Animal Resources Laboratories, Madison, WI) weighing 180-230 g were divided into four groups of at least six rats each and fasted 24 h before administration of 2.0 mmol/kg (po) of disulfiram. Twenty-four hours after the administration of disulfiram, the separate groups were administered 8.0 mmol/kg of the AcH-sequestering agents **3**, **4**, **6**, or saline (control) ip, followed 1 h later by ethanol (43.5 mmol/kg, ip, as a 20% aqueous solution). The animals were sacrificed by cervical dislocation 1 h after ethanol, and blood was collected by open-chest cardiac puncture. Blood AcH, as well as blood ethanol, was measured using the head-space gas chromatographic procedure as above.

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Halogenated L-Fucose and D-Galactose Analogues: Synthesis and Metabolic Effects[1]

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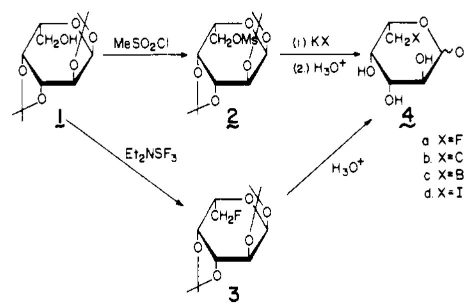
Several new analogues of L-fucose modified in the 2 position and the 5-methyl group have been synthesized as potential plasma-membrane glycoconjugate inhibitors or modifiers, and their biological effects have been studied. 2-Chloro-, 2-bromo-, and 2-iodo-2-deoxy-L-fucose (**9a**, **9b**, and **13**, respectively) have been prepared by addition of the appropriate halogen to 3,4-di-*O*-acetyl-L-fucal, followed by hydrolysis of the anomeric halogen and the acetyl groups. A series of four halogenated 5-methyl analogues of L-fucose (**4**, X = F, Cl, Br, and I) have been obtained starting from 1,2:3,4-di-*O*-isopropylidene-L-galactose. The synthesis of this latter compound has been improved. A corresponding series of 6-deoxy-6-halo-D-galactose analogues, which are enantiomers of the 5-(halomethyl)-L-fucose analogues, has also been synthesized. Analogues **4b**, **4c**, and **9b** at 1×10^{-3} M specifically inhibited the incorporation of L-[^{3}H]fucose into macromolecular components of SW613 human mammary tumor cells. Analogue **13** inhibited the growth of L1210 murine leukemic cells with an IC_{50} of 6×10^{-5} M in culture. 6-Deoxy-6-fluoro-D-galactose and its enantiomer **4a** were found to be effective inhibitors of D-[^{3}H]galactose and L-[^{3}H]fucose incorporation, respectively, into macromolecular components of human mammary tumor cells. The effectiveness of inhibition was reduced with an increase in size of the halogen atom. Analogue **4a** and its enantiomer have been tritiated at C-1 and both were found to be activated to a nucleotide sugar, which was followed by incorporation into the macromolecular fraction of SW613 human mammary tumor cells in vitro.

The plasma membrane is involved in a number of cellular properties which are implicated in neoplasia, such as increased invasiveness, breakdown in cell to cell communication, impairment of differentiation, and antigenic modulation. Extensive investigation has shown that changes occur in the plasma membrane following oncogenic transformation in terms of its modified properties and composition.[2-4] Many, if not most, of these changes involve cell-surface glycoconjugates. These molecules, and particularly their carbohydrate moieties, appear to develop structural and functional differences potentially exploitable for chemotherapeutic attack.

L-Fucose is a terminal sugar and, hence, an inviting target for chemotherapeutic intervention, especially since Seltzer et al.[5] have shown that following its continuous intravenous infusion a decrease in mammary tumor size occurred in rats. In addition, Wassenaar and Tator[6] have demonstrated that both L-fucose and D-galactose are preferentially taken up in brain tumor.

Scheme I



A consideration of the intermediates of L-fucose metabolism and the pathways that lead to GDP–L-fucose has provided a basis for the rational design of its potential inhibitors or modifiers. GDP–L-fucose in cells is synthesized either from appropriate D-mannose precursors by a de novo pathway or from L-fucose starting with phosphorylation of its anomeric carbon, followed by condensation of the resulting 1-phosphate sugar with GTP. Thus, while L-fucose analogues are likely to affect the latter ("salvage") pathway, analogues of D-mannose are potential inhibitors of the de novo biosynthetic pathway.

In the present study, the synthesis and biological activities of several L-fucose analogues, modified at the 2 and 6 positions, are described. These compounds may interfere with the salvage pathways utilizing fucose. Modification of the 6 position should not greatly affect the substrate activity for appropriate enzymes, such as L-fucose kinase, enabling conversion to nucleotide sugars and incorporation into glycoconjugates. Conversely, modification of the 2 position, because of its proximity to the anomeric center,

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Table I. ^{1}H NMR Parameters of 2-Deoxy-2-halo-α-L-fucopyranoses at 100 MHz[a]

no.	chem shifts, δ, and multiplicities[b] of signals					
	H-1	H-2	H-3	H-4	H-5	H-6 (CH_3)
9b	5.10 (d)	4.14 (q)	3.79 (q)	3.54 (q)	4.12 (m)	1.05 (d)
9a	5.06 (d)	4.00 (q)	3.73 (q)	3.52 (q)	4.04 (m)	1.08 (d)

no.	abs values of spin-spin coupling constants,[c] Hz				
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
9b	3.25	11.0	3.1	1.25	6.5
9a	3.3	11.0	3.4	1.3	6.6

[a] Samples were dissolved in Me_2SO-d_6 with Me_4Si as internal standard. [b] Multiplicities (in parentheses) are designated d, doublet; q, quartet; m, multiplet. [c] Coupling constants are first-order determinations.

could lead to the development of inhibitors of L-fucose-anabolizing enzymes.

