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Carboxymethylated cyclodextrin derivatives as chiral NMR discriminating agents

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Abstract—Procedures to prepare cyclodextrins with carboxymethyl groups incorporated selectively at the primary (6-position) or secondary (2-position) are described. Complexation properties of the primary and secondary carboxymethylated derivatives of α-, β-, and γ-cyclodextrins are compared to native cyclodextrins and indiscriminately substituted carboxymethylated cyclodextrins, using pheniramine, chlorpheniramine, and brompheniramine as substrates. The stoichiometry of association of these substrates with the α-cyclodextrins is 1:1, whereas with the γ-cyclodextrins, a 2:1 substrate:cyclodextrin complex forms. Data for the β-cyclodextrins suggest that there is a mix of 1:1 and 2:1 substrate–cyclodextrin complexes. The position of the carboxymethyl groups on the cyclodextrin does not appear to alter the geometry of substrate–cyclodextrin association. The effectiveness of the carboxymethylated cyclodextrins as chiral NMR discriminating agents is compared with the native cyclodextrins. In all cases, the indiscriminately substituted α-, β-, and γ-cyclodextrins are more effective at enantiodistinction with the cationic substrates than native cyclodextrins or the derivatives with carboxymethyl groups at the primary or secondary positions. Among α-, β-, and γ-indiscriminately substituted cyclodextrins, there was no clearly optimal candidate for chiral NMR discrimination studies. The indiscriminately substituted carboxymethyl cyclodextrins are effective water-soluble chiral NMR discrimination reagents for cationic substrates.

1. Introduction

Cyclodextrins are cyclic oligosaccharides consisting of various numbers of D-glucose subunits connected by glycosidic ether linkages. The most common are α-, β- and γ-cyclodextrins, containing six, seven, and eight subunits, respectively. These basket-like molecules can serve as hosts for smaller molecules that insert into the cavity of the cyclodextrin. One of the important applications of this host–guest association behavior is enantiodiscrimination, which allows the separation of enantiomers by gas chromatography, liquid chromatography, or capillary electrophoresis, as well as the analytical determination of enantiomeric excess by NMR spectroscopy. Understanding the factors and mechanisms that drive the associations between cyclodextrins and their guests is at the heart of improving the methods currently available for the separation and discrimination of enantiomeric substances.

Native and derivatized cyclodextrins can be used to produce chiral discrimination in NMR spectroscopy.[1–20] Subsequent work showed that the small degree of enantiomeric discrimination, that is often observed in the spectrum of substrates in the presence of cyclodextrins, can be enhanced by coupling a paramagnetic lanthanide ion to the system.[21–23] Covalent attachment of a diethylenetriaminepentaacetic acid moiety to the cyclodextrin through an amide bond provided the lanthanide binding site.

Another strategy for enhancing the discrimination induced in the NMR spectrum of cationic substrates is to use cyclodextrins with anionic substituent groups.[15–18] In particular, commercially available sulfated or carboxymethylated derivatives can be used for this purpose.[24,25] The sulfate and carboxymethylate moieties also provide binding sites for paramagnetic lanthanide ions, and addition of appropriate lanthanide ions to the systems often enhances the enantiomeric discrimination in the NMR spectrum.[24,25]

Trends in the association of native and carboxymethylated α-, β-, and γ-cyclodextrins with cationic pheniramine **1**,

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chlorpheniramine **2**, and brompheniramine **3** (Fig. 1) will be described in this work. Modified carboxymethylated α-, β-, and γ-cyclodextrins were prepared with selective derivatization at the primary or 6-position (α-CDCM-6, β-CDCM-6, γ-CDCM-6) and secondary or 2-position (α-CDCM-2, β-CDCM-2, γ-CDCM-2) (Fig. 2). Derivatives in which the primary and secondary sides were indiscriminately carboxymethylated (α-CDCM-Ind, β-CDCM-Ind, γ-CDCM-Ind) were also prepared and characterized. Aspects of the stoichiometry and geometry of association, as well as the utility of these reagents for chiral NMR discrimination, are described.

2. Results and discussion

2.1. Synthesis and characterization

Preparation of the indiscriminate and secondary-substituted cyclodextrins was achieved by following established procedures. Native α-, β-, or γ-cyclodextrins were reacted with sodium iodoacetate in aqueous sodium hydroxide solution at room temperature to yield the indiscriminately carboxymethylated cyclodextrins. Indiscriminate carboxymethylation refers to derivatives with the carboxymethyl groups at the 2-, 3-, and 6-positions, although prior work has shown that the 2-position tends to have the highest extent of substitution.[26] The 2-position hydroxyls were readily and selectively functionalized with carboxymethyl groups via sodium hydride and sodium iodoacetate at room temperature in DMF.[27] Increasing the reaction time or temperature generally led to an increase in the degree of substitution (DS) for both the indiscriminate and secondary derivatives. It was found that broadening of the carboxymethyl and cyclodextrin resonances in the proton NMR spectra was greatly intensified at higher DS because of the large number of different derivatives in the mixture.

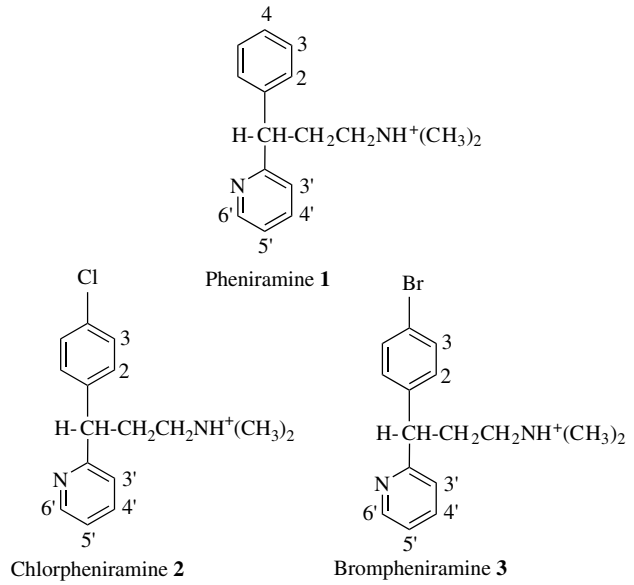

Figure 1. Structures of the cationic pheniramine substrates.

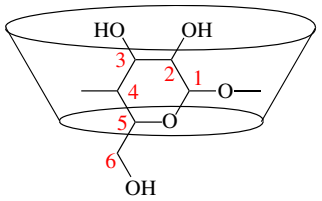

Figure 2. Cone-shaped representation of a cyclodextrin superimposed with one D-glucose subunit. Hydroxyls at the 2- and 3-positions define the wider secondary side of the molecule, while the 6-position hydroxyl is situated at the more narrow side.

A synthetic route to the selective primary carboxymethylation of α-, β-, and γ-CD was established. It has been shown that bases, such as pyridine or imidazole, activate the primary hydroxyl groups of cyclodextrins, rendering them open to electrophillic attack.[28] Reactions using chloroacetic acid and sodium iodoacetate as electrophiles were explored, and it became apparent that the cleanest reactions were obtained with sodium iodoacetate. Pyridine proved to be the best activating agent, provided it was used stoichiometrically in DMF and not neat, as had been reported for other 6-position syntheses. The use of a minimum volume of DMF as a solvent appeared to increase the solubility of the reagents dramatically. The reaction of cyclodextrin and sodium iodoacetate in the presence of pyridine in DMF did not go forward at room temperature. Heating to 80 °C for at least 24 h was necessary, and was accompanied by a characteristic change from clear colorless to cloudy red-brown. The excess pyridine/pyridinium proved very difficult to remove, even with the use of size exclusion chromatography. The best reduction in NMR-visible impurities was achieved through successive washes with acetone.

