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SYNTHESES OF (α2-9) AND (α2-8) LINKED NEURAMINYLNEURAMINIC ACID DERIVATIVES[1]

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Abstract: A newly prepared glycosyl donor, the acetyl protected 2β-bromo-3β-hydroxy-N-acetylneuraminic ester was condensed with the protected 2-deoxy-2,3-dehydroneuraminic ester having a free hydroxy group at 9- or 8-position to form NeuAc(α2-9)NeuAc and NeuAc(α2-8)-NeuAc linkage, respectively, which are involved in the group C meningococcal polysaccharides and gangliosides. The obtained 3β-hydroxy disaccharides were phenoxythiocarbonylated, reduced with tri-n-butylstannane, and deprotected to give the free glycosides in high yields.

N-Acetylneuraminic acid (NeuAc) located at the non-reducing ends of carbohydrate chains of glycoproteins and glycolipids plays a role in biological recognition.[2,3] Many attempts have been done with little success in the synthesis of NeuAc linked carbohydrate chains. In the previous papar,[4] we have shown that the 2β-bromo-3β-hydroxy-NeuAc methyl ester **1**[5] was a useful glycosyl donor to afford NeuAc(α2-3)Gal or NeuAc(α2-3')Lac glycosides. We report here a successful application of this approach to the syntheses of NeuAc(α2-9)NeuAc and NeuAc(α2-8)NeuAc linkages involved in the group C meningococcal polysaccharides[6] and gangliosides. This is the first synthesis of the NeuAc(α2-8)NeuAc linkage.

The glycosylation of the 9-unprotected 2,3-dehydro-NeuAc methyl ester **4**[7] (1.1 equiv) with the 2-bromo-3-hydroxy derivative of peracetylated NeuAc methyl ester **1** in toluene-1,2-dichloroethane (1:1) in the presence of silver triflate (AgOTf) at 0 °C gave in 71% isolated yield a mixture of 2-9 linked di-NeuAc derivatives. The mixture was separated by the repeated silica gel column chromatography (benzene-acetone-methanol, gradient elution from 30:30:0 to 30:30:2) or by preparative ODS HPLC (methanol-water, 46:54) to the protected NeuAc-

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(α2-9)NeuAc glycoside **6**[9] (mp 137-139 °C, 42% yield) and the corresponding β-glycoside **10**[9] (21% yiled). As demonstrated earlier, the anomeric configuration was determined by analysis of ^{1}H-NMR spectrum. Thus, the $J_{7,8}$ coupling constant of NeuAc units of **6** and **10** showed 8.4 and 2.7Hz, respectively, and $\Delta\delta|$H-9'-H-9$|$ values of them were 0.17 and 0.59ppm, respectively. These values agreed with those deduced from the empirical rule.[10]

In the above glycosylation condition, the bromide **1** reacted with the 8-unprotected 2,3-dehydro-NeuAc methyl ester **5**[11] (1.0 equiv) giving a mixture of 2-8 linked di-NeuAc derivatives in 43% yield. The mixture was separated by preparative ODS HPLC (methanol-water, 45:55) to the NeuAc(α2-8)NeuAc glycoside **14**[9] (26% yield) and the NeuAc(β2-8)NeuAc glycoside **18**[9] (8% yield). The anomeric configuration was determined by the fact that in ^{1}H-NMR spectra the $J_{7,8}$ coupling constant of **14** and **18** showed 7.5 and 1.8Hz, respectively, and $\Delta\delta|$H-9'-H-9$|$ values of them were 0.41 and 0.91ppm, respectively.

