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THE SYNTHESIS AND CYTOTOXIC ACTIVITY OF 1,3,4-TRI-*O*-ACETYL-2,6-DIDEOXY-L-*arabino*- AND -L-*lyxo*-HEXOPYRANOSE

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ABSTRACT

Methyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside (**6**) was prepared from L-rhamnose in five steps. Hydrolysis of **6** with 50% aqueous acetic acid gave 2,6-dideoxy-L-*arabino*-hexopyranose. Treatment of 3,4-di-*O*-acetyl-L-rhamnal with acetic acid in the presence of acetic anhydride and 2% sulfuric acid afforded 1,2,3-tri-*O*-acetyl-2,6-dideoxy-L-*arabino*-hexopyranose in 65% yield. Selective benzoylation and subsequent mesylation of **6** afforded methyl 3-*O*-benzoyl-2,6-dideoxy-4-*O*-mesyl-α-L-*arabino*-hexopyranoside, which was treated with sodium benzoate and sodium azide in hexamethylphosphoric triamide to give the corresponding 3,4-dibenzoyl **9** and 4-azido **11** analogs. Hydrogenation and *N*-acetylation of **11** afforded the 4-acetamido derivative **12**. Deprotection of **9** and **12** gave 2,6-dideoxy-L-*lyxo*-hexopyranose and 4-acetamido-2,4,6-trideoxy-L-*lyxo*-hexopyranose, which were characterized as their peracetates. The free and corresponding peracetylated derivatives were assayed for their ability to inhibit the growth of P388 leukemia cells in culture. Although the free sugars did not inhibit the replication of these tumor cells under the conditions employed, their peracetylated derivatives demonstrated significant activity.

INTRODUCTION

In a recent review, Robbins and Nicolson[1] have described a variety of structural and compositional differences that exist between normal cells and those transformed by oncogenic viruses and chemical carcinogens. In an effort to exploit such differences and thereby accomplish a chemotherapeutic attack directed at the membranes of tumor cells, we are employing derivatives of the membrane carbohydrate L-fucose. A number of derivatives of L-fucose modified at C-4 have been synthesized and tested[2]. In an extension of this work we now report the synthesis and cell cytotoxicity in tissue culture of some 2-deoxy derivatives of L-fucose.

RESULTS AND DISCUSSION

Methods available for the synthesis of methyl α- and β-D-glycosides from the

corresponding glycals have been reviewed by Ferrier[3,4] and include the acid-catalyzed addition of methanol and the methoxymercuration of glycals and their acetates. The synthesis of methyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside (**6**), an intermediate essential for the syntheses reported here, was previously described by Marsh *et al.*[5] who used the methoxymercuration pathway. Sztaricskai *et al.*[6] have also described the synthesis of a mixture of the α- and β-glycosides by a similar procedure. Although the addition of methanol appears to be a less effective method than the methoxymercuration procedure, we were interested in determining whether the use of protecting groups might influence the course of the former reaction, and thus help eliminate the by-products previously reported. Thus, we investigated the addition of methanol to L-rhamnal and to the 3,4-di-*O*-acetyl and 3,4-di-*O*-benzoyl derivatives in the presence of AG 50W-X8 cation-exchange resin.

As 1,5-anhydro-3,4-di-*O*-benzoyl-2,6-dideoxy-L-*arabino*-hex-1-enitol (**2**) may be readily obtainable by treatment of 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-mannopyranosyl bromide with zinc, the following procedure was adopted rather than the more lengthy synthesis described by Lundt and Pedersen[7] *via* L-rhamnal[8]. Benzoylation of L-rhamnose gave the 1,2,3,4-tetrabenzoate as a syrupy product in good yield. Further treatment with hydrogen bromide in acetic acid afforded crystalline 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-mannopyranosyl bromide[9]. However, since this material did not appear to be very stable when left in contact with the atmosphere, synthesis of the required glycal was continued *in situ*, after neutralization and further treatment with zinc dust. Elimination took place readily and 1,5-anhydro-3,4-di-*O*-benzoyl-2,6-dideoxy-L-*arabino*-hex-1-enitol (**2**) was isolated in ~40% overall yield from L-rhamnose. The structure of **2** was supported by the 270-MHz ^{1}H-n.m.r. spectrum, which was similar to that reported previously[7]. As an alternative synthesis of **2**, 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-L-*arabino*-hex-1-enitol (**1**) was hydrolyzed with sodium methoxide to give L-rhamnal which, upon benzoylation afforded **2** in 74% overall yield.

