SYNTHESIS OF ISOMERIC AND ENANTIOMERIC O-PHOSPHONYLMETHYL DERIVATIVES OF 9-(2,3-DIHYDROXYPROPYL)ADENINE*

Antonín HOLÝ and Ivan ROSENBERG

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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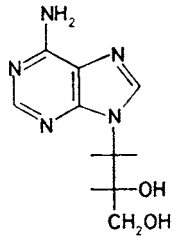
Reaction of 9-(*S*)-(2,3-dihydroxypropyl)adenine (*I*) with chloromethanephosphonyl chloride (*VII*) in pyridine or triethyl phosphate, or with chloromethyl(pyridinio)phosphonate (*IX*) in pyridine, afforded a mixture of 2′-(*IV*) and 3′-O-chloromethanephosphonate (*V*) which were separated on anion exchange resin or alkylsilica gel. Treatment of compounds *IV* and *V* with aqueous alkaline hydroxide, followed by deionization, gave 9-(*S*)-(2-hydroxy-3-phosphonylmethoxypropyl)adenine (*VI*) and 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (*III*) (HPMPA), respectively. The (*R*)- and (*RS*)-forms of *III* and *VI* were prepared analogously from the respective (*R*)-enantiomer and racemate of *I*. 9-(*S*)-(2,3-Dihydroxypropyl)-N^6-benzoyladenine (*XIV*) was converted into 3′-O-(dimethoxytrityl) derivative *XVII* and further into 2′,N^6--dibenzoyl derivative *XIX*. Reaction of compound *XVII* with *IX*, followed by acid hydrolysis and alkaline cyclization, afforded pure isomer *VI* whereas pure *III* was prepared from *XIX* by reaction with *VII* in triethyl phosphate and subsequent alkaline cyclization.

During the past several years we have systematically studied the so-called chiral acyclic nucleoside analogues, *i.e.* N-alkyl derivatives of heterocyclic bases in which the chiral hydroxyl-bearing alkyl substituent represents part of the nucleoside sugar moiety. Among them we have found several types of compounds exhibiting interesting biological activities. All active compounds are adenine derivatives which inhibit methylation reactions *via* S-adenosyl-L-homocysteinase (*SAHase*) inhibition[1]. These derivatives do not undergo metabolic transformations or phosphorylation *in vitro* or *in vivo*. One of the interesting compounds of this series is *e.g.* 9-(*S*)-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA) (*I*) which exhibits an antiviral activity[2], particularly against (−)-stranded RNA viruses, as well as the whole spectrum of other biological activities (for a review see ref.[1]). Phosphoric acid esters of acyclic adenosine analogues have no significant biological effects and any activity observed is due to the parent nucleoside analogue formed *in vivo* by dephosphorylation[3].

The *in vivo* dephosphorylation of nucleotide derivatives represents a key catabolic process, influencing the activity of those biologically effective antimetabolites –

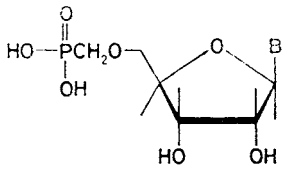
* Part I in the series Acyclic Nucleotide Analogues.

nucleoside analogues – whose mechanism of action depends on phosphorylation. Therefore, we set out to investigate isopolar and isosteric nucleotide analogues, resistant to dephosphorylation reactions. Very interesting in this respect are O-phosphonylmethyl ethers of nucleosides (*II*) (see ref.[4]): although these compounds inhibit many enzymes of nucleic acid metabolism, neither analogues of ribonucleoside 5′-, 2′- or 3′-phosphates[5,6] nor related compounds derived from arabinofuranosyl derivatives[7] have antiviral or cytostatic effects. Our interest was therefore attracted by a new, hitherto not studied group of nucleotide analogues which combine both the above-mentioned structural features: acyclic nucleotide analogues which, instead of phosphoric acid ester bond, contain a phosphonylmethyl group ether-bonded to hydroxyl functionalities of the alkyl side chain. In such compounds, *in vivo* stability of bonds to the heterocyclic base as well as to the phosphonic acid residue is *a priori* guaranteed. Recently we described[8] an interesting potent antiviral activity of one member of this series, 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine

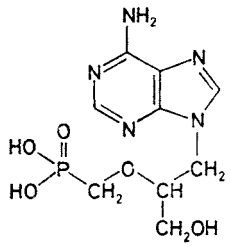


I

(*S*)-DHPA



II

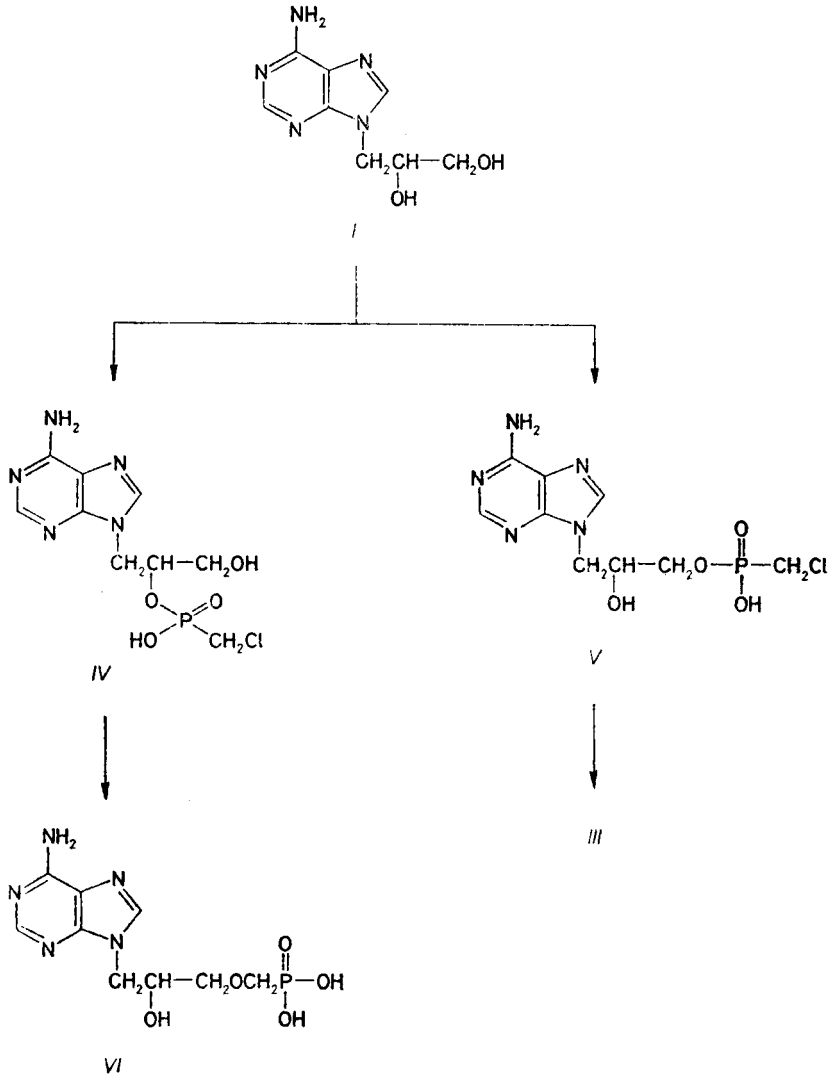


III

(HPMPA)

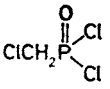
(*III*) (HPMPA). This compound, derived from the above-mentioned antiviral *I*, is effective specifically against DNA-viruses. Of the possible structural isomers of compound *I*, only the (*S*)-enantiomer of the 2′-isomer is biologically active. Our present paper describes alternative syntheses and characterization of compound *III* and its isomers.

