A Convenient Synthesis of 2'-Deoxy-6-thioguanosine, *Ara*-Guanine, *Ara*-6-Thioguanine and Certain Related Purine Nucleosides by the Stereospecific Sodium Salt Glycosylation Procedure [1]

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A simple and high-yield synthesis of biologically significant 2'-deoxy-6-thioguanosine (**11**), *ara*-6-thioguanine (**16**) and *ara*G (**17**) has been accomplished employing the stereospecific sodium salt glycosylation method. Glycosylation of the sodium salt of 6-chloro- and 2-amino-6-chloropurine (**1** and **2**, respectively) with 1-chloro-2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranose (**3**) gave the corresponding N-9 substituted nucleosides as major products with the β-anomeric configuration (**4** and **5**, respectively) along with a minor amount of the N-7 positional isomers (**6** and **7**). Treatment of **4** with hydrogen sulfide in methanol containing sodium methoxide gave 2'-deoxy-6-thioinosine (**10**) in 93% yield. Similarly, **5** was transformed into 2'-deoxy-6-thioguanosine (β-TGdR, **11**) in 71% yield. Reaction of the sodium salt of **2** with 1-chloro-2,3,5-tri-*O*-benzyl-α-D-arabinofuranose (**8**) gave N-7 and N-9 glycosylated products **13** and **9**, respectively. Debenzylation of **9** with boron trichloride at -78° gave the versatile intermediate 2-amino-6-chloro-9-β-D-arabinofuranosylpurine (**14**) in 62% yield. Direct treatment of **14** with sodium hydrosulfide furnished *ara*-6-thioguanine (**16**). Alkaline hydrolysis of **14** readily gave 9-β-D-arabinofuranosylguanine (*ara*G, **17**), which on subsequent phosphorylation with phosphorus oxychloride in trimethyl phosphate afforded *ara*G 5'-monophosphate (**18**).

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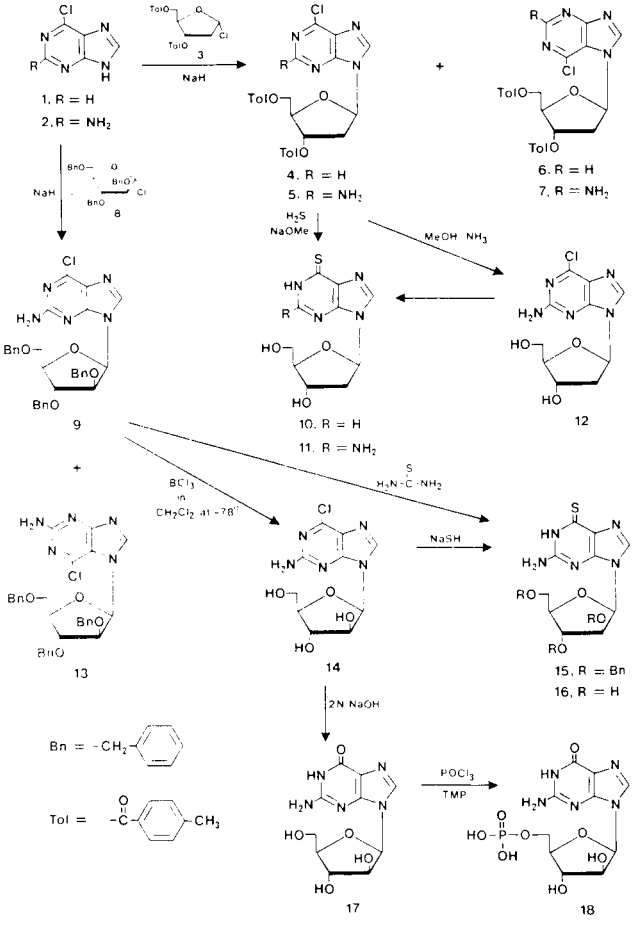
Over the years, several 6-thioguanine nucleosides have been prepared [2-8] and evaluated for their antitumor properties [8-18]. The most important of these are the α- and β-anomers of 2-amino-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine-6-thione (α- and β-TGdR) [4, 19-21]. Although α-TGdR is less toxic than the β-anomer (**11**) [22], the β-TGdR is a more potent and useful antitumor agent. It has been found that, in general, the β-anomeric configuration is required for the nucleosides to exhibit potent biological activity [23]. In the course of a program designed to synthesize certain 6-thiopurine nucleoside analogs with the β-anomeric configuration as potential anticancer agents, we recently required β-TGdR in large quantity.

