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PAPER

Synthesis of alanine-based colorimetric sensors and enantioselective recognition of aspartate and malate anions†

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Two chiral colorimetric sensors (**1,2**) were synthesized and characterized by spectroscopic techniques and their enantioselective recognition of chiral dicarboxylic anions (D/L-aspartate and D/L-malate) was examined by UV-vis and ^{1}H NMR spectroscopy. Interaction of the receptors **1** and **2** with the enantiomers of aspartate or malate caused different color changes, and they act as optical chemosensors for the recognition of D-aspartate *vs.* L-aspartate and D-malate *vs.* L-malate. Receptor **1** exhibits high enantioselective binding for aspartate anions [$K_{A(D)}/K_{A(L)} = 12.15$].

Introduction

The need for new synthetic chiral receptors for the molecular recognition of biologically important anions has prompted research on new chiral receptors with selective recognition of anions.[1] The development of a chiral artificial receptor could find applications in the development of pharmaceuticals, enantioselective sensors, catalysts, enzyme models, and other molecular devices.[2] Many artificial receptors of different kinds have been synthesized for selective anion recognition, which include chiral macrocyclic, acyclic polyamine, chiral calixarene, and chiral cyclodextrin derivatives.[3] The main objective for the molecular design of a chemosensor is to achieve specific molecular recognition and transfer of the recognition event into a signal. The has been intense interest in naked eye-based chemosensors which can detect and discriminate enantiomers.[4]

Commonly L-aspartic acid is found in nature, while recent research suggests that D-aspartate is an essential intriguing molecule found within the neuron system of animals ranging from mollusks to vertebrates.[5] High concentrations of L-aspartate have also been observed in the early-onset dementia of the Alzheimer type.[6] Hence, considerable attention has been paid to the synthesis of enantioselective receptors for aspartate anions. On the other hand, malic acid usually exists in nature in only one enantiomeric form, L-malic acid. The synthetic mixture of malic acid is used as an acidulant in pharmaceutics, but the abnormal levels of its concentration leads to some diseases.[7] On the other hand, D-malic acid is found in appreciable concentration only in the metabolism of some micro-organisms. Therefore, some efforts have been developed to distinguish between malate enantiomers in order to detect malpractice in the food industry.[8] The chiral discrimination of malic acid enantiomers has been achieved through CPL,[9a] HPLC,[9b,c] electrophoresis,[9d,e] NMR,[9f] absorption spectroscopy,[9g] and fluorescence.[9h] Although there has been a lot of effort reported on the chiral recognition of malic acid, the selective color change for enantiomers of malic acid is still rare.[10]

Herein, we report the preparation of new chiral chromogenic receptors **1** and **2** and their utility in the enantioselective colorimetric discrimination between certain enantiomers (D/L-aspartate and D/L-malate) (Chart 1). The differentiation of the enantiomers is very difficult because of their similar chemical and physical properties. Hence, in this work, the anthraquinone chromogenic unit was chosen as a scaffold to link the chiral barrier (L-alanine group) and thiourea as the binding site (Scheme 1). Mostly, alanine is employed as a chiral source in building the desired chiral molecule.[11] Here, the enantioselective recognition ability for chiral dicarboxylate anions has been investigated by ^{1}H NMR and UV-vis spectroscopy. Different color changes were observed when receptor **1** interacts with the enantiomers of aspartate, which illustrates that **1** has a good enantioselective recognition ability for aspartate anions.

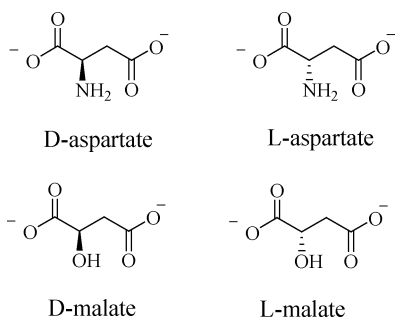

Chart 1

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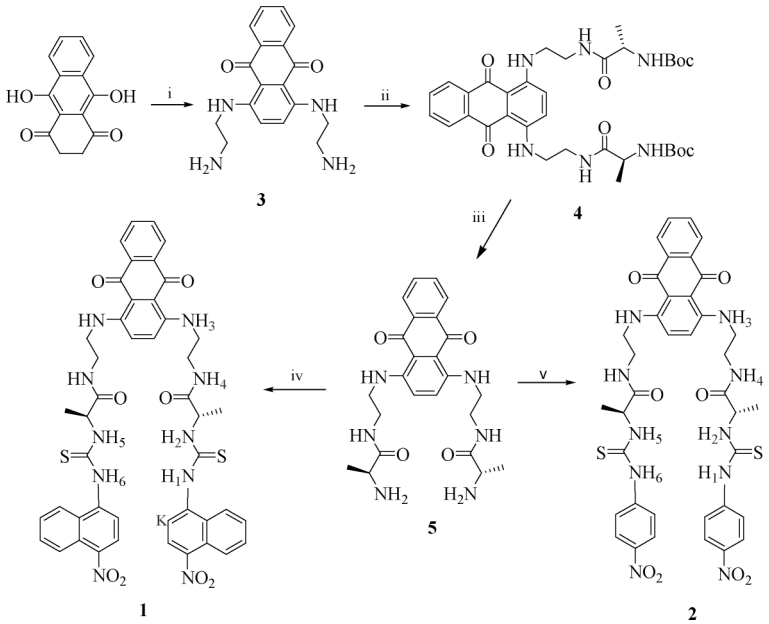
† Electronic supplementary information (ESI) available: Color changes for titration of **1** with hydroxide. 2D COSY spectrum of sensors **1–2** and NMR titration data of receptors **1–2** with D/L aspartate and D/L-malate. See DOI: 10.1039/c1ob05135k



Scheme 1 *Reagents and conditions*: (i) ethylenediamine, 50 °C, 1 h, 37%; (ii) N-Boc-L-alanine, CDI (1,1′-carbonyldiimidazole), rt, 48 h; (iii) H_2SO_4, CH_2Cl_2, 6 h; (iv) *p*-nitronaphthyl isothiocyanate, THF, reflux, 72 h; (v) *p*-nitrophenyl isothiocyanate, THF, reflux, 72 h.

