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Sensitive fluorescent sensors for malate based on calix[4]arene bearing anthracene

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Abstract—Two chiral fluorescence receptors **3a** and **3b** based on calix[4]arenes were synthesized, and their chiral recognition properties for enantiomeric malate were studied by fluorescence and ^{1}H NMR spectra in $CHCl_3$. The addition of either L- or D-malate caused obvious fluorescence quenching of the host solution. Different fluorescent responses demonstrate that the two receptors have good enantioselective recognition abilities towards malate.

1. Introduction

The chemical and more importantly biological activity of any chiral substance depends on its stereochemistry. This is why the design, synthesis and structure–activity relationships of enantioselective receptors are still vital areas of research. Chiral synthetic ligands can open new possibilities in enantioselective catalysis and enantioseparations of racemic chiral compounds; they can be active in different parts of membrane transport and help us in understanding many vital processes in the biological world.[1] Recently, much research effort has been devoted to the synthesis of chiral fluorescent hosts, which are capable of distinguishing between the enantiomers of chiral guest molecules, due to their high sensitivity and potential applications in analytical, biological, clinical and biochemical environments.[2]

Malate, a model for numerous chiral compounds possessing an optically active α-hydroxy group, which exists abundantly in Nature, is used as a general purpose acidulant in pharmaceutics, and serves as a convenient starting material for the synthesis of various (homo) chiral compounds.[3] Chiral discrimination and resolution of malates and other hydroxy acids have been achieved mainly through CPL,[4a] HPLC,[4b,c] electrophoresis,[4d,e] NMR,[4f] absorption[4g] spectroscopy and fluorescence.[4h] Although a lot of work has been reported on the chiral recognition of malate, receptors based on a selective fluorescent change for enantiomers of malate are still rare.[5]

Herein, we chose calixarenes as the building blocks to design fluorescence sensors for malate, because of their capabilities of being modified at both the upper and the lower rims. The functionalized calixarenes can exhibit some specific excellent properties.[6] Recently, four novel two-armed calix[4]arenes have been synthesized in our laboratory, containing amino acid chiral moieties and hydrazine or thiourea binding sites, and supplied with dansyl or *p*-nitro-benzene indicator groups, respectively.[7] Fluorescence or UV–vis titration experiments demonstrated that these hosts can bind chiral amine acid anions or phenylglycine anion enantioselectively and indicate it via substantial spectral change.

Herein, two chiral fluorescence calix[4]arene receptors **3a** and **3b** were synthesized by introducing the more sensitive anthracene fluorophore as the signal units. Obviously fluorescence quenching and different fluorescent responses with the addition of small amounts of chiral malate were observed, which indicate that these two receptors **3a** and **3b** can be used as sensitive chemosensors for malate.

2. Results and discussion

2.1. Synthesis

The chiral fluorescence receptors **3a** and **3b** were synthesized by the reaction of intermediates **2a**, **2b** and

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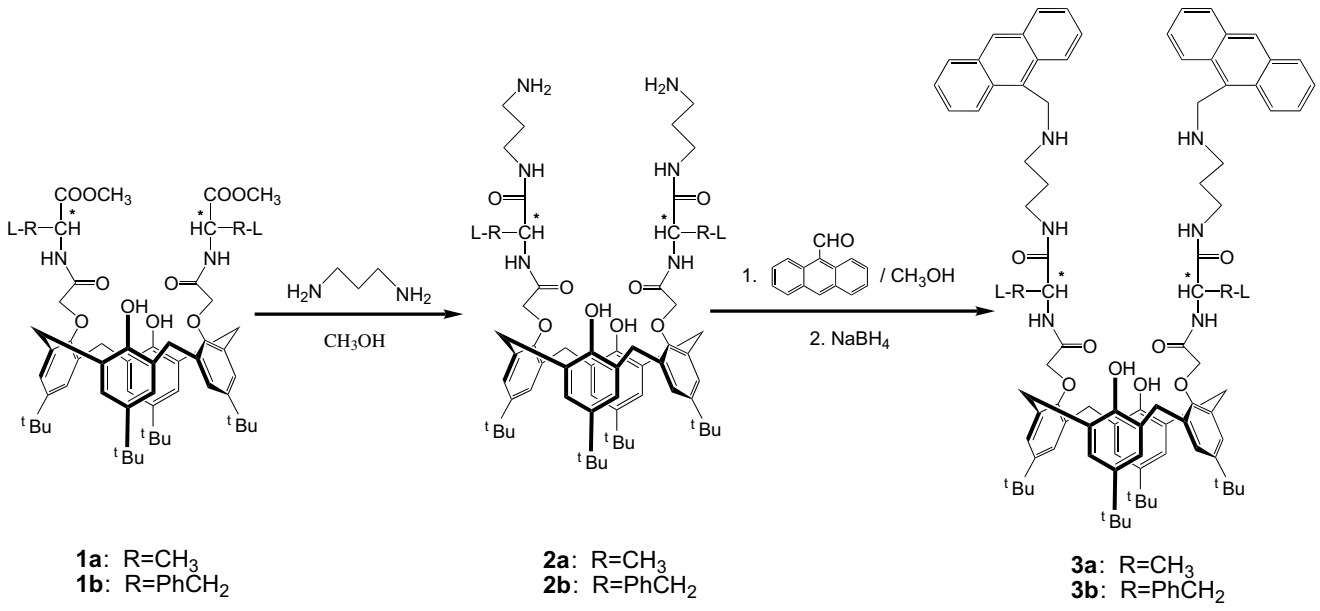

Scheme 1. The synthesis of receptors **3a** and **3b**.