Considerable effort has been expended in the development of simpler methods for the preparation of 2'-deoxy-β-D-ribofuranosyl derivatives of 6-thiopurines. The potent antitumor agent [24] 9-(2-deoxy-β-D-ribofuranosyl)purine-6-thione (**10**) has previously been prepared by enzymatic [25,26] as well as by chemical methods [27,28]. However, each of these procedures gave **10** in relatively low yield. The prior glycosylation procedure [27] for introducing the 2-deoxy-β-D-*erythro*-pentofuranosyl moiety *via* chloromercuri-6-chloropurine suffered from the need to separate the anomers at some stage of the synthetic sequence, whereas the conversion [28] of 2'-deoxyinosine to **10** require the availability of the preformed 2'-deoxyribonucleoside. Use of the stereospecific sodium salt glycosylation technique, developed recently in our laboratory [29-32] has now been found to be highly useful for the synthesis of **10** as well as 2'-deoxy-6-thioguanosine (**11**) and *ara*-6-thioguanine (**16**), which is the subject of this report. The intermediate 2-amino-9-(β-D-arabinofuranosyl)-6-chloropurine (**14**) provided an alternate route to the biologically significant *ara*-guanine (*ara*G, **17**).

The commercially available [33] 6-chloropurine (**1**) and 2-amino-6-chloropurine (**2**) were used for glycosylation studies in an effort to obtain the corresponding nucleoside intermediates, which could then readily be converted into the desired 2'-deoxy-6-thiopurine nucleosides by direct nucleophilic displacement. The sodium salt of **1**, generated *in situ* by the treatment of sodium hydride in acetonitrile, was reacted with 1-chloro-2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranose (**3**) [34] at ambient temperature under a nitrogen atmosphere. Two nucleosidic products were formed (Scheme I). After silica gel column chromatography, a 61% yield of crystalline (from ethanol) 6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**4**) (mp 108-110° dec) and the corresponding N-7 glycosyl isomer **6** (mp 152-153°) in 13% yield were isolated. No formation of the α-anomers was detected by tlc or hplc techniques. The isolated 61% yield of crystalline **4** is far superior to the 14% yield of the same compound prepared by the chloromercuri salt procedure [27]. When **4** was treated with hydrogen sulfide in 1*M* sodium methoxide in methanol at reflux temperature, deprotection of the carbohydrate moiety with concomitant nucleophilic displacement of the 6-chloro function to the 6-thio group occurred to give 9-(2-deoxy-β-D-*erythro*-

pentofuranosyl)purine-6-thione (2′-deoxy-6-thioinosine, **10**). Compound **10** was isolated as crystalline needles in over 93% yield, and was found to be identical in all respects with an authentic sample of 2′-deoxy-6-thioinosine previously prepared [27]. However, the overall yield of **10** by the presently described procedure is much superior to the previously reported method [27].

Scheme I



A similar glycosylation of the sodium salt of 2-amino-6-chloropurine (**2**) [33] with **3** gave a mixture of crystalline (mp 173-175°, from ethanol) 2-amino-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**5**) in 51% yield, and the corresponding N-7 glycosyl isomer (**7**) in 9% yield as crystalline needles of mp 120-122°. Compounds **5** and **7** were separated on a silica gel column using chloroform:methanol (98:2) as the eluent. Again, no formation of the α-anomers was detected in this reaction, and the 51% yield of crystalline **5** obtained by this procedure is quite superior to the 31.5% yield reported by Goodman *et al.* [4] by the mercury salt method. Subsequent treatment of **5** with hydrogen sulfide in 1*M* sodium methoxide in methanol, as in the case of **10**, readily provided 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine-6-thione (β-TGdR, **11**) in 71% yield. Compound **11** was identical with an authentic sample of β-TGdR [4]. Deprotection of **5** with methanolic ammonia (saturated at 0°) at room temperature gave 2-amino-6-chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**12**) in 68% yield. Treatment of **12** with sodium hydrogen sulfide in boiling methanol furnished yet another route to the synthesis of β-TGdR.

