Complexation of Hydrophobic Sugars and Nucleosides in Water with Tetrasulfonate Derivatives of Resorcinol Cyclic Tetramer Having a Polyhydroxy Aromatic Cavity: Importance of Guest-Host CH-π Interaction

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Abstract: Relatively hydrophobic monosaccharide derivatives (some aldopentoses and deoxy and methylated sugars) including nucleoside cytidine and its 5'-phosphate, as well as simple aliphatic alcohols (ethanol through hexanol), are bound to the tetrasulfonate derivatives **1a-c** of resorcinol cyclic tetramer via a well-defined host-guest complexation in water. On the other hand, unmodified aldohexoses, disaccharides, and the nucleoside uridine show much lower affinities. The ^{1}H NMR complexation-induced shift data indicate that the hydrophobic moiety, e.g., methyl, of the guest is incorporated in the polyhydroxy aromatic cavity of the host. In respect to the change in substituent X on 2-C of the benzene rings of the host, both **1b** (X = CH_3) and **1c** (X = OH) exhibit higher binding affinities than the parent host **1a** (X = H). These results suggest that guest-host CH-π interaction involving electron-rich benzene rings of the host as π-bases is at least partially responsible for the present host-guest complexation in water. The implications of the present findings are discussed in light of biological sugar-binding processes.

Introduction

Selective binding of sugars is a growing area of molecular recognition.[2] A general strategy is to use multiple hydrogen-bonding interactions in apolar organic media.[2a-d] In view of biological molecular recognition of sugars, and especially cell-surface oligosaccharides,[3] much attention should also be paid to the sugar binding in water. This is, however, not an easy task.[4] The host-guest hydrogen bonding becomes far less effective in aqueous media. In addition, simple sugar derivatives are neutral and highly hydrophilic. They could not be *good* guests, so far as the two major association forces in water, i.e., the so-called hydrophobic effects[5] and electrostatic interactions,[6] are concerned. In fact, aqueous host-guest complexation, so far investigated by using cyclodextrins,[7] cyclophanes,[6,8-10] and polyazamacrocycles[11] as hosts, has been mostly concerned with the binding of ions and relatively hydrophobic molecules as guests, including those of biological origin such as amino acids,[12] steroids,[13] terpenes,[14] and nucleotides.[6,11] Although scattered information is available as to the sugar-cyclodextrin[15] and sugar-cyclophane interactions,[16] there is at present no rational guide for the design of artificial sugar-binding hosts working in aqueous media. A hint may be provided by a survey of the X-ray structures of sugar-protein complexes,[17] where both polar (hydrogen bonding) and apolar (CH-π) interactions are involved.

Resorcinol cyclic tetramer **1** is a metacyclophane having a bowl-shaped[2b] aromatic cavity made up of four electron-rich dialkyldihydroxybenzene rings. The lipophilic compound **1d** has been shown to extract sugars into apolar organic media upon

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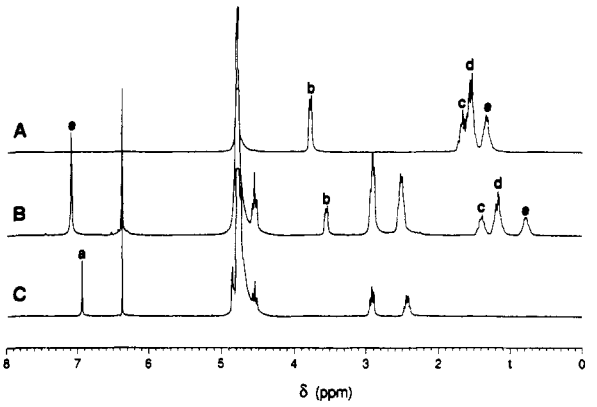
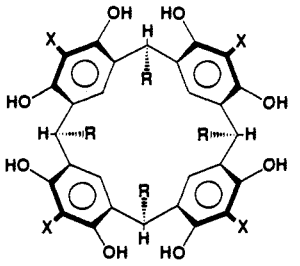


Figure 1. ^{1}H NMR spectra of (A) guest **7c** (40 mM), (C) host **1a** (40 mM), and (B) an equimolar mixture of **1a** (40 mM) and **7c** (40 mM) in D_2O at 25 °C. Assignments: (a) aromatic 5-H of **1a**; (b) 1-H and 2-H of **7c**; (c) and (d) two types of 3-H and 6-H of **7c**; (d) and (e) two types of 4-H and 5-H of **7c**.

formation of hydrogen-bonded host–guest complexes.[2a-c] It also forms a stable monolayer to which sugars in water bind selectively.[16] In the present work, we have prepared water-soluble tetrasulfonate derivatives **1a–c**.[18] We report here that sugars, especially deoxy and methylated derivatives, are bound to the present artificial receptors in water via a well-defined host–guest complexation, where guest–host CH–π interaction plays an important role.

Results and Discussion

Tetrasulfonated Hosts. Acid-catalyzed condensation of sodium 2-formylethane-1-sulfonate, generated in situ from its trimethylene cyclic acetal, with resorcinol afforded a tetrasulfonate derivative **1a** of resorcinol cyclic tetramer. Similar condensation with 2-methylresorcinol or pyrogallol (1,2,3-trihydroxybenzene) followed by careful purification[19] gave an analogous compound **1b** or **1c** having four additional methyl or hydroxyl groups, respectively. Hosts **1a–c** are quite readily soluble in water with solubilities ≥0.4 M at room temperature. The resulting aqueous solutions showed no sign of bubbling when shaken. In addition, the surface tension of water at 10 °C (λ = 74.2 dyn/cm) was not much affected by the presence of even **1b**: λ = 72.0 and 68.9 at [**1b**] = 23.3 and 46.6 mM, respectively. These results indicate that hosts **1a–c** are too hydrophilic to be surface-active or micelle-forming.[21]



1a: R = $(CH_2)_2SO_3Na$; X = H

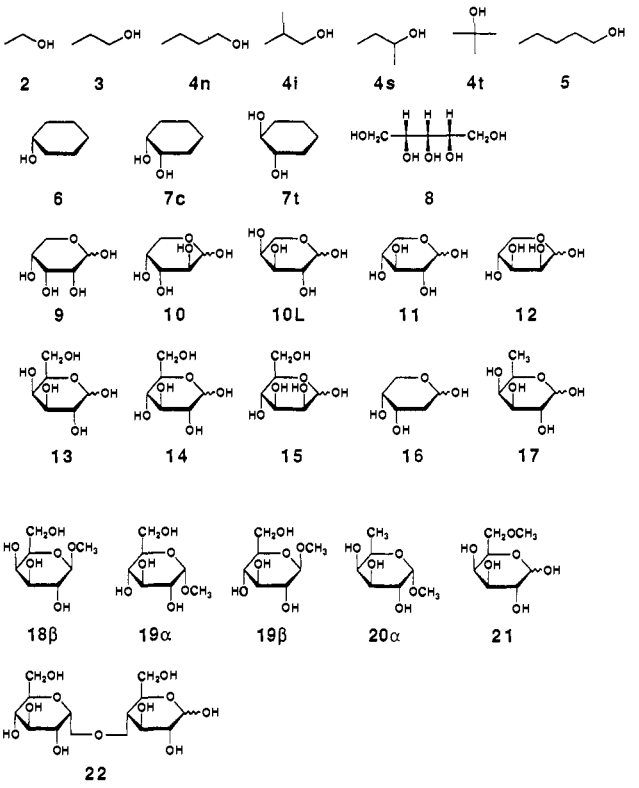
1b: R = $(CH_2)_2SO_3Na$; X = CH_3

1c: R = $(CH_2)_2SO_3Na$; X = OH

1d: R = $(CH_2)_{10}CH_3$; X = H

The pK_a value for the dissociation of the OH groups of compound **1a** in H_2O was determined by pH titration of the buildup

