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Cyclomaltooligosaccharide-assisted spectroscopic discrimination of phthalimido-derived amino acids through the formation of molecular aggregates

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Abstract—Spectroscopic evidence was used to demonstrate the formation of molecular associates in an aqueous solution of phthalimido tryptophan. These molecular associates are loosely formed through π–π aromatic stacking, properties that are not sufficient to cause NMR spectroscopic enantiomeric discrimination. A cyclomaltooligosaccharide with a larger cavity, such as cyclomaltooctaose (γ-cyclodextrin), is capable of forming a ternary complex with these molecular associates and enhances π–π aromatic stacking interactions, resulting in NMR enantiomeric discrimination. Electrospray-ionization mass spectroscopy (ESIMS) and NOESY two-dimensional NMR spectroscopic methods were used to study these complexes. Association constants and thermodynamic data for these cyclomaltooligosaccharide complexes were also estimated.

Keywords: Cyclomaltooligosaccharide; Enantiomeric discrimination; Molecular aggregation; ESIMS

1. Introduction

Since the first report of chiral recognition by a cyclomaltooligosaccharide almost a half a century ago,[1] there has been a substantial amount of research done that shows that cyclomaltooligosaccharides (commonly known as cyclodextrins and herein referred to as CDs) can function as chiral environments that can be used for enantiomeric recognition.[2] However, this research has proved to be of limited success. It has been postulated that the major reason for the somewhat lackluster results associated with CDs in enantiomeric recognition was due to the higher symmetry of CDs and their limited interactions (mainly hydrophobic) between the guest molecule and the CD skeleton, due to the fact that the hydroxyl groups of CD mostly interact with the surrounding aqueous environment. For these reasons, functionalized (modified) CDs show superior enantiomeric recognition in comparison with their unmodified counterparts.[2] Even so, non-functionalized CDs immobilized on polymers have been successfully used as a stationary phase for chiral chromatography.[3]

Considering that the cavity of non-functionalized CDs cannot provide 'classical' three-point interactions that are so often used in the explanation of enantiomeric recognition[4] and enantiomeric discrimination associated with modified CDs, these essential three-point interactions should come from the guest molecule. One possibility is that enantiomeric guest molecules form molecular aggregates in an aqueous solution through several non-bonding interactions. If this occurs then the small molecular aggregates that form, which are primarily dimers, should bind into a large CD cavity and enforce the formation of molecular associates and cause enantiomeric spectroscopic non-equivalence. Our preliminary results suggest that these interactions occur between properly derived amino acids in an aqueous solution. The presence of certain cyclomaltooligosaccharides (notably cyclomaltooctaose (γ-CD)) makes these interactions stronger, as demonstrated by NMR

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spectroscopic study.[5] Herein we explore the CD-assisted spectroscopic discrimination of enantiomers of amino acid derivatives.

2. Results and discussion

It is well documented that aromatic compounds bind into the CD cavity.[6] On the other hand, amino acids are an abundant source of chirality in organic chemistry, yet the majority of them do not form strong inclusion complexes with CDs. Therefore, introducing an aromatic ring through derivatizations of amino acids seemed to be an appropriate method to employ in our proposed studies. To further reinforce the logic of using molecular systems derived from aromatic compounds and amino acids, we found it significant that it has been well established that aromatic compounds tend to bind into peptide cavities that have aromatic amino acids with complementary electronic properties.[7] To explore the importance of aromatic–aromatic interactions in the formation of chiral molecular aggregates and their binding into CDs, three amino acids (alanine, phenylalanine, and tryptophan) and two carboxylic acid anhydrides (phthalic anhydride and cyclohexane-1,2-dicarboxylic anhydride) were selected in the preparation of six *R*-isomeric and six *S*-isomeric imino derivatives. The first group of chiral molecules used in our study of molecular associates consisted of molecular systems with a contribution from π–π aromatic interactions and are shown in Scheme 1.

Our reasoning for preparing *N*-phthalimidotryptophan (**1a**) was simple: these molecules should form molecular aggregates in aqueous media, and the aromatic moieties of the molecular aggregates should bind into the CD cavity to form a strong inclusion complex. There are many structural possibilities to consider in the formation of molecular complexes between amino acid derivatives **1** and CDs, and a few are presented in Figure 1. It is reasonable to assume that all of these molecular complexes are in dynamic equilibrium in aqueous media. It is also known that an NMR spectroscopic study would give an average chemical shift (signals) for all molecular aggregates in equilibrium.[8]

All NMR spectra of **1a** with the enantiomeric composition of $R{:}S = 1{:}4$ (enantiomer non-equivalence) and in concentration range from 10^{-4} to 10^{-1} are identical. We demonstrated in some of our earlier studies of chiral amides in chloroform solution that NMR non-equivalency of enantiomers can be observed if one of the enantiomers is present in excess.[9] This was explained by the formation of weak diastereomeric complexes between molecules of the excess enantiomer acting as a resolving agent.[9] According to our NMR study, this is not the case with compound **1a**, presumably due to very weak non-bonding interactions between enantiomers in bulk water media.

