Fluorescent Recognition of L- and D-Tryptophan in Water by Micelle Probes

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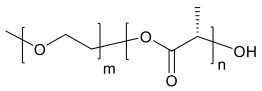
A series of BINOL-based monoaldehydes have been designed and synthesized as fluorescent probes for L- and D-tryptophan. It is found that in the presence of a diblock copolymer PEG-PLLA, these probes can be encapsulated into micelles which in combination with Zn^{2+} have exhibited chemo- and enantioselective fluorescent enhancement with tryptophan in water media.

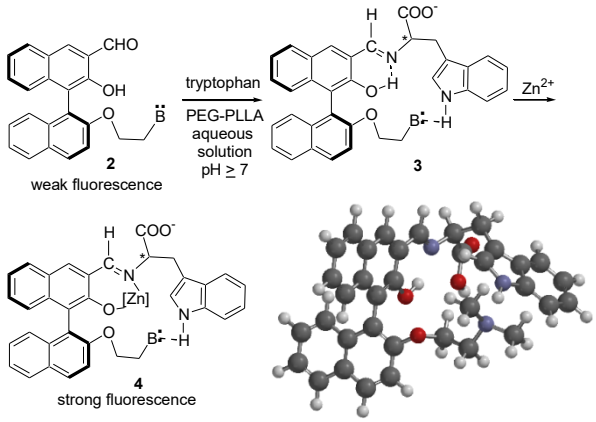
Chemoselective and enantioselective fluorescent detections of free amino acids are potentially useful because both enantiomers of amino acids are found to play diverse roles in biological systems.[1-4] Tryptophan is an essential amino acid and is important in many metabolic functions.[5-10] The level of tryptophan can be used to diagnose various metabolic disorders and associated diseases. Supplementation with this amino acid is considered in the treatment of depression and sleep disorders. While most related studies focused on L-tryptophan,[5-10] the importance of D-tryptophan has also been realized in cancer cell, bacteria and mice.[11-14] For example, D-tryptophan was recently found in the blood samples of patients with gastric cancer but not in those of healthy people.[11] It may be used as a biomarker for gastric cancer and possibly other cancers.

Previously, Tang and coworkers reported a 1,1'-bi-2-naphthol (BINOL)-based compound with good chemo- and enantioselectivity in the fluorescent recognition of tryptophan in ethanol solution.[15] This probe however was not reported to be able to conduct selective detection of tryptophan in aqueous solution. Recently, we reported that in the presence of the biodegradable diblock copolymer polyethylene glycol–poly L-lactic acid (PEG–PLLA, **1**),[16] a water *insoluble* probe can be used to conduct fluorescent recognition of lysine in aqueous solution.[17] The polymer PEG-PLLA forms micelles in aqueous solution which can encapsulate the water insoluble probe into its hydrophobic core to allow the interaction of the probe with the substrate to generate the desired fluorescent response.



PEG-PLLA **1**

In order to develop fluorescent probes for *chemo*- and *enantioselective* recognition of tryptophan in aqueous solution, we have designed a new type of fluorescent probe as represented by **2** in the present study (Scheme 1). The probes **2** contain a Lewis basic site as a hydrogen bonding acceptor. These compounds are expected to be insoluble in water. They should have weak fluorescence because of the excited state proton transfer between the hydroxyl and the aldehyde groups.[18] In the presence of PEG-PLLA, they can be encapsulated inside the hydrophobic core of the micelles

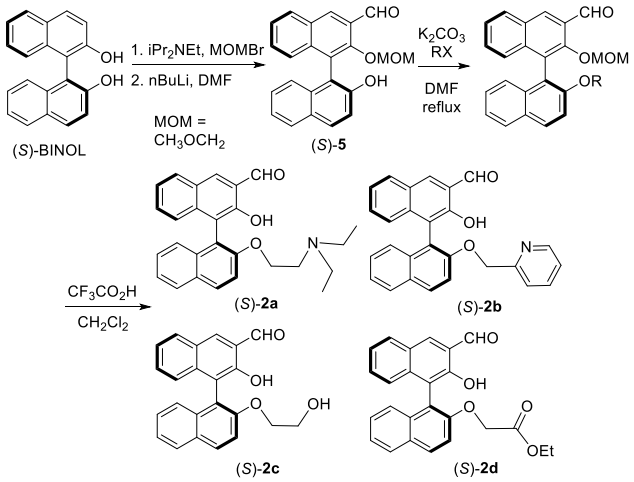


Scheme 1. Design of the fluorescent probes **2** for the selective recognition of tryptophan

