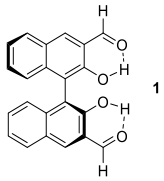
Highly Chemoselective and Enantioselective Recognition of Serine by a Fluorescent Probe

Yalin Wang[a], Jun Tian[a], Feng Zhao[a,b], Yu Chen[a], Bingyi Huo[a], Shanshan Yu*[a], Xiaoqi Yu[a], Lin Pu*[b]

Abstract A diarylacetylene-containing 1,1'-bi-2-naphthol derivative (*R*)-**2** is designed and synthesized. This compound in combination with Zn^{2+} is found to be the first chemoselective as well as enantioselective fluorescent probe for the biologically important amino acid serine. It is found that L-serine can greatly enhance the fluorescence of the probe at λ = 471 nm but D-serine and 17 other common amino acids cannot. The enantioselective fluorescence enhancement ratio [ef = $(I_L-I_0)/(I_D-I_0) = \Delta I_L/\Delta I_D$] up to 15 was observed for the response of (*R*)-**2**+Zn^{2+} toward serine.

The amino acid L-serine has been shown to play a central role in cell proliferation.[1] In human body, L-serine is produced by several biosynthetic processes and it can also be obtained by dietary intake. The synthetic pathway of L-serine is found to be essential in the function and development of the central nervous system.[2] In 1992, Nishikawa and coworkers discovered high concentration of D-serine, the enantiomer of L-serine, present in the mammalian brain.[3] Since then, considerable efforts of researchers have been conducted to understand the origin and function of D-serine.[4] Astrocytes in the central nervous system of rodents and humans uptake glucose from the blood vessels and then convert it to L-serine. In the neuron, serine racemase changes L-serine to D-serine. Thus, D-serine is found in many regions of brain and plays various functions.[5-7] For example, it can regulate the activation of N-methyl-D-aspartate receptors which contributes to the regulation of synaptic activities, learning and memory.[8-10] The serum levels of D-serine in patients with schizophrenia were found to be significantly lower than normal.[11] Patients with Alzheimer's disease were reported to have higher levels of D-serine in their cerebrospinal fluid.[12,13] A number of analytical methods such as bioelectro sensors, HPLC, and others have been developed for the detection of D-serine.[4]

Our laboratory is interested in developing fluorescent probes for chemo- and enantioselective recognition of the biologically significant amino acids like serine because of the easily available instrument, multiple detecting modes and the ability for high throughput analysis and real time imaging. Although many fluorescent probes have been developed for amino acid detection,[14] none of them can recognize serine with both chemoselectivity and enantioselectivity. For example, in 2014, we discovered that the 1,1'-bi-2-naphthol (BINOL)-based compound **1** in combination with Zn^{2+} exhibits enantioselective fluorescent enhancement in the presence of several amino acids but it shows no chemoselectivity for serine.[15]



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Previously, McFarland and Finney demonstrated that restricting the rotation of diarylacetylenes can greatly enhance their fluorescence and this process can be used as a responding mechanism for fluorescent assay.[16] We have thus proposed to incorporate diarylacetylene units into the BINOL-based compound **1** to prepare compound (*R*)-**2** for chemoselective as well as enantioselective fluorescent recognition of amino acids (Scheme 1). Compound (*R*)-**2** contains two diarylacetylene fragments at the 3,3'-position of the central BINOL unit. The imine product **3** formed from the reaction of (*R*)-**2** with L- or D-serine may form inter- or intramolecular complexes Zn^{2+} to restrict the rotation of the diarylacetylene units to generate enhanced fluorescence. In this paper, we are delighted to report our discovery that compound (*R*)-**2** [and its enantiomers (*S*)-**2**] shows highly chemoselective as well as enantioselective fluorescent response toward serine.

Scheme 1. Design of a fluorescent probe (*R*)-**2**.

