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A naphthoimidazolium-cholesterol derivative as a ratiometric fluorescence based chemosensor for the chiral recognition of carboxylates†

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Fluorescent chemosensors for sensing chiral molecules have been actively studied in recent years. In the current study, we report a naphthoimidazolium-cholesterol derivative (NI-chol 1) as a fluorescence based chemosensor for chiral recognition, in which the naphthoimidazolium serves not only as a fluorophore but also as a recognition moiety for anions *via* imidazolium (C–H)$^+$–anion binding and the cholesterol unit acts as a chiral barrier. In particular, NI-chol 1 displayed unique and distinct ratiometric changes with Boc-D-Phe, on the other hand, Boc-L-Phe induced a negligible change. Furthermore, a distinct downfield shift (from 9.64 ppm to 9.96 ppm) of the imidazolium C–H peak was observed for Boc-D-Phe (5 eq.) with severe broadening, which indicates strong ionic hydrogen bonding between the C–H proton and the carboxylate.

Since amino acids, the basic fundamental unit for peptides and proteins, are chiral, important processes in biology involve chiral interactions. Chiral fluorescence based chemosensors can convert enantioselective recognition into fluorescence changes,[1,2] which results in simple and easy detection methods for chiral recognition compared to conventional methods, such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), gas chromatography (GC) or NMR. Accordingly, fluorescence-based recognition for chiral anions has been extensively studied. Host–guest chemistry and molecular recognition have greatly affected the development of chiral fluorescence based chemosensors.[3] The main requirement for the fluorescence based approaches to evaluate enantiomeric compositions is chiral sensors with the ability to differentially interact with opposite enantiomers of a chiral target in a manner that gives rise to different optical signal outputs.

On the other hand, imidazolium based fluorescent chemosensors have been actively studied since they can show strong ionic hydrogen bonding interactions such as imidazolium (C–H)$^+$–anions.[4] Over the last decade, fluorescence based imidazolium derivatives have found application in the area of sensing simple anions,[5] nucleotides,[6] IP_3 and IP_6,[7] RNA or DNA,[8] anionic surfactants,[9] bacteria[10] and CO_2 gas,[11] *etc.*[12] Our group has actively investigated imidazolium based fluorescent chemosensors for various targets.[8] However, there are a paucity of papers for chiral recognition using fluorescence changes using imidazolium groups.[13,14] Yu and coworkers introduced an imidazolium moiety into BINOL (1,1′-bi-2-naphthol), which displayed red shifts with fluoride and acetate in acetonitrile and large fluorescence quenching effects with *t*-Boc Ala anions producing a K_L/K_D value of 4.5 in acetonitrile.[13a]

Compared to binaphthyl based chemosensors, there are relatively few examples in which the cholesterol moiety has been used as a chiral barrier.[14] In the current study, we report the example of a naphthoimidazolium-cholesterol derivative as a chiral fluorescence based chemosensor. Naphthoimidazoliums are inherently fluorescent, so an extra fluorophore is not required as in the case with imidazolium based fluorescence based chemosensors. **NI-chol 1** displayed a unique ratiometric change with Boc-D-Phe, while Boc-L-Phe induced very little change compared to that of the D-isomer. Similar but less distinct changes were also observed for Boc-D-Val over Boc-L-Val. In addition, a distinct downfield shift (from 9.64 ppm to 9.96 ppm) of the imidazolium C–H peak of **NI-chol 1** was observed for Boc-D-Phe (5 eq.) with severe broadening, which can be attributed to the strong ionic hydrogen bonding interaction between the C–H proton and the carboxylate.

