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Synthesis of cholate-based pyridinium receptor and its recognition toward L-tryptophan

Dazhi Li, Yunxu Yang *, Chao Yang, Biwei Hu, Baolong Huo, Lingwei Xue, Aizhi Wang, Feifei Yu

Department of Chemistry and Chemical Engineering, School of Chemistry and Biological Engineering, University of Science and Technology Beijing, Beijing 100083, China

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ABSTRACT

Four cholate-based pyridinium compounds were synthesized and their binding abilities toward unmodified amino acids were investigated by UV spectroscopy and fluorescence emission spectroscopy. Studies revealed that the recognition process involved hydrogen bonding, electrostatic force, and π–π interaction. The receptor **4a** was found to recognize L-tryptophan specifically, and the complex was studied by ^{1}H NMR spectroscopy. The receptors **4b** and **4c** showed very little recognition ability toward L-tryptophan, indicating the important role of the benzyl group at pyridinium ring.

1. Introduction

Among the numerous kinds of molecular recognition, amino acid is one of the most preferred targets for molecular recognition due to its central role in natural living systems. In trying to evaluate the underlying intermolecular forces between amino acids and other reciprocal artificial host compounds, many efforts have been expended. It has been found that non-covalent forces, such as hydrogen bonding, π–π stacking, and hydrophobic interactions provide the selectivity observed in recognition process.[1] But most of the investigated recognitions require derivation of amino acids, such as methyl esterification[2] and quaternization,[3] due to the low solubility in organic solvent. For this reason, neither under their original forms involved in life process nor under appropriate aqueous solution could be studied for most of amino acids.

In recent years, supramolecules containing cholate groups have received much attention[2d,4] for their good performance in molecular recognition. Under changes of general environmental, the cholate derivates are able to respond with conformational change, which likely lead to molecular complementarity. Thus, the supramolecules can recognize the guests with its biding sites in a particular environment, such as specific pH, solvent polarity, temperature etc. by one main conformation.[5] On the other hand, the molecular structure of amine acid coexists with hydrogen bond donor ($-NH_2$) and hydrogen bond acceptor ($-COO^-$). The carboxylate in the molecule head also results in high negative charge under physiological conditions. Taking the detailed structural information into consideration, we tried to design a water-soluble, tweezer-like derivate of cholic acid for the recognition. Finally, a polar benzyl pyridinium **4a** featuring cholate groups that contains backbones of concave hydrophilic face and convex hydrophobic face, has been synthesized in our present work. The receptor would provide positive center to bind with the negatively charged amine acid via electrostatic interaction, and provide a benzyl group that could probably induce the π–π stacking with substrates. Meanwhile, three corresponding compounds **4b**, **4c**, and **6** were also synthesized as model hosts to illustrate the integrant of benzyl group and the importance of tweezer-like polycholate backbones applied in multipoint interactions in our design.

2. Results and discussion

2.1. Synthesis

3-Amino methyl cholate **2** was prepared from the start material of cholic acid according to the literature procedures[6] and then reacted with 3,5-dinicotinic acid or 3-nicotinic acid under the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) to give dinicotinamide **3** or

* Corresponding author. E-mail address: yxyang63@aliyun.com (Y. Yang).

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monomer **5**. Hosts **4a**–**c** were obtained by quaternized **3** with benzyl bromide, 9-(bromomethyl)-anthracene, and methyl iodide, respectively. Nicotinamide **5** was quaternized with benzyl bromide to give host **6**.

2.2. Binding studies of 4a with amino acids

*2.2.1. UV–vis absorption and fluorescence binding studies of **4a** with amino acids.* Nineteen α-amino acids (as shown in Fig. 1, respectively) were used to evaluate the binding properties of **4a** in water/ethanol (1/1, v/v, phosphate buffer solution (PBS), 5 mM, pH=7.2).[4f,7] The fluorescence spectra were obtained by excitation of **4a** at 247 nm. In order to make **4a** fully conjugate with guests, amino acids were added in tenfold excess to **4a**. As shown in Fig. 1, **4a** showed an originally maximum absorption peak at 204 nm (log ε=4.49) and a minor broad peak at 341 nm (log ε=3.88). After addition of amino acids to the system of **4a**, the main absorption reduced and accompanied with a slightly red-shift at 204 nm; at 341 nm only a little decrease of the absorption occurred. But the maximum absorption changes of **4a** were quite obvious after adding L-phe, L-His, L-Tyr, L-**Trp**, which reduced to almost a half and gave a significant red-shift ($\Delta\lambda_{max}$=34 nm for L-**Trp**). These results indicated that the π–π interaction truly plays an important role in the recognition process between **4a** and the substrate amino acids.[8] With the absorption results, the nineteen substrate amino acids could be categorized into three groups: group I contains eleven amino acids, which have small size and most of them are aliphatic amino acids; Group II were L-Cys, L-Met, L-Arg, and L-Lys; Group III possesses L-phe, L-His, L-Tyr, and L-**Trp**, all of which are aromatic amino acids.

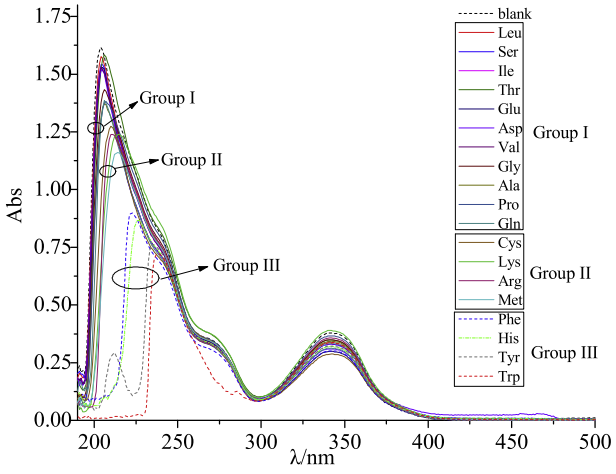


Fig. 1. The UV spectra of **4a** (5.00×10^{-5} M) in water/ethanol (1/1, v/v) phosphate buffer solution (5 mM, pH=7.2) upon addition of different amino acid (5.00×10^{-4} M), corresponding amino acid solution as reference.

