Studies on Sialic Acids. XVIII. Synthesis of Aryl-α-glycosides of 3-Deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic Acid (KDN)

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Condensation of sodium phenoxide, sodium *p*-nitrophenoxide, and sodium 4-methylumbelliferonate with benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chlorid)onate under the Williamson reaction conditions gave the corresponding α-glycosides in good yields. Deprotection reaction of these α-glycosides gave sodium salts of phenyl-, *p*-nitrophenyl-, and 4-methylumbelliferonyl-α-glycosides of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (KDN). The structure and stereochemistry of the glycosylation products were determined by proton nuclear magnetic resonance (^{1}H-NMR) and circular dichroism (CD) spectral analysis.

Keywords KDN; sialic acid; aryl glycoside; ^{1}H-NMR; CD

Recently, we have reported the synthesis of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (**1**, KDN) by base-catalyzed condensation and the synthesis of 2-*O*-methyl and 2-*O*-cholesterol derivatives of **1** under Koenigs–Knorr-like reaction conditions.[1] In studies of sialic acids, the aryl-α-glycosides of sialic acid are useful chromogenic substrates for tracing and quantifying sialidase (EC 3.2.1.18, α-*N*-acetylneuraminosyl glycohydrolase) in biological materials.[2,3] The phenols released in the enzymatic hydrolysis can be determined spectrophotometrically, either directly or after a specific transformation. The *p*-nitrophenyl-,[4] the *m*-methoxyphenyl-,[5] and the 4-methylumbelliferyl-α-glycosides[6] of sialic acid are known as appropriate substrates. In this paper, we wish to report the synthesis of aryl-α-glycosides of **1** under Williamson reaction conditions.[7] The structure and stereochemistry of the glycosylation products were determined by proton nuclear magnetic resonance (^{1}H-NMR) and circular dichroism (CD) spectral analysis.

In the previous paper,[1] we examined the Koenigs–Knorr-like reaction, using benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl bromid)onate (**3**) as a glycosyl donor without purification, because of its instability. This time, we prepared the corresponding 2-chloro derivative, benzyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chlorid)onate (**4**), as a new glycosyl donor which is stable enough to purify on a silica gel column. Compound **4** was synthesized by treatment of benzyl 2,4,5,7,8,9-hexa-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**2**) with acetic acid saturated with dry hydrogen chloride gas. The structure of **4** was elucidated by ^{1}H-NMR comparison with **3**. Condensation of **4** with sodium salts[8] of phenol, *p*-nitrophenol, and 4-methylumbelliferone in *N*,*N*-dimethylformamide gave the corresponding α-glycosides, benzyl (phenyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**5a**), benzyl (*p*-nitrophenyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**5b**), and benzyl (4-methylumbelliferonyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**5c**) in 31—77% yields, respectively. These compounds were deprotected with 0.1 N sodium hydroxide to give sodium (phenyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**6a**), sodium (*p*-nitrophenyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**6b**), and sodium (4-methylumbelliferonyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**6c**) in 80—87% yields, respectively.

In the ^{1}H-NMR spectra, the chemical shifts at the 3-H″ (eq) double-doublet resonance of the protected derivatives

