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A novel route towards cycle-tail peptides using oxime resin: Teaching an old dog a new trick

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Abstract

Two anabaenopeptins, Schizopeptin 791 and anabaenopeptin NZ825, have similar structural features and have been synthesized via a novel acid-catalyzed head-to-side-chain concomitant cyclization/cleavage reaction on oxime resin. The methodology gave rapid access to the anabaenopeptin scaffold by taking advantage of a combined solid-phase/solution-phase synthetic strategy. Also, as side-products of the synthesis, large C_2-symmetric 38-member cyclic peptides ring bearing two endocyclic lysine side-chains were isolated, constituting a novel cyclic peptide scaffold.

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Introduction:

The identification of new natural products with useful bioactivities is a growing field of research, driven by the needs of drug companies for compounds with unique structures.[1] Cyanobacteria are organisms that produce a rich diversity of secondary metabolites with a variety of biological activities.[2] Their secondary metabolites contain abundant variable peptide scaffolds, which are often cyclic and usually contain nonproteinogenic and endocyclic amino acids.[3]

A significant number of cyanobacteria peptides share the common feature of a macrocyclic depsipeptide core linked to a linear peptide chain.[4] Among the diverse peptides that microorganisms produce, cycle-tail peptides are a privileged scaffold. Well known cycle-tail peptides such as daptomycin[5] and polymyxin[6] are widely used antibiotics on the market. Among phytochemical cyclic peptides, the secondary metabolites known as anabaenopeptins (hereafter APs) are characterized by a 19-membered ring with endocyclic D-Lysine or L-lysine.[7]

Natural macrocyclic products obtained by the lactamization between a terminal carboxylic acid and the epsilon primary amine of the *N*-terminus of a lysine have an exocyclic α-amine linked to different substituents, such as an ureido chain (tail), as exemplified by schizopeptin 791[7t] and similarly-structured anabaenopeptin NZ825[7e], isolated from cyanobacteria *Schizotrix sp*. and *Anabaena sp*. respectively (Figure 1). In addition to their unique structure, APs are of great interest due to their inhibition of proteolytic enzymes and protein phosphatases. Interestingly, schizopeptin 791 inhibited the serine protease trypsin with an IC_{50} of 45.0 μg/mL. In contrast, no activity have been reported for anabaenopeptin NZ825, most probably due to its very low availability. Therefore, there is a need to develop a rapid synthetic procedure towards these compounds. In fact, few synthetic methodologies have been developed towards anabaenopeptin scaffold.[8]

By exploiting the nucleophilic cleavage susceptibility of the Kaiser/DeGrado oxime resin,[9] we achieved a convergent on-resin head-to-side-chain concomitant cyclization/cleavage to access the AP scaffold. This strategy relies on the selective deprotection of orthogonally protected amino acids (Boc/Cbz), then uses the free side chain amino group as a cleavage nucleophile. Indeed, as the oxime ester linkage is stable in both acidic and non-nucleophilic basic conditions, anchoring linear peptides by an oxime ester bond allows peptide elongation on solid-support media with a Boc strategy. After *N*-Boc removal, the susceptibility of the oxime resin to nucleophile attack allows an easy release of the protected peptide via acid-catalyzed *N*-terminal macrocyclization.

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Though oxime resin has been used to prepare a rich diversity of small[10] and large[11] cyclic head-to-tail peptide rings, the on-resin synthetic method we report here is a new way to rapidly access the AP scaffold by a head-to-side-chain concomitant cyclization/cleavage reaction.

