mmol), DMF (6 mL), and ethylenimine (0.5 mL) was stirred at room temperature for 3 h. The addition of MeOH (6 mL) to the mixture gave crystallines and recrystallization from DMF–MeOH (1:1) yielded orange prisms: 0.18 g (29%); mp 217 °C (dec); UV λ_{max} (50% EtOH) 317 nm (log ϵ 4.25), 336 (4.15); IR ν (Nujol) 3450–3210 (NH_2), 1730 (carbamate), 1650 cm^{-1} (quinone). Anal. ($C_{18}H_{24}O_8N_4$) C, H, N.

2-(2,5-Dimethoxy-4-methylphenyl)-2-methoxyethyl 2,3,-4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (17). A mixture of **16** (2.90 g, 12.8 mmol), Ag_2O (3.60 g, 15.3 mmol), anhydrous $CaSO_4$ (10 g), and $CHCl_3$ (25 mL) was stirred at room temperature for 0.5 h. After the addition of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (5.80 g, 14.1 mmol), the mixture was stirred for 30 h and filtered. The filtrate was evaporated to dryness and the residue was chromatographed on silica gel eluting with C_6H_6–EtOAc. The eluate (C_6H_6–EtOAc, 3:1) gave **17** as a powder: 3.0 g (43%); IR ν (Nujol) 1755 cm^{-1} (C=O). Anal. ($C_{26}H_{36}O_{13}$) C, H.

2-Methoxy-2-(5-methyl-*p*-benzoquinon-2-yl)ethyl 2,3,-4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (18). To a cooled mixture of **17** (3.0 g, 5.4 mmol) and HOAc (15 mL) was added dropwise 60% HNO_3 (2.0 mL). The mixture was stirred at room temperature for 0.5 h, poured into 100 mL of ice–water, and extracted with Et_2O. The extracts were dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed over silica gel eluting with C_6H_6–EtOAc. The eluate (C_6H_6–EtOAc, 3:1) gave **18** as a powder: 1.12 g (39.5%); IR ν (Nujol) 1740–1780 (ester), 1655 (quinone), 1620 cm^{-1} (C=C). Anal. ($C_{24}H_{30}O_{13}$) C, H.

2-[3,6-Bis(1-aziridinyl)-5-methyl-*p*-benzoquinon-2-yl]-2-methoxyethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (19). To a cooled mixture of **18** (1.24 g, 2.36 mmol), EtOH (9 mL), and EtOAc (1 mL) was added ethylenimine (1 mL). The mixture was stirred at room temperature for 2 h and evaporated to dryness. The residue was chromatographed over silica gel eluting with C_6H_6–EtOH. The eluate (C_6H_6–EtOH, 10:1) gave **19** as red crystallines: 0.10 g (7.0%); mp 60–63 °C; IR ν (Nujol) 1755 (ester), 1645 (quinone), 1580 cm^{-1} (C=C); UV λ_{max} (EtOH) 333 nm (log ϵ 4.15). Anal. ($C_{28}H_{36}N_2O_6$) C, H, N.

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Alkylating Nucleosides 1. Synthesis and Cytostatic Activity of *N*-Glycosyl(halomethyl)-1,2,3-triazoles. A New Type of Alkylating Agent

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1,3-Dipolar cycloaddition of benzyl azide or peracetylated glucopyranosyl azides to propargyl halides or 1,4-dihalobutynes yielded 1-benzyl- or 1-glycosyl(halomethyl)-1,2,3-triazoles. Alkylating chloromethyl- bromomethyl- and iodomethyl-1,2,3-triazoles were also obtained from the corresponding hydroxymethyl derivatives by treatment with $(C_6H_5)_3P/CCl_4$, $(C_6H_5O)_3P/Br_2$, and $(C_6H_5O)_3P/I_2$, respectively. 1-Benzyl-4-(fluoromethyl)-1,2,3-triazole was obtained from 1-benzyl-4-(bromomethyl)-1,2,3-triazole by treatment with KF and 18-crown-6. Chloromethyl-, bromomethyl-, and iodomethyl-1,2,3-triazole derivatives inhibited the "in vitro" growth of HeLa cells. Some of these compounds increased the life span of mice bearing tumors.

Alkylating agents have been extensively studied in connection with cancer chemotherapy.[1,2] One of the classical major disadvantages of such drugs is their low selectivity for neoplastic tissue.[1] However, recent studies have shown the effectiveness and specificity of some alkylating agents, such as cyclophosphamide or merophan in the treatment of Burkitt's lymphoma[3,4] or the specificity of 5-aziridino-2,4-dinitrobenzamide on Walker tumor cells.[5] These and other examples[2] should encourage the search for new and more selective alkylating agents as anticancer drugs.