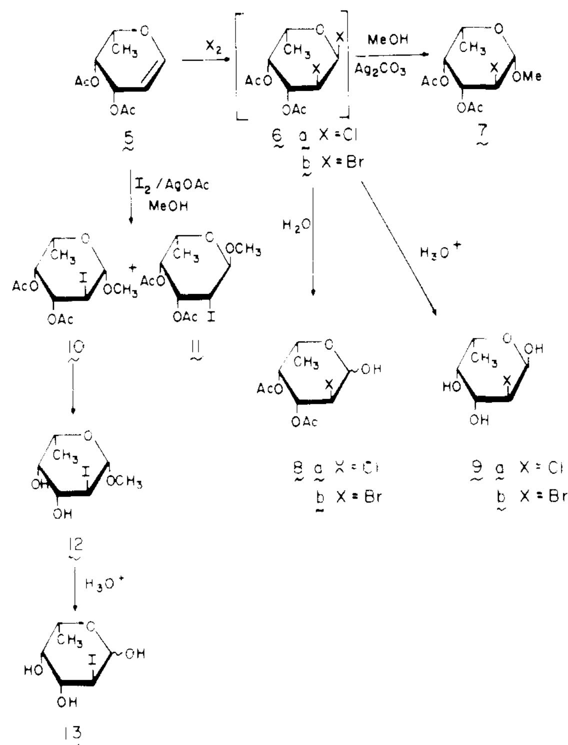
D-Galactose is likewise phosphorylated at C-1 prior to being converted to UDP-galactose. Therefore, we have synthesized 6-deoxy-6-halo-D-galactose analogues which are enantiomers of the corresponding 5-(halomethyl)-L-fucose analogues. The effects of these analogues on various biological characteristics of different tumor cells were determined, particularly with respect to their incorporation into glycoproteins and their interference with the incorporation of their corresponding natural sugars.

Chemistry. The synthesis of 6-halogenated L-fucose analogues is shown in Scheme I. Preparation of 1,2:3,4-di-*O*-isopropylidene-L-galactopyranose (1), the key intermediate for the modification of the 5-methyl group of L-fucose, has been simplified. This intermediate was obtained directly by reduction of L-galactono-1,4-lactone as described earlier,[7] which was followed by acetonation, thus avoiding the isolation of L-galactose. The same procedure has been adapted to a simplified synthesis of L-galactose, thus avoiding a time-consuming ion-exchange purification step. Replacement of the mesyl group in 2 with halogen was accomplished by treatment with appropriate halide salts. This was followed by the hydrolysis of the isopropylidene group, which gave the 6-halogenated L-fucose analogues (**4b–d**) with the exception of the 6-fluoro derivative (4a). The latter was obtained directly from 1 by fluorination with diethylaminosulfur trifluoride[8] to give the blocked derivative 3, which on acid hydrolysis gave the desired fluoro analogue 4a. Although for the purpose of proper nomenclature these 6-modified L-fucose analogues are to be considered as L-galactose derivatives, their designation as 6-halofucoses is to be preferred when their biological properties are considered.

The corresponding series of 6-halogenated enantiomeric D-galactose analogues has also been prepared by the application of similar methods to 1,2:3,4-di-*O*-isopropylidene-D-galactose.

Synthesis of 2-deoxy-2-halo-L-fucopyranose analogues is shown in Scheme II. Addition of chlorine to di-*O*-acetyl-L-fucal (**5**; 1,5-anhydro-3,4-di-*O*-acetyl-1,2,6-trideoxy-L-*lyxo*-hex-1-enitol)[9] in carbon tetrachloride gave the 1,2-dichloride **6a**, which was shown to be a single isomer by NMR spectroscopy. The latter was not further characterized but was converted to the methyl glycoside **7** in order to establish the configuration at C-2. The coupling constant $J_{2,3}$ = 7.5 Hz, which was determined from the ^{1}H NMR spectrum of **7** (Table I), established the relationship between the H-2 and H-3 protons as diaxial.

Scheme II



Thus, the 2-chloro-substituent is equatorial and **7** has the desired L-$^1C_4$ conformation. In addition, another coupling constant, $J_{1,2}$ = 8 Hz, indicates that **7** is a β-L-methyl glycoside. The anomeric chlorine atom in **6a** was hydrolyzed in wet acetone in the presence of silver carbonate to yield the partially acetylated derivative **8a**. The completely deacetylated analogue **9a** was obtained by refluxing the dichloro derivative **6a** with 0.1 N HCl.

In contrast to the chlorination of **5** which gave essentially a single product, bromination gave four different isomers. Hydrolysis of the anomeric bromine occurred on the passage of the crude reaction mixture through a dry silica gel column yielding **8b**, while the other isomer was not isolated. The completely deacetylated analogue **9b** was obtained on refluxing the crude 1,2-dibromo addition product **6b** with dilute HCl. Configurational assignments of the target compounds have been secured by ^{1}H NMR spectroscopy (Table I).

In order to introduce iodine into the 2 position, di-*O*-acetyl-L-fucal (**5**) was iodomethoxylated, a reaction which has been shown to proceed by trans addition,[10] giving the two trans adducts **10** and **11**, respectively, with the L-*talo*

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Table II. Effects of Several Fucose Analogues on Cell Growth and Macromolecular Biosynthesis

		human mammary tumor SW613[a]		
		incorp. (5 h), % control		
fucose analogues	leukemia L1210: IC_{50},[b] M	fucose	leucine	growth,[d] % control
2-Cl-2-deoxy-L-fucose (**9a**)	2 × 10^{-4}	75	84	83
2-Br-2-deoxy-L-fucose (**9b**)[c]	no effect at 1 × 10^{-3}	37	106	95
2-deoxy-2-I-L-fucose (13)	6 × 10^{-5}	8	3	69
6-F-L-fucose (4a)[c]	slight effect at 1 × 10^{-3}	9	66	102
6-Cl-L-fucose (**4b**)[c]	slight effect at 1 × 10^{-3}	14	81	113
6-Br-L-fucose (4c)[c]	4 × 10^{-4}	21	79	98
6-I-L-fucose (4d)	1.8 × 10^{-4}	92	99	93

[a] Approximately 5 × 10^6 SW613 human mammary tumor cells were grown as a monolayer in 35-mm Petri dishes containing 1 × 10^{-3} M fucose analogue. [^{3}H]Fucose, 0.5 μCi (50 Ci/mmol, New England Nuclear), or [^{3}H]leucine, 2.5 μCi (58 Ci/mmol), was added 1 h after the addition of the fucose analogues and incubated for another 5 h. Incorporation into an acid-precipitable fraction was measured (ref 10). All tests were run in duplicate on at least three separate occasions. [b] IC_{50} values are the concentration of compound necessary to reduce by 50% the growth of L1210 leukemic cells grown in tissue culture for 44 h. [c] Specifically reduces [^{3}H]fucose incorporation by >75% at 10^{-3} M. [d] Growth of cell cultures in the presence of 10^{-3} M sugar analogue was monitored after 48 h. Protein determinations (ref 14) were performed and control growth was set to 100%.