generated from this diblock copolymer. Condensation of **2** with tryptophan inside the micelles should form the imine product **3** in which the Lewis basic site can form an intramolecular hydrogen bonding interaction with the indole N-H hydrogen in the hydrophobic environment of the micelle core. An energy minimized molecular modeling structure of **3**, where B = NMe_2 and tryptophan configuration is L, obtained by semi-empirical calculation by using Spartan (PM3) program is shown in Scheme 1 which features a hydrogen bonding interaction between the amine nitrogen and the indole N-H group. On the basis of our previous studies on the BINOL-aldehyde-based fluorescent probes,[18] we expect that the fluorescence intensity of **3** should not be strong because of both the excited state proton transfer between the hydroxyl group and the imine nitrogen and the excited state isomerization of the imine C=N bond. However, in the presence of Zn^{2+}, a complex like **4** could be generated with greatly enhanced fluorescence because Zn^{2+} coordination can inhibit the fluorescence quenching processes.[18] Herein, our synthesis

and study of several derivatives of **2** are reported. We have discovered that in the presence of PEG-PLLA, chemoselective as well as enantioselective fluorescent recognition of tryptophan in aqueous solution by using these probes can be achieved.

Scheme 2 shows an efficient synthesis of the BINOL-based probes (*S*)-**2a-d**. Treatment of (*S*)-BINOL with 1 equiv MOMBr (MOM = CH_3OCH_2) in the presence of a base gave a monoMOM protected BINOL which upon deprotonation with excess nBuLi and reaction with DMF gave the monoaldehyde product (*S*)-**5** in 62% yield after acidic workup.[19] Reaction of (*S*)-**5** with an alkyl halide RX in the presence of K_2CO_3 gave (*S*)-**6** whose MOM group was then removed by treatment with trifluoroacetic acid to give the desired products (*S*)-**2a-d** in 40 – 92% yields. Among these products, compounds (*S*)-**2a** and (*S*)-**2b**[19c] contain nitrogen-based Lewis base sites and compounds (*S*)-**2c** and (*S*)-**2d** contain oxygen-based Lewis base sites. All these compounds gave two singlets at δ 10.2 – 10.6 for the proton signals of their aldehyde groups as well as the adjacent hydroxyl groups, indicating strong intramolecular hydrogen bonding interaction. Compound (*S*)-**2a** was isolated as its HCl salt since its neutral form was less stable.

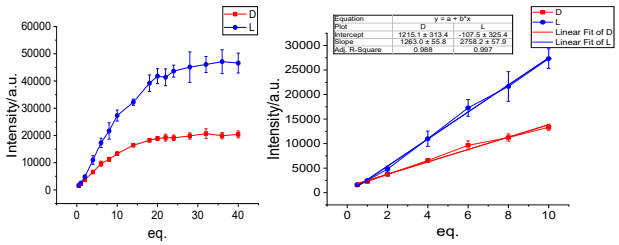


Scheme 2. Synthesis of the fluorescent probes (*S*)-**2a-d**

In order to use compounds (*S*)-**2a-d** to conduct fluorescent recognition of amino acids, we encapsulated them into PEG-PLLA to make (*S*)-**2a-d**@PEG-PLLA micelle solutions (2.0 x 10^{-5} M) (see SI for detailed experiments). The fluorescence responses of the (*S*)-**2a-d**@PEG-PLLA micelle solutions toward the two enantiomers of 19 amino acids in the presence of $Zn(OAc)_2$ (2.0 equiv) were investigated. For each measurement, 1 mL of a (*S*)-**2a-d**@PEG-PLLA solution was mixed with 1 mL carbonate buffer (CBS, 25mM), 10 equiv amino acid (10 μL, 20 mM in water) and 2.0 equiv $Zn(OAc)_2$ (10 μL, 4 mM in water) which was allowed to stand at room temperature for 3 h. The final concentration of the probes in the solutions was 1.0 x 10^{-5} M with pH = 10.1. Figure S2 in SI gives an example of the fluorescence spectra of (*S*)-**2a-d**@PEG-PLLA in the presence of the enantiomers of 19 amino acids. The micelle solution (*S*)-**2c**@PEG-PLLA showed very weak fluorescence upon excitation at 430 nm. When it was treated with most of the amino acids, only very small fluorescence enhancement was observed. However, both D-tryptophan and L-tryptophan greatly enhanced the fluorescence at λ = 545 nm. The fluorescence intensity with L-tryptophan was found to be 60% greater than that with D-tryptophan. Several basic buffer solutions were screened and the carbonate buffer solution provided the optimal results (See Figure S6). The reaction of (*S*)-**2c**@PEG-PLLA with D- and L-Trp showed stabilization of the fluorescence intensity after 3 h at room temperature, which allowed stable measurements (See Figure S7).

