Selective deprotection of phenacyl, benzyl and methyl esters of *N*-protected amino acids and dipeptides and *N*-protected amino acids benzyl ester linked to resins with bis(tributyltin) oxide



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Phenacyl, methyl and benzyl esters of various N- α -Boc, N- α -Cbz or N,N-dimethylamino protected amino acids and dipeptides, as well as esters of N- α -protected amino acids linked to Wang and Pam resins have been efficiently and chemoselectively cleaved by bis(tributyltin) oxide in aprotic solvents to give the corresponding carboxylic acids in good yields. Moreover, the absence of racemization during the deprotection has been demonstrated. A limitation of the method is the instability of the N- ϵ -Fmoc group in the amino acid esters 8 and 10, N- α -Fmoc-L-alanine linked to Wang resin 23 and the Cbz protecting groups in N- α -Boc-N- ϵ -Cbz-L-lysine benzyl and methyl esters (5 and 7), respectively, and N- α -Cbz-L-alanyl-L-alanine methyl ester 19. In the case of N- α -protected dipeptides, there was no evidence of free amino acid which indicates that the peptide bond is unaffected.

The selective cleavage of carboxylic esters with retention of the chiral integrity of the stereogenic centres is of critical importance in 'many synthetic sequences.^{1,2} Since many molecules of synthetic interest are multifunctional it is imperative to have protecting groups which can be removed under mild conditions both selectively and in the presence of other sensitive moieties. This is especially true in methodologies where the design of protection strategies requires two or three independent dimensions of orthogonality.[†]

Development of a mild and efficient reagent for deprotection of carboxylic esters has been the focus of our interest.⁴ In this connection, we have developed the use of bis(tributyltin) oxide (BBTO) for the chemoselective cleavage of primary alkyl and aromatic carboxylic esters as well as double esters such as (pivaloyloxy)methyl carboxylates without affecting a large range of functional groups within the molecule. Particularly we found that amides and β -lactams were completely inert to BBTO. Recently Pérez and Maier have successfully used this reagent for the selective deprotection of esters of steroids.⁵

Peptide synthesis in solution (liquid-phase peptide synthesis, LPPS) and solid-phase peptide synthesis (SPPS),^{6.7} always need specific combinations of temporary and permanent protection of the peptide fragment. The cleavage of 4-nitrobenzyl, 2,2,2-trichloroethyl (Tce) and phenacyl (Pac) esters of *N-tert*-butoxycarbonyl (Boc) or *N*-benzyloxycarbonyl (Cbz) protected amino acids and dipeptides, using tetrabutylammonium fluoride in THF (tetrahydrofuran), DMF (dimethylformamide) or DMSO (dimethyl sulfoxide) as solvent, was reported by Rinehart and co-workers.⁸ Waldmann and co-workers reported a selective cleavage of heptyl esters, used for carboxyl protection in *N*-Cbz, *N*-Boc and *N*-allyloxycarbonyl (Alloc) dipeptides, by enzymatic hydrolysis with a lipase from *Rhizopus niveus*.⁹

The rapidly growing field of combinatorial solid-phase organic synthesis¹⁰ involving libraries of peptides,¹¹ peptidomimetic¹² and organic molecules¹³ has markedly renewed interest in the chemical protection strategy to allow selective orthogonal protection and deprotection of reactive groups in the monomers and in the selective cleavage from the resin to give final products.¹⁴ The benzyl ester linkage is the most frequent mode of attachment of the first amino acid to the resins of polystyrene crosslinked with divinylbenzene such as Merrifield,¹⁵ Wang,¹⁶ Pam¹⁷ and Sasrin¹⁸ resins. The two most widely-used protection strategies in SPPS are the N- α -Boc/O-side chain Bz- in Merrifield and Pam resins and the N-a-Fmoc/O-side chain Bu' strategies in conjunction to Wang and Sasrin resins. Successful cleavage of the final peptide or nonpeptide product represent one of the most crucial steps in the synthetic cycle. In the traditional Merrifield protocol,^{7a} the preferred method for cleavage of the benzyl ester linkage is by use of anhydrous hydrofluoric acid (HF)¹⁹ to yield the free parent peptide acid (unprotected peptide). The cleavage step in the more acid-labile resins, such as Wang resin in the Fmoc strategy are effected by milder acids, such as trifluoroacetic acid (TFA), leading to fully deprotected peptides.²⁰ There are several drawbacks that must be considered when using acidolytic deprotection methods in SPPS. These are: (i) side reactions typical of strongly acidic conditions; (ii) cleavage of sensitive amino acids and protecting groups to yield a mixture of products as a result of partial hydrolysis; (iii) the inability to effect synthetic manipulations such as segment condensations (convergent solid-phase peptide synthesis)^{21,22,‡} and/or the preparation of cyclic peptides. Non-acidolytic methods are useful in these cases. Two such methods for the cleavage of benzyl esters are: (i) catalytic hydrogenation ²⁴ and (ii) catalytic transfer hydrogenation.²⁵ A limitation of these methods is the deprotection of benzyl ethers and other hydrogenolizable moieties. For method (i) there is also a problem of the intolerance of palladium catalyst to the presence of divalent sulfur in the substrate. Tetrabutyl carbonate 26 has been utilized to release peptides in an $N-\alpha$ -protected form from their Merrifield resin support.

