An Improved One-pot Synthesis of N^{α}-(*tert*-Butoxycarbonyl)-*O*-(*O'*,*O''*-dialkylphosphoro)-L-tyrosines Using Dialkyl *N*,*N*-Diethylphosphoramidites

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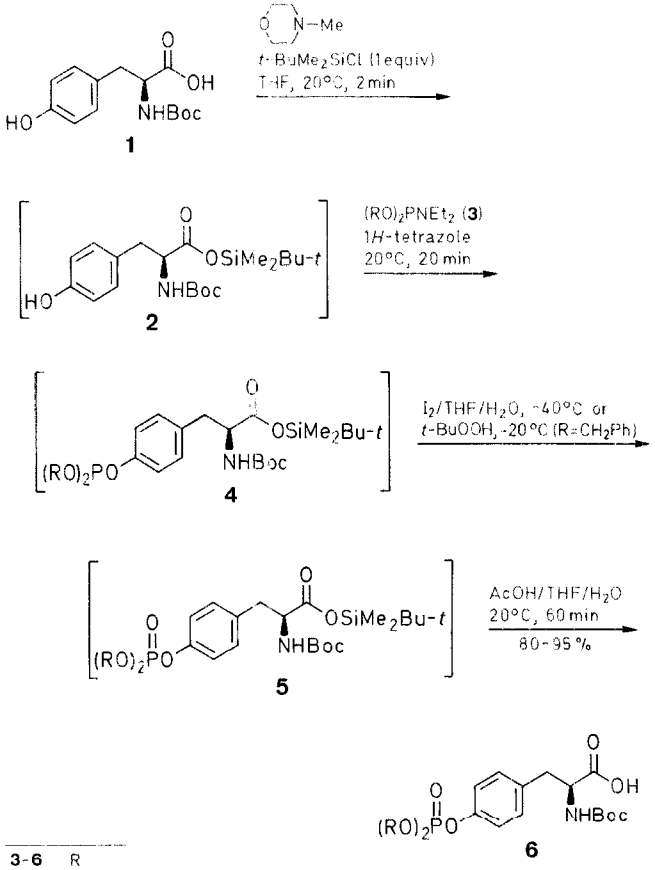
N^{α}-(*tert*-Butoxycarbonyl)-*O*-(*O'*,*O''*-dialkylphosphoro)-L-tyrosines **6a–c** were prepared in high yields by an efficient one-pot procedure, which involved initial *tert*-butyldimethylsilyl protection of the carboxy terminus of *N*-Boc tyrosine **1** followed by successive *in situ* phosphitylation of the tyrosyl hydroxyl group, oxidation of the resultant phosphite triester and deprotection.

N^{α}-(*tert*-Butoxycarbonyl)-*O*-(*O'*,*O''*-dialkylphosphoro)-L-tyrosines **6a–c** have been of particular use in the Boc (*tert*-butoxycarbonyl) mode of solution-phase peptide synthesis for the preparation of *O*-phosphotyrosine containing peptides.[1–4] However, previous syntheses of these derivatives have required a laborious three-step procedure, which involved (a) initial protection of the carboxy terminus of *N*-Boc tyrosine (**1**) with the 4-nitrobenzyl or 2-methylanthraquinone group, (b) phosphorylation of the tyrosine hydroxyl group using either the sodium hydride/dialkyl phosphorochloridate procedure or the two-step (i) dialkyl *N*,*N*-diethylphosphoramidite/1*H*-tetrazole-(ii) *m*-chloroperoxybenzoic acid procedure followed by (c) hydrogenolytic or sodium dithionite reduction of the carboxyl protecting group.[1–4]

Although we have also reported a simple one-pot procedure for the preparation of **6b** by phosphorylation of *N*-Boc tyrosine (**1**) with diethyl phosphorochloridite in the presence of pyridine,[5] the procedure lacks generality due to the unavailability of dialkyl phosphorochloridites. While diethyl phosphorochloridite is commercially available and stable over prolonged storage, dimethyl phosphorochloridite is difficult to prepare and disproportionates on storage,[6] and a synthetic preparation of dibenzyl phosphorochloridite has yet to be reported. As we have previously used dialkyl *N,N*-diethylphosphoramidites **3a, c** for the efficient "phosphite triester" phosphorylation of alcohols[7,8] and *N*-Boc protected tyrosine derivatives,[9,10] we recognized that the development of a one-pot procedure using such reagents would simplify the synthetic procedure and be more economical.[10]

