Reaction Products from Fischer's Methylation of 3-Deoxy-D-*glycero*-D-*galacto*-2-nonulosonic Acid (KDN)

Toshitsugu KAI,* Xue-Long SUN, Makoto TANAKA, Hiroaki TAKAYANAGI, and Kimio FURUHATA

School of Pharmaceutical Sciences, Kitasato University, 5–9–1 Shirokane, Minato-ku, Tokyo 108, Japan.

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Several compounds were isolated from the Fischer's methylation reaction mixture of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN) with hydrochloric acid or methanesulfonic acid in methanol. The major product was methyl (methyl 3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (2), which we have already reported. New furan derivatives and a 4,8-anhydro product were also isolated. The structures of these compounds were elucidated by nuclear magnetic resonance spectroscopy and X-ray crystallographic analysis.

Key words sialic acid; Fischer's glycosylation; furan derivative; 4,8-anhydro product; crystalline KDN; β-elimination

Deaminoneuraminic acid, 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN, **1**), was isolated from polysialoglycoprotein (PSGP) of rainbow trout eggs.[1] It has been synthesized,[2,3] and we have also synthesized various analogues.[3-8] These KDN analogues are of particular relevance to the investigation of neuramidase inhibitors,[9] and they also provide interesting carbohydrate templates for further transformation. KDN methyl ester and its methyl β-glycoside are extremely useful materials for the synthesis of KDN analogues. In a previous paper, we described their synthesis under Fischer's conditions.[6] In this report, we describe the isolation of minor products from the reaction mixture and determination of their structures by means of mass and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and X-ray crystallographic analysis.

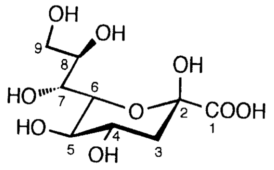

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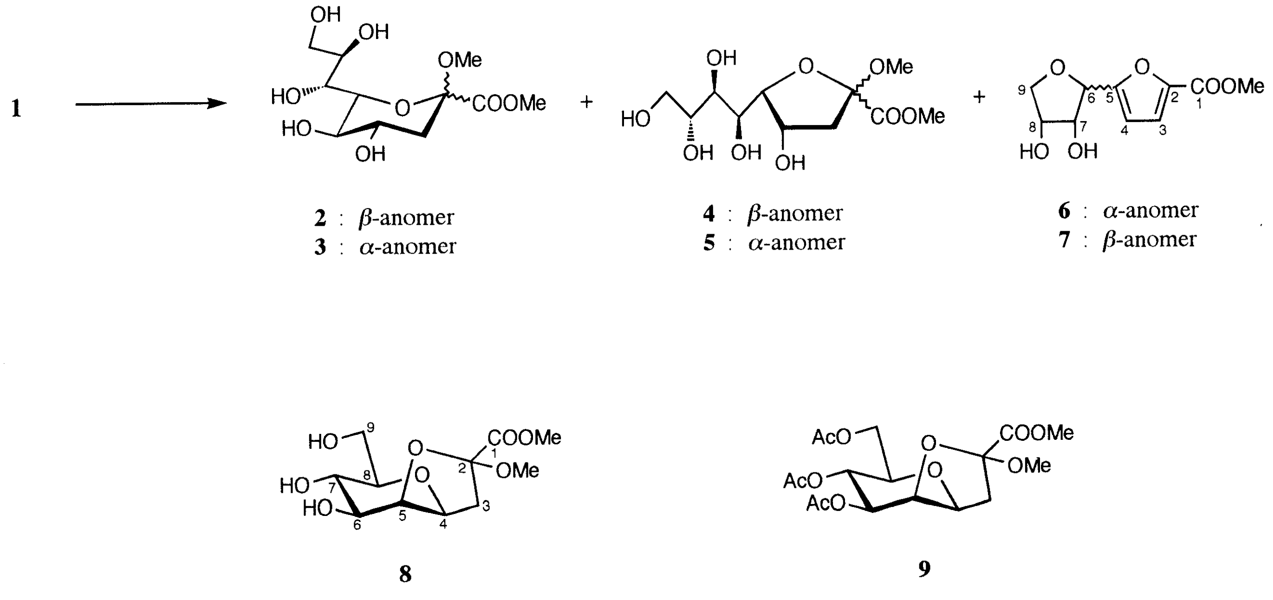
Fig. 1

Results and Discussion

Synthesis of carbohydrates requires a large quantity of starting material, because the overall yield is usually low. We developed an improved chemical synthesis of KDN by base-catalyzed condensation of oxalacetic acid with D-mannose,[2] obtaining 100 g quantities,[3] and we succeeded in crystallizing the β-pyranose form of **1**.

