SYNTHESIS OF 3-AMINO- AND 3-AZIDOANALOGS OF 9-(3-HYDROXY--2-PHOSPHONYLMETHOXYPROPYL)ADENINE (HPMPA)*

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1-Azidopropane-2,3-diol (*IIb*) reacts with *p*-toluenesulfonyl chloride to give the tosyl derivative *IIIa* which, on acid catalyzed condensation with 2,3-dihydropyran, afforded 1-azido-2-(tetrahydropyran-2-yloxy-3-(*p*-toluenesulfonyloxy)propane (*IIIb*). Treatment of adenine sodium salt with *IIIb* resulted in the intermediate *IV* which was transformed by acid hydrolysis to 9-(*RS*)--(3-azido-2-hydroxypropyl)adenine (*V*). Catalytic hydrogenation of *V* led to 9-(*RS*)-(3-amino-2--hydroxypropyl)adenine (*VI*). 9-(*RS*)-(3-Azido-2-hydroxypropyl)-N^6-benzoyladenine (*VII*) was obtained from *V* by chlorotrimethylsilane/benzoyl chloride treatment. Reaction of the compound *VII* with dimethyl *p*-toluenesulfonyloxymethanephosphonate (*VIII*) in the presence of excess sodium hydride, followed by alkaline hydrolysis, afforded methyl 9-(3-azido-2-phosphonylmethoxypropyl)adenine (*IXa*) which was transformed to the parent acid *IXb* by bromotrimethylsilane treatment. Hydrogenolysis of *IXb* yielded 9-(*RS*)-(3-amino-2-phosphonylmethoxypropyl)-adenine (*X*).

The significant antiviral activity of 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine (HPMPA, *I*), directed specifically against DNA viruses[1], prompted us to investigate the structure-activity relationship in the series of acyclic nucleotide analogs. The previous paper of this series[2] describes the synthesis of a large variety of adenine 9-phosphonylmethoxyalkyl and 9-phosphonylalkyl analogs as isomers, isosters, homologs and carba analogs of the parent structure *I*. In addition to the parent compound[3], N-(2-phosphonylmethoxyethyl) derivatives of adenine[4] and other heterocyclic bases[5] were found to be active against a series of DNA viruses[6,7] as well as against human immunodeficiency (HIV) virus[8]. All the structural variations examined so far preserved the hydroxyl function as the necessary structural element or replaced it by an alkoxy group or hydrogen atom. In this paper we describe the synthesis of two HPMPA analogs in which the hydroxyl function in the side chain is replaced by an azido or amino group.

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The introduction of phosphonylmethyl grouping onto a hydroxyl function at the side chain can be performed either by an intramolecular etherification reaction of chloromethanephosphonic acid esters of vicinal diols which proceeds on the hydroxyl group at the neighbouring position or by reaction of *p*-toluenesulfonyloxymethanephosphonic acid esters with alkoxide anion generated from an isolated hydroxyl group at the side chain[3]. The first alternative is obviously not applicable for the present purpose. Neither is the condensation with phosphonic acid synthon suitable for use with a 2-aminoalcohol type substrate. To eliminate the side reactions, the amino group would have to be double-protected or replaced by a suitable precursor, e.g. azido group. The second approach was chosen as a potentially optimal solution, since it gives rise simultaneously to two series of analogs both of which are worth of examination, namely, azido- and aminoanalogs of HPMPA.

The key-compound of the synthesis, 9-(3-azido-2-hydroxypropyl)adenine (*V*), has been synthesized earlier[9] by a substitution reaction of 9-(2-hydroxy-3-*p*-toluenesulfonyloxypropyl)adenine with sodium azide. Since this method was not thought to be efficient for scaling-up the synthesis of compound *V*, an alternative procedure was developed for the purpose. 1-Azido-2,3-propanediol (*IIb*) is easily accessible by reaction of 1-chloro-2,3-propanediol (*IIa*) with sodium azide in aqueous solution[10]. This compound was tosylated by treatment with an equimolar amount of *p*-toluenesulfonyl chloride and a slight excess of pyridine in acetonitrile. The oily ester *IIIa* was purified by silica gel chromatography and characterized by its ^{1}H NMR spectrum which reveals the presence of a single secondary hydroxyl group, as well as of protons corresponding to the —CH_2N_3, CH—O and CH_2O grouping and the typical signals of 4-methylphenyl group. To exclude the possible elimination reaction during the forthcoming condensation, the remaining free 2-hydroxyl was protected by tetrahydropyran-2-yl group. The fully protected synthon *IIIb* which was obtained by acid catalyzed reaction of *IIa* with 2,3-dihydropyrane was used in the further step without excessive purification.

