trans-dihydride form was observed in $[CpRu(Ph_2PCH_2CH_2CH_2PPh_2)H_2]^+$ and $[CpRu(PR_3)_2H_2]^+$. They also found that a 2:1 mixture of dihydride and dihydrogen cations could be obtained for $[CpRu(Ph_2PCH_2CH_2PPh_2)H_2]^+$ and claimed that they did not interconvert. Chinn and Heinekey[11c,d] observed irreversible isomerization of the dihydrogen form to the dihydride for $[CpRu(PPh_3)_2(H_2)]BF_4$ and suggest that protonation of $CpRuL_2H$ complexes always gives the dihydrogen form initially. The latter then, depending on the ligand set and thermodynamic factors, either remains as an H_2 complex, converts to an equilibrium system (L_2 = dmpe), or isomerizes all the way to the transoid dihydride form.

The low solubility and broad line widths of III relative to those of I and II prevented our examining the relative effect of the greater steric bulk and greater basicity of the PCy_3 ligand on the stability of the dihydrogen versus dihydride complexes. $PCyp_3$ has a similar cone angle as P-*i*-Pr_3 and is expected to have a slightly greater basicity than P-*i*-Pr_3. It is reasonable that the thermodynamic parameters are identical for I and II within estimated error.

The $\Delta S^\ddagger$ of -20 eu for the oxidative addition of η^2-H_2 in IA to form the dihydride tautomer is consistent with a well-ordered, late transition state. Theoretical calculations have shown that, in the concerted oxidative addition of dihydrogen to the d^8 metal system, $Pt(PH_3)_2$, the H-H bond is retained until late in the transition state when the H atoms suddenly separate.[28] The relatively high negative value of $\Delta S^\ddagger$ may partially be accounted for by a loss of the rotational degree of freedom of η^2-H_2 in the transition state as the metal-H bond strength increases. Inelastic neutron scattering[29] and solid-state NMR[5b] studies have shown that the H_2 ligand in $W(CO)_3(P\text{-}i\text{-}Pr_3)_2(H_2)$ undergoes rapid hindered rotation in a plane perpendicular to the W-H_2 axis with a barrier of only 2.4 kcal/mol.

The activation parameters for formation of the dihydride complex from the η^2-H_2 complex in I [$\Delta H^\ddagger$ = 10.1 (1.8) kcal/mol, $\Delta S^\ddagger$ = -20 (6) eu, $\Delta G^\ddagger$ = 16.0 (0.2) kcal/mol (298 K)] can be compared to the activation parameters obtained for intramolecular exchange in the d^6 cationic complexes *trans*-$[M(\eta^2\text{-}H_2)(H)(depe)_2]BPh_4$ (M = Fe, Ru, Os) [Fe, $\Delta H^\ddagger$ = 12.4 (0.4) kcal/mol, $\Delta S^\ddagger$ = -2 (1) eu, $\Delta G^\ddagger$ = 13.0 (0.2) kcal/mol; Os, $\Delta H^\ddagger$ = 9.0 (0.5), $\Delta S^\ddagger$ = -12 (4) eu, $\Delta G^\ddagger$ = 12.6 (0.2) kcal/mol].[3] In these complexes, the unique hydride is exchanged with the two equivalent hydrogen atoms of the coordinated η^2-H_2 and the H-H bond interaction decreases in the order Ru > Fe > Os. If the mechanism for exchange involves breaking of the H-H bond and formation of a fluxional seven-coordinate trihydride intermediate, the enthalpy of activation for the exchange would represent the energy of the conversion of the dihydrogen hydride complex to a trihydride complex. The stronger Lewis acid character of Os^{2+} (d^6) system compared to W^0 (d^6) in $W(CO)_3(P\text{-}i\text{-}Pr_3)_2(\eta^2\text{-}H_2)$ is expected to cause stronger $\sigma(H_2) \rightarrow M$ bonding in the Os^{2+} complex. This may be counterbalanced by the greater σ-donor ability of the ancillary ligands in the Os complex relative to the three π-accepting CO ligands in the W complex, in producing comparable values for activation enthalpies for the conversion of a dihydrogen into a dihydride ligand. Further work is in progress in defining the transition state in this process.

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Cooperative Binding by Aggregated Mono-6-(alkylamino)-β-cyclodextrins

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Abstract: Mono-6-(hexadecylamino)-β-cyclodextrin (**5**) and mono-6-(octylamino)-β-cyclodextrin (**4**) have been titrated against organic guests (4-nitrophenol, 4-hydroxybenzoic acid, cyclohexanecarboxylic acid, etc.) in aqueous solution to give sigmoidal binding isotherms. Replotting of these data according to the Hill equation gives Hill coefficients > 1.0 (2.2 in the case of **5** and 4-nitrophenol). In contrast, both β-cyclodextrin (β-CD) and mono-6-amino-β-cyclodextrin (**3**) both exhibit hyperbolic binding isotherms, leading to Hill coefficients close to unity. Hill coefficients greater than one are the experimental hallmark of cooperativity, in which initial binding events render subsequent binding events more favorable. The degree of cooperativity is sensitive to the concentration of the hosts (**4** and **5**) and the solvent (cooperativity is suppressed in DMSO). These results are discussed in terms of the aggregation properties of the amphipathic hosts (cmc = 200 and 1.4 μM, for **4** and **5**, respectively), which were studied by conductivity, dynamic light scattering, and NMR. Two possible mechanisms to account for the observed cooperativity are presented and discussed.

Allosteric interaction between recognition sites is a ubiquitous regulatory mechanism in biological macromolecules, including enzymes,[1] receptors,[2] and ribosomes.[3] Allostery refers to the modulation of binding or catalysis at an active site as a consequence of binding at a remote effector site. When the active and effector sites are equivalent, the allostery is termed cooperativity (also "homotropic allostery"),[4] for which hemoglobin is the par-

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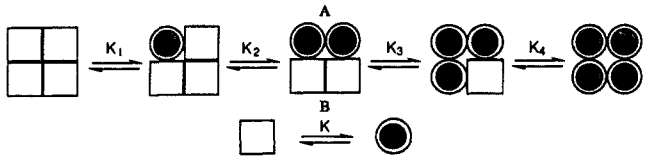

Figure 1. Schematic for (A) cooperative binding of O_2 to hemoglobin ($K_1 \cong K_2 \cong K_3 < K_4$, $h = 2.8$) and (B) noncooperative binding of O_2 by myoglobin or a dissociated subunit of hemoglobin.

adigmatic example.[5-8] Only recently has allosteric behavior been mimicked in synthetic host molecules, probably because the fabrication of specific and interactive recognition sites remains problematic. Nevertheless, allosteric hosts have been reported by Rebek and co-workers who appended crown ethers to 2,2'-bipyridyl[9-11] and biphenyl[9,11,12] to observe heterotropic (nonequivalent recognition sites) and homotropic interactions, respectively. Beer[13] has also used crown ethers for the construction of hosts which exhibit heterotropic allosteric interactions in the binding of various ions. Traylor[14] and Tabushi[15] have observed allosteric binding on the part of synthetic heme analogues. In related work Shinkai,[16] Gokel,[17] and others[18] have made "molecular switches" in which binding of guests is modulated by external factors such as heat, light, pH, and oxidation potential. An allosterically regulated synthetic catalyst for sequence-selective cleavage of duplex DNA has been reported by Dervan.[19]

An especially common strategy for establishing allosteric interactions in the biological setting is to assemble a protein aggregate in which information about binding is communicated from one recognition site to another by conformational changes at the interfaces between subunits.[20] Thus, the allosteric entity in such a circumstance is the aggregate rather than the individual subunits. For example, while hemoglobin, an aggregate of four subunits, binds O_2 cooperatively,[5,21] its dissociated subunits are noncooperative (see Figure 1).[22] Detailed structural analysis by X-ray crystallography and site-directed mutagenesis has begun to reveal the mechanics of the allosteric transitions in hemoglobin,[5,23] aspartate transcarbamoylase,[24] and other multisubunit enzymes.[1,25] An especially interesting recent finding by Newton and Koshland[26] describes the dependence of protein kinase C cooperativity (Hill coefficients of 8–11) on intimate association with several phosphatidylserine molecules.

