Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 7893–7897
Bioorganic & Medicinal Chemistry

2-Deoxy-2,3-didehydro-*N*-acetylneuraminic acid analogues structurally modified at the C-4 position: Synthesis and biological evaluation as inhibitors of human parainfluenza virus type 1

Kiyoshi Ikeda,[a,*] Kazuki Sato,[a] Satoru Kitani,[a] Takashi Suzuki,[b,c] Naoyoshi Maki,[b] Yasuo Suzuki[b,c] and Masayuki Sato[a]

[a]*Department of Organic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan*
[b]*Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, and COE Program in the 21st Century, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan*
[c]*CREST, Japan Science and Technology Agency, Saitama, Japan*

Received 21 June 2006; revised 25 July 2006; accepted 26 July 2006

Abstract—To explore the influence of binding to human parainfluenza virus type 1 (hPIV-1), a series of 4-*O*-substituted Neu5Ac2en derivatives **6a**–**e** was synthesized and tested for their ability to inhibit hPIV-1 sialidase. Among compounds **6a**–**e**, the 4-*O*-ethyl-Neu5Ac2en derivative **6b** showed the most potent inhibitory activity (IC_{50} 6.3 μM) against hPIV-1 sialidase.

1. Introduction

N-Acetylneuraminic acid (Neu5Ac) and various related derivatives, sialic acids, play an important role in various biochemical and biological processes.[1] Influenza sialidase, a key enzyme responsible for propagation of the influenza virus, is a target of drug design. Various 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu5Ac2en, **1**) analogues have been synthesized as competitive sialidase inhibitors.[2] Among them, 2,3-didehydro-2,4-dideoxy-4-guanidinyl-*N*-acetylneuraminic acid (zanamivir) (**2**) and its analogues showed the most potent inhibitory activity against sialidase.[3] Human parainfluenza virus type 1 (hPIV-1) is an important pathogen causing upper and lower respiratory disease in infants and young children,[4] however, there are no known potential inhibitors of hPIV-1 infection.

Neu5Ac2en derivatives with structural modifications at C-4 position are particular candidates for the design of potent inhibitors against various sialidases.[5] We recently found that 4-*O*-thiocarbamoylmethyl-Neu5Ac2en (**3**)[6] has strong inhibitory activity toward hPIV-1 sialidase compared with **1** (Fig. 1).

As part of a program aimed at new sialidase inhibitors against hPIV-1, we report here the synthesis of 4-*O*-substituted Neu5Ac2en derivatives **6a**–**e** and their inhibition of hPIV-1.

2. Results and discussion

2.1. Chemical synthesis

For the synthesis of 4-*O*-substituted analogues of Neu5Ac2en **6a**–**e**, methyl 5-acetamido-8,9-*O*-isopropylidene-2,3,5-tri-deoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate **4**[7] was chosen as the starting material. Selective 4-*O* alkylation of **4** with 1.5 molar equivalent of methyl iodide in the presence of 5.0 molar equivalent of silver oxide and 0.5 molar equivalent of tetra-*n*-butylammonium iodide (TBAI) in dimethylformamide (DMF) at room temperature gave one major product, 4-*O*-methyl derivative **5a**, in 56% yield (Table 1, entry 1). The structural assignment of **5a** was based on its ^{1}H NMR spectrum which showed the presence of one additional methoxy group (δ 3.40) and on the well-

Keywords: Sialic acid; 4-*O*-alkylated Neu5Ac derivative; Human parainfluenza virus type 1 sialidase inhibition.
* Corresponding author. Tel./fax: +81 54 264 5108; e-mail: ikeda@ys2.u-shizuoka-ken.ac.jp

