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SYNTHETIC STUDY OF α(2,8) OLIGOSIALOSIDE USING *N*-TROC SIALYL *N*-PHENYLTRIFLUOROIMIDATE

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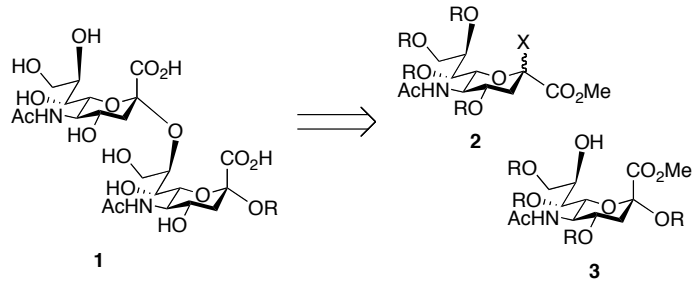
Abstract – An effective approach for the synthesis of oligo-α(2,8) sialosides using *N*-Troc sialyl donors is described. Glycosylation of *N*-Troc sialoside with *N*-Troc sialyl *N*-phenyltrifluoroimidate and phosphites smoothly proceeded to provide α(2,8) disialosides in good yield and selectivity.

Sialic acids such as Neu5Ac, Neu5Gc and KDN, are often located at the non-reducing end of glycoconjugates on the cell surface through α-glycosidic bonds, and play a central role in cell surface recognition phenomena.[1] Recently, poly- and oligo-sialosides composed of the α(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.[2] 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 α(2,8) sialosides.



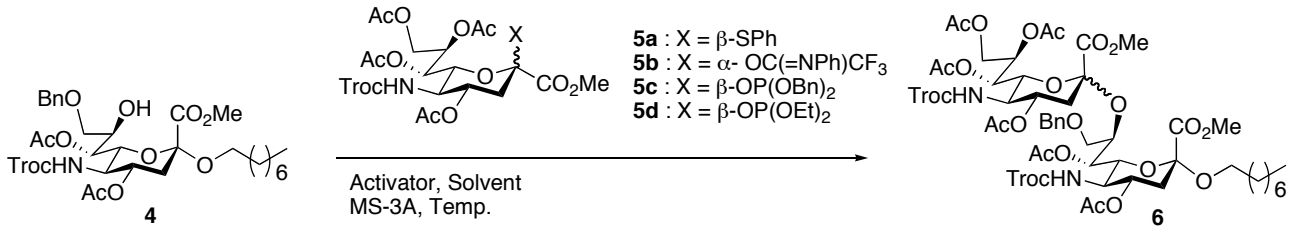
Scheme 1 Strategy for the synthesis of oligo-α(2,8) sialosides

The synthesis of α(2,8) disialyl unit (**1**) is one of the most problematic processes in chemical oligosaccharide synthesis.[3] 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 α-glycosidic linkages, and promotes β-elimination during glycosidation. Recently, the sialy donors possessing *N*,*N*-diacetyl,[4] azido,[5] *N*-TFA,[6] *N*-Troc,[7,8] *N*-trichloroacetyl[8] and *N*-Fmoc[8] 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.[8] 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 poition 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.[9,10] In addition, *N*-phenyl trifluoroimidate would be an effective leaving group for glycosylation of low reactive acceptors.[11] Treatment of acceptor (**4**) with three equivalents of thioglycoside[12] (**5a**) in the presence of NIS and TfOH in MeCN at -35 ºC provided disaccharides (**6**) in 36% yield with good α-selectivity (Entry 1). Activation of trifluoroimidate (**5b**) with a catalytic amount of TMSOTf at -35 ºC resulted in the improved yield of disaccharide (**6**). Glycosidation of trifluoroimidate (**5b**) at -78 ºC 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**)[13] resulted in good yield of **6**. Structure determination of α-sialoside (**6α**) was achieved by ^{1}H NMR spectral analysis based on the empirical roles.[14]