This general and stereospecific procedure has also been found to be equally applicable to the preparation of the antiviral [35-38] and antitumor [39-45] agent 9-β-D-arabinofuranosylguanine (*ara*G, **17**). *Ara*G was initially synthesized by Reist and Goodman [46] by the condensation of 2,6-dichloropurine with tetraacetylxylofuranose, followed by chemical transformation of the resulting triacetyl nucleoside. Subsequently, a coupling [8,47], cyclonucleoside interconversion [48,49], sequential oxidation-reduction conversion of ribo- to the arabinonucleoside [50], enzymatic [51,52] and a combination of chemical and enzymatic procedures [35,53,54] have been used to prepare *ara*G. However, attempts to prepare *ara*G by the aluminum chloride catalyzed condensation of 2-acylguanine and tetra-*O*-acetyl-D-arabinofuranose resulted in the formation of the α-anomer as the major product [55]. In our desire for a simple synthetic procedure which is suitable for the preparation of large quantities of *ara*G as well as *ara*-6-thioguanine, we have reacted the sodium salt of **2** with 1-chloro-2,3,5-tri-*O*-benzyl-α-D-arabinofuranose (**8**) [56] having nonparticipating blocking groups, in anhydrous acetonitrile (Scheme I). A clean reaction was observed at room temperature and the two isomeric nucleosides thus formed were readily separated by flash silica gel column chromatography to give 2-amino-6-chloro-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**9**) in 68% yield, and the corresponding N-7 positional isomer **13** in 11% yield. No formation of the α-anomers was detected. The isolated yield of the key intermediate **9** (68%) by this procedure compares very favorably to the reported 33% yield of **9** obtained by the condensation of the trimethylsilyl derivative of **2** with the α-halogenose **8** in the presence of molecular sieve [47]. Debenzylation of **9** with boron trichloride in dichloromethane at -78°, followed by purification of the reaction product on a C_{18} reverse phase column using 10% aqueous methanol as the eluent gave crystalline 2-amino-6-chloro-9-β-D-arabinofuranosylpurine (**14**) in 62% yield. Direct treatment of **14** with 2*N* sodium hydroxide in dioxane at reflux temperature furnished a 71% yield of crystalline *ara*G (**17**), which was found to be identical in all respects (uv, ir, ^{1}H nmr, mp, mixed mp and tlc in three different solvent systems) with an authentic sample of **17** prepared by the reported procedure [47]. Direct phosphorylation of the unprotected *ara*G with phosphorus oxychloride in trimethyl phosphate at 5-15°, accordingly to the general procedure of Yoshikawa and co-workers [57] gave 9-β-D-arabinofurano-

sylguanine 5'-monophosphate (**18**), which was isolated as the free acid in 48% yield. The purity of **18** was assured by homogeneity in several tlc systems, and confirmed by ^{1}H nmr and elemental analyses.

Nucleophilic displacement of the 6-chloro group of **9** was accomplished by the treatment with thiourea in boiling ethanol to afford crystalline intermediate 2-amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine-6-thione (**15**) in 85% yield. However, treatment of unprotected **14** with sodium hydrosulfide in boiling ethanol gave crystalline 2-amino-9-β-D-arabinofuranosylpurine-6-thione (*ara*-6-thioguanine, **16**) in 74% yield. Compound **16** was identical to previously reported *ara*-6-thioguanine [8].

Thus, this simple high-yield methodology provided a facile and convenient route to the large-scale preparation of biologically significant purine nucleosides, such as 2'-deoxythioguanosine, *ara*-thioguanine and *ara*G.

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. The presence of water as indicated by elemental analysis was verified by ^{1}H nmr spectroscopy. Thin layer chromatography (tlc) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (E. Merck, 230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components in tlc was by uv light, and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30°. Infrared (ir) spectra were recorded in potassium bromide with a Perkin-Elmer 1420 spectrophotometer and ultraviolet spectra (uv) were recorded on a Beckman DU-50 spectrophotometer. Nuclear magnetic resonance (^{1}H nmr) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as the internal standard. The signals are described as s (singlet), d (doublet), t (triplet), and m (multiplet).

6-Chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**4**) and 6-Chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**6**).