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.07.045

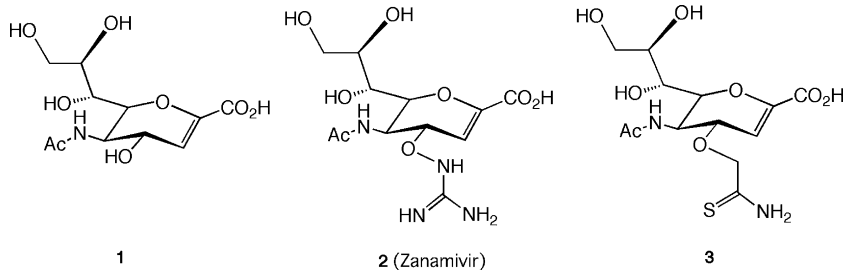
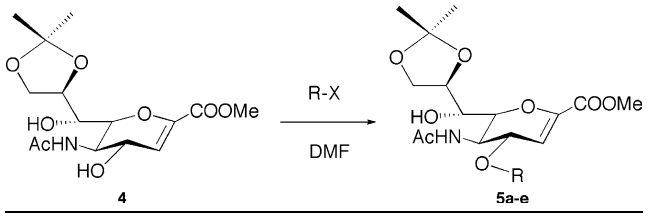


Figure 1.

Table 1. Subsititution of 4-hydroxy group of **4**

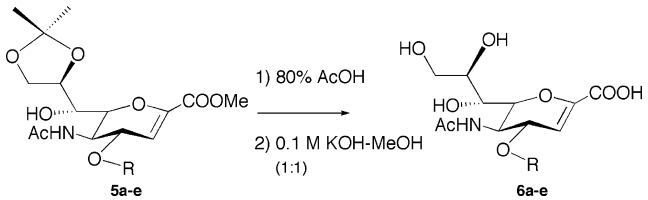


Entry	R-X	Condition	Product	Yield (%)
1	MeI	Ag_2O, TBAI	**5a**	56
2	EtBr	Ag_2O, TBAI	**5b**	45
3	*n*-PrBr	Ag_2O, TBAI	**5c**	41
4	Allyl bromide	Ag_2O, TBAI	**5d**	69
5	Propargyl bromide	Ag_2O, TBAI	**5e**	N.R.[a]
6	Allyl bromide	NaH	**5d**	52
7	Propargyl bromide	NaH	**5e**	56

[a] N.R., No reaction.

known reactivity of OH-4 in compound **4**.[8] In a similar manner, 4-*O* alkylations of **4** with ethyl bromide, *n*-propyl bromide, and allyl bromide in the presence of silver oxide gave **5b**, **5c**, and **5d** in 45%, 41%, and 69% yields, respectively (entries 2–4); however, the reaction of **4** with propargyl bromide in the presence of silver oxide was unsuccessful (entry 5). The reactions of **4** with 2.0 molar equivalent of allyl bromide and propargyl bromide using 1.3 molar equivalent of sodium hydride as a base in DMF at 0 °C successfully gave **5d** and **5e** in 52% and 56% yields, respectively (entries 6 and 7). Compounds **5a**–**e** showed a one-proton doublet in their ^{1}H NMR spectrum at δ 6.02–6.07 ($J_{3,4}$ 2.3–3.0 Hz, H-3), characteristic of the 2,3 double bond.

Table 2. Deprotection of **5a**–**e** and inhibitory activities of **6a**–**e**



Entry	R	Product	Yield (%)	IC_{50} (μM)[a]
1	–Me	**6a**	quant.	274
2	–Et	**6b**	59	6.3
3	–*n*-Pr	**6c**	85	26
4	$-CH_2CH{=}CH_2$	**6d**	81	83
5	$-CH_2C{\equiv}CH$	**6e**	97	29

[a] Inhibitory activities were determined by the method according to Ref. 6b.

Hydrolysis of **5a**–**e** with 80% aqueous acetic acid for 1 h at 80 °C, followed by treatment with 0.1 M KOH–MeOH (1:1) for 12 h at room temperature afforded, **6a**–**d**[9] and **6e** in quant., 59%, 85%, 81%, and 97% yields in two steps, after purification by chromatography on silica gel and then desalting, followed by lyophilization from a H_2O suspension (Table 2).