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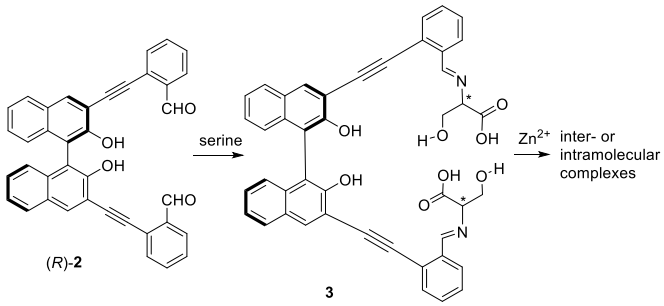
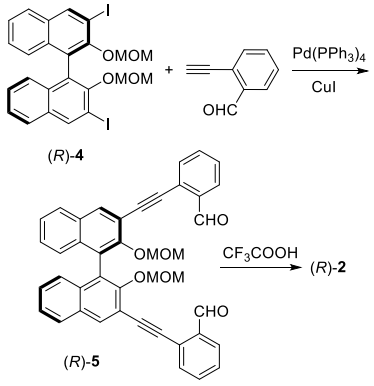
Supporting information for this article, including NMR and mass spectra of the new compounds and additional optical spectra, is given via a link at the end of the document



The 3,3'-diodoBINOL compound (*R*)-**4** was prepared from (*R*)-BINOL in two steps according to the literature.[17,18] From the Sonogashira coupling[19] of (*R*)-**4** with 2-ethynylbenzaldehyde,[20] compound (*R*)-**5** was obtained in 82% yield (Scheme 2). Removal of the MOM group of (*R*)-**5** with trifluoroacetic acid gave the desired compound (*R*)-**2** as a yellow solid in 95% yield. The ^{1}H NMR spectrum of (*R*)-**2** in $CDCl_3$ exhibits a hydroxyl signal at δ 7.41 confirmed by its disappearance with addition of D_2O. This is very different from the hydroxyl proton signal of **1** which appeared at δ 10.5[21], much more down-field because of its intramolecular hydrogen bonds with the adjacent aldehyde groups. The significantly upfield chemical shift of the hydroxyl proton signal of (*R*)-**2** indicates that this compound should not have as strong intramolecular hydrogen bonds as those in **1**.

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



The optical spectra of (*R*)-**2** are compared with those of (*R*)-**1**. As shown in Figure 1a, (*R*)-**1** gives stronger absorption at wavelength > 400 nm than (*R*)-**2**. This can be attributed to the charge transfer band due to the donor-acceptor conjugation of the hydroxyl group of (*R*)-**1** with its adjacent aldehyde group and its intramolecular hydrogen bonding. The absorptions of (*R*)-**2** between 300 and 400 nm are more intense than those of (*R*)-**1** which can be attributed to the π-π* transition of the more extended π system of (*R*)-**2**. A dramatic difference between the fluorescence spectra of (*R*)-**1** and (*R*)-**2** is observed as shown in Figure 1b. Going from (*R*)-**1** to (*R*)-**2** gives a greatly enhanced fluorescence signal at λ > 400 nm, indicating that the extended conjugation of the diarylacetylene units of (*R*)-**2** has produced a stronger fluorophore. The emission wavelength of (*R*)-**2** occurs at a longer wavelength than that of BINOL (at λ_{max} = 368 nm and λ_{exc} = 278 nm)[22] because of the more extended π system.

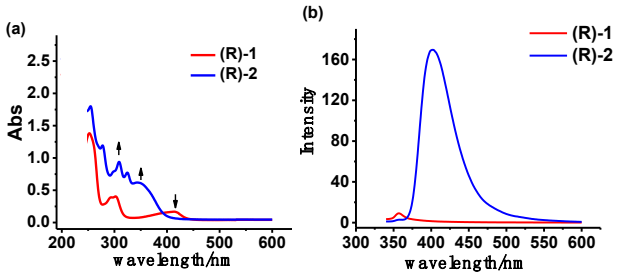
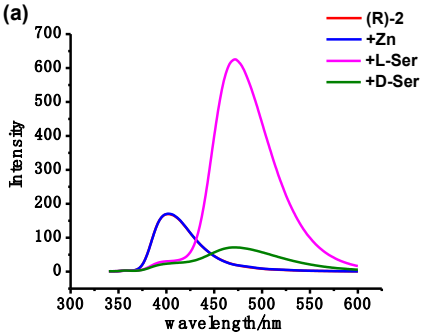


Figure 1. UV/Vis (a) and fluorescence (b) spectra of (*R*)-**1** and (*R*)-**2** (concentration: 2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2. λ_{exc} = 320 nm, slit 5/5 nm, int. time 0.5 s)

