A Metal-Free Fluorescent Probe for Selective Detection of Histidine

Jun Tian,[a] Kai Lu,[a] Yalin Wang,[a] Yu Chen,[a] Binyi Huo,[a] Yixuan Jiang,[a] Shanshan Yu,*[a] Xiaoqi Yu,*[a] Lin Pu*[b]

Abstract. A highly selective fluorescent probe has been discovered for the recognition of L-histidine (His) without the need to use a metal cation. Mechanistic studies including 1D and 2D ^{1}H and ^{13}C NMR analyses and DFT calculation have revealed that the 2-formyl group of the 1,1'-binaphthyl-based probe reacts with His to form a multicyclic product and the structural rigidity of this product should have contributed to the greatly enhanced fluorescence. The reactions of all the other amino acids with the probe cannot generate such a structurally rigid product and thus exhibit no or much smaller fluorescence enhancement. This probe can also be easily modified to allow the selective detection of His to be conducted in aqueous or fluorous solution.

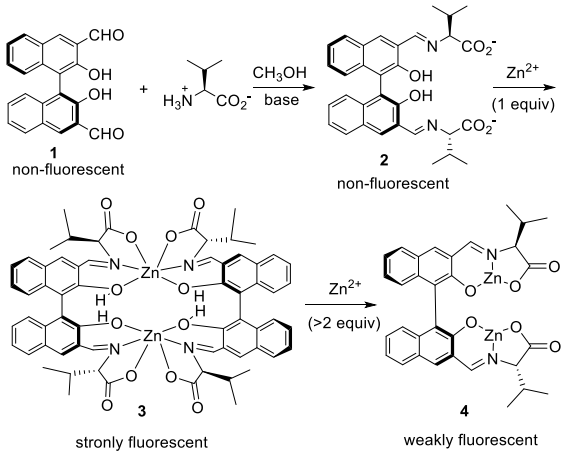
Keywords: fluorescent recognition, histidine, 1,1'-binaphthyl, aldehyde

Introduction

L-Histidine (His) plays important roles in biological systems including animals and humans. It can not only act as a neurotransmitter or neuromodulator of human muscle and nervous systems, but also assist metal element transfer because of its unique imidazole group.[1-3] Recently, medical research has revealed that a continuous deficiency of histidine can cause chronic kidney disease, rheumatoid arthritis and other malfunctions,[4] while abnormally elevated concentrations can be associated with asthma, liver cirrhosis[5] and Alzheimer's disease.[6] Therefore, detection of His is highly valuable in diagnosis and monitoring of various diseases A variety of methods have been developed to identify His which include gas and liquid chromatography, supercritical fluid chromatography, electrochromatography, UV-vis spectroscopy, fluorescence spectroscopy, etc.[7,8,9] Among these studies, the use of fluorescence-based molecular probes has attracted special attention because of the high sensitivity, easily available instruments, multiple sensing modes, and the potential for rapid and remote observation and analysis. Most of the currently reported examples for the fluorescent recognition of His rely on the chelate coordination of this amino acid with metal ions.[8]

In recent years, our laboratory has studied the use of the 1,1'-bi-2-naphthol (BINOL)-based aldehydes, such as compound **1**, as fluorescent probes for the recognition of amino acids in the presence of Zn^{2+}.[10] Both the BINOL-dialdehyde **1** and its condensation product with an amino acid, the diimine compound **2**, were non-fluorescent (Scheme 1). When **2** was treated with 1 equiv Zn^{2+}, greatly enhanced fluorescence was observed which was attributed to the formation of the dimeric complex **3**.[10b] The structural rigidity of **3** due to the restricted rotation of the two naphthalene rings around the 1,1'-bond in each BINOL unit is used to account for its strong fluorescence. When **3** was treated with an excess amount of Zn^{2+}, the dimeric structure was found to dissociate to the monomeric complex **4** with significantly reduced fluorescence. In **4**, its two naphthalene rings in the BINOL unit has regained flexibility for rotation around the 1,1'-bond which can relax the excited state energy via nonradiative decay. Thus, using compound **1** as a fluorescent probe for amino acids requires the addition of Zn^{2+} not only to coordinate to the imine product formed with an amino acid but also to generate the dimeric complex like **3** to increase the structural rigidity for fluorescence enhancement.

Scheme 1. Reaction of the aldehyde-based probe **1** with an amino acid and Zn^{2+}.



