New Application of Peptide Cyclization on an Oxime Resin (the PCOR Method): Preparation of Lanthionine Peptides

George Ösapay and Murray Goodman*

Department of Chemistry, University of California at San Diego, La Jolla, CA 92093-0343, USA

Peptides containing an orthogonally protected lanthionine amino acid unit at the N-terminus, prepared on a Kaiser-oxime resin using Boc-chemistry and the BOP peptide coupling method, have been cyclized *via* peptide cyclization on an oxime resin.

In the method of Peptide Cyclization on an Oxime Resin (the PCOR method[1]), the cyclization reaction takes place *via* amide bond formation by intramolecular reaction of a backbone or side-chain amine with the carboxy group activated as an oxime ester bond on a Kaiser-resin. We have applied this method to develop a novel synthetic route to prepare lanthionine peptides.

Lanthionine is a monosulfide analogue of cystine: two alanine-like units (abbreviated as Ala_L)† are bridged side chain to side chain by a thioether bond. For the synthesis of lanthionine peptides (*e.g.*, nisin segments), a desulfurization reaction of disulfide bridges with hexaethylphosphorus triamide was successfully applied by Shiba and Wakamiya.[2] The application of this sulfur extrusion method is limited in the presence of different structural elements, such as base sensitive protecting groups.[3] Recently, we described another synthetic route[4] simulating the biosynthesis of lanthione peptides through the addition of a peptidyl cysteine-SH to the double bond of a dehydroalanine residue in a given sequence. Our experiments provided only one of the possible diastereoisomeric products, although, Michael addition reactions are known to be non-stereoselective. The sterically hindering solid support might be responsible for the fact that one of the diastereoisomers is favoured by this method.

The recently developed method for peptide cyclization on an oxime resin is an efficient tool for ring formation of peptide chains of different types and sizes.[1,5] Here, we demonstrate its utility for the preparation of thioether bridged peptides by synthesizing a few examples of building blocks for biologically active peptides.

The synthetic strategy for cyclic lanthionine peptides requires an orthogonally protected lanthionine unit **1**‡ to be coupled to the N-terminal residue to the peptide chain. The peptide chain is initially assembled on an oxime resin[6]§ by using Boc-chemistry and BOP activation.[5,7] The head-part of the lanthionine unit (Fig. 1) is then coupled to the peptide chain. After removal of the Boc protecting group from the 'tail-part' of the lanthionine followed by neutralization of the

† *Abbreviations*: $Ala_L \cdots Ala_L$, lanthionine unit; Boc, *tert*-butyloxycarbonyl; BOP; benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; Z; benzyloxycarbonyl; Bzl; benzyl; DIEA; *N*,*N*-diisopropylethylamine; For; formyl; TFA; trifluoroacetic acid; $Tmac_2O$; trimethylacetic or pivalic anhydride; Tos; toluene-*p*-sulfonyl; DMF, dimethylformamide; DCC, 1,3-dicyclohexylcarbodiimide; DCM, dichloromethane. All amino acids used were of the L configuration except tryptophan (D).

‡ Compound **1** was prepared from Z-serine β-lactone[10] by its reaction with Boc-Cys-OMe in DMF in the presence of Cs_2CO_3 (same molar quantities) during 1 h at room temp. The product was subjected to silica gel column chromatography (eluent: chloroform–methanol, 10:1). Yield: 50%; R_F(toluene–pyridine–acetic acid, 80:10:1) = 0.16; R_F(butanol–acetic acid–water, 4:1:1), 0.79; $[\alpha]_D^{24}$ −22.7 (*c* 1.5, MeOH); FAB-MS, *m/z* 457 [MH^+].

