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HIGHLY SEQUENCE SELECTIVE NONMACROCYCLIC TWO-ARMED RECEPTORS FOR PEPTIDES

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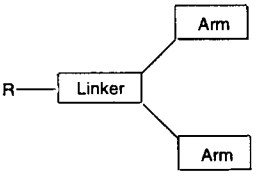
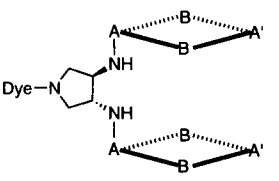
Abstract: Simple nonmacrocyclic two armed receptors have been synthesized to create a new class of sequence-selective receptors for peptides. Screening several examples of these simple compounds against a 24,389-member library of N-acetyl tripeptides revealed novel binding properties.

Much progress has been achieved in the effort to design simple receptors for the sequence-selective binding of peptides.[1] Our laboratory previously described the synthesis and screening of several highly selective two-armed receptors for which the basic design is a conformationally restricted "linker" covalently linking two functionalized "arms" from a cavity in which complementary pairs of a substrate is bound.[2] By varying linkers and arms combinatorially, two-armed receptor libraries can be readily prepared.[3]

One of our most successful designs is the receptor **1**, where a dye labeled pyrrolidine linker is used to orient two macrocyclic "arms".[2a] This oligomeric receptor displayed remarkable selectivity upon screening against one of our combinatorial tripeptide libraries. Out of the possible 3375 members in the library this receptor showed a distinct binding affinity toward just two of the members: (D)Pro-(L)Val-(D)Gln and (L)Lys-(L)Val-(D)Pro. However, subsequent attempts to alter the binding affinity of the receptor significantly have met with limited success as the various derivatives examined have shown a proclivity for binding (L)Val-containing peptides. Furthermore, the low yields that often characterize macrocyclizations coupled with the slow work in creating novel derivatives makes design of **1** less desirable than it could be when considering the task of creating a library of receptors.



Two-Armed Receptor



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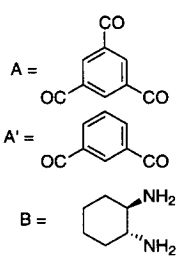

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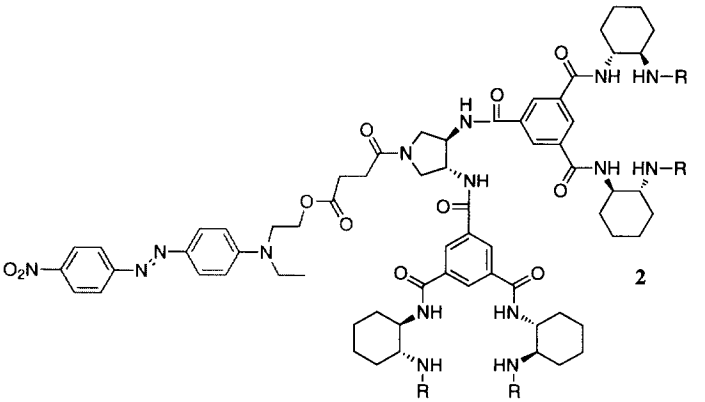
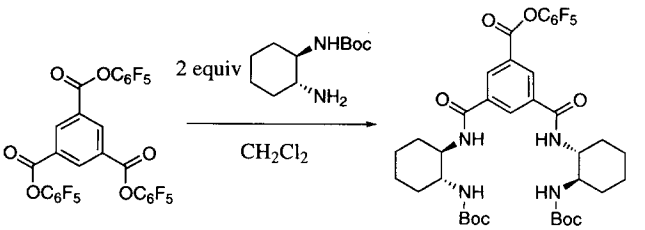
In this communication we describe a novel type of two-armed receptor **2** in which the macrocycles are replaced by more flexible and more easily derivatizable "open arms". As we will show, this type of receptor shows unique binding selectivities. Furthermore, these binding selectivities can be altered easily by changing the nature of R.



