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

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ABSTRACT

4-*O*-Thiocarbamoylmethyl-Neu5Ac2en **3** has strong inhibitory activity toward human parainfluenza virus type 1 (hPIV-1) sialidase compared with the parent Neu5Ac2en **2**. We synthesized analogs having thiocarbamoylethyl- **4** and thiocarbamoylpropyl group **5** at the C-4 position of **2**. The inhibition degrees of **4** and **5** were weaker than that of thiocarbamoylmethyl analog **3**, indicating a remarkable effect of the carbon chain length in thiocarbamoylalkyl groups at the C-4 position on inhibitory activities against hPIV-1 sialidase.

1. Introduction

Human parainfluenza virus type 1 (hPIV-1), which belongs to genus *Respirovirus*, family *Paramyxovididae*, is a serious human pathogen causing upper and lower respiratory disease and is known to be a cause of laryngotracheobronchitis (croup) in infants and young children[1]; however, there is no known potential inhibitor of hPIV-1 infection. *N*-Acetylneuraminic acid (**1**, Neu5Ac) and various related derivatives, sialic acids, play an important role in various biochemical and biological processes.[2] Influenza sialidase, a key enzyme responsible for propagation of the influenza virus, is a target of drug design. A variety of 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (Neu5Ac2en) analogs (**2**) have been synthesized as competitive sialidase inhibitors.[3] Neu5Ac2en derivatives with structural modifications at the C-4 position are particular candidates for the design of potent inhibitors against anti-paramyxovirus agents.[4] We found that 4-*O*-thiocarbamoylmethyl-Neu5Ac2en (**3**)[5] has strong inhibitory activity toward hPIV-1 sialidase compared with **2** (Fig. 1).

We were interested in comparing inhibitory activity against hPIV-1 sialidase of a few thiocarbamoylalkyl groups in the hydroxyl group at C-4 position. As part of a program aimed at new sialidase inhibitors against hPIV-1, we describe herein the synthesis of analogs having thiocarbamoylethyl- **4** and thiocarbamoylpropyl group **5** at the C-4 position of Neu5Ac2en **2** and their inhibitory activities against hPIV-1 sialidase.

2. Results and discussion

2.1. Chemical synthesis

As outlined in Scheme 1, the synthesis of compounds **4** and **5** began with compounds **7** and **8** as the key intermediate, respectively.

For the synthesis of 4-*O*-cyanoalkylated analogs of Neu5Ac2en **7** and **8**, methyl 5-acetamido-8,9-*O*-isopropylidene-2,3,5-tri-deoxy-D-*glycero*-D-*galacto*-non-2-enopyranoso-nate **6**[6] was chosen as the starting material. First, for the synthesis of **7**, the reaction of **6** with 3-bromopropionitrile in the presence of sodium hydride in dimethylformamide (DMF) at room temperature resulted in the recovery of **6** by β-elimination of 4-*O*-thiocarbamoylethyl moiety of **7**, due to *retro*-Michael reaction under strongly basic conditions (Table 1, entry 1). Selective 4-*O*-cyanoethylation of **6** with 3-bromopropionitrile in the presence of silver oxide and a catalytic amount of tetra-*n*-butylammonium iodide (TBAI) in DMF at 50 °C successfully gave 4-*O*-cyanoethyl derivative **7** in 58% yield (entry 2).

