Special Issue Article

Synthesis and Utilization of Trialkylammonium-Substituted Cyclodextrins as Water-Soluble Chiral NMR Solvating Agents for Anionic Compounds

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Abstract Cationic trialkylammonium-substituted α-, β-, and γ-cyclodextrins containing trimethyl-, triethyl-, and tri-*n*-propylammonium substituent groups were synthesized and analyzed for utility as water-soluble chiral nuclear magnetic resonance (NMR) solvating agents. Racemic and enantiomerically pure (3-chloro-2-hydroxypropyl)trimethyl-, triethyl-, and tri-*n*-propyl ammonium chloride were synthesized from the corresponding trialkyl amine hydrochloride and either racemic or enantiomerically pure epichlorohydrin. The ammonium salts were then reacted with α-, β-, and γ-cyclodextrins at basic pH to provide the corresponding randomly substituted cationic cyclodextrins. The ^{1}H NMR spectra of a range of anionic, aromatic compounds was recorded with the cationic cyclodextrins. Cyclodextrins with a single stereochemistry at the hydroxy group on the (2-hydroxypropyl)trialkylammonium chloride substituent were often but not always more effective than the corresponding cyclodextrin in which the C-2 position was racemic. In several cases, the larger triethyl or tri-*n*-propyl derivatives were more effective than the corresponding trimethyl derivative at causing enantiomeric differentiation. None of the cyclodextrin derivatives were consistently the most effective for all of the anionic compounds studied. *Chirality 28:299–305, 2016.*

KEY WORDS: cyclodextrins; chiral solvating agents; NMR; enantiomeric differentiation; chiral analysis

Nuclear magnetic resonance (NMR) spectroscopy is frequently used for the analysis of enantiomeric purity and the assignment of absolute configuration.[1–6] NMR methods for chiral analysis rely on the utilization of chiral derivatizing agents, solvating agents, metal complexes, or liquid crystals to achieve nonequivalent environments for enantiomeric compounds leading to differences in chemical shifts in the NMR spectrum. Among the different NMR systems, chiral solvating agents are noteworthy because of their ease of use and less stringent need for complete enantiomeric purity of the chiral reagent. Also, whereas there is often a concern that reaction with a chiral derivatizing agent could result in some loss of configuration of the analyte, this rarely if ever occurs with chiral solvating agents that associate through noncovalent interactions. Enantiomeric differentiation in NMR spectra of substrates with chiral solvating agents occurs because the complexes are diastereomeric and/or the association constants of the two enantiomers with the chiral solvating agent are different. It is important that the exchange of bound and unbound substrate with the chiral solvating agent be fast on the NMR time scale.

Cyclodextrins are an especially versatile family of chiral NMR solvating agents.[2,3,5–16] Cyclic oligosaccharides comprised of D-glucose rings, the different sizes of the cavity in α-, β-, and γ-cyclodextrin accommodate different size analytes. Derivatization of the hydroxyl groups expands the versatility of cyclodextrins by providing chiral reagents with different solubility properties and different functionalities, allowing the cyclodextrin to be tailored for certain families of analytes. Native, underivatized cyclodextrins are soluble in water and have been used in NMR spectroscopy to enantiomerically differentiate a range of compounds. Many of the water-soluble organic analytes previously examined with cyclodextrins are charged species.

Prior studies have shown that anionic cyclodextrins containing sulfate[17,18] or carboxymethylate[18–21] groups are often much more effective chiral NMR solvating agents for cationic substrates than the corresponding native cyclodextrin. Similarly, cationic cyclodextrins containing protonated amino,[22–26] bis(6-trimethylammonium),[27] or mono(6-xylylethyenediamine)[28] groups were found to be more effective chiral NMR solvating agents for anionic substrates than the corresponding native cyclodextrin. A more recent report examined cyclodextrins with higher degrees of substitution of trimethylammonium groups and found that these were much more effective chiral

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NMR solvating agents for anionic substrates than native cyclodextrins and derivatives with only two trimethylammonium substituent groups.[29] Of the cationic cyclodextrins, an advantage of the trimethylammonium derivative is that it contains quaternary amines that are cationic at all pH values. Cyclodextrin derivatives with primary amine groups require careful adjustment of pH to maintain the cyclodextrin as a cationic species and analyte as an anionic species.

The procedure used to synthesize cyclodextrins that contain a trimethylammonium group is shown in Figure 1. Commercially available glycidyltrimethylammonium chloride was reacted with the cyclodextrin for several days at basic pH. Prior reports of these cationic cyclodextrins as chiral NMR solvating agents involved the use of derivatives prepared using racemic glycidyltrimethylammonium chloride.[27,29] Opening of the epoxide ring in the reaction produces a new stereocenter at the carbinol group on the substituent. When racemic glycidyltrimethylammonium chloride is used in the reaction, the stereochemistry of the carbinol group is racemic as well. It is possible that having only a single stereochemistry of the carbinol group may enhance the enantiomeric recognition properties of the trimethylammoniated cyclodextrins by providing another site for enantiomeric differentiation. Also, since the degree of enantiomeric differentiation is often dependent on the quality of the fit of the substrate into the cyclodextrin cavity, derivatives with ammonium groups containing larger ethyl or *n*-propyl groups may have varying degrees of effectiveness as chiral NMR solvating agents.

