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Sulfated and carboxymethylated cyclodextrins and their lanthanide complexes as chiral NMR discriminating agents

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Abstract—Sulfated and carboxymethylated cyclodextrins are effective at causing enantiomeric discrimination in the ^{1}H NMR spectra of water-soluble organic cations. The anionic derivatives are more soluble in water than native cyclodextrins and the enantiomeric discrimination is usually larger than observed with native cyclodextrins. Lanthanide ions such as dysprosium(III) and ytterbium(III) associate at the anionic sulfonate and carboxymethylate groups and often cause considerable enhancements in the enantiomeric discrimination in the ^{1}H NMR spectrum of the substrate. The enantiomeric discrimination caused by addition of lanthanide ions is large enough that much lower concentrations of the cyclodextrin can be used compared to conventional analyses with chiral solvating agents. Sulfated and carboxymethylated cyclodextrins are commercially available, which facilitates their use in NMR applications.

1. Introduction

Cyclodextrins are cyclic oligosaccharides comprised of six (α), seven (β), and eight (γ) D-glucose rings bonded through α(1-4) linkages. The cyclodextrin cavity is tapered with the secondary hydroxyl groups at the 2- and 3-positions of each glucose ring at the wider opening. The primary hydroxyl groups at the 6-position are at the narrower opening of the cavity. The different cavity sizes of α-, β-, and γ-cyclodextrin, as well as a diverse variety of derivatives that can be prepared by modification at the 2-, 3-, or 6-position, have resulted in an extensive literature on cyclodextrin host–guest compounds.[1] The relatively hydrophobic nature of the cavity provides an environment suitable for inclusion of aromatic rings and hydrophobic aliphatic chains. Native or various derivatized cyclodextrins have found extensive application as chiral discriminating agents in chromatographic,[2–6] capillary electrophoretic (CE),[7–10] or NMR spectroscopic methods.[8–26] Water-soluble and organic-soluble cyclodextrins are available, and the range of compounds for which cyclodextrins represent useful chiral discriminating agents is quite large.

Even though cyclodextrins are effective chiral discriminating agents for NMR spectroscopy, the extent of enantiomeric distinction in the NMR spectrum is often small or non-existent. We have shown that it is possible to enhance the enantiomeric discrimination by coupling paramagnetic lanthanide ions to cyclodextrins.[27,28] Coupling was achieved by attaching a diethylenetriaminepentaacetic acid (DTPA) ligand to either the primary or secondary side of cyclodextrin. Reaction of DTPA dianhydride with the appropriate ethylenediamino- or aminocyclodextrin provided derivatives with different length tethers for lanthanide binding. In a similar system, a cyclodextrin-lanthanide couple was obtained by attachment of the macrocyclic ligand 1,4,7,10-tetraaza-1,4,7-tri(carboxymethyl)cyclododecane.[29] None of these lanthanide-binding cyclodextrins are commercially available, and each requires a synthetic procedure that involves several steps.

For CE separations, charged cyclodextrins are desirable and sulfated (CD-S) or carboxymethylated (CD-CM) derivatives are commonly used.[7–10] CD-S and CD-CM derivatives are available from a number of commercial suppliers, and are interesting candidates for use as chiral discriminating agents in NMR spectroscopy. Underivatized β-CD is not highly soluble in water, and the presence of the anionic groups increases its solubility. Furthermore, the ion-pairing interaction between organic cations and the sulfate or carboxymethylate groups of CD-S and CD-CM derivatives may enhance the association constants and enantiomeric discrimination in the NMR spectrum. Enhanced discrimination

