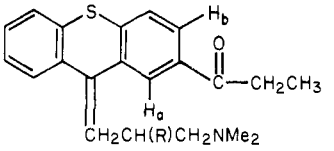
Table VII. Chemical Shifts (δ, ppm) and ΔEu values (ppm) for compounds in $CDCl_3$/internal standard Me_4Si (1%)



no.	sample wt, mol	H_a	H_b	$COCH_2CH_3$	NMe_2	H_c	stereochem assignment
34	1.48×10^{-5}	8.03 (d) [-3.8][a]	7.70 (q) [-2.3]	3.02 (q) [-2.4]	2.11 (s) [-10.0]	5.65 (d) [-3.5]	1:1 *Z* & *E*
		8.15 (d) [-6.5]	7.85 (q) [-2.3]	3.02 (q) [-2.7]	2.14 (s) [-10.0]	5.77 (d) [-5.2]	isomeric mix.
36	1.6×10^{-5}	8.03 (d) [-5.3]	7.75 (q) [-1.5]	2.97 (q) [-2.2]	2.20 (s) [-8.1]	5.95 (t) [-3.7]	2:1 *Z* & *E* isomeric mix.
35	1.59×10^{-5}	8.03 (d) [-5.3]	7.75 (q) [-1.5]	2.98 (q) [-2.2]	2.30 (s) [-8.2]	5.97 (t) [-3.9]	*Z* isomer

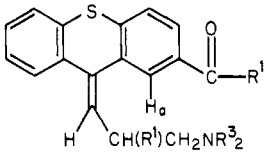
[a] ΔEu values are in brackets.

Catalytic Hydrogenation of Olefins (Table V). This method is exemplified by the hydrogenation of **30**. The olefin **30**, as its free base (3.8 g), in ethanol (150 mL) was hydrogenated at 25 °C at an initial pressure of 300 psi, over 5% Pd/C catalyst (0.3 g). After the uptake was complete (24 h), the mixture was filtered and the solvent removed in vacuo. The residual oil was dissolved in dry ether and treated with ethereal HCl to give the thioxanthene **52** as the hydrochloride.

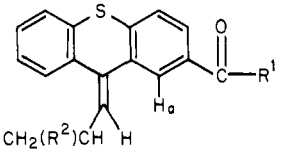
Isolation of the Carbinols (Table V). The preparation of **50** is an example of this procedure. The carbinol (7.0 g) was refluxed in methanol (100 mL), containing *p*-toluenesulfonic acid (0.2 g), for 2 h. Evaporation of the solvent in vacuo gave a brown gum (6.2 g), which was dissolved in ether, and the solution was extracted with acetic acid (2 N). The acid extract was made alkaline with NaOH solution and the mixture extracted with ether. The ether extract wad dried and on evaporation gave an oil (1.4 g). The oil was chromatographed on silica gel, using chloroform as eluent, to give the carbinol **50**, which was isolated as the oxalate salt (1.0 g) in the usual manner.

Separation of the Geometrical Isomers. The separation of the isomers **32** and **33** is an example of this procedure. The isomeric mixture (as the HCl salts) was fractionally crystallized from alcohol, isomer **32** being the less-soluble isomer.

^{1}H NMR Determinations. ^{1}H NMR spectra[21] of the thioxanthenes (Table IV) were initially determined at 30 °C using a Varian A60D spectrometer. This demonstrated, in the majority of cases, the presence of approximately equal mixtures of the *Z* and *E* isomers. (H_a in the *Z* isomer showed as a doublet downfield



Z isomer



E isomer

from the H_a doublet of the *E* isomer.) This was confirmed for certain compounds using an europium-shift reagent as follows.

Solutions were prepared by adding approximately 5 mg of the test compound (as the free base) to 0.5 mL of $CDCl_3$. ^{1}H NMR spectra were obtained at 30 °C using a Varian CAT 20 spectrometer. Tris(dipivalomethanato)europium [$Eu(DPM)_3$] was then added to the solution, and the spectra were recorded again. The ΔEu value[22] for each relevant proton was derived from the slope of the straight line obtained by plotting the magnitude of the shift induced at various values of the molar ratio of $Eu(DPM)_3$ to solute (see Table VII).

Acknowledgment. The authors thank Dr. M. L. Podmore for the spectral measurements and Mrs. A. Kalsi, B. Doggett, and J. Patrick for technical assistance.

(21) Solutions were prepared by adding approximately 50 mg of the test compound (as its free base) to 0.5 mL of $CDCl_3$.