The addition of methanol to L-rhamnal and to its 3,4-diacetate **1** was conducted under reflux in the presence of AG 50W-X8 cation-exchange resin. In both cases, t.l.c. examination of the reaction mixture revealed the formation of several products. In the case of **1**, no single component was isolated in a yield higher than 5%. In marked contrast, treatment of the dibenzoate **2** with methanol and AG 50W-X8 cation-exchange resin for 24 h under reflux afforded, in ~70% yield, a mixture of two components, as indicated by t.l.c. analysis. The ^{1}H-n.m.r. spectrum at 60 MHz also indicated the formation of two major components, which were tentatively identified as the methyl α- and β-L-glycosides formed by the addition of methanol to the glycal double bond. Complete separation of the two components by column chromatographic fractionation on silica gel was not achieved; however, the first component eluted from the column was identified by its 270-MHz ^{1}H-n.m.r. spectrum (Table I) as methyl 3,4-di-*O*-benzoyl-2,6-dideoxy-α-L-*arabino*-hexopyranoside (**5**). After hydrolysis of the mixture with sodium methoxide, methyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside (**6**) was obtained in ~40% yield from **2**. The 270-MHz ^{1}H-n.m.r.

TABLE I

FIRST ORDER ^{1}H-N.M.R. DATA OF 5–12[a]

Compd.	*Chemical shifts (δ values)*[b]							*Coupling constants (Hz)*								
	H-1	*H-2a*	*H-2e*	*H-3*	*H-4*	*H-5*	*H-6*	$J_{1,2a}$	$J_{1,2e}$	$J_{2a,2e}$	$J_{2a,3}$	$J_{2e,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	*OMe*
5	4.84	1.93	2.48	5.55	5.18	5.05	1.35	2.9	~0.8	−12.9	11.4	5.1	9.6	9.6	6.2	3.37
6	4.73	1.68	2.13	3.89	3.08	3.60	1.29	3.3		−12.5	12.5	5.1	9.2	9.2	6.3	3.22
7	4.80	1.89	2.31	5.33	3.41	3.77	1.36	3.3		−12.5	11.8	5.5	9.5	9.2	6.3	3.35
8[c]	4.80	1.87	2.45	5.57	4.60	3.96	1.41	3.7	~1.0	−12.9	11.4	5.2	9.6	9.6	6.5	3.37
9[d]	4.97	2.26	2.10	5.63–5.53		4.22	1.24	3.0		−12.4	12.4	5.2		<1.0	6.6	3.41
10[e]	4.78	1.77	1.92	4.00	3.63	3.90	1.28	2.9			11.5	5.5	2.9	<1.0	6.6	3.32
11[f]	4.86	2.21	2.05	5.59	3.88	4.07	1.32	3.3	~1.0	−12.2	12.2	5.2	3.3	1.0	6.3	3.35
12[g]	4.84	1.86	2.15	5.44	4.50	4.19	1.18	3.3	~1.0	−12.2	12.2	5.1	3.7	1.0	6.6	3.36

[a]Determined on solutions in chloroform-*d* at 270 MHz. [b]The mean points of the resonances were taken as their chemical shifts, even where second-order features were observed. [c]OMs 2.88. [d]H-2e resonance peaks showed broadening, indicating a possible $J_{1,2e}$ 1.0 Hz. [e]H-2a resonance peaks showed $J_{2a,2e}$ = $J_{2a,3}$ 11.8–13.2 Hz and $J_{1,2a}$ 4.0 Hz, indicating some second-order distortion. [f]$J_{2e,4}$ long-range W coupling ~1.0 Hz. [g]$J_{NH,4}$ 5.90 (10.3 Hz), NAc 2.07.

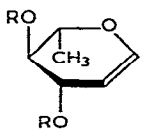
spectrum of **6** (Table I) was in accord with the data recorded previously at 60 MHz for the D isomer[10]; however, whereas the H-1 resonance was previously reported as a doublet of doublets having splittings of $J_{1,2e}$ 1.5 Hz and $J_{1,2a}$ 3.5 Hz, the spectrum recorded for **6** showed H-1 to be a doublet with a splitting of $J_{1,2a}$ 3.3 Hz. Hydrolysis of **6** with 50% aqueous acetic acid gave 2,6-dideoxy-L-*arabino*-hexopyranose (**4**) in 63% yield. Treatment of 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-L-*arabino*-hex-1-enitol (**1**) with acetic acid and acetic anhydride under acidic conditions[11] afforded 1,3,4-tri-*O*-acetyl-2,6-dideoxy-L-*arabino*-hexopyranose (**3**) as a syrupy product in 65% yield. The 270-MHz ^{1}H-n.m.r. spectrum of **3** displayed two H-1 resonance signals in the ratio of 3:4, which were assigned to the α-L and β-L anomers, respectively.