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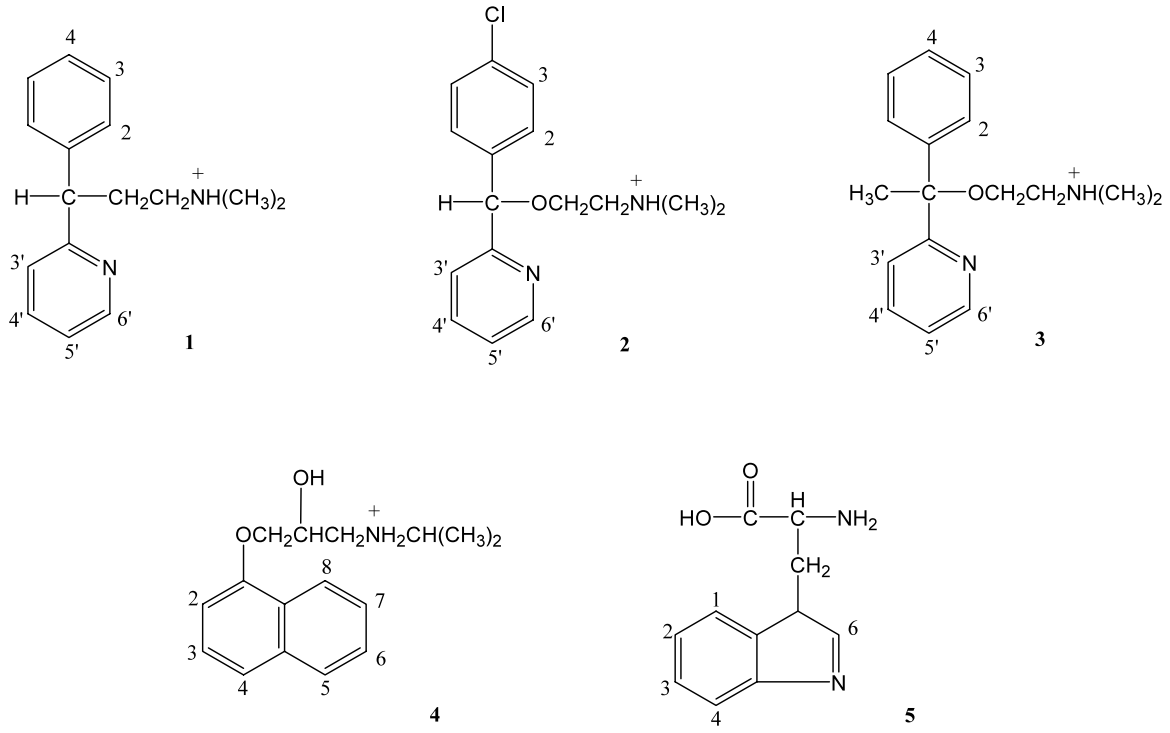

Figure 1. Structure of substrates.

with charged cyclodextrins has been observed in many CE separations, and in a few instances when the discrimination of organic cations with anionic cyclodextrin derivatives was also examined using NMR spectroscopy.[8–11] The incorporation of cationic moieties into organic pharmaceuticals to enhance their water-solubility is a common strategy, and many of these compounds have functional groups that form inclusion complexes with cyclodextrins. What is especially relevant to this study is that the sulfate or carboxymethylate groups provide sites for binding of lanthanide(III) ions, which can then cause further enhancements in enantiomeric discrimination.

2. Results and discussion

The particular CD-S and CD-CM derivatives that we have investigated involve an indiscriminate addition of sulfate or carboxymethylate groups to cyclodextrin. Substituent groups are attached at both primary and secondary hydroxyl sites. The β-CD-S has an average of nine sulfate groups per cyclodextrin. The β-CD-CM has an average of 3.5 carboxymethyl groups per cyclodextrin. The procedure commonly used to prepare CD-CM derivatives leads to predominant substitution of carboxymethyl groups at the secondary hydroxyl sites, but some substitution at the primary hydroxyl groups as well.[30] Carboxylate groups are known to associate more strongly with lanthanide ions than sulfate groups, but the presence of multiple sulfate groups in the CD-S derivative may facilitate chelate complexation of lanthanide ions.