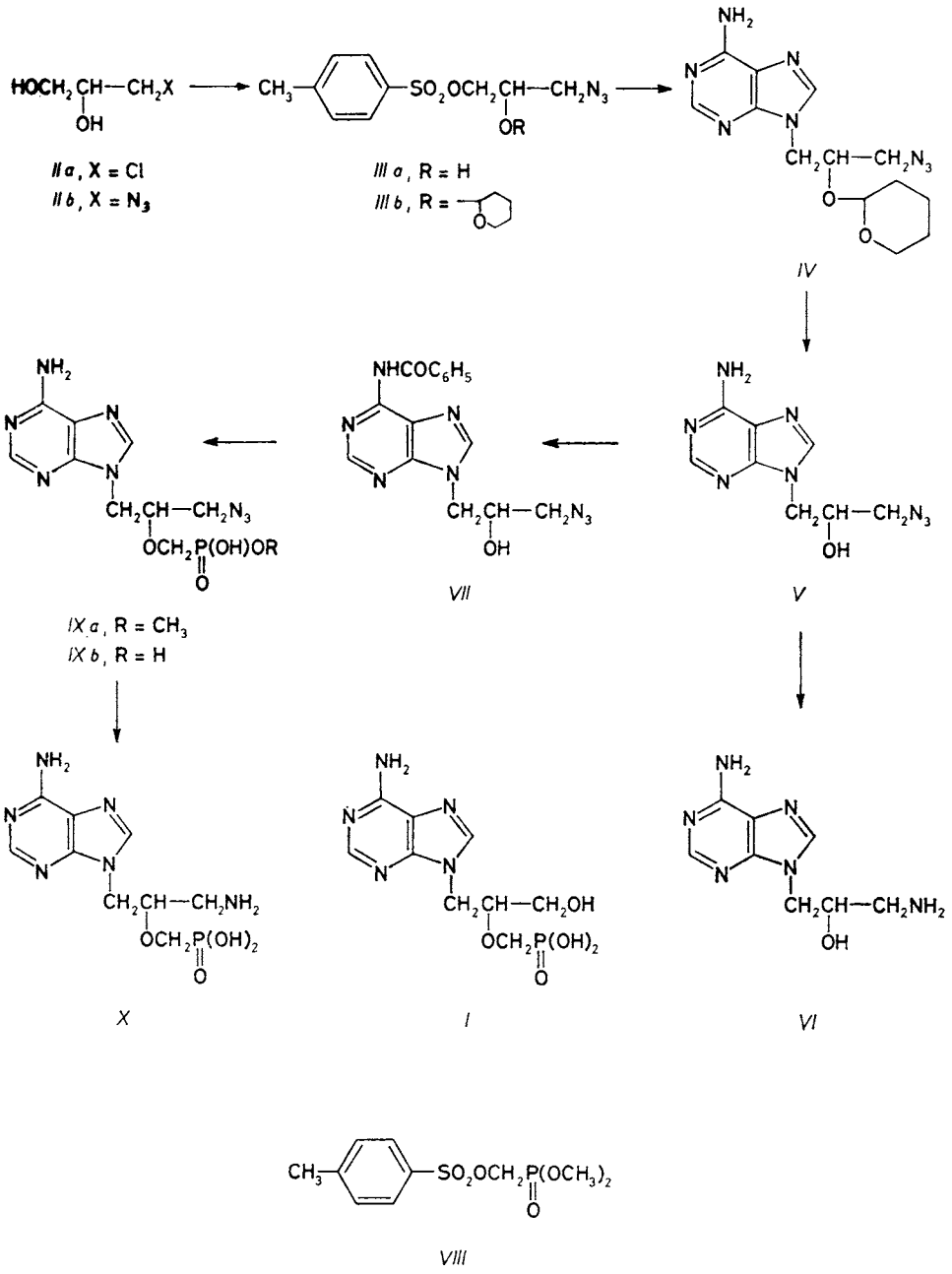
This reaction consisted in condensation with adenine sodium salt generated in situ by treatment of the heterocyclic base with sodium hydride in dimethylformamide solution. 9-(3-Azido-2-(tetrahydropyran-2-yl)oxypropyl)adenine (*IV*) was isolated as the main product of this condensation in a respectable yield. The ^{1}H NMR spectrum of the compound *IV* gave all the expected signals and revealed the presence of two diastereoisomers in an approximately 1 : 1 ratio.

