Journal of Peptide Science
J. Peptide Sci. **4**: 138–141 (1998)

SHORT COMMUNICATION

An Efficient and Convenient Procedure for the Synthesis of N^{α}-Fmoc-*O*-Monobenzyl Phosphonotyrosine

B.K. HANDA[a,*] and C.J. HOBBS[b]

[a] Physical Methods Department, Roche Discovery Welwyn, Welwyn Garden City, Hertfordshire AL7 3AY, UK
[b] Medicinal Chemistry Department, Roche Discovery Welwyn, Welwyn Garden City, Hertfordshire AL7 3AY, UK

Received 18 September 1997
Accepted 18 October 1997

Abstract: An efficient procedure is described for the synthesis of N^{α}-Fmoc-*O*-monobenzyl phosphonotyrosine from the corresponding dibenzyl derivative by monodebenzylation in the presence of sodium iodide. A simple work up procedure removes the by-products and the monobenzylated phosphono product is obtained in high yield. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: monobenzyl phosphotyrosine; phosphotyrosine; Fmoc phosphotyrosine; phosphonotyrosine; monobenzyl phosphonotyrosine

We are engaged in the synthesis of phosphotyrosine containing peptides which are required for a number of therapeutic areas currently being investigated at our research centre. In order to synthesise these peptides by a solid phase strategy we have employed Fmoc-Tyr (PO_3H_2)-OH to incorporate phosphotyrosine [1]. Although this building block is very useful, on several occasions the syntheses either failed or yielded crude products which required extensive purification resulting in markedly reduced yields. Considerable improvement in the synthesis is often achieved when Fmoc-Tyr(PO_3H_2)-OH is replaced by the monobenzyl derivative Fmoc-Tyr[PO(OBzl)OH]-OH which has recently become available, but unfortunately there are long delays in obtaining this material from the only commercial supplier. We therefore decided to develop a convenient and efficient synthesis of this extremely useful intermediate.

A recent report describes the use of the monobenzyl phosphoderivative of tyrosine, along with that of threonine and serine, in the synthesis of a range of phosphorylated peptides, but does not describe the synthesis of these reagents [2]. There are, however, published methods for the synthesis of Fmoc-Ser[PO(OBzl)OH]-OH and Fmoc-Thr[PO(OBzl)OH]-OH [3,4] which are long and laborious, requiring selective protection for the phosphate group. Additionally, the C-terminal carboxyl requires protection during the synthesis. It is well known that bis-protected *O*-phosphoryl amino acids are susceptible to monodealkylation under acidic as well as basic conditions [3,5]. This observation could be exploited if the monodealkylation were achieved in high yield and under mild conditions. Highly efficient and selective dealkylation of phosphorus esters under a variety of different conditions has been widely described [6–8]. In this paper we wish to report an efficient method which we have developed to synthesize N^{α}-Fmoc-*O*-monobenzyl phosphonotyrosine by monodeben-

Abbreviations: ^{1}H-NMR, proton nuclear magnetic resonance; TBDMSCl, *tert*-butyldimethylsilyl chloride; Fmoc-ONSu, Fmoc-*N*-hydroxysuccinimide; NMM, *N*-methylmorpholine.

* Correspondence to: Physical Methods Department, Roche Discovery Welwyn, Broadwater Road, Welwyn Garden City, Hertfordshire AL7 3AY, UK.

CCC 1075–2617/98/020138-04$17.50

zylation of N^{α}-Fmoc-*O*-dibenzyl phosphonotyrosine. When the dibenzylated derivative is heated to reflux in acetone in the presence of two equivalents of sodium iodide, complete monodebenzylation occurs within 2 h. A simple work up procedure which is carried out to isolate the product provides highly pure monobenzylphospho tyrosine in 80% yield. The dibenzyl phosphotyrosine used as starting material is a known compound and was conveniently prepared from Fmoc-tyrosine as previously described [9,10].