The reaction in Scheme 1 demonstrates that restricting the rotation around the 1,1'-bond of the binaphthyl unit can generate fluorescence enhancement. We propose to move the 3-formyl group in **1** to the 2-position of the binaphthyl structure to build a new probe like **5** for fluorescent recognition of amino acids. Introduction of the 2-formyl group in **5** brings the aldehyde group much closer to the lower naphthalene ring which can potentially make it easier to restrict the rotation of the 1,1'-binaphthyl unit around the 1,1'-bond upon substrate

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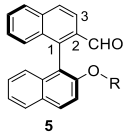
binding to generate a more rigid structure with enhanced fluorescence.[11] We have discovered that this compound shows greatly enhanced fluorescence in the presence of His without the need to add a metal cation like Zn^{2+}. It can be used for selective detection of His. Herein, this result is reported.



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Results and Discussion

1. Synthesis and Characterization of Compound 5a

Scheme 2 shows our synthesis of compound **5a**. Starting from (*S*)-BINOL, we prepared compound **6** in two steps which was then subjected to the Kumada coupling with MeMgI followed by bromination with NBS to give compound **7**.[11-13] Conversion of **7** to **8** was conducted by applying the Kornblum reaction followed by deprotection with dilute hydrochloric acid.[11,13] The reaction of the mono aldehyde **8** with bromoethane in the presence of a base in refluxing acetonitrile gave the desired product **5a** in 97% yield.

Scheme 2. Synthesis of compound (*S*)-**2**.

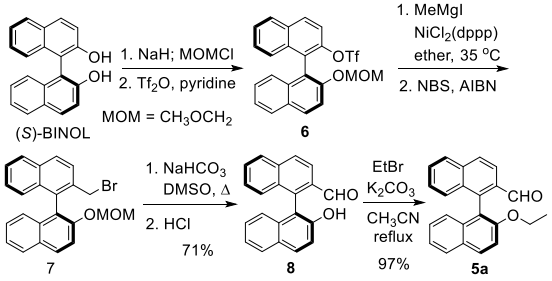


Figure 1 gives the UV-vis absorption and fluorescence spectra of compound **5a** (2.0×10^{-5} M in ^{i}PrOH/MeOH = 99:1, v/v). It shows that when **5a** was excited at 285 nm, there was weak emission at 366 nm. The UV-vis spectrum displays absorptions at λ (ε) = 252 (5.2×10^{-4}), 284 (1.7×10^{-4}), and 336 (5.7×10^{-5}) nm.

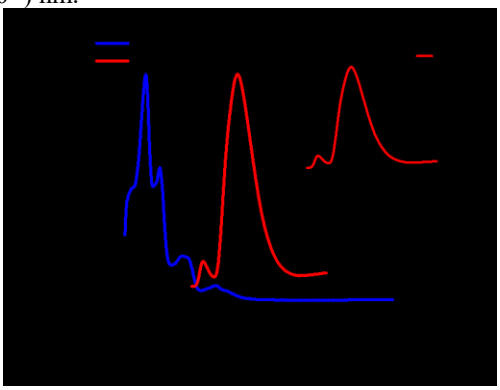


Figure 1. (a) Normalized UV-vis and fluorescence spectra of **5a.** (b) Fluorescence spectrum of **5a** (2.0×10^{-5} M in ^{i}PrOH/MeOH = 99:1, v/v, λ_{exc} = 285 nm).

2. Fluorescent Responses of 5a toward L-Histidine and Other Amino Acids

We investigated the fluorescent response of **5a** toward the tetrabutylammonium (TBA) salts of 19 common amino acids in the absence of a metal ion. In these experiments, an ^{i}PrOH solution of **5a** (2.0×10^{-3} M, 25 μL) was mixed with each amino acid salt (25 μL 10.0 equiv) in methanol at 30.0 °C for 3.0 h which was then diluted with ^{i}PrOH to a final concentration of **5a** at 2.0×10^{-5} M (2.5 mL, ^{i}PrOH/MeOH = 99:1, v/v). When the compound was treated with various amino acids, His produced significant fluorescence enhancement at λ = 365 nm, but all the other amino acids showed much smaller or non fluorescence enhancement (Figure 2a,b). As shown in Figure 2b, His greatly enhanced the fluorescence of **5a** at λ = 365 nm with I/I_0-1 of 37.9. The fluorescence quantum yield (Φ_F) of the reaction between **5a** and His (10 equiv) was found to be ≥ 91.0%. We also studied the fluorescence response of **5a** toward the enantiomer of His, D-histidine, which showed only small enantioselectivity (Figure S3 in SI).

