Nucleic acid related compounds. 33. Conversions of adenosine and guanosine to 2,6-dichloro, 2-amino-6-chloro, and derived purine nucleosides[1]

MORRIS J. ROBINS and BOGDAN UZNAŃSKI[2]

Department of Chemistry, The University of Alberta, Edmonton, Alta., Canada T6G 2G2

Received March 2, 1981

MORRIS J. ROBINS and BOGDAN UZNAŃSKI. Can. J. Chem. **59**, 2601 (1981).

Enzymatic deamination of adenosine 1-*N*-oxide gave 1-hydroxyinosine (**2***a*) which was acetylated and then chlorinated to give 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**3**). Ammonia in dry 1,2-dimethoxyethane converted **3** into 2-chloroadenosine triacetate (**4***a*). Treatment of **4***a* with trimethylamine at elevated temperatures in acetonitrile resulted in formation of 2-*N*,*N*-dimethylaminoadenosine triacetate (**4***b*). Guanosine (**5**) was acetylated smoothly by an improved procedure. The resulting triacetate (**6**) was chlorinated in ~85% yield to give 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**7**). Treatment of **7** with trimethylamine at ambient temperature for 28 hours gave the 6-*N*,*N*-dimethylamino compound (**8***d*). However, potassium fluoride or sodium azide with catalytic quantities of trimethylamine in DMF or acetonitrile gave the 2-amino-6-fluoro (**8***a*) or 2-amino-6-azido (**8***b*) products in yields of greater than 90%.

MORRIS J. ROBINS et BOGDAN UZNAŃSKI. Can. J. Chem. **59**, 2601 (1981).

La désamination enzymatique du *N*-oxyde-1 d'adénosine conduit à l'hydroxy-1 inosine (**2***a*) qui par une acétylation, suivie d'une chloration donne la dichloro-2,6 (tri-*O*-acétyl-2,3,5 β-D-ribofurannosyl)-9 purine (**3**). Le composé **3** dans l'ammoniac avec le diméthoxy-1,2 éthane anhydre se transforme en triacétate de chloro-2 adénosine (**4***a*). Ce dernier, à haute température, réagit avec la triméthylamine dans l'acétonitrile pour donner le triacétate de *N*,*N*-diméthylamino-2 adénosine (**4***b*). Grâce à une méthode convenable on a acétylé facilement la guanosine (**5**). La chloration du triacétate (**6**) ainsi obtenu conduit avec un rendement de 85% à l'amino-2 chloro-6 (tri-*O*-acétyl-2,3,5 β-D-ribofurannosyl)-9 purine (**7**). Le composé **7** réagit avec la triméthylamine pendant 28 heures à la température de la pièce en donnant le composé *N*,*N*-diméthylamino-6 (**8***d*). Cependant le fluorure de potassium ou l'azoture de sodium en présence de quantités catalytiques de triméthylamine dans le DMF ou dans l'acétonitrile conduit aux composés fluoro-6 (**8***a*) ou amino-2 azido-6 (**8***b*) avec des rendements supérieurs à 90%.

[Traduit par le journal]

Potent effects on blood pressure and platelet aggregation are exerted by 2-chloroadenosine and related nucleoside derivatives (2). The cytotoxic 2-fluoroadenosine and modified sugar analogues have been studied as anticancer agents (3). Paterson and co-workers have observed recently that 6-*S*-(*p*-nitrobenzyl)thioinosine, a strong competitive inhibitor of nucleoside transport across cell membranes, exerts protection against toxic effects of certain nucleoside antibiotics and anticancer agents in animals (4). This observation stimulated our interest in evaluation of host protection against toxicity of 2-halopurine nucleosides. We have explored alternative routes to 2- and 6-chloro 2,6-disubstituted purine nucleosides from adenosine and guanosine. We now describe improved syntheses of acetyl-protected 6-amino-2-chloro- and 2-amino-6-chloropurine ribonucleosides. These have been converted to useful related 2 and 6 disubstituted compounds by nucleophilic replacements.

Syntheses of 2-halopurine nucleosides usually have employed classical methods of coupling a purine base with an activated carbohydrate derivative (5). The Fischer–Helferich condensation of the silver salt of 2,8-dichloroadenine with tetraacetylglucosyl bromide (6) was the original prototype for synthesis of the naturally occurring purine ribonucleosides (7). Nucleophilic replacements and hydrolytic diazotizations at C-2 and C-6 provided the natural amino and oxo substituents. Unwanted halo groups were removed reductively. More recent coupling methods have employed 2 and 6 disubstituted purines to give dihalo and aminohalo nucleosides (5,8). Montgomery and co-workers have used 2,6-dichloro- (**3***b*) and 2,6-diacetamidopurine (9) in coupling reactions followed by transformations to give fluoropurine nucleosides. Robins and co-workers have performed 6-chloro- to 6-fluoropurine conversions using silver fluoride in hot xylene (10). They also have utilized diazotization in aqueous acid for the introduction of chloro and fluoro groups (11). Kiburis and Lister have reported displacement of trimethylamine from 6-*N*-purinyltrimethylammonium chloride salts using potassium fluoride (12), a procedure applied successfully by ourselves for the synthesis of the 6-fluoropurine 2′-deoxyribonucleoside (13).

Owing to the high cost and limited availability of

[1]For the previous paper in this series see ref. 1.

