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Chemoenzymatic synthesis of neuraminic acid analogs structurally varied at C-5 and C-9 as potential inhibitors of the sialidase from influenza virus

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Abstract

The 9-amino or 9-*N*-acyl-5-trifluoroacetyl methyl α-ketosides (**1a–c**) and their 2,3-didehydro analogs (**2a–c**) have been synthesized through Neu5Ac aldolase-catalyzed aldol reaction of 6-azido-2-benzyloxycarbonylamino-2-deoxy-D-mannose with sodium pyruvate. The six compounds were investigated as inhibitors of sialidase from influenza virus. Compound **2b**, a 2,3-didehydro type, showed the most potent inhibitory activity ($IC_{50} > 7.8$ μM) against the enzyme, whereas, compounds **1a–c** as the methyl α-glycosides were found to be practically inactive ($IC_{50} > 100$ μM).

Keywords: Sialidases; Influenza virus; Inhibitors; Neuraminic acid analogs; Neu5Ac aldolase

1. Introduction

Sialidase cleaves terminal α-ketosidically linked sialic acid from influenza virus receptors. Because of this enzyme, influenza virus is released from infected cells [1]. Inhibition of the enzyme might therefore restrict the establishment and progression of infection by influenza virus. A variety of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enonic acid (Neu5Ac2en) analogs has been synthesized as competitive sialidase inhibitors [2]. Among them, 4-guanidino-Neu5Ac2en analogs showed very potent inhibitory activity against sialidase [3]. The best substrate–enzyme

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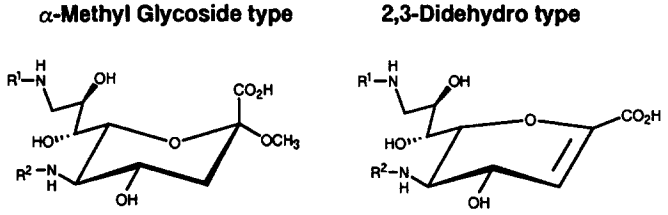


1a; R^1 = H, R^2 = CF_3CO
1b; R^1 = CH_3CO, R^2 = CF_3CO
1c; R^1 = CF_3CO, R^2 = CF_3CO

2a; R^1 = H, R^2 = CF_3CO
2b; R^1 = CH_3CO, R^2 = CF_3CO
2c; R^1 = CF_3CO, R^2 = CF_3CO

Scheme 1.

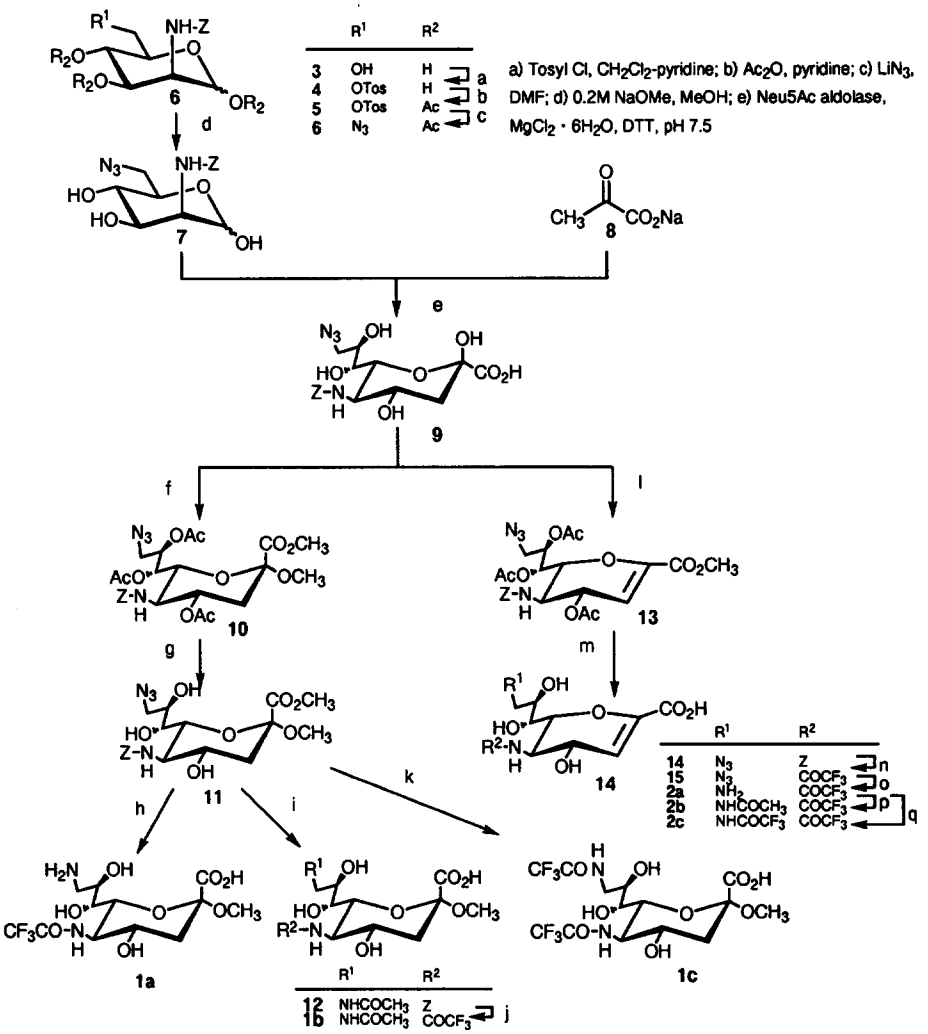
interaction was achieved by replacing the *N*-acetyl group at C-5 in Neu5Ac2en by an *N*-trifluoroacetyl group [2a]. In addition, the 9-hydroxyl group is important for binding to sialidase [1b,c,4].

