Synthesis and Biological Activity of Potential Antimetabolites of L-Fucose

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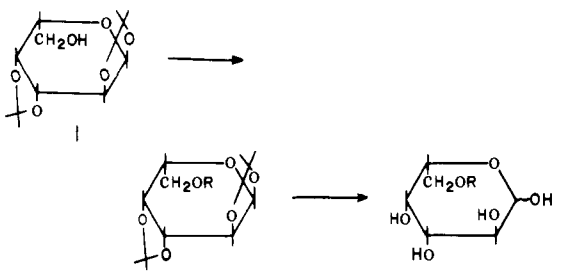
6-Substituted 6-deoxy-L-galactose (L-fucose) derivatives were synthesized as potential antimetabolites of L-fucose. The cytotoxic effects of these compounds were evaluated on P388 leukemia cells in culture. The L-fucose analogues which showed the most potent growth inhibition were the sulfonyl ester, bromo, and iodo derivatives; since these compounds were all capable of alkylation, it is conceivable that their cytotoxic action is a consequence of this property. In agreement with this interpretation, none of the agents synthesized showed specific inhibition of the incorporation of L-[^{3}H]fucose into glycoprotein.

A large number of the most successful anticancer drugs in current clinical use appear to act by inhibiting the biosynthesis of DNA in neoplastic cells; thus, the rapidly proliferating cancers of man are the neoplasms which are most responsive to existing agents. The relative resistance of the more slowly proliferating solid tumors to the presently available chemotherapeutic armamentarium suggests that the development of agents which primarily affect other metabolic processes may yield useful drugs for the treatment of these malignant tumors.

Considerable effort has been expended in the past few years in an attempt to better understand the role of the plasma membrane in cellular behavior and function in both normal and malignant cells. It has become apparent that the plasma membrane is intimately involved in a variety of phenomena which play an important role in cancer.[1-3] These include cell-growth regulation, cell-recognition phenomena, invasion, metastasis, host immune surveillance, and differentiation.[4] Modification of cell-surface components, such as glycoproteins and glycolipids, offers the potential of altering one or more of these properties of tumor cells. Such alteration could be realized conceptually by interference with the biosynthesis of glycoproteins or glycolipids in a manner that leads to the formation of incomplete glycoconjugates (e.g., by inhibition of the biosynthesis of nucleotide sugars or by alteration of glycosyltransferase or glycosidase activities). In addition, incorporation of structural analogues or specific monosaccharides into these macromolecules may provide a means of obtaining changes in glycoprotein and/or glycolipid structure and function. Whether the differences observed[1-3] between the cell-surface components of normal and malignant cells are of sufficient magnitude to permit chemotherapeutic exploitation through such an approach remains to be determined.

In an attempt to ascertain the influence of structural modification of monosaccharides on the properties of neoplastic cells, we have initiated a program designed to develop analogues of L-fucose. A variety of factors makes this carbohydrate appear to be a particularly inviting target for chemotherapeutic modification. These include the following: (a) L-fucose, which appears as a nonreducing terminus in glycoconjugates, is one of the last sugars added to the growing glycopeptide prior to transport to the cell surface;[3-6] (b) exogenous L-fucose can be utilized in glycoprotein and glycolipid synthesis;[7] (c) L-fucose does not enter the glycolytic pathway and, therefore, is not appreciably metabolized to other sugars which might create considerable host toxicity;[8,9] (d) L-fucose caused marked inhibition of the growth of murine 3T3 fibroblasts in culture, which appeared to be analogous to contact inhibition,[10] and a continuous intravenous infusion of this sugar resulted in significant growth inhibition of a transplanted rat mammary tumor and an increase in the L-fucose content of the tumor;[11] (e) significantly elevated levels of fucosyltransferase and fucosidase activities were observed in implanted rat hepatomas relative to liver,[12] indicating that differences in L-fucose metabolism can exist between normal tissues and certain tumors. Elevation of total fucosyltransferase activity in the plasma of cancer patients[13] has recently been shown to be due to a specific elevation of plasma GDP-L-fucose:β-D-galactosyl-α-2-L-fucosyltransferase.[14] The relationship of this enzyme to the increased L-fucose content reported to be present in the serum of patients with certain malignant tumors remains to be delineated.[15]

Scheme I



2a, R = $SO_2C_6H_4$-p-CH_3 4a, R = $SO_2C_6H_4$-p-CH_3
b, R = $SO_2C_6H_5$ b, R = $SO_2C_6H_5$
c, R = SO_2CH_3 c, R = SO_2CH_3

3a, R = CH_3 5a, R = CH_3
b, R = CH_2CH_3 b, R = CH_2CH_3
c, R = $CH_2C_6H_5$ c, R = $CH_2C_6H_5$

L-Fucose is the most lipophilic of the monosaccharides found in glycoproteins and glycolipids. This lipophilicity can be attributed to the presence of a methyl group at position 5 of the pyranose ring. This hydrophobic region of the molecule may play an important role as a binding site or as a determinant of tertiary structure in the interaction of L-fucose-containing glycoproteins with other molecules. Thus, it was of particular interest to prepare a variety of L-fucose analogues with modifications in the methyl group in an effort to evaluate how changes in this region would affect the biological properties of L-fucose in an experimental tumor cell line.

