Glycosidic Coupling of Regiospecifically Synthesized Xantho[2,3-*g*]tetralin Aglycones to Afford Moderately Antileukemic but Redox Inactive Structures Related to Anthracyclines

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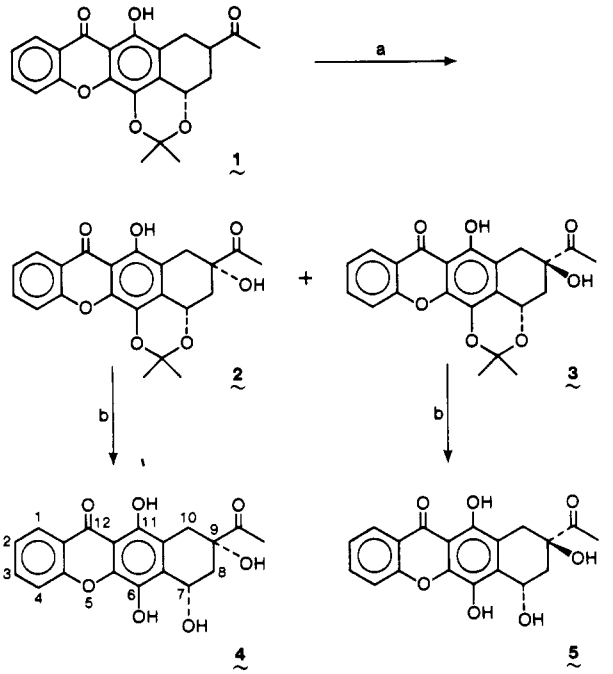
Synthetic routes are described to several chromophore modified glycosides related to the anthracyclines and bearing a nonreducible xantho[2,3-*g*] moiety in place of rings B and C bearing the quinone. Glycosidic coupling was effected by a modified Koenigs–Knorr procedure affording derivatives of several representative sugars required for the hepatic microsomal oxygen consumption studies. The structure of 7β-[3′,4′-di-*O*-acetyl-2′,6′-dideoxy-L-*arabino*-hexopyranosyl]-6,11-dihydroxyxantho[2,3-*g*]tetralin was confirmed and its configuration and molecular geometry were determined by single-crystal X-ray diffraction. This determination reveals a slight bending of the chromophore, as a result of introduction of the ring oxygen, a switch of the hydrogen bond involving the 6-OH from its normal position to the flanking carbonyl to the O-5′ of the sugar, and a change in molecular dihedral angle by 1.5°. The new glycosides are very difficult to reduce electrochemically and display low (1–12%) augmentation of hepatic microsomal oxygen consumption relative to doxorubicin. Significant differences in the latter values, in contrast to the very similar redox potentials, suggest a sensitivity of the substrate requirements of the metabolizing enzymes to the shapes of the glycosides. The observation of cytotoxicity in this series of novel glycosides against L1210 leukemia suggests that the quinone moiety, which is implicated in cardiotoxicity, may not be essential for useful biological activity.

The principal clinical limitation of the widely prescribed anthracycline antitumor antibiotics[1-3] continues to be the severe risk of cardiotoxicity.[4,8] Consequently there is an urgent need to effect a separation of cytotoxic and cardiotoxic effects in these valuable agents by appropriate structural modification. Some progress has been made based on evidence that the onset of cardiotoxicity is related to the property of the anthracycline quinone moiety to undergo microsomal enzymatic reduction followed by reaction with molecular oxygen to generate reactive oxygen species.[7-11] The latter may result in lipid peroxidation in cardiac tissue which is susceptible to oxidative lesions owing to the suppressed levels of catalase[12,13] and possibly superoxide dismutase[7] in that organ. Based on this premise, structural modification of the chromophore in a series of daunorubicin derivatives to suppress the tendency toward enzymatic reduction effects a 20-fold improvement in cardiotoxicity in animals with no sacrifice in anticancer properties.[11] A further indication of the validity of this approach of rational chromophore modification is the absence of any evidence for reductive deglycosidation during the metabolism of 5-iminodaunorubicin,[14] a reaction characteristic of daunorubicin and doxorubicin.[1-3] As a consequence of these studies 5-iminodaunorubicin and related drugs are undergoing preclinical evaluations in the U.S.[15] However many questions remain to be answered. These include to what extent, if any, does the redox activity of the chromophore contribute to the cytotoxic action? Secondly how severe are the structural and stereochemical constraints on chromophore modification imposed by the requirement for intercalative binding to DNA? There are, of course, many other questions but we

Scheme I[a]



[a] Reaction conditions: (a) K-*t*-OBu, *t*-BuOH, DMF, nitrogen atmosphere, room temperature for 30 min, then cool to −25 °C, and then triethyl phosphite, O_2, −15 to −25 °C for 2 h; (b) THF, HCl, H_2O, room temperature for 6 h.

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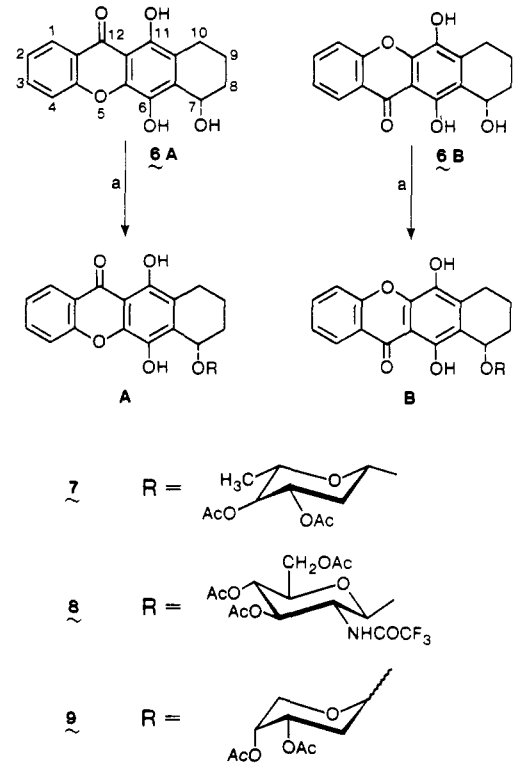
address these particular questions in the present report. We recently described the regiospecific synthesis of 9-acetyl-6,9,11-trihydroxyxantho[2,3-*g*]tetralins which showed the desired low hepatic microsomal oxygen consumption indicative of minimal in vivo redox activity.[16] We report further studies of the unique chemical properties of these novel structures and their glycosidic coupling to a series of model sugars. X-ray diffraction examination of one of the resulting glycosides provided evidence for a slight bending of the chromophore and a change in dihedral angle between the aglycone and the sugar which is a direct consequence of a specific hydrogen bond shift. We also report cytotoxicity data against L1210 leukemia, electrochemical redox potentials, and augmentation of hepatic microsomal oxygen uptake.