With the development of a great number of handles or linker-

 $[\]dagger$ An orthogonal system is defined as 'a set of completely independent classes of protecting groups, such that each class of groups can be removed in any order in the presence of all other classes'.³

[‡] Protected peptide fragments are synthesized on polymer support, and cleaved from the resins in such a way that the N- α -protecting group, are preserved and that the C-terminal of the segment is either the free carboxylic acid or a derivative of this which is suitable for coupling. For a recent account of the synthesis of fully protected peptide fragments see ref. 23.

| Entry | Starting material | Product | Conditions | Yield (%) |
|-------|---|---|----------------------|------------------------|
| 1 | $N-\alpha$ -Boc- $N-\varepsilon$ -Cbz-L-lysine phenacyl ester 1 | $N-\alpha$ -Boc- $N-\epsilon$ -Cbz-L-lysine 2 | Toluene, 70 °C, 36 h | 80 |
| 2 | N-Boc-L-phenylalanyl-L-proline phenacyl ester 3 | N-Boc-L-phenylalanyl-L-proline 4 | Toluene, 90 °C, 48 h | 76 |
| 3 | $N-\alpha$ -Boc- $N-\epsilon$ -Cbz-1-lysine benzyl ester 5 | 2 | Toluene, 70 °C, 36 h | 60 <i>ª</i> |
| 4 | N-Boc-L-phenylalanyl-L-proline benzyl ester 6 | 4 | Toluene, 90 °C, 96 h | 69 <i>^b</i> |

^a N-α-Boc-L-lysine was obtained in 10% yield and 3% of the starting ester was recovered. ^b Recovery of starting ester was 20%.

resins,§ many groups have been designed to permit the release of carboxylic acids from the resin under milder conditions by a great variety of reagents. High yields for the cleavage of Fmoc-protected peptide segments from an allyloxycarbonyl handle -OCH2CH=CHCH2OCH2CO- may be obtained using tributyltin hydride in the presence of [Pd(Ph₃P)₂Cl₂].²⁸ Very recently Albericio and co-workers reported that treatment of peptide resins with the weak base morpholine in DMF renders protected peptides in high yields and purities.²⁹ Soft bases such as lithium mercaptoethanol³⁰ have been used to release peptides bound to carboxamidomethyl ester resin. Hard-base cleavage by tetrabutylammonium fluoride $[F^{-}]^{31,32}$ have been used to release peptides bound to Pam resin,³¹ phenacyl ester linkage peptide-resin³² and silicon-containing handle resins.^{33,34} Tetrafluoroboric acid in trifluoroacetic acid, in the presence of thioanisole has been found to cleave amino acid and Fmoc based peptide from Wang resins.³⁵ The use of the orthonitrobenzyl unit as a handle for the photolytic cleavage of peptides from solid support has recently received considerable attention.³⁶ The use of hexafluoroisopropanol, as very weak acid has been recently reported for cleaving protected peptide fragments from 2-chlorotrityl resin with a minimal amount of racemization.37 The hard acid trimethylsilyl trifluoromethanesulfonate in TFA or HF, in the presence of thioanisole has been found to cleave the benzyl ester from Pam resin.38 Protected peptide segments may also be detached from resins by ammonolysis or hydrazinolysis of the C-terminal peptide resin benzyl ester linkage. This gives rise either to the protected C-terminal amides or hydrazides.39

