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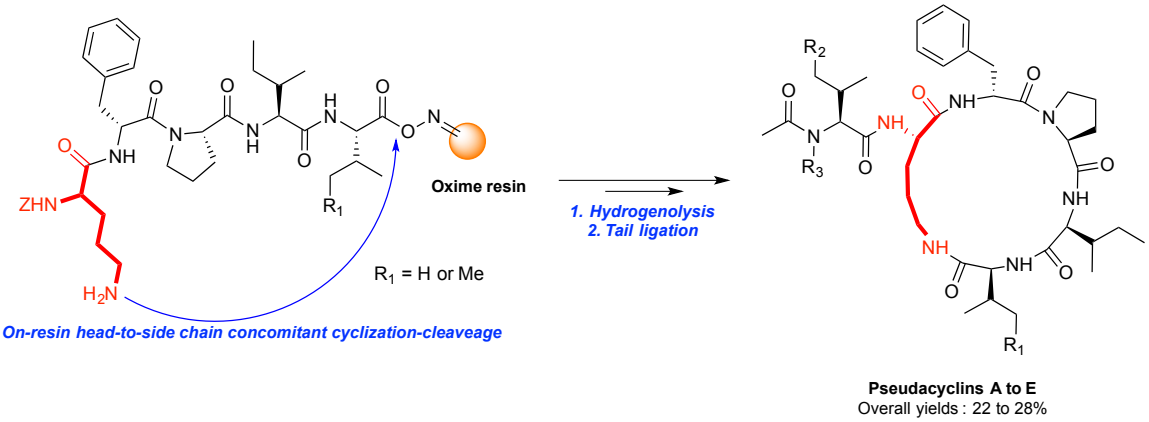
Graphical Abstract:



Abstract:

Taking advantage of the nucleophile-sensitive ester link of oxime resin, a novel synthetic strategy was applied to the first synthesis of a type of cyclic peptides known as pseudacyclins A-E. The endocyclic ornithine side-chain part was incorporated by an on-resin acid-catalyzed concomitant cyclization-cleavage reaction after a selective deprotection of orthogonally protected ornithine. The synthetic methodology gives high macrocyclization yields and low oligomerization side-products. The combination used of solid-phase/solution-phase strategy was efficient to prepare pseudacyclins and could prove useful to prepare other natural cycle-tail peptides.

Key words: Oxime resin, cyclic peptides, pseudacyclins, cycle-tail peptides, peptide synthesis, peptide cyclization

Total synthesis of pseudacyclins A-E by an on-resin head-to-side chain concomitant cyclization-cleavage reaction

Introduction:

Cycle-tail peptides are one of the most versatile molecular scaffolds to be found among the rich diversity of bioactive peptides found in nature,[1,2]. For example, cycle-tail peptides daptomycin[3-5] and polymyxin[6,7] are well-known and widely used antibiotics.[8] Various synthetic methodologies for cycle-tail peptides have been devised;[7,9-11] generally, the final step is a solution macrocyclization between an amine and the *C*-terminal carboxylic acid. However, this strategy often requires high dilution to counterbalance entropic loss by cyclization. Also, activation of the *C*-terminus by highly electrophilic ester species have to be used to cause cyclization, thus increasing the probability of racemization. Although, agents can be used to minimize the epimerization process, it is always a concerned in solution.[12]

Though any macrocyclization process is always challenging, considering that undesired oligomerization and epimerization side reactions can occur, anchoring the linear peptide on a solid support can reduce the rate of diffusion and decrease the probability of dimerization and cyclodimerization (*pseudodilution effect*).[13,14]

