of the two components are illustrated. However, in [3]pseudorotaxanes the guests must be extended. The above rationalizations are consistent with the observation of stronger dipolar interactions of the [2]pseudorotaxanes with the cavity.

Another piece of information came from electrospray ionization MS of aqueous solutions of αCD/**2** in ratios 2:1 and 1:1. The base peak in both solutions was that of αCD (αCD + Na^+). Intense peaks (30% of the base peak) corresponding to the protonated 1:1 adduct (*m*/*e* 1188)

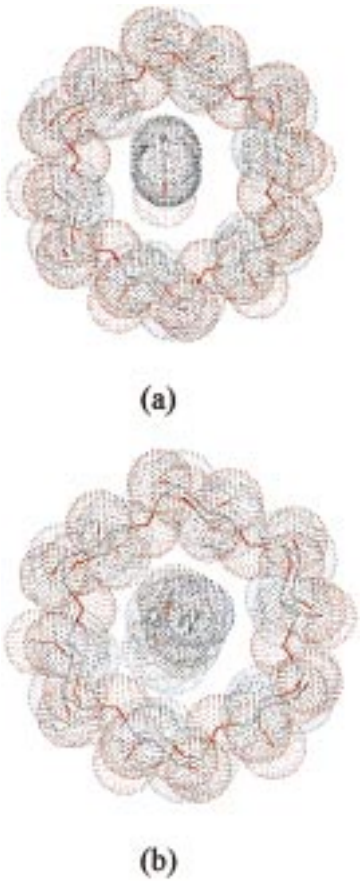


Figure 10. View of the αCD cavity with (a) extended **2** and (b) skewed **2**, as calculated[11] using the crystallographic coordinates of αCD.

were observed in both solutions, and weak peaks (4% of the base peak) of the 2:1 adduct (*m*/*e* 2159) were found, along with peaks corresponding to dimeric αCD (*m*/*e* 1968, 2αCD + Na^+, 33% of the base peak), and to higher adducts possibly formed in situ, as deduced from the extensive fragmentation observed above *m*/*e* 2160.

Equilibrium Constants. Calculation of the equilibrium constants from the NMR spectroscopic data (Figure 1) was not straightforward. Similar systems have been treated on the basis of the assumption that 1:1 adducts are present.[6,8] Wenz and co-workers treated αCD/**1** as a stepwise binding process of two αCD rings[1b] using microcalorimetric data. We have just documented the presence of 2:1[1b] and 1:1 adducts in all cases investigated. Furthermore, the presence of isomeric [2]- and possibly [3]pseudorotaxanes in the solution adds to the complication of the system. We treated the systems as fast exchanging with respect to αCD H3 (bearing in mind that this feature was not strictly exhibited by the other αCD protons) and going through the stepwise process of threading to one (K_{11}) and then to a second (K_{12}) αCD ring. The observed orientational isomerism was not explicitly treated. The equation that was obtained (see the Experimental Section) requires fitting with four variables, and thus many combinations may satisfy the model. The values obtained should be regarded as indicating trends rather than strict numbers but are in general agreement with the qualitative information displayed in Figure 1. Thus, K_{11} ranged from 8.8 to 9.6 $\times 10^3$ M^{-1}, and K_{12} varied from 0.95 to 2.1 $\times 10^3$ M^{-1}, the order being αCD/**1** < αCD/**2** < αCD/**3**. The values are of the same order of magnitude as those calculated for αCD/**1** previously[1b,6] and in agreement with reported association constants between αCD and aliphatic amines and alcohols,[18] according to which *K* increases with increase in the aliphatic chain length. Data for **4** were insufficient for any quantitative treatment to be undertaken.

