Pergamon

Tetrahedron 58 (2002) 2985–2996

TETRAHEDRON

6-Guanidinopurine nucleosides and their analogues

Michal Česnek,* Antonín Holý and Milena Masojídková

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Praha 6, Czech Republic

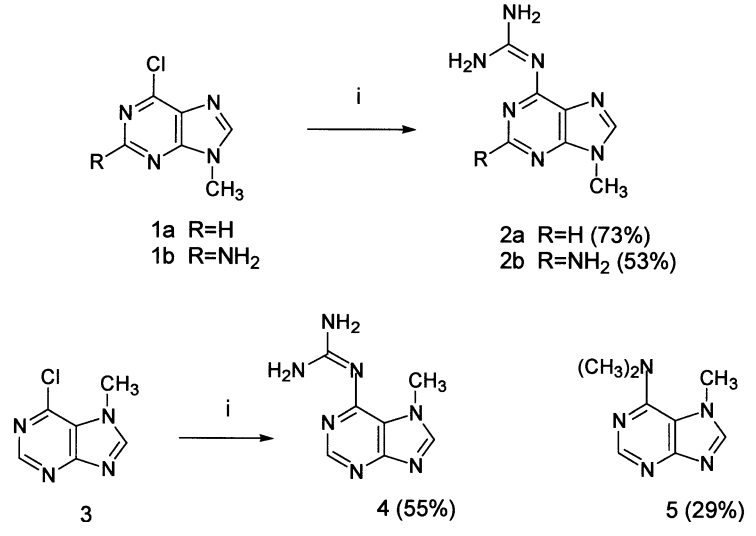
Received 5 November 2001; accepted 21 February 2002

Abstract—A general synthetic approach to 6-guanidinopurines, their ribonucleosides, 2-deoxyribonucleosides, acyclic nucleoside analogues and acyclic nucleoside phosphonates was developed. The approach consists in the reaction of the 6-chloropurine derivatives with guanidine solution in DMF under DABCO catalysis. This method was used to synthesize 6-guanidinopurine and 2-amino-6-guanidinopurine derivatives. Acyclic nucleosides and acyclic nucleoside phosphonates were also obtained by alkylation of the 6-guanidinopurine bases.

1. Introduction

The important role of the 6-amino group in purine derivatives is documented not only by its Watson–Crick base-pairing capacity in nucleic acids. Its presence is essential in purinoceptor agonists of A-type[1] and P-type,[2] in substrate/inhibitor binding to enzymes of purine metabolism[3] as well as in important regulatory pathways.[4,5] Our detailed study of the structure-antiviral activity relationship of the base-substituted 9-[2-(phosphonomethoxy)ethyl]-purines recently revealed a similar key-role of the 6-amino group at the purine base of these compounds.[6] In an attempt to elucidate whether this effect is not due to the basic character of this group we also prepared the corresponding analogues containing a 6-aminomethyl or a 6-amidino function at the purine ring;[7] however, they were devoid of the biological activity exerted by their parent compound.

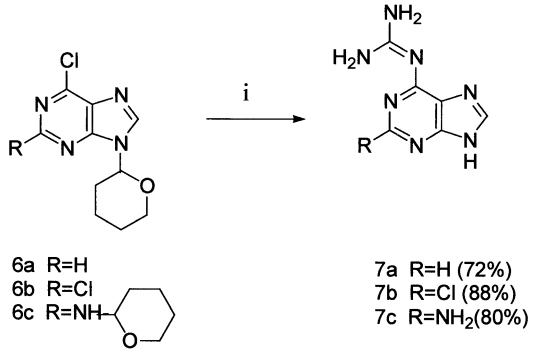
The guanidino group is closely related to the amino function as far as its basic character; in fact, it is more basic than the latter one. Among biologically active compounds bearing this function there are cytostatics,[8] e.g. the minor groove binding agent netropsin, CHS828 (Ref. 9), and its analogues with antiviral and antitumor activity,[10] inhibitors of polyamine synthesis [methylglyoxalbis(guanylhydrazone)],[9] and



Scheme 1. *Reagents*: (i) DABCO, guanidine (see Method A).

Keywords: 6-guanidinopurines; purine ribonucleosides; purine 2′-deoxyribonucleosides; acyclic nucleoside phosphonates; acyclic nucleosides; guanidine derivatives.
* Corresponding author. Fax: +4202-24310090; e-mail: cesnekm@uochb.cas.cz

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00186-2

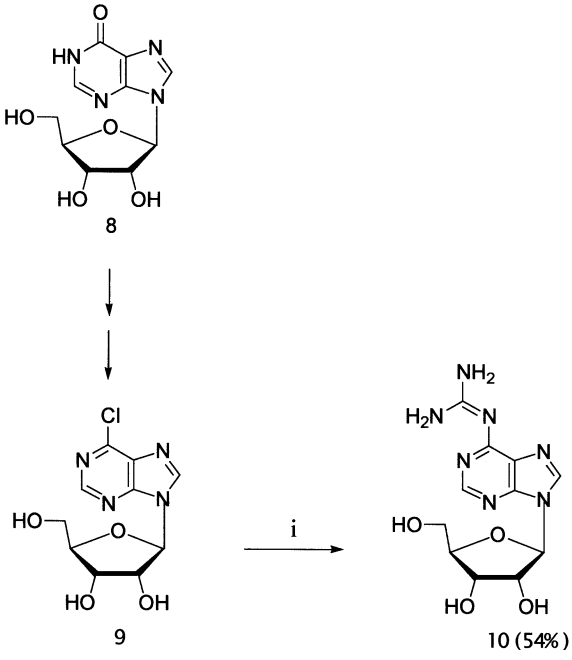


Scheme 2. *Reagents*: (i) DABCO/guanidine (see Method A).

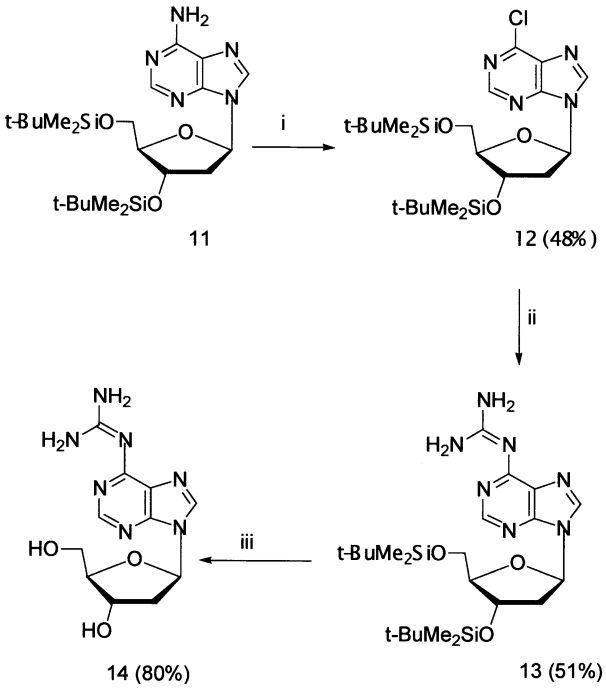
inhibitors of the influenza virus neuramidinase.[11] Replacement of the amino group by the guanidino function in amino acids[12] finds applications in peptide chemistry. It is thus surprising that not much attention has been paid to the introduction of the guanidino group in place of amino functions in nucleoside and nucletide chemistry. Recently, the synthesis of 4-guanidinopyrimidin-2-one nucleosides was described in the literature[13] and the analogues were incorporated into synthetic oligonucleotides.[14] In this paper we describe the synthesis of 6-guanidinopurines and their derivatives relevant to our ongoing studies.

2. Results

There are two distinct approaches for introduction of the guanidino function in the 6-position of the purine moiety: (a) it is built-up from an existing amino function in adenine derivatives and (b) replacement of an active leaving group at the 6-position by guanidine. Due to the extreme basicity of guanidine and the danger of pyrimidine ring destruction in the purine system, the former alternative is preferable. Several model adenine compounds were therefore subjected



Scheme 3. *Reagents*: (i) DABCO, guanidine, 60°C, 6 h (see Method B).

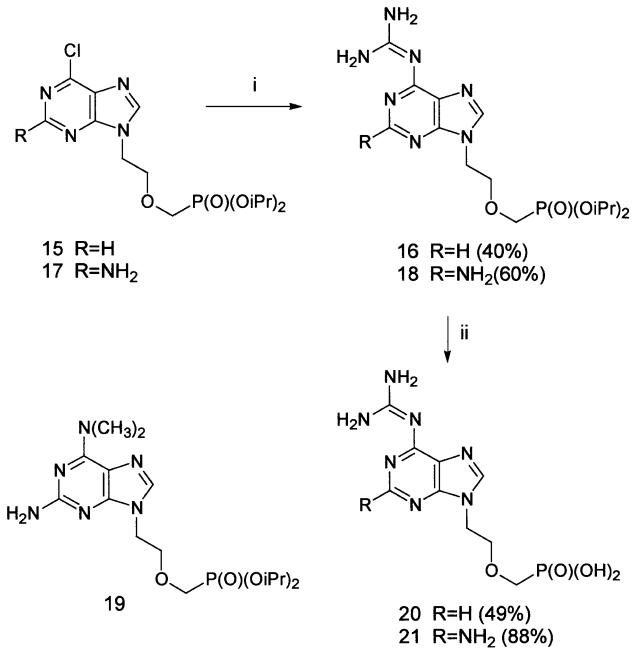


Scheme 4. *Reagents* (i) (1) CCl_4, $Et_4N^+Cl^-$ (1 M in CH_2Cl_2), *t*BuONO, 1 h at 0°C, (2) 0.5 h at 50°C; (ii) DABCO, guanidine, 3 h rt (see Method A); (iii) $Bu_4N^+F^-$ in THF, 2 h rt.

to conditions used in amino acid chemistry for the amino→guanidino group conversion: 9-tetrahydropyranyladenine failed to react with (1*H*)-pyrazol-3-carboxamidine;[15] we did not observe any reaction of 9-(2,3-dihydroxypropyl) adenine or its isopropylidene derivative with *S*-methylisothiourea in the presence of triethylamine in water, butanol or DMF.[16] No reaction was recorded during treatment of the same isopropylidene derivative with formamidinesulfonic acid in MeOH.[17] Evidently, all these conversions are limited to highly nucleophilic amino functions, such as aliphatic amines. Similar reluctance was registered also by other authors in the attempts to convert a cytosine derivative into 4-guanidino-2-pyrimidinone using reaction with benzoylisothiocyanate and subsequent treatment with amines. Although the nucleophility of the amino group in cytosine is much higher compared to that of adenine, the reaction proceeded with a low conversion rate only.[13]

We encountered similar problems in our attempts to activate the hypoxanthine derivative (2′,3′,5′-tri-*O*-acetylinosine) by formation of the intermediary 6-(2,3,5-triisopropylbenzenesulfonyloxy) purine nucleoside and its subsequent treatment with guanidine in DMF.[13] Although the conversion was detectable, it was far from being practical. Unfortunately, an analogous activation by transforming this inosine derivative to the 6-(1-triazolyl)purine nucleoside followed by guanidine treatment was not promising either.[14] In both attempts, the only identifiable product was inosine.

Thus, we have investigated the conversion of 6-chloropurine derivatives to 6-guanidinopurines by reaction with guanidine solutions, prepared by treatment of guanidine hydrochloride in DMF or DMF/acetonitrile mixtures with



Scheme 5. (i) DABCO, guanidine (see Method B): (1) 80°C, 1 h; (2) 100°C, 15 h; (ii) TMSBr, CH_3CN, rt overnight.

