# SYNTHESIS OF 9-(2-PHOSPHONYLMETHOXYETHYL)ADENINE AND RELATED COMPOUNDS*

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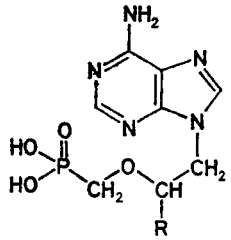
Diethyl 2-hydroxyethoxymethanephosphonate (*VIII*) was converted into diethyl 2-halogenoethoxymethanephosphonates *IXa* and *IXb* by reaction with triphenylphosphine and tetrachloromethane or tetrabromomethane; analogous reaction of *VIII* with *p*-toluenesulfonyl chloride afforded diethyl 2-(*p*-toluenesulfonyloxy)ethoxymethanephosphonate (*IXc*). Reaction of sodium salt of adenine with compounds *IX* led to 9-(2-diethoxyphosphonylmethoxyethyl)adenine (*X*). Compound *X* was converted into 9-(2-phosphonylmethoxyethyl)adenine (*II*) by treatment with bromotrimethylsilane whereas alkaline hydrolysis of *X* gave ethyl ester *Vb*. Reaction of 9-(2-hydroxyethyl)adenine (*IIIa*) or its $N^6$-benzoyl derivative *IIIb* with dimethyl *p*-toluenesulfonyloxymethanephosphonate (*IV*) in the presence of sodium hydride, followed by alkaline hydrolysis yielded methyl ester *Va*. Morpholide *XI* reacted with an inorganic phosphate and diphosphate to give 9-(2-phosphorylphosphonylmethoxyethyl)adenine (*XII*) and 2-(diphosphorylphosphonylmethoxyethyl)adenine (*XIII*), respectively.

In the first communication of this series[1] we have described preparative methods leading to 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (*I*), the first acyclic nucleotide analogue with an exceptionally high antiviral activity[2]. This compound contains an isosteric isopolar phosphonylmethyl ether moiety instead of the phosphate grouping. Within the framework of our studies of structure–activity relationships in the series of acyclic adenine nucleotide analogues we found and described[2,2] the antiviral activity of a related compound, 9-(2-phosphonylmethoxyethyl)adenine (*II*) (PMEA), which also contains the mentioned characteristic grouping in its molecule. The structural similarity of both compounds is evident from their formulae (*I*, *II*). Interestingly enough, whereas the biological activity of compound *I* is connected exclusively with the (*S*)-enantiomer and thus conditioned by a chiral interaction, compound *II* contains no center of chirality. Therefore, the mechanism of action of both compounds may be different.

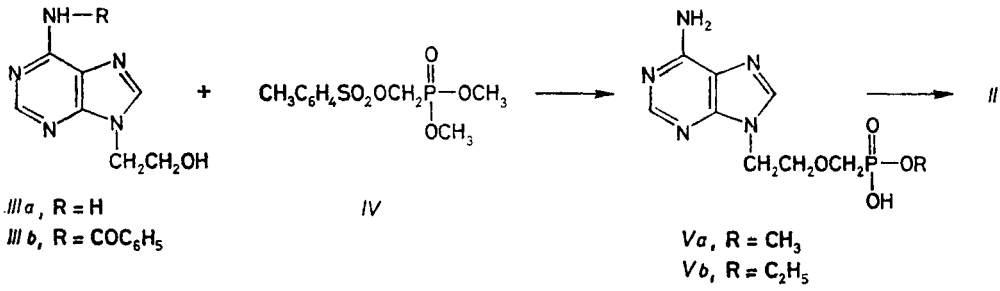
The present paper concerns synthetic methods leading to compound *II* and its simple derivatives, as well as preparation of the diphosphate and triphosphate analogues as potential metabolites which could arise from *II* in the cells.

* Part III in the series: Acyclic Nucleotide Analogues; Part II: Collect. Czech. Chem. Commun. *52*, 2792 (1987).