Chemistry. Acetalation of L-galactose[16] using acetone, copper sulfate, and sulfuric acid provided 1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (1). Reaction of 1 with several sulfonyl chlorides gave the sulfonate esters **2a–c**, which, after hydrolysis of the isopropylidene protecting groups with 80% aqueous trifluoroacetic acid, gave the free sugars **4a–c** in good yields (Scheme I). The 6-methyl and 6-ethyl ethers **3a,b** were prepared in good yield from 1 using the appropriate alkyl iodide in the presence of Ag_2O. These alkyl ethers were also prepared using thallium(I)

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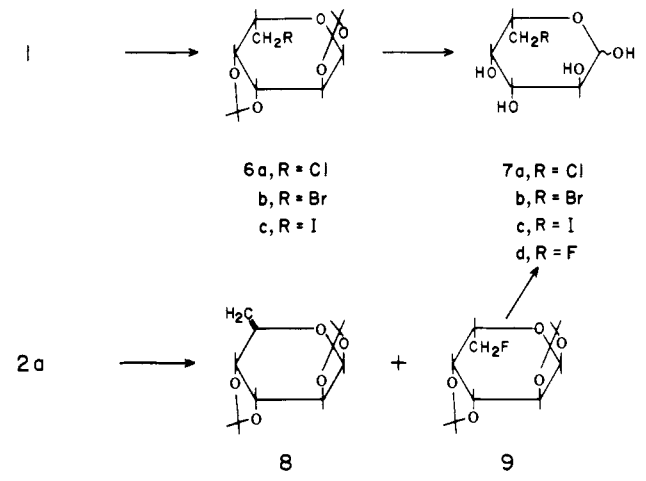
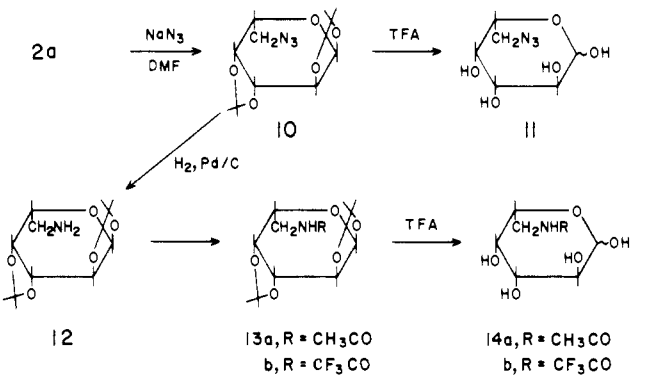
Scheme II



Scheme III

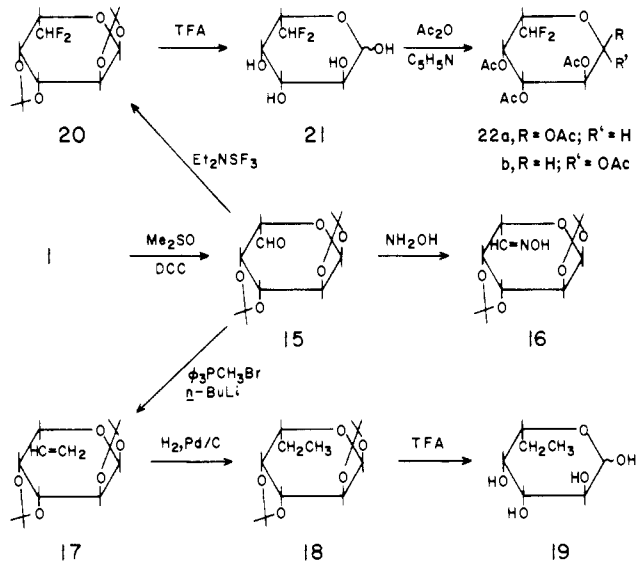


ethoxide/alkyl halide but only in very low yield. The 6-benzyl ether **3c** was formed from 1 using benzyl chloride and NaH in Me_2SO. Deacetalation of **3a–c** with trifluoroacetic acid provided the corresponding free sugars **5a–c**.

Treatment of **2a** with tetrabutylammonium fluoride in MeCN gave a mixture of the desired 6-fluoro derivative **9** and the elimination product **8** in approximately a 2:1 ratio, with a small quantity of unreacted **2a** remaining (Scheme II). Chromatographic separation of this mixture on silica gel, followed by removal of the acetal groups from **9**, gave 6-deoxy-6-fluoro-L-galactose (**7d**). The R_f values observed for **8** and **9** (0.51 and 0.53, respectively) on silica gel using a mixture of cyclohexane and AcOEt (4:1) as solvent were very similar. However, hydrolysis of the isopropylidene groups of a mixture of **8** and **9**, followed by chromatographic separation of the products, provided a better route for the isolation of **7d**. The 6-chloro derivative **6a** was prepared from 1 in good yield using sulfuryl chloride in pyridine. The 6-bromo compound **6b** was obtained from 1, using the triphenylphosphine/*N*-bromosuccinimide procedure of Hanessian and co-workers.[17] Treatment of 1 with the Rydon reagent[18,19] methyltriphenoxyphosphonium iodide in DMF gave the 6-iodo derivative **6c**. Hydrolysis of these 6-halo intermediates (**6a–c**) with trifluoroacetic acid gave the free sugars **7a–c**.

Reaction of **2a** with sodium azide in DMF gave the 6-azido derivative **10** (Scheme III), which was deprotected as described above to yield 6-azido-6-deoxy-L-galactose (11). Catalytic hydrogenation of **10** provided the 6-amino sugar **12**. Acylation of **12** with the appropriate anhydride

Scheme IV



gave the 6-acetamido and the 6-(trifluoroacetamido) derivatives **13a** and **13b**, respectively. Removal of the isopropylidene groups from **13a,b** produced the free sugars **14a,b**.

Oxidation of 1 with a mixture of Me_2SO and DCC in the presence of pyridinium hydrochloride[20] provided the corresponding aldehyde 1,2:3,4-di-*O*-isopropylidene-α-L-*galacto*-hexodialdo-1,5-pyranose (**15**), as shown in Scheme IV. This reaction did not go to completion, which resulted in the presence of a small amount of 1 following initial purification by distillation; however, this small quantity of 1 did not interfere substantially with the subsequent reaction, eliminating the need for further purification. Characterization of **15** was attained by conversion to the oxime **16**. Reaction of the aldehyde **15** with methylenetriphenylphosphorane, generated from methyltriphenylphosphonium bromide using methylsulfinyl carbanion in Me_2SO, failed to give the desired product. However, when the Wittig reagent methylenetriphenylphosphorane was generated from methyltriphenylphosphonium bromide using *n*-butyllithium in Et_2O and reacted with **15**, the desired product **17** was obtained. Catalytic hydrogenation of **17** gave a near quantitative yield of 6,7-dideoxy-1,2:3,4-di-*O*-isopropylidene-α-L-*galacto*-heptopyranose (**18**), which, following removal of the protecting groups with trifluoroacetic acid, gave 6,7-dideoxy-L-*galacto*-hepto-1,5-pyranose (**19**).