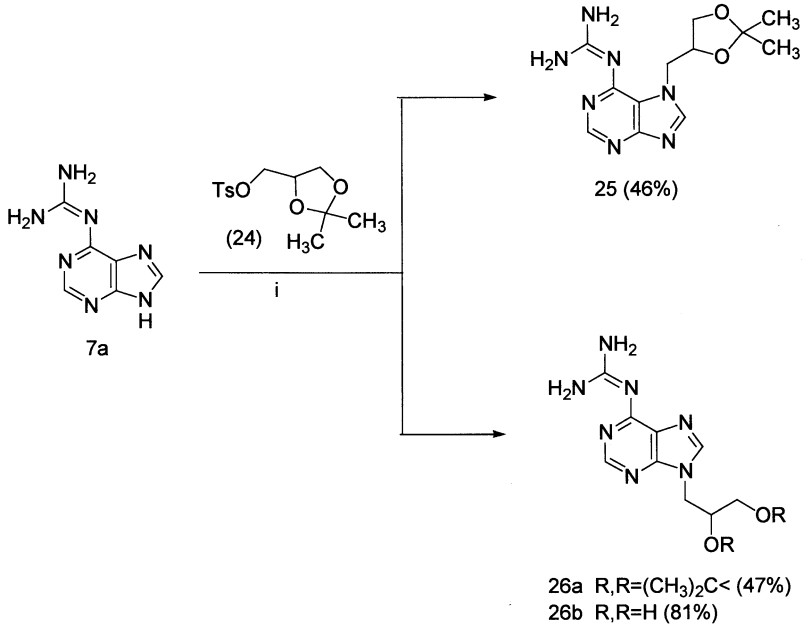
the theoretical amount of NaH. In the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO), this reaction gave nearly quantitative conversion of the 6-chloro derivatives and the compounds could be isolated in fair yields. It should be stressed that, contrary to the original method which makes use of a two-step reaction converting the 6-chloro derivatives first to the quaternary intermediates,[18] in the present case it is essential to add both components, i.e. DABCO and guanidine, to the chloro derivative simultaneously.

2-Amino-6-chloro-9-methylpurine (**1b**) was prepared by methylation of 2-amino-6-chloropurine with methyl iodide in the presence of NaH.[19] Its treatment with guanidine/DABCO gave the corresponding 2-amino-6-guanidino-9-methylpurine (**2b**). Also 6-chloro-9-methylpurine (**1a**) and 6-chloro-7-methylpurine (**3**) obtained by methylation of 6-chloropurine gave accordingly the 6-guanidino-9-methylpurine (**2a**) and its 7-methyl isomer (**4**) (Scheme 1).

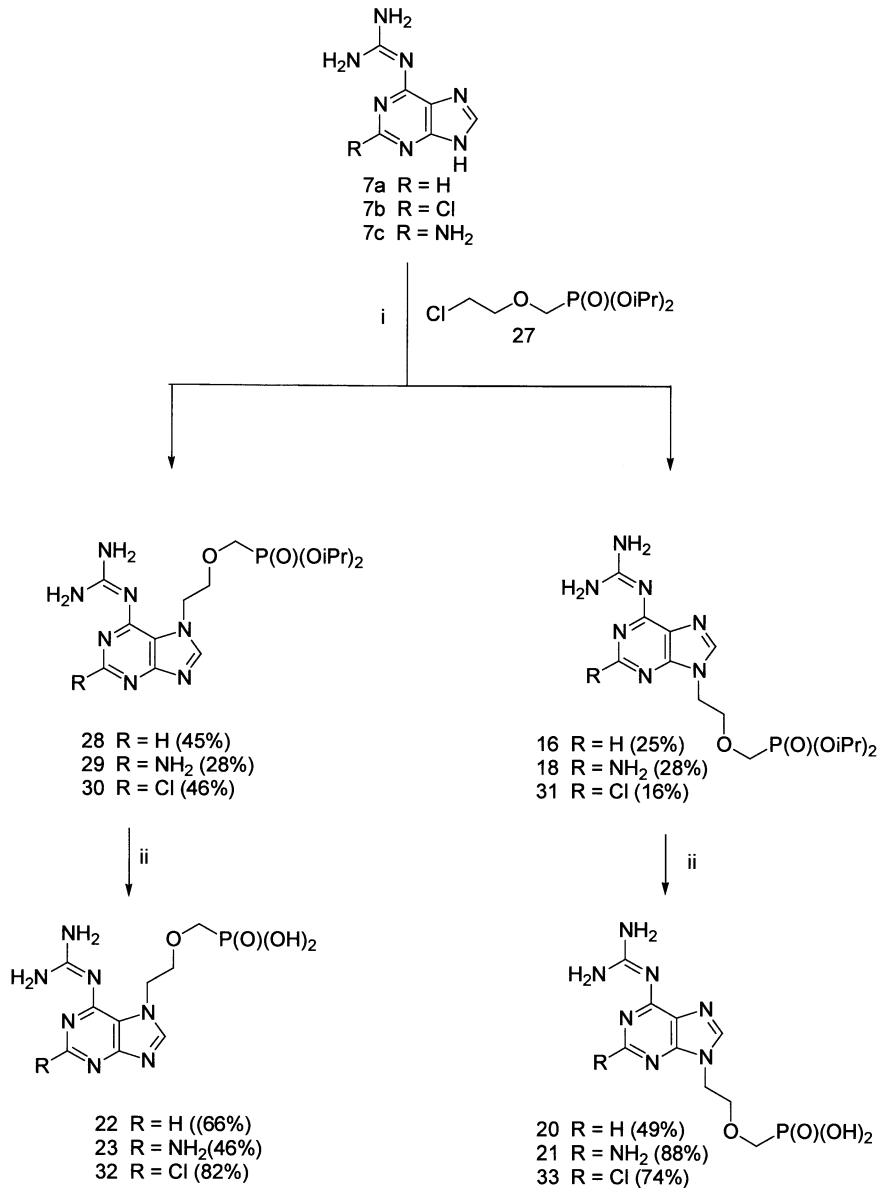
In order to prepare 6-guanidinopurine (**7a**), 6-chloro-9-tetrahydropyranylpurine (**6a**) was treated with guanidine/DABCO to give, after removal of excess reagents by Dowex 50 (H^+-form), with simultaneous cleavage of the protecting group compound **7a**. Similar conversion was achieved by treatment of 2,6-dichloro-9-tetrahydropyranylpurine (**6b**) with guanidine/DABCO: in accord with the expectations, the reaction took place at the more reactive 6-chloro position only. The product isolated after Dowex 50 deionization was 2-chloro-6-guanidinopurine (**7b**). 2-Amino-6-guanidinopurine (**7c**) was isolated from a similar reaction of the tetrahydropyranyl derivative **6c** with guanidine (Scheme 2).

During the guanidinolysis of the 7-methyl derivative **3** to compound **4** we have isolated and identified by-product, 6-dimethylamino-7-methylpurine (**5**). Formation of 6-dimethylaminopurine derivatives as side-products was noted in most other cases as well, albeit to a smaller extent. It is evidently due to the evolution of dimethylamine from DMF during the liberation of guanidine from its salt, or, due to guanidinolysis of dimethylformamide itself. These side reactions were eventually suppressed or prevented by replacing DMF with DMF/acetonitrile mixture, or, using *N*-methylpyrrolidone as a solvent.

Protection of the carbohydrate moiety was not necessary in the synthesis of the adenosine analogue, 9-(β-D-ribofuranosyl)-6-guanidinopurine (**10**): 6-chloropurine riboside (**9**) (easily available from 2′,3′,5′-tri-*O*-acetylinosine (**8**)) gave on reaction with guanidine/DABCO compound **10** in a fair



Scheme 6. *Reagents*: (i) NaH, DMF; (1) 50°C, 1 h; (2) 100°C, 15 h.



Scheme 7. *Reagents*: (i) Cs_2CO_3 in DMF, 100°C, 15 h; (ii) TMSBr, CH_3CN, rt overnight.

yield (Scheme 3). The strongly basic character of the guanidino derivatives makes purification of the unprotected 6-guanidinopurine nucleosides difficult. Due to this fact, as well as to the known increased lability of the nucleosidic linkage in 2′-deoxynucleosides we performed the synthesis in the 2-deoxyribo series with sugar-protected nucleosides. 3′,5′-Di-*O*-(*t*-butyldimethylsilyl)-2′-deoxyadenosine[20] (**11**) was converted by the known procedure[21] to the 6-chloropurine derivative **12** which gave on treatment with guanidine/DABCO the protected guanidino derivative **13**. Its deprotection with tetrabutylammonium fluoride afforded 9-(2-deoxy-β-D-ribofuranosyl)-6-guanidinopurine (**14**) (Scheme 4).

This method of conversion of 6-chloropurine derivatives to their 6-guanidino counterparts was also applied to an interesting class of nucleotide analogues, the acyclic nucleoside phosphonates[23] which display antiviral and/or cytostatic activity.[24] Ester-protected 6-chloropurine (**15**) or 2-amino-6-chloropurine (**17**) intermediates gave on treatment with guanidine/DABCO the 6-guanidinopurine diesters **16** and **18**, respectively. Their transformation to the free phosphonic acids **20**, **21** was performed under standard conditions,[6] i.e. by reaction with bromotrimethylsilane followed by hydrolysis (Scheme 5). The zwitterionic free phosphonates were purified by ion exchange chromatography. From the reaction of compound **17** with guanidine we were able to isolate and characterize the side product of this reaction, the 2-amino-6-dimethylaminopurine derivative **19** (vide supra).

With the aim of comparing the directive effects of the 6-guanidino group with those of the 6-amino group in purines we also examined alkylation reactions of 6-guanidinopurine (**7a**), 2-chloro-6-guanidinopurine (**7b**) and 2-amino-6-guanidinopurine (**7c**). Treatment of 6-guanidinopurine (**7a**) with 4-(*p*-tolylsulfonyloxymethyl)-1,3-dioxolane (**24**)

in the presence of an equivalent of NaH in DMF gave regioisomeric isopropylidene derivatives **25** and **26a** (Scheme 6).

Contrary to the alkylation of adenine with the same reagent wherein the main product is the corresponding 9-isomer,[25] the ratio of regioisomers is in this case shifted in favor of the 7-isomer **25**. Deprotection of **26a** gave the 6-guanidinopurine analogue **26b** of a broad-spectrum methylation inhibitor, 9-(2,3-dihydroxypropyl)adenine (DHPA).[26]

Analogous alkylations of 6-guanidinopurines **7a–c** with diisopropyl 2-chloroethoxymethylphosphonate (**27**) in DMF in the presence of Cs_2CO_3 gave the already known 9-isomers **16**,**18**, identical with materials obtained by guanidinolysis (vide supra) and the analogous 2-chloro derivative **31**. However, also in all these cases, the 7-isomers **28–30** were formed in quantities exceeding those known from alkylations of adenine or 2,6-diaminopurine with compound **27** under comparable conditions[6] (Scheme 7). The deprotection by $BrSiMe_3$ treatment gave 7-isomers **22**, **23** and **32**, or 9-isomers **20**, **21** and **33**, respectively.

