Carbohydrate Research 343 (2008) 2459–2462

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Regioselective synthesis of 4azido-Neu2en5,7Ac$_2$1Me and its intramolecular transformation to 4azido-Neu2en5,9Ac$_2$1Me

Qingjie Zhao [a], Yuanyuan Lou [b], Ruisheng Xiong [a], Haihong Li [c], Jingshan Shen [a,c,*]

[a] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China
[b] College of Materials Science and Engineering, Nanjing University of Technology, Nanjing 210009, PR China
[c] Topharman Shanghai Co., Ltd, Shanghai 201209, PR China

ARTICLE INFO

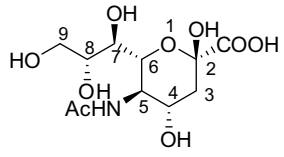
Article history:
Received 30 January 2008
Received in revised form 11 June 2008
Accepted 12 June 2008
Available online 20 June 2008

Keywords:
Neu5Ac
Analogues
Acetyl migration
Azides
X-ray crystal structure
Carbohydrates

ABSTRACT

Methyl 5-*N*-acetyl-7-*O*-acetyl-4-azido-2,3-didehydro-2,4-dideoxy-neuraminic acid (4azido-Neu2en5,7Ac$_2$1Me) was synthesized regioselectively starting from 4azido-Neu2en5Ac1Me in high yield. The transformation of 4azido-Neu2en5,7Ac$_2$1Me to the corresponding thermodynamically stable 4azido-Neu2en5,9Ac$_2$1Me via intramolecular acetyl migration was confirmed by single-crystal X-ray diffraction analysis. The proposed rearrangement mechanism is discussed.

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranoso nic acid (Neu5Ac, **1**) and its derivatives are significant carbohydrates that play important roles in the construction of biological molecules.[1–3] In an effort to learn more about the involvement of Neu5Ac derivatives substituted in the glycerol chain in biological processes with retention of amino or guanido groups at C-4,[4–10] an efficient preparation of this class of compounds would provide easy access to various structural congeners required for further structure–activity relationships study. 4Azido-Neu2en5Ac1Me (**2**) was a versatile intermediate for such kind of research since it can be easily transformed into amino or guanido derivatives. Herein, we report our efforts on the first efficient and regioselective synthesis of the 7-O-acetylated form of 4azido-Neu2en5Ac1Me (Schemes 1 and 2).



1
Neu5Ac

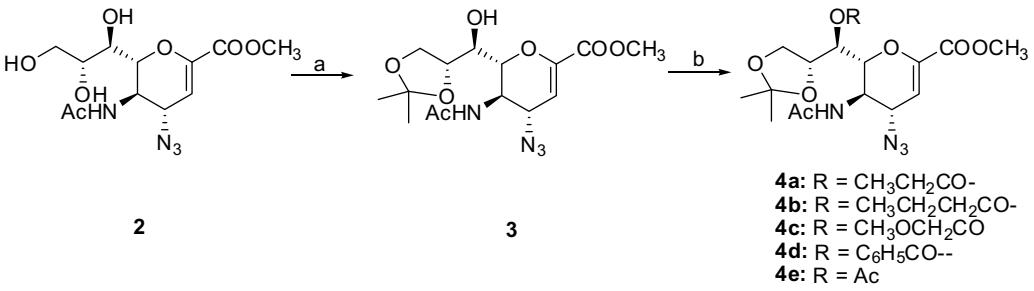
4-Azido-Neu2en5,7,8,9Ac$_4$1Me, prepared from Neu5Ac in good yield,[11] was deacetylated with NaOMe in MeOH to give compound **2**[12] in 96% yield. Without separation, compound **2** was reacted with acetone using Dowex 50W-X8 (H^+) resin as a catalyst to give protected compound **3**[13] in 80% yield. Treatment of compound **3** with either propionic, butyric, methoxyacetic, benzoic or acetic anhydride in pyridine under the catalysis of 4-dimethylaminopyridine (DMAP)[14] afforded the corresponding acylated compounds **4a–e** in 95–98% yield.

