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Chiral cyclohexane based fluorescent chemosensors for enantiomeric discrimination of aspartate

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Abstract

Some new chiral cyclohexyl based fluorescent anion receptors have been synthesized and their absolute configuration has been determined by using circular dichroism (CD). Complexation experiments have been carried out with several dicarboxylates, and stoichiometries and complexation constants for the corresponding complexes have been determined. The chiral discrimination ability of these ligands for chiral dicarboxylates has been studied and the best results have been obtained with TMA aspartate.

1. Introduction

Over the last years, the development of chemosensors capable of recognizing anionic species has aroused great interest.[1] However, enantioselective recognition and even more sensing of biologically relevant molecules, such as amino acids, remain a major challenge for host–guest chemists.[2] Although several receptors have been developed for chiral dicarboxylates[3] less examples of enantioselective chemosensors have been described.[4] In the design of efficient chemosensors, fluorescence is one of the most interesting properties to be used in the transduction process because it provides many advantages such as high sensitivity, rapid on-site evaluation and low cost[5] and among the fluorescent mechanisms developed in the signaling process for anion sensing, excimer/exciplex formation has been successfully used.[6] As excimer formation is strongly dependent on geometry the binding unit to be used should be carefully designed. In that sense, cyclohexane derivatives with the appropriate configuration have been successfully used in recognition and it has been established that the rigidity of this system can be used to control the complex geometry.[7] During several years we have been studying the complexing properties of ligands derived from *trans-transoid-trans*-1,2,4,5-tetrasubstituted cyclohexanes and their possible application in sensing, and we have reported the preparation of two ligands useful in the selective recognition of maleate versus fumarate anions.[8]

In our efforts to develop fluorescent chemosensors for dicarboxylates of biological interest, now we would like to report the preparation of four enantiomerically pure cyclohexane based chiral ligands (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** and their behavior in the enantiomeric discrimination of aspartate (**3**) glutamate (**4**), and tartrate (**5**) anions (Chart 1).

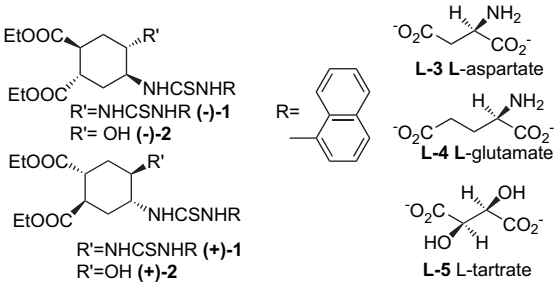

Chart 1.

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2. Results and discussion

2.1. Synthesis and stereochemistry determinations

Ligands (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** were synthesized as described in Schemes 1 and 2. Thus, compounds (−)-**9** and (+)-**9** were separately obtained from racemic *trans*-1,2-bis(ethoxycarbonyl)-4-cyclohexene oxide[9] following the procedure described by Chandrasekar et al.[10] Reaction with naphthylisothiocyanate yielded the corresponding thioureas (+)-**1** and (−)-**1**. The relative configuration of the stereocentres in addition to the main conformation of the ligands in DMSO solutions was perfectly established by NMR techniques and are showed in Chart 2.

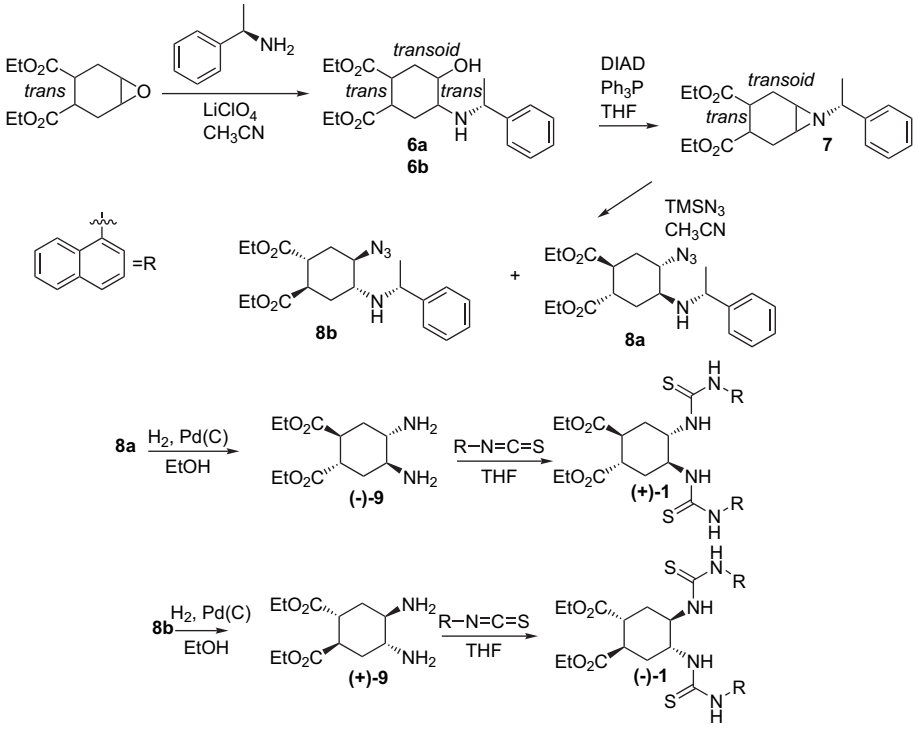
Similarly, (+)-**2** and (−)-**2** were prepared as shown in Scheme 2 from the previously separated compounds **6b** and **6a**, respectively. The relative configuration of (+)-**2** and (−)-**2** and the main conformation in DMSO solutions for these ligands were also established by using NMR techniques (Chart 2) (see Supplementary data).