All these derivatives were prepared starting from the racemate or both enantiomers of 9-(2,3-dihydroxypropyl)adenine (*I*), accessible by previously described routes. The phosphonylmethyl ether bond was introduced into the side chain of this compound mostly by intramolecular cyclization of its chloromethanephosphonyl esters *IV* and *V* in aqueous alkali (the method was originally described for the synthesis of 2′(3′)-O-phosphonylmethylribonucleosides[6]). The reaction requires the presence of neighbouring hydroxyl group of the original vicinal diol which is substituted by the phosphonylmethyl ether bond (Scheme 1).

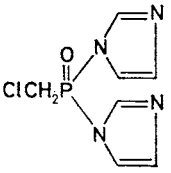


SCHEME 1

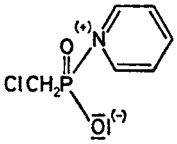
The key intermediates *IV* and *V* can be prepared from compound *I* or its protected derivatives by esterification with chloromethanephosphonic acid derivatives. Esterification with free chloromethanephosphonic acid in the presence of activating reagents of the carbodiimide type[6] was not satisfactory because it required an excess of the reagent and tedious purification of the formed monoester. The chloromethanephosphonic acid diimidazolide (*VIII*), prepared *in situ* by reaction of chloromethanephosphonyl dichloride (*VII*) with excess imidazole in dimethylformamide, was not very effective as esterification reagent: the reaction with (*RS*)-*I* gave only 5% of compounds *IV* and *V*. The only reagents of preparative utility were the dichloride *VII* or a product of its hydrolysis with equimolar amount of water in pyridine, probably *IX* (see ref.[9]). These two reagents react sufficiently rapidly at room



VII



VIII



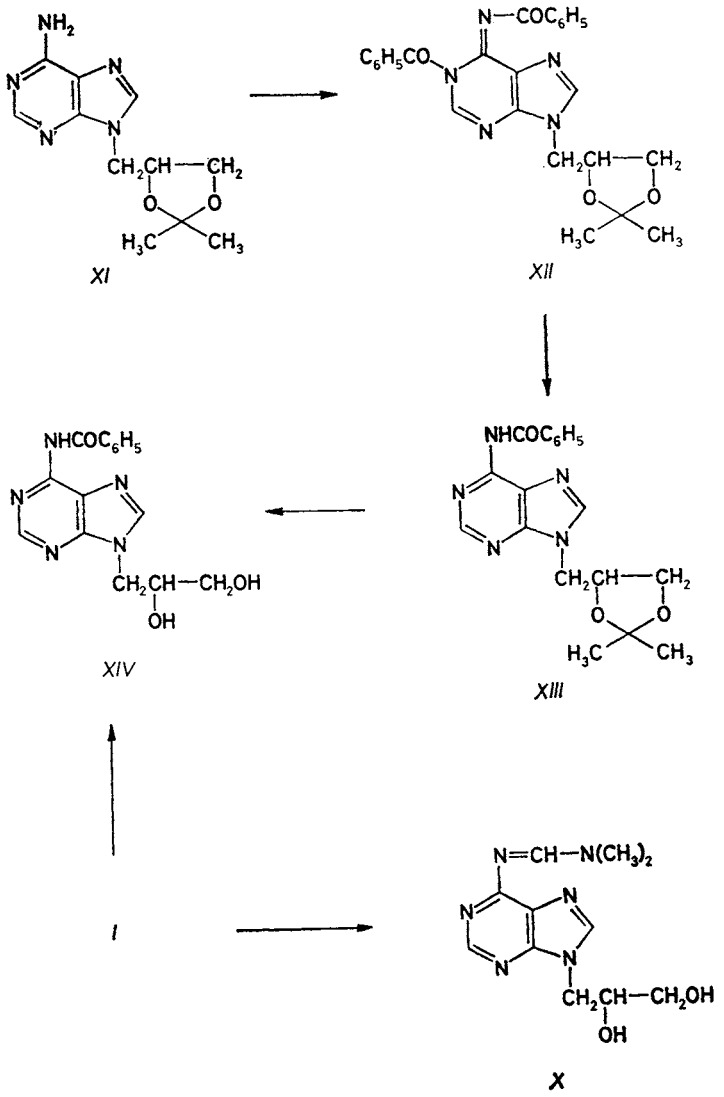
IX

temperature in pyridine which binds the arising hydrogen chloride. Under these conditions the reaction with *I* was sometimes accompanied by the N(1)- or N(6)--substitution; therefore, we prepared the esters *IV* or *V* from N^6-substituted derivatives of *I*. The N^6-dimethylaminomethylene derivative *X* was prepared by reaction of *I* with dimethylformamide dimethylacetal[10] followed by controlled hydrolysis (Scheme 2). Treatment of this compound with the reagent *VII* or *IX* and subsequent work-up with aqueous ammonia (to which chloromethanephosphonyl esters are resistant) afforded in relatively high yields (80%) a mixture of isomeric compounds *IV* and *V* with the 3'-isomer *V* predominating. Column chromatography of this mixture on Dowex 50 (H^+ form) in water separated both isomers from each other as well as from the unreacted *I* which remained bonded to the resin. This procedure was applied to the preparation of the isomeric derivatives *IV* and *V* from (*R*)-*I*, (*S*)-*I* and (*RS*)-*I*. Both free acids *IV* and *V* crystallized from water as stable hydrates and their isomeric purity was easily checked by HPLC in a neutral buffer in which they completely separated. This technique was also used for their isolation on a micro or semimicro scale (*e.g.* for the preparation of ^{14}C-labelled compounds[11]). Separation of *IV* from *V* on both ion exchange resin and alkyl silica gel is more efficient than that of the phosphonylmethyl ether *I* from *VI*.

The chloromethanephosphonyl esters *IV* and *V* were still more efficiently prepared by direct reaction of unprotected 9-(2,3-dihydroxypropyl)adenine (*I*) with the dichloride *VII* in triethyl phosphate. This procedure is analogous to the phosphoryla-

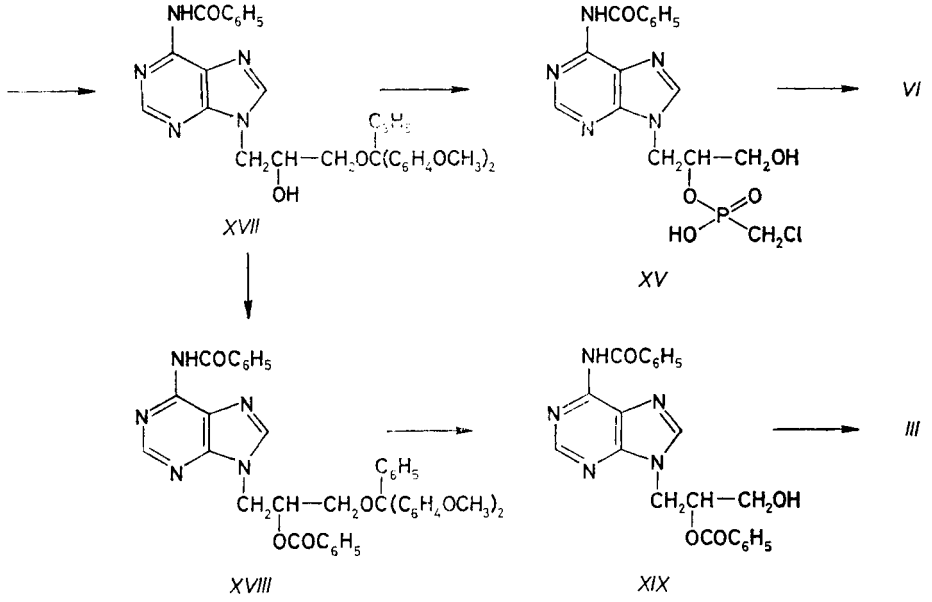
tion of nucleosides with phosphorus oxychloride[12] and affords intermediate ester--chlorides of chloromethanephosphonic acid which undergo autocatalyzed acid hydrolysis to give a mixture of *IV* and *V* in high yields. Also in this case the 3'-isomer *V* markedly predominates.

