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Synthesis of cyclic peptidosulfonamides as scaffolds for MC4 pharmacophoric groups

Bas Wels,[a] John A. W. Kruijtzer,[a] Keith M. Garner,[b] Roger A. H. Adan[b] and Rob M. J. Liskamp[a,*]

[a]*Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands*

[b]*Department of Pharmacology and Anatomy, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands*

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Abstract—The synthesis of potential β-turn mimetics based on cyclic sulfonamide peptoid/peptoid hybrids is described. These are readily synthesized using a solid phase protocol followed by cyclization in solution, and their suitability to combinatorial approaches is illustrated by the synthesis of a small but diversely functionalized library.

We are interested in the development of relatively small scaffolds for the attachment of peptide side chains, targeted at the MC4 receptor (MC4-R), which is a member of the melanocortin receptor family. There are five melanocortin receptor subtypes (MC1-R–MC5-R), which are all seven-transmembrane receptors coupled to the adenylate cyclase pathway.[1] The MC4-R is of great current interest, as it is believed to be involved in energy homeostasis,[2] obesity syndrome,[3] and recently erectile activity.[4] It was hypothesized that the binding conformation of the naturally occurring peptide ligands involves a β-turn, centered on a His-Phe-Arg-Trp tetrapeptide.[5] Therefore, β-turn mimetics as scaffolds for the side chains of the naturally occurring peptides are very interesting, as was demonstrated by previous efforts yielding β-turn mimetics capable of binding at the MC4-R[6] and the related MC1-R.[7] We have developed the synthesis of potential β-turn mimetics **1**, closely related to cyclic tripeptides.[8] In this communication, we wish to report the synthesis of a small library of a novel macrocyclic system **2** in Figure 1, which improves on cyclic ($\alpha_2\beta$)-tripeptides **1**. The latter turned out to be somewhat unstable, decomposing under treatment with TFA, which led to difficulties in side chain deprotection of functionalized derivatives of **1**. We therefore set out to synthesize cyclic peptidosulfonamides, with improved stability.

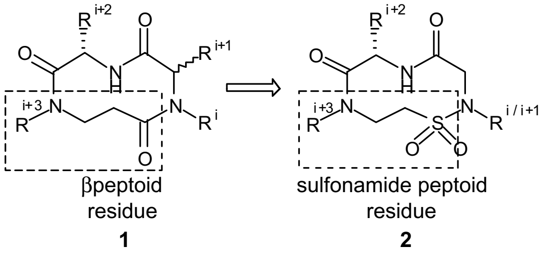

Figure 1. Cyclic ($\alpha_2\beta$)-tripeptides **1** and cyclic sulfonamide peptoid–peptide hybrids **2**.

In view of the limited stability of cyclic $\alpha_2\beta$ tripeptides, a system was designed with an alternative backbone structure. Small cyclic tripeptides are known to be unstable, decomposing to cyclols[9] unless completely N-alkylated.[10] Cyclols of 10-membered cyclopeptides also exist,[11] and presumably decomposition of **1** takes place through the formation of similar structures, despite the presence of N-alkylation. Therefore, a more peptidomimetic system seemed attractive, that is replacing the

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Keywords: MC4-agonists; Cyclic peptide; Solid phase synthesis; Peptidosulfonamide; Peptidomimetic; Peptoid.

* Corresponding author. Tel.: +31 30 253 7396; fax: +31 30 253 6655; e-mail: r.m.j.liskamp@pharm.uu.nl

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β-peptoid residue in **1** by a sulfonamide peptoid residue.[12] The sulfonamide peptoids are a class of peptidomimetics that are compatible with solid phase synthesis, and are thus suitable for application in β-turn library synthesis. Moreover, to our knowledge only one example of a 10-membered sulfonamide containing macrocycle has been described in the literature.[13] Preliminary investigations showed that: (i) The presence of an N-alkyl substituent on the sulfonamide bond was essential to facilitate cyclization, and (ii) The presence of an R^{i+1} side chain (analogously to **1**) prevented coupling of the sulfonamide peptoid monomer, probably because of steric hindrance. Therefore, a peptoid residue was incorporated at this position, which led to structure **2**. The devised synthesis allowed any desired side chain to be introduced using a minimum of synthetic steps, leading to a considerable molecular diversity of potential β-turn mimetics.

