A combinatorial approach towards pharmaceutically relevant cyclic peptides
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Summary

A Combinatorial Approach Towards Pharmaceutically Relevant Cyclic Peptides

In the search for more selective and specific drugs and therapies for diseases the pharmaceutical industry is constantly seeking for new chemical entities. Cyclic peptides are quite abundant in nature and belong to the so-called privileged structures, with significantly more hit rates in biological screenings. Consequently, a library of these compounds would be a perfect tool to for lead-finding purposes. Moreover, the pharmacophoric diversity of a cyclic peptide library may be varied easily by the nature and configuration of the amino acid side chain moieties. This thesis deals with several combinatorial approaches for the synthesis of cyclic peptides 1 and pseudopeptides 2-3, ranging from the smallest homodiketopiperazines to cyclic pentapeptides (Figure 1).

Figure 1 Target cyclic peptides and pseudopeptides.

Chapter 1 provides an overview on the combinatorial synthesis of cyclic peptides. The concepts of peptide and cyclic peptide synthesis are discussed and an overview on the classification of cyclic peptides is given. The difficulties in the synthesis, mainly due to the unfavourable transoid character of the intermediate amide bond(s), are outlined together with several strategies to overcome the cyclization problems. The concept of combinatorial chemistry is introduced with its advantages compared to classical synthesis. Finally, several approaches on the combinatorial synthesis of cyclic peptides are outlined discussing the advantages and disadvantages.

Chapter 2 deals with the synthesis of bis(lactams) via an auxiliary-mediated approach (Scheme 1). Based on earlier work, the auxiliary was optimized for the sequence independent synthesis of several seven and eight-membered bis(lactams) (8). The auxiliary was inserted into the backbone of the linear peptide facilitating the mutually reactive C- and N-terminal groups to approach one another for the macrocyclization reaction (7). A subsequent ring-contraction mechanism led to the desired bis(lactams) with the remainings of the auxiliary still attached.
Scheme 1 Auxiliary-mediated synthesis of bis(lactams).

In the optimal auxiliary (6a) a bulky isopropyl substituent was installed next to the phenolic esters providing just enough steric hindrance to prevent unwanted aminolysis, but still allow for successful esterifications. The second part deals with the modification of the auxiliary with an alkyne enabling anchoring to an azide-functionalized Merrifield resin via a copper-catalyzed azide-alkyne cycloaddition reaction (6c). Before moving to the solid phase, the whole synthetic sequence was first validated in solution (6b). However, the solid phase synthesis of bis(lactams) failed and extensive experimentation revealed that the simultaneous on resin N-Allo/Allyl deprotection was the bottleneck in this strategy.

Chapter 3 describes the synthesis of triazole-containing cyclic pseudopeptides via a backbone amide linkage strategy (Scheme 2). An alkyne functionalized acid-labile benzylic amine-type linker (9) was elongated with standard N-Fmoc based chemistry and was terminated by coupling of an azido acid (10). In solution, these peptides were cyclized with a copper-catalyzed azide-alkyne cycloaddition reaction. After translation of the chemistry to the solid phase it was found that the pseudo peptides had to be cleaved prior to cyclization. With this linker strategy, an analogue of cyclo-[Pro−Val−Pro−Tyr] was made by the solid phase/solution phase method. An analogue of segetalin B, a natural cyclic pentapeptide with estrogen-like activity, was prepared as well via the backbone amide linker method as an example of a cyclic pentapeptide analogue.

Scheme 2 Backbone amide linker strategy for the synthesis of triazole-containing cyclic tetra- and pentapeptide.
Evaluation of the conformation of the cyclic peptide analogue by NMR and modelling techniques revealed a similar type of β-turn motif compared to the natural peptide. However, the arrangement of the side chains of the amino acids in the region of the structural motif which is considered to be involved in the biological activity proved to be different in the triazole-containing analogue. Biological testing should reveal the influence of the incorporation of the triazole motif on the biological activity.

**Figure 2** 1,5-Disubstituted triazole-containing analogues of cyclo-[Pro–Val–Pro–Tyr].

In Chapter 4 the synthesis of several analogues of the peptide cyclo-[Pro–Val–Pro–Tyr] containing 1,5-disubstituted triazoles (12–13) has been described (Figure 2). Coupling of the proper azide-containing dipeptides to alkyne-containing (di)peptides via a recently discovered ruthenium-catalyzed azide-alkyne cycloaddition reaction resulted in the formation of the subsequent linear precursors. A classical head-to-tail macrolactamization resulted in the formation of the cyclic peptides, or in the formation of dimers depending on the closure site.

**Scheme 3** Combined Ugi-4CR and azide-alkyne cycloaddition reaction (CuAAC) for the synthesis of small cyclic pseudopeptides and pseudo tetrapeptides.

Chapter 5 focuses on the combination of a multicomponent reaction for the synthesis of the linear peptide precursor together with the copper-catalyzed azide-alkyne cycloaddition
reaction for the macrocyclization reaction for the synthesis of triazole-containing cyclic peptides (Scheme 3). After some variation on the structure of the isonitrile linked to the alkyne, the aliphatic isonitrile (17) proved to be optimal and reaction with amines (15), aldehydes (14) and azido-acids (16) resulted in the clean formation of the linear cyclization precursors (18). The peptides could be efficiently cyclized with a copper-catalyzed azide-alkyne cycloaddition reaction to obtain the cyclic pseudopeptides (19) in good yields in just two steps.

A library of small cyclic pseudopeptides was made in solution with the combined Ugi-4CR/azide-alkyne cycloaddition reaction. Moreover, the addition of azido acid dipeptides (20) to amines, aldehydes and a cyclohexyl-based isonitrile (21), followed by copper-catalyzed azide-alkyne cycloaddition reaction resulted in the formation of triazole-containing cyclic tetrapeptides (23). This method was finally applied in the synthesis of a triazole-containing analogue of chlamydacin, a potent histone deacetylase inhibitor. A new strategy was developed for the introduction of the active side chain of the cyclic peptide by cross metathesis.

Finally, Chapter 6 comprises an evaluation of the previous chapters and includes some suggestions for future research.