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A new generation of reversible backbone-amide protection for the solid phase synthesis of difficult sequences

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Abstract

A versatile safety catch backbone-amide protection system (**1**, **2**) has been developed to inhibit interchain aggregation during solid phase peptide synthesis. The strategy utilises the N^{α}-Fmoc derivative of an *N*-(3-methylsulfinyl-4-methoxy-6-hydroxybenzyl) (SiMB, **2**) substituted amino acid (**5b**), a group which exhibits excellent all round coupling kinetics. The value of these improved derivatives is demonstrated through the syntheses of leucine enkephalinamide (**9**) and the well known 'difficult sequence' from the acyl carrier protein, residues (65–74) (**10**) via standard uronium activation and pentafluorophenyl ester chemistry.

Keywords: amino acids and derivatives; solid phase synthesis; peptides and polypeptides.

In solid phase peptide synthesis 'difficult sequences'[1] are characterised by the sudden appearance of sluggish deprotection and acylation reactions. It has been proposed that this is a consequence of resin-bound peptide chain self-association leading to the formation of aggregated secondary structures such as β-sheets.[1] The dramatic reduction in acylation and deprotection rates, which may persist throughout the remainder of a synthesis, frequently yields poor quality crude products. A major contributing factor to 'aggregation' is interchain backbone-amide hydrogen bonding between growing peptide chains.[2] Based upon these observations, we have previously described the *N*-(2-hydroxy-4-methoxybenzyl) (Hmb, **3**) backbone-amide protecting group,[3] for the temporary protection of the secondary amide bond. This was designed to be compatible with the Fmoc/*t*-butyl† approach to solid phase peptide synthesis.[4] The judicial use of Hmb backbone protection inhibits the formation of aggregated secondary structures and has provided excellent improvements in the quality of crude product from many previously poor syntheses.[5]

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† BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; Fmoc, fluoren-9-ylmethoxycarbonyl; Fmoc–Cl, fluoren-9-ylmethoxycarbonylchloroformate; HOBt, 1-hydroxybenzotriazole; MeTf, methyl triflate; NMM, *N*-methylmorpholine; TFA, trifluoroacetic acid; TMS–Cl, trimethylsilyl chloride.

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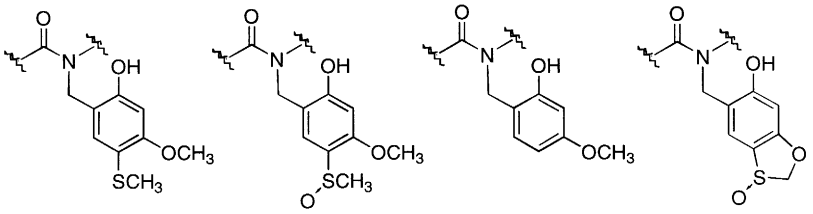
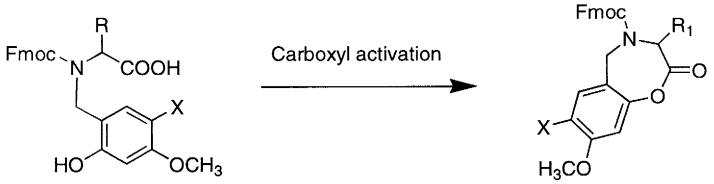


(**1**) (**2**) (**3**) (**4**)

Hmb protection is introduced into a peptide chain via *N*,*O*-bisFmoc-N^{α}-(2-hydroxy-4-methoxybenzyl) amino acid derivatives.[3] Direct N^{α}-acylation of the incorporated N^{α}-(Hmb) residue is hindered, but is mechanistically facilitated by initial acylation of the 2-hydroxyl function followed by intramolecular O→N acyl transfer.[3] The optimal reagent for *O*-acylation of N^{α}-Hmb protected residues is the symmetric anhydride, which, despite the acyl transfer mechanism, may require extended reaction times for quantitative coupling.[3] Thus, for routine use, the major shortcomings of the Hmb group are the time-consuming preparation of amino acid derivatives and their slow (albeit quantitative) *N*-acylation. Building upon the undoubted success of the Hmb group, we have incorporated new design elements into a second generation of amide-bond protection, SiMB (**2**), which provides the synthetic benefits afforded by backbone protection together with improved acylation kinetics.

