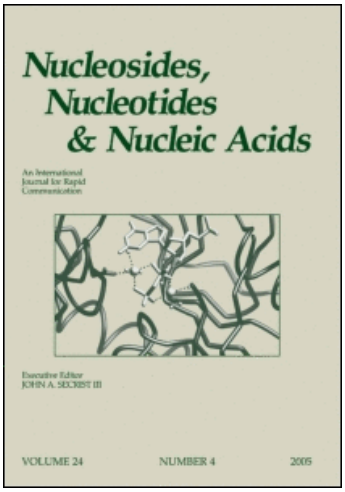

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STEREOSELECTIVE SYNTHESIS OF 6-METHYL-9-(2-DEOXY-β-D-*ERYTHRO*-PENTOFURANOSYL)PURINE

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Abstract: The coupling of the sodium salt of 6-methylpurine with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride in acetonitrile gives the di -*O*-*p*-toluoyl protected 9-β nucleoside regio- and stereo-selectively in good yield. Methoxide deprotection followed by preparative hplc then affords pure 6-methyl-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine.

Introduction. 6-Methyl-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**1**) [trivially named 6-methylpurine 2'-deoxyribofuranoside or 6MPDR] is a compound that is not toxic to mammalian cells *per se* as the purine nucleoside phosphorylase [PNPase] which they contain differs from the PNPases of certain bacteria, mycoplasma and some parasites in its utilization of substrate. But, mammalian cells expressing the prokaryotic gene become susceptible to treatment with 6MPDR as it is metabolized to 6-methylpurine and 6-methylpurine riboside which are toxic. This approach has been used to effect killing of colonic carcinoma cells[1] as an alternative to the use of ganciclovir in tandem with Herpes virus thymidine kinase.[2] We required a convenient chemical source of high-purity 6MPDR to further exploit its biological properties.

Results and Discussion. 6-Methyl-9-(2-deoxy-β-D-*erythro*-pentofuranosyl) purine (**1**) has been prepared in 13% yield by the fusion of 6-methylpurine with tri-*O*-acetyl-2-deoxy-D-ribofuranose.[3] This low yield was a result of the coupling reaction producing more of the α than the β anomer. In an earlier report[4] only the

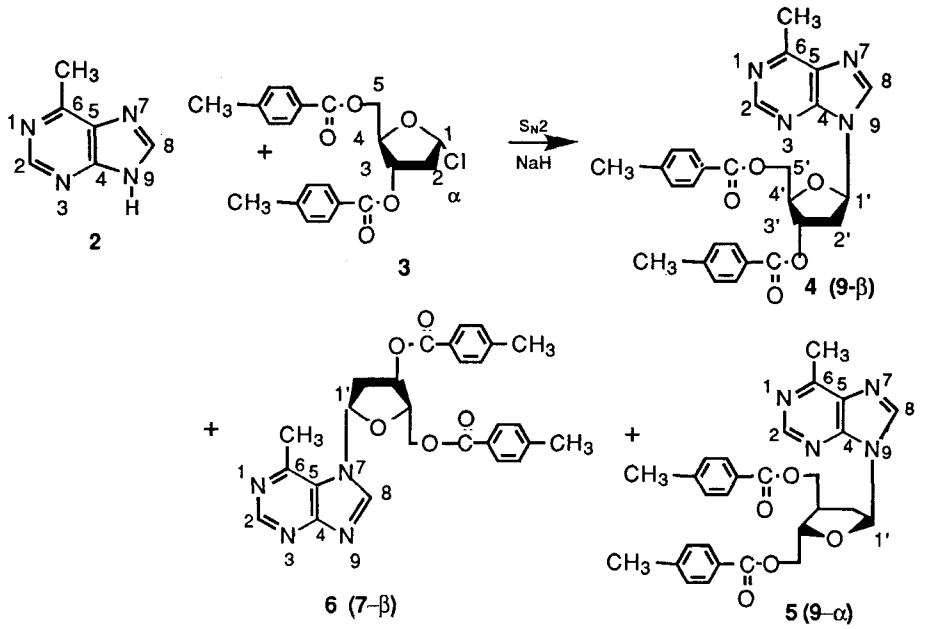
α anomer was isolated. Another source[5] of **1** is by the biotransformation of 6-methylpurine with 2'-deoxyuridine in the presence of thymine-dependent Escherichia coli mutant cells encapsulated in alginate gel with a conversion of 55%.

