Synthesis of 2′-Aminomethyl Derivatives of *N*-(2-(Phosphonomethoxy)ethyl) Nucleotide Analogues as Potential Antiviral Agents

Hana Dvořáková,*[,†] Milena Masojídková,[†] Antonín Holý,[†] Jan Balzarini,[‡] Graciela Andrei,[‡] Robert Snoeck,[‡] and Erik De Clercq[‡]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

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A series of purine and pyrimidine *N*-(2-(phosphonomethoxy)ethyl) derivatives bearing aminomethyl, (dimethylamino)methyl, morpholinomethyl, and (trimethylammonio)methyl groups at the 2′-position were synthesized. The compounds were prepared by alkylation of the heterocyclic bases with appropriately substituted (aminoalkyl)oxiranes followed by condensation of the resulting intermediates with dialkyl ((*p*-tolylsulfonyl)oxy)methanephosphonate and subsequent treatment of the obtained diester with bromotrimethylsilane. 9-(3-Amino-2-(phosphonomethoxy)propyl)adenine (**2a**) proved active against varicella zoster virus (VZV), cytomegalovirus (CMV), and Moloney murine sarcoma virus (MSV) in the concentration range of 7−35 μg/mL. None of the other aminoalkyl derivatives demonstrated significant antiviral activity against herpes simplex virus type 1 and 2 (HSV-1 and HSV-2), VZV, (CMV), vaccinia virus (VV), MSV, and human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2).

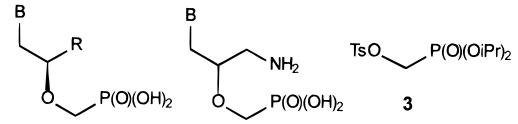
Introduction

Acyclic (phosphonomethoxy)alkyl derivatives of pyrimidine and purine bases and their aza and deaza analogues (**1**, Chart 1) exhibit antiviral and cytostatic activities.[1] Our recent structure−activity relationship study demonstrated that the margin for structural alteration of both the heterocyclic base and the side chain is very narrow.[2]

The choice of the base is limited to adenine, 2-aminopurine, 2,6-diaminopurine, guanine, their 8-aza[3] and 3-deaza derivatives,[4] and cytosine.[2] The structure of the side chain bearing such bases determines the range of antiviral effects. While the (*S*)-3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) derivatives (**1b**) inhibit DNA viruses, the effect of 3-fluoro-2-(phosphonomethoxy)-propyl (FPMP) (**1c**) and (*R*)-2-(phosphonomethoxy)-propyl (PMP) (**1d**) compounds[5] is selectively antiretroviral. 2-(Phosphonomethoxy)ethyl derivatives (PME) (**1a**) lacking the substituent at the 2′-position are active against both DNA and retroviruses.[1] The influence of the 2′-substituent on the biological activity is the subject of our continuous investigation. We have recently reported[6] that except for the 2′-methyl derivatives **1d** none of the 2′-alkyl, -cycloalkyl or -aralkyl derivatives of the type **1** had any antiviral activity. Introduction of the aminomethyl group to the 2′-position of the parent structure **1a** led to the moderately active adenine derivative **2**,[1] and some activity was also reported for the guanine counterpart.[7]

In this paper we report on the synthesis of acyclic phosphonate analogues of nucleotides bearing aminomethyl, (dimethylamino)methyl, and (trimethylammonio)methyl groups at the 2′-position of the (phosphonomethoxy)ethyl chain. We synthesized systematically adenine and cytosine derivatives, though in some cases we extended our studies also to 2,6-diaminopurine and guanine derivatives.

Chart 1



1a, R= H
1b, R= CH_2OH
1c, R= CH_2F
1d, R= CH_3

2 **3**

Chemistry

The original method[8] for the preparation of racemic aminomethyl derivative **2** made use of 9-(3-azido-2-hydroxypropyl)adenine which was transformed into the phosphonate by the reaction with bis(2-propyl) ((*p*-tolylsulfonyl)oxy)methanephosphonate (**3**) and converted into the final amino derivative **2** by hydrogenation. This procedure was replaced by an alternative strategy avoiding hydrogenation due to competing side reaction on the heterocycle (e.g. cytosine). The synthetic route for preparation of aminomethyl derivatives of adenine, cytosine and 2,6-diaminopurine is shown in Scheme 1. The starting phthalimido derivatives **4a**, **4b**, and **4c** are easily accessible by alkylation of the heterocyclic base with phthalimidomethyloxirane.[9] The amino function at the heterocyclic base was then protected by conversion into the *N*-((dimethylamino)-methylene) derivative by the action of dimethylformamide dimethyl acetal in dimethylformamide.[10] The protected derivatives **5a**, **5b**, and **5c** were treated with the tosylate **3** in the presence of excess sodium hydride in dimethylformamide[11] followed by removal of the amidine protecting group by treatment with aqueous ammonia. The resulting phthalimido diesters **6a**, **6b**, and **6c** lost simultaneously their ester and phthalimido protecting groups on treatment with bromotrimethylsilane in acetonitrile and subsequent hydrolysis. The phosphonic acids **2a**, **2b**, and **2c** were isolated by ion exchange chromatography. In the case of adenine and

† Academy of Sciences of the Czech Republic.
‡ Katholieke Universiteit Leuven.
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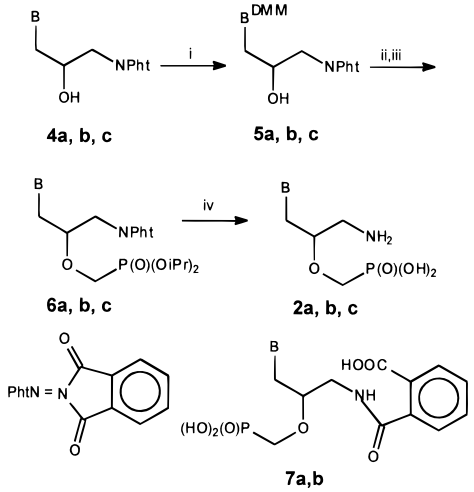
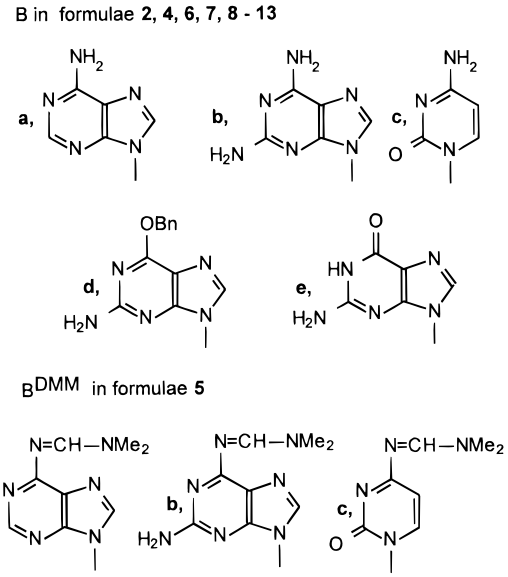
Scheme 1[a]



[a] (i) $Me_2NCH(OMe)_2$, DMF; (ii) **3**, NaH, DMF; (iii) NH_3, H_2O, MeOH, (iv) Me_3SiBr, MeCN.

