Heterocyclic *N*-Glycosyl Derivatives. 15. Synthesis of Unsaturated 6-Methylthiopurine Nucleosides

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The recognition of the significant anticancer activity and the clinical importance of 6-mercapto- and 6-methylthiopurine ribofuranosides[1] has stimulated the synthesis of new nucleosides of these and related bases having mercapto groups.[2] As part of an investigation on the preparation of nucleosides of potential biological interest, we have now studied the reaction of 6-methylthiopurine with 3,4-di-*O*-acetyl-D-xylal and 3,4,6-tri-*O*-acetyl-D-glucal. The ability of acylated glycals to react with purine derivatives in the presence of acid catalyst is well documented.[3] Glycosidation of 6-methylthiopurine (I) with 3,4-di-*O*-acetyl-D-xylal (II) was carried out according to the method previously described[4] to give a mixture of the unsaturated nucleosides IIIa, IVa, and Va in 64% total yield. Products were separated by thick-layer chromatography and identified on the basis of analysis and spectroscopic data. The magnetic parameters obtained by first-order analysis of the nmr spectra of these nucleosides are shown in Table I. The chemical shifts and coupling constants when compared with those of the nucleosides obtained from the reaction of 6-chloropurine and 3,4-di-*O*-acetyl-D-xylal,[4] namely 9-(4-*O*-acetyl-1,2,3-trideoxy-D-*threo*-pent-1-enopyranos-3-yl)-6-chloropurine and 9-(4-*O*-acetyl-2,3-dideoxy-α- and -β-D-*glycero*-pent-2-enopyranosyl)-6-chloropurine, provided conclusive evidence on the conformations of nucleosides IIIa, IVa, and Va. On the basis of this comparison the compound with glycal structure IIIa and the α anomer nucleoside IVa were considered to exist in chloroform solution as mixtures of the two chair conformers in equilibrium, with the H1 form preponderating. On the other hand, the β anomer Va adopts almost exclusively the 1H conformation (Scheme I).

In a similar manner, reaction of 6-methylthiopurine with 3,4,6-tri-*O*-acetyl-D-glucal afforded the anomeric unsaturated nucleoside pair VIIa and VIIIa in 77% yield, with the α anomer predominating. The H1 conformation for each of these nucleosides was assigned from the values of $J_{4',5'}$ (8.3 and 8.7 Hz, respectively) which are characteristic of a quasi-axial–axial relationship of H-4′ and H-5′. Furthermore, the close resemblance between the reported chemical shifts for 9-(4,6-di-*O*-acetyl-2,3-dideoxy-α- and -β-D-*erythro*-hex-2-enopyranosyl)-6-chloropurine, which are known to adopt the H1 conformation,[5] and those obtained for nucleosides VIIIa and VIIa (Table II) corroborated the above conformational assignment and established the anomeric configuration of VIIIa and VIIa as α and β, respectively.

The glycosidation site in all the above nucleosides was established by uv spectroscopy. Absorption maxima and extinc-

Scheme I

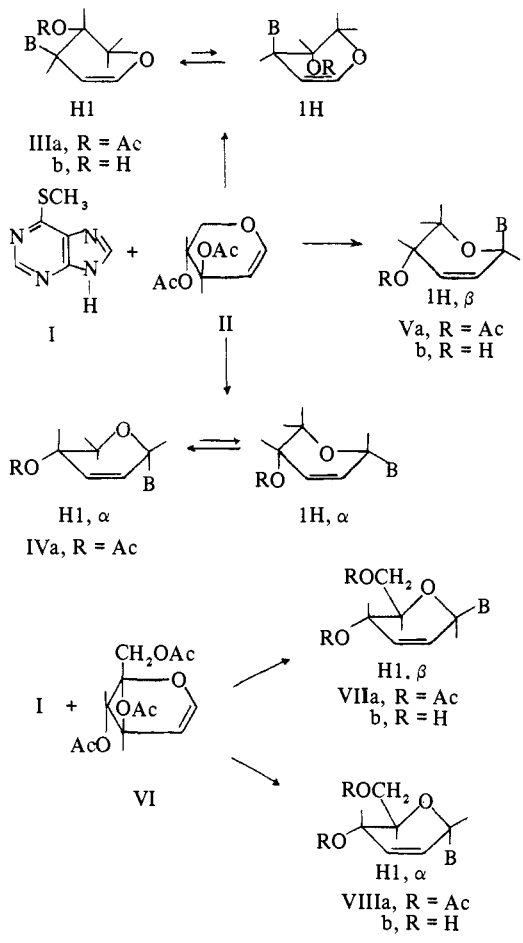

Table I. Nmr Parameters for Nucleosides IIIa, IVa, and Va and Related 9-(4-*O*-Acetyl-1,2,3-trideoxy-D-*threo*-pent-1-enopyranos-3-yl)-6-chloropurine (IX)[a] and 9-(4-*O*-Acetyl-2,3-dideoxy-α- and -β-D-*glycero*-pent-2-enopyranosyl)-6-chloropurine (X and XI),[a] 60 MHz, $CDCl_3$