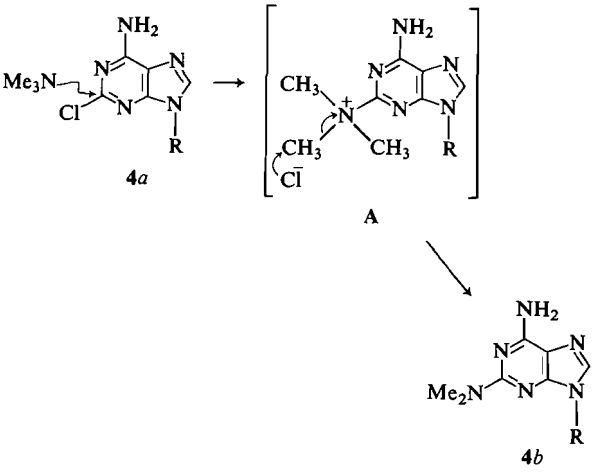
[2]Postdoctoral Fellow 1979–1980. On leave from the Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, 90–362 Łódź, Boczna 5, Poland.

0008-4042/81/172601-07$01.00/0

2,6-dichloropurine plus the yield and isomer considerations involved in base–sugar coupling methods (5,9), we opted for transformations of naturally occurring nucleosides (14). A sequence for conversion of adenosine (**1**) to 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**3**) had been reported (15). Oxidation of **1** with *m*-chloroperoxybenzoic acid in aqueous acetic acid/dioxane gave adenosine 1-*N*-oxide in 96% yield.[3] Kawashima and Kumashiro used chemical deamination of this product to give 1-hydroxyinosine (**2***a*) in 56% crude yield (15). However, we employed adenosine deaminase to effect this conversion cleanly in 88% crystallized yield without formation of colored by-products. Acetylation of **2***a* to the tetraacetate (**2***b*) followed by treatment with aqueous pyridine gave 2′,3′,5′-tri-*O*-acetyl-1-hydroxyinosine (**2***c*). Phosphoryl chloride in hot 2-picoline converted **2***c* → **3** in 73% yield. With these improvements, the overall yield from **1** → **3** was 61%.[4]

Treatment of **3** with anhydrous ammonia in dry 1,2-dimethoxyethane (DME) effected nucleophilic replacement of the 6-chloro function without ammonolysis of the ester groups.[5] The resulting 2-chloroadenosine triacetate (**4***a*) was treated with trimethylamine in acetonitrile at elevated temperatures in a sealed pressure vessel. The sole nucleoside product isolated was 2-*N*,*N*-dimethylaminoadenosine triacetate (**4***b*). Carefully purified (dimethylamine-free) trimethylamine neat or in acetonitrile also converted **4***a* → **4***b*. The presumed intermediate 2-*N*-purinyltrimethylammonium chloride salt (**A**) was not observed. Temperatures required to effect displacement of the 2-chloro function from **4***a* apparently result in the facile displacement of amine nitrogen by attack of chloride on a methyl carbon of **A** to give **4***b*. Attempted *in situ* generation of **A** from **4***a* using excess potassium fluoride in the presence of trimethylamine again resulted in formation of **4***b*.[6] However, the *in situ* procedure functions smoothly at C-6 (*vide infra*).

A simple acetylation of guanosine (**5**) was reported in 1947 (19). However, this often quoted preparation employed inconvenient quantities of acetic anhydride and pyridine and can give rigid suspensions of incompletely acetylated products in larger scale work. An improved procedure that utilizes acetic anhydride and pyridine in *N*,*N*-dimethylformamide (DMF) to give clear solutions and yields of ~87% of 2′,3′,5′-tri-*O*-acetylguanosine (**6**) is described in the Experimental.



Chlorination of **6** has been studied previously (20) and improved procedures have been reported (21). However, the reaction conditions, especially heating, were difficult to duplicate with different sized preparations. Decomposition occurred in the heated mixtures and our yields of the crystalline 2-amino-6-chloro product (**7**) using those procedures seldom exceeded 50%.

We have examined this reaction utilizing ^{31}P nmr spectroscopy. A reference sample of $POCl_3$ in acetonitrile gave a broad ^{31}P singlet at 4.18 ppm from external H_3PO_4. Chlorination reaction conditions were simulated in an nmr tube containing phosphoryl chloride, *N*,*N*-dimethylaniline, and **6** in acetonitrile in a probe heated at 70°C. A second ^{31}P signal (sharp singlet) at −1.17 ppm (upfield from H_3PO_4) was observed immediately. A third singlet at −7.9 ppm increased steadily over a period of one hour. During this time, the peak at −1.17 ppm diminished to an insignificant intensity. Several aromatic phosphorodichloridates give ^{31}P singlets in the range of 1 to −2.5 ppm (22). A sample of dichlorophosphoric acid in acetonitrile gave a ^{31}P signal in the range of −2.96 to −8.85 depending on the amount of *N*,*N*-dimethylaniline present.

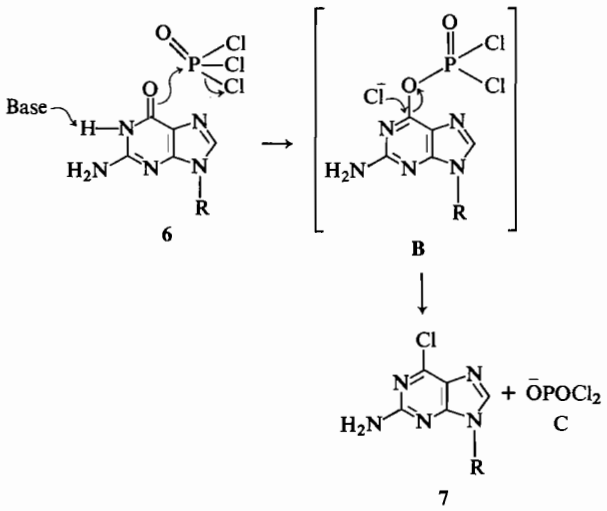
These data are compatible with phosphorylation of **6** to give the aromatic phosphorodichloridate intermediate (**B**) followed by attack of chloride at C-6 of **B** to give **7** plus dichlorophosphate (**C**). A higher concentration of external chloride anions should favor conversion of **B** → **7** before decompo-

[3]This modification of the procedures of Brown and MacCoss and their co-workers (16) will be published as part of an unrelated study with Dr. F. Hansske.