We report herein the preparation of cyclodextrin derivatives where the carbinol in the substituent group has only a single stereochemistry and where the substituent has trimethyl-, triethyl-, or tri-*n*-propylammonium groups. The utility of these cationic cyclodextrins as chiral NMR solvating agents for a range of anionic analytes is reported.

MATERIALS AND METHODS

Reagents

Substrate compounds for NMR analysis were obtained from commercial sources either as a sodium salt or neutral compound. Epichlorohydrin, (*R*)-epichlorohydrin, triethylammonium chloride, tri-*n*-propylamine, (*S*)-(3-chloro-2-hydroxypropyl)trimethylammonium chloride, α-, β-, and γ-cyclodextrin and tetrabutylammonium iodide were obtained from Sigma-Aldrich (St. Louis, MO). Spectra/Por Cellulose Ester dialysis tubing, 100–500 MWCO was purchased from Spectrum Laboratories (Rancho Dominguez, CA).

Synthetic Procedures

Synthesis of racemic (3-chloro-2-hydroxypropyl)triethylammonium chloride. Racemic (3-chloro-2-hydroxypropyl)triethylammonium chloride was synthesized by a modification of a published procedure.[30] In a 250-mL round bottom flask, 11.60 g (126 mmol) of racemic epichlorohydrin, 17.34 g (126 mmol) of triethylammonium chloride, and 50 mL of chloroform were added. The solution was stirred at 65°C under nitrogen for 2–5 days. The reaction was monitored for completion by ^{1}H NMR spectroscopy in $CDCl_3$. The product was extracted into 30 mL distilled water and used directly in a reaction with the appropriate quantity or α-, β-, or γ-cyclodextrin.

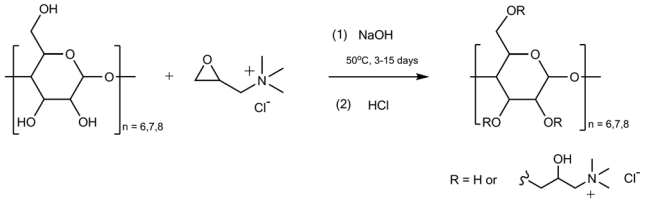

Fig. 1. Reaction of glycidyltrimethylammonium chloride with α-, β-, or γ-cyclodextrin.

Synthesis of (*R*)-(3-chloro-2-hydroxypropyl)triethylammonium chloride. The procedure was identical to that used to prepare racemic (3-chloro-2-hydroxypropyl)triethylammonium chloride except that (*R*)-epichlorohydrin was used in the reaction. The reaction was monitored for completion by ^{1}H NMR spectroscopy in $CDCl_3$.

Synthesis of racemic (3-chloro-2-hydroxypropyl)tri-*n*-propylammonium chloride. Tri-*n*-propylamine (18.08 g, 126 mmol) was added dropwise to 60 mL of 2 M HCl in a 250-mL round-bottom flask at 0°C. Rotary evaporation provided tri-*n*-propylammonium chloride as a white solid. The product was verified by ^{1}H NMR spectroscopy in $CDCl_3$. Racemic (3-chloro-2-hydroxypropyl)tri-*n*-propylammonium chloride was prepared by a procedure identical to that used to prepare racemic (3-chloro-2-hydroxypropyl)triethylammonium chloride except that tri-*n*-propylammonium chloride was used in the reaction. The reaction, which typically required 4–7 days, was monitored for completion by ^{1}H NMR spectroscopy in $CDCl_3$. The product was separated using 30 mL distilled water. The top, clear, aqueous layer contained the product and was subsequently used to react with cyclodextrins.

Synthesis of (*R*)-(3-chloro-2-hydroxypropyl)tri-*n*-propylammonium chloride. The procedure was identical to that used to prepare racemic (3-chloro-2-hydroxypropyl)tri-*n*-propylammonium chloride except that (*R*)-epichlorohydrin was used in the reaction. The reaction was monitored for completion by ^{1}H NMR spectroscopy in $CDCl_3$.

Synthesis of (*rac*-2-hydroxypropyl)triethylammonium-α-cyclodextrin. Racemic (2-hydroxypropyl)triethylammonium-α-cyclodextrin was synthesized by a modification of a published procedure.[31] To a 250-mL round bottom flask, 4.67 g (4.8 mmol) of α-cyclodextrin and a catalytic amount of tetrabutylammonium iodide was added to the 30 ml aqueous solution of racemic (3-chloro-2-hydroxypropyl)triethylammonium chloride (126 mmol). The pH of the solution was raised to 12 with 4 M sodium hydroxide. The reaction mixture was stirred at 50°C. The pH was monitored during the first 10 hours of the reaction and often more sodium hydroxide was needed to maintain the pH at 12. The reaction was monitored by ^{1}H NMR spectroscopy until the desired degree of substitution was achieved (7–10 days). The reaction mixture was placed on ice and the pH adjusted to 6 by the addition of 2 M HCl and the total volume was brought up to 75 mL with distilled water. A 30–40 cm long piece of Spectra/Por CE tubing was allowed to soak in distilled H_2O for 0.5 h. The reaction mixture was added to the dialysis tubing and dialyzed against distilled H_2O for 10 h. Fresh distilled water was added and dialysis was continued for an additional 6 h. The volume of the liquid in the dialysis tube was reduced by rotary evaporation and then in a vacuum desiccator to afford either a clear, colorless syrup or white crystalline material. Integration of the appropriate signals in the ^{1}H NMR spectrum was used to determine the degree of substitution.

