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Synthesis of 2′,3′-anhydronucleosides of pyrazolo[3,4-*d*]pyrimidine as antiviral agents

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4,6-Bismethylthio-N-1-(2′,3′-anhydro-β-D-lyxofuranosyl), and 4,6-bismethylthio-N-2-(2′,3′-anhydro-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidines (**10** and **17**) have been synthesized by 2′,3′-transelimination of the corresponding N-1(**9**)- and N-2(**12**)-β-D-xylofuranosylpyrazolo[3,4-*d*]pyrimidines. The compounds and their intermediates have been screened for their antiviral activities against RDV and EMCV exhibiting 10-100% activity.

Recently the synthesis and biological evaluation of modified nucleosides, both with respect to base and sugar[1], particularly the sugar modified nucleosides[2,3] have been greatly emphasized. These compounds are reported to interfere with the viral encoded enzymes selectively[4-6]. Further, the anhydronucleosides serve as very good synthons[7] for the synthesis of nucleosides of *trans*-β-hydroxy functionality deoxy and dideoxy nucleoside, a class of compounds for RT inhibition[8,9]. The pyrazolo[3,4-*d*]pyrimidine nucleosides have shown potent-antiparasitic[10] and antiviral[11,12] activities. Keeping the above points in view, we undertook the synthesize 2′,3′-anhydro-nucleosides of pyrazolo[3,4-*d*]pyrimidine as antiviral agents.

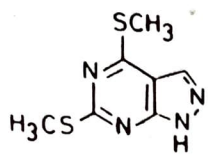
Results and Discussion

The fusion of 4,6-bismethylthiopyrazolo[3,4-*d*]pyrimidine (**1**) and 1,2,3,5-tetra-acetyl-D-xylofuranose (**2**) in the presence of *p*-toluenesulphonic acid gave a mixture of products, two of which were isolated by column chromatography over silica gel, and characterized as 4,6-bismethylthio-N-2-(2′,3′,5′-tri-O-acetyl- α-D-xylofuranosyl- and N-1-(2′,3′5′-tri-O-acetyl-β-D-xylofuranosyl)-pyrozolo[3,4-*d*]pyrimidines (**3** and **4**) in 16 and 45% yields, respectively.

The structures of **3** and **4** were assigned on the basis of their mass (m/z 471, M^+) and ^{1}H NMR spectral data. In the ^{1}H NMR spectrum of **3**, the H-1 protons[1] appeared as a doublet at δ 6.3 ($J_{1'2'}$ = 4.5 Hz) while in **4** it appeared as a singlet merged with the H-2′ multiplet between δ 5.9 and 6.2. Such findings have earlier been reported in xylofuranosyl nucleosides[13]. However, the position of glycosideration and anomeric configurations in the above nucleosides were further ascertained on the basis of ^{13}C NMR and ^{1}H NMR data of the corresponding deblocked nucleosides, 4,6-bismethylthio-N-β-D- and N-2-α-D-xylofuranosyl-pyrazolo[3,4-*d*]pyrimidines **6** and **5** obtained by the reaction of **4** and **3** with MeOH, NH_3 at 0°C. In the ^{13}C NMR spectra of **6** and **5**, the C-3 appeared at δ 124.5 and 133.1 respectively, a characteristic of N-1 and N-2 substituted pyrazolo[3,4-*d*]pyrimidines[14]. In the ^{1}H NMR spectrum, the H-1′ proton appeared as a doublet at δ 6.2 (J = 4 Hz) in **6** while in **5** it appeared as a doublet at δ 6.0 (J = 3 Hz) confirming the structural assignments[15].

Reaction of **6** with acetone in the presence of iodine at room temperature gave 4,6-bismethylthio-N-1-(3′,5′-isopropylidene- β-D-xylofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (**7**). Appearance of *gem*-dimethyl in the ^{1}H NMR spectrum at δ 1.29 and 1.31 alongwith H-1′ at δ 6.0 as a singlet is characteristic of the structure. Reaction of **7** with methanesulphonyl chloride afforded the corresponding 2′-O-methanesulphonyl derivative (**8**), which on heating with aqueous acetic acid at 100° gave 4,6-bismethylthio- N-1-(2′-O-methanesulphonyl-β-D-xylofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**9**). Reaction of **9** with NaOMe/NaOH at 0°C gave 4,6-bismethylthio-N-1- (2′,3′-anhydro-β-D-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**10**). The structure of **10** was supported by its mass (326, M^+) and ^{1}H NMR spectral data where H-1′ proton appeared as a singlet at δ 6.4 along with the usual signals.