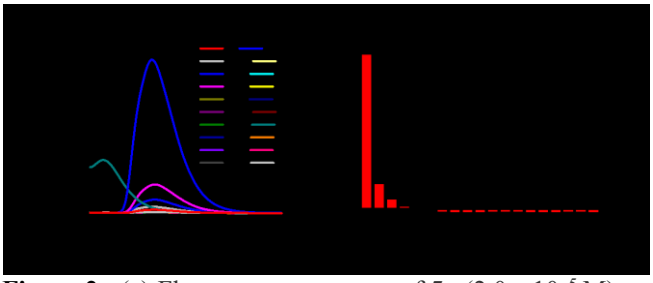


Figure 2. (a) Fluorescent responses of **5a** (2.0×10^{-5} M) toward 10 equiv of various amino acids. (b) Plot of the fluorescence enhancement I/I_0-1 at 365 nm. (Solvent: ^{i}PrOH/MeOH = 99/1, v/v. λ_{exc} = 285 nm, slits = 5/5 nm. I_0: Fluorescence intensity of **5a** at 365 nm in the absence of an amino acid).

We investigated the influence of the reaction time before dilution on the fluorescent response. As shown in Figure 3a and 3b, the fluorescence enhancement of **5a** by His (10 equiv) became stable after 150 min. Thus, all the following fluorescence measurements were conducted after 180 min of reaction. The fluorescence response of **5a** to various equivalents of His was measured. As shown in Figure 3c and 3d, when the solution of **5a** was treated with His, the fluorescence intensity at λ = 365 nm was greatly enhanced. The fluorescence intensity reached maximum at 10 equiv of His. As shown in Figure S1 in SI, the limit of detection (LOD) for His by using **5a** was determined to be 7.03×10^{-8} M. We also investigated the fluorescent responses of **5a** toward His (7.0 equiv) in the presence of other amino acids (3.0 equiv.). As shown by Figure S2 in SI, no significant interference on the fluorescence measurement was observed except with Lys, Hcy or Cys.

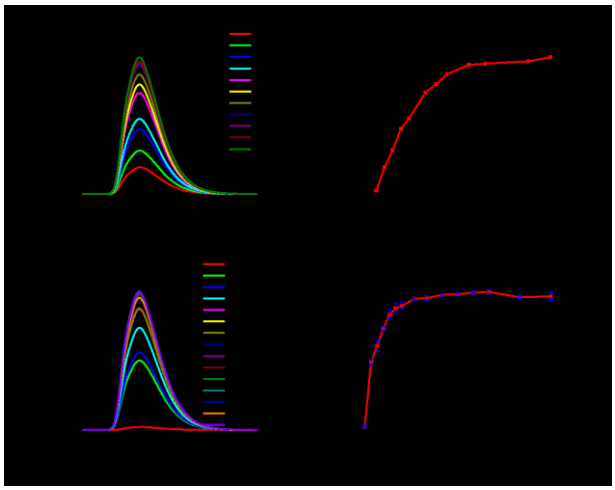
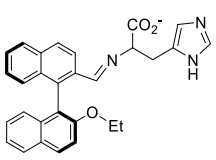


Figure 3. (a) Fluorescence spectra of **5a** (2.0×10^{-5} M) with 10 equiv of His at 15, 30, 45, 60, 90, 110, 130, 170, 200, 280 and 320 min. (b) Fluorescence intensity at 365 nm. (c) Fluorescence spectra of **5a** with 0 – 30 equiv of His. (d) Fluorescence intensity of **5a** versus the equivalence of His at 365 nm. (The error bars were obtained with three independent measurements. Solvent: ^{i}PrOH/MeOH = 99/1, v/v. λ_{exc} = 285 nm, slits = 5/5 nm.)

3. Study the Reactions of 5a with His and Other Analogs by NMR and Mass Spectroscopic Analyses and Molecular Modeling

To understand the origin of the fluorescent response of **5a** toward His, we performed a ^{1}H NMR titration experiment. Figure 4 gives the ^{1}H NMR spectra when **5a** (6.0 mM) was treated with 0 – 10 equiv of the TBA salt of His in a mixed solvent of CD_3CN:CD_3OD (1:9). It was found that with the addition of His, the aldehyde proton signal at δ 9.60 decreased, and the signals at δ 5.61 and δ 7.75 gradually increased. The 2D NMR HSQC spectrum of **5a** + His (10 equiv) (Figure S4a in SI) gives a cross peak between the imine proton signal δ 7.73 and the imine carbon signal at δ 160.5 which confirms the formation of the imine product **9** from the condensation of the aldehyde group of **5a** with the amine group of His. The cross peak between the proton signal δ 5.61 and the carbon signal at δ 70.22 in the HSQC spectrum can be assigned to the proton H_a and its bonded carbon in another product **10** formed from the intramolecular nucleophilic addition of the imidazole NH group to the imine carbon of **9**.[14] The 2D NMR HMBC spectrum (Figure S4b in SI) shows a cross peak between the H_a signal at δ 5.61 and the carbon signals at δ 123.8 (C_α) and δ 134.6 (C_β) which can be attributed to the correlation of H_a with C_α and C_β respectively in **10**.