EXPERIMENTAL PART

Nuclear magnetic resonance spectra were recorded on a Bruker AC400 instrument, while the mass spectra were obtained on either a Finnigan MAT95S or a Finnigan TSQ7000 spectrometer. Microanalyses were performed on a Perkin Elmer 2400 elemental analyser and the optical rotations were measured on a Perkin Elmer 241 instrument. Thin layer chromatography was performed on Merck Keiselgel 60 F_{254} plates using chloroform/methanol/acetic acid/water (A) 120:15:3:2, (B) 60:18:2:3. The compounds were visualised directly under UV light (254 nm) and by spray solution of 20% phosphomolybdic acid in ethanol (Aldrich) and heating strongly (dark blue colour). Analytical RP-HPLC was performed with an ABI model 151A system on an Aquapore C8 RP-300 column (7 μm, 30 × 4.6 mm). The elution gradient comprised 95% A–95% B over 12 min, where A = 0.1% TFA in water and B = 0.085% TFA in 70% acetonitrile and the flow rate was 2 ml/min.

Fmoc-*N*-hydroxy succinimide was purchased from Sigma-Aldrich. Anhydrous THF, *N*-methyl morpholine, TBDMSCl, tetrazole and dibenzyl diisopropyl phosphoramidite were from Aldrich (Dorset, England). The commercial source for N^{α}-Fmoc-*O*-monobenzyl-L-phosphonotyrosine was Novabiochem (UK) and the accompanying data sheet was used to compare the analytical data of the synthetic material.

N^{α}-Fmoc-L-Tyr-OH 1

To a solution of Tyrosine (5 g, 27.6 mmol) in 10% $NaHCO_3$ (45 ml) was added a solution of Fmoc-ONSu (9.3 g, 27.6 mmol) in acetone (85 ml). The resulting suspension was stirred at room temperature overnight. Most of the acetone was removed by evaporation *in vacuo*, ether (100 ml) was added and the solution was allowed to stand at room temperature. After 30 min, the solid which had separated (sodium salt of Fmoc-tyrosine) was collected by filtration, washed thoroughly with ether, water and ethyl acetate. The solid was suspended in water (80 ml) and acidified to pH 1 with 6N HCl. The product was extracted with ethyl acetate (2 × 75 ml). The combined ethyl acetate extracts were washed with a saturated solution of sodium chloride (2 × 50 ml) and dried over anhydrous sodium sulphate. On evaporation, a gum was obtained which crystallized from ethyl acetate/hexane to give **1** (7.5 g, 68%).

R_F (A) 0.35. $[M+H]^+ m/z$ 404, $[M+Na]^+ m/z$ 426. Elementary analysis: Calculated for $C_{24}H_{21}NO_5$ (403.44): C, 71.45; H, 5.25; N, 3.47%; Found: C, 71.08; H, 5.32; N, 3.27.

N^{α}-Fmoc-*O*-Dibenzylphosphono-L-Tyr-OH 2

To a solution of Fmoc-L-tyrosine **1** (4.04 g, 10 mmol) in anhydrous THF (40 ml) was added NMM (1.01 g, 10 mmol) and TBDMSCl (1.51 g, 10 mmol) under nitrogen. After 15 min dibenzyl diisopropylphosphoramidite (6.9 g, 20 mmol) and tetrazole (2.12 g, 30 mmol) were added. The reaction mixture was stirred at room temperature for 3 h, then cooled to 0°C and 70% *tert*-butyl hydroperoxide (3.88 ml, 30 mmol) was added. After stirring at 0°C for 2 h, 10% $Na_2S_2O_5$ (50 ml) was added and stirring continued for a further half hour. The product was extracted with ethyl acetate (3 × 50 ml) and the combined extracts were washed with a 5% solution of $KHSO_4$ (3 × 85 ml), brine and dried over anhydrous sodium sulphate. Upon evaporation the crude product, obtained in the form of a viscous oil, was chromatographed on a silica gel column (4 × 50 cm) eluting with chloroform, 5% methanol in chloroform, 5% acetic acid in 5% methanol/chloroform. The fractions containing the desired product were evaporated to give an oil. The oil was dissolved in ethyl acetate (100 ml) and washed with water (3 × 50 ml), dried over sodium sulphate and evaporated to afford **2** (5.5 g, 83%) as an oil. The oil partially solidified on repeated evaporation with dichloromethane *in vacuo*.