Hydrolysis of **4a–d** with water in the presence of Dowex 50W-X8 (H^+) resin yielded the corresponding 7-O-acylated compounds. Due to their unstable character, these compounds were difficult to purify via crystallization and column chromatography, as indicated by LC–MS. Fortunately, compound **4e** could be converted into pure compound **5** by hydrolysis and crystallization from EtOAc. Compound **5** was stable when stored in desiccators or in common solvents such as MeOH, CH_2Cl_2, and EtOAc at room temperature, while it was unstable in acidic media. It is interesting to note that compound **5** in MeOH began to transform into the thermodynamically stable compound **6** at 40 °C (Scheme 2). Furthermore, no 8-O-acetylated product was found during the rearrangement. It was postulated that the intramolecular transfer of the acetyl group proceeded via the cyclic intermediates of orthoesters **7** and **8** (Scheme 3).[15]

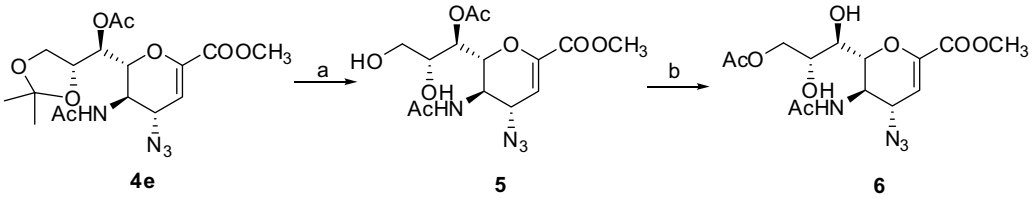
All structures of new compounds were confirmed by elemental analysis, ^{1}H NMR, and ^{13}C NMR spectroscopy. The evident

* Corresponding author.
E-mail address: jsshen@mail.shcnc.ac.cn (J. Shen).

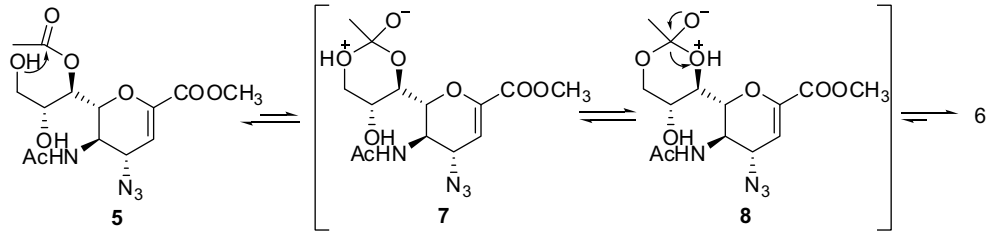
0008-6215/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.carres.2008.06.016



Scheme 1. Reagents and conditions: (a) Dowex 50W-X8 (H^+) resin, Me_2CO, 80%; (b) anhydrides, Py, DMAP, 10–24 h; 95–98%.



Scheme 2. Reagents and conditions: (a) Dowex 50W-X8 (H^+) resin, water, 76%; (b) MeOH, reflux, 80%.



Scheme 3. Plausible mechanism for the transformation of **5** to thermodynamically stable **6**.

Table 1
Chemical shifts (ppm) in the ^1H NMR spectra (Me_2SO-d_6) of **2**,[12] **5**, and **6**

	4-H	5-H	6-H	7-H	8-H	9-H_a	9-H_b
2	4.50	3.95	4.15	3.44	3.65	3.65	3.44
5	4.30	4.02	4.86	4.37	3.79	3.33	3.20
6	4.42	3.94	3.41	4.17	3.83	4.27	3.94

difference between compounds **2**,[12] **5**, and **6** was elucidated by the chemical shifts of C–H in the ^1H NMR spectra (Table 1). The obvious deviation of proton chemical shift was consistent with introduction and the position change of the *O*-acetyl group. An unambiguous confirmation of compound **6** was provided by X-ray crystal analysis. Figure 1 illustrates the structure, conformation, and atom numbering system of compound **6**.