Results and discussion

The preparation of chiral receptors **1** and **2** is depicted in Scheme 1. A synthetic intermediate, 1,4-bis(2-aminoethylamino)-anthracene-9,10-dione (**3**) was prepared from 9,10-dihydroxy-2,3-dihydroanthracene-1,4-dione.[12] Reaction of **3** with N-boc-L-alanine in the presence of CDI (1,1′-carbonyldiimidazole) gave the intermediate **4**, which was then treated with H_2SO_4 to afford the compound **5**. Subsequently, reaction of **5** with *p*-nitronaphthyl isothiocyante or *p*-nitrophenyl isothiocyanate gave the corresponding receptors **1** and **2**, respectively, in good yields. All of these compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS.

Anion binding studies

The colorimetric enantioselective sensing ability of the receptors **1** and **2** with D- and L-aspartate anions in the solvent system DMSO/H_2O (4/1, v/v) was monitored by UV-vis absorption and naked eye experiments. The anions were added as tetrabutylammonium salts to the DMSO/H_2O (4/1, v/v) solutions of the receptors **1** and **2** (5×10^{-5} M). The receptor **1** showed three absorption bands in DMSO/H_2O (4/1, v/v) at 384, 595, and 639 nm, respectively. The interaction of receptor **1** with D-aspartate anion was investigated in detail through the UV-vis spectroscopic titration, and the spectral behavior was observed (Fig. 1a). Upon addition of D-aspartate to receptor **1** in DMSO/H_2O (4/1, v/v), the intensities of the absorption peaks at 384, 595, and 639 nm gradually decreased, while a new absorption band at 520 nm evolved (Fig. 1a).

In the naked eye experiments, a color change of sky blue to dark magenta was observed for receptor **1** (Fig. 2c). Two clear isosbestic points at 429 and 559 nm indicate that there is a balance between the complex and host–guest in solution.[13] By plotting the changes in the absorbance intensity of **1** at 520 nm as a function of

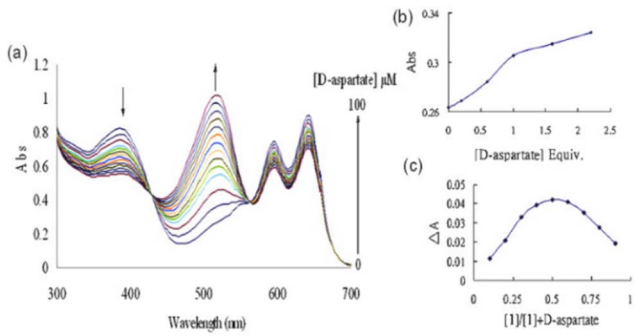


Fig. 1 A series of spectra taken over the course of the titration of 5×10^{-5} M DMSO/H_2O (4/1, v/v) in **1** with a standard solution of D-aspartate at 25 °C. The titration profile (insert) indicates the formation of a 1 : 1 complex.

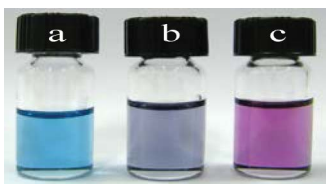


Fig. 2 Color changes of complex **1** upon addition of various anions in DMSO/H_2O (4/1, v/v): (a) **1** only; (b) **1** + 2.0 equiv. of L-aspartate; (c) **1** + 2.0 equiv. of D-aspartate.

D-aspartate anion concentration, a sigmoidal curve was obtained, which is shown in the inset Fig. 1b. To corroborate the 1 : 1 ratio between **1** and D-aspartate, Job's plot analysis was also executed. The measured absorbance variation ($\Delta A = A_{abs} - A_i$) reaches a maximum when the molar fraction of ([**1**]/[**1**] + [D-aspartate]) is 0.5, confirming the 1 : 1 stoichiometry (Fig. 1c).

In studies of anion recognition, it is of primary importance of discriminate whether the process is a hydrogen bond complexation

or a proton transfer of a Brønsted-type acid–base equilibrium. Initially a genuine H-bond complex was formed and further the formation of deprotonated L^-, which can be ascribed to a "frozen" proton release from the donor (the acid) to the acceptor (the base) (eqn (1)) and the more advanced proton release process: [14]