Several theoretical approaches have been used in the design of alkylating drugs.[6] One of the most fruitful approaches involves the attachment of the alkylating agent to a carrier that is related to substances normally involved in cell growth. Carriers which have been employed are naturally occurring amino acids,[7,8] carbohydrates,[9,10] steroids,[11] nucleic acid components, bases,[12,13] nucleosides,[14-20] and nucleotides.[16] Some of the alkylating derivatives of nucleic acid components have been reported to show anticancer activity. Thus, 5-[bis(2-chloroethyl)amino]uracil and its 6-methyl derivative (Dopan) are effective against tumors of the hematopoietic system,[21,22] 9-alkyl- and 9-ribofuranosyl derivatives of 6-(1-aziridinyl)purines show activity against adenocarcinoma 755,[14] and 5-[[bis(2-chloroethyl)amino]methyl]uridine is active against leukemia.[15]

Several functional groups have been commonly used as the active moiety of alkylating agents. These are nitrogen mustards, sulfur mustards, aziridines, epoxides, alkanesulfonates, and nitrosoureas. There are, however, some other chemically efficient alkylating groups, such as allylic-

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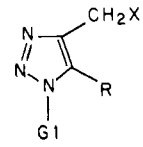
or benzylic-type halides, that have been little used as active moieties of antitumor drugs.

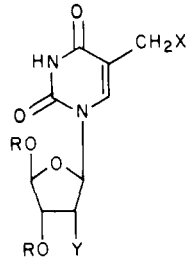
As part of our program for the search of new anticancer agents, we have studied the synthesis and cytostatic activity of a new type of alkylating agent, namely, 1-glycosyl(halomethyl)-1,2,3-triazole (1) in which the al-



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

kylating moiety is a benzylic-type halide. These alkylating structures resemble that of nitrogen mustards, in that both have the same atom sequence NCCX.

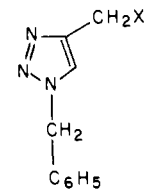
Several halomethyl derivatives of nucleic acid bases are known,[12] but only a few cases of nucleoside derivatives[15,17] have been described, e.g., **3** and **5**, the cytostatic activities of which have not been reported. These pyrimidine nucleosides have been synthesized by bromination of the thymidine **2** with NBS to give **3** or by displacement of the



2, X = H; Y = H; R = AcO
3, X = Br; Y = H; R = AcO
4, X = $OCH_2C_6H_5$; Y = OBz; R = Bz
5, X = Br; Y = OBz; R = Bz

benzyl ether group of **4** with hydrogen bromide to give **5**. We have introduced the halomethyl group on the triazole ring by direct cycloaddition of glycosyl azides to the triple bond of propargyl halides or by halogenation of 1-glycosyl(hydroxymethyl)-1,2,3-triazole (1, X = OH).

Chemistry. The synthesis of alkylating nucleoside 1 was preceded by the synthesis of the nonglycosidic derivatives 1-benzyl-4-(halomethyl)-1,2,3-triazoles **6**. The



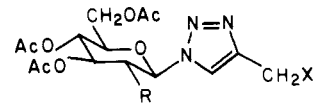
6a, X = F
b, X = Cl
c, X = Br
d, X = I
e, X = OH

preparation, as model compounds, of these benzyl derivatives **6** would allow, on the one hand, to test on these readily accesible compounds the synthetic methods to be used later on nucleosides, and, on the other hand, to study the effect of the glycosidic or nonglycosidic nature of the N-1 substituent on biological activity.

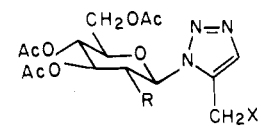
The 4-(chloromethyl)- and 4-(bromomethyl)-1,2,3-triazoles **6b** and **6c** were obtained by 1,3-dipolar cycloaddition of benzyl azide to propargyl chloride and propargyl bromide, respectively. Because the acetylene was asymmetric, two possible adducts could be formed: the 1-benzyl-4-(halomethyl)-1,2,3-triazole, **6**, and the 1-benzyl-5-(halomethyl)-1,2,3-triazole. However, only the sterically less hindered 4-(halomethyl) derivative **6** was detected on TLC.