2.2. Biological evaluation

The behavior of compounds **6a**–**e** toward hPIV-1 sialidase was tested by our previously reported method.[6b] As may be seen in Table 2, interestingly, 4-*O*-ethyl-Neu5Ac2en **6b** showed the most potent inhibitory activity (IC_{50} 6.3 μM), although the degree of inhibition was the same as that of **3** (IC_{50} 7.5 μM). Compounds **6c** and **6e** showed almost the same inhibitory effects as **1** (IC_{50} 30 μM). In the previous study,[6b] 4-guanidino-Neu5Ac2en **2** had much lower inhibitory activity toward hPIV-1 sialidase than that of **1**. Compound **6d** exhibited decreased sialidase inhibition compared with **6b**, **6c**, **6e**, and compound **6a** was practically inactive against sialidase.

In conclusion, 4-*O*-alkylated Neu5Ac2en derivatives **6a**–**e** having methyl, ethyl, *n*-propyl, allyl, and propargyl groups at the C-4 position were synthesized via the key compound **4**. These compounds had inhibitory activities against hPIV-1. The reason for the higher inhibitory activity of compound **6b** is unclear in this study, but it is possible that hPIV-1 has a microsphere that interacts with the C-4 position of Neu5Ac2en in the cavity of the catalytic pocket in HN glycoprotein. These findings should provide useful information for the development of anti-human parainfluenza virus compounds.

3. Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO P-1030 (Japan) digital polarimeter. IR spectra were recorded on a SHIMADZU IRPrestige-21 (Japan) spectrometer. ^{1}H NMR spectra were recorded with a JEOL ECA-500 (500 MHz) (Japan) instrument. ^{13}C NMR spectra were recorded with a JEOL ECA-500 (126 MHz) (Japan)

instrument. Chemical shifts are expressed in ppm relative to Me_4Si ($\delta = 0$) in $CDCl_3$ and in D_2O referenced to HOD (4.85 ppm) as internal standards. Fast-atom-bombardment (FAB) mass spectra were obtained with a JEOL JMS-700 (Japan) mass spectrometer in the positive-ion mode using an NBA and thioglycerol matrix. High-resolution mass spectra (HR-MS) were recorded on a JEOL JMS-700 (Japan) instrument under Fab conditions. Column chromatography was performed on Silica Gel 60 (70–230 mesh, Merck). Desalting was carried out with an ASAHI CHEMICAL Micro Acylizer G1. All reactions were monitored using TLC (Silica Gel $60F_{254}$, E. Merck, Germany) by charring after spraying 5% H_2SO_4 in MeOH and then heating.

3.1. Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-methyl-D-*glycero*-D-*galacto*-non-2-enonate (5a)

Under argon compound **4** (90 mg, 0.26 mmol) was dissolved in anhydrous DMF (5 mL) and stirred for 1 h with freshly activated MS 4 Å (0.30 g). To the mixture were added methyl iodide (55 mg, 0.39 mmol), Ag_2O (302 mg, 1.3 mmol), and TBAI (48 mg, 0.13 mmol), and the mixture was stirred for 2 days in the dark under Ar. Insoluble materials were filtered off through Celite 545 and the filtrate was concentrated to dryness. The residue was purified by silica gel chromatography using AcOEt/*n*-hexane (2:1) to give the 4-*O*-methyl compound **5a** (52 mg, 56%) as an amorphous powder. $[\alpha]_D^{22}$ 68 (*c* 1.7, $CHCl_3$). IR (neat, cm^{-1}): 1732, 1630. ^1H NMR (500 MHz, $CDCl_3$) δ 1.36, 1.40 (s, each 3H, Me_2C), 2.01 (s, 3H, NHAc), 3.40 (s, 3H, OMe), 3.80 (s, 3H, OMe), 4.08 (dd, 1H, $J_{8,9a} = 4.5$, $J_{9a,9b} = 11.5$ Hz, H-9a), 4.12 (m, 2H, H-6 and H-7), 4.26 (m, 2H, H-4 and H-5), 5.65 (d, $J_{5,NH} = 8.0$ Hz, NH), 6.07 (d, 1H, $J_{3,4} = 3.0$ Hz, H-3). FABHRMS *m/z* Calcd for $C_{16}H_{26}NO_8$ $(M+H)^+$ 360.1658. Found: 360.1648.

