SYNTHESIS OF ACYCLIC NUCLEOTIDE ANALOGUES DERIVED FROM N^3-SUBSTITUTED ISOGUANINE

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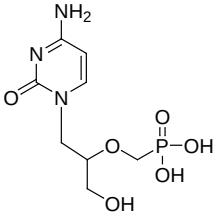
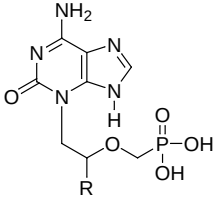
Reaction of 9-benzyl-6-{[(dimethylamino)methylidene]amino}purin-2(3*H*)-one (**7**) with ethylene carbonate gave a mixture of 9-benzyl-2-(2-hydroxyethoxy)purin-6-amine (**10**) and 2-amino-9-benzyl-3-(2-hydroxyethyl)purin-2(3*H*)-one (**11**). This mixture reacted with diisopropyl (tosyloxymethyl)phosphonate in the presence of NaH followed by catalytic hydrogenation and bromotrimethylsilane treatment to afford isomeric 6-amino-3-[2-(phosphonomethoxy)ethyl]purin-2(3*H*)-one (**3**) and 2-[2-(phosphonomethoxy)ethoxy]purin-6-amine (**15**). Similar treatment of compound **7** with tritylglycidol gave two isomeric 2-hydroxy-3-(trityloxy)propyl derivatives **18**, **20** which were subsequently condensed with diisopropyl (tosyloxymethyl)phosphonate to afford protected diester intermediates **21** and **22**; these compounds were transformed by hydrogenolysis and ester cleavage with bromotrimethylsilane to the isomeric 6-amino-3-[3-hydroxy-2-(phosphonomethoxy)propyl]-purin-2(3*H*)-one (**2**) and 2-[3-hydroxy-2-(phosphonomethoxy)propoxy]purin-6-amine (**24**). None of the free phosphonates **2**, **3**, **15** or **24** exhibited any antiviral or cytostatic activity.
Key words: Purines; Nucleosides; Nucleotides; Acyclic analogs; Phosphonates; Alkylation; Antivirals.

Out of the large and permanently expanding group of acyclic nucleoside phosphonates, which exhibit numerous biological activities[1] such as antiviral, anticancer[2], antiparasitic[3] and/or immunomodulatory effects[4], the most prominent representative is a cytosine derivative (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (**1**, HPMPC, Cidofovir)[5] which was approved in the U.S.A. and Europe for treatment of CMV-retinitis in AIDS patients (Vistide®)[6]; it also shows therapeutic activity against papillomavirus-induced larynx warts[7] and anogenital warts[8], against herpes simplex infections[9], molluscum contagiosum[10], progressive multifocal leukoencephalopathy[11] and others[12]. As a part of our studies on structure–activity relationship in this series of compounds, we were interested in synthesising

its isoguanine (2-hydroxypurine-6-amine) counterpart. Isoguanine derivatives combine the heteroaromatic ring of purine with the spatial orientation of 6-amino and 2-oxo groups which is similar to that in cytosine derivatives. This applies in particular to the N^3-substituted derivatives that can be considered similar to 5,6-disubstituted cytosine derivatives. Isoguanosine and its derivatives were also shown to possess extraordinary base-pairing properties[13].



1, HPMPC



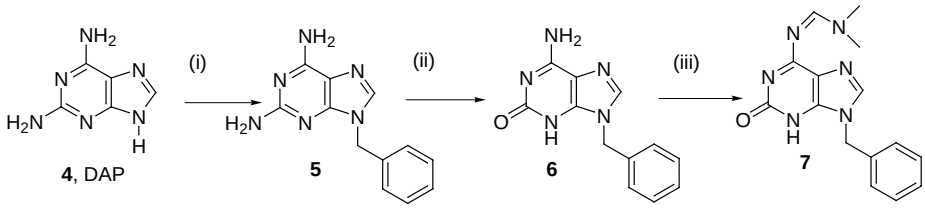
2, R = CH_2OH HPMPisoG

3, R = H PMEisoG

Therefore, we were interested in the synthesis of 3-substituted isoguanine derivatives **2** and **3** bearing the 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP), and/or (2-phosphonomethoxy)ethyl (PME), acyclic phosphonate chain in the N^3-position.

Although various heterocyclic ring closure[14] or photosubstitution[15] approaches to 3-alkylisoguanine derivatives were reported, we first selected a straightforward approach to 3-substituted isoguanine, *i.e.* alkylation of isoguanine with appropriate alkylation agents bearing a protected phosphonate moiety and a suitable leaving group. Alkylation of isoguanine or isoguanosine was reported[16] to afford mixtures of products including N^1-, N^3-, N^9-, *O*-alkyl and N^6-alkyl derivatives arising probably from subsequent Dimroth rearrangement of the originally formed N^1-alkyl intermediate[17]. Therefore we started from the N^9,N^6-protected derivative of isoguanine **7** prepared by oxodeamination of the corresponding protected 2,6-diaminopurine derivative **5** (ref.[18]) (Scheme 1). The nitrous acid oxodeamination[19] afforded N^9-benzylisoguanine **6** which, after protection of the primary amino group, afforded intermediate **7** in fair yield.