Among different synthetic strategies to cyclize peptides, our research group focused on the use of oxime resin. Discovered by Kaiser and DeGrado in the early 80s, the oxime resin has a unique oxime ester link that can be cleaved by a nucleophilic displacement to release the peptide.[15,16] As the oxime ester linkage is stable to both acidic and non-nucleophilic basic conditions, anchoring an amino acid on oxime resin allows peptide elongation using *N*-Boc strategy. After the desired peptide synthesis and *N*-Boc removal, the susceptibility of oxime resin to nucleophiles allows an easy release of the free or protected peptide via acid-catalyzed *N*-terminal macrocyclization in a buffered AcOH/DIEA mixture. The macrocyclic peptide can then be recovered by simple filtration and evaporation. This strategy was used for the preparation of Boc/Bzl/Cbz-protected peptide segments and cyclic peptides.[17-20] However, oxime resin was only exploited to afford cyclic peptides in a head-to-tail fashion to yield cyclic dipeptides[21-26] and larger peptides macrocycles.[27,28] Also, a dimerization-cyclization-cleavage sequence was used to prepare C_2-symmetrical cyclic peptides[29] such as the well-known antibiotics tyrocidine A[30] and gramicidin S[20]. However, only two examples reported by Taylor described the use of head-to-side chain cyclization on oxime resin to prepare amphiphilic α-helical peptides with multiple lactam bridges between Lys^{i} + Glu^{i+4} and Lys^{i} + Asp^{i+4}.[31-33] Here, we report the first total synthesis of

pseudacyclins A-E (Figure 1) by a head-to-side chain concomitant cyclization-cleavage reaction on oxime resin. Interestingly, pseudacyclin A exhibits cytotoxic and antiproliferative activities against human lymphocytes. However, no bioactivity studies have been reported for pseudacyclins B-E, probably due to the lack of materials required for such studies..[34]

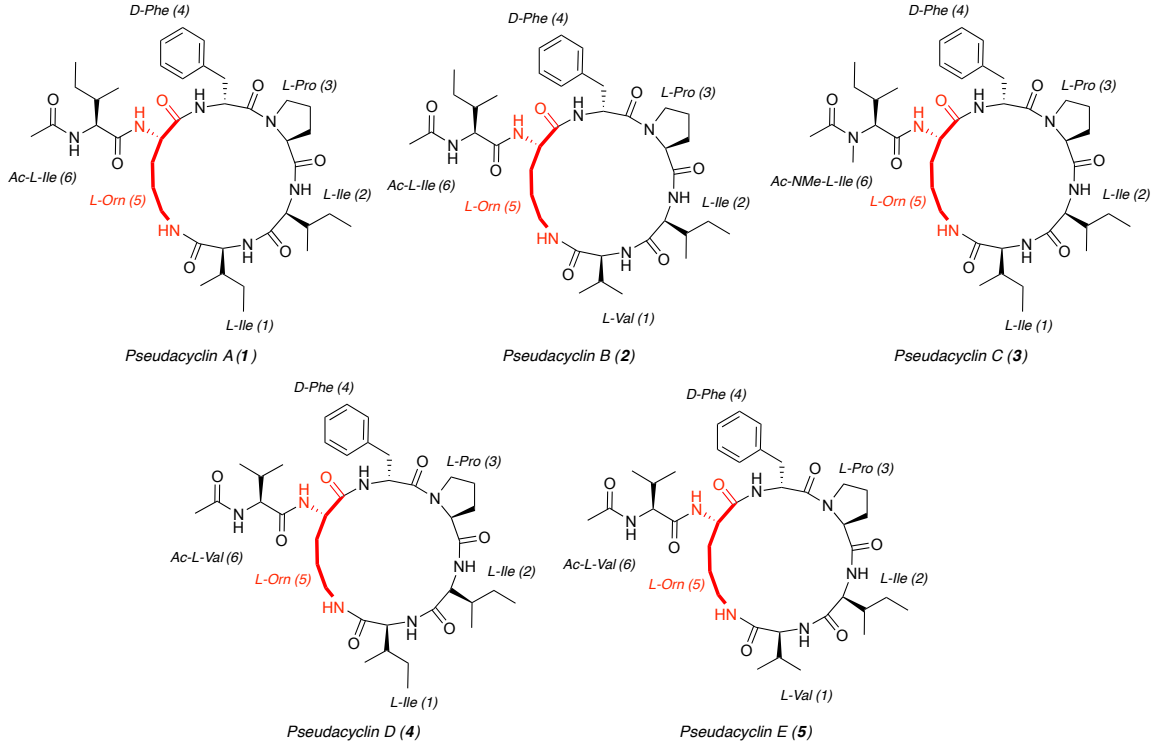


Figure 1: Structures of pseudacyclins **A-E** (red: endocyclic ornithine)