In order to obtain derivatives at C-4 of **6**, selective esterification of OH-3 was a prerequisite to the subsequent introduction of a mesyloxy group. Study of the selective benzoylation of methyl 6-deoxy-α-D-glucopyranosides by Kondo *et al.*[12] clearly indicated that the reactivities of the secondary hydroxyl groups were in the order OH-2 > OH-3 > OH-4. In addition, Marsh *et al.*[5] have reported that selective sulfonylation of methyl 2,6-dideoxy-α-L-*arabino*-hexopyranose occurs principally at O-3. Accordingly, selective benzoylation of **6** was carried out at low temperature, and the 3-benzoate **7** was obtained in 62% yield. The n.m.r. spectrum of **7** (Table I) showed a multiplet resonating at δ 5.33 ($J_{3,4}$ 9.5, $J_{2e,3}$ 5.5, and $J_{2a,3}$ 11.8 Hz), which was assigned to H-3. Further mesylation of **7** gave methyl 3-*O*-benzoyl-2,6-dideoxy-4-*O*-mesyl-α-L-*arabino*-hexopyranoside (**8**).

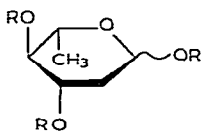
When a sulfonyloxy group is located adjacent to a β-*trans*-axial substituent, its displacement by a nucleophilic group is impaired[13]. For example, it has previously been demonstrated that treatment of methyl 6-deoxy-2,3-*O*-isopropylidene-α-D- (ref. 14) and -α-L-mannopyranoside[15,16] with azide anion gives rise to ring-contraction products, namely methyl 5-azido-5,6-dideoxy-2,3-*O*-isopropylidene-α-D- and -α-L-talofuranosides, respectively, together with smaller proportions of the corresponding allofuranosides. These ring-contraction reactions take place presumably because of the β-*trans*-axial effect[13] between the axial group at C-2 and the methylsulfonyloxy group at C-4, and because of the antiperiplanar relationship between the C-5 ring-oxygen bond and the C-4 sulfonate bond, a configuration well suited for an intramolecular back-side attack at C-4. In the case of methyl 3-*O*-benzoyl-2,6-dideoxy-4-*O*-mesyl-α-L-*arabino*-hexopyranoside (**8**), no axial substituent is at C-2, and therefore we felt confident that nucleophilic displacement would take place at C-4 and not an analogous ring contraction. Accordingly, conversion of the 4-mesylate **8** to the corresponding 3,4-dibenzoate **9**, with inversion of configuration, occurred readily by heating **8** in hexamethylphosphoric triamide with sodium benzoate for ~24 h at 120°; **9** was isolated as a syrup in 53% yield. Evidence supporting the formation of **9** was obtained from the $J_{4,5}$ coupling constant (< 1.0 Hz), whereas the corresponding allo- and talo-furanosides[16] would be expected to have $J_{4,5}$ coupling constants of ~10.0 Hz and 3.5 Hz, respectively.

Although it is generally observed[17–19] that an equatorial proton resonates at a field lower than that of a chemically similar but axially oriented proton, specific

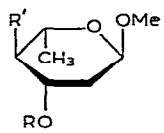
structural and configurational features may cause exceptions to this rule. For example, it was originally suggested by Lemieux and Stevens[20] and later confirmed by Angyal and Pickles[21] that the anomeric forms of D-altropyranose represent such an exception. A similar case was also shown to exist for D-idopyranose[21] and 2-deoxy-D-*erythro*-pentopyranose[20]. The effect of a neighboring carbonyl group, as in steroidal α-haloketones, has also been demonstrated to reverse the usual axial–equatorial relationship[22], as has the effect of an adjacent *syn*-axial acetyl group in *cis*- and *trans*-cyclohexane derivatives[23]. We now report a similar observation for methyl 3,4-di-*O*-benzoyl-2,6-dideoxy-α-L-*lyxo*-hexopyranoside (**9**). The n.m.r. spectrum (Table I) of **5** showed H-2a as a triplet of doublets and H-2e as a doublet of doublets centered at δ 1.93 and δ 2.48, respectively. In the case of **9**, where a *syn*-axial relationship between BzO-4 and H-2a is present, the chemical shifts are reversed, with the net effect being shielding H-2e (δ 2.10) and deshielding H-2a (δ 2.26).



