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Stereoselective Synthesis of Oligo-α-(2,8)-Sialic Acids

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Polysialic acids composed of the α(2,8) and/or α(2,9) Neu5Ac units **1** and **2** are frequently located at the nonreducing end of biologically active glycoconjugates such as glycoproteins and glycolipids (Figure 1).[1] Recent progress in glycobiology suggests that such oligomers may play important roles in biological events on the cell surface.[2] In terms of structure−activity relationship studies, the use of chemically pure synthesized oligosialosides as biochemical probes would be highly desirable because the possibility that such isolated glycoconjugates could be heterogeneous and/or contaminated with antigenic compounds cannot be excluded. However, oligosialosides **1** and **2** represent difficult and challenging synthetic targets. The presence of the C1 carboxyl group of sialic acids reduces the reactivity of the anomeric position toward glycosidation. A lack of C3 substitutions readily promotes the 2,3 elimination of a sialyl donor during glycosidation, making the formation of α-isomers difficult.[3] Furthermore, the C8 hydroxyl group on the exocyclic chain exhibits low reactivity toward glycosylation.

To overcome these problems, the sialyl donors containing a stereodirecting group at the C3 position, namely OH or SPh, have been developed for indirect synthetic methods and permit the preparation of various α(2,8)-disialosides.[4] The substituent at the C3 position on the donors should be stereoselectively installed and must be removed at the end of the synthesis. On the other hand, the conversion of the acetamide group at the C5 position of the sialyl donor to *N*,*N*-diacetyl,[5] azido,[6] *N*-TFA,[7] *N*-Troc,[8,9] *N*-Fmoc,[9] *N*-trichloroacetyl,[9] and *N*-phthalimide[10] groups has been reported to be effective for direct α-selective sialylation. The coupling of *N*,*N*-diacetyl,[7] *N*-TFA,[11] and *N*-Troc[12] sialyl donors and acceptors directly provided α(2,8)-disialosides in moderate yields and good selectivity. Furthermore, the 1,5-lactamization of sialic acids was developed as an alternative route for enhancing the reactivity of the C8 hydroxyl groups toward glycosylation.[13] In these methods, the use of acetonitrile as a solvent is critical for achieving α-selective glycosidation.[14] However, the utility of these methodologies in direct α-sialylation has been mainly demonstrated in the synthesis of α(2,8)-disialosides. Therefore, an effective method for the synthesis of oligosialosides continues to be needed.

In this communication, we report on an effective α-selective sialylation using the 5-*N*,4-*O*-carbonyl-protected sialyl donor **3** and its application to the synthesis of α(2,8)-tetrasialoside **1a**. As illustrated in Table 1, treatment of the 5-*N*,4-*O*-carbonyl-protected α-sialoside **4a**[15] containing C7 and C8 dihydroxyl groups and 1.5 equiv of 5-*N*,4-*O*-carbonyl- and 7,8-*O*-dichloroacetyl-protected sialy donor **3** with NIS and a catalytic amount of TfOH in CH_2Cl_2 at −78 °C provided α(2,8)-sialoside **5a** in 86% yield with complete α-selectivity. The chloroacetyl protecting groups at the *O*-7,8 positions proved to be effective for improving the reactivity of the sialyl donor **3** and can be selective removed to provide 7,8-diols for the next glycosylation.[16] It should be noted that the protection of the 5-*N*,4-*O*-carbonyl group on the thiosialoside enables

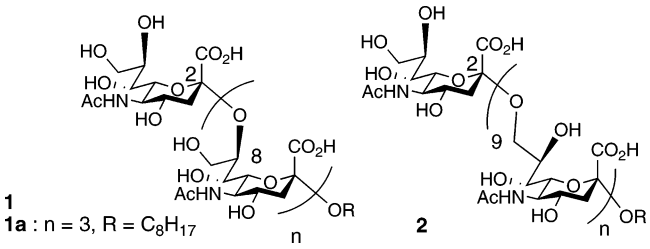
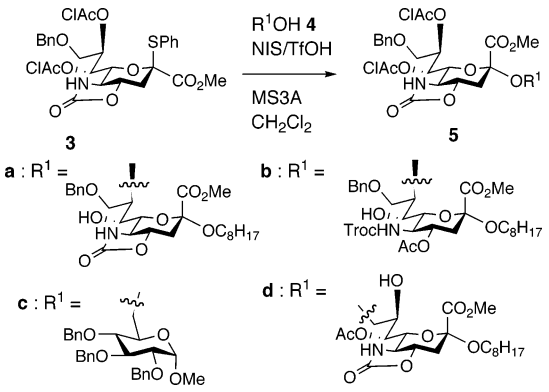


Figure 1. Structure of α(2,8)- and α(2,9)-sialosides **1** and **2**.