[4]An alternative synthesis was developed which requires only three steps from guanosine (**5**) and gives **3** in 64% overall yield (17).

[5]Ammonia in alcohol or liquid ammonia, used without drying, effects concomitant ammonolysis of the ester groups (18).

[6]An alternative approach employing nonaqueous diazotization has been developed for convenient fluorination at C-2 (17).



sition occurred. Indeed, addition of tetraethylammonium chloride to an identically prepared nmr experiment resulted in disappearance of the intermediate ^{31}P singlet at -1.49 ppm within 10 minutes. A singlet at -10.65 ppm increased steadily over the 10 minute period. Use of analogous carefully anhydrous reaction conditions described in the Experimental gave readily repeatable yields of ~85% of crystalline **7**.

Treatment of **7** with excess trimethylamine at room temperature for an extended time resulted in formation of the 6-*N*,*N*-dimethylamino derivative (**8***d*). A fluorescent salt-like product was formed with complete disappearance of **7** (tlc) within one hour at room temperature. Treatment of this hygroscopic solid with potassium fluoride in DMF at elevated temperature resulted in formation of a mixture of 2-amino-6-fluoro (**8***a*) and 2-amino-6-chloro (**7**) nucleosides. However, treatment of **7** with a catalytic quantity of trimethylamine and excess potassium fluoride in DMF at room temperature resulted in clean conversion to **8***a* in 93% yield.

Analogous conversion of **7** to **8***b* was effected quantitatively using sodium azide in acetonitrile with trimethylamine as catalyst. The nmr spectra of **8***b* were compatible with a 6-azido ⇄ cyclic tetrazole equilibrium (23). This phenomenon was not investigated further since smooth hydrogenolysis of **8***b* occurred to give 2-aminoadenosine triacetate (**8***c*) in 98% crude yield overall from **7**.

The present improved syntheses of **6** and **7** allow convenient and high yield preparation of the useful intermediates **7** and **8***a*–*c*. The solid-solution phase transfer catalysis employed in the conversions of **7** → **8***a* or **8***b* is a useful improvement over the stoichiometric quaternary salt procedure of Lister (12). The soluble nucleoside **7** reacts with trimethylamine to give the quaternary ammonium salt. Transfer of chloride to the solid phase as its less soluble sodium or potassium salt occurs concomitantly with anion exchange for azide or fluoride. Nucleophilic replacement at C-6 by the solubilized anion releases trimethylamine for the catalytic cycle. The ambient temperature allows selective displacement at C-6 without competing formation of the 6-*N*,*N*-dimethylamino by-product (**8***d*). However, this catalytic process did not function successfully at C-2. The reduced reactivity at the 2-position results in favored nucleophilic attack at the methyl carbon of the quaternary salt leading to formation of the 2-*N*,*N*-dimethylamino compound (**4***b*) as the exclusive product. Therefore, nonaqueous diazotization reactions have been developed to provide convenient access to the 2-halopurine nucleosides (17).

Experimental

General

Melting points were obtained on a Reichert microstage block and are uncorrected. Ultraviolet (uv) spectra were recorded using a Cary 15 spectrophotometer. The ^1H nmr spectra were determined using Bruker 90 or Varian 100-MHz spectrometers with Me_4Si as internal reference and $CDCl_3$ as solvent unless Me_2SO-d_6 is specified. Acetyl methyl singlets and base proton peaks are given. Sugar proton multiplets were as expected. The ^{19}F nmr spectra were determined in $CDCl_3$ unless Me_2SO-d_6 is specified. ^{19}F chemical shifts are expressed in δ ppm upfield (minus sign) from CCl_3F as internal reference. Mass spectra (ms) were measured at 70 eV with an AEI MS-50 instrument with coupled computer analysis using direct probe sample introduction at 150–230°C by the mass spectral laboratory of this department. The molecular ion (M^+), 2,3,5-tri-*O*-acetyl-D-ribofuranosyl ion ($M^+ - B = C_{11}H_{15}O_7 = 259.0818$), and the substituted purinyl ion (B) or a protonated species (BH or BH_2) are given with their % relative intensities if observed in the spectrum.

