PREPARATION OF PHOSPHONOMETHYL ETHERS DERIVED FROM 2-PHENYLETHANOL AND ITS AMINO DERIVATIVES

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Received January 4, 1995
Accepted February 14, 1995

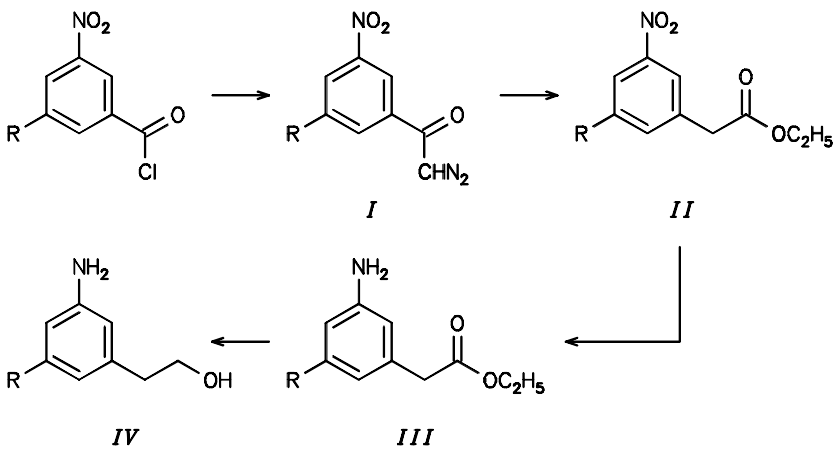
A series of compounds derived from the acyclic nucleoside antiviral 9-(2-phosphonomethoxyethyl)adenine (PMEA), in which the adenine ring is replaced by phenyl, 4-aminophenyl, 3-aminophenyl or 3,5-diaminophenyl group, has been prepared starting from the corresponding phenethyl alcohols. 2-(3-Aminophenyl)ethanol was prepared from 3-nitrobenzoyl chloride using the Arndt–Eistert reaction. The primarily formed diazoketone *Ia* was converted into ethyl 3-nitrophenylacetate (*IIa*) which on catalytic hydrogenation afforded ethyl 3-aminophenylacetate (*IIIa*). Compound *IIIa* was reduced with lithium aluminium hydride to give 2-(3-aminophenyl)ethanol (*IVa*). 2-(3,5-Diaminophenyl)ethanol (*IVb*) was prepared analogously from 3,5-dinitrobenzoyl chloride. After protection of the amino group with dimethylaminomethylene group, the alcohol *IVa* was converted to diisopropyl 2-(3-aminophenyl)ethoxymethylphosphonate (*XII*) by reaction with sodium hydride and diisopropyl *p*-toluenesulfonyloxymethanephosphonate, followed by deprotection of the amino group by treatment with ammonia. Reaction of diisopropyl ester *XII* with bromotrimethylsilane gave free 2-(3-aminophenyl)-ethoxymethylphosphonic acid (*XVII*). The same procedure, applied to the corresponding aminophenethyl alcohols, afforded: 2-(4-aminophenyl)ethoxymethylphosphonic acid (*XVI*) and 2-(3,5-diaminophenyl)ethoxymethylphosphonic acid (*XVIII*). The synthesized compounds were tested in vitro on cell cultures for the cytostatic and antiviral activity (HSV-1, HSV-2, VSV, VZV, CMV). No antiviral activity has been found for any of the compounds.

For several years, the systematic study of phosphonomethyl ethers of acyclic nucleoside analogs has belonged to the main research interests of our group. From the very beginning, this interest has been stimulated first of all by the discovery of high in vivo antiviral activity of two adenine nucleotide analogs: 9-(2-phosphonomethoxyethyl)adenine (PMEA) and (S)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine (HPMPA) (refs[1,2]). Both the compounds are active against a whole series of DNA viruses[1,3,4]; moreover, PMEA is also significantly active against retroviruses[5,6] and recently undergoes clinical investigations in the therapy of AIDS, either free or in the form of its diester[7,8]. In the course of several years, some tens of related phosphonomethoxy compounds, which were variously modified in the aliphatic as well as heterocyclic part of the molecule, were synthesized and tested and their structure–activity relationships were studied[3,9]. Of the large number of such modified derivatives one can

mention e.g. phosphonomethyl ethers derived from *N*-(3-azido-2-hydroxypropyl), *N*-(3-amino-2-hydroxypropyl) and *N*-(3-fluoro-2-hydroxypropyl) derivatives of various heterocyclic bases[10,11], 9-(2-*C*-alkyl-2-phosphonomethoxyethyl)adenines[12] or 2-phosphonomethoxyalkyl derivatives of modified nucleobases (refs[13,14] and references therein).

