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Polycations. IX. Polyammonium Derivatives of Cyclodextrins: Syntheses and Binding to Organic Oxyanions

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ABSTRACT: *A series of polycationic derivatives of α- and β-cyclodextrin have been synthesized. Their interactions for inclusive binding of series of organic phosphorus oxyanions and anions of biological α-amino acids have been investigated using NMR techniques. Determinations of association constants and orientation of guest anion binding to the host polycationic cyclodextrin derivatives have been performed.* © 2000 John Wiley & Sons, Inc. Heteroatom Chem 11:546–555, 2000

INTRODUCTION

For some time our laboratory has been concerned with the synthesis and investigation of intermolecular association characteristics of polycationic organic salts [1a–j]. Polycationic species of interest have included dendrimers, "strings," and polymer-bound ion-exchange materials, as well as a variety of ring systems. In the present report we turn our attention to the synthesis of polycationic derivatives of the α- and β-cyclodextrins and their capability to serve as host species for interactions with series of oxyanions, specifically, biologically important phosphorus oxyanions and anions related to the biological α-amino acids.

The functionalization of cyclodextrins for specific introduction of substituents has been thoroughly established [2–8]. Our particular interest has been with the preparation of cyclodextrin derivatives where each of the hydroxyl groups at the 6-position has been replaced with a cation-bearing substituent. Cyclodextrin derivatives substituted at each of the 6-position sites are available via reactions performed on the tosylated cyclodextrins [2], and our present effort uses this approach for the introduction of the cation-bearing substituents.

It was envisioned that species so substituted at the smaller rim of the conical section defined by the cyclodextrin structure (schematically shown in Figure 1) could exhibit particular binding characteristics toward organic anions, serving as host species for a variety of anions. These species would bear, in addition to the cylindrical cationic region, two hydrophobic regions, one within the cyclodextrin cone and the other on the opposite side, above the cationic region.

The polycationic cyclodextrin derivatives thus synthesized could be imagined to have a general tube shape. Each cationic region would be flanked on either side by relatively hydrophobic regions, the interior of the parent cyclodextrin unit, and the so-called tails attached above. The tubular nature of these derivatives would be variable; while the lower

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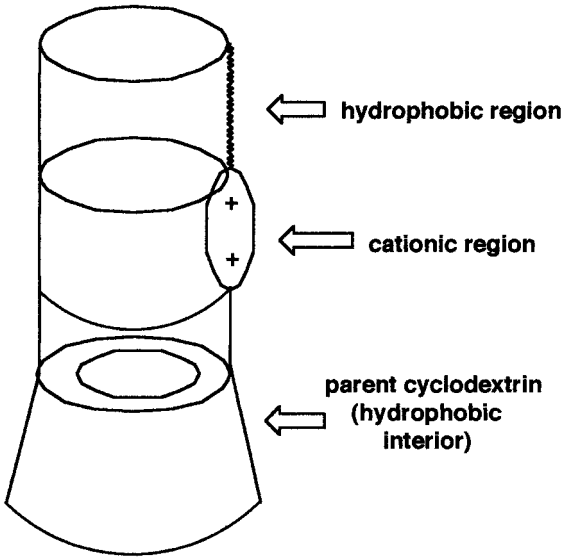
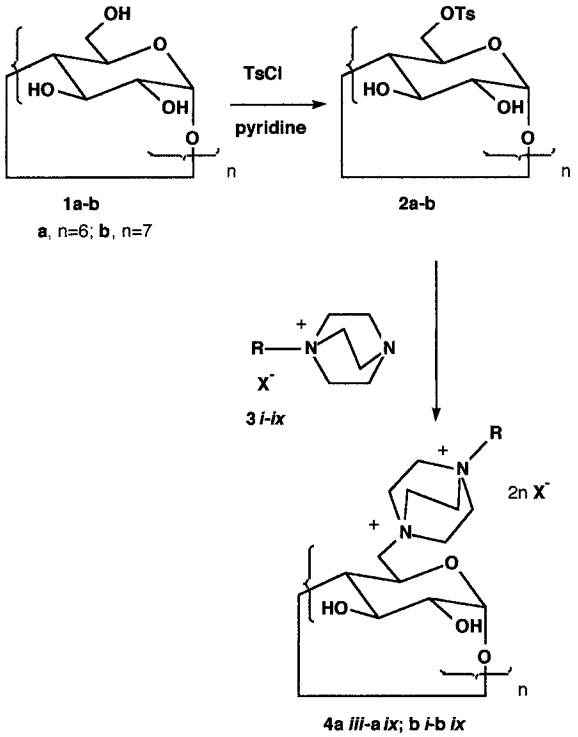


FIGURE 1



i, R = -CH$_2$CH$_2$OH; *ii*, R = -CH$_2$CH$_2$CH$_2$OH; *iii*, R=-CH$_2$(CH$_2$)$_4$CH$_2$OH; *iv*, R=-CH$_2$(CH$_2$)$_6$CH$_2$OH; *v*, R=-CH$_2$(CH$_2$)$_8$CH$_2$OH; *vi*, R=-CH$_2$(CH$_2$)$_9$CH$_2$OH; *vii*, R=-CH$_2$C$_6$H$_5$; *viii*, R=-CH$_2$(CH$_2$)$_{10}$CH$_3$; *ix*, R=-CH$_2$CH$_2$CH$_2$CH$_2$CN

FIGURE 2

portion would be rigid, the top portion may be envisioned as long flexible ropes, forming a tube when these ropes come together. The lipophilic interaction of these ropes is assisted by hydrophobic interactions with the external water.