Few synthetic allosteric hosts emulate this strategy by assembling interacting subunits into a functional aggregate. Collman and co-workers[27] observed cooperative binding of O_2 by a crystalline synthetic heme analogue; when this aggregated form of the host is disrupted by dissolution in solvent, the cooperative

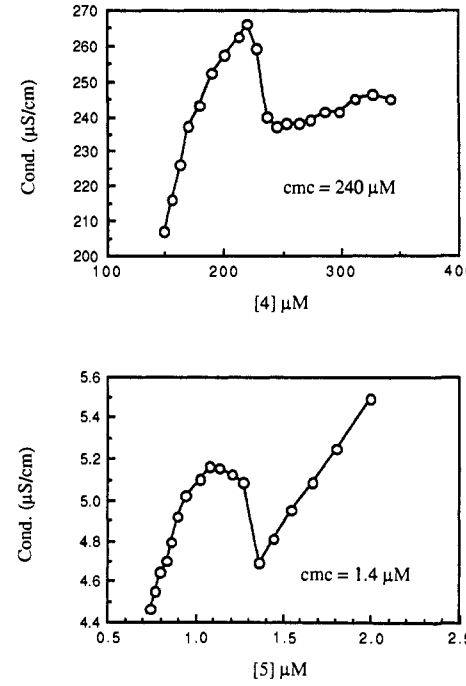

Figure 2. Determination of critical micelle concentration for glycolipids **4** (A) and **5** (B) in H_2O at 23 °C. Each glycolipid was dissolved in ultrapure H_2O (Millipore-Q), filtered through a 0.2-μm polycarbonate filter, suspended in a constant temperature bath, and diluted by incremental addition of H_2O while the conductivity of the solution was monitored.

(4) "Homotropic allostery" refers to interaction between equivalent sites; positive homotropic allostery is often referred to simply as "cooperativity", while negative homotropic allostery is termed "negative cooperativity" or "anti-cooperativity." "Heterotropic" allostery refers to interactions between nonequivalent sites.

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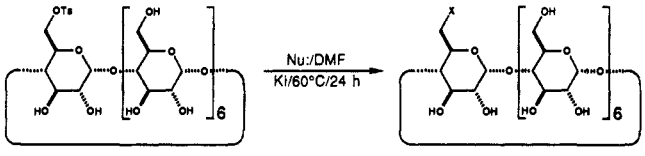
aspect of the binding is lost. Piszkiewicz[28] has noted that micelle-catalyzed reactions exhibit a sigmoidal dependence of rate upon surfactant concentration. We have observed cooperative binding of organic guests by an aggregated amphipathic cyclodextrin[29] and provide further observations on the mechanism of this cooperativity below.

Results

The amphipathic cyclodextrins (glycolipids) incorporating a single alkyl chain derive in all cases from mono-6-deoxy-6-(*p*-tolylsulfonyl)-β-cyclodextrin (**2**), prepared from β-cyclodextrin (**1**, β-CD). Treatment of **2** with hexadecylamine in DMF at 60 °C (24 h) in the presence of catalytic KI and DMAP gave glycolipid **5**, after ion exchange (IRA-400(Cl^-) or Sephadex CM-25) and purification by filtration through activated carbon. Glycolipids **4** and **6** were prepared in the same manner with octylamine and didecylamine, respectively. Further purification of **5** was possible by warming a concentrated (5 mg/mL) aqueous solution and collecting the white precipitate; glycolipid **4** would not precipitate under these conditions. Mono-6-deoxy-6-amino-β-cyclodextrin hydrochloride (**3**) was prepared by previously reported methods.[30] The ammonium linkage proved essential to the water solubility of **4** and **5**; glycolipids **7** (prepared from $C_{12}H_{25}SH$ and **2**) and **8** (prepared from stearic acid and **3**) were relatively insoluble in water.



2

3 X=NH_3^+ Cl^-
4 X=$NH_2^+C_8H_{17}$ Cl^-
5 X=$NH_2^+C_{16}H_{33}$ Cl^-
6 X=$NH^+(C_{10}H_{21})_2$ OAc^-
7 X=$SC_{12}H_{25}$
8 X=$NHCO(C_{15}H_{31})$

The pK_a of the ammonium group of **5** was determined, by titration against $HClO_4$ and KOH, to fall in the range 7.5–8.0 in H_2O at 25 °C. As the pH of the solution rose above 8.0 the glycolipid would fall out of solution. Cyclodextrin **3** gave a similar value (7.5–8.2) and remained in solution at high pH. Diethanolamine was used as a standard and gave pK_a 8.96 with our apparatus (lit.[31a] pK_a 8.88). The relatively low pK_a values for **5** and **3** may reflect the hydrophobic environment provided by the cyclodextrin. The pK_a of 4-nitrophenol was not significantly altered by addition of **3**: pK_a 7.13 (lit.[31b] pK_a 7.15) in H_2O at 25 °C, 7.06 in the presence of **3** (1 equiv).

Critical micelle concentrations (cmc) were determined for **4** (240 μM) and **5** (1.4 μM) by measuring conductivity vs. the concentration of ionic glycolipid in water at 23 °C (see Figure 2). The conductivity of a 25.7 mM aqueous solution of **5** (filtered through a 0.2-μm filter) dropped significantly over the course of 50 h at 23 °C; this was accompanied by a slow increase in the turbidity of the sample culminating in the formation of a white precipitate. This solid was recovered by filtration, dissolved in DMSO-d_6 with sonication, and demonstrated to be **5** by ^1H NMR. The same precipitate could be produced more rapidly by warming of an aqueous solution of **5**.

Dynamic light scattering experiments corroborated the cmc determinations: **5** in water (12.7 mM filtered through a 0.2-μm polycarbonate filter) was irradiated (HeCd laser at 441.6 nm) and the scattering angle varied from 27.5 to 96.7°. A hydrodynamic radius of 20 Å and a relatively low polydispersity (0.0734) suggest a spherical aggregate. With time (several hours) the effective diameter of these aggregates grew to roughly 60 Å with a high polydispersity (0.1–0.2), suggesting a nonspherical structure for the growing aggregate. In another experiment the effective diameter of the aggregates ([**5**] = 25.7 mM) increased over the course of 1.5 h from 56.0 Å to 63.1 Å (see Figure 3).

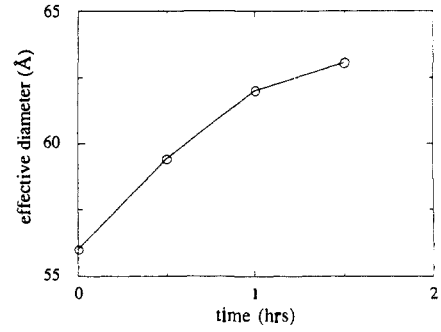


Figure 3. Effective diameter of aggregates of **5** (25.7 mM) as a function of time as determined by dynamic light scattering (441.6 nm at 27.5 or 96.7° angle) in H_2O at ambient temperature.

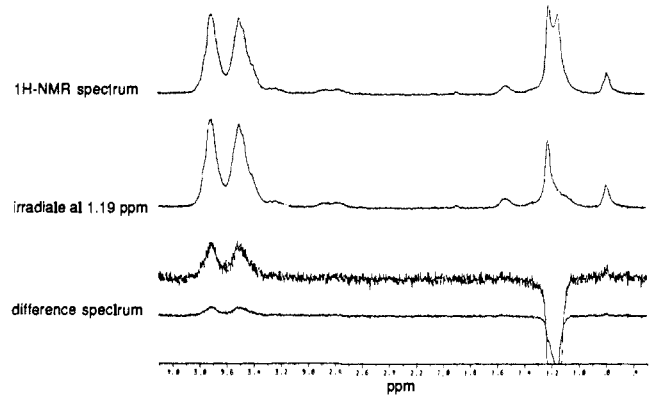


Figure 4. Difference NOE spectrum of **5** (2.79 mM in D_2O at 12 °C) irradiated at δ 1.19 ppm to yield a 12% enhancement in the cyclodextrin protons.

^1H NMR spectra of **5** in D_2O (6.45 mM) contain a pair of resonances which correspond to the alkyl chain: a narrow peak at δ 1.26 ppm (TSP as reference) and a broad peak at δ 1.19 ppm. This feature of the spectrum is acutely dependent on the temperature; as the temperature of the sample is increased from 15 to 90 °C the broad, upfield peak begins to sharpen and move downfield (see supplementary material). Irradiation of this resonance ([**5**] = 2.79 mM in D_2O at 12 °C) produces a 12% nuclear Overhauser enhancement of the CD protons (see Figure 4). Poor resolution precludes more specific assignment of which CD protons are subject to enhancement. However, similar irradiation of the sharp, downfield peak yields no NOE. In DMSO-d_6 ([**5**] = 39.3 mM, 25 °C) only one, narrow peak (δ 1.22 ppm, TMS reference) is observed and irradiating it also affords no NOE of the CD protons. Introduction of 4-NP (1 equiv in D_2O) alters the spectrum of **5**; the δ 1.26 resonance increases in area at the expense of the broader, upfield resonance. In this system, irradiation of the peak at δ 1.19 produces only a 2.7% NOE. Glycolipid **4** exhibits a similar pattern: in D_2O (1 mM) a broad peak is observed at δ 1.17 ppm, accounting for most of the alkyl chain methylenes, while in DMSO-d_6 the resonance is quite sharp and has shifted to δ 1.23 ppm. A 2D NOESY spectrum of **4** in D_2O (1 mM) suggests that there is contact between the terminal methyl group of the alkyl chain and the CD, whereas **4** in DMSO exhibits contact between the alkyl chain methylenes and the CD. Glycolipid **6**, having two C_{10} chains, is virtually insoluble in water and even in DMSO-d_6 there are two resonances for the alkyl chains, one broad and one sharp.

