Sialic acids are a family of monosaccharides comprising about 40 members which have in common neuraminic acid (Neu) (5-amino-3,5-dideoxy-D-glycero-D-galacto-nonulosonic acid). Since the first discovery of N-acetylneuraminic acid (Neu5Ac, 1) from brain glycolipids or salivary mucins, many of sialic acids have been known to occur in animal and some microorganisms. Because they occupy the terminal position on macromolecules and cell membranes and thus are involved with essential functional roles in many biological and pathological phenomena, sialic acids have become a family of the most important molecules of life. The lead member of the family, Neu5Ac (1), which is found in important naturally occurring glycoconjugates and oligosaccharides, is typically linked to galactose through an α→3 or an α→6 linkage or is polymerized in the form of an α→2 or an α→9 linkage. Over the years, a number of strategies for the stereoselective construction of the equatorial α-sialyl linkages have been developed, and the main focus was on the utilization of various leaving groups on sialyl donors such as phosphite, hydroxy group, N-phenyltrifluoracetimidate, xanthate, halide, sulfide, and 1-adamantanyl thiosialoside. We introduced previously four new glycosyl donors having new leaving groups for stereoselective glycosylations: 2-carboxybenzyl (CB) glycosides, 2′-(benzoxycarbonyl)benzyl glycosides, glycosyl p-bromophenyl pthalates, and glycosyl pthalates. The efficiency and stereoselectivity of sialylations with sialyl donors having these new leaving groups such as CB sialosides, 2′-(benzoxycarbonyl)benzyl sialosides, sialyl p-bromophenyl pthalates, and sialyl pthalates were not satisfactory. Fortunately, however, we were able to achieve the stereoselective α-sialylation employing the glycosyl pentenoate/PhSeOTf method, which also we introduced previously for the stereoselective β-mannosylation and other glycosylations. Herein we report the efficient, stereoselective α-sialylation method by using N-acetylneuraminyl (Neu5Ac) pentenolate and N-trifluoroacetylneuraminyl (Neu5TFA) pentenolate as donors with PhSeOTf as a promoter. Although there has been a report on the preparation of sialylation with 2 by other workers, they reported the poor stereoselectivity of the sialylation of only one benzyl alcohol with 2 employing other promoters but failed to report the sialylation of sugars. Sialyl pentenolates 2 and 3 were readily prepared by esterification of corresponding sialyl anomeric hydroxy sugars and respectively, with 4-pentenoic acid using N,N-diisopropylcarbodiimide (DIC) in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) as shown in Scheme 1. At first, we examined sialylations of primary hydroxy acceptor 6 with the donor 2 employing PhSeOTf as the activating agent in the presence of 2,4,6-tri-tert-butylpyrimidine (TTBP) and 4A molecular sieves at −78 or −40 °C to 0 °C in four different solvents. The sialylation of 6 with 2 in dichloromethane was not α-selective but rather β-selective, yielding sialyl disaccharides 7 (α/β = 1:13.4) with a large excess of the β-anomer in 72% yield (entry 1 in Table 1). 1,4-Dioxane and toluene, however, were found to be not proper solvents for the present sialylation (entries 2 and 3 in Table 1), whereas the sialylation in acetonitrile produced sialic acid disaccharide 7 (α/β = 3:3:1), favoring the α-anomer in 54% yield (entry 4). In this stage, we realized that the base TTBP might not be necessary for the present sialylation, even though it was essential for trapping triflic acid generated during glycosylations in our original work, because there were no acid-labile protecting groups in both donors and acceptors in the present work. The sialylation of 6 with 2 in acetonitrile without TTBP at −40 °C to 0 °C was more satisfactory than those under other conditions, affording sialoside 7 (α/β = 5.5:1) with an excess of the α-anomer in 91% yield (entry 5). Acetonitrile is known to facilitate the formation of equatorial glycosyl bonds in several glycosylation reactions. Acetonitrile, in the present study, might interact with sialyl oxocarbonium ions generated during the sialylations and the resulting axial β-sialyl nitrilium ion might be one of reactive inter-

