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Versatile intermediates in the selective modification of the amino function of 2-amino-2-deoxy-D-mannopyranose and the 3-position of 2-acetamido-2-deoxy-D-mannose: potential membrane modifiers in neoplastic control

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Abstract

A general method has been developed to selectively modify the amino group of 2-amino-2-deoxy-D-mannopyranose (D-mannosamine), a precursor of the terminal membrane sugar, sialic acid. 1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-α-D-mannopyranose oxalate was prepared via two routes that allowed introduction of various acyl groups onto the amino function. These compounds were evaluated for their antineoplastic properties. The most significant preclinical therapeutic finding was the antileukemic activity found in mice for tetra-*O*-acetyl-2-*epi*-streptozotocin (the acetylated α-mannosamine epimer of streptozotocin). Administration of 50 mg/kg/day × 5 to leukemia L1210-bearing DBA/2Ha mice resulted in 5/5 35-day survivors. Neutralization of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-mannopyranose oxalate under aqueous conditions led to 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranose, the oxidation of which gave 2-acetamido-4,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-D-*erythro*-hex-1-en-3-ulose. This agent demonstrated an IC_{50} [2] of 25 μM with a murine L1210 cell culture. Administration of 100 mg/kg/day × 5 resulted in 42% ILS[3] in DBA/2 mice with ip L1210 leukemia. Several other nonacetylated

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[2] IC_{50} = concentration for 50% inhibition of cell growth.

[3] ILS = increased life span.

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derivatives were also prepared by direct *N*-acylation, producing, for example, fluorescently tagged *N*-dansylmannosamine.

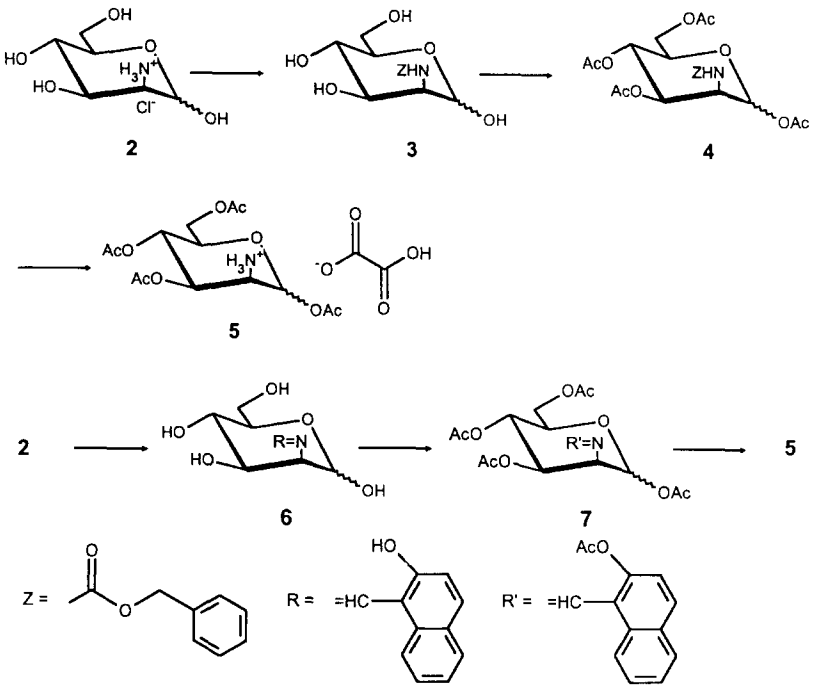
Keywords: D-Mannose, 2-amino-2-deoxy-; D-Mannose, 2-acetamido-2-deoxy-; Sialic acid; Membrane modifiers; Antileukemia testing, in vivo

1. Introduction

The importance of *N*-acetylmannosamine (2-acetamido-2-deoxy-D-mannopyranose, **1**) is realized in its multistep conversion to cytidine monophosphate-*N*-acetylneuraminic acid (CMP–NANA) and the eventual transfer of sialic acid to the terminal position of glycoprotein chains found on the plasma membrane of cells. Selective interference with the biosynthesis of NANA and its transfer to glycoproteins is a potential target for the treatment of neoplastic and viral diseases [1–6]. 3-*O*-Methyl-*N*-acetyl-D-glucosamine inhibits both rat liver *N*-acetylglucosamine kinase and *N*-acetylmannosamine kinase (early NANA metabolic enzymes) at 17 μM and 80 μM, respectively, in vitro [7]. Incorporation of [1-^{14}C]-*N*-acetylglucosamine and [1-^{14}C]-*N*-acetylmannosamine into cellular glycoproteins of human hepatoma cells (HepG2) was inhibited 88% and 70%, respectively, by 3-*O*-methyl-*N*-acetylglucosamine at 1 mM [7]. Similarly, *N*-propanoylglucosamine 6-phosphate inhibits *N*-acetylmannosamine kinase competitively and is metabolized to *N*-propanoylneuraminic acid [8]. Kayser et al. [8] studied the incorporation of [1-^{14}C]-*N*-propanoylglucosamine and [1-^{14}C]-*N*-propanoylmannosamine analogues into sialic acid in both membranes of different rat organs and serum glycoproteins. They observed an increased rate of incorporation for the *N*-propanoylmannosamine derivative into both serum glycoproteins and liver and lung membrane glycoproteins with respect to *N*-acetylglucosamine and *N*-acetylmannosamine. This demonstrates that it is possible to change the composition of cell-surface glycoproteins by incorporation of modified sialic acid precursors. Reduced or altered NANA composition in the plasma membrane may affect tumor growth and metastatic potential. Also, the review by Schirrmacher et al. [9] on the importance of cell-surface carbohydrates with respect to cancer cell adhesion, invasion and metastasis, along with the work of Ota et al. [10] on reduced sialylation of peanut agglutinin-binding sugar chains in metastatic B-16 melanoma cells, suggests various roles of sialic acid in neoplasms.

Hadfield et al. [11,12] have prepared 2-*N*-$COCF_3$-mannosamine analogues by direct acylation using ethyl trifluoroacetate in methanol, while Fondy and Emlich [13] have prepared haloacetamido analogs of mannosamine using aqueous methanol or aqueous pyridine conditions. Both of these approaches to modify the nitrogen function of mannosamine are limited to reagents that can endure polar solvent conditions.

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-D-mannopyranose (**5**) has been prepared (Scheme 1), which allows introduction of various acyl groups into the 2-amino group of *O*-acetylated mannosamine (Scheme 2). The extent of N-acylation is limited by the competition of O → N acetyl migration versus the rate of nucleophilic attack of the liberated 2-amino group in **5** on the acylating reagent (Scheme 2). Several unprotected mannosamine analogs were prepared by direct N-acylation under neutral aqueous conditions (Scheme 3).

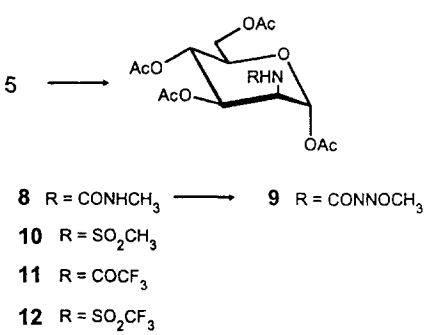


Scheme 1.

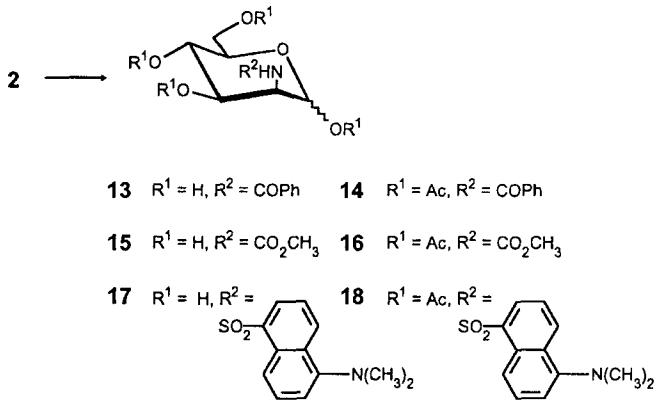
Neutralization of the oxalate salt of **5** under aqueous conditions (Scheme 4) gave 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranose (**22**), another potentially useful intermediate in the preparation of precursors to modified sialic acids.