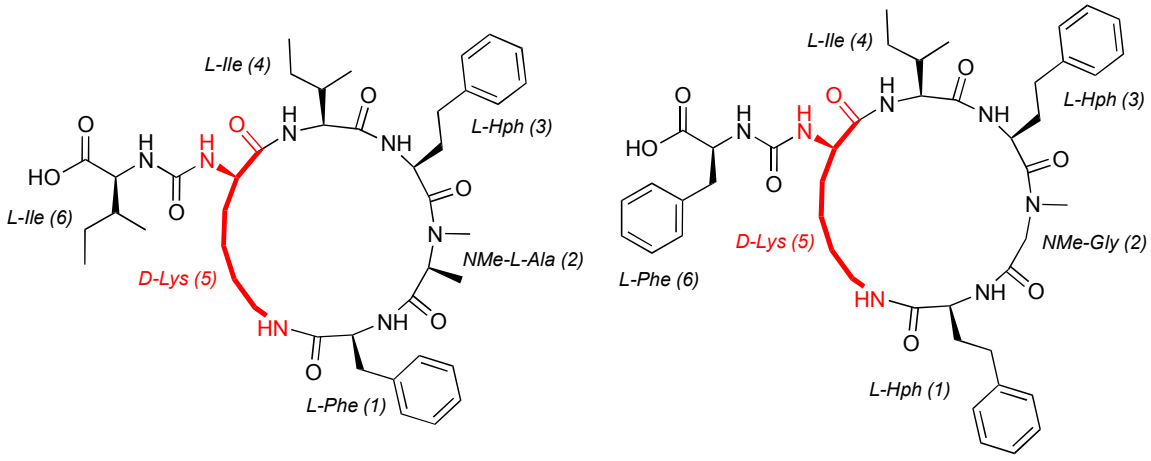

Figure 1: Chemical structures of schizopeptin 791 and anabaenopeptin NZ825 (red: endocyclic lysine)

Results and discussion

We designed our method to give a rapid and convergent synthesis of the AP scaffold. Our retrosynthetic approach is described in Figure 2. We envisioned that APs could be prepared by an exocyclic late-stage ligation of a *p*-nitrophenylcarbamate amino benzyl ester and the free α-amino group appendage of AP's cyclic peptide core.

The latter compound can be obtained by on-resin acid-catalyzed head-to-tail concomitant cyclization/cleavage on oxime resin using the epsilon-amino group of a lysine as a nucleophile. The strategy used orthogonally protected D-lysine and *N*-Boc protected amino acids as starting materials. The linear peptide chain elongation involves the use of *N*-Boc protected amino acids coupled together using well-known coupling strategies to assemble the first four amino acids on the resin. The key step is the insertion of Z-D-Lys(Boc)-OH at the fifth position, which allows selective side-chain lysine *N*-Boc deprotection. The free epsilon-amino group can then participate in the head-to-side-chain cyclization/cleavage reaction, while the *N*-terminal amine is unreactive due to the benzyloxycarbonyl (Cbz) protection, thus making it impervious to undesired head-to-tail cyclization side products.

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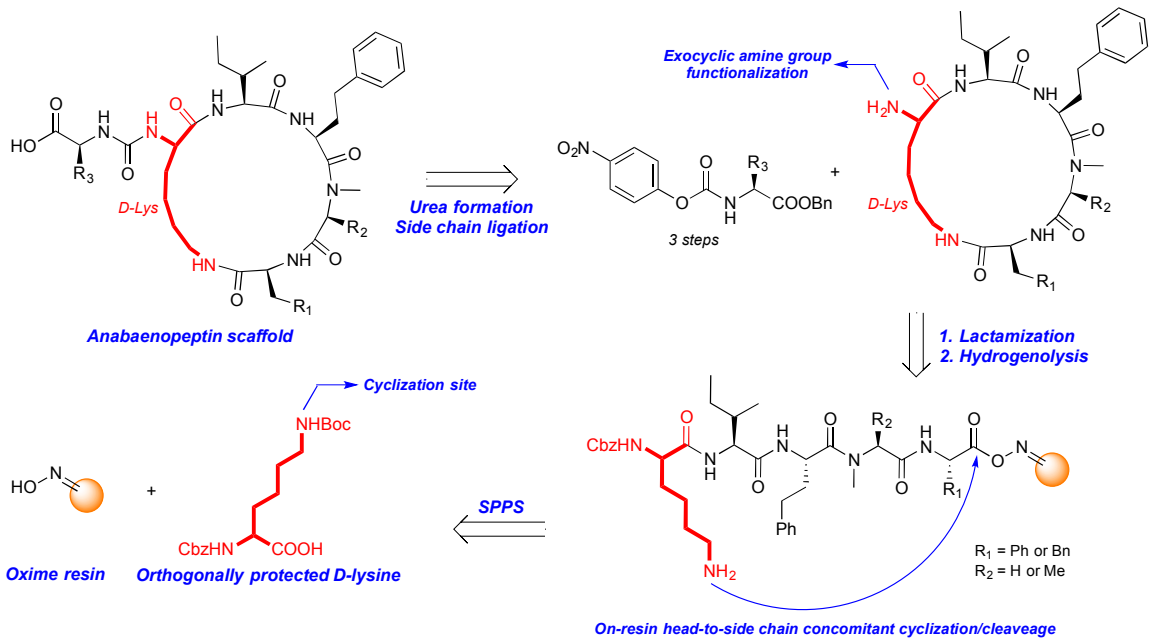