To explore the influence on sialidase binding of replacing the *N*-acetyl group at C-5 and the hydroxyl group at C-9 in *N*-acetylneuraminic acid derivatives by the *N*-acetyl or *N*-trifluoroacetyl group, we now report the chemoenzymatic synthesis of the the 9-amino or 9-*N*-acyl-5-*N*-trifluoroacetyl methyl α-ketosides (**1a–c**) and their 2,3-didehydro-2,3-dideoxy analogs (**2a–c**) (Scheme 1) and their behavior towards sialidase from the influenza virus.

2. Results and discussion

The synthetic strategy adopted for the preparation of **1** and **2** required a key intermediate **9** bearing chemoselective functional groups on C-5 and C-9, namely benzyloxycarbonyl and azido groups, synthesized from 6-azido-2-benzyloxycarbonylamino-2-deoxy-D-mannose (**7**) with sodium pyruvate under catalysis by Neu5Ac aldolase [5] (Scheme 2).

Compound **7** was obtained in four steps from 2-benzyloxycarbonylamino-2-deoxy-D-mannoses (**3**). Compound **3**, prepared from D-mannosamine hydrochloride, benzyl chloroformate, and saturated aqueous $NaHCO_3$, was treated with *p*-toluenesulfonyl chloride in pyridine–CH_2Cl_2, and then acetylated with Ac_2O and pyridine to give **5** in 43% yield. Treatment of **5** with LiN_3 in DMF afforded **6** in 54% yield. Deacetylation with 0.2 M NaOMe in MeOH gave **7** in 70% yield. Neu5Ac aldolase-catalyzed condensation of **7** with sodium pyruvate and dithiothreitol (DTT) and $MgCl_2 \cdot 6H_2O$ in potassium phosphate buffer (pH 7.5) provided, after ion-exchange chromatography, the key intermediate **9** in moderate yield (45%). Methyl esterification of **9** with Amberlite IR-120 (H^+) resin in MeOH and peracetylation and treatment with HCl in AcCl, followed by conversion into the methyl α-glycoside **10** by silver salicylate [6] afforded the methyl α-ketoside **10** in 59% yield in four steps. The ^1H NMR spectrum of **10** showed a one-proton doublet of doublets at δ 2.59 ($J_{3ax,3eq}$ 12.6, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), characteristic of the α-sialyl linkage. The IR spectrum of **10** showed absorption bands at 2102 and 1744 cm^{-1}, characteristic of the azido and ester groups, respectively.



Scheme 2.

Deacetylation of **10** with 0.1 M NaOMe in MeOH afforded the diol **11** in 80% yield. For the synthesis of **1a**, selective removal of the benzyloxycarbonyl group of **11** with trifluoroacetic acid and thioanisole [7], subsequent trifluoroacetylation with tifluoroacetic

Table 1
Inhibition of sialidase from influenza virus A/PR/8/34 (H1N1)

Compound	IC_{50} (M)
2a	5.25×10^{-4}
2b	7.77×10^{-6}
2c	1.21×10^{-5}

acid methyl ester and triethylamine [8], and then hydrogenation with Pd-black in MeOH gave **1a**, {FABMS: m/z 377 $(M+H)^+$, 399 $(M+Na)^+$} in 11% yield in four steps. Selective reduction of the azido group in **11** by 1,3-propanedithiol in MeOH [9] and subsequent *N*-acetylation with Ac_2O in MeOH gave **12** in 19% yield in two steps. Compound **12** was hydrogenated with Pd-black in MeOH and trifluoroacetylated to give **1b** {FABMS: m/z 419 $(M+H)^+$, 441 $(M+Na)^+$} in 47% yield. Saponification of the methyl ester of **11** with 0.1 M aqueous KOH in MeOH and hydrogenolysis and subsequent trifluoroacetylation gave **1c** {FABMS: m/z 495 $(M+Na)^+$} in 19% yield in three steps.

The synthesis of the target compounds **2a–c** was achieved as follows. Compound **9** was transformed into **13** by elimination of HCl of the glycosyl chloride with 1,8-diazabicyclo[5.4.0]undecen-7-ene (DBU) [10] in 51% yield in four steps, showing in its ^1H NMR spectrum a one-proton doublet at δ 5.97 ($J_{3,4}$ 3.0 Hz, H-3), characteristic of the 2,3-double bond. *O*-Deacetylation and subsequent hydrolysis of the methyl ester group of **13** gave **14** in almost quantitative yield. Removal of the benzyloxycarbonyl group from compound **14** followed by *N*-trifluoroacetylation to give **15** in 62% yield in two steps. Reduction of **15** with by Pd-black, H_2 in MeOH afforded **2a** {FABMS: m/z 345 $(M+H)^+$, 367 $(M+Na)^+$} in 68% yield. Acylation of **2a** with Ac_2O in MeOH furnished **2b** {FABMS: m/z 409 $(M+Na)^+$} in 90% yield. Trifluoroacetylation of **2a** with trifluoroacetic acid methyl ester and triethylamine gave **2c** {FABMS m/z 441 $(M+H)^+$, 463 $(M+Na)^+$} in 78% yield.

Compounds **1a–c** and **2a–c** were evaluated as inhibitors of influenza A sialidase. As shown in Table 1, among **2a–c** as 2,3-didehydro types, compound **2b** showed most potent inhibitory activity against the enzyme. In addition, the 9-amido analogs **2b** and **c** inhibited more than did the 9-amino analog **2a**. However, **1a–c**, methyl α-glycoside types, exhibited poor inhibitory activity up to 100 μM concentration. Detailed results of the biological investigation will be reported elsewhere.

