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Sequence-Selective Visual Recognition of Nonprotected Dipeptides

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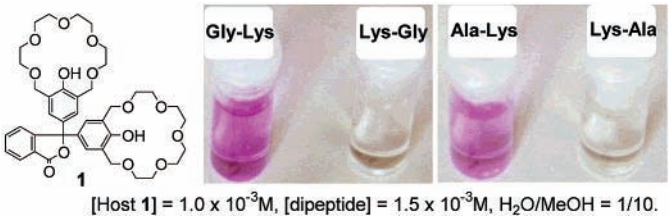
ABSTRACT



A receptor 1 with phenolphthalein and two crown ethers in the molecule develops brilliant purple color in the presence of dipeptides with a specific amino acid-sequence containing a C-terminal lysine. This type of color development could be extended to the detection of oligopeptides of a specific sequence at the N-terminal such as scyliorhinin I and APP_{770}(394−410).

The recognition and transmission of information in protein−protein interactions play an essential role in living systems. The development of synthetic receptors for the sequence-selective recognition of peptides is a challenging and potentially information-rich topic, as with further understanding of weak noncovalent interactions within a supramolecular framework, insight into many vital biological functions could be gained. Recently, several synthetic receptors for peptides have been reported which mostly used protected peptides as guest molecules.[1] Additionally, the visual recognition of various attributes of guest molecules has also been reported.[2] In this paper, we report the sequence-selective visual recognition of nonprotected dipeptides in a protic solvent system.

The synthetic receptors require at least three functions for visual recognition of the peptide sequence. They should be able (1) to detect the length of the peptide, (2) to discriminate the size of peptide side chains, and (3) to transform the invisible interaction into a visual form. Recently, we reported that receptor **1** selectively recognized the chain lengths of α,ω-diamines and linear triamines and revealed information through the use of color.[2g,h] Receptor **1** is a hybrid molecule consisting of phenolphthalein (**4**), which is commonly used as a pH indicator, and two crown ether moieties (Figure 1). In methanol solution, hybrid molecule **1** developed striking color changes that could be detected by the naked eye upon the addition of 1,8-diaminooctane or 1,9-diaminononane. Receptor **1** has been proposed to develop this color variation by recognition of the distance between an N-terminal amino group and the basic functional group in the side chains of dipeptides, with color changes occurring only if the distance between the two amino groups lies within an appropriate range. For instance, the dipeptide Gly-(L)Lys (**5**) has eight atoms between the terminal amino groups, while (L)Lys-Gly (**6**) exhibits only five carbon atoms. Thus, color development

(1) (a) For recent review: Still, W. C. *Acc. Chem. Res*. **1996**, *29*, 155−163. (b) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev*. **2000**, *100*, 2479−2494. (c) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc*. **1998**, *120*, 3536−3537. (d) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc*. **1998**, *120*, 11208−11209. (e) Chen. C.-T.; Wagner, H.; Still, W. C. *Science*. **1998**, *279*, 851−853. (f) Fessmann, T.; Kilburn, J. D. *Angew. Chem., Int. Ed*. **1999**, *38*, 1993−1996. (g) Schneider, H.-J.; Sirish, M. *Chem. Commun*. **1999**, 907−908. (h) Braxmeier, T.; Demarcus, M.; Fessmann, T.; McAteer, S.; Kilburn, J. D. *Chem. Eur. J*. **2001**, *7*, 1021−1031. (i) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc*. **2001**, *123*, 11296−11297.