**Key Words:** Glycosyl pentenoate, α-Sialylation, Glycosylation, Phenylselenyl triflate

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This paper is dedicated to Professor Sunggak Kim on the occasion of his honorable retirement.
Table 1. Sialylations of acceptor 6 with sialyl donor 2 in different solvents

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Reaction Time</th>
<th>Base</th>
<th>Yield (%)</th>
<th>α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂Cl₂</td>
<td>-78 °C → 0 °C</td>
<td>15 min 1 h</td>
<td>TTBP</td>
<td>72</td>
<td>1:13.4</td>
</tr>
<tr>
<td>2</td>
<td>1,4-Dioxane</td>
<td>-78 °C → rt</td>
<td>15 min 1 h</td>
<td>TTBP decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Toluene</td>
<td>-78 °C → rt</td>
<td>15 min 1 h</td>
<td>TTBP decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>CH₃CN</td>
<td>-40 °C → 0 °C</td>
<td>15 min 1 h</td>
<td>TTBP</td>
<td>54</td>
<td>3.3:1</td>
</tr>
<tr>
<td>5</td>
<td>CH₃CN</td>
<td>-40 °C → 0 °C</td>
<td>30 min 8 h</td>
<td>No base</td>
<td>91</td>
<td>5.5:1</td>
</tr>
</tbody>
</table>

* Determined after isolation. The ratio was determined by LC-mass.

To improve the efficiency and stereoselectivity of the sialylation with sialyl pentenolates, we considered Neu5TFA pentenolate 3 as a sialyl donor having the 5-N-trifluoroacetyl (TFA) group instead of Neu5Ac pentenolate 2 having 5-N-acetyl (Ac) group. In fact, the trifluoroacetyl group has been previously recognized as the better protecting group for the 5-amino group in the sialylation with thiolsialosides. With Neu5TFA pentenolate 3 as the sialyl donor, sialylations of primary hydroxy acceptors 8 and 18 bearing benzyl-protecting groups were highly α-selective, yielding exclusively α-disaccharides 20 and 21, respectively, in high yields (entries 1 and 2 in Table 3). Sialylations of primary hydroxy acceptors 9 and 19 possessing benzyl-protecting groups with 3 as the sialyl donor, on the other hand, were less α-selective, providing sialyl disaccharides 22 (α/β = 4.2:1) and 23 (α/β = 3.6:1), respectively, with an excess of α-anomers (entries 3 and 4). The sialylation of dihydroxy acceptor 10 with 3 was regio-selective as anticipated and α-selective as well, yielding sialyl(2→6)galactoside 24 (α/β = 5.8:1) with an excess of the α-anomer in 88% yield (entry 5). Sialylations of secondary dihydroxy acceptors 11 and 12 were also regio- and stereoselective, giving sialyl(2→3)galactosides 25 (α/β = 2.6:1) and 26 (α/β = 4.0:1), respectively, with an excess of α-anomers (entries 6 and 7). The results indicate that the trifluoroacetyl group at the N-5 position in the sialyl pentenolate, indeed, makes the sialylation more α-selective than the acetyl group. Although it has been suggested that the stronger electron-withdrawing trifluoroacetyl group would reduce the nucleophilicity of the amino group, thereby suppressing possible side reactions in glycosylations, the origin of the enhanced α-selectivity by trifluoroacetyl group is unclear as yet. Nevertheless, it could be mentioned that electron-withdrawing groups even at remote positions of donors mediates. The better result without the base, namely, TTBP might be ascribed to the interference of the base with activation of pentenolates by PhSeOTf in the present sialylation.
were found to strongly affect the outcome of stereoselectivity in mannopyranosylations by destabilizing the mannosyl oxocarbenium ion intermediates.26 It is also difficult to explain the present fact that the a-selectivity of the benzyl-protected acceptors 8 and 18 is more pronounced than that of the benzoyl-protected acceptors 9 and 19.27

In summary, a new efficient sialylation method was developed employing N-trifluoroacetyliminouramyl pentenoate 3 as a glycosyl donor and PhSeOTf as a promoter. a-Selectivity was enhanced with trifluoroacetyl-protecting group at the 5-amino functionality of the donor as compared to that with the acetyl group. Acetonitrile was chosen as the solvent with sialyl group. Tri fluoromethylation was more pronounced than that of the benzoyl group.18 It is also difficult to explain the present fact that the a-selectivity of the benzyl-protected acceptors 8 and 18 is more pronounced than that of the benzoyl-protected acceptors 9 and 19.27

In summary, a new efficient sialylation method was developed employing N-trifluoroacetyliminouramyl pentenoate 3 as a glycosyl donor. Acetonitrile was chosen as the solvent for the present sialylation. The synthesis of the GPI anchor of the scrapie prion protein employing the present sialylation method is currently underway.