The 3β-hydroxy groups of the glycosides **6**, **10**, **14**, and **18** were reduced to the corresponding 3-deoxy glycosides in the follwing way. The glycosides were treated with phenyl chlorothionocarbonate and 4-dimethylaminopyridine[12] in acetonitrile for **6** and **10**, in DMF for **14**, and in DMSO for **18** to give the thionocarbonates **7**[9] (91% yield, $J_{7,8}$=8.9Hz, $\Delta\delta|$H-9'-H-9$|$=0.06ppm), **11**[9] (83%, 4.0Hz, 0.62ppm), **15**[9] (85%, 7.5Hz, 0.42ppm), and **19**[9] (80%, 2.7Hz, 0.85ppm), respectively. Reduction of the thionocarbonates with tri-n-butylstannane afforded **8**[9] (97% yield, $J_{7,8}$=8.9Hz, $\Delta\delta|$H-9'-H-9$|$=0.18ppm), **12**[9] (96%, 3.4Hz, 0.67ppm), **16**[9] (95%, 7.8Hz, 0.53ppm), and **20**[9] (96%, 2.7Hz, 0.91ppm), in which **20** was identical with the previously obtained authentic sample.[11] The $J_{7,8}$ coupling constant and $\Delta\delta|$H-9'-H-9$|$ value remained unaltered in each series of α- and β-glycosides, even if the C-3 was substituted with hydroxy or phenoxythiocarbonyl group. Deacetylation (t-BuOK in methanol at room temp) and saponification (1N NaOH in methanol at room temp) of **8**, **12**, and **16** afforded the deprotected di-NeuAc **9**,[9] **13**,[9] and **17**,[9] quantitatively.

In conclusion, we found that the 2β-bromo-3β-hydroxy-NeuAc derivative **1** is a useful donor for the glycosylation of the 8- or 9-unprotected peracetyl 2,3-dehydro-NeuAc methyl ester since the 3β-hydroxy group of the bromide **1** prevents the dehydrohalogenation reaction and assists the glycosylation reaction.[13] The 2,3-double bond present in the glycosylation products can be used for further glycosylation via the corresponding 2β,3β-bromohydrin.[5]

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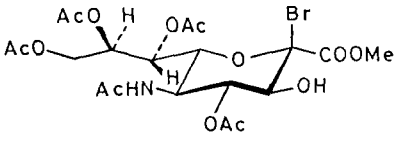

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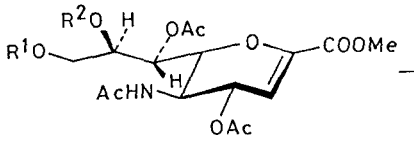
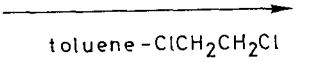
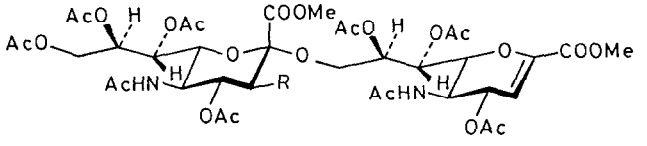


4 R^1 = H , R^2 = Ac

5 R^1 = Ac , R^2 = H



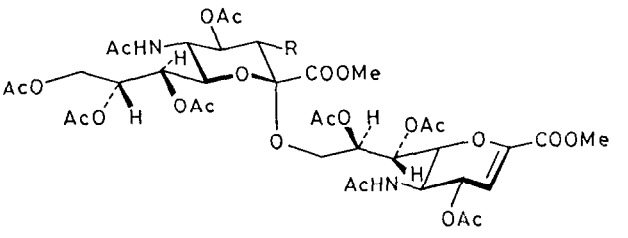


6 R = OH (42 %)

7 R = OC(=S)OPh (91 %)

8 R = H (97 %)

9 deprotected 8 (97 %)

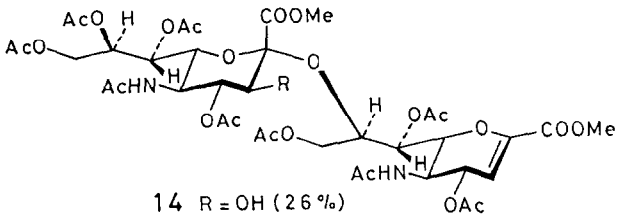


10 R = OH (21 %)

11 R = OC(=S)OPh (83 %)

12 R = H (96 %)

13 deprotected 12 (96 %)

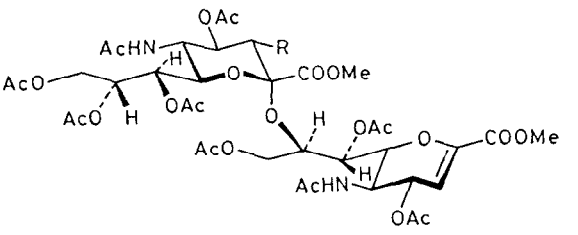


14 R = OH (26 %)

15 R = OC(=S)OPh (85 %)

16 R = H (95 %)

17 deprotected 16 (97 %)



18 R = OH (8 %)

19 R = OC(=S)OPh (80 %)

20 R = H (96 %)

this issue.

