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Fluorescent chemosensors based on cyclohexane: selective sensing of succinate and malonate versus their longer or shorter homologues

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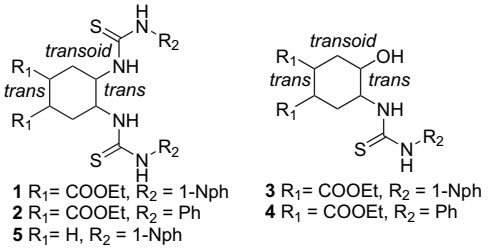
ABSTRACT

The sensing properties towards aliphatic α,ω-dicarboxylates of five cyclohexane-based ligands are described. The studied ligands have been designed following the binding site-signalling unit approach and they all possess thiourea groups as recognition moieties. Ligands **1** and **3** containing naphthalene units can be used as fluorescent sensors as they form intra- or intermolecular excimers in the presence of appropriate dicarboxylates. Selective sensing of succinate and malonate versus their longer or shorter homologues has been observed.

1. Introduction

Over the last years, the development of chemosensors capable of recognizing anionic species has aroused great interest.[1] Among the organic anions, dicarboxylates are very attractive targets because of their biological importance. For this reason, a large effort has been directed towards the preparation of chemosensors containing in their structures binding sites for complexing dicarboxylates.[2] Among the different types of signalling units used in the design of chemosensors, those based on light (fluorescent or chromogenic systems) have demonstrated to be the most interesting due to their high sensitivity and ease of manipulation. During the last years we have been interested in the use of cyclohexane systems to design fluorescent chemosensors.[3] Thus, we have previously reported on the diastereoselective recognition and sensing of maleate versus fumarate anions by compound **1** (Chart 1).[3a] The sensing properties of **1** are mainly related to the conformational change that complexation induces in the cyclohexane moiety, which upon irradiation gives rise to the formation of an excimer involving both naphthalene units. Following the same idea we now report the synthesis of ligands **1**–**4** and their use in the selective recognition and sensing of several α,ω-dicarboxylates. On the other hand, in order to evaluate the influence of the ester groups on the anion complexation, ligand **5** was also prepared.



1 R_1= COOEt, R_2 = 1-Nph
2 R_1= COOEt, R_2 = Ph
5 R_1= H, R_2 = 1-Nph

3 R_1= COOEt, R_2 = 1-Nph
4 R_1 = COOEt, R_2 = Ph

Chart 1.

2. Results and discussion

2.1. Synthesis and conformational studies

Ligands **1** and **2** were prepared as shown in Scheme 1. Cyclohexene oxide **6**[4] was opened with benzylamine in the presence of $LiClO_4$ as a catalyst to give the racemic *trans-transoid-trans* aminoalcohol **7**. This compound was transformed into the aziridine **8** by reaction with triphenylphosphine and DIAD through a Mitsunobu reaction. Ring opening with trimethylsilylazide ($TMSN_3$) in acetonitrile[5] gave rise to compound (±)-**9**, which was converted into *trans*-diamine **10** by reaction with hydrogen and Pd(C). From this compound, both **1** and **2** were easily obtained by reaction with the corresponding isothiocyanate.

Compounds **3** and **4** were synthesized following the pathway indicated in Scheme 2. Reduction of compound **7** gave rise to aminoalcohol **11**, which was converted into (±)-**3** and (±)-**4** by reaction with the corresponding isothiocyanates. Finally, **5**