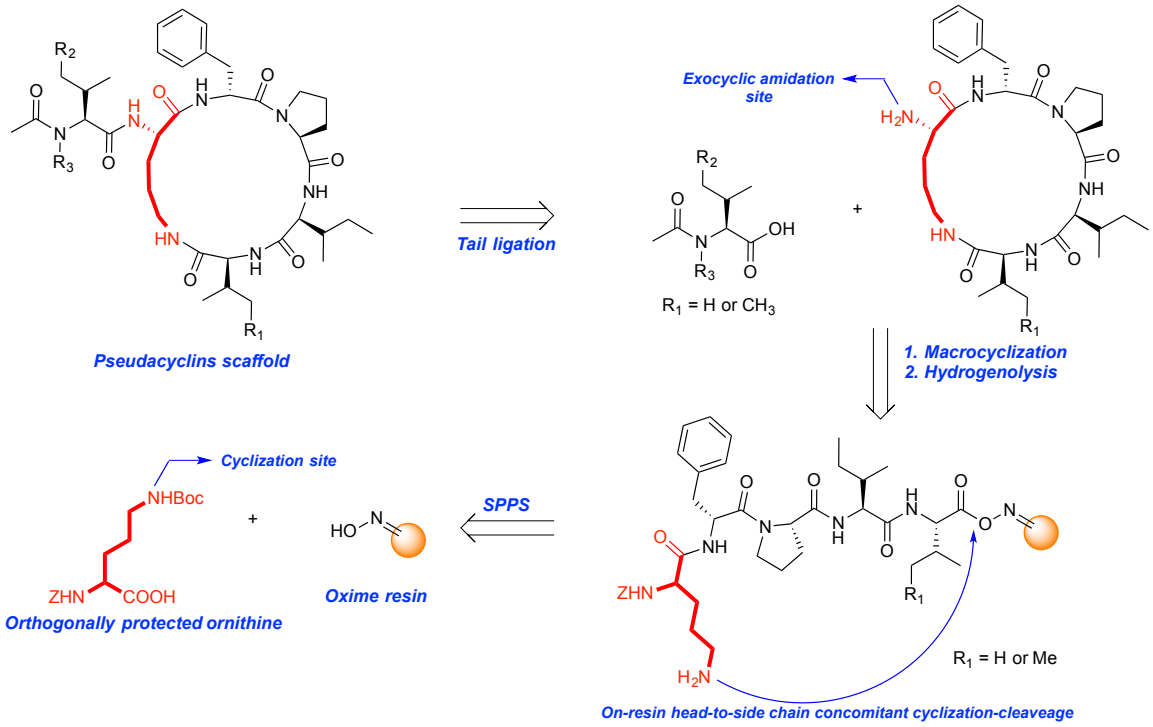


Figure 2: Retrosynthetic approach towards pseudacyclin cycle-tail peptide scaffold

Results and discussion

Pseudacyclins A-E (**1-5**) are characterized by 18-membered cyclic peptides that incorporate an endocyclic L-ornithine side-chain. The exocyclic α-amino group is linked to a tail: an acetylated valine or isoleucine. Our retrosynthetic approach is described in Figure 2. We envisioned that pseudacyclins A-E (**1-5**) could be prepared at the final step by the ligation of the *N*-acetylated amino acid to the free amine macrocycle, and that the latter could be obtained by on-resin acid-catalyzed head-to-side chain concomitant cyclization-cleavage of the required peptide using suitably protected ornithine as a nucleophile.

