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Studies on Sialic Acids. XIV. Lactone Derivatives of *N*-Acetylneuraminic Acid

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Benzoylation of *N*-acetylneuraminic acid (Neu5Ac) gave a variety of partially benzoylated bicyclic 1,7-lactone derivatives and a perbenzoylated bicyclic 1,4-lactone derivative in good yields. The structures of these compounds were elucidated mainly by means of proton nuclear magnetic resonance spectroscopy. Further, pivaloylation and ethoxycarbonylation of Neu5Ac also gave the corresponding acylated bicyclic lactone derivatives. These results allowed us to postulate a mechanism for the formation of bicyclic lactone derivatives in the acylation of Neu5Ac. Furthermore, treatment of Neu5Ac with diazomethane in an acidic methanol solution gave methyl α-D-neuraminoside methyl ester and a monocyclic 2-*O*-methyl-γ-lactone derivative in 18% and 29% yields, respectively. These results provided information about the equilibrium of Neu5Ac in basic and acidic media.

Keywords——*N*-acetylneuraminic acid; benzoylation; diazomethane; ^{1}H-NMR; bicyclic 1,4-lactone; bicyclic 1,7-lactone; monocyclic γ-lactone; equilibrium

Introduction

N-Acetylneuraminic acid (Neu5Ac) (**1**) is expected to undergo intramolecular lactonization under acidic or basic conditions, giving the corresponding lactone derivative. It was reported by Derevitskaya *et al.*[1] and Khorlin and Privalova[2] in the 1960's that **1** gave a bicyclic or a monocyclic 1,4-lactone derivative on condensation using *N*,*N'*-dicyclohexylcarbodiimide or on direct acetylation, but the structures of the products were not elucidated in detail. In the case of 3-deoxy-D-*manno*-2-octulosonic acid (KDO), which is a keto-aldosonic acid similar to **1**, there have already been reports on the formation of several lactone derivatives under conditions of acylation and esterification, and detailed elucidation of their structures by means of proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy.[3,4]

We have examined the syntheses of biologically active derivatives of sialic acid.[5] Protection of carboxyl and hydroxyl groups of **1** is very important to prepare key intermediates. Therefore, it was also necessary to examine in detail the lactone derivatives formed in the protection reaction of functional groups of **1**, and the results of such examinations have been reported in previous papers.[6,7] We now wish to report new results concerning the formation of the lactone derivatives of **1**, and to summarize the behavior of **1** in basic and acidic media. That is, a variety of partially benzoylated bicyclic 1,7-lactone derivatives and a new perbenzoylated bicyclic 1,4-lactone derivative were obtained in good yields by benzoylation of **1** under various conditions, and these products led us to propose a mechanism for the formation of bicyclic lactone derivatives during acylation of **1**. Further, other acylations of **1** such as pivaloylation and ethoxycarbonylation also gave mainly the corresponding bicyclic lactone derivatives. Furthermore, in the preparation of Neu5Ac methyl ester by treatment of **1** with diazomethane, further addition of diazomethane in an acidic solution gave a monocyclic 2-*O*-methyl-γ-lactone derivative besides methyl α-D-neuraminoside methyl ester.

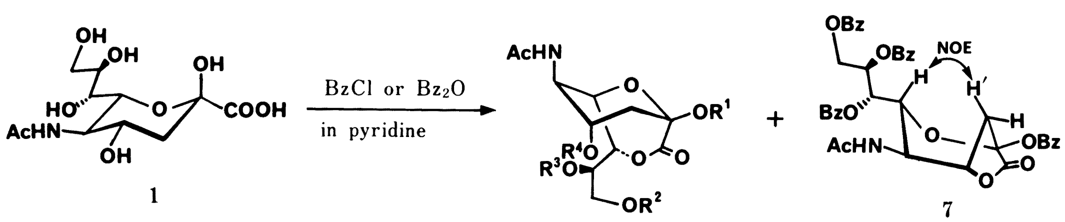
Results and Discussion

We have already reported in previous papers[6,7] that 5-acetamido-2,4,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosono-1,7-lactone (**2**) was formed as a by-product in direct acetylation of **1** and alkyl ester derivatives of **1** were formed during esterification of the carboxyl group of **1** with cesium carbonate and alkyl halides. Further, the esters were intramolecularly lactonized by addition of an excess of reagents to form the corresponding more stable monocyclic γ-lactone derivatives of **1**, such as 5-acetamido-2-*O*-methyl-, -allyl-, or -benzyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**3**, **4**, or **5**) as shown in Chart 4. The structures of these compounds, including the absolute configuration, have been elucidated mainly by means of ^{1}H-NMR spectroscopy and X-ray analysis.

We also examined the synthesis of bicyclic lactone derivatives by benzoylation of **1**. Acetylation of **1** using acetic anhydride in pyridine gave a bicyclic 1,7-lactone derivative (**2**) in only 6% yield, but the benzoylation of **1** gave mainly bicyclic lactone derivatives. Benzoylation of **1** with benzoyl chloride in pyridine gave the per-*O*-benzoylated bicyclic 1,7-lactone derivative (**6**) as a main product, together with a new per-*O*-benzoylated bicyclic 1,4-lactone derivative (**7**), and 2,8,9-tri-*O*-benzoylated bicyclic 1,7-lactone derivative (**8**), in 80% total yield. The sterically hindered 7- or 4-OH was not benzoylated owing to low reactivity to benzoyl chloride and it was attacked intramolecularly by the mixed anhydride of 1-COOH to form the δ- or γ-lactone. Furthermore, under the condition of benzoylation of **1** with benzoic anhydride in pyridine, the use of 1.1 eq of benzoic anhydride gave only the 2-*O*-benzoylated bicyclic 1,7-lactone derivative (**10**) in 47% yield, while the use of 3.3 eq of benzoic anhydride gave **8**, the 2,9-di-*O*-benzoylated bicyclic 1,7-lactone derivative (**9**), and **10**. These results suggest that the bicyclic lactone derivatives are formed as shown in Chart 2.

