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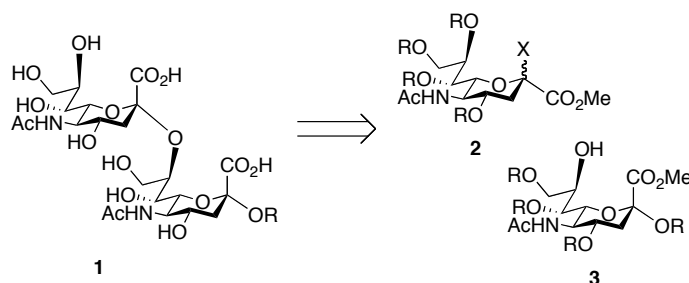
## SYNTHETIC STUDY OF $\alpha(2,8)$ OLIGOSIALOSIDE USING *N*-TROC SIALYL *N*-PHENYLTRIFLUOROIMIDATE

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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.<sup>1</sup> 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.<sup>2</sup> 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.



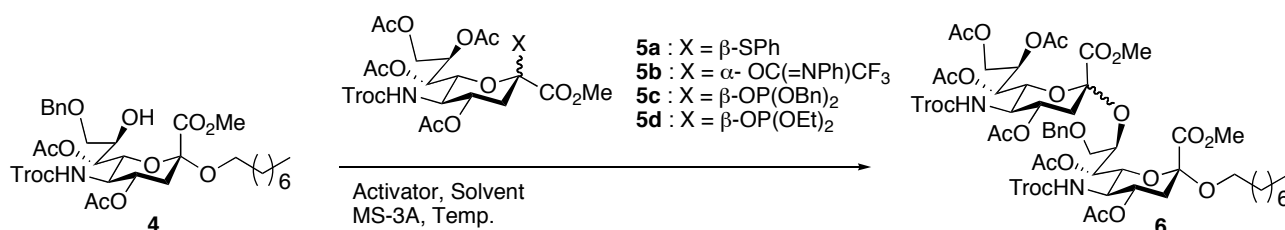
**Scheme 1** Strategy for the synthesis of oligo- $\alpha(2,8)$  sialosides

The synthesis of  $\alpha(2,8)$  disialyl unit (**1**) is one of the most problematic processes in chemical oligosaccharide synthesis.<sup>3</sup> 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 anomeric position makes it difficult to stereoselectivity form the thermodynamically unstable  $\alpha$ -glycosidic linkages, and promotes  $\beta$ -elimination during glycosidation. Recently, the sialy donors possessing *N,N*-diacetyl,<sup>4</sup> azido,<sup>5</sup> *N*-TFA,<sup>6</sup> *N*-Troc,<sup>7,8</sup> *N*-trichloroacetyl<sup>8</sup> and *N*-Fmoc<sup>8</sup> 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  $\beta$ -thiosialoside.<sup>8</sup> 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  $\alpha(2,8)$  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 sialyl donors at low reaction temperature.<sup>9,10</sup> In addition, *N*-phenyl trifluoroimidate would be an effective leaving group for glycosylation of low reactive acceptors.<sup>11</sup> Treatment of acceptor (**4**) with three equivalents of thioglycoside<sup>12</sup> (**5a**) in the presence of NIS and TfOH in MeCN at -35 °C provided disaccharides (**6**) in 36% yield with good  $\alpha$ -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  $\alpha$ -selectivity ( $\alpha$ : $\beta$  = 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**)<sup>13</sup> resulted in good yield of **6**. Structure determination of  $\alpha$ -sialoside (**6a**) was achieved by <sup>1</sup>H NMR spectral analysis based on the empirical roles.<sup>14</sup>