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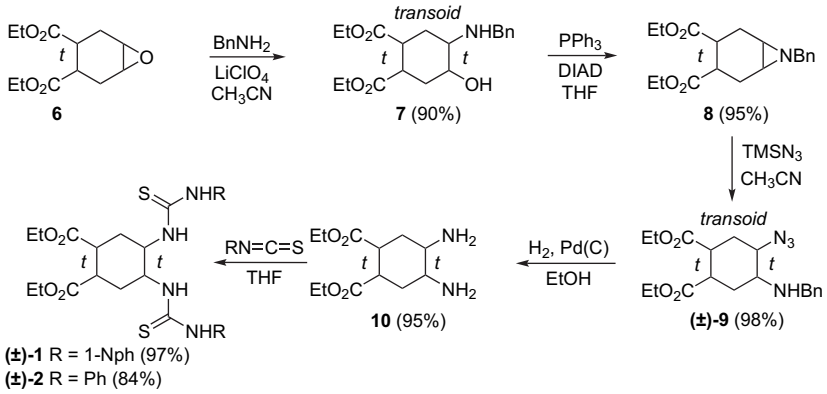
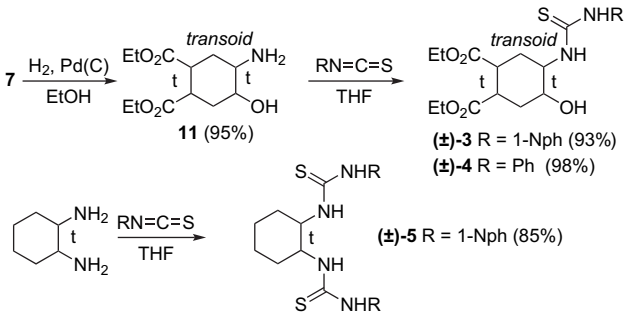


Scheme 1.

was directly prepared in 85% yield from commercial (±)-*trans*-1,2-diaminocyclohexane, by reaction with 2 equiv of 1-naphthylisothiocyanate.



Scheme 2.

The preferred conformation of these ligands in DMSO solution was unambiguously established by ^{1}H NMR techniques.[3] As shown in Figure 1 ligands **1**–**4** present the cyclohexane moiety mainly in a chair conformation, while for ligands **1** and **2** both ester groups are in the axial positions and both thiourea groups in the corresponding diequatorial disposition, for ligands **3** and **4** the opposite situation applies. In addition, modellization studies carried out with ligand **1** and **3** by using PcModel 8.0[6] showed that these conformations correspond to a relative minimum of energy. As it was expected **5** was found to be in a chair conformation with both substituents in equatorial positions.

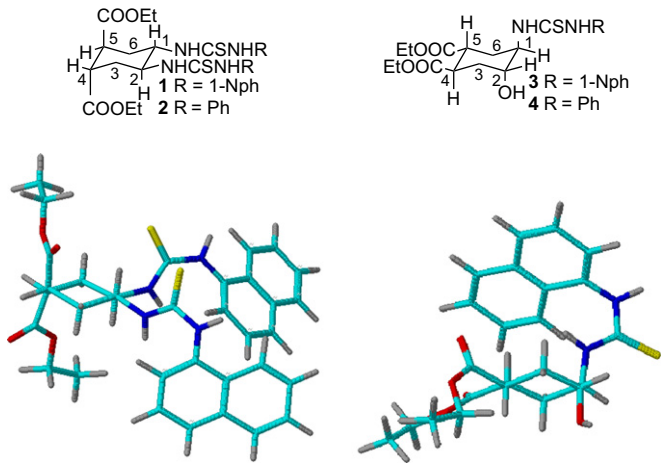


Figure 1. Up: main conformation of ligands **1**–**4** in DMSO solutions. Bottom: chair conformations for ligands **1** and **3** calculated by using PcModel 8.0.

The UV spectra of ligands **1** and **3** showed an absorption band at λ_{max}=270 and 267 nm, respectively, and a broad shoulder at λ=300 nm. By contrast, ligands **2**, **4** and **5** showed only a band at λ_{max}=272, 274 and 271 nm, respectively. In addition, ligands **1**, **3** and **5** presented fluorescent properties with an emission band around 400 nm (λ_{exc}=290 nm).

2.2. Binding studies

Complexation experiments with aliphatic α,ω-dicarboxylates of different chain lengths (oxalate (**DC2**), malonate (**DC3**), succinate (**DC4**), glutarate (**DC5**) and adipate (**DC6**) all of them as their TMA salts) were carried out in DMSO using fluorescence and UV spectroscopies. Tritiation experiments gave information about the stoichiometry of the complexes as well as the complexation constant values. All these results are summarized in Table 1.

Ligands **1** and **2** have in their structure two thiourea groups and for this reason the 1:1 stoichiometry of the complexes formed with **DC3**–**DC6** can be easily explained by considering that each thiourea group of the ligand is involved in the complexation of one carboxylate of the studied guest (Chart 2a). By contrast **DC2** formed complexes with a 1:2 stoichiometry. In this case, the small size and rigidity of oxalate preclude the coordination of both carboxylate groups by both thioureas because the coordination of the first carboxylate places the second one faraway from the second thiourea group and thus the 1:2 complex becomes the more stable possibility (Chart 2b).