*I*, R = (*S*)—$CH_2OH$
*II*, R = H

The first approach to compound *II* consists in the condensation of 9-(2-hydroxyethyl)adenine (*IIIa*) alkoxide anion with a phosphonic acid synthon, dimethyl *p*-toluenesulfonyloxymethanephosphonate[3] (*IV*). This reaction was carried out in the same manner as described[3] for the preparation of nucleoside 5'-O-phosphonylmethyl ethers; because of possible side-reaction with the heterocyclic moiety, suitable protection of its amino group, *e.g.* transformation into the $N^6$-benzoyl derivative *IIIb*, proved to be advantageous. The reaction components reacted in dimethylformamide in the presence of an excess of sodium hydride, generating the alkoxide ion. The primarily arising diester of *II* was not isolated but directly converted by alkaline hydrolysis into the monomethyl ester *Va*. The ester-bonded methyl group was removed by treatment with iodotrimethylsilane in dimethylformamide and the compound *II* was isolated by chromatography on an anion exchanger (Scheme 1).



*IIIa*, R = H
*IIIb*, R = $COC_6H_5$

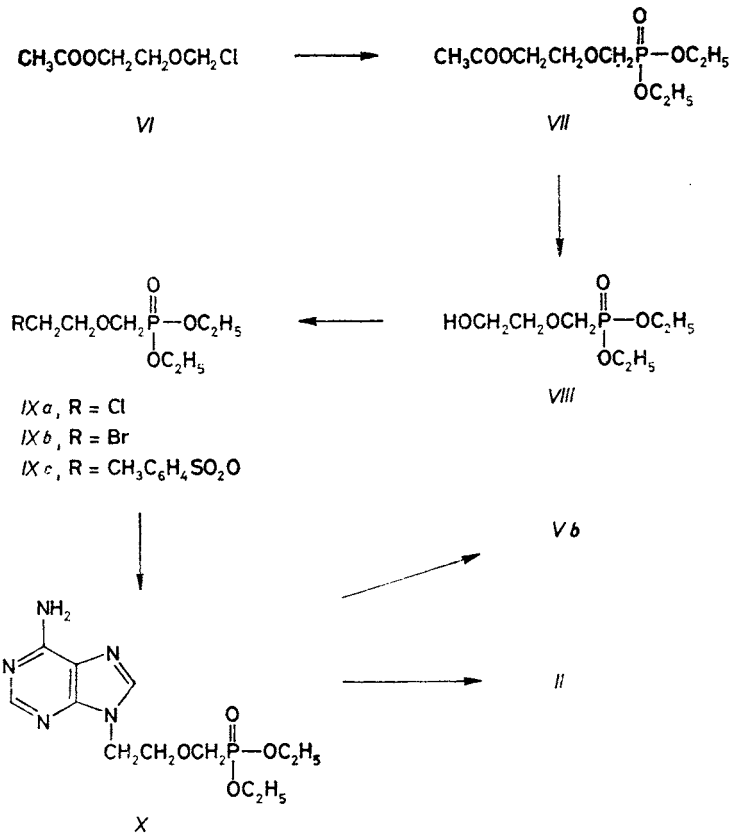
*IV*

*Va*, R = $CH_3$
*Vb*, R = $C_2H_5$

SCHEME 1

The described procedure does not afford high yields and requires the preparation of 9-(2-hydroxyethyl)adenine (*IIIa*) and its benzoylation to compound *IIIb*. Much more satisfactory was condensation of adenine (as its alkali metal salt) with a synthon, containing a structural element of ethoxymethanephosphonic acid substituted in the position 2 with a suitable leaving group. According to the existing experience, such procedure requires use of neutral compounds (esters of phosphoric or phosphonic

acids) since organophosphorus synthons of the mono- or dianion type are markedly less reactive. As suitable synthons we used compounds of the type *IX* (Scheme 2),