Chart I



of phenolate ion, as monitored by UV absorbance at 302 nm at 25 °C;[22] pK_a = 10.0, which was close to pK_a = 9.4 for compound **1** (R = CH_3 and X = H) in acetone-d_6–D_2O (3:5).[10d] The ^{1}H NMR spectrum of **1a** (1–2 mM) in D_2O showed a pair of sharp singlets at δ 6.38 (aromatic 2-H) and 6.94 (aromatic 5-H) and a sharp triplet at 4.55 (benzylic H) (Figure 1C). The corresponding resonances for other hosts (1–2 mM) appeared at δ 6.95 (**1b**) or 6.63 (**1c**) (aromatic 5-H) and 4.68 (**1b**) or 4.63 (**1c**) (benzylic H). The assignments of the two aromatic protons in **1a** were confirmed on the basis of the NOE correlation[23] between aromatic 5-H, benzylic H, and $CHCH_2CH_2SO_3Na$. The NMR results thus show that the four benzene rings and the four benzylic moieties of tetrasulfonate derivatives **1a–c** are equivalent, as in parent compound **1d**.[2a] This is taken as evidence that **1a–c** in water also take on a symmetrical (C_{4v}) bowl-shaped conformation.[24] The ^{1}H NMR spectra for hosts **1a** and **1c** showed practically no concentration dependence, while those for **1b** were somewhat dependent on [**1b**] (vide infra).

Complexation in Water. The interaction of various sugars and alcohols as references with host **1a** in water was investigated by means of ^{1}H NMR spectroscopy. The guests studied (Chart I) include aliphatic alcohols [ethanol (**2**), 1-propanol (**3**), 1-butanol (**4n**), 2-methyl-1-propanol (**4i**), 2-butanol (**4s**), 2-methyl-2-propanol (**4t**), and 1-pentanol (**5**)], cyclic mono- and diols [cyclohexanol (**6**) and *cis*- and *trans*-1,2-cyclohexanediol (**7c** and **7t**)], a sugar alcohol related to ribose [adnitol (**8**)], aldopentoses [ribose (**9**), arabinose (**10**), xylose (**11**), and lyxose (**12**)], aldohexoses [galactose (**13**), glucose (**14**), and mannose (**15**)], deoxy sugars [2-deoxyribose (**16**) and fucose (6-deoxygalactose, **17**)], methylated sugars [methyl β-galactopyranoside (**18**β), methyl α- and β-glucopyranoside (**19**α and **19**β), methyl α-fucopyranoside (**20**α), and 6-*O*-methylgalactose (**21**)], and a disaccharide [maltose (**22**)]. All of the sugars investigated were D enantiomers. In the case of arabinose (**10**), the L enantiomer **10L** was also used.

Figure 1 shows the ^{1}H NMR spectra of diol **7c** as a representative guest (A), host **1a** (C), and their 1:1 mixture (B) in D_2O.

(18) For the interaction of the tetradeprotonated species of **1** with ammonium guests in water, see refs 6 and 10.

(19) Crude products of symmetric hosts **1b** and **1c** were contaminated with their conformational isomers of lower symmetry.[10f,20] These had to be removed.

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(24) The tetradeprotonated tetraanion of **1** (R = CH_3 and X = H) was shown to possess strong hydrogen bonds between ArOH and ArO^- groups.[10]

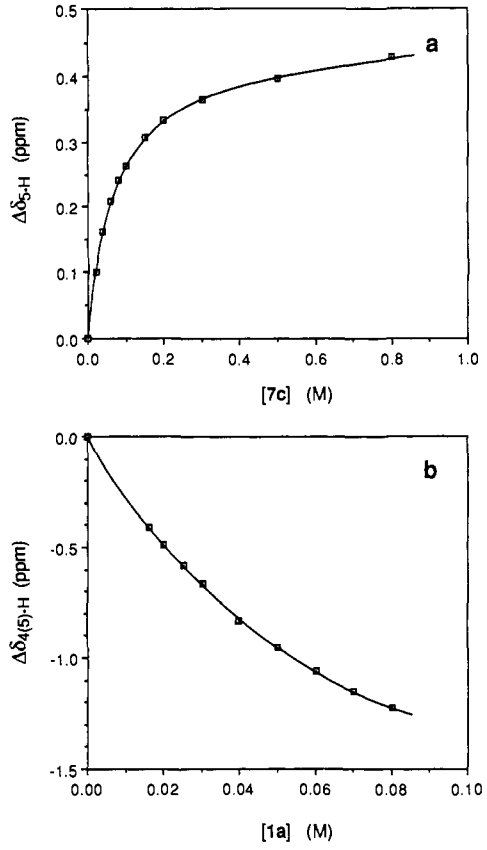


Figure 2. Complexation-induced shifts (negative value indicates an upfield shift) (a) for aromatic 5-H (a, referring to Figure 1) of host **1a** (2 mM) as a function of [**7c**] and (b) for the higher field component of 4-H and 5-H (e, referring to Figure 1) of guest **7c** (2 mM) as a function of [**1a**] in D_2O at 25 °C.

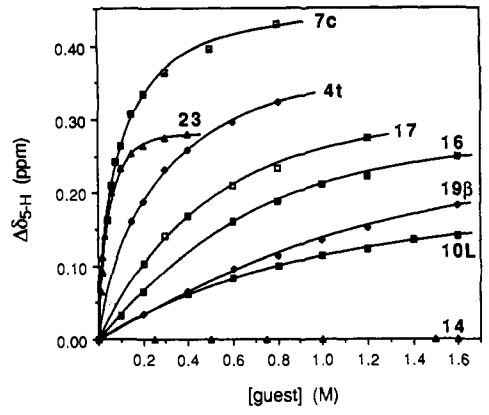


Figure 3. Complexation-induced shifts (negative value indicates an upfield shift) for aromatic 5-H of host **1a** (2 mM) in D_2O at 25 °C as a function of guest concentration.