Even though the values of the complexation constants between ligand **1** and **DC3**–**DC6** were similar, the corresponding complexes showed a clearly different behaviour in the UV and fluorescence spectra. Figure 2 shows the UV spectrum of free ligand **1** in DMSO (10^{-5} M) and the spectra of the same solution after addition of 3.0 equiv of **DC3**–**DC6** all of them as their TMA salts. As can be seen,

Table 1
Stoichiometry and log β (determined by using the SPECFIT program[7]) for ligands **1**–**5** with TMA salts of oxalate (**DC2**), malonate (**DC3**), succinate (**DC4**), glutarate (**DC5**) and adipate (**DC6**) in DMSO by UV–vis spectroscopy

Ligand		DC2	DC3	DC4	DC5	DC6
1	log β	4.9±0.2	2.8±0.2	3.41±0.01	3.3±0.2	3.86±0.03
	L:DC	1:2	1:1	1:1	1:1	1:1
2	log β	3.66±0.03	3.8±0.4	—	—	3.1±0.4
	L:DC	1:2	1:1	—	—	1:1
3	log β	6.9±0.1	5.8±0.2	5.9±0.1	6.4±0.2	6.84±0.02
	L:DC	2:1	2:1	2:1	2:1	2:1
4	log β	3.19±0.07	5.41±0.09	6.9±0.1	6.9±0.1	6.8±0.2
	L:DC	2:1	2:1	2:1	2:1	2:1
5	log β	—	11.1±0.1	8.1±0.5	—	9.4±0.3
	L:DC	—	2:1	2:1	—	2:1

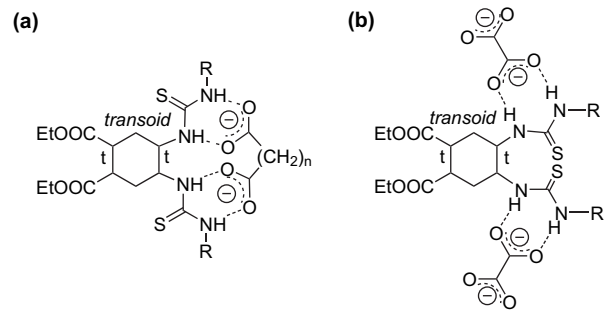


Chart 2.

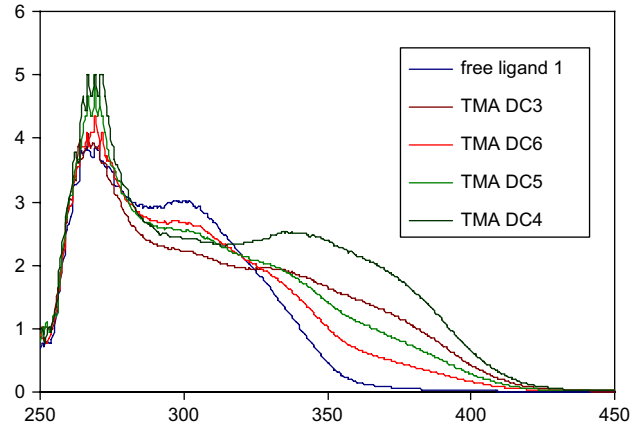


Figure 2. UV–vis spectra in DMSO of (a) ligand **1** (10^{-5} M), (b) ligand **1**+3 equiv **DC6**, (c) ligand **1**+3 equiv **DC5**, (d) ligand **1**+3 equiv **DC4** and (e) ligand 1+3 equiv **DC3**.