Synthesis. (a) Chromophores. Treatment of *trans*-9-acetyl-6,7,11-trihydroxyxantho[2,3-*g*]tetralin 6,7-acetonide (**1**)[16,17] with potassium *tert*-butoxide in DMF and *tert*-butyl alcohol in the presence of triethyl phosphite with molecular oxygen[18] on a larger scale than we reported previously[16] permitted the isolation not only of the *trans*-7-oxo-9-acetyl derivative **2**[16] but also the epimeric *cis*-7-oxo-9-acetyl compound **3** which results from attack by molecular oxygen on either side of the intermediate anion (Scheme I). Mild acid treatment of **2** affords the single deprotected and fully developed aglycone **4** and similarly mild acid treatment of **3** afforded only **5**. The stereochemistry of **2** and **4** has been established previously.[16] The trans relationship of the 7-oxygen function and 9-acetyl in **2** and **4** follows from the inability of **4** and the precursor (**42** in ref 16) to form an intramolecular ketal in the presence of methanol bridging positions 7 and 9. This contrasts with the facile cyclic ketal formation with methanol of a 7-epimer (structure **49** in ref 16). It follows that the stereochemistry of **3** and **5** have the 7-oxygen function and 9-acetyl groups in a cis relationship.

(b) Glycosidic Coupling of Chromophores. Glycosides Required for Hepatic Microsomal Oxygen Consumption Studies. A key step in the metabolic activation of anthracyclines[1,2] leading to cardiotoxicity is their enzymatic reduction followed by reaction with oxygen to generate reactive oxygen species which lead to lipid peroxidation and cardiac damage.[7-11] A critical requirement of this process may be the substrate specificity of the enzymes. Therefore in order to examine this effect systematically with the new chromophores we required several representative glycosides bearing different sugars.

A number of methods were explored for the glycosidic coupling step employing initially the 6,11,7(or 10)-trihydroxyxantho[2,3-*g*]tetralins. These included coupling of the chromophore with protected halo sugars in the presence of silver carbonate[19] or cadmium carbonate[20,21] in the presence of calcium sulfate. As in our previous experience[17] the silver carbonate procedure tended to effect primarily oxidation of ring B. The alternative cadmium carbonate[20,21] catalyzed reaction while obviating this problem produced only low yields (~2.5%) of the desired products and is more suitable for coupling the model sugar acetobromoglucose than the sugars of more direct interest such as daunosamine. Finally it was found that a modified

Scheme II[a]



[a] Reaction conditions: (a) acetobromo(or chloro) sugar, $HgBr_2$, $Hg(CN)_2$, THF, molecular sieves (3 Å), 55 °C.

Koenigs–Knorr procedure employing mercuric bromide and mercuric cyanide as catalysts in the presence of the protected sugars[22] afforded the desired glycosides in acceptable yields. Protected glycosidic derivatives of the 6,11,7(or 10)-trihydroxyxantho[2,3-*g*]tetralin were prepared by this procedure (Scheme II) incorporating the following sugar moieties: **7** employs 3′,4′-di-*O*-acetyl-2′,6′-dideoxy-β-L-*arabino*-hexopyranosyl;[23] **8** employs 3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(trifluoroacetamido)-β-D-glucopyranosyl;[24] **9** employs 3′,4′-di-*O*-acetyl-2′-deoxy-β-D-*erythro*-pentopyranosyl.[25,26] In the first two cases the regioisomeric glycosides were separated by column chromatography.

The individual regioisomers were assigned initially on the basis of the ^1H NMR. The phenolic protons in **7A** appear at δ 9.40 and 12.12 whereas in the case of **7B** they appear at δ 8.36 and 12.17. The assignment of **7A** was subsequently confirmed by single-crystal X-ray analysis[27] (Figure 1). The configurations of the sugar moieties in **7B**, **8A**, and **8B** were assigned β on the basis of the characteristic $J_{1',2'}$ coupling constants, i.e., **7B**, $J_{1'a',2'a'}$ = 9.8 Hz, $J_{1'a',2'e'}$ = 1.9 Hz, **8A**, $J_{1',2'}$ = 8 Hz, and **8B**, $J_{1',2'}$ = 9 Hz. Severe overlap of the ^1H NMR signals for $C_{1'}H$ and $C_{1'}H$ for **7A** prevented an assignment of the sugar conformation in this compound by this procedure. However as demonstrated in Figure 1 the X-ray determination confirmed that this also possesses the β-configuration.

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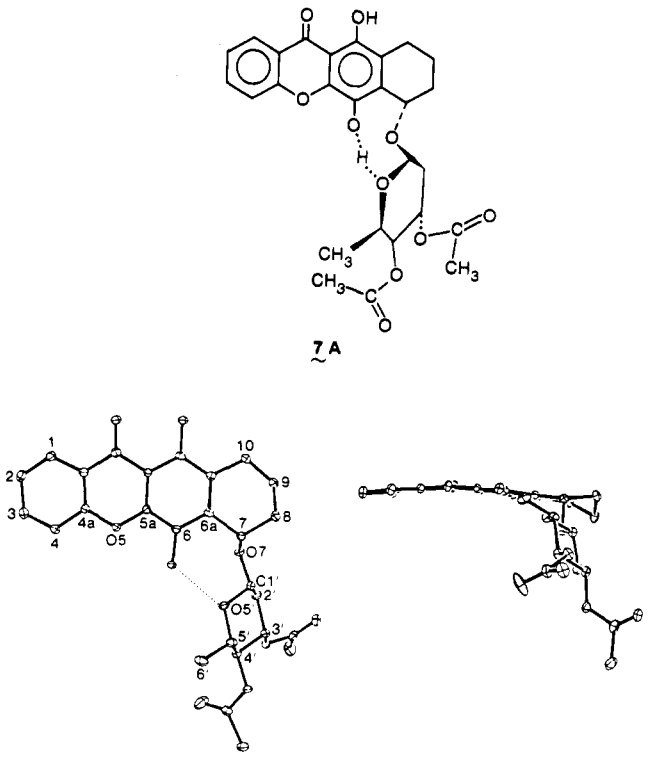