We studied the fluorescent response of (*R*)-**2** toward the two enantiomers of 18 common amino acids in the presence of $Zn(OAc)_2$ at room temperature. In order to improve the solubility of the substrates in methanol, all the amino acids used herein were their tetrabutylammonium (TBA) salts, which were obtained by treating the amino acids with TBAOH (2.0 equiv). When (*R*)-**2** (2.0×10^{-5} M in MeOH) was mixed with $Zn(OAc)_2$ (4.0 equiv), little change was observed for the fluorescence at 402 nm (λ_1) (Figure 2a). When the (*R*)-**2** (2.0×10^{-5} M) + $Zn(OAc)_2$ (4.0 equiv) solution was treated with L-Ser-TBA (10.0 equiv), a large fluorescent enhancement at 471 nm (λ_2) was observed as shown in Figure 2a. However, when the same (*R*)-**2** (2.0×10^{-5} M) + $Zn(OAc)_2$ sensor solution was treated with D-Ser-TBA (Figure 2a) and 17 pairs of the other amino acid enantiomers (Figure 2b), little or no fluorescence enhancement was observed at 471 nm. We examined the effect of the reaction time on the fluorescence response. As shown in Figure S1, the fluorescence enhancement of (*R*)-**2**+$Zn(OAc)_2$ with L-Ser-TBA and D-Ser-TBA (10 equiv) became stable after 5 h. We have thus conducted all the fluorescence measurements after 5 h of the reaction. It was found that under these conditions, L-Ser-TBA enhanced the fluorescence of (*R*)-**2**+$Zn(OAc)_2$ at 471 nm to 31.3 folds and D-Ser-TBA only increased it to 3.0 folds, giving an enantiomeric fluorescence enhancement ratio (*ef* = $[I_L-I_0]/[I_D-I_0]$) of 15.



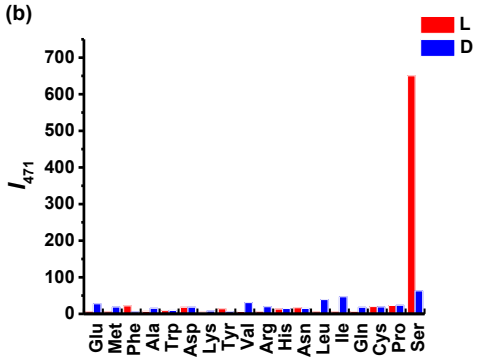


Figure 2. (a) Fluorescence spectra of (*R*)-**2** with $Zn(OAc)_2$ in the presence of L/D-Ser-TBA (10 equiv). (b) Fluorescence intensity of (*R*)-**2** with $Zn(OAc)_2$ and 18 D-/L-amino acid-TBAs (10 equiv). [(*R*)-**2**: 2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2. $Zn(OAc)_2$: 4 equiv. Reaction time: 5 h at rt. λ_{exc} = 320 nm, slit 5/5 nm, int. time 0.5 s; y-axis: intensity/arbitrary unit]

Figure 3 displays the effects of the concentration of L-/D-Ser-TBA on the emission intensities of the (*R*)-**2**+Zn^{2+} sensor solution at λ = 471 nm. Highly enantioselective fluorescent response was observed. As shown in Figure 3b, the fluorescence intensity I_{471} of (*R*)-**2**+Zn^{2+} increased greatly as the concentration of L-Ser-TBA increased from 0 to 10 equiv after which it slowly decreased with the concentration. D-Ser-TBA caused much smaller fluorescence enhancement over the entire concentration range.

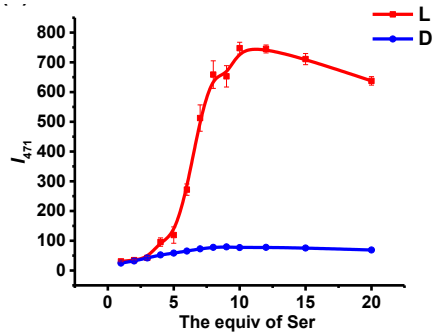


Figure 3. Plot of I_{471} for (*R*)-**2** (2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2) + Zn^{2+} (4 equiv) in the presence of varying concentrations of D- and L-Ser-TBA (0 - 40×10^{-5} M). (Reaction time: 5 h at rt. λ_{exc} = 320 nm, slit 5/5 nm, int. time 0.5 s)