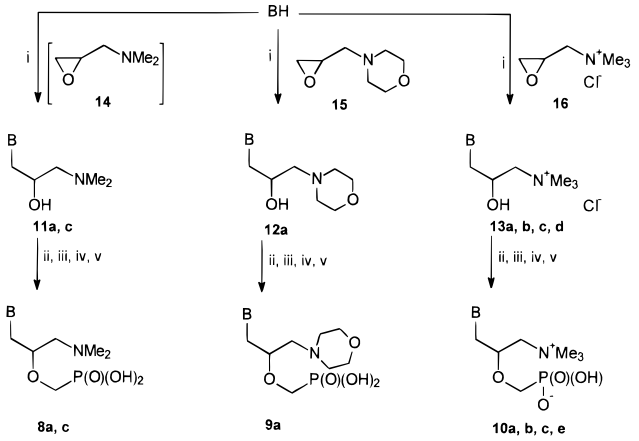
Chart 2



2,6-diaminopurine the phthalamoyl derivatives **7a** and **7b** were isolated as side products.

A similar strategy was applied for the synthesis of N-substituted derivatives of the type **8**, **9**, and **10.** The starting 2-hydroxypropyl derivatives **11, 12**, and **13** were prepared by alkylation of the purine or pyrimidine base, respectively, by a suitably substituted oxirane (**14, 15**, and **16**). The alkylation of the bases with oxiranes **14, 15** and **16** was performed in dimethylformamide in the presence of a catalytic amount of cesium carbonate (Scheme 2). This method proved to be useful earlier in alkylations of adenine as well as other purine and pyrimidine bases.[11] The alkylation gave predominantly N-9 isomers in the purine series and N-1 isomer with cytosine. In those cases where the starting oxirane is not available or is not sufficiently stable, it is possible to apply the Mitsunobu reaction,[12] which generates the oxirane *in situ* from the corresponding 1,2-diol. This alternative was used for the synthesis of 3-((dimethylamino)methyl) derivatives **8**. Treatment of 1-(dimethylamino)propane-2,3-diol with ethyl azodicarboxylate and triphenylphosphine in benzene gave the unstable oxirane **14**, which was directly used after evaporation of

Scheme 2[a]



[a] (i) Cs_2CO_3, DMF; (ii) $Me_2NCH(OMe)_2$, DMF; (iii) **3**, NaH, DMF; (iv) NH_3, H_2O, MeOH; (v) Me_3SiBr, MeCN.

the solvent for the alkylation of the heterocyclic base. The resulting 3-(dimethylamino)-2-hydroxypropyl derivatives **11** were purified by HPLC. The reaction proceeded smoothly with compound **15**.[13] The resulting *N*-(2-hydroxy-3-morpholinopropyl)adenine (**12**) was easily obtained by crystallization.

The preparation of quaternary (trimethylammonio)methyl derivatives **13** followed an identical route which made use of the commercially available ((trimethylammonio)methyl)oxirane salt **16**. The alkylation of heterocyclic bases with this reagent proceeded smoothly; however, the high polarity of the condensation products did not allow the separation of regioisomers N-9 and N-3 either by ion exchange chromatography or by preparative HPLC. Therefore, the crude products **13a** and **13b** were used in the next reaction sequence. For the synthesis of guanine (trimethylammonio)methyl derivative **10e** we used 2-amino-6-(benzyloxy)purine; this precursor proved to be useful already earlier[9] in the preparation of *N*-(3-phthalimido-2-hydroxypropyl)-guanine. The alkylation of this base with the oxirane **16** afforded again a nonseparable mixture of regioisomers N-9 and N-7 **13d**, which had to be used in the next reaction steps.

The amidine protection of 2-hydroxypropyl derivatives **11, 12**, and **13,** their condensation with the synthon **3**, ammonolysis, and removal of the ester functionalities of protected phosphonates was performed in the same manner as for the compounds **4a, 4b**, and **4c**. The resulting phosphonic acids **8, 9**, and **10** were isolated and purified by ion exchange chromatography as free acids. Using anion exchange chromatography we succeeded in separating the mixtures of regioisomers. However, the yields of the compounds **10a**, **10b**, and **10e** were very low.

The structure of the compounds was proved by ^1H NMR spectra and in some cases ^{13}C NMR data. The presence of the phthalimide group in hydroxy derivatives **4a** and **4b** is indicated by four signals of eight sp^2-carbon atoms of the symmetrical phthalimide moiety in the ^{13}C NMR spectrum.[9] The number of signals in the (carboxybenzoyl)amino derivatives **7a** and **7b** demonstrates loss of the symmetry of the phthalimide part as a result of opening the phthalimide to the phthalamic acid **7a** and **7b.** The position of the substituent at the base (N-9 substitution on the purine, or N-1 substitution

Table 1. Antiviral Activity in Vitro

	minimum inhibitory concentration (mg/mL)[a]												
virus	**2a**	**2b**	**2c**	**7a**	**7b**	**8a**	**8c**	**9a**	**10a**	**10b**	**10c**	**10e**	HPMPA	PMEA
HSV-1 (KOS)	150	300	>400	>400	>400	>400	>400	70	>100	>400	>400	>400	0.2	7
HSV-1TK$^-$(B2006)	150	>400	>400	>400	>400	>400	>400	>100	>100	>400	300	>400	0.1	20
HSV-2 (G)	300	ND	>400	300	>400	>400	>400	>100	>100	>400	>500	>400	0.10	4
CMV (AD-169)	15	>20	>50	>50	>50	>20	>50	>50	>50	>50	ND	>50	0.15	28
CMV (Davis)	35	>20	>50	>50	>50	>20	ND	>50	>50	ND	ND	ND	0.2	29
VZV TK$^+$ (YS)	7	>50	>50	>50	>50	>20	>50	>50	>50	>50	>50	>50	0.05	31
VZV TK$^+$(OKA)	11	>50	>50	>50	>50	>20	>50	>50	>50	>50	ND	>50	0.03	25
VZV TK$^-$ (07−1)	ND[b]	>50	ND	>50	>50	>20	ND	>50	>50	ND	>50	ND	0.04	14
VZV TK$^-$(YS/R)	7	>50	>50	>50	>50	>20	>50	>50	>50	>50	>50	>50	0.01	14
VV	300	>400	>400	300	−	>400	>400	>100	>100	>400	−	>400	0.70	300
MSV	9.7 ± 2.5	152 ± 52	>200	153 ± 21	126 ± 2	>200	>200	25 ± 14	>200	>200	−	>200	3.7	0.34
HIV-1 (III_B)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>20	1.5
HIV-2 (ROD)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>20	1.5

