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MACROCYCLIC TRIAMINES AS LINKERS IN TWO-ARMED RECEPTORS FOR PEPTIDES

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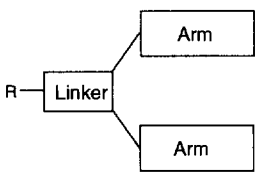
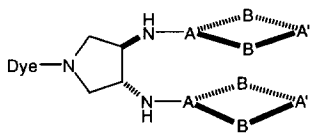
Abstract: Commercially available triazamacrocycles have been substituted with trimesic acid/1,2-diamine cyclooligomers to create a new class of sequence-selective receptors for peptides. Screening of these compounds against a 3375-member library of N-acetyl tripeptides revealed novel peptide-binding properties. Copyright © 1996 Elsevier Science Ltd

Significant progress has been made recently in the design and preparation of sequence-selective synthetic receptors for peptidic substrates.[1] Some such receptors have the complex, cage-like structures that characterize typical host molecules, but a different and particularly simple structural motif has also emerged as frequently having sequence-selective peptide-binding properties. We describe one subset of such molecules as "two-armed receptors" and its general structure diagrammed below. In this structure, the *linker* is typically a conformationally restricted moiety that covalently links and directs two functionalized, substrate-binding *arms* toward one another to form a binding cleft. The arms are typically oligomeric structures that carry functionality appropriate for binding desired substrates. In our experience, the best arms are conformationally restricted in some way (e.g. macrocyclic)[2] though flexible (e.g. linear)[3] oligomers can also be used. By varying linkers and arms combinatorially, two-armed receptor libraries can be readily prepared.[3a,d,e]

One of the first conformationally restricted two-armed receptors we studied is **1**.[2a] Here a dye-substituted pyrrolidine diamine functions as linker and AB_2A' macrocycles represent the arms. By screening this receptor using a combinatorial library of tripeptide substrates, it was found that **1** had a strong preference for binding only two of the 3375 different tripeptide sequences in the library: (D)Pro-(L)Val-(D)Gln and (L)Lys-(L)Val-(D)Pro. Since then we have prepared many derivatives of **1** in which the AB_2A' macrocyclic arms were varied in an attempt to change its sequence selectivity. However, unless substantially different arms were used, the corresponding receptors retained a high preference for (L)Val-containing peptides.



Two-Armed Receptor



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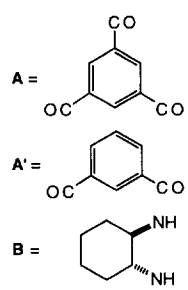
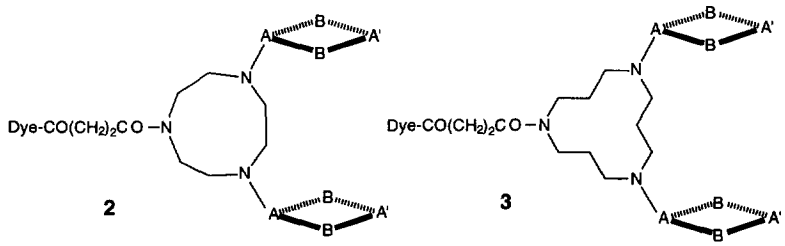
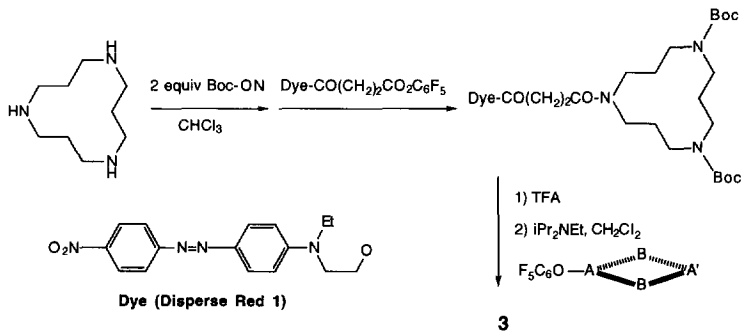


In this communication, we describe new two-armed receptors (**2** and **3**) having the diaminopyrrolidine linker of **1** replaced by commercially available macrocyclic triamines. As we will show, these receptors are also highly sequence-selective binders of peptides and the consensus sequences they preferentially bind no longer contains Val.



Synthesis of both receptors was straightforward and is outlined below for **3**. Thus 1,5,9-triazacyclododecane was first diprotected and then coupled to the azo dye Disperse Red 1 using its succinate pentafluorophenyl ester.[2a] Deprotection with trifluoroacetic acid and diacylation with pentafluorophenyl-activated AB_2A' gave **3** in an the overall yield of 33%. Receptor **2** was made in an analogous fashion in 52% overall yield from di-Boc 1,4,7-triazacyclononane.[4]



The new receptors were first screened as described previously for binding against an encoded library of 3375 (15^3) sidechain-protected tripeptides of the form Ac-AA3-AA2-AA1-$NH(CH_2)_5CONH$-polystyrene.[5] Roughly four copies of the tripeptide library (~1.5 mg of beads) were equilibrated for three days with a dilute chloroform solution of **2** or **3**. After this time, <1% of the beads had acquired the red color of the dye indicating binding of the peptide on the bead to the receptor. In these assays, receptor **3** displayed higher peptide-binding selectivity with only 1 out of every ~3000 beads strongly binding the dye-labeled receptor. In contrast, receptor **2** associated strongly with 1 out of every ~1000 members of the library. The equilibrium concentrations of receptors **2** and **3** in these assays were 14 μM and 26 μM, respectively, which corresponded to binding constants on the order of 10^4. The red beads were picked and decoded by electron capture gas chromatography.[6] Tables 1 and 2 give the sequences (N→C) of the

peptides that were most tightly bound (the frequencies give the percentage of the red beads picked that had the indicated sequence).

