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Development of calixarene-based host molecules for peptides in aqueous media

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Abstract—We synthesized a peptidocalix[4]arene library consisting of 1000 members that was suitable for peptide recognition in aqueous media. Some peptidocalix[4]arenes in the library were host molecules for guest peptides. Electrostatic interaction between the host and guest molecules was the most important factor for binding in aqueous media.

Peptide recognition is an essential biological process and oligopeptides are important targets.[1–3] Development of synthetic receptors for specific amino acid sequences under physiological conditions is a challenging task for researchers in the field of molecular recognition.[4] One of the keys to specific recognition is the ingenious construct of hydrogen bonding and ionic interaction between the host and guest molecules. Although a large number of receptors for peptides have been reported in organic solvents, the success of specific recognition in aqueous media is still limited because these interactions are very weak in aqueous solution.[5]

Calixarenes are widely used as a platform for artificial receptors.[6] Their potential to function as receptors depends on the modification of the core calixarene. Previously, we synthesized peptidocalix[4]arene libraries consisting of 3500–50000 members that had various peptides substituted at the lower and upper rims.[7,8] In those studies, we demonstrated that the split synthesis of libraries based on calixarenes is an attractive approach for discovering the new host molecules. These binding studies, however, were performed in $CHCl_3$ solution. Here, we report the synthesis of a peptidocalix[4]arene library suitable for binding peptides in hydrophilic media and for binding studies of the library for some peptides in aqueous solutions.[9]

Peptide libraries designed to discover the host molecule in aqueous media are shown in Figure 1. The upper rim of the calix[4]arene was substituted with four tetrapeptides. Ten amino acids were chosen as building blocks, which mainly consist of polar, acidic, or basic amino acids to enhance the hydrophilicity of the host molecule. Amphiphilic resin was chosen as a solid support to synthesize the library in organic media and to screen with peptides in aqueous media.

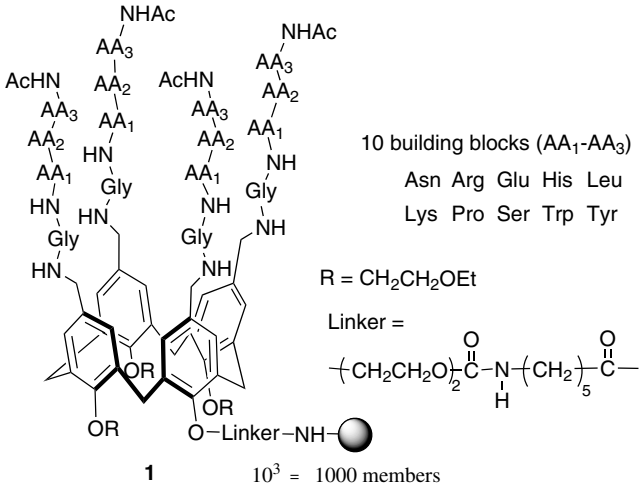

Figure 1. Hydrophilic peptidocalix[4]arene library.

