Synthesis and Antitumor Activity of Certain 3-β-D-Ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazines Related to Formycin Prepared via Ring Closure of a 1,2,4-Triazine Precursor

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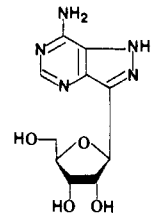
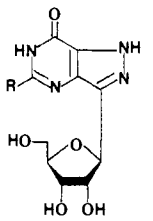
Several 3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazines related to formycin were prepared and tested for their antitumor activity in cell culture. Dehydrative coupling of 3-amino-6-hydrazino-1,2,4-triazin-5(4*H*)-one (**5**) with 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid (**6a**) and further ring closure of the reaction product (**7**) provided 6-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-f]-1,2,4-triazin-8(7*H*)-one (**8**). Condensation of **5** with 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid chloride (**6b**), followed by ring annulation, also gave **8** in good yield. Debenzoylation of **8** furnished the guanosine analogue 6-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazin-8(7*H*)-one (**4b**). Thiation of **8** with P_2S_5, followed by debenzoylation of the thiated product (**11a**), afforded 6-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazin-8(7*H*)-thione (**11b**). Methylation of the soldium salt of **11a** gave the 8-methylthio derivative (**10**), which on ammonolysis furnished 6,8-diamino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazine (**9**). Diazotization of **10** with *tert*-butyl nitrite (TBN) and $SbCl_3$ in 1,2-dichloroethane gave the corresponding 6-chloro derivative (**12a**). Reaction of **10** with TBN in THF in the absence of a halogen source gave 8-(methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-f]-1,2,4-triazine (**12b**). Ammonolysis of **12b** gave the azaformycin A analogue 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazine (**3**), which on deamination afforded 3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-f]-1,2,4-triazin-8(7*H*)-one (**4a**). The azaformycin A analogue (**3**) showed pronounced inhibitory effects against L1210, WIL2, and CCRF-CEM cell lines with ID_{50} values ranging from 5.0 to 7.3 μM.

Since the isolation[1,2] and structural elucidation[3,4] of the naturally occurring *C*-nucleoside antibiotics formycin A (7-amino-3-β-D-ribofuranosyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (**1**)) and formycin B (3-β-D-ribofuranosylpyrazolo[4,3-*d*]-pyrimidin-7(1*H*,6*H*)-one (**2a**)) from *Nocardia interforma*, a number of reports have appeared in the literature describing their biological and physicochemical properties.[5-21] Formycin A, a cytotoxic analogue of adenosine, is readily deaminated by the catabolic enzyme adenosine deaminase (ADA) to the less active inosine analogue formycin B (**2a**).[22] However, interest in the potential of formycin A as an antitumor agent has been rekindled by the observation that, in combination with an ADA inhibitor, compound **1** is superior to 9-β-D-arabinofuranosyladenine (*ara*-A) in prolonging the life of mice infected with L1210 leukemia.[23] Moderate antiviral activity with **1** has also been observed in cell culture.[24-26] Recently, it has been demonstrated in mammalian cells[27,28] and in *Leishmania*[29,30] that the adenylosuccinate synthetase/lyase system provides a pathway for the metabolic regeneration of formycin A from formycin B.

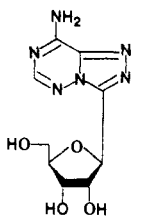
Important biological activity of aza/deaza analogues of guanine and its metabolites is anticipated[31] from ever increasing knowledge of guanine nucleotide metabolism in microbial and mammalian systems.[32,33] The guanosine analogue related to formycin B, 5-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(1*H*, 6*H*)-one (**2b**), has recently been prepared.[17] In an effort to obtain metabolically more stable *C*-nucleoside analogues, it was planned to synthesize the aza congeners of formycin A, formycin B, and **2b** (**3**, **4a**, and **4b**, respectively). Although the synthesis of a carbon-linked acyclovir analogue, 6-amino-3-[(2-hydroxyethoxy)methyl]-1,2,4-triazolo[3,4-f]-1,2,4-triazin-8(7*H*)-one, has recently been described,[34] the total synthesis of ribonucleosides of the 1,2,4-triazolo-[3,4-f]-1,2,4-triazine ring system has not been realized. As part of an ongoing synthetic program directed toward the preparation of *C*-nucleosides,[20,21,35] we now report the synthesis and in vitro antitumor activity of **3**, **4a**, and **4b**. It is of particular interest that unlike the natural *N*-nu-



