Cyclisation of Totally Unprotected Peptides in Aqueous Solution by Oxime Formation

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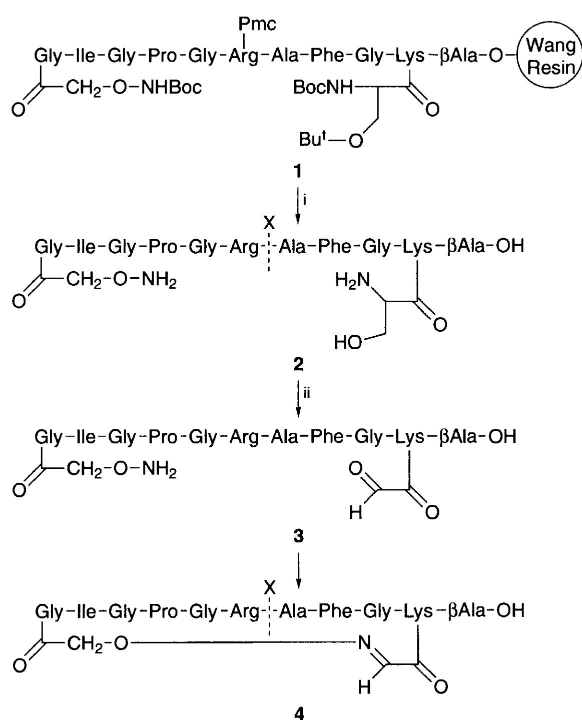
A practical method for the cyclisation of totally unprotected peptides in aqueous buffer using an intramolecular oxime formation.

Conformationally constrained peptides produced by cyclisation are useful to provide important, biologically active, topological features. Cyclic peptides have been used to mimic the surface antigens of proteins for immunological studies,[1] and to induce selectivity in receptor binding.[2] Conventional methods for peptide cyclisation involve lactam[3] or disulfide formation.[4] Cyclic lactam formation, in organic solution or on a solid-phase support, requires the protection of all side chain functionalities prior to the cyclisation and a subsequent deprotection step to remove these protecting groups. Disulfide formation requires thiol manipulation and the resulting bond is not metabolically stable. We propose a novel and direct strategy, using unprotected peptide segments, that avoids the inconvenience of lactam formation but gives a metabolically stable bond. Previously, we reported methods exploiting carbonyl chemistry for the intermolecular ligation of unprotected peptide fragments in aqueous buffer.[5] These ligations required an aldehyde on one peptide segment and a weak base on the other. Here we describe an intramolecular variation of this methodology that allows the facile synthesis of cyclic peptides from stable linear precursors derived from commercially available starting materials.

To illustrate this method an end-to-side chain cyclisation was used. A serine residue (an aldehyde precursor) was coupled to a lysyl side chain and at the N-terminus an N^{α}-hydroxylamine was used as a weak base (Scheme 1). Previous results[5] have shown that, under the mildly acidic reaction conditions, this weak base is the only reactive nucleophile present. All other functional groups on the amino acid side chains, including the N^{ε}-amine of lysine, are protonated or form reversable Schiff bases in the reaction conditions. The test peptide sequence was IGPGRAF which contains a type II β turn from the V3 loop of gp 120[6] and a neutralising determinant targeted for vaccine development. The lysine residue was incorporated as the Dde-Lys(Fmoc)-OH derivative.[7] The Fmoc was then removed with DBU and Boc-Ser(Bu^t)-OH was coupled to the peptide resin. Removal of the Dde group with 1% hydrazine followed by stepwise assembly using standard Fmoc/Bu^t chemistry and BOP† activation[8] gave the desired peptide-resin **1**. The peptide was cleaved from the resin and all protecting groups removed by treatment with TFA‡ and appropriate scavengers to give the unprotected linear precursor **2** in excellent yield. After HPLC purification, the 1,2-aminoalcohol of the serine-peptide **2** was oxidised with sodium periodate[9] in sodium phosphate buffer, pH 6.8, to give the N^{α}-glyoxylyl derivative **3** which then rapidly underwent an intramolecular cyclisation with the hydroxylamine weak base to give the oxime **4**. The reaction was followed by RP-HPLC (Fig. 1) and shown to be complete within 2 min in >90% yield based on the HPLC data.§ The peptide concentration was 0.8 mmol dm^{-3}, several hundred times less dilute than the conventional high-dilution methods utilising organic solvents. The minor side product observed in the RP-HPLC of the reaction products was shown to be due to the formaldehyde generated by the serine oxidation reacting with the hydroxylamine to give a linear oxime. The separation of the oxime geometric isomers was not discernable in the RP-HPLC analysis.



