A Facile Synthesis of 2-Deoxy-2,3-didehydroneuraminic Acid Derivatives

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The 2-thio- or 2-selenoglycosides of *N*-acetylneuraminic acid methyl ester were transformed by successive treatment with dimethyl(methylthio)sulfonium triflate (DMTST) and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) to give the corresponding methyl 2-deoxy-2,3-didehydroneuraminates in excellent yields. Their acids and their analogues are sialidase inhibitors of pharmaceutical interest.

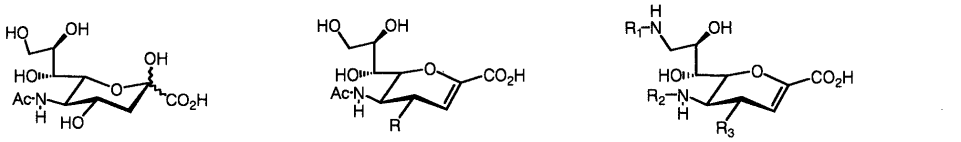
Key words methyl 2-thio-*N*-acetylneuraminate; 2-deoxy-2,3-didehydroneuraminic acid; DMTST; sialidase inhibitor

A sialidase has been considered a suitable and effective target for designing chemotherapeutic agents against influenza viruses.[1] The synthesis of a number of 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (Neu5Ac2en, **2**) analogues, which might be a transition state analogue binding to the active site of sialidase,[2] has received considerable attention over the past decade. Recently, using molecular modelling, von Itzstein and co-workers reported the design and biological evaluation of 2,3-didehydro-2,4-dideoxy-4-amino-*N*-acetylneuraminic acid (4-amino-Neu5Ac2en, **3**) and its guanidino analogue (4-guanidino-Neu5Ac2en, **4**). It was suggested that these compounds showed very potent antiviral activity against the influenza virus enzyme.[3] As a part of program aimed at the synthesis of new sialidase inhibitors, we have reported the chemoenzymatic synthesis of 5,9-di-*N*-acyl-2,3-didehydro-2,3-dideoxyneuraminic acids (**5a**—**c**)[4a] and 5,9-di-*N*-Ac-4-carbamoylmethyl-2,3-didehydro-2,3-dideoxyneuraminic acid (**6**)[4b] and their behaviour towards the sialidase from the influenza virus. For the synthesis of sialidase inhibitors of various deoxy analogues of Neu5Ac2en as a transition-state analogue, we now describe a novel and convenient dimethyl(methylthio)sulfonium triflate (DMTST)[5]-1,8-diazabicyclo[5.4.0]-7-undecene (DBU) promoted transformation of the 2-thio and 2-selenoglycosides of *N*-acetylneuraminic acid suitably modified at C-4 and C-9 into the corresponding 2-deoxy-2,3-didehydroneuraminic acid derivatives.

Results and Discussion

Several methods for introduction of double bond into the C-2 and C-3 positions in *N*-acetylneuraminic acids have been reported.[6] However, the formation of a 4,5-oxazoline derivative resulting from the intramolecular substitution of the 4-acetoxy group by the adjacent *N*-acetamido function as a by-products proved serious problem, when peracetylated methyl esters of *N*-acetylneuraminic acid were treated with trimethylsilyl trifluoromethanesulfonate. Thus, change of the configuration at C-4 has often been observed, while phenyl thioglycosides of *N*-acetylneuraminic acid are readily available and stable. Therefore, usage of the thioglycosides allows for easy manipulation, purification, and storage prior to activation. The sulfur atom in 2-thioglycosides of *N*-acetylneuraminic acid, which is a soft nucleophile, can be selectively activated by a variety of soft electrophiles, such as heavy metal salts, halogens, and alkylating or acylating reagents, without the intermediacy of any glycosyl halide, to form a reactive glycosylating species for creation of a new glycosidic bond. For the facile synthesis of 2-deoxy-2,3-didehydroneuraminic acid derivatives as transition-state analogues, we examined a mild and efficient method for the selective activation of the thiophenyl and selenophenyl groups in more elaborate systems which are of biological interest, such as **5** and **6**. The activation of **10** by NIS (*N*-iodosuccinimide)-TBAOTf (*tert*-butylammonium triflate)[7] and subsequent treatment with DBU resulted in recovery of unchanged starting material. In the case of the activation by NBS (*N*-bromosuccinimide)-I_2-TBAOTf,[8] NBS-Br_2-TBAOTf or NIS-TfOH (trifluoromethanesulfonic acid),[9] followed by the treatment with DBU in CH_2Cl_2 gave the expected compound **16** (FABMS: *m/z* 529 $[M+H]^+$, 551 $[M+Na]^+$) in 49, 52 and 49% yields, respectively, showing a one-proton doublet at δ 6.14 ($J_{3,4}$=3.0 Hz, H-3), characteristic of the 2,3-double bond in its ^{1}H-NMR spectrum. The best results were obtained when the methyl ester **10** was treated with DMTST (3 eq), the most extensively used as a sulfenium ion-like reagent, in CH_2Cl_2 at −20 °C, and subsequently with DBU (3 eq). These results are summarized in Table 1.

