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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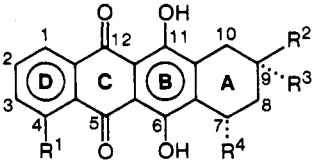
10-Acetyl-7,8-dihydroxyxantho[2,3-*f*]tetralin is obtained by photo-Fries rearrangement of an acylated and double ketal protected tetralin followed by sodium thiocresylate catalyzed rearrangement of the resulting benzoyltetralin. Introduction of the 10-hydroxy function with base, triethyl phosphite, 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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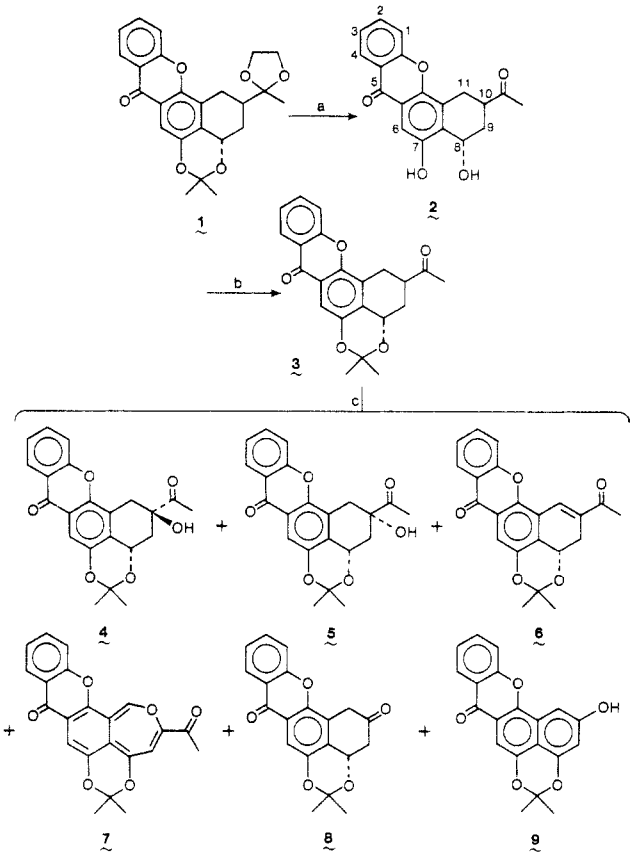
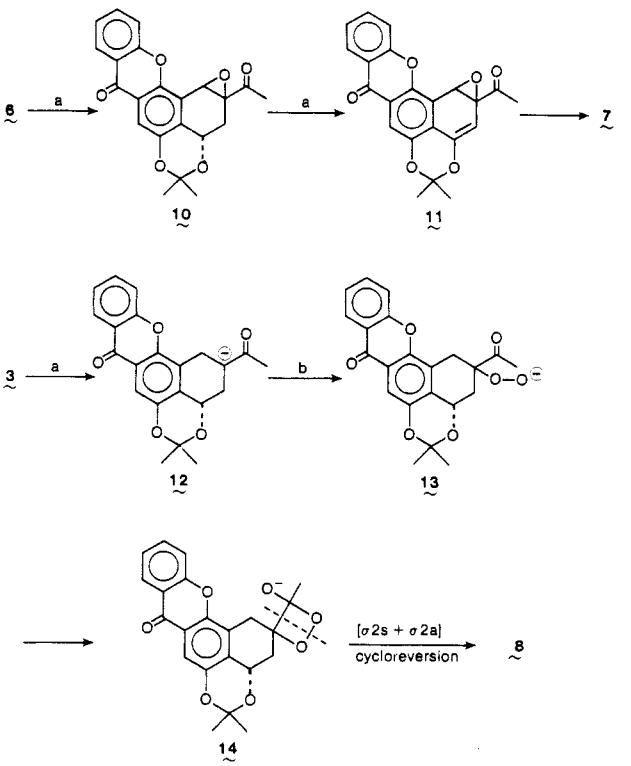
Scheme I[a]



[a] Reaction conditions: (a) THF, HCl, H_2O, room temperature for 16 h; (b) 2,2-dimethoxypropane, THF, *p*-TSA, room temperature for 15 h; (c) K-*t*-OBu, *t*-BuOH, DMF, nitrogen atmosphere, room temperature for 30 min, then cool to −25 °C, triethylphosphite, O_2, −15 °C to −25 °C for 2 h.

Scheme II[a]



[a] Reaction conditions: (a) K-*t*-OBu, DMF, *t*-BuOH, nitrogen atmosphere, room temperature for 30 min, then cool to −25 °C, triethyl phosphite, O_2, −15 °C to −25 °C for 2 h, leading to dehydrative elimination and valence tautomerism; (b) K-*t*-OBu, DMF, nitrogen atmosphere, room temperature for 30 min; (c) cool to −25 °C and then triethyl phosphite, O_2, at −15 °C to −25 °C for 2 h followed by intramolecular nucleophilic addition and cycloreversion.

preferentially in cardiac tissue.[5-9]

Certain chromophore-modified anthracyclines show a correlation between the suppression of redox activity and concomitant cardiotoxic effects.[9,10] Examples to date include 5-iminodaunorubicin[11] and 5-iminodoxorubicin.[12] In this regard we recently described the synthesis and study of the properties of glycosides of 9-acetyl-6,7,9,11-tetrahydroxyxantho[2,3-*g*]tetralin.[13] The observation of cytotoxicity in this series of novel glycosides, which are virtually devoid of redox activity, against L1210 leukemia[13] suggests that the quinone moiety, which is implicated in cardiotoxicity,[5,8] may not be essential for biological activity.[14] While a number of structural, stereochemical, and pharmacological questions remain unanswered, it appears that exploration of appropriate chromophore-modified structures may contribute to a separation of cytotoxic and cardiotoxic effects.

In the present report we further explore this approach and describe the synthesis and properties of 10-acetyl-10-hydroxyxantho[2,3-*f*]tetralin-8-glycosides as angular chromophore analogues of the anthracyclines. Their low redox activity, measured both polarographically and by the augmentation of hepatic microsomal oxygen consumption, as well as their activity against L1210 leukemia, is discussed.

Synthesis. (a) Chromophores. The angular xantho-[2,3-*g*]tetralin chromophore **1** was obtained from photo-Fries rearrangement of a benzoyltetralin followed by a nucleophile-catalyzed ring closure.[15] Deprotection of **1** using hydrogen chloride in aqueous THF gave **2** (Scheme I) in 60% yield and in which the C_8-H is equatorial as is evident from the $\nu_{1/2}$ = 3.0 Hz in the ^{1}H NMR.[16] Compound **2** was then converted into the 7,8-acetonide **3** in 76% yield by treatment with dimethoxypropane in the presence of *p*-toluenesulfonic acid. Treatment of compound **3** under conditions designed to introduce the C_{10}-OH group (i.e., reaction with potassium *tert*-butoxide in *tert*-butyl alcohol and DMF in the presence of triethyl phosphite followed by reaction with molecular oxygen[17])

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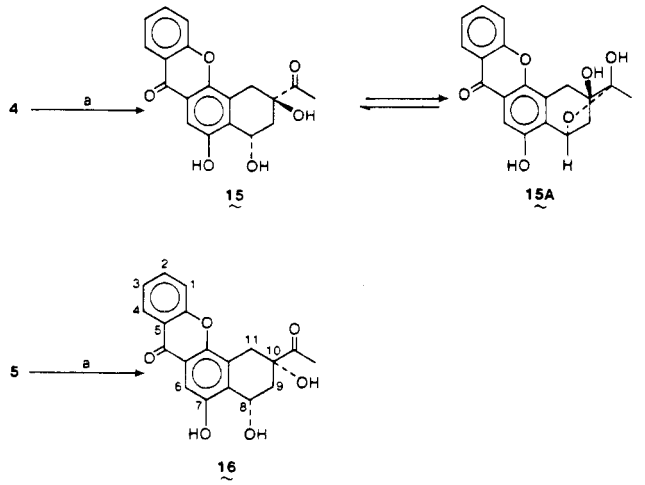
Scheme III[a]



[a] Reaction conditions: (a) THF, HCl, H_2O, room temperature for 8 h.