In order to have an initial indication of the three-dimensional structure of **2**, preliminary Molecular Modeling was performed using the MacroModel program[14] (*v* 7.0). The structure previously obtained for **1a** using Monte Carlo conformational searching was converted into the corresponding sulfonamide macrocycle **2a**, shown in Figure 2. Subsequent minimalization resulted in a structure with a highly similar conformation to **1a**, as is apparent from the RMS of the superimposition of the backbone atoms (0.055 Å).

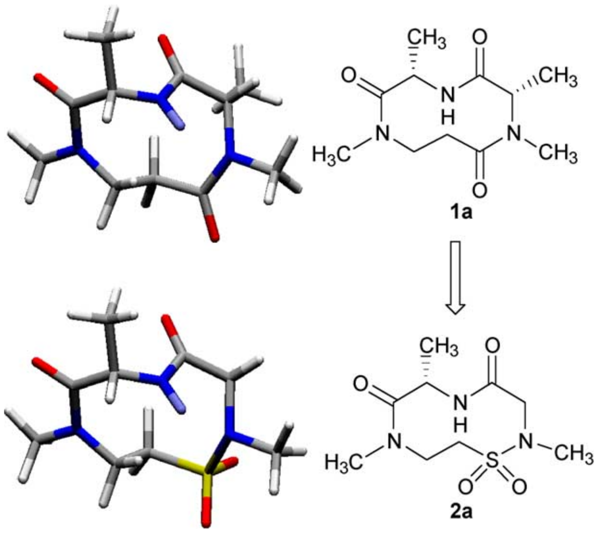
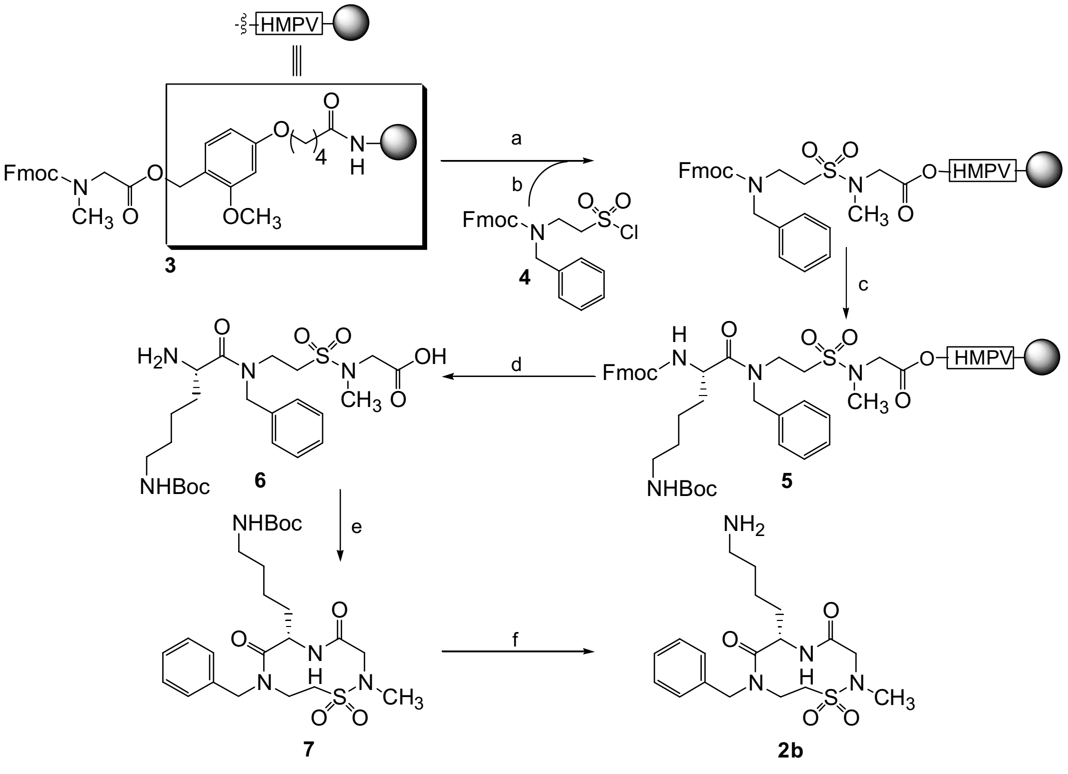


Figure 2. Molecular modeling of **1a** and **2a** showing their structural similarity.