$$LH + M^{2-} \rightleftharpoons L^- + HM^- \quad (1)$$

The band that develops at 520 nm might be due to the deprotonated receptor L^- (**1** = LH), which was confirmed by the Brønsted acid–base reaction by adding 1.0 equivalent of strong base [n-Bu_4N]OH (*cf*. SI-1, see the ESI†). The spectral behavior revealed that deprotonation of the NH fragment by D-aspartate is responsible for the drastic color change, as a result of a charge transfer interaction between the nitrogen atom of the thiourea unit and the electron deficient 4-nitronaphthyl moiety. Such a deprotonation is mainly related to the intrinsic acidity of LH[15] and the stability of HM^- in solution.[16] The deprotonation of receptor **1** with D-aspartate was corroborated by ^{1}H NMR titration experiments carried out in DMSO-d_6/H_2O (4/1, v/v) (Fig. 3). It was found that the proton signal of N–H_1 (δ = 10.21 ppm), which is closer to the 4-nitronaphthyl group (signals of N–H protons were assigned with respect to the 2D NOESY spectrum of **1**) (*cf*. SI-2a, see the ESI†), broadened significantly early in the titration and underwent downfield shifts with increasing D-aspartate concentration. The N–H_1 peak disappeared after addition of 1.0 equivalent of D-aspartate, whilst a new signal was observed at δ = 12.34 ppm. This suggest that there might be the formation of a $[HM]^-$ (M = D-aspartate anion) species. The monodeprotonation is also signalled by the significant upfield shift of the proton of the naphthyl group (C–H_K). Such an effect could be derived from the through-bond propagation onto the naphthyl framework of the electronic charge generated on N–H deprotonation. When D-aspartate was added, the thiourea (N–H_2) signal was found to undergo downfield shift. The results implied that the complex formation of the receptor **1** with D-aspartate leads to the monodeprotonation of the receptor. In evidence for this supposition, the intermolecular N···H···O hydrogen bond distances were calculated at the HF/6-31G(d) level using *ab initio* calculations (Fig. 4). This could be explained that four protons of thiourea were directed toward anion ligands, but each hydrogen-bond distance is different as shown in SI-Table 1 (see the ESI†). Among them, the proton (H_1) that is connected to the 4-nitronaphthyl group has a much shorter distance to the carboxylic group than a typical hydrogen-bond distance, which ranges between 1.86 and 2.16 Å.[17]

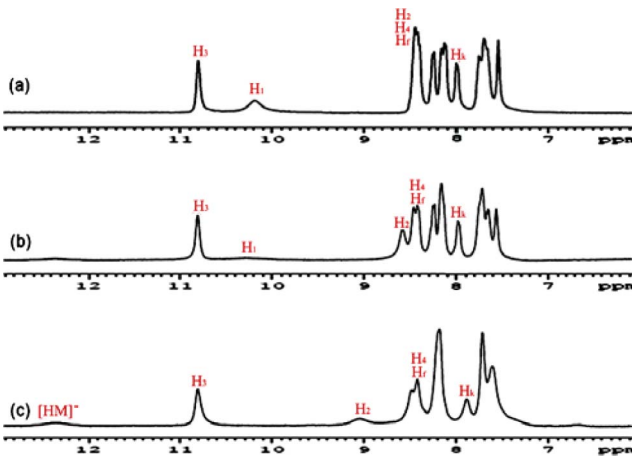


Fig. 3 ^{1}H NMR (400 Hz) spectra of sensor **1** (10 mM) in solution (DMSO-d_6/H_2O = 4/1, v/v) upon addition of various quantities of D-aspartate: (a) 0 eq.; (b) 0.2 eq.; (c) 1.0 eq.

Table 1 K_a values, correlation coefficients (R) and enantioselectivities (K_D/K_L) of receptors **1** and **2** with anions

Receptor	Anion[c]	K_a/M[a]	R[b]	K_D/K_L
1	D-aspartate	$(6.27 \pm 0.03) \times 10^3$	0.9964	12.15
	L-aspartate	$(5.16 \pm 0.02) \times 10^2$	0.9922	
	D-malate	$(5.03 \pm 0.05) \times 10^3$	0.9962	7.89
	L-malate	$(6.38 \pm 0.04) \times 10^2$	0.9931	
2	D-aspartate	$(2.58 \pm 0.04) \times 10^3$	0.9952	5.59
	L-aspartate	$(4.62 \pm 0.03) \times 10^2$	0.9927	
	D-malate	$(2.42 \pm 0.06) \times 10^3$	0.9986	3.10
	L-malate	$(7.83 \pm 0.03) \times 10^2$	0.9952	

[a] The data were calculated from UV-vis titration in DMSO/H_2O (4/1, v/v). [b] The data values for R were obtained by the results of nonlinear curve fitting. [c] The anions were used as their tetrabutylammonium salts.

These two mechanisms (hydrogen bond complex or proton transfer) could also be explained by the ^{1}H NMR dilution experiment.[18] If the process is a deprotonation, the equilibrium is independent of the concentration of host and guest because the equilibrium constant is dimensionless. On the other hand, if the binding process is a hydrogen bonding interaction, the equilibrium is shifted to the dissociation direction. The results revealed that the C–H proton of the naphthyl group did not undergo any significant chemical shift to the direction of dissociation by

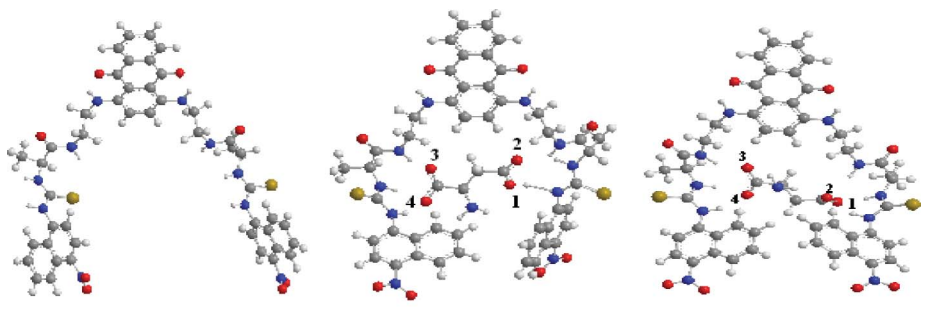


Fig. 4 Optimized geometries from *ab initio* HF/6-31G(D) calculations.

dilution. Therefore, it can be clearly demonstrated that this process is due to deprotonation rather than hydrogen bond complexation (*cf*. SI-3, see the ESI†).