That is, 1-COOH of **1** first forms a mixed anhydride [A], which is attacked by 2-OH, to give 2-*O*-benzoylated Neu5Ac [B]. The intermediate [B] is again converted into the mixed anhydride [C]. The intermediate [C] is in equilibrium among $^{1}C_{4}$(D), $^{5,2}B$(D), and $^{4}C_{1}$(D) conformers, and the 7- or 4-OH participates in intramolecular lactonization. The bicyclic 1,4-lactone derivative predictable from the $^{4}C_{1}$(D) conformation is presumably not formed owing to the large strain in its structure. Subsequently, 9-, 8-, and 4-OH are successively benzoylated.

Next, we examined acylation of **1** using pivaloyl chloride as a bulky acylating agent, which is sterically hindered. However, that gave the corresponding pivaloylated bicyclic 1,7-lactone derivatives (**11**—**13**) in 96% total yield, as shown in Chart 3. Similarly we examined acylation



Bz = benzoyl

	R^1	R^2	R^3	R^4
6	Bz	Bz	Bz	Bz
8	Bz	Bz	Bz	H
9	Bz	Bz	H	H
10	Bz	H	H	H

Chart 1. Benzoylation of Neu5Ac (**1**)

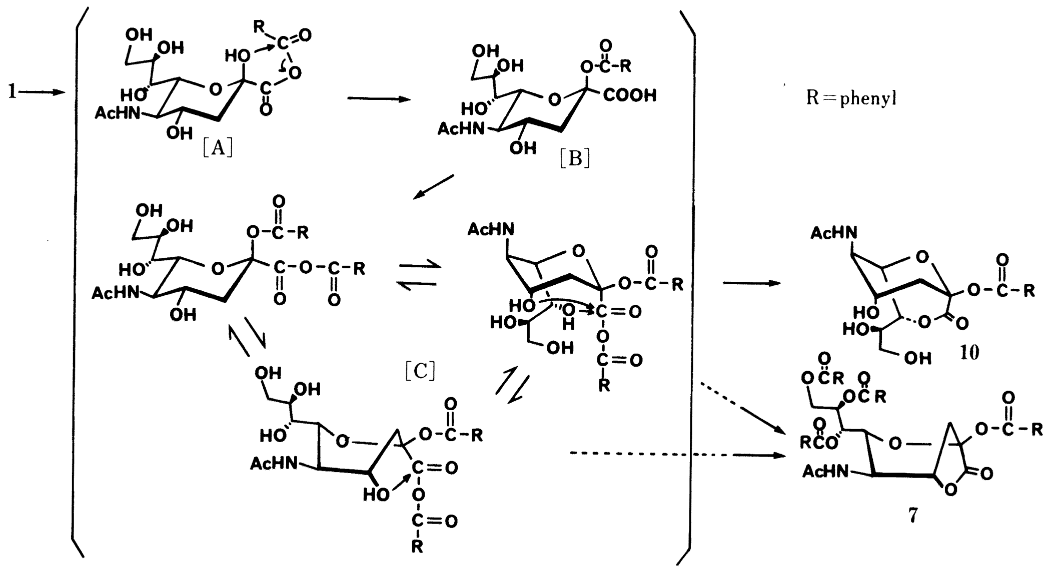
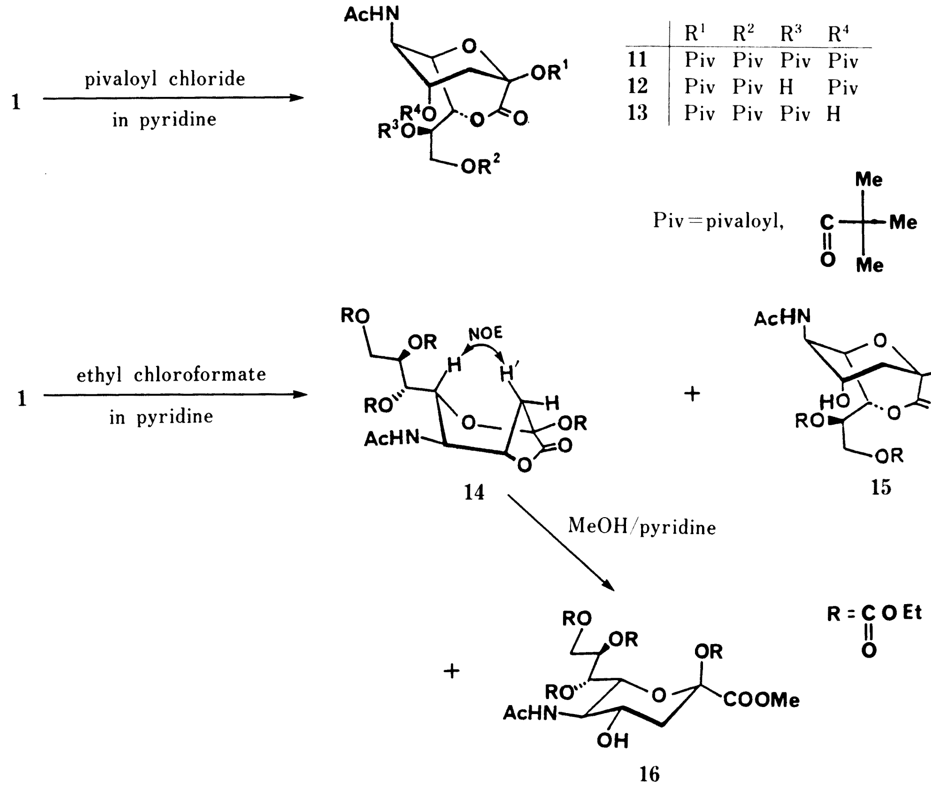