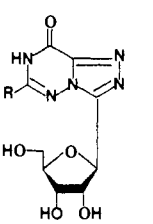
Formycin A
1



2a, R = H
b, R = NH_2



3



4a, R = H
b, R = NH_2

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cleosides of the heterocyclic analogues of purines containing a bridgehead nitrogen atom,[36] the azaformycin B analogues (**4a** and **4b**) retain the Watson–Crick type hydrogen-bonding sites (N_7H) of the aglycon moiety.

Chemistry. The synthesis of the guanosine analogue 6-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-one (**4b**) was first approached by the ring annulation of the glycosyl derivative of an appropriately functionalized 1,2,4-triazine. We envisaged this approach as the straightforward and well-suited route to **4b**. The synthesis of such a substituted 1,2,4-triazine, 3-amino-6-hydrazino-1,2,4-triazin-5(4*H*)-one (**5**), was accomplished as reported.[37] Dehydrative coupling of **5** with 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid (**6a**)[38] in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) gave the viable intermediate **7** (Scheme I), which on further heating in ethylene glycol at 200 °C ring closed to furnish 6-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-one (**8**) in rather low yield. In an effort to obtain **8** in respectable yield, compound **5** was treated with 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid chloride (**6b**)[39] in THF in the presence of 2 M equiv of KOH. A 60% yield of the desired **8** was obtained after ring closure of the intermediate **7**. Debenzoylation of **8** with NaOMe/MeOH at room temperature furnished **4b** in excellent yield. The fact that compound **4b** is indeed the desired 1,2,4-triazolo[3,4-*f*]-1,2,4-triazine ribonucleoside and not the triazinooxadiazine (if compound **7** ring closed on exocyclic oxygen) was established by comparison of the UV absorption spectra (λ_{max} (pH 1 and 7) 245 and 275 nm; λ_{max} (pH 11) 240 and 280 nm) of **4b** with that reported for the model methyl compound 6-amino-3-methyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-one,[37] which are virtually identical. Thiation of **8** with P_2S_5 in boiling dioxane containing 4-(*N*,*N*-dimethylamino)pyridine (DMAP) gave a monothiated product, which was purified chromatographically and recrystallized from acetone. This product was characterized as 6-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo-[3,4-*f*]-1,2,4-triazin-8(7*H*)-thione (**11a**), which on debenzoylation with NH_3/MeOH gave the 6-thioguanosine analogue 6-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-thione (**11b**) in excellent yield. The formation of **11a** lends additional support for the structural assignment of **8**.

Scheme I

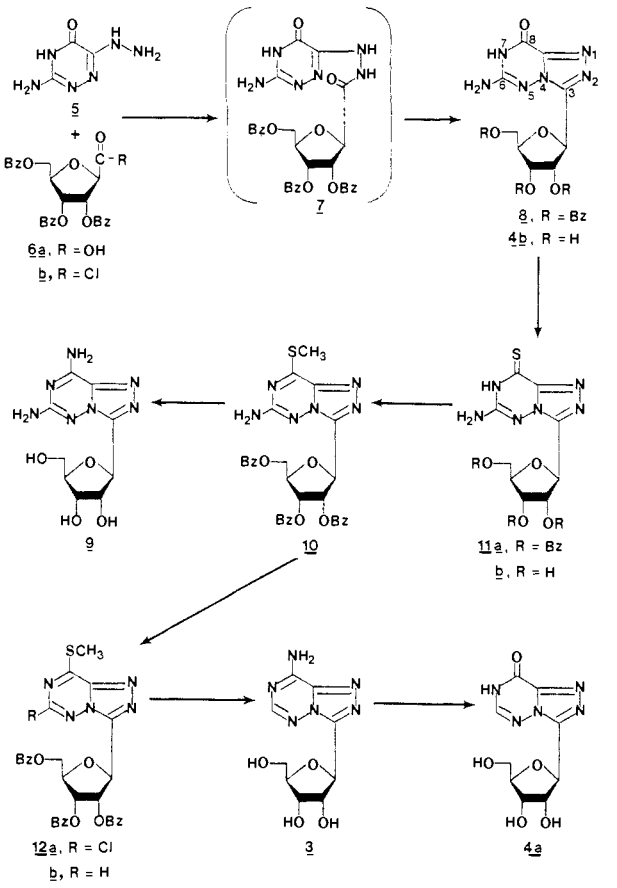

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Table I. Effect of Formycin A, Formycin B, and Certain 3-β-D-Ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazines on the Growth of L1210, WIL2, and CCRF-CEM Leukemia Cells in Culture

