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Enantioselective sensing of dicarboxylates. Influence of the stoichiometry of the complexes on the sensing mechanism

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ABSTRACT

Two new cyclohexane-based thiourea chiral ligands have been synthesized in their enantiomerically pure forms. Both the ability of these ligands in the complexation of chiral dicarboxylates and their sensing properties have been studied. The influence of the stoichiometry of the formed complexes on the fluorescent properties of the systems has been established. The effect of additional substitution in the cyclohexyl moiety was considered by comparing the properties of the newly prepared ligands with those of similar compounds previously described.

1. Introduction

Chirality lies in the origin of life and many chiral molecules are involved in biochemical processes. Amongst these molecules, chiral anions play a wide number of roles in different areas such as biology, medicine, and environmental chemistry. For this reason, the development of chemosensors able to detect these species is a topic that has had continuous development in recent years.[1–5] Among the different anions, carboxylates are of particular interest because they are present in amino acids as well as many other organic compounds with biological activity. For this reason, great effort has been directed toward the preparation of chemosensors containing in their structures binding sites for complexing carboxylates and more specifically dicarboxylates.[6–11] Due to the different biological activity that a pair of enantiomers can exhibit, the synthesis of enantioselective chemosensors has become an important challenge in recent years.[12–15] However, the number of publications related to enantiomeric sensing is still limited.

In our studies on dicarboxylate recognition by using cyclohexane-based naphthylthioureas, we previously reported the use of racemic compounds **1** and **2** (Chart 1) in sensing of homologous dicarboxylates.[16] These studies have demonstrated that the ethoxycarbonyl groups present in ligand **2** have a strong influence not only on the complexes stoichiometry but also on the sensing response. Thus, whereas racemic compound **2** formed complexes with a 1:1 stoichiometry with TMA succinate, racemic compound **1** gave rise to a complex with a 2:1 stoichiometry with the same anion. The observed difference seems to be related to the influence that the ethoxycarbonyl groups have on the stability of the cyclohexane moiety. Whereas in the free ligand, both ethoxycarbonyl groups are in a *trans*-diaxial arrangement giving rise to strong 1,3-diaxial repulsions, in the 1:1 complexes, the cyclohexane adopts a boat type conformation, where the ethoxycarbonyl groups are twisted. As a consequence, 1,3-diaxial steric hindrance is reduced, making the boat conformation more stable than a possible chair-like complex. This effect does not occur in the absence of the ethoxycarbonyl groups and for this reason, the 2:1 complexes in which the cyclohexane is in a typical chair conformation are formed. On the other hand, it has been established not only in homologous but also in diastereoisomeric dicarboxylates that the cyclohexane conformation in the complexes is responsible for the sensing signal. Thus, only the complexes with a boat conformation (1:1 stoichiometry) that allows both naphthyl groups to be close in the space show an excimer band in the fluorescence spectrum (see Chart 2 for maleate and fumarate).[16,17]

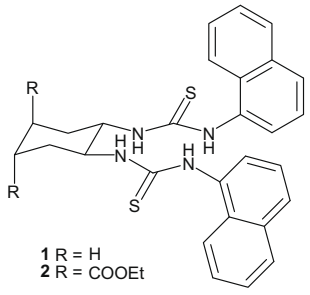

Chart 1.

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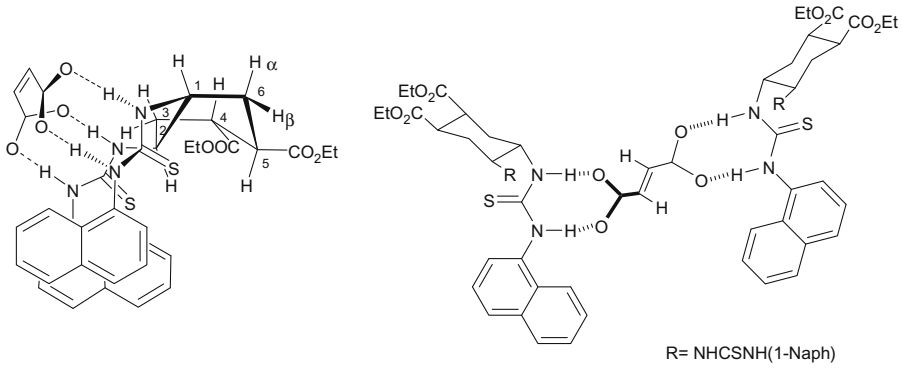

Chart 2.