Similarly, in the fluorescence spectra, the addition of amino acids to **4a** would cause a fluorescence quenching under λ_{ex} at 247 nm. The intensities of **4a** with L-**Cys** and L-**Lys** belonging to the group II of amino acids were relatively lower compared to other 16 amino acids. While, the fluorescence intensity change of **4a** with L-**Trp** was the most obvious, which decreased by more than a half; the spectrum had a slight blue-shift from 410 nm to 400 nm, as shown in Fig. 2.

Based on the above results, we could see that there is specific selectivity of **4a** to L-**Trp** by UV–vis absorption and fluorescence spectroscopy. Host **4a** is a potential receptor for L-**Trp**.

*2.2.2. UV–vis and fluorescence titration of **4a** with L-**Trp**.* In order to investigate the dependence of the absorption and fluorescence

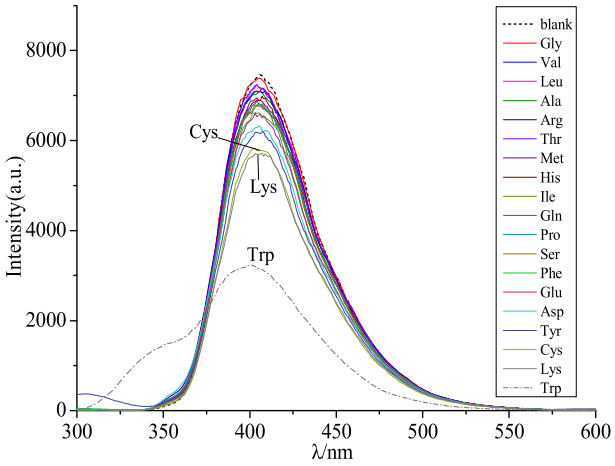


Fig. 2. The emission spectra of solution of **4a** (5.0×10^{-5} M) under water/ethanol (1/1, v/v) phosphate buffer solution (5 mM, pH=7.2) upon addition of different amino acids (5.0×10^{-4} M), λ_{ex}=247 nm.

intensities to the concentration of L-**Trp**, we carried out absorption and fluorescence titration experiments by the addition of increasing amount of L-**Trp** into the solution of **4a**.

As shown in Fig. 3, In UV–vis titration, the original main absorption of **4a** at 204 nm reduced after L-**Trp** was gradually added, and after 2 equiv excess, the absorption remained the same. Similarly, in fluorescence titration, **4a** exhibits strong emission spectra (λ_{em}=410 nm) in the absence of L-**Trp**. However, the gradually addition of L-**Trp** (from 0.00 to 6.50×10^{-4} M) to the solution of **4a** would cause a fluorescence quenching and lead to a fluorescence enhancement synchronously at 350 nm, as shown in Fig. 4.

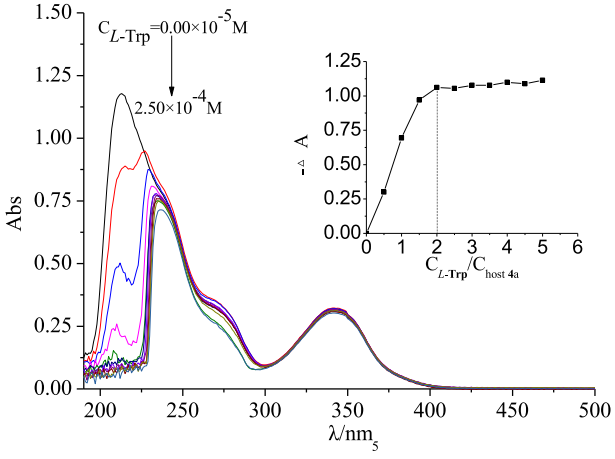


Fig. 3. The UV spectra of **4a** (5.0×10^{-5} M) in water/ethanol (1/1, v/v) phosphate buffer solution (5 mM, pH=7.2) upon addition of different amount of L-**Trp** (0.00, 2.50, 5.00, 7.50, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, 25.0×10^{-5} M); Inset: the difference of absorption value ($-\Delta A=A_0-A$) at 204 nm as a function of $C_{L\text{-}Trp}/C_{4a}$, corresponding L-**Trp** solution as reference.

*2.2.3. Binding constant and stoichiometry of **4a** with L-**Trp**.* As shown in Fig. 3, the UV–vis absorption intensity of **4a** at 204 nm was found decreased gradually with the increasing concentration of L-**Trp** from 0.00 to 1.00×10^{-4} M and then was stable after 1.00×10^{-4} M. This phenomenon further confirmed the interaction of **4a** with L-**Trp** and the formation of 1:2 complex of **4a**/L-**Trp** after the excess added L-**Trp** (a 1:1 complex would probably form at first at the insufficient addition of L-**Trp**[4e]). On the basis of the 1:2

