SYNTHESIS OF 8-AMINO- AND *N*-SUBSTITUTED 8-AMINOADENINE DERIVATIVES OF ACYCLIC NUCLEOSIDE AND NUCLEOTIDE ANALOGS

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8-Aminoadenine derivatives **2** were obtained from 8-bromoadenines **1** in one-pot reaction *via* 8-azidoadenines. Reaction of 8-bromoadenines **1** with methylamine or dimethylamine in ethanol afforded the corresponding N^9-substituted 8-(methylamino)adenines **3** and 8-(dimethylamino)adenines **4**. Alkylation of 8-aminoadenine (**2a**) with diverse alkylation agents afforded N^9-substituted 8-aminoadenine derivatives **2**, and alkylation of 8-(dimethylamino)adenine (**4a**) gave mixtures of the corresponding N^9-substituted 8-(dimethylamino)adenines **4** and their N^3-substituted regioisomers **5**. 8,3′-*N*-Anhydro derivatives **7** were prepared by tosylation of (*S*)-8-bromo-9-{2-[(diisopropoxyphosphoryl)-methoxy]-3-hydroxypropyl}adenine (**1c**) followed by treatment with methanolic ammonia or methylamine solution.

Keywords: Purines; Acyclic nucleotide analogues; Amines; Alkylation; *N*-Cyclonucleosides; Nucleosides; Antivirals.

The importance of 8-substituted purine derivatives in nucleoside and nucleotide chemistry and their potency as biologically active compounds is well-known. Of them, 8-amino derivatives (*e.g.* 8-aminoguanosine[1] or 8-amino-9-benzylguanine[2]) are potential immunomodulators. 8-Aminoadenosine, which is resistant to adenosine deaminase, inhibits growth of sarcoma 180 ascites cells[3]. 8-Aminopurine nucleosides also attract interest because of their structural similarity to a paralytic marine toxin, saxitoxin[4].

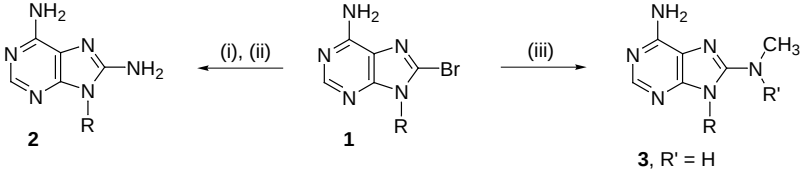
This work is a continuation of the structure–biological activity relationship (SAR) studies in the series of *N*-(2,3-dihydroxypropyl) (DHP), *N*-[2-(phosphonomethoxy)ethyl] (PME), and (*S*)-*N*-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) derivatives of heterocyclic bases[5]. The synthesis of the DHP, PME and HPMP derivatives with various substituents in position 8 of adenine was undertaken in order to obtain information about the effect of substitution in this position on the biological activity in these series.

Our recent studies were aimed at compounds derived from 8-hydroxyadenine (6-amino-7*H*-purine-8(9*H*)-one)[6], 8-sulfanyladenine (6-amino-7*H*-purine-8(9*H*)-thione)[7], and 8-(methylsulfanyl)adenine[7]. This work deals with analogous compounds bearing amino or substituted amino group in position 8 of the purine base. It is continuation of a recent preliminary study dealing with 8-aminoadenine derivatives made in this Laboratory[8].

RESULTS AND DISCUSSION

In analogy to the previous papers mentioned[6,7], two principal approaches for the synthesis of 8-amino derivatives were used in this study: (i) modification of the corresponding acyclic nucleotide derivatives in position 8 of the purine moiety or (ii) preparation of the 8-amino and 8-(dimethylamino) adenine base and their subsequent alkylation with a suitable reagent. Diverse alkylation agents were used in order to investigate regioselectivity of the alkylations: methyl tosylate, diisopropyl [(2-chloroethoxy)methyl]phosphonate (PME reagent)[9] and (*S*)-[(trityloxy)methyl]-oxirane [(*S*)-*O*-tritylglycidol], in DMF and with sodium hydride or cesium carbonate as a base.

8-Aminoadenine derivatives of acyclic nucleotide analogs **2** were prepared in one-pot reaction *via* 8-azido derivatives in satisfactory yields[10]: Treatment of the corresponding 8-bromoadenine derivatives **1** with lithium azide in DMF at 110 °C followed by catalytic hydrogenation in methanol at room temperature afforded 8-aminoadenine compounds **2** (Scheme 1)[11].



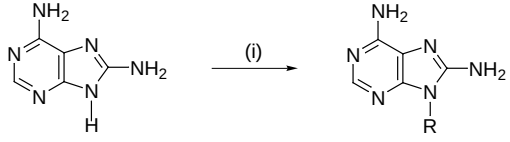
(i) LiN_3, DMF, 110°C; (ii) H_2, Pd/C, MeOH; (iii) CH_3NH_2 or $(CH_3)_2NH$/EtOH, 100°C; (iv) TMSBr, MeCN

SCHEME 1

Synthesis of compounds containing methylamino or dimethylamino group in position 8 of the purine moiety was performed by the reaction of the appropriate 8-bromo derivative **1** with solution of methylamine or dimethylamine in ethanol in an autoclave at 100 °C. The phosphonate diesters afforded by cleavage under standard conditions using bromo(trimethyl)silane (TMSBr) in acetonitrile followed by hydrolysis the free phosphonates which were isolated by ion-exchange chromatography.

The modified adenine bases needed for subsequent alkylations were prepared under similar reaction conditions as mentioned for transformation of acyclic nucleotide analogs (Scheme 1). 8-Bromoadenine (**1a**) gave, on treatment with lithium azide in DMF followed by catalytic hydrogenation, 8-aminoadenine (**2a**) in 51% overall yield. So far, 8-aminoadenine was prepared by other methods and characterized as its salts[12]. 8-(Dimethylamino)adenine (**4a**) was obtained by the reaction of the bromo derivative **1a** with ethanolic dimethylamine in an autoclave (yield 90%). Alternatively, compound **4a** was also prepared by heating of **1a** with benzylamine in DMF (ref.[8]). In this case, the reaction took about half of the time needed for the above mentioned procedure while the yield of 8-(dimethylamino)adenine amounted to 66%. This reaction can be explained by transfer of formyl group from DMF to benzylamine followed by reaction of liberated dimethylamine with 8-bromoadenine (**1a**).

In the alkylation reaction of 8-aminoadenine (**2a**) with the above alkylation reagents, the N^9-subtituted derivatives were successfully isolated from the reaction mixture as the only regioisomers (Scheme 2). Thus, the N^9-methyl derivative **2f**, the PME derivative **2b** identical with the authentic



2a **2b,d,f-h**

2f, R = CH_3
2b, R = $CH_2CH_2OCH_2P(O)(OiPr)_2$
2d, R = $CH_2CH_2OCH_2P(O)(OH)_2$
2g, R = (*S*)-$CH_2CH(OH)CH_2OTr$
2h, R = (*S*)-$CH_2CH(OH)CH_2OH$

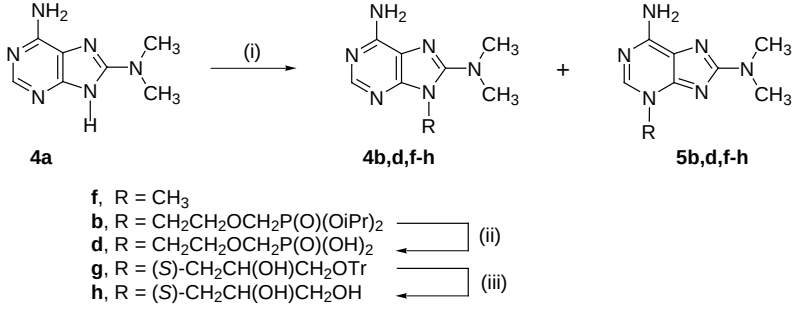
(ii)
(iii)

(if) MeOTs, DMF, NaH; (ib) $ClCH_2CH_2OCH_2P(O)(OiPr)_2$, DMF, NaH;
(ig) (*S*)-O-tritylglycidol, DMF, Cs_2CO_3; (ii) TMSBr, MeCN; (iii) Dowex 50 (H^+), 50% aq. MeOH

SCHEME 2

compound prepared by base modification of the corresponding acyclic nucleotide analog (Scheme 1), and (*S*)-8-amino-9-(2,3-dihydroxypropyl)adenine (**2h**) (ref.[13]; deals with the racemic compound prepared by different procedures) after deprotection of the trityl derivative **2g** were obtained.

