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Carbohydrate Research 340 (2005) 1413–1418
Carbohydrate RESEARCH

Note

Cyclomaltooligosaccharide (cyclodextrin)-assisted enantiomeric recognition of benzo[*lmn*][3,8]phenanthroline-derived amino acids

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Received 7 December 2004; accepted 2 March 2005
Available online 9 April 2005

Abstract—Formation of self-assembly molecular aggregates and cyclomaltooligosaccharide (cyclodextrin) molecular aggregates with benzo[*lmn*][3,8]phenanthroline-derived amino acids is presented. The nature of the molecular aggregates was studied by negative-ion electrospray-ionization mass spectrometry (ESIMS). The enantiomeric recognition was demonstrated by NMR enantiomeric discrimination of the amino acid derivatives in aqueous solutions of cyclodextrins.

Keywords: Cyclomaltooligosaccharide; Cyclodextrin; Enantiomeric recognition; Molecular aggregation; Electrospray-ionization mass spectrometry; ESIMS

There are many drugs that are marketed as racemic mixtures. Preparing enantiomerically pure compounds is crucial for the pharmaceutical industry.[1] It is known that cyclomaltooligosaccharides (hereafter, 'cyclodextrins') are capable of forming stable complexes with aromatic compounds.[2] Therefore, it is reasonable to propose that cyclodextrins can form diasteriomeric inclusion complexes with racemic aromatic compounds. Based on the knowledge that diastereomers have different physical properties, it is reasonable to expect that one of the diasteriomeric inclusion complexes will crystallize out from a solution of the racemic compound complexed with a cyclodextrin in solution more readily than the other.

Here we would like to explore the possibility of forming diasteromeric cyclodextrin inclusion complexes with racemates that have two chiral centers, one relatively large aromatic acceptor, and two smaller electron-rich aromatic moieties. A graphical representation of our target molecule is presented in Figure 1. Considering structural properties, one can postulate the formation of chiral molecular self-assembly.[3]

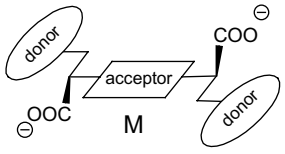

Figure 1. Chiral molecule with two donors and one acceptor.

Some of the most utilized starting materials for the preparation of optically active molecules are natural amino acids. We chose three amino acids from which to prepare our compounds for the study of formation of molecular aggregates (Scheme 1). Each of the molecular aggregates synthesized were designed to explore structural parameters necessary to obtain enantiomeric recognition through formation of diasteromeric molecular aggregates. The central portions of the target molecules contain a naphthalene ring with four carbonyl groups, structural moieties that make this aromatic portion of the molecule electron deficient. The electron-rich part of the molecule was supplied by either the indole moiety of tryptophan (compound **1c**) or the phenyl moiety of phenylalanine (compound **1b**). Alanine was selected for preparation of derivative **1a**, which does not contain an additional aromatic moiety, but was

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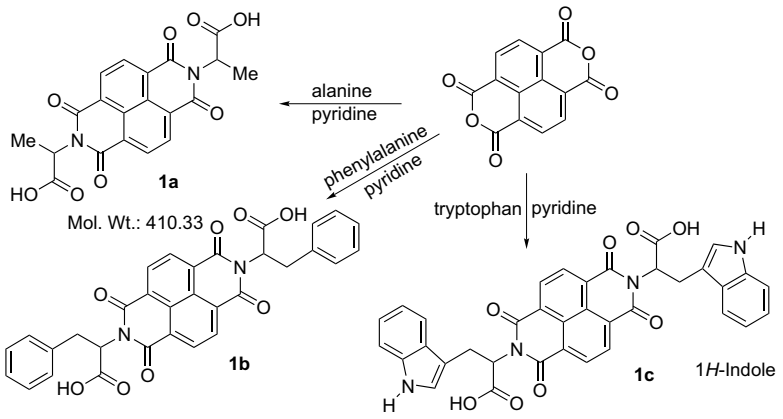
doi:10.1016/j.carres.2005.03.002