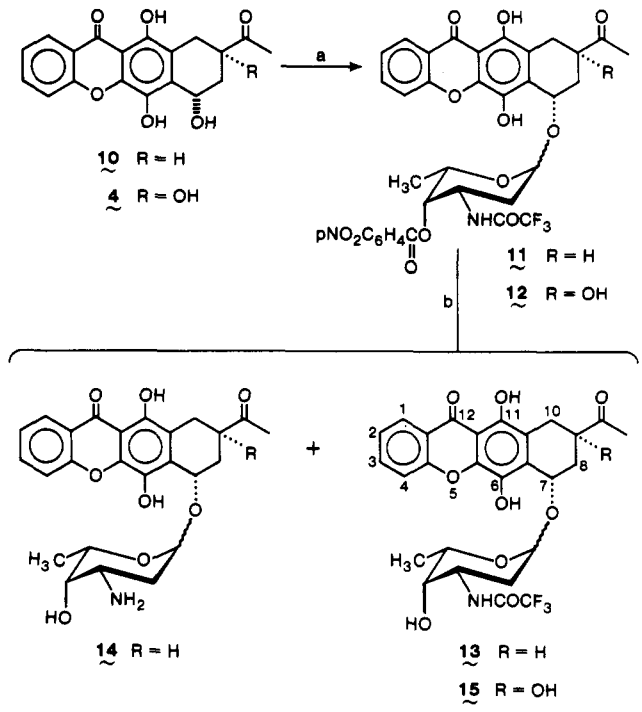
Figure 1. Structure and single-crystal X-ray diffraction derived ORTEP[33] drawings of the glycoside **7A**. The latter crystallizes with space group $P2_1$ appropriate for an optically active compound, with lattice parameters a = 5.97 Å, b = 12.40 Å, c = 16.79 Å, and β = 95°. The calculated unit cell volume corresponds to two molecules of the glycoside. Ellipsoids of 10% probability are shown.

While conventional EI-MS failed to detect the molecular ions of the glycosides, owing to extensive fragmentation,[2] the FAB-MS technique[28] employing xenon and sulfolane as carriers permitted detection of the molecular ions in each case and confirmed the molecular formulae. All the glycosides (including those of the fully functionalized chromophores described below) exhibited common fragmentations corresponding to MH^+ – (sugar), M^+ – (sugar), MH^+ – (O-sugar), M^+ – (O-sugar), and M^+ – [(O-sugar) + H] which assisted in confirming the structures.

(c) Single-Crystal X-ray Determination of Glycoside Structure, Configuration, and Geometry. The glycoside 6,7,11-trihydroxyxantho[2,3-*g*]tetralinyl 3′,4′-di-*O*-acetyl-2′,6′-deoxy-β-L-*arabino*-hexopyranose (**7A**) crystallizes from (1:1) THF–petroleum ether with space group $P2_1$ and with lattice parameters a = 5.97 Å, b = 12.40 Å, c = 16.49 Å and β = 95°.[27] The calculated unit cell volume corresponds to two molecules of the glycoside. The X-ray determination confirms the β-configuration of the glycoside (Figure 1) and reveals structural changes that are pertinent to possible DNA interaction. The elevation drawing (Figure 1) shows that the hydrogen bond involving the 6-OH switches from its normal position to the flanking carbonyl of the O-5′ of the sugar. The side view (Figure 1) demonstrates a slight bend in the chromophore and a dihedral angle of 95° representing an increase of 1.5° from the value in daunorubicin.[1,2]

(d) Coupling of Fully Functionalized Chromophores to Protected Daunosamine. The preferred procedure for glycosidic coupling[22] determined from the above studies was then applied to the coupling of the fully functionalized chromophores to the daunosamine moiety of the parent antibiotic. When 2,3,6-trideoxy-3-(trifluoroacetamide)-(α,β)-L-*lyxo*-hexopyranose was prepared from daunosamine hydrochloride by treatment with (*S*)-ethyl trifluorothioacetate, initially a mixture of α- and β-isomers was obtained. However when the solution of the products in ether was set aside at room temperature for 2 days the α-isomer was slowly converted into the β-isomer which, since it is much less soluble, separated from solution in pure form. The progress of this isomerization was monitored by ^{1}H NMR.

Scheme III[a]



[a] Reaction conditions: (a) protected chlorodaunosamine, $HgBr_2$, $Hg(CN)_2$, THF, molecular sieves (3 Å), 55–60 °C, 42 h; (b) acetone, 0.1 N NaOH, nitrogen atmosphere, room temperature 30 min.

The corresponding chloro sugar was prepared by passing anhydrous hydrogen chloride into a suspension of 2,3,6-trideoxy-1,4-di-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-α-L-lyxopyranose,[23] and then it was coupled with the modified Koenigs–Knorr procedure[22] to the chromophores **10** and **4** to give the glycosides **11** and **12**, respectively, in 57% and 49% yield (Scheme III). The ^{1}H NMR spectra of **11** and **12** showed doubling of the principal peaks indicating the presence of both α- and β-isomers in each case.

When compound **11** was treated with 0.1 N sodium hydroxide in acetone at ambient temperatures for 30 min the partially deprotected **13** and the fully deprotected glycoside **14** were obtained and were separated cleanly by preparative TLC. The structures were proven by spectral means employing ^{1}H NMR, IR, and FAB-MS.[28] As in the case of **11** and **12** the ^{1}H NMR of the pure **13** and **14** showed doubling of the peaks permitting assignment to individual α- and β-isomers of the deprotected glycosides. Similar treatment of **12** with base afforded the partially deprotected glycoside **15** in 25% yield.

In a control experiment to determine the effects of chromophore stereochemistry on coupling, the (*cis*-7-hydroxy-9-acetyl)-9-acetyl-6,7,9,11-tetrahydroxyxantho-

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(29) We are indebted to Dr. J. A. Plambeck and D. Rajani of the Department of Chemistry, University of Alberta, for the electrochemical measurements.

[2,3-g]tetralin (5) was subjected to the modified Koenigs–Knorr procedure with protected chlorodaunosamine. In contrast to the efficient coupling of 4 (which possesses the same stereochemistry about positions 7 and 9 as daunorubicin) attempted coupling of 5 gave only trace amounts of the corresponding glycoside detected by FAB-MS. This indicates strict stereochemical requirements for the coupling reaction.