Deprotection of the intermediate *IV* was effected by an acid treatment. 9-(3-Azido-2-hydroxypropyl)adenine (*V*) was characterized by ^{1}H NMR spectrum and by comparison with an authentic specimen[9]. Its structure was further confirmed by transformation to the known[9] 9-(3-amino-2-hydroxypropyl)adenine (*VI*) by a palladium-catalyzed hydrogenolysis. This reaction sequence is more practical for syntheses of racemic compounds *V* and *VI* compared with the procedures starting from 9-(2,3-dihydroxypropyl)adenine[9].

According to our previous experience it is essential to protect the 6-amino group in order to exclude side-reactions at this site. This protection was achieved by the selective N-benzoylation which utilizes consecutive reactions of the compound *V* with chlorotrimethylsilane and benzoyl chloride followed by ammonolysis[11]. The N^6-benzoyladenine derivative *VII* which was isolated in a fair yield also shows the presence of all proton signals in the ^{1}H NMR spectrum which corresponds to the proposed structure (in particular one benzoyl group, NH group and a secondary OH group). The compound *VII* was then condensed with dimethyl *p*-toluenesulfonyloxymethanephosphonate (*VIII*) (ref.[12]) in dimethylformamide solution. The alkoxide anion formation from the secondary hydroxyl group was enforced by the pretreatment of compound *VII* with sodium hydride. An excess of the reagent (3 equivalents) was necessary to achieve an efficient condensation reaction. This requirement, already observed earlier[3], can be interpreted by the presence of additional acidic hydrogen (NH-benzoyl) in the molecule. The reaction takes place at room temperature and its progress is rather slow. An attempt to accelerate the esterification by excess of reagent *VIII* resulted in a formation of side-product(s).

An alkaline treatment of the crude reaction product causes simultaneous hydrolysis of the N-benzoyl group and one of the ester groups of the intermediate. The resulting monomethylester *IXa* resists further alkaline hydrolysis; unlike in HPMPA (*I*) synthesis[3], the azido function cannot participate in an intramolecular cyclisation-ring opening reaction of the monoester. The ester *IXa* was effectively purified by ion-exchange chromatography. Its ^{1}H NMR spectrum agrees completely with the proposed structure and confirms the presence of P—OCH_3 as well as P—CH_2 groupings. The cleavage of the ester group was achieved by chlorotrimethylsilane treatment and subsequent hydrolysis of the unstable intermediate[3]. 9-(3-Azido-2-phosphonylmethoxypropyl)adenine (*IXb*) was purified by ion-exchange chromatography and crystallization to HPLC-homogeneity. The presence of characteristic N_3CH_2, PCH_2 and OCH_2 signals, as well as typical singlets of adenine protons in its ^{1}H NMR spectrum substantiates the structure which unequivocally follows from the reaction intermediates. Furthermore, the ultraviolet spectrum which fully corresponds to 9-substituted adenine derivatives totally excludes alternative isomeric structures, in particular the presence of any substituent at the 6-amino group.

The final step of the synthesis (Scheme 1) consists in hydrogenolysis of the azido group in the compound *IXb*. This reaction can be accomplished rather easily in an aqueous solution on palladium/charcoal catalyst. The autocatalytic effect of the acid group in compound *IXb* plays an important role in accelerating this reaction which does not require addition of any external acid. 9-(3-Amino-2-phosphonylmethoxypropyl)adenine (*X*) 3-amino analog of HPMPA, was isolated by ion exchange chromatography. In addition to the ^{1}H NMR spectrum, the structure of this product was verified by its electrophoretic behaviour (the lower mobility than that of compound *IVb* at neutral pH reflects the presence of additional strongly basic amino



SCHEME 1

group) and by positive ninhydrin reaction proving the occurence of primary amino group at the side-chain (adenine amino group, e.g. in compound *IXb*, gives a slight color response in this reaction).