The enantioselectivity of receptor **1** towards D-aspartate was compared with that towards L-aspartate. When receptor **1** was treated with L-aspartate under the same conditions, similar UV-vis spectral behavior was observed (Fig. 5a). During the process, the most pronounced effect is the L-aspartate anion-induced color change from sky blue color to medium purple color (Fig. 2b). In order to investigate the contribution of the N–H deprotonation effects with the anion-induced effects, ^{1}H NMR spectral analyses were carried out in DMSO-d_6/H_2O (4/1, v/v). A notable feature of these titrations is that the proton signals of thiourea underwent downfield shifts during the complex formation of receptors **1** with L-aspartate. After the addition of 1.0 equivalent of L-aspartate, the signal of N–H_1 disappeared but a $[HM]^-$ (M = L-aspartate anion) peak was not observed in the range of 11–13 ppm. This result indicates that the complex is formed through multiple hydrogen bonds and is inconsistent with a deprotonation process between the receptor and L-aspartate. This could be explained by the result of the ^{1}H NMR dilution experiment. In this, the CH proton of the naphthyl group underwent downfield shift in the direction of dissociation by dilution (*cf*. SI-4, see the ESI†). Besides that, the *ab initio* calculations indicate that the process is *via* hydrogen bond complexation rather than proton transfer (*cf*. SI-Table 1, see the ESI†). From the UV-vis titrations, the binding of L-aspartate allowed the Job's plot method (Fig. 5) for the determination of binding stoichiometry, which was found to be a 1 : 1 host–guest complexation. The association constant was calculated and shown in Table 1. Comparison of the UV-vis absorption spectra of complex **1** upon addition of either D- or L-aspartate anion is shown in Fig. 6. Apparently, receptor **1** has higher enantioselectivity and sensitivity recognition for D-aspartate over L-aspartate anions in DMSO-d_6/H_2O (4/1, v/v).

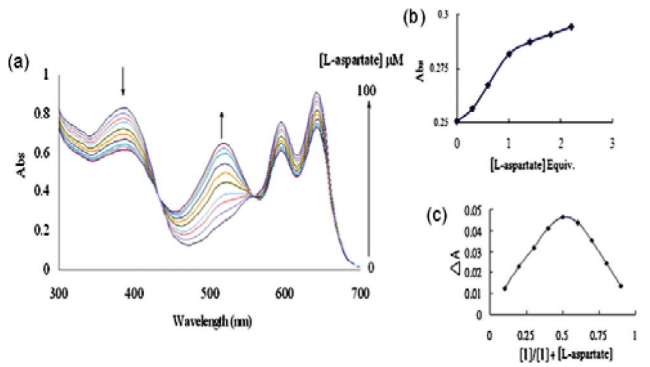


Fig. 5 A series of spectra taken over the course of the titration of 5 × 10^{-5} M DMSO/H_2O (4/1, v/v) solution of **1** with a standard solution of L-aspartate at 25 °C. The titration profile (insert) indicates the formation of a 1 : 1 complex.

The interaction of receptor **1** with D- or L-aspartate was also corroborated by ^{1}H NMR titration experiment. Fig. 7 shows the spectra of **1** and its complexes with equal amounts of D- or L-aspartate in DMSO-d_6/H_2O (4/1, v/v). In the presence of L-aspartate, the characteristic peaks of N–H_1 of **1** disappeared, while N–H_2 had a downfield shift from 8.43 to 8.81 ppm; when treated with D-aspartate, the N–H_2 peak shifted from 8.43 to

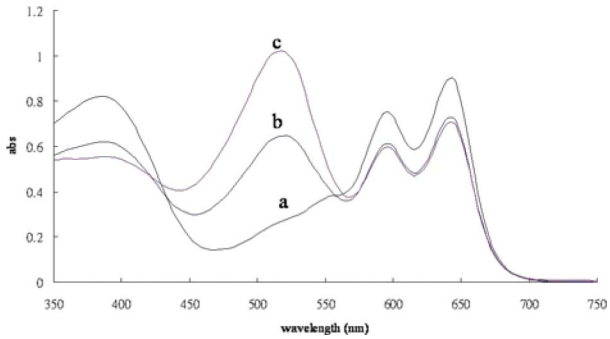


Fig. 6 UV-vis spectral change of **1** (5 × 10^{-5} M) operated in DMSO-d_6/H_2O (4/1, v/v) after the addition of 2.0 equivalents of anions: (a) **1** only; (b) **1** + L-aspartate; (c) **1** + D-aspartate.

