SYNTHESIS OF POTENTIAL PRODRUGS AND METABOLITES OF 9-(*S*)-(3-HYDROXY-2-PHOSPHONYLMETHOXYPROPYL)ADENINE*

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Received April 1st, 1987

Reaction of 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (*I*) with N,N'-dicyclohexylcarbodiimide afforded the cyclic phosphonate *II*. The same compound was also obtained by controlled alkaline hydrolysis of 3'-O-chloromethanephosphonyl-9-(*S*)-(2,3-dihydroxypropyl)-adenine (*III*). Methanolysis of compound *II* or *III* by sodium methoxide gave methyl ester *VII*. Isomeric cyclic ester *V* and methyl ester *VIII* were obtained from 3'-O-phosphonylmethyl ether *IV* or 2'-O-chloromethanephosphonyl ester *VI* by the same reactions. Compound *I* was transformed into morpholide *IX* which afforded the P-diphosphoryl derivative *X* by treatment with inorganic diphosphate. The P-phosphoryl derivative *XII* was obtained from compound *I* by successive protection with dimethoxytrityl chloride, activation with diphenyl chlorophosphate, treatment with inorganic phosphate and acid deprotection.

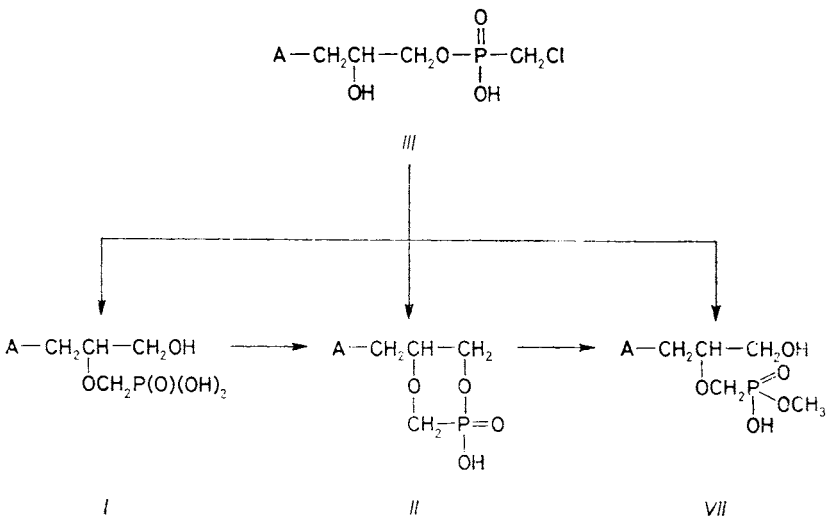
In our first communication of this series[1] we have described the preparation of 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (*I*), the first acyclic nucleotide analogue of significant biological activity. This compound (HPMPA) exhibits a pronounced antiviral activity againt DNA viruses, particularly against the herpes virus group (including acyclovir resistant TK^- mutants), adenoviruses and retroviruses[2], and is the subject of continued biological investigation. The penetration of compound *I* into cells, indispensable for its biological activity, is surprising in itself. Although, according to quantitative evaluation[3], the intracellular level of *I* amounts to a small fract on of its extracellular concentration, it is sufficient to induce an exceptionally large biological effect (ID_{50} about $10^{-7}-10^{-8}$ mmol l^{-1}). One of the ways how to increase the transport of compounds of the type *I* into cells is to develop prodrugs which contain a reduced charge and can generate the active compound *I* in the cell under physiological conditions. Our present paper describes synthesis leading to two types of such potential prodrugs of *I*: cyclic and acyclic esters.