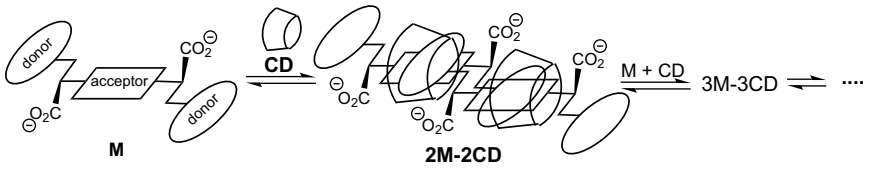
Scheme 1. Synthetic transformation of three amino acids into **1a**, **1b**, and **1c**.

synthesized to demonstrate the necessity of second aromatic moiety (Scheme 1).

Considering that compound **1c** has electron-deficient and electron-rich aromatic moieties, it should form molecular associates through dimerization, trimerization, etc. Due to the statistical factor, it is reasonable to believe that formation of high-order molecular associates in solutions is unlikely. We have postulated that the stability of these molecular associates can be increased through their cyclodextrin inclusion complexes (Scheme 2).

One excellent method for use in the study of molecular associates is electrospray-ionization mass spectrometry (ESIMS).[4] Combinations of an aqueous solution of each of the three cyclodextrins, α, β, and γ (cyclomaltohexaose, -heptaose, and -octaose), and the six enantiomers (RR and SS of **1a**, **1b**, and **1c**) were the subject of electrospray mass spectrometric studies. Even without the presence of cyclodextrins, these molecules form dimers (**2M**) as was demonstrated with negative-ion ESIMS of **1a** in methanol–water solution (Fig. 2). This finding supports our postulation that molecular aggregation of **1** in solution can occur spontaneously.

The questions remaining are (a) what is the influence of cyclodextrin on the molecular aggregation of **1** and (b) can a cyclodextrin form molecular aggregates with these compounds? Regardless of which combination of molecule **1** and cyclodextrin (α-, β-, or γ-cyclodextrin)



Scheme 2. Possible molecular associates in aqueous cyclodextrin solutions.

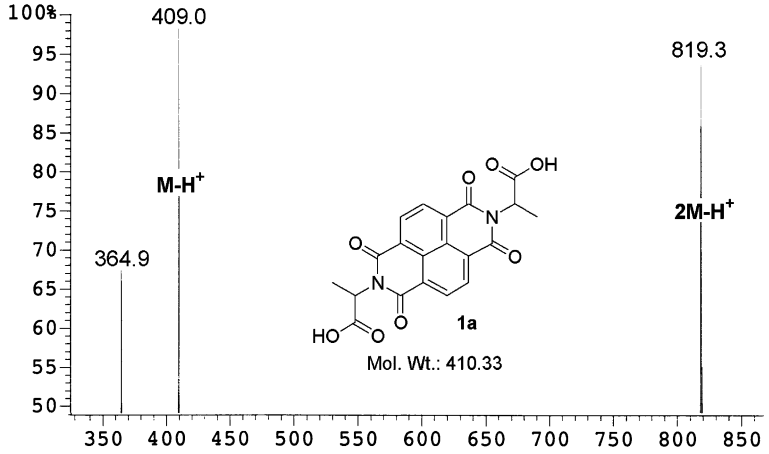


Figure 2. Negative-ion ESIMS of **1aSS** in methanol–water.

was used, both self-assembly molecular aggregates and aggregates with cyclodextrins in aqueous media were observed. This is demonstrated with the negative-ion electrospray mass spectra of **1a** in an aqueous solution of α-cyclodextrin (Fig. 3). The signal intensity of the molecular dimer of **1a** increased with reference to the molecular signal (Fig. 2). Formation of the molecular trimer (**3M**−$\mathbf{H}^+$ = *m*/*z*1229) was also observed. We were unable to observe higher order of molecular aggregates. Molecule **1a** cannot form self-assembly molecular aggregates through aromatic stacking interactions, as these can only form through hydrogen-bonding interactions present in two carboxylic acid groups.