Contrary to compound **2a**, alkylation of 8-(dimethylamino)adenine (**4a**) gave a mixture of N^9-substituted derivatives **4** and N^3-substituted derivatives **5** in an approximate ratio 1 : 1 to 1 : 2 (Scheme 3). With sodium hydride as a base for alkylation, N^3-alkyl isomers were isolated as salts: compound **5f** as tosylate and compound **5b** as hydrochloride. The trityl derivatives **4g** and **5g** gave on acid deprotection compounds **4h** and **5h**, respectively.



(if) MeOTs, DMF, NaH; (ib) $ClCH_2CH_2OCH_2P(O)(OiPr)_2$, DMF, NaH;
(ig) (*S*)-*O*-tritylglycidol, DMF, Cs_2CO_3; (ii) TMSBr, MeCN; (iii) Dowex 50 (H^+), 50% aq. MeOH

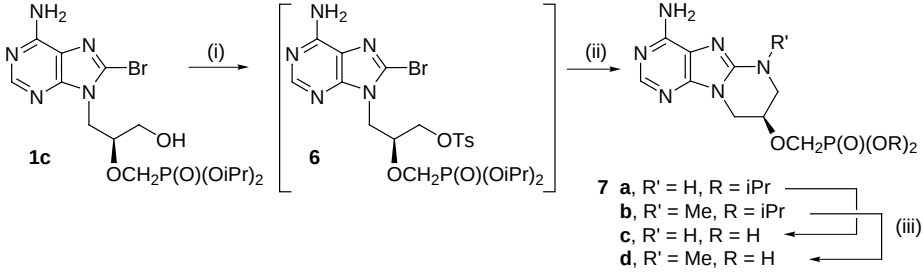
SCHEME 3

Syntheses of numerous purine *N*-cyclonucleosides were reported in the literature[14]. Starting compounds for the cyclizations were 8-bromopurine nucleosides bearing a leaving group (Ms or Ts) on a carbohydrate moiety.

In the preceding studies[6,7], we have described the preparation of the acyclic nucleotide analogs derived from HPMPA, with *O*-anhydro or *S*-anhydro linkage. Analogous compounds containing NH-anhydro or $N(CH_3)$-anhydro linkages **7c** and **7d**, respectively, were also obtained in moderate overall yields (17 and 26%, respectively) by tosylation of the bromo derivative **1c** followed by treatment of the crude mixture with alcoholic ammonia or methylamine, respectively, in an autoclave followed by cleavage of the resulting diesters **7a** and **7b** (Scheme 4).

The ultimate cleavage of phosphonate diesters **2b**, **2c**, **3b**, **3c**, **4b**, **4c**, **5b**, **7a**, and **7b** was achieved by standard treatment with bromo(trimethyl)sil-

ane (TMSBr) in acetonitrile followed by hydrolysis. The isolation of the free phosphonates **2d**, **2e**, **3d**, **3e**, **4d**, **4e**, **5d**, **7c**, and **7d** was achieved by deionisation and ion-exchange chromatography. The purified compounds were finally crystallized from water.



(i) TsCl, pyridine, 0°C, 2 days; (ii) NH_3/MeOH or CH_3NH_2/EtOH, 80°C; (iii) TMSBr, MeCN

SCHEME 4

All new compounds were fully characterized by ^{1}H NMR (and ^{13}C NMR), MS and HR-MS or microanalysis. The structures of the compounds prepared by alkylation of starting compounds **2a** and **4a** were determined on the basis of proton-coupled ^{13}C NMR spectra, and N^9-substituted derivatives **2b** and **4b** were compared with the authentic materials prepared by the modification of acyclic nucleotide analogs in position 8 of the purine moiety.

An example of proton-coupled ^{13}C NMR spectra is given for the pair of isomers **4h** and **5h** obtained from 8-(dimethylamino)adenine (**4a**) by alkylation with (*S*)-[(trityloxy)methyl]oxirane after detritylation (Scheme 3). The structure of the N^9-alkyl derivative **4h** was confirmed by the signal multiplicity: doublets of C-2 (δ 150.33, ^1J(C-2,H-2) = 198.2) and C-6 (δ 153.70, ^3J(C-6,H-2) = 11.7), triplet of C-5 (δ 116.38, ^3J(C-5,NH_2) = 3.9), doublet of triplet of C-4 (δ 151.31, ^3J(C-4,H-2) = 11.7, ^3J(C-4,H-1′) = 2.9), and multiplet of C-8 (δ 156.03, ^3J(C-8,CH_3) = ^3J(C-8,H-1′) = 2.9). On the other hand, the structure of the N^3-alkyl derivative **5h** was determined by the presence of alkylation effects (upfield shifts (–9 ppm) at C-2 and (–5 ppm) at C-6 and downfield shifts (9 ppm) at C-8 and (5 ppm) at C-1′) and by the change in the signal multiplicity of C-2 (δ 141.23, ^1J(C-2,H-2) = 209.0, ^3J(C-2,H-1′) = 4.9), C-4 (δ 151.99, ^3J(C-4,H-2) = 6.8, ^3J(C-4,H-1′) = 3.9), and C-8 (δ 164.79, ^3J(C-8,CH_3) = 2.9). Similarly, the N^9-alkyl derivative **2h** prepared by the alkylation of 8-aminoadenine (**2a**) (Scheme 2) is characterized by the following signal multiplicity: doublets of C-2 (δ 148.72, ^1J(C-2,H-2) =

198.25) and C-6 (δ 152.23, ^3J(C-6,H-2) = 10.7), triplets of C-5 (δ 116.99, ^3J(C-5,NH_2) = 3.9) and C-8 (δ 152.84, ^3J(C-8,H-1′) = 2.9), and doublet of triplet of C-4 (δ 150.18, ^3J(C-4,H-2) = 10.7, ^3J(C-4,H-1′) = 3.9).

Hydrochloride **5b** and tosylate **5f** gave appropriate elemental analysis. Singlets of NH group were observed in ^{1}H NMR spectra: 12.01 for compound **5b** and 11.60 for compound **5f**. Protonation at the nitrogen atom N^7 follows from the significantly altered chemical shifts of C-5 (δ 108.275 and 108.24) and C-8 (δ 157.17 and 157.39) in the ^{13}C NMR spectra compared with *e.g.* the N^3-alkyl derivative **5h** (C-5 (δ 118.53) and C-8 (δ 164.79)).

The UV spectra constitute additional evidence of the substituent type in position 8 of the purine moiety as well as of the character of the corresponding alkyl regioisomers. Thus, it is possible to distinguish between (average values of λ_{max} and ε_{max} are given, respectively): N^9-substituted 8-aminoadenine derivatives **2** (pH 2, 271 nm, 13 000; pH 12, 274 nm, 15 000); N^9-substituted derivatives of 8-(methylamino)adenine **3** (pH 2, 274 nm, 12 700; pH 12, 279 nm, 15 400); N^9-substituted derivatives of 8-(dimethylamino)adenine **4** (pH 2, 281 nm, 14 000; pH 12, 278 nm, 15 000); and N^3-substituted derivatives of 8-(dimethylamino)adenine **5** (pH 2, 307 nm, 23 100 and 235 nm, 21 200; pH 12, 321 nm, 16 300 and 235 nm, 11 400).

The CD spectra of enantiomers of the chiral acyclic nucleoside and nucleotide analogs **2e**, **2h**, **3e**, **4e**, **4h**, and **5h** do not exhibit a marked Cotton effect; this reflects the flexible character of the aliphatic side chain bearing a chiral carbon atom. However, in analogy to the *O*-anhydro and *S*-anhydro derivatives of HPMPA (refs[6,7]), the CD spectra of the NH-anhydro derivative **7c** and the N(CH_3)-anhydro derivative **7d** containing rigid tricyclic systems show significant Cotton bands: (λ (Δε)): 283 (1.24), 219 (–6.48) and 195 (–3.10) for compound **7c**, and 282 (–3.40), 223 (3.04) and 201 (–1.17) for **7d**. Inverse Cotton bands around the approximately similar wavelength are evidence of the essentially different conformation of the new rings in compounds **7c** and **7d**. Thus, the character of the substitution at the nitrogen atom of the anhydro derivatives affects the resulting conformation of the molecule.