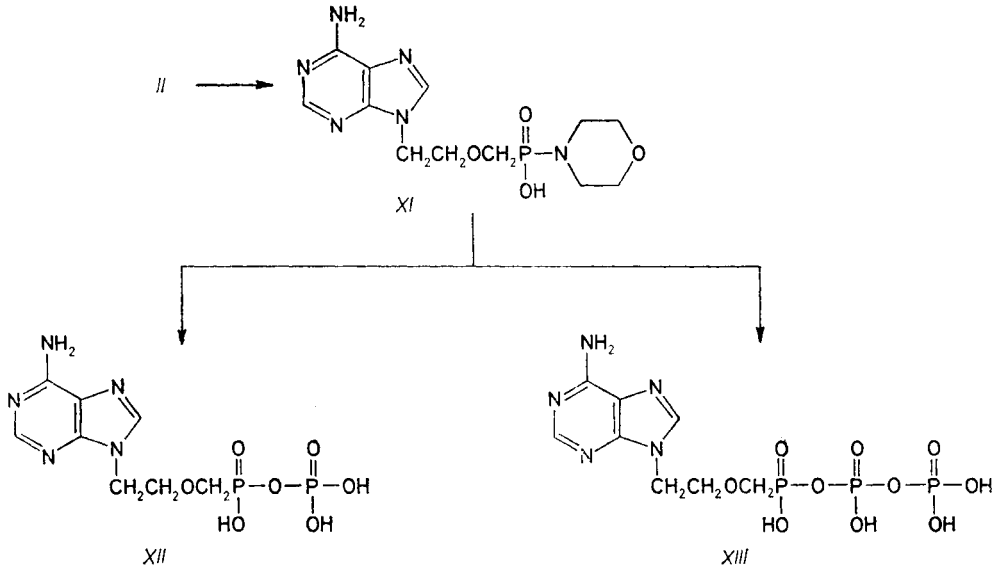

SCHEME 2

prepared from diethyl 2-acetoxyethoxymethanephosphonate (*VII*). Compound *VII* was prepared by Arbuzov reaction of 2-acetoxyethoxymethyl chloride (*VI*) (which in turn was accessible by reaction of 1,3-dioxolane with acetyl chloride) with triethyl phosphite[4]. Acid-catalyzed cleavage (in the presence of a cation exchanger) of *VII* afforded high yield of diethyl 2-hydroxyethoxymethanephosphonate (*VIII*), a key intermediate in the syntheses of PMEA (*II*). Compound *VIII* was converted into the reactive synthons in two ways: *a*) by reaction with triphenylphosphine and tetrachloromethane or tetrabromomethane, leading to the respective 2-chloroethyl (*IXa*) and 2-bromoethyl (*IXb*) derivatives, or *b*) by esterification of the 2-hydroxyethyl group in *VIII* with *p*-toluenesulfonyl chloride in the presence of a base, affording

the 2-*p*-toluenesulfonyloxyethyl derivative *IXc*. From the practical standpoint, the alternative *b*) is more advantageous because it does not use triphenylphosphine and does not involve the relatively uneconomical removal of side-products arising from the triphenylphosphine adducts. However, the use of all the three synthons *IX* in further reactions is comparable.

The alkylation itself was carried out with sodium salt of adenine and practically equimolar amount of the synthon *IX*. At elevated temperatures and anhydrous conditions, the reaction was relatively rapid, leading predominantly to the $N^9$-isomer. In all cases, the reaction product was 9-(2-diethoxyphosphonylmethoxyethyl)adenine (*X*) which was isolated by chromatography on silica gel.

Compound *X* was converted into 9-(2-phosphonylmethoxyethyl)adenine (*II*) by reaction with bromotrimethylsilane, *e.g.* in acetonitrile. Since this reagent also silylated the adenine ring in *X*, we tried to reduce its consumption by a pre-silylation with hexamethyldisilazane. This reagent itself was incapable of splitting ester functionalities and was moreover used as reaction medium for the dealkylation with bromotrimethylsilane. The cleavage was performed at room temperature and the silyl ester intermediates were hydrolyzed in a slightly alkaline buffer. After deionization, the compound *II* was isolated by chromatography on an anion exchanger in a volatile organic acid (acetic acid) and crystallized from water as the sparingly soluble, completely pure free acid. The stable product was characterized by its $^1H$ and $^{13}C$ NMR spectra (Scheme 2).




SCHEME 3

As expected, compound *X* was hydrolyzed exclusively to the first stage, affording the monoethyl ester *Vb*. However, this partial hydrolysis offers no advantage in the preparation of compound *II*.

