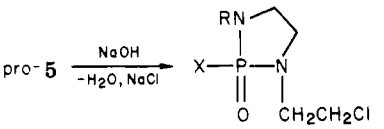
mechanisms of base-catalyzed hydrolysis, pro-5 representatives 9–13 are unique in that deprotonation of the amidic position can be followed by intramolecular cyclization via displacement of chlorine in the mustard group (pro-5 → i), which is a reaction mode having precedence in the



i

chemistry known for 1.[21] In addition, these compounds offer the possibility for direct displacement of chlorine by hydroxide. Our unpublished ^{31}P NMR kinetic studies of 2 [T. W. Engle, G. Zon, and W. Egan] have indicated a half-life of ca. 15 min (pH ≥7, 37 °C) for intramolecular chlorine displacement to aziridinium ion 4 and, consequently, monitoring the rate of chloride production during the present studies of 9–13 was not pursued as mechanistically informative data. In any event, the aforementioned considerations do not substantially affect the composite nature of k^* and the linear correlation with k'.

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(22) M. J. P. Harger, *J. Chem. Soc., Perkin Trans. 1*, 605 (1977).
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(24) Instruction 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., 1973, and subsequent insert pages for more recent updating.

Alkylating Nucleosides. 2.[1] Synthesis and Cytostatic Activity of Bromomethylpyrazole and Pyrazole Nitrogen Mustard Nucleosides

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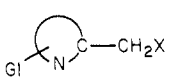
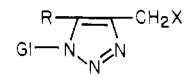
Glycosylation of ethyl 3(5)-(bromomethyl)pyrazole-5(3)-carboxylate (3) and 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide (4) with poly-*O*-acetylated sugars via an acid-catalyzed fusion method afforded the corresponding ethyl 3-(bromomethyl)pyrazole-5-carboxylate and 3-(bromomethyl)pyrazole-5-carboxamide substituted nucleosides 5 and 7, respectively. In some cases, the positional isomers 6 and 8 were also obtained. Treatment of 5 and 7 with methanolic ammonia gave the deprotected 3-(aminomethyl)pyrazole-5-carboxamide nucleosides 9. Reaction of 3–5 and 7 with bis(2-chloroethyl)amine led to the corresponding pyrazole nitrogen mustards 10–13. All the bromomethylpyrazole nucleosides described showed significant cytostatic activity against HeLa cell cultures.

With the aim of obtaining new types of possible anticancer drugs, we have in our laboratories several ongoing research programs concerning the synthesis, cytostatic evaluation, and mode of action of *N*-glycosyl heterocyclic compounds in which a halomethyl group, as alkylating moiety, is attached to the heteroaromatic ring (1).



1, X = halogen
Gl = glycosyl

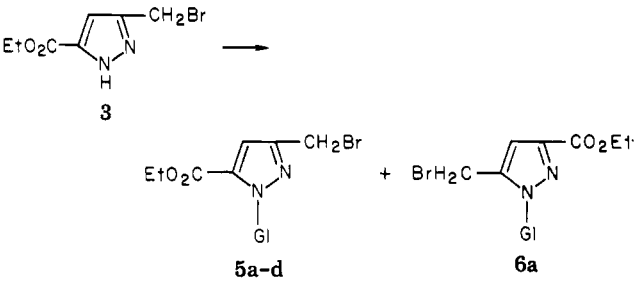


2, X = halogen
R = H or CH_2X
Gl = glycosyl

In the first paper of this series,[1] the synthesis of several *N*-glycosylhalomethyl-1,2,3-triazoles, 2, by cycloaddition of glycosyl azides to propargyl halides or by halogenation of the corresponding *N*-glycosylhydroxymethyl-1,2,3-triazoles was reported. These and other halomethyl-1,2,3-triazole nucleosides, having different sugar residues, were evaluated as cytostatic agents.[1,2] In general, all those bromo- and iodomethyl derivatives showed significant "in vitro" activities. 4-(Bromomethyl)- and 4-(iodomethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2, R = H, Gl = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) were also effective against ECA and P388 tumor systems. A study on the mode of action of these two latter compounds has demonstrated that they really act as alkylating agents.[2]

We now report the synthesis and cytostatic activity of nucleosides of ethyl 3(5)-(bromomethyl)pyrazole-5(3)-carboxylate and 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide (3 and 4) via an acid-catalyzed fusion method. We also describe the preparation of *N*-glycosyl-3-(aminomethyl)- and *N*-glycosyl-3-[[bis(2-chloroethyl)amino]-methyl]pyrazoles by transformation of the corresponding *N*-glycosyl-3-(bromomethyl) derivatives.

Scheme I



a, Gl = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl
b, Gl = 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl
c, Gl = 2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl
d, Gl = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl

Chemistry. The bromomethylpyrazoles 3 and 4 were prepared by treating ethyl 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxylate and 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxamide, respectively,[3] with phosphorus tribromide.