Previously, we examined Fischer's methyl glycosylation of **1** using the strong cation exchange resin, Dowex-50 (H^+), and we found an anomeric equilibrium between α- and β-furanose and pyranose.[6] Thus we suspected that the β-pyranoside of methyl (methyl 3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**2**) would be the only product if **1** were treated with a strong acid, such as hydrochloric acid or methanesulfonic acid, in methanol



2 : β-anomer
3 : α-anomer

4 : β-anomer
5 : α-anomer

6 : α-anomer
7 : β-anomer

8

9

Chart 1

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* To whom correspondence should be addressed.

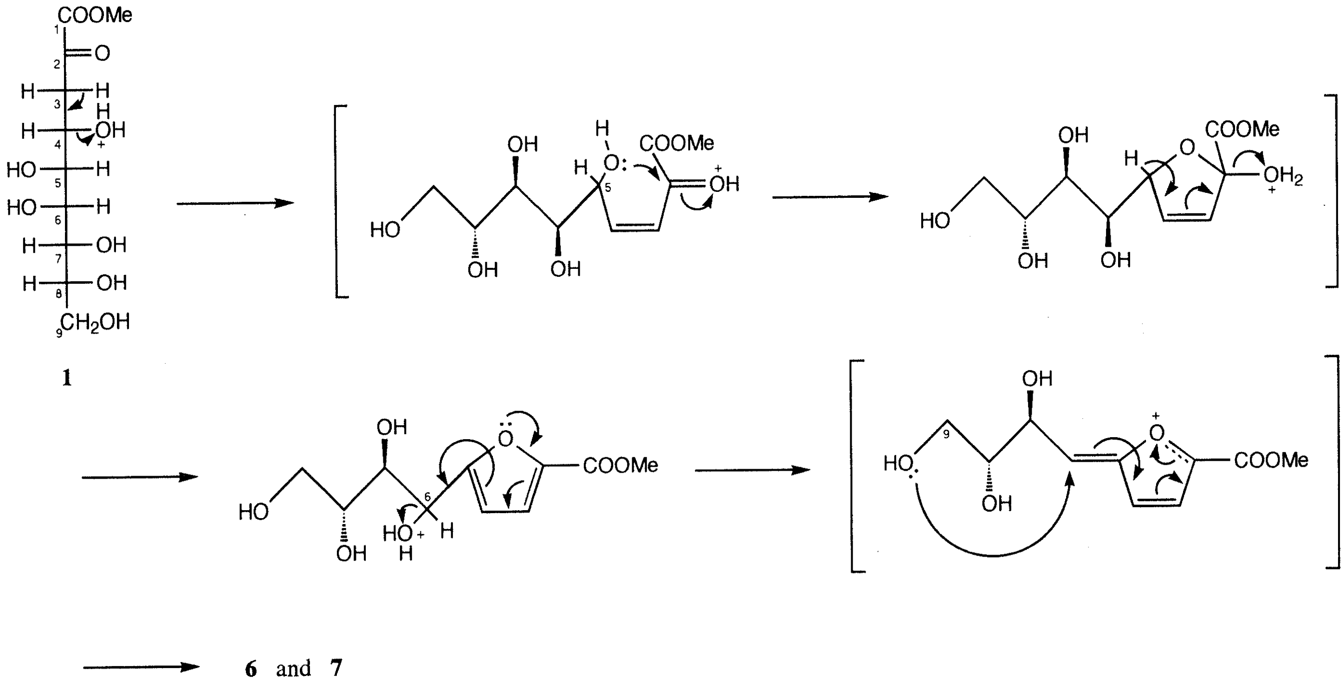

Chart 2. Possible Mechanism for the Formation of **6** and **7**

and heated. We thus found a facile method for the preparation of **2** as new crystals. Furthermore, from the reaction mixture, new furan derivatives and a 4,8-anhydro compound were isolated as minor products.