The enantiomeric discrimination in the NMR spectrum of several substrates (Fig. 1) with β-CD-S, β-CD-CM, and β-CD was compared. Portions of the spectra of pheniramine maleate **1** (0.025 M) in the presence of β-CD, β-CD-CM, and β-CD-S (0.025 M) are shown in Figure 2. The magnitude of the shifts in the spectrum of

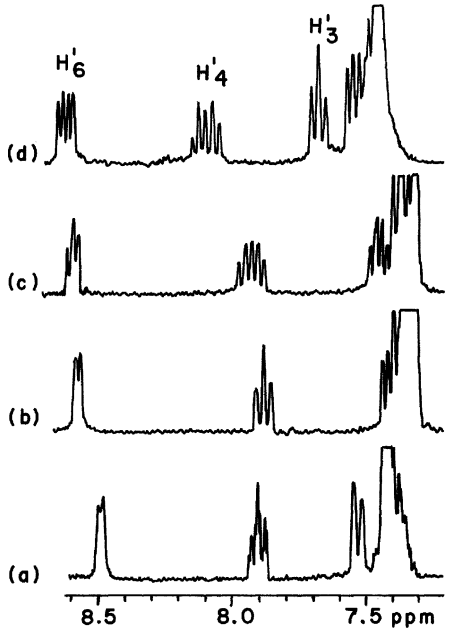

Figure 2. ^{1}H NMR spectrum (300 MHz) of (a) pheniramine maleate, **1**, (0.025 M) in deuterium oxide at 20°C with (b) β-CD (0.025 M), (c) β-CD-CM (0.025 M), and (d) β-CD-S (0.025 M).

1 is greater with the most anionic β-CD-S and least with the neutral β-CD. More importantly, whereas no enantiomeric discrimination is apparent in the spectrum with β-CD, the H'_4 and H'_6 resonances show enantiomeric distinction in the presence of β-CD-CM (H'_4, 14.3 Hz; H'_6, 7.1 Hz) and β-CD-S (H'_4, 15.8 Hz; H'_6, 10.5 Hz). Furthermore, the H'_3 resonance overlaps with others in the presence of β-CD or β-CD-CM, but is enantiomerically discriminated in the presence of β-CD-S (7.5 Hz). There are also interesting differences in the shifts of the diastereotopic methylene resonances of **1** in the presence of the different cyclodextrins. Only one pair of the diastereotopic methylene hydrogens is resolved in the presence of β-CD and β-CD-S, whereas both are fully resolved in the presence of β-CD-CM.

Table 1 provides comparative data on enantiomeric discrimination in the NMR spectra of **1**, carbinoxamine maleate **2**, doxylamine succinate **3**, and propranolol hydrochloride **4** with β-CD, β-CD-CM, and β-CD-S. With few exceptions, the most anionic β-CD-S is the most effective at causing enantiomeric discrimination. Presumably the anionic derivatives stabilize the association of cationic substrates in the cavity, which enhances the enantiomeric distinction relative to neutral β-CD.

Association of substrates within the cavity of cyclodextrins can be ascertained by examining the shifts of the resonances of the internal H_3 and H_5 hydrogens. Inclusion of aromatic substrates in the cavity causes pronounced upfield shifts of these resonances. The NMR spectrum of the highly substituted β-CD-S derivative has resonances shifted downfield relative to that of β-CD, and is complex because of the lack of symmetry. The H_3 and H_5 resonances are not distinguishable in the spectrum of β-CD-S and it is impossible to monitor whether they shift in the presence of substrates. For β-CD-CM, the H_3 resonances occur at 3.89 ppm and are downfield of the overlapped H_5 and H_6 resonances centered at 3.81 ppm. In the presence of all four organic cations listed in Table 1, the H_3 resonance of β-CD-CM shifts upfield and overlaps with H_6. With substrates that do not have interfering resonances, it is apparent that the H_5 resonance of β-CD-CM shifts upfield as well. The shifts of the H_3 and H_5 resonances indicate that the cationic substrates associate with β-CD-CM by insertion in the cavity, and not by an ion pairing mechanism in which the substrate is outside the cavity. Presumably the same occurs with β-CD-S.