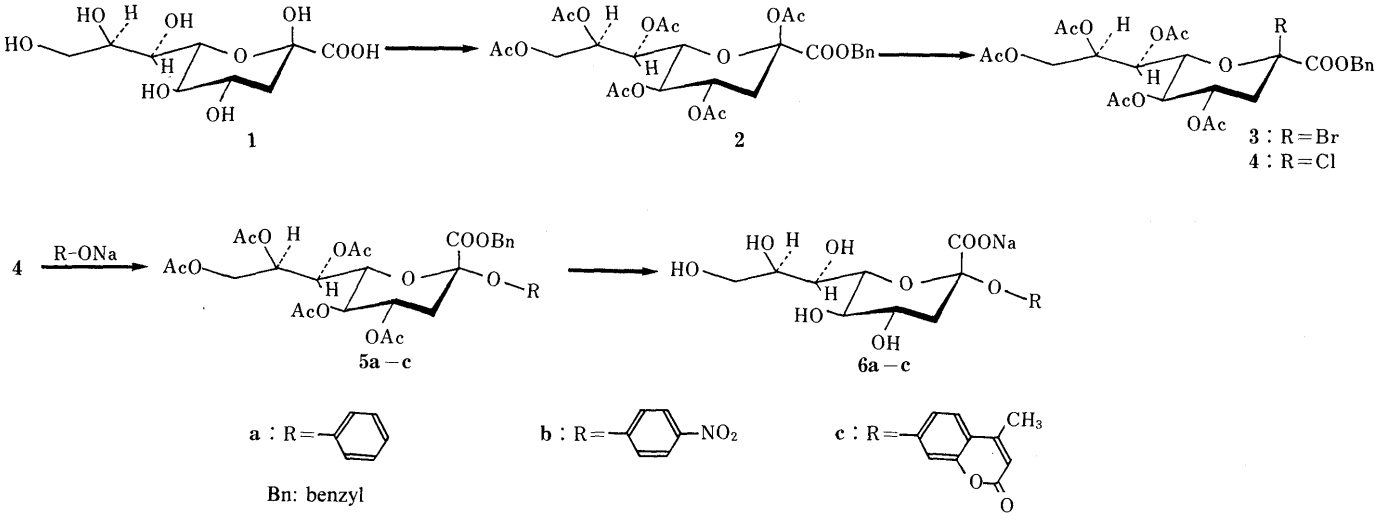

Chart 1

TABLE I. Proton Chemical Shifts and Spin-Coupling Data for **5a**,[a] **5b**,[a] **5c**,[a] **6a**,[b] **6b**,[b] and **6c**[b]

Compound	Spin couplings (Hz)									
	KDN moiety									
	$J_{3a,3e}$	$J_{3a,4}$	$J_{3e,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
5a	13.0	11.8	4.5	9.6	9.6	—	—	4.1	2.0	12.8
5b	13.0	11.9	4.6	9.5	9.5	—	—	4.2	2.0	12.5
5c	13.0	11.9	4.6	9.6	9.6	—	—	3.8	2.0	12.1
6a	12.5	11.5	4.8	9.5	10.0	0.8	—	—	—	—
6b	12.8	11.8	4.8	9.5	9.5	1.0	—	—	—	—
6c	12.8	11.8	4.7	9.5	10.0	1.0	—	—	—	—

Compound	Chemical Shifts (δ)											
	KDN moiety											
	3-H_{ax}	3-H_{eq}	4-H	5-H	6-H	7-H	8-H	9-H	9′-H	$COCH_3$	CH_2Ph	Aromatic
5a	2.21	2.83	4.97	4.91	4.56	5.40	5.40	4.19	4.29	2.01—2.14	5.03	6.97—7.41
5b	2.27	2.84	4.98	5.20	4.74	5.38	5.38	4.12	4.21	2.03—2.06	4.92, 5.18	6.96—8.02
5c	2.26	2.85	4.99	4.94	4.67	5.40	5.40	4.16	4.27	2.03—2.20	4.96, 5.18	6.83—7.35
6a	1.81	2.76	—	3.52	3.75	—	—	—	—			
6b	1.90	2.72	—	3.56	4.04	—	—	—	—			
6c	1.89	2.74	—	3.56	3.96	—	—	—	—			

a) Recorded for a solution in $CDCl_3$ at 300 MHz. *b*) Recorded for a solution in D_2O at 400 MHz.

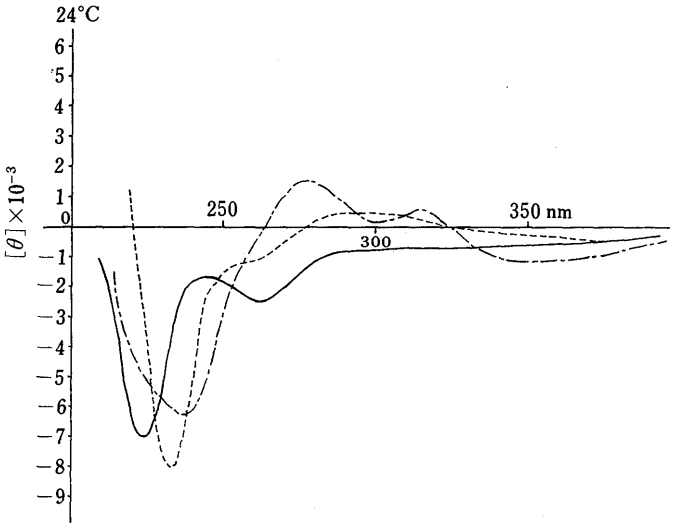

Fig. 1. CD Curves of **6a** (——), **6b** (– – – –), and **6c** (— · —) in MeOH

(**5a**, **5b**, and **5c**) were 2.83—2.85 ppm, and those of the deprotected compounds (**6a**, **6b**, and **6c**) were 2.72—2.76 ppm as shown in Table I. Empirical studies of *N*-acetyl-D-neuraminic acid derivatives[9,10] indicated that for α-anomers the chemical shift of 3-H″ (eq) varies between 2.6 and 2.8 ppm. For β-anomers, the range is 2.1—2.5 ppm. The chemical shifts of the above compounds were therefore considered to be those of the α-anomers.

