Synthesis of Phosphonic Acid Analogues of Sialic Acids (Neu5Ac and KDN) as Potential Sialidase Inhibitors

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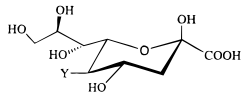
Received October 7, 1996 (Revised Manuscript Received March 4, 1997[⊗]*)*

Five phosphonic acid analogues of *N*-acetylneuraminic acid (Neu5Ac) and 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN) have been synthesized. The synthesis was accomplished using an indium-mediated coupling of *N*-acetylmannosamine or mannose with dimethyl (3-bromopropen-2-yl)phosphonate in aqueous media. The potential of these phosphonate acid analogues of sialic acids as sialidase inhibitors was evaluated and found to show moderate biological acitivities.

Introduction

The sialic acids, of which *N*-acetylneuraminic acid (Neu5Ac, **1a**) and 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN, **1b**) are members, are a class of carbohydrate molecules of some biological significance. Neu5Ac has emerged to be a key biomolecule in the regulation of many biological phenomena.[1] For example, binding to terminal α-glycosides of Neu5Ac on cell-surface glycoproteins and glycolipids is the initiating process of cell infection by certain viruses.[2] In an inflammatory response to injury, it is the interaction between selectins and sialylated oligosaccharides which is believed to be involved in the early stage of adhesion of leukocytes to activated endothelial cells.[3] Gangliosides, with one or more residues of Neu5Ac, are receptor molecules located on the outer surface of vertebrate cell membranes interacting with external biological factors such as toxin proteins.[4] In many cases, it is believed that the carboxylic acid group of **1a**, in its anionic form, is essential for binding.[5] On the other hand, while neuraminic acid and its simple modifications are widely found in glycoconjugates in nature, the deaminated parent compound, KDN, **1b**, has only been recently isolated from rainbow trout egg polysialoglycoprotein (PSGP).[6] The KDN residue was located at the nonreducing end in PSGP. It was suggested that KDN, on terminal capping of the oligo(poly)-sialyl chain, may serve to protect the oligosialyl groups from the action of sialidases. Sialidases are glycohydrolases that catalyze the hydrolysis of the terminal sialic acids linked to the glycoproteins, glycolipids, and polysaccharides by an α-ketosidical bond. Recently, the development of sialidase inhibitors has generated considerable interest because of their potential as antiviral drugs.[7] X-ray crystallographic studies of the influenza virus sialidase/Neu5Ac complex have been reported.[8] Several analogues of Neu5Ac have been synthesized, and their potential as sialidase inhibitors have been examined.[9] Structural modification has been focused so far on replacement of the hydroxyl functions with azido or guanidino groups. Here, we report the synthesis of novel phosphonic acid analogues of **1** in the expectation that the phosphonic acid group should play the same role as the carboxylic acid in providing the negative charge for binding.[10]



1a: Y=NHAc, Neu5Ac
1b: Y=OH, KDN

2a, R=H or Me or Et **2b**

Synthesis

Many chemical syntheses of Neu5Ac and KDN have been reported.[11] Alternately, enzymic synthesis of both naturally-occurring and unnatural sialic acids has been achieved with the use of Neu5Ac aldolase.[12] Recently, we[13] and others[14] have developed a concise syntheis of Neu5Ac and KDN based on indium-mediated coupling of carbohydrates with (bromomethyl)acrylates (**2a**) in

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.