Fusion of 3 with penta-*O*-acetyl-β-D-glucopyranose in the presence of *p*-toluenesulfonic acid provided a mixture of

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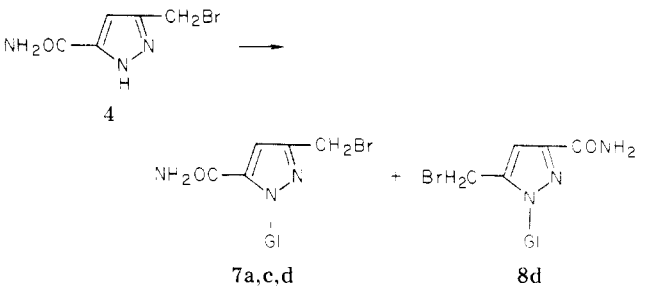
Table I. ^{1}H NMR Data

no.	solvent	δ (H-1′)	$J_{1',2'}$, Hz	δ (CH_2Br)
5a	$CDCl_3$	6.38	9	4.40
5a	Me_2SO	6.44	9	4.54
5b	$CDCl_3$	7.16	6	4.52
5b	Me_2SO	7.00	6	4.66
5c	$CDCl_3$	6.60	9	4.41
5c	Me_2SO	6.53	9	4.61
5d	$CDCl_3$	6.86	2	4.41
5d	Me_2SO	6.77	2	4.66
6a	$CDCl_3$	5.80	9	4.56
6a	Me_2SO	6.16	9	4.85
7a	$CDCl_3$	6.48	9	4.40
7a	Me_2SO	6.68	9	4.54
7c	$CDCl_3$	6.61	9	4.49
7c	Me_2SO	6.74	9	4.54
7d	$CDCl_3$	6.92	1.5	4.45
7d	Me_2SO	7.01	2	4.64
8d	$CDCl_3$	6.08	<1	4.54
8d	Me_2SO	6.24	<1	4.84

three isomeric nucleosides, which were separated chromatographically and identified as ethyl 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(bromomethyl)pyrazole-5-carboxylate (**5a**), its α anomer **5b**, and ethyl 1-(2,3,-4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(bromomethyl)-pyrazole-3-carboxylate (**6a**) in 24, 7, and 22% yield, respectively (Scheme I). Structural assignments of these nucleosides were made on the basis of their ^{1}H NMR (Table I) and UV spectra. The anomeric configuration was clearly ascertained as β for **5a** and **6a** and α for **5b** on the basis of their corresponding values of $J_{1',2'}$. The site of glycosylation of the positional isomers **5a** and **6a** was established by comparison of their ^{1}H NMR spectra with each other. Thus, the signal for the anomeric proton of **5a** appeared at lower field than that of **6a**. This downfield shift of the anomeric proton of **5a** is consistent with the presence of an anisotropic carbethoxy group adjacent to the site of glycosylation.[4] On the other hand, the signal for the methylenic protons of the bromomethyl group of **6a** showed a downfield shift as compared with that of **5a** as a consequence of the deshielding effect of the adjacent glycosyl moiety.[5] As will be seen later, in the case of obtaining only one isomer the δ values for these methylenic protons in **5a** and **6a** were very useful in assigning the site of glycosylation of additional bromomethylpyrazole nucleosides. Compound **5b** was established as a 3-(bromomethyl)-5-carboxylate substituted nucleoside, since its UV spectrum was identical with that of **5a** and different from that of **6a**. As expected,[6] the anomeric proton of this α-glucopyranosyl nucleoside **5b** occurred at lower field than that of the corresponding β-anomer **5a**.

Similar fusion reactions of **3** with tetra-*O*-acetyl-β-D-ribopyranose and tetra-*O*-acetyl-β-D-ribofuranose gave only one product in each case, namely, ethyl 1-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)- (**5c**) and ethyl 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(bromomethyl)pyrazole-5-carboxylate (**5d**) in 36 and 65% yield, respectively (Scheme I). The chemical shift for the bromomethylenic protons of these nucleosides was quite comparable with that of **5a** (Table I); thus, they were assigned as substituted 3-(bromomethyl)-5-carboxylates. The β anomeric configuration of the ribopyranosyl derivative **5c** was established on the basis of its large coupling constant, $J_{1',2'}$. In the case of the ribofuranosyl derivative **5d**, the value of the coupling constant ($J_{1',2'}$ = 2 Hz) suggested a β configuration. This suggestion was further supported by the δ value for the anomeric proton, which is very close to that of the related nucleoside 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(bromomethyl)pyrazole-5-carboxamide (**7d**), which will be described later. Finally, the structure of **5d** was confirmed by chemical evidence. As described later, treatment of **5d** or **7d** with methanolic ammonia yielded the same deblocked 3-(aminomethyl)-5-carboxamide substituted nucleoside **9g**.

