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Solid-phase synthesis of homodetic cyclic peptides from Fmoc-MeDbz-resin

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ABSTRACT

Cyclic homodetic peptides are very appealing for medicinal chemistry programs. In addition to the high efficiency and selectivity inherently associated with peptides, a cyclic structure totally formed by amide bonds increases their stability under physiological conditions. Here Fmoc-MeDbz-resin was studied for the preparation of these peptides. Our results demonstrate the usefulness of this strategy for the preparation of cyclic "head-to-side chain" peptides through cyclative cleavage (simultaneous cyclization and release from the resin). In contrast, for the synthesis of the "head-to-tail" counterparts, the cyclization-cleavage should be carried out in the presence of thiophenol.

Introduction

Cyclic peptides are excellent drug candidates for a broad range of diseases, including cancer, infections, and diseases of the immune system.[1–3] The high efficiency and selectivity and low toxicity of these molecules, together with their superior stability in physiological conditions when compared with lineal counterparts makes them highly attractive for medicinal chemistry programs.[2,4] Homodetic cyclic peptides, which show the greatest stability, are formed by only amide bonds, while heterodetic peptides also have other bonds, mainly disulfides and esters.[5–7]

As the most convenient method for the synthesis of peptides is the solid-phase approach,[8,9] the cyclization step can take place at three timepoints of the synthesis: (i) on the resin before cleavage;[10] (ii) in solution after cleavage;[11–13] and (iii) when the cyclization takes place concomitantly with cleavage, namely *Cyclative Cleavage*.[14,15] In the later strategy, cleavage is usually achieved by the attack of a nucleophile on the peptide resin (linker) bond.

Diketopiperazine (DKP) formation,[16] which is a paradigmatic case of cyclative cleavag, is driven by the stability of the six-member ring. In fact, DKP formation is a spontaneous and severe side reaction that commonly occurs during the synthesis of *C*-terminal peptides. However, other less facile cyclic peptides have been also obtained thanks to this strategy.

A controlled approach for cyclative cleavage requires the concourse of a safety-catch linker. The linker is stable to the elongation of the peptide chain, and in the last step before the cleavage, it undergoes a modification that makes it labile to intranucleophilic attack.[14]

Our group recently reported the synthesis of cyclothiodepsipeptides using 3-(Fmoc-amino)-4-(methylamino) benzoic acid (Fmoc-MeDbz) linker.[16] This is the second generation of the diaminobenzoic acid linker developed by Blanco-Canosa and Dawson.[17] Both linkers are extensively used for the preparation of peptide thioesters required in Native Chemical Ligation (NCL) using Fmoc-chemistry.

Fmoc-MeDbz linker allows the elongation of the peptide chain on the amino function in position 3 only of the aromatic ring, since

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the presence of the Me group in the amino of position 4 makes it less reactive. Once the peptide chain has been built, the reaction of the peptide linker with *p*-nitrophenyl chloroformate, followed by treatment with a base such as diisopropylethylamine (DIEA), render a *N*-acyl-*N′*-methyl-benzimidazolinone (MeNbz) resin. This resin can undergo nucleophilic attack because it is an activated *N*-acylurea (*N*-acyl-*N′*-methylurea).

The use of this strategy for the synthesis of cyclothiodepsipeptides involves the deprotection of the thiol of Cys followed by nucleophilic attack to render the thioester with concomitant release of the peptide from the resin. Later and independently, the same strategy was explored by Olsen's group for the preparation of Autoinducing Peptides (AIPs), which are cyclothiodepsipeptides.[18]

More recently, Stockdill's team reported the use of Fmoc-MeDbz linker for the preparation of *C*-terminal-modified peptides,[19] paying special attention to the case of Cys *C*-terminal peptides, which are highly prone to epimerization.[20] In all these cases, the linker is released through an intermolecular reaction, using nucleophiles, including amino acids and alcohols in the presence of base. In some of Stockdill's work, the nucleophilic attack takes place in solution once MeNbz, which is the activated linker, has been released from the solid support via treatment with trifluoroacetic acid (TFA) because of the presence of an acid labile linker (Wang or Rink) between the MeDbz/MeNbz and the solid support. In all these cases, excess of nucleophiles (10 equiv.) is used.[19]

Here we report an extension of our strategy, which was first developed for the preparation of cyclothiodepsipeptides using MeDbz linker through cyclative cleavage. In this regard, here we prepared homodetic cyclic peptides using our strategy, in which the intramolecular reaction that causes cyclative cleavage is through an amine attack. First of all, we attempted the synthesis of head-to-side chain cyclic peptides. As a model, we used the same ones as in our previous work, but diaminopropionic acid (Dap) was used instead of Cys. Among the ω-amino amino acids, Dap is more demanding for this kind of reactions than Orn or Lys, because it is less basic (see below for α-amino acids) and shows less flexibility, which can jeopardizes the cyclative cleavage. The amino side-chain of Dap was protected in the form of *tert*-butoxycarbonyl (Boc) (Scheme 1).

