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Carbohydrate Research 330 (2001) 31–41

CARBOHYDRATE RESEARCH

Synthesis of 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid analogues modified at the C-4 and C-9 positions and their behaviour towards sialidase from influenza virus and pig liver membrane

Kiyoshi Ikeda*,[a,1] Kimihiko Sano,[a] Masaki Ito,[b] Megumi Saito,[b] Kazuya Hidari,[c] Takashi Suzuki,[c] Yasuo Suzuki,[c] Kiyoshi Tanaka*[a,2]

[a]*Department of Synthetic Organic Chemistry and Medicinal Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*

[b]*Department of Clinical Pharmacology and Therapeutics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*

[c]*Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*

Received 31 May 2000; accepted 10 September 2000

Abstract

The synthesis of novel 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid analogues structurally varied at C-4 and C-9 by transformation from versatile key intermediates and their inhibitory activity against sialidase from influenza virus A and pig liver membrane are described.

Keywords: Sialic acid; Sialidase inhibitor; Cyanomethyl group; Influenza virus sialidase; Mammalian sialidase

1. Introduction

Influenza virus sialidase is a surface enzyme that is essential for infection of the virus. Sialidase catalyzes the hydrolysis of α-glycosidic bonds to the terminal sialic acid residues of surface glycoconjugates on hemagglutinin during virus budding and on the host cell virus receptor to assist virus release and prevent aggregation.[1] Therefore, a sialidase has been considered as a suitable and effective target for designing chemotherapeutic agents against influenza virus.[2] Several groups have previously reported the inhibition of various sialidase activities by a variety of 2-deoxy-2,3 - didehydro - *N* - acetylneuraminic acid (Neu5Ac2en, **1**) derivatives.[3] Compound **1** is postulated to be a transition-state analogue binding to the active site of sialidase.[4] By molecular modelling techniques, von Itzstein and his co-workers reported the design and biological evaluation of 4-amino-2,3-didehydro-2,4-dideoxy-*N*-acetylneuraminic acid (4-amino-Neu5Ac2en, **2**) and its guanidino analogue (4-guanidino-Neu5Ac2en, **3**), which exhibited selective inhibition for the viral over mammalian sialidase.[5] As a part of our ongoing program aimed at the synthesis of new sialidase inhibitors, we reported the chemoen-

[1] *Corresponding author. Tel.: +81-54-2645742; fax: +81-54-2645745; e-mail: ikeda@ys2.u-shizuoka-ken.ac.jp

[2] *Corresponding author.

0008-6215/01/$ - see front matter
PII: S0008-6215(00)00267-6

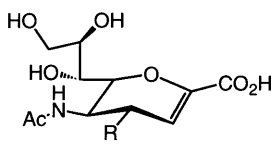
zymatic synthesis of 5,9-di-*N*-acyl-2,3-didehydro-2,3-dideoxyneuraminic acids[6a] and 5,9-di-*N*-acetyl-4-carbamoylmethyl-2,3-didehydro-2,3-dideoxyneuraminic acid[6b] and their behavior towards the sialidase from the influenza virus. In this study, in order to ascertain the importance of the 4- and 9-hydroxyl groups in *N*-acetylneuraminic acid, replacement of the hydroxyl groups at C-4 in *N*-acetylneuraminic acid analogues by the cyanomethyl, carbamoylmethyl, amidinomethyl, methoxyiminomethyl, and thiocarbamoylmethyl groups was carried out. Furthermore, the hydroxyl group at C-9 was also modified by azido or *N*-acetyl groups. Here we describe the synthesis of these novel 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid analogues modified at the C-4 and C-9 positions and their inhibitory activities against sialidase from both viral and mammalian sources (Scheme 1).

2. Results and discussion

Compounds **11**, **15** and **17** bearing a cyanomethyl group on C-4 position were selected as the key intermediates for the preparation of **4a**–**c**, **5a**–**d**, and **6a**–**d** in our synthesis. The cyanomethyl group is a versatile functional group for the preparation of sugar amides and amidine salts,[7] which may potentially induce new electrostatic and/or hydrogen-bonding interactions. The synthesis of the C-4 modified Neu5Ac2en (**4a**–**c**) compounds was accomplished as shown in Scheme 2. The Zemplén O-deacetylation of **7**[8] with 0.1 M NaOMe in MeOH–CH_2Cl_2, followed by protection by an isopropylidene group at C-8,9 by dry acetone and IR120 (H^+) resin gave compound **8** in 81% yield. Selective introduction of the cyanomethyl group at C-4 of **8** by $BrCH_2CN$, Ag_2O, and t-Bu_4NI,[9] and successive removal of the isopropylidene group with aqueous 80% AcOH furnished the triol **9** in 47% overall yield. Acetylation of **9** with Ac_2O and pyridine provided compound **10** in 98% yield. The methylene protons of the cyanomethyl group in the ^1H NMR spectrum of **10** resonated at δ 4.11, 4.35 (d, J_{gem} 16.5 Hz).

Compound **10** was transformed by treatment with dimethyl(methylthio)sulfonium triflate (DMTST) and then 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[10] to give the key compound **11** in 97% yield, showing in its ^1H NMR spectrum, a one-proton doublet at δ 6.17 ($J_{3,4}$ 3.5 Hz, H-3) in 97% yield.

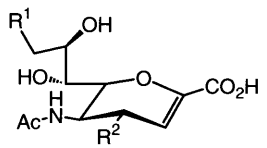
The key intermediate **11** thereby in hand was transformed into **4a**–**c** using methodology similar to that described by Malet and Hindsgaul.[7] Thus, initial deacetylation of **11** and hydrolysis of the methyl ester were carried out with 0.1 M KOH–MeOH, and chromatographic purification on silica gel gave the cyanomethyl compound **4a** {FABMS: m/z 353 $[M + Na]^+$} in 49% yield. Next, for the synthesis of the carbamoylmethyl compound **4b**,