In conclusion, the chemoenzymatic synthesis of 9-amino or 9-*N*-acyl-5-*N*-trifluoroacetyl methyl α-ketosides (**1a–c**) and their 2,3-didehydro analogs (**2a–c**) was accomplished via key intermediate **9**. In addition, **2b,c** are potential sialidase inhibitors.

3. Experimental

General methods.—Melting points are uncorrected. Optical rotations were measured with a Jasco DIP-140 digital polarimeter. IR spectra were recorded on a Jasco IR-810 spectrometer. ^1H NMR spectra were recorded with a Jeol JNM-EX 270 [^1H (270 MHz)]

spectrometer. Chemical shifts are given in ppm relative to internal Me_4Si ($\delta = 0$) in $CDCl_3$ or CD_3OD, or sodium 4,4-dimethyl-4-silapentane-1-sulfonate hydrate (DSS, $\delta = 0$ in D_2O) as internal standards at ambient temperature. Fast atom bombardment (FAB) mass spectra were obtained with a Jeol JNM SX-102 mass spectrometer in the positive ion mode using an NBA matrix. Column chromatography was performed on Silica Gel Merck 60 (70–230 mesh) and Bio-Gel P-2 (200–400 mesh Bio-Rad). Ion-exchange resins Amberlite CG-400 (formate, 100–200 mesh) was purchased from Organo. TLC was performed on aluminium sheets coated with Silica Gel $60F_{254}$ (Merck). Glycolipids containing sialic acid were visualized with resorcinol reagent. The bands of lipids containing sialic acid were stained blue.

1,3,4-Tri-O-*acetyl-2-benzyloxycarbonylamine-2-deoxy-6*-O-p-*tolylsulfonyl*-D-*mannose* (**5**).—A solution of *p*-toluenesulfonyl chloride (3.85 g, 20.2 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a cooled solution of **3** (5.76 g, 18.4 mmol) in pyridine (30 mL) and CH_2Cl_2 (50 mL) at 0–5°C over 30 min, and the mixture was stirred for 7 h at room temperature. Acetic anhydride (20 mL) was then added to the mixture at 0°C which was stirred for 10 h at room temperature and then poured into CH_2Cl_2 (200 mL). The organic layer was successively washed with water, N HCl, aqueous $NaHCO_3$, and aqueous NaCl, and dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel using 2:1 hexane EtOAc to give **5** (4.76 g, 43%); $[\alpha]_D$ + 43.7° (*c* 1.0, $CHCl_3$); ν_{max} 1756 (OAc) and 1218 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$): δ 1.92, 2.02, 2.15 (s, each 3 H, AcO), 2.43 (s, 3 H, SO_3PhCH_3), 4.00–4.14 (m, 3 H, H-5, H-6a, and H-6b), 4.32 (ddd, 1 H H, $J_{1,2}$ 2.0, $J_{2,3}$ 4.3, $J_{2,NH}$ 9.6 Hz, H-2), 5.07–5.18 (m, 3 H, H-4 and CH_2Ph), 5.26 (d, 1 H, H-1), 7.29–7.38 (m, 7 H, CH_2Ph and SO_3PhCH_3), and 7.76 (d, 2 H, *J* 8.3 Hz, SO_3PhCH_3). Positive FABMS (NBA): $(M + H)^+$ *m/z* 594.

1,3,4-Tri-O-*acetyl-6-azido-2-benzyloxycarbonylamino-2,6-dideoxy*-D-*mannose* (**6**).—A mixture of **5** (4.76 g, 8.0 mmol) and LiN_3 (0.59 g, 12 mmol) in DMF (20 mL) was heated with stirring for 3 h at 70–80°C. The mixture was cooled to room temperature and diluted with EtOAc (200 mL). The mixture was washed with water and the aqueous layer extracted with EtOAc. The combined extracts were dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel using 3:1 hexane–EtOAc to give **6** (2.00 g, 54%), $[\alpha]_D$ + 57.1° (*c* 1.0, $CHCl_3$); ν_{max} 2100 (N_3), 1713 (OAc), and 1601 cm^{-1} (carbamate); ^1H NMR ($CDCl_3$): δ 1.96, 2.05, 2.18 (s, each 3 H, AcO), 3.31 (dd, 1 H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 13.5 Hz, H-6a), 3.40 (dd, 1 H, $J_{5,6b}$ 3.0 Hz, H-6b), 3.96 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 4.37 (ddd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 4.0, $J_{2,NH}$ 9.2 Hz, H-2), 5.10–5.13 (m, 2 H, CH_2Ph), 5.19 (t, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.30 (dd, 1 H, H-3), 6.12 (d, 1 H, H-1), and 7.20–7.41 (m, 5 H, CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ *m/z* 465.

6-Azido-2-benzyloxycarbonylamino-2,6-dideoxy-D-*mannose* (**7**).—To a solution of compound **7** (1.86 g, 4.00 mmol) in dry MeOH (80 mL) was added methanolic 0.1 M NaOMe (20 mL), and the resulting solution was stirred for 3 h at 0°C, and then treated with Amberlite IRC-50 (1.0 g) resin to remove sodium ions, filtered, and concentrated to dryness. The residue was chromatographed on silica gel using 10:1 CH_2Cl_2–MeOH to give **7** (950 mg, 70%), $[\alpha]_D$ + 31.3° (*c* 0.6, CH_3OH); ν_{max} 3338 (OH), 2098 (N_3), and 1700 cm^{-1} (carbamate); ^1H NMR (CD_3OD): δ 3.32–3.38 (m, 1 H, H-4), 3.44 (dd, 1 H, $J_{5,6a}$ 5.6, $J_{6a,6b}$ 13.2 Hz, H-6a), 3.48–3.54 (m, 1 H, H-6b), 3.58–3.68 (m, 1 H, H-3), 3.90 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6a}$ 5.6, $J_{5,6b}$ 3.0 Hz, H-5), 3.95–4.00 (m, 1 H, H-2),

5.05–5.12 (m, 2 H, CH_2Ph), 5.13 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), and 7.25–7.32 (m, 5 H, CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ m/z 339.