The search for efficient methods to effect the cleavage of the peptides from polymeric resins under mild conditions continues to attract synthetic chemists. The use of BBTO as deprotecting reagent of esters in peptide synthesis has not been explored, although it has been used to obtain tributyltin esters as carboxylic acid protecting group of amino acids, and *N*-benzoylglycine.⁴⁰

In order to further demonstrate the utility of BBTO methodology, the deprotection of phenacyl, benzyl and methyl esters of amino acids and dipeptides in the presence of the carbamate-amino protecting groups Boc, Cbz and Fmoc, was undertaken as well as the deprotection of resin-linked benzyl esters of model N- α -Boc and N- α -Fmoc protected amino acids.

Results and discussion

Phenacyl esters of $N-\alpha-N-\varepsilon$ -amino protected amino acids and $N-\alpha$ -amino protected dipeptides. Upon treatment with BBTO, $N-\alpha$ -Boc- $N-\varepsilon$ -Cbz-L-lysine phenacyl ester 1 is cleaved at 70 °C to afford the corresponding $N-\alpha$ -Boc- $N-\varepsilon$ -Cbz-L-lysine 2 in good yield (80%) (Table 1, entry 1). It is interesting to note the usefulness of BBTO for chemoselective deprotection of the phenacyl ester in the presence of $N-\alpha$ -Boc and $N-\varepsilon$ -Cbz as permanent protecting groups.

The pac ester of N- α -Boc-L-phenylalanyl-L-proline **3** was cleaved in good yield by BBTO treatment in toluene (Table 1, entry 2). An advantage of using phenacyl esters as protecting groups for carboxylic acids lies in the facile, convenient and high-yielding method for their synthesis by using BBTO to prepare tributyltin carboxylates which react with phenacyl bromides in the presence of quaternary ammonium salts.⁴¹ The synthesis and cleavage of phenacyl esters of protected amino acids and peptides mediated by BBTO may prove to be a new, convenient and alternative method for carboxyl group protection and deprotection.

Benzyl esters of N- α -N- ϵ -amino protected amino acids and N- α -amino protected dipeptides

The benzyl ester of $N-\alpha$ -Boc- $N-\varepsilon$ -Cbz-L-lysine **5** showed similar reactivity to phenacyl esters, although some simultaneous cleavage of the $N-\varepsilon$ -Cbz protecting group was observed, as a small amount of $N-\alpha$ -Boc-L-lysine (10%) was detected in the reaction mixture (Table 1, entry 3). Thus, the chemoselectivity is not perfect for benzyl esters *versus* the N-benzyl carbamate protecting group but, nevertheless, is excellent with respect to the N-Boc protecting group. The benzyl ester group of $N-\alpha$ -Boc-L-phenylalanyl-L-proline **6** was selectively cleaved by BBTO in 69% isolated yield (83% based on starting recovered material; entry 4). This result also shows that BBTO is a useful reagent for the cleavage of a benzyl ester in the presence of Boc protecting amine group.

Methyl esters of $N-\alpha$ - $N-\epsilon$ -amino protected amino acids and $N-\alpha$ -amino protected dipeptides

The results of ester cleavage by BBTO of the methyl ester of $N-\alpha$ -Boc- $N-\epsilon$ -Cbz-L-lysine 7, $N-\alpha$ -Boc- $N-\epsilon$ -Fmoc-L-lysine 8, $N-\alpha$ -Fmoc- $N-\epsilon$ -Cbz-L-lysine 10 and several methyl esters of $N-\alpha$ -amino protected dipeptides, such as Boc, Cbz and dimethylamino groups are given in Table 2.

In assessing the efficacy of cleavage by BBTO we observed that the methyl ester 7 was cleaved to 2 in 65% yield, 10% of benzyl alcohol was obtained and 3% of the starting ester was recovered. In the present study, much greater selectivity was observed for the Pac ester 1 (compare entries 1 and 5). As a limitation of the method using BBTO, the instability of the Cbz group in N-Cbz-L-alanyl-L-alanine methyl ester 19 towards this reagent was also noted (entry 12).