9-anthraldehyde, and then reduced by $NaBH_4$ (Scheme 1). Compounds **1a** and **1b** were synthesized according to the literature.[7a] Receptors **2a**, **2b**, **3a** and **3b** are easily soluble in common organic solvents, such as $CHCl_3$, CH_3CN, CH_3OH, DMSO and DMF. The structures of these compounds were characterized by IR, ESI-MS, ^1H NMR, ^{13}C NMR spectra and elemental analysis.

The ^1H NMR spectra of **3a** and **3b** show two sets of AB quartets for the bridging methylene protons and two sets of singlets for the *tert*-butyl group. This indicates that the two receptors are in the cone conformation in $CHCl_3$. The ^1H NMR spectra of **3a** and **3b** also exhibit one set of doublets for the $ArOCH_2$ protons. This splitting pattern may relate to the introduction of the chiral moieties in the molecules, as seen in other chiral calix[4]arenes.[7,8]

2.2. Fluorescence and UV–vis spectra study

The fluorescence spectra were recorded from a solution of **3a** or **3b** in the absence and presence of malate; the counter cation was tetrabutylammonium. Considering that malate and the two receptors have a good solubility in the $CHCl_3$, $CHCl_3$ was chosen as the solvent in which receptors **3a** and **3b** both have good fluorescence response.

Figures 1 and 2 show the fluorescence emission spectra of a mixture of **3a** and different concentrations of L- or D-malate in $CHCl_3$ ($\lambda_{ex} = 373$ nm), respectively. When gradually increasing the concentration of malate, the fluorescence emission intensities of **3a** (5.0×10^{-5} mol L^{-1}) at 417 nm ($\lambda_{ex} = 373$ nm) decreased obviously. The quenching efficiency was about 40% with the addition of 2.7 equiv of L-malate (Fig. 1), while it was 70% by 2.7 equiv of D-malate. The different quenching efficiencies ($\Delta I_D/\Delta I_L = 1.75$) indicated that receptor **3a** has a good enantioselective recognition ability between D- and L-malate.

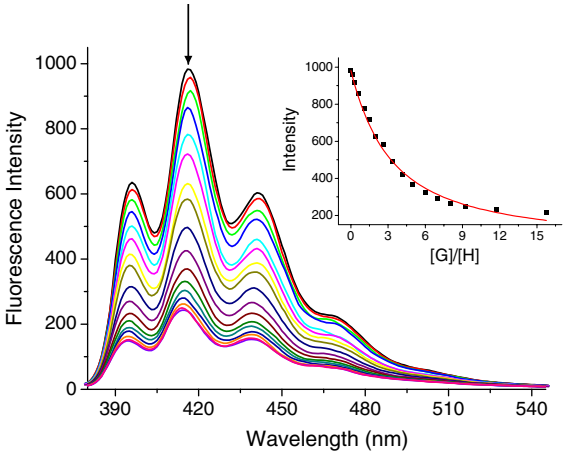

Figure 1. Fluorescence spectra of receptor **3a** (5.0×10^{-5} mol L^{-1}) with L-malate in $CHCl_3$. The equivalents of guest are: 0, 0.1, 0.3, 0.6, 1.1, 1.5, 2.0, 2.7, 3.2, 4.2, 5.0, 6.0, 7.0, 8.0, 9.2, 11.8 and 15.8. $\lambda_{ex} = 373$ nm. Inset: changes of fluorescence intensity of **3a** at 417 nm upon addition of L-malate. The line is fitting curve. The correlation coefficient (R) of non-linear curve fitting is 0.9936.

This fluorescence quenching phenomena can be attributed to the classical photoinduced electron transfer (PET) mechanism. In the absence of malate, the fluorescence quenching of receptor **3a** most likely arose from the change of free energy (ΔG_{PET}) of electron transfer between the photoexcited anthracene units and the bonding units of the receptor. When the malate interacted with receptor **3a**, an anion: receptor complex was formed through hydrogen bonding between the anion and receptor. This interaction would significantly enhance the reduction potential of the receptor, which would enhance the ratio of electron transfer from HOMO orbit of complex to the excited anthracene units and in turn would lead to the intramolecular PET process being easier.[9] Therefore, an anion-induced fluorescence

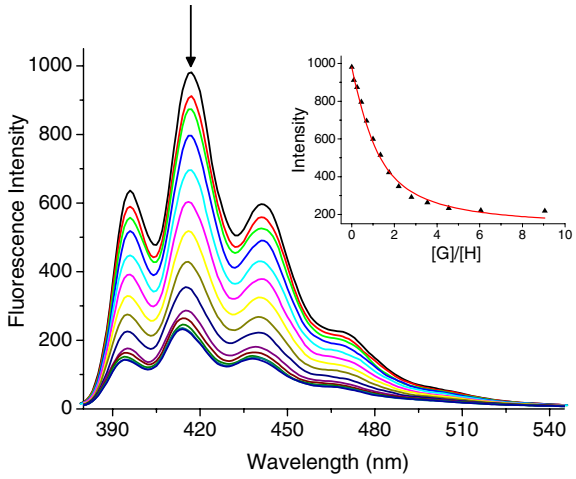

Figure 2. Fluorescence spectra of receptor **3a** (5.0×10^{-5} mol L^{-1}) with D-malate in $CHCl_3$. The equivalents of guest are: 0, 0.1, 0.25, 0.45, 0.7, 1.0, 1.35, 1.75, 2.2, 2.7, 3.5, 4.5, 6.0 and 9.0. $\lambda_{ex} = 373$ nm. Inset: changes of fluorescence intensity of **3a** at 417 nm upon addition of D-malate. The line is fitting curve. The correlation coefficient (R) of non-linear curve fitting is 0.9933.

quenching was observed. The UV–vis spectra study confirmed this explanation (see Fig. 7). The changes in the absorption spectra of the anthracene moiety were only minor for **3a** (peaks at 334, 350, 368, 388 nm) in the presence of L-malate, which implied that a PET process occurred with anion bonding.[10] Similar phenomena have been observed when **3a** interacted with D-malate. The presence of a methylene spacer acting as the insulating role minimized any ground interactions between the fluorophore and the anion receptor. It was proposed that upon anion recognition, the rate of the electron transfer from the HOMO orbit of the anion–receptor complex to the anthracene excited state enhanced, which caused the fluorescence emission to be quenched, with a little change of UV–vis spectra in the complexation.

