Lanthanide–Cyclodextrin Complexes as Probes for Elucidating Optical Purity by NMR Spectroscopy

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Abstract: A multidentate ligand is bonded to cyclodextrins by the reaction of diethylenetriaminepentaacetic dianhydride with 6-mono- and 2-mono(ethylenediamine) derivatives of cyclodextrin. Adding Dy(III) to the cyclodextrin derivatives enhances the enantiomeric resolution in the ^{1}H NMR spectra of carbinoxamine maleate, doxylamine succinate, pheniramine maleate, propranolol hydrochloride, and tryptophan. The enhancement is more pronounced with the secondary derivative. The Dy(III)-induced shifts can be used to elucidate the geometry of cyclodextrin–substrate inclusion complexes. Lanthanide-induced shifts are reported for complexes of aspartame, tryptophan, propranolol, and 1-anilino-8-naphthalenesulfonate with cyclodextrins, and the relative magnitudes of the shifts agree with previously reported structures of the complexes.

Introduction

The cyclodextrins are a series of cyclic oligosaccharides consisting of six (α), seven (β), and eight (γ) D-glucose units bound through α(1–4) linkages. The molecules are pail-shaped with the two secondary hydroxyl groups (2- and 3-positions) on each glucose ring situated at the mouth of the wider opening and the one primary hydroxyl group (6-position) on each ring situated at the mouth of the narrower opening. The three hydroxyl groups exhibit different reactivities, which facilitates the preparation of a wide range of cyclodextrin derivatives.

Of particular significance is the ability of cyclodextrins to accommodate guest molecules in the cavity. The different cavity sizes of the α (4.7–5.2 Å), β (6.0–6.4 Å), and γ (7.5–8.3 Å) derivatives facilitate inclusion complexation with compounds exhibiting a diversity of shapes, sizes, and structures. The inside of the cavity is relatively hydrophobic and provides a favorable environment for the inclusion of phenyl and naphthyl rings in aqueous solutions[1] and hydrophobic aliphatic chains.[2] Cyclodextrin inclusion complexes have been utilized as model systems for enzymatic studies[3,4] and as drug delivery systems.[2,4]

The cyclodextrins are optically active and offer the potential for discrimination of enantiomeric substrates. Most often, the discrimination involves inclusion complexation within the cavity. Association usually involves the formation of hydrogen bonds between the substrate and cyclodextrin. The proximity of hydrogen-bonding moieties can vary markedly for a pair of enantiomers, with a concomitant difference in the association constants. Cyclodextrin stationary phases have been widely employed in gas and liquid chromatography for the separation of enantiomers.[1,5] Alternatively, the complexes formed between a pair of enantiomers and cyclodextrin are diastereomers. In this case, even with comparable association constants, enantiomeric resolution may be observed using NMR spectroscopy. Chiral resolution in the NMR spectra of enantiomers in the presence of cyclodextrins has been observed for a variety of compounds, and is a useful means of determining enantiomeric excess.[6–13]

Pirkle and co-workers[14,15] were the first to demonstrate that, when the association constants between a pair of optical isomers and a chiral resolving agent differ, the extent of chiral resolution observed in the NMR spectra of the enantiomers could be enhanced by the addition of a paramagnetic lanthanide ion. This technique was more recently applied to other chiral resolving agents, including cyclodextrins, although the enhancement in enantiomeric resolution with cyclodextrins was small.[16]

An alternative to mixing a lanthanide species with the chiral resolving agent is to covalently bond the lanthanide to the resolving agent. Covalent attachment reduces some of the complexities of the equilibria associated with lanthanide–chiral resolving agent–substrate mixtures. Because of the predictability of lanthanide-induced shifts, there is also the possibility of gaining information on the geometry of association between the substrate and the chiral resolving agent.[17] To our knowledge, this paper represents the first report of the use of directly coupled lanthanide–chiral resolving agent complexes to enhance enantiomeric resolution.

Experimental Section

Reagents. Diethylenetriaminepentaacetic dianhydride (DTPAA), ethylenediaminetetraacetic acid, β-cyclodextrin hydrate, *p*-toluenesulfonyl chloride, propranolol hydrochloride (**IV**), ethylenediamine, anhydrous dimethyl sulfoxide, anhydrous dimethylformamide, tetraethylammonium chloride, anhydrous pyridine, deuterium oxide, and sodium hydride (95%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Carbinoxamine maleate (**I**), pheniramine maleate (**II**), doxylamine succinate (**III**), γ-cyclodextrin, 1-anilino-8-naphthalenesulfonic acid potassium salt (**V**), ibuprofen (**VI**), tryptophan (**VII**), aspartame (**VIII**), fenoprofen calcium salt (**IX**), and 2-toluidino-6-naphthalenesulfonic acid potassium salt were obtained from Sigma Chemical Corp. (Saint Louis, MO).

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Lanthanides were obtained as their metal oxides and converted to the nitrates using established procedures.[18] Bio-gel P2 steric exclusion chromatography gel (200/400 mesh) was obtained from Bio-Rad (Richmond, CA).

Preparation of Cyclodextrin Derivatives. Coupling of DTPAA to the 6-Mono(ethylenediamine) Derivative of β-Cyclodextrin (6-β-CD-EN-DTPA). Cyclodextrin was dried in vacuo for 24 h at 100 °C prior to use. The 6-mono(O-tosylate) derivative of cyclodextrin was prepared and converted to the 6-mono(ethylenediamine) derivative (6-β-CD-EN) as described by Chao.[19] The 6-mono(O-tosylate) was used in crude form after drying in vacuo at room temperature for 24 h. The 6-mono-(ethylenediamine) derivative was used in crude form after drying in vacuo over P_4O_{10} at room temperature for 24 h. Coupling of diethylenetri-aminepentaacetic acid (DTPA) to the 6-mono(ethylenediamine) derivative was accomplished by modification of a literature procedure used to couple DTPA to other amines.[20]

A solution of DTPAA (0.36 g, 1 mmol) and triethylamine (0.15 mL) in anhydrous dimethyl sulfoxide (6 mL) was placed into a 150-mL, three-neck, round-bottomed flask equipped with a nitrogen purge and addition funnel. A solution of dry 6-β-CD-EN (1.2 g, 1 mmol) in anhydrous dimethyl sulfoxide (6 mL) was added dropwise with stirring over 30 min. The solution was stirred for an additional 30 min, after which distilled water (5 mL) was added. Stirring was continued for 30 min, and the solution was poured into a beaker containing 200 mL of acetone. The yellow-brown precipitate that formed was collected by suction filtration.

