SYNTHETIC STUDY OF $\alpha$(2,8) OLIGOSIALOSIDE USING $N$-TROC SIALYL $N$-PHENYLTRIFIluORoIMIDATE

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Abstract – An effective approach for the synthesis of oligo-$\alpha$(2,8) sialosides using $N$-Troc sialyl donors is described. Glycosylation of $N$-Troc sialoside with $N$-Troc sialyl $N$-phenyltrifluoroimidate and phosphites smoothly proceeded to provide $\alpha$(2,8) disialosides in good yield and selectivity.

Sialic acids such as Neu5Ac, Neu5Gc and KDN, are often located at the non-reducing end of glycoconjugates on the cell surface through $\alpha$-glycosidic bonds, and play a central role in cell surface recognition phenomena. Recently, poly- and oligo-sialosides composed of the $\alpha$(2,8) disialyl unit (1) are found in many glycoproteins as well as glycolipids, and would be important fragments to bind proteins as well as monomeric sialic acid. However, low availability of the pure sialo-containing glycoconjugates from the natural sources makes it difficult to elucidate their biological activity, and requires an effective methodology for the synthesis of $\alpha$(2,8) sialosides.

Scheme 1 Strategy for the synthesis of oligo-$\alpha$(2,8) sialosides

The synthesis of $\alpha$(2,8) disialyl unit (1) is one of the most problematic processes in chemical oligosaccharide synthesis. The reactivity of the C8 hydroxyl group on sialoside (3) towards glycosylation is dramatically reduced by the C1 carboxyl and/or the C5 acetamide group. In addition, the C1 carbonyl group reduces the reactivity of sialyl donor (2) towards glycosidation. Furthermore, lack of the
stereo-directing group adjacent to the anomic position makes it difficult to stereoselectivity form the thermodynamically unstable α-glycosidic linkages, and promotes β-elimination during glycosidation. Recently, the sialy donors possessing N,N-diacyl, azido, N-TFA, N-Troc, N-trichloroacetyl and N-Fmoc groups at the C5 position have been reported to exhibit the improved reactivity towards sialylation. We have already reported the one-pot synthesis of sialo-containing oligosaccharides using the N-Troc β-thiosialoside. The N-Troc protected sialyl donors were effective for the synthesis of sialo-containing amino acids and peptides because modification of the N-Troc protecting group to the NHAc group can be achieved without racemization of the amino acids and peptides. Herein we report an efficient synthesis of α(2,8) sialosides by glycosidation of N-Troc sialyl donors.

We first investigated glycosylation of sialoside at the C8 position with N-Troc sialyl donors varying the leaving groups (Table 1). We selected N-phenyl trifluoroimidate and phosphites as leaving groups, which enable activation of the N-acetyl sialy donors at low reaction temperature. In addition, N-phenyl trifluoroimidate would be an effective leaving group for glycosylation of low reactive acceptors. Treatment of acceptor (4) with three equivalents of thioglycoside (5a) in the presence of NIS and TfOH in MeCN at -35 °C provided disaccharides (6) in 36% yield with good α-selectivity (Entry 1). Activation of trifluoroimidate (5b) with a catalytic amount of TMSOTf at -35 °C resulted in the improved yield of disaccharide (6). Glycosidation of trifluoroimidate (5b) at -78 °C provided disaccharide (6) in 67% yield with good α-selectivity (α:β = 81:19). Glycosyl phosphites (5c) and (5d) resulted in the reduced yields of 6 in comparison with imidate (5b). Use of 1.5 equivalents of glycosyl donor (5b) resulted in good yield of 6. Structure determination of α-sialoside (6α) was achieved by 1H NMR spectral analysis based on the empirical roles.

