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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

jmfang@ntu.edu.tw

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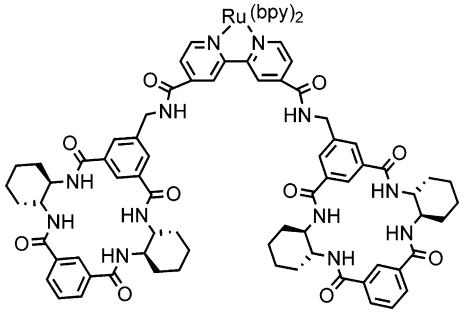

Molecule sensor **1** is devised by incorporating the reporting unit of ruthenium(II) complex and two recognition motifs of chiral cyclotetraamides on the sidearms. The target binding tripeptides for sensor **1** were readily identified by using an encoded library screening method. This solid-phase screening 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-$NHC_{12}H_{25}$ in the solution phase showed a consistent trend for the stereoselective recognition of the DD-isomer over the LD-, DL-, and LL-isomers.

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

[†] National Taiwan University.
[‡] Academia Sinica.

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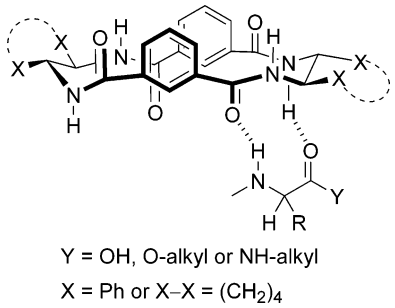

FIGURE 1. A_2B_2 motif formed by two units of isophthalic acid (**A**) and two units of vicinal diamine (**B**) in recognition of α-amino acid derivatives via double hydrogen bondings.

consisting of 2,6-diaminopyridine, phenylalanine, succinic acid, (4-aminomethyl)phenol, and (4′-bromomethylbiphenyl)acetic acid.[10] This receptor displays a selective binding to dipeptide *N*-Cbz-(*β*-Ala)-Ala. A similar macrocyclic hexaamide receptor is derived from a core structure of 1,3,5-trimercaptobenzene to form a confined deep-basket shape that well accommodates the guest molecule of tripeptide cPr-Ala-Pro-Ala-$NHC_{12}H_{25}$.[11] A podant ionophore[12] having a three-dimensional framework also shows a sequence-selective recognition of L-Arg-L-Phe-D-Asp. Cyclodextrins[13] and Kemp's acid amide derivatives[14] are also suitable motifs for peptide recognition. The receptor molecules constructed by incorporation of such motifs exhibit high affinity toward linear and cyclic peptides. A multiloop receptor has been built by extension of four hydrophilic cyclic peptide loops, Gly-Asp-Gly-Asp, on a hydrophobic calix[4]arene core.[7] This molecular receptor thus provides multiple electrostatic and hydrogen bonding interactions to bind with the surface peptide of cytochrome *c* that contains abundant lysine residues.[7]

In view of sensing peptides in an enantioselective fashion, of special interest are the molecular receptors with chiral recognition sites, e.g., the A_2B_2 motif of an octadecacyclotetramide structure (Figure 1). The A_2B_2 motif is readily constructed by two units of isophthalic acid and two units of (1*R*,2*R*)-1,2-diphenylethanediamine[15] [or (1*R*,2*R*)-1,2-cyclohexanediamine],[16] in which 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 studies[15,16d] have revealed that chiral columns using A_2B_2 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 A_4B_6 motif also serves as a receptor for dipeptide and tripeptide derivatives.[11, 17]

As for the signal transduction, the molecular receptors of A_2B_2 and A_4B_4 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 A_2B_2 motifs as the recognition units[16a,d] and a tris(bipyridine) ruthenium(II) complex as the signal transduction unit (Figure 2). The two macrocyclic A_2B_2 units are linked to 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 A_2B_2 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-$(bpy)_2RuCl_2$ (**6**). When diamine **3** was treated with Boc_2O, 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 **7** (Scheme 1).[20] Thus, diamine **3** was first converted to the *N*,*N*′-bis-Cbz derivative, which was then reacted selectively with