It was found that in the above-mentioned groups of compounds those derivatives are active in which the phosphonomethoxyalkyl group is bound to a nucleobase containing an amino group. A question arises, however, whether the compound should really contain the complete nucleobase or whether the interaction of the phosphonate with the given viral enzymes requires in general only the presence of an amino group bound to an aromatic heterocycle or even to a single aromatic nucleus. A paper, describing the preparation of such guanine analogs with an open imidazole ring, appeared recently[15].

We have now synthesized a series of compounds analogous to PMEA and its 2,6-diaminopurine analog in which, instead to the purine ring, the phosphonomethoxyethyl moiety is bound to a phenyl, 4-aminophenyl, 3-aminophenyl and 3,5-diaminophenyl group. As starting compounds in the synthesis we used the corresponding phenethyl alcohols of which 2-phenylethanol and 2-(4-aminophenyl)ethanol are commercially available; 2-(3-aminophenyl)ethanol and 2-(3,5-diaminophenyl)ethanol were prepared from the corresponding nitrobenzoyl chlorides using the Arndt–Eistert reaction as the key synthetic step (Scheme 1). Thus, reaction of 3-nitrobenzoyl chloride with diazo-

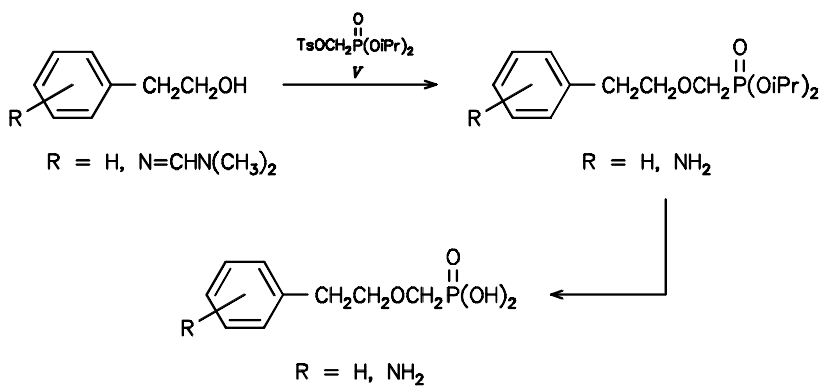


In formulae *I* – *II* : *a*, R = H; *b*, R = NO_2
III – *IV* : *a*, R = H; *b*, R = NH_2

SCHEME 1

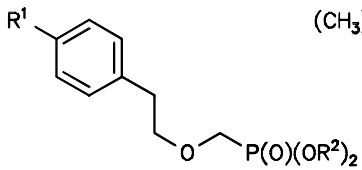
methane afforded diazoketone *Ia* which on boiling with ethanol in the presence of silver oxide afforded ethyl (3-nitrophenyl)acetate (*IIa*) in high yield. Catalytic hydrogenation of the acetate *IIa* gave ethyl (3-aminophenyl)acetate (*IIIa*) which on reduction with lithium aluminium hydride was converted into the desired 2-(3-aminophenyl)ethanol (*IVa*). This preparation of *IVa* appears to be better than the older ways such as catalytic reduction of ethyl 3-nitro-*O*-benzoylmandelate[16] or preparation from 3-aminoacetophenone by Wilgerodt reaction[17]. Analogously to *IVa*, we prepared also 2-(3,5-diaminophenyl)ethanol (*IVb*) from 3,5-dinitrobenzoyl chloride, however, in this case, ethyl (3,5-diaminophenyl)acetate (*IIIb*) was reduced with sodium in boiling ethanol because the reduction with lithium aluminium hydride afforded only very low yields of the product. Even the sodium reduction gave relatively low yields (30 – 40%) due to extreme instability of the product (exposure to air or light resulted in oxidation of the amino groups and its isolation and chromatographic purification was accompanied by severe losses).

Reaction of sodium salts of the corresponding phenethyl alcohols with diisopropyl *p*-toluenesulfonyloxymethanephosphonate (*V*, ref.[18]) afforded the phosphonates as diisopropyl esters (Scheme 2). However, the preparation of sodium salt of 2-phenylethanol was complicated by its strong propensity to elimination under formation of styrene. It was therefore necessary to perform the reaction of 2-phenylethanol with sodium hydride and tosylate *V* at low temperature (–20 °C) and to follow the reaction course. The obtained diisopropyl ester *VI* was isolated and then converted into the free phosphonomethyl derivative *VII* by reaction with bromotrimethylsilane.