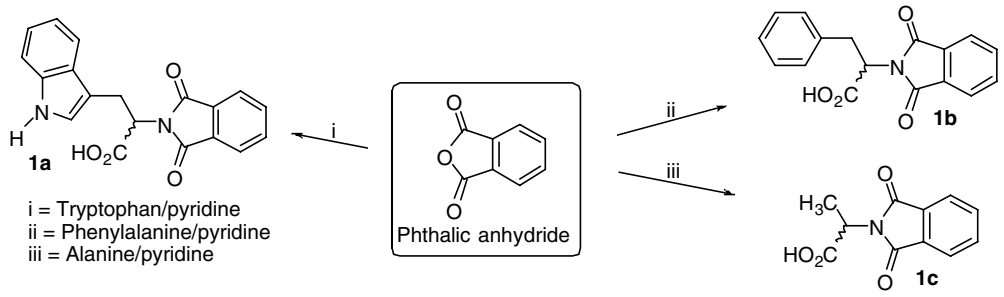
Electrospray-ionization mass spectroscopy (ESIMS) has been used extensively for the detection of the formation of molecular associates in aqueous solutions.[5,10] Negative-ion ESIMS spectroscopy (sodium salt) of **1a*S*** in aqueous solution clearly indicates the formation of molecular associates, and notably, the formation of the molecular dimer with MW = 667.1 (Fig. 2). One can argue that the failure to observe the NMR enantiomer non-equivalence is due to a weak enantiomer association constant.[†]

If the association constant is very small, then the formation of **1a** molecular associate can be enforced by CD encapsulation (binding) as proposed in Figure 1. The CD cavity should be of sufficient size to accommodate two aromatic rings and in this way form a ternary complex with a molecular associate of **1a**. Therefore, NMR enantiomeric non-equivalence should be observed in γ-CD, the cyclomaltooligosaccharide with the largest cavity studied here. Our ^{1}H NMR spectroscopic studies fully agree with this reasoning (Fig. 3). Although there are some chemical shift changes (noticeable between 7.0 and 7.2 ppm), indicating interactions between α-CD and racemic **1a**, it is unlikely that cyclomaltohexaose (α-CD) can form a ternary (three molecules) or higher degree cyclomaltooligosaccharide complex with a **1a** dimer, as indicated in Figure 1. On the other hand, enantiomeric discrimination is observed in cyclomaltoheptaose (β-CD), notably a doubling of some signals between 7.0 and 7.2 ppm, but not to a high degree. We believe that this observation is due to the formation of a 'normal' 1:1 complex between racemic **1a** and β-CD. The NMR spectra of racemic **1a** in γ-CD clearly shows the NMR enantiomer non-equivalence of aromatic protons (Fig. 3).

Not only does the presence of γ-CD cause enantiomeric NMR spectroscopic non-equivalence (discrimination), but it is also possible to assign the signals to the appropriate enantiomer, as well as determine the enantiomeric composition. For example, there is a little influence of γ-CD on the chemical shift of **1a*S*** (compare the **1a*RS*** spectra from Fig. 3 with **1a*S*** spectra from Fig. 4); however, there is a substantial upfield chemical shift of indole hydrogens (6.8–7.5 ppm) and downfield chemical shift (7.7 ppm) for phthalimido hydrogens (Fig. 3). Based on this observation, one can suggest a preferential formation of the γ-CD complex with **1a*R*** in comparison with **1a*S***.

Considering that the best results of NMR spectroscopic non-equivalence (chiral recognition) were observed in an aqueous γ-CD of racemic **1a**, we turned

[†] This point will be addressed in a separate manuscript that will extensively study the formation of these molecular associates in micellar media.



Scheme 1. Phthalimido amino acid derivatives.

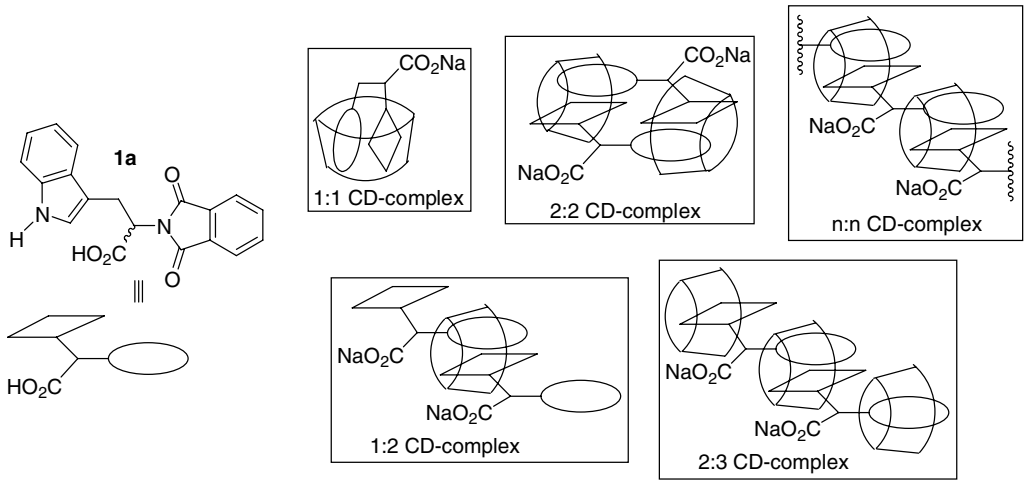


Figure 1. Possible π–π aromatic molecular complexes between CD and **1a**.