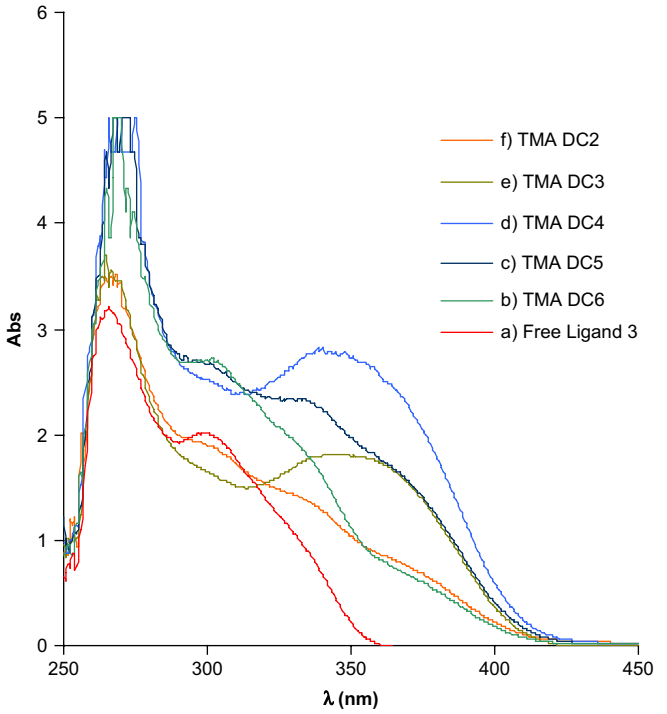


Figure 3. UV–vis spectra in DMSO (ligand concentration 10^{-5} M) of (a) ligand **3**, (b) ligand **3**+3.0 equiv of TMA **DC6**, (c) ligand **3**+3.0 equiv of TMA **DC5**, (d) ligand **3**+3.0 equiv of TMA **DC4**.

the same effect is observed with the three dicarboxylates, a new absorption band emerges at 335 nm, but the stronger effect is observed with TMA succinate (TMA **DC4**).

Compounds **3** and **4**, both possessing only one thiourea group lead in all cases (see Table 1) to a 2:1 stoichiometry on UV titration with the aliphatic α,ω-dicarboxylates **DC2**–**DC6** in DMSO solutions. As can be seen in Table 1, complexation constant values are high, except for **4** with oxalate, and only small changes are observed depending on the chain length. Following the trend observed for compound **1**, a new band (ca. 350 nm) appears in the UV spectra of compound **3** on complexation with dicarboxylates whose intensity depends on the length of the chain (Fig. 3) reaching a maximum for **DC4**.

More interesting differences were observed in their fluorescence spectra. Studies carried out with ligand **1** demonstrated that in the presence of TMA adipate and glutarate (**DC6** and **DC5**) no modification was observed in the emission band (Fig. 4a for TMA **DC5**). By contrast in the presence of succinate (**DC4**) and malonate (**DC3**) a new band appears around 490 nm (Fig. 4b for **DC4**). The intensity of this new band could be related to the formation of an excimer species induced by the complexation, as observed in previous work.[3]

The stoichiometry of the complexes with these four dicarboxylates is the same (1:1) but the excimer band is only observed with succinate and malonate, which suggests that the length of the aliphatic chain in the dianion is very important and for values higher than four carbon atoms both naphthyl groups are placed in the complex too far one from the other to allow the excimer formation. As it was expected, TMA oxalate with the 1:2 stoichiometry only induces a small enhancement of the ligand fluorescence (see Supplementary data). Ligand **2** is poorly fluorescent and for this reason its utility as chemosensor is very limited.

Studies carried out with ligand **3** demonstrated that in the presence of TMA adipate and glutarate (TMA **DC6** and **DC5**) no modification was observed in the band of emission (Fig. 5a for TMA **DC5**). By contrast in the presence of TMA malonate and succinate (**DC3** and **DC4**) a new band appears at 495 nm (Fig. 5b). The wavelength of this new band correlates with that assigned above for the intramolecular excimer formed on complexation of **1** with **DC4**. However, only one thiourea group is present in compound **3** and thus, an *intermolecular* excimer is postulated (Chart 3) in this case. It is worth noting that, as above, TMA malonate and even better succinate have an appropriate chain length to place both naphthalene moieties at the correct distance to give rise to the excimer band.

Studies carried out with ligand **5** showed that the presence of the two ethoxycarbonyl groups is essential for ligand **1** to act as a chemosensor. Thus, ligand **5** formed a completely different type of complexes than ligand **1**. In this case complexes with a 2:1 stoichiometry were observed, which did not exhibit any excimer band in their fluorescence spectra. Only a very small shift in the emission band of the ligand was observed (see Supplementary data). This fluorescent behaviour precludes the use of ligand **5** in sensing experiments.