Finally, studies developed with (+)-(1*R*,2*R*)-**2** and (−)-(1*S*,2*S*)-**2** have demonstrated that by following a similar mechanism, these enantiomerically pure ligands are able to work as enantiomeric sensors for D- and L-aspartate.[18] In order to explore the enantiomeric recognition of chiral cyclohexane-based thiourea ligands, we herein report the more simple enantiomerically pure cyclohexane-based naphthylthioureas (+)-(1*R*,2*R*)-**1** and (−)-(1*S*,2*S*)-**1** which can also be used in chiral sensing by means of both fluorescence and UV spectroscopies. In addition, these ligands will allow us to have additional information about the influence of the ethoxycarbonyl groups both in the stoichiometry of the complexes formed and in the sensing properties.

2. Results and discussion

The synthesis of ligands (+)-(1*R*,2*R*)-**1** and (−)-(1*S*,2*S*)-**1** was easily carried out from commercial (−)-(1*R*,2*R*)- and (+)-(1*S*,2*S*)-*trans*-1,2-diaminocyclohexane, respectively, and 1-naphthylisothiocyanate. The ^{1}H NMR studies showed that, as it was expected, the cyclohexane moiety showed a chair conformation with the thiourea substituents in equatorial positions. From a photophysical point of view, these ligands showed two bands in the UV spectra at 257 and 300 nm in DMSO solutions corresponding to the thiourea moiety and the naphthyl group, respectively.[19] On the other hand, the fluorescence spectrum consists of an emission band at 410 nm (λ_{exc} = 270 nm). These data are in agreement with those of (+)-(1*R*,2*R*)-**2** and (−)-(1*S*,2*S*)-**2** showing that the ethoxycarbonyl groups present in compounds **2** have no influence on the photophysical properties of the synthesized naphthylthiourea cyclohexane ligands.[18]

2.1. Complexation experiments

The first studied dicarboxylates were D- and L-aspartate and D- and L-glutamate all of them as TMA salts. Titration studies by using fluorescence spectroscopy allowed us to determine both the stoichiometries and the complexation constants for the anions studied (Table 1). The behavior of ligands (+)-(1*R*,2*R*)-**1** and (−)-(1*S*,2*S*)-**1** was according to the previously reported results obtained for (±)-**1,** with TMA succinate leading to complexes with 2:1 stoichiometry. These types of complexes are not expected to induce any conformational changes in the cyclohexane moiety and as a consequence, no excimers should be observed after the anion complexation. In contrast, ligands (+)-(1*R*,2*R*)-**2** and (−)-(1*S*,2*S*)-**2** form 1:1 complexes with the same anions and as a consequence the excimer band was observed as has been previously reported (Fig. 1).

In order to obtain additional information about the influence of the dicarboxylate rigidity on the stoichiometry of the complexes, both enantiomers of camphoric acid as a TMA salt **3** were studied. The choice of this dicarboxylate was based on its rigid structure that precludes both carboxylate groups to be placed close in space. The expected complex was modeled at low level molecular mechanics (PCMODEL 8.0[20]) and, as shown in Figure 2, no excimers are likely to form. Experimental data confirmed this point and all the ligands studied formed complexes with a 2:1 stoichiometry while no excimers were observed in their fluorescence spectra.