(22) P. V. Demarco, K. K. Elzey, R. B. Lewisard, and E. Wenkert, *J. Am. Chem. Soc.*, **92**, 5734 (1970).

(23) K. Sindelar, J. O. Jileh, J. Pomykacak, Z. Sedivy, and M. Protiva, *Collect. Czech. Chem. Commun.*, **43** (2), 471 (1978).

Alkylating Nucleosides. 4. Synthesis and Cytostatic Activity of Chloro- and Iodomethylpyrazole Nucleosides

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The synthesis and cytostatic activity of several chloromethyl- and iodomethylpyrazole nucleosides are described. Glycosylation of ethyl 3(5)-(chloromethyl)pyrazole-5(3)-carboxylate (**3**) and 3(5)-(chloromethyl)pyrazole-5(3)-carboxamide (**4**) with poly(O-acetylated) sugars via an acid-catalyzed fusion method gave the corresponding 3-(chloromethyl)-5-carboxylate and 3-(chloromethyl)-5-carboxamide substituted nucleosides **7** and **9**, respectively. From the reaction of **4** with tetra-*O*-acetyl-β-D-ribofuranose, the 5-(chloromethyl)-3-carboxamide-substituted derivative **11** was also obtained. Reaction of **7**, **9**, and **11** with sodium iodide in acetone provided the related iodomethylpyrazole nucleosides **8**, **10**, and **12**. In general, chloromethyl-substituted nucleosides showed moderate activities against HeLa cells, while all the corresponding iodomethyl derivatives exhibited high activities. Some of these latter compounds increased the life span of mice bearing ECA tumor.

In previous papers of this series,[1-4] we have reported the synthesis, cytostatic activity, and mode of action of several halomethyl-1,2,3-triazole and bromomethylpyrazole nucleosides **1** and **2**, as a new type of alkylating agents. The

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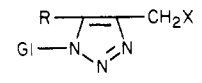
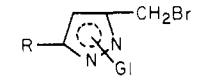
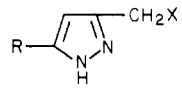


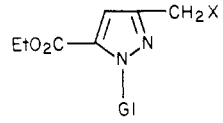
1, X = halogen
R = H or CH_2X
Gl = glycosyl



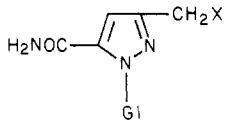
2, R = CO_2Et, $CONH_2$, CH_3
Gl = glycosyl



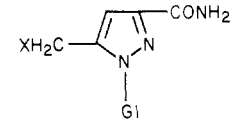
3, X = Cl; R = CO_2Et
4, X = Cl; R = $CONH_2$
5, X = I; R = CO_2Et
6, X = I; R = $CONH_2$



7, X = Cl 8, X = I
7a-c 8a,c



9, X = Cl 10, X = I
9a-c 10a,c



11, X = Cl 12, X = I
11c 12c

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 = 2,3,5-tri-*O*-acetyl-β-D-ribofuranose

design of these nucleosides as potential antitumor drugs was based on the use of the chemically alkylating halomethyl group as the active moiety of such compounds. In fact, it could be observed in the series of halomethyl-1,2,3-triazoles, **1**, that both cytostatic activity and chemical alkylating ability increased in the same order. In the "in vivo" tests, 4-(bromomethyl)- and 4-(iodomethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (**1**, X = Br, I; R = H) were reported to be effective against ECA and P388 tumor systems.[4] Significant "in vitro" activities were also found in the bromomethylpyrazole nucleosides described. Concerning the "in vivo" activity of these latter nucleosides, those carboxamide-substituted derivatives (**2**, R = $CONH_2$) produced a significant increase in the life span of mice bearing ECA tumor.[5]

All of these facts and our interest in structure–activity relationships prompted us to study iodomethylpyrazole nucleoside analogues of **2**.

The present paper describes the synthesis and cytostatic activity of nucleosides of ethyl 3(5)-(iodomethyl)-pyrazole-5(3)-carboxylate and 3(5)-(iodomethyl)pyrazole-5(3)-carboxamide (**5** and **6**) from the corresponding chloromethylpyrazole nucleosides. It also describes the synthesis and cytostatic activity of these latter nucleosides.