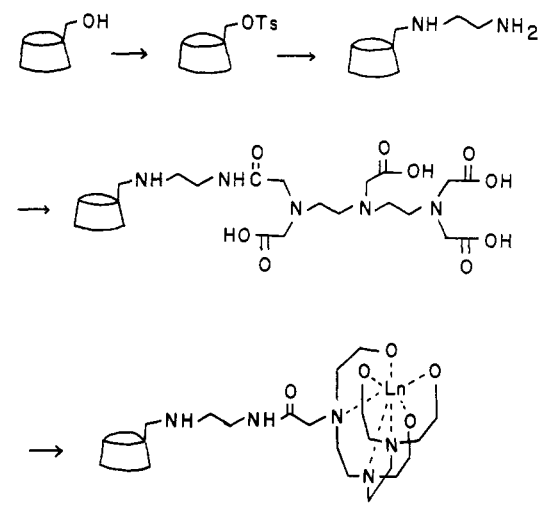
The crude 6-β-CD-EN-DTPA was purified by steric exclusion chromatography using Bio-Gel P2 (200/400 mesh) as the stationary phase and 0.050 M ammonium bicarbonate as the mobile phase. By using a 35-cm bed height of a 2.5-cm diameter column, 1-g quantities of the crude product could be purified. Gas pressure was used to maintain a flow rate of 5 mL/min. Fractions 10–15 (5-mL volume) contained approximately 0.6 g of the product as its triammonium salt. Unreacted starting materials eluted later. Formation of the desired product was confirmed by ^{1}H NMR and elemental analysis. ^{1}H NMR in D_2O: δ 3.0–3.5 (m, 14H), 3.56 (t, 7H), 3.62 (dd, 7H), 3.81 (t, 7H), 3.86 (m, 22H), 3.95 (t, 7H), 5.08 (d, 7H). Anal. Calcd for $(NH_4)_3$-$C_{58}H_{94}N_5O_{43}\cdot 8H_2O$: C, 39.86; H, 7.04; N, 6.41. Found: C, 39.88; H, 7.27; N, 6.53. The fully protonated form was obtained by addition of excess of hydrochloric acid to a solution of the triammonium salt in water. The precipitate that formed was collected and dried over P_4O_{10} at room temperature. Anal. Calcd for $C_{58}H_{97}N_5O_{43}\cdot 10H_2O$: C, 40.21, H, 6.81; N, 4.04. Found: C, 39.83; H, 6.35; N, 4.22.

Coupling of DTPAA to the 6-Mono(ethylenediamine) Derivative of γ-Cyclodextrin (6-γ-CD-EN-DTPA). The synthesis and purification of 6-γ-CD-EN-DTPA as its triammonium salt were accomplished by the same procedure as used for the β-cyclodextrin derivative. Formation of the desired product was confirmed by ^{1}H NMR spectroscopy and elemental analysis. ^{1}H NMR in D_2O: δ 3.0–3.5 (m, 14H), 3.50–3.75 (m, 16H), 3.75–3.85 (m, 32H), 3.90 (t, 8H), 5.08 (d, 8H). Anal. Calcd for $(NH_4)_3$-$C_{64}H_{104}N_5O_{48}\cdot 9H_2O$: C, 39.88; H, 7.01; N, 5.81. Found: C, 39.83; H, 6.88; N, 6.15.

Coupling of DTPAA to the 2-Mono(ethylenediamine) Derivative of β-Cyclodextrin (2-β-CD-EN-DTPA). The 2-mono(*O*-tosylate) derivative of β-cyclodextrin was obtained by a reported procedure.[21] Derivation at the 2-position was confirmed by the carbohydrate region of the ^{13}C NMR spectrum.[21] ^{13}C NMR in D_2O: δ 59.8 (s, 7C), 69.2 (s, 1C), 71.9 (s, 7C), 72.3 (s, 6C), 72.9 (s, 6C), 79.6 (s, 1C), 80.9 (s, 1C), 81.4 (s, 6C), 98.1 (s, 1C), 101.8 (s, 6C). ^{1}H NMR in D_2O: δ 7.55 (d, 2H), 7.91 (d, 2H). The crude 2-(tosylate) derivative was dried at room temperature in vacuo for 24 h. It was then converted to the ethylenediamine derivative and reacted with DTPAA by procedures analogous to that described for the primary derivative. Purification of the product as its triammonium salt was achieved by steric exclusion chromatography. Formation of the desired product was confirmed by ^{1}H NMR data and elemental analysis. ^{1}H NMR in D_2O: δ 3.0–3.5 (m, 14H), 3.51 (t, 7H), 3.77 (dd, 7H), 3.80–3.85 (m, 29H), 3.89 (t, 7H), 5.02 (d, 7H). Anal. Calcd for $(NH_4)_3$-$C_{58}H_{94}N_5O_{43}\cdot 8H_2O$: C, 39.86; H, 7.04; N, 6.41. Found: C, 39.97; H, 6.94; N, 6.12.

Preparation of Na[Dy(EDTA)]. Ethylenediaminetetraacetic acid disodium salt (2.00 g, 5.1 mmol) and sodium bicarbonate (0.857 g, 10.2 mmol) were added to 30 mL of water. The mixture was warmed with stirring for 30 min to effect evolution of carbon dioxide. A solution of dysprosium(III) nitrate hexahydrate (2.33 g, 5.1 mmol) in 10 mL of water was added, and the solution was stirred at room temperature for 30 min. Addition of the reaction mixture to acetone (200 mL) resulted in the formation of a fine white precipitate. The precipitate was collected by centrifugation and decantation of the supernatant, washed three times with acetone, and dried overnight in vacuo over P_4O_{10}.

Scheme 1



Apparatus and Procedures. NMR spectra were recorded on a General Electric 300-MHz spectrometer. Unless otherwise noted, all spectra were recorded with equal (0.025 M) concentrations of substrate and CD-EN-DTPA derivative. The Dy(III) complexes of the CD-EN-DTPA derivatives were generated *in situ* by addition of dysprosium(III) nitrate to solutions of the CD-EN-DTPA derivative and substrate in D_2O. The Dy(III) could either be added as a solid or as aliquots from a concentrated stock solution (0.188 M) in D_2O. NMR spectra were usually recorded at 50 °C, unless otherwise specified, and the sample was allowed to equilibrate for at least 15 min in the probe prior to recording the spectrum. When the magnitudes of lanthanide-induced shifts were determined, chemical shifts were recorded relative to the center peak of the methyl triplet of tetraethylammonium chloride.

