Immobilised, Activated Peptides as Reagents for Cyclic and Derivatised Peptide Libraries

David Tumelty,* Dirk Vetter and Valery V. Antonenko

Dept. of Chemistry, Affymax Research Institute, 4001 Miranda Avenue, Palo Alto, CA 94304, USA

Cyclic peptides have been synthesised from an immobilised, activated intermediate, where activation of the immobilised peptide is carried out with the N^{α}-protecting group intact, its subsequent removal leading to cyclisation.

Cyclic peptides are of interest because of the conformational constraints present in their structure. The comparison of a series of linear sequences with their corresponding cyclic forms may help in the drug design process.[1] In recent years, several schemes have been put forward to facilitate the synthesis of cyclic peptides on solid supports.[2] While such methods were primarily designed to enable the cyclized products to be cleaved from the support and subsequently purified, they may also be used to form immobilised cyclic peptides.† We have investigated the use of orthogonally-protected, dicarboxylic amino acids as trifunctional templates, with the view to synthesising immobilised cyclic (and other) peptide libraries using our VLSIPS[3] and ESL[4] formats.

Initially, we investigated the synthesis and cyclisation of several simple sequences on ground and amino-functionalised glass (glass is the support used in the VLSIPS strategy), and on conventional resins‡ using this trifunctional template strategy. In these studies, we observed that the linear peptides were partially modified by some of the coupling reagents used rather than forming the cyclic products. Since minute amounts of peptides (in the picomole range) are present on a VLSIPS chip, we were concerned that such side-reactions would occur when using coupling reagents to activate a carboxyl on an immobilised peptide in the presence of its free amino terminus. This led to the concept of activating the immobilised peptide while the N^{α}-protecting group was still present: subsequent removal of this group from the carboxyl-activated intermediate would lead to cyclization.§ In an extension of the concept, treatment of the immobilised, activated peptide(s) with a variety of nucleophilic reagents can easily lead to the formation of derivatised peptides or a peptide library.

We tested these methodologies using conventional solid-phase peptide synthesis (Scheme 1). The cyclisation template used was Fmoc-Glu(OAl)-OH,¶ attached through either a Wang[6] or HMPB[7] linker to a PEG-PS resin.[8] These linkers enabled the intermediate stages of the procedure to be examined and characterised following acidolysis. The hexamer H-Phe-Gly-Gly-Phe-Ala-Gly-OH was assembled using Fmoc amino acids,[9] except that the final residue was incorporated with either a photolabile[10] or a hyper-acid labile[12]‖ group as its N^{α}-protection. The allyl group was removed by agitating the resin with $Pd(0)(PPh_3)_4$ in $CHCl_3$–morpholine (9:1), containing PPh_3, for 2 h. Pentafluorophenyl ester formation on the unmasked side-chain was achieved using an excess of pentafluorophenyl trifluoroacetate–pyridine–DMF (2:1:1, 60 equiv.)[12] for 2 h.

After extensive DMF washes, samples of the resins were treated as follows: those with photolabile protection were suspended in dioxane or acetonitrile and photolysed for 45 min at 365 nm; those with hyper-acid labile protecting groups were treated with 2% TFA–DCM (CH_2Cl_2) for 30 min., then with 5% DIEA–DMF for a further 30 min. On cleavage from their respective resins, the crude peptides contained 50–65% of the monomeric cyclized product [*cyclo*(Phe–Gly–Gly–Phe–Ala–Gly–Glu)] when using the photolabile groups and 95% when using the hyper-acid labile group.**

Treatment of the immobilised, 'active ester' intermediate with a variety of nucleophilic species can lead to the formation of side-chain or C-terminally derivatized peptides. To illustrate this approach, the synthesis of a simple glycopeptide was undertaken. The resin-bound, pentafluorophenyl ester-activated intermediate was treated with an excess of galactosylamine (50 equiv.) in DMSO for 5 h. After acidolysis, a new product was obtained in 80% yield. Isolation and characterization of this product†† showed it to be the desired glycopeptide.

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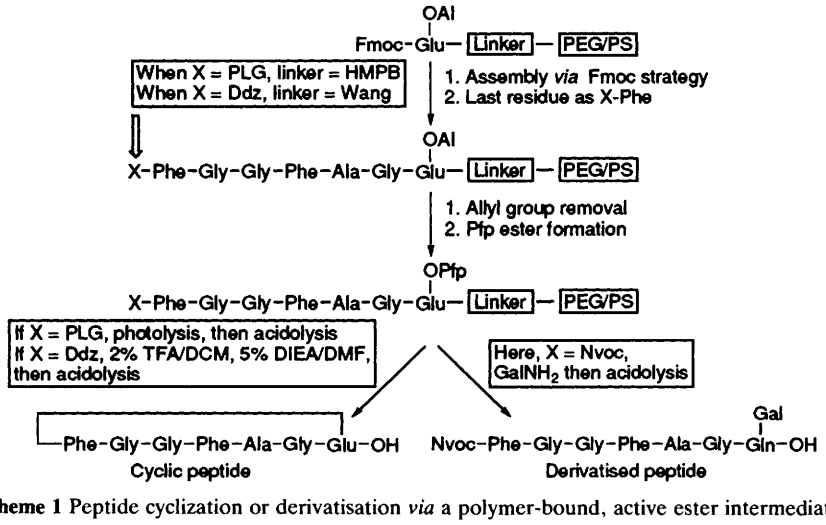
Footnotes

† By selective protection of either the α- or side-chain carboxyl function, followed by deprotection and cyclization, a 'head-to-tail' amide bond or 'head-to-side-chain' lactam may be formed.

‡ Ground glass (200–400 μm) was functionalized with an aminopropyl-



Scheme 1 Peptide cyclization or derivatisation *via* a polymer-bound, active ester intermediate

silane linker[3] to approx. 0.05 mmol/g substitution. Commercially available PEG-PS resin had 0.24 mmol/g substitution. The sequences FGGFAGQ and RQFKVVTQ were synthesised and cyclised according to the literature.[2b/2c] DIPCDI/HOBt, HBTU/HOBt or PyBOP/HOBt were used in the cyclisation step. After cyclisation, the products were cleaved from the supports and the products analysed by reversed-phase HPLC and FABMS.

§ Although a similar strategy has been successfully applied to solution-phase peptide synthesis,[5] this method has not, to our knowledge, been demonstrated previously on a solid support.

¶ Commercially available from Propeptide (SNPE, France). The α-allyl ester was similarly used in these studies, having been obtained as previously reported.[2b/2c]

‖ Nvoc[10a], Menvoc[10b] or Menpoc[10b] groups were used with equivalent results. These groups are removed by ultraviolet irradiation (365 nm, 10 W cm^{-2}) for periods of up to 90 min.

** For the photolabile protecting group method, activated resin (50 mg) was placed in a crystallizing dish (15 cm diameter), covered with solvent (to 1 cm depth) then irradiated for 45–90 min. The major product was isolated by HPLC purification in each case and gave the correct FABMS ($M + H^+ = 666.3$). Using previously described methods,[2b/2c] the same sequence with PyBOP/HOBt cyclisation gave 87% crude product by HPLC.

†† FABMS gave correct accurate mass of 1106.3932 ($M + Na^+$).

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