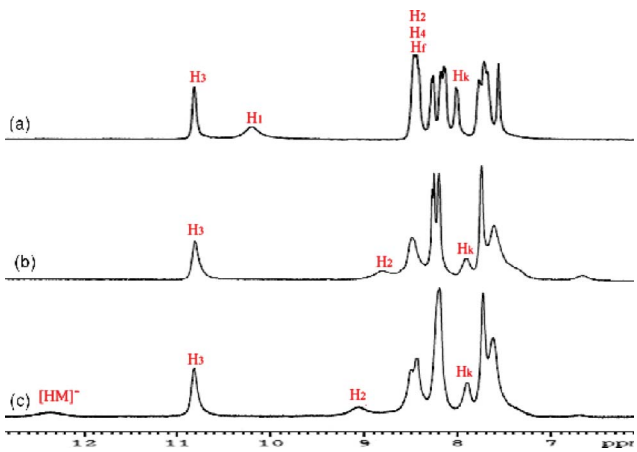


Fig. 7 ^{1}H NMR spectra change of **1** operated in the solution (DMSO-d_6/H_2O = 4/1) after the addition of 2.0 equivalents of anions: (a) **1** only; (b) **1**+ L-aspartate; (c) **1**+ D-aspartate.

9.05 ppm. The difference observed in the change of chemical shift for the enantiomers of aspartate demonstrated that **1** has a stronger interaction with D-aspartate than with L-aspartate. In addition, the association constant (K_a) of **1** with D-aspartate is $(6.27 \pm 0.03) \times 10^3$ $(\text{mol L}^{-1})^{-1}$, while that of **1** with L-aspartate is $(5.16 \pm 0.02) \times 10^2$ $(\text{mol L}^{-1})^{-1}$, and the enantioselectivity [K_D/K_L] is 12.15 (Table 1). Since receptor **1** has a unique color change and higher enantioselectivity for D-aspartate than L-aspartate, it can act as an optical chemosensor for recognition of D-aspartate *vs.* L-aspartate. The change in color and different enantioselectivity with D- and L-aspartate could be related to the stereochemistry of the receptor and the structure complementarity with guest. The possible structure of **1** was calculated by quantum chemical calculation at the DFT level of theory and the minimum energy structure was shown in Fig. 4. The receptor **1** might have a conformation with two functional arms on the 1,4-anthraquinone unit with a minimum distance between the two naphthyl groups of 13.831 Å, while the distance between the two sulfurs is 13.270 Å. Based on this model, a suitable conformation was assumed when it interacts with **1**. The D-aspartate anion could fit more perfectly into the complex inducing a conformation change in the receptor. The NH of the thiourea group on receptor **1** might have a strong base and acid interaction with the oxygen of the CO_2^- group in D-aspartate (pK_{a_2} value of the aspartic acid is 3.86 in H_2O).[19] Hence,

the enantioselective recognition depends on the steric effect and the structure complementarity of the receptor with the guest.

The high enantioselectivity of receptor **1** for D-aspartate compared to that for the L-aspartate prompted us to investigate the enantiomers of malate, D- and L-malate anions. A similar phenomenon in the UV-vis absorption spectra was observed in Fig. 8. The absorption spectrum (a) was measured in the absence of anion. As shown in spectra (b) and (c), the CT absorption bands appeared at 520 nm and the solution color changes from sky blue color to purple for L-malate and from sky blue color to pansy for D-malate, respectively (Fig. 9). It is apparent that receptor **1** showed a selective color change for D-malate *vs.* L-malate. The enantioselectivity of **1** for the recognition of these anions could also be rationalized on the basis of the stereochemistry of the receptor and the structure complementarity with guest. The dramatic color changes could also be attributed to the occurrence of the deprotonation of the N–H proton of the thiourea moiety with anion (pK_{a_2} value of malic acid is 5.20 in H_2O).[19] This observation was similarly confirmed by ^1H NMR titration experiments with D-malate in which the peak of $[HM]^-$ (M = D-malate anion) appeared at 12.34 ppm (*cf.* SI-5, see the ESI†). But when L-malate was treated against receptor **1**, the peak of N–H_1 disappeared but no $[HM]^-$ (M = L-malate anion) peak was observed in the range of 11–13 ppm. This result implied that receptor **1** might only bind with L-malate through multiple hydrogen bonds. The binding process of receptor **1** with L-malate might be explained when a ^1H NMR dilution experiment of **1** with L-malate was performed. The result has demonstrated that the process is *via* hydrogen bond interactions rather than a deprotonation (*cf*. SI-6, see the ESI†).

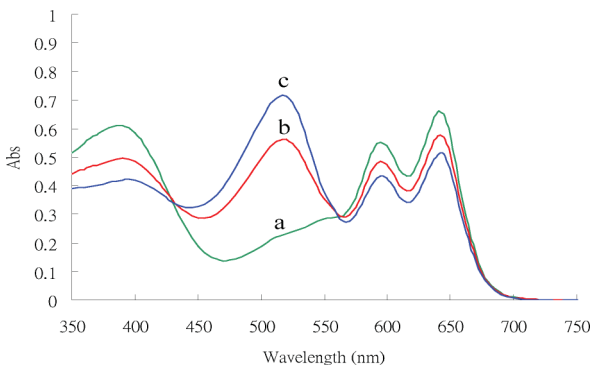


Fig. 8 UV-vis spectral change of **1** (5×10^{-5} M) operated in DMSO/H_2O (4/1, v/v) after addition of 2.0 equivalents of anions: (a) **1** only; (b) **1** + L-malate; (c) **1** + D-malate.