Scheme II

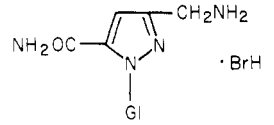


A similar series of reactions was achieved with 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide (**4**; Scheme II). Acid-catalyzed fusion of **4** with penta-*O*-acetyl-β-D-glucopyranose or tetra-*O*-acetyl-β-D-ribopyranose provided only one compound in each case, namely, 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- (**7a**) or 1-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)-3-(bromomethyl)pyrazole-5-carboxamide (**7c**) in 46 and 20% yield, respectively. The glycosylation site of these compounds was determined by comparing the δ values for the anomeric and bromomethylenic protons with those of **5a** and **5c**, respectively (Table I). Further support for these structures will be given later by the transformation of **5a** or **7a** and **5c** or **7c** into the same deprotected 3-(aminomethyl)-5-carboxamide substituted nucleosides **9e** and **9f**, respectively.

In the case of the fusion reaction of **4** with tetra-*O*-acetyl-β-D-ribofuranose, two isomeric compounds were obtained, which were identified as 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(bromomethyl)pyrazole-5-carboxamide (**7d**) and 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-5-(bromomethyl)pyrazole-3-carboxamide (**8d**) in 45 and 22% yield, respectively. As described earlier for the positional isomers **5a** and **6a**, the glycosylation site of the pair **7d** and **8d** was determined on the basis of the differences of chemical shifts for the anomeric and bromomethylenic protons between both compounds (Table I). Thus, while the bromomethylenic protons of **7d** appeared at higher field than those of **8d**, the anomeric proton of **7d** appeared at lower field. The anomeric configuration of **8d** was unequivocally determined by the value $J_{1',2'}$ < 1 Hz. The small value $J_{1',2'}$ = 1.5 Hz suggested a β configuration for **7d**. Therefore, it was assigned as β since the value of the difference of the chemical shift for H-1′ between **7d** and **8d** ($\Delta\delta_{CDCl_3}$ = 0.84 and $\Delta\delta_{Me_2SO}$ = 0.77 ppm), as a consequence of the anisotropic effect of the carboxamide group in **7d**, was very close to that of similar positional isomer nucleosides having the same anomeric configuration, such as 3-methyl-1-(β-D-ribofuranosyl)-1,-2,4-triazole-5-carboxamide and 5-methyl-1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide.[7] Hence, a still larger downfield shift for the anomeric proton of **7d** would be expected in the case of an α configuration.

Most of the bromomethylpyrazole nucleosides described in this paper were treated with methanolic ammonia for two reasons. Firstly, due to the reactivity of the benzylic-type bromomethyl group toward nucleophilic agents, substitution of the bromine atom by the amino group was expected. Then it was of interest to compare the cytostatic activity of the resulting aminomethylpyrazole derivatives with that of the related bromomethylpyrazoles. The second reason was to chemically confirm the structures of **5d**, **7a**, and **7c**. Treatment of the blocked 3-(bromo-

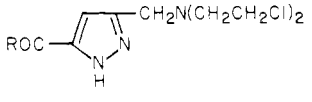
Scheme III



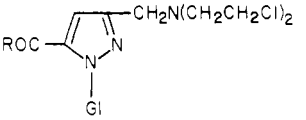
5a or **7a** → **9e**, Gl = β-D-glucopyranosyl
5c or **7c** → **9f**, Gl = β-D-ribopyranosyl
5d or **7d** → **9g**, Gl = β-D-ribofuranosyl

methyl)-5-(ethoxycarbonyl) and 3-(bromomethyl)-5-carboxamide substituted nucleosides **5a**, **5c**, and **5d** and **7a**, **7c**, and **7d** with methanolic ammonia led to the following deblocked nucleosides as hydrobromides (Scheme III): 1-(β-D-glucopyranosyl)-3-(aminoethyl)pyrazole-5-carboxamide (**9e**), from **5a** and **7a**; 1-(β-D-ribopyranosyl)-3-(aminomethyl)pyrazole-5-carboxamide (**9f**), from **5c** and **7c**; and 1-(β-D-ribofuranosyl)-3-(aminomethyl)-pyrazole-5-carboxamide (**9g**), from **5d** and **7d**. These results confirmed that the site of glycosylation and the anomeric configuration of each pair (**5a**,**7a**; **5c**,**7c**; **5d**,**7d**) were the same.