Table 1. Glycosidation of 5-*N*,4-*O*-Carbonyl-Protected Sialoside **3**



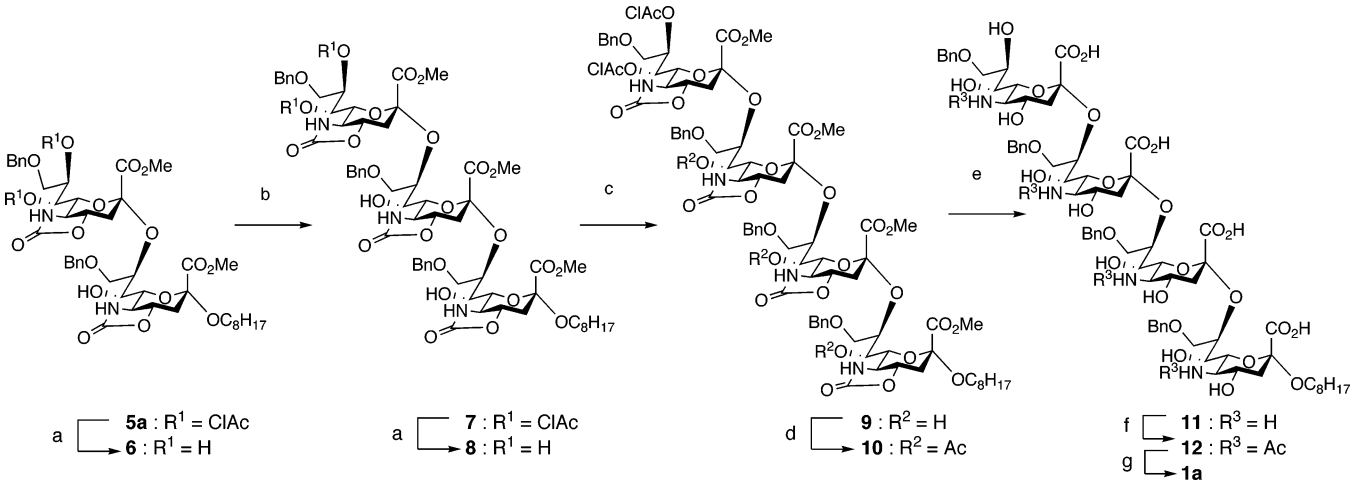
entry	equivalent of donor **3** (equiv)	acceptor	product	yield (%)	α:β
1	1.5	**4a**	**5a**	86	α only[a]
2	1.5	**4b**	**5b**	20	74:26[b]
3	1.0	**4c**	**5c**	quant	α only[a]
4	1.0	**4d**	**5d**	96	α only[a]

[a] The α:β ratio was estimated by analysis of ^{1}H NMR spectra. [b] The α:β ratio was estimated by HPLC analysis based on refractive index detection.

α-sialylation to proceed without acetonitrile effects or neighboring-group participation by any of the auxiliaries.[17] The glycosylation of *N*-Troc sialoside **4b** with donor **3** resulted in a reduced coupling yield of **5b** with moderate α-selectivity. These results indicate that the cyclic protecting group effectively improved the reactivity of the C8 hydroxyl group toward glycosylation.[18] We next carried out the glycosylation of primary alcohols in **4c** and **4d** with **3**. The sialylation of primary alcohols based on the acetonitrile effects frequently results in poor α-selectivity, compared to the sialylation of secondary hydroxyl groups such as the C3 hydroxyl group on galactosides. Treatment of the primary hydroxyl group of **4c** with 1.0 equiv of sialyl donor **3** under the same reaction conditions provided α-sialosides **5c** in quantitative yield and excellent selectivity. Furthermore, glycosylation of the 8,9-dihydroxyl sialyl acceptor **4d** with donor **3** afforded α(2,9)-disialoside **5d** in 96% yield as a single product. These results indicated that donor **3** was especially effective for sialylation of the primary alcohols.