Compound *I* is a derivative of 9-(*S*)-(2,3-dihydroxypropyl)adenine whose secondary hydroxyl group is replaced by a phosphonylmethyl ether functionality. Its primary hydroxyl in position 3' is free and can be thus intramolecularly esterified by the

* Part II in the series Acyclic Nucleotide Analogues; Part I: Collect. Czech. Chem. Commun. *52*, 2775 (1987).

neighbouring substituted phosphonic acid functionality to give the derivative *II*. This compound contains a six-membered dioxaphosphorinane ring which is chemically sufficiently stable[4] and, contrary to *I*, dissociates to the monoanion at physiological pH. However, since it is relatively easily opened in an alkaline medium to give specifically the starting phosphonylmethyl ether *I* (see also analogous compounds of the ribo-series[4]), it might afford the active compound on reaction with nucleophiles inside the cell.

The simplest route to the cyclic phosphonate *II* consists in cyclization of compound *I* by treatment with activating reagents, *e.g.* N,N'-dicyclohexylcarbodiimide. This reaction can be performed with the free acid *I* as well as its ammonium, pyridinium or N,N'-dicyclohexylcarboxamidinium salts. It proceeds at elevated temperature *e.g.* in pyridine, dimethylformamide or its mixture with tert-butyl alcohol. Unlike the analogous preparation of six-membered 3',5'-cyclic phosphates in the ribonucleotide series, the reaction of *I* takes place even in the presence of water. The product *II* is easily isolated by chromatography on a medium basic anion exchanger (*e.g.* Sephadex A-25) and stored *e.g.* in the form of its well soluble sodium salt (Scheme 1).



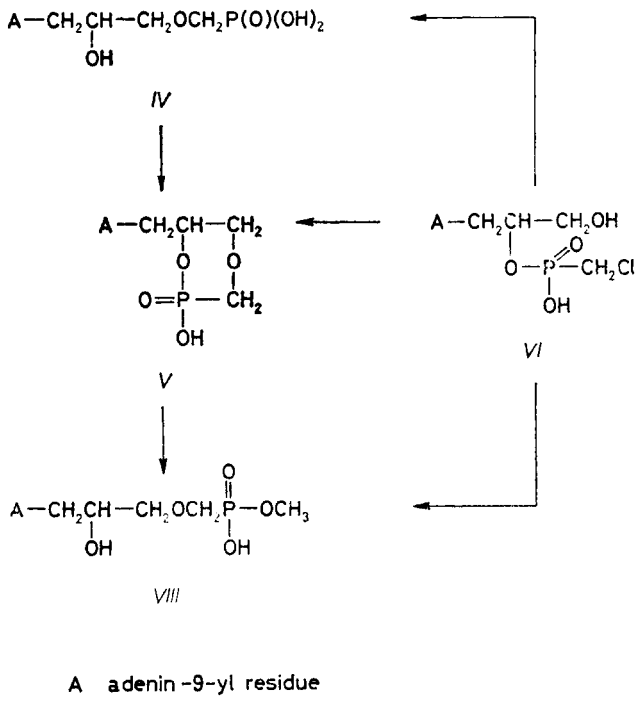
A adenin-9-yl residue

SCHEME 1

Compound *II* also arises as an intermediate in the preparation of phosphonylmethyl ether *I* by intramolecular cyclization of 3'-O-chloromethanephosphonyl ester

of 9-(*S*)-(2,3-dihydroxypropyl)adenine[1] (*III*). Under suitably controlled conditions the reaction of *III* with aqueous alkali metal hydroxide[1] can be performed so as to suppress the subsequent alkaline hydrolysis of *II* to the acyclic form *I*. However, it is advantageous to cyclize the compound *III* under anhydrous conditions, *e.g.* in dimethyl sulfoxide with potassium tert-butoxide as the cyclization reagent. It is essential to neutralize the reaction mixture as rapidly as possible, *e.g.* with Dowex 50 in pyridinium form. In both cases the product *II* was isolated by chromatography on anion exchanger in a neutral volatile buffer (Scheme 1).