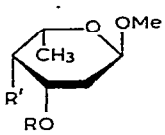
1 R = Ac
2 R = Bz



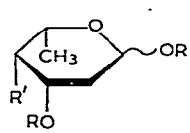
3 R = Ac
4 R = H



5 R = Bz, R' = OBz
6 R = H, R' = OH
7 R = Bz, R' = OH
8 R = Bz, R' = OMs



9 R = Bz, R' = OBz
10 R = H, R' = OH
11 R = Bz, R' = N_3
12 R = Bz, R' = NHAc
13 R = H, R' = NHAc



14 R = H, R' = OH
15 R = Ac, R' = OAc
16 R = H, R' = NHAc
17 R = Ac, R' = NHAc

Hydrolysis of the benzoyl groups of **9** necessitated heating with M methanolic sodium methoxide under reflux for at least 24 h; this procedure afforded **10** in 65% yield. The n.m.r. spectrum (Table I) showed the resonance signals due to H-2a as a triplet of doublets and to H-2e proton as a doublet of doublets centered at δ 1.77 and δ 1.92, respectively ($J_{1,2a}$ 2.9, $J_{2a,2e} = J_{2a,3}$ 11.8–13.2, and $J_{2e,3}$ 5.5 Hz). Despite the second-order nature of the H-2a and H-2e resonances, the respective coupling constants and chemical shifts with H-2e to lower field of H-2a are in agreement with previously reported data[17–19]. In a recent publication[24] of the ^{1}H-n.m.r. spectrum of methyl 2,6-dideoxy-α-L-*lyxo*-hexopyranoside recorded at 300 MHz in

TABLE II

EFFECT OF DERIVATIVES **3**, **4**, AND **14–17** ON THE GROWTH OF P388 LEUKEMIA CELLS IN CULTURE[a]

Compound	*Conc.* (mM)	*Inhib.* (%)
4	0.5	0
14	0.5	0
16	0.5	0
3	0.01	48
15	0.01	45
17	0.1	77

[a]Log phase P388 cells were treated with the indicated concentrations of the various derivatives. The cells were inoculated at a concentration of 10^4 cells/ml in Fischer's medium supplemented with horse serum and incubated at 37°. At 72 h, cell numbers were determined with a model ZBI Coulter counter.

deuterium oxide, the chemical-shift assignments for H-2a and H-2e were reversed from those reported here; however, the highly second-order nature of the spectrum presented precludes any meaningful first-order analysis. Crystalline 2,6-dideoxy-L-*lyxo*-hexopyranose (**14**) was obtained in 67% yield by hydrolysis of **10** in 50% aqueous acetic acid, and acetylation under normal conditions afforded the respective 1,3,4-triacetate **15** as a syrup.

Considerable interest in the 3-amino-2,3,6-trideoxy-L-hexoses has developed in recent years due to their occurrence in natural materials. 3-Amino-2,3,6-trideoxy-L-*lyxo*-hexopyranose (daunosamine) has been found as a constitutent of the glycosidic anthracycline antibiotics: daunomycin[25], adriamycin[26], carminomycin[27], cinerubine A[28], and rhodomycin[29]. The 4-epimeric sugar, 3-amino-2,3,6-trideoxy-L-*arabino*-hexopyranose (acosamine), has been found in the actinoidin group of antibiotics[30], and 3-amino-2,3,6-trideoxy-L-*ribo*-hexopyranose (ristosamine) is a constituent of the antibiotic ristomycin[31]. The chemical syntheses of daunosamine[5,32], acosamine[33], and ristosamine[34] have also been reported. In contrast, the corresponding 4-amino-2,4,6-trideoxy-L-hexoses have received little attention. We now describe the synthesis of such a derivative, namely 4-acetamido-2,4,6-trideoxy-L-*lyxo*-hexopyranose (**16**).

Treatment of the 4-mesylate **8** with sodium azide in hexamethylphosphoric triamide displaced the sulfonyloxy group with inversion of configuration to give **11** ($J_{4,5}$ 1.0 Hz) as an analytically pure syrup in 88% yield. As discussed previously, an equatorial proton normally resonates at a field lower than that of an axial proton at the same location, although exceptions have been observed. Another exception was found in the ^{1}H-n.m.r. spectrum (Table I) of **11**: the anisotropic deshielding effect on H-2a caused by the adjacent *syn*-axial azido group resulted in H-2a (δ 2.21) resonating at a field lower than that of the geminal H-2e proton (δ 2.05).

Hydrogenation of **11** and subsequent *N*-acetylation afforded the corresponding crystalline 4-acetamido derivative **12** in 76% yield. The structure of **12** was supported

by its n.m.r. spectrum (Table I). Removal of the benzoyl group by treatment with methanolic sodium methoxide gave **13** in 86% yield which, upon treatment with aqueous acetic acid, afforded crystalline 4-acetamido-2,4,6-trideoxy-L-*lyxo*-hexopyranose (**16**) in 70% yield. The diacetate **17** was isolated as a syrup upon acetylation.