Antileukemic Cytotoxicity, Augmentation of Hepatic Microsomal Oxygen Uptake, and Redox Characteristics of Ring C Modified Glycosides. Cyclic voltammetry and polarographic studies on the glycosides **7A**, **7B**, **8A**, and **9A,B** confirmed that the chromophores were, as expected, extremely resistant to reduction, with $E_{1/2}$ −1.320, −1.390, −1.420, and −1.500 V vs. SCE, respectively, compared with the readily reducible doxorubicin or daunorubicin which have $E_{1/2}$ = −0.66 V.[10,11,17]

The augmentation of normal oxygen uptake by fresh rat liver microsomes may be used as a measure of in vivo redox activity compared with the parent antibiotic doxorubicin.[11] The oxygen uptake by representative examples of the new glycosides was uniformly low: **7A**, **7B**, **8A**, **8B**, and **9A,B** were 2%, 1%, 4%, 6%, and 12% of the activity of doxorubicin under standard conditions, respectively. Since the reproducibility and estimated precision of this assay is ±2%[30] the differences between **7B** at 1% and **9A,B** at 12% are significant. The polarographic redox potentials are not appreciably different and neither is the chemical nature of the attached monosaccharide moieties. A possible interpretation of the difference may be the sensitivity of the substrate requirements of the activating reductase enzyme to changes in the shape of the glycosides. This kind of effect may have a bearing on the comparable in vivo reductive enzymatic activation of natural anthracyclines alluded to earlier.[1,2,17]

Compounds **12–15** all exhibited cytotoxicity against leukemia L1210 cells grown in culture with ID_{50} values in a range up to 10 μg/mL.[31] The antileukemic activity is lower than the parent antibiotics daunorubicin and doxorubicin.[1,2] Nevertheless the cytotoxicity of these prototype aglycone modified compounds combined with their evident low redox activity in cell cultures suggests the quinone moiety is not essential for cytotoxicity. The results further suggest development along these lines may effect a useful separation of cytotoxicity and cardiotoxicity.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Only the principal sharply defined IR peaks are reported. ^{1}H NMR spectra were recorded on approximately 5–15% (w/v) solutions in appropriate deuterated solvents with tetramethylsilane as internal standard. Line positions are recorded in ppm from the reference. Wherever possible, and where overlap of signals did not interfere, the positions of the individual protons in particular regioisomers were assigned. In MS spectrometers peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15 000. Kiesel gel 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. Electrochemical measurements of redox potentials were made as described previously.[10,11]

9-Acetyl-6,7,9,11-tetrahydroxyxantho[2,3-*g*]tetralin 6,7-Acetonide (2) and 9-Acetyl-6,7,9,11-tetrahydroxyxantho[2,3-*g*]tetralin 6,7-Acetonide (3). A mixture of dimethylformamide (300 mL) (freshly distilled from CaH_2) and dry *tert*-butyl alcohol (120 mL) was treated with potassium *tert*-butoxide (2.57 g, 23 mmol) and the reaction mixture was stirred at room temperature under nitrogen for 30 min and then cooled to −25 °C. Triethyl phosphite (60 mL) and compound 1 (1.14 g, 3 mmol) were added to the mixture and dry oxygen was bubbled through the solution for 2 h while the temperature was kept between −15 and −25 °C.[19] The reaction mixture was diluted with 200 mL of ice cold water, acidified with dilute hydrochloric acid, and extracted exhaustively with ethyl acetate. The ethyl acetate layer was washed thoroughly with water and dried (Na_2SO_4). Removal of solvent under reduced pressure and treatment with ether caused a solid to separate as reported previously.[16]

The solvent was removed in vacuo from the filtrate and the residual solid was subjected to column chromatography and eluted with ether–petroleum ether (25:75). The first few fractions gave a solid which was further purified by recrystallization from THF–ether (1:1) to give **3**: 216 mg (18% yield); mp 195–198 °C; ^{1}H NMR ($CDCl_3$) δ 1.7 (d, 6 H, CH_3 + CH_3), 2.0 (t, 1 H, C_8H), 2.18 (m, C_8H'), 2.28 (s, 3 H, $COCH_3$), 2.75 (d, 1 H, $C_{10}H_{e'}$, $J_{10e'a'}$ = 18 Hz), 3.25 (d, 1 H, $C_{10}H_{a'}$, $J_{10a'e'}$ = 18 H), 4.30 (s, 1 H, C_9OH exch), 5.25 (q, 1 H, $C_7H_{a'}$, $J_{7a'8a'}$ = 11 Hz, $J_{7a'8e'}$ = 5.5 Hz), 7.40 (m, 1 H, Ar), 7.62 (m, 1 H, Ar), 7.76 (m, 1 H, Ar), 8.3 (dd, 1 H, Ar), 12.48 (s, 1 H, C_{11}OH exch); IR ($CHCl_3$) ν_{max} 3460 (OH), 1710 ($COCH_3$), 1650 (γ-pyrone), 1610 and 1590 cm^{-1} (Ar); MS, *m/z* (relative intensity) 397.1237 (1.1, M^+ + 1), 396.1216 (3.02, calcd for $C_{22}H_{20}O_7$ 396.1215), 338.0788 (15.9, $M^+ - CH_3COCH_3$), 321.0718 [19.70, $M^+ - (CH_3COCH_3 + OH)$], 320.0682 [92.7, $M^+ - (CH_3COCH_3 + H_2O)$], 295.0607 [60.8, $M^+ - (CH_3COCH_3 + CH_3CO)$], 278.0570 (73.9, 321.0718 − CH_3CO), 277.0501 (100, 320.0682 − CH_3CO), 58.0440 (3.3, CH_3COCH_3). Further elution of the column and removal of solvent gave **2** which has been reported earlier.[16]

(*cis*-7,9-Dihydroxy)-9-acetyl-6,7,9,11-tetrahydroxyxantho[2,3-*g*]tetralin (4) and (*trans*-7,9-Dihydroxy)-9-acetyl-6,7,9,11-tetrahydroxyxantho[2,3-*g*]tetralin (5). A solution of **3** (50 mg, 0.126 mmol) in 100 mL of THF containing concentrated HCl (1 mL) and water (1 mL) was stirred at room temperature for 6 h, then diluted with 200 mL of water, and extracted exhaustively with ethyl acetate. The organic layer was washed with sodium bicarbonate and then water and dried (Na_2SO_4). Removal of the solvent in vacuo gave a yellow solid. The latter was subjected to preparative TLC on silica gel with ether as eluent to give first some unreacted **3** (5 mg) and then the slower moving deprotected compound **5** which was purified by recrystallization from THF–ether (1:1): 21.5 mg (53% yield on the basis of unrecovered **3**); mp 200 °C; ^{1}H NMR (Me_2SO-d_6 and $CDCl_3$, 1:1) δ 2.12 (t, 1 H, C_8H), 2.30 (t, 1 H, C_8H'), 2.38 (s, 3 H, $COCH_3$), 2.96 (s, 2 H, $C_{10}H_{a'}H_{e'}$), 5.1 (s, 1 H, C_9OH exch), 5.4 (m, 1 H, $C_7H_{a'}$, after D_2O exchange multiplet changes to a quartet with $J_{7a',8a'}$ = 10 Hz, $J_{7a',8e'}$ = 6 Hz), 6.12 (d, 1 H, C_7OH, exch), 7.40 (t, 1 H, Ar), 7.60 (d, 1 H, Ar), 7.78 (m, 1 H, Ar), 8.25 (dd, 1 H, Ar), 9.42 (s, 1 H, C_6OH exch), 12.24 (s, 1 H, C_{12}OH exch); IR (Nujol) ν_{max} 3450–3300 (OH), 1680 ($COCH_3$), 1620 (γ-pyrone), 1580 cm^{-1} (Ar); MS, *m/z* (relative intensity) 357.0948 (0.4, M^+ + 1), 356.0911 (2.41, calcd for $C_{19}H_{16}O_7$ 356.0909), 338.0790 (4.36, $M^+ - H_2O$), 321.0718 [8.40, $M^+ - (H_2O + HO)$], 320.0683 (4.77, $M^+ - 2H_2O$), 295.0608 [100, $M^+ - (H_2O + CH_3CO)$], 294.0527 (13.81, 295.0608 − H), 277.0499 [39.88, $M^+ - (2H_2O + CH_3CO)$], 104.0261 (2.11, C_7H_4O). Similarly deprotection of **2** gave **4** as reported previously.[16]