In conclusion, two independent methods were used for preparation of alkylated adenines bearing amino (or substituted amino) group in position 8: (i) syntheses of 8-aminoadenines **2**, 8-(methylamino)adenines **3**, and 8-(dimethylamino)adenines **4** from the corresponding 8-bromoadenines **1**, (ii) alkylation of 8-aminoadenine (**2a**) and 8-(dimethylamino)adenine (**4a**) with diverse alkylation agents. The former method is direct and convenient, affording good yields of products. The yields of the base alkylations

are generally lower; two regioisomers are formed in an approximately equimolar ratio in the alkylations of 8-(dimethylamino)adenine (**4a**): N^3-alkyl derivatives **5** and N^9-alkyl derivatives **4**. The N^9-substituted analogs **2b** and **4b** were prepared by two independent routes. 8,3′-*N*-Anhydro derivatives **7** were also prepared. Biological activities of the final compounds will be examined.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi Melting Point B-545 and are uncorrected. Analytical TLC were performed on Silufol UV 254 plates (Kavalier Votice, Czech Republic) in the systems chloroform–methanol (9 : 1) (S1), chloroform–methanol (85 : 15) (S2), chloroform–methanol (8 : 2) (S3), water–ethanol–acetone–ethyl acetate (1 : 1 : 1 : 4) (S4), isopropyl alcohol–ammonia–water (7 : 1 : 2) (S5). Preparative TLC were carried out on 40 × 17 × 0.4 cm loose-layer plates of silica gel containing UV indicator (made in the Service Laboratory of the Institute). Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogencarbonate (TEAB) at pH 7.5; the electrophoretical mobilities are referenced to uridine 3′-phosphate. NMR spectra were measured on Varian Unity 500 spectrometer (500 MHz for ^{1}H and 125.7 MHz for ^{13}C NMR) in hexadeuteriodimethyl sulfoxide (DMSO-d_6) referenced to the solvent signals (2.5 ppm for ^{1}H and 39.7 ppm for ^{13}C NMR), or in deuterium oxide containing sodium deuteroxide with sodium 3-(trimethylsilyl)propane-1-sulfonate as an internal standard for ^{1}H NMR and dioxane as an external standard for ^{13}C NMR (δ(dioxane) 66.86 ppm). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). UV absorption spectra were measured on a UV mini-1240 Shimadzu spectrometr, CD spectra on a Jobin–Yvon–Mark V instrument, and IR spectra on a FT-IR spectrometr Bruker IFS 88.

Starting Materials and Reagents

Bromo(trimethyl)silane, cesium carbonate, and methylamine and dimethylamine solutions were purchased from Fluka (Switzerland), methyl tosylate from Aldrich. Dimethylformamide was distilled from P_2O_5 and stored over molecular sieves (4 Å). Acetonitrile was refluxed with CaH_2 and distilled over molecular sieves (4 Å).

8-Aminoadenine Derivatives **2**. General Procedure

A mixture of the corresponding 8-bromo derivative[6] **1** (1.5 mmol), lithium azide (4.5 mmol) and DMF (15 ml) was heated to 100 °C for 7 h. The mixture was evaporated *in vacuo*, and the residue was codistilled with toluene (2 × 15 ml) and methanol (2 × 15 ml). The residue was hydrogenated in methanol (60 ml) in the presence of 5% Pd-C (0.3 g) at 25 °C overnight. The mixture was then filtered through a Celite layer and the solvent was evaporated. The residue was purified by preparative TLC in chloroform–methanol (5 : 1) and crystallized from mixture ethanol–ether.

8-Amino-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}adenine (**2b**). White crystals, m.p. 146 °C, yield 40%, R_F 0.28 (S3). FAB MS, *m/z* (rel.%): 373 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 1.12 (d, 6 H, J(CH_3,CH) = 6.1, CH_3); 1.17 (d, 6 H, J(CH_3,CH) = 6.1, CH_3); 3.79 (d, 2 H, J(P,CH) = 8.3, PCH_2); 3.80 (t, 2 H, J(2′,1′) = 5.3, H-2′); 4.14 (t, 2 H, J(1′,2′) = 5.3, H-1′); 4.49 (m, 2 H, POCH); 6.41 (brs, 2 H, NH_2); 6.45 (brs, 2 H, NH_2); 7.90 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 23.76 (d, 2 C, J(P,C) = 4.9, CH_3); 23.92 (d, 2 C, J(P,C) = 3.9, CH_3); 40.20 (C-1′); 64.86 (d, J(P,C) = 164.1, PC); 70.06 (d, J(P,C) = 11.7, C-2′); 70.38 (d, 2 C, J(P,C) = 5.9, POC); 117.06 (C-5); 148.73 (C-2); 150.10 (C-4); 152.15 (C-6); 152.165 (C-8). For $C_{14}H_{25}N_6O_4P$ (372.4) calculated: 45.16% C, 6.77% H, 22.57% N, 8.32% P; found: 45.08% C, 6.87% H, 22.28% N, 8.60% P.

(S)-8-Amino-9-{2-[(diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}adenine (**2c**). White crystals, m.p. 158 °C, yield 58%, R_F 0.24 (S2). FAB MS, *m/z* (rel.%): 403 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 1.15 (d, 3 H, J(CH_3,CH) = 6.1, CH_3); 1.18 (d, 6 H, J(CH_3,CH) = 6.1, CH_3); 1.21 (d, 3 H, J(CH_3,CH) = 6.1, CH_3); 3.47 (m, 2 H, H-3′); 3.82 (m, 1 H, H-2′); 3.84 (dd, 1 H, J(P,CHb) = 8.6, J(gem) = 13.9, PCHb); 3.91 (dd, 1 H, J(P,CHa) = 8.6, J(gem) = 13.9, PCHa); 4.03 (dd, 1 H, J(1′b,2′) = 5.4, J(gem) = 15.1, H-1′b); 4.07 (dd, 1 H, J(1′a,2′) = 6.3, J(gem) = 15.1, H-1′a); 4.52 (m, 2 H, POCH); 5.07 (brs, 1 H, OH); 6.36 (brs, 2 H, NH_2); 6.47 (brs, 2 H, NH_2); 7.91 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 23.75 (d, J(P,C) = 4.9, CH_3); 23.81 (d, J(P,C) = 4.9, CH_3); 23.90 (d, J(P,C) = 3.9, CH_3); 23.96 (d, J(P,C) = 3.9, CH_3); 41.71 (C-1′); 60.52 (C-3′); 64.01 (d, J(P,C) = 165.0, PC); 70.45 (d, J(P,C) = 6.9, POC); 70.50 (d, J(P,C) = 5.9, POC); 80.24 (d, J(P,C) = 10.8, C-2′); 116.80 (C-5); 148.85 (C-2); 150.17 (C-4); 152.21 (C-6); 152.46 (C-8). For $C_{15}H_{27}N_6O_5P$ (402.4) calculated: 44.77% C, 6.76% H, 20.89% N, 7.70% P; found: 44.58% C, 6.56% H, 20.68% N, 7.53% P.

8-Aminoadenine (**2a**)

The procedure is the same as described for compounds **2b** and **2c**. The residue after hydrogenation was dissolved in water and applied onto a column of Dowex 50X8 (H^+ form, 50 ml), washed with water and eluted with 5% aqueous ammonia. After evaporation of an appropriate UV absorbing fraction the residue was dried over P_2O_5. Yield 51%. Analytical sample was crystallized from a mixture water–ethanol to afford yellowish crystals of **2a**; m.p. >350 °C, R_F 0.49 (S4). FAB MS, *m/z* (rel.%): 151 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 6.27 (s, 2 H, NH_2); 6.52 (brs, 2 H, NH_2); 7.90 (s, 1 H, H-2); 10.80 (br, 1 H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 114.50 (C-5); 149.30 (C-4); 150.31 (C-2); 152.50 (C-6); 154.78 (C-8). For $C_5H_6N_6$ (150.1) calculated: 40.00% C, 4.03% H, 55.97% N; found: 39.74% C, 4.01% H, 55.55% N. Exact mass (FAB HRMS) found: 151.0732; calculated for $C_5H_7N_6$ [M + H]: 151.0732.