Reaction of **15** with freshly prepared diethylaminosulfur trifluoride under the mild conditions reported for the conversion of aldehyde or ketone carbonyl groups to the corresponding geminal difluoride[21] provided 6-deoxy-6,6-difluoro-1,2:3,4-diisopropylidene-α-L-galactopyranose (**20**) in good yield.[22] Deprotection of **20** with aqueous trifluoroacetic acid gave the desired 6-deoxy-6,6-difluoro-L-galactopyranose (**21**) as a crystalline solid. Acetylation of **21** produced a mixture of peracetylated products; separation of this mixture by column chromatography provided crystalline 6-deoxy-6,6-difluoro-1,2,-3,4-tetra-*O*-acetyl-α-L-galactopyranose (**22a**) and an inseparable mixture consisting of 6-deoxy-6,6-difluoro-1,2,3,4-tetra-*O*-acetyl-β-L-galactopyranose (**22b**), 6-deoxy-6,6-difluoro-1,2,3,5-tetra-*O*-acetyl-α-L-galactofuranose (**23a**), and 6-deoxy-6,6-difluoro-1,2,3,5-tetra-*O*-acetyl-β-L-galactofuranose (**23b**).

It is interesting to note the long-range fluorine–proton coupling observed in the ^1H NMR spectrum of **20** between

Table I. Effects of Some 6-Substituted L-Fucose Derivatives on the Growth of P388 Leukemia Cells in Culture[a]

		% inhibition			
no.	6-substit	1 × 10^{-3} M	5 × 10^{-4} M	1 × 10^{-4} M	1 × 10^{-5} M
	OH	10		16	14
4a	$OSO_2C_6H_4$-p-CH_3	96	94	25	14
4b	$OSO_2C_6H_5$	97	95	52	
4c	OSO_2CH_3	96	86	56	
5a	OCH_3	73		16	12
5b	OC_2H_5	23	31	25	
5c	$OCH_2C_6H_5$	26	21	17	
7a	Cl	15		10	10
7b	Br	97	87	33	
7c	I	91	71	44	
7d	F	8		10	3
11	N_3	20		12	7
14a	$NHCOCH_3$	16		9	15
14b	$NHCOCF_3$	36		14	7
19	CH_3	2			
21	F_2	0	0	0	

[a] P388 leukemia cells (1.1 × 10^4 cells/mL) were incubated with various concentrations of 6-substituted L-fucose derivatives at 37 °C in Fischer's medium containing 10% horse serum. Seventy-two hours later, cell numbers were determined in triplicate using a Coulter electronic particle counter.

F(6) and H(1) ($^5J_{F(6),H(1)}$ = 1.8 Hz) and between F(6) and H(3) ($^5J_{F(6),H(3)}$ = 1.3 Hz); no fluorine–proton coupling was observed for H(2). Fluorine coupling to other protons was not discernable due to poorly resolved multiplets. Similar long-range coupling was not seen for **21** or **22a,b**.

Biology. The compounds synthesized in this study were evaluated for their effects on the growth of P388 leukemia cells in culture (Table I). The L-fucose analogues which showed the most potent growth inhibition were the sulfonyl esters **4a–c** and the bromo and iodo derivatives **7b** and **7c**, respectively. Some growth inhibition was observed for the methyl ether **5a** but only at a relatively high concentration. None of the other deprotected sugars showed significant cytotoxicity. The compounds which produced demonstrable growth inhibition have the potential to act as alkylating agents, suggesting that their cytotoxic action might be a consequence of this property.

Recent reports[23–27] have demonstrated an increased cytotoxicity for peracetylated carbohydrates relative to that of the corresponding free sugars. The peracetylated derivative of a representative member of the present series, **21**, which displayed no cytotoxicity, was evaluated for growth inhibition. Both **22a** and the mixture of **22b** and the hexofuranoses **23a,b** showed a similar level of growth inhibition of P388 leukemia cells in culture at 2.5 × 10^{-4} M of 89% and 87%, respectively. The similar cytotoxicity produced by these peracetylated derivatives of **21** suggests that the compounds are serving as precursors of a common active species, most probably the free sugar generated by hydrolytic cleavage of the acetyl groups, possibly after transport of the peracetylated derivatives into the neoplastic cells.

It was also of interest to determine if the free sugars had effects on the incorporation of exogenous L-fucose into cellular glycoproteins. To accomplish these measurements, the effects of the various analogues at concentrations of 10^{-4} or 10^{-3} M on the incorporation of L-[^{3}H]fucose and [^{14}C]glucosamine (employed as a control for nonspecific effects) into glycoproteins of P388 leukemia cells were evaluated in culture. None of the free sugars reported in this study showed specific inhibition of the incorporation of radioactive L-fucose into glycoprotein under the conditions employed, even at high levels of analogue relative to that of radioactive fucose. This finding suggests that the analogues did not function as antimetabolites of L-fucose and implies a high degree of specificity in the hydrophobic portion of L-fucose which does not permit much molecular modification in this region.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses and optical rotations were performed by Baron Consulting Co., Orange, Conn. Where analyses are indicated only by symbols of the elements, the analytical results for those elements were within ±0.4% of theoretical values. Evaporations were performed under reduced pressure at 40 °C with a rotary evaporator, unless otherwise stated. TLC was conducted on glass plates coated (0.25 mm) with silica gel (Anasil G, Analabs), and compounds were visualized by treatment with sulfuric acid followed by heating. Column chromatography was carried out using silica gel (EM Merck 7729 or 7734). Proton magnetic resonance spectra were obtained with Varian T60 and Bruker 270HX spectrometers (solutions in Me_2SO-d_6, pyridine-d_5, or $CDCl_3$ with Me_4Si as internal standard) with chemical shift values reported in δ relative to the internal standard; NMR data were compatable with the proposed structures (see paragraph at the end of this paper concerning Supplemental Material). Radioactivity was determined with a Packard scintillation spectrometer.