General Procedure for Glycosidic Coupling. Synthesis of 7- and 10β-[3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-L-*arabino*-hexopyranosyl]-6,11-dihydroxyxantho[2,3-*g*]tetralins (7A,B). A mixture of 6,11,7(or 10)-trihydroxyxantho[2,3-*g*]tetralins (**6A** and **6B**) (0.298 g, 1 mmol) was dissolved in dry THF (15 mL) to

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(31) 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% air:5% CO_2. Cells are dispensed at 10^5 cells/mL and drug added at 10, 1, 0.1 or 0.01 μg/mL final concentration. Cell concentration is measured 72 h later by using a Coulter Counter and the ID_{50} value (the theoretical drug concentration required to inhibit cell growth by 50%) determined. We are grateful to Dr. Robert Newman of the M. D. Anderson Tumor Institute, Houston, and Dr. Miles Hacker of the Vermont Regional Cancer Center, Burlington, for these data.

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which was added 3 Å molecular sieve (~2 g), mercuric bromide (0.360 g, 1 mmol), mercuric cyanide[22,32] (25 mg, 1 μmol), and 2-deoxyacetochlororhamnose (0.500 g, 2 mmol); the reaction mixture was heated under reflux for 72 h, during which time a further quantity of the chloro sugar (250 mg, 1 mmol) was added at 24-h intervals. The reaction mixture was diluted with two volumes of THF and filtered and the inorganic material washed with THF. The filtrate was concentrated and subjected to chromatography on silica with ether–petroleum ether (1:1) as eluant. The early fractions contained the elimination product[17] and then subsequent elution with ether accompanied by TLC monitoring of the fractions afforded **7A** which was further purified by recrystallization from THF–petroleum ether (1:1) to give pure **7A** (4.5% yield): mp 202 °C. (For the purposes of discussion of the ^{1}H NMR spectra please refer to the numbering system in Scheme III.) Carbons and the oxygen in the sugar are designated with a prime. In addition geminal protons either in ring A or in the sugar are designated as H and H′. In the cases of **9A,B** the ^{1}H NMR spectra gave signal integrations corresponding to fractional protons owing to the presence of pairs of regioisomers. ^{1}H NMR ($CDCl_3$) δ 1.32 (d, 3 H, CH_3) 1.80 (m, 3 H), 2.00 (m + s, 4 H, $OCOCH_3$ + H) 2.05 (s, 3 H, $OCOCH_3$), 2.35 (m, 2 H), 2.50 (m, 1 H), 2.94 (m, 1 H), 3.60 (m, 1 H, C′$_5$H), 4.80 (t, 1 H, C′$_4$H), 5.0 (m, 2 H, C′$_1$H + C′$_3$H anomeric proton, overlap prevents determination of coupling constant), 5.15 (t, 1 H, C_7H_e, $J_{7e',8e'}$ = $J_{7e',8e'}$ = 3.5 Hz), 6.16 (s, 1 H), C_6OH exch), 7.35 (t, 1 H, Ar), 7.50 (d, 1 H, Ar), 7.75 (t, 1 H, Ar), 8.26 (dd, 1 H, Ar), 12.12 (s, 1 H, C_{11}OH exch). NMR indicates C′$_1$H and C′$_3$H overlap so it was difficult to know if it an α- or β-anomer but from X-ray studies it is indicated that it is the β-anomer. IR ($CHCl_3$) ν_{max} 3400 (OH), 1750 ($OCOCH_3$), 1650 (C=O), 1610 (Ar) cm^{-1}; MS (FAB, xenon–sulfolane), m/z 513 (14.8, MH^+), 512 (10.3, M^+), 298 (29.6, MH^+ – sugar), 297 (12.9, M^+ – sugar), 282 [51.1, MH^+ – (O-sugar)], 281 [100, M^+ – (O-sugar)], 280 [90, M^+ – (O-sugar) – H^+]. Anal. Calcd for $C_{27}H_{28}O_{10}$: C, 63.3; H, 5.5. Found: C, 63.2; H, 5.5.

Further elution of the column with ether gave **7B** which was further purified by crystallization from THF–ether (7% yield): mp 220 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.18 (d, 3 H, CH_3), 1.60 (m, 2 H), 1.75 (m, 1 H), 1.97 (s, 3 H, $OCOCH_3$), 2.04 (s, 3 H, $OCOCH_3$), 2.18 (m, 2 H), 2.40 (m, 1 H), 2.85 (m, 1 H), 3.75 (m, 1 H, C′$_5$H), 4.58 (t, 1 H, C′$_4$H), 5.04 (m, 1 H, C′$_3$H), 5.12 (q, 1 H, C′$_1$H$_{a'}$, $J_{1'a,2'a'}$ = 9.8 Hz, and $J_{1'a',2'e'}$ = 1.9 Hz, indicates a β anomer), 5.16 (t, 1 H, C_7H_e, $J_{7e,8a}$ = $J_{7e',8e'}$ = 2.6 Hz), 7.5 (dt, 1 H, Ar) 7.68 (d, 1 H, Ar), 7.92 (dt, 1 H, Ar), 8.20 (dd, 1 H, Ar), 8.36 (s, 1 H, C_{12}OH exch), 12.17 (s, 1 H, C_6OH exch); IR (KBr) ν_{max} 3400 (H), 1750 ($OCOCH_3$), 1660 (C=O), 1615 (Ar) cm^{-1}; MS (FAB, xenon–sulfolane), m/z (relative intensity) 513 (100, MH^+), 512 (56.7, M^+), 298 (10.2, MH^+ – sugar), 297 (7.4, M^+ – sugar), 282 [15.6, MH^+ – (O-sugar)], 281 [54.4, M^+ – (O-sugar)], 280 [35.0, M^+ – (O-sugar) – H]. Anal. Calcd for $C_{27}H_{28}O_{10}$: C, 63.3; H, 5.5. Found: C, 63.3; H, 5.5.