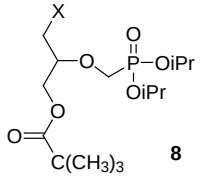
Direct alkylation of compound **7** using precursors of the HPMP (ref.[20]) type **8** or PME (ref.[21]) type **9** in DMF, resulted in *O*-alkyl derivatives accompanied by only traces of *N*-alkyl products, irrespective of whether in the presence of potassium carbonate or cesium carbonate, sodium hydride or sodium *tert*-butoxide, or in the absence of base. The stepwise build-up of



(i) BnBr, K_2CO_3, DMF; (ii) AcOH/$NaNO_2$, H_2O; (iii) DMADMF/ DMF

SCHEME 1

the phosphonate-bearing side chain, as illustrated in Schemes 2 and 3, resulted in a more favourable, roughly 1 : 1 ratio of *N*- and *O*-alkylation. Alkylation of compound **7** using ethylene carbonate or 2,3-epoxypropyl trityl ether (tritylglycidol) in DMF in the presence of potassium carbonate afforded in both cases mixtures of *N*- and *O*-alkyl products in overall yields around 50%. Lower susceptibility of the isoguanine derivative to alkylation



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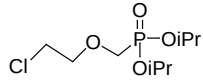

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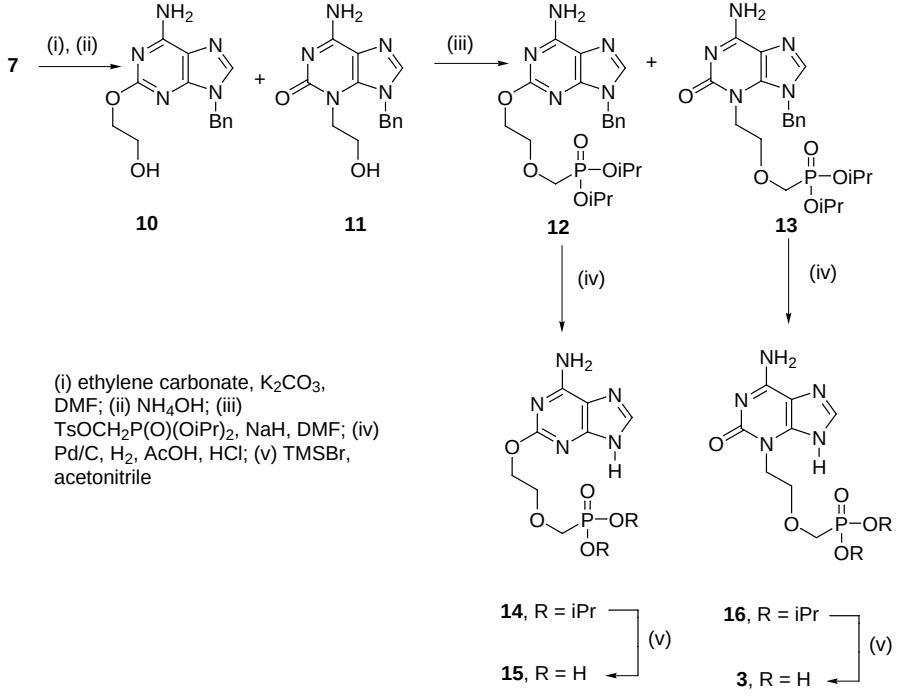
required elevated temperature of 140 °C during the reaction. Sodium hydride and sodium *tert*-butoxide were too drastic and resulted in complex reaction mixtures. On the other hand, cesium carbonate did not promote the reaction. Under the reaction conditions, partial deprotection of 6-amino group occurred in both cases; full deprotection of 6-amino group was achieved by treatment with ammonia. The intermediary *N*- and *O*-isomers **10** and **11**, as well as **18** and **20** were difficult to separate at this stage; the mixture of intermediates was used in further reaction, but the NMR samples were purified by preparative TLC. Compound **19** later proven as 2′-*O*-isomer of compound **18** was also isolated as a minor side product (less than 5%) of the alkylation step. Whether it rises from the nucleophile attacking the 2′-position of 2,3-epoxypropyl trityl ether, or it is a result of migration between the two neighbouring propane hydroxy groups under relatively drastic conditions was not studied. The phosphonate moiety was introduced by reaction with diisopropyl (tosyloxymethyl)phosphonate in the presence of NaH to give the intermediates **12**, **13**, **21** and **22**.

