A Novel Amino Protection–Deprotection Procedure and Its Application in Solid Phase Peptide Synthesis

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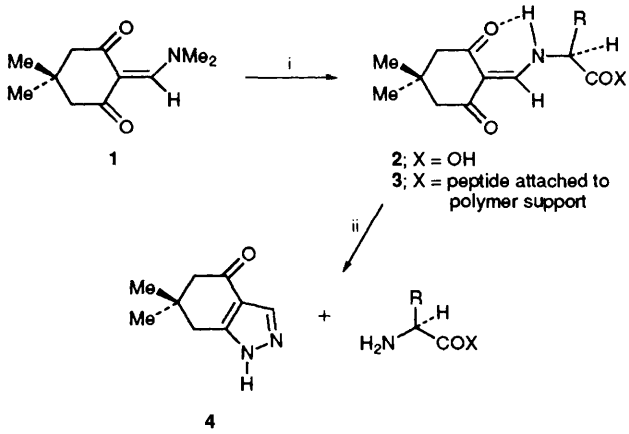
N-4,4-Dimethyl-2,6-dioxocyclohexylidenemethyl (Dcm) amino acids are readily prepared and the protecting group can be removed with hydrazine at room temperature; their potential in solid phase peptide synthesis is illustrated.

For a variety of reasons amino group protection procedures employed in peptide synthesis and in particular solid phase peptide synthesis (SPPS) are almost exclusively based on the chemistry of urethane derivatives.[1] A particularly effective strategy employed in SPPS capitalises on the base lability of the fluorenylmethoxycarbonyl group (Fmoc);[2] this allows side-chain functionalities to be orthogonally blocked with acid-labile groups, all of which can subsequently be removed concomitant with the release of the peptide from the support.

We now report the prototype of a novel and alternative amino protection procedure which can be used independently or in conjunction with the Fmoc–SPPS strategy. 5,5-Dimethyl-2-(dimethylaminomethylene)cyclohexane-1,3-dione **1** is readily prepared from dimedone and dimethylformamide dimethylacetal[3] and reacts at room temperature with amino acids to afford stable, crystalline derivatives† **2**. Dcm amino acids can then be coupled to the free amino terminal of a polymer-bound amino acid or peptide **3** using standard diisopropylcarbodiimide–hydroxybenzotriazolyl (DIPCDI–HOBt) conditions. The structure of the Dcm protecting group precludes racemisation *via* the usual oxazolone mechanism and, as determined by standard procedures,‡ none is detectable with the coupling conditions described above. For



Scheme 1 *Reagents and conditions*: i, amino acid in ethanol, room temp.; ii, 1% hydrazine in dimethylformamide, room temp.

† The amino acid is stirred usually overnight with **1** in ethanol at room temperature. After removal of solvent, the residue is triturated with diethyl ether and the resulting solid partitioned between aqueous $KHSO_4$ and EtOAc. The *N*-4,4-dimethyl-2,6-dioxocyclohexylidenemethyl (Dcm) amino acid is obtained from the organic phase and recrystallised (EtOAc–light petroleum). All the principle amino acids (with side-chain protection where appropriate), with the exception of proline, have been prepared and fully characterised: for example, Dcm-Val-OH (yield 76%), m.p. 200–201 °C, $[\alpha]_D^{25}$ (*c* 1.0, MeOH) – 27.9, ^{1}H NMR (250 MHz, $CDCl_3$): δ 11.48 (dd, J_1 9.0, J_2 14.0 Hz, NH), 9.70 (s, CO_2H), 8.14 (d, *J* 14.0 Hz, C=CH), 3.92 (dd, J_1 4.0, J_2 9.0 Hz, α-CH), 2.40 (s, CH_2), 2.37 (s, CH_2), 2.33 (m, β-CH), 1.06 (s, CH_3) 1.05 (s, CH_3), 1.02 (d, *J* 6.0 Hz, γ-CH_3) and 0.99 (d, *J* 6.0 Hz, γ-CH_3).

‡ The absence of detectable racemisation was established by HPLC and NMR comparisons of Dcm dipeptides (Dcm-Phe-Ala-OBzl and Dcm-D-Phe-Ala-OBzl) of established chirality against those obtained from coupling regimes.

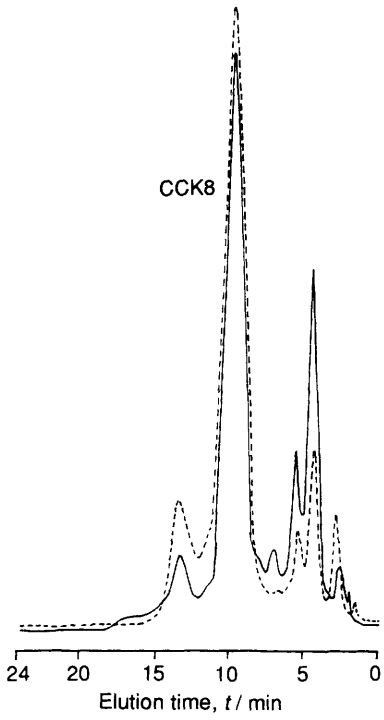


Fig. 1 HPLC of crude CCK8 **6** prepared from Dcm-amino acids (dotted trace) and Fmoc-amino acids (solid trace), C_{18} μ-Bondapak column using 0.1% CF_3CO_2H in H_2O–MeCN (75:25 v/v) as the eluent. CCK8 from Dcm preparation was isolated and showed to be identical with authentic material.

primary amino acids the structure of the Dcm derivative is stabilised by an intramolecular hydrogen bond (see NMR data). This is not the case for secondary amino acids such as proline and *N*-methylamino acids and although these can be prepared they are not sufficiently stable for use in peptide synthesis.