9-Azido-5-benzyloxycarbonylamino-3,5,9-trideoxy-D-glycero-*β*-D-galacto-*2-nonulopyranosonic acid* (**9**).—A solution of Neu5Ac aldolase (E.C.4.1.3.3, 20 u), $MgCl_2 \cdot 6H_2O$ (2 mg) and dithiothreitol (DTT) (7 mg) in distilled water (1 mL) was added to a solution of **7** (950 mg, 2.81 mmol) and sodium pyruvate (3.09 g, 28.1 mmol) in 0.1 M potassium phosphate buffer (pH 7.5) (200 mL), and the mixture was stirred for 5 days at room temperature. For purification, the whole solution was loaded onto an ion-exchange column containing Amberlite CG-400 (200–400 mesh, formate form) resin, washed with water and then eluted with a gradient of 0–1 M aqueous NH_4HCO_3. Fractions containing the product, detected by TLC, were collected and purified on a column of Bio-Gel P-2 gel using water as eluant. After freeze-drying, **9** (564 mg, 45%) was obtained, $[\alpha]_D$ − 1.7° (*c* 1, H_2O); ν_{max} 3350 (OH), 2102 (N_3), 1700 (CO), and 1685 cm^{-1} (carbamate); ^1H NMR (D_2O): δ 1.68 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.2 Hz, H-3*ax*), 2.07 (dd, 1 H, $J_{3ax,3eq}$ 12.2, $J_{3eq,4}$ 5.0 Hz, H-3*eq*), 3.29 (dd, 1 H, $J_{8,9a}$ 5.3, $J_{9a,9b}$ 13.2 Hz, H-9a), 3.39–3.55 (m, 3 H, H-5, H-7, and H-9b), 3.72–3.80 (m, 1 H, H-4), 3.88–3.93 (m, 1 H, H-8), 4.98, 5.06 (d, each 1 H, J_{gem} 12.5 Hz, CH_2Ph), and 7.20–7.41 (m, 5 H, CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ m/z 427.

*Methyl [methyl 4,7,8-tri-*O*-acetyl-9-azido-5-benzyloxycarbonylamino-3,5,9-trideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosid]onate* (**10**).—A solution of compound **9** (112 mg, 0.25 mmol) in anhydrous MeOH (10 mL) was treated with Amberlite IR-120B (H^+ form) (100 mg) resin and stirred for 5 h at room temperature, filtered and concentrated. The residue was redissolved in pyridine (10 mL) and treated with Ac_2O (10 mL) and stirred for 30 min at 0°C, and then for 10 h at room temperature. The mixture was dissolved in CH_2Cl_2 (100 mL) and successively washed with water, N HCl, aqueous $NaHCO_3$, aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was dissolved in AcCl (20 mL) and treated with a stream of anhydrous HCl gas for 20 min. The mixture was kept for 12 h at 4°C, concentrated to dryness, and the residue was dissolved in anhydrous MeOH (10 mL). To the mixture was added silver salicylate (149 mg, 0.38 mmol) and it was stirred in the dark for 5 h at room temperature. The solids were filtered off and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 and successively washed with aqueous $NaHCO_3$, aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was purified by silica-gel chromatography using 5 : 2 hexane EtOAc to give **10** (86 mg, 59%), $[\alpha]_D$ + 3.3° (*c* 1, $CHCl_3$); ν_{max} 2102 (N_3) and 1744 cm^{-1} (CO); ^1H NMR data ($CDCl_3$): δ 1.83 (3 H, s, AcO), 1.88 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.6 Hz, H-3*ax*), 2.17 (s, 3 H, AcO), 2.19 (s, 3 H, AcO), 2.59 (dd, 1 H, $J_{3ax,3eq}$ 12.6, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.27–3.35 (m, 4 H, OCH_3 and H-9a), 3.59 (dd, 1 H, $J_{8,9b}$ 3.1, $J_{9a,9b}$ 13.4 Hz, H-9b), 3.72–3.88 (m, 4 H, CO_2CH_3 and H-5), 4.10 (dd, 1 H, $J_{5,6}$ 10.7, $J_{6,7}$ 2.0 Hz, H-6), 4.55 (br d, 1 H, NH), 4.82 (ddd, 1 H, $J_{3ax,4}$ 12.6, $J_{3eq,4}$ 4.6, $J_{4,5}$ 10.4 Hz, H-4), 4.93, 5.15 (d, each 1 H, J_{gem} 12.2 Hz, CH_2Ph), 5.34–5.39 (m, 1 H, H-8), 5.42 (dd, 1 H, $J_{6,7}$ 2.0, $J_{7,8}$ 7.9 Hz, H-7), and 7.27–7.36 (m, 5 H, CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ m/z 581, $(M + Na)^+$ 603.

