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Extending the possibility of an *N*-Troc-protected sialic acid donor toward variant sialo-glycoside synthesis☆

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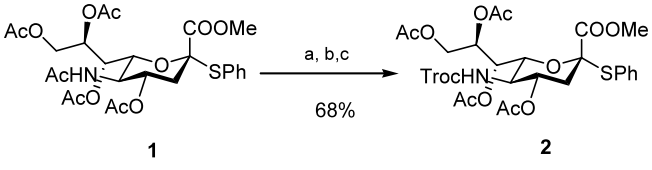
Abstract—The potential of an *N*-Troc-protected sialic acid donor, equipped with phenylsulfenyl functionality as a leaving group, has been explored. As a result, the entitled donor was proven to be highly reactive and to have broad applicability toward the synthesis of variant sialo-glycans, which have *N*-glycolyl, de-*N*-acetyl, 1,5-lactam and 8-*O*-sulfo sialic acid analogs.

Efficient chemical synthesis of fine sialic acid-containing glycoconjugates is a central subject in carbohydrate science, due to their diverse biological roles[1] relevant to cell–cell interaction including those such as pathogen–host recognition, tumor metastasis, toxin–receptor interaction, cell differentiation and proliferation, malignant alteration, neural network formation and so on. The focal point of sialo-glycoside synthesis is the construction of stereoelectronically disfavored α-glycoside adjacent to its deoxy structure. Efforts to solve this problematic issue over a few decades gave rise to several practical methodologies for the construction of α-sialosides, including our approach[2] that employs a combination of an alkylthioglycoside donor of sialic acid with a nitrile solvent effect, by which numerous sialo-oligosaccharides were successfully synthesized so far.[3] However, in respect of the diversity of sialic acids found in nature, such as *N*-glycolyl, de-*N*-acetyl, 1,5-lactam, *O*-acetyl and *O*-sulfo analogues, which also correlate to the polymorphous functions of sialo-glycoconjugates,[4] most of these approaches that built up *N*-acetyl sialic acid-containing molecules are not impeccable. In this context, a single expedient synthetic approach to oligosaccharides having various analogs of sialic acids is essential for scrutiny of the biological significance of the entire sialo-glycoconjugates.[5] Here we report a novel versatile synthetic approach toward glycans having *N*-glycolyl, de-*N*-acetyl, 1,5-lactam, 8-*O*-sulfo sialic acid analogs.

We envisioned that an *N*-2,2,2-trichloroethoxycarbonyl (Troc) protected-sialic acid donor would be feasible for the pivotal approach to variant sialosides, considering the acid-resistant nature and selective cleavability under mild condition of the Troc group. Conversion of the reported α-phenylthioglycoside of *N*-acetyl sialic acid derivative (**1**)[6] into the corresponding *N*-Troc derivative (**2**) was easily accomplished by following Higuchi's one-pot methodology[5e] (68% in three steps) (Scheme 1). In a comparison between the ^{1}H NMR spectra of compound **2** and its β-isomer,[†] it was observed that the chemical shift of H-3$_{eq}$ of the α-isomer (δ 2.88) was at



1 **2**

Troc: 2,2,2-Trichloroethoxycarbonyl

Scheme 1. Preparation of α-phenylthioglycoside of *N*-Troc sialic acid **2**. *Reagents and conditions*: (a) MsOH/MeOH, reflux, 24 h; (b) TrocCl, Et_3N/MeOH, 0°C→rt 30 min; (c) Ac_2O, pyr./rt, 16 h.

Keywords: *N*-Troc group; sialidation; sialic acid analogs.

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† The corresponding β-isomer of **2** has been first reported by Wu and co-workers, which was utilized for Neu5Ac-α(2→5)-Neu5Gc linkage, but they did not refer to the availability of *N*-Troc donor for the synthesis of diverse sialyl oligosaccharides. Ref. 3g.

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lower field than that of the β-isomer (δ 2.73), and H-4 of the α-isomer (δ 4.98) was shifted higher than that of the β-isomer (δ 5.45), and the coupling constant of $J_{7,8}$ of the α-isomer (8.0 Hz) was bigger than that of the β-isomer (2.5 Hz), which were in accordance with the empirical rule to define the anomeric configuration of *N*-acetyl sialic acid glycosides.[7]