2. Results and discussion

Two methods of synthesis of **5** were developed. In the first (Scheme 1), **2** was *N*-acylated to the *N*-benzyloxycarbonyl (CBZ) derivative **3** in 64% yield [14]. Com-



Scheme 2.

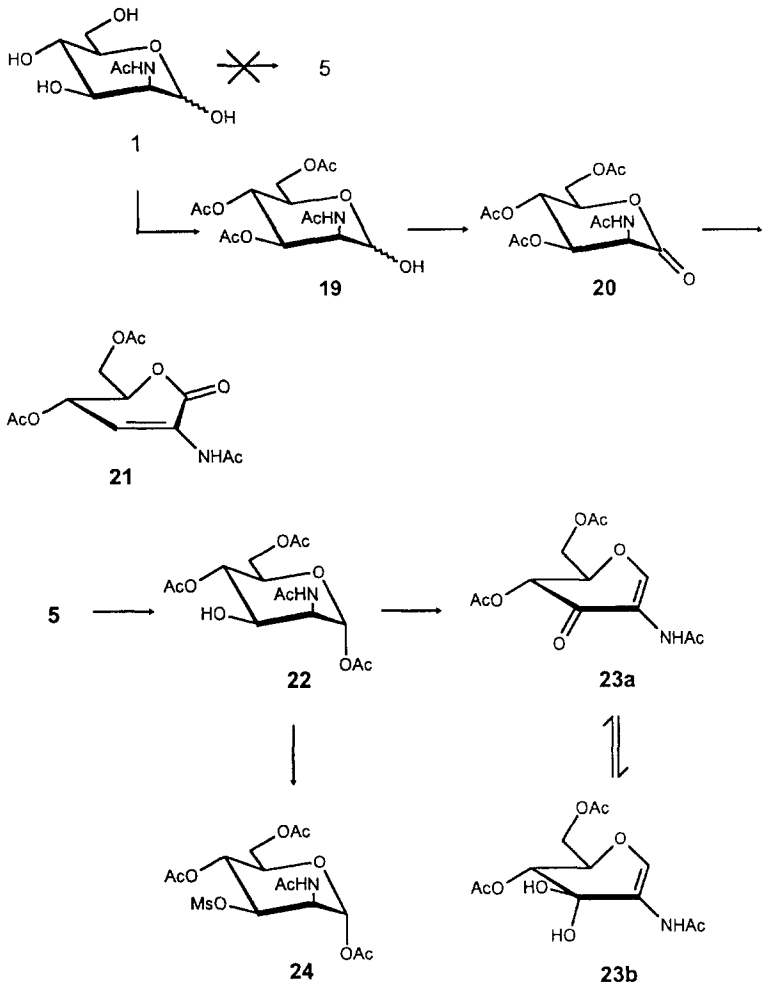


Scheme 3.

pound **3** was treated with acetic anhydride–pyridine to give **4** in 93% yield (anomeric ratio 78 α : 22 β). Hydrogenolysis of **4** with PtO_2 in acetic acid and excess oxalic acid gave **5** in 76% yield for an overall yield of 45% starting with **2**. In the second approach (Scheme 1), **2** was converted to the Schiff base **6** in 45% yield [15,16] and treated with Ac_2O–pyridine to give the acetylated derivative **7** in an anomeric ratio of $\alpha : \beta = 67 : 33$ with a yield of 75%. Treatment of **7** with oxalic acid in moist acetone resulted in the formation of **5** in 99% yield for an overall yield of 33% starting with **2**. Various acids were tried as trapping agents both in the hydrogenolysis of **4** and the cleavage of the Schiff base **7**, but oxalic acid gave the cleanest product, which was stable for many months at 4°C. With the protected mannosamine derivative **5** (oxalate or acetate), a series of 2-*N*-acyl-*O*-acetyl derivatives of mannosamine was prepared in varying yields (Scheme 2, 17–49%).

Anomeric assignment in the mannopyranose series using ^1H NMR spectroscopy is difficult when only one anomer is available since both anomers have similar coupling constants, $J_{1,2} \sim 1$–2 Hz, and in the ^{13}C NMR spectrum the C-1's of *N*-acetylmannosamine are almost coincidental [17]. Examination of **4** via ^1H NMR ($CDCl_3$, δ) indicated H-1α at 6.11 and H-1β at 5.87, with $J_{1,2}$ values of 1.85 Hz and 1.90 Hz, respectively. ^{13}C NMR ($CDCl_3$, δ) indicated the C-1's at 91.9 ($J_{C1,H1}$ 178.4 Hz) and 90.7 ($J_{C1,H1}$ 166.7 Hz), proving the predominant α anomer at lower field. The α anomer has a larger $J_{C1,H1}$ by ~ 10 Hz [18]. Evaluation of another derivative **9** indicated the following: ^1H NMR ($CDCl_3$, δ) 6.22 (H-1, $J_{1,2} = 2.11$ Hz) and ^{13}C NMR ($CDCl_3$, δ) 91.3 ($J_{C1,H1}$ 175.9 Hz). Thus, the α anomer was the only isomer isolated during synthesis of **8**. Preparation of **6** and **7** also gave predominantly the α anomer (see ^1H NMR data, Experimental section) so that **5** was α-enriched via either synthetic pathway.

In the case of the 2-benzamido derivative **13** and the 2-*N*-dansyl analogues **17** and **18**, the anomeric protons are not sufficiently resolved to give accurate anomeric ratios. The ratio of acetylated anomers also depends on the method of preparation, i.e., whether the acetylating mixture is pre-mixed before addition to the compound or whether the compound is allowed to anomerize in pyridine before addition of acetic anhydride.



Scheme 4.

Deacetylation of the protected analogues was problematic because of the possible epimerization and/or *N*-deacylation under basic conditions and furanose formation under acidic conditions. For example, we observed that **10** and **11** both decomposed on attempted deacetylation under basic conditions. The ureido compound **8** after deacetylation might be expected to rearrange to the 1,2-cyclic urea, and in fact it did rearrange or decompose upon silica gel (SiO_2) chromatography.

Compound **19** (refs [19] and [20]) was produced in only 12% yield by treating 2-acetamido-2-deoxy-D-mannopyranose (**1**) with acetyl bromide in an unsuccessful attempt to prepare 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-mannopyranosyl bromide as Micheel and Lengsfeld [21] had done for *N*-acetylglucosamine (Scheme 4). Compound **19** then was oxidized with CrO_3–HOAc to give the D-mannono-1,5-lactone **20**

Table 1
Effects of various 2-*N*-acyl derivatives of 2-amino-2-deoxy-D-mannopyranose on L1210 leukemia growth in cell culture and therapeutic efficacy *in vivo*

Compound	IC_{50} (L1210, μM)	Dose (MKD×5) [a]	%ILS (L1210) [b]
3	> 1,000	—	N.T. [c]
4	22	200	25
8	28% inhibition at 1,000 [d] μM	—	N.T.
9	50	50	5/5, 35-day survivors [e]
10	6.5	50	N.E. [f]
11	20	100	N.E.
12	480	50	13
13	N.E. at 1,000 μM	—	N.T.
14	N.E. at 100 μM [g]	200	21 [h]
16	71	1,000	14
17	18% inhibition at 100 μM	1,000	12
18	N.T.	—	N.T.
19	25	50	12
20	> 300	100	11
21	500	10	18
22	N.E. at 1,000 μM	—	N.T.
23a	25	100	42
24	5	500	26

[a] Mg kg^{-1} d^{-1} ×5 days×5 mice. ip (intraperitoneally). [b] A total of 10^6 cells 24 h before first injection; DBA/2 Ha female mice. [c] Not tested. [d] For 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-N^1-methylureido-β-D-glucopyranose [29], N.E. at 1,000 μM. [e] For 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-N^1-methyl-N^1-nitrosoureido-β-D-glucopyranose [29], 4/5 40 day survivors at 30 mkd, DBA/2 Ha mice. Streptozotocin: 5/5 Survivors, DBA/2 Ha at 50 mkd×5. [f] No effect. [g] Similar results for 1,3,4,6-tetra-*O*-acetyl-2-benzamido-2-deoxy-β-D-glucopyranose. [h] For 1,3,4,6-tetra-*O*-acetyl-2-benzamido-2-deoxy-β-D-glucopyranose, 19.5% ILS at 200 mkd×5 ip, 3 mice.

(ref. [22]), which upon treatment with K_2CO_3/EtOAc or chromatography on SiO_2 gave the 2,3-unsaturated-1,5-lactone **21** (ref. [22]) (Scheme 4).