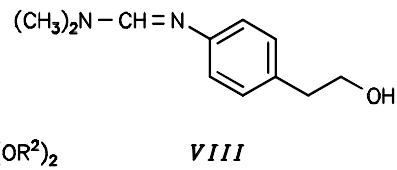


SCHEME 2

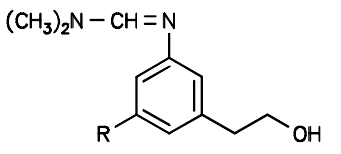
Prior to the condensation with tosylate *V*, the amino group of the aminophenethyl alcohols was protected by reaction with dimethylformamide dimethyl acetal which gave dimethylaminomethylene derivatives *VIII*, *IX* and *X*. The presence of the dimethylaminomethylene-protected amino group suppressed strongly the mentioned elimination of the phenethyl alcohols so that the reaction of compounds *VIII*, *IX* and *X* with sodium hydride, and subsequently with diisopropyl *p*-toluenesulfonyloxymethanephosphonate (*V*) could be performed at room temperature, i.e., under conditions usual for preparing phosphonomethyl derivatives of nucleosides and their acyclic analogs. The originally arising diisopropylphosphonomethyl derivatives with the protected amino group were in situ deprotected by reaction with ammonia in aqueous alcohol to give the amino derivatives *XI*, *XII* and *XIII*.



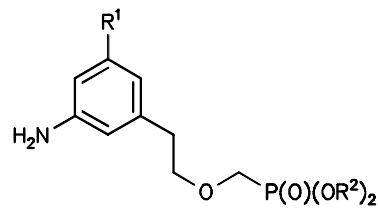
	R^1	R^2
VI	H	$CH(CH_3)_2$
VII	H	H
XI	NH_2	$CH(CH_3)_2$
XVI	NH_2	H



VIII



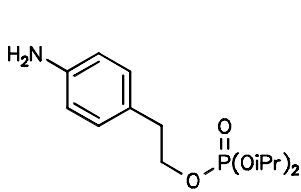
	R
IX	H
X	N = CH – $N(CH_3)_2$



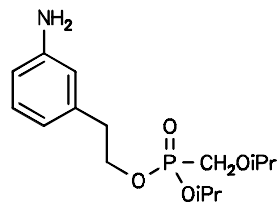
	R^1	R^2
XII	H	$CH(CH_3)_2$
XIII	NH_2	$CH(CH_3)_2$
XVII	H	H
XVIII	NH_2	H

An alternative protection of the amino group in 2-(4-aminophenyl)ethanol by benzoylation did not give satisfactory results because the removal of the benzoyl group required either potassium hydroxide or sodium methoxide. Under such conditions the ester groups underwent partial hydrolysis and the obtained product mixtures were not easily separated. Although the reaction with ammonia removed the dimethylaminomethylene group very slowly, there was no hydrolysis of the ester groups and therefore the condensation product could be easily isolated as the diisopropyl ester.

The preparation of phosphonomethyl derivatives of the mentioned types was accompanied by several side products some of which we isolated in the pure state and identified. Thus, e.g., in the preparation of diisopropyl phosphonomethyl derivative of 4-aminophenethyl alcohol *XI* we also isolated the phosphate *XIV*; in the preparation of diisopropyl phosphonomethyl derivative of 3-aminophenethyl alcohol *XII* the side product was isopropoxymethanephosphonate *XV*.



XIV



XV

Diisopropyl esters of phosphonomethyl derivatives *XI*, *XII* and *XIII* were deblocked by reaction with bromotrimethylsilane to give the free phosphonates *XVI*, *XVII* and *XVIII*.

Since all the prepared compounds with free amino group were very sensitive to oxidation, their isolation and purification on columns of silica gel or ion-exchanger was accompanied with significant losses. Particularly unstable were all the 3,5-diaminophenyl derivatives.

The antiviral activity and cytotoxicity of the free phosphonomethyl derivatives was tested in cell cultures. Compounds *VII*, and *XVI – XVIII* were inactive towards herpes simplex viruses (HSV-1, HSV-2), vesicular stomatitis virus (VSV) and RNA viruses; compounds *XVI* and *XVII* were inactive also towards Varicella zoster virus (VZV) and cytomegalovirus[19]. None of the compounds was cytotoxic. No cytostatic effects have been found on L-1210, L-929 and HeLa S3 cell cultures[20].

