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Synthesis of (R)-2, 2'- (1, 1'-binapthyl-2, 2'-diylbis (oxy) bis (*N*-9, 10-dioxo-9, 10, -dihydroanthracen-2-yl) acetamide) as Fluorescent Sensor for Amino Acids

Apinya Chuenka, Apisit Songsasen and Boontana Wannalerse*

ABSTRACT

A new fluorescent sensor, (R)-2, 2'- (1, 1'-binaphthyl-2, 2'-diylbis (oxy) bis (*N*-(9, 10-dioxo-9, 10, -dihydroanthracen-2-yl) acetamide), L1 was synthesized and characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR), Electrospray ionization mass spectroscopy (ESI-MS) and elemental analysis (EA). L1 exhibits significant fluorescence quench in the presence of various amino acids (D-Ala, L-Ala, D-Leu, L-Leu, D-Phe, L-Phe D-Trp and L-Trp) at concentrations of 5×10^{-5} M. From fluorescence titration studies, D-Trp is able to clearly decrease the fluorescence intensity of L1, when compared to other amino acids. The association constant, of L1 and D-Trp is 1.26×10^{3} M^{-1} using a Benesi-Hildebrand plot. Job analysis shows that L1 forms a complex with D-Trp in a 1: 1 fashion. A highly selective response of L1 is useful for the selective fluorescence recognition of D-Trp. In addition, upon addition of D-Trp to the deuterated dimethyl sulfoxide solution of L1, a considerable shift of the signal of NH protons was found in the ^{1}H nuclear magnetic resonance (NMR) spectrum. Moreover, the aromatic region was found to shift upfield. Therefore, the receptor L1 selectively recognized D-Trp over other amino acids. The complexation of L1 and D-Trp is formed via the hydrogen bonding and π-π stacking interactions.

Keywords: fluorescent sensor, amino acids

INTRODUCTION

Amino acids are the basis of biological systems and provide a necessary function in the transport and storage of all nutrients. (Akitomi *et al*., 2013). Moreover, amino acids perform an important role in the maintenance, growth, reproduction and regulation of immunity in metabolic pathways. (Zhang *et al*., 2013) A number of techniques for amino acid studies and detections have been reported, including high-performance liquid chromatography (Devall *et al*., 2007; Gheshlaghi *et al*., 2008; Ilisz *et al*., 2008), electrochemistry (Huang *et al*., 1996), capillary electrophoresis (Vespalec *et al*., 2000; Zhang *et al*., 2007), absorption spectrometry (Argirova *et al*., 1999), gas chromatography-mass spectrometry (Kaspar *et al*., 2008) and mass spectrometry (Yao *et al*., 2000). These techniques are quite cumbersome and expensive. Thus, host-guest chemistry, a method which studies the molecular recognition between the synthesis compound and amino acids, can be applied to solve the problem. Many researchers synthesize chiral molecules that

Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: fscibnw@ku.ac.th

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can selectively bind amino acids via hydrogen bonding interactions (Qin *et al*., 2008; Sánchez-Hernández *et al*., 2011; Fu *et al*., 2012). He *et al*. (2009) prepared the benzylaminomethyl groups at the 3,30-position of 1, 10-bi-2-naphthol (BINOL), which could be a enantioselective fluorescent sensor for N-Boc-proline and other amino acid derivatives. Yang *et al*. (2011) synthesized and investigated the unsymmetrical salan fluorescent sensors for N-Boc protected amino acid. Then, Wu *et al*. (2014) prepared a chiral fluorescent sensor based on BINOL bearing hexahydropyrrolo [1,2-*c*] imidazol-1-one units for the recognition of N-Cbz-protected amino acids. The current study designed and synthesized an amino acid fluorescent sensor based on 2-aminoanthraquinone with binapthol and investigated complexation between the receptor L1 and amino acids using ^{1}H nuclear magnetic resonance (NMR) and fluorescence spectroscopy.

MATERIALS AND METHODS

Materials

All reagents for synthesis and amino acids were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA) and used without further purification.