Neutralization of **5** with aqueous NaOAc (Scheme 4) led to a rearrangement product, 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranose (**22**). The 3-OH substituent of **22** could be oxidized via modification of a method by Paquette and Wise [23] with concomitant 1-*O*-acetyl elimination giving 2-acetamido-4,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-D-*erythro*-hex-1-en-3-ulose (**23a**), a new amino sugar. Compound **23a** had an IC_{50} of 25 μM in L1210 cell culture. The hydrate **23b** forms to some extent on SiO_2 chromatography of **23a** with attendant spectral (IR and NMR) changes. Compound **22** was mesylated under usual conditions giving **24**. The biological results obtained for these compounds are summarized in Table 1.

To ensure that none of the mannosamine derivatives were converted to the furanose form during direct acylation, we prepared the 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-5,6-*O*-isopropylidene-α-D-mannofuranose by the method of Hasegawa and Kiso [24]; α-mannofuranose ^{1}H NMR ($CDCl_3$) δ 6.09 (d, > 1 H, H-1, J 3.57 Hz, plus partially exchanged NH) [25]. This J-value is almost twice that observed for the pyranose series.

The 2-*epi*-streptozotocin acetate derivative **9** (half-life of 2.1 h in 4:1 0.01 M $KHPO_4$–ethanol, pH 7.4, at room temperature protected from light) was shown to be

10-fold more toxic in β-cell culture than in pancreatic fibroblasts despite its moderate antileukemic activity [26,27]. It may be interesting to determine how this streptozotocin-like compound functions in the presence of a new *C*-methyl flavanone recently isolated from plants (2′-hydroxymatteucinol). The flavanone has a very strong hypoglycemic activity in streptozotocin-induced diabetic rats [28]. In a detailed biological study of **9**, Bernacki et al. [26,27] found that, at 3×10^{-4} M, 24 h after addition of **9** to L1210 cells, both protein and glycoprotein biosyntheses were significantly lowered; streptozotocin had little or no effect on macromolecular biosynthesis after 24 h, even up to 1 mM. We also prepared the acetylated β-streptozotocin compound [29]. [See Table 1 (footnote e) for comparison to the acetylated α-manno epimer, **9**.] Lastly, Sosnovsky and Rao [30] prepared an α,β-mixture of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-N^1-methyl-N^1-nitrosoureido-D-mannopyranose but found only 10% ILS against P388 lymphocytic leukemia in CD_2F_1 male mice (10^6 cells) at 16 mkd for 9 days (ip).

Further evaluation of these new mannosamine analogues may provide useful information to exploit the biosynthetic pathway of neuraminic acid to the detriment of cancer cells. Additionally, 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-mannopyranose (**5**), and 2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranose (**22**) are structurally novel compounds, which should prove to be extremely useful intermediates for the synthesis of additional carbohydrate analogues.

3. Experimental

All melting points were measured on a Mel-Temp apparatus and are uncorrected. Organic extracts were dried over Na_2SO_4 except as noted, and the products were purified with Bio-Sil A (100–200 mesh, Bio-Rad) or Silica Gel-60 (SiO_2) (0.04–0.063 mm mesh, E. Merck) column chromatography. Thin-layer glass plates were 2.5 × 10 cm and supplied by Analtech (Silica Gel GF, 250 μm). Compounds were detected by charring with 10% H_2SO_4–CH_3OH spray. UV spectra were determined on a Beckman model 25 spectrophotometer. IR spectra were measured on a Perkin–Elmer model 197 spectrometer in solid KBr pellets or in NaCl liquid cells. Optical rotations were made on a Perkin–Elmer model 141 polarimeter. ^{1}H NMR spectra were determined on a Varian EM-390 or Varian XL-100 spectrometer. ^{13}C NMR spectra were made on a Varian XL-100 spectrometer using the deuterated solvents indicated with $(CH_3)_4Si$ as internal standard or external $(CH_3)_4Si$ for D_2O samples. The 200 Mz spectrum was performed on a Bruker instrument. Analyses for samples reported are to within ±0.3%. Polyvinylpyridine was from Reilly Industries, Inc. and streptozotocin was purchased from Sigma Chemical Co.

*2-[(Benzyloxycarbonyl)amino]-2-deoxy-*D*-mannopyranose* (**3**).—To a solution of **2** ([31] and [32]) (6.00 g, 27.8 mmol) in water (15 mL) were added sodium acetate (4.55 g, 55.6 mmol), followed by *N*,*N*-dimethylacetamide (DMA, 30 mL). The solution was cooled to −20°C, and benzyloxycarbonylchloride (4.17 mL, 27.8 mmol) was added dropwise for 10 min, followed by DMA (8 mL). The bath temperature was raised to 0°C for 1 h and then stirred at 4°C for 2 days. After removal of solvents, the residue was dissolved in dry acetone, filtered to remove NaCl, evaporated, dissolved in 1:4

methanol–toluene, applied to an SiO_2 column (4 × 58 cm, 200–400 mesh) and eluted with the same solvent. Fractions with R_f 0.3 on TLC (1:4 CH_3OH–toluene) were combined, evaporated and redissolved in a minimum volume of acetone and precipitated with petroleum ether to yield 5.56 g (63.9%) of **3**: mp 65°C (dec) $[\alpha]_D^{25.5}$ −3.8° → −0.5° (16 h; *c* 1, MeOH). IR (KBr, cm^{-1}) 3350 (br OH), 2910 (CH), 1690 (NHCO). ^1H NMR (D_2O): δ 7.89 (s, 5, ArH), 5.59 (s, > 2H, PhCH_2 and H-1α, 53.5%), 5.43 (d, < 1H, *J* 1.44 Hz, H-1β, 46.5%). [Lit. ^1H NMR [14] (D_2O): δ 7.88 (5, ArH), 5.60 (PhCH_2 and H-1α), 5.44 (*J* 2 Hz, H-1, β); anomeric ratio α : β = 60 : 40; $[\alpha]_D^{25}$ −4° (*c* 1.0, EtOH]. ^{13}C NMR (acetone-d_6): δ 158.7 and 157.7 (C=O), 137.6 (C′-1), 128.9 (C′-2, C′-4), 128.5 (C′-4), 128.4 (C′-3, C′-5), 94.5 (C-1 coincident anomers), other ring carbons and $PhCH_2$ at 73.0, 70.0, 68.3, 68.0, 66.9, 62.2, 56.2. Anomeric ratio of α : β = 4.2 : 1 based on height of C=O peaks. Anal. Calcd for $C_{14}H_{19}NO_7$: C, 53.67; H, 6.11; N, 4.47. Found: C, 53.69; H, 6.35; N, 4.22.

1,3,4,6-Tetra-O-*acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy*-D-*mannopyranose* (**4**). —To a cooled (0°C) suspension of **3** (5.56 g, 17.2 mmol) in dry pyridine (100 mL) was added acetic anhydride (100 mL), and the mixture stirred for 6 h. After removal of the volatiles in vacuo, the residue was coevaporated with toluene and chromatographed on a 4 × 58 cm SiO_2-column using 98 : 2 CH_2Cl_2–CH_3OH as eluant. Fractions containing **4** were dissolved in anhyd ether (Et_2O) and coevaporated with petroleum ether and dried over P_2O_5 to give a glassy solid; yield 7.49 g (93.2%): mp 50°C (dec). IR (KBr, cm^{-1}) 3335 (br NH), 2945 (br C–H), 1760 (br C=O). ^1H NMR ($CDCl_3$): δ 7.38 (s, 5, ArH), 6.11 (d, 0.75 H, $J_{1,2}$ = 1.85 Hz, H-1α), 5.87 (d, 0.25 H, $J_{1,2}$ = 1.9 Hz, H-1β), 5.13 (s, 2, PhCH_2), 4.17 (s, 2 H, H-6), 2.17, 2.07, 2.03, 1.95 (4 × s, 12, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 170.5, 169.9, 169.5, 168.3, 168.0 CH_3C=O), 156.5, 155.9, (carbamate C=O), 136.0 (C′-1), 128.4, 128.1 (C′-2, 4; C′-3,5) 91.9 (C-1α, 78%, $J_{C1,H1}$ = 178.4 Hz), 90.7 (C-1β, 22%, $J_{C1,H1}$ = 166.7 Hz), 73.2, 71.3, 70.2, 69.1 (ring carbons), 67.1 ($PhCH_2$), 65.6 (ring carbon), 62.3 and 62.2 (C-6's), 20.6 (CH_3CO). Anal. Calcd for $C_{22}H_{27}NO_{11}$: C, 54.88; H, 5.65; N, 2.91. Found: C, 54.80; H, 5.78; N, 2.75.