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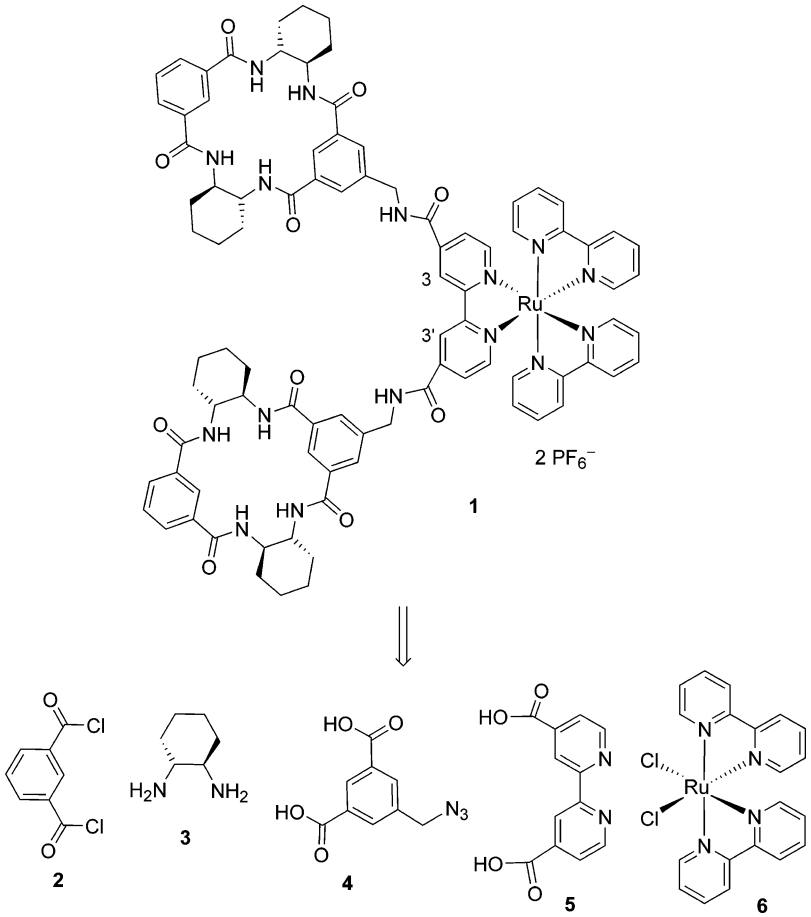
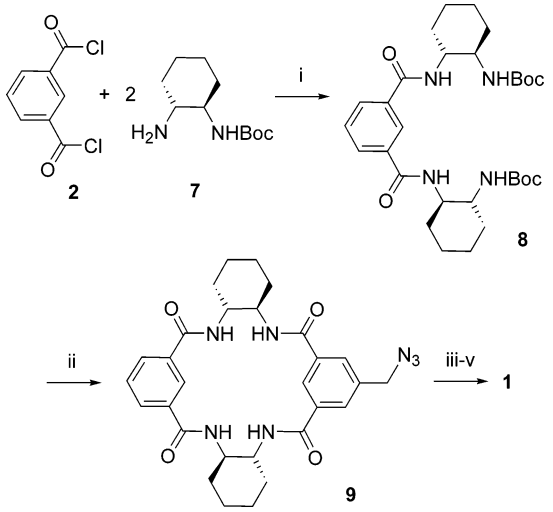


FIGURE 2. Construction of molecular sensor **1** by five building blocks: isophthalic acid bis-chloride (**2**, two units), (1*R*,2*R*)-cyclohexane-1,2-diamine (**3**, four units), 5-(azidomethyl)isophthalic acid (**4**, two units), 2,2′-bipyridine-4,4′-dicarboxylic acid (**5**, one unit), and cis-$(bpy)_2RuCl_2$ (one unit).

