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SYNTHESIS OF 4-PHOSPHONO- AND OF 4-(PHOSPHONOMETHYL)-DL-PHENYLALANINE, TWO ANALOGUES OF O-PHOSPHOTYROSINE

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Abstract : 4-Phosphono-DL-phenylalanine **1** was synthetized from 4-bromo-DL-phenylalanine or from 4-(bromomethyl)-bromobenzene ; 4-(phosphonomethyl)-DL-phenylalanine **14** was prepared from methyl *p*-toluate. The interest of these phosphonic analogues arises from their possible interference in the metabolism of O-phosphotyrosine.

INTRODUCTION

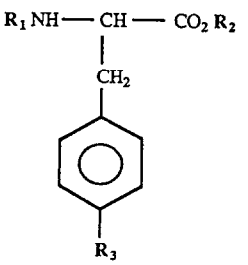
Phosphorylation of a serine or of a threonine residue is a classical post-translational modification of a protein ; the formation of phosphoserine is associated with the hormonal regulation of several enzymic activities by either protein kinase A, activated by the cyclic AMP cascade[1] or by protein kinase C activated by the phosphoinositide chain[2]. The discovery, in 1979, of O-phosphotyrosine in the peptide chains of immunoprecipitates[3,4] and in the proteins of animal cells[5] as the result of a stable yet reversible modification implies the existence of tyrosine protein kinases[6,7] (TPK) and of specific protein phosphatases[8,9] (TPP) capable of hydrolyzing the phosphoric phenol ester. The widespread occurence of O-phosphotyrosine has opened an extensive field of research both in normal and tumor cells[10-12].

The synthesis and degradation of O-phosphotyrosine may be affected by specific inhibitors of TPK or of TPP. These lines have been only scantily investigated : some analogues of ATP[13], a few halomethylketones[14], quercitine[15] and amiloride[16] show weak inhibitory properties versus TPK.

In this paper, we describe two molecules possessing a structural relationship with O-phosphotyrosine : i) 4-phosphono-DL-phenylalanine, recently mentioned among numerous new products as potential competitive N-methyl-D-aspartic acid (NMDA) antagonists[17], was synthetized by two different methods, ii) a new synthesis of 4-(phosphonomethyl)-DL-phenylalanine[18].

RESULTS AND DISCUSSION

The first phosphonic analogue of O-phosphotyrosine, 4-phosphono-DL-phenylalanine **1** was obtained from p-bromo-DL-phenylalanine **1a** which was first converted to its N-(*tert*-butyloxycarbonyl)-derivative **1b**, following the classical method of Moroder *et al.* ; the N-protected bromo amino acid was treated by methyl iodide to give the fully protected compound **1c**.



1 : $R_1 = R_2 = H$; $R_3 = PO_3H_2$

1a : $R_1 = R_2 = H$; $R_3 = Br$

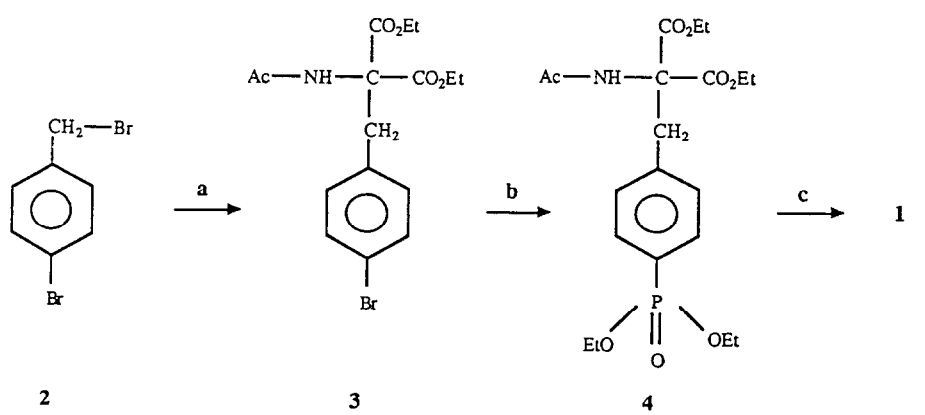
1b : $R_1 = BOC$- ; $R_2 = H$; $R_3 = Br$

1c : $R_1 = BOC$- ; $R_2 = Me$; $R_3 = Br$

1d : $R_1 = BOC$- ; $R_2 = Me$; $R_3 = PO_3Et_2$

The subsequent phosphonylation of bromide **1c** was performed in toluene with diethylphosphite, in the presence of a catalytic amount of *tetrakis*-triphenylphosphine-palladium[19], in accordance with Hirao's process. The purified phosphonate **1d** was obtained with 78 % yield. Attempts to perform this phosphonylation through a $S_{RN}1$ photostimulated substitution, in acetonitrile at room temperature[19], was unsuccessful. The simultaneous release of the three protective groups in **1d** was carried out by acidic hydrolysis (boiling 6 N HCl) ; 4-phosphono-DL-phenylalanine **1** was finally liberated from the hydrochloride by treatment with 1,2-epoxypropane (56 % yield).