The inclusion of anion guests within the tube of the cyclodextrin derivatives would be anticipated to modify the NMR chemical shifts of the host cyclodextrin derivative in a predictable manner. Titration of the host species with added anion guest species would be expected to provide association constants for the systems, as well as indications of the orientation of host/guest interactions. We herein report on the results of this type of NMR investigation, as well as the synthesis and characterization of the polycationic cyclodextrin derivatives.

RESULTS AND DISCUSSION

Two commercially available cyclodextrins (α- and β-cyclodextrin, **1a** and **1b**, respectively) have been per-tosylated at the 6-position hydroxyl groups using a standard method [2]. The resultant species, **2a** from α-cyclodextrin and **2b** from β-cyclodextrin, so activated for substitution, have been treated with a series of monocationic derivatives **3i–3ix** of 1,4-diazabicyclo [2.2.2]octane (DABCO) bearing a single free tertiary amine site that can perform a substitution reaction for the tosylate functionality to produce the polycationic cyclodextrin derivatives **4aiii–4aix** and **4bi–4bix** (see Figure 2).

The preparation of several monocationic DABCO derivatives has been reported previously from this laboratory [1b,g,i]. Additional monocationic DABCO derivatives (specifically **3iii, 3v–3ix** used in the syntheses of the newly reported cyclodextrin derivatives) are also reported here. All monocationic DABCO derivatives thus prepared exhibited NMR (^{13}C, ^{1}H, and COSY) in accord with their proposed structures, as well as combustion analyses in accord with the formulae for hydrated forms of the target salts. All of the polycationic salts are significantly hydroscopic, rapidly absorbing water from the atmosphere upon even short exposure times. The NMR, analytical, and yield data for the newly synthesized monocationic DABCO derived salts are given in Table 1.

Addition of the per-tosylated cyclodextrin to a heated acetonitrile solution of each of the monocationic DABCO derivatives results in the precipitation of the target polycationic salts derived from cyclodextrin. Intriguingly, selective precipitation of the polyhalide salt product occurs, leaving in solution the functionalized cyclodextrin with tosylate anion. A consequence of this result is the isolation of the target material in less than 50% yield, albeit pure with regard to the negative counterion. Presumably, the association of the tosylate (organic) anions with the polycationic cyclodextrin derivatives renders the

TABLE 1 Yield, NMR, and Elemental Analysis for Newly Synthesized Monoalkylated DABCO Salts

Compound	*Synthesis*	*Yield*	*^{1}H NMR (δ) (Hz)*	*^{13}C NMR (δ)*	*Elem. Anal.*		
3iii	$-(CH_2)_6OH$	88%	1.29, br, 4H 1.45, br, 2H 1.67, br, 2H 3.09, br, 6H 3.15, t, 2H, $J = 4$ 3.29, br, 6H 3.49, t, 2H, $J = 8$	19.78, 23.24, 24.03, 29.63, 42.88, 50.73, 60.22, 63.22	$C_{12}H_{25}N_2OCl \cdot 2(H_2O)$ Calcd: Found: 	C H C H	50.60% 10.26% 50.33% 10.61%
3v	$-(CH_2)_{10}OH$	88%	1.20, br, 12H 1.43, br, 2H 1.64, br, 2H 3.11, br, 6H 3.16, br, 2H 3.28, br, 6H 3.49, t, 2H, $J = 8$	21.42, 25.33, 25.85, 28.41, 28.66, 28.75, 28.81, 31.59, 44.52, 52.37, 62.21, 65.02	$C_{16}H_{33}N_2OBr \cdot 2(H_2O)$ Calcd: Found: 	C H C H	49.87% 9.68% 49.55% 9.91%
3vi	$-(CH_2)_{11}OH$	81%	1.18, br, 14H 1.42, br, 2H 1.63, br, 2H 3.09, br, 6H 3.13, br, 2H 3.27, br, 6H 3.46, t, 2H, $J = 5$	23.60, 27.53, 28.04, 30.61, 30.88, 31.00, 31.11, 31.20, 33.78, 46.70, 54.54, 54.58, 64.40	$C_{138}H_{264}N_{12}O_{30}Br_{12} \cdot 4(H_2O)$ Calcd: Found: 	C H C H	46.01% 7.61% 45.86% 7.83%
3vii	$-CH_2C_6H_5$	86%	2.98, br, 6H 3.26, br, 6H 4.69, s, 2H 7.41, br, 5H	46.85, 54.44, 70.48, 128.47, 131.66, 131.97, 135.65	$C_{13}H_{19}N_2Cl \cdot 0.5(H_2O)$ Calcd: Found: 	C H C H	55.13% 7.11% 54.88% 7.19%
3viii	$-(CH_2)_{11}CH_3$	89%	0.75, br, 3H 1.17–1.24, br, 20H 3.08, br, 6H 3.28, br, 6H	12.68, 20.34, 21.33, 24.78, 27.37, 27.67, 27.78, 27.83, 27.99, 28.02, 30.48, 43.43, 51.24, 63.90	$C_{18}H_{37}N_2Br$ Calcd: Found: 	C H C H	54.40% 10.40% 54.62% 10.28%
3ix	$-(CH_2)_4CN$	72%	1.47, br, 2H 1.64, br, 2H 2.32, t, 2H, $J = 6$ 2.93, br, 6H 3.03, t, 2H, $J = 4$ 3.14, br, 6H	16.55, 20.98, 22.02, 44.59, 52.58, 63.81, 121.57	$C_{11}H_{20}N_3Cl$ Calcd: Found: 	C H C H	53.32% 8.95% 53.43% 8.87%

species more soluble in the organic medium (acetonitrile) and facilitates precipitation and isolation of the desired organic cationic salts with only halide gegenions.

