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Conversion of the carboxy group of sialic acid donors to a protected hydroxymethyl group yields an efficient reagent for the synthesis of the unnatural beta-linkage

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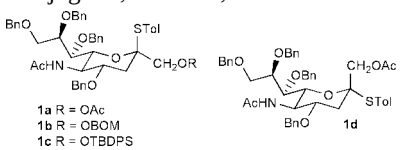
New sialyl donors with a protected hydroxymethyl group at the anomeric center are over 1000 times more reactive than the normal ester containing sialylation reagent and give excellent yield (> 90%) with unusually high β-stereoselectivity in sialylation.

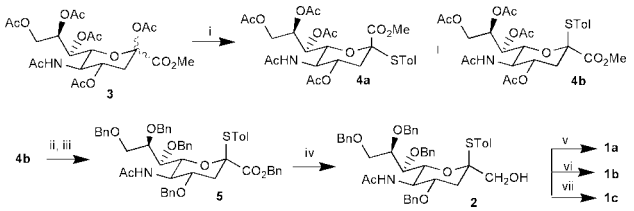
N-Acetylneuraminic acid (sialic acid) residues are often located at the non-reducing end of glycoconjugates, and play important roles in many biological recognition events such as cancer metastasis and bacterial or viral infection.[1,2] The synthesis of sialyl glycoconjugates, however, remains a challenge.[3]



In a typical sialylation reaction, the electron withdrawing carboxy group at the anomeric center of sialic acids significantly destabilizes the oxonium ion forming transition state thus lowering the reactivity of sialyl donors towards nucleophiles. Furthermore, the steric hinderance at the anomeric center leads to elimination and low yields of sialylation. Various methods have been developed to overcome these problems, including, for example, introduction of an additional *N*-acetyl moiety,[4,5] installation of an anchimeric assisting group at the C3 position[5–8] or a carboxy equivalent at the anomeric center.[9,10] The latter approach includes utilizing a less electron withdrawing furyl substituent as the carboxy surrogate.[9] This method, however, failed to yield any disaccharides with secondary sugar alcohols. Increased reactivities towards *N*-iodosuccinimide (NIS) have also been observed with the reduced 2,3-didehydro sialic acid derivatives, but these reactions gave very low yields (~ 20%).[10]

To tackle the aforementioned problems, we converted the carboxy group of sialic acid to the hydroxymethyl group and prepared derivatives **1a**–**1d** for investigation (Scheme 1). The peracetylated sialic acid **3**[11] was treated with *p*-thiocresol to give thio-sialic acid **4b** in 75% yield together with 20% of the α isomer **4a**. The protective groups of **4b** were exchanged for benzyl groups to give compound **5** in two steps in 55% yield. Reduction of the ester moiety was accomplished with $LiBH_4$ in 90% yield to give the hydroxymethyl sialic acid derivative **2**, the hydroxy group of which was protected with the acetyl, benzyloxymethyl (BOM) or *tert*-butyldiphenylsilyl (TBDPS) group to give sialyl donors **1a**–**1c**. The α sialyl donor **1d** was prepared from **4a** in a similar manner as the β sialyl donor **1a**.

The relative reactivity values (RRV) of known and new sialic acid donors **1a**–**1d**, **2**, **4b** and **5** were measured as previously described[12] and shown in Table 1. Compared to the benzyl protected sialic acid **5**, over *three orders of magnitude* increase in RRV was observed with all the reduced sialic acid derivatives **1a**†–**1d** and **2** (RRV for **1a**, **1b**, **1c**, **1d** and **2** are 4.0×10^4, 2.3×10^5, 7.8×10^4, 8.0×10^4 and 3.3×10^5 respectively). The reactivities of all these reduced sialic acids (**1a**–**1d** and **2**) are

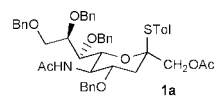
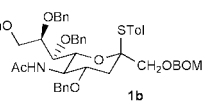
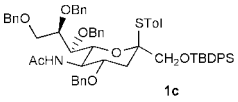
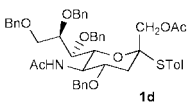
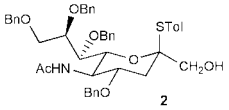
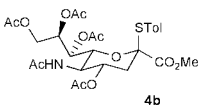
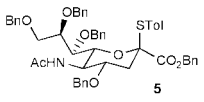
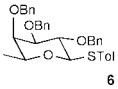