Our own early investigations and those of others, noted that mono-N^{α}-Fmoc-N^{α}-(Hmb)amino acids (**5a**), although relatively simple to prepare, formed, upon carboxyl activation, a 4,5-dihydro-8-methoxy-1,4-benzoxapin-2(3*H*)-one (**6a**).[6] Species **6a** was poorly reactive, which necessitated the use of *N*,*O*-bisFmoc-N^{α}-(Hmb)amino acid residues for quantitative Hmb amino acid coupling.[3] In order to exploit the relative ease of synthesising N^{α}-Fmoc-N^{α}-substituted amino acids, we reasoned that addition of an electron withdrawing substituent *para* to the phenolic hydroxyl functionality should improve acylation kinetics of intermediate benzoxapin-2(3*H*)-ones (**6a**→**6b**) and yield synthetically useful activated amino acid derivatives. In addition, the rate of intramolecular O→N acyl transfer would also be expected to increase significantly. An attractive candidate for this purpose, compatible with other procedures encountered during solid phase peptide synthesis, was an oxidation–reduction system based upon the readily reversible sulphide (TFA labile, poorly active benzoxapin-2(3*H*)-one) to sulphone (TFA stable, highly active benzoxapin-2(*3H*)-one)[7] transition. We initially introduced this safety catch concept through the 6-hydroxy-5-methyl-1,3-benzoxathiolyl group (**4**), which gave excellent coupling kinetics and low epimerisation as the C-terminal residue of a protected peptide segment.[8]

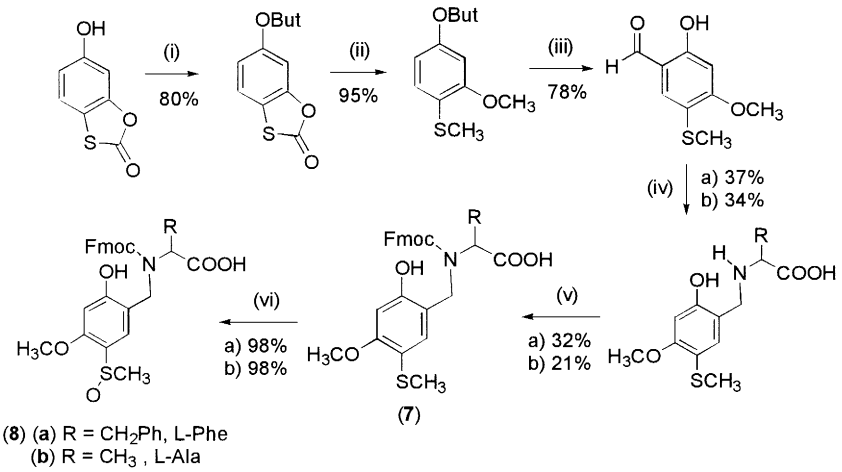


(**5**)
R = amino acid side-chain
(**a**) X = H, (**b**) X = $SOCH_3$

(**6**) (**a**,**b**)

Recently, we have found that the open chain version of (**4**), i.e. Fmoc-(SiMB)amino acids (**8a** and **8b**) are readily prepared (Scheme 1), from the commercially available 6-hydroxybenzoxathialone (Aldrich), yielding stable, crystalline solids.[9]

The performance of derivatives **8a** and **8b** has been investigated in the preparation of two test sequences (**9** and **10**). The two diastereoisomers of (**9**) were initially prepared by standard solid phase



Scheme 1. Reagents and conditions: (i) isobutylene/MeTf; (ii) CH_3I/Cs_2CO_3/2-butanone; (iii) $POCl_3$/DMF; (iv) (a) H-amino acid-OH/NaOH; (b) $NaBH_4$; (v) (a) TMS-Cl/DIEA; (b) Fmoc-Cl; (vi) $NaIO_4$

peptide synthesis techniques,[10] to enable the chiral integrity and acylation kinetics of N^{α}-Fmoc-N^{α}-(SiMB)-L-phenylalanine (**8a**) to be examined. As expected, amino acid derivative **8a** was incorporated using a standard coupling method (BOP/HOBt/NMM, 5 equiv.) within 45 min. Following N^{α}-Fmoc deprotection, the anticipated increase in the rate of acylation was observed with quantitative coupling of Phe onto N^{α}-(SiMB)Phe using pentafluorophenyl ester chemistry in 45 mins. It should be noted that the coupling of Phe to N^{α}-(Hmb)Phe requires the use of symmetric anhydrides for expended periods (24 h).[3] Completion of the assembly followed by cleavage using reductive acidolysis[11] gave chirally intact crude product (Fig. 1a,b).