1,2:3,4-Di-*O*-isopropylidene-α-L-galactopyranose (1). To a suspension of L-galactose[16] (11 g, 61 mmol) and anhydrous $CuSO_4$ (24 g) in Me_2CO (300 mL) was added concentrated H_2SO_4 (1.1 mL) and this mixture was stirred at room temperature for 36 h. The reaction mixture was filtered through Celite and the filter pad washed well with Me_2CO. The filtrate was treated with $NaHCO_3$ (65 mL of a saturated aqueous solution) and the mixture was evaporated. The residue was extracted with $CHCl_3$ (4 × 150 mL), and the combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to an amber syrup (14.5 g). This syrup was dissolved in a minimal amount of $CHCl_3$ and applied to a silica gel column (Merck 7734, 6.5 × 28 cm), which was eluted with $CHCl_3/Me_2CO$ (18:3, v/v). Fractions containing **3** were combined and evaporated to a pale amber syrup: yield 9.1 g (58%); $[\alpha]^{25}_D$ −55.0° (*c* 1.00, $CHCl_3$), [lit.[28] for D derivative $[\alpha]_D^{29}$ -55° (*c* 3.6, $CHCl_3$)]. Anal. ($C_{12}H_{20}O_6$) C, H.

1,2:3,4-Di-*O*-isopropylidene-6-*p*-toluenesulfonyl-α-L-galactopyranose (2a). A solution of 1 (6.1 g, 23.4 mmol) in pyridine (40 mL) was cooled (0–5 °C) and *p*-toluenesulfonyl chloride (5.5 g, 29 mmol) was added. The solution was warmed to ambient temperature and stirred for 15 h. The reaction mixture was cooled and H_2O (8 mL) was added. The resulting clear solution was poured into an ice–water mixture, and, after standing (ca. 1 h), the crystalline solid was collected by filtration, washed with H_2O (75 mL), dissolved in toluene, and evaporated to a syrup, which was coevaporated with toluene until no pyridine remained. The addition of EtOH (5 mL) resulted in crystallization. Recrystallization from EtOH gave 8.4 g (86%) of **2a**: mp 86–88 °C; $[\alpha]^{25}_D$ −112° (*c* 1.00, $CHCl_3$). Anal. ($C_{19}H_{26}O_8S$) C, H.

1,2:3,4-Di-*O*-isopropylidene-6-benzenesulfonyl-α-L-galactopyranose (2b). A solution of 1 (520 mg, 2 mmol) in pyridine (2 mL) was treated with freshly distilled benzenesulfonyl chloride (0.28 mL, 2.2 mmol) as described for **2a** but at a temperature of 7 °C. The resulting syrup was purified by silica gel column chromatography (Merck 7734, 3.5 × 15 cm) to give **2b** as a viscous syrup, which resisted crystallization: yield 750 mg (93%); $[\alpha]^{25}_D$ −49.4° (*c* 1.00, $CHCl_3$). Anal. ($C_{18}H_{29}O_8S$) C, H.

1,2:3,4-Di-*O*-isopropylidene-6-methanesulfonyl-α-L-galactopyranose (2c). A solution of 1 (520 mg, 2.0 mmol) in pyridine (2 mL) was treated with methanesulfonyl chloride (0.15 mL, 2 mmol) as described for **2a** but at a temperature of 7 °C. The resulting solid was recrystallized from EtOH to give **2c**: yield 500 mg (83%); mp 118–120 °C; $[\alpha]^{25}_D$ +83° (*c* 1.00, $CHCl_3$). Anal. ($C_{13}H_{22}O_8S$) C, H.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-methyl-α-L-galactopyranose (3a). A solution of 1 (1.04 g, 4.0 mmol) in MeI (33 mL) to which Ag_2O (3.1 g) had been added was stirred at ambient

temperature. After 2.5 days, an additional quantity of Ag_2O (1.1 g) and MeI (10 mL) was added and stirring was continued for 5.5 days. The reaction mixture was filtered, the separated solid material was washed with AcOEt, and the filtrate was evaporated to a syrup. This syrup was purified by silica gel (Merck 7734) column chromatography using cyclohexane/AcOEt (4:1, v/v) as eluent, which provided 700 mg (64%) of **3a** as a syrup: $[\alpha]^{25}_D$ +71.6° (*c* 1.00, $CHCl_3$). Anal. ($C_{13}H_{22}O_6$) C, H.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-ethyl-α-L-galactopyranose (3b). A solution of 1 (520 mg, 2.0 mmol) in EtI (15 mL) was treated with Ag_2O (1.2 g followed by 0.8 g) as described for **3a**. Column chromatography provided 525 mg (86%) of **3b** as a colorless syrup: $[\alpha]^{25}_D$ +65° (*c* 1.00, $CHCl_3$). Anal. ($C_{14}H_{24}O_6 \cdot 0.25H_2O$) C, H.

6-*O*-Benzyl-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (3c). A solution of 1 (780 mg, 3.0 mmol) in Me_2SO (1.5 mL) was added to a suspension of NaH (200 mg of a 50% oil dispersion washed with C_6H_6) in Me_2SO (3.5 mL). This mixture was stirred at ambient temperature for 1 h, at which point benzyl chloride was added and stirring was continued for 20 h. The reaction mixture was poured into an ice–water mixture (50 mL) and the resulting syrup extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ extracts were concentrated to a small volume (ca. 3 mL), which was diluted with AcOEt (40 mL). The AcOEt solution was extracted with H_2O (3 × 10 mL), dried (Na_2SO_4), and evaporated to a syrup. The syrup was dissolved in $CHCl_3$ (1 mL) and applied to a silica gel column (Merck 7734, 2.5 × 20 cm), which was eluted with $CHCl_3/Me_2CO$ (99:1, v/v). Fractions containing **3c** were combined to afford 675 mg (65%) of **3c** as a syrup: $[\alpha]^{25}_D$ +71.1° (*c* 1.00, $CHCl_3$). Anal. ($C_{19}H_{26}O_6$) C, H.

