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Synthesis and biological evaluation of a radiolabeled analog of methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside directed towards influencing cellular glycosaminoglycan biosynthesis

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Abstract

Two methods are presented for the synthesis of methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside. The first method employs the Barton–McCombie deoxygenation methodology, and the second method utilizes an oxidation–β-elimination methodology that allows for the incorporation of hydrogen isotopes into the title compound. Hence, methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**4**) and methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside-6-*t* (**14**) were synthesized and evaluated for their ability to inhibit hepatocyte, cell-surface glycosaminoglycan biosynthesis and to incorporate a [^{3}H] radiolabel into isolated glycosaminoglycans, respectively. Compound **4**, at a concentration of 1.0 mM, demonstrated a reduction of D-[^{3}H]glucosamine and [^{35}S]sulfate incorporation into isolated glycosaminoglycans by 69 and 59%, of the control cultures, respectively. At 10 and 20 mM, **4** demonstrated a maximum inhibition of incorporation of both radiolabels to approximately 10% of the control cultures. Compound **14** demonstrated a maximum incorporation of a [^{3}H] radiolabel into isolated cell-surface glycosaminoglycans at 10 and 20 mM. The mechanism of inhibition of glycosaminoglycan biosynthesis is due, in part, to the incorporation of a 4-deoxy moiety into glycosaminoglycan chains resulting in premature chain termination.

Keywords: Glycosaminoglycan; Methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside; Radiolabel; Incorporation

1. Introduction

Proteoglycans are very important constituents of cell-surface membranes and have been demonstrated to play a role in several cellular functions including cell migration, differentiation, communication, and proliferation.[1] In a disease state, alterations in cellular proteoglycan synthesis can affect the structure and functions of these glycoconjugates. These proteoglycans have been implicated in several pathologies including those associated with Alzheimer's disease,[2–7] cancer,[8–10] diabetes,[11–15] and the herpes simplex virus.[16–18] We have launched a program to investigate the role of monosaccharides in influencing proteoglycan biosynthesis in culture.[19–22]

The proteoglycans of interest are those which contain heparan sulfate as the glycosaminoglycan (GAG) portion and are localized on the cell-surface of mouse hepatocytes.[21,23] Heparan sulfate is composed of a repeating disaccharide unit of a D-glucosamine residue linked (1→4) to a uronic acid residue (either D-glucuronic acid or L-iduronic acid) and can be variably *N*- and *O*-sulfonated. This GAG is linked to a core protein through a conserved tetrasaccharide region consisting of -D-GlcA-β-(1→3)-D-Gal-β-(1→3)-D-Gal-β-(1→4)-D-Xyl-β- attached to a serine or threonine residue of the core protein. Monosaccharide analogs of the components of the tetrasaccharide region including 3-deoxy-D-*xylo*-hexopyranose (3-deoxy-D-galactose) and a 4-chloro-4-deoxy analog of D-glucuronic acid, namely

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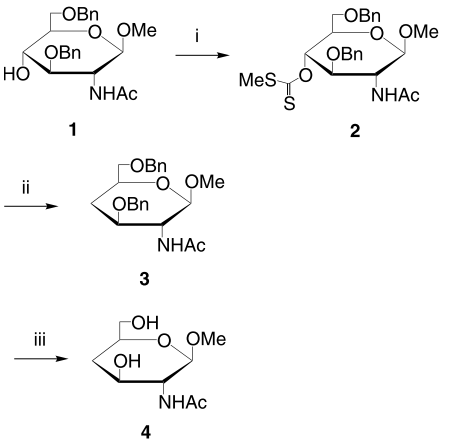
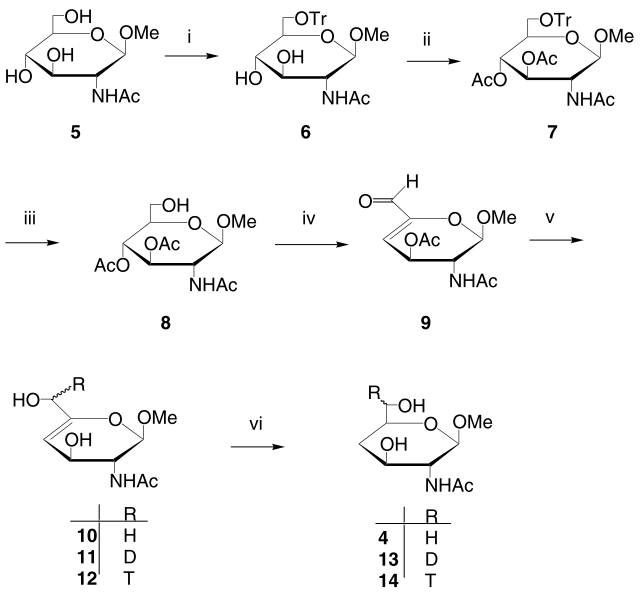
methyl (methyl 4-chloro-4-deoxy-β-D-galactopyranosid)uronate, demonstrated impaired cellular GAG chain elongation of primary hepatocytes in culture.[21] However, no inhibition of heparan sulfate synthesis was observed with mouse hepatocytes exposed to 4-deoxy-L-*threo*-pentose, although inhibition of neuronal and astrocytic proteoglycans was observed.[22] Monosaccharide analogs of the components of the repeating disaccharide unit of heparan sulfate, including 4-deoxy analogs of 2-acetamido-2-deoxy-D-glucose[20] and 4-deoxy-4-fluoro analogs of 2-acetamido-2-deoxy-D-hexose,[19] have demonstrated significant inhibition of GAG biosynthesis. Furthermore, Bernacki and coworkers[24–26] have described the inhibition of tumorigenic cell growth by treatment separately with several deoxy and deoxyfluoro analogs of 2-acetamido-2-deoxy-D-hexose. However, the mechanism of inhibition of GAG synthesis is not known for these analogs. Herein we describe the synthesis of methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside and the radiolabeled analog, namely methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside-6-*t*, and their effects on GAG biosynthesis.