R_F (A) 0.6. $[M+H]^+ m/z$ 664, $[2M+H]^+ m/z$ 1327.7. Elementary analysis: Calculated for $C_{38}H_{34}NO_8P$. 0.5 H_2O (663.67. 0.5H_2O) C, 67.82; H, 5.24; N, 2.11%; Found: C, 67.50; H, 5.41; N, 2.77.

N^{α}-Fmoc-*O*-Monobenzyl Phosphono-L-Tyr-OH 3

To a solution of N^{α}-Fmoc-*O*-dibenzylphosphono-L-tyrosine **2** (3.1 g, 4.67 mmol) in acetone (50 ml) was

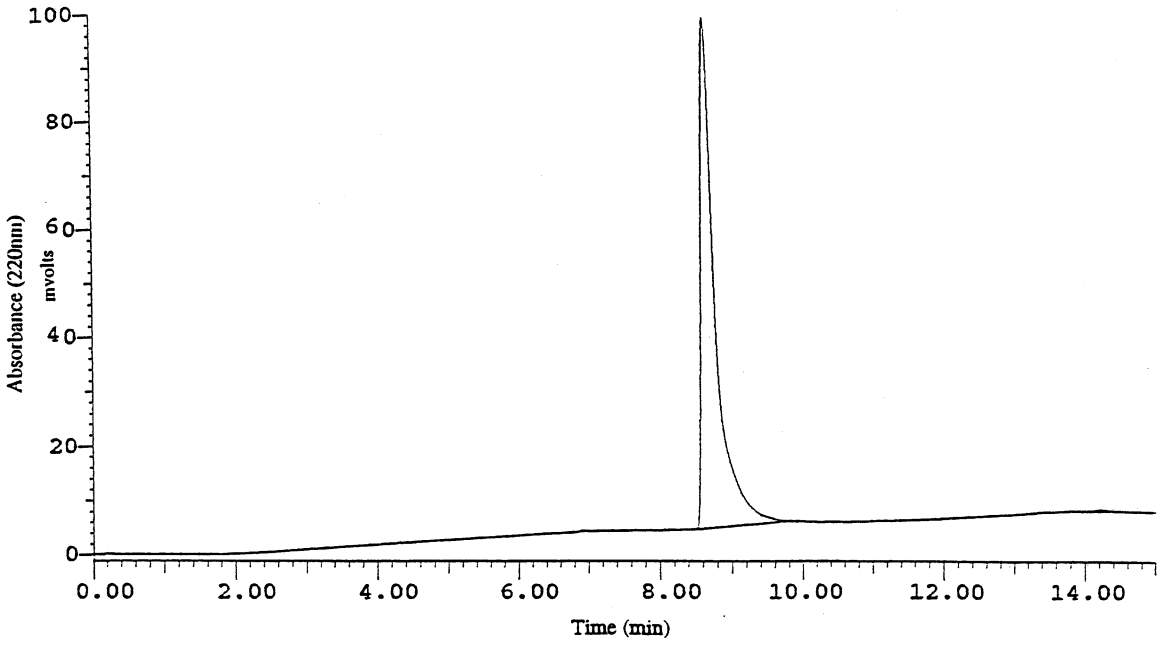

Figure 1 RP-HPLC of N^{α}-Fmoc-*O*-monobenzyl phosphono-L-Tyr-OH. For conditions see experimental.