Scheme 1 The synthesis of cyclic peptides by oxime formation. *Reagents and conditions*: i, TFA/Scavengers; ii, $NaIO_4$, pH 6.8, 2 min, X = trypsin cleavage site.

To demonstrate that the product was a cyclic peptide, the linear precursor **2** and the reaction product **4** were treated with trypsin, which hydrolyses peptides at the Arg-Ala bond. Treatment of the linear peptide **2** with trypsin¶ gave the expected two products (Fig. 1), while the cyclic peptide **4** gave a single product. MS analysis|| confirmed the identity of the fragments showing that cyclisation had occurred.

In conclusion, the intramolecular cyclisation by oxime formation is highly chemoselective and flexible. The reactive weak base-aldehyde pair can be placed in several configurations, allowing side-chain to side-chain, end to end, and end to side-chain cyclisation. It also has the advantage of not requiring

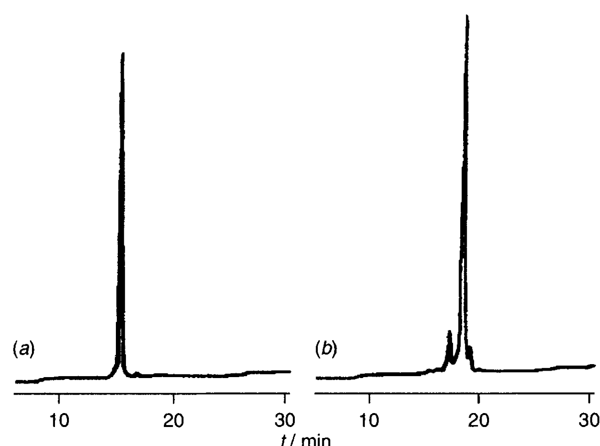


Fig. 1 Analytical HPLC showing the cyclisation by oxime formation. (*a*) Peptide **2**, elution time = 15.3 min. Column, Vydac C_{18} (5 mm × 250 mm), buffer A 0.045% aq. TFA; buffer B 0.039% aq. TFA, 60% aq. MeCN; linear gradient 10–100% B over 30 min, flow rate 1 ml min^{-1} detection 225 nm. (*b*) Reaction products 2 min after addition of $NaIO_4$. Peptide **4**, elution time = 18.4 min. Same conditions as (*a*).

protecting groups and subsequent deprotection steps, permitting the products to be used directly for antigen scanning and in constrained peptide libraries.

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Footnotes

† Abbreviations used: BOP, Benzotriazole-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate. Dde, 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl, TIS, Triisopropylsilane. MALDI-MS, Matrix Assisted-Laser Desorption/Ionisation-Mass Spectrometry.

‡ The peptide-resin was treated with TFA–thioanisole–water–TIS, 92.5 : 2.5 : 2.5 : 2.5, at room temperature, 1 h.

§ MALDI-MS, **4** *m/z* 1140.0 [Calc. 1140.0244 for $C_{50}H_{75}N_{16}O_{15}$].

¶ Trypsin was purchased from Sigma (T1005) and used 1 equivalent of trypsin to 100 equivalents of peptide in Tris buffer at pH 7.8.

|| MALDI-MS, hydrolysis of **2** gave *m/z* 628.3 (Calc. 628.6863 for $C_{25}H_{44}N_{10}O_9$) N^{α}-hydroxylamine fragment, and *m/z* 579.1 (Calc. 579.6539 for $C_{26}H_{41}N_7O_8$) C-terminal fragment; hydrolysis of the cyclic peptide **4** gave *m/z* 1158.3 (calc. 1158.2596 for $C_{50}H_{77}N_{16}O_{16}$) linear oxime.

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