8-(Methylamino) Derivatives **3** and 8-(Dimethylamino) Derivatives **4** of Adenine Acyclic Nucleotide Analogues. General Procedure

A mixture of 8-bromo derivative **1b** or **1c** (2 mmol) and methylamine or dimethylamine in ethanol (33%, 30 ml) was heated in an autoclave at 100 °C for 15 h. The solvent was evaporated, the residue was codistilled with ethanol (2 × 15 ml) and dried over phosphorus pentoxide overnight. The residue was directly treated with TMSBr under standard condition. For the overall yields of products **3d**, **3e**, **4d**, **4e**, see Deprotection of Phosphonates with TMSBr.

8-(Dimethylamino)adenine (**4a**)

Procedure A: A mixture of 8-bromoadenine (**1a**; 3 g, 14 mmol) and dimethylamine solution in ethanol (33%, 60 ml) was heated in autoclave to 120 °C for 18 h. The solid was filtered off, washed with ethanol (3 × 30 ml) and ether (3 × 20 ml). The filtrate was concentrated *in vacuo* and the precipitated solid was again filtered off and washed with ethanol and ether. The combined portions of solid were dried over phosphorus pentoxide to give yellowish powder (2.25 g, 90%) of compound **4a**, decomposition >300 °C, R_F 0.52 (S4). FAB MS, *m/z* (rel.%): 179 (100) [M + H]. ^{1}H NMR spectrum is identical with the authentic material[8]. For $C_7H_{10}N_6$ (178.2) calculated: 47.18% C, 5.66% H, 47.16% N; found: 47.08% C, 5.72% H, 47.04% N.

Procedure B (ref.[8]): A mixture of 8-bromoadenine (**1a**; 3 g, 14 mmol), DMF (80 ml), and benzylamine (5 ml) was heated to 130 °C for 9 h. The solvent was taken down *in vacuo* and codistilled with toluene (3 × 20 ml) and methanol (2 × 20 ml). Crystallization from methanol afforded yellowish crystals (1.62 g, 66%) of compound **4a**, decomposition >300 °C.

Alkylation of 8-Aminoadenine (**2a**) with Methyl Tosylate

A mixture of compound **2a** (0.3 g, 2 mmol), and sodium hydride (80 mg of 60% dispersion, 2 mmol) in DMF (15 ml) was stirred at 100 °C for 0.5 h. Methyl tosylate (0.36 ml, 2.4 mmol) was added and the mixture was stirred at this temperature for another 5 h. The solvent was evaporated and the residue purified by preparative chromatography on a silica gel plate in isopropyl alcohol–ammonia–water (S5) and crystallized from mixture water–ethanol. Yield 0.20 g (61%) of compound **2f**.

8-Amino-9-methyladenine (**2f**). Yellowish crystals, m.p. 182–185 °C, R_F 0.62 (S5). EI MS, *m/z* (rel.%): 164 (100) [M]. FAB MS, *m/z* (rel.%): 165 (80) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 3.43 (s, 3 H, CH_3); 6.40 (brs, 2 H, NH_2); 6.44 (brs, 2 H, NH_2); 7.91 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 27.41 (CH_3); 117.18 (C-5); 148.77 (C-2); 150.15 (C-4); 152.10 (C-6); 152.65 (C-8). Exact mass (FAB HRMS) found: 165.0900; calculated for $C_6H_9N_6$ [M + H]: 165.0889. UV, λ_{max} (ε_{max}) (MeOH): 274 (14 800).

Alkylation of **2a** with Diisopropyl [(2-Chloroethoxy)methyl]phosphonate

A mixture of compound **2a** (0.2 g, 1.3 mmol) and sodium hydride (54 mg of 60% dispersion, 1.3 mmol) in DMF (15 ml) was stirred at 110 °C for 0.5 h. Diisopropyl [(2-chloroethoxy)methyl]phosphonate[9] (0.5 ml, 1.5 mmol) was added and the mixture was stirred for another 10 h. The solvent was evaporated and the residue codistilled with toluene (2 × 15 ml). Preparative chromatography on a silica gel plate in chloroform–methanol mixture (4 : 1) followed by crystallization from ethanol–ether mixture afforded 0.22 g (45%) of compound **2b**.

8-Amino-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}adenine (**2b**). White crystals, m.p. 142–143 °C, R_F 0.28 (S3). FAB MS, ^{1}H NMR and ^{13}C NMR spectra are identical with the authentic compound.

Alkylation of **2a** with (*S*)-[(Trityloxy)methyl]oxirane [(*S*)-*O*-Tritylglycidol]

A mixture of 8-aminoadenine (**2a**; 0.8 g, 5.3 mmol), DMF (30 ml), (*S*)-*O*-tritylglycidol (1.7 g, 5.3 mmol), and cesium carbonate (86 mg, 0.26 mmol) was stirred at 110 °C for 20 h. The

hot suspension was filtered over Celite and the solvent evaporated. Column chromatography of the residue on silica gel followed by crystallization from acetone–methanol mixture gave 1.18 g (47%) of compound **2g**.

(S)-8-Amino-9-[2-hydroxy-3-(trityloxy)propyl]adenine (**2g**). Yellowish crystals, m.p. 145 °C, R_F 0.54 (S3). FAB MS, *m/z* (rel.%): 467 (20) [M + H]; 243 (100) [Tr]. ^{1}H NMR (500 MHz, DMSO-d_6): 2.93 (dd, 1 H, *J*(3′b,2′) = 4.9, *J*(gem) = 9.5, H-3′b); 3.03 (dd, 1 H, *J*(3′a,2′) = 5.4, *J*(gem) = 9.5, H-3′a); 3.90 (dd, 1 H, *J*(1′b,2′) = 7.6, *J*(gem) = 13.9, H-1′b); 4.06 (dd, 1 H, *J*(1′a,2′) = 4.2, *J*(gem) = 13.9, H-1′a); 4.11 (m, 1 H, H-2′); 5.52 (d, 1 H, *J*(OH,2′) = 5.6, OH); 6.24 (brs, 2 H, NH_2); 6.36 (brs, 2 H, NH_2); 7.24 (t, 3 H, arom); 7.31 (t, 6 H, arom); 7.40 (d, 6 H, arom); 7.89 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 44.79 (C-1′); 66.24 (C-3′); 68.63 (C-2′); 86.13 (C-Ph); 117.07 (C-5); 127.12 (3 C, arom); 127.98 (6 C, arom); 128.49 (6 C, arom); 143.90 (3 C, arom); 148.81 (C-2); 150.12 (C-4); 152.14 (C-6); 152.59 (C-8).

(*S*)-8-Amino-9-(2,3-dihydroxypropyl)adenine (**2h**)

A mixture of the trityl derivative **2g** (0.4 g, 0.9 mmol), Dowex 50X8 (H^+) (5 ml), methanol (10 ml), and water (10 ml) was refluxed for 3 h, then filtered while hot and the resin was washed with methanol (2 × 10 ml), ether (3 × 10 ml), and 5% aqueous ammonia. The ammonia fraction was evaporated and the residue crystallized from water to afford white crystals (0.15 g, 78%) of compound **2h**; m.p. 256 °C (ref.[13], not melting up to 260 °C), R_F 0.10 (S3). FAB MS, *m/z* (rel.%): 225 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 3.45 (m, 2 H, H-3′); 3.83 (m, 1 H, H-2′); 3.84 (dd, 1 H, *J*(1′b,2′) = 7.3, *J*(gem) = 13.2, H-1′b); 4.04 (dd, 1 H, *J*(1′a,2′) = 2.5, *J*(gem) = 13.2, H-1′a); 4.92 (t, 1 H, *J*(OH,3′) = 5.7, OH); 5.24 (d, 1 H, *J*(OH,2′) = 4.6, OH); 6.25 (s, 2 H, NH_2); 6.41 (s, 2 H, NH_2); 7.90 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 44.25 (C-1′); 63.46 (C-3′); 70.41 (C-2′); 116.99 (C-5); 148.72 (C-2); 150.18 (C-4); 152.23 (C-6); 152.84 (C-8). Exact mass (FAB HRMS) found: 225.1085; calculated for $C_8H_{13}N_6O_2$ [M + H]: 225.1099. UV, λ_{max} (ε_{max}): (pH 2) 272 (13 700); (pH 12) 274 (15 800); (MeOH) 274 (17 000). CD, λ ($\Delta\varepsilon$) (MeOH): 255 (0.20), 222 (0.45).

