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Chemoenzymatic synthesis of an *N*-acetylneuraminic acid analogue having a carbamoylmethyl group at C-4 as an inhibitor of sialidase from influenza virus

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Abstract

5,9-Diacetamido-2,6-anhydro-*O*-4-carbamoylmethyl-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (**1**) was synthesized via a key intermediate **2** through the Neu5Ac aldolase [E.C.4.1.3.3]-catalyzed aldol reaction of 2-acetamido-2,6-dideoxy-6-azido-D-glucose with sodium pyruvate operating under alkaline conditions (pH 10.5) in order to accelerate epimerization C-2 of *N*-acetyl-D-glucosamine (D-GlcNAc) derivatives. Compound **1** showed inhibitory activity against sialidase.

Keywords: Chemoenzymatic synthesis; Sialic acid; Neu5Ac aldolase; Sialidase inhibitor; Carbamoylmethyl group; Influenza virus

1. Introduction

The synthesis of inhibitors for influenza virus sialidase has been of great interest to us [1], because inhibition of sialidase might permit limiting the establishment and progression of infection by the influenza virus [2]. In particular, derivatives of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac2en), which are thought to be transition-state analogues of the enzyme reaction [3], have received considerable attention. In addition, the C-4 position near the anomeric center in sialic acids seems to play an important role in enzyme–substrate interactions [4]. 5-Acetamido-2,6-anhydro-4-guanidino-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (4-deoxy-4-guanidino-Neu5Ac2en) is a potent and selective inhibitor of influenza virus sialidase [5]. As part of a program aimed at the new sialidase inhibitors, we have described the chemoenzymatic synthesis of neuraminic acid analogs structurally varied at C-5 and C-9 and their inhibitory activities against the enzyme [6]. This paper reports a facile chemoenzymatic synthesis of a 5,9-di-*N*-acetyl-2,3-didehydro-2,3-dideoxy compound **1** bearing a carbamoylmethyl group, which is a good affinity ligand for proteases [7], at the C-4 position of a

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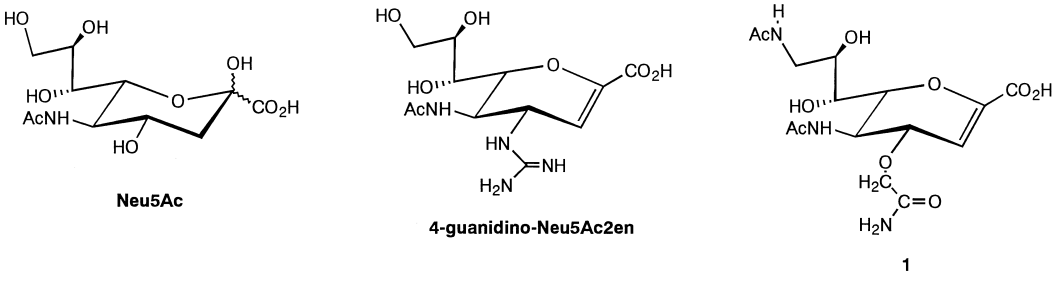
sialic acid derivative, prepared from a D-glucosamine derivative, and its behavior towards the sialidase of the influenza virus (Scheme 1).

2. Results and discussion

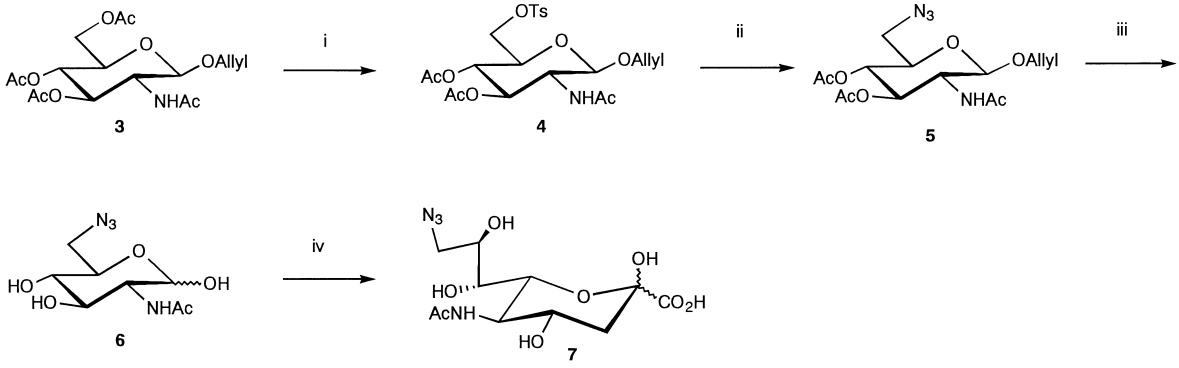
The natural substrate for enzymatic synthesis of sialic acid is *N*-acetyl-D-mannosamine (D-ManNAc), however this compound is expensive. The base-catalyzed C-(2) epimerization of D-GlcNAc to D-ManNAc has been extensively studied [8]. The rate of epimerization of D-GlcNAc to D-ManNAc is highly pH-dependent, and operation at pH 10.5–11 is necessary for a reasonable rate of conversion. The enzyme was stable at pH 7.2–7.5 through the incubation period, however, at alkaline pH there was a substantial loss of activity. Tsukada and Ohta reported the protective role of D-GlcNAc and sodium pyruvate on the enzyme stability under alkaline conditions [9]. The synthetic strategy adopted for the preparation of **1** required a key intermediate (**2**) which was synthesized from 2-acetamido-6-azido-2-deoxy-D-glucose (**6**) with sodium pyruvate catalyzed by Neu5Ac aldolase under the Ca^{2+}- catalyzed epimerization at 2-position of the D-GlcNAc derivative at pH 10.5 [8b,c].