Keywords: Calixarene; Combinatorial library; Recognition in aqueous media.
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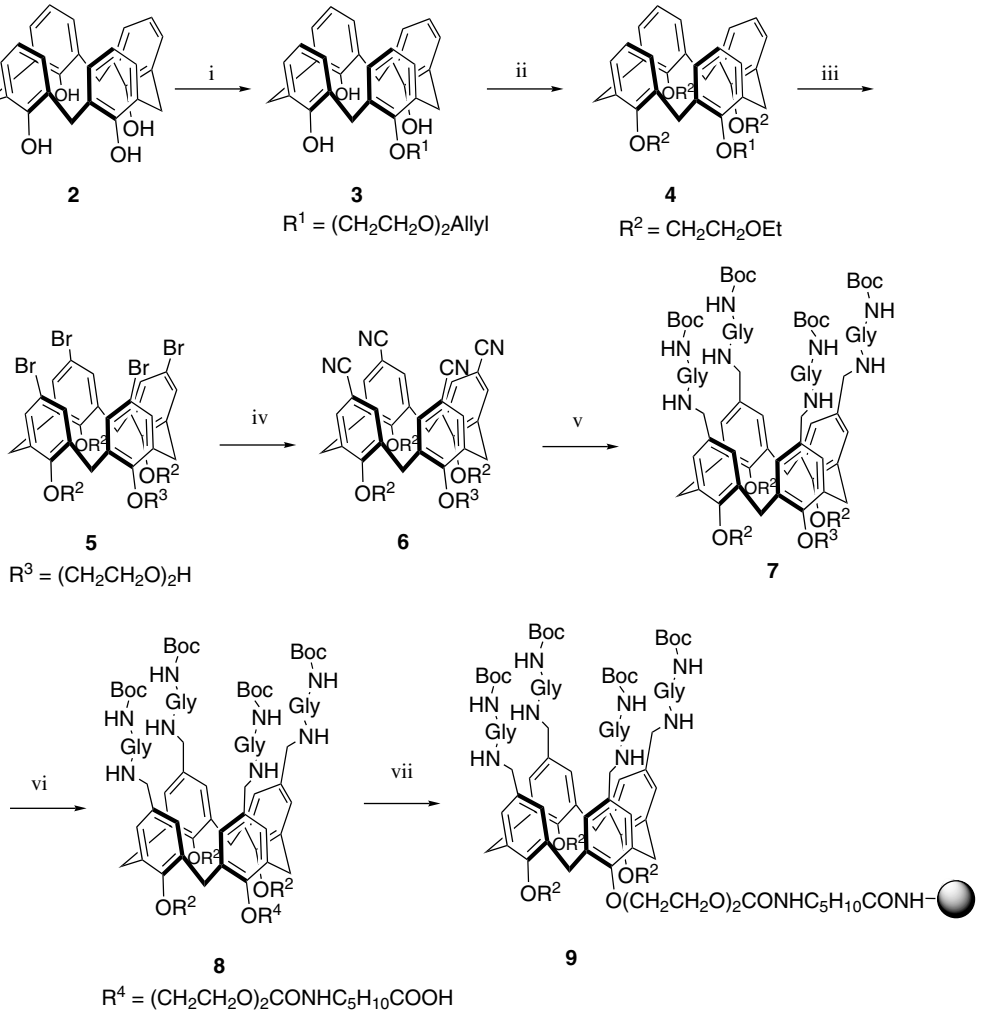
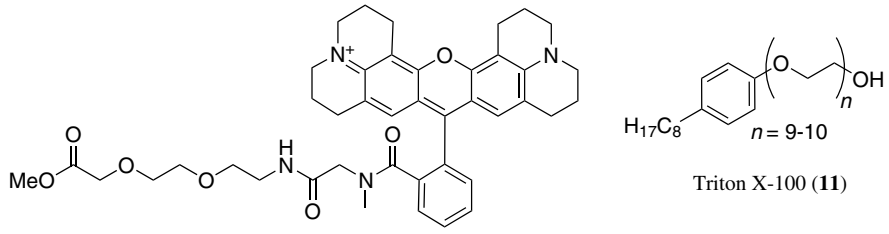
Core-compound **9** was prepared according to Scheme 1. Starting from calix[4]arene **2**, one phenolic hydroxyl group was alkylated to give allyloxyethoxyethyl ether **3** and the remaining phenolic alcohols were converted to ethoxyethyl ether. After deprotection of the ally group, the upper rim was brominated to produce lower rim-alkylated tetrabromocalix[4]arene **5**. Palladium-catalyzed cyanation[10] of **5** was achieved in good yield. The cyano group on **6** was reduced by alane[11] and the resulting tetraamine was condensed with Boc-glycine to give **7**. The hydroxy group at the lower rim was coupled with benzyl 6-aminohexanoate via alkoxycarbonylimidazole to install a linker for solid phase synthesis. Finally, the benzylester was hydrogenated and the resulting carboxylic acid was condensed with aminomethylated NoveSyn™ TG resin[12] (110 μm beads, 0.3 mmol/g NH_2) to prepare the peptide library on a solid support.

The peptide library was produced using a split encoding method based on a previously reported procedure.[7,8] After removal of the *N*-Boc groups in **9**,[13] three steps of an encoded split/pool combinatorial synthesis[14] were run on the upper rim to append four tripeptides (total tetrapeptide including glycine as the first common amino acid) using 10 Fmoc-amino acids as building blocks. Because 10 amino acids were used as building blocks, this library consisted of $10^3 = 1000$ peptidocalixarenes. After removal of the N-terminal Fmoc group, the resulting amino group was acetylated. Finally, **1** was obtained by trifluoroacetic acid treatment to remove the protective groups on the side chain of the peptides.[15]

Some dye-labeled tripeptides were prepared to test the binding abilities for the library members in water. Certain tripeptide sequences bind simple dye molecules selectively in water.[16] We selected the fluorescent dye



