Alkylating Nucleosides. 3 (1). The Reaction of 3,5-Dimethylpyrazole Nucleosides with *N*-Bromosuccinimide

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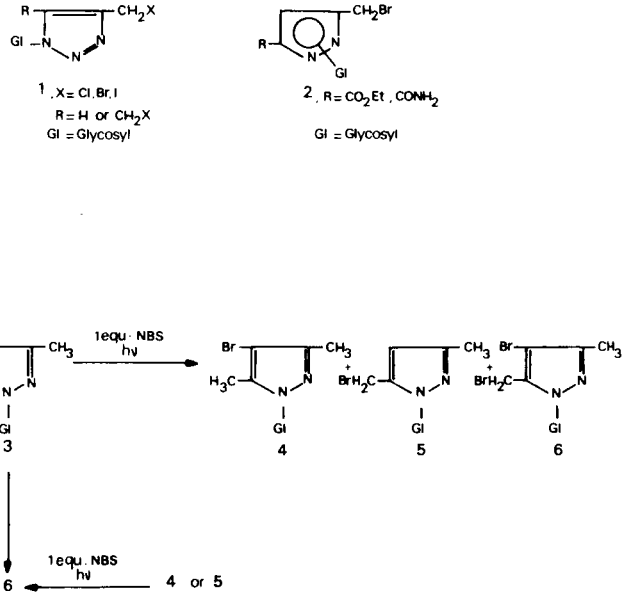
Received July 25, 1979

Bromination of 3,5-dimethylpyrazole nucleosides with *N*-bromosuccinimide gave the corresponding 4-bromo-3,5-dimethylpyrazole, 3-methyl-5-(bromomethyl)pyrazole and 4-bromo-3-methyl-5-(bromomethyl)pyrazole nucleosides. Structural assignments were made on basis of analytical and ^{1}H nmr spectral data. All of the bromomethylpyrazole nucleosides described showed cytostatic activity against HeLa cell sultures.

J. Heterocyclic Chem., **17**, 113 (1980).

In previous papers (1,2) the synthesis of *N*-glycosylhalomethyl pentahererocyclic compounds, **1**, was reported by cycloaddition of glycosyl azides to propargyl halides or by halogenation of *N*-glycosylhydroxymethyl-1,2,3-triazoles (2), and **2** by glycosylation of the corresponding bromomethylpyrazoles *via* the acid-catalyzed fusion method (1). Due to the chemical alkylating ability of the benzylic type halomethyl group, these compounds were designed as potential cytostatic drugs. In fact, most of bromo- and iodomethyl derivatives showed significant "in vitro" activities. In the "in vivo" tests some 4-bromomethyl- and 4-iodomethyl-1,2,3-triazoles (**1**, X = Br, I; R = H) (3) as well as those carboxamide substituted bromomethylpyrazole nucleosides (**2**, R = $CONH_2$) were effective against certain tumor systems (4). Interest in structure-activity relationships prompted us to investigate the preparation of new substituted *N*-glycosylbromomethylpyrazoles.

With the aim of following a similar route to that described for the synthesis of **2** (1), our initial efforts were directed toward obtaining 3(5)-methyl-5(3)-(bromomethyl)-pyrazole and 3,5-bis(bromomethyl)pyrazole, which by glycosylation would lead to 3(or 5)methyl- and bromomethyl substituted analogues of **2**. However, all attempts to obtain such bromomethylpyrazol bases were unsuccessful. Thus, bromination of 3,5-dimethylpyrazole with one equivalent of *N*-bromosuccinimide under illumination led to the electrophilic substitution at C-4 and no reaction on side chain took place. When the reaction was performed with two equivalents of NBS, mixtures of brominated compounds which decomposed on standing and on tlc were obtained. Similar intractable mixtures resulted by treatment of 3,5-bis(hydroxymethyl)pyrazole with phosphorus tribromide. We had already noticed (2) the difficulty in obtaining other *N*-unsubstituted bromomethylpyrazoles, such as 3(5)-(bromomethyl)pyrazole and 3,4-bis(bromomethyl)pyrazole. This fact is attributed to the instability of such compounds due to their high reactivity. In view of this problem, we decided to explore



a: Gl = 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl

b: Gl = 2,3,5-tri-O-acetyl-β-D-ribofuranosyl

a different route involving the introduction of the alkylating bromomethyl group on suitable 3- and/or 5-substituted pyrazole nucleosides previously formed. For this purpose we studied the reaction of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- and 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3,5-dimethylpyrazole (**3**) with *N*-bromosuccinimide as an alternative route for the synthesis of bromomethylpyrazole nucleosides.