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/2 kPa and compounds were dried over phosphorus pentoxide at 13 Pa. Thin-layer chromatography was performed on Silufol UV 254 foils (Kavalier, The Czech Republic). The solvent systems are given in the text. Spots were detected by UV light at 254 nm or by spraying with 0.5% 4-(4-nitrobenzyl)pyridine in ethanol with subsequent heating and exposure to ammonia vapours. Preparative column chromatography was carried out on silica

gel (30 – 60 μm, Service Laboratories of the Institute), reversed phase chromatography on octadecyl-silica gel (20 μm, Laboratorni Pristroje, Prague) with a gradient of methanol; detection with a Uvicord 4701 A (LKB, Sweden) instrument at 254 nm. Electrophoresis was performed on a Whatman No. 3MM paper in 0.1 M triethylammonium hydrogen carbonate for 1 h at 20 V/cm. The electrophoretic mobilities given in the text (E_{AMP}) are referenced to adenosine 5′-phosphate. ^{1}H NMR spectra (δ, ppm; *J*, Hz) were measured on Varian UNITY 200 (200.01 MHz) and Varian UNITY 500 (499.8 MHz) instruments in hexadeuteriodimethyl sulfoxide with tetramethylsilane or in deuterium oxide and sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as the respective internal standards. Mass spectra were taken on a ZAB-EQ (VG Analytical) spectrometer using EI (electron energy 70 eV) nebo FAB (xenone, 8 kV) techniques with glycerol (G) or thioglycerol (TG) as matrices.

Preparation of Esters *IIa*, *IIb*, General Procedure

3-Nitrobenzoyl chloride or 3,5-dinitrobenzoyl chloride (50 mmol) was added at 0 °C to a vigorously stirred solution of diazomethane (freshly prepared by the usual procedure from 23 g (222 mmol) nitrosomethylurea in 230 ml of ether) during 40 min. The mixture was then stirred under cooling for another 45 min. The deposited diazo ketone *Ia* or *Ib* was collected, crystallized from cyclohexane–ethanol and air-dried.

The dry ketone *Ia* or *Ib* was dissolved in a hot mixture of absolute ethanol (250 ml) and toluene (150 ml) and Ag_2O (prepared from 0.33 g of NaOH and 1.29 g of $AgNO_3$ according to ref.[21]) was added. The mixture was refluxed for 2 h, and then another portion of Ag_2O (same as above) was added and the reflux was continued for another 2 h. The reaction mixture was concentrated to 1/3 of the original volume and filtered through a layer of celite. The clear filtrate was evaporated and the residue was dried in vacuo at 50 °C for 1 h.

Ethyl (3-nitrophenyl)acetate (IIa). Yield 9.02 g (86%), yellowish liquid, R_F 0.41 (toluene–ethyl acetate 10 : 1). For $C_{10}H_{11}NO_4$ (209.2) calculated: 57.41% C, 5.30% H, 6.70% N; found: 57.60% C, 5.28% H, 6.58% N. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.19 t, 3 H, $J(CH_3,CH_2)$ = 7.2 (CH_3); 3.84 s, 2 H (CH_2); 4.10 q, 2 H (**CH**$_2$CH$_3$); 7.58 – 7.79 m, 2 H (H-arom.); 8.10 – 8.19 m, 2 H (H-arom.).

Ethyl (3,5-dinitrophenyl)acetate (IIb). The pure product was obtained by chromatography on silica gel (800 ml) in light petroleum–ethyl acetate (6 : 1); R_F 0.40. Yield 3.5 g (27.5%), yellow viscous oil. For $C_{10}H_{10}N_2O_6$ (254.21) calculated: 47.25% C, 3.97% H, 11.02% N; found: 47.48% C, 3.97% H, 10.88% N. Mass spectrum (FAB; bis(hydroxyethyl) disulfide, DMF), *m/z*: 255 (M + H). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.22 t, 3 H, $J(CH_3,CH_2)$ = 7.0 (CH_3); 4.09 s, 2 H (CH_2); 4.13 q, 2 H (**CH**$_2$CH$_3$); 8.65 m, 2 H (H-arom.); 8.76 m, 1 H (H-arom.).

Ethyl (3-Aminophenyl)acetate (*IIIa*)

Chloroform (5 ml) and 10% palladium on carbon (0.9 g) were added to a solution of *IIa* (7.79 g, 37.2 mmol) in absolute ethanol (150 ml) and the mixture was hydrogenated at atmospheric pressure and room temperature for 4 h. The suspension was filtered through celite, the filtrate was mixed with methanolic ammonia (4 ml) and evaporated. The residue was chromatographed on silica gel (550 ml) in toluene–ethyl acetate (3 : 1), R_F 0.30. During the chromatography the column and the collecting flasks were wrapped with an aluminium foil. Yield 5.58 g (84%) of yellow liquid. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.17 t, 3 H, $J(CH_3,CH_2)$ = 7.0 (CH_3); 3.44 s, 2 H (CH_2); 4.06 q, 2 H (**CH**$_2$CH$_3$); 5.06 bs, 2 H (NH_2); 6.34 – 6.48 m, 3 H (H-arom.); 6.90 – 6.99 m, 1 H (H-arom.).