Treatment of $N-\alpha$ -Boc- $N-\varepsilon$ -Fmoc-L-lysine methyl ester **8** showed that the methyl ester can be removed completely while the Boc group was preserved under these conditions, partial loss of Fmoc protecting group being detected (entry 6). However, under these conditions BBTO partially removed the Fmoc and Cbz groups of $N-\alpha$ -Fmoc- $N-\varepsilon$ -Cbz-L-lysine methyl ester **10** and the yield of the corresponding carboxylic acid **11** was low (entry 7).

Treatment of the dipeptides 12^{42} and 14^{43} with BBTO in benzene at 80 °C for 18 h furnished, after isolation, the pure dipeptide acids 13 (63%) and 15 (60%) respectively (entries 8 and 9). In each of these cases there was no evidence for the presence of free L-leucine or L-phenylalanine as examined on an amino acid analyser and it was concluded that the peptide bond is unaffected by BBTO under these conditions.

The methyl ester of N-Boc-L-phenylalanyl-L-proline 16 was cleaved in 69% isolated yield (84 % based on starting recovered

[§] Barany and Merrifield have defined a 'handle' (or 'linkage agent' or 'linker') as a bifunctional spacer which serves to attach the first amino acid to the polymeric support in two discrete steps. One end of the handle incorporates features of a smoothly cleavable protecting group, and the other end allows facile coupling to a previous functionalized support. See refs. 7a, 21a and 27.

| Entry | Starting material | Product | Conditions | Yield (%) |
|-------|---|--|---------------------------|------------------------|
| 5 | N_{α} -Boc- N_{ϵ} -Cbz-L-lysine methyl ester 7 | 2 | Toluene, 70 °C, 36 h | 65 <i>ª</i> |
| 6 | $N-\alpha$ -Boc- $N-\epsilon$ -Fmoc-L-lysine methyl ester 8 | N-α-Boc-N-ε-Fmoc-L-lysine 9 | Toluene, 100 °C, 24 h | 67 <i>^b</i> |
| 7 | $N-\alpha$ -Fmoc-N- ϵ -Cbz-L-lysine methyl ester 10 | N-α-Fmoc-N-ε-Cbz-L-lysine 11 | Toluene, 100 °C, 24 h | 23° |
| 8 | <i>N</i> , <i>N</i> -Dimethyl-L-phenylalanyl-L-leucine methyl ester 12 | <i>N</i> , <i>N</i> -Dimethyl-L-phenylalanyl-L-leucine 13 | Benzene, 80 °C, 16 h | 63 |
| 9 | N.N-Dimethyl-L-aspartyl-L-phenylalanine Me ester 14 | N,N-Dimethyl-L-aspartyl-L-phenylalanine 15 | Benzene, 80 °C, 16 h | 60 |
| 10 | N-Boc-L-phenylalanyl-L-proline methyl ester 16 | 4 | Toluene, 90 °C, 36 h | 69 ^{<i>d</i>} |
| 11 | N-Boc-L-leucyl-L-proline methyl ester 17 | N-Boc-L-leucyl-L-proline 18 | Toluene, 90 °C, 96 h | 68 |
| 12 | N-Cbz-L-alanyl-L-alanine methyl ester 19 | N-Cbz-L-alanyl-L-alanine 20 | Acetonitrile, 85 °C, 48 h | 52 ^e |

^{*a*} Benzyl alcohol was obtained in 10% yield and 3% of the starting ester was recovered. ^{*b*} Partial loss of Fmoc protecting group was detected. ^{*c*} The reaction was incomplete, 30% of the starting ester being recovered, along with partial loss of the Fmoc and Cbz protecting groups. ^{*d*} Recovery of starting ester was 10%. ^{*e*} Benzyl alcohol was obtained in 20% yield.



Fig. 1 The reaction of Merrifield, Wang and Pam resins with BBTO in CH_3Cl

material). The methyl ester of the *N*-Boc protected dipeptide **17** was also deprotected in good yield (entry 11). The absence of racemization during the deprotection of $N-\alpha$ -Boc- $N-\epsilon$ -Fmoc-L-lysine methyl ester **8**, and of the dipeptides *N*-Boc-L-phenylalanyl-L-proline methyl ester **16** and *N*-Cbz-L-alanyl-L-alanine methyl ester **19** was checked; the products were found to be enantiomerically and diastereoisomerically pure (see Experimental section).