The satisfactory result (the correlation coefficient is over 0.99) of the non-linear curve fitting confirmed that **3a** and L- or D-malate formed a 1:1 complex (see the top right plot of Figs. 1 and 2). For the complex of 1:1 stoichiometry, an association constant K_{ass} can be calculated by using the following equation in Origin 7.0:[11,12]

$$I = I_0 + \frac{I_{lim} - I_0}{2C_0}\left\{C_H + C_G + 1/K_{ass} - \left[(C_H + C_G + 1/K_{ass})^2 - 4C_H C_G\right]^{1/2}\right\}$$

where I represents the fluorescence intensity, and C_H and C_G are the corresponding concentrations of host and guest. C_0 is the initial concentration of host. The association constants (K_{ass}) and correlation coefficients (R) obtained by a non-linear least-squares analysis of I versus C_H and C_G are listed in Table 1.

Figures 3 and 4 show the changes in the fluorescence spectra of receptor **3b** (5.0×10^{-5} mol L^{-1}) with the addition of L- or D-malate. Receptor **3b** was more sensitive to malate compared with **3a**, when a small amount of L- or D-malate was introduced to the solution of **3b** in $CHCl_3$, the fluorescence emission of **3b** was quenched remarkably. The quenching efficiency was 36% with the addition of 1.8 equiv of L-malate, while it was 55% by 1.8 equiv of D-malate. The fluorescence quenching mechanism can be attribute to the PET process;[9] the changes in the absorption spectra of the anthracene moiety were also minor for **3b** in the presence of L-malate (Fig. 7). The result of a non-linear curve fitting (at 416 nm) indicates that a 1:1 complex was formed between receptor **3b** and L- or D-malate (see the top right plot of Figs. 3 and 4). In addition, the association constants (K_{ass}) are different; the association constant of **3b** with L-malate is $(1.96 \pm 0.28) \times 10^4$ M^{-1}; while that of **3b** with D-malate is $(2.04 \pm 0.32) \times 10^5$ M^{-1}, which corresponds to the D/L-selectivity ($K_{ass(D)}/K_{ass(L)}$) of 10.41 for malate.

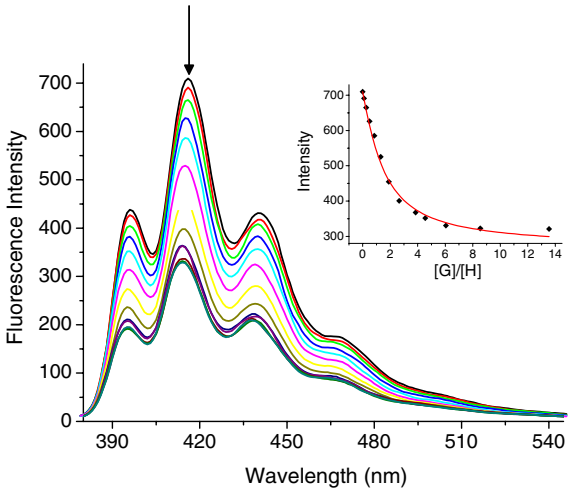

Figure 3. Fluorescence spectra of receptor **3b** (5.0×10^{-5} mol L^{-1}) with L-malate in $CHCl_3$. The equivalents of guest are: 0, 0.1, 0.25, 0.5, 0.85, 1.3, 1.8, 2.6, 3.8, 4.5, 6.0, 8.5 and 13.5. $\lambda_{ex} = 373$ nm. Inset: changes of fluorescence intensity of **3b** at 416 nm upon addition of L-malate. The line is fitting curve. The correlation coefficient (R) of non-linear curve fitting is 0.9908.

Table 1. Association constants (K_{ass}), correlation coefficients (R) and enantioselectivities K_D/K_L of receptors **3a** and **3b** with D- or L-malate in $CHCl_3$ at 25 °C

Anion[a]	Receptor **3a**			Receptor **3b**		
	K_{ass}[b] (M^{-1})	R	K_D/K_L	K_{ass}[b] (M^{-1})	R	K_D/K_L
D-malate	$(3.24 \pm 0.41) \times 10^4$	0.9933	4.34	$(2.04 \pm 0.32) \times 10^5$	0.9961	10.41
L-malate	$(7.46 \pm 0.63) \times 10^3$	0.9936		$(1.96 \pm 0.28) \times 10^4$	0.9908	

[a] The anions were used as their tetrabutylammonium salts.
[b] All error values were obtained by the results of non-linear curve fitting.

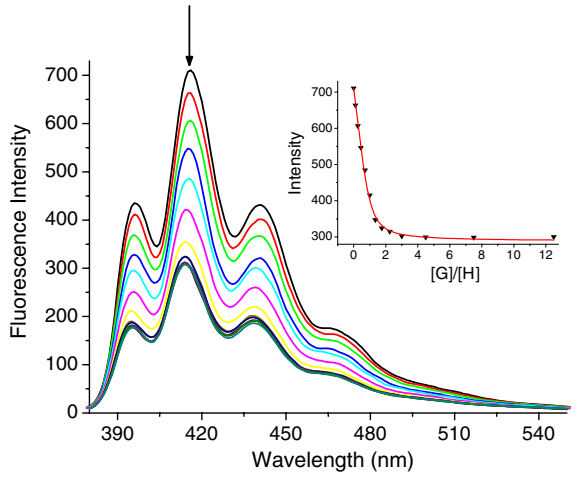

Figure 4. Fluorescence spectra of receptor **3b** (5.0×10^{-5} mol L^{-1}) with D-malate in $CHCl_3$. The equivalents of guest are: 0, 0.1, 0.25, 0.45, 0.7, 1.0, 1.35, 1.8, 2.2, 3.0, 4.5, 7.5 and 12.5. $\lambda_{ex} = 373$ nm. Inset: changes of fluorescence intensity of **3b** at 416 nm upon addition of D-malate. The line is fitting curve. The correlation coefficient (R) of non-linear curve fitting is 0.9961.