Under these conditions, the desired compound **16** was obtained in 93% isolated yield after purification by silica gel chromatography. In contrast, no reaction occurred when corresponding methylthio glycoside was used as a substrate. As can be seen from the table, phenyl α-selenoketoside of *N*-



Neu5Ac (**1**)

Neu5Ac2en (**2**) R = OH

4-Amino-Neu5Ac2en (**3**) R = NH_2

4-Guanidino-Neu5Ac2en (**4**) R = $NHC(NH)NH_2$

5a: R_1 = H, R_2 = CF_3CO, R_3 = OH

5b: R_1 = CH_3CO, R_2 = CF_3CO, R_3 = OH

5c: R_1 = CF_3CO, R_2 = CF_3CO, R_3 = OH

6: R_1 = CH_3CO, R_2 = CH_3CO, R_3 = $-OCH_2CONH_2$

Chart 1

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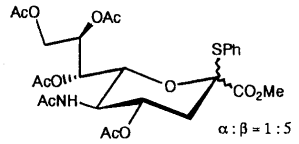
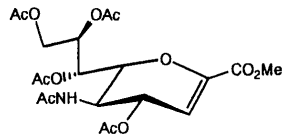
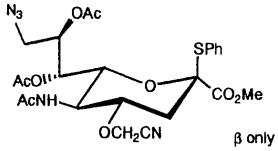
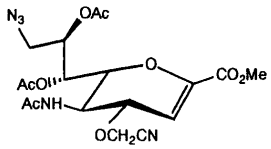
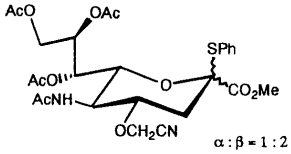
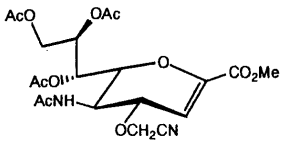
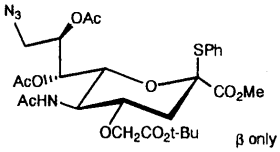
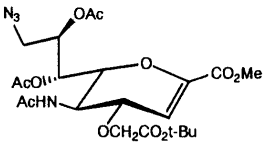
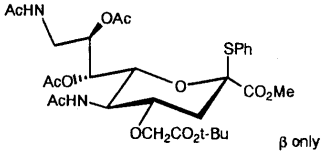
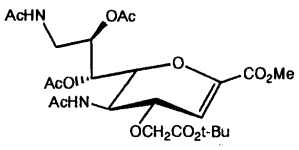
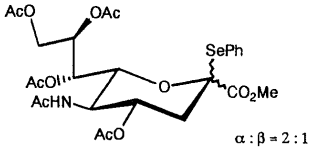
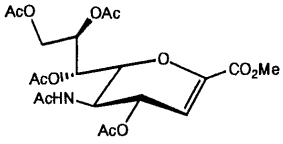
Table 1. Conversion of 2-Thio- and 2-Selenoneuraminic Acids to Their 2-Deoxy-2,3-didehydro Derivatives

Entry	Substrate	Product	Yield (%)
1	 α : β = 1 : 5 **7**	 **13**	95
2	 β only **8**	 **14**	99
3	 α : β = 1 : 2 **9**	 **15**	97
4	 β only **10**	 **16**	93
5	 β only **11**	 **17**	98
6	 α : β = 2 : 1 **12**	 **13**	92

acetylneuraminic acid (**12**)[10] was smoothly converted to 2-deoxy-2,3-didehydroneuraminic acid derivative **13** in 92% yield. The former compound is a versatile donor that may provide the desired selectivity in glycosidation reactions.[11]

In conclusion, the present procedure may provide useful methodology for the preparation of a variety of the 2-deoxy-2,3-didehydroneuraminic acid derivatives. Further studies on development of more potent and selective sialidase inhibitors using this method are in progress.

