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A Convenient Synthesis of Phosphonate Isosteres of Serine Phosphates

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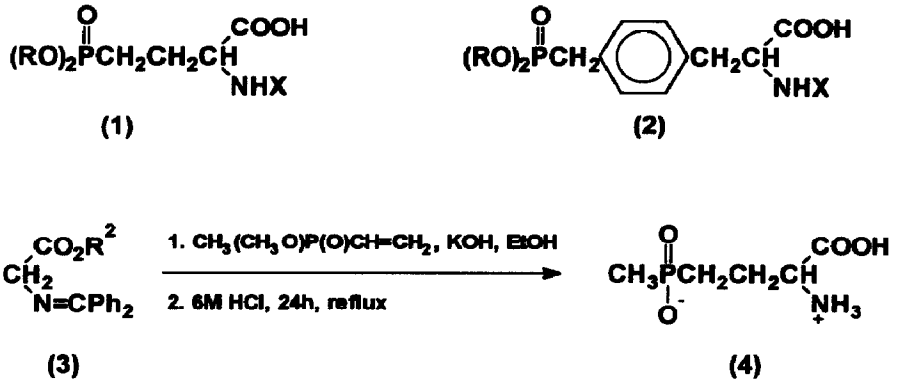
Abstract: Serine phosphonate isosteres (**7**), suitably protected for direct use in peptide synthesis, have been prepared from vinylphosphonate diesters in three steps and high overall yield.

The addition of inorganic esters, especially phosphate[1] and sulphate, to peptides and proteins is an important mechanism for the control of biochemical processes both at the enzyme/substrate level and also in some agonist/receptor interactions. In the case of phosphorylation, for example, it seems likely that aberration in kinase and phosphatase activity plays an important role in tumorigenesis[2]. In particular, the subversion of growth control by a number of oncogenes appears to reside in their capacity to up-regulate kinase, especially tyrosine kinase, activity[3]. There is substantial interest in the synthesis of suitably protected phosphonate analogues (**1**)[4] and (**2**)[5] of, respectively, serine phosphate and tyrosine phosphate since such isosteres possess the hydrolytically stable P-C bond[6] and if incorporated into peptides should allow more precise definition of the contribution of an individual kinase or phosphatase to a metabolic process. Such studies may offer rational routes to novel antitumour agents.

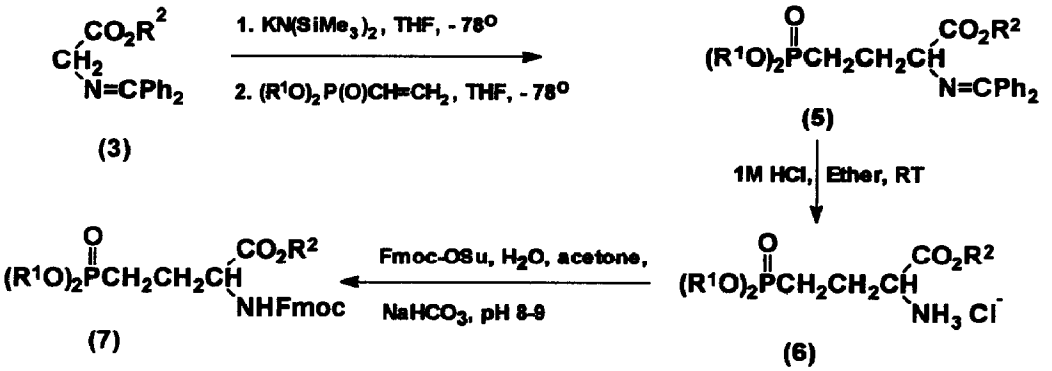
Currently available syntheses of (**1**) are generally long, the shortest reported involves seven isolated intermediates,[4b] and mostly do not provide derivatives appropriately protected at both phosphorus and nitrogen but with the free carboxylic acid function required for peptide synthesis. We now report a highly convenient route to derivatives which can be used directly in peptide synthesis.

Minowa has reported[7] that *fully deprotected* phosphinothricin (**4**) can be prepared by the base-induced addition of the glycine Schiff base (**3**) to methyl methylvinylphosphinate followed by acid hydrolysis (Scheme 1).