Neu5Ac2en (**1**) R = -OH

4-Amino-Neu5Ac2en (**2**) R = $-NH_2$

4-Guanidino-Neu5Ac2en (**3**) R = $-NHC(=NH)NH_2$



4a: R^1 = -OH, R^2 = $-OCH_2CN$

4b: R^1 = -OH, R^2 = $-OCH_2C(=O)NH_2$

4c: R^1 = -OH, R^2 = $-OCH_2C(=NH)NH_2$

5a: R^1 = $-N_3$, R^2 = $-OCH_2CN$

5b: R^1 = $-N_3$, R^2 = $-OCH_2C(=O)NH_2$

5c: R^1 = $-N_3$, R^2 = $-OCH_2C(=NH)NH_2$

5d: R^1 = $-N_3$, R^2 = $-OCH_2C(=NH)OMe$

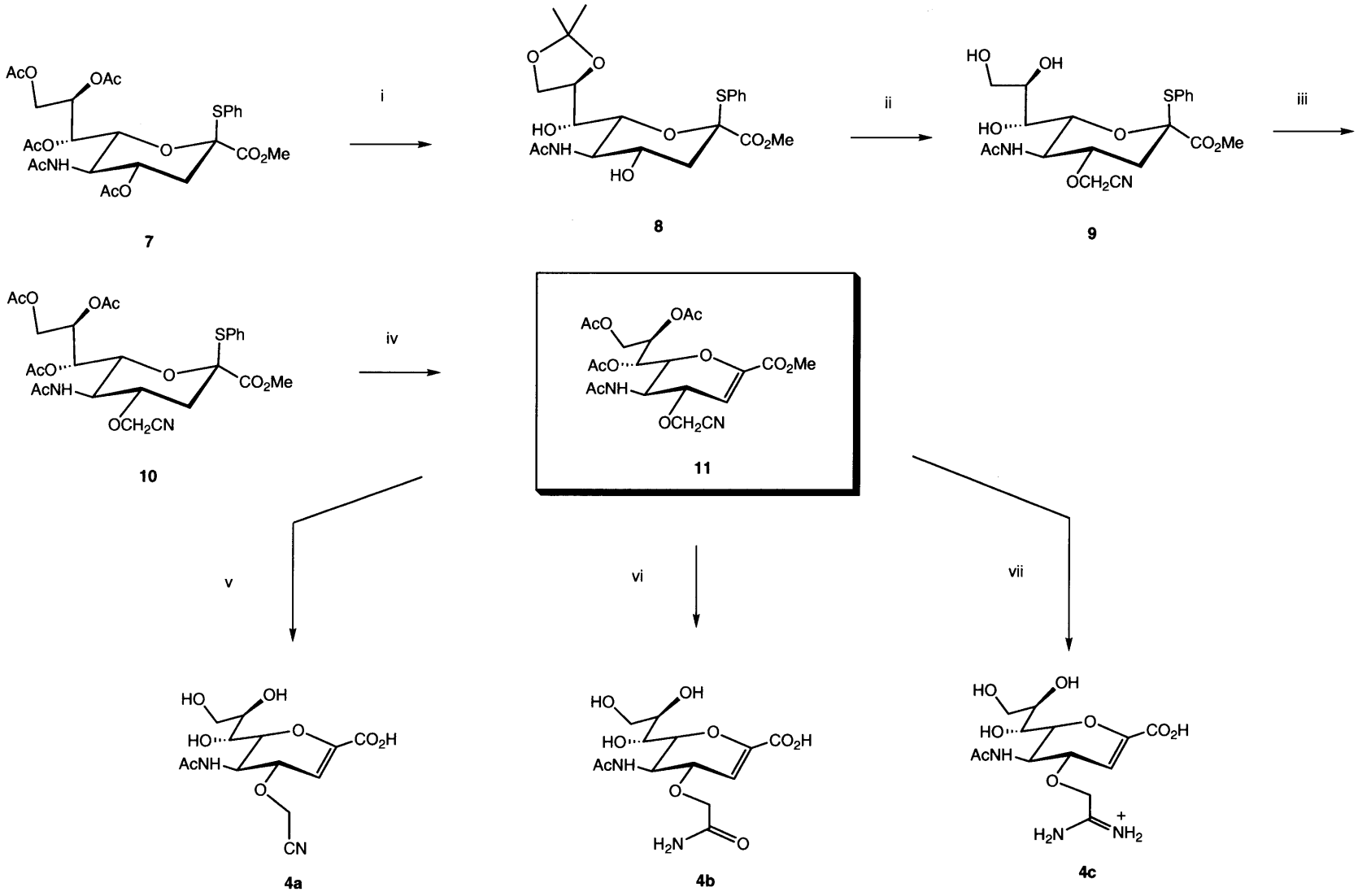
6a: R^1 = -NHAc, R^2 = $-OCH_2C(=S)NH_2$

6b: R^1 = -NHAc, R^2 = $-OCH_2CN$

6c: R^1 = -NHAc, R^2 = $-OCH_2C(=O)NH_2$

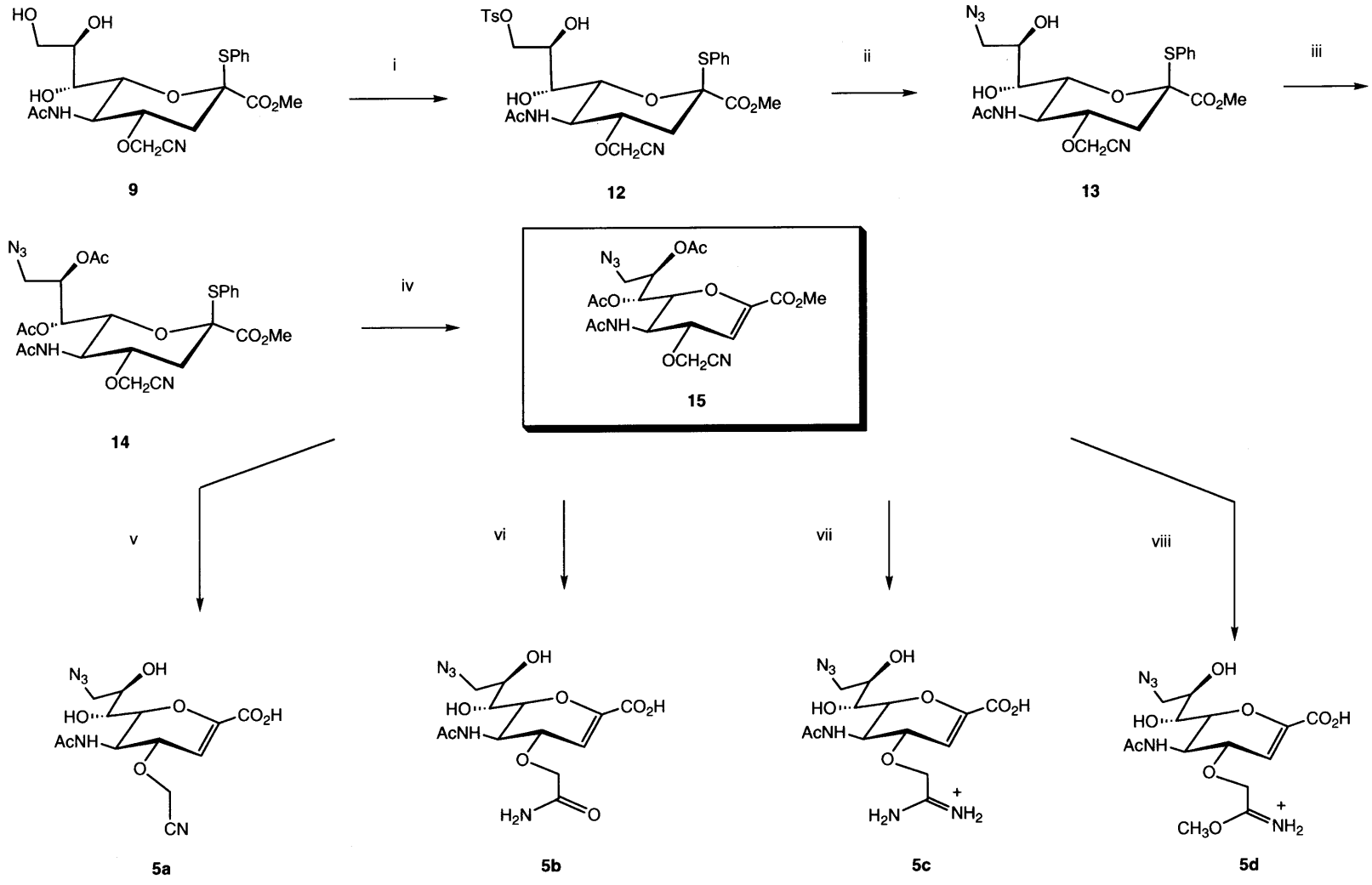
6d: R^1 = -NHAc, R^2 = $-OCH_2C(=NH)NH_2$

Scheme 1.