In the synthesis of diphosphate and triphosphate analogues *XII* and *XIII*, compound *II* was first converted into 9-(2-morpholinophosphonylmethoxyethyl)adenine (*XI*) by the usual reaction with morpholine in the presence of N,N'-dicyclohexylcarbodiimide as activating reagent. The 2-phosphorylphosphonyl (*XII*) and 2-diphosphorylphosphonylethoxymethyl (*XIII*) derivatives were then obtained from *XI* by reaction with tri(n-butylammonium)phosphate and diphosphate, respectively, in dimethyl sulfoxide, *i.e.* under conditions usual for synthesis of ribonucleoside 5'-diphosphates and 5'-triphosphates[5]. Both compounds were purified by chromatography on an anion exchanger in an acid medium in which they were sufficiently stable, and were isolated as homogeneous lithium salts (Scheme 3).

The described syntheses of compounds *II*, particularly those using the reactive organophosphorus synthons *IX* for the preparation of intermediates *X*, are of general use and have been widely applied to the preparation of analogous compounds of the type *II*, modified both in the heterocyclic base and in the side-chain.

## EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Unless otherwise stated, the solvents were evaporated at 40°C/2 kPa and the compounds dried over phosphorus pentoxide at 13 Pa. Paper chromatography was performed on a Whatman No. 1 paper in the system S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), paper electrophoresis (20 V/cm) on a Whatman No. 3MM paper in 0·1 mol $l^{-1}$ triethylammonium hydrogen carbonate, pH 7·5 ($E_{Up}$ denotes mobility, referred to uridine 3'-phosphate). Thin-layer chromatography (TLC) was performed on Silufol UV 254 silica gel-coated plates (Kavalier, Czechoslovakia) in the systems S2, chloroform–methanol (4 : 1), and S3, ethyl acetate. Liquid chromatography (HPLC) was carried out on a 200 × 4 mm column of Separon SGX (7 μ; Laboratorní přístroje, Prague) in the system S4, 2% acetonitrile in 0·05 mol $l^{-1}$ triethylammonium hydrogen carbonate, pH 7·5 (detection at 254 nm, elution rate 1 ml $min^{-1}$). UV spectra were measured in aqueous solutions on a Specord UV-VIS spectrophotometer (Carl Zeiss, Jena, G.D.R.), NMR spectra were obtained with a Varian XL-200 instrument (chemical shifts in ppm, coupling constants in Hz).

9-(2-*Hydroxyethyl*)-$N^6$-*benzoyladenine* (*IIIb*) was prepared from 9-(2-hydroxyethyl)adenine[6] (*IIIa*) by reaction with chlorotrimethylsilane and benzoyl chloride in pyridine[7] in 81% yield; m.p. 184–186°C. For $C_{14}H_{13}N_5O_2$ (283·3) calculated: 59·36% C, 4·63% H, 24·72% N; found: 59·41% C, 4·68% H, 24·68% N. $^1$H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·81 brq, 2 H (O—$CH_2$, $J$ = 5·3); 4·33 t, 2 H (N—$CH_2$, $J(CH_2CH_2)$ = 5·5); 5·06 t, 1 H (OH, $J(CH_2CH_2)$ = 5·2); 8·42 s, 1 H (H-2); 8·73 s, 1 H (H-8); 7·50–7·70 m, 3 H and 8·00–8·10 m, 2 H (aromatic protons).

*Dimethyl p-toluenesulfonyloxymethanephosphonate* (IV) was prepared as described earlier[3].

*Diethyl 2-acetoxyethoxymethanephosphonate* (VII) was obtained from 2-acetoxyethoxymethyl chloride (*VI*) by a described[4] procedure.

