A Novel 4-Aminobenzyl Ester-based Carboxy-protecting Group for Synthesis of Atypical Peptides by Fmoc–Bu^t Solid-phase Chemistry

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4-{*N*-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl ester (ODmab) is a new carboxy-protecting group that is based on the safety-catch principle and can be used orthogonally with Fmoc–Bu^t peptide chemistry, but is completely removable with 2% *v/v* hydrazine·H_2O–DMF at room temperature within minutes.

Recently we reported the use of *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (*N*-Dde)[1] as a new amino-protecting group which is quasi-orthogonal with *N*-Fmoc and finding increasing application in the synthesis of atypical peptides[2] assembled by Fmoc–Bu^t solid-phase methodologies. By applying the 'safety-catch' principle we reasoned that *N*-Dde[1] and its conditions for deprotection, when coupled with the known lability of 4-aminobenzyl esters,[3] could be adapted to develop a novel carboxy-protecting strategy.

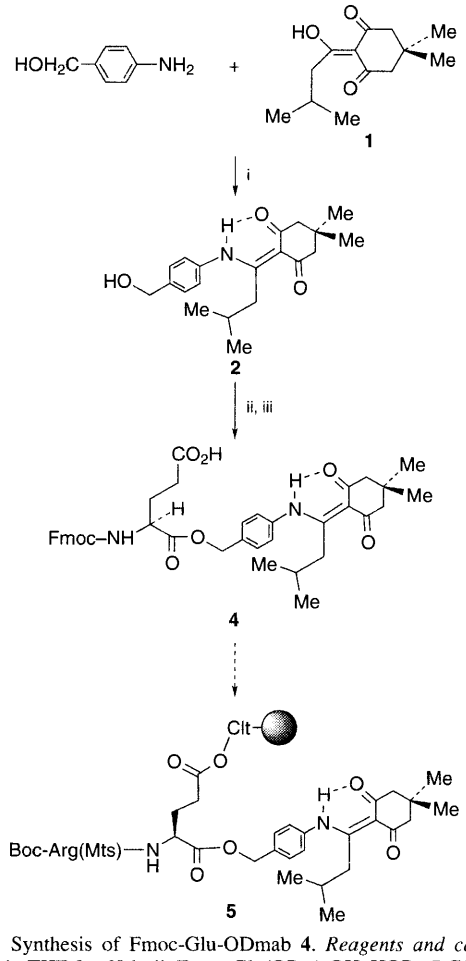
A high fidelity selective protection–deprotection procedure for either the side-chain or α-carboxylic functionality of either aspartyl or glutamyl residues within a resin-bound peptide would offer a valuable approach for the construction of complex modified peptides, *e.g.* branched, head-to-tail and side-chain lactam-bridged cyclic and glyco-peptides. Furthermore, such methodology would have significant implications, not only for individually identified target molecules, but also for the multiple synthesis of modified peptides and non-peptide entities. To date only the allyl ester[4] and trimethylsilyl ethyl ester[5] have found general applications in this context, but both have limitations.†

The crystalline adduct derived from the condensation of 2-acetyldimedone with 4-aminobenzyl alcohol readily reacts with Fmoc-Glu(OBu^t)-OH and Fmoc-Asp(OBu^t)-OH *via* 1,3-dicyclohexylcarbodiimide (DCC) activation to yield esters which on mild acidic treatment afford the corresponding acids Fmoc-Glu 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene) ethyl]amino}benzyl ester (ODab) and Fmoc-Asp-ODab in 60–70% overall yields.[6] These prototype esters based on Dde are indeed stable to TFA and are quantitatively deprotected with 2% *v/v* hydrazine–DMF at room temperature within minutes. Treatment with a dinucleophile reagent initially removes the *N*-Dde group which is followed by a spontaneous collapse, *via* a 1,6-electron pair shift, of the resultant 4-aminobenzyl ester to afford the required free acid. However, the ODab esters displayed a small but significant degree of instability to the *N*-Fmoc deprotection conditions (13% lost after 1 h continuous-flow treatment with 20% piperidine– DMF).

