Assembly of cyclic peptide dendrimers from unprotected linear building blocks in aqueous solution

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Sequential weak base–aldehyde condensations are used to cyclise unprotected linear peptide fragments and then to assemble the cyclic products onto a dendrimeric antigen presenting molecule.

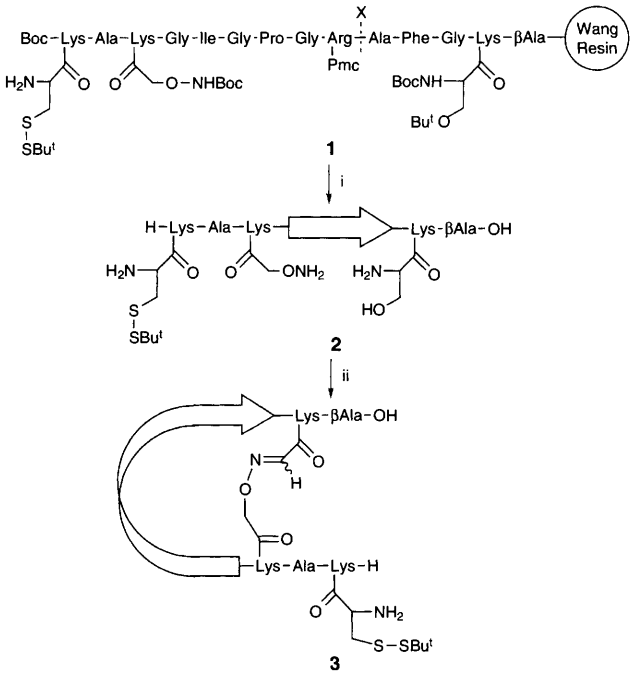
Peptide dendrimers are a class of biomolecules finding extensive use in immunology and other biomedical applications. They consist of a defined number of copies of a bioactive peptide attached to a lysyl core matrix. An example is found in multiple antigenic peptides (MAPs),† which usually contain four or eight copies of the peptide and have been used as immunogens,[1] diagnostic antigens,[2] intracellular delivery agents[3] and as artificial enzymes.[4] Previously we have reported methods for the intermolecular ligation of unprotected peptide fragments in aqueous buffer using carbonyl chemistry with weak bases to give an oxime, hydrazone, thiazolidine or thiaproline bond.[5] We have used these techniques[6] to assemble linear bioactive peptides onto dendrimeric core molecules to give artificial proteins with molecular weights in excess of 24 kD.

Recently we have described a facile method for preparing cyclic peptides from unprotected linear precursors by utilising an intramolecular oxime formation.[7] The oxime is formed by reacting an *N*-terminal *O*-alkylhydroxylamine with an N^{ε}-lysyl glyoxylaldehyde obtained by the oxidation of an N^{ε}-lysyl serine.[8] The hydroxylamine and the serine may be placed either at the *N*-terminal, or on a lysyl side chain at any position in the sequence allowing side chain-to-side chain or *N*-terminal-to-side chain cyclisations.

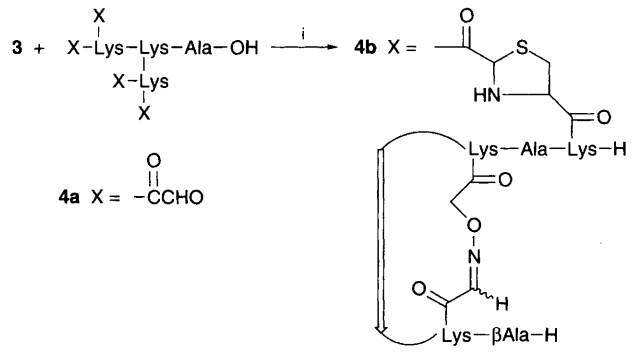
Here we describe an intergrated approach using two weak bases on a linear peptide to affect sequentially, a cyclisation and then an attachment to a dendrimeric core. Since the MAP core contains cyclic antigens that may mimic similar structural arrangements of the native protein it is likely that the antibodies induced by the MAP molecule will have a higher affinity for the native protein than those induced by a MAP with linear antigens. The target amino acid sequence was IGPGRAF, a neutralising determinant from the V3 loop of gp120. In the native protein this sequence contains a type II β-turn[8] and is a target for vaccine development.

This procedure utilises the side chains of three lysyl residues (Scheme 1). On the sidechain of the first lysine was coupled an *O*-alkylhydroxylamine and on the second lysine was coupled a serine residue. The 1,2-amino alcohol of the serine residue is an aldehyde precursor, which together with the hydroxylamine weak base was to be used to form the cyclic oxime. On the third lysyl residue was coupled a Cys(SBu^t) moiety. This is the second weak base, a 1,2-amino thiol, which was to be used to attach the cyclic peptide to the core matrix by thiazolidine formation (Scheme 2). The thiol functionality of the cysteine was protected as the *S-tert*-butylsulfenyl derivative during the cyclic oxime formation. This protecting group was chosen because of its stability to TFA cleavage and to periodate oxidation, and for the ease of conversion to the active 1,2-amino thiol by mild oxidation with a phosphine derivative.