However, molecule **1c** is capable of forming molecular associates through aromatic stacking interactions, and γ-cyclodextrin (largest cavity size) might stabilize these molecular associates by forming inclusion complexes between the aggregates and cyclodextrin. Our negative-ion electrospray mass spectra fully support our expectations (Fig. 4). Besides normal molecular dimers formed (proof of self-assembly), there are signals that demonstrate the formation of molecular complexes with cyclodextrin that are of higher order than 1:1, such as a 2:1 (2M + 1γCD) and a 1:2 (1M + 2γCD) complex (Fig. 4). This suggests that due to the formation of high-order diasteriomeric molecular complexes, spectroscopic

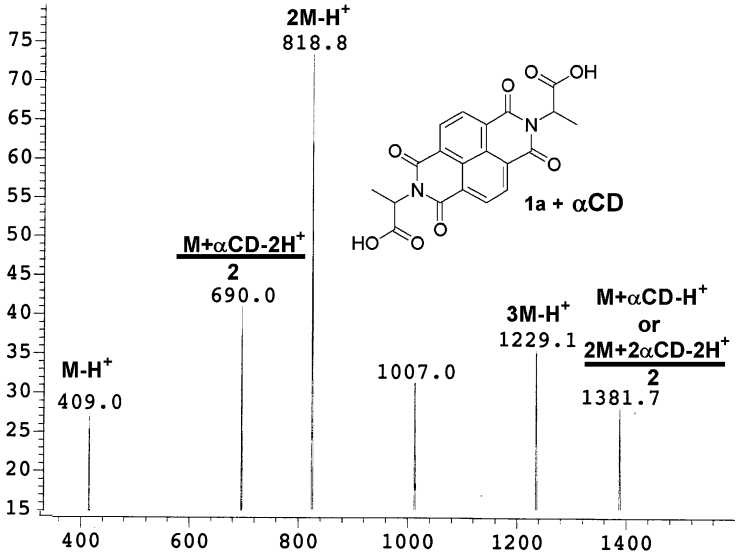


Figure 3. Negative-ion ESIMS of **1aSS** in aqueous α-cyclodextrin solution.

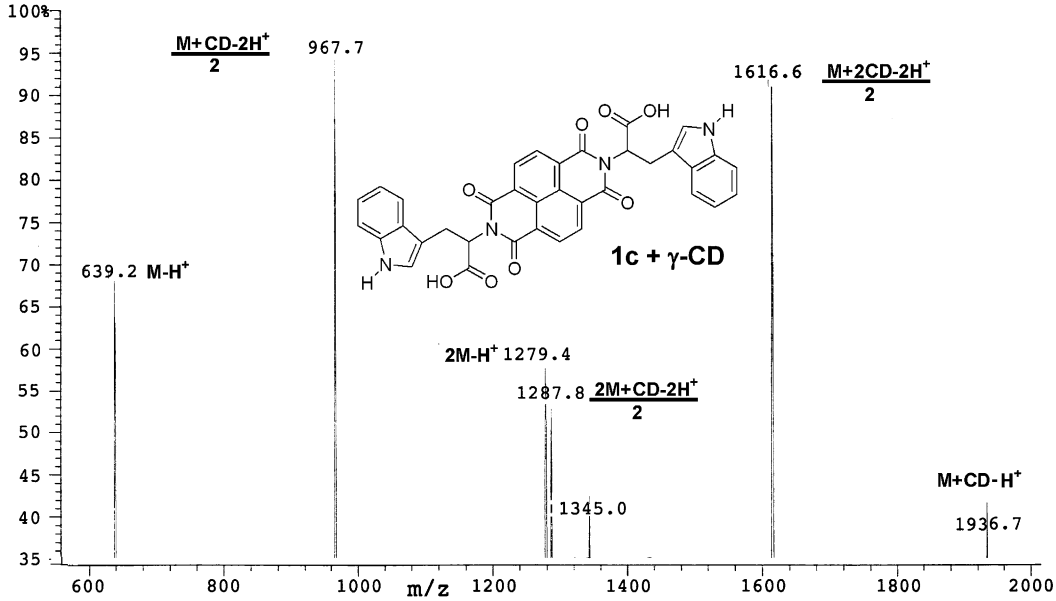


Figure 4. Negative-ion ESIMS of **1cS** (0.001 M) in Me_2SO (1 drop) and γ-cyclodextrin (γ-CD, 10^{-2} M).