Scheme 1. Reagents and conditions: (i) NaH, THF, CS_2, MeI (93.5%); (ii) Bu_3SnH, AIBN, toluene (70.0%); (iii) H_2, Pd–C, MeOH (86.5%).



Scheme 2. Reagents and conditions: (i) TrCl, pyr (79.1%); (ii) Ac_2O, pyr (89.3%); (iii) HCO_2H, Et_2O (89.9%); (iv) SO_3·pyr, Et_3N, Me_2SO (87.0%); (v) $NaBH_4$, MeOH (87.4%); (vi) H_2, Pd–C, MeOH (73.0%).

2. Results and discussion

Chemical synthesis.—The 4-deoxy analogs of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside were synthesized as shown in Schemes 1 and 2. Several methods exist for the deoxygenation of carbohydrates. These have been reviewed by Hartwig,[27] albeit many of the conventional methods are applicable only to unhindered primary alcohols. Following the Barton–McCombie deoxygenation procedure,[28] compound **1**[29] in tetrahydrofuran was sequentially treated with sodium hydride, carbon disulfide, and methyl iodide to afford the 4-*O*-methylxanthyl derivative, namely methyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[(methylthio)-thiocarbonyl]-β-D-glucopyranoside (**2**), in 93.5% yield following column chromatography. ^{1}H NMR spectroscopy of **2** showed that the signal for the H-4 proton had shifted downfield by 2.3 ppm relative to the corresponding signal in the spectrum of **1**. Treatment of **2** with tri-*n*-butyltin hydride in toluene at refluxing temperature afforded the deoxygenated product, namely methyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**3**), in 70.0% yield following column chromatography and recrystallization. Structural confirmation of **3** was obtained by ^{1}H NMR spectroscopy; signals for two protons were observed at 1.4 and 2.2 ppm corresponding to the two protons at C-4. Also, ^{13}C NMR spectroscopy of **3** exhibited an upfield shift by 43.6 ppm of the signal for C-4 relative to the corresponding signal in the spectrum of **1**.

Removal of the benzyl protecting groups of **3** was accomplished using palladium-on-carbon (Pd–C) and hydrogen to afford methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**4**) in 86.5% yield following column chromatography. Compound **4** was evaluated for the inhibition of hepatocyte cell-surface GAG synthesis.

The synthesis of a radiolabeled analog of **4** (Scheme 2) was begun by the treatment of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**5**)[30] with triphenylmethyl chloride in pyridine to afford methyl 2-acetamido-2-deoxy-6-*O*-triphenylmethyl-β-D-glucopyranoside (**6**) in 79.1% yield following crystallization of the crude reaction product. Acetylation of **6** proceeded smoothly using acetic anhydride in pyridine to afford methyl

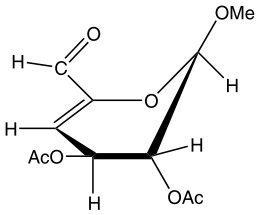


Fig. 1. Possible molecular structure of **9** as proposed by Perlin and co-workers[34] for an analog of **9**.

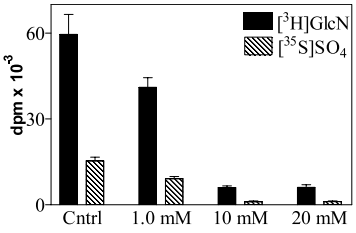


Fig. 2. Effect of increasing concentration of methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**4**) on cellular glycosaminoglycan biosynthesis. Hepatocyte cultures were incubated with D-[^{3}H]glucosamine and [^{35}S]sulfate for 24 h in the absence (control) or presence of compound **4** at 1, 10, and 20 mM. The values represent the mean ± S.D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that control vs. 1 mM, $P < 0.01$; control vs. 10 mM, $P < 0.01$; control vs. 20 mM, $P < 0.01$.