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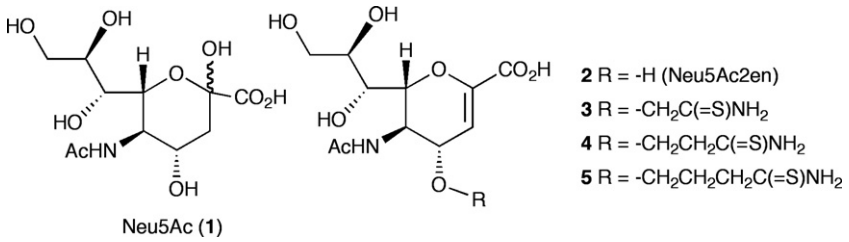
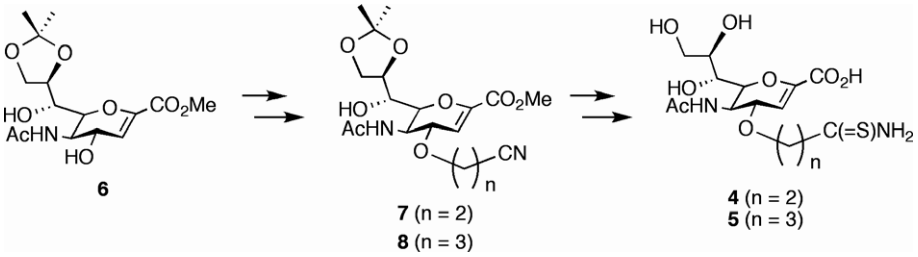
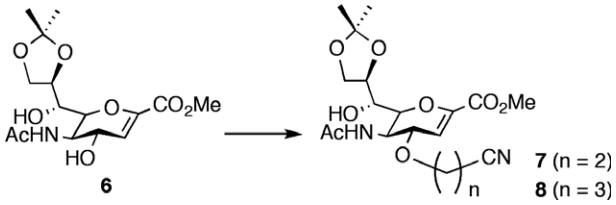


Figure 1.



Scheme 1. Synthesis of **4** and **5**.

Table 1
Synthesis of **7** and **8**



Entry	Conditions	Time (h)	Products	Yields[a] (%)
1	$BrCH_2CH_2CN$, NaH, DMF, rt	1	**7**	N.R.[b]
2	$BrCH_2CH_2CN$ Ag_2O, TBAI, DMF, 50 °C	12	**7**	58
3	CH_2=CHCN, DBU, CH_3CN, 0 °C	12	**7**	70
4	$BrCH_2CH_2CH_2CN$, NaH, DMF, rt	1	**8**	50
5	$BrCH_2CH_2CH_2CN$, Ag_2O, TBAI, DMF, rt	12	**8**	70

[a] Isolated yields alter purification.
[b] No reaction.

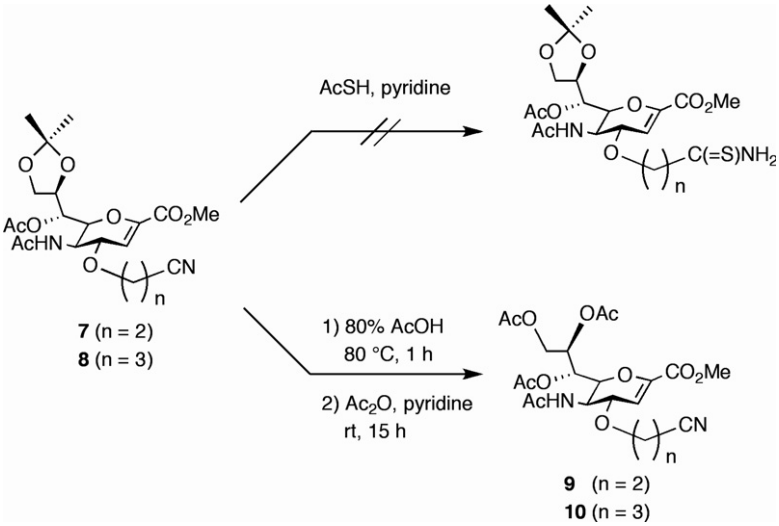
Interestingly, the Michael addition reaction of **6** with 10 molar equivalent of acrylonitrile in the presence of 1.0 molar equivalent of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile at 0 °C smoothly proceeded to give **7** in 70% yield (entry 3). For the preparation of **8**, 4-*O*-cyanopropylation of **6** with 4-bromobutyronitrile in the presence of sodium hydride in DMF at room temperature successfully afforded 4-*O*-cyanopropyl derivative **8** in 50% yield (entry 4). The reaction of 10 molar equivalent of 4-bromobutyronitrile and 5.0 molar equivalent of silver oxide in DMF gave compound **8** in 70% yield (entry 5).

Thiocarbamoylalkylation of the C-4 position of **7** and **8** by a previously reported method[7] with AcSH–pyridine did not proceed. Therefore, the isopropylidene group of **7** and **8** was removed with 80% AcOH at 80 °C for 1 h and the resulting alcohols were subsequently acetylated to give cyanoalkyl compounds **9** and **10** in 92% and 99% yields over two steps, respectively (Scheme 2).