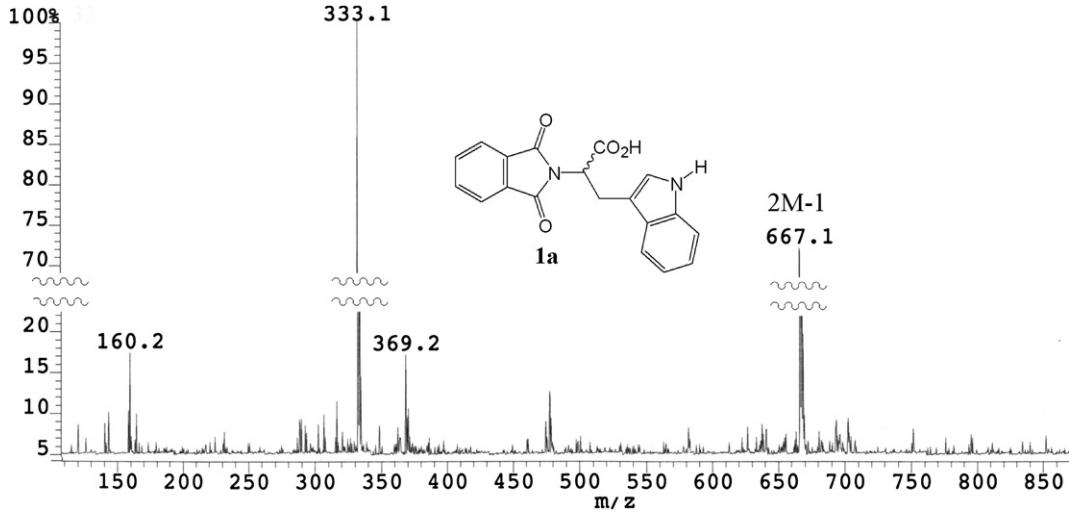


Figure 2. Negative-ion ESIMS of the sodium salt of **1a** in an aqueous solution.

our attention to two-dimensional NMR spectroscopy. In a pivotal experimental study by Amato et al. on the application of ^{1}H NMR, two-dimensional, molecular modeling, and molecular dynamic simulation on CD enantiomeric discrimination,[11] it was found that both *R* and *S* enantiomers of the racemic drug CI-933 were deeply inserted into the host cavity. The diastereomeric complexes thus formed exhibited a different distribution of intermolecular hydrogen bonds. In our case, the molecular associates that formed bind in the

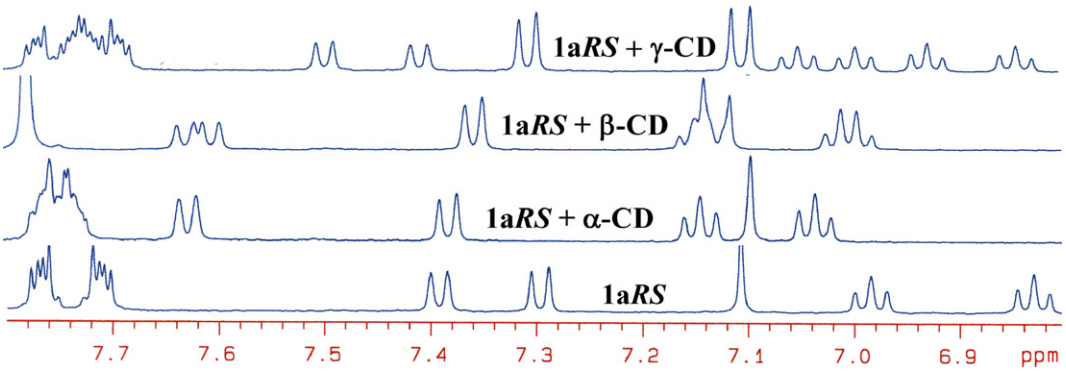


Figure 3. Aromatic portion of the ^{1}H NMR spectra of racemic **1a** in aqueous CDs.