Synthesis and characterization of (A)-1 and receptor L1

The synthetic procedure is shown in Figure 1. 2-Aminoanthraquinone (1.0 g) was dissolved in 30 mL of $CHCl_3$. The reaction was stirred for 5 min under N_2 atmosphere, then pyridine (0.5 mL) was added to the solution, and the mixture was stirred for 30 min. A solution of 2- chloroacetylchloride (2.0 mL) was added dropwise to the mixture. The reaction mixture was stirred until the product was formed as a solid (20 h). The reaction mixture was poured into water and extracted with dichloromethane. The organic layer was dried with anhydrous Na_2SO_4. The product was purified by recrystallizing from hexane (Wannalerse *et al*., 2008). The resultant parameters were: yield 22.63%; melting point (m.p.) 223.9 °C; ^{1}H nuclear magnetic resonance (NMR) (400 MHz, deuterated chloroform; $CDCl_3$), δ (parts per million; ppm): 4.30 (s, 2H), 7.85 (m, 2H), 8.26 (s, 1H), 8.36 (m, 4H), 8.64 (s, 1H); IR (KBr, cm^{-1}) *v*: 3348 (s, N-H), 3314 (m, N-H), 2943 (m, C-H), 1720 (s, C=O), 1665 (s, C=C), 1589 (s, N-H), 1534 (s, N-H), 1294 (s, N-H), 1232 (s, C-N), 714 (s, C-Cl), 617 (s, C-Cl); and electrospray ionization mass spectroscopy (ESI-MS) calculated for $C_{16}H_{10}ClNO_3$ [(A)-1 + Na]$^+$: 322.7; found: 322.7.

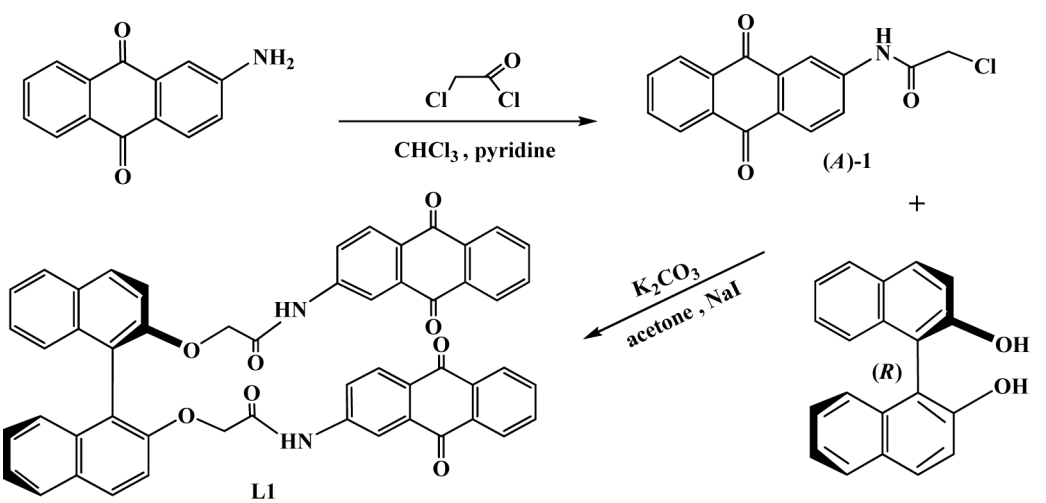

Figure 1 Synthesis route of L1.