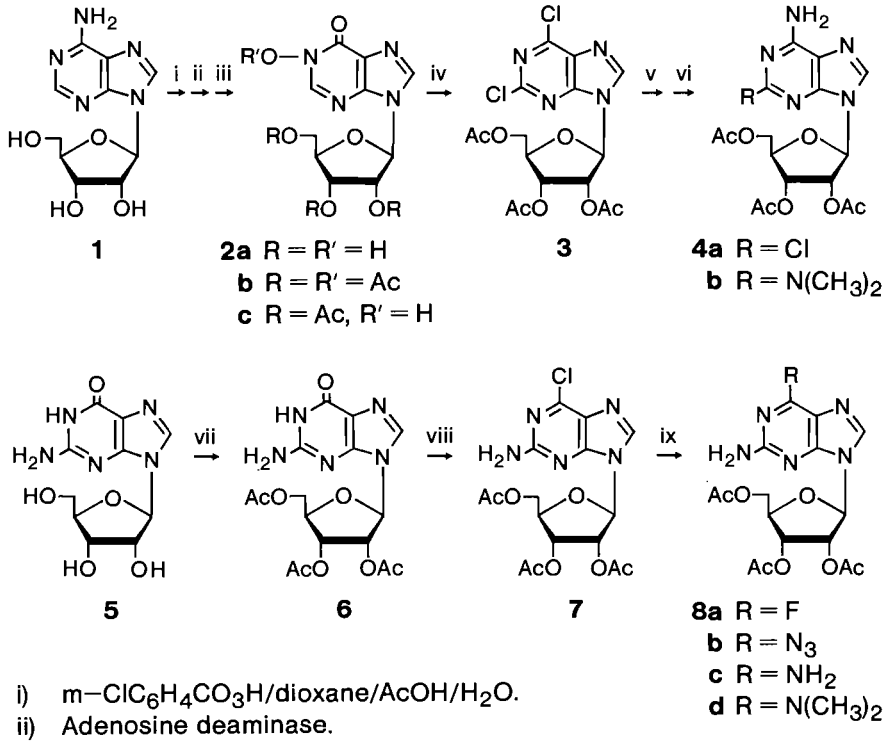
Trimethylamine was purified by heating with acetic anhydride, distillation *in vacuo*, and storage over CaH_2. Reagent grade acetonitrile and DMF were heated with and then distilled directly *in vacuo* from P_2O_5 into a flame-dried reaction flask assembly to provide rigorously anhydrous solvents. Chloroform, pyridine, 1,2-dimethoxyethane, 1,4-dioxane, dibromomethane, dichloromethane, 1,2-dichloroethane, and methanol were distilled and stored over drying agents. Silica refers to Mallinckrodt CC-7 silica gel for chromatography.

Evaporations were effected wtih a Buchler rotary evaporator equipped with a Dewar Dry-Ice condenser at mechanical oil pump (*in vacuo*) or water aspirator vacuum at room temperature.

1-Hydroxyinosine (2a)

Adenosine deaminase (100 mg, Sigma type II, crude from calf intestinal mucosa) was added to a solution of 1.0 g (3.5 mmol) of adenosine 1-*N*-oxide (16) in 200 mL of 0.05 *M* $NaH_2PO_4/Na_2HPO_4/H_2O$ buffer at pH 7.5. The solution was allowed to stand for 3 days at room temperature and was then evaporated to ~50 mL *in vacuo*. This concentrate was applied to a column of 120 mL of Amberlite IR-120 (H^+) resin and the column was developed with

Reaction Scheme



i) m−$ClC_6H_4CO_3H$/dioxane/AcOH/H_2O.
ii) Adenosine deaminase.
iii) Ac_2O/Pyridine, then H_2O.
iv) $POCl_3$ /2-picoline.
v) NH_3 / DME.
vi) $Me_3N/CH_3CN/\Delta$.
vii) Ac_2O/Pyridine/DMF.
viii) $POCl_3$ /$PhNMe_2$ /Et_4NCl/MeCN.
ix) a) KF/Me_3N/DMF.
b) NaN_3 /Me_3N/CH_3CN followed by
c) H_2 /Pd·C.
d) NMe_3 /CH_3CN.

SCHEME 1

H_2O. Appropriate fractions (~3L, uv detector) were pooled and evaporated *in vacuo*. The residue was crystallized from 95% EtOH to give 875 mg (88%) of colorless crystals of **2***a*, mp ~210°C dec.; uv (0.1 *N* HCl) max:251 nm (ε 9500); (0.1 *N* NaOH) max:294, 256, 228 nm (ε 4200, 6800, 29 700); ms *m*/*z*:284.0760 (3.5%, M^+ [$C_{10}H_{12}N_4O_6$] = 284.0756), 152.0331 (100%, BH = 152.0334). Literature mp > 200°C dec (15); > 400°C with decomposition above 170°C (24); lit. uv (pH 3) max:251 nm (ε 9100); (pH 9) max:294, 256, 229 nm (ε 4000, 6300, 30 000) (24).

2′,3′,5′-Tri-O-acetyl-1-hydroxyinosine (2c)

A 1 g sample of adenosine 1-*N*-oxide was deaminated by stirring with adenosine deaminase in 100 mL of pH 7.5 buffer for 35 h at room temperature. A 50 mL portion of 1-butanol was added and the mixture was evaporated *in vacuo*. The residue was dried for 15 h at 0.1 Torr and then 10 mL of pyridine and 20 mL of Ac_2O were added. The mixture was sonicated for 1 h and then stirred at room temperature for 9 h. The mixture was cooled and 50 mL of 1-butanol was added. The volume was reduced to ~30 mL and 3 successive portions of 50 mL of 1-butanol were added and evaporated *in vacuo*. Chloroform (50 mL) was added to the heavy suspension which was filtered using a membrane filter. This filtrate contained 2′,3′,5′-tri-*O*-acetyl-1-*N*-acetyloxyinosine (**2***b*) as indicated by four ^1H nmr acetyl methyl singlets at δ 2.10, 2.12, 2.14, and 2.45 in addition to the other expected proton signals. Compound **2***b* gave ms *m*/*z*:452 (3.6%, M^+ [$C_{18}H_{20}N_4O_{10}$] = 452), 259 (100%, M^+ − B) at low resolution at 200°C and 17 eV.