On the other hand, it is known that the nitrogen mustard group attached to several purine[8] and uracil[9] derivatives gives active anticancer agents. In some of these compounds, such as certain 8-[[bis(2-chloroethyl)-amino]methyl]-*N*-methylpurines,[10] 5-[[bis(2-chloroethyl)amino]methyl]uracil,[11] and 5-[[bis(2-chloroethyl)-amino]methyl]uridine,[12] the bis(2-chloroethyl)amino moiety is attached to the heterocyclic ring through a methylene group. Based on this fact, we were interested in preparing pyrazole nitrogen mustard derivatives from the corresponding bromomethylpyrazoles described. This would allow us to compare the relative cytostatic effect that two different alkylating groups [bromomethyl and bis-(2-chloroethyl)aminomethyl] have on the same carrier. Reaction of the free bromomethylpyrazole bases **3** and **4** with bis(2-chloroethyl)amine afforded ethyl 3(5)-[[bis(2-chloroethyl)amino]methyl]pyrazole-5(3)-carboxylate (**10**)



10, R = OEt
11, R = NH_2



12a,**c**,**d**, R = OEt
13a,**c**,**d**, R = NH_2

and 3(5)-[[bis(2-chloroethyl)amino]methyl]pyrazole-5(3)-carboxamide (**11**). In the same way, the 3-(bromomethyl)pyrazole nucleosides **5a**,**c**,**d** and **7a**,**c**,**d** were transformed into the corresponding pyrazole nitrogen mustard nucleosides, namely, ethyl 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- (**12a**), ethyl 1-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)- (**12c**), and ethyl 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-[[bis(2-chloroethyl)-amino]methyl]pyrazole-5-carboxylate (**12d**) and 1-(2,3,-4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- (**13a**), 1-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)- (**13c**), and 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-[[bis(2-chloroethyl)-amino]methyl]pyrazole-5-carboxamide (**13d**). The structures of all these pyrazole nitrogen mustards were established on the basis of their analytical and spectroscopic data.

Attempts to obtain 3(5)-(bromomethyl)pyrazole and 3,4-bis(bromomethyl)pyrazole, which by glycosylation would lead to analogues of the *N*-glycosyl(bromomethyl)-1,2,3-triazoles previously described,[1] were unsuccessful. Thus, cycloaddition of diazomethane to propargyl bromide and 1,4-dibromobutyne afforded intractable mixtures of compounds whose ^1H NMR spectra did not show the expected signals for the desired compounds. The same results were obtained by treatment of 3(5)-(hydroxymethyl)pyrazole and 3,4-bis(hydroxymethyl)pyrazole with phosphorus tribromide. This fact can be probably attributed to the instability of such compounds, due to their high reactivity. Other methods for the preparation of these latter bromomethylpyrazole nucleosides are being considered.

Table II. Cytostatic Activity against HeLa Cells

bromomethyl-pyrazoles		aminomethyl-pyrazoles		pyrazole nitrogen mustards	
no.	ED_{50}, μg/mL	no.	ED_{50}, μg/mL	no.	ED_{50}, μg/mL
3	>100	9e	>100	10	18
4	>100	9f	>100	11	20
5a	5	9g	>100	12a	>100
5b	5			12c	>100
5c	2			12d	>100
5d	2.5			13a	>100
6a	6			13c	50
7a	4			13d	35
7c	3				
7d	2				
8d	2				

Cytostatic Activity. All the compounds reported in this paper were evaluated as cytotoxic agents against HeLa cell cultures (Table II). All the bromomethylpyrazole nucleosides described showed significant activities, similar to those of the bromomethyl- and iodomethyl-1,2,3-triazole nucleosides previously described,[1,2] while the corresponding bromomethylpyrazolic bases were completely inactive. In contrast to this, most of the related pyrazole nitrogen mustard nucleosides were inactive, except for **13c** and **13d** which showed a slight activity similar to that of the non-nucleosidic-mustards **10** and **11**. Substitution of the bromine atom of the bromomethyl group by the amino group caused the total loss of activity. The "in vivo" cytostatic evaluation and the mode of action of these bromomethylpyrazole nucleosides are presently being carried out.

Experimental Section

Chemical Methods. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded at 100 MHz on a Varian XL spectrometer using Me_4Si as internal standard. UV absorption spectra were taken with a Perkin-Elmer 350 or 402 spectrophotometer. Analytical thin-layer chromatography was performed on glass plates coated with a 0.25-mm layer of silica gel GF_{254} (Merck) and preparative layer chromatography on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254} (Merck). The compounds were detected with UV light (254 nm) or by spraying the plates with 30% sulfuric acid in ethanol and heating at ca. 110 °C.