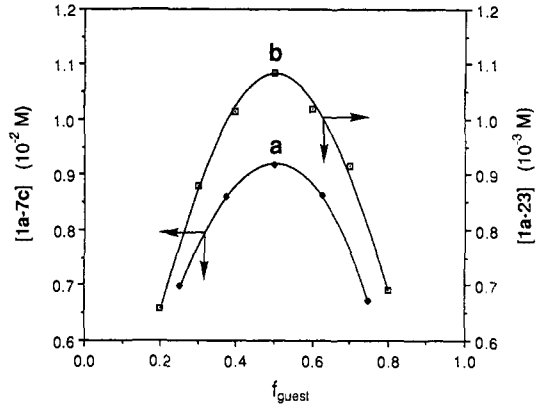


Figure 4. Job plots of [complex] vs mole fractions of guest (f_{guest}) for the complexation of host **1a** and guest **7c** (a) or **23** (b) in D_2O at 25 °C under conditions where [**1a**] + [guest] is maintained at 80 and 10 mM for **7c** and **23**, respectively.

Upon mixing, the guest signals (b–e) undergo upfield shifts, while that of the aromatic 5-H (a) of the host is shifted downfield. These shifts, also observed for other guests, are due to specific host–guest complexation and are not due to nonspecific change in the solvent properties upon addition of a guest or host. This is supported by a significant body of evidence as follows. (1) Both the guest-induced downfield shifts of 5-H (a, referring to Figure 1) of the host (Figure 2a) and the host-induced upfield shifts of the higher field component of 4-H and 5-H (e, referring to Figure 1) of the guest (Figure 2b) exhibit saturation with increasing [guest] or [host]. (2) There is a remarkable selectivity among guests. Thus, fucose (**17**) readily gives rise to complexation-induced shifts of the host signal in a similar manner as **7c**, while closely related galactose (**13**) and glucose (**14**) bring about little and no shifts, respectively. Arabinose (**10L**) shows an intermediate behavior. The selectivity in the sugar binding cannot be explained by medium effects. The titration data are shown in Figure 3, together with those for other typical guests such as **4t**, **7c**, **16**, and **19β**. (3) There is a site specificity for a given guest (vide infra). Thus, guest **7c** undergoes the largest **1a**-induced upfield shifts at 4-H and 5-H, followed by 3-H and 6-H; the hydroxymethine protons (1-H and 2-H) exhibit the smallest shifts (Figure 1). (4) In marked contrast to host **1a**, the ^1H NMR chemical shifts of resorcinol as reference are practically not affected by the present guests. (5) There is a competition between guests. Thus, for example, the extent of upfield shift for the methyl proton resonance of fucose (**17**, 2 mM) as induced by host **1a** (50 mM) is reduced in the presence of guest **7c** as a competitor; $\Delta\delta_{obsd} = -0.23$ and -0.11 at [**7c**] = 0 and 50 mM, respectively (negative value indicates an upfield shift).

The titration curves in Figure 2 are consistent with a 1:1 host–guest complexation. Benesi–Hildebrand analysis of the shifts in $\delta_{5\text{-H}}$ for the host (Figure 2a, i.e., under conditions of constant [**1a**] with varying [**7c**]) gave a binding constant $K = 14\ M^{-1}$ which was in reasonable agreement with $K = 12\ M^{-1}$, obtained by a similar treatment of the shifts in $\delta_{4(5)\text{-H}}$ for the guest (Figure 2b, i.e., under conditions of constant [**7c**] with varying [**1a**]).[25] The 1:1 stoichiometry was confirmed by the continuous variation (Job) plots of the concentrations of complex **1a**–**7c** vs mole fraction of **7c** (f_{guest}) under conditions where [**1a**] + [**7c**] was kept constant at 80 mM (Figure 4); the maximum occurs at $f_{guest} = 0.5$.

Hosts **1b** and **1c** behaved in a manner similar to the parent host **1a**; they exhibited characteristic complexation-induced shifts in $\delta_{5\text{-H}}$. Among **1a**–**c**, only **1b** showed concentration-dependent ^1H NMR spectra. The resonances for aromatic 5-H and 2-CH_3 of **1b** underwent downfield and upfield shifts, respectively, with an increase in [**1b**]; $\delta_{5\text{-H}} = 6.95, 6.95, 6.97, 7.00, 7.04, 7.10$, and 7.15 and $\delta_{2\text{-methyl}} = 2.06, 2.05, 2.02, 1.94, 1.84, 1.65$, and 1.49 at [**1b**] = 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 mM, respectively. These results indicate that tetramethylated host **1b** undergoes aggregation or self-binding at higher concentrations, but only to a negligible extent at ≤2 mM.

All of the binding constants were evaluated by the Benesi–Hildebrand analysis (referring to eq 1) of the shifts in $\delta_{5\text{-H}}$ for the host (Figure 3) under conditions of fixed [host] and varying [guest] in unbuffered D_2O at 25 °C; in eq 1, $\Delta\delta_{obsd} = \delta_{obsd} - \delta_{1a}$ and $\Delta\delta_{sat} = \delta_{sat} - \delta_{1a}$ are the observed and saturation shifts, respectively, of the chemical shifts of 5-H in the presence of a guest. The host and guest concentrations were chosen so as to

$$\frac{1}{\Delta\delta_{obsd}} = \frac{1}{\Delta\delta_{sat}} + \frac{1}{\Delta\delta_{sat}K[\text{guest}]_t} \qquad (1)$$

meet the Benesi–Hildebrand conditions, i.e., $[\text{guest}]_t/[\text{host}]_t \geq 10$ (t = total). In every case, plots of $1/\Delta\delta_{obsd}$ vs $1/[\text{guest}]_t$ according

(25) Analysis of the titration data for 3(6)-H of guest 7c (resonance c in Figure 1) gave a similar binding constant, $K = 13\ M^{-1}$.

Table I. Binding Constants (K)[a] for the Complexation of Host **1a**[b] with Various Guests and Saturation Shifts ($\Delta\delta_{sat}$) for the Aromatic 5-H of the Host[c]

guest	K (M^{-1})	$\Delta\delta_{sat}$ (ppm)	guest	K (M^{-1})	$\Delta\delta_{sat}$ (ppm)
2	<1[d]	0.46	**14**	~0	
3	≤1.7	0.36	**15**	~0	
4n	≤2.7	0.41	**16**	≤1.2	0.38
4i	≤3.1	0.42	**17**	≤1.8	0.40
4s	≤3.5	0.37	**18β**	<1[d]	0.53
4t	≤4.2	0.42	**19α**	<1[d]	0.42
5	≤5.7	0.39	**19β**	<1[d]	0.47
6	16	0.43	**20α**	≤1.8	0.46
7c	14	0.46	**21**	≤2.3	0.41
7t	14	0.42	**22**	~0	
8	~0		**23**	26	0.30
9	<1[d]	0.26	**24**	≤1.2	0.30
10	<1[d]	0.27	**25**	≤3.6	0.32
10L	<1[d]	0.27	**26**	20	0.33
11	~0		**27**	29	0.38
12	~0		**28**	~0	
13	~0				

[a] See ref 26 for the treatment of small binding constants. [b] [**1a**] = 2 mM in D_2O at 25 °C. [c] Positive value indicates a downfield shift. [d] The actual values obtained by the Benesi–Hildebrand analyses are 0.27 (**2**), 0.44 (**9**), 0.85 (**10**), 0.85 (**10L**), 0.39 (**18β**), 0.41 (**19α**), and 0.40 (**19β**).

