Haloacetamido Analogues of 2-Amino-2-deoxy-D-mannose. Syntheses and Effects on Tumor-Bearing Mice

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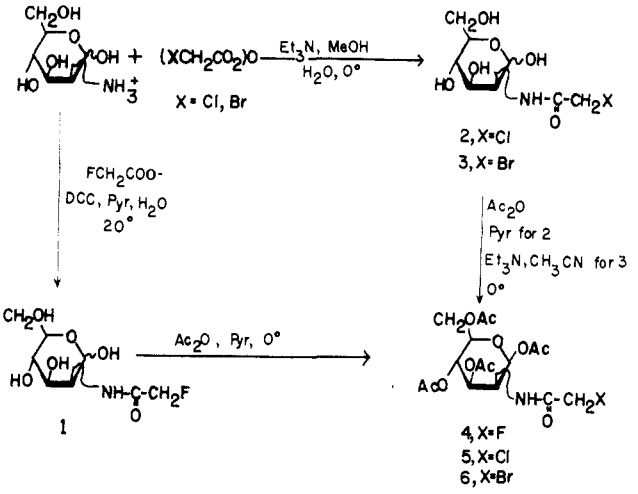
Haloacetamido analogues (fluoro, chloro, and bromo) of 2-deoxy-2-acetamido-D-mannose and their tetra-*O*-acetates were prepared from D-mannosamine hydrochloride, with either chloroacetic or bromoacetic anhydride or by dicyclohexylcarbodiimide-activated condensation with fluoroacetate followed by acetylation. Comparative specific rotations and ^{13}C and ^{1}H NMR spectra were consistent with a β configuration for the tetra-O-acetylated derivatives. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(bromoacetamido)-β-D-mannose and the corresponding analogue of glucose inhibited [^{3}H]thymidine incorporation into mouse L1210 leukemia cells by 50% (IC_{50}) at concentrations between 6 and 9 μM. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(chloroacetamido)-β-D-mannose was 3-fold more active in the thymidine-incorporation assay (143 ± 24 μM, IC_{50}) than was the corresponding analogue in the glucose series (425 ± 62 μM; $p = 0.005$). All of the haloacetamido free sugars, as well as the tetra-*O*-acetates of the fluoroacetamido analogues in the glucose, galactose, and mannose series, were inactive in the thymidine incorporation assay at 1 mM. In the mannose series the tetra-O-acetylated chloroacetamido and bromoacetamido analogues, as well as the bromoacetamido free sugar, could be administered at relatively high in vivo tolerated doses compared to the corresponding analogues in the galactose and glucose series. These three mannose analogues produced high proportions of cures of Ehrlich tumor-bearing B6D2F$_1$ mice, whereas in the galactose and glucose series only the tetra-O-acetylated bromoacetamido analogues had previously produced in vivo chemotherapeutic activity.

Carbohydrate analogues with the potential for altering cell-surface carbohydrate biochemistry might be useful for cancer chemotherapy in vivo or for producing altered host response to tumor cells treated in vitro and reimplanted. To test this approach, we synthesized haloacetamido analogues of 2-deoxy-2-acetamido-β-D-glucose and of 2-deoxy-2-acetamido-β-D-galactose and studied their effects in vitro against Friend murine erythroleukemia cells[1] and in vivo in mice challenged with Ehrlich ascites tumor.[2] *N*-Bromoacetamido analogues in both the glucose and galactose series when administered as the tetra-*O*-acetates produced a high proportion of cures after only a single injection. Drug-induced host participation in the curative effect was suggested because increased infiltration of host polymorphonuclear leucocytes into the peritoneal site of tumor challenge and drug treatment was observed with the carbohydrate analogues but not with other lipophilic alkylating agents.[2,3] These results may be related to studies indicating that receptors for cell-surface carbohydrate residues are important in the functioning of macrophages[4] and polymorphonuclear leucocytes.[5]

The results with the glucose and galactose analogues suggested that synthesis and evaluation of similar analogues in the mannose series would be useful for determination of the carbohydrate specificity of the effects obtained with the hexosamine analogues. Moreover, 2-deoxy-2-acetamido-D-mannose is a metabolic precurser for sialic acid, which in turn plays an especially critical role in cell-surface biochemistry.