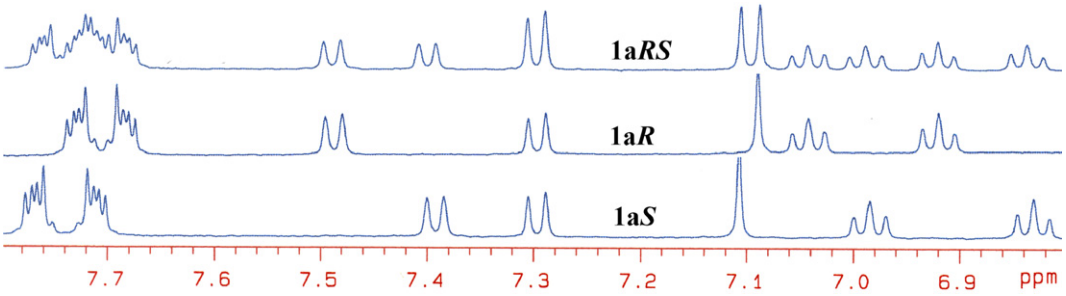


Figure 4. Comparison of γ-CD induced NMR non-equivalency of **1a**.

largest CD cavity to enhance diastereomeric discrimination.

This was perfectly demonstrated on the NOESY spectra of **1a*S*** in water. There are strong space coupling interactions between the phthalimido (H_a–H_d) moiety of **1a*S*** and indole (H_e–H_i) moiety of **1a*S***. This indicates that π–π aromatic stacking interactions form molecular aggregates in aqueous media. When γ-CD was added to the aqueous solution, additional cross coupling signals were observed between the aromatic moieties of **1a*S*** and the CD hydrogens. The major cross coupling interactions were observed between the phthalimido moiety of **1a*S*** and methylene groups of the γ-CD ring (Fig. 5).

The formation of a ternary complex should also be observed in negative-ion ESIMS spectra. In basic solution compound **1a** bears at least one negative charge (carboxylic salt). Considering the γ-CD cavity is of sufficient size[‡] to form an inclusive complex with a **1a** dimer, it should not be possible to detect a 'free' **1a** dimer (*m*/*z* 667) in the solution, but rather it should be complexed with one γ-CD (*m*/*z* 981.9), two γ-CD (*m*/*z* 1630), and three γ-CD (*m*/*z* 2278.6) molecules, respectively. This is perfectly demonstrated in Figure 6.

To better understand the binding of guest compound **1a** with host CDs, the association constants (K_a, mol^{-1}) were measured by ^{1}H NMR spectroscopy (500 MHz). The solution of **1a** (10^{-3} M) in aqueous $NaHCO_3$ (3×10^{-3} M) was titrated with the host solution at a different temperature, and each time the change in chemical shift was measured. Nonlinear regression analysis using Origin 6.1 (Aston Scientific Ltd) was used to generate the association constant K_a according to the equation:[12]

$$\Delta = \frac{\Delta_{max} K_a [H]}{1 + K_a [H]}$$

where Δ is the peak shift in parts per million, Δ_{max} the maximum peak shift in parts per million, and [H] the concentration of host. At least two experiments were performed for each system. Using the above equation, the binding isotherm for guest **1a*S*** to all three CDs (α, β, and γ-CD) were used to calculate the association constant and standard free energy ($\Delta G^0 = -RT \ln K_a$; where $R = 8.3144$ J K^{-1} mol^{-1}).

The highest association constant was measured for γ-CD and **1a*S*** (Table 1). These results are in agreement with the above-mentioned requirements for spectroscopic discrimination of enantiomers in aqueous CD media: mainly that the CD cavity should be sufficiently large to accommodate the formation of a ternary complex with two guest molecules in the cavity. On the other

[‡] The CD cavity sizes are 174, 262, and 427 Å^3 for α-CD, β-CD, and γ-CD, respectively. For further information see Ref. 6.

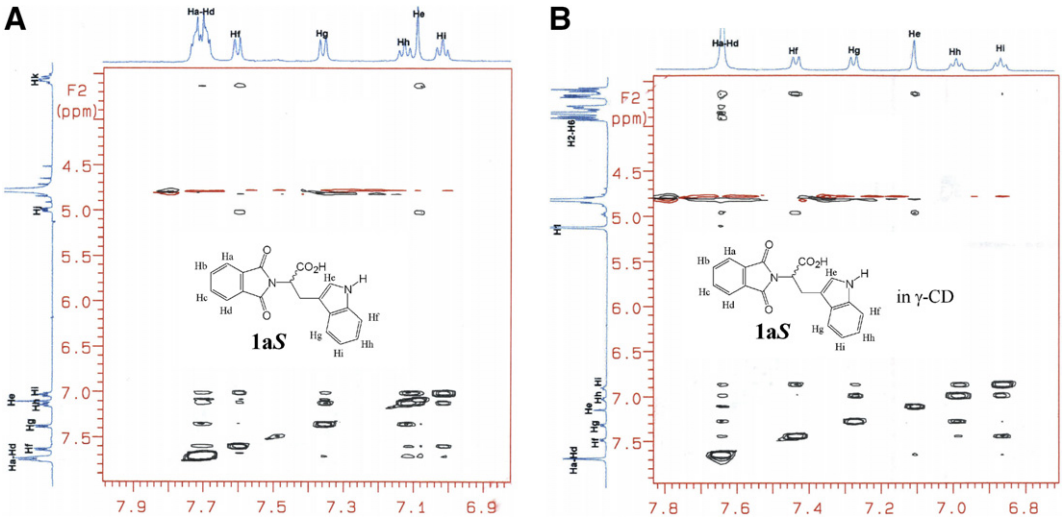


Figure 5. The NOESY spectra of 10^{-2} M **1a*S*** in D_2O (**A**) and 10^{-2} M γ-CD (**B**).