[a] Concentration required to reduce virus-induced cytopathicity or cell growth by 50%. [b] Not determined.

on the pyrimidine) was confirmed for most of the compounds studied from comparison of their UV and ^{1}H and ^{13}C NMR spectra with previously described derivatives.[9] The major products in the mixtures of N-9 and N-3 isomers **13a** and **13b** exhibit characteristic alkylation effects for the N-9 substitution, while for the minor products we observed markedly different effects (for C-8 and C-1′ lowfield shift about 10.5 and 6.0 ppm and for C-2 upfield shift about 9 ppm) indicating N-3 alkylation.[14] On the other hand, the minor product in the mixture **13d** is characterized by lowfield shift for C-4 and C-8 (about 9 and 6 ppm) and upfield shift for C-5 (about 8 ppm), indicating N-7 alkylation of the base.[15]

Biological Results

The title compounds were evaluated for their inhibitory effect against HSV-1, HSV-2, thymidine kinase (TK)-deficient HSV-1/TK$^-$ and vaccinia virus (VV) replication in E_6SM cells, cytomegalovirus and varicella zoster virus (TK$^+$ and TK$^-$) replication in HEL cells, Moloney murine sarcoma virus (MSV)-induced transformation of C3H/3T3 cells, and human immunodeficiency virus (HIV-1- and HIV-2)-induced cytopathicity in MT-4 cells. None of the compounds proved markedly inhibitory at subtoxic concentrations to HSV-1 and HSV-2, VV, CMV, and HIV (except for **9a** that was inhibitory to HSV-1 at 70 μg/mL and for **2a** that inhibited CMV and VZV replication at a concentration ranging from 7 to 35 μg/mL). Also, **2a** and **9a** inhibited MSV-induced transformation of C3H cell cultures at compound concentrations (9.7 and 25 μg/mL, respectively) that were clearly lower than the toxicity threshold. Other compounds such as **2b**, **7a**, and **7b** proved only minimally active against MSV. The observation that **2a** and **9a** were inhibitory to MSV but not HIV may be suggestive of a different structure−activity requirement of the 2′-aminomethyl-substituted PMEA derivatives for murine and human retroviruses.

Experimental Section

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 Kofler block and are uncorrected. TLC was performed on Silufol UV_{254} plates (Kavalier Votice, Czech Republic). Preparative TLC was carried out on 40 × 17 × 0.4 cm loose layer plates of silica gel containing UV indicator. Liquid chromathography (HPLC) was performed on columns (250 × 4mm) of C18 silica gel Separon SGX (7 μm), elution rate 1 mL min^{-1}, detection at 254 nm. The solvent systems and capacity ratio $k = (t_r - t_0)/t_0$ (t_r, retention time; t_0, hold-up time) are mentioned below. Paper electrophoresis was performed on a Whatman No. 3 MM paper at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogen carbonate (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 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)propanesulfonate (DSS) as internal standard for ^{1}H and dioxane as 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 Beckman DU-65 spectrometer in aqueous solutions. Bromotrimethylsilane, cytosine, and (2,3-epoxypropyl)trimethylammonium chloride were purchased from Fluka (Switzerland), 3-(dimethylamino)-1,2-propanediol was obtained from Aldrich (Germany), adenine, dimethylformamide, and cesium carbonate were purchased from Janssen (Belgium), and 2,6-diaminopurine was from Tokyo Kasei Co. (Japan). Dimethylformamide was distilled from P_2O_5 and stored over molecular sieves (4 Å). Acetonitrile was refluxed with CaH_2 and distilled.

General Procedure for Preparation of Compounds 2a−c, 8a,c, 9a, and 10a−c,e. A mixture of hydroxy derivatives **4a−c, 11a,c, 12a, and 13a−d** (10 mmol); dimethylformamide (50 mL); and *N*,*N*-dimethylformamide dimethyl acetal (10 mL, 75 mmol) was allowed to stand at 20 °C for 24 h in a stoppered flask. In the case of cytosine derivatives, *N*,*N*-dimethylformamide dineopentyl acetal (14 mL, 50 mmol) was used. After evaporation of the reaction mixture and codistillation with dimethylformamide (3 × 50 mL), the residue was stirred with solid carbon dioxide and pyridine−water (1:1, 100 mL) for 30 min. The mixture was then evaporated and codistilled with pyridine (3 × 50 mL) and with dimethylformamide (3 × 50 mL). The crude (dimethylamino)methylene derivative was dissolved in dimethylformamide (50 mL); bis-(2-propyl) ((*p*-toluylsulfonyl)oxy)methanephosphonate **(3)** (4.2 g, 12 mmol) was added and the mixture cooled down to −20 °C. After addition of sodium hydride (1.2 g, 30 mmol, 60% dispersion), the mixture was allowed to warm to room temperature and stirred under exclusion of moisture until the starting compound disappeared (24 h). The reaction mixture was neutralized with acetic acid, and the condensation product was deblocked by standing with ammonia−water−methanol (100 mL, 1:1:1) overnight. The mixture was taken down and deionized on Dowex 50 × 8 (H^+ form, 100 mL). The crude bis-(2-propyl) ester was dried over phosphorus pentoxide and then used directly in the deprotection step.

The material from the preceding step was dissolved in acetonitrile (80 mL), bromotrimethylsilane (8 mL) was added, and the mixture was set aside in a stoppered flask at 20 °C for 24 h. After evaporation, the residue was codistilled with acetonitrile (3 × 50 mL), mixed with water (50 mL), and adjusted to pH 8 with triethylamine. The mixture was allowed to stand for 1 h and then evaporated *in vacuo*, and the residue

was deionized on Dowex 50 × 8 (H^+ form, 100 mL). The obtained crude product was purified by chromatography on a column of Dowex 1 × 2 (acetate form, 100 mL). The product was eluted with a linear gradient of acetic acid (0 - 0.5 mol/L, 1 L each). Crystallization from 80% ethanol afforded the pure phosphonate.