As a consequence of a systematic evaluation,‡ we now report that 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl ester (ODmab) retains all the desired chemical properties, as well as being completely stable to the *N*-Fmoc deprotection conditions, *i.e.* 20% piperidine–DMF. The application of the ODmab ester as an orthogonal protecting group is demonstrated by the synthesis of a model cyclic peptide (based on a tachykinin antagonist[7]) by on-resin head-to-tail cyclisation of the corresponding linear peptide assembled using Fmoc-Glu-ODmab and standard Fmoc/Bu^t procedures[8] on 2-chlorotrityl polystyrene resin.[9]

The desired protected amino acid, Fmoc-Glu-ODmab, was prepared in three simple steps (Scheme 1) from commercially available Fmoc-Glu(OBu^t)-OH. The key component, 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino}benzyl alcohol **2** was readily obtained as pale yellow crystals in *ca.* 70% yield (mp 154–157 °C) from the reaction of 4-aminobenzyl alcohol and 2-(3-methylbutyryl)dimedone **1** in refluxing THF. The esterification of **2** with Fmoc-Glu(OBu^t)-OH was accomplished by activation with DCC to yield Fmoc-Glu(OBu^t)-ODmab **3**, which on treatment with TFA–CH_2Cl_2 gave the required Fmoc-Glu-ODmab‡ **4** as off-white crystals in an overall yield of *ca.* 55%.

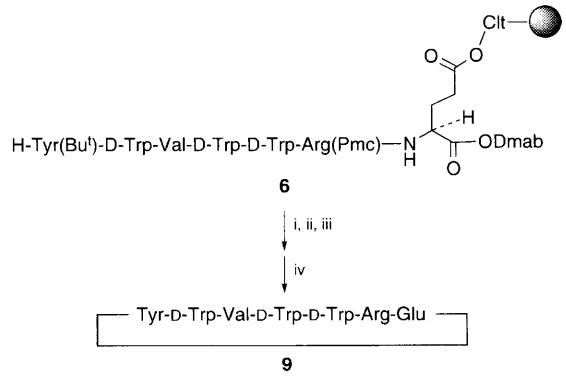
The resin-bound model dipeptide **5** was used initially to establish the relative stability of the ODmab and related esters to 20% piperidine–DMF. Compound **5** was prepared by loading **4** *via* the side chain carboxy group onto a 2-chlorotrityl chloride polystyrene,[9] Fmoc-deprotecting, and acylating with Boc-Arg(Mts)-OH under standard conditions.[8]§ The resin **5** was then subjected to continuous-flow treatment with 20% piperidine–DMF (1 ml min-1) for 4 h. Reverse phase HPLC analysis¶ of the crude cleavage (using 0.5% TFA–CH_2Cl_2, 1–2 min) product showed exclusively Boc-Arg(Mts)-Glu-ODmab (>99%). Having established the excellent stability of the ODmab ester, **4** was utilised for the synthesis of our model cyclic peptide **9**.



Scheme 1 Synthesis of Fmoc-Glu-ODmab **4**. *Reagents and conditions*: i, refluxed in THF for 60 h; ii, Fmoc-Glu(OBu^t)-OH–HOBt–DCC–DIPEA (1 : 1 : 1 : 2, 0.7 equiv) in CH_2Cl_2, 18 h; iii, 50% TFA–CH_2Cl_2, 2 h.

The peptide-resin **6** was assembled using standard automated Fmoc–Bu^t procedures.§ Treatment of **6** with 1% TFA–CH_2Cl_2, and with 2% hydrazine·H_2O–DMF (5 min) followed by 1% TFA–CH_2Cl_2 gave exclusively the desired peptidic molecules H-Tyr(Bu^t)-D-Trp-Val-D-Trp-D-Trp-Arg(Pmc)-Glu-ODmab **7** and H-Tyr(Bu^t)-D-Trp-Val-D-Trp-D-Trp-Arg(Pmc)-Glu-OH **8**, respectively, in excellent yields (>95%). The general applicability of our carboxy-protecting group for the on-resin synthesis of cyclic peptides is demonstrated as follows (Scheme 2): **6** was treated with 2% hydrazine·H_2O–DMF, washed with 5% *N,N*-diisopropylethylamine (DIPEA)–DMF, followed by *in situ* cyclisation by activation using 1-hydroxy-7-azabenzotriazole (HOAt)–DCC,[10] and finally simultaneous deprotection–cleavage from solid-support with TFA–H_2O–$HSCH_2CH_2SH$–Pr^i_3SiH (90:5:4:1% *v/v*) to afford the cyclic peptide **9** in good purity and yield (Fig. 1).