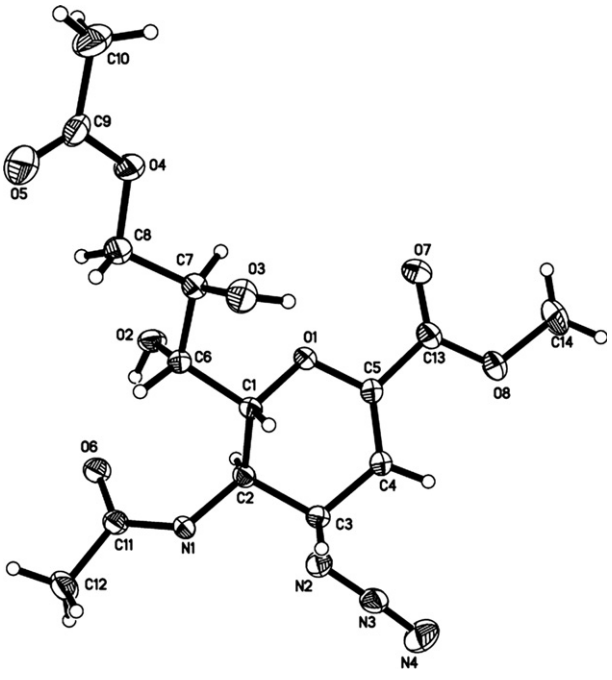


Figure 1. Crystal structure of **6** showing 50% probability displacement for ellipsoids.

1. Experimental

1.1. General methods

The reactions were performed with the use of commercial reagents and distilled solvents purified according to standard procedures. Optical rotations were recorded using a Perkin–Elmer 241 polarimeter. ^1H NMR spectra and ^{13}C NMR spectra were obtained in $CDCl_3$ or Me_2SO-d_6 on a Bruker AMX-400/600 equipment at 300 MHz or 400 MHz using TMS as an internal standard at room temperature. The chemical shifts (δ) are recorded in ppm relative to $CDCl_3$ ($\delta = 7.26$) or Me_2SO-d_6 ($\delta = 2.50$) for proton and $CDCl_3$ ($\delta = 77.0$) or Me_2SO-d_6 ($\delta = 39.5$) for carbon. Melting points were determined by using the capillary method on a Buchi-510 melting point apparatus and are uncorrected. The mass spectrum was

recorded on a Finnigan MAT-95/711spectrometer. High-resolution mass spectra (HRMS) were recorded on an FTMS spectrometer. Elemental analyses were performed on a Carlo Erba 1106 analyzer.

1.2. Preparation of methyl 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid (3)[13]

Methyl 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid **2** (724 g, 2.7 mmol) was dissolved in acetone (360 mL), and Dowex 50W × 8X (H^+) resin (10 g) was added to the resulting soln while stirring at room temperature to adjust pH to 4. The mixture was then stirred for 3 h. Then, the soln was filtered and evaporated to give an oil, then saturated aq sodium hydrogen carbonate (100 mL) and CH_2Cl_2 (200 mL) were added. After separation, the aq layer was extracted with CH_2Cl_2 (3 × 100 mL). All organic layers were combined, washed with brine (200 mL), dried over anhyd sodium sulfate, and the solvent was removed by distillation under diminished pressure to give a white solid, which was of sufficient quality for use in the next step. An analytically pure sample was obtained via silica gel column chromatography (1:2 hexane–EtOAc). Yield: 21.5 g (80%); $[\alpha]_D^{20}$ +158 (*c* 0.42, $CHCl_3$), lit.[13] +161; mp 95–97 °C; R_f 0.55 (20:1 CH_2Cl_2–MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 5.95 (d, 1H, *J* 2.5 Hz, H-3), 5.85 (d, 1H, *J* 8.0 Hz, NH), 5.35 (dd, 1H, *J* 2.0, 5.3 Hz, H-7), 4.67 (dd, 1H, *J* 2.5, 9.0 Hz, H-4), 4.64 (dd, 1H, *J* 1.9, 11.2 Hz, H-6), 4.38 (dd, 1H, *J* 6.1, 11.7 Hz, H-8), 4.14 (dd, 1H, *J* 6.2, 9.0 Hz, H-9), 3.96 (dd, 1H, *J* 6.1, 8.8 Hz, H-9), 3.81 (s, 3H, CO_2Me), 3.60 (dd, 1H, *J* 8.7, 18.6 Hz, H-5), 2.14 (s, 3H, OAc), 2.01 (s, 3H, NAc), 1.37 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.73, 20.85, 23.30, 48.67, 52.58, 57.56, 61.96, 67.73, 70.82, 75.69, 107.50, 145.15, 161.50, 170.42, 170.64; HRESIMS: calcd for $C_{15}H_{22}N_4O_7Na$ $[M+Na]^+$: 393.1386; found: *m*/*z* 393.1397.