Scheme 1. Synthesis of core-compound **9** of library **1**. Reagents and conditions: (i) $TsO(CH_2CH_2O)_2Allyl$, K_2CO_3, CH_3CN, 140 °C, 70% based on recovered **2**; (ii) $BrCH_2CH_2OEt$, NaH, DMF, rt, 45%; (iii) 1. Pd/C, TsOH, EtOH/H_2O, 10/1, 80 °C; 2. $HPyBr_3$, $CHCl_3$/MeOH, 10/1, 0 °C then rt, 92% (two steps); (iv) $Zn(CN)_2$, DPPF, $Pd_2(dba)_3$, DMF, 130 °C, 100%; (v) 1. AlH_3, Et_2O, 0 °C; 2. $BocNHCH_2COOH$, HOBt, EDC, CH_2Cl_2, rt; 3. K_2CO_3, MeOH/H_2O, 10/1, 99% (three steps); (vi) 1. *N*,*N*′-carbonyldiimidazole, DMAP, CH_2Cl_2, rt; 2. $BnOCO(C_6H_{12})NH_2$, DIEA, CH_2Cl_2/DMF, 80 °C; 3. H_2, $Pd(OH)_2$ Dioxane, rt, 77% (three steps); (vii) aminomethylated Nove Syn™ TG resin (loading: 0.3 mmol/g), DIC, HOBt, CH_2Cl_2; Abbreviations: DMF = *N*,*N*′–dimethylformamide, Ts = *p*-toluenesulfonyl, Py = pyridine, dba = dibenzylideneacetone, DPPF = 1,1-bis(diphenylphosphino)ferrocene, EDC = *N*-ethyl diisobutylcarbodiimide, HOBt = 1-hydroxybenzotriazole, DMAP = 4-dimethylaminopyridine, DIEA = diisopropylethylamine.



10

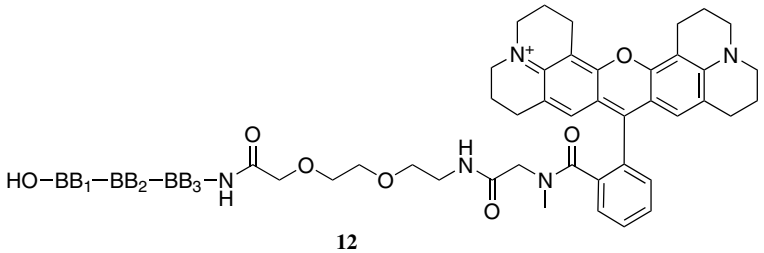
Triton X-100 (**11**)

Figure 2. Structures of dye-linked methylester (control compound) and Triton X-100.

rhodamine 101 over other dyes, because this dye has no significant interaction with specific peptides in water.[4f] Prior to the binding studies of the library, a dye-linked ester **10**, which has no peptides was screened with the peptidocalixarene library **1** to investigate label-induced artifacts. Unexpectedly, almost 10% of the beads were fluorescence-active in pH 6.86 phosphate buffer. This result indicates that a large number of peptidocalixarenes bind the dye. To suppress the interaction, we investigated the use of the non-ionic surfactant Triton X-100 (**11**), which solubilizes membrane proteins. Following the addition of the Triton X-100 (5% final concentration), the fluorescent beads disappeared, indicating that the interaction was suppressed. Therefore, screening of the library **1** for binding rhodamine 101-labeled tripeptides was run in pH 6.86 phosphate buffer containing 5% Triton X-100 (Fig. 2).

Six different kinds of peptides **12** were prepared by solid phase synthesis. The results of the screening are shown in Table 1.[17] A neutral peptide **12a** and a peptide **12b** containing one Asp residue did not bind any library members. On the other hand, some library members were bound to a tripeptide **12c** that contained two Asp residues, but the selectivity of the library members was not very high. Fourteen relatively bright fluorescent beads were isolated and their peptide sequences were analyzed (Table 2).[18] The sequences of all the beads contained one or two Arg residues and the remaining amino acid residues contained mainly aromatic amino acids (Trp or Tyr). A tripeptide **12d** containing one Lys residue, which is a basic amino acid, also bound to specific library members. Its sequence selectivity was higher than that of **12c**. All fluorescent library members had one or two Glu residues and aromatic amino acid residues (Trp or Tyr). There were brighter beads in the screening of **12e**, which has a Lys residue instead of a Tyr residue at BB_3 in **12d**. The sequences on the active beads mainly consisted of two or three Glu and an aromatic amino acid. The order in the peptide sequences of the guest molecules was changed from **12e** to **12f**. Three host molecules had common peptide sequences for binding **12e** and **12f** (Table 2, entries 1, 2, 4, and 5 for screening with guest **12f**). When two Lys residues in **12e** were replaced with Arg (**12g**), the binding selectivity for the library members was slightly reduced. Almost half of the active beads had common sequences for binding **12e** and **12g**. The library members that were found in the screening of these basic peptides, resembled each other but some host molecules had a unique peptide sequence. The most striking feature in all the screenings was that acidic library members prefer basic guest peptides and basic library members prefer acidic peptides. These findings suggested that the binding nature in water is dominated by electrostatic interactions.[19] Interestingly, acidic guest **12c** preferentially binds hosts with a basic Arg residue, but not Lys or His. This finding indicates that **12c** clearly distinguishes between these basic amino acid residues. In all binding assays, Tyr and Trp frequently appeared in addition to acidic or basic amino acid residues. The reason for the preference of aromatic residues on the library members is not clear.