1,3,4,6-Tetra-O-*acetyl-2-amino-2-deoxy*-α-D-*mannopyranose* · *(COOH)*$_2$ (**5**).—To a solution of **4** (0.050 g, 0.104 mmol) in glacial acetic acid (4 mL) were added oxalic acid (0.05 g, 0.4 mmol) and PtO_2 (5 mg), and the solution was hydrogenated for 2 h. After filtration (Celite), the solution was evaporated at 25°C and dried in vacuo over P_2O_5 and KOH. The residue was suspended in an anhyd acetone–ether mixture, filtered and washed with Et_2O, yielding 33 mg (73%) of **5**: mp 140–142°C. The analytical sample was prepared by dissolving **5** in warm, dry acetone, filtering and evaporating to a minimum volume. The crystals were washed with Et_2O: mp 154–155.5°C (dec); $[\alpha]_D^{25}$ +31.5° → +29.5° (24 h; *c* 1, HOAc). IR (KBr, cm^{-1}) 3400 br, 3150 sh, 2900 br (amine salt), 1730 (ester C=O). ^{13}C NMR (CD_3CO_2D): δ 178.0 ($COCH_3$), 163.0 ($HO_2CCO_2^-$), 90.1 ($J_{C\text{-}1,H\text{-}1}$ = 177.2 Hz, C-1α), 71.2, 68.1, 66.4, 63.3, 52.4 (ring C's), 20.7 ($COCH_3$). Anal. Calcd for $C_{14}H_{21}NO_9 \cdot (COOH)_2$: C, 43,94; H, 5.30; N, 3.20. Found: C, 43.68; H, 5.41; N, 2.93.

Compound **5** *via* **7** (*see below for preparation*).—To 0.182 g (0.319 mmol) of **7** (α anomer) in dry acetone (4.5 mL) chilled to 0°C were added oxalic acid (0.18 g, 1.43 mmol) and acetone (2.0 mL, not dried). A flocculant precipitate formed immediately, and the mixture was stirred at room temperature for 40 min, then chilled to 0°C, filtered,

washed with anhyd Et_2O and dried in vacuo. TLC of the product (6:4 Et_2O–petroleum ether) suspended in acetone indicated it to be free of starting material and aldehyde. The yield was 0.138 g (99%): mp 147–149°C (dec). IR identical to that of **5**.

Preparation of 2-deoxy-2[(2-hydroxynaphthylmethylidene)amino]-D-*mannopyranose* (**6**).—To **2** (0.108 g, 0.501 mmol) in water (0.1 mL) were added sodium acetate (0.045 g, 0.55 mmol), 2-hydroxylnaphthaldehyde (0.086 g, 0.50 mmol) and methanol (8 mL). After 3 h the reaction mixture was cooled to 0°C, filtered through Whatman #1 paper and washed with ice-cold water, then CH_2Cl_2, and lastly, Et_2O. Compound **6** [yield: 0.075 g (45%)] was recrystallized from hot CH_3OH: mp 191.5–193.5°C (dec). [Lit. 196–199°C (dec)] [15]. $[\alpha]_D^{23}$ −229° (*c* 0.54, dry pyridine). IR (KBr, cm^{-1}) 1630 (CH=N). ^1H NMR (Me_2SO-d_6 + drop D_2O): δ 9.02 and 8.93 (2s, 2H, 2 × (CH=N), 7.37 (m, 6 H, ArH), 5.17 and 4.99 (2 s, 0.7 H, H-1α and 0.3 H-1β), 3.57 (m, 6 H, carbohydrate-H)

2-[(2-Acetoxynaphthylmethylidene)amino]-1,3,4,6-tetra-O-*acetyl-2-deoxy*-α-D-*mannopyranose* (**7**).—To dry pyridine (100 mL) and acetic anhydride (100 mL) at 0°C was added **6** (4.30 g, 12.9 mmol). The mixture was stirred for 16 h at room temperature, evaporated at 30°C in vacuo to dryness and then coevaporated 1 × with toluene. The residue was dissolved in CH_2Cl_2 (400 mL) and washed 3 × with H_2O (Na_2SO_4). SiO_2 chromatography (4 × 29 cm) was performed eluting first with 6:4 Et_2O–petroleum ether to remove aldehyde and then 7:3 Et_2O–petroleum ether yield 1.42 g (20%) of the faster moving α anomer, followed by 4.16 g of the α,β-mixture for a total yield of 75.8%. A portion (0.22 g) of the α,β-mixture from above was chromatographed on a SiO_2 column (4 × 29 cm) eluting first with anhyd 6:4 Et_2O–petroleum ether, then with anhyd 7:3 Et_2O–petroleum ether, and finally with anhyd 8:2 Et_2O–petroleum ether. Crystals of **7** (0.085 g, 39%) were obtained from CCl_4–petroleum ether. Compound **7** was recrystallized from CCl_4: mp 157.5–160°C; $[\alpha]_D^{24}$ −137° (*c* 1, $CHCl_3$). IR (KBr, cm^{-1}), 1750 (CH_3C=O), 1643 (CH=N–). ^1H NMR ($CDCl_3$): δ 9.0 (d, 1H, ArH), 8.71 (s, 1 H, CH=N), 7.93 (t, 2 H, ArH), 7.59 (t, 2 H, ArH), 7.19 (d, 1H, ArH), 6.05 (d, 1 H, $J_{1,2}$ = 1.65 Hz, H-1α), 5.73 (t, 1 H), 5.24 (d, d, 1 H), 4.17 (m, 4 H), 2.38 (s, 3, $ArCH_3C$=O), 2.08 (s, 9, 3 × CH_3C=O), 1.98 (s, 3, CH_3C=O). Anal. Calcd for $C_{27}H_{29}NO_{11}$: C, 59.66; H, 5.38; N, 2.58. Found: C, 59.94; H, 5.58; N, 2.70.

1,3,4,6-Tetra-O-*acetyl-2-deoxy-2-*(N^1-*methylureido*)-α-D-*mannopyranose* (**8**).—A suspension of **5** (1.0 g, 2.3 mmol) in dry CH_2Cl_2 (50 mL) was cooled to −20°C. Methyl isocyanate (0.150 mL, 2.52 mmol) was added, followed by 1.08 g (9 mequiv) of polyvinylpyridine resin. The mixture was stirred at room temperature for 20 h, after which time it was filtered (Celite) and washed with dry CH_2Cl_2. The product was chromatographed on a silica gel column (2.5 × 39 cm), eluting with 9:1 EtOAc–toluene. Fractions with R_f 0.4 on TLC were combined and recrystallized from CH_2Cl_2–Et_2O yielding 0.286 g (31%) of **8**: mp 127°C (dec); $[\alpha]_D^{25.5}$ +25.9° (*c* 1, chloroform). IR (KBr, cm^{-1}) 3375 and 3270 (NHs), 2950 (CH), 1740 (ester and urea C=O). ^1H NMR ($CDCl_3$): δ 6.05 (d, 1 H, $J_{1,2}$ = 1.79 Hz, H-1α), 5.20 (m, 4 H), 4.52 (d, 1 H), 4.15 (m, 2 H), 2.78 (d, 3 H, $J_{N'\text{-}H,N'\text{-}CH_3}$ = 4.8 Hz, N'-CH_3), 2.15, 2.05, 2.03, 2.01 (4, 12 H, ester CH_3). Anal. Calcd for $C_{16}H_{24}N_2O_{10}$: C, 47.52, H, 5.98; N, 6.93. Found: C, 47.47; H, 6.20; N, 6.72.