In this article we detail the syntheses of fluoro, chloro, and bromo analogues of 2-deoxy-2-(haloacetamido)-D-mannose as the free sugars and as the tetra-*O*-acetates. Biological activity of these analogues and of the corresponding analogues in the glucose and galactose series is determined in vitro by inhibition of tritiated thymidine incorporation into L1210 leukemia cells. In vivo toxicities and effects against Ehrlich tumor cell challenges are determined for the mannose analogues in B6D2F$_1$ mice in order to permit comparison with similar data obtained for the glucose and galactose analogues in this and earlier[1,2] work. Animals tolerated higher doses of the bromoacetamido and chloroacetamido analogues in the mannose series compared to doses previously used successfully with the corresponding analogues in the glucose and galactose series.[1,2] High proportions of cures of Ehrlich tumor-bearing animals were obtained not only with the tetra-O-acetylated derivatives of the bromoacetamido analogue but also with the tetra-O-acetylated chloroacetamido analogue and with the bromoacetamido free sugar derivative.

Scheme I



Chemistry. The chloroacetamido and bromoacetamido analogues of 2-deoxy-2-acetamido-D-mannose (**2** and **3**) were prepared (Scheme I) by reacting the corresponding haloacetic anhydride with D-mannosamine hydrochloride in aqueous methanol–triethylamine. The fluoroacetamido analogue (**1**) was prepared by the method of Dwek et al.[6] for the preparation of the glucose analogue by dicyclohexylcarbodiimide-activated condensation of fluoroacetic acid with D-mannosamine hydrochloride in aqueous pyridine. The free sugars were obtained as the monohydrates and were tetra-*O*-acetylated with acetic anhydride (Scheme

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Table I. Yields and Chemical Characterization of 2-Deoxy-2-(haloacetamido)-D-mannoses

compd	no.	mp, °C	yield,[b] %	formula	anal.[c]	M_r
ManNFAc[a]	1	61-63	38	$C_8H_{14}NO_6F$	C, H, N; F[d]	238
ManNClAc·H_2O	2	96.5-98	20	$C_8H_{16}NO_7Cl$	C, H, N, O, Cl	273
ManNBrAc·H_2O	3	98-99	28	$C_8H_{16}NO_7Br$	Br	317
$ManNF(Ac)_5$	4	146-147	11	$C_{16}H_{22}NO_{10}F$	C, H, N, F	406
$ManNCl(Ac)_5$	5	179.5-180	38	$C_{16}H_{22}NO_{10}Cl$	C, H, N, O, Cl	422
$ManNBr(Ac)_5$	6	176-176.5	31	$C_{16}H_{22}NO_{10}Br$	H, N; C;[e] Br[f]	467

[a] ManNFAc (1) was obtained as the freeze-dried anhydrous powder. The hydrate could not be crystallized. [b] Yields for the free sugars are based on Man·HCl as starting material and are for the highest purity recrystallized material obtained for compounds 2 and 3. For compound 1 the yield is for the anhydrous nonrecrystallized product. Yields for the tetra-*O*-acetates are based on the corresponding free sugars as starting material and are for the highest purity recrystallized material obtained. [c] Due to interference between oxygen and bromine analyses experienced by Galbraith Laboratories, bromide analysis for compounds 6 and 3 were performed by us using a halide amperometric titrator. Other elemental analyses for compound 6 courtesy of DuPont Chemical Co., Wilmington, DE. Structure of compound 6 confirmed by mass spectrometry (see Experimental Section). [d] F: calcd, 7.93; found, 7.48. [e] C: calcd, 41.1; found, 40.6. [f] Br: calcd, 17.1; found by halide titration, 16.1.

Table II. Specific Rotations of 2-Deoxy-2-(haloacetamido)-D-hexoses and Their 1,3,4,6-Tetra-*O*-acetates

