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REGIOSELECTIVITY OF GLYCOSYLATION REACTION OF 4,6-BIS(METHYLTHIO)-PYRAZOLO-[3,4-d]PYRIMIDINE WITH D-XYLOFURANOSYL- AND D-GLUCOFURANOSYL SUGARS+

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Abstract : *Steric control offered by the 3-substituents in D-xylofuranosyl- and D-glucofuranosyl sugars has been exploited for the regioselective N-2-glycosylation of 4,6-bis(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (1), to generate key intermediates suitable for later transformations to pyrazolo[3,4-d]pyrimidine nucleosides.*

Nucleoside analogues are the centre of current interest as antiviral chemotherapeutic agents[1]. Potent antileishmanial activity of allopurinol riboside has generated considerable interest in pyrazolo[3,4-d]pyrimidine nucleosides and the subject has been reviewed[2]. Recently, we have reported a convenient method[3] for the regioselective N-2-alkylation of 4,6-bis(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (**1**) which is an important intermediate in the synthesis of pyrazolo [3,4-d]pyrimidine nucleosides with an objective[4] of comparing their biological activities with corresponding N-1-isomer. In order to compare the biological activity of isomeric N-1- and N-2-nucleosides of pyrazolo[3,4-d]pyrimidine, there was need for a method which could be utilised for the regioselective N-2-glycosylation of an appropriate pyrazolo[3,4-d]pyrimidine base. In this report we describe our approach to achieve this goal (Scheme).

Ribosylation of 4,6-bis(methylthio)-1H-pyrazolo[3,4-d]pyrimidine has been reported by a Russian group[5]. The method produced a mixture of N-1- and N-2-nucleosides (as triacetates) in a ratio of 2:1. Use of higher temperature produced a much better yield of the thermodynamically more stable, N-1-isomer at the cost of N-2-isomer[5]. Several other groups[6] have utilised **1** and closely related pyrazolo[3,4-d]pyrimidines for ribosylation reactions, yielding the N-1-isomer as the major product.

Recently, we have had an occasion to synthesise[7] 4-amino-6-methylthio-1-(3'-deoxy-β-D-ribofuranosyl)-1H-pyrazolo[3,4-d]pyrimidine for biological evaluation. Thus, condensation of **1** with 1,2-di-O-acetyl-5-O-benzoyl-3-deoxyribofuranose produced N-1- and N-2-isomers in a ratio of 30:1 (entry 2). Under identical conditions ribosylation gave N-1- and N-2-nucleosides in a ratio of 6:1 (entry 1). Analysis of these results and examination of molecular models lead us to believe that use of an appropriate sugar like D-xylofuranose which has 3-hydroxy group in the β-configuration as opposed to the α-configuration in D-ribofranose should favour the formation of N-2-β-anomer for steric reasons. Similar logic has been given to explain the formation of unexpected 9-α-anomer in the condensation of 8-azapurine with 1,2,3,5-tetra-O-acetyl-D-xylofuranose[8]. We have observed that glycosylation of **1** with 1,2,di-O-acetyl-3,5-di-O-benzoyl-D-xylofuranose under similar conditions gave N-2-β-isomer as the major product along with N-1-α-isomer (entry 3)[9]. Interestingly, introduc-

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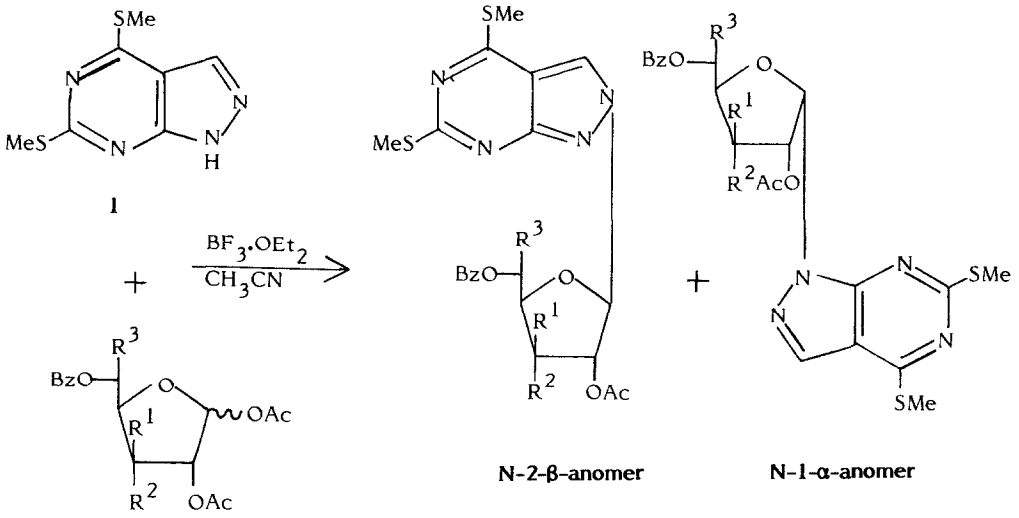