The filtrate was evaporated and the residual **2***b* was dissolved in 1,4-dioxane (40 mL), pyridine (5 mL), and water (2 mL). The resulting solution was stirred for 48 h at room temperature to ensure complete hydrolysis of the 1-*N*-acetyloxy function. Addition of 50 mL of 1-butanol was followed by slow evaporation of solvents to ~30 mL, during which crystallization of product occurred. The crystals were filtered, washed with 2-propanol, and dried *in vacuo* to give 1.12 g of **2***c*. The filtrate

was concentrated to ~1 mL and a further 138 mg of product was collected to give 1.26 g (88%) of **2***c*, mp 194–195°C (mp values of 203–205°C and 206–210°C also were observed depending on the rate of heating); lit. (15) mp 194.5–195.5°C; uv (0.1 *N* HCl/MeOH 9:1) max:251 nm (ε 9000); (0.1 *N* NaOH/MeOH 9:1) max:294, 256 nm (ε 4400, 6200); (MeOH) max:270 (sh), 251, 245 nm (ε 4100, 7600, 7600); ^{1}H nmr δ:2.08, 2.12, 2.14 (OAc's), 8.01 (s, 1, H-2), 8.40 (s, 1, H-8); ms *m/z*:410.1084 (2.3%, M^+ $[C_{16}H_{18}N_4O_9]$ = 410.1074, 259.0818 (100%, M^+–B), 153.0412 (14%, BH_2 = 153.0412).

*9-(2,3,5-Tri-*O*-acetyl-*β*-*D*-ribofuranosyl)-2,6-dichloropurine (**3**)*

A mixture of 0.9 g (2.2 mmol) of **2***c*, 3 mL of dried 2-picoline, and 10 mL of freshly distilled phosphoryl chloride was heated at reflux for 1 h. The red-brown solution was evaporated and the brown oily residue was dissolved in 10 mL of $CHCl_3$ and applied to a column of 30 mL of silica. The product was eluted with acetone/chloroform/cyclohexane 2:3:1. The orange eluate was evaporated and the residue again dissolved in 10 mL of $CHCl_3$ and applied to a layered column (1.5 cm dia) of 0.5 cm of silica, 5 cm of decolorizing carbon, and 1 cm of silica. The product was eluted with $CHCl_3$ and the 200 mL of eluate was evaporated. Addition of 3 mL of 1-butanol and partial evaporation resulted in separation of crystals which were filtered, washed with cold ether, and dried *in vacuo* to give 716 mg (73%) of **3**, mp 162–163°C; lit. (15) mp 160–161°C; uv (MeOH) max:273, 252 nm (ε 9100, 5100); ^{1}H nmr δ:2.04, 2.09, 2.12 (OAc's), 8.28 (s, 1, H-8); ms *m/z*:446.0392 (1.1%, M^+ $[C_{16}H_{16}Cl_2N_4O_7]$ = 446.0396), 259.0823 (100%, M^+–B).

*2',3',5'-Tri-*O*-acetyl-2-chloroadenosine (**4**a)*

Dry ammonia gas (passed through a drying tube packed with CaH_2) was bubbled slowly through a solution of 1.0 g (2.2 mmol) of **3** (dried at 60°C *in vacuo*) in 20 mL of dry 1,2-dimethoxyethane (distilled directly from NaH into the dried reaction flask) at room temperature for 8 h. The solution was evaporated and the colorless residue was treated with 10 mL of $CHCl_3$. This suspension was applied to a column of silica (50 g, 2 × 45 cm). Chloroform/acetone with an increasing gradient of acetone was used for elution. The appropriately pooled fractions were evaporated and the resulting colorless solid foam was dissolved in chloroform/2-propanol. The product crystallized upon slow evaporation of the solution. It was filtered, washed with ether, and dried to give 857 mg (91%) of **4***a*, mp 151–152°C; uv (MeOH) max:263 nm (ε 15 500); ^{1}H nmr δ:2.11, 2.17, 2.18 (OAc's) 7.97 (s, 1, H-8); ms *m/z*:427.0906 (6.1%, M^+ $[C_{16}H_{18}ClN_5O_7]$ = 427.0895), 259.0823 (100%, M^+–B). The corresponding deacylated nucleoside has a reported uv (pH 7) max:264 nm (ε 15 200) (8*e*).

*2',3,5'-Tri-*O*-acetyl-2-*N,N*-dimethylaminoadenosine (**4**b)*

A sealed ampule containing 130 mg (0.3 mmol) of **4***a* (dried *in vacuo* at 100°C for 2 h), 350 mg (6 mmol) of purified trimethylamine, and 1 mL of rigorously dried acetonitrile was heated at 100°C for 6 h inside a steel pressure bomb. After cooling to room temperature the ampule was opened and the mixture was filtered through a short column (1 × 1 cm) of silica. The column was washed with 50 mL of $CHCl_3$ and the combined eluate was evaporated to dryness. The residue was crystallized from 1-propanol, washed with ether, and dried to give 120 mg (92%) of **4***b*, mp 169–170°C; uv (MeOH) max:292, 264, 228 nm (ε 9000, 13 000, 22 400); (0.1 *N* HCl/MeOH 8:2) max:306, 260 nm (ε 9000, 16 400); (0.1 *N* NaOH/MeOH 8:2) max:294, 262 nm (ε 8400, 12 800); ^{1}H nmr δ:2.06, 2.12, 2.13 (OAc's), 3.16 (s, 6, NMe_2), 7.52 (s, 1, H-8); ms *m/z*:436.1711 (47%, M^+ $[C_{18}H_{24}N_6O_7]$ = 436.1706), 259.0817 (6.1%, M^+–B), 178.0964 (100%, BH = 178.0966). The corresponding deacylated nucleoside has reported uv (pH 1) max:305, 261 nm (ε 8760, 16 200); (pH 13) max:295, 262 nm (ε 8320, 12 500) (8*c*).