Donor **2**, in hand, was then subjected to coupling reaction with a range of acceptors in order to evaluate its efficacy as a glycosyl donor. All condensations were promoted by the *N*-iodosuccinimide–acid[8] system with the assistance of the nitrile solvent effect,[2] and stereochemistry of the newly-formed glycosides was assigned according to the empirical rule and, for some compounds, further confirmed by the reported method using HMBC technique.[3f] In entries 1, 2, 3 and 4, it was noted that donor **2** was rapidly glycosidated to selectively give α-anomeric outcomes in relatively high yields, whereas condensation of the conventional *N*-acetyl donor **1** with diol galactose acceptor **6** was sluggish and afforded α-sialoside **12** in poor yield. Encouraged by these interesting results, we next examined the reactivity of the *N*-Troc donor **2** by competitive coupling reaction. As competitors, the corresponding *N*,*N*-diacetyl and *N*-trifluoroacetyl (TFAc) sialic acid donors (**3** and **4**) were selected based on Boon's reports,[3e,9] and then coupled with acceptor **5**, respectively. Coupling reaction of both donors was as rapid as that of donor **2** to give sialosides in high yield, but with unexpected low α-selectivity in the case of *N*,*N*-diacetyl donor **3** (entries 5 and 6). Interestingly, the competitive coupling of donors **2**, **3** and **4** with acceptor **5** yielded **9** (*N*-Troc) in 40% yield, **13** (*N*,*N*-diacetyl) in 22% yield and **14** (*N*-TFAc) in 15% yield, respectively, suggesting the hierarchy of reactivity among these sialyl donors is *N*-Troc>*N*,*N*-diacetyl>*N*-TFAc (entry 7). Thereby, in entry 8, armed–disarmed like coupling reaction[10] could be performed between the potent **2** and less reactive phenylthioglycoside of *N*-acetyl sialic acid derivative **8** to furnish sialyl-(2→9)sialic acid dimer **15** in 43% (α/β = 24/19). In most of the events, α/β ratios of the sialylated products were around 4/1 and independent of the anomeric configuration of donor **2** (data not shown). Isolation of α-products from concomitantly produced β-isomer by silica gel column chromatography, which is usually an arduous procedure, was readily accomplished in all cases because of bigger ΔR_f (ca. 0.2) between the α- and β-isomers, compensating for the medium α-selectivity. Thus, the practical utility of compound **2** as a sialyl donor has been demonstrated (Figs. 1 and 2 and Table 1).

As expected, the selective deblocking of the Troc group of sialyl galactoside (**16**) with zinc in acetic acid proceeded smoothly to give a free amino derivative, which, on successive treatment with acylating reagents, acetoxyacetyl chloride and trifluoroacetic anhydride, afforded the corresponding *N*-acetylglycolyl derivative (**17**) as *N*-glycolyl sialoside precursor[5d] and *N*-TFAc derivative (**18**) as 5-amino sialoside precursor[5h] in high yields, respectively. The amino intermediate could be

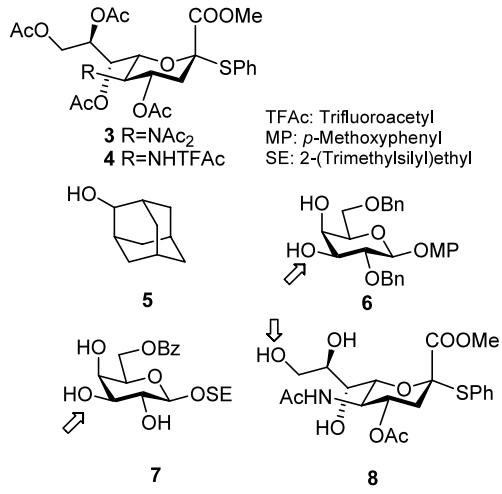


Figure 1. Donors and acceptors used in this study. Arrows point at the glycosylation site.