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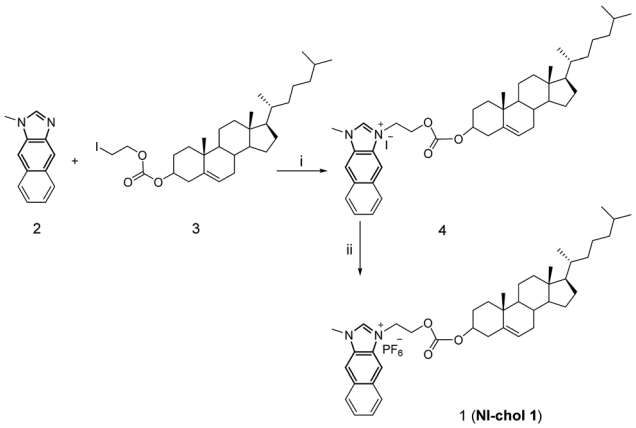

Scheme 1 The synthetic procedure of **NI-chol 1**. (i) Acetonitrile, N_2, reflux, 24 h, 81.1%; (ii) DMF, KPF_6, 98.6%.

Compound **2** was synthesized according to literature procedures.[5c] Compound **3** was prepared from cholesteryl chloroformate and 2-iodoethanol in the presence of pyridine in a yield of 80.8% after column chromatography using hexane/dichloromethane as eluents (1 : 1, v/v). Then, **2** and **3** were refluxed in acetonitrile to afford **4** as shown in Scheme 1, which was then treated with aqueous saturated KFP_6 to afford **1** (**NI-chol 1**) as a white solid. Detailed synthetic procedures are given in the Experimental section. All new compounds were fully characterized by ^1H and ^{13}C NMR as well as high-resolution mass spectroscopy (Fig. S1–S9, ESI†).

Fig. 1 illustrates the design strategy towards chiral probe **1**, in which naphthoimidazolium serves not only as a fluorophore but also as a recognition moiety for anions *via* imidazolium $(C–H)^+$–anion binding and the cholesterol unit acts as a chiral barrier.

The enantioselectivity of **NI-chol 1** for Boc-D-Phe detection was investigated through fluorescence emission spectroscopy by adding various amino acids to a solution of compound **NI-chol 1** in CH_3CN–DMSO (95 : 5, v/v). The chiral recognition was examined with tetrabutylammonium salts of Boc-D- and Boc-L-amino acid anions. In the absence of amino acids, **NI-chol 1** displayed an emission at 452 nm and the fluorescence quantum yield (Φ_f) of **NI-chol 1** was 0.27, using 9,10-diphenylanthracene standard with a Φ_f of 0.97 in cyclohexane (Fig. S10, ESI†). Among phenylalanine (Phe), valine (Val), serine (Ser) glutamine (Glu), leucine (Leu) and alanine (Ala), as shown in

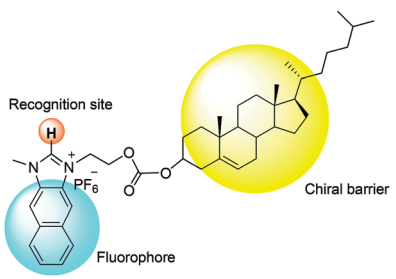

Fig. 1 Design strategy of **NI-chol 1**.

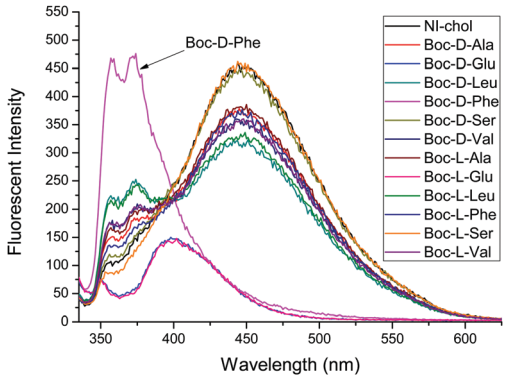

Fig. 2 Fluorescence changes of **NI-chol 1** (10 μM) in CH_3CN–DMSO (95 : 5, v/v) with D and L isomers of Ala, Glu, Leu, Phe, Ser and Val (500 μM). (λ_{ex} = 325 nm, slit 3 × 5).