The free (**4**, **14**, and **16**) and corresponding acetylated (**3**, **15**, and **17**) derivatives were tested for their capacity to inhibit the growth of P388 leukemia cells in culture. The free sugar derivatives, each tested up to a concentration of 0.5mM, did not produce any inhibition of cellular growth (Table II). In contrast, the peracetylated derivatives of 2,6-dideoxy-L-*arabino*-hexopyranose (**3**) and 2,6-dideoxy-L-*lyxo*-hexopyranose (**15**) exhibited significant inhibition of growth at a concentration of 0.01mM. The di-*O*-acetyl derivative of 4-acetamido-2,4,6-trideoxy-L-*lyxo*-hexopyranose (**17**), however, required a significantly higher concentration in order to produce cytotoxicity. The greater inhibitory activity of the peracetates as compared to that of their corresponding free sugars suggests differences in transport properties or cytotoxic mechanisms of action.

EXPERIMENTAL

General methods. — All evaporations were performed under diminished pressure. Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. Hexamethylphosphoric triamide was dried over calcium hydride and then distilled under diminished pressure. Column chromatography was performed on Silica gel, Merck 7734 (70-235 mesh), while all reactions were monitored by t.l.c. on Silica gel G (E. Merck A.G., Darmstadt, Germany). Petroleum ether refers to a fraction having b.p. 35–60°. Elemental analyses and optical rotations were performed by Baron Consulting Company, Orange, CT (U.S.A.).

*1,3,4-Tri-*O*-acetyl-2,6-dideoxy-*L-arabino-*hexopyranose* (**3**). — To a cooled solution of **1** (Pfanstiehl Lab. Inc., Waukegan, IL 60085, U.S.A.; 0.5 g) in acetic acid (20 ml) was added acetic anhydride (20 ml) containing 2% (v/v) of sulfuric acid. Within 3 h, t.l.c. (3:1, v/v, petroleum ether–ethyl acetate) showed complete conversion to a single product. The reaction mixture was diluted with chloroform (100 ml) and extracted with water (2 × 50 ml). The chloroform layer was evaporated and the remaining solvent codistilled with water and then alcohol. Fractionation of the residue on a column of silica gel with 6:1 (v/v) petroleum ether–ethyl acetate as eluent afforded **3** (0.35 g, 65%) as a clear syrup, $[\alpha]_D^{25}$ −98° (*c* 1, chloroform).

Anal. Calc. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.57. Found: C, 52.38; H, 6.52.

*1,5-Anhydro-3,4-di-*O*-benzoyl-2,6-dideoxy-*L-arabino-*hex-1-enitol* (**2**). — *A. From 1,2,3,4-tetra-*O*-benzoyl-*L-*rhamnopyranose.* To a solution of 1,2,3,4-tetra-*O*-benzoyl-L-rhamnopyranose (17 g) in ether (50 ml) was added acetic acid (250 ml). The solution was treated with hydrogen bromide until saturation and then maintained at room temperature for a further 16 h, when t.l.c. (6:1, v/v, petroleum ether–ethyl acetate) showed the reaction to be complete. After the remaining hydrogen bromide was neutralized by the addition of excess sodium acetate, the solution was diluted 10–15%

(v/v) with water and then treated with zinc dust (10 g) with vigorous stirring. T.l.c. (10:1, v/v, petroleum ether–ethyl acetate) indicated the reaction to be complete within 0.5 h and, after filtration, the solution was diluted with chloroform and washed with water. The chloroform layer was evaporated together with water until the remaining acetic acid was removed. The impure syrupy residue was chromatographed on a column of silica gel, and elution with 20:1 (v/v) petroleum ether–ethyl acetate afforded **2** (7.8 g, 79%) as an analytically pure syrup, $[\alpha]_D^{25}$ +230° (*c* 1, chloroform).

B. From **1**. A solution of **1** (1 g) in methanol saturated with ammonia (50 ml) was maintained for 16 h at room temperature, when t.l.c. (2:1, v/v, petroleum ether–ethyl acetate) indicated completion. The solvent was evaporated and chloroform was added and distilled to afford a syrupy residue. This was immediately dissolved in pyridine (50 ml) and, after cooling (−10°), treated with benzoyl chloride until t.l.c. (10:1, v/v, petroleum ether–ethyl acetate) showed the reaction to be complete. The reaction mixture was diluted with water (50 ml) and chloroform (50 ml), and treated with a concentrated aqueous solution of sodium hydroxide until the aqueous layer remained basic. The chloroform layer was evaporated and the remaining pyridine codistilled with water. The impure, syrupy residue was chromatographed on a column of silica gel, and elution with 20:1 (v/v) petroleum ether–ethyl acetate gave **2** (1.167 g, 74%) as a mobile syrup, $[\alpha]_D^{25}$ +239° (*c* 1, chloroform); lit.[7] $[\alpha]_D^{23}$ +221° (*c* 6.1, chloroform).