*2',3',5'-Tri-*O*-acetylguanosine (**6**)*

A mixture of 14.2 g (0.05 mol) of guanosine (**5**) (pre-dried *in vacuo* at 100°C for 2 days over P_2O_5), 30 mL of acetic anhydride, 15 mL of dry pyridine, and 40 mL of DMF was heated at 75°C with stirring for 3.75 h. The resulting clear solution was filtered while hot, cooled to room temperature, and evaporated *in vacuo* to a heavy crystalline suspension. The residue was treated with 50 mL of 2-propanol and filtered. The filter cake was washed with 2-propanol and dried to give 20.9 g (94%) of white crystals of **6** as the hemi-solvate (^{1}H nmr δ 2.82 (d, 3, 0.5 DMF)). This product was added to 750 mL of boiling 2-propanol with vigorous magnetic stirring. The partially dissolved suspension was stirred at reflux for 5 min and then was refrigerated at −5°C overnight. The product was filtered, washed with 2-propanol, and dried *in vacuo* at 80°C over P_2O_5 to give 17.88 g (87.4%) of tlc homogeneous **6**, mp ~230–233°C (softening from 195°C); lit. (19) mp 232°C; uv (MeOH) max:256 nm (ε 14 500); ^{1}H nmr (Me_2SO-d_6) δ:2.04 (s, 6, two OAc's), 2.10 (s, 3, OAc), 6.5 (s, 2, NH_2), 7.96 (s, 1, H-8).

*9-(2,3,5-Tri-*O*-acetyl-*β*-*D*-ribofuranosyl)-2-amino-6-chloropurine (**7**)*

Acetonitrile (50 mL) was distilled directly from P_2O_5 into a flask containing 10.23 g (0.025 mol) of pre-dried **6** and 8.3 g (0.05 mol) of Et_4NCl (pre-dried *in vacuo* at 85°C overnight over P_2O_5). *N,N*-Dimethylaniline (3.2 mL, 0.025 mol, dried over and distilled from CaH_2) and phosphoryl chloride (13.7 mL, 0.15 mol, freshly distilled) were added to the stirred solution at room temperature. The flask was placed in an oil bath preheated to 100°C and the solution was heated with stirring at reflux for 10 min. Volatile materials were flash evaporated immediately *in vacuo*. The resulting yellow foam was dissolved in 150 mL of $CHCl_3$ and stirred vigorously with crushed ice for 15 min. The layers were separated and the aqueous phase was extracted with 5 × 50 mL of $CHCl_3$. The combined organic phase was kept cold by addition of crushed ice. It was washed with 6 × 30 mL of cold water, 5% $NaHCO_3/H_2O$ to pH ~7, dried over $MgSO_4$ for 1 h, and filtered. A 60 mL portion of 2-propanol was added and the combined filtrate was slowly evaporated *in vacuo* to ~40 mL. The crystalline product that had separated was filtered, washed with 2-propanol (20 mL), and dried *in vacuo* overnight to give 9.31 g (87%) of crude **7**. This product was recrystallized from 90 mL of boiling 2-propanol to give 9.03 g (85%) of purified **7**, mp 152–153°C; lit. (20) mp 147.5–148.5°C; uv (MeOH) max:311, 249 nm (ε 7700, 7800); lit. (20) uv (EtOH) max:310, 249 nm (ε 7900, 8600); ^{1}H nmr δ:2.06, 2.08, 2.14 (OAc's), 5.70 (br, 2, NH_2), 7.94 (s, 1, H-8).

*9-(2,3,5-Tri-*O*-acetyl-*β*-*D*-ribofuranosyl)-2-amino-6-*N,N*-dimethylaminopurine (**8**d)*

A solution of 214 mg (0.5 mmol) of **7** (dried for 2 days over P_2O_5 in a desiccator) and 200 mg (3.4 mmol) of purified Me_3N in 4 mL of rigorously dried acetonitrile was allowed to stand at room temperature for 28 h in a sealed flask. The solution was evaporated to dryness. The residue was treated with 2 mL of $CHCl_3$ and applied to a silica column (1 × 5 cm) which was eluted with 100 mL of $CHCl_3$. The eluate was evaporated to a heavy syrup which crystallized upon exposure to ether overnight in a sealed desiccator (diffusion crystallization). The crystals were filtered, washed with ether, and dried to give 192 mg (88%) of **8***d*, mp 127–128°C; uv (MeOH) max:285, 267 (sh), 230 nm (ε 14 200, 10 600, 20 200); (0.1 *N* HCl/MeOH 8:2) max:300, 257 nm (ε 13 000, 12 000); (0.1 *N* NaOH/MeOH 8:2) max:284, 267 (sh) nm (ε 14 600, 10 400); ^{1}H nmr δ:2.05, 2.08, 2.10 (OAc's), 3.40 (s, 6, NMe_2), 4.67 (br s, 2, NH_2), 7.55 (s, 1, H-8); ms *m/z*:436.1710 (55%, M^+ $[C_{18}H_{24}N_6O_7]$ = 436.1706),259.0818 (5.7%, M^+–B), 178.0962 (100%, BH = 178.0966). The corres-

ponding deacylated nucleoside has reported uv (H_2O) max:284, 268 (sh), 228 nm (ε 15 600, 11 500, 19 100) (25).