Biological Methods. Cytostatic Activity. The previously described method[13] was followed. Minimal Eagle's medium[14] (Difco, code 5675) supplemented with 10% fetal calf serum (Difco) was used. HeLa cells (10^5 cells/mL) were incubated at 37 °C in Leighton tubes. After 2–3 h, the cells were attached to the glass, and the compound to be tested, suspended in sterile saline containing 0.05% (v/v) Tween 80, was then added. The volume of this suspension was 10% of the final incubation mixture. Incubation was carried out at 37 °C for 72 h. As a positive control, 6-mercaptopurine was always included ($ED_{50} \approx 0.1$ μg/mL). Cell growth was estimated by measuring the cell proteins following the colorimetric method of Oyama and Eagle.[15]

Ethyl 3(5)-(Bromomethyl)pyrazole-5(3)-carboxylate (3). Phosphorus tribromide (6.5 g, 23 mmol) was slowly added to a stirred suspension of ethyl 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxylate[3] (3.4 g, 20 mmol) in benzene (100 mL). The mixture was kept at room temperature overnight while stirring

and then ice–water (20 mL) was added, and the mixture was neutralized with solid hydrogen carbonate and extracted with ether (100 mL; three times). The organic layer was washed with water and dried over sodium sulfate. Evaporation of the solvent gave a solid, which was recrystallized from cyclohexane to afford 4.23 g (91%) of **3**: mp 74–75 °C; UV λ_{max} (ethanol) 222 nm (ϵ 11 700); ^{1}H NMR ($CDCl_3$) δ 6.86 (s, 1, H-4), 4.56 (s, 2, CH_2Br). Anal. ($C_7H_9N_2O_2Br$) C, H, N.

3(5)-(Bromomethyl)pyrazole-5(3)-carboxamide (4). Phosphorus tribromide (6.5 g, 23 mmol) was slowly added to a stirred suspension of 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide[3] (2.8 g, 20 mmol) in 1,2-dimethoxyethane (100 mL). The mixture was kept at room temperature overnight and worked up as above. Evaporation of the ether gave a solid, which was recrystallized from ethyl acetate to give 3.47 g (80%) of **4**: mp 136–137 °C; UV λ_{max} (ethanol) 218 nm (ϵ 13 500); ^{1}H NMR (Me_2SO-d_6) δ 6.98 (s, 1, H-4), 4.82 (s, 2, CH_2Br). Anal. ($C_5H_6N_3OBr$) C, H, N.

Ethyl 1-(2,3,4,6-Tetra-*O*-acetyl-β- and -α-D-glucopyranosyl)-3-(bromomethyl)pyrazole-5-carboxylate (5a and 5b) and Ethyl 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(bromomethyl)pyrazole-3-carboxylate (6a). A mixture of ethyl 3(5)-(bromomethyl)pyrazole-5(3)-carboxylate (**3**; 1.16 g, 5 mmol) and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (3.90 g, 10 mmol) was heated at 150 °C in the presence of *p*-toluenesulfonic acid (35 mg) under reduced pressure for 15 min. The resulting mixture was chromatographed on plates using ethyl acetate–petroleum ether–chloroform (10:45:45). The fastest moving band yielded a solid product which after crystallization from cyclohexane gave 0.21 g (7.5%) of **5b**: mp 134–135 °C; $[\alpha]^{25}_D$ +94° (*c* 0.5, chloroform); UV λ_{max} (ethanol) 222 nm (ϵ 10 300), 240 (ϵ 6700) (sh). Anal. ($C_{21}H_{27}N_2O_{11}Br$) C, H, N.

The second fastest moving band gave 0.90 g of a syrup which was rechromatographed using ethyl acetate–petroleum ether–chloroform (1:3:6), to afford 0.67 g (24%) of **5a** as a foam which crystallized on standing: mp 105–106 °C (from carbon tetrachloride–hexane); $[\alpha]^{25}_D$ 0° (*c* 0.56, chloroform); UV λ_{max} (ethanol) 222 nm (ϵ 10 500), 240 (ϵ 6800) (sh). Anal. ($C_{21}H_{27}N_2O_{11}Br$) C, H, N.

The slowest moving band yielded 0.61 g (22%) of **6a**: mp 126–127 °C (from ethyl acetate–petroleum ether); $[\alpha]^{25}_D$ +12° (*c* 0.5 chloroform); UV λ_{max} 214 nm (ϵ 15 300). Anal. ($C_{21}H_{27}N_2O_{11}Br$) C, H, N.

Ethyl 1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-3-(bromomethyl)pyrazole-5-carboxylate (5c). By a similar method to that described above, **3** (1.16 g, 5 mmol) was allowed to react with 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose (3.18 g, 10 mmol) in the presence of *p*-toluenesulfonic acid (35 mg). The reaction mixture was purified by preparative TLC using ethyl acetate–petroleum ether (1:1). Elution of the major band afforded 0.89 g (36%) of **5c**: mp 132–133 °C (from carbon tetrachloride); $[\alpha]^{25}_D$ −42° (*c* 0.5, chloroform); UV λ_{max} 230 nm (ϵ 10 100), 245 (ϵ 7600) (sh). Anal. ($C_{18}H_{23}N_2O_9Br$) C, H, N.