Table 1
Complexation constants and stoichiometry of the formed complexes (fluorescence, DMSO)

	Ligand	(+)-**1**	(−)-**1**	(+)-**2**	(−)-**2**
L-Aspartate	Log β	6.0 ± 0.3	6.2 ± 0.2	3.8 ± 0.2[a]	3.6 ± 0.3[a]
	Stoichio.	2:1	2:1	1:1	1:1
D-Aspartate	Log β	5.9 ± 0.2	6.5 ± 0.3	3.5 ± 0.3°	3.8 ± 0.3[a]
	Stoichio.	2:1	2:1	1:1	1:1
L-Glutamate	Log β	5.5 ± 0.2	6.2 ± 0.2	3.6 ± 0.1°	3.5 ± 0.3[a]
	Stoichio.	2:1	2:1	1:1	1:1
D-Glutamate	Log β	5.9 ± 0.2	6.3 ± 0.2	3.6 ± 0.2[a]	3.6 ± 0.1[a]
	Stoichio.	2:1	2:1	1:1	1:1
(+)-Camphorate	Log β	5.7 ± 0.1	6.2 ± 0.2	6.7 ± 0.2	6.3 ± 0.1
	Stoichio.	2:1	2:1	2:1	2:1
(−)-Camphorate	Log β	5.6 ± 0.1	6.2 ± 0.2	6.3 ± 0.1	6.3 ± 0.1
	Stoichio.	2:1	2:1	2:1	2:1

[a] These data appear in Ref. 18.

2.2. Chiral sensing

The complexation constant values shown in Table 1 for ligands (+)-(1*R*,2*R*)-**1** and (−)-(1*S*,2*S*)-**1** indicate little enantioselectivity [for example, the complexation constant between (+)-(1*S*,2*S*)-**1** and TMA (+)-camphorate was around three times higher than that calculated for TMA (−)-camphorate with the same ligand]. Even though the differences in complexation constant values are moderate, the ligands studied can be used as UV and fluorescent chemosensors. Thus, Figure 3 shows titration experiments with ligand (−)-(1*S*,2*S*)-**1** and both enantiomers of TMA camphorate. As can be seen in Figure 3, UV spectra can be used to discriminate both enantiomers.

Thus, whereas the UV spectra of the (−)-**1**-(+)-camphorate complex show very small changes than those of the corresponding free ligand, the UV spectra of the (−)-**1**-(−)-camphorate complex show an absorbance increment at around 290 nm which can be used for the identification of (−)-camphorate. As was expected, the same behavior was observed with the corresponding enantiomeric complexes (+)-1-(−)-camphorate and (+)-1-(+)-camphorate, respectively (Fig. 4).

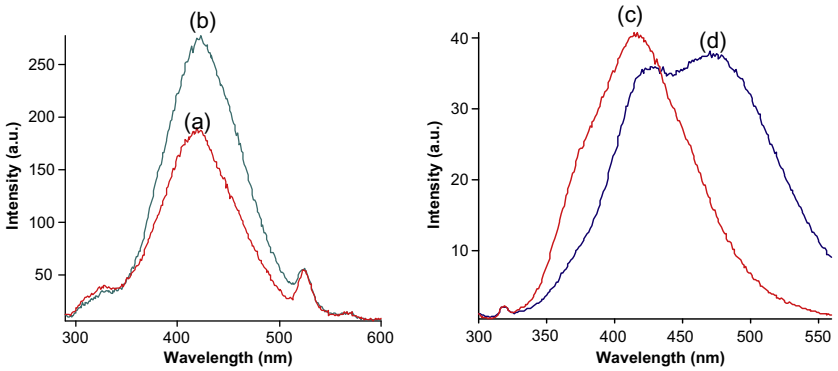

Figure 1. Fluorescence spectra of (a) (−)-**1**, (b) (−)-**1** + 3.0 equiv of TMA L-aspartate, (c) (−)-**2**, and (d) (−)-**2** + 3.0 equiv of TMA L-aspartate (ligand 10^{-5} M in DMSO).