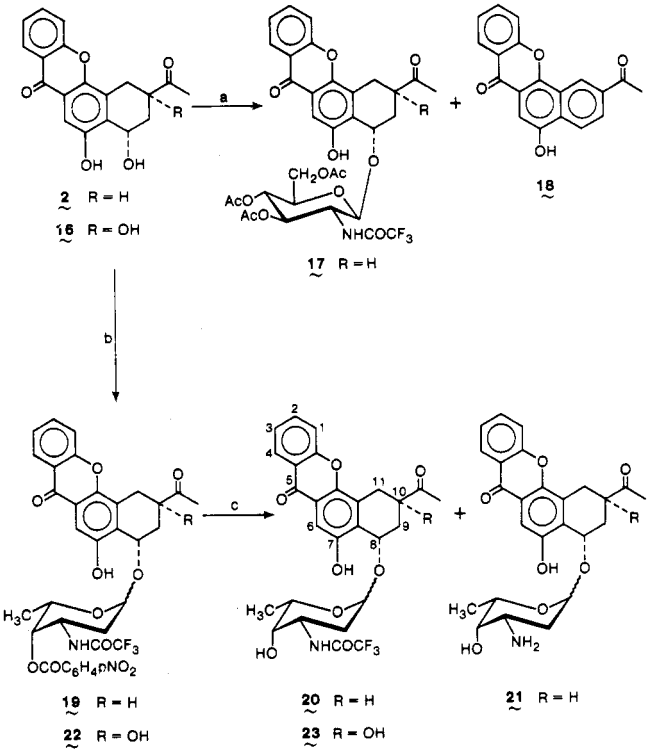
afforded six products **4–9**, which are readily separable by chromatography. The structures of these compounds were assigned by ^1H NMR, IR, and MS and by comparison with related structures previously identified.[15] Generation of the carbanion **12** (Scheme II) at C-10 of **3** followed by oxidation gives the epimers **4** and **5**, the stereochemistries of which were assigned by ^1H NMR.[13] Compound **6** can arise from the dehydration of either **4** or **5**.

The ^1H NMR spectrum of **7** shows singlets at δ 7.66 and 8.76 ascribed to the protons H_{12} and H_9, respectively ($J_{12,9}$ = 1.5 Hz). The other ^1H NMR, IR, and MS data are in accord with the assigned structure. Compound **7** may arise from **6** by the formation of an epoxide **10** at the C_{10}–C_{11} position of the conjugated ketone under basic conditions[18] (Scheme II). Generation of an anionic center at C_8 followed by hydroxylation and elimination (as in the conversion of **8** to **9**) forms **11**, which is than subject to a valence tautomerism[19] to give **7** (Scheme II).

Compound **8**, in which the acetyl side chain has been eliminated, plausibly arises via the obligatory intermediate peroxide anion **13** implicated in the formation of **4** and **5**.[17] Ring closure of the peroxide could form the transient 1,2-dioxetane anion **14**, and subsequent formal [$\sigma 2_s + \sigma 2_a$] cycloreversion leads to **8** and the acetate anion (Scheme II). There are several precedents for the suggested facile cleavage of 1,2-dioxetanes to yield carbonyl compounds.[20-22] The phenolic compound **9** can be formed from **8** by the generation of the anion at C_8 followed by hydroxylation, by interaction with molecular oxygen, and then dehydration leading to a double-bond insertion at positions C_8–C_9 and enolization of the ketone. The IR spectrum shows an absorption at 3400–3100 cm^{-1} (phenolic OH), while the ^1H NMR reveals seven aromatic protons in accord with the assigned structure for **9**.

Deprotection of compounds **4** and **5** under mild acid conditions afforded **15** and **16**, respectively (Scheme III). The stereochemistry of the analogous epimeric linear xantho[2,3-*g*]tetralins has been established previously by chemical means.[13,15] In compound **16** the C_8-H is pseudoequatorial because it exhibits couplings of $J_{8e',9e'}$ = 2.5 Hz and $J_{8e',9a'}$ = 5 Hz requiring that the C_8-OH and C_{10}-OH groups are cis diaxial. In contrast compound **15** exhibits the C_8-H as $J_{8a',9a'}$ = 9.5 Hz an $J_{8a',9e'}$ = 6 Hz. It follows that the the C_8-H in **15** is pseudoaxial requiring, by comparison with **16**, that the C_8-OH and C_{10}-OH groups in **15** are trans. It may be noted that C_{10}-hydroxylation of **3** and acid-catalyzed deprotection of the resulting angular compounds **4** and **5** follows the same stereochemical course as the linear chromophore (xantho[2,3-*g*]tetralin system[13]) leading to the formation fo **15** and **16** (Scheme IV). The relative stereochemistry of **15** and **16** thus inferred is in accord with the facile glycosidation of **16** in contrast to **15** (see below).

Scheme IV[a]



[a] Reaction conditions: (a) protected bromo sugar, $HgBr_2$, $Hg(CN)_2$, THF, molecular sieve (3 Å), 65–70 °C, 118 h; (b) protected chlorodaunosamine, $HgBr_2$, $Hg(CN)_2$, THF, molecular sieve (3 Å), 55–60 °C, 40 h; (c) acetone, 0.1 N NaOH, nitrogen atmosphere, room temperature, 30 min.

(b) Glycosidic Coupling of Chromophores. Initially attempts were made to effect glycosidic coupling with the protected chlorodaunosamine in the presence of cadmium carbonate,[23,24] in the presence of calcium carbonate, or with silver carbonate.[25] In general these methods proved unsatisfactory as in the case of the xantho[2,3-*g*]tetralin derivatives.[13] However, satisfactory glycosidic coupling could be effected with the modified Koenigs–Knorr procedure.[26] The 10-acetylxantho[2,3-*f*]tetralin (**2**) was coupled with 3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(trifluoroacetamido)-β-D-glucopyranosyl bromide and mercuric cyanide in THF to afford the glycoside **17** together with the fully aromatized compound **18** (Scheme IV). The susceptibility of ring A in this system to aromatize has been

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Table I. Augmentation of Hepatic Microsomal Oxygen Uptake by 10-Acetyl-10-hydroxyxantho[2,3-*f*]tetralin 8-Glycosides

compd	oxygen consumption, % activity of doxorubicin[a]	compd	oxygen consumption, % activity of doxorubicin[a]
17	4	21	10
19	6	22	10
20	6	23	3

[a] Reference 14.

noted above in the conversion of **8** to **9**. Coupling of the chromophore **2** with protected chlorodaunosamine was carried out under similar conditions of the modified Koenigs–Knorr reaction[26] to afford the glycoside **19**. Alkaline deprotection of **19** and separation of the products by preparative thin-layer chromatography then gave the partially deprotected glycoside **20** together with the fully deprotected glycoside **21** (Scheme IV).