SCHEME

tion of a methoxy group at 3-position with only slightly different steric and electronic requirement as compared to hydroxy yielded exclusively N-2-β-isomer (entry 4).

Considerable interest exists in the preparation of 'hexofuranosyl nucleosides', primarily because of the structural similarity of these substances to the naturally occurring ribose nucleosides. The presence of a furanose ring in the latter compounds is an obvious feature of the 'hexofuranosyl nucleosides' which would be desirable to retain in preparing analogues which might act as metabolic inhibitors and possess antiviral or antitumour properties[10b]. Thus, a variety of 'hexofuranosyl nucleosides' of purines have been studied[10]. A series of hexofuranosyladenine nucleosides have been tested as substrates and inhibitors of adenosine deaminase and some of these have shown activity[10c]. This coupled with the observation that no 'hexofuranosyl nucleosides' of pyrazolo[3,4-d] pyrimidines appears to have been studied, has prompted us to extend our studies to include this class as well.

D-Glucofuranose which can be viewed as 5-hydroxymethyl-D-xylofuranose, was thus expected to behave like D-xylofuranose during glycosylation reactions. Experimental results (entries 5, 6 and 7) have confirmed that indeed it follows the same course of reaction. To our knowledge these are the first examples of 'hexofuranosyl nucleosides' involving a pyrazolo[3,4-d]pyrimidine ring system having an isosteric relationship with the purine system.

Conversion of many of these protected nucleosides to appropriate nucleosides is in progress and will be reported in due course along with their biological activities.

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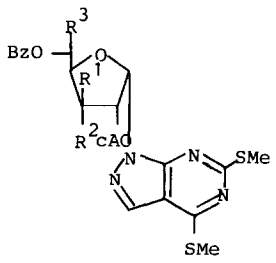
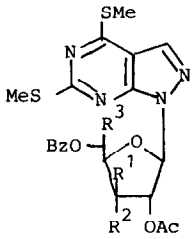
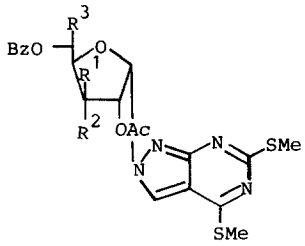
Table : Glycosylation of 4,6-bis(methylthio)-1H-pyrazolo[3,4-d]pyrimidine with D-xylofuranosyl- and D-glucofuranosyl sugars



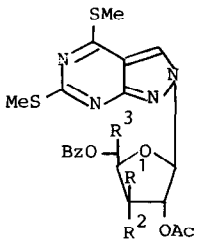
N-1-α



N-1-β



N-2-α



N-2-β

No.	R^1	R^2	R^3	Yield* %	N-1 α- ($J_{1',2'}$)	N-1 β- ($J_{1',2'}$)	N-2 α- ($J_{1',2'}$)	N-2 β- ($J_{1',2'}$)
1.	H (D-ribosyl)	OBz	H	70	-	60 (1 Hz)	-	10 (0 Hz)
2.	H (3-deoxy-D-ribosyl)	H	H	62	-	60 (0 Hz)	-	2 (0 Hz)
3.	OBz (D-xylosyl)	H	H	60	20 (4 Hz)	-	-	40 (0 Hz)
4.	OMe (D-xylosyl)	H	H	56	-	-	-	56 (0 Hz)
5.	OBz (D-glucofuranosyl)	H	CH_2-OBz	68	38 (3.5 Hz)	-	-	30 (0 Hz)
6.	OMe (D-glucofuranosyl)	H	CH_2-OBz	61.5	-	-	9.5 (3 Hz)	52 (0 Hz)
7.	OMs (D-glucofuranosyl)	H	CH_2-OBz	40	10 (3 Hz)	-	-	30 (0 Hz)

* Yields are of isolated products, calculated on the basis of reacted base (1) and have not been optimised. All the new compounds have been fully characterised by ^{1}H NMR, IR, UV, MS and elemental analyses.