The absolute configurations were determined from the CD spectra with the help of the exciton chirality rule.[11] As can be seen in Figure 1a, there is a negative exciton couplet in the spectrum of (+)-**1** (and a positive one in that of (−)-**1**) with an inflection point at the same wavelength where the maximum of the most intense band can be observed in the UV absorption spectrum. The couplet is non-symmetric similar to the case of some other *trans*-1,2-disubstituted cyclohexanes with identical chromophore substituents.[12] Quantum chemical calculations[13] were performed on the electronic spectrum of 1-naphthylthiourea, which indicated that these features arise from a pair of coupled transitions of the naphthalene moieties with transition moments close to parallel with the long axes of the naphthalene rings. (They correspond to the excitation of unsubstituted naphthalene to its 21B3u state.)[14] A conformational analysis, based on molecular mechanics calculations,[15] was carried out for (+)-**1** and led to the identification of 24 conformers whose energy was less than 3 kcal/mol higher than that of the most stable one. It was found that in all these conformers the long axes of the naphthalene units close negative angles, predicting negative couplets in their CD spectra (see Fig. 1b). Thus, the signs of the couplets in the observed spectra suggest the configurations shown in Chart 1 for (+)-**1** and (−)-**1**.

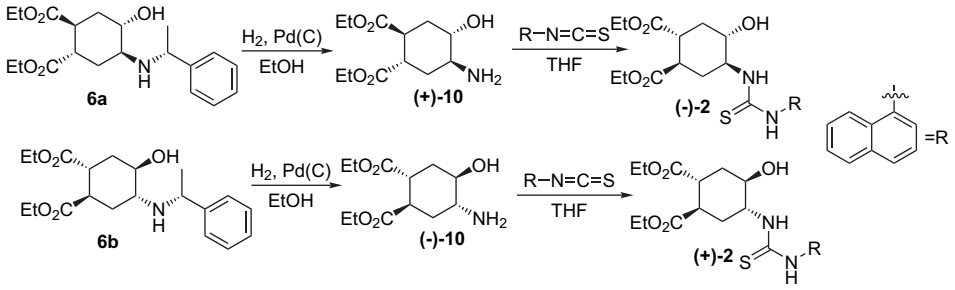
2.2. Complexation experiments

Complexation experiments with L-aspartate (L-**3**), D-aspartate (D-**3**), L-glutamate (L-**4**), D-glutamate (D-**4**), L-tartrate (L-**5**), and D-tartrate (D-**5**) all as their tetramethylammonium (TMA) salts were performed in DMSO. The stoichiometries and equilibrium constants were determined by fitting[16] the UV spectra of equilibrium mixtures (Fig. 2 for (+)-**1** and (+)-**2** with L-aspartate and for other ligands and dicarboxylates see Supplementary data) and the obtained results are summarized in Table 1.

The stoichiometry of the complexes formed by ligands (+)-**1** and (−)-**1** was 1:1 with all the studied dicarboxylates. The complexation constant values were also very similar. By contrast, ligands (+)-**2** and (−)-**2** showed a 2:1 stoichiometry in their complexes with L- and D-aspartate and L- and D-glutamate, and a 1:1 stoichiometry with L- and D-tartrate. In addition, complexes formed with L- and D-glutamate showed complexation constant values higher than complexes with L- and D-aspartate.



Scheme 1.



Scheme 2.

In order to obtain information about the structure of the formed complexes, ^{1}H NMR studies were carried out. The studies developed with (+)-**1** and (−)-**1** in the presence of 1 equiv of L- and D-aspartate and L- and D-glutamate demonstrated that both thioureas of the ligand were involved in the complexation and that a conformational change was induced in the cyclohexane moiety. In the complex, both axial and equatorial hydrogens (H_α and H_β) appear at the same value of δ (Fig. 3c), suggesting a boat-type conformation of the cyclohexyl moiety similar to the one previously observed in the complex formed between the racemic (±)-**1** and TMA maleate.[9] By contrast, when the anion present was L- or D-tartrate no conformational changes were observed after the addition of 1 equiv (Fig. 3b). In this case the 1:1 complex seems to be formed by involving only one thiourea group.

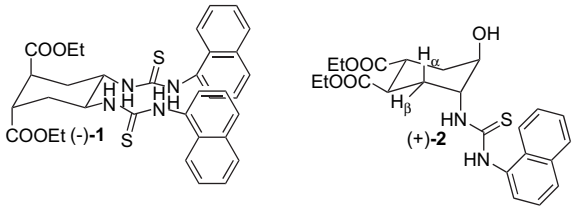


Chart 2.

By contrast, ^{1}H NMR studies carried out with (+)-**2** and (−)-**2** and L- and D-aspartate and L- and D-glutamate showed that with these ligands no conformational changes were induced in the cyclohexane moiety after complexation. This behavior was expected due to the 2:1 stoichiometry of the complexes that allow the dicarboxylate to be bound to two thiourea groups

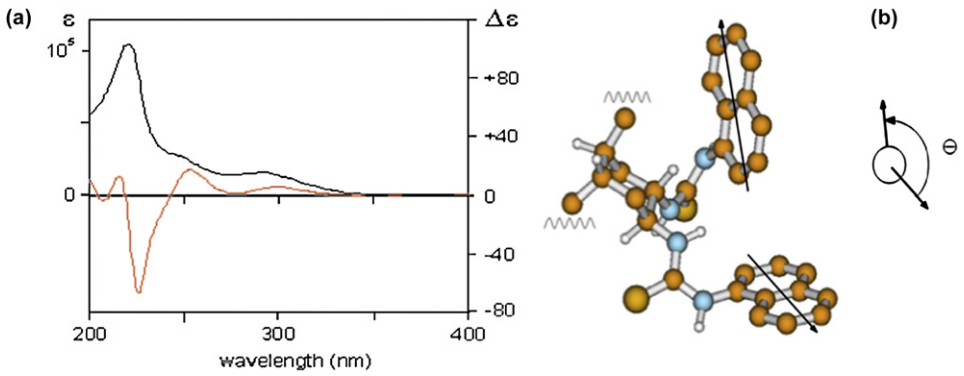


Figure 1. (a) UV absorption and CD spectra of (+)-**1**. (b) Direction of the coupling transition dipoles in the lowest energy conformation.

