SYNTHESIS AND PROPERTIES OF 2-GUANIDINOPURINES

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Received July 11, 2006
Accepted August 4, 2006

2-Guanidinopurines were prepared as derivatives of 2,6-diamino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine (PMEDAP) (**1**), which shows an important antiviral activity. It completes earlier described synthesis of 6-guanidinopurine derivatives. The title compounds were obtained by the reaction of the corresponding 2-chloropurines with guanidine. 2- And 6-guanidinopurines were used as model compounds for determination of dissociation constants (pK_a) of their ionogenic groups by capillary zone electrophoresis. The pK_a values of ionogenic groups of the above compounds were compared with those of the corresponding aminopurines. The pK_a of guanidino group at the purine moiety varies from 7.77 to 10.32. There is no protonation of N^1-position in contrast to aminopurines. None of these compounds showed any antiviral activity.

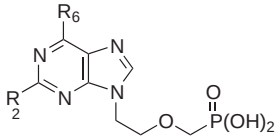
Keywords: Purines; Nucleosides; Guanidines; Basicity; Dissociation constants; PMEDAP analogues; Acyclic nucleoside phosphonates; Electrophoresis.

The antiviral activity of 2,6-diamino-9-[2-(phosphonomethoxy)ethyl]purine (PMEDAP)[1] (**1**) is well known (Chart 1). In the last few years we carried out a study of replacement of the amino group at the position 6 in the purine moiety by the guanidino group and studied the effect of this replacement on antiviral activity of these new analogues. The consequence of this replacement was either a complete loss of or a significant decrease in antiviral activity. On the other hand, several compounds bearing guanidino group in the purine moiety possess interesting immunomodulatory activity[2]. Within the programme of our structure–activity relationship studies in the series of guanidinopurine derivatives of acyclic adenine nucleotide analogues we are now going to describe the synthesis and properties of the corresponding isomeric compounds, i.e. 2-guanidinopurine derivatives and comparing their antiviral activity with the corresponding 2-aminopurines.

doi:10.1135/cccc20061303

We attempted to elucidate whether an antiviral effect would be preserved on substitution of the amino group of 2,6-diaminopurine at the position 2 by the guanidino group. This paper describes the synthesis of 2-guanidinopurine analogues of PMEDAP and guanidino analogues of PMEMAP; both these parent compounds show antiviral activity[1a,1b].

The antiviral activity of acyclic nucleotides analogues depends on the following principles: (i) Penetration of the active compound through the cellular membrane; (ii) activation by the cellular enzyme by phosphorylation and (iii) the inhibition of the target viral enzymes by these anabolites. Evidently the presence of the guanidino group itself does not prevent the passage. It is evident from the active transport of arginine.



1, $R^2 = R^6 = NH_2$, (PMEDAP)

2, $R^2 = H$; $R^6 = NH_2$, (PMEA)

3, $R^2 = NH_2$; $R^6 = H$, (PMEMAP)

CHART 1

Hydrogen bonding plays an important role in the latter two steps. On replacement by the guanidino group the probability of hydrogen bond formation and thereby the mutual interaction of the molecules is even higher. These reasons lead us for the synthesis of guanidinopurines.

The basicity of compound can also play the signifficant role in the substrate–enzyme activity. Obviously the replacement of amino by guanidino group increases the overall basicity of the purine system. The size of this effect is so far unknown. We have attempted to elucidate whether such a replacement causes any changes in the protonation of the purine moiety.

A determination of pK_a values of newly synthesized amino- and (amino)guanidinopurine nucleotide analogues[3] as analogues of PMEDAP by potentiometric titration did not afford satisfactory results, therefore we have applied the capillary zone electrophoresis. We attempted to elucidate how the acid-base dissociation constants of guanidinopurines differ from those of aminopurines.

Such a comparison of acid-base dissociation constants between 2- and 6-guanidinopurines was not yet published in the literature. The data of dissociation constants of the guanidino-group-containing compounds described so far in the literature concerns arylguanidines[4] and heteroarylguanidines[5].

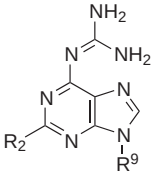
Aiming at the comparison of the acid-base dissociation constants of guanidinopurines with aminopurines we have prepared the corresponding guanidinopurine compounds derived from PMEA (**2**) {6-amino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine}, PMEMAP (**3**) {2-amino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine} and PMEDAP (**1**) (Chart 1).

Furthermore, in order to compare the basicity of 2- and 6-guanidino groups we have also synthesized a spectrum of 2- and 6-guanidinopurines bearing at the position 9 functional groups other than phosphonates.

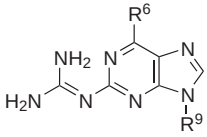
RESULTS AND DISCUSSION

Synthesis

6-Guanidinopurines **4** and **5** necessary for comparison of basicity of the 2- and 6-guanidino regioisomers were prepared from the corresponding 6-chloropurine derivatives according to the literature[2]. At the position 9 there is no functional group in these compounds, which could be protonated at different pH. On the other hand, 6-guanidinopurines **6** and **7**, which were prepared in accord with the literature[6] by similar procedure can be protonated at the side chain bound at the position 9 of the purine moiety. Apart from the strongly basic (cationogenic) guanidino group, compounds **5** and **7** contain also another (cationogenic) amino group susceptible to protonation in position 2 (Chart 2).

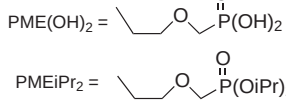


4, R^2 = H; R^9 = CH_3
5, R^2 = NH_2; R^9 = iPr
6, R^2 = H; R^9 = $PME(OH)_2$,
7, R^2 = NH_2; R^9 = $PME(OH)_2$,



12, R^6 = NH_2; R^9 = $PMEiPr_2$
13, R^6 = NH_2; R^9 = $PME(OH)_2$
15, R^6 = NH_2; R^9 = iPr
20, R^6 = H; R^9 = iPr
24, R^6 = H; R^9 = $PMEiPr_2$,
25, R^6 = H; R^9 = $PME(OH)_2$,