NMR spectroscopy was relied upon for the characterization of the carboxymethylated cyclodextrins. ^{1}H NMR in deuterium oxide clearly showed the methylene resonances of the carboxymethyl groups as broadened singlets between 4.0 and 4.4 ppm. The cyclodextrin H_1 resonance around 5.1 ppm splits into two more broad signals. ^{13}C NMR spectra were essential in confirming the carboxymethylation, as a diagnostic peak arising from the carboxylate carbon appears around 179 ppm in all of the carboxymethylated species. Other changes in the carbon spectrum depended upon the position of the carboxymethyl group. For CDCM-Ind, the resonances were severely broadened. CDCM-2 gives rise to an additional C_2 resonance at approximately 70 ppm, indicating the addition of a carboxymethyl group at the 2-position, and a new peak at 62 ppm that was identified as the methylene carbon of the carboxymethyl group. Addition of Yb(III)nitrate to the sample caused severe broadening of the carbon signals at 62 and 179 ppm, confirming their assignment as those of the carboxymethyl group. CDCM-6 did not show an additional peak at 70 ppm, though it did give rise to a new signal at about 62 ppm, which was attributed to the methylene protons of the carboxymethyl groups.

In all cases, the DS was not completely uniform, meaning that a mixture of cyclodextrins with varying degrees of carboxymethylation was obtained. The average degree of sub-

stitution is easily calculated from the integration of the NMR spectrum. The highest DS achieved for the CDCM-6 derivatives was 2. The CDCM-2 and CDCM-Ind derivatives were more highly carboxymethylated, with typical DS values of 4 and 8, respectively. An NMR spectroscopic method was also used to determine the waters of hydration of the CDCM-Ind derivatives. Values of 8, 11, and 9 were obtained for batches of the α-, β-, and γ-CDCM-Ind, respectively.

MALDI-MS could be used to determine the highest DS present for a particular sample. However, due to the facile fragmentation of the carboxymethylated cyclodextrin samples, MALDI-MS was unable to provide information about the relative abundance of cyclodextrins with varying DS. MS–MS experiments were employed to demonstrate this fragmentation phenomenon. The MALDI-MS data were able to provide an indication of the highest degree of carboxymethylation present (Fig. 3).

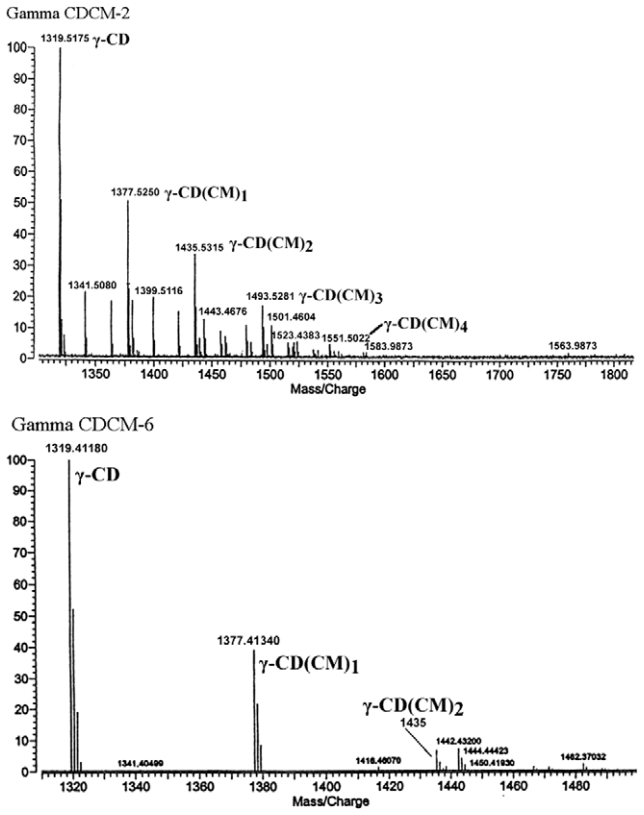

Figure 3. MALDI-MS data from γ-CDCM-2 and γ-CDCM-6 with evidence for carboxymethylation labelled.

2.2. Stoichiometry

The stoichiometry of the association between α-, β- and γ-cyclodextrins and substrates (+)-**2** and (+)-**3** was determined by Job's method,[29–31] and found to be dependent upon the size of the cyclodextrin. However, the stoichiometry of the association of **2** and **3** with α-, β-, and γ-cyclodextrins was independent of the substitution pattern of carboxymethyl groups. Job plots of the α-CDs with **2** and **3** clearly indicate the formation of a 1:1 complex (Fig. 4a), as evidenced by the peak at 0.5. The γ-CDs clearly associate with the substrates in a 1:2 ratio of host-to-guest (Fig. 4b), as evidenced by the peak at about 0.66. The peaks with the largest $\Delta\delta$ values were used in constructing the Job plots, and are indicated in Figure 4. Job plots for β-CD were more difficult to interpret, and based on various spectral phenomena and a prior report,[32] we believe that the complicated Job plots are the result of a mixture of 1:1 and 1:2 complexes.

NMR spectral data provide further evidence for the proposed stoichiometries. In the case of the β-CDs, ^{1}H NMR spectra of CD:substrate mixtures approaching 1:1 in molar concentration exhibit a distinct broadening that is not seen in the spectra of either the α- or γ-CDs under the same conditions. This indicates that the exchange of substrate in the β-cyclodextrin cavity slows to an intermediate rate at a 1:1 ratio, but is fast at all other concentration ratios. The H_2 and H_3 proton resonances of **1**, **2**, and **3** shift to higher frequency in the presence of the α-CDs, to lower frequency with the γ-CDs, and exhibit small shifts with the β-CDs (Fig. 5). It is reasoned that the H_2 and H_3 protons of **1**, **2**, and **3** are deshielded when they enter the cavity of the cyclodextrin, as is clearly demonstrated for the 1:1 complex with the α-CDs. The shift to a lower frequency of the H_2 and H_3 resonances of **1**, **2**, and **3** with the γ-CDs is reasonable since the two substrate aromatic rings in the cavity are expected to shield each other. The small shifts of the H_2 and H_3 resonances of **1**, **2**, and **3** in the presence of the β-CDs are likely accounted for by the presence of 1:1 (deshielded) and 2:1 (shielded) complexes countering each other. Further evidence for a 2:1 substrate-to-γ-CD complex is observed for a series of spectra taken with increasing concentrations of the γ-CD species. The shifts in the ^{1}H NMR spectra of **1**, **2**, and **3** (10 mM) increase until the concentration of the γ-CD species is raised to 5 mM and then level off at higher concentrations of the γ-CD. In contrast, for the α- and β-derivatives, the shifts only begin to level off when cyclodextrin concentrations of 10 mM are reached.