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-fluoropurine (8a)

A suspension of 4 g (69 mmol) of KF (dried *in vacuo* over a cold flame for 0.5 h) in a solution of 127 mg (2.1 mmol) of purified Me_3N (distilled directly into the flask *in vacuo* from CaH_2) and 2 g (4.7 mmol) of dried **7** in 45 mL of DMF (distilled directly into the flask *in vacuo* from CaH_2) was stirred vigorously at room temperature for 14 h. The mixture was evaporated to dryness *in vacuo*, the residue was treated with 50 mL of $CHCl_3$, and the suspension was filtered using a membrane filter. The filtrate was evaporated partially and 20 mL of 1-propanol was added. This was evaporated partially and a second 20 mL of 1-propanol was added. Evaporation was continued to ~10 mL. The separated crystalline product was filtered and washed with 1-propanol and then ether to give 1.78 g of **8***a*. An additional 55 mg of product was recovered from the filtrate. The combined product was recrystallized from 14 mL of boiling 2-propanol to give 1.78 g (93%) of purified **8***a*, mp 151–152°C; lit. (10) mp 144–146°C; uv (MeOH) max:289, 245 nm (ε 6800, 10 800); lit (10) uv (95% EtOH) max:289, 246 nm (ε 7000, 11 100); ^{1}H nmr δ:2.06, 2.07, 2.13 (OAc's), 5.30 (br s, 2, NH_2), 7.84 (d, $^5J_{H\text{-}8\text{-}F\text{-}6}$ = 0.45 Hz, 1, H-8); ^{19}F nmr (Me_2SO-d_6/$CDCl_3$) δ:−71.6 (s, 1, F-6); ms *m/z*:411.1190 (18%, M^+ [$C_{16}H_{18}FN_5O_7$] = 411.1190), 259.0822 (100%, M^+–B), 153.0457 (22%, BH = 153.0451).

9-(2,3,5,-Tri-O-acetyl-β-D-ribofuranosyl)-2-amino-6-azidopurine (8b)

A. A suspension of 0.5 g (7.7 mmol) of NaN_3 (dried *in vacuo* at room temperature for 3 days over P_2O_5) in a solution of 1 g (2.3 mmol) of dried **7** and 0.1 g (1.7 mmol) of purified Me_3N in 30 mL of rigorously dried acetonitrile was stirred at room temperature overnight. The mixture was evaporated *in vacuo*, the residue was treated with 30 mL of $CHCl_3$, and the suspension was applied to a column of silica (1 × 3 cm). The column was eluted with $CHCl_3$, the eluate was evaporated to dryness, and the residue was dissolved in 40 mL of 1,4-dioxane. This solution was frozen and lyophilized to give 1.09 g (quantitative) of a photosensitive white powder. The ^{1}H nmr spectrum of this powder indicated an equilibrium of two products (6-azido⇄ cyclic tetrazole) (23). Compound(s) **8***b* had uv (MeOH) max: 299, 271, 238 nm; ^{1}H nmr δ:7.78 (s, 0.25, H-8), 7.95 (s, 0.75, H-8); ms *m/z*:434.1299 (13%, M^+ [$C_{16}H_{18}N_8O_7$] = 434.1299), 259.0814 (72%, M^+–B), 176.0532 (2.4%, BH = 176.0559). This material was hydrogenolyzed to a single product.

B. Alternatively, this reaction can be completed in 1.5 h at room temperature using 0.6 g (10.2 mmol) of Me_3N and 1.0 g (15.4 mmol) of NaN_3 with 0.5 g (1.2 mmol) of **7** in 30 mL of acetonitrile.

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2,6-diaminopurine (8c)

A solution of 1.46 g (3.4 mmol) of **8***b* in 50 mL of dry 1,4-dioxane was hydrogenolyzed at 30 psi with 0.5 g of 5% Pd/C catalyst at room temperature for 18 h in a Parr shaker. The mixture was filtered through a short silica column (2 × 1 cm) and the column was washed with acetone/$CHCl_3$ 1:1. The combined filtrate was evaporated to dryness and the tlc pure solid foam was dried *in vacuo* for 2 days to give 1.35 g (98%) of **8***c*. A crystalline sample was obtained by diffusion of ether into a solution of **8***c* in 1,4-dioxane. This sample had mp 105–106°C; uv (MeOH) max:282, 257 nm (ε 9400, 10 000); ^{1}H nmr δ:2.10 (s, 6, two OAc's), 2.14 (s, 3, OAc), 5.02 (br s, 2, 2-NH_2), 5.9 (br s overlapped by sugar protons, 2, 6-NH_2), 7.64 (s, 1, H-8); ms *m/z*:408.1385 (28%, M^+ [$C_{16}H_{20}N_6O_7$] = 408.1393), 259.0809 (M^+–B), 150.0647 (100%, BH = 150.0654). This compound has been reported previously as an amorphous intermediate (26). The corresponding deacylated nucleoside has reported uv (H_2O) max:280, 256 nm (ε 11 100, 10 700) (25).

Acknowledgements

We thank the Natural Sciences and Engineering Research Council of Canada (A5890), the National Cancer Institute of Canada, and the University of Alberta for generous financial support.