Resin-linked N-protected amino acids

The ester chemical linkage of the growing peptide chain to the resin is crucial in solid phase synthesis. It has to be easily formed, stable to repeated cycles of acylation and deprotection, and yet easily cleaved at the end of the synthesis without damage to newly formed peptide bonds. To evaluate the use of BBTO in the cleavage of esters from the resin linked *N*- α -protected amino acids, we studied *N*- α -Boc-*O*-benzyl-L-serine linked to Merrifield resin **21**, *N*- α -Fmoc-L-alanine linked to Wang-resin **23** and *N*- α -Boc-L-alanine linked to Pam resin **26** (Fig. 1).

The results of Table 3 show the role of the solid support. Initial attempts to cleave the benzyl ester of $N-\alpha$ -Boc-O-benzyl-L-serine bound directly to the crosslinked polystyrene-codivinylbenzene (Merrifield resin) using BBTO conditions met with little success, only 12% of N-a-Boc-O-benzyl-L-serine 22 being obtained after 5 days of reflux in chloroform. The reaction failed, presumably owing to the lack of accessibility to the carboxyl and carbinol carbon reactive sites by the bulky BBTO reagent. Indeed, problems associated with steric hindrance around the carboxyl and carbinol carbons have been previously described by us.4a This difficulty was overcome by use of linker-resins (handles) such as Wang or *p*-alkoxybenzyl alcohol resin and Pam or 4-(oxymethyl)phenylacetamidomethyl resin (see Fig. 1). Treatment of N-a-Fmoc-L-alanine bound to the Wang resin 23 with BBTO liberated 40% of L-Alanine 24, determined on an amino acid analyser and 18% of N-a-Fmoc-L-

alanine 25. From the N- α -Fmoc-L-alanine the Fmoc group was removed quantitatively with 5% piperidine in DMF,⁴⁴ and Lalanine was examined on an amino acid analyser (Table 3). As mentioned previously, the N- α -Fmoc protecting group was partially lost under the conditions employed.

The benzyl ester of *N*-Boc-L-alanine Pam resin **26** was cleaved with BBTO yielding the *N*- α -Boc-L-alanine **27** in 63% yield. This orthogonal deprotection with BBTO which did not affect the *N*- α -*tert*-butoxycarbonyl group is in accord with earlier ones mentioned above in the case of protected dipeptides.

In conclusion, this methodology is very useful for the deprotection of phenacyl, methyl and benzyl esters, of peptides particularly in the presence of N- α -Boc protecting groups. Moreover, because of the compatibility of bis(tributyltin) oxide with a variety of acid-sensitive functional groups including N- α -Boc, O-benzyl and O-tert-butyl¶ this method provides easy access to protected peptide fragments from Pam and Wang resins which can be used in convergent solid-phase peptide synthesis.^{21,23} Conversely, the N- α -Boc and O-tert-butyl groups can be removed under standard conditions without affecting the benzyl ester groups.

The results presented above seem to be particularly attractive for the attachment of non-peptide carboxylic acid functional molecules to Wang and Pam resins, followed by a sequence of reactions and a final selective detachment with BBTO under neutral non-aqueous reaction conditions. Moreover, such a sequence may have potential for combinatorial solid-phase organic synthesis.¹⁰

Notable advantages of the present method are: (a) lack of racemization of amino acid and peptide products; (b) BBTO is a cheap reagent which does not require special handling techniques and equipment and (c) Wang and Pam resins are commercially available. Although cleavage of carboxylic esters give moderate to excellent product yields, a major disadvantage of this method is the neccessary purification of the final products to remove completely the tin impurities; this results in considerable lowering of the isolated product yields. However, ¹H NMR analyses indicated that all products were >97%. Currently, work is underway to extend the use of this reagent to cleave model peptides linked to Wang and Pam resins as well as phenacyl resin and the use of microwave irradiation to accelerate the deprotection of peptides bound to resins.