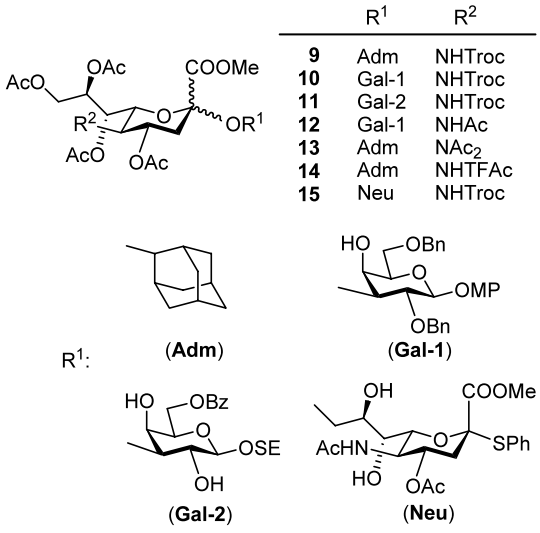


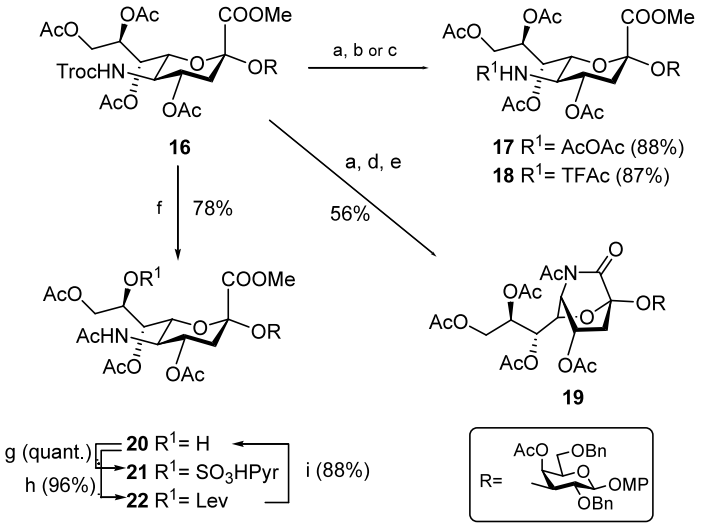
Figure 2. Sialylated products.

also transformed into 1,5-lacatamized bicyclo form under basic conditions in refluxing methanol, which was isolated as acetylated form (**19**) in moderate yield. The precursor **19** was confirmed to be deacetylated in almost quantitative yield via two-step manipulation: *N*-Acetyl deblocking by $NH_2NH_2{\cdot}AcOH$ in THF and de-*O*-acetylation under the Zemplén condition. In addition, it was found that the selective cleavage of Troc protection effected by zinc in 10% acetic acid solution in 1,4-dioxane enhanced regioselective migration of the acetyl group to the generating free amino group. This treatment of compound **16** led to producing the 8-hydroxyl-*N*-acetyl derivative (**20**) in high yield, which was subjected to 8-*O*-sulfation and levulinoylation[11] to give the corresponding 8-*O*-sulfo and 8-*O*-levulinoyl derivative in high yields, **21** and **22**, respectively. Removal of the levulinoyl group of **22** effected by $NH_2NH_2{\cdot}AcOH$[11] was also highly efficient, suggesting that capping of 8-hydroxyl with levulinoyl group will work well for the 8-*O*-sulfated-sialyl oligosaccharide synthesis (Scheme 2).

Table 1. Coupling reaction of sialyl donors with various acceptors[a]

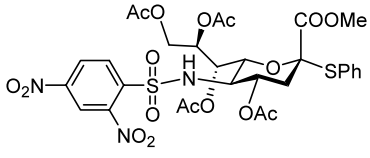
Entry	Donor	Acceptor	Promoter[b]	Temp. (°C)	Time	Product	Yield (α/β)(%)[c]
1	**2**	**5**	A	−30	10 min	**9**	76/19
2	**2**	**6**	B	−50	20 min	**10**	54/<10[d]
3	**2**	**7**	A	−30	5 min	**11**	35/<8[d]
4	**1**	**6**	B	−25	13 h	**12**	23 (α)
5	**3**	**5**	A	−30	10 min	**13**	42/42[e]
6	**4**	**5**	A	−30	10 min	**14**	74/16[e]
7[f]	**2**, **3**, **4** (1.0 equiv. each)	**5**	A	−30	16 h	**9**	32/8[e]
						13	11/11[e]
						14	12/3[e]
8	**2**	**8**	A	−40	3 h	**15**	24/19

[a] All reactions were conducted in CH_3CN–CH_2Cl_2(5/1) except entry 2 (EtCN), and the mole ratio of donor to acceptor was 1.2 to 1 unless otherwise specified.
[b] (A) NIS (1.5 equiv. of donor) and TESOTf (0.15 equiv. of donor); (B) NIS (1.5 equiv. of donor) and TfOH (0.15 equiv. of donor).
[c] Isolated yield.
[d] Including inseparable impurity.
[e] Ratio was calculated from relative signal intensity in ^{1}H NMR spectra.
[f] Competitive coupling reaction. Yields are mean value of three trials.