Alkylation of 8-(Dimethylamino)adenine (**4a**) with Methyl Tosylate

A mixture of compound **4a** (0.65 g, 3.6 mmol) and sodium hydride (0.22 g of 60% dispersion, 5.4 mmol) in DMF (20 ml) was stirred at 100 °C for 30 min. Methyl tosylate (0.8 ml, 5.4 mmol) was added and the mixture was stirred 110 °C for another 16 h. The solvent was evaporated and the residue purified by preparative chromatography on a silica gel plate in chloroform–methanol (S2) followed by crystallization from ethanol to afford 0.13 g (19%) of compound **4f** and 0.20 g (15%) of compound **5f**.

8-(Dimethylamino)-9-methyladenine (**4f**). White crystals, m.p. 261–262 °C, R_F 0.51 (S2). FAB MS, *m/z* (rel.%): 193 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 2.91 (s, 6 H, NCH_3); 3.56 (s, 3 H, NCH_3); 6.69 (brs, 2 H, NH_2); 8.00 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 29.68 (NCH_3); 41.46 (2 C, NCH_3); 116.35 (C-5); 150.43 (C-2); 151.18 (C-4); 153.48 (C-6); 155.78 (C-8). For $C_8H_{12}N_6$ (192.2) calculated: 49.99% C, 6.29% H, 43.72% N; found: 49.97% C, 6.43% H, 43.71% N. UV, λ_{max} (ε_{max}) (MeOH): 276 (15 400).

8-(Dimethylamino)-3-methyladenine tosylate (**5f**). White crystals, m.p. 280 °C, R_F 0.20 (S2). FAB MS, *m/z* (rel.%): 193 (100) [8-(dimethylamino)-3-methyladenine + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 2.29 (s, 3 H, CH_3-arom); 3.15 (s, 6 H, NCH_3); 3.83 (s, 3 H, NCH_3); 7.12 (d, 2 H, H-arom); 7.49 (d, 2 H, H-arom); 7.70 (br, 2 H, NH_2); 8.45 (s, 1 H, H-2); 11.60 (brs, 1 H,

NH). ^{13}C NMR (125 MHz, DMSO-d_6): 20.94 (CH_3-arom); 35.72 (NCH_3); 37.85 (2 C, NCH_3); 108.24 (C-5); 125.63 (2 C, C-arom); 128.29 (2 C, C-arom); 138.01 (C-arom); 145.51 (C-arom); 145.70 (C-2); 147.77 (C-6); 151.08 (C-4); 157.39 (C-8). IR (KBr): 3 358, 3 184 (NH_2); 2 817, 1 448 (CH_3 in NMe_2); 1 671, 1 646, 1 583, 1 525 (NH_2 and ring). For $C_{15}H_{20}N_6O_3S$ (364.4) calculated: 49.44% C, 5.53% H, 23.06% N, 8.80% S; found: 49.25% C, 5.54% H, 22.75% N, 8.72% S. Exact mass (FAB HRMS) found: 193.1234; calculated for $C_8H_{13}N_6$ [M – TsOH + H]: 193.1202. UV, λ_{max} (ε_{max}) (MeOH): 309 (8 500).

Alkylation of **4a** with Diisopropyl [(2-Chloroethoxy)methyl]phosphonate

A mixture of compound **4a** (0.25 g, 1.4 mmol) and sodium hydride (62 mg of 60% dispersion, 1.5 mmol) in DMF (10 ml) was stirred at 110 °C for 0.5 h. Diisopropyl [(2-chloroethoxy)methyl]phosphonate[9] (0.35 ml, 1.5 mmol) was added and the mixture was stirred for another 15 h. The solvent was evaporated and the residue codistilled with toluene (2 × 15 ml). Preparative chromatography on a silica gel plate in chloroform–methanol mixture (9 : 1) followed by crystallization from ethyl acetate afforded 90 mg (16%) of compound **4b** and 185 mg (30%) of compound **5b**.

9-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}-8-(dimethylamino)adenine (**4b**). Yellowish crystals, m.p. 152 °C, R_F 0.36 (S2). FAB MS, *m/z* (rel.%): 401 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 1.09 (d, 6 H, J(CH_3,CH) = 6.1, CH_3); 1.14 (d, 6 H, J(CH_3,CH) = 6.1, CH_3); 2.90 (s, 6 H, NCH_3); 3.70 (d, 2 H, J(P,CH) = 8.4, PCH_2); 3.97 (t, 2 H, J(2′,1′) = 5.6, H-2′); 4.21 (t, 2 H, J(1′,2′) = 5.6, H-1′); 4.43 (m, 2 H, POCH); 6.77 (s, 2 H, NH_2); 8.01 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 23.68 (d, 2 C, J(P,C) = 3.9, CH_3); 23.87 (d, 2 C, J(P,C) = 3.9, CH_3); 42.19 (NCH_3); 42.65 (C-1′); 64.85 (d, J(P,C) = 163.1, PCH_2); 69.22 (d, J(P,C) = 11.7, C-2′); 70.25 (d, 2 C, J(P,C) = 6.8, POC); 116.38 (C-5); 150.43 (C-2); 151.10 (C-4); 153.58 (C-6); 155.68 (C-8). For $C_{16}H_{29}N_6O_4P$ (400.4) calculated: 47.99% C, 7.30% H, 20.99% N, 7.74% P; found: 47.75% C, 7.28% H, 21.22% N, 7.59% P.

3-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}-8-(dimethylamino)adenine hydrochloride (**5b**). White crystals, m.p. 165–167 °C, R_F 0.20 (S2). FAB MS, *m/z* (rel.%): [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 1.12 (d, 6 H, J(CH_3,CH) = 6.2, CH_3); 1.16 (d, 6 H, J(CH_3,CH) = 6.2, CH_3); 3.17 (s, 6 H, NCH_3); 3.79 (d, 2 H, J(P,CH) = 8.3, PCH_2); 3.94 (t, 2 H, J(2′,1′) = 5.0, H-2′); 4.46 (t, 2 H, J(1′,2′) = 5.0, H-1′); 4.47 (m, 2 H, POCH); 8.00 (br, 2 H, NH_2); 8.42 (s, 1 H, H-2); 12.01 (brs, 1 H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 23.72 (d, 2 C, J(P,C) = 4.9, CH_3); 23.86 (d, 2 C, J(P,C) = 3.9, CH_3); 38.02 (NCH_3); 48.32 (C-1′); 64.66 (d, J(P,C) = 163.1, PC); 69.05 (d, J(P,C) = 10.7, C-2′); 70. 27 (d, 2 C, J(P,C) = 6.8, POC); 108.275 (C-5); 145.60 (C-2); 147.82 (C-6); 150.425 (C-4); 157.17 (C-8). IR (KBr): 3 299, 3 145 (NH_2); 2 827, 1 450 (CH_3 in NMe_2); 1 678, 1 642, 1 577, 1 520 (NH_2 and ring); 1 391, 1 376 (CH_3 in iPr); 1 178, 1 142 (iPr); 1 219 (P=O); 1 105 (COC); 1 012, 992 (POC). For $C_{16}H_{30}ClN_6O_4P$ (436.9) calculated: 43.99% C, 6.92% H, 8.12% Cl, 19.24% N, 7.09% P; found: 43.79% C, 6.86% H, 8.03% Cl, 18.93% N, 7.24% P.

Alkylation of **4a** with (*S*)-[(Trityloxy)methyl]oxirane [(*S*)-*O*-Tritylglycidol]

A mixture of compound **4a** (0.7 g, 4 mmol), DMF (20 ml), (*S*)-*O*-tritylglycidol (1.37 g, 4.5 mmol), and cesium carbonate (0.13 g, 0.4 mmol)) was stirred at 110 °C for 15 h. The hot suspension was filtered over Celite and evaporated. The residue afforded, on preparative thick layer chromatography on silica gel (S2), 0.37 g (17%) of compound **4g** and 0.41 g (21%) of compound **5g**.