The reaction with compound *VII* in triethyl phosphate was also employed for the preparation of N^6-benzoyl derivatives of compounds *IV* and *V*. The starting com-



SCHEME 2

pound in this synthesis, 9-(2,3-dihydroxypropyl)-N^6-benzoyladenine ((*R*)-, (*S*)-, and (*RS*)-*XIV*), was prepared by two independent routes (Scheme 2). The 2',3'-O-isopropylidene derivative *XI* (which is an intermediate in the synthesis of *I*, *cf.* refs[13,14]) was benzoylated in pyridine to give N-dibenzoyl derivative *XII*. Controlled ammonolysis of *XII* afforded N^6-monobenzoate *XIII* whose acid hydrolysis led to N^6-benzoyl derivative *XIV*. A simpler approach to this compound consists in selective N-benzoylation of *I* by its successive reaction with chlorotrimethylsilane and benzoyl chloride in pyridine according to the method described for N-benzoylation of nucleosides[15]. An excess of both the reagents employed and the resulting large amount of salts in the reaction mixture, complicating the isolation of *XIV*, represent a drawback of this method. As expected, compound *XIV* reacted with chloromethanephosphonyl dichloride *VII* to give a mixture of chloromethanephosphonates *XV* and *XVI*. However, in an acidic aqueous solution these derivatives lost the N^6-benzoyl group thus yielding the isomeric compounds *IV* and *V*.

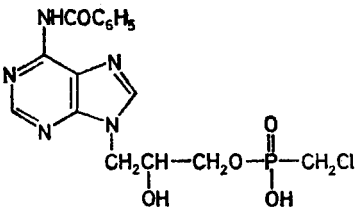


SCHEME 3

The structural proof of isomers *IV* and *V* or *XV* and *XVI* is based on their elemental composition, ^{1}H NMR spectra and electrophoretical behaviour (one dissociable group in a neutral solution). The structure is further supported by their cyclization in aqueous alkali metal hydroxides affording the above isomerically pure phosphonylmethyl ethers *III* and *VI*. The reaction proceeds quantitatively by mere heating of compounds *IV* and *V* or *XV* and *XVI*, or their mixtures, with 1–2 mol l^{-1} sodium

or lithium hydroxide to 70–80°C for 1–3 h. No side products were formed in this reaction (except sodium chloride and benzoic acid salts in the case of *XV* and *XVI*) so that the end products (*III*, *VI*) could be isolated simply by deionization, *e.g.* on Dowex 50 (in H^+ form). Small amounts of coloured contaminants can be removed by chromatography on an anion exchanger (such as Dowex 1 or Sephadex A-25). The products can be isolated either as sparingly soluble free acids, or as their well-soluble sodium salts. This method was applied to the preparation of the isomerically pure 2'-phosphonylmethyl (*III*) and 3'-phosphonylmethyl (*VI*) ethers in the racemic as well as in both enantiomeric series. It should be noted that optical purity of the (*R*)- and (*S*)-enantiomers of compounds *III* and *VI* cannot be directly proven because their ether bond is not easy to degrade. However, since the reaction mechanism excludes even a partial racemization, we can safely assume that the original absolute configuration is retained during the transformation of compound *I* into the reaction products *III* or *VI*.

The structure of isomers *III* and *VI* has been definitely confirmed by the comparison with products obtained from independent syntheses starting from isomerically homogeneous O-chloromethanephosphonates. Such intermediates were prepared from the derivatives of *XIV*, protected specifically on the primary or secondary hydroxyl group in the side chain. Thus, reaction of the (*S*)-enantiomer of N^6-benzoyl derivative *XIV* with bis(*p*-methoxyphenyl)phenylmethyl chloride afforded the 3'-O-dimethoxytrityl derivative *XVII*. The reaction with the dichloride *VII* in triethyl phosphate could not be directly applied in this case because the arising hydrogen chloride would have cleaved the trityl protecting group. Therefore, the esterification was carried out with compound *IX* in pyridine and the trityl protecting group was removed in a slightly acidic medium to yield the N^6-benzoyl derivative *XV*. This compound was converted with aqueous sodium hydroxide into the isomerically pure 3'-O-phosphonylmethyl ether *VI* (Scheme 3).



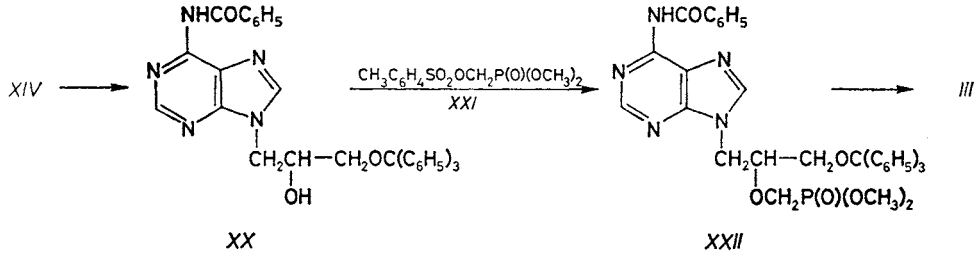
XVI

The same starting compound *XVII* was reacted with benzoyl cyanide[16] to yield 2'-O-benzoyl derivative *XVIII*. Mild hydrolysis of *XVIII* in trifluoroacetic acid led to N^6,2'-O-dibenzoyl derivative *XIX* whose structure was confirmed by ^1H NMR spectrum. Reaction of *XIX* with the dichloride *VII* in triethyl phosphate, followed by base-catalyzed intramolecular cyclization, afforded directly the pure 2'-O-phos-

phonylmethyl ether *III* (HPMPA) (Scheme 3). The isomers *III* and *VI*, prepared by these unequivocal synthetic procedures, were used not only for identification of the compounds in HPLC but also for an additional indirect verification of HPLC parameters of O-chloromethanephosphonyl derivatives *IV* and *V* obtained directly from the unprotected compound *I* or the N^6-benzoyl derivatives *XV* and *XVI*.

The preparation of compound *III* from the dibenzoyl derivative *XIX* is suitable for preparation of larger amounts of the biologically active geometric isomer *III* of the absolute configuration (*S*) from 9-(*S*)-(2,3-dihydroxypropyl)adenine (*I*) in sufficiently high yields, without any time-consuming separation of the isomers *IV* and *V* on ion-exchanger columns.