compd	ID_{50},[a] μM		
	L1210	WIL2	CCRF-CEM
3	6.3	7.3	5.0
4a	7.0	15.0	23.0
4b	47% inhibn at 100 μM	35% inhibn at 100 μM	30% inhibn at 100 μM
9	16.3	45.8	44.7
11b	*b*	*b*	67.0
formycin A (1)	27.6	6.2	6.1
formycin B (**2a**)	31.6	7.5	8.4

[a] Inhibitory dose 50 (ID_{50}) is the concentration of compound that produced 50% inhibition of tumor cell growth as compared to the untreated controls. Values shown are from a single representative experiment and were confirmed in a second independent test. [b] Inactive at 100 μM.

Compound **11a** was found to be a convenient starting material for the synthesis of the adenosine and 2-aminoadenosine analogues (**3** and **9**, respectively). Methylation of the sodium salt of **11a**, generated in situ by the treatment of sodium hydride in DMF/CH_2Cl_2, with methyl iodide at ambient temperature gave an almost quantitative yield of 6-amino-8-(methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (**10**) after flash column chromatography. When compound **10** was treated with MeOH/NH_3 (saturated at 0 °C) at elevated temperature and pressure, deprotection of the carbohydrate moiety with concomitant nucleophilic displacement of the 8-methylthio function of the aglycon occurred to give 6,8-diamino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (**9**) in good yield. The reaction was clean, and the product was purified by crystallization.

Application of the nonaqueous diazotization procedure, employed with 2-aminopurine nucleosides and *tert*-butyl nitrite (TBN), to introduce a chloro group at C-2 of certain purine nucleosides,[40] was found to be remarkably successful with **10**. Thus treatment of **10** with TBN and antimony trichloride in 1,2-dichloroethane at −10 °C for 2.5 h gave 6-chloro-8-(methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (**12a**) in 77% yield. However, when the reaction of **10** with TBN was carried out in dry THF in the absence of a halogen source there was obtained a nucleoside product, which after purification was characterized as 8-(methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (**12b**) on the basis of elemental analysis and spectral studies. This reductive deamination of **10** apparently proceeds through the intermediacy of purinyl radicals, which abstract hydrogen atoms from solvent molecules.[41,42] Ammonolysis of **12b** with MeOH/NH_3 in a steel reaction vessel at 100 °C for 12 h gave the desired azaformycin A analogue 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (**3**). Deamination of **3** with aqueous nitrous acid gave the crystalline azaformycin B analogue 3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-one (**4a**), in rather low yield. While this work was in progress, an abstract appeared[43] in which the synthesis of **3** has been described employing a 1,2,4-triazine precursor and benzyl (2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)thioformimidate.

Biological Studies. The azaformycin analogues synthesized during this study were tested in parallel with **1** and **2a** for their inhibitory effects on the growth of L1210, WIL2, and CCRF-CEM leukemias in cell culture. The results of the evaluation are summarized in Table I. It is evident that some of the *C*-nucleoside derivatives exhibited significant inhibitory effects on the growth of these cell lines in culture. The azaformycin A derivative **3** showed pronounced inhibitory effects against all the cell lines tested with ID_{50} values ranging from 5.0 to 7.3 μM. The azaformycin B analogue **4a**, as expected, was less effective than **3** in inhibiting the growth of these cells, with ID_{50} values ranging from 7.0 to 23.0 μM. Both **3** and **4a** are 4-fold more active than formycin A and formycin B against L1210 in cell culture. However, the diamino compound **9** was considerably less effective, compared to either **3** or **4a**. Although compound **4b** exhibited moderate inhibitory effects on the growth of these cell lines at 100 μM, **11b** was inactive at that concentration. The remaining compounds were inactive against L1210, WIL2, and CCRF-CEM leukemias at 100 μM.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were determined at 89.6 MHz with a JEOL FX-90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of H_2O as indicated by elemental analysis was verified by ^1H NMR. Infrared (IR) spectra were obtained on a Beckman acculab 2 spectrophotometer, and ultraviolet (UV; sh = shoulder) spectra were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 (EM Reagents) plates. J. T. Baker silica gel (70–230 mesh) was used for column chromatography. All solvents used were reagent grade. Tetrahydrofuran, dioxane, and DMF were stored over molecular sieves (4A) prior to use. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30 °C.