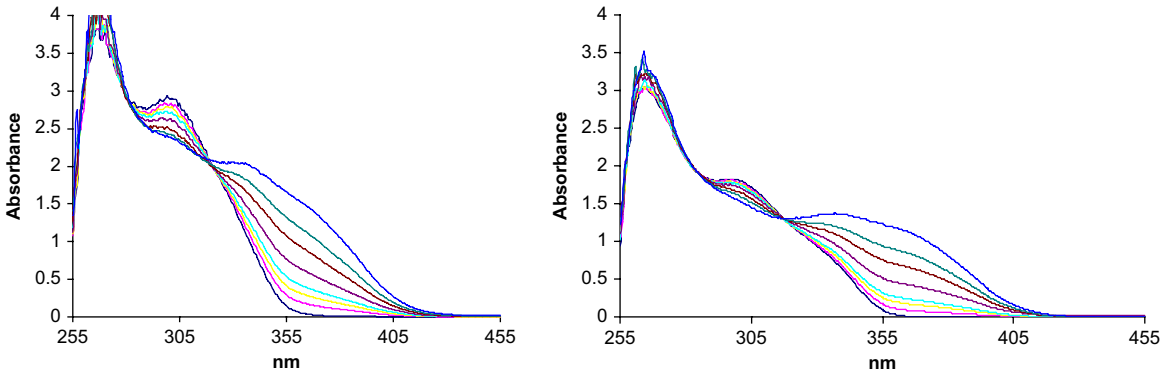


Figure 2. UV spectra in DMSO of (+)-**1** (10^{-5} M)+TMA L-aspartate (left) and (+)-**2** (10^{-5} M)+TMA L-aspartate (right).

Table 1
Equilibrium constants (log β) and stoichiometries (L:A) for the complexes formed between (+)-**1**, (−)-**1**, (+)-**2** and (−)-**2** with L- and D-aspartate, L- and D-glutamate, and L- and D-tartrate

		Host			
		(+)-**1**	(−)-**1**	(+)-**2**	(−)-**2**
Stoichio.	L-**3**	1:1	1:1	2:1	2:1
log β		3.8±0.2	3.6±0.3	3.7±0.1	3.2±0.1
Stoichio.	D-**3**	1:1	1:1	2:1	2:1
log β		3.5±0.3	3.8±0.3	3.6±0.2	3.4±0.3
Stoichio.	L-**4**	1:1	1:1	2:1	2:1
log β		3.6±0.1	3.5±0.3	6.9±0.1	6.8±0.1
Stoichio.	D-**4**	1:1	1:1	2:1	2:1
log β		3.6±0.2	3.6±0.3	7.0±0.1	7.1±0.1
Stoichio.	L-**5**	1:1	1:1	1:1	1:1
log β		3.4±0.3	4.0±0.4	3.4±0.1	3.4±0.1
Stoichio.	D-**5**	1:1	1:1	1:1	1:1
log β		4.1±0.4	3.0±0.2	3.3±04	3.3±04

UV−vis titrations in DMSO at 25 °C.

(one from each ligand) without involving the cyclohexane moiety. With these ligands, L- and D-tartrate formed complexes with 1:1 stoichiometries, which involved only one molecule of ligand. The complexation induced clear changes in the aromatic zone of the ^{1}H NMR spectra but no changes were observed in the cyclohexane signals because in this case no conformational modification is needed for the coordination process.

2.3. Enantiomeric discrimination of chiral dicarboxylates

The utility of ligands (+)-**1** and (−)-**1** and (+)-**2** and (−)-**2** for chiral discrimination was studied with the above-cited chiral dicarboxylates by using fluorescence. Thus, the fluorescence spectrum of (−)-**1** (λ_{exc}=290 nm) substantially changes upon the addition of L-**3** (see Fig. 4a). The band of the pure ligand shifts from 410 nm to longer wavelengths and a new band emerges at 495 nm. The appearance of this new band may be related to the formation of an excimer. Thus, L-**3** dianion with its (*R*) configuration perfectly fits in the complex inducing a conformational change in the ligand. This change places both naphthalene

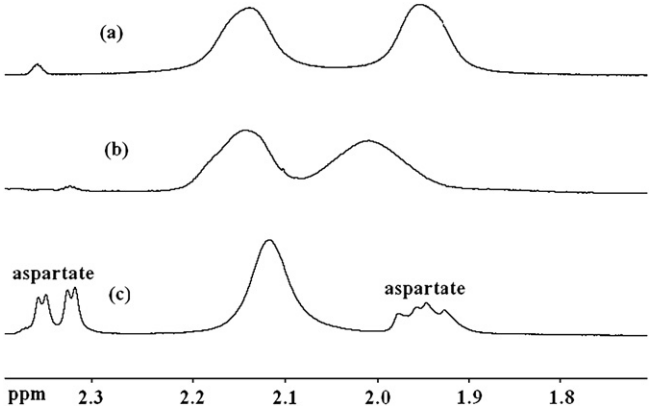


Figure 3. ^{1}H NMR spectra (cyclohexyl moiety: axial and equatorial protons) in DMSO-d_6 of (a) free ligand (+)-**1**, (b) (+)-**1**+1 equiv of TMA L-tartrate, and (c) (+)-**1**+1 equiv of TMA L-aspartate.

groups close and almost parallel giving rise to the excimer emission. The latter feature is not so strong when D-**3** is added to the solution (see Fig. 4b) due to the absolute configuration of this dicarboxylate that precludes the effective proximity of both naphthyl groups. As can be expected, the opposite behavior can be observed with ligand (+)-**1** (see Supplementary data).

A possible structure of the complex is displayed in Figure 5, which is similar to that previously described for a related complex.[8] The complex is stabilized by four hydrogen bonds formed between the hydrogens of the thiourea group and the carboxylate oxygens. This proposed structure turned out to be a local minimum in the molecular mechanics calculations.[15]

When L- and D-glutamate was added to solutions of (+)-**1** and (−)-**1**, the results obtained were very similar to those described for both enantiomers of aspartate. Thus, (+)-**1** shows excimer formation with D-glutamate and (−)-**1** with L-glutamate. However, the intensity of the excimer band was in these cases lower than with aspartate. This difference can be due to the longer chain of this dicarboxylate that precludes both naphthyl groups to be as close as in the complexes formed with aspartate. In conclusion, (+)-**1** and (−)-**1** show different fluorescence behavior depending on the absolute configuration of the amino acid. On the other hand, the effect of the length of the chain in the dicarboxylate gives rise to stronger modifications with aspartate than with glutamate.