Even though the complexes of the substrates with the γ-CDs have a 2:1 stoichiometry, the exchange of substrate molecules within the cavity is fast at all concentration ratios studied. Distinct resonances are never observed for a bound and unbound form of the substrate. The resonances of **1**–**3** never broadened at any of the concentration ratios studied with the γ-CDs. Analysis of **2** and **3** enriched in one enantiomer showed the expected enrichment of one resonance of those that exhibited enantiomeric discrimination, which is also indicative of fast exchange conditions. The spectrum of a single enantiomer of **2** and **3** showed only one set of substrate resonances at all concentrations of the γ-CDs.

Scatchard plots were used in an attempt to determine the association constants for (+)-**2** and (+)-**3** with certain of the cyclodextrins. Values could be determined for complexes of the α-CDs with **2** and **3**, but very poor data were obtained for the β- and γ-CDs because of the presence of the 2:1 complexes, rendering the Scatchard method unsuitable for such a determination.[32–36] The association constants of the cationic (+)-**2** and (+)-**3** with α-CDCM-Ind

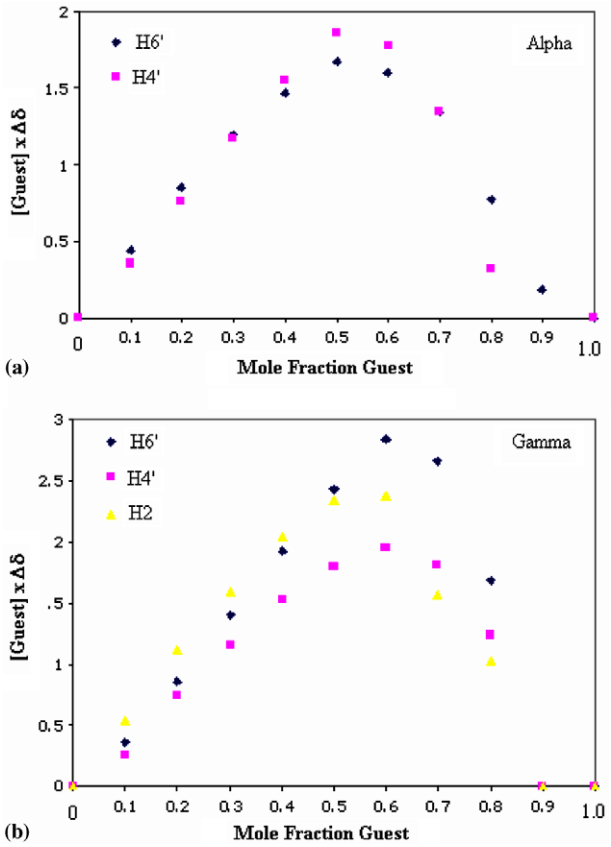

Figure 4. (a) Job plot of α-CDCM-Ind with (+)-chlorpheniramine maleate; (b) Job plot of γ-CDCM-Ind with (+)-brompheniramine maleate.

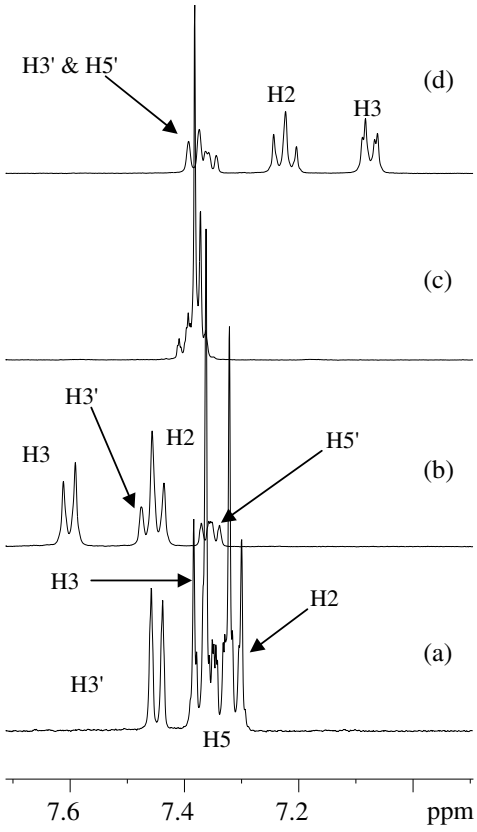

Figure 5. ^{1}H NMR (400 MHz, D_2O) of (a) 10 mM chlorpheniramine with (b) 10 mM native α-CD; (c) 10 mM native β-CD; and (d) 10 mM native γ-CD.

were much larger (ca. 650 M^{-1}) than with the native α-CD (ca. 30 M^{-1}), indicating the importance of ion pair association between the cationic moiety of the substrate and the anionic carboxymethyl group.

2.3. Geometry of association

Shifts of the cyclodextrin resonances can be used as diagnostic signals to analyze the geometry of association between a substrate and the cyclodextrin. Aromatic guest molecules that enter the cavity of the cyclodextrin induce shifts to lower frequencies in the resonances of the internal H_3 and H_5 protons. Since the H_3 proton is located nearer to the secondary face and the H_5 proton is located nearer to the primary face of the cyclodextrin, monitoring shifts in these resonances can indicate the opening through which the substrate enters and the depth of the insertion into the cavity. The magnitude of the shifts of the internal cyclodextrin protons can also indicate how complementary the fit is for a substrate in the cavity. A loose fit of a substrate into the cavity will cause smaller shielding than one in which the ring size and cavity size are more evenly matched. ROESY experiments indicate protons of the substrate and cyclodextrin that are in close spatial proximity, and can be used to give a more complete picture of the geometry of association between the cyclodextrin and substrate.

A 1:1 mixture of α-CD with **2** and **3** exhibits shielding of the internal H_3 proton (ca. 0.2 ppm) and a slight deshielding of H_5 (ca. 0.05 ppm). A mixture of α-CD with **1** exhibits shielding (ca. 0.1 ppm) of H_3 and no shift in H_5. ROESY spectra were obtained for mixtures of 10 mM of **2** with native α-CD at both 4 mM and 30 mM, and no interaction was detected between any of the pyridyl protons and the cyclodextrin protons. In contrast, ROESY interactions are observed between the protons on the aromatic ring and the protons on the interior of α-CD. The data indicate that the aromatic ring of **1**, **2**, and **3** inserts into the cavity of the α-CDs rather than the pyridyl ring. In all likelihood, the nitrogen atom of the pyridyl ring is involved in hydrogen-bonding with the hydroxyl groups at the face of the cyclodextrin. The data further suggest that the H_3 proton of **2** inserts deeply into the cavity, and interacts with both the H_3 and H_5 protons of α-CD, whereas the H_2 proton of the substrate inserts only far enough to interact with H_3 of the CD. A schematic drawing of the geometry of association of **1**, **2**, and **3** with the α-CDs is shown in Figure 6. The spatial proximity of the aromatic ring of the substrate and H_3 of the cyclodextrin results in shielding and the shift to lower frequency. Presumably, the spatial proximity between the halogen in **2** and **3** and H_5 of the α-CD causes deshielding and accounts for the small shift to higher frequency of the H_5 resonance (Fig. 6). The deshielding of H_5 of the cyclodextrin is more pronounced for **3** than **2**, presumably because the larger bromine atom is closer to H_5.