Results and Discussion

Lanthanide ions are coupled to cyclodextrin as shown in Scheme 1. The carbonyl oxygen atoms are removed from the final structure for the sake of clarity. The DTPA moiety was selected as an appropriate ligand because it is known from previous work to effectively encapsulate lanthanide ions and prevent association of other substrates.[22] Modification at either the primary or secondary site of the cavity is achieved by varying the conditions of the tosylation reaction. Derivatization at the secondary side is verified by recording the ^{13}C NMR spectrum. The large downfield shift of carbon 2 (6.7 Hz) and smaller upfield shifts of carbons 1 (–3.7 Hz), 3 (–3.1 Hz), and 4 (–0.5 Hz) indicate substitution at a secondary site.[21] The product is purified as a triammonium salt by steric exclusion chromatography in an ammonium bicarbonate mobile phase. The triammonium salt is the expected form on the basis of the pH of the ammonium bicarbonate mobile phase and the pK_a values of the products. These pK_a values are expected to be similar to those of EDTA.

The NMR spectra of the final products show discernible peaks for the H_{1-4} hydrogens of the cyclodextrin.[23,24] Several of the EN-DTPA resonances appear as an overlapping set of peaks in the region from 3.0 to 3.5 ppm. The complexity of this region is caused by the loss of symmetry of the EN-DTPA group in the CD-EN-DTPA derivative. When the lanthanide-induced shifts

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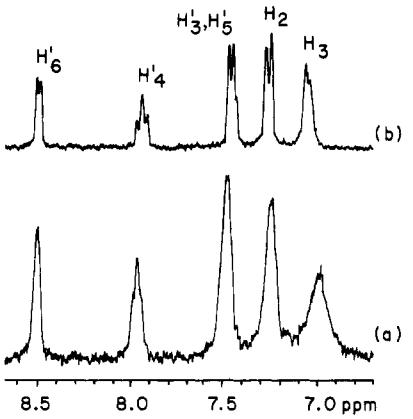

Figure 1. ^{1}H NMR spectra (300 MHz, D_2O) of carbinoxamine maleate (0.025 M), 6-β-CD-EN-DTPA (0.025 M), and dysprosium(III) nitrate (0.005 M) at a) 20 °C and b) 50 °C.

are measured, the intensities of the CD-EN-DTPA resonances are reduced proportionately to the equivalents of added Dy(III). The reduction in intensity indicates that Dy(III) exhibits slow exchange with CD-EN-DTPA and causes severe broadening of the CD-EN-DTPA resonances.

There is no agreement on the appropriate zero reference for use with lanthanide shift reagents in aqueous solution.[17] Compounds such as *tert*-butyl alcohol, dioxane, acetone, 3-(trimethylsilyl)propionate, and 2,2-dimethyl-2-silapentane-5-sulfonate have been applied as zero references, but there are indications that many of these associate with lanthanide species.[17] Previous work recommended tetraethylammonium iodide as a better reference than sulfonate salts for use with lanthanide ions.[25] We evaluated tetraethylammonium chloride (TEA) as a reference for use with the CD-EN-DTPA-Dy^{III} complexes. The minimal broadening in the spectrum of TEA compared to that in the spectra of CD-EN-DTPA and the substrate is indicative of weak or no association between the TEA and dysprosium(III). In addition, the distance between the methylene and methyl resonances is constant within 0.1 ppm with increasing concentration of dysprosium(III), also indicative of minimal association.

The ^{1}H NMR spectra of substrates at 300 MHz and ambient probe temperatures (20 °C) with CD-EN-DTPA-Dy^{III} are broadened. The broadening may be the result of either the enhanced relaxation rates that occur in the presence of the Dy(III) or exchange broadening. In previous work, it was noted that the contribution from exchange broadening to lanthanide–chiral resolving agent mixtures was significant at 300 MHz.[16] Warming the solution significantly reduced the exchange broadening;[16] however, association between the chiral resolving agent and substrate was retained so that enantiomeric resolution was still observed.

Figure 1 shows the spectra of carbinoxamine maleate at 20 and 50 °C with 6-β-CD-EN-DTPA and 0.2 equiv of Dy(III). The reduction in broadening at 50 °C allows for resonances to be assigned on the basis of coupling data. Furthermore, enantiomeric resolution is still observed in the spectra of **I–IV** at 50 °C, although the extent of resolution is reduced compared to that at 20 °C. Table 1 reports the Dy(III)-induced and relative Dy(III)-induced shifts in the spectra of carbinoxamine maleate at 20 and 50 °C with 6-β- or 2-β-CD-EN-DTPA and 0.3 equiv of Dy(III). The similarity of the relative shifts at 20 and 50 °C indicates that the geometry of the cyclodextrin–carbinoxamine complex does not change appreciably over this temperature range.

Enhancement of Enantiomeric Resolution. Certain resonances in the NMR spectra of **I–IV** exhibit enantiomeric resolution in

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Table 1. Dy(III)-Induced Shifts and Relative Dy(III)-Induced Shifts in the ^{1}H NMR Spectra (300 MHz, D_2O) of Carbinoxamine Maleate (0.025 M) in the Presence of 6-β-CD-EN-DTPA (0.025 M) or 2-β-CD-EN-DTPA (0.025 M) with Dy(III) (0.0075 M) at 20 and 50 °C[a]

	6-β-CD-EN-DTPA		2-β-CD-EN-DTPA	
	20 °C	50 °C	20 °C	50 °C
H_2	−0.41 (0.55)	−0.38 (0.58)	−0.56 (0.67)	−0.38 (0.66)
H_3	−0.74	−0.66	−0.83	−0.58
H_3'	−0.07 (0.09)	−0.10 (0.15)	−0.15 (0.18)	−0.07 (0.12)
H_4'	−0.05 (0.07)	−0.08 (0.12)	−0.09 (0.11)	−0.07 (0.12)
H_5'	−0.07 (0.09)	−0.10 (0.15)	−0.16 (0.19)	−0.10 (0.17)
H_6'	−0.14 (0.19)	−0.17 (0.26)	−0.21 (0.25)	−0.17 (0.29)
CH	−0.19 (0.26)	−0.18 (0.27)	*b*	−0.25
CH_3	0.06 (0.08)	0.02 (0.03)	0.10 (0.12)	0.08 (0.14)

[a] Values in parentheses are the ratios of the magnitudes of Dy(III)-induced shifts relative to those of H_3. [b] Peak obscured by cyclodextrin resonances.