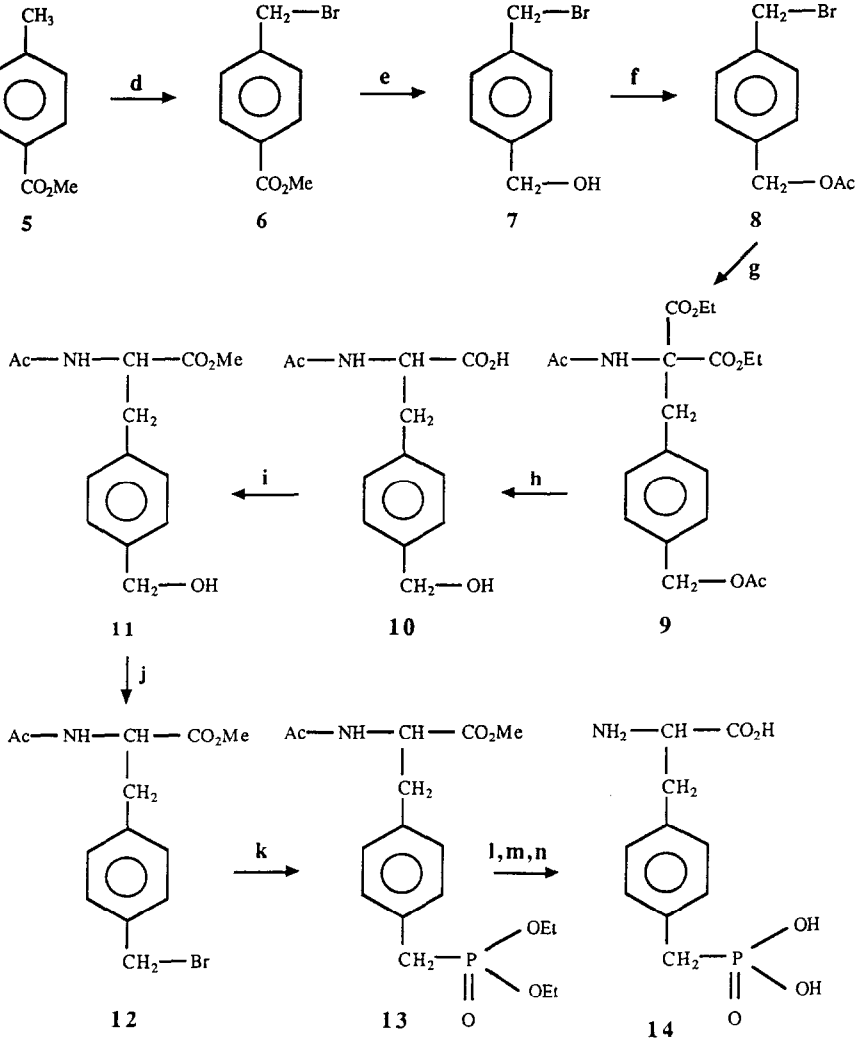
Another expeditious way to **1**, from the readily available 4-(bromomethyl)-bromobenzene **2** is outlined in Scheme 1.



Scheme 1. Second way to 4-phosphono-DL-phenylalanine **1**.

The nucleophilic substitution of the benzylic bromide **2**, by the carbanion of diethyl acetamidomalonate (step *a*) was accomplished in the conditions of liquid-solid phase transfer catalysis[20] (79 % yield). Phosphonylation of **3** (step *b*, 79 % yield) and hydrolysis of **4** (step *c*, 64 % yield) were performed as above. The low cost of **2**, compared to **1a**, renders this second way very attractive. All the reported yields concern chromatography purified products.

The methods for preparing 4-(phosphonomethyl)-DL-phenylalanine are outlined in Scheme 2.



Scheme 2. Synthesis of 4-(phosphonomethyl)-DL-phenylalanine **14**.