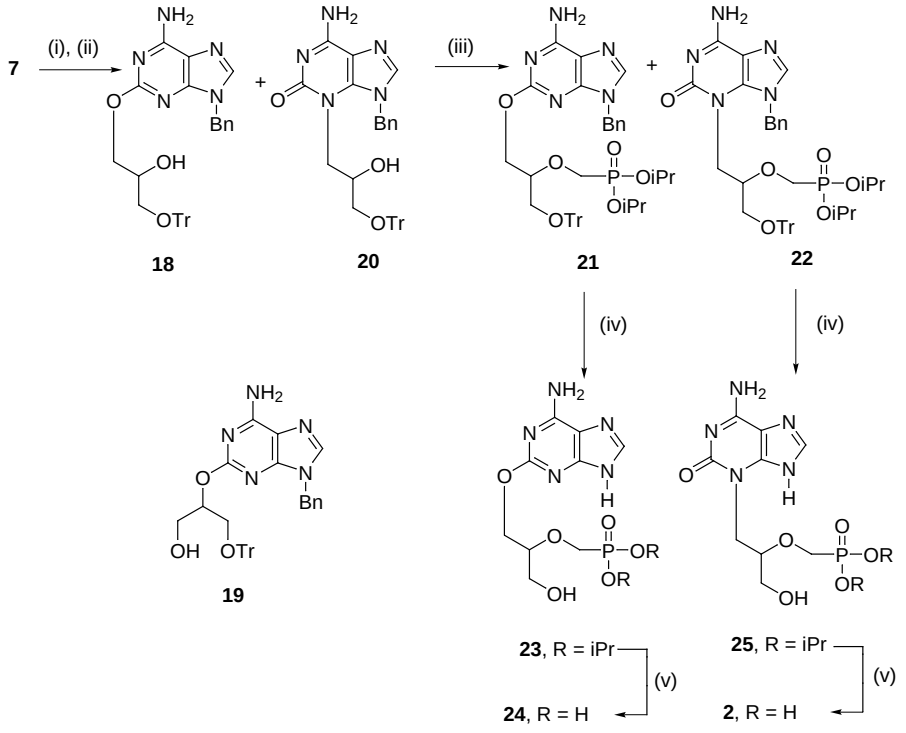
Deprotection of the intermediates proceeded by a common reaction sequence. The N^9-benzyl group of PME derivatives **12** and **13** was removed by catalytic hydrogenation in glacial acetic acid. The same conditions were used to deprotect both *O*-trityl and N^9-benzyl groups in HPMP derivatives **21** and **22**. Full separation of *N*- and *O*-isomers both in the PME and HPMP series was achieved at the stage of diisopropyl esters **12**, **13**, **23** and **25** by chromatography on silica gel.



SCHEME 2

Final removal of the phosphonate isopropyl ester groups was performed using bromo(trimethyl)silane treatment followed by hydrolysis[21]. Final purification of phosphonic acids **2**, **3**, **15** and **24** was performed by a combination of ion exchange and reverse phase chromatography.

The structures of all compounds were confirmed by NMR spectra. ^{1}H NMR spectra provided basic information on the presence of the purine base and their substituents in the molecule. The bonding of alkyl substituent to N- and/or O-atom could be distinguished in ^{13}C NMR spectra on the basis of a



(i) Tritylglycidol, K_2CO_3, DMF; (ii) NH_4OH; (iii) $TsOCH_2P(O)(OiPr)_2$, NaH, DMF; (iv) Pd/C, H_2, AcOH, HCl; (v) TMSBr, acetonitrile

SCHEME 3

characteristic chemical shift difference between N-CH and O-CH carbon signals[22–24] (compare $\delta \approx 47$ ppm in compounds **2**, **3** and **20** with δ 63–68.5 ppm in **15**, **18**, **19**, **21** and **24**). The presence of phosphonate group in compounds **2**, **3**, **12**–**16** and **23**–**25** is manifested by J(P,H) and J(P,C) couplings in ^{1}H and ^{13}C NMR spectra (see Experimental). The differentiation between alternative *N*-alkylated regioisomers is possible using heteronuclear long-range ^3J(C,H) couplings of N-CH protons to carbon atoms of the purine moiety in proton-coupled ^{13}C NMR spectra[24–26]. Experimental evidence for alkyl groups in the N^3-position in the present series of *N*-alkylated nucleosides is based on the observation of ^3J(C,H) couplings of N-CH protons to C-2 and C-4 carbons (Fig. 1a). Similarly, benzyl group at N^9 is manifested by ^3J(C,H) coupling of its N-CH_2 protons to C^4 and C^8 carbons (Fig. 1b). For comparison, the *O*-alkyl derivatives are characterised by ^3J(C,H) coupling of

O-CH protons to C-2 carbon (Fig. 1c). Typical values of the ^3J(C,H) couplings are 3–4.5 Hz.

Compounds **2**, **3**, **15** and **24** were inactive in cytostatic[22] [*in vitro* inhibition of cell growth in mouse leukemia L1210 cell line (ATCC CCL 219); murine L929 cell line (ATCC CCL 1)] and antiviral[23] [DNA viruses HSV-1, HSV-2, CMV, VZV and vaccinia virus and retroviruses HIV-1, HIV-2 and MSV] assays.

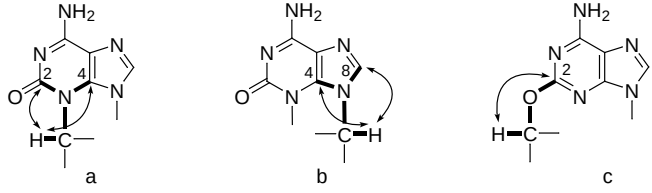


FIG. 1
Heteronuclear ^3J(C,H) couplings characteristic of alkyl substituent bonded to nitrogen N^3 (a), nitrogen N^9 (b) and oxygen atom at C^2 (c)

EXPERIMENTAL

Unless stated otherwise, solutions were evaporated at 40 °C/ 2 kPa and compounds were dried at 13 Pa over phosphorus pentoxide. Melting points were determined on a Kofler block and are uncorrected. TLC was performed on Silufol UV_{245} sheets in the solvent systems: S1 chloroform–methanol (95 : 5), S2 chloroform–methanol (90 : 10), S3 chloroform–methanol (4 : 1), S4 propan-2-ol–concentrated aqueous ammonia–water (7 : 1 : 2). For column chromatography 70–230 mesh silica gel 60 from Aldrich was used, while preparative reverse phase HPLC was performed on a 2 × 35 cm C-18 column. NMR samples were isolated using Aldrich preparative TLC plates. NMR spectra were recorded on a Varian Unity-500 spectrometer (^{1}H at 500 MHz, ^{13}C at 125.7 MHz); chemical shifts are referenced to internal tetramethylsilane in hexadeuteriodimethyl sulfoxide and deuteriochloroform. The free phosphonic acids were measured in deuterium oxide containing sodium deuteroxide with sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. UV absorption spectra (λ in nm) were measured on a Beckmann DU-65 spectrometer. Mass spectra were taken on a ZAB-EQ spectrometer (VG Analytical) using EI (electron energy 70 eV) and FAB (ionisation by Xe, accelerating voltage 8 kV) techniques. 2,6-Diaminopurine (**4**) was purchased from Tokyo Kasei Co. (Japan), bromotrimethylsilane, cesium carbonate, 10% Pd/C and benzyl bromide were products of Aldrich, ethylene carbonate was obtained from Fluka. Diisopropyl (tosyloxymethyl)phosphonate was prepared according to ref.[5].