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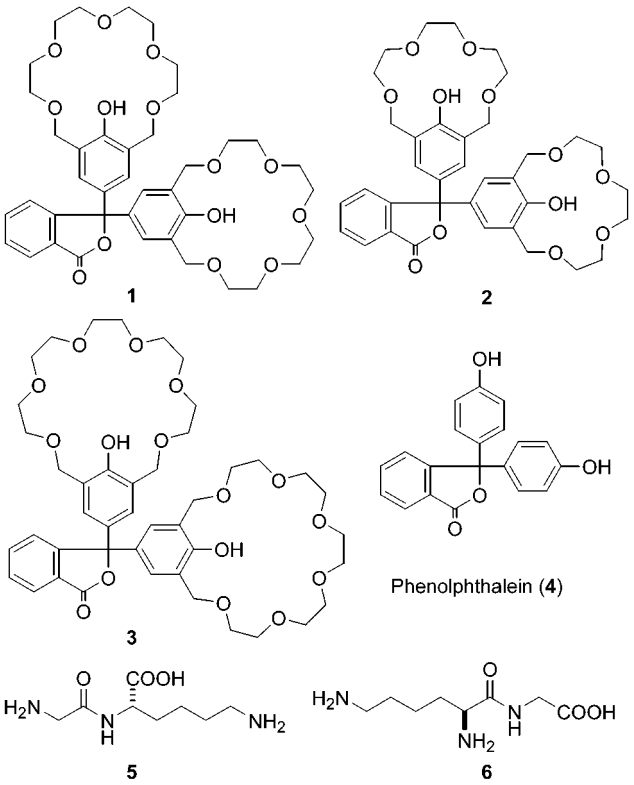


Figure 1. Structures of hosts and dipeptides.

by **1** is expected to selectively occur in the presence of **5** and not at all for **6**, in which the amino acid sequence is reversed.

To estimate the importance of the ring size of the crown ether groups, receptors **2** and **3**, with 15- and 21-membered crown rings, respectively, were synthesized by a procedure similar to that for **1**.[2g] The binding ability of the receptors **1**−**3** to dipeptides **5** and **6** was analyzed by mixing the receptor/dipeptide pairs in a methanol/water = 10/1 solution with a large excess of *N*-ethylpiperidine. As expected, dramatic color development was observed with **1** in the presence of **5** (Figure 2). No other combination of hosts **2** or **3** with guests **5** and **6** generated color development.[3] These results indicated that the ring size of the crown ether plays a crucial role in the color development process.

Interactions between the receptor **1** and commercially available dipeptides containing lysine were studied, and results are shown in Figure 3. Strong color development was observed with several dipeptides having Lys as the C-terminus (nos. 7 and 13−15). Interestingly, the dipeptide with the reverse sequence generated no color change (compare no. 3 with 13, 4 with 14, and 5 with 15). These observations indicate that the amino group in the side chain of Lys plays a crucial role in color development. We also found that the guanidine group of Arg does not shift in this system due to steric factors, though it is more basic than the primary amino group (see no. 5). Furthermore, color development is sensitive to the steric environment of the N-terminal amino group. Thus receptor **1** has the ability to discriminate based on the size of peptide side chain (see nos. 13, 14, and 20).

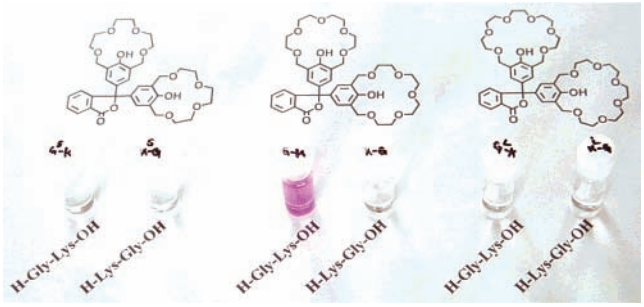


Figure 2. Color development by the hosts **1**−**3**. The concentration of hosts were 1.0×10^{-3} M and those of dipeptides were 1.5×10^{-3} in the presence of *N*-ethylpiperidine (2.5×10^{-2} M) in methanol/water = 10/1.

