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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 7857–7860

Fluorescent enantioselective receptor for *S*-mandelate anion based on cholic acid

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Received 23 March 2006; revised 5 September 2006; accepted 6 September 2006

Abstract—A chiral fluorescent receptor **1** based on cholic acid was designed and synthesized. The enantioselective recognition ability of **1** to mandelate anion was studied by the fluorescence in CH_3CN and a ^1H NMR spectroscopic method. The results indicate that **1** exhibited a good enantioselectivity to the enantiomer of the mandelate anion in CH_3CN.

The development of molecule-based enantioselective fluorescent sensors is receiving growing research attention.[1–3] The application of this method will be of great significance in the combinatorial high through-put assay of chiral drugs and catalysts. L-Mandelic acid presents as a metabolite of phenylalanine in mammalian cells, plays an important role in biological systems.[4] When the hydroxylation of phenylalanine to tyrosine is defective, for example, in phenylketo-nuria (PKU), the former is metabolized by alternative pathways in which mandelate is produced.[5] Therefore, the synthesis of enantioselective receptors for the mandelate anion has attracted considerable interest.[6–17] Among the works on the fluorescence-based chiral recognition, only limited reports about the enantioselectivity of the mandelate anion have appeared.[7,16,17]

Cholic acid has recently emerged as a promising natural material to construct supramolecular systems for molecular recognition.[18–20] By virtue of its inherent chiral scaffold, it is extensively used as the skeleton of chiral receptors for sequence-selective peptide recognition and enzyme modelling.[21–23] The viability of being chemically modified on C3, C7, C12 and even C24[24] permits us to conduct chiral receptors by an exquisite design and masterly synthesis. By the extraction method, Davis's group reported a chiral receptor based on cholic acid, which showed a significant enantioselectivity to phenylalanine derivatives.[25] The use of fluorescence-based enantioselective sensors is particularly appealing because fluorescence can potentially provide both a high sensitivity and real-time measurement. To continue our interest in fluorescent chemosensor development, we have undertaken the design and synthesis of a cholic acid-based chiral chemosensor for mandelate. Introducing a suitable binding subunit on C3 and two signal display units on C7 and C12 perhaps will lead to a chiral receptor for mandelate. The thiourea group is a strong H-bond donor, also structurally complementary to the carboxylate group, and widely used in design receptors for anions.[26] On the other hand, the pyrenyl group, an excellent fluorophore,[27] could be appended onto the C7 and C12 of cholic acid and its bulky size may create a binding site favouring chiral recognition when introduced onto the C7 and C12 of cholic acid. With the backdrop of this strategy, we synthesized receptor **1** (Scheme 1) and control compound **2**.

The treatment of pyrene-2-carbonyl chloride with **3**[28] afforded the key intermediate **4** in a mixture of xylene and dichloromethane (Scheme 1). The resulting compound **4** was reduced by zinc dust and followed by the condensation reaction with 4-trifluoromethylphenyl-isothio-cyanate affording the target compound **1**. When excited at 352 nm in CH_3CN, receptor **1** (1.0×10^{-6} M) displayed a monomer emission at 388 nm and an excimer emission at 488 nm, with an intensity ratio of

Keywords: Cholic acid; Mandelate; Fluorescence; Enantioselectivity.
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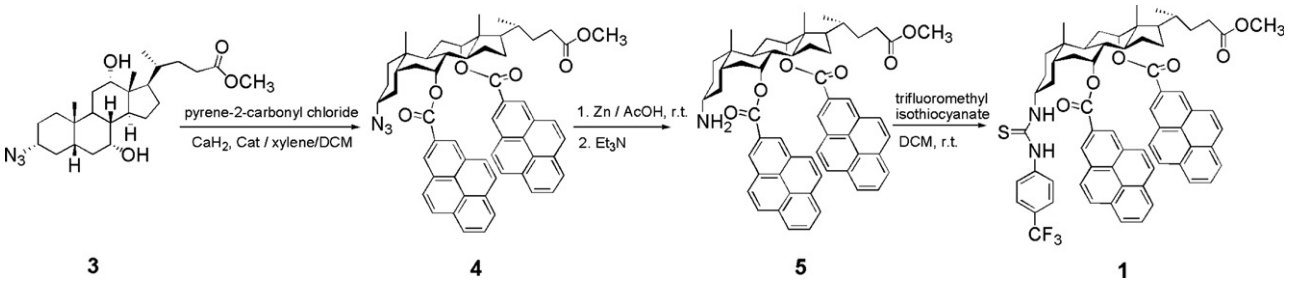
doi:10.1016/j.tetlet.2006.09.031



Scheme 1. Synthetic routes for receptor **1**.