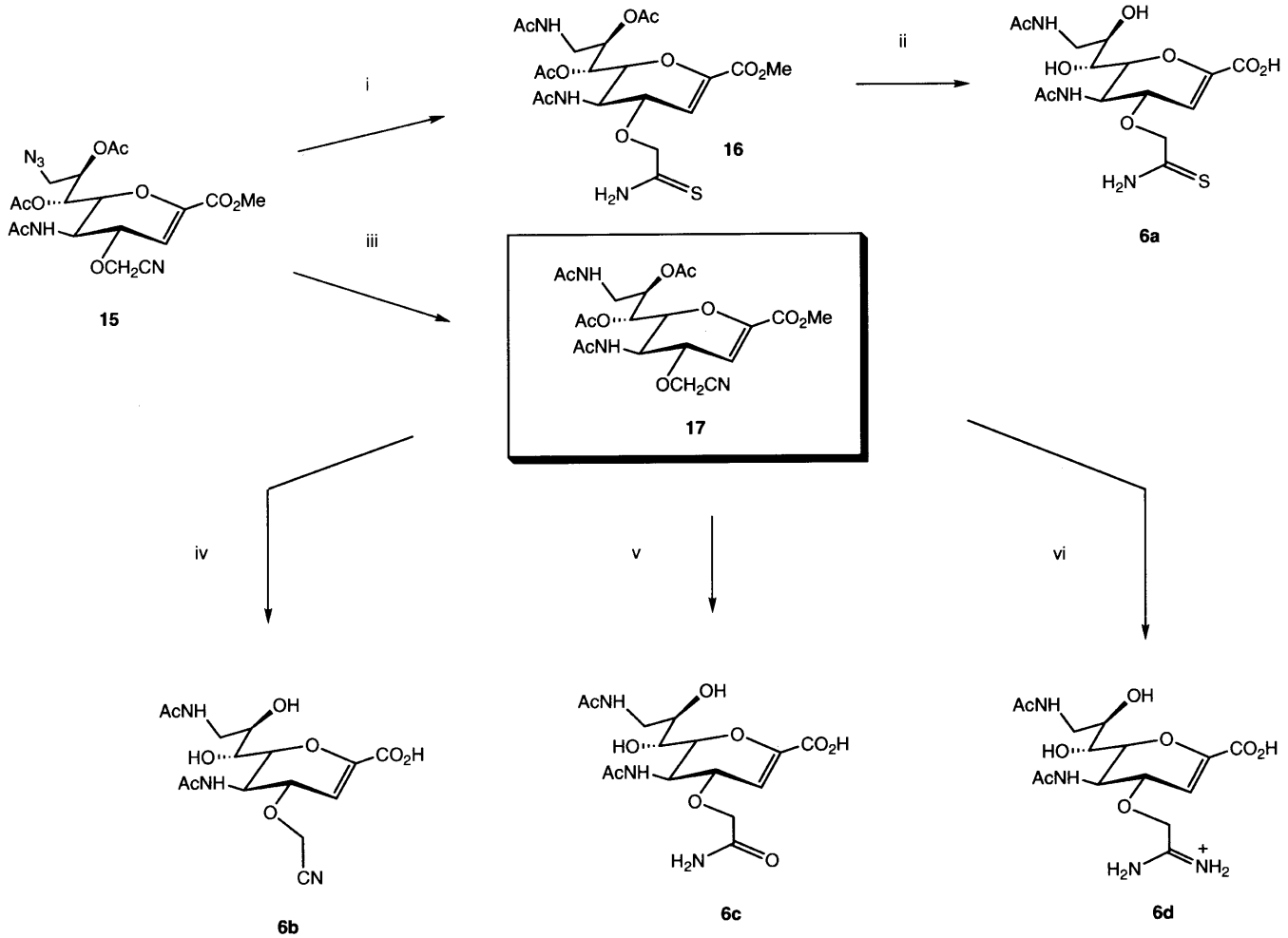


i) 1) 0.1M NaOMe-CH_2Cl_2-MeOH, 2) IR120(H^+), acetone; ii) 1) $BrCH_2CN$, Ag_2O, ī BAI, 2) 80%AcOH; iii) Ac_2O, pyridine; iv) 1) DMTST, 2) DBU; v) 0.1MKOH-MeOH ; vi) 1) 0.1M NaOMe-MeOH, 2) aq. $NaHCO_3$; vii) 1) 0.1M NaOMe-MeOH, 2) anhydr. NH_4Cl, 3) 0.1M KOH-MeOH.

Scheme 2.


HO
OH
SPh
HO
AcNH
O
CO2Me
OCH2CN
9
i
TsO
OH
SPh
HO
AcNH
O
CO2Me
OCH2CN
12
ii
N3
OH
SPh
HO
AcNH
O
CO2Me
OCH2CN
13
iii
N3
OAc
SPh
AcO
AcNH
O
CO2Me
OCH2CN
14
iv
N3
OAc
AcO
AcNH
O
CO2Me
OCH2CN
15
v
vi
vii
viii
N3
OH
HO
AcNH
O
CO2H
CN
5a
N3
OH
HO
AcNH
O
CO2H
O
H2N
O
5b
N3
OH
HO
AcNH
O
CO2H
O
H2N
+
NH2
5c
N3
OH
HO
AcNH
O
CO2H
O
CH3O
+
NH2
5d

Scheme 3.



Scheme 4.

11 was treated with 0.1 M NaOMe–MeOH, followed by aqueous $NaHCO_3$ to give **4b** {FABMS: m/z 371 $[M + Na]^+$, 387 $[M + K]^+$} in 55% yield in two steps. For the synthesis of the amidinomethyl compound **4c**, **11** was successively treated with 0.1 M NaOMe–MeOH, anhydrous NH_4Cl, and then 0.1 M KOH–MeOH to afford **4c** {FABMS: m/z 349 $[M + H]^+$, 371 $[M + Na]^+$} in 57% yield in three steps.

For the synthesis of **5a**–**d** bearing an azide group at C-9, compound **9** was converted into the common intermediate **15** (Scheme 3). Thus, selective tosylation at C-9 of **9** with *p*-toluenesulfonyl chloride in pyridine–CH_2Cl_2 (64% yield) and subsequent treatment of **12** with NaN_3 and 18-crown-6[11] in DMF afforded **13** in 74% yield. The IR spectrum of **13** showed absorption bands at 2106 and 1736 cm^{-1}, characteristic of the azido and ester groups, respectively. Acetylation of **13** with Ac_2O and pyridine provided compound **14** in 95% yield, which was similarly treated with DMTST and then DBU to give **15** {FABMS: m/z 454 $[M + H]^+$} in 99% yield. In the same way as described for the preparation of **4a**–**c**, compound **15** was converted into the corresponding cyanomethyl compound **5a** {FABMS: m/z 396 $[M + K]^+$}, carbamoylmethyl compound **5b** {FABMS: m/z 396 $[M + Na]^+$}, and amidinomethyl compound **5c** {FABMS: m/z 345 $[M]^+$} in 80, 67, and 60% yields, respectively. Additionally, **15** was treated with 0.1 M NaOMe–MeOH, and then 0.1 M KOH–MeOH to give **5d** {FABMS: m/z 411 $[M + Na]^+$} in 76% yield.

