STUDIES ON SIALIC ACIDS XXIV. SYNTHESIS OF 2,7-ANHYDRO-*N*-ACETYLNEURAMINIC ACID

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The first synthesis of 2,7-anhydro-*N*-acetylneuraminic acid, a sialic acid, has been completed efficiently by intramolecular glycosidation of the *S*-(1-phenyl-1*H*-tetrazol-5-yl) glycoside derivative of *N*-acetylneuraminic acid (5) using silver triflate - bis(acetonitrile)palladium(II) chloride as a promoter.

KEYWORDS sialic acid; 2,7-anhydro-*N*-acetylneuraminic acid; intramolecular glycosidation; silver triflate; bis(acetonitrile)Pd(II) chloride; benzylation; *S,S'*-bis(1-phenyl-1*H*-tetrazol-5-yl) dithiocarbonate

Sialic acid containing intramolecular glycoside, 2,7-anhydro-*N*-acetylneuraminic acid (1), was isolated in a free form from cerumen of the wet type by Suzuki *et al.*¹⁾ Moreover, Li *et al.* recently found that leeches contain a novel sialidase which releases 1 almost quantitatively from α -sialosylglycoconjugate.²⁾ Because it is available only in a small quantity from natural sources, 1 has to be synthesized in order to study its biological activities. As a part of the research program on glycosylation of sialic acid, we now report the first synthesis of 2,7-anhydro-*N*-acetylneuraminic acid and the confirmation of its structure.

Several methods are available for preparing 1,6-anhydrohexopyranoses,^{3,4,5)} but it is very difficult to synthesize 1 by those methods. We have developed a facile method for the synthesis of 1 by the treatment of *S*-(1-phenyl-1*H*-tetrazol-5-yl) glycoside with silver triflate and palladium(II) as a promoter.⁶⁾ This procedure gave a satisfactory yield of 1. The starting material, methyl 5-acetamido-3,5-dideoxy-8,9-isopropylidene- β -D-glycero-D-galacto-2-nonulopyranosonate (2) was prepared as reported in a previous paper.⁷⁾ Benzylation of 2 using benzoic an-

hydride in pyridine gave regioselectively 4-*O*-benzoyl derivative of 2 (3),⁸⁾ colorless needles, mp 151-153°C, $[\alpha]_D^{19} -4.6^\circ$ ($c=1$ MeOH), $C_{22}H_{29}NO_{10}$, in 85% yield. The sterically hindered 2- or 7-OH of 2 was not benzylation owing to low reaction with benzoic anhydride.⁹⁾ The treatment of 3 with *S,S'*-bis(1-phenyl-1*H*-tetrazol-5-yl) dithiocarbonate (4)¹⁰⁾ in acetonitrile in the presence of 4-*N,N*-dimethylaminopyridine gave the stable *S*-glycoside, methyl [*S*-(1-phenyl-1*H*-tetrazol-5-yl) 5-acetamido-4-benzoyl-3,5-dideoxy-8,9-isopropylidene- β -D-glycero-D-galacto-2-nonulopyranosid]onate (5),¹¹⁾ colorless prisms, mp 177-178°C, $[\alpha]_D^{19} -187.6^\circ$ ($c=1$ MeOH), $C_{29}H_{33}N_5O_9S$, in 75% yield. The gated proton-decoupled ^{13}C -NMR experiments evidenced the β -configuration of the anomeric center of 5 as the coupling constant $J_{1-C,3-H_{ax}} = 1.0$ Hz was observed at δ 166.7 (1-C).¹²⁾

Intramolecular glycosidation of 5 was performed in dichloromethane in the presence of silver triflate - bis(acetonitrile)palladium(II) chloride, and molecular sieves 4A to give methyl (5-acetamido-2,7-anhydro-4-*O*-benzoyl-3,5-dideoxy-8,9-isopropylidene- α -D-glycero-D-galacto-2-nonulopyranosid)onate (6),¹³⁾ white powder, $[\alpha]_D^{19} +91.4^\circ$ ($c=1$ MeOH), $C_{22}H_{27}NO_9$, in 45% yield and 2-deoxy-2,3-dehydro derivative of 3 (7), colorless

needles, mp 224–225°C, $[\alpha]_D^{19} +123.5^\circ$ ($c=1$ MeOH), in 40% yield. In this reaction, the yield of 6 in the presence of palladium(II) was higher than that in only silver triflate. The structure of 6 was deduced from a comparison of the ^1H -NMR data of 3 and 6. The vicinal coupling constants on the pyranose ring in 6 were all small (1–4.5 Hz), reflecting the structural change in 3, that is, the conversion of the pyranose ring in 6 from $^2\text{C}_5(\text{D})$ to $^5\text{C}_2(\text{D})$. These results indicated that the intramolecular glycosyl linkage was formed between 2-C and 7-OH.