Treatment of **1** with hydrochloric acid in methanol at 70 °C gave one major and five minor products. The major product, **2**, was crystallized in 28% yield from the concentrated reaction mixture, and the filtrate was separated by column chromatography on silica gel to give three known compounds, the α-isomer of **2** (**3**), and methyl (methyl 3-deoxy-β- and -α-D-*glycero*-D-*galacto*-2-nonulofuranosid)onates (**4** and **5**) in 26% total yield, together with two new compounds, methyl 5-(α- and β-D-erythrofuranosyl)furan-2-carboxylates (**6**, 0.2% yield and **7**, 0.9% yield). The structures of **6** and **7** were elucidated by ^{1}H-NMR spectral analysis. The configuration at C-6 of the furan derivatives (**6** and **7**) was deduced from nuclear Overhauser effect (NOE) experiments. That is, on irradiation of H-6, NOEs were observed at H-9 in both the α- and β-anomers (α-anomer **6**, 1.1%; β-anomer **7**, 1.7%). But, on irradiation of H-9, NOE was observed at H-6 only in the β-anomer (**7**, 2.3%), and not in the α-anomer. Furthermore, the structures of **6** and **7** were confirmed by comparison of the ^{1}H-NMR spectral properties with those of methyl 5-(2′,3′-di-*O*-acetyl-α- and -β-D-erythrofuranosyl)pyrrole-2-carboxylates, which are thermal degradation products of sodium *N*-acetylneuraminate.[10] The reaction mechanism is considered to involve the formation of a furan ring through acid-catalyzed β-elimination of hydroxy groups and nucleophilic attack of the hydroxy group at C-9 upon the C-6 position, as shown in Chart 2.

In the case of treatment with methanesulfonic acid, instead of hydrochloric acid, in methanol at 70 °C, **1** was converted into a mixture of **2**, **3**, **4**, and **5** in 67% total yield, and a new compound, methyl (methyl 4,8-anhydro-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulofuranosid)onate (**8**) was also isolated in 3% yield. Acetylation of **8** with acetic anhydride and pyridine afforded methyl (methyl 6,7,9-tri-*O*-acetyl-4,8-anhydro-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**9**). The structures of **8** and **9** were elucidated by ^{1}H-NMR spectral analysis, as shown in Table 1. The coupling constants between H-5 and H-6 (J=3.5 Hz), H-6 and H-7 (J=9.5 Hz), and H-7 and H-8 (J=9.5 Hz) indicate a manno-type proton network for **8**. Further, the coupling between H-4 and H-5 (J=2.0 Hz) shows H-4 to be axial. Three singlet signals at δ 2.03, 2.06, and 2.10 of **9** indicate the presence of three newly introduced *O*-acetyl groups.

Table 1. ^{1}H-NMR Data for **8** and **9**

Proton	**8**	**9**
3-H	2.47 (dd, J=4.0, 14.5 Hz)	2.28 (dd, J=4.0, 14.5 Hz)
3-H′	2.85 (dd, J=2.0, 14.5 Hz)	2.60 (dd, J=2.0, 14.5 Hz)
4-H	4.27 (dd, J=2.0, 4.0 Hz)	4.22 (dd, J=2.0, 4.0 Hz)
5-H	4.69 (dd, J=2.0, 3.5 Hz)	4.45 (dd, J=2.0, 3.5 Hz)
6-H	4.23 (dd, J=3.5, 9.5 Hz)	5.14 (dd, J=3.5, 9.5 Hz)
7-H	4.52 (t, J=9.5 Hz)	5.37 (t, J=9.5 Hz)
8-H	3.77 (dt, J=3.0, 9.5 Hz)	3.56 (dt, J=4.0, 9.5 Hz)
9-H	4.17 (dd, J=3.0, 9.5 Hz)	4.08 (2H, d, J=4.0 Hz)
9-H′	4.53 (dd, J=3.0, 9.5 Hz)	
2-OMe	3.36 (s)	3.27 (s)
2-COOMe	3.65 (s)	3.82 (s)
OAc	—	2.03 (s), 2.06 (s), 2.10 (s)

Spectra were measured in C_5D_5N for **8** and in $CDCl_3$ for **9** at 300 MHz. Chemical shifts are given in δ (ppm). Coupling constants are given in parentheses.

