A Convenient Synthesis of *N*-Phosphoryldipeptide Acids by Direct Phosphorylation

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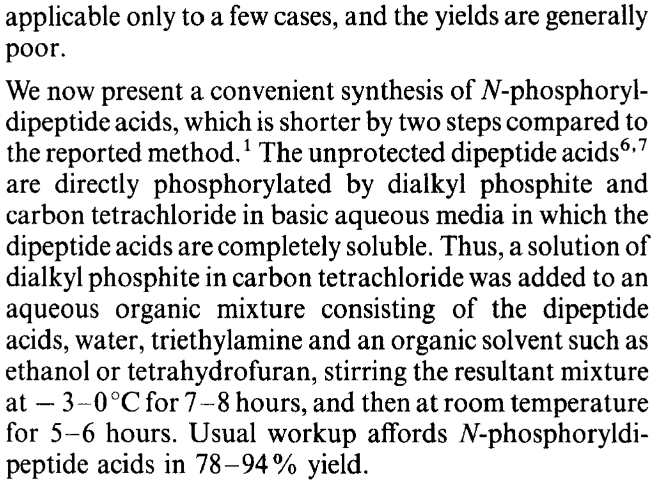
Dipeptide acids are directly phosphorylated with dialkyl phosphite and triethylamine in carbon tetrachloride/water under mild conditions to give the title compounds in high yields.

The pharmacological properties such as anti-hypertension[1,2] and anti-bacterial collagenase[3] activities of *N*-phosphorylated small peptide acids have stimulated investigation of the synthesis of these compounds.[4] The known syntheses involve the phosphorylation of peptide esters with phosphoryl chlorides with dialkoxy or diaryl- oxy(chloro)phosphines in non-phosphorylpeptide esters.[1-5] However, these procedures are tedious and applicable only to a few cases, and the yields are generally poor.

We now present a convenient synthesis of *N*-phosphoryldipeptide acids, which is shorter by two steps compared to the reported method.[1] The unprotected dipeptide acids[6,7] are directly phosphorylated by dialkyl phosphite and carbon tetrachloride in basic aqueous media in which the dipeptide acids are completely soluble. Thus, a solution of dialkyl phosphite in carbon tetrachloride was added to an aqueous organic mixture consisting of the dipeptide acids, water, triethylamine and an organic solvent such as ethanol or tetrahydrofuran, stirring the resultant mixture at − 3–0 °C for 7–8 hours, and then at room temperature for 5–6 hours. Usual workup affords *N*-phosphoryldipeptide acids in 78–94% yield.



3	R^1	R^2	R^3	3	R^1	R^2	R^3
a	Bu	H	H	**d**	*i*-Pr	Me	Me
b	*i*-Pr	H	H	**e**	Bu	Me	$CH_2C_6H_4OH$-4
c	Bu	Me	Me	**f**	*i*-Pr	*i*-Bu	$CH_2C(O)NH_2$

In order to obtain high yields of *N*-phosphoryldipeptide acids, the optimum ratio of the precursors dipeptide acid[6,7]/dialkyl phosphite/carbon tetrachloride/triethylamine is 1 : 1.05 : 2.5 : 2.5, and the dialkyl phosphite must be added dropwise slowly at below 0 °C. It was found that the byproducts in this reaction were dialkyl phosphate and tetraalkyl pyrophosphate which could be removed by washing the basic reaction mixture with diethyl ether or ethyl acetate after removal of the organic solvent with a rotary evaporator. Then the aqueous layer was acidified carefully with dilute hydrochloric acid to pH 3 in an ice bath to avoid decomposition of the product. At last the pure product could be afforded by simple extraction and washing (Table).