Chart 2. Benzoylation Mechanism



	R^1	R^2	R^3	R^4
11	Piv	Piv	Piv	Piv
12	Piv	Piv	H	Piv
13	Piv	Piv	Piv	H

Chart 3. Pivaloylation and Ethoxycarbonylation of Neu5Ac (**1**)

of **1** using ethyl chloroformate as an acylating agent, which has an electrophilically weakened carbonyl group. However, that also gave mainly the ethoxycarbonylated bicyclic 1,4- and 1,7-lactone derivatives (**14**, **15**), as shown in Chart 3. Compound **16** might arise from the

Table I. Correlation between the Stereochemistry of Protons and the Conformation of Neu5Ac

	${}^{1}C_{4}$ (D)	${}^{4}C_{1}$ (D)	${}^{5,2}B$ (D)
H-4	ax	eq	eq
H-5	ax	eq	ax
H-6	ax	eq	ax

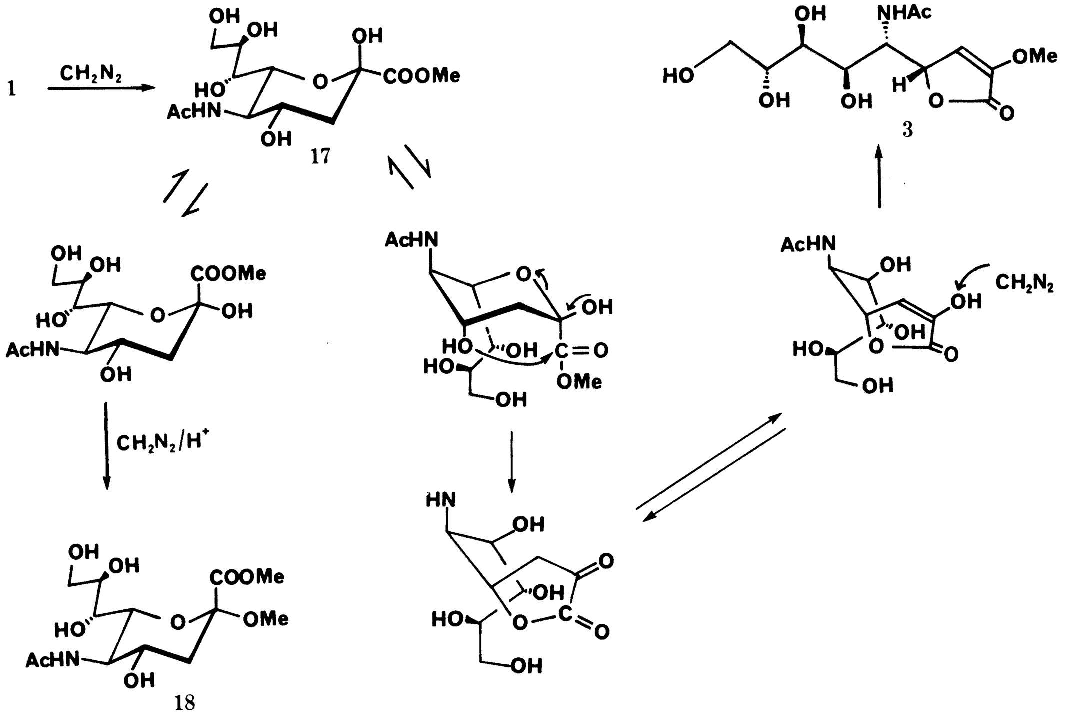


Chart 4. Esterification of Neu5Ac (**1**) by Diazomethane (CH_2N_2)

treatment of **14** with methanol at the termination of the reaction. In fact, we confirmed by thin layer chromatography (TLC) that **14** was converted into **16** on adding methanol to **14** in pyridine. We established that 4-OH of **16** was not ethoxycarbonylated as follows. In the ^{1}H-NMR spectrum of **17** obtained by acetylation of **16** with acetic anhydride and pyridine, the methine proton H-4 was shifted to lower field at 1.22 ppm as compared with that of **16**. Thus it was proved that **1** is acylated at its anomeric hydroxyl group and then gives the bicyclic lactone derivatives even when acylating agents are used which cannot readily form intermediate [B] in Chart 2.

The ^{1}H-NMR and the other data for all synthesized lactone derivatives are summarized in Tables II—IV. The assignment of ^{1}H-NMR signals of each bicyclic 1,7-lactone derivative was easy because the observed pattern was similar to that of **2**.[6] That is, a characteristic ^{1}H-NMR pattern of a bicyclic 1,7-lactone derivative due to the ${}^{4}C_{1}$(D) conformation was observed. The signals of the methylene protons of C-3 were closer to each other and were broader than those of the compounds in ${}^{1}C_{4}$(D) conformation, **11** and **12**. The couplings between H-3 and H-4 ($J_{3,4}=1.7$—4.8 Hz), and H-3′ and H-4 ($J_{3',4}=0$—3.5 Hz) show H-4 to be equatorial. Further, the couplings between H-4 and H-5 ($J_{4,5}=0$—1.5 Hz), H-5 and H-6

TABLE II. ^{1}H-NMR Data for Synthesized Neu5Ac Derivatives in $CDCl_3$ or DMSO-d_6[a)]

