ORGANIC LETTERS

2007
Vol. 9, No. 21
4283−4286

# Macrocyclic Diketopiperazine Receptors: Effect of Macrocyclization on the Binding Properties of Two-Armed Receptors

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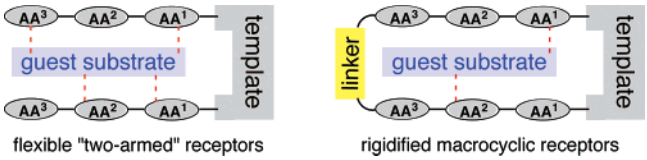
**Received August 3, 2007**

## ABSTRACT




**The synthesis of macrocyclic two-armed diketopiperazine receptors and their binding properties toward peptides is described. Macrocyclization with short linkers led to receptors with significantly modified binding properties compared to their flexible open chain parent receptors, whereas with long linkers the original binding selectivities were largely retained.**

Over the past decade, several molecular receptors consisting of a template and two peptidic side chains as recognition modules have been developed.[1] Despite their structural flexibility, many of these "two-armed" or "tweezer-like" receptors bind peptidic guests with good to excellent selectivities. Within the receptor structure, the template is crucial either as a recognition site for a certain functional group[2] or as a rigid conformationally well-defined scaffold that directs the peptidic side chains in a defined orientation away from the template.[3−5] The peptidic "receptor arms" are responsible for the binding selectivity, and even small changes in their structure can lead to significantly modified binding properties. Several studies investigated and demonstrated the importance of the rigid template for the selective recognition of substrates by two-armed receptors.[6] In contrast, little is known about the importance of the conformational flexibility within the peptidic receptor arms for selective substrate binding.

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(2) For examples see: (a) Shepherd, J.; Gale, T.; Jensen, K. B.; Kilburn, J. D. *Chem. Eur. J.* **2006**, *12*, 713−720. (b) Wright, A. T.; Anslyn, E. V.; McDevitt, J. T. *J. Am. Chem. Soc.* **2005**, *127*, 17405−17411. (c) Wright, A. T.; Anslyn, E. V. *Org. Lett.* **2004**, *6*, 1341−1344. (d) Arienzo, R.; Kilburn, J. D. *Tetrahedron* **2002**, *58*, 711−719. (e) Jensen, K. B.; Braxmeier, T. M.; Demarcus, M.; Frey, J. G.; Kilburn, J. D. *Chem. Eur. J.* **2002**, *8*, 1300−1309. (f) Botana, E.; Ongeri, S.; Arienzo, R.; Demarcus, M.; Frey, J. G.; Piarulli, U.; Potenza, D.; Kilburn, J. D.; Gennari, C. *Eur. J. Org. Chem.* **2001**, 4625−4634. (g) Braxmeier, T.; Demarcus, M.; Fessmann, T.; McAteer, S.; Kilburn, J. D. *Chem. Eur. J.* **2001**, *7*, 1889−1898. (h) Fessmann, T.; Kilburn, J. D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1993−1996. (i) Davies, M.; Bonnat, M.; Guillier, F.; Kilburn, J. D.; Bradley, M. *J. Org. Chem.* **1998**, *63*, 8696−8703.

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(5) For recent examples of other receptors with peptidic recognition modules, see: (a) Kubo, M.; Nishimoto, R.; Doi, M.; Kodama, M.; Hioki, H. *Chem. Commun.* **2006**, 3390−3392. (b) Schmuck, C.; Wich, P. *Angew. Chem., Int. Ed.* **2006**, *45*, 4277−4281. (c) Schmuck, C.; Heil, M. *Chem. Eur. J.* **2006**, *12*, 1339−1348. (d) Chamorra, C.; Liskamp, R. M. J. *J. Comb. Chem.* **2003**, *5*, 794−801.