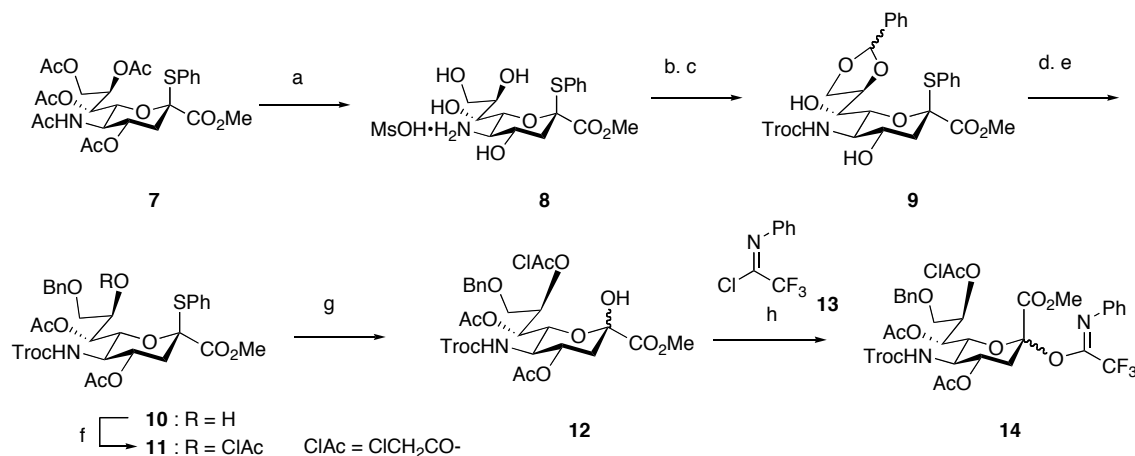
**Table 1** Glycosylation of sialoside at the C8 position with *N*-Troc sialyl donors varying the leaving groups.



Entry	Donor	eq.	Activator	Solvent	Temp (°C)	Yield (%)	$\alpha$ : $\beta$ <sup>a</sup>
1	<b>5a</b>	3.0	NIS(6.0 eq.)/TfOH(0.1 eq.)	MeCN	-35	36	73 : 27
2	<b>5b</b>	3.0	TMSOTf (0.3 eq.)	MeCN	-35	71	67 : 33
3	<b>5b</b>	3.0	TMSOTf (0.3 eq.)	MeCN/CH <sub>2</sub> Cl <sub>2</sub> = 2/3	-78	67	81 : 19
4	<b>5b</b>	1.5	TMSOTf (0.15 eq.)	MeCN/CH <sub>2</sub> Cl <sub>2</sub> = 2/3	-78	61	83 : 17
5	<b>5c</b>	3.0	TMSOTf (0.3 eq.)	MeCN/CH <sub>2</sub> Cl <sub>2</sub> = 2/3	-78	57	85 : 15
6	<b>5d</b>	3.0	TMSOTf (0.3 eq.)	MeCN/CH <sub>2</sub> Cl <sub>2</sub> = 2/3	-78	47	84 : 16

<sup>a</sup>The  $\alpha$ : $\beta$  ratio was determined by HPLC analysis based on refractive index detection