An attempted modified Koenigs–Knorr coupling of the *trans*-C_8–C_{10}-dihydroxychromophore **15** with the protected chlorodaunosamine afforded only traces of the glycoside detected by FABMS. It appears plausible that under the mild acid conditions employed in the coupling that the facile intramolecular hemiketal formation of **15A** from **15** prevents normal glycosidic coupling. By contrast the glycosidation of **16** proceeds normally. This provides an additional example comparable to the linear chromophore cases[13] of the strict stereochemical requirements in functionalized chromophores for effective coupling.

Accordingly, glycosidic coupling of the *cis*-8,10-dihydroxychromophore (**16**) with protected chlorodaunosamine proceeded normally affording **22** in 20% yield. The magnitudes of the coupling constants in glycoside **22** are in accord with an exclusive α-chromophore configuration, ang therefore **22** has a stereochemistry corresponding to daunorubicin[1,2] (I) and doxorubicin (III).[14] Alkaline deprotection of **22** gave **23** in 44% yield. The ^{1}H NMR spectrum of **23** confirmed that the α-configuration was retained.

Results and Conclusion

Antileukemic Cytotoxicity and Augmentation of Hepatic Microsomal Oxygen Uptake and Redox Characteristics of 10-Acetyl-10-hydroxyxantho[2,3-*f*]tetralin 8-Glycosides. Polarographic and cyclic voltammetry studies on compounds **2**, **3**, **16**, **17**, **19–21**, and **23** confirmed that the chromophores were, as expected, extremely resistant to reduction, with half-wave potentials at or negative of −1.25 V vs. SCE, compared with the readily reducible doxorubicin, which has a half-wave potential of −0.66 V.[10] All of these chromophore reductions were shown to be irreversible by cyclic voltammetry. Compound **19** alone exhibited additional reductions at −0.385 V and −1.005 V, which are due to the nitro group.

The augmentation of normal oxygen uptake by fresh rat liver microsomes has been used as a measure of in vivo redox activity of anthracycline derivatives and analogues compared with the parent anthracycline doxorubicin.[7,9] A correlation has been observed between low activity in this test and corrsponding low in vivo cardiotoxicity.[9] The oxygen uptake by representative examples of the protected **17**, partially deprotected **23**, and fully deprotected **21** glycosides were 4, 3, and 10%, respectively, compared with doxorubicin (Table I). A group of the new synthetic glycosides derived from the xantho[2,3-*g*]tetralins (compounds **12–15** of ref 13) as well as the xantho[2,3-*f*]tetralin derivatives in the present series (Table II) all exhibit low cytotoxicity against leukemia L1210 cells grown in culture with ID_{50} values in the range of 1–10 μg/mL. This result for the xantho[2,3-*f*]tetralin glycosides provides a further example that cytotoxicity may be expressed in agents bearing nonreducible chromophores and without the quinone moiety of the parent anthracycline.

Table II. Cytotoxicity of 10-Acetyl-10-hydroxyxantho-[2,3-*f*]-tetralin 8-Glycosides against L1210 Leukemia

compd	ID_{50}, μg/mL	compd	ID_{50}, μg/mL
17	>10	21	8
19	10	22	8
20	10	23	>10

Experimental Section

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 FT spectrophotometer, and only the principal sharply defined peaks are reported. The ^{1}H NMR spectra were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spectrometers or on Bruker WH-200 and WH-400 spectrometers. The spectra were recorded on approximately 5–15% (w/v) solutions, depending upon the spectrometers, and in appropriate deuterated solvents with tetramethylsilane as internal standard. Line positions are recorded in parts per million from reference. Electron impact and FAB mass spectra[27] were determined on an Associated Electrical Industries (AEI) MS-9 double-focusing high-resolution mass spectrometer. The peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15000. Kieselgel DF-5 (Camag, Switzerland) and Eastman Kodak precoated sheets were used for thin-layer chromatography. In the workup procedures reported for the various syntheses described, solvents were removed with a rotary evaporator under reduced pressure without heating. Kieselgel (Fluka, Switzerland) was used for column chromatography.

10-Acetyl-7,8-dihydroxyxantho[2,3-*f*]tetralin (2). Compound **1**[13] (408 mg, 1 mmol) was dissolved in 80 mL of THF, and to the solution was added 16 mL of concentrated HCl and 16 mL of water. The reaction mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate. The ethyl acetate layer was washed with water, sodium bicarbonate, and again with water, and then dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was recrystallized from THF/chloroform (1:1) to afford 195 mg of **2** (60% yield): mp 215–218 °C; ^{1}H NMR (Me_2SO-d_6/$CDCl_3$, 1:1) δ 1.82 (m, 1 H), 2.40 (s + m, 4 H, $COCH_3$ + H), 2.90 (q, 1 H), 3.22 (m, 1 H), 3.42 (dd, 1 H), 4.3 (s, 1 H, C_8-OH exch), 5.10 (bs, 1 H, C_8-$H_{e'}$ $\nu_{1/2}$ = 3.0 Hz), 7.35 (t, 1 H, Ar), 7.55 (m, 2 H, Ar), 7.75 (m, 1 H, Ar), 8.28 (dd, 1 H, Ar), 9.55 (bs, 1 H, C_7-OH exch); IR (KBr) ν_{max} 3540–3380 (OH), 1700 ($COCH_3$), 1635 (γ-pyrone), 1610 and 1585 (Ar) cm^{-1}; MS, *m/z* (rel int) 325.1034 (1.19, M^+ + 1), 324.0994 (5.79, M^+; calcd for $C_{19}H_{16}O_5$, 324.0993), 307.0925 (2.9, M^+ − OH), 306.0889 (13.4, $M^+ - H_2O$), 264.0764 (40.8, $M^+ - (OH + CH_3CO)$), 263.0703 (100, $M^+ - (H_2O + CH_3CO)$), 262.0629 (29.5, 263.0703 − H).