enantiomeric discrimination of **1c** should occur. As useful as ESIMS is in proving the formation of molecular complexes, this method cannot demonstrate the existence of enantiomer recognition or discrimination. For this purpose an NMR spectroscopic study of the cyclodextrin aqueous solution of racemic **1a**, **1b**, and **1c** was performed.

We were not able to observe evidence of NMR discrimination of alkaline aqueous solutions of racemic **1a** and **1b** with or without the presence of α-, β-, and γ-cyclodextrin. This is a very interesting finding, considering that molecular complexes of these compounds are formed, as demonstrated in the example of the ESIMS spectra of **1a** and α-cyclodextrin (Fig. 3). If we assume that inclusion complexes with cyclodextrin (inclusion complex is formed when part of the molecule enters the cyclodextrin cavity) are necessary for NMR spectroscopic enantiomer discrimination, then one can argue that these molecular aggregates are not formed by inclusion cyclodextrin complexation, but rather by hydrogen-bonding interactions on the outside of the cyclodextrin cavity. The NMR spectrum represents the average association of a molecule that is in fast equilibrium with many different aggregates. It is therefore important that molecule **1** form a strong diastereomeric complex, preferably with cyclodextrin as the molecular dimer. For instance, formation of a ternary complex between **1a** in α-cyclodextrin is not evident in the ESIMSs. On the other hand there is a strong signal for the ternary β-cyclodextrin complex with the **2cS** dimer (Fig. 5).

This finding is in agreement with our NMR spectroscopic study of the enantiomeric discrimination of **1c** in aqueous cyclodextrin solution (Fig. 6). The racemic mixture of **1cRR** and **1cSS** in water shows only one set of signals, belonging to both **1cRR** and **1cSS**. The molecule is too big to enter the α-cyclodextrin cavity; therefore, a diastereomeric inclusion complex cannot be formed, and the enantiomer cannot be observed by NMR spectroscopy as was demonstrated in Figure 6. The cavity of β-cyclodextrin can accommodate the molecular size; therefore, strong diastereomeric inclusion complexes are formed and NMR recognition is observed (Fig. 6). The γ-cyclodextrin has the largest cavity of the three studied cyclodextrins; therefore, it can form a ternary inclusion complex (two molecules and one cyclodextrin). Formation of a strong ternary complex also results in its lower water solubility and difficulty in NMR characterization (Fig. 6).