A second procedure was also used for the synthesis of chloro-, bromo-, and iodomethyl-1,2,3-triazoles **6b–d**, consisting in the treatment of the known[23] 1-benzyl-4-(hydroxymethyl)-1,2,3-triazole **6e** with triphenylphosphine[24–27] or triphenyl phosphite[28–30] halogenating reagents. These methods have been extensively used to transform primary or secondary hydroxyl groups of sugars and nucleosides to halo derivatives. Reaction of **6e** with triphenylphosphine–carbon tetrachloride[24–27] in acetonitrile afforded the chloromethyl derivative **6b**. Treatment of **6e** with triphenyl phosphite–bromine[28] in 1,2-dimethoxyethane gave the bromo analogue **6c**, and a similar treatment with triphenyl phosphite–iodine[28–30] in 1,2-dimethoxyethane afforded the iodomethyl-1,2,3-triazole **6d**. Although the obtainment of 1-benzyl-4-(fluoromethyl)-1,2,3-triazole (**6a**) from **6e** could be conceivably achieved by using phosphorous fluorinating agents,[31] compound **6a** was easily prepared from the bromomethyl derivative **6c** by mild treatment in dry acetonitrile with anhidrous KF and in the presence of 18-crown-6.[32,33]

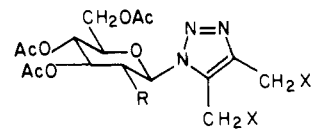
Following the synthetic scheme described above for benzyltriazoles, alkylating nucleosides **9–13** were similarly



9a, R = OAc; X = Cl
b, R = OAc; X = Br
c, R = OAc; X = I
d, R = OAc; X = OH
10a, R = NHAc; X = Cl
b, R = NHAc; X = Br
c, R = NHAc; X = OH



11a, R = OAc; X = Cl
b, R = OAc; X = Br
c, R = OAc; X = OH
12a, R = NHAc; X = Cl
b, R = NHAc; X = OH



13a, R = OAc; X = Cl
b, R = OAc; X = Br
c, R = OAc; X = OH
14, R = NHAc; X = OH

obtained by two procedures. The first one consisted in the 1,3-dipolar cycloaddition of glycosyl azides, such as 2,3,-4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**7**)[34] or 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**8**),[35] to halomethyl acetylenes, such as propargyl chloride, propargyl bromide, 1,4-dichlorobutyne, or 1,4-dibromobutyne. Thus, reaction of azide **7** with propargyl chloride or propargyl bromide afforded a mixture of 4-(halomethyl) and 5-(halomethyl) derivatives **9** and **11**, respectively, in which the former predominated. Similarly, cycloaddition of azide **8** to propargyl chloride gave a mixture of 4- and 5-(chloromethyl)-1-glycosyl-1,2,3-triazoles **10a** and **12a**, respectively. However, the reaction of **8** with propargyl bromide afforded an intractable complex mixture from which no definite product could be isolated. Similar complex mixtures were also formed when the synthesis of other bromomethyl or iodomethyl derivatives of 1-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole was attempted. The formation of such mixtures from glucosamine derivatives only, and not from glucose derivatives, may be due to the reaction of the C-2′ acetamide AcNH– group of the tetraacetylglucosamine residue with the $-CH_2X$ group of propargyl halide or halomethyl-1,2,3-triazole.

1,3-Dipolar cycloaddition of azide **7** to symmetric di-

polarophiles 1,4-dichlorobutyne or 1,4-dibromobutyne afforded difunctional alkylating nucleosides **13a** or **13b**. However, reaction of azide **8** and dihalobutynes gave intractable complex mixtures.

The second procedure for the obtention of alkylating nucleosides **9–13** consisted of two steps. The first one was the 1,3-dipolar cycloaddition of glycosyl azides **7** and **8** to propargyl alcohol or to 1,4-dihydroxybutyne to give hydroxymethyl- or bis(hydroxymethyl)-1,2,3-triazoles, respectively. Thus, reaction of azides **7** or **8** with propargyl alcohol afforded mixtures of 4- and 5-(hydroxymethyl)-1,2,3-triazoles **9d** and **11c** or **10c** and **12b**, respectively. The former isomeric mixture (**9d** and **11c**) was chromatographically homogeneous in several solvent systems and could not be separated. 1,3-Dipolar cycloaddition of 1,4-dihydroxybutyne and glycosyl azides **7** or **8** afforded 4,5-bis(hydroxymethyl)-1,2,3-triazoles **13c** or **14**, respectively.

The second step consisted of the treatment of hydroxymethyl derivatives, obtained in the first step, with triphenylphosphine or triphenyl phosphite halogenating reagents. Thus, reaction of the 4-hydroxymethyl derivative **10c** with triphenyl phosphite–bromine in 1,2-dimethoxyethane afforded 4-(bromomethyl)-1,2,3-triazole **10b**, which could not be obtained by the first procedure. The 4- and 5-(hydroxymethyl) derivatives, **9d** and **11c**, mixture obtained before was treated with triphenyl phosphite–iodine in 1,2-dimethoxyethane in the hope that iodinated-resulting products could be separated. However, after preparative TLC, only the 4-(iodomethyl)-1,2,3-triazole **9c** was obtained, in addition to several decomposition polymeric products. The yield of **9c** in relation to the 4-(hydroxymethyl) isomer content in the starting mixture was good (>80%), which indicated that decomposition products were formed mainly from 5-substituted derivatives.