(*S*)-**2**, the enantiomer of (*R*)-**2**, was also used to interact with $Zn(OAc)_2$ and D-/L-Ser-TBA. Under the same conditions, the fluorescence responses of (*S*)-**2**+Zn^{2+} toward D-/L-Ser-TBA are close to a mirror image relationship with those when (*R*)-**2** was used (Figure S4). This confirms the chiral recognition process for the fluorescent response of the probe toward serine. We then investigated the fluorescence response of both (*R*)- and (*S*)-**2** toward serine at various enantiomeric composition. As shown in Figure 4, the fluorescence intensity at 471 nm for each enantiomeric

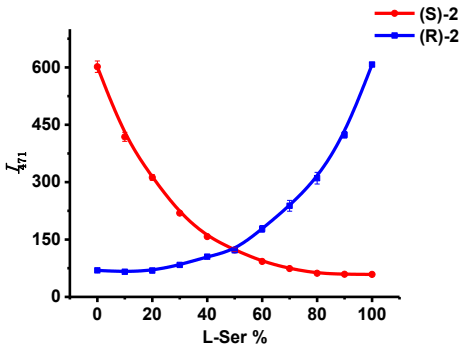


Figure 4. Fluorescence intensity at λ_2 = 471 nm versus [L-Ser]% for the reaction of (*S*)- or (*R*)-**2** (2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2) with $Zn(OAc)_2$ (4.0 equiv) and Ser-TBA (10.0 equiv, L-Ser: from 0 to 100%). (The error bars were obtained from three independent experiments. Reaction time: 5 h at rt. λ_{exc} = 320 nm, slit 5/5 nm, int. time 0.5 s; x-axis: *ee*%; y-axis: intensity/arbitrary unit)

probe versus the [L-Ser] % values of serine resembles a mirror image relationship. These plots can be used to determine the enantiomeric composition of serine by fluorescence measurement.

We further studied the fluorescence response of (*R*)-**2**+Zn^{2+} toward the serine analogs L-/D-threoine and L-/D-*allo*-threoine (Figure 5). As shown in Figure 6, under the same conditions as those used for the interaction with serine, the TBA salts of L-threoine and L-*allo*-threoine (10 equiv) greatly enhanced the fluorescence of (*R*)-**2**+Zn^{2+} with 29.6 folds for L-threoine at 468 nm and 37 folds for L-*allo*-threoine at 470 nm. The fluorescence enhancements by the TBA salts of D-threoine and D-*allo*-threoine were only 3.3 and 10.9 folds respectively.

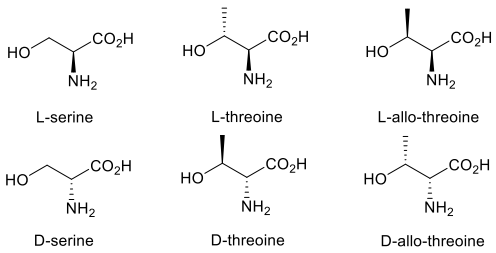
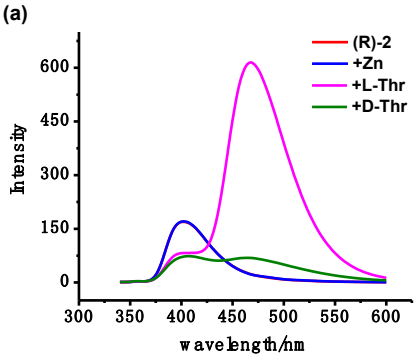


Figure 5. Structures of serine and its analogs threoine and *allo*-threoine.



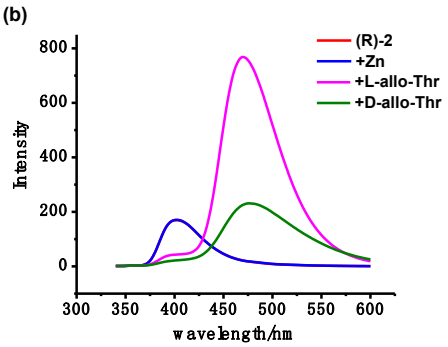


Figure 6. Fluorescence spectra of (*R*)-**2** (2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2) with $Zn(OAc)_2$ (4 equiv) in the presence of the TBA salts of (a) L-/D-Thr (10 equiv) and (f) L-/D-allo-Thr (10 equiv). (Reaction time: 5 h at rt. λ_{exc} = 320 nm, slit 5/5 nm, int. time 0.5 s)

The above studies demonstrate that the chemoselectivity of (*R*)-**2**+Zn^{2+} for serine is mostly determined by the β-hydroxy group of the amino acids. Without this hydroxyl group, almost no fluorescence enhancement can be observed (see Figure 2b). It also shows that the enantioselectivity is mostly determined by the α-chiral amine center and the chiral configuration of the β-hydroxyl group only plays a minor role.