The opposite trend is seen when the sodium salt of tryptophan **5** is mixed with β-CD, β-CD-CM, and β-CD-S. The spectrum of **5** with the highly anionic β-CD-S shows almost no shifts or enantiomeric discrimination, suggesting that weak association occurs. The shifts in the spectrum with β-CD are much larger and a slight degree of enantiomeric discrimination is observed for the resonances of the diastereotopic methylene hydrogens, although the multiplet is too complicated to accurately determine the extent of discrimination. The spectrum of **5** with β-CD-CM exhibits intermediate shifts and no apparent enantiomeric discrimination.

Addition of Dy(III)nitrate (0.025 M) to mixtures of β-CD-S (0.025 M) with organic cations (0.025 M) causes the β-CD-S resonances to shift upfield by about three ppm. The spectra of the organic cations in these mixtures show pronounced dysprosium-induced upfield shifts as well. In some cases, significant enhancements in enantiomeric discrimination are observed. Figure 3 shows the spectrum of **2** (0.025 M) with β-CD-S as increasing concentrations of Dy(III)nitrate are added. In the spectrum without Dy(III), partial enantiomeric discrimination of H'_4, H'_6, and the methine resonance is observed. The other resonances overlap and it is impos-

Table 1. Enantiomeric discrimination (Hz) in the ^{1}H NMR spectra (300 MHz) of cationic substrates (0.025 M) in the presence of β-CD, β-CD-CM, or β-CD-CD-S (0.025 M) in deuterium oxide at 20°C

Pheniramine **1**	β-CD	β-CD-CM	β-CD-S
H'_3	_[a]	_[a]	7.5
H'_4	0	14.3	15.8
H'_6	0	7.1	10.5
Carbinoxamine **2**			
H'_4	0	7.0	13.5
H'_6	0	0	7.5
CH	0	0	15.6
Doxylamine **3**			
H'_4	6.6	8.8	4.9
Propranolol **4**			
H_2	0	0	8.5
H_5	0	0	3.4
H_8	0	0	7.2

[a] Overlaps with another resonance.

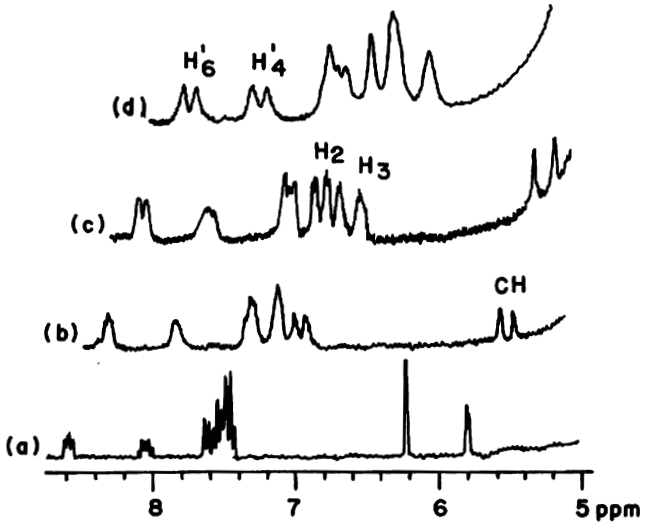

Figure 3. A portion of the ^{1}H NMR spectrum (300 MHz) of (a) carbinoxamine maleate, **2**, (0.025 M) and β-CD-S (0.025 M) in deuterium oxide at 20°C with (b) dysprosium-(III)nitrate (0.010 M), (c) dysprosium(III)nitrate (0.015 M), and (d) dysprosium(III)nitrate (0.025 M).