This second procedure has also been used to prepare, from **13c**, alkylating nucleosides **13a** and **13b**, obtained by the first one. The overall yields of the latter compounds from the glucopyranosyl azide **7** were lower in the second procedure than in the first, due to the low yield obtained in the synthesis of the 4,5-bis(hydroxymethyl) derivative **13c**.

As expected, the conformation and anomeric configuration of the sugar moiety of all alkylating nucleosides described were, as in the starting azides, C1 and β, respectively, as could be deduced from the high value of the coupling constants, J = 9–10 Hz, of the D-glucopyranose ring protons. The 4 or 5 substitution of compounds **6** and **9–12** was determined by ^{1}H NMR on the basis of the differences in chemical shifts of the H-4 or H-5 triazole aromatic protons in different solvents, such as chloroform and dimethyl sulfoxide, $\Delta\delta = \delta_{(CD_3)_2SO} - \delta_{CDCl_3}$ (see Tables I and II, supplementary material). In a study[36] on the chemical shifts of H-4 and H-5 protons of 1-methyl-1,2,3-triazole, it has been shown that the chemical shifts of the proton adjacent to the substituted nitrogen (H-5) is more sensitive than H-4 to solvent and substituent changes.

In those reactions in which the two possible 4- or 5-substituted triazoles were obtained, the 4-substituted derivative was assigned the isomer having the larger $\Delta\delta$ value and the 5-substituted derivative was assigned the isomer having the smaller $\Delta\delta$ value (Table II). This is in agreement with the existence of a substituted nitrogen atom adjacent to the aromatic proton (H-5) in the former case. When only one isomer was obtained, as in the case of 1-benzyl-1,2,3-triazole derivatives **6**, the assignment was made by comparison of their δ and $\Delta\delta$ values, in $CDCl_3$ and $(CD_3)_2SO$, with those of 1-methyl-1,2,3-triazole[36] (Table I, supplementary material).

The two methylene groups of 4,5-disubstituted compounds **13** and **14** appeared in the NMR spectra as separate signals. The band appearing at lower field was assigned to the CH_2X (5) group, since this signal in compounds **13a** and **13b** appeared as a typical AB spin system. The signal corresponding to the other methylene group, $CH_2X(4)$, appeared at higher field as a singlet. The magnetic nonequivalence of the two protons of the $CH_2X(5)$ group may be due, among other factors, to restricted rotation around the C-5–CH_2X bond produced by the closeness of $CH_2X(4)$ group and to the presence of the asymmetric sugar moiety.

Cytostatic Activity. The cytostatic activity of these halomethyl-1,2,3-triazole derivatives against HeLa cells has been evaluated. The more active compounds in the in vitro test were assayed for their cytostatic activity in mice bearing Ehrlich carcinoma ascites (ECA) tumor[37] (Table III). In all the compounds studied, the cytostatic activity depended on the $-CH_2X$ group attached to the triazole ring and on the N-1 substituent.

Cytostatic activity increased with the $-CH_2X$ alkylating ability, i.e., RCH_2OH, $RCH_2F < RCH_2Cl < RCH_2Br < RCH_2I$. This biological activity was significative in the in vitro test for the bromo and iodo derivatives **6c**, **6d**, **9b**, **9c**, **11b**, and **13b**. Since 1-benzyl-4-(fluoromethyl)-1,2,3-triazole, **6a**, showed no activity, due to its low alkylating capacity, no additional glycosyl derivatives of fluoromethyl-1,2,3-triazole have been prepared. In the in vivo test, compounds **6c**, **6d**, **11b**, and **13b** did not show activity. Only bromomethyl and iodomethyl compounds **9b** and **9c** produced a significant increase in the life span of mice bearing ECA tumor. Compound **9b** has also been studied at the National Cancer Institute and was found to produce a significant increase in the life span of mice bearing P388 lymphocytic leukemia. At a dose of 50 mg/kg, a T/C × 100 value of 163 was observed.

Cytostatic activity also depended on the N-1 substituent. When this group was benzyl or 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl, activities against HeLa cells were greater than when this group was 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl. The N-1 substituent dependence was even greater in the in vivo test, since only tetracetyl-β-D-glucopyranosyl, but not benzyl derivatives, were found to be active.