1. M. J. Robins and J. S. Wilson. J. Am. Chem. Soc. **103**, 932 (1981).
2. (*a*) G. V. R. Born, R. J. Haslam, M. Goldman, and R. D. Lowe. Nature, **205**, 678 (1965); (*b*) J. A. Angus, L. B. Cobbin, R. Einstein, and M. H. Maguire. Brit. J. Pharmacol. **41**, 592 (1971).
3. (*a*) J. A. Montgomery and K. Hewson. J. Am. Chem. Soc. **79**, 4559 (1957); (*b*) J. Med. Chem. **12**, 498 (1969); (*c*) R. W. Brockman, F. M. Schabel, Jr., and J. A. Montgomery. Biochem. Pharmacol. **26**, 2193 (1977).
4. (*a*) A. R. P. Paterson, S. Yang, E. Y. Lau, and C. E. Cass. Mol. Pharmacol. **16**, 900 (1979); (*b*) A. R. P. Paterson, J. H. Paran, S. Yang, and T. P. Lynch. Cancer Res. **39**, 3607 (1979).
5. L. Goodman. *In* Basic principles in nucleic acid chemistry. Vol. I. *Edited by* P. O. P. Ts'o. Academic Press, New York. 1974. pp. 93–208.
6. E. Fischer and B. Helferich. Chem. Ber. **47**, 210 (1914).
7. J. Davoll, B. Lythgoe, and A. R. Todd. J. Chem. Soc. 967 (1948); 1685 (1948).
8. (*a*) J. Davoll and B. A. Lowy. J. Am. Chem. Soc. **74**, 1563 (1952); (*b*) G. B. Brown and V. S. Weliky. J. Org. Chem. **23**, 125 (1958); (*c*) H. J. Schaeffer and H. J. Thomas. J. Am. Chem. Soc. **80**, 3738 (1958); (*d*) T. Sato, T. Shimadate, and Y. Ishido. Nippon Kagaku Zasshi, **81**, 1440 (1960); **81**, 1442 (1960); Chem. Abstr. **56**, 11692g (1962); (*e*) J. A., Montgomery and K. Hewson. J. Heterocycl. Chem. **1**, 213 (1964); (*f*) W. W. Lee, A. P. Martinez, L. Goodman, and D. W. Henry. J. Org. Chem. **37**, 2923 (1972); and references therein.
9. J. A. Montgomery, S. D. Clayton, and A. T. Shortnacy. J. Heterocycl. Chem. **16**, 157 (1979).
10. J. F. Gerster, A. G. Beaman, and R. K. Robins. J. Med. Chem. **6**, 340 (1963).
11. (*a*) J. F. Gerster and R. K. Robins. J. Am. Chem. Soc. **87**, 3752 (1965); (*b*) J. Org. Chem. **31**, 3258 (1966).
12. J. Kiburis and J. H. Lister. J. Chem. Soc. C, 3942 (1971).
13. M. J. Robins and G. L. Basom. Can. J. Chem. **51**, 3161 (1973).
14. M. J. Robins. *In* Bioorganic chemistry. Vol. III. Macro- and multimolecular systems. *Edited by* E. E. van Tamelen. Academic Press, New York. 1977. Chapt. 11.
15. H. Kawashima and I. Kumashiro. Bull. Chem. Soc. Jpn. **40**, 639 (1967).
16. (*a*) M. A. Stevens, D. I. Magrath, H. W. Smith, and G. B. Brown. J. Am. Chem. Soc. **80**, 2755 (1958); (*b*) M. MacCoss, E. K. Ryu, R. S. White, and R. L. Last. J. Org. Chem. **45**, 788 (1980).
17. M. J. Robins and B. Uznański. Can. J. Chem. **59**, 2608 (1981).
18. L. F. Christensen, A. D. Broom, M. J. Robins, and A. Bloch. J. Med. Chem. **15**, 735 (1972).
19. H. Bredereck and A. Martini. Chem. Ber. **80**, 401 (1947).
20. J. F. Gerster, J. W. Jones, and R. K. Robins. J. Org. Chem. **28**, 945 (1963).

21. (*a*) J. F. GERSTER, A. F. LEWIS, and R. K. ROBINS. *In* Synthetic procedures in nucleic acid chemistry. Vol. 1. *Edited by* W. W. Zorbach and R. S. Tipson. Wiley-Interscience, New York. 1968. pp. 242–243; (*b*) S. J. MANNING and L. B. TOWNSEND. *In* Nucleic acid chemistry: improved and new synthetic procedures, methods, and techniques. Part 2. *Edited by* L. B. Townsend and R. S. Tipson. Wiley-Interscience, New York. 1978. pp. 589–594.
22. M. M. CRUTCHFIELD, C. H. DUNGAN, J. H. LETCHER, V. MARK, and J. R. VAN WAZER. ^{31}P Nuclear magnetic resonance; topics in phosphorus chemistry. Vol. 5. Wiley-Interscience, New York. 1967. p. 287.
23. J. A. JOHNSON, JR., H. J. THOMAS, and H. J. SCHAEFFER. J. Am. Chem. Soc. **80**, 699. (1958).
24. J. C. PARHAM, J. FISSEKIS, and G. B. BROWN. J. Org. Chem. **31**, 966 (1966).
25. T. NAITO, K. UENO, and F. ISHIKAWA. Chem. Pharm. Bull. **12**, 951 (1964).
26. J. A. MONTGOMERY and K. HEWSON. J. Org. Chem. **33**, 432 (1968).