3. Biological activity

The resulting 6-guanidinopurine bases, nucleosides and acyclic nucleoside phosphonates could be considered analogues of adenine or 2,6-diaminopurine compounds. None of the 6-guanidinopurines **7** and their nucleosides **10**, **14** exhibited under standard conditions[27] any cytotoxicity in vitro in L929, L1210, HeLaS3 and CCRF CEM cells. Neither of the 6-guanidinopurine analogues **20**, **21** of cytotoxic 9-[2-(phosphonomethoxy)ethyl] derivatives of adenine (PMEA) and 2,6-diaminopurine (PMEDAP) compounds inhibited growth of the cell cultures. We cannot exclude the possibility that the compounds are not transported over the cellular membranes, due to the presence of the strongly basic guanidino function.

4. Conclusion

We have developed a general method for the synthesis of 6-guanidinopurines, their nucleosides and acyclic nucleoside phosphonates which consists in the treatment of the corresponding 6-chloropurine derivatives with guanidine and DABCO. None of the tested derivatives showed any noticeable cytotoxicity.

5. Experimental

Unless otherwise stated, solvents were evaporated at 40°C/2 kPa and compounds were dried overnight at 2 kPa over P_2O_5. Melting points were determined on a Büchi Melting Point B-545 apparatus. TLC was performed on Silufol UV254 plates (Kavalier Votice, Czech Republic) in systems A, ethyl acetate–methanol (9:1), B, ethyl acetate–ethanol–acetone–water (4:1:1:1), C, chloroform–methanol (9:1), D, chloroform–methanol (95:5). 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, pH 7.5.

^{1}H NMR spectra were recorded at 500 MHz on a Varian UNITY-500 instrument in CD_3SOCD_3, D_2O or D_2O+NaOD solutions with tetramethylsilane (TMS) or sodium disilapentanesulfonate (DSS) as the respective internal standards. Proton chemical shifts and coupling constants were obtained by the first order analysis of the spectra. ^{13}C NMR spectra were recorded at 125 MHz on a Varian UNITY-500 instrument in CD_3SOCD_3 with the solvent signal as an internal reference (δ 39.7) or in D_2O with dioxane as an external standart (δ 66.86). Carbon chemical shifts and ^{13}C–^{31}P coupling constants were obtained from 'normal' proton-decoupled spectra or 'attached proton test' spectra. Proton-coupled ^{13}C NMR spectra with NOE enhancement were used to obtain J(C,H) coupling constants. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). IR spectra were measured on a Equinox 55 apparatus. UV spectra were measured on a Shimadzu type UV 1240 Mini spectrophotometer in aqueous solutions.

5.1. Materials

Bromotrimethylsilane, 6-chloropurine, sodium hydride, DABCO, methyl iodide and cesium carbonate were purchased from Aldrich (Praha, Czech Republic), 2-amino-6-chloropurine and 2,6-dichloropurine from Monelli (Olomouc, Czech Republic), guanidine hydrochloride, dihydropyrane, Dowex 50×8 and Dowex 1×2 were obtained from Fluka (Switzerland). Dimethylformamide was distilled from P_2O_5 in vacuo. Acetonitrile was refluxed with CaH_2 and distilled. All solvents were stored over molecular sieves (4 Å).

5.2. Preparation of guanidine solution

Method A: 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 a mixture of acetonitrile (42 mL) and DMF (21 mL), and the mixture was stirred at room temperature overnight under exclusion of moisture. The resulting slurry was directly used for transformations.

Method B: Mixture of guanidine hydrochloride (0.96 g, 10 mmol) and 60% NaH (0.40 g, 10 mmol) in DMF (10 mL) was stirred at room temperature overnight. The resulting slurry was used in further steps.

5.2.1. 6-Chloro-9-methyl-9*H*-purine (1a) and 6-chloro-7-methyl-7*H*-purine (3) (cf. Ref. 19). The title compounds were prepared by the procedure described for methylation of 2-amino-6-chlorpurine.[19] Yield of **1a**: 0.72 g (66%) white solid, mp 138–140°C; (**3**) yield 0.19 g (17%) white crystals, mp 195–196°C. Their ^{1}H NMR spectra are consistent with the literature.[31a,b]

5.2.2. 6-Guanidino-9-methyl-9*H*-purine (2a). The mixture of compound **1a** (1.88 g, 11.1 mmol), DABCO (1.25 g, 11.1 mmol) and guanidine solution prepared by Method A (166.5 mL, 55.5 mmol) was stirred at room temperature for 15 h. The resulting mixture was evaporated in vacuo, codistilled with toluene (3×30 mL) and the residue in

water was neutralized with Dowex 50×8 (H^+-form). The suspension was applied onto the column of Dowex 50×8 (H^+-form), the column was washed with water, eluted with a mixture of H_2O–MeOH–conc. aqueous NH_3 (6:3:1) and the eluate was evaporated in vacuo. White crystalline solid was filtered, the filtrate was evaporated and the residue was crystallized from water to give white crystals (1.56 g, 73%), mp 272–275°C; R_F(D)=0.11. FAB MS, *m/z* (rel. %): 192 (100) [M+H]. IR (KBr) 3397, 3321, 3203 (NH_2, NH), 1648, 1635, 1595, 1569, 1521, 1438, 1425, 1408 cm^{-1} (NH_2, C=N, C=C). δ_H (500 MHz, DMSO-d_6): 8.04 and 8.28 (2×1H, 2×s, H-2 and H-8); 7.45 (4H, br, N*H*); 3.72 (3H, s, C*H*$_3$). δ_C (125 MHz, DMSO-d_6): 160.19 (N–C); 160.05 (C-6); 150.75 (C-2); 150.51 (C-4); 141.57 (C-8); 125.25 (C-5); 29.39 (CH_3);. Anal. calcd for $C_7H_9N_7$ (191.20): 43.97, C; 4.74, H; 51.28, N; found: 43.73, C; 4.89, H; 50.72, N.

5.2.3. 2-Amino-6-guanidino-9-methyl-9*H*-purine (2b). Compound **1b** (Ref. 19) (0.91 g, 4.7 mmol), DABCO (0.53 g, 4.7 mmol) and solution of guanidine prepared by Method A (75 mL, 25 mmol) was stirred at room temperature for 16 h. The reaction mixture was worked up as described for compound **2a**. The column of Dowex 50×8 (H^+-form) was washed with water, then eluted with diluted aqeous ammonia (1:10). Solid white crystals, which separated were filtered, the filtrate was evaporated and crystallized from water to give another portion of the product. White crystals (0.54 g, 53%), mp 273–274°C; R_F(B)=0.20. FAB MS, *m/z* (rel. %): 207 (100) [M+H]. IR (KBr) 3471, 3349, 3284, 3187, 3118 (NH_2, NH), 1664, 1626, 1597, 1581, 1522, 1441, 1423, 1395 cm^{-1} (NH_2, C=N, C=C). δ_H (500 MHz, DMSO-d_6): 7.61 (1H, s, H-8); 7.50 (4H, br, N*H*); 5.96 (2H, brs, N*H*$_2$); 3.54 (3H, s, C*H*$_3$). δ_C (125 MHz, DMSO-d_6): 160.39 (C-2); 160.18 (N–C); 159.13 (C-6); 152.63 (C-4); 138.17 (C-8); 119.30 (C-5); 29.01 (CH_3). Anal. calcd for $C_7H_{10}N_8{\cdot}H_2O$ (206.21): 37.50, C; 5.39, H; 49.97, N; found: 37.57, C; 5.52, H; 49.91, N. UV spectrum: (pH 2) λ_{max}=305 nm (ε_{max}=10,100); λ_{max}=245 nm (ε_{max}=6600); λ_{max}=221.2 nm (ε_{max}=33,800).

5.2.4. 6-Guanidino-7-methyl-7*H*-purine (4) and 6-(dimethylamino)-7-methyl-7*H*-purine (5). 6-Chloro-7-methyl-7*H*-purine (**3**) (3 mmol) was treated with DABCO (0.34 g, 3 mmol) and guanidine solution prepared by Method A (45 mL, 15 mmol) and worked up similarly as described for compound **2a**. Chromatography on silica gel in chloroform–methanol mixture gave *6-(dimethylamino)-7-methyl-7H-purine* (**5**) as yellowish crystals (0.15 g, 29%), mp 109–111°C. R_F(D)=0.37. FAB MS, *m/z* (rel. %): 178 (100) [M+H]. IR (KBr) 2808 (CH_3, NMe_2), 1612, 1590, 1576, 1553, 1543, 1483, 1469 (purine-ring), 1179, 1141 (NMe_2), 1064 cm^{-1} (Me–N–Me). δ_H(500 MHz, DMSO-d_6): 8.34 and 8.36 (2×1H, 2×s, H-2 and H-8); 3.98 (3H, s, C*H*$_3$); 3.06 (6H, s, C*H*$_3$); δ_C (125 MHz, DMSO-d_6): 160.91 (C-4); 155.05 (C-6); 151.02 (C-2); 147.86 (C-8); 114.86 (C-5); 41.45 and 35.16 (2C, CH_3). Exact mass (FAB HRMS) found: 178.2235; calcd for $C_8H_{12}N_5$ [M+H] 178.2241.

Further elution of the column gave *6-guanidino-7-methyl-7H-purine* (**4**) as yellowish crystals (0.31 g, 55%), mp 218–222°C; R_F(D)=0.09. FAB MS, *m/z* (rel. %): 192 (40) [M+H]. IR (KBr) 3350, 3313, 3113 (NH_2, NH), 1673, 1610, 1595, 1561, 1519, 1437, 1398 cm^{-1} (NH_2, C=N, C=C). δ_H (500 MHz, DMSO-d_6): 8.12 and 8.25 (2×1H, 2×s, H-2 and H-8); 7.30 (4H, br, N*H*); 4.10 (3H, s, C*H*$_3$). δ_C (125 MHz, DMSO-d_6): 159.68 (N–C); 158.93 (C-4); 157.04 (C-6); 150.99 (C-2); 145.42 (C-8); 116.92 (C-5); 34.04 (CH_3). Exact mass (FAB HRMS) found 192.0987; calcd for $C_7H_{10}N_7$ [M+H] 192.0998.

5.2.5. 6-Guanidinopurine (7a). Guanidine solution prepared by Method A (63 mL, 21 mmol) was added to the flask containing compound **6a** (1 g, 4.2 mmol) and DABCO (0.47 g, 4.2 mmol), the mixture was stirred for 4 h at room temperature and evaporated in vacuo. The residue was codistilled with toluene (3×30 mL), dissolved in water and the solution was neutralized with Dowex 50×8 (H^+-form). The mixture was acidified with aq. HCl, stirred for 1 h at room temperature and applied onto the column of Dowex 50×8 (H^+-form). The column was washed with water and then eluted with diluted aqueous ammonia (1:10). The UV absorbing eluate was evaporated and the residue crystallized from water to give white crystals (0.53 g, 72%), mp>284°C (dec.); R_F(B)=0.20. FAB MS, *m/z* (rel. %): 178 (100) [M+H]. IR (KBr): 3426 (NH_2), 3328, 3190, 3176, 3146, 3120 (NH_2, 3×NH), 3052 (=C–H), 1645, 1629, 1620, 1570, 1561, 1541, 1534, 1493, 1467, 1434, 1405 (NH_2, C=N, C=C), 1243, 939, 721 (guanid.). δ_H (500 MHz, DMSO-d_6): 12.60 (1H, br, NH); 8.29 and 8.12 (2×1H, 2×s, H-2 and H-8); 7.33 (4H, br, N*H*). δ_C (125 MHz, DMSO-d_6): 159.84, 2C (C-6 and C-1); 157.34 (C-4); 150.60 (C-2); 141.75 (C-8); 120.00 (C-5). Anal. calcd for $C_6H_7N_7$ (177.17): 40.68, C; 3.98, H; 55.34, N; found: 40.44, C; 4.01, H; 54.80, N. UV spectrum: (pH 2) λ_{max}=270 nm (ε_{max}=15,900); (pH 7) λ_{max}=296 nm (ε_{max}=21,600).