SCHEME 1. Synthesis of Molecular Sensor 1[a]



[a] Reagents and conditions: (i) *i*-Pr_2NEt, CH_2Cl_2, 25 °C, 3 h, 95%; (ii) TFA, CH_2Cl_2, 25 °C, 1.5 h, then diC_6F_5-ester of **4**, *i*-Pr_2EtN, THF, 25 °C, 12 h, 99%; (iii) Ph_3P, THF, reflux, 2 h, H_2O, THF, reflux, 14 h, 87%, or H_2, 10% Pd/C, MeOH, 25 °C, 8 h, 99%; (iv) diacid **5**, EDC, HOBt, DMF, rt, 49 h, 87%; (v) **6** (hydrate), EtOH, H_2O, reflux, 6 h, NH_4PF_6, 83%.

Boc_2O to give exclusively the *N*-Boc-*N*,*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**.[16b] After removal of the Boc groups by CF_3COOH, 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 Ph_3P/H_2O (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-$Ru(bpy)_2Cl_2$, followed by exchange of Cl^- with PF_6^- counterions, thus culminated in the synthesis of sensor **1** with a Ru reporter and double A_2B_2 recognition motifs. Because one of bipyridyl ligands contains the chiral A_2B_2 substituents, the Ru(II) compound **1** likely exists in two diastereomeric forms with Δ- or Λ-configurations at the ruthenium center. This speculation is supported by the NMR study (vide infra).

The Ru(II) compound **1** (as the PF_6^- salt) appeared as orange red solids. Its CH_2Cl_2 solution showed the characteristic absorption bands at $\lambda_{max} = 289$ nm ($\epsilon = 73\,200$ $M^{-1}cm^{-1}$) for the bipyridyl ligand and at $\lambda_{max} = 457$ nm ($\epsilon = 15\,100$ $M^{-1}cm^{-1}$) attributable to the metal to ligand

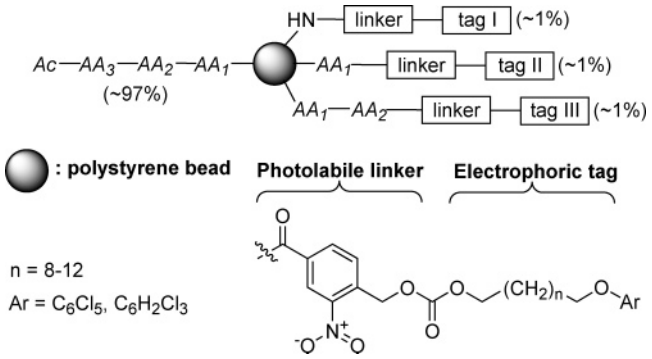


FIGURE 3. Bead-bound tripeptide library with electrophoric tags. Tripeptide AA_1-AA_2-AA_3 represents any combination of 15 amino acids: Gly, L-Ala, D-Ala, L-Val, D-Val, L-Ser, D-Ser, L-Pro, D-Pro, L-Asn, D-Asn, L-Gln, D-Gln, L-Lys, and D-Lys.

charge transfer (MLCT).[19] In more polar media [e.g., a mixed solution of MeOH/$CHCl_3$ (1:9)], the absorption maxima shifted to longer wavelengths, 299 nm (ϵ = 71 100 $M^{-1}cm^{-1}$) and 465 nm (ϵ = 15 300 $M^{-1}cm^{-1}$). Upon excitation at 457 nm, compound **1** (2×10^{-6} M) exhibited an emission band with peak wavelengths at 595 nm (Φ ~0.054) and 630 nm in CH_2Cl_2 and MeOH/$CHCl_3$ (1:9), respectively. The relatively long lifetime of 1.2 μs measured in aerated CH_2Cl_2 leads us to unambiguously assign the 595-nm emission (or 630 nm in MeOH/$CHCl_3$) to a phosphorescence manifold. In degassed CH_2Cl_2 the lifetime increases to 1.7 μs. Taking O_2 concentration of 2.6×10^{-3} M in aerated CH_2Cl_2,[21] an O_2 quenching rate constant of $\sim 9.0 \times 10^7$ M^{-1} s^{-1} was thus deduced, which is ~20 times smaller than 1/9 of the diffusion controlled rate of 1.8×10^{10} M^{-1} s^{-1} calculated in CH_2Cl_2.[22] Note that the O_2-triplet quenching rate is generally derived from the theory of electron-exchange type of energy transfer, in which the overall spin must be conserved upon forming a collisional complex. Accordingly, the possibility of each collision generating $^1O_2$ is statistically 1/9. The relatively small O_2 quenching constant of 9.0×10^7 M^{-1} s^{-1} in **1** clearly indicates that the ^{3}MLCT emission is less effectively quenched by O_2 due to the introduction of two chiral cyclotetraamide motifs on the sidearms.