Experimental

General

¹H and ¹³C NMR instruments and column chromatographic conditions have been described earlier.^{4a} All N- α -N- ϵ -protected amino acids, N- α -protected peptides and Merrifield, Wang and Pam resin-linked protected amino acids were purchased from Bachem Bioscience Inc. The protected amino acids esters used

[¶] The *O*-tert-butyl group from $N-\alpha$ -Fmoc-*O*-tert-butyl-L-serine linked to Wang resin was stable under BBTO benzyl ester cleavage conditions (E. G. Mata and O. A. Mascaretti, unpublished results).

| Entry | Starting material | Product | Conditions | Yield (%) |
|-------|--|---|---------------------------|-----------|
| 13 | N-Boc-O-benzyl-L-serine Merrifield-resin linked 21 | N-Boc-O-benzyl-L-serine 22 | Chloroform, 61 °C, 5 days | 12 |
| 14 | N-Fmoc-L-alanine Wang- resin linked 23 | L-Alanine 24 N-Fmoc-L-alanine | Chloroform, 61 °C, 60 h | 58 " |
| 15 | N-Boc-L-alanine Pam-resin linked 26 | N-Boc-L-alanine 27 | Chloroform, 61 °C, 96 h | 63 |

^a Overall yield. 40% of Alanine and 18% of Fmoc-L-alanine (determined as L-alanine after removal of Fmoc group with piperidine²⁶).

were synthesized according to standard procedures.⁴⁵ *N*,*N*-Dimethyl-L-phenyl-alanyl-L-leucine methyl ester was prepared according to the literature procedure.⁴² L-Aspartyl-L-phenyl-alanine methyl ester was purchased from Fluka Chemie AG and *N*,*N*-dimethylated according to the procedure reported in the literature.⁴³ All yields are reported for purified isolated products free of organotin compounds, checked by ¹H NMR.

General procedure for the deprotection of esters in *N*-protected amino acids and dipeptides by BBTO

The N-protected amino acid or dipeptide ester (0.3 mmol) was added to a solution of BBTO (0.3 cm³, 0.58 mmol) in toluene or benzene (10 cm³). The mixture was heated at 70-100 °C (see Tables 1 and 2) for 24-36 h (checked by TLC) after which it was evaporated under reduced pressure. The reaction products were pre-purified by C-18 reverse-phase silica-gel column chromatography (eluent: acetonitrile-water, 70:30) after which, the impure fraction was dissolved in EtOAc (10 cm³) and extracted with 5% aq. NaHCO₃ (3 \times 5 cm³). The aqueous phase was acidified to pH 4 with 10% aq. KHSO₄ and extracted with EtOAc $(3 \times 5 \text{ cm}^3)$. The combined extracts were washed with brine $(2 \times 5 \text{ cm}^3)$, dried (Na_2SO_4) and evaporated under reduced pressure. The structures of the products were confirmed by comparison of their physical and spectroscopic data with those in the literature. The absence of racemization was checked during the deprotection of compounds 8, 16 and 19: Compound 9 [α]_D 5.2 (c 1.0, CHCl₃) [lit.,¹¹ 5.1 (c 1.0 in CHCl₃)]; compound 4 $[\alpha]_D$ -31.5 (c 1.0 in EtOH) [lit.,¹¹ -33.0 (c 1.0 in EtOH)]; compound **20** [α]_D -33.0 (c 1.0 in EtOH) [lit.,¹¹ - 33.2 (*c* 1.0 in EtOH)].||

General procedure for the cleavage of resin-linked esters of N- α -Boc amino acids by BBTO

N- α -Boc amino acid resin (0.05 mmol) was suspended in chloroform (1.5 cm³) and treated with BBTO (59 mg, 0.1 mmol). The mixture was refluxed for several hours (see Table 3, entries 13 and 15), after which it was filtered and the resin washed with chloroform. The combined filtrates were evaporated to dryness and the residue purified in a similar way to that used for deprotection of esters of amino acids and dipeptides by BBTO (see above).

General procedure for the cleavage of resin-linked esters of N- α -Fmoc amino acids by BBTO

N- α -Fmoc amino acid resin (0.07 mmol) was suspended in chloroform (1.5 cm³) and treated with BBTO (83 mg, 0.14 mmol). The suspension was refluxed for 60 h (see Table 3, entry 14) after which it was filtered and the resin washed with chloroform. After evaporation of the combined filtrates under reduced pressure the residue was taken up in EtOAc (2 cm³) and extracted with water (2 × 1 cm³) to remove the free amino acid present in this fraction. The used resin was suspended in water—methanol (1:1), refluxed for 6 h and filtered. This filtrate, containing part of the free amino acid, was combined with the aqueous phases, evaporated to dryness and examined on an amino acid analyser. The organic phase, containing N- α -Fmoc

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amino acid, was treated with 5% piperidine in DMF⁴⁴ to give the free amino acid which was examined on an amino acid analyser.

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