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Synthesis of 4-deoxy analogues of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-xylose and their effects on glycoconjugate biosynthesis

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Abstract

4-Deoxy analogues of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-xylose were synthesized and evaluated as inhibitors of glycoconjugate biosynthesis. Methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**11**) showed a reduction in [^{3}H]GlcN and [^{14}C]Leu incorporation into hepatocyte cellular glycoconjugates by 89 and 88%, of the control cells, respectively, at 20 mM, whereas the free sugars, 2-acetamido-2,4-dideoxy-α,β-D-*xylo*-hexopyranoses (**15**), showed a reduction of [^{3}H]GlcN and [^{14}C]Leu incorporation by 75 and 64%, respectively, at 20 mM. The acetylated analogues of **11** and **15**, namely methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside and 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-α,β-D-*xylo*-hexopyranoses, showed a greater inhibition of [^{3}H]GlcN and [^{14}C]Leu incorporation at 1 mM compared with their non-acetylated counterparts, but were toxic to hepatocytes at concentrations of 10 and 20 mM. Corresponding derivatives of 2-acetamido-2,4-dideoxy-L-*threo*-pentopyranose showed no biological effect up to 20 mM, suggesting that the C-6 substituent is important for the biological activity.

Keywords: Glycoconjugates; 2-Acetamido-2,4-dideoxy-D-*xylo*-hexopyranose derivatives; 2-Acetamido-2,4-dideoxy-L-*threo*-pentopyranose derivatives

1. Introduction

The structure and synthesis of glycoproteins and proteoglycans have in recent years become very active areas of research, reflecting the growing recognition of their importance in structural and functional biology and biochemistry. Nevertheless, analogues of the natural precursors of these glycoconjugates are limited in number. Such analogues not only provide tools to perturb glycoconjugate metabolism, but in so doing they shed light on the physiological roles of these biological molecules. Among these roles are aspects of cell growth and division, cellular mobility, cell–cell recognition and communication, and cellular differentiation [1–3]. The analogues also provide clues, or leads, to the development of therapeutic agents for pathological processes in which glycoconjugates are involved. These processes include aspects related to important disorders such as cancer [4,5], arthritis [6], and amyloid-based diseases such as Alzheimer's [7] and adult-onset diabetes [8].

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Alterations in glycoprotein and proteoglycan biosynthesis provide a possibility of affecting the function of the whole molecule [9]. Synthetic analogues of proteoglycans, including glycosaminoglycans (GAGs), could provide some insight into the metabolism of these glycoconjugates. Previously, an analogue of D-galactose, namely 3-deoxy-D-*xylo*-hexose (3-deoxy-D-galactose), was found [10] to act on heparan sulfate proteoglycan (HSPG) biosynthesis without affecting protein synthesis in mouse hepatocytes in culture. Also, Sharma et al. [11] have reported the effects of a series of 4- and 6-fluoro derivatives of 2-acetamido-2-deoxy-D-hexopyranoses on L1210 leukemia cell growth in vitro and found significant inhibition of both protein and glycoconjugate biosynthesis.

Herein, we report the synthesis and biological evaluation of 2-acetamido-2,4-dideoxy-D-*xylo*-hexose and 2-acetamido-2,4-dideoxy-L-*threo*-pentose, and derivatives of each, directed towards influencing cellular glycoconjugate biosynthesis.

2. Results and discussion

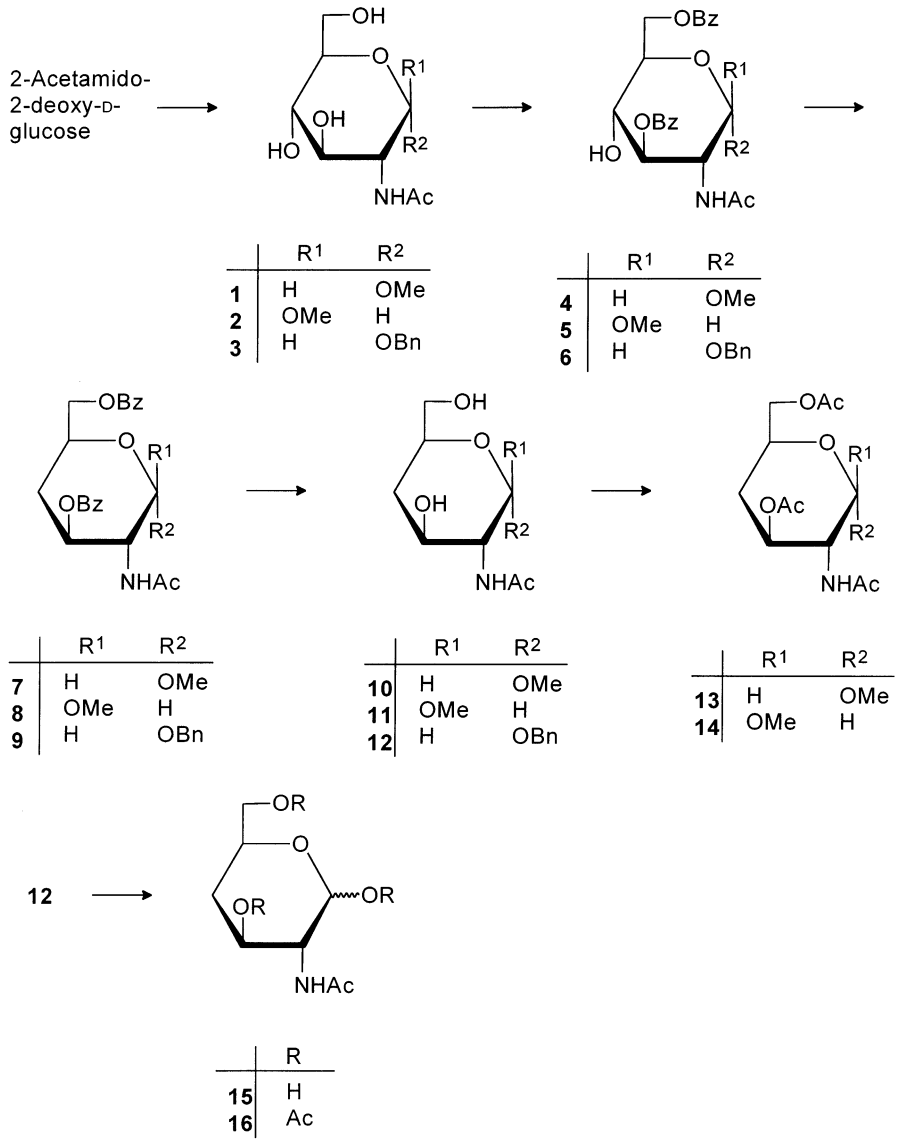
Chemical synthesis.—The 4-deoxy analogues of 2-acetamido-2-deoxy-D-glucose were synthesized as shown in Scheme 1. Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**) [12] was selectively benzoylated using a freshly prepared solution of benzoyl chloride (2.1 equivalents) in pyridine at −40 °C. Thin-layer chromatography (TLC) indicated the presence of two minor components assumed to be the mono- and per-*O*-benzoylated analogues. Chromatographic resolution of the mixture afforded the 3,6-di-*O*-benzoyl derivative **4** in 82.4% yield. Compound **4** was subjected to the Barton–McCombie deoxygenation procedure [13] using 1,1′-thiocarbonyldiimidazole, followed by treatment with tri-*n*-butyltin hydride in toluene in the presence of azoisobutyronitrile (AIBN), to afford the 4-deoxy derivative **7** in 94.4% yield. In a separate experiment, isolation of the intermediate thioimidazolide provided corroborating evidence for the presence of a hydroxyl group at C-4 in **4**; the ^{1}H NMR spectrum showed a downfield shift of the H-4 signal from 3.86 to 6.23 ppm. Wessel et al. [14] observed a downfield shift of nearly 2.5 ppm in the H-4′ signal of a maltoside derivative bearing an *O*-thiocarbonylimidazolyl group at C-4′.

O-Debenzoylation of **7** using potassium hydroxide in methanol afforded methyl 2-acetamido-2,4-dideoxy-α-D-*xylo*-hexopyranoside (**10**) in 93.9% yield. Treatment of **10** with acetic anhydride in pyridine gave the di-*O*-acetyl derivative **13** in 87.6% yield. The overall yield for the four-step conversion of **1** into **13** was 64.0%.

Similarly, compounds **2** [12] and **3** [15] were each selectively benzoylated to produce the 3,6-di-*O*-benzoylated derivatives, **5** and **6** [16], in 70.9 and 76.9% yields, respectively. Recently, Wang and Lee [17] reported a similar procedure that afforded compound **5** in 84% yield, but as a hydrated form.

Compounds **5** and **6** were each subjected to the Barton–McCombie deoxygenation procedure [13] to afford the 4-deoxy analogues, **8** (92.9%) and **9** [16b] (76.2%), respectively. O-Debenzoylation of each afforded compounds **11** (92.6%) and **12** ([16b]; see also Ref. [18]) (90.9%), respectively. Compound **11** was acetylated to produce crystalline **14** in 87.0% yield (overall 53.1% from **2**). Catalytic hydrogenolysis (Pd–C) of **12** afforded **15** [18–21] (99.1%) as an oil, shown by ^{1}H NMR spectroscopy to be a mixture of α and β anomers in a ratio of 3:1, respectively. Acetylation of **15** produced **16** (93.1%, overall 49.1% from **3**) as an oil that was shown by ^{1}H NMR spectroscopy to be a mixture of α and β anomers in a ratio of 2:1, respectively.