iPr = $CH(CH_3)_2$



Model compounds for the determination of basicity guanidinopurines

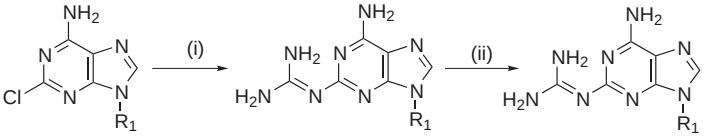
CHART 2

Experience from the synthesis of 6-guanidinopurines was used for the preparation of 2-guanidinopurines. It is well known that the reactivity of C–Cl group in 6- and 2-chloropurines differs significantly; thus, we have selected the synthesis of 6-amino-2-guanidinopurine (**10**) to verify the applicability of our standard method in the preparation of the guanidino derivatives, which was originally developed for the synthesis of 6-guanidinopurines[6]. This approach starts from the easily available 6-amino-2-chloro-9-(tetrahydropyran-2-yl)purine[7] (**8**). The reactivity of the 2-chloro atom at the purine moiety in the nucleophilic attack by guanidine is much lower compared to the 6-chloro atom[8]. While the 6-chloro atom in the purine moiety undergoes guanidinolysis already at room temperature, no reaction during 24 h was observed in the case of 2-chloropurine derivatives at ambient temperature. Therefore, we have applied the conditions analogous to the reaction of 2-chloro derivatives with amines[9]. The 2-guanidino derivative **9** was obtained by reaction of the 6-amino-2-chloropurine derivative **8** with guanidine in the presence of DABCO (1,4-diazabicyclo[2.2.2]octane) as a base. Dimethylformamide was not appropriate as a solvent, the high basicity of guanidine solution and high temperature caused its decomposition and subsequent formation of by-products. Therefore, *N*-methylpyrrolidone was used instead DMF as a solvent. 6-Amino-2-guanidinopurine (**10**) was obtained by acid hydrolysis, which cleavaged off the tetrahydropyran-2-yl protecting group in the intermediate **9**.

The transformation of 6-amino-2-chloropurine derivative[1b] **11** was used for the preparation of the 2-guanidinopurine analogue of PMEDAP [1a] **13** by guanidinolysis with a guanidine solution in the presence of DABCO in *N*-methylpyrrolidone. The intermediate **12** gave, after removal of the protecting isopropyl groups with bromotrimethylsilane in CH_3CN, 6-amino-2-guanidino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine (**13**) in a satisfactory yield.

The synthesis of 6-amino-2-guanidino-9-isopropyl-9*H*-purine (**15**) was performed as follows: 2,6-dichloropurine **16** was treated with isopropyl iodide in dimethyl sulfoxide in the presence of K_2CO_3 to give the isopropyl derivative **17** [10]. Its ammonolysis with methanolic ammonia gave 6-amino-2-chloro-9-isopropyl-9*H*-purine[8] (**14**) and the following guanidinolysis gave the desired compound **15** (Scheme 1).

For the preparation of 2-guanidinopurines lacking amino group at the position 6 in the purine moiety we have used several methods. The first method consisted in conversion of the 6-chloro atom to the 6-hydrazino

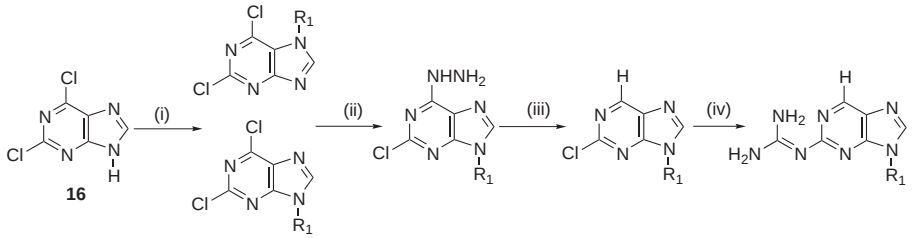


8, R^1= *O*-THP **9**, R^1= *O*-THP 1. **10**, R^1= H
11, R^1= PMEiPr$_2$ **12**, R^1= PMEiPr$_2$ 2. **13**, R^1= PME(OH)$_2$
14, R^1= iPr **15**, R^1= iPr

(i) guanidine in NMP, DABCO, 120 °C; (ii) 1. H_2SO_4, 60 °C for **9**; 2. TMSBr, CH_3CN, r.t. for **12**

SCHEME 1

group and its subsequent decomposition by Ag_2O. The starting 2,6-dichloropurine **16** was alkylated[10] with isopropyl iodide in the presence of K_2CO_3 in DMSO. The thus obtained intermediate **17** was transformed into 2-chloro-6-hydrazino-9-isopropyl derivative **18** with hydrazine hydrate in methanol and subsequently by reaction with Ag_2O in methanol to 2-chloro-9-isopropylpurine derivative **19** as described in Experimental (procedure *A*). This compound gave the desired 2-guanidino derivative **20** (Scheme 2) by the procedure similar to that for other 2-guanidino derivatives.



16

17, R^1 = iPr **18**, R^1= iPr **19**, R^1= iPr **20**, R^1= iPr
21a, R^1 = 9-PMEiPr$_2$ **22**, R^1= PMEiPr$_2$ **23**, R^1= PMEiPr$_2$ **24**, R^1= PMEiPr$_2$
21b, R^1 = 7-PMEiPr$_2$ **25**, R^1= PME(OH)$_2$

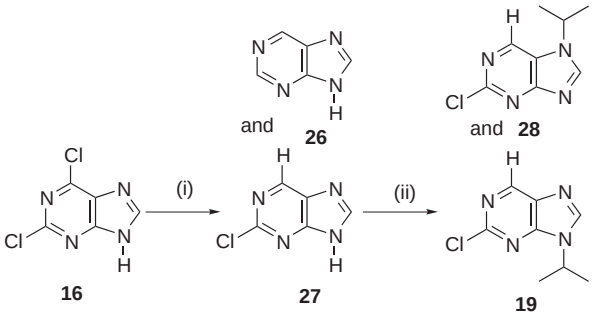
(i) 1. isopropyl iodide, K_2CO_3, DMSO, r.t. (7-regioisomer was not found); 2. 2-[(diisopropoxyphosphoryl)methoxy]ethyl 4-methylbenzene-1-sulfonate, NaH, DMF, 100 °C; (ii) $NH_2NH_2.H_2O$, MeOH, r.t.; (iii) Ag_2O, dioxane, H_2O, reflux; (iv) guanidine in NMP, DABCO, 120 °C; (v) TMSBr, CH_3CN, r.t.

SCHEME 2

The second method was direct catalytic dechlorination with subsequent alkylation. Contrary to the literature[11], the catalytic dechlorination of 2,6-dichloropurine **16** on Pd/C led to purine **26** as the major product. Also

the modification of this method using aqueous K_2CO_3 afforded purine (**26**) as a major product. The desired product **19** was obtained by alkylation of a minor product **27** as described in Experimental (procedure *B*) (Scheme 3). Catalytic dehalogenations of 2,6-dichloropurine **16** substituted with isopropyl in position 9 gave solely 9-isopropylpurine.

Due to the better yields, reaction conditions and workup of the reaction mixture, the removal of chlorine atom by hydrazinolysis and subsequent decomposition of this compound by Ag_2O, a similar reaction scheme as described above for procedure *A* was used for the synthesis of 2-guanidinopurine derivative **25** from the protected diester **24**.