Table III. Effects of Acetylation on Biological Activities of 2-Halogeno-L-fucose Analogues[a]

		P288 lymphoma		
			incorp., (5 h), % control	
compd	leukemia L1210: IC_{50}, M	growth (24 h), % control	$GlcNH_2$	Leu
3,4-$(OAc)_2$-2-Cl-2-deoxy-L-fucose (**8a**)	4 × 10^{-5}	59	45	51
2-Cl-2-deoxy-L-fucose (9a)	2 × 10^{-4}	58	98	91
3,4-$(OAc)_2$-2-Br-2-deoxy-L-fucose (8b)	6 × 10^{-5}	47	55	45
2-Br-2-deoxy-L-fucose (9b)	>10^{-3}	65	91	100

[a] Conditions of testing are described under Experimental Section.

Table IV. Effects of D-Galactose Analogues on Cell Growth and Macromolecular Biosynthesis[a]

		P288 murine lymphoma			
X	leukemia L1210: IC_{50}, M	growth, % control	D-Gal	D-Glc-NH_3	L-Leu
F[b]	>10^{-3}	100	39	89	84
Cl	1 × 10^{-3}	100	87	78	86
Br	1.3 × 10^{-4}	94	70	97	85
I	>1 × 10^{-3}	94	71	88	92
Ms	7.5 × 10^{-5}	100	72	91	91

[a] Conditions of testing described under Experimental Section. [b] Specifically reduces [^{3}H]galactose incorporation by >50% at 10^{-3} M.

Table V. Effects of Fluorinated Membrane Sugar Analogues on Human Mammary Tumor Cell Macromolecular Biosynthesis[a]

	% control, cpm/μg of protein	
analogue plus competing sugar	uptake	incorp.
L-[^{3}H]fucose + 5 mM 6-F-L-fucose	18	5
6-F-[^{3}H]fucose + 5 mM fucose	10	7
[^{3}H]Gal + 5 mM 6-F-Gal	44	45
6-F-[^{3}H]Gal + 5 mM Gal	100	85

[a] SW613 human mammary tumor cells were incubated in RPMI 1640 plus 10% fetal calf serum plus the indicated [^{3}H]sugar or fluoro[^{3}H]sugar (1 μCi/mL) for 2 h in the presence or absence of 5 mM unlabeled sugar or sugar analogue. Total radioactivity in phosphate-buffered saline washed cells was measured for uptake, and radioactivity present in a washed 10% TCA pellet was a measure of incorporation. Results were the mean of triplicate cultures.

isomer **11** predominating and the L-*fuco* isomer **10** in only 8% yield. The latter was deacetylated to give the methyl glycoside **12**, and converted to the free 2-deoxy-2-iodo-L-fucose **13** by acid hydrolysis. The assignment of **10** and **11** was based on $J_{1,2}$ constants of 1.0 and 8.9 Hz, respectively, corresponding to 1,2 diequatorial protons in **11** and 1,2 diaxial protons in **10**. The upfield shift of the 1-methoxyl protons (δ 3.40) in **11** as compared to those of **10** (δ 3.59) likewise indicates their respective axial and equatorial positions,[10] which is consistant with the L-$^1C_4$ conformations of the iodo derivatives **10** and **11**.

Biological Activity. Cell Culture Studies. The effects of L-fucose and D-galactose analogues on growth, viability, and incorporation of D-glucosamine, D-galactose, L-fucose, and L-leucine into cellular proteins and glycoconjugates were evaluated in murine leukemia L1210, murine lymphoma P288, and human mammary tumor SW613 cells in vitro. The L1210 cells were generally more sensitive to cytotoxic agents due to their shorter doubling time but were found to be unsuitable for incorporation studies, due to the low incorporation rate of L-fucose. Human mammary tumor cells, on the other hand, were more suitable for these incorporation studies, since this cell line incorporated relatively large amounts of L-[^{3}H]-fucose. Table II summarizes the IC_{50} values (50% growth inhibitory concentrations) of halogenated L-fucose analogues which were tested against leukemia L1210 cells and also provides results of the ability of these analogues to inhibit incorporation of L-fucose and L-leucine into macromolecules.

Table III compares the effects of acetylation on the IC_{50} values in leukemia L1210 cells, as well as on the growth and incorporation of precursors into lymphoma P288 cells. The effects of halogen substitution at the 6 position of D-galactose on IC_{50} values in leukemia L1210 and on the

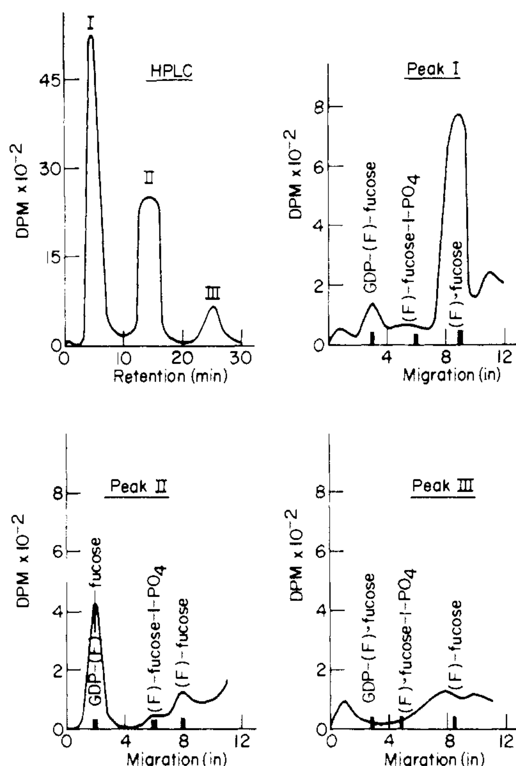

Figure 1. Chromatographic analysis of the metabolites of 6-fluoro[^{3}H]fucose. Labeled L1210 cells were extracted with perchloric acid, and the acid-soluble fraction was analyzed on high-pressure LC. Fractions were collected off the column and counted for radioactivity with a Packard scintillation counter. Three radioactive peaks (I, II, and III) were observed. Aliquots of each peak were analyzed further with paper chromatography. Peak I consisted primarily of 6-fluoro[^{3}H]fucose, peak II consisted primarily of GDP–6-fluoro[^{3}H]fucose, and peak III, the washout fraction, contained no readily identifiable material.

incorporation of D-galactose, D-glucosamine, and L-leucine have been determined (Table IV).