The geometry displayed in Figure 6 appears to be adopted for all of the α-CDs, regardless of the position of the carb-

oxymethyl groups. It might be expected that the α-CDCM-6 would attract the positively charged pheniramine substrates through the primary side opening, so as to allow ion pairing with the anionic carboxymethylate group. Since the H_3 and H_5 resonances of the α-CD and α-CDCM-6 exhibit identical trends in the presence of **1**, **2**, and **3**, it can be concluded that these substrates associate through the secondary side of the cyclodextrin only and that the position of the carboxymethyl group does not alter the geometry of association adopted between the substrates and the α-CDs. The association constants of **2** and **3** with α-CDCM-6 is similar in magnitude to that with native α-CD, which also agrees with the association at the secondary side of the cavity.

Both the H_3 (ca. 0.17 ppm) and H_5 (ca. 0.12 ppm) resonances of the β-CDs exhibit substantial shielding upon association with **1**, **2**, and **3** at 1:1 ratios, indicating that the aromatic ring inserts more deeply into the cavity than with the α-CDs. ROESY data further supports this assertion because, unlike with the α-CDs where there was no detected interaction between H_2 of **2** and the internal H_5 of the cyclodextrin, interactions are detected between the internal H_3 and H_5 protons of β-CD and the H_2 and H_3 protons of the aromatic ring of **2**. Similar to the findings with the α-CDs, there is no evidence for an alteration in the association geometry of the substrate with β-CDCM-6 because the ^{1}H NMR spectra of native β-CD and β-CDCM-6 with **1**, **2**, and **3** are essentially identical.

Possible configurations of two substrate molecules inside the γ-CD cavity are illustrated in Figure 7. In one configuration (Fig. 7b), both substrates enter the cavity from the same side. This geometry would seem unlikely based upon the steric considerations as well as the repulsive forces between the two halogen atoms. An alternative configuration (Fig. 7a) in which one substrate enters from the primary side and the other enters from the secondary side seems more likely, and is supported by NMR shift and preliminary ROESY data. The H_3 (ca. 0.1 ppm) and H_5 (ca. 0.1 ppm) resonances of the γ-CDs exhibit upfield shifts in the presence of **1**, **2**, and **3** at 1:1 ratios, although they were not as large as those with β-CD. The smaller shift of the internal γ-CD proton resonances relative to those with β-CD likely reflects a looser fit of the substrate in the cavity, even though it forms a 2:1 complex with the γ-CD. Similar to the β-CD, ROESY data show clear interactions between the H_2 and H_3 protons of **2** and the H_3 and H_5 protons of the γ-CD. Interestingly, a weak interaction between the H_6 proton of the γ-CD (primary side of molecule, Fig. 2) and the H_3' and H_5' pyridyl protons appears to show up in the ROESY spectrum, which is suggestive of the configuration in Figure 7a. As with the α- and β-CDs, the association geometry for **1**, **2**, and **3** appears to be the same with γ-CD, γ-CDCM-6, γ-CDCM-2 and γ-CDCM-Ind, although the shifts of the substrate resonances are smaller with the native γ-CD than with the carboxymethyl derivatives. Given the geometry shown in Figure 7a, carboxymethylation at the primary or secondary side would stabilize the association of cationic substrates. One other observation, which is consistent with the geometry shown in Figure 7a is that the upfield shift of the H_3 resonances of **1**, **2**, and **3** with the γ-CDs is larger than that of the H_2 resonance. The alignment of two aromatic rings in the γ-CD cavity is expected to cause more shielding of the H_3 proton of **1**, **2**, and **3**. Further efforts to characterize the 2:1 substrate–γ-CD complexes either through crystallographic data or additional ROESY data are underway, and the proposed geometry of the complexes shown in Figure 7a must still be considered tentative.

2.4. Substrate resonances

The effect of α-CD, β-CD, and γ-CD and each of their carboxymethylated derivatives in causing shifts and enantiomeric discrimination in the ^{1}H NMR spectra of **1**, **2**, and **3** was examined. Figures 8–10 provide a representative comparison of the effects of the different cyclodextrins on the aromatic portion of the ^{1}H NMR spectrum of **2**. The effect of the presence of the anionic carboxymethyl groups on the secondary face of the α-CD has pronounced effects on the extent of enantiomeric discrimination in the spectrum. The addition of α-CD and α-CDCM-6 causes almost no discernible enantiomeric discrimination in the ^{1}H NMR spectrum of **2** (Fig. 8). The addition of α-CDCM-2 causes a slight enantiomeric discrimination of the H_3' and H_4' resonances. The addition of the highly substituted α-CDCM-Ind causes baseline discrimination of the H_4' and H_3' resonances and partial discrimination of the methine resonance of the aliphatic group. Even though the resonances of the protons on the aromatic ring of **2** show the larger shifts in the presence of the α-CDs, the substantial enantiomeric discrimination of the H_3' and H_4' resonances on the pyridyl ring demonstrates the significance of the association of this ring with the chiral sites on the secondary face.

As with the α-CD series, β-CD and β-CDCM-6 cause almost no discernible enantiomeric discrimination in the

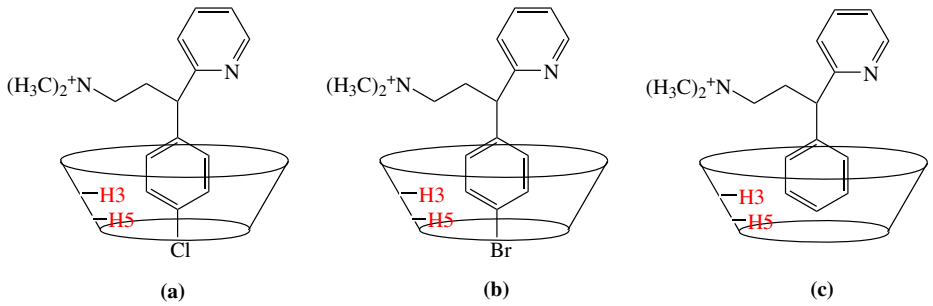

Figure 6. Schematic drawing of the geometry of association of (a) **2**; (b) **3**; and (c) **1** with α-CD.

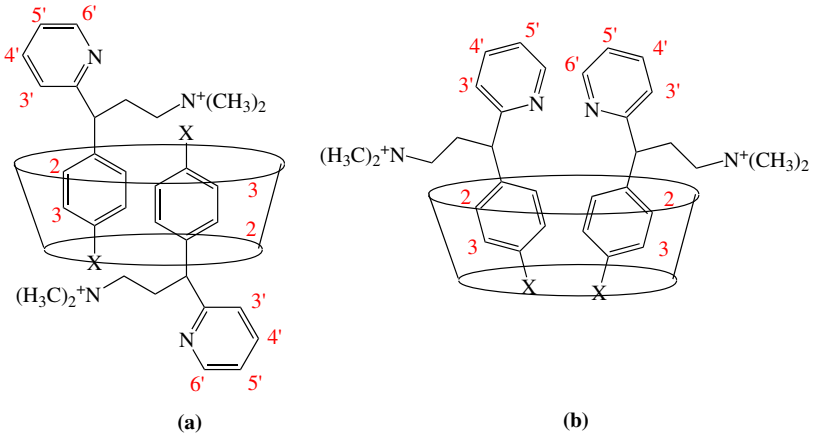

Figure 7. Two possible geometrical arrangements of pheniramine substrates with the γ-CDs.

^{1}H NMR spectrum of **2**, whereas β-CDCM-2 causes partial discrimination of the H'_4 and H'_6 resonances, and β-CDCM-Ind causes baseline enantiomeric discrimination of the H'_4 and H'_6 resonances (Fig. 9).