Synthesis of the 2-acetamido-2,4-dideoxy-L-*threo*-pentopyranose series of compounds was initiated with D-xylose (see Scheme 2). Following the methods described by Jennings and Jones [22,23], 1,2,3-tri-*O*-acetyl-4-chloro-4-deoxy-α,β-L-arabinopyranoses (**17**) were prepared from D-xylose in a four-step procedure. Thus, 4-chloro-4-deoxy-L-arabinopyranosyl chloride 2,3-di(chlorosulfate) was obtained from D-xylose on treatment with sulfuryl chloride in pyridine and chloroform. The conversion of the di(chlorosulfate) into 4-chloro-4-deoxy-L-arabinopyranose was achieved by treatment with 20% sodium iodide in 50% aqueous methanol, followed by acid-catalyzed

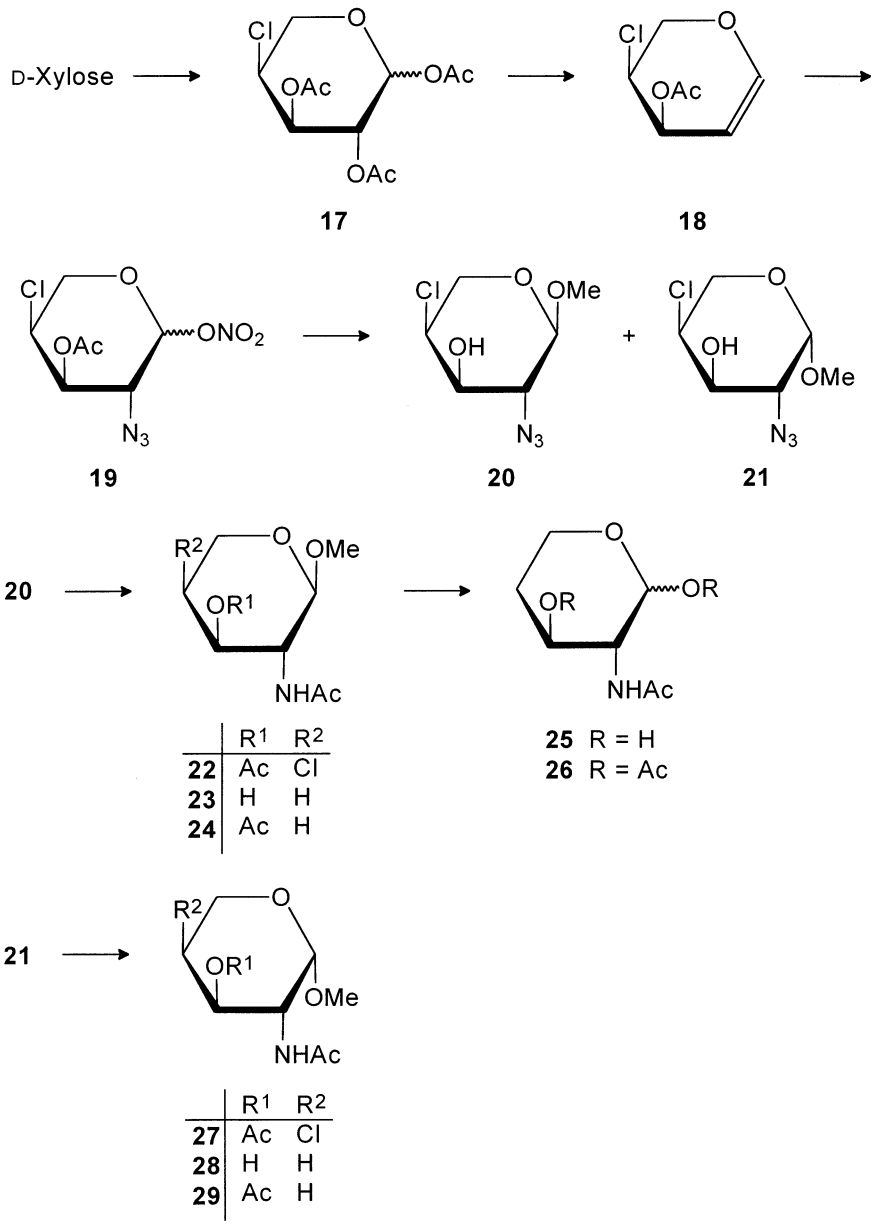


Scheme 1.

hydrolysis of the resultant methyl glycosides. Acetylation of the product using acetic anhydride in pyridine gave **17** as a mixture of α and β anomers in a ratio of 1:8.4, as determined by ^{1}H NMR spectroscopy, in 47.5% yield from D-xylose.

The conversion of **17** into the corresponding glycal, namely 3-*O*-acetyl-4-chloro-4-deoxy-L-arabinal (**18**), was achieved in a two-step procedure that was adapted from the method described by Roth and Pigman ([24]; see also Ref. [25]). Addition of a solution of the intermediate acetobromo derivative of **17**, prepared by treatment of **17** with hydrogen bromide in glacial acetic acid, to a mixture of zinc dust in 50% aqueous acetic acid gave **18** in 60.3% yield. The regio- and stereoselective introduction of nitrogen at C-2 to produce the L-*arabino* configuration was achieved by azidonitration of **18**. Following the method of Lemieux and Ratcliffe [26], a mixture of 3-*O*-acetyl-2-azido-4-chloro-2,4-dideoxy-α- and β-L-arabinopyranosyl nitrates (**19**) was formed upon treatment of the glycal **18** with sodium azide and ceric ammonium nitrate in acetonitrile. Treatment of the mixture of nitrates with sodium methoxide in methanol gave a mixture of methyl 2-azido-4-chloro-2,4-dideoxy-α- (**20**) and β-L-arabinopyranoside (**21**), which was resolved by flash chromatography.

Compounds **20** and **21** were converted into methyl 2-acetamido-3-*O*-acetyl-4-chloro-2,4-



Scheme 2.

dideoxy-α- (**22**) and -β-L-arabinopyranoside (**27**), respectively, by hydrogenation of each over palladium-on-carbon, followed by acetylation of the resultant 2-amino products. Reductive dechlorination of **22** and **27**, using Raney nickel in ethanol containing sodium hydroxide, gave methyl 2-acetamido-2,4-dideoxy-α- (**23**) and β-L-*threo*-pentopyranoside (**28**) in 41.6 and 67.4% yield, respectively; the corresponding 3-*O*-acetyl derivatives, namely **24** and **29**, were also prepared. A synthesis of DL modifications of compounds **23**, **24**, **28**, and **29** starting from tetrahydro-2-methoxypyran-3-one has been reported [27].

Acid-catalyzed hydrolysis of **24** using Amberlite IR-120 (H^+) resin in water gave 2-acetamido-2,4-dideoxy-L-*threo*-pentopyranose (**25**) in 54.5% yield. Treatment of **25** with acetic anhydride in pyridine gave 2-acetamido-1,3-di-*O*-acetyl-2,4-dideoxy-L-*threo*-pentopyranose (**26**) as a mixture of α and β anomers in a ratio of 9.8:1, respectively, as determined by ^1H NMR spectroscopy.

Biological evaluation.—Murine hepatocytes were isolated and placed in culture as described previously [28,29] and treated with D-[^{3}H]glucosamine hydrochloride and L-[^{14}C]leucine in the presence or absence of sugar analogues for 24 h. The 4-deoxy-D-glucosamine analogue **10** showed no inhibitory effects on [^{3}H]GlcN and [^{14}C]Leu incorporation up to 20 mM (see Table 1). The corresponding β anomer **11** showed an enhanced inhibition of glycoconjugate biosynthesis by a reduction of [^{3}H]GlcN and [^{14}C]Leu incorporation by 33 and 18%, respectively, at 10 mM and by 89 and 88%, respectively, at 20 mM. A similar reduction was observed for the free-sugar analogue **15**, in which case [^{3}H]GlcN and [^{14}C]Leu incorporation was reduced by 34 and 33%, respectively, at 10 mM and by 75 and 64%, respectively, at 20 mM (Fig. 1).

Bernacki et al. [30] have reported that acetylated sugar derivatives show greater inhibition of glycoconjugate biosynthesis than that of the corresponding non-acetylated derivatives. They suggested that the acetylated sugar analogues are able to gain access into cells by passive diffusion through the plasma membrane rather than by a glucose-transport mechanism. In the present work, it was found that the acetylated glucosamine analogues **13**, **14**, and **16** showed greater inhibition of incorporation of [^{3}H]GlcN and [^{14}C]Leu at 1.0 mM than that of the corresponding non-acetylated analogues; the latter did not exhibit any inhibition at 1.0 mM. However, at 10 and 20 mM, compounds **13**, **14**, and **16** were cytotoxic to hepatocytes.

Table 1
Effect of sugar analogues on glycoconjugate biosynthesis in mouse hepatocytes in vitro

Compound	Concentration (mM)	% Reduction in incorporation	
		[^{3}H]GlcN	[^{14}C]Leu
10	1	0 ± 1	12 ± 14
	10	3 ± 14	11 ± 10
	20	2 ± 9	17 ± 5
11	1	0 ± 3	21 ± 8
	10	33 ± 13	18 ± 2
	20	89 ± 16	88 ± 5
13	1	31 ± 10	20 ± 1
	10	CD [a]	CD
	20	CD	CD
14	1	30 ± 12	20 ± 5
	10	CD	CD
	20	CD	CD
15	1	0 ± 1	6 ± 7
	10	34 ± 11	33 ± 1
	20	75 ± 19	64 ± 2
16	1	66 ± 13	41 ± 5
	10	CD	CD
	20	CD	CD

[a] CD refers to cell death.

The 4-deoxy analogues of 2-acetamido-2-deoxy-D-xylose, namely **23**–**26**, **28**, and **29**, showed only small inhibitory effects on [^{3}H]GlcN and [^{14}C]Leu incorporation into glycoconjugates, even at 20 mM. These results suggest that the C-6 functionality is important for the inhibition of glycoconjugate biosynthesis.

The mechanism of action of the monosaccharide derivatives described in the present work is under investigation.

3. Experimental

General methods.—Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter for solutions in a 1-dm cell at room temperature (rt). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer at 400.1 and 100.6 MHz, respectively. The signals due to residual protons in the deuterated solvents were used as internal standards. Chemical shifts (δ) are reported in ppm downfield from Me_4Si for ^{1}H and ^{13}C NMR spectra. Infrared (IR) spectra were recorded on a Bomem MB-series FTIR spectrophotometer. TLC was performed using glass plates precoated with EM Science Silica Gel 60 F_{254}. Flash chromatography was performed using EM Science Silica Gel 60 (230–400 mesh).