(i) H_2, 5% Pd/C, CH_3COONa, or H_2, 5% Pd/C, K_2CO_3, H_2O, r.t.; (ii) NaH, isopropyl bromide, DMF, r.t.

SCHEME 3

The starting material for this synthesis was prepared by the reaction of 2,6-dichloro-9*H*-purine (**16**) with 2-[(diisopropoxyphosphoryl)methoxy]-ethyl 4-methylbenzene-1-sulfonate in the presence of NaH in DMF. The 9-regioisomer **21a** and 7-regioisomer **21b** were isolated by column chromatography and detemined by NMR spectroscopy. The thus obtained intermediate **21a** was treated with hydrazine hydrate in methanol to give 2-chloro-6-hydrazino derivative **22** which was further transformed to the 2-chloro intermediate **23** by the same procedure as described for compound **19**. Guanidination of this 2-chloro derivative was carried out using the same procedure as in the case of **12** to obtain the desired product **24**. The final step was removal of protecting isopropyl ester groups by the reaction with bromotrimethylsilane in CH_3CN to give the free 2-guanidino derivative **25**.

Determination of pK_a Values of Amino- and Guanidinopurines and Their Analogues by Capillary Zone Electrophoresis

The determination was carried out using the capillary zone electrophoresis (CZE). The pK_a values were determined by non-linear regression analysis of the pH-dependence of their effective electrophoretic mobilities measured by CZE. The pK_a values were determined for compounds **4**–**7**, **12**, **13**, **15**, **20**, **24**, and **25**.

Comparison of 6-guanidino derivatives with their 2-guanidino counterparts shows that compounds containing 6-guanidino group are less basic compared to their 2-isomers (Chart 3, Table I). In the group of 6-guanidino derivatives, the values strongly depend on the presence of additional functional groups (in particular the amino group on the base and phosphonate group in the aliphatic chain). The lowest basicity showed compound **4** which did not contain amino group in position 2 (pK_a = 7.77), the basicities of compounds **5** and **6** with amino or anionic phosphonate group are comparable (pK_a = 8.23 and 8.29, respectively). The most basic compound in the 6-guanidino series is **7** (pK_a = 8.84).

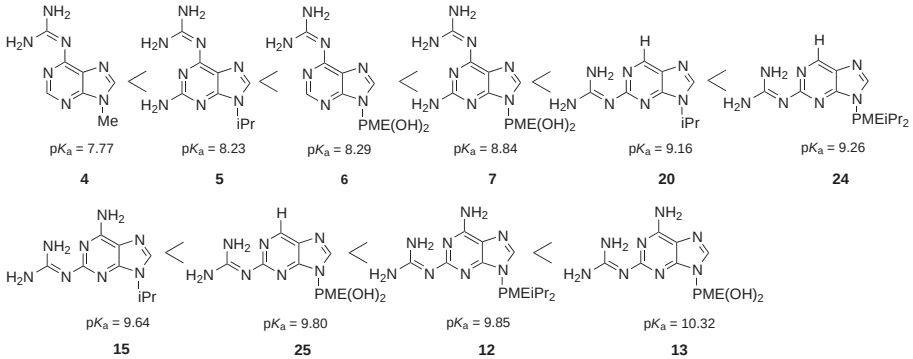


CHART 3

Contrary to the 9-alkyl derivatives the presence of free phosphonate group in the side chain increases basicity of the guanidino group. It is probably consequence of zwitterion formation. In the 2-guanidinopurines the lowest value was found for compound **20** (pK_a = 9.16), which does not bear any other ionogenic group. The situation is different in compound **25** (pK_a = 9.80). In spite of the fact that it does not contain amino group at position 6, it is more basic compared with the 6-amino-9-alkyl derivative **15** (pK_a = 9.64). The highest value of pK_a was determined in compound **13**

(pK_a = 10.32) with two ionogenic groups (6-amino and 2-guanidino groups). Whilst purine phosphonates with guanidino group at the position 2, i.e. compounds **25** (pK_a = 9.80), **12** (pK_a = 9.85) and **13** (pK_a = 10.32) are the most basic compounds, the phosphonates bearing guanidino group at position 6 are less basic (**6** (pK_a = 8.29) and **7** (pK_a = 8.84)). When comparing basicity of free acids and their diisopropyl esters (with the some purine moiety is the same), the lower basicity is always characteristic of diisopropyl esters. The corresponding pairs of compounds are: **24** (pK_a = 9.26) with **25** (pK_a = 9.80) and **12** (pK_a = 9.85) with **13** (pK_a = 10.32). Higher basicity was in both cases observed in esters of phosphonic acids (**24** and **12**) than in the corresponding alkyl derivatives (**20** and **15**).

TABLE I

Thermodynamic pK_a values of ionogenic groups of analyzed compounds (at 25 °C) $(N1)H^+/(N1)$, dissociation of protonated nitrogen 1 (N1) in the purine moiety; $P(O)(OH)O^-/P(O)(O)_2^{2-}$, dissociation of phosphonic acid group to the second degree (–2); $(G)H^+/(G)$, dissociation of protonated nitrogen guanidinyl group; n.d., not determined; n.p., not present.

Compd	pK_a		
	$(N1)H^+/(N1)$	$P(O)(OH)O^-/P(O)(O)_2^{2-}$	$(G)H^+/(G)$
4	n.d.	n.d.	7.77 ± 0.02
5	n.d.	n.d.	8.23 ± 0.03
6	n.d.	6.86 ± 0.06	8.29 ± 0.06
7	n.d.	6.77 ± 0.04	8.84 ± 0.05
20	n.d.	n.d.	9.16 ± 0.02
24	n.d.	n.d.	9.26 ± 0.02
PMEMAP	4.13 ± 0.04	7.46 ± 0.06	n.d.
PMEDAP	4.89 ± 0.05	7.37 ± 0.07	n.d.
PMEG	n.d.	7.29 ± 0.06	10.02 ± 0.07 (enol)[a]
15	n.d.	n.d.	9.64 ± 0.03
25	n.d.	6.64 ± 0.08	9.80 ± 0.08
12	n.d.	n.d.	9.85 ± 0.03
13	n.d.	6.77 ± 0.03	10.32 ± 0.11

[a] The value for the dissociation of the enol hydroxy group of PMEG (no guanidinyl group is present in PMEG), 9-[2-(phosphonomethoxy)ethyl]-9*H*-guanine.

Comparison of 6- or 2-amino compounds with their guanidinopurine counterparts showed a different mechanism of protonation of the purine moiety. The protonation of adenines at N^1 is well known[12]. Obviously the protonation of guanidinopurine takes place at the guanidino group because no protonation of N^1 was observed.