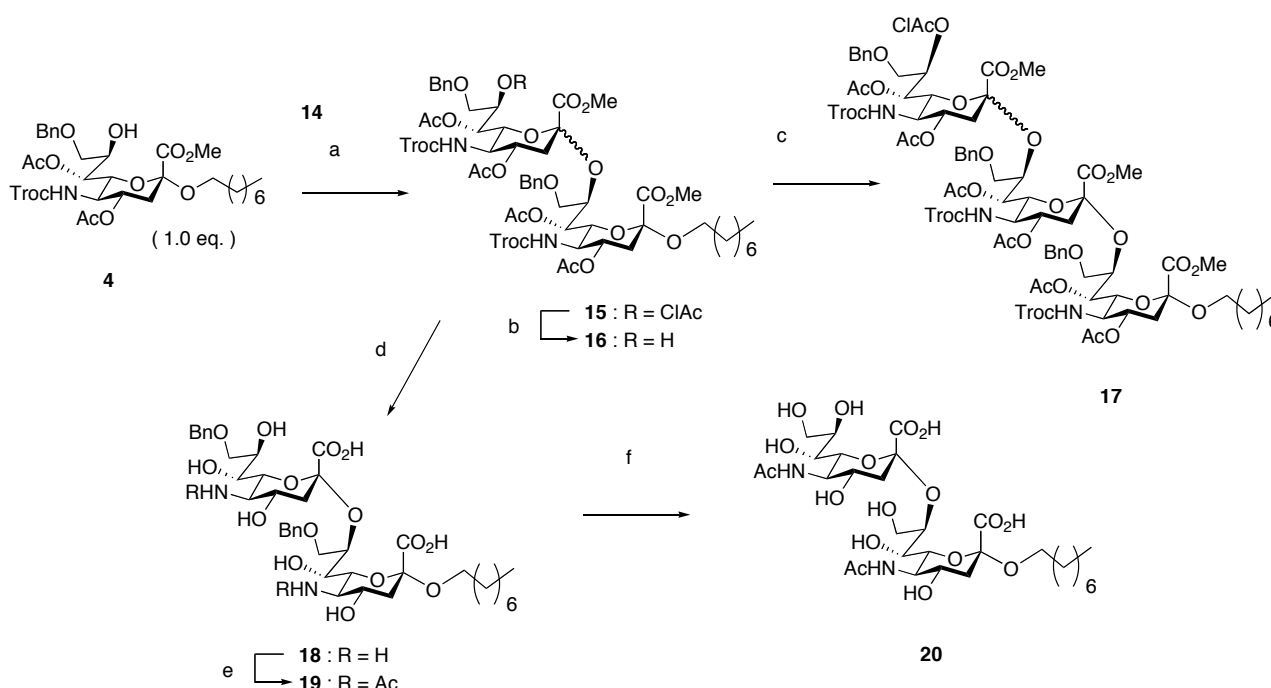
Next, we investigated the synthesis of oligo- $\alpha(2,8)$ -sialosides. The glycosyl trifluoroimidate (**11**) protected with a chloroacetyl group at the C8 hydroxyl group was designed for the synthesis of oligo- $\alpha(2,8)$  sialosides. The chloroacetyl protecting group can be selectively removed after glycosidation. The synthesis of the sialyl donor (**11**) is shown in Scheme 2. Treatment of tetraacetyl *N*-acetyl  $\beta$ -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 trifluoroimidate (**14**) in good yield.



**Scheme 2** Reagents and conditions: (a) MsOH, MeOH, 60 °C. (b) TrocOSu, NEt<sub>3</sub>, MeCN, H<sub>2</sub>O, rt. (c) PhCH(OMe)<sub>2</sub>, CSA, MeCN, rt. (d) Ac<sub>2</sub>O, py, CH<sub>2</sub>Cl<sub>2</sub>, DMAP, (e) BH<sub>3</sub>•NMe<sub>3</sub>, AlCl<sub>3</sub>, THF, MS4A, 0 °C, 54% from **7**. (f) chloroacetyl chloride, py, CH<sub>2</sub>Cl<sub>2</sub>, rt, 98%. (g) NBS, acetone, H<sub>2</sub>O, 0 °C, 90%. (h) Cs<sub>2</sub>CO<sub>3</sub>, acetone, 0 °C, 80%,  $\alpha$ : $\beta$  = 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 CH<sub>3</sub>CN at -78 °C provided disaccharides (**6**) in 58% yield with good  $\alpha$ -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- $\alpha(2,8)$  sialoside (**17**). Treatment of the acceptor (**16**) with three equivalents of glycosyl imidate (**14**) under the same glycosidation conditions unfortunately provided trisaccharide (**17**) in only 6% as an anomeric mixture, whose structure was confirmed by MS and <sup>1</sup>H 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  $\alpha(2,8)$  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  $\alpha(2,8)$ -disialoside (**19**) possessing the two benzyl groups in 77% yield based on **15**. Hydrogenolysis of the benzyl ethers in the presence of Pd(OH)<sub>2</sub> provided the fully deprotected  $\alpha(2,8)$ -disialoside (**20**)<sup>15</sup> in 96% yield. The coupling constants <sup>3</sup>J<sub>Cl-H3ax</sub> (5.8 and 5.1 Hz) of **19** indicate that the two glycosidic linkages are  $\alpha$ -configuration.<sup>16</sup>