Figure 3. Visible recognition of the sequence of dipeptides by the host **1**. The concentration of **1** was 1.0×10^{-3} M and those of dipeptides were 1.5×10^{-3} in the presence of *N*-ethylpiperidine (2.5×10^{-2} M) in methanol/water = 10/1. 1; Host **1** only, 2; Gly-Gly, 3; Lys-Gly, 4; Lys-Ala, 5; Lys-Arg, 6; Lys-Met, 7; Lys-Lys, 8; Lys-Trp, 9; Lys-Glu, 10; Lys-Pro, 11; Lys-Val, 12; Lys-Tyr, 13; Gly-Lys, 14; Ala-Lys, 15; Arg-Lys, 16; Met-Lys, 17; Trp-Lys, 18; Glu-Lys, 19; Pro-Lys, 20; Val-Lys, 21; Tyr-Lys.

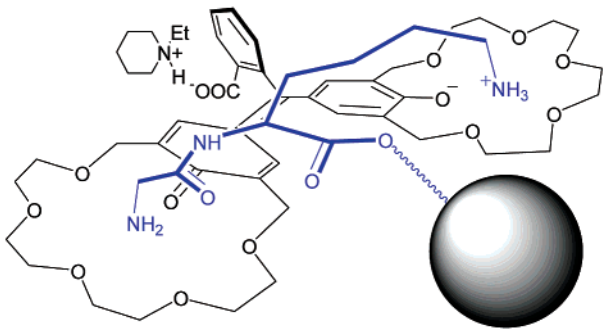
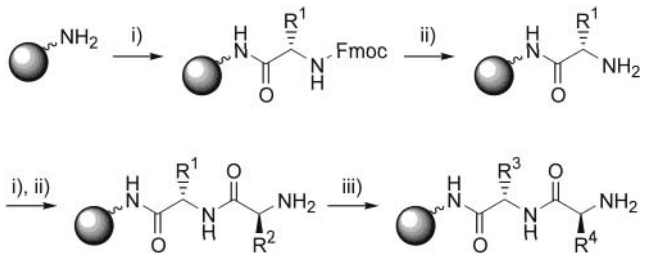


Figure 4. Image of the colored complex.

Although receptor **1** was sensitive to bulkiness in the vicinity of terminal amino groups of the guest molecule, we expected that receptor **1** would exhibit vague recognition against bulkiness extending from the inner carboxyl group of guest dipeptide (Figure 4). On the basis of this model, we examined the interaction between receptor **1** and 400 polymer-supported dipeptides. As shown in Scheme 1, the polymer-supported dipeptides were synthesized by the standard parallel method. NH_2-PEG-resin (Watanabe Chemical) and Fmoc-amino acids were used as starting materials. Condensation reactions were carried out under WSC/HOBT/DMF conditions, and deprotection of Fmoc groups was undertaken with 20% piperidine in DMF. It is noteworthy that removal of the protective group on the side chain was achieved by Node's odorless thiol method without special ventilation.[4,5] The color changes generated by interactions between receptor **1** and the 400 polymer-supported dipeptides are presented in Figure 5. Strong color development was observed for only six of the 400 dipeptides (Gly-Lys, Ala-Lys, Ser-Lys, Thr-Lys, Arg-Lys, and Lys-Lys). The six dipeptides may be classified into three groups based on their side chain compositions. The first group has small alkyl substituents (Gly-Lys and Ala-Lys) and the second group has a hydroxy substituent (Ser-Lys and Thr-Lys), while the third group exhibits a relatively large but remote functional group (Arg-Lys and Lys-Lys). In the second group, a hydrogen bond should be formed between the phenolate oxygen and the hydroxy group of the side chain, which is expected to stabilize the colored complex.[6]

Scheme 1. Preparation of the Polymer-Supported Amino Acids[a]