6-Chloro-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (6a). Sulfuryl chloride (0.7 mL) was added to a solution of 1 (1.5 g, 5.7 mmol) in pyridine (10 mL) which had been cooled to 5 °C, and this solution was stirred at 4 °C for 18 h. Water (25 mL) was slowly added to the reaction mixture, which was allowed to warm to room temperature. This solution was extracted with $CHCl_3$ (5 × 10 mL), and the combined fractions were extracted with H_2O (3 × 5 mL), dried (Na_2SO_4), and evaporated to a syrup which was coevaporated with toluene. The resulting residue was dissolved in a mixture of cyclohexane/AcOEt (4:1, v/v), applied to a silica gel column (Merck 7734, 2.5 × 8 cm), and eluted with the same solvent. The fractions containing **6a** were combined and evaporated to provide 900 mg (57%) of a syrup: $[\alpha]^{25}_D$ +61.8° (*c* 1.00, $CHCl_3$). Anal. ($C_{12}H_{19}ClO_5$) C, H, Cl.

6-Bromo-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (6b). To a solution of 1 (1.3 g, 5 mmol) in DMF at 5 °C was added *N*-bromosuccinimide (930 mg, 5.2 mmol) followed by Ph_3P (1.4 g, 5.2 mmol) in several portions. This solution was heated at 70 °C for 2 h. Methanol (10 mL) was added to the warm solution which was then evaporated to a dark syrup and dissolved in AcOEt (40 mL). This solution was filtered, and the filtrate was extracted with saturated aqueous $NaHCO_3$ (3 × 20 mL) and H_2O (3 × 25 mL). The dried Na_2SO_4 solution was evaporated to a residue which was triturated with Et_2O (10 mL) and the Ph_3PO was removed by filtration. The filtrate was evaporated to a syrup and dissolved in warm cyclohexane, and the additional quantity of Ph_3PO which crystallized was removed by filtration. The filtrate was evaporated to a syrup, dissolved in a minimal amount of cyclohexane, applied to a silica gel column (3.5 × 10 cm), and eluted with cyclohexane/AcOEt (6:1, v/v). The appropriate fractions were combined and evaporated to provide 1.3 g (81%) of **6b** as a syrup: $[\alpha]^{25}_D$ +95.5° (*c* 1.00, $CHCl_3$). Anal. ($C_{12}H_{19}BrO_5$) C, H, Br.

6-Deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (6c). A solution of 1 (3.4 g, 13 mmol) and methyltriphenoxyphosphonium iodide[18,19] (5.2 g, 12.3 mmol) in DMF (45 mL) was stirred at ambient temperature for 9 h. Methanol (5 mL) was added and the mixture diluted with AcOEt (100 mL). This solution was extracted with 10% aqueous $Na_2S_2O_3$ (3 × 25 mL) and H_2O (3 × 25 mL) and dried (Na_2SO_4). Evaporation provided a syrup, which was dissolved in cyclohexane, applied to a silica gel column (Merck 7734, 3.5 × 19 cm), and eluted with a mixture of cyclohexane/AcOEt (9:1, v/v). Tubes containing **6c** were combined and evaporated to a thin syrup, which was dissolved in toluene and extracted with a 10% solution of NaOH (5 × 25 mL) and H_2O (3 × 25 mL), dried (Na_2SO_4), and evaporated to a syrup which crystallized to provide 3.5 g (72%) of **6c**: mp 56–58 °C, $[\alpha]^{25}_D$ +69° (*c* 1.00, $CHCl_3$). Anal. ($C_{12}H_{19}IO_5$) C, H; I: calcd, 34.32; found, 34.77.

6-Deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (9). To a solution of tetrabutylammonium fluoride (prepared from 15 g of the clathrate) in MeCN (35 mL) was added **2a** (1.25 g, 3.02 mmol), and this solution was heated at reflux temperature for 24 h. The reaction mixture was diluted with $CHCl_3$ (60 mL), and then H_2O (50 mL) was added. The $CHCl_3$ layer was removed and the aqueous layer extracted with $CHCl_3$ (3 × 20 mL). The combined fractions were extracted with H_2O (3 × 2.5 mL), dried (Na_2SO_4), and evaporated to a dark syrup. A solution of this syrup in AcOEt was evaporated in the presence of silica gel (2.5 g). This mixture was placed on a silica gel column (Merck 7729, 3.5 × 10 cm), which was eluted with a mixture of cyclohexane/AcOEt (5:1, v/v) to provide a partial separation of **8** and **9** [R_f values of 0.51 and 0.54, respectively, on silica gel TLC developed in cyclohexane/AcOEt (4:1, v/v)]. Fractions containing the unresolved mixture of **8** and **9** were rechromatographed on a second column.

Evaporation of the combined fractions containing only the initial compound to be eluted provided 6-deoxy-1,2:3,4-di-*O*-isopropylidene-D-*arabino*-hex-5-enopyranose (**8**): yield 200 mg (25%); mp 75 °C; $[\alpha]^{25}_D$ +129.2° (*c* 1.00, $CHCl_3$). Anal. ($C_{12}H_{18}O_5$) C, H.

Fractions containing only the second compound to be eluted were combined and evaporated to provide **9** as a syrup: yield 430 mg (35%); $[\alpha]^{25}_D$ +44.7° (*c* 1.00, $CHCl_3$). Anal. ($C_{12}H_{19}FO_5$) C, H, F.

6-Azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (10). Sodium azide (2.6 g, 40 mmol) was added to a solution of **2a** (4.14 g, 10 mmol) in DMF (70 mL), and this mixture was heated at 125 °C for 15 h. The reaction was cooled and filtered through Celite, the filtrate was concentrated to 25 mL, and H_2O (50 mL) was added. The aqueous solution was extracted with $CHCl_3$ (4 × 25 mL), and the combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to an amber syrup. This syrup was dissolved in a minimal amount of cyclohexane/AcOEt (85:15, v/v), applied to a silica gel column (Merck 7734, 4.5 × 10 cm), and eluted with the same solvent. The fractions containing **10** were combined and evaporated to a syrup: yield 2.6 g (94%); $[\alpha]^{25}_D$ +103.4° (*c* 1.00, $CHCl_3$). Anal. ($C_{12}H_{19}N_3O_5$) C, H, N.