The position of the $-CH_2X$ group (whether at the 4 or 5 position) in the monofunctional derivatives **9–12** or the presence of a second alkylating group, such as in **13a** and **13b**, did not significantly affect the in vitro cytostatic activity. However, in the in vivo assays in mice bearing ECA tumor, only some monosubstituted 4-(halomethyl) derivatives were found to be active.

Studies[37] on the mode of action of these agents have shown that they inhibited DNA synthesis by ECA cells, blocked di[^{3}H]methyl sulfate incorporation by ECA cells, and promoted the release of radioactivity from [8-^{3}H]-guanosine-labeled nucleic acids but not from [*methyl*-^{3}H]thymidine-labeled DNA. From these studies, it has been suggested that these halomethyl-1,2,3-triazoles act as alkylating agents.

Experimental Section

Chemical Methods. Melting points were observed on a Gallenkamp capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance were recorded at 100 MHz on a Varian XL-100 spectrometer using Me_4Si as internal standard. UV absorption spectra were taken with a Perkin-Elmer

Table III. Analytical and Biological Data of Halomethyl-1,2,3-triazoles

compd	formula	anal.[a]	cytostatic activity: HeLa cells: ED_{50}, μg/mL	cytostatic activity in mice bearing ECA tumor: optimal dosage, mg/kg[b]	anim wt diff[c]	T/C × 100[d]
6a	$C_{10}H_{10}FN_3$	C, H, F, N	>100			
6b	$C_{10}H_{10}ClN_3$	C, H, Cl, N	18			
6c	$C_{10}H_{10}BrN_3$	C, H, Br, N	2			
6d	$C_{10}H_{10}IN_3$	C, H, I, N	2			
9a	$C_{17}H_{22}ClN_3O_9$	C, H, Cl, N	37			
9b	$C_{17}H_{22}BrN_3O_9$	C, H, Br, N	3.5	75	−0.5	145
9c	$C_{17}H_{22}IN_3O_9$	C, H, I, N	2	100	−1.1	195
9d	$C_{17}H_{23}N_3O_{10}$	C, H, N	>100[e]			
10a	$C_{17}H_{23}ClN_4O_8$	C, H, Cl, N	20			
10b	$C_{17}H_{23}BrN_4O_8$	C, H, Br, N	20			
10c	$C_{17}H_{24}N_4O_9$	C, H, N	>100			
11a	$C_{17}H_{22}ClN_3O_9$	C, H, Cl, N	25			
11b	$C_{17}H_{22}BrN_3O_9$	C, H, Br, N	4			
11c	$C_{17}H_{23}N_3O_{10}$	C, H, N	>100[e]			
12a	$C_{17}H_{23}ClN_4O_8$	C, H, Cl, N	25			
12b	$C_{17}H_{24}N_4O_9$	C, H, N	>100			
13a	$C_{18}H_{23}Cl_2N_3O_9$	C, H, Cl, N	6			
13b	$C_{18}H_{23}Br_2N_3O_9$	C, H, Br, N	3			
13c	$C_{18}H_{25}N_3O_{11}$	C, H, N	>100			
14	$C_{18}H_{26}N_4O_{10}$	C, H, N	>100			

[a] Analytical results are within ±0.4% of the theoretical values. [b] Administered once daily for 9 consecutive days beginning 24 h after tumor implantation. [c] The difference (in grams) between the weights of test and control animals. [d] T/C is the ratio (expressed as a percentage) of the median survival time of the treated group of mice divided by the median survival time of the control group. A value of T/C × 100 ≥ 125 is considered a statistically significant indication of the antitumor activity of the compound.[38] [e] Values taken from a 1:1 mixture of 9d and 11c.

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 was prepared on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254} (Merck). Compounds were detected with a UV light (254 nm) or by spraying the plate with an ethanol–sulfuric acid (3:7) mixture and heating. Column chromatography was performed on glass columns filled with silica gel 60, 70–230 mesh (Merck).

1-Benzyl-4-(fluoromethyl)-1,2,3-triazole (6a). To a solution of 18-crown-6 (0.50 g, 0.0019 mol) and dry acetonitrile (12 mL), 0.58 g (0.01 mol) of anhydrous KF was added. The mixture was stirred for 30 min at room temperature and 1.26 g (0.005 mol) of **6c** was added. The resulting mixture was heated to reflux, in the absence of humidity, for 33 h, then concentrated in vacuo, and purified by chromatography using a mixture of EtOAc–petroleum ether (1:1) as eluant. From the faster UV-absorbing band 0.3 g (30%) of **6a** as a white solid was obtained: mp 46–48 °C (ethanol); UV λ_{max} (EtOAc) 228 nm (ε 990).