Methyl Ester of 9-(2-Phosphonylmethoxyethyl)adenine (*Va*)

Benzoyl derivative *IIIb* (4·1 g; 14·5 mmol) was dissolved in warm dimethylformamide (70 ml), the solution was cooled in ice and sodium hydride (0·72 g; 30 mmol) was added. The mixture was stirred at room temperature for 30 min and compound *IV* (4·3 g; 14·5 mmol) was added. After dissolution, the mixture was allowed to stand at room temperature for 48 h, diluted with 0·5 mol $l^{-1}$ sodium hydroxide (100 ml), kept at 50°C for 6 h, cooled and acidified by addition of Dowex 50X8 ($H^+$ form). The suspension was poured on a column (100 ml) of the same resin. The column was washed with 50% aqueous methanol to disappearance of UV absorption and the product was eluted with 5% aqueous ammonia. The UV-absorbing eluate was taken down and the product was purified by chromatography on a column of Dowex 1X2 (acetate form; 200 ml); elution with linear gradient of acetic acid (0—0·5 mmol $l^{-1}$, 2 × 2 l). The product fraction was taken down, the residue codistilled with water (3 × 50 ml) and crystallized from aqueous ethanol; yield 1·4 g (34%) of *Va* (free acid); $R_F$ 0·51 (S1), $E_{Up}$ = 0·47. $^1$H NMR spectrum (deuterium oxide): 3·40 d, 3 H (P—$OCH_3$, $J$(P—OCH) = 10·3); 3·65 d, 2 H (P—$CH_2$, $J$(P—CH) = = 8·4); 3·94 t, 2 H (O—$CH_2$, $J$ = 5·0); 4·40 t, 2 H (N—$CH_2$, $J$ = 5·0); 8·14 s, 1 H (H-2); 8·15 s, 1 H (H-8).

The reaction was carried out in the same way with 4 mmol of *IIIa*, affording 0·30 g (26%) of *Va*, identical with the product obtained above.

Ethyl Ester of 9-(2-Phosphonylmethoxyethyl)adenine (*Vb*)

A solution of *X* (0·66 g; 2 mmol) in 0·5 mol $l^{-1}$ sodium hydroxide (25 ml) was heated to 80°C for 6 h and the reaction mixture was processed as described for *Va* (in the deionization step, the ion exchanger was first washed with water). Chromatography on Dowex 1X2 (acetate form) in a gradient of acetic acid, followed by crystallization from aqueous ethanol, afforded 0·45 g (74·5%) of *Vb* (free acid). For $C_{10}H_{16}N_5O_4P$ (301·3) calculated: 39·86% C, 5·35% H, 23·25% N, 10·30% P; found: 40·10% C, 5·47% H, 23·11% N, 10·32% P. $R_F$ 0·53 (S1); $E_{Up}$ = 0·47.

Diethyl 2-Hydroxyethoxymethanephosphonate (*VIII*)

A mixture of diethyl ester *VII* (137·3 g; 0·54 mmol), ethanol (600 ml), and Dowex 50X8 ($H^+$-form, pre-washed with ethanol) was refluxed with stirring until the reaction was complete (30 h; TLC in S3). The mixture was filtered, the resin washed with ethanol and the combined filtrates were taken down *in vacuo*. The residue was codistilled with toluene (2 × 100 ml) at 40°C/13 Pa and dried at 60°C/12 Pa over phosphorus pentoxide; yield 113·5 g (99%) of oily *VIII*; $R_F$ 0·20 (*VII*: $R_F$ 0·40) in S3.

Diethyl 2-Chloroethoxymethanephosphonate (*IXa*)

A mixture of *VIII* (15·9 g; 75 mmol), tetrachloromethane (150 ml), and triphenylphosphine (22·3 g; 85 mmol) was refluxed for 4 h. After evaporation, the residue was stirred with ether–light petroleum (1 : 1, 100 ml), the suspension was filtered, the solid washed with the same solvent mixture, and the combined extracts were taken down. The product was purified by chromatography on a column of silica gel (250 ml) in ethyl acetate. Elution with ethyl acetate–methanol (95 : 5), evaporation and drying *in vacuo* afforded 8·6 g (50%) of *IXa* as an oil which was directly used in the next reaction step. $R_F$ 0·60 (S3).