The structure proposed for **3a–f** is in agreement with the results of the spectroscopic data and microanalyses. It is worth noting that the phosphoryl group offers an improvement in sensitivity of the FAB mass spectra of *N*-phosphoryldipeptide acids by factor of 4–29 as compared to the corresponding dipeptide acid. The fragmentations of the *N*-phosphoryldipeptide acids were characterized by the successive losses of alkylene from the dialkyloxy groups.[8,9]

In conclusion, we have revealed a practical synthesis of *N*-phosphoryldipeptide acids. This method is advantageous over the previous procedure in terms of simple workup, easily available reagents, short step and high yield. It has successfully been used for the direct phosphorylation of pentapeptide acids in our lab, and further studies on the phosphorylation of longer peptide acids such as nonapeptide acid are in progress.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are not corrected. Positive-ion FAB-MS data were obtained on a KYKY Zhp-5 or a VG-ZAB-HS double-focussing mass spectrometer and VG11-250 data system. IR spectra were recorded on a Shimadzu 430 spectrophotometer. ^1H, ^{31}P and ^{13}C NMR spectra were obtained using a JEOL FX-100 spectrometer. Optical rotations were measured with WXG-4 polarimeter made by Daqing Optical Instrument Factory (Shanghai, China).

N-(Diisopropoxyphosphoryl)glycylglycine (3b); Typical Procedure: To an ice-salt-cold solution of glycylglycine (**2a**; 3.0 g, 22.7 mmol) in H_2O (20 mL), EtOH (20 mL) and Et_3N (7.9 mL, 56.8 mmol) was added dropwise a solution of diisopropyl phosphite (**1b**; 3.9 mL, 23.9 mmol) in CCl_4 (5.5 mL, 56.8 mmol) over a period of 40 min. The resultant mixture was stirred at − 3–0 °C for 7–8 h, and then at r.t. for 5–6 h. The mixture was diluted with H_2O (20 mL) and the organic solvent was removed by distillation in vacuo. The aqueous phase was washed with EtOAc (2 x 10 mL), acidified to pH 3 with dilute HCl, and then extracted with EtOAc (5 x 10 mL). The combined extracts are washed with 20% citric acid solution (3 x 10 mL) and sat. aq NaCl (3 x 10 mL), respectively, dried ($MgSO_4$), and concentrated in vacuo to give a colorless viscous oil, which solidified on standing below − 10 °C. A small amount of solvent was removed under reduced pressure at r.t. The crude product was crystallized from $EtOAc/Et_2O$ to afford colorless crystals **3b**; yield: 6.3 g (94%); mp 83–84 °C. (Table).

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Table. *N*-Phosphoryldipeptide Acids **3a–f** Prepared[a]