9-(3-Amino-2-(phosphonomethoxy)propyl)adenine (2a): yield 1.1 g (34%); mp >250 °C; $k = 1.9$ (0.05 M TEAB); E_{Up} 0.44; ^{1}H NMR (D_2O + NaOD) δ 8.27 and 8.15 (2 × H, 2 × s, H-2, H-8), 4.42 (dd, 1H, J(1′a,2′) = 4.8, J_g = 14.7, 1′a-CH_2), 4.32 (dd, 1H, J(1′b,2′) = 5.2, J_g = 14.7, 1′b-CH_2), 3.77 (m, 1H, OCH), 3.57 (dd, 1H, J(P,CH) = 9.5, J_g = 12.2, PCH_2), 3.50 (dd, 1H, J(P,CH) = 9.5, J_g = 12.2, PCH_2), 2.70 (dd, 1H, J(3′a,2′) = 4.0, J_g = 13.7, 3′a-CH_2), 2.49 (dd, 1H, J(3′b,2′) = 7.3, J_g = 13.7, 3′b-CH_2); FABMS m/z MH^+ 303; UV spectrum (λ_{max} (ϵ)) at pH 2 259.0 (10 600), at pH 7 261.0 (12 000), at pH 12 261.0 (12 000). Anal. ($C_9H_{15}N_6O_4P\cdot H_2O$) C, H, N, P.

9-(3-Amino-2-(phosphonomethoxy)propyl)-2,6-diaminopurine (2b): yield 0.43 g (13%); mp 243−245 °C; $k = 2.3$ (0.05 M TEAB); E_{Up} 0.42; ^{1}H NMR (D_2O + NaOD) δ 7.91 (s, 1H, H-8), 4.32 (dd, 1H, J(1′a,2′) = 4.6, J_g = 15.1, 1′a-CH_2), 4.24 (dd, 1H, J(1′b,2′) = 5.6, J_g = 15.1, 1′b-CH_2), 4.09 (m, 1H, 2′-CH), 3.72 (dd, 1H, J(P,CH) = 8.1, J_g = 12.7, PCH_2), 3.32 (dd, 1H, J(P,CH) = 8.5, J_g = 12.7, P-CH_2); 3.22 (dd, 1H, J(3′a,2′) = 2.9, J_g = 13.4, 3′a-CH_2), 2.86 (dd, 1H, J(3′b,2′) = 9.5, J_g = 13.4, 3′b-CH_2); FABMS m/z MH^+ 318.4; UV spectrum (λ_{max} (ϵ)) at pH 2 290.0 (10 000), 252.0 (10 400); at pH 7 281.0 (9700), 255.0 (8400), at pH 12: 281.0 (9700), 255.0 (8100). Anal. ($C_9H_{16}N_7O_4P\cdot H_2O$) C, H, N, P.

1-(3-Amino-2-(phosphonomethoxy)propyl)cytosine (2c): yield 0.33 g (11%); mp 240 °C (with decomposition); k = 2.1 (0.05 M TEAB); E_{Up} 0.54; ^{1}H NMR (D_2O + NaOD) δ 7.65 (d, 1H, J(5,6) = 7.5, H-6), 6.03 (d, 1H, J(5,6) = 7.5, H-5), 4.05 (dd, 1H, J(1′a,2′) = 3.9, J_g = 13.7, 1′a-CH_2), 3.94 (dd, 1H, J(1′b,2′) = 5.9, J_g = 13.7, 1′b-CH_2), 4.00 (m, 1H, 2′-CH), 3.73 (dd, 1H, J(P,CH) = 8.1, J_g = 12.7, PCH_2), 3.26 (dd, 1H, J(P,CH) = 8.8, J_g = 12.7, PCH_2); 3.22 (dd, 1H, J(3′a,2′) = 2.7, J_g = 13.4, 3′a-CH_2), 2.95 (dd, 1H, J(3′b,2′) = 9.3 J_g = 13.4, 3′b-CH_2); FABMS m/z MH^+ 279.2; UV spectrum (λ_{max} (ϵ)), at pH 2 282.0 (13 500), at pH 7 273.0 (10 100), at pH 12 275.0 (9900). Anal. ($C_8H_{15}N_4O_5P\cdot H_2O$) C, H, N, P.

9-[3-((*o*-Carboxybenzoyl)amino)-2-(phosphonomethoxy)propyl]adenine (7a) and 9-[3-((*o*-carboxybenzoyl)-amino)-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (7b). Compounds **7a** and **7b** were obtained after elution of amino derivatives **2a** and **2b** from the column of Dowex 1 × 2 (acetate form) by continued washing with acetic acid (1 M) and processed analogously.

7a: yield 0.34 g; mp >250 °C; k = 5.1 (0.05 M TEAB/10 min, grad. 10% acetonitrile/10 min); E_{Up} 0.70; ^{1}H NMR (D_2O + NaOD) δ 8.34 and 8.18 (2 × s, 2 × 1H, H-2, H-8), 7.30−7.65 (m, 4H, arom), 4.49 (2 × dd, 2H, J(1′a,2′) = 4.6, J(1′b,2′) = 5.1, J_g = 14.9, 1′-CH_2), 4.04 (m, 1H, OCH); 3.47−3.70 (m, 4H, PCH_2, 3′-CH_2); ^{13}C NMR (D_2O) δ 177.1 and 174.05 (C═O), 156.57 (C-6), 153.53 (C-2), 150.28 (C-4), 144.83 (C-8), 138.82, 135.41, 131.37, 130.35, 129.02, and 128.28 (arom C), 119.29 (C-5), 78.70 (d, J(P,C) = 12.2, C-2′), 69.24 (d, J(P,C) = 149.5, P-C), 45.68 (C-1′), 41.00 (C-3′). Anal. ($C_{17}H_{19}N_6O_7P$) C, H, N, P.

7b: yield 0.2 g; mp >250 °C; k = 6.4 (0.05 M TEAB/10 min, grad. 10% acetonitrile/10 min); E_{Up} 0.70; ^{1}H NMR (D_2O + NaOD) δ 8.03 (s, 1H, H-8), 7.34−7.61 (m, 4H, arom), 4.36 (dd, 1H, J(1′a,2′) = 4.9, J_g = 14.9, 1′a-CH_2), 4.31 (dd, 1H, J(1′b,2′) = 5.6, J_g = 14.9, 1′b-CH_2), 4.01 (brpent, 1H, 2′-CH), 3.60 (dd, 1H, J(P,CH) = 9.8, J_g = 11.7, PCH_2), 3.54 (dd, 1H, J(P,CH) = 9.3, J_g = 11.7, PCH_2), 3.58 (dd, 1H, J(3′a,2′) = 4.0, J_g = 14.2, 3′a-CH_2), 3.52 (dd, 1H, J(3′b,2′) = 4.9, J_g = 14.2, 3′b-CH_2), ^{13}C NMR (D_2O) δ 177.09 and 174.04 (C═O), 161.12 (C-2), 157.22 (C-6), 152.46 (C-4), 142.48 (C-8), 138.82, 135.47, 131.37, 130.37, 129.03 and 128.32 (arom C), 113.87 (C-5), 78.70 (d, J(P,C) = 12.2, C-2′), 69.21 (d, J(P,C) = 151.1, P-C), 45.25 (C-1′), 41.00 (C-3′). Anal. ($C_{17}H_{20}N_7O_7P$) C, H, N, P.