In conclusion, we have demonstrated an efficient and simple protection–deprotection procedure for carboxylic acids that is orthogonal to both acid (TFA)- and 20% piperidine- labile protecting groups used in standard and automated peptide chemistry, and complements our previously reported *N*-Dde for the protection of Lys side-chain amine functionality.



Scheme 2 Fmoc–Bu^t solid-phase synthesis of a model cyclic peptide utilising **4**. *Reagents and conditions*: i, 2% *v/v* hydrazine·H_2O–DMF, 7 min; ii, 5% *v/v* DIPEA–DMF, 10 min; iii, DCC, HOAt (1.2 molar excess) in DMF, 18 h; iv, TFA–H_2O–$HSCH_2CH_2SH$–Pr^i_3SiH (90:5:4:1% *v/v*), 30 °C, 2 h.

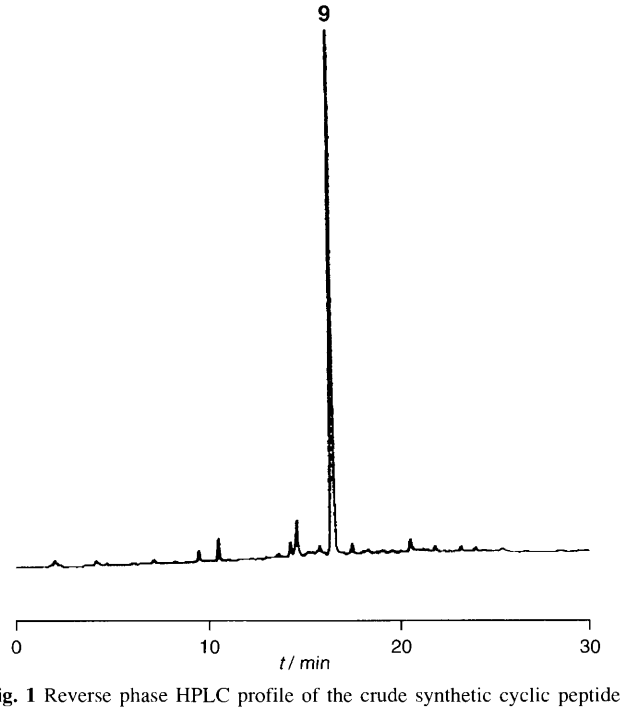


Fig. 1 Reverse phase HPLC profile of the crude synthetic cyclic peptide **9**

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Footnotes

† The selective removal of the allyl ester requires the use of complex mixtures such as $Pd(Ph_3P)_4$–AcOH–*N*-methylmorpholine–$CHCl_3$ over an extended period of 2 h. The trimethylsilylethyl ester (OTmse) is a valuable protecting group but is unstable to mild acidic conditions, *e.g.* TFA.

‡ The synthesis and comparative evaluation of esters based on 2-acetyldimedone, 2-propionyldimedone, 2-(4-methylvaleryl)dimedone and 2-(3-methylbutyryl)dimedone will be reported in detail elsewhere. *Selected data* for *N*-Fmoc-Glu-ODmab **4**: mp 93–95 °C; δ_H ($CDCl_3$, 250 MHz) 0.73 [6 H, d, *J* 6.70 Hz, (CH_3)$_2$–CH], 1.05 (6 H, s, 2 CH_3), 1.86 (1 H, m, Me_2–CH), 2.05, 2.28 (2 H, 2 m, Glu C$^\beta H_2$), 2.38 (2 H, s, CH_2), 2.46 (2 H, s, CH_2), 2.47 (2 H, m, Glu C$^\gamma H_2$), 2.96 (2 H, d, *J* 6.84 Hz, CH_2–CH), 4.18 (1 H, m, Fmoc CH), 4.38 (2 H, m, Fmoc CH_2), 4.45 (1 H, m, Glu C$^\alpha H$), 5.18 (2 H, s, Bzl CH_2), 5.55 (1 H, d, *J* 7.98 Hz, Glu NH), 7.08, 7.36 (4 H, 2 d, *J* 8.3 Hz, Bzl Arom. CH), 7.24–7.40, 7.55, 7.74 (8 H, m, Fmoc Arom. CH), 15.24 (1 H, s, NH); electrospray (ES)-MS, MH^+ calc. 681.8, found 681.4. The corresponding derivative *N*-Fmoc-Asp-ODmab, mp 107–112 °C, has also been synthesised.