Methyl 2-acetamido-3,6-di-O-*benzoyl-2-deoxy*-α-D-*glucopyranoside* (**4**).—To a solution of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside [12] (**1**) (4.37 g, 18.6 mmol) in anhyd pyridine (40 mL) at −40 °C was added freshly distilled benzoyl chloride (2.1 equiv, 3.41 mL) dropwise over 30 min. The reaction mixture was warmed to rt, and after 15 h MeOH (5 mL) was added and the mixture was

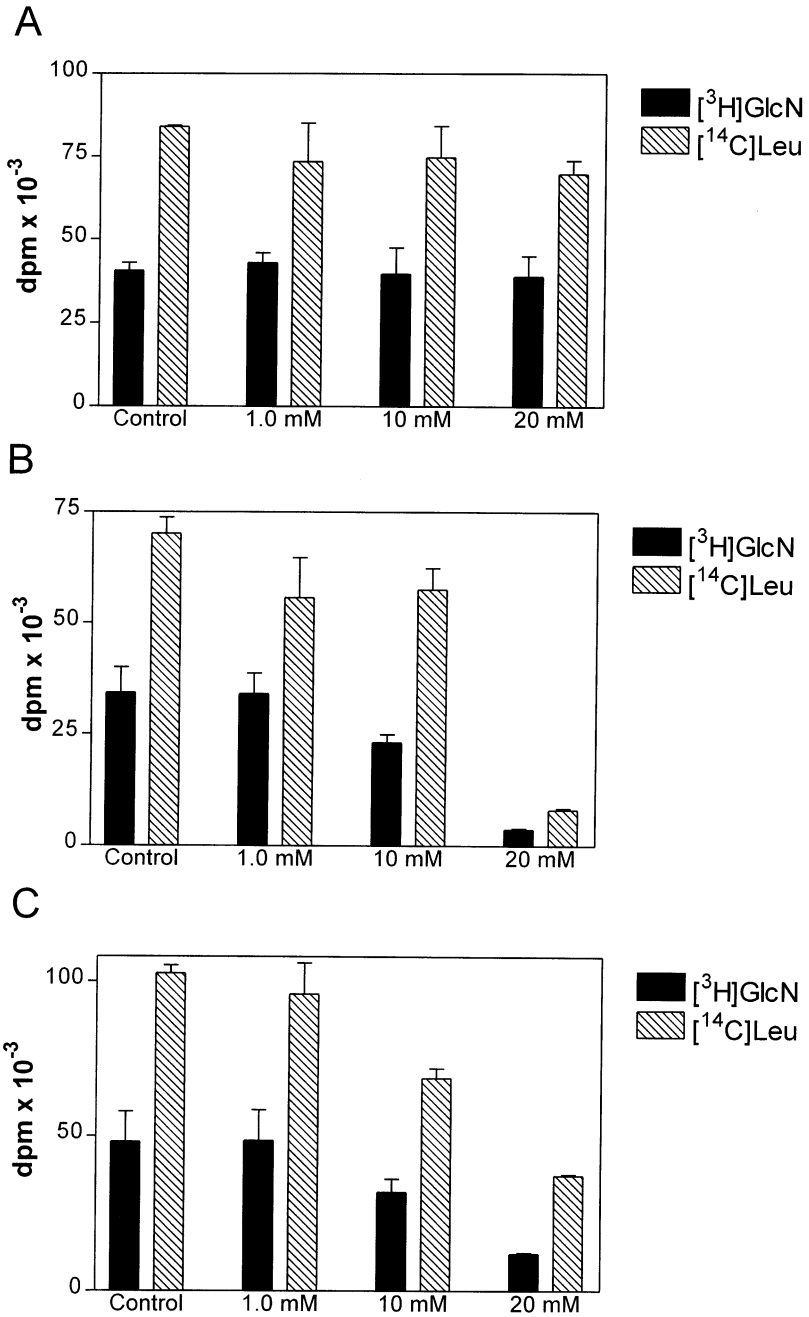


Fig. 1. Effects of increasing concentrations of methyl 2-acetamido-2,4-dideoxy-α-D-*xylo*-hexopyranoside (**10**) (panel A), methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**11**) (panel B), and 2-acetamido-2,4-dideoxy-α,β-D-*xylo*-hexopyranoses (**15**) (panel C), on cellular glycoconjugate biosynthesis. Cultures were incubated in the absence (control) or presence of 1, 10, and 20 mM of the analogue. The values represent the mean ± S.D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that in panel A, control vs. 1 or 10 mM, not significant; control vs. 20 mM, $P \leq 0.05$. In panel B, control vs. 1 mM, not significant; control vs. 10 mM, $P \leq 0.05$ ([^{14}C]Leu only); control vs. 20 mM, $P \leq 0.01$. In panel C, control vs. 1 mM, not significant; control vs. 10 mM, $P \leq 0.05$; control vs. 20 mM, $P \leq 0.01$.

concentrated under reduced pressure and co-evaporated with toluene to dryness. Flash chromatography on silica gel (1:99 MeOH–$CHCl_3$) afforded a white solid, which was recrystallized from EtOAc–petroleum ether to give pure **4** (6.79 g, 82.4%) as tiny, white needles: R_f 0.37 (1:2 hexanes–EtOAc); mp 147–149 °C; $[\alpha]_D$ +110.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.81 (s, 3 H, NAc), 3.40 (s, 3 H, OMe), 3.83 (apparent t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.98 (m, 1 H, $J_{5,6}$ 2.2, $J_{5,6'}$ 4.2 Hz, H-5), 4.45 (apparent dt, 1 H, $J_{2,3}$ 10.9 Hz,

H-2), 4.56 (dd, 1 H, $J_{6,6'}$ 12.1 Hz, H-6), 4.70 (dd, 1 H, H-6′), 4.76 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.34 (dd, 1 H, H-3), 5.99 (d, 1 H, $J_{2,NH}$ 9.7 Hz, NH), 7.35–7.56 (6 H, Ph), 7.95–8.05 (4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.0 (NCO*C*H$_3$), 51.6 (C-2), 55.2 (OMe), 63.4 (C-6), 69.0 (C-4), 70.2 (C-5), 74.7 (C-3), 98.4 (C-1), 128.1–133.4 (Ph), 166.9 and 167.8 (2 Ph*C*=O), 170.1 (NC=O). Anal. Calcd for $C_{23}H_{25}NO_8$: C, 62.30; H, 5.68; N, 3.16. Found: C, 62.43; H, 5.46; N, 3.14.

Methyl 2-acetamido-3,6-di-O-*benzoyl-2-deoxy-β*-D-*glucopyranoside* (**5**).—To a solution of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside [12] (**2**) (3.29 g, 14.0 mmol) in anhyd pyridine (30 mL) at −40 °C was added freshly distilled benzoyl chloride (2.1 equiv, 3.41 mL) dropwise over 30 min. After 15 h the mixture was processed as described for the preparation of **4** to give pure **5** (4.40 g, 70.9%) as tiny, white crystals: R_f 0.20 (2:3 hexanes–EtOAc); mp 185–187 °C; $[\alpha]_D$ +6.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.84 (s, 3 H, NAc), 3.50 (s, 3 H, OMe), 3.75–3.85 (m, 2 H, $J_{4,5}$ 9.6 Hz, H-4, H-5), 4.13 (m, 1 H, H-2), 4.57 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.63 (dd, 1 H, $J_{5,6}$ 2.1, $J_{6,6'}$ 12.1 Hz, H-6), 4.72 (dd, 1 H, $J_{5,6'}$ 4.3 Hz, H-6′), 5.39 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.6 Hz, H-3), 5.96 (d, 1 H, $J_{2,NH}$ 9.2 Hz, NH), 7.38–7.59 (6 H, Ph), 7.99–8.06 (4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.3 (NCO*C*H$_3$), 54.0 (C-2), 56.7 (OMe), 63.6 (C-6), 69.3 (C-4), 74.3 (C-5), 76.0 (C-3), 102.0 (C-1), 128.5–133.6 (Ph), 167.1 and 167.6 (2 Ph*C*=O), 170.5 (NC=O). Anal. Calcd for $C_{23}H_{25}NO_8$: C, 62.30; H, 5.68; N, 3.16. Found: C, 62.24; H, 5.62; N, 3.11.

Benzyl 2-acetamido-3,6-di-O-*benzoyl-2-deoxy-α*-D-*glucopyranoside* (**6**).—To a solution of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside [15] (**3**) (10.0 g, 32.1 mmol) in anhyd pyridine (150 mL) at −40 °C was added freshly distilled benzoyl chloride (2.1 equiv, 5.48 mL) dropwise over 30 min. After 11 h the mixture was processed as described for the preparation of **4** to afford a crude oil that was purified by flash chromatography on silica gel (1:1 hexanes–EtOAc) to give pure **6** (12.8 g, 76.9%) as a foamy oil: R_f 0.40 (1:1 hexanes–EtOAc); $[\alpha]_D$ +115.9° (*c* 1.75, $CHCl_3$), lit. +106° (*c* 0.24, MeOH) [16a], lit. +107.2° (*c* 1.0, $CHCl_3$) [16b], lit. +117.7° (*c* 0.485, $CHCl_3$) [16c]; ^{1}H NMR ($CDCl_3$): δ 1.78 (s, 3 H, NAc), 3.41 (br s, 1 H, OH), 3.88 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 4.08 (m, 1 H, H-5), 4.45–4.54 (m, 3 H, J_{gem} 12.0 Hz, H-2, H-6, PhC*H*), 4.73 (dd, 1 H, $J_{5,6'}$ 4.4, $J_{6,6'}$ 12.0 Hz, H-6′), 4.76 (d, 1 H, PhC*H*), 4.98 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.43 (dd, 1 H, $J_{2,3} = J_{3,4}$ 10.3 Hz, H-3), 6.06 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH), 7.25–7.58 (9 H, Ph), 7.98–8.07 (6 H, Ph); ^{13}C NMR ($CDCl_3$): δ 22.9 (NCO*C*H$_3$), 51.6 (C-2), 63.4 (C-6), 68.9 (C-4), 69.7(Ph*C*H$_2$), 70.6 (C-5), 74.6 (C-3), 96.6 (C-1), 128.0–136.6 (Ph), 166.9 and 167.7 (2 Ph*C*=O), 170.4 (NC=O). Anal. Calcd for $C_{29}H_{29}NO_8$: C, 67.04; H, 5.63; N, 2.70. Found: C, 67.25; H, 5.50; N, 2.54.