Diethyl 2-Bromoethoxymethanephosphonate (*IXb*)

A mixture of *VIII* (21·2 g; 0·1 mol), dioxane (200 ml), triphenylphosphine (26·3 g; 0·1 mol), and tetrabromomethane (33·2 g; 0·1 mol) was refluxed for 4 h and taken down *in vacuo*. The residue was stirred with ether (250 ml) for 20 min and the supernatant was decanted. This procedure was repeated and the combined ethereal extracts were taken down. The remaining oil was stirred with ether–light petroleum (1 : 1; 300 ml) and after decanting the solvent was evaporated. Chromatography on a column of silica gel (300 ml; 30—40 μ) in benzene, evaporation of the product fractions and drying at 13 Pa over phosphorus pentoxide gave 17·6 g (64%) of chromatographically homogeneous (S3, $R_F$ 0·65) *IXb*.

Diethyl 2-*p*-Toluenesulfonyloxyethoxymethanephosphonate (*IXc*)

A solution of *p*-toluenesulfonyl chloride (121·5 g; 0·64 mol) in dichloromethane (250 ml) was added during 30 min to a stirred solution of *VIII* (114 g; 0·54 mol) and triethylamine (88·4 ml) in dichloromethane (250 ml). After stirring for 2 h and addition of 4-dimethylaminopyridine (611 mg; 5 mmol), the mixture was stirred for further 4 h and the solvent was evaporated. The residue was taken up in benzene (600 ml), filtered, the solid washed with benzene and the filtrate concentrated *in vacuo*. The residue was dissolved in benzene (100 ml) and chromatographed on a column of silica gel (30—40 μ, 500 ml) in benzene. After washing out the excess sulfonyl chloride, the product was eluted with benzene–ethyl acetate (9 : 1). Evaporation of solvent and drying over phosphorus pentoxide at 13 Pa afforded 145 g (73%) of oily *IXc*. For $C_{14}H_{23}O_7PS$ (366·4) calculated: 45·89% C, 6·33% H, 8·47% P, 8·75% S; found: 46·04% C, 6·38% H, 8·32% P, 8·42% S. $^1$H NMR spectrum ($C^2HCl_3$): 1·32 m and 4·15 m, 10 H (P—$OC_2H_5$); 3·78 d, 2 H (P—$CH_2$); 3·81 t, 2 H (O—$CH_2$); 4·15, 2 H ($SO_2OCH_2$); 2·45 s, 3 H (arom. $CH_3$); 7·36 m and 7·79 m, 4 H (aromatic protons). $R_F$ 0·60 (S3).

9-(2-Diethoxyphosphonylmethoxyethyl)adenine (*X*)

*A*) A stirred suspension of adenine (1·35 g; 10 mmol) and sodium hydride (0·24 g; 10 mmol) in dimethylformamide (80 ml) was heated to 80°C for 1 h under exclusion of moisture. A solution of *IXb* (2·75 g; 10 mmol) in dimethylformamide (10 ml) was added dropwise at this temperature during 3 h. The mixture was heated for further 12 h to 90°C (clear solution), taken down at 40°C/13 Pa, and the residue was extracted with boiling chloroform (3 × 100 ml). The extract was filtered through Celite, the solvent evaporated *in vacuo* and the residue chromatographed on a column of silica gel (300 ml) in chloroform. Elution with chloroform–ethanol (95 : 5) afforded *X* which was crystallized from ethyl acetate–light petroleum; yield 2·1 g (64%), m.p. 137°C; $R_F$ 0·57 (S2). For $C_{12}H_{20}N_5O_4P$ (329·4) calculated: 43·76% C, 6·12% H, 21·27% N, 9·42% P; found: 44·01% C, 6·22% H, 21·42% N, 9·18% P. $^1$H NMR spectrum ($C^2HCl_3$): 1·29 t, 6 H (C—$CH_3$, $J$ = 7·05); 3·78 d, 2 H (P—$CH_2$, $J$(P—CH) = 8·3); 3·94 t, 2 H (H-2′, $J$(1′, 2′) = 4·9); 4·10 dq, 4 H ($CH_2CH_3$, $J$(P—OCH) = 8·2); 4·41 t, 2 H (H-1′); 5·78 brs, 2 H ($NH_2$); 7·94 s, 1 H (H-8); 8·35 s, 1 H (H-2).