Synthesis of (*rac*-2-hydroxypropyl)triethylammonium-β-cyclodextrin, (*rac*-2-hydroxypropyl)triethylammonium-γ-cyclodextrin, (*rac*-2-hydroxypropyl)tri-*n*-propylammonium-α-cyclodextrin, (*rac*-2-hydroxypropyl)tri-*n*-propylammonium-β-cyclodextrin and (*rac*-2-hydroxypropyl)tri-*n*-propylammonium-γ-cyclodextrin. The procedure was identical to that used to prepare (*rac*-2-hydroxypropyl)triethylammonium-α-cyclodextrin with similar equivalents of reactants except the appropriate ammonium salt and cyclodextrin were used in the reaction.

Synthesis of ((*S*)-2-hydroxypropyl)trimethylammonium-β-cyclodextrin. The procedure was identical to that used to prepare (*rac*-2-hydroxypropyl)triethylammonium-α-cyclodextrin except that (*S*)-(3-chloro-2-hydroxypropyl)trimethylammonium chloride was used in the reaction.

Synthesis of ((*R*)-2-hydroxypropyl)triethylammonium-β-cyclodextrin and ((*R*)-2-hydroxypropyl)tri-*n*-propylammonium-β-cyclodextrin. The procedure was identical to that used to prepare (*rac*-2-hydroxypropyl) triethylammonium-α-cyclodextrin except that (*R*)-(3-chloro-2-hydroxypropyl) triethyl- or tri-*n*-propylammonium chloride and the appropriate cyclodextrin were used in the reaction.

Procedures for Chiral Differentiation Studies

Concentrated solutions (0.10 M) of substrates enriched in one enantiomer (when available) were prepared in deuterium oxide. When the substrate was available as a neutral compound instead of its sodium salt, a stoichiometric equivalent of sodium deuteroxide in deuterium oxide was added to the solution. Each 600 μL NMR sample contained the necessary weight of each cyclodextrin to provide a 10 mM, 20 mM, or 30 mM concentration as needed. Additionally, 540 μL of deuterium oxide and 60 μL of the 0.1 M stock solution of a substrate were added to provide a 10 mM concentration of substrate. ^{1}H NMR spectra were obtained using a Bruker Avance (Billerica, MA) 400 MHz NMR spectrometer with eight scans at ambient temperature.

RESULTS AND DISCUSSION

Synthesis of Cationic Cyclodextrins

Racemic (2-hydroxypropyl)trimethylammonium-substituted α-, β-, and γ-cyclodextrin (*rac*-TMA) is commonly prepared by the reaction of commercially available glycidyltrimethylammonium chloride with the cyclodextrin (Fig. 1).[29,31] The starting ammonium salts needed for a similar preparation of the racemic (2-hydroxypropyl)triethyl- and tri-*n*-propylammonium and enantiomerically pure (2-hydroxypropyl)-trimethyl-, triethyl-, and tri-*n*-propyl ammonium cyclodextrins are not commercially available. Initial attempts at preparing these other ammonium salts involved the reaction of either racemic- or (*R*)-epichlorohydrin with triethylamine or tri-*n*-propylamine (Fig. 2). Two different previously published procedures were used in these attempts.[32,33] A solution of trimethylamine in ethanol, which is commercially available, was used in the reaction with (*R*)-epichlorohydrin. The reaction in Figure 2 was ineffective for our purposes, as we were unable to avoid the production of substantial quantities of an alkene byproduct (*N*-(3-hydroxy-1-propenyl)trialkylammonium chloride – $(CH_3)_3N^+CH = CHCH_2OH\ Cl^-$) with all three amines. The precedence for forming the alkene has been previously observed.[34] The yield of the desired trialkylammonium epoxide using the reaction shown in Figure 2 was too low to merit a subsequent reaction with cyclodextrin.

An alternative procedure to prepare (2-hydroxypropyl)-trimethylammonium-substituted cyclodextrins with a single stereochemistry at the carbinol of the substituent group involves the reaction of commercially available (*S*)-(3-chloro-2-hydroxypropyl)trimethylammonium chloride with α-, β-, or γ-cyclodextrin at basic pH, as shown in Figure 3.[31] This procedure was effective at producing the desired (*S*)-2-hydroxypropyl)trimethylammonium-substituted cyclodextrins ((*S*)-TMA). Typical degrees of substitution for the (*S*)-(2-hydroxypropyl)trimethylammonium derivatives of β-cyclo-dextrin ranged from 5 to 12.

Racemic- and (*R*)-(3-chloro-2-hydroxypropyl)triethyl- and tri-*n*-propylammonium chloride salts were prepared as shown in Figure 4.[30] The progress of the reaction can be monitored by the diminishment of the trialkylamine methyl resonance and growth of the trialkylammonium methyl resonance in the ^{1}H NMR spectrum. The (2-hydroxypropyl)trialkylammonium salt was separated from unreacted trialkylamine and

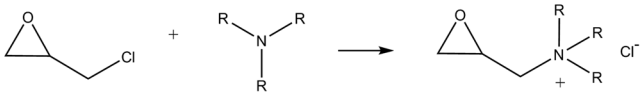

Fig. 2. Reaction used in an attempt to prepare glycidyltrialkylammonium chloride salts.