Both L-tartrate and D-tartrate did not induce any change in the fluorescence spectra of (+)-**1** or (−)-**1**. This behavior can be related with the geometry of the 1:1 complexes formed with these anions in which only a thiourea is involved in coordination and no modification in the conformation of the cyclohexyl moiety was observed. Thus, both naphthyl groups are faraway one to the other and no excimer is observed in the fluorescence spectrum.

Whereas ligands (+)-**1** and (−)-**1** showed an enantiomeric behavior ((+)-**1** gives the stronger excimer with L-aspartate and (−)-**1** with D-aspartate), the behavior of (+)-**2** and (−)-**2** was clearly different. Thus, both (+)-**2** and (−)-**2** give the stronger modification of the fluorescence spectrum in the presence of TMA L-aspartate (the spectra for (−)-**2** are displayed in Fig. 6 and for (+)-**2** see Supplementary data). With these ligands the formed excimer should be intermolecular in agreement with the 2:1 stoichiometry showed in the complexes. This stoichiometry could also be responsible for the similar effect that the same enantiomer has with both ligands because the role played for the stereochemistry of the stereocentre in this case can be only related to steric factors.

In addition, studies carried out with L- and D-glutamate show that with both enantiomers, the ligands (+)-**2** and (−)-**2** give rise to excimer bands with practically the same intensity. The longer chain of glutamate determines that the configuration of the α carbon has small influence on the geometry of complexation.

With these ligands, the results observed with tartrate were the same as those showed by (+)-**1** and (−)-**1**, no excimer bands being observed, and this behavior can be explained by taking into account that the complexes formed have a 1:1 stoichiometry that does not induce any change in the conformation of the cyclohexyl moiety.

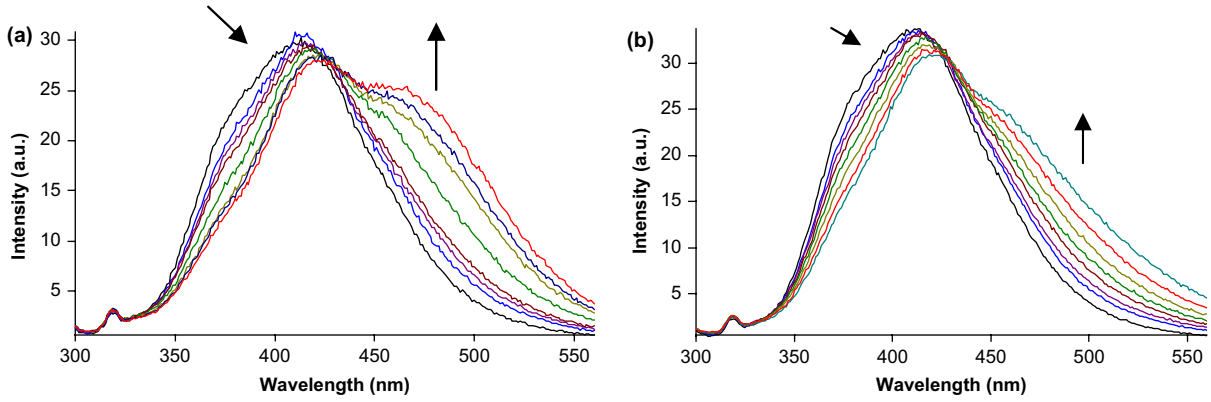


Figure 4. Fluorescence spectra in DMSO of (a) (−)-**1** (10^{-5} M)+TMA L-aspartate and (b) (−)-**1** (10^{-5} M)+TMA D-aspartate (λ_{exc}=290 nm).

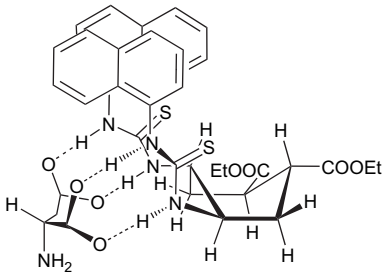


Figure 5. Structural proposal for the complex formed between ligand (−)-**1** and TMA (*R*)-aspartate.

In order to evaluate the utility of the prepared ligands in chiral recognition, an additional experiment was carried out with ligand (−)-**2**, the one which gave the stronger differences between both enantiomers of TMA aspartate. In this experiment, to a solution of 10^{-5} M of the ligand 40 equiv of L-aspartate was added and its fluorescence spectrum was registered. This patron solution was then divided into two samples and 15 equiv of L-aspartate was added to the first sample and the fluorescence spectrum was registered. As it was expected, no changes were observed because the sample was already saturated in this dicarboxylate. To the second sample, 15 equiv of D-aspartate was added. The fluorescence spectrum in this case showed a clear decrease in the band. This behavior clearly supports the utility of this patron solution for enantiomeric discrimination between L- and D-aspartate (Fig. 7).

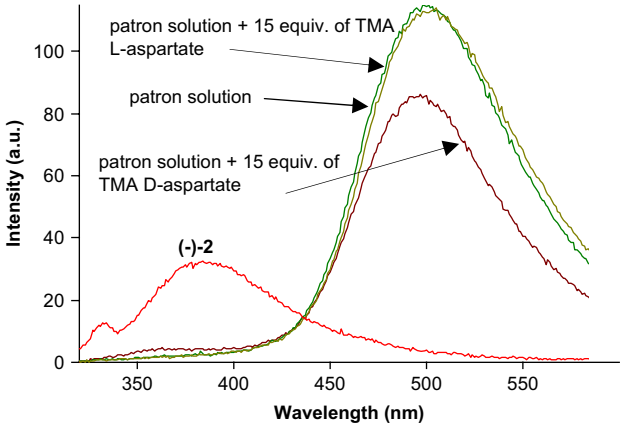


Figure 7. Enantiomeric recognition of TMA L- and D-aspartate by using a patron solution ((−)-**2**+40 equiv of TMA L-aspartate in DMSO) (λ_{exc}=290 nm).