(S)-8-(Dimethylamino)-9-[2-hydroxy-3-(trityloxy)propyl]adenine (**4g**). Yellowish crystals, m.p. 198–199 °C, R_F 0.37 (S1). FAB MS, *m/z* (rel.%): 495 (40) [M + H]; 243 (100) [Tr]. ^{1}H NMR (500 MHz, DMSO-d_6): 2.87 (s, 6 H, NCH_3); 2.87 (dd, 1 H, *J*(3′b,2′) = 5.4, *J*(gem) = 9.5, H-3′b); 2.98 (dd, 1 H, *J*(3′a,2′) = 5.4, *J*(gem) = 9.5, H-3′a); 4.04 (dd, 1 H, *J*(1′b,2′) = 7.9, *J*(gem) = 14.2, H-1′b); 4.10 (dd, 1 H, *J*(1′a,2′) = 5.1, *J*(gem) = 14.2, H-1′a); 4.37 (m, 1 H, H-2′); 5.43 (d, 1 H, *J*(OH,2′) = 5.6, OH); 6.73 (brs, 2 H, NH_2); 7.24 (t, 3 H, H-arom); 7.31 (t, 6 H, H-arom); 7.35 (d, 6 H, H-arom); 7.97 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 42.14 (2 C, NCH_3); 46.93 (C-1′); 66.27 (C-3′); 67.02 (C-2′); 86.10 (CPh_3); 116.39 (C-5); 127.12 (3 C, C-arom); 127.99 (6 C, C-arom); 128.40 (6 C, C-arom); 143.83 (3 C, C-arom); 150.36 (C-2); 151.09 (C-4); 153.70 (C-6); 155.72 (C-8). Exact mass (FAB HRMS) found: 495.2441; calculated for $C_{29}H_{31}N_6O_2$ [M + H]: 495.2509.

(S)-8-(Dimethylamino)-3-[2-hydroxy-3-(trityloxy)propyl]adenine (**5g**). White crystals, m.p. 249 °C, R_F 0.13 (S1). FAB MS, *m/z* (rel.%): 495 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 2.87 (dd, 1 H, *J*(3′b,2′) = 6.7, *J*(gem) = 9.3, H-3′b); 3.02 (dd, 1 H, *J*(3′a,2′) = 5.0, *J*(gem) = 9.3, H-3′a); 3.06 (s, 6 H, NCH_3); 4.03 (dd, 1 H, *J*(1′b,2′) = 8.7, *J*(gem) = 13.4, H-1′b); 4.24 (m, 1 H, H-2′); 4.58 (dd, 1 H, *J*(1′a,2′) = 3.0, *J*(gem) = 13.4, H-1′a); 5.56 (d, 1 H, *J*(OH,2′) = 5.0, OH); 6.82 (brs, 2 H, NH_2); 7.26 (t, 3 H, H-arom); 7.33 (t, 6 H, H-arom); 7.43 (d, 6 H, H-arom); 7.88 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 38.28 (2 C, NCH_3); 52.85 (C-1′); 65.76 (C-3′); 67.24 (C-2′); 86.12 (CPh_3); 120.76 (C-5); 127.20 (3 C, C-arom); 128.06 (6 C, C-arom); 128.39 (6 C, C-arom); 139.85 (C-2); 143.89 (3 C, C-arom); 148.48 (C-6); 152.16 (C-4); 166.53 (C-8). Exact mass (FAB HRMS) found: 495.2561; calculated for $C_{29}H_{31}N_6O_2$ [M + H]: 495.2509.

Deprotection of Trityl Derivatives **4g** and **5g**. General Procedure

The same procedure as described for compound **2h**, using Dowex 50X8 (H^+) followed by crystallization from ethanol afforded compounds **4h** (72%) and **5h** (81%).

(S)-9-(2,3-Dihydroxypropyl)-8-(dimethylamino)adenine (**4h**). White crystals, m.p. 221 °C, R_F 0.62 (S4). FAB MS, *m/z* (rel.%): 253 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 2.91 (s, 6 H, NCH_3); 3.32 (dt, 1 H, *J*(3′b,2′) = *J*(3′b,OH) = 5.9, *J*(gem) = 11.2, H-3′b); 3.41 (ddd, 1 H, *J*(3′a,2′) = 5.0, *J*(3′a,OH) = 5.8, *J*(gem) = 11.2, H-3′a); 3.99 (dd, 1 H, *J*(1′b,2′) = 8.8, *J*(gem) = 14.3, H-1′b); 4.07 (dd, 1 H, *J*(1′a,2′) = 3.8, *J*(gem) = 14.3, H-1′a); 4.15 (m, 1 H, H-2′); 4.82 (t, 1 H, *J*(OH,3′) = 5.8, OH); 5.12 (d, 1 H, *J*(OH,2′) = 5.0, OH); 6.75 (s, 2 H, NH_2); 8.00 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 42.23 (2 C, NCH_3); 46.90 (C-1′); 64.08 (C-3′); 68.89 (C-2′); 116.38 (C-5); 150.33 (C-2); 151.31 (C-4); 153.70 (C-6); 156.03 (C-8). For $C_{10}H_{16}N_6O_2$ (252.3) calculated: 47.61% C, 6.39% H, 33.31% N; found: 47.56% C, 6.47% H, 33.16% N. UV, λ_{max} (ε_{max}): (pH 2) 282 (14 900); (pH 7) 278 (15 600); (pH 12) 278 (15 000). CD, λ ($\Delta\varepsilon$) (H_2O): 312 (–0.08), 277 (2.43), 209 (–3.19).

(S)-3-(2,3-Dihydroxypropyl)-8-(dimethylamino)adenine (**5h**). White crystals, m.p. 168 °C, R_F 0.53 (S4). FAB MS, *m/z* (rel.%): 253 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 3.06 (s, 6 H, NCH_3); 3.21 (dd, 1 H, *J*(3′b,2′) = 6.6, *J*(gem) = 11.4, H-3′b); 3.38 (dd, 1 H, *J*(3′a,2′) = 5.2, *J*(gem) = 11.4, H-3′a); 3.94 (m, 1 H, H-2′); 4.11 (dd, 1 H, *J*(1′b,2′) = 7.2, *J*(gem) = 13.7, H-1′b); 4.34 (dd, 1 H, *J*(1′a,2′) = 3.5, *J*(gem) = 13.7, H-1′a); 6.80 (br, 2 H, OH); 7.24 (brs, 2 H, NH_2); 7.96 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 38.33 (2 C, NCH_3); 52.02 (C-1′); 63.10 (C-3′); 68.88 (C-2′); 118.53 (C-5); 141.23 (C-2); 148.71 (C-6); 151.99 (C-4); 164.79 (C-8). For $C_{10}H_{16}N_6O_2$ (252.3) calculated: 47.61% C, 6.39% H, 33.31% N; found: 47.47% C, 6.53% H, 33.08% N. UV, λ_{max} (ε_{max}): (pH 2) 307 (16 600), 236 (15 900); (pH 7) 307 (15 800), 236

(15 000); (pH 12) 322 (11 800), 236 (8 200). CD, λ (Δε) (H_2O): 300 (0.10), 225 (–1.67), 208 (–1.69).

Synthesis of 8,3′-*N*-Anhydro Derivatives **7**. General Procedure

A mixture of compound **1c** (0.47 g, 1 mmol), 4-(dimethylamino)pyridine (30 mg), and pyridine (20 ml) was cooled to –10 °C and tosyl chloride (0.23 g, 1.3 mmol) was added under exclusion of moisture. The mixture was stirred at –10 °C for 2 h, left to stand at 0 °C for 24 h and then at room temperature for 24 h. Methanol (10 ml) was added and, after 30 min, the mixture was evaporated at 30 °C and partitioned between ethyl acetate and water. The organic phase was dried over anhydrous magnesium sulfate, filtered, the solvent was evaporated, codistilled with toluene (2 × 20 ml) and the residue was dried over P_2O_5. The dry oil was dissolved in 30% methanolic ammonia solution or in solution of methylamine in ethanol (≈5.6 mol l^{-1}, 30 ml) and the mixture was heated in an autoclave at 80 °C for 6 h. The solvent was evaporated and the crude products **7a** and **7b** were directly treated with TMSBr under standard conditions (see compounds **7c** and **7d**, respectively). Analytical sample of compound **7b** was prepared by TLC purification on silica gel.

