An Efficient One-Pot Synthesis of *N*-Protected α-Amino-β-dialkoxyphosphinyloxy(diphenoxyphosphinyloxy)-carboxylic Acids (Phosphate-Group Esters of *O*-Phosphorylated *N*-Protected α-Amino-β-hydroxyamino Acids)

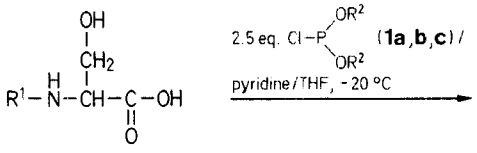
John W. PERICH, Paul F. ALEWOOD*, R. B. JOHNS**

Department of Chemistry, University of Melbourne, Parkville, Victoria 3052, Australia

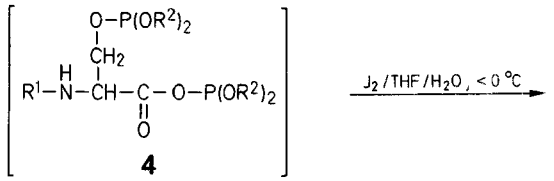
Dialkyl or diphenyl phosphorochloridites readily phosphitylate *N*-Boc- and *N*-Z-serine in the presence of pyridine to give *N*-protected *O,O'*-disubstituted phosphoserines in high yields after iodine/water oxidation. This procedure was also extended to the preparation of *N*-protected *O,O'*-disubstituted phosphothreonines and *N*-protected *O,O'*-disubstituted phosphotyrosines.

The phosphorylation of various endogenous proteins is now widely recognized to play a major regulatory role in cell metabolism[1]. Until recently, the preparation of simple phosphopeptide substrates[2] for chemical and biochemical studies has been limited due to the lack of an efficient synthetic protocol. We have developed a strategy which involves the preparation of suitably protected phosphoamino acids[3,4] followed by their direct incorporation into peptides[5].

Previous syntheses of phosphate-group esterified *N*-protected phospho-L-serines (**5a, b, c, 6a, b, c**) started with commercially available *N*-protected serine (**2** or **3**) and involved carboxy-group protection, *O*-phosphorylation, and a final deprotection[3,4]. Although this three-step procedure gave the desired products in fair overall yield, several manipulations and silica-gel chromatography were required. In view of these technical difficulties, we recognized that an improvement in synthetic procedures would be advantageous if it obviated these time-consuming steps and afforded phosphoserine products in high yields and in shorter reaction times. Our approach to this problem was to investigate the possibility of obtaining **5a, b, c** and **6a, b, c** by the direct phosphorylation of the precursors **2** and **3**.



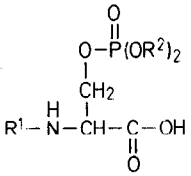
2 R¹ = Boc
3 R¹ = Z



4

1,5,6	R²
a	CH_3
b	C_2H_5
c	C_6H_5

Boc = *t*-butoxycarbonyl
Z = benzoxycarbonyl

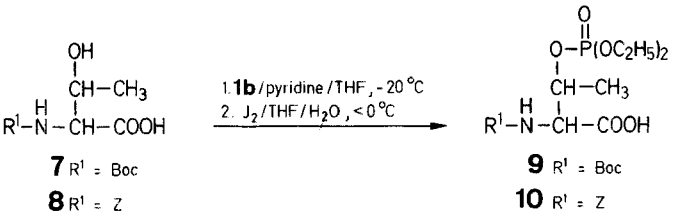


5a,b,c R¹ = Boc
6a,b,c R¹ = Z

Recent work[6,7] militated against the use of dialkyl and diaryl phosphorochloridates for such one-pot conversions because of the preferential formation of the mixed anhydride which, in the presence of an alcohol, gave the carboxylic ester. However, we considered that the more reactive dialkyl and diaryl phosphorochloridites (**1a, b, c**) would readily form the intermediate bis-phosphite **4** and give the desired phosphoserine derivative after an aqueous oxidative work-up.