1-Benzyl-4-(chloromethyl)-1,2,3-triazole (6b). Method 1. A mixture of benzyl azide (1.80 g, 0.0136 mol), propargyl chloride (1.80 g, 0.024 mol), and benzene was heated to reflux for 5 h and then evaporated to dryness, and the residue was crystallized from ethanol to give 0.3 g (10%) of **6b**: mp 114–116 °C; UV λ_{max} (EtOH) 231 nm (ε 1270).

Method 2. To a solution of **6e**[23] (1.89 g, 0.01 mol) in anhydrous acetonitrile (10 mL), triphenylphosphine (2.88 g, 0.011 mol) and CCl_4 (3 mL) were added. The mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was stirred for 30 min with a mixture (100 mL) of ethyl ether–petroleum ether (1:1). The solution was decanted from the precipitate which formed and evaporated to dryness, and the residue was crystallized from ethanol to give 0.72 g (47%) of **6b** identical to that obtained by method 1.

1-Benzyl-4-(bromomethyl)-1,2,3-triazole (6c). Method 1. In a flask fitted with a reflux condenser and a calcium chloride tube, a mixture of benzyl azide (2 g, 0.015 mol), propargyl bromide (3.32 g, 0.028 mol), and anhydrous toluene was refluxed for 5 h. Then, the solution was evaporated to dryness and the residue crystallized to give 0.36 g (10%) of **6c**: mp 124–126 °C (ethanol); UV λ_{max} (EtOH) 236 nm (ε 2270).

Method 2. Triphenyl phosphite (3.1 g, 0.01 mol) was added to a solution of **6e**[23] (1.89 g, 0.01 mol) in dry 1,2-dimethoxyethane (10 mL). The resulting mixture was stirred at 0 °C for 30 min, and 1.60 g (0.01 mol) of bromine was added dropwise, while the temperature was kept at 0 °C. Then, the solution was stirred at room temperature for 4 days, concentrated in vacuo, and purified by chromatography using a mixture of $CHCl_3$–petroleum ether (3:1) as solvent. Extraction of the main band, as observed with UV light, gave a white solid which was crystallized from ethanol to give 0.66 g (27%) of **6c** identical to that obtained by method 1.

1-Benzyl-4-(iodomethyl)-1,2,3-triazole (6d). To a cooled (ice) solution of **6e** (1.88 g, 0.01 mol) in anhydrous 1,2-dimethoxyethane (20 mL), triphenyl phosphite (2.10 g, 0.01 mol) was added. After stirring this mixture at 0 °C for 30 min, iodine (2.54 g, 0.01 mol) was added and the stirring continued at room temperature for 24 h. Then, the solution is concentrated in vacuo and the residue purified by chromatography with $CHCl_3$–petroleum ether (3:1) as solvent. Extraction with EtOAc of the main band gave 2.24 g (76%) of **6d**: mp 124–125 °C (ethanol); UV λ_{max} (EtOH) 241 nm (ε 3980).

1-Benzyl-4-(hydroxymethyl)-1,2,3-triazole (6e). A mixture of propargyl alcohol (2.8 g, 0.05 mol), benzyl azide (6.7 g, 0.05 mol), and toluene (24 mL) was refluxed for 8 h. On cooling, **6e** crystallized: yield 36%; mp 76–77 °C (benzene), lit.[23] mp 76–76.5 °C.

1-Glycosyl-1,2,3-triazoles Obtained by 1,3-Dipolar Cycloaddition of Glycosyl Azides to Acetylenes. General Procedure. In a 100-mL flask, fitted with reflux condenser and calcium chloride tube, a mixture of glycosyl azide (0.01 mol), acetylene (0.04 mol), and anhydrous toluene (20 mL) was refluxed until complete reaction of glycosyl azide, usually 4–15 h. Then, the solution was evaporated to dryness and the residue crystallized or chromatographed as indicated in each case.

4- and 5-(Chloromethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (9a and 11a). The residue obtained from the reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide and propargyl bromide following the general procedure was chromatographed with EtOAc–petroleum ether (1:1). With a UV light, two bands were observed, which were extracted and the products eluted with EtOAc. The faster running band gave 2.10 g (48%) of **9a**: mp 169–170 °C (ethanol); UV λ_{max} (EtOH) 226 nm (ε 2340). The slower band yielded 0.80 g (18%) of **11a**: mp 129–130 °C (methanol); UV λ_{max} (EtOH) 232 nm (ε 2340).