Two-dimensional NMR studies were carried out to have information about the conformation of the complexes formed by ligand **1** with TMA **DC4**, **DC5** and **DC6** in DMSO solutions, and the results agree with the proposed geometries obtained by modellization using PcModel 8.0. Thus, in samples prepared with ligand **1** (1 equiv) and tetramethylammonium succinate (**DC4**) (1.5 equiv) it was observed that the cyclohexyl moiety is far from the chair conformation and a twisted-boat or even a boat conformation agrees better with the observed results. Thus, COSY experiments show a strong correlation between H6α and H6β; H6α also exhibits a clear correlation with H5 whereas coupling with H1 is weaker. H6β also exhibits a weak correlation with H5. In addition, NOESY experiments show that H6α correlates with H1 and H4 whereas H6β only exhibits a weak correlation with H5. The signals corresponding to H2 and H5 are too close to allow observation of the NOE correlation. With all this information a structural proposal like this showed in Chart 4 can be acceptable. This structural proposal

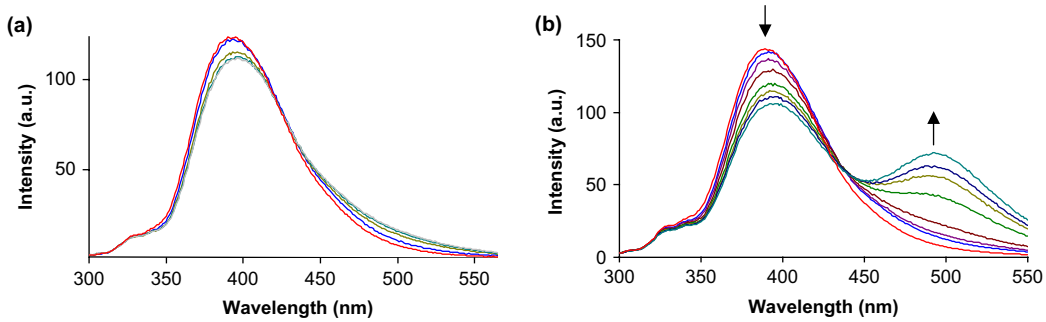


Figure 4. Fluorescence spectra (in DMSO 10^{-5} M, λ_{exc}=290 nm) of (a) ligand **1**+increasing amounts of TMA **DC5**, (b) ligand **1**+increasing amounts of TMA **DC4**.

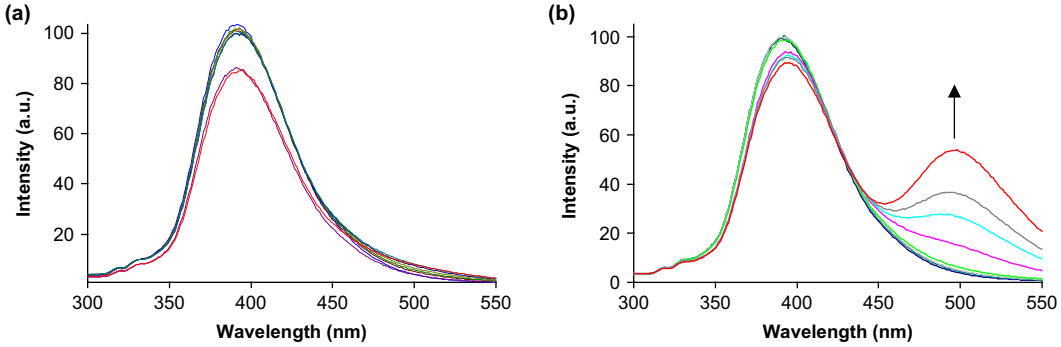


Figure 5. Fluorescence spectra of (a) ligand **3**+increasing amounts of TMA **DC5**, (b) ligand **3**+increasing amounts of TMA **DC4** (in DMSO 10^{-5} M, λ_{exc}=290 nm).

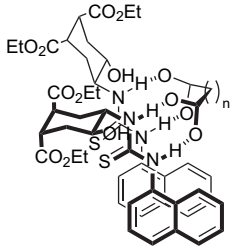


Chart 3.