Table II. Binding Constants (K)[a] for the Complexation of Hosts **1b** and **1c**[b] with Various Guests and Saturation Shifts ($\Delta\delta_{sat}$) of the Aromatic 5-H of the Host[c]

	host			
	1b		**1c**	
guest	K (M^{-1})	$\Delta\delta_{sat}$ (ppm)	K (M^{-1})	$\Delta\delta_{sat}$ (ppm)
4t	19	0.42	24	0.39
6	125	0.41	64	0.42
7c	80	0.45	80	0.42
10	≤2.1	0.39	≤2.5	0.35
16	≤4.9	0.47	≤3.9	0.42
17	≤6.0	0.38	≤8.4	0.38
19β	<1[d]	0.38	≤2.4	0.26
23	68	0.30	47	0.28
24	≤4.6	0.30		
27	110	0.30		

[a] See ref 26 for the treatment of small binding constants. [b] [**1a**] = 2 mM and [**1b**], [**1c**] = 2, 1, or 0.5 mM in D_2O at 25 °C. [c] Positive value indicates a downfield shift. [d] The actual value obtained by the Benesi–Hildebrand analysis is 0.60.

to eq 1 yielded an excellent straight line with a correlation coefficient $r \geq 0.997$. In Table I are summarized the binding constants (K) together with saturation shifts ($\Delta\delta_{sat}$) of the aromatic 5-H of host **1a**. The K and $\Delta\delta_{sat}$ values of hosts **1b** and **1c** for selected guests are shown in Table II. Small numbers for K are given with limiting values. This is because of possible deviation of the thermodynamically relevant activity from the concentrations at higher guest concentrations required for the binding assays.[26] The $\Delta\delta_{sat}$ values of hosts **1a–c** for a given guest are rather constant.

The binding behaviors were also studied for nucleosides [cytidine (**23**), uridine (**24**), and thymidine (**25**)] and reference compounds of **23** [cytidine 5′-phosphate (**26**), cytosine (**27**), and ribose 5′-phosphate (**28**)] (Chart II). The ^1H NMR spectra of nucleoside **23** as a representative guest (A), host **1b** (C), and their 1:1 mixture (B) are shown in Figure 5. In this case, the cytosine ring protons (b and c) and 1-H′ (d) of the sugar moiety show complexation-induced shifts, while other sugar CH proton resonances undergo almost no shifts. The Job plot for this complexation (also shown in Figure 4) is consistent, again with a 1:1 host–guest stoichiometry. The binding constants obtained above are also shown in Table I.

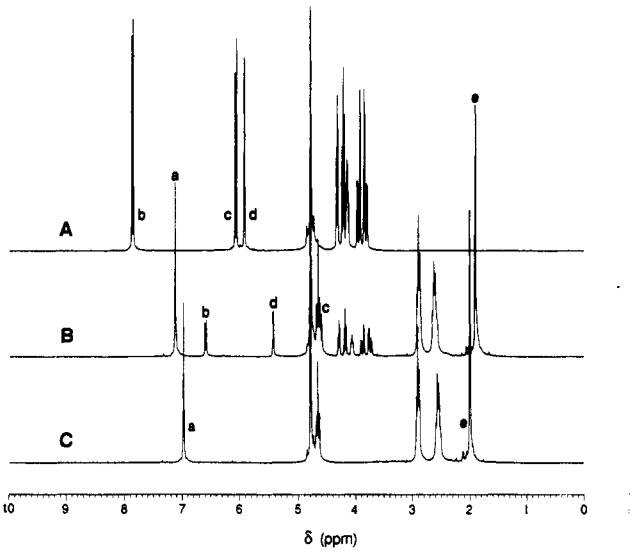
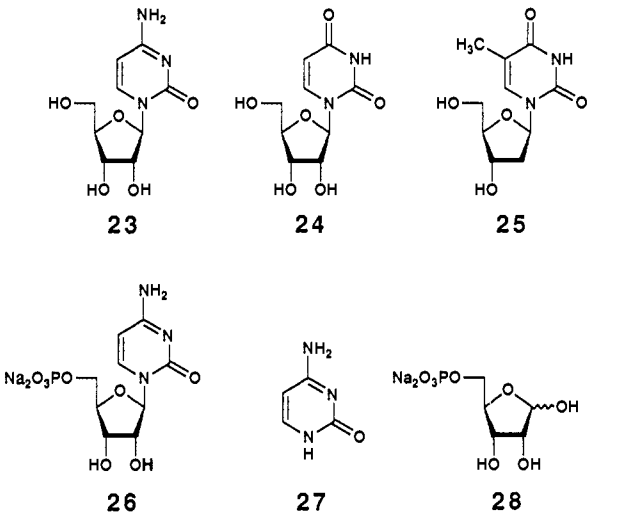


Figure 5. ^1H NMR spectra of (A) guest **23** (25 mM), (C) host **1b** (25 mM), and (B) an equimolar mixture of **1b** (25 mM) and **23** (25 mM) in D_2O at 25 °C. Assignments: (a) aromatic 5-H of **1b**; (b) 6-H of **23**; (c) 5-H of **23**; (d) 1′-H of **23**; (e) aromatic 2-CH_3 of **1b**.