compd	no.	$[\alpha]^{22}_D$, deg found	$[\alpha]^{22}_D$, deg lit.	ref
		A. Free Sugars[a]		
ManNFAc	1	+14.4		
GlcNFAc		ND	+23, +31	6, 8
GalNFAc		+69.3		
ManNClAc·H_2O	2	+7.6		
GlcNClAc		+33.6	+25	8
GalNClAc		+72.1		
ManNBrAc·H_2O	3	+3.3		
GlcNBrAc		+32.5	+27	9
GalNBrAc		+69.3		
ManNAc·H_2O		+11.2	+10	10
GlcNAc		+41.4	+40.9, +41.2	11, 12
GalNAc		+85	+80 (50 h)	13
			+85	14
		B. Tetra-*O*-acetates[b]		
$ManNF(Ac)_5$	4	-9.3		
$GlcNF(Ac)_5$		+1.8	-1.2, -5.4	6, 15
$GalNF(Ac)_5$		+12.3		
$ManNCl(Ac)_5$	5	-27.4		
$GlcNCl(Ac)_5$		+12.1		
$GalNCl(Ac)_5$		+17.3		
$ManNBr(Ac)_5$	6	-31.4		
$GlcNBr(Ac)_5$		+12.7		
$GalNBr(Ac)_5$		+16.0		
$ManN(Ac)_5$		-19.2	[for β-L-$ManN(Ac)_5$: +19]	10
$GlcN(Ac)_5$		+93	+92 (α), +1.2 (β)	12
$GalN(Ac)_5$		ND	+102 (α), +7 (β)	13

[a] Measured as 1% solutions, in H_2O, after 2 h. Loss of bromide or chloride measured by quantitative amperometric titration was negligible after 2 to 4 days. [b] Measured as 1% solutions in $CHCl_3$.

I). 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(fluoroacetamido)-β-D-mannose (4) and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(chloroacetamido)-β-D-mannose (5) were prepared with pyridine as base, but the bromoacetamido group reacted with pyridine requiring that 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(bromoacetamido)-β-D-mannose (6) be prepared in acetonitrile with triethylamine as base. Table I lists the compounds synthesized, the yields obtained, and analytical characterization. The specific rotations are presented separately in Table II to facilitate comparison with the corresponding analogues in the glucose and galactose series. This table also presents corrected values for some of the specific rotations in the glucose and galactose series which were incorrectly reported to us from a commercial laboratory for our previous publication[1] and have now been corrected in our laboratory, and our corrections have been confirmed by reanalyses conducted by the original laboratory.

Results and Discussion

Comparative in Vivo Toxicities. Table III presents the in vivo toxicity determinations for the newly synthesized mannose analogues in comparison with the corresponding analogues in the glucose and galactose series. In general, the mannose free sugars 1–3 appear to be less toxic than the corresponding analogues in the other two series. Tetra-*O*-acetylation of the fluoroacetamido analogue 1 to produce 4 generates a 4-fold increase in host toxicity, an increase which is not observed when the corresponding analogues in the glucose and galactose series are administered as the tetra-*O*-acetates.[1] Compounds 5 and 6 are significantly less toxic than the corresponding derivatives in the galactose series and appear to be similar in toxicity to the analogues in the glucose series.

Effects on L1210 Leukemia Cells in Vitro. All of the newly synthesized mannose analogues and most of the corresponding analogues in the glucose and galactose series were tested for in vitro inhibition of tritiated thymidine incorporation into L1210 leukemia cells. The free sugars have no measureable effect on thymidine incorporation until a concentration of 2.5 mM is used. Table III shows that even at this high concentration the free sugars are either only modestly inhibitory or have no measureable effect. The tetra-O-acetate derivatives, on the other hand, show a pattern of in vitro activity consistent with that previously observed when analogues in the glucose and galactose series were tested for their ability to inhibit proliferation of dividing tumor cells in culture.[1] Table III shows that the tetra-O-acetylated derivatives of the fluoroacetamido analogues are inactive at concentrations up to 1 mM. The chloroacetamido analogues are intermediate in their inhibitory activity, while the bromoacetamido analogues produce 50% inhibition of thymidine incorporation at concentrations between 6 and 9 μM. It is noteworthy that 5 is 3-fold more active in vitro than is the corresponding analogue in the glucose series, and the difference if highly significant ($p = 0.005$).