The isomer of HPMPA, 3'-O-phosphonylmethyl ether *IV*, is biologically inactive[2]. Since its inactivity might be due to the inability of the compound *IV* to penetrate the cellular membrane, we converted this compound[1] into cyclic phosphonate *V* by the above-mentioned dicyclohexylcarbodiimide cyclization. Compounds *II* and *V* exhibit very similar chemical properties, particularly the capability of affording the respective phosphonylmethyl derivatives *I* and *IV* by base-catalyzed hydrolysis. Moreover, for comparison we also obtained a mixture of racemic compounds *II* and *V* by controlled base-catalyzed cyclization of an easily accessible[1] mixture of isomeric racemates *III* and *VI* (Scheme 2).



A adenin-9-yl residue

SCHEME 2

As models for acyclic esters of the phosphonylmethyl derivative *I* and its isomer *IV* we chose the methyl esters *VII* and *VIII*. A mixture of both racemic isomers was obtained by reaction of racemic *III* and *VI* with sodium methoxide in methanol. Even in this case one had to remove rapidly the excess of base during the work-up to suppress product hydrolysis. The methyl esters *VII* and *VIII* were isolated by chromatography on an anion exchanger or on octadecylsilica gel. The reaction intermediates, cyclic phosphonates *II* and *V*, were specifically opened by the methoxide anion. This mechanism has been directly proven (similarly as in the ribo-series[4]) by reaction of the cyclic phosphonate *II* with sodium methoxide which afforded the expected methyl ester *VII* as the only product (Scheme 1).

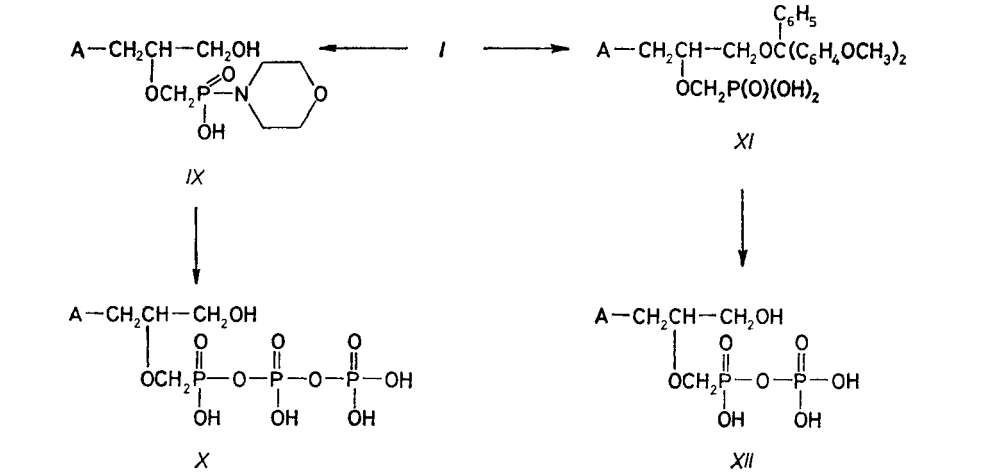
The cyclic phosphonates *II* and *V* as well as the methyl esters *VII* and *VIII*, prepared by the mentioned methods, were homogeneous according to HPLC and their electrophoretic mobilities corresponded to the presence of one dissociated group at neutral pH. Their UV spectra corresponded to N^6-substituted adenine derivatives. An additional structural proof was given also by their alkaline hydrolysis to the corresponding isomeric phosphonylmethyl ethers *I* or *IV* as sole reaction products.

Within the framework of this study we also investigated the synthesis of analogues of di- and triphosphates derived from the isomeric phosphonylmethyl ethers *I* and *IV*. Since compound *I* apparently affects the *de novo* synthesis of viral (and also cellular) DNA[2], it is highly probable that the compounds of the mentioned type are the actual active antimetabolites. Such derivatives had to be unequivocally synthesized in order to prove their formation from *I* under *in vitro* as well as *in vivo* conditions, and to investigate their stability and possible further *in vitro* transformations. The investigation of their stability is particularly important, because these compounds are activated forms of phosphonylmethyl derivatives which might easily undergo various intramolecular transformations.