10-Acetyl-7,8-dihydroxyxantho[2,3-*f*]tetralin 7,8-Acetonide (3). Compound **2** (324 mg, 1 mmol) was dissolved in 75 mL of THF, and to the solution was added dimethoxypropane (8 mL) and *p*-toluenesulfonic acid monohydrate (10 mg). The reaction mixture was stirred at room temperature for 15 h, and the solvent was removed under reduced pressure. The residue was subjected to column chromatography and eluted with ether/petroleum ether (1:1) to give a white solid, which was further purified by recrystallization from THF/ether (1:1) affording 277 mg of **3** (76% yield): mp 185 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (d, 6 H, CH_3 + CH_3), 1.95 (m, 1 H), 2.39 (s, 3 H, $COCH_3$), 2.65 (m, 1 H), 3.28 (m, 3 H),

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4.78 (q, 1 H, C_8-$H_{a'}$, $J_{8a',9a'}$ = 11 Hz, $J_{8a',9e'}$ = 6 Hz), 7.38 (m, 1 H, Ar), 7.50 (d, 1 H, Ar), 7.60 (s, 1 H, Ar), 7.74 (m, 1 H, Ar), 8.35 (dd, 1 H, Ar); IR ($CHCl_3$) ν_{max} 1710 ($COCH_3$), 1655 and 1625 (γ-pyrone), 1610 and 1595 (Ar) cm^{-1}; MS, m/z (rel int) 365.1356 (1.1, M^+ + 1), 364.1324 (3.99, M^+; calcd for $C_{22}H_{20}O_2$, 364.1323), 306.0897 (30.43, $M^+ - CH_3COCH_3$), 263.0702 (100, $M^+ - (CH_3COCH_3 + CH_3CO)$), 262.0634 (42.6, 263.0702 - H), 58.0443 (1.52, CH_3COCH_3).

Oxidation of 10-Acetyl-7,8-dihydroxyxantho[2,3-*f*]tetralin 7,8-Acetonide (3) to Afford 4–9. Compound **3** (3.64 g, 0.01 mol) was oxidized by use of the experimental procedure described previously[13] to give a mixture of crude products that was subjected to column chromatography. The column was first eluted with ether/petroleum ether (10:90), and a yellow band was collected, which on removal of the solvent gave a yellow-colored product that was purified by recrystallization from ether/THF (1:1) to give 20 mg of **7** (0.53% yield): mp 232–235 °C; ^1H NMR (Me_2SO-d_6) δ 1.65 (s, 6 H, $CH_3 + CH_3$), 2.80 (s, 3H, $COCH_3$), 7.45 (s, 1 H, Ar), 7.55 (m, 1 H, Ar), 7.66 (d, 1 H, Ar), 7.95 (dd, 1 H, Ar), 8.22 (dt, 1 H, Ar), 8.76 (d, 1 H, Ar); IR ($CHCl_3$) ν_{max} 1685 ($COCH_3$), 1650 (γ-pyrone), 1610 and 1585 (Ar) cm^{-1}; MS, m/z (rel int) 377.0982 (0.62, M^+ + 1), 376.0946 (2.97, M^+; calcd for $C_{22}H_{16}O_6$, 376.0946), 360.1015 (100, M^+ - O), 317.08 (25.47, $M^+ - (O - COCH_3)$). Further elution of the column with ether/petroleum ether (50:50) and collection of a fluorescent band afforded a solid residue after removal of the solvent. The solid was shaken with ether and collected. The insoluble product was further washed with ether and recrystallized from THF/ether (1:1) to give 6 mg of compound **9** (0.18% yield): mp 300 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.65 (s, 6 H, $CH_3 + CH_3$), 6.82 (d, 1 H, Ar), 7.16 (s, 1 H, Ar), 7.55 (m, 2 H, Ar), 7.90 (m, 2 H, Ar), 8.26 (dd, 1 H, Ar); IR (Nujol) ν_{max} 3400–3100 (OH), 1620 (γ-pyrone), 1605 and 1595 (Ar) cm^{-1}; MS, m/z (rel int) 335.0878 (21.77, M^+ + 1), 334.0843 (100, M^+; calcd for $C_{20}H_{14}O_5$, 334.0843), 333.0763 (3.83, M^+ - H), 319.0620 (11.62, $M^+ - CH_3$), 317.0826 (4.28, M^+ - OH).

The filtrate was evaporated under vacuum, and the residue was recrystallized from petroleum ether/ether (1:1) to give 14 mg of a crystalline product **6** (0.4% yield): mp 185 °C; ^1H NMR (Me_2SO-d_6) δ 1.60 (d, 6 H, $CH_3 + CH_3$), 2.30 (dt, 1 H, C_7-$H_{a'}$), 2.55 (s, 3 H, $COCH_3$), 3.14 (q, 1 H, C_9-$H_{e'}$), 5.25 (q, 1 H, C_8-$H_{a'}$, $J_{8a',9e'}$ = 7.5 Hz, $J_{8a',9a'}$ = 16 Hz), 7.5 (m, 2 H, Ar), 7.80 (m, 1 H, Ar), 7.90 (m, 1 H, Ar), 8.05 (d, 1 H, C_{11}-H, $J_{11,H_{9a'}}$ = 2.8 Hz), 8.20 (dt, 1 H, Ar); IR (CH_2Cl_2) ν_{max} 1660 ($COCH_3$), 1625 (γ-pyrone), 1610 and 1595 (Ar) cm^{-1}; MS, m/z (rel int) 362.1185 (1.35, M^+; calcd for $C_{22}H_{18}O_5$, 362.1182), 304.0742 (100, $M^+ - CH_3COCH_3$), 289.05 (28.68, $M^+ - (CH_3COCH_3 + CH_3)$), 261.0550 (11.48, $M^+ - (CH_3COCH_3 + CH_3CO)$), 58.0552 (2.28, CH_3COCH_3).

Further elution of the column with ether/petroleum ether (50:50) gave a white crystalline compound, which was recrystallized from $CHCl_3$ to give 12 mg of **8** (0.36% yield): mp 225 °C; ^1H NMR ($CDCl_3$) δ 1.65 (d, 6 H, $CH_3 + CH_3$), 2.65 (q, 1 H, C_9-$H_{a'}$, $J_{9a',9e'}$ = 16 Hz, $J_{9a',8a'}$ = 12.5 Hz), 3.15 (q, 1 H, C_9-$H_{e'}$, $J_{9e',9a'}$ = 16 Hz, $J_{9e',8a'}$ = 5.5 Hz), 3.60 (d, 1 H, C_{11}-$H_{a'}$, $J_{11a',11e'}$ = 21.5 Hz), 4.24 (d, 1 H, C_{11}-$H_{e'}$, $J_{11e',11a'}$ = 21.5 Hz), 5.30 (q, 1 H, C_8-$H_{a'}$, $J_{8a',9a'}$ = 12.5 Hz, $J_{8a',9e'}$ = 5.5 Hz), 7.42 (t, 1 H, Ar), 7.5 (d, 1 H, Ar), 7.75 (m, 2 H, Ar), 8.35 (dd, 1 H, Ar); IR (Nujol) ν_{max} 1715 (CO), 1645–1650 (γ-pyrone), 1595 (Ar) cm^{-1}; MS, m/z (rel int) 337.1034 (0.80, M^+ + 1), 336.1001 (3.6, M^+; calcd for $C_{20}H_{16}O_5$, 336.1001), 278.0585 (86.9, $M^+ - CH_3COCH_3$), 250.0635 (100, $M^+ - (CH_3COCH_3 + CO)$), 249.0550 (31.5, 250.0635 - H), 222.0677 (41.8, 250.0635 - CO), 221.0603 (37.0, 249.0550 - CO), 58.0443 (4.7, CH_3COCH_3).