4- and 5-(Bromomethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (9b and 11b). According to the general procedure, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide and propargyl bromide reacted to give a residue which was chromatographed using the mixture EtOAc–petroleum ether (1:1) as solvent. With a UV light, two bands were observed. The faster one gave compound **9b**: yield 0.74 g, 22%; mp 151–152 °C (methanol); UV λ_{max} (EtOH) 235 nm (ε 2440). From the band closer to the origin, compound **11b** was obtained as a syrup, yield 10%. An analytically pure sample was obtained after a second chromatography.

4- and 5-(Hydroxymethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (9d and 11d). The residue obtained from the reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide and propargyl alcohol was chromatographed with EtOAc–$CHCl_3$ (2:1). The main band, observed with UV light, yielded 2.49 g (58%) of a chromatographically homogeneous (1:1) mixture of **9d** and **11d**, which was crystallized from 1-butanol: mp (mixture) 148–150 °C; UV λ_{max} (EtOH; mixture) 228 nm (ε 2150).

4-(Iodomethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (9c). A 1:1 mixture of **9d** and **11d** (4.29 g, 0.01 mol) was treated with $(C_6H_5O)_3P$–I_2 in 1,2-dimethoxyethane as indicated for the obtention of **6d** and chromatographed using as eluent EtOAc–petroleum ether (1:1). Extraction of the faster band of the two observed gave 2.3 g (45%) of **9c**: mp 155–157 °C (ethanol); UV λ_{max} (EtOH) 239 nm (ε 4150). The lower band yielded 1.1 g (22%) of a polymeric unidentified substance.

1-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-4- and -5-(chloromethyl)-1,2,3-triazole (10a and 12a). A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide and propargyl chloride reacted following the general procedure, and the resulting residue was chromatographed with EtOAc–$CHCl_3$ (3:1). Two bands were observed on charring. The faster moving band afforded 2.12 g (46%) of **10a**: mp 180–182 °C (ethanol); UV λ_{max} (EtOH) 225 nm (ε 1700). From the slower band, 1.15 g (26%) of **12a** was isolated: mp 175–177 °C (ethanol); UV λ_{max} (EtOH) 224 nm (ε 4515).

1-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-4- and -5-(hydroxymethyl)-1,2,3-triazole (10c and 12b). The residue obtained from the reaction of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide and propargyl alcohol was chromatographed with chloroform–ethanol (10:1), giving two bands on the plates. The faster moving band gave 1.35 g (32%) of **12b**: mp 180–182 °C (ethanol); UV λ_{max} (EtOH) 229 nm (ε 2280). From the slower band was obtained 1.71 g (40%) of **10c**: mp 211–212 °C (ethanol); UV λ_{max} (EtOH) 228 nm (ε 2540).

1-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-4-(bromomethyl)-1,2,3-triazole (10b). Compound **10c** was treated with $(C_6H_5O)_3P$–Br_2 in 1,2-dimethoxyethane as indicated in method 2 for the obtention of **6c**. The reaction residue was chromatographed using EtOAc as solvent, to give compound **10b**: yield 21%; 143–145 °C (ethanol); UV λ_{max} (EtOH) 230 nm (ε 2540).

4,5-Bis(hydroxymethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (13c). Following the general procedure, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide and 1,4-dihydroxybutyne reacted to give, after chromatographic purification using EtOAc–methanol (9:1) as solvent, 1.31 g (28%) of **13c**: mp 158–160 °C (1-butanol); UV λ_{max} (EtOH) 233 nm (ε 1700).

4,5-Bis(chloromethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (13a). Method 1. A mixture of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide and 1,4-dichlorobutyne reacted, according to the general procedure, to give, after chromatography using EtOAc–petroleum ether (1:1) as solvent, 2.33 g (45%) of **13a**: mp 184–186 °C (methanol); UV λ_{max} (EtOH) 236 nm (ε 3100).

Method 2. Compound **13c** was treated with $(C_6H_5)_3P$–CCl_4 in dry acetonitrile, as indicated in method 2 for the obtainment of **6b**, and the reaction residue was chromatographed with EtOAc–petroleum ether (1:1) to give **13a** identical with that obtained before, yield 80%.

4,5-Bis(bromomethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (13b). Method 1. 1,3-dipolar cycloaddition of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide to 1,4-dibromobutyne according to the general procedure gave, after chromatography with EtOAc–petroleum ether (1:1), 0.70 g (13%) of **13b**: mp 154–156 °C (ethanol); UV λ_{max} (EtOH) 254 nm (ε 4300).

Method 2. Compound **13c** reacted with $(C_6H_5O)_3P$–Br_2 in dry 1,2-dimethoxyethane as indicated in method 2 for the obtainment of **6c**, and the reaction residue was chromatographed with $CHCl_3$–EtOH (10:1) to give **13b** identical with that obtained before, yield 21%.