2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-triphenylmethyl-β-D-glucopyranoside (**7**) in 89.3% yield following crystallization of the crude reaction product. In a separate experiment, **5** was converted into **7** in a one-pot fashion in 91.4% yield.

Removal of the 6-*O*-triphenylmethyl group by acid-catalyzed hydrolysis was considered to be possibly problematic owing to the potential for acetyl group migration. Randazzo et al.[31] have described the use of metal ions for the selective removal of triphenylmethyl groups under conditions that avoided hydrolysis or migration of other groups. Using similar reaction conditions, **7** was treated with anhydrous copper sulfate in toluene at reflux temperature for 1 week; however, TLC indicated that no reaction had occurred.

A promising method for the selective detritylation of protected primary hydroxyl groups in carbohydrates was developed by Bessodes et al.[32] and involves the use of formic acid in Et_2O. The advantage of this method is the short reaction time (10–15 min), which allows for the presence of other acid-labile protecting groups. In the present investigation, **7** was treated with formic acid in Et_2O for 1.5 h, at which time TLC showed the presence of only one new component, namely methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**8**), that was isolated in 89.9% yield by column chromatography.

The method of Cree et al.[33] was followed for the oxidation–β-elimination process in which **8** was treated with a mixture of Me_2SO, sulfur trioxide–pyridine complex, and triethylamine to afford methyl 2-acetamido-3-*O*-acetyl-2,4-dideoxy-α-L-*threo*-hex-4-enodialdo-1,5-pyranoside (**9**) in 87.0% yield. In the ^{1}H NMR spectrum of **9**, the H-4 signal appeared as a doublet of doublets rather than as simply a doublet, owing to long-range coupling (*J* 1.0 Hz) to H-2, an observation that was confirmed by NOE experiments. Perlin et al.[34] also described long-range coupling between H-2 and H-4 in similar 4-enopyranoside systems, and the preferred conformation of **9** may be similar to the envelope form suggested by Perlin et al. for methyl 2,3-di-*O*-acetyl-4-deoxy-α-L-*threo*-hexo-4-enodialdo-1,5-pyranoside (Fig. 1). Compound **9** was treated with sodium borohydride in MeOH for 20 min to afford methyl 2-acetamido-2,4-dideoxy-α-L-*threo*-hex-4-enopyranoside (**10**) in 87.4% yield. Compound **10** was subjected to a hydrogen-balloon pressure in the presence of Pd–C for 3 days. TLC showed the formation of a major and a minor component; the major component was isolated by column chromatography and characterized as the desired product **4** in a yield of 73.0%. The minor component was not isolated but was presumed to be the C-5 epimer.

For the incorporation of hydrogen isotopes into the title compound, **9** was treated with sodium borodeuteride, which allowed for the incorporation of deuterium at C-6 to afford **11**. In the ^{1}H NMR spectrum, the H-6 proton appeared as a one-proton singlet at 3.84 ppm; also, the ^{13}C NMR spectrum of **11** exhibited the expected triplet for C-6, and the ^{2}H NMR spectrum exhibited a singlet at 3.14 ppm. Hydrogenation of **11** in the presence of Pd–C afforded **13** (49.4% from **9**); the ^{13}C NMR spectrum exhibited the expected triplet for C-6.

For the synthesis of the radiolabeled analog of **4**, compound **9** was treated with sodium borotritiide (1 mCi) followed by sodium borohydride. The crude product was subjected to a hydrogen-balloon pressure in the presence of Pd–C until TLC showed the complete disappearance of the starting material (3 weeks). Compound **14** was isolated as a chromatographically homogeneous product and was used as a probe in cell cultures to determine the mechanism of biological inhibition of cellular GAGs.

Biological evaluation.—Hepatocyte cultures treated with D-[^{3}H]glucosamine (GlcN) and [^{35}S]sulfate (SO_4) are known to incorporate and express both radiolabels into cellular heparan sulfate glycosaminoglycans. Compound **4** was evaluated for its inhibitory effect on hepatocyte GAG synthesis at 1, 10, and 20 mM (Fig. 2). The addition of **4** to hepatocyte cultures at 1.0 mM caused a decrease in both [^{3}H]GlcN and [^{35}S]SO_4 incorporation to 69 and 59% of the control cells, respectively. At 10 and 20 mM, the incorporation of both radioisotopes was decreased in each case to approxi-

mately 10% of the control cells. These results demonstrate that **4**, at varying concentrations, perturbs cellular GAG synthesis. The mechanism of action of **4** on cellular GAG synthesis may involve the following: (1) inhibition of various enzymatic steps which would transform **4** into a UDP-analog; (2) inhibition of the corresponding UDP-transferase which would affect GAG chain-elongation; (3) incorporation of **4**, or a metabolite derived from **4**, into GAGs causing premature chain termination owing to the 4-deoxy moiety of **4**.