Ethyl 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-(bromomethyl)pyrazole-5-carboxylate (5d). By a method similar to that described above, **3** (1.16 g, 5 mmol) was allowed to react with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.18 g, 10 mmol) in the presence of *p*-toluenesulfonic acid (35 mg). Preparative TLC of the crude reaction product using chloroform–petroleum ether (1:1) gave 1.60 g (65%) of **5d** as a homogeneous syrup: $[\alpha]^{25}_D$ −7° (*c* 0.54, chloroform); UV λ_{max} (ethanol) 235 nm (ϵ 10 300), 245 (ϵ 9200) (sh). Anal. ($C_{18}H_{23}N_2O_9Br$) C, H, N.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(bromomethyl)pyrazole-5-carboxamide (7a). By a method identical with that described above, 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide (**4**; 1.02 g, 5 mmol) was allowed to react with 1,-2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (3.90 g, 10 mmol) in the presence of *p*-toluenesulfonic acid (35 mg). The crude reaction product was chromatographed on preparative plates using ethyl acetate–petroleum ether (9:1) to give 1.22 g (46%) of **7a**: mp 217–218 °C (from ethyl acetate); $[\alpha]^{25}_D$ 0° (*c* 0.5, chloroform); UV λ_{max} (ethanol) 218 nm (ϵ 12 300). Anal. ($C_{19}H_{24}N_3O_{10}Br$) C, H, N.

1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-3-(bromomethyl)pyrazole-5-carboxamide (7c). By a procedure similar to that described above, **4** (1.02 g, 5 mmol) was allowed to react with 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose (3.18 g, 10 mmol) in the presence of *p*-toluenesulfonic acid (35 mg). Preparative TLC of the crude reaction product using ethyl acetate–chloroform (1:1) yielded 0.46 g (20%) of **7c** as a homogeneous foam: $[\alpha]^{25}_D$ −15° (*c* 0.6, chloroform); UV λ_{max} (ethanol) 226 nm (ϵ 7100). Anal. ($C_{16}H_{20}N_3O_8Br$·AcOEt) C, H, N.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-(bromomethyl)pyrazole-5-carboxamide (7d) and 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-5-(bromomethyl)pyrazole-3-carboxamide (8d). By a method identical with that described above, **4** (1.02 g, 5 mmol) was allowed to react with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.18 g, 10 mmol) in the presence of *p*-toluenesulfonic acid (35 mg). The reaction mixture was chromatographed by preparative TLC using ethyl acetate–chloroform (1:1). The fastest moving band gave 0.42 g (45%) of **7d** as a foam, which crystallized on standing: mp 120–121 °C (from ethyl acetate–petroleum ether); $[\alpha]^{25}_D$ −24° (*c* 0.54, chloroform); UV λ_{max} (ethanol) 230 nm (ϵ 9100). Anal. ($C_{16}H_{20}N_3O_8Br$) C, H, N.

The slowest moving band afforded 0.39 g (22%) of **8d** with mp 171–172 °C (from ethyl acetate): $[\alpha]^{25}_D$ −71° (*c* 0.48, chloroform); UV λ_{max} (ethanol) 223 nm (ϵ 8400). Anal. ($C_{16}H_{20}N_3O_8Br$) C, H, N.

1-(β-D-Glucopyranosyl)-3-(aminomethyl)pyrazole-5-carboxamide Hydrobromide (9e). From 5a. A solution of **5a** (0.22 g, 0.4 mmol) in methanolic ammonia (25 mL) was allowed to stand at room temperature for 20 h. The residue obtained by evaporation of the solvent was repeatedly treated with anhydrous ether and coevaporated to give **9e** as a homogeneous solid in quantitative yield: mp 230 °C; $[\alpha]^{25}_D$ +6° (*c* 0.54, water); ^{1}H NMR (Me_2SO-d_6–D_2O) δ 7.01 (s, 1, H-4), 6.24 (d, 1, $J_{1',2'}$ = 9 Hz, H-1'), 4.10 (s, 2, CH_2NH_2). Anal. ($C_{11}H_{19}N_4O_6Br$) C, H, N.

From 7a. Treatment of **7a** (0.21 g, 0.4 mmol) with methanolic ammonia in a similar way to that described above gave quantitatively **9e**, identical in all respects with that obtained from **5a**.