Chemistry. Our initial route for the synthesis of the mentioned iodomethypyrazole nucleosides was that employed for the preparation of the related bromomethylpyrazole nucleosides **2** (R = CO_2Et, $CONH_2$).[2] This route consisted of glycosylation of the corresponding halomethylpyrazolic bases via an acid-catalyzed fusion method. The iodomethylpyrazoles **5** and **6** were prepared by reaction of ethyl 3(5)-(chloromethyl)pyrazole-5(3)-carboxylate and 3(5)-(chloromethyl)pyrazole-5(3)-carboxamide (**3** and **4**) with sodium iodide in acetone. Compounds **3** and **4** were obtained by treating the corresponding hydroxymethyl derivatives, namely, ethyl 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxylate and 3(5)-(hydroxymethyl)pyrazole-5-(3)-carboxamide, respectively,[6] with thionyl chloride. Use of the fusion procedure with ethyl 3(5)-(iodomethyl)-pyrazole-5(3)-carboxylate (**5**) or 3(5)-(iodomethyl)-pyrazole-5(3)-carboxamide (**6**) and penta-*O*-acetyl-β-D-glucopyranose in the presence of *p*-toluenesulfonic acid gave, in both cases, very poor yields of nucleosidic material. However, simultaneous attempts to glycosylate the related chloromethylpyrazoles **3** and **4** by an identical procedure gave acceptable results. Therefore, we approached the synthesis of the desired iodomethyl-substituted nucleosides by glycosylation of the chloromethylpyrazole derivatives **3** and **4**, followed by chlorine–iodine exchange.

Table I. ^1H NMR and UV Data of Chloromethyl Derivatives

no.	UV λ_{max} (EtOH), nm (ε)	^1H NMR solvent	δ (H-1′)	$J_{1',2'}$, Hz	δ (CH_2Cl)
3	232 (5750)	$CDCl_3$			4.75
4	233 (4000)	Me_2SO			4.80
7a	230 (10 600), 240 (8300)(sh)	$CDCl_3$	6.38	9.5	4.52
7b	230 (7800), 240 (5800)(sh)	$CDCl_3$	7.16	6	4.65
7c	230 (17 200), 240 (12 900)(sh)	Me_2SO	6.85	2	4.80
9a	227 (9500)	Me_2SO	6.85	9	4.76
9b	227 (9700)	Me_2SO	7.43	6	4.89
9c	226 (10 300)	$CDCl_3$	7.04	2	4.64
11c	218 (7850)	$CDCl_3$	6.19	0	4.75

Fusion of **3** with penta-*O*-acetyl-β-D-glucopyranose in the presence of *p*-toluenesulfonic acid provided ethyl 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(chloromethyl)pyrazole-5-carboxylate (**7a**) and its α anomer **7b** in a ratio of 10:1. Structural assignments of these nucleosides were made on the basis of their ^1H NMR spectra (Table I). The anomeric configuration was clearly ascertained as β for **7a** and α for **7b** on the basis of the corresponding values of $J_{1',2'}$. The chemical shift for the anomeric proton was identical in each case with that of the related bromomethylpyrazole nucleosides, namely, ethyl 1-(2,3,4,6-tetra-*O*-acetyl-β- and -α-D-glucopyranosyl)-3-(bromomethyl)pyrazole-5-carboxylate.[2] Thus, **7a** and **7b** were established as 1-glycosyl-3-(chloromethyl)-5-carboxylates.

Ribosylation of **3** by the same procedure gave ethyl 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(chloromethyl)-pyrazole-5-carboxylate as the only reaction product. This compound was assigned as a 3-(chloromethyl)-5-carboxylate-substituted nucleoside, since its UV spectrum was identical with that of **7a** and **7b**, respectively (Table I). The small 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 was very close to that of the analogous 3-(bromomethyl)-5-carboxylate-substituted nucleoside,[2] which has a β configuration.

Reaction of **7a** and **7c** with sodium iodide in acetone afforded the corresponding iodomethyl nucleosides, namely, ethyl 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(**8a**) and ethyl 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(iodomethyl)pyrazole-5-carboxylate (**8c**), in high yields (Table II).

(1) de las Heras, F. G.; Alonso, R.; Alonso, G. *J. Med. Chem.* **1979**, *22*, 496.
(2) García-Lõpez, M. T.; Herranz, R.; Alonso, G. *J. Med. Chem.* **1979**, *22*, 807.
(3) García-Lõpez, M. T.; Herranz, R.; Alonso, G. *J. Heterocycl. Chem.* **1980**, *17*, 113.
(4) Contreras, A.; Sãnchez-Pérez, R. M.; Alonso, G. *Cancer Chemother. Pharmacol.* **1978**, *1*, 243.
(5) Contreras, A.; Sãnchez-Pérez, R. M.; Alonso, G. unpublished results.
(6) Muganini, E.; Grunnanger, P. *Atti. Accad. Naz. Lincei, Cl. Sci. Fis. Mat. Nat., Rend.* **1953**, *14*, 958; *Chem. Abstr.* **1954**, *48*, 2684.

