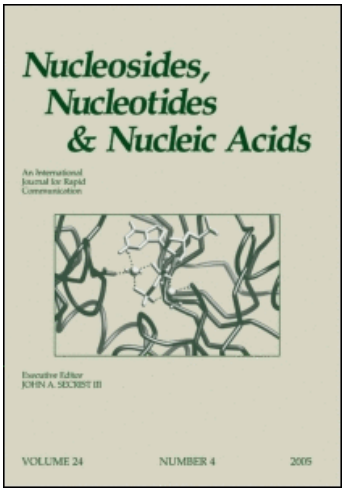

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SYNTHESIS OF NOVEL BICYCLIC NUCLEOSIDES RELATED TO NATURAL GRISEOLIC ACIDS

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SYNTHESIS OF NOVEL BICYCLIC NUCLEOSIDES RELATED TO NATURAL GRISEOLIC ACIDS

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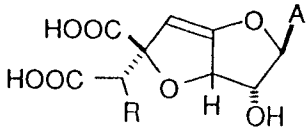
Abstract: Synthesis of novel, bicyclic nucleosides related to natural griseolic acids is described. The synthetic approach involves nucleobase construction at the C-2' position of 1,4:3,6-dianhydro-D-mannitol. The carbohydrate precursor used in the synthesis, 1,4:3,6-dianhydro-D-glucitol, can be prepared easily from D-glucitol. Bicyclic analogues of five naturally occurring nucleosides have been prepared. The single crystal X-ray structure of a representative example is presented and discussed.

INTRODUCTION

The few compounds that are known to contain the bicyclic carbohydrate system, 1,4:3,6-dianhydrohexitol, have been found to display a wide range of interesting biological properties. For example, the naturally occurring compounds called griseolic acids A, B, and C (**1** and **2**) isolated from the cultured broths of *Streptomyces griseoaurantiacus*[1] have been shown to have inhibitory activity against cyclic nucleotide-phosphodiesterase and structural analogues of these compounds have been found to have greater activity and selectivity.[1,2] The dinitrates of bicyclic sugars such as that of the isosorbide system have potent vasodilatory effects.[3]

Our interest in the discovery of unusual nucleoside analogues with the potential for antiviral activity, led to an investigation on the synthesis of nucleoside analogs containing the 1,4:3,6-dianhydrohexitol sugar moiety (**3**). A literature search revealed that to date, only two such nucleoside analogues have been prepared and assessed for antiviral activity: the first example is a C-1' purine isosorbide derivative which was inadvertently prepared by Holy and coworkers[4], and was found to have significant activity against the vaccinia virus and with low toxicity; the second is a 1,4:3,6-dianhydrohexitol nucleoside in which the base



R=OH, R=H

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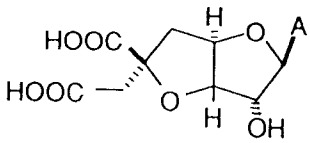

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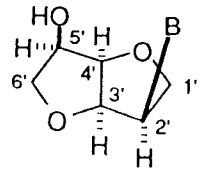


3

is appended at the C-5' hydroxyl group of the hexitol by a methylene linker.[5] Compounds related to the former have been prepared[6] for examination of the effects of conformational restriction in oligonucleotides caused by conformationally restricted carbohydrate moieties. In this report, we describe synthetic approaches to novel bicyclic nucleoside analogs derived from 1,4:3,6-dianhydro-D-mannitol in which the nucleobase is attached directly to the dianhydrohexitol at the C-2' position. Other nucleoside analogs in which the base is transposed to positions other than C-1' on the sugar moiety have been found to have interesting biological properties, notably potent antiviral activity.[7-10] Additionally, these C-2' based nucleosides have been found to possess greater stability *in vitro*, thereby reducing toxicity associated with decomposition to toxic metabolites.[7] The bicyclic nucleoside analogs of this paper and their phosphorylated derivatives are expected to have "glycosyl" bond stability and they are of potential biological interest in studying if the effect of the greater rigidity of the carbohydrate unit resulting in a degree of structural pre-organization would facilitate more specific interaction with viral enzymes.

RESULTS AND DISCUSSION

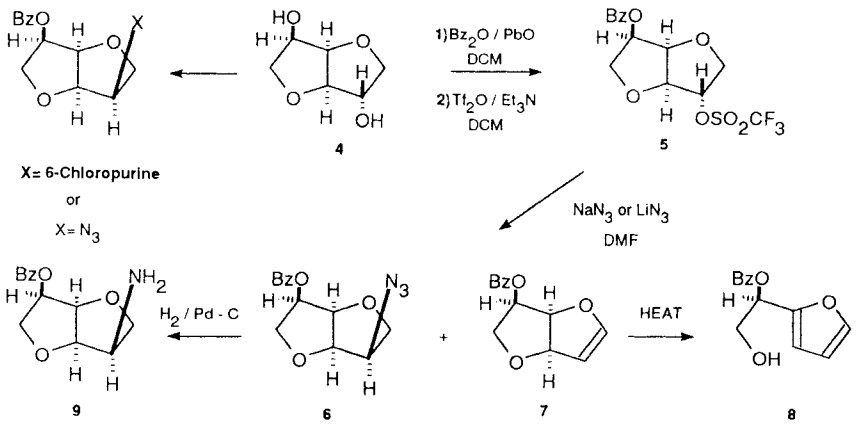
The approach to preparing such nucleoside analogues was initiated from the readily available starting material, 1,4:3,6-dianhydro-D-glucitol (**4**) (Scheme 1), prepared from D-glucitol by treatment with acid[11]. Selective protection of the C-5 hydroxyl group was accomplished by taking advantage of the greater reactivity of this hydroxyl group to give 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol. Direct conversion to a purine nucleoside analogue was first attempted by Mitsunobu coupling of 6-chloropurine to the protected isosorbide under a variety of conditions (DEAD, PPh_3 or DIAD, PPh_3).[12-14] Unfortunately, none of the reactions attempted yielded any nucleoside product. Similarly, direct displacement of a leaving group with the potassium salt of 6-chloropurine in the presence of 18-crown-6 ether also met with failure. In both of the above cases, excessive steric crowding of the *endo* face of the ring system together with the adverse electronic effect of the endocyclic oxygen lone pairs was thought to be responsible for the absence of product formation.

An alternative approach which involved conversion of the C2-hydroxyl group to a mesylate, followed by displacement with azide anion, with the intention of constructing the desired nucleobase, met with only limited success. The C2-*endo* azide **6** was eventually prepared from 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol triflate **5**. Interestingly, unlike most triflates, compound **5** is stable, can be crystallized, and is remarkably easy to handle. However, on stirring at room temperature with lithium azide in DMF for 2.5 h, compound **5** was converted to the azide **6** (48%) and the elimination product **7** (26% yield) (Scheme 1). The latter converts easily to the furan derivative **8**. This unwanted by-product proved difficult to remove by chromatography, and it was necessary to convert it to its acetate in order to facilitate chromatographic purification of the azide **6** which was then smoothly converted to the amine **9** by catalytic reduction.