1-(β-D-Ribopyranosyl)-3-(aminomethyl)pyrazole-5-carboxamide Hydrobromide (9f). From 5c. Treatment of **5c** (0.24 g, 0.5 mmol) with a saturated solution of methanolic ammonia (25 mL) for 20 h, followed by evaporation of the solvent and coevaporation with anhydrous ether, gave **9f** in quantitative yield as a foam: $[\alpha]^{25}_D$ −16° (*c* 0.7, water); ^{1}H NMR (Me_2SO-d_6–D_2O) δ 7.00 (s, 1, H-4), 6.42 (d, 1, $J_{1',2'}$ = 9 Hz), 4.10 (s, 2, CH_2NH_2), 3.33 (s, 3, CH_3OH). Anal. ($C_{10}H_{17}N_4O_5Br$·CH_3OH) C, H, N.

From 7c. By an identical method with that described above, compound **7c** (0.23 g, 0.5 mmol) gave quantitatively **9f**, identical in all respects with that obtained from **5c**.

1-(β-D-Ribofuranosyl)-3-(aminomethyl)pyrazole-5-carboxamide Hydrobromide (9g). From 5d. Treatment of **5d** (0.24 g, 0.5 mmol) with methanolic ammonia (25 mL) following the procedure described for **9e** and **9f** gave **9g** in quantitative yield as a syrup: $[\alpha]^{25}_D$ −22° (*c* 0.75, water); ^{1}H NMR (Me_2SO-d_6–D_2O) δ 7.00 (s, 1, H-4), 6.85 (d, 1, $J_{1',2'}$ = 4 Hz), 4.10 (s, 2, CH_4NH_2). Anal. ($C_{10}H_{17}N_4O_5Br$) C, H, N.

From 7d. By an identical method with that described above, compound **7d** (0.23 g, 0.5 mmol) gave quantitatively **9g**, identical in all respects with that obtained from **5d**.

Ethyl 3(5)-[[Bis(2-chloroethyl)amino]methyl]pyrazole-5(3)-carboxylate (10). A solution of **3** (1.16 g, 5 mmol) in dry acetone (20 mL) was treated with bis(2-chloroethyl)amine (1.42 g, 10 mmol) freshly liberated from the hydrochloride, the whole was allowed to stand at room temperature for 24 h, and then the solvent was evaporated. The residue was treated with ether and filtered, and the filtrate was evaporated to give a syrup which was purified by preparative TLC using ethyl acetate–petroleum ether (1:1). Elution of the major band afforded 1.15 g (73%) of **10** as a syrup: ^{1}H NMR ($CDCl_3$) δ 6.83 (s, 1, H-4), 4.00 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.65 (t, 4, CH_2Cl), 3.05 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{11}H_{17}N_3O_2Cl_2$) C, H, N.

3(5)-[[Bis(2-chloroethyl)amino]methyl]pyrazole-5(3)-carboxamide (11). A solution of **4** (0.50 g, 2.5 mmol) in dry acetone (50 mL) was treated with bis(2-chloroethyl)amine (0.70 g, 5 mmol) freshly liberated from the hydrochloride and was allowed to stand at room temperature for 24 h. The solvent was removed and the residue was purified by preparative TLC using ethyl acetate–chloroform (1:1) to give 0.49 g (75%) of **11**: mp

123–124 °C (from ethyl acetate); ^{1}H NMR (Me_2SO-d_6) δ 6.72 (s, 1, H-4), 3.85 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.67 (t, 4, CH_2Cl), 2.90 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_9H_{14}N_4OCl_2$) C, H, N.

Ethyl 1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[[bis(2-chloroethyl)amino]methyl]pyrazole-5-carboxylate (12a). A solution of **5a** (0.56 g, 1 mmol) in dry acetone (20 mL) was treated with bis(2-chloroethyl)amine (0.28 g, 2 mmol) recently liberated from the hydrochloride, and the whole was allowed to stand at room temperature for 24 h. The solvent was removed and the residue extracted with ether. The ether was evaporated to give a syrup, which was purified by preparative TLC using ethyl acetate–petroleum ether (1:1). Elution of the major band gave 0.44 g (70%) of **12a** as a homogeneous syrup: $[\alpha]^{25}_D$ −6° (*c* 0.52, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1, H-4), 6.55 (d, 1, $J_{1',2'}$ = 9 Hz, H-1'), 3.85 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.60 (t, 4, CH_2Cl), 2.98 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{25}H_{35}N_3O_{11}Cl_2$) C, H, N.

Ethyl 1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-3-[[bis(2-chloroethyl)amino]methyl]pyrazole-5-carboxylate (12c). By a method identical with that described for **12a**, **5c** (0.30 g, 0.75 mmol) and bis(2-chloroethyl)amine (0.21 g, 1.50 mmol) led to **12c** (0.28 g, 82%) as a homogeneous syrup: $[\alpha]^{25}_D$ −4° (*c* 0.5, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1, H-4), 6.75 (d, 1, $J_{1',2'}$ = 9 Hz, H-1'), 3.85 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.60 (t, 4, CH_2Cl), 3.03 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{22}H_{31}N_3O_9Cl_2$) C, H, N.