6-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-one (8). Method 1. A solution of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid[38] (**6a**; 10.11 g, 20 mmol) and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 5.40 g, 20 mmol) in absolute ethanol (200 mL) was added to a suspension of 3-amino-6-hydrazino-1,2,4-triazin-5(4*H*)-one[37] (**5**; 2.80 g, 20 mmol) in water/ethanol (1:9, v/v, 500 mL), and the mixture was heated at 60–70 °C for 18 h. After cooling to room temperature, unreacted **5** was removed by filtration and the filtrate was evaporated to dryness. The residual intermediate **7** was used without purification for the ring-closure reaction.

The above intermediate (**7**) was dissolved in ethylene glycol (200 mL) and heated at 200 °C (bath temperature) for 2 h. The reaction mixture was cooled, poured into water (800 mL), and extracted with ethyl acetate/*n*-butyl alcohol (5:2, v/v) mixture (2 × 500 mL). The organic phase was washed with saturated brine solution (100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified on a flash silica gel column (2.5 × 50 cm) with chloroform/acetone (6:1) as the eluent. Fractions containing the desired product were pooled and evaporated to dryness. Crystallization of the residue from methanol gave 2.0 g (17%) of analytically pure **8**: mp 120 °C; IR (KBr) ν 1720 (C=O), 3200–3400 (NH) cm^{-1}; UV λ_{max} (pH 1) 226 nm (ε 39 300), 270 (11 300), 282 (8900); UV λ_{max} (pH 7) 226 nm (ε 49 000), 270 (6500); UV λ_{max} (pH 11) 224 nm (ε 50 600), 270 (5900); ^1H NMR (Me_2SO-d_6) δ 5.60 (d, 1, *J* = 7.0 Hz, $C_1'H$), 6.60 (br s, 2, NH_2), 7.30–8.80 (m, 15, 3 C_6H_5), and other sugar protons. Anal. ($C_{30}H_{24}N_6O_8 \cdot H_2O$) C, H, N.

Method 2. To a cooled (0–5 °C) solution of **5** (1.40 g, 10 mmol) in water (20 mL) containing KOH (1.12 g, 20 mmol) was added a solution of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonic acid chloride[39] (**6b**, prepared from 5.40 g, 11 mmol, of **6a**) in dry THF

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(200 mL) during 30 min with stirring. Stirring was continued for an additional 30 min before the reaction mixture was diluted with cold saturated brine solution (100 mL). The semisolid that separated was collected by filtration and triturated with ether (5 × 50 mL). The solid that formed was again collected to obtain **7**, which was ring closed to **8** by heating in ethylene glycol (200 mL) at 200 °C for 2 h and worked up as described in method 1 to yield 3.60 g (60%): mp 120 °C. This product was found to be identical with that prepared by method 1.

6-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-one (4b). To a solution of **8** (3.0 g, 5 mmol) in anhydrous methanol (200 mL) was added freshly prepared 1 M sodium methoxide in methanol unitl the pH of the solution was 9. The mixture was stirred at room temperature for 18 h. The solution was acidified with Dowex-50 H^+ resin to pH 4, and the resin was removed by filtration. The resin was washed with warm methanol (2 × 50 mL). The combined filtrate and washings were evaporated to dryness. Crystallization of the residue from water gave 1.15 g (90%) of pure **4b** as white needles: mp 240–242 °C; IR (KBr) ν 1710 (C=O), 3200–3320 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 245 nm (sh) (ϵ 13600), 275 (sh) (7500); UV λ_{max} (pH 7) 245 nm (ϵ 11900), 275 (sh) (5700); UV λ_{max} (pH 11) 240 nm (ϵ 13600), 280 (7400); ^1H NMR (Me_2SO-d_6) δ 5.0 (d, 1, J = 7.2 Hz, $C_1'H$), 6.60 (br s, 2, NH_2), and other sugar protons. Anal. ($C_9H_{12}N_6O_5$) C, H, N.