Figure 1 shows the CD spectra of the deprotected derivatives (**6a**, **6b**, and **6c**). In the previous paper, we reported the CD spectra of several derivatives of **1**,[1] and the peak around 220—230 nm was assigned to the n-π* Cotton effect of the carboxyl group. The negative sign of the Cotton effect was assigned to the α-anomer and the positive sign to the β-anomer. In Fig. 1, all curves show a negative n-π* Contton effect around 220—230 nm, and this result supports the assignment of as the α-anomers.

In conclusion, the glycosylation of **4** with several sodium phenoxides under the Williamson reaction conditions stereospecifically gave α-glycosides in high yields. The stereochemistry of these derivatives could be determined on the basis of ^{1}H-NMR and CD spectral analysis.

Experimental

Melting points were measured with a Yamato melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. Thin layer chromatography (TLC) was performed on Silica gel GF254 (Merck) plates; detection was done under ultraviolet (UV) irradiation and by spraying 5% sulfuric acid solution. Fast atom bombardment mass spectra (FAB-MS) and infrared (IR) spectra were measured with JEOL JMA-3100 and JASCO IR-A2 instruments, respectively. CD spectra were measured in a 0.1 cm cell with a JASCO J-20 spectrometer. ^{1}H-NMR spectra were measured with Varian VXR-300 and XL-400 spectrometers. Tetramethylsilane (TMS) in $CDCl_3$ or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) in D_2O was used as an internal reference. Column chromatography was conducted on Silica gel 60 (70—230 mesh).

Benzyl (4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chlorid)onate (4) A solution of **2** (3.0 g, 4.9 mmol) in acetic acid (30 ml) and acetyl chloride (1 ml) was saturated with dry hydrogen chloride in an ice-water bath. After 18 h at room temperature, the reaction mixture was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography with ether : hexane = 2 : 1 to give **4** (2.8 g, 97%) as a colorless amorphous solid. $[\alpha]_D^{25}$ −61.9° (c = 0.32, $CHCl_3$). MS (EI) m/z: 586 (M^+). *Anal.* Calcd for $C_{26}H_{31}ClO_{13}$: C, 53.20, H, 5.32. Found: C, 53.12; H, 5.42. IR ν_{max}^{KBr} cm^{-1}: 2950, 1740, 1430, 1360. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.00, 2.03, 2.03, 2.05, 2.07 (3H × 5, s × 5, $COCH_3$), 2.24 (1H, dd, J = 14.0, 11.3 Hz, 3-H_{ax}), 2.84 (1H, dd, J = 14.0, 5.0 Hz, 3-H_{eq}), 4.10 (1H, dd, J = 12.5, 5.0 Hz, 9-H), 4.34 (1H, dd, J = 12.5, 2.5 Hz, 9′-H), 4.48 (1H, dd, J = 10.5, 2.3 Hz, 6-H), 4.95 (1H, t, J = 10.0 Hz, 5-H), 5.22 (1H, m, 8-H), 5.22, 5.34 (1H × 2, d × 2, J = 12.0 Hz, CH_2Ph), 5.44 (1H, m, 4-H), 5.47 (1H, dd, J = 8.0, 2.3 Hz, 7-H), 7.40 (5H, m, aromatic H).

Benzyl (Phenyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (5a) A solution of sodium phenoxide (100 mg, 0.85 mmol) and **4** (500 mg, 0.85 mmol) in dry *N*,*N*-dimethylformamide (20 ml) was stirred for 2 h at room temperature. Then the reaction mixture was poured into water (100 ml) and extracted with ethyl acetate. The combined extracts were evaporated *in vacuo* to give a syrup, which was chromatographed on a silica gel column with ethyl acetate : hexane = 1 : 1 to give **5a** (170 mg, 31%) as an amorphous powder. $[\alpha]_D^{25}$ −15.3° (c = 0.31,

$CHCl_3$). MS (EI) *m/z*: 644 (M^+). *Anal.* Calcd for $C_{32}H_{36}O_{14}$: C, 59.62; H, 5.63. Found: C, 59.52; H, 5.75. IR ν_{max}^{KBr} cm^{-1}: 2945, 1750, 1375. The ^{1}H-NMR data are summarized in Table I.