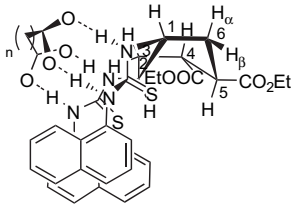


Chart 4.

also would explain the fluorescent properties observed with this anion because in this complex both aromatic systems lie almost parallel allowing the excimer formation.

3. Conclusions

Five ligands able to complex dicarboxylates have been prepared and their utility in sensing has been evaluated. The results obtained in comparative experiments demonstrate that the ethoxycarbonyl groups in the cyclohexane moiety have a strong influence on the complexes' stoichiometries and in consequence on the sensing properties. On the other hand, ligands **1** and **3** can be used in selective sensing of α,ω-dicarboxylates. The excimer band that only appears in the presence of TMA succinate and malonate allows to differentiate these dicarboxylates from their shorter (oxalate) or longer (glutarate and adipate) homologues.

4. Experimental section

4.1. General procedures and materials

trans-1,2-Bis(ethoxycarbonyl)-4-cyclohexene oxide (**6**) was prepared following the procedure described in the literature.[4] 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 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 (thermostatted). Fluorescence spectra were carried out in a Varian Cary Eclipse Fluorimeter.

*4.1.1. trans-transoid-trans-5-(Benzylamino)-1,2-bis(ethoxycarbonyl)-4-hydroxycyclohexane (**7**)*

Benzylamine (0.46 mL, 4.2 mmol) was added dropwise to a solution of **6** (1.02 g, 4.2 mmol) and $LiClO_4$ (0.451 g, 4.2 mmol) in CH_3CN (10 mL) at 0 °C. After 18 h under reflux, the mixture was allowed to cool at room temperature. Water (10 mL) was added and the mixture was extracted with dichloromethane. The combined organic phases were dried over Na_2SO_4. Solvent was evaporated and the resulting crude was subjected to column chromatography on silica gel (hexane/ethyl acetate) to obtain **7** as a yellow oil

(1.32 g, 90%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33–7.25 (m, 5H), 4.15 (q, J=7.2 Hz, 4H), 3.93 (d, J=11.5 Hz, 1H), 3.72 (d, J=11.5 Hz, 1H), 3.51–3.49 (m, 1H), 3.26–3.12 (m, 2H), 2.56–2.50 (m, 1H), 2.41–2.24 (m, 2H), 1.78–1.65 (m, 1H), 1.60–1.50 (m, 1H), 1.26 (t, J=7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.3, 174.1, 128.9, 128.6, 127.6, 69.8, 61.3, 60.8, 58.7, 51.3, 40.6, 40.5, 31.5, 28.6, 21.5, 14.6.

4.1.2. *N-Benzyl-trans-3,4-bis(ethoxycarbonyl)-7-azabicyclo[4.1.0]heptane (**8**)*

DIAD (1.9 mL, 9.7 mmol) was slowly added under argon to a stirred solution of **7** (2.35 g, 6.72 mmol) and Ph_3P (2.54 g, 9.7 mmol) in THF (23 mL) at 0 °C, and the mixture was stirred at room temperature for 18 h. Removal of the organic solvent followed by column chromatography of the residual oil (hexane/ethyl acetate as eluent) led to **8** as colourless oil (2.25 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34–7.24 (m, 5H), 4.16–4.12 (m, 4H), 3.49 (d, J=13.8 Hz, 1H), 3.42 (d, J=13.8 Hz, 1H), 2.83 (dt, J_1=11.5 Hz, J_2=8.2 Hz, 1H), 2.53–2.28 (m, 3H), 1.91–1.66 (m, 4H), 1.26–1.23 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.8, 175.0, 139.8, 128.7, 127.9, 127.2, 64.2, 60.9, 47.7, 39.6, 38.9, 36.6, 28.3, 27.7, 14.6, 14.5.

4.1.3. *trans-transoid-trans-5-(Benzylamino)-1,2-bis(ethoxycarbonyl)-4-azidocyclohexane (**9**)*

$TMSN_3$ (0.48 mL, 3.65 mmol) was added dropwise to a 0 °C solution of **8** (0.856 g, 2.45 mmol) in CH_3CN (20 mL) under argon, and the resulting mixture was stirred overnight at room temperature. Then, the excess of azide and the solvent were evaporated under low pressure to give **9** as colourless oil (0.90 g, 98%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34–7.22 (m, 5H), 4.17 (m, 4H), 3.84 (d, J=12.7 Hz, 1H), 3.73 (d, J=12.7 Hz, 1H), 3.65–3.58 (m, 1H), 3.12–3.00 (m, 2H), 2.79–2.76 (m, 1H), 2.26–2.05 (m, 2H), 1.92–1.86 (m, 1H), 1.82–1.70 (m, 1H), 1.50 (br s, 1H), 1.26–1.24 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.6, 137.2, 128.6, 128.0, 126.9, 64.3, 60.4, 53.4, 49.9, 38.0, 37.9, 24.6, 14.2.