Methyl 2-acetamido-3,6-di-O-*benzoyl-2,4-dideoxy-α*-D-xylo-*hexopyranoside* (**7**).—A solution of compound **4** (1.20 g, 2.71 mmol) and 1,1′-thiocarbonyldiimidazole (2 equiv, 0.96 g) in 1,2-dichloroethane (20 mL) was heated at reflux temperature for 24 h; TLC showed that the reaction was complete and the presence of a component having R_f 0.21 (1:5 hexanes–EtOAc). The solvent was removed under reduced pressure, toluene (125 mL) was added, and the crude reaction solution was degassed using argon gas for 30 min. The solution was then added to a solution, at reflux temperature, of tri-*n*-butyltin hydride (5 equiv, 3.64 mL) in toluene (150 mL), containing a catalytic amount of AIBN (100 mg), which had also been degassed with argon for 30 min. After 9 h the reaction mixture was cooled, concentrated under reduced pressure, and co-evaporated with MeOH. A solution of the crude product in CH_3CN (100 mL) was washed with *n*-pentane (5 × 25 mL). The solvent was removed under reduced pressure to give a yellowish solid that was purified by flash chromatography on silica gel (1:1 hexanes–EtOAc) to give **7** (1.09 g, 94.4%) as an amorphous white solid, which was recrystallized from EtOAc to afford tiny, white needles: R_f 0.47 (1:3 hexanes–EtOAc); mp 166–167 °C; $[\alpha]_D$ +106.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.81–1.90 (apparent q, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 12.6 Hz, H-4ax), 1.88 (s, 3 H, NAc), 2.28 (ddd, 1 H, $J_{3,4eq}$ 4.9, $J_{4eq,5}$ 2.1 Hz, H-4eq), 3.40 (s, 3 H, OMe), 4.24 (m, 1 H, H-5), 4.39 (apparent d, 2 H, *J* 4.8 Hz, H-6, H-6′), 4.42 (apparent dt, 1 H, H-2), 4.82 (d, 1

H, $J_{1,2}$ 3.5 Hz, H-1), 5.34 (apparent dt, 1 H, $J_{2,3} = J_{3,4ax}$ 11.0 Hz, H-3), 5.82 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH), 7.39–7.58 (6 H, Ph), 7.99–8.05 (4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.3 (NCO*C*H$_3$), 52.1 (C-4), 55.1 (OMe), 65.8 (C-5), 66.1 (C-6), 69.5 (C-3), 99.2 (C-1), 128.4–133.2 (Ph), 165.2 and 166.6 (2 Ph*C*=O), 170.0 (NC=O). Anal. Calcd for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.89; N, 3.28. Found: C, 64.87; H, 5.99; N, 3.31.

Methyl 2-acetamido-3,6-di-O-*benzoyl-2,4-dideoxy-β*-D-xylo-*hexopyranoside* (**8**).—A solution of compound **5** (0.91 g, 2.05 mmol) and 1,1′-thiocarbonyldiimidazole (2 equiv, 0.73 g) in 1,2-dichloroethane (10 mL) was heated at reflux temperature for 12 h; TLC showed that the reaction was complete and the presence of a component having R_f 0.16 (1:5 hexanes–EtOAc). The solvent was removed under reduced pressure, toluene (150 mL) was added, and the crude reaction solution was degassed with argon for 30 min. The solution was then added to a solution, at reflux temperature, of tri-*n*-butyltin hydride (10 equiv, 5.50 mL) in toluene (100 mL), containing a catalytic amount of AIBN (100 mg), which had also been degassed with argon for 30 min. After 9 h the reaction mixture was processed as described for the preparation of **7**. The crude solid was purified by flash chromatography on silica gel (1:1 hexanes–EtOAc) to give pure **8** (0.81 g, 92.9%) as a white solid: R_f 0.33 (1:5 hexanes–EtOAc); mp 217–220 °C; $[\alpha]_D$ +1.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.84–1.92 (apparent q, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 12.1 Hz, H-4ax), 1.91 (s, 3 H, NAc), 2.33 (ddd, 1 H, $J_{3,4eq}$ 5.0, $J_{4eq,5}$ 1.7 Hz, H-4eq), 3.53 (s, 3 H, OMe), 4.01 (m, 1 H, H-5), 4.10 (m, 1 H, H-2), 4.45 and 4.49 (dq, 2 H, $J_{5,6}$ 4.4, $J_{5,6'}$ 5.7, $J_{6,6'}$ 11.5 Hz, H-6, H-6′), 4.55 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.34 (apparent dt, 1 H, $J_{2,3}$ 10.9 Hz), 5.75 (d, 1 H, $J_{2,NH}$ 9.2 Hz, NH), 7.42–7.50 (6 H, Ph), 7.99–8.10 (4 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.4 (NCO*C*H$_3$), 33.3 (C-4), 54.7 (C-2), 56.4 (OMe), 66.0 (C-6), 69.6 (C-5), 71.2 (C-3), 102.2 (C-1), 128.4–133.4 (Ph), 166.2 and 166.5 (2 Ph*C*=O), 170.4 (NC=O). Anal. Calcd for $C_{23}H_{25}NO_7$: C, 64.63; H, 5.89; N, 3.28. Found: C, 64.57; H, 6.05; N, 3.24.

Benzyl 2-acetamido-3,6-di-O-*benzoyl-2,4-dideoxy-α*-D-xylo-*hexopyranoside* (**9**).—A solution of compound **6** (4.0 g, 7.70 mmol) and 1,1′-thiocarbonyldiimidazole (2 equiv, 2.74 g) in 1,2-dichloroethane (40 mL) was heated at reflux temperature for 12 h. TLC showed the reaction was complete and the presence of a component having R_f 0.58 (1:3 hexanes–EtOAc). The solvent was removed under reduced pressure, toluene (300 mL) was added, and the crude reaction solution was degassed with argon for 30 min. The solution was then added to a solution, at reflux temperature, of tri-*n*-butyltin hydride (5 equiv, 10.4 mL) in toluene (250 mL), containing a catalytic amount of AIBN (150 mg), which had also been degassed with argon for 30 min. After 6 h the reaction mixture was processed as described for the preparation of **7**. The crude solid was recrystallized from MeOH to give pure **9** (2.95 g, 76.2%) as tiny, white needles: R_f 0.70 (1:3 hexanes–EtOAc); mp 179–182 °C; $[\alpha]_D$ +129.2° (*c* 1, $CHCl_3$), lit. +61.9° (*c* 0.5, $CHCl_3$) [16b]; ^{1}H NMR ($CDCl_3$): δ 1.84 (s, 3 H, NAc), 1.80–1.93 (m, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.7 Hz, H-4ax), 2.30 (m, 1 H, $J_{4eq,5}$ 1.5 Hz, H-4eq), 4.33 (m, 1 H, H-5), 4.36–4.40 (m, 2 H, H-6, H-6′), 4.45 (apparent dt, 1 H, $J_{2,3} = J_{2,NH}$ 10.0 Hz, H-2), 4.51 (d, 1 H, J_{gem} 11.8, PhC*H*), 4.76(d, 1 H, PhC*H*) 5.03 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.39 (apparent dt, 1 H, $J_{3,4eq}$ 4.9 Hz, H-3), 7.31–7.58 (9 H, Ph), 7.99–8.07 (6 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.2 (NCO*C*H$_3$), 33.0 (C-4), 52.0 (C-2), 66.0 (C-6), 66.1 (C-5), 69.5 (C-3), 69.6 (Ph*C*H$_2$), 97.4 (C-1), 128.0–133.2 (Ph), 166.2 and 166.5 (2 Ph*C*=O), 170.0 (NC=O). Anal. Calcd for $C_{29}H_{29}NO_7$: C, 69.17; H, 5.80; N, 2.78. Found: C, 69.58; H, 6.00; N, 2.39.

Methyl 2-acetamido-2,4-dideoxy-α-D-xylo-*hexopyranoside* (**10**).—A mixture of compound **7** (0.6 g, 1.40 mmol), KOH (1.2 g), and MeOH (50 mL) was stirred for 5 min. Neutralization using Amberlite IR-120 (H^+ form) resin, followed by filtration and concentration under reduced pressure, afforded a crude solid. Recrystallization from MeOH–EtOAc afforded pure **10** (0.289 g, 93.9%) as a white solid: R_f 0.61 (1:3 MeOH–$CHCl_3$); mp 153–155 °C; $[\alpha]_D$ +186.7° (*c* 0.95, MeOH); ^{1}H NMR (D_2O): δ 1.35 (apparent q, 1 H,

$J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.8 Hz, H-4ax), 1.86–1.93 (m, 1 H, H-4eq), 1.89 (s, 3 H, NAc), 3.23 (s, 3 H, OMe), 3.47 and 3.54 (dq, 2 H, $J_{5,6}$ 6.2, $J_{5,6'}$ 3.2, $J_{6,6'}$ 12.0 Hz, H-6, H-6′), 3.65 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.4 Hz, H-2), 3.77–3.84 (m, 2 H, $J_{3,4eq}$ 5.0 Hz, H-3, H-5), 4.75 (d, 1 H, H-1); ^{13}C NMR (D_2O): δ 24.8 (NCOCH_3), 37.4 (C-4), 57.9 (C-2, OMe), 66.6 (C-6), 68.5 (C-3), 71.5 (C-5), 101.6 (C-1), 177.5 (NC=O). Anal. Calcd for $C_9H_{17}NO_5$: C, 49.30; H, 7.82; N, 6.39. Found: C, 49.30; H, 7.58; N, 6.26.

*Methyl 2-acetamido-2,4-dideoxy-β-*D-xylo-*hexopyranoside* (**11**).—A mixture of compound **8** (0.8 g, 1.40 mmol), KOH (1.2 g), and MeOH (50 mL) was stirred for 5 min. Neutralization using Amberlite IR-120 (H^+ form) resin, followed by filtration and concentration under reduced pressure, afforded a crude solid. Recrystallization from MeOH–acetone afforded pure **11** (0.380 g, 92.6%) as a white solid: R_f 0.40 (MeOH–$CHCl_3$); mp 200–201 °C; $[\alpha]_D$ −33.2° (*c* 0.16, MeOH); ^{1}H NMR (D_2O): δ 1.28 (apparent q, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.8 Hz, H-4ax), 1.85–1.91 (m, 1 H, H-4eq), 1.88 (s, 3 H, NAc), 3.35 (s, 3 H, OMe), 3.35–3.40 (m, 1 H, H-2), 3.48–3.57 (m, 3 H, H-5, H-6, H-6′), 3.63 (apparent dt, 1 H, $J_{2,3}$ 10.8, $J_{3,4eq}$ 5.0 Hz, H-3), 4.20 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1); ^{13}C NMR (D_2O): δ 24.7 (NCOCH_3), 37.0 (C-4), 59.5 (C-2, OMe), 66.1 (C-6), 71.4 (C-3), 75.1 (C-5), 104.8 (C-1), 177.3 (NC=O). Anal. Calcd for $C_9H_{17}NO_5$: C, 49.30; H, 7.82; N, 6.39. Found: C, 49.44; H, 7.86; N, 6.28.