9-(3-(Dimethylamino)-2-(phosphonomethoxy)propyl)-adenine (8a): yield 0.34 g (11%); mp >250 °C; E_{Up} 0.53; ^{1}H NMR (D_2O + NaOD) δ 8.34 and 8.21 (2 × s, 2 × 1 H, H-2 and H-8), 4.49 (dd, 1H, J(1′a,2′) = 4.1, J_g = 14.9, 1′a-CH_2), 4.35 (dd, 1H, J(1′b,2′) = 4.6, J_g = 14.9, 1′b-CH_2), 4.03 (m, 1H, 2′-CH), 3.70 (dd, 1H, J(P,CH) = 9.3, J_g = 12.0, PCH_2), 3.45 (dd, 1H, J(P,CH) = 9.5, J_g = 12.0, PCH_2), 2.50 (d, 2H, J(3′,2′) = 5.9, 3′-CH_2), 2.36 (s, 6H, $N(CH_3)$); FABMS m/z MH^+ 331.3. Anal. ($C_{11}H_{19}N_6O_4P$) C, H, N, P.

1-(3-(Dimethylamino)-2-(phosphonomethoxy)propyl)-cytosine (8c): yield 0.41 g (24%); mp >250 °C; k = 1.0 (0.05 M TEAB); E_{Up} 0.46; ^{1}H NMR (D_2O + NaOD) δ 7.61 (d, 1H, J(5,6) = 7.3, H-6), 6.03 (d, 1H, J(5,6) = 7.3, H-5), 4.05 (dd, 1H, J(1′a,2′) = 3.9, J_g = 14.7, 1′a-CH_2), 4.00 (dd, 1H, J(1′b,2′) = 5.6, J_g = 14.7, 1′b-CH_2), 4.20 (m, 1H, 2′-CH), 3.86 (dd, 1H, J(P,CH) = 8.5, J_g = 13.2, PCH_2), 3.50 (dd, 1H, J(P,CH) = 9.0, J_g = 13.2, PCH_2), 3.28 (dd, 1H, J(3′a,2′) = 2.4, J_g = 13.4, 3′a-CH_2), 3.20 (dd, 1H, J(3′b,2′) = 10.7 J_g = 13.4, 3′b-CH_2); 2.92 (s, 6H, $N(CH_3)_2$); FABMS m/z MH^+ 307.1. Anal. ($C_{10}H_{19}N_4O_5P$) C, H, N, P.

9-(3-Morpholino-2-(phosphonomethoxy)propyl)adenine (9a): yield 1.1 g (35%); mp 197−200 °C; k = 2.7 (4% acetonitrile/0.05 M TEAB); E_{Up} 0.64; ^{1}H NMR (D_2O + NaOD) δ 8.21 and 8.14 (2 × s, 2 × 1H, H-2, H-8), 4.47 (dd, 1H, J(1′a,2′) = 3.2, J_g = 14.0, 1′a-CH_2), 4.34 (dd, 1H, J(1b′,2′) = 5.6, J_g = 14.0, 1′b-CH_2), 4.32 (m, 1H, 2′-CH), 3.94 (m, 4H, OCH_2), 3.73 (dd, 1H, J(P,CH) = 8.3, J_g = 12.9, P-CH_2), 3.31 (dd, 1H, J(P,CH) = 7.3, J_g = 12.9, PCH_2), 3.18 and 3.06 (2 × m, 2 × 2H, NCH_2), 3.06 (m, 1H, 3′a-CH_2), 2.92 (dd, 1H, J(3′a,2′) = 10.0, J_g = 13.7, 3′b-CH_2); FABMS m/z MH^+ 373.2; UV spectrum (λ_{max} (ϵ)), at pH 2 259.0 (11 900), at pH 7 261.0 (12 300), at pH 12 262.0 (12 700). Anal. ($C_{13}H_{21}N_6O_5P$) C, H, N, P.

9-(2-(Phosphonomethoxy)-3-(trimethylammonio)propyl)adenine (10a): yield 0.34 g (10%); mp 270−273 °C; k = 0.5 (0.05 M TEAB), E_{Up} 0.28; ^{1}H NMR (D_2O + NaOD) δ 8.35 and 8.25 (2 × s, 2 × 1 H, H-2, H-8), 4.64 (dd, 1H, J(1′a,2′) = 4.4, J_g = 14.9, 1′a-CH_2), 4.53 (m, 1H, 2′-CH), 4.48 (dd, 1H, J(1′b,2′) = 3.2, J_g = 14.9, 1′b-CH_2), 3.84 (dd, 1H, J(P,CH) = 9.3, J_g = 12.2, PCH_2), 3.60 (dd, 1H, J(P,CH) = 9.8, J_g = 12.2, PCH_2), 3.42 (dd, 1H, J(3′a,2′) = 1.5, J_g = 14.2, 3′a-CH_2), 3.24 (dd, 1H, J(3′b,2′) = 9.8 J_g = 14.2, 3′b-CH_2), 3.19 (s, 9H, $N^+(CH_3)_3$); FABMS m/z MH^+ 345.2; UV spectrum (λ_{max} (ϵ)) at pH 2 258.0 (11 700), at pH 7 261.0 (11 800), at pH 12 261.0 (11900). Anal. ($C_{12}H_{22}N_6O_4P$) C, H, N, P.

9-(2-(Phosphonomethoxy)-3-(trimethylammonio)propyl)-2,6-diaminopurine (10b): yield 0.37 g (8%); mp >250 °C, k = 0.7 (0.05 M TEAB), E_{Up} 0.39; ^{1}H NMR (D_2O + NaOD) δ 8.00 (s, 1H, H-8), 4.43 (dd, 1H, J(1′a,2′) = 4.9, J_g = 15.1, 1′a-CH_2), 4.30 (dd, 1H, J(1′b,2′) = 2.9, J_g = 15.1, 1′b-CH_2), 4.48 (m, 1H, 2′-CH), 3.81 (dd, 1H, J(P,CH) = 9.2, J_g = 12.2, PCH_2), 3.58 (dd, 1H, J(P,CH) = 9.8, J_g = 12.2, PCH_2), 3.40 (dd, 1H, J(3′a,2′) = 1.5, J_g = 13.9, 3′a-CH_2), 3.28 (dd, 1H, J(3′b,2′) = 9.5, J_g = 13.9, 3′b-CH_2), 3.19 (s, 9H, $N^+(CH_3)_3$); UV spectrum (λ_{max} (ϵ)) at pH 2 281.0 (9400), 255.0 (7900), at pH 7 280.0 (9800), 256.0 (8300), at pH 12 2861.0 (9700), 256.0 (8200). Anal. ($C_{12}H_{22}N_6O_4P$) C, H, N, P.