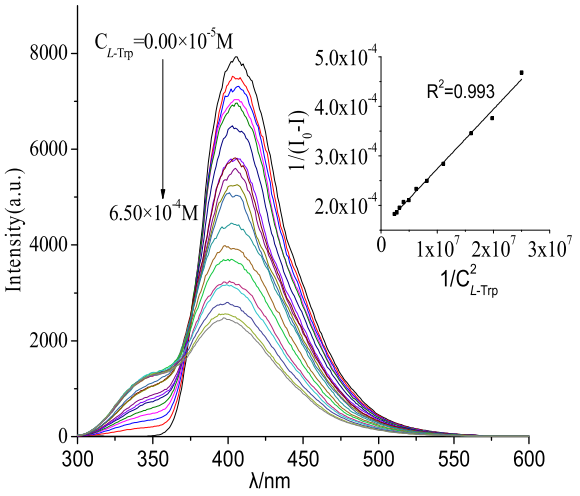


Fig. 4. The emission spectra of **4a** (5.0×10^{-5} M) in water/ethanol (1/1, v/v) phosphate buffer solution (5 mM, pH=7.2) upon addition of different amount of L-**Trp** (0.00, 0.250, 0.500, 0.750, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50×10^{-4} M), λ_{ex}=247 nm. Inset: at 406 nm, $1/(I_0-I)$ as a function of $1/C^2_{L\text{-Trp}}$.

stoichiometry, we calculated the value of the binding constant for the complex according to Chen Y. L.,[4e] the equation was adopted as follow:

$$\frac{1}{I_0-I}=\frac{1}{K(I_0-I_2)[G]^2}+\frac{1}{I_0-I_2}$$

Where I_0 and I is the fluorescence intensity of host solution and the solution of host with guest; I_2 is the fluorescence intensity of the 1:2 complex. [G] is the total concentration of guest. K is complex constant. When the equation was applied, $1/(I_0-I)$ versus $1/[G]^2$ should give a straight line. The straight line (R^2=0.993) was displayed in Fig. 4 (inset), and K=1.27×10^7 M^{-2}.

2.3. Binding studies of 4a using ^{1}H NMR spectroscopy

Further binding studies between **4a** and L-**Trp** was carried out by detecting the change of proton magnetic resonance signal using ^{1}H NMR spectroscopy. L-Trytophan sodium salt was added as guest in two and half fold excess to **4a** in CD_3OD/D_2O (5:1, v/v). With the gradual addition of L-**Trp** to **4a**, the ^{1}H NMR signals of **4a** and L-**Trp** produced substantial movements with the protons on pyridinium ring, benzyl, cholate arms, indole ring, and the amino acid side chain. The titration spectra of **4a**, L-**Trp** and the complex **4a**/L-**Trp**, which provided preliminary information of the interreactions on the complex formation, were displayed in Fig. 5.

Initially, when 0.5fold L-**Trp** was added to **4a** solution, the overall signals of **4a** showed a little change as shown in Fig. 5C. The signals intensity of $C_{2'}$–H, $C_{4'}$–H, and $C_{9'}$–H diminished; the split of $C_{5'}$–H, $C_{6'}$–H, and $C_{8'}$–H changed from double peak to single peak; C_{12}–H changed from single peak to double peak; C_7–H, C_3–H displayed a slight upfield shift; $H_{23\alpha}$, C_{21}–H, C_{19}–H changed from multiplet to broad multiplet and also showed slight upfield shift. On the other hand, the movements of the L-**Trp** signals varied considerably. The signals of C_a–H, C_d–H, C_h–H, C_i–H diminished, and C_d–H, C_h–H, C_i–H also showed downfield shift; C_g–H changed from double peak to broad multiplet, C_k–H diminished following an obvious upfield shift; the two double peaks of H_{j1} disappeared and gave a multiplet; the dd peaks of H_{j2} diminished and displayed an obvious downfield shift. These results indeed demonstrated the formation of **4a**/L-**Trp** complex and revealed the interactions via hydrogen bonding and electrostatic interaction between **4a** and L-**Trp**.