*B*) Sodium salt of adenine was prepared from adenine (5·4 g; 40 mmol) and sodium hydride (0·96 g; 40 mmol) in dimethylformamide (200 ml) as described under *A*). A solution of *IXa* (8·6 g; 37·3 mmol) in dimethylformamide (20 ml) was added, the mixture was stirred at 80°C for 15 h and worked up as described under *A*) to give 5·7 g (46%) of *X*, identical with the product prepared above.

*C*) Sodium salt of adenine (40 mmol) was prepared as described under *B*), a solution of *IX* (13·6 g; 37·2 mmol) in dimethylformamide (30 ml) was added, the mixture was stirred at 100°C

for 12 h and worked up according to *A*). Yield 7·3 g (60%) of *X*, identical with the product obtained under *A*).

9-(2-Phosphonylmethoxyethyl)adenine (*II*)

*A*) Bromotrimethylsilane (10·2 g; 66 mmol) was added to a suspension of *X* (9·9 g; 30 mmol) in acetonitrile (30 ml). The resulting solution was set aside at room temperature for 16 h in a stoppered flask and taken down *in vacuo*. The residue was codistilled with acetonitrile (20 ml) and methanol (2 × 50 ml) and dissolved in 0·4 mol $l^{-1}$ triethylammonium hydrogen carbonate pH 7·5 (100 ml). After standing for 1 h, the solution was taken down, the residue codistilled with methanol (2 × 100 ml), taken up in water (100 ml) and acidified by addition of Dowex 50 ($H^+$ form). The suspension was poured on a column of the same resin (200 ml), the column was washed with water to disappearance of acid reaction and UV absorption of eluate, and then with 2·5% aqueous ammonia. The UV-absorbing ammonia fractions were taken down, the residue was dissolved in water (50 ml), made alkaline with ammonia and applied on a column of Dowex 1X2 (acetate form, 300 ml). The column was washed with water until UV absorption and conductivity dropped, the resin was transferred into a 2 l flask and stirred for 30 min with refluxing 2 mol $l^{-1}$ acetic acid (1 l). The hot mixture was filtered, the solid washed with the same solution (500 ml) and the filtrate taken down *in vacuo*. The residue was coevaporated with water (3 × 100 ml) and crystallized from water. The separated product was filtered, washed successively with water, ethanol, and ether and dried. The mother liquor was taken down and again crystallized from water. The combined crops afforded 6·0 g (73%) of *II*, not melting up to 250°C. For $C_8H_{12}N_5O_4P$ (273·3) calculated: 35·16% C, 4·43% H, 25·63% N, 11·36% P; found: 35·59% C, 4·38% H, 26·02% N, 11·42% P. UV spectrum (pH 2, 7, 12): $\lambda_{max}$ = 262 nm, $\varepsilon_{max}$ 13 400. $^{13}C$ NMR spectrum (deuterium oxide, ref. dioxane = 66·86 ppm): 153·02 s (C-2); 149·42 s (C-4); 118·88 s (C-5); 156·04 s (C-6); 44·47 s (C-1'); 71·38 d (C-2', $J$(C, P) = 17·6); 69·78 d (C-3', $J$(C, P) = 122·1). $^1H$ NMR spectrum ($^2H_2O$): 3·49 t, 2 H (P—$CH_2$, $J$(P—CH) = = 8·5); 3·94 t, 2 H (O—$CH_2$, $J$ = 5·0); 4·39 t, 2 H (N—$CH_2$, $J$ = 5·0); 8·13 s, 1 H (H-2); 8·22 s, 1 H (H-8). HPLC (S4): $k$ = 0·83 (*XI*, $k$ = 3·24). $E_{Up}$ = 0·86, $R_F$ = 0·19 (S1).