The following glycosides were also prepared using the general procedure: **7β-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(trifluoroacetamido)-D-glucopyranosyl]-6,11-dihydroxyxantho[2,3-*g*]tetralin (8A)** (overall yield 3%): mp 226 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.60 (m, 1 H, C_8H'), 1.75 (m, 1 H, C_9H'), 1.90 (m + s, 4 H, $OOCOCH_3H_3^+$, C_9H), 2.00 (s, 3 H, $OCOCH_3$), 2.10 (s, 3 H, $OCOCH_3$), 2.16 (m, 1 H, C_8H), 2.45 (m, 1 H, $C_{10}H'$), 2.75 (m, 1 H, $C_{10}H$), 3.70 (q, 1 H, C′$_2$H), 3.78 (m, 1 H, C′$_5$H), 4.15 (dd, 1 H, C′$_6$H′), 4.32 (dd, 1 H, C′$_6$H), 4.90 (t, 1 H, C′$_4$H), 4.98 (d, 1 H, C′$_1$, $J_{1'2'}$ = 8.0 Hz indicates it to be a β-anomer), 5.28 (t, 1 H, C′$_3$H), 5.35 (t, 1 H, $C_7H_{e'}$, $J_{7e',8a'}$ = $J_{7e,8e}$ = 2.5 Hz), 7.52 (m, 1 H, Ar), 7.70 (d, 1 H, Ar) 7.95 (t, 1 H, Ar), 8.24 (dd, 1 H, Ar), 9.05 (d, 1 H, NH exch), 9.18 (s, 1 H, C_6OH exch), 12.12 (s, 1 H, C_{11}OH exch); IR ($CHCl_3$) ν_{max} 3590 (NH), 3325 (OH), 1750, 1745, 1705 ($OCOCH_3$), 1650 ($NHCOCF_3$), 1625 (CO), 1610 and 1590 (Ar) cm^{-1}; MS (FAB exon–sulfolane), m/z (relative intensity) 682 (6.2, MH^+), 6.81 (2.7, M^+), 384 (0.9, M^+ – 297), 298 (4.9, MH^+ – sugar), 297 (8.9, M^+ – sugar), 282 [35.3, MH^+ – (O-sugar)], 281 [100, M^+ – (O-sugar)], 280 [92.2, M^+ – (O-sugar) – H]. Anal. Calcd for $C_{31}H_{30}O_{13}NF_3$: C, 54.6; H, 4.4; N, 2.0. Found: C, 54.6; H, 4.5; N, 2.0.

10β-[3′,4′,6′-Tri-*O*-acetyl-2′-deoxy-2′-(trifluoroacetamido)-D-glucopyranosyl]-6,11-dihydroxyxantho[2,3-*g*]tetralin (8B): yield, 0.8%; mp 225 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.6 (m, 3 H), 1.94 (s, 3 H, $OCOCH_3$), 2.02 (s, 3 H, $OCOCH_3$), 2.06 (s, 3 H, $OCOCH_3$), 2.20 (m, 1 H), 2.40 (m, 1 H), 2.80 (dd, 1 H), 3.75 (q, 1 H), 4.0 (m, 2 H), 4.26 (q, 1 H), 4.93 (t, 1 H), 5.18 (d, 2 H, C′$_1$H, $J_{1',2'}$ = 9 Hz indicate it to be the β-anomer), 5.22 (s, 1 H, C_7H_e, $J_{1/2}$ = 3 Hz), 5.28 (t, 1 H), 7.50 (m, 1 H, Ar), 7.65 (d, 1 H, Ar), 7.95 (m, 1 H, Ar), 8.20 (dd, 1 H, Ar), 8.50 (s, 1 H, C_{11}OH exch), 9.72 (d, 1 H, NH, exch), 12.14 (s, 1 H, C_6OH, exch); IR ($CHCl_3$) ν_{max} 3490 (NH), 3300 (OH), 1745, 1705 ($OCOCH_3$), 1650 ($NHCOCF_3$), 1625 (CO), 1610 (Ar) cm^{-1}; MS (FAB xenon–sulfolane), m/z (relative intensity) 682 (3.8, MH^+), 681 (2.1, M^+), 384 (6.1, M^+ – 297), 298 (5.2, MH^+ – sugar), 297 (9.7, M^+ – sugar), 282 [28.9, MH^+ – (O-sugar)], 281 [100, M^+ – (O-sugar)], 280 [81.0, M^+ – (O-sugar) – H].

7(and 10)α-[3′,4′-Di-*O*-acetyl-2′-deoxy-D-*erythro*-pentopyranosyl-6,11-dihydroxyxantho[2,3-*g*]tetralins (9A,B): yield 27%; mp 160–165 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.6–2.2 (2 ds, m, 11 H, 2 ds = 2 × $OCOCH_3$), 2.37 (m, 1 H), 2.86 (m, 1 H), 3.71 (m, 1 H), 4.00 (m, 1 H), 4.50 (d, <1 H), 5.11 (m, 3 H), 5.34 (t, <1 H), 5.52 (s, <1 H), 7.50 (m, 1 H, Ar), 7.65 (dd, 1 H, Ar), 7.95 (m, 1 H, Ar), 8.20 (dd, 1 H, Ar), 9.34 (s, <1 H, OH), 9.40 (s, <1 H, OH), 12.14 (d, 1 H, OH), the ratio of 9.34 and 9.40 signals is 1.2; IR ($CHCl_3$) ν_{max} 3400 (OH), 1745 ($OCOCH_3$), 1645 (CO), 1610, and 1590 (Ar) cm^{-1}; MS (FAB, xenon–sulfolane), m/z (relative intensity) 499 (14.3, MH^+), 498 (7.7, M^+), 298 (15.9, MH^+ – sugar), 297 (7.3, M^+ – sugar), 282 [29.8, MH^+ – (O-sugar)], 281 [100, M^+ – (O-sugar)], 280 [86.9, M^+ – (O-sugar)– H]. Anal. Calcd for $C_{26}H_{26}O_{10}$: C, 62.7; H, 5.2. Found: C, 63.0; H, 5.5.