Diisopropyl (R)-{[(4-amino-8,9-dihydro-7H-pyrimido[1,2-e]purin-8-yl)oxy]methyl}phosphonate (**7b**). R_F 0.31 (S1). FAB MS, *m/z* (rel.%): 399 (100) [M + H]. ^{1}H NMR (500 MHz, DMSO-d_6): 1.12 (d, 3 H, J(CH_3,CH) = 6.1, CH_3); 1.13 (d, 3 H, J(CH_3,CH) = 6.1, CH_3); 1.17 (d, 3 H, J(CH_3,CH) = 6.1, CH_3); 1.18 (d, 3 H, J(CH_3,CH) = 6.1, CH_3); 3.08 (s, 3 H, NCH_3); 3.53 (dd, 2 H, J(P,3′) = 1.6, J(3′,2′) = 3.2, H-3′); 3.88 (dd, 1 H, J(P,Hb) = 9.3, J(gem) = 13.9, PCHb); 3.94 (dd, 1 H, J(P,Ha) = 8.8, J(gem) = 13.9, PCHa); 3.98 (dd, 1 H, J(1′b,2′) = 3.2, J(gem) = 13.2, H-1′b); 4.15 (ddd, 1 H, J(1′a,P) = 1.3, J(1′a,2′) = 2.8, J(gem) = 13.2, H-1′a); 4.24 (quintet, 1 H, J = 3.1, H-2′); 4.52 (dsept, 2 H, J(CH,CH_3) = 6.1, J(P,OCH) = 7.8, POCH); 6.46 (s, 2 H, NH_2); 7.90 (s, 1 H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): 23.665 (d, J(P,C) = 4.9, CH_3); 23.70 (d, J(P,C) = 4.9, CH_3); 23.89 (d, J(P,C) = 3.9, CH_3); 23.92 (d, J(P,C) = 3.9, CH_3); 43.19 (C-1′); 49.47 (C-3′); 62.70 (d, J(P,C) = 165.0, PC); 70.02 (d, J(P,C) = 12.7, C-2′); 70.44 (d, J(P,C) = 6.9, POC); 70.495 (d, J(P,C) = 6.9, POC); 117.19 (C-5); 148.66 (C-2); 149.89 (C-8); 150.11 (C-4); 151.99 (C-6). Exact mass (FAB HRMS) found: 399.1889; calculated for $C_{16}H_{28}N_6O_4P$ [M + H]: 399.1909.

Deprotection of Phosphonates with TMSBr. General Procedure

A mixture of a phosphonate diester (1 mmol), TMSBr (1 ml) and acetonitrile (5 ml) was stirred overnight at ambient temperature, then evaporated and codistilled with acetonitrile (10 ml). The residue was dissolved in water and made alkaline with aqueous ammonia. After evaporation *in vacuo*, the residue was dissolved in water and applied on a column of Dowex 50X8 (H^+ form, 50 ml); the column was washed with water and eluted with 2.5% aqueous ammonia. After evaporation of the UV absorbing fraction, the product was purified on a Dowex 1X2 (acetate) column (50 ml) by elution with linear gradient of acetic acid (0–0.5 mol l^{-1}, 1 l each). The UV absorbing fractions were evaporated *in vacuo* and the residue was crystallized from water.

8-Amino-9-[2-(phosphonomethoxy)ethyl]adenine (**2d**). White crystals, slow decomposition over 250 °C, yield 90%, E_{Up} 0.76. FAB MS, *m/z* (rel.%): 289 (100) [M + H]. ^{1}H NMR (500 MHz, D_2O): 3.51 (d, 2 H, J(P,CH) = 8.5, PCH_2); 3.87 (t, 2 H, J(2′,1′) = 5.1, H-2′); 4.17 (t, 2 H, J(1′,2′) = 5.1, H-1′); 7.96 (s, 1 H, H-2). UV, λ_{max} (ε_{max}): (pH 2) 271 (10 900); (pH 7) 273 (11 300); (pH 12) 274 (14 300).

(S)-8-Amino-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (**2e**). White crystals, m.p. >250 °C, yield 58%, E_{Up} 0.78. FAB MS, *m/z* (rel.%): 319 (100) [M + H]. ^{1}H NMR (500 MHz, D_2O): 3.55 (dd, 1 H, *J*(P,CHb) = 8.8, *J*(gem) = 12.9, PCHb); 3.59 (dd, 1 H, *J*(3′b,2′) = 3.9, *J*(gem) = 12.2, H-3′b); 3.68 (dd, 1 H, *J*(P,CHa) = 9.5, *J*(gem) = 12.9, PCHa); 3.82 (dd, 1 H, *J*(3′a,2′) = 3.9, *J*(gem) = 12.2, H-3′a); 3.85 (m, 1 H, H-2′); 4.14 (dd, 1 H, *J*(1′b,2′) = 6.6, *J*(gem) = 15.4, H-1′b); 4.19 (dd, 1 H, *J*(1′a,2′) = 4.2, *J*(gem) = 15.4, H-1′a); 8.01 (s, 1 H, H-2). ^{13}C NMR (125 MHz, D_2O): 42.65 (C-1′); 59.75 (C-3′); 66.73 (d, *J*(P,C) = 154.3, PCH_2); 80.17 (d, *J*(P,C) = 10.7, C-2′); 115.11 (C-5); 148.87 (C-2); 149.28 (C-4); 151.11 (C-6); 153.71 (C-8). For $C_9H_{15}N_6O_5P$ (318.2) calculated: 33.97% C, 4.75% H, 26.41% N, 9.73% P; found: 33.71% C, 4.68% H, 26.15% N, 9.48% P. UV, λ_{max} (ε_{max}): (pH 2) 271 (12 900); (pH 7) 273 (14 100); (pH 12) 274 (15 400). CD, λ (Δε) (H_2O): 263 (0.68), 213 (0.63).

8-(Methylamino)-9-[2-(phosphonomethoxy)ethyl]adenine (**3d**). White crystals, m.p. >300 °C, yield 81%, E_{Up} 0.79. FAB MS, *m/z* (rel.%): 303 (100) [M + H]. ^{1}H NMR (500 MHz, D_2O): 2.99 (s, 3 H, CH_3); 3.49 (d, 2 H, *J*(P,CH) = 8.3, PCH_2); 3.83 (t, 2 H, *J*(2′,1′) = 5.3, H-2′); 4.08 (t, 2 H, *J*(1′,2′) = 5.3, H-1′); 7.94 (s, 1 H, H-2). For $C_9H_{15}N_6O_4P$ (302.2) calculated: 35.77% C, 5.00% H, 27.81% N, 10.25% P; found: 35.50% C, 5.00% H, 27.58% N, 10.25% P. UV, λ_{max} (ε_{max}): (pH 2) 274 (12 700); (pH 12) 279 (15 200).

(S)-9-[3-Hydroxy-2-(phosphonomethoxy)propyl]-8-(methylamino)adenine (**3e**). White crystals, m.p. 215 °C, yield 58%, E_{Up} 0.75. FAB MS, *m/z* (rel.%): 333 (20) [M + H]; 165 (100) [8-(methylamino)adenine + H]. ^{1}H NMR (500 MHz, D_2O): 2.94 (s, 3 H, NCH_3); 3.42 (dd, 1 H, *J*(3′b,2′) = 4.6, *J*(gem) = 12.2, H-3′b); 3.48 (d, 2 H, *J*(P,CH) = 8.7, PCH_2); 3.70 (dd, 1 H, *J*(3′a,2′) = 3.2, *J*(gem) = 12.2, H-3′a); 3.73 (m, 1 H, H-2′); 3.98 (d, 2 H, *J*(1′,2′) = 5.6, H-1′); 7.86 (s, 1 H, H-2). ^{13}C NMR (125 MHz, D_2O): 28.63 (NCH_3); 42.12 (C-1′); 60.08 (C-3′); 68.30 (d, *J*(P,C) = 150.2, PCH_2); 79.73 (d, *J*(P,C) = 9.7, C-2′); 115.80 (C-5); 148.50 (C-2); 149.43 (C-4); 150.50 (C-6); 154.17 (C-8). For $C_{10}H_{17}N_6O_5P$ (332.3) calculated: 36.15% C, 5.16% H, 25.29% N, 9.32% P; found: 35.99% C, 5.38% H, 25.06% N, 9.18% P. UV, λ_{max} (ε_{max}): (pH 2) 274 (12 700); (pH 12) 279 (15 500). CD, λ (Δε) (H_2O): 266 (1.37), 216 (1.20).