For the synthesis of L1, (R)-(+)-1, 1′-Bi (2-naphthol) (0.5 g) and potassium carbonate (K_2CO_3, 0.4834 g) were dissolved in 10 mL of CH_3CN. The mixed solution was stirred for 30 min under N_2 atmosphere. Then, the solution of (A)-1 (1.0489 g) and sodium iodide (catalyst amount) in 20 mL of CH_3CN was added dropwise to the mixture. The reaction was stirred until the product was formed as a solid (10 h). The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried with anhydrous Na_2SO_4. L1 was purified using silica gel column chromatography with CH_2Cl_2: $C_4H_8O_2$ = 2: 8 as the eluent to obtain the product as a light yellow powder. The resultant parameters were: yield 24.24%; melting point (m.p.) 174.9 ˚C; ^{1}H nuclear magnetic resonance (NMR) (400 MHz, deuterated dimethyl sulfoxide; DMSO-d_6), δ (parts per million; ppm): 4.97 (s, 2H), 7.05 (d, 1H), 7.30 (t, 2H), 7.74 (t, 4H), 7.87 (d, 1H), 7.90 (d, 3H), 7.99 (d, 1H), 8.18 (d, 1H) 9.89 (s, 1H), ^{13}C-NMR (400 MHz, deuterated chloroform; $CDCl_3$), δ (parts per million; ppm): 68.28, 114.48, 116.74, 119.28, 124.03, 124.77, 125.26, 127.13, 127.28, 127.96, 128.49, 128.91, 129.89, 130.12, 131.44, 133.30, 133.57, 133.60, 133.90, 134.20, 134.40, 141.81, 152.04, 166.00, 181.93, 182.53; IR (KBr, cm^{-1}) *v*: 3368 (s, N-H), 3062 (m, C-H), 1704 (s, C=O), 1673 (s, C=C), 1591 (s, C=C), 1528 (s, C=C), 1474 (m, C-H), 1328 (s, N-H), 1295 (s, C-O), 1219 (m, C-N), 1152 (s, C=O), 1097 (m, C=O); and analytical calculated for $C_{52}H_{32}N_2O_8$: C, 76.84; H, 3.97; N,3.45; found: C, 76.72; H, 3.62; N, 3.59; ESI-MS calculated for $C_{52}H_{32}N_2O_8$ $[L1 + Na]^+$: 835.82; found: 835.5.

Fluorescence measurement

A fluorimetric quartz cell was filled with 3.5 mL fluorophore L1 (5.0×10^{-5} M) in dimethyl sulfoxide solution. Then, an emission spectrum was taken from the solution. This solution was titrated with various amino acids solution and the fluorescence intensity of the system was measured. The emission intensity, at an excitation wavelength of 431 nm, was measured. Spectral bandwidths of monochromators for excitation and emission were 10.0 and 20.0 nm, respectively.

Nuclear magnetic resonance studied on complexation

The nuclear magnetic resonance experiments were measured in deuterated dimethyl sulfoxide. The 5×10^{-3} M solution of receptor L1 which was added with four equivalents of various amino acids (such as D-Ala, L-Ala, D-Leu, L-Leu, D-Phe, L-Phe D-Trp and L-Trp) was prepared. The ^{1}H nuclear magnetic resonance (NMR) spectra were recorded.

RESULTS AND DISCUSSION

Fluorescence emission studies in dimethyl sulfoxide solution

The fluorescence properties of receptor L1 toward various amino acids were investigated using fluorescence titrations. From the excited wavelength at 431 nm, the receptor L1 displayed the emission band at 555 and 602 nm. The addition of various amino acids (0-20 equivalents) to the solution of receptor L1 induced decreasing fluorescence emission. The corresponding fluorescence titrations spectra of receptor L1 with D-Trp are shown in Figure 2. It was found that the fluorescence intensity of the receptor L1 decreased 1.5 folds, compared to the fluorescence decrease of the receptor L1 toward other amino acids as shown in Figure 3. It could be observed that D-Trp bar exhibited the lowest fluorescence intensity. These results indicate that receptor L1 could be used as a fluorescence sensor for D-Trp. The selective fluorescence quenching of D-Trp may have been due to the complexation between the receptor L1 and D-Trp under hydrogen bonding interactions (Xu *et al*., 2009).