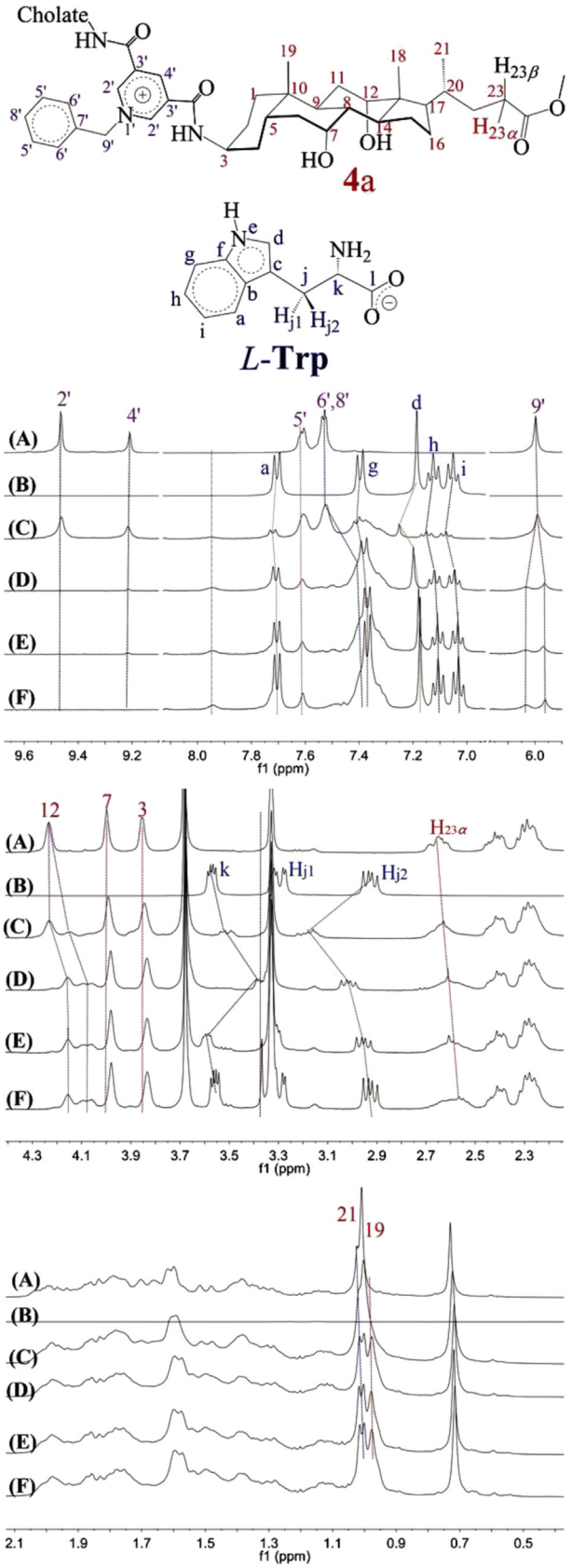


Fig. 5. The ^{1}H NMR spectra of (A) **4a**; (B) L-**Trp**; (C) **4a**+0.5 equiv L-**Trp**; (D) **4a**+1.0 equiv L-**Trp**; (E) **4a**+1.5 equiv L-**Trp** and (F) **4a**+2.5 equiv L-**Trp** in CD_3OD/D_2O=5/1 (v/v) at room temperature. The L-**Trp** was in the form of sodium salt.

Upon the addition of L-**Trp** (1.0 equiv, 1.5 equiv, and 2.5 equiv) to **4a** (as shown in Fig. 5D–F), due to the classical π–π interaction[8,9] between indole ring of L-**Trp** and pyridinium-benzyl group of **4a**, the signals of $C_{2'}$–H, $C_{4'}$–H disappeared; the signals intensity of $C_{5'}$–H, $C_{6'}$–H, $C_{8'}$–H, and $C_{9'}$–H diminished, and $C_{9'}$–H splited into two peaks as well (The split of $C_{9'}$–H should also be contributed by CH–π conjugation, electrostatic interaction from positive ammonium salt units and the carboxylate ions of L-**Trp**); but the signals intensity on C_a–H, C_g–H, C_d–H, C_h–H, C_i–H had been regaining their proper position along with the addition of L-**Trp** to **4a**. Moreover, owing to the multiple hydrogen bonding, hydrophobic force, π–π, and electrostatic interaction, the signals of C_{12}–H, C_{21}–H, and C_{19}–H not only exhibited upfield shift but also splited, and finally to multiplet in C_{12}–H, to double peak in C_{21}–H; Furthermore, C_k–H changed back to their proper position from upfield shift to downfield; H_{j1}, H_{j2} changed back to their proper position from downfield shift with the addition of L-**Trp**. It was also important to point out that, due to the continued multiple hydrogen bonding on C_{12}–OH, C_7–OH, C_3–NH, and the conformational changes of the cholate arms, the ^{1}H signals of C_{12}–H, C_7–H, C_3–H, and $H_{23\alpha}$ showed a stable, sustained changes along with the addition of L-**Trp** to **4a**.

2.4. Binding studies of host 4b, 4c, 6 with UV–vis and fluorescence spectroscopy

The biding investigations of **4b**, **4c**, **6** were carried out with L-**Trp** directly to illustrate the structure activity relation in recognition. Unexpectedly, the UV–vis absorption and the fluorescence of **4b** showed very little change upon addition of different amount of L-**Trp** even though the maximum absorbance of **4b** was greater than that of **4a**.[10] What's more, the **4b**'s fluorescence intensity was 70% lower than **4a**. These hint that a larger size of anthracene ring probably hindered the interaction of the recognition between **4b** and guests. On the other hand, **4c** had 66% lower fluorescent intensity than that of **4a** and only showed a little fluorescent change with the addition of L-**Trp** under maximum excitation wavelength;[11a] **6** has no fluorescence at all, although its UV–vis absorption showed a similar change to **4a** upon addition of different amount of L-**Trp**.[11b] Thus, compound **4b**, **4c**, and **6** should have interactions toward L-tryptophan, but they are not suitable for fluorescence detection.

2.5. Selectivity of 4a to L-Trp in the presence of other coexisting amino acids

It is particularly important to evaluate the selectivity of **4a** for L-**Trp** over other amino acids. The interference of coexisting amino acids to L-**Trp** detection was investigated by fluorescence spectra. As shown in Fig. 6, no significant fluorescence intensity changes of **4a**/L-**Trp** solution could be observed in the presence of the coexisting amino acids (4.00×10^{-5} M, 1.0 equiv to L-**Trp**), indicating that **4a** exhibits high selectivity to L-**Trp** over other amino acids. These results showed that **4a** has good sensing selectivity to L-**Trp**, which made its suitable of practical L-**Trp** detection.

2.6. Sensing mechanism

The strong evidence for the electrostatic interaction and multiple hydrogen bonds between **4a** and L-**Trp** came from the UV–vis absorption, fluorescence spectra, and ^{1}H NMR analysis. Significant changes on the absorption, fluorescence spectra of **4a** can be well explained by the L-**Trp**-induced conformational change of backbone with multiple hydrogen bond donors. Cholic acid has two faces: α-face with three hydrophilic hydroxy groups and β-face with three hydrophobic methyl groups. Under different polar condition, the behavior and conformation of cholates would be different. If **4a** is placed in a polar solvent, the α-faces will turn outside with the help of formation of hydrogen bonding, and the two β-faces will face to each other to create a micro hydrophobic cave environment. The original constricted conformation of **4a**, which the two ester groups were folded into the hydrophobic cave,[12] helped to reduce the collision between **4a** and solvent molecule. It could favor the enhancement of fluorescence intensity.