Deacetylation of allyl glycoside **3** [10] with NaOMe in MeOH and selective tosylation at C-6 with *p*-toluenesulfonyl chloride in pyridine–CH_2Cl_2, and then acetylation with Ac_2O and pyridine provided compound **4** in 71% yield in three steps. Treatment of **4** with NaN_3 and 18-crown-6 ether [11] in DMF afforded **5** in 85% yield. The IR spectrum of **5** showed absorption bands at 2098 and 1745 cm^{-1}, characteristic of the azido and ester groups, respectively (Scheme 2).

Successful removal of the allyl group of **5** with SeO_2 in AcOH and 1,4-dioxane [12], followed by 0.2M NaOMe in MeOH gave **6** in 62% yield in two steps. Incubation of Neu5Ac aldolase with **6** and sodium pyruvate in distilled water (pH 10.5) at room temperature for 3 days provided, after ion-exchange chromatography and desalting, compound **7** [13] FABMS: *m/z* 335 $(M)^+$, 357 $(M+Na)^+$ in 23% yield. Methyl esterification of **7** with Amberlite IR-120 (H^+) resin in MeOH and peracetylation with acetic anhydride and pyridine gave compound **8** {FABMS: *m/z* 517 $(M+H)^+$, 539 $(M+Na)^+$} in 81% yield in two steps. The



Scheme 1.



i) 1) NaOMe, MeOH, 2) TsCl, pyridine, 3) Ac_2O, pyridine; ii) NaN_3, 18-crown-6 ether, DMF; iii) 1) $PdCl_2$, AcOH-THF, 2) NaOMe, MeOH; iv) Neu5Ac Aldolase, sodium pyruvate, pH10.5.

Scheme 2.