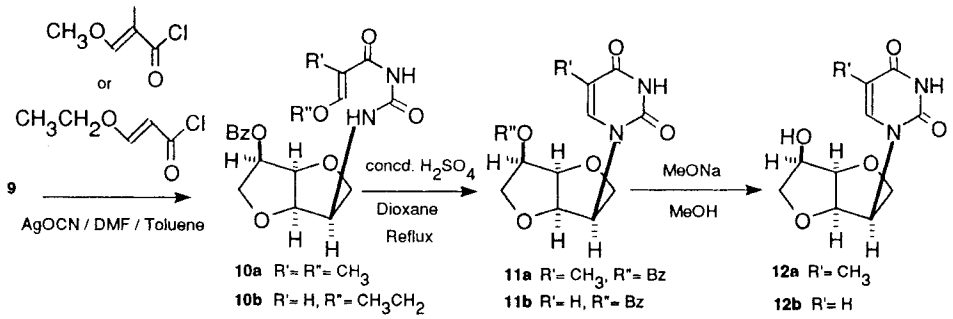
Conversion of the amine **9** to the target bicyclic pyrimidine nucleosides was completed by using a base-construction methodology. For example, reaction of the amine **9** (Scheme 2) with 3-methoxy-2-methacryloyl chloride in the presence of silver cyanate, gave the acryloyl urea **10a**.[15] Conversion of urea **10a** to the protected bicyclic nucleoside **11a** was achieved by refluxing with 2N sulfuric acid in dioxane.[16] Deprotection of the C-5'-hydroxyl group was effected by stirring the nucleoside with freshly prepared sodium methoxide in methanol for 1 h to yield 2-deoxy-2-[3,4-dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro-D-mannitol (**12a**). The uridine analog **12b** was synthesized by a related route (Scheme 2).

Preparation of the cytidine analogue **16** (Scheme 3) was initially attempted from nucleoside **11b** *via* conversion to the 4-triazol-1-ylpyrimidine **13** by reaction with triazole and $POCl_3$ in pyridine.[17] This reaction, however, gave a large number of unidentified side products in addition to the desired intermediate. An alternative route to **16** involved conversion of the uridine to the 4-thiouridine **15** with Lawesson's reagent[18] in refluxing dichloroethane. Amination and deprotection to yield (**16**) was accomplished with NH_3 / methanol at 100 °C.

Synthesis of the purine nucleoside analogues was also achieved by application of the base construction methodology.[19] Conversion of the amine **9** (Scheme 4) to the 5,6-diamino-4-chloropyrimidine derivative **17** by condensation with 5-amino-4.6-dichloropyrimidine under basic conditions proceeded in moderate yields. Ring-closure to complete the purine ring system by reaction of **17** in acidified triethylorthoformate followed



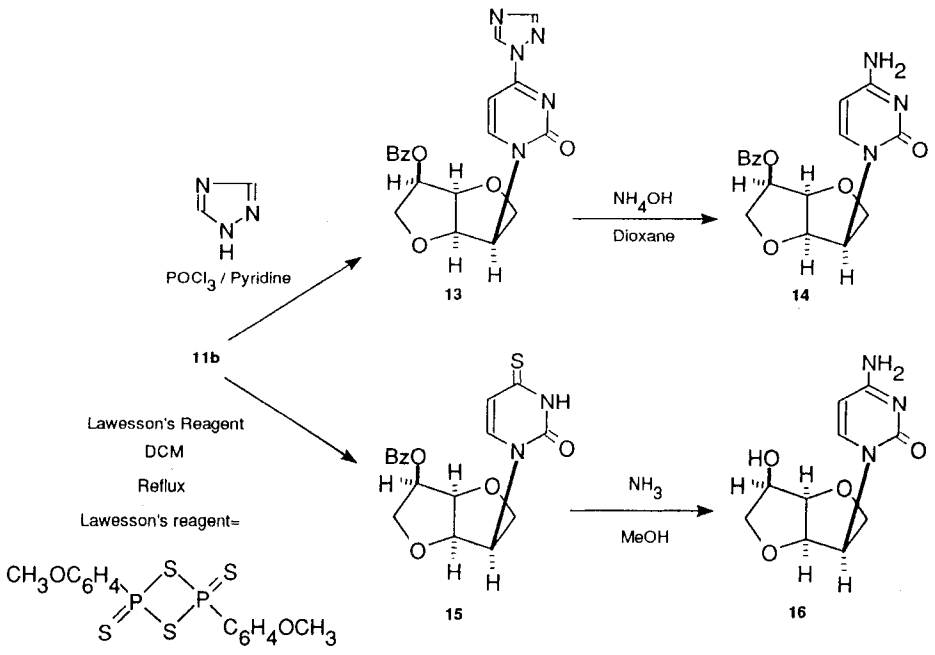
Scheme 1



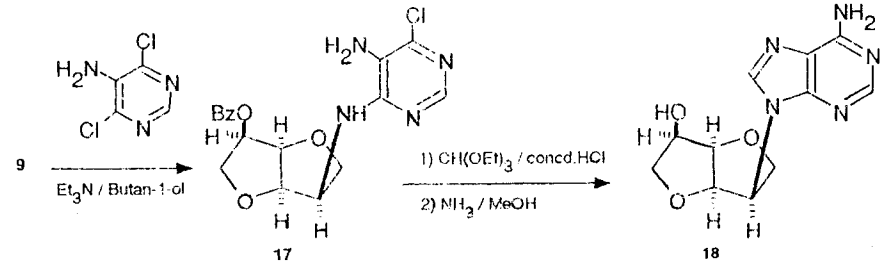
Scheme 2

by amminolysis of the resulting 6-chloropurine intermediate afforded, after concomitant deprotection, the 1,4:3,6-dianhydromannitol adenine nucleoside **18** as a white solid.

Reaction of the amine **9** with the isomeric pyrimidine, 2-amino-4,6-dichloropyrimidine, produced the 2,6-diamino-4-chloropyrimidine derivative **19** (Scheme 5). Coupling of **19** with 4-chlorobenzenediazonium chloride gave an orange diazo compound **20**, which was smoothly reduced to the 2,5,6-triamino-4-chloropyrimidine **21** with zinc and acetic acid at 90°C. Cyclization of **21** to complete the imidazo ring was accomplished with triethyl orthoformate in the presence of HCl. While the cyclization did occur, the product of this reaction was found to be mostly the 2-formamidopurine compound despite the use of methodology intended to remove any unwanted formylation products.[20] However,

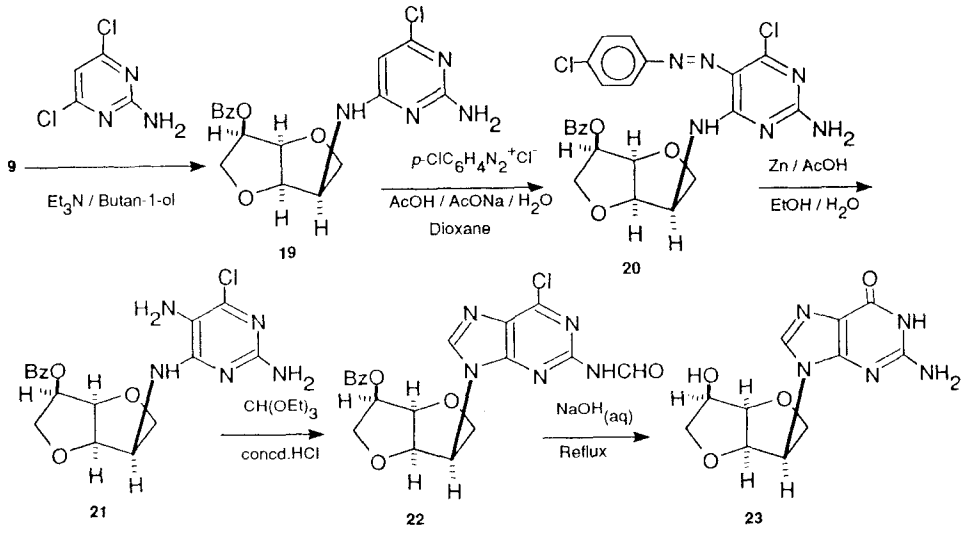


Scheme 3



Scheme 4

treatment of this product with aqueous sodium hydroxide resulted in conversion to the guanine nucleoside **23.** The N-formyl group was easily cleaved under these conditions. Determination of the stereochemistry of the bicyclic nucleoside products was achieved through extensive ^{1}H NMR homonuclear decoupling experiments performed on the azide **6**. Comparison of the coupling constants observed for the C-2, C-3, and C-4 protons, which all posses the *exo* configuration in the azide, with the same protons in the triflate and other



Scheme 5

isosorbide derivatives having the C-2 proton *endo*, show that much larger coupling constants are associated with the all *exo* configuration. Comparison of the coupling constants of the nucleosides obtained *via* base construction with that of the precursor azide showed that the stereochemical integrity was preserved at the C-2 position during synthesis. Percentage NOE measurements (Figure 1) obtained from difference spectra provided further support for the stereochemical assignments. Values of NOE's of 6 - 9% were observed for protons on the *exo* face of the bicyclic ring system, with an enhancement of 6% between C-6H of the base and the *endo* C-1'H.