Other 9-β 2'-deoxyribonucleosides have been prepared[6-12] by reacting a protected α-deoxyribofuranosyl halide with the sodium salt of the purine in a polar, aprotic solvent; giving the products in good yield with high regio- and stereoselectivity. We have now developed this method to provide a high-yielding, high-purity source of 6MPDR (**1**).

The necessary starting material for the 6MPDR (**1**) is 2-deoxy-α,β-*erythro*-pentofuranose (2-deoxy-D-ribose). Hoffer[13] described its conversion first to 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranoside, and thence to the crystalline 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**3**).[13,14] Portionwise addition of 1 equivalent of the α-glycosyl chloride **3** over 1 hour to a suspension of the anion formed by treatment of 6-methylpurine (**2**) with 1.15 equivalents of sodium hydride, in dry acetonitrile under nitrogen gave a mixture of the 9-β, 9-α and 7-β nucleosides **4**, **5** and **6**: in a ratio of *ca* 8:2:1 respectively (by ^{1}H nmr analysis) (**SCHEME 1**). The desired blocked 9-β nucleoside **4** was purified by radial thin layer chromatography and isolated in 69% yield. This reaction could be readily carried out on a scale of several grams. The separation was such that flash chromatography should be equally useful here.

Purification at this stage required unambiguous identification of the isomers and this was achieved by analysis of ^{1}H nmr chemical shifts (see **TABLE I**). N-7 purine nucleosides are typically differentiated from their N-9 isomers by the characteristic downfield chemical shifts[6,12] for the anomeric 1'-H and the purine 8-H resonances. The assignment of configuration at the anomeric carbon of **4** and **5** as β and α respectively was based on both that 2-H and 8-H resonances of the α anomer are downfield from their β counterparts,[12] and that for the isolated pure 3',5'-diacyl derivative **4** there was the characteristic[12] small chemical shift difference between 4'-H and 5',5''-H resonances.

Isomer **6** also exhibits a similar pattern and was assigned the β configuration. The standard criterion for assignment of stereochemistry - that of comparison[6,12] of the splitting pattern of the 1'-H proton - could not be applied because the appearances of the anomeric resonances of all three di-*O*-*p*-toluoyl compounds were similar.



SCHEME 1

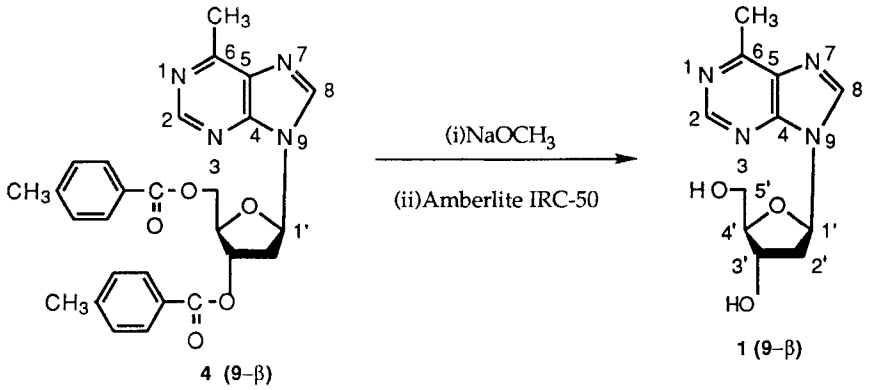
TABLE I. Partial ^1H nmr Chemical Shifts Of 6-Methyl-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-D-ribofuranosyl)purines in $CDCl_3$

compd	isomer	H-8	H-2	H-1′	H-3′	H-4′	H-5′5″
4	9-β	8.67	8.12	6.48 (dd*)	5.73	4.66	4.55
5	9-α	8.83	8.32	6.58 (t)	5.64	4.78	4.58
6	7-β	8.90	8.41	6.83 (t)	5.67	4.73	4.58