A mixture of 6-chloropurine (**1**, 11.5 g, 75 mmoles) [33] and sodium hydride (60% in oil, 3.35 g, 83.75 mmoles) in anhydrous acetonitrile (750 ml) was stirred at ambient temperature for 30 minutes under a nitrogen atmosphere. Dry, powdered 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**3**, 29.9 g, 77 mmoles) [34] was added portionwise with stirring during 20 minutes and stirring was continued for further 18 hours. A small amount of insoluble material was removed by filtration. Evaporation of the filtrate gave an oily residue, which was purified on a silica gel column (5 x 60 cm) using toluene:acetone (9:1, v/v) as the eluent. The following two nucleosides were isolated in the order listed: 6-Chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**4**) was isolated from the initial fractions and crystallized from ethanol to yield 23.2 g (61%), mp 108-110° dec (lit [27] 107-109°); ^{1}H nmr (DMSO-d_6): δ 2.41 (s, 3, CH_3), 2.43 (s, 3, CH_3), 6.76 (t, 1, C$_1$'H, peak width 14.0 Hz), 7.36 and 7.94 (m, 8, 2 phenyls), 8.80 (s, 1, C$_2H$) and 9.00 (s, 1, C$_8H$).

Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$ (506.9): C, 61.60; H, 4.57; N, 11.05. Found: C, 61.73; H, 4.72; N, 11.03.

The N-7 glycosyl isomer 6-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**6**) was isolated from subsequent fractions and crystallized from ethanol to yield 4.9 g (13%), mp 152-153°; ^{1}H nmr (DMSO-d_6): δ 2.41 (s, 3, CH_3), 2.43 (s, 3, CH_3), 6.96 (t, 1, C$_1$'H, peak width 14.5 Hz), 7.36 and 7.94 (m, 8, 2 phenyls), 8.94 (s, 1, C$_2H$) and 9.26 (s, 1, C$_8H$).

Anal. Calcd. for $C_{26}H_{23}ClN_4O_5$ (506.9): C, 61.60; H, 4.57; N, 11.05. Found: C, 61.55; H, 4.49; N, 11.05.

9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)purine-6-thione (**10**).

A suspension of **4** (15.2 g, 30 mmoles) in anhydrous methanol (1.4 l) was saturated with anhydrous hydrogen sulfide gas at room temperature and heated to reflux with the exclusion of moisture. While a slow stream of hydrogen sulfide was maintained, 1*M* sodium methoxide in methanol (presaturated with hydrogen sulfide, 80 ml) was added to the refluxing solution. After refluxing for 2 hours, the flow of hydrogen sulfide was stopped and refluxing was continued for an additional 15 minutes. Methanolic sodium methoxide (1*M*, 45 ml) was added to the reaction mixture and heated under reflux for an additional 1 hour. The solvent was removed and the residue was partitioned between water and chloroform (200 ml each). The aqueous layer was separated, washed with chloroform (2 x 200 ml), filtered and the clear filtrate was rendered neutral with acetic acid and kept in the refrigerator (0-5°) overnight. The crystalline solid that separated was collected by filtration, washed with cold water (3 x 25 ml) and air-dried. Recrystallization from aqueous methanol gave 7.5 g (93%) of the title compound, mp 184-186° (lit [27] 184-186°); uv (*p*H 1): λ max 223 nm (ε 9,300), 323 (21,200); (*p*H 7): 227 nm (ε 10,500), 317 (25,000); (*p*H 11): 232 nm (ε 15,600), 310 (23,900); ^{1}H nmr (DMSO-d_6): δ 6.31 (t, 1, C$_1$'H, peak width 14.0 Hz), 8.21 and 8.50 (2s, 2, C$_2H$ and C$_8H$), 13.80 (s, 1, N$_1H$).

Anal. Calcd. for $C_{10}H_{12}N_4O_3S$ (268.3): C, 44.78; H, 4.51; N, 20.89; S, 11.93. Found: C, 44.60; H, 4.48; N, 20.82; S, 12.14.

2-Amino-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-*toluoyl*-β-D-*erythro*-pentofuranosyl)purine (**5**) and 2-Amino-6-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**7**).