Benzyl (*p*-Nitrophenyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (5b) A solution of sodium *p*-nitrophenoxide (165 mg, 0.85 mmol) and **4** (500 mg, 0.85 mmol) in dry *N,N*-dimethylformamide (20 ml) was stirred for 2 h at room temperature. The mixture was processed as described for **5a** to give **5b** (450 mg, 77%) as an amorphous powder. $[\alpha]_D^{25}$ −16.2° (*c*=0.33, $CHCl_3$). MS (EI) *m/z*: 689 (M^+). *Anal.* Calcd for $C_{32}H_{35}NO_{16}$: C, 55.73; H, 5.12; N, 2.03. Found: C, 55.70; H, 5.13; N, 1.89. IR ν_{max}^{KBr} cm^{-1}: 2970, 1740, 1600, 1520, 1370, 1350. The ^{1}H-NMR data are summarized in Table I.

Benzyl (4-Methylumbelliferonyl 4,5,7,8,9-Penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (5c) A solution of sodium 4-methylumbelliferonate (170 mg, 0.85 mmol) and **4** (500 mg, 0.85 mmol) in *N,N*-dimethylformamide (20 ml) was stirred for 2 h at room temperature. The mixture was processed as described for **5a** to give **5c** (410 mg, 66%) as an amorphous powder. $[\alpha]_D^{25}$ −31.4° (*c*=0.28, $CHCl_3$). MS (EI) *m/z*: 727 (M^+). *Anal.* Calcd for $C_{36}H_{38}O_{16}$: C, 59.50; H, 5.27. Found: C, 59.74; H, 5.43. IR ν_{max}^{KBr} cm^{-1}: 2940, 1750, 1620, 1380. The ^{1}H-NMR data are summarized in Table I.

Sodium (Phenyl 3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (6a) A stirred solution of **5a** (150 mg, 0.23 mmol) in methanol (15 ml) was treated with 0.1 N sodium hydroxide (15 ml) at room temperature. After 1 h, the solution was cooled to 0 °C, neutralized with Dowex-50 (H^+) resin, filtered and concentrated. The residue was freeze-dried to give **6a** (65 mg, 81%) as an amorphous powder. $[\alpha]_D^{26}$ −12.6° (*c*=0.13, MeOH). MS (FAB) *m/z*: 367 (M^++1). *Anal.* Calcd for $C_{15}H_{19}NaO_9 \cdot 2H_2O$: C, 44.78; H, 5.76. Found: C, 44.55; H, 5.66. IR ν_{max}^{KBr} cm^{-1}: 3370, 1600, 1400. The ^{1}H-NMR data are summarized in Table I.

Sodium (*p*-Nitrophenyl 3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (6b) A stirred solution of **5b** (300 mg, 0.44 mmol) in methanol (30 ml) was treated with 0.1 N sodium hydroxide (30 ml) at room temperature and the mixture was processed as described for **6a** to give **6b** (147 mg, 87%) as an amorphous powder. $[\alpha]_D^{26}$ +27.4° (*c*=0.39, MeOH). MS (FAB) *m/z*: 412 (M^++1). *Anal.* Calcd for $C_{15}H_{18}NNaO_{11} \cdot 2H_2O$: C, 40.28; H, 4.96; N, 3.13. Found: C, 40.26; H, 4.89; N, 3.00. IR ν_{max}^{KBr} cm^{-1}: 3370, 1720, 1590, 1490, 1340. The ^{1}H-NMR data are summarized in Table I.

Sodium (4-Methylumbelliferonyl 3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (6c) A stirred solution of **5c** (300 mg, 0.41 mmol) in methanol (30 ml) was treated with 0.1 N sodium hydroxide (30 ml) at room temperature and the mixture was processed as described for **6a** to give **6c** (140 mg, 80%) as an amorphous powder. $[\alpha]_D^{26}$ +21.5° (*c*=0.54, MeOH). MS (FAB) *m/z*: 449 (M^++1). *Anal.* Calcd for $C_{19}H_{21}NaO_{11} \cdot 2H_2O$: C, 47.11; H, 5.20. Found: C, 47.64; H, 4.88. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1610, 1390. The ^{1}H-NMR data are summarized in Table I.

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