As previously described for compound **3a** (5), the 1-β-D-ribofuranosyl derivative **3b** was obtained in 80% yield by glycosylation of 3,5-dimethylpyrazole (6) *via* mercuric cyanide-nitromethane method. Bromination of **3** with one equivalent of NBS in carbon tetrachloride and irradiating with a 200 watt lamp gave a mixture of three compounds which were separated chromatographically

0022-152X/80/010113-03$02.25

and identified as 1-glycosyl-4-bromo-3,5-dimethylpyrazole (**4**), 1-glycosyl-3-methyl-5-(bromomethyl)pyrazole (**5**) and 1-glycosyl-3-methyl-4-bromo-5-(bromomethyl)pyrazole (**6**). Although the total yield of bromination for **3a** and **3b** was similar, the ratio of monobrominated compounds on the side chain or on the pyrazole ring varied with the starting material. Thus, in the case of the 1-glucosyl derivative **3a**, the 5-bromomethyl substituted compound **5a** predominated. However, the 1-ribosyl derivative **3b** gave the 4-bromo substituted compound **4b** as the major product. Bromination of **3** with two equivalents of NBS under the same conditions as above gave essentially the dibrominated compound **6** along with very minor amounts of **4** and **5**. Compound **6** was also obtained by treatment of **4** or **5** with one equivalent of the brominating agent. Attempts to introduce a second bromomethyl group by adding larger amounts of NBS furnished intractable mixtures of compounds.

Structural assignments were made on basis of analytical and spectral data (Table I). The ^{1}H nmr of **4** indicated clearly the absence of the H-4 pyrazole proton and the presence of two separate singlets corresponding to the methyl groups. As in the case of the non-brominated compound **3**, the signal for CH_3-5 appeared at lower field than that for CH_3-3 as a consequence of the deshielding effect of the glycosyl moiety (7). The 5-bromomethyl substitution of compounds **5** and **6** was determined by comparing the chemical shift value for the remaining methyl group with that for CH_3-3 and CH_3-5 of the 3,5-dimethyl substituted nucleosides **3** and **4**. The β-anomeric configuration of **4a, 5a, 5b, 6a** and **6b** was clearly demonstrated on the basis of the corresponding values of $J_{1'2'} \simeq 9$ Hz for the 1-glucopyranosyl derivatives **4a, 5a** and **6a**, and $J_{1'2'} < 1$ Hz for the 1-ribofuranosyl derivatives **5b** and **6b**. In the case of **3b** and **4b**, $J_{1'2'} = 2.5$ Hz. Although this value does not allow an unequivocal assignment, they were assigned as β since the δ value for the anomeric proton is very close in each case, to that of the related compounds **5b** and **6b**. On the other hand, the mercuric cyanide method, used for the preparation of **3b**, gives in general only 1'2'-*trans*-nucleosides.

All the compounds reported in this paper were evaluated as cytotoxic agents againts HeLa cell cultures. As shown in Table II, cytostatic activity depended on the presence of the alkylating bromomethyl group. Thus, while those bromomethylpyrazole nucleosides **5** and **6** showed significant "in vitro" activities, those 3,5-dimethylpyrazole nucleosides **3** and **4** were inactive or, as in the case of **4a**, showed very slight activity.

EXPERIMENTAL

Melting points were determined on a kofler apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded at 100 MHz on a Varian XL-spectrometer using TMS as the internal standard and ultraviolet spectra were recorded with a Perkin-Elmer 402 spectrophotometer. Analytical thin layer chromatography was performed on aluminum sheets coated with a 0.2 mm layer of silica gel $60F_{254}$ (Merck), and preparative layer chromatography on 20 x 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck), The compounds were detected with uv light of 254 nm or by spraying with sulfuric acid in ethanol, 30%.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3,5-dimethylpyrazole (**3b**).

To a mixture of 5.89 g. (0.02 mole) of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (8), 5.04 g. (0.02 mole) of mercuric cyanide and molecular sieve in 75 ml. of dry nitromethane was added 0.96 g. (0.01 mole) of 3,5-dimethylpyrazole (6). The mixture was refluxed for 6 hours. After this, it was filtered while still hot, in order to remove the insoluble residue which was washed with more hot nitromethane. The filtrate was evaporated to dryness *in vacuo* and the residue obtained was treated with chloroform and filtered to separate the solid formed. The chloro-

Table I

^{1}H Nmr Data at 100 MHz with TMS as Internal Standard

Compound	Solvent	Chemical Shifts (δ) H-4	H-1'	CH_3-5	CH_3-3	CH_2Br	$J_{1'2'}$ (Hz)
3a	Deuteriochloroform	5.84	5.70	2.30	2.18	–	9.5
3a	DMSO	5.90	5.86	2.26	2.06	–	9.5
3b	Deuteriochloroform	5.82 (a)		2.26	2.18	–	(a)
3b	DMSO	5.91	5.92	2.23	2.12	–	2.5
4a	Deuteriochloroform	–	5.55	2.37	2.22	–	9
4a	DMSO	–	5.72	2.26	2.06	–	9
4b	Deuteriochloroform	–	5.80 (b)	2.30	2.20	–	(b)
4b	DMSO	–	6.03	2.25	2.14	–	2.5
5a	Deuteriochloroform	6.15	5.83	–	2.22	4.55	9.5
5a	DMSO	6.20	5.93	–	2.08	4.74	9.5
5b	Deuteriochloroform	6.12	6.00	–	2.23	4.49	<1
6a	Deuteriochloroform	–	5.83	–	2.21	4.50	9
6a	DMSO	–	6.10	–	2.11	4.73	9
6b	Deuteriochloroform	–	5.98	–	2.22	4.49	<1

(a) Unresolved multiplet including H-4, H-1', H-2' and H-3'. (b) Unresolved multiplet including H-1', H-2' and H-3'.