Compound	Chemical shifts (δ) (multiplicities)										
	H-3	H-3′	H-4	H-5	H-6	H-7	H-8	H-9	H-9′	NH	NAc
6	2.55	2.64	5.56	4.47	4.45	5.07	6.04	4.90	5.13	6.26	2.02
	(br dd)	(dd)	(br d)	(ddd)	(dd)	(dd)	(ddd)	(dd)	(dd)	(d)	(s)
7	2.64	4.13	5.01	3.54	5.13	5.78	5.88	4.48	4.89	6.29	2.08
	(dd)	(d)	(d)	(dd)	(dd)	(dd)	(ddd)	(dd)	(dd)	(d)	(s)
8	2.29	2.44	4.31	4.29	4.43	5.08	5.88	4.82	5.13	6.37	1.96
	(br dd)	(br dd)	(br d)	(br d)	(s)	(d)	(ddd)	(dd)	(dd)	(d)	(s)
9[a)]	2.12	2.46	4.01	4.11	4.56	4.49	4.21	4.43	4.57	8.42	1.91
	(dd)	(br dd)	(br d)	(br d)	(s)	(d)	(ddd)	(dd)	(dd)	(d)	(s)
10[a)]	2.10	2.42	3.96	3.91	4.52	4.31	3.83	3.59	3.69	8.36	1.89
	(dd)	(br dd)	(br s)	(br d)	(s)	(d)	(ddd)	(dd)	(dd)	(d)	(s)
11	2.20	2.31	5.14	4.19	4.12	4.64	5.44	4.31	4.74	6.12	2.04
	(dd)	(br dd)	(br m)	(br m)	(s)	(d)	(ddd)	(dd)	(dd)	(d)	(s)
12	2.20	2.32	5.14	4.18	4.63	4.15	4.16	4.18	4.53	6.06	2.06
	(dd)	(br dd)	(br m)	(br dt)	(br s)	(m)	(m)	(m)	(dd)	(d)	(s)
13	2.10	2.26	4.22	4.14	4.13	4.73	5.36	4.30	4.74	6.19	2.03
	(dd)	(br dd)	(br s)	(br d)	(s)	(d)	(ddd)	(dd)	(dd)	(d)	(s)
14	2.54	3.98	5.03	3.49	4.71	5.05	5.14	4.28	4.52	6.11	2.03
	(dd)	(d)	(d)	(dd)	(dd)	(dd)	(ddd)	(dd)	(dd)	(d)	(s)
15	2.11	2.31	4.25	4.16	4.29	4.84	5.25	4.47	4.74	6.34	2.03
	(dd)	(br dd)	(ddd)	(ddd)	(d)	(d)	(ddd)	(dd)	(dd)	(d)	(s)
16	1.89	2.60	4.05	3.85	4.08	5.32	5.02	4.30	4.67	5.99	2.02
	(dd)	(dd)	(ddd)	(dd)	(dd)	(dd)	(ddd)	(dd)	(dd)	(d)	(s)
17	2.12	2.57	5.27	4.29	4.12	5.30	5.02	4.25	4.63	5.36	2.01
	(dd)	(dd)	(ddd)	(ddd)	(dd)	(dd)	(ddd)	(dd)	(dd)	(br d)	(s)
18[a)]	1.72	2.03	3.85	3.50	3.75	3.18	3.50	3.29	3.60	8.10	1.90
	(dd)	(dd)	(ddd)	(dd)	(dd)	(dd)	(ddd)	(dd)	(dd)	(d)	(s)
19[a)]	1.55	2.46	3.54	3.54	3.42	3.26	3.63	3.40	3.63	7.86	1.87
	(dd)	(dd)	(—[b)])	(—[b)])	(—[b)])	(br d)	(ddd)	(dd)	(dd)	(d)	(s)
3[a)]	6.29		5.44	4.22	3.83	3.26	3.51	3.42	3.62	7.73	1.77
	(d)		(t)	(ddd)	(d)	(d)	(ddd)	(dd)	(dd)	(d)	(s)

($J_{5,6}=0$—1.0 Hz), and H-6 and H-7 ($J_{6,7}=0$—1.0 Hz) were very small, which suggests that the methine protons of C-5 and C-6 are equatorial, that is, there is a change from $^1C_4$(D) to $^4C_1$(D) conformation, as shown in Table III. Furthermore, the positions of benzoylation were assigned from the number of benzoyl groups and by comparing the chemical shifts of methine protons with those of compound **6**, the hydroxyl groups of which were all benzoylated. Compound **8** was proved to be the 2,8,9-tri-*O*-benzoylated bicyclic 1,7-lactone derivative (three benzoyl groups and only H-4 was shifted to higher field at 1.25 ppm when compared with that of **6**). In a similar way, **9** and **10** were proved to be 2,9-di-*O*-, and 2-*O*-benzoylated bicyclic 1,7-lactone derivatives, respectively. In infrared (IR) spectra of these bicyclic 1,7-lactone derivatives (**6**, **8**, **9**, and **10**), only the lactone carbonyl stretching band of compound **10** overlapped with the ester carbonyl stretching band at 1735 cm^{-1}, while those of the other compounds were observed at 1765—1772 cm^{-1}.

Compound **7** afforded characteristic IR and ^{1}H-NMR data different from those of the other bicyclic 1,7-lactone derivatives. In the IR, the lactone carbonyl stretching band was observed at 1820 cm^{-1}, higher wave number in comparison with that of bicyclic 1,7-lactone derivatives. This suggests that compound **7** consists of a smaller-membered ring than the six-membered ring of the bicyclic 1,7-lactone derivative. In the ^{1}H-NMR, all protons of **7** were assigned on the basis of decoupling experiments. The methylene protons of C-3 were observed

Table II. (continued)