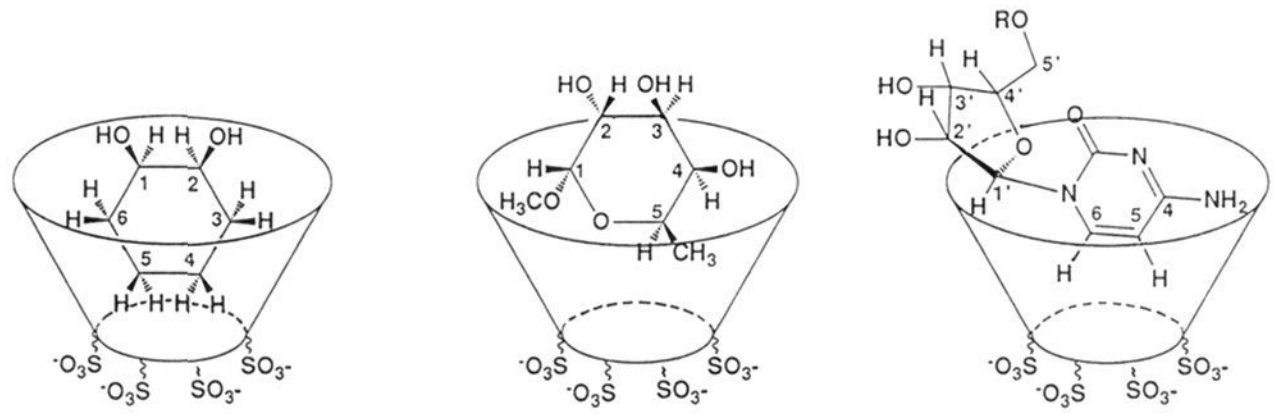
Chart II



Guests, especially sugars, at higher concentration ranges as used here might be aggregated. This is, however, not the case. Evidence for this includes the following. (1) Neither ^1H nor ^{13}C NMR resonances for the CH moieties of glucose (**14**) in a wide concentration range showed any concentration dependence. The actual data for 1-H and 1-C are δ_H, δ_C (concentration) = 5.25 (α) and 4.66 (β), 92.9 (α) and 96.7 (β) (0.4 M), and 5.25 (α) and 4.66 (β), 92.9 (α) and 96.7 (β) (1.6 M). (2) The linearity of the Benesi–Hildebrand plot indicates that there is only one complexation-responsible species for the sugar in the concentration range used (≤1.6 M). (3) *cis*-1,2-Cyclohexanediol (**7c**) is more hydrophobic and hence more susceptible to aggregation than sugars. The K's for **7c** obtained in two different ways (refer to Figure 2a,b) are in agreement with each other, indicating that complexation-inhibiting self-aggregation of guest **7c** up to 0.8 M (Figure 2a) is not taking place. (4) Pyrimidine nucleosides such as cytidine (**23**), uridine (**24**), and thymidine (**25**) as hydrophobic

(26) The actual data for sucrose, a disaccharide, in water indicate that deviation of the activity from the concentrations occurs at a concentration range ≥1 M (Moore, W. J. *Physical Chemistry*, 4th ed.; Prentice-Hall: Englewood Cliffs, NJ, 1972; Chapter 8). Let X be the value for the binding constant obtained from Benesi–Hildebrand analysis. A guest concentration range ≤1 M covered a good percent complexation to give X above 1 M^{-1}. In this case, X must be close to the activity-based true binding constant. For smaller X's, i.e., <1 M^{-1}, however, the deviation may be more significant; a higher guest concentration range (≥1 M) had to be used only to cover a lower complexation range (≤55%). Thus, depending on the magnitude of X, the binding constant K in Tables I and II is expressed as X when $10 < X$, ≤X when X is between 1 and 10, <1 when X is between 0 and 1, or ~0 in the case where the complexation shift is practically zero or too small to allow Benesi–Hildebrand treatment. Also see the Experimental Section.

Chart III



29 30 31

sugar derivatives undergo dimerization via stacking. The K's for dimerization, however, are very small,[27] i.e., 0.87 (**23**), 0.61 (**24**), and 0.91 M^{-1} (**25**).[28]

Apolar Guest Binding Sites. The binding constants for host **1a** (Table I) suggest that apolar CH moieties of the guests provide the primary binding sites. Supporting evidence for this is summarized as follows. (1) The binding constants generally decrease with decreasing hydrophobicities[29,30] of the guests; pentanol (**5**) > butanol (**4**) > propanol (**3**) > ethanol (**2**) and cyclohexanediol (**7**) > aldopentoses (**9–12**) ≥ aldohexoses (**13–15**). (2) Deoxy sugars exhibit enhanced affinities as compared with the parent oxy sugars, e.g., deoxyribose (**16**) > ribose (**9**). A clearer illustration of this comes from the decreasing binding constants: fucose (**17**, R = CH_3) > arabinose (**10L**, R = H) > galactose (**13**, R = CH_2OH); these differ only in the substituents (R) at 5-C. (3) Methyl substitution of a sugar OH group either at 6-C or 1-C is affinity-enhancing. Thus, 6-methylgalactose (**21**), methyl galactopyranoside (**18**), and methyl glucopyranoside (**19**) can be bound significantly more tightly than the parent monosaccharides **13** and **14**. The enhanced binding ability of thymine derivative **25** over uracil derivative **24** is in accord with this. The self-binding of tetramethylated host **1b** is interesting in this connection.

Binding Geometries. The ^1H NMR shift data are also consistent with the view that the apolar portion of a guest is incorporated into the aromatic cavity of the host so as to be subject to the diamagnetic shielding effects of the benzene rings. The complexation-induced shifts at saturation binding, $\Delta\delta_{sat}$ (ppm, negative value indicates an upfield shift), for three typical guests **7c**, **20α**, and **23** are as follows: for **7c** with **1a** as host, −2.5 and −1.6 (two types of H's on 4-C and 5-C), −1.6 and −1.3 (two types of H's on 3-C and 6-C), −1.1 (1-H and 2-H); for **20α** with **1a** as host,[31] −1.1 (1-OCH_3), −0.1 (2-H), −0.1 (3-H), −0.9 (4-H), −1.6 (5-H), −1.6 (5-CH_3); for **23** with **1b** as host, −1.7 (5-H), −1.5 (6-H), −0.6 (1′-H). The corresponding $\Delta\delta_{sat}$ values for the methyl groups of guests **4t** and **17** are −1.7 and −2.4, respectively. The largest values of $\Delta\delta_{sat}$ for a given guest are in the range −(1.5–2.5) ppm and are comparable with those for cyclophane–arene complexes in water[32] as well as for hydrogen-bonded complexes of host **1d** in organic media.[2b,33] The geometries of complexes, which are consistent with NMR shift data, are shown schematically in structures **29–31** (Chart III). In Figure 6 are shown the CPK models for host **1a** (top view) and guests **7c** and **20α** that allow a comparison of the sizes of host and guest.

Cyclic 1,2-diol **7c** undergoes the largest shifts at 4-H and 5-H. The simultaneous interaction of these vicinal hydrogens (structure **29**) may have some relevance to the selectivity among aldopentoses (**9–12** and **16**); only those (**9**, **10**, and **16**) where 3-H and 4-H are cis show substantial affinities. In fact, these cis-hydrogens in arabinose (**10L**) were shown by X-ray crystallography to simultaneously interact with the indole ring of Trp-16 of an arabinose-binding protein.[17c] In the case of methyl fucoside (**20α**), 5-H and 5-CH_3 undergo the largest shifts, while the shift for the other methyl group in 1-OCH_3 is significant too. The most hydrophobic 5-$CH(CH_3)$ moiety appears to be deeply incorporated into the cavity,[34] with the 1-OCH_3 group only weakly interacting with the host (structure **30**). This is consistent with an examination of CPK models. This might also explain why methyl fucoside (**20α**) having two CH_3 groups shows a binding constant (Table I) similar to that for fucose (**17**) having only one. As for the geometrical requirements of the binding, it is also interesting to note that ribose-related *linear* sugar alcohol **8** and a bulky glucose dimer **22** show no affinity to the host.

Nucleoside **23** in water takes a preferred anti conformation (structure **23**).[35] The complexation-induced shift data for this nucleoside suggest that **23** is bound edge-to-face in the syn conformation (structure **31**, R = H); otherwise, 5-H and 6-H on the cytosine ring and 1′-H on the sugar moiety are in an anti orientation and their simultaneous interaction with the host could not be expected.[36] The anti-to-syn conformational change upon binding is most likely for steric reasons; if *anti*-**23** were to be bound at the site of cytosine ring 5-H and 6-H, a severe steric interaction between the sugar moiety of **23** and the host would be encountered.