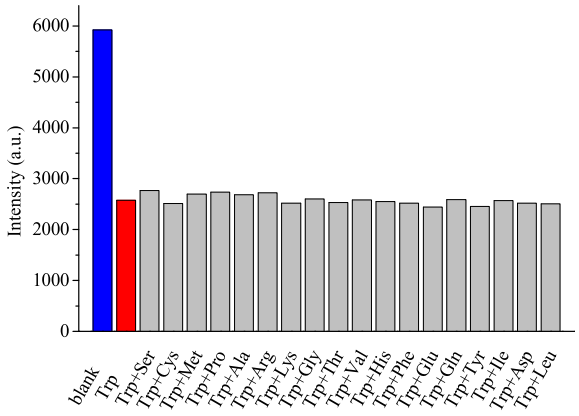
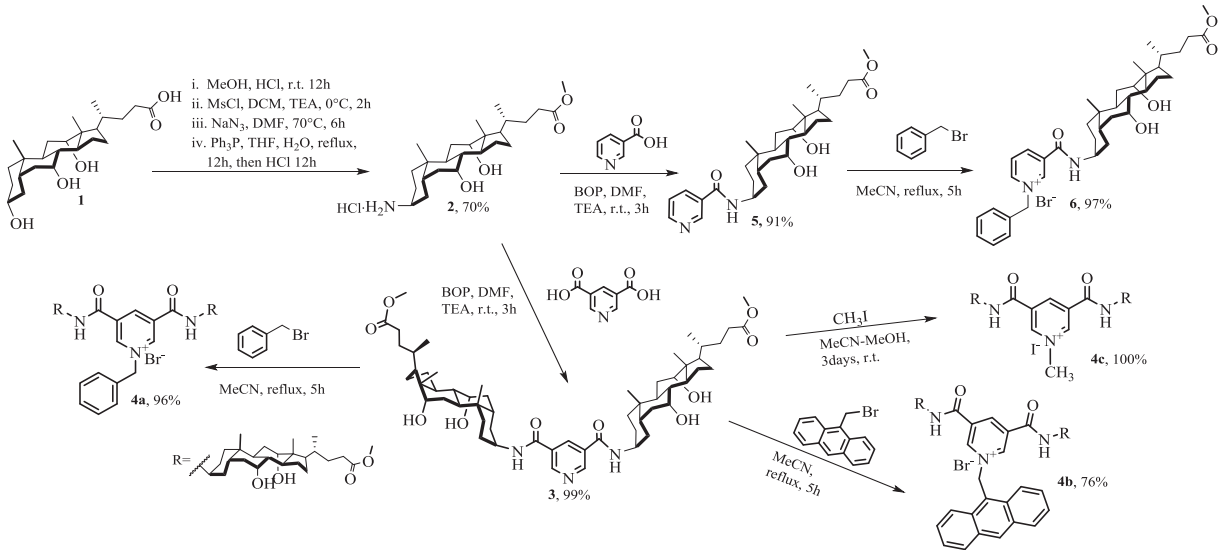


Fig. 6. Selectivity of **4a** (4×10^{-5} M) to L-**Trp** (4×10^{-4} M) in water/ethanol (1/1, v/v) phosphate buffer solution (5 mM, pH=7.2) in the presence of other coexisting amino acids (4×10^{-4} M).

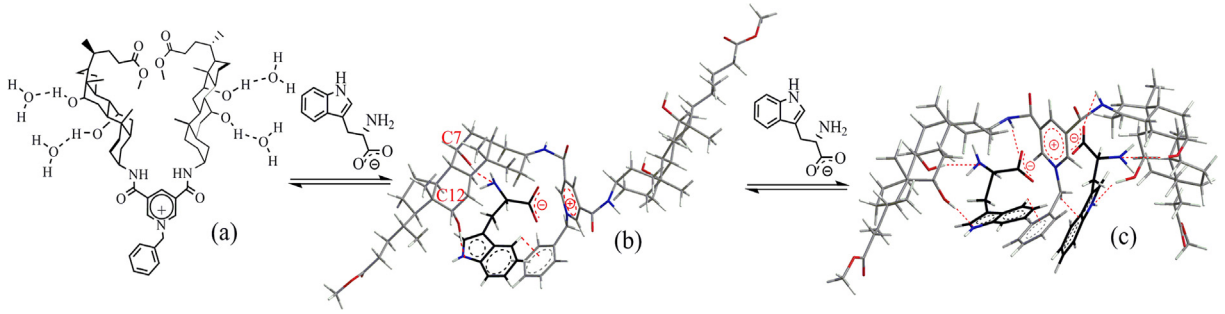
It was plausible to suppose that the electrostatic interaction force between **4a** and L-**Trp** should be treated as the prime driving factor in recognition. The close approach of carboxylate group and amine group units in L-**Trp** may be driven by electrostatic, hydrogen bonding, and CH–π interactions, which would contribute to the overall binding energies of the complex. When L-**Trp** carboxylate ions closed to positive pyridinium ring driven by the electrostatic force, L-**Trp** would approach to **4a** from its α-face and lies in the cleft between benzyl group and cholate arms of **4a**. Meanwhile, owing to the tweezer-like derivative **4a** possesses hydroxyl groups at each arms, it is able to form multiple hydrogen bonding with the amino groups and carboxylate ions of L-**Trp**. Thus, the hydrophobic cave in **4a** would break down along with the complex formation, and indeed, the quenching of fluorescence occurred with the addition of L-**Trp** to **4a**. The fluorescence intensity of **4a** decreased by more than a half and the emission spectra showed a slight blue-shift from 410 to 400 nm after the addition of L-**Trp**, as shown in Fig. 2. This indicated that more energy consumption of the excited state energy of **4a** had happened with the energy transmitting to guest molecules through resonance energy transfer.[13] The complex conformation of **4a**/L-**Trp** is proposed in Scheme 2.

Besides, the apparent changes of the ^{1}H NMR signal on pyridinium ring, phenyl, and indole rings supported and illustrated the electrostatic interaction, CH–π, and π–π interactions between host and guest. What's more, the ^{1}H signals on C_3, C_7, and C_{12} clearly demonstrated the formation of hydrogen bonding, and the changes on C_k–H, C_j–H, C_{19}–H, C_{21}–H, and C_{23}–H implied the conformational changes of cholate arms.