It can be concluded that the introduction of guanidino group led to an increase in basicity and probably to the total change of protonation of the purine moiety. Contrary to the aminopurine phosphonates with zwitterionic character of molecule, which bear two negative charges under the weakly basic conditions zwitterionic purine compounds with the guanidino group are characteristic by solely one negative charge in the weakly basic conditions. This may be play important role for the bioavailability of guanidinopurines.

Biological Activity

None of the title 2-guanidinopurines showed any significant antiviral activity against DNA viruses, RNA viruses and or retroviruses, nor exhibited any cytotoxicity in vitro in L929, L1210, HeLaS3 and CCRF CEM cells[13] under standard conditions. These tests show that the substitution of the amino function by the guanidino group either in position 6 or in position 2 of the purine moiety, leads to a decrease in or complete loss of antiviral activity.

Conclusion

A general method for synthesis of 2-guanidinopurine derivatives was developed. The monosubstituted 2-chloropurine derivatives were prepared by the transformation of 6-chloro function in 2,6-dichloropurine derivative via hydrazine intermediate. The catalytic dehydrochlorination shows very weak regioselectivity.

Basicity of the guanidino derivatives with pK_a values in the range 7.77–10.32 is much higher (by three to six orders of magnitude) than that of the amino derivatives **3** and **1** with pK_a equal to 4.13 and 4.89, respectively. The stronger basicity of the guanidino derivatives compared to amino derivatives is reflected by the increased acidity of phosphonic acid residue in the former derivatives. Basicity of the derivatives with guanidinyl group in position 2 of purine is significantly larger (ca. by 1.5 unit difference in pK_a) than that of the compounds bearing guanidinyl group in position 6 of purine. Basicity of the aminoguanidino derivatives is higher than

that of the guanidino derivatives (ca. by 0.5 unit difference in pK_a). Similarly, the basicity of PMEDAP (**1**) with two amino groups in positions 2 and 6 of purine is higher than that of PMEMAP (**3**) with single amino group at position 2 of purine.

CZE has proved to be a suitable and useful method for determination of dissociation constants (pK_a) of amino and (amino)guanidinopurine nucleotide analogues, such as acyclic nucleoside phosphonate, acyclic nucleoside phosphonate diesters and other related compounds, in a microscale, applying only few nanoliter sample volumes of 0.1 mM analyte solution per analysis. It was proved the change in protonation in the purine moiety between guanidinopurines and aminopurines. The bioavailability of these basic compounds will be further examined.

EXPERIMENTAL

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried over P_2O_5 at 2 kPa. Melting points were determined on a Büchi melting point B-545 apparatus and are uncorrected. Chromatography systems S1: $CHCl_3$–MeOH (95:5); S3: EtOAc–EtOH–acetone–H_2O–NH_3 (4:1:1:1:0.25)]; S4: EtOH–EtOAc (10:90) containing 1% of NH_3; S5: EtOAc–EtOH–acetone–H_2O (6:1:1:0.5) with 1% of NH_3; S6: EtOAc–EtOH–acetone–H_2O (4:1:1:1) with 1% NH_3; S7: EtOAc–EtOH–acetone–H_2O (4:1:1:1). Preparative TLC was carried out on 40 × 17 × 0.4 cm loose layers of silica gel containing a UV indicator. Paper electrophoresis was performed on Whatman paper No. 3 MM at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogencarbonate (TEAB) at pH 7.5 and the electrophoretic mobilities (E_{Up}) are referenced to uridine 3′-phosphate. Mass spectra were measured on a spectrometer ZAB-EQ (VG Analytical) using FAB ionization by Xe (accelerating voltage 8 kV, glycerol matrix) or EI electron energy 70 eV techniques. NMR spectra (*J*, Hz; δ, ppm) were measured on a Bruker DRX 500 (500 MHz for ^{1}H, 125.7 MHz for ^{13}C NMR spectra) in DMSO-d_6. Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4Å). Preparative HPLC purifications were performed on columns packed with 7 μm C18 reversed phase (Waters Delta 600 chromatograph column), 17 × 250 mm, in ca. 200 mg batches of mixtures using a linear gradient of 0.025 M tetraethylammonium hydrogencarbonate buffer in H_2O–CH_3OH (0 to 100% CH_3OH) or linear gradient MeOH–H_2O (1:4 to 9:1) as eluent.

Deionisation was performed on Dowex 50X8 (H^+ form) columns by the following procedure: after application of crude product the column was washed with water until the UV absorption dropped. Thereafter, the column was eluted with 2.5% aqueous NH_3 or with a MeOH–Et_3N–H_2O (1:1:3) mixture. Chromatography on Dowex 1X2 (acetate form) was made as follows: After application of the aqueous solution of the crude product onto the column, it was washed with water until the UV absorption dropped. The column was then eluted with a gradient of dilute acetic acid (0–1 mol/l).

Guanidine Solution

Guanidine hydrochloride (2.01 g, 21 mmol) was added to a suspension of sodium hydride (60% suspension in paraffin oil; 0.84 g, 21 mmol) in *N*-methylpyrrolidone (21 ml) and the mixture was stirred at room temperature overnight under exclusion of moisture. The resulting slurry was directly used for transformations.

Cleavage of the Phosphonate Esters. General Procedure

The ester (1 mmol) was suspended in acetonitrile (30 ml) and TMSBr (3 ml) was added dropwise. The reaction mixture was stirred at room temperature overnight. After evaporation of the volatiles and co-distillation with acetonitrile (20 ml), the residue was treated with water (20 ml) and 35% aqueous NH_3 (3 ml) for 5 min and evaporated. The residue was dissolved in water and applied onto a column of Dowex 50X8 (H^+ form). The column was washed with water and eluted with 2.5% aqueous NH_3. The product-containing UV-absorbing fractions were evaporated, the residue was dissolved in 35% aqueous NH_3 and applied onto a column of Dowex 1X2 (acetate form), washed with water and eluted with a linear gradient of aqueous acetic acid (0–1 mol/l). The product containing fractions were evaporated, codistilled with water (5 × 30 ml) and the residue was crystallized to give a pure free phosphonate. This procedure affords compounds **13** and **25**.

6-Amino-2-chloro-9-(tetrahydropyran-2-yl)-9*H*-purine (**8**)

The compound was prepared according to lit.[7], yield 95%.