monomer to excimer emission (I_M/I_E) is 2.84. Similarly, **2** (also excited at 352 nm) gave a monomer emission at 388 nm and an excimer emission at 488 nm, respectively, and the I_M/I_E is 0.84. Compared with **2**, **1** emitted a much weaker emission (Fig. 1), which indicated that the thiourea group was responsible for the quenching of both the monomer and excimer of receptor **1**. This is conceivably attributable to the fact that the thiourea groups take part in the photo-induced electron transfer (PET) process in which an electron is transferred from a lone-pair electron of the sulfur atom to two excited pyrenyl units of **1**.[29] After binding the anion, the electron-donating power of the thiourea group in the host perhaps can be enhanced, leading to a stronger PET process from the thiourea to the pyrenyl units (vide supra). Thus a fluorescent transduction of the binding behaviour between the host and the guest can be created. In CH_3CN, the intensity of the ratio of the monomer to the excimer is barely changed in the concentration range of 10^{-7}–10^{-4} M, implicating that its excimer emissions result from an intramolecular excimer but not from an intermolecular excimer.

To investigate the enantioselective ability of receptor **1**, the fluorescence titration of **1** (1.0×10^{-6} M) upon addition of *R*- and *S*-mandelate (used as their tetrabutylammonium salts) were performed in CH_3CN. Experimental results revealed that, with a gradual increase in the concentration of *S*-mandelate anion, both the monomer and excimer emissions of **1** can be quenched. When treated with a 500 equiv of the *S*-mandelate anion, the interaction of receptor **1** and the guest can be equilibrated with the monomer and the excimer quenched ca. 20% and 30%, respectively (Fig. 2). While treated with 500 equiv of the *R*-mandelate anion, in great contrast, the florescence of **1** could just be quenched less than 5% (Fig. 3). In this context, receptor **1** exhibited enantioselective factors of larger than 5.0 towards two antipodal forms of mandelate.

Assuming the complex stoichiometry was 1:1, the complexation of the guest *S*-mandelate anion with **1** could be calculated from a non-linear least squares fitting method[30] and gave an association constant (K_{ass}) of the complex of a value of 3.43×10^3 M^{-1}. The excellent relative coefficient ($R = 0.999$) demonstrated that the 1:1 complex between **1** and *S*-mandelate had been formed.[30–32] The change of the fluorescence intensity of **1** with *R*-mandelate is too small to calculate the association constant with this method. Obviously, **1** could

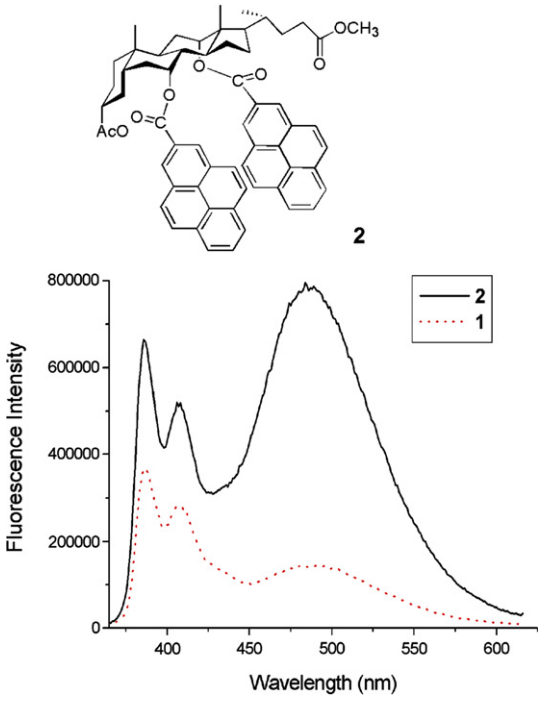


Figure 1. The fluorescence emission of spectra of **1** and **2** in CH_3CN (1.0×10^{-6} M), $\lambda_{ex} = 352$ nm.

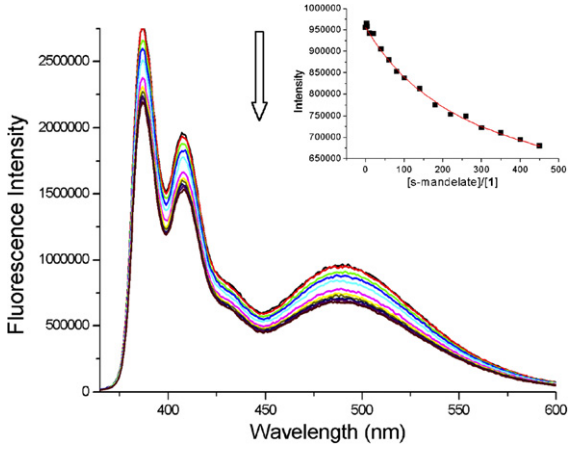


Figure 2. The fluorescence emission spectra of **1** (1.0×10^{-6} M) with *S*-mandelate in CH_3CN, $\lambda_{ex} = 352$ nm. Inset: the fluorescence intensity of **1** at 488 nm with different amounts of *S*-mandelate.