8-(Dimethylamino)-9-[2-(phosphonomethoxy)ethyl]adenine (**4d**). White crystals, m.p. 183 °C, yield 76%, E_{Up} 0.84. FAB MS, *m/z* (rel.%): 317 (100) [M + H]. ^{1}H NMR (500 MHz, D_2O): 3.05 (s, 6 H, CH_3); 3.46 (d, 2 H, *J*(P,CH) = 8.3, PCH_2); 3.96 (t, 2 H, *J*(2′,1′) = 5.2, H-2′); 4.30 (t, 2 H, *J*(1′,2′) = 5.2, H-1′); 8.01 (s, 1 H, H-2). For $C_{10}H_{17}N_6O_4P$ (316.3) calculated: 37.98% C, 5.42% H, 26.57% N, 9.79% P; found: 37.94% C, 5.70% H, 26.30% N, 9.55% P. UV, λ_{max} (ε_{max}): (pH 2) 281 (15 300); (pH 12) 278 (15 700).

(S)-8-(Dimethylamino)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine (**4e**). White crystals, m.p. 147–148 °C, yield 67%, E_{Up} 0.80. FAB MS, *m/z* (rel.%): 347 (100) [M + H]. ^{1}H NMR (500 MHz, D_2O): 3.01 (s, 6 H, NCH_3); 3.27 (dd, 1 H, *J*(P,CHb) = 9.9, *J*(gem) = 12.2, PCHb); 3.39 (dd, 1 H, *J*(3′b,2′) = 5.5, *J*(gem) = 12.6, H-3′b); 3.40 (dd, 1 H, *J*(P,CHa) = 8.6, *J*(gem) = 12.2, PCHa); 3.62 (dd, 1 H, *J*(3′a,2′) = 2.3, *J*(gem) = 12.6, H-3′a); 3.91 (m, 1 H, H-2′); 4.13 (dd, 1 H, *J*(1′b,2′) = 6.1, *J*(gem) = 14.9, H-1′b); 4.26 (dd, 1 H, *J*(1′a,2′) = 7.3, *J*(gem) = 14.9, H-1′a); 8.02 (s, 1 H, H-2). ^{13}C NMR (125 MHz, D_2O): 40.84 (2 C, NCH_3); 43.58 (C-1′); 60.61 (C-3′); 68.28 (d, *J*(P,C) = 150.6, PCH_2); 78.83 (d, *J*(P,C) = 9.7, C-2′); 115.76 (C-5); 149.94 (C-2); 150.30 (C-4); 152.11 (C-6); 156.91 (C-8). For $C_{11}H_{19}N_6O_5P$ (346.3) calculated: 38.15% C, 5.53% H, 24.27% N, 8.94% P; found: 37.70% C, 5.62% H, 23.96% N, 8.85% P. UV, λ_{max} (ε_{max}): (pH 2) 281 (13 500); (pH 12) 278 (13 900). CD, λ (Δε) (H_2O): 278 (2.87), 232 (2.05), 204 (–2.80).

8-(Dimethylamino)-3-[2-(phosphonomethoxy)ethyl]adenine (**5d**). White crystals, m.p. 183 °C, yield 76%, E_{Up} 0.72. FAB MS, *m/z* (rel.%): 317 (100) [M + H]. ^{1}H NMR (500 MHz, D_2O): 3.08

(s, 6 H, NCH_3); 3.45 (d, 2 H, *J*(P,CH) = 8.6, PCH_2); 3.94 (t, 2 H, *J*(2′,1′) = 5.0, H-2′); 4.44 (t, 2 H, *J*(1′,2′) = 5.0, H-1′); 8.12 (s, 1 H, H-2). ^{13}C NMR (125 MHz, D_2O): 37.95 (2 C, NCH_3); 49.14 (C-1′); 68.84 (d, *J*(P,C) = 150.4, PC); 69.01 (d, *J*(P,C) = 9.7, C-2′); 119.96 (C-5); 141.41 (C-2); 147.46 (C-6); 152.24 (C-4); 166.755 (C-8). IR (KBr): 3 291, 3 138 (NH_2); 2 809, 1 448 (CH_3 in NMe_2); 2 647 (OH in POH); 1 682, 1 634, 1 576, 1 520 (NH_2 and ring); 1 140 (P=O); 1 101 (COC). Exact mass (FAB HRMS) found: 317.1155; calculated for $C_{10}H_{18}N_6O_4P$ [M + H]: 317.1127. UV, λ_{max} (ε_{max}): (pH 2) 307 (23 100), 235 (21 200); (pH 12) 321 (16 300), 235 (11 400).

(R)-{[(4-Amino-8,9-dihydro-7H-pyrimido[1,2-e]purin-8-yl)oxy]methyl}phosphonic acid (**7c**). White crystals, m.p. 295–297 °C, overall yield 17%, E_{Up} 0.73. FAB MS, *m/z* (rel.%): 301 (100) [M + H]. ^1H NMR (500 MHz, D_2O): 3.53 (dd, 1 H, *J*(3′b,2′) = 2.0, *J*(gem) = 13.4, H-3′b); 3.55 (dd, 1 H, *J*(P,CHb) = 8.7, *J*(gem) = 12.6, PCHb); 3.61 (dd, 1 H, *J*(P,CHa) = 8.9, *J*(gem) = 12.6, PCHa); 3.76 (ddd, 1 H, *J*(3′a,P) = 2.3, *J*(3′a,2′) = 3.4, *J*(gem) = 13.4, H-3′a); 4.00 (dd, 1 H, *J*(1′b,2′) = 3.3, *J*(gem) = 13.2, H-1′b); 4.30 (dt, 1 H, *J*(1′a,2′) = *J*(1′a,P) = 2.5, *J*(gem) = 13.2, H-1′a); 4.37 (m, 1 H, H-2′); 7.95 (s, 1 H, H-2). ^{13}C NMR (125 MHz, D_2O): 44.44 (C-1′); 46.44 (C-3′); 69.36 (d, *J*(P,C) = 149.4, PC); 71.23 (d, *J*(P,C) = 9.8, C-2′); 119.40 (C-5); 151.59 (C-2); 152.29 (C-4); 153.76 (C-8); 153.89 (C-6). Exact mass (FAB HRMS) found: 301.0721; calculated for $C_9H_{14}N_6O_4P$ [M + H]: 301.0814. UV, λ_{max} (ε_{max}): (pH 2) 274 (14 100); (pH 7) 281 (18 000); (pH 12) 281 (17 400). CD, λ ($\Delta\varepsilon$) (H_2O): 283 (1.24), 262 (0.12), 246 (0.57), 219 (–6.48), 205 (0.00), 195 (–3.10).

(R)-{[(4-Amino-6-methyl-8,9-dihydro-7H-pyrimido[1,2-e]purin-8-yl)oxy]methyl}phosphonic acid (**7d**). White crystals, m.p. 238–240 °C, overall yield 26%, E_{Up} 0.74. FAB MS, *m/z* (rel.%): 315 (100) [M + H]. ^1H NMR (500 MHz, D_2O): 3.22 (s, 3 H, NCH_3); 3.70 (dd, 1 H, *J*(3′b,2′) = 1.8, *J*(gem) = 13.6, H-3′b); 3.80 (dd, 1 H, *J*(P,CHb) = 9.9, *J*(gem) = 12.9, PCHb); 3.81 (dt, 1 H, *J*(3′a,2′) = *J*(3′a,P) = 2.6, *J*(gem) = 13.6, H-3′a); 3.90 (dd, 1 H, *J*(P,CHa) = 9.3, *J*(gem) = 12.9, PCHa); 4.02 (dd, 1 H, *J*(1′b,2′) = 3.3, *J*(gem) = 13.9, H-1′b); 4.42 (m, 1 H, H-2′); 4.43 (dt, 1 H, *J*(1′a,2′) = *J*(1′a,P) = 2.2, *J*(gem) = 13.9, H-1′a); 8.09 (s, 1 H, H-2). Exact mass (FAB HRMS) found: 315.0867; calculated for $C_{10}H_{15}N_6O_4P$ [M + H]: 315.0970. UV, λ_{max} (ε_{max}): (pH 2) 280 (14 700); (pH 7) 284 (18 300); (pH 12) 285 (18 800). CD, λ ($\Delta\varepsilon$) (H_2O): 320 (0.08), 282 (–3.40), 240 (1.04), 223 (3.04), 201 (–1.17).

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