The above synthetic procedure enables to synthesize in large quantities the azido (*IXb*) and amino analogs of HPMPA (*X*) which are not only interesting as potential antivirals but can also serve as suitable intermediates for further syntheses. The compounds are racemates; however, they can be used to obtain the essential biological information. The same sequence would be applicable for the preparation of the corresponding enantiomers from optically active compounds *V*.

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 dried over phosphorus pentoxide at 10—3 Pa. Chromatography on silica gel sheets (Silufol UV 254, Kavalier, Czechoslovakia) was performed in chloroform (S1) or chloroform–methanol 4 : 1 (S2), paper chromatography on a Whatman No. 1 paper in 2-propanol–conc. ammonia–water (7 : 1 : 2) (S3), HPLC on a 200 × 4 mm column packed with Separon SGX C18 (5μ) in 0·05M triethylammonium hydrogen carbonate pH 7·5, containing the following amount (v/v) of acetonitrile: 2% (S4), 4% (S5), 6% (S6) and 9% (S7). Paper electrophoreses were carried out in the same buffer on a Whatman No. 3 MM paper at 20 V/cm. The electrophoretic mobilities (E_{Up}) refer to uridine 3′-phosphate. Proton NMR spectra were measured on a Varian XL 200 instrument in hexadeuterodimethyl sulfoxide (unless stated otherwise) with tetramethylsilane as internal standard; chemical shifts are given in ppm, coupling constants in Hz.

Chemicals and reagents. Silica gel for column chromatography (30—40μ) was the product of Service Laboratories of the Institute. Dimethylformamide and acetonitrile were dried by distillation from phosphorus pentoxide, pyridine by calcium hydride and distillation. The solvents were kept over molecular sieves. 1-Chloro-2,3-epoxypropane and sodium azide were obtained from Lachema (Czechoslovakia), benzoyl chloride, chlorotrimethylsilane, dihydropyran and 10% Pd/C catalyst were Merck (F.R.G.) products, and adenine and bromotrimethylsilane were purchased from Janssen (Belgium.)

1-Azido-3-(*p*-toluenesulfonyloxy)propan-2-ol (*IIIa*)

A stirred mixture of 1-chloro-2,3-epoxypropane (92·5 ml; 1 mol), water (60 ml) and Dowex 50X8 (H^+ form; 25 ml) was refluxed (bath temperature 120°C) for 18 h, filtered and washed with water (50 ml). The filtrate was taken down in vacuo and the residue was distilled, affording 85·0 g (77%) of 1-chloropropane-2,3-diol (*IIa*), b.p. 126—130°C/2 kPa. A solution of this product (0·77 mol) in water (300 ml) was refluxed with sodium azide (84·5 g; 1·3 mol) for 1 h. After cooling, the mixture was taken down in vacuo, the residue was suspended in acetone (120 ml), filtered, the solid was washed with acetone (2 × 120 ml) and the combined filtrates were evaporated in vacuo. The residue was dissolved in ether (300 ml), the solution was dried over magnesium sulfate, filtered and the ether was evaporated in vacuo. Distillation of the residue in vacuo (bath temperature up to 120°C) afforded 78·3 g (89%) of 1-azidopropane-2,3-diol (*IIb*), b.p. 86°C/6 Pa.

A solution of *p*-toluenesulfonyl chloride (95·3 g; 0·5 mol) in acetonitrile (300 ml) was added under ice cooling to a stired solution of *IIb* (58·5 g; 0·5 mol) and pyridine (44·5 ml; 0·55 mol)

in acetonitrile (200 ml). The mixture was set aside at 0°C for 3 days, water (20 ml) was added and, after standing for 1 h at room temperature, the mixture was concentrated to a half. The residue was diluted with ethyl acetate (500 ml), washed with water (3 × 100 ml) and dried over magnesium sulfate. After evaporation in vacuo, the residue was chromatographed on a column of silica gel (400 ml) in chloroform. The combined product fractions were taken down and the product was dried in vacuo; yield 90 g (66%) of *IIIa* as colourless oil, R_F 0·12 (S1). For $C_{10}H_{13}N_3 . SO_4$ (271·3) calculated: 44·27% C, 4·83% H, 15·49% N, 11·82% S; found: 44·43% C, 4·86% H, 15·52% N, 11·64% S. ^{1}H NMR spectrum: 2·40 s, 3 H (CH_3); 3·24 brd, 2 H, J = 6·0 (NCH_2); 3·93 m, 3 H (OCH_2 + OCH); 5·64 br, 1 H (OH); 7·46 + 7·81, 4 H (arom. protons).