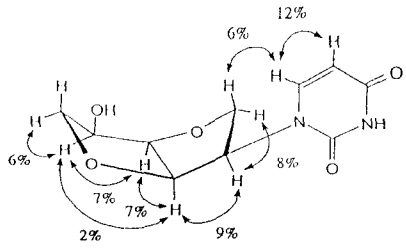


Figure 1. Differential NOE data observed for compound **12b**

Further confirmation of the relative and absolute stereochemistry of these bicyclic nucleosides was obtained through single crystal X-ray diffraction and NOE studies. The ORTEP plot of the bicyclic uridine **12b** from the X-ray data (Figure 2) showed that the

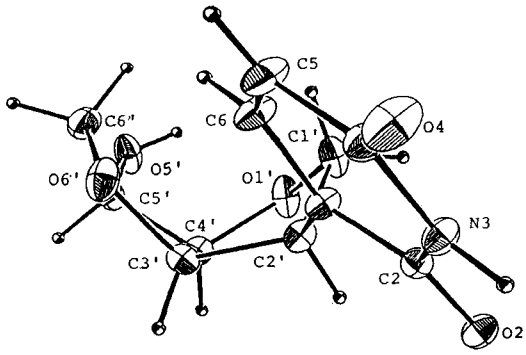


Figure 2. ORTEP plot of compound **12b**.

anhydro ring A (C1'-C2'-C3'-C4'-O1') is in the C-2' envelope conformation (C-2' *exo*) and anhydro ring B (C3'-C4'-C5'-C6'-O6') is in the C-6' envelope conformation (C-6' *endo*). The concave nature of this carbohydrate moiety is clearly apparent. The base and the C-5' hydroxyl group were accordingly found in pseudoequatorial positions, the base displaying a C2-N1-C2'-C1' bond torsional angle of -107°. Calculation of the pseudo-rotational phase angle (*P*) for anhydro ring A using the standard equation for nucleosides,[21] produced a value of $P = 24°$. The value of *P* for the majority of active anti-HIV compounds has been shown to be in the range $P = -165$ to $-220°$, which is generally consistent with the C-3' *exo* conformation ($P = -198°$). However, these correlations are not necessarily valid for bicyclic dideoxynucleosides or their isomeric analogs. Comparison of the C2-N1-C2'-C1' torsional angle with the glycosidic bond torsional angle (χ) of known antiviral nucleosides showed that the observed angle was somewhat smaller than values of χ for anti-HIV nucleosides, but was not outside the normal range.[22] The solid state packing structure of **12b** exhibited considerable inter- and intra- molecular hydrogen bonding (Figure 3). Chain formation *via* N3—HN3···O4 hydrogen bonds was augmented by an unusual bifurcated hydrogen bonding system involving the O-5' hydroxyl group, which participates in an intramolecular hydrogen bond to the O-1' position (O5'—HO5'···O1') and an intermolecular hydrogen bond to the O-2 position (O5'—HO5'···O2) of the neighboring molecule. All hydrogen bonds were found to be of normal length. The hydrogen bonding of C-5'OH to O-1' has a marked effect on the reactivity of this hydroxyl group as previously described.

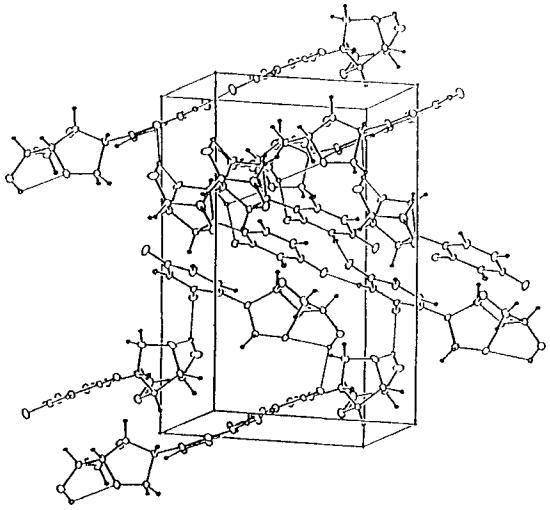


Figure 3. Crystal packing structure for compound **12b**, showing inter- and intramolecular hydrogen bonding arrangements within the unit cell.

In summary, approaches to the synthesis of novel bicyclic nucleosides related to the natural griseolic acids are described. The precursor for the syntheses described was 1,4:3,6-dianhydro-D-glucitol synthesized readily from D-glucitol by treatment with acid. The methodology involved construction of the appropriate nucleobases at the C-2' position of the concave face of the 1,4:3,6-dianhydro-D-mannitol ring system derived from the aforementioned precursor. NMR and X-ray crystallographic data provided unequivocal support for the relative and absolute stereochemistry of the target compounds and also furnished information on the conformation of the bicyclic sugar moiety. Compounds **12a**, **12b**, **16**, **18**, and **23** are currently undergoing antiviral evaluation. These results will be published elsewhere.

EXPERIMENTAL

Melting points reported were recorded on a Thomas Hoover apparatus fitted with a microscope and are uncorrected. NMR spectra were recorded on a Bruker AC-300 pulse Fourier transform spectrometer with tetramethylsilane as internal standard. Ultraviolet absorption spectra were recorded on a Gilford Response spectrophotometer. Infrared spectra were recorded on a Mattson Cygnus 25 Fourier transform spectrophotometer, with

samples as neat liquids or nujol mull between sodium chloride plates. Single crystal X-ray data was obtained on an Enraf-Nonius CAD-4 diffractometer at the University of Iowa. Elemental analyses were performed at Desert Analytics, Tucson, AZ and Supersun Technology, Stony Brook, NY. Preparative thin layer chromatography was carried out on plates prepared with E. Merck PF_{254} silica gel. Flash column chromatography was performed using columns packed with 230 - 400 mesh silica gel. Solvents and reagents were dried and purified according to standard procedures.