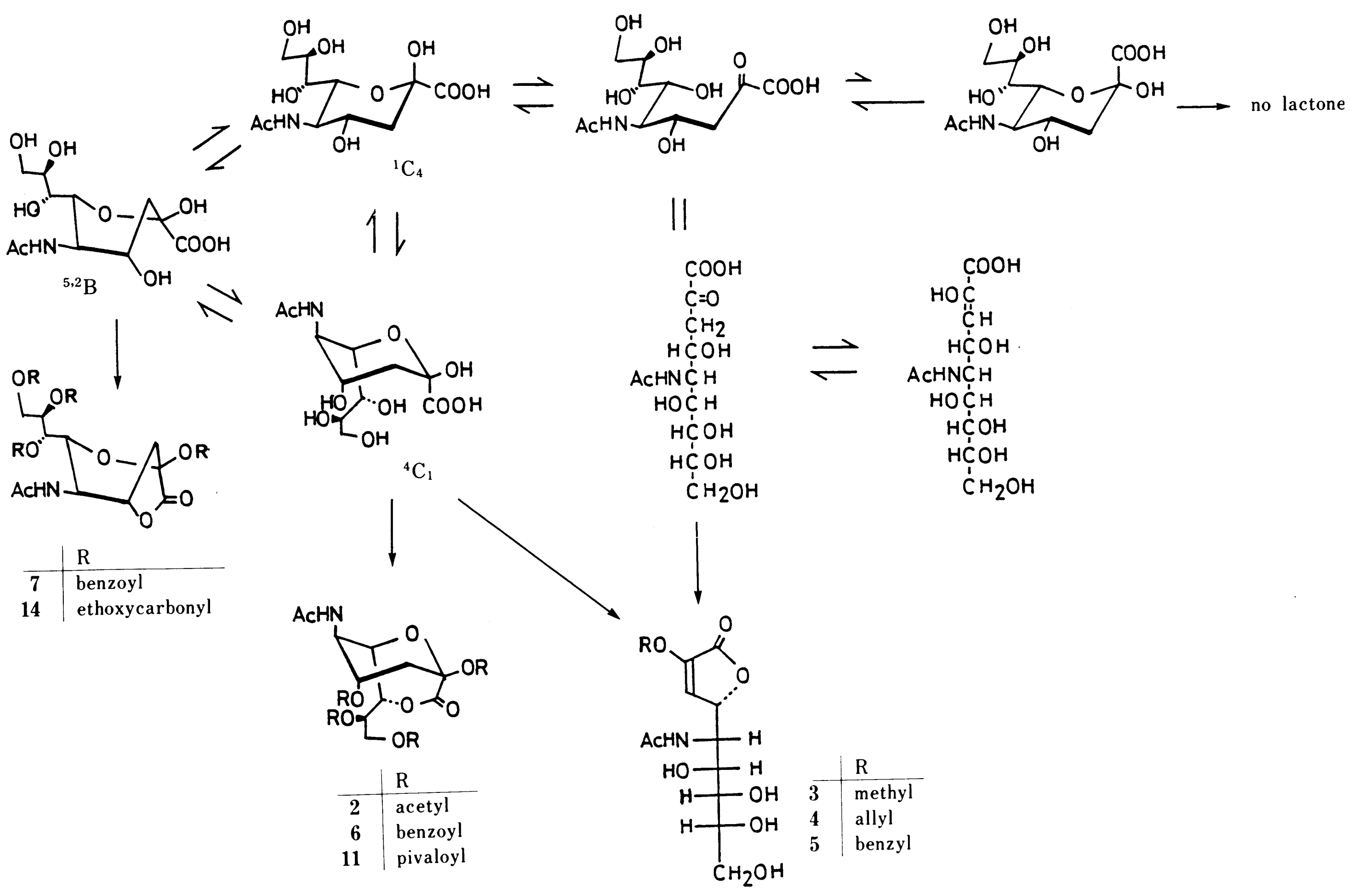
Compound	Chemical shifts (δ) (multiplicities)								
	OMe-2	COOMe	OH-2	OH-4	OH-6	OH-7	OH-8	OH-9	Others
6									7.25—8.07 (m × 2, phenyl × 4)
7									7.35—8.05 (m × 2, phenyl × 4)
8				1.87 (br s)					7.34—8.03 (m × 2, phenyl × 3)
9[a)]				5.91 (d)			5.73 (d)		7.54—8.08 (m × 4, phenyl × 2)
10[a)]				5.69 (br s)			5.26 (d)	4.70 (t)	7.57—7.99 (m × 3, phenyl × 1)
11									1.21, 1.20, 1.19, 1.18 (s × 4, OPiv × 4)
12							3.21 (d)		1.23, 1.22, 1.20 (s × 3, OPiv × 3)
13				3.88 (br s)					1.22 (s × 1, OPiv × 1), 1.18 (s × 1, OPiv × 2)
14									1.26—1.37 (t × 4), 4.14—4.28 (q × 4), OCOOEt × 4
15				4.03 (br s)					1.31, 1.32, 1.33 (t × 3), 4.20—4.25 (q × 3), OCOOEt × 3
16				3.40 (br d)					1.28—1.34, 4.17—4.22 (t × 3, q × 3, OCOOEt × 3), 3.81 (s, COOMe)
17									1.24—1.36, 4.14—4.25 (t × 3, m, OCOOEt × 3), 3.80 (s, COOMe), 1.90 (s, OAc)
18[a)]		3.71 (s)	6.43 (d)	4.83 (d)		4.55 (d)	4.33 (d)	4.22 (t)	
19[a)]	3.26 (s)	3.75 (s)		4.50 (d)		5.02 (d)	3.94 (d)	4.22 (t)	
3[a)]	3.68 (s)				4.73 (d)	4.30 (d)	4.49 (d)	4.39 (t)	

a) Compounds in DMSO-d_6 were measured at 60 °C; after adding D_2O, their splitting patterns were assigned. *b*) Not assigned owing to the complexity of the spectrum.

in very low field ($\delta_{H\text{-}3}$ 2.64 ppm, $\delta_{H\text{-}3'}$ 4.13 ppm) compared with those of bicyclic 1,7-lactone derivatives. No coupling between H-3′ and H-4 ($J_{3',4}$) or H-4 and H-5 ($J_{4,5}$) was observed. The coupling between H-3 and H-4 ($J_{3,4}=4.4$ Hz) was small. These results show H-4 to be equatorial. The coupling between H-5 and H-6 ($J_{5,6}=10.3$ Hz) proves H-5 and H-6 to be in axial conformation. Therefore compound **7** is suggested to take ^{5,2}B(D) conformation (Table I). The ^{5,2}B(D) conformation was confirmed by means of nuclear Overhauser effect (NOE) experiments. That is, on irradiation of H-6, NOE was observed at H-3′ (6%) but not at H-3 (0%). On irradiation of H-3′, NOEs were observed at H-3 (14%) and H-6 (11%). On irradiation of H-3, NOE was observed at H-3′ (13%), but not at H-6 (0%). These results show that NOE is present between H-3′ and H-6, as shown in Chart 1. Therefore, it is established that compound **7** takes the bicyclic ^{5,2}B(D) conformation.