1.3. General procedure for the preparation of methyl 5-acetamido-7-*O*-acyl-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid (4a–e)

A suspension of methyl 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid **3** (100 mg, 0.27 mmol) in pyridine (1.0 mL) was treated with DMAP (4 mg). The resulting mixture was cooled in an ice-water bath while propionic anhydride, butyric anhydride, methoxyacetic anhydride, benzoic anhydride, or acetic anhydride (0.54 mmol) was added dropwise, respectively. After stirring for 10–24 h at room temperature the mixture was concentrated to give an oil which was dissolved in EtOAc (6 mL) and washed sequentially with 1 M hydrochloric acid (2 × 3 mL), saturated aq sodium hydrogen carbonate (3 × 3 mL), and finally brine (3 mL). The organic layer was then dried over $MgSO_4$, concentrated, and the residues were purified by column chromatography or recrystallization.

1.3.1. Methyl 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-7-*O*-propionyl-D-*glycero*-D-*galacto*-non-2-enonic acid (4a)

The residue was chromatographed on silica gel with 1:1 EtOAc–petroleum ether to give **4a** (111 mg, 96%); white solid; $[\alpha]_D^{20}$ +48.1 (*c* 0.48, MeOH); mp 63–64 °C; R_f 0.28 (1:1 EtOAc–petroleum ether); ^1H NMR (400 MHz, $CDCl_3$): δ 5.95 (d, 1H, *J* 2.6 Hz, 3-H), 5.85 (d, 1H, *J* 7.7 Hz, NH), 5.37 (dd, 1H, *J* 1.9, 5.2 Hz, 7-H), 4.72 (dd, 1H, *J* 2.7, 9.1 Hz, 4-H), 4.66 (dd, 1H, *J* 1.8, 10.6 Hz, 6-H), 4.37 (dd, 1H, *J* 6.2, 11.6 Hz, 8-H), 4.14 (dd, 1H, *J* 6.1, 9.0 Hz, 9-Ha), 3.95 (dd, 1H, *J* 6.3, 9.0 Hz, 9-Hb), 3.80 (s, 3H, CO_2Me), 3.53 (dd, 1H, *J* 8.7, 18.5 Hz, 5-H), 2.43 (m, 2H, CH_2), 2.01 (s, 3H, NAc), 1.36 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.16 (t, 3H, *J* 7.6 Hz, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 8.94, 23.39, 25.21, 26.41, 27.43, 49.81, 52.46, 56.73, 65.52, 68.77, 74.72, 75.27, 107.48, 108.86, 145.01, 161.57, 170.82, 174.27; HRESIMS: calcd for $C_{18}H_{26}N_4O_8Na$ $[M+Na]^+$: 449.1648; found: *m*/*z* 449.1636. Anal. Calcd for $C_{18}H_{26}N_4O_8$: C, 50.70; H, 6.15; N, 13.14. Found: C, 50.71; H, 6.19; N, 12.88.

1.3.2. Methyl 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-7-*O*-*n*-butyryl-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid (4b)