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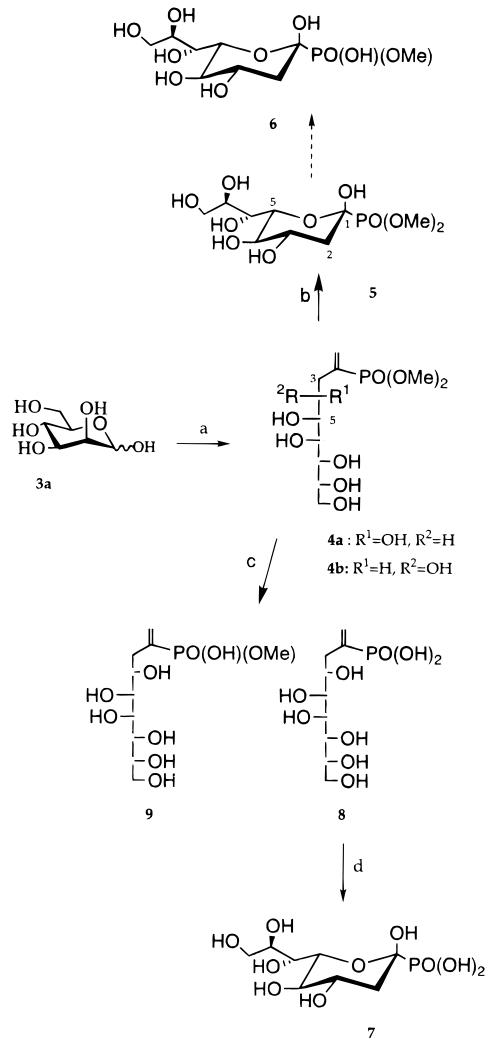
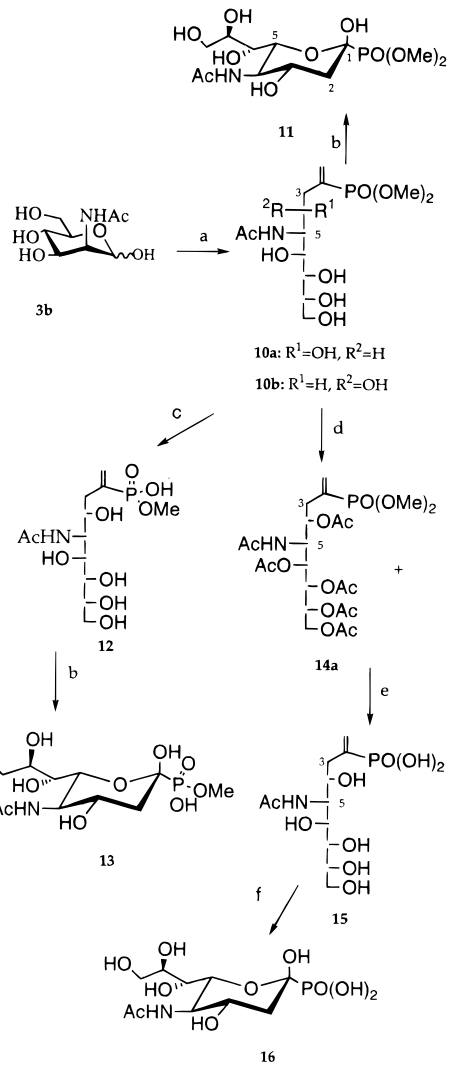
S0022-3263(96)01891-9 CCC: $14.00

Scheme 1[a]



[a] (a) In, **2b**, H_2O; (b) O_3, MeOH−CH_2Cl_2, then DMS; (c) NaOH (1 N), MeOH; (d) O_3, MeOH−H_2O, then DMS.

Scheme 2[a]



[a] (a) In, **2b**, H_2O; (b) O_3, MeOH−CH_2Cl_2, then DMS; (c) NaOH (1 N), MeOH; (d) Ac_2O, pyridine, DMAP; (e) (1) bromotrimethylsilane, CH_2Cl_2, (2) NaOH, MeOH; (f) O_3, MeOH−H_2O, then DMS.

aqueous media.[15] One advantages of the indium chemistry is that the water-soluble carbohydrate molecules can react directly without the protection−deprotection often required in conventional carbohydrate chemistry. We have therefore extended this approach for the synthesis of the phosphonic acid analogues of sialic acids.

(a) Synthesis of Phosphono-KDN (Scheme 1). Dimethyl (3-bromopropen-2-yl)phosphonate (**2b**) was synthesized in good yield using a literature procedure.[16] The coupling of D-mannose (**3a**) with **2b** and indium in water gave the polyhydroxyl phosphonate compounds **4a** and **4b** as an epimeric mixture. It is interesting to note that, in this reaction, the coupling of mannose with **2a** or **2b**, the stereoselectivity was much better with **2b** (syn:anti = 20:1) than with **2a** (syn:anti = 5:1). The desired isomer, compound **4a**, can be obtained in 94% yield after flash chromatography. Ozonolysis of compounds **4a** in methanol−dichloromethane at −78 °C gave the keto phosphonate which cyclized spontaneously to the pyranose form **5**. Compound **5** existed as one anomer on the basis of its ^1H, ^{13}C, and ^{31}P NMR, which all showed one set of signals. The phosphorus group in compound **5** is assigned to the equatorial position on the basis of the relatively small H−P coupling constant (∼5.5 Hz) of H_{2ax} of the 2-deoxy position with phosphorus. An axial phosphorus group would be expected to give rise to a larger P−H_{ax} coupling of about 13 Hz.[17] All attempted hydrolyses of **5** to give the monohydrogen phosphonate **6** or the dihydrogen phosphonate **7**, under acidic or basic conditions, or on treatment with trimethylsilyl bromide, gave complicated reaction mixtures. On the other hand, hydrolysis of **4a** with sodium hydroxide (1 N in water) in methanol at room temperature gave the phosphonic acid **8** in quantitative yield. The intermediate monomethyl phosphonate **9** was not observed to any significant extent. Ozonolysis of **8** in methanol gave compound **7** in high yield. The phosphorus group in **7** also retained the P-equatorial position.