Coupling of *trans*-9-Acetyl-6,7,11-trihydroxyxantho[2,3-*g*]tetralin (10) with 1-Chloro-2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranose. Compound **10** (85 mg, 0.25 mmol) was taken up in dry THF (25 mL) and to the solution was added $Mg(CN)_2$ (520 mg), $MgBr_2$ (236 mg), and powdered molecular sieves 3 Å (2 g). The reaction mixture was stirred at 55–60 °C for 2 h. The freshly prepared protected chlorodaunosamine (4 equiv) was added in four equal portions in CH_2Cl_2 (5 mL) at 0, 4, 16, and 29 h while the temperature was maintained at 55–60 °C for a total period of 42 h. The reaction mixture was then filtered and the inorganic material washed thoroughly with THF. The solvent was removed and the residue was subjected to column chromatography. Elution with ether–petroleum ether (50:50) gave some unreacted sugar and then further elution by ether, with monitoring of the fractions by TLC, gave the glycoside. The latter was washed thoroughly with ether and purified by recrystallization from THF–ether (1:1) to give **11**: 102 mg (yield 57%); mp 205–215 °C; ^{1}H NMR (Me_2SO-d_6, $CDCl_3$ 1:1) δ 1.22 (d, 3 H, C′$_5$$CH_3$), 1.70 (m, 2 H), 2.35 (m + d, 4 H, $COCH_3$ + H), 2.60 (m, 2 H), 3.25 (m, 2 H), 4.34 (q, <1 H), 4.55 (m, 1 H), 472 (q, H), 5.3 (t, <1 H, C_7H_e, $J_{7e',8a'}$ = $J_{7e',8e'}$ = 2.5 Hz), 5.45 (m, 2 H), 5.60 (bd, <1 H), 7.40 (t, 1 H, Ar), 7.62 (q, 1 H, Ar), 7.80 (m, 1 H, Ar), 8.10 (d, 1 H, C_6OH exch), 8.30 (m + s, 5 H, Ar), 8.60 (t, 1 H, NH exch), 12.25 (d, 1 H, C_{11}OH exch). The doublet at 2.35 for $COCH_3$ and the doublets for C_6 and C_{11}OH at 8.10 and 12.25 indicate that it is a mixture of two isomers, i.e., α- and β-isomers and from the intensities of the doublets at 8.10 they are in the ratio of 3:2. IR ($CHCl_3$) ν_{max} 3300 (b, OH, NH), 1730 (OCO), 1700 ($COCH_3$), 1650 ($NHCOCF_3$), 1625 (γ-pyrone), 1610, and 1585 cm^{-1} (Ar); MS (FAB glycerol–sulfolane), m/z (relative intensity) 715 (2.3, MH^+), 714 (1.0, M^+), 391 (0.6, O-sugar), 375 (13.3, sugar), 340 (25.5, MH^+ – sugar), 339 (8.8, M^+ – sugar), 324 [32.6, MH^+ – (O-sugar)], 323 [88.8, M^+ – (O-sugar)], 322 [50.7, M^+ – (O-sugar + H)], 321 (18.3, 322 – H), 320 (5.1, 321 – H), 307 (8.1, 322 – CH_3), 279 (100, 322 – $COCH_3$), 278 (38.8, 279 – H), 263 (17.5, 375 – $NHCOCF_3$).

Coupling of (*cis*-7,9-Dihydroxy)-9-acetyl-6,7,9,11-tetrahydroxyxantho[2,3-*g*]tetralin (4) with Protected Chlorodaunosamine. Following the same procedure as described above (except that the heating was continued for 40 h) and purifying the product by column chromatography and recrystallization from THF–ether (1:1) gave the glycoside **12** (49% yield): mp 179–85 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.08 (q, 3 H, C$_5$·CH_3), 1.75 (m, 1 H), 2.02 (m, 1 H), 2.25 (ds + m, 5 H, $COCH_3$ + 2 H), 2.98 (m, 2 H), 4.35 (m, 1 H), 4.60 (q, 1 H), 5.15 (t, <1 H), 5.30 (dd, 1 H), 5.45 (bd, 2 H, after D_2O exch this signal integrates for more than one proton which indicates that a part of the C_9OH is included in this signal), 5.60 (s, H, C_9OH, exch), 7.50 (t, 1 H, Ar), 7.70 (t, 1 H, Ar), 7.95 (m, 1 H, Ar), 8.25 (m, 3 H, Ar), 8.44 (d, 2 H, Ar), 9.5 (m, 2 H, C′$_3$NH and C_6OH exch), 12.18 (d, 1 H, C_{11}OH, exch). [The doubling of peaks for $COCH_3$, C′$_5$$CH_3$, C_6OH, C_{11}OH, and C′$_3$NH and the fact that certain signals integrate for partial protons is

due to the presence of both α- and β-isomers.] IR ($CHCl_3$) ν_{max} 3340 (NH, OH), 1730 (OCO), 1710 ($COCH_3$), 1650 (γ-pyrone), 1610, and 1585 cm^{-1} (Ar); MS (FAB), m/z (relative intensity) 731 (0.2, MH^+), 730 (0.1, M^+) 391 (0.1, O-sugar), 356 (0.8, MH^+ - sugar), 355 (0.3, M^+ - sugar), 340 [0.8, MH^+ - (O-sugar)], 339 [1.8, M^+ - (O-sugar)], 338 (0.9, 339 - H), 321 (1.2, 338 - OH), 320 (1.9, 338 - H_2O), 279 [5.1 (391 - $NHCOCF_3$)], 225 (2.8, 391 - $C_7H_4NO_4$), 122 (8.3, $C_6H_4NO_2$), 121 (100, 122 - H), 104 (15.3, C_7H_4O).

