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Synthesis of fluorescence-labeled peptidocalix[4]arene library and its peptide sensing ability

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Abstract—The fluorescence spectrum of the peptidocalix[4]arene **5**, which was found in the screening of library **1** against the target peptide **2a**, was dependent on the concentration of **2b**.

The development of chemosensors has raised considerable interest for it's use as a convenient analytical method in many different fields. A large number of optical and electrochemical sensors for metal or organic ions have been reported in the last two decades.[1] In contrast, there are few chemosensors for neutral organic molecules[2] because of the difficulty of rational receptor design for target molecules. Combinatorial methods are a powerful approach for making chemosensors for organic molecules. Recently, an ATP sensor,[3] amino acid sensors,[4] and peptide sensors[2b] were developed using the combinatorial approach.

In this communication, we describe a calixarene-based chemosensor[5] for a peptide developed using combinatorial methodology. Jin and co-workers reported a sodium ion-selective fluorescent sensor that possesses two fluorescent pyrenyl groups at the end of the sodium ion binding site in the calix[4]arene derivative.[6] We hypothesized that this system could be applied for organic molecules. We synthesized a fluorescence-labeled peptide library following a previously reported procedure[7] (Fig. 1). Two pyrenyl groups were attached at the peptide N-terminal ends. When the host molecule against the target molecule was found in this library, the discovered library members acted as a sensor against the target in a manner similar to that reported by Jin.[6] Library **1** consists of $15^3 = 3375$ peptidocalixarenes because 15 amino acids as building blocks are attached onto both arms of a calix[4]arene.

The ^{5}Leu enkephalin derivative **2a** in Table 1 was chosen as an analyte.[8,9] For screening of the library, approximately 3.0×10^{-5} mol dm^{-3} dye-labeled peptide **2a** was incubated with 5 mg of library **1** on the beads in $CHCl_3$ for 3 days. Only a few beads turned red after equilibration, signifying that only a few library members bind peptide **2a**. All colored beads were isolated

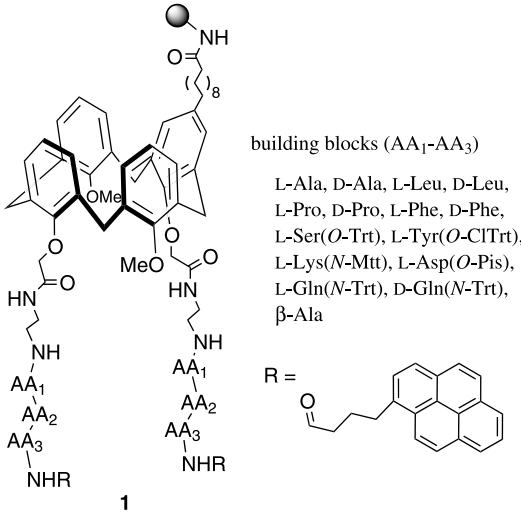

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Figure 1. Fluorescence-labeled library based on calix[4]arene. Trt = triphenylmethyl, ClTrt = 2-chloro-triphenylmethyl, Mtt = 4-methyl-triphenylmethyl, Pis = 2-phenylisopropyl.

Keywords: calixarenes; combinatorial library; chemosensors; fluorescence change.

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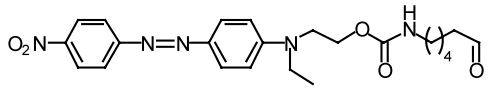
Table 1. Peptide sequences of the analytes R′-NH-BB_1-BB_2-BB_3-BB_4-L-Leu-OMe

Compound	R′	BB_1	BB_2	BB_3	BB_4
2a	Dye[a]	L-Tyr(*O*-*t*-Bu)	Gly	Gly	L-Phe
2b	Pal[b]	L-Tyr(*O*-*t*-Bu)	Gly	Gly	L-Phe
3a	Dye	L-Tyr(*O*-*t*-Bu)	L-Tyr(*O*-*t*-Bu)	L-Tyr(*O*-*t*-Bu)	L-Phe
3b	Pal	L-Tyr(*O*-*t*-Bu)	L-Tyr(*O*-*t*-Bu)	L-Tyr(*O*-*t*-Bu)	L-Phe
4a	Dye	L-Ser(*O*-*t*-Bu)	L-Ser(*O*-*t*-Bu)	L-Phe	L-Val
4b	Pal	L-Ser(*O*-*t*-Bu)	L-Ser(*O*-*t*-Bu)	L-Phe	L-Val

[a] Dye:



[b] Pal: Palmitoyl.