**Experimental Section**

**Table 3. Sialylations of various acceptors 8-12, 18, and 19 with sialyl donor 3 in acetonitrile**

<table>
<thead>
<tr>
<th>entry</th>
<th>acceptor</th>
<th>product</th>
<th>yield (%)</th>
<th>α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>20</td>
<td>85</td>
<td>α only</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>21</td>
<td>85</td>
<td>α only</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>22</td>
<td>87</td>
<td>4:2:1</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>23</td>
<td>93</td>
<td>3:6:1</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>24</td>
<td>88</td>
<td>5.8:1</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>25</td>
<td>85</td>
<td>2:6:1</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>26</td>
<td>88</td>
<td>4.0:1</td>
</tr>
</tbody>
</table>

aDetermined after isolation. bThe ratio was determined by LC-mass.

A solution of glycosyl donor (0.12 mmol) and glycosyl acceptor (0.18 mmol) in CH3CN (3 mL) was then added and the resulting solution was stirred at 0 °C for 8 h, the reaction mixture was quenched with saturated aqueous NaHCO3 (5 mL), diluted with CH2Cl2, and filtered through Celite®. The filtrate was washed with saturated aqueous NaHCO3 and brine, dried over MgSO4, and concentrated in vacuo. The residue was purified by flash column chromatography. The α/β ratios of sialylation products were determined on the basis of the integrations of H3eq peaks of their NMR spectra of the mixtures of α- and β-anomers and determined more precisely by LC-mass employing with C18 silica HPLC column and CH3CN/H2O (7:3 or 8:2) eluent.

**General procedure for sialylations with sialyl pentenoates 2 and 3.** A solution of PhSeBr (0.61 mmol) and AgOTf (0.61 mmol) in CH3CN (5 mL) in the presence of 4A molecular sieves was stirred for 30 min at room temperature and cooled to -40 °C. A solution of glycosyl donor (0.12 mmol) and glycosyl acceptor (0.18 mmol) in CH3CN (3 mL) was then added and the resulting solution was stirred at -40 °C for 30 min. After being stirred at 0 °C for 8 h, the reaction mixture was quenched with saturated aqueous NaHCO3 (5 mL), diluted with CH2Cl2, and filtered through Celite®. The filtrate was washed with saturated aqueous NaHCO3 and brine, dried over MgSO4, and concentrated in vacuo. The residue was purified by flash column chromatography. The α/β ratios of sialylation products were determined on the basis of the integrations of H3eq peaks of their NMR spectra of the mixtures of α- and β-anomers and determined more precisely by LC-mass employing with C18 silica HPLC column and CH3CN/H2O (7:3 or 8:2) eluent.

**Acknowledgments.** This work was supported by a grant from Korea Science and Engineering Foundation through Center for Bioactive Molecule Hybrids (CBMH). B.-Y.L and D.-H.S. thank the fellowship of the BK 21 program from the Ministry of Education and Human Resources Development.

**Supporting Information.** General Methods and characterization data are available.

**References**


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27. Although the protecting group effects of acceptors on the glycosylation stereochemistry have been observed before, the origin of the effects is unclear as yet. Rationalizations for the protecting group effect on the glycosylation stereochemistry have been mostly donor-based considerations. For examples, see: (a) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* 1988, 110, 5583-5584. (b) Fraser-Reid, B.; Lopez, J. C.; Radhakrishnan, K. V.; Mach, M.; Schluter, U.; Gomez, A. M.; Uriel, C. *J. Am. Chem. Soc.* 2002, 124, 3198-3199.