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10.1021/ol701892x CCC: $37.00 © 2007 American Chemical Society
**Published on Web 09/20/2007**

We have addressed this question by connecting the termini of the receptor arms with different linkers and examining the binding properties of the resulting rigidified, macrocyclic receptors. Herein we present the effect of macrocyclization on the binding properties of diketopiperazine receptors as examples of two-armed receptors. We demonstrate (a) that macrocyclization can alter the binding properties of two-armed receptors significantly and (b) that the extent of altered binding selectivities depends on the length of the linker used to connect the receptor arms.

Diketopiperazine receptors are based on a structure-directing diketopiperazine derived from hydroxyproline, which serves as a structure-directing template and anchor for the two peptidic side chains (Figure 1a).[4,6a] Combinatorial

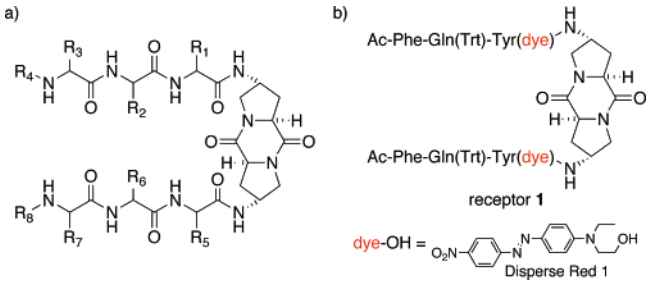


**Figure 1.** (a) General structure of diketopiperazine receptors and (b) receptor **1** (the dye is attached via an ether linkage to the Tyr residue).

binding studies revealed that these two-armed receptors bind peptidic substrates with high selectivities and binding affinities of up to $\Delta G = -6$ kcal mol$^{-1}$ both in organic solvents and in aqueous environment.[7,8] Conformational analysis demonstrated that the diketopiperazine adopts a well-defined turn-conformation that proved crucial for selective peptide binding.[6a] As for other two-armed receptors, analysis of the conformation adopted by the receptor arms has not been straightforward due to their structural flexibility.

Receptor **1** was chosen as a receptor prototype to analyze the effect of macrocyclization on the binding properties of diketopiperazine receptors (Figure 1b). **1** had previously been found to bind tripeptides like Ac-D-Hph-D-Hph-D-His, Ac-L-Hph-L-Gln-D-Hph, and Ac-D-Gln-D-Hph-D-Hph (Hph = hydrophobic amino acid, either Ala, Val, Leu, or Phe) with binding affinities of $\Delta G \leq -4.7$ kcal mol$^{-1}$ in chloroform.[4a,7] These binding selectivities are neither very pronounced nor very low compared to other diketopiperazine receptors. Thus, we felt that there was enough room for improved, decreased, or simply altered binding selectivities upon structural modifications of **1**.

To test for the effect of macrocyclization, the peptidic side chains were connected by using linkers that differed in length and chemical functionality. The termini of the receptor arms were linked by (a) an olefin, (b) amide bonds, and (c) a disulfide bond (Figure 2). The first two linkers were designed

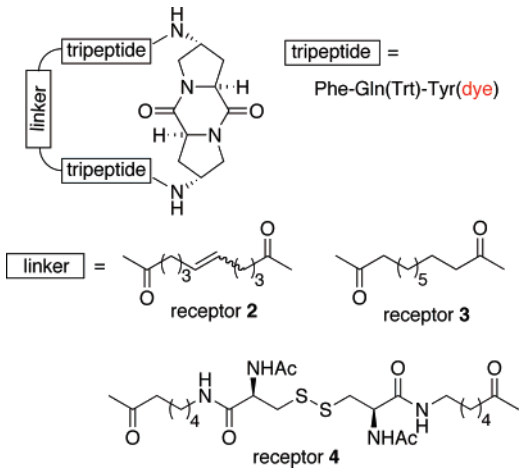


**Figure 2.** Macrocyclic diketopiperazine receptors **2**−**4**.

to have the same number of atoms (ten), thus allowing testing to determine whether functional groups within the linker would have an influence on the binding properties of macrocyclic diketopiperazine receptors. The disulfide linker contains 12 additional atoms, thus forming a larger macrocycle and allowing for a comparison of the effect of different ring sizes on the binding properties (Figure 2).