(b) Synthesis of Phosphono-Neu5Ac (Scheme 2). Similar coupling of *N*-acetylmannosamine (**3b**) and **2b** with indium in water gave the epimeric mixture **10a** and **10b**. In this case, the stereoselectivity was syn:anti = 5:1. The same stereoselectivity was observed in the coupling of **3b** with **2a**. The desired epimer **10a** could be obtained by recrystallization from methanol−ethyl

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acetate (1:2) with a yield of 32%. Ozonolysis of **10a** in methanol−dichloromethane at −78 °C gave the pyranophosphonate **11**, also with the phophono group equatorial. All attempts to hydrolyze **11** also met with failure. Base hydrolysis of **10a** gave interestingly only the monomethyl phosphonate ester **12**. Attempts to hydrolyze both methyl groups of compound **10a** under basic condition by extending the reaction times, or by raising the reaction temperature, gave only complicated mixtures, in which no desired compound could be obtained. Ozonolysis of **12** gave the pyranose methyl phosphonate **13** in excellent yield. In order to prepare the fully hydrolyzed phosphonic acid, the epimeric mixture **10a** and **10b** was first acetylated under conventional conditions, after purification by chromatography, to give the major isomer **14a** which can be isolated by recrystallization in 51% yield. After treatment with 4 equiv of bromotrimethylsilane and then base hydrolysis, the completely demethylated phosphonic acid **15** was obtained in 74% yield. The same ozonolysis of **15** led finally to compound **16** again as a single anomer with the phosphono group equatorial.

Biological Evaluation

All five Neu5Ac and KDN phophonate analogues, compounds **5**, **7**, **11**, **13**, and **16**, have been tested for the inhibition of both viral and bacterial sialidases using standard procedures[7] with 2′-(4-methylumbelliferyl)-*N*-acetylneuraminic acid as substrate. The results are summarized in the following table of IC_{50} values against two different sialidases:

against influenza virus sialidase (**N2**)		against *Vibrio cholerae* sialidase	
compd **7**	2×10^{-4} M	compd **7**	5×10^{-3} M
compd **5**	1×10^{-3} M	compd **5**	2×10^{-2} M
compd **11**	6×10^{-5} M	compd **11**	1×10^{-3} M
compd **13**	2×10^{-4} M	compd **13**	1×10^{-3} M
compd **16**	2×10^{-4} M	compd **16**	5×10^{-4} M

Discussion

Compounds **5**, **7**, **11**, **13**, and **16** displayed weak to moderate inhibition of sialidase from both *Vibrio cholerae* and influenza virus (N2). Interestingly and perhaps unexpectedly, compound **11**, the bismethyl ester of **16**, was as active as the partially or completely de-esterified compounds. Typically, it would be expected that the negatively-charged phosphonic acid group would form strong charge−charge interactions with the conserved triarginyl cluster within the active sites of these sialidases, as is observed for the carboxylate of Neu5Ac derivatives.[7] The fact that phosphonic acid esters also inhibit these sialidases may well suggest that the compounds could be interacting with the active site in a different mode to that observed for other Neu5Ac and Neu5Ac2en derivatives.[18] It is also worth noting from these results that little difference, if any, in sialidase inhibition between phospha KDN (**5** and **7**) and phospha Neu5Ac (**11**, **13**, and **16**) analogues was observed. This suggests that neither the C-5 *N*-acetyl group (**11**, **13**, and **16**) nor the hydroxyl group (**5** and **7**) play a significant role in the binding of these compounds to the active site and that the phosphorus substituent has a predominating effect. This explanation is not unreasonable, as it is well-known that Neu5Ac-recognizing sialidases require an acylamino group for recognition.[19] Vasella *et al*. have reported the synthesis of three phospha analogues of 2-deoxy-*N*-acetylneuraminic acid, two anomeric phosphonates, and an α,β-unsaturated phosphonate as potential inhibitors of *V*. cholerae sialidase and found these compounds to also hold moderate activity.[20]