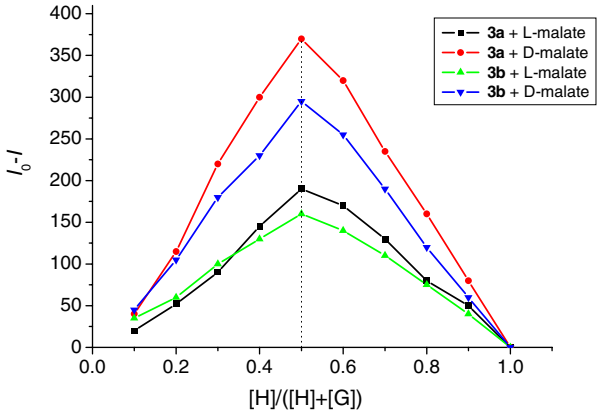

Figure 5. Job plots of **3a** or **3b** with L- and D-malate (at 416 nm). The total concentration of the host and guest is 1.0×10^{-5} mol L^{-1} in $CHCl_3$. I_0: fluorescence intensity of **3a** or **3b**; I: fluorescence intensity of **3a** or **3b** in the presence of the guest.

The continuous variation methods were employed to determine the stoichiometric ratio of receptor **3a** or **3b** with guest (L- or D-malate). The total concentration of the host and guest was constant (1.0×10^{-5} mol L^{-1}) in $CHCl_3$, with a continuously variable molar fraction of host ([H]/([H] + [G])). Figure 5 shows the Job plots of receptor **3a** or **3b** with L- or D-malate (at 416 nm). When the molar fraction of the host was 0.50, the fluorescence intensity reached a maximum, which demonstrated that receptors **3a** or **3b** formed a 1:1 complex with L- or D-malate, respectively.[13]

Figure 6 exhibits the fluorescence change of receptor **3a** or **3b** with L- or D-malate in $CHCl_3$. Different fluorescent responses for the chiral malate indicate that **3a** and **3b** both have good enantioselective recognition abilities for malate. The data in Table 1 illustrate that the two receptors can bind D- or L-malate in the same order: that is two receptors have stronger interaction with D-malate than with L-malate; receptor **3b** has more selectivity for recognition of D-malate. Although a lot of sensitive anion fluorescent sensors have been reported before, many of them focus their interests in haloid anion, dicarboxylic anion, dihydrogen phosphate or ATP/ADP.[9b,14] Sensitive chiral anion fluorescent sensors are still rare. Receptors **3a** and **3b** exhibit a very sensitive response to the malate, even the addition of 1.0 equiv of malate to the solution can induce obvious fluorescence quenching. We presume that maybe due to the strong interaction between the malate and receptors, the anthracene with excellent photophysical properties[15] could transduce the recognition process into fluorescent signal properly.

2.3. ^{1}H NMR study

^{1}H NMR experiments were undertaken to assess the chiral recognition properties between receptors **3a** and **3b** and the chiral malate because it can provide directly, structural and dynamic information.[16] Studies on the chiral recognition were carried out on a 300 MHz NMR spectrometer using the compounds **3a** and **3b** as chiral solvating agents in $CDCl_3$. Since the signal of chiral CH proton of malate has serious superposition with the signal of the hosts, we were unable to clarify the cleavage conditions of CH

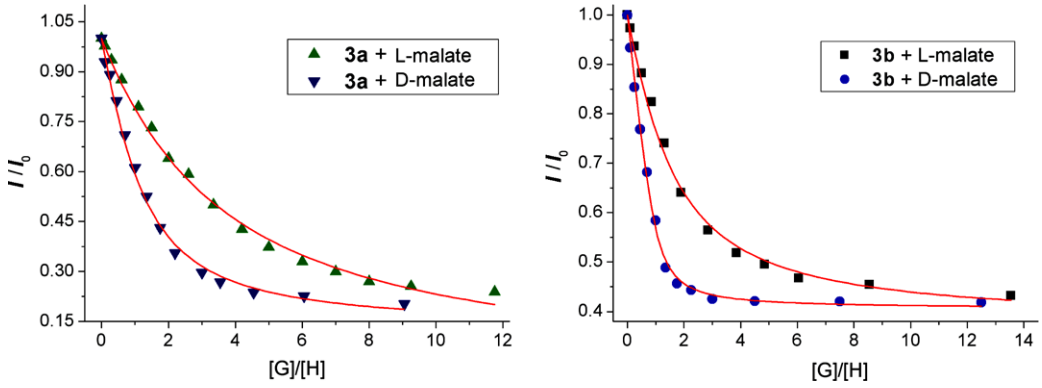

Figure 6. Fluorescence intensity change of receptor **3a** (left) or **3b** (right) (5.0×10^{-5} mol L^{-1}) with L-or D-malate in $CHCl_3$, the line is fitting curve.