4.1.4. *trans-transoid-trans-4,5-Diamino-1,2-bis(ethoxycarbonyl)cyclohexane (**10**)*

A mixture of **9** (0.900 g, 2.40 mmol) and a catalytic amount of 10% Pd(C) in ethanol (150 mL) was stirred for 2 days under an H_2 atmosphere (56 psi). The mixture was then filtered through Celite and the solvent evaporated to afford diamine **10** as colourless oil (0.589 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.17 (q, J=7.1 Hz, 4H), 3.48 (br s, 4H), 3.22 (br s, 2H), 2.86–2.84 (m, 2H), 2.36–2.31 (m, 2H), 1.69–1.66 (m, 2H), 1.27 (t, J=7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.6, 173.3, 61.2, 52.1, 31.3, 21.6, 14.2; EI-HRMS calcd for $C_{12}H_{22}N_2O_4$: 258.1580; found: 258.1556.

4.1.5. *trans-transoid-trans-1,2-Bis(ethoxycarbonyl)-4,5-bis-(3-(naphthalen-1-yl)thioureido)cyclohexane (**1**)*

1-Naphthylisothiocyanate (0.826 g, 4.46 mmol) was added dropwise to a solution of **10** (0.575 g, 2.23 mmol) in THF (15 mL) and the resulting solution was refluxed for 16 h. Then the mixture was allowed to cool to room temperature and was poured over hexane (25 mL), yielding **1** as a white precipitate (1.18 g, 84%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.63 (s, 2H), 7.94–7.91 (m, 8H), 7.86–7.49 (m, 8H), 4.50–4.37 (m, 2H), 4.16–4.06 (m, 4H), 3.02 (br s, 2H), 2.44–2.40 (m, 2H), 1.51–1.39 (m, 2H), 1.25 (t, J=7.2 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.6, 173.0, 134.9, 130.1, 129.2, 128.8, 127.5, 127.0, 126.2, 125.9, 122.3, 61.5, 40.0, 14.4; EI-HRMS calcd for $C_{34}H_{36}N_4O_4S_2$: 628.2178; found: 628.2173.

4.1.6. *trans-transoid-trans-1,2-Bis(ethoxycarbonyl)-4,5-bis-(3-phenylthioureido)cyclohexane (**2**)*

Phenylisothiocyanate (0.23 mL, 1.90 mmol) was added dropwise to a solution of **10** (0.245 g, 0.95 mmol) in THF (5 mL) and the resulting solution was refluxed for 16 h. Then the mixture was cooled at room temperature and poured over hexane (25 mL) from where **2** precipitated as a white solid (0.489 g, 97%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.51 (m, 2H), 8.00 (s, 2H), 7.49 (d, J=12.8 Hz, 4H), 7.29 (t, J=12.8 Hz, 4H), 7.07 (t, J=12.8 Hz), 4.52 (br s, 2H), 4.09–4.00 (m, 4H), 2.91 (br s, 2H), 1.99 (br s, 4H), 1.20 (t, J=7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.3, 173.3, 139.8, 128.8, 126.5, 124.1, 61.2, 52.1, 31.3, 21.6, 14.2; EI-HRMS calcd for $C_{26}H_{32}N_4O_4S_2$: 528.1865; found: 528.1882.