A mixture of 2-amino-6-chloropurine (**2**, 0.50 g, 3 mmoles) [33] and sodium hydride (60% in oil, 0.125 g, 3.3 mmoles) in anhydrous acetonitrile (50 ml) was stirred at ambient temperature for 30 minutes under a nitrogen atmosphere. Dry, powdered **3** (1.20 g, 3.3 mmoles) was added portionwise with stirring during 5 minutes and stirring was continued for further 20 hours. A small amount of insoluble material was removed by filtration. Evaporation of the filtrate gave an oily residue, which was purified on a silica gel column (2 x 20 cm) using chloroform:methanol (98:2, 97.3, v/v) as the eluent. The following two nucleosides were isolated in the order listed: 2-Amino-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**5**) was isolated from the initial fractions and crystallized from ethanol to yield 0.77 g (51%), mp 173-175°; uv (*p*H 1): λ max 224 nm (ε 18,000), 246 (20,000), 313 (10,000); (*p*H 7): 226 nm (ε 19,000), 248 (21,000), 316 (11,000); (*p*H 11): 226 nm (ε 25,000), 247 (26,000), 313 (10,000); ^{1}H nmr (DMSO-d_6): δ 2.40 (s, 3, CH_3), 2.44 (s, 3, CH_3), 6.40 (t, 1, C$_1$'H, peak width 13.66 Hz), 7.03 (s, 2, NH_2, exchanged with deuterium oxide, 7.30-7.93 (m, 8, 2 phenyls) and 8.35 (s, 1, C$_8H$).

Anal. Calcd. for $C_{26}H_{24}ClN_5O_5$ (521.94): C, 59.83; H, 4.63; N, 13.42; Cl, 6.79. Found: C, 59.85; H, 4.55; N, 13.20; Cl, 6.84.

The N-7 glycosyl isomer 2-amino-6-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)purine (**7**) was isolated from subsequent fractions and crystallized from ethanol to yield 0.15 g (9%), mp 120-121°; uv (*p*H 1): λ max 242 nm (ε 29,600), 324 (8,300); (*p*H 7): 244 nm (ε 30,000), 326 (8,300); (*p*H 11): 244 nm (ε 36,000), 327 (8,800); ^{1}H nmr (DMSO-d_6): δ 2.41 (s, 3, CH_3), 2.43 (s, 3, CH_3), 6.70 (t, 1, C$_1$'H, peak width 13.66 Hz), 6.74 (s, 2, NH_2, exchanged with deuterium oxide), 7.20-7.92 (m, 8, 2 phenyls) and 8.72 (s, 1, C$_8H$).

Anal. Calcd. for $C_{26}H_{24}ClN_5O_5$ (521.94): C, 59.83; H, 4.63; N, 13.42; Cl, 6.79. Found: C, 59.83; H, 4.73; N, 13.12; Cl, 6.60.

2-Amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-thione (**11**).

A suspension of **5** (0.52 g, 1 mmole) in anhydrous methanol (50 ml) was saturated with dry hydrogen sulfide gas at room temperature and heated to reflux with the exclusion of moisture. While a slow stream of hydrogen sulfide was maintained, 1*M* sodium methoxide in methanol (presaturated

with hydrogen sulfide, 2.7 ml) was added to the refluxing solution. After refluxing for 2 hours, the flow of hydrogen sulfide was stopped and refluxing was continued for an additional 15 minutes. Methanolic sodium methoxide (1*M*, 1.5 ml) was added to the reaction mixture and refluxed under anhydrous condition for an additional 1 hour. The solvent was evaporated to dryness and the residue was partitioned between water and chloroform (10 ml each). The aqueous phase was separated, washed with chloroform (2 x 10 ml), filtered and the clear filtrate rendered neutral with acetic acid. After storing the solution in the refrigerator (0-5°) overnight, the crystalline solid that deposited was collected by filtration, washed with cold water (2 x 1 ml) and air-dried. Recrystallization of the solid from aqueous methanol gave 0.20 g (71%) of the title compound, mp 190-192° dec (lit [4] 190° dec); uv (*p*H 1): λ max 264 nm (ε 12,500), 346 (36,800); (*p*H 7): 259 nm (ε 13,100), 342 (39,700); (*p*H 11): 252 nm (ε 22,200), 270 (11,100), 320 (31,800); ^{1}H nmr (DMSO-d_6): δ 6.10 (t, 1, $C_1'H$, peak width 13.92 Hz), 6.85 (s, 2, NH_2, exchanged with deuterium oxide), 8.11 (s, 1, C_8H) and 12.1 (br s, 1, N_1H, exchanged with deuterium oxide).