Starting from methyl *p*-toluate **5**, bromination with N-bromosuccinimide (step *d*) gave the methyl ester of 4-(bromomethyl)- benzoic acid **6** which is converted to 4-(bromomethyl)benzyl alcohol **7** (step *e*). As lithium aluminium hydride led to a mixture of approximatively equal parts of **7** and of 4-methylbenzyl alcohol, we used aluminium hydride prepared in the reaction medium as a reducing agent for step *e* ; 4-(bromomethyl)benzyl alcohol, the major reduction product thus obtained, was acetylated (step *f*). The classical acetylation using acetyl chloride in pyridine was avoided, as the organic base reacted with the bromomethyl group ; 4-(bromomethyl)benzyl acetate **8** was obtained with 65 % yield, using acetic anhydride and gaseous HCl as catalyst[21].

Alkylation of the bromomethyl side chain, via the stable Schiff base derived from glycine ethyl ester and benzophenone, under the phase-transfer conditions of O'Donnell *et al.*[22], was unsuccessfull. Treatment of **8** with diethyl acetamidomalonate (step *g*) gave 9 with a good yield. The triester, treated with sodium carbonate, underwent both saponification and a partial decarboxylation in step *h* ; the resulting sodium salt of N-acetyl-4(hydroxymethyl)-DL-phenylalanine was acidified and purified by ion exchange chromatography to give **10**, from which **11** was prepared. The reagents necessary for esterifying the carboxylic group (step *i*) must not react with the hydroxylic side chain, thus eliminating the use of methyl iodide or of dimethyl sulphate. Treatment of **10** with methanol in the presence of thionyl chloride, giving directly the chloro analogue of **11**, could be an interesting shunt : unfortunately, the yield of this reaction was low ; a quantitative esterification of **10** was achieved with gazeous diazomethane. Bromination of the benzyl alcohol derivative **11** by classical methods was ineffective ; step *j* was performed according to the boron trifluoride etherate/halide ion method of Mandal and Mahajan[23] : an excellent yield was obtained with tetraethylammonium bromide, whereas sodium bromide proved to be inefficient.

Introduction of the phosphonic group was made according to Arbusov[24] (step *k*) ; the protected compound **13** was finally hydrolyzed to give the hydrobromide of **14**, through three successive cleavages : saponification of the carboxylic ester by sodium hydroxyde (step *l*) ; attack of the phosphonic ester by HBr (step *m*) ; deamidification with 6 N HCl (step *n*). Free 4-(phosphonomethyl)-DL-phenylalanine **14** was finally obtained after a treatment with 1,2-epoxybutane.

Our first biological assays were limited to studying the cytostatic activity of compounds **1**, **14** and **13**. Human leukemia K562 cells were selected for studying an eventual cytostatic activity. These cells have a 9:22 chromosomal translocation involving the *c-abl* gene[25], a common characteristic of chronic myeloid leukemia patients[26,27] ; they contain an altered *c-abl* protein with associated tyrosine kinase activity[28].

No cytostatic activity could be observed, either with 4-phosphono-DL-phenylalanine or with 4-(phosphonomethyl)-DL-phenylalanine, at a concentration of 250 $\mu g\ ml^{-1}$. On the contrary, compound **13**, the protected form of the inactive aminoacid **14**, inhibited cell growth in the range 250 μg-1 mg ml^{-1}, with a ID_{50} 750 $\mu g.ml^{-1}$. The difference between compounds **13** and **14** may be linked to the difficulties encountered by a highly polar molecule to enter a cell[29] ; incorporation of **14** in an oligopeptide thus appears to be a promising development of our work.

EXPERIMENTAL

General procedures

Two kinds of precoated TLC plates from Merck (Darmstadt, FRG) were used : Kieselgel 60 F_{254}, 0,2 mn x 20 cm x 20 cm and cellulose DC-Alufolien n° 5552. Solvent systems were diethyl ether/*n*-hexane (50:50, v/v) for silica gel and *n*-butanol/isopropanol/formic acid/water (3:1:1:1, v/v) for cellulose TLC sheets. The spots were visualized with UV, ninhydrin or phosphomolybdic reagents. Ion-exchange chromatography was performed on a 3 cm x 60 cm column containing Dowex-50 WX 8 (20-50 mesh) resin in H^+ form, eluted with water. A Waters apparatus was used for HPLC.

UV and IR spectra were determined respectively on Varian DMS 90 and on Beckman Acculat 1 spectrophotometers. Structures were checked by NMR spectroscopy ('H NMR : Hitachi Perkin-Elmer R-24B, Varian T-60, 60 MHz ; Bruker AC 200, 200 MHz and Bruker AC 250, 250 MHz ; ^{13}C NMR : Bruker AC 200, 50.32 MHz and Bruker AC 250, 62.95 MHz ; ^{31}P NMR : Bruker WP 90, 36.44 MHz) using TMS, TSP or H_3PO_4 as references. Mass spectra were determined with a VG Micromass 16 F. Microanalyses were performed on Carlo Erba 1106 analyser and analyses for C, H, N and P by the Microanalytical Department of CNRS Vernaison, France.

