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Tetrahedron Letters 45 (2004) 561–564
Tetrahedron Letters

Synthesis of calix[4]arene library substituted with peptides at the upper rim

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Received 14 July 2003; revised 8 October 2003; accepted 31 October 2003

Abstract—A fluorescence-labeled calix[4]arene library substituted with peptides at the upper rim was synthesized. Screening of the library for binding a dye-labeled oligopeptide indicated that some peptidocalix[4]arenes selectively bind the oligopeptide. The chemosensitivity of the library members for a target peptide was also investigated.

Calixarenes are widely used as a platform for artificial receptors.[1] Their potential to function as receptors depends on the modification of the core calixarene. Recently, we synthesized a fluorescence-labeled peptidocalix[4]arene library[2,3] substituted with peptides at the lower rim (phenolic oxygen side of calixarene). In the library, there were the host molecules that only selectively bind the target peptide but also act as chemosensors[4] for the target. In that study, we demonstrated that the split synthesis of libraries based on calixarenes is an attractive approach for discovering the new host molecules.[2,5] Although most organic molecules bind calixarene-based receptors at the upper rim (aromatic nuclei side of calixarene), split or parallel synthesis of the upper rim-modified calixarene libraries have not yet been reported.[6] In this communication, we report the split synthesis of a fluorescence-labeled calix[4]arene library substituted with peptides[7] at the upper rim, and a binding assay for a pentapeptide. The chemosensitivity for the pentapeptide is also reported.

The fluorescence-labeled peptide library is shown in Figure 1. The substrate binding site, consisting of two-armed tripeptides was directly attached to the aminomethylated calix[4]arene at the upper rim. Two pyrenyl groups were attached at the peptide N-terminal. The

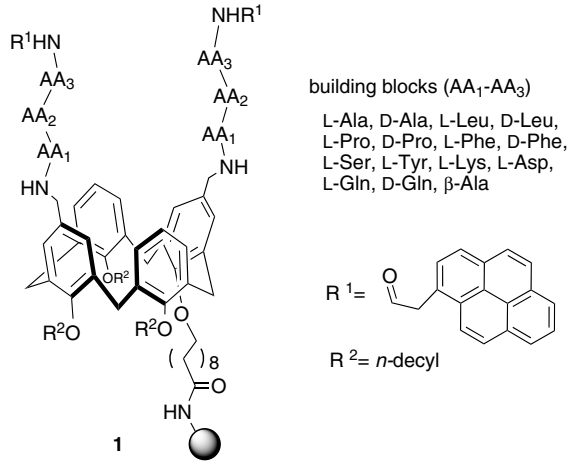

Figure 1. Upper rim-modified peptidocalix[4]arene library.

conformational change of the peptidocalix[4]arene induced by binding the substrate was expected to change the fluorescence spectra in the same manner as previously reported.[2]

Core-compound **6** was prepared according to Scheme 1. Starting from dibenzyloxycalix[4]arene **2**[8], *n*-decylation and deprotection of benzyl groups at the lower rim followed by selective bromination at upper rim afforded **3**. Compound **3** was subjected to a third *n*-decylation at the lower rim. After the remaining phenolic OH was alkylated with MPM-protected 6-bromo-*n*-decanol, the MPM group was removed by DDQ (2,3-dichloro-5,

Keywords: Calixarenes; Combinatorial library; Binding assay; Chemosensors.

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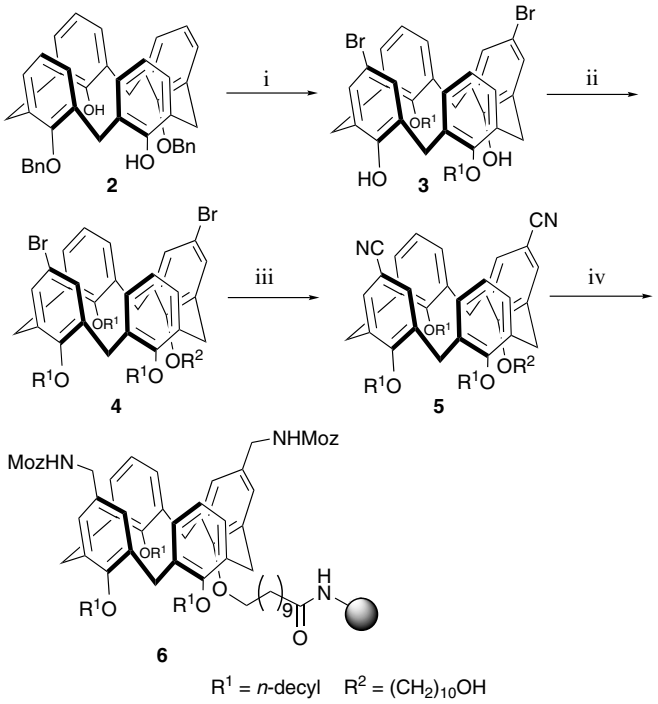
doi:10.1016/j.tetlet.2003.10.215