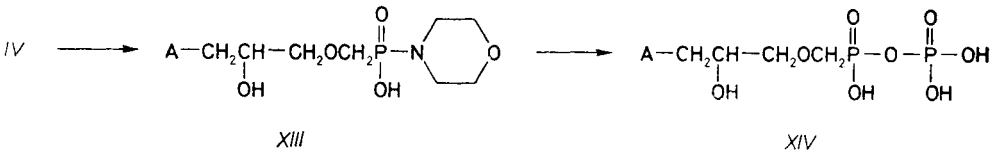
The P-diphosphoryl derivative *X* was synthesized, analogously to ribonucleoside 5'-triphosphates[5], *via* the active intermediate *IX*. The preparation of *IX* is difficult because the reaction of *I* with morpholine in the presence of an activating reagent gives rise to cyclic phosphonate *II* as side-product which does not react with either inorganic mono- or diphosphate. Under high-dilution conditions, compound *II* becomes the principal product; on the other hand, with a substantially increased concentration of reactants (ten times higher than usual[5]), compound *I* was quantitatively converted into a mixture of *II* and *IX* with the latter markedly predominating. Reaction of *IX* with an inorganic diphosphate in dimethyl sulfoxide[5] afforded the expected triphosphate analogue *X* which was isolated by chromatography on an anion exchanger in acidic medium.

The perturbing influence of the neighbouring primary hydroxyl in reactions requiring activation of the phosphorus atom can be suppressed by its protection. Thus, compound *I* was tritylated with chlorodi(*p*-methoxyphenyl)phenylmethane in pyridine to give the 3'-protected derivative *XI* which was activated by *in situ*

formation of mixed anhydride with diphenyl phosphate[6] and subsequently reacted with an inorganic phosphate. Desalting and acid-catalyzed deprotection afforded the diphosphate analogue, 2'-O-phosphorylphosphonylmethyl derivative *XII* (Scheme 3).



A adenin-9-yl residue



A adenin-9-yl residue

SCHEME 3

Activation of the 3'-O-phosphonylmethyl group in compound *IV via* the morpholide *XIII* is easier than the reaction of the isomeric compound *I*. This behaviour is evidently caused by a lower nucleophilicity of the secondary hydroxyl in compound *IV*; the morpholide *XIII* reacted with inorganic phosphate in dimethyl sulfoxide to give the diphosphate analogue *XIV* which was isolated by chromatography on an anion exchanger in acidic medium (Scheme 3). The above potential metabolites of the biologically active compound *I* were used in a study of its metabolism in cells. It appeared that the phosphorylation can be effected *in vitro* by treatment with the cell-free extract of L-1210 mice leukemia cells (containing nucleotide kinases) and

that compounds *X* and *XII* arise in significant amount by metabolic transformations of compound *I* *in vivo*[3].

The biological effect of methyl esters *VII* and *VIII* is only marginal; however, the described methods of their synthesis are also applicable to the preparation of additional ester types some of which could have advantageous pharmacological parameters as prodrugs of compound *I*. The cyclic phosphonate *II*, derived from *I*, exhibits a pronounced antiviral effect, in some cases at least comparable with that of *I*. A detailed description of these effects is the subject of another communication. So far, there is no evidence whether this activity is an intrinsic property of the compound *II* or whether it results from its pharmacologically improved parameters and subsequent hydrolysis to *I* as the actual active principle.

EXPERIMENTAL

Unless stated otherwise, the solvents were evaporated at 40°C/2 kPa and compounds were dried at 13 Pa over phosphorus pentoxide. Paper chromatography was performed in the system S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2) on a Whatman No. 1 paper. Paper electrophoresis was carried out on a Whatman No. 3MM paper, 20 V/cm in S2, 0·1 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5, and S3, 0·05 mol l^{-1} sodium dihydrogen citrate. The E_{Up} and E_{ATP} values are electrophoretical mobilities related to uridine 3′-phosphate and adenosine 5′-triphosphate, respectively. HPLC analyses were carried out on 4 × 200 mm columns of Separon SGXC18 (7 μ) in 0·05 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5, containing 5% (S4) and 10% (S5) of methanol; flow rate 1 ml min^{-1}, detection at 254 nm. NMR spectra were measured on a Varian XL-200 instrument with tetramethylsilane as internal standard; chemical shifts are given in ppm (δ-scale), coupling constants in Hz.