Compd	Anomeric confign	Chemical shifts, τ values									
		H-1′	H-2′	H-3′	H-4′	H-5′e[b]	H-5′a[b]	H-2	H-8	S-CH_3	AcO
IIIa		3.11	5.02	4.44–4.87		5.56–6.27		1.23	1.83	7.26	7.89
IVa	α	3.45	3.80	3.58	4.65	5.91	6.14	1.22	1.85	7.24	7.85
Va	β	———	3.27–3.82	———	4.73	6.0		1.20	1.92	7.25	7.85
IX		3.15	5.02	4.70	4.74	5.82	5.96	1.24	1.70		7.84
X	α	3.42	3.78	3.54	4.65	5.90	6.13	1.20	1.66		7.87
XI	β	3.37	3.67	3.46	4.74	5.99		1.20	1.76		7.88

		Coupling constants, Hz							
		$J_{1',2'}$	$J_{1',3'}$	$J_{2',3'}$	$J_{2',4'}$	$J_{3',4'}$	$J_{4',5'a}$[b]	$J_{4',5'e}$[b]	$J_{5'a,5'e}$[b]
IIIa		6.0	−1.1	3.8	1.5				
IVa	α	2.8	−1.4	9.9	−1.1	3.1	5.2	4.3	−12.3
Va	β						Sum 5.8		
IX		6.0	−1.0	3.9	1.0	4.0	2.7	4.8	−12.0
X	α	2.03	−2.03	10.00	−1.24	3.52	5.21	4.25	−12.20
XI	β	3.13	−1.83	10.08	−1.13	4.53	Sum 5.76		

[a] See ref 4. [b] H-5′a and H-5′e refer to the more abundant conformation.

Table II. Comparison of the Chemical Shifts (τ Values) for Nucleosides VIIa and VIIIa with Those for 9-(4,6-Di-*O*-acetyl-2,3-dideoxy-α- and -β-D-*erythro*-hex-2-enopyranosyl)-6-chloropurine (XIII and XII),[a] 60 MHz, $CDCl_3$

Compd	Anomeric confign	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-6′	H-2	H-8	SCH_3	AcO	
VIIa	β	3.29	3.86	3.74	4.46	———	5.74	———	1.29	1.87	7.29	7.86	7.96
VIIIa	α	3.37	3.73	3.64	4.52	6.07	5.77	5.91	1.2	1.85	7.24	7.84	8.00
XII	β	3.24	3.89	3.70	4.44	5.78	5.72	5.74	1.16	1.68		7.82	7.92
XIII	α	3.35	3.76	3.60	4.52	6.05	5.75	5.90	1.18	1.67		7.85	8.00

[a] See ref 5.

tion coefficients were in the range of those reported for the model compound 9-methyl-6-methylthiopurine.[6]

Nucleosides IIIb and Vb and the acetylated derivatives IIIa, IVa, Va, VIIa, and VIIIa were evaluated for their *in vitro* cytotoxic activity against HeLa tumoral cells according to the criteria established by the CCNSC.[7] Compounds IIIa, IIIb, Va, and Vb were found to be active at a 50% inhibiting dose (ID_{50}) of 62, 23, 54, and 19 μg/ml, respectively. The ID_{50} of all the other compounds screened was higher than 100 μg/ml.

Experimental Section

Melting points are uncorrected. Nmr spectra were recorded on a Perkin-Elmer R-12 spectrometer with TMS as the internal standard. Uv spectra were recorded on a Perkin-Elmer 350 spectrophotometer. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter. Preparative layer chromatography was performed on glass plates (20 × 20 cm) coated (2 mm thickness) with silica gel PF_{254} (Merck). Silica gel GF_{254} (Merck) was used for tlc (0.25 mm thickness). Spots were visualized with uv light (254 nm).

Reaction of 6-Methylthiopurine with 3,4-Di-*O*-acetyl-D-xylal. A mixture of 1.66 g (0.01 mol) of 6-methylthiopurine (I) and 4 g (0.02 mol) of 3,4-di-*O*-acetyl-D-xylal (II) in 50 ml of EtOAc containing a few drops of trifluoroacetic acid was heated at 95° in a sealed tube under continuous agitation for 96 hr. After this time a few additional drops of trifluoroacetic acid were added and the reaction was continued for 24 hr. The solvent was evaporated under reduced pressure to a thick syrup which was dissolved in chloroform and the solution applied to 22 preparative plates. The plates were developed ten times with a mixture of Et_2O–petroleum ether (3:1) resulting in the separation of three major bands.