All solvents were distilled before use according to the classically admitted procedures.

Synthesis of 4-phosphono-DL-phenylalanine **1**

Method A

Compound **1b** : N-(*tert*-butyloxycarbonyl)-4-bromo-DL-phenylalanine

4-Bromo-DL-phenylalanine **1a** (2.44 g, 10 mmol), purchased from Bachem (Bubendorf, Switzerland), was suspended at room temperature under magnetic stirring in a mixture of dimethylformamide (25 ml) and triethylamine (7.5 ml). Melted di-*tert*-butyl-dicarbonate (2.4 g, 11 mmol) was added dropwise ; the suspension was clarified by adding water (ca. 10 ml). After 10 min, evaporation under reduced pressure at 55°C left an aqueous phase, which was extracted at 0°C by AcOEt after acidification to pH 2-3 with a saturated aqueous solution of $KHSO_4$. The organic layer was separated and the aqueous layer re-extrated with AcOEt. The combined organic layers, after beeing washed with water, were concentrated under reduced pressure at 55°C ; the oily residue was moistened with water and lyophilized to give a white powder (3.5 g ; almost quantitative yield). The complete protection of the amino group was confirmed by a negative ninhydrin test.

Compound **1c** : N-(*tert*-butyloxycarbonyl)-4-bromo-DL-phenylalanine methylester

Compound **1b** (3.45 g ; 10 mmol) was suspended at room temperature in hexamethylphosphoramide (50 ml) and stirred until complete dissolution. An aqueous 25 % NaOH solution (1.76 ml, 11 mmol) was added and the mixture left 1 h under stirring. An excess of methyl iodide (5.68 g, 40 mmol) was delivered dropwise in the mixture by means of a syringe and stirring was maintained for 3 h. The reaction medium, mixed with an ice-cold aqueous solution satured with NaCl and containing 5 % HCl (75 ml), was extracted with Et_2O (3 x 25 ml) ; the organic layer, washed successively with brine and with aqueous sodium thiosulphate, was dried (Na_2SO_4) and a white-yellowish solid (2.65 g ; 73 % yield) was obtained after removing the solvent under reduced pressure. Crystalline **1c** (2.27 g, 63 % yield) was obtained by dissolving the crude product in hot methanol and precipitation with water, followed by a final recrystallisation in methanol. The purity of **1c** was checked by HPLC (C_{18} μ Bondapak ; methanol/water, 70:30 v/v ; detection at 220 nm).

Compound **1d** : N-(*tert*-butyloxycarbonyl)-4-(diethoxyphosphono)-DL-phenylalanine methylester (**1d**)

A mixture of **1c** (2 g ; 5.6 mmol), diethylphosphite (0.85 g ; 6.1 mmol), triethylamine (0.62 g ; 6.1 mmol) and fresly prepared *tetrakis*-triphenylphosphine-palladium catalyst (0.35 g ; 0.3 mmol) was stirred at 90°C, in toluene (5 ml), under a slight nitrogen stream, over a period of 12 h. The mixture was cooled and

filtered. The precipitate was washed with diethylether (10 ml). The filtrate was evaporated to give an oily residue, which was dissolved in diethylether (20 ml) and allowed to stand overnight in refrigerator. The filtered mixture after an additionnal evaporation step, furnished a crude oil (2.3 g), which was chromatographed over silica gel with ethylacetate as eluent to give pure **1d** (1.81 g ; yield 78 %). Relevant 'H NMR data (60 MHz, CCl_4) : δ 1.35 (d, J = 7 Hz, 6H, $2CH_3CH_2O$), 1.4 (s, 9H, ter-C_4H_9), 3.1 (d, J = 5 Hz, 2H, CH_2CH), 3.7 (s, 3H, CH_3O), 4.05 (q, J = 7 Hz, 4H, $2CH_3CH_2O$), 4.2 to 5.0 (br s, 2H, $CHCH_2$ and NH), 7.0-7.9 (m, 4H, aromatic protons).