6-Amino-2-guanidino-9-(tetrahydropyran-2-yl)-9*H*-purine (**9**)

A guanidine solution prepared as above (18 ml, 18 mmol) was added to a flask containing compound **8** (2.5 g, 9 mmol) and DABCO (1 g, 9 mmol). The mixture was stirred at room temperature for 24 h and then heated at 120 °C for 8 h. The reaction mixture was triturated with ether (160 ml), filtered and the precipitate was dissolved in MeOH, filtered through Cellite and evaporated in vacuo. The residue was extracted with a $CHCl_3$–MeOH mixture (3:1), the extract was adsorbed on silica gel and purified on a column of silica gel (50 g, $CHCl_3$–MeOH, 88:12) to give compound **9** (1 g, 83%). White crystals; m.p. 217–220 °C (EtOH). FAB MS, *m/z* (%): 277 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 1.56 m, 2 H, 1.71 m, 1 H, 1.95 m, 2 H and 2.23 m, 1 H (C-CH_2); 3.64 m, 1 H and 4.00 m, 1 H (O-CH_2); 5.54 dd, 1 H, *J* = 2.1 and 10.7 (N-CH-O); 7.77 brs, 2 H (NH_2); 8.20 s, 1 H (H-8); 8.37 br, 3 H (NH, NH_2); 10.44 s, 1 H (NH). ^{13}C NMR (DMSO-d_6): 22.60, 24.68, 30.04, 67.80 and 81.00 (N-THP); 115.73 (C-5); 138.80 (C-8); 149.49 (C-4); 152.43 (C-6); 155.62 and 155.74 (C-2 a N-C). Exact mass (FAB HRMS) found: 277.1525; for $C_{11}H_{17}N_8O$ [M + H] calculated: 277.1469.

6-Amino-2-guanidinopurine (**10**)

Compound **9** (0.70 g, 2.5 mmol) was dissolved in water (20 ml) and treated with a 1 M H_2SO_4 (5 ml) at room temperature overnight. The reaction mixture was neutralized with $Ba(OH)_2$ and boiled for a short time. The precipitate was filtered off and the filtrate was evaporated in vacuo to give compound **10** (0.34 g, 70%). White crystals; m.p. >318 °C (dec.) (H_2O). FAB MS, *m/z* (%): 193 (70), [M + H]. ^{1}H NMR (DMSO-d_6): 8.06 s, 1 H (H-8); 7.62 brs, 2 H, 8.20 br, 3 H, 10.15 br, 1 H and 12.90 br, 1 H (NH, NH_2). ^{13}C NMR (DMSO-d_6): 114.90

(C-5); 139.65 (C-8); 149.00 (C-4); 151.20 (C-6); 154.90 (C-2); 155.30 (N-C). Exact mass (FAB HRMS) found: 192.0836; for $C_6H_9N_8$ [M + H] calculated: 192.0872.

2,6-Dichloro-9-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-9*H*-purine (**21a**) and 2,6-Dichloro-7-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}-7*H*-purine (**21b**)

Sodium hydride (60% suspension in paraffin oil; 0.32 g, 8 mmol) was added to a solution of 2,6-dichloropurine (**16**; 1.5 g, 8 mmol) in DMF (25 ml) and the reaction mixture was stirred at room temperature for 1 h; 2-[(diisopropoxyphosphoryl)methoxy]ethyl 4-methylbenzene-1-sulfonate was added dropwise and the reaction mixture was stirred at 100 °C for 13 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3 × 30 ml), extracted with chloroform and purified by column chromatography (50 g, $CHCl_3$–MeOH, 97:3) to give compound **21a** (2.09 g, 64%) as yellow oil. FAB MS, *m/z* (%): 411 (35), [M + H]. ^{1}H NMR (DMSO-d_6): 1.09 d, 6 H, *J*(CH_3,CH) = 6.1 and 1.14 d, 6 H, *J*(CH_3,CH) = 6.1 (CH_3); 3.77 d, 2 H, *J*(P,CH) = 8.2 (P-CH_2); 3.92 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 4.46 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 4.44 m, 2 H (POCH); 8.69 s, 1 H (H-8). ^{13}C NMR (DMSO-d_6): 23.68 d, 2 C, *J*(P,C) = 4.4 (CH_3); 23.82 d, 2 C, *J*(P,C) = 4.4 (CH_3); 43.78 (N-CH_2); 64.78 d, *J*(P,C) = 164.1 (P-CH_2); 69.90 d, *J*(P,C) = 11.7 (PO-CH_2); 70.37 d, 2 C, *J*(P,C) = 6.3 (P-OCH); 130.49 (C-5); 148.85 (C-8); 149.68 (C-6); 151.10 (C-2); 153.75 (C-4). Exact mass (FAB HRMS) found: 411.0747; for $C_{14}H_{22}Cl_2N_4O_4P$ [M + H] calculated: 411.0756.

Further elution of column gave the 7-regioisomer **21b**. FAB MS, *m/z* (%): 411 (50), [M + H]. ^{1}H NMR (DMSO-d_6): 1.09 d, 6 H, *J*(CH_3,CH) = 6.1 and 1.14 d, 6 H, *J*(CH_3,CH) = 6.1 (CH_3); 3.76 d, 2 H, *J*(P,CH) = 8.1 (P-CH_2); 3.93 t, 2 H, *J*(2′,1′) = 4.9 (H-2′); 4.44 m, 2 H (P-OCH); 4.67 t, 2 H, *J*(1′,2′) = 4.9 (H-1′); 8.81 s, 1 H (H-8). ^{13}C NMR (DMSO-d_6): 23.72 d, 2 C, *J*(P,C) = 4.4 (CH_3); 23.82 d, 2 C, *J*(P,C) = 3.9 (CH_3); 46.52 (C-1′); 64.78 d, *J*(P,C) = 164.1 (P-C); 70.20 d, 2 C, *J*(P,C) = 6.4 (P-OC); 70.89 d, *J*(P,C) = 11.2 (C-2′); 122.05 (C-5); 143.44 (C-6); 150.91 (C-2); 153.06 (C-8); 163.41 (C-4). Exact mass (FAB HRMS) found: 411.0762; for $C_{14}H_{22}Cl_2N_4O_4P$ [M + H] calculated: 411.0756.