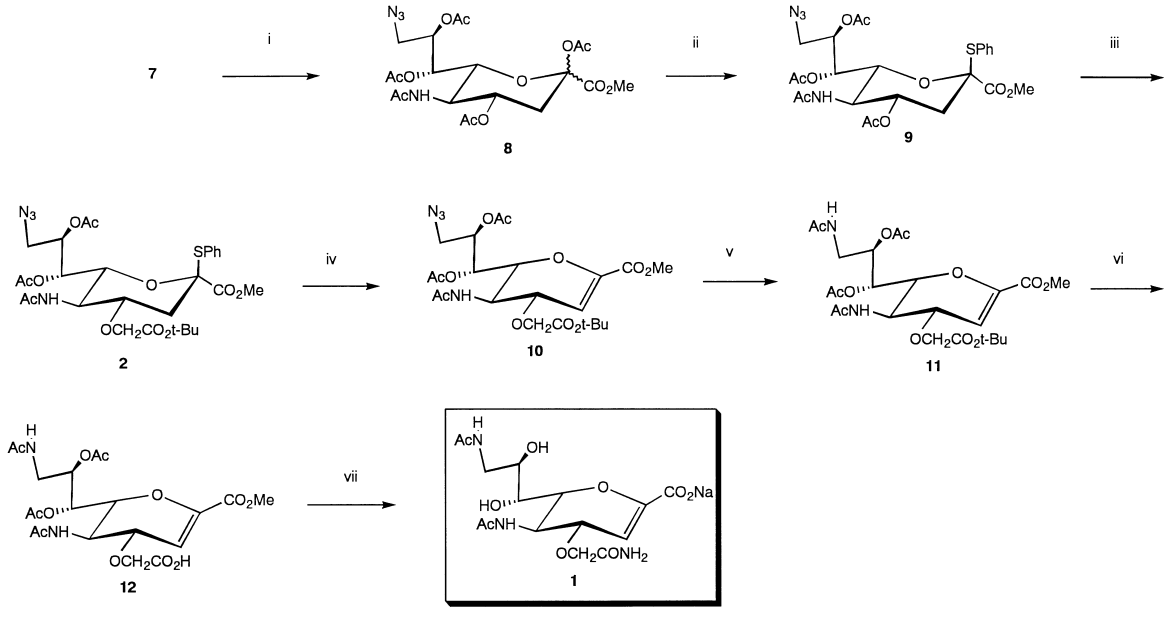
^{1}H NMR spectrum of **8** showed a one-proton doublet of doublets at δ 2.69 ($J_{3ax,3eq}$ 13.8, $J_{3eq,4}$ 5.0 Hz, H-3*eq*). Conversion of **8** to β-thio-ketoside **9** with thiophenol and boron trifluoride etherate in CH_2Cl_2 gave the β-2-thioglycoside **9** {FABMS: *m*/*z* 567 $(M+H)^+$} in 82% yield. Deacetylation **9** with NaOMe in MeOH (75% yield), followed by selective introduction of the *t*-butoxycarbonylmethyl group at O-4 by $BrCH_2CO_2t$-Bu, Ag_2O, and *t*-Bu_4NI [14], (30% yield) and then acetylation with Ac_2O and pyridine provided the key intermediate (**2**) {FABMS: *m*/*z* 639 $(M+H)^+$, 661 $(M+Na)^+$} (98% yield). The methylene protons of the *t*-butoxy carbonylmethyl group in the ^{1}H NMR spectrum of **2** showed at δ 3.86, 4.03 (d, J_{gem} 17.3 Hz). For the synthesis of **1**, compound **2** was transformed into **10** by treatment with *N*-bromosucciimide (NBS), I_2, and *t*-butylammonium triflate (TBAOTf), followed by 1,8-diazabicyclo[5.4.0] undecen-7-ene (DBU) [15] to give **10** {FABMS: *m*/*z* 529 $(M+H)^+$} in 49% yield in two steps, showing in its ^{1}H NMR spectrum a one-proton doublet at δ 6.14 ($J_{3,4}$ 3.0 Hz, H-3), characteristic of the 2,3-double bond. Selective reduction and simultaneous acetylation of the azido group of **10** by AcSH–pyridine [16] gave **11** {FABMS: *m*/*z* 545 $(M+H)^+$, 567 $(M+Na)^+$} in 45% yield. Removal of the *t*-butyl group of **11** by CF_3CO_2H–CH_2Cl_2 gave **12** {FABMS: *m*/*z* 489 $(M+H)^+$, 511 $(M+Na)^+$} in quantitative yield. Compound **12** was treated with oxalyl chloride and then 28% aqueous NH_4OH, followed by Dowex 50W-X8 (Na^+) to give the target molecule **1** {FABMS (glycerol-thioglycerol 1:2): *m*/*z* 451 $(M+K+H)^+$, *m*/*z* 455 $(M+2Na)^+$} in quantitative yield (Scheme 3).

In a preliminary examination of inhibitory activity against the sialidase of influenza virus A/PR/8/34(H1N1) [17], compound **1** inhibited the sialidase by 7% at 0.01 μM. In contrast to **1**, 4-deoxy-4-guanidino-Neu5Ac2en showed an inhibitory effect of 45% at the same concentration.

In conclusion, the chemoenzymatic synthesis of 5,9-diacetamido-4-carbamoylmethyl-2,3-didehydro compound **1** was accomplished via key intermediate **2**. Compound **1** showed only weak inhibitory activity on the influenza A virus sialidase.

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. ^{1}H NMR spectra were recorded with a Jeol JNM-EX 270 [^{1}H (270 MHz)] spectrometer. ^{1}H Chemical



Scheme 3.

shifts are given in ppm relative to 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. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Fast atom bombardment (FAB) mass spectra were obtained with a Jeol JNM SX-102 mass spectrometer in the positive ion mode using NBA matrix. Column chromatography was performed on Silica Gel Merck 60 (70-230 mesh) and Bio-Gel P-2 (Pharmacia). 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). The spots were visualized by spraying the plates with 5% aqueous H_2SO_4 in MeOH and then heating. Glycolipids containing sialic acid were visualized with resorcinol reagent. The bands of lipids containing sialic acid were stained blue.