1,3,4,6-Tetra-O-*acetyl-2-deoxy-2-(*N^1-*methyl-*N^1-*nitrosoureido)*-α-D-*mannopyranose*

(**9**).—Compound **8** (0.17 g, 0.42 mmol), followed by sodium nitrite (0.032 g, 0.46 mmol), was added to rapidly stirring concentrated formic acid (5 mL) at 0°C. After 10 min the solvent was evaporated at 20–25°C, and the residue dissolved in CH_2Cl_2, washed with water and dried (Na_2SO_4) and chromatographed on a silica gel column (1.2 × 35 cm) using 98:2 $CHCl_3$–CH_3OH as eluant. The product was detected by UV quenching on TLC plates using the same solvents. Fractions containing product were evaporated, and the residue was dissolved in Et_2O, filtered and petroleum ether was added. The solution was evaporated to a hygroscopic foam to give 0.173 g (95%) of **9**: $[\alpha]_D^{24}$ +6.0° (*c* 1, $CHCl_3$); mp 55°C (dec). IR (KBr, cm^{-1}) 3370 br (NH), 2950 br (CH), 1740 (ester C=O), 1480 (*N*-nitroso). ^1H NMR ($CDCl_3$): δ 7.26 (br d, 1 H, N-H), 6.22 (d, 1 H, $J_{1,2}$ = 2.1 Hz, H-1α), 5.35 (m, 2 H), 4.75 (m, 1 H), 4.16 (s with m at base, 3 H, H-6s and other proton), 3.18 (s, 3 H, NCH_3), 2.18, 2.05, 2.03, 2.01 (ester CH_3's). ^{13}C NMR ($CDCl_3$): δ 169.9, 169.2 (ester C=O), 91.3 (C-1α), $J_{C\text{-}1,H\text{-}1}$ = 175.9 Hz), 70.4, 69.1, 65.0, 61.5, 50.7 (ring C's), 26.7 (NCH_3), 20.8, 20.6 (ester CH_3's). Anal. Calcd for $C_{16}H_{23}N_3O_{11}$: C, 44.34, H, 5.35, N, 9.70. Found: C, 44.59, H, 5.62; N, 9.53.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-methylsulfonamido-α-*D*-mannopyranose* (**10**).—Methanesulfonyl chloride (0.18 mL, 2.35 mmol) in CH_2Cl_2 (2 mL) was added over a period of 5 min to 1,3,4,6-tetra-*O*-acetyl-D-mannopyranose · CH_3COOH [0.4 g, 0.98 mmol, prepared in the same manner as **5**, but without $(COOH)_2$ present] in dry CH_2Cl_2 (10 mL) contained in a three-necked flask swept with N_2 at −60°C. Dry pyridine (0.37 mL, 4.61 mmol) was then added and the temperature was allowed to rise gradually. After 2.5 h the reaction mixture was diluted with CH_2Cl_2 and washed 4 × with H_2O (Na_2SO_4). The residue was chromatographed on a 2.2 × 50 cm SiO_2 column using initially 99:1 CH_2Cl_2–CH_3OH then 98:2 CH_2Cl_2–CH_3OH for elution. The yield of **10** was 0.21 g (49.5%). The product was twice crystallized from CH_2Cl_2–anhyd Et_2O; to give pure **10**: mp 147–149°C (dec); $[\alpha]_D^{25}$ +31.6° (*c* 1H, $CHCl_3$). IR (KBr, cm^{-1}) 3275, 3220 (NH), 2950 (CH), 1745 (ester C=O). ^1H NMR ($CDCl_3$): δ 6.13 (d, 1 H, $J_{1,2}$ = 1.88 Hz, H-1α), 5.61 (d, 1 H, $J_{H\text{-}2,NH}$ = 9.4 Hz, NH), 3.08 (s, 3 H, $NHSO_2CH_3$), 2.20, 2.11, 2.10, 2.09 (4 × s, 12 H, 4 × $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 170.7, 170.0, 169.7, 168.2 (4 × C=O), 92.6 (C-1α), 70.6, 68.8, 65.4, 62.3, 53.3 (ring carbons), 42.0 ($NHSO_2CH_3$), 20.8 (4 × $COCH_3$). Anal. Calcd for $C_{15}H_{23}NO_{11}S$: C, 42.35; H, 5.45; N, 3.29. Found: C, 42.23; H, 5.64, N, 3.27.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-*D*-mannopyranose* (**11**).—To 1,3,4,6-tetra-*O*-acetyl-D-mannopyranose · CH_3COOH (0.328 g, 0.8 mmol, prepared without oxalic acid during reduction of **4** in dry CH_2Cl_2 (10 mL) contained in a flask swept with N_2 at −70°C was added dry Et_3N (0.4 mL, 2.88 mmol). Trifluoroacetic anhydride (0.2 mL, 1.42 mmol) in CH_2Cl_2 (4 mL) was next added over a 5 min period. The reaction mixture was stirred at room temperature for 1 h, diluted with CH_2Cl_2 and washed 4 × with H_2O (Na_2SO_4). The residue was chromatographed on a column of SiO_2 (2.2 × 50 cm) using 98:2 CH_2Cl_2–CH_3OH for elution. The combined fractions were evaporated, dissolved in anhydrous Et_2O, filtered, concentrated to a minimum volume and dropped into rapidly stirring petroleum ether (100 mL). The yield was 0.107 g or 30.1% of **11**: mp 137–139.5°C; $[\alpha]_D^{25}$ +69.1° (*c* 1, $CHCl_3$). IR (KBr, cm^{-1}) 3310 (NH), 1730 (ester C=O), 1549 ("amide II band"). ^1H NMR ($CDCl_3$): δ 6.59 (d, 1 H, NH), 6.13 (d, 1 H, $J_{1,2}$ = 1.84 Hz, H-1α), 5.33 (m, 2 H) 4.67 (m, 1 H, H-5), 4.19 (m, 3

H), 2.22, 2.11, 2.08, 2.02 (4 × s, 12, 4 × CH_3C=O). Anal. Calcd for $C_{16}H_{20}F_3NO_{10}$: C, 43.34; H, 4.55; F, 12.86. Found: C, 43.12; H, 4.71; F, 12.80.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-trifluoromethylsulfonamido-α-D-mannopyranose (**12**).—To 1,3,4,6-tetra-*O*-acetyl-D-mannopyranose · CH_3COOH (1.0 g, 2.46 mmol, prepared without oxalic acid during reduction of **4** in dry CH_2Cl_2 (10 mL) contained in a flask swept with N_2 at −70°C were added trifluoromethanesulfonic acid anhydride (0.6 mL, 3.67 mmol) in CH_2Cl_2 (2 mL), followed by dry Et_3N (1.5 mL, 10.8 mmol) over a 5 min period. Stirring was continued for 15 min, the cooling bath was removed and stirring was continued for another 15 min. The reaction mixture was diluted with CH_2Cl_2 and washed 5 × with H_2O (Na_2SO_4). The residue was chromatographed 2 × on a SiO_2 (2.2 × 50 cm) column eluting with 98:2 CH_2Cl_2–CH_3OH, then over a column of SiO_2 of the same size packed in CH_2Cl_2, eluting initially with same solvent and then with 98:2 CH_2Cl_2–CH_3OH. The combined fractions were evaporated, dissolved in CH_2Cl_2, treated with a small amount of charcoal for 15 min, filtered through Celite and evaporated. Crystals of **12** were obtained from a minimum volume of anhyd Et_2O; yield 0.202 g (17.2%). Recrystallization from Et_2O gave pure **12**: mp 160–164°C; $[\alpha]_D^{24}$ + 30.3° (*c* 0.99, $CHCl_3$). IR (KBr, cm^{-1}) 3170 (NH), 1750 and 1710 (CH_3C=O). ^1H NMR ($CDCl_3$): δ 6.14 (d, 1 H, $J_{1,2}$ = 1.75 Hz, H-1 α), 5.38 (m, 2 H), 4.22 (m, 4 H) 2.22, 2.13, 2.12 (3 × s, 12 H, CH_3C=O). Anal. Calcd for $C_{15}H_{20}F_3NO_{11}S$: C, 37.58; H, 4.21; F, 11.89. Found: C, 37.86; H, 4.31; F, 12.10.