For the conversion of cyanoalkyl groups of **9** and **10** to the thiocarbamoyl group, we examined the thiocarbamoylation of **9** and **10** using AcSH–pyridine in CH_2Cl_2; however, the reaction did not proceed (Table 2, entries 1 and 5). When using $BF_3{\cdot}OEt_2$ instead of pyridine, the reaction gave the expected compound **12** in 63% yield, in contrast to the result of **9** (entries 2 and 6). The thiolysis of nitrile group of **10** with AcSH and $BF_3{\cdot}OEt_2$ involves the intermediacy of the acylthioimidate.[8] Treatment of **9** with AcSH–trimethylsilyl chloride in CH_2Cl_2 resulted in the recovery of **9** (entry 3). Interestingly, the thiocarbamoylation of **9** with AcSH–$BnNH_2$ gave **11** in 33% yield (entry 4).

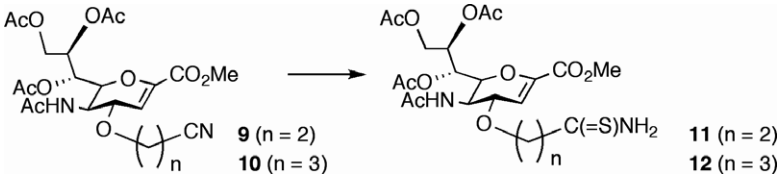
Treatment of **12** with 0.1 M KOH–MeOH (1:1) for 12 h at room temperature afforded the expected compound **5** in 70% yield; however, hydrolysis of **11** with 0.1 M KOH–MeOH (1:1) for 12 h at room temperature led to β-elimination of 4-*O*-thiocarbamoylethyl moiety to give **2** under strong basic conditions. Therefore, we focused on the search for an efficient method in mild conditions for the hydrolysis of methyl ester residue of **11** without β-elimination of 4-*O*-thiocarbamoylethyl moiety.

Pig liver esterase (PLE)[9] has been successfully used in the smooth hydrolysis of β-substituted methyl esters, specifically in substrates susceptible to β-elimination under strong basic conditions.[10] We examined the enzymatic hydrolysis of methyl ester group of **13** by PLE in neutral aqueous conditions. Thus, deprotection of acetyl groups of **11** with trimethylsilyl chloride in MeOH was performed to afford **13** in 76% yield. Hydrolysis of **13** with



Scheme 2. Synthesis of **9** and **10**.

Table 2
Synthesis of **11** and **12**



Entry	Substrate	Conditions	Product	Yield[a] (%)
1	**9**	AcSH, pyridine, CH_2Cl_2, 12 h	**11**	N.R.[b]
2	**9**	AcSH, BF_3OEt_2, CH_2Cl_2, 12 h	**11**	N.R.
3	**9**	AcSH, TMSCl, CH_2Cl_2, 12 h	**11**	N.R.
4	**9**	AcSH, $BnNH_2$, 48 h	**11**	33
5	**10**	AcSH, pyridine, CH_2Cl_2, 12 h	**12**	N.R.
6	**10**	AcSH, BF_3OEt_2, CH_2Cl_2, 12 h	**12**	63

[a] Isolated yields after purification.
[b] No reaction.

PLE (Sigma) in 0.01 M potassium phosphate buffer, pH 7.0, at 35 °C for 20 h successfully afforded **4** in quantitative yield, after purification by chromatography on silica gel and then desalting by Bio-Gel P-2, followed by lyophilization from a H_2O suspension (Scheme 3). This is the first example of enzymatic hydrolysis of methyl ester of sialic acids using PLE in neutral aqueous conditions.