References and Notes

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9. In the light of present results we would like to revise our earlier reported: N-1-β-nucleosides to N-2-β-isomers in D-xylofuranosyl series, ref. Hasan, A.; Tripathi, R.P.; Ram Pratap; Bhakuni, D.S.; Pal, R.; Mishra, A.; Guru, P.Y.; Katiyar, J.C. *Ind. J. Chem.*, **1989**, *28B*, 403.
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11. *Typical experimental procedure : 4,6-Bis(methylthio)-2-(2'-O-acetyl-3'-O-methyl-5',6'-di-O-benzoyl-α-glucofuranosyl-2H-pyrazolo[3,4-d]pyrimidine(N-2-α-anomer) and 4,6-bis(methylthio)-2-(2'-O-acetyl-3'-O-methyl-5',6'-di-O-benzoyl-β-glucofuranosyl)-2H-pyrazolo[3,4-d]pyrimidine (N-2-β-anomer).*

A mixture of **1** (9.0 g, 42.0 mmol) and 1,2-di-O-acetyl-3-O-methyl-5,6-di-O-benzoyl-glucofuranose (20.6 g, 42.4 mmol) in anhydrous acetonitrile (300 ml) was stirred at 70° for 1 h. The mixture was cooled to 50° and freshly distilled borontrifluoride etherate (5.2 ml, 42.3 mmol) was added to it and the reaction mixture was refluxed for 2 h. The solvent from the resulting mixture was removed under reduced pressure and the residue taken up in ethyl acetate, washed with aqueous $NaHCO_3$, H_2O and dried (Na_2SO_4). The product obtained after removal of solvent was purified by silica gel column chromatography. Elution with $CHCl_3$ gave N-2-α-anomer (2.0 g, yield 9.5%); mp 109°C (EtOAc-hexane); UV (MeOH) : 263, 278; IR (KBr) : 1740 (C=O), 1570; MS : 241 (B+30); PMR ($CDCl_3$) : δ 2.0 (s, 3H, $COCH_3$), 2.53 (s, 3H, SCH_3), 2.56 (s, 3H, SCH_3), 3.37 (s, 3H, OCH_3), 3.97 (m, 1H, H-3'), 4.3-4.8 (m, 3H, H-4', H-6', H-6''), 5.6-6.0 (m, 2H, H-2', H-5'), 6.27 (d, J = 3 Hz, 1H, H-1'), 7.2-7.5 (m, 6H, ArH), 7.7-8.1 (m, 5H, H-3, ArH). Anal. calcd. for $C_{30}H_{30}N_4O_8S_2$: C, 56.4; H, 4.7; N, 8.8%. Found : C, 56.1; H, 4.9; N, 8.5%.

Further elution with $CHCl_3$-EtOAc (98:2, v/v) gave N-2-β-anomer (11.0 g, yield 52%); mp 142°C (EtOAc-hexane); UV (MeOH) : 262, 281; IR (KBr) : 1740, 1600, 1240; MS : 241 (B+30); PMR ($CDCl_3$) : δ 2.20 (s, $COCH_3$), 2.62 (s, 3H, SCH_3), 2.70 (s, 3H, SCH_3), 3.22 (s, 3H, OCH_3), 3.85 (d, J = 3.5 Hz, 1H, H-3'), 4.68-4.75 (m, 3H, H-4', H-6'), 5.03 (dd, J = 2.5, 12Hz, 1H, H-6''), 5.61 (s, 1H, H-2'), 5.94 (m, 1H, H-5'), 6.21 (s, 1H, H-1'), 7.42-7.49 (m, 4H, ArH), 7.58 (q, J = 7.5 Hz, 2H, ArH), 8.05 (t, J = 7.5 Hz, 4H, ArH), 8.26 (s, 1H, H-3, ArH). Anal. calcd. for $C_{30}H_{30}N_4O_8S_2$: C, 56.4; H, 4.7; N, 8.8%. Found : C, 56.2; H, 4.6; N, 8.4%.

Finally 2.0 g of unreacted **1** was also eluted from the column.