6-Amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (12). A solution of **10** (1.1 g, 3.8 mmol) in MeOH (30 mL) was hydrogenated at 35 psi in the presence of 10% palladium on carbon (300 mg) for 2 h. Celite was added and the mixture was filtered. The filtrate was evaporated to a syrup which was dissolved in a minimal amount of $CHCl_3$, applied to a silica gel column (Merck 7729, 2.5 × 19 cm) and eluted with $CHCl_3$/MeOH (85:15, v/v). The appropriate fractions were combined and evaporated to provide **12** as a pale amber syrup: yield 900 mg (90%); $[\alpha]^{25}_D$ +48.6° (*c* 1.00, CH_3OH). Anal. ($C_{12}H_{21}NO_5$) C, H, N.

6-Acetamido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (13a). Acetic anhydride (0.55 mL) was added to a solution of **12** (350 mg, 1.3 mmol) in pyridine (2 mL) at 5 °C and stirred at room temperature for 3 h. Water (0.5 mL) was added and the solution was evaporated to a syrup, which was coevaporated with toluene. The syrup was dissolved in $CHCl_3$ and applied to a silica gel column (Merck 7734, 2.5 × 12 cm), which was eluted with $CHCl_3$/EtOH (39:1, v/v). Combination and subsequent evaporation of the appropriate fractions gave 260 mg (60%) of **13a** as a syrup: $[\alpha]^{25}_D$ +8.4° (*c* 1.00, $CHCl_3$). Anal. ($C_{14}H_{23}NO_6$) C, H, N.

6-Deoxy-1,2:3,4-di-*O*-isopropylidene-6-(trifluoroacetamido)-α-L-galactopyranose (13b). Trifluoroacetic anhydride (0.50 mL) was added to a solution of **12** (300 mg, 1.16 mmol) in pyridine (1.8 mL) and treated as described for **13a**. Column chromatography ($CHCl_3/Me_2CO$, 19:1, v/v) provided 320 mg (78%) of **13b**: $[\alpha]^{25}_D$ +15.9° (*c* 1.00, pyridine). Anal. ($C_{14}H_{20}F_3NO_6$) C, H, F, N.

1,2:3,4-Di-*O*-isopropylidene-α-L-*galacto*-1,5-dialdohexopyranose (15). The procedure followed was essentially that of Howarth et al.[20] A solution of 1 (6.5 g, 25 mmol) in Me_2SO (50 mL) was added to a flask containing pyridinium chloride (25 mmol, freshly prepared from pyridine and anhydrous HCl) and

Table II. Physicochemical Data for 6-Substituted L-Galactopyranoses

no.	$[\alpha]^{25}_D$,[a]	mp, °C	yield, %	purification[e]	formula	anal.
4a	−18.0	108–110	86	A, EtOH	$C_{13}H_{19}O_8S\cdot 1.0C_2H_5OH$	C, H
4b	−68.7[b]	112–114	83	A, EtOH	$C_{12}H_{16}O_8S\cdot 1.0H_2O$	C, H
4c	−41.7[b]	d	81	B (3:1)	$C_7H_{14}O_8S$	C, H
5a	−80.6[b]	122–125	95	B (4:1)	$C_7H_{14}O_6$	C, H
5b	−78.5[b]	d	91	B (4:1)	$C_8H_{16}O_6\cdot 1.0H_2O$	C, H
5c	−47.0[b]	71–73	95	B (5:1)	$C_{13}H_{18}O_6\cdot 1.0H_2O$	C, H
7a	−54.6	118–120	51	B (4:1)	$C_6H_{11}ClO_5$	C, H, Cl
7b	−65.7[b]	d	85	B (5:1)	$C_6H_{11}BrO_5\cdot 0.5H_2O$	C, H
7c	−56.5	106–108	70	A, EtOH	$C_6H_{11}IO_5\cdot 1.0C_2H_5OH$	C, H, I[f]
7d	−74.0	164–166	72	B (4:1)	$C_6H_{11}FO_5$	C, H, F[g]
11	−56.4	135–137	78	A, MeOH	$C_6H_{11}N_3O_5$	C, H, N
14a	−71.7	50–52	74	B (5:3)	$C_8H_{15}NO_6$	C, H, N
14b	−43.0	d	92	B (3.5:1)	$C_8H_{12}FNO_6$	C, H, F, N
19	−26.2[b]	d	81	B (4:1)	$C_7H_{14}O_5\cdot 0.75H_2O$	C, H
21	−82.0[c]	125 dec	85	A, Me_2CHOH	$C_6H_{10}F_2O_5$	C, H, F

[a] At equilibrium at c 1.00 in H_2O. [b] At equilibrium at c 1.00 in pyridine. [c] c 0.87, H_2O. [d] Syrup. [e] A, recrystallization from solvent indicated; B, chromatographed on silica gel with a mixture of $CHCl_3$/MeOH as indicated. [f] I: calcd, 37.80; found, 38.22. [g] F: calcd, 10.44; found, 10.94.

this mixture was stirred at room temperature until dissolution was complete. DCC (15.5 g, 75 mmol) was added and the resulting mixture was stirred at room temperature for 20 h. A solution of oxalic acid (6.5 g) in MeOH (20 mL) was added and the reaction was stirred at room temperature until cessation of gas evolution. The reaction mixture was poured into H_2O saturated with NaCl (250 mL) and filtered. The solids were washed with H_2O (60 mL), the filtrate was extracted with $CHCl_3$ (5 × 70 mL), and the combined $CHCl_3$ fractions were extracted with H_2O (3 × 50 mL), dried (Na_2SO_4), and evaporated to an amber syrup. Distillation of this crude syrup provided 4.7 g (73%) of **15** containing a trace of **1** as indicated by thin-layer chromatography.