Table 2. Enantiomeric discrimination (Hz) in the ^{1}H NMR spectra (300 MHz) of substrates (0.025 M) in the presence of β-CD-S or β-CD-CM (0.025 M) in deuterium oxide at 20°C with Dy(III)nitrate or Yb(III)nitrate

Carbinoxamine **2**	β-CD-S	β-CD-CM	
CH	42 (Dy = 0.025 M)	152 (Dy = 0.002 M)	50 (Yb = 0.020 M)
H_2	48		
H_3	76		
H'_4	30		
H'_6	32		
Pheniramine **1**			
CH			46 (Yb = 0.020 M)
Doxylamine **3**			
CH_3		127 (Dy = 0.003 M)	25 (Yb = 0.025 M)

sible to determine whether enantiomeric discrimination occurs. On addition of Dy(III)nitrate, the methine and four of the aromatic resonances (H_2, H_3, H'_4, and H'_6) show enantiometric discrimination at some point in the series of spectra. The enantiomeric discrimination increases as the concentration of Dy(III) is raised. Overlap of some of the substrate resonances at different concentrations of Dy(III) demonstrates the value of measuring a series of spectra with increasing concentrations of lanthanide. The presence of Dy(III) causes some broadening in the spectrum of the substrate, but it is not severe enough to restrict the improvements realized by the addition of a lanthanide ion. The magnitudes of the enantiomeric discrimination observed for resonances of **2** in the presence of β-CD-S and Dy(III) are provided in Table 2.

Addition of Dy(III)nitrate to mixtures of β-CD-CM and cationic substrates broadens the resonances of β-CD-CM but does not shift them much from their original position. The resonances of the organic cations show some unusual behavior in that certain of them shift downfield, whereas others shift upfield. For example, the pyridyl resonances of **1**–**3** shift downfield, whereas the resonances for the other phenyl ring, which is known to insert into the cavity, shift upfield. This same general trend in the direction of dysprosium-induced shifts in the spectrum of substrates such as **2** was reported in a prior CD-DTPA system with an amine tether.[28] In that report, evidence suggested that the pyridine moiety was protonated when mixed with the CD-DTPA, and a cooperative association occurred in which the phenyl ring was included in the cyclodextrin cavity and the pyridinium nitrogen associated with the negatively charged lanthanide–DTPA system. In mixtures with dysprosium(III) and β-CD-CM, it is likely that the pyridine nitrogen atom, which is a hard Lewis base donor, undergoes a direct association with dysprosium ions bonded at carboxylate groups on the secondary face of the cavity, thereby accounting for the unusual lanthanide-induced shifts.

Addition of equivalent concentrations of Dy(III) to mixtures of the substrates and β-CD-CM (0.025 M) causes pronounced broadening in the spectrum of the substrate. Direct bonding of the pyridyl nitrogen in **1**–**3** to the dysprosium ion bound at the carboxymethylate groups would explain this significant broadening. Addition of Dy(III)nitrate or Yb(III)nitrate to mixtures of the sodium salt of tryptophan with β-CD-S or β-CD-CM causes pronounced broadening of the resonances of **5**, indicating that the anionic **5** binds directly to the lanthanide ion as well.

At low concentrations of Dy(III) relative to β-CD-CM (0.025 M) and substrate (0.025 M), less broadening and significant enhancements in enantiomeric discrimination are observed for some resonances. For example, the methine resonance of **2** exhibits an enantiomeric discrimination of 152 Hz at a Dy(III) concentration of 0.002 M. The methyl resonance of **3** shows an enantiomeric discrimination of 127 Hz at a Dy(III) concentration of 0.003 M. Since the lanthanide-induced shifts in the spectra of the substrates are so appreciable, a smaller shifting lanthanide ion such as Yb(III) can be used. Less broadening occurs with Yb(III), yet enhancements in enantiomeric discrimination are still observed. Figure 4 shows an example for the methyl resonance of **3** (0.025 M) with β-CD-CM (0.025 M) and increasing concentrations of Yb(III)nitrate. Figure 5