For the synthesis of **6a**–**d** having an NAc group at C-9 (Scheme 4), the transformation to the common intermediate **17** from **15** was examined. After unfruitful attempts to reduce

the azide group of **15** using Pd–C, H_2, selective reduction of the azide group of **15** was effected with simultaneous acetylation by AcSH–pyridine[12] giving **16** {FABMS: m/z 504 $[M+H]^+$, 526 $[M+Na]^+$} in 79% yield. Compound **16** was treated with 0.1 M KOH–MeOH to give thiocarbamoylmethyl compound **6a** {FABMS: m/z 406 $[M+H]^+$, 428 $[M+Na]^+$} in 67% yield. The azido group of **15** was successfully reduced by use of PPh_3,[13] and subsequent acetylation with Ac_2O and pyridine afforded compound **17** in 85% yield. In the same way as already described, compound **17** was transformed into the corresponding cyanomethyl derivative **6b** {FABMS: m/z 470 $[M+H]^+$}, the carbamoylmethyl compound **6c** {FABMS: m/z 412 $[M+Na]^+$}, and amidinomethyl compound **6d** {FABMS: m/z 391 $[M+2\ H]^+$} in 41, 60, and 52% yields, respectively.

The behaviour of the compounds newly synthesized in this study towards sialidase from the influenza virus A/Memphis/1/71(H3N2)[14] and pig liver membrane[15] was examined in comparison to Neu5Ac2en (**1**) (Table 1). As may be seen from the table, compounds **5d**, **6c**, and **6d** showed comparable inhibitory activities against both enzymes, although the degree of inhibition was less than that of **1**. Interestingly, compound **6a** exhibited selective inhibition against the viral over mammalian sialidase, whereas the inhibitory effects of **5b**,**c** were only on mammalian sialidase.

Table 1
Inhibition of sialidase by analogues of Neu5Ac2en modified at the C-4 and C-9 positions [a]

	Sialidase	
	A/Memphis/1/71(H3N2)	p.g. liver membrane
4a	++	+
4b	++	+
4c	++	+
5a		+
5b		++
5c		++
5d	++	++
6a	++	
6b	+	+
6c	++	++
6d	++	++
1	++++	++++

[a] Inhibition of sialidase at 1.0 mM inhibitor; +, <10%; ++, 10–30%; +++, 30–90%; ++++, >90%.

In conclusion, the synthesis of 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid analogues modified at the C-4 and C-9 positions was achieved via structurally similar common key intermediates. The compounds thus synthesized showed inhibitory activities against the influenza virus and mammalian sialidase.

3. Experimental

General procedure.—Optical rotations were measured with a JASCO DIP-140 (Japan) digital polarimeter. IR spectra were recorded on a JASCO IR-810 (Japan) spectrometer. ^1H NMR spectra were recorded with a JEOL EX 270 (Japan) instrument. Chemical shifts are expressed in ppm relative to Me_4Si ($\delta = 0$) in $CDCl_3$, CD_3OD and in D_2O referenced to HOD (4.85 ppm) as the internal standard. Fast-atom-bombardment (FAB) mass spectra were obtained with a JEOL SX-102 (Japan) mass spectrometer in the positive-ion mode using an NBA 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 Merck 60 (70–230 mesh) and LH-20 (Pharmacia). All reactions were monitored by TLC (Silica Gel 60-F_{254}, E. Merck, Germany) by charring after spraying with 5% H_2SO_4 in MeOH and then heating.

*Methyl (phenyl 5-acetamido-3,5-dideoxy-8,9-O-isopropylidene-2-thio-*D*-glycero-β-*D*-galacto-2-nonulopyranosid)onate* (**8**).—To a soln of **7** (6.79 g, 11.6 mmol) in dry MeOH (150 mL) was added 0.2 M NaOMe (20 mL), and the mixture was stirred for 2 h at 0 °C, and neutralized by the addition of Amberlite IRC-50 (3.5 g) resin, and the mixture was filtered and the filtrate concd. The residue was dissolved in dry acetone (100 mL) with Amberlite IRA120 (H^+) (5 g) resin and the mixture was stirred for 13 h at rt, filtered, and concd. Purification of the residue by column chromatography on silica gel with 10:1 CH_2Cl_2–MeOH yielded **8** (4.25 g, 81%),

$[\alpha]_D$ − 169.6° (*c* 0.30, $CHCl_3$); ν_{max} ($CHCl_3$): 1736, 1663, 851, and 779 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.40, 1.42 (s, each 3 H, Me_2C), 2.08 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4} = 13.5$ Hz, H-3ax), 2.08 (s, 3 H, AcN), 2.80 (dd, 1 H, $J_{3eq,4}$ 4.5 Hz, H-3eq), 3.44 (s, 3 H, MeO), 3.57 (m, 1 H, H-7), 3.93 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH} = 8.6$ Hz, H-5), 3.97 (m, 1 H, H-8), 3.98 (dd, 1 H, $J_{8,9a}$ 5.1, $J_{9a,9b}$ 10.5 Hz, H-9a), 4.07 (ddd, 1 H, H-4), 4.15 (dd, 1 H, $J_{6,7} = 2.0$ Hz, H-6), 4.27 (m, 1 H, H-9b), 6.33 (d, 1 H, NH), 7.24–7.38, and 7.51–7.54 (m, 5 H, Ph); positive ion FABMS (NBA): *m/z* 456 $[M + H]^+$, 478 $[M + Na]^+$.

*Methyl (phenyl 5-acetamido-4-*O*-cyanomethyl-3,5-dideoxy-2-thio-*D*-glycero-β-*D*-galacto-2-nonulopyranosid)onate* (**9**).—Compound **8** (686 mg, 1.5 mmol) was dissolved in CH_2Cl_2 (20 mL) and to the mixture were added 4 Å molecular sieves (1.0 g) and bromocyanonitrile (540 mg, 4.5 mmol). After stirring for 1 h, to the mixture were added Ag_2O (1.04 g, 4.5 mmol) and *n*-Bu_4NI (550 mg, 1.5 mmol) and the mixture was stirred for 2 days in the dark under Ar. The insoluble materials were filtered off through Celite 545 and the filtrate was concd to dryness. The residue was chromatographed on silica gel using 20:1 CH_2Cl_2–MeOH to give the 4-*O*-cyanomethyl compound, which was dissolved in 80% AcOH (10 mL) and heated at 80–90 °C for 0.5 h and concd to dryness. Purification of the residue by column chromatography on silica gel with 10:1 CH_2Cl_2–MeOH gave **9** (319 mg, 47%); $[\alpha]_D$ − 88.7° (*c* 0.68, $CHCl_3$); ν_{max} ($CHCl_3$): 3427, 1738, and 1661 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.99 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4} = 13.9$ Hz, H-3ax), 2.06 (s, 3 H, AcN), 2.78 (dd, 1 H, $J_{3eq,4}$ 3.8 Hz, H-3eq), 3.55 (s, 3 H, MeO), 3.72–3.82 (m, 3 H, H-8, H-9a,b), 4.00–4.06 (m, 2 H, H-4, H-6), 4.28, 4.42 (d, each 1 H, J_{gem} 16.5 Hz, –OCH_2CN), 4.48 (m, 1 H, H-5), 4.70 (m, 1 H, H-7), 7.24–7.38, and 7.53–7.56 (m, 5 H, Ph); positive ion FABMS (NBA): *m/z* 455 $[M + H]^+$, 477 $[M + Na]^+$.