Allyll 2-acetamido-3,4-di-O-*acetyl-2-deoxy-6*-O-*p-tolylsulfonyl*-β-D-*glucopyranoside* (**4**).—Compound **3** (3.1 g, 8.0 mmol) was added to a solution of 28% aqueous NH_4OH–MeOH (5:95) (50 mL) and the mixture was stirred for 8 h at room temperature and evaporated. The residue was chromatographed on silica gel using 5:1CH_2Cl_2-MeOH to give the diol (1.80 g, 86%), a solution of which in pyridine (10 mL) and CH_2Cl_2 (15 mL) was treated with *p*-toluenesulfonyl chloride (1.58 g, 8.3 mmol) at −50 °C under Ar, and the mixture was stirred for 12 h at the same temperature, and evaporated. The residue was dissolved in (2:1) pyridine–Ac_2O (15 mL), and the mixture was kept for 15 h at room temperature, and then evaporated to dryness. The residue was chromatographed on silica gel using 50:1 CH_2Cl_2–MeOH to give **4** (2.8 g, 82% from the diol) as prisms, m.p. 168–170 °C, $[\alpha]_D$ +0.6° (*c* 1.0, $CHCl_3$); ν_{max} 1746 (OAc), 1653, and 1560 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.93 (s, 3 H, AcNH), 2.00, 2.01 (s, each 3 H, AcO), 2.45 (s, 3 H, PhCH_3), 3.71–3.87 (m, 2 H, H-2, H-5), 4.01 (dd, 1 H, *J* 13.2, *J* 6.2 Hz,–CH_2CH = CH_2), 4.07–4.13 (m, 2 H, H-6), 4.25 (dd, 1 H, *J* 5.1 Hz, –CH_2CH = CH_2), 4.65 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.89 (dd, 1 H, $J_{3,4}$ 11.9, $J_{4,5}$ 9.9 Hz, H-4), 5.21 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-3), 5.56 (d, 1 H, $J_{2,NH}$ 8.6 Hz, NH), 5.75–5.89 (m, 1 H, –CH_2CH = CH_2), 7.35, 7.78 (d, each 2 H, *J* 8.3 Hz, $PhCH_3$). Anal. Calcd for $C_{22}H_{28}NO_9S$ H_2O: C, 52.80; H, 6.04; N, 2.80. Found: C, 52.20; H, 5.65; N, 2.27.

Allyl 2-acetamido-3,4-di-O-*acetyl-6-azido-2-deoxy*-β-D-*glucopyranoside* (**5**).—A mixture of **4** (5.1 g, 10.2 mmol), NaN_3 (3.32 g, 51.0 mmol) and 18-crown-6 ether (0.898 g, 3.4 mmol) in DMF (50 mL) was heated at 40–50 °C with stirring for 40 h under Ar. The mixture was cooled to room temperature, and filtered in order to remove insoluble material, and the filtrate was concentrated. The residue was chromatographed on silica gel using 30:1 CH_2Cl_2–MeOH to give **5** (3.2 g, 85%) as prisms, m.p. 174–176 °C, $[\alpha]_D$ −39° (*c* 1.0, $CHCl_3$); ν_{max} 2098 (N_3), 1745 (OAc),1653, and 1560 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.93 (s, 3H, AcH), 2.04 (s, 6 H, AcO), 3.18 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 13.4 Hz, H-6a), 3.44 (dd, 1 H, $J_{5,6b}$ 7.6 Hz, H-6b), 3.72 (ddd, 1 H, $J_{4,5}$ 10.1 Hz, H-5), 3.92 (ddd, 1 H, $J_{1,2}$ and $J_{2,NH}$ 8.6, $J_{2,3}$ 10.8 Hz, H-2), 4.12 (dd, 1 H, *J* 13.0, *J* 6.2 Hz,–CH_2CH = CH_2), 4.37 (dd, 1 H, *J* 5.2 Hz,–CH_2CH = CH_2), 4.77 (d, 1H, $J_{1,2}$ 8.6 Hz, H-1), 4.97 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-4), 5.21 (dd, 1H, *J* 10.3, *J* 1.6 Hz,–CH_2CH = CH_2), 5.29 (dd, 1H, *J* 17.0 Hz,–CH_2CH = CH_2), 5.32 (dd, 1H, H-3), 5.86 (m, 1H, CH_2CH = CH_2), 5.95 (d, 1H, NH). Anal. Calcd for $C_{15}H_{22}N_4O_7$: C, 48.65; H, 5.99; N, 15.13. Found: C, 48.67; H, 5.97; N, 14.88.