The phosphonyl derivatives *I* and *IV* or their mixtures, as well as chloromethanephosphonyl esters *III* and *VIII*, were prepared according to the described[1] procedure.

Cyclic Esters *II* and *V*

A) Dimethylformamide (50 ml) and pyridine (100 ml) were added to a solution of (*S*)-*IV* (303 mg; 1 mmol; free acid) and morpholino-N,N′-dicyclohexylguanidine (293 mg; 1 mmol) in water (10 ml). The obtained solution was added dropwise during 2 h to a refluxing solution of N,N′-dicyclohexylcarbodiimide (4·12 g; 20 mmol) in pyridine (250 ml). The mixture was then refluxed for 8 h, cooled and the solvents were evaporated *in vacuo*. The residue was taken up in water, filtered through Celite and the filtrate was concentrated *in vacuo*. The compound was purified by chromatography on a column of DEAE-Sephadex (HCO_3^- form; 50 ml), elution with a linear gradient of aqueous triethylammonium hydrogen carbonate, pH 7·5 (0—0·15 mol l^{-1}; 2 × 500 ml). After evaporation of the product fractions and codistillation of the residue with ethanol (4 × 50 ml), the product was converted into the sodium salt using Dowex 50 (Na^+ form); yield 290 mg (91%) of sodium salt of (*S*)-*V*; R_F 0·45 (S1), $E_{Up} = 0{\cdot}48$ (S2), $k = 3{\cdot}61$ (S4).

The racemic and isomeric mixture of (*RS*)-*II* and (*RS*)-*V* was prepared similarly, starting from (*RS*)-*I* and (*RS*)-*IV* (ref.[1]; 327 mg; 1·1 mmol). The morpholino-N,N′-dicyclohexylguanidinium salt was prepared in pyridine (100 ml), however, a homogeneous solution was obtained only after 4 days of stirring. Yield 300 mg (88%) of sodium salt. The physical data are given with the pure isomers.

B) A solution of N,N'-dicyclohexylcarbodiimide (1·7 g; 8 mmol) in tert-butyl alcohol (24 ml) was added dropwise during 1 h to a refluxing solution of (*S*)-*I* (500 mg; 1·6 mmol; free acid) and morpholino-N,N'-dicyclohexylguanidine (470 mg; 1·6 mmol) in aqueous tert-butyl alcohol (48 ml). After refluxing for 6 h, the mixture was worked up as described under *A*); yield 440 mg (89%) of sodium salt of (*S*)-*II*; R_F 0·45 (S1), $E_{Up} = 0·48$ (S2), $k = 3·14$ (S4). The method was also used for preparation of (*S*)-*V* from (*S*)-*IV* (2 mmol) in the same yield.