Table 2. Peptide sequences of colored beads in **1** for **2a**

Compound	AA_1	AA_2	AA_3	Frequency[a]
1a	D-Leu	L-Ser(*O*-Trt)	L-Ala	8
1b	D-Leu	L-Ser(*O*-Trt)	L-Lys(*O*-Mtt)	4
1c	L-Ser(*O*-Trt)	L-Phe	L-Lys(*O*-Mtt)	3
1d	L-Leu	L-Phe	L-Ser(*O*-Trt)	2
		Others		11

[a] Number of beads isolated.

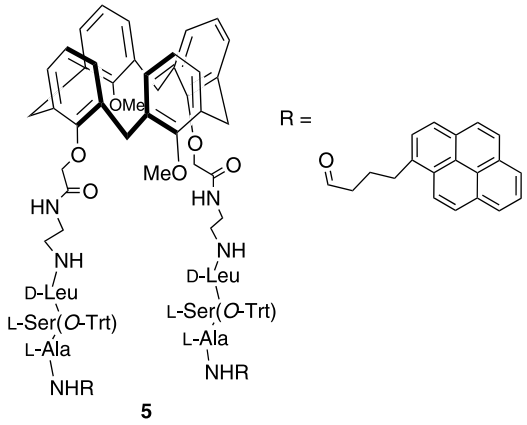


Figure 2. Chemosensor for the analyte **2b**.

and decoded to identify their amino acid sequences, which are shown in Table 2.

The solid support free **5** in Fig. 2,[10] which possesses the most frequently appearing peptide sequence (D-Leu-L-Ser(*O*-Trt)-L-Ala in AA_1-AA_2-AA_3) in the screening, was selected as the chemosensor for analyte **2b**. Analyte **2b** possesses the palmitoyl group at the N-terminal (R′) instead of the dye moiety in **2a**. The reason for changing the N-terminal from the dye to the palmitoyl group is that the fluorescence emission of **5** overlaps with the absorption of **2a**.

Fluorescence spectra of **5** in the presence and absence of analyte **2b** are shown in Fig. 3.[11] The addition of **2b** to the solution of **5** enhances the fluorescence of both the monomer and the eximer emission depending on the concentration of **2b**. The addition of 1.6×10^{-3} mol dm^{-3} of **2b** to the 1.0×10^{-6} mol dm^{-3} of **5** in $CHCl_3$ induced a 2- and 2.5-fold increase in fluorescence at the monomer and the eximer, respectively. On the other hand, addition of the control pentapeptides **3b** or **4b**, which have different peptide sequences[12] to **5** affected the fluorescence intensity less (Fig. 4). This result indicates that compound **5** does not only bind **2b** selectively, but also acts as a chemosensor for **2b**. The association constant (K_{ass}) of **5** against peptide **2b** was estimated to be about 2.0×10^3 mol dm^{-3} from the eximer fluorescence intensity using the Benesi–Hildebrand plot.[13] The mechanism of fluorescence enhancement is unclear at present. It is possible that the sidechain protecting trityl group and/or the lone-pair of the peptide on **5** could quench the fluorescence in the absence of the analyte.

In conclusion, we developed calixarene-based chemosensors for neutral molecules using the combinatorial approach. The design and synthesis for water soluble and more sensitive chemosensors are currently under investigation.