Scheme 1

We have found that the addition of vinylphosphonate diesters to a solution of potassium (*N*-diphenylmethylene) glycine esters (**3**, R^2 = Et, ^{t}Bu) in THF at -78°C provides the corresponding fully protected serine phosphonate isosteres (**5**)[8] in excellent yield (>90%) (Scheme 2).[9]



Scheme 2

(a) R^1=Et, R^2=^{t}Bu; (b) R^1=R^2=Et; (c) R^1=Me, R^2=Et; (d) R^1=Et, R^2=H; (e) R^1=Me, R^2=H

^{1}H Nmr monitoring of the reaction of (**5a**, **b**, and **c**) with ethereal hydrochloric acid indicated that, while the phosphonate ester remained unaffected, both the imine and the carboxylic ester, including surprisingly the ethyl ester group, were hydrolysed under such conditions[10] to give 4-(diethylphosphono)- (**6d**) and 4-(dimethylphosphono))- (**6e**) (2-amino)butanoic acid hydrochlorides[11]. This approach, after appropriate *N*-protection, provides a three-step, high yield route to the serine phosphonate isosteres 4-(diethylphosphono)- (**7d**) and 4-(dimethylphosphono)- (**7e**) 2-{*N*-(9-fluorenylmethoxy-

carbonyl)amino}butanoic acids[12] in a form suitable, albeit racemic, for direct use in peptide synthesis. In no case did competition from phosphonate ester hydrolysis present a problem although small amounts (less than 5%) of ester exchange occurred in some mixed ester cases.

By using shorter reaction times it was possible to remove the imine group to give (**6b** and **6c**)[10] without hydrolysis of the carboxylic ester. Following Fmoc protection and mild alkaline hydrolysis,[13] (**6b** and **6c**) gave the free carboxylic acid derivatives (**7d** and **7e**).

We have successfully incorporated the serine derivative (**7d**)[14] into peptide sequences and, following dealkylation using iodotrimethylsilane,[15] obtained the free phosphonic acid peptide derivatives which separated into two diastereoisomers on reverse phase HPLC.[16]

In conclusion, this approach provides a very convenient route to racemic phosphonoserine derivatives[17] suitably protected for ready incorporation into peptides. We are currently investigating the synthesis of single enantiomers of both serine[18] and tyrosine phosphonates by routes based on our general approach to the racemic derivatives.

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7. N. Minowa, S. Fukatsu, T. Niida, M. Takada, and K. Sato, *Tetrahedron Letters*, 1983, **24**, 2391; this synthesis is reported as a one-pot reaction, in moderate yield and with few details.
8. (**5a**): ^{1}H NMR (300MHz, $CDCl_3$) δ : 1.31 (t, 6H, $OCH_2\underline{CH_3}$, J =7.01Hz), 1.44 (s, 9H, $C(CH_3)_3$), 1.77 (m, 2H, PCH_2), 2.15 (m, 2H, βCH_2), 3.95 (t, 1H, αH), 4.08 (quin, 4H, OCH_2, J = 7.15Hz), 7.17-7.64 (m, 10H, aromatic). (**5b**): ^{1}H NMR (500MHz, $CDCl_3$) δ : 1.25 (t, 3H, J =7.09 Hz, $COCH_2\underline{CH_3}$), 1.28 (t, 3H, J = 7.09Hz, $POCH_2\underline{CH_3}$), 1.32 (t, 3H, J =7.09Hz, $POCH_2\underline{CH_3}$), 1.75 (m, 2H, PCH_2), 1.90 (m, 1H, βCH_2), 2.17 (m, 1H, βCH_2), 4.03-4.18 (m, 4H, $POCH_2$), 4.19 (q, 2H, J =7.09 Hz, $COCH_2$), 4.15 (m, 1H, αCH),7.16-7.82 (m, 10H, aromatic). MS: m/z 431 $(M)^+$. (**5c**): ^{1}H NMR (300MHz, $CDCl_3$) δ: 1.25 (t, 3H, $OCH_2\underline{CH_3}$, J =7.09Hz), 1.81 (m, 2H, PCH_2), 2.17 (m, 2H, βCH_2), 3.70 (d, 3H, OCH_3, J_{PCH}=10.80Hz), 3.72 (d, 3H, OCH_3, J_{PCH}=10.79Hz), 4.16 (q, 2H, $O\underline{CH_2}CH_3$ J =7.09Hz), 4.08 (m, 1H, αCH), 7.16-7.82 (m, 10H, aromatic). MS m/z 403 $(M)^+$.
9. Attempts to generate the anion of (**3**) using a variety of other conditions were less successful; for example, only traces of (**5**) could be isolated when lithium di*iso*propylamide was used as the base.
10. Ester hydrolysis may be assisted by involvement of either the phosphoryl or the imine group. However, in contrast to the serine analogues, treatment of the tyrosine isostere triesters with acid to remove the imine-protecting group does not lead to simultaneous hydrolysis of the carboxylate ester.[15] This may indicate that anchimeric assistance of the phosphoryl group, rather than the imine group, is involved in the facile hydrolysis of the serine phosphonate triester (**5**).