*Methyl 2,3-di-*O*-benzoyl-2,6-dideoxy-α-*L-arabino-*hexopyranoside* (**5**). — A solution of **2** (1.24 g) in methanol (50 ml) was heated under reflux with AG 50W-X8 cation-exchange resin (0.5 g) for 16 h, when t.l.c. (6:1, v/v, petroleum ether–ethyl acetate) indicated that only partial reaction had taken place. Further resin (1.5 g) was added and the reaction continued for 24 h, when t.l.c. indicated a mixture of products having similar R_F values. After filtration and evaporation, the syrupy residue was chromatographed on a column of silica gel and eluted by the sequential application of petroleum ether containing 1, 2, and 3% (v/v) of ethyl acetate. The first product isolated from the column was shown by its n.m.r. spectrum (Table I) to be the syrupy methyl α-L-glycoside **5** (0.38 g, 28%), $[\alpha]_D^{25}$ −250° (*c* 1, chloroform).

Anal. Calc. for $C_{21}H_{22}O_6$: C, 68.11; H, 5.95. Found: C, 67.87; H, 6.09.

*Methyl 2,6-dideoxy-α-*L-arabino-*hexopyranoside* (**6**). — To a solution of **2** (0.6 g) in methanol (30 ml) was added AG 50W-X8 cation-exchange resin (3.0 g). The reaction mixture was heated under reflux for 20 h, when t.l.c. (10:1, v/v, petroleum ether–ethyl acetate) indicated completion of the reaction. The suspension was filtered, the filtrate evaporated and the residue applied to a silica gel column; elution with 20:1 (v/v) petroleum ether–ethyl acetate afforded a mixture of the α- and β-glycosides (0.426 g, 70%). A methanolic solution (20 ml) was then treated with sodium (0.1 g) and heated under reflux for 0.5 h, when t.l.c. showed two products whose composition was unaffected by further heating. The solution was neutralized with AG 50W-X8 cation-exchange resin and, after filtration and evaporation, the product was extracted between petroleum ether and water to remove the methyl benzoate

formed in the hydrolysis. The aqueous solution was evaporated and the product chromatographed on a silica gel column with 1:1 (v/v) petroleum ether–ethyl acetate as eluent. The first component to be isolated (0.063 g, 22%) was tentatively identified by the 60-MHz n.m.r. spectrum as the methyl β-L-glycoside; n.m.r.: δ 4.60 (dd, $J_{1,2e}$ 3, $J_{1,2a}$ 9.5 Hz, H-1). Further elution with 1:2 (v/v) petroleum ether–ethyl acetate gave the methyl α-L-glycoside **6** (0.121 g, 42%) as an analytically pure syrup, $[\alpha]_D^{25}$ −71° (*c* 1, chloroform); lit.[10] (D series) $[\alpha]_D^{23}$ +87° (*c* 1.2, water).

2,6-Dideoxy-L-arabino-*hexopyranose* (**4**). — A solution of **6** (1.0 g) in water (25 ml) and acetic acid (25 ml) was heated for 3 h at 90°, when t.l.c. (ethyl acetate) showed one major product. The reaction mixture was evaporated, and the syrupy product was eluted from a column of silica gel with ethyl acetate to afford **4** (0.58 g, 63.4%) as a syrup, $[\alpha]_D^{25}$ −98° (*c* 1, chloroform).

Anal. Calc. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.57. Found: C, 52.38; H, 6.52.

Methyl 3-O-benzoyl-2,6-dideoxy-α-L-arabino-hexopyranoside (**7**). — A solution of **6** (3.6 g) in pyridine (200 ml) was maintained below 0° while benzoyl chloride was slowly added. The reaction was monitored by t.l.c. (2:1, v/v, petroleum ether–ethyl acetate) until optimal conversion to a mono-*O*-benzoyl derivative was indicated; the excess reagent was then destroyed by the addition of water. The solution, further diluted with water (200 ml) and chloroform (200 ml), was treated with a concentrated aqueous solution of sodium hydroxide until the aqueous layer remained basic. The chloroform phase was then codistilled with water to remove pyridine. The residue was chromatographed on a column of silica gel with 10:1 (v/v) petroleum ether–ethyl acetate as eluent to give **7** as a syrup (3.64 g, 61.6%), $[\alpha]_D^{25}$ −82° (*c* 1, chloroform).