3.2. Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-ethyl-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (5b)

The reaction was carried out using compound **4** (102 mg, 0.30 mmol) and ethyl bromide (164 mg, 1.5 mmol) in a manner similar to the preparation of **5a** to give **5b** (50 mg, 45%) as an amorphous powder, $[\alpha]_D^{22}$ 34 (*c* 0.11, $CHCl_3$). IR (neat, cm^{-1}): 3298, 1734, 1637. ^1H NMR (500 MHz, $CDCl_3$) δ 1.19 (t, 3H, $J = 6.8$ Hz, OCH_2CH_3), 1.34, 1.38 (s, each 3H, Me_2C), 2.05 (s, 3H, NHAc), 3.53–3.59 (m, 2H, H-7 and OCH_2CH_3), 3.65 (m, 1H, OCH_2CH_3), 3.78 (s, 3H, OMe), 4.06 (dd, 1H, $J_{8,9} = 5.2$, $J_{gem} = 12.0$ Hz, H-9a), 4.12–4.15 (m, 3H, H-4, H-6, and H-9b), 4.21 (dd, 1H, $J_{4,5} = 14.9$, $J_{5,NH} = 7.5$ Hz, H-5), 4.31 (ddd, 1H, $J_{7,8} = 11.5$, $J_{8,9b} = 8.0$ Hz, H-8), 4.59 (d, 1H, $J_{7,OH} = 2.9$ Hz, OH), 5.86 (d, 1H, NH), 6.03 (d, 1H, $J_{3,4} = 2.9$ Hz, H-3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 15.5, 23.2, 25.3, 27.0, 48.1, 52.5, 63.5, 67.3, 71.4, 72.3, 74.4, 77.0, 107.5, 109.2, 145.8, 162.4, 171.8. FABHRMS *m/z* Calcd for $C_{17}H_{28}NO_8$ $(M+H)^+$ 374.1815. Found: 374.1790.

3.3. Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-*n*-propyl-D-*glycero*-D-*galacto*-non-2-enonate (5c)

The reaction was carried out using compound **4** (111 mg, 0.32 mmol) and *n*-propyl bromide (198 mg, 1.6 mmol) in a manner similar to the preparation of **5a** to give **5c** (51 mg, 41%) as an amorphous powder, $[\alpha]_D^{23}$ 27 (*c* 0.16, $CHCl_3$). IR (neat, cm^{-1}): 3257, 1720, 1650. ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (t, 3H, $J = 7.5$ Hz, OCH_2CH_3), 1.35, 1.40 (s, each 3H, Me_2C), 1.55–1.62 (m, 2H, CH_2CH_3), 2.06 (s, 3H, NHAc), 3.47 (m, 1H, OCH_2CH_3), 3.54–3.79 (m, 2H, H-7 and OCH_2CH_3), 3.79 (s, 3H, OMe), 4.07 (dd, 1H, $J_{8,9a} = 5.2$, $J_{gem} = 12.0$ Hz, H-9a), 4.11–4.16 (m, 3H, H-4, H-6 and H-9b), 4.24 (dd, 1H, $J_{4,5} = 14.9$, $J_{5,NH} = 7.5$ Hz, H-5), 4.33 (ddd, 1H, $J_{7,8} = 11.5$, $J_{8,9b} = 8.0$ Hz, H-8), 4.57 (d, 1H, $J_{7,OH} = 3.5$ Hz, OH), 5.61 (d, 1H, NH), 6.04 (d, 1H, $J_{3,4} = 2.9$ Hz, H-3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 10.4, 23.0, 23.1, 25.2, 26.9, 47.9, 52.4, 67.2, 69.7, 71.4, 72.4, 74.3, 76.9, 107.3, 109.1, 145.7, 162.3, 171.7. FABHRMS *m/z* Calcd for $C_{18}H_{30}NO_8$ $(M+H)^+$ 388.1971. Found: 388.1974.