6-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-thione (11a). A mixture of **8** (3.60 g, 6.04 mmol), purified P_2S_5 (3.50 g, 15.7 mmol), and 4-(*N*,*N*-dimethylamino)pyridine (0.50 g, 4 mmol) in anhydrous dioxane (350 mL, peroxide free) was heated under gentle reflux for 25 min. Another portion of P_2S_5 (2.5 g) was added and refluxed for additional 45 min. The reaction mixture was cooled, poured into ice-water (300 mL), and extracted with chloroform (after saturating the aqueous solution with solid NaCl). The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was purified on a flash silica gel column (4 × 20 cm) with chloroform/acetone (6:1) as the eluent. The fractions containing the homogeneous product were pooled and evaporated to dryness, and the residue was crystallized from acetone containing chloroform to yield 2.50 g (70%) of the title compound: mp 261–263 °C; IR (KBr) ν 1250 (C=S), 1715 (C=O), 3300–3400 (NH) cm^{-1}; UV λ_{max} (pH 1) 240 nm (ϵ 30000), 330 (sh) (8000); UV λ_{max} (pH 7) 232 nm (ϵ 38000), 334 (8000); UV λ_{max} (pH 11) 232 nm (ϵ 39000), 334 (8600); ^1H NMR (Me_2SO-d_6) δ 5.63 (d, 1, J = 6.2 Hz, $C_1'H$), 6.68 (br s, 2, NH_2), 7.32–8.04 (m, 15, $3C_6H_5$), and other sugar protons. Anal. ($C_{30}H_{24}N_6O_7S$) C, H, N, S.

6-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8(7*H*)-thione (11b). A solution of **11a** (4.50 g, 7.35 mmol) in MeOH/NH_3 (100 mL, saturated at 0 °C) was stirred at ambient temperature for 18 h in a pressure bottle. The residue, after evaporation of the solvents, was purified on a flash silica gel column (5 × 30 cm) with chloroform/methanol (8:2) as the eluent. Fractions containing the desired homogeneous product were pooled and evaporated to dryness, and the residue was crystallized from aqueous ethanol to yield 2.0 g (91%) of **11b**: mp 268–270 °C; IR (KBr) ν 1280 (C=S), 3200–3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 230 nm (ϵ 19500), 327 (11400); UV λ_{max} (pH 7) 230 nm (ϵ 20100), 327 (14400); UV λ_{max} (pH 11) 230 nm (ϵ 19800), 327 (14400); ^1H NMR (Me_2SO-d_6) δ 4.98 (d, 1, J = 6.2 Hz, $C_1'H$), 6.62 (br s, 2, NH_2), and other sugar protons. Anal. ($C_9H_{12}N_6O_4S$) C, H, N, S.

6-Amino-8-(methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (10). To a solution of **11a** (0.30 g, 0.49 mmol) in DMF/CH_2Cl_2 (1:1, 10 mL) was added NaH (60% in oil; 24 mg, 0.60 mmol), and the mixture was stirred at room temperature for 30 min. Methyl iodide (0.08 g, 0.55 mmol) was added to the solution, and the mixture was stirred at room temperature for 18 h. EtOAc/H_2O (1:1, 2 mL) was added, and the mixture was evaporated to dryness. The residue was dissolved in water, the pH of the solution adjusted to 4 with 1 N HCl, and the solution extracted with EtOAc (2 × 50 mL). The organic phase was washed successively with water (2 × 25 mL), 1 N $NaHSO_4$ solution (25 mL), water (25 mL), and saturated brine solution (2 × 10 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent and purification of the residue on a flash silica gel column using chloroform/acetone (8:2) as the eluent gave 0.30 g (98%) of the title compound as homogeneous foam: IR (KBr) ν 1310 (SCH_3), 1710 (C=O), 3200–3400 (NH_2) cm^{-1}; UV λ_{max} (MeOH) 227 nm (ϵ 44500), 270 (13800); ^1H NMR ($CDCl_3$) δ 2.56 (s, 3, SCH_3), 3.60–4.00 (m, 5, NH_2 and 3 sugar protons), 5.88 (d, 1, J = 6.3 Hz, $C_1'H$), 7.20–8.08 (m, 15, $3C_6H_5$), and other sugar protons. Anal. ($C_{31}H_{26}N_6O_7S$) C, H, N, S.

