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SYNTHESIS OF PROTECTED DERIVATIVES OF O-PHOSPHOTYROSINE INCORPORATION IN A HEPTAPEPTIDE

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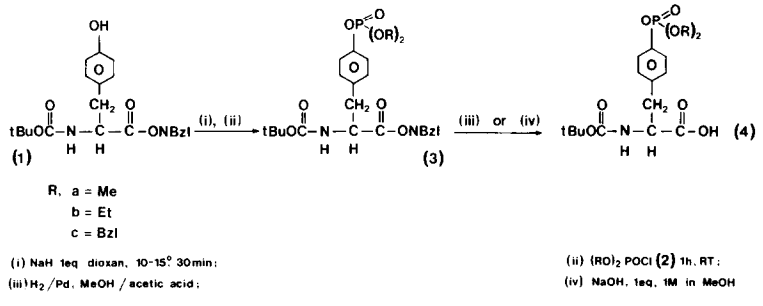
Abstract: The solid phase synthesis of the phosphopeptide Leu-Arg-Arg-Ala-Tyr(P)-Leu-Gly is reported via the stepwise incorporation of the protected phosphoamino acid N^{α}-*tert*-butyloxycarbonyl-O-dimethyl-phosphono-L-tyrosine.

It is now recognized that phosphorylation of protein tyrosine residues may play an important regulatory role in a number of cellular processes including the action of several peptide hormone receptors[1] and the retroviral transformation of cells[2]. In view of the importance of these processes we have sought to develop procedures for the preparation of O-phosphotyrosine[3] and synthetic peptides containing this phosphoamino acid to facilitate more detailed chemical studies. Two syntheses[4,5] of small peptides containing O-phosphotyrosine have been reported. However, the methods employed are tedious and lack in general applicability.

Our communication describes the synthesis of several protected O-phosphotyrosine derivatives (Scheme I) and the incorporation of 4a into a peptide sequence.

SCHEME 1.



Phosphorylation of the sodium salt of tyrosine derivative 1 with the phosphochloridates $(RO)_2POCl$ 2(a-c) gave the phosphate esters 3(a-c) as light yellow oils in excellent yield (90-95%, homogenous by TLC, ethyl acetate/pentane, 1:1). (Table 1, Scheme 1).

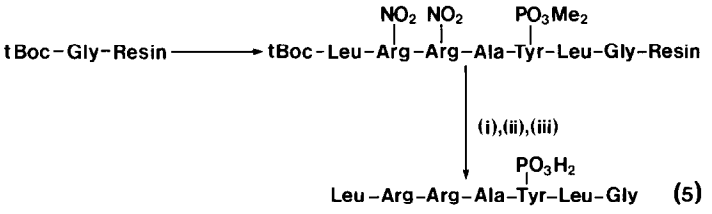
Table 1

Derivative	Yield	^{31}PNMR ($CHCl_3$)	α_D^{20} (C=1, MeOH)	IR (neat)
3a	90	-3.8,s	-10.0	3350,br,NH; 1750, ester; 1715, amide.
3b	96	-6.3,s	-10.5	3400,br,NH; 1750, ester; 1715, amide.
3c	90	-6.3,s	-0.17	3400,br,NH; 1750, ester; 1715, amide.
4a	98	-3.7,s	-0.10	3300,br,NH; 1715, amide, acid.
4b	98	-6.7,s	-0.21	3400,br,NH; 1720, amide, acid.
4c	75	-6.6,s	+0.05	3400,br,NH; 1710, amide, acid.

The esters 3a and 3b were converted to the free acids 4a and 4b by catalytic hydrogenolysis of the p-nitrobenzyl group (H_2/Pd, MeOH/CH_3CO_2H). The benzyl derivative 4c was obtained by selective alkaline hydrolysis of the ester 3c (NaOH,1eq, RT 90min) (Table 1). All the reported compounds gave ^{13}C, ^{1}H and ^{31}P NMR spectra in accordance with the assigned structures. The protected phosphoamino acids[6] are stable at room temperature, and have shown no signs of decomposition after 12 months.

The O-phosphotyrosine derivative 4a was used[7] in the solid phase synthesis of heptapeptide (5) (scheme (II)).

SCHEME 2.



(i) HBr / CF_3COOH / anisole, 90 min RT
(ii) H_2 / $Pd(OAc)_2$, 60psi, 24h
(iii) 45% HBr / CH_3COOH, 15h

The peptide sequence corresponds to the cAMP-dependent protein kinase substrate with tyrosine substituted for the serine[8]. 1.3g of t-Boc-Gly-Resin (0.9mmol/g) yielded 2.3g of peptide-resin after coupling the six residues in the sequence[9]. Cleavage from the resin (1g) and amino-terminal deprotection was achieved with HBr/TFA[10] and this followed by hydrogenation (H_2/$Pd(OAc)_2$, 90% CH_3COOH) to reduce the nitroarginine residues (Scheme II). A final treatment of crude peptide (100mg) with 45% HBr/CH_3CO_2H (2ml) removed the methyl protecting groups. Chromatography on SP-sephadex[11] yielded the free heptapeptide (5) as a white powder after lyophilization (40mg). The homogeneity, composition and phosphorous content of the product have been established[12].

Thus we have demonstrated the synthesis of protected O-phosphotyrosine derivatives, and their incorporation into a peptide sequence. Investigation of the properties of O-phosphotyrosine peptides is presently being undertaken.

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6. Attempts to prepare crystalline DCHA salts of 4(a-c) were unsuccessful.

7. Compound 4a had the following spectral data:-
^{1}HNMR $\delta(CDCl_3)$: 1.41 (s,9H,CH_3), 3.71 (m,2H,$\underline{CH_2}$-Ar), 3.85 (d,6H,PO$\underline{CH_3}$, J_{PH}=11.5Hz), 4.54 (m,1H,CH), 5.17 (br,1H,NH), 7.15 (s,4H,Ar), 9.37 (s,1H,$CO_2\underline{H}$).
^{13}CNMR $\delta(CDCl_3)$: 174.3 (s), 155.8 (s), 149.8 (d,J_{PC}=7.33Hz), 134.1 (s), 131.3 (s), 120.2 (s), 80.5 (s), 55.6 (d,J_{PC}=5.85Hz), 54.6 (s), 28.7 (s).

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11. The heptapeptide was eluted using a pyridinium acetate gradient (0.05M, pH2.5 to 2M, pH5.0).

12. Peptide 5 had the following properties:
TLC (butanol/acetic acid/water/pyridine; 5:1:4:3), Rf = 0.25, single spot.
It moved as a single spot towards the positive electrode (pH2.8) in electrophoresis (relative mobility to lysine 0.9).
Phosphate analysis gave 1.08 mole of inorganic phosphate per mole of peptide after treatment with alkaline phosphatase.
Amino acid analysis (5.7M HCl, 110°, 24h): Leu 1.97 (2); Arg 1.98 (2); Ala 1.03 (1); Tyr 0.94 (1); Gly 1.02 (1).
^{31}PNMR $\delta(H_2O$, pH 7.0): -0.5.

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