Experimental Section

General Methods. Melting points (mp) are uncorrected. Optical rotations were measured with a DIP-140 (Jasco) polarimeter at 20°. NMR spectra (δ in ppm, relative to tetramethylsilane) were recorded with a Varian 200 or 500 MHz spectrometer. Low- and high-resolution mass spectral analyses were performed by the Mass Spectrometry Service, Department of Chemistry, or the Biomedical Mass Spectrometry Unit of Faculty of Medicine, McGill University. Analytical thin layer chromatography were performed on silica gel 60 F_{254} plastic back plates (Aldrich) and was visualized by dipping into a solution of ammonium molybdate (2.5 g) and ceric sulfate (1 g) in concentrated H_2SO_4/H_2O (10 mL/90 mL) and heating with a heat gun.

Dimethyl (4,5,6,7,8,9-D-*glycero*-D-*galacto*-Hexahydroxynon-1-en-2-yl)phosphonate (4a). Indium (764 mg, 6.6 mmol) was added to a solution of D-mannose (200 mg, 1.11 mmol) and **2b** (1.53 g, 6.6 mmol) in water (35 mL). The mixture was stirred at room temperature for 20 h. After filtration and evaporation *in vacuo*, compounds **4a** and **4b** was purified by flash chromatography (methanol−dichloromethane = 1:4). Compound **4a** (344 mg, 94%) was obtained as a syrup: $[\alpha]_D$ −6.7 (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 2.42 (ddd, 1H, J = 9.0, 14.5, 20.0 Hz), 3.34 (ddd, 1H, J = 4.5, 14.5, 14.5 Hz), 3.38 (d, 1H, J = 9.5 Hz), 3.52 (dd, 1H, J = 11.5, 6.5 Hz), 3.58 (d, 3H, J_{H-P} =11.0 Hz), 3.59 (d, 3H, J_{H-P} = 11.0 Hz), 3.60 (m, 2H), 3.69 (dd, 1H, J = 11.5, 3.0 Hz), 3.72 (d, 1H, J = 9.5 Hz), 3.99 (dd, 1H, J = 4.5, 9.0 Hz), 5.96 (d, 1H, J_{H-P} = 50.0 Hz), 5.99 (d, 1H, J_{H-P} = 23.5 Hz); ^{13}C-NMR (50 MHz, D_2O) δ 40.08 (d, J_{C-P} = 11.7 Hz), 56.90 (d, J_{C-P} = 11.7Hz), 66.74, 71.19 (d, J_{C-P} = 3.5 Hz), 71.92, 72.69, 74.32, 74.52, 134.92 (d, J_{C-P} = 170.0 Hz), 137.54 (d, J_{C-P} = 19.0 Hz); ^{31}P-NMR (109 MHz, D_2O with 85% H_3PO_4 as reference) δ 27.10; MS (FAB) m/z 331 (M + 1); HRMS (FAB) calcd for $C_{11}H_{24}O_9P$ (M + 1) 331.115 80, found 331.115 80.

Dimethyl (5-Acetamido 4,6,7,8,9-D-*glycero*-D-*galacto*-pentahydroxynon-1-en-2-yl)phosphonate (10a). Indium (622 mg, 5.4 mmol) was added to a solution of *N*-acetylmannosamine (200 mg, 0.9 mmol) and **2b** (1.24 g, 5.4 mmol) in water (50 mL). The reaction mixture was stirred at ambient temperature for 20 h. After filtration and evaporation *in vacuo*, compounds **10a** and **10b** (302 mg, 90%) were obtained as an epimeric mixture (syn:anti = 5:1). The major isomer **10a** was obtained by crystallization from methanol−dichloromethane (1:2) as a solid (107 mg, 32%): mp 134−135 °C; $[\alpha]_D$ = −59 (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.90 (s, 3H), 2.25 (m, 2H), 3.30 (d, 1H, J = 8.5 Hz), 3.46 (dd, 1H, J = 6.5, 12.0 Hz), 3.57 (m, 1H), 3.60 (d, 6H, J_{H-P} = 11.5 Hz), 3.67 (dd, 1H, J = 12.0, 3.0 Hz), 3.78 (m, 2H), 4.21 (t, 1H, J = 7.0 Hz), 5.93 (d, 1H, J_{H-P} = 50 Hz), 5.99 (d, 1H, J_{H-P} = 23 Hz); ^{13}C-NMR (50 MHz, D_2O) δ 25.86, 40.49 (d, J_{C-P} = 11.7 Hz), 56.78, 56.94 (d, J_{C-P} = 5.7 Hz), 66.70, 70.26 (d, J_{C-P} = 3.6 Hz), 71.17, 72.75, 74.08, 134.58 (d, J_{C-P} = 170.2 Hz), 137.65 (d, J_{C-P} = 4.3 Hz), 176.49; ^{31}P-NMR (109 MHz, D_2O) δ 25.00; MS (FAB) m/z 372 (M + 1); HRMS (FAB) calcd for $C_{13}H_{27}NO_9P$ (M + 1) 372.142 35, found 372.142 28.