1-Azido-2-(tetrahydropyran-2-yloxy)-3-(*p*-toluenesulfonyloxy)propane (*IIIb*)

To a stirred mixture of *IIIa* (81·0 g; 0·3 mol), dihydropyran (40 ml; 37 g; 0·44 mol) and dioxane (150 ml) was added 4M-HCl in dimethylformamide (5 ml) under ice-cooling. The stirring at 0°C was continued until the starting compound disappeared (5 h, monitored in S1). The mixture was made alkaline with triethylamine, taken down in vacuo and the residue was dissolved in ethyl acetate (500 ml), washed with water (3 × 100 ml) and dried over magnesium sulfate. Evaporation in vacuo and drying afforded 101 g (95%) of chromatographically homogeneous product *IIIb* as a yellowish oil which was used in the next reaction.

An aliquot of *IIIb* (5·0 g) was chromatographed on a column of silica gel (100 g) in chloroform to give 4·5 g of the product, R_F 0·47 (S1). For $C_{15}H_{21}N_3O_5S$ (355·4) calculated: 50·69% C, 5·96% H, 11·83% N, 9·02% S; found: 50·67% C, 6·20% H, 12·01% N, 8·93% S.

9-(3-Azido-2-(tetrahydropyran-2-yloxy)propyl)adenine (*IV*)

Sodium hydride (3·6 g; 0·15 mol) was added to a suspension of adenine (20·25 g; 0·15 mol) in dimethylformamide (700 ml) and the stirred mixture was heated to 80°C for 1 h under exclusion of moisture. A solution of *IIIb* (53·3 g; 0·15 mol) in dimethylformamide (50 ml) was added and the mixture was stirred at 100°C for 12 h. After evaporation at 50°C/2 kPa and codistillation with toluene (2 × 200 ml), the residue was extracted with boiling chloroform (4 × 300 ml), filtered, taken down in vacuo and chromatographed on a column of silica gel (400 ml) in chloroform. The product fractions were taken down and crystallized from ethyl acetate (with light petroleum added to turbidity) to give 32·0 g (67%) of *IV*, m.p. 150–152°C. R_F 0·65 (S2). For $C_{13}H_{18}N_8O_2$ (318·4) calculated: 49·04% C, 5·70% H, 35·20% N; found: 49·17% C, 5·61% H, 35·47% N. ^{1}H NMR spectrum ($CDCl_3$): 1·50 m, 6 H; 3·05–3·65 m, 4 H; 4·0–4·50 m, 4 H; 6·11 br, 2 H (NH_2); 7·84 s, 1 H (H-2); 8·36 s, 1 H (H-8) (1 : 1 diastereoisomeric mixture).

9-(3-Azido-2-hydroxypropyl)adenine (*V*)

A mixture of compound *IV* (31·8 g; 0·1 mol) and 0·25M-H_2SO_4 was stirred to homogeneity and heated to 80°C for 48 h. After neutralization with saturated solution of barium hydroxide, the hot mixture was filtered through Celite and the solid was washed with boiling water (2 000 ml). The filtrate was concentrated in vacuo to about 200 ml and cooled in ice. The separated product was collected, washed with water, acetone, ether and dried in vacuo. Another portion of the pure product was obtained from the mother liquor; total yield 21·7 g (86%) of compound *V*, m.p. 189–190°C, R_F 0·30 (S2), 0·71 (S3). HPLC: k 7·0 (S6). For $C_8H_{10}N_8O.H_2O$ (252·3) calculated: 38·08% C, 4·30% H, 47·85% N; found: 38·14% C, 4·05% H, 44·52% N. ^{1}H NMR spectrum: 3·30 m, 2 H (N_3CH_2); 4·14 m, 3 H (NCH_2 + OCH); 5·64 d, 1 H, J(OH, CH) = 4·7 (OH); 7·19 br, 2 H (NH_2); 8·05 s, 1 H (H-2); 8·13 s, 1 H (H-8).