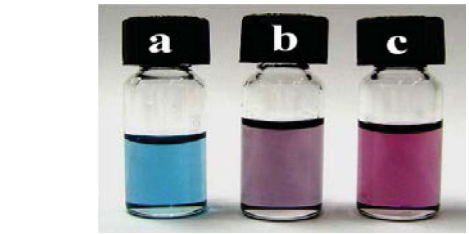


Fig. 9 Color changes of complex **1** upon addition of various anions in DMSO/H_2O (4/1, v/v): (a) **1** only; (b) **1** + 2.0 equiv. of L-malate; (c) **1** + 2.0 equiv. of D-malate.

From the UV-vis titrations, the Job's plot method showed the formation of a 1 : 1 stoichiometry complex of **1** with either D- or L-malate (*cf*. SI-7 and SI-8, see the ESI†). The association constants of **1** with D- or L-malate were calculated as $(5.03 \pm 0.05) \times 10^3$ (mol $L^{-1})^{-1}$ and $(6.38 \pm 0.04) \times 10^2$ (mol $L^{-1})^{-1}$, respectively. The D/L $[K_D/K_L]$ selectivity was found to be 7.89 for malate (Table 1). To elucidate the interaction between D- or L-malate with receptor **1**, *ab initio* calculations of the [**1**·D-malate] complex and the [**1**·L-malate] complex were calculated. These calculations clearly showed that only the proton (H_1) of the D-malate has a much shorter distance to the dicarboxylic group than a typical H-bond distance (*cf*. SI-Table 1, see the ESI†).

The influence of the different substituents of the thiourea group on the anion binding and sensing property was studied. Receptor **2** was examined by replacing the 4-nitronaphthyl group with the weaker electron-withdrawing 4-nitrophenyl group. With the progressive addition of either D- or L-aspartate to receptor **2**, the intensity of the absorption peak at 354 nm gradually decreased and a new band at 471 nm concomitantly evolved. Two clear isosbestic points at 390 and 544 nm were observed (Fig. 10a, and *cf*. SI-9, see the ESI†). These changes were accompanied by the color changes from blue to green for D-aspartate and from blue to cornflower blue for L-aspartate (Fig. 11). The color changes caused by the introduction of either D- or L-aspartate may be ascribed to the formation of the hydrogen bond complexes between the receptor **2** and the aspartate anions. From the ^1H NMR titration experiment, it was found that when receptor **2** formed a complex with D-aspartate, both the proton signals of N–H_1 (10.35 ppm) and N–H_2 (8.18 ppm) underwent downfield shifts to 11.25 and 9.10 ppm, respectively, after increasing the concentration of D-aspartate (*cf*. SI-10, see the ESI†). On the other hand, when receptor **2** forms a complex with L-aspartate, both the proton

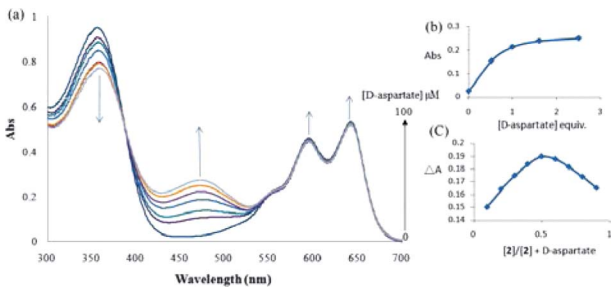


Fig. 10 A series of spectra taken over the course of the titration of a 5×10^{-5} M DMSO/H_2O (4/1, v/v) solution of **2** with a standard solution of D-aspartate at 25 °C. The titration profile (insert) indicates the formation of a 1 : 1 complex.

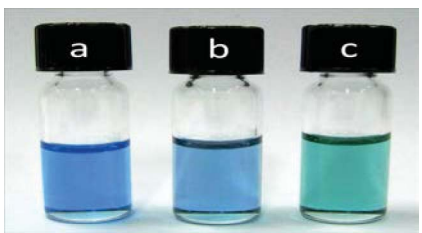


Fig. 11 Color changes of complex **2** upon addition of various anions in DMSO/H_2O (4/1, v/v): (a) **2** only; (b) **2** + 2.0 equiv. of L-aspartate; (c) **2** + 2.0 equiv. of D-aspartate.

signals of N–H_1 (10.35 ppm) and N–H_2 (8.18 ppm) similarly underwent downfield shifts to 10.91 and 8.80 ppm, respectively, with increasing concentration of L-aspartate (*cf.* SI-11, see the ESI†). The relatively small downfield shifts indicate that the complex is formed through weak hydrogen bonding interactions and is inconsistent with a deprotonation process between D- or L-aspartate and the receptor **2**. To further support the supposition of the hydrogen bonding interactions, the dilution experiments (*cf*. SI-12 and SI-13, see the ESI†) and the intermolecular N···H···O hydrogen bonded distances were calculated by *ab initio* calculation (*cf*. SI-Table 1, see the ESI†). The association constant of **2** with D- or L-aspartate is calculated as shown in Table 1. The data in Table 1 illustrated that the association constants of **1** are much higher than **2** with aspartate anions. The results demonstrated that the introduction of the *p*-nitronaphthyl group enhances the acidity of the thiourea NH, which provides an effective intramolecular charge transfer and enhances the hydrogen bonding ability, resulting in strong anion binding.[20] All the results indicate that the receptor **2** is interacting with D- or L-aspartate anions through hydrogen bonding interactions. The comparison of the ^{1}H NMR and absorption spectra of complex **2** upon addition of either D- or L-aspartate is shown in the ESI (Fig. SI-14).† Receptor **2** has a unique color change and higher enantioselectivity recognition for D-aspartate than L-aspartate, and can act as an optical chemosensor for recognition of D- *vs.* L-aspartate.