In the development of a successful one-pot procedure, it was realized that temporary protection of the carboxyl moiety was necessary, since previous work in the literature indicated that the treatment of a carboxylic acid with a dialkyl *N,N*-dialkylphosphoramidite resulted in the formation of a carboxyl *N,N*-dialkylamide.[11,12] Of several silyl protecting groups commonly used in organic syntheses, the *tert*-butyldimethylsilyl group was selected on the basis of its known resistance to undergo silyl transfer and its ready hydrolytic cleavage under mild acidic conditions.[13]

In our present work, we describe the efficient one-pot preparation of *N*-Boc protected *O*-(*O'*,*O''*-dialkylphosphoro)tyrosines **6a–c** using dimethyl, diethyl, and dibenzyl *N,N*-diethylphosphoramidite **3a–c**.[2,10] Thus, products **6a** and **6b** were obtained in 80 and 95% yield, respectively, by the initial treatment of **1** with (a) *N*-methylmorpholine and *tert*-butyldimethylsilyl chloride followed by successive *in situ* phosphitylation of the tyrosine hydroxyl of the resultant silyl ester intermediate **2** with (b) **3a** or **3b**/1*H*-tetrazole, and (c) oxidation of the resultant phosphite-triester intermediate **4** with aqueous iodine; acid hydrolysis of the silyl group from **5** occurring during the mild oxidation step. The products are obtained by simple sodium hydrogen carbonate extraction, subsequent acidification to pH 2 and a final dichloromethane extraction.



3-6	R
a	CH_3
b	Et
c	CH_2Ph

As aqueous iodine cannot be used for the oxidation of dibenzyl phosphite triesters due to iodide-mediated debenzylation of the iodo dibenzylphosphonium iodide intermediate,[7] the use of an alternative non-nucleophilic mild oxidation procedure became necessary. Hence, the above phosphorylation of **1** using **3c** as phosphitylating reagent was modified by the use of 14% aqueous *tert*-butyl hydroperoxide for oxidation of the dibenzyl phosphite triester **4c** and 50% aqueous acetic acid for the final hydrolytic cleavage of the silyl group from **5c** to give **6c** in 91% yield.

The advantages of this one-pot synthesis are that the procedure is simple and economical, dialkyl *N,N*-diethylphosphoramidites are easily prepared and can be stored for prolonged periods without extensive oxidation, products are obtained in a short time (< 1 hour), in high yield, and are homogeneous as judged by ^{13}C- and ^{31}P-NMR spectroscopy (> 99.5% pure).

All reagents were of analytical grade quality from freshly opened containers. Boc-Tyr-OH was purchased from Vega Chemical Co. *N*-Methylmorpholine, *tert*-butyldimethylsilyl chloride, 1*H*-tetrazole and iodine were purchased from Aldrich Chemical Co. Aqueous *tert*-Butyl hydroperoxide (14%) was purchased from Merck-Schuchardt Chemical Co. Analytical reagent grade THF was dried by successive drying over 4 Å sieves and analytical reagent grade AcOH was used without further purification. Optical rotations at the Na-D line were obtained using a Perkin-Elmer 241MC polarimeter.

N^{α}-(*tert*-Butoxycarbonyl)-*O*-(*O'*,*O''*-dimethylphosphoro)-L-tyrosine (6a) and (*S*)-N^{α}-(*tert*-Butoxycarbonyl)-*O*-(*O'*,*O''*-diethylphosphoro)-L-tyrosine (6b):

N-Methylmorpholine (0.303 g, 3.0 mmol) in dry THF (1 mL) and *tert*-butyldimethylsilyl chloride (0.450 g, 3.0 mmol) in THF (1 mL) are successively added to a solution of **1** (0.843 g, 3.0 mmol) in THF (9 mL) at 20°C. After 2 min, a solution of **3a** (0.66 g, 4.0 mmol) or **3b** (0.72 g, 4.0 mmol) in THF (1 mL) is added at 20°C followed by the addition of 1*H*-tetrazole (0.63 g, 9.0 mmol) in one portion. After 20 min, the solution is cooled to −40°C and a solution of iodine (1.02 g, 4.0 mmol) in THF/H_2O (3:1, 4 mL) added. After 10 min, an aq. solution of 10% $Na_2S_2O_5$ (10 mL) is added, the solution stirred for 10 min at 20°C and then transferred to a separating funnel using Et_2O (40 mL). The aqueous phase is discarded and the organic phase washed with 10% $Na_2S_2O_5$ (1 × 15 mL) and then extracted with 5% $NaHCO_3$ solution (3 × 10 mL). The combined aqueous extract is washed with Et_2O (30 mL), acidified to pH 2 with 30% HCl, and extracted with CH_2Cl_2 (3 × 30 mL). The organic extract is dried (Na_2SO_4) and the solvent is then evaporated under reduced pressure to give **6a**; yield: 0.93 g (80%) or **6b**; yield: 1.19 g (95%) as a clear colorless oil.