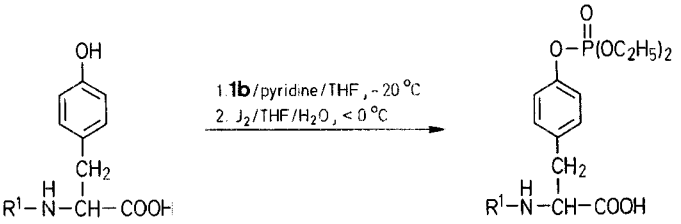
In our present work, we describe the one-pot preparation of phosphoserines **5a, b, c** and **6a, b, c** by the low-temperature treatment of *N*-protected serine (**2** or **3**) with 2.5 equivalents of dimethyl[8], diethyl[9], or diphenyl[10] phosphorochloridite (**1a, b, c**) followed by oxidative work-up (iodine/water[11]) and sodium hydrogen carbonate extraction. Subsequent acidification to pH 3 and a final dichloromethane extraction gives the desired phosphoserine derivatives **5a, b, c** and **6a, b, c** in 70–90% yields, the yields being a function of the purity of phosphorochloridous esters **1a, b, c**.

In addition, we report that this procedure is also suitable for the preparation of *N*-protected phospho-L-threonine derivatives **9** and **10** and *N*-protected phospho-L-tyrosine derivatives **13**[12] and **14**. This procedure is demonstrated by the one-pot phosphorylation of *N*-protected threonine (**7** and **8**) and *N*-protected tyrosine (**11** and **12**) with commercially available phosphorochloridite **1b**.



7 R¹ = Boc
8 R¹ = Z

9 R¹ = Boc
10 R¹ = Z



11 R¹ = Boc
12 R¹ = Z

13 R¹ = Boc
14 R¹ = Z

The advantage of this one-pot procedure lies in the good yields (70–90%), the homogeneity of the products (> 95% pure by ^{31}P-N.M.R.), and the short times required (< 1 hour).

***N*-Protected α-Amino-β-dialkoxyphosphinyloxy (or diphenoxyphosphinyloxy)-carboxylic Acids (5a, b, c, 6a, b, c, 9, 10) and *N*-Protected *O*-(Diethoxyphosphinyl)-tyrosine (13, 14); General Procedure:**
Diethyl phosphorochloridite (**1b**; 4.0 ml, ~25.0 mmol) (or dimethyl or diphenyl phosphorochloridite, **1a** or **1c**) is rapidly injected by syringe into a rapidly stirred solution of the *N*-protected amino acid (10.0 mmol) and pyridine (6 ml) in dry tetrahydrofuran (30 ml) at –20 °C under nitrogen. The temperature of the solution rises to 0° and there is an immediate precipitation of pyridine hydrochloride. The mixture is stirred at 0° for 15 min and then cooled to –30°. A solution of iodine (6.33 g, 25.0 mmol) in tetrahydrofuran/water (9/1; 10 ml) is rapidly added so that the temperature is maintained below 0° (at this stage, manual shaking of the mixture may be necessary to