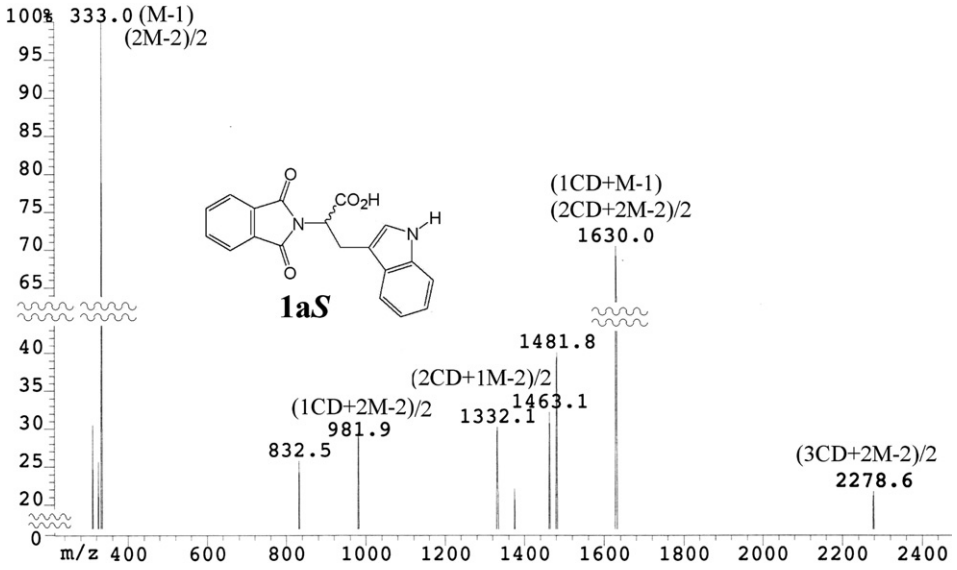


Figure 6. Negative-ion ESIMS of **1a*S*** (10^{-3} M) in γ-CD (10^{-2} M).

Table 1. Association constant and standard free energy ΔG^0 for the binding of guest molecule **1a*S*** to host α, β and γ-CD

CD	Association constant (K_a/mol^{-1})	Standard free energy (ΔG^0/kJ mol^{-1})
α-CD	66.7 ± 13	−10.41 ± 0.5
β-CD	105.1 ± 14	−11.54 ± 0.3
γ-CD	226.2 ± 13	−13.44 ± 0.2

hand, it was demonstrated that the ***R*** isomer of **1a** forms a slightly stronger ternary complex with γ-CD than does its ***S*** isomer. The estimated association constant for **1a*R*** is approximately 4.64 times higher than that of **1a*S*** with γ-CD.

Listed in Table 2 are the association constant K_a, standard free energy ΔG^0, standard entropy term $T\Delta S^0$ and standard enthalpy ΔH^0 of guest **1a*S*** with γ-CD at different temperatures. Due to a higher molecular motion (molecular dynamics) at a elevated temperature, the formation of molecular associates at elevated temperatures decreased, which is reflected in the value of the association constant.

From the given data, it appears that **1a** is a good molecular system to form molecular associates in aqueous media and that a CD with large cavity, such as γ-CD will enforce the formation of these molecular aggregates by forming ternary inclusion complexes with them. If we assume that molecular associates between **1a** are formed through π–π stacking in aqueous media, then by replacing the indole ring with a phenyl ring or a hydrogen atom, these interactions should be diminished or removed. Indeed, after experimental analysis, we

Table 2. Thermodynamic parameters for the γ-CD binding of **1a*S***

Temperature (K)	K_a (mol^{-1})	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	$T\Delta S^0$ (kJ mol^{-1})
298	226.2 ± 13	−13.44 ± 0.2	−24.4 ± 2	−10.96 ± 1.8
313	186.7 ± 8	−12.96 ± 0.1	−24.4 ± 2	−11.44 ± 1.9
333	74.1 ± 6	−10.67 ± 0.2	−24.4 ± 2	−13.73 ± 1.8

Table 3. Association constants and free energies for the CD binding with **1c*S***

CD	Association constant K_a (mol^{-1})	Standard free energy ΔG^0 (kJ mol^{-1})
α-CD	63.5 ± 10	−10.29 ± 0.4
β-CD	227.1 ± 17	−13.45 ± 0.2
γ-CD	82.1 ± 6	−10.93 ± 0.2

could discern by NMR spectroscopy no enantiomer discrimination in any of the three CD aqueous solutions of racemic **1b**. On the other hand, compound **1c** has only one aromatic group; therefore, the formation of π–π stacking that is present in **1a** (phthalimido–indole aromatic interactions) are not possible. Considering the size of the phthalimide ring, **1c** should form the strongest CD inclusion complex with β-CD.[2] This was perfectly demonstrated with the estimated association constants between **1c** and α-, β-, and γ-CD (Table 3). The phthalimido ring of **1c** is too big to enter the small α-CD cavity. On the other hand, it fits relatively well into the β-CD cavity, while the γ-CD cavity is too large for the phthalimido group of **1c**. The formation of the ternary complex with two molecules of **1c** and one CD is not likely due to the lack of aromatic (electronic) complementarity observed in **1a**.