The synthesis of a representative functionalized example of **2** (**2b**) is shown in Scheme 1. The solid phase chemistry was performed on polystyrene resin functionalized with the highly acid labile 4-hydroxymethyl-3-methoxyphenoxy-valeric acid (HMPV) linker. This allows cleavage of the linear peptide from the resin using 1% TFA in DCM while keeping side chain functionalities protected. The linear tripeptide was assembled from resin bound Fmoc-protected peptoid **3**. This was deprotected, and the sulfonamide peptoid moiety was introduced using an Fmoc-protected sulfonyl chloride **4**. After another deprotection/coupling cycle, the linear trimer **5** was



Scheme 1. Synthesis of peptoid based mimetic **2b**. Reagents: (a) 20% piperdine/DMF; (b) Fmoc-NPhe^{S}-Cl **4**, NMM, DCM; (c) i. 20% piperidine/DMF, ii. Fmoc-Lys(Boc)-OH, HBTU/HOAt, DIEA, DMF; (d) i. 20% piperidine/DMF, ii. 1% TFA/DCM; (e) EDC/HOAt, DIEA; (f) TFA.

deprotected, and cleaved from the resin in 84% yield. The cyclization was subsequently performed using HATU/HOAt activation in 57% yield. The resulting cyclic trimer **7** was stable to TFA deprotection, yielding the deprotected functionalized cyclic product **2b** in an overall yield of 17% after HPLC purification; the products of dimerization and tetramerization were also obtained in overall yields of 10% and 9%, respectively.

In initial attempts at library synthesis, a major side reaction was observed resulting from TFA acylation of the TFA salt of linear trimer **6**. This could be prevented by synthesizing the linear trimer on 2-chlorotrityl resin and cleaving using hexafluoroisopropanol,[15] thus circumventing the use of TFA. However, this resulted in contamination with the dipeptide deletion product because of poor coupling of the sulfonyl chloride. Therefore, the trimers were assembled on HMPV resin, and all TFA salts resulting from the cleavage step removed prior to the cyclization using a solid phase extraction (SPE) column.

As an illustration that essentially any desired functionality can be introduced into **2** using the above methods, a small library of functionalized turn mimetics was subsequently synthesized. The library was targeted at the MC4 receptor, which requires a wide range of side chain functionalities to be introduced. The composition of the library is shown in Figure 3. As library members, Phe, Trp, and Arg were used, because these are present in the minimal sequence that retains melanotropic activity, that is Ac-DPhe-Arg-Trp-NH_2.[16] At the R^1 position the peptoid of homotryptophan (NhTrp) was used, since this is synthetically more accessible than NTrp. Additionally, Lys was incorporated into the peptide as an additional basic residue since it was previously found in active compounds, and improved selectivity.[7,17a]

All 12 compounds shown were synthesized in a parallel fashion on polystyrene-HMPV resin as was shown previously and desalted by SPE. TLC analysis indicated good to excellent purities, and ESI MS in each case confirmed the identity of the crude linear peptide. Cyclization with EDC/HOAT subsequently took place at 1 mM concentration in DCM solution. Coupling reagents and remaining linear species were removed by acid/base extraction, and the cyclic products were checked for purity and identity by TLC and ESI MS, again indicating good purity and the formation of the desired product in each case. The crude cyclic trimers were finally deprotected using TFA with H_2O/EDT/TIS as scavengers, precipitated from an MTBE/hexane mixture and lyophilized.

The library synthesis results showed that indeed the above method is versatile and robust; ESI MS analysis confirmed the expected $[M+H]^+$ molecular ions as the most abundant peak in the majority of the samples, with