2-Acetamido-6-azido-2,6-dideoxy-D-*glucopyranose* (**6**).—A solution of **5** (1.96 g, 5.3 mmol) and SeO_2 (0.88 g, 7.9 mmol) in AcOH (0.50 mL) and 1,4-dioxane (40 mL) was heated under reflux for 9 h. The reaction mixture was then filtered in order to remove insoluble material. After evaporation of the filtrate, the residue was dissolved in dry MeOH (40 mL) and to the mixture was added methanolic 0.1 M NaOMe (20 mL), and the resulting solution was stirred for 2 h at 0 °C, and 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 5:1CH_2Cl_2–MeOH to give **6** (808 mg, 62%), as a white powder, $[\alpha]_D$ +102° (*c* 1.0, $CHCl_3$); ν_{max} 2110 (N_3), 1627, and 1542 cm^{-1} (CONH); ^1H NMR (CD_3OD): δ 1.90 (s, 3 H, AcNH), 3.20–3.22 (m, 1 H, H-4), 3.30 (dd, 1 H, $J_{5,6a}$ 5.6, $J_{6a,6b}$ 13.2 Hz, H-6a), 3.42 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 3.59 (dd, 1H, $J_{2,3}$ 10.9, $J_{3,4}$ 8.9 Hz, H-3), 3.77 (dd, 1H, $J_{1,2}$ 3.6 Hz, H-2), 3.82–3.89 (m, 1 H, H-5), 5.01 (d, 1 H, H-1). Positive f.a.b.-m.s. (NBA): $(M + H)^+$ *m/z* 247.

5-Acetamido-9-azido-3,5,9-trideoxy-D-glycero-D-galacto-*2-nonulopyranosonic acid* (**7**).—A solution of Neu5Ac aldolase [E.C.4.1.3.3, 100 unit] in distilled water (1.0 mL) was added to a solution of **6**

(680 mg, 2.76 mmol) and sodium pyruvate (3.04 g, 27.6 mmol) in distilled water (10 mL) at pH 10.5 adjusted by aqueous $Ca(OH)_2$ solution, and the mixture was stirred for 3 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 0–0.5 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 filteration chromatography using water as eluant. After freeze drying, **7** (224 mg, 23%) was obtained, $[\alpha]_D -0.1°$ (*c* 1.0, H_2O); ν_{max} 2102 (N_3), 1738 (carboxyl), 1653, and 1542 cm^{-1} (CONH); ^1H NMR (CD_3OD): δ 1.66 (dd, 1 H, $J_{3ax,3eq}$ 13.1, $J_{3ax,4}$ 11.1 Hz, H-3*ax*), 1.89 (s, 3 H, AcNH), 2.05 (dd, 1 H, $J_{3eq,4}$ 4.7 Hz, H-3*eq*), 3.30 (dd, 1 H, $J_{8,9a}$ 5.9, $J_{9a,9b}$ 13.2 Hz, H-9a), 3.35 (dd, 1 H, *J* 8.9 Hz, H-7), 3.44 (d, 1 H, $J_{8,9b}$ 3.0 Hz, H-9b), 3.70–3.91 (m, 4H, H-4, 5, 6, 8). Positive f.a.b.-m.s. (NBA): $(M)^+$ 335 *m/z*, $(M+Na)^+$ 357.

Methyl (5-acetamido-4,7,8-tri-O-*acetyl-9-azido-3,5,9-trideoxy*-D-glycero-D-galacto-*2-nonulopyranoso)onate* (**8**).—A solution of compound **7** (167 mg, 0.48 mmol) was dissolved in anhydrous MeOH (12 mL) with Amberlite IR-120B (H^+ form) (200 mg) resin and stirred at room temperature for 10 h, filtered and concentrated. The residue was redissolved in pyridine (3 mL) and treated with Ac_2O (2 mL) and stirred at 0 °C for 30 min, and then for 10 h at room temperature. The mixture was dissolved in CH_2Cl_2 (20 mL) and successively washed with water, 1N HCl, aqueous $NaHCO_3$, aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel using 50:1 CH_2Cl_2–MeOH to give **8** (200 mg, 81%) as a white powder, m.p. 153–154 °C, $[\alpha]_D -26.5°$ (*c* 1.0, $CHCl_3$); ν_{max} 2102 (N_3), 1738 (AcO), 1653, and 1542 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.89 (s, 3H, AcNH), 2.04, 2.07, 2.17 (s, 3H, AcO), 2.52 (dd, 1H, $J_{3ax,3eq}$ 13.0, $J_{3eq,4}$ 5.0 Hz, H-3*eq*), 3.37 (dd, 1 H, $J_{8,9a}$ 8.0, $J_{9a,9b}$ 13.4 Hz, H-9a), 3.80 (s, 3H, CO_2Me), 3.83 (dd, 1 H, $J_{8,9b}$ 2.6 Hz, H-9b), 4.08 (dd, 1H, $J_{5,6}$ 10.6, $J_{6,7}$ 2.0 Hz, H-6), 4.18 (ddd, 1H, $J_{4,5}$ 10.5, $J_{5,NH}$ 9.7 Hz, H-5), 4.90 (ddd, 1 H, $J_{7,8}$ 3.0 Hz, H-8), 5.25 (ddd, 1 H, $J_{3ax,4}$ 10.5 Hz, H-4), 5.41 (dd, 1 H, H-7), 5.63 (s, 1 H, AcNH). Positive f.a.b.-m.s. (NBA): $(M+H)^+$ 517 *m/z*, $(M+Na)^+$ 539.