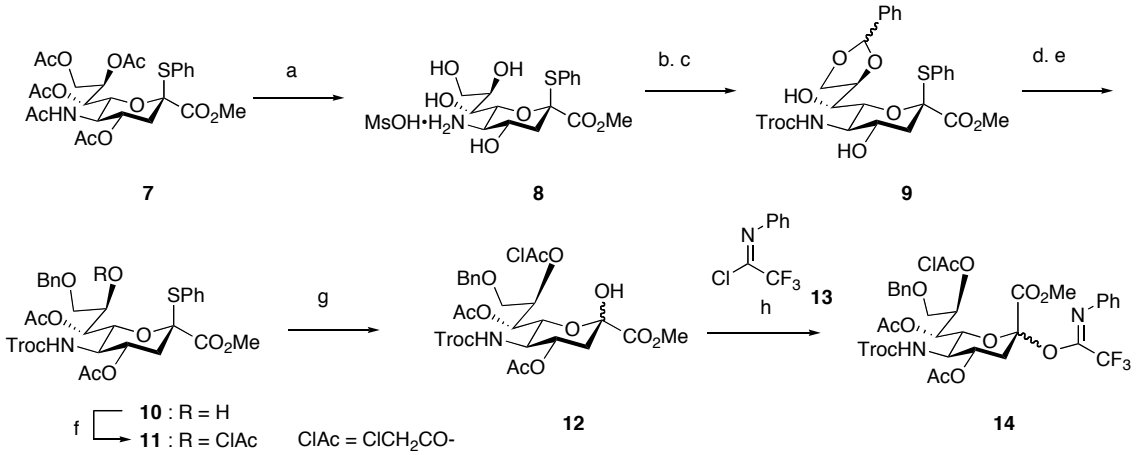
Table 1 Glycosylation of sialoside at the C8 position with *N*-Troc sialyl donors varying the leaving groups.



Entry	Donor	eq.	Activator	Solvent	Temp (ºC)	Yield (%)	α : β[a]
1	**5a**	3.0	NIS(6.0 eq.)/TfOH(0.1 eq.)	MeCN	-35	36	73 : 27
2	**5b**	3.0	TMSOTf (0.3 eq.)	MeCN	-35	71	67 : 33
3	**5b**	3.0	TMSOTf (0.3 eq.)	MeCN/CH_2Cl_2 = 2/3	-78	67	81 : 19
4	**5b**	1.5	TMSOTf (0.15 eq.)	MeCN/CH_2Cl_2 = 2/3	-78	61	83 : 17
5	**5c**	3.0	TMSOTf (0.3 eq.)	MeCN/CH_2Cl_2 = 2/3	-78	57	85 : 15
6	**5d**	3.0	TMSOTf (0.3 eq.)	MeCN/CH_2Cl_2 = 2/3	-78	47	84 : 16

[a]The α:β ratio was determined by HPLC analysis based on refractive index detection

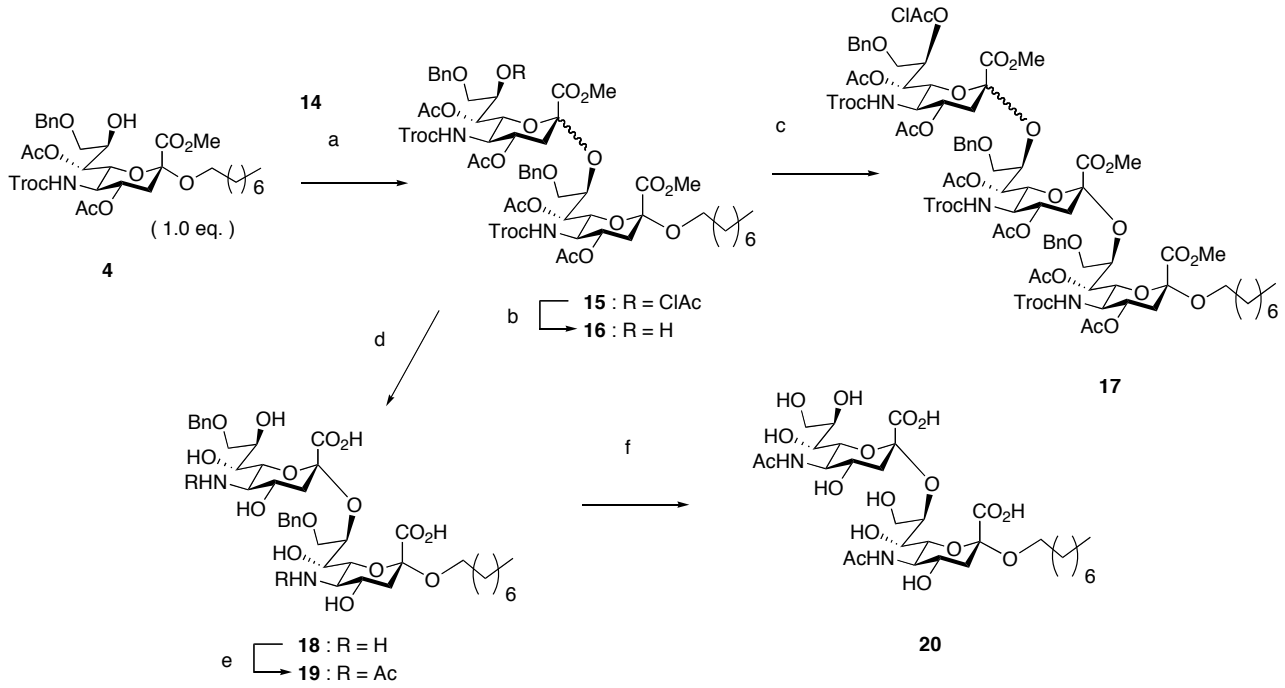
Next, we investigated the synthesis of oligo-α(2,8)-sialosides. The glycosyl trifluoroimidate (**11**) protected with a chloroacetyl group at the C8 hydroxyl group was designed for the synthesis of oligo-α(2,8) sialosides. The chloroacetyl protecting group can be selectively removed after glycosidation. 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 trifluoroimidate (**14**) in good yield.