6,8-Diamino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (9). Compound **10** (0.50 g, 0.79 mmol) was combined with MeOH/NH_3 (75 mL, saturated at 0 °C) and heated in a steel bomb at 95–100 °C for 12 h. The reaction mixture was evaporated to dryness, and the residue was triturated with hot benzene (2 × 25 mL). Two crystallizations of the residue from aqueous ethanol gave 0.15 g (66%) of analytically pure **9**: mp 273–275 °C; IR (KBr) ν 3200–3400 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 232 nm (ϵ 11500), 276 (5600); UV λ_{max} (pH 7) 230 nm (ϵ 12100), 280 (4900); UV λ_{max} (pH 11) 231 nm (ϵ 12100), 278 (5000); ^1H NMR (Me_2SO-d_6) δ 5.10 (d, 1, J = 5.5 Hz, $C_1'H$), 6.62 and 8.82 (2 br s, 4, $2NH_2$), and other sugar protons. Anal. ($C_9H_{13}N_7O_4$) C, H, N.

6-Chloro-8-(methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (12a). To a cooled (−10 °C) solution of **10** (0.15 g, 0.24 mmol) in dry 1,2-dichloroethane (10 mL) was added a solution of $SbCl_3$ (77 mg, 0.34 mmol) in 1,2-dichloroethane (10 mL). After the solution was stirred for 5 min, *tert*-butyl nitrite (TBN; 0.14 mL, 1.2 mmol) was added and the resulting suspension was stirred at −10 °C for 2.5 h. The reaction mixture was poured into ice-water (50 mL), and the aqueous mixture was extracted with $CHCl_3$ (2 × 50 mL). The combined organic phase was washed successively with water (4 × 20 mL), 5% aqueous $NaHCO_3$ (3 × 25 mL), and saturated brine solution (20 mL) and dried over anhydrous Na_2SO_4. The residue, after evaporation of the solvent, was purified on a flash silica gel column (2 × 20 cm) using hexane/acetone (8:2) as eluent to yield 0.12 g (77%) of **12a** as a homogeneous foam: IR (KBr) ν 790 (C–Cl), 1300 (SCH_3), 1710 (C=O) cm^{-1}; UV λ_{max} (pH 1) 240 nm (ϵ 12300), 312 (5800); UV λ_{max} (pH 7) 242 nm (ϵ 13000), 315 (5800); UV λ_{max} (pH 11) 245 nm (ϵ 7800), 325 (3900); ^1H NMR ($CDCl_3$) δ 2.80 (s, 3, SCH_3), 5.92 (d, 1, J = 6.5 Hz, $C_1'H$), 7.62–8.12 (m, 15, $3C_6H_5$), and other sugar protons. Anal. ($C_{31}H_{24}ClN_5O_7S$) C, H, N, Cl, S.

8-(Methylthio)-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (12b). To a stirred solution of **10** (0.15 g, 0.24 mmol) in dry THF (15 mL) was added TBN (0.2 mL, 1.68 mmol) in an atmosphere of dry nitrogen, and the mixture was stirred at 50 °C for 24 h. The reaction mixture was evaporated to dryness, and the residue was purified on a flash silica gel column (3 × 25 cm) using hexane/acetone (8:2) as eluent to yield 0.12 g (82%) of **12b** as analytically pure foam: IR (KBr) ν 1305 (SCH_3), 1720 (C=O) cm^{-1}; UV λ_{max} (MeOH) 240 nm (ϵ 11900), 310 (5200); ^1H NMR ($CDCl_3$) δ 2.86 (s, 3, SCH_3), 5.86 (d, 1, J = 7.0 Hz, $C_1'H$), 7.23–8.19 (m, 15, $3C_6H_5$), 8.21 (s, 1, C_6H), and other sugar protons. Anal. ($C_{31}H_{25}N_5O_7S$) C, H, N, S.