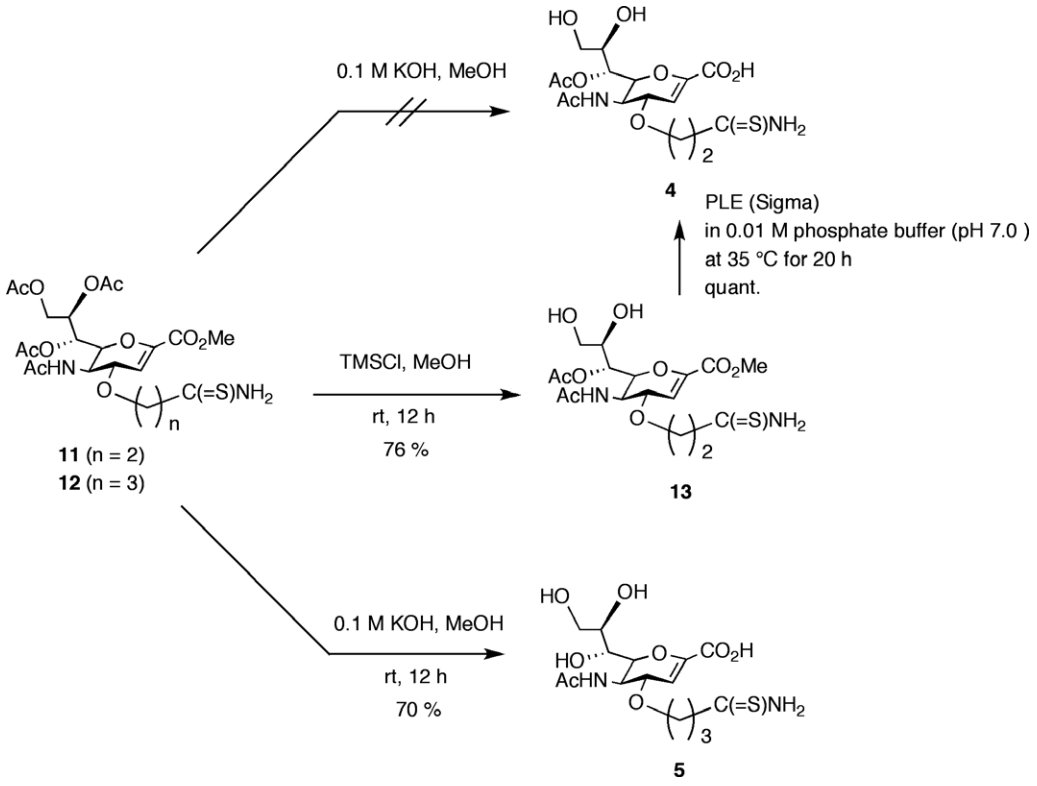
2.2. Biological evaluation

The behavior of compounds **4** and **5** toward hPIV-1 sialidase was tested by our previously reported method.[5b] As can be seen in Table 3, 4-*O*-thiocarbamoylethyl- **4** and 4-*O*-thiocarbamoylpropyl-Neu5Ac **5** had inhibitory activities IC_{50} 68 μM and IC_{50} 102 μM, respectively. However, the degree of inhibition was lower toward hPIV-1 sialidase than 4-*O*-thiocarbamoylethyl-**3** (IC_{50} 9 μM).

In conclusion, 4-*O*-thiocarbamoylethyl-**4** and 4-*O*-thiocarbamoylpropyl-Neu5Ac **5** were synthesized via the key compound **6**. Compounds **4** and **5** exhibited decreased sialidase inhibition compared with **3**. The reason for the lower inhibitory activity of compounds **4** and **5** is unclear in this study, but it was found that the difference in the carbon chain length of the substituent in the hydroxyl group at the C-4 position of **2** might affect inhibitory activities against hPIV-1 sialidase. 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. ^1H 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. 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.



Scheme 3. Synthesis of **4** and **5**.

Table 3
Inhibitory activities of **4** and **5**

Entry	Compound	IC_{50}[a] (μM)
1	**2**	300
2	**3**	9
3	**4**	68
4	**5**	102

[a] Inhibitory activities were determined by the method according to Ref. 5b.