Fig. 2, **NI-chol 1** displayed unique ratiometric changes with Boc-D-Phe with a Φ_f of 0.30. The blue shifted emission peak at 370 nm increases while the original emission at 452 nm decreases. A clear iso-emissive point appears at 401 nm. Even though a relatively smaller effect was observed for D-Val, a ratiometric change was clear enough to be observed. The blue shift to 370 nm from 452 nm can be attributed to the imidazolium C–H interaction with the carboxylate group of the amino acid. Electron withdrawing effects of the imidazolium moiety decrease when strong ionic hydrogen bonding occurs. Naphthoimidazolium has a donor–acceptor system and can undergo internal charge transfer (ICT) from naphthalene to the imidazolium upon excitation by light, with the imidazolium acting as an acceptor. The electron withdrawing effect of the imidazolium moiety decreases when strong ionic hydrogen bonding occurs.[5a] Hence, **NI-chol 1** behaves as a ratiometric fluorescence based probe for Boc-D-Phe against Boc-L-Phe.

Fig. 3 and 4 illustrate the fluorescence titrations of Boc-D-Phe and Boc-L-Phe. The blue shift to 370 nm from 452 nm can be attributed to the imidazolium C–H interaction with the carboxylate group of the amino acid. The association constant (K_D) was calculated using the software (SigmaPlot 2000) to be 2.13 × 10^3 M^{-1} based on the ratio of I_{370nm}/I_{452nm} for Boc-D-Phe.

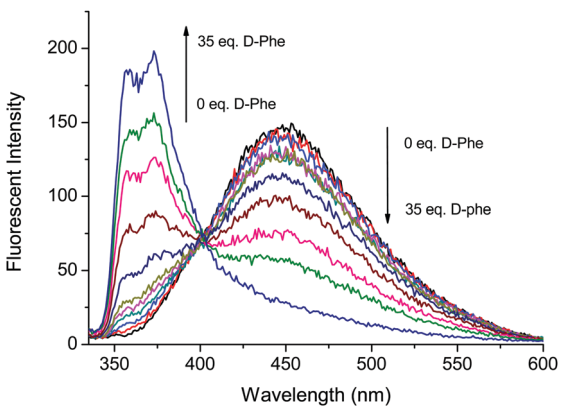

Fig. 3 Fluorescence based titrations of **NI-chol 1** (10 μM) in CH_3CN–DMSO (95 : 5, v/v) with various equivalents of Boc-D-Phe.

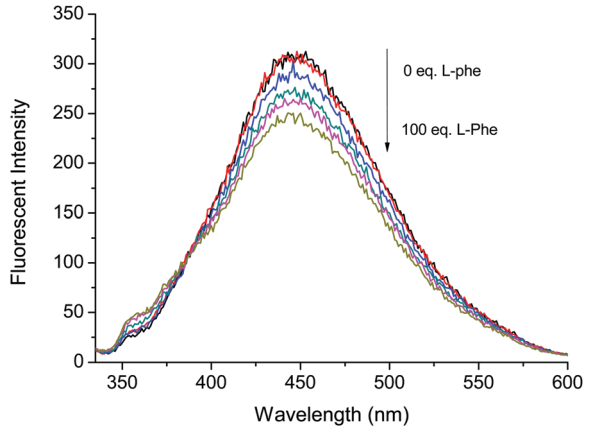

Fig. 4 Fluorescence based titrations of **NI-chol 1** (10 μM) in CH_3CN–DMSO (95 : 5, v/v) with various equivalents of Boc-L-Phe.

However, the addition of Boc-L-Phe induced only a small change as illustrated in Fig. 4. The association constant (K_L) for Boc-L-Phe was 1.5×10^2 M^{-1}. Thus, the enantioselectivity could be obtained *via* K_D/K_L as *ca.* 14, which is higher than those for most of the reported imidazolium-based chiral sensors. In addition, the fluorescence titrations with D-Val are illustrated in the ESI† (Fig. S11 and S12). Even though the changes were smaller than those with Boc-D-Phe, similar ratiometric changes were observed at 370 nm and 452 nm.