The structures of the bicyclic lactone derivatives (**11**—**16**) produced by pivaloylation and ethoxycarbonylation were also established in a similar manner.

Next, we examined the esterification of **1** using diazomethane. Treatment of **1** with diazomethane in aqueous methanol solution gave Neu5Ac methyl ester (**17**) in 78% yield. Further treatment of **17** with diazomethane in a hydrochloric acid–methanol solution



	R
7	benzoyl
14	ethoxycarbonyl

	R
2	acetyl
6	benzoyl
11	pivaloyl

	R
3	methyl
4	allyl
5	benzyl

Chart 5. Equilibrium of Neu5Ac (**1**) and Lactone Derivatives

TABLE III. ^{1}H-NMR Data for Synthesized Neu5Ac Derivatives in $CDCl_3$ or DMSO-d_6[a)]

Compound	First-order coupling constants (Hz)										
	3,3′	3,4	3′,4	4,5	5,6	6,7	7,8	8,9	8,9′	9,9′	5,NH
6	−14.8	4.8	1.0	1.5	1.0	1.0	9.1	3.5	2.4	−12.8	8.5
7	−13.2	4.4	0	0	10.3	1.5	7.7	5.5	1.9	−12.5	7.3
8	−13.8	3.0	−0[b)]	−0[b)]	0	0	6.7	5.7	2.4	−12.7	8.2
9[a)]	−13.5	1.7	3.3	−0[b)]	0	0	9,.2	4.9	2.5	−11.5	7.7
10[a)]	−13.5	1.8	3.5	−0[b)]	0	0	9.1	4.9	2.8	−11.5	7.9
11	−15.0	3.9	−1.5	—[c)]	0	0	8.5	3.5	2.3	−12.8	8.5
12	−14.8	4.0	−1.5	1.2	1.2	0	8.0	—[c)]	—[c)]	−13.2	8.5
13	−14.2	3.3	−1.5	−0[b)]	−0[b)]	−0[b)]	7.0	5.0	2.5	−12.5	8.5
14	−13.3	5.5	0	0	10.7	1.5	8.6	3.5	2.2	−12.5	6.9
15	−14.0	3.7	2.4	2.7	1.1	−0[b)]	8.1	4.1	2.3	−12.9	8.4
16	−13.6	11.2	4.8	10.3	1.8	1.8	6.0	6.0	2.2	−12.4	8.4
17	−13.5	11.5	5.0	10.5	10.5	2.1	6.0	6.0	2.5	−12.5	10.5
18[a)]	−12.8	11.7	5.1	10.7	10.5	1.6	8.9	6.8	2.7	−11.0	8.4
19[a)]	−12.5	12.0	3.5	—[c)]	—[c)]	0	9.5	6.2	2.8	−12.2	6.5
3[a)]		1.8		1.8	10.2	0	8.4	6.0	3.1	−11.2	9.5

Compound	First-order coupling constants (Hz)					
	3,OH-2[d)]	4,OH-4	6,OH-6	7,OH-7	8,OH-8	9,OH-9
8		−0[b)]				
9[a)]		5.1			0	
10[a)]		−0[b)]			6.3	5.2
12					4.0	
13		−0[b)]				
15		−0[b)]				
16		4.8				
18[a)]	2.2	6.2		4.8	5.5	6.0
19[a)]		5.2		4.4	4.6	5.5
3[a)]			8.1	7.3	5.5	5.6

a) See Table I. *b*) Signal was broadened. *c*) Not assigned owing to the complexity of the spectrum. *d*) Observed between OH-2 and H-3_{ax}.

(pH = 1) gave several products on TLC. They were chromatographed on silica gel, giving mainly methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)-onate (**18**) and 5-acetamido-2-*O*-methyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**3**), in 18% and 29% yields, respectively. This result suggests that the α-anomer of **17** affords **18**, and the β-anomer of **17** does not afford methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate, but a more stable monocyclic γ-lactone derivative (**3**). The formation mechanism of these products is suggested to be as shown in Chart 4. That is, a monocyclic γ-lactone derivative (**3**) is formed from the β-anomer of **17** through a change from $^{1}C_{4}$(D) to $^{4}C_{1}$(D) conformation and then by intramolecular ester exchange reaction with retention of C-4 stereochemistry.[7)]

Conclusion

Dreiding models demonstrate that only the β-anomer is subject to natural intramolecular lactonization in sialic acids.[8)] We could efficiently synthesize all lactone derivatives of Neu5Ac