3.1. Methyl 5-acetamido-2,6-anhydro-4-*O*-(2-cyanoethyl)-3, 5-dideoxy-8,9-*O*-isopropylidene-D-*glycero*-D-*galacto*-non-2-enonate (7)

Compound **6** (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 3-bromopropionitrile (55 mg, 0.39 mmol), Ag_2O (302 mg, 1.3 mmol), and TBAI (48 mg, 0.13 mmol), and the mixture was allowed to stir for 48 h in the dark under an argon atmosphere. Insoluble materials were filtered through a Celite 545 and the filtrate was concentrated to dryness. The resulting residue was chromatographed by silica gel with $CHCl_3$–MeOH (50:1) to give **7** (52 mg, 56%). $[\alpha]_D^{24}$ +48.4 (*c* 0.33, $CHCl_3$). IR (KBr): 3286, 2243, 1724, 1645 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.35, 1.39 (s, each 3H), 2.09 (s, 3H), 2.55–2.68 (m, 2H), 3.56 (dd, 1H, *J* = 4.6, 7.5 Hz), 3.66 (ddd, 1H, *J* = 4.6, 5.2, 9.8 Hz), 3.79 (s, 3H), 3.89 (ddd, 1H, *J* = 4.6, 5.2, 9.8 Hz), 4.07 (d, 1H, *J* = 10.9 Hz), 4.09 (dd, 1H, *J* = 4.6, 8.6 Hz), 4.14–4.20 (m, 2H), 4.45 (m, 1H), 4.37 (dd, 1H, *J* = 2.3, 8.6 Hz), 4.52 (d, 1H), 5.73 (d, 1H), 6.00 (d, 1H, *J* = 2.3 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 19.4, 23.1, 25.2, 27.0, 48.1, 52.4, 62.3, 67.2, 70.0, 74.1, 75.0, 77.3, 106.4, 109.2, 118.2, 146.1, 162.1, 173.1. Positive-ion FABMS (NBA): *m*/*z* 399 $[M+H]^+$, 421 $[M+Na]^+$. Positive-ion HR-FABMS (NBA) (*m*/*z*) Calcd for $C_{18}H_{27}N_2O_8$ $[M+H]^+$: 399.1767, Found 399.1744.

For the preparation of **7** using acrylonitrile[11] and DBU, to a solution of **6** (111 mg, 0.32 mmol) and acrylonitrile (170 mg, 3.2 mmol) in CH_3CN (2 mL) was added DBU (49 mg, 0.32 mmol) at 0 °C, and the mixture was allowed to stir for 12 h at the same temperature under Ar. After the addition of saturated aqueous NH_4Cl solution to the reaction mixture, the mixture was extracted with chloroform and the organic layer was washed with saturated aqueous NaCl solution, dried over anhydrous $MgSO_4$, and concentrated to dryness. The residue was chromatographed by silica gel with $CHCl_3$–MeOH (50:1) to give **7** (89 mg, 70%).

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

Under argon, sodium hydride (22 mg, 0.92 mmol) at 0 °C was added to a solution of **6** (245 mg, 0.71 mmol), propargyl bromide (172 mg, 1.42 mmol) in anhydrous DMF (5 mL) was added, and the mixture was stirred for 1 h at the same temperature. After the addition of MeOH (1 mL), the solvent was concentrated to dryness. The residue was chromatographed by silica gel with 50:1 $CHCl_3$–MeOH to give **8** (142 mg, 52%). IR (KBr): 3275, 2254, 1724, 1645 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ:1.33, 1.38 (s, each 3H), 1.83–1.96 (m, 2H), 2.08 (s, 3H), 2.39–2.54 (m, 2H), 3.52–3.56 (m, 2H), 3.73–3.81 (m, 1H), 3.76 (s, 3H), 4.02 (m, 1H), 4.07 (dd, 1H, *J* = 5.2, 9.2 Hz), 4.12–4.18 (m, 2H), 4.23 (dd, 1H, *J* = 2.3, 8.6 Hz), 4.32 (ddd, *J* = 6.3, 5.2, 2.9 Hz), 4.58 (br, 1H), 6.01 (d, 1H, *J* = 2.3 Hz), 6.09 (d, 1H, *J* = 7.5 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ:13.8, 14.1, 23.1, 25.2, 25.3, 27.1, 48.4, 52.5, 65.6, 67.2, 70.1, 74.3, 74.3, 107.2, 109.2, 119.6, 145.7, 162.3, 173.0. Positive-ion FABMS (NBA): *m*/*z* 413 $[M+H]^+$, 435 $[M+Na]^+$. Positive-ion HR-FABMS (NBA) (*m*/*z*) Calcd for $C_{19}H_{29}N_2O_8$ $[M+H]^+$: 413.1924, Found 413.1942.