To confirm the stereochemistry of **9**, X-ray crystallography was conducted. Figure 2 shows the crystal structure of **9**. From this ORTEP view, the pyranoside takes chair form (${}^7C_4$), and the furanoside takes twist form (${}^0T_4$), and the anomeric configuration is α.

We assume that **8** is also formed from the open structure of KDN methyl ester in a multistep process, as shown in

Chart 3. The initial step could involve acid-catalyzed β-elimination of the hydroxy group at C-4 and the formation of an α,β-unsaturated keto moiety. Subsequent steps would include nucleophilic attack of the hydroxy group at C-8 on the C-4 position, resulting in a new anhydro ring through intramolecular Michael reaction, and the formation of the furanosyl ring. In this process, none of the original configuration of **8** is changed.

In conclusion, we have developed a facile method for the preparation of crystalline KDN and its β-methyl glycoside, which are required for syntheses of sialic acid analogues. The formation of bicyclic derivatives (**6**, **7**, and **8**) on Fischer methylation is a consequence of the characteristic properties of KDN.

Experimental

Melting points were measured with a Yazawa BY-10 melting point apparatus without correction. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) plates, and spots were detected under ultraviolet (UV) light and by spraying with 5% sulfuric acid. Fast atom bombardment mass spectra (FAB-MS), and infrared (IR) spectra were measured with JEOL JMS-DX300 and JASCO A-102 instruments, respectively. The ^{1}H-NMR spectra were measured with Varian VXR-300 spectrometer. Tetramethylsilane (TMS) in $CDCl_3$ and C_5D_5N or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) in D_2O were used as internal references. Column chromatography was conducted on Silica gel 60 (70—230 mesh, Merck).

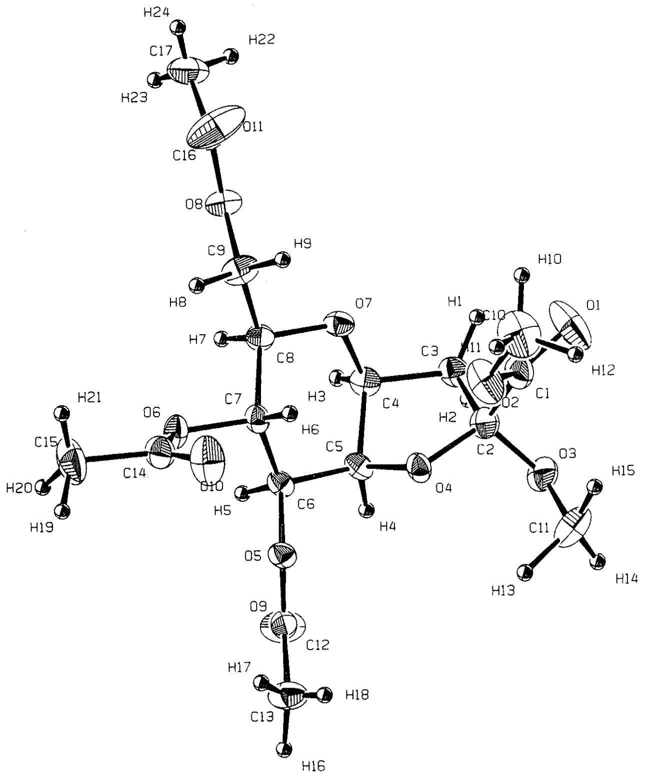

Fig. 2. Crystal Structure of **9**

Purification of KDN A solution of **1** (10.0 g, 37.3 mmol) in water (100 ml) was passed over the anion exchange resin Dowex-1 ($HCOO^-$, 350 ml), and **1** was eluted with 0—1 N formic acid. The eluate was concentrated under reduced pressure. The residue was left at room temperature for 7 d, to afford colorless crystals (4.65 g, 47%). mp 144—147 °C. $[\alpha]_D^{30}$ −49.7° ($c=1$, H_2O). *Anal.* Calcd for $C_9H_{16}O_9$: C, 40.30; H, 6.01. Found: C, 40.39; H, 5.89. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3440, 2960, 1730. ^{1}H-NMR (300 MHz, D_2O) δ: 1.75 (1H, dd, $J=11.5$, 12.5 Hz, 3-H_{ax}), 2.18 (1H, dd, $J=5.0$, 12.5 Hz, 3-H_{eq}), 3.51 (1H, t, $J=9.5$ Hz, 5-H), 3.58 (1H, dd, $J=6.0$, 11.5 Hz, 9-H), 3.67 (1H, ddd, $J=3.0$, 6.0, 9.0 Hz, 8-H), 3.79 (1H, dd, $J=1.5$, 9.0 Hz, 7-H), 3.79 (1H, dd, $J=3.0$, 11.5 Hz, 9-H'), 3.91 (1H, dd, $J=1.5$, 9.5 Hz, 6-H), 3.92 (1H, ddd, $J=5.0$, 9.5, 11.5 Hz, 4-H).

