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

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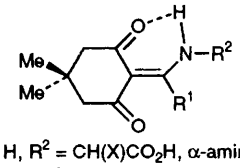
A new amine protecting group which can be used orthogonally with both Fmoc and Boc protection is reported; by employing lysine protected appropriately as the branching motif, a 34 residue di-epitopic peptide has been constructed by continuous flow solid phase peptide synthesis.

Currently solid phase peptide synthesis (SPPS) of multiple antigen peptide systems[1] (MAPs) containing different peptide sequences, *e.g.* both B and T cell epitopes, is only feasible using Merrifield methodology.[2]† In the preceding communication[3] 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-acetyldimedone 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 test[5] 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.§



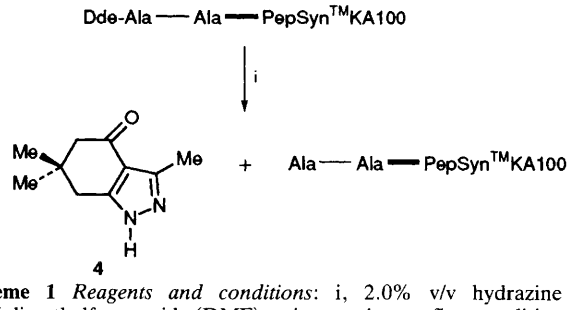
1; R^1 = H, R^2 = $CH(X)CO_2H$, α-amino acid
2; R^1 = Me, R^2 = $CH(X)CO_2H$, α-amino acid
3; R^1 = Me, R^2 = $[CH_2]_4CH(Fmoc\text{-}NH)CO_2H$

† 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 $[\alpha]_D^{24}$ + 3.1 (*c* 2.0, MeOH); FAB-MS *m/z* 533 (M^+ +H); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.00 (6H, s, Dde Me_2), 1.54 (2H, m, $C^{\gamma}H_2$), 1.72 (2H, m, $C^{\delta}H_2$), 1.81, 1.97 (2H, 2 × m, $C^{\beta}H_2$), 2.36 (4H, s, Dde 2 × CH_2), 2.53 (3H, s, Dde C=CMe), 3.40 (2H, m, $C^{\varepsilon}H_2$), 4.19 (1H, t, *J* 6.95 Hz, Fmoc CH), 4.36 (2H, d, *J* 6.96 Hz, Fmoc CH_2), 4.46 (1H, m, $C^{\alpha}H$), 5.81 (1H, d, *J* 8.21 Hz, $N^{\alpha}H$), 7.27, 7.37, 7.58, 7.73 (8H, Fmoc ArH) 9.10 (1H, br s, $C^{\alpha}O_2H$), 13.30 (1H, m, $N^{\varepsilon}H$). 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 mammary[6] and intestinal[7] epithelial mucin. Acylation of Polyhipe SU 500 amide resin with Fmoc-Lys(Dde)-OH was followed by the construction at the N^{α}-lysine position of the mammary antigenic sequence PDTRPAPGSTAP-PAHGVTSA, by standrd Fmoc continuous flow strategy. This peptide was terminated with Boc-Pro and following Dde deprotection¶ with 2% v/v hydrazine in DMF, the intestinal



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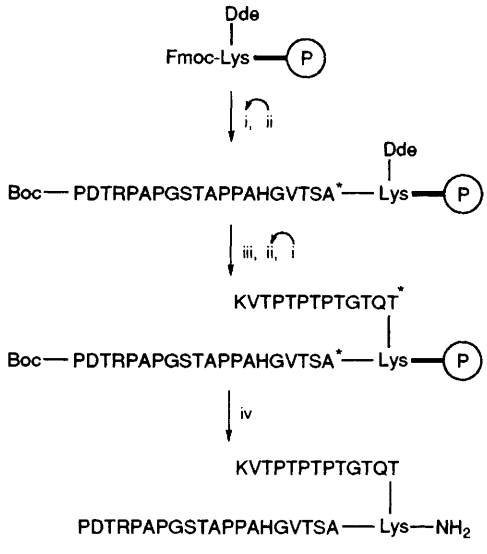
Scheme 1 *Reagents and conditions*: i, 2.0% v/v hydrazine in *N,N*-dimethylformamide (DMF), using continuous flow conditions (3 cm^3 min^{-1}) and monitoring at 300 nm; a sharp peak is observed demonstrating that deprotection is complete after 3 min



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(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, CF_3CO_2H–MeOH–H_2O–Et_3SiH (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 cm^3 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 antibodies[8] confirming the presence of both epitopes within the molecule.

We are grateful to Drs S. Hoare and T. White, and NovaBiochem for materials and assistance. We also acknowledge the SERC for the postgraduate student award to N. D. H.

Received, 23rd December 1992; Com. 2/06825G

References

1 J. P. Tam, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 5409.
2 R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149.
3 B. W. Bycroft, W. C. Chan, S. R. Chhabra, P. H. Teesdale-Spittle and P. M. Hardy, *J. Chem. Soc., Chem. Commun.*, preceding communication.
4 H. Machleidt and V. Hartmann, *Liebigs Ann. Chem.*, 1964, **679**, 9; H. Smith, *J. Chem. Soc.*, 1953, 803.
5 W. S. Hancock and J. E. Battersby, *Anal. Biochem.*, 1976, **71**, 260.
6 M. R. Price, J. A. Pugh, F. Hudecz, W. Griffiths, E. Jacobs, I. M. Symonds, A. J. Clarke, W. C. Chan and R. W. Baldwin, *Brit. J. Cancer*, 1991, **61**, 681.
7 J. R. Gum, J. C. Byrd, J. W. Hicks, N. W. Toribard, D. T. A. Lamport and Y. S. Kim, *J. Biol. Chem.*, 1989, **264**, 6480.
8 M. R. Price, unpublished results.