Table II. Physical Data of Iodomethyl Derivatives

no.	mp, °C	recrystn solvent[a]	yield, %	formula[b]	UV λ_{max} (EtOH), nm (ϵ)	^{1}H NMR solvent	δ (H-1′)	$J_{1',2'}$, Hz	δ (CH_2I)
5	106-107	A	60	$C_7H_9N_2O_2I$	232 (12 200)	$CDCl_3$			4.52
6	152-153	B	77	$C_5H_6N_3OI$	238 (11 600), 290 (3150), 360 (1650)	Me_2SO			4.56
8a	130-132	C	80	$C_{21}H_{27}N_2O_{11}I$	228 (18 800), 240 (12 000) (sh)	$CDCl_3$	6.48	10	4.68
8c	syrup		90	$C_{18}H_{23}N_2O_9I$	228 (20 200), 240 (13 450) (sh)	Me_2SO	6.84	2	4.52
10a	204-206	C	78	$C_{19}H_{24}N_3O_{10}I$	228 (12 700)	$CDCl_3$	6.58	10	4.40
10c	122-124	C	82	$C_{16}H_{20}N_3O_8I$	227 (17 500)	$CDCl_3$	6.97	2	4.40
12c	184-186	C	90	$C_{16}H_{20}N_3O_8I$	221 (15 600), 240 (10 900) (sh)	$CDCl_3$	6.12	0	4.50

[a] A, cyclohexane; B, MeOH; C, EtOAc-petroleum ether. [b] Analytical results for C, H, and N were within 0.4% of the theoretical values.

Similar fusion reactions were achieved with 3(5)-(chloromethyl)pyrazole-5(3)-carboxamide (**4**). Acid-catalyzed fusion of **4** with penta-*O*-acetyl-β-D-glucopyranose gave 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(chloromethyl)pyrazole-5-carboxamide (**9a**). TLC of the crude reaction product showed the presence of a very small amount of other nucleosidic material, which was identified as 1-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-3-(chloromethyl)pyrazole-5-carboxamide (**9b**) on the basis of its ^{1}H NMR and UV spectra. As described earlier for **7a** and **7b**, the glycosylation site of **9a** was determined by comparing the δ value for the anomeric proton with that of the 3-(bromomethyl)pyrazole-5-carboxamide nucleoside previously reported.[2] In the case of the α anomer **9b**, the site of glycosylation was assigned by comparing its UV absorption spectrum, which was superimposable, with that of the β-anomer **9a** (Table I).

Ribosylation of **4** with tetra-*O*-acetyl-β-D-ribofuranose by the same procedure, followed by chromatographic separation, afforded 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(chloromethyl)pyrazole-5-carboxamide (**9c**) and its positional isomer 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-5-(chloromethyl)pyrazole-3-carboxamide (**11c**) in a ratio of ca. 1:1. Evidence for the structures of these compounds stems from comparing their ^{1}H NMR and UV spectra with each other (Table I), as well as with those of the related 3-(bromomethyl)pyrazole nucleosides.[2] In both cases, the chemical shifts of the H-1′ signal of **9c** and **11c** were in good agreement with those of the 3-(bromomethyl)-5-carboxamide and 5-(bromomethyl)-3-carboxamide substituted nucleosides, respectively, with a β configuration. As in that case, H-1′ of the 5-carboxamide derivative **9c** appeared at lower field than that of the 3-carboxamide derivative **11c** as a consequence of the anisotropic carboxamide group adjacent to the site of glycosylation.[7] On the other hand, the UV spectra of **9c** and **11c** showed a difference from each other similar to that reported with respect to the corresponding bromomethyl derivatives.[2] The anomeric configuration of **9c** could not be unequivocally established by the value $J_{1',2'}$ = 2 Hz. However it was assigned as β on the basis of the δ value for the anomeric proton as indicated.

As described earlier, replacement of chlorine by iodine in **9a,c** and **11c** provided the corresponding iodomethylpyrazole nucleosides, namely, 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)- (**10a**), 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-(iodomethyl)pyrazole-5-carboxamide (**10c**) and 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-5-(iodomethyl)pyrazole-3-carboxamide (**12c**), respectively (Table II).