4.1.7. *trans-transoid-trans-5-Amino-1,2-bis(ethoxycarbonyl)-4-hydroxycyclohexane (**11**)*

Catalytic hydrogenation (65 psi) of **7** (0.645 g, 1.85 mmol) in ethanol (100 mL) in the presence of 10% Pd(C) for 2 days yielded aminoalcohol **11** as colourless oil (0.456 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.15 (q, J=7.1 Hz, 4H), 3.46–3.40 (m, 1H), 3.25–3.12 (m, 2H), 2.81–2.72 (m, 1H), 2.44 (br s, 3H), 2.29–2.21 (m, 2H), 1.72–1.55 (m, 2H), 1.25 (t, J=7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 173.9, 71.0, 61.5, 53.1, 51.1, 41.0, 40.6, 31.6, 31.3, 14.6; EI-HRMS calcd for $C_{12}H_{21}NO_5$: 259.1420; found: 259.1413.

4.1.8. *trans-transoid-trans-1,2-Bis(ethoxycarbonyl)-4-hydroxy-5-(3-(naphthalen-1-yl)thioureido)cyclohexane (**3**)*

1-Naphthylisothiocyanate (0.588 g, 3.18 mmol) was added dropwise to a solution of **11** (0.804 g, 3.10 mmol) in THF (10 mL) and the resulting mixture was refluxed for 16 h. Then organic solvent was evaporated and **3** (1.28 g, 93%) was afforded as a white solid after column chromatography (hexane/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.99–7.90 (m, 3H), 7.57–7.46 (m, 4H), 5.71 (m, 1H), 4.51 (m, 1H), 4.18–4.16 (m, 2H), 3.98 (q, J=7.6 Hz, 2H), 3.56 (m, 1H), 3.11–3.00 (m, 2H), 2.69 (br s, 1H), 2.25–2.21 (m, 1H), 1.90–1.71 (m, 2H), 1.53–1.49 (m, 1H), 1.29–1.26 (m, 3H), 1.16 (t, J=7.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.6, 173.7, 172.9, 134.5, 129.5, 128.4, 127.3, 126.9, 125.6, 124.9, 122.4, 69.2, 61.1, 60.8, 60.3, 55.7, 40.1, 39.5, 31.7, 28.1, 30.9, 14.1, 13.9; EI-HRMS calcd for $C_{23}H_{26}N_2O_4S$ (M−H_2O): 426.1613; found: 426.1502.

4.1.9. *trans-transoid-trans-1,2-Bis(ethoxycarbonyl)-4-hydroxy-5-(3-phenylthioureido)cyclohexane (**4**)*

Following the same procedure described in the synthesis of **3**, the reaction of **11** (288 mg, 0.82 mmol) in THF (4 mL) with phenylisothiocyanate (0.1 mL, 0.84 mmol) gave **4** (0.326 g, 98%) as a yellow oil after column chromatography (hexane/ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.46–7.23 (m, 5H), 6.12 (s, 1H), 4.48 (s, 1H), 4.24–4.09 (m, 4H), 3.71 (dt, J=9.2 and 4.6 Hz, 1H), 3.24–3.22 (m, 1H), 2.93 (br s, 1H), 2.40–2.10 (m, 3H), 1.88–1.75 (m, 1H), 1.73–1.67 (m, 1H), 1.31–1.26 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.2, 173.3, 130.6, 126.0, 125.8, 125.4, 61.8, 61.5, 40.8, 36.0, 32.5, 28.9, 14.6, 14.5; EI-HRMS calcd for $C_{19}H_{24}N_2O_4S$ (M−H_2O): 376.1457; found: 376.1441.

4.1.10. *trans-1,2-Bis-(3-(naphthalen-1-yl)thioureido)cyclohexane (**5**)*

Following the same procedure described for the synthesis of compound **1**, the reaction of *trans*-1,2-diaminocyclohexane (254 mg, 2.23 mmol) in THF (5 mL) with 1-naphthylisothiocyanate (826 mL, 4.5 mmol) gave **5** as a white solid (0.917 g, 85%). Mp 139–142 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.67 (s, 2H, NH), 7.95 (d, J=8.7 Hz, 2H), 7.90 (d, J=7.5 Hz, 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), 2.2 (m, 2H), 1.65 (m, 2H), 1.25–1.21 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.8, 134.7, 134.4, 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 $C_{28}H_{28}N_4S_2$: 484.1755; found: 484.1756.

4.2. Binding studies

Binding constants of ligands **1**–**5** towards 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.[7]

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Supplementary data

It includes fluorescent titration of ligand **1** with TMA oxalate and malonate; fluorescence of ligand **3** with TMA **DC3**; fluorescence of ligand **5** with TMA **DC4**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.05.064.

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