5-*O*-Benzoyl-2-trifluoromethanesulfonyl-1,4:3,6-dianhydro-D-glucitol (5). A solution of 5-*O*-benzoyl-1,4:3,6-dianhydro-D-glucitol (2.0 g, 8.0 mmol) prepared from the dianhydro-D-glucitol[11] in dry dichloromethane (40 mL) was treated with triethylamine (3.24 g, 32 mmol) and then cooled to -78°C with stirring under an atmosphere of nitrogen. Trifluoromethanesulfonic anhydride (2.24 g, 8.0 mmol) was then added slowly to the cooled, stirred mixture. After stirring for 1 h, the mixture was allowed to warm to room temperature and was then diluted with chloroform. The mixture was washed once with brine and the organic layer separated and dried over magnesium sulfate. After filtration, the solution was concentrated under vacuum to give an orange crystalline solid. This solid was dissolved in a small amount of ethyl acetate, silica gel was added to the solution and the solvent evaporated to give a free running orange powder. This powder was added to the top of a silica gel column and then eluted with hexanes-ethyl acetate (80:20). Concentration of the appropriate fractions gave 2.37 g (91%) of the title compound as fine white needles: mp 123-126 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (2 H, m, OBz), 7.59 (1 H, m, OBz), 7.47 (2 H, m, OBz), 5.44 (1 H, dd, $J_{5,6}$ = 10.5 Hz, $J_{5,4}$ = 6 Hz, H5), 5.36 (1H, m, H2), 5.18 (1 H, dd, $J_{4,5}$ = 6Hz,$J_{4,3}$ = 6Hz,H4), 4.74 (1 H, dd, $J_{3,4}$ = 6Hz, H3), 4.20 (1 H, d, $J_{6a,b}$ = 10.5Hz, H6), 4.13-3.97 (3 H, m, H1, H6); ^{13}C NMR ($CDCl_3$) δ 165.6, 129.7, 129.1, 128.5, 120.5, 116.2, 89.2, 85.6, 81.2, 73.8, 72.8, 71.1; ^{19}F NMR ($CDCl_3$) δ 75.38. Anal. Calcd for $C_{14}H_{13}F_3O_7S$: C, 43.99; H, 3.43; S, 8.39. Found: C, 44.32; H, 3.48; S, 8.10.

5-*O*-Benzoyl-2-deoxy-2-azido-1,4:3,6-dianhydro-D-mannitol (6). A solution of 5-*O*-benzoyl-2-trifluoromethanesulfonyl-1,4:3,6-dianhydro-D-glucitol (2.14 g, 6.6 mmol) in dry DMF (40 mL) was treated with lithium azide (0.59 g, 12.12 mmol). The resulting solution was stirred for 2.5 h at room temperature. After this time, the mixture was diluted with

chloroform, washed with brine and the organic extract dried over magnesium sulfate. The filtrate was concentrated under vacuum and the last traces of DMF removed by distillation under high vacuum. The resulting orange oil was chromatographed with hexanes-ethyl acetate (70:30) to give the pure azide as a pale yellow oil (0.80g, 48%): IR (nujol mull) 2108 (N_3), 1729 (Bz) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (2 H, m, OBz), 7.59 (1 H, m, OBz), 7.46(2 H, m, OBz), 5.39 (1 H, dd, $J_{5,6a,b}$ = 10.5 Hz, $J_{5,4}$ = 6Hz, H5), 4.89 (1 H, dd, $J_{4,5}$ = 6 Hz, $J_{4,3}$ = 5.7 Hz, H4), 4.68 (1 H, dd, $J_{3,4}$ = 5.7 Hz, H3), 4.13 (2 H, m, H6), 4.03 (1 H, dd, $J_{1a,2}$ = 6.9 Hz, $J_{1a,b}$ = 7.8 Hz, H1b), 3.86 (1 H, m, H2), 3.73 (1 H, dd, $J_{1,b,2}$ = 6.9 Hz, $J_{1a,b}$ = 7.8 Hz, H1a); ^{13}C NMR ($CDCl_3$) 165.7, 133.1, 129.6, 128.3, 82.4, 81.4, 74.3, 71.3, 69.8, 61.6. Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.27. Found: C, 56.20 ; H, 4.35 ; N, 14.78.

5-*O*-Benzoyl-2-[(3-methoxy-2-methyl-1-oxo-2-propenyl)amino]carbonyl]amino]-1,4:3,6-dianydro-D-mannitol (10a). A solution of 5-*O*-benzoyl-2-deoxy-2-azido-1,4:3,6-dianhydro-D-mannitol **(6)** (0.66 g, 2.4 mmol) in ethanol (30 mL) was hydrogenated at 30 p.s.i. for 18 h over Pd-C (70 mg). The catalyst was filtered off, and the solvent evaporated to give a gray solid. The product, 5-*O*-benzoyl-2-deoxy-2-amino-1,4:3,6-dianhydro-D-mannitol (**9**), was purified by flash column chromatography with MeOH-triethylamine (95:5) to give 5-*O*-benzoyl-2-deoxy-2-amino-1,4:3,6-dianhydro-D-mannitol (**9**) as pale yellow crystals (0.49 g, 83%): ^1H NMR ($CDCl_3$) δ 8.08 (2 H, m, OBz), 7.59 (1 H, m, OBz), 7.45 (2 H, m, OBz), 5.41 (1 H, dd, $J_{5,6a,b}$ = 9.3 Hz, $J_{5,4}$ = 5.7 Hz, H5), 4.89 (1 H, d, $J_{4,5}$ = 5.7 Hz, $J_{4,3}$ = 5.5 Hz, H4), 4.32 (1 H, dd, $J_{3,4}$ = 5.5 Hz, $J_{3,2}$ = 5.7 Hz, H3), 4.02 (2 H, m, H6), 3.96 (1 H, dd, $J_{1a,,2}$ = 6.9 Hz, $J_{1a,b}$ = 7.8, H1b), 3.52 (1 H, m, H2), 3.35 (1 H, dd, $J_{1b,2}$ = 6.9 Hz, $J_{1a,b}$ = 7.8 Hz, H1a), 1.64 (2 H, s, br, exchangeable, NH_2); ^{13}C NMR ($CDCl_3$) δ 165.9, 133.1, 129.7, 128.3, 82.9, 81.5, 75.2, 73.4, 71.2, 55.7.

A solution of 3-methoxy-2-methacryloyl chloride (0.7 g, 5.12 mmol) was refluxed in dry toluene with silver cyanate (1.4 g, 9.2 mmol) under an atmosphere of nitrogen. After 30 min, the mixture was allowed to cool and was the transferred *via* a syringe to a solution of 5-*O*-benzoyl-2-deoxy-2-amino-1,4:3,6-dianhydro-D-mannitol (0.4 g, 1.6 mmol) in dry DMF. After stirring at ambient temperature for 24 h, the solid residue was filtered off and the filtrate concentrated under vacuum. Final traces of DMF were distilled off under high vacuum to yield a cloudy yellow oil. Purification by flash chromatography gave pure 5-*O*-benzoyl-2-deoxy-2-[(3-methoxy-2-methyl-1-oxo-2-propenyl)amino]carbonyl]-amino]-

1,4:3,6-dianhydro-D-mannitol (**10a**) as a pale yellow gum (0.58 g, 93%): ^{1}H NMR ($CDCl_3$) δ 9.40 (1 H, d, J = 9 Hz, N^{1}H), 8.70 (1 H, s, br, exchangeable, N^{3}H), 8.06 (2 H, m, OBz), 7.59 (1 H, m, OBz), 7.45 (2 H, m, OBz), 5.44 (1 H, dd, $J_{5,6a,b}$ = 10 Hz, $J_{5,4}$ = 5.7 Hz, H5'), 4.94 (1 H, dd, $J_{4,5}$ = 5.7 Hz, $J_{4,3}$ = 5.5 Hz, H4'), 4.56 (1 H, dd, $J_{3,4}$ = 5.5 Hz, $J_{3,2}$ = 5.7 Hz, H3'), 4.47 (1 H, m, H2'), 4.12 (2 H, m, H6'), 3.85 (3 H, s ,OCH_3), 2.94, 2.89 (2H, m ,H1'), 1.80 (3 H ,s, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.8, 169.5, 158.7, 154.2, 133.1, 129.8, 129.1, 128.1, 107.2, 81.0, 74.9, 71.5, 71.1, 61.2, 52.9, 8.8. Anal. Calcd for $C_{19}H_{21}N_2O_7$: C, 58.46; H, 5.68; N, 7.18. Found: C, 58.14; H, 5.36 ; N, 6.78.