In the last method, also leading to the pure isomer *III*, the phosphonylmethyl ether group is attached directly to the free 2'-hydroxyl group in a suitably protected derivative of *I*. For this reason, N^6-benzoyl derivative *XIV* was first transformed into 9-(2-hydroxy-3-trityloxypropyl)-N^6-benzoyladenine (*XX*) by treatment with trityl chloride. In the presence of sodium hydride, this compound reacted with dimethyl *p*-toluenesulfonyloxymethanephosphonate[5] (*XXI*) under formation of diester of the completely blocked compound *III* (*XXII*). This intermediate *XXII* was not isolated and after removal of the trityl group by acid hydrolysis, was converted directly into compound *III* with alkali. Under these conditions the otherwise stable ester functionalities were hydrolyzed with participation of the neighbouring primary hydroxyl groups[6]. After desalting (and removal of the formed benzoic acid), the compound *III*



SCHEME 4

was isolated by ion exchange chromatography. In spite of a comparatively high conversion of the starting compound, this method of preparing the isomerically pure *III* (Scheme 4) requires double protection of the starting compound *I* and preparation of the synthon *XXI* (see ref.[5]). Therefore, it is not likely to compete with the above-mentioned methods which made use of the commercially available chloromethanephosphonyl dichloride (*VII*); on the other hand, the advantage of this synthesis consists in stability of the key intermediate *XX* which cannot undergo migration of the protecting group, as *e.g.* in the dibenzoyl derivative *XIX*.

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Unless stated otherwise, the solvents were evaporated at 40°C/2 kPa and the compounds were 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 on a Whatman No. 2 paper in system S2, 0·1 mol l^{-1} triethylammonium hydrogen carbonate pH 7·5 at 20 V cm^{-1}. Thin-layer chromatography was carried out on plates of Silufol UV 254 (Kavalier, Czechoslovakia) in system S3, chloroform–methanol (95 : 5). Analytical liquid chromatography (HPLC) was performed on 0·4 × 20 cm column of Separon SGX C18 (Laboratorni přístroje, Prague), flow rate 0·7 ml min^{-1} in 0·05 ml l^{-1} triethylammonium hydrogen carbonate, pH 7·5, containing 5% of methanol (S4) or 10% of acetonitrile (S5), detection at 254 nm. Capacity factor $k = (t_r - t_0)/t_0$ (t_r retention time, t_0 hold-up time). Column chromatography on silica gel was carried out on silica gel (30—40 μ) (made by Service Laboratories of the Institute) in chloroform–methanol mixtures. Ultraviolet absorption spectra were measured in aqueous solutions on a Specord UV-VIS spectrophotometer (Carl Zeiss, Jena, G.D.R.). ^{1}H NMR spectra were obtained with a Varian XL-200 instrument in deuteriochloroform or hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants in Hz.

Starting Compounds and Reagents

9-(*S*)-(2,3-Dihydroxypropyl)adenine and its (*R*)-enantiomer were prepared according to ref.[9], the racemic compound as described in ref.[10]. Prior to use, these compounds were codistilled three times with anhydrous pyridine, triturated with anhydrous ether and dried *in vacuo* over phosphorus pentoxide. Chloromethanephosphonyl dichloride was an Aldrich product, benzoyl cyanide and imidazole were purchased from Merck. Chlorobis(*p*-methoxyphenyl)phenylmethane was prepared as described[17]. Triethyl phosphate was distilled *in vacuo*, dimethylformamide was distilled from phosphorus pentoxide *in vacuo*, pyridine was purified with *p*-toluenesulfonyl chloride and distilled *in vacuo*. Pyridine and dimethylformamide were stored over molecular sieves. Triethylamine was boiled with sodium borohydride and distilled.

Reagent IX

A solution of water in pyridine (1 mol l^{-1}; 10 ml) was added dropwise during 5 min to an ice-cooled and stirred solution of compound *VII* (1·67 g; 10 mmol) in pyridine (10 ml). After stirring for 20 min at room temperature, the separated salt was collected by filtration or centrifugation with exclusion of moisture and the thus-obtained 0·5 mol l^{-1} solution of *IX* was immediately used.

9-(2,3-Dihydroxypropyl)-N^6-dimethylaminomethyleneadenine (*X*)

A mixture of hydrate of *I* (*R*, *S* or *RS*; 4·54 g; 20 mmol), dimethylformamide (50 ml), and dimethylformamide dimethylacetal (20 ml) was stirred in a stoppered flask overnight at room temperature. After evaporation at 40°C/13 Pa, the residue was codistilled with dimethylformamide (20 ml) under the same conditions and 70% aqueous pyridine (70 ml) was added. Solid carbon dioxide was gradually added so as to maintain its excess for 20 min. The solution was taken down and the residue codistilled with pyridine (3 × 50 ml) at 40°C/13 Pa. The product was dissolved in pyridine (50 ml), precipitated by adding dropwise into ether (150 ml), collected on filter, washed with ether and dried *in vacuo*. The obtained *X* (5·1 g; 96%) was used without further purification.

Reaction of *I* with Chloromethanephosphonic Acid Diimidazolide (*VIII*)

Compound *VII* (0·40 ml; 3·9 mmol) was added to a stirred solution of imidazole (1·1 g; 16 mmol) in dimethylformamide (10 ml) and the mixture was stirred for 1 h at room temperature in a stoppered flask. Dried (*RS*)-*I* (625 mg; 3 mmol) was added and the mixture was stirred at room temperature for 24 h. HPLC analysis of the reaction mixture (in S4) found 1·1% of *IV* and 4·2% of *V*, in addition to the starting *I*.

Chloromethanephosphonates *IV* and *V*

A) A freshly prepared solution of *IX* in pyridine (40 mmol, *vide supra*) was added to (*RS*)-*X* (10 mmol). After standing for 2 h at room temperature, 1 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5 (150 ml) was added and the solution was taken down *in vacuo*. The residue was codistilled with methanol (3 × 100 ml), allowed to stand with 5% aqueous ammonia (100 ml) overnight, taken down *in vacuo* and the residue in water (100 ml) was applied on a column of Dowex 50X8 (H^+ form; 400 ml). Water (4 ml min^{-1}) eluted first the acids and then a mixture of *IV* and *V* which, after evaporation *in vacuo*, was crystallized from water (with addition of ethanol to turbidity). The obtained product contained hydrates of the isomeric racemic *IV* and *V* (not melting up to 250°C) in the ratio 37 : 63. For $C_9H_{15}N_5O_5$ (308·7) calculated: 35·01% C, 4·90% H, 11·49% Cl, 22·74% N, 10·07% P; found: 35·14% C, 5·12% H, 11·55% Cl, 22·57% N, 9·82% P. *IV*: R_F 0·47 (S1), $k = 4·39$ (S4); *V*: R_F 0·47 (S1), $k = 5·73$; $E_{Up} = 0·52$.

B) Compound (*RS*)-*I* (1·25 g; 6 mmol) was dissolved in warm triethyl phosphate (20 ml), the solution was cooled with ice and *VII* (0·80 ml; 7·8 mmol) was added under stirring. The mixture was stirred overnight at room temperature and diluted with ether (140 ml). The product was collected on filter, washed with ether (200 ml) and dried at 2 kPa. The obtained compound was refluxed with water (40 ml) for 14 h with stirring. The reaction mixture, containing (analysis in S4) 11% of the starting compound, 23% of (*RS*)-*IV* and 66% of (*RS*)-*V*, was directly applied on a column of Dowex 50X8 (100 ml) and eluted with water (4 ml min^{-1}). The UV-absorbing fraction containing mixture of *IV* and *V* was taken down and crystallized from water (ethanol added to turbidity). Monohydrates of free acids (*RS*)-*IV* and (*RS*)-*V* were obtained in 78% yield as a 24·5 : 75·5 mixture.