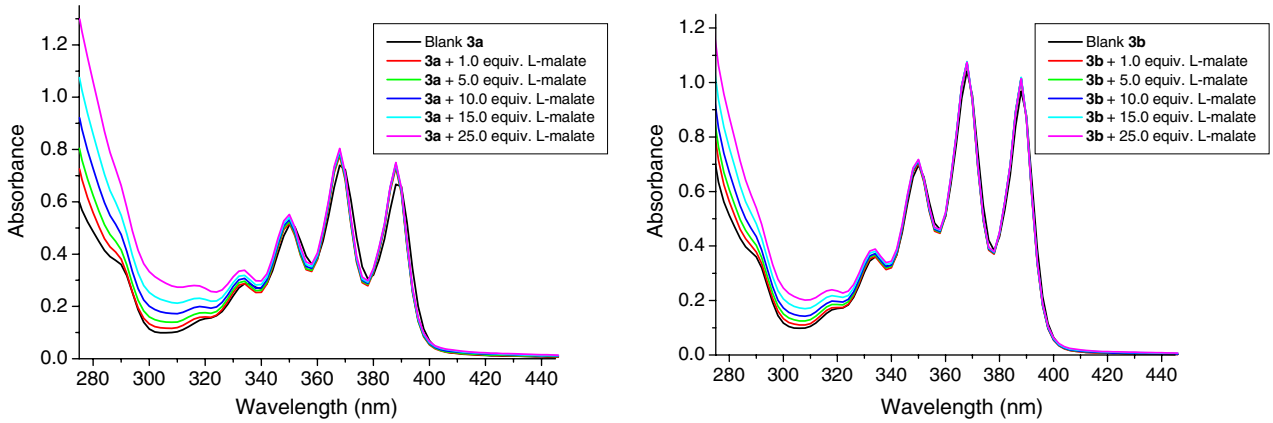

Figure 7. UV–vis spectra of receptors **3a** (left) or **3b** (right) (5.0×10^{-5} mol L^{-1}) with the addition of L-malate in $CHCl_3$. The equivalents of anion are: 0, 1.0, 5.0, 10, 15 and 25.

proton when the receptors interacted with racemic malate, so we did not list the corresponding spectra.

Figure 8 exhibits the spectra of receptor **3a** and its complex with equimolar amounts of L- or D-malate in $CDCl_3$. When treated with L-malate, the characteristic peak of amide (N***H*** **a**) had a upfield shift from 9.773 to 9.358 ppm, while the other amide (N***H*** **b**) became much weakened; when treated with D-malate, the peak of amide (N***H*** **a**) shifted to 9.255 ppm, while the amide (N***H*** **b**) nearly disappeared. The phenolic proton also had an obvious upfield shift from 8.484 to 7.645 ppm or 7.723 ppm for L- or D-malate, respectively. The peaks of the CH_2 protons linked with calix[4]arene skeleton showed obvious shrinkage and the chiral proton of receptor **3a** also was upfield shifted from 4.773 to 4.546 or 4.480 ppm when L- or D-malate was added to the solution. The above results indicate that **3a** has a stronger interaction with D-malate than with L-malate; while the structure of **3a** also underwent an obvious change when it interacted with malate, which maybe due to the strong hydrogen bonding interaction between the malate and the amides of receptor caused by the two branches of **3a**. Multiple hydrogen bonding interaction and structure-complementary between host and guest may result in good chiral recognition of **3a** for the enantiomers of malate.

^{1}H NMR spectra of **3b** in the absence and presence of malate are shown in Figure 9. Upon adding an equimolar amount of L- or D-malate to a solution of **3b**, the characteristic peaks of amide (N***H*** **b**) disappeared, while the other amide (N***H*** **a**) had an upfield shift from 9.285 to 9.004 or 8.972 ppm for L- or D-malate, respectively, which suggested that the interaction between host and guest mainly happened through multiple hydrogen bonding.[6b,7,17] Compared with **3a**, the structure of **3b** had no obvious change when it interacted with L- or D-malate, except some proton signals cleaved into more complicated signals, which suggested that **3b** has a more rigid structure than that of **3a**. The spatial hindrance caused by the aromatic phenyl groups of phenylalanine units might limit the two branches of **3b** to interact with the anion guest. The rigidity effect is

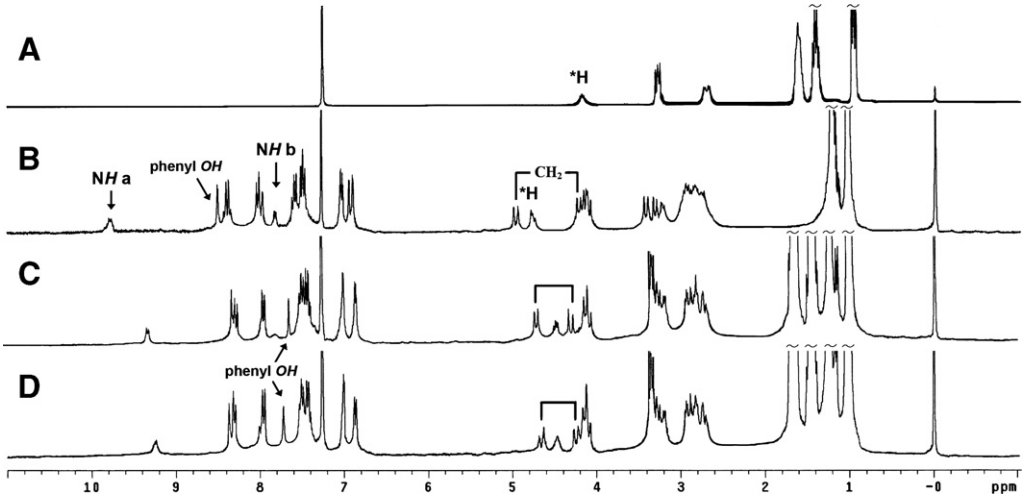

Figure 8. The ^{1}H NMR spectra of **3a** and its guest complex at 25 °C ([**3a**] = [guest] = 2.0×10^{-3} M) in $CDCl_3$ at 300 MHz: (A) racemic malate; (B) receptor **3a**; (C) receptor **3a** + L-malate; (D) receptor **3a** + D-malate.