The open chain compounds **1a**−**c** served as precursors for the macrocyclic receptors **2**−**4** (Scheme 1). For the synthesis of **2**, 5-hexenoic acid was coupled onto **1b** to yield **1a**. Macrocyclization by a metathesis reaction[9] was effected by using 0.8 equiv of Grubb's catalyst at a concentration of 1 mM in a mixture of $CH_2Cl_2$ and $CH_3OH$ in 71% yield.[10] Receptor **3** was obtained by macrocyclization of **1b** with the bis-pentafluorophenyl (Pfp) ester of sebacic acid. Slow addition of the bis-Pfp ester and the bisamine **1b** to a solution of Hünig's base in THF ($c$ = 1 mM) allowed for isolation of receptor **3** in a yield of 32%. For the synthesis of receptor **4**, 6-aminohexanoic acid and Acm-protected cysteine were coupled onto **1b** to yield **1c**. Exposure of **1c** to iodine in a solution of $CH_2Cl_2$, methanol, and water ($c$ = 1 mM)[11] provided receptor **4** in 40% yield.

The binding properties of the macrocyclic receptors **2**−**4** were evaluated by combinatorial binding assays with a resin-

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(10) A mixture of *E* and *Z* isomers was obtained in a ratio of 2:1 that were separated by preparative HPLC. Combinatorial binding studies (see below) were performed with both isomers and revealed similar binding selectivities. Thus, all further studies were performed with the mixture of *E*/*Z*-isomers.

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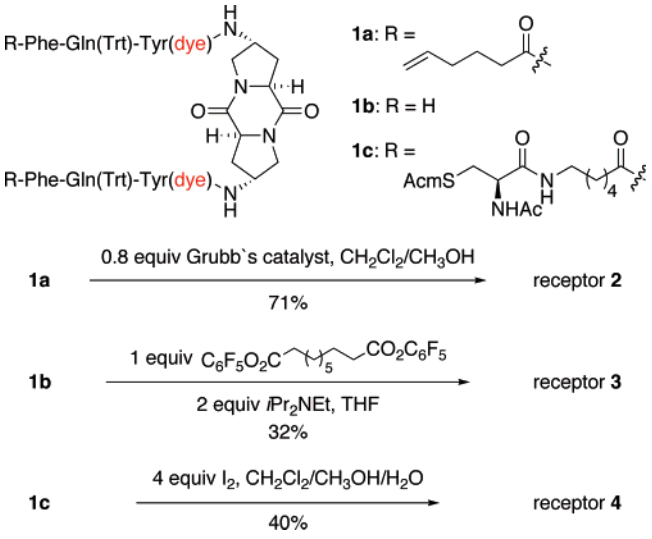
**Scheme 1.** Macrocyclizations to Yield Receptors **2**−**4**




bound tripeptide library of the general structure Ac-AA3-AA2-AA1-NH$(CH_2)_5$CONH-resin. The library was prepared on polystyrene resin by encoded[12] split and mix synthesis[13] and had already been used for the analysis of the binding properties of the open chain receptor **1**.[4] Twenty nine different amino acids were used in each of the three positions, resulting in maximally $29^3 = 24389$ different acylated tripeptides as members of the library.[14] To ensure a representative screening result, an amount corresponding to at least five theoretical copies of the library was used per assay.[15]

Upon mixing of the library with dilute solutions of the receptors (∼50−100 $\mu$M) in chloroform and equilibrating the mixtures for at least 24 h, several beads picked up the red color of the receptor in all three assays (Figure 3). In the assay of receptor **2**, approximately 1 out of 200 beads turned bright orange. The assays of receptors **3** and **4** indicated higher levels of binding selectivity since only 1 bead out of ∼400 and ∼1000, respectively, had turned orange.