H-Tyr-Phe-(D/L)Phe-Gly-Leu-NH_2 (**9**)

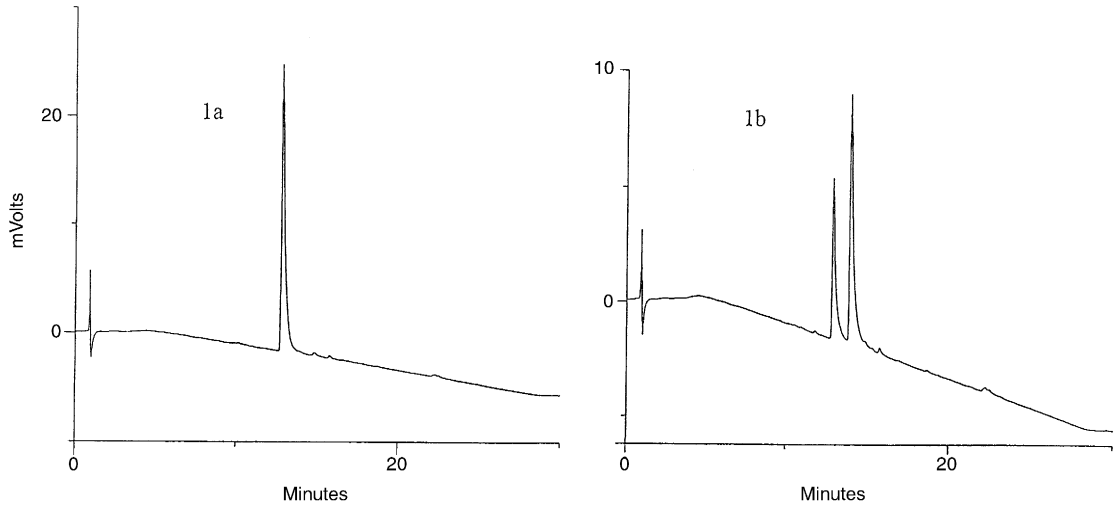


Fig. 1. Analytical HPLC (Phenomenex Jupiter C4 column: 10% B in A to 90% B in A over 25 min: A=0.1% aq. TFA, B=90% CH_3CN, 10% A: 1.5 ml min^{-1} flow rate monitoring at 215 nm). Compound **1a** crude [Leu^5]-enkephalinamide (**9**: L-Phe): **1b** crude [Leu^5]-enkephalinamide (**9**: L+D-Phe; co-injection of enantiomers)

The second test peptide was chosen to highlight the use of N^{α}-Fmoc-N^{α}-(SiMB)-L-alanine (**8b**) in the synthesis of the well known difficult sequence from the acyl carrier protein, residues (65–74) (**10**).[12]

ACP (65–74) is known to aggregate significantly on deprotection of the penultimate glutamine residue. Incorporation of the final valine residue is often incomplete, typically reaching 85–90% via traditional solid phase procedures.[12]

H- Val^{65}-Gln-Ala-Ala^{68}-Ile^{69}-Asp-Tyr-Ile-Asn-Gly^{74}-NH_2 (**10**)

Using standard pentafluorophenol ester coupling conditions,[10] the peptide was assembled upto Ile^{69}. Ala^{68} was incorporated by coupling N^{α}-Fmoc-N^{α}-(SiMB)-L-alanine (**8b**) via BOP/HOBt/NMM (5 equiv. 45 mins) activation. Removal of the Fmoc group was followed by acylation of the secondary amine using Fmoc-Ala-OPfp/HOBt in a standard 45 min coupling. Continuation of the sequence gave the desired peptide, with no detectable level of the desVal nonapeptide[13] (Fig. 2). Amino acid analysis confirmed the quantitative incorporation of the final valine residue.

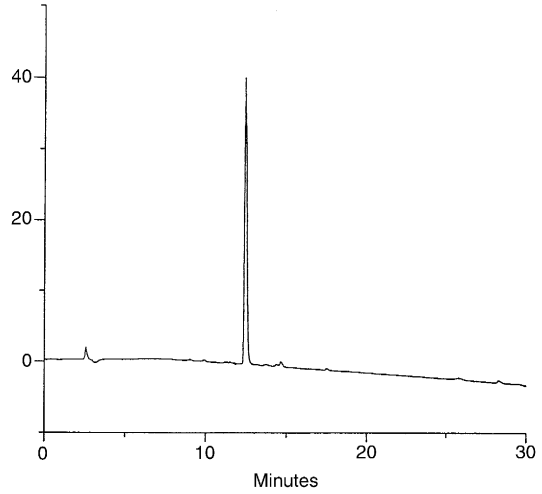


Fig. 2. Analytical HPLC (Phenomenex Jupiter C4 column: 10% B in A to 40% B in A over 25 min: A=0.1% aq. TFA, B=90% CH_3CN, 10% A: 1.5 ml min^{-1} flow rate, monitoring at 215 nm). Crude ACP (65–74) (**10**)