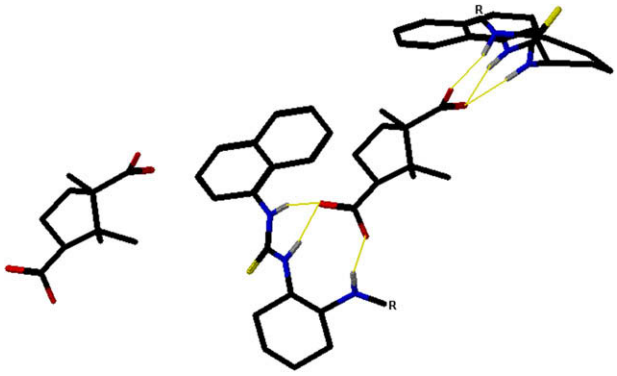

Figure 2. Structural proposal for camphorate and its 2:1 complex with ligand **1**.[20]

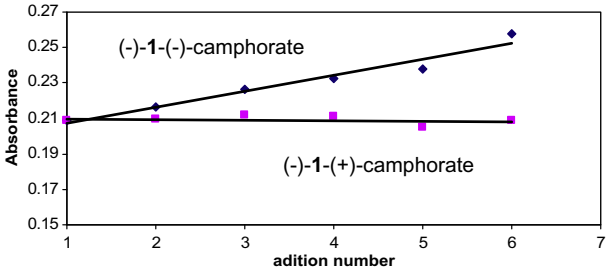

Figure 4. Absorbance variation at 290 nm with the addition of increasing amounts of TMA (+)-and (−)-camphorate to solutions of ligand (−)-**1** in DMSO.

Similar results were obtained for TMA D- and L-aspartate and TMA D- and L-glutamate, however with these dicarboxylates smaller differences between the diastereoisomeric complexes were observed. This fact suggests that the rigidity of the chiral anion is an important factor in enantiomeric sensing. The results obtained in the fluorescence studies (Fig. 4) demonstrated that this technique can also be used for discriminating both enantiomers. Whereas (+)-camphorate and ligand (−)-**1** led only to an enhancement of the fluorescence, the same ligand and (−)-camphorate led to an enhanced red-shifted emission band after complexation (Fig. 5).

These data demonstrate that ligands (+)-(1*R*,2*R*)-**1** and (−)-(1*S*,2*S*)-**1** can be used as enantiomeric sensors for both rigid and flexible dicarboxylates. However, (+)-(1*R*,2*R*)-**2** and (−)-(1*S*,2*S*)-**2** are only adequate for flexible dicarboxylates through a different mechanism. The latter compounds showed excimer formation as the sensing mechanism whereas the former gives rise to a red shift in the emission band with only one of the enantiomers.

3. Conclusions

Two new cyclohexane-based naphthylthiourea chiral ligands (+)-(1*R*,2*R*)-**1** and (−)-(1*S*,2*S*)-**1** have been prepared. These ligands can be used as enantioselective sensors for different dicarboxylates by UV and fluorescence spectroscopies. Comparative studies carried out with these ligands and compounds (+)-(1*R*,2*R*)-**2** and (−)-(1*S*,2*S*)-**2** demonstrated that the presence of the ethoxycarbonyl groups in these latter compounds is essential to give rise to complexes with a 1:1 stoichiometry. In addition, complex stoichiometry determines the fluorescent properties of the complex. Thus, whereas 1:1 complexes show excimer bands in the fluorescence spectra, this type of emission is not present with 2:1 stoichiometries. Finally, dicarboxylate rigidity makes the enantiomeric sensing more selective.

4. Experimental

4.1. General procedures and materials

All other reagents were commercially available, and used without purification. THF was distilled from Na/benzophenone under

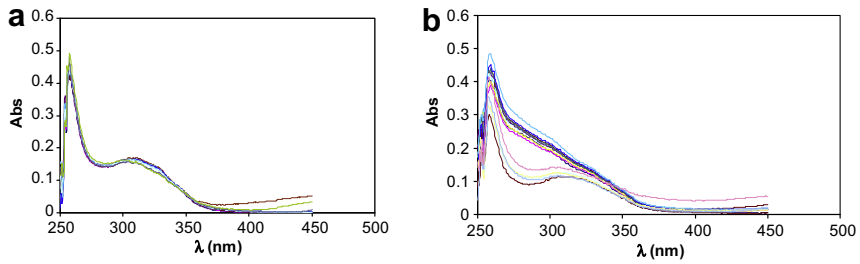

Figure 3. UV titration experiments of (−)-(1*S*,2*S*)-**1** with (a) TMA (+)-camphorate and (b) TMA (−)-camphorate (10^{-5} M in DMSO).