The γ-CDs produce far less discrimination of the pyridyl resonances than the α- and β-CD series, but rather large shifts and discrimination of the H_2 and H_3 resonances (Fig. 10). The different enantiodiscriminating behavior of the γ-CDs relative to the α- and β-CDs likely reflects the differences caused by the 2:1 complexes with the γ-CDs. The γ-CDCM-6 causes partial enantiomeric discrimination of H_3. The γ-CDCM-2 is more effective and causes partial enantiomeric discrimination of H_2, H_3, and H'_4. The discrimination is greatest with γ-CDCM-Ind, which in addition to causing partial enantiomeric discrimination of H_2 and H'_4, is unique in causing baseline discrimination of the H_3 resonance.

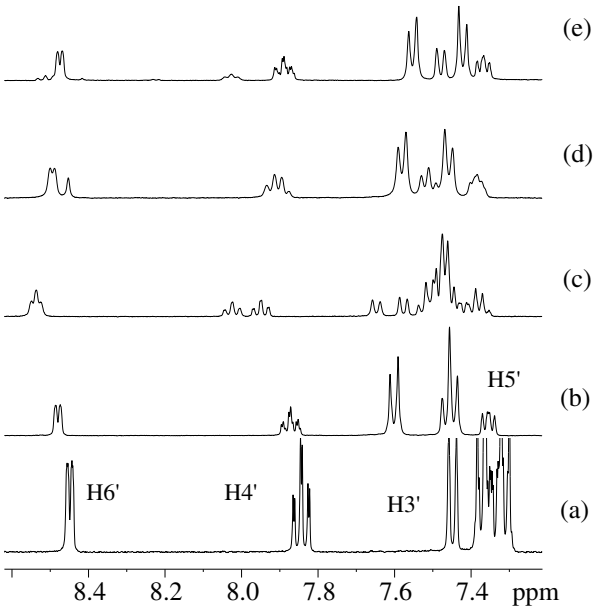

Figure 8. ^{1}H NMR (400 MHz, D_2O) of (a) 10 mM chlorpheniramine with 10 mM (b) α-CD, (c) α-CDCM-Ind, (d) α-CDCM-2, and (e) α-CDCM-6.

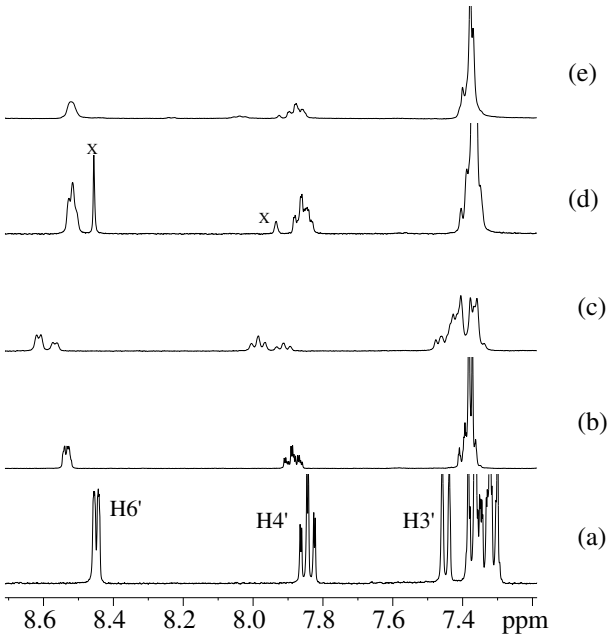

Figure 9. ^{1}H NMR (400 MHz, D_2O) of (a) 10 mM chlorpheniramine with 10 mM (b) β-CD, (c) β-CDCM-Ind, (d) β-CDCM-2, and (e) β-CDCM-6. Impurities are marked by 'x'.

Enantiomeric discrimination in the ^{1}H NMR spectra of **1**, **2**, and **3** (10 mM) with the various CDs (10 mM) are provided in Table 1. Data are only provided for those protons that had distinct resonances in the spectrum. There are likely instances when a resonance did exhibit enantiomeric discrimination that was obscured by its overlapping with other resonances in the spectrum. The general effectiveness of the highly anionic indiscriminately substituted cyclodextrins at causing enantiomeric discrimination relative to the native and selective primary and secondary derivatives is apparent, although none of the α-, β- and γ-CDCM-Ind derivatives are consistently the most effective.

For example, the β-CDCM-Ind is unique in causing baseline discrimination of the H'_6 resonance of **1**, **2**, and **3**, whereas the α-CDCM-Ind is unique in causing baseline dis-

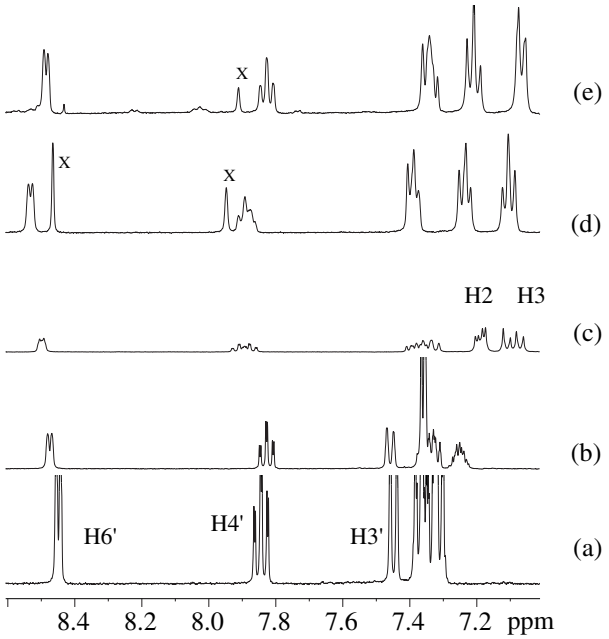

Figure 10. ^{1}H NMR (400 MHz, D_2O) of (a) 10 mM chlorpheniramine with 10 mM (b) γ-CD, (c) γ-CDCM-Ind, (d) γ-CDCM-2 and (e) γ-CDCM-6. Impurities are marked by 'x'.

crimination of the H_3' resonance of **1**, **2**, and **3**. Both the α- and β-CDCM-Ind cause baseline discrimination of the H_4' resonance of **1**, **2**, and **3**. The γ-CDCM-Ind is unique in causing baseline discrimination of the H_3 resonance of **2** and **3**.

The cyclodextrins also have different effects on the resonances of the aliphatic groups of **1**, **2**, and **3**. The diastereotopic hydrogen atoms of the methylene group α to the nitrogen atom are not resolved in the ^{1}H NMR spectra of all three substrates, and appear as a triplet at about 3.1 ppm. The resonances of the diastereotopic hydrogen atoms on the methylene group β to the nitrogen atom are resolved, and two complex multiplets centered at about 2.5 and 2.7 ppm appear in the spectrum. For **1**, the addition of various α-CDs produces no significant changes in the resonances of the aliphatic group. The β-CDs cause a substantial resolution of the diastereotopic hydrogen atoms of the α-methylene group and enhance the diastereotopic resolution of the β-methylene group. Finally, the addition of the γ-CDs causes no significant changes to the resonances of the α-methylene group and only a slight improvement in the resolution of the β-methylene hydrogen resonances. These data suggest that the complex of **1** with the β-CDs constrains the motion of the aliphatic chain more so than the α- or γ-CDs, thereby accounting for the greater resolution of the diastereotopic hydrogen atoms.