Table 1. Structure of rhodamine 101-labeled guest **12** and screening results with a peptidocalix[4]arene library



12

Compound	BB_1	BB_2	BB_3	Screening result
12a	Ser	Ser	Tyr	No binding
12b	Ser	Asp	Tyr	No binding
12c	Ser	Asp	Asp	Selective binding
12d	Ser	Lys	Tyr	Selective binding
12e	Ser	Lys	Lys	Selective binding
12f	Lys	Ser	Lys	Selective binding
12g	Ser	Arg	Arg	Selective binding

Table 2. Peptide sequences of library members binding to guest tripeptide **12c–g**

Entry	AA_1	AA_2	AA_3	Freq.[a]
Guest **12c**				
1	Trp	Trp	Arg	3
2	Tyr	Tyr	Arg	3
3	Trp	Tyr	Arg	2
4	Tyr	Trp	Arg	2
5	Arg	Trp	Arg	1
6	Arg	Leu	Arg	1
7	Tyr	Arg	Arg	1
8	Tyr	Arg	Tyr	1
9	Leu	Arg	Trp	1
10	Arg	Tyr	Tyr	1
11	Trp	Arg	Trp	1
12	Leu	Trp	Arg	1
Total				18
Guest **12d**				
1	Tyr	Glu	Trp	5
2	Trp	Glu	Trp	3
3	Glu	Glu	Trp	2
4	Trp	Glu	Tyr	2
5	Tyr	Glu	Tyr	1
6	Glu	Trp	Tyr	1
7	Trp	Tyr	Glu	1
Total				15
Guest **12e**				
1	Glu	Glu	Trp	4
2	Trp	Glu	Glu	4
3	Glu	Glu	Glu	2
4	Glu	Tyr	Glu	2
5	Glu	Tyr	Tyr	2
6	Glu	Glu	Leu	2
7	Tyr	Glu	Glu	1
8	Tyr	Glu	Trp	1
9	Glu	Glu	Trp	1
10	Glu	Trp	Glu	1
Total				20
Guest **12f**				
1	Glu	Glu	Trp	3
2	Glu	Trp	Glu	3
3	Tyr	Glu	Tyr	2
4	Trp	Glu	Glu	1
5	Tyr	Glu	Glu	1
6	Trp	Glu	Tyr	1
7	Trp	Ser	Glu	1
8	Tyr	Ser	Glu	1
Total				13
Guest **12g**				
1	Trp	Glu	Glu	5
2	Tyr	Tyr	Glu	4
3	Tyr	Glu	Trp	2
4	Tyr	Glu	Tyr	2
5	Glu	Tyr	Trp	2
6	Tyr	Glu	Glu	1
7	Glu	Trp	Tyr	1
8	Glu	Glu	Trp	1
9	Trp	Glu	Tyr	1
10	Leu	Glu	Trp	1
11	Trp	Tyr	Glu	1
Total				21

[a] Number of beads having the indicated sequence.

In summary, we synthesized a four-armed hydrophilic peptidocalix[4]arene library consisting of 1000 members. Studies of the screening conditions in water revealed that a neutral surfactant reduces the interaction between fluorescent dye and specific peptides. Electrostatic interaction between the host and guest molecules is the most important factor for binding in the aqueous media. Further binding studies directed toward highly selective host molecules and investigation of the binding mode of action are in progress.

Acknowledgement

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11. Reduction by $LiAlH_4$ did not give the corresponding tetraamine.
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15. Protective groups on the side chains were cleaved by treating twice with $H_2O/i Pr_3SiH$/TFA, 1/1/30 at room temperature for 1 h.
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17. The screening conditions were as follows: 5 mg of the solid supported library (ca. three copies for each sequence) was mixed in a 0.6 mL Eppendorf tube with 0.6 mL of a guest peptide (5 μmol L^{-1}) in pH 6.86 phosphate buffer containing 5% Triton X-100. After agitation for 24 h, red colored beads were isolated manually under low-power microscope.
18. Peptide sequence on colored beads was identified as follows: Tag release was modified from the original procedure (Ref. 14). A single selected bead was placed in a capillary tube and washed with DMF. The bead was then suspended in 1.5 μL of 6 M NaOH aqueous solution and 3 μL of hexane. The capillary was sealed and placed in a water bath at 60 °C for 0.5 h to release the tag alcohols. The tube was subsequently sonicated in an ultrasonic bath for 30 min at room temperature to extract the tag into hexane. The tube was opened and the hexane phase was mixed with bis(trimethylsilyl)acetamide (ca. 0.1 μL). The tag solution was injected directly into an electron capture detector, capillary gas chromatograph for analysis. The intensity of the resulting peaks produced by this alkaline hydrolysis was increased ca. 50% compared with original photolysis.
19. For electrostatic interaction of host–guest complex in water, see: Ref. 5.