5.2.6. 2,6-Dichloro-9-(tetrahydropyran-2-yl)-9*H*-purine (6b). The title compound was prepared as described in literature;[28] mp 120–122 °C (Ref. 28 gives 119–120°C); yield, 92%.

5.2.7. 2-Chloro-6-guanidinopurine (7b). Solution of guanidine prepared by Method A (54 mL, 18.3 mmol), was added to 2,6-dichloro-9-tetrahydropyranyl-9*H*-purine (**6b**) (1 g, 3.7 mmol) and DABCO (0.41 g, 3.7 mmol) and the mixture was stirred at room temperature for 5 h. The reaction mixture was worked up as described for compound **6a**, and the product was crystallized from aqueous ethanol; white crystals (0.68 g, 88%). Not melting <395°C; R_F(D)=0.12. FAB MS, *m/z* (rel. %): 212 (55) [M+H]; 93 (100). IR (KBr) 3484 3465, 3321, 3086, 2964, 2805 (NH_2, NH), 1710, 1660, 1634, 1579, 1523, 1441 cm^{-1} (NH_2, C=N, C=C). δ_H (500 MHz, DMSO-d_6): 12.80 (1H, br, N*H*); 8.14 (1H, s, H-8); 7.29 (1H, brs, N*H*). δ_C (125 MHz, DMSO-d_6): 160.04 (N–C); 157.46 (brs, C-6); 153.0 (br, C-4); 150.89 (C-2); 142.47 brs (C-8); 118.0 br (C-5). Exact mass (FAB HRMS) found: 212.0497; calcd for $C_6H_7ClN_7$ [M+H] 212.0491.

5.2.8. 2-Amino-6-guanidinopurine (7c). The solution of guanidine prepared by Method A (80 mL, 26.5 mmol) was added to the flask containing mixture of compound **6c** (Ref. 29) (1.8 g, 5.3 mmol) and DABCO (0.60 g, 5.3 mmol) and

the mixture was stirred at room temperature for 5 h. The reaction was worked up as described for **7a**, and the residue was crystallized from aqueous ethanol to give yellowish crystals (0.82 g, 80%). Mp>310°C (dec.); R_F(C)=0.15. FAB MS, *m/z* (rel. %): 193 (75) [M+H]; 93 (100). IR (KBr): 3492, 3410, 3322, 3129, 2855 (NH_2, NH); 1663, 1616, 1574, 1520, 1492, 1462, 1431, 1380 (NH_2, C=N, C=C). δ_H (500 MHz, DMSO-d_6): 12.10 (1H, br, N*H*); 7.69 (1H, s, H-8); 7.24 (4H, br, NH); 5.76 (2H, brs, NH_2). Anal. calcd for $C_6H_8N_8{\cdot}H_2O$ (192.18): 34.28, C; 4.80, H; 53.31, N; found: 34.25, C; 4.80, H; 52.74, N.

5.2.9. 9-(β-D-Ribofuranosyl)-6-guanidino-9*H*-purine (10). A mixture of 9-(β-D-ribofuranosyl)-6-chloropurine (**9**) (0.57 g, 2 mmol), DABCO (0.22 g, 2 mmol) and guanidine solution (Method B, 10 mL, 10 mmol) was heated at 60°C for 6 h. After evaporation of the solvent in vacuo and codistillation with toluene (3×30 mL) and the residue in water (30 mL) was neutralized with Dowex 50×8 (H^+-form). The suspension was applied onto the column of Dowex 50×8 (H^+-form), the column was washed with water and eluted with diluted aqueous ammonia (1:10). The product was evaporated and crystallized from water to give white crystals (0.33 g, 54 mp 128–132°C; R_F(B)=0.26. FAB MS, *m/z* (rel. %): 310 (100) [M+H]. IR (KBr): 3344, 3217, 3110 (OH, NH_2, NH); 2928 (CH_2); 1708, 1631, 1590, 1565, 1525, 1455, 1436, 1401 (NH_2, C=N, C=C); 1356, 1330, 1226 (C–N); 1117, 1106, 1080, 1056, 1025 cm^{-1} (C–O, C–OH). δ_H(500 MHz, DMSO-d_6): 8.30 and 8.34 (2×1H, 2×s, H-2 and H-8); 7.60 (4H, br, NH); 5.91 (1H, d, $J_{1',2'}$=6.2 Hz, H-1′); 5.40 (3H, br, O*H*); 4.61 (1H, dd, $J_{2',3'}$=4.9 Hz, $J_{2',1'}$=6.2 Hz, H-2′); 4.15 (1H, dd, $J_{3',4'}$= 3.1 Hz, $J_{3',2'}$=4.9 Hz, H-3′); 3.96 (1H, td, $J_{4',3'}$=3.1 Hz, $J_{4',5'}$=3.5 Hz, H-4′); 3.67 (1H, dd, $J_{5'a,4'}$=3.5 Hz, J_{gem}= 12.2 Hz, H-5′a); 3.56 (1H, dd, $J_{5'b,4'}$=3.5 Hz, J_{gem}=12.2 Hz, H-5′b). δ_C (125 MHz, DMSO-d_6): 159.97 and 159.48 (C-6 and N–C); 150.79 (C-2); 149.83 (C-4); 140.26 (C-8); 125.65 (C-5); 87.90 (C-1′); 85.96 (C-4′); 73.72 (C-2′); 70.83 (C-3′); 61.83 (C-5′). Exact mass (FAB HRMS) found 310.1195; calcd for $C_{11}H_{16}N_7O_4$ [M+H] 310.1214. UV spectrum: (pH 2) λ_{max}=270 nm (ε_{max}=17,100); (pH 7) λ_{max}=293 nm (ε_{max}=28,300), λ_{max}=223 nm (ε_{max}= 13,000).

5.2.10. 6-Amino-9-[2-deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-9*H*-purine (11). The titled compound was prepared as described in Ref. 20. Mp 128–129°C (Ref. 20 128–130°C); yield 95%. ^{1}H NMR spectrum is consistent with the literature.[20]

5.2.11. 9-[2-Deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-chloro-9*H*-purine (12) (cf. Ref. 21). A solution of compound **11** (0.24 g, 0.5 mmol) in CCl_4 (8 mL) was stirred at 0°C and tetraethylammonium chloride (1 M solution in CH_2Cl_2, 2 mL, 2 mmol) was added dropwise, followed by dropwise addition of *t*-butyl nitrite (0.3 mL, 2.5 mmol). The resulting mixture was stirred for 1 h at 0°C, warmed to room temperature during 0.5 h and stirred at 50°C for 3 h. The solvents were removed in vacuo and the residue was chromatographed on a silica gel plate [MeOH–CH_2Cl_2 (3:97)], to give yellow oil (0.12 g, 48%). ^{1}H and ^{13}C NMR data are consistent with literature.[22]

5.2.12. 9-[3,5-Di-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl]-6-guanidino-9*H*-purine (13). Guanidine solution prepared by Method A (54 mL, 18.1 mmol) was added to compound **12** (1.81 g, 3.6 mmol) and DABCO (0.41 g, 3.6 mmol). The mixture was stirred at room temperature for 3 h, neutralized by acetic acid and concentrated to half of the volume in vacuo. Water (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3× 50 mL). Combined organic layers were dried with $MgSO_4$, the volatiles were evaporated in vacuo and the residue was codistilled with toluene. The residue gave by chromatography on a silica gel column [(50 g, EtOAc–EtOH (90:10)]. Compound **13** (0.97 g, 51%) as white crystals, mp 111–113°C; R_F(B)=0.29. FAB MS *m/z* (rel. %): 522 (20) [M+H]; 73 (100). IR ($CHCl_3$): 3479, 3298, 3126 (NH_2, NH), 1700, 1627, 1587, 1565, 1520 (NH_2, C=N, C=C), 2956, 2898, 1411, 1259, 838 ($SiMe_2$), 1392, 1360, (*t*-Bu), 1129, 1109, 1095, 1070 cm^{-1} (C–O). δ_H (500 MHz, DMSO-d_6): 8.30 and 8.29 (2×1H, 2×s, H-2 and H-8); 7.50 (3H, br, N*H*); 6.35 (1H, t, $J_{1',2'}$=6.8 Hz, H-1′); 4.61 (1H, brdt, $J_{3',4'}\sim J_{3',2'b}$=3.2 Hz, $J_{3',2'a}$=5.7 Hz, H-3′); 3.85 (1H, ddd, $J_{4',3'}$=3.0 Hz, $J_{4',5'b}$=4.4 Hz, $J_{4',5'a}$=6.1 Hz, H-4′); 3.80 (1H, dd, $J_{5'a,4'}$=6.1 Hz, J_{gem}=10.9 Hz, H-5′a); 3.65 (1H, dd, $J_{5'b,4'}$=4.4 Hz, J_{gem}=10.9 Hz, H-5′b); 2.91 (1H, ddd, $J_{2'a,3'}$=5.7 Hz, $J_{2'a,1'}$=7.2 Hz, J_{gem}=13.2 Hz, H-2′a); 2.30 (1H, ddd, $J_{2'b,3'}$=3.4 Hz, $J_{2'b,1'}$=6.4 Hz, J_{gem}=13.2 Hz, H-2′b); 0.90 and 0.85 (2×9H, 2×s, C(CH_3)$_3$); 0.11 (6H, s, Si–CH_3); 0.02 and 0.01 (2×3H, 2×s, Si–CH_3). δ_C (125 MHz, DMSO-d_6): 159.79 (C-6); 159.39 (N–C); 150.85 (C-2); 149.84 (C-4); 139.83 (C-8); 125.46 (C-5); 87.13 (C-4′); 83.24 (C-1′); 72.26 (C-3′); 62.75 (C-5′); 36.69 (C-2′); 25.93, 3C, 25.87, 3C, 18.14 and 17.90 (C(CH$_3$)$_3$); −4.76 and −5.34 (2×2C, Si–CH$_3$). Exact mass (FAB HRMS) found: 522.3035; calcd for $C_{23}H_{44}N_7O_3Si$ [M+H]: 522.3044.