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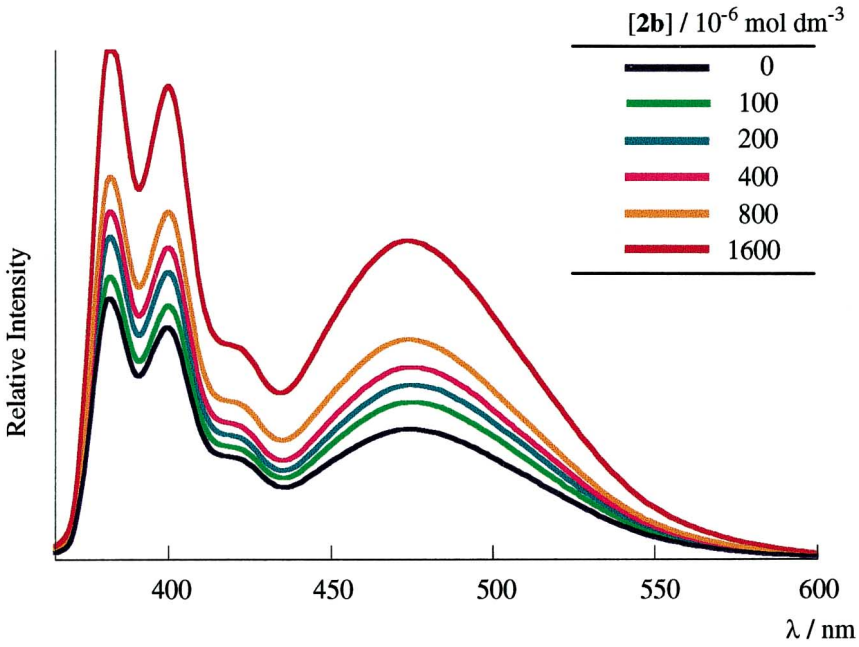

Figure 3. Fluorescence emission spectroscopic change of **5** in $CHCl_3$ upon addition of peptide **2b** at 20°C. [**5**] = 1.0×10^{-6} mol dm^{-3}. Excitation wavelength: 344 nm.

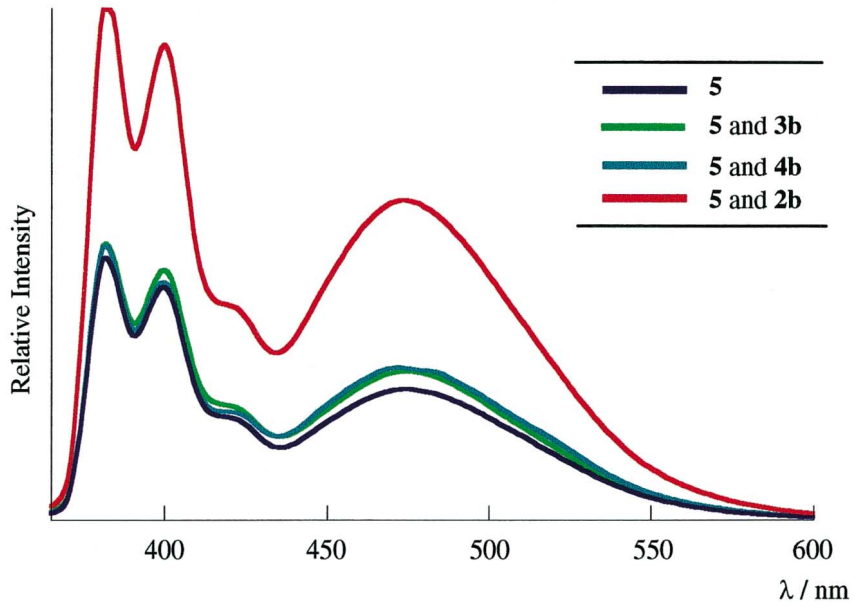

Figure 4. Fluorescence emission spectroscopic change of **5** in $CHCl_3$ upon addition of peptide **2b**, **3b** or **4b** at 20°C. [**5**] = 1.0×10^{-6} mol dm^{-3}. [**2b**] = [**3b**] = [**4b**] = 1.6×10^{-3} mol dm^{-3}. Excitation wavelength: 344 nm.