Methyl (methyl 9-azido-5-benzyloxycarbonylamino-3,5,9-trideoxy-D-glycero-*α*-D-galacto-*2-nonulopyranosid)onate* (**11**).—To a solution of compound **10** (387 mg, 0.67 mmol) in dry MeOH (5 mL) was added methanolic 0.1 M NaOMe (10 mL). The

mixture was stirred for 5 h at 0°C, made neutral with Amberlite IRC-50 (0.5 g) resin, filtered, and concentrated. The residue was purified by silica-gel chromatography using 30:1 CH_2Cl_2–MeOH to give **11** (241 mg, 80%), $[\alpha]_D$ + 7.6° (*c* 1.0, CH_3OH); ν_{max} 3388 (OH), 2100 (N_3), 1726 (ester), and 1693 cm^{-1} (carbamate); ^1H NMR (CD_3OD): δ 1.88 (dd, 1 H, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 11.6 Hz, H-3*ax*), 2.78 (dd, 1 H, $J_{3ax,3eq}$ 12.9, $J_{3eq,4}$ 4.3 Hz, H-3*eq*), 3.33 (s, 3 H, OCH_3), 3.44 (dd, 1 H, $J_{8,9a}$ 13.2, $J_{9a,9b}$ 5.6 Hz, H-9a), 3.55–3.65 (m, 3 H, H-4, H-5, and H-6), 3.78 (d, 1 H, $J_{8,9b}$ 3.0 Hz, H-9b), 3.88 (s, 3 H, CO_2CH_3), 4.00–4.12 (m, 1 H, H-8), 4.18 (d, 1 H, $J_{6,7}$ 5.0 Hz, H-7), 4.90 (d, 1 H, J 7.6 Hz, NH), 5.08, 5.18 (d, each 1 H, J_{gem} 12.2 Hz, CH_2Ph), and 7.27–7.41 (m, 5 H, CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ *m/z* 455, (M + Na) + 477.

Methyl (methyl 9-amino-3,5,9-trideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-*2-nonulopyranosid)onate* (**1a**).—Compound **11** (44 mg, 0.10 mmol) was dissolved in a solution of 0.1 M NaOH (5 mL) and MeOH (5 mL). The mixture was stirred for 5 h, made neutral with Amberlite IRC-50 resin, and concentrated. The residue was dissolved in trifluoroacetic acid (10 mL), thioanisole (458 μL, 3.9 mmol) was added, and the mixture was stirred for 15 h at room temperature. Ether was added to the mixture, the ether-insoluble materials were washed with MeOH and the filtrate and washings were concentrated. The residue was dissolved in anhydrous MeOH (2 mL), and to the mixture were added Et_3N (0.3 mL) and methyl trifluoroacetate (1 mL). After stirring for 2 h at room temperature, the mixture was concentrated, and the residue was dissolved in MeOH (3 mL) containing Pd-black (20 mg), and the flask was fitted with a balloon of hydrogen. After stirring for 12 h, the mixture was filtered, and the filtrate concentrated and purified by silica-gel chromatography using 3:1 CH_2Cl_2–MeOH to give **1a** (4 mg, 11%), ν_{max} 1774 and 1685 cm^{-1} (CO); ^1H NMR (D_2O): δ 1.61 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.2 Hz, H-3*ax*), 2.67 (dd, 1 H, $J_{3ax,3eq}$ 12.2, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 2.91 (dd, 1 H, $J_{8,9a}$ 10.2, $J_{9a,9b}$ 13.2 Hz, H-9a), 3.26–3.36 (m, 4 H, H-9b and OCH_3), 3.46 (d, 1 H, $J_{7,8}$ 8.6 Hz, H-7), 3.63–3.77 (m, 1 H, H-4), 3.87–3.93 (m, 2 H, H-5 and H-6), and 3.94–4.08 (m, 1 H, H-8). Positive FABMS (NBA): $(M + H)^+$ *m/z* 377, $(M + Na)^+$ 399.

Methyl 9-acetamido-5-benzyloxycarbonylamino-3,5,9-trideoxy-D-glycero-α-D-galacto-*2-nonulopyranosidonic* (**12**).—To a solution of **11** (161 mg, 0.36 mmol) in anhydrous MeOH (4 mL) was added Et_3N (196 μL, 1.42 mmol) and 1,3-propanedithiol (143 μL, 1.42 mmol) and the mixture was stirred for 48 h at room temperature. The product was eluted from Amberlite 120B (H^+ form) with a gradient of 0–10% NH_4OH–MeOH, and purified with Sephadex LH-20 gel-filtration chromatography. Fractions containing the 9-amino compound were collected and evaporated to dryness. The residue was dissolved in anhydrous MeOH (3 mL) and a solution of in 200 μL of 1:9 Ac_2O–MeOH was added. The mixture was stirred for 90 min at room temperature, and concentrated, and purified by silica gel chromatography using 3:1 CH_2Cl_2–MeOH to give **12** (30 mg, 19%), $[\alpha]_D$ − 1.9° (*c* 0.6, CH_3OH); ν_{max} 1632 cm^{-1} (CO); ^1H NMR data (CD_3OD): δ 1.47 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ = 12.2 Hz, H-3*ax*), 1.85 (s, 3 H, AcO), 2.67 (dd, 1 H, $J_{3ax,3eq}$ 12.2, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.08 (dd, 1 H, $J_{8,9a}$ 8.6, $J_{9a,9b}$, 13.5 Hz, H-9a), 3.21 (s, 3 H, OCH_3), 3.29 (d, 1 H, $J_{7,8}$ 8.6 Hz, H-7), 3.38 (t, 1 H, $J_{4,5} = J_{5,6}$ = 9.6 Hz, H-5), 3.45–3.58 (m, 3 H, H-4, H-6, and H-9b), 3.79 (ddd, 1 H, $J_{7,8} = J_{8,9a}$ = 8.6, $J_{8,9b}$ 2.6 Hz, H-8), 4.98, 5.04 (d, each 1 H, J_{gem} 12.2 Hz, CH_2Ph), and 7.15–7.35 (m, 5 H,

CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ m/z 457, $(M + Na)^+$ 479, $(M + K)^+$ 495.