Ethyl (3,5-Diaminophenyl)acetate (*IIIb*)

The compound was prepared from dinitro derivative *IIb* (2.88 g, 11.3 mmol) analogously as described for compound *IIIa*. The hydrogenation was complete in 20 h. The crude product was chromatographed on silica gel (500 ml) in ethyl acetate, R_F 0.40. Yield 1.55 g (70.6%) of a yellow liquid, darkening on the light. Mass spectrum (EI), *m*/*z*: 194 (M^+). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.17 t, 3 H, $J(CH_3,CH_2)$ = 7.1 (CH_3); 3.25 s, 2 H (CH_2); 4.04 q, 2 H (**CH**$_2$$CH_3$); 4.70 bs, 4 H (2 × NH_2); 5.68 m, 3 H (H-arom.).

2-(3-Aminophenyl)ethanol (*IVa*)

Lithium aluminium hydride (2.85, 75 mmol) was added to a solution of ester *IIIa* (4.59 g, 25.6 mmol) in dry dioxane (50 ml) and the mixture was stirred under argon at ambient temperature overnight. After quenching with ethyl acetate (20 ml), methanol (30 ml) was added dropwise, followed by water (20 ml). The mixture was stirred for 30 min and then filtered through celite. The solvent was evaporated, the residue partitioned between ethyl acetate (250 ml) and water (150 ml) and the organic layer was dried over magnesium sulfate. After evaporation of the solvent, the residue was chromatographed on silica gel (750 ml) in toluene–acetone 1 : 1 (R_F 0.33). Yield 1.4 g (40%) of a yellow viscous oil that crystallized on longer standing. Mass spectrum (EI), *m*/*z*: 137.2 (M^+), 119.2 (M – H_2O), 106.1 (3-aminobenzyl). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 2.56 t, 2 H, $J(CH_2,CH_2)$ = 7.3 (2 × H-2); 3.54 m, 2 H (2 × H-1); 4.58 t, 1 H, $J(OH,CH_2)$ = 5.2 (OH); 4.91 bs, 2 H (NH_2); 6.38 m, 3 H (H-arom.); 6.89 t, 1 H (H-arom.).

2-(3,5-Diaminophenyl)ethanol (*IVb*)

A. The compound was prepared from ester *IIIb* (583 mg, 3 mmol) analogously as described for compound *IVa*. Chromatography of the crude product on silica gel (150 ml) in ethyl acetate–acetone–ethanol–water (18 : 3 : 1 : 1) afforded 50 mg (11%) of a yellowish viscous oil, R_F 0.17. Mass spectrum (EI), *m*/*z*: 152 (M^+), 122 (3,5-diaminotoluene), 121 (3,5-diaminobenzyl). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 2.41 t, 2 H, $J(CH_2,CH_2)$ = 8.0 (2 × H-2); 3.48 m, 2 H (2 × H-1); 4.50 bt, 1 H (OH); 4.86 bs, 4 H (2 × NH_2); 5.64 m, 3 H (3 × H-arom.).

B. Sodium (250 mg) was added to a solution of ester *IIIb* (320 mg, 1.65 mmol) in absolute ethanol (1 ml), the stirred mixture was refluxed for 30 min, diluted with ethanol (10 ml) and neutralized with Dowex 50 (H^+ form). The neutral solution, together with the ion-exchanger used for the neutralization, was applied onto a column of Dowex 50 (H^+ form; 100 ml). After washing the column with water, the product was eluted with 5% aqueous ammonia. The combined product fractions were concentrated and the residue was chromatographed on silica gel (50 ml) as described in procedure *A*. Yield 120 mg (48%) of compound *IVb*.

Bis(2-propyl) 2-Phenylethoxymethylphosphonate (*VI*)

Tosylate *V* (3.20 g, 9.1 mmol) was added to a solution of 2-phenylethanol (800 mg, 6.55 mmol) in dimethylformamide (16 ml), the solution was cooled to –20 °C and sodium hydride (800 mg, 20 mmol, of 60% suspension in oil) was added. The mixture was stirred at –20 °C for 30 min and then the temperature was raised to 15 °C during 1 h. After neutralization with acetic acid (1.5 ml), the solvent was evaporated, the residue was diluted with ethyl acetate (200 ml) and washed with water (2 × 100 ml). The organic layer was dried over magnesium sulfate and the solvent was evaporated. Chromatography on silica gel (400 ml) in toluene–ethyl acetate (1 : 1; R_F 0.20) gave 689 mg (35%) of colourless liquid. For $C_{15}H_{25}O_4P$ (300.3) calculated: 60.00% C, 8.39% H, 10.31% P; found: 59.70% C, 8.38% H, 10.28% P. Mass spectrum (FAB; G + CH_3OH), *m*/*z*: 301.4 (M + H). ^{1}H NMR spectrum