6-Amino-9-benzyl-9*H*-purin-2(3*H*)-one (**6**)

To a stirred solution of $NaNO_2$ (1.2 g, 17.4 mmol) in a mixture of H_2O (50 ml) and dioxane (20 ml), 9-benzylpurine-2,6-diamine[18] **5** (1.2 g, 5 mmol) was added at 50 °C. Acetic acid

(1.8 ml, 31.2 mmol) was added dropwise and the stirring was continued for 30 min. Concentrated NH_3 was added to pH 8 and the mixture was concentrated *in vacuo*; the residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (9 : 1) as eluent. Compound **6** (0.820 g, 68%) was isolated as a white solid, R_F 0.4 (S3), m.p. 180–197 °C (dec.). FAB MS, *m/z* (rel.%): 242.2 (100) [M + H]. ^{1}H NMR (DMSO-d_6): 10.50 brs, 1 H (NH); 7.58 s, 1 H (H-8); 7.60–7.50 m, 2 H and 7.35–7.25 m, 3 H (arom. H); 7.25 brs, 2 H (NH_2); 5.11 s, 2 H (N-CH_2 arom.). ^{13}C NMR (DMSO-d_6): 157.5 (C-6); 155.00 (C-2); 152.62 (C-4); 138.84 (C-8); 137.68 and 128.75, 2 C 127.69, 127.64, 2 C (arom. C); 108.87 (C-5); 45.42 (N-CH_2 arom.). For $C_{12}H_{11}N_5O \cdot H_2O$ (259.3) calculated: 55.59% C, 5.05% H, 28.36% N; found: 55.42% C, 5.12% H, 28.24% N.

9-Benzyl-6-{[(dimethylamino)methylidene]amino}-9*H*-purin-2(3*H*)-one (**7**)

A solution of **6** (1.205 g, 5 mmol) in DMF (20 ml) and dimethylformamide dimethylacetal (5 ml) was stirred at room temperature for 16 h. After concentrating the mixture *in vacuo*, the residue was chromatographed on a column of silica gel using $CHCl_3$–MeOH (95 : 5) as eluent. Compound **7** (1.38 g, 93%) was isolated as a white solid, R_F 0.6 (S3). FAB MS, *m/z* (rel.%): 297.3 (100) [M + H]. ^{1}H NMR (DMSO-d_6): 9.82 brs, 1 H (N3-H); 8.43 s, 1 H (H-8); 8.31 s, 1 H ($(CH_3)_2$N-C-H); 7.85–7.28 m, 5 H (arom. H); 5.22 s, 2 H (N-CH_2 arom.); 3.14 s and 3.03 s, 2 × 3 H (N-$(CH_3)_2$). For $C_{15}H_{16}N_6O$ (296.3) calculated: 60.80% C, 5.44% H, 28.36% N; found: 60.92% C, 5.38% H, 28.48% N.

6-Amino-9-benzyl-2-(2-hydroxyethoxy)-9*H*-purin-6-amine (**10**)
and 6-Amino-9-benzyl-3-(2-hydroxyethyl)-9*H*-purin-2(3*H*)-one (**11**)

A mixture of **7** (1.48 g, 5 mmol), K_2CO_3 (2.07 g, 15 mmol) and ethylene carbonate (0.88 g, 10 mmol) in DMF (30 ml) was refluxed at 140 °C for 8 h. After cooling to room temperature, methanol (10 ml) was added and the mixture was stirred at ambient temperature for 16 h. After concentrating the mixture *in vacuo*, the residue was chromatographed on silica gel using $CHCl_3$–MeOH (9 : 1) as eluent. A mixture of **10** and **11** (0.82 g, 58%, ratio *ca* 1 : 1) was isolated as a white solid, R_F 0.4 (S3). NMR samples were isolated by preparative HPLC (MeOH, H_2O).

Compound **10**. ^{1}H NMR (DMSO-d_6): 7.95 s, 1 H (H-8); 7.30 m, 5 H (arom. H); 7.18 brs, 2 H (NH_2); 5.11 s, 2 H (N-CH_2 arom.); 5.03 t, 1 H, *J*(OH,2′) = 5.6 (OH); 4.27 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 3.73 brq, 2 H, *J*(2′,1′) = 5.3, *J*(2′,OH) = 5.6 (H-2′).

Compound **11**. ^{1}H NMR (DMSO-d_6): 8.04 s, 1 H (H-8); 7.32 m, 5 H (arom. H); 7. 20 brs, 2 H (NH_2); 5.25 s, 2 H (N-CH_2 arom.); 4.81 t, 1 H, *J*(OH,2′) = 5.6 (OH); 3.92 t, 2 H, *J*(1′,2′) = 5.3 (H-1′); 3.67 brq, 2 H, *J*(2′,1′) = 5.3, *J*(2′,OH) = 5.6 (H-2′).