The residue was chromatographed on silica gel with 3:7 EtOAc–petroleum ether to give compound **4b** (114 mg, 96%); white solid; $[\alpha]_D^{20}$ +41.6 (*c* 0.65, MeOH); mp 126–128 °C; R_f 0.41 (1:1 EtOAc–petroleum ether); δ 5.95 (d, 1H, *J* 7.9 Hz, NH), 5.94 (d, 1H, *J* 2.8 Hz, 3-H), 5.36 (dd, 1H, *J* 1.7, 5.1 Hz, 7-H), 4.71 (dd, 1H, *J* 2.7, 9.1 Hz, 4-H), 4.65 (dd, 1H, *J* 2.0, 10.4 Hz, 6-H), 4.37 (dd, 1H, *J* 6.1, 11.5 Hz, 8-H), 4.13 (dd, 1H, *J* 6.4, 9.0 Hz, 9-Ha), 3.93 (dd, 1H, *J* 6.2, 9.0 Hz, 9-Hb), 3.80 (s, 3H, CO_2Me), 3.52 (dd, 1H, *J* 8.7, 17.4, 18.7 Hz, 5-H), 2.37 (m, 2H, CH_2), 2.00 (s, 3H, NAc), 1.65 (m, 2H, CH_2), 1.35 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 0.95 (s, 3H, *J* 7.2 Hz, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 13.66, 18.25, 23.45, 25.25, 26.50, 35.99, 49.80, 52.53, 56.84, 65.61, 68.73, 74.75, 75.37, 107.55, 108.93, 145.07, 161.65, 170.93, 173.47; HRESIMS: calcd for $C_{19}H_{28}N_4O_8Na$ $[M+Na]^+$: 463.1805; found: *m*/*z* 463.1804. Anal. Calcd for $C_{19}H_{28}N_4O_8$: C, 51.81; H, 6.41; N, 12.72. Found: C, 51.92; H, 6.34; N, 12.57.

1.3.3. Methyl 5-acetamido-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-7-*O*-methoxylacetyl-D-*glycero*-D-*galacto*-non-2-enonic acid (4c)

The residue was chromatographed on silica gel with EtOAc (60%) in petroleum ether to give compound **4c** (116 mg, 97%); white solid; $[\alpha]_D^{20}$ +42.0 (*c* 0.23, MeOH); mp 80–81 °C; R_f 0.15 (1:1 EtOAc–petroleum ether); ^1H NMR (400 MHz, $CDCl_3$): δ 6.50 (d, 1H, *J* 8.5 Hz, NH), 5.92 (d, 1H, *J* 2.4 Hz, 3-H), 5.48 (dd, 1H, *J* 1.5, Hz, 7-H), 4.60 (dd, 1H, *J* 1.5, 10.5 Hz, 6-H), 4.56 (dd, 1H, *J* 2.6, 9.5 Hz, 4-H), 4.36 (dd, 1H, *J* 5.9, 10.7 Hz, 8-H), 4.19 (d, 1H, *J* 16.8 Hz, CH–H), 4.13 (dd, 1H, *J* 6.4, 9.1 Hz, 9-Ha), 4.02 (d, 1H, *J* 16.8 Hz, CH–H), 3.95 (dd, 1H, *J* 6.2, 9.1 Hz, 9-Hb), 3.78 (s, 3H, CO_2Me), 3.70 (dd, 1H, *J* 9.2, 19.0 Hz, 5-H), 3.45 (s, 3H, OCH_3), 1.99 (s, 3H, NAc), 1.32 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.30, 25.09, 26.42, 49.69, 52.48, 56.81, 59.40, 65.40, 69.36 (2C), 74.67, 75.23, 107.65, 108.85, 144.99, 161.49, 170.25, 170.99; HRESIMS: calcd for $C_{18}H_{26}N_4O_9Na$ $[M+Na]^+$: 465.1597; found: *m*/*z* 465.1579. Anal. Calcd for $C_{18}H_{26}N_4O_9$: C, 48.87; H, 5.92; N, 12.66. Found: C, 48.89; H, 5.95; N, 12.49.