*Methyl (phenyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-*O*-cyanomethyl-3,5-dideoxy-2-thio-*D*-glycero-β-*D*-galacto-2-nonulopyranosid)onate* (**10**).—Compound **9** (330 mg, 0.72 mmol) was dissolved in pyridine (5 mL) and treated with Ac_2O (2.5 mL). The mixture was stirred at 0 °C for 30 min, and then stirred overnight at rt. The soln was diluted with CH_2Cl_2 and successively washed with water, 1 M HCl, aq satd $NaHCO_3$, and brine, dried ($MgSO_4$), and then concd. The residue was chromatographed on silica gel using 20:1 CH_2Cl_2–MeOH to give **10** (410 mg, 98%); $[\alpha]_D$ − 38.5° (*c* 1.0, $CHCl_3$); ν_{max} ($CHCl_3$): 1740 and 1684 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.98 (s, 3 H, AcN), 2.02, 2.08, 2.11 (s, 9 H, AcO), 2.76 (dd, 1 H, $J_{3ax,3eq}$ 13.8, $J_{3eq,4}$ 4.5 Hz, H-3eq), 3.62 (s, 3 H, MeO), 3.93 (ddd, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH} = 10.0$ Hz, H-5), 3.99–4.12 (m, 2 H, H-4, H-9a), 4.11, 4.35 (d, each 1 H, J_{gem} 16.5 Hz, –OCH_2CN), 4.48 (dd, 1 H, $J_{8,9b}$ 1.9, $J_{9a,9b}$ 12.4 Hz, H-9b), 4.70 (dd, 1 H, $J_{6,7}$ 2.4 Hz, H-6), 4.97–5.00 (m, 1 H, H-8), 5.47–5.49 (m, 1 H, H-7), 5.59 (d, 1 H, NH), and 7.34–7.52 (m, 5 H, Ph); positive ion FABMS (NBA): *m/z* 581 $[M + H]^+$, 603 $[M + Na]^+$.

*Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-*O*-cyanomethyl-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enonate* (**11**).—DMTST (147 mg, 0.57 mmol) was added to a stirred mixture of compound **10** (110 mg, 0.19 mmol) and 4 Å molecular sieves (0.5 g) in dry CH_2Cl_2 (4 mL) at −20 °C under Ar. After stirring for 2 h at the same temperature, a soln of DBU (87 mg, 0.57 mmol) in dry CH_2Cl_2 (0.5 mL) was added at −20 °C, and the mixture was stirred for 1 h at the same temperature, and then overnight at rt. The precipitates were filtered off through Celite 545 and the filtrate was washed with aq satd $NaHCO_3$ and brine, dried ($MgSO_4$), and concd. Column chromatography on silica gel with 10:1 CH_2Cl_2–MeOH gave **11** (87 mg, 97%) as an amorphous powder, $[\alpha]_D$ + 10° (*c* 1.2, $CHCl_3$); ν_{max} ($CHCl_3$): 1748, 1686, and 1526 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.05 (s, 3 H, AcN), 2.13, 2.19 (s, 9 H, AcO), 3.87 (s, 3 H, MeO), 4.21 (dd, 1 H, $J_{8,9a}$ 7.8, $J_{9a,9b}$ 14.6 Hz, H-9a), 4.21–4.35 (m, 2 H, H-4, 6), 4.40–4.50 (m, 1 H, H-5), 4.44, 4.55 (d, each 1 H, J_{gem} 16.5 Hz, –OCH_2CN), 4.61 (dd, 1 H, $J_{8,9b}$ 3.5 Hz, H-9b), 5.41 (m, 1 H, H-8), 5.87 (d, 1 H, $J_{NH,5}$ 8.4 Hz, NH), and 6.13 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-3); positive ion FABMS (NBA): *m/z* 471 $[M + H]^+$.

*5-Acetamido-2,6-anhydro-4-*O*-cyanomethyl-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enonic acid* (**4a**).—Compound **11** (41 mg, 0.087 mmol) was dissolved in a 1:1 soln of 0.1 M KOH in MeOH (4 mL). The mixture was stirred for 15 h at rt and neutralized with Amberlite 120 (H^+). The precipitates were filtered off through Celite 545 and the filtrate was concd. The residue was chromatographed on silica gel using 6:6:1 $CHCl_3$–MeOH–H_2O to give **4a** (14 mg, 49%) as a powder; ^{1}H NMR (D_2O): δ 2.10 (s, 3 H, AcN), 3.54–3.70 (m, 2 H, H-8, H-9a), 3.91, 4.29 (d, each 1 H, J_{gem} 11.9 Hz, –OCH_2CN), 3.93–3.99 (m, 2 H, H-7, H-9b), 4.24 (br t, 1 H, $J_{4,5} = J_{5,6} = 11.1$ Hz, H-5), 4.49–4.53 (m, 2 H, H-4, H-6), and 5.82 (d, 1 H, $J_{3,4}$ 2.2 Hz, H-3); positive ion FABMS (NBA): m/z 353 $[M + Na]^+$. HR-FABMS Anal. Calcd for $C_{13}H_{18}N_2NaO_8$: m/z 353.0961 $[M + Na]^+$, Found: 353.0958.

*5-Acetamido-2,6-anhydro-4-*O*-carbamoylmethyl-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enonic acid* (**4b**).—Compound **11** (66 mg, 0.14 mmol) was dissolved in dry MeOH (3 mL), and methanolic 0.1 M NaOMe (3 mL) was added to the mixture at 0 °C under Ar. After being stirred for 12 h at rt, satd aq $NaHCO_3$ (1 mL) was added to the mixture, which was stirred for 5 days at rt. The mixture was adjusted to pH 2 with Amberlite 120 (H^+), the resin was filtered through Celite 545, the filtrate was subjected to gel filtration, and the aq soln was concd. The residue was dissolved in a 1:1 soln of 0.1 M KOH in MeOH (3 mL) at 0 °C and the mixture was stirred for 12 h at rt. The mixture was adjusted to pH 2 with Amberlite 120 (H^+), the resin was filtered through Celite 545, and the filtrate was desalted with an AC Micro Acylizer G1 and the resulting aq soln was concd. The residue was chromatographed on silica gel using 65:35:5 $CHCl_3$–MeOH–H_2O to give **4b** (27 mg, 55%), after lyophilization; ^{1}H NMR (D_2O): δ 2.10 (s, 3 H, AcN), 3.66–3.71 (m, 2 H, H-8, H-9a), 3.90–3.97 (m, 1 H, H-9b), 4.13, 4.26 (d, each 1 H, J_{gem} 15.7 Hz, –OCH_2CONH_2), 4.29–4.33 (m, 1 H, H-5), 4.52 (m, 1 H, H-4), and 5.98 (br s, 1 H, H-3); positive ion FABMS (NBA): m/z 371 $[M + Na]^+$. HRFABMS Anal. Calcd for $C_{13}H_{20}N_2NaO_9$: m/z 371.1067 $[M + Na]^+$, Found: 371.1052.