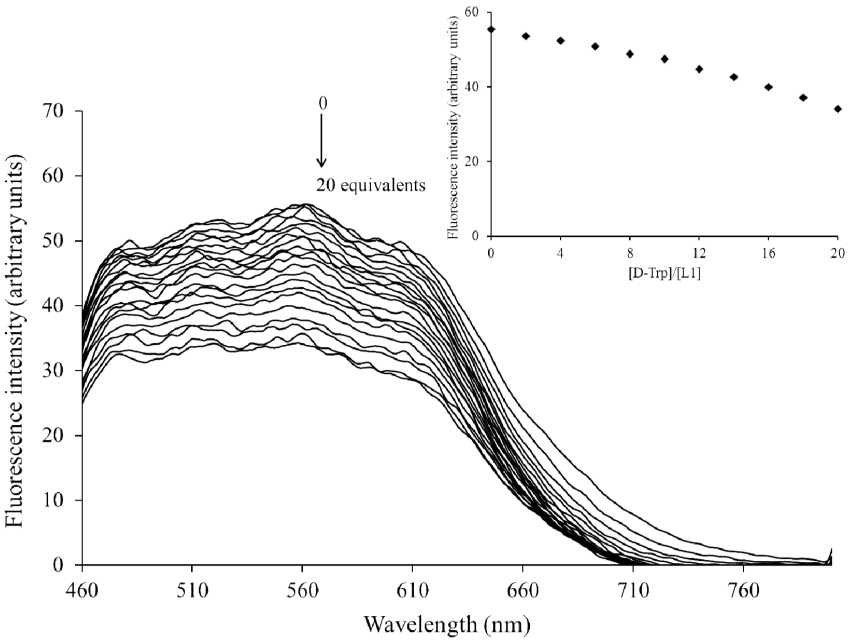

Figure 2 Variation of fluorescence intensity recorded for L1 (5×10^{-5} M) upon gradual addition of D-Trp (0-20 equivalents; equiv.) in dimethyl sulfoxide. The nonlinear fitting curve of change in fluorescence intensity of L1 with respect to the amount of D-Trp is shown in the inset.

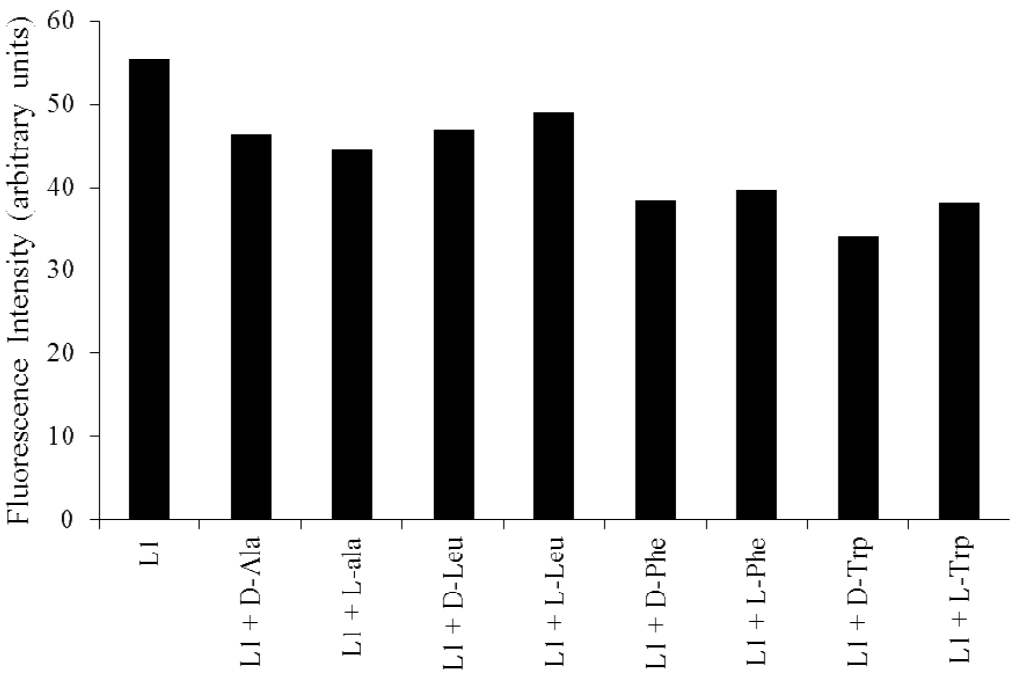

Figure 3 Comparison of the binding experiments of sensor L1 (5×10^{-5} M) with various amino acids (1.33×10^{-3} M) in tetrabutylammonium hexafluorophosphate.