1.3.4. Methyl 5-acetamido-2,6-anhydro-4-azido-7-*O*-benzoyl-3,4,5-trideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid (4d)

The residue was chromatographed on silica gel with 2:3 EtOAc–petroleum ether to give **4d** (126 mg, 98%); white solid; $[\alpha]_D^{20}$ +51.0 (*c* 0.24, MeOH); mp 166–167 °C; R_f 0.37 (1:1 EtOAc–petroleum ether); ^1H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, 2H, *J* 7.2 Hz, 2-H and 2′-H), 7.61 (t, 1H, *J* 7.5 Hz, 4′-H), 7.47 (d, 1H, *J* 7.4 Hz, 3-H and 3′-H), 6.04 (d, 1H, *J* 7.1 Hz, NH), 5.94 (d, 1H, *J* 2.7 Hz, 3-H), 5.62 (d, 1H, *J* 1.6, 4.7 Hz, 7-H), 5.08 (dd, 1H, *J* 2.7, 9.3 Hz, 4-H), 5.00 (dd, 1H, *J* 1.5, 10.3 Hz, 6-H), 4.51 (dd, 1H, *J* 6.0, 11.0 Hz, 8-H), 4.23 (dd, 1H, *J* 6.2, 9.1 Hz, 9-Ha), 4.16 (dd, 1H, *J* 6.1, 8.9 Hz, 9-Hb), 3.82 (s, 3H, CO_2Me), 3.20 (ddd, 1H, *J* 7.3, 9.6, 17.1 Hz, 5-H), 2.06 (s, 3H, NAc), 1.34 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.57, 25.28, 26.47, 51.78, 52.49, 55.80, 65.62, 69.93, 74.89, 75.29, 107.70, 109.04, 128.64 (2C), 129.03, 130.01 (2C), 133.74, 145.05, 161.66, 166.37, 171.34; HRESIMS: calcd for $C_{22}H_{26}N_4O_8Na$ $[M+Na]^+$: 497.1648; found: *m*/*z*

497.1649. Anal. Calcd for $C_{22}H_{26}N_4O_8$: C, 55.69; H, 5.52; N, 11.81. Found: C, 55.83; H, 5.63; N, 11.55.

1.3.5. Methyl 5-acetamido-7-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid (4e)

The residue was crystallized with EtOAc–petroleum ether to give **4e** (106 mg, 95%); white solid; $[\alpha]_D^{20}$ +60.8 (*c* 0.38, MeOH); mp 155–156 °C; R_f 0.55 (20:1 CH_2Cl_2–MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.95 (d, 1H, *J* 2.7 Hz, H-3), 5.77 (d, 1H, *J* 8.1 Hz, NH), 5.35 (dd, 1H, *J* 1.9, 5.4 Hz, H-7), 4.70 (dd, 1H, *J* 2.6, 9.1 Hz, H-4), 4.65 (dd, 1H, *J* 1.6, 10.4 Hz, H-6), 4.38 (dd, 1H, *J* 6.2, 10.6 Hz, H-8), 4.14 (dd, 1H, *J* 6.3, 9.0 Hz, H-9), 3.96 (dd, 1H, *J* 6.3, 9.0 Hz, H-9), 3.81 (s, 3H,CO_2Me), 3.57 (dd, 1H, *J* 8.7, 18.3 Hz, H-5), 2.14 (s, 3H, OAc), 2.01 (s, 3H, NAc), 1.37 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ 20.94, 23.36, 25.31, 26.47, 52.54, 52.71, 57.40, 65.62, 68.96, 74.81, 75.66, 107.62, 108.96, 145.18, 161.66, 170.85, 170.99; HRESIMS: calcd for $C_{17}H_{25}N_4O_8$: 413.1672 $[M+H]^+$; found: *m*/*z* 413.1651. Anal. Calcd for $C_{17}H_{24}N_4O_8$: C, 49.51; H, 5.87; N, 13.59. Found: C, 49.17; H, 6.07; N, 13.59.