C) Solid potassium tert-butoxide (975 mg) was added to a solution of (*RS*)-*III* (650 mg; 2 mmol; free acid) in dry dimethyl sulfoxide (35 ml) and the suspension was stirred at room temperature for 16 h. The obtained clear solution was added under stirring to an ice-cooled suspension of Dowex 50 (pyridinium form; 20 ml) in 50% aqueous pyridine (30 ml). After stirring for 5 min, the suspension was filtered, the resin washed with aqueous pyridine and the combined filtrates were taken down *in vacuo*. Further work-up was the same as described under *A*), yielding 600 mg (96·5%) of sodium salt of (*RS*)-*II*. An analogous reaction in dimethylformamide afforded the product in 80% yield. ^{1}H NMR spectrum in ^2H_2O: *II*: 3·97 dd, 1 H (P—CH, J(P—CH) = 8·4; $J_g = -14·0$); 3·71 dd, 1 H (P—CH, J(P—CH) = 2·4, $J_g = -14·0$); 8·09 s, 1 H (H-2); 8·10 s, 1 H (H-8); 4·00—4·40 m, 5 H. *V*: 4·36 m, 2 H (H-1'); 4·75 m, 1 H (H-2'); 4·00 br dt, 1 H (H-3', J(3', 2') ~ 2·0; J(3', P) = 1·0; $J_g = -12·0$); 3·32 dd, 1 H (H-3'', J(3'', 2') ~ 10·0; $J_g = -12·2$); 3·92 dd, 1 H (P—CH', J(P—CH') = 8·8; $J_g = -14·0$); 3·59 dd, 1 H (P—CH'', J(P—CH'') = 2·0; $J_g = -14·0$); 8·09 s, 1 H (H-2); 8·13 s, 1 H (H-8). (50°C): 4·39 m, 2 H (H-1'); 4·78 m, 1 H (H-2', $\sum J = 25·7$; J(P—CH) = 4·6; J(2', NCHa) = 6·0; J(2', NCHb) = 2·8); 4·00 dq, 1 H (H-3', J(3', 2') = 2·5, J(3', P) = 1·4, $J_g = -12·5$); 3·34 dd, 1 H (H-3'', J(3'', 2') = 9·8, $J_g = -12·5$); 3·92 dd, 1 H (P—CH_2, J(P—CH') = 8·6, $J_g = -14·0$); 3·61 dd, 1 H (P—CH_2, J(P—CH'') = 2·2; $J_g = -14·0$); 8·17 s, 2 H (H-2, H-8). ^{13}C NMR spectrum (APT): ^1J(P—CH) = 144·3 (P—CH_2); ^2J(P—CH) = 6·7 (O—CH); ^3J(P—CH) = 6·1 (N—CH_2); ^3J(P—C) = 3·5 (O—CH_2).

Methyl Esters *VII* and *VIII*

A) A solution of racemic isomeric *III* and *VI* (200 mg; 0·62 mmol) in 1 mol l^{-1} sodium methoxide (20 ml) was refluxed for 6 h and then added to a stirred suspension of Dowex 50 (pyridinium form; 50 ml) in 20% aqueous pyridine (50 ml). After 10 min the suspension was filtered, the filtrate taken down *in vacuo* and the residue codistilled with 5% triethylamine in methanol (2 × 50 ml). The product was purified by chromatography on Dowex 1X2 (acetate form; 100 ml); elution with a linear gradient of acetic acid in water (0—0·5 mol l^{-1}; 2 × 750 ml). Yield 180 mg (92%) of free acids (*RS*)-*VII* and (*RS*)-*VIII*. R_F 0·45 (S1), $E_{Up} = 0·49$ (S2), $k = 2·01$ (S4) (*VII*); $k = 2·35$ (S4) (*VIII*).

B) The reaction was carried out with (*RS*)-*II* (0·6 mmol) as described under *A*); yield 80% of (*RS*)-*VII*, pure according to HPLC.

9-(*S*)-(2-Hydroxy-3-morpholinophosphonylmethoxypropyl)adenine ((*S*)-*XIII*)

A solution of N,N'-dicyclohexylcarbodiimide (2·1 g) in tert-butyl alcohol (15 ml) was added dropwise at 75°C during 1 h to a stirred solution of (*S*)-*IV* (303 mg; 1 mmol; free acid) and morpholine (1 ml) in aqueous tert-butyl alcohol (1 : 1; 30 ml). After stirring and heating to 75°C for 8 h, the mixture was cooled and taken down *in vacuo*. The residue was dissolved in water (50 ml) and N,N'-dicyclohexylurea was filtered through Celite. The filtrate was extracted with ether (3 × 20 ml) and the aqueous phase was taken down. The residue was dried by codistillation with ethanol (3 × 20 ml) and the crude product was precipitated from methanol with ether