After removal of the isopropylidene group in 6 with 80% AcOH–H₂O at 40°C for 2 h, the product was subjected to debenzoylation with 2% MeONa–MeOH at 25°C for 4 h and the reaction mixture was neutralized at pH 5.0 with Dowex-50 (H^+) to provide a methyl ester derivative of 1 (8),¹⁴ colorless needles, mp 95–96°C, $[\alpha]_D^{19} +77.8^\circ$ ($c=1$ MeOH), $\text{C}_{12}\text{H}_{19}\text{NO}_8 \cdot \text{H}_2\text{O}$, in 90% yield. After saponification of 8 with 0.5N NaOH at 35°C for 3 h, subsequent treatment with Dowex-50 (H^+) afforded 2,7-anhydro-*N*-acetylneuraminic acid (1, 5-acetamido-2,7-anhydro-3,5-dideoxy- α -D-*glycero*-D-*galacto*-2-nonulopyranosonic acid),¹⁵ white powder, $\text{CD}(\text{MeOH})$ $[\theta]_{235}^{23} +3550$, $[\theta]_{213}^{23} -350$, $\text{C}_{11}\text{H}_{17}\text{NO}_8$, in 95% yield, which was identified as 2,7-anhydro-*N*-acetylneuraminic acid by comparing its negative-ion (matrix; ethanolamine) FAB-MS, GC-MS,¹⁶ and its chromatographic behavior (TLC)¹ with those of an authentic sample of 1.

- a) benzoic anhydride / pyridine b) DMAP / MeCN c) AgOTf (2 eq) / $(\text{MeCN})_2\text{Pd}(\text{II})\text{Cl}_2$ (1 eq) / MS 4A / CH_2Cl_2 d) 80% AcOH–H₂O ; 2% MeONa–MeOH e) 0.5N NaOH ; Dowex-50 (H^+)