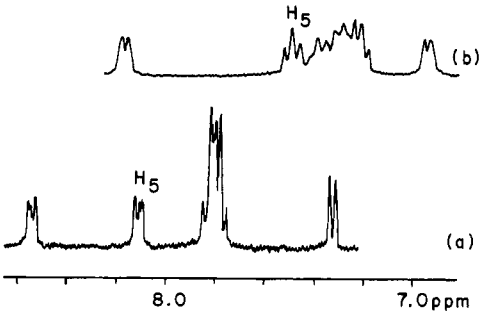
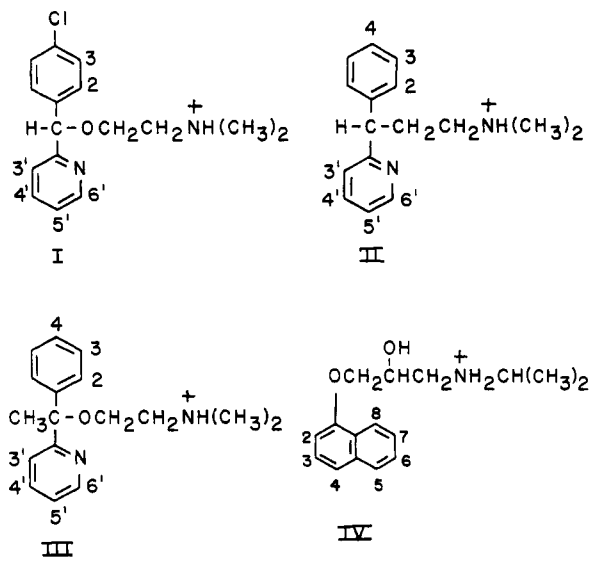
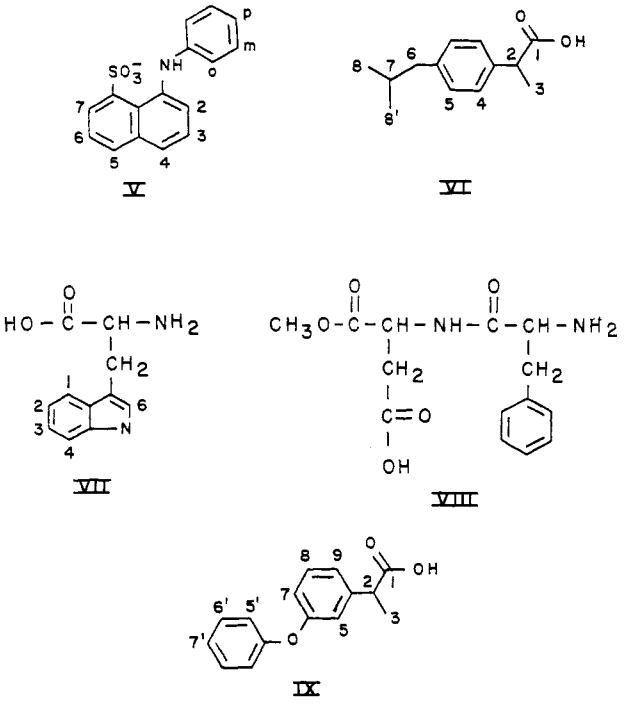


Figure 2. ^{1}H NMR spectra (300 MHz, D_2O) of propranolol hydrochloride (0.025 M) with 6-β-CD-EN-DTPA (0.025 M) and (a) no dysprosium(III) nitrate and (b) dysprosium(III) nitrate (0.005 M).

the presence of cyclodextrins.[8,9] The NMR spectra of **I–IV** with 6-β-CD-EN-DTPA shift when Dy(III) is added. No enhancement in enantiomeric resolution is observed in the spectra of pheniramine and doxylamine for 0.1–0.5 equiv of Dy(III). Concentrations of Dy(III) higher than 0.5 equiv are not practical because of the broadening in the spectra. For carbinoxamine, a doubling of the resonance for H_3, corresponding to a resolution of 4.5 Hz at 0.3 equiv of Dy(III), is observed. The most significant enhancement in enantiomeric resolution using 6-β-CD-EN-DTPA is observed for H_5 of propranolol. As seen in the spectra shown in Figure 2, the doublet corresponding to H_5 resolves to an apparent triplet on addition of 0.2 equiv of Dy(III), with corresponding enantiomeric resolution of 6.9 Hz.





Nonequivalent hydrogen bonding at the secondary hydroxyl groups of cyclodextrins is often responsible for the distinction between enantiomers. It is known from previous work with chiral lanthanide shift reagents that enantiomeric resolution is often more pronounced for resonances of nuclei that are close to both the chiral center and the lanthanide.[17] It was therefore anticipated that attachment of a lanthanide ion at the secondary face might have a more pronounced effect on the degree of enantiomeric resolution.

The NMR spectra of **I–III** in the presence of 2-β-CD-EN-DTPA show a marked enhancement of enantiomeric resolution on addition of Dy(III). For doxylamine in the presence of β-cyclodextrin or 2-β-CD-EN-DTPA, the triplet for H_4' is an apparent quartet with enantiomeric resolution of 4.2 Hz at 300 MHz. Addition of 0.3 equiv of Dy(III) to a solution containing 2-β-CD-EN-DTPA changes the resonance to a multiplet of five peaks with enantiomeric resolution of 12.6 Hz. On addition of 0.4 equiv of Dy(III), the doublet for H_3' changes into a multiplet of three peaks with enantiomeric resolution of 7.8 Hz. The methyl resonances in the spectrum of doxylamine split into two singlets on addition of Dy(III), although it is not possible to determine whether the splitting is the result of enantiomeric resolution or resolution of the diastereotopic methyl groups.[9] A similar splitting was observed for the methyl resonances in the spectrum of carbinoxamine.

Enantiomeric resolution is not observed in the H_4' and H_6' resonances of the spectrum of pheniramine maleate in the presence of β-cyclodextrin or 2-β-CD-EN-DTPA at 300 MHz. As seen in Figure 3, addition of Dy(III) to a solution containing 2-β-CD-EN-DTPA causes enantiomeric resolution of both signals. The extent of resolution improves with increasing concentration of Dy(III), although broadening limits the concentration of Dy-(III) that can be employed. At 0.5 equiv of Dy(III), the enantiomeric resolutions are 11.1 Hz for H_4' and 10.2 Hz for H_6'.

The series of spectra recorded for the aromatic portion of carbinoxamine maleate in the presence of 2-β-CD-EN-DTPA with increasing amounts of Dy(III) is shown in Figure 4. Essentially complete enantiomeric resolution is achieved for all of the aromatic hydrogens at 0.4 or 0.5 equiv of Dy(III). The enantiomeric resolutions are 25.8 Hz for H_2, 25.2 Hz for H_3, 19.2 Hz for H_3', 30.0 Hz for H_4', 18.3 Hz for H_5', and 20.1 Hz for H_6'.