Screening of Tripeptide Library. To find the peptides best fitted to molecule **1**, we screened a 15 × 15 × 15 tripeptide library (3375 different combinations) that has been established by Still and co-workers using encoded split combinatorial chemistry (Figure 3).[11,16a,23] The tripeptide-bound polystyrene beads incorporate a photocleavable linker (*o*-nitrobenzyl carbonate) and varied electrophoric tags (chlorinated phenoxyalkanes) for encoding specific sequences of tripeptides.

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 $CHCl_3$ (0.5 mL). A solution of sensor **1** (40 μL, 1×10^{-3} M) in MeOH/$CHCl_3$ (1:9) was added, and

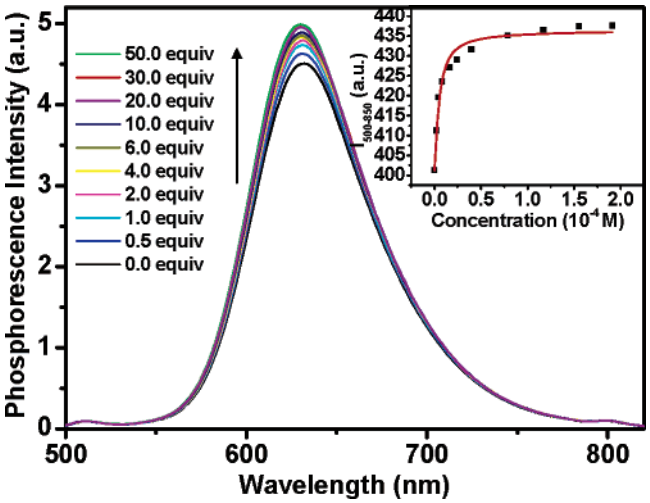


FIGURE 4. Phosphorescence titration spectra of **1** (4.0×10^{-6} M) by addition of various amounts of the tripeptide derivative **10**-DD [as a 4.0×10^{-3} M solution in MeOH/$CHCl_3$ (1:9)] at 293 K, λ_{ex} = 466 nm. Inset: A curve fitting of the data using the nonlinear least-squares method. K_a is derived to be 2.9×10^5 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 between **1** and peptides derived from alanine residues.

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)-$NHC_{12}H_{25}$ (**10**-DD), Ac-(L-Ala)-Gly-(D-Ala)-$NHC_{12}H_{25}$ (**11**-LD), Ac-(D-Ala)-Gly-(L-Ala)-$NHC_{12}H_{25}$ (**12**-DL), and Ac-(L-Ala)-Gly-(L-Ala)-$NHC_{12}H_{25}$ (**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** (4.0×10^{-6} M) in MeOH/$CHCl_3$ (1:9, 2 mL) at 20 °C were added aliquots of a specific tripeptide derivative, e.g., **10**-DD (as a 4.0×10^{-3} M solution) in MeOH/$CHCl_3$ (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,[24] the non-

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TABLE 1. Association Constants and Free Energy Changes for 1/Tripeptide Complexation in MeOH/$CHCl_3$ (1:9) Solution[a]