Table 1 Glycosylation of sialoside at the C8 position with N-Troc sialyl donors varying the leaving groups.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>eq.</th>
<th>Activator</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Yield (%)</th>
<th>α:β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5a</td>
<td>3.0</td>
<td>NIS(6.0 eq.)/TfOH(0.1 eq.)</td>
<td>MeCN</td>
<td>-35</td>
<td>36</td>
<td>73 : 27</td>
</tr>
<tr>
<td>2</td>
<td>5b</td>
<td>3.0</td>
<td>TMSOTf (0.3 eq.)</td>
<td>MeCN</td>
<td>-35</td>
<td>71</td>
<td>67 : 33</td>
</tr>
<tr>
<td>3</td>
<td>5b</td>
<td>3.0</td>
<td>TMSOTf (0.3 eq.)</td>
<td>MeCN/CH₂Cl₂ = 2/3</td>
<td>-78</td>
<td>67</td>
<td>81 : 19</td>
</tr>
<tr>
<td>4</td>
<td>5b</td>
<td>1.5</td>
<td>TMSOTf (0.15 eq.)</td>
<td>MeCN/CH₂Cl₂ = 2/3</td>
<td>-78</td>
<td>61</td>
<td>83 : 17</td>
</tr>
<tr>
<td>5</td>
<td>5c</td>
<td>3.0</td>
<td>TMSOTf (0.3 eq.)</td>
<td>MeCN/CH₂Cl₂ = 2/3</td>
<td>-78</td>
<td>57</td>
<td>85 : 15</td>
</tr>
<tr>
<td>6</td>
<td>5d</td>
<td>3.0</td>
<td>TMSOTf (0.3 eq.)</td>
<td>MeCN/CH₂Cl₂ = 2/3</td>
<td>-78</td>
<td>47</td>
<td>84 : 16</td>
</tr>
</tbody>
</table>

α:β ratio was determined by HPLC analysis based on refractive index detection.
Next, we investigated the synthesis of oligo-α(2,8)-sialosides. The glycosyl trifluorooimidate (11) protected with a chloroacetyl group at the C8 hydroxyl group was designed for the synthesis of oligo-α(2,8) sialosides. The chloroacetyl protecting group can be selectively removed after glycosidation. The synthesis of the sialyl donor (11) is shown in Scheme 2. Treatment of tetraacetyl N-acetyl β-thiosialoside (7) with methanesulfonic acid provided amine (8). N-Acylation of amine (8) with TrocOSu, followed by acetalization of the C8 and C9 hydroxyl groups afforded diol (9). The remaining hydroxyl group was protected with the acetyl group, followed by regioselectively reductive opening of the benzylidene acetal provided monool (10) in 54% overall yield from 7. Protection of the resulting hydroxyl group with chloroacetyl group provided fully protected thioglycoside (11) in 98% yield. Hydrolysis of the thioglycoside, followed by coupling with imidoyl chloride (13) afforded the sialyl trifluorooimidate (14) in good yield.

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**Scheme 2** Reagents and conditions: (a) MsOH, MeOH, 60 °C. (b) TrocOSu, NEt3, MeCN, H2O, rt. (c) PhCH(OMe)2, CSA, MeCN, rt. (d) Ac2O, py, CH2Cl2, DMAP, (e) BH3•NMMe3, AlCl3, THF, MS4A, 0 °C, 54% from 7. (f) chloroacetyl chloride, py, CH2Cl2, rt, 98%. (g) NBS, acetone, H2O, 0 °C, 90%. (h) Cs2CO3, acetone, 0 °C, 80%, α:β = 88:12.

Coupling of the sialyl donor (14) with acceptor (4) was examined (Scheme 3). Treatment of acceptor (4) with three equivalents of the glycosyl imidate (14) in the presence of a catalytic amount of TMSOTf in CH2CN at -78 °C provided disaccharides (6) in 58% yield with good α-selectivity. Removal of the chloroacetyl group with thiourea provided the disaccharide acceptor (16) in 80% yield. Next, the synthesis of trisialoside (17) was examined. Next we examined the synthesis of tri-α(2,8) sialoside (17). Treatment of the acceptor (16) with three equivalents of glycosyl imidate (14) under the same glycosidation conditions unfortunately provided disaccharide (17) in only 6% as an anomeric mixture, whose structure was confirmed by MS and 1H NMR spectra. The ratio of the anomeric isomers could not be estimated by the information. These results indicate that reactivity of disaccharides (16) towards glycosylation was dramatically reduced in comparison with monosialoside (4).
Finally, deprotection of α(2,8) disialoside was examined. The N-Troc and acetyl groups and methyl esters of 16 were spontaneously hydrolyzed under the standard basic conditions to provide amino acid (18). Treatment of 18 with acetic anhydride, followed by exposure to NaOMe in MeOH afforded α(2,8)-dissialoside (19) possessing the two benzyl groups in 77% yield based on 15. Hydrogenolysis of the benzyl ethers in the presence of Pd(OH)$_2$ provided the fully deprotected α(2,8)-dissialoside (20) in 96% yield. The coupling constants $^3J_{1Cl-H3a}$ (5.8 and 5.1 Hz) of 19 indicate that the two glycosidic linkages are α-configuration.

