Stereoselective Recognition of Tripeptides Guided by Encoded Library Screening: Construction of Chiral Macrocyclic Tetraamide Ruthenium Receptor for Peptide Sensing

Kuei-Hua Chang,† Jen-Hai Liao,† Chao-Tsen Chen,*,† Barun K. Mehta,† Pi-Tai Chou,*, and Jim-Min Fang*,†,‡

Department of Chemistry, National Taiwan University, Taipei 106, Taiwan, and Genomic Research Center, Academia Sinica, Taipei 115, Taiwan

Received September 15, 2004

Introduction

Fundamental studies of peptide recognition provide a way to investigate the more complicated protein interactions, which are often addressed in chemical biology and drug discovery research. The main binding forces between a peptide and its host receptor include hydrogen bonding, electrostatic, and hydrophobic interactions.1 For example, vancomycin is a potent antibacterial agent, which displays quadruple hydrogen bindings with the D-Ala-D-Ala fragment that is essential for formation of bacterial cell wall.2 The new strain of bacteria by mutation of the D-Ala-D-Ala dipeptide into D-Ala-D-lactate can resist the attack of vancomycin, presumably due to elimination of a crucial hydrogen bonding and the increase of repulsive electronic interactions between the lactate portion and vancomycin.2

An efficient peptide receptor must exhibit a spacious recognition site in order to accommodate the relatively large guest molecule. For this purpose, acyclic and macrocyclic structures are often applied as the recognition motifs, to which crown ethers can be incorporated to enhance the interaction with the terminal ammonium group of the peptide.3 Acyclic peptide receptors are generally constructed by implanting two peptide or multiple-amide chains on a structurally defined backbone, such as steroid,4,5 calixarene,6,7 diketopiperazine,8 and dibenzofuran.9 Conversely, a macrocyclic tetraamide receptor has been prepared by using five building blocks

Molecule sensor 1 is devised by incorporating the reporting unit of ruthenium(II) complex and two recognition motifs of chiral cyclotetraamides on the sidearms. The target binding tripeptides for sensor 1 were readily identified by using an encoded library screening method. This solid-phase screening indicated a preferable binding of molecule 1 with D-alanine over the L-isomer. The optical and NMR studies for the binding events of 1 with tripeptides Ac-Ala-Gly-Ala-NH2 in the solution phase showed a consistent trend for the stereoselective recognition of the DD-isomer over the LD-, DL-, and LL-isomers.

NH of the amido group and C=O of the vicinal amido group form an ideal hydrogen donor/acceptor pair suitable for bonding with α-amino acid derivatives.15,16 This chiral receptor is expected to exert different complexation strengths with respect to D- and L-amino acid derivatives. Previous HPLC studies15,16 have revealed that chiral columns using A2B2 derivatives as a stationary phase are applicable to the separation of derivatized isomers of α-amino acids and tripeptides. In this regard, the cage compound with an A2B2 motif also serves as a receptor for dipeptide and tripeptide derivatives.11,17

As for the signal transduction, the molecular receptors of A2B2 and A2B4 scaffolds are elaborated to detect peptide derivatives by attaching suitable fluorophore/quencher pairs, e.g., dansyl and dabcyl, via the fluorescence resonance energy transfer (FRET) mechanism.18 In the absence of guest molecule, the free receptor shows rather weak fluorescence due to the efficient FRET, followed by the dominant nonradiative deactivation pathways. Upon complexation with the target peptide, an enhanced fluorescence is observed as the fluorophore and quencher are pushed apart.

On the basis of the protocol of two-armed receptors devised by Still’s group,11,16,17 we demonstrate herein a novel molecule sensor 1 that is used to detect tripeptides with a high sequence- and stereoselectivity. Compound 1 is designed to incorporate two A2B2 motifs as the recognition units16,4 and a tris(bipyridine) ruthenium-(II) complex as the signal transduction unit (Figure 2). The two macroyclic A2B2 units are linked by a bipyridine moiety via two amide bonds. Thus, the multiple-amide unit provides a desirable semirigid structure and sufficient hydrogen bonding sites for peptide recognition. The appropriate disposition of two A2B2 motifs in molecule 1 may also enhance the binding with the target peptides in a cooperative manner.16 The Ru(II) center is sensitive to the binding event so that the changes of luminescence properties and redox potential can be readily monitored and correlated to the binding strength.19