Our thermodynamic data are in agreement with our NMR studies. Only small but noticeable enantiomeric discrimination of racemic **1c** was observed in aqueous β-CD (Fig. 7).

A similar effect should be observed if the phthalimido group of **1a** is reduced to a cyclohexane ring, in which case the formation of π–π aromatic stacking is not possible, and enantiomeric spectroscopic non-equivalence must come only from a 1:1 CD complex with the indole moiety in the CD cavity. Again, due to the size of the CD cavity, only β-CD can induce spectroscopic enantiomeric non-equivalence. To perform this study, the required cyclohexanedicarboimides **2** were prepared by following the reaction outlined in Scheme 2.

As expected, we were not able to observe any spectroscopic non-equivalence with racemic **2b** and **2c** in the presence of any of the three CDs. Partial enantiomer signal separation was observed for the indole protons of racemic **2a** in aqueous β-CD (Fig. 8).

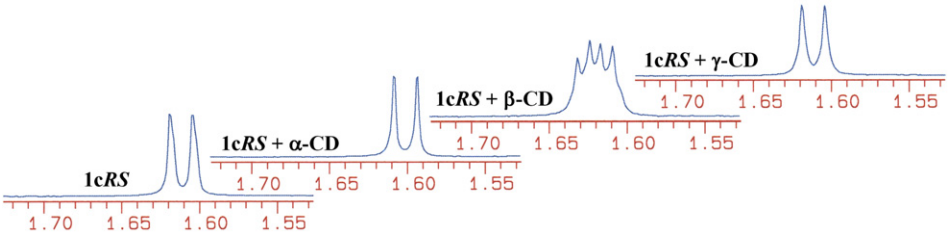
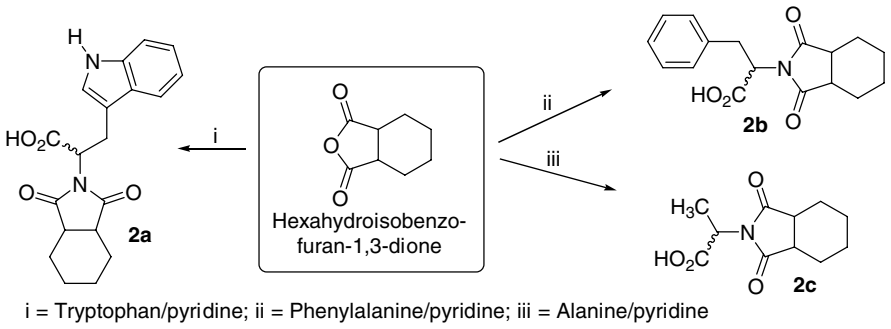


Figure 7. The NMR methyl signal for **1c*RS*** in aqueous CDs.



Scheme 2. Preparation of cyclohexane-1,2-dicarboxylimido derivatives of amino acids.

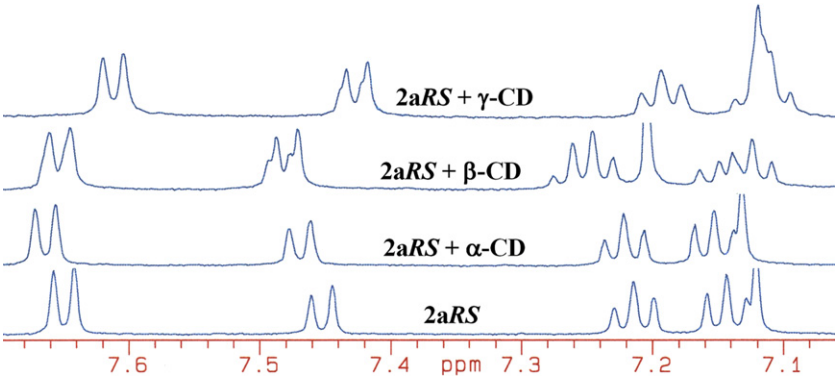


Figure 8. ^{1}H NMR spectrum of the aromatic portion of racemic **2a** in aqueous CDs.

3. Conclusions

In conclusion, we can state that, although in general, aqueous CDs produce a poor chiral environment for spectroscopic discrimination of racemic mixtures in comparison with functionalized CDs, this might not be true if racemic molecules can form molecular associates. In this case, the binding of these molecular associates into the CD cavities enhance spectroscopic enantiomeric discrimination. This was perfectly demonstrated in the case of **1a** and γ-CD. If the requirements for the formation of a molecular associate are eliminated, the spectroscopic discrimination is diminished or non-existing. The evidence of the formation of a ternary CD complex is provided by electrospray-ionization mass spectroscopy (ESIMS).