(hexadeuteriodimethyl sulfoxide): 1.19 d and 1.22 d, 6 H and 6 H, $J(CH_3,CH)$ = 6.1 (CH_3); 2.81 t, 2 H, $J(CH_2,CH_2)$ = 6.9 (CH_2Ph); 3.73 d, 2 H, $J(P,CH)$ = 8.4 (PCH_2); 3.76 t, 2 H (OCH_2); 4.55 2 × d sept, 2 H, $J(P,OCH)$ = 7.8 (POCH); 7.15 – 7.30 m, 5 H (H-arom.).

2-Phenylethoxymethylphosphonic Acid (*VII*)

Bromotrimethylsilane (2.4 ml, 18.2 mmol) was added to a solution of phosphonate *VI* (547 mg, 1.82 mmol) in acetonitrile (8 ml) and the solution was stirred in the dark under argon at ambient temperature for 24 h. After addition of 1 M triethylammonium hydrogen carbonate to slightly alkaline reaction, the solvent was evaporated, the residue was codistilled with water (4 × 70 ml) and then subjected to reversed phase chromatography (C_{18}). The column was washed first with water and then with a gradient 0 – 100% methanol, the product being eluted at the concentration 30% of methanol. The combined product fractions were evaporated and the amorphous residue was dissolved in water (5 ml) and applied onto a column of Dowex 50 (Li^+ form; 100 ml). The column was washed with water and the UV-absorbing fractions were concentrated. The residue was codistilled with ethanol, then mixed with ethanol and set aside at 4 °C overnight. The white solid product was collected, washed with ether and dried in vacuo at 50 °C for 1 h. Yield 256 mg (62%) of acid *VII* as dilithium salt. Mass spectrum (FAB; G + methanol), *m/z*: 229.0 (M + H, $C_9H_{11}Li_2O_4P$), 223 (M + H, $C_9H_{12}LiO_4P$), 77 (C_6H_5). ^{1}H NMR (D_2O): 2.94 t, 2 H, $J(CH_2,CH_2)$ = 6.9 (CH_2Ph); 3.50 d, 2 H, $J(P,CH_2)$ = 8.6 (PCH_2); 3.84 t, 2 H (OCH_2); 7.37 m, 5 H (H-arom.).

Bis(2-propyl) 2-(4-Aminophenyl)ethoxymethylphosphonate (*XI*)

Dimethylformamide dimethyl acetal (15 ml) was added to a solution of 2-(4-aminophenyl)ethanol (1.5 g, 10.93 mmol) in dry dimethylformamide (25 ml) and the solution was stirred at ambient temperature for 20 h. After evaporation of the solvent, the residue was codistilled with dimethylformamide and mixed with crushed dry ice (about 10 g). Then, 50% aqueous pyridine (45 ml) was added and the mixture was stirred for 1 h. The solvent was evaporated, the residue was codistilled with dry pyridine (50 ml) and toluene (2 × 25 ml) and dried at room temperature for 1 h. The thus-obtained protected derivative *VIII* was dissolved in dimethylformamide (60 ml) and stirred with sodium hydride (60% suspension; 1.31 g, 32.8 mmol) for 30 min. Tosylate *V* (5.74 g, 16.4 mmol) was added and the mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of crushed dry ice and water (0.5 ml). After stirring for 30 min and evaporation of the solvent, the residue was allowed to stand with a 3 : 1 mixture of 25% aqueous ammonia and methanol (100 ml) at room temperature for 4 days. The solvent was evaporated and the residue was partitioned between ethyl acetate (150 ml) and water (150 ml). The aqueous phase was once more extracted with the same volume of ethyl acetate, the combined organic extracts were dried over magnesium sulfate and the solvent was evaporated. Chromatography of the residue on silica gel (500 ml) in toluene–acetone (3 : 2) afforded 1.24 g (36%) of a lightly yellow viscous oil, R_F 0.46 (toluene–acetone 3 : 2). For $C_{15}H_{26}NO_4P$ (315.4) calculated: 57.13% C, 8.31% H, 4.44% N, 9.82% P; found: 56.86% C, 8.37% H, 4.52% N, 9.77% P. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.22 d and 1.23 d, 6 H and 6 H, $J(CH_3,CH)$ = 6.3 (CH_3); 2.62 t, 2 H, $J(CH_2,CH_2)$ = 7.2 (CH_2Ph); 3.60 t, 2 H (OCH_2): 3.71 d, 2 H, $J(P,CH)$ = 8.3 (PCH_2); 4.56 m, 2 H, $J(P,OCH)$ = 7.3 (POCH); 4.84 bs, 2 H (NH_2); 6.47 d and 6.86 d, 2 H and 2 H, J = 8.1 (H-arom.).