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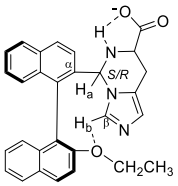

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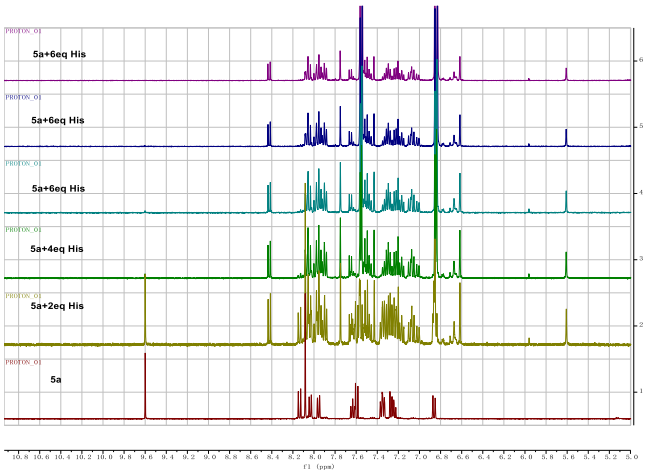


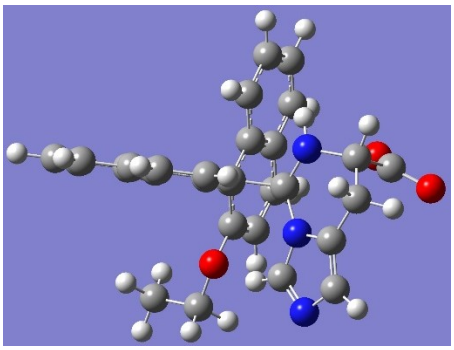
Figure 4. ^{1}H NMR spectra of (*S*)-**2** (6 mM) titrated with His (CD_3CN:CD_3OD = 1:9) (The ^{1}H NMR spectra were recorded after the solution was allowed to stand at 30 °C for 3 h).

On the basis of the HSQC and HMBC spectra (Figure 4a and 4b in SI), the proton signals at δ 3.94 and 4.01 can be assigned to the CH_2 protons of the CH_3CH_2O group. One of the proton signals at δ 7.47 gives a cross peak with a carbon signal at δ 134.6 in the HSQC spectrum which can be assigned to the H_b on the imidazole ring of **10**. In the 2D NOESY and ROSEY spectra of the reaction mixture (Figure S4c and S4d in SI), cross peaks between the signals at δ 3.94 and 4.01 and that at δ 7.47 were observed. This supports a hydrogen bonding between H_b and the ethoxy oxygen as depicted in the structure of **10**.

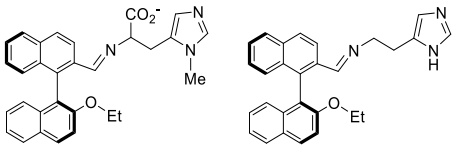
The reaction of **5a** with His (10 equiv) at 30 °C over 0 – 4 h was monitored by ^{1}H NMR analysis. As shown in Figure S5, the two peaks at δ 7.73 and 5.61 appeared at the same time from the reaction corresponding to the formation of the products **9** and **10** respectively. The ratio of the two products (**9**:**10** = 1:0.85) did not change from 30 to 240 min, suggesting that an equilibrium between these two compounds was reached. In Figure 4, there is also a small peak at δ 5.96. It can be attributed to a minor diastereomer of **10** due to the newly formed chiral carbon bearing H_a. The integration of this signal versus that of the signal at δ 5.61 is about 1:5. That is, the intramolecular nucleophilic cyclization of **9** to form **10** is a stereoselective process.

We conducted a density functional theory (DFT) calculation on the two diastereomers of the product **10** (the counter ion is NMe_4^+) by using the Gaussian program (GaussianView 6.0.16 method: DFT, B3LYP. Basis set: 6-31G). It shows that the diastereomer with a S configuration at the chiral carbon is more stable than the one with a R configuration by 4.2 kcal/mole. Figure 5 gives the molecular modeling structure of the more stable diastereomer. In this structure, there should be an intramolecular hydrogen bonding interaction between the H-C(N)$_2$ proton of the imidazole ring and the EtO oxygen with H…O distance of 2.326 Å. There might also be a weak hydrogen bonding interaction between the NH proton and the carboxylate oxygen with the H...O distance of 2.733 Å.

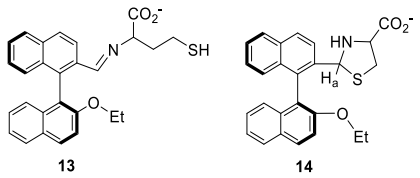
Figure 5. The molecular modeling structure of the more stable diastereomer of **10** by DFT calculation (The counter ion NMe_4^+ is omitted).