Table IV. Physical Data for Synthesized Neu5Ac Derivatives

Compound	Formula	MS	Analysis (%) Calcd (Found)			mp (°C)	Rf	$[\alpha]_D$	(Temp.) (Conc.) (Solv.)	IR ν_{max}^{KBr} cm^{-1}				
			C	H	N					NH, OH	Lactone	Ester	Amide I	Amide II
6	$C_{39}H_{33}NO_{12}$	707 (M$^+$)[a]	66.20 (65.63	4.67 4.78	1.98 1.93)	—[d]	0.71[f]	+23°	23 °C $c=1$ MeOH	3340	1772	1723	1670	1530
7	$C_{39}H_{33}NO_{12}$	707 (M$^+$)[a]	66.20 (65.69	4.67 5.23	1.98 1.79)	130— 132	0.64[f]	+45°	23 °C $c=1$ MeOH	3280	1820	1740	1665	1540
8	$C_{32}H_{29}NO_{11}$	604 (M$^+$)[b]	63.68 (63.87	4.81 4.93	2.32 2.26)	145— 147	0.50[f]	+0.9°	25 °C $c=1$ MeOH	3360	1770 (sh)	1725	1660	1530
9	$C_{25}H_{25}NO_{10}$	500 (M+H)[c]	60.12 (60.23	5.01 5.05	2.81 2.83)	197— 199	0.31[f]	+41.4°	26 °C $c=1$ DMSO	3470 3330	1765	1720	1670	1550
10	$C_{18}H_{21}NO_9$	396 (M+H)[c]	54.68 (54.39	5.32 5.48	3.54 3.45)	180— 181	0.50[g]	+24.7°	19 °C $c=1$ MeOH	3450 3250		1735	1640	1550
11	$C_{31}H_{49}NO_{12}$	628 (M+H)[b]	59.33 (58.67	7.81 7.77	2.23 2.33)	—[d]	0.48[h]	+43.6	19 °C $c=1$ $CHCl_3$	3370	1760	1740	1680	1535
12	$C_{26}H_{41}NO_{11}$	544 (M+H)[b]	57.46 (56.49	7.55 7.53	2.58 2.46)	—[d]	0.23[h]	+35.5	19 °C $c=1$ $CHCl_3$	3510 3370	1760	1740	1667	1540
13	$C_{26}H_{41}NO_{11}$	544 (M+H)[b]	57.46 (55.72	7.55 7.39	2.58 2.43)	—[d]	0.18[h]	+22.1°	19 °C $c=1$ $CHCl_3$	3510 3350	1755	1742	1662	1540
14	$C_{23}H_{33}NO_{16}$	580 (M+H)[b]	47.67 (47.76	5.70 5.66	2.42 2.29)	171— 172	0.25[h]	+26.3°	19 °C $c=1$ $CHCl_3$	3270	1820	1758	1658	1562
15	$C_{20}H_{29}NO_{14}$	508 (M+H)[b]	47.34 (47.60	5.72 5.87	2.76 2.76)	—[d]	0.14[h]	+8.0°	19 °C $c=1$ $CHCl_3$	3470 3350	1758		1660	1535
16	$C_{24}H_{37}NO_{17}$	536 (M−75)[b]	47.14 (46.82	6.06 5.98	2.29 2.10)	163— 164	0.17[h]	−9.8°	19 °C $c=1$ $CHCl_3$	3470 3375		1755	1662	1540
17	$C_{26}H_{39}NO_{18}$	653 (M$^+$)[a]	47.78 (47.25	5.97 6.26	2.14 2.02)	—[d]	0.40[h]	−23.8°	19 °C $c=0.2$ $CHCl_3$	3250		1755	1660	1562
18	$C_{12}H_{21}NO_9$	324 (M+H)[c]	44.58 (44.47	6.55 6.54	4.33 4.22)	179— 181[e] (dec.)	0.20[g]	−27.6°[e]	18 °C $c=1$ H_2O	3450 3300		1745	1640	1545
19	$C_{13}H_{23}NO_9$	338 (M+H)[c]	46.29 (46.26	6.82 6.89	4.15 3.93)	181— 182	0.37[g]	−5.1°	26 °C $c=1$ MeOH	3460 3225		1730	1645	1560
3	$C_{12}H_{19}NO_8$	306 (M+H)[c]	47.21 (47.40	6.23 6.34	4.59 4.39)	169— 170	0.28[g]	−79.0°	24 °C $c=1$ MeOH	3480 3300	1770		1645	1535

a) EI-MS. *b*) FAB-MS; Xenon as a carrier gas and glycerol as a matrix. *c*) FD-MS. *d*) Not recrystallized. *e*) Lit. 9); mp 180—182 °C (dec.), $[\alpha]_D^{20}$ −28.0° ($c=1$, H_2O). *f—h*) Solvent system was chloroform : methanol = 10 : 1, 2.5 : 1, and 20 : 1, respectively.

predictable from these Dreiding models under acylation and esterification conditions. A mechanism is proposed for their formation. These results including those in previous papers,[6,7] suggest that the equilibrium of Neu5Ac in basic and acidic media, and all lactone derivatives derived from it, are as shown in Chart 5. This chart may provide clues for studies of the behavior of sialic acid *in vivo*.

Experimental

General Method——Melting points (mp) were determined with Yamato melting point apparatus, and the results are uncorrected. Optical rotations ($[\alpha]_D$) were recorded on a JASCO JIP-4 digital polarimeter. TLC was performed on Silica gel 60 F254 plates (Merck, Art. 5719) and spots were detected under ultraviolet (UV) irradiation or by spraying the plate with 5% sulfuric acid in an aqueous methanol solution and heating on a hot plate. Electron impact mass spectra (EI-MS), field desorption mass spectra (FD-MS), fast atom bombardment mass spectra (FAB-MS), and IR spectra were measured with JEOL JMS D-100, JEOL JMS DX-300, JEOL JMA-3100, and JASCO IR-A 2 instruments, respectively. NMR were obtained with Varian 300 and 400 instruments in the FT mode. Chemical shifts (δ) were expressed in parts per million from internal tetramethylsilane in chloroform-*d* ($CDCl_3$) or in dimethyl sulfoxide-d_6 (DMSO-d_6). Column chromatography was conducted on silica gel; Wakogel C-200 (100—200 mesh) and C-300 (200—300 mesh).

Benzoylation of Neu5Ac (1) with Benzoyl Chloride——Benzoyl chloride (3.0 ml, 25.8 mmol) was added dropwise to a stirred suspension of **1** (1.02 g, 3.3 mmol) in dried pyridine (20 ml) at 0 °C. The mixture was stirred for 16 h at room temperature, then poured into ice-cold water and extracted with chloroform. The chloroform extract was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to a syrup. The syrupy residue was separated and purified by silica gel column chromatography (chloroform–methanol, 100 : 1—50 : 1). The first eluate was evaporated *in vacuo* to give 5-acetamido-2,4,8,9-tetra-*O*-benzoyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**6**, 0.93 g, 40%) as a white powder. The second eluate was evaporated *in vacuo* and the residue was recrystallized from ethyl acetate to give 5-acetamido-2,7,8,9-tetra-*O*-benzoyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,4-lactone (**7**, 0.46 g, 20%) as colorless needles. The third eluate gave a product which was recrystallized from ethanol to provide 5-acetamido-2,8,9-tri-*O*-benzoyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**8**, 0.27 g, 20%) as colorless prisms.