Effects on Ehrlich Tumor-Bearing Hosts. The increased in vitro activity of compound 5 in comparison with the corresponding analogue in the glucose series indicated that 5 might exhibit in vivo antitumor activity not previously observed for the corresponding analogues in the glucose series. Moreover, the lower host toxicities of compounds 2, 3, and 5 compared to host toxicities of the corresponding analogues in the other series suggested that levels of drug administration could be reached in vivo with the mannose analogues that could not be attained with corresponding analogues in the other series. The higher

Table III. Effects of 2-Deoxy-2-(haloacetamido)-D-hexoses on B6D2F$_1$ Mice and on L1210 Leukemia Cells in Vitro

compd	no.	LD_{50},[a] mmol/kg	% of control[f] at 2.5 mM
A. Free Sugars			
ManNFAc	1	1.6[b]	93
GlcNFAc		0.18[c]	ND[g]
GalNFAc		0.74[c]	ND[g]
ManNClAc·H_2O	2	>3.7	97
GlcNClAc		2.0[c]	97
GalNClAc		1.5[c]	51
ManNBrAc·H_2O	3	>3.2	79
GlcNBrAc		>2.4[c]	95
GalNBrAc		1.7[c]	68
B. Tetra-O-acetates			IC_{50},[h] μM
$ManNF(Ac)_5$	4	0.42 (0.28-0.63)[d]	>1000
$GlcNF(Ac)_5$		0.17[c]	>1000
$GalNF(Ac)_5$		0.98[c]	>1000
$ManNCl(Ac)_5$	5	1.60 (1.27-2.02)[d]	143 ± 24[i] (4)
$GlcNCl(Ac)_5$		1.12 (0.80-1.57)[d]	425 ± 62[i] (3)
$GalNCl(Ac)_5$		0.83 (0.67-1.00)[d]	220 (1)
$ManNBr(Ac)_5$	6	0.36 (0.29-0.46)[d]	9.4 ± 5 (2)
$GlcNBr(Ac)_5$		0.36 (0.18-0.53)[e]	6.5 (1)
$GalNBr(Ac)_5$		0.24 (0.19-0.29)[e]	ND[g]

[a] Single dose (ip) that killed 50% of the animals. [b] Calculated by the method of Weil[16] using four animals per group and a dose increment of 3. [c] Values are taken from ref 1. [d] Calculated by the method of Weil[16] using four animals per group and a dose increment of 1.5. Values in parentheses are 95% confidence intervals. [e] Average of separate determinations [four for $GlcNBr(Ac)_5$ and three for $GalNBr(Ac)_5$] using two animals per group and a dose increment of 2. Values in parentheses are 95% confidence intervals based on the standard error of the separate determinations. [f] Thymidine incorporation assay in vitro. [g] ND designates value not determined. [h] Concentration that inhibited thymidine incorporation in vitro by 50%. Number of determinations in parentheses. Values for multiple determinations are means and SE for three or more determinations, range for two determinations. [i] Significantly different from each other ($p = 0.005$ by two-tailed Student's t test).

doses possible with compounds **2**, **3**, and **5** might enable these mannose analogues to produce antitumor activity. In previous studies with analogues in the glucose and galactose series, only the tetra-O-acetylated bromoacetamido analogues, corresponding to **6** in the mannose series, had exhibited in vivo activity against Ehrlich tumor.[2] Table IV shows that the chloroacetamido analogue **5** does produce a high proportion of in vivo cures, as does the free sugar derivative **3**, but **2** is inactive. Compound **6** retains the in vivo activity previously observed with the corresponding glucose and galactose analogues, although a slightly higher dose of this mannose analogue is required compared to the effective dose of the corresponding analogue in the other two series.

In the earlier work,[2] the tetra-O-acetylated chloroacetamido derivative in the galactose series was ineffective at a dose of 0.25 mmol/kg, whereas in this present work the chloroacetamido analogue in the mannose series, compound **5**, required a dose of 0.7 mmol/kg to produce measureable in vivo activity. Subsequent work with the chloroacetamido analogue in the galactose series has shown that 0.8 mmol/kg, the limit of host toxicity, does not

Table IV. In Vivo Chemotherapy of Ehrlich Tumor in B6D2F$_1$ Mice with Derivatives of 2-Deoxy-2-(haloacetamido)-D-mannose[a]

compd	no.	dose,[b] (mmol/kg)/day	days after tumor challenge	60-day survivors, no. of survivors/no. treated
$ManNBr(Ac)_5$	6	0.22	1	4/4
		0.19	1	1/8
			2	1/8
			3	1/8
		0.13	1	3/8
$ManNCl(Ac)_5$	5	0.71	1	1/8
		0.71	1	4/8
		0.35	3	
		0.71	1, 5	8/8
$ManNF(Ac)_5$	4	0.12	1	0/5
ManNBrAc·H_2O	3	3.2	1, 4	2/4
		2.5	1	5/8
		2.5	1, 5	3/8
ManNClAc·H_2O	2	3.7	1, 4	1/5
ManNFAc	1	0.42	1, 2	0/5
controls				
untreated				0/12
saline vehicle			1, 5	0/8
Tween-saline vehicle			1, 5	0/9