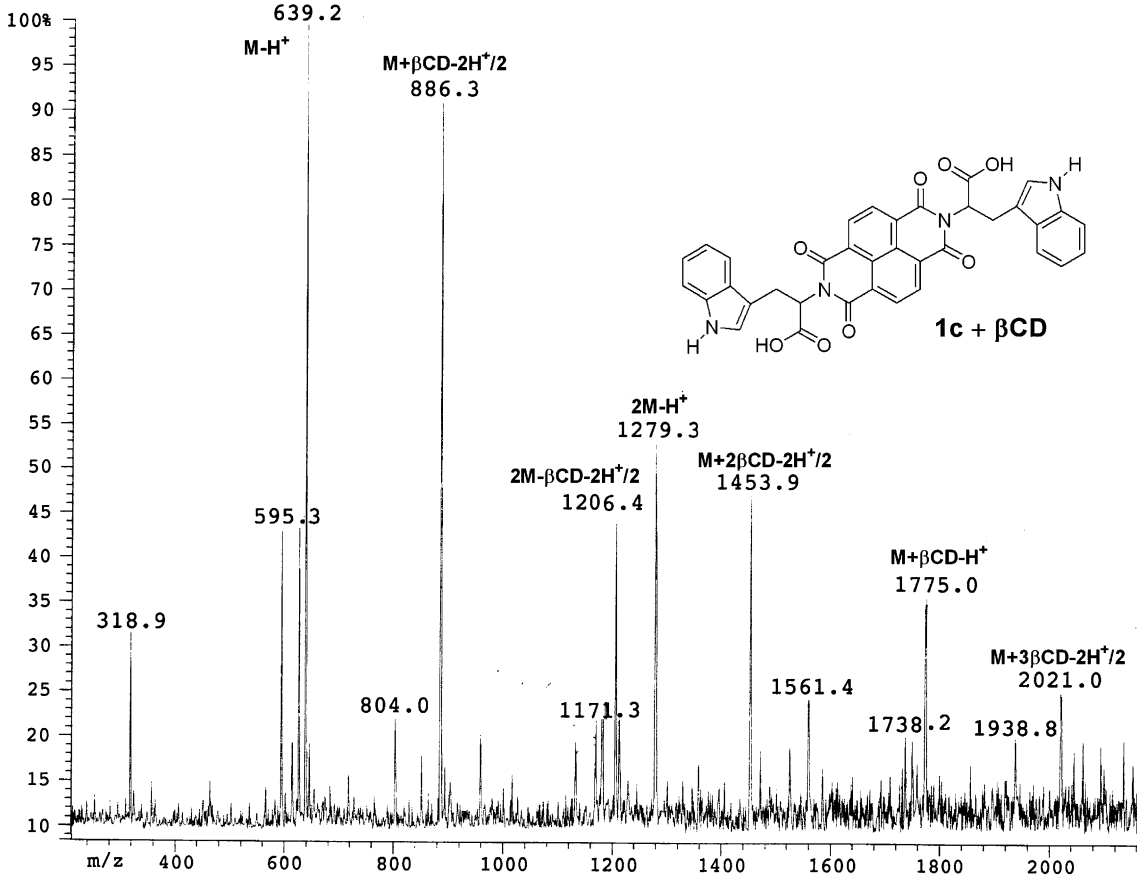


Figure 5. Negative-ion ESIMSs of **1cSS** in aqueous β-cyclodextrin solution.

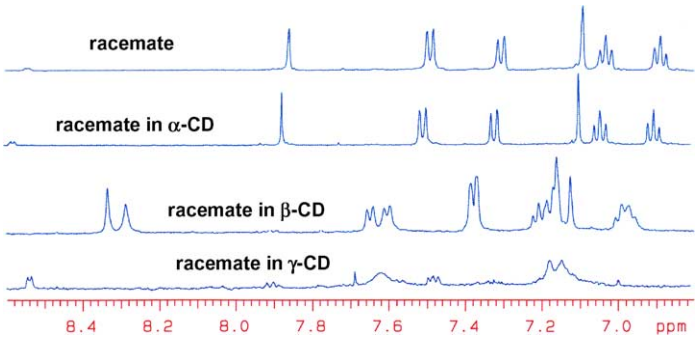


Figure 6. A portion of the NMR spectra of **1c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α-, β-, γ-CD (0.01 M).

1. Conclusions

In conclusion, there is spectroscopic evidence for the formation of molecular aggregates of **1** in aqueous solution. In cases when only one electron-deficient aromatic moiety exists, the aggregate formation occurs through carboxylic hydrogen-bonding interactions as demonstrated by ESIMS. This interaction is not sufficient for enantiomeric discrimination by NMR spectroscopy. It is necessary to form molecular aggregates through aromatic stacking interactions present in **1c**. This aggregate binds into the cyclodextrin cavity, forming strong diastereomeric complexes. As a result of this complexation, NMR spectroscopic discrimination of **1cSS** and **1cRR** is observed.