9-Benzyl-2-{2-[(diisopropyloxyphosphoryl)methoxy]ethoxy}-9*H*-purin-6-amine (**12**)
and 6-Amino-9-benzyl-3-{2-[(diisopropyloxyphosphoryl)methoxy]ethyl}-9*H*-purin-2(3*H*)-one (**13**)

A solution of **10** and **11** (1.43 g, 5 mmol) and NaH (0.80 g, 20 mmol) in DMF (30 ml) was stirred at 0 °C for 0.5 h. Diisopropyl [(tosyloxyl)methyl]phosphonate (1.75 g, 5 mmol) was added and the mixture was stirred at 0 °C for 2 h. After neutralisation with glacial acetic acid, the mixture was concentrated *in vacuo* and chromatographed on silica gel using $CHCl_3$–MeOH (97 : 3) as eluent.

Compound **12** (0.81 g, 34.9%) was isolated as a white amorphous solid, R_F 0.6 (S2). ^{1}H NMR (DMSO-d_6): 8.05 s, 1 H (H-8); 7.36–7.28 m, 5 H (arom. H); 7.24 brs, 2 H (NH_2); 5.26 s, 2 H (N-CH_2 arom.); 4.66–4.50 m, 2 H (POCH); 4.36 t, 2 H, J(1′,2′) = 5.1 (H1′); 3.81 t, 2 H, J(2′,1′) = 5.1 (H2′); 3.79 d, 2 H, J(P,CH_2) = 9.0 (PCH_2); 1.19 d and 1.26 d, 2 × 6 H, J(CH,CH_3) = 6.2 (CH_3). For $C_{21}H_{30}N_5O_5P$ (463.5) calculated: 54.42% C, 6.52% H, 15.11% N, 6.68% P; found: 54.37% C, 6.43% H, 15.01% N, 6.51% P.

Compound **13** (0.68 g, 29.3%) was isolated as a white amorphous solid, R_F 0.55 (S2). ^{1}H NMR (DMSO-d_6): 7.98 s, 1 H (H-8); 7.35–7.28 m, 5 H (arom. H); 7.18 brs, 2 H (NH_2); 5.08 s, 2 H (N-CH_2 arom.); 4.64–4.48 m, 2 H (POCH); 4.40 t, 2 H, J(1′,2′) = 5.1 (H1′); 4.02 t, 2 H, J(2′,1′) = 5.1 (H2′); 3.65 d, 2 H, J(P,CH_2) = 9.0 (PCH_2); 1.12 d and 1.21 d, 2 × 6 H, J(CH,CH_3) = 6.2 (CH_3). For $C_{21}H_{30}N_5O_5P$ (463.5) calculated: 54.42% C, 6.52% H, 15.11% N, 6.68% P; found: 54.28% C, 6.39% H, 14.97% N, 6.47% P.

6-Amino-3-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-9*H*-purin-2(3*H*)-one (**16**)

A solution of **13** (0.926 g, 2 mmol) in glacial acetic acid (20 ml) with one drop of concentrated HCl was stirred under H_2 in the presence of 10% Pd/C catalyst (0.05 g) for 16 h. The mixture was filtered and concentrated *in vacuo*. The residual acetic acid was removed by codistillation with 5% ethanol in toluene. The residue was dissolved in NH_3–MeOH, reconcentrated *in vacuo* and chromatographed on silica gel using $CHCl_3$–MeOH (90 : 10) as eluent. Compound **16** (0.63 g, 84%) was isolated as a white solid, R_F 0.45 (S2), m.p. 203–205 °C. FAB MS, *m/z* (rel.%): 374.3 (100) [M + H]. ^{1}H NMR (DMSO-d_6): 12.10 brs, 1 H (N9-H); 8.12 s, 1 H (H-8); 7.03 s, 2 H (NH_2); 4.64 m, 2 H (POCH); 4.43 t, 2 H, J(1′,2′) = 5.0 (H-1′); 3.96 t, 2 H, J(2′,1′) = 5.0 (H-2′); 3.68 d, 2 H, J(P,CH_2) = 8.1 (PCH_2); 1.20 d, 12 H, J(CH,CH_3) = 6.2 (CH_3). For $C_{14}H_{24}N_5O_5P$ (373.3) calculated: 45.04% C, 6.48% H, 18.76% N, 8.30% P; found: 45.32% C, 6.53% H, 18.61% N, 6.25% P.

6-Amino-3-[2-(phosphonomethoxy)ethyl]-9*H*-purin-2(3*H*)-one (**3**)