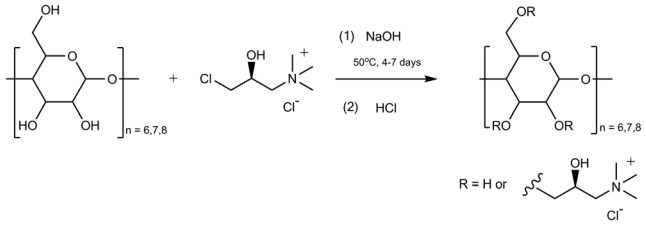

Fig. 3. Reaction of (*S*)-(3-chloro-2-hydroxypropyl)trimethylammonium chloride with α-, β-, or γ-cyclodextrin.

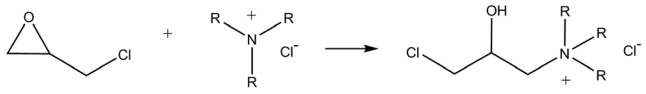

Fig. 4. Reaction used to prepare (3-chloro −2-hydroxypropyl)triethylammonium chloride and (3-chloro-2-hydroxypropyl)tri-*n*-propylammonium chloride.

epichlorohydrin by extraction into water. The resulting aqueous solution was reacted with the cyclodextrin as in Figure 3. A large excess of the (3-chloro-2-hydroxypropyl)trialkylammonium salt is used relative to the cyclodextrin to raise the degree of substitution. A peak at about 4.4 ppm in the ^{1} H NMR spectrum of the product corresponds to the methine resonance of the substituent group. Representative ^{1}H NMR spectra obtained for *rac*-TEA-α and *rac*-TEA-γ are shown in Figure 5a,b, respectively. The area of the peak at 4.4 ppm relative to either the area of the H1 or H2-H6 resonances of the cyclodextrin can be used to determine the degree of substitution (DS). Typical DS ranged from 7 to 12 for the triethylammonium (*rac*-TEA and (*R*)-TEA) and tri-*n*-propylammonium-substituted cyclodextrins (*rac*-TPA and (*R*)-TPA). Only derivatives with a DS in the range of 10–12 were used in studies aimed at producing enantiodifferentiation in the NMR spectra of substrates. Purification of the cationic cyclodextrins was achieved by dialysis using membranes with a 500 MW cutoff.

NMR Studies of Anionic Substrates

The effectiveness of *rac*-TMA-β, *rac*-TEA-β, and *rac*-TPA-β as chiral NMR solvating agents is compared to (*S*)-TMA-β (*R*)-TEA-β and (*R*)-TPA-β. Thirteen different substrates (**1–13**, Fig. 6) that span a range of compound classes and are anionic when deprotonated as herein were examined. Enantiomeric differentiation in the ^{1}H NMR spectrum of **1–13** with

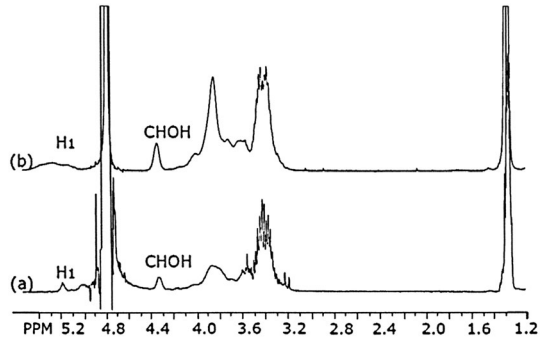

Fig. 5. ^{1}H NMR spectrum (400 MHz, D_2O, 25°C) of (**a**) *rac*-TEA-α and (**b**) *rac*-TEA-γ. The peak at about 4.4 ppm corresponds to the methine hydrogen of the substituent group.

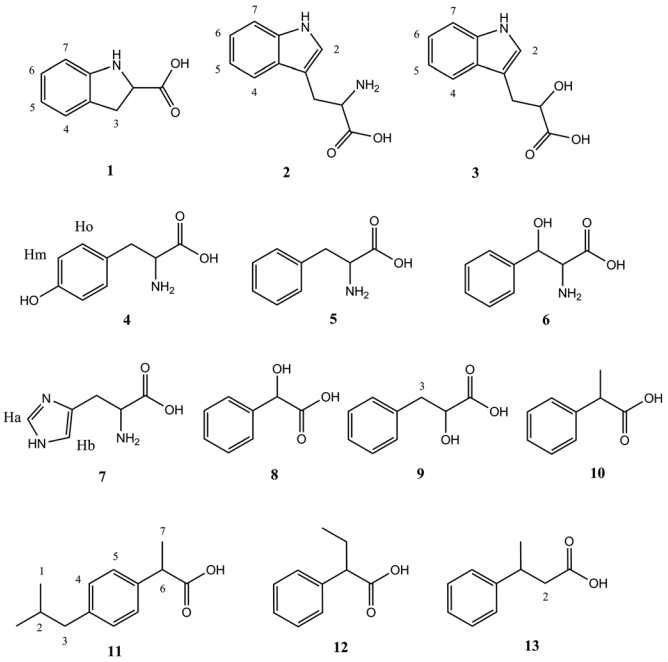

Fig. 6. Structure of substrates: indoline-2-carboxylic acid (**1**), tryptophan (**2**), 3-indolelactic acid (**3**), tyrosine (**4**), phenylalanine (**5**), phenylserine (**6**), histidine (**7**), mandelic acid (**8**), 3-phenyllactic acid (**9**), 2-phenylpropionic acid (**10**), ibuprofen (**11**), 2-phenylbutyric acid (**12**), 3-phenylbutyric acid (**13**). All substrates were examined as their sodium salts.