3. Conclusions

Four new chiral ligands have been prepared and their configuration and main conformations in DMSO solutions have been established. Complexation constants and stoichiometries have been determined for L- and D-aspartate, L- and D-glutamate, and L- and D-tartrate all of them as TMA salts. Ligands containing two thiourea groups form 1:1 complexes with all the studied dicarboxylates. By contrast, ligands containing only one thiourea group show 2:1 stoichiometries except with tartrate. Solutions of

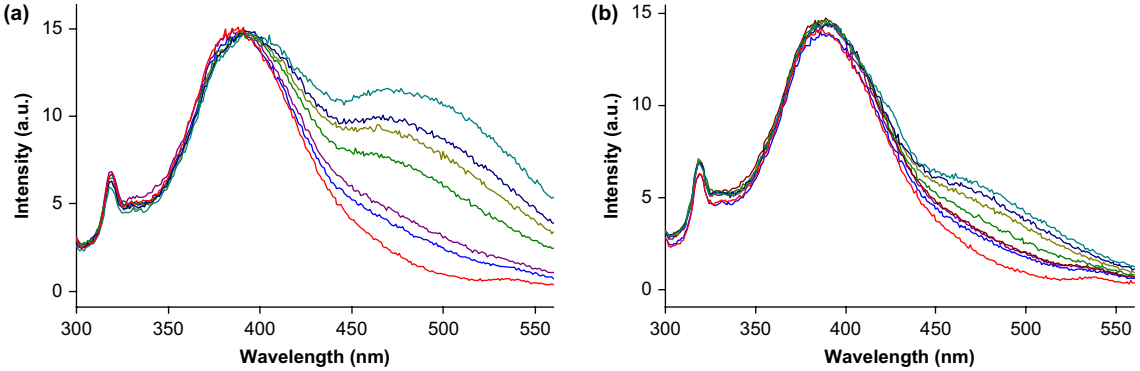


Figure 6. Fluorescence spectrum in DMSO of (−)-**2** (10^{-5} M) (a) in the presence of TMA L-aspartate and (b) TMA D-aspartate (0–3 equiv) (λ_{exc}=290 nm).

either (−)-**2** with a large excess of L-aspartate or (+)-**2** with a large excess of D-aspartate can be used for enantiomeric discrimination between L- and D-aspartate.

4. Experimental section

4.1. General procedures and materials

trans-1,2-Bis-(ethoxycarbonyl)-4-cyclohexene oxide was prepared by following the procedure described in the literature.[9] All other reagents were commercially available and were used without purification. THF was distilled from Na/benzophenone under Ar prior to use. Column chromatography was performed with silica gel 60 (230–400 mesh, Merck). Silica gel 60 F_{254} (Merck) plates were used for TLC. ^{1}H and ^{13}C NMR spectra were recorded with the deuterated solvent as the lock and residual solvent as the internal reference. High-resolution mass spectra (FAB) were recorded in the positive ion mode. UV–vis spectra were recorded using a 1 cm path length quartz cuvette. All measurements were carried out at 293 K (thermostatted). Fluorescence spectra were recorded in a Varian Cary Eclipse Fluorimeter.

4.2. trans-transoid-trans-5-((R)-1-Phenylethylamino)-1,2-bis-(ethoxycarbonyl) 4-cyclohexanol (**6a**+**6b**)

(*R*)-(+)-1-Phenylethylamine (1.63 mL, 12.59 mmol) was added dropwise to a solution of racemic *trans*-1,2-bis-(ethoxycarbonyl)-4-cyclohexene oxide (3.05 g, 12.59 mmol) and $LiClO_4$ (1.34 g, 12.59 mmol) in acetonitrile (10 mL) at 0 °C. After 18 h under reflux, the mixture was allowed to cool at room temperature. After addition of water (10 mL), it was extracted with dichloromethane. The combined organic phases were dried (anhydrous Na_2SO_4) and evaporated to yield a crude mixture of diastereoisomers that were separated by column chromatography (silica gel) to give **6a** (R_f (dichloromethane/ethyl acetate 1:1)=0.69, 1.65 g, 36%) and **6b** (R_f (dichloromethane/ethyl acetate 1:1)=0.46, 1.70 g, 37%) both as pale yellow oils. Compound **6a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.24 (m, 5H), 4.16–4.12 (dq, J_1=7.3 Hz, J_2=2.9 Hz, 4H), 3.90 (q, J=7.0 Hz, 1H), 3.48 (dt, J_1=7.7 Hz, J_2=3.7 Hz, 4H), 3.22 (q, J=7.1 Hz, 1H), 3.12 (q, J=7.1 Hz, 1H), 2.62–2.54 (m, 1H), 2.36–2.26 (m, 1H), 2.24–2.15 (m, 1H), 1.78–1.69 (m, 1H), 1.42–1.33 (m, 1H), 1.35 (d, J=7.0 Hz, 3H), 1.25 (dt, J_1=7.3 Hz, J_2=2.9 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 173.9, 146.3, 128.6, 127.2, 126.5, 69.3, 60.9, 60.9, 56.7, 55.5, 40.5, 40.1, 31.0, 29.1, 23.9, 14.3, 14.3; MS (EI) found: 363.1973, $C_{20}H_{29}NO_5$ requires: 363.2046. Compound **6b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32–7.23 (m, 5H), 4.17–3.95 (m, 5H), 3.43–3.36 (m, 1H), 3.21–3.11 (m, 2H), 2.38–2.21 (m, 3H), 1.64–1.48 (m, 2H), 1.35 (d, J=7.0 Hz, 1H), 1.27 (t, J=7.3 Hz, 1H), 1.17 (t, J=7.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 173.7, 145.3, 128.5, 127.1, 126.7, 69.9, 60.9, 60.8, 56.0, 54.7, 40.3, 40.1, 31.0, 28.0, 25.4, 14.2, 14.1; MS (EI) found: 363.1973, $C_{20}H_{29}NO_5$ requires: 363.2046.

4.3. trans-3,4-Bis-(ethoxycarbonyl)-7-((R)-1-phenylethyl)-7-aza-bicyclo[4.1.0]heptane (**7**)

DIAD (2.1 mL, 10.70 mmol) was slowly added over a mixture of the diastereoisomers **6a** and **6b** (2.71 g, 7.45 mmol), and Ph_3P (2.81 g, 10.70 mmol) in THF (23 mL) at 0 °C under Ar. The mixture was stirred for 18 h at room temperature. After evaporation of the solvent, the crude was purified by column chromatography, hexane/ethyl acetate (7:3) as eluent, to give a mixture of diastereoisomers **7** (2.50 g, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.23 (m, 5H), 4.17–4.06 (m, 4H), 2.93–2.85 (m, 1H), 2.55–2.32 (m, 3H), 2.23–2.17 (m, 1H), 1.82–1.57 (m, 4H), 1.37–1.19 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.7, 174.9, 145.3, 128.4, 128.3, 126.9, 126.8, 126.7, 72.4, 69.6, 60.9, 41.5, 39.5, 38.5, 37.7, 36.1, 35.6, 28.7, 27.6, 14.3; MS (EI) found: 345.1915, $C_{20}H_{27}NO_4$ requires: 345.1940.