Figure 1 summarizes the fluorescence responses of all the micelle solutions (*S*)-**2a-d**@PEG-PLLA toward the two enantiomers of 19 amino acids under the same conditions. It shows that among the four probes, (*S*)-**2a**@PEG-PLLA and (*S*)-**2c**@PEG-PLLA exhibited better chemoselectivities than (*S*)-**2b**@PEG-PLLA and (*S*)-**2d**@PEG-PLLA, and the chemoselectivity and enantioselectivity of (*S*)-**2c**@PEG-PLLA is slightly better than (*S*)-**2a**@PEG-PLLA. In the presence of (*S*)-**2c**@PEG-PLLA, L-tryptophan generated 19.6 fold fluorescence enhancement at λ = 545 nm and D-tryptophan generated 12.3 fold fluorescence enhancement, that is the enantioselective fluorescence enhancement ratio *ef* = 1.65 [*ef* = $(I_L\text{-}I_0)/(I_D\text{-}I_0)$, where I_0 is the fluorescence of the probe without the amino acid]. (*S*)-**2d**@PEG-PLLA also showed good enantioselective fluorescent response toward phenylalanine, tryptophan and lysine, and (*S*)-**2b**@PEG-PLLA showed good enantioselective fluorescent response toward histidine. Figure S8 gives the enantioselective fluorescent responses of (*S*)-**2d**@PEG-PLLA with L and D-phenylalanine. It shows that the fluorescence enhancement of (*S*)-**2d**@PEG-PLLA with L-phenylalanine continuously increased even when measured at 5 °C.

We then studied the effect of the concentration of tryptophan on the fluorescence response of (*S*)-**2c**@PEG-PLLA. As shown in Figure 2a, the fluorescence intensity increased with increasing tryptophan concentration until 20 equiv L- or D-tryptophan at which the fluorescence



(a) (b)

Figure 2. (a) Fluorescence intensity of (*S*)-**2c**@PEG-PLLA (10 μM) and $Zn(OAc)_2$ (2 equiv) at λ = 545 nm versus the stoichiometry of tryptophan. (b) Expanded view of 0.5 to 10 equiv tryptophan. (λ_{ex} = 430 nm, slit = 3/3 nm, integration time = 0.1 s).

enhancement was saturated. In the range of 0.5 to 10 equiv of either D- or L-tryptophan, first-order linear fit was applied and R square value more than 0.99 was found (Figure 2b). The limit of detection (LOD) was thus determined to be 5.68 μM for D-tryptophan and 2.60 μM for L-tryptophan (LOD = 3*SD/k. SD, standard deviation of noise. k, slope of calibration curve). With a tryptophan detection

