Pergamon

Tetrahedron Letters 40 (1999) 6821–6825

TETRAHEDRON LETTERS

Peptide library based on calix[4]arene

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Received 16 June 1999; revised 5 July 1999; accepted 9 July 1999

Abstract

A calix[4]arene library consisting of ca. 50 000 members was synthesized using 15 amino acids. Screening of the library for binding dye-labeled oligopeptides indicated that some peptidocalix[4]arenes selectively bind specific oligopeptides.

Keywords: calixarenes; peptides; combinatorial chemistry; molecular recognition.

Calixarenes are useful building blocks in supramolecular chemistry. A number of calixarene derivatives have been synthesized to search for host molecules that tightly and selectively bind to the target substrate.[1] Remarkable progress has been achieved in this decade. Calixarene-based receptors were found to form complexes with not only metal or simple organic ions but also a variety of organic compounds.[2] Furthermore, calixarene derivatives can be used as more sophisticated molecules, for example, they are used as chemical sensors,[3] enzyme mimetics,[4] and allosteric molecules.[5] Despite their potential use in supramolecules, desired receptors are sometimes difficult to access because of their design. On the other hand, applications of combinatorial chemistry are now widespread. In the field of molecular recognition, combinatorial libraries are a powerful tool for investigation of host–guest interaction.[6] A number of ligand libraries were screened for receptors to examine their binding abilities and substrate selectivity. In contrast to ligand libraries, only a few receptor libraries have been reported.[7] Still and co-workers created peptidosteroid libraries that consist of 10 000 members.[7a,7b] These libraries have been screened against enkephalins. Some peptidosteroids not only bind enkephalins but also distinguish between closely-related peptide sequences. In this communication, we report the construction of a peptide library based on calix[4]arene that provides access to the desired receptors for target molecules.

Peptide libraries designed based on calix[4]arene are shown in Fig. 1. To create a library by encoding split synthesis on a solid support, calixarenes were attached to polystyrene resin at the upper-rim through a methylene chain. Two acetylated dipeptides were used as substrate binding sites suspended from the distal (1,3) position at the lower-rim of the calix[4]arene scaffold through an aminoethylated acetamide linkage. Because 15 amino acids are used as building blocks, this library consists of 15^4=50625 peptidocalixarenes. As lower-rim modifications by amide groups have a strong affinity for alkali metal

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0040-4039/99/$ - see front matter
PII: S0040-4039(99)01368-4

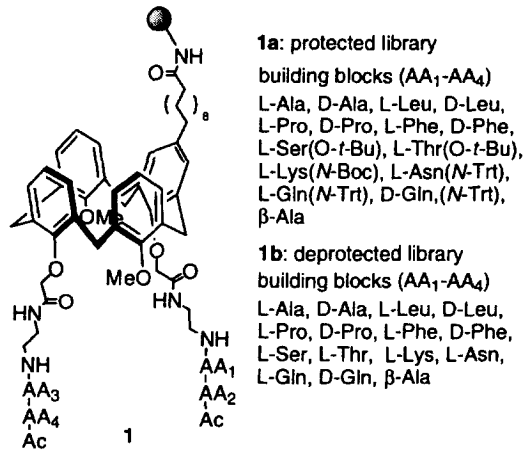
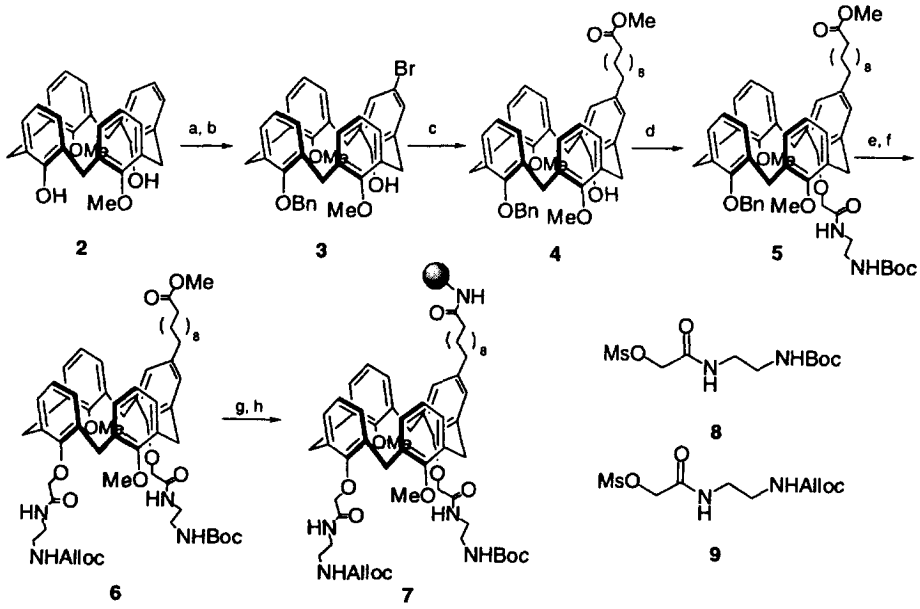