4.4. trans-transoid-trans-5-((R)-1-Phenylethylamino)-1,2-bis-(ethoxycarbonyl)-4-azidocyclohexane (**8a**+**8b**)

To a solution of **7** (2.50 g, 7.24 mmol) in acetonitrile (60 mL) at 0 °C under Ar, $TMSN_3$ (1.41 mL, 10.66 mmol) was added dropwise. The mixture was stirred overnight at room temperature. Then, the excess of azide and the solvent were evaporated under low pressure to give a diastereoisomeric mixture of **8a** (R_f (dichloromethane/ethyl acetate 95:5)=0.50, 1.94 g, 69%) and **8b** (R_f (dichloromethane/ethyl acetate 95:5)=0.29, 453 mg, 16%) as colorless oils. Compound **8a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.24 (m, 5H), 4.20–4.07 (m, 4H), 3.86 (t, J=7.0 Hz, 1H), 3.69–3.64 (m, 1H), 3.00–2.98 (m, 1H), 2.70–2.65 (m, 1H), 2.22–2.16 (m, 1H), 1.95–1.88 (m, 2H), 1.58–1.50 (m, 1H), 1.37 (d, J=7.0 Hz, 3H), 1.30 (t, J=7.6 Hz, 3H), 1.23 (t, J=7.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 174.0, 145.7, 128.7, 127.3, 126.5, 72.4, 61.0, 60.5, 60.1, 56.2, 53.3, 39.7, 29.9, 27.8, 24.5, 21.8, 14.3; MS (EI) found (M+H): 389.2189, $C_{20}H_{29}N_4O_4$ requires: 389.2190. Compound **8b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35–7.21 (m, 5H), 4.19–4.02 (m, 4H), 3.87 (q, J=7.0 Hz, 1H), 3.37–3.34 (m, 1H), 3.08–2.98 (m, 2H), 2.53–2.48 (m, 1H), 2.21–2.04 (m, 2H), 1.82–1.62 (m, 2H), 1.50 (s, 1H), 1.37 (d, J=7.0 Hz, 3H), 1.26 (t, J=7.5 Hz, 3H), 1.18 (t, J=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 173.9, 145.3, 128.7, 127.2, 126.5, 69.5, 61.1, 61.0, 55.7, 53.5, 39.8, 28.6, 27.7, 25.3, 14.3, 14.2; MS (EI) found (M+H): 389.1678, $C_{20}H_{29}N_4O_4$ requires: 389.2190.

4.5. (−)-4,5-Diamino-1,2-bis-(ethoxycarbonyl)-cyclohexane ((−)-**9**)

A mixture of **8a** (1.05 g, 2.71 mmol) and a catalytic amount of Pd(C) 10% in ethanol (75 mL) was stirred for 2 days under an H_2 atmosphere (65 psi). After filtration through Celite, the solvent was evaporated to give (−)-**9** (644 mg, 92%) as a colorless wax. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.13 (q, J=7.6 Hz, 4H), 3.17 (br s, 2H), 2.54–2.51 (m, 2H), 2.20–2.16 (m, 2H), 1.58–1.50 (m, 2H), 1.35 (s, 4H), 1.25 (t, J=7.6 Hz,

6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 60.9, 53.3, 40.6, 32.6, 21.8, 14.3; $[\alpha]_D^{20}$ −5.9 (*c* 3.4, $CHCl_3$); MS (EI) found (M+H): 258.1582, $C_{12}H_{22}N_2O_4$ requires: 258.1580.

*4.6. (+)-4,5-Diamino-1,2-bis-(ethoxycarbonyl)-cyclohexane ((+)-**9**)*

A mixture of **8b** (453.4 mg, 1.17 mmol) and a catalytic amount of Pd(C) 10% in ethanol (75 mL) was stirred for 2 days under an H_2 atmosphere (65 psi). After filtration through Celite, the solvent was evaporated to give (+)-**9** (268 mg, 89%) as a colorless wax. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.15 (q, *J*=7.8 Hz, 4H), 3.18 (br s, 2H), 2.55−2.52 (m, 2H), 2.21−2.16 (m, 2H), 1.65 (s, 4H), 1.57−1.51 (m, 2H), 1.26 (t, *J*=7.8 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 60.9, 53.3, 40.6, 32.6, 21.8, 14.3; $[\alpha]_D^{20}$ +5.8 (*c* 4.1, $CHCl_3$); MS (EI) found: 258.1562, $C_{12}H_{22}N_2O_4$ requires: 258.1580.

*4.7. (+)-trans-transoid-trans-1,2-Bis-(ethoxycarbonyl)- 4,5-bis-(3-(naphtalen-1-yl)thioureid)cyclohexane ((+)-**1**)*

To a solution of (−)-**9** (644.8 mg, 2.50 mmol) in THF (20 mL) at room temperature, 1-naphthylisothiocyanate (926.3 mg, 5.00 mmol) was added and the mixture was heated under reflux for 16 h. Then the mixture was allowed to cool to room temperature and was poured over hexane (25 mL). From this mixture, (+)-**1** (1.35 g, 86%) precipitated as a white solid. (Found: C, 64.5; H, 6.0; N, 8.7; S, 9.9. $C_{34}H_{36}N_4O_4S_2$: C, 64.9; H, 5.7; N, 8.9; S, 10.2.) Mp: 122−125 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.62 (s, 2H), 7.96−7.92 (m, 8H), 7.82−7.47 (m, 8H), 4.54 (m, 2H), 4.18−4.04 (m, 4H), 3.01 (br s, 2H), 2.13−1.96 (m, 4H), 1.22 (t, *J*=7.4 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.8, 173.2, 134.7, 133.9, 129.5, 128.1, 126.1, 125.5, 124.7, 122.7, 60.5, 52.4, 28.9, 25.1, 22.1, 14.1; $[\alpha]_D^{20}$ +46.8 (*c* 1.1, acetone); MS (EI) found: 628.2227, $C_{34}H_{36}N_4O_4S_2$ requires: 628.2178.