rac-TMA-β or (*S*)-TMA-β) (Table 1), *rac*-TEA-β or (*R*)-TEA-β (Table 2), and *rac*-TPA-β or (*R*)-TPA-β (Table 3) are provided. In each case, the substrate concentration was 10 mM and spectra with cyclodextrin were recorded at concentrations typically ranging from 10 to 30 mM. The values of enantiomeric differentiation reported in Tables 1–3 are the maximum observed among the different cyclodextrin concentrations where no overlap with other substrate or cyclodextrin resonances occurred.

TABLE 1. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic β-TMA-cyclodextrin species

		rac-TMA-β[a,b]	(*S*)-TMA-β
1	CH_2	0.032 (20 mM)	0.020 (10 mM)
	H4	0.019 (20 mM)	0.015 (30 mM)
	H6	0	0.017 (30 mM)
3	H2	0	0.006 (20 mM)
	H4	0	0.006 (20 mM)
	H5	0	0.005 (20 mM)
	H6	0	0.005 (20 mM)
	H7	0	0.006 (20 mM)
	CH	0.021 (20 mM)	0.011 (30 mM)
	CH_2	0.037 (20 mM)	0
4	Ho	0.021 (20 mM)	0
	Hm	0	0.010 (20 mM)
	CH	0	0.006 (10 mM)
	CH_2	0	0.019 (10 mM)
6	CHOH	0	0.003 (30 mM)
7	Ha	0	0.002 (10 mM)
	CH_2	0.004 (20 mM)	0
	CH_2'	0.005 (20 mM)	0
8	CH	0.006 (20 mM)	0.010 (30 mM)
9	CH	0.020 (20 mM)	0.013 (30 mM)
10	CH_3	0.005 (20 mM)	0.008 (30 mM)
11	H5	0.009 (20 mM)	0
	H7	0.006 (20 mM)	0.006 (20 mM)

[a]The concentration of the cyclodextrin is indicated in parentheses.
[b]Data from reference 29.

TABLE 2. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic β-TEA-cyclodextrin species

		rac-TEA-β[a]	(*R*)-TEA-β
3	CH	0.010 (20 mM)	0.010 (20 mM)
4	Hm	0.008 (20 mM)	0.011 (30 mM)
	CH_2'	0.030 (20 mM)	0.013 (20 mM)
7	Ha	0.002 (10 mM)	0
9	CH	0.005 (10 mM)	0.011 (30 mM)
10	CH_3	0.018 (30 mM)	0
11	H5	0	0.008 (20 mM)
	H7	0.007 (20 mM)	0.008 (20 mM)
12	CH_3	0.010 (20 mM)	0.014 (30 mM)
13	CH_2	0.010 (10 mM)	0.010 (30 mM)
	CH_2'	0.053 (20 mM)	0.063 (30 mM)

[a]The concentration of the cyclodextrin is indicated in parentheses.

TABLE 3. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic β-TPA-cyclodextrin species

		rac-TPA-β[a]	(*R*)-TPA-β
4	Hm	0	0.014 (20 mM)
6	CHOH	0	0.006 (30 mM)
9	CH	-[b]	0.014 (30 mM)
11	H7	0.005 (20 mM)	0.006 (30 mM)
12	CH_3	0	0.013 (30 mM)
13	CH_2	0.011 (30 mM)	0.016 (30 mM)
	CH_2'	0.042 (20 mM)	0.065 (30 mM)
	CH_3	0	0.009 (30 mM)

[a]The concentration of the cyclodextrin is indicated in parentheses.
[b]Resonance obscured by another resonance in the NMR spectrum.

The results for TMA-β and TEA-β show that neither the racemic nor enantiomerically pure derivative is consistently more effective at causing enantiomeric differentiation in the NMR spectrum (Tables 1, 2). The inconsistency in effectiveness between cyclodextrin derivatives with a racemic or enantiomerically pure carbinol in the substituent group even occurs for different resonances of the same substrate. For example, (*S*)-TMA-β causes enantiomeric differentiation for the H6 resonance of **1** that is not observed with *rac*-TMA-β; however, the enantiomeric differentiation of one of the methylene hydrogen atoms and H4 of **1** with *rac*-TMA-β is larger than that with (*S*)-TMA-β. (*S*)-TMA-β causes enantiomeric differentiation of several hydrogen resonances in **3** and **4** that is not observed with *rac*-TMA-β. However, *rac*-TMA-β produces larger enantiomeric differentiation of one or two resonances of **3** and **4** than (*S*)-TMA-β. More resonances show larger enantiomeric differentiation with (*R*)-TEA-β (six overall) than with *rac*-TEA-β (three overall) (Table 2). However, with **7** and **10**, enantiomeric differentiation is only observed with the *rac*-TEA-β. Enantiomeric differentiation was always larger with the (*R*)-TPA-β than with *rac*-TPA-β, although fewer

resonances show any differentiation with the TPA derivatives than with the TMA or TEA derivatives (Table 3).