10.1021/ja0613613 CCC: $33.50

Scheme 1 [a]



[a] Reagents and conditions: (a) thiourea, 2,6-lutidine, DMF, 70 °C, 85% for **6**, 82% for **8**. (b) **3** (3.0 equiv), NIS (3.6 equiv), TfOH (0.3 equiv), MS3A, −78 °C, 89%, α only. (c) **3** (3.0 equiv), NIS (3.6 equiv), TfOH (0.30 equiv), MS3A, −78 °C, 57% α only. (d) Ac_2O, Py, CH_2Cl_2, −50 °C. quant (e) $LiOH{\cdot}H_2O$, H_2O, EtOH, 80 °C, 88%. (f) Ac_2O, $NaHCO_3$, H_2O, 0 °C then NaOMe, MeOH, 64%. (g) $Pd(OH)_2$, H_2 (1 atm), MeOH, H_2O, 70%.

The structures **5a**−**d** were confirmed as follows. The α configuration of sialosides **5a**, **5c**, and **5d** was determined on the basis of the $^3J_{C1-H3ax}$ coupling constants of the corresponding hydrolysates of **5a**, **5c**, and **5d**.[19] The regioselectivity in the glycosylation of diols **4a** and **4d** was estimated by ^{1}H NMR analysis of the acetylated products from **5a** and **5d.** The hydrolysate of the *N*-Troc derivative **5b**-α was identical to the hydrolysate of **5a** (details are shown in Supporting Information).

We next conducted the synthesis of α(2,8)-tetrasialosides **1a** via **5a** (Scheme 1). The removal of the two chloroacetyl groups at the C7 and C8 positions of **5a** provided triol **6** in 85% yield. Treatment of triol **6** and the 5-*N*,4-*O*-carbonyl-protected sialoside **3** (1.5 equiv) with NIS/TfOH in CH_2Cl_2 at −78 °C provided α(2,8)-trisialoside **7** in 68%. The use of 3.0 equiv of the glycosyl donor **3** resulted in the disappearance of acceptor **6** and provided trisaccahride **7** in 89% yield with complete α selectivity. Deprotection of the chloroacetyl groups on **7** afforded the tetraol acceptor **8** in 82% yield. Tetrasaccharide formation from **8** using 3.0 equiv of donor **3** under the same reaction conditions provided tetrasaccharide **9** in 57% yield with complete α-selectivity along with the recovered acceptor **8** (31%). The coupling constants $^3J_{C1-H3ax}$ (5.9, 5.8, 5.4, and 5.3 Hz) for **9** indicated that the configuration of all glycosidic linkages was α. In addition, the regioselectivity of each glycosylation step was confirmed by ^{1}H NMR analysis of the acetylated product **10**.

Deprotection of the α(2,8)-tetrasialoside **9** was examined. Exposure of the protected tetrasialoside **9** to basic conditions provided amino acids **11** (88%). Acetylation the resulting amines provided the *N*-acetyl derivative **12** in 64% yield. Finally, removal of the benzyl ethers on **12** by hydrogenolysis using a palladium catalyst afforded the fully deprotected tetrasialosides **1a**.

In conclusion, an efficient and elegant synthesis of α(2,8)-oligosialosides is described. The 5-*N*,4-*O*-carbonyl-protected sialyl donor undergoes α-sialylation in CH_2Cl_2 to provide α(2,8)- and α(2,9)-disialosides in excellent yields. The 5-*N*,4-*O*-carbonyl protecting group was effective for improving the reactivity of the C8 hydroxyl groups toward glycosylation. Using the sialyl building block, the synthesis of tetra-α(2,8)-sialic acid could be accomplished using a simple glycosidation and deprotection protocol. This coupling method allows the synthesis of the various oligosaccharides containing α(2,8)- and α(2,9)-oligosialoside units, which are effective biochemical probes for elucidating their biological activities.

Supporting Information Available: Experimental procedures for the α-sialylation and full characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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