Results and Discussion

Synthesis. Molecular sensor 1 was constructed by five building blocks: isophthalic acid bis-chloride (2), (R,R)-cyclohexane-1,2-diamine (3), 5-(azidomethyl)isophthalic acid (4), 2,2′-bipyridine-4,4′-dicarboxylic acid (5), and cis(bpy)RuCl2 (6). When diamine 3 was treated with Boc2O, a mixture of mono-Boc and di-Boc derivatives were obtained. To avoid this complication, an indirect method was applied to prepare the mono-Boc derivative (Scheme 1).20 Thus, diamine 3 was first converted to the N,N′-bis-Cbz derivative, which was then reacted selectively with

FIGURE 1. A2B2 motif formed by two units of isophthalic acid (A) and two units of vicinal diamine (B) in recognition of α-amino acid derivatives via double hydrogen bondings.

![Figure 1](image-url)
Boc₂O to give exclusively the N-Boc-N'-Cbz derivative. Attachment of the second Boc group was prohibited presumably due to the sterically demanding environment. After the Cbz groups were removed by catalytic hydrogenation, the mono-Boc derivative 7 was obtained in 67% overall yield. The coupling reaction of 7 (2 equiv) with isophthalic acid bis-chloride (2) occurred smoothly in the presence of Hünig base to give diamide 8. After removal of the Boc groups by CF₃COOH, the resulting amine (as the TFA salt) was treated with the pentafluorophenyl diester of 5-(azidomethyl)isophthalic acid in the presence of Hünig base to give a cyclotetraamide 9. The azido group of 9 was reduced by catalytic hydrogenation or by using Ph₃P/H₂O (Staudinger reaction); and the amine product (2 equiv) was then reacted with 2,2'-bipyridine-4,4'-dicarboxylic acid (5) using EDC and HOBt as the coupling reagents. The subsequent reaction with cis-Ru(bpy)₂Cl₂, followed by exchange of Cl⁻ with PF₆⁻ counterions, thus culminated in the synthesis of sensor 1 with a Ru reporter and double A₂B₂ recognition motifs. Because one of bipyridyl ligands contains the chiral A₂B₂ substituents, the Ru(II) compound 1 likely exists in two diastereomeric forms with ⧧- or ⧨-configurations at the ruthenium center. This speculation is supported by the NMR study (vide infra).

The Ru(II) compound 1 (as the PF₆⁻ salt) appeared as orange red solids. Its CH₂Cl₂ solution showed the characteristic absorption bands at λₘₐₓ = 289 nm (ε = 73 200 M⁻¹·cm⁻¹) for the bipyridyl ligand and at λₘₐₓ = 457 nm (ε = 15 100 M⁻¹·cm⁻¹) attributable to the metal to ligand

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FIGURE 2. Construction of molecular sensor 1 by five building blocks: isophthalic acid bis-chloride (2, two units), (1R,2R)-cyclohexane-1,2-diamine (3, four units), 5-(azidomethyl)isophthalic acid (4, two units), 2,2'-bipyridine-4,4'-dicarboxylic acid (5, one unit), and cis-(bpy)₂RuCl₂ (one unit).

SCHEME 1. Synthesis of Molecular Sensor 1

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TABLE 1. Analyses

Reagents and conditions: (i) t-Pr₂NEt, CH₂Cl₂, 25 °C, 3 h, 95%; (ii) TFA, CH₂Cl₂, 25 °C, 1.5 h, then diC₆F₅-ester of 4, t-Pr₂EtN, THF, 25 °C, 12 h, 99%; (iii) Ph₃P, THF, reflux, 2 h, H₂O, THF, reflux, 14 h, 87%; or H₂, 10% Pd/C, MeOH, 25 °C, 8 h, 99%; (iv) diacid 5, EDC, HOBt, DMF, rt, 49 h, 87%; (v) 6 (hydrate), EtOH, H₂O, reflux, 6 h, NH₄PF₆, 83%.