2-(4-Aminophenyl)ethyl bis(2-propyl) phosphate (*XIV*) (130 mg; 4%) was obtained as side product; yellowish viscous oil, R_F 0.51 (toluene–acetone 3 : 2). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.21 d and 1.22 d, 6 H and 6 H, $J(CH_3,CH)$ = 6.3 (CH_3); 2.44 m, 2 H, $J(P,OCH)$ = 7.0 (POCH); 2.72 t, 2 H, $J(CH_2,CH_2)$ = 7.0 (CH_2Ph); 3.98 q, 2 H, $J(P,OCH)$ = 7.0 (OCH_2); 4.89 bs, 2 H (NH_2); 6.49 d and 6.88 d, 2 H and 2 H, J = 8.3 (H-arom.).

Bis(2-propyl) 2-(3-Aminophenyl)ethoxymethylphosphonate (*XII*)

The title compound was prepared from amino alcohol *IVa* (1.4 g, 10.2 mmol) analogously as described for ester *XI*. The crude product was chromatographed on silica gel (300 ml) in toluene–acetone (1 : 1) (R_F 0.52) to give 700 mg (22%) of amorphous product. Mass spectrum (FAB; T + G + dimethyl sulfoxide), *m*/*z*: 316 (M + H), 274 (M – isopropyl), 232 (M – 2 × isopropyl). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.23 d and 1.21 d, 3 H and 3 H, $J(CH_3,CH)$ = 6.1 (CH_3); 1.26 d, 6 H, $J(CH_3,CH)$ = 6.3 (CH_3); 2.65 t, 2 H, $J(CH_2,CH_2)$ = 7.1 (CH_2Ph); 3.65 t, 2 H (OCH_2); 3.72 d, 2 H, J(P,CH) = 8.3 (PCH_2); 4.57 d sept, 2 H, J(P,OCH) = 7.8 (POCH); 4.96 bs, 2 H (NH_2); 6.35 – 6.42 m, 3 H (H-arom.); 6.90 t, 1 H (H-arom.).

2-(3-Aminophenyl)ethyl 2-propyl 2-propyloxymethylphosphonate (*XV*) was identified as side product. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 1.08 d, 6 H, $J(CH_3,CH)$ = 6.1 (CH_3); 1.22 d and 1.27 d, 3 H and 3 H, $J(CH_3,CH)$ = 6.1 (CH_3); 2.74 t, 2 H (CH_2Ph); 4.10 bq, 2 H, $J(CH_2,CH_2)$ = 7.1, J(P,OCH) = 7.6 (OCH_2); 4.14 d and 4.15 d, 1 H and 1 H, J(P,CH) = 9.0 (PCH_2); 4.52 sept, 1 H, J = 6.2 ($CH_2O\mathbf{CH}$); 4.64 d sept, 1 H, J(P,OCH) = 7.3 ($PO\mathbf{CH}(CH_3)_2$); 4.96 bs, 2 H (NH_2); 6.35 – 6.42 m, 3 H (H-arom.); 6.90 t, 1 H (H-arom.).

2-(4-Aminophenyl)ethoxymethylphosphonic Acid (*XVI*)

The compound was prepared by reaction of diisopropyl ester *XI* (700 mg, 2.22 mmol) with bromotrimethylsilane (2.6 ml, 20 mmol), analogously as described for the compound *VII*. The crude product was chromatographed on a reversed phase (C_{18}). After washing with water, the elution was performed with a gradient 0 – 20% methanol, the product being eluted with 3 – 8% methanol. Second chromatography under the same conditions afforded 110 mg (21.4%) of the product, white light-sensitive solid, R_F 0.19 (2-propanol–25% aqueous ammonia–water 7 : 1 : 2); E_{AMP} 1.07. Mass spectrum (FAB; G + CH_3OH + CF_3COOH), *m*/*z*: 232 (M + H). ^{1}H NMR spectrum (D_2O + NaOD): 2.82 t, 2 H, $J(CH_2,CH_2)$ = 7.1 (CH_2Ph); 3.49 d, 2 H, J(P,CH) = 8.6 (PCH_2); 3.76 t, 2 H (OCH_2); 6.82 d and 7.16 d, 2 H and 2 H, J = 8.3 (H-arom.).