References

1. For selected reviews of chemosensors, see: (a) Czarnik, A. W. *Fluorescent Chemosensors for Ion and Molecule Recognition*; American Chemical Society: Washington DC, 1992; (b) Prasanna de Silva, A.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566; (c) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, 197–202; (d) Bissel, R. A.; Prasanna de Silva, A.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; Sandanayake, K. R. A. S. *Chem. Soc. Rev.* **1992**, 187–195; (e) Diamond, D.; McKervey, M. A. *Chem. Soc. Rev.* **1996**, 15–24; (f) Lockhart, J. C. *Comprehensive Supramolecular Chemistry*; Pergamon Press: Oxford, 1996; Vol. 1, pp. 605–634.
2. Examples of chemosensors for peptides or saccharides sensors: (a) Chen, C.-T.; Wagner, H.; Still, W. C. *Science* **1998**, *279*, 851–853; (b) Iorio, E. J.; Shao, Y.; Chen, C.-T.; Wagner, H.; Still, W. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1635–1638; (c) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910–1922; (d) Davis, C. J.; Lewis, P. T.; McCarroll, M. E.; Read, M. W.; Cueto, R.; Strongin, R. M. *Org.*

Lett. **1999**, *1*, 331–334; (e) Wang, W.; Gao, S.; Wang, B. *Org*. *Lett*. **1999**, *1*, 1209–1212.
3. Schneider, S. E.; O'Neil, S. N.; Anslyn, E. V. *J*. *Am*. *Chem*. *Soc*. **2000**, *122*, 542–543.
4. Leipert, D.; Nopper, D.; Bauser, M.; Gauglitz, G.; Jung, G. *Angew*. *Chem*., *Int*. *Ed*. **1998**, *37*, 3308–3311.
5. For reviews on calixarene-based chemosensors, see: Diamond, D.; Nolan, K. *Anal*. *Chem*. **2001**, *73*, 22A–29A.
6. Jin, T.; Ichikawa, K.; Koyama, T. *J*. *Chem*. *Soc*., *Chem*. *Commun*. **1992**, 499–501.
7. Hioki, H.; Yamada, T.; Fujioka, C.; Kodama, M. *Tetrahedron Lett*. **1999**, *40*, 6821–6825.
8. Development of peptidosteroidal receptors for the ^{5}Leu enkephalin derivatives by combinatorial approach has been reported: (a) Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. C. *J*. *Am*. *Chem*. *Soc*. **1994**, *116*, 7955–7956.; (b) Cheng Y.; Suenaga, T.; Still, W. C. *J*. *Am*. *Chem*. *Soc*. **1996**, *118*, 1813–1814.
9. The analytes **2**, **3** and **4** were synthesized by general Fmoc solid-phase peptide synthesis, see: Chan, W. C.; White, P. D. *Fmoc Solid-Phase Peptide Synthesis*: *A Practical Approach*; Oxford University Press: New York, 2000.
10. Starting from dimethoxycalix[4]arene, **5** was prepared by solution-phase synthesis via *O*-alkylation by *N*-Boc protected aminoethylated chloroacetamide followed by Fmoc peptide synthesis and capping with 1-pyrenebutyric acid.
11. In the absence of **2b**, the fluorescence spectrum of **5** showed dual emission resulting from the monomer (382 and 400 nm) and the excimer (470 nm). The intensity of the monomer is comparable to that of the excimer. The ratio (monomer/excimer) is not affected by the concentration of **5** in the range of 10^{-5} to 10^{-7} mol dm^{-3}. The result indicates that the emission at 470 nm is assigned to intramolecular excimer.
12. Compound **1a** on the beads was not stained red after incubation with **3a** or **4a**, which indicates that both **3b** and **4b** do not bind sensor **5**.
13. Connors, K. A. *Binding Constants*; Wiley-Interscience, 1987; p. 141.