Mono-cholate backbone **6** was used to estimate the arms' function of cholate pyridinium salt, and **4b**, **4c** were used to estimate the steric hindrance effect nearby the positive charge of pyridinium units. The results indicated that **4b**, **4c**, and **6** had no selectivity or specificity binding capability to L-**Trp** through fluorescence spectra. The structure–activity relationship of **4a**–**c** and **6** further declared that both pyridinium-aryl unit and tweezer-like cholate arms in **4a** play very important roles in the selectivity/sensitivity of L-**Trp** over other amino acids.



Scheme 1. The synthesis routes of receptors.



Scheme 2. A proposed dynamic conformation of the complex of **4a**/L-**Trp** in water/ethanol phosphate buffer solution (5 mM, pH=7.2).

3. Conclusion

In summary, we have designed a tweezer-like quaternary ammonium salt derivative of cholate **4a**, a highly sensitive and selective fluorescence receptor for L-**Trp** over other α-amino acids in ethanol aqueous solution. The appealing performance of the receptor was demonstrated to originate from the electrostatic and multiple hydrogen bonding cooperative interactions, being synergetic with conformational change of cholate backbone along with signal diminution. The structure recognition relationships showed that pyridinium-aryl group and tweezers-like cholate arms play very important roles in the selectivity/sensitivity of L-**Trp**.

4. Experimental

4.1. General

4.1.1. Materials. All reagents and solvents were commercially available and were used without further purification unless otherwise stated. The water used for investigation was distilled prior to use. Cholic acid was purchased from Alfa Aesar. All α-amino acids were L-configuration and were purchased from Beijing Chemical Regent Company. 9-(Bromomethyl)anthracene was prepared according to literature[14] from 9-methanolanthracene.

4.1.2. Instruments. Optical rotations were measured at 25 °C with WZZ-3 automatic polarimeter (Shanghai Physical Optical Instrument Co., Ltd). Infrared spectra were recorded on a Shimadzu IR-8400S spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded by Bruker of 400 MHz on a Varian Gemin-400. ESI-TOF Mass spectra were obtained on Bruker spectrometers. All UV–vis and fluorescence spectra in this work were recorded in Pgeneral TU-1901 and Hitachi F-4500 fluorescence spectrometers. All pH values were measured with a Model pHS-3C pH meter (Shanghai, China).

4.1.3. Synthesis. The synthetic routes of **4a**–**c** and **6** were outlined in Scheme 1, and the details were described below.

*4.1.3.1. Synthesis of methyl 3β-amino-7α, 12α-dihydroxy-5β-cholan-24-oate hydrochloric salt **2**.* Methyl cholate was synthesized firstly according to the literature,[6a] followed by sulfonylation with methane sulfonyl chloride,[6b] then substitution by azido group.[6b] Azido methyl cholate was reduced by triphenylphosphine,[6c] and finally gave amino methyl cholate hydrochloric salt by addition of concentrated hydrochloric acid at 0 °C. The mixture was filtered to give a white solid **2**, overall yield: 70%; mp: ~210 °C (decomposition); $[\alpha]_D^{25}$ +25.0 (*c* 1.0, MeOH); IR (KBr, cm^{-1}) 3539, 3458, 3026, 2945, 2881, 1726, 1633, 1514, 1461, 1382, 1311, 1202, 1172, 1035; ^{1}H NMR (DMSO-d_6) δ 0.57 (s, 3H, 18-H), 0.86 (s, 3H, 19-H), 0.90 (d, *J*=6.0 Hz, 3H, 21-H), 1.10–1.50 (m, 11H), 1.55–1.86 (m, 8H), 1.95–2.00 (m, 1H), 2.05–2.10 (m, 1H), 2.10–2.22 (m, 1H), 2.25–2.35 (m, 1H), 2.65 (t, *J*=12.6 Hz, 1H), 3.56 (s, 3H, –COOCH_3), 3.60 (s, 1H, 7-H), 3.76 (s, 1H, 12-H), 4.19 (br s, 2H, –O*H*), 7.92 (s, 3H, –NH_3^+).

4.1.3.2. Synthesis of N,N′-dicholate-3,5-dinicotin-amide ***3***. A similar method was adopted to synthesize the titled compound according to literature.[15] A mixture of 3,5-dinicotinic acid (0.084 g, 0.50 mmol), amino methyl cholate hydrochloric salt (0.458 g, 1.00 mmol), BOP (0.464 g, 1.05 mmol), DMF (5 mL), and TEA (0.42 mL, 3.0 mmol) was stirred at room temperature for 24 h. Then, the mixture was added into $NaHCO_3$ (30 mL, 1 M) at 0 °C. The white precipitate was filtered and washed with water (100 mL), dried in air at 50 °C, purified with flash chromatography, yield: 0.482 g, 99%. R_f: 0.71 (EA/EtOH, 3/1); mp: 158–162 °C; $[\alpha]_D^{25}$ +38.0 (*c* 1.0, MeOH); IR (KBr, cm^{-1}) 3443, 2939, 2868, 1724, 1649, 1528, 1437, 1377, 1270, 1038; ^1H NMR (CD_3OD) δ 0.72 (s, 6H, 18-H), 0.99 (s, 6H, 19-H), 1.01 (s, 6H, 21-H), 1.25 (t, *J*=7.2 Hz, 2H), 1.95–2.10 (m, 7H), 2.20–2.40 (m, 7H), 2.69 (td, J_1=14.4 Hz, J_2=4.0 Hz, 2H), 3.60 (q, *J*=7.2 Hz, 2H), 3.64 (s, 6H, −COOCH_3), 3.81 (s, 2H, 7-H), 3.96 (s, 2H, 12-H), 4.08 (q, *J*=6.8 Hz, 2H), 4.20 (s, 2H), 8.45 (s, 1H, Py−H), 8.99 (d, *J*=2.0 Hz, 2H, Py−H); ESI-TOF MS *m*/*z*: calcd for $[C_{57}H_{87}N_3O_{10}+Na]^+$: 996.6289; found: 996.6133; calcd for $[4M+3Na]^{3+}$: 1321.8442; found: 1321.8235.