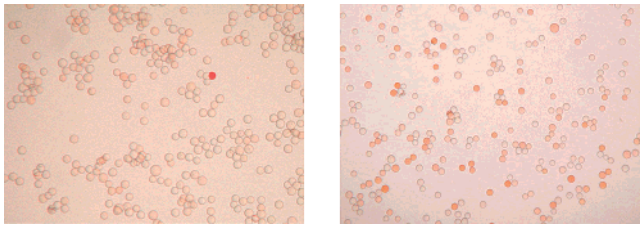


**Figure 3.** Combinatorial binding assays of diketopiperazine receptor **1** (left) and macrocyclic receptor **2** (right) with the tripeptide library Ac-AA3-AA2-AA1-NH$(CH_2)_5$CONH-resin.

These qualitative results of the binding assay revealed that all macrocyclic diketopiperazine receptors are able to bind to peptides selectively. In comparison to the binding assay with receptor **1**, the darkest beads in the assays of **2**−**4** were not nearly as intensely colored as those observed in the assay of **1** (Figure 3). Isolation of several of the colored beads (∼30 per assay) and analysis of their respective encoding electrophoric tags by gas chromatography, using electron capture detection, revealed the peptide binding selectivities (Table 1).

**Table 1.** Peptide Binding Selectivities of Receptors **1**−**4** for Tripeptides within the Library Ac-AA3-AA2-AA1-NH$(CH_2)_5$CO-PS Resin

| | AA3 | AA2 | AA1 | freq found[a] | freq expected[a] |
|---|---|---|---|---|---|
| **1**[b] | D-Ala/D-Val | D-Hph[c] | D-His | 34 | 0.04 |
| | L-Ala/L-Leu | L-Gln | D-Hph | 37 | 0.04 |
| | D-Gln | D-Hph | D-Val/D-Leu | 20 | 0.04 |
| **2** | X[d] | L-Pro/Lys | L/D-Pro | 30 | 0.71 |
| | D-Pro/L-Lys | L/D-Pro | X | 20 | 0.48 |
| | D-Phe/D-Ser | L/D-Lys | X | 20 | 0.48 |
| **3** | X | L/D-Pro | L/D-Pro | 35 | 0.23 |
| | D-Lys | D-Pro | L-Pro | 23 | 0.004 |
| **4** | L/D-Ala | L-Gln | L-Ala | 17 | 0.008 |
| | L-Hph | L-Gln | D-Hph | 13 | 0.01 |
| | D-Gln | D-Hph | D-Hph | 37 | 0.07 |

[a] The column frequency found lists the percentage of beads selected in the combinatorial assay for the indicated peptide sequence. The column frequency expected lists the expected frequency for the particular tripeptide sequence upon random bead picking. The comparison between "freq found" and "freq expected" is therefore a measure for the selectivity level of the receptor. [b] Data taken from ref 4a. [c] Hph = hydrophobic amino acid, either Ala, Leu, Val or Phe. [d] X = random amino acid

Receptors **2** and **3** with the olefin and alkane linkers bind predominantly to Lys and Pro rich peptides. Their binding selectivities are clearly different from those observed for the parent open chain receptor **1**. Interestingly, receptors **2** and **3** have slightly different binding selectivities suggesting that not only the macrocycle but also the type of linker can affect the binding selectivities.

Macrocyclic receptor **4** with the longer linker has binding selectivities that are similar to those of the open chain receptor **1**. **4** binds to two out of the three peptide motives that were also selected by **1** (Ac-L-Hph-L-Gln-D-Hph and Ac-D-Gln-D-Hph-D-Hph) (Table 1).

The third motif selected by **1** (Ac-D-Hph-D-Hph-D-His) is, however, not selected by the macrocyclic receptor. These results suggest that the ring size of macrocycle **4** is large enough to accommodate a similar conformation as in the

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(14) AA1−AA3 = Gly, D-Ala, L-Ala, D-Val, L-Val, D-Leu, L-Leu, D-Phe, L-Phe, D-Pro, L-Pro, D-Ser, L-Ser, D-Thr, L-Thr, D-Asp, L-Asp, D-Glu, L-Glu, D-Asn, L-Asn, D-Gln, L-Gln, D-His, L-His, D-Lys, L-Lys, D-Arg, L-Arg.