Inclusion complexation of organic guests is a widely exploited property of CDs.[32] Binding titrations of 4-NP with CDs **1**, **2**,

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Table I. Summary of Titration Data

host	solvent	titrant[a]	[titrant], mM	guest[b]	[guest], mM	K_a (M^{-1})	h	method
β-CD	D_2O	β-CD	0.036–0.90	4-NP	0.36	1300[c]	1.0 ± 0.1	NMR, UV[h]
3	D_2O	CCA[d]	0.036–0.99	4-NP	0.36	3300[c,e]	1.1 ± 0.1	NMR
4	D_2O	**4** (high conc)	0.050–1.90	4-NP	0.29	–	(1.7 ± 0.2)[f]	NMR, UV[h]
4	H_2O	**4** (low conc)	0.003–0.033	TNS	0.0033	(200 000)[c]	1.4 ± 0.2	fluorescence[i]
5	D_2O	**5** (high conc)	0.036–0.90	4-NP	0.36	3700[g]	2.2 ± 0.1	NMR, UV[h]
5	D_2O	CCA (high conc)[d]	0.046–0.89	4-HBA	0.36	880[g]	2.3 ± 0.1	NMR
5	D_2O	**5** (high conc)	0.068–0.89	D-*N*-AcPhe	0.36	1500[g]	1.6 ± 0.2	NMR
5	D_2O	**5** (high conc)	0.068–0.89	D-*N*-AcTrp	0.36	2300[g]	1.5 ± 0.2	NMR
5	D_2O	**5** (low conc)	0.0028–0.040	4-NP	0.036	(62 000)[c]	1.3 ± 0.1	UV
5	H_2O	**5** (low conc)	0.0008–0.0067	TNS	0.0033	(333 000)[c]	1.4 ± 0.1	fluorescence[i]
5	DMSO-d_6	**5**	0.004–0.89	4-NP	0.37	(80 000)[c]	1.1 ± 0.1	NMR

[a] In UV experiments H_2O was substituted; all aqueous solutions were buffered at pH (or pD) = 7 with Tris or phosphate and maintained at 30 °C; the concentration range of the titration is described qualitatively in relation to the cmc of the pure host glycolipid. [b] 4-NP = 4-nitrophenol; TNS = 2-*p*-toluidino-6-naphthalenesulfonate; 4-HBA = 4-hydroxybenzoic acid; D-*N*-AcPhe = D-*N*-acetylphenylalanine; D-*N*-AcTrp = D-*N*-acetyltryptophan. [c] Determined by fitting data to eq 1 by using HOSTEST;[34] values in parentheses are apparent association constants, but probably reflect induced aggregation rather than inclusion complexation. [d] CCA = cyclohexanecarboxylic acid. [e] Competitive binding experiment in which CCA is the titrant and [host] = [chromophoric guest] = a constant. [f] Hill coefficient was calculated by assigning the maximum $\Delta\delta_i$ obtained as $\Delta\delta_{max}$; no saturation could be observed at accessible concentrations of **4**. [g] Estimated as $[host]^{-1}$ at Y = 0.5 for sigmoidal binding isotherms. [h] Value from NMR experiment shown. [i] $\lambda_{excitation}$ = 320 nm; bandwidth = 10 nm; monitored emission λ_{max} (478–454 nm).

4, and **5** were monitored by absorbance (400 nm) and ^1H NMR at 30 °C at pH 7.0 (Tris or phosphate buffers) to determine the equilibrium constants (K_a) for inclusion complexation. Titrations of the amphipathic hosts were complicated by their aggregation properties, so several methods were used: (A) Varying [guest] while monitoring the chemical shift of the interior H3 and H5 CD protons. This proved problematic because the CD protons of the glycolipids are broadened in aqueous solution. Nevertheless, ^1H NMR spectra of excess 4-NP in the presence of **5** exhibited shifts in the interior H3 and H5 CD protons of **5** of 0.016 ppm upfield, which is comparable to the same effect observed for 4-NP and β-CD (0.018 ppm upfield). In general though, this was method insufficiently accurate for titration experiments. (B) Varying [host] while monitoring a spectroscopic observable of the guest. Though a convenient method, observation of the saturation point ($\Delta\delta_{max}$) often proved difficult or impossible due the limited solubility of the glycolipids in aqueous solutions. When necessary the $\Delta\delta_{max}$ was estimated from the value obtained from titration of the aromatic guest with β-CD. (C) Varying the concentration of a competing guest, typically cyclohexanecarboxylic acid (CCA), against constant concentrations of host and chromophoric guest. The K_a for inclusion of CCA in β-CD has been estimated[33] at 2400 M^{-1}.

Binding isotherms for hosts β-CD and **3** with 4-NP in aqueous buffer (pH 7.0, 5.0 mM phosphate or Tris) gave hyperbolic graphs (see supplementary material). In these cases the association constants were calculated by direct fitting of the titration data by regression analysis to eq 1 with the use of HOSTEST, a program developed by Wilcox and Cowart[34] in which $\sum = G_0^1 + k_d$, $\Delta = G_0 - K_d$, G_0 = initial guest concentration, H_0 = initial host concentration, and $\Delta\delta$ is the change in an observable parameter (chemical shift, absorbance, or fluorescence intensity) which is assumed to correlate with molecular association. Alternatively, replotting of the saturation curve according to the Eadie–Hofstee approximation (eq 2) allowed estimation of the equilibrium

$$\Delta\delta = \frac{\Delta\delta_{max}}{G_0}\left[\frac{H_0}{2} + \frac{\sum}{2}\left[1 - \sqrt{\frac{H_0^2 - 2H_0\Delta}{\sum^2} + 1}\right]\right] \quad (1)$$

$$\Delta\nu_{obs} = -K_D\frac{\Delta\nu_{obs}}{[X]} + \Delta\nu_{max} \quad (2)$$

constant for association ($K_a = K_D^{-1}$) from the slope of the resulting linear graph. Both β-CD and **3** bound 4-NP with $K_a \approx 10^3$ M^{-1}. This is consistent with previous determinations.[35]

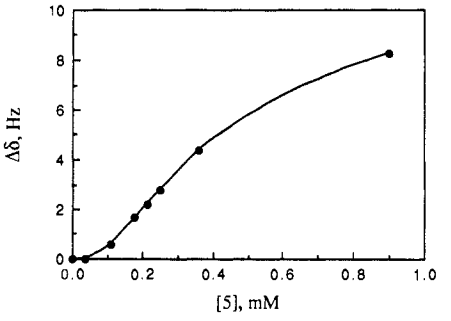

Figure 5. Binding isotherm at 30 °C for **5** titrated against 4-NP (0.36 mM). Tris (5.15 mM at pH 7.0) in D_2O was the solvent.

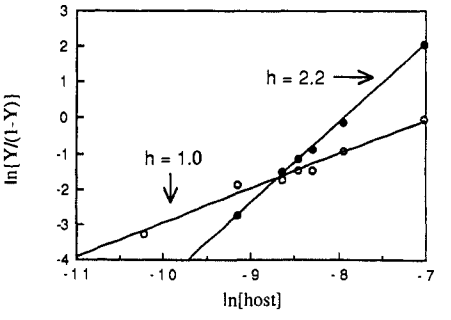

Figure 6. Hill replot of binding isotherm at 30 °C of **5** titrated against 4-NP (0.36 mM, see Figure 5), giving a Hill coefficient of 2.2, as compared to a similar analysis of the binding of β-CD to 4-NP which gives h = 1.0.

Titrations of **5** against constant concentrations of 4-NP, 4-HBA, D-*N*-acetylphenylalanine, and D-*N*-acetyltryptophan led to sigmoidal rather than hyperbolic graphs (see Figure 5) and replotting of the data according to Eadie–Hofstee gave nonlinear graphs. Scatchard analysis[36] afforded concave-down graphs, whereas linear graphs emerged from the same analysis of titration data for β-CD

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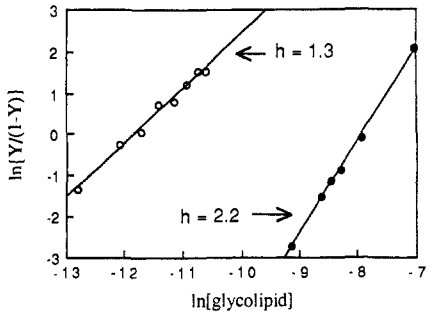

Figure 7. Hill plots for titration of **5** (2.8–40 μM) against 4-NP (36 μM) in a lower concentration regime (monitored Δabs of 4-NP at 410 nm), giving h = 1.3, in contrast to h = 2.2 for **5** in a higher concentration regime from Figure 6.