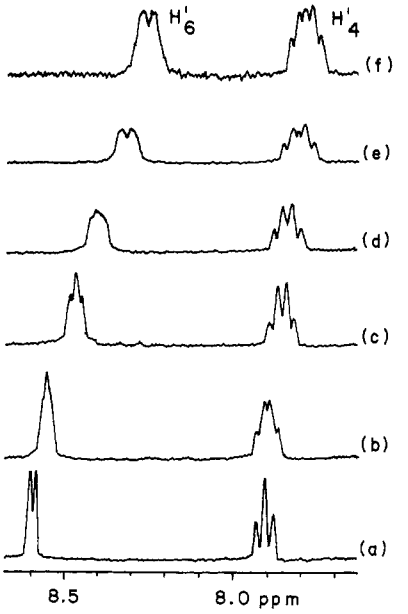


Figure 3. ^1H NMR spectra (300 MHz, D_2O) of pheniramine maleate (0.025 M) with 2-β-CD-EN-DTPA (0.025 M) and (a) no dysprosium-(III) nitrate, (b) 0.0025 M dysprosium(III) nitrate, (c) 0.0050 M dysprosium(III) nitrate, (d) 0.0075 M dysprosium(III) nitrate, (e) 0.010 M dysprosium(III) nitrate, and (f) 0.0125 M dysprosium(III) nitrate.

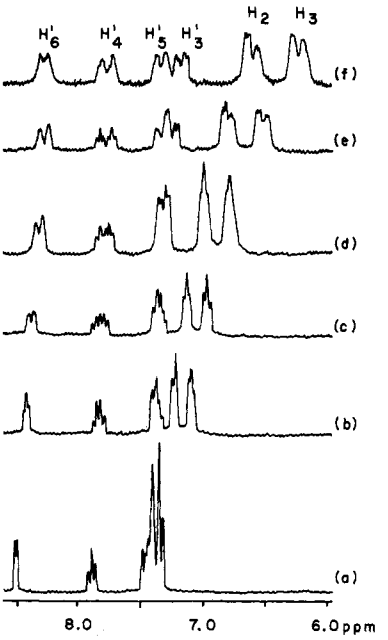


Figure 4. ^1H NMR spectra (300 MHz, D_2O) of carbinoxamine maleate (0.025 M) with 2-β-CD-EN-DTPA (0.025 M) and (a) no dysprosium-(III) nitrate, (b) 0.0025 M dysprosium(III) nitrate, (c) 0.005 M dysprosium(III) nitrate, (d) 0.0075 M dysprosium(III) nitrate, (e) 0.010 M dysprosium(III) nitrate, (f) 0.0125 M dysprosium(III) nitrate.

There are several mechanisms that might possible account for the enhancement is enantiomeric resolution caused by addition of Dy(III) to CD–EN–DTPA–substrate mixtures. The first, represented in Figure 5a, is a situation in which the substrate associates with Dy(III) in solution. If different association constants are assumed for the two substrate configurations with the cyclodextrin, enhancement in enantiomeric resolution can occur as noted in previous work of Pirkle and others.[14-16] The association constants of lanthanide ions are lower with DTPA amides (10^{19})[26] than with DTPA (10^{22})[27] but are still sufficiently

(26) Lauffer, R. B.; Brady, T. J. *Magn. Reson. Imaging* **1985**, *3*, 11–16.

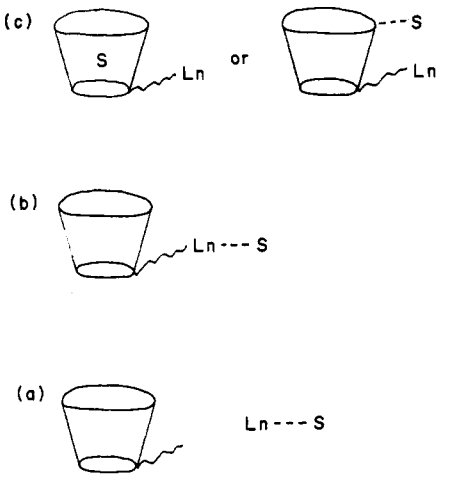


Figure 5. Possible mechanisms of association between a substrate and the CD-EN-DTPA–lanthanide system.

Figure 6. ^{1}H NMR Spectra (300 MHz, D_2O) of (a) carbinoxamine maleate (0.025 M) with (b) β-cyclodextrin (0.025 M), (c) 6-β-CD-EN-DTPA (0.025 M), and (d) 2-β-CD-EN-DTPA (0.025 M).

large that it is unlikely that an appreciable concentration of free lanthanide ion would be present in solution.

The mechanism in Figure 5a also fails to account for the differences in enantiomeric resolution observed for the primary and secondary derivatives, unless derivatization with DTPA at the primary and secondary sites causes a significant change in the relative association constants between the two enantiomers and cyclodextrin. Such a situation should cause pronounced differences in enantiomeric resolution of the substrates with the primary and secondary derivative without Dy(III). Such differences are not observed for any substrate employed in this study.

Further evidence that **I–VIII** bind similarly to cyclodextrin, 2-β-CD-EN-DTPA, and 6-β-CD-EN-DTPA is seen in the aromatic regions of the spectra of carbinoxamine maleate shown in Figure 6. These spectra are typical of those observed for **I–VIII**. Association of the substrate with the cyclodextrin causes shifts in the aromatic region. The magnitudes of the shifts are virtually identical in the presence of each of the three cyclodextrins, indicative of similar geometries of association and association constants.

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Table 2. Dy(III)-Induced Shifts in the ^{1}H NMR Spectra of Carbinoxamine Maleate (0.025 M) in the Presence of (A) Dysprosium(III) Nitrate (0.0075 M), (B) Na[Dy(EDTA)] (0.0075 M), (C) 6-β-CD-EN-DTPA (0.025 M) and Dy(III) (0.0075 M), and (D) 2-β-CD-EN-DTPA (0.025 M) and Dy(III) (0.0075 M) in D_2O

	A	B	C	D
H_2	0.02	−0.08	−0.38	−0.38
H_3	0.02	−0.08	−0.66	−0.58
H_3'	0.02	−0.01	−0.10	−0.07
H_4'	0.11	−0.02	−0.08	−0.07
H_5'	0.11	−0.05	−0.10	−0.10
H_6'	0.05	−0.07	−0.17	−0.17
CH	0.08	−0.03	−0.20	−0.25
NCH_3	0.00	0.03	0.02	0.08

Shifts were recorded in the ^{1}H NMR spectra of carbinoxamine maleate, pheniramine maleate, propranolol hydrochloride, and 1-anilino-8-naphthalenesulfonate in the presence of dysprosium(III) nitrate. Data shown for carbinoxamine maleate in Table 2 are representative of the data for the four substrates studied. The shifts with dysprosium(III) nitrate are negligible and indicate that association of free Dy(III) with carbinoxamine is weak at best. The relative shifts in the spectrum of carbinoxamine with dysprosium(III) nitrate are different from those with the dysprosium derivatives of CD-EN-DTPA. Binding with free Dy(III) cannot explain the enantiomeric resolution or shifts observed with the CD-EN-DTPA-Dy^{III} derivatives.