3.4. Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-(2-propenyl)-D-*glycero*-D-*galacto*-non-2-enonate (5d)

The reaction was carried out using compound **4** (166 mg, 0.48 mmol) and allyl bromide (174 mg, 5.5 mmol) in a manner similar to the preparation of **5a** to give **5d** (128 mg, 69%) as an amorphous powder, $[\alpha]_D^{24}$ 32 (*c* 1.0, $CHCl_3$). IR (KBr, cm^{-1}): 1732, 1662. ^1H NMR (500 MHz, $CDCl_3$) δ 1.36, 1.40 (s, each 3H, Me_2C), 2.02 (s, 3H, NHAc), 3.58 (dd, 1H, $J_{6,7} = 0.7$, $J_{7,8} = 8.0$ Hz, H-7), 3.80 (s, 3H, OMe), 4.09, 4.94 (m, 2H, H-6 and H-9a), 4.14–4.17 (m, 2H, H-8 and H-9a), 4.20–4.23 (m, 2H, H-5 and H-9b), 4.34 (dd, 2H, $J_{1a,2} = 2.3$, $J_{1b,2} = 5.0$, $J_{gem} = 13.5$ Hz, $CH_2{=}CHCH_2{-}$), 5.24 (dd, 1H, $J_{2,3a} = 10$, $J_{3a,3b} = 16.5$ Hz, $CH_2{=}CH CH_2{-}$), 5.32 (dd, 1H, $J_{2,3b} = 2.0$ Hz, $CH_2{=}CHCH_2{-}$), 5.64 (d, 1H, $J_{5,NH} = 6.5$ Hz, NH), 5.89 (m, 1H, H-2), 6.05 (d, 1H, $J_{3,4} = 2.5$ Hz, H-3). FABHRMS *m/z* Calcd for $C_{18}H_{28}NO_8$ $(M+H)^+$ 386.1815. Found: 386.1837.

For the preparation of **5d** using allyl bromide and sodium hydride, sodium hydride (22 mg, 0.92 mmol) at 0 °C under Ar was added to a solution of **4** (245 mg, 0.71 mmol) and propargyl bromide (172 mg, 1.42 mmol) in DMF (5 mL) was added, and the mixture was stirred for 1 h. After the addition of MeOH (1 mL), the solvent was concentrated to dryness. The residue was chromatographed on silica gel using 50:1 $CHCl_3$/MeOH to give **5d** (142 mg, 52%).

3.5. Methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-(2-propynyl)-D-*glycero*-D-*galacto*-non-2-enonate (5e)

The reaction was carried out using compound **4** (270 mg, 0.78 mmol) in a manner similar to the preparation of **5d** using sodium hydride as a base to give **5e** as an amorphous powder, $[\alpha]_D^{24}$ 48 (*c* 0.90, $CHCl_3$). IR

(KBr, cm^{-1}): 3275, 1724, 1635. ^1H NMR (500 MHz, $CDCl_3$) δ 1.36, 1.40 (s, each 3H, Me_2C), 2.09 (s, 3H, NHAc), 2.53 (t, 1H, $J = 2.3$ Hz, C≡CH), 3.55 (dd, 1H, $J_{7,OH} = 2.9$, $J_{7,8} = 8.0$ Hz, H-7), 3.79 (s, 3H, OMe), 4.05–4.11 (m, 2H, H-6 and H-9a), 4.13–4.18 (m, 2H, H-5 and H-9b), 4.23, 4.33 (dd, each 1H, $J = 2.3$, $J_{gem} = 16.7$ Hz, OCH_2C≡), 4.36 (m, 1H, H-8), 4.42 (dd, 1H, $J_{3,4} = 2.3$, $J_{4,5} = 8.0$ Hz, H-4), 4.61 (br, 1H, OH), 5.64 (d, 1H, $J_{5,NH} = 7.5$ Hz, NH), 6.02 (d, 1H, H-3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 23.3, 25.3, 27.1, 48.6, 52.5, 56.0, 67.3, 70.3, 72.6, 74.2, 75.5, 77.5, 79.7, 106.9, 109.2, 146.0, 162.3, 172.7. FABHRMS *m/z* Calcd for $C_{18}H_{26}NO_8$ $(M+H)^+$ 384.1658. Found: 384.1663.