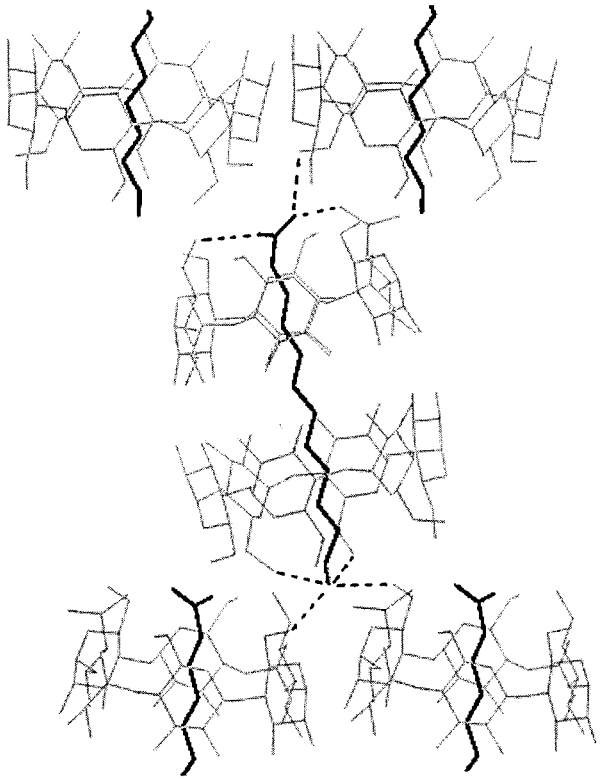


Figure 11. Crystal structure of αCD/12-aminoundecanoic acid: αCD/**2** [3]pseudorotaxanes and their packing along the *c* crystallographic axis plotted using the program "O".[25]

Solid-State Results. Colorless crystals were obtained for αCD/**2** from water at a 2:1 ratio of [αCD]:[**2**], and crystal data was collected at room temperature.[19] The αCD molecules form head to head dimers (Figure 11) via the O3 atoms of the secondary hydroxyl. One guest molecule lies across the length of an αCD dimer, forming a [3]pseudorotaxane. Each dimer is surrounded by 14.4 water molecules distributed over 22 positions. The glucosidic bridging oxygen atoms of each αCD monomer form nearly perfect hexagons parallel to each other. The conformation of the αCD monomer is stabilized by intramolecular H-bonds connecting the secondary hydroxyl groups at position 3 (O3*n*) with neighboring secondary hydroxyl groups at position 2 [O2(n + 1)] with an average distance of 2.91 Å. This is longer than the corresponding distance (2.78 Å)[20] of the βCD dimeric

(18) Ross, P. D.; Rekharsky, M. V. *Biophys. J.* **1996**, *71*, 2144−2154.

(19) Crystal data for αCD/**2**, $(C_{36}H_{60}O_{30})_2 \cdot C_{12}H_{25}O_2N \cdot (C_2H_6O)_{0.6} \cdot H_2O)_{14.4}$: orthorhombic $P2_12_12_1$, a = 14.07(1), b = 27.44(2), c = 31.20(2) Å, V = 12 050(13) Å^3, Z = 1, ρ_{calcd} = 1.33 g cm^{-3}. $2\theta_{max}$ = 44°, μ (Mo $K\alpha$)= 0.12 mm^{-1}, T = 296 K, R_1= 0.0809 for 6994 reflections (6972 were independent). Lp but no absorption correction was applied. A total of 6947 reflections were used up to 2θ 44^o to solve the structure with the molecular replacement method PATSEE[22] by using the coordinates of the skeleton atoms of αCD and of the αCD/barium polyiodide complex[23] and refined with full matrix least-squares on F^2 using the program SHELXL.[24] The goodness of fit on F^2 was 1.049, and the residual electron density was 0.656 e Å^{-3}.

(20) Mentzafos, D.; Mavridis, I. M.; LeBas, G.; Tsoucaris, G. *Acta Crystallogr.* **1991**, *B47*, 746−57.

complexes, indicating that the stabilization in the αCD monomer is weaker than in *β*CD. The αCD dimers are formed by H-bonding between the O3*n* hydroxyl groups of the two monomers with an average intermolecular distance of 2.82 Å, as in the structure of αCD/**3**.[7] The dimers aligned along the *c* crystallographic axis form layers related by a 2-fold screw axis, which make an angle of 9° with the *ab* crystallographic plane (Figure 11). The centers of dimers of consecutive layers are laterally shifted by 9.5 Å. This crystal packing is observed for the first time in αCD complexes. Structures of αCD inclusion complexes determined up to now have shown that dimeric complexes form channels,[7] whereas monomeric complexes form either head to tail channels or cage-type packing. 12-Aminododecanoic acid (**2**), fully extended, spans the entire length of αCD dimer parallel to its 6-fold axis and is very well localized. The end amino and carboxyl groups, emerging from the two primary faces of the dimer, are involved in H-bonds with primary hydroxyl groups (O6) of the enclosing macrocycle or of αCD molecules of consecutive layers, as well as with water molecules. There is no interaction between guest molecules of neighboring dimers. The same lack of interaction between amino and carboxyl groups of neighboring amino acid molecules is observed in the crystal structure of pure 11-aminoundecanoic acid.[21] Attempts to crystallize αCD/**1** have failed up to now.