and purified on a column of DEAE-Sephadex A25 (HCO_3^- form; 50 ml) by elution with a linear gradient of aqueous triethylammonium hydrogen carbonate (0—0·15 mol l^{-1}; 2 × 500 ml). The product fractions were combined, taken down *in vacuo*, the residue was codistilled with ethanol (4 × 20 ml) and dried over phosphorus pentoxide *in vacuo*; yield 370 mg (78%) of triethylammonium salt of (*S*)-*XIII*. $E_{Up} = 0·48$ (S2), $k = 3·67$ (S5).

9-(*S*)-(3-Hydroxy-2-morpholinophosphonylmethoxypropyl)adenine ((*S*)-*IX*)

A) Compound (*S*)-*I* (1 mmol) was reacted in analogous way as described for (*S*)-*XIII* and the reaction was monitored by HPLC in the system S5. After 48 h the composition of the reaction mixture remained constant: (*S*)-*IX* 40%, (*S*)-*II* 22%, and starting (*S*)-*I* 38%. This mixture was further utilized without additional purification.

B) A solution of N,N′-dicyclohexylcarbodiimide (824 mg) in tert-butyl alcohol (2·1 ml) was added dropwise at 80°C in the course of 3 h to a stirred solution of (*S*)-*I* (303 mg; 1 mmol; free acid) in a mixture of water (0·5 ml),tert-butyl alcohol (0·5 ml) and morpholine (0·34 ml) and refluxed for 5 h. HPLC analysis: (*S*)-*IX* 45%, (*S*)-*II* 47%, starting (*S*)-*I* 3%. The reaction mixture was dissolved in water (50 ml), filtered through Celite and the filtrate extracted with ether (3 × 10 ml). The aqueous phase was taken down *in vacuo* and the residue was coevaporated with pyridine (3 × 10 ml) and finally with dimethylformamide (3 × 10 ml). The thus-obtained product was used directly in further reactions; $E_{Up} = 0·48$ (S2), $k = 4·30$ (S5).

9-(*S*)-(3-Dimethoxytrityloxy-2-phosphonylmethoxypropyl)adenine ((*S*)-*XI*)

A suspension of (*S*)-*I* (600 mg; 2 mmol; free acid) in pyridine (20 ml) was sonicated for 45 min. After addition of another portion of pyridine (100 ml), the sonication was continued for 30 min. Dimethoxytrityl chloride (3·42 g; 10 mmol) was added and the mixture was stirred for 48 h at room temperature. The homogeneous solution was left to stand with methanol (1 ml) for 2 h and the solvent was evaporated *in vacuo*. The residue was taken up in chloroform (100 ml), washed with 5% aqueous sodium hydrogen carbonate (2 × 20 ml) and water (2 × 20 ml), pyridine was added to the chloroform layer and the organic phase was concentrated *in vacuo* to a small volume. This concentrate was added dropwise into ether (300 ml) under vigorous stirring, the precipitate was collected, washed with ether and dried *in vacuo* to afford 1·3 g (95%) of sodium salt of (*S*)-*XI*. ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide); 2·99 m, 2 H (3′-CH_2); 3·71 s, 6 H (OCH_3); 4·15—4·90 m, 6 H (1′-CH_2, 2′-CH); 6·70—7·0 m and 7·45 m, 13 H (arom. protons); 7·87 s and 8·14 s, 2 H (H-2, H-8).