3.6. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-methyl-D-*glycero*-D-*galacto*-non-2-enonic acid (6a)

Compound **5a** (17 mg, 0.047 mmol) was dissolved in 80% aqueous AcOH (2 mL). After stirring for 1 h at 80 °C, the reaction solution was concentrated to dryness, the residue was dissolved in a solution of 0.1 M KOH in MeOH (1:1) (2 mL) at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction solution was adjusted to pH 2 with Amberlite 120 (H^+), the solution was filtered to remove the resin, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography using $CHCl_3$/MeOH/H_2O (65:35:5) to give **6a** (14 mg, quant.) after desalting with an AC Micro Acylizer G1 and lyophilization from a H_2O suspension. IR (neat, cm^{-1}): 3263, 1718, 1637. ^1H NMR (500 MHz, D_2O) δ 1.93 (s, 3H, NHAc), 3.29 (s, 3H, OMe), 3.47–3.53 (m, 2H, H-7 and H-9a), 3.76 (dd, 1H, $J_{8,9b} = 1.7$, $J_{gem} = 12.0$ Hz, H-9b), 3.81 (m, 1H, H-8), 3.98 (dd, 1H, $J_{4,5} = 6.3$, $J_{5,6} = 12.0$ Hz, H-5), 4.18 (d, 1H, H-6), 4.28 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-4), 5.71 (d, 1H, H-3). FABHRMS *m/z* Calcd for $C_{12}H_{19}NO_8Na$ $(M+Na)^+$ 328.1008. Found: 328.1079.

3.7. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-ethyl-D-*glycero*-D-*galacto*-non-2-enonic acid (6b)

The reaction was carried out using compound **5b** (50 mg, 0.13 mmol) in a manner similar to the preparation of **6a** to give **6b** (25 mg, 59%) as an amorphous powder, $[\alpha]_D^{25}$ 90 (*c* 0.12, CH_3OH). IR (neat, cm^{-1}): 3392, 1735, 1624. ^1H NMR (500 MHz, D_2O) δ 1.19 (t, 3H, $J = 6.8$ Hz, OCH_2CH_3), 1.96 (s, 3H, NHAc), 3.43 (m, 1H, OCH_2CH_2), 3.53–3.61 (m, 3H, H-7, H-9a, and OCH_2CH_2), 3.79 (dd, 1H, $J_{8,9b} = 2.3$, $J_{gem} = 12.0$ Hz, H-9a), 3.84 (ddd, 1H, $J_{7,8} = 9.2$, $J_{8,9b} = 5.8$ Hz, H-8), 4.11 (dd, 1H, $J_{4,5} = 8.6$, $J_{5,6} = 10.9$ Hz, H-5), 4.17 (d, 1H, H-6), 4.28 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-4), 5.85 (d, 1H, H-3). FABHRMS *m/z* Calcd for $C_{13}H_{21}NO_8Na$ $(M+Na)^+$ 342.1165. Found: 342.1163.