(4,5,6,7,8,9-D-*glycero*-D-*galacto*-Hexahydroxynon-1-en-2-yl)phosphonic Acid (8). To a solution of **4a** (150 mg, 0.45 mmol) in methanol (15 mL) was added at 0 °C a solution of

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sodium hydroxide (1 N in water, 1 mL). The reaction mixture was stirred for 15 h at room temperature and then neutralized by Dowex-50 resin until pH = 3−4. The resin was filtered, and the solution was concentrated *in vacuo*. Compound **8** (137 mg, 100%) was precipitated by acetone as white powder: mp 94−96 °C dec; $[\alpha]_D = +10$ (c = 1, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 2.39 (m, 2H), 3.42 (d, 1H, J = 9.0 Hz), 3.52 (dd, 1H, J = 11.5, 6.5 Hz), 3.57 (ddd, 1H, J = 9.0, 6.5, 2.0 Hz), 3.60 (d, 1H, J = 9.0 Hz), 3.68 (dd, 1H, J = 11.5, 2.0 Hz), 3.72 (d, 1H, J = 9.0 Hz), 4.04 (dd, 1H, J = 6.5, 7.0 Hz), 5.62 (d, 1H, J_{H-P} = 46.5 Hz), 5.78 (d, 1H, J_{H-P} = 22.0 Hz); ^{13}C-NMR (50 MHz, D_2O) δ 40.20 (d, J_{C-P} = 12.0 Hz), 66.75, 71.98 (d, J_{C-P} = 3.7 Hz), 71.96, 72.75, 74.24, 74.35, 131.80 (d, J_{C-P} = 8.6 Hz), 141.00 (d, J_{C-P} = 156.5 Hz); ^{31}P-NMR (109 MHz, D_2O) δ 16.40; MS (FAB) m/z 303 (M + 1); HRMS (FAB) calcd for $C_9H_{20}O_9P$ (M + 1) 303.084 50, found 303.084 43.

2-Deoxy-D-*glycero*-D-*galacto*-β-octopyranosono-1-phosphonic Acid (Phosphono-KDN, 7). To a solution of **8** (50 mg, 0.17 mmol) in methanol−water (8:1, 10 mL) was bubbled ozone for 20 min at −78 °C. TLC indicated no presence of starting material at this time. Dimethyl sulfite (0.5 mL) was added, and the reaction mixture was stirred for another 10 h at room temperature. After concentration *in vacuo*, compound **7** (45 mg, 90%) was precipitated from acetone as a white powder: $[\alpha]_D = -25$ (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.78 (ddd, 1H, J = 12.6, 12.6, 5.0 Hz, H-2_{ax}), 2.43 (dd, 1H, J = 13.0, 5.0 Hz, H-2_{eq}), 3.53 (dd, 1H, J = 9.3, 9.3 Hz), 3.63 (dd, 1H, J = 11.0, 5.4 Hz), 3.74−3.88 (m, 3H), 3.94−4.02 (m, 2H); ^{13}C-NMR (50 MHz, D_2O) δ 36.99 (d, J_{C-P} = 8.8 Hz, C-2), 63.27, 68.01, 68.52 (d, J_{C-P} = 11.8 Hz), 70.01, 70.36, 70.79 (d, J_{C-P} = 11.4 Hz), 96.36 (d, J_{C-P} = 200.6 Hz, C-1); ^{31}P-NMR (109 MHz, D_2O) δ 15.10; MS (FAB) m/z 305 (M + 1); HRMS (FAB) calcd for $C_8H_{18}O_{10}P$ (M + 1) 305.063 76, found 305.063 83.