Bromo(trimethyl)silane (0.66 ml, 5.0 mmol) was added dropwise to a solution of diisopropyl ester **16** (0.373 g, 1 mmol) in dry acetonitrile (10 ml) at room temperature under nitrogen. After stirring for 16 h, the solvents were removed *in vacuo* and the residual yellow oil was first taken up in dry acetonitrile (20 ml), reconcentrated, and then dissolved in methanol (30 ml) and reconcentrated. A solution of the residue in water (5 ml) was applied to a column of Dowex 1 (AcO^- form) (20 ml) and eluted first with water (200 ml) and then with 0.5 M acetic acid. Product-containing fractions were concentrated *in vacuo*, purified by reverse phase chromatography (C-18, water) and crystallised from H_2O–acetonitrile to give white crystals of **3** (0.249 g, 86%), R_F 0.2 (S4), m.p. 241–243 °C. FAB MS, *m/z* (rel.%): 290.2 (100) [M + H]. ^{1}H NMR (D_2O): 8.12 s, 1 H (H-8); 4.56 t, 2 H, J(1′,2′) = 4.6 (H-1′); 4.14 t, 2 H, J(2′,1′) = 4.6 (H-2′); 3.63 d, 2 H, J(P,CH) = 8.1 (PCH_2). ^{13}C NMR (D_2O): 158.81 (C-2); 156.23 (C-4); 152.52 (C-6); 146.02 (C-8); 111.92 (C-5); 69.27 d, J(P,C) = 7.4 (C-2′); 66.48 d, J(P,C) = 145.1 (P-C); 47.40 (C-1′). UV, λ_{max} (ε) (H_2O): (pH 1) 280 (10 100); (pH 7) 273 (8 700); (pH 13) 273 (8 600). For $C_8H_{12}N_5O_5P \cdot 1.5\ H_2O$ (316.2) calculated: 30.37% C, 4.78% H, 22.14% N, 9.81% P; found: 30.60% C, 4.72% H, 22.28% N, 9.78% P.

2-{2-[(Diisopropoxyphosphoryl)methoxy]ethoxy}-9*H*-purin-6-amine (**14**)

In a manner similar to that described for **16** was the benzyl derivative **12** converted to **14** which was isolated as a white solid in 87% yield, R_F 0.40 (S2), m.p. 218–220 °C. ^{1}H NMR (DMSO-d_6): 12.53 brs, 1 H (N9-H); 7.89 s, 1 H (H-8); 7.11 s, 2 H (NH_2); 4.58 m, 2 H (POCH); 4.31 t, 2 H, J(1′,2′) = 5.0 (H-1′); 3.81 d, 2 H, J(P,CH_2) = 8.0 (PCH_2); 3.81 t, 2 H, J(2′,1′) = 5.0 (H-2′); 1.22 d, 12 H, J(CH,CH_3) = 6.2 (CH_3). For $C_{14}H_{24}N_5O_5P$ (373.3) calculated: 45.04% C, 6.48% H, 18.76% N, 8.30% P; found: 45.15% C, 6.55% H, 18.65% N, 8.25% P.

2-[2-(Phosphonomethoxy)ethoxy]-9*H*-purin-6-amine (**15**)

In a manner similar to that described for **3** was diisopropyl ester **14** converted to **15**, isolated as a white solid in 83% yield, R_F 0.2 (S4), m.p. 248–250 °C. FAB MS, *m/z* (rel.%): 290.2 (100) [M + H]. ^{1}H NMR (D_2O): 7.82 s, 1 H (H-8); 4.44 t, 2 H, J(1′,2′) = 4.5 (H-1′); 3.94 t, 2 H, J(2′,1′) = 4.5 (H-2′); 3.59 d, 2 H, J(P,CH) = 8.3 (PCH_2). ^{13}C NMR (D_2O): 161.01 (C-2); 159.67 (C-4); 154.77 (C-6); 151.82 (C-8); 115.86 (C-5); 70.43 d, J(P,C) = 8.7 (C-2′); 68.85 d, J(P,C) = 150.4 (P-C); 65.77 (C-1′). UV, λ_{max} (ε) (H_2O): (pH 1) 275 (9 400), 241 (6 200); (pH 7) 267 (8 600), 245 (5 500); (pH 13) 267 (8 500), 245(5 500). For $C_8H_{12}N_5O_5P \cdot H_2O$ (307.2) calculated: 31.28% C, 4.59% H, 22.80% N, 10.08% P; found: 31.05% C, 4.63% H, 22.65% N, 9.95% P.

9-Benzyl-2-{[2-hydroxy-3-(trityloxy)propyl]oxy}-9*H*-purin-6-amine (**18**),
9-Benzyl-2-{[1-hydroxy-3-(trityloxy)propan-2-yl]oxy}-9*H*-purin-6-amine (**19**)
and 6-Amino-9-benzyl-3-[2-hydroxy-3-(trityloxy)propyl]-9*H*-purin-2(3*H*)-one (**20**)

A solution of **7** (1.48 g, 5 mmol), K_2CO_3 (0.69 g, 5 mmol) and tritylglycidol (1.58 g, 5 mmol) in DMF (30 ml) was stirred at 120 °C for 8 h. After cooling to room temperature, methanol (10 ml) was added and the mixture was stirred for 16 h. After concentrating the mixture *in vacuo*, the residue was chromatographed on silica gel using $CHCl_3$–MeOH (95 : 5) as eluent to furnish mixture of compounds **18** and **20** (1.53 g, 55%, ratio *ca* 1 : 1) as a white solid followed by compound **19** (140 mg, 5%). NMR samples of **18** and **20** were isolated by preparative HPLC (C-18, water–methanol).