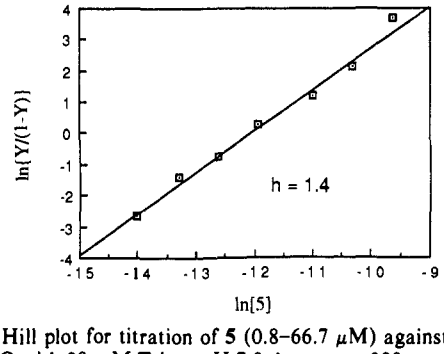

Figure 8. Hill plot for titration of **5** (0.8–66.7 μM) against TNS (3.3 μM) in H_2O with 20 mM Tris at pH 7.0; $\lambda_{excitation}$ = 320 nm (bandwidth = 10 nm); λ_{max} for emission varied from 478 to 454 nm.

and **3**. Replotting of both sets of titration data (hyperbolic and sigmoidal) according to eq 3 (the Hill equation) gave linear graphs

$$\ln [Y/1 - Y] = h \ln [X] - \ln K_D \qquad (3)$$

$$Y = \Delta\delta_i/\Delta\delta_\infty$$

$[X]$ = concentration of ligand (host or guest)

K_D = overall dissociation constant (= K_a^{-1})

(see Figure 6) where the slope is defined as the "Hill coefficient" (h), which is construed as an index of cooperativity.[36a] Noncooperative systems exhibit $h \simeq 1.0$, positively cooperative systems $h > 1.0$ (but less than the number of interacting binding sites), and negatively cooperative systems $h < 1.0$. As a point of reference, hemoglobin has h = 2.8, while myoglobin (having a single O_2 binding site) has h = 1.0.[5] Glycolipid **5** gave h = 2.2 ± 0.2 with 4-NP, while binding of 4-NP to β-CD and **3** gave Hill coefficients of 1.0 ± 0.1 and 1.1 ± 0.1, respectively. 4-HBA, D-*N*-acetylphenylalanine, and D-*N*-acetyltryptophan also gave Hill coefficients greater than unity (see Table I).

All the titration experiments described so far were performed in a concentration regime in which **5** was clearly aggregated according to both conductivity and DLS results. The degree of cooperativity proved to be strongly dependent on the concentration of the host. With use of absorbance spectrometry, a titration of 4-NP (0.036 mM) was performed with [**5**] = 2.5–30 μM, resulting in h = 1.3 (see Figure 7). Fluorophoric guests are commonly used to probe host–guest interactions in CDs because inclusion in the CD enhances the fluorescence intensity; 2-*p*-toluidino-6-naphthalenesulfonate (TNS) forms an inclusion complex with β-CD (K_a = 1800)[37] and was used to corroborate the absorbance data monitoring either the intensity or the wavelength (478–454 nm) of the emission maximum ($\lambda_{excitation}$ = 320 nm, bandwidth = 10 nm). TNS (10 μM in 20 mM Tris buffer, pH 7.0) was exposed to incremental additions of **5** (1–75 μM), giving h = 1.4 (see Figure 8). Of particular interest is that titration of TNS against a constant concentration of **5** (50 μM) produces a leveling

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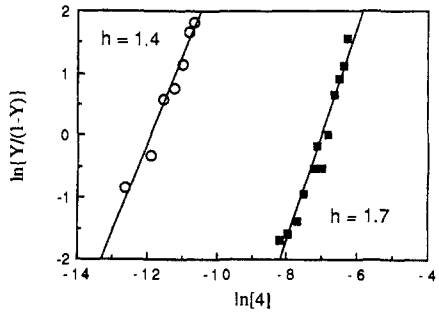

Figure 9. Hill analysis of binding titrations (30 °C) of **4** against 4-NP (0.29 mM, [Tris] = 5.15 mM, pH 7.0, monitored $\Delta\delta$ of H2 of 4-NP or Δabs at 410 nm, h = 1.7) and TNS (3.3 μM, [Tris] = 20 mM, pH 7.0, monitored emission λ_{max} ($\lambda_{excitation}$ = 320 nm), h = 1.4).

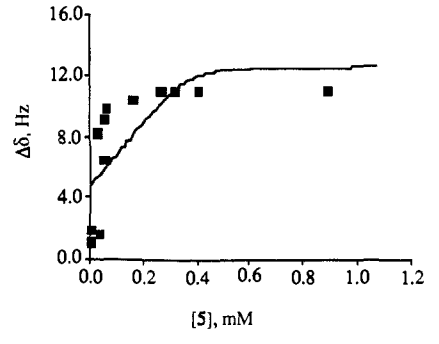

Figure 10. Binding titration of **5** against 4-NP (0.37 mM) in DMSO-d_6. The curve corresponds to the best fit to eq 1 provided by HOSTEST.

off of ΔF at ~1 equiv, followed by a sharp rise in ΔF as TNS addition is continued.

Glycolipid **4**, having only eight carbons in its alkyl chain, was prepared to explore the possible cooperative behavior of a structure intermediate between **3** (noncooperative) and **5** (positively cooperative). When the concentration of **4** was varied from 0.25 to 2.5 mM in the presence of 4-NP (0.286 mM in Tris at 30 °C), Hill analysis afforded h = 1.7; the range of the titration was limited by the solubility of **4**, so saturation was estimated from comparable titrations with **3** and **5**. When the concentration of **4** was varied from 2.5 to 25 μM (titrated against 10 μM TNS in 20 mM Tris) yielded h = 1.4 (see Figure 9). On some occasions a break in the titration data would be observed in the μM region, and the data for titration of **4** in general suffered from greater scatter than that of **5**. It may be that the cmc and aggregate structure are not as clearly defined for **4**.

It was initially assumed that in DMSO there was no aggregation, largely on the basis of the ^{1}H NMR spectrum of **5** in this solvent. Indeed, binding titrations of **5** against 4-NP in DMSO yielded h = 1.1 ± 0.1, consistent with a noncooperative binding process. However, the clearly hyperbolic binding isotherm reaches a saturating value for $\Delta\delta$ well before a full equivalent of the titrant (**5**) has been added. Figure 10 shows the titration data for **5** and 4-NP in DMSO-d_6 as compared to a theoretical binding curve based on eq 1.

Discussion

Though sigmoidal binding titrations can be construed as prima facie evidence for a cooperative event, the nature and mechanism of the cooperative event would remain in question. The binding titrations do not represent simple proton transfer events. Sigmoidal titrations were observed regardless of the pK_a of the guest (phenoxide, carboxylate, or sulfonate) and in the presence as well as absence of buffer. The pH of the solution remained unchanged (pH 7.0) throughout the titration and glycolipid **5** is in any event insufficiently acidic (pK_a 7.5–8.0) to alter appreciably the 4-nitrophenol $\rightleftharpoons$ 4-nitrophenoxide equilibrium. The relative insolubility of the neutral glycolipids **7** and **8** prevented exploration of binding in systems where proton transfer would not be possible. The relatively low pK_a values of the ammonium groups of **3** and

5 may reflect the proximity of the hydrophobic CD cavity.

The aggregation of surfactants into micellar aggregates is a cooperative phenomenon.[38] If the allosteric transitions of **4** and **5** corresponded to a guest-induced aggregation, the observation of cooperative binding would seem less remarkable and a poor model of protein subunit interactions. This is the mechanism adduced by Piszkiewicz[28] to account for the sigmoidal dependence of reaction rates on the concentration of micellar surfactants. In an effort to determine binding of guests to monomeric **4** and **5**, as opposed to their aggregates, we sought to perform titrations such that the [host] is less than its cmc, obtaining low Hill coefficients (<1.5) and nearly hyperbolic curves. However, the cmc values for **4** and **5** (240 and 1.4 μM, respectively) were determined in pure water, but the titration experiments were performed in the presence of buffer and ionic guests. In addition, the graphs obtained from these titrations reached saturation well below 1 equiv of added host (or competing guest) relative to the guest. This last observation is in fact most striking when DMSO is the solvent (see Figure 10). To reach saturation with less than 1 equiv of host requires more than one binding site per host; aggregated host would provide numerous potential hydrophobic sites for the guest in addition to the CD cavities. We believe that the buffer and guest in fact cause such an aggregation of the glycolipid and that we have never actually succeeded in titrating monomeric amphipathic host.

There is, nevertheless, a clear dependence of the Hill coefficient on the overall concentration regime in which the titration is performed, perhaps reflecting a dependence on the size and shape of the aggregates which may in turn depend on the concentration of the host. It should be noted, however, that variation in the concentration of the amphipathic host itself is not the source of cooperativity—competitive binding titrations in which the concentration of the host remains unchanged also exhibit Hill coefficients near 2. That the aggregates function as cooperative entities is secure, but the apparently inaccessible monomers of **4** and **5** cannot be used for comparison; β-CD and **3** must serve as surrogates for these monomers and they are in fact noncooperative in their binding of guests. The binding of 4-NP and **5** in DMSO-d_6 is noncooperative ($h = 1.1 \pm 0.1$, a value indistinguishable from that of β-CD) even though **5** is aggregated here as well. The structure of the aggregate in DMSO is unknown, but clearly differs sharply from that in aqueous solution, as evidenced by its ^{1}H NMR spectrum and NOE experiments which indicate that the alkyl chains of **5** do not occupy the interior of the CDs.