*B*) A mixture of *X* (16·5 g; 50 mmol), hexamethyldisilazane (200 ml), and ammonium sulfate (1 g) was refluxed for 6 h (calcium chloride protecting tube). After cooling, the mixture was allowed to stand with bromotrimethylsilane (19·9 g; 130 mmol) at room temperature for 16 h and evaporated. The residue was codistilled with dioxane (2 × 50 ml), mixed with 0·2 mol $l^{-1}$ triethylammonium hydrogen carbonate pH 7·5 (200 ml), made alkaline with triethylamine and stirred at room temperature for 2 h, the pH being rendered alkaline with triethylamine. The mixture was taken down, the residue codistilled with methanol (3 × 100 ml) and further worked up as described under *A*). Chromatography on Dowex 1X2 and crystallization from water afforded 10·6 g (78%) of *II*, pure according to HPLC (99%) and identical with the product prepared under *A*).

*C*) Iodotrimethylsilane (4 ml; 28 mmol) was added to a solution of *Va* (1·4 g; 4·9 mmol) in dimethylformamide (50 ml) at −50°C. After standing at room temperature for 16 h, the solution was mixed with 2 mol $l^{-1}$ triethylammonium hydrogen carbonate (50 ml), warmed to 80°C until it decolorized, cooled and taken down *in vacuo*. The residue was codistilled with methanol (3 × 50 ml), deionized on a column of Dowex 50X8 ($H^+$ form, 100 ml) and purified on Dowex 1X2 by elution with gradient of acetic acid (0—0·75 mol $l^{-1}$, 2 × 1 l as described under *A*); yield 1·25 g (93%) of *II*, identical with the product prepared by procedure *A*).

9-(2-Morpholinophosphonylmethoxyethyl)adenine (*XI*)

A solution of N,N'-dicyclohexylcarbodiimide (4 g; 19 mmol) was added dropwise during 3·5 h to a stirred refluxing solution of *II* (1·2 g; 4·4 mmol) and morpholine (1·7 ml; 19 mmol) in aqueous tert-butyl alcohol (1 : 1; 132 ml). The mixture was then refluxed for 8 h and morpholine (0·85 ml; 9·7 mmol) and N,N'-dicyclohexylcarbodiimide (2 g; 9·7 mmol) in tert-butyl alcohol (50 ml) were added. After 3 h of refluxing, the solvent was evaporated, the residue in water (100 ml) was filtered through Celite, the filtrate was extracted with ether (3 × 50 ml) and the aqueous phase was taken down. The residue was codistilled with ethanol (3 × 50 ml), precipitated with ether from ethanol, washed with ether and dried over phosphorus pentoxide, affording 2·7 g (96%) of N,N'-dicyclohexylcarboxamidinium salt of *XI*, electrophoretically uniform ($E_{Up} = 0{\cdot}47$), which was used in further reactions.

9-(2-Phosphorylphosphonylmethoxyethyl)adenine (*XII*)

A solution of bis(tri-n-butylammonium)phosphate in dimethyl sulfoxide (0·5 mol $l^{-1}$; 12·8 ml) was added to a mixture of *XI* (1·35 g; 2·1 mmol) and tri-n-butylamine (1·5 ml; 6·3 mmol). After shaking at room temperature for 3 days, the mixture was shaken with ether (60 ml) and the ethereal phase was decanted. The residue was extracted once more with ether (60 ml) and traces of ether were removed by evacuation. Water (50 ml) was added and the mixture was applied on a column of Dowex 1X2 ($Cl^-$ form; 100 ml), elution with linear gradient of 0—0·4 mol $l^{-1}$ lithium chloride in 0·01 mol $l^{-1}$ hydrochloric acid (2 × 1 000 ml). The product fractions were combined, neutralized with 0·5 mol $l^{-1}$ lithium hydroxide to pH 6·7—6·8 and concentrated *in vacuo* with addition of ethanol almost to dryness. The thick suspension was mixed with the same volume of acetone and centrifuged. The sediment was washed with acetone–ethanol (1 : 1; 4 × 30 ml), ether (2 × 30 ml) and dried *in vacuo*; yield 590 mg (76%) of the lithium salt of *XII*, homogeneous according to HPLC in S4.

9-(2-Diphosphorylphosphonylmethoxyethyl)adenine (*XIII*)

The title compound (as lithium salt) was prepared in a 76% yield from *XI* (2·1 mmol) and 0·5 mol . $l^{-1}$ tributylammonium diphosphate following the procedure described for the compound *XII*. The product was homogeneous according to HPLC (S4).

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