Compound **18** was isolated as a white amorphous solid. ^{1}H NMR (DMSO-d_6): 8.06 s, 1 H (H-8); 7.20–7.42 m, 22 H (20 arom. H and NH_2); 5.25 s, 2 H (N-CH_2-Ph); 4.35 dd, 1 H, J(1′a,2′) = 4.6, J(gem) = 11.0 (H-1′a); 4.25 dd, 1 H, J(1′b,2′) = 5.9, J(gem) = 11.0 (H-1′b); 4.01 m, 1 H (H-2′); 3.07 dd, 1 H, J(3′a,2′) = 5.6, J(gem) = 9.3 (H-3′a); 3.04 dd, 1 H, J(3′b,2′) = 5.6, J(gem) = 9.3 (H-3′b). ^{13}C NMR (DMSO-d_6): 161.69 (C-2); 156.97 (C-6); 151.30 (C-4); 139.58 (C-8); 115.35 (C-5); 144.04 (3 × C), 128.49 (6 × C), 128.00 (6 × C), 127.12 (3 × C) and 85.99 (O-Tr); 137.36, 128.79 (2 × C), 127.82 (3 × C) and 46.18 (N-Bn); 68.47 (C-1′); 68.18 (C-2′); 65.30 (C-3′).

Compound **19** was isolated as a white amorphous solid. ^{1}H NMR (DMSO-d_6): 8.04 s, 1 H (H-8); 7.17–7.41 m, 22 H (20 arom. H and NH_2); 5.30 m, 1 H (H-2′); 5.24 s, 2 H (N-CH_2-Ph); 3.68 dd, 1 H, J(3′a,2′) = 5.8, J(gem) = 11.3 (H-3′a); 3.63 dd, 1 H, J(3′b,2′) = 5.9, J(gem) = 11.3 (H-3′b); 3.23 dd, 1 H, J(1′a,2′) = 3.7, J(gem) = 9.7 (H-1′a); 3.12 dd, 1 H, J(1′b,2′) = 5.6, J(gem) = 9.7 (H-1′b). ^{13}C NMR (DMSO-d_6): 161.50 (C-2); 156.96 (C-6); 151.41 (C-4); 139.60 (C-8); 115.32 (C-5); 143.91 (3 × C), 128.38 (6 × C), 127.92 (6 × C), 127.05 (3 × C) and 85.85 (O-Tr); 137.34, 128.70 (2 × C), 127.63 (3 × C) and 46.05 (N-Bn); 75.62 (C-2′); 62.94 (C-1′); 60.64 (C-3′).

Compound **20** was isolated as a white amorphous solid. ^{1}H NMR (DMSO-d_6): 7.84 s, 1 H (H-8); 7.21–7.43 m, 22 H (arom. H + NH_2); 5.11 s, 2 H (N-CH_2 arom.); 4.16 dd, 1 H, *J*(1′a,2′) = 3.8, *J*(gem) = 14.0 (H-1′a); 4.04 m, 1 H (H-2′); 3.82 dd, 1 H, *J*(1′b,2′) = 9.0, *J*(gem) = 14.0 (H-1′b); 3.09 dd, 1 H, *J*(3′a,2′) = 5.9, *J*(gem) = 9.3 (H-3′a); 2.94 dd, 1 H, *J*(3′b,2′) = 5.2, *J*(gem) = 9.3 (H-3′b). ^{13}C NMR (DMSO-d_6): 154.73 (C-2); 153.04 (C-4); 152.27 (C-6); 139.01 (C-8); 108.78 (C-5); 144.04 (3 × C), 128.49 (6 × C), 127.99 (6 × C), 127.10 (3 × C) and 86.04 (O-Tr); 137.57, 128.77 (2 × C), 128.02, 127.70 (2 × C) and 45.38 (N-Bn); 68.56 (C-2′); 66.51 (C-3′); 46.92 (C-1′).

9-Benzyl-2-({2-[(diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}oxy)-9*H*-purin-6-amine (**21**) and 9-Benzyl-3-{2-[(diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}-9*H*-purin-2(3*H*)-one (**22**)

Diisopropyl [(tosyloxy)methyl]phosphonate (1.75 g, 5 mmol) was added at 0 °C to a solution of the above mixture of **18** and **20** (1.53 g, 2.75 mmol) in anhydrous DMF (20 ml) and pretreated with sodium hydride (60% suspension in mineral oil, 0.4 g, 10 mmol) for 30 min. The mixture was stirred at 0 °C for 4 h before quenching with acetic acid and concentrated *in vacuo*. The residue was partitioned between chloroform (50 ml) and water (50 ml), and the aqueous layer was further extracted with chloroform (2 × 20 ml). The combined organic phases were dried (anhydrous Na_2SO_4), filtered and concentrated *in vacuo* affording a mixture of **21** and **22** as viscous yellow-brown foam (1.72 g, 2.34 mmol) which was further used in the next reaction step.

2-({2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}oxy)-9*H*-purin-6-amine (**23**) and 3-{2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}-9*H*-purin-2(3*H*)-one (**25**)

A mixture of **21** and **22** (1.72 g, 2.34 mmol) in glacial acetic acid (20 ml) was stirred under H_2 in the presence of 10% Pd/C (0.05 g) and catalytic amount (1 drop) of saturated methanolic HCl for 16 h. The mixture was filtered and concentrated *in vacuo*. The residual acetic acid was removed by codistillation with 5% ethanol in toluene. The residue was dissolved in NH_3–MeOH, reconcentrated *in vacuo* to give a crude mixture of phosphonates **23** and **25**. This mixture was chromatographed on silica gel with $CHCl_3$–MeOH (97 : 3) as eluent. Compounds **23** and **25** were isolated as white amorphous solids.