5.2.13. 9-(2-Deoxy-β-D-ribofuranosyl)-6-guanidino-9*H*-purine (14). Compound **13** (0.55 g, 1.1 mmol) in 1 M tetrabutylammonium fluoride in THF (4.2 mL, 4.2 mmol) was stirred at room temperature for 2 h, concentrated to half of the volume in vacuo and partitioned between ether and water. The aqueous phase was alkalized with a drop of aqueous ammonia and applied on Dowex 1×2 (OH^-) column (50 mL); the column was eluted with water and the eluate evaporated in vacuo. The residue was recrystallized from aqueous ethanol to give white crystals (0.25 g, 80%), mp>255°C (dec.); R_F(C)=0.22; FAB MS, *m/z* (rel. %): 294 (25) [M+H], 242 (100). IR (KBr) 3350, 3220, 3120 (NH_2, NH); 1699, 1628, 1590, 1566, 1524, 1457, 1440 (NH_2, C=N, C=C); 1095, 1059 (C–OH); 907 cm^{-1} (THF-ring breathing). δ_H (500 MHz, DMSO-d_6): 8.32 and 8.28 (2×1H, 2×s, H-2 and H-8); 7.60 (4H, br, N*H*); 6.36 (1H, dd, $J_{1',2'b}$=6.1 Hz, $J_{1',2'a}$=7.8 Hz, H-1′); 5.30 (2H br, OH); 4.41 (1H, dt, $J_{3',4'}\sim J_{3',2'b}$=2.6 Hz, $J_{3',2'a}$=5.7 Hz, H-3′); 3.88 (1H, td, $J_{4',3'}$=2.4 Hz, $J_{4',5'a}\sim J_{4',5'b}$=4.3 Hz, H-4′); 3.62 (1H, dd, $J_{5'a,4'}$=4.1 Hz, J_{gem}=11.8 Hz, H-5′a); 3.52 (1H, dd, $J_{5'b,4'}$=4.1 Hz, J_{gem}=11.8 Hz, H-5′b); 2.72 (1H, ddd, $J_{2'a,3'}$=5.7 Hz, $J_{2'a,1'}$=7.8 Hz, J_{gem}=13.2 Hz, H-2′a); 2.26 (1H, ddd, $J_{2'b,3'}$=2.8 Hz, $J_{2'b,1'}$=6.1 Hz, J_{gem}= 13.2 Hz, H-2′b). δ_C (125 MHz, DMSO-d_6): 160.02 (N–C); 159.63 (C-6); 150.78 (C-2); 149.64 (C-4); 139.87 (C-8); 125.65 (C-5); 88.13 (C-4′); 83.94 (C-1′); 71.19 (C-3′); 62.11 (C-5′); 39.66 (C-2′). Anal. calcd for

$C_{11}H_{15}N_7O_3 \cdot 2.5H_2O$ (293.28): 39.05, C; 5.90, H; 28.98, N; found: 39.29, C; 5.50, H; 28.98, N. UV-spectrum: (pH 2) λ_{max}=270 nm (ε_{max}=17,500); (pH 7) λ_{max}=222 nm (ε_{max}=13,000), λ_{max}= 293 nm (ε_{max}=28,600).

5.2.14. 9-[2-(Diisopropylphosphorylmethoxy)ethyl]-6-guanidino-9*H*-purine (16). A mixture of compound **15** (Ref. 6) (1.22 g, 3.2 mmol), DABCO (0.36 g, 3.2 mmol) and guanidine solution (Method B: 16 mL, 16 mmol) was stirred for 5 h at room temperature, evaporated in vacuo and the residue was codistilled with toluene (3×30 mL). The residue in water was neutralized by Dowex 50×8 (H^+-form) and the mixture was applied onto the column of the same resin. The column was washed with water and then eluted with diluted aqueous ammonia (1:10). The residue of the ammonia eluate was chromatographed on silica gel (40 g, EtOAc–EtOH, 85:15) to give compound **16** (0.51 g, 40%) as a yellowish oil. R_F(D)=0.13. FAB MS, *m/z* (rel. %): 400 (100) [M+H]; IR (KBr) 3395, 3337, 3217, 3106 (NH_2, NH); 1701, 1650, 1628, 1580, 1560, 1524, 1430, 1414 (NH_2, C=N, C=C); 1227, 1235 (P=O); 1106, 1119 (C–O–C); 1011, 995 (P–O–C); 1386, 1375 (*i*Pr–CH_3); 1170, 1140 cm^{-1} (C–O-C). δ_H (500 MHz, DMSO-d_6): 8.30 and 8.08 (2×1H, 2×S, H-2 and H-8); 7.55 (4H, br, N*H*); 4.48 (2H, m, POC*H*); 4.34 (2H, t, $J_{1',2'}$= 5.1 Hz, H-1′); 3.90 (2H, t, $J_{2',1'}$=5.1 Hz, H-2′); 3.78 (2H, d, $J_{P,CH}$=8.4 Hz, PCH_2); 1.16 and 1.12 (2×6H, 2×d, J_{CH_3},CH=6.2 Hz, CHCH_3). δ_C (125 MHz, DMSO-d_6): 159.72 (N–C); 159.05 (C-6); 150.66 (C-2); 150.30 (C-4); 141.61 (C-8); 124.85 (C-5); 70.49 (d, $J_{P,C}$=12.2 Hz, C-2′); 70.33 (2C, d, $J_{P,C}$=6.3 Hz, P–OC); 64.68 (d, $J_{P,C}$=164.6 Hz, P–C); 42.50 (C-1′); 23.91 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.77 (2C, d, $J_{P,C}$=4.9 Hz CH_3). Exact mass (FAB HRMS) found: 400.1862; calcd for $C_{15}H_{27}N_7O_4P$ [M+H]: 400.1875.

5.2.15. 2-Amino-9-[2-(diisopropylphosphorylmethoxy)-ethyl]-6-guanidino-9*H*-purine (18) and 2-amino-9-([2-(diisopropylphosphorylmethoxy)ethyl])-6-(dimethylamino)-9*H*-purine (19). Guanidine solution prepared by Method B (19 mL, 19 mmol) was added to the mixture of compound **17** (Ref. 6) (1.5 g, 3.8 mmol) and DABCO (0.43 g, 3.8 mmol). The mixture was stirred at room temperature for 4 h, evaporated in vacuo and the residue was codistilled with toluene (3×30 mL). The residue in water was neutralized by Dowex 50×8 (H^+-form) and the suspension was applied onto the column of the same resin (100 mL). The column was washed with water and then eluted with diluted aqeous ammonia (1:10). The UV-absorbing product was chromatographed on a column of silica gel (50 g, EtOAc–EtOH–acetone–H_2O, 4:1:1:1) to give *2-amino-9-([2-(diisopropylphosphorylmethoxy)ethyl]-6-(dimethylamino)-9H-purine* (**19**) (0.20 g, 13%) as a white foam, R_F(C)=0.83. FAB MS, *m/z* (rel. %): 401 (100) [M+H]. IR (KBr) 3527, 3419, 3492, 3326, 3197 (NH_2); 1638, 1600, 1592, 1581. 1527, 1500, 1467 (NH_2, C=N, C=C); 1243 (P=O); 1012, 998) P–O–C); 1388, 1377 (*i*Pr–CH_3); 1123, 1104 (C–O–C); 1179, 1142 (C–C–C); 1051 cm^{-1} (Me–N–Me). δ_H (500 MHz, DMSO-d_6): 7.66 (1H, s, H-8); 5.80 (2H, brs, NH_2); 4.51 (2H, m, POC*H*); 4.13 (2H, t, $J_{1',2'}$=5.2 Hz, H-1′); 3.81 (2H, t, $J_{2',1'}$=5.2 Hz, H-2′); 3.76 (2H, d, $J_{P,CH}$=8.4 Hz, PCH_2); 3.37 (6H, brs, NHCH_3); 1.19 and 1.16 (2×6H, 2×d, J_{CH_3},CH=6.1 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 159.61 (C-2); 154.90 (C-6); 152.83 (C-4); 136.89 (C-8); 113.70 (C-5); 70.45 (d, $J_{P,C}$=11.7 Hz, C-2′); 70.35 (2C, d, $J_{P,C}$=5.9 Hz, P–OC); 64.78 (d, $J_{P,C}$=164.1 Hz, P–C); 42.08 (C-1′); 40.25 and 37.80 (NH–CH_3); 23.94 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.81 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Exact mass (FAB HRMS) found 401.2070; calcd for $C_{16}H_{30}N_6O_4P$ [M+H] 401.2066.

Further elution of the column gave *2-amino-9-[2-(diisopropylphosphorylmethoxy)ethyl]-6-guanidino-9H-purine* (**18**) (0.95 g, 60%) as a colorless oil; R_F(C)=0.37. FAB MS, *m/z* (rel. %): 415 (100) [M+H]. IR (KBr) 3480, 3392, 3335, 3199, 3140 (NH_2, NH); 1700, 1643, 1620, 1577, 1528, 1510, 1469, 1438, 1421 (NH_2, C=N, C=C); 1119, 1105 (C–O–C); 1386, 1375 (*i*Pr–CH_3); 1179, 1140 (C–O–C); 1011, 991 cm^{-1} (P–O–C). δ_H (500 MHz, DMSO-d_6): 7.62 (1H, s, H-8); 7.40 (4H, br, NH_2); 5.93 (2H, brs, NH_2); 4.52 (2H, m, P–OC*H*); 4.12 (2H, t, $J_{1',2'}$=5.1 Hz, H-1′); 3.82 (2H, t, $J_{2',1'}$=5.1 Hz, H-2′); 3.76 (2H, d, $J_{P,CH}$=8.4 Hz, PCH_2); 1.20 and 1.16 (2×6H, 2×d, J_{CH_3},CH=6.2 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 160.22 (N–C); 160.02 (C-2); 159.04 (C-6); 152.17 (C-4); 137.86 (C-8); 119.17 (C-5); 70.65 (d, $J_{P,C}$=11.7 Hz, C-2′); 70.37 (2C, d, $J_{P,C}$= 5.98 Hz, P–OC); 64.78 (d, $J_{P,C}$=165.0 Hz, P–C); 41.91 (C-1′); 23.95 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.82 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Anal. calcd for $C_{15}H_{27}N_8O_4P \cdot 0.5H_2O$ (414.40): 42.55, C; 6.67, H; 26.46, N; 7.32, P; found: 42.69, C; 6.69, H; 26.31, N; 7.11, P.

5.2.16. Alkylation of 6-guanidinopurine (7a) with compound 24. NaH (0.16 g, 4 mmol, 60% dispersion in mineral oil) was added to compound **7a** (0.70 g, 4 mmol) in DMF (50 mL) and the mixture was stirred at 50°C for 1 h. Compound **24** (Ref. 30) (1.37 g, 4.8 mmol) in DMF (7 mL) was added dropwise and the resulting mixture was stirred at 100°C for 15 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3×20 mL) and extracted by hot $CHCl_3$. The residue gave by silica gel column chromatography [30 g, $CHCl_3$–MeOH, 86:14] and crystallization of the appropriate fractions from methanol *7-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-6-guanidino-7H-purine* (**25**). Yield 0.53 g (46%) of white crystals, mp 208–212°C; R_F(C)=0.54. FAB MS, *m/z* (rel. %): 292 (100) [M+H]. IR (KBr) 3455, 3406, 3350, 3125, 2804 (NH_2, NH); 1702, 1627, 1598, 1563, 1521, 1484, 1449 (NH_2, C=N, C=C); 1382, 1372 (>C(Me)$_2$); 1169, 1070 (ring-1,3-dioxolane); 950 cm^{-1} (ring breathing). δ_H (500 MHz, DMSO-d_6): 8.28 and 8.10 (2×1H, 2×s, H-2 and H-8); 7.30 (4H, br, NH); 4.73 (1H, dd, $J_{1'a,2'}$=3.5 Hz, J_{gem}=13.8 Hz, H-1′a); 4.65 (1H, dd, $J_{1'b,2'}$=6.6 Hz, J_{gem}=13.8 Hz, H-1′b); 4.44 (1H, m, H-2′); 4.07 (1H, dd, $J_{3'a,2'}$=6.5 Hz, J_{gem}=8.8 Hz, H-3′a); 4.06 (1H, dd, $J_{3'b,2'}$=6.5 Hz, J_{gem}=8.8 Hz, H-3′b); 1.25 and 1.22 (2×3H, 2×s, CH_3). δ_C (125 MHz, DMSO-d_6): 159.60 (N–C); 158.85 (C-4); 156.44 (C-6); 150.74 (C-2); 145.94 (C-8); 116.30 (C-5); 109.26 (C–*i*Pr); 74.92 (C-2′); 65.89 (C-3′); 48.65 (C-1′); 26.63 and 25.56 (2×CH_3). Exact mass (FAB HRMS) found: 292.1524; calcd for $C_{12}H_{18}N_7O_2$ [M+H] 292.1522.