Scheme 2 Reagents and conditions: (a) MsOH, MeOH, 60 ºC. (b) TrocOSu, NEt_3, MeCN, H_2O, rt. (c) $PhCH(OMe)_2$, CSA, MeCN, rt. (d) Ac_2O, py, CH_2Cl_2, DMAP, (e) BH_3•NMe_3, $AlCl_3$, THF, MS4A, 0 ºC, 54% from **7**. (f) chloroacetyl choride, py, CH_2Cl_2, rt, 98%. (g) NBS, acetone, H_2O, 0 ºC, 90%. (h) Cs_2CO_3, acetone, 0 ºC, 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 CH_3CN at -78 ºC 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 trisaccharide (**17**) in only 6% as an anomeric mixture, whose structure was confirmed by MS and ^{1}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 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**)[15] in 96% yield. The coupling constants $^3J_{C1\text{-}H3ax}$ (5.8 and 5.1 Hz) of **19** indicate that the two glycosidic linkages are α-configuration.[16]



Scheme 3 Reagents and conditions: (a) **14** (3.0 eq.) TMSOTf (0.3 eq.), CH_2Cl_2/MeCN (2/3), MS3A, -78 ºC, 58%, α:β = 83:17. (b) thiourea, 2,6-lutidine, DMF, 70 ºC. (c) **14** (3.0 eq.) TMSOTf (0.3 eq.), CH_2Cl_2/MeCN (2/3), MS3A, -78 ºC, 6% as anomeric mixture. (d) LiOH•H_2O, EtOH, H_2O, 80 ºC. (e) Ac_2O, MeOH, then NaOMe, MeOH, 77% from **15**. (g) $Pd(OH)_2$, H_2 (1 atm), H_2O, MeOH, 96%.

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

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13. Spectra of **5b**: ^{1}H NMR (400 MHz, $CDCl_3$) 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, $J_{6,7}$ = 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, $J_{3ax.,4}$ = 10.1 Hz, $J_{3eq.,4}$ = 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, CH_2CCl$_3$, J = 12.1 Hz), 4.37 (dd, 1H, $J_{8,9}$=2.4 Hz, J_{gem}=12.6 Hz), 4.13 (dd, 1H, H-9', $J_{8,9'}$ = 5.8 Hz, J_{gem} = 12.6 Hz), 3.80 (s, 3H, OMe), 3.78 (m, 1H), 2.78 (dd, 1H, H-3eq., $J_{3eq.,4}$= 5.3 Hz, J_{gem}= 13.5 Hz), 2.29 (dd, 1H, $J_{3ax.,4}$ = 10.1 Hz, J_{gem}= 13.5 Hz), 2.18, 2.04, 1.99, 1.98 (4s, 12H, Ac); ^{13}C NMR (100 MHz, $CDCl_3$) 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}).
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15. Spectra of **20**: $[\alpha]_D^{27}$ +6.4º (c 1.00, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 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, OCH_2, J = 7.25 Hz, J = 8.70 Hz), 2.73 (dd, 1H, H-3'eq., $J_{3'ax.,3'eq.}$ = 12.1 Hz, $J_{3'eq.,4'}$ = 4.35), 2.61 (dd, 1H, H-3eq., $J_{3ax.,3eq.}$ = 12.1 Hz, $J_{3eq.,4}$ = 4.35 Hz), 2.04, 2,00 (2s, 6H, Ac), 1.71 (dd, 1H, H-3'ax., $J_{3'ax.,3'eq.}$ = 12.1 Hz, $J_{3'ax.,4'}$ = 12.1), 1.50 – 1.58 (m, 3H, H- 3ax, OCH$_2$CH_2), 1.25 (br s, 10H, CH_2), 0.83 (t, 3H, J = 6.8 Hz) ; ^{13}C NMR (100 MHz, D_2O, acetone-d_6) δ 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^{-1}). MS (ESI-TOF) calcd for $C_{30}H_{53}N_2O_{17}Na$ $[M+Na]^+$ 713.3, found 713.5.

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