6-Deoxy-6-fluoro-L-[1-^{3}H]galactose and 6-deoxy-6-fluoro-D-[1-^{3}H]galactose were incorporated into SW613 human mammary tumor cells (Table V). The uptake and incorporation of 6-deoxy-6-fluoro-L-[1-^{3}H]galactose were blocked by the addition of 5 mM L-fucose, while the addition of 5 mM D-galactose reduced the incorporation of 6-deoxy-6-fluoro-D-[1-^{3}H]galactose by only 15%.

In Vivo Studies. A continuous iv infusion[11] of 6-deoxy-6-fluoro-D-galactose at levels of 1500 and 2000 (mg/kg)/day did not increase the survival time of mice bearing L1210 leukemia. These levels are just below the observed lethal level of 2300 (mg/kg)/day; D-galactose administered at identical dosage levels was also without chemotherapeutic benefit. Preliminary studies with this analogue and **8b** in which graded doses were administered ip., daily for 5 days, to mice with L1210 ascites tumor, prolonged their life span by 32 and 25%, respectively, at optimal dosage levels.

Ribonucleotide Pool Size Analysis. L1210 cells (1 $\times 10^{-7}$) incubated in the presence of 1 mCi of 6-deoxy-6-fluoro-L-[1-^{3}H]galactose for 5 h in 10 mL of RPMI 1640 medium containing 10% heat-inactivated fetal calf serum were extracted with 6% perchloric acid (100 μL/10^7 cells). This acid-soluble extract was analyzed using high-pressure liquid chromatography (LC). The fractions were collected and counted for radioactivity with a scintillation counter. The radioactivity profile is shown in Figure 1. Three radioactive peaks labeled I, II, and III observed on high-pressure LC were characterized further using paper chromatography. Aliquots of each peak were applied to S + S orange paper and chromatographed in a solvent system consisting of pyridine–glacial acetic acid–ethyl acetate–water (5:1:5:3). Peak I cochromatographed with authentic L-fucose or 4a ("6-fluorofucose"), which are not separated in this system; peak II cochromatographed with authentic GDP-fucose, indicating the formation of GDP-6-fluoro[^{3}H]fucose, while peak III (the washout peak on high-pressure LC) had no distinct peaks on the paper chromatogram.

Discussion

Replacement of one hydrogen of the 5-methyl group of L-fucose by a halogen atom is expected to have no significant effect on the conformation of the pyranose ring of the parent sugar and a relatively small electronic effect on the anomeric center. In comparing the effects of this series of analogues on the incorporation of precursors into the macromolecular fraction (Table II), it was found that 4a at 1 mM reduced specifically [^{3}H]fucose incorporation to 9% of control levels while it did not affect L-leucine incorporation or growth of leukemia L1210 cells to a significant extent. With increasing size of the halogen atom, the effect on L-fucose incorporation is progressively reduced, with a concomitant increase of cytotoxicity. In order to study the metabolism of 4a, it has been tritiated. It is converted to GDP–6-fluorofucose and eventually incorporated into glycoprotein and glycolipid. The substitution of the 2-hydroxyl group with halogen on L-fucose could be expected to affect significantly the conformation of the molecule. Nevertheless, a careful comparison of the ^{1}H NMR coupling constants (Table I) for the 2-chloro and 2-bromo derivatives indicates that the 2-halogen atom has little effect on the conformation of the parent sugar. It could, however, exert its effect on the charge density of the anomeric hydroxyl group, as well as sterically hindering its phosphorylation. These effects may have found their expression in the significant cytotoxicity of the 2-iodo derivative in both L1210 and SW613 cells and the concomitant inhibition of the incorporation of L-fucose and L-leucine. All these effects are diminished with the decrease in the size of the halogen atom (Table II).

Effects of the O-acetylation of the 3 and 4 positions of the 2-halogenated L-fucose analogues have also been determined (Table III). The O-acetylated derivatives were found to be more potent inhibitors of growth than their nonacetylated counterparts. This is particularly evident for the 2-bromo-2-deoxy analogues. The extent of the inhibition of precursor incorporation into the macromolecular fraction of P288 lymphoma cells appears to parallel that of growth inhibition (Table III). The substantially greater inhibition of the O-acetylated derivatives could be related to the greater lipid solubility and, therefore, cellular permeability of the acetylated sugars, as has been postulated to occur for some acetylated 2-amino-2-deoxy sugars, such as peracetylated D-glucosamine.[12]

The inhibition of L1210 cell growth in vitro due to 5 $\times 10^{-5}$ M 3,4-di-*O*-acetyl-2-bromo-2-deoxy-L-fucose (**8b**) was reversed by only 5 and 10% upon addition of 1 $\times 10^{-3}$ and 5 $\times 10^{-3}$ M L-fucose, respectively. Therefore, its growth-inhibitory properties probably are not related to the inhibition of fucose-related pathways. This compound, upon daily ip administration of 200 (mg/kg)/day for 5 days to

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mice bearing L1210 leukemia, increased life span by 25%.