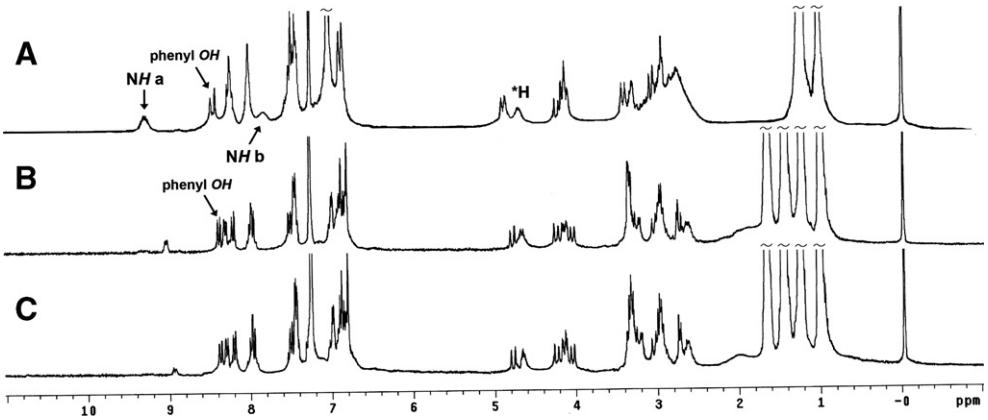

Figure 9. The ^{1}H NMR spectra of **3b** and its guest complex at 25 °C ([**3b**] = [guest] = 2.0×10^{-3} M) in $CDCl_3$ at 300 MHz: (A) receptor **3b**; (B) receptor **3b** + L-malate; (C) receptor **3b** + D-malate.

an important factor in the design of receptors; many chiral macrocyclic or binaphthyl derivatives with rigid structures have been synthesized and proved their good enantiomeric recognition abilities.[1c,2a,18] We presume that receptor **3b** exhibits better selectivity for the enantiomers of malate in the fluorescence titration and thus can be attributed to its much more rigid structure than receptor **3a**.

3. Conclusion

In conclusion, two chiral fluorescence receptors **3a** and **3b** have been synthesized. The enantioselective recognition of the receptors was studied by fluorescence and ^{1}H NMR spectra. Receptors **3a** and **3b** exhibit good chiral recognition abilities towards the enantiomers of D- and L-tetrabutylammonium malate, and formed a 1:1 complex between the host and guest; receptor **3b** has a better enantioselective recognition ability than receptor **3a**. The receptors' good preorganization property, steric effect, relative rigidity, structure-complementary with guest and multiple hydrogen bonding may be responsible for the enantioselective recognition of malate. Sensitive fluorescent responses and good enantioselective recognition abilities reveal that these two receptors can be used as fluorescent chemosensors for malate.

4. Experimental

4.1. General

Propylenediamine was distilled before use. $CHCl_3$ was washed with water and dried from $CaCl_2$, Et_3N was dried and distilled from CaH_2 and KOH, respectively. All other commercially available reagents were used without further purification. Melting points were determined with a Reichert 7905 melting-piont apparatus and are uncorrected. Optical rotations were taken on a PerkinElmer Model 341 polarimeter. IR spectra were obtained on a Nicolet 670 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$, on a Varian Mercury VX-300 MHz spectrometer. ^{13}C NMR spectra were recorded on a Varian Inova unity-600 MHz spectrometer. Mass spectra were recorded on a Finnigan LCQ advantage mass spectrometer. Elemental analysis was determined with a Carlo-Erba 1106 instrument. Fluorescence spectra were obtained on a Shimadzu RF-5301 spectrometer. The UV–vis spectra were performed with a TU-1901 spectro-photomer. The malate was used as their tetrabutylammonium salts. Compounds **1a**, **1b** and 9-anthraldehyde were synthesized according to the methods reported in the literature, respectively.[7a,19]

4.2. Synthesis

General procedure for the synthesis of the calix[4]arene derivatives **2a** and **2b**: a solution (20 mL) of **1a** or **1b** (1.5 mmol) in CH_3OH was added dropwise to a stirred solution of propylenediamine (0.66 g, 9.0 mmol) in CH_3OH (10 mL). The mixture was stirred for 48 h under N_2 at room temperature. The solvent and excess propylenediamine were removed under reduced pressure and the residue was dried in vacuo to give product **2a** or **2b** as a yellow solid, no further purification for the next reaction.

4.2.1. 5,11,17,23-Tetra-4-*tert*-butyl-25,27-bis(aminopropylamino-L-alanine-*N*-carbonylmethoxy)-26,28-dihydroxycalix[4]arene 2a. Pure product was obtained as a yellow powder (1.50 g) in 98% yield; mp 152–154 °C; $[\alpha]_D^{20} = +5.2$ (*c* 0.015, $CHCl_3$); IR (KBr/cm^{-1}) *v*: 3438, 3046, 2959, 2871, 1655, 1602, 1546, 1483, 1443, 1385, 1361, 1303, 1244, 1204, 1125, 1044, 872, 819, 629, 573; ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 18H, Bu^t), 1.33 (s, 18H, Bu^t), 1.49 (d, $J = 7.2$ Hz, 6H, CCH_3), 2.80–2.86 (m, 8H, $CH_2CH_2NH_2$), 3.33–3.45 (m, 8H, $CONHCH_2CH_2$), 3.48 (d, $J = 13.2$ Hz, 4H, $ArCH_2Ar$), 4.23 (d, $J = 12.9$ Hz, 4H, $ArCH_2Ar$), 4.49 (d, $J = 15.3$ Hz, 2H, OCH_2CO), 4.67–4.72 (m, 2H, NC^*HCO), 4.80 (d, $J = 15.6$ Hz, 2H, OCH_2-CO), 6.95 (s, 4H, ArH), 7.10 (s, 4H, ArH), 7.52 (s, 2H, $CONHCH_2$), 7.80 (s, 2H, ArOH), 9.34 (d, $J = 8.1$ Hz, 2H, CONH); ^{13}C NMR ($CDCl_3$): δ (ppm) 18.2, 18.6, 31.2, 31.8, 32.4, 34.0, 34.3, 36.5, 37.7, 39.7, 40.0, 45.6, 48.9, 49.3, 75.0, 125.5, 125.6, 126.2, 126.5, 127.3, 127.4, 132.5, 132.6, 143.0, 148.3, 149.6, 149.7, 150.1, 169.2, 169.4, 172.4, 172.7. Elemental Anal. Calcd for