The X-ray structure of αCD/**3** has been solved in our laboratory previously.[7] Likewise, the diamine lies across an αCD head to head dimer, and there is no direct association between guests of consecutive dimers in the lattice. Finally, the structure of *β*CD/**4** is very similar to the above, in that the guest diacid extends through the *β*CD dimer.[9]

We therefore observe that the adducts that do crystallize are [3]pseudorotaxanes only. Association via the guest termini is not observed, as hydrogen-bonding requirements of the functional groups are satisfied within each αCD dimer or with neighboring molecules and the length of the guests is well-fit in the elongated hydrophobic space provided by the dimer.

Conclusions

All long linear bifunctional molecules studied in this article form [2]- *and* [3]pseudorotaxanes with αCD in aqueous solution. At basic pH, the 1:1 complexes are present in concentrations higher than those at neutral pH. The longer guests tend to form complexes enriched in the 2:1 adduct at both pHs. There are clear indications that the [2]pseudorotaxanes are present in two isomeric forms having the macrocyclic host threaded in two orientations. The presence of isomeric forms for the [3]-pseudorotaxanes, too, cannot be ruled out. Various exchange rate regimes are observed, but in neutral solutions the formation of the 1:1 complexes is fast in the NMR time scale, whereas the threading of a second αCD ring is a slower process. The sequential organization of the αCD rings along the guests' backbones in solution has thus been clearly characterized, whereas in the solid state only [3]pseudorotaxanes have been crystallized. This study, therefore, bridges the solution with the solid-state results and demonstrates that the often conveniently assumed 1:1 stoichiometry in similar systems is an oversimplification of the real situation.

Experimental Section

11-Aminoundecanoic acid, 12-aminododecanoic acid, and 1,-11-dodecanediamine, 1,13-tridecanoic diacid were purchased from Aldrich, α-Cyclodextrin was obtained from Janssen and used as received. Buffered solutions were prepared from sodium dihydrogen phosphate−potassium hydrogen phosphate salts in D_2O for neutral (pH = 7.3) solutions and from sodium dihydrogen phosphate−sodium hydroxide for basic (pH = 13.6) solutions. Job plots were constructed by mixing varying volumes of equimolar solutions (pH = 13.6) of αCD and of a guest to a constant final volume, so that the resulting mixtures ranged from 90% to 10% αCD. The respective concentrations were αCD/**1**, 30 mM; αCD/**2**, 10 mM; and αCD/**4**, 80 mM.

Calculation of Equilibrium Constants. The observed nucleus is H3 of αCD, the chemical shifts of which at 298 K seem to obey the fast exchange regime (concentration-dependent). The total concentration of αCD (C_A) is held constant, whereas the total concentration of the substrate (guest) (C_S) is gradually increased from 0.1 to nearly 1.2 equiv. The expression for the stepwise equilibrium constants, K_{11} and K_{12}, of an aqueous solution where 1:1 and 2:1 [host]:[guest] complexes are present, is derived as follows:

$$\alpha + S \overset{K_{11}}{\rightleftharpoons} \alpha S,\ K_{11} = [\alpha S]/[\alpha][S] \Rightarrow [\alpha S] = K_{11}[\alpha][S] \quad (1)$$

$$\alpha S + \alpha \overset{K_{12}}{\rightleftharpoons} \alpha_2 S\ K_{12} = [\alpha_2 S]/([\alpha S][\alpha]) \Rightarrow [\alpha_2 S] = K_{11}K_{12}[\alpha]^2[S] \quad (2)$$

$$\text{mass balance gives } C_A = [\alpha]_{free} + [\alpha S] + 2[\alpha_2 S] \quad (3)$$