Peptide **1** was synthesised on a *p*-(benzyloxy)benzylalcohol resin (Wang resin). The first lysine residue was introduced as the Fmoc-Lys(Mtt)-OH derivative. The Mtt protecting group[10] was removed with 1% TFA, 5% TIS in CH_2Cl_2 and Boc-Ser(Bu^t)-OH coupled to the peptide resin. Stepwise synthesis of the rest of the peptide was performed using standard Fmoc/Bu^t chemistry and HBTU activation. The second lysine residue was also incorporated as the Fmoc-Lys(Mtt)-OH derivative and Boc-(aminooxy)acetic acid coupled to the sidechain using the procedure just described. The final lysine was coupled as the Boc-Lys(Fmoc)-OH derivative, the Fmoc protecting group removed and Fmoc-Cys(SBu^t)-OH coupled to the side chain. After the removal of the Fmoc protecting group the peptide was cleaved from the resin with TFA‡ to give the linear peptide **2**.



Scheme 1 The synthesis of the cyclic peptide building block. *Reagents and conditions*: i, TFA/Scavengers; ii, $NaIO_4$, pH 7.0, 1 min, X = trypsin cleavage site.



Scheme 2 The assembly of the cyclic building blocks onto a dendrimeric core. *Reagents and conditions*: i, $P(CH_2CH_2CO_2H)_3$, pH 5.

After purification by HPLC the serine-peptide **2** was dissolved in sodium acetate buffer (0.01 mol dm^{-3}, pH 7.0) to give a final concentration of 0.8 mmol dm^{-3}. The 1,2-amino alcohol was then oxidised with sodium periodate to give the glyoxyl derivative that rapidly underwent intramolecular condensation to give the cyclic oxime **3** in 34% isolated yield.§ As previously reported, the geometric isomers of the cyclic oxime were not discernable by HPLC analysis. Tryptic digest¶ and MALDI-MS analysis of the fragments produced showed that **3** was the desired cyclic peptide.‖

The tetravalent dendrimeric core was synthesised on a β-alanyl-Wang resin using Fmoc-Lys(Fmoc)-OH[11] and then Boc-Ser(Bu^t)-OH, the carbonyl precursor, was coupled to each of the four branches of the α,ε-amines of the MAP core. Cleavage with TFA gave the $Ser_4Lys_2Lys\beta Ala$-OH (Ser_4MAP). Without purification, the Ser_4MAP was converted to the $Glyoxyl_4$MAP **4a** by oxidation with sodium periodate (8 equiv.) in sodium phosphate buffer (0.01 mol dm^{-3}, pH 8.0). After 5 min the reaction mixture was purified by HPLC and the $Glyoxyl_4$MAP lyophilised and stored at −70 °C.

The *S-tert*-butylsulfenyl protecting group was then removed from the peptide **3** (0.5 mg, 0.3 μmol) with tris-(2-carboxyethyl)phosphine (5 equiv.) in sodium acetate buffer (0.05 mol dm^{-3}, pH 6, 0.5 ml). The deprotection, followed by HPLC analysis, was complete within 1 h.** The $Glyoxyl_4$MAP (26 μg, 0.125 equiv.) was then added to the reaction mixture and the pH adjusted to 5.0 using AcOH. After 36 h the product **4b** was purified by HPLC and MALDI-MS analysis showed the expected molecular ion.††

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Footnotes

† HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Mtt = 4-methyltrityl; TIS = triisopropylsilane; MALDI-MS = Matrix Assisted-Laser Desorption/Ionisation-Mass Spectrometry; MAP = multiple antigenic peptide.

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

§ MALDI-MS, *m/z* 1659.2 [1658.8 calculated for $C_{72}H_{118}N_{22}O_{19}S_2$]

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

‖ MALDI-MS, hydrolysis of **3** gave *m/z* 1678.3 (1677.86 calculated for MH^+ $C_{72}H_{121}N_{22}O_{20}S_2$) linear oxime; hydrolysis of **2** gave *m/z* 1148.6 (1147.61 calculated MH^+ for $C_{47}H_{86}N_{16}O_{13}S_2$) N^{α}-hydroxylamine fragment, and *m/z* 578.9 (579.65 calculated for $C_{26}H_{41}N_7O_8$) *C*-terminal fragment.

** MALDI-MS, *m/z* 1572.9 (1571.82 calculated for MH^+ $C_{68}H_{111}N_{22}O_{19}S$).

†† MALDI-MS, *m/z* 6909.1 (6908.47 calculated for $C_{301}H_{475}N_{95}O_{85}S_4$).

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