**Scheme 3** Reagents and conditions: (a) **14** (3.0 eq.) TMSOTf (0.3 eq.), CH<sub>2</sub>Cl<sub>2</sub>/MeCN (2/3), MS3A, -78 °C, 58%,  $\alpha$ : $\beta$  = 83:17. (b) thiourea, 2,6-lutidine, DMF, 70 °C. (c) **14** (3.0 eq.) TMSOTf (0.3 eq.), CH<sub>2</sub>Cl<sub>2</sub>/MeCN (2/3), MS3A, -78 °C, 6% as anomeric mixture. (d) LiOH·H<sub>2</sub>O, EtOH, H<sub>2</sub>O, 80 °C. (e) Ac<sub>2</sub>O, MeOH, then NaOMe, MeOH, 77% from **15**. (g) Pd(OH)<sub>2</sub>, H<sub>2</sub> (1 atm), H<sub>2</sub>O, MeOH, 96%.

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

## ACKNOWLEDGEMENTS

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13. Spectra of **5b**:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  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,  $J_{6,7} = 1.5$  Hz,  $J_{7,8} = 6.8$  Hz), 5.27 (d, 1H, NH,  $J = 9.7$  Hz), 5.26 (m, 1H), 5.21 (ddd, 1H,  $J_{3\text{ax},4} = 10.1$  Hz,  $J_{3\text{eq},4} = 5.3$  Hz,  $J_{4,5} = 9.7$  Hz), 4.92 (d, 1H,  $J = 12.1$  Hz), 4.75 (dd, 1H,  $J_{5,6} = 10.6$  Hz,  $J_{6,7} = 1.5$  Hz), 4.51 (d, 1H,  $\text{CH}_2\text{CCl}_3$ ,  $J = 12.1$  Hz), 4.37 (dd, 1H,  $J_{8,9} = 2.4$  Hz,  $J_{\text{gem}} = 12.6$  Hz), 4.13 (dd, 1H, H-9',  $J_{8,9'} = 5.8$  Hz,  $J_{\text{gem}} = 12.6$  Hz), 3.80 (s, 3H, OMe), 3.78 (m, 1H), 2.78 (dd, 1H, H-3eq.,  $J_{3\text{eq},4} = 5.3$  Hz,  $J_{\text{gem}} = 13.5$  Hz), 2.29 (dd, 1H,  $J_{3\text{ax},4} = 10.1$  Hz,  $J_{\text{gem}} = 13.5$  Hz), 2.18, 2.04, 1.99, 1.98 (4s, 12H, Ac);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  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 ( $\text{cm}^{-1}$ ).
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15. Spectra of **20**:  $[\alpha]_{\text{D}}^{27} +6.4^\circ$  (c 1.00,  $\text{H}_2\text{O}$ );  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  4.16 (m, 1H, H-8), 4.10 (dd, 1H, H-9a,  $J_{8,9a} = 3.9$  Hz,  $J_{9a,9b} = 11.6$  Hz), 3.49 – 3.88 (m, 13H), 3.39 (dt, 1H,  $\text{OCH}_2$ ,  $J = 7.25$  Hz,  $J = 8.70$  Hz), 2.73 (dd, 1H, H-3'eq.,  $J_{3'\text{ax},3'\text{eq}} = 12.1$  Hz,  $J_{3'\text{eq},4'} = 4.35$ ), 2.61 (dd, 1H, H-3eq.,  $J_{3\text{ax},3\text{eq}} = 12.1$  Hz,  $J_{3\text{eq},4} = 4.35$  Hz), 2.04, 2.00 (2s, 6H, Ac), 1.71 (dd, 1H, H-3'ax.,  $J_{3'\text{ax},3'\text{eq}} = 12.1$  Hz,  $J_{3'\text{ax},4'} = 12.1$ ), 1.50 – 1.58 (m, 3H, H-3ax,  $\text{OCH}_2\text{CH}_2$ ), 1.25 (br s, 10H,  $\text{CH}_2$ ), 0.83 (t, 3H,  $J = 6.8$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ , acetone- $d_6$ )  $\delta$  175.0 x 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<sup>-1</sup>). MS (ESI-TOF) calcd for C<sub>30</sub>H<sub>53</sub>N<sub>2</sub>O<sub>17</sub>Na [M+Na]<sup>+</sup> 713.3, found 713.5.

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