

A Novel Lysine-protecting Procedure for Continuous Flow Solid Phase Synthesis of Branched Peptides

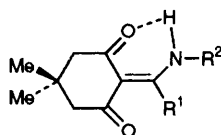
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A new amine protecting group which can be used orthogonally with both Fmoc and Boc protection is reported; by employing lysine protected appropriately as the branching motif, a 34 residue di-epitopic peptide has been constructed by continuous flow solid phase peptide synthesis.

Currently solid phase peptide synthesis (SPPS) of multiple antigen peptide systems¹ (MAPs) containing different peptide sequences, e.g. both B and T cell epitopes, is only feasible using Merrifield methodology.^{2†} In the preceding communication³ we described a novel amino protecting group, Dcm 1, and its application in continuous flow SPPS. We now report that the methyl derivative 2, unlike its precursor, is stable to the deprotection conditions for the Fmoc group and can therefore be used as orthogonal protection to Fmoc in SPPS of branched peptides, in particular MAPs.

Fmoc-Lys-OH reacts readily with 2-acetyldimmedone to afford the *N*^ε-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) derivative 3.‡ The lability of the Dde group to 2% v/v hydrazine in DMF under continuous flow conditions was demonstrated with the model peptide Dde-Ala-Ala-Pepsyn KA(100). Deprotection which results in the formation of 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indazole 4 (Scheme 1) and can be monitored by the absorption at 300 nm was complete after 3 min. Exposure of Fmoc-Lys(Dde)-OH to neat trifluoroacetic acid (TFA) followed by periodic monitoring of the amine concentration using the picrylsulfonic acid amine test⁵ modified for quantitative analysis resulted in only ca. 0.1% loss of Dde after 24 h. Stability of Dde to 20% piperidine in DMF under continuous flow conditions was investigated using the model peptide Boc-Ala-Lys(Dde)-Ala-Tyr(Bu^t)-Gly-Gly-Phe-Leu-Polyhipe SU 500 amide resin. A loss of ca. 3.0 and 6.5% was observed after 2 and 4 h respectively as monitored by HPLC analysis of the cleaved peptide. A contact time of 2 h with 20% piperidine represents 17 × 7 min Fmoc deprotection cycles, allowing the synthesis of medium size peptide chains without significant loss of the Dde group.§



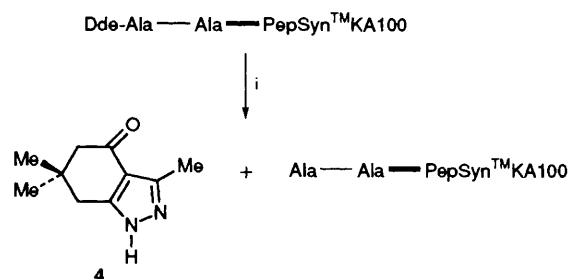
- 1; R¹ = H, R² = CH(X)CO₂H, α-amino acid
 2; R¹ = Me, R² = CH(X)CO₂H, α-amino acid
 3; R¹ = Me, R² = [CH₂]₄CH(Fmoc-NH)CO₂H

† This requires the orthogonal protection of the α- and ε-amino group of a lysine residue with the Fmoc and Boc group and elaboration in turn of a linear peptide from each amino group by Merrifield methodology.