Further, condensation of 1,2-di-O-acetyl-3-O-



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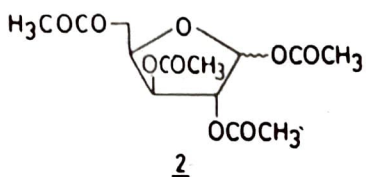

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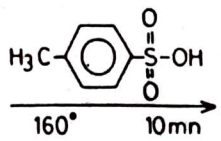
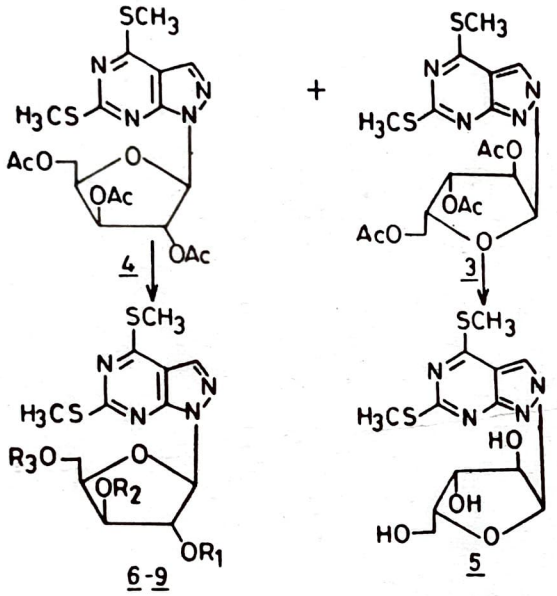
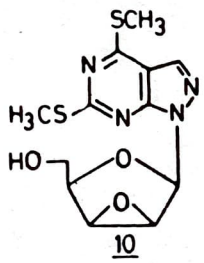