§ The oxime resin (*p*-nitrobenzophenone oxime polymer) was prepared from a 1% crosslinked polystyrene resin (Bio-Beads SX1, BioRad, Inc.) according to the literature.[6] The resulting solid support contained 0.65 mequiv. oxime groups on 1 g of resin. The peptide cyclization on an oxime resin requires low substitution level (0.2–0.3 mmol g^{-1}) to avoid intermolecular side reactions. For this reason, 0.4 mmol Boc-amino acid was used for loading on 1 g of oxime resin with DCC in methylene chloride over 3 h. The obtained substitution levels (0.24–0.32 mmol g^{-1}) were determined by picric acid titration.

Peptide couplings were carried out by using BOP activation. A DMF solution of Boc-amino acids and BOP reagent were added in 3-fold excess to the deprotected (with 25% TFA–DCM, 30 min) and neutralized (2.5% DIEA–DCM) peptidyl resin. After 1 h the completeness of couplings was monitored by the Kaiser-test.[11]

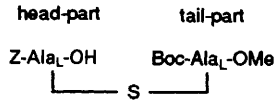
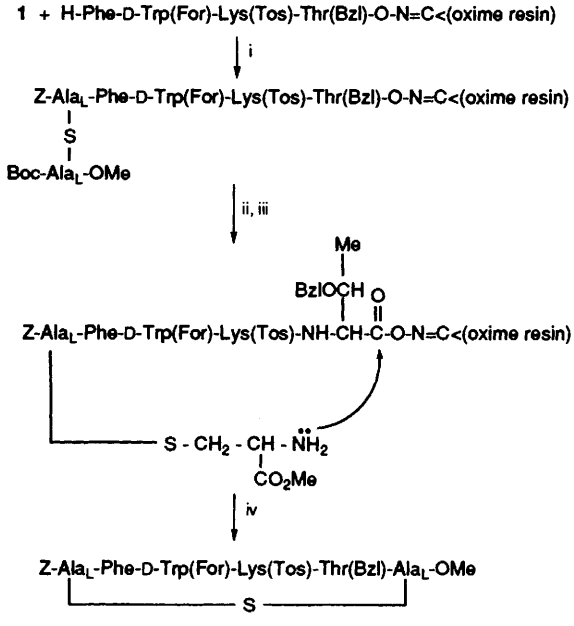


Fig. 1 Structure of an orthogonally protected lanthionine amino acid **1**



Scheme 1 Synthesis of a fully protected lanthionine hexapeptide **4** by the PCOR method; *reagents and conditions*: i, BOP–DMF, 1 h; ii, 25% TFA–DCM, 30 min, then regular washing steps; iii, 2.5% DIEA–DCM; iv, 10 mol equiv. AcOH in DMF–DCM, 24–48 h

N-terminus, an internal cyclization takes place through nucleophilic cleavage of the peptidyl oxime ester linkage (Scheme 1). The cyclization reaction is accelerated when 10 mol equiv. acetic acid are added as a catalyst.[8] The cyclic product is released into solution in a fully protected form and the solid phase is removed by filtration from the reaction mixture. The solution contains the product with only very small amounts of impurities. The crude product is obtained in high yield with 90–95% purity after precipitation from DMF–water (Table 1).¶

Compounds **2–6** are orthogonally protected key intermediates for syntheses of different biologically active analogues of peptide hormones. Compounds **2** and **3** are intermediates for the synthesis of lanthionine opioids, such as enkephalins and dermorphins. Peptide segments **4** and **5** are constituents of lanthionine analogues of somatostatin derivatives. The cyclic lanthionine dipeptide (**6**) contains a *cis*-amide bond which represents a novel synthon for incorporation into bioactive peptides. The lanthionine bridge in these structures represents an unusual conformational constraint. It has a stronger stabilizing effect on conformations of short peptides than the analogous disulfide bridge.[4] As a result higher bioactivities are frequently observed. In addition, lanthionine peptides generally exhibit increased stability towards enzymatic degradation.[9]

¶ The purity of the peptides was determined by reversed-phase HPLC. (Vydac C-18 semipreparative column; eluent: acetonitrile–water mixture containing 0.1% TFA.)