For the preparation of **8** using 4-bromobutylonitrile and Ag_2O, compound **6** (200 mg, 0.58 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 4-bromobutylonitrile (522 mg, 3.53 mmol), Ag_2O (670 mg, 2.89 mmol), and TBAI (110 mg, 0.29 mmol), and the mixture was allowed to stir for 12 h in the dark under argon. Insoluble materials were filtered through a Celite 545 and the filtrate was concentrated to dryness. The residue was chromatographed by silica gel with $CHCl_3$–MeOH (50:1) to give compound **8** (154 mg, 64%).

3.1.2. Methyl 5-Acetamido-2,6-anhydro-4-*O*-(2-cyanoethyl)-3,5-dideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-2-enonate (9)

Compound **7** (104 mg, 0.261 mmol) was dissolved in 80% aqueous AcOH (3 mL). After stirring for 1 h at 80 °C, the reaction solution was concentrated to dryness, the residue was dissolved in a solution of pyridine (3 mL) and acetic anhydride (1.5 mL) at 0 °C and the mixture was allowed to stir for 12 h at room temperature. The reaction mixture was evaporated to dryness. The resulting residue was chromatographed by silica gel with $CHCl_3$–MeOH (50:1) to give **9** (116 mg, 92%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.96, 2.02, 2.02, 2.09 (s, each 3H), 2.61 (dd, 2H, J = 5.7, 6.3 Hz), 3.73–3.85 (m, 2H), 3.77 (s, 3H), 4.14 (dd, 1H, J = 7.5, 12.0 Hz), 4.19–4.20 (m, 2H), 4.41 (dd, 1H, J = 6.9, 5.2 Hz), 4.52 (dd, 1H, J = 3.5, 12.0 Hz), 5.34 (ddd, 1H, J = 7.5, 3.5, 4.6 Hz), 5.51 (dd, 1H, J = 5.2, 4.6 Hz), 5.96 (br s, 1H), 6.10 (d, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 19.0, 20.7, 20.7, 20.9, 23.2, 47.0, 52.6, 61.9, 63.4, 68.0, 69.9, 72.9, 76.0, 108.3, 117.9, 144.2, 161.9, 170.0, 170.1, 170.4, 170.7. Positive-ion FABMS (NBA): m/z 485 $[M+H]^+$, 507 $[M+Na]^+$. Positive-ion HR-FABMS (NBA) (m/z) Calcd for $C_{21}H_{29}N_2O_{11}$ $[M+H]^+$: 485.1771, Found 485.1786.

3.1.3. Methyl 5-Acetamido-2,6-anhydro-4-*O*-(3-cyanopropyl)-3,5-dideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-2-enonate (10)

The reaction was carried out using compound **8** (61 mg, 0.148 mmol) in a manner similar to the preparation of **9** using sodium hydride as a base to give **10** (99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.86–1.91 (m, 2H), 1.98, 2.04, 2.04, 2.11 (s, each 3H), 2.39–2.52 (m, 2H), 3.64 (ddd, 1H, J = 5.2, 5.7, 12.0 Hz), 3.76 (ddd, 1H, J = 5.7, 6.3, 12.0 Hz), 3.80 (s, 3H), 4.06 (dd, 1H, J = 3.4, 6.3 Hz), 4.16 (dd, 1H, J = 7.4, 12.0 Hz), 4.24 (ddd, 1H, J = 6.3, 6.9, 8.6 Hz), 4.38 (dd, 1H, J = 6.9, 5.2 Hz), 4.53 (dd, 1H, J = 3.4, 12.0 Hz), 5.36 (ddd, 1H, J = 7.4, 3.4, 4.1 Hz), 5.52 (dd, 1H, J = 5.2, 4.1 Hz), 5.70 (d, 1H, J = 8.6 Hz), 6.13 (d, 1H, J = 3.4 Hz). ^{13}C NMR (126 MHz, $CDCl_3$) δ:14.1, 20.7, 20.8, 20.9, 23.3, 25.5, 47.2, 52.6, 61.9, 66.6, 67.9, 70.0, 73.0, 76.1, 108.5, 119.5, 143.9, 162.0, 169.9, 170.1, 170.2, 170.6. Positive-ion FABMS (NBA): m/z 499 $[M+H]^+$, 521 $[M+Na]^+$. Positive-ion HR-FABMS (NBA) (m/z) Calcd for $C_{22}H_{31}N_2O_{11}$ $[M+H]^+$: 499.1928, Found 499.1951.