The behavior of the resonances of the diastereotopic hydrogen atoms of the aliphatic groups of **2** and **3** shows a different trend than that observed for **1**. For example, the addition of α-CDs to **2** and **3** causes substantial resolution of the diastereotopic α-methylene hydrogen resonances but diminishes the resolution of the diastereotopic β-methylene resonances. The β-CDs and γ-CDs cause enhancements in the resolution of both sets of diastereotopic methylene resonances, although the extent was greater with the β-CDs. Obviously, there are a variety of subtle and different interactions of these cationic aliphatic substituent groups with the carboxymethyl and hydroxyl groups of the different sized cyclodextrins. In all cases, the resonances were too complex to reliably use them in analyzing for the presence of enantiomeric discrimination.

3. Conclusions

Among the native, CDCM-6, CDCM-2, and CDCM-Ind derivatives, the best enantiomeric discrimination was always observed with the most highly anionic, indiscriminately carboxymethylated cyclodextrins. CDCM-2 derivatives were more similar to CDCM-Ind in their ability to cause discrimination in the NMR spectra of pheniramine type substrates. CDCM-6 derivatives were most similar to the native cyclodextrins in their effect on the NMR spectra of the substrates. Incorporation of the carboxymethyl groups on the primary face did not appear to alter the association of pheniramine-type substrates with the cyclodextrins. Data indicate that these substrates form 2:1 complexes with the γ-CDs, 1:1 with the α-CDs, and a mixture of 2:1 and 1:1 with the β-CDs. No particular CDCM-Ind derivative was most effective at causing enantiomeric discrimination for all of the substrates. Evaluating all three of the CDCM-Ind compounds, is therefore, warranted for applications with other substrates.

4. Experimental

4.1. Reagents

All reagents were obtained from major commercial suppliers. The α-, β-, and γ-cyclodextrins were dried in an Abderholden using phosphorus pentoxide as the drying agent and 1-butanol as the solvent.

4.2. Instrumentation

All ^{1}H (32 scans) and ^{13}C spectra (1024 scans) were collected on a Bruker Avance 400 MHz NMR spectrometer. Samples were run in D_2O at ambient probe temperature, and ^{1}H NMR spectra were calibrated using the HOD peak at 4.79 ppm. The ^{13}C NMR spectra were calibrated with a small quantity of internal methanol as a standard. When necessary, assignments were confirmed using 2D-COSY spectra. ROESY spectra were obtained using the standard parameters incorporated into the Bruker software with a mixing time of 200 ms. Mass spectra were collected on an IonSpec (Irvine, CA) HiRes MALDI-FTMS equipped with a 4.7 T magnet.

4.3. Preparation of the indiscriminately substituted cyclodextrins

4.3.1. α-CDCM-Ind. α-Cyclodextrin (5.0 g, 5.14 mmol) was diluted with 70 mL of distilled water and stirred, while NaOH (4.93 g, 123.3 mmol) dissolved in 300 mL distilled

Table 1. Enantiomeric discrimination in ppm in the ^{1}H NMR spectrum (400 MHz) of **1**, **2**, and **3** with different cyclodextrin derivatives (* indicates baseline discrimination of the resonance)

1	CH	H_4	H'_3	H'_4	H'_6	
α-CDCM-2	0.010	—	0.010	0.019	—	
α-CDCM-Ind	0.039	—	0.075*	0.080*	0.014	
β-CDCM-6	—	—	0.010	—	—	
β-CDCM-2	—	—	—	0.010	0.008	
β-CDCM-Ind	—	—	—	0.075*	0.042*	
γ-CD	—	0.019	—	—	—	
γ-CDCM-6	—	0.019	—	—	—	
γ-CDCM-2	—	0.008	—	0.005	—	
γ-CDCM-Ind	—	0.018	0.008	0.028	0.013	
2	CH	H_2	H_3	H'_3	H'_4	H'_6
α-CDCM-6	—	—	—	—	0.007	—
α-CDCM-2	—	—	—	0.019	0.019	—
α-CDCM-Ind	0.022	—	—	0.075*	0.075*	—
β-CD	—	—	—	—	0.009	—
β-CDCM-2	—	—	—	—	0.016	0.010
β-CDCM-Ind	—	—	—	—	0.074*	0.048*
γ-CD	0.030	0.011	0.014	—	—	—
γ-CDCM-6	0.020	0.021	—	—	—	—
γ-CDCM-2	—	0.016	0.018	—	0.018	—
γ-CDCM-Ind	—	0.013	0.041*	—	0.032	—
3	CH	H_2	H_3	H'_3	H'_4	H'_6
α-CD	—	—	—	—	0.006	—
α-CDCM-2	—	—	—	0.018	0.020	—
α-CDCM-Ind	0.021	—	0.020	0.069*	0.069*	0.010
β-CD	—	0.018	0.009	0.012	0.012	—
β-CDCM-6	0.008	0.015	0.007	—	0.010	0.009
β-CDCM-2	—	0.021	0.008	0.017	0.020	0.009
β-CDCM-Ind	—	0.034	0.016	0.021	0.074*	0.047*
γ-CD	0.020	—	—	—	—	—
γ-CDCM-6	0.020	—	—	—	—	—
γ-CDCM-2	—	0.023	—	0.017	0.008	—
γ-CDCM-Ind	—	0.030	0.040*	0.021	0.020	—

H_2O was added. Sodium iodoacetate (25.6 g, 123.3 mmol) was added as a solid, and the mixture was stirred at room temperature for 18–24 h. Methanol was added to precipitate a white solid, which was washed with acetone and dried. ^{1}H NMR (D_2O): $\delta = 5.3$ and 5.1 ppm (2s, br, H_1); 4.4–4.05, CH_2COONa; 4.05–3.8 (m, $H_{3,5,6}$); 3.65–3.47 (m, H_4); 3.5–3.4 (m, br, H_2). ^{13}C NMR (D_2O): 178–179 ppm (*C*OONa); 102, 100, 82, 80, 73–70 (m, br), 61.

4.3.2. β-CDCM-Ind. β-Cyclodextrin (1.0 g, 2.2 mmol) was suspended in 30 mL of distilled water and stirred, while NaOH (0.99 g, 25 mmol) and sodium iodoacetate (5.13 g, 25 mmol) were added as solids. The mixture was stirred at room temperature overnight, then reduced in volume by rotary evaporation. Acetone (30 mL), followed by methanol (10 mL) was added to precipitate the product. The sticky white solid was collected by filtration and stirred in acetone for 36 h to yield a more free-flowing white solid that was collected by filtration and stored in a vacuum dessicator. ^{1}H NMR (D_2O): $\delta = 5.3$ and 5.1 ppm (2s, br, H_1); 4.4–4.05, CH_2COONa; 4.0–3.85 (m, $H_{3,5,6}$); 3.5–3.47 (m, H_4); 3.5–3.4 (m, br, H_2). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 165, 102, 82, 73–70 (m, br), 60, 50.