5-*O*-Benzoyl-2-[3,4-dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro -D-mannitol (11a). A solution of the acryloyl urea (**10a**) (0.56g, 1.44 mmol) in dioxane (10 mL), was treated with 2N H_2SO_4 and then refluxed for 18 h. The solution was then neutralized with 2N NaOH, and then concentrated under vacuum to give a viscous oil. Purification of the product by flash column chromatography yielded the title compound as a white foam after concentration of the appropriate fractions (0.21 g, 41%): ^{1}H NMR (DMSO-d_6) δ 10.30 (1 H, s, br, N^{3}H), 8.04 (2H, m, OBz), 7.71 (1 H, m, OBz), 7.58 (2 H, m, OBz), 7.50 (1 H, s, H6), 5.49 (1 H, dd, $J_{5',6'a,b}$ = 11.3 Hz, $J_{5',4'}$ = 5.1 Hz, H5'), 4.88 (2 H, m, H4', H2'), 4.65 (1 H, dd, $J_{3',4'}$ = 6.3 Hz ,$J_{3',2'}$ = 6 Hz, H3'), 4.18-3.98 (4 H, m, H1', H6'), 1.80 (3 H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ 165.2, 163.7, 151.2, 129.3, 128.9, 108.0, 104.2, 81.2, 80.7, 74.6, 71.1, 67.7, 60.1, 56.6, 12.2; UV λ_{max} = 268.0 nm (ε = 7377). Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.02; H, 5.01; N, 7.54.

2-Deoxy-2-[3,4-dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro-D-mannitol (12a). Sodium metal (0.1g) was stirred in dry methanol (12 mL) under an atmosphere of nitrogen until complete dissolution of the metal had occurred. A solution of the protected nucleoside (**11a**) (0.20 g, 0.58 mmol) in methanol was then added slowly to the stirred solution. After stirring for 1 hour, the mixture was neutralized with 0.1 M HCl. The solvents were then removed under vacuum to give an off white residue which was titurated with ethanol. Concentration of the filtrate gave a clear glass which was purified by preparative t.l.c. (chloroform-methanol 4:1) (0.13 g, 93%): ^{1}H NMR (DMSO-d_6) δ 11.32 (1 H, s, exchangeable, N^{3}H), 7.49 (1H, s, H6), 5.18 (1 H, d, J = 7.5 Hz, br, exchangeable, OH), 4.91 (1 H, m, H2'), 4.57 (1 H, dd ,$J_{3',4'}$ = 6 Hz, $J_{3',2'}$ = 6 Hz, H3'), 4.38

(1 H, dd, $J_{4',5'}$ = 6 Hz, $J_{4',3'}$ = 6 Hz, H4'), 4.20 (1 H, m, H1'), 4.15-3.93 (3 H, m, H1', H6'), 3.81 (1 H, dd, $J_{5',6'a,b}$ = 6 Hz, $J_{5',4'}$ = 6 Hz, H5'), 1.78 (3 H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.2, 150.8, 128.5, 107.1, 103.6, 81.9, 80.0, 71.1, 68.0, 56.8, 11.8; UV λ_{max} = 267.0 (ε= 6268). Anal. Calcd. for $C_{11}H_{14}N_2O_5 \cdot H_2O$: C, 48.53; H, 5.92; N, 10.29. Found: C, 49.08; H, 5.53; N, 9.79.

5-*O*-Benzoyl-2-deoxy-2-[[[3-ethoxy-1-oxo-2-propenyl)amino]carbonyl]amino]-1,4:3,6-dianhydro-D-mannitol (10b). A solution of 3-ethoxyacryloyl chloride (0.64 g, 4.7 mmol) was refluxed in dry toluene (10 mL) with silver cyanate (1.12 g, 7.4 mmol) under an atmosphere of nitrogen. After 30 min, the mixture was allowed to cool and was then transferred *via* a syringe to a solution of 5-*O*-benzoyl-2-deoxy-2-amino-1,4:3,6-dianhydro-D-mannitol (**9**) (0.4 g, 1.6 mmol) in dry DMF (5 mL). After stirring at ambient temperature for 24 h, the solid residue was filtered off and the filtrate concentrated under vacuum. Final traces of DMF were removed under high vacuum. Purification by flash column chromatography (95:5 chloroform-methanol) gave the pure compound as a pale yellow solid (0.40 g, 63%): mp = 196-198°C; ^1H NMR ($CDCl_3$) δ 9.60 (1 H, s, br, exchangeable, N^3H), 9.23 (1 H, d, br, N^1H), 8.09 2 H, m, OBz), 7.69 (1 H, d, J = 15 Hz, H6), 7.58 (1 H, m, OBz), 7.45 (2 H, m, OBz), 5.46 (1 H,dd, $J_{5',6'a,b}$ = 11.4 Hz $J_{5',4'}$ = 6.2 Hz, H5'), 5.34 (1 H, d, J = 15 Hz, H5), 4.93 (1 H, dd, $J_{4',5'}$ = 6.2 Hz, $J_{4',3'}$ = 6.2 Hz, H4'), 4.57 (1 H, dd, $J_{3',4'}$ = 6.2 Hz, $J_{3',2'}$ = 6.2 Hz, H3'), 4.48 (1 H, m, H6b'), 4.13 (2 H, m, H1b', H6a'), 3.97 (2 H, q, J = 6 Hz, 12 Hz, ethyl), 3.53 (1 H, dd, $J_{1'b,2'}$ = 9.3 Hz, $J_{1'a,b}$ = 11.3 Hz ,H1a'), 1.37 (3 H, t, J= 12 Hz, ethyl); ^{13}C NMR ($CDCl_3$) δ 168.1, 165.9, 163.1, 155.1, 133.2, 129.7, 129.4, 128.4, 97.8, 81.4, 81.2, 74.9, 71.6, 71.3, 67.6, 52.9, 14.4; UV λ_{max} = 235.5 (ε = 23551) λ_{shl} = 243.0 (ε = 20194). Anal. Calcd for $C_{19}H_{21}N_2O_7$: C, 58.45; H, 5.68; N, 7.18. Found: C, 57.95; H, 5.63; N, 6.95.