A series of D-galactose analogues substituted in the 6 position by a halogen atom has also been prepared. These analogues are enantiomers of the corresponding L-fucose analogues and have been tested for their cytotoxicity against L1210 cells, inhibition of growth, and inhibition of incorporation of precursors into the macromolecular fraction of P288 cells (Table IV). The most cytotoxic D-galactose derivative was found to be the 6-methanesulfonyl derivative, which was followed by 6-bromo-6-deoxy-D-galactose. Although 6-deoxy-6-fluoro-D-galactose was found to be devoid of cytotoxicity at 1 mM, it was shown to be selective in inhibiting the incorporation of D-galactose into the macromolecular fraction at this concentration. In this regard, it behaves analogously to the corresponding L-fucose enantiomer. When 6-deoxy-6-fluoro-D-galactose was administered ip to mice bearing L1210 leukemia at a dose of 25 mg/kg × 5, their life span was increased by 32%. The life span of mice inoculated with P288 cells after pretreatment with the analogue was also prolonged by 24% in the transplantability test system.[13] These results prompted further in vivo studies with the compound.

Constant infusion of 6-deoxy-6-fluoro-D-galactose for 68 h at doses of 1500 and 2000 (mg/kg)/day in mice bearing L1210 leukemia did not result in any measured increase in life span. Toxicity was evident at doses above 2000 (mg/kg)/day, with 75% of the mice dead on day 3 following an infusion of 2300 (mg/kg)/day of 6-deoxy-6-fluoro-D-galactose. Preliminary indications of liver toxicity were evident at this dosage. Infusions of D-galactose at similar levels had no discernable biological effect.

Experimental Section

General Chemical Methods. Melting points were performed on a Mel-Temp apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 457 spectrophotometer. NMR spectra were recorded on a Varian XL-100 spectrometer using Me_4Si as an internal standard. Optical rotations were measured with a Perkin-Elmer 141 polarimeter and are reported as equilibrium values unless otherwise noted. Elemental analyses were determined by Robertson Laboratory, Florham Park, N.J. Evaporations were carried out on a rotary evaporator under reduced pressure. Dry column chromatography[14] was carried out using silica gel Woelm for dry-column chromatography, activity III/30 mm (ICN Pharmaceuticals). Approximately 50 g of silica gel per gram of product to be separated was uniformly packed into a glass column (14.5 × 600 mm). The product was dissolved in ether or methanol, and a small amount of silica gel, sufficient to cause complete adsorption, was added. This silica gel preparation was placed on the rotary evaporator and, after solvent removal, it was applied to the top of the column; elution was carried with a suitable solvent system. All thin-layer chromatography was performed on silica gel plates, and spots were detected using sulfuric acid–methanol spray. Tritiated **4a** (4.7 Ci/mmol) and its enantiomer (9.43 Ci/mmol) were prepared by Amersham, London, England, from the appropriate precursors.

Methods for Biological Evaluation. General. The cell lines in these studies were murine L1210 leukemia and P288 lymphoma and human SW613 mammary tumor maintained in vitro.

Cell Cultures. L1210 leukemia cells were grown in stationary tube cultures in RPMI 1640 medium[15] containing 10% heat-inactivated fetal calf serum. Murine P288 lymphoma cells were maintained as an ascites tumor in DBA/2J female mice. Periodically, cells were removed from mice, under aseptic conditions, washed twice in RPMI 1640 medium, and cultured in RPMI 1640 containing 10% fetal calf serum. These cultures were grown in stationary tube cultures in a 90% air/10% CO_2 incubator. Cells maintained their viability in culture and in log phase had a generation time of approximately 13 h. They were maintained in culture for periods of 1 to 3 months, whereupon new in vitro cultures were initiated from the in vivo line. In this manner, constant selective genetic pressure was maintained on the cell line. SW613 breast tumor cells were supplied by Dr. E. Jenson, EG & G/Mason Research Institute, and cultured in RPMI 1640 plus 10% heat-inactivated fetal calf serum.

Biological Testing Systems: In Vitro Studies. L1210. An inoculum of 5×10^4 cells in 1 mL of RPMI 1640 medium containing 10% heat-inactivated fetal calf serum and 20 mM Hepes buffer was supplemented with 1 mL of the same medium containing the compound to be tested. The tubes were incubated in an upright position for 3 days, and growth was estimated either by protein assay[16] or cell counts using a Coulter counter. The growth in control cultures varied from six- to tenfold after 3 days. Each concentration was tested in triplicate. For compounds found inhibitory, the tests were repeated at least twice. Variation between different tests was within ± 10% for the 50% inhibitory concentration. Results have been expressed in terms of IC_{50}, which denotes the molar concentration of the sugar analogue in the nutrient medium leading to 50% inhibition of cell growth as compared with the drug-free control.

P288. (A) Cell Growth and Viability. For routine testing, P288 murine leukemic cells were suspended at approximately 10^6 cells/mL in fresh RPMI 1640 without glucose and containing 10% heat-inactivated fetal calf serum. One-milliliter aliquots were then transferred to disposable polyethylene test tubes and placed in a CO_2/air incubator. One hour later, sugar analogues were added to a final concentration of 1 mM (or as otherwise stated). Cell growth and viability were monitored at later times by use of a Coulter cell counter and Trypan blue dye exclusion, respectively.

(B) Macromolecular Biosynthesis. [^{14}C]Glucosamine, 2 μM (1.1×10^6 dpm, Amersham), and [^{3}H]leucine, 0.37 mM (2.6×10^6 dpm, New England Nuclear), or [^{3}H]galactose, 1.5 μM (5.5×10^6 dpm, New England Nuclear), were added to cell cultures to assess the effects of sugar analogues on protein and glycoprotein biosynthesis. Incubations were terminated 5 h later by the addition of 2 mL of 10% trichloroacetic acid, and the resulting pellet was dissolved in NaOH and its radioactivity quantitated by scintillation counting methods.

SW613. (A) Cell Growth. Human mammary tumor cells were grown in 35-mm Petri dishes (Flow Labs) with 2 mL of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum. Sugar analogues were added at 1 mM final concentration and the plates incubated for 48 h. Cell growth was estimated by the increase in cellular protein[16] during this time period.

(B) Macromolecular Biosynthesis. [^{3}H]Fucose or [^{3}H]leucine was added 1 h after the addition of sugar analogues. Five hours later macromolecular incorporation was measured as outlined earlier for P288 cells.