2-Benzamido-2-deoxy-D-mannopyranose (**13**).—Compound **2** (0.5 g, 2.31 mmol) and sodium acetate (0.4 g, 4.88 mmol) were dissolved in water (2 mL), followed by the addition of DMA (5 mL). The reaction mixture was chilled to 0°C, then benzoyl chloride (0.3 mL, 2.60 mmol) in DMA (2 mL) was added dropwise over a 5 min period, and stirring was continued for 1.5 h. The solvents were evaporated at 40°C in vacuo. The residue was dissolved in 8:2 $CHCl_3$–CH_3OH, applied to a 2 × 50 cm column of Bio-Sil A (200–400 mesh) and eluted with the same solvent. The combined fractions were redissolved in a minimum volume of acetone, filtered, and the filtrate was added dropwise to rapidly stirring anhydrous Et_2O (250 mL). The precipitate was filtered and washed with Et_2O and dried to give 0.473 g (72.3%) of **13**: mp 90–95°C (dec); $[\alpha]_D^{26}$ − 15.9° → − 17.8° (*c* 1, water). IR (KBr, cm^{-1}) 3350 (OH), 2900 (CH), 1635, sh 1600 (NHCO). ^1H NMR (acetone-d_6): δ 7.87 and 7.48 (2 × m, 5 H, ArH), 5.30 (d, 1 H, *J* = 1.52 Hz, H-1), 5.06 (br s, 1 H), 3.79 (br s, 2 H, H-6s). Anal. Calcd for $C_{13}H_{17}NO_6$: C, 55.16; H, 6.05; N, 4.95. Found: C, 55.24; H, 6.32; N, 4.67.

1,3,4,6-Tetra-O-acetyl-2-benzamido-2-deoxy-D-mannopyranose (**14**).—Compound **13** (0.375 g, 1.33 mmol) in dry pyridine (10 mL) was chilled to 0°C, acetic anhydride (10 mL) was added, and the mixture was stirred for 16 h at room temperature. Solvents were evaporated in vacuo, and the residue then coevaporated 2 × with toluene. The product was dissolved in CH_2Cl_2 and washed 3 × with water (Na_2SO_4). The mixture obtained after evaporation was chromatographed on a 2 × 58 cm SiO_2 column eluting with 49.5:49.5:1 toluene–$CHCl_3$–CH_3OH. The fractions containing product were dissolved in Et_2O, filtered, concentrated to a minimum volume and added dropwise to 250 mL of rapidly stirring petroleum ether. The product was filtered and dried in vacuo, giving a yield of 0.425 g (71.2%): mp 73° (dec). IR (KBr, cm^{-1}) 3350 (NH), 2945 (CH), 1737 (ester C=O), 1660 (amide C=O). ^1H NMR ($CDCl_3$): δ 7.81, 7.51 (2 × m, 5 H, ArH),

6.18 (d, 0.6 H, $J_{1,2} = 2.0$ Hz, H-1 α), 5.98 (d, 0.4 H, $J_{1,2} = 1.67$ Hz, H-1 β), 4.21 (br s, 2 H, H-6's), 2.19, 2.08, 2.05, 2.00 ($4 \times$ s, 12, $4 \times CH_3C{=}O$). Anal. Calcd for $C_{21}H_{25}NO_{10}$: C, 55.87; H, 5.58; N, 3.10. Found: C, 55.79, H, 5.32; N, 3.05.

*2-Deoxy-2-[(methyloxycarbonyl)amino]-*D*-mannopyranose* (**15**).—Compound **2** (2 g, 9.28 mmol) and sodium acetate (2.28 g, 27.8 mmol) were dissolved in water (5 mL), and DMA (50 mL) was added. The temperature was lowered to −20°C, followed by dropwise addition of methyl chloroformate (0.860 mL, 11.1 mmol) while maintaining the temperature below 0°C. After 1 h the reaction mixture was evaporated in vacuo at 35°C to give a residue that solidified upon treatment with acetone. The solid was used in the preparation of **16** without further purification.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[(methyloxycarbonyl)amino]-α-*D*-mannopyranose* (**16**).—To the dry crude solid **15** from the preceding preparation, dry pyridine (75 mL) and acetic anhydride (75 mL) previously mixed at 0°C were added. After 2 h at room temperature, the solvents were evaporated in vacuo at 35°C, then coevaporated with toluene. The residue was dissolved in CH_2Cl_2 and washed $2\times$ with water (Na_2SO_4). The residue obtained by evaporation was chromatographed on a 4×30 cm column of SiO_2 eluting with ether. About 0.08 g of product obtained from the combined fractions was rechromatographed on a column of SiO_2 (2×30 cm) eluting with 90:9:1 CH_2Cl_2–Et_2O–CH_3OH. Crystals of product obtained from CCl_4–petroleum ether were washed with petroleum 95:5 ether–CCl_4, then with petroleum ether and dried in vacuo. The remainder of the product was purified in the same manner for a total yield of 1.62 g (42.6%, based on **2**): mp 118–120°C; $[\alpha]_D^{26}$ +44.4° (*c* 1, $CHCl_3$). IR (KBr, cm^{-1}) 3330 (NH), 2990, 2995 (CH), 1730 (ester C=O and carbamate C=O) ^{1}H NMR ($CDCl_3$): δ 6.07 (d, 1 H, $J_{1,2} = 1.89$ Hz, H-1α), 5.22 (m, 2 H, ring-H), 5.07 (br s, 1 H, NH exchanges), 4.18 (m, 4 H, ring-H), 3.71 (s, 3 H, $NHCO_2CH_3$), 2.17, 2.08, 2.05, 2.02 ($4 \times$ s, 12 H, $4 \times COCH_3$). Anal. Calcd for $C_{16}H_{23}NO_{11}$: C, 47.41; H, 5.72; N, 3.46. Found: C, 47.16; H, 5.82; N, 3.17.

*2-Deoxy-2-(5-dimethylamino-1-naphthylsulfonamido)-*D*-mannopyranose* · H_2O (**17**). —To 0.432 g (2.00 mmol) of **2** and 0.504 g (6.14 mmol) of sodium acetate in water (1 mL) and DMA (5 mL) chilled to −15°C, was added 0.522 g (1.93 mmol) of dansyl chloride. The bath temperature was allowed to reach 0°C and stirring was continued for 20 h at room temperature. Solvents were evaporated in vacuo, and the product was chromatographed on a SiO_2 column (2×58 cm) using 8:2 CH_2Cl_2–CH_3OH as eluant. The precipitate obtained from CH_2Cl_2–petroleum ether was filtered, washed with Et_2O and dried to give 0.30 g (35%) of **17**: mp 117–120°C (dec); $[\alpha]_D^{25}$ + 18.8° (*c* 1, MeOH). IR (KBr, cm^{-1}) 3300 br (OH), 2920, 2815, 2760 (CH). ^{1}H NMR (acetone-d_6): δ 8.50, 7.63, 7.10 ($3 \times$ m, 7 H, ArH and NH), 5.02 (d, 1 H, $J_{1,2} = 1.5$ Hz, H-1), 2.89 (s, 6 H, $N(CH_3)_2$. Anal. Calcd for $C_{18}H_{24}N_2O_7S \cdot H_2O$: C, 50.22; H, 6.09; N, 6.51. Found: C, 50.06; H, 5.91; N, 6.53.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(5-dimethylamino-1-naphthylsulfonamido)-*D*-mannopyranose* (**18**).—To 0.14 g (0.33 mmol) of **17** in dry pyridine (1 mL) cooled to 0°C, was added acetic anhydride (1 mL). The reaction was stirred for 6 h at room temperature, and the solvent was evaporated in vacuo. The residue was dissolved in $CHCl_3$ and washed $3\times$ with H_2O (Na_2SO_4). The product was chromatographed on a SiO_2 column (1.2×17 cm) using 95:5 $CHCl_3$–CH_3OH as eluant, and then $2\times$ on a