Compound **1** : 4-Phosphono-DL-phenylalanine

Compound **1d** (1.7 g ; 4.1 mmol) was dissolved in aqueous 6 M HCl (20 ml). The solution was refluxed for 10 h. The cooled mixture was evaporated ; the residue was dissolved in water (15 ml) and the solution evaporated again ; this operation was repeated three times. The last residue was dissolved in ethanol (10 ml). 1,2-Epoxypropane was progressively added to this solution until complete precipitation. The precipitate was filtered, washed with acetone, then dried to give **1** as a white solid (0.563 g ; yield 56 % ; m. p. > 260°C). Relevant 'H NMR data (60 MHz, D_2O) : δ 3.2-3.6 (m, 2H, CH_2CH), 4.4 (t, J = 5 Hz, 1H, CH_2CH), 7.3-8.1 (m, 4H, aromatic protons). ^{31}P NMR (36.44 MHz, D_2O) : δ 19.3 ppm. A sample was recrystallized from water/ethanol (80/20, v/v) and subjected to microanalysis. Anal. Calcd. for $C_9H_{12}NO_5P$: C, 44.08 ; H, 5.71 ; N, 4.90. Found : C, 43.9 H, 5.5 ; N, 4.9.

Method B

Compound **3** : Diethylacetamido *p*-bromobenzyl malonate

A mixture of *p*-bromobenzyl bromide **2** (4 g ; 16 mmol), diethyl acetamidomalonate (3.8 g ; 17.5 mmol), dry K_2CO_3 (4.4 g ; 32 mmol), and benzyl-triethylammonium chloride (TEBAC, 0.1 g : 0.4 mmol), in dry acetonitrile (40 ml), was refluxed for 2 h. The cooled reaction medium was filtered and the filtrate evaporated. The residue dissolved in CH_2Cl_2 (20 ml) was washed twice with water (2 x 5 ml). The organic layer, dried over $MgSO_4$, was evaporated to give a crude yellow solid (6.1 g), which was chromatographed over silica gel (eluent : CH_2Cl_2) to give **3** as a white solid (4.9 g ; yield 79 %). Relevant 'H NMR data (60 MHz, CCl_4) : δ 1.3 (t, J = 7 Hz, 6H, $2CH_3CH_2O$), 2.0 (s, 3H, CH_3CO), 3.55 (s, 2H, CH_2Ar), 4.25 (q, J = 7 Hz, 4H, $2CH_3CH_2O$), 6.45 (s, 1H, NH), 6.7-7.5 (m : AA'BB', 4H, aromatic protons). Anal. Calcd. for $C_{16}H_{20}BrNO_5$: C, 49.72 ; H, 5.18 ; N, 3.63. Found : C, 50.0 ; H, 5.4 ; N, 3.7.

Compound **4** : Diethyl acetamido (4-diethoxyphosphono)-benzyl malonate

Compound **3** (4 g ; 10.3 mmol) was phosphonylated as described above to furnish a crude oil which was purified by flash chromatography (silica gel ; eluent : ethylacetate) to give **4** (3.6 g ; yield 79 %). Relevant 'H NMR data (60 MHz, CCl_4) : δ 1.3 (t, J = 7 Hz, 12H, $4CH_3CH_2O$), 2.0 (s; 3H, CH_3CO), 3.6 (s, 2H, CH_2Ar), 3.7-4.45 (m, 8H, $4CH_3CH_2O$), 6.95 (s, 1H, NH), 6.95-7.8 (m, 4H, aromatic protons). ^{31}P NMR (36.44 MHz, $CDCl_3$) : δ 18.16 ppm.

Compound **1** : 4-Phosphono-DL-phenylalanine (from 4, scheme 1)

Phosphonate **4** (3 g ; 6.8 mmol) was hydrolyzed in the same operating conditions as for hydrolysis of **1d**. This treatment led to a white solid (1.06 g ; yield 64 %), showing spectroscopic and analytical data in accordance with structure **1**.

Synthesis of 4-(phosphonomethyl)-DL-phenylalanine **14**

Compound **6** : 4-(Bromomethyl)benzoic acid methyl ester

This compound was prepared as described earlier, starting from methyl *p*-toluate (30 g, 0.2 mol) **5** dissolved in tetrachloromethane (400 ml) ; N-bromosuccinimide (NBS, 0.23 mol) and azobis(isobutyro)-nitrile (125 mg) were added and the mixture stirred under illumination by a 200 w bulb ; the illumination was stopped when the reflux began ; the reaction then proceeded to completion as NBS was converted to succinimide (3 h), to yield a white solid (33.2 g, 72,5 %). Relevant 'H NMR data (60 Mhz, CCl_4) : δ 3.8 (s, 3H, CO_2CH_3), 4,4 (s, 2H, CH_2Br), 7.25-7.95 (m, 4H, aromatic protons).