§ Solid-phase assembly of peptides was accomplished by Fmoc continuous-flow procedures using a Millipore PepSynthesizer® 9050. Carboxy activation was achieved by the mixture *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)–DIPEA–1-hydroxybenzotriazole (HOBt) (1:2:1 molar ratio). Mts = N^G-2,4,6-trimethylbenzenesulfonyl.

¶ Purified synthetic intermediates, derivatised amino acids and crude synthetic peptides were analysed by reverse phase HPLC on Hypersil Pep300 C_3 column (4.6 × 150 mm). The elution gradient was either 20 to 70% **B** in 30 min or 20 to 100% **B** in 35 min at 1.20 ml min^{-1} (**A** = 0.06% aq.TFA, **B** = 0.06% TFA in 90% aq. MeCN) and the eluate monitored at 220 nm. All purified synthetic peptides gave expected plasma desorption-MS data: **7** calc. MH^+ 1759.7, found 1761.2; **8** calc. 1447.7, found 1448.1; **9** calc. 1106.2, found 1107.7.

References

1 B. W. Bycroft, W. C. Chan, S. R. Chhabra and N. D. Hone, *J. Chem. Soc., Chem. Commun.*, 1993, 778.

2 G. B. Bloomberg, D. Askin, A. R. Gargaro and M. J. A. Tanner, *Tetrahedron Lett.*, 1993, **34**, 4709; H. F. Brugghe, H. A. M. Timmermans, L. M. A. Van Unen, G. J. T. Hove, G. Van de Werken, J. T. Poolman and P. Hoogerhout, *Int. J. Peptide Protein Res.*, 1994, **43**, 166; W. C. Chan, B. W. Bycroft, D. J. Evans and P. D. White, in *Peptides: Chemistry, Structure and Biology*, ed. R. S. Hodges and J. A. Smith, ESCOM, Leiden, 1994, p. 727; B. W. Bycroft, W. C. Chan, N. D. Hone, S. Millington and I.A. Nash, *J. Am. Chem. Soc.*, 1994, **116**, 7415; J.-C. Truffert, O. Lorthioir, U. Asseline, N. T. Thuong and A. Brack, *Tetrahedron Lett.*, 1994, **35**, 2353; P. Dumy, I. M. Eggleston, S. Cervigni, U. Sila, X. Sun and M. Mutter, *Tetrahedron Lett.*, 1995, **36**, 1255; N. Ahlborg, *J. Immunol. Methods*, 1995, **179**, 269.

3 C. S. Pande, S. K. Gupta and J. D. Glass, *Indian J. Chem.*, 1987, **26B**, 957.

4 M. H. Lyttle and D. Hudson, in *Peptides: Chemistry and Biology*, ed. J. Smith and J. E. Rivier, ESCOM, Leiden, 1992, p. 583; A. Loffet and H. X. Zhang, *Int. J. Peptide Protein Res.*, 1993, **42**, 346; S. A. Kates, N. A. Solé, C. R. Johnson, D. Hudson, G. Barany and F. Albericio, *Tetrahedron Lett.*, 1993, **34**, 1549; S. A. Kates, S. B. Daniels, N. A. Solé, G. Barany and F. Albericio, in *Peptides: Chemistry, Structure and Biology*, ed. R. S. Hodges and J. A. Smith, ESCOM, Leiden, 1994, p. 113.

5 C. K. Marlowe, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 437.

6 W. C. Chan, B. W. Bycroft, D. J. Evans and P. D. White, in *Peptides 1994*, ed. H. L. S. Maia, ESCOM, Leiden, 1995, p. 153.

7 P. Rovero, V. Pestellini, C. A. Maggi, R. Patacchini, D. Regoli and A. Giachetti, *Eur. J. Pharmacol.*, 1990, **175**, 113.

8 E. Atherton and R. C. Sheppard, *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford, 1989; G. B. Fields and R. L. Noble, *Int. J. Peptide Protein Res.*, 1990, **35**, 161.

9 K. Barlos, O. Chatzi, D. Gatos and G. Stavropoulos, G. *Int. J. Peptide Protein Res.*, 1991, **37**, 513.

10 L. A. Carpino, *J. Am. Chem. Soc.*, 1993, **115**, 4397.