Based on a sound understanding of backbone protection, as applied to solid phase peptide synthesis, we have designed and prepared a novel amide protecting group, SiMB (**2**). N^{α}-Fmoc-(N^{α}-SiMB) amino acid derivatives (**5b**, **8a** and **8b**) are prepared readily and couple quantitatively through standard uronium carboxyl activation (via the intermediate benzoxapin-2(3*H*)-one). Efficient (quantitative) acylation of N^{α}-SiMB amino acids is achieved with commercially available active esters opening the way for fully automated synthesis of 'difficult sequences'.

References

1. (a) Kent, S. B. H. In *Peptides, Structure and Function; Proceedings of the 9th American Peptide Symposium*; Deber, C. M.; Hruby, V. J.; Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; 407. (b) Atherton, E.; Sheppard, R. C. *ibid*, 415.
2. Kent, S. B. H.; Alewood, D.; Alewood, P.; Baca, M.; Jones, A.; Schnollzer, M. In *Innovations and Perspectives in Solid Phase Synthesis, 2nd International Symposium*; Epton, R., Ed.; Intercept: Andover, 1992; 1.
3. Johnson, T.; Quibell, M.; Sheppard, R. C. *J. Pept. Sci.* **1995**, *1*, 11. A range of derivatives are commercially available from Calbiochem-Novabiochem.
4. Atherton, E.; Sheppard, R. C. In *Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press, Oxford, 1989; p. 1.
5. Hyde, C.; Johnson, T. J.; Owen, D.; Quibell, M.; Sheppard, R. C. *Int. J. Pept. Protein Res*. **1994**, *43*, 431.
6. (a) Nicolas, E.; Pujades, M.; Bacardit, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 2317. (b) Quibell, M., unpublished observations, 1993.

7. (a) Patek, M.; Lebl, M. *Tetrahedron Lett*, **1991**, *32*, 3891. (b) Kiso, Y.; Fukui, T.; Tanaka, S.; Kimura, T.; Akaji, K. *Tetrahedron Lett.* **1994**, *35*, 3571.
8. Offer, J.; Johnson, T.; Quibell, M. *Tetrahedron Lett.* **1997**, *38*, 9047.
9. Compound (**8a**) was prepared in 37% yield from resorcinol. Analytical HPLC showed the presence of a single peak with ESMS 586.1 (MH^+); 1171.3 ($2MH^+$), calculated mass: $C_{33}H_{31}NO_7S$, 585.6.
10. All work was carried out using Novasyn® TGR resin (Novabiochem). Couplings were carried out in DMF using 5 equiv. activated amino acid derivatives. Coupling yields were quantified by amino acid analysis (Biochrom 20 analyser).
11. Reductive acidolysis conditions; peptide-resin was treated with $SiCl_4$:TFA:anisole:EDT (5:90:2.5:2.5 v/v) for 2 h at room temperature. Following filtration, TFA was removed by nitrogen sparging, peptides were precipitated and washed with *tert*-butylmethyl ether precipitation. Peptide **9** via derivative **8a**, gave a single species (see Fig. 1a) with ESMS 645.5 (MH^+) and 1288.9 ($2MH^+$) (expected MH^+ 645.8). Amino acid analysis: Gly 1.05 (1), Leu 1.01 (1), Tyr 1.00 (1), Phe 1.93 (2).
12. (a) Hancock, W. S.; Prescott, D. J.; Vagelos, P. R.; Marshall, G. R. *J. Org. Chem*. **1973**, 38, 774. (b) Johnson, T. J.; Quibell, M.; Owen, D.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun*. **1993**, *4*, 369.
13. Peptide **10** via derivative **8b** gave a single species (see Fig. 2) with ESMS 1062.7 (MH^+) (expected MH+ 1063.2). Amino acid analysis: found (expected): Asp 1.96 (2), Glu 0.92 (1), Gly 1.05 (1), Ala 2.05 (2), Val 0.98 (1), Ile 2.08 (2), Tyr 1.04 (1).