In conclusion, we reported an effective approach for the synthesis of oligo-α(2,8) sialosides using N-Troc sialyl donors. Glycosylation of the N-Troc sialoside (4) with the N-Troc sialyl N-phenyltrifluoroimidates provided α(2,8) sialosides in good yield and selectivity. The coupling methodology would be effective for the synthesis of α(2,8) sialo-containing glycoconjugates such as glycosyl amino acids and peptides. Synthesis of various oligosaccharide containing the α(2,8) disialyl unit is in progress.

**ACKNOWLEDGEMENTS**

This work was supported by a Grand-in Aid for Scientific Research on Priority Area (S) from the Ministry of Education, Culture, Sports, Science, and Technology. (Grant-in-aid No.14103013).
REFERENCES AND NOTES

13. Spectra of 5b: 1H NMR (400 MHz, CDCl3) δ 7.28 (br s, 2H), 7.11 (dd, 1H, J = 7.7, 7.7 Hz), 6.74 (d, 2H, J = 7.7 Hz), 5.42 (dd, 1H, J6,7 = 1.5 Hz, J7,8 = 6.8 Hz), 5.27 (d, 1H, NH, J = 9.7 Hz), 5.26 (m, 1H), 5.21 (ddd, 1H, J3ax,4 = 10.1 Hz, J3eq,4 = 5.3 Hz, J4,5 = 9.7 Hz), 4.92 (d, 1H, J = 12.1 Hz), 4.75 (dd, 1H, J5,b = 10.6 Hz, J6,b = 1.5 Hz), 4.51 (d, 1H, CH2CCl3, J = 12.1 Hz), 4.37 (dd, 1H, J8,9 = 2.4 Hz, Jgem = 12.6 Hz), 4.13 (dd, 1H, H-9′, J8,9′ = 5.8 Hz, Jgem = 12.6 Hz), 3.80 (s, 3H, OMe), 3.78 (m, 1H), 2.78 (dd, 1H, H-3eq, J3eq,4 = 5.3 Hz, Jgem = 13.5 Hz), 2.29 (dd, 1H, J3ax,4 = 10.1 Hz, Jgem = 13.5 Hz), 2.18, 2.04, 1.99, 1.98 (4s, 12H, Ac); 13C NMR (100 MHz, CDCl3) δ 170.7, 170.4, 170.3, 170.0, 167.3, 154.2, 142.6, 128.8, 124.8, 120.6, 119.4, 97.9, 95.5, 74.6, 73.5, 70.0, 67.9, 67.7, 61.8, 53.0, 51.5, 36.7, 20.9, 20.8, 20.7; IR (KBr) 3324, 3027, 2958, 1746, 1539, 1333, 737 (cm⁻¹).
15. Spectra of 20: [α]D²⁷ +6.4° (c 1.00, H₂O); 1H NMR (400 MHz, D₂O) δ 4.16 (m, 1H, H-8), 4.10 (dd, 1H, H-9a, J8,9a = 3.9 Hz, J9a,9b = 11.6 Hz ), 3.49 – 3.88 (m, 13H ), 3.39 (dt, 1H, OCH₂, J = 7.25 Hz, J = 8.70 Hz), 2.73 (dd, 1H, H-3′ eq., J3′ax,3′eq. = 12.1 Hz, J3′ eq,4′ = 4.35), 2.61 (dd, 1H, H-3′ eq., J3ax,3′ eq. = 12.1 Hz, J3eq,4 = 4.35 Hz), 2.04, 2.00 (2s, 6H, Ac), 1.71 (dd, 1H, H-3′ ax., J3′ax,3′ eq. = 12.1 Hz, J3′ eq,4′ = 12.1 ), 1.50 – 1.58 (m, 3H, H- 3ax, OCH₂CH₂ ), 1.25 (br s, 10H, CH₂), 0.83 (t, 3H, J = 6.8 Hz); 13C NMR (100 MHz, D₂O, acetone-d₆) δ 175.0 × 2, 173.5, 173.4, 101.3, 100.5, 78.7, 74.2, 72.8, 71.8,
69.8, 68.5, 68.3, 68.0, 65.2, 62.8, 61.8, 52.6, 51.9, 49.0, 40.6, 31.2, 28.6, 28.5, 25.3, 22.4, 22.1 x 2, 13.5; IR (KBr) 3530, 1650, 1418, 1378, 1071, 778 (cm\(^{-1}\)). MS (ESI-TOF) calcd for C\(_{30}\)H\(_{53}\)N\(_2\)O\(_{17}\)Na [M+Na]** 713.3, found 713.5.