In order to investigate whether the structurally similar in chiral dicarboxylates could be differentiated by color change, the binding of receptor **2** with D- and L-malate was studied. Consistent with the above results of the titration of D- and L-aspartate, the titration of **2** with D- and L-malate gave similar phenomena in their UV-vis absorption spectra (Fig. 12) as well as the same color changes (*cf*. SI-15, see the ESI†). It appeared that receptor **2** has the same propensity; formation of hydrogen bonding interactions occurred upon addition of increasing concentrations of either D- or L-malate. This supposition is similarly proved by the ^{1}H NMR titration experiments (*cf*. SI-16–18, see the ESI†) and dilution experiments (*cf*. SI-19 and SI-20, see the ESI†). The hydrogen-bond distances were also reflected in the *ab initio* calculations (*cf*. SI-Table 1, see the ESI†). The higher enantioselectivity and the color change of receptor **2** toward D- or L-malate mean that **2** can also be used as an optical chemosensor for recognition of D- or L-malate anions.

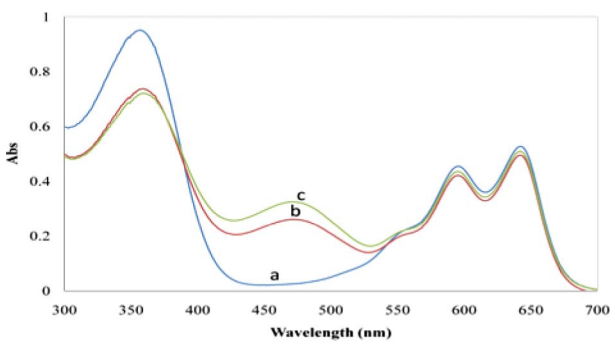


Fig. 12 UV-vis spectral change of **2** (5×10^{-5} M) operated in DMSO/H_2O (4/1, v/v) after addition of 2.0 equivalents of anions: (a) **2** only; (b) **2** + L-malate; (c) **2** + D-malate.

Conclusion

In conclusion, two new chiral colorimetric receptors were synthesized. Both receptors show good sensitivity and enantioselectivity for the discrimination of D/L-aspartate and D/L-malate by dramatic color changes in DMSO/H_2O (4/1, v/v). Thus, both **1** and **2** can be used as optical chemosensors for recognition of D- *vs.* L-aspartate or D- *vs.* L-malate anions. To the best of our knowledge, receptor **1** is the first synthetic host that can selectively recognize either D/L-aspartate or D/L-malate by color changes. Future work will continue to develop practical colorimetric sensors for the recognition of other chiral enantiomers.

Experimental

General

The chemical reagents were purchased from Acros or Aldrich Corporation and utilized as received. All solvents were purified by standard procedures. Melting points were measured on a Yanaco MP-S3 melting-point apparatus. The infrared spectra were performed on a Perkin Elmer System 2000 FT-IR spectrophotometer. UV-Vis spectra were measured on a Cary 300 spectrometer. All NMR spectra were measured on a Bruker spectrometer at 400 (^{1}H) and 100 MHz (^{13}C) and Varian Unity Inova-600 spectrometers at 600 (^{1}H) and 150 MHz (^{13}C) with DMSO-d_6 as solvent. High-resolution mass spectra were measured with a Finnigan/Thermo Quest MAT 95XL instrument.

1,4-Bis(2-aminoethylamino)anthracene-9,10-dione (3). To a suspension of of 9,10-dihydroxy-2,3-dihydroanthracene-1,4-dione (10.5 g, 42 mmol) in MeOH (100 mL), ethylenediamine (29 mL, 0.42 mol) was slowly added at 5 °C. The mixture was stirred under N_2 at 50 °C for 1 h, and then left stirring at room temperature overnight. The solution was evaporated under vacuum and the residue was subsequently washed with acetonitrile and ether to give **3** (5.2 g, 37% yield) as a dark blue solid. The spectral data of **3** were consistent with the literature data.[12]

Di-*tert*-butyl (2*S*,2′*S*)-1,1′-(2,2′-(9,10-dioxo-9,10-dihydroanthracene-1,4-diyl) bis(azanediyl)bis(ethane-2,1-diyl)) bis(azanediyl) bis(1-oxopropane-2,1-diyl)dicarbamate (4). To an ice-cooled solution of *N*-Boc-L-alanine (0.80 g, 4.2 mmol) in dry CH_2Cl_2 (25 mL), 1,1′-carbonyldiimidazole (CDI) (0.82 g 5.0 mmol) was slowly added. The resulting mixture was stirred for 2 h, then a solution of **3** (0.65 g, 2.0 mmol) in CH_2Cl_2 (200 mL) was slowly added at 0 °C, and then it was left stirring at room temperature for 48 h. The solution was poured into 50 mL of 10% HCl and extracted with CH_2Cl_2. The organic layer was washed with $NaHCO_3$, water, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure. The residue was purified on a flash column of silica gel (CH_2Cl_2/ethyl acetate/MeOH = 3.5/0.5/0.1) to give the pure product **4** (0.22 g, 42% yield) as a blue solid. mp: 206–207 °C. $[\alpha]_D^{25} = -12$ [$c = 0.008$, DMSO], HRMS(FAB) calcd for $C_{34}H_{46}O_8N_6$ [M^+] 666.3377; found 666.3371.