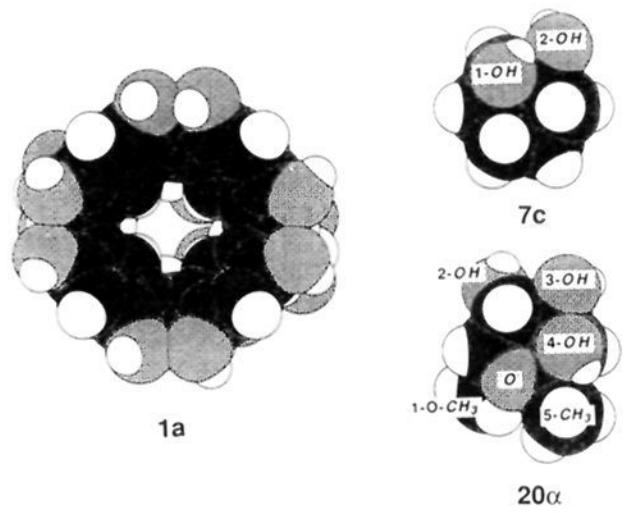


Figure 6. CPK models of host **1a** (top view) and guests **7c** and **20α**.

(27) Adenosine as a purine nucleotide undergoes more facile dimerization via self-stacking. The complexation of adenosine and host **1a** did not exhibit ideal behavior as a consequence.

(28) Ts'o, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. 1, pp 453–584.

(29) (a) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525. (b) Matsui, Y.; Mochida, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2808.

(30) (a) Janado, M.; Yano, Y. *J. Solution Chem.* **1985**, *14*, 891. (b) Miyajima, K.; Machida, K.; Nakagaki, M. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2595. (c) Yano, Y.; Tanaka, K.; Doi, Y.; Janado, M. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2963.

(31) The 1-H resonance for bound **20α** could not be identified because of overlap with other resonances.

(32) Ferguson, S. B.; Sanford, E. M.; Seward, E. M.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5410.

(33) (a) Tanaka, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1990**, *112*, 2807. (b) Kikuchi, Y.; Kato, Y.; Tanaka, Y.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1991**, *113*, 1349.

(34) CPK models indicate that only one methyl group can be deeply incorporated into the cavity of the host (refer to Figure 6).

(35) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984; Chapter 4.

(36) For nucleotide–cyclodextrin interactions, see: Komiyama, M.; Sawata, S.; Takeshige, Y. *J. Am. Chem. Soc.* **1992**, *114*, 1070.

Nucleotide **26** can also be bound in this way with a binding constant similar to that for nucleoside **23** (Table I). The anionic sulfonate and phosphate groups in the host and guest are well separated, and they cause almost no electrostatic inhibition of binding (structure **31**, R = PO_3^{2-}).

"Apolar" Interaction. There are a number of recent observations which seem to be relevant to the present findings. (1) According to the available X-ray crystallographic structures of sugar–protein complexes, there are two types of major sugar–protein interactions.[17] One is hydrogen bonding involving the sugar OH groups. The other is *stacking* of the CH moieties of a bound sugar with aromatic amino acid side chains. (2) Biological oligosaccharides on the cell surface and those found in DNA-binding antitumor antibiotics often contain deoxy (especially 6-deoxy, such as fucose) and alkylated sugars.[37-39] (3) There is an important recent suggestion that the direct oligosaccharide–oligosaccharide interaction in the intercellular adhesion is promoted by a hydrophobic association.[3] (4) Sugars and their derivatives are adsorbed on polystyrene with varying affinities, depending on their hydrophobicities.[30]

Sugars thus have both hydrophilic and hydrophobic characters, the latter becoming more important upon deoxygenation, alkylation, or oligosaccharide formation. There is little doubt that the hydrophobic character plays important roles, probably in collaboration with polar hydrogen-bonding interactions, in the biological sugar-binding processes, and in molecular recognition of sugars associated therewith. The present work demonstrates that this is also true in the sugar binding with a synthetic receptor having a polyhydroxy aromatic cavity.

Nature of "Apolar" Host–Guest Association: Importance of CH–π Interaction. Apolar host–guest association in water is promoted by the "hydrophobic" forces[5,40] or the van der Waals interactions,[6,40b,41] or more probably by a combination of these. A deeper insight into the driving forces for the present complexation may be provided by examining the effects of substituents X on the benzene rings of the host. As shown in Table *II*, both **1b** (X = CH_3) and **1c** (X = OH) exhibit higher binding abilities than the parent host **1a** (X = H) for every guest. Thus, both methyl groups (highly hydrophobic and moderately electron-donating) and hydroxyl groups (highly hydrophilic and highly electron-donating) promote guest binding. Inspection of Table II suggests that $K_{1b} > K_{1c}$ for relatively hydrophobic guests **6** and **23** and $K_{1c} \geq K_{1b}$ for relatively hydrophilic sugar derivatives **10**, **16**, **17**, and **19**β. These results are interpreted as suggesting that hydrophobic forces, if any, cannot be the sole governing factor and the electronic effects of the substituents come into play. The most reasonable interpretation is that guest–host CH–π interaction involving electron-rich benzene rings of the host as π-bases is at least partially responsible for the present host–guest complexation in water. We have convincing evidence that such a CH–π interaction is important also in the hydrogen-bonded complexes of host **1d** in apolar organic media.[42]

Recent molecular recognition studies using cyclophane hosts show the importance of electrostatic contribution to the aromatic–aromatic,[9c,32,43] either face-to-face or edge-to-face, and aromatic–cation interactions,[9b] where the cation is of the form $CH_3N^+\equiv$. The CH–π interaction involving aliphatic CH moieties is also well documented[44] as either a conformation-controlling intramolecular process or an intermolecular force that governs heterogeneous molecular recognition.[45] A concept of C—H···X hydrogen bonding has been advanced.[46] The present finding indicates that even a nonactivated methyl group can be subject to such a CH–π interaction in a homogeneous association process in water. One essential feature is that the present hosts **1a–c** are composed of exceptionally electron-rich benzene rings having two or three hydroxyl groups and two or three alkyl groups. As for the guests, the HCO moieties may make an important contribution to the stabilities of the sugar complexes. As far as polarization of the *H–C–*O and *H–C–*N^+ bonds are concerned, the neutral oxygen and the positively charged nitrogen functionalities have similar effects, judging from the ^1H NMR chemical shift for CH_3OH (δ 3.34) and $(CH_3)_4N^+Cl^-$ (δ 3.20) in D_2O.