In Vivo Studies. L1210. Cells, 10^6, were transplanted ip into DBA/2 Ha female mice. 6-Fluoro-D-galactose or **8b** was administered ip in daily doses from day 1 through 5 following tumor inoculation. There were five mice in the untreated control group and three mice in each treated group who received graded dosages of the analogues. The effectiveness of these compounds was measured by comparison of the mean survival time of the treated group with that of the control group.

L1210 Constant Infusion Studies. Cells, 10^6, were transplanted ip into DBA/2 Ha female mice on day 0. On day 1, infusions of various concentrations of 6-fluorogalactose, dissolved in bicarbonate-buffered saline, were begun. Infusions continued for 68 h (up to day 4) and were administered using a Harvard pump via cannulation of the tail vein (9 mL/day).[11] The animals had free access to food and water during this period. Following the infusion period, the cannula were removed and the survival time for each animal was recorded.

Ribonucleotide Pool Size Analysis. Ribonucleotide pool size analysis was performed on L1210 cells treated with tritiated

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4a for 5 h. Approximately 10^7 treated cells were washed twice with 2 mL of RPMI 1640, extracted with 6% perchloric acid (100 $\mu L/10^7$ cells), and centrifuged at 800*g* for 2 min. The supernatant (acid-soluble fraction) was neutralized with 2 N KOH to a pH of 7.0. The precipitate was removed by centrifugation, and an aliquot of the resulting supernatant was analyzed for its ribonucleotide content using a Dupont 830 high-pressure liquid chromatographic system equipped with a 254-nm detector. Ten microliters of the supernatant was absorbed onto an ABX column (1 m × 2 mm, U shaped) and eluted at room temperature using a phosphate buffer gradient (0.002–0.05 M, pH 3.0) at a flow rate of about 1 mL/min.[12] Fractions were collected and the identity of the peaks was established by using retention times of authentic standards for comparison. Areas under the peaks were integrated on Spectra-Physics Model 23,000-010 Autolab Minigrator. Aliquots of fractions were counted in 10 mL of ACS cocktail (Amersham) in a Packard scintillation counter. Radiolabeled metabolites were identified by descending paper chromatography using pyridine–glacial acetic acid–ethyl acetate–water (5:1:5:3).

D-**Galactopyranose Derivatives.** 1,2:3,4-Di-*O*-isopropylidene-6-*O*-menthanesulfonyl-D-galactopyranose was prepared from D-galactose by established procedures[17,18] and was converted to the following D-galactopyranose derivatives: 6-deoxy-6-iodo-D-galactopyranose by the method of Raymond and Schroeder;[19] 6-bromo-6-deoxy-D-galactopyranose by the method of Barnett et al.;[20] 6-chloro-6-deoxy-D-galactopyranose by the method of Buck and Foster[21] and Wood et al.;[22] and 6-deoxy-6-fluoro-D-galactopyranose by the method of Taylor and Kent.[23]

L-**Fucopyranose Derivatives. 1,2:3,4-Di-*O*-isopropylidene-L-galactopyranose (1).** L-Galactose was prepared from L-galactono-1,4-lactone by reduction with sodium amalgam using a modification of the method described by Frush and Isbell.[7] The product was not purified by ion-exchange chromatography, as described by these authors. Instead, a syrupy product obtained from solvent evaporation of water and methanol was dried thoroughly under vacuum with heating and then stirred with 100 mL of dry acetone and 1 mL of concentrated sulfuric acid per gram of product at room temperature for 20 h, as described by Ballou and Fischer.[17] Neutralization with ammonia (gas) was followed by filtration and solvent evaporation. The product was purified by fractional distillation on a Kugelrohr apparatus at bp 115–112 (0.2 mm) (35% yield). The infrared spectrum of 1 was identical with that of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-methanesulfonyl-L-galactopyranose (2). As described in the preceding experiment, an 8.0-g sample of L-galactono-1,4-lactone was reduced with sodium amalgam and converted to 1, which, without purification, was stirred for 4 h at 0 °C with 75 mL of dry pyridine and 5 mL of methanesulfonyl chloride. The reaction mixture was poured onto ice and stirred to give the precipitated product. Recrystallization from methanol–water gave 7.3 g (48%) of **2**: mp 124–125 °C, lit.[18] mp 124–125 °C (for the D enantiomer). The following compounds were prepared from **2** by the same procedures used to prepare the corresponding D-galactopyranose derivatives: 6-deoxy-6-iodo-L-galactopyranose (**4d**), 6-bromo-6-deoxy-L-galactopyranose (**4c**), and 6-chloro-6-deoxy-L-galactopyranose (**4b**). They were characterized by their melting points and IR spectra, which were identical with those obtained for their respective enanantiomeric D-galactopyranose derivatives. In all cases, the optical rotations of **4c**, **4d**, and **4b** at equilibrium were of equal magnitude but opposite sign of those obtained for the corresponding D-galactopyranose derivatives.

L-**Galactose.** A sample of **1** (600 mg) was refluxed for 18 h in methanol (10 mL) containing 0.02 N H_2SO_4 (10 mL). The reaction mixture was neutralized with $BaCO_3$, filtered, and evaporated. The product was purified on a dry silica gel column (eluant: ethanol–ethyl acetate, 1:1) and recrystallized from methanol after treatment with charcoal to yield 222 mg (47%); mp 163–166 °C, lit.[7] mp 165–168 °C; $[\alpha]^{22}_D$ -79.3° (*c* 0.41, H_2O), lit.[7] $[\alpha]^{22}_D$ -80° (*c* 4, H_2O).