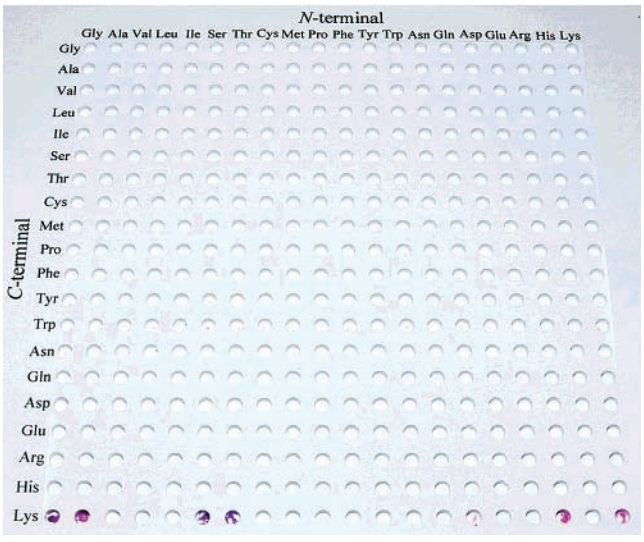


Figure 5. Color development between host **1** and 400 polymer-supported dipeptides. Polymer-supported dipeptides were rinsed with a solution of host **1** (1.0×10^{-3} M) and *N*-ethylpiperidine (2.5×10^{-2} M) in methanol.

UV−visible spectra of pertinent dipeptides are shown in Figure 6. No color was found to develop using Gly-Orn.

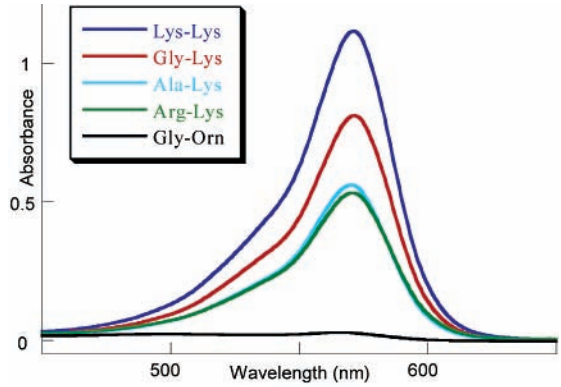


Figure 6. UV−vis spectra of **1** with the dipeptides. The concentration of **1** was 1.0×10^{-3} M and those of dipeptides were 1.5×10^{-3} in the presence of *N*-ethylpiperidine (2.5×10^{-2} M) at 25 °C in methanol/water = 10/1.

From previous results with α,ω-diamines,[2g] Gly-Orn was expected to weakly develop color, as there are seven atoms between the two amino groups. The seven atoms including the amide bond, however, are slightly shorter and more rigid than in 1,7-diaminoheptane. This explains why color development was not observed with Gly-Orn and indicates that the strict 1,8-relationship between the two amino groups is indispensable for the recognition of dipeptides. Among possible combinations of essential amino acids, only dipeptides with Lys at the C-terminus meet this requirement.

The apparent association constants and molar absorption coefficients were measured by UV−visible titration in methanol/water = 10/1 in the presence of *N*-ethylpiperidine (5.0×10^{-2} M) by adding dipeptide stock solutions ($5 \times$

(3) No color development was observed between phenolphthalein and guests **5** and **6** under the same conditions.

(4) (a) Nishide, K.; Ohsugi, S.; Shiraki, H.; Tamakita, H.; Node, M. *Org. Lett.* **2001**, *3*, 3121−3124. (b) Node, M.; Kumar, K.; Nishide, K.; Ohsugi, S.; Miyamoto, T. *Tetrahedron Lett.* **2001**, *42*, 9207−9210.

(5) As for the side chain protected amino acids; Fmoc-Ser(Trt)-OH, Fmoc-Thr(Trt)-OH, Fmoc-Cyr(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Arg(Mtr)-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH.

(6) Similar hydrogen bonding between phenolcrown hosts and ethanolamine derivatives were reported. (a) Naemura, K.; Ueno, K.; Takeuchi, S.; Tobe, Y.; Kaneda, T.; Sakata Y. *J. Am. Chem. Soc.* **1993**, *115*, 8475−8476. (b) Hirose, K.; Ogasahara, K.; Nishioka, K.; Tobe, Y.; Naemura, K. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1984−1993.