Determination of the association constanst and stoichiometry

The binding stoichiometry between receptor L1 and D-Trp was calculated using job plot analysis as shown in Figure 4. The total concentration of receptor L1 and D-Trp was fixed at 50 μM and mixed in the solution. The maximum emission ratio of [L1]/([L1]+[D-Trp]) was 0.5. This indicated that the stoichiometric complexation of L1: D-Trp was 1:1. The association constants of receptor L1 with various amino acids were determined through the Benesi-Hildebrand equation (Benesi *et al*., 1949) as listed in Table 1. The association constants (K_a) of receptor L1 with amino acids were in the order D-Trp ~ L-Trp > D-Phe ~ L-Phe > D-Ala ~ L-Ala. The results showed that receptor L1 prefers aromatic amino acids over aliphatic amino acids. The association constants of L1 toward aromatic amino acids were higher than for aliphatic amino acids probably due to the complementary π-π stacking interactions. Nevertheless, the association constant of L1 and D- and L-Leu could not be calculated because of unsuitable molecular geometry.

^{1}H nuclear magnetic resonance studies in deuterated dimethyl sulfoxide solution

To further get more information on the complexation between receptor L1 and various amino acids, ^{1}H nuclear magnetic resonance experiments were also carried out in deuterated dimethyl sulfoxide, as shown in Figure 5. It was noticed that the peak at 9.89 ppm was assigned to the –NH proton of receptor L1. The chemical shift

Table 1 Association constants of receptor L1 with various amino acids.

Amino acids	Association constant (K_a, M^{-1})
D-Trp	1.26×10^3
L-Trp	1.10×10^3
D-Phe	9.33×10^2
L-Phe	8.51×10^2
D- Ala	3.01×10^2
L-Ala	2.88×10^2
D- Leu	ND
L- Leu	ND

ND = Not determined.

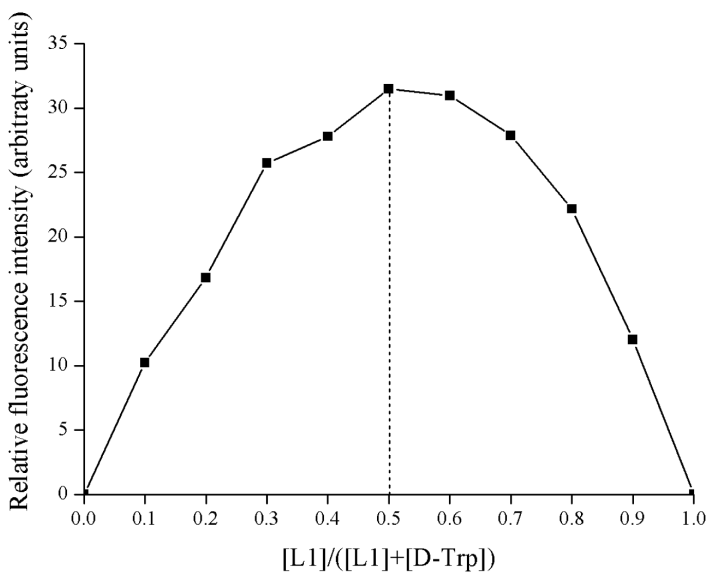

Figure 4 Job plot of receptor L1 and D-Trp in dimethyl sulfoxide. The dotted line indicates the maximum relative fluorescence intensity.