1-(2-(Phosphonomethoxy)-3-(trimethylammonio)propyl)cytosine (10c): yield 0.6 g (6%); mp >250 °C; k = 2.4 (0.05 M TEAB); E_{Up} 0.4; ^{1}H NMR (D_2O + NaOD) δ 7.63 (d, 1H, J(5,6) = 7.3, H-6), 6.04 (d, 1H, J(5,6) = 7.3, H-5), 4.37 (brpent, 1H, 2′-CH), 4.13 (dd, 1H, J(1′a,2′) = 4.6, J_g = 14.7, 1′a-CH_2), 4.03 (dd, 1H, J(1′b,2′) = 4.6, J_g = 14.7, 1′b-CH_2), 3.82 (dd, 1H, J(P,CH) = 9.8, J_g = 12.7, PCH_2), 3.69 (dd, 1H, J(P,CH) = 10.7, J_g = 12.7, PCH_2), 3.52 (d, 2H, J(3′,2′) = 5.9, 3′-CH_2), 3.23 (s, 9H, $N^+(CH_3)_3$); FABMS m/z MH^+ 321.2; UV spectrum (λ_{max} (ϵ)) at pH 2 281.0 (13 900), at pH 7 273.0 (10 400), at pH 12 273.0 (10700). Anal. ($C_{11}H_{21}N_4O_5P\cdot 2H_2O$) C, H, N, P.

9-(2-(Phosphonomethoxy)-3-(trimethylammonio)propyl)guanine (10e): yield 0.3 g (18%); mp >250 °C; k = 0.6 (0.05 M TEAB); E_{Up}0.50; ^{1}H NMR (D_2O + NaOD) δ 7.86 (s, 1H, H-8), 4.45 (m, 1H, 2′-CH), 4.37 (dd, 1H, J(1′a,2′) = 4.9, J_g = 15.1, 1′a-CH_2), 4.29 (dd, 1H, J(1′b,2′) = 3.4, J_g = 15.1, 1′b-CH_2), 3.82 (dd, 1H, J(P,CH) = 9.5, J_g = 11.7, PCH_2), 3.58 (dd, 1H, J(P,CH) = 10.5, J_g = 11.5, P-CH_2), 3.39 (brd, 1H, J(3′a,2′) = 0.5, J_g = 13.9, 3′a-CH_2), 3.31 (dd, 1H, J(3′b,2′) = 9.3, J_g = 13.9, 3′b-CH_2), 3.19 (s, 9H, $N^+(CH_3)_3$); FABMS m/z MH^+ 361.3; UV spectrum (λ_{max} (ϵ)) at pH 2 254.0 (7700), at pH 7 252.0 (9100), at pH 12 269.0 (7400). Anal. ($C_{12}H_{22}N_6O_5P\cdot 2H_2O$) C, H, N, P.

Preparation of (2,3-Epoxypropyl)dimethylamine (14) *in Situ*. To a solution of triphenylphosphine (13.4 g, 50 mmol) in benzene (100 mL) was added dropwise diethyl azodicarboxylate (7.5 mL, 50 mmol), and the mixture was stirred for 15 min at room temperature. After addition of 3-(dimethylamino)-1,2-propanediol (6.0 g, 50 mmol) in benzene (50 mL), the mixture was refluxed for 4 h. The mixture was taken down, and the residue was dissolved in dimethylformamide (20 mL) and used directly for the alkylation.

General Procedure for Preparation of 2-Hydroxypropyl Derivatives 11ac, 12a, 13a−d. A mixture of heterocyclic base (20 mmol), cesium carbonate (0.4 g, 1.2 mmol), the appropriate oxirane (24 mmol) [(2,3-epoxypropyl)dimethylamine (**14**), (2,3-epoxypropyl)morpholine (**15**) or (2,3-epoxypropyl)trimethylammonium chloride (**16**)], and dimethylformamide (100 mL) was heated at 120 °C for 16 h under stirring and exclusion of moisture (TLC chloroform−methanol, 7:3, for **11**, **12** or electrophoresis and HPLC for **13**).

9-(3-(Dimethylamino)-2-hydroxypropyl)adenine (11a). The reaction mixture was concentrated *in vacuo*, the residue was codistilled with toluene (3 × 100 mL), and the product was purified by preparative HPLC in water and crystallized from ethanol with addition of ether: yield 0.9 g (20%); mp 112−114 °C; k = 5.2 (0.05 M TEAB); ^{1}H NMR ((CD$_3$)$_2$SO) δ 8.14 and 8.04 (2 × s, 2 × 1H, H-2, H-8), 7.22 (brs, 2H, NH$_2$), 5.05 (br, 1H, OH), 4.31 (m, 1H and 3.93−3.98 m, 2H, 1′-CH$_2$ and 2′-CH), 2.28 (dd, 1H, J(3′a,2′) = 6.4, J_g = 11.9, 3′a-CH$_2$), 2.22 (dd, 1H, J(3′b,2′) = 5.9, J_g = 11.9, 3′b-CH$_2$), 2.18 (s, 6H, N-CH$_3$); FABMS m/z MH$^+$ 237.1; UV spectrum (λ_{max} (ϵ)), at pH 2 258.0 (16 400), at pH 7 261.0 (14 500), at pH 12 261.0 (16 000). Anal. (C$_{10}$H$_{16}$N$_6$O·$_2$O) C, H, N.

1-(3-(Dimethylamino)-2-hydroxypropyl)cytosine (11c). The reaction mixture was concentrated *in vacuo*, the residue was codistilled with toluene (3 × 100 mL), and the product was purified by preparative HPLC in water and crystallized from methanol with addition of ether: yield 1.0 g (23%); mp 90−91 °C; k = 2.8 (0.05 M TEAB); ^{1}H NMR ((CD$_3$)$_2$SO) δ 7.44 (d, 1H, J(5,6) = 7.5, H-6), 5.60 (d, 1H, J(5,6) = 7.5, H-5), 7.00 and 6.95 (2 × br, 2 × 1H, NH$_2$), 4.85 (brs, 1H, OH), 4.03 (dd, 1H, J(1′a,2′) = 3.0, J_g = 13.4, 1′a-CH$_2$), 3.79 (m, 1H, 2′-CH), 3.17 (dd, 1H, J(1′b,2′) = 8.6, J_g = 13.4, 1′b-CH$_2$), 2.21 (dd, 1H, J(3′a,2′) = 6.6, J_g = 12.2, 3′a-CH$_2$), 2.17 (dd, 1H, J(3′b,2′) = 6.2, J_g = 12.2, 3′b-CH$_2$), 2.15 (s, 6H, N-(CH$_3$)$_2$); FABMS m/z MH$^+$ 213.2; UV spectrum (λ_{max} (ϵ)) at pH 2 282.0 (10 800), at pH 7 274.0 (8000), at pH 12 274.0 (6900). Anal. (C$_9$H$_{16}$N$_4$O$_2$·H$_2$O) C, H, N.