$C_{60}H_{86}N_6O_8$: C, 70.70; H, 8.50; N, 8.24. Found: C, 70.68; H, 8.62; N, 8.23.

4.2.2. 5,11,17,23-Tetra-4-*tert*-butyl-25,27-bis(aminopropyl-amino-L-phenylalanine-*N*-carbonylmethoxy)-26,28-dihydroxycalix[4]arene 2b. Pure product was obtained as a yellow powder (1.70 g) in 97% yield; mp 157–158 °C; $[\alpha]_D^{20} = -11.3$ (*c* 0.015, $CHCl_3$); IR (KBr/cm^{-1}) *v*: 3439, 3058, 2959, 2867, 1654, 1602, 1547, 1483, 1431, 1385, 1360, 1301, 1236, 1204, 1124, 1047, 872, 738, 700, 573; ^1H NMR ($CDCl_3$): δ 1.01 (s, 18H, Bu^t), 1.28 (s, 18H, Bu^t), 2.57–2.64 (m, 4H, CH_2NH_2), 2.76–2.81 (m, 8H, $CH_2CH_2NH_2$), 3.10–3.24 (m, 8H, $ArCH_2$, $CONHCH_2$), 3.26–3.31 (m, 2H, $ArCH_2Ar$), 3.40 (d, $J = 13.5$ Hz, 2H, $ArCH_2Ar$), 4.05 (d, $J = 13.2$ Hz, 2H, $ArCH_2Ar$), 4.14 (d, $J = 13.2$ Hz, 2H, $ArCH_2Ar$), 4.26 (d, $J = 14.7$ Hz, 2H, OCH_2CO), 4.79 (d, $J = 14.7$ Hz, 2H, OCH_2CO), 4.87–4.89 (m, 2H, NC^*HCO), 6.84 (s, 4H, ArH), 7.04 (s, 4H, ArH), 7.15–7.18 (m, 10H, ArH), 7.69 (s, 2H, $CONHCH_2$), 7.77 (s, 2H, ArOH), 9.28 (d, $J = 8.4$ Hz, 2H, CONH), 9.79 (s, 2H, CONH); ^{13}C NMR ($CDCl_3$): δ (ppm) 31.1, 31.9, 32.3, 32.8, 34.2, 36.3, 37.8, 39.1, 40.2, 45.0, 53.6, 54.6, 56.3, 74.6, 125.9, 126.4, 126.7, 127.0, 127.9, 128.2, 128.5, 128.9, 129.5, 130.2, 132.5, 132.7, 137.5, 142.4, 147.8, 150.2, 150.6, 169.1, 171.0. Elemental Anal. Calcd for $C_{72}H_{94}N_6O_8$: C, 73.81; H, 8.09; N, 7.17. Found: C, 73.75; H, 8.12; N, 7.15.

4.3. General procedure for the synthesis of 3a and 3b

A mixture of **2a** or **2b** (1 mmol) and 9-anthraldehyde (0.45 g, 2.2 mmol) in CH_3OH (10 mL) was stirred for 48 h at room temperature, and then $NaBH_4$ (0.20 g) was poured into the solution. The mixture was stirred for 24 h under N_2 protection at ambience temperature. The mixture was then heated to 50 °C and stirred for 2 h. The solvent was removed under reduced pressure; the residue was washed with water. The crude product was purified by column chromatography on silica gel using $CHCl_3$/CH_3OH as eluant to obtain pure products **3a** and **3b**, respectively.

4.3.1. 5,11,17,23-Tetra-4-*tert*-butyl-25,27-bis(*N*-2-((anthracen-9-yl)methylamino)propyl-amino-L-alanine-carbonylmethoxy)-26,28-dihydroxycalix[4]arene 3a. Pure product (0.57 g) was obtained by column chromatography on silica gel (eluant: $CHCl_3/CH_3OH = 10:1$ (v/v)) as a yellow powder in 40.7% yield; mp 168–170 °C; $[\alpha]_D^{20} = -12.5$ (*c* 0.015, $CHCl_3$); IR (KBr/cm^{-1}) *v*: 3420, 3054, 2958, 2863, 1709, 1652, 1599, 1544, 1483, 1447, 1384, 1361, 1299, 1224, 1202, 1159, 1124, 1089, 1038, 887, 735, 602, 532; ^1H NMR ($CDCl_3$): δ 1.04 (s, 18H, Bu^t), 1.24 (s, 18H, Bu^t), 1.18 (d, $J = 5.1$ Hz, 6H, CCH_3), 2.65–2.96 (m, 12H, $CONHCH_2CH_2CH_2$, 2H, Anthr-CH_2NH), 3.25 (d, $J = 13.2$ Hz, 4H, Anthr-CH_2), 3.38 (d, $J = 14.7$ Hz, 2H, $ArCH_2Ar$), 3.46 (d, $J = 7.5$ Hz, 2H, $ArCH_2Ar$), 4.08 (d, $J = 7.6$ Hz, 2H, $ArCH_2Ar$), 4.13 (d, $J = 14.8$ Hz, 2H, $ArCH_2Ar$), 4.19 (d, $J = 13.2$ Hz, 2H, OCH_2CO), 4.69–4.76 (m, 2H, NC^*HCO), 4.92 (d, $J = 12.8$ Hz, 2H, OCH_2CO), 6.88 (s, 2H, ArH), 6.92 (s, 2H, ArH), 7.00 (s, 2H, ArH), 7.02 (s, 2H, ArH), 7.45–7.49 (m, 6H, Anthr-H), 7.55 (d, $J = 8.1$ Hz, 4H, Anthr-H), 7.81 (s, 2H, $CONHCH_2$), 7.99 (d, $J = 8.1$ Hz, 4H, Anthr-H), 8.35 (d, $J = 8.7$ Hz, 4H, Anthr-H), 8.47 (s, 2H, ArOH), 9.72 (s, 2H, CONH); ^{13}C NMR (600 MHz, $CDCl_3$): δ (ppm) 19.2, 27.9, 31.1, 31.8, 32.5, 34.0, 34.2, 37.7, 44.2, 45.4, 46.5, 48.8, 74.9, 123.9, 125.4, 125.8, 126.7, 126.9, 127.1, 127.4, 129.1, 129.4, 131.1, 131.6, 132.7, 132.8, 134.2, 142.6, 148.1, 149.7, 150.4, 169.1, 172.0; ESI-MS *m*/*z* (%): 1399.7 (M^+, 100). Elemental Anal. Calcd for $C_{90}H_{106}N_6O_8$: C, 77.22; H, 7.63; N, 6.00. Found: C, 77.19; H, 7.65; N, 5.98.