*$J_{1'2'}$ = 6Hz, $J_{1'2''}$ = 8Hz

Treatment of 6-methyl-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pento furanosyl)purine (**4**) with 1.3 equiv of sodium methoxide in dry methanol, followed by neutralization with Amberlite IRC-50 (H^+) ion exchange resin gave the 9-β purine 2′-deoxyribonucleoside **1**, which was purified by chromatography on silica. Recystallization provided a solid with a comparable melting point to that reported for the previously prepared 9-β compound,[3] however careful reversed-phase hplc showed the presence of minor impurities. Importantly, one of the impurities was the cytotoxic substrate 6-methylpurine (**2**), probably carried

through from the initial coupling reaction. This material was pure enough for normal synthetic purposes however our application required complete removal of the 6-methylpurine(**2**). Preparative hplc provided



6-methyl-9-(2-deoxy-β-D-*erythro*-pentofuranosyl) purine (**1**) of >99% purity.

This provides a superior laboratory scale method for the synthesis of 6-methyl-9-(2-deoxy-β-D-*erythro*-pentofuranosyl) purine (**1**),an important nucleoside with potential for therapeutic application.

Experimental.

General. Melting points were taken in open capillary tubes on a Gallenkamp 5 6797 melting point apparatus and are uncorrected. ^{1}H and ^{13}C nmr spectra were recorded at 200.13 and 50.323 MHz respectively in 5 mm tubes on a Bruker AC 250 spectrometer, using an internal lock. Chemical ionization accurate masses were obtained on a JEOL JMS-DX 303 Mass Spectrometer. Room temperature was 20-25°. Silica gel 60 PF_{254} containing gypsum was used for radial thin-layer chromatography. Plates used for this chromatography were coated with a 75mm band of silica of 4mm thickness. For reversed-phase preparative hplc a Waters 590 programmable hplc pump with an Alltech Econosil C18 (10μm) 250 X 22.5mm column was used, with uv detection at 240nm. Anhydrous methanol was freshly distilled from magnesium methoxide. Ether was dried with sodium wire; and acetonitrile with 3Å molecular sieves. 6-Methylpurine (**2**) and 2-deoxy-D-ribose were purchased from the Aldrich Chemical Company.

2-Deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride (3) This, 7.2g was prepared from 4.9g of 2-deoxy-D-ribose(51%) by Hoffer's procedure,[13] as a white powder, mp 121-123° (d) (lit[13] 109°). (This unstable product must be washed until free of acid. It is best stored *in vacuo* in the presence of soda lime at 0-5° for at least several days before use and then used within two weeks

subsequent to that[15]. Decomposition is autocatalytic. ^{13}C nmr ($CDCl_3$) δ 21.70, Ar-CH_3; 44.50, C-2; 63.49, C-5; 73.54, C-3; 84.70, C-4; 95.35, C-1; 126.64, ArC-4; 126.77, ArC-4; 129.21, ArC(-2,6); 129.65, ArC(-2,6); 129.89, ArC(-2,6); 144.04, ArC-1; 144.27, ArC-1; 166.04, C(O); 166.37, C(O). ^1H nmr ($CDCl_3$)δ 2.40, s, 6H, CH_3; 2.73, d, $J_{2,2'}$ = 15Hz, 1H, H-2; 2.80, ddd, $J_{2,2'}$ = 15Hz, $J_{2,3'}$ = 7Hz, $J_{2',1}$ = 5Hz, 1H, H-2; 4.59, ABX, $J_{5,5'}$ = 12Hz, $J_{5,4}$ = 4.5Hz, 1H, H-5; 3.85, ABX, $J_{5,5'}$ = 12Hz, $J_{5',4}$= 3Hz, 1H, H-5'; 4.86, q, J = 4Hz, 1H, H-4; 5.57, dm, J = 7Hz, 1H, H-3; 6.48, d, $J_{1,2}$ = 4.5Hz, 1H, H-1; 7.22, d, J = 8Hz, 2H, Ar-H(3,5); 7.25, d, J = 9Hz, 2H, Ar-H(3,5); 7.90, d, J = 8Hz, 2H, Ar-H(2,6); 8.00, d, J = 8Hz, 2H, Ar-H(2,6).