Table II

Cytostatic Activity Against HeLa Cells

Compound	ED_{50} (μg./ml.)
3a	>100
3b	>100
4a	70
4b	>100
5a	4
5b	15
6a	8
6b	7

form extract was washed with 30% aqueous potassium iodide, water and then dried over anhydrous sodium sulfate. The residue obtained after removing the solvent was purified by preparative tlc (1:1 ethyl acetate-petroleum ether). Elution of the major band gave 2.83 g. (80%) of **3b** as a syrup; uv λ max (ethanol): 225 nm (ε 5200).

Anal. Calcd. for $C_{16}H_{22}N_2O_7$: C, 54.23; H, 6.25; N, 7.90. Found: C, 54.13; H, 6.13; N, 7.52.

Bromination Reactions. General Procedure.

To a solution of 3,5-dimethylpyrazole nucleoside **3** (4 mmoles) in 40 ml. of carbon tetrachloride was added *N*-bromosuccinimide (Method A: 4 mmoles; Method B: 8 mmoles) and the mixture was irradiated by suspending the flask one centimeter above a 200 watt lamp. After this, it was filtered while still hot and the filtrate evaporated to dryness to give a residue which was purified as specified in each case.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-bromo-3,5-dimethylpyrazole (**4a**), 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-methyl-5-(bromomethyl)pyrazole (**5a**) and 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-methyl-4-bromo-5-(bromomethyl)-pyrazole (**6a**).

Method A.

The crude reaction product was chromatographed by preparative tlc (chloroform). The fastest moving band afforded 0.225 g. (10%) of **6a** with m.p. 242-243° (from carbon tetrachloride-petroleum ether); uv λ max (ethanol): 250 nm (ε 13,200).

Anal. Calcd. for $C_{19}H_{24}N_2O_9Br_2$: C, 39.04; H, 4.13; N, 4.79; Br, 27.39. Found: C, 39.39; H, 4.19; N, 5.04; Br, 27.64.

The intermediate moving band furnished 0.67 g. (33%) of **4a** with m.p. 119-121° (from carbon tetrachloride-petroleum ether); uv λ max (ethanol): 235 nm (ε 9900).

Anal. Calcd. for $C_{19}H_{25}N_2O_9Br$: C, 45.14; H, 4.98; N, 5.54; Br, 15.80. Found: C, 45.06; H, 4.88; N, 5.63; Br, 16.20.

The slowest moving band yielded 0.83 g. (41%) of **5a** with m.p. 170-171° (from carbon tetrachloride-petroleum ether); uv λ max (ethanol) 238 nm (ε 9200).

Anal. Calcd. for $C_{19}H_{25}N_2O_9Br$: C, 45.14; H, 4.98; N, 5.54; Br, 15.80. Found: C, 45.22; H, 5.05; N, 5.46; Br, 16.02.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-methyl-4-bromo-5-(bromomethyl)pyrazole (**6a**).

Method B.

The crude reaction product was purified by preparative tlc (chloroform). Elution of the major band gave 1.75 g. (75%) of **6a** with physical properties identical with those above described for that compound.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-bromo-3,5-dimethylpyrazole (**4b**), 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-methyl-5-(bromomethyl)pyrazole (**5b**) and 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-methyl-4-bromo-5-(bromomethyl)pyrazole (**6b**).

Method A.

The residue obtained after removing the solvent was chromatographed by tlc (4:1 chloroform-hexane). The fastest moving band afforded 0.24 g. (12%) of **6b** as a syrup; uv λ max (ethanol): 247 nm (ε 7500).

Anal. Calcd. for $C_{16}H_{20}N_2O_7Br_2$: C, 37.50; H, 3.93; N, 5.46; Br, 31.20. Found: C, 37.87; H, 4.02; N, 5.30; Br, 31.38.

The intermediate band gave 0.78 g. (45%) of **4b** as a syrup; uv λ max (ethanol): 233 nm (ε 6300).

Anal. Calcd. for $C_{16}H_{21}N_2O_7Br$: C, 44.34; H, 4.88; N, 6.46; Br, 18.45. Found: C, 44.60; H, 4.92; N, 6.25; Br, 18.37.

The slowest moving band yielded 0.39 g. (23%) of **5b** as a syrup: uv λ max (ethanol): 238 nm (ε 8500).

Anal. Calcd. for $C_{16}H_{21}N_2O_7Br$: C, 44.34; H, 4.88; N, 6.46; Br, 18.45. Found: C, 44.08; H, 5.08; N, 6.68; Br, 18.53.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-methyl-4-bromo-5-(bromomethyl)pyrazole (**6b**).

Method B.

The crude reaction product was purified by tlc (4:1 chloroform-hexane). Elution of the major band gave 1.36 g. (66%) of **6b** identical in all respects to that above described.

Acknowledgment.

The authors are grateful to Miss Emilia Bayo for her technical assistance.

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