Compound 7 : 4-(Bromomethyl)benzyl alcohol
This product was obtained from **6** (11.45 g, 0.05 mol) in anhydrous cold (between -10°C and -15°C) diethyl ether (300 ml) by reduction with aluminium hydride (0.1 mol), respecting an exact ratio of 1:2 between the substrate and the reducing agent. AlH_3 had to be prepared in the reaction flask from a 3:1 molar mixture of lithium aluminium hydride (0,075 mol) and of aluminium chloride (0.025 mol). After two hours at room temperature, the reaction mixture was cooled (-10°C) and hydrolysed using an aqueous solution (25 ml) saturated with Na_2SO_4 ; the alcohol **7** was liberated. The inorganic salts were filtered off, the organic layer dried over anhydrous sodium sulphate and evaporated under reduced pressure without any heating. The white solid **7** (7 g ; yield : 70 %) was unstable and had to be rapidly used for the following step. Relevant ^1H NMR data (60 MHz, $CDCl_3$) : δ 2.0 (s,1H, OH) 4.4(s, 2H, CH_2Br), 4.6 (s, 2H, CH_2OH), 7.3 (s, 4H, aromatic protons).

Compound 8 : 4-(Bromomethyl)benzyl acetate
Following the work of Smith and Sloane[21], a solution of compound **7** (3.65 g, 0.018 mol) in acetic anhydride (20 ml) was cooled (-10°C), saturated with dry gaseous HCl and stirred overnight at room temperature. Distillation under reduced pressure left **8** as an oily residue, with bp = 110°C/0.5 mm (2.8 g ; yield : 65 %). Relevant ^1H NMR data (60 MHz, CCl_4) : δ 2.0 (s, 3H, CH_3CO_2), 4.4 (s, 2H, $PhCH_2Br$), 5.0 (s, 2H, $PhCH_2OAc$), 7.2 (s, 4H, aromatic protons).

Compound 9 : Diethyl[4-(acetoxymethyl)benzyl]acetamidomalonate
Diethylacetamidomalonate (8.68 g, 0.04 mol) dissolved in anhydrous benzene (50 ml) was added to a suspension of sodium hydride (0.04 mol, 80 % dispersion in mineral oil) in freshly distilled DMF (70 ml) ; the mixture was heated to 50°C and maintained at this temperature to complete the formation of the sodio derivative (cessation of hydrogen evolution). The solution, freed from the unreacted sodium hydride by filtration, is added under argon to **8** (0.0396 mol), dissolved in anhydrous benzene (50 ml). After 4 hours at 50°C ,the mixture is left two days at room temperature to allow the precipitation of sodium bromide, which was eliminated by adding a small quantity of water. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to obtain a white solid (14.2 g) which was crystallized from hexane/diethyl ether mixture (50:50, v/v), to give finally the pure triester **9** with a 54 % yield (8.1 g). Relevant ^1H NMR data (60 MHz ,$CDCl_3$) : δ, 1.15 (t , 6H, $2CH_3CH_2O$), 1.9 (s, 3H, CH_3CONH), 2.0 (s, 3H,CH_3CO_2), 3.5 (s, 2H, $PhCH_2C$), 4.1 (q, 4H, $2CH_3CH_2O$), 4.9 (s, 2H, $PhCH_2OAc$), 6.45 (s, 1H, CONH), 6.75-7.15 (m, 4H, aromatic protons).

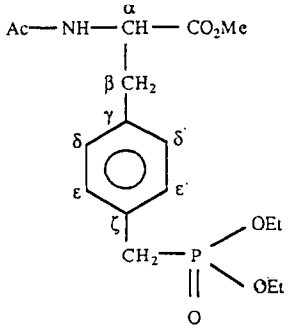
Compound 10 : N-Acetyl(4-hydroxymethyl)-DL-phenylalanine
A mixture of **9** (5.68 g, 0.015 mol), Na_2CO_3 (0.045 mol) and water (100 ml) was refluxed during 8 h and left at room temperature. The solution was chromatographed on Dowex 50 (H^+ form) ; the resin was eluted with water (500 ml) and the acidic eluates collected until a neutral pH was reached. A hydroscopic white solid (2.52 g, 71 % yield) was obtained by freeze drying. Relevant ^1H NMR data (250 MHz, CD_3COCD_3) : δ 1.93 (s, 3H, CH_3CONH), 3.08 (2H, $PhCH_2CH$, AB part of ABX system, J_{AB} = 13.90 Hz), 4.58 (s, 2H, CH_2OH), 4.73 (m, 1H, $PhCH_2CH$, X part of ABX system), 7.20-7.29 (m, 4H, aromatic protons). Relevant ^{13}C NMR data (62.95 MHz, CD_3COCD_3) : δ 173.06 (COOH), 171.60 (NHCO), 141.26 and 136.58 (C_Q aromatic), 127.54 and 129.87 (CH aromatic), 64.24 (CH_2OH), 54.51 (CH), 37.61 (CH_2CH), 22.36 (CH_3).