Experimental

General Methods Melting points are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded on a JASCO IR-810 spectrometer. ^{1}H-NMR spectra were recorded with a JEOL JNM-EX 270 [^{1}H (270 MHz)] spectrometer. ^{1}H chemical shifts are given in ppm relative to Me_4Si (δ=0) in $CDCl_3$ or CD_3OD as internal standards at ambient temperature. Fast atom bombardment (FAB) mass spectra were obtained with a JEOL JNM SX-102 mass spectrometer in the positive ion mode using NBA matrix. Column chromatography was performed on silica gel Merck 60 (70—230 mesh). TLC was performed on aluminum sheets coated with silica gel $60F_{254}$(Merck). The spots were visualized by spraying the plates with 5% aqueous sulfuric acid in MeOH and then heating.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (13) General Procedures for the Preparation of 2-Deoxy-2,3-didehydroneuraminic Acids DMTST (71 mg, 0.27 mmol) was added to a stirred solution of compound **7** (53 mg, 0.091 mmol) and MS 4 Å (0.5 g) in dry CH_2Cl_2 (2 ml) at −20 °C under Ar. After stirring for 2 h at the same temperature, to the mixture was added a solution of DBU (41 mg, 0.27 mmol) in dry CH_2Cl_2 (0.5 ml) at −20 °C, and the mixture was stirred for 1 h at the same temperature, and then overnight at room temperature. The precipitates were filtered off through Celite 545 and the filtrate was washed with aqueous saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. Column chromatography on silica gel with10 : 1 CH_2Cl_2–MeOH gave 2-deoxy-2,3-didehydroneuraminic acid (**13**) (41 mg, 95%) as amorphous powders. $[\alpha]_D$ +56° (c=1.3, $CHCl_3$). IR ν_{max} ($CHCl_3$): 1750, 1690, 521 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.93 (3H, s, AcNH), 2.04, 2.06, 2.07, 2.13 (each 3H, s, AcO), 3.81 (3H, s, CO_2Me), 4.20 (1H, dd, $J_{8,9a}$=7.3, $J_{9a,9b}$=12.2 Hz, H-9a), 4.38—4.43 (2H, m, H-5,6), 4.64 (1H, dd, $J_{8,9b}$= 3.2 Hz, H-9b), 5.34—5.38 (1H, m, H-8), 5.51—5.53 (2H, m, H-4,7), 5.93

(1H, br d, J=8.1 Hz, AcNH), 6.00 (1H, d, $J_{3,4}$=3.0 Hz, H-3). Positive FAB-MS m/z: 474 $[M+H]^+$, 496 $[M+Na]^+$.

Transformation of **12** (52 mg, 0.082 mmol) according to the general procedure gave compound **13** (36 mg, 92 %) as amorphous powders.

Methyl 5-Acetamido-7,8-di-*O*-acetyl-2,6-anhydro-9-azido-4-*O*-cyanomethyl-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (14) Transformation of **8** (30 mg, 0.053 mmol) according to the general procedure gave compound **14** (24 mg, 99%) as amorphous powders. $[\alpha]_D$ +41° (c=0.68, $CHCl_3$). IR ν_{max} ($CHCl_3$): 2108,1744, 1680, 1525 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.01 (3H, s, AcNH), 2.09, 2.14 (each 3H, s, AcO), 3.49 (1H, dd, $J_{8,9a}$= 7.6, $J_{9a,9b}$=13.5 Hz, H-9a), 3.80 (1H, dd, $J_{8,9b}$=4.0 Hz, H-9b), 3.82 (3H, s, CO_2Me), 4.16 (1H, ddd, $J_{4,5}$=7.0, $J_{5,6}$=8.6 Hz, H-5), 4.43, 4.46 (each 1H, d, J_{gem}=16.2 Hz, $-OCH_2CN$), 4.35—4.47 (2H, m, H-4,6), 5.20 (1H, ddd, $J_{7,8}$= 4.0 Hz, H-8), 5.50 (1H, dd, $J_{6,7}$=4.0 Hz, H-7), 5.79 (1H, d, $J_{NH,5}$=8.6 Hz, AcNH), 6.09 (1H, d, $J_{3,4}$=3.3 Hz, H-3). Positive FAB-MS m/z: 454 $[M+H]^+$.