3.1.4. Methyl 5-Acetamido-2,6-anhydro-4-*O*-(2-thiocarbamoylethyl)-3,5-dideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-2-enonate (11)

Compound **9** (74 mg, 0.152 mmol) was dissolved in thioacetic acid (2 mL). To the solution was added benzylamine (1 mL), and the mixture was stirred for 48 h at room temperature. The reaction mixture was concentrated to dryness. The residue was chromatographed by silica gel with $CHCl_3$–MeOH (50:1) to give **11** (26 mg, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.97, 2.04, 2.06, 2.11 (s, each 3H), 2.61 (m, 2H), 3.81 (s, 3H), 3.90–3.94 (m, 2H), 4.03 (m, 1H), 4.18 (dd, 2H, J = 8.0, 12.0 Hz), 4.35 (dd, 1H, J = 5.7, 6.3 Hz), 4.39 (dd, 1H, J = 6.3, 5.2 Hz), 4.55 (dd, 1H, J = 8.0, 3.4, 12.0 Hz), 5.45 (ddd, 1H, J = 8.0, 3.4, 4.6 Hz), 5.55 (dd, 1H, J = 5.2, 4.6 Hz), 5.64 (d, 1H), 6.20 (d, 1H), 7.53 (br s, 1H), 7.99 (br s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 20.6, 20.7, 20.9, 23.2, 45.4, 46.8, 52.6, 61.6, 67.8, 68.0, 69.7, 72.3, 75.8, 107.5, 143.4, 161., 170.0, 170.1, 170.4, 170.7, 207.7. Positive-ion FABMS (NBA): m/z 519 $[M+H]^+$, 541 $[M+Na]^+$.

3.1.5. Methyl 5-Acetamido-2,6-anhydro-4-*O*-(3-thiocarbamoylpropyl)-3,5-dideoxy-7,8,9-tri-*O*-acetyl-D-*glycero*-D-*galacto*-non-2-enonate (12)

To a solution of compound **10** (170 mg, 0.341 mmol) and thioacetic acid (129 mg, 1.70 mmol) was added $BF_3{\cdot}OEt_2$ (126 mg, 0.89 mmol) at 0 °C and the mixture was stirred for 12 h at room temperature. The reaction mixture was concentrated to dryness. The residue was chromatographed by silica gel with $CHCl_3$–MeOH (50:1) to give **11** (26 mg, 33%). ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.96–2.00 (m, 2H), 1.96, 2.02, 2.02, 2.06 (s, each 3H), 2.71 (dd, 2H, J = 6.9, 7.5 Hz), 3.54 (m, 1H), 3.69 (m, 1H), 3.79 (s, 3 H), 3.88 (dd, 1H, J = 4.0, 4.0 Hz), 4.13 (dd, 1H, J = 7.5, 12.0 Hz), 4.34 (dd, 1H, J = 4.6, 6.9 Hz), 4.40 (ddd, 1H, J = 4.0, 4.6, 4.6 Hz), 4.51 (dd, 1H, J = 4.0, 12.0 Hz), 5.46 (ddd, J = 3.5, 7.5, 4.0 Hz), 5.60 (dd, 1H, J = 6.9, 3.5 Hz), 5.96 (br d, 1H, J = 4.6 Hz), 6.20 (d, 1H, J = 4.0 Hz), 7.66 (br s, 1H), 8.14 (br s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 20.7, 20.8, 20.9, 23.2, 29.2, 41.6, 47.0, 52.6, 61.8, 67.3, 68.3, 69.7, 71.3, 76.0, 108.2, 143.3, 162.0, 170.1, 170.3, 170.3, 170.8, 210.3. Positive-ion FABMS (NBA): m/z 533 $[M+H]^+$, 555 $[M+Na]^+$. Positive-ion HR-FABMS (NBA) (m/z) Calcd for $C_{22}H_{33}N_2O_{11}S$ $[M+H]^+$: 533.1805, Found 533.1793.