Scheme 1. Synthesis of the core-compound **6** of library **1**. Reagents and conditions: (i) 1. NaH, *n*-decylbromide, DMF, 86%; 2. H_2, $Pd(OH)_2$, EtOH/benzene 2/1, 80%; 3. $HPyBr_3$, $CHCl_3$/MeOH, 10/1, 98%; (ii) 1. NaH, *n*-decylbromide, DMF, 72%; 2. NaH, $MPMOC_{10}H_{20}Br$, DMF, 82%; 3. DDQ, aq $NaHCO_3/CH_2Cl_2$ 1/10, 100%; (iii) $Zn(CN)_2$, DPPF, $Pd_2(dba)_2$, DMF, 140 °C, 74%; (iv) 1. BH_3·THF, THF, reflux; 2. 6 M HC1, reflux, ; 3. Moz-*S*, K_2CO_3, l,4-dioxane/H_2O 4/1, 78% in three steps; 4. IBX, DMF, 98%; 5. $NaClO_2$, $NaHPO_4{\cdot}2H_2O$, 2-methyl-2-butene *t*-BuOH/H_2O (15/4) 82%; 6. aminomethylated polystyrene resin, DIC, HOBt, CH_2C1_3. Abbreviations: DMF = *N*,*N*′-dimethylformamide, MPM = *p*-methoxyphenylmethyl, DDQ = 2,3-dichloro-5,6-dicyano-l,4-benzoquinone, DPPF = 1,1′-bis(diphenylphosphino)ferrocene, Moz-*S* = *S*-*p*-methoxybenzyloxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine, Moz = *p*-methoxybenzyloxycarbonyl, DIC = 1,3-diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole.

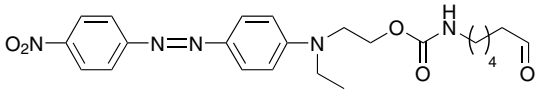
6-dicyano-1,4-benzoquinone) to give **4**. Compound **4** has a cone conformation,[9] which was determined by ^1H NMR analysis. Palladium-catalyzed cyanation of **4** was achieved in good yield.[10] The cyano group was reduced by borane and the resulting two aminomethyl groups were protected by Moz (*p*-methoxybenzyloxycarbonyl) groups. Finally, the hydroxy group in R^2 was oxidized and the resulting carboxylic acid was condensed with aminomethylated polystyrene resin to prepare the peptide library on a solid support.

The peptide library was produced using the previously reported procedure.[2,5] After removal of the *N*-Moz groups in **6**,[11] a split synthesis was run on both sides of the arms to append a tripeptide using 15 Fmoc–amino acids as building blocks. Because 15 amino acids were used as building blocks, this library consists of $15^3 = 3375$ peptidocalixarenes. After removal of the N-terminal Fmoc group in the tripeptide, the library was condensed using 2-pyreneacetic acid as a fluorophore. Finally, **1** was obtained by treating with trifluoroacetic acid to remove the protective groups on the side chain of the peptides.[12]

The Leu[5] enkephalin derivative **7** in Figure 2 was chosen as an analyte. For the binding study of the library **1**, approximately 3.0×10^{-5} mol dm^{-3} dye-labeled peptide **7a** was incubated with ca. 5 mg of the library on beads in $CHCl_3$ for 3 days. Only a few beads turned red, signifying that only a few of the library members bind peptide **7a**. All the colored beads were isolated and decoded to identify their amino acid sequences (Table 1). Remarkably, only 4 of 3375 library members were identified and all the colored beads had an L-Tyr at AA_2.D-Phe frequently appeared in AA_3.

R-NH-L-Tyr(*O*-*t*-Bu)-Gly-Gly-L-Phe -L-Leu-OMe

7a: R =



7b: R = $CH_3(CH)_{14}CO$

Figure 2. Structure of the analytes **7a** and **7b**.

The solid support free **8** in Figure 3, which possessed most frequently appearing peptide sequences in the screening shown in Table 1, was selected as the chemosensor for analyte **7b**.[13] Compound **8** was prepared in solution phase.