All salts thus prepared exhibited NMR (^{13}C, ^{1}H, and COSY) in accord with their proposed structures, as well as combustion analyses in accord with the formulae for hydrated forms of the target salts. (All of the polycationic salts derived from the cyclodextrins are also significantly hydroscopic adsorbing varying amounts of water rapidly upon exposure to the atmosphere. Combustion analyses are thus for varying hydrated forms of the salts.) The NMR, analytical, and yield data for the newly synthesized cyclodextrin derived salts are given in Table 2.

A selected series of these salts have been investigated for their ability to act as hosts for interaction with organic anionic guests. A summary and discussion of the methods available for the determination of host/guest association constants is available [9].

Determinations of association constants using UV-vis or fluorescence techniques are thwarted by the absence within the structures of the cyclodextrin derivatives of suitable chromophores or other probes. Similarly, only a few of the guest species of interest have such probes available. Variation in the NMR spectrum of the host species upon interaction with the guests presents itself as the most viable approach toward determination of association constants without the introduction of structural features

TABLE 2 Yield, NMR, and Elemental Analysis Data for Newly Synthesized Polycationic Cyclodextrin Species

Compound	*R*	*Yield*	*^{1}H NMR (δ) (Hz)*	*^{13}C NMR (δ)*	*Elem. Anal.*
4bi	$-(CH_2)_2OH$	40%	3.48–3.55, m 2H(x7) 3.64, m, 4H(x7) 3.76, m, 6H(x7) 3.96, m, 6H(x7) 4.04, m, 4H(x7) 4.95, d, 1H(x7), $J = 3$	44.43, 52.21, 55.42, 60.66, 66.98, 72.33, 72.58, 73.64, 81.51, 102.36	$C_{98}H_{182}N_{14}O_{35}Cl_{14} \cdot 3(H_2O)$ Calcd: C 44.14% H 7.02% Found: C 44.31% H 7.11%
4bii	$-(CH_2)_3OH$	37%	2.00, m, 2H(x7) 3.56, t, 2H(x7), $J = 8$ 3.63, t, 2H(x7), $J = 7$ 3.76–3.98, br, 18H(x7) 4.96, d, 1H(x7), $J = 8$	23.50, 42.97, 50.28, 56.80, 59.21, 61.97, 70.84, 71.09, 72.15, 80.05, 100.88	$C_{105}H_{196}N_{14}O_{35}Cl_{14} \cdot 5(H_2O)$ Calcd.: C 44.87% H 7.66% Found: C 45.02% H 7.41%
4aiii	$-(CH_2)_6OH$	41%	1.33, br, 4H(x6) 1.47, m, 2H(x6) 1.73, m, 2H(x6) 3.41, t, 2H(x6), $J = 8$ 3.48–3.58, brm, 6H(x6) 3.67, br, 6H(x6) 3.73–3.81, brm, 8H(x6) 4.96, d, 1H(x6), $J = 3.5$	23.01, 26.24, 26.79, 32.61, 45.48, 52.35, 61.75, 63.07 66.69, 73.12 73.52, 75.21 82.91, 103.16	$C_{108}H_{204}N_{12}O_{30}Cl_{12} \cdot 3(H_2O)$ Calcd.: C 49.32% H 8.05% Found: C 49.20% H 8.17%
4biii	$-(CH_2)_6OH$	39%	1.33, br, 4H(x7) 1.47, m, 2H(x7) 1.75, br, 2H(x7) 3.50, t, 2H(x7), $J = 7.5$ 3.52–3.81, brm, 20H 4.98, d, 1H, (x7), $J = 4$	20.78, 23.96, 24.56, 30.37, 43.89, 50.50, 59.61, 60.86, 64.52, 71.25, 71.38, 72.58, 80.52, 101.25	$C_{126}H_{238}N_{14}O_{35}Cl_{14} \cdot 2(H_2O)$ Calcd.: C 50.20% H 8.10% Found: C 49.96% H 8.37%
4aiv	$-(CH_2)_8OH$	32%	1.34, br, 8H(x6) 1.49, br, 2H(x6) 1.75, br, 2H(x6) 3.37, t, 2H(x6), $J = 8.7$ 3.50–3.88, brm, 20H(x6) 5.02, d, 1H(x6), $J = 3.4$	22.18, 26.48, 26.92, 29.71, 29.80, 32.83, 45.65, 52.99, 61.79, 63.29, 73.46, 73.59, 73.63, 74.76, 82.71, 103.48	$C_{120}H_{228}N_{12}O_{30}Cl_{12} \cdot 4(H_2O)$ Calcd.: C 51.17% H 8.44% Found: C 51.01% H 8.62%
4biv	$-(CH_2)_8OH$	41%	1.35, br, 8H(x7) 1.47, br, 2H(x7) 1.77, br, 2H(x7) 3.36, t, 2H(x7), $J = 9$ 3.50–3.83, brm, 20H(x7) 5.02, d, 1H(x7), $J = 3.5$ 81.38, 102.1	21.79, 25.19, 25.78, 28.52, 28.56, 31.60, 44.26, 51.35, 60.36, 61.80, 65.35, 72.18, 72.22, 73.56	$C_{140}H_{266}N_{14}O_{35}Cl_{14} \cdot 3(H_2O)$ Calcd.: C 51.64% H 8.42% Found: C 51.37% H 8.65%
4av	$-(CH_2)_{10}OH$	40%	1.32, br, 12H(x6) 1.47, br, 2H(x6) 1.76, br, 2H(x6) 3.43, t, 2H(x6), $J = 8$ 3.51–3.81, brm, 20H(x6) 5.03, d, 1H(x6), $J = 3.5$	20.82, 24.65, 24.86, 27.71, 28.02, 28.18, 28.21, 30.86, 43.41, 50.39, 61.27, 64.68, 71.07, 71.27, 71.48, 73.28, 81.53, 101.85	$C_{132}H_{252}N_{12}O_{30}Cl_{12} \cdot 4(H_2O)$ Calcd.: C 53.11% H 8.78% Found: C 52.90% H 9.13%
4bv	$-(CH_2)_{10}OH$	40%	1.25, br, 12H(x7) 1.45, br, 2H(x7) 1.76, br, 2H(x7) 3.35, t, 2H(x7), $J = 9$ 3.49–3.81, brm, 20H(x7) 5.02, d, 1H(x7), $J = 3.5$	21.33, 24.06, 24.91, 25.34, 28.08, 28.17, 28.35, 31.20, 43.88, 50.90, 60.08, 61.65, 65.10, 71.57, 71.95, 73.30, 81.00, 101.93	$C_{154}H_{294}N_{14}O_{35}Cl_{14} \cdot 5(H_2O)$ Calcd.: C 53.05% H 8.73% Found: C 53.12% H 8.60%