Table 1. Sidechain-protected peptide sequences selectively bound by **2** (14 μM).

AA3	AA2	AA1	Frequency Found
(L)Asn(*N*-trityl)	(L)Pro	(D)Val, (D)Ala, (D)Gln(*N*-trityl)	38%
(D)Gln(*N*-trityl)	(D)Gln(*N*-trityl)	(D)Val, (L)Asn(*N*-trityl)	38%
(L)Gln(*N*-trityl)	(D)Gln(*N*-trityl)	(D)Gln(*N*-trityl)	23%

Table 2. Sidechain-protected peptide sequences selectively bound by **3** (26 μM).

AA3	AA2	AA1	Frequency Found
(L)Gln(*N*-trityl)	(D)Gln(*N*-trityl)	(D)Gln(*N*-trityl)	100%

These results indicate that the new receptors **2** and, especially, **3** have significant sequence-selective peptide binding properties that differ considerably from the consensus selectivity found with **1** for (L)Val-containing peptides. While both **2** and **3** show a strong preference for various *N*-trityl-carboxyamide-substituted peptides (Asn and Gln), **3** binds a single sequence - (L)Gln(*N*-trityl)-(D)Gln(*N*-trityl)-(D)Gln(*N*-trityl) - under the conditions of our assay. Interestingly, the (L)Asn-(L)Pro-(D)Gln sequence found with **2** has been previously observed to bind, *inter alia*, a rather unselective analog of **1** having acyclic diamine as a linker.[2a]

Receptors **2** and **3** also bind sidechain-deprotected peptides sequence-selectively as shown below.

Table 3. Deprotected peptide sequences selectively bound by **2**.

AA3	AA2	AA1	Frequency Found
Basic $CHCl_3$ (134 μM):			
(L)Ala	(L)Pro	(L)Lys	40%
(L)Lys	(D)Ser	(D)Pro	30%
Acidic $CHCl_3$ (210 μM):			
X	(L)Pro	(L)Lys	50%
(L)Ala	(L)Pro	(L)Lys	21%
(L)Lys	X	(D)Pro	36%

Table 4. Deprotected peptide sequences selectively bound by **3**.

AA3	AA2	AA1	Frequency Found
Basic $CHCl_3$ (77 μM):			
(L)Pro	(D)Ala	(L)Ser	33%
(D)Pro	(L)Lys	(D)Ser	22%
Acidic $CHCl_3$ (220 μM):			
X	(L)Pro	(L)Lys	36%
(L)Ala	(L)Pro	(L)Lys	14%
(L)Lys	(L)Ala	(L)Pro	29%

To clarify the protonation state of the Lys-containing members in the peptide library used in screening, we carried out assays both under acidic (containing 1% HOAc) and basic (containing 1% Et_3N) conditions. As indicated in the tables, binding strength was diminished approximately ten-fold and the preference for binding peptides containing Asn and Gln found with the protected library was lost; however, other sequences were selectively bound instead. Most of these sequences included one (L)Lys that was commonly associated with the dipeptide sequence (L)Pro-(L)Lys. In many instances, this dipeptide was part of the tripeptide sequence (L)Ala-(L)Pro-(L)Lys. With **3**, the same sequence was bound in acid along with the isomeric sequence (L)Lys-(L)Ala-(L)Pro that corresponds to an approximate frame shift. A similar approximate frame shift relationship is found under basic conditions where **2** and **3** bind (L)Lys-(D)Ser-(D)Pro and (D)Pro-(L)Lys-(D)Ser respectively. Thus **2** and **3** show selective binding at both the di- and tripeptide levels for similar, but not identical, peptide sequences.

These results indicate that macrocyclic polyamines can provide viable linkers for use in the construction of two-armed receptors for peptides. The two receptors (**2**, **3**) made here from 1,5,9-triazacyclododecane and 1,4,7-triazacyclononane showed binding selectivities that were closely related to one another but that differed substantially those from their pyrrolidine-linked cousins (*e.g.* **1**). These results suggest triazamacrocycle-linked, two-armed receptors should be valuable components of receptor libraries and further imply that commercially available tetraaza-macrocycles (*e.g.* cyclen) might be useful precursors of analogous three-armed receptors.

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6. Ohlmeyer, M.H.J.; Swanson, R.N.; Dillard, L.W.; Reader, J.C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W.C. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922.

7. For technical reasons, the deprotected tripeptide library was not N-acetylated but instead was N-acylated with a variety of simple organic acylating agents (listed in ref 5). In our binding assays here, virtually no selectivity for the N-acyl group was observed.

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