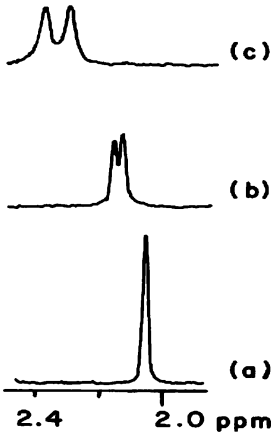

Figure 4. The methyl resonance in the ^{1}H NMR spectrum (300 MHz) of (a) doxylamine succinate, **3**, (0.025 M) and β-CD-CM (0.025 M) in deuterium oxide at 20°C with (b) ytterbium(III)nitrate (0.010 M) and (c) ytterbium(III)nitrate (0.020 M).

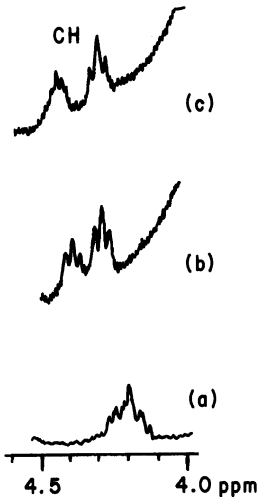

Figure 5. The methine resonance in the ^{1}H NMR spectrum (300 MHz) of (a) pheniramine maleate, **1**, (0.025 M) and β-CD-CM (0.025 M) in deuterium oxide at 20°C with (b) ytterbium(III)nitrate (0.004 M) and (c) ytterbium(III)nitrate (0.010 M).

shows another example for the methine resonance of **1** with β-CD-CM (0.025 M) and increasing concentrations of Yb(III)nitrate.

When performing NMR analyses, the concentration of the chiral solvating agent is almost always set equal to or greater than the concentration of substrate to increase the likelihood of obtaining enantiomeric discrimination. The significant enhancements in enantiomeric discrimination caused by the addition of lanthanide ions to β-CD-CM allows the use of much lower β-CD-CM to substrate ratios. The lower concentration of β-CD-CM reduces the extent of lanthanide-induced broadening and the cost of the reagent needed for analysis.

A typical example is illustrated by the data in Table 3 for **1** (0.025 M) with varying amounts of β-CD-CM and a fixed amount of Dy(III) (0.005 M). Even at a β-CD-CM to **1** ratio of 1:10, the presence of Dy(III) causes significant enantiomeric discrimination of the methine, H'_4 and H'_6 resonances. At a β-CD-CM concentration of 0.0025 M, the Dy(III)-induced shifts and enantiomeric discrimination are essentially constant for concentrations of Dy(III) of 0.005 M or greater, indicating that bonding of the Dy(III) to β-CD-CM is saturated at these conditions. As seen by the data in Table 3, greater enantiomeric discrimination is observed with higher concentrations of β-CD-CM. One reason is because a higher proportion of substrate can associate with the β-CD-CM. For samples with a higher concentration of β-CD-CM than Dy(III), the increase in dysprosium-induced shifts and enantiomeric discrimination as a function of increasing β-CD-CM concentrations indicates that more than one β-CD-CM associates with each Dy(III) ion. At 0.025 M β-CD-CM, 0.005 M Dy(III)nitrate, and 0.025 M **1**, the broadening due to Dy(III) is so severe that it is impossible to determine if enantiomeric discrimination occurs.

Table 3. Enantiomeric discrimination (Hz) in the ^{1}H NMR spectra (300 MHz) of pheniramine maleate, **1**, (0.025 M) in the presence of β-CD-CM and Dy(III)nitrate (0.005 M) in deuterium oxide at 20°C

[β-CD-CM]	CH	H'_3	H'_4	H'_6
0.0025 M	17	–[a]	25	27
0.0075 M	60	67	62	66
0.0125 M	87	80	93	95
0.0250 M	–[b]	–[b]	–[b]	–[b]

[a] Overlaps with another resonance.
[b] Resonances too broadened to accurately measure the chemical shift.