6-Methyl-9-(2-deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)purine (4). To a suspension of 6-methylpurine (**2**) (0.67g, 5.0mmol) in dry acetonitrile (25ml) was added portionwise sodium hydride (60% in oil, 0.23g, 5.8mmol) and the mixture was stirred at room temperature under a nitrogen atmosphere for 1h. 2-Deoxy-3,5-di-*O-p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**3**) (1.94g, 4.99mmol) was then added in small aliquots over 1 hour with stirring, and the whole then stirred for 4 h before pouring into a mixture of brine (100ml) and potassium dihydrogen orthophosphate (0.78g, 5.73mmol) in water (5ml). The mixture was extracted with ether (3×50ml), washed with brine (2×50ml), dried and concentrated *in vacuo* to leave a coloured gum, 1.84g, which was largely a mixture of *6-methyl-9-(2-deoxy-3,5-di-*O-p-*toluoyl-β-D-*erythro-*pentofuranosyl)-purine (4), 6-methyl-9-(2-deoxy-3,5-di-*O-p-*toluoyl-α-D-*erythro-*pentofuranosyl)purine (5)* and *6-methyl-7-(2-deoxy-3,5-di-*O-p-*toluoyl-β-D-*erythro-*pentofuranosyl)purine (6).* By ^1H nmr analysis of the purine 2-H and 8-H signals, and the sugar 1'-H and 3'-H resonances, the ratio of ***4:5:6*** was *ca* 8:2:1.

The mixture was purified by radial thin-layer chromatography on silica, in 6 portions, starting with dichloromethane then using dichloromethane/ methanol (with an increasing gradient of polarity up to 20:1) as eluent to afford **4** (1.68g, 69%) as an ochre *gum.* (Found: $MH^{+\cdot}$, 487.1979. $C_{27}H_{26}N_4O_5$ requires $MH^{+\cdot}$, 487.1981.) ^{13}C nmr (CD_3OD) δ 19.38, Het-CH_3; 21.65, Ar-CH_3; 37.56, C-2'; 63.89, C-5';.75.06, C-3'; 82.99, C-4'; 84.81, C-1'; 126.31 and 126.57, ArC-4; 129.18,129.54 and 129.71, Ar(C-2,6); 133.53, C-5; 142.00, C-8; 144.03 and 144.42, ArC-1; 149.95, C-6 or C-4; 152.16, C-2; 159.44, C-4 or C-6; 165.84, C(O); 166.04, C(O);.^1H nmr ($CDCl_3$) δ 2.26, s, 3H, Ar-CH_3; 2.31, s, 3H, Ar-CH_3; 2.72, s, 3H, Het-CH_3; 2.76, m, 1H, H-2' or H-2"; 3.12, m, 1H, H-2' or H-2"; 4.55, m, 2H, H-5'/5"; 4.66, dd, J = 5.7Hz, 1H, H-4'; 5.73, d, J = 5Hz, 1H, H-3'; 6.48, t, J = 6.8Hz, 1H, H-1'; 7.08, d, J = 8Hz, 2H, Ar-H(3 or 5); 7.15, d, J = 8Hz, 2H, Ar-H(3 or 5); 7.78, d, J = 8Hz, 2H, Ar-H(2,6); 7.85, d, J = 8Hz, 2H, Ar-H(2,6); 8.12, s, 1H, H-2; 8.67,.s, 1H, H-8.

^{1}H nmr analysis, s, 8.38 and s, 8.82 (obscured) indicated the presence of 6-methylpurine (**2**) at a level of 1-2%.