3.8. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-*n*-propyl-D-*glycero*-D-*galacto*-non-2-enonic acid (6c)

The reaction was carried out using compound **5c** (51 mg, 0.13 mmol) in a manner similar to the preparation of **6a** to give **6c** (37 mg, 85%) as an amorphous powder, $[\alpha]_D^{25}$ 48 (*c* 0.25, CH_3OH). IR (neat, cm^{-1}): 3313, 1717, 1630. ^1H NMR (500 MHz, D_2O) δ 0.80 (t, 1H, $J = 7.5$ Hz, CH_2CH_3), 1.43–1.50 (m, 2H, CH_2CH_3), 1.96 (s, 3H, NHAc), 3.43 (m, 1H, OCH_2CH_2), 3.53–3.61 (m, 3H, H-7, H-9a, and OCH_2CH_2), 3.79 (dd, 1H, $J_{8,9b} = 2.3$, $J_{gem} = 12.0$ Hz, H-9a), 3.84 (ddd, 1H, $J_{7,8} = 9.2$, $J_{8,9b} = 5.8$ Hz, H-8), 4.11 (dd, 1H, $J_{4,5} = 8.6$, $J_{5,6} = 10.9$ Hz, H-5), 4.17 (d, 1H, H-6), 4.28 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-4), 5.85 (d, 1H, H-3). FABHRMS *m/z* Calcd for $C_{14}H_{24}NO_8$ $(M+H)^+$ 334.1502. Found: 334.1501.

3.9. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-(2-propenyl)-D-*glycero*-D-*galacto*-non-2-enonic acid (6d)

The reaction was carried out using compound **5d** (50 mg, 0.13 mmol) in a manner similar to the preparation of **6a** to give **6d** (35 mg, 81%) as an amorphous powder. IR (neat, cm^{-1}): 3298, 1734, 1637. ^1H NMR (500 MHz, D_2O) δ 1.92 (s, 3H, NHAc), 3.50–3.53 (m, 2H, H-7 and H-9a), 3.69 (s, 3H, OMe), 3.74 (dd, 1H, $J_{8,9} = 1.7$, $J_{gem} = 12.0$ Hz, H-9b), 3.79 (m, 1H, H-8), 3.96 (dd, 1H, $J_{4,5} = 6.3$, $J_{5,6} = 12.0$ Hz, H-5), 4.06–4.10 (m, 2H, H-6 and C=CCH_2–), 4.17 (d, 1H, C=CCH_2–), 4.30 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-4), 5.14 (d, 1H, $J = 117.2$ Hz, =CH_2), 5.19 (d, 1H, $J = 10.3$ Hz, =CH_2), 5.78 (m, 1H, CH=CH_2), 6.05 (d, 1H, H-3). FABHRMS *m/z* Calcd for $C_{14}H_{21}NO_8Na$ $(M+Na)^+$ 354.1165. Found: 354.1172.

3.10. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-(2-propynyl)-D-*glycero*-D-*galacto*-non-2-enonic acid (6e)

The reaction was carried out using compound **5e** (41 mg, 0.107 mmol) in a manner similar to the preparation of **6a** to give **6e** (34 mg, 97%) as an amorphous powder, $[\alpha]_D^{25}$ 31 (*c* 0.12, CH_3OH). IR (neat, cm^{-1}): 3257, 1734, 1637. ^1H NMR (500 MHz, D_2O) δ 1.97 (s, 3H, NHAc), 2.83 (t, 1H, $J = 2.3$ Hz, C≡CH), 3.53–3.57 (m, 2H, H-7 and H-9a), 3.78 (dd, 1H, $J_{8,9} = 2.3$, $J_{gem} = 12.0$ Hz, H-9a), 3.84 (ddd, 1H, $J_{7,8} = 9.2$, $J_{8,9b} = 6.3$ Hz, H-8), 4.09 (dd, 1H, $J_{4,5} = 8.6$, $J_{5,6} = 10.9$ Hz, H-5), 4.21 (d, 1H, H-6), 4.24 (dd, each 1H, $J_{gem} = 16.7$ Hz, OCH_2C), 4.47 (dd, 1H, $J_{3,4} = 2.3$ Hz, H-4), 5.89 (d, 1H, H-3). FABHRMS *m/z* Calcd for $C_{14}H_{20}NO_8$ $(M+H)^+$ 330.1189. Found: 330.1261.