In order to gain further understanding on the interaction of the (*R*)-**2**+Zn^{2+} fluorescent sensor system with chiral amino acids, we conducted additional spectroscopic studies for the reaction of(*R*)-**2** with serine in the presence of Zn^{2+}.

UV−vis Study.

The UV-vis spectrum of the solution of (*R*)-**2**+$Zn(OAc)_2$ in methanol displays absorptions at λ_{max} (ε) = 254 (1.19×10^5), 278 (8.54×10^4), 309 (6.71×10^4), 324 (5.84×10^4) and 340 nm (4.66×10^4) (Figure 7). The presence of $Zn(OAc)_2$ had no significant effect on the UV absorption of (*R*)-**2**. When (*R*)-**2** (2.0×10^{-5} M in MeOH/0.8% CH_2Cl_2) was treated with D- or L-Ser-TBA (10 equiv), the absorptions at λ_{max} = 254, 309, and 340 nm decreased with a new absorption emerged at λ_{max} = 401 nm.

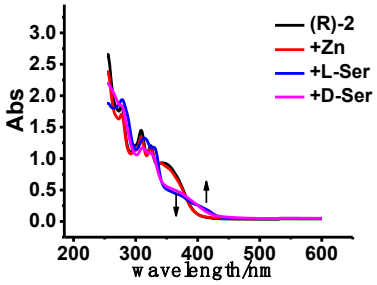
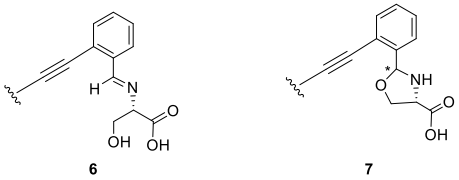


Figure 7. UV/vis absorption spectra of (*R*)-**2** (2.0×10^{-5} M in CH_3OH/0.8% CH_2Cl_2) + $Zn(OAc)_2$ (4 equiv) with/ without D- and L-Ser-TBA (10 equiv). (Reaction time: 5 h at rt)

NMR Study.

We studied the reaction of (*R*)-**2** (5 mM) with L-/D-Ser-TBA (10 equiv) in $CDCl_3/CD_3OD$ = 2:3 (v/v) by ^1H NMR spectroscopy. The TBA salts of L-/D-serine were prepared by completely dissolving each amino acid and TBAOH in methanol in 1:2 ratio followed by removal of the solvent. The solution of (*R*)-**2** in $CDCl_3/CD_3OD$ showed two small ^1H NMR signals at δ 5.98 and 5.99, which can be attributed to a partial formation of mono- and di-hemiacetals from the addition of CD_3OD to the aldehyde groups of (*R*)-**2** (Figure 8). When L-Ser-TBA was added to this solution, in 0.5 – 2 h (*R*)-**2** was completely consumed with the disappearance of the aldehyde signal at δ 10.45. A new peak appeared at δ 9.05 which can be assigned to the imine proton signal of the structural unit **6** in the product. The HMQC spectrum (Figure S7) supports this assignment with a cross peak observed for the imine ^1H signal with the ^{13}C signal at δ 161.4. At δ 6.41 and 5.90, new peaks also appeared which can be attributed to the oxazolidine fragment **7** (Figure 8a).[23] This is supported by the HMQC spectrum with the cross peaks of between these proton signals and the ^{13}C signals at δ 91.13 and 91.04 respectively. The reaction of (*R*)-**2** with D-Ser-TBA gave similar ^1H NMR signals.