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Boc₂O to give exclusively the N-Boc-N'-Cbz derivative. Attachment of the second Boc group was prohibited...
charge transfer (MLCT). In more polar media [e.g., a mixed solution of MeOH/CHCl₃ (1:9)], the absorption maxima shifted to longer wavelengths, 299 nm ($\epsilon = 71,100 \text{ M}^{-1} \text{cm}^{-1}$) and 465 nm ($\epsilon = 15,300 \text{ M}^{-1} \text{cm}^{-1}$). Upon excitation at 457 nm, compound 1 (2 $\times 10^{-4}$ M) exhibited an emission band with peak wavelengths at 595 nm ($\tau = 1.2 \text{ s}$). Taking O₂ concentration of 2.6 $\times 10^{-3}$ M in aerated CH₂Cl₂, we screened a 15 amino acids: Gly, L-Ala, D-Ala, L-Val, D-Val, L-Ser, D-Ser, L-Pro, D-Pro, L-Asn, D-Asn, L-Gln, D-Gln, L-Lys, and D-Lys.

The phosphorescence titrations were undertaken to probe the titration of the binding of 1 with surface tripeptides. In this experiment, to a solution of 1 ($4.0 \times 10^{-6}$ M) in MeOH/CHCl₃ (1:9, 2 mL) at 20 °C were added aliquots of a specific tripeptide derivative, e.g., 10-DD (as a 4.0 $\times 10^{-3}$ M solution) in MeOH/CHCl₃ (1:9), and the phosphorescence spectra (with excitation at 466 nm) were recorded on individual titration (Figure 4). Despite negligible changes of the absorption spectra, the phosphorescence intensity gradually increases as the concentration of 10-DD increases. This indicates the enhancement of phosphorescence yield upon complexation between 1 and the tripeptide. Two plausible mechanisms were proposed in an attempt to rationalize the experimental results. Theoretically, via the rigidification effect, the non-

**FIGURE 3.** Bead-bound tripeptide library with electrophoric tags. Tripeptide AA₁-AA₂-AA₃ represents any combination of 15 amino acids: Gly, L-Ala, D-Ala, L-Val, D-Val, L-Ser, D-Ser, L-Pro, D-Pro, L-Asn, D-Asn, L-Gln, D-Gln, L-Lys, and D-Lys.

**FIGURE 4.** Phosphorescence titration spectra of 1 ($4.0 \times 10^{-6}$ M) by addition of various amounts of the tripeptide derivative 10-DD [as a $4.0 \times 10^{-3}$ M solution in MeOH/CHCl₃ (1:9)] at 293 K, $\lambda_{ex} = 466$ nm. Inset: A curve fitting of the data using the nonlinear least-squares method. $K_{d}$ is derived to be $2.9 \times 10^{10} \text{ M}^{-1}$. The mixture was agitated for 40 h to ensure equilibrium.

The 22 deep red beads that refer to the binding of 1 with the surface tripeptides were picked out under a microscope. The selected beads were then irradiated with 365-nm light to remove the photolabile linkers, and the released electrophoric tags were decoded by gas chromatography using an electron capture detector.

Based on this approach, we quickly identified 19 tripeptides having high affinity toward the host molecule 1 (see the Supporting Information). This solid-phase screening showed preference for three peptide sequences: Ac-(D-Ala)-Gly-(D-Ala), Ac-Gly-(D-Ala)-Gly and Ac-(D-Asn)-(L-Ser)-(L-Pro). The binding preference for D-Ala over L-Ala suggests the stereoselective recognition in the solid-phase system.

**Confirmation of Stereoselective Recognition in Solution System.** It is also crucial to evaluate whether the stereoselective recognition in the solid-phase system can also be applied in solution. We thus synthesized four tripeptide isomers, Ac-(D-Ala)-Gly-(D-Ala)-NHCH₂H₂S (10-DD), Ac-(L-Ala)-Gly-(D-Ala)-NHCH₂H₂S (11-LD), Ac-(D-Ala)-Gly-(L-Ala)-NHCH₂H₂S (12-DL), and Ac-(L-Ala)-Gly-(L-Ala)-NHCH₂H₂S (13-LL), and examined their binding behaviors with compound 1.

The phosphorescence titrations were undertaken to probe the binding of receptor 1 with tripeptides. In this experiment, to a solution of 1 ($4.0 \times 10^{-6}$ M) in MeOH/CHCl₃ (1:9, 2 mL) at 20 °C, we added aliquots of a specific tripeptide derivative, e.g., 10-DD (as a 4.0 $\times 10^{-3}$ M solution) in MeOH/CHCl₃ (1:9), and the phosphorescence spectra (with excitation at 466 nm) were recorded on individual titration (Figure 4). Despite negligible changes of the absorption spectra, the phosphorescence intensity gradually increases as the concentration of 10-DD increases. This indicates the enhancement of phosphorescence yield upon complexation between 1 and the tripeptide.