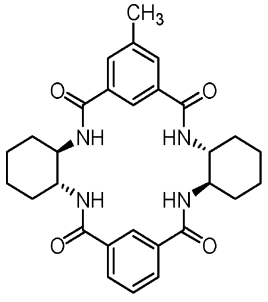
entry	peptide derivative	K_a[b] (10^3 M^{-1})	ΔG[c] (kJ mol^{-1})
1	Ac-D-Ala-Gly-D-Ala-$NHC_{12}H_{25}$ (**10**-DD)	294 ± 33	−30.7
2	Ac-L-Ala-Gly-D-Ala-$NHC_{12}H_{25}$ (**11**-LD)	170 ± 5	−29.3
3	Ac-D-Ala-Gly-L-Ala-$NHC_{12}H_{25}$ (**12**-DL)	154 ± 27	−29.1
4	Ac-L-Ala-Gly-L-Ala-$NHC_{12}H_{25}$ (**13**-LL)	58 ± 9	−26.7

[a] Receptor **1** (4.0×10^{-6} M) was titrated with tripeptide (4.0×10^{-3} 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 $\Delta G = -RT \ln K_a$.

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 complexation between **1** and 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 concentration of tripeptide. The 1:1 stoichiometry and binding constant (K_a) of complex (Table 1) were determined by curve fitting using the nonlinear least-squares method, taking into account the dilution effect.[25] The binding strength toward receptor **1**, according to the deduced association constants (see Table 1), follows an order of **10**-DD > **11**-LD ~ **12**-D; > **13**-LL. This result clearly indicated that receptor **1** favorably binds tripeptide **10**-DD which contains two D-Ala residues. The binding affinity of **10**-DD is 5- and 2-fold stronger than its antipode **13**-LL and diastereomers **11**-LD and **12**-DL, respectively. The resulting stereoselectivity in solution is in good agreement with that obtained in the solid-phase library screening.

^{1}H NMR titration experiments provided supportive evidence to the binding modes. When receptor **1** was titrated with tripeptide **10**-DD in $CH_3OD/CDCl_3$ (1:9), the NH on the tetraamide rings and the $C_3/C_{3'}$ protons on the bipyridinediamide rings (see structure **1** in Figure 2 for numbering) showed significant changes in chemical shifts (Figure 5). This result indicated that the bipyridinediamide moiety, in addition to the A_2B_2 motifs, also contributed considerable hydrogen bondings to the complexation with the tripeptide substrate. The NMR analysis indicated that compound **1** might exist as a mixture of two diastereomers (~1:1) with different configurations (Δ or Λ) at the ruthenium center. Upon addition of more than 22 equiv of **10**-DD, the $C_3/C_{3'}$ protons (at ~9.35 ppm) shifted downfield and split into two signals, which might account for the two diastereomeric complexes of **1/10**-DD. 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_2B_2 molecule **14** was prepared and subjected to ^{1}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 protic solvents, e.g., $CD_3OD/CDCl_3$ (1:9), due to the complicated exchange of amido protons.



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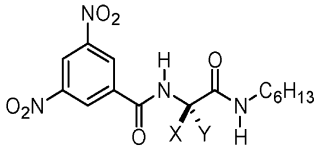

15-D X = Me, Y = H
15-L X = H, Y = Me

We also prepared the analogues of **10**-DD and **11**-LD by replacing the acetyl (Ac) capping group with a bulky *tert*-butyloxycarbonyl (*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-$NHC_{12}H_{25}$ via a deliberately designed chiral receptor. The two-armed receptor **1** incorporating a bipyridinediamide moiety and two chiral cylcotetraamide motifs achieves an effective multiple hydrogen bondings recognition with tripeptides in a stereoselective manner. The attachment

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(25) Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