[a] Mice weighing 18–24 g, challenged ip day 0 with 2.5×10^7 Ehrlich tumor cells, treated ip as indicated. Compounds 4–6 were administered as suspensions in 0.85% NaCl solution that was 1% in Tween-80. Compounds 1–3 were administered in 0.85% NaCl solution. [b] Doses shown caused no drug-induced deaths and include the highest in vivo tolerated doses.

produce activity against Ehrlich tumor (McCarthy and Fondy, unpublished). In the earlier work,[2] a cumulative dose of 1.2 mmol/kg of the tetra-O-acetylated chloroacetamido analogue in the glucose series produced some tumor reduction in vivo but no long-term cures. Subsequent studies have shown that a single dose of 1.1 mmol/kg is also ineffective. Thus, the tetra-O-acetylated chloroacetamido analogue in the mannose series (**5**) produces significant activity against Ehrlich tumor in vivo, whereas the galactose analogue is inactive to the limit of host toxicity, and the glucose analogue is not markedly active at a dose level comparable to that at which **5** is effective.

Experimental Section

General. Melting points were determined with a Buchi Model M-50 apparatus and are uncorrected. Proton NMR spectra were obtained on a Varian A-60-A spectrometer and ^{13}C NMR spectra on a Varian XL-100-15 spectrometer with tetramethylsilane as internal reference. Reference spectra for 1,3,4,6-tetra-O-acetyl-2-deoxy-2-acetamido-β-D-mannose gave the following results: ^1H NMR ($CDCl_3$) δ 5.90 (d, 1 H, H-1, $J_{1,2}$ = 2 Hz), 4.28–4.12 (d, 2 H, H-6, H-6'), 2.10–2.00 (15 H, $5CH_3CO$); ^{13}C NMR ($CDCl_3$) δ 91.09 (C-1), 73.77 (C-5), 71.57 (C-3), 65.79 (C-4), 62.37 (C-6), 49.79 (C-2), 23.35 ($NCOCH_3$), 20.73 ($OCOCH_3$). Mass spectrometry was performed on **6** through the courtesy of DuPont Chemical Co., Wilmington, DE. NMR data on all compounds and mass spectrometric analysis of **6** were consistent with the proposed structures. Microanalyses for the fluoroacetamido and chloroacetamido analogues were obtained from Galbraith Analytical Laboratories, Knoxville, TN. However, Galbraith Laboratories was unable to provide consistent and reproducible analyses of the bromoacetamido analogues and obtained erratic total recoveries of the five atomic constituents. Therefore, we performed quantitative bromide analysis on **3** and **6** in our laboratory with an Aminco-Cotlove halide titrator. Elemental analysis for C, H, and N was provided for **6** through the courtesy of DuPont Chemical Co.

Optical rotations were measured at 22 °C in a Perkin-Elmer Model 141 spectropolarimeter set at 589 nm. The free sugars were prepared as 1% solutions in water and the measurements were begun immediately and followed for 2 h. Additional readings were taken daily for 2 to 4 days. The values reported in Table II are at 2 h. Additional change in optical activity after that point was negligible. No measureable halide loss was detected by quantitative analysis with the halide titrator. The tetra-*O*-acetate derivatives were measured as 1% solutions in $CHCl_3$. In order to confirm our results, we reexamined several of the glucose and galactose analogues whose rotations had been provided to us by a commercial consulting company and had been presented in our previous publication.[1] We found that most of those values were questionable. We therefore remeasured all of the previously published values and confirmed our procedures by measuring the optical activity of 2-deoxy-2-acetamido-D-hexoses and their tetra-*O*-acetates whose specific rotations are well established. The specific rotations of the newly synthesized mannose analogues, the revised values for the glucose and galactose analogues, and the relevant literature values are presented in Table II. Melting point, percentage yields, and analytical determinations are given in Table I. TLC of the free sugars was carried out as previously described.[1] TLC of the tetra-*O*-acetates was performed on silica gel plates with ethyl acetate as developing solvent. Compounds were visualized by sulfuric acid spray or by sugar acetate spray reagent prepared from hydroxylamine hydrochloride and ferric nitrate.[7] R_f values for the tetra-*O*-acetates were as follows: **4**, 0.80; **5** and **6**, 0.52; $ManN(Ac)_5$, 0.64.