11. **(6b)**: ^{1}H NMR (300MHz, D_2O) δ : 1.29 (3xt, 9H, $OCH_2\underline{CH_3}$), 1.85-2.48 (m, 4H, PCH_2), 4.13 (m, 6H, $O\underline{CH_2}CH_3$), 4.56 (q, 1H, αCH). MS: m/z 267 $(M)^+$. **(6c)**: ^{1}H NMR (300MHz, D_2O) δ : 1.29 (t, 3H, $OCH_2\underline{CH_3}$, J =7.10Hz), 1.9-2.6 (m, 4H, $-CH_2\underline{CH_2}$-), 3.77 (d, 6H, OCH_3, J_{POCH} =10.96Hz), 4.21 (t, 1H, αCH), 4.31 (q, 2H, $O\underline{CH_2}CH_3$, J =7.07Hz). **(6d)**: ^{1}H NMR (300MHz, D_2O) δ : 1.26 (t, 6H, $OCH_2\underline{CH_3}$, J =7.06Hz), 1.90-2.60 (m, 4H, $-CH_2\underline{CH_2}$-), 4.07 (m, 1H, αCH), 4.09 (q, 4H, OCH_2, J =7.26Hz). MS m/z 194 $(M+H)^+$-OEt. **(6e)**: ^{1}H NMR (500MHz, D_2O) δ : 2.0-2.2 (m, 4H, $-CH_2\underline{CH_2}$-), 3.76 (d, 6H, $-OCH_3$, J_{POCH}=10.74Hz), 4.08 (t, 1H, αCH).
12. **(7b)**: ^{1}H NMR (300MHz, $CDCl_3$) δ : 1.32 (3xt, 9H, OCH_2CH_3), !.77 (m, 2H, PCH_2), 1.96 (m, 1H, βCH), 2.18 (m, 1H, βCH), 4.09 (m, 4H, $POCH_2$), 4.21 (m, 3H, $COCH_2$ and FMOC CH), 4.39 (m, 3H, αCH and FMOC CH_2), 5.57 (d, 1H, NH, J =7.84Hz), 7.32 (t, 2H, fluorenyl H2,H7, J =7.05Hz), 7.40 (t, 2H, fluorenyl H3,H6, J =7.34Hz), 7.58 (m, 2H, fluorenyl H4,H5), 7.77 (d, 2H, fluorenyl H1,H8, J =7.45Hz). **(7c)**: ^{1}H NMR (300MHz, $CDCl_3$) δ : 1.28 (t, 3H, $OCH_2\underline{CH_3}$, J =7.14Hz), 1.74-1.99 (m, 3H, PCH_2 and βCH), 2.19 (m, 1H, βCH), 3.74 (d, 6H, OCH_3, J_{PCH}=10.56Hz), 4.21 (m, 3H, FMOC CH and $O\underline{CH_2}CH_3$), 4.38 (m, 3H, FMOC CH_2 and αCH), 5.60 (d, 1H, NH, J =7.82Hz), 7.31 (t, 2H, fluorenyl H2,H7, J =7.44Hz), 7.4 (t, 2H, fluorenyl H3,H6, J =7.34Hz), 7.60 (m, 2H, fluorenyl H1,H8), 7.76 (d, 2H, fluorenyl H4,H5, J =7.48Hz). MS m/z 462 $(M+H)^+$. **(7d)**: ^{1}H NMR (300 MHz, $CDCl_3$) δ : 1.31 (t, 3H, $OCH_2\underline{CH_3}$, J =7.07Hz), 1.33 (t, 3H, OCH_2CH_3, J =6.87Hz), 1.85 (m, 2H, PCH_2), 2.06 (m, 1H, βCH), 2.23 (m, 1H, βCH), 4.11 (m, 4H, $O\underline{CH_2}CH_3$), 4.21 (t, 1H, FMOC CH, J =6.91Hz), 4.37 (m, 3H, αCH and FMOC CH_2), 5.79 (d, 1H, NH, J =7.24Hz), 7.30 (t, 2H, fluorenyl H2, H7, J =7.15Hz), 7.39 (t, 2H, fluorenyl H3,H6, J =7.38Hz), 7.59 (m, 2H, fluorenyl H4,H5), 7.76 (d, 2H, fluorenyl H1,H8, J =7.38Hz). **(7e)**: ^{1}H NMR (500MHz, $CDCl_3$) δ : 1.81-2.00 (m, 2H, PCH_2), (m, 1H, βCH), (m, 1H, βCH), 3.76 (d, 3H, OCH_3, J_{POCH}=10.82Hz), 3.78 (d, 3H, OCH_3, J_{POCH}=10.8Hz), 4.22 (t, 1H, FMOC CH), 4.36-4.45 (m, 3H, FMOC CH_2 and αCH), 5.78 (d, 1H, NH, J=7.21Hz), 7.31 (t, 2H, fluorenyl H2, H7, J=7.21Hz), 7.40 (t, 2H, fluorenyl H3, H6, J=7.21Hz), 7.60 (t, 2H, fluorenyl H1, H8, J=7.20Hz), 7.76 (d, 2H, fluorenyl H4, H5, J=7.21Hz). MS m/z 432 $(M-1)^+$, 439 $(M-1+Li)^+$.
13. Na_2CO_3, H_2O, CH_3CN, 18°C, 1 hour
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