Anal. Calcd. for $C_{10}H_{13}N_5O_3S$ (283.31): C, 42.39; H, 4.62; N, 24.72; S, 11.31. Found: C, 42.26; H, 4.74; N, 24.98; S, 11.46.

2-Amino-6-chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**12**).

A solution of **5** (0.26 g, 0.5 mmole) in methanolic ammonia (saturated at 0°, 20 ml) was allowed to stand at room temperature for 20 hours. The solvent was evaporated to dryness and the residue was purified on a flash silica gel column (1.5 x 20 cm) using dichloromethane:methanol (95:5, 90:10, v/v) as the solvent to provide 0.10 g (68%) of analytically pure **12**, mp 120° dec; uv (*p*H 1): λ max 246 nm (ε 7,500), 309 (8,800); (*p*H 7): 247 nm (ε 8,100), 306 (9,800); (*p*H 11): 246 nm (ε 8,100), 306 (9,800); ^{1}H nmr (DMSO-d_6): δ 6.22 (t, 1, $C_1'H$, peak width 13.4 Hz), 6.97 (s, 2, NH_2, exchanged with deuterium oxide) and 8.35 (s, 1, C_8H).

Anal. Calcd. for $C_{10}H_{12}ClN_5O_3$ (285.69): C, 42.04; H, 4.24; N, 24.52; Cl, 12.41. Found: C, 41.98; H, 3.99; N, 24.34; Cl, 12.16.

2-Amino-6-chloro-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**9**) and 2-Amino-6-chloro-7-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**13**).

A mixture of 2-amino-6-chloropurine (**2**, 1.01 g, 6 mmoles) and sodium hydride (60% in oil, 0.25 g, 6.25 mmoles) in anhydrous acetonitrile (50 ml) was stirred at ambient temperature for 30 minutes under a nitrogen atmosphere. A solution of 1-chloro-2,3,5-tri-*O*-benzyl-α-D-arabinofuranose (**8**) [56] [freshly prepared from 2,3,5-tri-*O*-benzyl-1-*O*-(*p*-nitrobenzyl)-D-arabinofuranose, 4.0 g, 7 mmoles] in acetonitrile (50 ml) was added dropwise. The reaction mixture was stirred at room temperature for 15 hours under a nitrogen atmosphere, and filtered to remove a small amount of insoluble material. Evaporation of the filtrate gave an oily residue, which was purified by flash silica gel column (2 x 15 cm) using chloroform:ethyl acetate (95:5, 90:10, v/v) as the solvent. The following two protected nucleosides were isolated in the order listed: 2-Amino-6-chloro-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**9**) was isolated from the initial fractions as homogeneous gum, 2.34 g (68%); uv (*p*H 1): λ max 319 nm (ε 15,200); (*p*H 7): 288 nm (ε 21,200), 312 (16,800); (*p*H 11): 314 nm (ε 14,900); ^{1}H nmr (deuteriochloroform): δ 6.32 (d, 1, J = 4.59 Hz, $C_1'H$), 6.97 (br s, 2, NH_2), 7.22-7.40 (m, 15, 3 phenyls) and 8.15 (s, 1, C_8H).

Anal. Calcd. for $C_{31}H_{30}ClN_5O_4$ (572.04): C, 65.20; H, 5.29; N, 12.20; Cl, 6.21. Found: C, 64.92; H, 5.53; N, 12.37; Cl, 6.23.

The N-7 glycosyl isomer 2-amino-6-chloro-7-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine (**13**) was isolated from subsequent fractions to yield 0.40 g (11%) as homogeneous gum; uv (*p*H 1): λ max 322 nm (ε 14,200); (*p*H 7): 326 nm (ε 17,000); (*p*H 11): 323 nm (ε 15,200); ^{1}H nmr (deuteriochloroform): δ 6.57 (d, 1, J = 4.08 Hz, $C_1'H$), 6.85 (br s, 2, NH_2), 7.17-7.40 (m, 15, 3 phenyls) and 8.40 (s, 1, C_8H).