Compound **23**. Yield 370 mg (40%), R_F 0.50 (S1). ^{1}H NMR (DMSO-d_6): 9.80 brs, 1 H (N^9-H); 8.03 s, 1 H (H-8); 7.31 brs, 2 H (NH_2); 4.78 sept, 2 H, *J*(CH_3,CH) = 6.1 (POCH); 4.60 t, 1 H, *J*(OH,3′) = 5.5 (OH); 4.23 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 11.0 (H-1′a); 4.11 dd, 1 H, *J*(1′b,2′) = 6.0, *J*(gem) = 11.0 (H-1′b); 3.76 m, 1 H (H-2′); 3.60 d, 2 H, *J*(P,CH) = 8.0 (PCH_2); 3.42 t, 2 H, *J*(3′,2′) = 5.5, *J*(3′,OH) = 5.5 (H-3′); 1.23 d and 1.33 d, 2 × 6 H, *J*(CH,CH_3) = 6.1 (CH_3). For $C_{14}H_{24}N_5O_5P$ (373.3) calculated: 45.04% C, 6.48% H, 18.76% N, 8.30% P; found: 44.78% C, 6.34% H, 18.62% N, 8.18% P.

Compound **25**. Yield 390 mg (43%), R_F 0.45 (S1). ^{1}H NMR (DMSO-d_6): 11.01 brs, 1 H (N^9-H); 7.86 s, 1 H (H-8); 7.02 brs, 2 H (NH_2); 4.84 t, 1 H, *J*(OH,3′) = 5.0 (OH); 4.68 sept, 2 H, *J*(CH_3,CH) = 6.1 (POCH); 4.06 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 13.7 (H-1′a); 3.87 dd, 1 H, *J*(1′b,2′) = 7.5, *J*(gem) = 13.7 (H-1′b); 3.80 m, 1 H (H-2′); 3.51 d, 2 H, *J*(P,CH) = 8.1 (PCH_2); 3.37 t, 2 H, *J*(3′,2′) = 5.0, *J*(3′,OH) = 5.0 (H-3′); 1.21 d and 1.32 d, 2 × 6 H, *J*(CH,CH_3) = 6.1 (CH_3). For $C_{14}H_{24}N_5O_5P$ (373.3) calculated: 45.04% C, 6.48% H, 18.76% N, 8.30% P; found: 44.82% C, 6.38% H, 18.65% N, 8.24% P.

2-[3-Hydroxy-2-(phosphonomethoxy)propoxy]-9*H*-purin-6-amine (**24**)

In a manner similar to that described for compound **3**, compound **23** was converted to **24** isolated as a white solid in 70% yield, R_F 0.3 (S4), m.p. 243–244 °C. FAB MS, *m/z* (rel.%): 320.2 (90) [M + H]. ^{1}H NMR (D_2O): 8.21 s, 1 H (H-8); 4.21 dd, 1 H, *J*(1′a,2′) = 4.0, *J*(gem) = 12.0 (H-1′a); 4.18 dd, 1 H, *J*(1′a,2′) = 5.0, *J*(gem) = 12.0 (H-1′b); 3.76 m, 1 H (H-2′); 3.55 d, 2 H, *J*(P,CH) = 8.0 (PCH_2); 3.38 t, 2 H, *J*(3′,2′) = 5.5, *J*(3′,OH) = 5.5 (H-3′). ^{13}C NMR (D_2O): 161.82 (C-2); 156.89 (C-6); 151.41 (C-4); 131.68 (C-8); 115.25 (C-5); 69.99 d, *J*(P,C) = 7.8 (C-2′); 68.48 (C-1′); 66.21 d, *J*(P,CH) = 152.7 (P-C); 63.09 (C-3′). UV, λ_{max} (ε) (H_2O): (pH 1) 275 (9 900), 243 (6 400); (pH 7) 270 (8 800), 245 (5 600); (pH 13) 270 (8 600), 245 (5 400). For $C_9H_{14}N_5O_6P\cdot H_2O$ (337.2) calculated: 32.06% C, 4.78% H, 20.77% N, 9.18% P; found: 31.86% C, 4.86% H, 20.32% N, 9.01% P.

6-Amino-3-[3-hydroxy-2-(phosphonomethoxy)propyl]-9*H*-purin-2(3*H*)-one (**2**)

In a manner similar to that described for compound **3**, compound **25** was converted to **2** isolated as white crystals in 72% yield, R_F 0.3 (S4), m.p. 255–258 °C. FAB MS, *m/z* (rel.%): 320.2 (100) [M + H]. ^{1}H NMR (D_2O): 8.14 s, 1 H (H-8); 4.34 dd, 1 H, *J*(1′a,2′) = 4.3, *J*(gem) = 12.8 (H-1′a); 4.18 dd, 1 H, *J*(1′b,2′) = 5.0, *J*(gem) = 12.8 (H-1′b); 3.84 m, 1 H (H-2′); 3.41 d, 2 H, *J*(P,CH) = 8.1 (PCH_2); 3.07 t, 2 H, *J*(3′,2′) = 5.5, *J*(3′,OH) = 6.0 (H-3′). ^{13}C NMR (D_2O): 155.04 (C-6); 153.13 (C-4); 152.43 (C-2); 139.68 (C-8); 118.26 (C-5); 70.06 (C-2′); 63.55 (C-3′); 64.85 d, *J*(P,C) = 142.1 (P-C); 46.51 (C-1′). UV, λ_{max} (ε) (H_2O): (pH 1) 279 (9 600); (pH 7) 272 (8 800); (pH 13) 272 (8 500). For $C_9H_{14}N_5O_6P\cdot H_2O$ (337.2) calculated: 32.06% C, 4.78% H, 20.77% N, 9.18% P; found: 31.78% C, 4.82% H, 20.41% N, 8.93% P.

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