Table 1 Lanthionine peptides cyclized on oxime resin[a]

	Compound	FAB-MS [MH$^+$]	Yield (%)
2	Z-c[Ala$_L$-Phe-Ala$_L$]-OMe	486	68
3	Z-c[Ala$_L$-Gly-Phe-Ala$_L$]-OMe	543	72
4	Z-c[Ala$_L$-Phe-D-Trp(For)-Lys(Tos)-Thr(Bzl)-Ala$_L$]-OMe	1173	65
5	Z-c[Ala$_L$-Tyr(Bzl)-D-Trp(For)-Lys(Tos)-Val-Ala$_L$]-OMe	1232	50
6	Z-c[Ala$_L$-Ala$_L$]-OMe	339	82

[a] Experimental protocol for the synthesis of lanthionine peptides by the PCOR method: (*a*) the protected peptide chain was assembled on an oxime resin at a peptide substitution level at *ca.* 0.3 mmol g^{-1} by using a standard synthetic protocol;[5] (*b*) excess oxime groups were capped with Tmac$_2$O;[1] (*c*) the Boc group was cleaved using 25% TFA–DCM, after extensive washing steps, the cyclization reaction was carried out in DMF–DCM (2:1, v/v) with DIEA for neutralization and addition of 10 mol equiv. acetic acid; (*d*) after a reaction time of 24 h, the reaction mixture was filtered, and the resin washed with DMF; the combined filtrates were concentrated and the product precipitated from DMF by addition of water, a portion of the product was purified by crystallization or chromatography for analytical purposes (all peptides were characterised by their amino acid analysed and by MS).

In summary, we present the synthesis of five new lanthionine peptides with 2–6 amino acid residues as an example of a new and successful application of the PCOR method. This paper describes an efficient route for preparing thioether bridged cyclic peptides in good yield and high purity.

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References

1 G. Ösapay and J. W. Taylor, *J. Am. Chem. Soc.*, 1990, **112**, 6046; G. Ösapay, M. Bouvier and J. W. Taylor, *Peptide Cyclization on Oxime Resin (PCOR)*, in *Techniques in Protein Chemistry II*, ed. J. J. Villafranca, Academic Press, San Diego, 1991, p. 221.
2 K. Fukase, M. Kitazawa, A. Sano, K. Shimbo, S. Horimoto, H. Fujita, A. Kubo, T. Wakamiya and T. Shiba, *Bull Chem. Soc. Jpn.*, 1992, **65**, 2227.
3 Author's unpublished results.
4 A Polinsky, M. G. Cooney, A. Toy-Palmer, G. Ösapay and M. Goodman, *J. Med. Chem.*, 1992, **35**, 4185.
5 G. Ösapay, A. Profit and J. W. Taylor, *Tetrahedron Lett.*, 1990, **31**, 6121; N. Nishino, M. Xu, H. Mihara, T. Fujimoto, M. Ohba, Y. Ueno and H. Kumagai, *J. Chem. Soc., Chem. Commun.*, 1992, 180.
6 W. F. DeGrado and E. T. Kaiser, *J. Org. Chem.*, 1980, **45**, 1295; S. H. Nakagawa and E. T. Kaiser, *J. Org. Chem.*, 1983, **48**, 678.
7 B. Castro, J.-R. Doromoy, G. Evin and C. Selve, *Tetrahedron Lett.*, 1975, 1219; J. T. Jarrett and P. T. Lansbury, *Tetrahedron Lett.*, 1990, **31**, 4561.
8 B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, 1972, **94**, 3102.
9 G. Ösapay, S. Wang, H. Shao and M. Goodman, *Peptide Chemistry 1992*, Proceedings of the 2nd Japanese Peptide Symposium, Shizuoka, Japan, 1992.
10 L. D. Arnold, T. H. Kalantar and J. C. Vederas, *J. Am. Chem. Soc.*, 1985, **107**, 7105.
11 E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.