The allosteric transition must therefore correspond to a change in the morphology of the aggregate. Our understanding of the morphology of aggregated **4** and **5** is rudimentary. The initial structure, as determined by DLS, seems to be a rather typical spherical micelle[39] with a hydrodynamic radius of 20 Å. Based on the cross-sectional area of a β-CD (217 Å^2, determined by film balance techniques),[40] the aggregation number would be ca. 23, perhaps higher if the CDs of **5** are canted relative to the micelle surface. With time, though, the micelles grow, becoming polydisperse, and elongated to a rodlike shape. Similar behavior has been reported by Menger[41] for an amphipathic β-CD having six appended chains with anionic sulfate at the terminus of each chain. In addition, both Ise[42] and Saenger[43] have noted the profound

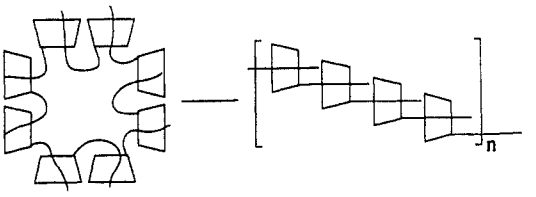

Figure 11. Hypothetical host–guest polymerization of **5** by intermolecular inclusion complexation of the alkyl chains.

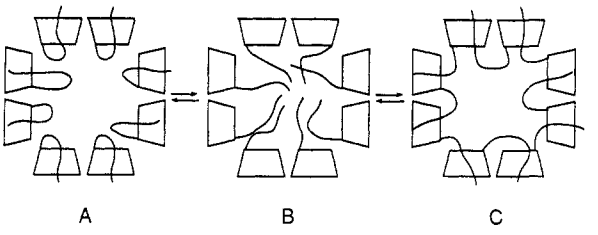

Figure 12. Possible structures of aggregates of **5** (for ease of presentation, only eight glycolipids are shown comprising the aggregate, the actual aggregation number is likely much higher): (A) intramolecularly complexed alkyl chains, (B) extended lipids not included in CD cavities, (C) intermolecular inclusion complexation of alkyl chains.

effect that added CDs have on the aggregation of surfactants such as sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide, *n*-dodecyl maltoside, β-octyl glucoside, and Triton X-100 and account for the observed effects by inclusion of the hydrophobic alkyl chains in the CD cavity. ^{1}H NMR spectra of **4** and **5** in aqueous solution, in conjunction with NOE experiments, make it plain that a portion of each attached alkyl chain takes refuge in the hydrophobic cavities provided by the CDs. It is possible that intermolecular inclusion of the alkyl chains forms the basis of the aggregate's structure and also accounts for the slow formation of long aggregates and ultimately precipitation in a structure that can be formulated as a host–guest polymer (see Figure 11). A possible driving force is that a linear polymer would enjoy hydrophobic binding of the alkyl chains by the CDs without introducing gauche interactions into the chain, such as would be required by intramolecular inclusion of the same chain. This sort of extended structure finds precedent in the work of Tabushi and Fujita[44] who have reported the X-ray structure of β-CD-SC_6H_5 which reveals a series of phenylthio-CDs, each forming an inclusion complex with the phenylthio group of the neighboring CD. Taken together the comments above suggest three extreme possible structures for aggregates of **5** (see Figure 12): intramolecular inclusion of all alkyl chains (A), intermolecular inclusion of all alkyl chains (C), and a presumably unstable intermediate state in which no alkyl chains are included. The actual structure of the aggregate is probably rather heterogeneous, encompassing all these possibilities.

In formulating a mechanism to account for cooperative binding it is important to verify that the CD cavity is in fact a recognition site for the guest. NOE difference experiments were not illuminating but ^{1}H NMR revealed that when [4-NP] > **5**, the 4-NP does reside in the CD cavity (as evidenced by shifts in the interior H3 and H5 protons). Similar experiments with **5** and excess SDS also showed 4-NP in the CD cavity, but no cooperativity is observed under these circumstances. This is not to say, however, that the guest does not also reside elsewhere in the aggregate. The titration of TNS against **5** in fact suggests that the [TNS] may build up in the aggregate interior (so that ΔF vs [TNS] levels off by virtue of intermolecular self-quenching) and ultimately induces a morphological change in the aggregate which frees up the CDs for binding.

The essence of positively cooperative binding is that the coupling free energy between recognition sites[45] is negative; that is, initial

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(42) (a) Okubo, T.; Kitano, H.; Ise, N. *J. Phys. Chem.* **1976**, 2661. See also: (b) Miyajima, K.; Ikuto, M.; Nakagaki, M. *Chem. Pharm. Bull.* **1987**, *35*, 389.
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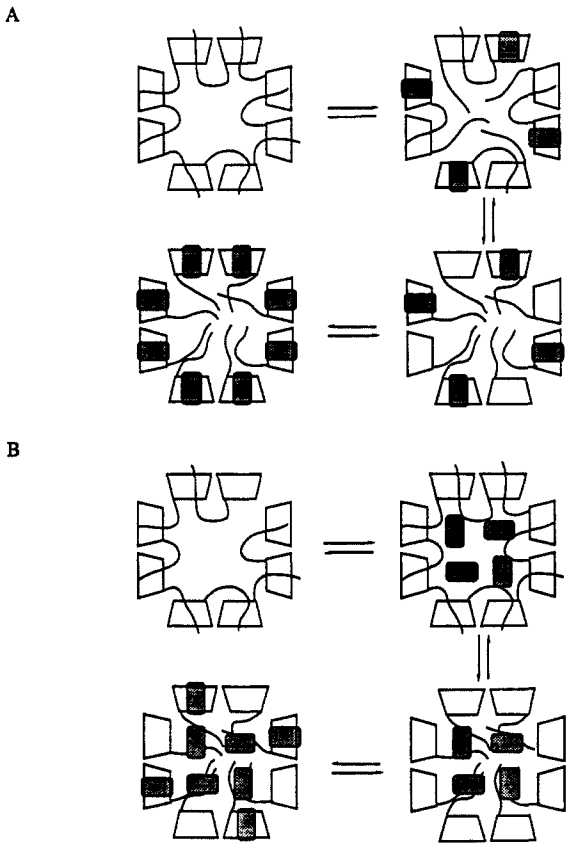

Figure 13. Proposed cooperativity mechanisms: (A) the "push" mechanism and (B) the "pull" mechanism (see text).

binding events render subsequent binding events more favorable. Two mechanisms are proposed which account for the data gathered so far: a "push" mechanism and a "pull" mechanism. In the push mechanism guest binds initially to the CD cavity in competition with the resident alkyl chain (see Figure 13a). The intruding guest at least partially pushes the alkyl chain from the CD cavity into the aggregate "interior". This would have the effect of rendering the aggregate more hydrophobic and thus more inviting to the other alkyl chains of the aggregate which occupy CDs. The in ⇌ out equilibrium of these chains would consequently be shifted slightly in favor of extension of the alkyl chain into the aggregate interior. This in turn would render the other CDs of the aggregate more available to incoming guests because the chains would compete less effectively for these sites. Alternatively, the initial host–guest interaction may take place in the hydrophobic interior of the aggregate; insofar as this increases the hydrophobicity of the interior, the in ⇌ out equilibrium of the alkyl chains would again be shifted so as to pull the alkyl chains partially out of the CDs (see Figure 13b). Subsequent guests could then bind either to the now more hydrophobic aggregate interior or into the CD cavities (as shown). Both mechanisms could converge on the same saturation state which places guests in both types of recognition site (see Figure 14). In view of the intermolecular self-quenching of TNS fluorescence at low concentration (≤1 equiv of TNS) in the presence of **5**, we favor the pull mechanism. If the initial binding were taking place in the CDs, ΔF would not level off as [TNS] approaches 1 equiv.

In conclusion, aggregates of amphipathic CDs **4** and **5** function as cooperative entities in their binding of simple anionic aromatic guests, emulating a strategy ubiquitous in nature. Strictly speaking, since there is likely interaction between two types of recognition site (CD cavity and aggregate interior), it should be termed an allosteric effect rather than a cooperative effect, the latter being restricted to symmetric systems. Insofar as binding can be converted into catalysis, it may be possible extend our mimicry to multisubunit synthetic catalysts.

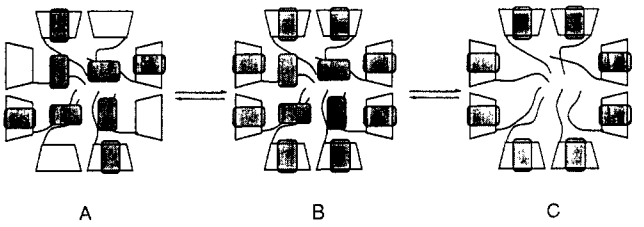

Figure 14. Approaches to saturation of all recognition sites (B) from the "push" mechanism (C) or the "pull" mechanism (A).