4. Experimental

4.1. General

Melting points were taken on an Electrothermal IA 9000 Digital Melting Point Apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were run on a Varian Unity 400 and a Varian INOVA 500-MHz spectrophotometer with DMSO-d_6 as the solvent and internal standard (2.50 and 35.91 ppm for ^{1}H and ^{13}C NMR, respectively). NOESY spectra were recorded on 500 MHz-spectrophotometer with D_2O as the solvent and the mixing time was kept 0.3 s. The mass spectra were recorded on a Micromass Quattro 2 Triple Quadropole Mass Spectrometer.

4.2. General procedure for the preparation of amino acid imides 1 and 2

4.2.1. Preparation of 2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(1*H*-indol-3-yl)propionic acid (1a*S*). A pyridine solution (600 mL) of phthalic anhydride (1.48 g; 0.01 mol) and L-tryptophan (2.04 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~5 mL, and the hot reaction mixture was added to ice-cooled HCl (150 mL water and 50 mL concd HCl). The solid precipitate that formed was separated by filtration, washed with water (3 × 20 mL), and dried in the oven at 60 °C for a few hours to afford pure **1a*S*** (2.8 g, 84%): mp 180–182 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 10.74 (1H, s, pyrrole ring NH) 7.79 (4H, s, benzene ring), 7.48 (1H, d, *J* 7.5 Hz), 7.26 (1H, d, *J* 7.5 Hz) 7.03 (1H, s, pyrrole ring), 7.00 (1H, t, *J* 8 Hz) 6.90 (1H, t, *J* 7.5 Hz) 5.12 (1H, dd, J_1 9.5 Hz; J_2 6.5 Hz) 3.58 (2H, ddd). ^{13}C NMR (125 MHz; DMSO-d_6): δ_C 170.5, 167.3 (carbonyls), 136.1, 134.9, 130.9, 127.0, 123.5, 123.4, 121.1, 118.5, 118.0, 111.6, 109.8 (11 aromatic carbons), 52.7 (chiral carbon CH), and 24.2 (methylene carbon). ESIMS^{-} (CH_3OH): *m*/*z* 333.1 ($M-H^+$,100%), 667.1 ($2M-H^+$, 70). Anal. Calcd for $C_{19}H_{14}N_2O_4$: C, 68.26; H, 4.22; N, 8.38. Found: C, 68.31; H, 4.22; N, 8.35.

4.2.2. Preparation of (*R*)-2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-(1*H*-indol-3-yl)propionic acid (1a*R*). The *R* stereoisomer was prepared in a 79% isolated yield by following the procedure described for the *S* stereoisomer. The NMR spectroscopic characteristics for this compound were identical to those of the *S* stereoisomer.

4.2.3. Preparation of (*S*)-2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionic acid (1b*S*). Phthalimide **1b*S*** was prepared by following the procedure for the preparation of **1a*S***. The isolated yield of **1b*S*** is 95% (2.8 g): mp 186–188 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.82 (4H, s) 7.15 (5H, m), 5.13 (1H, dd, J_1 11.5 Hz; J_2 4.5 Hz), 3.49 (1H, dd, J_1 15.5 Hz; J_2 4.5 Hz), 3.35 (1H, dd, J_1 14 Hz; J_2 11.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 170.1, 167.1 (carbonyls), 137.3, 134.9, 130.7, 128.7, 128.3, 126.6, 123.4 (7 aromatic carbons), 52.9 (chiral carbon CH), and 33.9 ppm (methylene carbon). ESIMS (CH_3OH): *m*/*z* 250.3 ($M-CO_2-H^+$, 15%), 294.2 ($M-H^+$, 100), 589.4 ($2M-H^+$, 25).

4.2.4. Preparation of (*R*)-2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionic acid (1b*R*). The *R* stereoisomer was prepared in 93% isolated yield by following the procedure for the preparation of **1a*S***. The NMR spectroscopic characteristics for this compound were identical to those of the **1b*S*** stereoisomer.

4.2.5. Preparation of (*S*)-2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propionic acid (1c*S*). Phthalimide **1c*S*** was prepared by following the procedure for the preparation of **1a*S***. Obtained product was further purified by crystallization from water to afford **1c*S*** (2.23 g, 91%): mp 148.5–150 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.85–7.90 (4H, m) 4.87 (1H, q, *J* 7 Hz), 1.54 (3H, d, J_2 7.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 171.1, 167.2 (carbonyls), 134.8, 131.3, 123.3 (3 aromatic carbons), 46.9 (chiral carbon CH), and 14.8 ppm (methyl carbon). ESIMS (CH_3OH): *m*/*z* 218.2 ($M-H^+$, 100%).

4.2.6. Preparation of (*R*)-2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propionic acid (1c*R*). The *R* stereoisomer was prepared in 88% isolated yield by following the procedure described for the *S* stereoisomer. The spectroscopic characteristics for this compound were identical to those of the *S* stereoisomer.