(7) Revankar, G. R.; Townsend, L. B. *J. Heterocycl. Chem.* **1970**, *7*, 1329.

Table III. Cytostatic Activity against HeLa Cells

chloromethylpyrazoles no.	ED_{50}, μg/mL	iodomethylpyrazoles no.	ED_{50}, μg/mL
3	>100	5	>100
4	>100	6	>100
7a	40	8a	2.5
7b	>100		
7c	32	8c	5.5
9a	18	10a	2
9b	12		
9c	26	10c	1.5
11c	12	12c	2

Table IV. Cytostatic Activity of Compounds 10a,c and 12c in Mice Bearing ECA Tumor (See Experimental Section for Methodology)

no.	daily dose,[a] mg/kg	toxicity day 5 survivors[b]	animal wt diff[c]	cures[d]	T/C × 100[e]
10a	50	7/7	−0.3	4	176
	25	7/7	−0.1	1	105
10c	50	7/7	−0.6		63
	25	7/7	−0.4	4	158
	12.5	7/7	−0.6	2	131
12c	50	7/7	−0.5	1	100
	25	7/7	−0.25	4	141
	12.5	7/7	0.30	2	121

[a] Nine ip doses were given at 24-h intervals, starting 24 h after ip injection of tumor cells. [b] Number of survivors on day 5/number of mice started on test. [c] Difference (g) between the weights of test and control animals. [d] Survivors on day of evaluation (day 30). [e] Percent increase in median survival time of treated mice as compared with controls. A value of T/C × 100 ≥ 125 is considered a statistically significant indication of the antitumor activity of the compound.[8]

Cytostatic Activity. All the compounds reported in this paper were evaluated as cytotoxic agents against HeLa cell cultures (Table III). The most active compounds in this test were assayed in mice bearing Ehrlich carcinoma ascites (ECA) tumor. As expected, cytostatic activity increased with the alkylating ability of the halomethyl group. Thus, the activities of the chloromethylpyrazole nucleosides were, in all cases, lower than those of the corresponding iodomethyl derivatives. All of these iodo nucleosides showed significant "in vitro" activities, similar

to those reported for the related bromomethylpyrazole nucleosides.[2] As in that case, the free halomethylpyrazolic bases **3–6** were completely inactive.

In the in vivo test, as in the case of the bromomethylpyrazole nucleosides,[5] those carboxamide-substituted iodomethylpyrazole nucleosides, **10a**, **10c**, and **12c**, produced a significant increase in the life span of mice bearing ECA tumor (Table IV). Although definite conclusions can not be drawn, it seems that the positions of the halomethyl and carboxamide groups (whether CH_2X at C-3 and $-CONH_2$ at C-5 or vice versa) do not affect significantly the cytostatic activity.

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.

Ethyl 3(5)-(Chloromethyl)pyrazole-5(3)-carboxylate (3). A solution of thionyl chloride (18 mL) in benzene (40 mL) was slowly added to a stirred suspension of ethyl 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxylate[6] (8.5 g, 50 mmol) in benzene (50 mL). The mixture was kept at room temperature overnight while stirring, and then ice–water (50 mL) was added. The mixture was neutralized with solid sodium bicarbonate and extracted with ether (200 mL; three times). The organic layer was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent left a solid, which was recrystallized from cyclohexane to give 8.58 g (91%) of **3**, mp 68–70 °C. Anal. ($C_7H_9N_2O_2Cl$) C, H, N.

3(5)-(Chloromethyl)pyrazole-5(3)-carboxamide (4). A solution of thionyl chloride (18 mL) in 1,2-dimethoxyethane (40 mL) was slowly added to a stirred suspension of 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxamide[6] (7.07 g, 50 mmol) in 1,2-dimethoxyethane (50 mL). The mixture was kept at room temperature overnight while stirring and worked up as above. Evaporation of the solvent gave a solid, which was recrystallized from ethyl acetate to give 8.27 g (100%) of **4**, mp 157–158 °C. Anal. ($C_5H_6N_3OCl$) C, H, N.