5-*O*-benzoyl-2-[3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro-D-mannitol (11b) and 2-deoxy-2-[3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro-D-mannitol (12b). The acryloyl urea **(10b)** (0.4 g, 1.02 mmol) was stirred in 2 N H_2SO_4 (10 mL) and dioxane (10 mL) under reflux for 18 h. After this time, the mixture was allowed to cool to ambient temperature and then neutralized with 2 N NaOH. The clear solution was then concentrated to give a white solid residue which was washed with ethanol and filtered. The filtrate was concentrated under vacuum to give an oil. Flash

column chromatography (95:5 chloroform-ethanol) allowed separation of nucleoside, **(12b)**, (Rf = 0.1) and protected nucleoside **11b** (Rf =0.45). Compound **12b**: Recrystallized from hot ethanol to give large, clear crystals (31 mg, 13%): mp= 195-200° C (dec.); ^{1}H NMR (DMSO-d_6) δ 11.29 (1 H, s, br, N^{3}H, exchangeable), 7.60 (1 H, d , J= 9 Hz, H6), 5.55 (1 H, d, J = 9 Hz, H5), 5.07 (1 H, d, J = 6.3 Hz, br, exchangeable, OH), 4.94 (1 H, m, H2'), 4.55 (1 H, dd, $J_{3',4'}$ = 5.4 Hz, $J_{3',2'}$ = 5.4 Hz, H3'), 4.38 (1 H, dd, $J_{4',5'}$ = 6 Hz, $J_{4',3'}$ = 5.4 Hz, H4'), 4.20 (1 H, m, H5'), 4.02 (1 H, s, H1'), 3.99 (1 H, s, H1'), 3.79 (1 H, dd, $J_{1'a,2'}$ = 6.6 Hz,$J_{1'a,b}$ = 8.4 Hz, H6b'), 3.49 (1 H, dd, $J_{1'b,2}$ = 6.6 Hz, $J_{1'a,b}$ = 8.4Hz, H6a'); ^{13}C NMR (DMSO-d_6) 165.5, 150.6, 142.9, 99.7, 82.2, 80.0, 71.7, 71.3, 67.9, 56.5; UV λ_{max} = 262.5 (ε = 10123); Anal. Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.00; N, 11.67. Found: C, 49.89; H, 4.92; N, 11.34. **Compound 11b:** White foam (0.22 g, 63%): ^{1}H NMR (DMSO-d_6) δ 11.37 (1 H, s, br, N^{3}H, exchangeable), 8.03 (2 H, m, OBz), 7.95 (1 H, d, J = 8.1 Hz, H6), 7.74-7.46 (3 H, m, OBz), 5.57 (1 H, d, J = 8.1 Hz, H5), 5.39 (1 H, dd, $J_{5',4'}$ = 5.4 Hz, $J_{5',6'a,b}$ = 10.9 Hz, H5'), 4.89 (1 H, m, H2'), 4.86 (1 H, dd, $J_{4',5'}$ = 5.4 Hz, $J_{4',3'}$ = 5.4 Hz, H4'), 4.63 (1 H, dd, $J_{3',4'}$ = 5.4 Hz, $J_{3',2'}$ = 4.9 Hz, H3'), 4.08-3.92 (4 H, m, H1', H6'); ^{13}C NMR (DMSO-d_6) δ 164.4, 162.5, 150.8, 143.0, 133.2, 128.8, 128.3, 128.0, 99.9, 80.8, 80.2, 73.9, 70.5, 67.2, 55.9; UV λ_{max}= 263.0 (ε = 8234), 227.5 (ε = 14711); Anal. Calcd for $C_{17}H_{16}N_2O_6$: C, 59.29; H, 4.65; N, 8.14. Found: C, 58.88; H, 4. 46; N, 7.74.

5-*O*-Benzoyl-2-deoxy-2-[3,4-dihydro-2-oxo-4-thio-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro-D-mannitol (15). The uridine nucleoside **(11b)** (0.22 g, 0.8 mmol) was stirred together with Lawesson's Reagent (0.18 g, 0.6 mmol) in refluxing dichloroethane (10 mL) under a nitrogen atmosphere for 24 h. After cooling, the mixture was concentrated under vacuum to give an oily residue. The residue was then washed with methanol, and the small amount of precipitate formed was filtered off. Concentration of the filtrate gave a pungent orange oil which was subjected to flash column chromatography (95:5 chloroform-methanol). The fractions containing the UV active product were collected and the solvent stripped off to give the title compound as an oil (0.18 g, 78%): ^{1}H NMR (DMSO-d_6) δ 12.8 (1 H, s, br, N^{3}H), 8.03 (2 H, m, OBz), 7.68 (1 H, m, OBz), 7.58 (2 H, m, OBz), 7.12 (1 H, d, br, H6), 6.35 (1 H, d, J = 6 Hz, H5), 5.40 (1 H, dd, $J_{5',6'a,b}$ = 11.4 Hz ,$J_{5',4'}$ = 5.4 Hz, H5'), 4.89 (1 H, m, H2'), 4.80 (1 H, dd, $J_{4',5'}$ = 5.4 Hz, $J_{4',3'}$ = 4.8 Hz, H4'), 4.69 (1 H, dd, $J_{3',4'}$ = 4.8 Hz, $J_{3',2'}$ = 4.8 Hz, H3'), 4.10-3.80 (4 H, m, H1', H6'); ^{13}C NMR (DMSO-d_6) δ 189.6, 165.0, 148.4, 139.1, 133.5, 129.2, 129.1, 128.8, 111.6, 81.3, 80.5, 74.3, 71.0,

65.8, 57.1; UV λ_{max}= 332.0 (ε = 2806), 230.0 (ε = 5639) λ_{shl}= 250.0. Anal. Calcd for $C_{17}H_{16}N_2O_5S$: C, 56.64; H, 4.47; N, 7.77. Found: C, 56.28; H, 4.35; N, 7.42.

2-Deoxy-2-[3,4-dihydro-2-oxo-4-amino-1(2*H*)-pyrimidinyl]-1,4:3,6-dianhydro-D-mannitol (16). A solution of the nucleoside **(15)** (0.12 g) in methanolic ammonia was heated in a bomb reactor at 100°C for 3 h. Concentration of the solution then gave a brown oil which was purified by preparative t.l.c. (4:1 chloroform-methanol). Collection of the product from the appropriate band gave the title compound as a white hygroscopic solid (20 mg, 25%): ^{1}H NMR (DMSO-d_6) δ 7.55 (1 H, d, J = 7.5 Hz, H6), 7.15-6.93 (2 H, 2s, br, NH_2), 5.66 (1H, d, J = 7.5 Hz, H5), 5.05 (1 H, s, br, OH), 5.03 (1 H, m, H2'), 4.52 (1 H, dd, $J_{3',4'}$ = 4.8 Hz, $J_{3',2'}$ = 4.8 Hz, H3'), 4.39 (1 H, dd ,$J_{4',5'}$ = 4.8 Hz, $J_{4',3'}$ = 4.8 Hz, H4'), 4.20 (1 H, m, H5'), 4.02 (1 H, m, H1'), 3.91 (1 H, m, H1'), 3.76 (1 H, m, H6'), 3.48 (1 H, m, H6'); ^{13}C NMR (DMSO-d_6) δ 165.2, 155.9, 143.9, 92.8, 82.5, 80.3, 72.1, 68.4, 57.4; UV λ_{max} = 273.5 (ε = 11980). Anal. Calcd for $C_{10}H_{13}N_3O_4.H_2O$ C, 46.69; H, 5.88; N 16.34. Found: C, 46.28; H, 5.76; N, 15.93.

5-*O*-Benzoyl-2-deoxy-2-[(5-amino-6-chloro-4-pyrimidinyl)amino]-1,4:3,6-dianhydro-D-mannitol (17). A solution of the amine **(9)** (0.5 g, 2 mmol) and 5-amino-4,6-dichloro-pyrimidine (0.36g, 2.2mmol) in 1:1 triethylamine-butan-1-ol (16 mL) was heated under reflux under an atmosphere of nitrogen for 72 h. After cooling, the mixture was treated with boiling ethyl acetate and the precipitate formed was filtered off. Concentration of the filtrate gave a brown oil which was purified by flash chromatography (95:5 chloroform-methanol) to give the title compound as a white foam (0.21 g, 28%): ^{1}H NMR ($CDCl_3$) δ 8.08 (1 H, s, H2), 8.06 (2 H, m, OBz), 7.71 (1 H, m, OBz), 7.58 (2 H, m, OBz), 5.52 (1 H, m, H5'), 5.41 (1 H, m, H2'), 4.75 (1 H, dd ,$J_{4',3'}$ = 4.3 Hz, $J_{4',5'}$ = 6.8 Hz, H4'), 4.62 (1 H, dd, $J_{3',4'}$ = 4.3Hz, $J_{3',2'}$ = 6Hz, H3'), 4.47 (1 H, m, H6'), 4.31(1 H, m, H6'), 4.18-4.02 (2 H, m, H1'), 3.57 (2 H, s, br, NH_2); ^{13}C NMR ($CDCl_3$) δ 165.8, 154.1, 149.3, 143.1, 133.3, 129.7, 129.3, 128.4, 122.2, 81.4, 81.1, 74.9, 71.5, 54.1; UV λ_{max} = 297.0 (ε = 10801), 261.0 (ε = 9857); Anal. Calcd for $C_{17}H_{17}$ Cl N_4O_4: C, 54.19; H, 4.55; N 14.87. Found: C, 53.76; H, 4.22; N, 14.53.