3.1.6. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-(3-thiocarbamoylpropyl)-D-*glycero*-D-*galacto*-non-2-enonic acid (5)

A solution of **12** (142 mg, 0.267 mmol) in 0.1 M KOH–MeOH (1:1) (2 mL) was allowed to stir at room temperature for 15 h, and then adjusted to pH 2–3 by Amberlite IRA-120 (H^+). The resin was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed by silica gel with $CHCl_3$/MeOH/H_2O (65:35:5, v/v), and then desalted with an AC Micro Acylizer G1 to give **5** (73 mg, 70%) after lyophilization from a H_2O suspension. ^{1}H NMR (500 MHz, D_2O) δ:1.87–1.93 (m, 2H), 1.97 (s, 3H), 2.57–2.65 (m, 2H), 3.50–3.57 (m, 3H), 3.66 (m, 1H), 3.78 (dd, 1H, J = 2.3, 12.0 Hz), 3.83 (ddd, 1H, J = 5.8, 8.0, 2.3 Hz), 4.11 (dd, 1H, J = 8.6, 10.9 Hz), 4.18 (d, 1H), 4.28 (dd, 1H, J = 2.3, 8.6 Hz), 5.96 (d, 1H). Positive-ion FABMS (NBA): m/z 393 $[M+H]^+$.

3.1.7. Methyl 5-acetamido-2,6-anhydro-4-*O*-(2-thiocarbamoylethyl)-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (13)

To a solution of compound **11** (64 mg, 0.124 mmol) in MeOH (3 mL) was added trimethylsilyl chloride (18 mg, 0.161 mmol) at room temperature and the mixture was allowed to stir for 3 h at the same temperature. The reaction mixture was concentrated to dryness. The residue was chromatographed by silica gel with $CHCl_3$/MeOH/H_2O (65:35:5, v/v) to give **13** (37 mg, 76%). ^{1}H NMR (500 MHz, D_2O) δ:1.97 (s, 3H), 2.72–2.81 (m, 2H), 3.53–3.57 (m, 2H), 3.73 (s, 3H), 3.78 (dd, 1H, J = 2.9, 12.0 Hz), 3.82 (ddd, 1H, J = 8.6, 2.9, 5.8 Hz), 3.87 (m, 1H), 3.99 (m, 1H), 4.10 (dd, 1H, J = 9.2, 10.9 Hz), 4.21 (d, 1H), 4.29 (dd, 1H, J = 2.3, 9.2 Hz), 6.11 (d, 1H, J = 2.3 Hz). Positive-ion FABMS (NBA): m/z 393 $[M+H]^+$. Positive-ion HR-FABMS (NBA) (m/z) Calcd for $C_{15}H_{25}N_2O_8S$ $[M+H]^+$: 393.1332, Found 393.1337.

3.1.8. 5-Acetamido-2,6-anhydro-3,5-dideoxy-4-*O*-(3-thiocarbamoylethyl)-D-*glycero*-D-*galacto*-non-2-enonic acid (4)

A solution of **13** (8.4 mg, 0.021 mmol) in 0.01 M potassium phosphate buffer (pH 7.0) (2 mL) was incubated with PLE (Sigma, 7.0 mg) at 35 °C for 22 h, the insoluble materials were filtered off and the filtrate was evaporated to dryness. The residue was chromatographed by silica gel with $CHCl_3$/MeOH/H_2O (65:35:5, v/v), and then desalted with an AC Micro Acylizer G1 to give **5** (7.9 mg, quant.) after lyophilization from a H_2O suspension. ^{1}H NMR (500 MHz, D_2O) δ:1.86 (s, 3H), 2.46 (m, 2H), 3.46 (m, 2H), 3.70 (m, 2H), 3.78 (m, 1H), 3.98 (m, 1H), 4.16 (m, 3H), 5.98 (d, 1H, J = 2.9 Hz). Positive-ion FABMS (NBA): m/z 401 $[M+Na]^+$.

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