column of SiO_2 (2 × 22 cm) using 9 : 1 CH_2Cl_2–Et_2O. The compound was dissolved in a minimum volume of dry CH_2Cl_2 and added dropwise to 50 mL of rapidly stirring petroleum ether. The precipitate was washed with petroleum ether and dried in vacuo to yield 0.170 g (89.9%) of **18**: mp 87–90°C (dec); $[\alpha]_D^{27}$ −14.9° (*c* 1, chloroform). IR (KBr, cm^{-1}) 3450 and 3260 (NH), 2900 (CH), 1735 (ester C=O). ^1H NMR ($CDCl_3$): δ 8.60, 8.30, 7.58, 7.23 (d, m, q, m, 6 H, ArH), 5.81 (d, 1 H, $J_{1,2}$ = 1.80 Hz, H-1α), 5.18 (m, 3 H, NH and H-2), 4.32–3.70 (m, 4 H, ring-H), 2.90 (s, 6 H, N $(CH_3)_2$), 2.12, 2.06, 2.02, 1.52 (4 × s, 12 H, ester CH_3). Anal. Calcd for $C_{26}H_{32}N_2O_{11}S$: C, 53.78; H, 5.56; N, 4.83. Found: 53.97; H, 5.81; N, 4.71.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-mannopyranose* (**19**).—To 1.00 g (4.52 mmol) of **1** (ref. [21]) in a 25 mL three-necked flask chilled to 0°C, was added dropwise acetyl bromide (2 mL, 27 mmol). The ice-bath was replaced with a 45°C bath for 1 h (with cooling when the reaction became too vigorous). The reaction mixture was transferred to a separatory funnel with $CHCl_3$ (20 mL) and water (4 mL) and neutralized with solid $NaHCO_3$ ($CaCl_2$). A stable hydrobromide salt of tetra-*O*-acetylmannosamine could not be obtained using this method. Thus the residue obtained by evaporation was applied to a column of SiO_2 (2 × 58 cm), eluting first with $CHCl_3$ and then with 9 : 1 $CHCl_3$–CH_3OH. Crystals of **19** were obtained from EtOH–anhyd EtO_2 yielding 0.19 g (12.1%): mp after recrystallization was 156–159°C; $[\alpha]_D^{25}$ +37.7° → +33.9° (23 h, *c* 1, $CHCl_3$). [Lit. [19,20] mp 142–148°C, $[\alpha]_D^{20}$ +33.6° (*c* 0.8, $CHCl_3$)]. IR (KBr, cm^{-1}) 3380 (OH and NH), 1735 (ester C=O), 1660 (amide C=O). ^1H NMR (100 MHz, $CDCl_3$): δ 5.92 (d, 1 H, NH exchanges), 5.51 and 5.40 (dd, 1 H, H-3), 5.20 (d, 1 H, *J* = 1.6 Hz, H-1), 2.12, 2.07, 2.01 (3 × s, 12 H, ester and amide CH_3). ^1H NMR (Me_2SO-d_6): δ 8.01 (d, 0.87, *J* = 9.3 Hz, exchanges, NH) 7.67, (d, 0.13 H, *J* = 9.8 Hz, exchanges, NH), 7.13 (d, 0.84 H, $J_{1,\ HO\text{-}1}$ = 4.6 Hz, exchanges, HO-1), 7.01 (d, 0.16, *J* = 6.5 Hz exchanges, HO-1), 4.93, 4.88 collapses to 4.90 (dd, collapses to d, 1 H, $J_{1,2}$ = 1.6 Hz after exchange, H-1), 2.04, 1.92 (2 × s, 2 × 6 ester and amide CH_3). Treatment of the product with phenylhydrazine produces an immediate pale yellow color in aq EtOH. Acetylation (acetic anhydride and pyridine produces a single spot on TLC with R_f identical to pentaacetylmannosamine. Anal. Calcd for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.09; N, 4.03. Found: C, 48.27; H, 6.09; N, 3.91.

*2-Acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-mannopyranose* (**22**).—To 0.10 g (0.23 mmol) of **5** in water (3 mL) was added sodium acetate (0.039 g, 0.48 mmol). When all had dissolved, the solution was transferred to a separatory funnel and extracted 2 × with CH_2Cl_2 (50 mL each, Na_2SO_4). Crystals of product were obtained from CH_2Cl_2–Et_2O or abs EtOH–anhyd Et_2O; crude yield: 0.071 g (89%). The product was chromatographed on a column of SiO_2 (1.5 × 25 cm) using 9 : 1 CH_2Cl_2–CH_3OH, yielding 0.057 g of **22**. Crystallization from abs EtOH–anhyd Et_2O gave 0.024 g (30%) of product: mp 182–184°C (dec); $[\alpha]_D^{25}$ + 46.6° (*c* 1, $CHCl_3$). IR (KBr, cm^{-1}) 3330 (OH and NH), 1732 (ester C=O), 1650 (amide C=O); negative phenylhydrazine test. ^1H NMR (100 MHz, $CDCl_3$): δ 6.07 (d, 1 H, $J_{1,2}$ = 2.04 Hz, H-1α), 5.98 (partial d, 1 H, NH exchanges), 4.49 (m, 1 H, $J_{2,3}$ = 4.42 Hz, H-2), 4.18 (m, 3 H, $J_{3,4}$ = 9.4–9.7 Hz, H-3, H-6, H-6'), 3.24 (d, 1 H, HO-3 exchanges), 2, 16, 2.13, 2.10 (3 × s, 12 H, ester and amide CH_3). ^{13}C NMR ($CDCl_3$): δ 171.9 and 170.4 (C=O), 91.8 (C-1), 70.2 (C-5), 69.0 (C-4), 68.3 (C-3), 62.4 (C-6), 52.1 (C-2), 23.1 (amide CH_3), 20.9 (ester CH_3).

Anal. Calcd for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.09; N, 4.03. Found: C, 48.46, H, 6.09; N, 3.75.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-mannono-1,5-lactone* (**20**).—To CrO_3 (7.5 g, 75 mmol) and acetic acid (75 mL) in a cold-water bath was added in small portions, 2.54 g (7.31 mmol) of **19**. After disappearance of starting material (TLC), solvent was removed in vacuo at 25°C. The residue was dissolved in CH_2Cl_2 (400 mL) and washed 5 × with H_2O (Na_2SO_4). The filtrate was treated with charcoal (2 g) for 10–15 min and filtered through Celite. The solvent was evaporated, and the residue was dissolved in H_2O and stirred with Chelex-100 (H^+ form, Bio-Rad) for several hours and then filtered and the resin washed with H_2O. The residue obtained by evaporation was crystallized from minimum volume of dry acetone and Et_2O and recrystallized from CH_2Cl_2–Et_2O to give 0.32 g of **20** with mp 182–183°C. Later crops gave an additional 0.78 g for a total yield of 44%; $[\alpha]_D^{23.5}$ +173° (*c* 0.5, chloroform). [Lit. [22] mp 146–147°C, $[\alpha]_D$ +188.3° (*c* 0.92, $CHCl_3$), 42% yield via Me_2SO/Ac_2O, crystallized from CH_3OH. IR (KBr, cm^{-1}) 1780 (C=O). ^1H NMR ($CDCl_3$): δ 6.30 (s, 1 H, NH), 2.17, 2.11, 2.03 (12 H, OAc and NAc), 60MHz]. IR (KBr, cm^{-1}) 3300 (NH), 1786 (lactone C=O), 1750 (ester C=O), 1651 (amide C=O). ^1H NMR (100 Mz, $CDCl_3$): δ 6.37 (d, 1 H, $J_{H\text{-}2,\,NH}$ = 6.88 Hz, N-H), 5.48 (dd, 1 H, $J_{3,4}$ = 1.52 Hz, H-3), 5.25 (m, 1 H, $J_{2,3}$ = 4.10 Hz, H-2), 5.07 (dd, 1 H, $J_{4,5}$ = 8.86 Hz, H-4), 4.63 (m, 1 H, H-5), 4.33 (m, 2 H, $J_{5,6}$ = ~ 5.20 Hz, $J_{5,6'}$ = ~ 3.3 Hz, H-6's), 2.18, 2.14, 2.12, 2.07 (4 × s, 12 H, ester and amide CH_3C=O). ^{13}C NMR ($CDCl_3$): δ 170.1, 169.9, 168.8, 167.8 (ester, amide, and C-1), 75.2, 71.7, 69.1, 61.9, 49.6 (ring carbons), 22.8 (amide CH_3C=O), 20.6 (ester CH_3C=O). Note: ^1H- and ^{13}C NMR in D_2O show ring-opening to the acid. Anal. Calcd for $C_{14}H_{19}NO_9$: C, 48.69; H, 5.55; N, 4.06. Found: C, 48.66; H, 5.64; N, 3.99.