The peptide chain elongation on the resin involves the use of peptide coupling strategies with *N*-Boc protected amino acids to assemble the four first amino acids. The key step involves the incorporation of Z-Orn(Boc)-OH at the fifth position. After removal of the *N*-Boc group under acidic conditions, head-to-side chain concomitant cyclization-cleavage could be performed, while keeping the Orn *N*-terminal protected by a carboxybenzyl group, thus avoiding undesired head-to-tail cyclization products. Then, hydrogenolysis liberates the α-amino group to achieve the exocyclic ligation with proper acetylated amino acids.

All peptides were synthesized on oxime resin with a low substitution level (0.3 mmol/g), using the protocols described in Figure 3 (see ESI for details). Low oxime functionalization on polystyrene was used, since higher resin loadings increased the occurrence of the oligomerization process.[29]

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 appropriate coupling/deprotection steps, the linear peptides were 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 precursor concentration of 10^{-2} M on the basis of the starting loading resin, leading to high macrocyclization yield (70–72%) of Z-protected cyclic peptides **6** and **7**.

As observed in the literature, incorporating an amino acid with a flexible aliphatic side-chain-bearing amine, such as ornithine in the present case, facilitates the macrocyclization process.[35] An HPLC-TOF-MS investigation was performed on the crude cyclization products. In both cases, results revealed only two peaks; the first was assigned to the cyclic monomer and the second to the dimer, the latter being obtained at 5% and 8% respectively in the preparation of **6** and **7** (see ESI for details). No other side products that might be expected to arise from higher oligomerization were identified in the cyclization-cleavage process.

Due to solubility problems, the desired cyclic monomers were purified from the cyclic dimers after the *N*-carboxybenzyl deprotection step. This deprotection of peptides **6** and **7** was performed by a catalytic palladium hydrogenation using mild conditions in EtOH with 1 atm of hydrogen to afford the desired macrocyclic peptides **8** and **9**.

Usually, HPLC preparative purification was used to separate the desired cyclic monomers from the cyclic oligomers formed during the cyclization process. This required a long purification time and yielded small quantities of the desired compounds. Therefore, we developed a powerful normal-phase chromatography method for rapid separation of **8** and **9** from their cyclic dimers. Purification was performed using medium-pressure liquid chromatography equipped with a 25 μm silica flash cartridge as the stationary phase and a gradient of dichloromethane and methanol

as eluent (see ESI for details). The pure desired peptides **8** and **9** were obtained with satisfying yields of 56% and 52% respectively.

We finally added the required tails to peptides **8** and **9** by a direct amidation between the exocyclic amine and the carboxylic acid from Ac-Val, Ac-Ile or Ac-NMe-Ile, leading to pseudacyclins A-E (**1-5**) using 6-Cl-HOBt, EDC and Et_3N. An HPLC semi-preparative purification afforded pure peptides **1-5** with purities over 95% and structures confirmed by high-resolution ESI-mass spectrometry with (M^+H^+) ions as the major species. Spectroscopic data of synthetic samples of **1-5** entirely matched those reported for the isolated natural pseudacyclins A-E (see ESI for details).[34]