We studied the reaction of **5a** with 3-methylhistidine under the same conditions as that with histidine. 3-Methylhistidine was not able to enhance the fluorescence of **5a** (Figure S6). The ^{1}H NMR spectroscopic study suggests that 3-methylhistidine should react with **5a** to give the corresponding imine product **11** without formation of a cyclization product like **10** (Figure S7). That is, the methyl group has prevented the intramolecular addition to the imine carbon. We also found that histamine cannot enhance the fluorescence of **5a** either (Figure S6). As shown by ^{1}H NMR analysis (Figure S7), the reaction of histamine with **5a** should give an imine compound **12** without an intramolecular cyclization to form a compound like **10**. This indicates that the intramolecular hydrogen bonding between the NH proton and the carboxylate group in **10** might be important for the cyclization.



Previously, a few aldehyde-based probes were reported for the selective detection of homocysteine (Hcy) and cysteine (Cys) due to the formation of the corresponding thiazinanes and thiazolidines respectively.[15-20] We studied the reactions of **5a** with Hcy and Cys respectively. It was found that Hcy cannot enhance the fluorescence of **5a** but instead reduces the fluorescence intensity (Figure S8). ^{1}H NMR studies suggest that Hcy should react with **5a** to give an imine product **13** without cyclization to form a thiazinane (Figure S9b). When **5a** was treated with Cys, only a small fluorescence enhancement was observed (Figure S8). As shown in the ^{1}H NMR spectrum, Cys reacted with **5a** probably formed the cyclization product thiazolidine **14** as evidenced by the appearance of the H_a signal at δ 5.17 (Figure S9a). Unlike those observed in other aldehyde-based probes,[15-17] formation of the thiazolidine product with Cys did not greatly increase the fluorescence of **5a**. This indicates that the rotation around the 1,1'-bond of the binaphthyl unit in **14** can relax the excited state energy to make it less emissive.



The above studies demonstrate that formation of the imine compounds such as **9**, **11**, **12** and **13** from the condensation of the aldehyde groups of **5a** with a primary amine cannot turn on the fluorescence of the probe. Formation of the thiazolidine product **14** from the reaction of **5a** with Cys only gave a modest fluorescence enhancement. Therefore, it is proposed that the greatly enhanced fluorescence for the interaction of **5a** with His should be due to the enhanced structural rigidity of the product **10** caused by its multicyclic structure and the intramolecular hydrogen bonding interaction between the imidazole $HC(N)_2$ hydrogen H_b and the ethoxy oxygen which restricts the rotation around the 1,1'-bainphthyl bond.

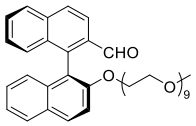
Although reaction of an aldehyde with an amino acid to form an imine product is generally expected, no subsequent intramolecular nucleophilic cyclization to form the imidazotetrahydropyrimidine product like **10** was reported before for the reaction of His with an aldehyde. We attribute the observed novel reactivity of His with **5a** under the mild conditions to the cooperation of the two intramolecular hydrogen bonds as depicted in the structure of **10**.

We monitored the reaction of **5a** with His in the presence of Lys or Cys by ^{1}H NMR spectroscopy (Figure S10 in SI). After a solution of **5a** (6.0 mM) in CD_3CN/CD_3OD (1:9, v/v) was treated with His (7.0 equiv) for 180 min, Lys (3.0 equiv) was added to react for 60 min. The ^{1}H NMR spectrum showed that the signals at δ 7.71 and 5.61 due to the products **9** and **10** from the reaction of **5a** with His diminished. This is probably because Lys contains a more nucleophilic amine group which can shift the equilibrium from the formation of **9** and **10** to the formation of a product from the reaction of **5a** with Lys. Under the same conditions when Cys (3.0 equiv) was added to the solution of **5a** and His, a significant new peak at δ 5.17 corresponding to the formation of the thiazolidine **14** appeared while the signals at δ 7.71 and 5.61 diminished. That is, the reaction of **5a** with Cys to form **14** should be more favorable over the formation of **9** and **10** with His. Therefore, the presence of Lys or Cys can interfere with the fluorescent detection of His with the probe **5a** because these amino acids contain a more nucleophilic amine or thiol group.