(15) Burgess, K.; Liaw, A. I.; Wang, N. Y. *J. Med. Chem.* **1994**, *37*, 2985−2987.

open chain receptor **1** that allows for binding to the Gln-containing peptides. It also suggests that the conformation of receptor **1** upon binding to peptides of the type Ac-D-Hph-D-Hph-D-His does not resemble any conformation that can be adopted by a macrocyclic receptor.[16]

To obtain a measure of the strength of the observed intermolecular interactions of macrocyclic receptors **2**−**4** and their preferred peptides, we determined relative binding affinities using a solid-phase binding assay (Table 2).[7]

**Table 2.** Relative Binding Affinities of Diketopiperazine Receptor **1** and Macrocyclic Receptors **2**−**4** to Solid Supported Peptides (Ac-peptide-NH$(CH_2)_5$CO-PS Resin)[a,b]

| receptor | peptide | $K_a$ ($M^{-1}$) | $\Delta G$ (kcal $mol^{-1}$) |
|---|---|---|---|
| **1**[c] | D-Val-D-Val-D-His | 2500 | −4.6 |
| **1** | D-Gln-D-Ala-D-Leu | 805 | −4.0 |
| **2** | D-Lys-D-Pro-L-Lys | 59 | −2.4 |
| **2** | L-Pro-L-Pro-D-Lys | 90 | −2.7 |
| **2** | L-Ser-D-Pro-D-Pro | 63 | −2.4 |
| **2** | D-Phe-L-Lys-D-Pro | 34 | −2.1 |
| **2** | D-Ser-D-Lys-L-Lys | 38 | −2.2 |
| **3** | D-Lys-D-Pro-L-Pro | 33 | −2.1 |
| **4** | L-Ala-L-Gln-L-Ala | 51 | −2.3 |
| **4** | D-Gln-D-Ala-D-Leu | 19 | −1.7 |

[a] The peptides were immobilized on polystyrene resin with a loading of ~0.33 mmol $g^{-1}$. [b] All measurements were repeated at least twice to ascertain the accuracy of the binding affinities within ±0.1 kcal$mol^{-1}$. [c] Data taken from ref 7.

These studies demonstrated that none of the macrocyclic receptors bind their preferred peptides with binding affinities that are comparable to those measured for the flexible parent diketopiperazine receptor **1**. These relative binding affinities are supported by the qualitative comparison of the color of the darkest beads in the assays of the macrocyclic receptors and the parent open-chain receptor **1**. None of the beads in the assays of the macrocycles **2**−**4** were as intensely colored as the darkest beads in the assays of the open chain receptor **1** (Figure 3), implying a weaker binding of the macrocyclic receptors to their selected peptides.

In conclusion, we demonstrated that the binding properties of two-armed receptors can be modified significantly by macrocyclization via the termini of their peptidic side chains. Macrocyclic receptors bind their peptidic substrates with lower binding affinities compared to flexible two-armed receptors. This shows that preorganization of two-armed receptors into their binding conformation is not trivial and reveals the importance of the conformationally flexible peptidic side chains for the highly selective binding properties of two-armed receptors. Binding selectivities change drastically when short linkers are employed whereas with longer linkers certain selectivities are retained. Macrocyclization can thus serve as a valuable tool to modify the binding properties of two-armed receptors.

**Acknowledgment.** This work was supported by the Swiss National Science Foundation and BACHEM. H.W. thanks BACHEM for an endowed professorship.

**Supporting Information Available:** Description of experimental procedures and characterization of receptors **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Noteworthily, the additional potential hydrogen bonding sites in the linker of receptor **4** do not modify the binding selectivities significantly compared to those of the open-chain receptor **1**.