Separation of *IV* and *V*

A) The mixture of (*S*)-*IV* and (*S*)-*V* (3 mmol) prepared by metod *B* (*vide supra*) was dissolved in water (1·5 ml) in the presence of the required amount of triethylamine. The excess base was removed *in vacuo* and the solution was applied in 0·3 ml (0·6 mmol) portions on a 8 × 500 mm column of octadecylsilica gel (*e.g.* Separon SIX C18; 7 μ) equilibrated with 0·05 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5. The column was washed with the same buffer with increasing amount of methanol (2·5%, 5% and 10%); detection at 254 nm. Compound *IV* was eluted first. Fractions, containing the separated isomers were combined and taken down *in vacuo*. The remaining triethylammonium salts were codistilled with methanol (2 × 25 ml), applied on a column of Dowex 50X8 (Na^+ form, 20 ml) and eluted with water. The UV-absorbing eluate was evaporated, the residue codistilled with ethanol, dissolved in methanol and precipitated with ether. The obtained sodium salts of compounds (*S*)-*IV* and (*S*)-*V* were more than 99% pure. For the R_F and k values see above.

B) A mixture of compounds (*S*)-*IV* and (*S*)-*V* (3 mmol), prepared by method *A*) or *B*), was dissolved in water (5 ml) with addition of ammonia and chromatographed on a 2 × 30 cm

column of Dowex 50X8 (H^+ form), eluent water (3 ml min^{-1}; fractions 10 min). The UV-absorbing fractions were analyzed by HPLC in the system S4, the pertinent fractions were combined and evaporated to dryness *in vacuo*. Total yield of (*S*)-*IV* and (*S*)-*V*, purer than 98%, was usually higher than 70% of the starting amount; the remaining unseparated mixture of compounds could be again separated under the same conditions.

Preparation of *IV* and *V via* Benzoyl Derivatives *XV* and *XVI*

Compound *VII* (2 ml; 19·8 mmol) was added with stirring to a suspension of (*RS*)-*XIV* (3·13 g; 10 mmol) in triethyl phosphate (40 ml), prepared by sonication for 10 min. After stirring for 15 h at room temperature, the formed gel was diluted with ether (200 ml) and stirring was continued for 2 h. Filtration under exclusion of moisture, washing with ether (3 × 50 ml) and drying over phosphorus pentoxide *in vacuo* afforded a mixture of 53% of *XV* (k = 4·74 in S5) and 47% of *XVI* (k = 5·31 in S5). This product was refluxed in water (60 ml) for 15 h, cooled with ice and the separated benzoic acid was removed by filtration through Celite. The filtrate was taken down *in vacuo*, the residue codistilled with ethanol (3 × 50 ml) and the product precipitated with ether (150 ml) from methanol (20 ml). The mixture of (*RS*)-*IV* and (*RS*)-*V* was separated on a 4·5 × 20 cm column of Dowex 50X8 (H^+ form) and both compounds were isolated as the free acids. Yield 380 mg (12%) of the 2′-isomer (*RS*)-*IV*, 1·6 g (50%) of the 3′-isomer (*RS*)-*V*, and 380 mg (12%) of unseparated mixture of both compounds. Isomeric purity of the products exceeded 96%.

(*RS*)-*IV*. ^{1}H NMR spectrum (^2H_2O; sodium 2,2-dimethyl-2-silapentane-5-sulfonate), (+^{2}HAc): 3·19 d, 2 H (P—CH_2, J(P—CH) = 9·6); 3·42 2× dd, 2 H (3′-CH_2, J(3′, 2′) = 5·0, J_g = −11·0); 4·34 m and 4·49 m, 3 H (1′-CH_2 and 2′-CH); 8·10 s, 1 H (H-2); 8·15 s, 1 H (H-8). ^{13}C NMR spectrum (^2H_2O, ref. dioxane): 35·89 d (C—P, ^1J(P—C) = 149·5); 46·38 d (C-1′, ^3J(P—C) = 5·8); 63·43 s (C-3′); 75·56 d (C-2′, ^2J(P—C) = 6·6); 119·13 s (C-5); 143·91 s (C-8); 149·90 s (C-4); 153·26 s (C-2); 156·22 s (C-6).

(*RS*)-*V*. ^{1}H NMR spectrum (^2H_2O; sodium 2,2-dimethyl-2-silapentane-5-sulfonate), (+^{2}HAc): 3·43 d, 2 H (P—CH_2, J(P—CH) = 9·7); 3·70—4·50 m, 3 H (N—CH_2, OCH); 8·09 s, 1 H (H-2); 8·16 s, 1 H (H-8). ^{13}C NMR spectrum (^2H_2O, ref. dioxane): 35·54 s (C—P, ^1J(P—C) = 118·1); 47·19 s (C-1′); 67·44 d (C-3′, ^2J(P—C) = 6·0); 69·47 d (C-2′, ^3J(P—C) = 7·0); 143·99 s (C-8); 149·62 s (C-4); 152·26 s (C-2); 155·44 s (C-6).

Compounds *III* and *VI* from Chloromethanephosphonates of *I* and *XIV*

A) A solution of (*S*)-*IV* (free acid or sodium salt; 3 mmol) in 2 mol l^{-1} sodium hydroxide (20 ml) was heated to 80°C for 10 h. The mixture was cooled, acidified by addition of Dowex 50X8 (H^+ form) and the suspension was poured on a column of the same resin (50 ml). After washing the column with water to drop of UV-absorption and acidity, the product was eluted with 2·5% aqueous ammonia. The UV-absorbing eluate was evaporated and the product analyzed by HPLC in S4 or by paper electrophoresis. Usually, the product was homogeneous and was converted to the sodium salt on a column of Dowex 50X8 (H^+ form) by elution with water, evaporation of the UV-absorbing fraction and precipitation of the residue (dried by codistillation with ethanol) with ether from methanol. Yield 80—90% of (*S*)-*VI* as sodium salt; R_F 0·22 (S1), k = 2·48 (S4); E_{Up} = 0·75.

When the product was not completely homogeneous, it was chromatographed on Sephadex A-25 in triethylammonium hydrogen carbonate or on Dowex 1X2 in a gradient of acetic acid under conditions described for the direct preparation of a mixture of *III* and *VI*.

In the same manner, compound (*S*)-*V* (free acid; 3 mmol) was converted to sodium salt of (*S*)-*III* in 75% yield; purity (HPLC) higher than 99%; R_F 0·22 (S1); $k = 2·75$ (S4); $E_{Up} = 0·75$.

B) A solution of sodium salt of (*RS*)-*XV* or (*RS*)-*XVI* (3 mmol) in 2 mol l^{-1} sodium hydroxide (20 ml) was processed as described under *A*) except that the elution of Dowex 50 in the deionization step was carried out with 25% aqueous methanol instead of water. Thus, compound (*RS*)-*XV* afforded sodium salt of (*RS*)-*VI* in 72% yield; compound (*RS*)-*XVI* gave sodium salt of (*RS*)-*XII* in 74% yield, isomeric purity of both products being higher than 98%.