Biology. Toxicities (LD_{50} values) were determined in $B6D2F_1$ mice using four animals per group and dose increments of 1.5 to 3 as noted in Table III. Ascites L1210 leukemia cells were obtained from DBA/2 passage hosts 5 days after intraperitoneal implantation with 10^6 cells, washed in 0.85% sterile NaCl solution, and suspended at 2×10^7 cells per milliliter in RPMI-1640 medium. One milliliter of cell suspension was pipetted into 15-mL sterile conical centrifuge tubes and equilibrated at 37 °C in 5% CO_2. Fifty micromoles of the tetra-*O*-acetate analogues were dissolved with gentle warming in 0.5 mL of 95% EtOH and added to 49.5 mL of RPMI-1640 medium that was 10% in fetal bovine serum and 2% in penicillin–streptomycin and had been preequilibrated to 37 °C. Aliquots of the analogues were pipetted immediately into the 1 mL of cell suspension, and the total volume in each tube was brought to 2.0 mL with temperature-equilibrated RPMI medium with fetal bovine serum and antibiotics as above. Tubes were incubated for 1 h with gentle swirling every 15 min, and then the cells were collected by centrifugation, washed 2 times with medium, and resuspended to 2.0 mL. Each well of a microtiter plate received 0.2 mL of RPMI-1640 medium with serum and antibiotics and 20 μL of cell suspension. The plates were equilibrated at 37 °C in 5% CO_2 for 1 h, 50 μL of a solution of [^{3}H]thymidine to give 1.0 μCi per well was added, and the incubation was extended for 15 h. Cells were harvested with a Bellco microharvester and washed with trichloroacetic acid, and radioactivity was determined in a scintillation counter. Control incubations received appropriate dilutions of 95% ethanol in RPMI-1640 medium and were treated in the same manner as tubes receiving the analogues. Effects of the free sugars were determined in the same manner as for the tetra-*O*-acetates, except that the compounds were dissolved directly into the incubation medium. In vivo chemotherapy testing was conducted as detailed earlier[1,2] by ip drug administration against ip challenges with 2.5×10^7 Ehrlich tumor cells, using dose schedules shown in Table IV.

Materials. D-(+)-Mannosamine hydrochloride was obtained from Sigma Chemical Co. Haloacetic anhydrides and fluoroacetic acid were obtained as previously given.[1] Tritium-labeled thymidine was obtained from ICN Chemical Co.

Syntheses. 2-Deoxy-2-(fluoroacetamido)-D-mannose (1). Fluoroacetic acid (volatile, toxic) (3.9 mL, 50 mmol) was dissolved in 10 mL of water at 0 °C and adjusted to pH 8 with chilled 3 N NaOH with stirring over a 15-min period, after which D-mannosamine hydrochloride (5.4 g, 25 mmol) was added. DCC (10.3 g, 50 mmol) dissolved in 80 mL of pyridine was added dropwise with stirring in an ice bath over a 1-h period, and then the reaction mixture was allowed to warm to room temperature and stirred for an additional 20 h. H_2O (300 mL) was added, the white precipitate was collected and washed with 50 mL of H_2O, and the combinated filtrate and washings were extracted with Et_2O (3×125 mL). The aqueous phase was concentrated on a flash evaporator not exceeding 40 °C to a syrupy amber residue, which was taken up in 15 mL of BuOH–EtOH–H_2O chromatography solvent and chromatographed on a microcrystalline cellulose column (3.5×100 cm). Fractions were collected and tested for material positive for Benedict's test, and aliquots of positive fractions were examined by TLC on cellulose with the same developing solvent. Fractions that eluted at approximately 2.2 L contained material with R_f of 0.45 and were combined, concentrated to approximately 15 mL, and pumped under reduced pressure overnight to remove additonal BuOH. The residue was extracted with 2×5 mL of H_2O, and the aqueous extract was applied to a column of Dowex-50 ion-exchange resin in the H^+ form (3.5×50 cm). The column was developed with H_2O, and fractions containing material with R_f 0.45 were identified as above, combined, and evaporated to a glassy solid (3.2 g). The monohydrate could not be crystallized from wet BuOH, so the glassy material was crushed to a fine white powder and 2.4 g was dissolved in anhydrous ethanol (6 mL), filtered, and precipitated with anhydrous Et_2O. The extremely hygroscopic white precipitate was collected under anhydrous conditions and dried in vacuo to yield 1.2 g.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(fluoroacetamido)-β-D-mannose (4) was prepared from 1.0 g (4.2 mmol) compound 1 dissolved in 7 mL of dry pyridine and treated with 4.8 mL of acetic anhydride at 0 °C. After 2 h the reaction mixture was treated with 8 mL of cold MeOH to stop the reaction, concentrated to low volume, dissolved in EtOAc, and chromatographed on a silica gel column with EtOAc. Fractions containing acetylated sugar were identified with sugar acetate spray reagent and examined for purity by TLC on silica gel with EtOAc as solvent, and fractions containing essentially pure material with R_f 0.8 were combined, concentrated, and pumped at reduced pressure overnight to yield 1.5 g of a yellowish syrup. The material was dissolved in 5 mL of CHC_3, treated with 45 mL of anhydrous Et_2O, and allowed to crystallize for 2 weeks at −5 °C, yield 175 mg (0.43 mmol) of **4**: ^{1}H NMR ($CDCl_3$) δ 5.95 (d, 1 H, H-1, $J_{1,2} = 2$ Hz), 4.88 (d, 2 H, FCH_2, $J_{H-F} = 48$ Hz), 4.33–4.15 (m, 2 H, H-6, H-6′), 2.10–2.00 (t, 12 H, $4CH_3CO$).