Experimental Section

Chemical shifts in D_2O are reported relative to internal TSP (sodium 3-(trimethylsilyl)propionate-d_4) (^{1}H NMR) or internal dioxane (^{13}C NMR). Elemental analyses were done at Galbraith Laboratories, Inc., Knoxville, TN. Water used for UV–vis and fluorescence spectra was ultrahigh purity from a Millipore Milli-Q purification apparatus and filtered through a 0.2-μm disposable filter. β-Cyclodextrin (purchased variously from Aldrich, CTC Organics, and American Maize Products) was recrystallized from water when used for binding experiments and used as received for preparation of monotosylate. 4-Nitrophenol and 4-hydroxybenzoic acid were recrystallized twice from water. TNS was purchased from Molecular Probes, Inc. and used as received.

TLC plates were E. Merck Silica 60 F-54 precoated, glass-backed, 0.25-mm-layer thickness, and reversed-phase plates used were RP-18 F_{254} S precoated, glass-backed, 0.25-mm-layer thickness from EM Science. TLC eluants are as follows: A = 7:7:5:4 EtOAc/2-propanol/concentrated NH_4OH/H_2O; B = 7:7:5 EtOAc/2-propanol/H_2O; C = 1:1 E/PAW = EtOAc/(55:20:25 pyridine/HOAc/H_2O); D = 1:1 CH_3CN/H_2O. TLC spots were visualized with the following indicators: for carbohydrates, 9.2 mL of *p*-anisaldehyde in 338 mL of 95% EtOH, 3.75 mL of glacial HOAc, and 12.5 mL of concentrated H_2SO_4 (10–20 min at 100–150 °C gives blue to black colors with cyclodextrins); for amines, 0.3% ninhydrin in 1-butanol containing 3% HOAc (10 min at 125–150 °C).

pK_a Determinations. pK_a values were determined by titrations run on a computer-controlled apparatus using a relatively slow responding pH electrode with a separate calomel reference cell and a salt bridge which was added to reduce stirring noise. A circulating water bath was used to maintain a constant temperature (25 °C). Primary data were stored as electrode voltage, then converted to pH by using the average of buffer calibrations made before and after the titration. Titrant additions ($HClO_4$ or KOH) were made in constant-volume aliquots by means of a compressed air, plunger-controlled pump activated by signals from the computer. Typically, 98 titrant additions were made at least 4 min apart. Upon completion of the titration, the computer converts the stored voltage readings to pH. A TITAN program[47] is used to fit pH vs titrant volume data by first numerically differentiating the titration data, then sorting the differentiated data to find the highest point. The pH at the point of maximum for these data is then taken as the starting value for pK_a in a one-component model.

Conductivity Experiments. Conductivity measurements were recorded on a Radiometer/Copenhagen CDM 83 conductivity meter with (variously) one of three conductivity cells: a PP1042 immersion conductivity cell, a CDC114 flow and pipet conductivity cell, or a DC314 flow and pipet conductivity cell. Aqueous glycolipid solutions ($[5]_{initial}$ = 1.11 mM; $[4]_{initial}$ = 1.72 mM) were filtered through a 0.2-μm disposable filter into separate vials and equilibrated to 23 °C. A conductivity electrode was lowered into each solution and the conductivity monitored as a function of [glycolipid], which was lowered by incremental addition of H_2O (also equilibrated to 23 °C).

DLS Experiments. Dynamic light scattering experiments were performed on a modified Wyatt Technology Dawn F light scattering photometer using a stabilized HeCd laser source (441.6 nm, Liconix Model 4210N, 0.3-mm beam diameter, Liconix Model 50SA power stabilizer). This photometer is a fixed multiangle fiber optic coupled instrument. Scattering angles of 27.5 and 96.7° were used. Samples were prepared by dissolving the glycolipid in ultrapure water and filtering through a 0.2-μm disposable polycarbonate filter into a cuvette and irradiating at ambient temperature (22–24 °C).

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NMR Experiments. Some spectroscopic data is presented in the supplementary material. The pulse program used for 1D NOE experiments was Brüker DISNMR microprogram NOEDIFF.AUR with a decoupling power of 0.1–1.0 mW on a 300-MHz instrument. The glycolipids were present in near saturating solution (in DMSO-d_6 or D_2O) and were degassed under aspirator vacuum for 30 min. Each sample was equilibrated at the designated temperature for 15 min. Net enhancements were calculated from peak area differences. To examine the effect of temperature on the NMR spectrum, **5** was dissolved in 1 mL of D_2O in an unsealed 5-mm NMR sample tube and degassed under aspirator vacuum for 30 min at 23 °C and then sealed with a cap and Teflon tape. ^1H NMR spectra were obtained at 30, 45, 60, 75, and 90 °C, allowing 15 min for equilibration at each temperature. The 2D NOE spectra were obtained on a 500-MHz instrument and the glycolipid ([**4**] = 1 mg/mL) was maintained at 21 °C. The pulse sequence was D1-90-D0-90-D9-90-FID (from Brüker DISNMR microprogram NOES.AUR) where D1 = prepulse delay time (1 s), the 90° pulse was 12.5 μs, D0 = the evolution time (initial delay of 3 μs, with an increment time of 438 μs for each of 256 files), and D9 is the mixing time (set at 0.6 s) with randomly varied time (V9 = 20%) to suppress zero-quantum *J*-coupling cross peaks. The data size was 1K words by 512 words with zero filling of 512 words and the data resolution was 4.459 Hz/point.

Titration Experiments. NMR experiments were performed at 300 MHz on Brüker instruments in D_2O with either internal TSP or external TMS as a reference. Internal TMS was used in the DMSO-d_6 titrations. Tris (5.15 mM) was adjusted to an apparent pH of 7.01 in D_2O. Individual samples were made up from stock solutions, agitated for 1 min with a Vortex-Genie, lowered into the NMR probe, and equilibrated for 5 min to 30 °C. Titration experiments using absorption spectroscopy were performed in a similar manner on an IBM-9420 double-beam spectrometer; Δabs was monitored against [titrant] and the temperature maintained at 30 °C with use of a circulating bath connected to a turret cell holder; each sample was allowed to equilibrate for 15 min prior to measurement. Fluorescence spectra were performed on a Shimadzu RF5000U spectrofluorophotometer with 2-*p*-toluidino-6-naphthalenesulfonate (TNS) as fluorophore with $\lambda_{excitation}$ = 320 nm.

Mono-6-deoxy-6-(*p*-tolylsulfonyl)-β-cyclodextrin (2). β-Cyclodextrin (60.0 g, 52.9 mmol) was suspended in 500 mL of water, and NaOH (6.57 g, 164.0 mmol) in 20 mL of water was added dropwise over 6 min. The suspension became homogeneous and slightly yellow before the addition was complete. *p*-Toluenesulfonyl chloride (10.08 g, 52.9 mmol) in 30 mL of acetonitrile was added dropwise over 8 min, causing immediate formation of a white precipitate. After 2 h of stirring at 23 °C the precipitate was removed by suction filtration and the filtrate refrigerated overnight at 4 °C. The resulting white precipitate was recovered by suction filtration and after drying for 12 h (0.005 mmHg) afforded 7.18 g of a pure white solid. A second crop gave 0.207 g which was indistinguishable from the first in all respects. The combined crops (11% yield) gave a white solid: mp 179 °C slow dec; TLC (SiO_2) one spot at R_f 0.61 (eluant A) and R_f 0.60 (eluant B), *p*-anisaldehyde, I_2, and UV positive; $[\alpha]^{23.5}_D$ +50.7°; ^1H NMR (300 MHz, DMSO-d_6) δ 7.74 (d, *J* = 8.07 Hz, 2 H), 7.42 (d, *J* = 8.02 Hz, 2 H), 5.87–5.58 (m, 14 H), 4.82 (br s, 4 H), 4.76 (br s, 3 H), 4.55–4.13 (m, 6 H), 3.74–3.43 (m, 28 H), 3.42–3.18 (m, overlaps with HOD), 2.42 (s, 3 H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 144.9 (s), 132.8 (s), 129.8 (d), 127.8 (d), 102.1 (m), 81.8 (d), 73.3–71.4 (m), 70.0, 68.7, 59.5 (t), 21.1 (q) ppm; IR (KBr) ν = 1320 cm^{-1}; UV (1% CH_3OH/H_2O) λ_{max} (log ε) = 262 (2.74), 267 (2.70), 273 nm (2.64); FAB-MS on a diethanolamine matrix,[48] *m/z* calcd for $C_{46}H_{80}NO_{36}$ (M − OTs + $(HOCH_2CH_2)_2NH_2^+$) 1222.45, measured 1222.25. Anal. Calcd for $C_{49}H_{76}O_{37}S{\cdot}3H_2O$: C, 43.81; H, 6.15; S, 2.39. Found: C, 43.64; H, 6.09; S, 2.38.