To understand the interaction of **NI-chol 1** with Boc-D-Phe, ^{1}H NMR titrations were performed in CD_3CN-DMSO-d_6 (95 : 5, v/v) (Fig. 5 and Fig. S13, ESI†). When 1.0 eq. of Boc-D-Phe was added to the solution of **NI-chol 1**, the imidazolium C–H peak at 9.64 ppm downfield shifted to 9.96 ppm, which indicates a hydrogen bonding interaction between the C–H proton and the carboxylate.

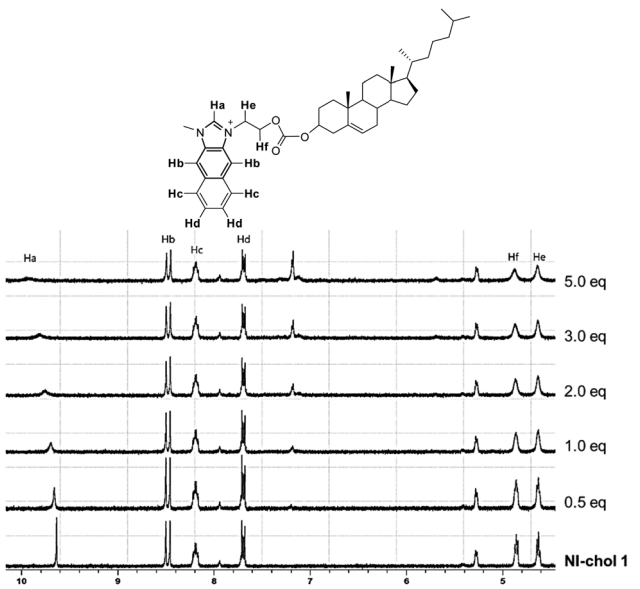

Fig. 5 Partial ^{1}H NMR spectra (300 MHz) of **NI-chol 1** (5 mM) upon the addition of Boc-D-Phe (tetrabutylammonium salt) in CD_3CN-DMSO-d_6 (95 : 5, v/v).

Furthermore, severe broadening of this peak was observed, which also supports the presence of strong ionic hydrogen bonding. With >5.0 eq. of Boc-D-Phe, the peak of imidazole C–H of **NI-chol 1** disappeared due to partial deprotonation. However, a smaller shift to 9.87 ppm was observed upon the addition of Boc-L-Phe (5 eq.) and the sharp singlet of Ha was preserved (Fig. S13, ESI†). In addition, there were slight downfield shifts for ethylene linkers between the naphthoimidazolium and the cholesteryl moiety. Since there are no significant changes in the ^{1}H NMR spectra for other peaks, it is not easy to predict the exact binding mode. Hydrogen bonding interactions between carboxylates and imidazolium C–H protons could be clearly deduced based on NMR data and the cholesteryl unit successfully served as a chiral barrier based on the selectivity for D-isomers of Phe and Val.

Furthermore, DFT calculations were used to understand the enantioselectivity of **NI-chol 1** for Boc-D-Phe and the ratiometric fluorescence change. First, the optimized structures of **NI-chol 1** with Boc-D-Phe or Boc-L-Phe were obtained as shown in Fig. 6. In the optimized structures, the oxygen atom in the carboxyl group of Phe is found to form a hydrogen-bond to H_a. This result agrees well with the NMR spectra in which the H_a proton is significantly influenced when **NI-chol 1** binds to Boc-D-Phe in Fig. 5. As shown in Fig. 6 and movie clips (ESI†), both phenyl and Boc groups of Boc-D-Phe interact with the cholesterol moiety of **NI-chol 1**. However, only the phenyl group of Boc-L-Phe interacts with the cholesterol moiety of **NI-chol 1**. The energy of **NI-chol 1** with Boc-D-Phe (Fig. 6(b)) is found to be 98.2 kcal mol^{-1} lower than that of **NI-chol 1** with Boc-L-Phe (Fig. 6(d)). This DFT calculation result indicates that the