First, Fmoc-MeDbz-OH linker was anchored to Chem-Matrix resin via a Gly spacer in order to separate the cleavable linker from the solid support (I). The Fmoc group was then removed using 20% piperidine in DMF, and the elongation of the peptide was carried out using DIC/OxymaPure for 1 h. The N^{α} of Dap was acetylated (II). Next, p-nitrophenyl chloroformate was added in dichloromethane (DCM), the resin was washed with DCM, and finally two treatments of 0.5 N DIEA in DMF rendered the activated *N*-acylurea (III).

The peptide-*N*-acylurea resin was treated with 50% TFA-DCM (IV). After extensive washings with DCM, the peptide resin was treated with 10% DIEA in DCM for 5 h at room temperature (V). The filtrates were collected and evaporated to dryness. HPLC and LC-MS showed the full formation of the two peptide models (**1** and **2** Fig. 1) with satisfactory purity in the filtrate (purity ≥ 95% SI Figs. S1 and S3).

Furthermore, the study of the kinetics of the cycle formation for peptide model **1** was accomplished using HPLC over 24 h (see SI for experimental procedure). Fig. 2 shows that no significant difference was observed after 7 h of reaction, To confirm that full cyclative cleavage had taken place, the remaining peptide resin was washed with large excess of ethylene diamine in the presence of DIEA. After evaporating the filtrates to dryness, HPLC analysis did not show any peak corresponding to the intermolecular cleavage,

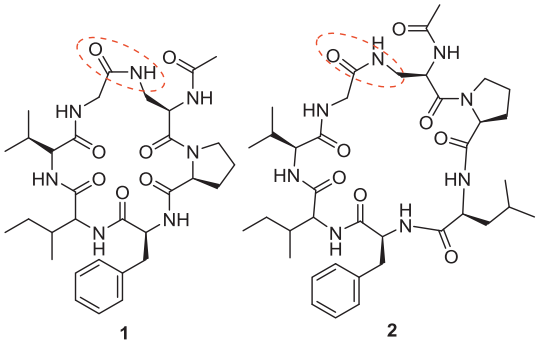
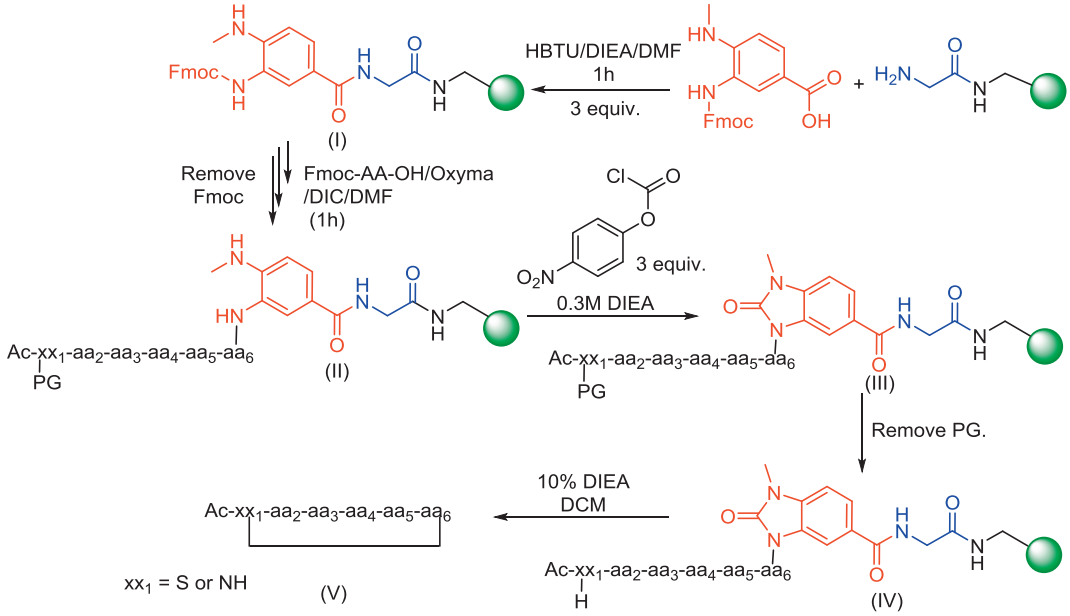


Fig. 1. Structure of peptide models (**1**, **2**). The red circle indicates where the cyclization took place.



Scheme 1. Cyclization of peptide models through nucleophilic intramolecular attack.