9-(4-*O*-Acetyl-1,2,3-trideoxy-D-*threo*-pent-1-enopyranos-3-yl)-6-methylthiopurine (IIIa). The fastest band gave 1.22 g of a syrup which was further purified by preparative tlc (five plates, EtOAc–petroleum ether 1:2) to give solid IIIa: mp 112–114° (from EtOAc–petroleum ether); $[\alpha]D$ −86.4° (*c* ~0.75, $CHCl_3$); uv λ_{max} (EtOH) 284 nm (ε 17,800), 291 (sh, 16,800); yield 40%. *Anal.* ($C_{13}H_{14}N_4O_3S$) C, H, N.

9-(4-*O*-Acetyl-2,3-dideoxy-α-D-*glycero*-pent-2-enopyranosyl)-6-methylthiopurine (IVa). Extraction of the second band followed by rechromatography using EtOAc-petroleum ether (1:2) afforded 0.25 g of IVa as a syrup: $[\alpha]D$ +50.4° (*c* ~0.5, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 18,190), 290 (sh, 17,300); yield 8.2%. *Anal.* ($C_{13}H_{14}N_4O_3S$) C, H, N.

9-(4-*O*-Acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enopyranosyl)-6-methylthiopurine (Va). The slowest band afforded 0.5 g of a syrup which was further chromatographed (tlc, EtOAc–petroleum ether 1:2) to give Va as an amorphous solid: $[\alpha]D$ +145° (*c* ~0.5, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 19,100), 290 (sh, 17,950); yield 16.5%. *Anal.* ($C_{13}H_{14}N_4O_3S$) C, H, N.

Reaction of 6-Methylthiopurine with 3,4,6-Tri-*O*-acetyl-D-glucal. A mixture of 1.66 g (0.01 mol) of 6-methylthiopurine and 5.44 g (0.02 mol) of 3,4,6-tri-*O*-acetyl-D-glucal in 70 ml of EtOAc containing trifluoroacetic acid as a catalyst was heated for 40 hr as above. After this time trifluoroacetic acid was added (4 drops) and the heating was continued for 30 hr. The crude reaction product was separated into two major fractions by preparative layer chromatography (40 plates) after 15 consecutive developments using Et_2O–petroleum ether (3:1).

9-(4,6-Di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIa). The fastest moving band afforded 1.46 g of a syrup which was rechromatographed using Et_2O–petroleum ether (3:1) to give 1.25 g of VIIa as a chromatographically homogenous syrup: $[\alpha]D$ +93.9° (*c* ~0.75, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 18,500), 290 (sh, 17,800); yield 33%. *Anal.* ($C_{16}H_{18}N_4O_5S$) C, H, N.

9-(4,6-Di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIIa). The slowest moving band afforded 2.26 g of a solid material which was further purified by preparative tlc (Et_2O–petroleum ether 3:1) to give 1.69 g of solid VIIIa: mp 119–120° (from EtOAc–petroleum ether); $[\alpha]D$ +35.3° (*c* ~0.6, $CHCl_3$); uv λ_{max} (EtOH) 283 nm (ε 18,350), 290 (sh, 17,400); yield 44%. *Anal.* ($C_{16}H_{18}N_4O_5S$) C, H, N.

9-(1,2,3-Trideoxy-D-*threo*-pent-1-enopyranos-3-yl)-6-methylthiopurine (IIIb). Treatment of IIIa (0.5 g) with 40 ml of methanolic ammonia (methanol saturated with ammonia at 0°) at room temperature for 24 hr caused the formation of IIIb which was purified by preparative tlc (EtOAc) to give solid IIIb: mp 176–177° (from EtOAc–Et_2O); $[\alpha]D$ −142.4° (*c* ~0.5, EtOH). *Anal.* ($C_{11}H_{12}N_4O_2S$) C, H, N, S.

9-(2,3-Dideoxy-β-D-*glycero*-pent-2-enopyranosyl)-6-methylthiopurine (Vb). Treatment of 0.16 g of Va as above afforded 40 mg of Vb: mp 170–171° (from EtOAc–petroleum ether); $[\alpha]D$ +145° (*c* ~0.5, EtOH). *Anal.* ($C_{11}H_{12}N_4O_2S$) C, H, N.

9-(2,3-Dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIb). Treatment of VIIa (0.47 g) as in the preceding cases afforded a crude compound which was purified by preparative tlc (EtOAc) to give VIIb: mp 131° (from EtOAc–petroleum ether); $[\alpha]D$ +130.7° (*c* 0.25, EtOH). *Anal.* ($C_{12}H_{14}N_4O_3S$) C, H, N.

9-(2,3-Dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-6-methylthiopurine (VIIIb). Deacetylation of VIIIa (0.34 g) as above afforded 0.21 g of solid VIIIb: mp 91–92° (from H_2O); $[\alpha]D$ −2° (*c* ~1, MeOH). *Anal.* ($C_{12}H_{14}N_4O_3S$) C, H, N, S.

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β-Chloroethylamines Related to Mescaline

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β-Haloethylamines are precursors of a variety of aziridines of interest as irreversible blocking agents. Current evidence indicates that a drug can be transformed into an alkylating analog without disturbing its receptor specificity. Examples