4.1.3.3. Synthesis of compound ***4a***. A mixture of *N,N′*-dicholate-3,5-dinicotinamide **3** (0.195 g, 0.200 mmol), BnBr (0.36 mL, 3.0 mmol) in acetonitrile (10 mL) was reflux for 5 h, and then the solution was concentrated to half volume and added into ethyl ether (15 mL). The white precipitate was collected and washed with ether, yield: 0.220 g, 96%. R_f: 0.03 (EA/EtOH, 10/1); mp: 183–185 °C; $[\alpha]_D^{25}$ +51.1 (*c* 0.3, MeOH); IR (KBr, cm^{-1}) 3431, 2979, 2868, 1734, 1670, 1533, 1456, 1377, 1271, 1198, 1173, 1095, 1038; ^1H NMR (CD_3OD) δ 0.71 (s, 6H, 18-H), 0.99 (s, 6H, 19-H), 1.01 (s, 6H, 21-H), 1.95–2.06 (m, 4H), 2.20–2.33 (m, 4H), 2.33–2.42 (m, 2H), 2.70 (td, J_1=14.4 Hz, J_2=3.0 Hz, 2H), 3.47 (q, *J*=7.2 Hz, 2H), 3.64 (s, 6H, −COOCH_3), 3.81 (s, 2H, 7-H), 3.96 (s, 2H, 12-H), 4.22 (s, 2H), 5.96 (s, 2H, N^+−CH_2−Ph), 7.49 (d, *J*=3.6 Hz, 3H, benzyl−H), 7.58 (d, *J*=4.0 Hz, 2H, benzyl−H), 9.22 (s, 1H, Py−H), 9.48 (s, 2H, Py−H); ^{13}C NMR (CD_3OD): δ 14.26, 18.87, 24.63, 25.50, 26.55, 28.83, 29.93, 31.01, 33.14, 33.40, 33.50, 35.45, 36.67, 37.60, 38.03, 39.60, 42.21, 44.27, 48.82, 49.26, 53.27, 67.41, 70.34, 75.24, 131.81, 132.04, 132.53, 135.14, 137.71, 145.30, 148.17, 164.45, 177.77; Anal. Calcd for $C_{64}H_{94}N_3O_{10}Br+5H_2O$: C, 62.19; H, 8.42; N, 3.40; found: C, 62.34; H, 8.04; N, 3.37.

4.1.3.4. Synthesis of compound ***4b***. A mixture of **3** (0.292 g, 0.300 mmol), 9-(bromomethyl)-anthracene (0.095 g, 0.35 mmol) in acetonitrile (5 mL) was reflux for 5 h until the reaction was complete monitored by TLC. After cooling, the mixture was filtered and the pale yellow solid was washed with cold MeCN (5 mL), yield: 0.283 g, 76%. R_f: 0.17 (EA/EtOH, 8/1); mp: 188–190 °C; $[\alpha]_D^{25}$ +47.3 (*c* 0.3, MeOH); IR (KBr, cm^{-1}) 3431, 3053, 2938, 2867, 1724, 1670, 1533, 1448, 1377, 1261, 1163, 1035; ^1H NMR (CD_3OD) δ 0.70 (s, 6H, 18-H), 0.93 (s, 6H, 19-H), 0.99 (d, *J*=6.4 Hz, 6H, 21-H), 2.10–2.30 (m, 4H), 2.30–2.40 (m, 2H), 2.65 (td, J_1=13.6 Hz, J_2=3.8 Hz, 2H), 3.65 (s, 6H, −COOCH_3), 3.78 (s, 2H, 7-H), 3.93 (s, 2H, 12-H), 4.10 (s, 2H), 7.01 (s, 2H, N^+−CH_2−Ar), 7.58 (t, *J*=7.4 Hz, 2H, Ar−H), 7.67 (t, *J*=7.4 Hz, 2H, Ar−H), 8.18 (d, *J*=8.4 Hz, 2H, Ar−H), 8.38 (d, *J*=8.4 Hz, 2H, Ar−H), 8.82 (s, 1H, Ar−H), 9.15(s, 1H, Py−H), 9.20 (s, 2H, Py−H). ^{13}C NMR (CD_3OD) δ=14.27, 18.89, 24.68, 25.49, 29.92, 30.98, 33.14, 33.49, 36.65, 37.55, 38.01, 39.55, 42.17, 44.24, 48.80, 49.25, 53.28, 59.96, 70.30, 75.21, 122.29, 124.97, 128.22, 131.30, 132.30, 134.18, 134.48, 134.67, 137.54, 145.11, 147.18, 164.33, 177.74; Anal. Calcd for $C_{72}H_{98}N_3O_{10}Br+3H_2O$: C, 66.51; H, 8.00; N, 3.23; found: C, 66.50; H, 7.80; N, 3.22.