Compound 11 : N-Acetyl(4-hydroxymethyl)-DL-phenylalanine methyl ester
An instant esterification was achieved by treating the N-acetylated aminoacid **10** (0.545 g, 2.3 mmol) in anhydrous THF (15 ml) by gaseous diazomethane, prepared from N-methyl-N-nitroso-p-toluenesulfonamide (Diazald®). The latter compound (1g, 4.6 mmol) was added to a solution of 2-(2-ethoxyethoxy)ethanol (Carbitol®, 3 ml) in anhydrous diethylether (3 ml) containing KOH (60 %, 4 ml). An apparatus consisting of four test tubes connected by their side arms was used. A stream of N_2, saturated with ether in the first tube, carries the diazomethane generated in the second tube into the third tube where the methylation occurs. The reaction was stopped when the nitrogen flow coloured in yellow the ether contained in the fourth tube. After evaporating the content of the third tube, 0.55 g of the ester **11** was

obtained (yield : 99 %). Relevant 'H NMR data (250 MHz, DMSO-d_6) : δ 1.79 (s, 3H, NHCOCH_3), 2.90 (2H, Ph CH_2CH, AB part of ABX system, J_{AB} = 13.57 Hz), 3.61 (s, 3H, COOCH_3), 4.42 (1H, PhCH$_2$C*H*, X part of ABX system), 4.46 (s, 2H, CH_2OH), 5.15 (brt, 1H, CH$_2$O*H*), 7.14-7.24 (m, aromatic protons), 8.34 (d, 1H, N*H*COCH$_3$, J = 7.73 Hz).

Compound **12** : N-Acetyl(4-bromomethyl)-DL-phenylalanine methyl ester
The benzylic alcohol **11** (1.004 g, 4 mmol), tetraethylammonium bromide (1.26 g, 6 mmol) and freshly distilled boron trifluoride etherate (0.8 ml, 6 mmol) were refluxed in dry $CHCl_3$ (80 ml) for 10 h. A saturated $NaHCO_3$ solution (20 ml) was added to the cooled reaction mixture. The organic layer was separated and the aqueous layer extracted with $CHCl_3$ (20 ml). The combined organic layers, washed successively with 10 % aqueous sodium thiosulphate and with a saturated brine solution, was dried over anhydrous sodium sulphate. The protected (4-bromomethyl)phenylalanine **12** (1.05 g, 83.5 % yield) was obtained by evaporating the solvent. Relevant 'H NMR data (250 MHz, $CDCl_3$) : δ 1.9 (s, 3H, NHCOCH_3), 3.05 (brd, 2H, PhCH_2CH), 3.65 (s, 3H, CO_2CH_3), 4.4 (s, 2H; PhCH_2Br), 4.8 (q, 1H, PhCH$_2$C*H*), 6.0 (brd, 1H, N*H*COCH$_3$), 6.9-7.3 (m, 4H, aromatic protons).

Compound **13** : N-Acetyl(4-diethoxyphosphonomethyl)-DL-phenylalanine
The bromo ester **12** (628 mg, 2 mmol) was refluxed in freshly distilled triethylphosphite (6 ml) for 6 h. Compound **13**, precipitated from the cooled reaction medium, was collected by filtration as a white solid ; remaining traces of triethylphosphite were removed under vacuum. The yield for step *h* was 67 % (500 mg). Mass spectrum, chief peaks, $^m/_z$ (%) : M^+ 312 (100), 242 (69), 241 (42), 175 (33), 105 (36), 104 (78), 81 (29), 43 (89), 29 (36). Relevant 'H NMR data (200 MHz, $CDCl_3$) : δ 1.2 (t, J = 7.05 Hz, 6H, 2CH_3CH$_2$O), 1.9 (s, 3H, NHCOCH_3), 3.0 (AB part of ABX system, J_{AB} = 14.3 Hz, 2H, PhCH_2CH), 3.1 (d, $^2J_{PH}$ = 21.35 Hz, 2H, CH_2P), 3.7 (s, 3H, CO_2CH_3), 4.0 [(m, $^3J_{HH}$ = 7.05 Hz, $^3J_{HP}$ = 5.4 Hz, 4H, P(O)(OCH_2CH$_3$)$_2$] 4.8 (X part of ABX system, 1H, PhCH$_2$C*H*), 6.45 (br s, 1H, N*H*COCH$_3$), 7.07-7.23 (m, 4H, aromatic protons). Relevant ^{13}C NMR data (50.32 MHz, $CDCl_3$) : 172.13 (*C* OOCH$_3$), 169.82 (*C* O-NH), 134.74 (d, $^5J_{CP}$ = 3.9 Hz, Cγ), 130.30 (d, $^2J_{CP}$ = 9.2 Hz, Cζ), 130.00 (d, $^3J_{CP}$ = 6.6 Hz, Cε, Cε'), 129.35 (d, $^4J_{CP}$ = 2.9 Hz, Cδ, Cδ'), 62.15 and 62.13 (2d, $^2J_{CP}$ = 6.5 Hz, O*C* H$_2$CH$_3$), 53.25 (Cα), 52.23 (CO-O*C* H$_3$), 37.45 (Cβ), 33.30 (d, $^1J_{CP}$ = 138.2 Hz, *C* H$_2$P), 22.90 (*C* H$_3$CONH), 16.37 (d, $^3J_{CP}$ = 5.9 Hz, OCH$_2$-*C* H$_3$).