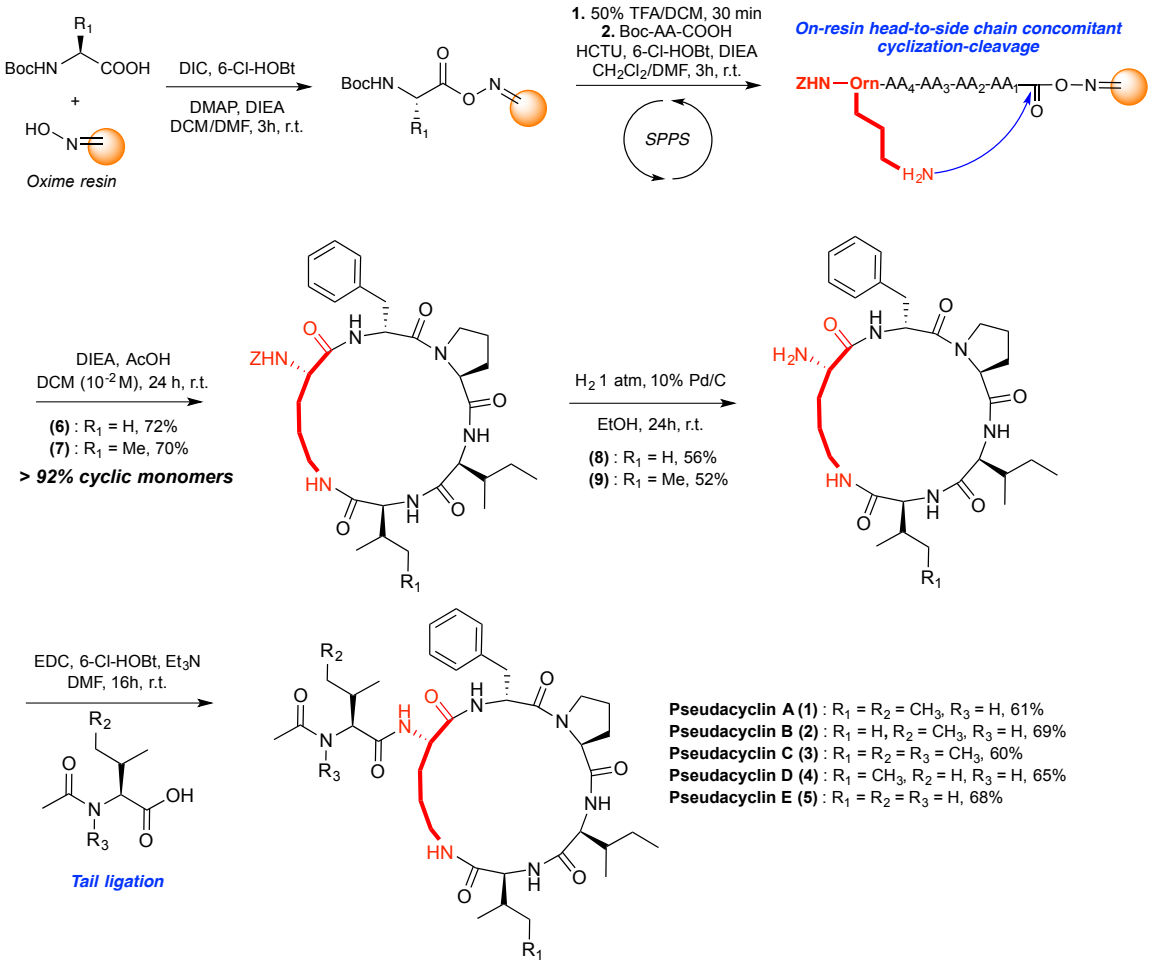


Figure 3: Procedure for the total synthesis of pseudacyclins A-E (**1-5**).

Conclusion

We achieved the first total synthesis of the cycle-tail peptides known as pseudacyclins A-E with high overall yields (22% to 28%). The cyclization process of the linear precursors is highly selective leading to over 92% of the desired cyclic monomer products, with less of 8% of the cyclic dimers. The strategy is based on the use of orthogonally protected ornithine to yield protected-cyclic peptides. Hydrogenolysis then gave the free exocyclic amine and side-chain ligation yielded pseudacyclins A-E. To the best of our knowledge, this constitutes the first report of an on-resin head-to-side chain concomitant cyclization-cleavage reaction using oxime resin to prepare naturally occurring peptide macrocycles. The procedure described could be exploited to prepare other cycle-tail peptides efficiently. Work is currently underway to prepare larger quantities of pseudocyclins A-E for bioactivity measurements and to demonstrate the generality of the synthetic strategy for the preparation of various ring-size cyclic peptides.

Acknowledgments

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