2-Deoxy-2-(chloroacetamido)-D-mannose (2). D-Mannosamine hydrochloride (7.3 g, 34 mmol) was dissolved in 8 mL of H_2O, added to 180 mL of MeOH and 6.3 mL of Et_3N, stirred for 20 min at 0 °C, then treated in an ice bath with 8.0 g (47 mmol) of chloroacetic anhydride with stirring, and allowed to react with stirring for 3 h at 0 °C. The reaction mixture was concentrated to about 15 mL on a flash evaporator without heating and then was applied directly to a microcrystalline cellulose column followed by a Dowex-50 H^+ column as detailed for compound 1: yield 3.55 g of a glassy material that appeared to be pure by TLC on cellulose. Recrystallization from 20 mL of *n*-BuOH saturated with H_2O at 0 °C was carried out without heating above 70 °C, yielding 1.82 g (6.6 mmol) of **2**, identified as the monohydrate by elemental analysis.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(chloroacetamido)-β-D-mannose (5) was prepared from 1.5 g (5.5 mmol) of **2** added to 10 mL of dry pyridine and stirred at room temperature until dissolved, then placed in an ice bath, and treated at 0 °C with

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acetic anhydride as described for 4: yield of white crystalline material after chromatography was 1.6 g; crystallization from $CHCl_3$–Et_2O afforded 900 mg (2.1 mmol) of compound **5**: ^1H NMR ($CDCl_3$) δ 5.95 (d, 1 H, H-1, $J_{1,2}$ = 2 Hz), 4.35–4.18 (m, 5 H, H-5, H-6, H-6′, $ClCH_2$), 2.15–2.05 (t, 12 H, $4CH_3CO$); ^{13}C NMR ($CDCl_3$) 90.51 (C-1), 73.47 (C-5), 71.51 (C-3), 65.18 (C-4), 61.80 (C-6), 50.19 (C-2), 42.77 ($NCOCH_2Cl$), 20.64 ($OCOCH_3$) ppm.