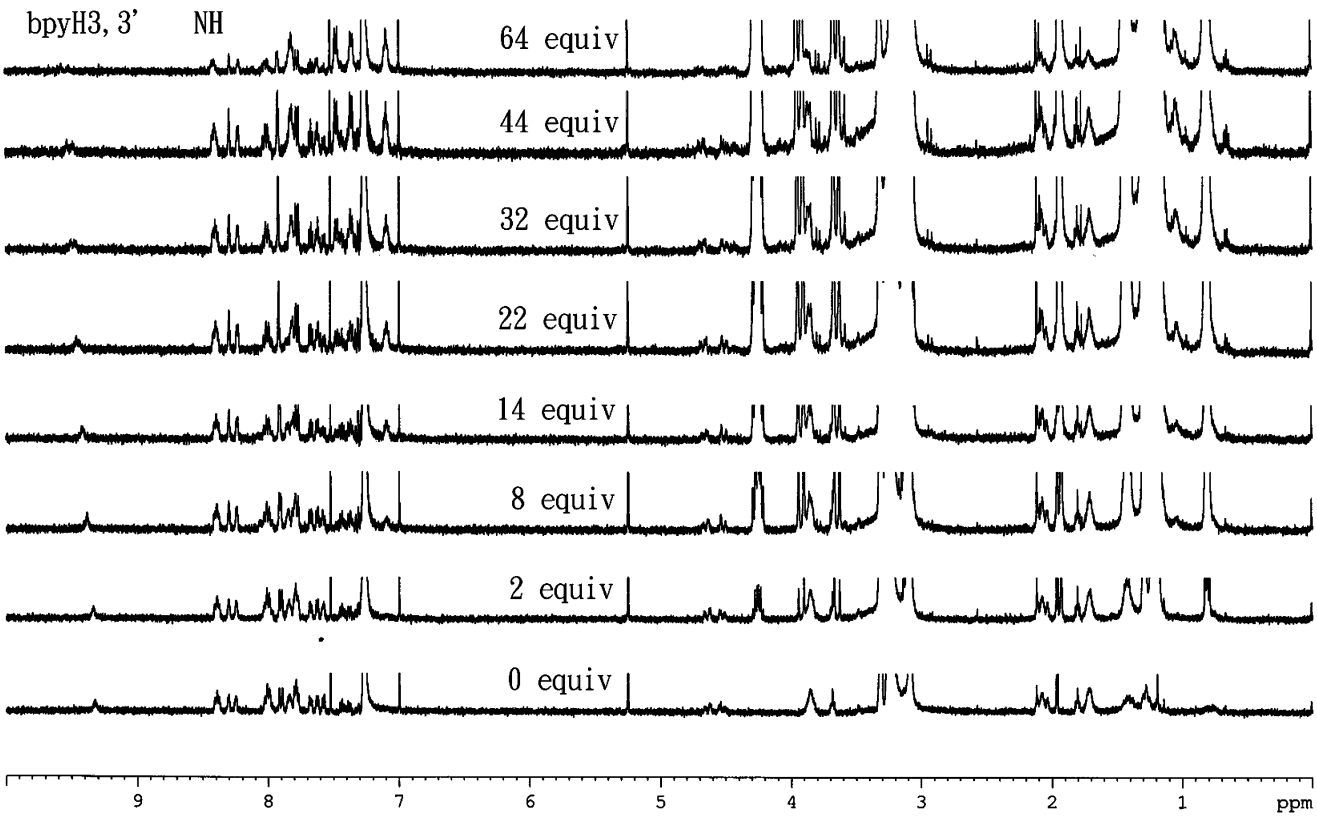

FIGURE 5. ^{1}H NMR titration of compound **1** (1×10^{-4} M) by addition of various amounts of **10**-DD [as 1×10^{-2} M solution in $CH_3OD/CDCl_3$ (1:9)].

of a Ru(II) reporter renders a real-time detection of the binding property based on the phosphorescence spectroscopy. This, in combination with the encoded library screening[21] technique, proves to be a rapid and efficient method for search and sensing of target peptides.