*Benzyl 2-acetamido-2,4-dideoxy-α-*D-xylo-*hexopyranoside* (**12**).—A mixture of compound **9** (2.10 g, 4.17 mmol), KOH (0.67 g), and MeOH (150 mL) was stirred for 40 min. Neutralization using Amberlite IR-120 (H^+ form) resin, followed by filtration and concentration under reduced pressure, afforded a crude solid. Recrystallization from acetone afforded pure **12** (1.12 g, 90.9%) as white needles: R_f 0.77 (1:3 MeOH–$CHCl_3$); mp 176–177 °C, lit. 170–171 °C [18]; $[\alpha]_D$ +243.9° (*c* 1, MeOH); ^{1}H NMR [$(CD_3)_2SO$]: δ 1.28 (apparent q, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 12.0 Hz, H-4ax), 1.83 (s, 3 H, NAc), 1.93 (m, 1 H, $J_{3,4eq}$ 4.9, $J_{4eq,5}$ 2.8 Hz, H-4eq), 3.35 and 3.39 (dq, 2 H, $J_{5,6}$ 4.7, $J_{5,6'}$ 5.6, $J_{6,6'}$ 11.3 Hz, H-6, H-6′), 3.57 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.71–3.77 (m, 2 H, H-3, H-5), 4.38 and 4.63 (dd, 2 H, J_{gem} 12.6 Hz, PhCH_2), 4.74 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 7.24–7.35 (m, 5 H, Ph), 7.76 (d, 1 H, $J_{2,NH}$ 8.1 Hz, NH); ^{13}C NMR [$(CD_3)_2SO$]: δ 22.6 (NCOCH_3), 36.9 (C-4), 55.5 (C-2), 63.9 (C-6), 64.3 (C-5), 67.7 (PhCH_2), 68.9 (C-3), 96.9 (C-1), 127.4–128.2 (Ph), 138.0 (Ph), 169.5 (NC=O). Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.36; H, 6.88; N, 4.67.

*Methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-α-*D-xylo-*hexopyranoside* (**13**).—To a solution of compound **10** (0.123 g, 0.29 mmol) in pyridine (2.0 mL) was added Ac_2O (1.0 mL) dropwise at −5 °C, the solution was then stirred for 8 h and allowed to warm to rt. The solution was concentrated and coevaporated with toluene under reduced pressure to afford a crude solid that was recrystallized from hexanes–EtOAc to give pure **13** (0.149 g, 87.6%) as tiny, white needles: R_f 0.67 (1:3 hexanes–EtOAc); mp 158–160 °C; $[\alpha]_D$ +88.8° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.65 (apparent q, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 12.0 Hz, H-4ax), 1.95, 2.02, and 2.08 (3 s, 9 H, NAc, 2 OAc), 1.99 (m, 1 H, $J_{4eq,5}$ 2.2 Hz, H-4eq), 3.36 (s, 3 H, OMe), 4.00 (m, 1 H, H-5), 4.12 (d, 2 H, *J* 4.78, H-6, H-6′), 4.17 (apparent dt, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 4.73 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.12 (apparent dt, 1 H, $J_{3,4eq}$ 4.99 Hz, H-3), 5.66 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.8 and 21.0 (2 OCOCH_3), 23.3 (NCOCH_3), 32.7 (C-4), 52.3 (C-2), 55.1 (OMe), 65.6 (C-5), 65.7 (C-6), 68.5 (C-3), 99.1 (C-1), 170.0, 170.7, and 171.2 (2 OCOCH$_3$, NC=O). Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.27; H, 7.12; N, 4.56.

*Methyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-β-*D-xylo-*hexopyranoside* (**14**).—To a solution of compound **11** (0.401 g, 1.83 mmol) in pyridine (5.0 mL) was added Ac_2O (3.0 mL) dropwise at −5 °C, the solution was then stirred for 8 h and allowed to warm to rt. The solution was concentrated and coevaporated with toluene under reduced pressure to afford a crude solid that was recrystallized from EtOAc to give pure **14** (0.483 g, 87.0%) as white needles: R_f 0.14 (1:3 hexanes–EtOAc); mp 203–205 °C; $[\alpha]_D$ −38.7° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.58 (apparent q, 1 H,

$J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.8 Hz, H-4ax), 1.94 (s, 3 H, NAc), 2.00–2.06 (m, 7 H, H-4eq, 2 OAc), 3.45 (s, 3 H, OMe), 3.70–3.79 (m, 2 H, H-2, H-5), 4.10–4.17 (dq, 2 H, $J_{5,6}$ 4.2, $J_{5,6'}$ 5.8, $J_{6,6'}$ 11.6 Hz, H-6, H-6′), 4.39 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.06 (apparent dt, 1 H, $J_{2,3}$ 10.8, $J_{3,4eq}$ 5.1 Hz, H-3), 5.64 (d, 1 H, $J_{2,NH}$ 8.9 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.8 and 21.0 (2 OCO*C*H$_3$), 23.4 (NCO*C*H$_3$), 33.0 (C-4), 54.8 (OMe), 56.4 (C-2), 65.5 (C-6), 69.3 (C-5), 70.2 (C-3), 101.9 (C-1), 170.3, 170.7, and 170.9 (2 O*C*OCH$_3$, NC=O). Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.27; H, 7.12; N, 4.56.

*2-Acetamido-2,4-dideoxy-α,β-*D-xylo-*hexopyranoses* (**15**).—A mixture of compound **12** (0.562 g, 1.90 mmol), AcOH (15.0 mL), and 10% Pd–C (0.5 g) was subjected to a hydrogen pressure (50 psig) for 3 days. The mixture was filtered through Celite 521 (Aldrich), and the filtrate was concentrated under reduced pressure to yield a crude oil. Flash chromatography on silica gel (1:3 MeOH–$CHCl_3$) afforded **15** as an oil (0.390 g, 99.1%). ^{1}H NMR spectroscopy indicated that the α and β anomers were present in a ratio of 3:1, respectively. R_f 0.35 and 0.23 (1:3 MeOH–$CHCl_3$); $[\alpha]_D$ +66.4° (*c* 1.58, H_2O, 24 h), lit. $[\alpha]_D$ +78° (*c* 1.58, H_2O) [19]. α Anomer: ^{1}H NMR (D_2O): δ 1.43–1.57 (m, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 12.0 Hz, H-4ax), 2.02–2.13 (m, 1 H, $J_{3,4eq}$ 4.9 Hz, H-4eq), 2.07 (s, 3 H, NAc), 3.54–3.83 (m, 3 H, H-2, H-6, H-6′), 4.01 (apparent dt, 1 H, $J_{2,3} = J_{3,4ax}$ 10.9 Hz, H-3), 4.13 (m, 1 H, H-5), 5.26 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1); ^{13}C NMR (D_2O): δ 22.0 (NCO*C*H$_3$), 34.8 (C-4), 55.5 (C-2), 63.6 (C-6), 65.2 (C-3), 68.5 (C-5), 91.5 (C-1), 174.6 (NC=O). β Anomer: ^{1}H NMR (D_2O): δ 1.43–1.57 (m, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.4 Hz, H-4ax), 2.02–2.13 (m, 4 H, H-4eq, NAc), 3.54–3.83 (m, 3 H, H-2, H-3, H-5), 4.64 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1); ^{13}C NMR (D_2O): δ 22.2 (NCO*C*H$_3$), 34.5 (C-4), 57.0 (C-2), 63.8 (C-6), 68.9 (C-3), 72.6 (C-5), 95.2 (C-1), 174.9 (NC=O).

*2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-α,β-*D-xylo-*hexopyranoses* (**16**).—To a solution of **15** (0.135 g, 0.66 mmol) in pyridine (5.0 mL) was added Ac_2O (3.0 mL) dropwise at −5 °C, and the solution was stirred for 9 h and allowed to warm to rt. The solution was concentrated and coevaporated with toluene under reduced pressure to afford a crude oil. Column chromatography on silica gel (1:3 hexanes–EtOAc) afforded **16** as a colorless, bubbly oil (0.203 g, 93.1%). ^{1}H NMR spectroscopy indicated that the α and β anomers were present in a ratio of 2:1, respectively. $[\alpha]_D$ +77.6° (*c* 1, $CHCl_3$). α Anomer: ^{1}H NMR ($CDCl_3$): δ 1.24–1.80 (m, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.8 Hz, H-4ax), 1.93 (s, 3 H, NAc), 2.01–2.15 (m, 10 H, H-4eq, 3 OAc), 4.06–4.15 (m, 3 H, H-5, H-6, H-6′), 4.30 (ddd, 1 H, $J_{2,NH}$ 9.1 Hz, H-2), 5.18 (apparent dt, 1 H, $J_{2,3} = J_{3,4ax}$ 11.1, $J_{3,4eq}$ 4.9 Hz, H-3), 5.50–5.54 (m, 1 H, NH), 6.17 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.8 and 21.0 (3 OCO*C*H$_3$), 23.2 (NCO*C*H$_3$), 32.5 (C-4), 51.6 (C-2), 65.3 (C-6), 67.6 (C-5), 67.7 (C-3), 91.8 (C-1), 168.9, 170.1, 170.7, and 171.5 (3 OCO*C*H$_3$, NC=O). β Anomer: ^{1}H NMR ($CDCl_3$): δ 1.24–1.80 (m, 1 H, H-4ax), 1.93 (s, 3 H, NAc), 2.01–2.15 (m, 10 H, H-4eq, 3 OAc), 3.85 (m, 1 H, H-6), 4.06–4.15 (m, 2 H, H-5, H-6′), 4.98 (apparent dt, 1 H, $J_{2,3} = J_{3,4ax}$ 11.0, $J_{3,4eq}$ 5.0 Hz, H-3), 5.50–5.54 (m, 1 H, NH), 5.58 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.8 and 21.0 (3 OCO*C*H$_3$), 23.2 (NCO*C*H$_3$), 32.5 (C-4), 51.6 (C-2), 65.3 (C-6), 67.6 (C-5), 70.1 (C-3), 93.2 (C-1), 168.9, 170.1, 170.7, and 171.5 (3 O*C*OCH$_3$, NC=O). Anal. Calcd for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23. Found: C, 50.72; H, 6.59; N, 4.21.