Removal of the Dcm group is readily achieved with a dilute solution of hydrazine in polar solvents at room temperature, the reaction being driven by the formation of the 1,2-diazole[4] **4** (Scheme 1).§ Alternatively the conditions for the removal of Fmoc, *i.e.* secondary bases such as piperidine and morpholine, are also effective. Schmitt–Walker assays of the free amino content of a sample of Dcm-Ala-Leu-KA(100)¶ following treatment with 1.0% and 0.1% v/v hydrazine hydrate in dimethylformamide (DMF) revealed essentially complete removal of Dcm after 10 and 20 min respectively, whereas 20% v/v piperidine and morpholine required 20 and 40 min, respectively, significantly longer time intervals than those employed to remove Fmoc.

All the acid-labile side-chain protecting groups conventionally used with the Fmoc strategy are stable to both sets of conditions, as are the various ester and amide linkages to the polymer supports. In particular no hydrazinolysis of the *tert*-butyl esters of Asp and Glu was detectable when polymer-bound peptides containing these residues were exposed to 1% hydrazine in DMF for 24 h at 25 °C. Dcm derivatives are stable to the acid conditions required for the cleavage of all the aforementioned groups.

H-Tyr–Gly–Gly–Phe–Leu-OH
5

H-Asp–Tyr–Met–Gly–Trp–Met–Asp–Phe-NH_2
6

To evaluate the efficacy of Dcm protection in SPPS, syntheses of [Leu[5]]-enkephalin **5** and the peptide amide CCK 8 **6** were undertaken and compared with standard Fmoc procedures. The initial manual batch synthesis of [Leu[5]]-enkephalin commenced with Fmoc-Leu-KA(100)¶ employing Dcm-Phe-OH and Dcm-Gly-OH but terminating with Fmoc-Tyr(OBu[t])-OH. The product purity and yield using a combination of normal Fmoc procedures and coupling and deprotection protocols for the Dcm group of either (*i*) DIPCDI–HOBt and 1% hydrazine hydrate (v/v) in DMF (30 min), or (*ii*) DIPCDI–HOBt and 20% morpholine in DMF (1 h) were comparable with those of a parallel standard Fmoc SPPS. This was the most effective of the coupling regimes investigated. In this context it should be noted that Dcm is not compatible with uronium salt coupling reagents. Similar product quality was obtained in an automated continuous flow synthesis again commencing with Fmoc-Leu-KA(100) but using all Dcm amino acids and the conditions (*i*) described above.‖

To ascertain whether the preparation of peptide amides was compatible with the methodology, CCK8 **6** was constructed using Dcm-amino acids (side-chain *tert*-butyl protection for Asp and Tyr) from Pepsyn KAM(100) under the coupling and deprotection regimes (*i*) described above. The peptide was cleaved from the support with trifluoroacetic acid–anisole–ethane-1,2-dithiol and the major product identified with authentic material. The HPLC pattern of the crude product was compared with that of **6** prepared by the standard Fmoc–SPPS procedure from H-KAM(100) (Fig. 1). Both contained a similar pattern of impurities but lower levels in the Dcm-based preparation.

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References

1 For reviews see: *The Peptides: Analysis, Synthesis and Biology*, vols. 1–9, ed. E. Gross and J. Meinhofer, Academic Press, New York, 1979; R. B. Merrifield, *Science*, 1986, **232**, 341; G. Barany, N. Kneib-Cordonier and D. G. Mullen, *Int. J. Peptide Protein Res.*, 1987, **30**, 705; E. Atherton and R. C. Sheppard, *Solid Phase Peptide Synthesis: a Practical Approach*, IRL Press, Oxford, 1989.

2 See: E. Atherton, M. J. Gait, R. C. Sheppard and B. J. Williams, *Bioorg. Chem.*, 1979, **8**, 351; E. Atherton and R. C. Sheppard in *Perspectives in Peptide Chemistry*, ed. A. Eberle, R. Geiger and Th. Wieland, Karger, Basle 1981; R. C. Sheppard, *Science Tools*, 1986, **33**, 9.

3 L. Mosti, G. Menozzi and P. Schenone, *J. Heterocycl. Chem.*, 1982, **19**, 1355.

4 L. Mosti, G. Menozzi and P. Schenone, *J. Heterocycl. Chem.*, 1983, **20**, 645, 649.

5 H. W. Schmitt and J. E. Walker, *FEBS Lett.*, 1977, **81**, 403.

§ The ease with which **1** reacts with a range of dinucleophiles to displace dimethylamine and forms aromatic heterocyclic systems[3,4] provided the rationale for the deprotection step. Hydrazine was subsequently adopted since its reaction with **1** appeared to be quantitative at room temperature.

¶ KA(100), polyamide on Kieselguhr support (Pepsyn KA) with a loading of 100 μmol g^{-1}.

‖ A particular advantage of the Dmc group is that, on cleavage with hydrazine, the product **4** has a strong absorption at 360 nm (ε 15 020 dm^3 mol^{-1} cm^{-1}, CH_2Cl_2), thereby allowing monitoring at the same wavelength used for the 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}. The resultant [Leu[5]]-enkephalin was identical in all respects to authentic material.