Monomethyl (5-Acetamido-4,6,7,8,9-D-*glycero*-D-*galacto*-pentahydroxynon-1-en-2-yl)phosphonate (12). Compound **10a** (100 mg, 0.27 mmol) was hydrolyzed in methanol (15 mL) as described for compound **4a**. After concentration *in vacuo*, compound **12** (96 mg, 100%) was obtained as a white wax without further purification: $[\alpha]_D = -19$ (c = 1, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.91 (s, 3H), 2.22 (m, 2H), 3.30 (d, 1H, J = 9.0 Hz), 3.37 (d, 3H, J_{H-P} = 11.0 Hz), 3.46 (dd, 1H, J = 12.0, 6.0 Hz), 3.59 (ddd, 1H), 3.68 (dd, 1H, J = 12.0, 2.5 Hz), 3.76 (d, 1H, J = 10.0 Hz), 3.81 (d, 1H, J = 10.0 Hz), 4.23 (t, 1H, J = 6.5 Hz), 5.60 (d, J_{H-P} = 44.5 Hz), 5.72 (d, J_{H-P} = 21.5 Hz); ^{13}C-NMR (50 MHz, D_2O) δ 21.91, 37.51 (d, J_{C-P} = 11.7 Hz), 51.73 (d, J_{C-P} = 5.1 Hz), 53.29, 63.25, 67.29 (d, J_{C-P} = 3.5 Hz), 67.58, 69.41, 70.67, 129.78 (d, J_{C-P} = 8.6 Hz), 137.71 (d, J_{C-P} = 165.7 Hz), 174.42; ^{31}P (109 MHz, D_2O) δ 20.28; MS (FAB) m/z 358 (M + 1); HRMS (FAB) calcd for $C_{12}H_{25}NO_9P$ (M + 1) 358.126 70, found 358.126 78.

Dimethyl 2-Deoxy-D-*glycero*-D-*galacto*-β-octopyranosono-1-phosphonate (5). A solution of **4a** (56 mg, 0.17 mmol) in methanol−dichloromethane (2:1, 12 mL) was used for the ozonolysis as described for compound **8**. Compound **5** (52 mg, 92%) was obtained as a wax: $[\alpha]_D = -36.5$ (c = 1, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.68 (ddd, 1H, J = 11.5, 11.5, 5.5 Hz), 2.12 (dd, 1H, J = 11.5, 5.2 Hz), 3.43 (ddd, 1H, J = 9.5, 4.5, 4.5 Hz), 3.51 (dd, 1H, J = 11.5, 6.5 Hz), 3.57 (ddd, 1H, J = 9.0, 9.0, 2.0 Hz), 3.63−3.73 (m, 3H), 3.72 (d, 3H, J_{H-P} = 12.5 Hz), 3.74 (d, 3H, J_{H-P} = 12.5 Hz), 3.86 (m, 1H); ^{13}C-NMR (50 MHz, D_2O) δ 36.78 (d, J_{C-P} = 9.6 Hz), 54.69 (d, J_{C-P} = 7.4 Hz), 55.19 (d, J_{C-P} = 6.6 Hz), 63.15, 67.87, 67.94, 69.88, 70.34, 71.44 (d, J_{C-P} = 13.2 Hz), 96.33 (d, J_{C-P} = 213.55 Hz); ^{31}P-NMR (109 MHz, D_2O) δ 21.57; MS (FAB) m/z 333 (M + 1); HRMS (FAB) calcd for $C_{10}H_{22}O_{10}P$ (M + 1) 333.095 06, found 333.095 02.

Dimethyl 4-Acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-β-octopyranosono-1-phosphonate (11). A solution of **10a** (42 mg, 0.11 mmol) in methanol−dichloromethane (2:1, 12 mL) was ozonized as described for compound **8**. Compound **11** (36 mg, 86%) was obtained as a wax: $[\alpha]_D = -25$ (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.71 (ddd, 1H, J = 12.0, 12.0, 4.5 Hz), 1.87 (s, 3H), 2.15 (dd, 1H, J = 12.0, 2.5 Hz), 3.40 (m, 2H), 3.55 (dd, 1H, J = 6.5, 6.5 Hz), 3.57 (m, 1H), 3.63−3.73 (m, 3H), 3.72 (d, 3H, J_{H-P} = 12.5Hz), 3.74 (d, 3H, J_{H-P} = 12.5 Hz), 3.91 (m, 1H); ^{13}C-NMR (50 MHz, D_2O) δ 26.03, 40.89 (d, J_{C-P} = 9.2 Hz), 55.65, 58.32 (d, J_{C-P} = 7.1 Hz), 58.74 (d, J_{C-P} = 7.0 Hz), 66.59, 69.31 (d, J_{C-P} = 7.0 Hz), 71.54, 73.56, 73.66 (d, J_{C-P} = 11 Hz), 99.45 (d, J_{C-P} = 211.25 Hz), 176.95; ^{31}P-NMR (109 MHz, D_2O) δ 21.33; MS (FAB) m/z 374 (M + 1); HRMS (FAB) calcd for $C_{12}H_{25}NO_{10}P$ (M + 1) 374.121 61, found 374.121 55.