To determine the mechanism of inhibition of cellular GAG synthesis, the [^{3}H] radiolabeled analog of **4**, namely **14**, was evaluated for its inhibitory effect on hepatocyte GAG synthesis. Compound **14** was evaluated at 1.0, 10, and 20 mM in the absence of radiolabeled GAG precursors (i.e., [^{3}H]GlcN and [^{35}S]SO_4). Fig. 3 demonstrates that addition of **14** to hepatocyte cultures caused a concentration-dependant incorporation of a [^{3}H] radiolabel into isolated GAGs. The maximum incorporation of a [^{3}H] radiolabel occurred at concentrations of 10 and 20 mM, a result which reflects the ability of **4**, at 10 and 20 mM, to have a maximum effect on the inhibition of [^{3}H]GlcN and [^{35}S]SO_4 into isolated GAGs.

These results suggest that the mechanism of action of **4** is due, in part, to the incorporation of a metabolite derived from **4** into GAG chains causing premature chain termination. The methyl aglycone of **4** can be hydrolyzed by glycosidases, and the resulting free sugar would be rapidly converted into a UDP-metabolite and incorporated into growing GAG chains. Incorporation of the 4-deoxy moiety into GAG chains would result in premature GAG-chain termination, and the resulting GAGs would have a decreased level of [^{3}H]GlcN and [^{35}S]SO_4 incorporation. Similar reductions of incorporations of isotopic labels were observed with 4-deoxy derivatives,[20] a 3-deoxy-3-fluoro derivative,[35,36] and 4-deoxy-4-fluoro derivatives[19,25,36] of 2-acetamido-2-deoxy-D-hexopyranoses.

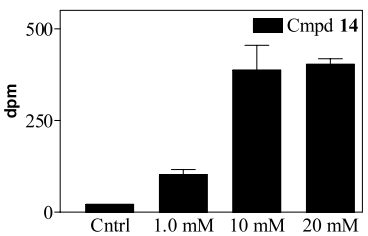


Fig. 3. Effect of increasing concentration of methyl 2-acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside-6-*t* (**14**) on hepatocyte cellular glycosaminoglycan synthesis. Hepatocyte cultures were incubated for 24 h in the absence (control) or presence of compound **14** at 1, 10, and 20 mM. The values represent the mean ± S.D. of triplicate cultures. Statistical analyses using an unpaired *t*-test revealed that control vs. 1 mM, $P < 0.01$; control vs. 10 mM, $P < 0.01$; control vs. 20 mM, $P < 0.01$.

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 rt. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400.1 and 100.6 MHz, respectively. The solvent was $CDCl_3$, unless stated otherwise. The signals owing 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. Thin-layer chromatography (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*-benzyl-2-deoxy-4-*O*-[(methylthio)thiocarbonyl]-β-*D*-glucopyranoside* (**2**).—A mixture of **1**[29] (0.098 g, 0.24 mmol), NaH (60% oily suspension, 3.3 equiv, 0.032 g), and three crystals of imidazole in THF (2 mL) was stirred for 1 h, and then CS_2 (10 equiv, 0.146 mL) was added. After 1 h, MeI (3 equiv, 0.045 mL) was added, and the reaction mixture was stirred overnight. The mixture was concentrated under reduced pressure to afford a yellowish oil that was subjected to flash chromatography (10:1 CH_2Cl_2–MeOH) to afford **2** (0.112 g, 93.5%) as a yellowish solid: R_f 0.45 (1:2 EtOAc–Et_2O); mp 133–134 °C; $[\alpha]_D$ + 63.3° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3 H, NAc), 2.54 (s, 3 H, SMe), 3.29 (m, 1 H, H-2), 3.51 (s, 3 H, OMe), 3.62 (m, 2 H, H-6, H-6′), 3.84 (m, 1 H, H-5), 4.41 (dd, 1 H, $J_{3,2} = J_{3,4}$ 8.9 Hz, H-3), 4.48–4.70 (m, 4 H, 2 PhCH_2), 4.95 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.65 (d, 1 H, NH), 5.98 (dd–t, 1 H, $J_{4,5}$ 9.0 Hz, H-4), 7.24–7.34 (m, 10 H, 2 Ph); ^{13}C NMR ($CDCl_3$): δ 19.5 (SMe), 23.6 (NCOCH_3), 57.1 (OMe), 57.3 (C-2), 69.4 (C-6), 73.5 (C-3), 73.6 and 74.2 (2 PhCH_2), 77.6 (C-4), 79.9 (C-5), 100.5 (C-1), 127.6–128.5 (Ph), 172.0 (NC=O), 201.0 (OC=S). Anal. Calcd for $C_{25}H_{31}NO_6S_2$: C, 59.38; H, 6.18; N, 2.77; S, 12.68. Found: C, 59.30; H, 6.18; N, 2.79; S, 12.46.

*Methyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside* (**3**).—A solution of **2** (0.400 g, 0.79 mmol), AIBN (0.8 equiv, 0.090 g), and Bu_3SnH (15 equiv, 3.18 mL) in toluene (30 mL) was heated at reflux temperature for 20 min. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel

(hexanes flush, then EtOAc–Et_2O gradient). Recrystallization from EtOAc–hexanes afforded pure **3** (0.221 g, 70.0%) as a white, crystalline solid: R_f 0.20 (1:2 EtOAc–Et_2O); mp 160–161 °C; $[\alpha]_D$ +17.8° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.43 (apparent q, 1 H, $J_{4ax,3} = J_{4ax,5} = J_{4ax,4eq}$ 11.4 Hz, H-4ax), 1.94 (s, 3 H, NAc), 2.20 (ddd, 1 H, $J_{4eq,3}$ 4.7, $J_{4eq,5}$ 1.5 Hz, H-4eq), 3.19 (m, 1 H, H-2), 3.48 (s, 3 H, OMe), 3.49–3.75 (m, 3 H, H-5, H-6, H-6′), 4.04 (ddd, 1 H, $J_{3,2}$ 10.6 Hz, H-3), 4.44 and 4.59 (2 d, 2 H, *J* 11.7 Hz, PhCH_2), 4.58 (s, 2 H, PhCH_2), 4.72 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.63 (dd, 1 H, $J_{NH,2}$ 6.8 Hz, NH), 7.29–7.36 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.8 (NCO*C*H_3), 34.1 (C-4), 56.6 (OMe), 58.3 (C-2), 71.1 (C-3, 6), 72.5 and 73.5 (2 Ph*C*H_2), 74.7 (C-5), 101.1 (C-1), 127.7–128.5, 139.4, and 139.5 (Ph), 170.5 (NC=O). Anal. Calcd for $C_{23}H_{29}NO_5$: C, 69.15; H, 7.32; N, 3.51. Found: C, 69.03; H, 7.23; N, 3.53.

*Preparation of methyl 2-acetamido-2,4-dideoxy-β-*D*-xylo-hexopyranoside* (**4**).—A mixture of **3** (0.120 g, 0.300 mmol) and 5% Pd–C (0.120 g) in ~2% hydrogen chloride in MeOH (7 mL) was subjected to a hydrogen pressure (50 psig) for 12 h. The mixture was neutralized with Amberlite IRA-400 (OH^-) resin and filtered through Celite 521 (Aldrich), and the filtrate was concentrated under reduced pressure to yield a crude solid. Flash chromatography on silica gel (1:9 MeOH–$CHCl_3$) afforded **4** as a solid that was recrystallized from MeOH–EtOAc to afford pure **4**[20] (0.057 g, 86.5%) as a white solid: R_f 0.18 (1:20 MeOH–CH_2Cl_2); mp 200–201 °C, lit. 200 °C;[20] $[\alpha]_D$ −33.2° (*c* 0.16, MeOH), lit. −33° (*c* 0.16, MeOH);[20] ^1H 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 (NCO*C*H_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.

*Preparation of methyl 2-acetamido-2-deoxy-6-*O*-triphenylmethyl-β-*D*-glucopyranoside* (**6**).—To a solution of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**5**)[30] (1.50 g, 6.38 mmol) in anhyd pyridine (20 mL) was added freshly prepared Ph_3CCl (1.1 equiv, 1.96 g). The mixture was stirred at rt until TLC indicated the complete disappearance of **5** (4 days). The mixture was added slowly to ice–water and extracted with CH_2Cl_2; the extracts were washed sequentially with aq $NaHCO_3$ (satd) and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was coevaporated with toluene to afford a creamy, white solid. Recrystallization of the residue from EtOH–hexanes afforded crystalline **6** (2.41 g, 79.1%): R_f 0.40 (1:9 MeOH–CH_2Cl_2); mp 194–196 °C, lit. 182–184 °C;[37] $[\alpha]_D$ −29.9° (*c* 1, MeOH), lit. −49.5° (*c* 1, $CHCl_3$);[37] ^1H NMR ($CDCl_3$): δ 1.91 (s, 3 H, NAc), 3.23 (m, 1 H, H-6), 3.30–3.25 (m, 4 H, H-3, H-4, H-5, H-6′), 3.48 (s, 3 H, OMe), 3.64 (m, 1 H, H-2), 4.30 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 7.12–7.43 (m, 15 H, Ph); ^{13}C NMR ($CDCl_3$): δ 23.0 (NCO*C*H_3), 56.7 (C-2), 57.3 (OMe), 64.7 (C-6), 72.4, 76.4, and 76.9 (C-3, C-4, C-5), 87.6 (*C*Ph_3), 103.4 (C-1), 128.0–130.0 (Ph), 145.5 (Ph), 173.8 (NC=O). Anal. Calcd for $C_{28}H_{31}NO_6$: C, 70.42; H, 6.54; N, 2.93. Found: C, 70.29; H, 6.40; N, 2.87.

*Methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-triphenylmethyl-β-*D*-glucopyranoside* (**7**).—Compound **6** (1.83 g, 3.83 mmol) was treated with Ac_2O (15 mL) in pyridine (20 mL) at 0 °C, and the reaction mixture was stirred overnight at rt. The solution was concentrated, and the residue was coevaporated with toluene under reduced pressure to afford a yellowish solid that was recrystallized from EtOAc to afford **7** (1.92 g, 89.3%) as tiny, white needles: R_f 0.72 (5:1 EtOAc–toluene); mp 253–255 °C; $[\alpha]_D$ +24.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.71 (s, 3 H, OAc), 1.95 and 2.01 (2 s, 6 H, OAc, NAc), 3.07 and 3.45 (dq, 2 H, $J_{6,5}$ 4.8, J_{gem} 10.4, $J_{6',5}$ 2.1 Hz, H-6, H-6′), 3.54 (m, 1 H, H-5), 3.56 (s, 3 H, OMe), 4.00 (m, 1 H, H-2), 4.52 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.15 (dd–t, 1 H, $J_{3,2} = J_{3,4}$ 9.3 Hz, H-3), 5.17 (dd–t, $J_{4,5}$ 10.0 Hz, H-4), 5.59 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 7.19–7.46 (m, 15 H, Ph); ^{13}C NMR ($CDCl_3$): δ 20.4 and 20.7 (2 OCO*C*H_3), 23.4 (NCO*C*H_3), 54.5 (C-2), 56.3 (OMe), 62.0 (C-6), 68.8 (C-4), 72.8 (C-3), 73.3 (C-5), 86.5 (*C*Ph_3), 101.6 (C-1), 126.9–128.6 (Ph), 143.6 (Ph), 168.9, 170.2, and 171.2 (2 O*C*OCH$_3$, NC=O). Anal. Calcd for $C_{32}H_{35}NO_8$: C, 68.44; H, 6.28; N, 2.49. Found: C, 68.60; H, 6.19; N, 2.55.

*Methyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**8**).—To compound **7** (1.50 g, 2.67 mmol) was added a solution of 1:1 HCO_2H–Et_2O (25 mL), and the mixture was stirred for 1.5 h at rt. The mixture was concentrated under reduced pressure, and the residue was coevaporated with toluene to afford a white solid that could not be recrystallized. Flash chromatography (EtOAc) on silica gel afforded **8** (0.767 g, 89.9%) as a white, amorphous solid: R_f 0.16 (EtOAc); mp 196–197 °C; $[\alpha]_D$ −14.3° (*c* 1, MeOH); IR (KBr): ν 3426, 1746, 1721, 1654, and 1555 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.94 (s, 3 H, NAc), 2.02 and 2.03 (2 s, 6 H, 2 OAc), 3.46–3.61 (m, 2 H, H-5, H-6), 3.49 (s, 3 H, OMe), 3.74 (dd, 1 H, $J_{6',5}$ 2.3, J_{gem} 12.5 Hz, H-6′), 3.89 (ddd, 1 H, H-2), 4.58 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.02 (dd–t, 1 H, $J_{4,3} = J_{4,5}$ 9.6 Hz, H-4), 5.28 (dd–t, 1 H, $J_{3,2}$ 10.1 Hz, H-3), 5.90 (d, 1 H, $J_{NH,2}$ 8.9 Hz, NH); ^{13}C NMR ($CDCl_3$): δ 20.7, 23.3, and 23.4 (2 OCO*C*H_3, NCO*C*H_3), 54.5 (C-2), 56.9 (OMe), 61.3 (C-6), 69.0 (C-4), 72.4 (C-3), 74.0 (C-5), 101.7 (C-1), 170.2, 170.5, and 171.1 (2 O*C*OCH$_3$, NC=O). Anal. Calcd for $C_{13}H_{21}NO_8 \cdot H_2O$: C, 47.56; H, 6.75; N, 4.27. Found: C, 47.90; H, 6.41; N, 4.38.