4. Fluorescent Responses of Compounds 5b and 5c in Aqueous Solution and Fluorous Phase Respectively

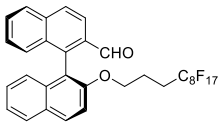
In order to conduct the fluorescent recognition of amino acids in aqueous solution, we prepared compound **5b** that contains a poly(ethylene glycol) group. From the reaction of **8** with $CH_3(OCH_2CH_2)_9OTs$ in the presence of K_2CO_3 in acetonitrile, **5b** was obtained in 91% yield. The fluorescent response of **5b** toward various amino acids in aqueous solution (1% DMSO) was investigated. In this study, the TBA salts of the amino acids were used which were prepared by mixing an amino acid and TBAOH (2 equiv versus the carboxylic group) in water. As shown in Figure 6a,b, in aqueous solution, **5b** also showed selective fluorescent response toward His. It was found that the fluorescence intensity of **5b** in the

presence of His (50 equiv) became stable after 240 min, and at this time the fluorescent enhancement reached maximum when more than 100 eq His was added (Figure S11 and S12 in SI).



5b

Because of the hydrophobic and lipophobic properties of highly fluorinated solvents,[21,22] conducting fluorescent sensing in the fluorous phase can avoid the interference of many organic and inorganic substances.[23-25] We prepared compound **5c** that contains a highly fluorinated alkyl group. From the reaction of **8** with $I(CH_2)_3C_8F_{17}$ in the presence of K_2CO_3 in acetonitrile, **5c** was obtained in 95% yield. This compound is soluble in the fluorous solvent 1H,1H,2H,2H-perfluoro-1-octanol (PFOH). We studied the fluorescent response of **5c** toward various amino acid TBA salts in PFOH (1% MeOH). The amino acid TBA salts were prepared by mixing an amino acid with TBAOH (2 equiv versus the carboxylic group) in MeOH. As shown in Figure 6c,d, in the fluorous solvent, **5c** also showed selective fluorescent response toward His. Figures S13 and S14 in SI show that the fluorescent response of **5c** with His (5 equiv) became stable after 150 min. When **5c** was treated with more than 10 equiv His for 180 min, the fluorescence intensity reached maximum.



5c

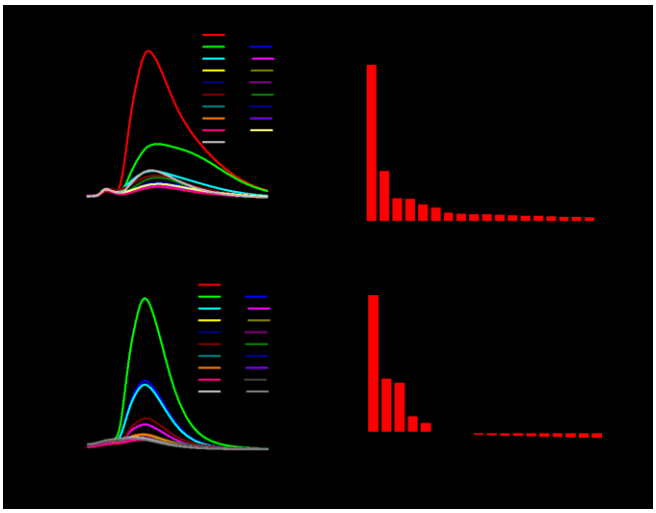


Figure 6. (a) Fluorescent responses of **5b** (2.0×10^{-5} M) toward 100 equiv of various amino acids. (b) Plot of the fluorescence enhancement I/I_0-1 of **5b** at 365 nm. (Solvent: H_2O/DMSO = 99/1, v/v.) (c) Fluorescent responses of **5c** (2.0×10^{-5} M) toward 5 equiv of various amino acids. (d) Plot of the fluorescence enhancement I/I_0-1 at 365 nm. (Solvent: PFOH/MeOH = 99/1, v/v. λ_{exc} = 285 nm, slits = 5/5 nm. I_0: Fluorescence intensity of **5c** at 365 nm in the absence of amino acids.)

We measured the LOD of these probes for His and their anti-interference ability. As shown in Figure S15 and S16 in SI, the LODs for His by using **5b** and **5c** were determined to be 2.90×10^{-7} M and 9.24×10^{-8} M, respectively. Figure S17 and S18 in SI show that the probes **5b** and **5c** exhibited good resistance to the interference of other amino acids. In addition, the fluorescence quantum yields of **5b** and **5c** with His (100 equiv) were determined to be $\Phi_F \geq 11.2\%$ and $\Phi_F \geq 4.0\%$ respectively.

5. Summary

In summary, a new fluorescent probe **5a** has been obtained for selective detection of His. Unlike the previously reported probes, this 1,1'-binaphthyl-based aldehyde shows greatly enhanced fluorescence with His in methanol solution in the *absence* of a metal salt. On the basis of 1D, 2D ^1H and ^{13}C NMR analyses and DFT calculation, it was proposed that **5a** reacts with His to form a multicyclic product with an intramolecular hydrogen bond that can restrict the rotation of the binaphthyl unit around the 1,1'-bond and increase the structural rigidity of the product to give greatly enhanced fluorescence. Other amino acids including Cys and Hcy cannot form such a structurally rigid product with **5a**, and thus cannot generate greatly enhanced fluorescence. Compound **5a** can also be easily modified to allow the selective detection of His to be conducted in aqueous or fluorous solution. To further develop these probes, structural modification will be conducted to increase the excitation and emission wavelengths for biological study.