4.3.2. 5,11,17,23-Tetra-4-*tert*-butyl-25,27-bis(*N*-2-((anthracen-9-yl)methylamino)propyl-amino-L-phenylalanine-carbonylmethoxy)-26,28-dihydroxycalix[4]arene 3b. Pure product (0.60 g) was obtained by column chromatography on silica gel (eluant: $CHCl_3/CH_3OH = 15:1$ (v/v)) as a yellow powder in 38.7% yield; mp 144–146 °C; $[\alpha]_D^{20} = -36.2$ (*c* 0.015, $CHCl_3$); IR (KBr/cm^{-1}) *v*: 3446, 3058, 2958, 2863, 1707, 1649, 1598, 1482, 1447, 1384, 1358, 1295, 1225, 1201, 1123, 1038, 878, 734, 696, 534; ^1H NMR ($CDCl_3$): δ 1.02 (s, 18H, Bu^t), 1.27 (s, 18H, Bu^t), 2.78–2.98 (m, 12H, $CONHCH_2CH_2CH_2$, 2H, Anthr-CH_2NH, 4H, $ArCH_2$), 3.05 (d, $J = 13.2$ Hz, 4H, Anthr-CH_2), 3.31–3.34 (m, 2H, $ArCH_2Ar$), 3.38 (d, $J = 13.8$ Hz, 2H, $ArCH_2Ar$), 4.10–1.17 (m, 4H, $ArCH_2Ar$), 4.20 (d, $J = 14.8$ Hz, 2H, OCH_2CO), 4.68–4.75 (m, 2H, NC^*HCO), 4.86 (d, $J = 15.6$ Hz, 2H, OCH_2CO), 6.85 (s, 4H, ArH), 6.92 (s, 4H, ArH), 7.00–7.02 (m, 10H, ArH), 7.02 (s, 2H, ArH), 7.44–7.52 (m, 10H, Anthr-H), 8.00 (s, 4H, Anthr-H), 7.84 (s, 2H, $CONHCH_2$), 8.21–8.26 (m, 4H, Anthr-H), 8.40 (s, 2H, ArOH), 9.28 (s, 2H, CONH); ^{13}C NMR (600 MHz, $CDCl_3$): δ (ppm) 31.3, 31.6, 32.1, 33.8, 34.1, 34.3, 37.1, 40.0, 40.4, 43.0, 46.8, 49.0, 53.6, 74.7, 121.7, 123.9, 125.7, 126.0, 126.5, 128.0, 128.3, 129.6, 130.6, 131.8, 132.9, 137.0, 142.3, 142.5, 147.9, 149.9, 150.4, 154.0, 169.5, 170.0, 171.6; ESI-MS *m*/*z* (%): 1551.8 (M^+, 100). Elemental Anal. Calcd for $C_{102}H_{114}N_6O_8$: C, 78.94; H, 7.40; N, 5.41. Found: C, 78.89; H, 7.43; N, 5.39.

4.4. Tetrabutylammonium salts

The tetrabutylammonium salts were prepared by adding 2 equiv of tetrabutylammonium hydroxide in methanol to a solution of the corresponding malic acid (1 equiv) in methanol. The mixture was stirred at room temperature for 2 h and evaporated to dryness under reduced pressure. The resulting syrup was dried at high vacuum and 50 °C for 24 h, checked by NMR and stored in a desiccator.

4.5. Binding studies

The host compounds **3a** and **3b** were prepared as a stock solution in $CHCl_3$ for 5×10^{-4} mol L^{-1}. Malate was prepared to approximate 0.0175 and 0.175 mol L^{-1} of stock solution in $CHCl_3$. The work solutions were prepared by adding different volumes of malate solution to a series of the test tubes, then, the same amount of stock solution of host compound was added into each of test tubes followed by dilution to 3.5 ml by $CHCl_3$. After being shaken for several minutes, the work solution could be measured immediately. The excited wavelengths were both 373 nm, the excitation slit width was 3 nm and the emission slit widths

were 5 nm (**3a**) or 3 nm (**3b**). The association constants were calculated by means of a non-linear least-square curve fitting method with Origin 7.0 (Origin-Lab Corporation). ^{1}H NMR studies were recorded as adding equivalent L- or D-malate into receptors (2.0×10^{-3} M).

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