Further elution gave *9-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-6-guanidino-9H-purine* (**26a**). Yield, 0.54 g (47%); white crystals, mp 201–202°C. R_F(C)=0.17. FAB

MS, *m/z* (rel. %): 292 (100) [M+H]. IR (KBr) 3400, 3317, 3155 (NH_2, NH); 1637, 1588, 1567, 1522, 1478, 1434 (NH_2, C=N, C=C); 1381, 1372 (>C(Me)$_2$); 1155, 1069 (ring-1,3-dioxolane); 945 cm^{-1} (ring breathing). δ_H(500 MHz, DMSO-d_6): 8.28 and 8.05 (2×1H, 2×s, H-2 and H-8); 7.50 (4H, br, N*H*); 4.48 (1H, m, H-2′); 4.31 (1H, dd, $J_{1'a,2'}$=4.5 Hz, J_{gem}=14.2 Hz, H-1′a); 4.24 (1H, dd, $J_{1'b,2'}$=6.2 Hz, J_{gem}=14.2 Hz, H-1′b); 4.01 (1H, dd, $J_{3'a,2'}$=6.6 Hz, J_{gem}=8.6 Hz, H-3′a); 3.75 (1H, dd, $J_{3'b,2'}$=5.4 Hz, J_{gem}=8.6 Hz, H-3′b); 1.27 and 1.22 (2×3H, 2×s, CH_3). δ_C (125 MHz, DMSO-d_6): 160.28 (N–C); 45.36 (C-1′); 66.21 (C-3′); 73.82 (C-2′); 109.10 (C–C(CH_3)$_2$); 125.00 (C-5); 141.61 (C-8); 150.33 (C-4); 150.84 (C-2); 160.08 (C-6); 26.70 and 25.27 (2×CH_3). Anal. calcd for $C_{12}H_{17}N_7O_2$ (291.31): 49.48, C; 5.88, H; 33.66, N; found: 49.11, C; 5.96, H; 33.28, N.

5.2.17. 9-(2,3-Dihydroxypropyl)-6-guanidino-9*H*-purine (26b). Conc. H_2SO_4 (0.05 mL) was added to the solution of compound **26a** (0.14 g, 0.5 mmol) in water (2.5 mL) and the mixture was stirred at room temperature overnight. It was neutralized with saturated $Ba(OH)_2$, filtered and washed with hot water (3×10 mL). The filtrate was evaporated in vacuo and the residue crystallized from water to give compound **26b** (89 mg, 81%); white crystals, mp 255–257°C. FAB MS, *m/z* (rel. %): 252 (100) [M+H]. IR (KBr) 3400, 3296, 3118 (NH_2, NH); 1663, 1636, 1603, 1565, 1524, 1437 (NH_2, C=N, C=C); 1115, 1047 cm^{-1} (C-OH). δ_H (500 MHz, DMSO-d_6): 8.27 and 8.00 (2×1H, 2×s, H-2 and H-8); 7.50 (4H, br, N*H*); 5.12 and 4.87 (2×1H, 2×brs, O*H*); 4.31 (1H, dd, $J_{1'a,2'}$=3.4 Hz, J_{gem}=13.9 Hz, H-1′a); 4.01 (1H, dd, $J_{1'b,2'}$=8.1 Hz, J_{gem}=13.9 Hz, H-1′b); 3.84 (1H, m, H-2′); 3.39 (1H, dd, $J_{3'a,2'}$=5.2 Hz, J_{gem}=11.0 Hz, H-3′a); 3.31 (1H, dd, $J_{3'b,2'}$=6.0 Hz, J_{gem}=11.0 Hz, H-3′b). δ_C (125 MHz, DMSO-d_6): 160.25 (N–C); 160.06 (C-6); 150.60 (C-2); 150.30 (C-4); 141.89 (C-8); 125.16 (C-5); 69.95 (C-2′); 63.73 (C-3′); 46.44 (C-1′). Exact mass (FAB HRMS) found: 252.1199; calcd for $C_9H_{14}N_7O_2$ [M+H] 252.1207.

5.2.18. Alkylation of 6-guanidinopurine (7a) with compound 27. Compound **27** (1.1 mL, 4.5 mmol, Ref. 6) was added to the suspension of 6-guanidinopurine (**7a**) (0.53 g, 3 mmol) and Cs_2CO_3 (0.49 g, 1.5 mmol) in DMF (20 mL) at 80°C. The resulting mixture was stirred at 100°C for 15 h, evaporated in vacuo and codistilled with toluene (3×30 mL). The residue was extracted by $CHCl_3$ and chromatographed on a silica gel column (30 g, EtOAc–EtOH–Et_3N, 85:14:1) to give *7-[2-(diisopropylphosphorylmethoxy)ethyl]-6-guanidino-7H-purine* (**28**) (0.54 g, 45%) as white crystals, mp 153–155°C; R_F(B)=0.38. FAB MS *m/z* (rel. %): 400 (100) [M+H]. IR (KBr) 3412, 3331, 3207, 3125 (NH_2, NH); 1635, 1595, 1561, 1522, 1436, 1414 (NH_2, C=N, C=C); 1233, 1221 (P=O); 1119, 1104 (C–O–C); 1014, 992 (PO–C); 1386, 1375 (*i*Pr–CH_3); 1177, 1143 cm^{-1} (C–O–C). δ_H (500 MHz, DMSO-d_6): 8.26 and 8.10 (2×1H, 2×s, H-2 and H-8); 7.30 (4H, br, N*H*); 4.74 (2H, t, $J_{1',2'}$=4.8 Hz, H-1′); 4.47 (2H, m, POCH); 3.89 (2H, t, $J_{2',1'}$=4.8 Hz, H-2′); 3.76 (2H, d, $J_{P,CH}$=8.2 Hz, PCH_2); 1.16 and 1.12 (2×6H, 2×d, J_{CH_3},CH=6.2 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 159.62 (N–C); 159.14 (C-4); 156.61 (C-6); 150.90 (C-2); 145.44 (C-8); 116.17 (C-5); 72.15 (d, $J_{P,C}$=11.8 Hz, C-2′); 70.29 (2C, d, $J_{P,C}$=6.4 Hz, P–OC); 64.68 (d, $J_{P,C}$=164.1 Hz, P–C); 46.06 (C-1′); 23.90 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.77 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Anal. calcd for $C_{15}H_{26}N_7O_4P{\cdot}0.3H_2O$ (399.39): 44.44, C; 6.63, H; 24.19, N; 7.64, P; found: 44.48, C; 6.69, H; 24.05, N; 7.47, P.

Further elution of the column gave *9-[2-(diisopropylphosphorylmethoxy)ethyl]-6-guanidino-9H-purine* (**16**) (0.30 g, 25%) as a yellowish oil. R_F(C)=0.22. FAB MS, *m/z* (rel. %): 400 (100) [M+H]. IR (KBr) 3392, 3336, 3214, 3106 (NH_2, NH); 1701, 1650, 1628, 1589, 1566, 1524, 1434, 1414 (NH_2, C=N, C=C); 1227, 1240 (P=O); 1119, 1105 (C–O–C); 1011, 990 (PO–C); 1386, 1375 (*i*Pr–CH_3); 1178, 1142 cm^{-1} (C–O–C). δ_H (500 MHz, DMSO-d_6): 8.30 and 8.09 (2×1H, 2×s, H-2 and H-8); 7.55 (4H, br, N*H*); 4.48 (2H, m, POC*H*); 4.34 (2H, t, $J_{1',2'}$=5.1 Hz, H-1′); 3.90 (2H, t, $J_{2',1'}$=5.1 Hz, H-2′); 3.78 (2H, d, $J_{P,CH}$=8.4 Hz, PCH_2); 1.16 and 1.12 (2×6H, 2×d, J_{CH_3}, CH=6.2 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 159.72 (N–C); 159.05 (C-6); 150.66 (C-2); 150.30 (C-4); 141.61 (C-8); 124.85 (C-5); 70.49 (d, $J_{P,C}$=12.2 Hz, C-2′); 70.33 (2C, d, $J_{P,C}$=6.3 Hz, P–OC); 64.68 (d, $J_{P,C}$=164.6 Hz, P–C); 42.50 (C-1′); 23.91 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.77 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Exact mass (FAB HRMS) found 400.1906; calcd for $C_{15}H_{27}N_7O_4P$ [M+H] 400.1899.

5.2.19. Alkylation of 2-chloro-6-guanidinopurine (7b) with compound 27. Compound **27** (1.7 mL, 7.2 mmol) was added to the mixture of 2-chloro-6-guanidinopurine (**7b**) (1.0 g, 4.7 mmol) and Cs_2CO_3 (0.78 g, 2.4 mmol) in DMF (46 mL) prewarmed to 80°C. The resulting mixture was stirred at 100°C for 12 h, evaporated in vacuo and codistilled with toluene (3×10 mL). The residue was extracted by $CHCl_3$ and chromatographed on a column of silica gel (30 g, MeOH–$CHCl_3$, 10:90] to give *2-chloro-6-guanidino-7-[2-(diisopropylphosphorylmethoxy)ethyl]-7H-purine* (**30**) (0.94 g, 46%) as white crystals, mp 168–170°C (EtOH–ether). R_F(B)=0.27. FAB MS, *m/z* (rel. %): 434 (100) [M+H]. IR (KBr) 3502, 3420, 3306, 3169, 3122 (NH_2, NH); 1644, 1636, 1594, 1556, 1510, 1488, 1420 (NH_2, C=N, C=C); 1261, 1253, 1228 (P=O); 1106, 1098 (C–O–C); 1012, 1000, 990, 975 (PO–C); 1386, 1378 (*i*Pr–CH_3); 1178, 1143 cm^{-1} (C–C–C). δ_H (500 MHz, DMSO-d_6): 8.14 (1H, s, H-8); 7.40 (4H, br, N*H*); 4.71 (2H, t, $J_{1',2'}$=4.8 Hz, H-1′); 4.47 (2H, m, POCH); 3.88 (2H, t, $J_{2',1'}$=4.8 Hz, H-2′); 3.76 (2H, d, $J_{P,CH}$=8.1 Hz, PCH_2); 1.16 and 1.12 (2×6H, 2×d, J_{CH_3},CH=6.2 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 160.19 (C-4); 159.84 (N–C); 156.72 (C-6); 150.98 (C-2); 146.24 (C-8); 115.08 (C-5); 71.90 (d, $J_{P,C}$=11.2 Hz, C-2′); 70.28 (2C, d, $J_{P,C}$=6.4 Hz, P–OC); 64.66 (d, $J_{P,C}$=163.6 Hz, P–C); 46.02 (C-1′); 23.88 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.76 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Anal. calcd for $C_{15}H_{25}ClN_7O_4P$ (433.83): 41.53, C; 5.81, H; 8.17, Cl; 22.60, N; 7.14, P. found: 41.47, C; 5.87, H; 8.29, Cl; 22.42, N; 7.19 P.