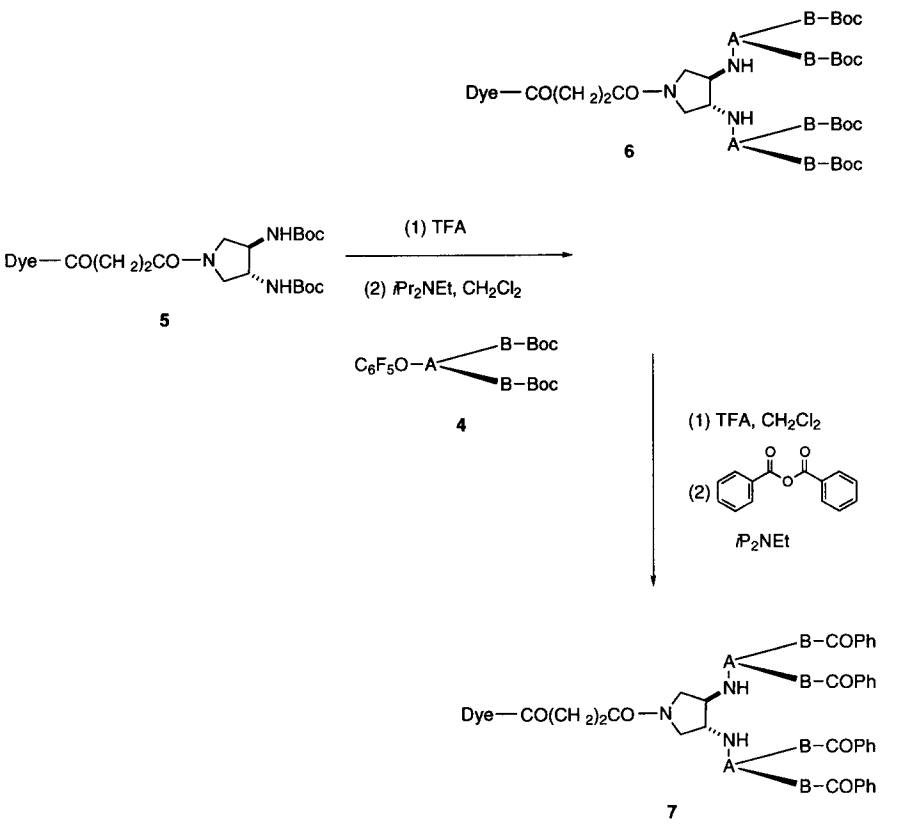
2

Synthesis of the receptor is very straightforward. The "open arm" portion **4** is made in one step upon simple addition of the mono-Boc protected (*R,R*)-diamino cyclohexane to the *tris* activated trimesic acid **3** in 44% yield.

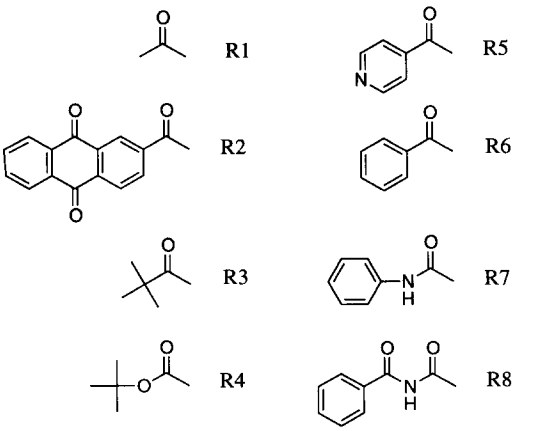


3 **4**

The arm is then coupled to the deprotected pyrrolidine-dye linker **5**.[2a] Finally, the Boc groups of **6** are removed and the desired capping group R is added. In the benzoyl case, the final receptor (**2**, R = COPh) was synthesized in 61% overall yield as outlined below.



Because of the branched linear structures of these open armed receptors, simply varying the final capping group allowed eight different receptors to be conveniently prepared.