*1,2,3-Tri-*O*-acetyl-4-chloro-4-deoxy-α,β-*L-*arabinopyranoses* (**17**).—Following the methods of Jennings and Jones [22,23], D-xylose (21.0 g, 0.140 mol) was treated with SO_2Cl_2 (4.5 equiv, 50 mL, 0.629 mol) to give 4-chloro-4-deoxy-L-arabinopyranosyl chloride 2,3-di(chlorosulfate) (44.8 g, 83.4%) as a syrup, which on treatment with 20% (w/v) NaI in 1:1 MeOH–water in MeOH (200 mL) was converted into methyl 4-chloro-4-deoxy-L-arabinopyranoside (14.6 g, 68.5%). Hydrolysis of the product (12 g, 65.7 mmol) with 1 N H_2SO_4 at reflux temperature gave 4-chloro-4-deoxy-L-arabinopyranose (10.1 g, 91.2%): mp 150–152 °C, lit. 150 °C [22]; $[\alpha]_D$ +122.9° (*c* 1.3, H_2O), lit. +119° (*c* 0.4, H_2O) [22]. To a solution of Ac_2O (14 mL) and pyridine (18

mL) at 0 °C was added the L-arabinopyranose (2.59 g, 15.3 mmol), and the reaction mixture was stirred for 18 h at rt. The solution was poured into ice-cold H_2O (50 mL), and the mixture was neutralized with $NaHCO_3$ and then extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were washed with H_2O (30 mL) and dried ($MgSO_4$); the solvent was removed under vacuum, and the yellow syrup was purified by flash chromatography on silica gel (2:1 hexanes–EtOAc) to give **17** as a colorless syrup (4.13 g, 91.2%): IR (NaCl): *ν* 2980, 2940, and 2880 (CH), and 1750 cm^{-1} (carbonyl). ^1H NMR spectroscopy indicated that the α and β anomers were present in a ratio of 1:8.4, respectively. α Anomer: R_f 0.41 (2:1 hexanes–EtOAc); ^1H NMR ($CDCl_3$): *δ* 2.00, 2.07, and 2.08 (3 s, 9 H, 3 OAc), 3.85 (dd, 1 H, $J_{4,5eq}$ 3.5 Hz, H-5eq), 4.05 (overlapping dd, 1 H, $J_{4,5ax}$ 8.5, $J_{5ax,5eq}$ 12.3 Hz, H-5ax), 4.41–4.38 (m, 1 H, H-4), 5.09 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 5.13 (dd, 1 H, $J_{3,4}$ 6.2 Hz, H-2), 5.73 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1); ^{13}C NMR ($CDCl_3$): *δ* 20.5 and 20.65 (3 $OCOCH_3$), 52.8 (C-4), 63.3 (C-5), 67.5 and 69.3 (C-2, C-3), 90.6 (C-1), 168.7, 168.8, and 169.5 (3 $OCOCH_3$). β Anomer: R_f 0.37 (2:1 hexanes–EtOAc); ^1H NMR ($CDCl_3$): *δ* 2.00, 2.09, and 2.11 (3 s, 9 H, 3 OAc), 3.87 (dd, 1 H, $J_{4,5ax}$ 2.3 Hz, H-5ax), 4.23 (dd, 1 H, $J_{4,5eq}$ 1.7, $J_{5eq,5ax}$ 13.1 Hz, H-5eq), 4.51–4.53 (m, 1 H, H-4), 5.26 (dd, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.38 (dd, 1 H, $J_{2,3}$ 10.1 Hz, H-2), 6.29 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1); ^{13}C NMR ($CDCl_3$): *δ* 20.4, 20.6, and 20.7 (3 $OCOCH_3$), 56.5 (C-4), 64.7 (C-5), 66.2 and 67.9 (C-2, C-3), 90.0 (C-1), 168.8, 169.4, and 170.1 (3 $OCOCH_3$). Anal. Calcd for $C_{11}H_{15}ClO_7$: C, 44.84; H, 5.13; Cl, 12.03. Found: C, 45.01; H, 5.12; Cl, 12.52.

3-O-Acetyl-4-chloro-4-deoxy-L-arabinal (**18**).—To a solution of **17** (6.06 g, 20.6 mmol) in CH_2Cl_2 (10 mL) was added 45% (w/v) HBr in AcOH (1 mL/g arabinal, 6 mL), and the solution was stirred for 12 h at rt. Powdered Zn dust (12 g) was added to ice-cold 50% aq AcOH (110 mL), and the mixture was stirred for 20 min. The solution of the acetobromo derivative was added dropwise and the reaction mixture was stirred for 5 h at 0 °C. The excess Zn was removed by filtration and the filtrate was extracted with cold CH_2Cl_2 (3 × 25 mL). The combined extracts were neutralized with satd aq $NaHCO_3$, washed with H_2O (25 mL), dried ($MgSO_4$), and concentrated under vacuum at ~30 °C to give a liquid. Purification by flash chromatography on silica gel (6:1 hexanes–EtOAc) gave **18** as a clear liquid (2.19 g, 60.3%) that solidified upon cooling: R_f 0.70 (2:1 hexanes–EtOAc); mp 34–35 °C; $[\alpha]_D$ −28.5° (*c* 1.79, $CHCl_3$); IR (NaCl): *ν* 3075, 3020, 2995, 2940, and 2890 (CH), 1735 (carbonyl), and 1640 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): *δ* 2.11 (s, 3 H, OAc), 4.01 (dd, 1 H, H-5ax), 4.13 (ddd, 1 H, $J_{5ax,5eq}$ 10.8 Hz, H-5eq), 4.30 (app dt, 1 H, H-4), 4.92 (overlapping dd, 1 H, H-2), 5.35 (overlapping ddd, 1 H, $J_{2,3}$ 5.6, $J_{3,4}$ 3.9, $J_{3,5eq}$ 1.5 Hz, H-3), 6.50 (d, 1 H, $J_{1,2}$ 6.0 Hz, H-1); ^{13}C NMR ($CDCl_3$): *δ* 20.9 ($OCOCH_3$), 52.8 (C-4), 63.6 (C-3), 65.2 (C-5), 98.0 (C-2), 147.4 (C-1), 170.3 ($OCOCH_3$). Anal. Calcd for $C_7H_9ClO_3$: C, 47.62; H, 5.14; Cl, 20.08. Found: C, 47.80; H, 5.05; Cl, 20.43.

Methyl 2-azido-4-chloro-2,4-dideoxy-α- (**20**) *and β-L-arabinopyranoside* (**21**).—To a solution of the glycal **18** (3.13 g, 17.7 mmol) in dry MeCN (65 mL) at −10 °C was added sodium azide (1.5 equiv, 1.73 g, 26.5 mmol), followed by ceric ammonium nitrate (3 equiv, 29.11 g, 53.1 mmol), and the mixture was stirred for 6 h at −10 °C. The mixture was diluted with cold Et_2O (30 mL), and the solids were dissolved with cold water (50 mL). The aq layer was extracted with Et_2O (4 × 25 mL). The combined organic layer and extracts were washed with water (25 mL) and dried ($MgSO_4$), the solvent was removed under vacuum, and the syrup was purified by flash chromatography on silica gel (10:1 hexanes–EtOAc) to give an anomeric mixture of 2-azido-2-deoxyglycosyl nitrates (**19**, 3.11 g, 62.5%) as a syrup: IR (NaCl): *ν* 3000, 2930, and 2860 (CH), 2120 (N_3), 1750 (carbonyl), 1670 and 1280 cm^{-1} (NO_2). ^1H NMR spectroscopy indicated that the α and β anomers were present in a ratio of 5:2, respectively. α Anomer: R_f 0.15 (10:1 hexanes–EtOAc); ^1H NMR ($CDCl_3$): *δ* 2.20 (s, 3 H, OAc), 3.93 (dd, 1 H, $J_{4,5eq}$ 2.3 Hz, H-5eq), 3.97 (dd, 1 H, H-2), 4.15 (dd, 1 H, $J_{4,5ax}$ 4.2, $J_{5ax,5eq}$ 13.0 Hz, H-5ax), 4.45–4.43 (m, 1 H, H-4), 4.95 (dd, 1 H, $J_{2,3}$ 8.5, $J_{3,4}$ 3.6 Hz, H-3), 5.64 (d, 1 H, $J_{1,2}$ 6.9 Hz,

H-1); ^{13}C NMR ($CDCl_3$): δ 20.6 ($OCOCH_3$), 53.7 (C-4), 57.3 (C-2), 65.6 (C-5), 71.5 (C-3), 98.0 (C-1), 169.8 ($OCOCH_3$). β Anomer: R_f 0.22 (10:1 hexanes–EtOAc); ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3 H, OAc), 3.95 (dd, 1 H, $J_{4,5ax}$ 2.0 Hz, H-5ax), 4.27 (dd, 1 H, H-2), 4.29 (dd, 1 H, $J_{4,5eq}$ 1.6, $J_{5ax,5eq}$ 13.3 Hz, H-5eq), 4.60–4.58 (m, 1 H, H-4), 5.17 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.7 Hz, H-3), 6.32 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.6 ($OCOCH_3$), 55.8 and 56.1 (C-2, C-4), 65.0 (C-5), 69.3 (C-3), 97.2 (C-1), 169.7 ($OCOCH_3$). To a solution of **19** (2.51 g, 8.94 mmol) in dry MeOH (30 mL) at 0 °C was added dropwise a solution of NaOMe [Na (0.7 g) in dry MeOH (50 mL)], and the reaction mixture was stirred for 2 h at rt. The solvent was removed under vacuum to give a residue that was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with H_2O (10 mL), dried ($MgSO_4$), and concentrated under vacuum to a syrup. The anomers were separated and purified by flash chromatography on silica gel (3:1 hexanes–EtOAc). The α anomer (**20**, 0.38 g, 20.5%) was crystallized from cyclohexane, whereas the β anomer (**21**, 0.89 g, 47.9%) was obtained as a syrup.

Compound **20**: R_f 0.31 (2:1 hexanes–EtOAc); mp 82 °C; $[\alpha]_D$ +4.5° (*c* 1.11, $CHCl_3$); IR (KBr): *v* 3370 (OH), 3020, 2940, and 2910 (CH), and 2120 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): δ 2.95 (d, 1 H, $J_{3,OH}$ 7.2 Hz, OH), 3.53 (s, 3 H, OMe), 3.62 (dd, 1 H, $J_{2,3}$ 8.4 Hz, H-2), 3.73 (dd, 1 H, $J_{4,5eq}$ 2.3 Hz, H-5eq), 3.71–3.74 (m, 1 H, H-3), 4.06 (dd, 1 H, $J_{4,5ax}$ 4.1, $J_{5ax,5eq}$ 12.9 Hz, H-5ax), 4.25 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 4.26 (m, 1 H, H-4); ^{13}C NMR ($CDCl_3$): δ 56.8 (OMe), 58.6 (C-4), 63.2 (C-2), 64.6 (C-5), 70.5 (C-3), 102.5 (C-1). Anal. Calcd for $C_6H_{10}ClN_3O_3$: C, 34.71; H, 4.85; Cl, 17.08; N, 20.24. Found: C, 35.06; H, 4.88; Cl, 16.87; N, 20.60.

Compound **21**: R_f 0.47 (2:1 hexanes–EtOAc); $[\alpha]_D$ +25.5° (*c* 1.6, $CHCl_3$); IR (NaCl): *v* 3450 (OH), 2920, and 2850 (CH), and 2110 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): δ 2.27 (d, 1 H, OH), 3.45 (s, 3 H, OMe), 3.58 (dd, 1 H, H-2), 3.83 (dd, 1 H, $J_{4,5eq}$ 2.5 Hz, H-5eq), 4.09 (dd, 1 H, $J_{4,5ax}$ 1.7, $J_{5ax,5eq}$ 13.0 Hz, H-5ax), 4.20 (ddd, 1 H, $J_{2,3}$ 9.7, $J_{3,4}$ 4.2, $J_{3,OH}$ 9.0 Hz, H-3), 4.36–4.40 (m, 1 H, H-4), 4.84 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 55.8 (OMe), 60.8 (C-4), 61.8 (C-2), 62.8 (C-5), 67.5 (C-3), 99.4 (C-1).