Compounds *5* and *6* were not separated (0.70g, 29%).

5: ^{1}H nmr partial ($CDCl_3$) δ 4.58, m, 2H, H-5′/5″; 4.78, m, 1H, H-4′; 5.64, m, 1H, H-3′; 6.56, t, *J* = 6Hz, 1H, H-1′; 8.32, s, 1H, H-2; 8.83,.s, 1H, H-8.

6: ^{1}H nmr partial ($CDCl_3$) δ 4.58, m, 2H, H-5′/5″; 4.73, m, 1H, H-4′; 5.67, d, *J* = 2Hz, 1H, H-3′; 6.48, t, *J* = 6Hz, 1H, H-1′; 8.41, s, 1H, H-2; 8.90,.s, 1H, H-8.

6-Methyl-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (1). To a solution of the nucleoside **4** (1.13g, 2.32mmol) in anhydrous methanol (30ml) was added a solution of sodium methoxide (3.0ml) which had been prepared previously by dissolving sodium metal (0.248g, 10.8mmol) in methanol (10ml). The resultant solution was then stirred for 2 h at room temperature, and neutralized with ion-exchange resin [Amberlite IRC-50(H^+), 3.00g of 10.0 meq/g min in *ca* 15 ml of methanol] that had been rinsed well with anhydrous methanol. The resultant mixture was stirred for 2 h (pH neutral), filtered and, after washing the resin well with methanol, the combined solutions were concentrated *in vacuo*. The residue was dissolved in methanol and subjected in 1 portion to radial thin-layer chromatography on silica starting with dichloromethane then using dichloromethane/methanol (with an increasing gradient of polarity up to 1:1) as eluent to afford a waxy off-white solid which was recrystallized from methanol/dichloromethane to give the nucleoside (**1**) as a waxy white solid (0.47g, 81%) mp 151.8 - 155.5° (d) (lit[3] 153°). (Found: $MH^{+\cdot}$, 251.1160. $C_{11}H_{14}N_4O_3$ requires $MH^{+\cdot}$, 251.1144.), ^{13}C nmr (CD_3OD) δ 19.18, Het-CH_3; 41.37, C-2′; 63.29, C-5′; 72.68, C-3′; 86.60, C-4′; 89.69, C-1′; 134.07, C-5; 145.71, C-8; 151.20, C-6 or C-4; 152.72, C-2; 160.17 C-4 or C-6. ^{1}H nmr (CD_3OD) δ 2.47, ddd, *J* = 3.5, 6.3, 13.5Hz, 1H, H-2′ or H-2″; 2.78, s, 3H, CH_3; 2.85, m, 1H, H-2′ or H-2″; 3.75, ABX, $J_{5',5''}$ = 14.0Hz, $J_{5',4'}$ = 3.9Hz, 1H, H-5′ or H-5″; 3.85, ABX, $J_{5',5''}$ = 12.2Hz, $J_{5',4'}$ = 3.4Hz, 1H, H-5′ or H-5″; 4.06, dd, *J* = 3.4, 6.8Hz, 1H, H-4′; 4.61, quintet, *J* = 3.1Hz, 1H, H-3′; 6.53, t, *J* = 6.8Hz, 1H, H-1′; 8.68, s, 1H, H-2; 8.73,.s, 1H, H-8.

Reversed-phase hplc - using an isocratic elution solution consisting of 20/80 v/v methanol/ 10mM ammonium hydrogen carbonate buffer solution - showed minor impurities. The most significant of these was 6-methylpurine, less than 1%, with other isomeric nucleoside(s) also present. Portions (40mg) of this material were purified by preparative hplc using ammonium formate buffer solution (removed from the product by vacuum) to provide a sample of >99% pure **1** (34mg), which crystallized from acetone in white needles mp 156.9 - 157.5°(d). $[\alpha]_D^{25}$ -12.6 ± 0.2 (*c* 0.45g, 0.90g/ 100ml of MeOH).

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