changes of the –NH proton of L1 toward various amino acids are listed in Table 2. It was found that D- and L-Trp induced the chemical shift changes rather than the other amino acids. After adding 4 equivalents of D- and L-Trp, the signal of the –NH proton of L1 was broadened and shifted downfield due to the hydrogen bonding interactions between the –COOH group of the amino acid and the –NH of the amide group. Moreover, the ArH region of receptor L1 moved to upfield due to the π-π interaction between the aromatic protons of receptor L1 and Trp. This indicated that a strong interaction would occur between receptor L1 and Trp. Upon the addition of D- and L-Phe, the –NH proton of L1 would shift slightly downfield and the aromatic region was slightly changed. This indicated that the complexations between receptor L1 and D- and L-Phe are a quite weak hydrogen bonding interaction. In the case of other amino acids (D-Ala, L-Ala, D-Leu and L-Leu), there were scarcely changes in the –NH proton and the aromatic region.

CONCLUSION

An easy-to-use fluorescent sensor was designed and synthesized bearing anthraquinone moiety as the recognition site and binaphthol moiety as the signaling group for D-Trp. This sensor showed high sensitivity and selectively

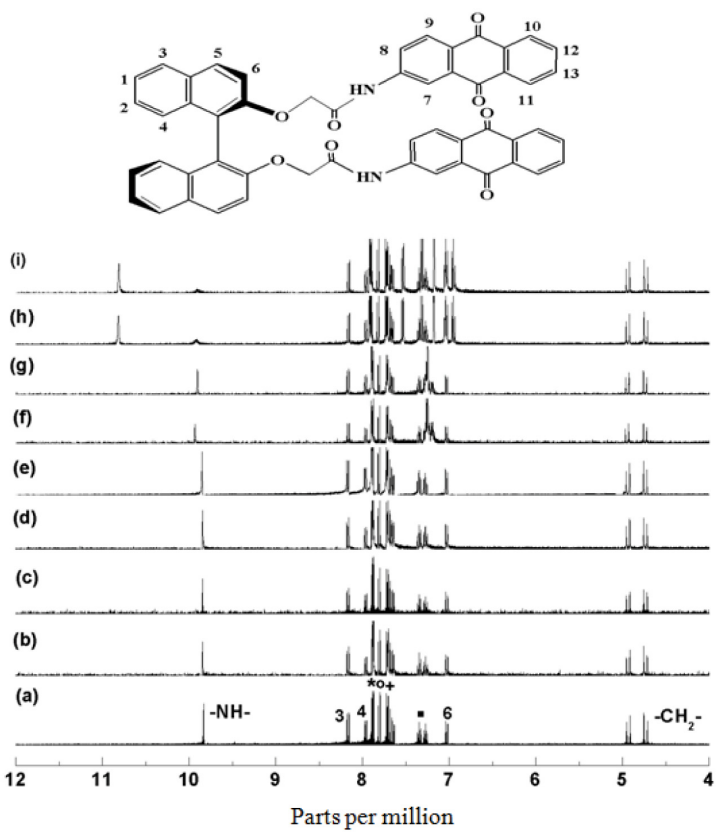

Figure 5 ^{1}H nuclear magnetic resonance spectra of receptor L1: (a) In deuterated dimethyl sulfoxide (5×10^{-3} M) upon addition of 4 equivalents of: (b) D-Ala; (c) L-Ala; (d) D-Leu; (e) L-Leu; (f) D-Phe; (g) L-Phe; (h) D-Trp and (i) L-Trp. (▪ = Assignment of protons at 1 and 2; ◦ = Assignment of protons at 5; * = Assignment of protons at 7, 8 and 9; + = Assignment of protons at 10, 11, 12 and 13)

Table 2 Chemical shift changes of –NH proton of L1 toward various amino acids.

Amino acid	Δδ (parts per million)
D-Trp	0.1947
L-Trp	0.1723
D-Phe	0.1036
L-Phe	0.0773
D- Ala	0.0353
L-Ala	0.0251
D- Leu	0.0212
L- Leu	0.0129

with D-Trp over other amino acids. The receptor L1 recognizes D-Trp by forming a stable 1:1 L1-(D-Trp) complex. The receptor L1 can be used as a fluorescent sensor for amino acids.

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