A typical screening procedure is described as follows. The combinatorial tripeptide beads (1 mg, containing about 4 copies of each tripeptide component) were suspended in CHCl₃ (0.5 mL). A solution of sensor 1 (40 μL, $1 \times 10^{-4}$ M) in MeOH/CHCl₃ (1:9) was added, and
radiative decay rates of free receptor 1 may be reduced upon multiple hydrogen bonding complexation with tripeptides. This, along with the possible stabilization due to the MLCT in such a supramolecular complex,19 may rationalize the phosphorescence enhancement during the titration. Alternatively, the complex formation may, to certain extents, hamper the penetration of the oxygen to proceed with the sensitization. Since the absorption spectral feature both in peak wavelength and absorbance remained unchanged during titration, the latter case seems to be favorable. Though an actual mechanism is pending for resolution, it is unambiguous that the spectral enhancement originates from the multiple hydrogen bonding with the tripeptides.

As a result, the corresponding changes of the phosphorescence intensity can be exploited to deduce the thermodynamic parameters upon complexation. The phosphorescence intensity, taken from the signal area between 500 and 850 nm, was then monitored against the excitation wavelength (ìex = 466 nm).24 The average value derived from the phosphorescence intensity change (as the signal area between 500 and 850 nm) of two independent titrations. The excitation wavelength ìex = 466 nm.

A similar phenomenon was also observed in the titration spectra of 1 with 13-LL (see the Supporting Information). Due to the complex proton configuration, further calculation of the binding constants on the basis of the concentration dependent chemical-shifts is impractical. Note that in the phosphorescence titration, to simplify the derivation, the emission yields of two diastereomeric complexes of 1/10-DD are assumed to be the same.

In comparison, a prototype A$_2$B$_2$ molecule 14 was prepared and subjected to $^3$H NMR titration with alanine derivatives in CDCl$_3$ solution. The results revealed that molecule 14 exhibited a weak binding with 3,5-dinitrobenzoylalanine hexylamide (15) albeit no apparent binding with Ac-Ala-OMe ester. The association constants for both 14/15-D and 14/15-L complexes at 295 K in CDCl$_3$ were estimated to be in a range of 50–150 M$^{-1}$, which are 3 orders of magnitude weaker than that of 1/10-DD. Unfortunately, a similar titration experiment for 14 using 10-DD was not feasible due to the sparse solubility of tripeptide in CDCl$_3$, neither could the titration experiments be conducted in prolinic solvents, e.g., CD$_3$OD/CDCl$_3$ (1:9), due to the complicated exchange of amido protons.

We also prepared the analogues of 10-DD and 11-LD by replacing the acetyl (Ac) capping group with a bulky tert-butloxy carbonyl (t-Boc) group. These t-Boc analogues turned out to have very weak affinity toward molecule 1, and no apparent change of the phosphorescence was observed. Since the bipyridinediamide moiety in molecule 1 also participated in the molecular recognition of tripeptides 10-DD and 11-LD (vide supra), introducing a bulky t-Boc group might not fit in the interior binding pocket of 1.

**Conclusion.** We have demonstrated the differentiation of four tripeptide isomers of Ac-Ala-Gly-Ala-NHC$_2$H$_5$ via a deliberately designed chiral receptor. The two-armed receptor 1 incorporating a bipyridinediamide moiety and two chiral cyclo tetramide motifs achieves an effective multiple hydrogen bondings recognition with tripeptides in a stereoselective manner. The attachment

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**TABLE 1. Association Constants and Free Energy Changes for 1/Tripeptide Complexation in MeOH/CHCl$_3$ (1:9) Solution**

<table>
<thead>
<tr>
<th>entry</th>
<th>peptide derivative</th>
<th>$K_a$ (10$^3$ M$^{-1}$)</th>
<th>$\Delta G^\circ$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ac-d-Ala-Gly-d-Ala-NHC$_2$H$_5$ (10-DD)</td>
<td>294 ± 33</td>
<td>-30.7</td>
</tr>
<tr>
<td>2</td>
<td>Ac-l-Ala-Gly-d-Ala-NHC$_2$H$_5$ (11-LD)</td>
<td>170 ± 5</td>
<td>-29.3</td>
</tr>
<tr>
<td>3</td>
<td>Ac-d-Ala-Gly-l-Ala-NHC$_2$H$_5$ (12-DL)</td>
<td>154 ± 27</td>
<td>-29.1</td>
</tr>
<tr>
<td>4</td>
<td>Ac-l-Ala-Gly-l-Ala-NHC$_2$H$_5$ (13-LL)</td>
<td>58 ± 9</td>
<td>-26.7</td>
</tr>
</tbody>
</table>