*5-Acetamido-4-*O*-amidinomethyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enonic acid* (**4c**).—Compound **11** (47 mg, 0.10 mmol) was dissolved in dry MeOH (4 mL) and methanolic 0.1 M NaOMe (4 mL) was added to the mixture at 0 °C under Ar. After being stirred for 12 h at rt, anhyd NH_4Cl (32 mg, 0.60 mmol) was added to the reaction mixture and the whole was heated for 2 h at 50 °C and evaporated. The residue was dissolved in H_2O, desalted and the aq soln was concd to dryness. The residue was dissolved in a 1:1 soln of 0.1 M KOH in MeOH (2 mL) at 0 °C and the mixture was stirred for 12 h at the rt. The mixture was adjusted to pH 2 with Amberlite 120 (H^+), the resin was filtered through Celite 545, the filtrate was desalted, and the aq soln was evaporated. The residue was chromatographed on silica gel using 65:35:5 $CHCl_3$–MeOH–H_2O to give **4c** (20 mg, 57%), after lyophilization; ^{1}H NMR (D_2O): δ 2.08 (s, 3 H, AcN), 3.66 (dd, 1 H, $J_{8,9a}$ 5.9, $J_{9a,9b}$ 11.6 Hz, H-9a), 3.67 (m, 1 H, H-7), 3.92 (dd, 1 H, $J_{8,9b}$ 2.7 Hz, H-9b), 3.96–4.00 (m, 1 H, H-8), 4.11, 4.24 (d, each 1 H, J_{gem} 15.9 Hz, –$OCH_2C(=NH)NH_2$), 4.27–4.29 (m, 1 H, H-5), 4.50 (m, 1 H, H-4), and 5.82 (d, 1 H, $J_{3,4}$ 2.2 Hz, H-3); positive ion FABMS (NBA): m/z 349 $[M + H]^+$, 371 $[M + Na]^+$. HRFABMS Anal. Calcd for $C_{13}H_{22}N_3NaO_8$: m/z 371.1303 $[M + Na]^+$, Found: 371.1305.

*Methyl (phenyl 5-acetamido-4-*O*-cyanomethyl-3,5-dideoxy-9-*p*-tolyesulfonyl-2-thio-*D*-glycero-β-*D*-galacto-2-nonulopyranosid)onate* (**12**).—To a soln of **9** (550 mg, 1.2 mmol) in pyridine (5 mL) and CH_2Cl_2 (10 mL) was added *p*-toluenesulfonyl chloride (686 mg, 3.6 mmol) at 0 °C under Ar, and the mixture was stirred for 12 h at rt, and then concd. The residue was chromatographed on silica gel using 10:1 CH_2Cl_2–MeOH to give **12** (470 mg, 64%); $[\alpha]_D - 79.2°$ (c 0.12, $CHCl_3$); ν_{max} ($CHCl_3$): 1738 and 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.95 (dd, 1 H, $J_{3ax,3eq}$ 13.9, $J_{3ax,4}$ 10.9 Hz, H-3ax), 2.11 (s, 3 H, AcN), 2.44 (s, 3 H, MePh), 2.80 (dd, 1 H, $J_{3eq,4}$ 4.3 Hz, H-3eq), 3.50 (s, 3 H, MeO), 3.94–4.14 (m, 3 H, H-8, H-9a,b), 3.97 (ddd, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH} =$ 10.6 Hz, H-5), 4.07 (ddd, 1 H, H-4), 4.15, 4.39 (d, each 1 H, J 16.7 Hz, –OCH_2CN), 6.02 (br

s, 1 H, NH), and 7.33–7.80 (m, 5 H, PhS); positive ion FABMS (NBA): m/z 609 $[M+H]^+$.

*Methyl (phenyl 5-acetamido-9-azido-4-*O*-cyanomethyl-3,5-dideoxy-2-thio*-D-glycero-D-*β*-galacto-*2-nonulopyranosid)onate* (**13**).—To a soln of **12** (669 mg, 1.1 mmol) in DMF (10 mL) was added sodium azide (357 mg, 5.6 mmol) and 18-crown-6 (146 mg, 0.56 mmol) and the mixture was heated at 60 °C with stirring for 20 h under Ar. The mixture was allowed to cool to rt, and concd to dryness. The residue was chromatographed on silica gel using 10:1 CH_2Cl_2–MeOH to give **13** (390 mg, 74%); $[\alpha]_D$ − 89.5° (*c* 1.0, $CHCl_3$); ν_{max} ($CHCl_3$): 3430, 2106, 1736, 1671, and 1553 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.97 (dd, 1 H, $J_{3ax,3eq}$ 13.9, $J_{3ax,4}$ 10.9 Hz, H-3ax), 2.13 (s, 3 H, AcN), 2.76 (dd, 1 H, $J_{3eq,4}$ 4.6 Hz, H-3eq), 3.49 (dd, 1 H, $J_{9a,9b}$ 12.5, $J_{8,9a}$ 7.8 Hz, H-9a), 3.56 (s, 3 H, MeO), 3.63 (dd, 1 H, $J_{8,9b}$ 3.5 Hz, H-9b), 3.84–3.95 (m, 1 H, H-5), 3.98–4.46 (m, 4 H, H-4, 6, 7, 8), 4.16, 4.42 (d, each 1 H, J_{gem} 16.5 Hz, –OCH_2CN), and 6.08 (d, 1 H, $J_{NH,5}$ 8.6 Hz, NH); positive ion FABMS (NBA): m/z 480 $[M+H]^+$.

*Methyl (phenyl 5-acetamido-7,8-di-*O*-acetyl-9-azido-4-*O*-cyanomethyl-3,5-dideoxy-2-thio*-D-glycero-*β*-D-galacto-*2-nonulopyranosid)onate* (**14**).—Compound **13** (70 mg, 0.146 mmol) was dissolved in pyridine (2 mL) and treated with Ac_2O (1 mL). The mixture was stirred at 0 °C for 30 min, and then overnight at rt. The soln was diluted with CH_2Cl_2 and successively washed with water, 1 M HCl, aq satd $NaHCO_3$, and brine, and dried ($MgSO_4$), and finally concd to dryness. The residue was chromatographed on silica gel using 10:1 CH_2Cl_2–MeOH to give **14** (78 mg, 95%); $[\alpha]_D$ − 94.3° (*c* 0.75, $CHCl_3$); ν_{max} ($CHCl_3$): 2105, 1746, 1682, and 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.92 (s, 3 H, AcN), 2.00, 2.03 (s, 6 H, AcO), 2.69 (dd, 1 H, $J_{3ax,3eq}$ 13.9, $J_{3eq,4}$ 4.6 Hz, H-3eq), 3.23 (dd, 1 H, $J_{9a,8}$ 9.2, $J_{9a,9b}$ 13.5 Hz, H-9a), 3.54 (dd, 1 H, $J_{8,9b}$ 2.3 Hz, H-9b), 3.57 (s, 3 H, MeO), 4.11 (q, 1 H, $J_{4,5} = J_{5,6} = J_{NH,5}$ = 10.6 Hz, H-5), 5.38 (m, 1 H, H-4), 4.15, 4.39 (d, each 1 H, J_{gem} 16.7 Hz, –OCH_2CN), 4.61 (dd, 1 H, $J_{6,7}$ 2.3 Hz, H-6), 4.95 (m, 1 H, H-8), 5.46 (m, 1 H, H-7), and 7.31–7.36 (m, 5 H, PhS); positive ion FABMS (NBA): m/z 564 $[M+H]^+$.