Direct Preparation of Mixture of *III* and *VI*

A) Pyridine (8 ml; 100 mmol) was added to a suspension of (*RS*)-*X* (5·3 g; 30 mmol) in 1,2-dichloroethane (200 ml). Compound *VII* (6·68 g; 40 mmol) in 1,2-dichloroethane (20 ml) was added under vigorous stirring. The stirring was continued for 2 h at room temperature, triethylamine (15 ml) and water (6 ml) were added and the mixture was evaporated to dryness. The residue was codistilled with dioxane (3 × 50 ml) and heated to 100°C with 2 mol l^{-1} lithium hydroxide (200 ml) for 6 h. The mixture was neutralized with hydrochloric acid to pH 7·2, and taken down *in vacuo*. The residue was codistilled with ethanol (5 × 100 ml) and stirred for 1 h with acetone–ethanol (1 : 1; 400 ml). The solid was filtered, washed with the same mixture, dried *in vacuo* and applied in water on a column of Dowex 1X2 (acetate form; 200 ml). The material was eluted with a linear gradient of 0—0·75 mol l^{-1} acetic acid (2 l each). The product-containing fraction was evaporated, codistilled with water (5 × 100 ml) and ethanol (3 × 100 ml). Precipitation with ether from ethanol yielded 2·8 g (46%) of a mixture of (*RS*)-*III* and (*RS*)-*VI* (ratio 43·5 : 56·5) as free acids. For the R_F and k values see above.

B) Compound *VII* (335 mg; 2 mmol) was added to a suspension of dried (*RS*)-*I* (1 mmol) in pyridine (10 ml) and the mixture was stirred at room temperature for 16 h. Water (10 ml) was added and after 1 h the solvents were evaporated. The dry residue was codistilled with 5% aqueous ammonia (20 ml) and dioxane (3 × 20 ml). Further treatment with lithium hydroxide and chromatography according to method *A*) afforded a mixture (200 mg; 66%) of free acids (*RS*)-*III* and (*RS*)-*VI* containing 44·0% of *III* and 56·0% of *VI*. For the R_F and k values see above.

C) A solution of *IX* (52 ml, *vide supra*) was added to dry (*S*)-*X* (2·3 g; 8·6 mmol) under exclusion of moisture. After standing at room temperature for 15 h, 2 mol l^{-1} triethylammonium hydrogen carbonate (40 ml) was added. The mixture was set aside for 20 min, taken down and the residue was codistilled with methanol (2 × 100 ml). Further treatment with lithium hydroxide and chromatography as described under *A*) afforded a mixture (1·3 g; 50%) of free acids (*S*)-*III* and (*S*)-*VI* containing 45·6% of *III* and 54·4% of *VI*.

Compounds (*R*)-*III* and (*R*)-*VI* were prepared similarly in 48% yield, the isomer composition being similar (45·5% of *III*, 54·5% of *VI*).

N^3,N^6-Dibenzoyl-2′,3′-O-isopropylidene-9-(*S*)-(2,3-dihydroxypropyl)adenine (*XII*)

Benzoyl chloride (13·5 ml; 116 mmol) was added dropwise at 0°C during 30 min to a mixture of *XI* (ref.[9], 12·0 g; 48 mmol), 4-dimethylaminopyridine (0·50 g), and pyridine (150 ml). The mixture was stirred at 0°C for 3 h and at room temperature overnight. Methanol (10 ml) was added and after 30 min the mixture was concentrated *in vacuo*. The concentrate was diluted with chloroform (500 ml), washed with water (4 × 100 ml), the organic phase was evaporated *in vacuo* and the residue was codistilled with toluene (4 × 100 ml). Crystallization from ethanol

(200 ml) afforded 19·8 g (90·3%) of *XII*, m.p. 210–212°C. For $C_{25}H_{23}N_5O_4$ (457·5) calculated: 65·63% C, 5·07% H, 15·31% N; found: 65·40% C, 5·13% H, 15·50% N. R_F 0·73 (S3). ^{1}H NMR spectrum (C^2HCl_3): 1·48 s, 6 H (isopropylidene); 3·50–4·65 m, 3 H (N—CH_2, O—CH); 8·20 s, 1 H (H-2); 8·67 s, 1 H (H-8); 7·10–7·60 m, 6 H and 7·75–8·0 m, 4 H (arom. protons).

N^6-Benzoyl-2′,3′-O-isopropylidene-9-(*S*)-(2,3-dihydroxypropyl)adenine (*XIII*)

Concentrated aqueous ammonia (75 ml) was added to a solution of *XII* (19·8 g; 43·3 mmol) in dioxane (450 ml), the mixture was stirred for 30 min and taken down. The residue was co-distilled with dioxane (2 × 100 ml) and crystallized from ethyl acetate (light petroleum added to turbidity), yielding 15·0 g (99%) of *XIII*, m.p. 145–147°C. For $C_{18}H_{19}N_5O_3$ (353·4) calculated: 61·17% C, 5·42% H, 19·82% N; found: 61·34% C, 5·44% H, 19·61% N. R_F 0·37 (S3). ^{1}H NMR spectrum (C^2HCl_3): 1·33 s, 3 H and 1·37 s, 3 H (isopropylidene); 3·70 dd, 1 H (H-3″, $J(3'', 2') = 5·8$; $J_g = -9·0$); 4·12 dd, 1 H (H-3′, $J(3', 2') = 6·5$); 4·30 dd, 1 H (H-3′, $J(3', 2') = 6·5$); 4·30 dd, 1 H (H-1″, $J(1'', 2') = 6·5$; $J_g = -14·5$); 4·48 dd, 1 H (H-1′, $J(1', 2') = 3·0$); 8·13 s, 1 H (H-2); 8·76 s, 1 H (H-8); 9·34 br s, 1 H (NH); 7·35–7·60 and 7·75–7·85 (arom. protons).

9-(*S*)-(2,3-Dihydroxypropyl)-N^6-benzoyladenine (*XIV*)

A) A solution of *XIII* (9·0 g; 25·5 mmol) in a mixture of dioxane (80 ml) and 0·25 mol l^{-1} sulfuric acid (80 ml) was set aside at room temperature for 48 h, neutralized with triethylamine and taken down *in vacuo*. The residue was mixed with chloroform (200 ml), filtered, washed with chloroform (50 ml) and crystallized from ethanol (250 ml), affording 4·87 g (61%) of *XIV*, m.p. 198–199°C. For $C_{15}H_{15}N_5O_3$ (313·3) calculated: 57·50% C, 4·82% H, 22·36% N; found: 57·43% C, 4·83% H, 22·17% N; R_F 0·28 (S3). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·38 dt, 1 H (H-3″, $J(3'', 2') = 6·0$); 3·47 dt, 1 H (H-3′, $J(3', 2') = 5·0$; $J_g = -11·0$); 3·92 m, 1 H (H-2′); 4·15 dd, 1 H (H-1″, $J(1'', 2') = 8·4$); 4·46 dd, 1 H (H-1′, $J(1', 2') = 3·3$; $J_g = -14·0$); 4·89 t, 1 H (3′-OH, $J(3', OH) = 5·6$); 5·18 d, 1 H (2′-OH, $J(2', OH) = 5·4$); 8·39 s, 1 H (H-2); 8·74 s, 1 H (H-8); 11·15 br s, 1 H (NH); 7·50–7·70 m, 3 H and 8·0–8·10 m, 2 H (arom. protons).