1,2:3,4-Di-*O*-isopropylidene-α-L-*galacto*-1,5-dialdohexopyranose 6-Oxime (16). A solution of **15** (500 mg) in MeOH (5 mL) was mixed with a solution of $NH_2OH\cdot HCl$ (200 mg, 2 mmol) in aqueous pyridine, and the resulting mixture was heated at reflux temperature for 7 h. Evaporation of the reaction mixture provided a syrup which dissolved in toluene. This solution was extracted sequentially with H_2O (2 × 10 mL), 0.25 N H_2SO_4 (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and H_2O (3 × 10 mL). The solution was dried (Na_2SO_4) and evaporated to a syrup which crystallized from hexanes. Recrystallization from cyclohexane provided 210 mg (40%) of **16**: mp 108–109 °C; $[\alpha]^{25}_D$ +128° (c 1.00, $CHCl_3$). Anal. ($C_{12}H_{19}NO_6$) C, H, N.

6,7-Dideoxy-1,2:3,4-di-*O*-isopropylidene-α-L-*galacto*-hept-6-enopyranose (17). To a suspension of methyltriphenylphosphonium bromide (5.18 g, 14.5 mmol) in Et_2O (75 mL) was added slowly with stirring a solution of *n*-BuLi in hexane (9.1 mL of a 1.6 M solution, 14.5 mequiv). The resulting solution was stirred at room temperature for 4 h under a stream of nitrogen. A solution of **15** (2.56 g, 9.8 mmol) in Et_2O (15 mL) was slowly added to the methylenetriphenylphosphorane solution and the resulting mixture was stirred for 5 h at room temperature. The reaction mixture was filtered and the separated salts were washed with Et_2O (50 mL). Evaporation of the filtrate gave a dark syrup which dissolved in Et_2O (15 mL), and the crystalline Ph_3PO was removed by filtration. The filtrate was evaporated and a $CHCl_3$ solution of the residual syrup was filtered through silica gel (Merck 7734). The solvent was removed and the residual syrup was dissolved in a minimal amount of $CHCl_3$ and applied to a silica gel column (Merck 7729, 3.5 × 15 cm) and eluted with a mixture of $CHCl_3$/AcOEt (98:2, v/v). Fractions containing only **17** were combined and evaporated to provide 830 mg (33%) of a thin syrup: $[\alpha]^{25}_D$ +110.5° (c 1.00, $CHCl_3$). Anal. ($C_{13}H_{20}O_5$) C, H.

6,7-Dideoxy-1,2:3,4-di-*O*-isopropylidene-α-L-*galacto*-heptopyranose (18). A solution of **17** (250 mg, 0.97 mmol) was dissolved in EtOH (7 mL) and hydrogenated at 40 psi in the presence of 10% palladium on carbon (50 mg) for 4 h. The reaction mixture was filtered through Celite and the filtrate was evaporated. The resulting syrup was dissolved in $CHCl_3$ (10 mL) and filtered. Evaporation of the filtrate provided 230 mg (92%) of **18**: $[\alpha]^{25}_D$ −45.8° (c 1.00, $CHCl_3$). Anal. ($C_{13}H_{22}O_5$) C, H.

6-Deoxy-6,6-difluoro-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (20). To a solution of **15** (1.16 g, 4.5 mmol) in CH_2Cl_2 (18 mL) under a dry nitrogen atmosphere was added diethylaminosulfur trifluoride[21] (1.2 mL, 2.1 equiv) dropwise (10 min). The resulting solution was stirred at ambient temperature (ca. 22 °C) for 24 h; TLC [cyclohexane/AcOEt (4:1); **15**, R_f 0.2; **20**, R_f 0.7] indicated the presence of only a small quantity of **15**. Water (5 mL) was slowly added to the reaction mixture, resulting in a slight warming of the solution. The organic layer was separated, washed with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL) and H_2O (3 × 5 mL), dried (Na_2SO_4), and evaporated to a dark syrup. This syrup was dissolved in 4 mL of a mixture of cyclohexane/AcOEt (4:1, v/v) and the insoluble material was removed by filtration. The filtrate was evaporated and the resulting syrup was redissolved in a minimal amount of a mixture of cyclohexane/AcOEt (5:1, v/v). This solution was applied to a silica gel column (Merck 7729, 2.5 × 15 cm, packed dry) and eluted with the same solvent. Fractions containing only the desired product were combined and evaporated to a pale amber syrup, which crystallized to give **20**: yield 775 mg (62%); mp 47–49 °C; $[\alpha]^{25}_D$ −40.1° (c 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.84 (1 H, qd, $J_{5,6}$ = 6.6 Hz, J_{6,F_a} = 57.5 Hz, J_{6,F_b} = 54.0 Hz, H-6), 5.56 (1 H, dd, $J_{1,2}$ = 4.9 Hz, $^5J_{1,F}$ = 1.8 Hz, H-1), 4.65 (1 H, qd, $J_{3,4}$ = 7.7 Hz, $^5J_{3,F}$ = 1.3 Hz, H-3), 4.36 (2 H, m, H-2, H-4), 3.90 (1 H, m, H-5), 1.55, 1.46, 1.35, 1.34 (12 H, 4 s, isopropylidene methyls). Anal. ($C_{12}H_{18}F_2O_5$) C, H; F: calcd, 13.57; found, 13.99.