4-Nitronaphthalenyl isothiocyanate (6). 4-Nitro-1-naphthylamine (0.56 g, 3.0 mmol), di-2-pyridylthionocarbonate (DPT) (1.10 g, 4.76 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.10 g, 0.82 mmol) in $CHCl_3/CH_3CN$ (3/1, v/v, 20 mL) were

combined and stirred at room temperature for 4 h. The resulting mixture was evaporated and the residue was purified on a flash column of silica gel (ethyl acetate/hexane = 1/5) to give **6** (0.47 g, 68% yield) as a yellow solid. mp: 93.0–94.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.44–7.46 (d, 1H, J = 8.0 Hz), 7.70–7.80 (m, 2H), 8.18–8.20 (d, 2H, J = 8.4 Hz), 8.58–8.60 (d, 1H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 144.4, 139.7, 133.6, 130.4, 129.5, 128.5, 125.9, 124.2, 123.7, 123.4, 121.8. HRMS(FAB) calcd for $C_{11}H_6N_2S$ [M$^+$] 230.0150; found 230.0146.

(2*S*,2′*S*)-*N*,*N*′-[2,2′-(9,10-Dioxo-9,10-dihydroanthracene-1,4-diyl)bis(azanediyl) bis(ethane-2,1-diyl)]bis{2-[3-(4-nitronaphthalen-1-yl)thioureido]propanamide} (1). A solution of **4** (2.65 g, 4.0 mmol) in CH_2Cl_2 (35 mL) was added into a chilled, well stirred mixture consisting of 96% H_2SO_4 (12 mmol) and CH_2Cl_2 (5.0 mL). The resulting mixture was stirred for 6 h at room temperature. Then the mixture was extracted with H_2O (2 × 100 mL). The aqueous phase was made alkaline by addition of 4 M NaOH (9.0 mL, 36.0 mmol), extracted with CH_2Cl_2 (2 × 100 mL), and dried with solid $MgSO_4$ (5.0 g). The combined organic phase was concentrated to give compound **5** in good yield. Without further purification, the blue solid and triethylamine (1.0 mL) were dissolved in dry CH_2Cl_2 (250 mL), and a solution of 4-nitronaphthyl isothiocyanate (1.84 g, 8.0 mmol) was added slowly. The mixture was stirred for 72 h at room temperature. The resulting precipitate was filtered and washed with CH_2Cl_2 to afford the pure product **1** (2.89 g, 78% yield), as a blue solid. mp: 195–196 °C. $[\alpha]_D^{25}$ = +45 [c = 0.002, DMSO], ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 10.83 (t, J = 6.3 Hz, 2H), 10.20 (s, 2H), 8.48 (d, J = 4.2 Hz, 2H), 8.47 (d, J = 4.2, 2H), 8.45 (s, 2H), 8.32 (d, J = 8.4 Hz, 2H), 8.22 (d, J = 3.6 Hz, 1H), 8.21 (d, J = 3.0 Hz, 1H), 8.18 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 7.80 (t, J = 7.8 Hz, 2H), 7.75 (d, J = 3.6 Hz, 1H), 7.74 (d, J = 3.0 Hz, 1H), 7.72 (t, J = 7.8 Hz, 2H), 7.6 (s, 2H), 4.91 (t, J = 6.9 Hz, 2H), 3.64–3.55 (m, 4H), 3.46–3.35 (m, 4H), 1.37 (d, J = 6.6 Hz, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): 181.3, 181.1, 172.8, 146.4, 142.8, 141.7, 134.3, 132.8, 130.1, 128.9, 127.8, 126.1, 125.7, 125.0, 124.9, 123.7, 123.3, 121.7, 109.3, 53.6, 41.7, 31.8, 19.5 ppm. HRMS(FAB) calcd for $C_{46}H_{43}O_8N_{10}S_2$; [M$^+$ + H] 927.2707; found 927.2706.

(2*S*,2′*S*)-*N*,*N*′-(2,2′-(9,10-Dioxo-9,10-dihydroanthracene-1,4-diyl)bis(azanediyl)bis(ethane-2,1-diyl))bis(2-(3-(4-nitrophen-yl)-thioureido)propanamide) (2). A similar procedure to the synthesis of **2** was carried out using **4** and 4-nitrophenyl isothiocyanate in place of 4-nitronaphthyl isothiocyanate. Yield: 2.41 g (73%), as a blue solid. mp: 191–193 °C. $[\alpha]_D^{25}$ = +37 [c = 0.003, DMSO], ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.80 (s, 2H), 10.41 (s, 2H), 8.48 (s, 2H), 8.31 (s, 2H), 8.19 (s, 2H), 8.15 (d, J = 8.8 Hz, 4H), 7.91 (d, J = 8.4 Hz, 4H), 7.73 (s, 2H), 7.55 (s, 2H), 4.83 (s, 2H), 3.57 (d, J = 4.0 Hz, 4H), 3.73 (s, 4H), 1.33 (d, J = 6.4 Hz, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): 181.3, 176.3, 172.6, 146.7, 146.4, 142.3, 134.3, 132.8, 126.1, 124.9, 124.8, 120.7, 109.3, 53.1, 41.6, 39.2, 19.3 ppm. HRMS (FAB) calculated for $C_{38}H_{38}O_8N_{10}S_2$ [M$^+$] 826.2315; found 826.2321.

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