9-(3-Amino-2-hydroxypropyl)adenine (*VI*)

Compound *V* (3·8 g; 15 mmol) in ethanol (400 ml) was hydrogenated on 10% Pd/C (Merck; 1·0 g) in the presence of conc. hydrochloric acid (4 ml) at room temperature and atmospheric pressure for 20 h. After filtration through Celite and washing the catalyst successively with water (200 ml), methanol and water, the mixture was neutralized with aqueous ammonia and taken down in vacuo. The residue in water (50 ml) was applied onto a column of Dowex 50X8 (H^+ form, 200 ml). The column was washed with water to drop of UV-absorption and conductivity to the original values and then the product was eluted with 2·5% aqueous ammonia. The UV-absorbing eluate was taken down in vacuo, the residue was dissolved in water (25 ml), applied onto a column of octadecylsilica gel (200 ml) and eluted with water (3 ml/min). The main UV-absorbing aqueous eluate was evaporated in vacuo, the residue was codistilled with ethanol (100 ml) and crystallized from 90% ethanol (ether added to turbidity) to give 2·2 g (65%) of *VI* monohydrate, m.p. 167°C, identical with an authentic specimen (ref.[9]) (R_F 0·42 in S3; HPLC: k 3·03 in S4).

9-(3-Azido-2-hydroxypropyl)-N^6-benzoyladenine (*VII*)

A suspension of compound *V* (12·6 g; 50 mmol) in pyridine (200 ml) was taken down in vacuo and the residue was resuspended in the same solvent (250 ml). Chlorotrimethylsilane (32 ml; 0·25 mol) was added with stirring. The mixture was stirred for 30 min, benzoyl chloride (29 ml; 0·25 mol) was added and the stirring was continued for 2 h under exclusion of moisture. After cooling with ice, the mixture was decomposed with ice-cold water (50 ml) and then with conc. aqueous ammonia (100 ml), stirred for 30 min and evaporated in vacuo. The residue was mixed with water (400 ml) and extracted with chloroform (5 × 200 ml). The extract was dried over magnesium sulfate, the solvent evaporated in vacuo and the product crystallized from ethyl acetate (ether added to turbidity) to afford 14·8 g (88%) of compound *VII*, m.p. 164°C; R_F 0·65 (S2). For $C_{15}H_{14}N_8O_2$ (338·4) calculated: 53·24% C, 4·17% H, 33·12% N; found: 53·46% C, 4·14% H, 33·03% N. ^{1}H NMR spectrum: 3·36 m, 2 H (N_3CH_2); 4·16 + 4·27 m, 1 H + 2 H (NCH_2 + OCH); 5·69 d, 1 H, J(OH, CH) = 4·3 (OH); 7·40—7·70 m, 3 H + 8·0—8·15 m, 2 H (arom. protons); 8·41 s, 1 H (H-2); 8·73 s, 1 H (H-8); 10·77 br, 1 H (NH).

9-(3-Azido-2-(methoxyphosphonylmethoxy)propyl)adenine (*IXa*)

Sodium hydride (2·16 g; 90 mmol) was added to a solution of compound *VII* (10·15 g; 30 mmol) in dimethylformamide (120 ml). After stirring at room temperature for 1 h, dimethyl *p*-toluenesulfonyloxymethanephosphonate (*VIII*; 8·9 g; 30 mmol) was added and the mixture was stirred at room temperature for 3 days. The solvent was evaporated in vacuo, the residue dissolved in methanol (150 ml), and set aside overnight. The mixture was neutralized by addition of Dowex 50X8 (H^+ form), made alkaline with triethylamine, filtered and the solid on the filter washed with methanol. After evaporation in vacuo, the residue was triturated with ether (3× 50 ml) decanted, and allowed to stand overnight with 0·5M-NaOH (200 ml). The mixture was again neutralized with Dowex 50X8 (H^+ form), made alkaline with aqueous ammonia, filtered and extracted with ether (3 × 50 ml). The aqueous phase was concentrated in vacuo to about 50 ml and applied onto a column of Dowex 50X8 (H^+ form, 300 ml). After washing the column successively with water and 50% methanol (1 000 ml of each), the product was eluted with 2·5% aqueous ammonia (1 500 ml), the eluate evaporated in vacuo, the residue dissolved in water (25 ml), made alkaline with ammonia and applied onto a column of Dowex 1X2 (acetate form, 250 ml). The elution was carried out with a linear gradient (à 2 000 ml) 0—1M of acetic acid (2·5 ml/min). The product fraction was taken down in vacuo and the residue was codistilled with water (3 × 50 ml) and dissolved in a minimum volume of boiling water. A five-fold volume of