Anal. Calc. for $C_{14}H_{18}O_5$: C, 63.16; H, 6.77. Found: C, 63.42; H, 6.66.

Methyl 3-O-benzoyl-2,6-dideoxy-4-O-mesyl-α-L-arabino-hexopyranoside (**8**). — To a solution of **6** (10 g) in pyridine (500 ml) cooled to −10° was added benzoyl chloride. The reaction was monitored and subsequently processed, as described for the preparation of **7**. The residue, after addition and evaporation of ethanol and chloroform, was dissolved in pyridine (500 ml), cooled to −10°, and treated with methanesulfonyl chloride (7 g). After maintaining the reaction temperature at 0° for 16 h, t.l.c. (6:1, v/v, petroleum ether–ethyl acetate) indicated completion of the reaction. The solution, diluted with chloroform (200 ml), was extracted with water (200 ml). The chloroform layer was evaporated together with water to remove the residual pyridine. The syrupy product crystallized from ethyl acetate–petroleum ether to afford **8** (8.0 g, 37.7%) as fine, white needles, m.p. 93–95°, $[\alpha]_D^{25}$ −11° (*c* 1, chloroform).

Anal. Calc. for $C_{15}H_{20}O_7S$: C, 52.33; H, 5.81; S, 9.30. Found: C, 52.11; H, 5.91; S, 9.02.

Methyl 3,4-di-O-benzoyl-2,6-dideoxy-α-L-lyxo-hexopyranoside (**9**). — To a solution of **8** (2.0 g) in hexamethylphosphoric triamide (40 ml) was added sodium benzoate (2 g). The reaction mixture was heated at 100° for 18 h, when t.l.c. (10:1, v/v, petroleum ether–ethyl acetate) indicated that ~50% of displacement had taken place. The temperature was raised to ~120° for a further 24 h, at which time t.l.c. indicated

completion of the reaction. The mixture was cooled, diluted with ethyl acetate (100 ml), and extracted with water (2 × 100 ml). The combined aqueous layers were re-extracted with ethyl acetate (50 ml) and the combined organic extracts were evaporated. Fractionation of the product on a column of silica gel with 20:1 (v/v) petroleum ether–ethyl acetate as eluent afforded **9** as a syrup (1.15 g, 53.4%), $[\alpha]_D^{25}$ −164° (*c* 1, chloroform).

Anal. Calc. for $C_{21}H_{22}O_6$: C, 68.11; H, 5.95. Found: C, 67.99; H, 6.09.

*Methyl 2,6-dideoxy-α-*L*-lyxo-hexopyranoside* (**10**). — To a solution of **9** (1.1 g) in methanol (10 ml) was added a solution of methanolic sodium methoxide (0.1M, 10 ml). The reaction mixture was kept for 24 h, when t.l.c. (ethyl acetate) indicated that the reaction was only partially complete. The concentration of sodium methoxide in the solution was increased to 0.1M, and the solution was heated under reflux for a further 24 h, when t.l.c. indicated completion of the reaction. The solution was neutralized with AG 50W-X8 cation-exchange resin and, after filtration and evaporation, the residue was dissolved in water. The solution was extracted with petroleum ether to remove the methyl benzoate formed in the hydrolysis. The aqueous solution was evaporated and the product applied to a column of silica gel. Elution with 2:1 (v/v) petroleum ether–ethyl acetate afforded **10** (0.372 g, 65%) as a syrup. Although the purity of this material was supported by t.l.c. and the n.m.r. spectrum, it failed to give a satisfactory analysis.

*2,6-Dideoxy-*L*-lyxo-hexopyranose* (**14**). — Compound **10** (0.238 g) was hydrolyzed as described for the preparation of **4**. Elution of the product from silica gel with 2:1 (v/v) ethyl acetate–petroleum ether afforded **14** (0.145 g, 66.8%); an analytical sample was obtained upon crystallization from acetone, m.p. 100–101.5°, $[\alpha]_D^{23}$ −75.6° (*c* 1, water); lit.[35] m.p. 103–106°, $[\alpha]_D^{25}$ −61.6° (*c* 1.039, water); lit.[36] m.p. 102–105°, $[\alpha]_D^{25}$ −51.5° (*c* 1.04, water).

The triacetate **15**, prepared with pyridine and acetic anhydride, was isolated as a syrup, $[\alpha]_D^{25}$ −58° (*c* 1, chloroform).

Anal. Calc. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.57. Found: C, 52.86; H, 6.83.