Further elution of the column with ether gave a mixture of **4** and **5** that was again subjected to column chromatography, and the column was eluted with ether/petroleum ether (15:85). Small fractions were collected, and their contents were monitored by TLC and then combined. Removal of the solvent from appropriate fractions gave a white solid, which was recrystallized from THF/ether (1:1) to give 1.25 g of compound **4** (33% yield): mp 222 °C; ^1H NMR ($CDCl_3$) δ 1.65 (d, 6 H, $CH_3 + CH_3$), 2.08 (t, 1 H, C_9-$H_{a'}$, $J_{9a',9e'}$ = 11 Hz), 2.25 (q, 1 H, C_9-$H_{e'}$, $J_{9e',9a'}$ = 11 Hz, $J_{9e',8a'}$ = 5 Hz), 3.08 (d, 1 H, C_{11}-$H_{a'}$, $J_{11a',11e'}$ = =18 Hz), 3.46 (d, 1 H, C_{11}-$H_{e'}$, $J_{11e',11a'}$ = 18 Hz), 4.40 (s, 1 H, C_{10}-OH exch) 5.30 (q, 1 H, C_8-$H_{a'}$, $J_{8a',9e'}$ = 5 Hz, $J_{8a',9a'}$ = 11 Hz), 7.42 (m, 2 H, Ar), 7.64 (s, 1 H, Ar), 7.72 (dt, 1 H, Ar), 8.35 (dd, 1 H, Ar); IR ($CHCl_3$) ν_{max} 3420 (OH), 1710 ($COCH_3$), 1645 (γ-pyrone), 1620 and 1590 (Ar) cm^{-1}; MS, m/z (rel int) 381.1295 (1.16, M^+ + 1), 380.1261 (5.21, M^+; calcd for $C_{22}H_{20}O_6$, 380.1261), 322.0844 (17.86, $M^+ - CH_3COCH_3$), 319.0973 (1.14, $M^+ - (H_2O + CH_3CO)$), 305.0773 (17.38, $M^+ - (CH_3COCH_3 + OH)$), 304.0739 (82.69, $M^+ - (CH_3COCH_3 + H_2O)$), 262.0633 (100, 305.773 - CH_3CO), 261.0554 (4.6, 319.0973 - CH_3COCH_3), 58.0442 (4.9, CH_3COCH_3).

Further elution of the column gave 0.95 g of compound **5** (25% yield): mp 210 °C; ^1H NMR ($CDCl_3$) δ 1.62 (d, 6 H, $CH_3 + CH_3$), 1.94 (q, 1 H, C_9-$H_{a'}$, $J_{9a',9e'}$ = 13 Hz, $J_{9a',8a'}$ = 10 Hz), 2.50 (s, 3 H, $COCH_3$), 2.80 (q, 1 H, C_9-$H_{e'}$, $J_{9e',9a'}$ = 13 Hz, $J_{9e',8a'}$ = 7 Hz), 3.08 (d, 1 H, C_{11}-$H_{a'}$, $J_{11a',11e'}$ = 16 Hz), 3.44 (d, 1 H, C_{11}-$H_{e'}$, $J_{11e',11a'}$ = 16 Hz), 3.56 (s, 1 H, C_{10}-OH, exch) 4.90 (q, 1H, C_8-$H_{a'}$, $J_{8a',9e'}$ = 7 Hz, $J_{8a',9a'}$ = 10 Hz), 7.36 (m, 2 H, Ar), 7.68 (m + s, 2 H, Ar), 8.30 (dd, 1 H, Ar); IR ($CDCl_3$) ν_{max} 3400 (OH), 1710 ($COCH_3$), 1640 (γ-pyrone), 1620 and 1590 (Ar) cm^{-1}; MS, m/z (rel ent) 381.1292 (0.52, M^+ + 1), 380.1262 (2.53, M^+; calcd for $C_{22}H_{20}O_6$, 380.1262), 322.0844 (17.6, $M^+ - CH_3COCH_3$), 305.0775 (17.6, $M^+ - (CH_3COCH_3 + OH)$), 304.0740 (84.5, $M^+ - (CH_3COCH_3 + H_2O)$), 289.0502 (10.3, 304.0740 - CH_3), 279.0658 (39.13, $M^+ - (CH_3COCH_3 + CH_3CO)$), 262.0631 (100, 279.0658 - OH), 261.0552 (5.4, 279.0658 - H_2O), 58.0441 (7.9, CH_3COCH_3).

(*trans*-8,10-Dihydroxy)-10-acetyl-7,8,10-trihydroxyxantho[2,3-*f*]tetralin (15) and (*cis*-8,10-Dihydroxy)-10-acetyl-7,8,10-trihydroxyxantho[2,3-*f*]tetralin (16). Compound **4** (190 mg, 0.5 mmol) was dissolved in THF (200 mL), and to this solution was added 2 mL of concentrated HCl and 2 mL of water. The reaction mixture was stirred at room temperature for 8 h. The reaction mixture was then diluted with water and extracted with ethyl acetate exhaustively. The ethyl acetate extract was washed with water and dried (Na_2SO_4). Removal of the solvent gave a solid residue, which was purified by column chromatography with elution by THF/ether (5:95) to give crude compound **15**. The latter was further purified by recrystallization from THF/ether (1:1) to give 63 mg of pure **15** (37% yield): mp 228–30 °C; ^1H NMR (Me_2SO-d_6/$CDCl_3$ 1:1) δ 2.16 (bt, 1 H, C_9-$H_{a'}$, this triplet after D_2O exchange changes to a sharp quartet with coupling constants $J_{9a',9e'}$ = 13 Hz, $J_{9a',8a'}$ = 9.5 Hz), 2.4 (d + s, 4 H, CH_3CO + C_9-$H_{e'}$), 3.30 (q, 2 H, C_{11}-$H_{a'}$, $H_{e'}$), 5.28 (s, 1 H, C_{10}-OH exch), 5.33 (bt, 1 H, C_8-$H_{a'}$, after D_2O exchange this broad triplet changes to a quartet with coupling constants $J_{8a',9a'}$ = 9.5 Hz, $J_{8a',9e'}$ = 6 Hz), 5.68 (bs, 1 H, C_8-OH exch), 7.40 (t, 1 H, Ar), 7.55 (m, 2 H, Ar), 7.75 (m, 1 H, Ar), 8.30 (d, 1 H, Ar), 9.76 (bs, 1 H, C_7-OH exch); IR (Nujol) ν_{max} 3320–3220 (OH), 1710 ($COCH_3$), 1618 (γ-pyrone), 1605 and 1580 (Ar) cm^{-1}; MS, m/z (rel int) 341.0982 (1.2, M + 1), 341.0947, 322.0841 (3.7, $M^+ - H_2O$), 305.0776 (13.8 $M^+ - (H_2O) + OH)$), 304.0742 (66.6, $M^+ - 2H_2O$), 289.0502 (22.5, $M^+ - (2H_2O + CH_3)$), 279.0656 (100, $M^+ - (H_2O + CH_3CO)$), 262.0631 (40.0, 279.0656 - OH), 261.0551 (7.9, $M^+ - (2H_2O + CH_3CO)$).