8-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazine (3). Compound **12b** (0.25 g, 0.41 mmol) was combined with MeOH/NH_3 (25 mL, saturated at 0 °C), and the resultant mixture was heated in a steel bomb at 100 °C for 12 h. The residue, after evaporation of the solvents, was triturated with hot benzene (2 × 10 mL) and crystallized from aqueous methanol as needles to yield 70 mg (64%) of **3**: mp 268–270 °C; IR (KBr) ν 3215–3400 (OH, NH_2) cm^{-1}; UV λ_{max} (pH 1) 226 nm (ϵ 12000), 272 (5500); UV λ_{max} (pH 7) 230 nm (ϵ 11300), 274 (4600); UV λ_{max} (pH 11) 232 nm (ϵ 11700), 273 (5400); ^1H NMR (Me_2SO-d_6) δ 5.28 (d, 1, J = 6.5 Hz, $C_1'H$), 8.10 (s, 1, C_6H), 8.82 (br s, 2, NH_2), and other sugar protons. Anal. ($C_9H_{12}N_6O_4$) C, H, N.

3-β-D-Ribofuranosyl-1,2,4-triazolo[3,4-*f*]-1,2,4-triazin-8-(7*H*)-one (4a). To a cooled (0 °C) and stirred solution of **3** (0.40 g, 1.49 mmol) in acetic acid/water (1:9, 100 mL) was added $NaNO_2$ (2.0 g) in small portions over a period of 30 min. After the addition of $NaNO_2$, the reaction mixture was stirred at 0 °C for 2 h and then allowed to stand at room temperature. After 10 h at room temperature and at 50 °C for 1 h, the reaction mixture was cooled to 0–5 °C, before an additional amount of acetic acid (5 mL) was added. The solution was evaporated to dryness, and the residue was coevaporated from dry toluene (3 × 25 mL). The resulting dry residue was boiled with 95% EtOH (20 mL), and the insoluble

material was removed by filtration. The filtrate was concentrated to ~15 mL and allowed to stand in the refrigerator overnight. The crystalline product that deposited was collected by filtration, washed with dry methanol (5 mL), and dried over P_2O_5 under vacuum. Two recrystallizations from ethanol gave 0.10 g (24.9%) of **4a**: mp 192–195 °C; IR (KBr) ν 1630, 1730 (HN–C=O), 3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 252 nm (sh) (ϵ 2300); UV λ_{max} (pH 7) 257 nm (ϵ 2400); UV λ_{max} (pH 11) 255 nm (ϵ 2700); ^1H NMR (Me_2SO-d_6) δ 4.92 (d, 1, J = 6.0 Hz, $C_1'H$), 8.10 (s, 1, C_6H), 12.54 (br s, 1, ring NH), and other sugar protons. Anal. ($C_9H_{11}N_5O_5$) H, N; C: calcd, 40.15; found, 38.09.

Cell Growth Inhibition Evalulation. Compounds were evaluated for their ability to inhibit the growth of L1210 murine lymphocytic leukemia, WIL2 human B-lymphoblastic leukemia, and CCRF-CEM human T-lymphoblastic leukemia, which were maintained in suspension cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and 20 mM Hepes buffer. Cells were free of mycoplasma contamination as determined by culturing under anaerobic conditions on broth/agar plates (Mycotrim-TC, NEN Products/Hana Media, Inc., Boston, MA) and by the Gen-Probe ribosomal RNA hybridization method (Gen-Probe, San Diego, CA). Test compounds were dissolved in deionized, distilled water at 2000 μM, sterilized by passage through a 0.2-μm filter (Gelman, Ann Arbor, MI), and diluted to 200 μM in growth media. Compounds **8** and **11b** were dissolved in Me_2SO at 20 μM and then serially diluted in growth media to 200 μM in 1% Me_2SO. A Me_2SO control containing 1% Me_2SO was prepared and tested in the same manner as the test compounds. Compounds were tested in triplicate on 96 well tissue culture plates. The highest concentration of compound (200 μM) was placed in the top row of the plate and seven 0.5 log serial dilutions were performed using a Cetus pipette (Cetus Corp., Emeryville, CA). Following serial dilution, wells contained 100 μL of test compound at concentrations ranging from 0.2 to 200 μM. Cells were adjusted to 1×10^5 cells/mL in growth media, and 100 μL was added to each well of test plates. This resulted in a final volume of 200 μL/well, a cell inoculum of 5×10^4/mL, and compound concentrations ranging from 0.1 to 100 μM. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