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$a$ Receptor 1 (4.0 $\times$ 10$^{-6}$ M) was titrated with tripeptide (4.0 $\times$ 10$^{-6}$ M) in MeOH/CHCl$_3$ (1:9) at 293 K. Two or three measurements were performed for each tripeptide substrate. $b$ The average value derived from the phosphorescence intensity change (as the signal area between 500 and 850 nm) of two independent titrations. The excitation wavelength $\lambda_{ex} = 466$ nm. $c$ Calculated from $\Delta G^\circ = -RT \ln K_a$.

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FIGURE 5. $^1$H NMR titration of compound 1 (1 x 10$^{-4}$ M) by addition of various amounts of 10-DD [as 1 x 10$^{-2}$ M solution in CH$_3$OD/CDCl$_3$ (1:9)].

experimental section

Synthesis of Cyclotetraamide 9. To a solution of 5-azidomethylisophthalic acid (301 mg, 1.36 mmol) in CH$_2$Cl$_2$ (35 mL) was added a solution of pentafluorophenol (563 mg, 3.06 mmol) and EDC (586 mg, 3.06 mmol) in CH$_2$Cl$_2$ (10 mL). The mixture was stirred for 3 h at room temperature, concentrated, and purified by flash chromatography on a silica gel column (EtOAc/hexane, 1:9) to afford 5-azidomethylisophthalic acid dipentafluorophenyl ester as white solids (741 mg, 98%).

Diamide 8b (1.75 g, 3.1 mmol) in CH$_2$Cl$_2$ (56 mL) was treated with TFA (14 mL) for 1.5 h at room temperature to remove the Boc protecting groups. The reaction mixture was concentrated under reduced pressure and triturated several times with Et$_2$O to give precipitates. The solids were collected and dried in vacuo for at least 6 h to afford the corresponding TFA salt (1.83 g, quantitative yield).

To a mixture of 5-azidomethyl-isophthalic acid dipentafluorophenyl ester (1.53 g, 2.77 mmol) and the above prepared TFA salt (1.60 g, 2.77 mmol) suspended in CH$_2$Cl$_2$ (65 mL) was added $i$-Pr$_2$NEt (2.85 g, 22 mmol) dropwise over a period of 10 min at room temperature. The mixture was stirred for 12 h, concentrated, and purified by chromatography on a silica gel column (CH$_3$OH/CH$_2$Cl$_2$, 1:19) to afford compound 9 as white solids (1.49 g, quantitative yield). The amine product (350 mg, 0.68 mmol) was added to a suspension of 2,2'-bipyridyl-4,4'-dicarboxylic acid (83 mg, 0.34 mmol) and HOBt (94 mg, 0.70 mmol) in DMF (10 mL) at 0 °C, followed by addition of EDC (142 mg, 0.74 mmol). The mixture was stirred for 1 h at 0 °C and 49 h at room temperature to give a pink suspension. The reaction mixture was concentrated under reduced pressure, dissolved in CH$_3$OH/CH$_2$Cl$_2$ (1:9), and washed twice with H$_2$O. The aqueous phase was extracted three times with CH$_3$OH/CH$_2$Cl$_2$ (1:9). The combined organic phase was dried over anhydrous Na$_2$SO$_4$ and filtered, and the filtrate was concentrated to give pale pink solids. The crude product was purified by chromatography on a silica gel column (CH$_3$OH/CH$_2$Cl$_2$, 1:9) to afford the two-arm bipyridyl ligand as white solids (367 mg, 87%).