Mono-6-deoxy-6-azido-β-cyclodextrin.[49] **Procedure A.** Powdered β-CDOTs (0.500 g, 0.388 mmol) was suspended in dry DMF (1.5 mL); after warming to 63 °C, the mixture became homogeneous. Crystalline KI (0.032 g, 0.194 mmol) and NaN_3 (0.252 g, 3.8 mmol) were added, and the reaction mixture was stirred at 60–65 °C 24 h. The reaction mixture was cooled to room temperature and treated with Amberlite MB-3 resin to remove salts. Acetone (100 mL) was added, producing a white precipitate. Recovery by suction filtration and drying overnight (10 mmHg) yielded a pure white solid (0.398 g, 88%): mp 206 °C slow dec; $[\alpha]^{22.0}_D$ + 108.2°.

Procedure B. β-CD-I (0.655 g, 0.526 mmol) was suspended in dry DMF (4.0 mL), NaN_3 (0.103 g, 1.5, mmol) was added, and the resulting suspension was warmed to 60–63 °C (which dissolved the solid components). After 24 h at elevated temperature the reaction mixture was cooled to 23 °C and treated with Amberlite MB-3 resin to remove unwanted salts. A pale white solid was obtained (0.548 g, 90%): mp 210 °C dec; $[\alpha]^{23.0}_D$ +83.3°.

TLC (SiO_2) R_f 0.71 (eluant A), *p*-anisaldehyde positive, ^1H NMR (300 MHz, DMSO-d_6) δ 5.84–5.60 (m, 14 H), 4.87 (shoulder, 1 H), 4.81 (br s, 6 H), 4.57–4.42 (m, 6 H), 3.80–3.45 (m, 28 H), 3.43–3.22 (br s, overlaps with HOD) ppm; ^{13}C NMR (75 MHz, D_2O) δ 102.1, 101.9, 82.3, 81.3, 73.3, 73.1, 72.1, 70.8, 60.3, 51.1 ppm; IR (KBr) ν = 2030 (N_3) cm^{-1}.

Mono-6-deoxy-6-amino-β-cyclodextrin Hydrochloride (3).[49,50] β-CD-N_3 (0.340 g, 0.293 mmol) was dissolved in 100 mL of water, 5% Pd/C was added, and the resulting suspension was subjected to 1 atm of H_2 (under a balloon). After 12 h at 23 °C the suspension was filtered through a bed of Celite and the clear filtrate lyophilized to give a fluffy, white solid (0.289 g, 87%): mp 203 °C slow dec; $[\alpha]^{23.0}_D$ +89.0°; TLC (SiO_2) R_f 0.40 (eluant A), ninhydrin and *p*-anisaldehyde positive; IR (KBr) disappearance of azide band at ν 2030 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 4.96 (shoulder, 1 H), 4.93 (br d, 6 H), 3.87–3.59 (m, 28 H), 3.59–3.32 (m, 14 H) ppm. This material (120 mg in 20 mL of H_2O) was subjected to chromatography on a Sephadex CM-25 ion-exchange column (2.5 × 10 cm) eluted with a water to 0.25 M aqueous NaCl gradient. Lyophilization of the appropriate fractions yielded a fluffy white solid which was desalted by filtration through Sephadex G-15 (2.5 × 65 cm) to give, after lyophilization, 71 mg of fluffy white solid (57%): mp 201 °C dec; TLC (SiO2) R_f 0.43, 0.13, 0.03 (eluants A, B, C) positive by ninhydrin and *p*-anisaldehyde; $[\alpha]^{23.0}_D$ +86.4°; ^1H NMR (300 MHz, D_2O) δ 5.07–4.99 (m, 7 H), 3.95–3.68 (m, 28 H), 3.68–3.41 (m, 14 H) ppm; ^{13}C NMR (75 MHz, D_2O): δ 101.9, 101.4, 82.9, 81.1, 80.7, 74.0–71.1, 67.9, 60.2 ppm; IR (KBr) ν = 2870–2865 (NH_3^+) cm^{-1}; FAB-MS, *m/z* calcd for $C_{42}H_{72}NO_{34}$ (M^+ − Cl) 1134.39, measured 1134.39. Anal. Calcd for $C_{42}H_{72}ClNO_{34}{\cdot}3H_2O{\cdot}NH_4Cl$: C, 39.47; H, 6.47; N, 2.20. Found: C, 39.42; H, 6.59; N, 2.49.

Mono-6-deoxy-6-(octylamino)-β-cyclodextrin Hydrochloride (4). 1-Octylamine (100 mg, 0.775 mmol) was dissolved in dry DMF (2.0 mL) and warmed to 70 °C. DMAP (97.0 mg, 0,795 mmol) and KI (32.2 mg, 0,194 mmol) were added, annd after 5 min powdered β-CDOTs (500 mg, 0.388 mmol) was added. The reaction was stirred for 36 h at 70 °C. The reaction was cooled to 23 °C, and the volatile materials were removed in vacuo to give a yellow, viscous oil. Cold water (30 mL) was added and the resulting precipitate collected. Approximately 15 mL of eluant C were added and vigorous stirring resulted in a slightly cloudy, yellowish solution. This was filtered through a Kim-wipe-stuffed pipet and the clear, yellowish filtrate purified by flash chromatography (SiO_2, 5.5 × 12.5 cm, eluant C). The proper fractions were combined and concentrated to a white solid by using alternating azeotropes of toluene and heptane. This solid was subjected to chromatography on a CM-25 ion-exchange column (2.5 × 5 cm bed) eluted with a water to 0.1 M aqueous NaCl (or NH_4Cl) gradient. Lyophilization of the appropriate fractions yielded a white solid which was desalted by filtration through Sephadex G-15 (2.5 × 60 cm bed eluted with H_2O) which in turn yielded after lyophilization 36.0 mg of a fluffy white solid (7%): mp 221 °C dec; TLC (SiO_2) R_f 0.65, 0.56, 0.15 (eluants A, B, C) positive by *p*-anisaldehyde; TLC (reversed phase) R_f 0.10 (eluant D), positive by *p*-anisaldehyde; $[\alpha]^{22.5}_D$ +14.3°; ^1H NMR (300 MHz, DMSO-d_6) δ 6.05–5.65 (m, 14 H), 4.81 (br d, 7 H), 4.60–4.50 (br s, 7 H), 3.80–3.45 (m, 28 H), 3.45–3.20 (overlaps with HOD); 3.12 (br t, 2 H), 2.85–2.75 (m, 2 H), 1.69–1.45 (m, 2 H), 1.40–1.30 (shoulder, 2 H), 1.23 (s, 6 H), 0.85 (t, *J* = 6.73 Hz, 3 H) ppm; ^1H NMR (300 MHz, D_2O) δ 5.05–4.94 (m, 3 H), 4.94–4.88 (m, 4 H), 3.85–3.58 (m, 21 H), 3.58–3.30 (m, 21 H), 3.20 (br t, 2 H), 2.70–2.55 (m, 2 H), 1.50–1.40 (m, 2 H), 1.31–1.21 (shoulder, 2 H), 1.17 (br s, 6 H), 0.81 (t, *J* = 6.78 Hz, 3 H) ppm; ^{13}C NMR (75 MHz, D_2O) δ 103.19, 103.17, 103.14, 100.05, 82.89, 81.24, 80.75, 79.95, 78.82, 74.0–70.06, 64.84, 60.06, 59.97, 47.37, 30.42, 28.77, 27.38, 22.78, 13.47 ppm; IR (KBr) ν = 2865 cm^{-1}; FAB-MS, *m/z* calcd for $C_{50}H_{88}NO_{34}$ (M^+ − Cl) 1246.52, measured 1246.15. Anal. Calcd for $C_{50}H_{88}ClNO_{35}{\cdot}3H_2O{\cdot}NaCl$: C, 43.04; N, 1.00; Cl, 5.08. Found: C, 42.98; N, 0.53; Cl, 5.33.