Methyl (phenyl 5-acetamido-4,7,8-tri-O-*acetyl-9-azido-2-thio-3,5,9-trideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosid)onate* (**9**).—A solution of **8** (185 mg, 0.36 mmol) and PhSH (43 mg, 0.39 mmol) in anhydrous CH_2Cl_2 (5 mL) was added $BF_3{\cdot}Et_2O$ (0.11 mL, 0.90 mmol) at 0 °C, and the mixture was kept overnight at room temperature, and then diluted with CH_2Cl_2, successively washed with aqueous $NaHCO_3$, aqueous NaCl, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel with 100:1 CH_2Cl_2–MeOH to give **9** (167 mg, 82%) as a powder, $[\alpha]_D -114°$ (*c* 0.5, $CHCl_3$); ν_{max} 2100 (N_3), 1735 (AcO), 1654, and 1542 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.90 (s, 3 H, AcNH), 2.04, 2.09, 2.12 (s, each 3 H, AcO), 2.69 (dd, 1 H, $J_{3ax,3eq}$ 13.8, $J_{3ax,4}$ 5.0 Hz, H-3*eq*), 3.33 (dd, 1 H, $J_{8,9a}$ 9.3, $J_{9a,9b}$ 13.2 Hz, H-9a), 3.65 (s, 3H, CO_2Me), 3.67 (dd, 1 H, $J_{8,9b}$ 2.3 Hz, H-9b), 4.14 (ddd, 1 H, $J_{4,5}$ and $J_{5,6}$ 10.5, $J_{5,NH}$ 9.9 Hz, H-5), 4.59 (dd, 1 H, $J_{6,7}$ 2.3 Hz, H-6), 4.77 (ddd, 1 H, $J_{7,8}$ 3.0 Hz, H-8), 5.38 (ddd, 1 H, $J_{3ax,4}$ 10.5 Hz, H-4), 5.45 (dd, 1 H, H-7), 5.71 (s, 1 H, AcNH), 7.37–7.44 (m, 5H, PhS−). Positive f.a.b.-m.s. (NBA): $(M+H)^+$ *m/z* 567. Anal. Calcd for $C_{28}H_{38}NO_4S$: C, 52.65; H, 5.99; N, 8.77. Found: C, 52.54; H, 6.10; N, 8.29.

Methyl (phenyl 5-acetamido-7,8-di-O-*acetyl-9-azido-4*-O-tert-*butoxycarbonylmethyl-3,5,9-trideoxy-2-thio*-D-glycero-D-galacto-*2-nonulopyranosid)onate* (**2**).—To a solution of compound **9** (154 mg, 0.27 mmol) in dry MeOH (5 mL) was added methanolic 0.2 M NaOMe (5 mL) and stirred for 5 h at 0 °C, and neutralized with Amberlite IRC-50 (0.5 g) resin and filtrated, and concentrated. The residue was eluted from a column of silica gel with 100:1 CH_2Cl_2–MeOH to give the triol (90 mg, 75%). The triol (80 mg, 0.18 mmol) was dissolved in dichloroethane (1.0 mL) and to the mixture was added MS 4 Å (200 mg) and bromoacetic acid *t*-butyl ester (43 mg, 0.22 mmol). After stirring for 1 h, to the mixture was added Ag_2O (126 mg, 0.54 mmol) and *n*-Bu_4NI (34 mg, 0.09 mmol) and the mixture was stirred for 12 h in the dark under Ar. The insoluble materials were filtered through Celite 545 and the filtrate was concentrated to dryness. The residue was purified by silica gel chromatography using 20:1 CH_2Cl_2–MeOH to give the diol (30 mg, 30% from the triol), which was dissolved in pyridine (1 mL) and treated with acetic anhydride (1 mL). The mixture was stirred at 0 °C for 30 min, and then overnight at room temperature. The solution was diluted with CH_2Cl_2 (50 mL) and successively washed with water, 1N HCl, aqueous saturated $NaHCO_3$, and brine, and dried ($MgSO_4$), and concentrated. The residue was