*Methyl 2-acetamido-3-*O*-acetyl-4-chloro-2,4-dideoxy-α-*L*-arabinopyranoside* (**22**).—To a solution of **20** (0.381 g, 1.83 mmol) in MeOH (25 mL) was added 10% Pd–C (0.20 g), and the mixture was subjected to a hydrogen pressure (50 psig) for 1 h at rt. The mixture was filtered through Celite 521 (Aldrich), and the filtrate was concentrated under vacuum to give a residue. The residue was dissolved in dry MeCN (10 mL) and dry pyridine (5 mL). The solution was cooled to 0 °C and Ac_2O (2.5 mL) was added, and the reaction mixture was stirred for 16 h at rt. The solution was poured into ice-cold water (30 mL), and the mixture was neutralized with $NaHCO_3$; the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with water (15 mL), dried ($MgSO_4$), and concentrated under vacuum to a white residue. Purification by flash chromatography on silica gel (1:4 hexanes–EtOAc) and recrystallization of the product from $CHCl_3$–MeOH gave **22** (0.323 g, 66.0%): R_f 0.61 (5:1 $CHCl_3$–MeOH); mp 198–199 °C; $[\alpha]_D$ +2.0° (*c* 0.6, MeOH); IR (KBr): *v* 3340 (NH), 2990, 2940, 2900, and 2850 (CH), 1740 (ester carbonyl), and 1660 cm^{-1} (amide); ^{1}H NMR ($CDCl_3$): δ 1.99 and 2.12 (2 s, 6 H, OAc, NAc), 3.48 (s, 3 H, OMe), 3.82 (dd, 1 H, $J_{4,5ax}$ 2.4 Hz, H-5ax), 3.93–3.99 (m, 1 H, H-2), 4.09 (dd, 1 H, $J_{4,5eq}$ 4.5, $J_{5eq,5ax}$ 12.7 Hz, H-5eq), 4.37–4.39 (m, 1 H, H-4), 4.65 (d, 1 H, $J_{1,2}$ 6.3 Hz, H-1), 5.39 (dd, 1 H, $J_{2,3}$ 8.8, $J_{3,4}$ 3.7 Hz, H-3), 5.58 (d, 1 H, $J_{2,NH}$ 7.8 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.8 and 23.5 ($OCOCH_3$, $NCOCH_3$), 51.7 (C-2), 55.7 (OMe), 56.5 (C-4), 64.4 (C-5), 69.6 (C-3), 101.2 (C-1), 170.1 and 170.2 ($OCOCH_3$, NC=O). Anal. Calcd for $C_{10}H_{16}ClNO_5$: C, 45.21; H, 6.07; Cl, 13.35; N, 5.27. Found: C, 45.18; H, 6.21; Cl, 13.16; N, 5.16.

*Methyl 2-acetamido-2,4-dideoxy-α-*L*-threo-pentopyranoside* (**23**).—To a solution of **22** (0.200 g, 0.753 mmol) in EtOH (30 mL) was added NaOH (0.2 g) and Raney Ni (0.2 g), and the mixture was subjected to a hydrogen pressure (40 psig) for 12 h at rt. The mixture was filtered through Celite 521 (Aldrich), and

the filtrate was neutralized with 6 N HCl and concentrated under vacuum to a residue. Purification by flash chromatography on silica gel (4:1 EtOAc–Me_2CO) and recrystallization of the product from MeOH gave **23** (59 mg, 41.6%): R_f 0.11 (5:1 EtOAc–Me_2CO); mp 134.5–136 °C; $[\alpha]_D$ −58.6° (*c* 0.64, $CHCl_3$); IR (KBr): *v* 3280 (OH and NH), 3100, 2960, 2930, and 2850 (CH), and 1650 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 1.61–1.69 (m, 1 H, H-4′), 1.93–2.00 (m, 2 H, H-4, OH), 2.01 (s, 3 H, NAc), 3.43 (s, 3 H, OMe), 3.43–3.51 (m, 1 H, H-2), 3.64–3.69 (m, 1 H, H-5′), 3.74–3.79 (m, 1 H, H-5), 3.99 (overlapping ddd, 1 H, $J_{2,3}$ 6.7, $J_{3,4}$ 4.5, $J_{3,4'}$ 11.4 Hz, H-3), 4.33 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1), 6.14 (d, 1 H, $J_{2,NH}$ 5.3 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 23.4 ($NCOCH_3$), 31.0 (C-4), 55.0 and 56.0 (C-2, OMe), 58.4 (C-5), 68.4 (C-3), 101.3 (C-1), 171.1 (NC=O). Anal. Calcd for $C_8H_{15}NO_4$: C, 50.79; H, 7.99; N, 7.40. Found: C, 50.52; H, 7.57; N, 7.22.

*Methyl 2-acetamido-3-O-acetyl-2,4-dideoxy-α-*L-threo-*pentopyranoside* (**24**).—To a solution of **22** (0.348 g, 1.31 mmol) in EtOH (40 mL) was added NaOH (0.35 g) and Raney Ni (0.2 g), and the mixture was subjected to a hydrogen pressure (40 psig) for 12 h at rt. The mixture was filtered through Celite 521 (Aldrich), and the filtrate was neutralized with 6 N HCl and concentrated under vacuum to a residue. The residue was dissolved in dry MeCN (5 mL) and dry pyridine (10 mL), and Ac_2O (6 mL) was added at 0 °C. The solution was stirred for 18 h at rt and then poured into ice-cold water (50 mL); the mixture was neutralized with $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with H_2O (15 mL), dried ($MgSO_4$), and concentrated under vacuum to give a white residue. Purification by flash chromatography on silica gel (1:10 hexanes–EtOAc) and recrystallization of the product from hexanes–EtOAc gave **24** (0.247 g, 81.9%): R_f 0.08 (1:4 hexanes–EtOAc); mp 146–149 °C; $[\alpha]_D$ −45.7° (*c* 1.06, $CHCl_3$); IR (KBr): *v* 3280 (OH and NH), 3090, 2960, 2930, and 2850 (CH), 1730 (ester carbonyl), and 1650 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 1.74–1.84 (m, 1 H, H-4′), 1.97 (s, 3 H, Ac), 1.95–2.00 (m, 1 H, H-4), 2.06 (s, 3 H, Ac), 3.45 (s, 3 H, OMe), 3.45–3.51 (m, 1 H, H-5′), 3.84–4.01 (m, 1 H, H-2), 4.03 (dt, 1 H, $J_{4,5}$ 4.2, $J_{5,5'}$ 12.2 Hz, H-5), 4.33 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1), 4.98 (dt, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 4.8 Hz, H-3), 5.41 (d, 1 H, $J_{2,NH}$ 8.4 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 21.1 and 23.5 ($OCOCH_3$, $NCOCH_3$), 30.0 (C-4), 53.7 (C-2), 56.2 (OMe), 60.1 (C-5), 70.1 (C-3), 102.3 (C-1), 169.9 and 170.9 ($OCOCH_3$, NC=O). Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.95; H, 7.41; N, 6.06. Found: C, 52.08; H, 7.66; N, 6.04.

*2-Acetamido-2,4-dideoxy-*L-threo-*pentopyranose* (**25**).—Amberlite IR-120 (H^+) resin (12 mL) was added to a suspension of **24** (0.47 g, 2.03 mmol) in water (70 mL). The mixture was heated for 48 h at 60–70 °C. The resin was removed by filtration and the solution was concentrated to dryness under vacuum. Separation of the products, **23** (18 mg, 5%) and **25** (0.180 g, 50.6%), was achieved by flash chromatography on silica gel (9:1 $CHCl_3$–MeOH) and **25** was recrystallized from EtOAc: R_f 0.54 (2:1 $CHCl_3$–MeOH); mp 139–141 °C; $[\alpha]_D$ +3.19° (*c* 0.72, H_2O, 5 min); $[\alpha]_D$ +5.97° (*c* 0.72, H_2O, 16 h); IR (KBr): *v* 3370 and 3140 (OH and NH), 2980, 2950, 2850, and 2790 (CH), and 1640 cm^{-1} (amide); ^1H NMR (D_2O): δ 1.60–1.74 (m, 1 H, H-4′), 1.96–2.10 (m, 1 H, H-4), 2.03 (s, 3 H, NAc), 3.48–3.55 (m, 1 H, H-2), 3.61–3.80 (m, 2 H, H-5, H-5′), 3.88–3.99 (m, 2 H, H-3, NH), 4.53 (d, 0.5 H, $J_{1\alpha,2}$ 8.5 Hz, H-1α), 5.17 (d, 0.5 H, $J_{1\beta,2}$ 2.5 Hz, H-1β); ^{13}C NMR (D_2O): δ 11.7 and 13.5 (2 C-4), 27.1 and 27.3 (2 $NCOCH_3$), 60.5 and 70.6 (2 C-2), 63.5 and 66.9 (2 C-5), 74.5 (C-3), 96.6 and 100.8 (2 C-1), 179.7 and 180.0 (2 NC=O). FABMS (+ion) expected for $C_7H_{13}NO_4$: 176.0923 [M + H]. Found: 176.0931 (M + H).