Preparation of Peracetylated 6-Deoxy-6,6-difluoro-L-galactoses. A solution of **20** (350 mg, 1.25 mmol) in 80% trifluoroacetic acid was stirred at room temperature for 1 h; evaporation of the reaction mixture gave a syrup which was coevaporated with H_2O (3 × 5 mL) and toluene (3 × 5 mL). The resulting syrup was dissolved in pyridine (4 mL) and cooled (4 °C), and acetic anhydride (1.5 mL) was added. This solution was stirred at 4 °C for 30 h. The reaction mixture was poured into an ice/water mixture, and the pasty residue was extracted into $CHCl_3$ (50 mL). The $CHCl_3$ layer was extracted sequentially with 0.5 N H_2SO_4 (3 × 10 mL), $NaHCO_3$ (3 × 10 mL), and H_2O (3 × 10 mL); dried (Na_2SO_4); and evaporated. The residual syrup was dissolved in a minimum amount of $CHCl_3$ and applied to a silica gel column (Merck 7729, 2.5 × 15 cm), which was eluted with a $CHCl_3$/Me_2CO (97:3) mixture. Fractions containing only the first compound to be eluted [R_f 0.59 on silica gel developed in a $CHCl_3$/Me_2CO (19:1) mixture] were combined and evaporated to give a syrup, which crystallized to provide 255 mg (55%) of 6-deoxy-6,6-difluoro-1,2,3,4-tetra-*O*-acetyl-α-L-galactopyranose (**22a**): mp 98–100 °C; $[\alpha]^{25}_D$ −69.3° (c 1.00, $CHCl_3$); ^1H NMR (Me_2CO-d_6) δ 6.39 (1 H, d, $J_{1,2}$ = 3.7 Hz, H-1), 6.02 (1 H, qd, $J_{5,6}$ = 5.9 Hz, J_{6,F_a} = 55.5 Hz, J_{6,F_b} = 54.4 Hz, H-6), 5.65 (1 H, m, H-4), 5.39 (1 H, dd, $J_{3,4}$ = 3.3 Hz, H-3), 5.25 (1 H, dd, $J_{2,3}$ = 10.7 Hz, H-2), 4.58 (1 H, m, H-5), 2.19, 2.17, 2.01, 1.96 (12 H, 4 s, acetyl methyls). Anal. ($C_{14}H_{18}F_2O_9$) C, H, F.

A second carbohydrate-containing band (R_f 0.53, TLC as above) was eluted; the appropriate fractions were combined and the solvent was evaporated to provide an opaque syrup (125 mg, 26%) which resisted crystallization. Anal. ($C_{14}H_{18}F_2O_9$) C, H, F. The ^1H NMR spectrum showed this syrup to consist of a mixture of

6-deoxy-6,6-difluoro-1,2,3,4-tetra-*O*-acetyl-β-L-galactopyranose (**22b**) [^{1}H NMR ($CDCl_3$) δ 5.78 (1 H, qd, $J_{5,6}$ = 5.5 Hz, J_{6,F_a} = 55.4 Hz, J_{6,F_b} = 54.4 Hz, H-6), 5.76 (1 H, d, $J_{1,2}$ = 8.46 Hz, H-1), 5.6 (1 H, m, H-4), 5.36 (1 H, dd, $J_{2,3}$ = 10.7 Hz, H-2), 5.10 (1 H, dd, $J_{3,4}$ = 3.3 Hz, H-3), 4.0 (1 H, m, H-5), 2.14, 2.13, 2.06, 2.00 (12 H, 4 s, acetyl methyls)], 6-deoxy-6,6-difluoro-1,2,3,5-tetra-*O*-acetyl-α-L-galactofuranose (**23a**) [^{1}H NMR δ 6.21 (s, $J_{1,2}$ = 0.0 Hz, H-1)], and 6-deoxy-6,6-difluoro-1,2,3,5-tetra-*O*-acetyl-β-L-galactofuranose (**23b**) [^{1}H NMR δ 6.33 (d, $J_{1,2}$ = 4.78 Hz, H-1)], in a ratio of approximately 66, 17, and 17%, respectively. Further separation of these isomers was not attempted.

General Procedure for Deacetalation. 6-Substituted 1,-2:3,4-di-*O*-isopropylidene derivatives were treated with aqueous trifluoroacetic acid (80%) at room temperature for 30–45 min. Reaction mixtures were evaporated to dryness and coevaporated with H_2O and then EtOH. The resulting residues were purified by either crystallization or column chromatography (silica gel, Merck 7729). Inspection of the NMR spectrum of each compound showed the presence of an anomeric mixture. Physicochemical data for the free sugars are listed in Table II.

Effects of Analogues on the Growth of P388 Leukemia Cells in Culture. Compounds were dissolved in 0.9% NaCl with or without 3% Me_2SO at ten times their final concentration. The solutions were sterilized by filtration and 0.5 mL of this solution was added to 4.5 mL of Fischer's medium supplemented with 10% horse serum containing 1.1×10^4 cells/mL. The tubes were incubated at 37 °C in a 5% CO_2 atmosphere. Cell numbers were determined in triplicate 72 h later with an electronic particle counter (Coulter counter, Model B).

Effects of Analogues on the Incorporation of Labeled Sugars into Glycoproteins. P388 leukemia cells were grown in culture to a concentration of 4×10^5 cells/mL (midlog phase) in Fischer's medium supplemented with 10% horse serum in flat bottles at 37 °C under a 5% CO_2 atmosphere. The cell number was determined, and cells were collected by centrifugation and resuspended in fresh medium at a concentration of 5×10^6 cells/mL. The resuspended cells were incubated at 37 °C for 2 h in a shaking water bath in the presence of the compound tested at a concentration of 10^{-4} or 10^{-3} M. After incubation, 0.2 mL of L-[^{3}H]fucose and 0.1 mL of [^{14}C]glucosamine were added to the cell suspension and the incubation was continued for 30 min. The reactions were terminated by the addition of 5 mL of ice-cold 10% trichloroacetic acid (TCA) and the resulting precipitate was collected by centrifugation. Precipitates were washed twice with 5 mL of ice-cold 5% TCA, extracted with 5 mL of cold $CHCl_3$/MeOH/Et_2O (2:2:1, v/v), and washed with 5 mL of MeOH. The precipitated glycoprotein pellets were hydrolyzed in 0.5 mL of 0.5 N NaOH for 30 min at 85 °C; the hydrolysates were neutralized with 0.5 mL of 0.5 N HCl and the radioactivity therein determined in Aquasol (New England Nuclear Corp.) by scintillation spectrometry.

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Supplementary Material Available: NMR data for 6-substituted 1,2:3,4-Di-*O*-isopropylidenes and 6-substituted L-galactopyranoses; also, effects of 6-substituted derivatives of L-fucose on the incorporation of L-[^{3}H]fucose and [^{14}C]glucosamine into acid-insoluble material of P388 leukemia cells in tissue culture (4 pages). Ordering information is given on any current masthead page.

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