2-Deoxy-2-(bromoacetamido)-D-mannose (3) was prepared as described for **2** from 10 g (46 mmol) of D-mannosamine hydrochloride in 11 mL of H_2O, 180 mL of MeOH, 6.3 mL of Et_3N, and 11 g (42 mmol) of bromoacetic anhydride. Cellulose and Dowex chromatography yielded, after evaporation and rigorous drying with anhydrous MeOH, lyophilization, and prolonged pumping in vacuo, 6.3 g of a glassy solid (mp 96–97.5 °C). Crystallization from 35 mL of wet *n*-BuOH was performed without heating above 64 °C to yield 2.8 g of **3**. The supernatant from the crystallization was concentrated, extracted into water, purified by Dowex-50 H^+ ion-exchange chromatography, and yielded upon crystallization from wet *n*-BuOH an additional 1.2 g: total yield 4.0 g (13 mmol). The product appeared to be the monohydrate based on bromide analysis.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(bromoacetamido)-β-D-mannose (6) was prepared from 1.0 g (3.2 mmol) of **3**, 5 mL of acetic anhydride, in 20 mL of CH_3CN, and 1.6 mL of Et_3N by reacting for 5.5 h at 0 °C. Silica gel chromatography was carried out in a cold room to minimize bromide loss and using a large column (3.5 × 100 cm) because TLC revealed the presence of a substantial contaminant with R_f 0.4 compared to the product with R_f 0.5. Early fractions from the silica gel column after solvent concentration afforded 280 mg of crystalline product **6**. The later fractions were rechromatographed to yield an additional 200 mg: combined yield 480 mg (1.0 mmol). The products were examined by TLC and found to be free of measureable contaminants. Recrystallization was carried out in EtOAc–Et_2O: ^1H NMR ($CDCl_3$) δ 5.96 (d, 1 H, H-1, $J_{1,2}$ = 2 Hz), 4.26–4.15 (t, 3 H, H-5, H-6, H-6′), 3.99 (s, 2 H, CH_2Br), 2.10–2.01 (t, 12 H, $4CH_3CO$); mass spectrum, *m/e* (relative intensity) 410 (0.99) [M, ^{81}Br – $OCOCH_3$], 408 (0.94) [M, ^{79}Br – $OCOCH_3$], 388 (3.22) (M – Br), 328 (3.61) [M – Br – $OCOCH_3$], 277 (2.61), 240 (4.17), 233 (4.87), 192 (5.44), 190 (5.65), 180 (3.89), 173 (3.85), 172 (2.57), 150 (3.74), 148 (11.45), 139 (7.18), 138 (7.34), 130 (2.83), 118 (2.76), 97 (6.0), 85 (2.7), 72 (3.7), 60 (2.8), 43 (100, CH_3CO).

Anomeric Configuration of 4–6. The β configuration was assigned to the tetra-O-acetylated haloacetamidomannose derivatives based on the following evidence: (a) 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-acetamido-D-mannose [$ManN(Ac)_5$] prepared for comparison with the haloacetamido analogues was shown to be the β anomer by reference to the specific rotation of the L enantiomer (see Table II). ^{13}C NMR analysis gave a chemical shift for C-5 of 73.77 ppm for this β anomer, while C-5 of **5** gave a value of 73.47 ppm. These values are consistent with literature values for C-5 of β-D-mannose and its methyl glycoside (76.7 and 77.5 ppm, respectively),[17] since the β effect of the acetate groups at C-4 and C-6 would produce an upfield shift of 2–3 ppm in the value for C-5. Values for C-5 in α-D-mannose and its methyl glycoside are 73.2 and 73.6 ppm, respectively.[17] The β effect of the acetate esters should produce chemical shifts in the C-5 absorption in the range of 71 ppm if **5** were the α anomer. (b) Coupling constants for H-1 cannot be used to determine the anomeric configuration from proton NMR spectra of the mannose derivatives, but chemical shift values can contribute to such a determination. $ManN(Ac)_5$ gave a chemical shift for H-1 of 5.90 ppm and compounds 4–6 gave values of 5.95 to 5.96 ppm. H-1 of the α anomers would be expected to give chemical shift values of 6.1 ppm.[18] (c) Specific rotations for 4–6 and for $ManN(Ac)_5$ are shown in Table II to be −9, −27, −31, and −19°, respectively. Comparative specific rotations of anomeric D-mannosyl derivatives show that the α anomers have values approximately 60° more dextrorotatory than the corresponding β anomers.[18] Thus, if compounds **4–6** were α anomers, the specific rotations for the β-anomers would have to be approximately −70 to −90° and would be inconsistent with the value of −19° for the nonhalogenated acetamido β anomer. Subsequent work (Srivastava and Fondy, unpublished) has afforded the other anomer of both **5** and **6** from the mother liquors that produced the anomers crystallized and used in this work. Specific rotation for the other anomer of **5** was +33.6° and for the other anomer of **6** was +29.1°, confirming that compounds **5** and **6** used in this work are the β anomers. ^1H and ^{13}C NMR data for the more dextrorotatory (α) anomers confirm the conclusions presented in a and b above.

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