These data indicate that in some cases the fixed stereochemistry at the carbinol of the substituent group is important in causing enantiomeric differentiation and is influential in the magnitude of the chemical shift difference between the two enantiomers. The inconsistency in cases where the *rac*-TMA-β caused larger enantiomeric differentiation for some resonances, whereas the (*S*)-TMA-β caused larger enantiomeric differentiation for others, suggests that the diastereomeric nature of the complexes species is more important than differences in the association constants in causing the enantiomeric differentiation.

A comparison of the effectiveness of the TMA-, TEA-, and TPA-substituted cyclodextrins is provided in Tables 4–6 for the *rac*-α, *rac*-β, and *rac*-γ derivatives, respectively. One of the advantages of cyclodextrins is that the different cavity sizes of α-, β-, and γ-cyclodextrin provide complementary fits for different sized substrates, thereby expanding the number of compounds that can be analyzed.

Among the three α-cyclodextrin derivatives, *rac*-TMA-α is often far more effective at causing enantiomeric differentiation in the ^{1}H NMR spectrum than *rac*-TEA-α and *rac*-TPA-α (Table 4). For those substrates studied with all three of the racemic cyclodextrins, enantiomeric differentiation of only one of the methylene hydrogen atoms of **9** is largest with *rac*-TEA-α and the H3 resonance of **11** is largest with *rac*-TPA-α. Given that the size of the cavity of α-cyclodextrin is the smallest of the three cyclodextrins, this suggests that the larger ethyl and *n*-propyl substituent groups likely block access of some substrates to the cavity.

For the β-cyclodextrin derivatives, *rac*-TMA-β and *rac*-TEA-β each exhibit complementary degrees of effectiveness and each is best for several resonances (Table 5). The general lack of enantiomeric differentiation in the ^{1}H NMR spectra of many substrates with *rac*-TPA-β suggests that the bulkier *n*-propyl groups likely restrict access of some substrates to the cavity. For the γ-cyclodextrin derivatives, the *rac*-TMA-γ, *rac*-TEA-γ, and *rac*-TPA-γ cause the largest enantiomeric differentiation of five, three, and four resonances, respectively (Table 6). These data show that the larger ethyl and *n*-propyl groups can be advantageous in enhancing the extent of enantiomeric differentiation, especially with β- and γ-cyclodextrin.

Enantiomeric differentiation of the diastereotopic H3 and H3' methylene resonances of **9** using *rac*-TEA-α, *rac*-TEA-β, and *rac*-TEA-γ is shown in Figure 7. This set of spectra shows the degree to which changes in the size of the cyclodextrin

TABLE 4. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic α-cyclodextrin species

		rac-TMA-α[a,b]	*rac*-TEA-α	*rac*-TPA-α
2	H4	0.007 (20 mM)	0	0
	H5	0.031 (20 mM)	0	0.013 (30 mM)
	H6	0.004 (20 mM)	0	0
	CH_2	0.037 (20 mM)	0	0
3	H4	0.031 (20 mM)	0.018 (30 mM)	0.017 (30 mM)
	H5	0.007 (20 mM)	0	0
	H7	0.030 (20 mM)	0.021 (20 mM)	0.019 (30 mM)
	CH	0.010 (20 mM)	0	0
	CH_2	0.010 (20 mM)	0	0
4	Hm	0.028 (20 mM)	0	0
	CH_2	0.037 (20 mM)	0	0
6	$CHNH_2$	0.025 (20 mM)	0	0
7	Hb	0.016 (20 mM)	0	0
9	CH	0.040 (20 mM)	0.030 (30 mM)	0.010 (10 mM)
	CH_2	0.015 (20 mM)	0.009 (30 mM)	0
	CH_2'	0	0.015 (20 mM)	0
11	H1	0.008 (20 mM)	0.001 (10 mM)	0
	H3	0	0	0.014 (20 mM)
	H7	0.008 (20 mM)	0	0
13	CH_2	-[c]	0.025 (30 mM)	0.033 (20 mM)
	CH_2'	-[c]	0.035 (30 mM)	0.016 (20 mM)
	CH_3	-[c]	0	0.007 (20 mM)

[a]The concentration of the cyclodextrin is indicated in parentheses.
[b]Data from reference 29.
[c]Sample not recorded.