Table. Compounds **5, 6, 9, 10, 13,** and **14** prepared

Product	Yield [%]	m.p. [°C][a]	Molecular Formula[b] or Lit. Data	$[\alpha]_D^{13}$[c] ($c = 1$, CH_2Cl_2)	^{13}C-N.M.R. ($CDCl_3/TMS_{int}$)[d] δ [ppm]	^{31}P-N.M.R. ($CDCl_3$/85% H_3PO_{4ext})[e] δ [ppm]
5a	79	68–70°	$C_{10}H_{20}NO_8P$ (313.2)	+ 43.1°	27.9, 53.5, 54.4, 67.5, 79.9, 155.1, 170.5	+ 0.5
5b	88	55–57°	55.5–57°[3]	+ 41.6°[f]	15.9, 28.2, 53.7, 64.5, 67.5, 80.1, 155.4, 170.5	− 2.0[f]
5c	72	62–64°	$C_{20}H_{24}NO_8P$ (437.4)	+ 40.0°	28.2, 53.7, 68.9, 80.4, 120.0, 125.6, 129.8, 150.1, 155.4, 170.7	−12.5
6a	76	oil	$C_{13}H_{18}NO_8P$ (347.3)	+ 27.6°	54.2, 54.8, 67.1, 67.5, 128.1, 128.5, 136.1, 156.0, 170.5	+ 0.1
6b	85	oil	$C_{15}H_{22}NO_8P$ (375.3)	+ 36.6°	15.8, 54.1, 64.5, 67.0, 67.2, 127.9, 128.4, 136.0, 156.0, 170.4	− 1.8
6c	70	oil	$C_{23}H_{22}NO_8P$ (471.4)	+ 34.8°	54.1, 67.2, 68.7, 119.9, 125.7, 127.9, 128.4, 129.8, 135.8, 150.0, 156.0, 170.4	−12.3
9	84	oil	$C_{13}H_{26}NO_8P$ (355.1)	+134.1°	15.9, 18.4, 28.2, 57.4, 64.4, 75.9, 80.0, 155.9, 171.1	− 2.9
10	84	oil	$C_{16}H_{24}NO_8P$ (389.1)	+ 21.0°	15.9, 18.4, 58.2, 64.5, 67.3, 75.8, 128.0, 128.5, 136.0, 156.7, 171.2	− 2.7
13	74	oil	$C_{18}H_{28}NO_8P$ (417.1)	+ 45.4°[g]	15.9, 28.1, 37.1, 54.1, 64.8, 79.9, 119.8, 130.7, 133.3, 149.3, 155.3, 173.8	− 6.8[h]
14	77	oil	$C_{21}H_{26}NO_8P$ (451.1)	+ 53.8°	15.9, 36.9, 54.6, 65.0, 66.8, 119.9, 127.9, 128.1, 128.4, 130.8, 136.2, 149.4, 156.0, 173.5	− 6.4

[a] Kofler melting point apparatus, uncorrected.
[b] Satisfactory microanalyses obtained for **5a, b, c:** C ±0.12, H ±0.27, N ±0.17, P ±0.21. Accurate mass obtained for **6a, b, c, 9, 10, 13, 14** (V.G. Micromass 7070 F mass spectrometer operating at 15 or 70 eV).
[c] Perkin-Elmer Model 241 MC Polarimeter.
[d] JEOL-FX 100 operating at 25.00 MHz.
[e] JEOL-FX 100 operating at 40.26 MHz.
[f] Ref.[3]; $[\alpha]^{27D}$: +37.2° (C = 1.4, $CHCl_3$); ^{31}P-N.M.R. ($CDCl_3/TMS_{int}$): $\delta = -2.0$ ppm.
[g] This corresponds well with $[\alpha]^{13D}$: +43.4° (C = 1, $CHCl_3$), obtained for authentic **13**[12].
[h] Ref.[12], ^{31}P-N.M.R. ($CDCl_3$/85% H_3PO_{4ext}): $\delta = -6.7$ ppm.

disperse the thick slurry). The mixture is then allowed to warm to room temperature and an aqueous 10% solution of sodium disulfite (100 ml) is added. The mixture is extracted with ether (2 × 75 ml). The yellow organic extract is washed with an aqueous 10% solution of sodium disulfite (100 ml) and is then extracted with 5% sodium hydrogen carbonate solution (3 × 30 ml). The combined aqueous extract is washed with ether (3 × 50 ml), acidified to pH 3 with 30% hydrochloric acid, and extracted with dichloromethane (3 × 75 ml). The organic extract is dried with sodium sulfate and the solvent is removed under reduced pressure to give the desired product as a clear colourless oil.

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* Current address: Victorian College of Pharmacy, Royal Parade, Parkville, Victoria 3052, Australia.
** Address for correspondence.

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