Following a similar procedure as was used for **4**, compound **5** was deprotected to give **16** (37% yield): mp 218 °C; ^1H NMR (Me_2SO-d_6/$CDCl_3$, 1:1) δ 2.20 (q, 1 H, C_9-$H_{a'}$, $J_{9a',9e'}$ = 15 Hz, $J_{9a',8e'}$ = 5.0 Hz), 2.40 (dt, 1 H, C_9-$H_{e'}$, $J_{9e',9a'}$ = 15 Hz, $J_{9e',8e'}$ = 2.5 Hz, $J_{9e',11e'}$ = 2 Hz; a long-range coupling between $C_{9e'}$ and $C_{11e'}$ is also observed in the ^1H NMR), 2.50 (s, 3 H, $COCH_3$), 3.22 (d, 1 H, C_{11}-$H_{a'}$, $J_{11a',11e'}$ = 17.5 Hz), 3.45 (dd, 1 H, C_{11}-$H_{e'}$, $J_{11e',11a'}$ = 17.5 Hz, $J_{11e',9e'}$ = 2 Hz), 4.65 (bs, 1 H, C_{10}-OH exch), 5.28 (bq, 1 H, C_8-$H_{e'}$, after D_2O exchange this signal changes to a quartet with coupling constants $J_{8e',9a'}$ = 5 Hz, $J_{8e',9e'}$ = 2.5 Hz), 5.40 (bs, 1 H, C_8-OH exch), 7.40 (t, 1 H, Ar), 7.54 (d, 1 H, Ar), 7.68 (s, 1 H, Ar), 7.75 (m, 1 H, Ar), 8.30 (dd, 1 H, Ar), 9.45 (bs, 1 H, C_7-OH exch); IR (Nujol) ν_{max} 3400–3300 (OH), 1705 ($COCH_3$), 1635 (γ-pyrone), 1615 and 1590 (Ar) cm^{-1}; MS, m/z (rel int) 341.0989 (2.6, M^+ + 1), 340.0952 (12.7, M^+; calcd for $C_{19}H_{16}O_6$, 340.0951), 322.0838 (5.96, $M^+ - H_2O$), 305.0779 (19.35 $M^+ - (H_2O + OH)$), 304.0743 (92.7, $M^+ - 2H_2O$), 297.0768 (1.6, $M^+ - CH_3CO$), 289.0500 (12.7, $M^+ - (2H_2O + CH_3)$), 280.0702 (22.9, 297.0768 - OH), 279.0659 (100, $M^+ - (H_2O + CH_3CO)$), 278.051 (21.2, 279.0659 - H), 262.0631 (87.2, 279.0659 - OH), 251.0710 (77.6, $M^+ - (H_2O + CO + CH_3CO)$).

Glycosidic Coupling of Chromophore 2 with the Bromotrifluoroacetamidoglucosamine. A similar procedure was followed as reported previously[13] except that 2 equiv of the sugar were added initially and additional equivalents were added after 20 and 44 h. Heating was continued for 118 h at 65–70 °C. The crude product was purified by column chromatogrphy using elution with ether to give the dehydration product **18**, which was

recrystallized from THF/ether (1:1) (yield 2.6%): mp 310 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.75 (s, 3 H, $COCH_3$), 7.55 (m + s, 2 H, Ar), 8.00 (m, 2 H, Ar), 8.25 (dt, 2 H, Ar), 8.40 (d, 1 H, Ar), 9.24 (d, 1 H, Ar), 10.25 (bs, 1 H, OH); IR ($CHCl_3$) ν_{max} 3300 (OH), 1670 ($COCH_3$), 1650, 1630 (γ-pyrone, 1610 and 1585 (Ar) cm^{-1}; MS, *m/z* (rel int) 305.0771 (21.34, M^+ + 1), 304.0736 (100, M^+; calcd for $C_{19}H_{12}O_4$, 304.0736), 289.0498 (55.38, $M^+ - CH_3$), 261.0548 (14.77, $M^+ - COCH_3$), 233.0600 (15.01, 261 - CO), 76.0314 (3.30, C_6H_4).

Further elution of the column with ether/THF (80:20) gave the glycoside **17**, which was recrystallized from THF/petroleum ether (1:1) to give pure **17** (yield 2.5%): mp 280–83 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.55 (m, 1 H), 1.90 (s, 3 H, $OCOCH_3$), 2.0 (s, 3 H, $OCOCH_3$), 2.08 (s, 3 H, $OCOCH_3$), 2.30 (s, 3 H, $COCH_3$), 2.60–2.96 (m, 2 H), 3.10–3.40 (m, 2 H), 3.72 (m, 1 H), 3.92 (m, 1 H), 4.21 (m, 2 H), 4.88 (t, 1 H), 5.15 (m, 2 H), 5.35 (t, 1 H), 7.46 (t, 2 H), 7.72 (d, 1 H), 7.86 (m, 1 H), 8.18 (dd, 1 H, Ar), 9.35 (d, 1 H, NH exch), 9.92 (s, 1 H, OH exch); IR (KBr) ν_{max} 3400–3300 (b, OH), 1750 ($OCOCH_3$), 1710 ($OCOCH_3$), 1655 (amide), 1625 (γ-pyrone), 1595 (Ar) cm^{-1}; MS (FAB, sulfolane), *m/z* (rel int) 709 (10.3, M^+ + 2), 708 (22.5, M^+ + 1), 707 (5.3, M^+), 400 (2.0, M^+ - 307), 384 (14.2, M^+ - 323), 323 (16.2, M^+ - sugar), 307 (10, M^+ - (O - sugar)), 306 (85.1, M^+ - (O - sugar + H)), 305 (18.5, 306 - H), 304 (6.4, 305 - H), 291 (10.7, 306 - CH_3), 289 (4.2, 304 - CH_3). Anal. Calcd for $C_{33}H_{32}O_{13}NF_3$: C, 56.0; H, 4.5; N, 2.0. Found: C, 55.8; H, 4.5; N, 2.1.

Glycosidic Coupling of Chromophore 2 with Protected Chlorodaunosamine. The procedure was followed as described previously,[13] and the product was isolated by column chromatography and elution with ether to give the glycoside **19**, which was purified by recrystallization from THF/ether (1:1) (yield 44%): mp 165–68 °C; ^{1}H NMR ($CDCl_3$) δ 1.2 (dd, 3 H, C_5'-CH_3), 1.7 (m, 1 H), 2.15 (m, 2 H), 2.4 (ds, 3 H, $COCH_3$), 2.55 (q, 1 H), 2.90 (q, 1 H), 3.3 (m, 1 H), 3.5 (m, 1 H), 4.45 and 4.6 (dq, 1 H), 4.7 (m, 1 H), 5.2 and 5.3 (dt, 1 H), 5.4 (s, <1 H), 5.5 (s, <1 H), 5.6 (s, <1 H), 6.65 (t, <1 H, Ar), 7.4 (m, 1 H, Ar), 7.55 (d, 1 H, Ar), 7.64 (bs, 1H, NH exch), 7.75 (m, 2 H, Ar), 8.3 (m, >4 H, Ar), 11.67, (s, 1 H, C_7-OH exch); IR ($CHCl[3]$ ν_{max} 3320 (b, NH, OH), 1725 (OCO), 1715 ($COCH_3$), 1650 (amide), 1620 (γ-pyrone), 1610 and 1595 (Ar) cm^{-1}; MS (FAB, glycerol/sulfolane) *m/z* (rel int) 699 (2.1, MH^+), 698 (0.1, M^+), 391 (0.2, O - sugar), 375 (2.0, sugar), 324 (3.5, MH^+ - sugar), 308 (5.3, MH^+ - (O - sugar)), 307 (6.8, M^+ - (O - sugar)), 306 (10.6, M^+ - (O - sugar + H)), 263 (31.5, 306 - $COCH_3$), 262 (5.2, 263 - H), 150 (30.3, $C_7H_4NO_3$), 122 (8.4, 150 - CO), 121 (100, 122 - H).