TABLE 2 (continued) Yield, NMR, and Elemental Analysis Data for Newly Synthesized Polycationic Cyclodextrin Species

Compound	*R*	*Yield*	*^{1}H NMR (δ) (Hz)*	*^{13}C NMR (δ)*	*Elem. Anal.*
4avi	$-(CH_2)_{11}OH$	36%	1.35, br, 14H(x6) 1.47, br, 2H(x6) 1.78, br, 2H(x6) 3.35, t, 2H(x6), $J = 9$ 3.51–3.88, brm, 20H(x6) 5.04, d, 1H(x6), $J = 3.5$	23.51, 27.61, 27.68, 29.75, 30.62, 31.88, 31.97, 32.04, 33.64, 46.01, 53.01, 61.77, 63.82, 67.20, 73.68, 74.04, 75.89, 83.39, 104.05	$C_{138}H_{264}N_{12}O_{30}Br_{12} \cdot 4(H_2O)$ Calcd.: C 46.01% H 7.61% Found: C 45.86% H 7.83%
4bvi	$-(CH_2)_{11}OH$	44%	1.33, br, 14H(x7) 1.45, br, 2H(x7) 1.77, br, 2H(x7) 3.36, t, 2H(x7), J = 9 3.49–3.81, brm, 20H(x7) 5.02, d, 1H(x7), $J = 3$	24.06, 27.56, 28.04, 30.79, 30.98, 31.08, 31.14, 33.91, 46.52, 53.46, 62.45, 64.26, 67.77, 74.48, 74.58, 75.94, 83.62, 104.61	$C_{161}H_{308}N_{14}O_{35}Br_{14} \cdot 3(H_2O)$ Calcd.: C 46.34% H 7.58% Found: C 46.31% H 7.82%
4avii	$-CH_2C_6H_5$	34%	3.66, br, 2H(x6) 3.70–3.92, brm, 18H(x6) 4.98, br, 1H(x6) 7.49–7.64, br, 5H(x6)	42.2, 49.3, 49.6, 59.1, 67.7, 70.3, 72.2, 80.1, 100.2, 123.6, 128.4, 130.3, 131.7	$C_{114}H_{168}N_{12}O_{24}Cl_{12} \cdot 3(H_2O)$ Calcd.: C 53.28% H 6.82% Found: C 53.16% H 6.99%
4bvii	$-CH_2C_6H_5$	31%	3.49, br, 2H(x7) 3.56–3.91, brm, 18H(x7) 4.98, d, 1H(x7), $J = 3.6$ 7.47–7.59, br, 5H(x7)	44.4, 51.1, 51.2, 60.5, 72.1, 72.3 73.4, 81.4, 102.2, 125.6, 130.0, 131.9, 133.3	$C_{133}H_{196}N_{14}O_{28}Cl_{14} \cdot 4(H_2O)$ Calcd.: C 53.12% H 6.84% Found: C 53.25% H 6.62%
4aviii	$-(CH_2)_{11}CH_3$	33%	0.87, t, 3H(x6), $J = 5$ 1.24, br, 18H(x6) 1.65, br, 2H(x6) 3.32, t, 2H(x6), $J = 9$ 3.59–3.88, brm, 18H(x6) 5.02, d, 1H(x6), $J = 3.5$	15.47, 23.37, 24.26, 24.32, 27.47, 30.46, 30.97, 31.39, 31.82, 31.90, 33.72, 45.91, 52.94, 61.58, 66.24, 72.13, 73.84, 75.77, 83.19, 103.98	$C_{144}H_{276}N_{12}O_{24}Br_{12} \cdot 4(H_2O)$ Calcd.: C 48.17% H 7.97% Found: C 48.08% H 8.13%
4bviii	$-(CH_2)_{11}CH_3$	29%	0.85, t, 3H(x7), $J = 5$ 1.16, br, 18H(x7) 1.60, br, 2H(x7) 3.25, t, 2H(x7), $J = 9$ 3.48–3.78, brm, 18H(x7) 4.96, d, 1H(x7), $J = 3.5$	13.74, 21.02, 21.72, 22.34, 25.82, 28.64, 28.76, 28.87, 28.92, 29.04, 31.48, 44.20, 51.20, 51.86, 60.03, 65.25, 72.17, 73.61, 81.26, 102.38	$C_{168}H_{322}N_{14}O_{28}Br_{14} \cdot 3(H_2O)$ Calcd.: C 48.51% H 7.95% Found: C 48.36% H 8.18%
4aix	$-(CH_2)_4CN$	38%	1.67, t, 2H(x6), $J = 8$ 1.88, m, 2H(x6) 2.53, t, 2H(x6)$J = 8$ 3.46–3.57, brm, 4H(x6) 3.68–3.85, brm, 16H(x6) 4.96, d, 1H(x6), $J = 4$	16.44, 21.15, 21.72, 44.27, 51.29, 60.47, 64.38, 71.94, 72.30, 73.74, 81.52, 101.75	$C_{102}H_{174}N_{18}O_{24}Cl_{12} \cdot 2(H_2O)$ Calcd.: C 49.04% H 7.18% Found: C 49.11% H 7.02%
4bix	$-(CH_2)_4CN$	42%	1.66, t, 2H(x7), $J = 8$ 1.88, m, 2H(x7) 2.51, t, 2H(x7) $J = 8$ 3.44–3.49, brm, 4H(x7) 3.54–3.85, brm, 16H(x7) 4.96, d, 1H(x7), $J = 4$	18.59, 23.26, 23.82, 46.43, 53.51, 62.70, 66.50, 74.27, 74.53, 75.55, 83.55, 104.32	$C_{119}H_{208}N_{21}O_{28}Cl_{14} \cdot 4(H_2O)$ Calcd.: C 48.54% H 7.22% Found: C 48.23% H 7.57%