Monomethyl 4-Acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-β-octopyrano-sono-1-phosphonate (13). A solution of **12** (30 mg, 0.084 mmol) in methanol−dichloromethane (2:1, 8 mL) was ozonized as described for compound **8**. Compound **13** was obtained in 87% (26 mg) yield: $[\alpha]_D = -27$ (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.67 (ddd, 1H, J = 12.5, 12.5 Hz, J_{H-P} = 5.5 Hz), 1.88 (s, 3H), 2.11 (dd, 1H, J = 4.5, 12.5 Hz), 3.34 (d, 1H, J = 9.5 Hz), 3.44 (dd, 1H, J =12.0, 6.0 Hz), 3.51 (d, 3H, J_{H-P} = 9.5 Hz), 3.60 (ddd, 1H, J = 9.5, 3.0, 6.0 Hz), 3.68 (dd, 1H, J = 12.0, 3.0 Hz), 3.73 (d, 1H, J = 10.0 Hz), 3.87 (m, 2H); ^{13}C-NMR (50 MHz, D_2O) δ 22.07, 37.50 (d, J_{C-P} = 8.8 Hz), 52.24, 53.40 (d, J_{C-P} = 5.9 Hz), 63.26, 66.63 (d, J_{C-P} = 11.4 Hz), 68.42, 69.54 (d, J_{C-P} = 11.1 Hz), 96.92 (d, J_{C-P} = 199.8 Hz), 174.74; ^{31}P-NMR (109 MHz, D_2O) δ 15.21; MS (FAB) m/z 360 (M + 1); HRMS (FAB) calcd for $C_{11}H_{23}NO_{10}P$ (M + 1) 360.105 96, found 360.105 96.

Dimethyl (5-Acetamido-4,6,7,8,9-D-*glycero*-D-*galacto*-pentaacetoxynon-1-en-2-yl)phosphonate (14a). To a solution of mixture of **10a,b** (150 mg, 0.4 mmol) in pyridine (10 mL) and acetic anhydride (5 mL) was added *p*-(dimethylamino)pyridine (10 mg). The reaction mixture was stirred for 15 h at room temperature. After concentration *in vacuo*, a syrup was obtained which was purified by flash chromatography (dichloromethane−acetone = 1:1) to give a mixture of compounds **14a** and **14b** (202 mg, 86%). The major isomer **14a** (119 mg, 51%) was obtained by recrystallization from cyclohexane−ethyl acetate (2:1): mp = 151 °C; $[\alpha]_D = -37$ (c = 0.5, methanol); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.64 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.41 (m, 2H), 3.74 (d, 3H, J_{H-P} = 11.5 Hz), 3.76 (d, J_{H-P} = 11.5Hz), 3.96 (dd, 1H, J = 12.5, 5.5 Hz), 4.25 (dd, 1H, J = 3, 12.5 Hz), 4.42 (ddd, 1H, J = 1.0, 10.0, 10.0 Hz), 5.03 (m, 1H), 5.10 (ddd, 1H, J = 7.0, 6.5, 1.0 Hz), 5.21 (dd, 1H, J = 2.5, 10.0 Hz), 5.32 (dd, 1H, J = 2.5, 8.0 Hz), 5.62 (d, 1H, J = 10.0 Hz), 5.92 (d, 1H, J_{H-P} = 47 Hz), 6.14 (d, 1H, J_{H-P} = 22.5 Hz); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 22.15, 22.27, 22.40, 22.47, 22.52, 24.78, 36.10 (d, J_{C-P} = 11.3 Hz), 49.78, 53.72 (d, J_{C-P} = 5.9 Hz), 53.93 (d, J_{C-P} = 5.6 Hz), 63.23, 68.98, 69.25, 70.53, 133.06 (d, J_{C-P} = 176.1 Hz), 134.91 (d, J_{C-P} = 8.7 Hz), 169.30, 169.72, 169.78, 169.83, 170.38; ^{31}P-NMR (109 HMz, $CDCl_3$) δ 21.46; MS (FAB) m/z 582 (M + 1); HRMS (FAB) calcd for $C_{23}H_{37}NO_{14}P$ (M + 1) 582.195 17, found 582.195 30.