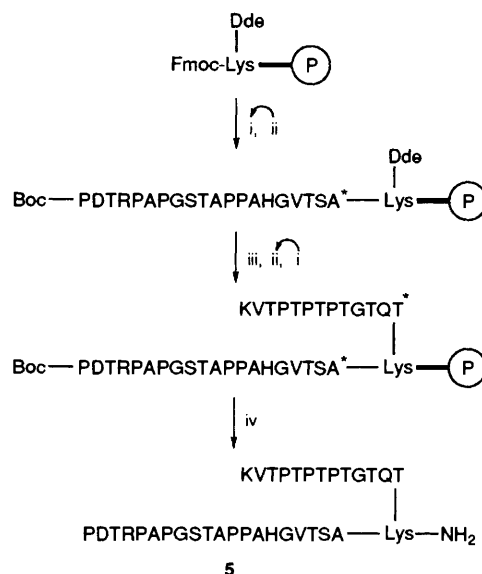
‡ Fmoc-Lys(Dde)-OH, m.p. 76–78 °C [α]_D²⁴ + 3.1 (c 2.0, MeOH); FAB-MS *m/z* 533 (*M*⁺ + H); ¹H NMR (250 MHz, CDCl₃) δ 1.00 (6H, s, Dde Me₂), 1.54 (2H, m, C^γH₂), 1.72 (2H, m, C^βH₂), 1.81, 1.97 (2H, 2 × m, C^βH₂), 2.36 (4H, s, Dde 2 × CH₂), 2.53 (3H, s, Dde C=Me), 3.40 (2H, m, C^εH₂), 4.19 (1H, t, *J* 6.95 Hz, Fmoc CH), 4.36 (2H, d, *J* 6.96 Hz, Fmoc CH₂), 4.46 (1H, m, C^αH), 5.81 (1H, d, *J* 8.21 Hz, N^εH), 7.27, 7.37, 7.58, 7.73 (8H, Fmoc ArH) 9.10 (1H, br s, C^αO₂H), 13.30 (1H, m, N^εH). The isomeric Dde-Lys(Fmoc)-OH is also readily prepared and both are now commercially available (Novabiochem UK Ltd, 3 Heathcoat Building, Highfields Science Park, Nottingham NG7 2QJ).

§ It is noteworthy that the Dde group unlike Dcm is compatible with uronium salt coupling reagents.

The potential of this protecting group for the synthesis of branched peptides is illustrated with the di-epitopic peptide 5 (Scheme 2) which contains antigenic sequences for both mammary⁶ and intestinal⁷ epithelial mucin. Acylation of Polyhipe SU 500 amide resin with Fmoc-Lys(Dde)-OH was followed by the construction at the N^ε-lysine position of the mammary antigenic sequence PDTRPAPGSTAP-PAHGVTSA, by standard Fmoc continuous flow strategy. This peptide was terminated with Boc-Pro and following Dde deprotection¶ with 2% v/v hydrazine in DMF, the intestinal



Scheme 1 Reagents and conditions: i, 2.0% v/v hydrazine in *N,N*-dimethylformamide (DMF), using continuous flow conditions (3 cm³ min⁻¹) and monitoring at 300 nm; a sharp peak is observed demonstrating that deprotection is complete after 3 min



5

(P) = Polyhipe SU500 amide resin; * Fmoc-amino acids employed had standard side chain protection

Scheme 2 Reagents and conditions: i, 20% v/v piperidine in DMF; ii, Fmoc-amino acid-*N*-hydroxybenzotriazole-diisopropylethylamine-2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; iii, 2% v/v hydrazine in DMF; iv, CF₃CO₂H-MeOH-H₂O-Et₃SiH (84:10:3:3)

¶ This deprotection can be monitored at the same wavelength used for removal of Fmoc with automated instruments such as NovaSyn Crystal, Millipore 9050 or ABI431A. Based on monitoring data, the deprotection step was optimised at 7.0 min with a flow rate of 3.5 cm³ min⁻¹.

antigenic sequence KVTPTPTPTGTQT was synthesised at the N^ε-lysine position once again by Fmoc methodology. The crude product, following TFA cleavage of the side-chain protection and release from the support, was purified by HPLC and characterised by plasma desorption mass spectrometry (PDMS) (MH⁺ requires *m/z* 3325.7, observed 3325.2), and amino acid analysis. The branched peptide binds independently to both C595 breast mucin, and 996/1 intestinal mucin monoclonal antibodies⁸ confirming the presence of both epitopes within the molecule.

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