significantly modifying the guest species from their biologically related form.

The most readily observed quantitative indicator of interaction of the host and guest species is the change in the ^{1}H NMR spectrum of the host polycationic cyclodextrin upon being challenged with the anionic guests. Specifically, an upfield shift of the signals for hydrogens in the vicinity of the cationic sites of the polycationic cyclodextrins is observed upon addition of the guest anionic species. This is in accord with those hydrogens being additionally shielded from the applied magnetic field by the additional high electron density associated with the guest anionic site. In addition, quite rapid exchange of guest species between the states of host-bound and free in solution is noted; only a single signal is observable for the polycationic cyclodextrins when challenged with the guest anionic species, rather than separate signals for associated and unassociated hosts.

Given this situation, some standard aspects commonly used in the determination of host/guest association constants and other binding characteristics are precluded. The variation of this change in chemical shift with changing of the relative concentrations of host and guest species can be used to determine association constants [10–12], but is not amenable to the direct determination of the stoichiometry of the association using continuous variation of mole concentration methods [13–14]. In the present instance, a 1:1 association of host with guest is deduced by the fit of the data with the Benesi-Hildebrand equation for such an association [9–10] and by calculations of host cavity and guest sizes using Chem 3D+.

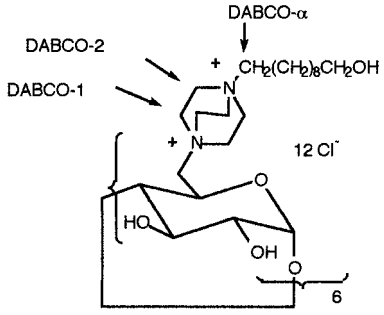
For measurement of the association constants, to measured neutral solutions (D_2O, pD = 7) of the polycationic cyclodextrin hosts were added varying amounts of measured solutions of the three series of biologically derived oxyanion guests, volumes being adjusted for standardization. The guests include a series of three phosphorus oxyanions from biological sources, six carboxylate salts of N-protected natural α-amino acids (and one sodium salt of a free amino acid), and two carboxylate salts of tetrapeptides. These guest species are indicated in Table 3. The added guest species were sufficiently dilute that no measurable change in pH could be observed over the range of concentrations of guests used (0.1–4.5 equivalent of guest compared to host). With the exception of the highest concentration of guest, with which was measured the limiting (maximum) change of chemical shift indicating fully complexed host, calculations of association constants were made using the lower concentrations of guests relative to hosts. It is with these concentrations that the greater, and thereby more accurate for purposes of association constant calculations, changes of chemical shift with increasing concentrations could be noted.

As previously noted, interaction of the organic anion guests with the polycationic functionalized cyclodextrin hosts could be noted through changes of the chemical shifts of ^{1}H-NMR signals derived from the hydrogens located near the cationic region of the host species. Specifically, upfield shifts in the signals for the hydrogens of the DABCO rings of the host species and adjacent sites (Figure 3, illustrated with **4aiv**) were noted as a result of shielding by association with the guest anionic species.

Representative spectra showing the changes in the chemical shifts of signals for hydrogens near the cationic sites of the host are shown in Figure 4. Specifically, representative spectra for the interactions of the polycationic cyclodextrin derivatives **4aiv** and **4av** with *N*-acetyl-L-phenylalanine (**8**) are shown.

TABLE 3 Guest Species Investigated with Host Polycationic Cyclodextrin Derivatives

Phosphorus Oxyanions (Disodium Salts)
phosphonomycin [(−)-(1R, 2S)-(1,2-epoxypropyl) phosphonic acid] (**5**)
2′-deoxyadenosine monophosphate (**6**)
2′-deoxythymidine monophosphate (**7**)
Amino acid derivatives (sodium salts)
N-acetyl-L-phenylalanine (**8**)
N-acetyl-L-cysteine (**9**)
N-acetyl-L-tyrosine (**10**)
N-acetyl-L-leucine (**11**)
N-acetyl-L-glutamic acid (**12**)
Folic acid [pteroylglutamic acid] (**13**)
L-lysine (**14**)
Peptides (Sodium Salts)
Phenylalanylglycylglycylphenylalanine (**15**)
Prolylphenylalanylglycyllysine (**16**)