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- 6) The details of mechanistic aspects of this glycosylation using AgOTf - (MeCN)₂PdCl₂ will be reported in due course.
- 7) H. Ogura, K. Furuhashi, S. Sato, K. Anazawa, M. Itoh, and Y. Shitori, *Carbohydr. Res.*, **167**, 77 (1987).
- 8) **3**: ¹H-NMR (400MHz, CDCl₃) δ; 1.34 and 1.40(both 3H, s, isopropylidene), 1.95(3H, s, NHAc), 2.43(1H, t, J=12.0Hz, 3-H_{ax}), 2.33(1H, dd, J=5.0, 12.5Hz, 3-H_{eq}), 3.49(1H, br dd, J=4.5, 8.5Hz, 7-H), 3.52(3H, s, COOMe), 4.05(1H, br.d, J=8.5Hz, 6-H), 4.15(1H, dd, J=8.0, 10.0Hz, 5-H), 4.15(2H, d, J=5.5Hz, 9-H₂), 4.20(1H, dt, J=5.5, 8.5Hz, 8-H), 4.59(1H, d, J=5.0Hz, 7-OH), 5.64(1H, dt, J=5.0, 11.0Hz, 4-H), 6.35(1H, d, J=8.0Hz, NH), and 7.42-7.98(5H, phenyl group).
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- 11) **5**: ¹H-NMR (300MHz, CDCl₃) δ; 1.33, 1.38(both 3H, s, isopropylidene), 2.06(3H, s, NAc), 2.34(1H, dd, J=11.0, 14.0Hz, 3-H_{ax}), 2.79(1H, dd, J=5.0, 14.0Hz, 3-H_{eq}), 3.35(3H, s, COOMe), 3.74(1H, dd, J=6.5, 8.5Hz, 9-H), 3.85(1H, br t, J=4.0Hz, 7-H), 3.99(1H, dd, J=5.5, 8.5Hz, 9-H'), 4.32(1H, dd, J=4.2, 10.0Hz, 6-H), 4.33(1H, q, J=10.5Hz, 5-H), 4.51(1H, ddd, J=4.0, 5.5, 6.5Hz, 8-H), 4.57(1H, d, J=4.8Hz, 7-OH), 5.66(1H, dt, J=5.0, 11.0Hz, 4-H), 6.82(1H, d, J=7.5Hz, NH), 7.36-7.90(10H, two phenyl groups). ¹³C-NMR (75MHz, CDCl₃) δ; 22.9(NCOMe), 37.0(3-C), 50.6(6-C), 53.3(COOMe), 64.9(9-C), 68.7(4-C), 69.0(7-C), 75.9(5-C), 76.0(8-C), 108.2(2-C), 147.4(5'-C), 124.8x2, 128.4, 128.5x2, 129.6x2, 130.4x2, 133.5, 133.8(two phenyl groups), 166.7(1-C), and 173.1(NCOMe). Proton assignments were made by ¹H-¹H COSY, and carbon shifts were assigned by DEPT, ¹H-¹³C COSY, and LSPD techniques.
- 12) H. Hori, T. Nakajima, Y. Nishida, H. Ohnishi, and H. Meguro, *Tetrahedron Lett.*, **29**, 6317 (1988).
- 13) **6**: ¹H-NMR (300Hz, CDCl₃) δ; 1.33, 1.42(both 3H, s, isopropylidene), 2.03(3H, s, NAc), 2.28(1H, dd, J=4.5, 15.5Hz, 3-H_{ax}), 2.33(1H, dd, J=1.5, 15.5Hz, 3-H_{eq}), 3.82(3H, s, COOMe), 4.12(1H, dt, J=3.0, 8.5Hz, 9-H), 3.96-4.20(2H, m, 8-H, 9-H'), 4.36(1H, br d, J=9.0Hz, 5-H), 4.50(1H, br dt, J=3.0, 7.9Hz, 7-H), 4.65(1H, br s, 6-H), 5.17(1H, br.s, 4-H), 6.24(1H, d, J=9.0Hz, NH), and 7.45-7.98(5H, two phenyl groups).
- 14) **8**: ¹H-NMR (400MHz, D₂O) δ; 1.92(3H, s, NAc), 1.98(1H, br.d, J=15.5Hz, 3-H_{ax}), 2.14(1H, dd, J=5.5, 15.5Hz, 3-H_{eq}), 3.44(1H, ddd, J=2.5, 6.0, 7.5Hz, 8-H), 3.47(1H, dd, J=6.0, 11.0Hz, 9-H), 3.62(1H, dd, J=2.5, 11.0Hz, 9-H'), 3.74(3H, s, COOMe), 3.85(1H, br s, 5-H), 3.87(1H, br d, J=5.5Hz, 4-H), 4.43(1H, d, J=7.5Hz, 7-H), and 4.56(1H, br s, 6-H).
- 15) **1**: ¹H-NMR (400MHz, D₂O) δ; 1.91(3H, s, NAc), 1.95(1H, d, J=15.5Hz, 3-H_{ax}), 2.10(1H, dd, J=5.5, 15.5Hz, 3-H_{eq}), 3.43(1H, ddd, J=2.5, 6.0, 7.5Hz, 8-H), 3.48(1H, dd, J=7.5, 11.0Hz, 9-H), 3.63(1H, dd, J=2.5, 6.0, 7.5Hz, 9-H'), 3.84(1H, br.s, 5-H), 3.87(1H, d, J=5.5Hz, 4-H), 4.38(1H, d, J=7.5Hz, 7-H), and 4.51(1H, br s, 6-H). ¹³C-NMR(75MHz, D₂O) δ; 22.0(NCOMe), 35.2(3-C), 52.0(5-C), 62.3(9-C), 66.7(4-C), 71.9(8-C), 77.1(7-C), 77.7(6-C), 104.8(2-C), 171.9(1-C), and 173.7(NCOMe).
- 16) This sample, methyl ester-trimethylsilyl derivative, of synthetic **1** was prepared as described in ref. 1.
- 17) The molecular composition of the compound was determined either by elemental analysis or by high resolution mass spectrometry.

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