(5-Acetamido-4,6,7,8,9-D-*glycero*-D-*galacto*-pentahydroxynon-1-en-2-yl)phosphonic Acid (15). To a solution of **14a** (97 mg, 0.17 mmol) in dry dichloromethane (20 mL) (88 μL, 4 equiv) was added bromotrimethylsilane at 0 °C. The reaction mixture was stirred for 10 h at room temperature. After concentration *in vacuo*, the residue was hydrolyzed in methanol (10 mL) with sodium hydroxide (1 N in water, 2 mL) to give compound **15** as a white powder (42 mg, 74%): mp = 172 °C dec; $[\alpha]_D = -45$ (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.92 (s, 3H), 2.76 (m, 2H), 3.29 (d, 1H, J = 9.0 Hz), 3.46 (dd, 1H, J = 11.5, 6.5 Hz), 3.58 (ddd, 1H, J = 2.5, 6.5, 9.0 Hz), 3.65 (dd, 1H, J = 2.5, 11.5 Hz), 3.76 (d, J = 10.0 Hz), 3.82 (dd, 1H, J = 10.0, 1.0 Hz), 4.20 (ddd, 1H, J = 4.0, 9.0, 1.0 Hz), 5.35 (d, 1H, J_{H-P} = 41.5 Hz), 5.59 (dd, 1H, J_{H-P} =19.5, 1.0 Hz); ^{13}C-NMR (50 MHz, D_2O) δ 25.92, 40.83 (d, J_{C-P} = 12.4 Hz), 56.74, 70.65 (d, J_{C-P} = 3.5 Hz), 66.72, 71.26, 72.08, 74.11, 132.61 (d, J_{C-P} = 8.8 Hz), 140.14 (d, J_{C-P} = 166.3 Hz), 176.58; ^{31}P-NMR (109 MHz, D_2O) δ 13.56; MS (FAB) m/z 344 (M + 1); HRMS (FAB) calcd for $C_{11}H_{23}NO_9P$ (M + 1) 344.111 05, found 344.111 15.

4-Acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-β-octopyranosono-1-phosphonic Acid (16). A solution of **15** (27 mg, 0.079 mmol) in methanol−water (4:1) was ozonized as described for compound **8**. Compound **16** (22 mg) was obtained in 84% yield: $[\alpha]_D = -23$ (c = 0.5, methanol); ^{1}H-NMR (500 MHz, D_2O) δ 1.69 (ddd, 1H, J = 13.0, 11.0 Hz, J_{H-P} = 4.5 Hz), 1.88 (s, 3H), 2.14 (dd, 1H, J = 13.0, 5.0 Hz), 3.35 (d, 1H, J = 9.0 Hz), 3.45 (dd, 1H, J = 11.5, 6.5 Hz), 3.65 (m, 1H), 3.69

(dd, 1H, J = 11.5, 2.5 Hz), 3.73 (d, 1H, J = 10.0 Hz), 3.89 (m, 2H); ^{13}C-NMR (50 MHz, D_2O) δ 22.11, 37.40 (d, J_{C-P} = 8.4 Hz), 52.27, 66.78 (d, J_{C-P} = 11.1 Hz), 68.48, 69.47 (d, J_{C-P} = 10.9 Hz), 70.14, 96.33 (d, J_{C-P} = 198.6 Hz), 174.77; ^{31}P-NMR (109 MHz, D_2O) δ 13.52; MS (FAB) m/z 346 (M + 1); HRMS (FAB) calcd for $C_{10}H_{21}NO_{10}P$ (M + 1) 346.090 31, found 346.090 46.

Acknowledgment. This work was supported by NSERC of Canada and FCAR of Quebec. We thank Kaylene Quelch for her skilled technical assistance in the biological evaluation of the synthesized compounds and the Monash University Research Fund for its financial support.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **4a**, **5**, **7**, **8**, **10a**, **11**, **12**, **13**, **14a**, **15**, and **16** (24 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961891P