1.4. Methyl 5-acetamido-7-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (5)

Methyl 5-acetamido-7-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonic acid **4e** (13 g, 37 mmol) was dissolved in water (390 mL), and then Dowex 50W × 8X (H^+) resin (5 g) was added to adjust pH to 4. After reaction for 3 h, the resulting soln was filtered and evaporated to give a foam, then EtOAc was added to crystallize the title compound **5** (8.9 g, 76%); white solid; $[\alpha]_D^{20}$ +55.9 (*c* 0.49, MeOH); mp 174–175 °C; R_f 0.18 (EtOAc); ^{1}H NMR (400 MHz, Me_2SO-d_6): δ 8.10 (d, 1H, *J* 9.8 Hz, NH), 5.85 (d, 1H, *J* 2.5 Hz, 3-H), 5.74 (s, 1H, OH), 4.86 (dd, 1H, *J* 1.9, 9.5 Hz, 6-H), 4.37 (dd, 1H, *J* 1.9, 10.3 Hz, 7-H), 4.30 (dd, 1H, *J* 2.6, 9.3 Hz, 4-H), 4.02 (ddd, 1H, *J* 8.6, 19.2 Hz, 5-H), 3.79 (ddd, 1H, *J* 3.7, 7.3, 10.6 Hz, 8-H), 3.74 (s, 3H, CO_2Me), 3.33 (dd, 1H, *J* 3.5, 11.5 Hz, 9-H), 3.20 (dd, 1H, *J* 7.3, 11.2 Hz, 9-H), 1.94 (s, 3H, OAc), 1.76 (s, 3H, NAc); ^{13}C NMR (400 MHz, Me_2SO-d_6): δ 20.84, 22.67, 46.26, 52.42, 59.05, 63.24, 68.25, 69.45, 75.97, 108.11, 145.14, 161.51, 169.25, 169.67; HRESIMS: calcd for $C_{14}H_{21}N_4O_8$: 373.1359 $[M+H]^+$; found: *m*/*z* 373.1356. Anal. Calcd for $C_{14}H_{20}N_4O_8$ 0.1 MeCOOEt: C, 45.38; H, 5.50; N, 14.70; Found: C, 45.67; H, 5.49; N, 14.47.

1.5. Methyl 5-acetamido-9-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (6)

A stirred soln of methyl 5-acetamido-7-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid **5** (300 mg, 0.81 mmol) in MeOH (50 mL) was heated at reflux for 48 h. The solvent was evaporated under diminished pressure, and the resulting mixture was chromatographed (silica gel, 50:1 CH_2Cl_2–MeOH) to give **6** as a white solid. Single crystals suitable for X-ray analysis were obtained by slow evaporation of an EtOAc–MeOH soln at 298 K. Yield: 240 mg (80%); $[\alpha]_D^{20}$ +74.2 (*c* 0.99, MeOH); mp 194–195 °C; R_f 0.36 (EtOAc); ^{1}H NMR (300 MHz, Me_2SO-d_6): δ 8.10 (d, 1H, *J* 9.8 Hz, NH), 5.85 (d, 1H, *J* 2.5 Hz, 3-H), 5.74 (s, 1H, OH), 4.86 (dd, 1H, *J* 1.9, 9.5 Hz, 6-H), 4.37 (dd, 1H, *J* 1.9, 10.3 Hz, 7-H), 4.30 (dd, 1H, *J* 2.6, 9.3 Hz, 4-H), 4.02 (ddd, 1H, *J* 8.6, 19.2 Hz, 5-H), 3.79 (ddd, 1H, *J* 3.7, 7.3, 10.6 Hz, 8-H), 3.74 (s, 3H, CO_2Me), 3.33 (dd, 1H, *J* 3.5, 11.5Hz, 9-H), 3.20 (dd, 1H, *J* 7.3, 11.2 Hz, 9-H), 1.94 (s, 3H, OAc), 1.76 (s, 3H, NAc); ^{13}C NMR (400 MHz, Me_2SO-d_6): δ 20.83, 22.68, 47.56, 52.34, 58.09, 66.76, 66.86, 68.21, 76.40, 107.27, 145.23, 161.65, 170.47, 171.92; HRESIMS: calcd for $C_{14}H_{21}N_4O_8$ $[M+Na]^+$: 373.1359; found: *m*/*z* 373.1359. Anal. Calcd for $C_{14}H_{20}N_4O_8$: C, 45.16; H, 5.41; N, 15.05. Found: C, 45.24; H, 5.34; N, 15.09.