4.1.3.5. Synthesis of compound ***4c***. MeI (2 mL) was added to the solution of compound **3** (0.195 g, 0.200 mmol) in MeCN/MeOH (5 mL/5 mL). The mixture was stirred at room temperature for 3 days, and then the solvent and excess MeI was removed to give a yellow solid, yield: 0.223 g, 100%. R_f: 0.27 (EA/EtOH, 3/1); mp: 196–197 °C; $[\alpha]_D^{25}$ +32.4 (*c* 0.3, MeOH); IR (KBr, cm^{-1}) 3447, 2941, 2868, 1720, 1668, 1533, 1437, 1377, 1252, 1221, 1028, 851, 762, 669; ^1H NMR (CD_3OD) δ 0.72 (s, 6H, 18-H), 1.01 (s, 12H, 19, 21-H), 2.72 (td, J_1=14.2 Hz, J_2=3.6 Hz, 2H), 3.49 (q, *J*=6.8 Hz, 1H), 3.61 (q, *J*=6.8 Hz, 1H), 3.64 (s, 6H, −COOCH_3), 3.82 (s, 2H, 7-H), 3.96 (s, 2H, 12-H), 4.24 (s, 2H), 4.50 (s, 3H, N^+−CH_3), 9.14 (s, 1H, Py−H), 9.41 (s, 2H, Py−H).

4.1.3.6. Synthesis of compound ***5***. A mixture of nicotinic acid (0.246 g, 1.00 mmol), amino methyl cholate hydrochloric salt **2** (0.458 g, 1.00 mmol), BOP (0.464 g, 1.05 mmol), DMF (5 mL), and TEA (0.42 mL, 3.0 mmol) was stirred at room temperature for 24 h. Then, the mixture was added into $NaHCO_3$ (30 mL, 1 M) at 0 °C. The white precipitate was filtered and washed with water (100 mL), dried in air at 50 °C, purified with flash chromatography, yield: 0.479 g, 91%. R_f: 0.67 (ethyl acetate/ethanol, 3/1); mp117–120 °C; $[\alpha]_D^{25}$ +34.9 (*c* 1.0, MeOH); IR (KBr, cm^{-1}) 3435, 2973, 2868, 1724, 1645, 1527, 1439, 1375, 1251, 1195, 1027; ^1H NMR ($CDCl_3$) δ 0.65 (s, 3H, 18-H), 0.93 (s, 6H, 19, 21-H), 1.06–1.10 (m, 2H), 2.63 (t, *J*=14 Hz, 1H), 3.62 (s, 3H, −COOCH_3), 3.83 (s, 1H, 7-H), 3.95 (s, 1H, 12-H), 4.07 (d, *J*=6.8 Hz, 1H), 4.27 (s, 1H), 6.43 (d, *J*=4.8 Hz, 1H, −CON*H*−), 7.35 (s, 1H, Py−H), 8.06 (d, *J*=6.8 Hz, 1H, Py−H), 8.66 (s, 1H, Py−H), 8.90 (s, 1H, Py−H); EI-TOF MS *m*/*z*: calcd for $[C_{31}H_{46}N_2O_5]^+$: 526.3406; found: 526.3509.

4.1.3.7. Synthesis of compound ***6***. A mixture of **5** (0.105 g, 0.200 mmol), BnBr (0.36 mL, 3.0 mmol) in acetonitrile (10 mL) was reflux for 5 h, and then the solution was concentrated to half volume and added into ethyl ether (15 mL). The white precipitate was collected and washed with ether, yield: 0.135 g, 97%. R_f: 0.02 (EA/EtOH, 10/1); mp: 139–143 °C; $[\alpha]_D^{25}$ +21.3 (*c* 1.0, EtOH); IR (KBr, cm^{-1}) 3420, 3063, 2937, 2868, 1734, 1663, 1539, 1497, 1456, 1375, 1198, 1177, 1028; ^1H NMR ($CDCl_3$) δ 0.65 (s, 3H, 18-H), 0.95 (d, *J*=5.6 Hz, 3H, 19-H), 0.98 (s, 3H, 21-H), 1.18 (t, *J*=7.2 Hz, 1H), 3.45 (q, *J*=7.2 Hz, 1H, 3-H), 3.63 (s, 3H, −COOCH_3), 3.80 (s, 1H, 7-H), 3.95 (s, 1H, 12-H), 4.20 (s, 1H), 4.48 (s, 1H), 6.11 (s, 2H, N^+−CH_2−Ph), 7.39 (s, 3H, benzyl−H), 7.57 (s, 2H, benzyl−H), 7.97 (s, 1H, Py−H), 8.65 (s, 1H, Py−H), 8.94 (s, 1H, Py−H), 8.98 (s, 1H, Py−H), 10.20 (s, 1H, −CON*H*−); Anal. Calcd for $C_{38}H_{53}N_2O_5Br+2.5H_2O$: C, 61.38; H, 7.80; N, 3.77; found: C, 61.86; H, 7.45; N, 3.76.

4.2. UV and fluorescence titrations of 4a and L-Trp

3.00 mL of **4a** (5.17×10^{-5} mol/L) phosphate buffer solution (water/ethanol=1/1, 0.005 M, pH=7.2) was put in a test tube with a cover, followed by addition of 100 μL different concentrated L-tryptophan solution. The UV spectra of **4a** in the resulting solution were recorded from 190 nm to 450 nm in 1 cm quartz cuvette. The blank solution of **4a** was used as reference. The fluorescence emission spectra for **4a** were obtained from 300 nm to 600 nm, λ_{ex}=247 nm. The procedures for other compounds were similar.

4.3. Binding studies of host 4a with L-Trp by ^1H NMR

The test samples were prepared by dissolving 15 mg of **4a** or 30 mg of L-**Trp** sodium in 0.5 mL CD_3OD/D_2O=5/1 (v/v) (0.026 M for **4a** and 0.27 M for L-**Trp**) in 5 mm NMR tube. After the host and guest solutions were measured individually, 25 μL of above L-**Trp** sodium solution was added into the host solution at each time and the mix was measured immediately at room temperature.

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Supplementary data

The UV and fluorescence titration spectra of **4b**, **4c**, and **6** with L-**Trp**, the characterization spectra of compounds are provided as supplementary data. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.12.068.

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