Experimental section.

General data. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. In the optical spectroscopic studies, all the solvents were either HPLC or spectroscopic grade. ^1H and ^{13}C NMR spectra were measured on a Bruker AM400 NMR spectrometer. ESI-MS and HRMS spectral data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer, respectively. Fluorescence spectra were obtained by using Hitech F-7000 spectrofluorometer at 298 K. UV-Vis absorption spectra were recorded on a Hitachi U1900 spectrometer. Fluorescence quantum yield (Φ) was determined by using Horiba Fluorolog-3.

Synthesis and Characterization of 8. A mixture of **7** (600 mg, 1.5 mmol) and $NaHCO_3$ (310 mg, 3.7 mmol, 2.5 equiv) in DMSO (20 mL) was heated at 90 °C for 6 h under nitrogen. After the reaction was complete, it was cooled to room temperature, and the mixture was extracted with CH_2Cl_2. The organic extracts were washed with H_2O. The combined organic extracts were dried with Na_2SO_4 and the solvent was removed under reduced pressure. Then, the residue was dissolved in CH_2Cl_2/EtOH (10 mL/10 mL) and concentrated hydrochloric acid (3 mL) was added. After heated at reflux for 2 h, the reaction mixture was poured into H_2O (20 mL) at rt and neutralized with saturated $NaHCO_3$ solution until no gas was evolved. It was then extracted with EtOAc (2×20 mL) and concentrated under reduced pressure to give **8** as a light yellow solid in 71% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 8.18 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 8.6 Hz, 1H),

7.98 (dd, J = 8.6, 6.9 Hz, 2H), 7.90 – 7.87 (m, 1H), 7.64 (ddd, J = 8.2, 6.6, 1.4 Hz, 1H), 7.44 (ddd, J = 8.6, 1.5, 0.8 Hz, 1H), 7.41 – 7.30 (m, 3H), 7.27 – 7.22 (m, 1H), 6.93 (d, J = 8.4 Hz, 1H), 4.87 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.32, 151.64, 138.71, 136.68, 134.42, 133.21, 132.57, 131.03, 129.79, 129.52, 128.78, 128.61, 128.19, 127.75, 127.40, 126.79, 124.64, 123.93, 122.63, 117.45, 113.55. HRMS: m/z calcd. for $C_{21}H_{14}O_2$ M+Na $^+$:321.0891. Found:321.0886.

Synthesis and Characterization of 5a. Compound **8** (100 mg, 0.34 mmol) and K_2CO_3 (93 mg, 0.67 mmol, 2 equiv) were mixed in MeCN (20 mL) at rt. EtBr (73 mg, 0.67 mmol, 2 equiv) was added and the resulting mixture was heated at reflux for 24 h. EtOAc (3×30 mL) was then used for extraction at rt. The organic extracts were washed with water and dried with Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (eluted with ethyl acetate/petroleum ether, 1:20) to afford **5a** as a white solid in 97% yield (105 mg). m.p. 116-117 ℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.67 (d, J = 0.9 Hz, 1H), 8.14 (d, J = 8.6 Hz, 1H), 8.01 (t, J = 9.4 Hz, 2H), 7.94 (dd, J = 8.2, 1.1 Hz, 1H), 7.88 (dd, J = 8.2, 1.2 Hz, 1H), 7.57 (ddd, J = 8.2, 6.3, 1.7 Hz, 1H), 7.42 (d, J = 9.1 Hz, 1H), 7.35 – 7.27 (m, 3H), 7.21 (ddd, J = 8.2, 6.8, 1.3 Hz, 1H), 6.94 (dt, J = 8.6, 0.9 Hz, 1H), 4.05 (m, 2H), 1.03 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.2, 154.5, 142.1, 136.4, 134.6, 132.8, 132.1, 130.6, 128.7, 128.7, 128.4, 128.3, 128.0, 127.3, 127.1, 126.7, 125.2, 123.9, 122.2, 117.6, 114.1, 64.6, 14.8. HRMS: m/z calcd. for $C_{23}H_{28}O_2$ M+H+:327.1385. Found:327.1387.