*4.8. (−)-trans-transoid-trans-1,2-Bis-(ethoxycarbonyl)-4,5-bis-(3-(naphthalen-1-yl)thioureid)cyclohexane ((−)-**1**)*

To a solution of (+)-**9** (268.5 mg, 1.04 mmol) in THF (7 mL) at room temperature, 1-naphthylisothiocyanate (385.3 mg, 2.08 mmol) was added and the mixture was heated under reflux for 16 h. Then the mixture was allowed to cool to room temperature and was poured over hexane (25 mL). From this mixture, (−)-**1** (542.8 mg, 83%) precipitated as a white solid. (Found: C, 64.3; H, 6.1; N, 8.2; S, 9.6. $C_{34}H_{36}N_4O_4S_2$: C, 64.9; H, 5.7; N, 8.9; S, 10.2.) Mp: 119−122 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.63 (s, 2H), 7.96−7.93 (m, 8H), 7.82−7.44 (m, 8H), 4.54 (m, 2H), 4.16−4.06 (m, 4H), 3.01 (br s, 2H), 2.12−1.96 (m, 4H), 1.22 (t, *J*=7.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.6, 173.0, 134.9, 131.3, 130.1, 129.2, 128.8, 127.5, 127.0, 126.1, 125.9, 122.3, 61.5, 55.7, 40.3, 30.1, 14.3; $[\alpha]_D^{20}$ −46.7 (*c* 4.7, acetone); MS (EI) found: 628.2173, $C_{34}H_{36}N_4O_4S_2$ requires: 628.2178.

*4.9. (−)-trans-transoid-trans-5-Amino-1,2-bis-(ethoxycarbonyl)-4-cyclohexanol ((−)-**10**)*

Catalytic hydrogenation (65 psi) of **6a** (1.65 g, 4.67 mmol) in ethanol (100 mL), mediated with 10% Pd(C), led to aminoalcohol (−)-**7** (993 mg, 82%) as a colorless wax. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.14 (t, *J*=7.2 Hz, 4H), 3.42−3.35 (m, 1H), 3.22−3.08 (m, 2H), 2.74−2.67 (m, 1H), 2.26−2.13 (m, 2H), 2.04 (br s, 3H), 1.72−1.50 (m, 2H), 1.23 (t, *J*=7.2 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.0, 173.9, 71.5, 61.0, 52.6, 40.5, 40.3, 32.0, 31.2, 14.3; $[\alpha]_D^{20}$ −8.9 (*c* 3.7, $CHCl_3$); MS (EI) found (M+H): 260.1482, $C_{12}H_{22}NO_5$ requires: 260.1497.

*4.10. (+)-trans-transoid-trans-5-Amino-1,2-bis-(ethoxycarbonyl)-4-cyclohexanol ((+)-**10**)*

Catalytic hydrogenation (65 psi) of **6b** (244.9 mg, 0.67 mmol) in ethanol (30 mL), mediated with 10% Pd(C), led to aminoalcohol (+)-**7** (170 mg, 99%) as a colorless wax. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.74 (br s, 3H), 4.15 (t, *J*=7.1 Hz, 4H), 3.46−3.40 (m, 1H), 3.25−3.12 (m, 2H), 2.80−2.69 (m, 1H), 2.16−2.10 (m, 2H), 1.72−1.56 (m, 2H), 1.27 (t, *J*=7.1 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.0, 173.9, 71.6, 61.0, 52.6, 40.5, 40.3, 32.1, 31.2, 14.3; $[\alpha]_D^{20}$ +9.1 (*c* 3.4, $CHCl_3$); MS (EI) found (M+H): 260.1496, $C_{12}H_{22}NO_5$ requires: 260.1497.

*4.11. (+)-trans-transoid-trans-5-(3-(Naphthalen-1-yl)-thioureid)-1,2-bis-(ethoxycarbonyl)-4-cyclohexanol ((+)-**2**)*

To a solution of (−)-**10** (971.4 mg, 3.75 mmol) in THF (5 mL) at room temperature, 1-naphthylthioisocyanate (712.6 mg, 3.85 mmol) was added dropwise, the mixture was heated under reflux for 16 h, and then, the solvent was evaporated to give (+)-**2** (1.62 g, 97%) as a pale yellow oil. (Found: C, 62.1; H, 6.4; N, 6.3; S, 7.2. $C_{23}H_{28}N_2O_5S$: C, 62.1; H, 6.3; N, 6.3; S, 7.2.) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11−7.87 (m, 4H), 7.60−7.44 (m, 4H), 5.70 (br s, 1H), 4.52 (br s, 1H), 4.21−3.97 (m, 4H), 3.55 (br s, 1H), 3.13−2.96 (m, 2H), 2.69 (br s, 1H), 2.25−2.20 (m, 1H), 1.92−1.72 (m, 2H), 1.54−1.49 (m, 1H), 1.29−1.16 (m, 3H), 1.16 (t, *J*=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.0, 173.8, 173.0, 134.8, 129.7, 129.3, 128.8, 127.7, 127.3, 125.9, 125.2, 122.5, 70.0, 61.4, 61.1, 60.5, 56.2, 40.4, 39.9, 32.1, 28.5, 21.2, 14.3; $[\alpha]_D^{20}$ +48.0 (*c* 3.3, acetone); MS (EI) found: 444.1755, $C_{23}H_{28}N_2O_5S$ requires: 444.1719.