Benzoylation of Neu5Ac (1) with 1.1 eq of Benzoic Anhydride——Benzoic anhydride (804 mg, 1.1 eq) was added to a stirred suspension of **1** (1.0 g, 3.3 mmol) in dried pyridine (10 ml). The mixture was stirred at room temperature for 3 d and methanol (10 ml) was added to it. The reaction mixture was stirred for 30 min and evaporated *in vacuo* to give a brown syrup, which was chromatographed on a silica gel column (chloroform–methanol, stepwise elution from 25 : 1 to 5 : 1) and crystallized from ethanol to give 5-acetamido-2-*O*-benzoyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopylanosono-1,7-lactone (**10**, 620 mg, 47.6%) as colorless prisms.

Benzoylation of Neu5Ac (1) with 3.3 eq of Benzoic Anhydride——Benzoic anhydride (804 mg, 1.1 eq) was added to a stirred suspension of **1** (1.02 g, 3.3 mmol) in dried pyridine (10 ml), and the mixture was stirred at room temperature for 1 d. A similar operation was repeated twice (total 3 d), then methanol (50 ml) was added. The reaction mixture was stirred for 30 min and evaporated *in vacuo* to a syrup, which was chromatographed on a silica gel column (chloroform–methanol, stepwise elution from 50 : 1 to 5 : 1) and crystallized from ethanol to give 5-acetamido-2,8,9-tri-*O*-benzoyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**8**, 119 mg, 6.0%), 5-acetamido-2,9-di-*O*-benzoyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**9**, 593 mg, 36.0%), and **10** (53 mg, 4.1%), all as colorless prisms.

Pivaloylation of Neu5Ac (1) with Pivaloyl Chloride——Pivaloyl chloride (1.5 ml) was added dropwise to a stirred suspension of **1** (0.5 g, 1.62 mmol) in dried pyridine (10 ml) at 0 °C, and the mixture was stirred for 16 h at room temperature. The reaction mixture was poured into ice-cold water and extracted with chloroform 3 times. The chloroform extract was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to a syrup. The syrupy residue was separated and purified by silica gel column chromatography (chloroform–methanol, 100 : 1—50 : 1). The first eluate was evaporated *in vacuo* to give 5-acetamido-2,4,8,9-tetra-*O*-pivaloyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**11**, 657 mg, 64.8%) as a white powder. The second eluate was evaporated *in vacuo* to give 5-acetamido-2,4,9-tri-*O*-pivaloyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**12**, 220 mg, 25.0%) as a white powder. The third eluate was evaporated *in vacuo* to give 5-acetamido-2,8,9-tri-*O*-pivaloyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**13**, 55 mg, 6.3%) as a white powder.

Ethoxycarbonylation of Neu5Ac (1) with Ethyl Chloroformate——Ethyl chloroformate (2.0 ml) was added dropwise to a stirred suspension of **1** (0.5 g, 1.62 mmol) in dried pyridine (10 ml) at 0 °C. The mixture was stirred for 2 d at room temperature and methanol (10 ml) was added to it. The reaction mixture was stirred for 30 min and evaporated *in vacuo* to give a brown syrup, which was dissolved in chloroform; the solution was washed with water and brine, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to a syrup. The syrupy residue was separated and purified by repeated silica gel column chromatography (once with chloroform–methanol (10 : 1), twice with ethyl acetate–acetone (10 : 1)). The first eluate was evaporated *in vacuo* and recrystallized from ethyl acetate to give 5-acetamido-2,7,8,9-tetra-*O*-ethoxycarbonyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,4-lactone (**14**, 336 mg, 35.8%) as colorless needles. The second eluate was evaporated *in vacuo* to give 5-acetamido-2,8,9-tri-*O*-ethoxycarbonyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosono-1,7-lactone (**15**, 225 mg, 27.4%). The third eluate was evaporated *in vacuo* and the residue was recrystallized from ethyl acetate to give methyl 5-acetamido-2,7,8,9-tetra-*O*-ethoxycarbonyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**16**, 125 mg, 12.6%) as colorless prisms.

Esterification of Neu5Ac (1) with Diazomethane——A solution of diazomethane in ether was added dropwise to a stirred solution of **1** (1.02 g, 3.3 mmol) in aqueous methanol (water 10 ml, methanol 20 ml) at room temperature until the yellow color of diazomethane persisted. Acetic acid (0.5 ml) was added, and the reaction mixture was stirred for 30 min then evaporated *in vacuo* to a syrup, which was crystallized from methanol to give methyl 5-acetamido-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**17**, 0.800 g, 76.0%).

Treatment of 17 with Diazomethane——A solution of **17** (1.00 g, 3.10 mmol) in methanol was treated dropwise with 2 N hydrochloric acid aqueous solution to adjust the pH to 1. Then a solution of diazomethane in ether was added dropwise in the same manner as described above and this operation was repeated at room temperature overnight until the starting material (**17**) was no longer detectable on TLC. The reaction mixture was evaporated *in vacuo* to give a syrup, which was chromatographed on a silica gel column (chloroform–methanol, stepwise elution from 10 : 1 to 3 : 1). The second eluate was evaporated *in vacuo* and the residue was crystallized from methanol to give methyl (methyl 5-acetamido-3,5-dideoxy-D-*glycero*-*α*-D-*galacto*-2-nonulopyranosid)onate (**18**, 188 mg, 18%) as white prisms. The residue from the third eluate was crystallized from 2-propanol to give 5-acetamido-2-*O*-methyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-noneno-1,4-lactone (**3**, 274 mg, 29%) as colorless prisms. The other eluate gave a mixture of other compounds as a syrup (220 mg), but the mixture was not separated any further.

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