Product	Yield[b] (%)	mp (°C)	Molecular Formula[c]	FAB-MS (MH+)	IR (KBr) ν (cm^{-1})	^{1}H NMR ($CDCl_3$) δ, *J* (Hz)	^{13}C NMR ($CDCl_3$/TMS, 25.14 MHz) δ, *J* (Hz)	^{31}P NMR[d] δ
3a	92	64–65	$C_{12}H_{25}N_2O_6P$ (324.3)	325	1650, 1720	0.9 (t, 6H, $2CH_3$), 1.1–1.9 (m, 8H, $2CH_2CH_2$), 3.5–3.8 (d, 2H, *J* = 11.4, CH_2N), 3.8–4.3 (m, 6H, $2CH_2O$, CH_2N), 7.4 (br s, 2H, 2NH), 10.4 (br s, 1H, CO_2H)	13.0 (CH_3), 18.2 ($CH_3\underline{C}H_2$), 31.7 (*J* = 5.9, CH_2), 40.7 (CH_2N), 44.1 (*J* = 6.5, CH_2N), 66.4 (*J* = 5.9, CH_2O), 170.3 (C=O), 170.9 (*J* = 5.9, C=O)	8.30
3b	94	83–84	$C_{10}H_{21}N_2O_6P$ (296,3)	297	1660, 1730	1.2 (d, 12H, $4CH_3$), 3.4–4.2 (m, 4H, $2CH_2N$), 4.3–4.8 (m, 2H, 2CHO), 7.5 (br s, 2H, 2NH), 10.6 (br s, 1H, CO_2H)	22.9 [*J* = 2.9, $(\underline{C}H_3)_2C$], 40.6 (CH_2N), 43.6 (*J* = 6.8, CH_2N), 71.5 [*J* = 5.9, $COH_3)_2\underline{C}$], 171.4 (*J* = 5.9, C=O), 172.1 (C=O)	8.26
3c	88	liquid	$C_{14}H_{29}N_2O_6P$ (352.4)	353	1655, 1780	0.6–1.1 (m, 6H, $2CH_3$), 1.2–1.9 (m, 14H, $2CH_2CH_2$, $2CH_3$), 3.6–4.3 (m, 6H, $2CH_2O$, 2CHN), 7.6 (br t, 2H, 2NH), 12.3 (br s, 1H, CO_2H)	12.9 (CH_3), 17.4 (CH_3), 18.2 ($CH_3\underline{C}H_2$), 31.9 (*J* = 5.9, CH_2), 47.8 (CHN), 50.6 (CHN), 66.4 (*J* = 9.0, CH_2O), 171.3 (*J* = 4.4, C=O), 174.2 (C=O)	7.63
3d	92	129–130	$C_{12}H_{25}N_2O_6P$ (324.3)	325	1634, 1725	1.2 (d, 18H, $6CH_3$), 3.6–4.3 (m, 2H, 2CHN), 4.4–4.8 (m, 2H, 2CHO), 7.6; 7.8 (2 br s, 2H, 2NH), 11.5 (br s, 1H, CO_2H)	17.5 (CH_3), 23.2 [$(\underline{C}H_3)_2C$], 47.7 (CHN), 50.6 (*J* = 7.0, CHN), 71.2 [*J* = 6.4, $(CH_3)_2\underline{C}$], 173.3 (*J* = 4.7, C=O), 174.4 (C=O)	5.55
3e	78	74–76	$C_{20}H_{33}N_2O_7P \cdot H_2O$ (462,5)	445	1700, 1770	0.8–1.0 (m, 6H, $2CH_3$), 1.0–1.8 (m, 13H, CH_3, CH_2, $2CH_2CH_2$), 2.8–3.2 (m, 2H, 2CHN), 3.5–4.3 (m, 4H, *J* = 7.1, $2CH_2O$), 4.7 (br s, 1H, OH), 6.6–7.1 (m, 4H_{arom}), 8.4 (br s, 2H, 2NH), 10.5 (br s, 1H, CO_2H)	13.4 (CH_3), 17.3 (CH_3), 18.6 ($CH_3\underline{C}H_2$), 32.2 (*J* = 5.9, CH_2), 36.7 ($\underline{C}H_2C_6H_4$), 51.1 (CHN), 53.6 (CHN), 66.9 (*J* = 2.9, CH_2O), 115.5, 126.8, 130.3 (C_{arom}), 168.7 (C=O), 173.5 (*J* = 5.9, C=O)	7.45
3f	82	83–85	$C_{16}H_{32}N_3O_7P$ (409.4)	410	1670, 1728	0.9 (t, 12H, $4CH_3$), 1.2 (d, 6H, $2CH_3$), 1.3–1.8 (m, 5H, $2CH_2$, CH), 2.8–3.0 (m, 2H, 2CHN), 4.5–4.8 (m, 2H, 2CHO), 6.8 (br d, 2H, 2NH), 8.0 (br s, 2H, NH_2), 11.5 (br s, 1H, CO_2H)	14.0 (CH_3), 21.8 ($CH_3\underline{C}H_2$), 23.8 [$(\underline{C}H_3)_2C$], 23.9 (CH_2), 37.0 ($\underline{C}H_2CO$), 49.1 (CHN), 54.1 (*J* = 3.1, CHN), 71.5 (CH_2O), 171.0 ($\underline{C}ONH_2$), 173.4 (*J* = 2.5, C=O), 174.2 (C=O)	6.25

[a] Optical rotations: **3e** $[\alpha]_D^{30} = 56.5°$ ($c = 0.9$, EtOAc), **3f**: $[\alpha]_D^{12} - 29.5°$ ($c = 1.90$, EtOAc); **3c,d** made from *dl*-alanine.

[b] Yield of pure isolated product; for **3d**, the yield is that obtained when NaOH was used as base.

[c] Satisfactory microanalyses obtained: C ± 0.48, H ± 0.29, N ± 0.28.

[d] ($CHCl_3/H_3PO_{4\,extern}$).

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