1-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-4,5-bis(hydroxymethyl)-1,2,3-triazole (14). A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide and 1,4-dihydroxybutyne reacted following the general procedure. The reaction residue was purified by column chromatography using EtOH as eluent, to give 3.02 g (66%) of compound **14** as a chromatographically homogeneous glass: UV λ_{max} (EtOH) 233 nm (ε 3200).

Biological Methods. In Vitro Cytostatic Activity. The previously described methods[38] were followed. Minimal Medium Eagle's[39] solution (Difco, code 5675) supplemented with 10% fetal calf serum (Difco) was used. HeLa cells (10^5 cell/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.[40]

In Vivo Cytostatic Activity. Compounds were tested for cytostatic activity in ICR swiss female mice weighing 19–22 g bearing Ehrlich carcinoma ascites tumor according to protocols 1100 and 1200 from the National Cancer Institute.[38] The compounds were suspended in 0.9% (w/v) NaCl solution containing 0.5% (v/v) Tween 80 and injected ip at a fixed volume of 0.4 mL. The treatment and control groups contained 6 and 30 mice, respectively. The positive control compound was 5-fluorouracil (20 mg/kg per injection).[37]

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Supplementary Material Available: Tables I and II containing NMR detailed data of 1-benzyl- and 1-glycosyl(halomethyl)-1,2,3-triazoles, respectively (3 pages). Ordering information is given on any current masthead page.

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Structural Modification Study of Bis(substituted aminoalkylamino)anthraquinones. An Evaluation of the Relationship of the [2-[(2-Hydroxyethyl)amino]ethyl]amino Side Chain with Antineoplastic Activity

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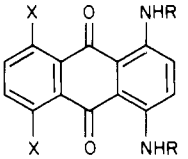
Several anthraquinones containing the [2-[(2-hydroxyethyl)amino]ethyl]amino, the [2-(dimethylamino)ethyl]amino, and the 2-(dimethylamino)ethoxy groups were prepared. Preparation of a lucanthone analogue, a 7-chloroquinoline derivative, and derivatives of naphthoquinones containing the [2-[(2-hydroxyethyl)amino]ethyl]amino side chain and related amino-substituted side chains was also conducted. It was found that the antineoplastic activity of anthraquinones containing the [2-[(2-hydroxyethyl)amino]ethyl]amino side chain is superior to those containing the tertiary amino side chain. However, the presence of the [2-[(2-hydroxyethyl)amino]ethyl]amino chain is an important, but not a sufficient, factor for good antineoplastic activity, as indicated by the lack of significant biological activity of other ring systems containing this side chain.

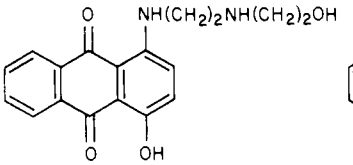
The outstanding antineoplastic activity displayed by a number of bis(substituted aminoalkylamino)anthraquinones,[2] particularly by 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (**1a**) promoted a structural modification study in this



1a, X = OH; R = $(CH_2)_2NH(CH_2)_2OH$
b, X = H; R = $(CH_2)_2NH(CH_2)_2OH$
c, X = H; R = $(CH_2)_3NH(CH_2)_2OH$
d, X = H; R = $(CH_2)_2NH(CH_2)_2CH_3$
e, X = H; R = $(CH_2)_2NHCH_2CH_3$
f, X = H; R = $(CH_2)_2NHCH_3$
g, X = H; R = $(CH_2)_2NH_2$

laboratory. In order to further evaluate the relationship of the [2-[(2-hydroxyethyl)amino]ethyl]amino side chain with biological activity, synthesis of several additional anthraquinones with both side chains replaced by a similar side chain or one of the side chains replaced by other functional groups was conducted. In addition, several other biologically interesting ring systems containing this side chain or its analogues were also prepared for this investigation.

A structural comparison between the substituents of the aminoanthraquinones and those of the antineoplastic antibiotic adriamycin suggested the preparation of compound **2**, wherein one of the side chains is replaced by a



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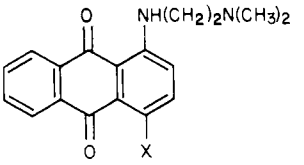
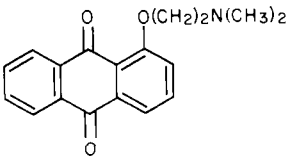


3a, X = $NH(CH_2)_2N(CH_3)_2$
b, X = OH



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hydroxyl group. To avoid the possibility of the formation of undesired cyclization products from the primary or the

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