Anal. Calcd. for $C_{31}H_{30}ClN_5O_4.H_2O$ (590.04): C, 63.05; H, 5.42; N, 11.86; Cl, 6.02. Found: C, 63.33; H, 5.46; N, 11.56; Cl, 5.92.

2-Amino-6-chloro-9-β-D-arabinofuranosylpurine (**14**).

To a solution of **9** (0.95 g, 1.66 mmoles) in dry dichloromethane (150 ml), cooled to -78° in dry ice/acetone bath under argon, was added boron trichloride (15 ml, 15 mmoles) during a period of 10 minutes. The reaction mixture was stirred at -78° for 4 hours and then at -20° to -30° for 2 hours. A mixture of methanol:dichloromethane (1:1, 50 ml) was added and stirred at -20° for 30 minutes before it was diluted with methanol (50 ml). The solution was neutralized with ammonium hydroxide and stirred at room temperature for 30 minutes. The precipitated solid was collected by filtration and washed with methanol (50 ml). The combined filtrates were evaporated to dryness. The residue was purified on a C_{18} reverse phase column using 10% aqueous methanol as the eluent. The homogeneous fractions were pooled and evaporated to dryness. The residue on crystallization from aqueous methanol gave 0.31 g (62%) of **14** as colorless crystals, mp >195° dec; ir: ν max 3200-3400 (NH_2, OH) cm^{-1}; uv (*p*H 1): λ max 220 nm (ε 27,200), 245 (7,200), 308 (8,400); (*p*H 7): 220 nm (ε 28,500), 245 (7,400), 305 (9,000); (*p*H 11): 221 nm (ε 29,200), 246 (7,500), 306 (9,100); ^{1}H nmr (DMSO-d_6): δ 6.12 (d, 1, J = 4.2 Hz, $C_1'H$), 6.96 (s, 2, NH_2) and 8.17 (s, 1, C_8H).

Anal. Calcd. for $C_{10}H_{12}ClN_5O_4$ (301.69): C, 39.81; H, 4.01; N, 23.20; Cl, 11.77. Found: C, 40.00; H, 3.98; N, 22.98; Cl, 12.05.

2-Amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)purine-6-thione (**15**).

A mixture of **9** (1.0 g, 1.75 mmoles) and thiourea (1.0 g, 13.1 mmoles) in absolute ethanol (100 ml) was heated under reflux for 3 hours. The reaction mixture was evaporated to dryness and the residue was purified by flash chromatography over silica gel using dichloromethane → ethyl acetate gradient as the eluent to give the title compound (0.85 g, 85%). An analytical sample was obtained by crystallization of the homogeneous product from a mixture of dichloromethane and acetone, mp 183-186°; ir: ν max 1250 (C=S), 3400 (NH_2) cm^{-1}; uv (methanol): λ max 204 nm (ε 34,000), 260 (6,000), 343 (21,100); ^{1}H nmr (DMSO-d_6): δ 4.52-4.62 (m, 6, 3CH_2Ph), 6.14 (d, 1, J = 4.8 Hz, $C_1'H$), 6.80-7.35 (m, 17, 3 phenyls + NH_2), 7.88 (s, 1, C_8H) and 11.96 (s, 1, N_1H).

Anal. Calcd. for $C_{31}H_{31}N_5O_4S$ (569.62): C, 65.36; H, 5.49; N, 12.29; S, 5.63. Found: C, 65.55; H, 5.46; N, 12.17; S, 5.49.

2-Amino-9-β-D-arabinofuranosylpurine-6-thione (**16**).

A mixture of **14** (7.5 g, 25 mmoles) and sodium hydrosulfide hydrate (3.0 g, 53.6 mmoles) in ethanol (350 ml) was heated under reflux for 6 hours. The reaction mixture was cooled to 0° and adjusted to *p*H 5 with 2*N* acetic acid. The precipitated solid was collected by filtration, washed with cold water (2 x 25 ml) and air-dried. Crystallization of the product from 95% aqueous ethanol gave 5.5 g (74% yield) of **16** as yellow needles, mp >250° dec (lit [8] 237-250°); ir: ν max 1580, 3200-3400 (NH_2, OH) cm^{-1}; uv (*p*H 1): λ max 208 nm (ε 15,200), 263 (5,100), 343 (13,300); (*p*H 7): 208 nm (ε 13,900), 226 (9,000), 257 (5,200), 339 (14,900); (*p*H 11): 250 nm (ε 8,100), 268 (sh) (4,400), 320 (12,000); ^{1}H nmr (DMSO-d_6): δ 5.99 (d, 1, J = 3.9 Hz, $C_1'H$), 6.62 (s, 2, NH_2), 7.94 (s, 1, C_8H) and 11.91 (s, 1, N_1H).