Ethyl 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-[[bis(2-chloroethyl)amino]methyl]pyrazole-5-carboxylate (12d). By a procedure identical with that described for **12a** and **12c**, **5d** (0.49 g, 1 mmol) and bis(2-chloroethyl)amine (0.28 g, 2 mmol) gave **12d** (0.44 g, 80%) as a homogeneous syrup: $[\alpha]^{25}_D$ −32° (*c* 0.65, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1, H-4), 7.04 (d, 2, $J_{1',2'}$ = 2 Hz), 3.90 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.62 (t, 4, CH_2Cl), 3.10 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{22}H_{31}N_3O_9Cl_2$) C, H, N.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-[[bis(2-chloroethyl)amino]methyl]pyrazole-5-carboxamide (13a). A solution of **7a** (0.51 g, 1 mmol) in dry acetone (20 mL) was treated with bis(2-chloroethyl)amine (0.28 g, 2 mmol) freshly liberated from its hydrochloride and allowed to stand at room temperature for 24 h. The solvent was removed, the residue was treated with ethyl acetate and filtered, and the filtrate was evaporated to give a syrup, which was purified by TLC using ethyl acetate–petroleum ether (9:1). Elution of the major band afforded **13a** (0.39 g, 65%) as a homogeneous syrup: $[\alpha]^{25}_D$ −17.5° (*c* 0.5, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.83 (s, 1, H-4), 6.68 (d, 1, $J_{1',2'}$ = 9 Hz, H-1'), 3.88 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.55 (t, 4, CH_2Cl), 3.10 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{23}H_{32}N_4O_{10}Cl_2$) C, H, N.

1-(2,3,4-Tri-*O*-acetyl-β-D-ribopyranosyl)-3-[[bis(2-chloroethyl)amino]methyl]pyrazole-5-carboxamide (13c). By a procedure identical with that described for **13a**, **7c** (0.17 g, 0.37 mmol) and bis(2-chloroethyl)amine (0.11 g, 0.75 mmol) led to **13c** (0.10 g, 50%) as a homogeneous syrup: $[\alpha]^{25}_D$ −12.5° (*c* 0.5, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.86 (s, 1, H-4), 6.64 (d, 1, $J_{1',2'}$ = 9 Hz, H-1'), 3.98 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.68 (t, 4, CH_2Cl), 3.05 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{20}H_{28}N_4O_8Cl_2$) C, H, N.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-[[bis(2-chloroethyl)amino]methyl]pyrazole-5-carboxamide (13d). By a procedure identical with that described for **13a** and **13c**, **7d** (0.17 g, 0.37 mmol) and bis(2-chloroethyl)amine (0.11 g, 0.75 mmol) led to **13d** (0.12 g, 60%) as a homogeneous syrup: $[\alpha]^{25}_D$ −10° (*c* 0.6, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 1, H-4), 7.02 (d, 1, $J_{1',2'}$ = 2 Hz, H-1'), 3.98 [s, 2, $CH_2N(CH_2CH_2Cl)_2$], 3.70 (t, 4, CH_2Cl), 3.09 [t, 4, $N(CH_2CH_2Cl)_2$]. Anal. ($C_{20}H_{28}N_4O_8Cl_2$·1AcOEt) C, H, N.

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2′-*O*-Acyl-6-thioinosine Cyclic 3′,5′-Phosphates as Prodrugs of Thioinosinic Acid[1]

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A series of 2′-*O*-acyl derivatives of 6-thioinosine cyclic 3′,5′-phosphate (6-HS-cRMP) were prepared and examined for their cytotoxic effects on S49 mouse lymphoma cells which were deficient in hypoxanthine–guanine phosphoribosyltransferase (HGPRTase). Cytotoxicity increased with the lipophilicity of the acyl group to a lowest EC_{50} of 65 μM for the 2′-*O*-palmityl derivative. Addition of a mutation in the gene for cAMP-dependent protein kinase to the HGPRTase-deficient cell line confers resistance to 2′-*O*-butyryl-cAMP but not to 2′-*O*-butyryl-6-HS-cRMP, indicating that the latter does not exert its toxic effect via activation of protein kinase. The time course of cell kill by 2′-*O*-palmityl-6-HS-cRMP resembled that of 6-mercaptopurine and not that of cyclic AMP in these cells. The data suggest that the intact cyclic nucleotides are penetrating the cells and being converted, by phosphodiesterase action and deacylation, to the first toxic metabolite of 6-mercaptopurine, thioinosinic acid.

All analogues of naturally occurring purine and pyrimidine bases and nucleosides currently in clinical use for cancer chemotherapy must be converted to their respective nucleotides to exert their cytostatic or cytotoxic effects.

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