acetone was gradually added, followed by ether (added to turbidity). The product *IXa* which crystallized on standing in a refrigerator, was collected, washed with ether and dried in vacuo; yield 4·8 g (47%), m.p. 233°C, R_F 0·57 (S3), E_{Up} 0·45; HPLC; k 2·16 (S7), 2·12 (S5). For $C_{10}H_{15}N_8O_4P$ (342·3) calculated: 35·08% C, 4·42% H, 32·74% N, 9·07% P; found: 34·96% C, 4·65% H, 32·83% N, 8·85% P. ^{1}H NMR spectrum (D_2O + NaOD): 3·38 d, 3 H, J(P, OCH) = 10·6 ($POCH_3$); 3·40–3·85 m, 4 H (N_3CH_2 + PCH_2); 3·99 br pent, 1 H (CHO); 4·45 d, 2 H, J(1′, 2′) = = 5·0 (NCH_2); 8·21 s, 2 H (H-2 + H-8).

9-(3-Azido-2-phosphonylmethoxypropyl)adenine (*IXb*)

Bromotrimethylsilane (4 ml; c. 30 mmol) was added to a suspension of compound *IXa* (3·42 g· 10 mmol) in acetonitrile (50 ml) and the solution was set aside overnight in a stoppered flask. The mixture was taken down in vacuo, the residue codistilled with acetonitrile (2 × 25 ml), dissolved in water (100 ml) and made alkaline with conc. aqueous ammonia. After standing for 1 h, the mixture was filtered through Celite, the filtrate was concentrated in vacuo to about 25 ml, made alkaline with ammonia and the solution was applied onto a column of Dowex 1X2 (acetate form; 200 ml). The column was washed with water to drop of UV absorption to the original value and then with a linear gradient (à 2 000 ml) of 0–1M acetic acid. The product was eluted at molarities 0·4–0·6. Evaporation in vacuo, codistillation with water (3 × 25 ml) and crystallization (as described for *IXa*) afforded 2·8 g (85%) of compound *IXb*, m.p. 213–215°C; R_F 0·18 (S3); HPLC: k 0·63 (S7), 0·54 (S5). For $C_9H_{13}N_8O_4P$ (328·3) calculated: 32·92% C, 3·99% H, 34·14% N, 9·45% P; found: 32·87% C, 4·13% H, 34·33% N, 9·26% P. ^{1}H NMR spectrum (D_2O + NaOD): 3·36 dd + 3·58 dd, 2 H, J(3′, 2′) = 5·0, J(gem) = −14·0 (N_3CH_2); 3·59 d, J(P, CH) = 9·5 (PCH_2); 3·98 br pent, 1 H (OCH); 4·45 d, J(1′, 2′) = 5·0 (NCH_2); 8·21 s, 1 H (H-8); 8·32 s, 1 H (H-2).

9-(3-Amino-2-phosphonylmethoxypropyl)adenine (*X*)

Compound *IXb* (2·63 g; 8 mmol) was hydrogenated in water (250 ml) on 10% Pd/C (1·0 g) at room temperature for 20 h. The mixture was made alkaline with aqueous ammonia and filtered through Celite which was then washed with 1% aqueous ammonia (200 ml). The filtrate was concentrated in vacuo to about 25 ml, made alkaline with ammonia, applied onto a column of Dowex 1X2 (acetate form, 150 ml) and eluted with water. The pure product fractions were taken down and crystallized from water. The collected product was washed with ethanol and ether and dried in vacuo; yield 2·1 g (87%) of chromatographically pure compound *X*, not melting up to 280°C. R_F 0·13 (S3), E_{Up} 0·38 (ninhydrin-positive); HPLC: k 0·40 (S5). For $C_9H_{15}N_6O_4P$ (302·3) calculated: 35·75% C, 5·00% H, 27·80% N, 10·27% P; found: 35·54% C, 5·12% H, 27·95% N, 10·43% P.

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