Anal. Calcd. for $C_{10}H_{13}N_5O_4S$ (299.31): C, 40.13; H, 4.38; N, 23.40; S, 10.71. Found: C, 40.06; H, 4.18; N, 23.19; S, 10.46.

9-β-D-Arabinofuranosylguanine (*ara*G, **17**).

A mixture of **14** (0.30 g, 1 mmole) and 2*N* sodium hydroxide solution (10 ml) in dioxane (10 ml) was heated under reflux for 6 hours. The reaction mixture was concentrated to 10 ml and diluted with 50 ml of water. The *p*H of the solution was adjusted to 6 with Dowex-50 (H^+) resin and filtered. The filtrate was evaporated to dryness. The residue was crystallized from water as needles to yield 0.20 g (71%) of **17**, mp >290° dec (lit [47] >290° dec); ir: ν max 1650 (C=O), 3200-3400 (NH_2, OH) cm^{-1}; uv (*p*H 1): λ max 255 nm (ε 11,900), 275 (sh) (8,400); (*p*H 7): 252 nm (ε 13,300), 273 (sh) (8,900); (*p*H 11): 257 nm (sh) (ε 11,600), 263 (sh) (δ 11,600); ^{1}H nmr (DMSO-d_6): δ 5.99 (d, 1, J = 4.2 Hz, $C_1'H$), 6.47 (br s, 2, NH_2), 7.76 (s, 1, C_8H) and 10.59 (br s, 1, N_1H).

Anal. Calcd. for $C_{10}H_{13}N_5O_5$ (283.24): C, 42.40; H, 4.62; N, 24.72. Found: C, 42.45; H, 4.60; N, 24.68.

9-β-D-Arabinofuranosylguanine 5'-Monophosphate (**18**).

*Ara*G (**17**, 0.50 g, 1.76 mmoles) was added with stirring to a precooled (0-5°, ice bath) mixture of freshly distilled trimethyl phosphate (8 ml) and phosphorus oxychloride (0.60 g). The temperature was monitored be-

tween 5-15°. After an hour, a clear solution was obtained, which was stored at 4° for 10 hours. The reaction mixture was poured slowly into ice-water (40 ml) containing sodium bicarbonate (0.50 g). Additional sodium bicarbonate was added periodically until the *p*H of the solution was stable at 5-6. Trimethyl phosphate was removed by extraction with ether (4 x 50 ml). Dissolved ether and excess water were removed by evaporation until salts began to crystallize. Enough water was added to achieve solution and the *p*H was checked (6-7) before application to the top of a Dowex 1 x 2 resin column (formate form, 100-200 mesh, 100 ml). The column was washed with water until no further uv-absorbing species were detected in the eluent. Gradient elution (water → 0.5 *M* formic acid) gave the product as a thick band. The appropriate homogeneous fractions were pooled and evaporated to about 25 ml. The remaining solution was frozen and lyophilized to obtain the title compound as white amorphous solid, yield 0.32 g (48%); ir: ν max 1660, 1710 (C=O), 3140-3300 (NH_2, OH) cm^{-1}; uv (*p*H 1): λ max 255 nm (ϵ 14,500), 275 (sh) (8,800); (*p*H 7): 252 nm (ϵ 13,300), 268 (sh) (10,700); (*p*H 11): 255 nm (ϵ 12,000), 265 (11,800); ^1H nmr (DMSO-d_6): δ 6.01 (d, 1, J = 4.5 Hz, C_1-*H*), 6.52 (br s, 2, NH_2) and 7.75 (s, 1, C_8-*H*).

Anal. Calcd. for $C_{10}H_{14}N_5O_6P.H_2O$ (363.24): C, 31.51; H, 4.23; N, 18.38. Found: C, 31.34; H, 4.17; N, 18.49.

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