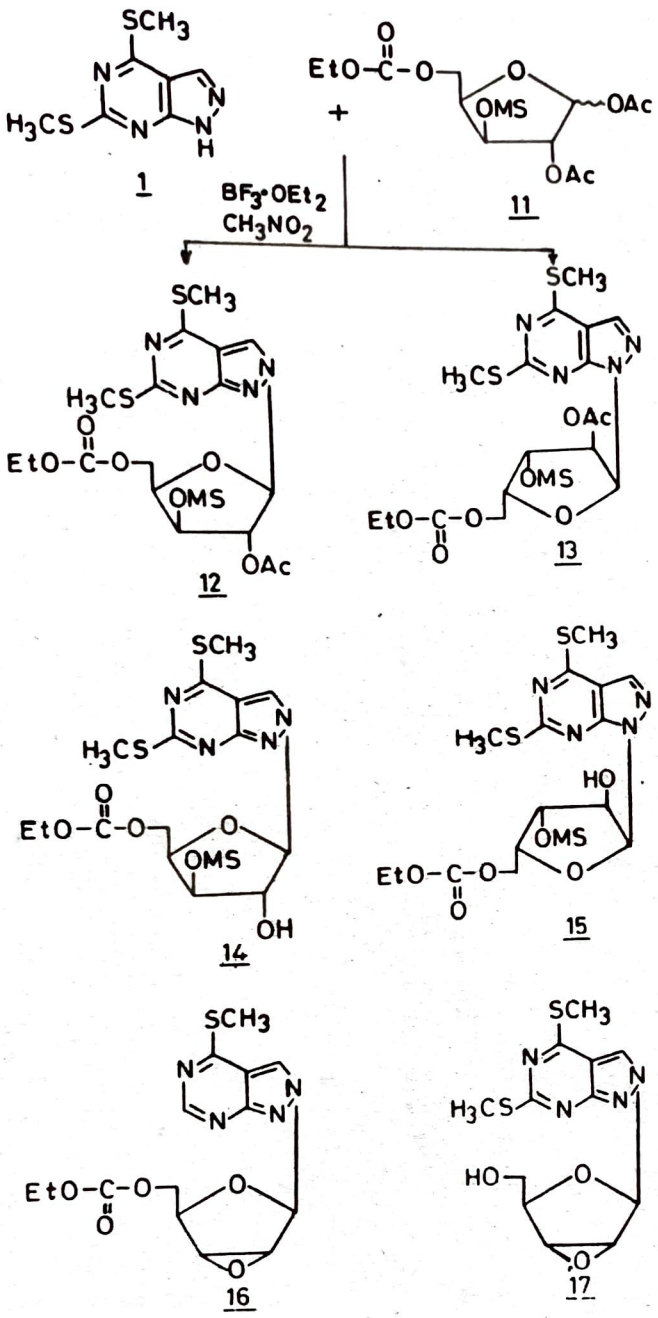


	R_1	R_2	R_3
6,	H	H	H
7,	H	$-(CH_3)_2$	–
8,	$-SO_2CH_3$	$-(CH_3)_2C-$	
9,	$-SO_2CH_3$	H	H



10

methanesulphonyl- 5-O-ethoxycarbonyl-D-xylofuranose (**11**) with **1** in refluxing nitromethane in the presence of $BF_3{\cdot}OEt_2$ gave a mixture of products. The main products isolated were characterised as 4,6-bismethylthio-N-2-(2′-O-acetyl-3′-O-methanesulphonyl-5′-O-ethoxycarbonyl-β-D-xylofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**12**) and 4,6-bismethylthio-N-1-(2′- O-acetyl-3′-O-methanesulphonyl-5′-O-ethoxycarbonyl-α-D-xylofuranosyl) pyrazolo-[3,4-*d*]pyrimidine (**13**). The structures of **12** and **13** were assigned on the basis of their mass (536 M^+) and ^1H NMR spectral data. In the ^1H NMR spectrum of **13**, H-1′ proton appeared as a doublet at δ 6.4 (J= 4 Hz) while in **12** it appeared as a singlet at δ 6.1. The position of glycosidation and the anomeric configurations were further ascertained on the basis of ^{13}C and ^1H NMR spectra of the corresponding deacetylated products. In 4,6-bismethylthio- N-2-(5′-ethoxycarbonyl-3′-O-methanesulphonyl-β-D-xylofuranosyl) pyrazolo[3,4-*d*]-pyrimidine (**14**) and 4,6-bismethylthio-N-1-(5′-O-ethoxycarbonyl-3′-O-methanesulphonyl-α-D-xylofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**15**), C-3 carbons appeared at δ 126 and 133 respectively indicating the correct assignments of the site of glycosidation. In the ^1H NMR spectrum of **14**, H-1′ proton appeared as a singlet at δ 5.9, and in **15** it appeared as a doublet at δ 6.3 (J= 5 Hz), characteristic of β- and α-xylofuranosyl nucleosides. Treatment of **12** with 1*N* NaOH in aqueous methanol at 0°C for 30 min gave a mixture of three products which were separated by column chromatography and characterised as 4,6-bismethylthio-N-2-(5′-ethoxycarbonyl- 3′-O-methanesulphonyl-β-D-



xylofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**14**), 4,6-bismethylthio-N-2-(5'- ethoxycarbonyl-2',3'-anhydro-β-D-ribofuranosyl)pyrazolo [3,4-*d*]pyrimidine (**16**) and 4,6-bismethylthio-N-2-(2',3'-anhydro-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**17**) in 10, 15 and 25% yields respectively. Compound **17** was the only major product when the reaction was carried out for 6 hr. The structure of these compounds were confirmed on the basis of their spectral data and elemental analysis.

The antiviral activities of nucleosides and their intermediates against RDV and EMCV were determined at concentration, and the results are given in Table I.

Table I—Biological activity of the various compounds synthesized

Compd	% Inhibition against	
	RDV	EMCV
3	30	—
4	15	—
6	50	30
10	80	90
12	40	20
13	60	30
14	60	50
15	62	40
16	40	30
17	40	100