Scheme 1 *Reagents and conditions*: (i) *p*-Thiocresol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 20% for **4a**, 75% for **4b**; (ii) NaOMe, MeOH, 100%; (iii) NaH, BnBr, DMF, 55%; (iv) $LiBH_4$, THF, MeOH, 90%; (v) Ac_2O, pyridine, 96%; (vi) BOMCl, Pr^i_2NEt, CH_2Cl_2, 86%; (vii) TBDPSCl, imidazole, DMF, 96%.

comparable to or even higher than perbenzylated L-fucose **6** which was the most reactive thioglycoside measured previously.[12]

With the RRV values in hand, sialylation of donors **1a**–**1d** was performed. The TBDPS protected sialyl donor **1c** failed to undergo glycosylation with galactose acceptor **7**, presumably due to the large TBDPS group. With the smaller acetyl protective group, donor **1a** underwent smooth sialylation with galactose acceptor **7** to give disaccharides **8** and **9** in 95% yield (**8**:**9** = 15:1) in acetonitrile using dimethyl(methylthio)sulfonium triflate (DMTST) as the promoter[13,14] (Scheme 2a). No elimination product was isolated. The hydroxymethyl moiety of the products can be subsequently unmasked after sialylation and selectively oxidized to the carboxy group in three high yielding steps as demonstrated by transformation of compound **8** to **10** (Scheme 2b) and **9** to **12** (Scheme 2c). However, quite unexpectedly the predominant product **8** formed in the sialylation contains a β linkage between the two monosaccharides. The near zero $^3J_{C1,H3a}$ value in the EXCIDE[5,15] spectra of **8** and **11**‡

Table 1 Relative reactivity values (RRV) of various thio-glycosides[a]

Compound	RRV	Compound	RRV
 **1a**	4.0×10^4	 **1b**	2.3×10^5
 **1c**	7.8×10^4	 **1d**	8.0×10^4
 **2**	3.3×10^5	 **4b**	2.55
 **5**	48.4	 **6**	7.2×10^4

[a] The RRV is based on the reactivity of 1-thiotolyl-2,3,4,6-tetraacetyl-β-D-mannopyranoside.

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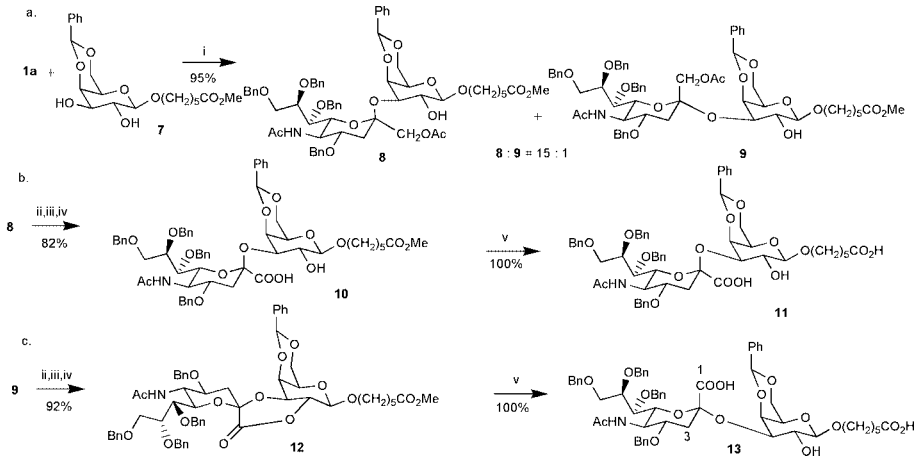
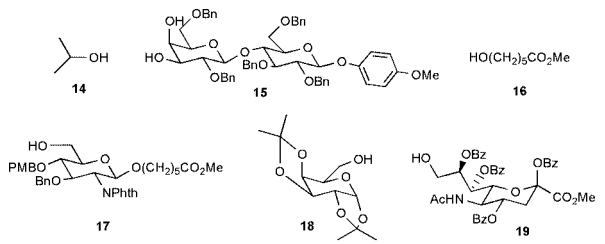


Scheme 2 *Reagent and conditions*: (i) DMTST, molecular sieves, CH_3CN, −40 °C to rt, overnight; (ii) NaOMe, MeOH, rt, 1 h; (iii) $PhI(OAc)_2$, TEMPO, rt, 12 h; (iv) $NaClO_2$, 2-methylbut-2-ene, rt, 4 h; (v) NaOH, H_2O, rt, 1 h.