9-(*S*)-(3-Hydroxy-2-phosphorylphosphonylmethoxypropyl)adenine ((*S*)-*XII*)

Tri-n-butylamine (0·45 ml), followed by diphenyl chlorophosphate (0·3 ml), was added to a solution of (*S*)-*XI* (750 mg; 0·89 mmol) in dioxane (7 ml). After stirring for 150 min at room temperature, the homogeneous solution was concentrated *in vacuo* at 30°C. Ether (20 ml) was added to the residue, the mixture was briefly sonicated and the suspension was filtered under exclusion of moisture. The solid residue was dried for 20 min *in vacuo* and shaken with a solution of bis-(tri-n-butylammonium)phosphate in pyridine (1 mol l^{-1}; 2 ml) for 12 h at room temperature. The solution was again taken down at 30°C *in vacuo* and the residue was codistilled with dioxane to remove pyridine. The residue was dissolved in water, applied on a column of octadecylsilica gel (200 ml) and eluted first with water to the drop of UV absorption and then with 50% aqueous methanol. The product-containing fractions of the latter eluate were taken down, the residue was dissolved in 20% aqueous acetonitrile (50 ml), mixed with conc. hydrochloric acid (1 ml) and

after 10 min at room temperature, neutralized with aqueous lithium hydroxide and evaporated *in vacuo*. The residue was suspended in ethanol–acetone (1 : 1; 50 ml) and centrifuged. Further work-up procedure (except the absorption on charcoal) was the same as described for (*S*)-*X* and gave 30 mg (8·5%) of trilithium salt of (*S*)-*XII*. E_{ATP} = 0·83 (S3).

9-(*S*)-(3-Hydroxy-2-diphosphorylphosphonylmethoxypropyl)adenine ((*S*)-*X*)

A solution of bis(tri-n-butylammonium)diphosphate in dimethyl sulfoxide (0·5 mol l^{-1}; 6·4 ml) was added to morpholide (*S*)-*IX* (crude product of 45% purity; 0·45 mmol). After shaking at room temperature for 3 days, the viscous solution was mixed with ether (50 ml) and the suspension was briefly sonicated. The ethereal portion was removed, the solid dried *in vacuo* at 30°C, dissolved in water (15 ml) and purified by chromatography on Dowex 1X2 (Cl^- form; 140 ml); elution with a linear gradient of lithium chloride in 0·01 mol l^{-1} aqueous hydrochloric acid (0—0·4 mol l^{-1}; 2 × 1 000 ml). The product fractions were combined and neutralized with aqueous lithium hydroxide (0·5 mol l^{-1}) to pH 6·7. The solution was concentrated *in vacuo* at 30°C to a small volume and after codistillation with ethanol (5 × 50 ml) the suspension was diluted with the same volume of acetone and centrifuged. The sediment was washed with acetone–ethanol (1 : 1; 4 × 50 ml) and ether (2 × 50 ml) and dried *in vacuo* over phosphorus pentoxide; yield 370 mg of material which (according to spectral analysis) contained 30% of the product (yield 51%; 0·23 mmol). This material was dissolved in water (20 ml), charcoal (Darko-Fluca; 1 g) was added and the suspension was adjusted to pH 3 with hydrochloric acid. The suspension was stirred for 20 min, filtered through Celite which was then washed with water (pH 3, 3 × 50 ml) and suspended in an ethanol–water–pyridine (2 : 2 : 1) mixture (50 ml). After stirring for 10 min at room temperature, the suspension was filtered and the filtrate was taken down *in vacuo*. The product was purified on Dowex 1X2 (Cl^- form) and further processing was carried out following the procedure given above. Yield 55 mg (24%) of trilithium salt of (*S*)-*X*; E_{ATP} = 1·00 (S3).

9-(*S*)-(2-Hydroxy-3-phosphorylphosphonylmethoxypropyl)adenine ((*S*)-*XIV*)

The reaction of (*S*)-*XIII* (0·5 mmol) with a solution of bis(tri-n-butylammonium)phosphate in dimethyl sulfoxide was performed as described for (*S*)-*X*. Yield of pure (*S*)-*XIV* (as lithium salt) was 65%. E_{ATP} = 0·83 (S3).

The authors are indebted to Dr M. Masojídková of this Institute for measurement and interpretation of the NMR *spectra.*

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Translated by M. Tichý.