Methyl 9-acetamido-3,5,9-trideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**1b**).—To a solution of **12** (30 mg, 0.066 mmol) in MeOH (2 mL) was added Pd-black (15 mg) and the mixture was kept under an atmosphere of H_2. After stirring for 12 h, the mixture was filtered and the filtrate was concentrated. The residue was dissolved in MeOH (1 mL), and Et_3N (0.3 mL) and methyl trifluoroacetate (1 mL) were added. After stirring for 1 h at room temperature the mixture was concentrated. The residue was purified by silica-gel chromatography using 3:1 CH_2Cl_2–MeOH to give **1b** (13 mg, 47%), $[\alpha]_D$ − 1.7° (*c* 0.24, CH_3OH); ν_{max} 1709 and 1624 cm^{-1} (CO); ^1H NMR (D_2O): δ 1.60 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4} = 12.2$ Hz, H-3*ax*), 1.95 (s, 3 H, AcO), 2.67 (dd, 1 H, $J_{3ax,3eq}$ 12.2, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.22 (dd, 1 H, $J_{8,9a}$, 7.3, $J_{9a,9b}$ 14.2 Hz, H-9a), 3.29 (s, 3 H, OCH_3), 3.39 (d, 1 H, $J_{7,8}$ 8.9 Hz, H-7), 3.52 (dd, 1 H, $J_{9a,9b}$ 14.2, $J_{8,9b}$ 2.6 Hz, H-9b), 3.69 (ddd, 1 H, $J_{3ax,4}$ 12.2, $J_{3eq,4}$ 4.6, $J_{4,5}$ 7.6 Hz, H-4), and 3.82–3.95 (m, 3 H, H-5, H-6, and H-8). Positive FABMS (NBA): $(M + H)^+$ m/z 419, $(M + Na)^+$ 441, $(M + 2Na-H)^+$ 463.

Methyl 3,5,9-trideoxy-5,9-bis(trifluoroacetamido)-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**1c**).—To a solution of **11** (47 mg, 0.1 mmol) in MeOH (5 mL) was added 0.1 M KOH (1 mL) and the mixture was stirred for 12 h at room temperature, made neutral with Amberlite IR-120B (H^+ form) resin, filtered, and the filtrate concentrated. The residue was dissolved in MeOH (3 mL) containing Pd-black (25 mg) and kept under an atmosphere of H_2. After stirring for 12 h, the mixture was filtered and the filtrate was concentrated, and dissolved in anhydrous MeOH (1 mL), and to the mixture were added Et_3N (0.3 mL) and methyl trifluoroacetate (1 mL). The mixture was stirred for 1 h and concentrated. The residue was purified by silica-gel chromatography using 3:1 CH_2Cl_2–MeOH to give **1c** (9 mg, 19%), $[\alpha]_D$ + 4.6° (*c* 0.12, CH_3OH); ν_{max} 1641 cm^{-1} (CO); ^1H NMR (CD_3OD): δ 1.49 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4} = 12.2$ Hz, H-3*ax*), 2.69 (dd, 1 H, $J_{3ax,3eq}$ 12.2, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.06–3.25 (m, 4 H, H-9a, and OCH_3), 3.29 (d, 1 H, $J_{7,8}$ 7.9 Hz, H-7), 3.56 (dd, 1 H, $J_{8,9b}$ 2.6, $J_{9a,9b}$ 13.5 Hz, H-9b), 3.61–3.71 (m, 1 H, H-4), 3.73–3.80 (m, 2 H, H-5, and H-6), and 3.87 (ddd, 1 H, $J_{7,8} = J_{8,9a} = 7.9$, $J_{8,9b}$ 2.6 Hz, H-8). Positive FABMS (NBA): $(M + Na)^+$ m/z 495, $(M + 2Na - H)^+$ 517.

Methyl 4,7,8-tri-O-*acetyl-2,6-anhydro-9-azido-5-benzyloxycarbonylamino-3,5,9-trideoxy*-D-glycero-D-galacto-*non-2-enonate* (**13**).—Compound **9** (564 mg, 1.27 mmol) was dissolved in anhydrous MeOH (20 mL) and stirred for 5 h with Amberlite IR-120B (H^+ form) (1 g) resin. The resin was filtered off and the filtrate concentrated. The residue was dissolved in pyridine (15 mL) and treated with Ac_2O (15 mL). The mixture was stirred for 30 min at 0°C, and then overnight at room temperature. The solution was diluted with CH_2Cl_2 (50 mL) and successively washed with water, N HCl, aqueous saturated $NaHCO_3$, and aqueous NaCl, and dried ($MgSO_4$), and concentrated. The residue was dissolved in AcCl (30 mL) and treated with a stream of anhydrous HCl gas for 20 min. The flask was kept for 12 h at 4°C, the mixture was concentrated, and the residue dissolved in CH_2Cl_2 (15 mL). After addition of DBU (208 μL, 1.39 mmol), the mixture was stirred for 1 h at room temperature and concentrated. The residue was chromatographed on a column of silica gel using 3:1 hexane–EtOAc to give **13** (356

mg, 51%), $[\alpha]_D$ + 43.2° (*c* 0.58, $CHCl_3$); ν_{max} 2098 (N_3), 1738, and 1659 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 1.99, 2.05, 2.16 (s, each 3 H, AcO), 3.48 (dd, 1 H, $J_{8,9a}$ 7.6, $J_{9a,9b}$ 13.5 Hz, H-9a), 3.80 (s, 3 H, CO_2CH_3), 3.83 (dd, 1 H, $J_{9a,9b}$ 13.5, $J_{8,9b}$ 3.0 Hz, H-9b), 4.01–4.13 (m, 1 H, H-5), 4.35 (dd, 1 H, $J_{5,6}$ 9.6, $J_{6,7}$ 3.0 Hz, H-6), 4.84 (d, 1 H, $J_{5,NH}$ 9.6 Hz, NH), 4.99, 5.14 (d, each 1 H, J_{gem} 12.2 Hz, CH_2Ph), 5.23 (ddd, 1 H, $J_{7,8}$ 3.6, $J_{8,9a}$ 7.6, $J_{8,9b}$ 3.0 Hz, H-8), 5.51 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 7.9 Hz, H-4), 5.55 (dd, 1 H, H-7), 5.97 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-3), and 7.28–7.40 (m, 5 H, CH_2PH). Positive FABMS (NBA): $(M + H)^+$ *m/z* 549.