2. Experimental

2.1. General

Melting points were taken on an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were run on Varian Unity 400 and Varian INOVA 500 MHz spectrophotometer with Me_2SO-d_6 as a solvent and internal standard (2.50 and 35.91 ppm for ^1H and ^{13}C NMR, respectively). The negative-ion electrospray-ionization mass spectra (ESIMSs) were recorded on a Micromass Quattro 2 triple quadropole mass spectrometer.

2.2. (*S*)-2-[7-(1-Carboxyethyl)-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1*H*-benzo[*lmn*][3,8]phenanthrolin-2-yl]-propionic acid (1aS)

A pyridine solution (600 mL) of 1,4,5,8-naphthalenetetracarboxylic dianhydride (1.34 g; 0.005 mol) and alanine (0.89 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~10 mL, and the hot reaction mixture was added to aq HCl (300 mL water and 100 mL concd HCl). The solid precipitate that formed was separated by filtration, washed with water (3 × 20 mL) and oven dried at 100 °C for a few hours to afford 1.86 g (91%) of pure product: mp 343–345 °C. ^1H NMR (Me_2SO-d_6, 500 MHz): δ 8.69 (s, 4H naphthalene ring) 5.59 (q, 2H, *J* 6.5 Hz) 1.57 (d, 3H, *J* 7 Hz). ^{13}C NMR (Me_2SO-d_6, 500 MHz): δ 171.2 (two equivalent carbonyls of carboxylic acid) 162.1 (four equivalent carbonyls), 131.1, 126.2 (aromatic carbons), 49.2 (chiral carbon), 14.5 (methylene carbon). ESIMS (negative ion, CH_3OH): *m/z* 364.9 (65%, $M-CO_2-H^+$), 410 (100%, $M-H^+$), 818 (95%, $2M-2H^+$). Anal. Calcd for $C_{20}H_{14}N_2O_8$ (534.47): C, 58.54; H, 3.44; N, 6.83. Found: C, 57.50; H, 3.49; N, 6.70. See Supplementary Data for the NMR and EIMS spectra.

2.3. (*R*)-2-[7-(1-Carboxyethyl)-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1*H*-benzo[*lmn*][3,8]phenanthrolin-2-yl]propionic acid (1aR)

The *R* stereoisomer was prepared in 90% yield by following the procedure for preparation of the *S* isomer. The NMR spectra and ESIMSs of the *R* and *S* stereoisomers are identical.

2.4. (*RS*)-2-[7-(1-Carboxy-2-phenylethyl)-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1*H*-benzo[*lmn*][3,8]phenanthrolin-2-yl]-3-phenylpropionic acid (1bRS)

A pyridine solution (500 mL) of 1,4,5,8-naphthalenetetracarboxylic dianhydride (1.34 g; 0.005 mol) and D,L-phenylalanine (1.65 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~10 mL, and the hot reaction mixture was added to aq HCl (300 mL water and 100 concd HCl). The yellow solid precipitate that formed was separated by filtration, washed with water (3 × 20 mL) and oven dried for a few hours to afford 2.81 g (96%) of pure product: mp 352–354 °C. ^1H NMR (Me_2SO-d_6, 500 MHz): δ 8.64 (s, 4H naphthalene ring), 7.16 (d, 4H phenyl ring, *J* 7 Hz) 7.11 (t, 4H, phenyl ring *J* 7.5 Hz) 7.04 (t, 2H phenyl ring, *J* 7 Hz) 5.86 (dd, 2H, J_1 5.5 Hz; J_2 9.5 Hz) 3.59 (dd, 2H, J_1 14 Hz; J_2 5.5 Hz) 3.32 (dd, 2H, J_1 14 Hz; J_2 9.5 Hz). ^{13}C NMR (Me_2SO-d_6, 400 MHz): δ 170.3 (two

equivalent carbonyls of carboxylic acid) 162.0 (four equivalent carbonyls), 137.8, 131.3, 128.9, 128.2, 126.4, 126.0, 125.7 (aromatic carbons), 54.6 (chiral carbon), 34.3 (methylene carbon). ESIMS (negative ion, CH_3OH): *m/z* 517.2 (85%, $M-CO_2-H^+$), 562 (100%, $M-H^+$). Anal. Calcd for $C_{32}H_{22}N_2O_8$ (562.53): C, 68.32; H, 3.94; N, 4.98. Found: C, 67.70; H, 3.89; N, 4.94. See Supplementary Data for NMR and EIMS spectra.