Further elution gave *2-chloro-6-guanidino-9-[2-(diisopropylphosphorylmethoxy)ethyl]-9H-purine* (**31**) (0.32 g, 16%) as a yellow oil, R_F(B)=0.10. FAB MS, *m/z* (rel. %): 434 (100) [M+H]. IR (KBr) 3493, 3403, 3330, 3160 (NH_2, NH); 1697, 1623, 1587, 1568, 1522, 1507, 1468, 1428 (NH_2, C=N, C=C); 1250, 1229 (P=O); 1120, 1106 (C–

O–C); 1010, 996 (PO–C); 1388, 1376 (*i*Pr–CH_3); 1177, 1142 cm^{-1} (C–O–C). δ_H (500 MHz, DMSO-d_6): 8.05 (1H, s, H-8); 7.45 (4H, br, N*H*); 4.48 (2H, m, POC*H*); 4.29 (2H, t, $J_{1',2'}$=5.0 Hz, H-1′); 3.87 (2H, t, $J_{2',1'}$=5.0 Hz, H-2′); 3.78 (2H, d, $J_{P,CH}$=8.4 Hz, PCH_2); 1.17 and 1.13 (2×6H, 2×d, $J_{CH_3,CH}$=6.2 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 160.21 (N–C); 159.87 (C-6); 151.18 (C-4); 151.04 (C-2); 141.75 (C-8); 124.00 (C-5); 70.45 (d, $J_{P,C}$=11.7 Hz, C-2′); 70.35 (2C, d, $J_{P,C}$=6.3 Hz, P–OC); 64.68 (d, $J_{P,C}$=164.1 Hz, P–C); 42.65 (C-1′); 23.89 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.75 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Exact mass (FAB HRMS) found: 434.1467; calcd for $C_{15}H_{26}ClN_7O_4P$ [M+H] 434.1472.

5.2.20. Alkylation of 2-amino-6-guanidinopurine (7c) with compound 27. Compound **27** (1.1 mL, 4.5 mmol) was added to the mixture of 2-amino-6-guanidinopurine (**7c**) (0.58 g, 3 mmol) and Cs_2CO_3 (0.49 g, 1.5 mmol) in DMF (20 mL) prewarmed to 80°C. The resulting mixture was stirred at 100°C for 15 h, evaporated in vacuo and codistilled with toluene (3×10 mL). The residue was extracted by $CHCl_3$ and chromatographed on a silica gel column [30 g, EtOAc–EtOH, 80:20] to give *2-amino-6-guanidino-7-[2-(diisopropylphosphorylmethoxy)ethyl]-7H-purine* (**29**) (0.35 g, 28%) as a white solid, mp 209–211°C; R_F(B)=0.26. FAB MS, *m/z* (rel. %): 415 (100) [M+H]. IR (KBr) 3492, 3392, 3329, 3203, 3108 (NH_2, NH); 1630, 1590, 1507, 1454 (NH_2, C=N, C=C); 1237, 1212 (P=O); 1119, 1106 (C–O–C); 1012, 991 (PO–C); 1387, 1375 (*i*Pr–CH_3); 1180, 1141 cm^{-1} (C–O–C). δ_H (500 MHz, DMSO-d_6): 7.94 (1H, s, H-8); 7.60 br, 4H, NH_2); 6.77 (2H, brs, NH_2); 4.89 (2H, m, POC*H*); 4.63 (2H, t, $J_{1',2'}$=4.8 Hz, H-1′); 3.86 (2H, t, $J_{2',1'}$=4.8 Hz, H-2′); 3.75 (2H, d, $J_{P,CH}$=8.2 Hz, PCH_2); 1.18 and 1.14 (2×6H, 2×d, $J_{CH_3,CH}$=6.1 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 160.35 (N–C); 156.86 (C-4); 156.15 (C-2); 154.69 (C-6); 144.16 (C-8); 71.72 (d, $J_{P,C}$=10.2 Hz, C-2′); 70.47 (2C, d, $J_{P,C}$= 6.3 Hz, P–OC); 64.68 (d, $J_{P,C}$=163.8 Hz, P–C); 46.00 (C-1′); 23.92 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.80 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Exact mass (FAB HRMS) found 415.1963; calcd for $C_{15}H_{28}N_8O_4P$ [M+H] 415.1971.

Further elution of the column gave *2-amino-6-guanidino-9-[2-(diisopropylphosphorylmethoxy)ethyl]purine* (**18**) (0.34 g, 28%) as colorless crystals, mp 117–121°C (aceton–ether). R_F(C)=0.15. FAB MS, *m/z* (rel. %): 415 (90) [M+H]; IR (KBr) 3470, 3386, 3317, 3203 (NH_2, NH); 1699, 1644, 1580, 1520, 1468, 1446, 1402 (NH_2, C=N, C=C); 1238, 1225 (P=O); 1119, 1106 (C–O–C); 1011, 993 (PO–C); 1387, 1376 (*i*Pr–CH_3); 1179, 1142 cm^{-1} (C–O–C). δ_H (500 MHz, DMSO-d_6): 8.20 (4H, br, N*H*); 7.74 (1H, s, H-8); 6.22 (2H, brs, NH_2); 4.51 (2H, m, POC*H*); 4.15 (2H, t, $J_{1',2'}$=5.1 Hz, H-1′); 3.83 (2H, t, $J_{2',1'}$=5.1 Hz, H-2′); 3.77 (2H, d, $J_{P,CH}$=8.4 Hz, P–CH_2); 1.19 and 1.15 (2×6H, 2×d, $J_{CH_3,CH}$=6.2 Hz, CH_3). δ_C (125 MHz, DMSO-d_6): 160.23 (N–C); 159.00 (C-2); 158.62 (C-6); 152.76 (C-4); 139.07 (C-8); 117.43 (C-5); 70.51 (d, $J_{P,C}$=11.7 Hz, C-2′); 70.36 (2C, d, $J_{P,C}$=5.9 Hz, P–OC); 64.76 (d, $J_{P,C}$=164.1 Hz, P–C); 42.08 (C-1′); 23.94 (2C, d, $J_{P,C}$=3.9 Hz, CH_3); 23.81 (2C, d, $J_{P,C}$=4.9 Hz, CH_3). Mass spectrum (FAB): 415 (100) (M+H); Exact mass (FAB HRMS) found 415.1953; calcd for $C_{15}H_{28}N_8O_4P$ [M+H] 415.1971.

5.3. Cleavage of the phosphonate diesters (general procedure)

The diester (1 mmol) in CH_3CN (10 mL) was treated dropwise with bromotrimethylsilane (1 mL). The mixture was stirred overnight at room temperature. After evaporation of the solvent and codistillation with CH_3CN (10 mL), the residue was treated with water (10 mL) and conc. NH_3 (3 mL) for 5 min. and evaporated. The residue was dissolved in water and applied onto the column of Dowex 50×8 (H^+-form) (25 mL). The column was washed with water and eluted with diluted aqueous ammonia (1:10). The UV-absorbing ammonia eluate was evaporated in vacuo and the residue in diluted aqueous NH_3 was applied onto the column of Dowex 1×2 (acetate form). The column was washed with water and eluted with a linear gradient of acetic acid (0–1 M, á 1 L). Fractions containing product were evaporated and crystallized. The following compounds were obtained by this procedure:

5.3.1. 6-Guanidino-9-[2-(phosphonomethoxy)ethyl]-9*H*-purine (20). From compound **16** (1.08 g, 2.7 mmol). White crystals from H_2O (0.42 g, 49%), mp 215–218°C; E_{Up}=0.60. FAB MS, *m/z* (rel. %): 316 (20) [M+H]; 91 (100). IR (KBr) 3400, 3363, 3188 (NH_2, NH); 2355 (OH in $PO(OH)_2$); 1702, 1630, 1591, 1572, 1510, 1457 (C=N^+, NH_2, C=N, C=C); 1145 (P=O); 1118 (C–O–C); 909 cm^{-1} (P–OH). δ_H (500 MHz, D_2O+NaOD): 8.24 and 8.22 (2×1H, 2×s, H-2 and H-8); 4.40 (t, 2H, $J_{1',2'}$=5.1 Hz, H-1′); 3.96 (2H, t, $J_{2',1'}$=5.1 Hz, H-2′); 3.52 (2H, d, $J_{P,CH}$= 8.4 Hz, PCH_2). δ_C (125 MHz, D_2O+NaOD): 162.91 (N–C); 161.95 (C-6); 154.02 (C-2); 152.27 (C-4); 145.93 (C-8); 127.10 (C-5); 72.98 (d, $J_{P,C}$=10.7 Hz, C-2′); 71.86 (d, $J_{P,C}$=149.4 Hz, P–C); 46.18 (C-1′). Anal. calcd for $C_9H_{14}N_7O_4P{\cdot}H_2O$ (315.23): 32.44, C; 4.84, H; 29.42, N; 9.29, P. found: 32.77, C; 4.84, H; 29.57, N; 9.26, P.

5.3.2. 2-Amino-6-guanidino-9-[2-(phosphonomethoxy)-ethyl]-9*H*-purine (21). From compound **18** (0.9 g, 2.2 mmol). The poorly soluble free acid form precipitated during purification on Dowex 1 column; it was decanted from resin and filtered. Yield (0.63 g, 88%); white crystals, mp>246°C (dec.); E_{Up}=0.39. FAB MS, *m/z* (rel. %): 331 (10) [M+H]; 91.3 (100). IR (KBr): 3470, 3388, 3338, 3219, 3124 (NH_2, NH); 2788, 2361 (OH); 1696, 1648, 1597, 1523, 1405 (NH_2, C=N, C=C); 1164 (P=O); 1128 (COC); 1055, 916 (P–OH, OH). δ_H (500 MHz, DMSO-d_6): 9.30 and 8.90 (2×2H, 2×br, N*H*); 8.15 (1H, s, H-8); 6.68 (2H, brs, NH_2); 4.16 (2H, t, $J_{1',2'}$=5.0 Hz, H-1′); 3.86 (2H, t, $J_{2',1'}$=5.0 Hz, H-2′); 3.54 (2H, d, $J_{P,CH}$=8.4 Hz, PCH_2). δ_C (125 MHz, D_2O+NaOD): 163.07, 162.61 and 161.94 (C-2, C-6 and N–C); 154.17 (C-4); 143.43 (C-8); 121.63 (C-5); 73.10 (d, $J_{P,C}$=10.7 Hz, C-2′); 71.91 (d, $J_{P,C}$=150.0 Hz, P–C); 45.63 (C-1′). Exact mass (FAB HRMS) found 331.0974; calcd for $C_9H_{16}N_8O_4P$ [M+H] 331.1032.

5.3.3. 6-Guanidino-7-[2-(phosphonomethoxy)ethyl]-7*H*-purine (22). From compound **28** (0.46 g, 1.2 mmol). White crystals from H_2O (0.24 g, 66%), mp 267–268°C (dec.); E_{Up}=0.75. FAB MS, *m/z* (rel. %): 316 (75), [M+H]. IR (KBr) 3475, 3440, 3228, 3046 (NH_2, NH); 2360, 2323 (OH in $PO(OH)_2$); 1708, 1699, 1655, 1619, 1600, 1569, 1529, 1499, 1465 (C=N^+, NH_2, C=N,

C═C); 1140 (P═O); 1112 (C–O–C); 919 cm^{-1} (P–OH). δ_H (500 MHz, D_2O+NaOD): 8.27 and 8.21 (2×1H, 2×s, H-2 and H-8); 4.65 (2H, t, $J_{1',2'}$=5.3 Hz, H-1′); 3.93 (2H, t, $J_{2',1'}$=5.3 Hz, H-2′); 3.52 (2H, d, $J_{P,CH}$=8.8 Hz, PCH_2). δ_C (125 MHz, D_2O+NaOD): 159.25 (N–C); 157.47 (C-4); 156.37 (C-6); 151.56 (C-2); 146.21 (C-8); 116.15 (C-5); 71.21 (d, $J_{P,C}$=10.2 Hz, C-2′); 68.96 (d, $J_{P,C}$=150.0 Hz, P–C); 46.37 (C-1′). Anal. calcd for $C_9H_{14}N_7O_4P{\cdot}0.5H_2O$ (315.23): 33.34, C; 4.66, H; 30.24, N; 9.55, P; found: 33.74, C; 4.67, H; 30.23, N; 9.42, P.

