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A novel chiral terpyridine macrocycle as a fluorescent sensor for enantioselective recognition of amino acid derivatives†

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Terpyridine macrocycle 1 is shown to be a strong chelating agent for organic ammonium salts and also a useful chromophore in fluorescent sensing. It exhibits very good enantioselectivity (K_{obs} (*S*)/K_{obs} (*R*) = 3.8) in chiral discrimination of α-phenylglycine methyl ester hydrochloride (PhEtOMe).

The study of enantiomeric recognition of biologically important substrates is a very important research area since it can provide valuable information for understanding the mechanism of molecular recognition in biological systems, and also the opportunity for developing useful molecular devices in biochemical and pharmaceutical studies, separation processes, catalysis and sensing.[1] In the past decade, chiral pyridine-containing macrocycles have been an attractive research area due to their chiral discrimination ability towards organic ammonium salts and amino acid derivatives.[2] Most of these enantiomeric recognition studies were carried out using a ^{1}H-NMR, FAB-MS, ESI-MS or UV-visible method. An attractive alternative method is fluorescent sensing due to its ease of measurement and high sensitivity.[3] Recently, a handful of fluorescent sensors based on binaphthol, acridine-containing crown and C_3-tripodal oxazoline have been developed for enantiomeric recognition of organic ammonium salts.[4] To design new fluorescent sensors for amino acid derivatives based on crown macrocycles, we have decided to incorporate a chiral 2,2′:6′,2″-terpyridine (tpy) unit into a crown ether macrocycle (Fig. 1). Though tpys are well-known chelating agents for various transition metals and their metal complexes have been employed as molecular signalling units in a number of fluorescent sensors,[5] the direct binding study of tpys with organic ammonium ions and the utility of metal-free tpy as a chromophore in fluorescent sensing remain unexplored. We, herein, report the study of tpy macrocycle **1** as a highly selective fluorescent sensor for chiral recognition of amino acid derivatives.

Tpy macrocycle **1** was readily prepared from a known chiral tpy derivative,[6] and the structure was assigned unambiguously by NMR experiments, ESI-MS and elemental analysis.‡ With this novel chiral tpy macrocycle in hand, we first examined its photophysical properties in CH_2Cl_2 (1.0×10^{-5} M). In the fluorescent spectrum, tpy macrocycle **1** exhibited a fluorescent emission peak at 355 nm (full line in Fig. 2). An excitation peak at the wavelength of 316 nm (data not shown) indicated a Stokes shift of 39 nm. In addition, two UV-visible absorption peaks at 264 nm and 297 nm can be observed in CH_2Cl_2 solution (dotted line in Fig. 2).

To investigate the enantioselectivity of macrocyclic host **1** in molecular recognition, (*S*)-α-phenylglycine methyl ester hydrochloride (PhEtOMe) was first used as the guest. The fluorometric titration experiments were carried out with the concentration of tpy macrocycle **1** fixed at 1.0×10^{-5} M in CH_2Cl_2 at room temperature, and the concentration of the guest was varied from 2.0×10^{-6} M to 1.0×10^{-4} M in CH_2Cl_2. The emission band of the macrocyclic host was excited at 316 nm with slit width of 2 nm, and the signal changes of the fluorescent emission intensity at 355 nm were recorded. As shown in Fig. 3, the fluorescent emission band at 355 nm was quenched gradually upon addition of the guest. A

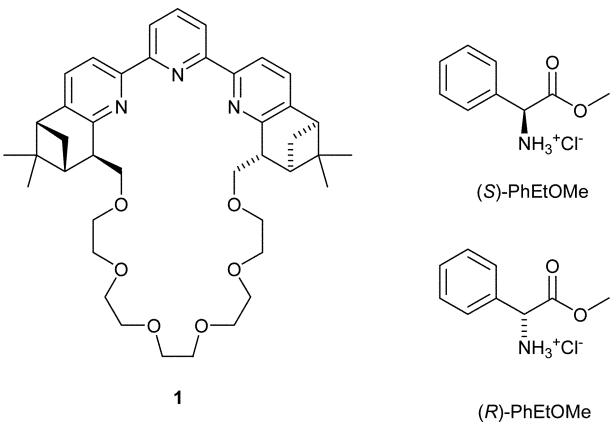

Fig. 1 Tpy macrocycle **1** (host) and PhEtOMe (guest).