2-Chloro-9-{2-[(diisopropylphosphoryl)methoxy]ethyl}-9*H*-purine (**23**)

After dropwise addition of hydrazine hydrate to a solution of compound **21a** (1.86 g, 4.5 mmol) in methanol (23 ml), the reaction mixture was stirred at room temperature for 30 min. The solvent was evaporated in vacuo and the residue was codistilled with methanol and purified by column chromatography on silica gel (40 g, S1). The desired product was dissolved in a mixture of dioxane–water 1:1 (40 ml) and Ag_2O (1.1 g) was added. The reaction mixture was then refluxed for 1 h. A thin layer of Cellite was used to filter off the silver salts and the filtrate was evaporated in vacuo. The residue was dissolved in ethyl acetate and extracted with aqueous solution of EDTA (3 × 30 ml). Combined organic layers were dried with anhydrous $MgSO_4$. Purification of the residue on a silica gel column (40 g, $CHCl_3$–MeOH, 93:7) gave compound **23** (0.9 g, 53%). FAB MS, *m/z* (%): 377 (75) [M + H]. 293 (100) [M – 2 iPr]. ^{1}H NMR (DMSO-d_6): 1.07 d, 6 H, *J*(CH_3,CH) = 6.1 and 1.13 d, 6 H (CH_3); 3.78 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.93 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 4.44 m, 2 H (P-OCH); 4.45 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 8.60 s, 1 H (H-8); 9.08 s, 1 H (H-6). ^{13}C NMR (DMSO-d_6): 23.74 d, 2 C, *J*(P,C) = 4.4 (CH_3) and 23.89 d, 2 C, *J*(P,C) = 3.9 (CH_3); 43.15 (C-1′); 64.69 d, *J*(P,C) = 164.1 (P-C); 70.12 d, *J*(P,C) = 11.8 (C-2′); 70.42 d, 2 C, *J*(P,C) = 6.3 (P-OCH); 133.21 (C-5); 148.57 (C-8); 149.91 (C-6); 152.97 (C-2); 153.47 (C-4). Exact mass (FAB HRMS) found: 377.1130; for $C_{14}H_{23}ClN_4O_4P$ [M + H] calculated: 377.1145.

9-{2-[(Diisopropylphosphoryl)methoxy]ethyl}-2-guanidino-9*H*-purine (**24**)

A guanidine solution (10 ml, 10 mmol) prepared as above was added to a flask containing compound **23** (0.8 g, 2.1 mmol) and DABCO (0.24 g, 2.1 mmol). The mixture was stirred at 120 °C for 2 h; after that the reaction mixture was evaporated in vacuo and the residue was codistilled with toluene (3 × 30 ml), dissolved in water, the solution was neutralized with Dowex 50X8 (H^+ form), and the resin was applied onto a column of Dowex 50X8. The column was washed with H_2O–MeOH (80:20), eluted with 2.5% aqueous ammonia, and then MeOH–Et_3N–H_2O (10:10:30). The combined eluates were evaporated in vacuo and the residue was purified on a column of silica gel (30 g, S5) to give compound **24** (0.54 g, 94%) as yellow foam. FAB MS, *m/z* (%): 400 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 1.10 d, 6 H, *J*(CH_3,CH) = 6.2 and 1.15 d, 6 H (CH_3); 3.78 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.89 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 4.32 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.48 d, sept, 2 H, *J*(CH,CH_3) = 6.2, *J*(P,CH) = 7.6 (P-OCH); 7.10 brs, 4 H (NH); 8.14 s, 1 H (H-8); 8.74 s, 1 H (H-6). ^{13}C NMR (DMSO-d_6): 23.74 d, 2 C, *J*(P,C) = 4.4 (CH_3) and 23.89 d, 2 C, *J*(P,C) = 3.9 (CH_3); 42.51 (C-1′); 64.71 d, *J*(P,C) = 164.1 (P-C); 70.33 d, *J*(P,C) = 11.7 (C-2′); 70.38 d, 2 C, *J*(P,C) = 6.3 (P-OC); 127.63 (C-5); 144.50 (C-8); 147.73 (C-6); 152.53 (C-4); 158.39 (N-C); 161.91 (C-2). For $C_{15}H_{26}N_7O_4P$ (399.4) calculated: 45.11% C, 6.56% H, 24.55% N, 7.76% P; found: 44.90% C, 6.90% H, 24.34% N, 7.45% P.

2-Guanidino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine (**25**)

Workup viz. cleavage of the phosphonate esters – general procedure; starting compound **24** (0.5 g, 1.25 mmol); Dowex 1X2 eluted with 0.25 M AcOH; yield 0.3 g (76%); white crystals; m.p. >312 °C (dec.) (H_2O). E_{Up} = 0.66. FAB MS, *m/z* (%): 316 (100), [M + H]. ^{1}H NMR (D_2O + NaOD): 3.53 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 3.95 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 4.36 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 8.29 s, 1 H (H-8); 8.65 s, 1 H (H-6). ^{13}C NMR (D_2O + NaOD): 42.77 (C-1′); 69.09 d, *J*(P,C) = 149.4 (P-C); 70.03 d, *J*(P,C) = 9.7 (C-2′); 127.35 (C-5); 146.04 (C-8); 148.46 (C-6); 151.92 (C-4); 159.25 (N-C); 161.51 (C-2). Exact mass (FAB HRMS) found: 316.0937; for $C_9H_{15}N_7O_4P$ [M + H] calculated: 316.0923.

6-Amino-2-chloro-9-{2-[(diisopropylphosphoryl)methoxy]ethyl}-9*H*-purine (**11**)

Compound **11** was prepared from 2-chloroadenine[14] according to lit.[1b] Yield 57%.

6-Amino-9-{2-[(diisopropylphosphoryl)methoxy]ethyl}-2-guanidino-9*H*-purine (**12**)

A guanidine solution prepared as above for guanidination (10 ml, 10 mmol) was added to the flask containing compound **11** (0.78 g, 2 mmol) and DABCO (0.22 g, 2 mmol). The mixture was stirred at 120 °C for 24 h, then triturated with ether and the precipitate was dissolved in MeOH and filtered through Cellite. The methanolic solution was adsorbed on silica gel, the crude compound was purified on a column of silica gel (30 g, EtOAc, then S7) to give compound **12** (0.77 g, 93%) as yellowish oil. FAB MS, *m/z* (%): 415 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 1.10 d, 6 H, *J*(CH_3,CH) = 6.1 and 1.15 d, 6 H (CH_3); 3.77 d, 2 H, *J*(P,CH) = 8.3 (P-CH_2); 3.86 t, 2 H, *J*(2′,1′) = 5.0 (H-2′); 4.28 t, 2 H, *J*(1′,2′) = 5.0 (H-1′); 4.46 m, 2 H (P-OCH); 8.02 s, 1 H (H-8); 7.70 brs, 2 H, 8.40 brs, 3 H and 10.45 brs, 1 H (NH, NH_2). ^{13}C NMR (DMSO-d_6): 23.77 d, 2 C, *J*(P,C) = 4.9 (CH_3) and 23.91 d, 2 C, *J*(P,C) = 3.9 (CH_3); 42.75 (C-1′); 64.71 d, *J*(P,C) = 164.1 (P-C); 70.38 d, 2 C, *J*(P,C) = 6.4 (P-OC); 70.48 d,

J(P,C) = 11.2 (C-2′); 115.68 (C-5); 141.13 (C-8); 149.96 (C-4); 152.27 (C-6); 155.73 and 155.79 (C-2 and N-C). Exact mass (FAB HRMS) found: 415.1982; for $C_{15}H_{28}N_8O_4P$ [M + H] calculated: 415.1971.