Methyl 5-Acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-*O*-cyanomethyl-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (15) Transformation of **9** (110 mg, 0.19 mmol) according to the general procedure gave compound **15** (87 mg, 97%) as amorphous powders. $[\alpha]_D$ +10° (c=1.2, $CHCl_3$). IR ν_{max} ($CHCl_3$): 1748 ,1686, 1526 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (3H, s, AcNH), 2.13, 2.19 (9H, s, AcO), 3.87 (3H, s, CO_2Me), 4.21 (1H, dd, $J_{8,9a}$= 7.8, $J_{9a,9b}$=14.6 Hz, H-9a), 4.44, 4.55 (each 1H, d, J_{gem}=16.5 Hz, $-OCH_2CN$), 4.61 (1H, dd, $J_{8,9b}$=3.5 Hz, H-9b), 5.41 (1H, ddd, $J_{7,8}$=3.8 Hz, H-8), 5.57 (1H, dd, $J_{6,7}$=4.6 Hz, H-7), 6.17 (1H, d, $J_{3,4}$=3.5 Hz, H-3), 6.23 (1H, d, $J_{NH,5}$=8.6 Hz, AcNH). Positive FAB-MS m/z: 471 $[M+H]^+$.

Methyl 5-Acetamido-7,8-di-*O*-acetyl-2,6-anhydro-9-azido-4-*O*-*tert*-butoxycarbonylmethyl-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (16) Transformation of **10** (49 mg, 0.077 mmol) according to the general procedure gave compound **16** (38 mg, 93%) as a syrup. $[\alpha]_D$ +25° (c=0.72, $CHCl_3$). IR ν_{max} ($CHCl_3$): 2110, 1742, 1682, 1527 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, *tert*-BuOCO), 2.01 (3H, s, AcNH), 2.09, 2.14 (each 3H, s, AcO), 3.49 (1H, dd, $J_{8,9a}$=8.1, $J_{9a,9b}$=13.5 Hz, H-9a), 3.81 (3H, s, CO_2Me), 4.03, 4.12 (each 1H, d, J_{gem}=16.5 Hz, $-OCH_2CO_2$*tert*-Bu), 4.09 (1H, ddd, $J_{4,5}$=7.3 Hz, $J_{5,6}$=8.6, $J_{5,NH}$=8.4 Hz, H-5), 4.35 (1H, dd, $J_{3,4}$=3.0 Hz, H-4), 4.43 (1H, dd, $J_{6,7}$=3.5 Hz, H-6), 5.23 (1H, ddd, $J_{7,8}$=3.5 Hz, H-8), 5.50 (1H, dd, H-7), 5.99 (1H, s, AcNH), 6.14 (1H, d, H-3). Positive FAB-MS m/z: 529 $[M+H]^+$, 551 $[M+Na]^+$.

Methyl 5-Acetamido-7,8-di-*O*-acetyl-2,6-anhydro-9-acetamido-4-*O*-*tert*-butoxycarbonylmethyl-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (17) Transformation of **11** (31 mg, 0.047 mmol) according to the general procedure gave compound **17** (25 mg, 98%) as amorphous powders. $[\alpha]_D$ +8° (c=0.56, $CHCl_3$). IR ν_{max} ($CHCl_3$): 1742, 1670, 1528 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, *tert*-BuOCO), 1.98, 1.99 (each 3H, s, AcNH), 2.04, 2.14 (each 3H, s, AcO), 3.81 (3H, s, CO_2Me), 4.04, 4.13 (each 1H, d, J_{gem}= 16.7 Hz, $-OCH_2CO_2$*tert*-Bu), 4.13—4.24 (1H, m, H-5), 4.34 (1H, dd, $J_{3,4}$= 3.0 Hz, H-4), 4.43 (1H, dd, $J_{6,7}$=3.5 Hz, H-6), 5.11 (1H, ddd, $J_{7,8}$=4.6 Hz, H-8), 5.42 (1H, dd, H-7), 6.15 (1H, d, H-3), 6.28 (1H, d, J=8.1 Hz, AcNH). Positive FAB-MS m/z: 545 $[M+H]^+$, 571 $[M+Na]^+$.

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