Mild Base Deprotection of 11 Affording the Glycosides 13 and 14. Compound 11 (85 mg) was dissolved in 15 mL of acetone, the solution was treated with 0.1 N NaOH (9 mL), and the reaction mixture was stirred for 30 min under a nitrogen atmosphere. The pH of the solution was adjusted to 8 by adding a few drops of 5% HCl and the solution was then extracted with ethyl acetate (5 × 25 mL). The ethyl acetate extract was washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was dissolved in acetone and applied to preparative TLC plates and eluted with $CHCl_3$–MeOH (4:1). Three bands appeared on the plates which were removed separately and the silica was extracted with acetone and stirred for 30 min and filtered. Removal of the solvent from band I gave **13**, which was purified by recrystallization from acetone to give the pure partially deprotected compound **13**: 14 mg (yield 18%); mp 270–285 dec; ^1H NMR (Me_2SO-d_6, $CDCl_3$ 1:1) δ 1.3 (t, 3 H, C'_5CH_3), 1.70 (m, 2 H), 2.10 (m, 1 H), 2.30 (d, 3 H, $COCH_3$), 2.55 (m, 2 H), 3.2 (m, 2 H), 3.60 (d, 1 H), 4.1 and 4.4 (q, both signals integrate for 1 H), 4.25 (m, 1 H), 4.7 and 4.85 (d, both signals integrate for 1 H, C'_4OH, exch), 5.2 and 5.4 (bs, 1 H, C_7H_e, $J_{1/2}$ = 3 Hz), 5.3 and 5.5 (d, 1 H), 7.45 (t, 1 H, Ar), 7.62 (dd, 1 H, Ar), 7.80 (m, 1 H, Ar), 8.25 (m, 2 H, one proton is exch), 8.8 (bs, 1 H, exch), 12.20 (s, 1 H, $C_{11}OH$, exch); IR ($CHCl_3$) ν_{max} 3300 (NH, OH), 1710 and 1695 ($COCH_3$), 1645 (amide), 1625 (γ-pyrone), 1605, and 1590 cm^{-1} (Ar); MS (FAB, glycerol), m/z (relative intensity) 566 (0.2, MH^+), 324 [1.4, MH^+ - (O-sugar)], 323 [4.2, M^+ - (O-sugar)], 322 (1.1, 323 - H), 279 (6.5, 322 - $COCH_3$), 278 [1.8, 322 - ($COCH_3$ + H)], 242 (0.6, O-sugar), 226 (0.9, 242 - O). The third TLC band upon workup gave compound 14 which was further purified by recrystallization from acetone–ether (1:1): 7 mg (yield 10%); mp 200–205 °C dec; IR (KBr) ν_{max} 3400 (NH_2, OH), 1700 ($COCH_3$), 1650 and 1625 (γ-pyrone), 1610, and 1590 cm^{-1} (Ar); MS (FAB, glycerol), m/z (relative intensity) 470 (1.9, MH^+), 469 (0.2, M^+), 335 (0.4, M^+ - sugar), 319 [0.5, M^+ - (O-sugar)], 318 (0.2, 319 - H), 303 (0.4, 318 - CH_3), 275 (0.7, 318 - $COCH_3$), 134 (0.5, sugar), 133 (1.4, 134 - H), 115 (18.7, 133 - H_2O), 99 (3.5, 115 - NH_2).

Mild Base Deprotection of 12 Leading to the Glycoside 15. When a procedure similar to that for the deprotection of 11 was used, compound **12** was deprotected and purified by TLC (on preparative silica plates) and eluted with ether to give **15** which was further purified by recrystallization from THF–ether (1:1) (yield 25%): mp 185 and 210 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.1 (q, 1 H, C'_5CH_3), 1.50 (m, 1 H), 2.20 (d + m, 6 H, $COCH_3$ + 3 H), 2.90 (m, 2 H), 3.50 (m, 2 H, one of the proton is exch), 4.02 (m, 1 H), 4.18 (q, 1 H), 4.95 (q, <1 H, exch), 5.10 (t, <1 H), 5.25 (m, 1 H), 5.40 (t, <1 H), 5.50 (d, <1 H, exch), 7.50 (t, 1 H, Ar), 7.65 (m, 1 H, Ar), 7.95 (m, 1 H, Ar), 8.20 (d, 1 H, Ar), 9.12 (t, 1 H, NH, exch), 9.40 (d, 1 H, C_6OH, exch), 12.16 (d, 1 H, COH, exch); IR ($CHCl_3$) ν_{max} 3400 (NH, OH), 1725, 1705 ($COCH_3$), 1650 (amide), 1625 (γ-pyrone), 1610, and 1590 cm^{-1} (Ar); MS (FAB, glycerol–sulfolane), m/z (relative intensity) 582 (0.5, MH^+), 581 (0.2, M^+), 356 (2.1, MH^+ - sugar), 355 (0.7, M^+ - sugar), 340 [1.6, MH^+ - (O-sugar)], 339 [3.5, M^+ - (O-sugar)], 338 [1.7, M^+ - (O-sugar)], 323 (1.0, 338 - CH_3), 321 (2.9, 338 - OH), 320 (4.2, 338 - H_2O), 295 (5.4, 323 - CO), 294 (2.3), 295 - H), 277 (39, 294 - OH), 242 (1.9, O-sugar), 241 (11.0, 242 - H), 226 (5.5, sugar), 225 (1.8, 226 - H), 104 (9.6, C_7H_4O).

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Supplementary Material Available: X-ray crystallographic data (16 pages). Ordering information is given on any current masthead page.

Expedient Chemical Synthesis of Sequence-Specific 2′,5′-Oligonucleotides

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A rapid chemical approach to the preparation of sequence-specific 2′,5′-oligonucleotides and analogues of 2-5A is described. For instance, reaction of the 5′-phosphoromorpholidate of adenosine (MopA, **14**) with the 5′-phosphoroimidazolidate of inosine (ImpI, **17**), under conditions of lead ion catalysis, gave MopA2′p5′I (**19a**) in 21% yield. Acid hydrolysis of **19a** gave pA2′p5′I, which then was converted to the corresponding 5′-phosphoroimidazolidate Imp5′A2′p5′I (**19a**) through redox condensation with triphenylphosphine, imidazole, and 2,2-dipyridyl disulfide. Lead ion catalyzed condensation of **19c** with Mop5′A (**14**) gave Mop5′a2′p5′A2′p5′I (**30**) in 17% yield. By acid hydrolysis, **30** could be converted to the corresponding 5′-monophosphate, or, by reaction with pyrophosphate in DMF, to the corresponding 2-5A analogue, ppp5′A2′p5′A2′p5′I (**5b**). The following oligonucleotides were prepared by using similar methodology: ppp5′A2′p5′A2′p5′A (**1b**), ppp5′A2′p5′I2′p5′A (**4b**), ppp5′I2′p5′A2′p5′A (**3b**), and ppp5′A2′p5′A2′p5′ (2′dA) (**2b**).

The natural occurrence of the 2′,5′-phosphodiester bond in 5′-triphosphoryladenylyl(2′→5′)adenylyl(2′→5′)-adenosine (2-5A or 2′,5′-oligo A), a mediator of interferon action,[2-4] and in pre-tRNA,[5] has led to increased interest in the synthesis of such unusually linked oligonucleotides.[6,7] For studies on the relationship of oligonucleotide structure

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