Figure 2: Retrosynthetic approach towards the AP scaffold

Following the retrosynthetic pathway, all peptides were prepared on oxime resin with a low substitution level (0.3 mmol/g) using the protocols illustrated in Figure 3 (see ESI for details). Low oxime functionalization on polystyrene has been used as higher resin loadings increase the undesired oligomerization process.[12] Briefly, the first amino acid was coupled for three hours using diisopropylcarbodiimide (DIC) as a coupling reagent. The *N*-Boc protecting group was removed using a mixture of 1:1 trifluoroacetic acid (TFA)/dichloromethane, while the second amino acid was activated with 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt) and 1-[*Bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HCTU). The Kaiser test showed that each coupling step on the resin was quantitative. After the appropriate deprotection step, the linear peptide was simultaneously cyclized and cleaved from the resin in the presence of diisopropylethylamine (DIEA; 2.5 equiv) and acetic acid (AcOH; 5 equiv) in dichloromethane at a respectable 10^{-2} M precursor concentration according to the starting loading of the resin, leading to high macrocyclization yield (72-74%) of Cbz-protected cyclic peptides **3** and **4**. As reported in the literature, incorporating a flexible aliphatic chain (we used lysine) facilitated the peptide folding and thus the macrocyclization process.[13]

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The crude cyclization/cleavage compounds contained mainly the desired cyclized peptides but also a proportion of cyclic peptide dimers. The C_2-symmetric cyclic dimers originate from the amino group attacking a neighboring monomer on the resin as the initial step. Then, the terminal lysine amino group cyclized on the resin to provide the cyclic dimers. Hence, lowering the resin loading could potentially decrease this side reaction. Although, more work is needed to confirm this hypothesis. The separation of the desired products from the cyclic dimers was difficult at this stage due to solubility problems. We decided to proceed with the next step towards the AP, the exocyclic amine deprotection by hydrogenolysis.

Peptides **3** and **4** were deprotected by catalytic palladium hydrogenation using mild conditions in EtOH with 1 atm of hydrogen to yield the two desired cyclic peptides **5** and **6**. HPLC-TOF-mass spectrometry was used to distinguish between the desired protected compounds **5** and **6** and their cyclic dimers, as reported previously.[11d] In both cases, only two peaks were revealed, both with identical masses (see ESI for details). However, the first peak possesses an isotopic *m/z* ratio equal to one, while the second peak has an isotopic *m/z* ratio equal to 0.5. The isotopic ratio distribution analysis confirms that the first peaks corresponded to the desired cyclic monomers **5** and **6,** while the second peaks of the chromatograms corresponded to the cyclic dimers **7** and **8**. The 38-member macrocyclic peptide side-products were obtained in 22% and 33% with dimerization followed by cyclization/cleavage.