purified by silica gel chromatography using 100:1 CH_2Cl_2–MeOH to give **2** (34 mg, 98% from the triol) as a white powder, m.p. 85–87 °C, $[\alpha]_D$ −62° (*c* 0.54, $CHCl_3$); ν_{max} 2100 (N_3), 1735 (AcO), 1654, and 1542 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.50 (s, 9 H, *t*-BuOCO), 1.99 (s, 3 H, AcNH), 2.10, 2.14 (s, each 3 H, OAc), 2.76 (dd, 1 H, $J_{3ax,3eq}$ 14.0, $J_{3ax,4}$ 3.8 Hz, H-3*eq*), 3.33 (dd, 1 H, $J_{8,9a}$ 9.2, $J_{9a,9b}$ 13.2 Hz, H-9a), 3.64 (s, 3 H, CO_2Me), 3.66 (dd, 1 H, $J_{8,9b}$ 2.2 Hz, H-9b), 3.89–3.97 (m, 2 H, H-4, H-5), 3.86, 4.03 (d, each 1 H, J_{gem} 17.3 Hz, *t*-BuOCOCH_2O–), 4.54 (dd, 1H, $J_{5,6}$ 9.9, $J_{6,7}$ 2.2 Hz, H-6), 4.81 (ddd, 1 H, $J_{7,8}$ 1.9 Hz, H-8), 5.41 (dd, 1H, H-7), 6.14 (d, 1 H, $J_{5,NH}$ 8.1 Hz, AcN*H*), 7.38–7.44 (m, 5 H, PhS-). Positive f.a.b.-m.s. (NBA): $(M+H)^+$ *m*/*z* 639, $(M+Na)^+$ 661.

Methyl 5-acetamido-7,8-di-O-*acetyl-2,6-anhydro-9-azido-4*-O-tert-*butoxycarbonylmethyl-3,5,9-trideoxy*-D-glycero-D-galacto-*non-2-enonate* (**10**).—To a solution of compound **2** (32 mg, 0.05 mmol) and MS 4 Å (0.3 g) in dry CH_2Cl_2 (2 mL) was added NBS (27 mg, 0.15 mmol) and I_2 (38 mg, 0.15 mmol) and TBAOTf (10 mg, 0.025 mmol) at −15 °C under Ar. After stirring for 1 h at the same temperature, to the mixture was added a solution of DBU (23 mg, 0.15 mmol) in dry CH_2Cl_2 (0.5 mL) at −15 °C, and the mixture was stirred overnight at room temperature. The suspension was filtered through Celite 545 and the filtrate was successively washed with aqueous 5% $Na_2S_2O_3$, and brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography using 20:1 CH_2Cl_2–MeOH to give **10** (13 mg, 49%) as a syrup, $[\alpha]_D$ +42° (*c* 0.94, $CHCl_3$); ν_{max} 2100 (N_3), 1750 (AcO), 1655, and 1560 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.47 (s, 9 H, *t*-BuOCO), 2.00 (s, 3 H, AcNH), 2.09, 2.15 (s, each 3 H, AcO), 3.48 (dd, 1 H, $J_{8,9a}$ 7.8, $J_{9a,9b}$ 13.5 Hz, H-9a), 3.81 (s, 3 H, CO_2Me), 4.09 (ddd, 1 H, $J_{4,5}$ 6.6, $J_{5,6}$ 8.6, $J_{5,NH}$ 8.4 Hz, H-5), 4.35 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 4.43 (dd, 1 H, $J_{6,7}$ 3.5 Hz, H-6), 5.24 (ddd, 1 H, $J_{7,8}$ 3.8 Hz, H-8), 5.50 (dd, 1 H, H-7), 5.90 (s, 1H, AcNH), 6.14 (d, 1 H, H-3). Positive f.a.b.-m.s. (NBA): $(M+H)^+$ *m*/*z* 529.