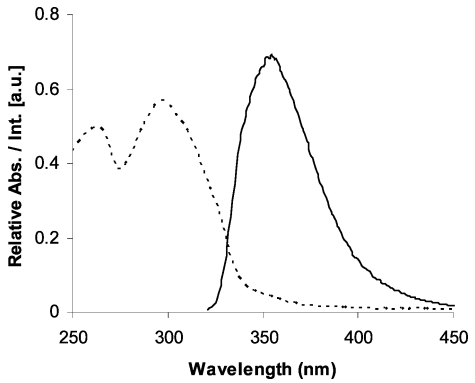

Fig. 2 Emission spectrum (full line) and normalized absorption spectrum (dotted line) of tpy macrocycle **1** in CH_2Cl_2 at room temperature.

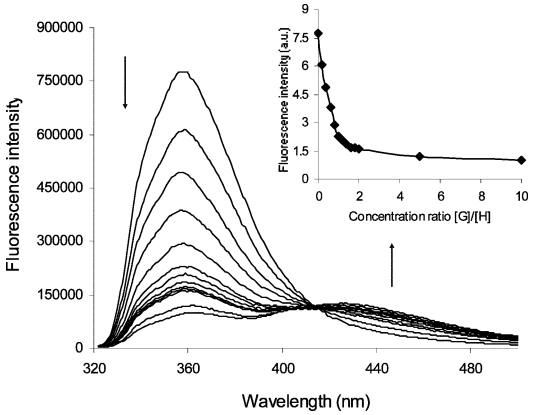

Fig. 3 Fluorometric titration of tpy macrocycle **1** (1.0×10^{-5} M in CH_2Cl_2, λ_{exc} = 316 nm, slit width = 2 nm) with (*S*)-PhEtOMe in CH_2Cl_2 at 25 °C. The insert shows the binding isotherm at 355 nm.

† Electronic supplementary information (ESI) available: plots for estimation of binding constants for tpy macrocycle **1** with (*R*)-PhEtOMe. See http://www.rsc.org/suppdata/cc/b3/b313960c/

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similar trend was observed for the (*R*)-enantiomer (data not shown). When the amount of the guest increased, a new but weak fluorescent emission peak at 430 nm started to appear. The binding of **1** with PhEtOMe could also be studied by UV-visible spectroscopy. The absorption peak of the macrocyclic host at 297 nm decreased gradually upon addition of the guest, and a new absorption peak at 350 nm was observed, indicating the formation of the host–guest complex.

The equilibrium constant was first estimated based on a 1 : 1 (host : guest) binding model. The plot with $I_o/(I - I_o)$ *versus* $[G]^{-1}$, where I and I_o are the fluorescent emission intensity at λ_{em} = 355 nm with concentration of PhEtOMe = [G] and 0 respectively, showed a poor linear relationship with R = 0.959 for (*S*)-PhEtOMe (Fig. 4a). The equilibrium binding constants (K) were obtained from the ratio of the *y*-intercept to the slope of the plots (1.8×10^5 and 2.1×10^4 M^{-1} for the (*S*)- and (*R*)-enantiomer respectively).[7] The lack of linearity of the Hildebrand–Benesi plots suggested that the stoichiometry of the inclusion complex may not be 1 : 1. Since there are two potential binding sites (the tpy unit and the crown ring) in **1**, it is reasonable to assume the stoichiometry is 1 : 2 for the host–guest complex. Thus the data were analysed using the modified Hildebrand–Benesi equation (eqn. (1)),[8] where a and b are constants.

$$\mathrm{H} + \mathrm{G} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathrm{HG}_1 + \mathrm{G} \overset{K_2}{\rightleftharpoons} \mathrm{HG}_2$$

$$K_{obs} = K_1 \times K_2 = \frac{[\mathrm{HG}_2]}{[\mathrm{H}][\mathrm{G}]^2} \qquad (1)$$

$$\frac{I_o}{I - I_o} = \frac{a}{b - a}\left(\frac{1}{K_{obs}}[\mathrm{G}]^{-2} + 1\right)$$