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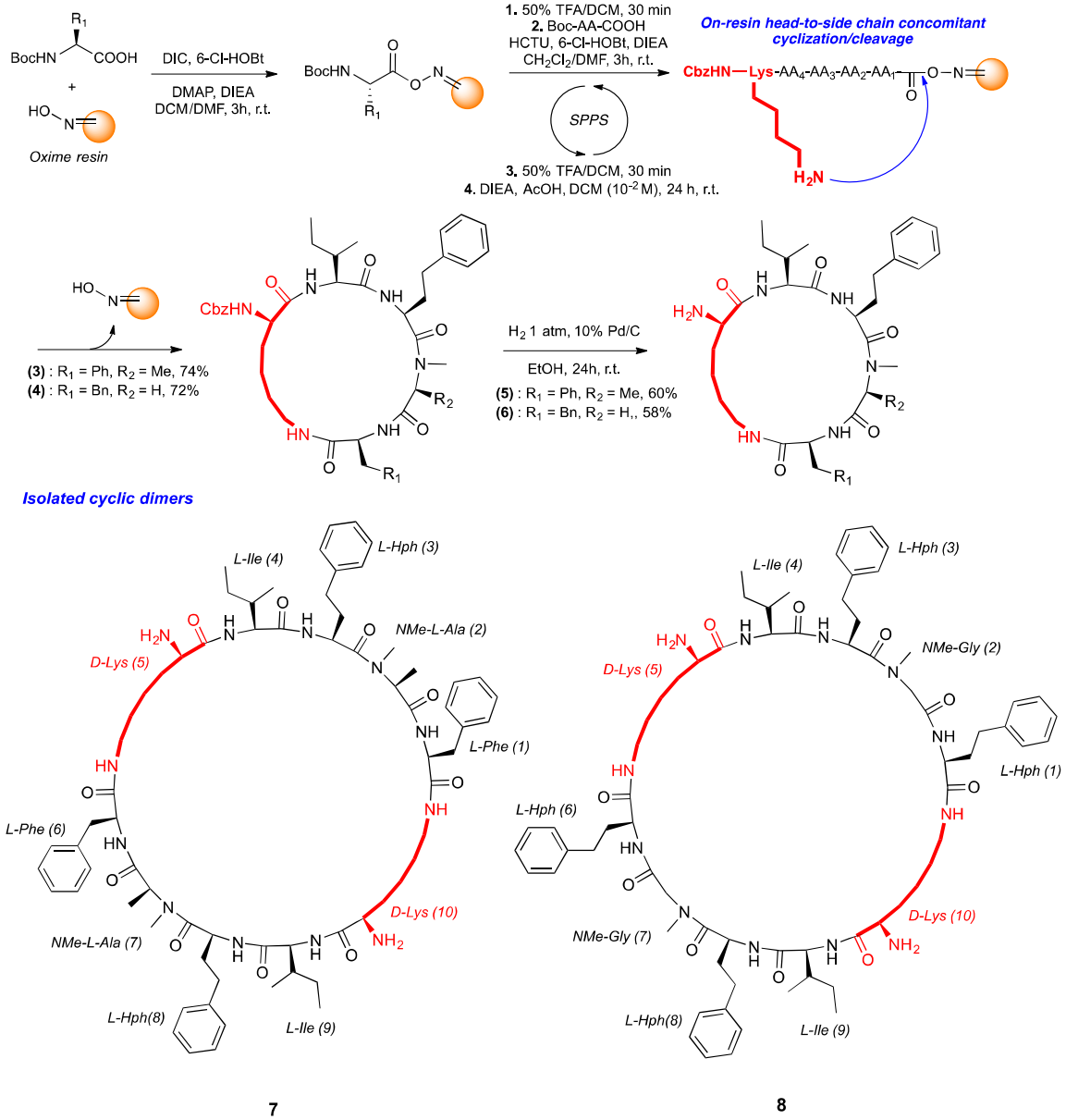

Figure 3: Synthesis of APs cyclic peptides **5** and **6** and their cyclic dimers isolated.