*4.12. (−)-trans-transoid-trans-5-(3-(Naphthalen-1-yl)-thioureid)-1,2-bis-(ethoxycarbonyl)-4-cyclohexanol ((−)-**2**)*

To a solution of (−)-**10** (1.09 g, 4.19 mmol) in THF (5 mL) at room temperature, 1-naphthylthioisocyanate (796.9 mg, 4.30 mmol) was added dropwise, the mixture was heated under reflux for 16 h, and then, the solvent was evaporated to give (+)-**2** (1.73 g, 93%) as a pale yellow oil. (Found: C, 62.3; H, 6.4; N, 6.3; S, 7.1. $C_{23}H_{28}N_2O_5S$: C, 62.1; H, 6.3; N, 6.3; S, 7.2.) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04−7.88 (m, 4H), 7.59−7.44

(m, 4H), 5.65 (br s, 1H), 4.51 (br s, 1H), 4.19–4.00 (m, 4H), 3.55 (br s, 1H), 3.14–2.92 (m, 2H), 2.70 (br s, 1H), 2.25–2.21 (m, 1H), 1.92–1.72 (m, 2H), 1.53–1.49 (m, 1H), 1.29–1.16 (m, 3H), 1.16 (t, J=7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.0, 173.8, 172.9, 134.8, 129.7, 128.8, 127.8, 127.3, 125.9, 125.2, 122.5, 70.1, 61.4, 61.1, 56.2, 40.4, 39.9, 32.1, 28.5, 31.2, 14.3; $[\alpha]_D^{20}$ −47.7 (*c* 3.4, acetone); MS (EI) found: 444.1722, $C_{23}H_{28}N_2O_5S$ requires: 444.1719.

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Supplementary data

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References and notes

1. (a) Fabbrizzi, L.; Licchelli, M.; Labaioli, G. *Coord. Chem. Rev.* **2000**, *205*, 85–108; (b) Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419–4476; (c) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486–516; (d) Gale, P. A.; García-Garrido, S. E.; Garric, J. *Chem. Soc. Rev.* **2008**, *37*, 151–190; (e) Suksai, C.; Tuntulani, T. *Chem. Soc. Rev.* **2003**, *32*, 192–202.
2. (a) James, T. D.; Samankumara Sandanayake, K. R. A.; Shinkai, S. *Nature* **1996**, *374*, 345–347; (b) Kubo, Y.; Maeda, S.; Tokita, S.; Kubo, M. *Nature* **1996**, *382*, 522–524; (c) Naemura, K.; Tobe, Y.; Kaneda, T. *Coord. Chem. Rev.* **1996**, *148*, 199–219; (d) Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313–3361; (e) Webb, T. H.; Wicox, C. S. *Chem. Soc. Rev.* **1993**, *22*, 383–395; (f) Finn, M. G. *Chirality* **2002**, *14*, 534–540.
3. See for example:. (a) Ragusa, A.; Rossi, S.; Hayes, J. M.; Stein, M.; Kilburn, J. D. *Chem.—Eur. J.* **2005**, *11*, 5674–5688; (b) Rossi, S.; Kyne, G. M.; Turner, D. L.; Wells, N. J.; Kilburn, J. D. *Angew. Chem., Int. Ed.* **2002**, *41*, 4233–4236; (c) Lee, C.-S.; Teng, P.-F.; Wong, W.-L.; Kwong, H.-L.; Chan, A. S. C. *Tetrahedron* **2005**, *61*, 7924–7930; (d) Alfonso, I.; Dietrich, B.; Rebolledo, F.; Gotor, V.; Lehn, J.-M. *Helv. Chim. Acta* **2001**, *84*, 280–295.
4. (a) Folmer, A. J.; Frantz, L.; Vincent, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 7986–7987; (b) Tsubaki, K.; Tanima, D.; Nuruzzaman, M.; Kusumoto, T.; Fuji, K.; Kawabata, T. *J. Org. Chem.* **2005**, *70*, 4609–4616; (c) Pagliari, S.; Corradini, R.; Galaverna, G.; Sforza, S.; Dossena, A.; Montalti, M.; Prodi, L.; Zaccheroni, N.; Marchelli, R. *Chem.—Eur. J.* **2004**, *10*, 2749–2758; (d) Wong, W.-L.; Huang, K.-H.; Teng, P.-F.; Lee, C.-S.; Kwong, H.-L. *Chem. Commun.* **2004**, 384–385.
5. De Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; Mc Coy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
6. (a) Nishizawa, S.; Kaneda, H.; Uchida, T.; Teramae, N. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2325–2328; (b) Nishizawa, S.; Kaneda, H.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463–9464.
7. (a) Samoshin, V. V. *Mini-Rev. Org. Chem.* **2005**, *2*, 225–235; (b) Amendola, V.; Boiocchi, M.; Esteban-Gómez, D.; Fabrizzi, L.; Monzani, E. *Org. Biomol. Chem.* **2005**, *3*, 2632–2639.
8. Costero, A. M.; Colera, M.; Gaviña, P.; Gil, S. *Chem. Commun.* **2006**, 761–763.
9. Costero, A. M.; Villarroya, J. P.; Gil, S.; Aurell, M. J.; Ramirez de Arellano, M. C. *Tetrahedron* **2002**, *58*, 6729–6734.
10. Chandrasekar, M.; Sekar, G.; Singh, V. K. *Tetrahedron Lett.* **2000**, *41*, 10079–10089.
11. Berova, N.; Nakanishi, N. *Circular Dichroism: Principles and Applications*, 2nd ed.; Berova, N., Nakanishi, N., Woody, R. W., Eds.; Wiley-VCH: New York, NY, 2000; pp 337–382.
12. (a) Kwit, M.; Gawronski, J. *Tetrahedron* **2003**, *59*, 9323–9331; (b) Boiadjiev, S. E.; Lightner, D. A. *Chirality* **2005**, *17*, 316–322.
13. The spectrum was calculated by ZINDO/S method (Hanson, L. K.; Fajer, J.; Thompson, M. A.; Zerner, M. C. *J. Am. Chem. Soc.* **1987**, *109*, 4728–4730) using Gaussian 03 program package (Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03, Revision B.01*; Gaussian: Pittsburgh, PA, 2003.).
14. Rubio, M.; Merchan, M.; Orti, E.; Roos, B. O. *Chem. Phys.* **1994**, *179*, 395–409.
15. Halgren (*J. Comput. Chem.* **1996**, *17*, 490–519) and simulated annealing algorithm were used. They were carried out by Spartan'02 package (*Spartan'02*; Wavefunction: Irvine, CA, USA, 2002).
16. *SPECFIT/32TM GLOBAL ANALYSIS SYSTEM v.3.0*; Spectrum Software Associates: Marlborough, MA, USA, 2000; www.bio-logic.info/rapid-kinetics/specfit.html.