B) Dried (*S*)-*I* (15·2 g; 72·9 mmol) in pyridine (400 ml) was sonicated for 15 min. Chlorotrimethylsilane (50·6 ml) was added to this suspension under stirring at room temperature. After stirring for 1 h, benzoyl chloride (45·8 ml) was added and stirring was continued for further 2 h at room temperature. The mixture was cooled with ice, treated with ice-cold water (79 ml) and stirred for 5 min. Concentrated aqueous ammonia (176 ml of 26% solution) was added dropwise during 5 min, the solution was stirred for 30 min and taken down *in vacuo*. The residue was dissolved in water (250 ml), washed with ethyl acetate (100 ml) and the aqueous phase was cooled with ice to incipient crystallization. After standing for 2 days at 4°C, the product was collected on filter, washed with a small amount of ice-cold water and suspended in acetone (250 ml). Ether (500 ml) was added with stirring, stirring was continued for 20 min, the product was filtered, washed with ether and dried; yield 18·5 g (81%) of *XIV*, chromatographically homogeneous and identical with the product obtained according to procedure *A*).

3′-O-Bis(*p*-methoxyphenyl)phenylmethyl-9-(*S*)-(2,3-dihydroxypropyl)-
-N^6-benzoyladenine (*XVII*)

Dimethoxytrityl chloride (9·2 g; 27 mmol) was added to a stirred suspension of compound *XIV* (7·54 g; 24·1 mmol, pre-dried by codistillation with 3 × 50 ml of pyridine) in pyridine (260 ml)

and the mixture was stirred at room temperature for 16 h. After addition of methanol (5 ml), the mixture was stirred for 3 h, poured into stirred saturated aqueous sodium hydrogen carbonate (300 ml) and extracted with chloroform (2 × 500 ml). The extract was washed with water (3 × 100 ml) and the solvent evaporated *in vacuo*. The residue was codistilled with toluene (3 × 100 ml) and dioxane (2 × 100 ml) to remove the pyridine and crystallized from toluene, affording 13·5 g (90·9%) of *XVII*, m.p. 110–111°C; R_F 0·45 (S3). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·01 m, 2 H (3'-CH_2); 3·74 s, 6 H (OCH_3); 4·05–4·50 m, 3 H (1'-CH_2, 2'-CH); 5·39 d, 1 H (2'-OH, J(OH, CH) = 5·4); 6·80–6·95 m and 7·15–7·70 m and 8·0–8·10 m, 18 H (arom. protons); 8·34 s, 1 H (H-2); 8·71 s, 1 H (H-8); 11·11 br s, 1 H (NH).

9-(*S*)-(2-Benzoyloxy-3-hydroxypropyl)-N^6-benzoyladenine (*XIX*)

A) Triethylamine (7 ml; 50·4 mmol), followed by a solution of benzoyl cyanide (6·6 g; 50·3 mmol) in acetonitrile (100 ml) was added to a solution of *XVII* (31 g; 43·3 mmol) in acetonitrile (400 ml). The mixture was set aside at room temperature for 16 h and methanol (0·4 ml) was added. After standing for 20 min and cooling with ice, trifluoroacetic acid (3·9 ml) was added, followed by 10% aqueous trifluoroacetic acid (110 ml). The mixture was allowed to stand for 4 h at room temperature, neutralized with triethylamine (21 ml) and taken down *in vacuo*. The residue was dissolved in chloroform (2 l), the solution was washed with water (3 × 400 ml) and the organic phase was taken down. The residue was codistilled with toluene, dissolved in hot chloroform (550 ml) and after addition of benzene (1 l) allowed to stand at 4°C for 16 h. The product was collected on filter, washed with benzene and dried *in vacuo*, affording 16·1 g (91%) of *XIX*, m.p. 174–175°C. For $C_{22}H_{19}N_5O_4$ (417·3) calculated: 63·30% C, 4·59% H, 16·78% N; found: 63·40% C, 4·65% H, 16·90% N; R_F 0·60 (S3). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·72 t, 2 H (3'-CH_2, J = 5·4); 4·69 d, 2 H (N—CH_2, J(1', 2') = 5·9); 5·23 t, 1 H (3'-OH, J(3·, OH) = 6·1); 5·44 pent., 1 H (2'-CH, J = 5·4); 8·48 s, 1 H (H-2); 8·68 s, 1 H (H-8); 11·05 br s, 1 H (NH); 7·35–8·15 m, 10 H (arom. protons).

B) Compound *XVII* (13·5 g; 21·9 mmol) was benzoylated in chloroform (150 ml) with benzoyl cyanide (3·7 g; 28 mmol) in the presence of triethylamine (3·9 ml) in the same manner as described under *A*). The mixture was treated with methanol (115 ml), trifluoroacetic acid (7·2 ml) and then with water (17·8 ml). After 15 min chloroform (800 ml) was added, the mixture was washed with water (100 ml) and the aqueous layer was extracted with chloroform (4 × 100 ml). The combined organic extracts were shaken with solid sodium hydrogen carbonate (50 g) for 20 min, filtered, the solids were washed with chloroform and the combined filtrates were taken down. The residue was refluxed with acetone (350 ml) for 2 min, cooled and mixed with ether (650 ml). After standing for 5 h at 4°C, the product was filtered, washed with a mixture of acetone and ether (1 : 1), and then with ether, and dried. Yield 8·1 g (89%) of *XIX*, identical with the product prepared according to *A*).

9-(*S*)-(3-Hydroxy-2-phosphonylmethoxypropyl)adenine ((*S*)-*III*)

A) A solution of *IX* (90 ml; 44·5 mmol) was added to (*S*)-*XIX* (3·7 g; 8·9 mmol). The mixture was set aside for 45 min at room temperature, cooled with ice and ice-cold 1 mol l^{-1} triethylammonium hydrogen carbonate pH 7·5 (100 ml) was added. After 30 min the mixture was extracted with chloroform (2 × 400 ml), the solvent was evaporated and the residue codistilled with toluene (3 × 50 ml) and dioxane (3 × 50 ml). The aqueous phase, after extraction with chloroform, was taken down and concentrated aqueous solution of the residue was applied on a column (200 ml) of octadecylsilica gel in water. Elution with water (4 ml min^{-1}) removed the salts.

Further portion of the product, identical with that in the chloroform extract, was obtained by elution of the column with 70% aqueous methanol. The combined portions of the 3'-O--chloromethanephosphonyl derivative of *XIX* were evaporated and heated with 1 mol l^{-1} aqueous sodium hydroxide (300 ml) to 75°C for 20 h. After acidification with Dowex 50X8 (H^+ form), the suspension was poured on a column of the same resin (200 ml) and washed with 25% aqueous methanol to disappearance of UV absorption of the eluate. Elution with 5% aqueous ammonia afforded crude (*S*)-*III* which was further purified by chromatography on Dowex 1X2 in acetic acid to give 2·3 g (84·6%) of free (*S*)-*III*, identical with the above-described products; $k = 2{\cdot}75$ (S4), $E_{Up} = 0{\cdot}75$. ^{13}C NMR spectrum (^2H_2O, ref. dioxane): 44·86 s (C-1'); 61·60 s (C-3'); 69·20 d (C—P, ^1J(P—C) = 150·3); 80·96 d (C-2', ^3J(P—C) = 11·0); 118·80 s (C-5); 144·27 s (C-8); 149·85 s (C-4); 153·25 s (C-2); 156·24 s (C-6).