Figure 1. Designed peptide libraries based on calix[4]arene

cations, these library members are designed to act as allosteric molecules regulated by alkali metal cations.

Core-compound **7** was prepared according to Scheme 1. Selective benzylation[8] and bromination of dimethylcalix[4]arene[9] **2** afforded **3**, which was subjected to Suzuki–Miyaura coupling with a boran prepared by hydroboration of methyl 10-undecenoate to produce **4** in high yield. The remaining phenolic OH was alkylated with *N*-Boc-protected mesylate.[8] After removal of the benzyl group, the resulting phenol was alkylated again with *N*-alloc-protected mesylate. [9] Finally, the methyl ester in **6** was hydrolyzed and the resulting carboxylic acid was condensed with aminomethyl polystyrene resin in order to prepare the peptide library on a solid support.



Scheme 1. Reagents and conditions: (a) BnBr, BaO, $Ba(OH)_2 \cdot 8H_2O$, DMF, 73%; (b) $HBr_3 \cdot Py$, MeOH, 73%; (c) Methyl 10-undecenoate, 9-BBN, then **3**, $PdCl_2(dppf)$, NaOMe, THF, reflux, 87%; (d) **8**, NaH, DMF, 73%; (e) H_2, $Pd(OH)_2$, EtOH, 81%; (f) **9**, NaH, DMF, 80%; (g) NaOH, H_2O, DMF 98%; (h) aminomethyl polystyrene resin, HOBt, disopropylcarbodiimide, CH_2Cl_2

Table 1
Dye-labeled oligopeptides

Dye-linker[a]-NH-R^1-R^2-R^3-R^4-R^5-CONH-*n*Bu

oligopeptide	R^1	R^2	R^3	R^4	R^5
10	L-Phe	L-Pro	L-Leu	—	—
11	L-Phe	L-Thr	L-Asn	—	—
12	L-Phe	L-Lys	L-Asn	—	—
13	L-Tyr	L-Gly	L-Gly	L-Phe	L-Leu

[a]Dye-linker :

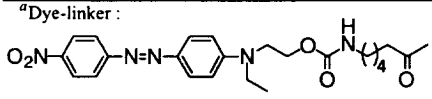


Table 2
Binding assay of libraries with oligopeptides

entry	oligopeptide	protected library	deprotected library
1	**10**	no binding	no binding
2	**11**	no binding	no binding
3	**12**	no binding	**some beads stained color**
4	**13**	**a few beads stained color**	half of beads stained color

A generation of the encoded peptide library was produced according to Still's method using halo aromatic tags.[10] After removal of the *N*-Boc group, a split synthesis was run on one side of the arms to append a dipeptide using 15 Fmoc-amino acids as building blocks (Fig. 1). After acetylation of the *N*-terminal in the dipeptide, the *N*-alloc group on the other arm was removed by a palladium catalyst. Again, combinatorial synthesis of the dipeptide and acetylation was performed. Side chains of the members in this library were protected (protected library: **1a**). Compound **1a** was treated with trifluoroacetic acid to remove the protective groups to obtain the deprotected library (**1b**).