2,6-Anhydro-9-azido-5-benzyloxycarbonylamino-3,5,9-trideoxy-D-glycero-D-galacto-*non-2-enonic acid* (**14**).—Compound **13** (356 mg, 0.65 mmol) was dissolved in 0.1 M NaOH (5 mL) and MeOH (5 mL), and stirred for 3 h at 0°C, in the presence of Amberlite IRC-50 resin. The resin was filtered off and the filtrate concentrated. The residue was purified by silica-gel chromatography using 10:1 CH_2Cl_2–MeOH to give **14** (274 mg, quant), $[\alpha]_D$ + 18.8° (*c* 1.0, CH_3OH); ν_{max} 2090 (N_3), 1700, and 1653 cm^{-1} (CO); ^1H NMR (CD_3OD): δ 3.38 (dd, 1 H, $J_{8,9a}$ 5.9, $J_{9a,9b}$ 12.9 Hz, H-9a), 3.55 (dd, 1 H, $J_{9a,9b}$ 12.9, $J_{8,9b}$ 2.6 Hz, H-9b), 3.60 (d, 1 H, $J_{7,8}$ 8.9 Hz, H-7), 3.74–3.82 (m, 4 H, H-5 and CO_2CH_3), 4.03 (ddd, 1 H, $J_{7,8}$ 8.9, $J_{8,9a}$ 5.9, $J_{9a,9b}$ 12.9 Hz, H-8), 4.19 (d, 1 H, $J_{5,6}$ 10.9 Hz, H-6), 4.41 (dd, 1 H, $J_{3,4}$ 2.3, $J_{4,5}$ 8.9 Hz, H-4), 5.09, 5.17 (d, each 1 H, J_{gem} 12.2 Hz, CH_2Ph), 5.93 9d, 1 H, $J_{3,4}$ 2.3 Hz, H-3), and 7.26–7.42 (m, 5 H, CH_2Ph). Positive FABMS (NBA): $(M + H)^+$ *m/z* 423, $(M + Na)^+$ 445.

2,6-Anhydro-9-azido-3,5,9-trideoxy-5-trifluoroacetamido-D-glycero-D-galacto-*non-2-enonic acid* (**15**).—Compound **14** (70 mg, 0.17 mmol) was dissolved in a solution of trifluoroacetic acid (10 mL) and thioanisole (780 μL, 6.6 mmol), and stirred for 15 h at room temperature. Ether was added to the mixture, the suspension was filtered. The precipitate was washed with MeOH and the filtrate and the washings were concentrated. The residue was dissolved in anhydrous MeOH (2 mL), and to the mixture were added Et_3N (0.3 mL) and methyl trifluoroacetate (1 mL). After stirring for 1 h at room temperature, the mixture was concentrated. The residue was chromatographed on silica gel with 2:1 CH_2Cl_2–MeOH to give **15** (38 mg, 62%), $[\alpha]_D$ + 2.0° (*c* 0.54, CH_3OH); ν_{max} 2106 (N_3), 1707, and 1654 cm^{-1} (CO); ^1H NMR data (D_2O): δ 3.45 (dd, 1 H, $J_{8,9a}$ 5.9, $J_{9a,9b}$ 12.9 Hz, H-9a), 3.52 (d, 1 H, $J_{7,8}$ 9.9 Hz, H-7), 3.60 (br d, 1 H, $J_{9a,9b}$ 12.9 Hz, H-9b), 4.06 (m, 1 H, H-8), 4.16 (dd, 1 H, $J_{4,5}$ 9.2, $J_{5,6}$ 10.9 Hz, H-5), 4.30 (d, 1 H, $J_{5,6}$ 10.9 Hz, H-6), 4.49 (br d, 1 H, $J_{4,5}$ 9.2, H-4), and 5.65 (br d, 1 H, H-3). Positive FABMS (NBA): $(M + H)^+$ *m/z* 371, $(M + Na)^+$ 393.

9-Amino-2,6-anhydro-3,5,9-trideoxy-5-trifluoroacetamido-D-glycero-D-galacto-*non-2-enonic acid* (**2a**).—Compound **15** (38 mg, 0.10 mmol) and Pd-black (14 mg) were dissolved in MeOH (5 mL). This mixture was hydrogenated at atmospheric pressure for 3 h and then filtered. The filtrate was evaporated to dryness, and the residue was chromatographed on a silica-gel column using 6:6:1 CH_2Cl_2–MeOH–H_2O. Fractions containing the product were concentrated and the residue was purified by Bio-gel P-2 gel filtration chromatography, with elution by water to give **2a** (24 mg, 68%), $[\alpha]_D$ + 15.9° (*c* 0.26, H_2O); ν_{max} 1712 and 1589 cm^{-1} (CO); ^1H NMR (D_2O): δ 2.89 (dd, 1 H, $J_{8,9a}$ 10.9, $J_{9a,9b}$ 12.2 Hz, H-9a), 3.38 (br d, 1 H, $J_{9a,9b}$ 12.2 Hz, H-9b), 4.47 (d, 1 H, $J_{7,8}$ 9.6 Hz, H-7), 4.10–4.04 (m, 1 H, H-8), 4.16 (dd, 1 H, $J_{4,5}$ 8.6, $J_{5,6}$ 11.2 Hz, H-5), 4.32 (d, 1 H, $J_{5,6}$ 11.2 Hz, H-6), 4.49 (br d, 1 H, $J_{4,5}$ 8.6 Hz, H-4), and 5.67 (br d, 1 H, H-3). Positive FABMS (NBA): $(M + H)^+$ *m/z* 345, $(M + Na)^+$ 367.