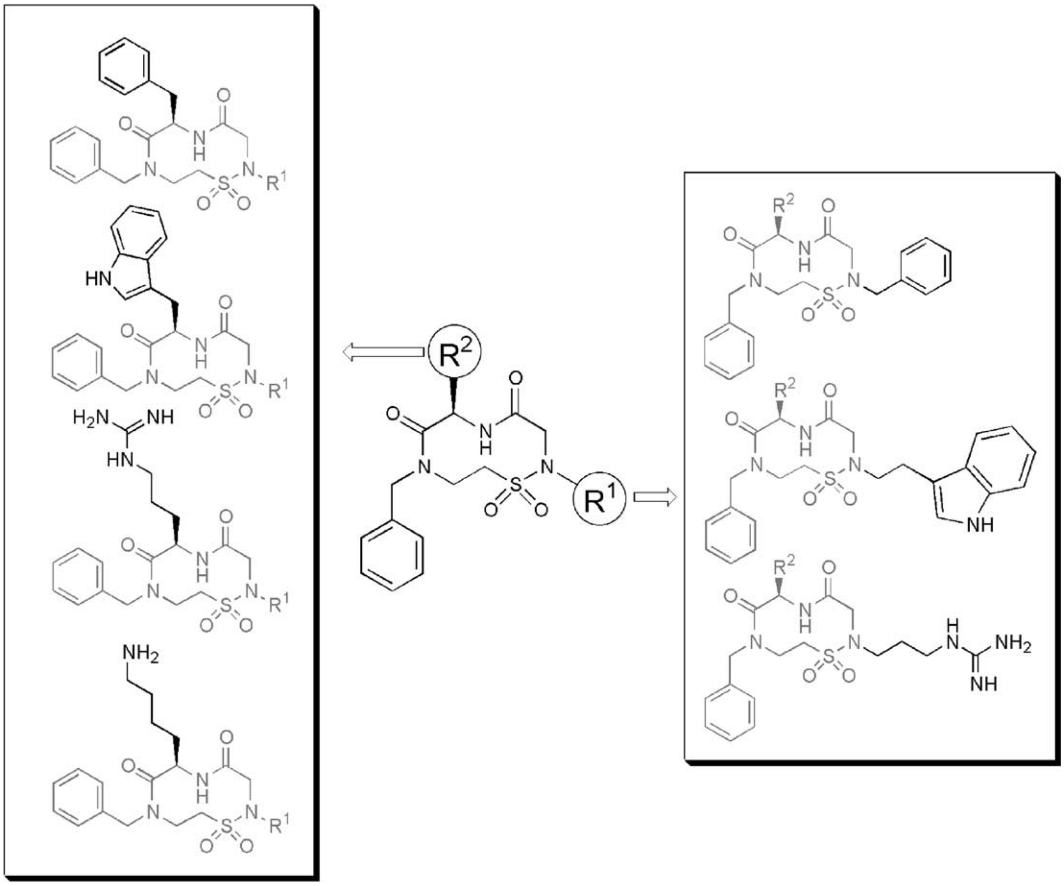


Figure 3. Side chain components of the cyclic sulfonamide peptidomimetic library consisting of 12 members.

the predominant side product being the head-to-tail cyclic dimers. Analysis by TLC showed reasonable purity of the library members, most having one dominant spot and a smaller spot of the head-to-tail dimer, except for the series containing NArg as the peptoid residue, which were too polar to be adequately analyzed by TLC. HPLC showed modest purities (10–70%) of all library members.

The modest HPLC purities and the relatively high amount of cyclic dimer formed in the cyclization reaction suggest that further optimization of the cyclization and deprotection is necessary. There seemed to be no consistent sequence dependence pattern to the cyclization results, except that polar library members containing one Arg or Lys side chain were obtained in better purity because they tend to precipitate relatively easily from MTBE/hexane. This demonstrated that the conditions used here are applicable to all molecules of type **2**.

All members of the library were subsequently screened for activation of the hMC3, hMC4 and hMC5 receptors in a cyclic AMP activation assay.[17] Additionally, all compounds were screened for antagonism of α-MSH activation of the hMC3 and hMC4 receptors. Unfortunately, none of the tested compounds showed activity or binding at concentrations up to 1 mM. Possibly, this reflects a dissimilarity of our compounds to the naturally occurring β-turn present in the α-MSH peptide, or it may simply indicate that the synthesized library was still not diverse enough to find a good hit.

In conclusion, we have developed a second generation of β-turn mimetics **2** encompassing cyclic peptidosulfonamides. These can be quickly and conveniently synthesized using a combined solid phase/solution chemistry approach, starting from easily obtained building blocks, and incorporating fully functional side chains. This was demonstrated by the synthesis of a library consisting of 12 members, incorporating a variety of side chain functionalities. Unfortunately, the resulting compounds were incapable of binding or activating melanocortin receptors. Research is underway to refine the methods described here in order to synthesize larger libraries. In particular, alternative routes to the sulfonamide peptoid-containing backbone skeleton, which should allow for more efficient cyclization, are being investigated. This will also allow structural characterization of the actual conformation adopted by these cyclic peptidosulfonamides.

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