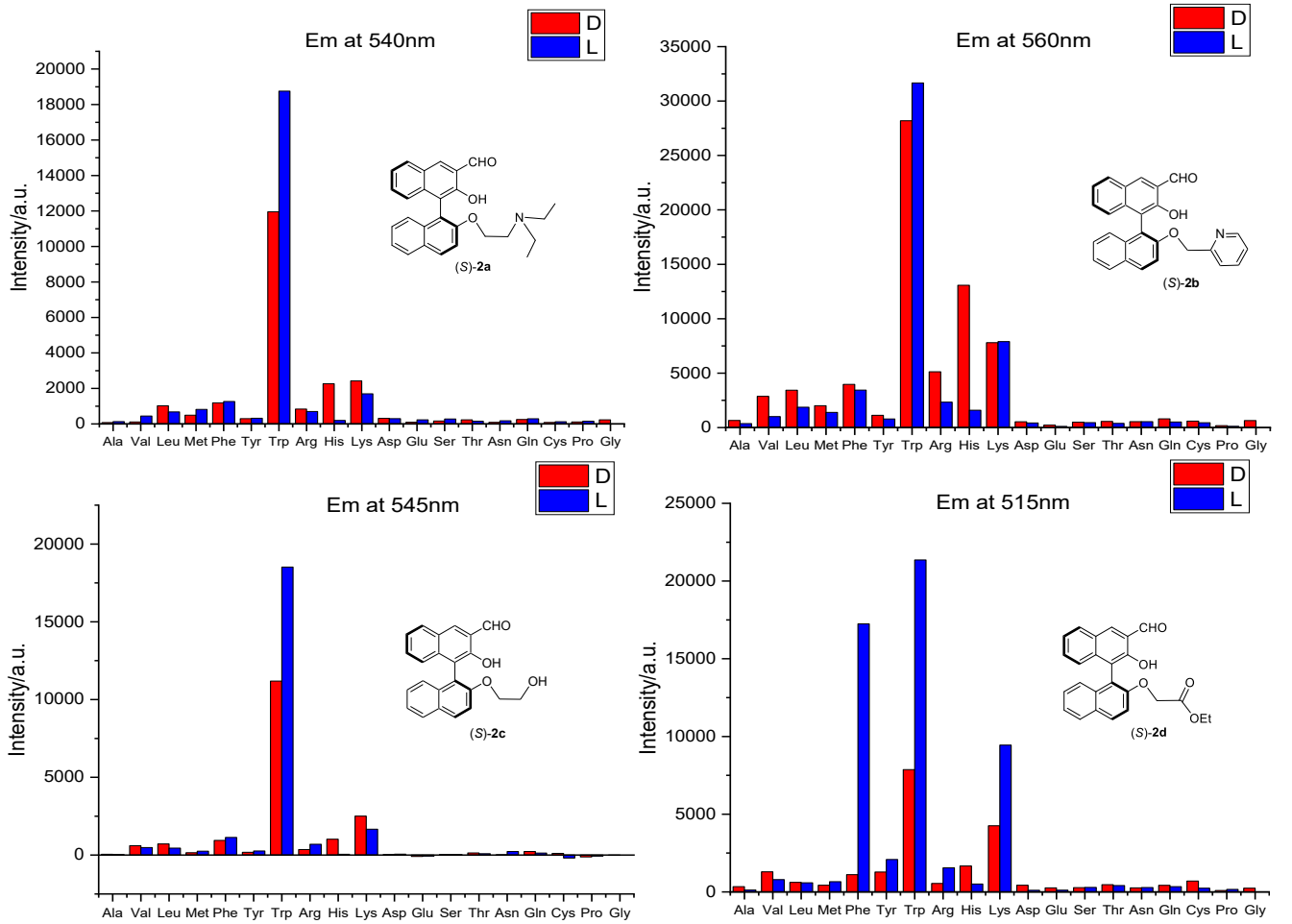


Figure 1. Fluorescence response of the micelle probes (*S*)-**2a**@PEG-PLLA, (*S*)-**2b**@PEG-PLLA, (*S*)-**2c**@PEG-PLLA and (*S*)-**2d**@PEG-PLLA towards various amino acids (Conditions: 10 μM micelle probe, 2 equiv $Zn(OAc)_2$, and 10 equiv amino acids in carbonate buffer solution. Peak intensities were used. Spectra were taken after 3 h of reaction at rt. λ_{ex} = 430 nm for (*S*)-**2a**, (*S*)-**2b**, and (*S*)-**2c**, and λ_{ex} = 400 nm for (*S*)-**2d**. slit = 3/3 nm, integration time = 0.1s.)

concentration range of 3~6 to 400 μM, (*S*)-**2c**@PEG-PLLA is potentially useful to measure the tryptophan concentrations in biological systems such as human serum, where the concentration of tryptophan was found to be 67.2±10.2 μM.[20]

(*R*)-**2c**, the enantiomer of (*S*)-**2c**, was synthesized by starting from (*R*)-BINOL. The diblock copolymer PEG-PDLA was also obtained from the D-lactic acid monomer. We thus prepared (*R*)-**2c**@PEG-PDLA as the enantiomeric analog of (*S*)-**2c**@PEG-PLLA and studied the fluorescence responses of this pair of enantiomeric probes at various enantiomeric excesses of tryptophan (*ee* = [D-L]/[D+L]). As shown in Figure 3, the fluorescence responses of (*R*)-**2c**@PEG-PDLA and (*S*)-**2c**@PEG-PLLA exhibit a close

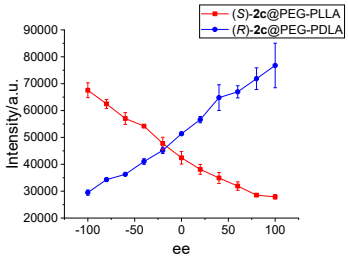


Figure 3. Fluorescence intensity of (*S*)-**2c**@PEG-PLLA/(*R*)-**2c**@PEG-PDLA (10 μM), $Zn(OAc)_2$ (2 equiv) and tryptophan (40 equiv) at λ = 545 nm versus the ee of tryptophan (λ_{ex} = 430 nm, slit = 3/3 nm, integration time = 0.1 s).

to mirror image relationship which confirms the observed enantioselective recognition. The imperfect matches are attributed to the intrinsic differences of the micelles, the random conformations of the polymers and their variable molecular weight distributions, and experimental errors.