Experimental Section

4,6-Bismethylthio-N-1-(3',5'-isopropylidene-β-D-xylofuranosylpyrazolo[3,4-*d*]pyrimidine (7). Iodine (0.5 g, 3.94 mmoles) was dissolved in anhydrous acetone (150 mL) and the solution stirred at 35-40°C. To this was added 4,6-bismethylthio-N-1-β-D-xylofuronosylpyrazolo[3,4-*d*]pyrimidine (**6**; 1.0 g, 2.9 mmoles) and stirring continued for 12 hr at the same temperature. Excess of iodine was quenched with aqueous sodium thiosulphate (10%) till the disappearance of colour. The solution was extracted with ethyl acetate, dried (Na_2SO_4) and evaporated *in vacuo* to give a white solid mass which was chromatographed over silica gel column. Elution of the column with MeOH-$CHCl_3$ (3:97) gave **7** as a colourless powder (0.77 g, 70%), m.p. 181° UV (MeOH): 248, 285; IR (KBr): 2920, 3350 cm^{-1} (OH); MS: m/z 384 (M^+); ^1H NMR ($CDCl_3$): δ 7.9 (s, 1H, H-3), 6.0 (s, 1H, H-1'), 4.8 (m, 1H, H-2'), 4.2-3.2 (m, 2H, H-3', H-4'), 3.1 (m, 2H, H-5'), 2.6 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3), 1.3 (s, 6H, $2\times CH_3$); ^{13}C NMR ($CDCl_3$): δ 168.7 and 164.5 (C-4 and C-6), 151.3 (C-7a), 131.8 (C-3), 108.4 (C-3a), 88.9 (C-1'), 84.8 (C-4'), 82.6 (C-2'), 79.6 (C-3'), 76.7 [$(CH_3)_2C<$], 59.9 (C-5') 27.2 and 26.8 [$>C(CH_3)_2$)], 14.8 and 13.2 ($2\times SCH_3$) (Found: C, 46.2; H, 4.9; N, 14.2. $C_{15}H_{20}N_4O_4S_2$ requires C, 46.8; H, 5.1; N, 14.6%).

4,6-Bismethylthio-N-1-(3'5'-isopropylidene-2-O-methylsulphonyl-β-D-xylofuranosyl)pyrazolo[3,4-*d*]-pyridine (8). Compound **7** (0.8 g, 2.07 mmoles) was dissolved in anhydrous pyridine (5 mL) and the solution stirred at 0°C. Methanesulphonyl chloride (1.2 mL) was added to it dropwise and stirring continued for 2 hr at 0°C and at room temperature for 24 hr. The reaction mixture was poured over crushed ice and extracted with $CHCl_3$. The extract was washed with aq. NaH-

CO_3, dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product which was purified by column chromatography to give **8** as a pale yellow foam (0.86 g, 90%); IR (KBr): 1200, 1380 cm^{-1} (SO_2CH_3); MS: m/z 464 (M^+); ^1H NMR ($CDCl_3$): δ 7.9 (s, 1H, H-3), 6.3 (d, 1H, J=2.5 Hz, H-1′), 5.9 (m, 1H, H-2′), 4.5 (m, 1H, H-3′), 4.2-3.9 (m, 3H, H-4′ and H-5′), 2.8 (s, 3H, $-SO_2-CH_3$), 2.6 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3), 1.4 (s, 6H, $2\times CH_3$) (Found: C, 41.27; H, 4.62; N, 12.67. $C_{16}H_{22}N_4O_6S_3$ requires C, 41.55; H, 4.76; N, 12.12%.

4,6-Bismethylthio-N-1-(2-O-methanesulphonyl-β-D-xylofuranosyl)pyrazolo[3,4-*d*]pyrimidine (9). Compound **8** (0.7 g, 1.5 mmoles) was dissolved in aq. glacial acetic acid (80%, 20 ml) and the solution heated at 100°C for 6 hr. The excess of solvent was evaporated and the residue purified over SiO_2 column. Elution of the column with MeOH-$CHCl_3$ (2:98) afforded **9** as an amorphous powder (0.42 g, 65%), m.p. 103°C; IR (KBr): 1185, 1290 cm^{-1}; MS: m/z 422 (M^+): ^1H NMR ($CDCl_3$): δ 8.0 (s, 1H, H-3), 6.5 (d, 1H, J=3Hz, H-1′), 6.1 (m, 1H, H-2′); 5.6 (m, 1H, H-3′); 4.6-4.3 (m, 3H, H-5′ and H-4′), 3.2 (s, 3H, SO_2CH_3), 2.6 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3) (Found: C, 36.35; H, 4.33; N, 12.85. $C_{13}H_{18}N_4O_6S_3$ requires C, 36.96; H, 4.26; N, 13.27%).

4,6-Bismethylthio-N-1-(2′,3′-anhydro-β-D-lyxofuranosyl)pyrazolo[3,4-*d*]pyrimidine (10). Compound **9** (0.49, 0.5 mmoles) was dissolved in anhydrous methanol (10 mL) and the solution stirred at 0°C. To this a solution of sodium methoxide (Na in anhydrous methanol) was added and the stirring continued for 8 hr. Excess of solvent was evaporated and the residue dissolved in $CHCl_3$ and washed with water. The organic layer was separated, dried (Na_2SO_4), evaporated *in vaccuo* and the residue crystallized from methanol as colourless needles (0.23 g, 75%), m.p. 180; MS: m/z 326 (M^+); ^1H NMR ($CDCl_3$): δ 7.9 (s, 1H, H-3), 6.4 (s, 1H, H-1′), 4.2-3.8 (m, 5H, H-2′, H-3′, H-4′ and H-5′), 2.6 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3) (Found: C, 43.50; H, 4.25; N, 17.40. $C_{12}H_{14}N_4O_3S_2$ requires 44.17; H, 4.29; N, 17.17%.