To test the binding ability and selectivity of these libraries, four types of dye-labeled peptides **10–13** were prepared, shown in Table 1. Approximately 30 μM of dye-labeled peptide was incubated with ca. 30 mg (ca. 10^5 beads) of the side chain protected or deprotected libraries on the beads in $CHCl_3$ for 3 days. The results are summarized in Table 2. In the screening of the side chain-protected library for binding to tripeptides **10–12**, no beads were stained, signifying that no library members bind these tripeptides. In the binding assay of the side chain deprotected library with tripeptide **10** and **11**, there were no colored beads. When the L-Thr residue in **11** was replaced with L-Lys (entry 3), some beads stained a deep red color. This result suggests that the amino group has an important role in binding the substrate. In screening of the deprotected library for dye-labeled ^{5}Leu enkephalin derivatives (**13**), almost half of the beads turned orange to red following incubation (entry 4). As a pentapeptide has more hydrogen bond donors and acceptors than a tripeptide, the selectivity of the deprotected library for binding to the pentapeptide should be reduced. In contrast, the selectivity was dramatically increased when the protected library was used. Only a few beads turned red after equilibration. These results indicated that the balance of hydrogen bonding between the receptor and ligand is very important for highly selective binding.

All colored beads were isolated and decoded to identify their amino acid sequences. The peptide

Table 3
Peptide sequences of colored beads in **1b** for **12**

compound	AA_1	AA_2	AA_3	AA_4	frequency[a]
14	L-Thr	L-Ser	L-Thr	L-Ser	4
15	L-Thr	L-Ser	L-Thr	L-Thr	3
16	L-Thr	L-Thr	L-Thr	L-Ser	2
17	L-Ser	L-Ser	L-Thr	L-Thr	1
18	L-Thr	L-Ser	L-Ser	L-Ser	1
19	L-Asn	L-Ser	D-Pro	L-Pro	1
20	L-Asn	D-Pro	L-Asn	L-Ser	1
21	D-Gln	D-Pro	D-Thr	D-Pro	1
22	D-Gln	D-Pro	D-Gln	D-Ala	1
23	L-Leu	L-Ala	L-Gln	L-Thr	1
24	L-Lys	L-Gln	D-Phe	L-Leu	1

[a] Number of beads isolated.

Table 4
Peptide sequences of colored beads in **1a** for **13**

compound	AA_1	AA_2	AA_3	AA_4	frequency[a]
25	L-Gln (*N*-Trt)	L-Leu	L-Asn (*N*-Trt)	L-Leu	2
26	L-Asn (*N*-Trt)	L-Leu	L-Gln (*N*-Trt)	L-Leu	2
27	L-Asn (*N*-Trt)	L-Thr (*O*-*t*-Bu)	L-Gln (*N*-Trt)	L-Leu	1
28	L-Gln (*N*-Trt)	L-Gln (*N*-Trt)	L-Asn (*N*-Trt)	L-Leu	1
29	L-Gln (*N*-Trt)	L-Lys (*N*-Trt)	L-Asn (*N*-Trt)	L-Ser (*O*-*t*-Bu)	1

[a] Number of beads isolated.

sequences of the colored beads in the deprotected library screened against **12** are shown in Table 3. L-Thr and L-Ser were frequently observed (compound **14–18**). As the linkers to the solid support in peptidecalixarenes do not participate in binding, the sequences in **15** and **16** are basically the same. The hydroxy groups of L-Thr and L-Ser could take part in binding **12** because no colored beads were observed when the corresponding protected library was used. On closer inspection of the sequences in **14–18**, binding to L-Thr was preferred over L-Ser as AA_1 and AA_3, and L-Ser was preferred over L-Thr as AA_2 and AA_4. Compound **12** roughly distinguishes between L-Ser and L-Thr. In assays of the protected library for **13** (Table 4), L-Gln (*N*-Trt) and L-Asn (*N*-Trt) were found in AA_1 and AA_3 of all colored beads. **25** and **26** are considered the same sequences. Although L-Leu was found frequently in AA_2 and AA_4, the selectivity was not as high. We estimated the binding constant of the solid-supported peptidocalixarenes **14** and dye-labeled peptides **12** using UV-monitored titration.[11] The constant was approximately 2×10^4 mol/l. Finally solid support free **14** was synthesized in solution phase to characterize the host–guest complex by NMR experiment. But solid support free **14** was too insoluble in $CDCl_3$ to evaluate the fashion of their complex. Synthesis of more soluble derivatives is in progress.

In summary, a peptide library based on calix[4]arene, consisting of ca. 50000 members was synthesized. To our knowledge, this is the first report of such a large calixarene-based library. Some peptidocalixarenes that bind dye-labeled oligopeptides were observed in the library. Receptors found

in the library can be extended to higher functional molecules by applying the accumulated results in calixarene chemistry.

Acknowledgements

This work supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (no. 11771405)

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