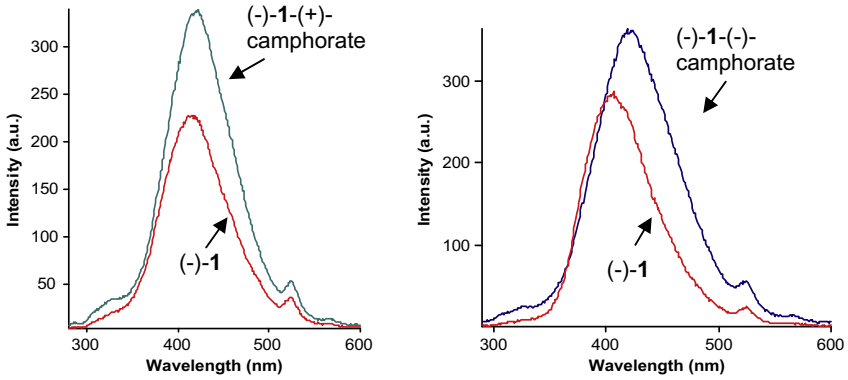

Figure 5. Fluorescence spectra of free ligand (−)-**1** and the diastereoisomeric complexes with camphorate (ligand solution 10^{-5} M in DMSO, 4 equiv of TMA anions).

Ar prior to use. Silica Gel 60 F254 (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 on a VG-AutoSpec. UV–vis spectra were recorded using a 1 cm path length quartz cuvette. All measurements were carried out at 293 K (thermostated). Fluorescence spectra were carried out in a Varian Cary Eclipse Fluorimeter. The TMA salts were obtained from the corresponding acid and TMA hydroxide.

4.1.1. (1*S*,2*S*)-1,2-Bis-(3-(naphthalen-1-yl)thioureido)cyclohexane (−)-(1*S*,2*S*)-1

1-Naphthylisothiocyanate (0.826 g, 4.46 mmol) was added dropwise to a solution of (1*S*,2*S*)-1,2-diaminocyclohexane (0.27 mL, 2.23 mmol) in THF (15 mL) and the resulting solution was refluxed for 16 h. The mixture was then allowed to cool to room temperature and was poured into hexane (25 mL), yielding (−)-**1** as a white precipitate (84% yield); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.67 (s, 2H, NH), 7.95 (d, J = 8.9 Hz, 2H), 7.90 (s, 2H), 7.82 (d, J = 7.8 Hz, 2H), 7.65 (s, 2H, NH), 7.53 (m, 4H), 7.45 (m, 4H), 4.25 (m, 2H), 4.20 (m, 2H), 1.65 (m, 2H), 1.25-1.21 (m, 4H); ^{13}C RMN (75 MHz, DMSO-d_6): δ 181.8, 134.7, 130.1, 128.5, 126.9, 126.7, 126.6, 126.1, 125.4, 123.3, 57.9, 32.1, 25,5; EI-HRMS calcd for $C_{28}H_{28}N_4S_2$: 484.17554; found: 484.17555; $[\alpha]_D^{20} = -0.39$ (c 0.019 M, DMSO).

4.1.2. (1*R*,2*R*)-1,2-Bis-(3-(naphthalen-1-yl)thioureido)cyclohexane (+)-(1*R*,2*R*)-1

This was obtained following the same procedure using (1*R*,2*R*)-1,2-diaminocyclohexane as a starting material. EI-HRMS calcd for $C_{28}H_{28}N_4S_2$: 484.17554; found: 484.17560; $[\alpha]_D^{20} = +0.40$ (c 0.022 M, DMSO).

4.2. Binding studies

Binding constants of ligands **1** and **2** toward tetramethylammonium dicarboxylates were evaluated by UV–vis titrations in DMSO. Typically, 10^{-5} M solutions of the receptors in DMSO (3 mL) were titrated by adding 2 μL aliquots of the envisaged carboxylates (as their TMA salts) in DMSO and registering the UV–vis spectrum after each addition. Log β was calculated by fitting all spectrophotometric titration curves with the SPECFIT program.[21]

Acknowledgments

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