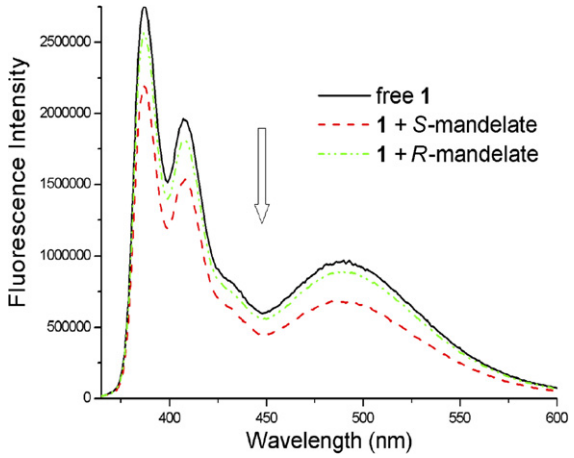


Figure 3. The fluorescence emission spectra of **1** (1.0×10^{-6} M) with 500 equiv of *S*-mandelate and *R*-mandelate in CH_3CN, respectively, $\lambda_{ex} = 352$ nm.

exert an enantioselective recognition of the racemic mandelate anion as demonstrated by the fluorescence titration experiments in CH_3CN. The chiral recognition is presumably attributed to the more complementary structure of *S*-mandelate with the receptor. A sample of racemic mandelate (1.0×10^{-4} M) with various enantiomeric compositions was prepared, and its interaction with receptor **1** (1.0×10^{-6} M) in CH_3CN was studied. A linear relationship between the fluorescence intensity and the enantiomeric composition of mandelate was obtained (Fig. 4). The finding corroborated well with the observed enantioselective recognition of mandelate by fluorescent receptor **1**. The enantioselectivity exhibited by the host towards mandelate was found to be highly solvent dependent. By changing the solvent to DMSO and chloroform, no quenching of host **1** was observed in their respective fluorescent titration with both enantiomers of mandelate (see the Supplementary data).

The ^{1}H NMR spectra further verified that **1** possesses enantioselectivity ability for mandelate anion. Because of the solubility problems, the ^{1}H NMR study could not be conducted in CH_3CN-d_3, instead it was carried out in DMSO-d_6. The protons of C*H* and O*H* in racemic mandelate coupled with each other and appeared as two doublet peaks at δ 4.33 and 5.12, respectively (see the Supplementary data). Treated with D_2O, the signal of the proton of O*H* disappeared and C*H* collapsed as a singlet at δ 4.52. When introducing 1 equiv of receptor **1**, the doublet signal at δ 4.33 of mandelate, becoming diastereomeric under the chiral influence of the host, was further split into two doublets at δ 4.29 and 4.25 ppm with an upfield shift of ca. $\Delta\delta$ 0.05. At the same time, the signal at δ 5.12 ascribed to the hydroxyl proton disappeared. On the other hand, the amido proton of N*H* of the host had significantly shifted from δ 9.24 to 9.69 as a result of complexation. In addition, the proton of C7 and C12 (at δ 5.38 and 5.56, respectively) was shifted slightly downfield. This strongly suggested that the interaction between host and guest had occurred through the formation of hydrogen bonding. Apparently, receptor **1** has exhibited enantioselectivity for the enantiomeric mandelate in ^{1}H NMR spectra.

To obtain more insight into the binding properties of **1** towards *S*-mandelate anions, some other carboxylate anions containing α- or β-functional group, such as L-phenylalanine, L-serine and lactate, were also subjected to the fluorescence study. Typical fluorescence change of **1** modulated with different amounts of acetate in CH_3CN was shown in Figure 5. Acetate can induce approximately 50% quenching of receptor **1**, which is more significant than *S*-mandelate. According to the fluorescence titration experiments, the association constants of **1** with other carboxylates were calculated and tabulated in Table 1. In contrast, when treated with all these anions, the fluorescence spectra of **2** had not changed, indicative of the lack of interaction between **2** and the guests.