6-Deoxy-6-fluoro-L-galactopyranose (4a). The fluorination procedure of Sharma and Korytnyk[8] was employed. To a solution of 1.93 mL of diethylaminosulfur trifluoride[24] in 5 mL of dry diglyme at -10 °C was added 814 mg of **1** in 5 mL of diglyme, dropwise with stirring. After warming slowly to room temperature, the reaction solution was heated at 60 °C for 2 h, cooled, poured onto ice, and extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried ($MgSO_4$), filtered, and evaporated to give a syrupy residue, which was purified by dry column chromatography (eluant: ether–benzene, 1:1). The 524 mg (73%) of **3** obtained had an IR spectrum identical with that of the enantiomeric 6-deoxy-6-fluoro-D-galactopyranose derivative. Hydrolysis using the same procedure described above for the L-galactose preparation gave 263 mg of **4a** (63% based on **3**) as a syrup. Dry column chromatography (eluant: ethanol–ethyl acetate, 1:1) and recrystallization from methanol–ether gave crystals: mp 163–164 °C; $[\alpha]^{22}_D$ -76.5° (*c* 0.23, H_2O) [mp 160 °C and $[\alpha]^{22}_D$ +76.5 (*c* 1, H_2O) for 6-deoxy-6-fluoro-D-galactopyranose have been reported by Taylor and Kent[23]].

6-Deoxy-6-fluoro-L-[1-^{3}H]galactopyranose was obtained directly from **4a** by catalytic tritiation carried out in Amersham's laboratories. The initial activity was 9.4 Ci/mmol. The compound was purified by paper chromatography.

3,4-Di-*O*-acetyl-2-chloro-2-deoxy-α-L-fucopyranosyl Chloride (6a). Di-*O*-acetylfucal (**5**; 3.0 g) was dissolved in carbon tetrachloride (150 mL), cooled to 0 °C and protected from light while chlorine was bubbled in until a yellow color persisted. Removal of excess chlorine and solvent by evaporation yielded 4.0 g (93%) of the adduct **6a** as a syrup. The NMR spectrum ($CDCl_3$) indicated the presence of only one addition product: δ 1.20 (d, 3, CH_3), 2.08 [s, 3, C(=O)CH_3], 2.19 [s, 3, C(=O)CH_3], 4.48 (m, 2 H, H-2 and H-5), 5.38 (m, 2 H, H-3 and H-4), 6.90 (d, 1, H-1, $J_{1,2}$ = 3.5 Hz).

Methyl 3,4-Di-*O*-acetyl-2-chloro-2-deoxy-β-L-fucopyranoside (7). The syrupy dichloro adduct (**6a**; 580 mg) in 25 mL of dry methanol was stirred with Ag_2CO_3 (2.76 g) at room temperature for 20 h. Filtration and solvent evaporation, followed by dry column chromatography (eluant: benzene–ether, 1:1), gave 144 mg (25%) of **7** as a light yellow syrup. Distillation using a Kugelrohr apparatus [bp 120–130 (0.3 Torr)] yielded an analytically pure sample: $[\alpha]^{22}_D$ -54.6° (*c* 0.44, H_2O); NMR ($CDCl_3$) δ 1.24 (d, 3, CH_3), 2.08 [s, 3, C(=O)CH_3], 2.18 [s, 3, C(=O)CH_3], 3.61 (s, 3, OCH_3), 3.91 (m, 2, H-2 and H-5, $J_{1,2}$ = 8.0 Hz), 5.01 (2 d, 1, H-3, $J_{2,3}$ = 7.9 Hz, $J_{3,4}$ = 3.5 Hz), 5.20 (2 d, 1, H-4, $J_{4,3}$ = 3.9 Hz, $J_{4,5}$ = 1.0 Hz). Anal. ($C_{11}H_{17}ClO_6$) C, H.

3,4-Di-*O*-acetyl-2-chloro-2-deoxy-L-fucopyranose (8a). The oily dichloro adduct (**6a**; 230 mg) was stirred in wet acetone (20 mL) with Ag_2CO_3 (600 mg) for 20 h. Filtration and solvent evaporation, followed by recrystallization from ether–petroleum ether, gave 200 mg (69%) of **8a** as white crystals: mp 164–165 °C; $[\alpha]^{22}_D$ -95.3 (*c* 0.15, H_2O). Anal. ($C_{10}H_{15}ClO_6$) C, H, Cl.

2-Chloro-2-deoxy-L-fucopyranose (9a). The hydrolytic procedure of Adamson and Foster[25] was employed. The dichloro derivative **6a** (3.0 g) was refluxed in 40 mL of 0.1 N HCl for 6 h, cooled, neutralized with Ag_2CO_3, and evaporated to give a syrupy residue which was dissolved in methanol and decolorized with charcoal. Dry column chromatography (eluant: ethanol–ethyl acetate, 1:1) yielded 1.33 g (73%) of syrup. Seed crystals were obtained by letting an aliquot of methanol solution slowly evaporate: mp 110–114 °C; $[\alpha]^{22}_D$ -66.7° (initial; *c* 0.2, H_2O) and -87.2° (equil). Anal. ($C_6H_{11}ClO_4$) C, H, Cl.

Recrystallization from acetonitrile (charcoal treatment) yielded the α anomer exclusively: mp 141–142 °C; $[\alpha]^{22}_D$ -133.0° (initial; *c* 0.2, H_2O) and -87.6° (equil). Anal. ($C_6H_{11}ClO_4$) C, H.

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3,4-Di-O-acetyl-2-bromo-2-deoxy-L-fucopyranose (8b). Bromination of di-O-acetylfucal (**5**; 1.0 g) was carried out by the same procedure outlined above for chlorination. TLC (eluant: benzene–ether, 2:1) of the crude dibromo addition product showed four distinct spots. Dry column chromatography was carried out (eluant: benzene–ether, 2:1). The combined fractions corresponding to the spot on TLC which had the highest R_f value yielded 0.38 g (26%) of white crystals. Recrystallization from ether gave **8b**: mp 180–182 °C; $[\alpha]^{22}_D$ -131° (c 0.1, H_2O). Anal. ($C_{10}H_{15}BrO_6$) C, H, Br.

2-Bromo-2-deoxy-α-L-fucopyranose (9b). Di-O-acetylfucal (**5**; 2.09 g) was brominated as described above, and the crude dibromo product was refluxed in 0.1 N HCl for 6 h, neutralized with Ag_2CO_3, filtered, and evaporated to give a syrupy residue. TLC (eluant: acetone–benzene 2:1) gave two spots, which were separated by dry column chromatography. The component of lower mobility crystallized. Recrystallization from acetonitrile with charcoal treatment gave 187 mg (9%) of **9b** as white crystals: mp 135–137 °C; $[\alpha]^{22}_D$ -90.8° (c 0.13, H_2O, equil); NMR, see Table I. Anal. ($C_6H_{11}BrO_4$) C, H, Br.