*2-Acetamido-1,3-di-*O*-acetyl-2,4-dideoxy-α,β-*L-threo-*pentopyranoses* (**26**).—To a solution of **25** (41 mg, 0.234 mmol) in pyridine (5 mL) was added Ac_2O (3 mL), and the solution was stirred for 18 h at rt. The solution was poured into ice-cold water (50 mL), and the mixture was neutralized with $NaHCO_3$. The aqueous solution was extracted with CH_2Cl_2 (3 × 25 mL), and the combined extracts were dried ($MgSO_4$) and concentrated under vacuum to give a white residue. Purification by flash chromatography on silica gel (1:4 hexanes–EtOAc) and recrystallization of the

product from $CHCl_3$–*i*-Pr_2O gave **26** (45 mg, 74.1%) as a white solid. ^1H NMR spectroscopy indicated that the α and β anomers were present in a ratio of 9.8:1, respectively. R_f 0.17 (1:4 hexanes–EtOAc); mp 143–144 °C; $[\alpha]_D$ +0.34° (*c* 1.19, $CHCl_3$); IR (KBr): *v* 3260 (NH), 3090, 2990, and 2960 (CH), 1760 and 1740 (ester carbonyls), and 1660 cm^{-1} (amide). α Anomer: ^1H NMR ($CDCl_3$): δ 1.79–1.91 (m, 1 H, H-4′), 1.95, 2.07, and 2.11 (3 s, 9 H, 2 OAc, NAc), 1.96–2.08 (m, 1 H, H-4), 3.60 (overlapping dt, 1 H, $J_{4,5'}$ 2.8 Hz, H-5′), 4.08 (dt, 1 H, $J_{4,5}$ 4.3, $J_{5,5'}$ 12.7 Hz, H-5), 4.10–4.16 (m, 1 H, H-2), 4.94 (dt, 1 H, $J_{2,3}$ 9.1, $J_{3,4}$ 4.7 Hz, H-3), 5.49 (d, 1 H, $J_{2,NH}$ 9.2 Hz, NH), 5.57 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.9, 21.0, and 23.2 (2 $OCOCH_3$, $NCOCH_3$), 29.4 (C-4), 52.3 (C-2), 61.1 (C-5), 69.8 (C-3), 93.4 (C-1), 169.7, 170.0, and 170.8 (2 $OCOCH_3$, NC=O). β Anomer: ^1H NMR ($CDCl_3$): δ 1.86–1.93 (m, 1 H, H-4′), 1.93, 2.05, and 2.14 (3 s, 9 H, 2 OAc, NAc), 1.96–2.06 (m, 1 H, H-4), 3.80–3.83 (m, 2 H, H-5, H-5′), 4.27 (ddd, 1 H, H-2), 5.13 (dt, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 5.0 Hz, H-3), 5.63 (d, 1 H, $J_{2,NH}$ 9.2 Hz, NH), 6.11 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.9, 21.0, and 23.1 (2 $OCOCH_3$, $NCOCH_3$), 30.4 (C-4), 51.8 (C-2), 59.8 (C-5), 68.1 (C-3), 92.2 (C-1), 169.2, 169.95, and 171.4 (2 $OCOCH_3$, NC=O). Anal. Calcd for $C_{11}H_{16}NO_6$: C, 51.17; H, 6.25; N, 5.42. Found: C, 51.13; H, 6.50; N, 5.54.

*Methyl 2-acetamido-3-*O*-acetyl-4-chloro-2,4-dideoxy-β-*L*-arabinopyranoside* (**27**).—Compound **21** (0.893 g, 4.30 mmol) was processed following the same procedure as for the preparation of **22** to afford a product as a white residue that was purified by flash chromatography on silica gel (1:3 hexanes–EtOAc) and then recrystallized from hexanes–EtOAc to give **27** (0.930 g, 81.4%): R_f 0.79 (5:1 $CHCl_3$–MeOH); mp 172–173 °C; $[\alpha]_D$ +21.0° (*c* 0.95, MeOH); IR (KBr): *v* 3410 (NH), 3260, 3080, 3010, 2950, and 2840 (CH), 1750 (ester carbonyl), and 1650 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 1.91 and 2.03 (2 s, 6 H, OAc, NAc), 3.34 (s, 3 H, OMe), 3.71 (dd, 1 H, $J_{4,5eq}$ 2.0 Hz, H-5eq), 4.02 (dd, 1 H, $J_{4,5ax}$ 1.3, $J_{5ax,5eq}$ 12.8 Hz, H-5ax), 4.32–4.33 (m, 1 H, H-4), 4.60 (ddd, 1 H, H-2), 4.69 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.09 (dd, 1 H, $J_{2,3}$ 10.9, $J_{3,4}$ 3.6 Hz, H-3), 5.83 (d, 1 H, $J_{2,NH}$ 9.4 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.7 and 23.0 ($OCOCH_3$, $NCOCH_3$), 47.5 (C-2), 55.3 (OMe), 57.5 (C-4), 62.6 (C-5), 68.7 (C-3), 98.9 (C-1), 169.8 and 170.8 ($OCOCH_3$, NC=O). Anal. Calcd for $C_{10}H_{16}ClNO_5$: C, 45.21; H, 6.07; Cl, 13.35; N, 5.27. Found: C, 45.14; H, 6.11; Cl, 13.31; N, 5.39.

*Methyl 2-acetamido-2,4-dideoxy-β-*L*-threo-pentopyranoside* (**28**).—Following the same procedure as for the preparation of **23**, **27** (0.203 g, 0.764 mmol) was converted into **28** (97.0 mg, 67.1%): R_f 0.16 (5:1 EtOAc–Me_2CO); mp 142–142.5 °C; $[\alpha]_D$ +99.2° (*c* 1.33, $CHCl_3$); IR (KBr): *v* 3300 (OH and NH), 3080, 3000, 2920, 2890, 2850, and 2830 (CH), and 1650 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 1.66–1.94 (m, 1 H, H-4′), 1.98–2.03 (m, 1 H, H-4), 2.06 (br s, 4 H, NAc, OH), 3.37 (s, 3 H, OMe), 3.55–3.71 (m, 2 H, H-3, H-5′), 3.78 (dt, 1 H, $J_{4,5}$ 4.8, $J_{5,5'}$ 10.7 Hz, H-5), 3.90 (ddd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 4.67 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.97 (d, 1 H, $J_{2,NH}$ 8.3 Hz, NH); ^{13}C NMR ($CDCl_3$, 50.323 MHz): δ 23.15 ($NCOCH_3$), 33.9 (C-4), 54.9 (C-2), 55.6 (OMe), 58.1 (C-5), 68.0 (C-3), 98.9 (C-1), 171.9 (NC=O). Anal. Calcd for $C_8H_{15}NO_4$: C, 50.79; H, 7.99; N, 7.40. Found: C, 50.53; H, 7.72; N, 7.27.

*Methyl 2-acetamido-3-*O*-acetyl-2,4-dideoxy-β-*L*-threo-pentopyranoside* (**29**).—Compound **27** (0.412 g, 1.55 mmol) was processed following the same procedure as for the preparation of **24** to afford a product as a white residue that was purified by flash chromatography on silica gel (1:4 hexanes–EtOAc) and then recrystallized from hexanes–EtOAc to give **29** (0.198 g, 55.2%): R_f 0.15 (1:4 hexanes–EtOAc); mp 146–147 °C; $[\alpha]_D$ +95.2° (*c* 1.74, $CHCl_3$); IR (KBr): *v* 3240 and 3210 (OH and NH), 3060, 2900, and 2850 (CH), 1730 (ester carbonyl), and 1650 cm^{-1} (amide); ^1H NMR ($CDCl_3$): δ 1.78–1.89 (m, 1 H, H-4′), 1.91–1.95 (m, 1 H, H-4), 1.95 and 2.02 (2 s, 6 H, OAc, NAc), 3.36 (s, 3 H, OMe), 3.66 (ddd, 1 H, $J_{4,5'}$ 1.8, $J_{4',5'}$ 5.3 Hz, H-5′), 3.74 (dt, 1 H, $J_{4,5}$ 2.6, $J_{5,5'}$ 11.6 Hz, H-5), 4.14 (dt, 1 H, H-2), 4.68 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.08 (dt, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 5.1 Hz, H-3), 5.69 (d, 1 H, $J_{2,NH}$ 9.0 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 21.1 and 23.35 ($OCOCH_3$,

$NCOCH_3$), 30.9 (C-4), 52.7 (C-2), 55.1 (OMe), 57.6 (C-5), 68.9 (C-3), 99.2 (C-1), 169.9 and 171.3 ($OCOCH_3$, NC=O). Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.95; H, 7.41; N, 6.06. Found: C, 52.20; H, 7.60; N, 6.14.

Biological evaluation

Materials. D-(1,6-$^3H_2$)Glucosamine-HCl (42.3 Ci/mmol) and L-[^{14}C]leucine (320 mCi/mmol) were purchased from Dupont and ICN Biomedicals, respectively. Sodium dodecyl sulfate (SDS), DL-leucine, EGTA, D-(+)-glucosamine hydrochloride, Hepes buffer, and trichloroacetic acid (TCA) were of reagent grade and were obtained from Sigma Chemical Co., Fisher Scientific Co., or BDH Chemicals. Williams' Medium E with L-glutamine, Ultroser G, and antibiotic–antimycotic mixtures were supplied by Gibco. Fibronectin and collagenase were purchased from Sigma Chemical Co.

Hepatocyte isolation and cell culture. Hepatocytes were obtained from 6–8-week-old female Swiss White mice (Charles River Canada, St. Constant, Quebec) by a procedure described previously [28,29]. Briefly, the liver was perfused with 50 mL of 0.01 M Hepes buffer (pH 7.4) containing 0.5 mM EGTA, followed by 50 mL of a collagenase type-IV solution (0.5 mg/mL) in 0.1 M Hepes (pH 7.6). The liver was removed, and the hepatocytes were separated from the capsule by gentle teasing. The pooled cells were centrifuged at 200 rpm at 5 °C for 5 min and washed once with fresh medium. After resuspension in 20 mL of Williams' Medium E and filtration through a Nitex 110 nylon membrane, the cells were exposed to Trypan Blue and counted on a hemocytometer to determine the viability and cell number. The viability was usually greater then 85%. The cells were plated in triplicate on fibronectin-coated tissue culture dishes (Falcon 35 × 10 mm) at a density of $2–2.5 \times 10^6$ cells per plate. They were incubated in 2 mL of Williams' Medium E containing 1% Ultroser G and 1% antibiotic–antimycotic mixtures. After 2 h, the nonadherent cells were removed, and the attached cells were fed with fresh plating medium for a 24-h period. The cells were then provided with fresh medium containing D-[^{3}H]glucosamine hydrochloride (2 μCi/mL), L-[^{14}C]leucine (0.5 μCi/mL), and monosaccharide derivative. The labeled cellular proteins and glycoconjugates were harvested 24 h later.

Glycoconjugate isolation. After the 24-h labeling period, the medium was separated from the cells, and the cell fractions were solubilized by treatment with 1% SDS (2 × 0.5 mL) and then combined with the medium. The proteins and glycoconjugates were precipitated by the addition of a 10% aq soln of TCA (3 mL) containing 10 mg/mL DL-leucine and 10 mg/mL of D-glucosamine hydrochloride. The samples were heated at 90 °C for 30 min to destroy leucine tRNA and then cooled at 4 °C for 30 min. Using a Millipore 1225 sampling manifold, each sample was passed through a glass microfiber filter (Whatman 934-AH), and the filter was washed three times with 5 mL of a cold, 5% aq soln of TCA containing 10 mg/mL DL-leucine and 10 mg/mL D-glucosamine hydrochloride, and once with cold EtOH (3 mL). The filters were air dried and immersed in 5 mL of scintillation solution, and the radioactivity was measured.

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