9-Acetamido-2,6-anhydro-3,5,9-trideoxy-5-trifluoroacetamido-D-glycero-D-galacto-*non-2-enonic acid* (**2b**).—To a solution of compound **2a** (7 mg, 0.02 mmol) in anhydrous MeOH (2 mL) was added a solution of Ac_2O (2.3 mg, 0.022 mmol) in 200 μL of MeOH. The solution was stirred for 40 min at room temperature, concentrated, and purified by silica-gel chromatography using 3 : 1 CH_2Cl_2–MeOH to give **12** (7 mg, 90%), $[\alpha]_D$ + 11.7° (*c* 0.14, CH_3OH); ν_{max} 1629 cm^{-1} (CO); ^1H NMR (D_2O): δ 1.96 (s, 3 H, AcO), 3.20–3.52 (m, 3 H, H-7, H-9a, and H-9b), 3.88–3.97 (m, 1 H, H-8), 4.13 (dd, 1 H, $J_{4,5}$ 8.9, $J_{5,6}$ 10.6 Hz, H-5), 4.27 (d, 1 H, $J_{5,6}$ 10.6 Hz, H-6), 4.47 (br d, 1 H, $J_{4,5}$ 8.9 Hz, H-4), and 5.65 (br, d, 1 H, H-3). Positive FABMS (NBA): $(M + Na)^+$ *m/z* 409, $(M + 2Na - H)^+$ 431.

2,6-Anhydro-3,5,9-trideoxy-5,9-bis(trifluoroacetamido)-D-glycero-D-galacto-*non-2-enonic acid* (**2c**).—To a solution of compound **2a** (10 mg, 0.03 mmol) in MeOH (1 mL) was added Et_3N (0.3 mL) and methyl trifluoroacetate (1 mL). The mixture was stirred for 40 min at room temperature, and concentrated. The residue was purified by silica-gel chromatography using 3 : 1 CH_2Cl_2–MeOH to give **2c** (10 mg, 78%), $[\alpha]_D$ + 6.7° (*c* 0.2, H_2O); ν_{max} 1712, and 1585 cm^{-1} (CO); ^1H NMR (D_2O): δ 3.39–3.48 (m, 2 H, H-7, and H-9a), 3.64 (d, 1 H, $J_{9a,9b}$ 14.2 Hz, H-9b), 3.96–4.07 (m, 1 H, H-8), 4.16 (dd, 1 H, ($J_{4,5}$ 8.6, $J_{5,6}$ 10.6 Hz, H-5), 4.32 (d, 1 H, $J_{5,6}$ 10.6 Hz, H-6), 4.49 (d, 1 H, $J_{4,5}$ 8.6 Hz, H-4), 5.66 (br d, 1 H, H-3). Positive FABMS (NBA): $(M + H)^+$ *m/z* 441, $(M + Na)^+$ 463.

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References

[1] (a) A.K.J. Chong, M.S. Pegg, N.R. Taylor, and M. von Itzstein, *Eur. J. Biochem.*, 207 (1992) 335–345; (b) N.R. Taylor and M. von Itzstein, *J. Med. Chem.*, 27 (1994) 616–624; (c) G. Herrler, A. Imhof, R. Brossmer, G. Milks, and J.C. Paulson, *J. Biol. Chem.*, 267 (1992) 12501–12505.

[2] (a) P. Meindl, G. Bodo, P. Palese, J. Schulman, and H. Tuppy, *Virology*, 58 (1974) 457–463; (b) P.-A. Driguez, B. Barrere, G. Quash, and A. Doutheau, *Carbohydr. Res.*, 262 (1994) 297–310; (c) T. Hagiwara, I. Kijima-Suda, T. Ido, H. Ohrui, and K. Tomota, *Carbohydr. Res.*, 263 (1994) 167–172.

[3] M. von Itzstein, W.-Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T.V. Phan, M.L. Smythe, H.F. White, S.W. Oliver, P.M. Colman, J.N. Varghese, D.M. Ryan, J.M. Woods, R.C. Bethell, V.J. Hotham, J.M. Cameron, and C.R. Penn, *Nature*, 363 (1993) 418–423.

[4] W.P. Burmeister, R.W.H. Ruigrok, and S. Cusack, *EMBO J.*, 11 (1992) 49–56.

[5] (a) M.A. Sparks, K.W. Williams, C. Lukacs, A. Schrell, G. Priebe, A. Spaltanstein, and G.M. Whitesides, *Tetrahedron*, 49 (1993) 1–12. (b) J.L.-C. Liu, G.-J. Shen, Y. Ichikawa, J.F. Rutan, G. Zapata, W.F. Wann, and C.-H. Wong, *J. Am. Chem. Soc.*, 114 (1992) 3901–3910.

[6] Dominicus J.M. van der Vliugel, W.A.R. van Heeswijk, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 102 (1982) 121.

[7] Y. Kiso, K. Ukawa, and T. Akita, *J. Chem. Soc. Chem. Commun.*, (1980) 101–1102.

[8] N.E. Byramova, A.B. Yuzikov, and N.V. Bovin, *Carbohydr. Res.*, 237 (1992) 161–175.

[9] H. Bayley, D.N. Standring, and J.R. Knowles, *Tetrahedron Lett.*, 39 (1978) 3633–3634.

[10] K. Okamoto, T. Kondo, and T. Goto, *Bull. Chem. Soc. Jpn.*, 60 (1987) 631–636.