The fluorescence spectrum of **8** at 1.0×10^{-6} mol dm^{-3} in $CHCl_3$ is shown in Figure 4. In the absence of **7b**, the fluorescence spectrum of **8** exhibited dual emission resulting from the monomer (382 and 400 nm) and the excimer (470 nm). The emission at 470 nm was assigned

Table 1. Peptide sequences of colored beads in **1** for **7a**

Compound	AA_1	AA_2	AA_3	Frequency[a]
1a	D-Leu	L-Tyr	D-Phe	14
1b	D-Leu	L-Tyr	D-Leu	4
1c	L-Tyr	L-Tyr	D-Phe	2
1d	L-Ala	L-Tyr	D-Phe	1

[a] Number of beads isolated.

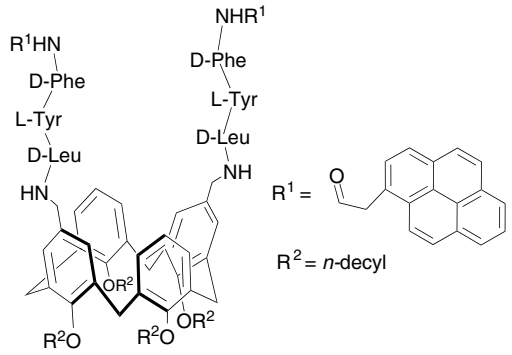


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Figure 3. Chemosensor for analyte **7b**.

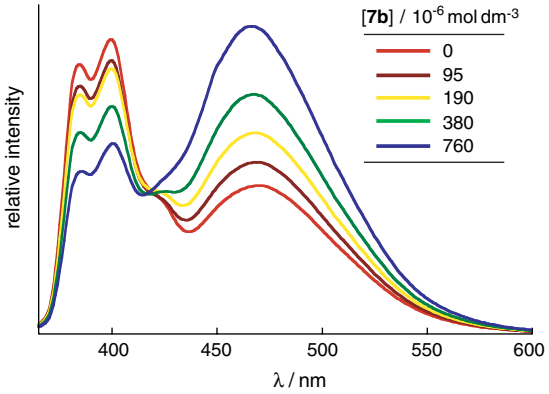

Figure 4. Fluorescence emission spectroscopic change of **8** in $CHCl_3$ upon addition of peptide **7b** at 20 °C. $[\mathbf{8}] = 1.0 \times 10^{-6}$ dm^{-3}. Excitation wavelength: 344 nm.

to the intramolecular excimer in this concentration.[14] The monomer emission was dominant compared with the excimer emission. The addition of analyte **7b** to the solution of **8** enhanced the fluorescence of excimer. In contrast, the intensity of the monomer emission decreased depending on the concentration of the analyte.[15] This result indicated that the analyte **7b** brings the two pyrenyl groups of **8** closer together. The spectral change was quite different from a previously reported lower rim-modified peptidocalixarene sensor, which enhances the fluorescence of both the monomer *and* excimer emission, depending on the concentration of **7b**. Both results were different from the sodium ion-selective fluorescence-labeled calixarene sensor reported by Jin et al.[16] Studies to clarify the difference in action, sensing abilities of the other analyte are in progress.

In conclusion, we synthesized a fluorescence-labeled calix[4]arene library substituted with peptides at the upper rim. The binding selectivity of **7a** for library members was higher than that of the lower rim-modified calixarene library. Solid supported core compound **6** might serve as a useful platform for split synthesis of libraries to produce artificial receptors using not only peptides but also various types of building blocks.

Acknowledgements

This work was supported in part by a SUNBOR GRANT from the Suntry Institute for Bioorganic Research and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (No. 15608002).

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11. Protective groups on the side chains were cleaved by treating twice with 5% iPr$_3$SiH–1% TFA/CH_2Cl_2 (v/v) at room temperature for 1 h.
12. *N*-Moz groups were cleaved by treating twice with 1% TFA/CH_2Cl_2 (v/v) in the presence of PhSH (30 equiv) at room temperature for 1 h.
13. Analyte **7b** possesses a palmitoyl group at the N-terminal (R) instead of the dye moiety in **7a**. The N-terminal was changed from the dye to the palmitoyl group because the fluorescence emission of **8** overlapped with the absorption of **7a**.
14. The ratio (monomer/excimer) was not affected by the concentration of **8** in the range of 10^{-6} to 10^{-8} mol dm^{-3}. The result indicates that the emission at 470 nm was assigned to an intramolecular excimer.
15. The association constant (K_{ass}) of **5** against peptide **2b** was estimated to be about 3.0×10^3 mol dm^{-3} from the eximer fluorescence intensity using the Benesi–Hildebrand plot[17].
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