4aiv

FIGURE 3

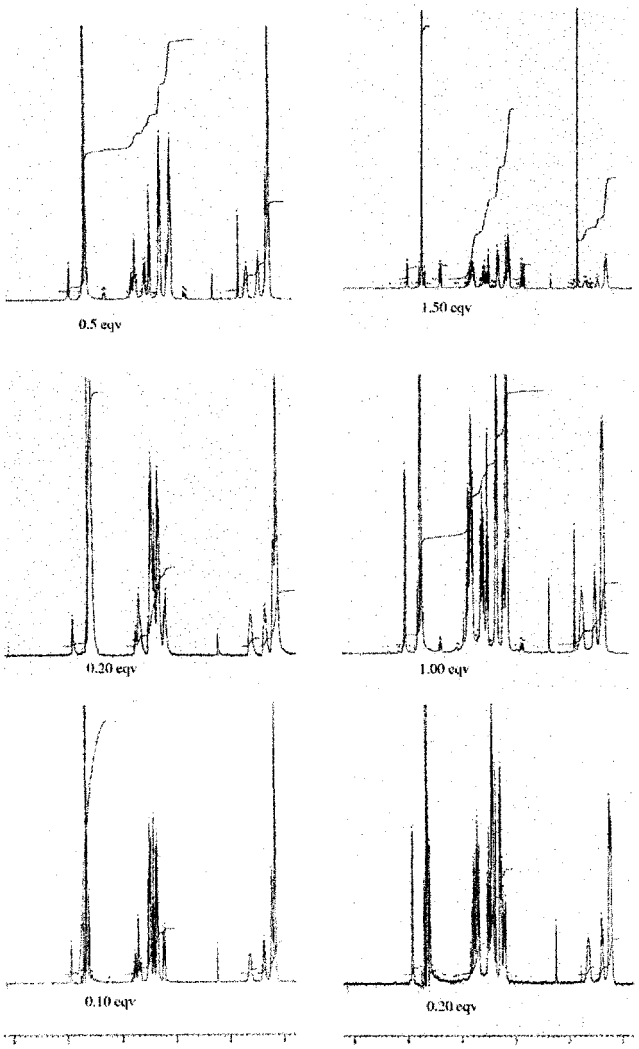


FIGURE 4 Effect of guest N-acetyl-L-Phe on ^{1}H NMR spectrum of guest polycationic cyclodextrin derivatives.

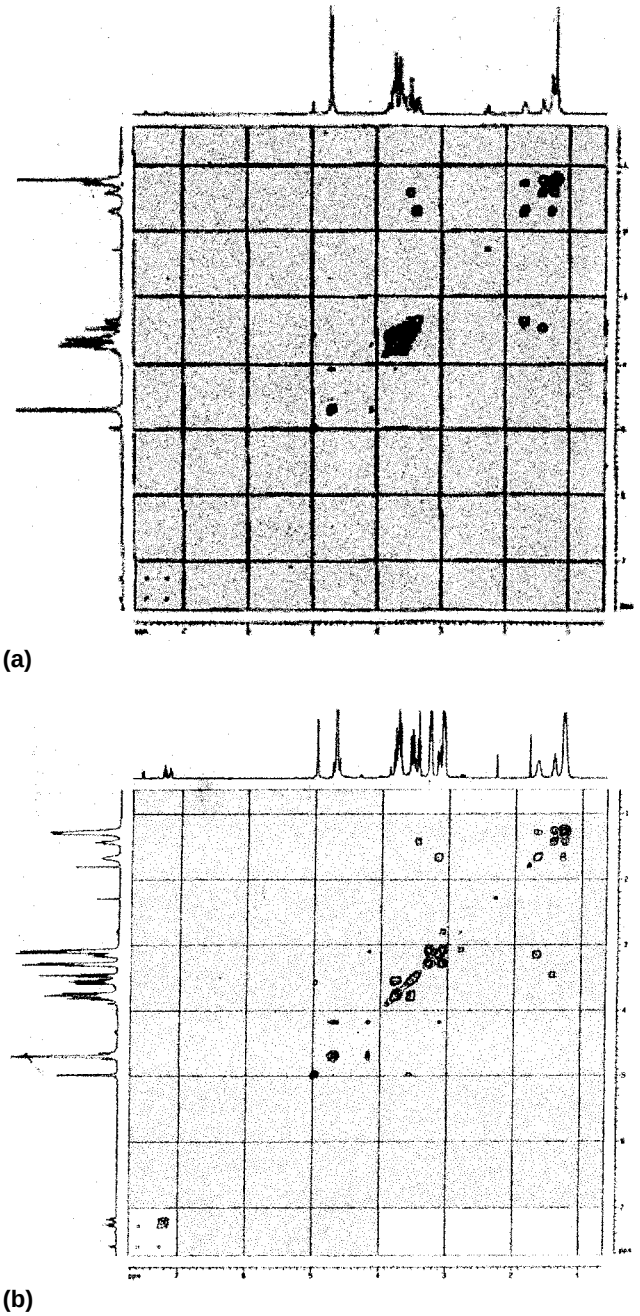


FIGURE 5 (a) COSY of **4avi**; (b) COSY of **4avi** with added 0.50 equiv. **8**.

Assignments of the shifted signals to specific sites have been made, confirmed through the use of ^{1}H-COSY 2D NMR measurements on the parent polycationic cyclodextrins and on the complexes. Representative COSY spectra for **4avi** and a solution of **4avi** with added *N*-acetyl-L-phenylalanine (**8**) are shown in Figure 5.