Comment on Polar Interaction. The apolar character or the hydrophobicity of a guest can be conveniently expressed by the partition coefficient (*P*) for it between an appropriate organic solvent (such as 1-octanol[29a] and diethyl ether[29b]) and water. Usually, the binding constants for host–guest association in water are more or less correlated with such hydrophobicity parameters. In the present case, however, the binding constants for host **1a** (Table I) are surprisingly insensitive to the hydrophilicities of the guests. (1) The *K*'s for acyclic alcohols from C_3 (**3**) through C_5 (**5**) change only by a factor of ~3. (2) Cyclic monool **6** and diol **7** have essentially identical *K*'s, although their hydrophobicities differ markedly. In fact, β-cyclodextrin binds monool **6** (K = 460[15] or 500[47] M^{-1}) an order of magnitude more tightly than diol **7** (K = 71 for **7c** and 61 M^{-1} for **7t**).[15] (3) The hydrophilicities of nucleobase **27**, nucleoside **23**, and nucleotide **26** must increase in this order, while they show similar *K*'s. (4) The most conspicuous case is sugars. Sugars **9**, **10**, and **16–21** are completely insoluble in octanol or ether, while alcohols **2–5** are miscible with it. Nevertheless, these sugars and alcohols exhibit similar binding affinities (Table I). This might be due to some compensating polar effects that promote the binding of a hydrophilic guest.

Although no supporting evidence is available, there can be two explanations of the "polar" effects at present; one is based on solvation/desolvation and the other on host–guest hydrogen bonding. In marked contrast to many other cyclophane hosts having a cylindrical, i.e., top–bottom symmetric, cavity, the present host has a bowl-shaped aromatic cavity (Figure 6), whose upper rim with many peripheral OH groups is wide open to bulk water. Owing to this structural characteristic, the polar OH or other polar groups of a hydrophilic guest may either be free from otherwise serious desolvation or undergo hydrogen-bonding interactions with the OH groups of the host.[48,49] This might also explain a re-

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(41) (a) VanEtten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242. (b) Bergeron, R. J.; Channing, M. A.; Gibeily, G. J.; Pillor, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 5146.

(42) Even in the hydrogen-bonded complex of lipophilic host **1d** with a simple monool, the guest seems to be bound in the cavity of the host (ref 33b). 2-Pentanol, for example, undergoes the largest ^1H NMR complexation shift (1.8 ppm upfield) at the terminal 5-H's (Kobayashi, K.; Kikuchi, Y.; Aoyama, Y. Unpublished results). This indicates that the otherwise flexible propyl group of bound 2-pentanol swings into the cavity of the host.

(43) (a) Muehldorf, A. V.; Van Engen, D.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561. (b) Ashton, P. R.; Odell, B.; Reddington, M. V.; Slawin, A. M. Z.; Stoddart, J. F.; Williams, D. J. *Angew. Chem.* **1988**, *100*, 1608; *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1550. (c) Zimmerman, S. C.; Vanzyl, C. M.; Hamilton, G. S. *J. Am. Chem. Soc.* **1989**, *111*, 1373. (d) Smithrud, D. B.; Diederich, F. *J. Am. Chem. Soc.* **1990**, *112*, 339. (e) Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 3910. (f) Rebek, J., Jr. *Angew. Chem.* **1990**, *102*, 261; *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245.

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(45) Ogura, K.; Uchida, T.; Noguchi, M.; Minoguchi, M.; Murata, A.; Fujita, M.; Ogata, K. *Tetrahedron Lett.* **1990**, *31*, 3331.

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(47) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99*, 7100.

(48) For the most recent and thorough studies on the detailed nature of host–guest interactions (including aromatic–aromatic, aromatic–cation, and hydrogen-bonding interactions) and solvent effects (including solvophobic and specific solvation/desolvation effects) in the host–guest association processes in various media, see: (a) Reference 32. (b) Smithrud, D. B.; Wyman, T. B.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5420. (c) Stauffer, D. A.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Org. Chem.* **1990**, *55*, 2762.

(49) For the hydrogen-bonding interactions involving the OH groups of cyclodextrins, see: Cramer, F.; Dietsche, W. *Chem. Ber.* **1959**, *92*, 1739. (b) Matsui, Y.; Naruse, H.; Mochida, K.; Date, Y. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1909.

markable selectivity of host **1a** for cytidine (**23**) over closely related uridine (**24**).[50] Characterization of this type of polar effects may be essential for a better understanding of molecular recognition of highly polar compounds in water.

Concluding Remarks

Molecular recognition of sugars may occupy a central position in the newer phase of glycoscience and glycotechnology. Sugars, even unmodified aldohexoses, have both hydrophobic ($C_6H_{12}O_6$) and hydrophilic characters ($C_6H_{12}O_6$). They become more hydrophobic when a OH group involved is deleted, either as such or in the form of CH_2OH, methylated, or hydrogen-bonded. The present work demonstrates that such *hydrophobic* sugars can be guests of synthetic cyclophane hosts in water. Biological oligosaccharides of enhanced hydrophobicities may have a much higher potential as guests. The binding constants obtained here are very small. This is not necessarily an essential problem; they may be increased by accumulating unit-binding sites.[51]

This work thus not only provides a clue for better understanding of the biological sugar-binding processes but also opens a door to molecular recognition of sugars with artificial receptors in aqueous media. It is of primary importance to further characterize the *apolar* host–guest interaction in the present system. At the same time, it appears to be essential to reveal possibly complicated roles of the peripheral OH groups of the host. Further work is now underway along these lines.

Experimental Section

General Procedures. ^{1}H NMR spectra at 270 MHz were taken with a JEOL JNM-GX 270 spectrometer; HDO (δ_H 4.80) in D_2O (CEA, 99.8% isotopic purity) was used as the internal standard. Assignments of complexation-shifted resonances were made by COSY correlations. IR spectra were obtained with a JASCO IR-810 spectrophotometer. Electronic absorption spectra were recorded with a Hitachi 320 spectrophotometer. Surface tensions of aqueous solutions were measured with a Shimadzu Du Nouy surface and interfacial tensionmeter. Elemental analyses were performed at the Microanalysis Center of Kyoto University. Methyl α-D-fucopyranoside (**20α**) was prepared by a slight modification of the literature method.[52] All other guest compounds **2–29** were commercial products. Water used in this study was doubly distilled and of the ion-exchange grade.

Tetrasulfonated Resorcinol Cyclic Tetramers 1a–c. A two-phase mixture of 2-(2-bromoethyl)-1,3-dioxane (2.0 g, 10 mmol) and an aqueous solution (10 mL) of Na_2SO_3 (2.5 g, 20 mmol) was stirred at 100 °C for 24 h. To the resulting homogeneous solution was added water (10 mL), and the mixture was washed with ether (20 mL × 2) to get rid of unreacted (bromoalkyl)-1,3-dioxane. To this were successively added ethanol (20 mL), resorcinol (2.0 g, 18 mmol), and concentrated HCl (3 mL). The mixture was stirred under nitrogen at 100 °C for 24 h. The solvent was evaporated, and the residue was taken in water (30 mL) and dialyzed three times against water (1 L) using a dialysis membrane having a transport critical molecular weight of 1000 (Spectra/Por membrane MWCO 1000) to remove inorganic salts.[53] Most of the water was removed in vacuo, and the residue was recrystallized from water–methanol to give hygroscopic tetrasulfonated compound **1a** (1.0 g, 40%):[54] mp 250 °C dec; ^{1}H NMR (D_2O) ([**1a**] = 3.1 mM) δ 2.44 (dt, J = 6.6 and 8.1 Hz, 8 H, $CHCH_2$), 2.92 (t, J = 6.6 Hz, 8 H, CH_2SO_3Na), 4.55 (t, J = 8.1 Hz, 4 H, $CHCH_2$), 6.38 (s, 4 H, aromatic 2-H), 6.94 (s, 4 H, aromatic 5-H); IR (KBr) 3425 (ν_{OH}), 1180 and 1050 cm^{-1} ($\nu_{S=O}$). Anal. Calcd for $C_{36}H_{36}O_{20}S_4Na_4{\cdot}4H_2O$: C, 40.00; H, 4.10. Found: C, 40.02; H, 4.15.