In the second mechanism (Figure 5b), the dysprosium ion is bound to the DTPA moiety but still has an available coordination site for substrate binding. The DTPA ligand has been shown to effectively encapsulate lanthanide ions, thereby preventing substrate molecules from binding.[22] Such encapsulation has facilitated the use of Gd^{III}DTPA as a water-soluble relaxation reagent[22] and image contrast reagent in magnetic resonance imaging.[28] The amide derivative, however, might not be expected to encapsulate the lanthanide as well.

Shifts in the spectra of carbinoxamine maleate, pheniramine maleate, propranolol hydrochloride, and 1-anilino-8-naphthalenesulfonate were recorded in the presence of Na[Dy(EDTA)], since this complex should most closely approximate the charge of the Dy(III) species in the CD-EN-DTPA derivatives. The shifts for carbinoxamine maleate with Na[Dy(EDTA)] are reported in Table 2 and are representative of those for all of the substrates studied. The shifts recorded with Na[Dy(EDTA)] are substantially smaller than those recorded with the CD-EN-DTPA derivatives. In addition, the relative magnitudes of the shifts are different from those with 6-β- and 2-β-CD-EN-DTPA-Dy^{III}.

Previous work has shown that tetracycline, when excited at 391 nm, is capable of transferring energy to Eu(III) through an intramolecular process.[29,30] Emission from Eu(III) at 613 nm is then observed. Intramolecular transfer is observed when Eu(III) is added either as a hydrated salt or as a complex with EDTA. Addition of equimolar and excess amounts of tetracycline to solutions of the 1:1 complex of Eu(III) with 6-β-CD-EN-DTPA does not produce any additional emission at 613 nm. The lack of additional Eu(III) emission indicates that Eu(III) is complexed with the DTPA moiety and that the DTPA moiety encapsulates the metal sufficiently to block complexation of an additional ligand such as tetracycline. Neither the NMR shift data with Na[Dy(EDTA)] nor the luminescence data support the situation represented in Figure 5b.

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(29) Hirschy, L. M.; Dose, E. V.; Winefordner, J. D. *Anal. Chem. Acta* **1983**, *147*, 311–316.
(30) Wenzel, T. J.; Collette, L. M.; Dahlen, D. T.; Hendrickson, S. M.; Yarmaloff, L. W. *J. Chromatogr.* **1988**, *433*, 149–158.

The mechanism shown in Figure 5c is therefore favored. In this case, the substrate associates with the cyclodextrin and not directly with the lanthanide. The mechanism represented by Figure 5c does not distinguish whether the substrate associates inside or outside the cavity, although evidence will be presented to support inclusion complexation of all of the substrates employed in our study. The lanthanide-induced shifts in the spectra of **V–VIII** with CD-EN-DTPA derivatives lend additional support for the situation in Figure 5c (*vide infra*). Compounds **V**, **VII**, and **VIII** have all been the focus of previous reports about the nature of their interaction with cyclodextrin.

To better understand the resolution of enantiomers of tryptophan using liquid chromatography with an α-cyclodextrin-bonded,[31] the association of tryptophan with α-cyclodextrin was studied by ^{1}H NMR spectroscopy and molecular mechanics calculations.[32] The *R*-(+) enantiomer of tryptophan associates favorably with the cavity because of better alignment for hydrogen-bond formation with the secondary hydroxyl groups.[32] We observed no enantiomeric resolution in the ^{1}H NMR spectrum of tryptophan at 300 MHz with β-cyclodextrin, 6-β-CD-EN-DTPA, or 2-β-CD-EN-DTPA. Addition of up to 0.5 equiv of Dy(III) to a mixture of 6-β-CD-EN-DTPA and tryptophan did not produce any observable enantiomeric resolution. With 2-β-CD-EN-DTPA, however, the two triplets for H_2 and H_3 split on addition of Dy(III), with corresponding enantiomeric resolution of 30.9 and 16.2 Hz, respectively, at 0.5 equiv of Dy(III).

Spiking with either the *R*-(+) or *S*-(−) enantiomer shows that the resonances for the *R*-(+) enantiomer shift further. Considering the favorable association of the R-(+) enantiomer with the cavity, the larger shift implies that differences in association constants between the two enantiomers and the cyclodextrin account for the enhancement in enantiomeric resolution in the presence of Dy(III). Differences in lanthanide-induced shifts caused by the diastereomeric nature of the *R*-(+) and *S*-(−) complexes, however, cannot be eliminated as a contributing factor.

Geometry of Cyclodextrin–Substrate Complexes. The shifts in the presence of a lanthanide ion are dipolar in origin and, under certain circumstances,[33] governed by eq 1.[34]

$$\Delta\delta = K(3\cos^2\theta - 1)/r^3 \qquad (1)$$

In this equation, $\Delta\delta$ is the lanthanide-induced shift, K is a constant for a particular lanthanide ion, r is the distance from the lanthanide ion to the nucleus of interest, and θ is the angle between the principal magnetic axis of the lanthanide complex and the line drawn from the lanthanide to the nucleus of interest. The shifts in the spectrum of a substrate in the presence of lanthanide–CD-EN-DTPA derivatives may be useful in elucidating the geometry of association between a substrate and cyclodextrin. To gain useful structural information, however, more must be understood about the precise positioning of the lanthanide ion relative to the cyclodextrin and substrate. Figure 7 depicts three possible situations. The arrow in the figure represents the positioning of a substrate in the cavity.

In parts a and b of Figure 7, the lanthanide is positioned respectively above and below the opening of the cavity. If the situation in Figure 7a occurred, the magnitudes of the shifts in the spectrum of a substrate should be markedly different for the primary and secondary derivatives. The shifts measured in the spectra of **I–IV** in the presence of 6-β- and 2-β-CD-EN-DTPA with 0.3 equiv of Dy(III) are reported in Table 3. Of particular significance is the similarity of the shifts in the spectra of these substrates with those of the primary and secondary derivatives. These data do not support the situation shown in Figure 7a.

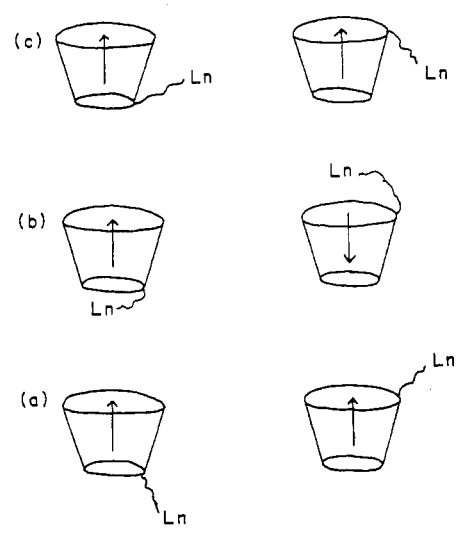

Figure 7. Possible orientations of the lanthanide ion relative to the cyclodextrin cavity. The arrows represent the orientations of the substrate in the cavity.