The three cell lines vary in their doubling times, so incubation times during the cytotoxicity assay were varied to allow approximately 4–4.5 population doublings during the course of the test. L1210 were incubated for 48 h, WIL2 for 72 h, and CCRF-CEM for 92 h. Following incubation, growth was determined by cell count on a Coulter Model ZM electronic cell counter. Growth in treated wells was expressed as a percentage of growth in untreated control wells. For wells treated with the compound dissolved in Me_2SO, growth was expressed as a percentage of growth in wells containing an equal concentration of Me_2SO. The percent control values were plotted vs. compound concentration, and the concentration that inhibits growth by 50% (ID_{50}) was determined.

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Registry No. **3**, 103959-89-3; **4a**, 103959-90-6; **4b**, 103959-83-7; **5**, 70481-88-8; **6a**, 23316-68-9; **6b**, 67560-74-1; **7**, 103959-81-5; **8**, 103959-82-6; **9**, 103959-86-0; **10**, 103980-83-2; **11a**, 103959-84-8; **11b**, 103959-85-9; **12a**, 103959-87-1; **12b**, 103959-88-2; EEDQ, 16357-59-8.

10-Acetyl-10-hydroxyxantho[2,3-*f*]tetralin 8-Glycosides as Angular Chromophore Analogues of Anthracyclines: Synthesis, Redox Properties, Microsomal Oxygen Consumption, and Antileukemic Evaluation

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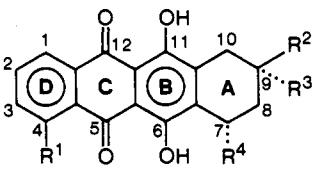
10-Acetyl-7,8-dihydroxyxantho[2,3-*f*]tetralin is obtained by photo-Fries rearrangement of an acylated and double ketal protected tetralin followed by sodium thiocresylate catalyzed rearrangement of the resulting benzoyltetralin. Introduction of the 10-hydroxy function with base, triethyl phosphite, and molecular oxygen affords six products. These include the desired epimeric 10-acetyl-7,8,10-trihydroxyxantho[2,3-*f*]tetralins in addition to products resulting from novel valence tautomerism and cycloreversion reactions in the oxidation reaction. Glycosidic coupling to the fully functionalized *cis*-8,10-dihydroxy epimer of the aglycon to protected chlorodaunosamine by a modified Koenigs–Knorr method proceeded satisfactorily. By contrast the epimeric *trans*-8,10-dihydroxy compound failed to undergo coupling under these conditions. This is attributed to facile competing intramolecular hemiketal formation in the latter case. The new angular glycosides are very resistant to electrochemical reduction and display very low (3–10%) augmentation of hepatic microsomal oxygen consumption relative to doxorubicin. The observed, albeit low, cytotoxicity against leukemia L1210 in cell culture provides an additional example where the presence of the quinone moiety in the parent anthracyclines, which is implicated in the clinical cardiotoxicity, may not be necessary for the expression of anticancer properties.

Efforts continue to be made to effect a separation of cytotoxic and cardiotoxic effects in the application of the anthracycline antitumor agents,[1,2] including daunorubicin (I), 4-demethoxydaunorubicin (II), and doxorubicin (III), which are widely used in the clinical treatment of a range of human malignancies.[1,2] Some encouraging results have been obtained based on evidence that the origin of the cardiotoxicity[3,4] may lie in the in vivo redox activity of the quinone-containing chromophore causing the generation of oxygen radicals and leading to lipid peroxidation



I $R^1 = OCH_3$; $R^2 = COCH_3$; $R^3 = OH$; R^4 = daunosaminyl

II $R^1 = H$; $R^2 = COCH_3$; $R^3 = OH$; R^4 = daunosaminyl

III $R^1 = OCH_3$; $R^2 = COCH_2OH$; $R^3 = OH$; R^4 = daunosaminyl

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