Similarly prepared were hygroscopic compounds **1b** (70%) and **1c** (10%), starting from 2-methylresorcinol and pyrogallol, respectively, in place of resorcinol. In these cases, however, crude products contained substantial amounts of conformational isomer(s): δ 7.33 and 6.12 for **1b** and 7.06, 6.51, 6.06, and 5.84 for **1c**. These byproducts were removed by repeated reprecipitation and/or recrystallization from water–methanol. Compound **1b**:[55] ^{1}H NMR (D_2O) ([**1b**] = 3.3 mM) δ 2.06 (s, 12 H, CH_3), 2.55 (dt, 8 H, $CHCH_2$), 2.91 (t, 8 H, CH_2SO_3Na), 4.68 (t, J = 8.1 Hz, 4 H, $CHCH_2$), 6.95 (s, 4 H, aromatic 5-H). Anal. Calcd for $C_{40}H_{44}O_{20}S_4Na_4{\cdot}4H_2O$: C, 42.25; H, 4.61. Found: C, 42.98; H, 4.80. Compound **1c**:[56] ^{1}H NMR (D_2O) ([**1c**] = 2 mM) δ 2.48 (dt, 8 H, $CHCH_2$), 2.92 (t, 8 H, CH_2SO_3Na), 4.63 (t, 4 H, $CHCH_2$), 6.63 (s, 4 H, aromatic 5-H). Anal. Calcd for $C_{36}H_{36}O_{24}S_4Na_4{\cdot}8H_2O$: C, 35.53; H, 4.31. Found: C, 35.44; H, 3.91.

pK_a Measurement. The pK_a value for the first ionization of the OH groups of host **1a** was determined spectrophotometrically by monitoring the absorbance at 302 nm for the phenolate ion for a series of degassed solutions of **1a** (0.005 mM) at various pH's; absorbance (pH): 0.030 (1.00), 0.032 (6.95), 0.032 (8.33), 0.035 (8.97), 0.040 (9.22), 0.042 (9.42), 0.049 (9.59), 0.050 (9.79), 0.058 (9.89), 0.068 (10.06), 0.077 (10.25), 0.097 (10.52), 0.098 (10.99), and 0.098 (13.80). The pK_a of 10.0 ± 0.1 was obtained as the midpoint of the absorbance–pH correlation.

Binding Constants, Saturation Shifts, and Job Plots. All binding assays, i.e., determinations of binding constants and saturation shifts, were carried out under Benesi–Hildebrand conditions. The host concentration for **1a** was 2 mM, while that of **1b** or **1c** was 2, 1, or 0.5 mM for low- (K < 30), medium- (30 < K < 80), or high-affinity (80 ≤ K) guests, respectively. In most cases, the guest concentration range for sugar derivatives was 0.2–1.6 M, i.e., 100–800-fold in excess of the host. Those for higher affinity guests were so chosen as to allow $[guest]_t$ to be at least 10-fold in excess of $[host]_t$. In every case, the double reciprocal plots according to eq 1 gave excellent linearity with a correlation coefficient $r \geq 0.997$. For every K, except for those expressed as <1 or ~0 (Tables I and II), at least either a 20–60 or 40–80% complexation was covered. The maximal coverage, however, was lower (≤55, but >30%) for K's < 1. Such guests as **8**, **11**, **14**, **15**, **22**, and **28** exhibited practically no complexation-induced shifts. Guests **12** and **13** gave complexation-induced shifts which were, however, too small to allow a Benesi–Hildebrand treatment. For these were given $K \approx 0$.

The saturation shifts for guests **7c** (with **1a** as host), **20α** (with **1a** as host), or **23** (with **1b** as host) were obtained directly for **23**, where saturation binding was practically reached, by a Benesi–Hildebrand analysis for **7c** (refer to Figure 2b), or by calculation for **20α** on the basis of the binding constant (Table I) and observed complexation shifts ($\Delta\delta_{obsd}$); K = [complex]/[guest][host] ≈ [complex]/[guest]$[host]_t$ (t = total) and $\Delta\delta_{obsd}/\Delta\delta_{sat}$ = [complex]/$[guest]_t$. Actually, $\Delta\delta_{obsd}$ = −0.14 (1-OCH_3), −0.01 (2-H), −0.01 (3-OH), −0.11 (4-OH), −0.20 (5-H), and −0.20 ppm (5-CH_3) (negative value indicates an upfield shift) under conditions $[\mathbf{20}]_t$ = 4 mM and $[\mathbf{1a}]_t$ = 80 mM. For guests **4t** and **17**, $\Delta\delta_{obsd}$ = −0.30 and −0.20, respectively, for the methyl groups under conditions [**4t**] = [**17**] = 2 mM and [**1a**] = 50 mM.

The concentration of a complex in solution, as required for the Job plots (Figure 4), was evaluated from $\Delta\delta_{obsd}$ for the guest, according to the equation, [complex] = $[guest]_t(\Delta\delta_{obsd}/\Delta\delta_{sat})$.

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(50) An examination of CPK models indicates that the amino group of guest **23** bound in such a way as shown in structure **31** is in contact with a pair of peripheral OH groups of the host, thus possibly allowing hydrogen bonding between these.

(51) Aoyama, Y. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; JAI Press: Greenwich, CT, 1992; Vol. 2, pp 65–92.

(52) Helferich, B.; Schafer, W. *Organic Synthesis Collect.*; Wiley, New York, 1941; Vol. I, pp 364–366.

(53) Inorganic salts could not satisfactorily be removed by treating crude **1a** with ion-exchange resins.

(54) 2,8,14,20-Tetra(2-sulfonatoethyl)pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]-octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaen-4,6,10,12,16,18,22,24-octol tetrasodium salt.

(55) 2,8,14,20-Tetra(2-sulfonatoethyl)-5,11,17,23-tetramethylpentacyclo-[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19-(26),21,23-dodecaen-4,6,10,12,16,18,22,24-octol tetrasodium salt.

(56) 2,8,14,20-Tetra(2-sulfonatoethyl)pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]-octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaen-4,5,6,10,11,12,16,17,18,22,24-dodecaol tetrasodium salt.