Table 3. Dy(III)-Induced Shifts in the ^{1}H NMR Spectra of Carbinoxamine Maleate (**I**), Pheniramine Maleate (**II**), Doxylamine Succinate (**III**), and Propranolol (**IV**) in D_2O (0.025 M β-CD-EN-DTPA, 0.025 M Substrate, 0.0075 M Dy(III))[a]

	I	II	III	IV
H_2	−0.38 (−0.38)	−0.22 (−0.26)	−0.22 (−0.28)	−0.36 (−0.43)
H_3	−0.66 (−0.58)	−0.31 (−0.33)	−0.34 (−0.38)	
H_5				−0.76 (−1.01)
H_8				−0.59 (−0.72)
H_3'	−0.10 (−0.07)	−0.08 (−0.07)	−0.14 (−0.13)	
H_4'	−0.08 (−0.07)	−0.10 (−0.08)	−0.08 (−0.06)	
H_5'	−0.10 (−0.10)	−0.08 (−0.07)	−0.08 (−0.05)	
H_6'	−0.17 (−0.17)	−0.18 (−0.20)	−0.12 (−0.11)	
CH	−0.18 (−0.25)	−0.16 (−0.19)		
NCH_3	0.02 (0.08)	−0.09 (−0.03)	−0.02 (0.05)	
NCH_2		−0.10 (−0.07)		
CCH_2		−0.12 (−0.14)		
CCH_3			−0.15 (−0.22)	
OCH_2				−0.26 (−0.25)

[a] Values in parentheses are those for the 2-β-CD-EN-DTPA derivative. Resonances not reported were obscured by cyclodextrin resonances or other resonances of the substrate.

Figure 7b depicts a situation in which the lanthanide either associates with the substrate to such a degree or blocks access to the entrance to the cavity so that the geometry of the substrate within the cavity is altered. Presumably, association of the lanthanide–DTPA moiety with **I–IV** would occur at the cationic portion of the molecule, the pyridyl nitrogen of **I–III**, or oxygen atoms of **I**, **III**, and **IV**. The data suggest that direct association of Dy(III) with the substrate does not occur, since the shifts for **I–IV** are largest for those nuclei on the aromatic rings and smallest for the pyridyl, N-substituted, and aliphatic portions of the compounds. For propranolol, the largest shifts are observed for those hydrogens (H_5, H_8) known from X-ray crystallographic data to be deepest in the cavity and farthest from any point at which the substrate could reasonably associate with a lanthanide ion.[1] Crystallographic data indicate that hydrogen bonding of the nitrogen atom to the secondary hydroxyl groups of cyclodextrin is important in stabilizing the inclusion complex of propranolol and causing the distinction between the enantiomers. The larger shift for the methyl group of propranolol with the 2-β derivative compared to the 6-β derivative is reasonable if hydrogen bonding of the nitrogen atom serves to direct this methyl group toward the point of attachment of the DTPA moiety in the secondary derivative.

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(32) Lipkowitz, K. B.; Raghothama, S.; Yang, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554–1562.
(33) McConnell, H. M.; Robertson, R. E. *J. Chem. Phys.* **1958**, *29*, 1361–1365.
(34) Briggs, J. M.; Moss, G. P.; Randall, E. W.; Sales, K. D. *J. Chem. Soc., Chem. Commun.* **1972**, 1180–1182.

Table 4. Dy(III)-Induced Shifts in the ^1H NMR Spectra of 1-Anilino-8-naphthalenesulfonic Acid Potassium Salt (**V**), Ibuprofen (**VI**), Tryptophan (**VII**), and Aspartame (**VIII**) in D_2O (0.025 M β-CD-EN-DTPA, 0.025 M Substrate, 0.0075 M Dy(III))[a]

	V	VI	VII	VIII
H_1			−0.42 (−0.49)	
H_2			−0.38 (−0.43)	
H_3			−0.37 (−0.41)	
H_4		−0.65	−0.35 (−0.35)	
H_5	−0.46 (−0.64)	−0.50		
H_6	−0.48 (−0.74)	−0.42	−0.20 (−0.17)	
H_7	−0.94 (−0.91)	−0.39		
H_8		−0.38		
H_8'		−0.36		
phenyl ortho	−0.78 (−0.81)			−0.26 (−0.38)
phenyl meta	−0.46 (−0.50)			−0.34 (−0.43)
phenyl para	−0.38 (−0.41)			−0.36 (−0.45)
Phe CH				−0.25 (−0.29)
Asp CH				−0.09
Phe CH_2				−0.10 (−0.29)
Asp CH_2				−0.12 (−0.11)
OCH_3				−0.16 (−0.23)

[a] Resonances not reported were obscured by cyclodextrin resonances or other resonances of the substrate.

Demarco and Thakkar[23] and others[24] have shown that the resonances of hydrogens on the inner surface of the cavity (H_3, H_5) shift more than those of the hydrogens on the outer surface (H_1, H_2, H_4) if inclusion complexation takes place. Addition of equimolar amounts of compounds **I–III** to β-cyclodextrin causes an upfield shift for H_3 between 30 and 40 Hz. We have been unable to determine the exact magnitude of this shift because of overlap with the H_6 resonance. The resonances for H_1 and H_4 exhibit negligible shifts (0–1 Hz), whereas H_2 exhibits downfield shifts for doxylamine (13 Hz), pheniramine (14 Hz), and carbinoxamine (12 Hz). The chemical shift of the H_5 resonance could not be assigned on addition of substrates **I–III** because of the complex nature of the spectra. The large shifts for H_3 support inclusion of these substrates in the host. The rather sizable shift of H_2 with doxylamine, pheniramine, and carbinoxamine may be the result of hydrogen bonding between the pyridyl nitrogen and the hydroxyl group at the 2-position.

If the situation in Figure 7b occurs, the cyclodextrin regions of the spectra should be different for 6-β- and 2-β-CD-EN-DTPA, because of differences in the shifts of H_3 and H_5 (H_3 is positioned nearer the secondary opening and H_5 nearer the primary opening). The cyclodextrin regions of these spectra are too complicated at 300 MHz to draw definitive conclusions. It is apparent that the addition of **I–IV** to 6-β- and 2-β-CD-EN-DTPA causes the triplet for H_3 to shift upfield and overlap with the resonance for H_6, much as observed for β-cyclodextrin. The available data suggest that the situation represented in Figure 7b is not occurring, and instead the lanthanide is positioned to the side of the cavity as shown in Figure 7c. Such an alignment would explain why the largest lanthanide-induced shifts are observed for nuclei imbedded in the cyclodextrin cavity for both the primary and secondary derivatives.