Methyl 5,9-diacetamido-7,8-di-O-*acetyl-2,6-anhydro-4*-O-tert-*butoxycarbonylmethyl-3,5,9-trideoxy*-D-glycero-D-galacto-*non-2-enonate* (**11**).—Compound **10** (26 mg, 0.05 mmol) was dissolved in a solution of thioacetic acid (7.4 mg, 0.10 mmol) in pyridine (1.0 mL) under argon. The mixture was stirred for 15 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography using (10:1) CH_2Cl_2–MeOH to give **11** (12 mg, 45%), $[\alpha]_D$ +42° (*c* 0.34, $CHCl_3$); ν_{max} 1744 (AcO), 1636, and 1559 cm^{-1} (CONH); ^1H NMR ($CDCl_3$): δ 1.47 (s, 9 H, *t*-BuOCO), 1.98, 1.99 (s, each 3 H, AcNH), 2.14 (s, 6 H, AcO), 3.32 (ddd, 1 H, $J_{8,9a}$ 7.8, $J_{9a,9b}$ 14.0 Hz, H-9a), 3.81 (s, 3H, CO_2Me), 4.04, 4.13 (d, each 1 H, J_{gem} 16.5 Hz, *t*-BuOCOCH_2O–), 4.16 (ddd, 1 H, $J_{4,5}$ 7.3 $J_{5,6}$ 8.4 $J_{5,NH}$ 8.4 Hz, H-5), 4.33 (dd, 1H, $J_{3,4}$ 3.0 Hz, H-4), 4.43 (dd, 1 H, $J_{6,7}$ 3.5 Hz, H-6), 5.11 (ddd, 1 H, $J_{7,8}$ 4.6 Hz, H-8), 5.43 (dd, 1 H, H-7), 6.10 (d, 1 H, *J* 8.4 Hz, AcN*H*), 6.15 (d, 1 H, H-3), 6.65 (t, 1 H, *J* 6.0 Hz, AcN*H*CH_2–). Positive f.a.b.-m.s. (NBA): $(M+H)^+$ *m*/*z* 545, $(M+Na)^+$ 567.

Methyl 5,9-diacetamido-7,8-di-O-*acetyl-2,6-anhydro-3,5,9-trideoxy-4*-O-*methoxycarbonyl*-D-glycero-D-galacto-*non-2-enonate* (**12**).—Compound **11** (12 mg, 0.02 mmol) was added to a solution of CF_3CO_2H–CH_2Cl_2 (1:2) (1.5 mL) at 0 °C, and the mixture was stirred for 15 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography using (5:1) CH_2Cl_2–MeOH to give **12** (13 mg, quant.); ^1H NMR ($CDCl_3$): δ 2.03, 2.12, 2.34 (s, 12H, AcO, AcNH), 3.82 (s, 3H, CO_2Me), 4.10–4.45 (m, 5 H, H-4, 5, 6, 9), 5.12 (m, 1 H, H-8), 5.44 (br s, 1 H, H-7), 6.13 (br s, 1 H, H-3). Positive f.a.b.-m.s. (NBA): $(M+H)^+$ *m*/*z* 489, $(M+Na)^+$ 511.

5,9-diacetamido-2,6-anhydro-4-O-*carbamoylmethyl-3,5,9-trideoxy*-D-glycero-D-galacto-*non-enonic acid* (**1**).—Compound **12** (12 mg, 0.02 mmol) was dissolved in oxayl chloride (0.5 mL), and the mixture was heated at 40–50 °C for 0.5 h. After coevaporation with toluene, the residue was dissolved in CH_2Cl_2 (1.0 mL) and to the mixture was added 28% aqueous NH_4OH (1.0 mL) at 0 °C. After stirring for 2 h, the solvent was evaporated to dryness, the residue was dissolved in H_2O (1.0 mL) and the mixture was treated with Dowex 50W-X8 (Na^+), the resin was filtered off through Celite 545, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography using (65:35:5) $CHCl_3$–MeOH–H_2O to give **1** (7 mg, quant.) as a powder; ^1H NMR (D_2O): δ 2.01 (s, 3 H, NH), 3.20–3.28 (m, 2 H, H-7 and H-9a), 3.54–3.78 (m, 2 H, H-8 and H-9b), 3.92–4.03 (m, 1 H, H-5), 4.10, 4.21 (d, each 1 H, J_{gem} 15.4 Hz, H_2NCOCH_2O–), 4.33–4.45 (m, 2 H, H-4 and H-6), 6.02 (d, 1 H, *J* 2.4 Hz, H-3). Positive f.a.b.-m.s. (glycerol-thioglycerol (1:2)): *m*/*z* 455 $(M+2Na)^+$, 451 $(M+K+H)^+$.

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