Ethyl 1-(2,3,4,6-Tetra-*O*-acetyl-β- and -α-D-glucopyranosyl)-3-(chloromethyl)pyrazole-5-carboxylate (7a and 7b). A mixture of ethyl 3(5)-(chloromethyl)pyrazole-5(3)-carboxylate (**3**; 1.88 g, 10 mmol) and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (5.85 g, 15 mmol) was heated at 150 °C in the presence of *p*-toluenesulfonic acid (70 mg) under reduced pressure for 15 min. The resulting mixture was chromatographed on plates using EtOAc–$CHCl_3$–petroleum ether (10:45:45). The fastest moving band yielded a solid, which was recrystallized from Et_2O–petroleum ether to give 0.155 g (3%) of **7b**, mp 162–163 °C. Anal. ($C_{21}H_{27}N_2O_{11}Cl$) C, H, N. The slowest moving band was eluted and rechromatographed using EtOAc–$CHCl_3$–petroleum ether (1:1:2), to afford 1.57 g (30%) of **7a** as a homogeneous syrup. Anal. ($C_{21}H_{27}N_2O_{11}Cl$) C, H, N.

Ethyl 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-(chloromethyl)pyrazole-5-carboxylate (7c). By a method similar to that described above, **3** (1.88 g, 10 mmol) was allowed to react with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (4.77 g, 15 mmol) in the presence of *p*-toluenesulfonic acid (70 mg). The reaction mixture was chromatographed by preparative TLC using $CHCl_3$. Elution of the major band gave an oil, which was rechromatographed using $CHCl_3$–EtOAc–petroleum ether (1:1:1), to give 1.85 g (42%) of **7c** as a homogeneous syrup. Anal. ($C_{18}H_{23}N_2O_9Cl$) C, H, N.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-(chloromethyl)pyrazole-5-carboxamide (9a). By a procedure similar to that described above, 3(5)-(chloromethyl)pyrazole-5(3)-carboxamide (**4**; 1.59 g, 10 mmol) was allowed to react with 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (5.85 g, 15 mmol) in the presence of *p*-toluenesulfonic acid (70 mg). The crude reaction product was purified by two consecutive preparative TLC, the first using $CHCl_3$ and the second EtOAc–$CHCl_3$ (1:1). Elution of the major band gave 1.46 g (30%) of **9a**, mp 222–223 °C (from EtOAc). Anal. ($C_{19}H_{24}N_3O_{10}Cl$) C, H, N.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-(chloromethyl)pyrazole-5-carboxamide (9c) and 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-5-(chloromethyl)pyrazole-3-carboxamide (11c). By a method identical with that described above, **4** (1.59 g, 10 mmol) was allowed to react with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (4.77 g, 15 mmol) in the presence of *p*-toluenesulfonic acid (70 mg). The reaction mixture was chromatographed by preparative TLC using EtOAc–$CHCl_3$ (1:1). The fastest moving band gave 1.86 g (44%) of **9c** as a syrup. Anal. ($C_{16}H_{20}N_3O_8Cl$) C, H, N. The slowest moving band afforded 1.47 g (35%) of **11c**, mp 153–154 °C (from EtOAc–petroleum ether). Anal. ($C_{16}H_{20}N_3O_8Cl$) C, H, N.

Preparation of Iodomethylpyrazole Derivatives 5, 6, 8, 10, and 12. General Procedure. A solution of 1 mmol of chloromethylpyrazole derivative (**3**, **4**, **7**, **9**, and **11**) and 1.5 mmol of NaI in 20 mL of dry acetone was heated to reflux until a precipitate of NaCl formed (~2 min). After cooling, the NaCl was removed by filtration and washed with dry acetone. The filtrate and washings were evaporated in vacuo. The residue was treated with a saturated solution of sodium thiosulfate and extracted with EtOAc (30 mL). The organic layer was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent yielded the corresponding iodomethyl derivatives **5**, **6**, **8**, **10**, and **12**.

In Vitro Cytostatic Activity. The previously described method[8] was followed. Minimal Eagle's medium[9] (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 ($ED_{50} \simeq 0.1$ μg/mL). Cell growth was estimated by measuring the cell proteins following the colorimetric method of Oyama and Eagle.[10]

In Vivo Cytostatic Activity. ICR Swiss female mice weighing 19–22 g were bred in the animal house of our Institute and used throughout this work. Ehrlich carcinoma was maintained in ascitic form by ip injection to mice of about 5×10^5 cells obtained from a donor mouse bearing a 7-day-old tumor.

Cytostatic activity in mice bearing Ehrlich carcinoma ascites (ECA) tumor was estimated according to protocols 1100 and 1200 from the National Cancer Institute.[8] Test compounds were suspended in 0.9% (w/v) NaCl solution containing 0.05% (v/v) Tween 80 and injected ip at a fixed volume of 0.4 mL. The treatment and control groups contained 7 and 30 mice, respectively. The positive control compound was 5-fluorouracil [20 (mg/kg)/injection].

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