Esterification and Glycosylation of 1 by Fischer's Method Using Hydrochloric Acid and Methanol Acetyl chloride (22.08 g, 0.281 mol) was added slowly to methanol (500 ml), then KDN (**1**, 10.0 g, 37.3 mmol) was added to the solution. The mixture was heated at 70 °C for 6 h, then evaporated, and the residual syrup was left at room temperature to afford methyl (methyl 3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**2**, 3.04 g, 28%) as colorless crystals. These were filtered off, and the filtrate was evaporated to dryness. The residual syrup was purified on a column of silica gel with chloroform–methanol (10 : 1) to give a mixture of methyl 5-(α- and β-D-erythrofuranosyl)furan-2-carboxylates (**6** and **7**, 216.9 mg, 3%), a mixture of methyl (methyl 3-deoxy-β- and -α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (**2** and **3**) and a mixture of methyl (methyl 3-deoxy-β- and -α-D-*glycero*-D-*galacto*-2-nonulofuranosid)onate (**4** and **5**) (2.9 g, 26%). The mixture of **6** and **7** was separated by silica gel TLC with 20 : 1 (v/v) chloroform–methanol (repeated 3 times) to give **6** (12.9 mg, 0.2%) and **7** (78.3 mg, 0.9%).

6: $[\alpha]_D^{30}$ −7.6° ($c=1$, MeOH). HRMS *m/z* Calcd for $C_{10}H_{12}NaO_6$: 251.0532 ($M^+ + Na$). Found: 251.0533 ($M^+ + Na$). IR $\nu_{\max}^{\mathrm{film}}$ cm^{-1}: 3420, 1740, 1610. ^{1}H-NMR (300 Mz, C_5D_5N) δ: 3.74 (3H, s, $-COOCH_3$), 4.22 (1H, dd, $J=6.5$, 8.5 Hz, 9-H), 4.30 (1H, dd, $J=6.5$, 8.5 Hz, 9-H'), 4.67 (1H, t, $J=4.5$ Hz, 7-H), 4.72 (1H, dt, $J=4.5$, 6.5 Hz, 8-H), 5.27 (1H, d, $J=4.5$ Hz, 6-H), 6.90 (1H, d, $J=3.5$ Hz, 4-H), 7.30 (1H, d, $J=3.5$ Hz, 3-H).

7: $[\alpha]_D^{30}$ −59.4° ($c=1$, MeOH). HRMS *m/z* Calcd for $C_{10}H_{12}NaO_6$: 251.0532 ($M^+ + Na$). Found: 251.0544 ($M^+ + Na$). IR $\nu_{\max}^{\mathrm{film}}$ cm^{-1}: 3450, 2980, 1740, 1660, 1540. ^{1}H-NMR (300 MHz, C_5D_5N) δ: 3.76 (3H, s, $-COOCH_3$), 4.26 (1H, dd, $J=2.5$, 9.0 Hz, 9-H), 4.42 (1H, dd, $J=5.0$, 9.0 Hz, 9-H'), 4.65 (1H, dt, $J=2.5$, 9.0 Hz, 8-H), 4.80 (1H, t, $J=5.0$, 7.0 Hz, 7-H), 5.34 (1H, d, $J=7.0$ Hz, 6-H), 6.66 (1H, d, $J=3.5$ Hz, 4-H), 7.24 (1H, d, $J=3.5$ Hz, 3-H).