9-(2-Hydroxy-3-morpholinopropyl)adenine (12a). The reaction mixture was concentrated *in vacuo*, the residue was codistilled with toluene (3 × 100 mL), and the product was crystallized from methanol with addition of ether: yield 3.0 g (54%); mp 165 °C; R_f 0.21 (chloroform−methanol, 8:2); ^{1}H NMR ((CD$_3$)$_2$SO) δ 8.13 and 8.05 (2 × s, 2 × 1H, H-2, H-8), 7.19 (brs, 2H, NH$_2$), 5.17 (br, 1H, OH), 4.28 (dd, 1H, J(1′a,2′) = 2.2, J_g = 12.2, 1′a-CH$_2$), 4.05 (m, 1H, 2′-CH); 4.02 (dd, 1H, J(1′b,2′) = 7.8, J_g = 12.2, 1′b-CH$_2$), 3.49−3.56 (m, 4H, OCH$_2$), 2.33−2.44 (m, 4H, NCH$_2$), 2.30 (2 × dd, 2H, J(3′a,2′) = 6.4, J(3′b,2′) = 5.9, J_g = 12.7, 3′-CH$_2$); FABMS m/z MH$^+$ 279.2; UV spectrum (λ_{max} (ϵ)) at pH 2 259.0 (12 900), at pH 7 261.0 (13 600), at pH 12 261.0 (13 200). Anal (C$_{12}$H$_{18}$N$_6$O$_2$·H$_2$O) C, H, N.

9-(2-Hydroxy-3-(trimethylammonio)propyl)adenine Chloride and 3-(2-Hydroxy-3-(trimethylammonio)propyl)adenine Chloride (13a). The product, which crystallized directly from the reaction mixture, was filtered off and washed with dimethylformamide and toluene: yield 4.85 g (85%) of the non-separable mixture of isomers N-9 and N-3 (ratio 3.2:1); E_{Up} 0.77; k = 2.4 (6% acetonitrile/0.05 M TEAB); ^{1}H NMR ((CD$_3$)$_2$SO) δ (N-9 isomer) 8.15 and 8.12 (2 × s, 2 × 1H, H-2, H-8), 7.24 (brs, 2H, NH$_2$), 6.23 (d, 1H, J(OH,CH) = 6.4, OH), 4.52 (m, 1H, 2′-CH), 4.24 (dd, 1H, J(1′a,2′) = 4.3, J_g = 14.3, 1′a-CH$_2$), 4.17 (dd, 1H, J(1′b,2′) = 6.7, J_g = 14.3, 1′b-CH$_2$), 3.51 (brd, 1H, J(3′a,2′) = 1.0, J_g = 12.4, 3′a-CH$_2$), 3.30 (dd, 1H, J(3′b,2′) = 9.8, J_g = 12.3, 3′b-CH$_2$), 3.13 (s, 9H, N$^+$(CH$_3$)$_3$), (N-3 isomer) 8.28 and 7.77 (2 × s, 2 × 1H, H-2, H-8), 7.90 and 7.96 (2 × br, 2H, NH$_2$), 6.33 (d, 1H, J(OH,CH) = 6.4, OH), 4.64 (m, 1H, 2′-CH); 4.43 (dd, 1H, J(1′a,2′) = 3.7, J_g = 13.7, 1′a-CH$_2$), 4.26 (dd, 1H, J(1′b,2′) = 7.9, J_g = 13.7, 1′b-CH$_2$), 3.62 (brd, 1H, J(3′a,2′) = 1.0, J_g = 12.2, 3′a-CH$_2$), 3.37 (dd, 1H, J(3′b,2′) = 9.8, J_g = 12.2, 3′b-CH$_2$), 3.15 (s, 9H, N$^+$(CH$_3$)$_3$); ^{13}C NMR ((CD$_3$)$_2$SO) δ (N-9 isomer) 156.16 (C-6), 152.60 (C-2), 149.89 (C-4), 141.76 (C-8), 118.72 (C-5), 67.68 (C-3′), 64.10 (C-2′), 53.64 (3C, CH$_3$), 47.22 (C-1′), (N-3 isomer) 155.31 (C-6), 152.35 (C-8), 149.94 (C-4), 144.52 (C-2), 120.53 (C-5), 66.39 (C-3′), 63.35 (C-2′), 53.64 (3C, CH$_3$), 53.18 (C-1′); FABMS m/z (MH − HCl)$^+$ 251.2. Anal. (C$_{11}$H$_{19}$ClN$_6$O) C, H, N, Cl.

9-(2-Hydroxy-3-(trimethylammonio)propyl)-2,6-diaminopurine Chloride and 3-(2-Hydroxy-3-(trimethylammonio)propyl)-2,6-diaminopurine Chloride (13b). The product, which crystallized directly from the reaction mixture, was filtered off and washed with dimethylformamide and toluene: yield 4.5 g (75%) of the nonseparable mixture of isomers N-9 and N-3 (ratio 6:1); E_{Up} −0.59; k = 2.7 (6% acetonitrile/0.05 M TEAB); ^{1}H NMR ((CD$_3$)$_2$SO) δ (N-9 isomer) 7.73 (s, 1H, H-8), 6.70 and 5.79 (2 × brs, 2 × 2H, NH$_2$), 4.50 (m, 1H, 2′-CH); 4.04 (dd, 1H, J(1′a,2′) = 4.6, J_g = 14.2, 1′a-CH$_2$), 4.00 (dd, 1H, J(1′b,2′) = 6.6, J_g = 14.2, 1′b-CH$_2$), 3.48 (dd, 1H, J(3′a,2′) = 1.2, J_g = 13.7, 3′a-CH$_2$), 3.32 (dd, 1H, J(3′b,2′) = 9.5, J_g = 13.7, 3′b-CH$_2$), 3.16 (s, 9H, N$^+$(CH$_3$)$_3$), (N-3 isomer) 7.39 (s, 1H, H-8), 7.23 and 6.73 (2 × br, 2 × 2H, NH$_2$), 4.58 (m, 1H, 2′-CH), 4.32 (dd, 1H, J(1′a,2′) = 7.1, J_g = 14.4, 1′a-CH$_2$), 4.21 (dd, 1H, J(1′b,2′) = 4.4, J_g = 14.4, 1′b-CH$_2$), 3.61 (brd, 1H, J(3′a,2′) = 1.0, J_g = 13.4, 3′a-CH$_2$), 3.49 (dd, 1H, J(3′b,2′) = 9.5, J_g = 13.4, 3′b-CH$_2$), 3.38 (s, 9H, N$^+$(CH$_3$)$_3$); ^{13}C NMR ((CD$_3$)$_2$SO) δ (N-9 isomer) 160.38 (C-2), 156.33 (C-6), 152.06 (C-4), 138.41 (C-8), 113.16 (C-5), 68.03 (C-3′), 64.14 (C-2′), 53.66 (3C, CH$_3$), 47.06 (C-1′), (N-3 isomer) 155.63 (C-6), 151.95 (C-4), 151.36 (C-2), 148.96 (C-8), 115.09 (C-5), 67.68 (C-3′), 64.14 (C-2′), 53.66 (3C, CH$_3$), 49.48 (C-1′); FABMS m/z (MH − HCl)$^+$ 266.1. Anal. (C$_{11}$H$_{20}$ClN$_7$O) C, H, Cl, N.