2-(3-Aminophenyl)ethoxymethylphosphonic Acid (*XVII*)

The compound was prepared from ester *XII* (700 mg, 2.2 mmol) and bromotrimethylsilane (2.6 ml, 20 mmol) analogously as described for compound *VII*. The crude product was applied onto a column of DEAE-Sephadex A-25 (300 ml; acetate form). The column was washed first with water (1 000 ml) and then with a gradient 0 – 0.4 M acetic acid (à 500 ml). The product was eluted with 0.3 M acetic acid. The product fractions were combined, evaporated, and the residue was codistilled with water (4 × 100 ml). The solid residue was triturated with a mixture of methanol and acetone (1 : 1), collected on filter, washed with acetone and ether and dried in vacuo. Yield 160 mg (31.5%) of white solid, R_F 0.13 (2-propanol–25% aqueous ammonia–water 7 : 1 : 2), E_{AMP} 1.27. For $C_9H_{14}NO_4P$ (231.2) calculated: 46.76% C, 6.10% H, 6.06% N, 13.40% P; found: 47.05% C, 6.12% H, 5.84% N, 13.10% P. Mass spectrum (FAB; T + G + H_2O), *m*/*z*: 232.2 (M + H), 120.2 (3-aminophenylethyl). ^{1}H NMR spectrum (D_2O): 2.89 t, 2 H, $J(CH_2,CH_2)$ = 6.7 (CH_2Ph); 3.63 d, 2 H, J(P,CH) = 8.6 (PCH_2); 3.83 t, 2 H (OCH_2); 6.95 m, 3 H (H-arom.); 7.28 t, 1 H (H-arom.).

2-(3,5-Diaminophenyl)ethoxymethylphosphonic Acid (*XVIII*)

Dimethylformamide dimethyl acetal (10 ml) was added to a solution of alcohol *IVb* (170 mg, 1.12 mmol) in dimethylformamide (10 ml) and the solution was set aside at room temperature for 20 h. The solvent was evaporated, the residue was codistilled with dimethylformamide (10 ml) and mixed

with dry ice (about 10 g), followed by 50% aqueous pyridine (10 ml). The mixture was stirred for 1 h, taken down and the residue was codistilled with dry pyridine and toluene (2 × 10 ml).

The thus-prepared intermediate *X* was dissolved in dimethylformamide (10 ml) and the solution was stirred with sodium hydride (60% suspension; 134 mg, 3.36 mmol) for 30 min. Tosylate *V* (588 mg, 1.68 mmol) was added and the mixture was stirred at ambient temperature for 4 h. After neutralization with acetic acid (0.3 ml), the solvent was evaporated and the residue was stirred with methanolic ammonia for 3 days. After evaporation, dry acetonitrile (10 ml) was added to the residue and bromotrimethylsilane (1.3 ml, 9.8 mmol) was added under argon. The suspension was stirred in the dark at room temperature for 20 h and neutralized with 1 M triethylammonium hydrogen carbonate. The solvent was evaporated and the crude product was applied onto a column of DEAE-Sephadex A-25 (160 ml; HCO_3^- form). The ion-exchanger was washed with water (350 ml) and then with a gradient of triethylammonium hydrogen carbonate (0 – 0.4 mol/l; 600 ml) the product being eluted at concentration 0.25 – 0.35 mol/l. The product-containing fractions were combined and evaporated in the dark at 30 °C. The crude product was twice chromatographed on a reversed phase (C_{18}-silica gel) in water. Fractions containing the pure product were combined, concentrated to 5 ml, applied onto a column of Dowex 50 (Li^+ form, 50 ml) and the lithium salt of compound *XVIII* was eluted with water. Evaporation and drying in vacuo in the dark over phosphorus pentoxide gave 50 mg (17%) of a grayish solid, R_F 0.26 (2-propanol–25% aqueous ammonia–water 7 : 1 : 1), E_{AMP} 0.78. Mass spectrum (FAB; T + G, CH_3OH), *m/z*: 247 (M + H, free *XVIII*, $C_9H_{15}N_2O_4P$), 259 (M + H, dilithium salt of *XVIII*, $C_9H_{13}Li_2N_2O_4P$).

The authors are indebted to Professor E. De Clercq, Dr J. Balzarini and Dr R. Snoeck, Rega Institute, Catholic University, Leuven, Belgium, for the antiviral tests. Their thanks are also due to Dr I. Votruba of this Institute for the cytostatic assays, and to Dr M. Masojidkova for measurement and interpretation of the NMR spectra. They also thank Mrs A. Sterecova for the technical assistance, the staff of the Analytical Laboratory (Dr V. Pechanec, Head) for the elemental analyses and the staff of the Central Laboratory of Mass Spectrometry (Dr K. Ubik, Head) for the mass spectral measurements. This work was supported by Grant No. 203/93/0118 of the Czech Grant Agency, Grant No. 455407 of the Grant Agency of the Czech Academy of Sciences and by Gilead Sciences (California, U.S.A.).

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