Synthesis and Characterization of 5b. (a) Preparation of the tosylate of $CH_3(OCH_2CH_2)_9OH$ (**15**). Compound **15**[26] (2.14 g, 5 mmol) and dry triethylamine (1.05 mL, 7.5 mmol) were dissolved in dry CH_2Cl_2 (60 mL) and p-toluenesulfonyl chloride (1.14 g, 6 mmol) was added. The reaction was stirred at rt for 20 h. The final mixture was washed with sat. aq. $NaHCO_3$, dried with anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel (eluted with ethyl acetate) to afford $CH_3(OCH_2CH_2)_9OTs$ (**16**) as a pale-yellow oil in 95% yield (2.76 g). ^{1}H NMR (400 MHz, Chloroform-*d*) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.16 (td, J = 4.8, 1.7 Hz, 2H), 3.85 – 3.44 (m, 35H), 3.38 (s, 2H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 144.82, 144.81, 132.78, 132.77, 129.82, 127.94, 72.67, 71.83, 70.64, 70.56, 70.49, 70.46, 70.41, 70.36, 70.05, 69.32, 69.25, 68.59, 61.54, 59.01, 21.64. HRMS: m/z calcd. for $C_{26}H_{24}O_{12}S$ M+Na $^+$:605.2608. Found:605.2583. (b) A procedure similar to the preparation of **5a** was used by reacting **16** with **8** which gave compound **5b** as a pale-yellow oil in 91% yield (215 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.67 (s, 1H), 8.14 (d, J = 8.6 Hz, 1H), 8.03 (dd, J = 16.2, 8.9 Hz, 2H), 7.96 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 7.59 (ddd, J = 8.1, 6.1, 1.9 Hz, 1H), 7.46 (dd, J = 9.1, 3.4 Hz, 1H), 7.38 – 7.31 (m, 3H), 7.24 (t, J = 7.7 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 4.16 (m, 3H), 3.85 – 3.51 (m, 26H), 3.47 (m, 4H), 3.38 (d, J = 1.7 Hz, 1H), 3.29 – 2.99 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 154.5, 141.9, 136.3, 134.5, 132.8, 132.2, 130.7, 129.8, 128.8, 128.4, 128.3, 128.1, 127.3, 127.2, 126.9, 125.1, 124.0, 122.1, 117.6, 114.1, 77.4, 72.7, 71.9, 70.7, 70.5, 70.5, 70.5, 70.4, 70.4, 70.3, 70.3, 70.2, 70.2, 70.1, 69.3, 69.3, 69.2, 69.2, 68.6, 61.6, 59.1, 29.7. HRMS: m/z calcd. for $C_{40}H_{52}O_{11}$ M+Na +:731,3407. Found: 731,3405.

Synthesis and Characterization of 5c. A procedure similar to the preparation of **5a** was used by reacting $I(CH_2)_3C_8F_{17}$ with **8**. Compound **5c** was obtained as a pale-yellow oil in 95% yield (241 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (s, 1H), 8.15 (d, J = 8.6 Hz, 1H), 8.05 (d, J = 9.1 Hz, 1H), 8.01 (d, J = 8.6 Hz, 1H), 7.93 (dd, J = 12.9, 8.2 Hz, 2H), 7.57 (ddd, J = 8.2, 6.0, 1.9 Hz, 1H), 7.43 – 7.35 (m, 2H), 7.33 – 7.24 (m, 3H), 7.07 (d, J = 8.5 Hz, 1H), 4.10 (dt, J = 9.2, 5.4 Hz, 1H), 3.96 (m, 1H), 1.69 (m, 2H), 1.51 – 1.34 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.9, 154.0, 141.5, 136.4, 134.5, 132.6, 132.2, 130.8, 129.0, 128.8, 128.6, 128.4, 128.1, 127.4, 126.9, 126.8, 125.2, 124.3, 122.1, 118.3, 114.0, 67.5, 27.3, 27.0, 26.8, 20.4, 1.02. HRMS: m/z calcd. for $C_{32}H_{19}F_{17}O_2$: M+Na +:781.1011. Found:781.1007.

Sample preparation for fluorescence measurement. A stock solution (2.0 mM) of a probe in various solvents was prepared. Stock solutions (10.0 mM) of the TBA salts of amino acids were prepared by mixing amino acids and TBAOH in methanol or water in situ. For optical analysis, solutions of a probe (25 μL each) were added to several test tubes. A solution of a TBA salt of an amino acid was added to each test tube. The resulting solution was allowed to stand at 308 K. After 3 h, the mixture in each test tube was diluted with methanol to obtain 2.0×10^{-5} M solutions of the probe for fluorescence measurements. Fluorescence spectra were recorded within 1 h of the sample preparation.

Supplementary Information Available: Additional experimental procedures and spectroscopic data are provided.

Key words: fluorescent recognition, histidine, 1,1'-binaphthyl, aldehyde

Conflicts of interest
There are no conflicts to declare.

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