TABLE 5. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic β-cyclodextrin species

		rac-TMA-β[a,b]	*rac*-TEA-β	*rac*-TPA-β
1	CH_2	0.032 (20 mM)	0	0
	H4	0.019 (20 mM)	0	0
3	CH	0.021 (20 mM)	0.010 (20 mM)	-[c]
	CH_2	0.037 (20 mM)	0	-[c]
4	Ho	0.021 (20 mM)	0	0
	Hm	0	0.008 (20 mM)	0
	CH_2'	0	0.030 (20 mM)	0
7	Ha	0	0.002 (10 mM)	0
	CH2	0.004 (20 mM)	0	0
	CH2'	0.005 (20 mM)	0	0
8	CH	0.006 (20 mM)	-[d]	-
9	CH	0.020 (20 mM)	0.005 (10 mM)	-[d]
10	CH_3	0.005 (20 mM)	0.018 (30 mM)	0
11	H5	0.009 (20 mM)	0	0
	H7	0.006 (20 mM)	0.007 (20 mM)	0.005 (20 mM)
12	CH_3	-[c]	0.010 (20 mM)	0
13	CH_2	-[c]	0.010 (10 mM)	0.011 (30 mM)
	CH_2'	-[c]	0.053 (20 mM)	0.042 (20 mM)

[a]The concentration of the cyclodextrin is indicated in parentheses.
[b]Data from reference 29.
[c]Sample not recorded.
[d]Resonance obscured by another resonance in the NMR spectrum.

TABLE 6. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic γ-cyclodextrin species

		rac-TMA-γ[a,b]	*rac*-TEA-γ	*rac*-TPA-γ
1	CH_2	0	0.005 (20 mM)	0
2	H4	0.010 (20 mM)	0	0
	H5	0.010 (20 mM)	0	0
	CH_2	0	0.040 (20 mM)	0
3	CH_2	0	0.010 (20 mM)	0.010 (20 mM)
4	Ho	0	0	0.007 (10 mM)
	CH_2	0.011 (20 mM)	0	0
5	CH_2	0.008 (20 mM)	0	0
8	CH	0.007 (20 mM)	-[c]	-[d]
9	CH	0.004(20 mM)	0.004 (20 mM)	0.006 (20 mM)
	CH_2	0.007 (20 mM)	0.007 (20 mM)	0.008 (20 mM)
13	CH_2'	-[d]	0.017 (20 mM)	0.017

[a]The concentration of the cyclodextrin is indicated in parentheses.
[b]Data from reference 29.
[c]Resonance obscured by another resonance in the NMR spectrum.
[d]Sample not recorded.

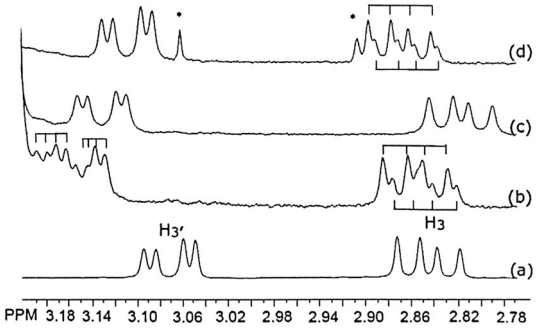

Fig. 7. ^{1}H NMR spectrum (400 MHz, D_2O, 25°C) of the diastereotopic H_3 and $H_{3'}$ methylene resonances of (**a**) **9** (10 mM, (D)-enriched) with 30 mM (**b**) *rac*-TEA-α, (**c**) *rac*-TEA-β, and (**d**) *rac*-TEA-γ. *Resonances from an impurity.

cavity can influence the degree of enantiomeric differentiation. The *rac*-TEA-α and *rac*-TEA-γ cause a small degree of enantiomeric differentiation for H3, whereas the *rac*-TEA-β does not. For H3', *rac*-TEA-α causes significant enantiomeric differentiation, whereas neither *rac*-TEA-β nor *rac*-TEA-γ causes any enantiomeric differentiation.

The effects of (*S*)-TMA-β, (*R*)-TEA-β, and (*R*)-TPA-β on the diastereotopic H2 and H2' methylene resonances of **13** are shown in Figure 8. All three cyclodextrins produce considerable enantiomeric differentiation of H2', the largest of which occurs with (*S*)-TMA-β. A lesser degree of enantiomeric differentiation is observed in the H2 resonance with all three of the cyclodextrins. The spectrum with (*R*)-TPA-β shows slightly more broadening than those with (*S*)-TMA-β and (*R*)-TEA-β. The broadening is likely caused by a slower exchange of substrate between its unbound and complexed form when mixed with (*R*)-TPA-β. This broadening, especially with the larger more sterically hindered TPA derivatives, is observed with some other substrates as well. One other feature worth noting is that the 3-bond coupling constants between H2 and H3 changes when comparing the spectrum of the substrate (Fig. 8a) with those with the cyclodextrins (Fig. 8b,c). Presumably, complexation of **13** with the cationic cyclodextrins influences the rotational motion within the substrate thereby changing the time-averaged dihedral angle between H2 and H3.

A comparison of the enantiomeric differentiation of the resonances of diastereotopic H2 and H2' of **13** with *rac*-TEA-α, *rac*-TEA-β, and *rac*-TEA-γ is shown in Figure 9. The largest enantiomeric differentiation of H2' is caused with *rac*-TEA-β, whereas the enantiomeric differentiation of H2 is slightly larger with *rac*-TEA-α. *rac*-TEA-γ causes only a slight

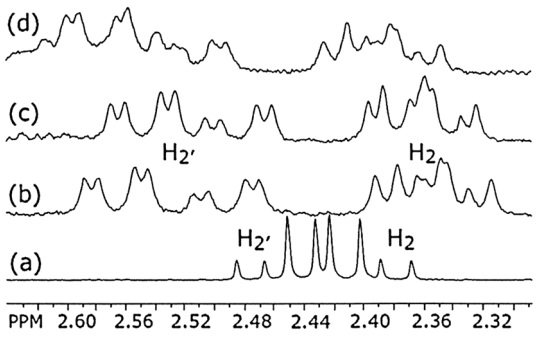

Fig. 8. ^{1}H NMR spectrum (400 MHz, D_2O, 25°C) of the diastereotopic H_2 and $H_{2'}$ methylene resonances of (**a**) **13** (10 mM, (*S*)-enriched) with 30 mM (**b**) (*S*)-TMA-β, (**c**) (*R*)-TEA-β, and (**d**) (*R*)-TPA-β.