Figure 6 shows the H'_3 and methine resonances of **1** (0.025 M) with β-CD-CM (0.0075 M) as increasing amounts of Yb(III)nitrate are added. The presence of the Yb(III) causes a distinct enantiomeric discrimination of both resonances with minimal peak broadening, further illustrating the potential benefits of using lower concentrations of the cyclodextrin reagent. The further enhancement in enantiomeric discrimination at a Yb(III) concentration of 0.025 M suggests that more than one Yb(III) ion binds to each β-CD-CM.

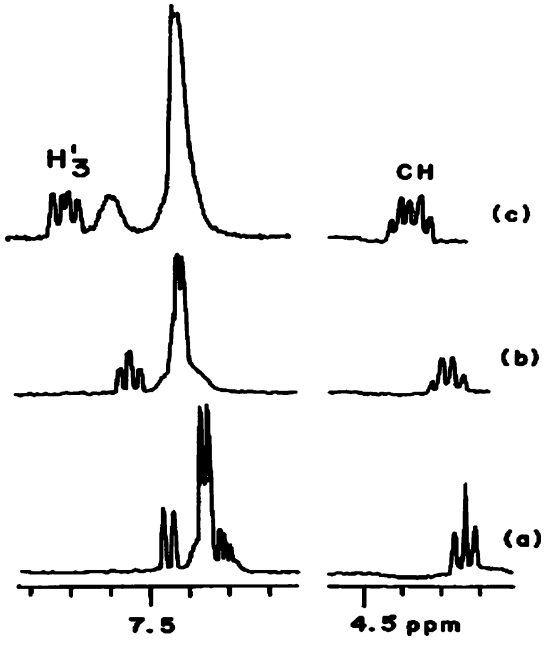

Figure 6. The H'_3 and methine resonances in the ^{1}H NMR spectrum (300 MHz) of (a) pheniramine maleate, **1**, (0.025 M) and β-CD-CM (0.0075 M) in deuterium oxide at 20°C with (b) ytterbium(III)nitrate (0.010 M) and (c) ytterbium-(III)nitrate (0.025 M).

3. Conclusions

Sulfated and carboxymethylated cyclodextrins are effective as water-soluble chiral solvating agents for organic cations. The anionic groups on the cyclodextrins also

provide binding sites for lanthanide ions. Addition of lanthanide ions such as Dy(III) and Yb(III) to mixtures of β-CD-S and β-CD-CM with organic cations causes shifts and often sizeable enhancements in enantiomeric discrimination in the ^{1}H NMR spectrum of the substrate. Whereas chiral NMR solvating agents are usually used at concentrations equivalent to or greater than that of the substrates, the enhancements with the lanthanides allow the use of much lower concentrations of solvating agent.

4. Experimental

4.1. Reagents

The β-CD-S was obtained from Sigma-Aldrich, Milwaukee, WI and has an average of nine sulfate groups per cyclodextrin. The β-CD-CM was a gift from Cerestar, Hammond, IN, and has an average of 3.5 carboxymethyl groups per cyclodextrin. Lanthanide nitrate salts and deuterated NMR solvents are available from several commercial sources.

4.2. NMR studies

NMR spectra were obtained by dissolving the appropriate amount of cyclodextrin and substrate in 1 ml of solvent. Unless otherwise stated, spectra were recorded with equimolar concentrations (0.025 M) of cyclodextrin and substrate. A series of spectra with increasing concentration of lanthanide ion were obtained by adding appropriate aliquots of the hydrated lanthanide(III)nitrate salt in deuterium oxide to the cyclodextrin-substrate samples. NMR spectra were recorded on a General Electric QE-PLUS 300 MHz Spectrometer at 20°C.

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