The magnitude of chemical shift changes, even at low loading of the guest species, along with the exhibition of only a single set of NMR signals, clearly indicate several characteristics of the interactions. First, there is a rapid equilibration of "free" and "bound" guest species. Further, this association is a strong one and occurs within the "tube" of the host rather than at the external surface. We understand this from the observations of only one set of signals for the affected hydrogens; the guest interacts equally with all of the charged sites of a particular chemical type. Interaction outside the tube would result in a significantly more complex NMR spectrum as all hydrogens of a particular chemical type would have different interactions with the anionic guest species.

Association constants for each guest-host inter-

action were determined through the measurement of the changes in chemical shift upon addition of increasing amounts of guest species using the Benesi-Hildebrand method (plot providing slope of K in Equation 1). The results are shown in Table 4. Calculations of $K(M^1 \times 10^3)$ in each instance were made on the basis of changes in chemical shift of the hydrogens of the DABCO ring more distant (DABCO-2) from the cyclodextrin unit. These hydrogens in all instances exhibited the greatest maximal change in chemical shift and thereby provided the most reliable indicator of binding.

$$K \times ([\text{Host}]_{\text{Stoic}} - \{\delta_{\text{obs}} - \delta_{\text{free}})/(\delta_{\max} - \delta_{\text{free}})\}$$
$$[\text{Host}]_{\text{Stoic}}) \times ([\text{Guest}]_{\text{Stoic}} - \{\delta_{\text{obs}} - \delta_{\text{free}})/$$
$$(\delta_{\max} - \delta_{\text{free}})\}[\text{Host}]_{\text{stoic}}) = \{(\delta_{\text{obs}} - \delta_{\text{free}})$$
$$/\delta_{\max} - \delta_{\text{free}})\}[\text{Host}]_{\text{stoic}} \quad (1)$$

Benesi-Hildebrand type graphs of the chemical shift data for DABCO-2 hydrogens for the systems **4avi** and **4av** with added *N*-acetyl-phenylalanine (**8**) are shown in Figure 6. The linearity of each of these plots (lines shown are least square fits of the data) is in accord only with a 1:1 association of host and guest.

There are sizable variations in the values of the association constants measured. First, the association constants noted for the phosphorus oxyanion species, while greater than unity, are generally less than 10^3 (4.8–460, with only one observed to be greater than 10^3: 1,200). Significantly stronger interactions are observed with the amino acid series (**8–14**) for which the general range is 10^3–10^4, with one instance (**4biv** interacting with **9**) more than 10^5. The cyclodextrin derivative most effective at serving as host for all of the amino acid series guests is **4biv**, that bearing the 8-hydroxyoctyl substituent on each of the DABCO rings. Significantly strong interactions are observed with each of the tetrapeptides (particularly **16**) and each of the host cyclodextrin derivatives examined. These results are promising for potential utility of the polycationic cyclodextrins as selective binding and transport agents for peptides.

While the binding of the entire series of amino acid derivatives and the tetrapeptides studied is quite strong, the orientation of binding is not the same for all of these guest species in a given host. As noted previously, the values for the association constants were determined through observation of the changes in chemical shift for the DABCO-2 hydrogens, a change that was quite similar for all of the host/guest systems. Observation of changes of all of the hydrogens within the cationic region of the cyclodextrin derivatives indicates different degrees of change in chemical shift for the DABCO-α hydrogens, depending on the specific host and guest species. Particularly, with the *N*-Ac-glutamic acid (**12**) and the folic acid (**13**) guest systems, with all host species, negligible change in the chemical shift of these DABCO-α hydrogens could be noted while the changes in chemical shifts for DABCO-1 and DABCO-2 hydrogens remained comparable to that for other systems studied. This was also observed for the remainder of the guests on interaction with hosts **4aviii** and **4bviii,** those with the dodecyl so-called tail lacking any functionality that could provide a polar or hydrogen-bonding interaction from the tail.

These latter results suggest that approach of the guest to the host can occur in two different ways, depending on the nature of any additional polar or anionic substituents on the guest and the ability of the host to accommodate such substituents.

In the normal situation, the guest enters the host

TABLE 4 Association Constants (K) for Binding of Anions with Polycationic Cyclodextrin Derivatives

	Host									
Guest	**4aiv**	**4av**	**4avi**	**4avii**	**4aviii**	**4biv**	**4bv**	**4bvi**	**4bvii**	**4bviii**
5	0.036	*	0.073	0.042	0.020	0.0048	*	0.38	0.031	*
6	0.099	0.080	1.2	0.025	*	0.39	0.012	2.6	*	*
7	0.086	0.049	*	*	*	0.46	0.22	*	*	*
8	1.0	5.6	24	3.4	1.1	4.2	1.8	7.6	4.0	5.6
9	5.6	15	28	7.6	67	120	7.2	34	76	18
10	0.16	2.2	1.1	5.3	0.24	44	0.72	2.0	1.7	1.6
11	3.1	2.2	0.89	1.0	0.43	4.0	3.3	0.86	7.3	0.55
12	1.4	1.9	1.0	1.0	1.1	3.9	1.3	0.45	1.4	0.71
13	2.6	2.3	3.6	2.5	2.9	3.4	6.2	1.3	2.1	0.28
14	2.8	1.0	1.5	4.7	0.72	3.2	31	1.3	1.7	0.50
15	1.3	0.89	1.3	1.0	0.91	23	2.7	1.7	12	0.62
16	36	4.2	11	27	25	39	13	12	33	5.4