At this stage, RP-HPLC preparative purification was used to separate the cyclic monomer from the cyclic dimer formed in the cyclization process. Unfortunately, this step required long purification times and had high losses, yielding small quantities of the desired compounds. However, we have developed a powerful normal-phase chromatography method that allows the separation of **5** and **6** from their cyclic dimers. The purification was performed using medium-

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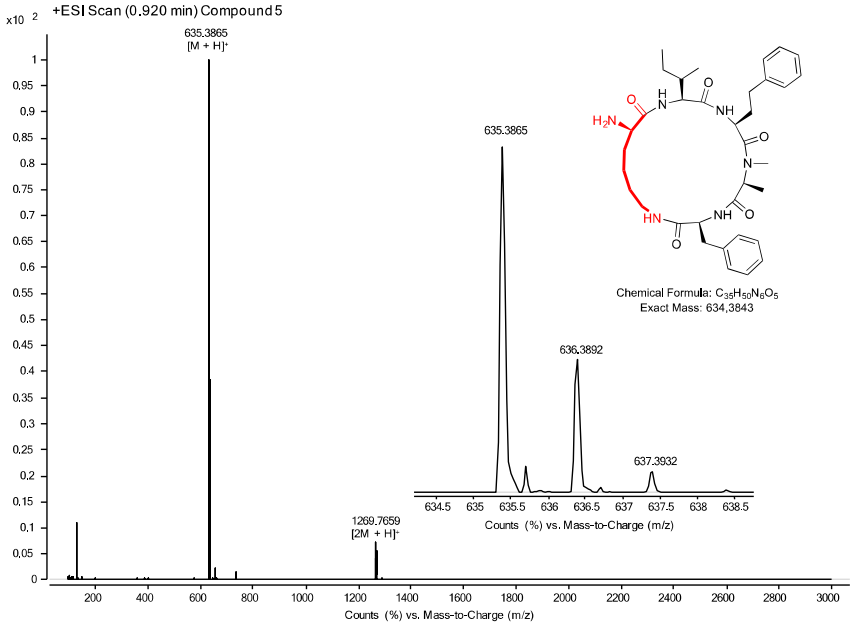
pressure liquid chromatography system equipped with a SiliaSep Premium (169 x 27 mm i.d.) flash cartridge loaded with silica as the stationary phase (25 µm). We used gradients of dichloromethane and methanol as eluents to afford the desired pure peptides **5** and **6** with satisfying isolated yields of 60% and 58% respectively. Also, we isolated the C_2-symmetric dimers **7** and **8** in 11% and 13% yield respectively**.** These cyclodimers possess a novel 38-membered macrocyclic peptides incorporating two endocyclic lysine side-chains. To the best of



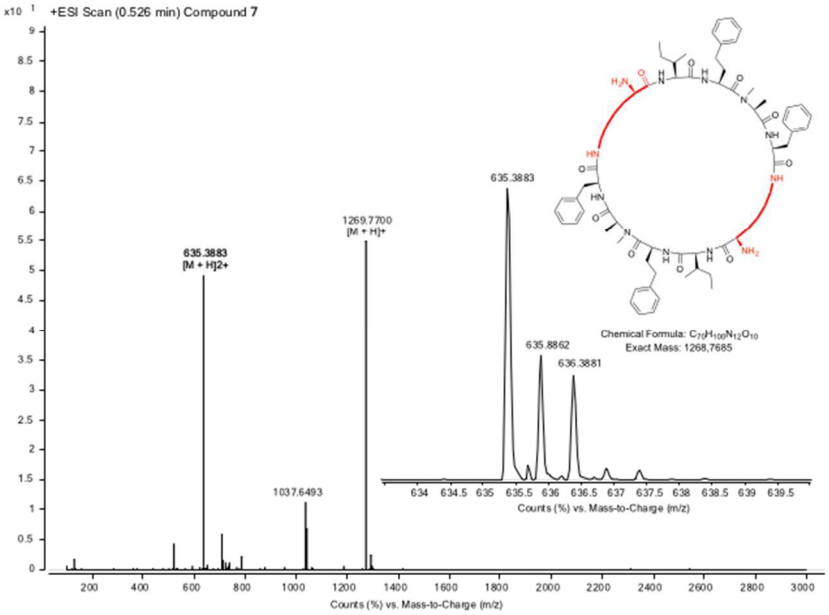


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Figure 4: ESI mass spectrum of cyclized peptide **5** obtained (above) and cyclic dimer **7** (below) with calculated monomer (**5**) and dimer (**7**) isotopic (inserts).