6-Amino-2-guanidino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine (**13**)

Workup viz. cleavage of the phosphonate esters – general procedure. Starting compound **12** (0.66 g, 1.6 mmol); Dowex 1X2 eluted with 0.5 M AcOH; yield 0.17 g (36%); white crystals; m.p. 266–268 °C (H_2O). E_{Up} = 0.49. FAB MS, *m/z* (%): 331 (10), [M + H]. ^{1}H NMR (D_2O + NaOD): 3.54 d, 2 H, *J*(P,CH) = 8.5 (P-CH_2); 3.92 t, 2 H, *J*(2′,1′) = 5.1 (H-2′); 4.25 t, 2 H, *J*(1′,2′) = 5.1 (H-1′); 7.96 s, 1 H (H-8). ^{13}C NMR (D_2O + NaOD): 42.74 (C-1′); 68.97 d, *J*(P,C) = 149.4 (P-C); 70.16 d, *J*(P,C) = 10.3 (C-2′); 113.51 (C-5); 141.28 (C-8); 150.46 (C-4); 155.34, 158.97 and 161.57 (C-6, C-2 and N-C). Exact mass (FAB HRMS) found: 331.1015; for $C_9H_{16}N_8O_4P$ [M + H] calculated: 331.1032.

Catalytic Dechlorination of 2,6-Dichloropurine. 2-Chloropurine (**27**) and Purine (**26**)

Compounds were prepared according to literature[11] with some modifications. The reaction mixture containing 2,6-dichloropurine (**16**; 1 g, 5.3 mmol), CH_3COONa (0.72 g, 8.7 mmol) and 5% Pd/C (0.3 g) in H_2O (50 ml) was treated with hydrogen at room temperature for 3 h. The insoluble material was filtered off from the reaction mixture with Cellite. The filtrate was evaporated in vacuo and purified on a column of silica gel (30 g, $CHCl_3$–MeOH, 95:5) to give two compounds:

Compound **27** (0.14 g, 17%); white crystals; m.p. 232–234 °C (H_2O). FAB MS, *m/z* (%): 155 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 8.68 s, 1 H (H-8); 9.04 s, 1 H (H-6); 13.40 br, 1 H (NH). ^{13}C NMR (DMSO-d_6): 129.04 br (C-5); 147.14 (C-6); 148.06 (C-8); 152.81 (C-2); 157.73 br (C-4). Exact mass (FAB HRMS) found: 155.0128; for $C_5H_4ClN_4$ [M + H] calculated: 155.0124.

Compound **26** (0.37 g, 58%); white crystals; m.p. 212–214 °C (H_2O). FAB MS, *m/z* (%): 121 (25), [M + H]. ^{1}H NMR (DMSO-d_6): 8.61 s, 1 H (H-8); 8.91 s, 1 H (H-2); 9.12 s, 1 H (H-6); 13.45 br, 1 H (NH). ^{13}C NMR (DMSO-d_6): 133.05 br (C-5); 145.80 br (C-6); 147.12 br (C-8); 152.21 (C-2); 152.50 br (C-4). Exact mass (FAB HRMS) found: 121.0518; for $C_5H_5N_4$ [M + H] calculated: 121.0514.

2-Chloro-9-isopropyl-9*H*-purine (**19**) and 2-Chloro-7-isopropyl-7*H*-purine (**28**)

Preparation of compound **19**. *Procedure A*: Hydrazine hydrate (0.26 ml) was added dropwise to a solution of compound **17** [10] (0.84 g, 3.6 mmol) in MeOH (18 ml) and the resulting mixture was stirred at room temperature for 1 h. Another portion of hydrazine hydrate (0.1 ml) was added and the reaction mixture was stirred at room temperature for additional 30 min. The solvent was removed under reduced pressure, the residue was codistilled with MeOH and purified on a short column of silica gel ($CHCl_3$–MeOH, 70:30). The product in 50% aqueous dioxane (40 ml) was treated with Ag_2O (1 g) and the reaction mixture was then heated to reflux for 1 h. The insoluble material was filtered off and the filtrate was taken down to dryness in vacuo. The residue was dissolved in EtOAc (70 ml) and extracted with a saturated solution of EDTA (3 × 40 ml). Combined organic layers were dried with anhydrous $MgSO_4$, evaporated in vacuo and purified on a column of silica gel (30 g, $CHCl_3$) to give compound **19** (0.48 g, 67%). Experimental data correspond to those of the product described in procedure *B*.

Procedure B: NaH (60% dispersion in paraffin oil; 0.04 g, 1 mmol) was added to a solution of compound **27** (0.15 g, 1 mmol) in DMF (2 ml) and the resulting mixture was stirred at room temperature for 1 h. Isopropyl bromide (0.18 ml, 2 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3 × 10 ml) and purified on a loose layer silica gel plate ($CHCl_3$–MeOH, 95:5) to give two compounds:

Compound **19** (80 mg, 41%); white solid. FAB MS, *m/z* (%): 197 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 1.55 d, 6 H, $J(CH_3,CH)$ = 6.7 (CH_3); 4.82 sept, 1 H, $J(CH,CH_3)$ = 6.7 (N-CH); 8.76 s, 1 H (H-8); 9.05 s, 1 H (H-6). ^{13}C NMR (DMSO-d_6): 22.02, 2 C (CH_3); 47.53 (N-CH); 133.62 (C-5); 146.53 (C-8); 149.98 (C-6); 152.65 (C-2); 152.82 (C-4). Exact mass (FAB HRMS) found: 197.0603; for $C_8H_{10}ClN_4$ [M + H] calculated: 197.0594.

Compound **28** (30 mg, 16%); white solid. FAB MS, *m/z* (%): 197 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 1.57 d, 6 H, $J(CH_3,CH)$ = 6.7 (CH_3); 4.89 pent, 1 H, $J(CH,CH_3)$ = 6.7 (N-CH); 8.87 s, 1 H (H-8); 9.25 s, 1 H (H-6). ^{13}C NMR (DMSO-d_6): 22.30, 2 C (CH_3); 49.68 (N-CH); 124.12 (C-5); 143.77 (C-6); 149.41 (C-8); 153.03 (C-2); 162.55 (C-4). Exact mass (FAB HRMS) found: 197.0599; for $C_8H_{10}ClN_4$ [M + H] calculated: 197.0594.