Experimental Section

Synthesis of Cyclotetraamide 9. To a solution of 5-azidomethylisophthalic acid (301 mg, 1.36 mmol) in CH_2Cl_2 (35 mL) was added a solution of pentafluorophenol (563 mg, 3.06 mmol) and EDC (586 mg, 3.06 mmol) in CH_2Cl_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 **8**[16b] (1.75 g, 3.1 mmol) in CH_2Cl_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_2O 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_2Cl_2 (65 mL) was added *i*-Pr_2NEt (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_3OH/CH_2Cl_2, 1:19) to afford compound **9** as white solids (1.49 g, 99%): mp > 320 °C; TLC ($MeOH/CHCl_3$ (1:19)) $R_f = 0.2$; IR (KBr) 3315, 3072, 2105, 1657 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.41 (2 H, d, $J = 7.6$ Hz), 8.36 (2 H, d, $J = 7.6$ Hz), 8.23 (1 H, s), 8.21 (1 H, s), 7.80 (2 H, dd, $J = 7.6$, 1.6 Hz), 7.78 (2 H, s), 4.55 (2 H, s), 3.89 (4 H, m), 1.96−1.93 (4 H, m), 1.77−1.74 (4 H, m), 1.60−1.50 (4 H, m), 1.35−1.25 (4 H, m); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.7 (C), 166.3 (C), 136.4 (C), 135.4 (C), 134.9 (C), 129.7 (CH), 129.4 (CH), 128.3 (CH), 126.7 (CH), 126.5 (CH), 53.8 (CH), 53.7 (CH), 53.0 (CH_2), 31.5 (CH_2), 24.7 (CH_2); FAB-MS *m/z* (rel intensity) 544 ($M + H^+$, 30); HRMS calcd for $C_{29}H_{34}N_7O_4$ ($M + H^+$) 544.2672, found 544.2665.

Synthesis of Molecular Sensor 1. To a solution of azide **9** (500 mg, 0.92 mmol) in THF (40 mL) was added solid PPh_3 (362 mg, 1.38 mmol) at room temperature. The mixture was refluxed for 2 h until no nitrogen evolved. The solution was cooled, and a mixture of H_2O (0.2 mL) and THF (9 mL) was added. The mixture was refluxed for an additional 14 h, concentrated, and purified by chromatography on a silica gel column ($CH_3OH/CHCl_3$, 1:4) to give the corresponding amine as white solids (433 mg, 87%).

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 $CH_3OH/CHCl_3$ (1:9), and washed twice with H_2O. The aqueous phase was extracted three times with $CH_3OH/CHCl_3$ (1:9). The combined organic phase was dried over anhydrous Na_2SO_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 ($CH_3OH/CHCl_3$, 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*-$Ru(bpy)_2Cl_2$ hydrate (25 mg, 0.052 mmol) in EtOH (8 mL) and H_2O (2 mL) was heated under reflux for 6 h. After removal of solvents, the residue was dissolved in CH_2Cl_2 and triturated with Et_2O. The solids were collected by filtration, washed with CH_2Cl_2/Et_2O (1:3), and dried in vacuo to afford the chloride salt of **1** (75 mg). The chloride salt was

dissolved in H_2O, and treated with saturated NH_4PF_6 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%); IR (KBr) 3422, 2942, 1654, 1540 cm^{-1}; ^1H NMR (DMSO-d_6, 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); ^{13}C NMR (DMSO-d_6, 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 (CH_2), 31.5 (CH_2), 24.7 (CH_2); FAB-MS *m/z* (rel intensity) 1802 ($M + H^+ - PF_6$), 1658 ($M + H^+ - 2\ PF_6$).

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/$CHCl_3$ (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/$CHCl_3$ (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 a 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 (K_a) is derived from the following equation.[22]

$$F = F_0 + (\Delta F_{max}/2C_0)\{1/K_a + C_0 + C - [(1/K_a + C_0 + C)^2 - 4C_0C]^{1/2}\}$$

F is the emission intensity, F_0 is the original emission intensity of the free receptor, and ΔF_{max} is the largest change of emission intensity after saturation with the substrate. C is the concentration of substrate; C_0 is the initial concentration of the receptor.

^{1}H 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 $CD_3OD/CDCl_3$ (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-$NHC_{12}H_{25}$) in $CD_3OD/CDCl_3$ (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 $C_3/C_{3'}$-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.

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