In producing APs, the penultimate step is the ligation between the cyclic peptides **5** or **6** and the protected *p*-nitrophenyl carbamate **9** or **10**. The final synthetic steps to obtain APs **1** and **2** are illustrated in Figure 5. The *p*-nitrophenylcarbamate amino acid benzyl esters **11** and **12** were prepared in three steps from commercially available *N*-Boc isoleucine or *N*-Boc phenylalanine. Carboxylic acid benzylation of *N*-Boc isoleucine and phenylalanine using benzyl bromide led to compounds **9** and **10** in 82% and 90% yields, respectively. After *N*-Boc removal using *p*-TsOH, the free amine was treated with *p*-nitrophenylchloroformate in the presence of Et_3N to give compounds **11** and **12** in 75% and 80% yields. Compound **12** was difficult to purifiy from traces of *p*-nitrophenol. Since it is used in excess in the tail ligation reaction, we decided to use it as is.

Notably, exocyclic ligation of cyclic peptides **5** or **6** with the *p*-nitrophenylcarbamate amino benzyl esters **11** or **12** in DCM or in THF provided the desired intermediates **13** and **14** in poor yields. However, same reaction between cyclic peptides **5** or **6** and **11** or **12** had good yields (55% and 51% respectively) using optimized conditions in DMF with the presence of DMAP (0.1 equiv.) and Et_3N (10 equiv.). Interestingly, performing the reaction without DMAP afforded intermediates **13** and **14** in low 24% and 20% yields respectively. Finally, hydrogenolysis using palladium on activated carbon in EtOH provided a 91% yield of schizopeptin 791 (**1**) and a 90% yield of anabaenopeptin NZ825 (**2**). HPLC semi-preparative purification afforded pure schizopeptin 791 (**1**) and anabaenopeptin NZ825 (**2**); their spectroscopic data matched perfectly with those reported for the isolated natural products (see ESI for details), confirming the first total synthesis of schizopeptin 791 (1) and anabaenopeptin NZ825 (2). Chemical shifts are identical within ± 0.05. The largest differences observed for NH chemical shifts are due to a concentration effect or to traces of water.

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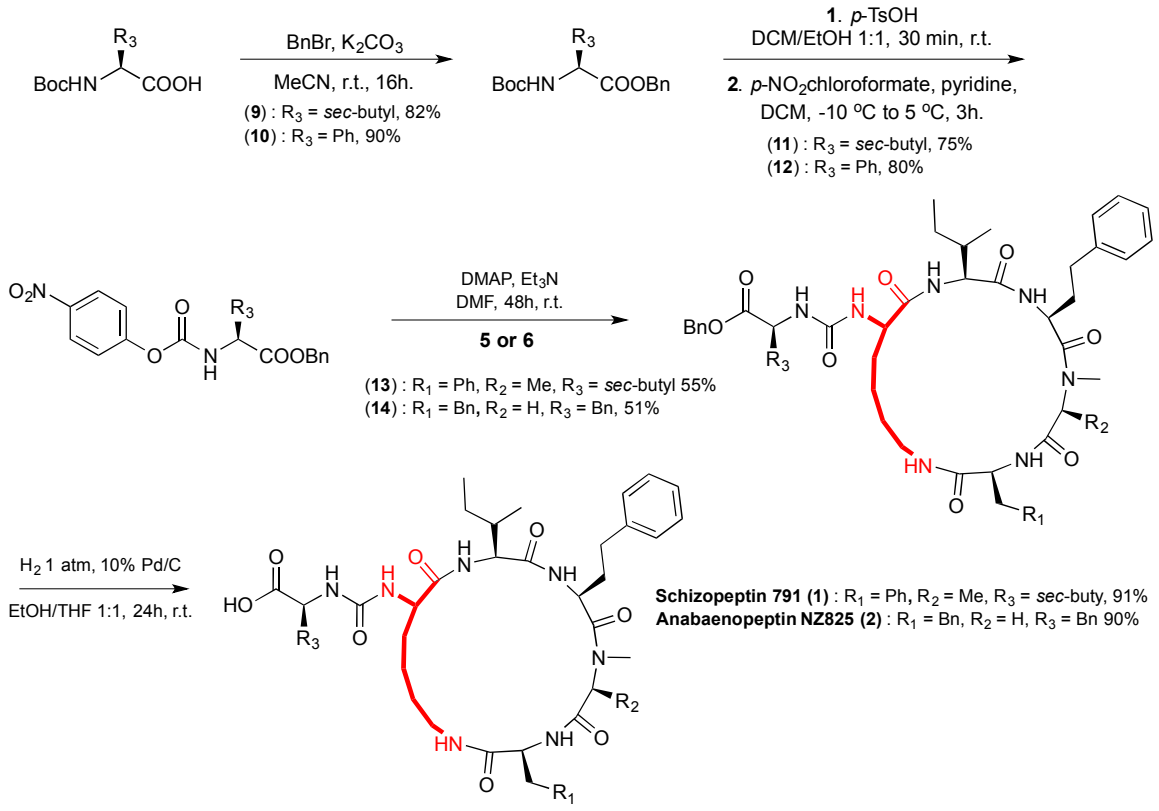