2-Guanidino-9-isopropyl-9*H*-purine (**20**)

Guanidine solution prepared as above (12 ml, 12 mmol) was added to the flask containing compound **19** (0.48 g, 2.4 mmol) and DABCO (0.27 g, 2.4 mmol) and the mixture was stirred at 120 °C for 2 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3 × 30 ml) and dissolved in water. The solution was neutralized with Dowex 50X8 (H^+ form) and the suspension was applied onto a column of Dowex 50X8 (20 ml). The column was washed with H_2O and eluted with 2.5% aqueous ammonia, and then with MeOH–Et_3N–H_2O (1:1:3). The combined eluates were evaporated in vacuo and the residue was purified on a column of silica gel (30 g, S3 and S5) and the final purification was carried out by HPLC (0.025 M TEAB in gradient 7–100% MeOH) to give compound **20** (0.35 g, 65%); white crystals; m.p. 252–253 °C (aqueous EtOH). FAB MS, *m/z* (%): 220 (100), [M + H]. ^{1}H NMR (DMSO-d_6): 1.50 d, 6 H, $J(CH_3,CH)$ = 6.7 (CH_3); 4.71 sept, 1 H, $J(CH,CH_3)$ = 6.7 (N-CH_3); 6.95 brs, 4 H (NH_2); 8.27 s, 1 H (H-8); 8.72 s, 1 H (H-6). ^{13}C NMR (DMSO-d_6): 22.08, 2 C (CH_3); 46.12 (N-CH); 127.92 (C-5); 142.12 (C-8); 147.72 (C-6); 152.13 (C-4); 158.72 (N-C); 162.45 (C-2). Exact mass (FAB HRMS) found: 220.1313; for $C_9H_{14}N_7$ [M + H] calculated: 220.1311. For $C_9H_{14}N_8 \cdot 0.5CO_3^{2-}$ (219.3) calculated: 49.30% C, 5.98% H, 44.72% N; found: 49.20% C, 5.84% H, 44.50% N.

6-Amino-2-guanidino-9-isopropyl-9*H*-purine (**15**)

A guanidine solution prepared as decribed above (10.5 ml, 10.5 mmol) was added to a flask containing 6-amino-2-chloro-9-isopropyl-9*H*-purine[10] (**14**; 0.46 g, 2.1 mmol), DABCO (0.23 g, 2.1 mmol) and the mixture was stirred at 120 °C for 21 h. The solvent was removed in vacuo; the residue was codistilled with toluene (3 × 30 ml), dissolved in water and the solution was neutralized with Dowex 50X8 (H^+ form). The suspension was applied onto a column of Dowex 50X8 (20 ml). The column was washed with H_2O and the product was eluted with a mixture of MeOH–Et_3N–H_2O (10:10:30). The eluate containing UV-absorbing fractions was evaporated in vacuo and the residue was purified on a column of silica gel (20 g, S4–S6) and the final purification was carried out by HPLC (0.025 M TEAB in gradient 7–100% MeOH) to give 0.27 g (53%) of white solid; m.p. >206 °C (dec.). FAB MS, *m/z* (%):

235 (20), [M + H]. ^{1}H NMR (DMSO-d_6): 1.47 d, 6 H, J(CH$_3$,CH) = 6.7 (CH$_3$); 4.63 sept, 1 H, J(CH,CH$_3$) = 6.7 (N-CH); 7.52 brs, 2 H (NH$_2$); 8.12 s, 1 H (H-8); 9.40 br, 4 H (NH). ^{13}C NMR (DMSO-d_6): 22.25, 2 C (CH$_3$); 46.40 (N-CH); 115.74 (C-5); 138.34 (C-8); 149.61 (C-4); 153.72 (C-6); 155.65 and 156.64 (C-2 and N-C); 176.42 (C=O). Exact mass (FAB HRMS) found: 235.1424; for $C_9H_{15}N_8$ [M + H] calculated: 235.1420. For $C_9H_{14}N_8 \cdot 0.5H_2O \cdot 0.5CO_3^{2-}$ (234.3) calculated: 41.75% C, 5.60% H, 41.00% N; found: 41.53% C, 5.99% H, 39.82% N.

Capillary Zone Electrophoresis – Instrumentation[3]

CZE analyses were carried out in the capillary electrophoretic analyzer P/ACE MDQ (Beckman Coulter, Fullerton (CA), U.S.A.) using the software 32 Karat System, version 7.0 (Beckman) for data acquisition and evaluation. Origin 6.1 (OriginLab Corp., Northampton (MA), U.S.A.) was used for data plotting and regression analysis. The analyzer was equipped with the internally uncoated fused silica capillary with outer polyimide coating, total/effective length 394/292 mm, ID/OD 75/360 μm (Polymicro Technologies, Phoenix (AR), U.S.A.). The analytes were detected by UV-VIS spectrophotometric photodiode array detector (190–600 nm) set to operate in the range 190–300 nm. Absorbance of analyzed compounds and of electroosmotic flow markers (DMSO or isophorone) was monitored at 225 and 250 nm, respectively. The analyses were performed at constant temperature of the BGE inside the capillary, 25 *C.

The new capillary was gradually flushed with water, 0.1 M NaOH, water and BGE, each washed for 5 min. Finally, the capillary was conditioned by a 20 min application of the high voltage to equilibrate the inner surface and to stabilize electroosmotic flow (EOF). Between runs under the same conditions, the capillary was rinsed with the BGE for 1 min. Prior to any change of the BGE the capillary was rinsed with 0.1 M NaOH for 5 min and then repeatedly stabilized. The samples were injected hydrodynamically, with pneumatically induced pressure 6.9–13.8 mbar for 5–10 s. The samples were dissolved in concentrations of 0.1 mmol/l in deionized water or in deionized water slightly alkalized by a small addition of NaOH to ensure solubility of some compounds.

BGE (background electrolytes) solutions with pH values covering a broad pH range (3.50–11.25) with 0.25 pH unit increment were prepared by mixing the appropriate amounts of stock solutions and then diluted to the constant ionic strength 25 mmol/l. The pH was measured at 25 °C by pH meter CyberScan pH 2100 (Oakton Instruments, Vernon Hills (IL), U.S.A.). The BGEs were filtered through a 0.45 μm pore nitrocellulose syringe filter (Millipore, Bedford (MA), U.S.A.) before use.

This work is a part of the research project No. Z40550506. It was supported by the Centre for New Antivirals and Antineoplastics (1M0508), by the Programme of Targeted Projects of Academy of Sciences of the Czech Republic (1QS400550501), by the Gilead Sciences, Inc. (Foster City (CA), U.S.A.), by the Grant Agency of the Czech Republic (grant No. 203/05/2539), and by the financial support of the European Commission (René Descartes Prize-2001, grant. No. HPAW-2002-100096). The authors' thanks are also due to the staff of the mass spectrometry and analytical departments of the Institute of Organic Chemistry and Biochemistry.

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