6a: $[\alpha]_D^{22}$ + 36.9° (*c* = 1, $CHCl_3$), Lit.[14] $[\alpha]_D^{24}$ + 36.3° (*c* = 1, $CHCl_3$).

6b: $[\alpha]_D^{13}$ + 44.4° (*c* = 1, $CHCl_3$), Lit.[5] $[\alpha]_D^{13}$ + 45.4° (*c* = 1, $CHCl_3$).

N^{α}-(*tert*-Butoxycarbonyl)-*O*-(*O'*,*O''*-dibenzylphosphoro)-L-tyrosine (6c):

N-Methylmorpholine (0.303 g, 3.0 mmol) in dry THF (1 mL) and *tert*-butyldimethylsilyl chloride (0.450 g, 3.0 mmol) in THF (1 mL) are successively added to a solution of **1** (0.843 g, 3.0 mmol) in THF (9 mL) at 20°C. After 2 min, a solution of **3c** (1.27 g, 4.0 mmol) in THF (1 mL) is added at 20°C followed by the addition of 1*H*-tetrazole (0.63 g, 9.0 mmol) in one portion. After 20 min, the solution is cooled to −20°C and 14% aq. *tert*-butyl hydroperoxide (2.6 mL, 4.0 mmol) added. After 10 min, an aq. solution of 10% $Na_2S_2O_5$ (10 mL) is added at −20°C and the solution is then transferred to a separating funnel using Et_2O (30 mL). The aqueous phase is then discarded and the organic phase washed with 10% $Na_2S_2O_5$ (15 mL). The solvent is removed by evaporation under reduced pressure and the residue dissolved in AcOH/H_2O/THF (2:1:1, 6 mL) and stirred for 60 min at

20 °C. The solvent is evaporated under reduced pressure, the oil dissolved in Et_2O (40 mL) and extracted with 5% $NaHCO_3$ solution (3 × 10 mL). The combined aqueous extract is washed with Et_2O (30 mL), acidified to pH 2 with 30% HCl, and extracted with CH_2Cl_2 (3 × 30 mL). The organic extract is dried (Na_2SO_4) and the solvent is evaporated under reduced pressure to give **6c** as a clear oil, which becomes a white solid on standing; yield: 1.48 g (91%); mp 91–93 °C, (Lit.[15] mp 91.5–92.5 °C).

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(1) Valerio, R.M., Alewood, P.F., Johns, R.B., Kemp, B.E. *Tetrahedron Lett.* **1984**, *25*, 2609.
(2) Valerio, R.M., Alewood, P.F., Johns, R.B., Kemp, B.E. *Int. J. Pept. Protein. Res.*, in press.
(3) Perich, J.W., Johns, R.B. *J. Org. Chem.* **1989**, *54*, 1750.
(4) Kitas, E.A., Perich, J.W., Johns, R.B., Tregear, G.W. *Tetrahedron Lett.* **1988**, *29*, 3591.
(5) Perich, J.W., Alewood, P.F., Johns, R.B. *Synthesis* **1986**, 572.
(6) Scherer, O.J., Thalacker, R.Z. *Z. Naturforsch. Teil B* **1972**, *27*, 1429.
(7) Perich, J.W., Johns, R.B. *Tetrahedron Lett.* **1987**, *28*, 101.
(8) Perich, J.W., Johns, R.B. *Synthesis* **1988**, 142.
(9) Perich, J.W., Johns, R.B. *Tetrahedron Lett.* **1988**, *29*, 2369.
(10) Perich, J.W. *Ph. D. Dissertation*, University of Melbourne, 1986.
(11) Burgada, R. *Bull. Soc. Chim. Fr.* **1972**, 4161.
(12) Gryaznov, P.I., Kibordin, A.M., Gazizov, T.Kh., Pudovik, A.N. *J. Gen. Chem. USSR (Engl. Transl.)* **1972**, *94*, 6190.
(13) Corey, E.J., Venkateswarlu, J. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(14) The optical rotation for **6a** has only been reported[1,2] in MeOH $[\alpha]_D^{20} -0.10°$ ($c = 1$, MeOH) and is not a useful comparison.
(15) Gibson, B.W., Falick, A.M., Burlingame, A.L., Nadasdi, L., Nguyen, A.C., Kenyon, G.L. *J. Am. Chem. Soc.* **1987**, *109*, 5343.