Methyl 2-acetamido-3-O-acetyl-2,4-dideoxy-α-L-threo-hex-4-enodialdo-1,5-pyranoside (**9**).—To a mixture of **8** (6.30 g, 19.7 mmol) in Me_2SO (80 mL) was added Et_3N (36 mL) at 0 °C. To this stirred solution was added dropwise a solution of sulfur trioxide–pyridine complex (6.6 equiv, 20.9 g) in Me_2SO (160 mL) over a 1.5-h period. The mixture was diluted with CH_2Cl_2 (400 mL) and washed sequentially with ice-cooled aq tartaric acid (satd), aq $NaHCO_3$ (satd), and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure to afford a residue. The residue was subjected to flash chromatography on silica gel (3:1 EtOAc–hexanes) to afford **8** (4.42 g, 87.0%) as a clear, bubbly oil that could not be crystallized and that decomposed upon standing: R_f 0.46 (EtOAc); $[\alpha]_D$ +32.9° (*c* 1, $CHCl_3$); IR (KBr): *v* 3470, 1738 (ester), 1705 (aldehyde), 1660 (C=C), and 1544 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.97 (s, 3 H, NAc), 2.09 (s, 3 H, OAc), 3.46 (s, 3 H, OMe), 4.46 (m, 1 H, H-2), 5.10 (d, 1 H, $J_{1,2}$ 2.4 Hz, H-1), 5.14 (dd, 1 H, $J_{3,2}$ 2.0, $J_{3,4}$ 4.6 Hz, H-3), 5.83 (d, 1 H, $J_{NH,2}$ 9.3 Hz, NH), 6.08 (dd, 1 H, $J_{4,2}$ 1.0 Hz, H-4), 9.25 (s, 1 H, CH=O); ^{13}C NMR ($CDCl_3$): δ 20.9 and 23.0 ($OCOCH_3$, $NCOCH_3$), 49.2 (C-2), 57.0 (OMe), 64.7 (C-3), 99.2 (C-1), 116.7 (C-4), 149.2 (C-5), 169.6 and 170.1 ($OCOCH_3$, NC=O), 186.6 (CH=O); FABMS (positive-ion): Calcd for $C_{11}H_{16}NO_6$: 258.0978. Found: 258.0975.

Methyl 2-acetamido-2,4-dideoxy-α-L-threo-hex-4-enopyranoside (**10**) *and the preparation of the deuterated analog* (**11**) *and the radiolabeled analog* (**12**).—To a solution of **9** (0.237 g, 0.921 mmol) in MeOH (7 mL) was added $NaBH_4$ (1 equiv, 0.035 g). The mixture was stirred for 20 min at rt and concentrated under reduced pressure, and the residue was coevaporated several times with MeOH. The resulting residue was subjected to flash chromatography on silica gel (1:9 MeOH–CH_2Cl_2) to afford **10** (0.175 g, 87.4%) as a clear, colorless oil which could not be crystallized and which decomposed upon standing: $[\alpha]_D$ −9.9° (*c* 1, MeOH); IR (KBr): *v* 3290, 1647, and 1548 cm^{-1}; ^1H NMR (MeOH-d_4): δ 1.90 (s, 3 H, NAc), 3.39 (s, 3 H, OMe), 3.83 (dd, 1 H, H-3), 3.91 (s, 2 H, H-6, H-6′), 4.04 (dd, 1 H, $J_{2,3}$ 3.9 Hz, H-2), 4.87 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1), 5.00 (d, 1 H, $J_{4,3}$ 4.2 Hz, H-4); ^{13}C NMR (MeOH-d_4): δ 22.6 ($NCOCH_3$), 53.5 (C-2), 56.6 (OMe), 62.4 (C-6), 65.8 (C-3), 100.5 (C-4), 100.8 (C-1), 152.6 (C-5), 173.2 (NC=O); FABMS (positive-ion): Calcd for $C_9H_{16}NO_5$: 218.1029. Found: 218.1006.

In a separate experiment, sodium borodeuteride (NaB^2H_4, 1 equiv, 0.037 g) was added to a solution of **9** (0.256 g, 0.995 mmol) in MeOH (7 mL). The mixture was stirred for 20 min at rt and concentrated under reduced pressure, and the residue was coevaporated several times with MeOH to afford crude **11** (0.347 g): ^1H NMR (MeOH-d_4): δ 1.82 (s, 3 H, NAc), 3.30 (s, 3 H, OMe), 3.78 (m, 1 H, H-3), 3.84 (s, 1 H, H-6, H-6′), 3.98 (m, 1 H, H-2), 4.70 (d, 1 H, H-1), 4.98 (d, 1 H, H-4); ^{13}C NMR (MeOH-d_4): δ 22.5 ($NCOCH_3$), 53.5 (C-2), 56.6 (OMe), 62.1 (t, C-6), 65.8 (C-3), 100.6 (C-4), 100.9 (C-1), 152.6 (C-5), 173.3 (NC=O); ^2H NMR (MeOH-d_4): δ 3.14. The compound decomposed on standing.