Scheme 2. Conversion of **16** into variant sialosides. *Reagents and conditions*: (a) Zn/AcOH, rt, 1∼2 h; (b) acetoxyacetyl chloride, Et_3N/THF, rt, 5 min; (c) $TFAc_2O$, Et_3N/THF, rt, 5 min; (d) NaOMe/MeOH, Drierite®, reflux, 2 days; (e) Ac_2O, pyr., DMAP/rt; (f) Zn/10% AcOH in 1,4-dioxane, rt, 24 h; (g) SO_3·pyr./pyr., rt, 1 h; (h) levulinic acid, DCC, DMAP/CH_2Cl_2, rt, 17 h; (i) NH_2NH_2·AcOH/ EtOH, rt, 4 h.

In conclusion, we have discovered the high potential of *N*-Troc protected sialyl donor **2**, and broadened its utility toward variant sialosides having *N*-glycolyl, de-*N*-acetyl, 1,5-lactam, and 8-*O*-sulfo sialic acid derivatives.[12] The reason underlying the enhanced reactivity of **2** by Troc group cannot be rationalized yet. Now at least, the possibility that an electron-withdrawing property rules the sialyl donor reactivity was eliminated by the coupling of the corresponding sialyl donor having a potent electron-withdrawal dinitrobenzenesulfonyl group[‡] and acceptors **6** and **7**



under similar conditions as mentioned above that took around 2 h to completion. This suggests that the reasons for the enhancement are plural. Since the synthesized sialyl-α(2→3)galactosides, **17**, **18**, **19**, and **22**, can be converted into the corresponding glycosyl donor blocks by the established protocol, this strategy will be integrated with various convergent approaches toward oligosaccharides.

[‡] For 2,4-dinitrobenzenesulfonyl group for amine protection: see: Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett*. **1997**, *38*, 5831.

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12. NMR data of selected compounds: For compound α-form of **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42–2.23 (m, 14H, Adamantane unit), 2.01, 2.04, 2.14 and 2.14 (4s, 12H, 4Ac), 2.70 (dd, 1H, $J_{gem}=12.5$, $J_{3,4}=4.5$ Hz, H-3*eq*^{Neu}), 3.65 (q, 1H, $J_{5,NH}=J_{4,5}=J_{5,6}=10.3$ Hz, H-5^{Neu}), 3.76 (s, 3H, COOMe), 4.09 (dd, 1H, $J_{gem}=10.7$, $J_{8,9a}=1.6$ Hz, H-9a^{Neu}), 4.13 (dd, 1H, $J_{6,7}=1.6$ Hz, H-6^{Neu}), 4.27 (dd, 1H, $J_{gem}=10.7$, $J_{8,9b}=2.0$ Hz, H-9b^{Neu}) 4.47 and 4.89 (2 d, 2H, Cl_3CCH_2O), 4.93 (m, 1H, H-4^{Neu}), 5.32 (m, 1H, H-8^{Neu}), 5.37 (dd, 1H, $J_{7,8}=8.4$ Hz, H-7^{Neu}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.79, 20.84, 20.88, 21.10, 26.95, 27.28, 31.41, 31.55, 33.04, 34.33, 36.35, 36.71, 37.50, 38.61, 51.64, 52.45, 62.20, 67.57, 68.64, 68.95, 71.82, 74.48, 76.75, 98.52, 154.16, 168.81, 169.91, 170.15, 170.47, 170.72. For compound β-form of **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45–2.11 (m, 14H, Adamantane unit), 2.01, 2.04, 2.06 and 2.14 (4s, 12H, 4Ac), 2.65 (dd, 1H, $J_{gem}=13.0$, $J_{3,4}=4.8$ Hz, H-3*eq*^{Neu}), 3.71 (q, 1H, $J_{5,NH}=J_{4,5}=J_{5,6}=10.3$ Hz, H-5^{Neu}), 3.75 (s, 3H, COOMe), 4.09 (dd, 1H, $J_{6,7}=2.3$ Hz, H-6^{Neu}), 4.09 (dd, 1H, $J_{gem}=12.3$, $J_{8,9a}=4.3$ Hz, H-9a^{Neu}), 4.46 (d, 1H, Cl_3CCH_2O), 4.89 (m, 2H, H-9b^{Neu} and Cl_3CCH_2O), 5.12 (m, 1H, H-8^{Neu}), 5.34 (m, 1H, H-4^{Neu}), 5.37 (dd, 1H, $J_{7,8}=2.7$ Hz, H-7^{Neu}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.79, 20.81, 20.95, 21.09, 26.88, 27.07, 31.28, 31.64, 32.27, 34.29, 36.45, 36.72, 37.24, 38.14, 51.93, 52.53, 62.73, 68.77 69.18, 72.04, 72.89, 74.53, 76.58, 97.52, 154.24, 168.15, 170.15, 170.49, 170.61, 170.80.