A mixture of the two-arm bipyridyl ligand (59 mg, 0.047 mmol) and cis-Ru(bpy)$_2$Cl$_2$ hydrate (25 mg, 0.052 mmol) in EtOH (8 mL) and H$_2$O (2 mL) was heated under reflux for 6 h. After removal of solvents, the residue was dissolved in CH$_2$Cl$_2$ and triturated with Et$_2$O. The solids were collected by filtration, washed with CH$_2$Cl$_2$/Et$_2$O (1:3), and dried in vacuo to afford the chloride salt of 1 (75 mg). The chloride salt was of a Ru(II) reporter renders a real-time detection of the binding property based on the phosphorescence spectrosopy. This, in combination with the encoded library screening technique, proves to be a rapid and efficient method for search and sensing of target peptides.
dissolved in H₂O, and treated with saturated NH₄PF₆ aqueous solution (5 mL). The mixture was stirred for 30 min at room temperature, filtered, and washed with cold water to give the phosphorus hexafluoride salt of Ru(II) sensor 1 as orange red solids: mp > 330 °C dec; yield 77 mg (83%); 1H NMR (DMSO-d₆, 500 MHz) δ 9.72 (2H, s), 9.24 (2H, s), 8.83 (6H, m), 8.37 (4H, m), 8.22–8.13 (8H, m), 7.90–7.67 (16H, m), 7.52–7.45 (8H, m), 4.45 (4H, m), 3.88 (8H, m), 1.96–1.90 (8H, d), 1.92 (8H, m), 1.74 (8H, m), 1.52 (8H, m), 1.31 (8H, m); 13C NMR (DMSO-d₆, 125 MHz) δ 166.6 (C), 166.5 (C), 166.4 (C), 162.9 (C), 157.1 (2 × C), 152.0 (CH), 151.6 (CH), 151.3 (CH), 141.4 (C), 139.0 (CH), 138.2 (CH), 135.1 (C), 134.9 (C), 129.6 (CH), 129.0 (CH), 128.3 (CH), 127.9 (CH), 126.7 (CH), 125.7 (CH), 125.4 (CH), 124.5 (CH), 122.0 (CH), 53.5 (CH), 42.8 (CH₂), 31.5 (CH₂), 24.7 (CH₂); FAB-MS m/z (rel intensity) 1802 (M⁺ + H) + 1658 (M⁺ + H⁺ - 2PF₆).

Phosphorescence Titration Studies. Phosphorescence spectra were recorded on AMINCO/Bowman Series 2 spectrometer. A solution of compound 1 (4.0 × 10⁻⁶ M) in MeOH/CHCl₃ (1:9, 2 mL) was placed in a quartz cuvette (1 cm width) at 293 K. Aliquots of tripeptide derivative (4.0 × 10⁻³ M) in MeOH/CHCl₃ (1:9) were added in an incremental fashion (0.5, 1, 2, 3, 4, 6, 10, 15, 30, 40, and 60 equiv). The phosphorescence spectra with 466-nm excitation were recorded for each addition. The phosphorescence intensity (as the signal area between 500 and 850 nm) was monitored as a function of tripeptide concentrations. Nanosecond-microsecond lifetime studies were performed by an Edinburgh FL 900 photon-counting system with a hydrogen-filled or nitrogen lamp as the excitation source. The emission decays were analyzed by the sum of exponential functions, which allows partial removal of the instrument time broadening and consequently renders a temporal resolution of ~200 ps.

The binding constant of complex and 1:1 stoichiometry were determined by curve fitting using the nonlinear least-squares method, and the association constant (Kₐ) is derived from the following equation.²²

\[ F = F₀ + (ΔF_{max}/2C₀)\left\{1/Kₐ + C₀ + C - \left[(1/Kₐ + C₀ + C)^2 - 4C₀C\right]^{1/2}\right\} \]

F is the emission intensity, F₀ is the original emission intensity of the free receptor, and ΔFₘₐₓ is the largest change of emission intensity after saturation with the substrate. C is the concentration of substrate; C₀ is the initial concentration of the receptor.

¹H NMR Titration Studies. ¹H NMR spectra were measured on a Bruker Avance-400 NMR spectrometer. A typical experiment was performed as follows. A solution of compound 1 (1.0 × 10⁻⁴ M) in CD₃OD/CDCl₃ (1:9, 0.5 mL) was placed in a 5-mm NMR tube. A small aliquot of tripeptide derivative (1.0 × 10⁻² M, e.g., Ac-D-Ala-Gly-D-Ala-NH₂C₆H₁₂) in CD₃OD/CDCl₃ (1:9) was added in an incremental fashion (2, 8, 14, 22, 32, 44, and 64 equiv), and their corresponding spectra were recorded. The chemical-shift changes of NH on the tetraamide rings and C₃/C₃'-H were monitored as a function of tripeptide concentrations.

Acknowledgment. We thank the National Science Council for financial support.

Supporting Information Available: Synthetic procedures, table of 19 tripeptides selected by encoded screening, and absorption, phosphorescence, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048368S