4,6-Bismethylthio-N-2-α- and β-(2′-O-acetyl-5′-ethoxycarbonyl-3′-methanesulphonyl-D-xylofuranosyl)pyrazolo[3,4-*d*]pyrimidine (13 and 12). 4,6-Bismethylthio-pyrazolo[3,4-*d*]pyrimidine (**1**) (2.59, 10 mmoles) and 1,2-di-O-acetyl-5-ethoxycarbonyl-3-O-methanesulphonyl-D-xylofuranose (**11**) (11 g, 31.25 mmoles) were refluxed in anhydrous nitromethane (150 mL). After 30 min, the temperature was lowered to 80°. To this was added $BF_3\cdot OEt_2$ (10 mL) and the reaction allowed to run at the same temperature for 1 hr and then excess of solvent removed *in vacuo*. The gummy residue was dissolved in ethyl acetate, washed with $NaHCO_3$ and water. The organic layer was separated and evaporated to give a number of isomers which were separated by column chromatography.

Compound 13: It was obtained as a colourless oil (16% yield); IR (Neat): 2900, 1750, 1270, 1190 cm^{-1}, MS: m/z 536 ($M^{+\cdot}$), 537 ($M^+ + 1$); ^1H NMR ($CDCl_3$): δ 8.0 (s, 1H, H-3), 6.4 (d, J=4 Hz, 1H, H-1′), 6.2 (dd, J=2 Hz, 1H, H-2′), 4.5 (m, 3H, H-5′ and H-4′), 4.1 (q, J=7 Hz, 2H $-O-CH_2CH_3$), 3.2 (s, 3H, SO_2CH_3), 2.6 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3), 2.1 (s, 3H, $COCH_3$), 1.2 (t, J=7 Hz, 3H, $-O-CH_2CH_3$).

Further elution of the column gave **12** as a colourless oil (46%); IR (Neat): 2900, 1750, 1190 cm^{-1}, MS: m/z 536 (M^+); ^1H NMR ($CDCl_3$): δ 8.3 (s, 1H, H-3), 6.1 (s, 1H, H-1′), 5.8 (m, 1H, H-2′), 5.1 (m, 2H, H-3′, H-4′), 4.1-4.5 (m, 4H, $-OCH_2-$, H-5′), 2.9 (s, 3H, SO_2CH_3), 2.6 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3), 2.1 (s, 3H, $COCH_3$), 1.2 (t, J=7 Hz, 3H, $-O-CH_2-CH_3$).

4,6-Bismethylthio-N-2-(2′3′- anhydro-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (17). A solution of **12** (3 g, 5.59 mmoles) in a mixture of methanol (140 mL) and water (25 mL) was stirred in an ice-bath. To this was added 1*N* NaOH (aq 7 mL) dropwise, and stirring continued for 6 hr at room temperature. The solvent was reduced to 1/4 of its volume *in vacuo*. The resulting solution was neutralized with cold aq. acetic acid carefully to the neutral *p*H when a gummy mass separated out. It was dissolved in $CHCl_3$ (200 mL) and washed with water (2×50 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to give a crude solid which was purified by column chromatography. Elution of the colum with $CHCl_3$-MeOH (97:3) afforded **17** as an amorphous solid, yield 70%, m.p. 170°; $[\alpha]_D^{25}$ +71.5°; UV: 243, 263, 281 nm; IR(KBr): 1594, 3200 cm^{-1}; MS: m/z 326 (M^+); ^1H NMR ($CDCl_3$): δ 8.1 (s, 1H, H-3), 6.0 (s, 1H, H-1′); 4.4 (m, 1H, H-2′), 4.1 (m, 2H, H-3′ and H-4′), 3.9 (m, 2H, H-5′), 2.6 (s, 6H, $2\times SCH_3$) (Found: C, 40.4; H, 4.36; N, 15.93. $C_{12}H_{14}N_4O_3S_2$ requires C, 44.17; H, 4.29; N, 17.17%).

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