The plot with $I_o/(I - I_o)$ *versus* $[G]^{-2}$ showed a very good linear relationship with R = 0.997 for (*S*)-PhEtOMe (Fig. 4b), which strongly supported the 1 : 2 (host : guest) binding model. The observed equilibrium binding constants (K_{obs}) were obtained from the ratio of the *y*-intercept to the slope of the plot (4.2×10^{10} and 1.1×10^{10} M^{-2} for the (*S*)- and (*R*)-enantiomer respectively).[7] The average equilibrium binding constant of **1** for one guest molecule is between 1.0 and 2.0×10^5 M^{-1}, which is higher than that of the analogous pyridine-containing macrocycles ($K < 10^4$ M^{-1}).[2a] Though the mode of binding in this system is not clear, the large equilibrium binding constants indicated that the tpy and the ether moiety in the crown macrocycle have a good environment for hydrogen bonding and π–π interaction with the guest molecule.[2] The ratio of K_{obs} for the (*S*)- and (*R*)-enantiomer is 3.8 ($\Delta\Delta G$ = −3.3 kJ mol^{-1}), which is considered as high among the known fluorescent sensors for chiral organic ammonium salts and ammonium salt derivatives.[4]

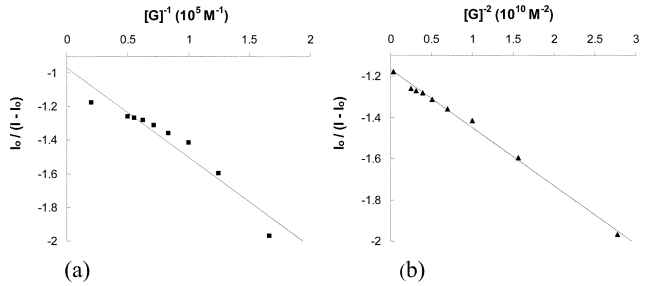

Fig. 4 Estimation of binding constants for tpy macrocycle **1** with (*S*)-PhEtOMe in CH_2Cl_2 at room temperature: (a) the plot based on the 1 : 1 binding model: $I_o/(I - I_o)$ *versus* $[G]^{-1}$; (b) the plot based on the 1 : 2 binding model: $I_o/(I - I_o)$ *versus* $[G]^{-2}$.

In summary, the new chiral tpy macrocycle (**1**) has been demonstrated to be a highly selective fluorescent sensor for PhEtOMe (K_{obs} (*S*)/K_{obs} (*R*) = 3.8). The chiral tpy unit of **1** was found to be a strong chelating agent for organic ammonium salts and also a useful chromophore in fluorescent sensing. Currently, studies of the binding between tpy macrocycle **1** and various amino acid derivatives are ongoing in our laboratory.

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Notes and references

‡ Tpy macrocycle **1** was isolated as a pale yellow solid. IR (KBr): 3447, 2925, 2867, 1561, 1430, 1107; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.69 (s, 6H), 1.30–1.42 (m, 2H), 1.45 (s, 6H), 2.30–2.50 (m, 2H), 2.54–2.68 (m, 2H), 2.78–2.88 (m, 2H), 3.52–3.90 (m, 24H), 4.10–4.24 (m, 1H), 4.36–4.46 (m, 1H), 7.30–7.50 (m, 2H), 7.86–7.99 (m, 1H), 8.20–8.54 (m, 4H); ^{13}C NMR (300 MHz, $CDCl_3$): δ 21.22, 26.30, 26.54, 28.84, 32.20, 35.13, 36.95, 40.56, 41.23, 42.30, 44.99, 47.04, 47.80, 51.42, 70.81, 118.37, 120.43, 130.21, 133.89, 137.70, 143.09, 145.72, 156.10; Anal. Calcd for $C_{41}H_{53}O_6N_3Na$: C, 70.86; H, 7.74; N, 5.55; Found: C, 69.67; H, 7.56; N, 5.94%; Positive ion MS (API) *m/z:* 684 ($M + H^+$), 706 ($M + Na^+$).

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