4.3.3. γ-CDCM-Ind. γ-Cyclodextrin (0.50 g, 0.385 mmol) was placed in a 100 mL rbf and dissolved in 6 mL distilled water. NaOH (0.493 g, 12.32 mmol) was dissolved in 20 mL distilled water and added to the cyclodextrin solution all at once. Sodium iodoacetate (2.56 g, 12.32 mmol) was then added as a solid to the reaction flask and stirred for 18 h at room temperature. The solvent was reduced to 20 mL by rotary evaporation. Methanol was added (45 mL) to precipitate out the product, which was collected by filtration on a glass frit and placed in a vacuum dessicator to dry. A quantitative yield was obtained. ^{1}H NMR (D_2O): $\delta = 5.4$–5.1 ppm (s, br, H_1); 4.4–4.0, CH_2COONa; 4.0–3.5 (br, $H_{3,5,6,2,4}$). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 165, 102, 82, 73–70 (m, br), 60, 50. ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 165, 102, 82, 73–70 (m, br), 60, 50.

4.4. Preparation of 2-position carboxymethylated cyclodextrins

4.4.1. α-CDCM-2. Dry α-cyclodextrin (1.0 g, 0.88 mmol) was diluted with anyhydrous DMF (20 mL) and degassed thoroughly. Sodium hydride (60% dispersion in mineral oil, 0.58 g, 14.5 mmol) was added to the mixture as a solid under a positive pressure of N_2, and stirred for 48 h while it formed a gel. Sodium iodoacetate (1.25 g, 6 mmol) was added as a solid and stirring was continued for an additional 48 h. The reaction mixture was filtered on a glass frit, and then washed with acetone for 24 h. Filtration yielded a white powder in quantitative yield. ^{1}H NMR (D_2O): $\delta = 5.03$ ppm (d, H_1); 4.3–4.1 (various peaks, CH_2COONa); 4.02–3.85 (m, $H_{3,5,6}$); 3.65–3.55 (m, H_2/H_4). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 104, 84, 80, 76, 74, 67, 64, 63, 62.

4.4.2. β-CDCM-2. Dry β-cyclodextrin (2.5 g, 2.2 mmol) was diluted with 100 mL of anhydrous DMF and degassed thoroughly. Sodium hydride (60% dispersion in mineral oil, 0.66 g, 3.2 mmol) was added under a positive pressure of N_2, and the mixture stirred for 24 h. Sodium iodoacetate (3.53 g, 17 mmol) was added to the gel-like mixture and the cloudy, yellow-tinted solution stirred for an additional 24 h at room temperature. The solvent was removed by rotary evaporation, and the resulting white residue was washed with acetone and then filtered through a glass frit to yield a white solid that was stored in a vacuum dessicator. ^{1}H NMR (D_2O): $\delta = 5.25$ and 5.15 ppm (2s, H_1); 4.45 (s, CH_2COONa); 4.0–3.8 (m, $H_{3,5,6}$); 3.6–3.4 (m, H_2/H_4). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 104, 84, 80, 76, 74, 67, 64, 63, 62.

4.4.3. γ-CDCM-2. Anhydrous DMF (30 mL) was added to a 100 mL three-neck rbf containing dried γ-cyclodextrin (1.0 g, 0.77 mmol). The flask was degassed and sodium hydride (60% dispersion in mineral oil, 0.26 g, 6.4 mmol) was added at once under positive pressure of N_2. The reaction was stirred for 24 h at room temperature, forming a white gel. Sodium iodoacetate (1.35 g, 6.4 mmol) was then added under a positive pressure of N_2. The solution turned yellowish and stirring was continued for an additional 24 h. The solvent was reduced by rotary evaporation, and a volume of acetone sufficient enough to produce precipitation of the product as a white solid was added. To loosen the product, the acetone mixture was stirred for 3 h after which the product was collected on a glass frit and then dried in a vacuum dessicator. A quantitative yield was obtained. ^{1}H NMR (D_2O): $\delta = 5.4$ ppm (br, H_1); 4.6–4.1 (s, CH_2COONa); 4.0–3.8 (m, $H_{3,5,6}$); 3.6–3.4 (m, H_2/H_4). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 104, 84, 80, 76, 74, 67, 64, 63, 62.

4.5. Preparation of 6-position carboxymethylated cyclodextrins

4.5.1. α-CDCM-6. Dry α-cyclodextrin (1.0 g, 0.88 mmol) was diluted with anyhydrous DMF (15 mL). Anhydrous pyridine (0.90 mL, 11 mmol) was syringed into the solution, followed by sodium iodoacetate (1.25 g, 5.6 mmol). The reaction was stirred at 80–90 °C for 48 h. The solvent was reduced in volume by rotary evaporation. The brown sticky residue was stirred in acetone for 48 h and then the resulting brown powder was filtered by vacuum filtration. ^{1}H NMR (D_2O): $\delta = 5.1$–5.0 ppm, (d, H_1), 4.6–4.3 (various peaks, CH_2COONa); 4.1–3.8 (m, $H_{3,5,6}$); 3.7–3.5 (m, H_2/H_4). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 103, 82, 74, 73, 72, 62, 61.

4.5.2. β-CDCM-6. Dry β-cyclodextrin (2.5 g, 2.2 mmol) was diluted with anhydrous DMF (100 mL) and degassed thoroughly. Anhydrous pyridine was added via a syringe (2.3 g, 30 mmol), followed by the addition of sodium iodoacetate (3.53 g, 17 mmol). The mixture was stirred at 90 °C for 24 h. The solvent was reduced by rotary evaporation, and then the sticky brownish solid was washed with acetone until it was free-flowing and suitable for filtration on a glass frit. The yellowish brown product was collected. Size exclusion chromatography with P-2 Biogel was used to purify the crude product, employing a 0.05 M ammonium bicarbonate buffer as the mobile phase. Yield 29%. ^{1}H NMR (D_2O): $\delta = 5.1$ ppm (s, H_1), 4.45 (s, CH_2COONa); 4.0–3.8 (m, $H_{3,5,6}$); 3.7–3.5 (m, H_2/H_4). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 104, 82, 78, 77, 76, 62, 60.

4.5.3. γ-CDCM-6. Dried γ-cyclodextrin (1.0 g, 0.771 mmol) was placed in a 50 mL rbf and dissolved under N_2 in 15 mL anhydrous DMF. Anhydrous pyridine (0.75 mL, 9.25 mmol) was added via a syringe. After 5 min, sodium iodoacetate (0.802 g, 0.386 mmol) was added as a solid under positive pressure of N_2. After thorough degassing, the reaction was heated to 90 °C for 24 h. The solvent was removed by rotary evaporation and acetone was added to the flask to loosen the sticky brown solid, and the acetone mixture was stirred overnight. The product was collected by vacuum filtration and dried in a vacuum dessicator. The crude product was purified by steric exclusion chromatography on P-2 Biogel in a 0.05 M ammonium bicarbonate buffer. Yield 34%. ^{1}H NMR (D_2O): $\delta = 5.1$–5.0 ppm (d, H_1), 4.6–4.3 (various peaks, CH_2COONa); 4.1–3.8 (m, $H_{3,5,6}$); 3.7–3.5 (m, H_2/H_4). ^{13}C NMR (D_2O): 180 ppm (*C*OONa); 103, 82, 74, 73, 72, 62, 61.

4.6. NMR spectra for chiral discrimination studies

Solutions of the chiral substrates (10 mM) in D_2O were prepared and enriched with one of the enantiomers when available. An appropriate weight of the cyclodextrin was added to a 600 μl sample in the NMR tube to obtain the desired concentration.