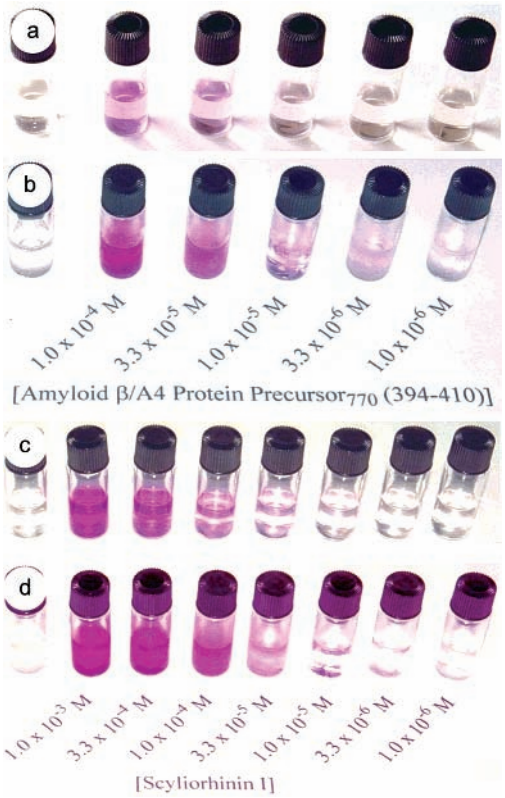


Figure 7. Color development of pertinent oligopeptides by the host **1**. The concentration of **1** was 1.0×10^{-2} M in the presence of *N*-ethylpiperidine (5×10^{-3} M) in methanol/water = 5/1. (a) APP at 25 °C, (b) APP at 0 °C, (c) scyliorhinin I at 25 °C, (d) scyliorhinin I at 0 °C.

10^{-3} M) into 5.0×10^{-4} M of receptor **1** at 25 ± 0.1 °C. Spectra were analyzed by the Rose−Drago method,[7] and the following values were obtained: Lys-Lys (K_{app} = 1020 ± 20, ϵ = 2120 ± 20),[8] Gly-Lys (K_{app} = 930 ± 30, ϵ = 1780 ± 30),[8] Ala-Lys (K_{app} = 1100 ± 30, ϵ = 1200 ± 20) and Arg-Lys (K_{app} = 830 ± 40, ϵ = 1230 ± 30). In exploring the analytical potential of this visualization, we applied this system to proteins with a (Gly, Ala, Ser, Thr, Arg, or Lys)-Lys sequence at their N-terminus. Amyloid β/A4 protein precursor, H-Ala-Lys-Glu-Arg-Leu-Glu-Ala-Lys-His-Arg-Glu-Arg-Met-Ser-Gln-Val-Met-OH, (APP_{770}(394−410)), a peptide with growth-promoting activity,[9,10] and scyliorhinin I, H-Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2, a peptide of the tachykinin family, were investigated for color development upon complexation with **1**.[10,11] Clear color development was observed with APP as well as scyliorhinin I at a concentration of 3.3×10^{-5} M at 25 °C (Figure 7a,c). Sensitivity increased as the temperature decreased to 0 °C (Figure 7b,d).

In conclusion, we have devised the clear sequence-selective visual recognition of nonprotected dipeptides in a protic solvent system with Lys at the C-terminus. This system may be used for the visual recognition of proteins with a (Gly, Ala, Ser, Thr, Arg, or Lys)-Lys sequence at their N-terminus.

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Supporting Information Available: Characterization data for hosts **2** and **3**, Job plot between **1** and dipeptides (Figure S1), and calibration curves for oligopeptides (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Job plots suggested that the host: guest ratio in this solution was 1:1 (Figure S1).

(9) Ninomiya, H.; Roch, J. M.; Sundsmo, M. P.; Otero, D. A. C.; Saitoh, T. *J. Cell. Biol.* **1993**, *121*, 879−886.

(10) Clear first-order calibration curves between oligopeptides and color development were observed (Figure S2).

(11) Conlon, J. M.; Deacon, C. F.; O'Toole. L.; Thim, L. *FEBS Lett.* **1986**, *200*, 111−116.