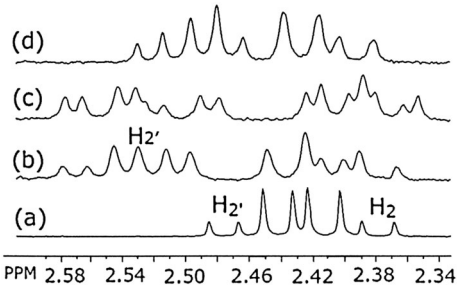

Fig. 9. ^{1}H NMR spectrum (400 MHz, D_2O, 25°C) of the diastereotopic H_2 and $H_{2'}$ methylene resonances of (**a**) **13** (10 mM, (*S*)-enriched) with 20 mM (**b**) *rac*-TEA-α, (**c**) *rac*-TEA-β, and (**d**) *rac*-TEA-γ.

enantiomeric differentiation of H2' and no enantiomeric differentiation of H2.

The cationic cyclodextrin responsible for the largest enantiomeric differentiation of each resonance of each substrate is reported in Table 7. A total of 38 resonances within the 13

TABLE 7. Enantiomeric differentiation (ΔΔδ) in ppm in the ^{1}H NMR spectrum of substrates (10 mM) in the presence of cationic cyclodextrin species

		ΔΔδ	CD[a]
1	CH_2	0.032	*rac*-TMA-β (20 mM)
	H4	0.019	*rac*-TMA-β (20 mM)
	H6	0.017	(*S*)-TMA-β (30 mM)
2	H4	0.010	*rac*-TMA-γ (20 mM)
	H5	0.031	*rac*-TMA-α (20 mM)
	H6	0.005	*rac*-TMA-α (20 mM)
	CH_2	0.040	*rac*-TEA-γ (20 mM)
3	H2	0.006	(*S*)-TMA-β (20 mM)
	H4	0.031	*rac*-TMA-α (20 mM)
	H5	0.007	*rac*-TMA-α (20 mM)
	H6	0.005	(*S*)-TMA-β (20 mM)
	H7	0.030	*rac*-TMA-α (20 mM)
	CH	0.021	*rac*-TMA-β (20 mM)
	CH_2	0.037	*rac*-TMA-β (20 mM)
4	Ho	0.021	*rac*-TMA-β (20 mM)
	Hm	0.028	*rac*-TMA-α (20 mM)
	CH	0.006	(*S*)-TMA-β (10 mM)
	CH_2	0.055	*rac*-TMA-α (20 mM)
	CH_2'	0.030	*rac*-TEA-β (20 mM)
5	CH_2	0.008	*rac*-TMA-γ (20 mM)
6	$CHNH_2$	0.025	*rac*-TMA-α (20 mM)
7	Ha	0.002	*rac*-TEA-β (10 mM)
	Hb	0.016	*rac*-TMA-α (20 mM)
	CH_2	0.004	*rac*-TMA-β (20 mM)
	CH_2'	0.004	*rac*-TMA-β (20 mM)
8	CH	0.010	(*S*)-TMA-β (30 mM)
9	CH	0.040	*rac*-TMA-α (20 mM)
	CH_2	0.015	*rac*-TMA-α (20 mM)
	CH_2'	0.015	*rac*-TEA-α (20 mM)
10	CH_3	0.018	*rac*-TEA-β (30 mM)
11	H1	0.008	*rac*-TMA-α (20 mM)
	H3	0.014	*rac*-TPA-α (20 mM)
	H5	0.009	*rac*-TMA-β (20 mM)
	H7	0.008	(*R*)-TEA-β (20 mM)
12	CH_3	0.018	(*S*)-TMA-β (20 mM)
13	CH_2	0.033	*rac*-TPA-α (20 mM)
	CH_2'	0.075	(*S*)-TMA-β (30 mM)
	CH_3	0.008	(*S*)-TMA-β (10 mM)

[a]Values reported are the best result obtained among all of the cationic cyclodextrins that were studied. The concentration of the cyclodextrin is indicated in parentheses.

substrates are enantiomerically differentiated. Overall, either *rac*-TMAs or (*S*)-TMA-β are most effective for the highest number of resonances. However, there are some examples where *rac*-TEA-α, *rac*-TEA-β, *rac*-TEA-γ, (*R*)-TEA-β, and *rac*-TPA-α produce the largest enantiomeric differentiation in the ^{1}H NMR spectrum. It is interesting to note that for every substrate in which more than one resonance shows enantiomeric differentiation, there is not a single cyclodextrin species that is more effective for each of those resonances. Much like prior work using cyclodextrins for chiral recognition, the differences in cavity size and nature of the substitutents means that no one cyclodextrin species is most effective for all substrates.

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