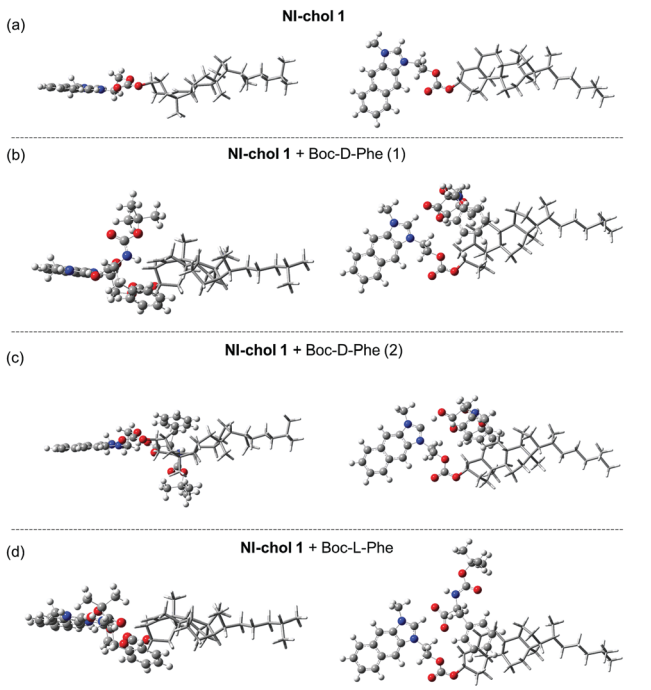

Fig. 6 The optimized structures of **NI-chol 1** (a) and **NI-chol 1** with Boc-D-Phe (b and c) or Boc-L-Phe (d). In (c), the H_a proton of the NI unit is significantly transferred to the carbonyl group of Boc-D-Phe. The cholesterol moiety of **NI-chol 1** is displayed in tube form for clarity.

interaction between Boc-D-Phe and **NI-chol 1** is much stronger than the interaction between Boc-L-Phe and **NI-chol 1**. Such a significant difference in the binding energy between **NI-chol 1** and Boc-D-Phe or Boc-L-Phe is responsible for the enantioselectivity of **NI-chol 1** for Boc-D-Phe.

Fig. 6(b and c) display the two optimized structures of **NI-chol 1** with Boc-D-Phe and their calculated absorption spectra are shown in Fig. S14 (ESI†). Note that the H_a proton of the NI unit is transferred to the carboxyl group of Boc-D-Phe, shown in Fig. 6(c). This result indicates that the absorption spectrum of NI is blue-shifted when the H_a proton moves significantly away from the NI unit. Similar results are expected from the emission spectra of NI units. To confirm this, the emission spectra of protonated and deprotonated NI units were calculated in Fig. S15(a) (ESI†). The calculated emission spectra of NI units agree well with the fluorescence spectra of **NI-chol 1** with and without Boc-D-Phe as shown in Fig. S15 (ESI†). This result indicates that after Boc-D-Phe binds to **NI-chol 1**, the NI unit is deprotonated by the carbonyl group of Boc-D-Phe and as a result, its fluorescence spectrum is blue-shifted.

In summary, we have developed a new naphthoimidazolium-cholesteryl derivative **NI-chol 1** for chiral anion recognition. The naphthoimidazolium moiety serves not only as a hydrogen bonding donor but also as a fluorescence unit. On the other hand, the cholesteryl moiety acts as a chiral barrier to induce enantiomer selectivity. Chiral fluorescence based receptor **1** displayed a unique ratiometric change in its emission with Boc-D-Phe. When Boc-D-Phe is added, the blue shifted emission at 370 nm increases with a concurrent decrease of the original emission at 452 nm, which results in a clear ratiometric fluorescence change. On the other hand, Boc-L-Phe did not induce any significant change. D-Val also induces similar but smaller ratiometric changes. The ionic hydrogen bonding interactions between the imidazolium C–H proton and carboxylate anions were confirmed by NMR analysis. Finally, the DFT calculations explain (1) the enantioselectivity of **NI-chol 1** for Boc-D-Phe, (2) emission spectra of **NI-chol 1** with and without Boc-D-Phe, and (3) the mechanism for ratiometric fluorescence detection of Boc-D-Phe by **NI-chol 1**.

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Conflicts of interest

There are no conflicts to declare.

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