Esterification and Glycosylation of 1 by Fischer's Method Using Methanesulfonic Acid and Methanol A solution of **1** (5.1 g, 19.0 mmol) and methanesulfonic acid (1 ml) in methanol was stirred for 8 h at 70 °C, then neutralized with dry Dowex-1 (OH^-) and filtered. The filtrate was evaporated and the residual syrup was chromatographed on a column of silica gel with chloroform–methanol (4 : 1) to give methyl (methyl

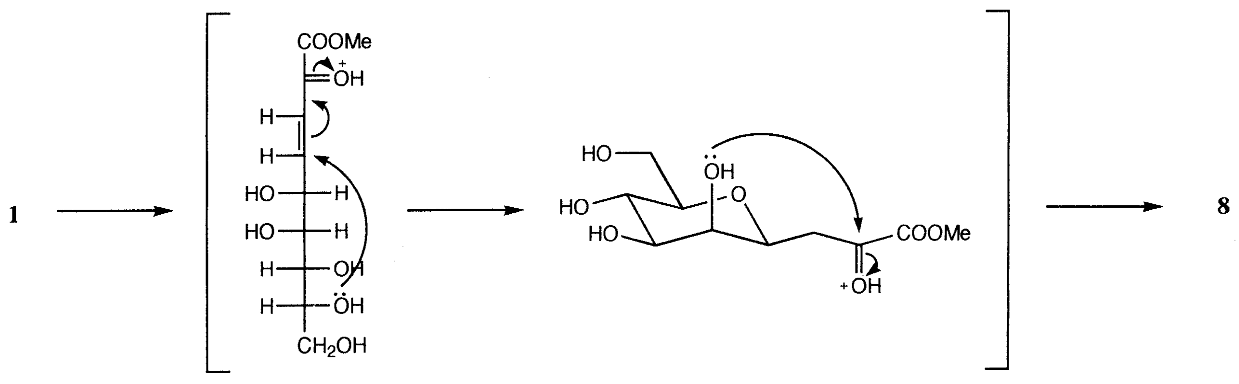

Chart 3. Possible Mechanism for the Formation of **8**

4,8-anhydro-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulofuranosid)onate (**8**, 140 mg, 3%) and a mixture of **2**, **3**, **4**, and **5** (3.8 g, 67%).

8: $[\alpha]_D^{23}$ −53.6° ($c=1$, MeOH). HRMS m/z Calcd for $C_{11}H_{19}O_8$: 279.1080 ($M^+ + H$). Found: 279.1104 ($M^+ + H$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1745. ^{1}H-NMR data are given in Table 1.

Acetylation of 8 Acetic anhydride (165 mg, 1.62 mmol) was added to a solution of **8** (50.0 mg, 0.180 mmol) in pyridine (128 mg, 1.62 mmol) at room temperature. The mixture was stirred for 16 h at room temperature, then concentrated, and the residue was purified on a column of silica gel with *n*-hexane–ethyl acetate (1 : 1) to give methyl (methyl 6,7,9-tri-*O*-acetyl-4,8-anhydro-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulofuranosid)onate (**9**, 30 mg, 41%) as colorless prisms. mp 133—135 °C, $[\alpha]_D^{23}$ −73.2° ($c=1$, MeOH). *Anal.* Calcd for $C_{17}H_{24}O_{11}$: C, 50.50; H, 5.94. Found: C, 50.49; H, 6.02. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750. ^{1}H-NMR data are given in Table 1.

Crystal Data for 9 A crystal with the dimensions of 0.3 × 0.3 × 0.3 mm^3 was used for the structure determination. The cell dimensions and diffraction intensities were measured on a Rigaku four-circle diffractometer (AFC-5R), using graphite-monochromated CuK_α radiation.

Crystal Data: $C_{17}H_{24}O_{11}$, orthorhombic, space group $P2_12_12_1$, $a=9.625(4)$, $b=25.930(9)$, $c=7.802(5)$ Å, $V=1947(1)$ Å^3, $Z=4$, $D_{calcd}=1.379$ g/cm^3. In total, 2053 independent reflections in the range of $2\theta=139.6°$ were collected by use of the $2\theta-\omega$ scan mode with a scanning rate of 16° min^{-1} (ω). In total, 1629 independent reflections with $I>3.00\sigma(I)$ were obtained and corrected for Lorentz and polarization factors, but not for absorption. The structure was solved by direct methods using the program MITHRIL.[11] The positions of all hydrogen atoms were located in the difference Fourier map. Atomic scattering factors were taken from the International Table for X-Ray Crystallography.[12] All calculations were performed using the TEXSAN[13] crystallographic software package of Molecular Structure Corporation. The final R value was 5.3%.

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