4.2.7. Preparation of (*S*)-2-(1,3-dioxo-1*H*-isoindol-2(3*H*,3a*H*,4*H*,5*H*,6*H*,7*H*,7a*H*)-yl)-3-(1*H*-indol-3-yl)propionic acid (2a*S*). This imide was prepared by following the procedure for the preparation of **1a*S*** in 93% (2.1 g isolated yield): mp 200–201 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 10.83 (1H, s, pyrrole ring NH) 7.44 (1H, d, *J* 8 Hz), 7.30 (1H, d, *J* 8 Hz) 7.04 (1H, s, pyrrole ring) 7.03 (1H, t) 6.95 (1H, t, *J* 7 Hz) 4.91 (2H, dd, J_1 11.5 Hz; J_2 10.5 Hz) 3.45 (2H, ddd), 2.72 (1H, q, J_1 7 Hz), 2.67 (1H, q, J_1 7 Hz), 1.50–0.95 (8H, m). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 178.9, 178.6, 170.3 (carbonyls), 136.0, 127.1, 123.6, 120.9, 118.4, 118.1, 111.4, 109.5 (8 aromatic carbons), 52.3, 38.8, 38.6, 23.4, 23.3, 22.3, 21.2, 21.0 (8 aliphatic carbon). ESIMS (CH_3OH) *m*/*z* 339.1 ($M-H^+$, 100%) 679.2 ($2M-H^+$, 100). Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.05; H, 5.92; N, 8.23. Found: C, 66.91; H, 5.92; N, 8.23.

4.2.8. Preparation of (*R*)-2-(1,3-dioxo-1*H*-isoindol-2(3*H*,3a*H*,4*H*,5*H*,6*H*,7*H*,7a*H*)-yl)-3-(1*H*-indol-3-yl)propionic acid (2a*R*). The **2a*R*** stereoisomer was prepared in a 92% isolated yield by following the procedure for the preparation of **1a*S***. The spectroscopic characteristics for this compound were identical to those of **2a*S***.

4.2.9. Preparation of (*R*,*S*)-2-(1,3-dioxo-1*H*-isoindol-2(3*H*,3a*H*,4*H*,5*H*,6*H*,7*H*,7a*H*)-yl)-3-(1*H*-indol-3-yl)propionic acid (2a*RS*). Racemic **2b** was prepared in a 92% isolated yield from racemic phenylalanine by following the procedure for the preparation of **1a*S***. The product was further purified by crystallization from water: mp 145–147 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 7.23 (2H, t, *J* 3 Hz), 7.17 (1H, t, *J* 7 Hz), 7.13 (2H, d, *J* 6.5 Hz), 4.93 (1H, dd, J_1 12 Hz; J_2 5 Hz), 3.36 (1H, dd, J_1 14 Hz; J_2 5 Hz), 3.26 (1H, dd, J_1 13.5 Hz; J_2 12 Hz), 2.75 (2H, m), 1.56–0.95 (8H, m). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 178.8, 178.6, 169.9 (carbonyls), 137.1, 128.9, 128.2, 126.6 (4 aromatic carbons), 52.9 (chiral carbon), 52.2, 38.6, 38.5, 33.3, 23.4, 22.4, 21.2, 21.1 ppm (aliphatic carbon). ESIMS$^-$ (CH_3OH): *m*/*z* 300.1 ($M-H^+$, 100%) 601.2 ($2M-H^+$, 20).

4.2.10. Preparation of (*R*,*S*)-2-(1,3-dioxo-1*H*-isoindol-2(3*H*,3a*H*,4*H*,5*H*,6*H*,7*H*,7a*H*)-yl)propionic acid (2a*RS*). Racemic **2c** was prepared from racemic alanine in a 90% isolated yield by the modification of the procedure for the preparation of **1a*S***. The pyridine solution (500 mL) of cyclohexane-1,2-dicarboxylic anhydride (3.2 g, 0.021 mol) and DL alanine (1.78 g, 0.02 mol) was refluxed for 10 h. The volume of the reaction mixture was reduced to ~5 mL and the hot reaction mixture was added to mixture of ethyl acetate (250 mL) and aq HCl (250 mL water and 50 mL concd HCl). The EtOAc layer was separated and washed with water (3 × 20 mL) and then removed under reduced pressure and then evaporated. The product was crystallized from water and dried in the air to afford **2c*RS*** (4.05 g, 90%): mp 148–149 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 4.62 (1H, q, *J* 7.5 Hz), 2.94 (2H, m), 1.73–1.61 (4H, m), 1.38 (3H, d, *J* 7.5 Hz), 1.29–1.26 (4H, m). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 178.9, 178.7, 170.9 (carbonyls), 46.9 (chiral carbon), 39.0, 23.1, 21.3, 21.3, 21.1, 14.1 (aliphatic carbon). ESIMS (CH_3OH): *m*/*z* 224.1 ($M-H^+$, 100%).

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