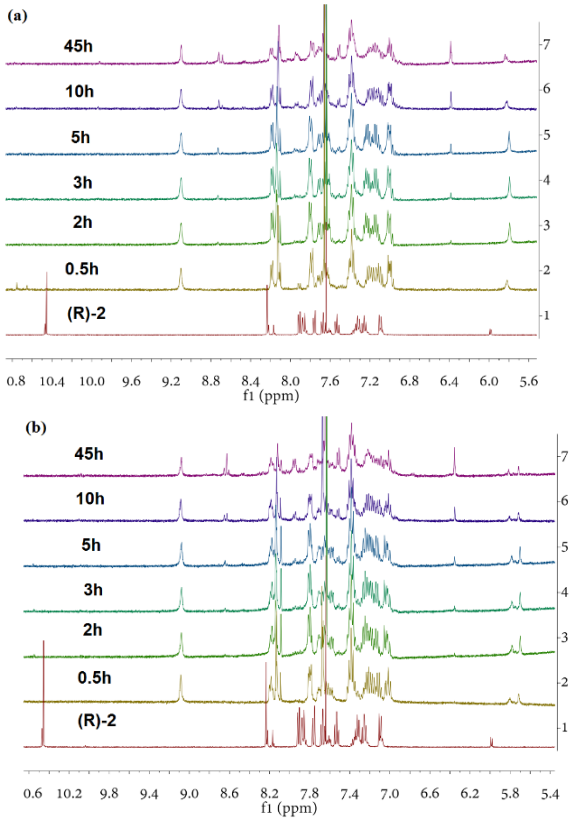


Figure 8. ^1H NMR spectra of the reaction mixture of (*R*)-**2** (5 mM) and (a) L-Ser-TBA or (b) D-Ser-TBA (10 equiv) in $CDCl_3$:CD_3OD = 2:3 (v/v) at various reaction times. For both plots: 1, (*R*)-**2**; 2-7, (*R*)-**2**+L- or D-Ser for 0.5 h, 2 h, 3 h, 5 h, 10 h, and 45 h, respectively).

Addition of 1 - 2 equiv Zn^{2+} ($ZnBr_2$ was used for its better solubility at the high concentration of the NMR experiments) to the above solutions led to the formation of precipitates with greatly reduced NMR signals (Figure S8). Under the fluorescence measurement conditions, however, no precipitate was observed. It is thus proposed that an intermolecular complexation of the imine units like **6** with Zn^{2+} at the high concentrations (5 mM) in the NMR experiments might occur to generate polymers or oligomers to precipitate out of the solution. Under the dilute conditions (2.0×10^{-5} M) of the fluorescence experiments, an intramolecular complexation with Zn^{2+} for the chirality matched probe and serine might form an intramolecular Zn^{2+} complex of **3** to restrict the rotation of the diarylacetylene units to give greatly enhanced fluorescence. When more than 2 equiv of Zn^{2+} was added to the above NMR samples (Figure S8), the aldehyde signal of (*R*)-**2** reappeared, indicating reversible reaction of (*R*)-**2** with serine in the presence of excess amount of Zn^{2+}. The coordination of the imine units with Zn^{2+} might be in competition with the coordination of free serine with Zn^{2+}. We have conducted mass spectroscopic analysis for the reaction mixture of (*R*)-**2**+Zn^{2+} with L- or D-Ser-TBA, but could not obtain direct evidence for the identities of the products due to the complex spectra. Thus, continuous research is needed in order to gain further understanding on the sensor-substrate reaction.

In summary, we have incorporated two diarylacetylene units into the BINOL structure to construct a novel fluorescent probe (*R*)-**2**. We have discovered that (*R*)-**2** in combination with Zn^{2+} is the first highly chemoselective as well as enantioselective fluorescent probe for serine. It was found that L-serine can generate large fluorescence enhancement at λ = 471 nm but not D-serine as well as 17 pairs of other amino acid enantiomers. It is proposed that the imine product formed from the condensation of (*R*)-**2** with L-serine could bind Zn^{2+} to form a structural rigid complex to generate the observed large fluorescence enhancement. D-Serine and other amino acids cannot cause such a structural rigidification induced fluorescence enhancement. We are continuously investigating the mechanism of the observed selective recognition, and are also developing the application of this probe.

Associated Content
Supporting Information
Additional synthesis and characterization data of compounds. Additional spectroscopic data.

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Notes
The authors declare no competing financial interest.

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Key words: fluorescent sensor, amino acids, serine, chemoselective, enantioselective

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