Figure 5: Side-chain synthesis, exocyclic ureamidation and deprotection procedures for the synthesis of **1** and **2**.

Conclusions

We achieved the first total synthesis of schizopeptin 791 (**1**) and anabaenopeptin NZ825[7e7e] (**2**) with high overall yields between 22% to 19%. The synthesis is based on the use of orthogonally protected lysine to graft endocyclic lysine by acid-catalyzed on-resin head-to-side-chain cyclization/cleavage. Linear precursor cyclization proceeded in excellent yields with up to 78% and 67% of the desired cyclic monomers. Hydrogenolysis gave the exocyclic free amine, followed by tail ligation and final deprotection, rapidly yielding **1** and **2**. In addition, we reported the first synthesis of a novel cyclic peptide scaffold, large C_2-symmetric 38-membered cyclic peptides bearing two endocyclic lysines. Overall, the work described can be applied to the preparation of a rich diversity of anabaenopeptins and other cycle-tail peptides. Evidently, performing the complete synthesis onto solid-phase is a very attractive and alternative synthetic approach towards natural cycle-tail peptides. We are exploring currently such approach using Alloc protected lysine for selective side chain deprotection. However, results have been disappointing so far.

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Conflicts of interest

There are no conflicts to declare.

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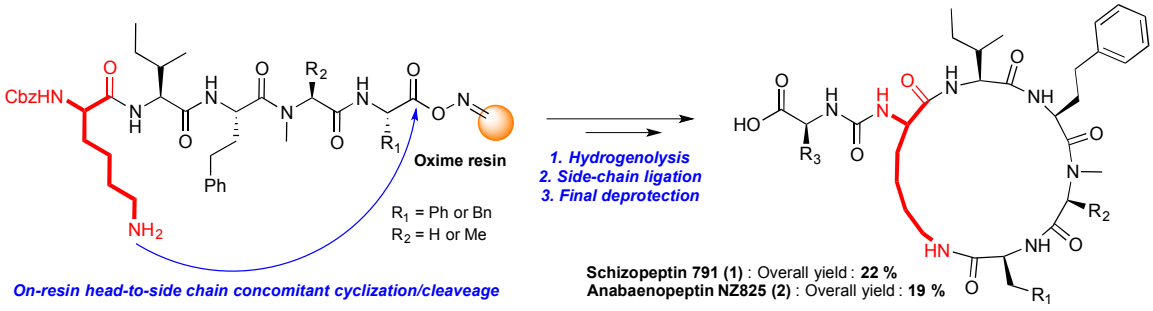

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