Mono-6-deoxy-6-(hexadecylamino)-β-cyclodextrin *p*-Toluenesulfonate. 1-Hexadecylamine (0.375 g, 1.55 mmol) was suspended in dry DMF (4.5 mL); warming to 63.0 °C gave a homogeneous, light yellow solution (10 min). Solid DMAP (0.194 g, 1.59 mmol), crystalline KI (0.0304 g, 0.183 mmol), and β-CDOTs (1.00 g, 0.776 mmol) were added, and the solution was stirred for 24 h. The reaction was terminated with the additon of 7 mL of cold water to form a white precipitate which was recovered by suction filtration and dried overnight over P_2O_5 at 0.01 mmHg in a desiccator to give 0.567 g of white solid. This solid was treated with 10 mL of eluant C, filtered, and divided into two equal volumes, and the two volumes were each successively subjected to flash chromatography on the

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same 5.5 × 12 cm column of SiO_2 eluted with eluant C. The proper fractions were combined and evaporated to a pale white solid by rotary evaporation using alternating azeotropes of 3 × 200 mL of toluene and 3 × 200 mL of *n*-heptane. Drying the isolated solid product overnight in vacuo (0.01 mmHg) followed by lyophilization from 25 mL of water gave 0.340 g (25%) of a light, pale-yellow solid: mp 205 °C slow dec; TLC (SiO_2) R_f 0.82 and 0.26 (eluants A and C), positive by ninhydrin, *p*-anisaldehyde, UV; $[\alpha]^{22.5}_D$ +16.5°; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.48 (d, J = 7.26 Hz, 2 H), 7.11 (d, J = 7.38 Hz, 2 H), 5.77 (br s, 14 H), 4.66 (shoulder, 1 H), 4.61 (br s, 6 H, H1), 4.53 (br s, 6 H), 3.75–3.43 (m, 28 H), 3.43–3.20 (m, overlaps with HOD), 3.13 (br t, 2 H), 2.77–2.51 (m, 2 H), 2.27 (s, 3 H), 1.62–1.37 (m, 2 H), 1.22 (s, 24 H), 0.84 (br t, J = 6.90 Hz, 3 H) ppm; ^{1}H NMR (300 MHz, D_2O) δ 7.53 (d, J = 7.73 Hz, 2 H), 7.21 (d, J = 7.86 Hz, 2 H), 5.03–4.90 (m, 7 H), 3.88–3.63 (m, 21 H), 3.63–3.20 (m, 21 H), 3.04 (br t, 2 H), 2.89–2.73 (m, 2 H), 2.26 (s, 3 H), 1.61–1.53 (m, 2 H), 1.22 (s, 14 H), 1.15 (br s, 10 H), 0.85 (t, J = 6.78 Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 145.4 (s), 133.1 (s), 127.9 (d), 125.4 (d), 101.9 (d), 83.5 (d), 81.6 (d), 74.6–71.1 (m), 70.5 (d), 59.9 (t), 49.4 (t), 31.2 (t), 29.5, 28.9, 28.6, 26.7, 22.0, 14.0 (br s) ppm; IR (KBr) ν = 1320 (O=S=O) cm^{-1}.

Mono-6-deoxy-6-(hexadecylamino)-β-cyclodextrin Hydrochloride (5). A Sephadex CM-25 ion-exchange column was prepared by suspending 10 g (dry weight) of the stationary phase in 300 mL 3:2 ethylene glycol/water (degassed in suction flask under aspirator vacuum for 300 min) for 2 h. The suspension was degassed in a similar manner (45 min) prior to forming a bed of 3 × 11.5 cm. After equilibration, β-CDNH$_2$C$_{16}^+$-OTs$^-$ (0.125 g) was dissolved in 5 mL of eluant and adsorbed onto the column and eluted with a gradient of 3:2 ethylene glycol/water to 3:2 ethylene glycol/0.25 M aqueous NaCl (or NH_4Cl). Fractions (12 mL each) were analyzed either by optical rotation or TLC. The appropriate fractions were combined and concentrated by rotary evaporation to a light yellow solution (ca. 60 mL). Dilution to 750 mL with acetone produced a cloudy suspension and suction filtration led to recovery of a white solid which was triturated rapidly with 25 mL followed by 5 mL followed by another 5 mL of cold water. The remaining residue was dried overnight in vacuo (0.01 mmHg), and the three supernatants were lyophilized. The remaining residue was revealed to be pure product (white solid; 0.108 g, 95%): mp 222 °C slow dec; TLC (SiO_2) R_f 0.77, 0.69, 0.24 (eluants A, B, C), ninhydrin and *p*-anisaldehyde positive, R_f 0.14 (reversed phase, eluant D), *p*-anisaldehyde positive; $[\alpha]^{21.5}_D$ +18.3°; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.80–5.61 (m, 14 H), 4.93 (br s, 1 H), 4.82 (br s, 6 H), 4.62–4.52 (m, 6 H), 3.85–3.45 (m, 28 H), 3.45–3.20 (m, overlaps with HOD), 3.10 (br t, 2 H), 2.85–2.72 (m, 2 H), 1.62–1.45 (m, 2 H), 1.22 (s, 24 H), 0.84 (t, J = 6.89 Hz, 3 H) ppm; ^{1}H NMR (300 MHz, D_2O) δ 5.04–4.97 (m, 3 H), 4.95 (br s, 4 H), 3.84–3.65 (m, 21 H), 3.63–3.41 (m, 21 H), 3.26 (br t, 2 H), 2.90–2.77 (m, 2 H), 1.61–1.51 (m, 2 H), 1.26 (s, 14 H), 1.19 (br s, 10 H), 0.82 (t, 3 H, J = 6.80 Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) 102.1 (d), 101.6 (s), 83.6 (d), 82.3 (s), 81.7 (d), 73.3–71.8 (m), 62.9 (d), 60.6 (s), 60.1 (t), 48.2 (t), 31.5 (t), 29.2, 26.3, 22.3 (br s), 14.2 (q) ppm; IR (KBr) ν = 2865 cm^{-1}; FAB-MS, m/z calcd for $C_{58}H_{104}NO_{34}$ (M^+ − Cl) 1358.64, measured 1358.54. Anal. Calcd for $C_{58}H_{104}ClNO_{34}\cdot 8H_2O\cdot 2NaCl$: C, 42.07; N, 0.85; Cl, 6.42. Found: C, 42.31; N, 0.90; Cl, 6.24.

Mono-6-deoxy-6-(didecylamino)-β-cyclodextrin Acetate (6). Didecylamine (462 mg, 1.55 mmol) was dissolved in dry DMF (4.0 mL) and the flask warmed to 75 °C. DMAP (194 mg, 1.59 mmol), KI (64.4 mg, 0.388 mmol), and β-CDOTs (1.00 g, 0.776 mmol) were added to the reaction mixture to give a clear yellow solution. After 24 h at 75 °C the reaction was cooled to 23 °C, and the volatile materials were removed in vacuo (0.01 mmHg, 8 H). The resulting yellow viscous oil was treated with 25 mL of cold water and the ensuing white precipitate collected by filtration. Allowing the clear filtrate to stand at 23 °C for 24 h afforded a second crop of precipitate. The combined crops were purified by flash chromatography (SiO_2, 5.5 × 13.5 cm, eluant C). A pale-white solid was obtained (277 mg, 24%): mp 212 °C dec; TLC (SiO_2) R_f 0.86, 0.70, 0.23 (eluants A, B, C) positive by *p*-anisaldehyde; TLC (reversed phase) R_f 0.24 (eluant D) *p*-anisaldehyde positive; $[\alpha]^{23.0}_D$ +13.3°; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.85–4.71 (br m, 7 H,), 3.80–3.45 (m, 28 H), 3.45–3.15 (br s, overlaps with HOD), 1.81–1.65 (br s), 1.53–1.39 (br m, 4 H), 1.23 (br s, 20 H), 1.19 (br s, 12 H), 0.86 (t, J = 6.77 Hz, 6 H) ppm; ^{1}H NMR (300 MHz, D_2O) δ 5.12–5.01 (br m, 7 H), 3.95–3.68 (m, 21 H), 3.68–3.42 (m, 21 H), 3.05 (br t, 4 H), 1.81–1.66 (br s), 1.52–1.32 (br m, 4 H), 1.23 (s, 24 H), 1.19 (br s, 8 H), 0.86 (t, J = 6.60 Hz) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.71, 102.39, 100.72, 84.00, 81.59, 74.50–71.11, 67.43, 61.19, 60.72, 50.40, 34.78, 28.01, 27.55, 23.39, 15.61 ppm; IR (KBr) ν = 2875 cm^{-1}; FAB-MS, m/z calcd for $C_{62}H_{112}NO_{34}$ (M − OAc) 1446.70, measured 1446.43.

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Supplementary Material Available: Six figures including a graph of conductivity vs time and ^{1}H NMR spectra of **5**, graph of binding isotherms for β-CD and CCA titrated against 4-NP and **3**, respectively, and NOESY and COSY spectra of **4** (6 pages). Ordering information is given on any current masthead page.

Symmetric Intramolecular Proton Transfers between Oxygen Atoms in Anionic Systems. An ab Initio Study

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Abstract: Three symmetric intramolecular proton exchanges involving oxygen atoms in negatively charged systems have been studied by ab initio methods. An important rearrangement of the electronic charge density takes place as the reactions proceed. The O–O bond is broken in the hydroperoxide anion at the transition state, the energy barrier being very large. In the water–hydroperoxide anion complex the water molecule acts as a bifunctional catalyst, the O–O bond remaining formed and the energy barrier being significantly lowered. Finally, the proton transfer in the glycolate anion occurs through a C_{2v} transition state whose total electronic charge density presents a ring point, the energy barrier being very small.

I. Introduction

Proton transfer reactions are one of the most important problems in chemical and biochemical systems.[1,2] Understanding and modeling of all factors involved in such transfers is a very interesting challenge for theoretical chemistry. An accurate description of the most relevant regions of the potential energy hypersurfaces is required as a first step, in this way supplying information that is not easily available from experiment and providing a starting point for a later modeling on the dynamics of the proton transfer. In recent years ab initio molecular orbital methods have become an important tool in the study of this field.

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