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7. The glycosyl acceptor **5** having a free hydroxy group at 9-position was prepared from 2-deoxy-2,3-dehydro-NeuAc methyl ester **2**[8] in the follwing two steps: (i) trityl chloride in pyridine at 60 °C for 2 h, then Ac_2O-pyridine at 60 °C for 1 h (4,7,8-tri-O-acetyl-9-O-Tr **3**[9], 84%); (ii) 90% AcOH at 60 °C for 1 h (9-ol **4**[9], 71%).
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9. Satisfactory elemental analyses were obtained for these compounds. $[\alpha]_D$ and ^{1}H-NMR data (non-reducing NeuAc unit in chloroform-d) are shown below.

Compound	$[\alpha]_D$[a]	Chemical shifts (δ) and $J_{7,8}$ coupling constants, Hz in ^{1}H-NMR											
		H-3eq (dd)	H-3ax (dd)	H-4 (dd)	H-5 (ddd)	H-6 (dd)	H-7 (dd)	H-8 (ddd)	H-9 (dd)	H-9' (dd)	Me ester (s)	NH (d)	$J_{7,8}$
3	+38.3°		6.05[b]	5.50	4.27	4.48	5.58	5.37	3.24	3.40	3.75	5.66	5.5
4	+58.7°		5.98[b]	5.52	4.49	4.39	5.46	5.11	3.61[c]	4.05	3.81	5.91	5.6
6	+29.9°		3.77	5.22	4.16	4.62	5.27	5.36	4.08	4.25	3.81[d]	5.73	8.4
7	+35.3°		5.68[b]	5.73	4.19	4.88	5.30	5.48	4.17	4.23	3.80[d]	5.72	8.9
8	+17.8°	2.53	1.89	4.94[c]	3.97	4.13	5.33	5.40	4.12	4.30	3.80[d]	5.37	8.9
9[e]	+12.7°[f]	2.70	1.80	3.77[c]	3.88[g]	3.81	3.54	3.87	3.62	3.85			8.9
10	+23.9°		3.81	5.24	4.16	4.29	5.37	5.05	4.08	4.67	3.82[d]		2.7
11	+35.4°		5.82[b]	5.54	4.38	4.44	5.42	5.19	4.09	4.71	3.80[d]	6.52	4.0
12	+35.1°	2.44	1.84	5.28[c]	4.05	4.30	5.41	5.17	4.13	4.80	3.81[d]	6.26	3.4
13[e]	+ 6.6°[f]	2.45	1.76	4.10[c]	3.93[g]	3.93	3.56	3.84	3.64	3.83			9.5
14	+27.4°		4.00	5.24	4.19	4.38	5.24	5.28	3.96	4.37	3.83[d]	5.67	7.5
15	+35.4°		5.89[b]	5.47	4.40	4.38	5.32	5.34	4.02	4.44	3.81[d]	5.48	7.5
16	+22.3°	2.65	2.19	4.88[c]	4.00	3.81	5.31	5.31	3.95	4.48	3.83[d]	5.14	7.8
17[e]	+35.7°[f]	2.71	1.78	3.66[c]	3.84[g]	3.67	3.54	3.78	3.62	3.83			9.0
18	+20.3°		3.85	4.99	4.21	4.66	5.35	5.32	3.95	4.86	3.82[d]	6.03	1.8
19	+22.9°		5.84[b]	5.26	4.38	4.72	5.38	5.33	4.00	4.85	3.79[d]	6.20	2.7
20	+31.2°	2.49	1.84	5.09[c]	4.08	4.62	5.37	5.31	4.01	4.92	3.78[d]	6.06	2.7

[a] Measured in chloroform. [b] Multiplicity: d. [c] Multiplicity: ddd. [d] Assignments may be interchanged with reducing unit. [e] Measured in D_2O (t-BuOH=1.23 ppm). [f] Measured in water. [g] Multiplicity: dd.

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13. When the 3-O-acetylated compound of **1** was used in this glycosylation, no glycosides was obtained.

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