*Methyl 5-acetamido-7,8-di-*O*-acetyl-2,6-anhydro-9-azido-4-*O*-cyanomethyl-3,5,9-trideoxy-*D-glycero-D-galacto-non-*2*-enonate (**15**).—Transformation of **14** (30 mg, 0.053 mmol) was carried out by the same procedure as described for **11** to give compound **15** (24 mg, 99%) as an amorphous powder, $[\alpha]_D$ + 41° (*c* 0.68, $CHCl_3$); ν_{max} ($CHCl_3$): 2108, 1744, 1680, and 1525 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 2.01 (s, 3 H, AcN), 2.09, 2.14 (s, each 3 H, AcO), 3.49 (dd, 1 H, $J_{8,9a}$ 7.6, $J_{9a,9b}$ 13.5 Hz, H-9a), 3.80 (dd, 1 H, $J_{8,9b}$ 4.0 Hz, H-9b), 3.82 (s, 3 H, MeO), 4.16 (q, 1 H, $J_{4,5} = J_{5,6} = J_{NH,5}$ = 8.6 Hz, H-5), 4.43, 4.46 (d, each 1 H, J_{gem} 16.2 Hz, –OCH_2CN), 4.35–4.47 (m, 2 H, H-4, 6), 5.20 (m, 1 H, H-8), 5.50 (m, 1 H, H-7), 5.79 (d, 1 H, NH), and 6.09 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-3); positive ion FABMS (NBA): m/z 454 $[M+H]^+$.

*5-Acetamido-2,6-anhydro-9-azido-4-*O*-cyanomethyl-3,5,9-trideoxy-*D-glycero-D-galacto-*non-2-enonic acid* (**5a**).—Transformation of **15** (40 mg, 0.088 mmol) was performed as described for **4a** to give compound **5a** (26 mg, 80%); ^1H NMR (D_2O): δ 2.09 (s, 3 H, AcN), 3.53–3.57 (m, 1 H, H-9a), 3.65–3.70 (m, 2 H, H-7, H-9b), 4.09–4.15 (m, 1 H, H-5), 4.12, 4.24 (d, each 1 H, J_{gem} 15.9 Hz, –OCH_2CN), 4.48 (br s, 1 H, H-4), and 5.81 (br s, 1 H, H-3); positive ion FABMS (NBA): m/z 396 $[M+K]^+$.

*5-Acetamido-2,6-anhydro-9-azido-4-*O*-carbamoylmethyl-3,5,9-trideoxy-*D-glycero-D-galacto-*non-2-enonic acid* (**5b**).—Transformation of **15** (40 mg, 0.088 mmol) was performed as described for **4b** to afford compound **5b** (22 mg, 67%); ^1H NMR (D_2O): δ 2.10 (s, 3 H, AcN), 3.55 (dd, 1 H, $J_{8,9a}$ 5.7, $J_{9a,9b}$ 13.2 Hz, H-9a), 3.66–3.72 (m, 1 H, H-7), 3.68 (dd, 1 H, $J_{8,9b}$ 2.4 Hz, H-9b), 4.08, 4.23 (d, each 1 H, J_{gem} 13.2 Hz, –OCH_2CONH_2), 4.09–4.25 (m, 2 H, H-6, 8), 4.22–4.29 (m, 1 H, H-5), 4.50 (br s, 1 H, H-4), and 5.83 (br s, 1 H, H-3); positive ion FABMS (NBA): m/z 396 $[M+Na]^+$. HR-FABMS Anal. Calcd for $C_{13}H_{19}N_5NaO_8$: m/z 396.1132 $[M+Na]^+$, Found: 396.1132.

*5-Acetamido-4-*O*-amidinomethyl-2,6-anhydro-9-azido-3,5,9-trideoxy-*D-glycero-D-galacto-*non-2-enonic acid* (**5c**).—Transformation of **15** (24 mg, 0.053 mmol) described for

4c gave compound **5c** (12 mg, 60%); ^{1}H NMR (D_2O): δ 2.10 (s, 3 H, AcN), 3.53–3.58 (m, 1 H, H-9a), 3.67–3.70 (m, 2 H, H-7, H-9b), 4.10–4.32 (m, 4 H, H-5, H-8, –OC*H*$_2$C(=NH)NH$_2$), 4.52 (br s, 1 H, H-4), and 5.93 (br s, 1 H, H-3); positive ion FABMS (NBA): *m*/*z* 373 $[M]^+$. HRFABMS Anal. Calcd for $C_{13}H_{19}N_5NaO_8$: *m*/*z* 396.1369 $[M+Na]^+$, Found: 396.1158.

*5-Acetamido-2,6-anhydro-9-azido-3,5,9-trideoxy-4-*O*-methoxyiminomethyl-*D*-glycero-*D*-galacto-non-2-enonic acid* (**5d**).—Compound **15** (40 mg, 0.088 mmol) was dissolved in dry MeOH (2 mL) and methanolic 0.1 M NaOMe (0.6 mL) was added to the mixture at 0 °C under Ar. After being stirred for 12 h at rt, the mixture was adjusted to pH 2 with Amberlite 120 (H^+), the resin was filtered through Celite 545, the filtrate was subjected to gel filtration, and then the aq soln was concd. The residue was dissolved in a 1:1 soln of 0.1 M KOH in MeOH (2 mL) at 0 °C and the mixture was stirred for 12 h at rt. The mixture was adjusted to pH 2 with Amberlite 120 (H^+), the resin was filtered through Celite 545, the filtrate was subjected to gel filtration and the aq soln was concd. The residue was chromatographed on silica gel using 65:35:5 $CHCl_3$–MeOH–H_2O to give **5d** (26 mg, 76%), after lyophilization; ^{1}H NMR (D_2O): δ 2.10 (s, 3 H, AcN), 3.58–3.70 (m, 3 H, H-7, H-9a,b), 3.80 (s, 3 H, –OCH_3), 4.27–4.35 (m, 4 H, H-5, H-8, –OC*H*$_2$C(=NH)OCH_3), 4.52–4.60 (m, 1 H, H-4), and 5.96 (br s, 1 H, H-3); positive ion FABMS (NBA): *m*/*z* 411 $[M+Na]^+$. HRFABMS Anal. Calcd for $C_{14}H_{22}N_5NaO_8$: *m*/*z* 411.1366 $[M+Na]^+$, Found: 411.1381.

*Methyl 5,9-diacetamido-7,8-di-*O*-acetyl-2,6-anhydro-3,5,9-trideoxy-4-*O*-thiocarbamoylmethyl-*D*-glycero-*D*-galacto-non-2-enonate* (**16**).—To a soln of **15** (47 mg, 0.10 mmol) in pyridine (0.5 mL) and CH_2Cl_2 (0.5 mL) was added thioacetic acid (38 mg, 0.50 mmol) at rt under Ar, and the mixture was stirred for 12 h. After removal of the solvent, the residue was chromatographed on silica gel using 10:1 CH_2Cl_2–MeOH to give **16** (40 mg, 79%); $[\alpha]_D + 101.8°$ (*c* 0.80, $CHCl_3$); ν_{max} ($CHCl_3$): 1731, 1665, and 1524 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.96, 1.98 (s, each 3 H, AcN), 2.04, 2.12 (s, each 3 H, AcO), 3.32 (ddd, 1 H, $J_{8,9a} = J_{NH,9a} = 5.7$, $J_{9a,9b}$ 14.6 Hz, H-9a), 3.84 (s, 3 H, MeO), 3.89 (ddd, 1 H, $J_{8,9b}$ 3.5, $J_{NH,9b}$ 6.2 Hz, H-9b), 4.17 (dd, 1 H, $J_{4,3}$ 2.7, $J_{4,5}$ 5.1 Hz, H-4), 4.32, 4.58 (d, each 1 H, J_{gem} 16.7 Hz, –OCH_2CN), 4.35–4.39 (m, 2 H, H-5, 6), 5.05 (m, 1 H, H-8), 5.46 (br s, 1 H, H-7), 6.08 (d, 1 H, H-3), 6.77 (dd, 1 H, NH), and 6.83 (d, 1 H, $J_{NH,5}$ 8.4 Hz, NH); positive ion FABMS (NBA): *m*/*z* 504 $[M+H]^+$, 526 $[M+Na]^+$.