indicated the β-configuration, while the $^3J_{C1,H3a}$ value of **13**§ was determined to be 6.1 Hz indicating its α-configuration. Comparison of the chemical shifts of H_{3eq} of compound **11** (2.64 ppm) and **13** (2.72 ppm)[16] further confirmed the assignment following the empirical rules of chemical shift.[4] The stereoselectivity does not vary much with different acceptors. Sialylation of various acceptors such as isopropyl alcohol **14**, lactose derivative **15** as well as primary alcohol **16**, glucosamine **17** and galactose **18**, with donor **1a** using DMTST as the promoter, gave predominantly the β-linked disaccharide[17] (β : α > 10 : 1) in high yields (> 90%). The exception was the sialic



acid derivative **19** which gave a ratio of 3 : 1 favoring β-disaccharide in 90% total yield. Sialylations in solvents such as ether, toluene and dichloromethane gave even more β anomer than those performed in acetonitrile. The acetonitrile effect[18] could not significantly alter the anomeric selectivity. Sialylation of galactose **7** with the BOM protected donor **1b** or α sialyl donor **1d** gave the product with similar yield and stereoselectivity to those with donor **1a**.

Sialylations with promoters other than DMTST were also tested. MeOTf[19] failed to activate sialyl donor **1a** while with PhSOTf[20] only the β isomer was isolated when galactose **18** was sialylated with **1a**. The use of NIS and triflic acid (TfOH) improved the α-selectivity (α : β = 1 : 2.5) when galactose acceptor **7** was sialylated with **1a** in 90% total yield.

In conclusion, it has been demonstrated that the reactivity of sialic donors can be dramatically increased by reducing the carboxy group at the anomeric center to the hydroxymethyl moiety. Subsequent sialylation with these novel sialyl donors proceeded in excellent yield (> 90%) but with unusually high β stereoselectivity, probably due to a significant anomeric effect. The hydroxymethyl moiety can be easily oxidized to the carboxy group in high yield. The high reactivity of sialyl donors could find uses in the preparation of enzymatically stable unnatural oligosaccharides containing β-sialic acid. Oligosaccharides with unnatural glycosidic linkage could have important biological implications, as illustrated in the study of CD-1 mediated T-cell activation.[21] The new glycosylation reagents can also be utilized in programmable one-pot synthesis, where the sialylation reaction often has to be the first and most reactive as sialic acid is often located at the non-reducing end of bio-active oligosaccharides.[12] Introduction of a C-3 auxiliary may give the α-linkage.

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Notes and references

† *Selected data* for **1a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (d, J = 7.9 Hz, 2H), 5.06 (d, J = 8.5 Hz, 1H), 3.69 (dd, J = 3.8, 10.4 Hz, 1H), 2.26 (s, 3H), 2.20 (dd, J = 3.7, 13.5 Hz, 1H), 1.92 (dd, J = 11.0, 13.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.44, 170.15, 88.86, 69.17, 68.18, 51.77, 35.62, 23.69, 21.07, 20.66; HRMS (M + Cs) calcd for $C_{48}H_{53}O_8NSCs$ 936.2546, found 936.2577.

‡ *Selected data* for **11**: ^{1}H NMR (400 MHz, CD_3OD) δ 5.32 (s, 1H), 2.66 (dd, J = 4.4, 13.2 Hz, 1H), 2.16 (t, J = 7.6 Hz, 2H), 1.77 (s, 3H), 1.72 (dd, J = 11.3, 13.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.36, 170.04, 102.54, 101.90, 100.26, 69.93, 69.19, 68.84, 52.04, 51.60, 35.92, 33.69, 28.65, 25.36, 24.25, 23.22; $^3J_{C1,H3a} \sim 0$ Hz; HRMS (M − H + 2 Na^+) calcd for $C_{58}H_{65}O_{16}NNa_2$ 1054.4196, found 1054.4202.

§ *Selected data* for **13**: ^{1}H NMR (600 MHz, CD_3OD) δ 5.32 (s, 1H), 2.72 (dd, J = 3.7, 12.6 Hz, 1H), 2.16 (t, J = 7.2 Hz, 2H), 1.94 (s, 3H), 1.88 (t, J = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 182.25, 174.40, 172.78, 103.45, 101.61, 101.28, 69.84, 68.80, 50.95, 38.15, 35.95, 29.43, 26.48; $^3J_{C1,H3a}$ = 6.1 Hz; HRMS (M − H + 2 Na^+) calcd for $C_{58}H_{65}O_{16}NNa_2$ 1054.4196, found 1054.4175.

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