B) Dibenzoate (*S*)-*XIX* (4·17 g; 10 mmol) was suspended in triethyl phosphate (50 ml) by sonication. Compound *VII* (3·35 g; 20 mmol) was added and the mixture was stirred at room temperature for 15 h. The resulting solution was added dropwise with stirring into ether (500 ml). After stirring for 10 min, the product was filtered, washed with ether (3 × 50 ml), dried for 10 h over potassium hydroxide at 13 Pa and heated with 2 mol l^{-1} sodium hydroxide (100 ml) to 75°C for 8 h. The mixture was deionized with Dowex 50 (H^+ form) as described under *A*). The crude product obtained from the ammonia eluate was chromatographed on a column of Sephadex A-25 (200 ml; HCO_3^- form), elution with a linear gradient of 0—0·03 mol l^{-1} of triethylammonium hydrogen carbonate, pH 7·5 (2 l each). The product fraction was evaporated, the residue codistilled with methanol (3 × 100 ml) and converted into the sodium salt on a column of Dowex (Na^+ form; 50 ml). Drying with ethanol and precipitation with ether from methanol afforded 2·5 g (71%) of sodium salt of (*S*)-*III* of purity (HPLC) exceeding 98%; $k = 2{\cdot}75$ (S4).

9-(*S*)-(2-Hydroxy-3-phosphonylmethoxypropyl)adenine ((*S*)-*VI*)

A solution of *IX* (295 ml; 150 mmol; *vide supra*) was added to the dried compound *XVII* (18·4 g; 30 mmol) and the mixture was set aside for 30 min at room temperature. After cooling with ice, ice-cold 1 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5 (800 ml), was added with stirring during 10 min and the stirring was continued for 10 min. The mixture was extracted with chloroform (1 500 ml and 500 ml), the combined extracts were washed with 0·1 mol l^{-1} triethylammonium hydrogen carbonate (2 × 200 ml) and the organic phase was taken down. The residue was codistilled with toluene (5 × 100 ml) and dioxane (3 × 100 ml) and dissolved in a mixture of dichloromethane–methanol (7 : 3; 500 ml). To this solution 75% aqueous trifluoroacetic acid (20 ml) was added under stirring. After standing at room temperature for 30 min, the mixture was made alkaline with triethylamine, taken down and partitioned between ether (200 ml) and water (500 ml). The aqueous phase was washed with ether (2 × 100 ml) and chloroform (2 × 100 ml). The residue after evaporation of the aqueous portion was taken up in 50% aqueous ethanol, mixed with Dowex 50X8 (Li^+ form; 150 ml) and stirred for 10 min. The mixture was filtered, the resin was washed with water and the combined filtrates were taken down. The residue was codistilled with ethanol and precipitated by dropwise adding its ethanolic solution (100 ml) to ether (900 ml). The product was filtered, washed with ethanol–ether (1 : 9; 3 × 50 ml), ether (3 × 100 ml) and dried *in vacuo*. Yield of lithium salt of (*S*)-*XV* was 13·8 g (86%).

This product was heated with 2 mol l^{-1} sodium hydroxide (100 ml) to 40°C for 15 h and to 60°C for 10 h. The mixture was deionized on Dowex 50X8 (300 ml) as described above (deionization with 25% aqueous methanol, elution of the product with 5% aqueous ammonia). The product was purified by chromatography on a column of Dowex 1X2 (acetate form; 300 ml) with a linear gradient of acetic acid (0—1 mol l^{-1}, 2 l each) and isolated as the free acid. Yield 6·1 g (77·6%)

of (*S*)-*VI*, pure according to HPLC; $k = 2{\cdot}48$ (S4); $E_{Up} = 0{\cdot}75$. ^{13}C NMR spectrum (^2H_2O, ref. dioxane): 47·43 s (C-1'); 69·36 s (C-2'); 70·64 d (C—P, ^1J(P—C) = 151·1); 74·83 d (C-3', ^3J(P—C) = 11·2); 118·86 s (C-5); 143·78 s (C-8); 149·55 s (C-4); 153·04 s (C-2); 155·98 s (C-6).

9-(*S*)-(3-Triphenylmethoxy-2-hydroxypropyl)-N^6-benzoyladenine (*XX*)

A solution of (*S*)-*XIV* (3·45 g; 11 mmol) and trityl chloride (4·2 g; 15 mmol) in pyridine (100 ml) was stirred for 76 h (after 30 h and 46 h further portions of trityl chloride (5 mmol each) were added). Methanol (10 ml) was added and, after 4 h, the mixture was taken down *in vacuo*. The residue was taken up in chloroform, washed with saturated solution of sodium hydrogen carbonate (2 × 100 ml), water (2 × 50 ml) and the organic solvent was evaporated *in vacuo*. The residue was codistilled with toluene (50 ml portions) until the pyridine was removed, boiled for 30 min with ethyl acetate (300 ml) and allowed to crystallize in a refrigerator overnight. The product was collected, washed with ethyl acetate and ether, and dried *in vacuo*. A further crop of the product was obtained by evaporation of the mother liquor and crystallization from acetone with light petroleum added; total yield 5·7 g (93%) of (*S*)-*XX*, m.p. 216—217°C. For $C_{34}H_{29}N_5O_3$ (555·6) calculated: 73·49% C, 5·26% H, 12·59% N; found 73·25% C, 5·23% H, 12·38% N. ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): 3·30 m, 2 H (N—CH_2); 4·10—4·50 m, 3 H (OCH_2, OCH); 5·44 d, 1 H (2'-OH, J(OH, CH) = 5·1); 8·34 s, 1 H (H-2); 8·71 s, 1 H (H-8); 11·12 br s, 1 H (NH); 7·20—7·70 m and 8·0—8·15 m, 20 H (arom. protons).

Reaction of (*S*)-*XX* with *XXI*

Sodium hydride (6 mmol) was added under exclusion of moisture to a solution of (*S*)-*XX* (1·11 g; 2 mmol) in dimethylformamide (8 ml). After stirring at room temperature for 1 h, compound *XXI* (0·58 g; 2 mmol) was added and the mixture was stirred at room temperature for 2 days. Acetic acid (0·36 ml) was added, the solvent was evaporated at 50°C/2 kPa and the residue was refluxed with 80% acetic acid (25 ml) for 1 h. The mixture was evaporated *in vacuo*, codistilled with water (4 × 50 ml) and the residue was heated with 2 mol l^{-1} sodium hydroxide (30 ml) to 80°C for 15 h. After cooling, filtration and washing the solid with water (20 ml), the filtrate was acidified by addition of Dowex 50X80 (H^+ form). The suspension was applied on a column of the same ion exchanger (100 ml) and eluted with 50% methanol to drop of UV absorption. The crude product was eluted with 2% aqueous ammonia and the eluate was taken down. The UV-absorbing residue in water (5 ml) was applied on a column of Dowex 1X2 (acetate form), eluted with water (2·5 ml/min) and then by linear gradient (à 1 l) of acetic acid (0—1 mol l^{-1}). The product-containing fraction was taken down, the residue codistilled with water and crystallized from aqueous ethanol, yielding 64% of (*S*)-*III*, identical with products prepared by the above-described methods; isomeric purity (HPLC) higher than 98%.

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