When (*S*)-**2c**@PEG-PLLA+Zn^{2+} was treated with D- and L-Trp, the UV-vis spectra only showed the increased signals of the added tryptophan but no change in the absorption of the probe (see Figure S13 in SI). This indicates that there was only a very small degree of reaction between the probe and the amino acid under this condition which however has led to the dramatically enhanced fluorescence in aqueous solution. We also found that when compounds such as (*S*)-**2b** and (*S*)-**2c** were used to interact with tryptophan in an organic solvent methanol in the absence of PEG-PLLA, there was much weaker fluorescent enhancement with almost no enantioselectivity. Thus, the polymer-based micelles not only allow the detection of tryptophan to be conducted in water, but also greatly enhance the fluorescence sensitivity and selectivity.

The sizes of (*S*)-**2c**@PEG-PLLA were characterized by using both dynamic light scattering (DLS) (See FigS14 in SI) and transmission electron cryomicroscopy (cryo-TEM). As shown by the cryo-TEM images in Figure 4, (*S*)-**2c**@PEG-PLLA forms nanoscale spherical structures. Although DLS results showed more structural changes after interaction with amino acids, cryo-TEM images supported that the micelle structures were mostly maintained after treated with either D-Trp or L-Trp.

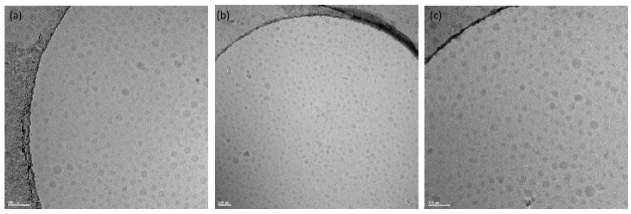
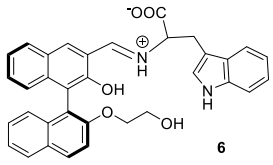


Figure 4. Cryo-TEM of micelle structures. (a) (*S*)-**2c**@PEG-PLLA; (b) (*S*)-**2c**@PEG-PLLA + D-Trp; (c) (*S*)-**2c**@PEG-PLLA + L-Trp. Scale bar: 100 nm.

Because of the interferences of the polymeric micelles, the ^{1}H NMR spectrum of (*S*)-**2c**@PEG-PLLA was not informative. We thus studied the reaction of (*S*)-**2c** with D- and L-Trp in methanol. ^{1}H NMR spectroscopic analyses of the reactions showed that both D- and L-Trp reacted with (*S*)-**2c** quickly in methanol to form the corresponding imine products with the disappearance of the aldehyde signal as well as the appearance of a new singlet at δ ~8.20 for the imine proton (See Figure S10 & S11 in SI). Mass spectroscopic analyses of the reaction mixture also supported the formation of the imine product **6** (calcd for **6**+H: 545.2076. m/z found for that from the reaction of D-Trp: 545.2092, and that from L-Trp: 545.2072. See Figure S19 & S20 in SI).



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In conclusion, a new class of molecular probes has been designed for the fluorescent recognition of tryptophan in aqueous solution. These compounds can be synthesized readily in four steps from the optically active BINOL. In the presence of the diblock copolymer PEG-PLLA and Zn^{2+}, the micelle probes formed from compounds (*S*)-**2a** and (*S*)-**2c** have shown excellent chemoselectivity for the fluorescent recognition of tryptophan in aqueous solution. In addition, the chirality of these probes has also made them enantioselective in their fluorescent response toward this amino acid. The micelle structure of the diblock copolymer not only allows the interaction of the water insoluble fluorescent probes with the amino acid to be conducted in aqueous solution, but also provides a hydrophobic interior to greatly enhance the fluorescence sensitivity and selectivity of the probes.

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Supplementary Information Available: Experimental procedures and additional spectroscopic data are provided.

Conflicts of interest. There are no conflicts of interest to declare.

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