In an analogous manner, **9** (0.200 g, 0.777 mmol) was treated with sodium borotritide (NaB^3H_4, 1 mCi) in MeOH at 0 °C. The mixture was stirred for 5 min followed by the addition of NaB^1H_4 (1 equiv, 0.029 g). After 30 min at rt, the mixture was concentrated under reduced pressure, and the residue was coevaporated several times with MeOH to afford crude **12**.

Preparation of methyl 2-acetamido-2,4-dideoxy-β-D-xylo-hexopyranoside (**4**) *from* **10** *and the preparation of the deuterated analog* (**13**) *and the radiolabeled analog* (**14**).—A mixture of **10** (0.205 g, 0.944 mmol) and 10% Pd–C (0.250 g) in MeOH (7 mL) was subjected to a hydrogen-balloon pressure for 3 days. The mixture was filtered through Celite 521 (Aldrich), the residue was washed with MeOH, and the combined filtrate and washings were concentrated under reduced pressure to give a residue which was purified by flash chromatography on silica gel (1:6 MeOH–CH_2Cl_2) to afford **4** (0.151 g, 73.0%) as a white solid. The ^1H and ^{13}C NMR spectra were identical to those obtained for a sample of **4** obtained by the route shown in Scheme 1.

In a separate experiment, a mixture of **11** (0.347 g, obtained in a crude form from **9**) and 10% Pd–C (0.300 g) in MeOH (10 mL) was subjected to a hydrogen-balloon pressure for 4 days. The reaction mixture was processed as described above for the preparation of **4** from **10** to afford **13** (0.107 g, 49.4% from **9**) as a white solid: R_f 0.37 (1:3 MeOH–$CHCl_3$); mp 198–202 °C; $[\alpha]_D$ − 32.0° (*c* 1, MeOH); ^1H NMR (MeOH-d_4): δ 1.30 (apparent q, 1 H, $J_{3,4ax} = J_{4ax,4eq} = J_{4ax,5}$ 11.8 Hz, H-4ax), 1.87–1.91 (m, 1 H, H-4eq), 1.90 (s, 3 H, NAc), 3.37 (s, 3 H, OMe), 3.40–3.46 (m, 2 H, H-2, H-5), 3.48 (m, 1 H, H-6), 3.57 (apparent dt, 1 H, $J_{2,3}$ 10.9, $J_{3,4eq}$ 5.0 Hz, H-3), 4.15 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1); ^{13}C NMR (MeOH-d_4): δ 23.0 ($NCOCH_3$), 37.0 (C-4), 56.9 (C-2), 58.8 (OMe), 65.2 (t, C-6), 70.8 (C-3), 73.9 (C-5), 103.9 (C-1), 174.0 (NC=O).

In an analogous manner, **12** (obtained in a crude form from **9**) was converted into the 6-tritio analog **14** by treatment with 10% Pd–C (0.200 g) in MeOH (10 mL) and a hydrogen-balloon pressure for 3 weeks. Compound **14** (0.062 g, 36.5%) was isolated as described for the preparation of **13**.

Biological evaluation

Materials. D-(6-^{3}H)Glucosamine–HCl (25.6 Ci/mmol), $Na_2^{35}SO_4$ (867 mCi/mL), and NaB^3H_4 (204.1 mCi/mmol) were purchased from either Dupont or ICN Biomedicals. EGTA, D-glucosamine hydrochloride, and Hepes buffer were of reagent grade and were obtained from Sigma Chemical Co., Fisher Scientific Co., or

BDH Chemicals. Williams' Medium E with L-glutamine, 10% fetal bovine serum, 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 the procedure described previously.[38,39] 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 10% fetal bovine serum and 1% antibiotic–antimycotic mixtures. After 2 h, the non-adherent cells were removed, and the attached cells were fed with fresh plating medium for a 24-h period. The cells were then treated with fresh medium containing D-[^{3}H]GlcN (2 μCi/mL) and [^{35}S]SO_4 (4 μCi/mL) in the presence or absence of monosaccharide derivative **4**, or with medium in the presence or absence of the monosaccharide derivative **14**. The labeled cellular GAGs were harvested 24 h later.

Glycosaminoglycan isolation. After the 24-h labeling period, the medium was separated from the cells, and the cells were solubilized in 4 M guanidine·HCl, 2% Triton X-100 in 0.05 M acetate buffer (pH 6.0). A GAG carrier (1 mg/mL each of chondroitin sulfate, hyaluronan, and heparin) was added to all of the samples. The isolation of radioactive GAGs was based on the cetylpyridinium chloride (CPC) precipitation technique described by Hronowski and Anastassiades.[40] Briefly, media and cell fractions were each subjected to papain digestion, and the GAGs were precipitated as sodium salts by CPC. After lyophilization, the samples were dissolved in a known volume of water for analysis. Media GAGs were analyzed only during preliminary experiments. Cellular GAGs served as the assay material for the effects of the sugar analogs.

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