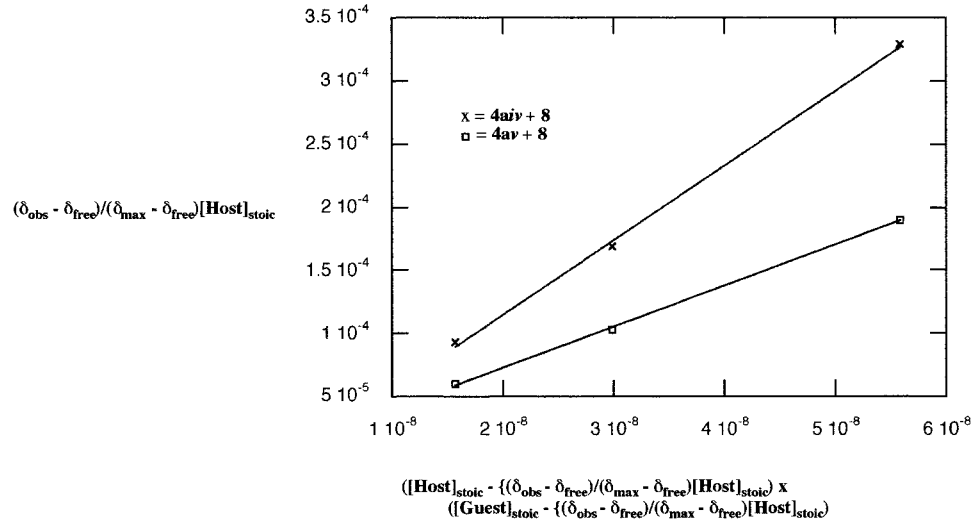


FIGURE 6 Plots using the Benesi-Hildebrand method for determination of *K*.

from the top of the tube. The anionic portion of the guest associates with the cationic region from the top, approaching closely to the DABCO-α region. We interpret the results to demonstrate that this mode of approach occurs with the guest species derived from Phe, Cys, Tyr, Leu, and Lys, as well as the two tetrapeptides studied.

Alternatively, approach of the guest to the host could occur from the bottom of the tube, the parent cyclodextrin side. The anionic region of the guest would thereby approach first the DABCO ring hydrogens, leaving the DABCO-α hydrogens relatively unshielded. This latter approach would be favored in two types of instances: (1) with all cyclodextrin derivatives, those systems for which an additional or anionic substituent is present on the guest, and (2) with all monoanionic guests interacting with a host (**4aviii** and **4bviii**) devoid of any capability of hydrogen bonding or ionic interaction involving the distal regions of the tail. The observations suggest that this approach occurs for *N*-acetyl-glutamic acid and folic acid species. Such systems involve relatively unfavorable interactions of the hydrophobic tail at the top of the host tube with any portion of the guest.

Overall, this new class of cyclodextrin derivative provides particularly intriguing capabilities for encapsulation of anionic species in aqueous solution. We may anticipate potential utility of such host species in applications requiring selectivity, separation of anionic species, transport in biologically related systems, and analyses.

EXPERIMENTAL

General

All chemicals used in syntheses, purification, and comparison analyses were of commercial reagent quality and were used without purification. The following compounds were prepared according to a previous report [2]: 1-azo-4-(2-hydroxyethyl) azoniabicyclo[2.2.2]octane chloride (**3i**), 1-azo-4-(3-hydroxypropyl)azoniabicyclo[2.2.2]octane chloride (**3ii**), and 1-azo-4-(8-hydroxyoctyl)azoniabicyclo [2.2.2]octane chloride (**3iv**). All NMR spectra were measured using a Brüker 400 MHz DPX400 instrument. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY.

*Preparation of 1-Azo-4-(substituted) azoniabicyclo[2.2.2]octane Salts (***3iii, 3v, 3vi, 3vii, 3viii, 3ix***)*

DABCO (1 equiv.) and the appropriate haloalkane (0.2 equiv.) were dissolved in ethyl acetate (4 $\times$ the combined masses of the reagents) in a round-bottomed flask and stirred at ambient temperature with a magnetic stirrer for 24 hours. After this time, the white precipitate was collected by suction filtration through sintered glass, washed with ethyl acetate (3 $\times$ 30 mL) and anhydrous ether (3 $\times$ 30 mL), and dried under high vacuum. Typical yields of products and analytical data are given in Table 1.

*Preparation of Polycationic Cyclodextrin Derivatives (***4aiii–4aix, 4bi–4bix***)*

To a solution of the appropriate **3** (0.005 mol) in acetonitrile (30 mL) in a 100 mL round-bottomed flask was added with magnetic stirring the appropriate per-tosylated cyclodextrin (**2**) (0.0007 mol) in acetonitrile (30 mL). The reaction mixture was stirred and heated at reflux for three days. After cooling, the

resultant white precipitate was collected by suction filtration, washed with ethyl acetate (3 × 30 mL) followed by ether (3 × 30 mL), and dried under high vacuum. Typical yields of products and analytical data are given in Table 2.

NMR Measurement of the Interaction of Guests with Polycationic Cyclodextrin Derivatives **4**

Stock solutions of the investigated polycationic cyclodextrin derivatives of concentration in the range 0.001–0.002 M were prepared in D_2O, and to these were added measured amounts of stock solutions of the guest species, prepared as the sodium salts of concentrations in the range 0.001–0.002 *M* in D_2O. Solutions were brought to standard volume. These mixtures (of approximate ratios of host/guest 1.0/0.1, 1.0/0.2, 1.0/0.5 and 1.0/1.0 equivalents) were prepared, and their ^{1}H NMR spectra were measured. These spectra were compared with those of pure host at 0.001 *M* in D_2O. Changes in chemical shift between the pure host signals for the two types of DABCO hydrogens and those α to the DABCO site along the pendant chain were measured and compared with the measured maximal shifts; maximal shifts were obtained by the addition of a large excess of guest to the solution until no additional change in chemical shift could be noted.

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