Each of the receptors generated were then screened at low concentrations against an encoded tripeptide library of 24,389 sidechain-protected tripeptides of the form Ac-AA3-AA2-AA1-NH$(CH_2)_5$CONH-polystyrene.[4] Approximately four copies of the tripeptide library (1.5 mg or 100,000 individual beads) were equilibrated for three days with a dilute solution of the receptor. After this time only a few beads had acquired the red color of the dye indicating selective binding of the peptide to the receptor. The red beads were picked and decoded by electron capture gas chromatography.[5]

As Table 1 shows, the various receptors generated by using capping groups R1–R8 displayed a variety of binding selectivities. The concentration of the unbound receptor at equilibrium is shown in parenthesis and sequences from beads of significantly diminished color are allocated to successive tiers. Tier 2 and tier 3 represent dark orange and orange beads respectively. In the R6 case, for example, the assay conditions allowed us to distinguish between Gly-(D)Ala-(L)Pro and Gly-(D)Lys-(L)Pro which appeared to bind with slightly less affinity. Although these beads were not the darkest beads they still were significantly darker then the general background. The Boc-protected receptor showed selectivity only for the two sequences (D)Lys-(D)Val-(L)Pro and (D)Lys-(D)Gln-(L)Pro, whereas the acetyl-capped receptor showed selectivity only for the tripeptide sequence (D)Val-(L)Asn-(D)Val. Furthermore, the receptor capped with R8 showed no binding at all. Thus we were able to create peptide receptors having a variety of binding affinities merely by switching the final capping group on the receptor.

Table 1. Sidechain-protected peptide sequences bound by receptor **2**.

R	AA3	AA2	AA1	# of beads picked
R1 (56 μM)	D-Val	L-Asn	D-Val	5
R3 (339 μM)	x	L-Pro	L-Pro	12
R4 (132 μM)	D-Lys	D-Val	L-Pro	3
	D-Lys	D-Gln	L-Pro	1
R5 (28 μM)	D-Val	L-Asn	L-Asn	5
	Gly	D-Ala	L-pro	3
	Tier 2			
	Gly	D-Lys	L-Pro	3
	L-His	L-Gln	L-Pro	2
	D-Asn	L-Gln	L-Pro	3
R6 (113 μM)	Gly	D-Ala	L-Pro	6
	Gly	Gly	L-Pro	1
	Tier 2			
	Gly	D-Lys	L-Pro	5
R7 (57 μM)	x	L-Pro	L-Pro	23

Receptor **2** also bound sidechain-deprotected tripeptide sequence selectively as tabulated above. To ensure more reproducible results the deprotected library was screened in the presence of trioctylamine (10 equiv) and trifluoroacetic acid (5 equiv) to buffer the solution. Although the changes in selectivity were not as pronounced as in the sidechain-protected case, we were still able to see some alterations in the selectivity upon varying the capping group (Table 2). The most marked example would be the strong selectivity of the benzoyl-capped receptor (R6) for Gly-(D)Ser-(L)Pro while the urea-capped receptor (R7) showed an affinity for (D)Asp-(L)Pro-(L)Pro.

Table 2. Sidechain-deprotected peptide sequences selectively bound by receptor **2**.

R	AA3	AA2	AA1	# of beads picked
R1(33 μM)	L-Arg	D-Ala	L-Pro	4
	Tier2			
	Gly	D-Ser	L-Pro	1
R2(146 μM)	L-Arg	D-Ala	L-Pro	4
R3(270 μM)	Gly	D-Ser	L-Pro	2
R4(133 μM)	L-Arg	D-Ala	L-Pro	2
R5(38 μM)	L-Arg	D-Ala	L-Pro	2
	Tier 2			
	L-Arg	D-Leu	L-Pro	2
	Gly	D-Ser	L-Pro	3
	Tier 3			
	Gly	D-Ala	L-Pro	4
	L-Arg	D-Ser	L-Pro	1
R6(86 μM)	Gly	D-Ser	L-Pro	2
	Tier 2			
	Gly	D-Ala	L-Pro	2
	Tier 3			
	Gly	D-Gln	L-Pro	2
R7(57 μM)	L-Glu	L-Pro	L-Pro	1
	D-Asp	L-Pro	L-Pro	2

These results indicate that an open armed receptor analogous to **1** can bind both sidechain-protected and deprotected tripeptides with significant sequence selectivity. Furthermore, the particular peptide sequences preferentially bound can be altered simply by changing the nature of the capping group in receptors such as ours allowing for the ready generation of a large number of receptors with a range of binding selectivities. It is likely that chiral amino acids could also be used at position R allowing for the generation of an even larger number of unique receptors.

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