1-(2-Hydroxy-3-(trimethylammonio)propyl)cytosine Chloride (13c). The product, which crystallized directly from the reaction mixture, was filtered off and washed with dimethylformamide and toluene: yield 3.0 g (57%); E_{Up} −0.58; k = 0.95 (0.05 M TEAB); ^{1}H NMR ((CD$_3$)$_2$SO) δ 7.57 (d, 1H, J(6,5) = 7.3, H-6), 7.30 and 7.02 (2 × br, 2 × 1H, NH$_2$), 6.20 (d, 1H, J(OH, CH) = 6.2, OH), 5.71 (d, 1H, J(5,6) = 7.3, H-5), 4.33 (m, 1H, 2′-CH), 3.82 (dd, 1H, J(1′a,2′) = 3.7, J_g = 13.4, 1′a-CH$_2$), 3.57 (dd, 1H, J(1′b,2′) = 7.1, J_g = 13.4, 1′b-CH$_2$); 3.46 (dd, 1H, J(3′a,2′) = 1.0, J_g = 13.4, 3′a-CH$_2$), 3.30 (dd, 1H, J(3′b,2′) = 9.8, J_g = 13.4, 3′b-CH$_2$), 3.15 (s, 9H, N$^+$(CH$_3$)$_3$); FABMS m/z (MH − HCl)$^+$ 227.2. Anal. (C$_{10}$H$_{19}$ClN$_4$O$_2$·3H$_2$O) C, H, Cl, N.

9-(2-Hydroxy-3-(trimethylammonio)propyl)-2-amino-6-benzyloxypurine Chloride and 7-(2-Hydroxy-3-(trimethylammonio)propyl)-2-amino-6-benzyloxypurine Chloride (13d). The reaction mixture was concentrated *in vacuo*, the residue was codistilled with toluene (3 × 100 mL), and the product was purified by chromatography on a column of Dowex 1 × 2 (carbonate form, 100 mL): yield 5.7 g (87%) of the nonseparable mixture of isomers N-9 and N-7 (ratio 1.8:1); E_{Up} −0.7; ^{1}H NMR ((CD$_3$)$_2$SO) δ (N-9 isomer) 7.92 (s, 1H, H-8), 7.32−7.55 (m, 5H arom), 6.42 (brs, 2H, NH$_2$), 5.49 (s, 2H, PhCH$_2$), 4.52 (m, 1H, 2′-CH), 4.07 (dd, 1H, J(1′a,2′) = 4.6, J_g = 14.1, 1′a-CH$_2$), 4.03 (dd, 1H, J(1′b,2′) = 6.8, J_g = 14.1, 1′b-CH$_2$), 3.23−3.45 (m, 2H, 3′-CH$_2$), 3.125 (s, 9H, N$^+$(CH$_3$)$_3$), (N-7 isomer) 8.10 (s, 1H, H-8), 7.32−7.55 (m, 5H arom), 6.16 (brs, 2H, NH$_2$), 5.52 (s, 2H, PhCH$_2$), 4.34 (m, 1H, 2′-CH), 4.22 (dd, 1H, J(1′a,2′) = 3.9, J_g = 13.9, 1′a-CH$_2$), 4.11 (dd, 1H, J(1′b,2′) = 8.3, J_g = 13.9, 1′b-CH$_2$), 3.23−3.45 (m, 2H, 3′-CH$_2$), 3.12 (s, 9H, N$^+$(CH$_3$)$_3$); ^{13}C NMR ((CD$_3$)$_2$SO) δ (N-9 isomer) 160.59 (C-2), 160.14 (C-6), 155.04 (C-4), 141.05 (C-8), 137.17 and 128.93 (2C) and 128.90 (2C) and 128.58 (arom C); 114.08 (C-5), 68.98 (C-3′), 67.43 (O-C arom), 64.40 (C-2′), 54.07 (3C, CH$_3$), 47.59 (C-1′), (N-7 isomer) 164.18 (C-4), 160.05 (C-2), 157.00 (C-6), 146.93 (C-8), 137.05 and 129.09 (2C), 128.97 (2C), and 128.72 (arom C), 106.31 (C-5), 68.21 (C-3′), 67.52 (O−C arom), 64.89 (C-2′), 53.79 (3C, CH$_3$), 51.15 (C-1′); FABMS m/z (MH − HCl)$^+$ 357.2. Anal. (C$_{18}$H$_{25}$ClN$_6$O$_2$) C, H, Cl, N.

Antiviral Assays. The antiviral assays, other than HIV-1, were based on inhibition of virus-induced cytopathicity in either E$_6$SM or HEL cell cultures, following previously established procedures.[16,17] Briefly, confluent cell cultures in mi-

crotiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1-h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ··· μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion on the control virus-infected cell cultures.

Inhibition of HIV-1-Induced Cytopathicity in MT-4 Cells. The methodology of the anti-HIV assays has been described previously.[18] Briefly, human MT-4 cells (5×10^5 cells mL^{-1}) were infected with 100 $CCID_{50}$ HIV-1 (strain $HTLV\text{-}III_B$)/mL and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds. After 5 days of incubation at 37 °C, the number of viable cells was determined in a blood cell counting chamber by trypan blue dye exclusion.

Inhibition of MSV-Induced Transformation of Murine C3H/3T3 Embryo Fibroblasts. The anti-MSV assay was performed as described previously.[18,19] Murine C3H/3T3 embryo fibroblast cells were seeded at 5×10^5 cells mL^{-1} into 1-cm^2 wells of a 48-well microplate. The cell cultures were infected 24 h later with 80 focus-forming units of MSV (prepared from tumours induced following intramuscular inoculation of 3-day-old NMRI mice with MSV, as described previously[20]) for 60−90 min at 37 °C. The medium was then replaced by 1 mL of fresh medium containing various concentrations of the test compounds. After 6 days, transformation of the cell culture was examined microscopically.

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