Controlled Base Deprotection of Glycoside 19 Leading to Glycosides 20 and 21. The deprotection of **19** was carried out in a similar way as reported previously,[13] and the reaction time was 1 h. After working up the solid thus obtained it was washed with THF and recrystallized from ethyl acetate to give **21** (α and β isomers (11.6% yield)): mp 252–258 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.2 (t, 3 H, C_5'-CH_3), 1.55 (m, 3 H), 2.35 (m + s, 4 H, $COCH_3$ + H), 2.86 (m, 2 H), 3.15 (m, 2 H), 3.96 (, 1 H), 4.4 (bs, 1 H, exch), 4.96 (s, 1 H), 5.30 (d, 1 H), 7.45 (s + t, 2 H, Ar), 7.70 (d, 1 H, Ar), 7.85 (t, 1 H, Ar) 8.15 (d, 1 H, Ar); IR ($CHCl_3$) ν_{max} 3520, 3400 (NH_2), 3340 (OH), 1710 ($COCH_3$), 1645, 1615 (γ-pyrone), 1595 (Ar) cm^{-1}; MS (FAB, glycerol/sulfolane), *m/z* (rel int) 455 (1.5, M^+ + 2), 454 (3.9, MH^+), 453 (0.3, M^+), 327 (0.4, M^+ - (O - sugar)), 326 (0.6, M^+ - (O - sugar + H)), 325 (2.2, 326 - H), 305 (3.3, M^+ - ($COCH_3$ + H + C_7H_4O)), 186 (43.1, $C_{12}H_{10}O_2$), 185 (100, 186 - H), 104 (2.2, C_7H_4O).

The filtrate was concentrated, and the residue was subjected to preparative TLC on silica plates eluted with THF/ether (20:80) giving trace amounts of **20**, which was identified by FABMS: mp 250 °C; IR ($CHCl_3$) ν_{max} 3300 (NH, OH), 1695 ($COCH_3$), 1665 (amide), 1630 and 1615 (γ-pyrone), 1605 and 1590 (Ar) cm^{-1}; MS (FAB, glycerol/sulfolane), *m/z* (rel int) 551 (4.4, M^+ + 2), 550 (12.8, MH^+), 549 (0.4, M^+), 324 (7.3, MH^+ - sugar), 323 (6.3, M^+ - sugar), 308 (24.8, MH^+ - (O - sugar)), 307 (23.7, M^+ - (O - sugar)), 306 (3.4, 307 - H), 291 (5.4, 306 - CH_3), 264 (28.6, 307 - $COCH_3$), 263 (100, 306 - $COCH_3$), 262 (13.6, 263 - H), 242 (1.4, O - sugar), 226 (10.3, sugar), 225 (5.8, 226 - H), 186 (7.2, $C_{12}H_{10}O_2$), 185 (78.3, 186 - H), 112 (10.6, $NHCOCF_3$).

Glycosidic Coupling of Chromophore 16 with Protected Chlorodaunosamine To Give Glycoside 22. The coupling of **16** with protected chlorodaunosamine was performed following the general procedure described previously[13] giving α-**22** (20% yield), which was recrystallized from THF/ether (1:1): mp 270 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.08 (d, 3 H, C_5'-CH_3), 1.68 (q, 1 H, C_2'-$H_{e'}$), 2.22 (m, 3 H, C_2'-$H_{a'}$, C_9-H,H'), 2.30 (s, 3 H, $COCH_3$), 3.23 (s, 2 H, C_{10}-H,H'), 4.35 (m, 1 H, C_3'-H), 4.60 (q, 1 H, C_5'-H), 5.0 (t, 1 H, C_8-$H_{e'}$, $J_{8e',9a'}$ = 5 Hz, $J_{8e',9e'}$ = 3.5 Hz), 5.31 (bs, 1 H, C_4'-H), 5.48 (d, 1 H, C_1'-$H_{e'}$, $J_{1'e',2'a'}$ = 2.5 Hz, $J_{1'e',2'e'}$ = 0 Hz), 5.60 (s, 1 H, C_{10}-H), 7.44 (m, 2 H, Ar), 7.72 (d, 1 H, Ar), 7.88 (m8 1 H, Ar), 8.16 (dd, 1 H, Ar), 8.30 (dd, 2 H, Ar), 8.40 (dd, 2 H, Ar), 9.5 (d, 1 H, NH exch), 10.2 (s, 1 H, C_7-OH exch); IR (Nujol) ν_{max} 3520 (NH), 3320 (OH), 1730 (OCO), 1700 (COH_3), 1645 (amide), 1620 (γ-pyrone), 1595 (Ar) cm^{-1}; MS (FAB, glycerol/sulfolane), *m/z* (rel int) 716 (4.2, M^+ + 2), 715 (10.8, M^+ + 1), 714 (1.2, M^+), 699 (0.7, $M^+ - CH_3$), 697 (M^+ - OH), 391 (0.6, O - sugar), 375 (12.8, sugar), 341 (43.7, 716 - sugar), 340 (15.9, MH^+ - sugar), 339 (6.2, M^+ - sugar), 324 (17.5, MH^+ - (O - sugar)), 323 (14.8, M^+ - (O - sugar)), 322 (4.2, M^+ - (O - sugar + H)), 307 (3.0, 322 - CH_3), 306 (7.3, 323 - OH), 305 (16.3, 323 - H_2O), 304 (13.4, 322 - H_2O), 289 (2.9, 304 - CH_3), 263 (39.6, 375 - NHCOCF3), 150 (23.2, $C_7H_4NO_3$), 122 (14.8, 150 - CO), 121 (100, 122 - H), 104 (44.3, C_7H_4O).