The lanthanide-induced shifts in the spectra of **V–VIII** in the presence of 6-β- and 2-β-CD-EN-DTPA with 0.3 equiv of Dy(III) are reported in Table 4. It was previously concluded that the indole ring of tryptophan inserted into the cavity of α-cyclodextrin.[32] Shift data for tryptophan with β-CD-EN-DTPA-Dy^{III} agree with such a structure, since the shifts for the phenyl ring are larger than the shift for H_6. The signals for the aliphatic portion of the molecule overlap with cyclodextrin peaks so that lanthanide-induced shifts can not be measured.

A previous study of the association of aspartame with β-cyclodextrin supported a structure in which the phenyl ring inserted into the cavity.[35] The Dy(III)-induced shifts are largest for the phenyl resonances. The Dy(III)-induced shifts for several resonances, including those of the phenyl ring, the CH_2 group of the phenylalanine moiety, and the methoxide group are larger with 2-β-CD-EN-DTPA than with 6-β-CD-EN-DTPA. It has been proposed that the aspartyl group of aspartame wraps over and interacts with exterior parts of the cyclodextrin.[35] Interaction of this group with the exterior portion apparently places the substrate closer to the dysprosium in the secondary derivative.

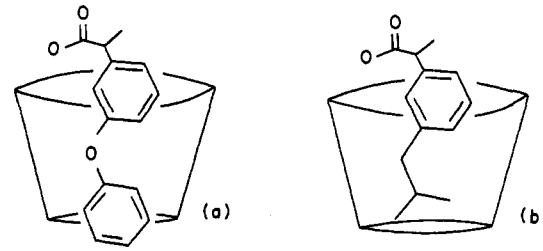

Figure 8. Representations of the inclusion complexes of (a) fenoprofen and (b) ibuprofen with cyclodextrin.

Table 5. Dy(III)-Induced Shifts in the ^1H NMR Spectra of 1-Anilino-8-naphthalenesulfonic Acid Potassium Salt (0.025 M) in D_2O with 6-β-CD-EN-DTPA (0.025 M) or 6-γ-CD-EN-DTPA (0.025 M) and Dy(III) (0.005 M)

	6-β-CD-EN-DTPA	6-γ-CD-EN-DTPA		6-β-CD-EN-DTPA	6-γ-CD-EN-DTPA
H_5	−0.33	−0.48	phenyl ortho	−0.56	−0.66
H_7	−0.69	−1.28	phenyl para	−0.26	−0.11

For ibuprofen, large shifts are observed for the resonances of the ring and isobutyl hydrogens. Enantiomeric resolution has been observed in the NMR spectra of ibuprofen in the presence of β-cyclodextrin,[9] although we observed no enhancement in enantiomeric resolution with the CD-EN-DTPA-Dy^{III} derivatives. Fenoprofen (**IX**) was shown by X-ray crystallography to imbed deeply into the cavity so that it extended out both sides as depicted in Figure 8a.[36] The solubility of fenoprofen in water was too low to permit the measurement of Dy(III)-induced shifts. Ibuprofen, because its structure is similar to that of fenoprofen, presumably binds to cyclodextrin by a similar mechanism (Figure 8b). Such a structure would account for the rather substantial shifts of the isobutyl resonances of ibuprofen on addition of Dy(III).

The geometry of association of 1-anilino-8-naphthalenesulfonate with β- and γ-cyclodextrin has been studied by NMR spectroscopy.[37] On the basis of NOE data, it was proposed that only the phenyl ring of 1-anilino-8-naphthalenesulfonate inserted into the cavity of β-cyclodextrin.[37] For γ-cyclodextrin, NOE data indicated that two different complexes could form, one involving insertion of the phenyl moiety and the other insertion of the naphthyl moiety. Dysprosium-induced shifts for 1-anilino-8-naphthalenesulfonate in the presence of 6-β- and 6-γ-CD-EN-DTPA with 0.2 equiv of Dy(III) are listed in Table 5.

The larger shifts of the naphthyl resonances relative to those of the phenyl resonances indicate that the naphthyl ring preferentially binds in the cavity. The shifts of the phenyl resonances are of sufficient magnitude, however, to indicate that a complex involving insertion of the phenyl ring occurs to some extent. The shifts of the naphthyl resonances are smaller for the β derivative than for the γ-derivative but comparable to those of the phenyl resonances. The implication is that 1-anilino-8-naphthalenesulfonate binds to β-cyclodextrin in two modes as well: one involving insertion of the phenyl ring and the other insertion of the naphthyl ring.

The resonances for H_5 and H_7 of 1-anilino-8-naphthalenesulfonate exhibit significantly different by Dy(III)-induced shifts

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with 2-β- and 6-β-CD-EN-DTPA-Dy^{III}. With the phenyl ring inserted in the cavity, the naphthyl ring is positioned above the cavity near the secondary hydroxyl groups. Involvement of the sulfonate moiety in hydrogen bonding to the secondary hydroxyl groups apparently positions the naphthyl ring closer to the Dy(III) in the secondary derivative.

The lanthanide-induced shifts in the spectra of analogous hydrogen resonances of **I–III** with β-CD-EN-DTPA derivatives are similar in magnitude. This similarity suggests that the geometries of association of these three substrates with cyclodextrin are essentially the same. The shifts are largest for the hydrogen atoms on the phenyl ring. Since the shifts of the cyclodextrin resonances indicate that these substrates form inclusion complexes, the lanthanide shift data suggest a structure in which the phenyl ring inserts into the cavity as shown in Figure 9. Presumably the pyridyl nitrogen is involved in hydrogen bonding with hydroxyl groups of the cyclodextrin, thereby stabilizing formation of the inclusion complex.

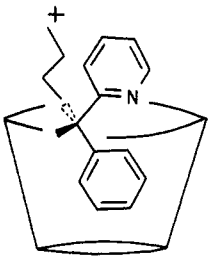

Figure 9. Proposed structure of the inclusion complexes of **I–III** with cyclodextrin.

Conclusions

Dysprosium complexes with CD-EN-DTPA derivatives enhance the enantiomeric resolution in the NMR spectra of substrates that associate with cyclodextrin. The secondary derivative is more effective at enhancing enantiomeric resolution than the primary derivative. Evidence indicates that the lanthanide-induced shifts of a substrate in the presence of lanthanide–CD-EN-DTPA derivatives are the result of substrate association with the cyclodextrin, rather than substrate association with the lanthanide. The largest lanthanide-induced shifts are observed for substrate nuclei imbedded within the cyclodextrin cavity. The magnitudes of the lanthanide-induced shifts can be used to analyze the geometries of cyclodextrin–substrate complexes.

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