*Preparation of 2-acetamido-4,6-di-*O*-acetyl-2,3-dideoxy-*D*-*erythro*-hex-2-enono-1,5-lactone* (**21**).—Compound **20** (1.85 g, 5.36 mmol) was dissolved in EtOAc (200 mL), followed by addition of several grams of potassium carbonate (K_2CO_3) with rapid stirring. Then three 0.2-mL portions of water were added in 15-min intervals until TLC indicated a faster moving UV quenching spot. The product was chromatographed on a column of SiO_2 (4 × 28 cm), eluting initially with 95:5 CH_2Cl_2–acetone, and finally with 9:1 CH_2Cl_2–acetone, to give 0.39 g (25%) of **21** as a syrup: $[\alpha]_D^{25}$ +132.5° (*c* 0.89, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) 3400 (NH), 1805; 1740 (ester C=O), 1700 (C=C), 1658 and 1540 (amide I and II). [Lit. [22], $[\alpha]_D$ + 144.5° (*c* 0.96, $CHCl_3$); IR (neat, cm^{-1}) 1760 (C=O), 1750 (ester C=O), 1700 (C=C), 1650 and 1540 amide I and II]. ^1H NMR ($CDCl_3$) was identical to that reported [22]. ^{13}C NMR ($CDCl_3$): δ 170.1 and 169.6 (ester and amide C=O), 159.9 (C-1), 126.9 and 117.9 (C-3 and C-2), 78.1, 63.8, 62.2 (ring carbons), 24.2 (amide CH_3), 20.7 and 20.5 (ester CH_3).

*2-Acetamido-4,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-*D*-erythro-hex-1-en-3-ulose* (**23a**). —To 2.00 g (5.76 mmol) of **22** in dry distilled Me_2SO (10 mL) was added 2.21 g (11.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide · HCl, followed by dry pyridine (0.62 mL, 7.67 mmol), and finally trifluoroacetic acid (0.47 mL, 6.1 mmol) [23]. After 3 h the mixture was diluted with CH_2Cl_2 (400 mL) and washed 1 × with satd NaCl. The aq portion was washed 2 × (100 mL each CH_2Cl_2; Na_2SO_4). The residue obtained after solvent evaporation was chromatographed on a column of SiO_2 (4 × 31 cm) eluting with EtOAc; R_f of product ~ 0.6 in EtOAc with UV quenching. The

compound also gave an orange color with 2,4-dinitrophenylhydrazine. The crude yield of product was 1.44 g (87.8%). Crystals formed (after 3 months at −20°C) from anhydr Et_2O–petroleum ether yielding 0.451 g (27.5%). Partial melting occurred below 100°C, with dec at 142°C UV (95% EtOH), λ max = 274 nm with br sh, ~295 nm. $[\alpha]_D^{24}$ + 234.4°C (*c* 1, dry CH_2Cl_2). IR (dry CH_2Cl_2, cm^{-1}) 3405 (NH), 2930 br (CH), 1755 (ester C=O), 1680 (amide C=O and 3-C=O), 1625 (sharp). IR (KBr, cm^{-1}) 3335 (NH), 2935 (CH), 1750 (ester C=O), 1680 (C=O), 1655 (amide C=O), 1625 (sharp). ^1H NMR ($CDCl_3$): δ 8.72 (s, 1 H, H-1), 7.22 (br s, 1 H, NH exchanges), 5.63 (d, 1 H, $J_{4,5}$ = 13.2 Hz, H-4), 4.56 (m, 1 H, H-5), 4.4 (m, 2 H, H-6 and H-6′), 2.18 and 2.10 (2 × s, 9 H, CH_3C=O). ^1H NMR. (200 MHz acetone-d_6): δ 8.53 (^1H, $J_{1,5}$ = 0.62 Hz, H-1), 5.60 ($J_{4,5}$ = 13.2 Hz, H-4), 4.73 ($J_{5,6}$ = 4.20 Hz, H-5), 4.46, 4.38 ($J_{5,6'}$ = 2.25 Hz; $J_{6,6'}$ = 12.85 Hz, H-6 and H-6′). ^{13}C NMR ($CDCl_3$): δ 183.7 (C-3), 170.1, 168.8, 168.0 (CH_3 C=O), 153.4 (C-1), 117.8 (C-2), 77.6 (C-5), 67.5 (C-4), 61.2 (C-6), 23.7 (amide CH_3), 20.6, 20.4 (ester CH_3). Anal. Calcd for $C_{12}H_{15}NO_7$: C, 50.53; H, 5.30; N, 4.91. Found: C, 50.35; H, 5.54; N, 5.01.

3-Hydrate (**23b**). During chromatography of **23a** above from another preparation when 72 : 28 petroleum ether–acetone was used for elution, a more polar compound was isolated that gave crystals from anhyd Et_2O in a yield of 0.05 g (12%): mp 148–155°C dec (R_f 0.43 in 95 : 5 CH_2Cl_2–CH_3OH). IR (KBr, cm^{-1}) 3400–3000 br (OH and NH), 1740, sh 1700 (ester C=O and C=C), 1650 (amide C=O). ^1H NMR ($CDCl_3$ + 0.1 mL acetone-d_6 + drop D_2O): δ 5.67 (s, 1 H, H-1), 5.33 (d, 1 H, $J_{4,5}$ ~ 9.9 Hz, H-4), 4.32 (m, 2 H, H-6 and H-6′), 4.20 (m, 1 H, H-5), 2.19, 2.17, 2.08 (3 × s, 9 H, ester and amide CH_3). Anal. Calcd for $C_{12}H_{15}NO_7 \cdot H_2O$: C, 47.53; H, 5.65; N, 4.62. Found: C, 47.25; H, 5.41; N, 4.86.

2-Acetamido-1,4,6-tri-O-acetyl-2-deoxy-3-O-methylsulfonyl-α-D-mannopyranose (**24**).—To 2.05 g (5.91 mmol) of **22** in dry pyridine (80 mL), which was cooled to −20°C, was added methanesulfonyl chloride (3.0 mL, 36 mmol). The temperature was allowed to rise to room temperature (TLC indicated one spot, R_f 0.2 in 8 : 2 Et_2O–EtOAc. The reaction mixture was cooled to −40°C, and 3.5 mL (> 6 equiv) of abs EtOH were added. When the temperature reached 0°C, the solvents were evaporated and coevaporated once with toluene. The residue was dissolved in CH_2Cl_2 (350 mL) and washed 3 × with H_2O (Na_2SO_4). The compound was applied to a column of SiO_2 (4 × 32 cm), eluting with 9 : 1 Et_2O–EtOAc. The fractions were dissolved in a minimum volume of dry CH_2Cl_2 and added dropwise into rapidly stirring petroleum ether (500 mL) to give, after drying, the resulting precipitate, 2.43 g (94.9%) of **24**: mp 75–80°C (dec); $[\alpha]_D^{25}$ + 59.8°C (*c* 1, $CHCl_3$). IR (KBr, cm^{-1}) 3310 (NH), 2960 (CH), 1735 (ester C=O), 1665 (amide C=O). ^1H NMR ($CDCl_3$): δ 6.05 (br s and s, 2 H, $J_{1,2}$ = 1.92 Hz, H-1 α; $J_{H\text{-}2,NH}$ = 9.16 Hz exchanges, N-H), 5.18 (dd, 2 H, H-3 and H-4), 4.75 (m, 1 H, H-2), 4.20 (m, 3 H, H-5 and H-6 and H-6′), 3.10 (s, 3 H, OSO_2CH_3), 2.19, 2.14, 2.10 (3 × s, 12 H, ester and amide CH_3). ^{13}C NMR ($CDCl_3$): δ 170.4, 169.9, 167.9 (ester and amide C=O), 91.8 (C-1), 75.1, 70.2, 65.5, 62.2, 49.9 (ring carbons), 39.1 (OSO_2CH_3), 23.1 (amide CH_3), 20.8, 20.7 (ester CH_3). Anal. Calcd for $C_{15}H_{23}NO_{11}S \cdot 0.5H_2O$: C, 41.47; H, 5.57; N, 3.22. Found: C, 41.57; H, 5.32; N, 3.08.

Determination of growth inhibitory effect on L1210 leukemia in vitro (Table 1)—Cultures were inoculated with 5 × 10^4 cells mL^{-1} in RPMI 1640 medium containing 10%

fetal bovine serum. The carbohydrate analogues were added, and the cells surviving were compared to control (no carbohydrate analogue added). The results were expressed as % control after 24 h. The IC_{50} (50% growth inhibitory concentration) was determined from the dose–response curve. All assays were performed in duplicate on at least two separate occasions.

Effect of the ip administration of mannosamine sugar analogue on the life span of DBA / 2Ha mice implanted with L1210 leukemia (Table 1)—DBA/2Ha female mice (20 g) were inoculated ip with 10^6 L1210 leukemia cells on day zero. Twenty-four hours later (day one), the mice were given a range of doses (5 mice per dose level) of the sugar analog ip once per day through day 5. Life span was monitored daily. The per cent increase in life span (ILS) was determined based on the life span of the control group, which consisted of 8–10 animals.

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