$$C_S = [S]_{free} + [\alpha S] + [\alpha_2 S] \quad (4)$$

Under fast exchange the chemical shift of H3 is given by

$$\delta_{obs} = \delta_\alpha f_{10} + \delta_{11} f_{11} + \delta_{12} f_{12} \quad (5)$$

where δ_α is the chemical shift of H3 in pure αCD, δ_{11} is the chemical shift of H3 in pure 1:1 complex, and δ_{12} is the chemical shift of H3 in pure 2:1 complex. Further, f_{10}, f_{11}, and f_{12} are the mole fractions of αCD pure, complexed as 1:1, and complexed as 2:1, respectively:

$$f_{10} = [\alpha]/C_A;\ f_{11} = [\alpha S]/C_A;\ f_{12} = 2[\alpha_2 S]/C_A \quad (6)$$

which, taking into account eqs 1−3, transform to:

$$f_{10} = [\alpha]/C_A = \frac{1}{1 + K_{11}[S] + 2K_{11}K_{12}[\alpha][S]} \quad (6a)$$

$$f_{11} = [\alpha S]/C_A = \frac{K_{11}[S]}{1 + K_{11}[S] + 2K_{11}K_{12}[\alpha][S]} \quad (6b)$$

$$f_{12} = [\alpha_2 S]/C_A = \frac{2K_{11}K_{12}[\alpha][S]}{1 + K_{11}[S] + 2K_{11}K_{12}[\alpha][S]} \quad (6c)$$

$$f_{10} + f_{11} + f_{12} = 1 \quad (7)$$

From eqs 5 and 7 we therefore obtain

(21) Aigouy, P. T.; Costeseque, P.; Sempere, R.; Senac, T. *Acta Crystallogr.* **1995**, B51, 55−61.
(22) Egert E.; Sheldrick, G. M. *Acta Crystallogr.* **1985**, *A41*, 262.
(23) Noltemeyer, M.; Saenger, W. *J. Am. Chem. Soc.* **1980**, *102*, 2710−2722.
(24) Sheldrick, G. M. *SHELXL93. Program for the Refinement of Crystal Structures*, University of Göttingen, Germany.
(25) Jones, T. A.; Kjeldgaad, M. *Molecular Modelling Program*; "*O*", *Version 5.9*, Upsala, Sweden, 1992.

$$\delta_{obs} = \delta_{\alpha}(1 - f_{11} - f_{12}) + \delta_{11}f_{11} + \delta_{12}f_{12} \Rightarrow \Delta\delta_{obs} = \Delta\delta^{\circ}_{11}f_{11} + \Delta\delta^{\circ}_{12}f_{12} \quad (8)$$

$\Delta\delta^{\circ}$ is the difference between the chemical shifts of free and complexed species.

$$\text{eq 8} \Rightarrow \Delta\delta_{obs} = \frac{K_{11}\Delta\delta^{\circ}_{11} + 2K_{11}K_{12}[\alpha]\Delta\delta^{\circ}_{12}}{1/[S] + K_{11} + 2K_{11}K_{12}[\alpha]}$$

In this equation, [S] is substituted as a function of [α], C_A, C_S, and the equilibrium constants.

$\Delta\delta_{obs}$ and C_S are experimentally measured, and $\Delta\delta^{\circ}_{11}$, $\Delta\delta^{\circ}_{12}$, K_{11}, and K_{12} are variables to be estimated; [α] is an intermediate variable whose value is iteratively calculated from C_A, C_S, K_{11}, and K_{12} for all points in the experimental curve. Nonlinear least-squares fitting was performed with GraphPad PRISM.

NMR Spectra. The spectra were acquired on either 250 or 400 MHz NMR instruments, using external DSS as reference. The ROESY spectra at 250 MHz were acquired using the decoupler to provide the spinlock pulse. The parameters used were D1 = 0.7−1 s, 512 or 1 K words experiment size, 128 or 512 scans, and a spinlock time of 250 ms. The spectral width and O1 used were 1818.2 Hz (7.26 ppm) and 4523.65 Hz, respectively, selected so as to minimize the appearance of TOCSY cross-peaks. At 400 MHz, the spinlock time was 300 ms and D1 = 1−2 s.

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Supporting Information Available: Crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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