*Methyl 4-azido-3-*O*-benzoyl-2,4,6-trideoxy-α-*L*-lyxo-hexopyranoside* (**11**). — To a solution of **8** (2.0 g) in hexamethylphosphoric triamide (20 ml) was added sodium azide (2.0 g). The reaction mixture was heated to ~120° for 24 h, when t.l.c. (6:1, v/v, petroleum ether–ethyl acetate) showed one major product. The solution was processed as described for the preparation of **9**, and the compound isolated was purified by fractionation on a column of silica gel with petroleum ether containing 2% (v/v) of ethyl acetate. The azide **11** (1.5 g, 86%) was obtained as a syrup, $[\alpha]_D^{25}$ −3° (*c* 1, chloroform).

Anal. Calc. for $C_{14}H_{17}N_3O_4$: C, 57.73; H, 5.84; N, 14.40. Found: C, 57.56; H, 5.59; N, 14.23.

*Methyl 4-acetamido-3-*O*-benzoyl-2,4,6-trideoxy-α-*L*-lyxo-hexopyranoside* (**12**). — A solution of **11** (0.165 g) in ethanol (10 ml) was hydrogenated in the presence of palladium-on-charcoal at 2.4 bar for 1 h; at this time, t.l.c. (6:1, v/v, petroleum ether–ethyl acetate) showed the reaction to be complete. The suspension was filtered and

the filtrate treated with acetic anhydride (0.2 ml). After being kept for 0.5 h, the solution was diluted with water (10 ml) and evaporated to a syrup that was chromatographed on a column of silica gel and eluted with 1:1 (v/v) ethyl acetate–petroleum ether. The 4-acetamido derivative **12** (0.133 g, 76.4%) crystallized from ethyl acetate–petroleum ether, m.p. 179–181°, $[\alpha]_D^{25}$ −100° (*c* 1, chloroform).

Anal. Calc. for $C_{16}H_{21}NO_5$: C, 62.54; H, 6.84; N, 4.56. Found: C, 62.31; H, 6.79; N, 4.43.

*Methyl 4-acetamido-2,4,6-trideoxy-α-*L*-lyxo-hexopyranoside* (**13**). — To a solution of **12** (0.193 g) in methanol (20 ml) was added sodium (0.07 g). The reaction mixture was heated under reflux for 1 h, when t.l.c. (ethyl acetate) indicated completion of the reaction. The solution was neutralized with AG 50W-X8 cation-exchange resin and, after filtration and evaporation, the product was chromatographed on a column of silica gel and eluted with 9:1 (v/v) ethyl acetate–methanol to afford **13** (0.109 g, 86%), which was crystallized from ethyl acetate–petroleum ether, m.p. 158–159°, $[\alpha]_D^{25}$ −55° (*c* 1, chloroform).

Anal. Calc. for $C_9H_{17}NO_4$: C, 53.20; H, 8.37; N, 6.90. Found: C, 53.48; H, 8.64; N, 6.83.

*4-Acetamido-2,4,6-trideoxy-*L*-lyxo-hexopyranose* (**16**). — A solution of **13** (0.157 g) in 50% aqueous acetic acid was heated to 90° when immediate decolorization was observed; after 0.5 h, t.l.c. (9:1, v/v, ethyl acetate–methanol) indicated completion of the reaction. The cooled solution was diluted with water (40 ml) and evaporated to ~10 ml. Ethanol (3 × 40 ml) was added to the solution and evaporated, reducing the volume each time to ~10 ml. The remaining material was diluted with ethanol (40 ml) and heated to reflux in the presence of activated carbon, whereupon, after filtration and evaporation, the residue was chromatographed on a silica gel column with 9:1 (v/v) ethyl acetate–methanol. Evaporation of the eluate afforded crystalline **16** (0.102 g, 70%); an analytical sample was obtained upon recrystallization from acetone, m.p. 148–149.5°, $[\alpha]_D^{25}$ −50° (*c* 1, water).

Anal. Calc. for $C_8H_{15}NO_4$: C, 50.79; H, 7.94; N, 7.41. Found: C, 50.90; H, 8.02; N, 7.34.

The diacetate **17**, prepared with pyridine and acetic anhydride, was isolated as a syrup, $[\alpha]_D^{25}$ −73° (*c* 1, chloroform).

Assay of cell culture activity. — This procedure was carried out as described previously[2]. Briefly, P388 leukemia cells were inoculated into Fischer's medium supplemented with horse serum at an initial concentration of 10^4 cells/ml. Following incubation at 37° for 72 h in the absence and presence of the synthesized compounds, cell numbers were determined with a model ZBI Coulter counter.

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