added sodium iodide (1.4 g, 9.3 mmol) and the reaction heated to reflux for 2 h by which time the starting material was no longer detected on TLC. The dark brown reaction mixture was allowed to cool and stand at room temperature for 1 h. A white solid separated, which was collected by filtration and washed several times with acetone and then ether. The solid was dissolved in water (40 ml) and acidified to pH 1 with 2N HCl. The white precipitate was collected by filtration, washed with cold water (3×5 ml), ether and dried over P_2O_5 *in vacuo* to yield **3** (2.2 g, 81%). R_F (B) 0.32. RP-HPLC Figure 1. $[M+H]^+ m/z$ 574, $[M+Na]^+ m/z$ 596, $[2M+H]^+ m/z$ 1147.3, $[2M+Na]^+ m/z$ 1169.3. ^{1}H-NMR (DMSO, d_6), δ, 2.83 (dd, 1H, β-CH_2 Tyr), 3.03 (dd, 1H, β-CH_2 Tyr), 4.1–4.23 (m, 4H, CH_2, CH Fmoc and α-CH Tyr), 4.97 (d, 2H, P-CH_2–C_6H_5), 7.08 (d, 2H, C_6H_4-Tyr), 7.21 (d, 2H, C_6H_4-Tyr), 7.25–7.42 (m, 7H, 2H-Fmoc and 5H P-Bz), 7.34–7.42 (m, 2H, Fmoc), 7.65 (t, 2H, Fmoc), 7.85 (d, 2H, Fmoc). Elementary analysis: Calculated for $C_{31}H_{28}NO_8P$ 1.7 H_2O (573.55. 1.7H_2O) C, 61.50; H, 5.25; N, 2.31%; Found: C, 61.40; H, 5.20; N, 2.10. $[\alpha]^{20} = -13.3°C$ ($c = 0.98\%$ in DMF) [lit. $-15.5 \pm 2.5°C$. m.p. 162–165°C [lit. 129–142°C][1].

[1] The analytical data for N^{α}-Fmoc-*O*-monobenzyl phosphono-L-tyrosine (m.p., R_F, ^{1}H-NMR, Mass, RP-HPLC) was compared with the data sheet obtained with the commercial sample.

REFERENCES

1. E.A. Ottinger, L.L. Shekels, D.A. Bornlohr and G. Barany (1993). Synthesis of phosphotyrosine containing peptides and their use as substrates for protein tyrosine phosphatases. *Biochemistry 32*, 4354–4361.
2. P. White and J. Beythien (1996). Preparation of phosphoserine, threonine and tyrosine containing peptides by the Fmoc methodology using preformed phosphoamino acid building blocks, in: *Innovations and perspectives in solid phase synthesis and combinatorial libraries*, R. Epton, Ed., p. 557–560, 4th International symposium, Edinburgh, September 1995.
3. T. Wakamiya, T. Nishida, R. Togashi, K. Saruta, J. Yasuoka and S. Kusumoto (1996). Preparation of N^{α}-Fmoc-*O*-[(benzyloxy)hydroxyphosphinyl]-β-hydroxy α-amino acid derivatives. *Bull. Chem. Soc. Jpn. 69*, 465–468.
4. H. Schmid, S. Vetter, W. Bannwarth and E. Kitas (1996). Diester building blocks of pSer and pThr suitable for Fmoc solid phase peptide synthesis, in: *Innovations and perspectives in solid phase synthesis and combinatorial libraries*, R. Epton, Ed., p. 525–528, 4th International symposium, Edinburgh, September 1995.
5. Z. Tian, C. Gu, R.W. Roeske, M. Zhou and R.L. Van Ettan (1993). Synthesis of phosphotyrosine containing peptides by solid phase method. *Int. J. Peptide Protein Res. 42*, 155–158.

6. M.D.M. Gray and D.J.H. Smith (1980). Selective demethylation of phosphorus esters. *Tetrahedron Lett. 21*, 859–860.
7. R. Kluger, A.S. Grant, S.L. Berne and M.R. Trachsel (1990). Dicarboxylic acid bis(methyl phosphates): Anionic biomimetics cross-linking agents. *J. Org. Chem 55*, 2864–2868.
8. L. Zervas and I. Dilaris (1955). Dealkylation and debenzylation of triesters of phosphoric acid. Phosphorylation of hydroxy and amino compounds. *J. Am. Chem Soc.77*, 5354–5357.
9. H.G. Chao, M.S. Bernatowicz, P.D. Reiss and G.R. Matsueda (1994). Synthesis and application of bis-silylethyl derived phosphate protected Fmoc-phosphotyrosine derivatives for peptide synthesis. *J. Org. Chem. 59*, 6687–6691.
10. E.A. Kitas, R. Knorr, A. Trzeciak and W. Bannwarth (1991). Alternative strategies for the Fmoc solid phase synthesis of 0^4-phosphono-L-tyrosine containing peptides. *Helv. Chim. Acta. 74*, 1314–1328.