2-Deoxy-2-[6-imino-(9*H*)-purin-9-yl]-1,4:3,6-dianhydro-D-mannitol (18). The pyrimidine **(17)** (0.20 g, 0.53 mmol) was stirred for 72 h at ambient temperature in triethyl

orthoformate (5 mL) acidified with concd. HCl (0.125 mL). The mixture was then concentrated under vacuum and the residue obtained eluted from a silica gel column (70:30 ethyl acetate-chloroform). Combination of the appropriate fractions and removal of the solvent gave a white powder which was recrystallized from hot methanol to give fine white needles of 5-*O*-benzoyl-2-deoxy-2-[6-chloro-(9*H*)-purin-9-yl]-1,4:3,6-dianhydro-D-mannitol (0.16 g, 76%): mp 194-196°C; ^{1}H NMR (DMSO-d_6) δ 8.83 (1 H, s, purine), 8.81 (1 H, s, purine), 8.08 (2 H, m, OBz), 7.71 (1 H, m, OBz), 7.58 (2 H, m, OBz), 5.45 (1 H, dd, $J_{5',6'a,b}$ = 9.6 Hz, $J_{5',4'}$ = 4.2 Hz, H5'), 5.28 (1 H, m, H2'), 5.06 (1 H, dd ,$J_{4',3'}$ = 3.6 Hz ,$J_{4',5'}$ = 4.2 Hz, H4'), 4.84 (1 H, dd, $J_{3',4'}$ = 3.6 Hz, $J_{3',2'}$ = 4.8 Hz, H3'), 4.40-4,28 (2 H, m, H6'), 4.15-4.00 (2 H, m, H1'); ^{13}C NMR (DMSO-d_6) δ 164.5, 151.8, 151.1, 148.6, 146.2, 132.8, 129.9, 128.7, 128.3, 80.9, 79.9, 74.2, 70.8, 67.8, 56.3; UV λ_{max}= 263.0 (ε = 13869), 226.0 (ε = 24968); Anal. Calcd for $C_{18}H_{15}Cl\,N_4O_4$: C, 55.89; H, 3.91; N 14.48. Found: C, 55.88; H, 4.14; N, 14.10.

A suspension of the above protected 6-chloropurine nucleoside (0.10 g) in methanolic ammonia (5 mL) was heated to 100°C in a bomb reactor for 3 h. The solution was then concentrated to give a white powder which was purified by preparative t.l.c. (3:1 chloroform-methanol). Collection of the appropriate UV active bands and elution with methanol gave the title compound as a white crystalline solid after removal of the solvent (40 mg, 59%): mp= 248-250 (dec.); ^{1}H NMR (DMSO-d_6) δ 8.18 (1 H, s, purine), 8.14 (1 H, s, purine), 7.23 (2 H, s, NH_2,exchangeable), 5.24 (1 H, m, H2'), 5.03 (1 H, s, OH, exchangeable), 4.68 (1 H, dd, $J_{3',4'}$ = 3.9Hz, $J_{3',2'}$ = 5.3 Hz, H3'), 4.55 (1H, dd, $J_{4',5'}$ = 4.6 Hz, $J_{4',3'}$ = 3.9Hz, H4'), 4.40-4.10 (3H, m, H1', H5'), 3.80 (1H, m, H6'), 3.57 (1H, m, H6'); ^{13}C NMR (DMSO-d_6) δ 155.3, 151.9, 139.6, 117.8, 112.9, 82.1, 79.9, 71.9, 71.8, 68.8, 56.2; UV λ_{max}= 259.5 (ε = 15384). Anal. Calcd for $C_{11}H_{13}N_5O_3$: C, 50.18; H, 4.97; N, 26.60. Found: C, 49.78; H, 4.65 ; N, 26.18.

5-*O*-Benzoyl-2-deoxy-2-[(2-amino-6-chloro-4-pyrimidinyl)amino]-1,4:3,6-dianhydro-D-mannitol (19). A solution of the amine **(9)** (0.73 g, 3.00 mmol) and 2-amino-4,6-dichloropyrimidine (0.54 g, 3.31 mmol) in 1:1 triethylamine - butan-1-ol (10 mL) was heated under reflux under nitrogen for 72 h. After cooling, boiling ethyl acetate was added, and a small amount of precipitate filtered off. The filtrate was then concentrated under vacuum to give a brown oil. Flash column chromatography of the crude product (70:30 chloroform-ethyl acetate) gave the title compound as a white foam. Yield = 0.47g

(43%); ^{1}H NMR (DMSO-d_6) δ 8.04 (2 H, m, OBz), 7.69 (1 H, m, OBz), 7.57 (2H, m, OBz) 7.25 (1 H, s, br, NH), 6.48 (2 H, s, br, NH_2, exchangeable), 5.98 (1 H, s, H5), 5.40 (1 H, dd, $J_{5',4'}$ = 4.8 Hz, $J_{5',6'a,b}$ = 8.1 Hz, H5'), 4.50 (2 H, m, H2', H4'), 4.88 (1H, dd, $J_{4',5'}$ = 4.8 Hz, $J_{4',3'}$ = 5.3 Hz, H3'), 4.08-3.91 (3 H, m, H6', H1'), 3.40 (1 H, m, H1'); ^{13}C NMR (DMSO-d_6) δ 164.5, 163.3, 162.4, 132.9, 128.9, 128.6, 128.3, 92.5, 80.5, 80.1, 74.6, 70.4, 69.0, 52.7; UV λ_{max}= 282.0 (ε = 16273), 227.5 (ε = 40515), 212.5 (ε=51273). Anal. Calcd for $C_{17}H_{17}ClN_4O_4$: C, 54.19; H, 4.55; N, 14.87. Found: C, 53.78; H, 4.32; N, 14.54.

5-*O*-Benzoyl-2-deoxy-2-[6-chloro-2-formamido-(9*H*)-purin-9-yl]-1,4:3,6-dianhydro-D-mannitol (22). A chilled solution of *p*-chlorophenyldiazonium chloride, prepared from *p*-chloroaniline (0.15 g, 1.16 mmol), sodium nitrite (0.08 g, 1.2 mmol), 3N HCl (2.2 mL) and water (1.5 mL), was added slowly to a solution of **19** (0.40 g), sodium acetate (2 g) and acetic acid (5 mL) in 1:1 water-dioxane (10 mL). The resulting solution was stirred at ambient temperature for 18 h. The orange precipitate was then filtered off under suction. Thin layer chromatography (1:1 hexanes-ethyl acetate) showed the product, 5-*O*-benzoyl-2-deoxy-2-{[2-amino-6-chloro-5-[(*p*-chlorophenyl)azo]-4-pyrimidinyl]-amino}-1,4:3,6 dianhydro-D-mannitol (**20**), to be homogenous and no further purification was performed (0.35 g, 55%): ^{1}H NMR ($CDCl_3$) δ 10.79 (1 H, d, *J*= 6Hz, br, NH), 7.96 (2 H, m, Cl-Ph), 7.75-7.48 (11H, m, OBz, Cl-Ph), 5.45 (1 H, dd, $J_{5',4'}$ = 5.4 Hz, $J_{5',6'a,b}$ = 9.6 Hz, H5'), 4.93 (1 H, dd, $J_{4',5'}$ = 5.4 Hz, $J_{4',3'}$ = 5.4 Hz, H4'), 4.71 (1 H, dd, $J_{3',4'}$ = 5.4Hz, $J_{3',2'}$ = 5.4 Hz, H3'), 4.59 (1H, m, H2'), 4.29-4.09 (2 H, m, H6'), 4.00 (1 H, m, H1'), 3.45 (1 H, m, H1'); UV λ_{max} = 388.5 (ε = 8146), 280.5 (ε = 6480), 225.5 (ε = 18274).