2.5. (*S*)-2-{7-[1-Carboxy-2-(1*H*-indol-3-yl)ethyl]-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1*H*-benzo[*lmn*][3,8]phenanthrolin-2-yl}-3-(1*H*-indol-3-yl)propionic acid (1cS)

A pyridine solution (600 mL) of 1,4,5,8-naphthalenetetracarboxylic dianhydride (1.34 g; 0.005 mol) and L-tryptophan (2.04 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~10 mL, and the hot reaction mixture was added to aq HCl (300 mL water and 100 concd HCl). The black solid precipitate that formed was separated by filtration, washed with water (3 × 20 mL) and oven dried for a few hours to afford 3.1 g (97%) of pure product: mp 286–288 °C. ^{1}H NMR (Me_2SO-d_6, 500 MHz): δ 10.65 (s, 2H pyrrol ring NH) 8.60 (s, 4H naphthalene ring), 7.47 (d, 2H, *J* 8 Hz), 7.19 (d, 2H, *J* 8.5 Hz) 7.05 (2H, s, pyrrol ring) 6.93 (t, 2H, *J* 7.5 Hz) 6.80 (t, 2H, *J* 7.5 Hz) 5.86 (dd, 2H, J_1 9.5 Hz; J_2 5.5 Hz) 3.69 (dd, 2H, J_1 14.5 Hz ; J_2 5.5 Hz) 3.49 (dd, 2H, J_1 15 Hz; J_2 9 Hz). ^{13}C NMR (Me_2SO-d_6, 400 MHz): δ 170.7 (two equivalent carbonyl of carboxylic acid) 162.1 (four equivalent carbonyls), 135.9, 131.2, 127.1, 125.9, 125.7, 123.8, 120.9, 118.3, 118.0, 111.4 and 110.2 (11 aromatic carbons), 54.3 (chiral carbon), 24.2 (methylene carbon). ESIMS (negative ion, CH_3OH): *m/z* 595.8 (65%, $M-CO_2-H^+$), 640.7 (100%, $M-H^+$), Anal. Calcd for $C_{36}H_{24}N_4O_8$ (640.6): C, 65.70; H, 3.78; N, 8.75. Found: C, 65.06; H, 4.08; N, 8.38. See Supplementary Data for NMR and EIMS spectra.

2.6. (*R*)-2-{7-[1-Carboxy-2-(1*H*-indol-3-yl)ethyl]-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1*H*-benzo[*lmn*][3,8]phenanthrolin-2-yl}-3-(1*H*-indol-3-yl)propionic acid (1cR)

The stereoisomer *R* was prepared in 94% yield by following the procedure for the preparation of the *S* isomer. The NMR and ESIMS spectra of *R* and *S* stereoisomers are identical.

Acknowledgements

We thank the Louisiana Board of Regents for their financial support (LEQSF (2001-04)-RD-B-12) for this work.

Supplementary data

The data include: (a) ^{1}H NMR spectrum of compound **1aS**; (b) ^{13}C NMR spectrum of compound **1aS**; (c) negative-ion ESIMS of compound **1aS** with γ-CD; (d) ^{1}H NMR spectrum of compound **1bRS**; (e) ^{13}C NMR spectrum of compound **1bRS**; (f) negative-ion ESIMS of compound **1bRS**; (g) negative-ion ESIMS of compound **1bRS** with γ-CD; (h) ^{1}H NMR spectrum of compound **1cS**; (i) ^{13}C NMR spectrum of compound **1cS**; (j) negative-ion ESIMS of compound **1cS**. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.carres.2005.03.002.

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