Acknowledgments

The authors thank Marukin Chuyu Co., Ltd. (Kyoto, Japan) for the generous gift of Neu5Ac. This work was partly supported by the Japan Health Science Foundation.

References and notes

1. Schauer, R.; Kelm, S.; Reuter, G.; Roggentin, P.; Shaw, L. Biochemistry and Role of Sialic Acids. In *Biology of the Sialic Acids*; Rosenberg, A., Ed.; Plenum Press: New York, 1995.
2. (a) Ikeda, K.; Sato, M.; Torisawa, Y. *Curr. Med. Chem.* **2004**, *3*, 339; (b) Wilson, J. C.; Thomson, R. J.; Dyason, J.

C.; Florio, P.; Quelch, K. J.; Abo, S.; von Itzstein, M. *Tetrahedron: Asymmetry* **2000**, *11*, 53; (c) Smith, P. W.; Starkey, I. D.; Howes, P. D.; Sollis, I. L.; Keeling, S. P.; Cherry, P. C.; von Itzstein, M.; Wu, W. Y.; Jin, B. *Eur. J. Med. Chem.* **1996**, *31*, 143.

3. (a) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418; (b) Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kobayashi, Y.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1921; (c) Honda, T.; Masuda, T.; Yoshida, S.; Arai, M.; Kaneko, S.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1925; (d) Honda, T.; Yoshida, S.; Arai, M.; Masuda, T.; Yamashita, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1929; (e) Masuda, T.; Shibuya, S.; Arai, M.; Yoshida, S.; Tomozawa, T.; Ohno, A.; Yamashita, M.; Honda, T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 669.
4. (a) Collins, P. L.; Chanock, R. M.; McIntosh, K. Parainfluenza Viruses. In *Fields Virology*; Fields, B. N., Knipe, D. M., Howley, P. M., Eds.; Lippincott-Raven: Philadelphia, 1996; p 1205; (b) Murphy, B. R. Parainfluenza Viruses. In *Infectious Diseases*; Gorbach, S. L., Bartlett, J. G., Blacklow, N. R., Eds.; W. B. Saunders: Philadelphia, 1998; p 2125.
5. (a) Beau, J. M.; Schauer, R. *Eur. J. Biochem.* **1980**, *106*, 531; (b) Zbiral, E.; Schreiner, E.; Kleineidam, R. G.; Schauer, R. *Liebigs Ann. Chem.* **1991**, 129; (c) Avraham, L. *Carbohydr. Res.* **1995**, *271*, 241; (d) Hanaoka, K.; Pritchet, T. J.; Takasaki, S.; Kochibe, N.; Sabesan, S.; Paulson, J. C.; Kobata, A. *J. Biol. Chem.* **1989**, *264*, 9842; (e) Alymova, I. V.; Taylor, G.; Takimoto, T.; Lin, T.-H.; Chand, P.; Babu, Y. S.; Li, C.; Xiong, X.; Portner, A. *Antimicrob. Agents Chemother.* **2004**, *48*, 1495.
6. (a) Ikeda, K.; Sano, K.; Ito, M.; Saito, M.; Hidari, K.; Suzuki, T.; Suzuki, Y.; Tanaka, K. *Carbohydr. Res.* **2001**, *330*, 31; (b) Suzuki, T.; Ikeda, K.; Koyama, N.; Hosokawa, C.; Kogure, T.; Takahashi, T.; Hidari, K.; Miyamoto, D.; Tanaka, K.; Suzuki, Y. *Glycoconjugate J.* **2001**, *18*, 331.
7. Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron* **1987**, *43*, 5909.
8. Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schmidt, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 5434.
9. Chand, P.; Babu, Y. S.; Rowland, S. R.; Lin, T.-H. PCT Intl. Patent Appl. WO 02/076971 A1, 2002.