A vigorously stirred mixture of the diazo nucleoside **(20)** (0.29 g, 0.56 mmol) in acetic acid (0.16 mL) , water (7.5 mL) and ethanol (7.5 mL) at 90°C, was treated with small portions of zinc dust (approx. 0.4 g) until all of the orange colored material was removed. The clear solution was then stirred at 90°C for a further 2 h before cooling to ambient temperature. The solution was then filtered to remove zinc and the filtrate concentrated under vacuum to give an oily residue. Purification of the oil by flash column chromatography (80:20 ethyl acetate-chloroform) gave 5-*O*-benzoyl-2-deoxy-2-[(6-chloro-2,5-diamino-4-pyrimidinyl)amino]-1,4:3,6-dianhydro-D-mannitol (**21**) as a white foam which darkened on exposure to light (0.16 g, 72%): ^{1}H NMR (DMSO-d_6) δ 8.01 (2 H, m, OBz), 7.69 (1 H, m, OBz), 7.56 (2 H, m, OBz), 6.36 (1 H, d, *J* = 7.5 Hz, br, N^6H,

exchangeable), 5.74 (2 H, s, br, N^5H_2, exchangeable), 5.40 (1 H, dd, $J_{5',4'}$ = 5.4 Hz ,$J_{5',6'a,b}$ = 9.6 Hz, H5'), 4.88 (1 H, dd, $J_{4',5'}$ = 5.4 Hz, $J_{4',3'}$ = 5.1 Hz, H4'), 4.55 (1 H, dd, $J_{3',4'}$ = 5.1 Hz, $J_{3',2'}$ = 4.5 Hz, H3'), 4.50 (1 H, m, H2'), 4.06-3.95 (3 H, m, H1', H6'), 4.00 (2 H, s ,br, N^2H_2, exchangeable), 3.50 (1 H, dd, $J_{1',2'}$ = 8.1 Hz, $J_{1'a,b}$ = 10.2 Hz, H1'a); ^{13}C NMR (DMSO-d_6) δ 164.5, 155.3, 154.4, 141.7, 133.0, 128.9, 128.7, 128.3, 112.9, 80.4, 79.8, 74.6, 70.4, 68.9, 53.4; UV λ_{max} = 304.5 (ε = 6712), 228.0 (ε = 22196), λshl = 245.5.

The pyrimidine nucleoside **(21)** in triethyl orthoformate, acidified with c. HCl was stirred at ambient temperature for 18 h. The solvent was then removed and the solid obtained treated with 0.5 N HCl for 1 h. The mixture was then neutralized with 3 N NaOH to pH 8. The precipitate was filtered off under suction and dried in a vacuum desiccator (0.11 g, 68%): ^1H NMR (DMSO-d_6) δ 11.25 (1 H, 2s ,NCHO), 9.42 (1H, d, br, N^2H), 8.62 (1 H, s, H8), 8.06 (2H, m, OBz), 7.68 (1 H, m, OBz), 7.57 (2 H, m, OBz), 5.43 (1 H, m, H5'), 5.13 (1 H, m, H2'), 5.03 (1 H, m, H4'), 4.82 (1 H, m, H3'), 4.32 (1 H, m, H6'), 4.23 (1 H, m, H6'), 4.11 (1 H, m, H1'), 4.05 (1 H, m, H1'); UV λ_{max} = 290.5 (ε = 10675), 227.5 (ε = 42100), λshl = 251.5; Anal. Calcd for $C_{19}H_{16}ClN_5O_5$: C, 53.09; H, 3.75; N, 16.29. Found: C, 52.72; H, 3.78; N, 15. 78.

2-Deoxy-2-[1,6-dihydro-6-oxo-2-imino-(9*H*)-purin-9-yl]-1,4:3,6-dianhydro-D-mannitol (23). A suspension of the purine nucleoside **(22)** (0.10 g) was heated under reflux in 0.33 N NaOH (10 mL) for 5 h. After 1 h complete dissolution had occurred. The solution was then allowed to cool to room temperature before neutralizing with dilute HCl. Removal of the solvent under vacuum gave a white solid residue which was dissolved in a small amount of methanol and added to a silica gel column and eluted with 4:1 chloroform-methanol. Combination of the appropriate fractions and removal of the solvent gave a white crystalline solid. Recrystallization from hot water gave fine white needles (0.07 g, 92%); ^1H NMR (DMSO-d_6) δ 10.59 (1 H, s, br, N^3H), 7.74 (1H, s, H8), 6.45 (2 H, s, br, N^2H), 5.04 (1 H, d, J = 6.6 Hz, br, OH), 4.83 (1 H, m, H2'), 4.59 (1 H, dd, $J_{3',4'}$ = 4.5 Hz, $J_{3',2'}$ = 4.8 Hz, H3'), 4.50 (1 H, dd, $J_{4',5'}$ = 4.5 Hz, $J_{4',3'}$ = 4.5 Hz, H4'), 4.28-4.19 (2 H, m, H1'), 4.06 (1 H, m, H6'), 3.80 (1 H, m, H6'), 3.52 (1H, m, H5'); ^{13}C NMR (DMSO-d_6) δ 156.8, 153.4, 151.3, 136.1, 115.3, 102.1, 82.0, 79.7, 71.8, 68.6, 56.0; UV λ_{max} (pH=7) 250.5 (ε = 8879), λ_{shl} = 269.0; λ_{max}(pH=1) 254.5 (ε = 7005), 277.0(ε = 4516), λ_{max}(pH = 14) 265.5 (ε = 6727), λ_{shl} = 255.5; Anal. Calcd for $C_{11}H_{13}N_5O_4$: C, 47.31; H, 4.69; N, 25.08. Found: C, 46.91; H, 4.67; N, 24.68.

Single Crystal X-ray Structure Determination of Compound 12b. A colorless crystal, 0.58mm x 0.41 mm x 0.28 mm, was used for the structure determination. Data were collected on an Enraf-Nonius CAD-4 diffractometer (Mo Kα, 0.7107 Å). A total of 2854 reflections were collected of which 2619 were unique reflections. Cell dimensions were determined from 23 reflections ($25 < 2\theta < 44$). The space group was $P2_12_12_1$. The structure was solved by direct methods and refined by full matrix least squares method (non-H anisotropic). All hydrogen atoms were located from difference maps and refined. Anisotropic refinements on all non-hydrogen atoms and isotropic refinements on hydrogen atoms gave $R = 0.033$ and $R_w = 0.039$. The standard deviation of unit weight was 0.98. A list of refined coordinates, estimated standard deviations, bond distances, and bond angles will be deposited at the Cambridge Crystallographic Data Center.

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