On the basis of the respective association constants, **1** has a high affinity to acetate than any other carboxylate anions. Generally, the association constant of **1** with the carboxylate anion increases with an increase of the pK_a of the conjugated acid. The stronger conjugated acid was the weaker binding behaviour anion exerted with receptor **1**. Consequently, receptor **1** showing a moder-

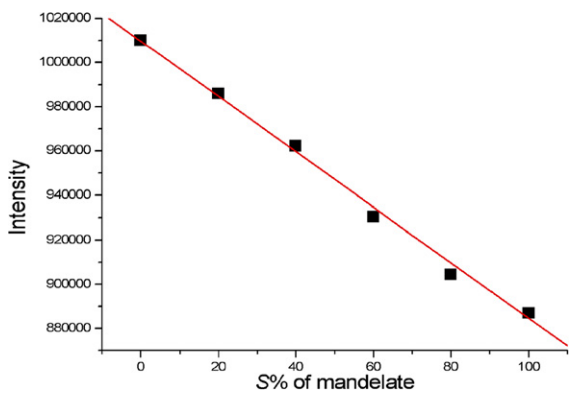


Figure 4. The fluorescence response of **1** (1.0×10^{-6} M) with mandelate (200 equiv) at various *S*-compositions in CH_3CN, $\lambda_{ex} = 352$ nm.

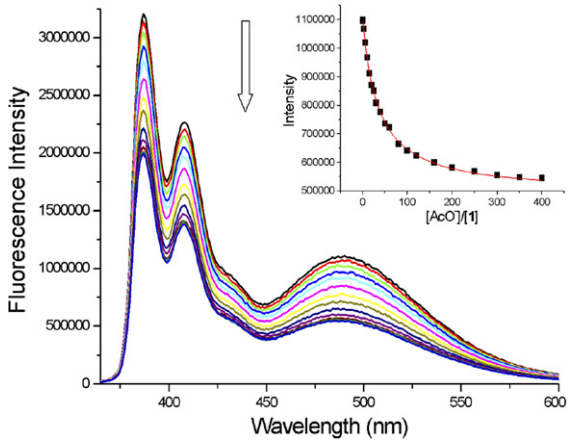


Figure 5. The fluorescence emission spectra of **1** (1.0×10^{-6} M) with acetate in CH_3CN, $\lambda_{ex} = 352$ nm. Inset: the fluorescence intensity of **1** at 488 nm with different amounts of acetate.

Table 1. Association constants K_{ass} (M^{-1}) and relative coefficients (R) of **1** with the carboxylate anion in CH_3CN

Anion	pK_a [a]	K_{ass} [b]	R [b]
Acetate[c]	4.74	$(2.85 \pm 0.09) \times 10^4$	0.9901
Benzonate[c]	4.19	$(1.04 \pm 0.06) \times 10^4$	0.9984
Lactate[c]	3.86	$(1.55 \pm 0.14) \times 10^3$	0.9991
L-Alanine[c]	3.60	$(2.55 \pm 0.21) \times 10^4$	0.9967
S-Mandelate[c]	3.37	$(3.43 \pm 0.30) \times 10^3$	0.9993
R-Mandelate[c]	3.37	—[d]	—[d]
L-Phenylalanine[c]	2.58	$(8.60 \pm 0.07) \times 10^3$	0.9981
L-Serine[c]	2.21	$(1.64 \pm 0.16) \times 10^3$	0.9975

[a] The values are the pK_as of the conjugated acid at 25 °C and came from the Merck Index.
[b] The values were calculated from the change of the fluorescence spectra.
[c] Anions were used as their tetrabutylammonium salts.
[d] The value is too small to be calculated.

ate affinity to *S*-mandelate likely gives rise from the decrease of proton-accepting ability of the carboxylate group affected by the α-hydroxyl group. The additional β-hydroxyl group in L-serine makes it more acidic as compared with alanine and leads to a much weaker binding interaction with host **1**. Interestingly, both the *S*-mandelic acid and L-phenylalanine are more acidic than lactic acid, while their conjugated anions show a stronger binding with receptor **1**. Consequently, the π–π interaction between the pyrenyl group of **1** and the phenyl group of *S*-mandelate or L-phenylalaine anion may be responsible for enhancing the binding interaction between the host and the guest.

In summary, a chiral fluorescent receptor **1** has been designed and synthesized. **1** showed a significant enantioselective ability to the enantiomers of the mandelate anion and gave an enantioselective discrimination of ca. 5.0. Fluorescent receptor **1** can be used to determine the compositions of the mandelate anion based on the linear relationship between the fluorescence intensity and the enantiomeric compositions of the guest. In addition, the binding ability of the host to carboxylate anions increasing with an increase in the pK_a of the conjugated acid was also demonstrated.

Acknowledgements

The financial support of the work by the grants from the University Grants Committee of the Hong Kong Special Administrative Region, China (AoE/P-10/01), and from the Hong Kong Batpist University (FRG/05-06/I-28) is acknowledged.

Supplementary data

The synthesis and characterization data of compounds **1**, **2**, **4** and **5**, the titration experiments, the NMR spectra of **1** and the ^1H NMR spectra of **1** with the racemic mandelate anion are available in a PDF format. The supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.09.031.

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