5.3.4. 2-Amino-6-guanidino-7-[2-(phosphonomethoxy)-ethyl]-7*H*-purine (23). From compound **29** (0.29 g, 0.7 mmol). White crystals from H_2O (0.11 g, 46%), mp 291–294°C (dec.); E_{Up}=0.62. FAB MS, *m/z* (rel. %): 331 (20) [M+H]. IR (KBr) 3500, 3425, 3335, 3172 (NH_2, NH); 2433 (OH in $PO(OH)_2$); 1666, 1632, 1520, 1488, 1449 (NH_2, C═N, C═C); 1136 (P═O); 1099 (C–O–C); 922 cm^{-1} (P–OH). δ_H (500 MHz, D_2O+NaOD): 8.04 (1H, s, H-8); 4.58 (2H, t, $J_{1',2'}$=5.1 Hz, H-1′); 3.92 (2H, t, $J_{2',1'}$=5.1 Hz, H-2′); 3.49 (2H, d, $J_{P,CH}$=8.7 Hz, PCH_2). δ_C (125 MHz, D_2O+NaOD): 159.25 (N–C); 157.47 (C-4); 156.37 (C-6); 151.56 (C-2); 146.21 (C-8); 116.15 (C-5); 71.21 (d, $J_{P,C}$=10.2 Hz, C-2′); 68.96 (d, $J_{P,C}$=150.0 Hz, P–C); 39.37 (C-1′). Exact mass (FAB HRMS) found 331.1059; calcd for $C_9H_{16}N_8O_4P$ [M+H] 331.1032.

5.3.5. 2-Chloro-6-guanidino-7-[2-(phosphonomethoxy)-ethyl]-7*H*-purine (32). From compound **30** (0.70 g, 1.6 mmol). White crystals from H_2O (0.46 g, 82%), mp 249–251°C; E_{Up}=0.74. FAB MS, *m/z* (rel. %): 350 (70), [M+H]. IR (KBr) 3525, 3314, 3269, 3208, 3111 (NH_2, NH); 2347 (OH in $PO(OH)_2$); 1708, 1699, 1665, 1617, 1561, 1524, 1491, 1466, 1426 (C═N^+, NH_2, C═N, C═C); 1127 (P═O); 1092 (C–O–C); 914 cm^{-1} (P–OH). δ_H (500 MHz, D_2O+NaOD): 8.27 (1H, s, H-8); 4.68 (2H, t, $J_{1',2'}$=5.2 Hz, H-1′); 4.00 (2H, t, $J_{2',1'}$=5.2 Hz, H-2′); 3.58 (2H, d, $J_{P,CH}$=8.7 Hz, PCH_2). δ_C (125 MHz, D_2O+NaOD): 159.54 (C-4); 158.54 (N–C); 156.44 (C-6); 151.79 (C-2); 146.37 (C-8); 114.94 (C-5); 71.22 (d, $J_{P,C}$=10.2 Hz, C-2′); 69.01 (d, $J_{P,C}$=149.9 Hz, P–C); 46.48 (C-1′). Anal. calcd for $C_9H_{13}CN_7O_4P{\cdot}0.25H_2O$ (349.67): 30.52, C; 3.84, H; 10.01, Cl; 27.68, N; 8.75, P; found: 30.47, C; 3.84, H; 10.21, Cl; 27.46, N; 8.51, P.

5.3.6. 2-Chloro-6-guanidino-9-[2-(phosphonomethoxy)-ethyl]-9*H*-purine (33). From compound **31** (0.32 g, 0.7 mmol). White crystals from H_2O (0.19 g, 74%), mp 254°C (dec.); E_{Up}=0.68. FAB MS, *m/z* (rel. %): 350 (100), [M+H]. IR (KBr) 3508, 3418, 3304, 3074 (NH_2, NH); 2368 (OH in $PO(OH)_2$); 1706, 1630, 1591, 1543, 1505, 1431 (C═N^+, NH_2, C═N, C═C); 1147 (P═O); 1131, 1117 (C–O–C); 914 cm^{-1} (P–OH). δ_H (500 MHz, D_2O+NaOD): 8.10 (1H, s, H-8); 4.28 (2H, t, $J_{1',2'}$= 5.0 Hz, H-1′); 3.94 (2H, t, $J_{2',1'}$=5.0 Hz, H-2′); 3.56 (2H, d, $J_{P,CH}$=8.7 Hz, PCH_2). δ_C (125 MHz, D_2O+NaOD): 160.18 (N–C); 158.88 (C-6); 151.61 (C-4); 150.13 (C-2); 142.86 (C-8); 122.89 (C-5); 70.02 (d, $J_{P,C}$=11.2 Hz, C-2′); 69.06 (d, $J_{P,C}$=149.9 Hz, P–C); 43.28 (C-1′). Anal. calcd for $C_9H_{13}CN_7O_4P{\cdot}0.25H_2O$ (349.67): 30.52, C; 3.84, H; 10.01, Cl; 27.68, N; 8.75, P; found: 30.37, C; 3.86, H; 10.14, Cl; 27.34, N; 8.62, P.

Acknowledgements

This study was performed as a part of research project # 4055905 of the Institute. It was supported by the Grant Agency of the Czech Republic (# 203/94/K001), by the programme of key-projects of the Academy of Sciences of the Czech Republic (S4055109), by the COST programme of the Ministry of Education (projects D.8.30. and D.13.8.) and by Gilead Sciences (Foster City, CA, USA). The authors' thanks are also due to the staff of the mass spectrometry and analytical departments of the Institute (Head, K. Ubik) and to Dr P. Fiedler for measuring the IR spectra.

References

1. (a) Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197–207. (b) Ha, S. B.; Melman, N.; Jacobson, K. A.; Nair, V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3085–3090. (c) Poulsen, S. A.; Quinn, R. J. *Bioorg. Med. Chem.* **1998**, *6*, 619–641.
2. (a) Jacobson, K. A. *Drug Dev. Res.* **2001**, *52*, 178–186. (b) Umino, T.; Yoshioka, K.; Saitoh, Y.; Minakawa, N.; Nakata, H.; Matsuda, A. *J. Med. Chem.* **2001**, *44*, 208–214.
3. Hampton, A. *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum: New York, 1991; Vol. 2, pp 399–450.
4. (a) Holý, A.; Votruba, I.; De Clercq, E. *Collect. Czech. Chem. Commun.* **1985**, *50*, 245–261. (b) Holý, A.; Votruba, I.; De Clercq, E. *Collect. Czech. Chem. Commun.* **1985**, *50*, 262–279.
5. (a) Pignot, M.; Pljevaljcic, G.; Weinhold, E. *Eur. J. Org. Chem.* **2000**, 549–555. (b) Wnuk, S. F.; Valdez, C. A.; Khan, J.; Moutinho, P.; Robins, M. J.; Yang, X. D.; Borchardt, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **2000**, *43*, 1180–1186.
6. Holý, A.; Günter, J.; Dvořáková, H.; Masojídková, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1999**, *42*, 2064–2086.
7. Hocek, M.; Masojídková, M.; Holý, A.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1525–1537.
8. Ekelund, S.; Nygren, P.; Larsson, R. *Biochem. Pharmacol.* **2001**, *61*, 1183–1193.
9. Ekelund, S.; Sjöholm, Ć.; Nygren, P.; Binderup, L.; Larsson, R. *Eur. J. Pharmacol.* **2001**, *418*, 39–45.
10. Lown, J. W.; Krowicki, K.; Balzarini, J.; Newman, R. A.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 2368–2375.
11. Sudbeck, E. A.; Jedrzejas, M. J.; Singh, S.; Brouillette, W. J.; Air, G. M.; Laver, W. G.; Babu, Y. S.; Bantia, S.; Chand, P.; Chu, N.; Montgomery, J. A.; Walsh, D. A.; Luo, M. *J. Mol. Biol.* **1997**, *267*, 584–594.
12. Lal, B.; Gangopadhyay, A. K. *Tetrahedron Lett.* **1996**, *37*, 2483–2486.
13. Doronina, S. O.; Behr, J. P. *Tetrahedron Lett.* **1998**, *39*, 547–550.
14. Robles, J.; Grandas, A.; Pedroso, E. *Tetrahedron* **2001**, *57*, 179–194.
15. Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497–2502.
16. Schenck, M.; Kirchhof, H. *Hoppe-Seyler's Z. Physiol. Chem.* **1926**, *158*, 90–110.
17. Keekyung, K.; Yi-Tsong, L.; Mosher, H. S. *Tetrahedron Lett.* **1988**, *29*, 3183–3186.

18. Linn, J. A.; McLean, E.; Kelley, J. L. *J. Chem. Soc., Perkin Trans. 1* **1994**, 913–914.
19. Norman, T. C.; Gray, N. S.; Koh, J. T.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 7430–7431.
20. Watkins, B. E.; Kiely, J. S.; Rapoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 5702–5708.
21. Gao, X.; Jones, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 1275–1278.
22. Maruenda, H.; Chenna, A.; Liem, L. K.; Singer, B. *J. Org. Chem.* **1998**, *63*, 4385–4389.
23. (a) Holý, A.; Dvořáková, H.; Jindřich, J. In *Antibiotics and Antiviral Compounds*; Krohn, K.; Kirst, H. A.; Maag, H., Eds.; Verlag Chemie: Weinheim/New York; 1993, pp 455–462. (b) Holý, A. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press, Inc.: Greenwich, CT (USA), 1994; pp 179–232.
24. (a) Naesens, L.; De Clercq, E. *Nucleosides Nucleotides* **1997**, *16*, 983–992. (b) Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neyts, J.; De Clercq, E. *Antivir. Chem. Chemother.* **1997**, *8*, 1–23.
25. Holý, A. *Collect. Czech. Chem. Commun.* **1978**, *43*, 2054–2061.
26. (a) De Clercq, E.; Descamps, J.; De Somer, P.; Holý, A. *Science* **1978**, *200*, 563–564. (b) De Clercq, E.; Holý, A. *J. Med. Chem.* **1979**, *22*, 510–513. (c) Votruba, I.; Holý, A. *Collect. Czech. Chem. Commun.* **1980**, *45*, 3039–3044. (d) De Clercq, E.; Leyten, R.; Sobis, H.; Matoušek, J.; Holý, A.; De Somer, P. *Toxicol. Appl. Pharmacol.* **1981**, *59*, 441–451.
27. Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817–1825.
28. Cassidy, F.; Olsen, R. K.; Robins, R. K. *J. Heterocycl. Chem.* **1968**, *5*, 461–465.
29. Hocek, M.; Holý, A. *Collect. Czech. Chem. Commun.* **1995**, *60*, 1386–1389.
30. Holý, A.; Ivanova, G. S. *Nucleic Acids Res.* **1974**, *1*, 19–34.
31. (a) Brown, D. J.; Ford, P. W. *J. Chem. Soc. C* **1969**, 2620–2623. (b) Badger, R. J.; Barlin, G. B. *J. Chem. Soc., Perkin Trans. 2* **1974**, 1854–1859.