Methyl 3,4-Di-O-acetyl-2-deoxy-2-iodo-β-L-fucopyranoside (10) and Methyl 3,4-Di-O-acetyl-2,6-dideoxy-2-iodo-α-L-talopyranoside (11). Di-O-acetylfucal (**5**; 2.14 g) in 70 mL of dry methanol was mixed with 2.35 g of silver acetate and cooled to 0 °C. Iodine (3.58 g) was added; after 1 h, the silver salts were filtered off and the methanolic filtrate was evaporated in vacuo. The syrupy residue was dissolved in chloroform and washed successively with aqueous sodium bicarbonate and sodium thiosulfate solutions. The chloroform solution was evaporated to dryness in vacuo, and the residue was dry column chromatographed (eluant: petroleum ether–ether, 2:1). Collected were 2.37 g (64%) of methyl 3,4-diacetyl-2,6-dideoxy-2-iodo-α-L-talopyranoside (**11**), 0.31 g (8%) of methyl 2-deoxy-3,4-diacetyl-2-iodo-β-L-fucopyranoside (**10**), and 0.43 g (11%) of an unresolved mixture of **11** and **10**.

Physical constants and analyses are as follows. For **11**: mp 40–42 °C; $[\alpha]^{22}_D$ -37.0 (c 0.53, methanol); NMR ($CDCl_3$) δ 5.22 (s, 1, H-1, $J_{1,2}$ = 1.0 Hz), 4.31 (d, 1, H-2, $J_{2,3}$ = 5.0 Hz), 4.90 (t, 1, H-3, $J_{3,4}$ = 3.6 Hz), 5.25 (s, 1, H-4, $J_{4,5}$ = 1.7 Hz), 4.19 (d, 1, H-5, $J_{5,6}$ = 6.7 Hz), 1.22 (d, 3, 5-CH_3), 3.40 (s, 3, 1-OCH_3), 2.08, 2.22 (s, 3, Ac, $J_{2,4}$ = 1.0 Hz). Anal. ($C_{11}H_{17}IO_6$) C, H, I.

For **10**: mp 111–112 °C; $[\alpha]^{22}_D$ -38.3 (c 0.12, methanol); NMR ($CDCl_3$) δ 4.34 (d, 1, H-1, $J_{1,2}$ = 8.9 Hz), 4.04 (q, 1, H-2, $J_{2,3}$ = 12.5 Hz), 5.10 (d, 1, H-3, $J_{3,4}$ = 3.2 Hz), 5.09 (s, 1, H-4, $J_{4,5}$ = 1 Hz), 3.85 (q, 1, H-5, $J_{5,6}$ = 6.5 Hz), 1.23 (d, 3, 5-CH_3), 3.59 (s, 1, OCH_3), 2.07, 2.16 (s, 3, Ac, $J_{2,4}$ = 1.1 Hz). Anal. ($C_{11}H_{17}IO_6$) C, H, I.

Methyl 2-Deoxy-2-iodo-β-L-fucopyranoside (12). The iodofucose derivative (**10**; 150 mg) was dissolved in 0.1 M methanolic sodium methoxide solution (20 mL) and allowed to stand at room temperature for 1 h. Neutralization with ion-exchange resin (Amberlite IR 120, H^+) and filtration, followed by evaporation, yielded a syrup, which was dissolved in hot ethyl acetate, decolorized with charcoal, filtered, and evaporated to give 80 mg (61%) of crystals: mp 114–116 °C; $[\alpha]^{22}_D$ -33.1° (c 0.13, methanol); NMR ($CDCl_3$) δ 4.46 (d, 1, H-1, J = 8.5 Hz), 3.56 (s, 1, OCH_3), 1.37 (d, 3, 5-CH_3). Anal. ($C_7H_{13}IO_4$) C, H, I.

2-Deoxy-2-iodo-L-fucopyranose (13). A solution of 74 mg of methyl 2-deoxy-2-iodo-β-L-fucopyranoside in 0.05 M H_2SO_4 (10 mL) was heated at 100 °C for 2 h, cooled, and neutralized with aqueous $Ba(OH)_2$ solution. Filtration and evaporation in vacuo was followed by dry column chromatography (eluant: ethyl acetate–ethanol, 3:1), which yielded a light yellow syrup. Crystallization was induced by slow evaporation from an acetonitrile–chloroform solution, giving 20 mg (30%) of yellow powder: mp 54–55 °C; $[\alpha]^{22}_D$ -91° (c 0.1, H_2O). Anal. ($C_6H_{11}IO_4$) C, H.

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Synthesis and Psychoanaleptic Properties of New Compounds Structurally Related to Diphenhydramine

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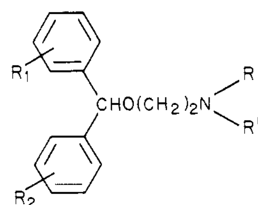
A new series of benzhydryloxyalkylpiperazines carrying a trivalent function has been synthesized and studied for its effects on the central nervous system. Most of the compounds exhibit unexpected nonamphetaminic psychoanaleptic properties. The structure–activity studies revealed the importance of the nature and the position of the substituents on the phenyl rings. However, no significant correlation between atropinic or antihistaminic effects and psychoanaleptic properties was observed.

The benzhydrol derivatives of diphenhydramine type (I) exhibit pronounced antihistaminic properties and some



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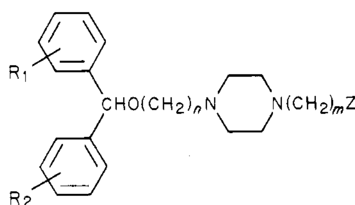

II, n = 2,3; m = 1–4; Z = CN, $COOC_2H_5$, COOH, $CONR_2$

atropinic effects.[1,2] It is also well known that these H_1 antagonists may exert both stimulating and sedative effects on the CNS.[3] This central excitation is associated with overdosage, as shown for pyribenzamine-induced activation of the EEG,[4] and is usually a stimulation of the convulsion type. However, the sedative effect appears mainly at therapeutic doses.

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