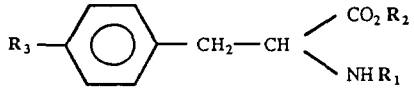


Anal. Calcd. for $C_{17}H_{26}NO_6P$: C, 54.97 ; H, 7.05 ; N, 3.77 ; P, 8.33. Found : C, 54.95 ; H, 6.85 ; N, 3.65 ; P, 8.12.

Compound **14** : 4-(Phosphonomethyl)-DL-phenylalanine
The fully protected amino acid **13** (185.5 mg, 0.5 mmol), sodium hydroxyde (1 N NaOH, 1.5 mEq) and a H_2O/EtOH mixture (12 ml, 50/50, v/v) were refluxed to obtain a homogeneous solution (3 h). Ethanol was then distilled off and the remaining solution lyophilized. The solid sodium carboxylate was treated under a nitrogen flow by a 33 % solution (5 ml) of hydrogen bromide in anhydrous acetic acid. After standing overnight at room temperature, the reagents were removed under vacuum and the solid N-acetylaminoacid

washed twice with dry diethyl ether. Step *n* was achieved by adding 6 N HCl (5 ml) to N-acetyl-4-(phosphonomethyl)phenylalanine in a sealed tube, which was heated (110°C, 20 h). After cooling, HCl was removed under reduced pressure and the salt of **14** dissolved in water and the solution lyophilized. The free aminoacid **14** was finally obtained from a solution of its salt in EtOH (5 ml) by precipitation with 1,2-epoxybutane (2 ml). After standing overnight at 4°C, the precipitate was collected and washed with diethyl ether. The total yield for the successive deprotection steps amounted to 60 %, giving 78 mg of a highly hygroscopic white solid. Steps *l, m,n* and the final precipitation were checked by TLC. Compound **14** contained traces of protected products, consisting mainly of the diethylphosphonic ester. Relevant ^{1}H NMR data (250 MHz, 20 % DCl in D_2O, TSP) : δ 3.25-3.40 (m, 2H, PhC*H*$_2$CH), 3.29 (d, $^2J_{PH}$ = 21.74 Hz, 2H, C*H*$_2$P) 4.42 (m, 1H, PhC*H*$_2$CH), 7.33 (s, 4H, aromatic protons) ; IR (KBr) υ 3600-2800 (OH), (NH), (CH), 1630 (CO), 1500 (NH), 1050 (PO_3^{2-}). UV spectra did not allow any discrimination between **14**, **1** and O-phospho-L-tyrosine, as the three products showed very similar peaks at respectively 173, 172 and 171 nm and shoulders at 202, 200 and 197 nm. The chromatographic characteristics of **14** are compared with four related aminoacids in Table I.

Table 1. Chromatographic data



N°	R_1	R_2	R_3	Rf *
1	H	H	PO_3H_2	0.34
14	H	H	$CH_2PO_3H_2$	0.44
13	CH_3CO	CH_3	$CH_2PO_3(C_2H_5)_2$	0.63
Tyr	H	H	OH	0.60
PhosphoTyr	H	H	OPO_3H_2	0.34

* On cellulose TLC sheets. Solvent system : n-butanol/isopropanol/formic acid/water (3:1:1, v/v).

Anal. Calcd for $C_{10}H_{14}O_5NP$, 3 H_2O : C, 38.34 ; H, 6.43 ; N, 4.47 ; P, 8.88. Found : C, 37.76 ; H : 5.42 ; N : 4.32 ; P, 8.86.

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