4.7. Determination of stoichiometry and association constants

Stoichiometries of complexes with the cyclodextrins were determined using Job's method.[29–31] The concentration of the cyclodextrin and substrate were continuously varied throughout the series, while maintaining a total concentration of 20 mM. Association constants were determined using the Foster–Fyfe adaptation of the Scatchard method.[33–36] This method was carried out by performing a series of infinite dilutions of the cyclodextrin, while maintaining

the concentration of the substrate at 2 mM. A 100 mM stock solution of the cyclodextrin was diluted with a 2 mM solution of the substrate to obtain cyclodextrin concentrations ranging from 50 to 1 mM.

4.8. Preparation of sample for mass spectrometry analysis

The mass spectra for the 2-CDCM and Ind-CDCM derivatives were obtained in a matrix of dihydroxybenzoic acid and sodium acetate in water–methanol. The spectrum for 6-CDCM was run in a less acidic 6-aza-2-thiothymine (ATT) matrix in acetone/water. MS–MS was used to confirm that fragments arose from the parent cyclodextrin compound.

4.9. Determination of waters of hydration of the cyclodextrin derivatives

The ^{1}H NMR spectrum of a 1 mL sample of anhydrous deuterated dimethyl sulfoxide (DMSO) with tetramethylsilane (0.1% v/v) was obtained, and the integrated area of the residual water resonance was compared to that of the TMS resonance. A quantitative amount of sample (usually about 25 mg) was then added to the NMR solvent, shaken, and centrifuged. The supernatant, which contained waters of hydration from the cyclodextrin, was removed and the increase in area of the water peak relative to that of TMS was determined. By appropriate calculations, the moles of water in the NMR tube and then the stoichiometry in the cyclodextrin could be determined. A comparison of the area of the resonance for the residual hydrogen atoms of the DMSO also served as a check on the calculations. The applicability of the method was confirmed by the analysis of a commercial β-cyclodextrin with known number of waters of hydration.

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References

1. D'Souza, V. T.; Lipkowitz, K. B. *Cyclodextrins, Chem. Rev.* **1998**, *98*, 1741–2076.
2. MacNicol, D. D.; Rycroft, D. S. *Tetrahedron Lett.* **1977**, 2173–2176.
3. Greatbanks, D.; Pickford, R. *Magn. Reson. Chem.* **1987**, *25*, 208–215.
4. Casy, A. F.; Mercer, A. D. *Magn. Reson. Chem.* **1988**, *26*, 765–774.
5. Saka, W.; Yamamoto, Y.; Inoue, Y.; Chujo, R.; Takahashi, K.; Hattori, K. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 3175–3182.
6. Brown, S. E.; Coates, J. H.; Lincoln, S. F.; Coghlan, D. R.; Easton, C. J. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 2699–2703.
7. Taylor, A.; Williams, D. A. R.; Wilson, I. D. *J. Pharm. Biomed. Anal.* **1991**, *9*, 493–496.
8. Dodziuk, H.; Sitkowski, J.; Stefaniak, L.; Jurczak, J.; Sybilska, D. *J. Chem. Soc., Chem. Commun.* **1992**, 207–208.
9. Taylor, A.; Blackledge, C. A.; Nicholson, J. K.; Williams, D. A. R.; Wilson, I. D. *Anal. Proc.* **1992**, *29*, 229–231.
10. Botsi, A.; Yannakopoulou, K.; Hadjoudis, E.; Perly, B. *J. Chem. Soc., Chem. Commun.* **1993**, 1085–1086.
11. Uccello-Barretta, G.; Balzano, F.; Caporusso, A. M.; Salvadori, P. *J. Org. Chem.* **1994**, *59*, 836–839.
12. Kitae, T.; Takashima, H.; Kano, K. *J. Inclusion Phenom. Macrocycl. Chem.* **1999**, *33*, 345–359.
13. Branch, S. K.; Holzgrabe, U.; Jefferies, T. M.; Mallwitz, H.; Oxley, F. J. R. *J. Chromatogr., A* **1997**, *758*, 277–292.
14. Uccello-Barretta, G.; Ferri, L.; Balzano, F.; Salvadori, P. *Eur. J. Org. Chem.* **2003**, 1741–1748.
15. Owens, P. K.; Fell, A. F.; Coleman, M. W.; Berridge, J. C. *J. Chromatogr., A* **1998**, *797*, 149–164.
16. Hellreigel, C.; Handel, H.; Wedig, M.; Steinhauer, S.; Sorgel, F.; Albert, K.; Holzgrabe, U. *J. Chromatogr., A* **2001**, *914*, 315–324.
17. Chankvetadze, B.; Schulte, G.; Bergenthal, D.; Blaschke, G. *J. Chromatogr., A* **1998**, *798*, 315–323.
18. Owens, P. K.; Coleman, M. W.; Berridge, J. C. *J. Inclusion Phenom. Macrocycl. Chem.* **2000**, *38*, 133–151.
19. Kano, K.; Hasegawa, H. *Chem. Lett.* **2000**, 698–699.
20. Kano, K.; Hasegawa, H.; Miyamura, M. *Chirality* **2001**, *13*, 474–482.
21. Wenzel, T. J.; Bogyo, M. S.; Lebeau, E. L. *J. Am. Chem. Soc.* **1994**, *116*, 4858–4865.
22. Wenzel, T. J.; Miles, R. D.; Zomlefer, K.; Frederique, D. E.; Roan, M. A.; Troughton, J. S.; Pond, B. V.; Colby, A. L. *Chirality* **2000**, *12*, 30–37.
23. Kean, S. D.; Easton, C. J.; Lincoln, S. F.; Parker, D. *Aust. J. Chem.* **2001**, *54*, 535–539.
24. Wenzel, T. J.; Amonoo, E. P.; Shariff, S. S.; Aniagyei, S. E. *Tetrahedron: Asymmetry* **2003**, *14*, 3099–3104.
25. Smith, K. J.; Wilcox, J. D.; Mirick, G. E.; Wacker, L. S.; Ryan, N. S.; Vensel, D. A.; Readling, R.; Domush, H. L.; Amonoo, E. P.; Shariff, S. S.; Wenzel, T. J. *Chirality* **2003**, *15*, S150–S158.
26. Reuben, J.; Rao, C. T.; Pitha, J. *Carbohydr. Res.* **1994**, *258*, 281–285.
27. Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275–4278.
28. Chao, Y. Ph.D. Thesis, Columbia University, 1972; pp 71–74.
29. Job, P. *Ann. Chem.* **1928**, *9*, 113–203.
30. Sahai, R.; Loper, G. L.; Lin, S. H.; Eyring, H. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1499–1503.
31. Gil, V. M. S.; Olivceira, N. C. *J. Chem. Ed.* **1990**, 473–478.
32. Chankvetadze, B.; Burjanadze, N.; Pintore, G.; Bergenthal, D.; Bergander, K.; Muhlenbrock, C.; Breitkreuz, J.; Blaschke, G. *J. Chromatogr., A* **2000**, *875*, 471–484.
33. Scatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660–672.
34. Foster, R.; Fyfe, C. A. *Trans. Faraday Soc.* **1965**, *61*, 1626–1631.
35. Foster, R.; Fyfe, C. A. *J. Chem Soc., Chem. Commun.* **1965**, 642.
36. Fielding, L. *Tetrahedron* **2000**, *56*, 6151–6170.