*5,9-Diacetamido-2,6-anhydro-3,5,9-trideoxy-4-*O*-thiocarbamoylmethyl-*D*-glycero-*D*-galacto-non-2-enonic acid* (**6a**).—Transformation of **16** (40 mg, 85 μmol) was performed as described for **4a** to give the compound **6a** (23 mg, 67%); ^{1}H NMR (D_2O) δ: 2.00, 2.04 (s, each 3 H, AcN), 3.27 (dd, 1 H, $J_{8,9a}$ 7.3, $J_{9a,9b}$ 14.0 Hz, H-9a), 3.50–3.60 (m, 2 H, H-7, 9b), 3.92–3.98 (m, 1 H, H-8), 4.43–4.47 (m, 1 H, H-4), 4.23–4.29 (m, 4 H, H-5, H-6, –OCH_2CN), and 5.74 (d, 1 H, $J_{3,4}$ 2.2 Hz, H-3), positive ion FABMS (NBA): *m*/*z* 406 $[M+H]^+$, 428 $[M+Na]^+$. HRFABMS Anal. Calcd for $C_{15}H_{23}N_3NaO_8S$: *m*/*z* 428.1104 $[M+Na]^+$, Found: 428.1096.

*Methyl 5,9-diacetamido-2,6-anhydro-4-*O*-cyanomethyl-3,5,9-trideoxy-*D*-glycero-*D*-galacto-non-2-enonate* (**17**).—To a soln of **15** (16 mg, 0.035 mmol) in 4:3:1 1,4-dioxane–MeOH–H_2O (1.0 mL) was added PPh_3 (14 mg, 0.053 mmol) at rt and the mixture was stirred for 18 h. After removal of the solvent, the residue was chromatographed on silica gel using 10:1 CH_2Cl_2–MeOH to give **17** (14 mg, 85%); $[\alpha]_D + 21.3°$ (*c* 0.36, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 1.98, 2.00 (s, each 3 H, AcN), 2.05, 2.13 (s, each 3 H, AcO), 3.31 (ddd, 1 H, $J_{8,9a}$ 4.6, $J_{NH,9a}$ 5.6, $J_{9a,9b}$ 14.6 Hz, H-9a), 3.75–3.89 (m, 1 H, H-9b), 3.83 (s, 3 H, MeO), 4.26 (1 H, ddd, $J_{4,5} = J_{5,6} = J_{5,NHAc} = 8.9$ Hz, H-5), 4.35, 4.47 (d, each 1 H, J_{gem} 16.7 Hz, –OCH_2CN), 4.41 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-4), 4.46 (dd, 1 H, $J_{6,7}$ 3.8 Hz, H-6), 5.08 (ddd, 1 H, $J_{8,7}$ 3.8 Hz, H-8), 5.42 (dd, 1 H, H-7), 6.09 (d, 1 H, H-3), 6.25 (br s, 1 H, NH), and 6.56 (br s, 1 H, NH); positive ion FABMS (NBA): *m*/*z* 470 $[M+H]^+$, 492 $[M+Na]^+$.

*5,9-Diacetamido-4-*O*-cyanomethyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enonic acid* (**6b**).—Transformation of **17** (31 mg, 0.066 mmol) was performed as described

for **4a** to give compound **6b** (10 mg, 41%); ^{1}H NMR (D_2O) δ: 2.20, 2.25 (s, each 3 H, AcN), 3.48 (dd, 1 H, $J_{8,9a}$ 7.3, $J_{9a,9b}$ 14.0 Hz, H-9a), 3.75 (dd, 1 H, $J_{8,9b}$ 10.5 Hz, H-9b), 4.16 (br s, 1 H, H-8), 4.36–4.50 (m, 3 H, H-5, 6, 7), 4.33, 4.46 (d, each 1 H, J_{gem} 16.5 Hz, –OCH_2CN), 4.64 (m, 1 H, H-4), and 5.97 (d, 1 H, $J_{3,4}$ 1.9 Hz, H-3); positive ion FABMS (NBA): m/z 470 $[M + H]^+$, 492 $[M + Na]^+$.

*5,9-Diacetamido-2,6-anhydro-4-*O*-carbamoylmethyl-3,5-dideoxy*-D-glycero-D-galacto-*non-2-enonic acid* (**6c**).—Transformation of **17** (30 mg, 0.064 mmol) as described for **4b** gave compound **6c** (15 mg, 60%); ^{1}H NMR (D_2O) δ: 2.21, 2.25 (s, each 3 H, AcN), 3.44–3.53 (m, 1 H, H-9a), 3.72–3.96 (m, 1 H, H-9b), 4.13–4.45 (m, 6 H, H-5, H-6, H-7, H-8, –OCH_2CONH_2), 4.64 (br s, 1 H, H-4), and 5.98 (d, 1 H, $J_{3,4}$ 3.0 Hz, H-3); positive ion FABMS (NBA): m/z 412 $[M + Na]^+$. HR-FABMS Anal. Calcd for $C_{15}H_{23}N_3NaO_9$: m/z 412.1332 $[M + Na]^+$, Found: 412.1320.

*5,9-Diacetamido-2,6-anhydro-4-*O*-amidinomethyl-3,5-dideoxy*-D-glycero-D-galacto-*non-2-enonic acid* (**6d**).—Transformation of **17** (28 mg, 0.060 mmol) as described for **4c** afforded the compound **6d** (12 mg, 52%); ^{1}H-NMR (D_2O) δ: 2.20, 2.24 (s, each 3 H, AcN), 3.44–3.52 (m, 1 H, H-9a), 3.72–3.81 (m, 1 H, H-9b), 3.88–3.96 (m, 1 H, H-8), 4.13–4.44 (m, 5 H, H-5, H-6, H-7, –$OCH_2C(=NH)NH_2$), 4.61 (t, 1 H, $J_{3,4} = J_{4,5} = 7.8$ Hz, H-4), and 5.97 (d, 1 H, H-3), positive ion FABMS (NBA): 391 m/z $[M + 2\ H]^+$. HRFABMS Anal. Calcd for $C_{15}H_{25}N_4NaO_8$: m/z 412.1570 $[M + Na]^+$, Found: 412.1555.

Acknowledgements

The authors thank Marukin Shoyu Co., Ltd. (Kyoto, Japan) for a generous gift of Neu5Ac. And also we wish to thank Mrs Tomoko Terada, Institute for Chemical Research, Kyoto University, for the measurement of HR-MS.

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