Controlled Base Deprotection of 22 To Give Glycoside 23. Compound **22** was stirred at room temperature with 0.1 N NaOH/acetone for 1 h. The usual workup and purification by column chromatography eluting with ether/THF (80:20) gave pure **23** in 46% yield: mp 232–35 °C; ^{1}H NMR (Me_2SOd_6) δ 1.1 (d, 3 H, C_5'-CH_3), 1.50 (q, 1 H, C_2'-$H_{e'}$), 2.05 (m, 1 H, C_2'-$H_{a'}$), 2.20 (m, 2 H, C_9-H,H'), 2.30 (s, 3 H, $COCH_3$), 3.2 (s, 2 H, C_{11}-H,H'), 3.52 (bs, 1 H, C_4'-H), 4.04 (m, 1 H, C_3'-H), 4.24 (q, 1 H, C_5'-H), 4.95 (t, 1 H, C_8-$H_{e'}$, $J_{8e',9a'}$ = 5 Hz, $J_{8e',9e'}$ = 4.5 Hz), 5.0 (d, 1 H, C_4'-OH exch) 5.25 (d, 1 H, C_1'-$H_{e'}$, $J_{1'e,2'a}$ = 2.3 Hz, $J_{1'e',2'e'}$ = 0 Hz), 5.50 (s, 1 H, C_{10}-OH exch), 7.45 (d+s, 2 H, Ar) 7.70 (d, 1 H, Ar), 7.85 (m, 1 H, Ar), 8.15 (dd, 1 H, Ar), 9.08 (d, 1 H, C_3'-NH exch), 10.20 (s, 1 H, C_7-OH exch); IR (Nujol) ν_{max} 3490 (NH), 3320 (OH), 1700 ($COCH_3$), 1635 (amide), 1620 (γ-pyrone), 1610 and 1595 (Ar) cm^{-1}; MS (FAB, glycerol/sulfolane), *m/z* (rel int) 567 (20.3, M^+ + 2), 566 (58.6, M^+ + 1), 565 (3.2, M^+), 548 (1.3, M^+ - OH), 522 (1.6, $M^+ - COCH_3$), 340 (22.6, MH^+ - sugar), 339 (11.4, M^+ - sugar), 324 (28.7, MG^+ - (O - sugar)), 323 (20.8, M^+ - (O - sugar)), 322 (5.0, M^+ - (O - sugar + H)), 307 (7.9, 322 - CH_3), 306 (18.0, 323 - OH), 305 (28.1, 322 - OH), 304 (23.3, 322 - H_2O), 280 (19.9, 323 - $COCH_3$), 279 (40.1, 322 - $COCH_3$), 263 (100, 280 - OH), 262 (19.2, 280 - H_2O), 226 (40.3, sugar), 114 (9.5, 226 - $NHCOCF_3$), 113 (73.5, 114 - H), 112 (1.9,$NHCOCF_3$).

Electrochemical Determination of Redox Properties. Polarographic analysis and cyclic voltammetry were carried out as described previously.[13,14] Aqueous solutions of the glycosides were prepared in 0.1 M potassium phosphate buffer, pH 7.0, and 0.1 M KCl supporting electrolyte. Addition of 10–20% CH_3CN by volume provided sufficient solubility for differential pulse polarography (except for compound **17**, which had to be studied in DMF).

Microsomal Oxygen Consumption Assay. Glycoside stimulation of microsomal oxygen consumption was determined by using a Clark electrode and a biological oxygen monitor (Model 53, Yellow Springs Instrument Co., Yellow Springs, OH). The following modifications of the method of Bachur et al.[28] were employed. Frozen microsomes were uniformly suspended in 2% Triton N-101 (Sigma) in 0.1 M, pH 7.5, potassium phosphate buffer at room temperature using gentle strokes (4–5) of an all-glass tissue homogenizer. Protein levels were determined by using the dye-binding method of Bradford[29] and the dye concentrate supplied by Bio-Rad Labs, Richmond, CA. Human albumin (fraction V, Sigma) served as the protein standard. The standard procedure for assaying each glycoside at a single concentration consisted of adding 3.6 mL of 0.2 M, pH 8.0, potassium phosphate buffer (gassed at 37 °C with laboratory air) to the incubation vial. An aliquot of microsomes (0.1 mL; approximately 10 mg of protein/mL) was added, the oxygen probe inserted, and the meter of the oxygen monitor set at 100% oxygen saturation. After 5 min at 37 °C, 0.1 mL of a solution of NADPH (P-L

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Biochemicals, Inc., Milwaukee, WI; 177 mg/mL in the above pH 8.0 buffer) was added to the chamber and allowed to equilibrate for 1.5 min, and then the endogenous oxygen consumption rate was determined for 1 min. After 0.5 min, 0.2 mL of the glycoside solution that was 2.0 mM in 80% polyethylene glycol 200 (PG 200, J. T. Baker Chemical Co., Phillipsburg, NJ) in water was added and allowed to equilibrate for 1 min, and then the rate of oxygen consumption was again measured for 1 min.

Antitumor Assays. Leukemia L1210 cells are grown in McCoy's 5A medium supplemented with glutamine, HCO_3^-, antibiotics, and 10% heat-inactivated horse serum at 37 °C in a humidified atmosphere of 95:5 air/CO_2. Cells are dispensed at 10^5 cells/mL, and drug is added at 10, 1, 0.1, or 0.01 μg/mL final concentration. Cell concentration is measured 72 h later using a Coulter Counter, and the ID_{50} value (the theoretical drug concentration required to inhibit cell growth by 50%) is determined.

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Registry No. 1, 104112-62-1; 2, 104070-16-8; 3, 104070-17-9; 4, 104070-18-0; 5, 104112-63-2; 6, 104070-19-1; 7, 104070-20-4; 8, 104070-21-5; 9, 104070-22-6; 15, 104070-23-7; 16, 104112-64-3; 17, 104070-24-8; 18, 104070-25-9; α-19, 104070-26-0; β-19, 104112-65-4; α-20, 104070-27-1; β-20, 104112-66-5; α-21, 104070-28-2; β-21, 104112-67-6; α-22, 104089-95-4; α-23, 104070-29-3; 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl bromide, 104070-30-6; 4-*O*-(*p*-nitrobenzoyl)-2,3,6-trideoxy-3-(trifluoroacetamido)-α-L-lyxopyranosyl chloride, 78548-38-6.

Synthesis and Antineoplastic Activity of Bis[[[(alkylamino)carbonyl]oxy]methyl]-Substituted 3-Pyrrolines as Prodrugs of Tumor Inhibitory Pyrrole Bis(carbamates)[1]

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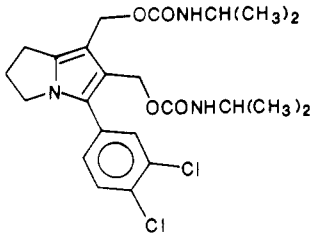
A series of bis[(carbamoyloxy)methyl]pyrrolines **2**–**4** were synthesized from either the appropriate α-silylated iminium salt, or an aziridine, or a 2*H*-azirine in a sequence involving 1,3-dipolar cycloaddition reactions. The antineoplastic activities of the pyrrolines were compared to the corresponding pyrroles. The C-2 *gem*-dimethyl-substituted pyrroline, **4**, which cannot be converted to the pyrrole in vivo, was inactive. The activity of the 2-phenyl-substituted pyrrolines **3** was markedly dependent on the nature of the phenyl substituent, although the corresponding phenylpyrroles all showed comparable activity. The differences in the activities of the pyrrolines **3** may be due to the rate of metabolic conversion of the pyrroline to the pyrrole. Electron-withdrawing substituents on the phenyl ring appear to retard this process.

The pyrrolizine **1** (NSC 278214) has been shown to possess significant reproducible activity against a broad range of experimental murine neoplasias and human tumor xenografts in nude athymic mice.[2] The compound was



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a potential candidate for human clinical trials, but one major problem impeded progress to human studies: the pyrrolizine **1** was very lipophilic (water insoluble) and was unstable in aqueous mixtures. This has led to very major problems in the development of an effective formulation of the agent.[3] One water-soluble prodrug of **1** was prepared, but the compound was unstable in aqueous solution and inactive in murine P388 lymphocytic leukemia test systems.[2c]

We have found that bis(carbamate) derivatives of bis(hydroxymethyl)-substituted pyrroles,[4] pyrrolizines,[5] and polycyclic benz-fused pyrroles[6] possess significant reproducible antineoplastic activity. The rationale employed

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