1.6. X-ray crystallographic data

Crystal data were collected using a Bruker SMART APEX CCD area detector diffractometer. A full sphere of the reciprocal space was scanned by phi–omega scans. Semi-empirical absorption correction based on redundant reflections was performed by the program SADABS.[16] The structures were solved by direct methods using SHELXS-97[17] and refined by full matrix least squares on F^2 for all data using SHELXL-97.[18] The hydrogen atom treatment varied depending on the crystal quality. All H atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms, with C–H = 0.93–0.96 Å, N–H = 0.86 Å, and U_{iso} = $1.2U_{eq}$(C) or (N). The crystal structure of **6** was refined to R_1 = 0.0413 (4579 reflections, all unique) and R_1 = 0.0364 (3007 reflections with $F_0 > 2\sigma(F_0)$) by the full-matrix least-squares method using the SHELXL-97[19] program based on 241 parameters.

Supplementary data

Complete crystallographic data for the structural analysis have been deposited (Deposition No. CCDC 676214) with the Cambridge Crystallographic Data Center. These data may be obtained, on request, from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (tel.: +44-1223-336408; fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or *via* www: http://www.ccdc.cam.ac.uk).

Acknowledgment

This work was supported by National Great Project of Scientific and Technical Supporting Programs Funded by Ministry of Science & Technology of China (No. 2006BAI01B02).

References

1. DeNinno, M. P. *Synthesis* **1991**, 583–593.
2. Hemeon, I.; Bennet, A. J. *Synthesis* **2007**, 1899–1926.
3. von Itzstein, M.; Keifel, M. J. *Chem. Rev.* **2002**, *102*, 471–490.
4. Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Starkey, I. D.; Cobley, K. N.; Weston, H.; Scicinski, J.; Merritt, A.; Whittington, A.; Wyatt, P.; Taylor, N.; Green, D.; Bethell, R.; Madar, S.; Fenton, R. J.; Morley, P. J.; Pateman, T.; Beresford, A. *J. Med. Chem.* **1998**, *41*, 787–797.
5. Taylor, N. R.; Cleasby, A.; Singh, O.; Skarzynski, T.; Wonacott, A. J.; Smith, P. W.; Sollis, S. L.; Howes, P. D.; Cherry, P. C.; Bethell, R.; Colman, P.; Varghese, J. *J. Med. Chem.* **1998**, *41*, 798–807.
6. Andrews, D. M.; Cherry, P. C.; Humber, D. C.; Jones, P. S.; Keeling, S. P.; Martin, P. F.; Shaw, C. D.; Swanson, S. *Eur. J. Med. Chem.* **1999**, *34*, 563–574.
7. Masuda, T.; Shibuya, S.; Arai, M.; Yoshida, S.; Tomozawa, T.; Ohno, A.; Yamashita, M.; Honda, T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 669–673.
8. Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1925–1928.
9. Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921–1924.
10. Smith, P. W.; Starkey, I. D.; Howes, P. D.; Sollis, S. L.; Keeling, S. P.; Cherry, P. C.; von Itzstein, M.; Wu, W.-Y.; Jin, B. *Eur. J. Med. Chem.* **1996**, *31*, 143–150.
11. Jin, W.-X.; Zhao, Q.-J.; Lai, Q.-L.; Wei, Y.-B. *Chinese J. Pharm.* **2007**, *38*, 321–324.
12. Chandler, M.; Bamford, M. J.; Conroy, R.; Lamont, B.; Patel, B.; Patel, V. K.; Steeples, I. P.; Storer, R.; Weir, N. G.; Wright, M.; Williamson, C. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1173–1180.
13. Honda, T.; Kobayashi, Y.; Masuda, T.; Yomashida, M.; Arai, M. U.S. Patent 6,340,702, 2002; *Chem. Abstr.*, **2003**, *136*, 118703.
14. Zhdanov, R. I.; Zhenodarova, S. M. *Synthesis* **1975**, 222–245.
15. Doerschuk, A. P. *J. Am. Chem. Soc.* **1952**, *74*, 4202–4203.
16. Sheldrick, G. M. SADABS; Bruker AXS: Madison, WI 53711, 2000.
17. Sheldrick, G. M. SHELXS-97: Program for Crystal Structure Solution; Göttingen 1997.
18. Sheldrick, G. M. SHELXS-97: Program for Crystal Structure Refinement, Release 2; Göttingen 1997.
19. Sheldrick, G. M. SHELXL-97. Program for Crystal Structure Refinement; University of Göttingen: Göttingen, Germany, 1997.