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Pre-activation based, highly alpha-selective O-sialylation with *N*-acetyl-5-*N*, 4-*O*-carbonyl-protected *p*-tolyl thiosialoside donor

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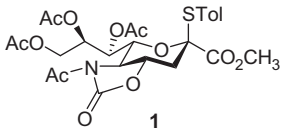
ABSTRACT

A new method for pre-activation of *N*-acetyl-5-*N*,4-*O*-carbonyl-protected *p*-tolyl thiosialoside donor with AgOTf and *p*-toluenesulfenyl chloride followed by coupling with other *p*-tolyl thioglycosides is reported. Excellent α-sialylation yields were achieved for these coupling reactions, as high as 91% yield of only α-isomer.

Sialic acid residues are frequently located at the nonreducing end of glycoconjugates and play important biological roles in many organisms.[1] *N*-Acetylneuraminic acid is the most common one and is present in a variety of glycosidic linkages, most typically α-(2,3) and α-(2,6) linkages to galactose or galactosamine,[2] as well as α-(2,8) or α-(2,9) linkages in polysialic acids.[3] The biological significance of sialoside receptors has driven research for their efficient synthesis. Significant efforts for improving α-sialylation have been made, including the use of anomeric leaving groups,[4] the introduction of an auxiliary group at C-1 and C-3,[5] the modification of the acetamide group at the 5th position of the sialyl donor into powerful electron-withdrawing groups,[6–14] or the optimized combinations of the leaving group with positional modification.[15] However, owing to the low reactivity and low stereoselectivity, highly efficient α-sialylation is still one of the most difficult and challenging processes in the chemical synthesis of oligosaccharides.

Huang's group developed a pre-activation based iterative one-pot synthesis method and successfully assembled numerous classes of complex oligosaccharides.[16] Comparing the traditional reactivity based one-pot syntheses, Huang's strategy works regardless of the reactivity of donor and acceptor, so it obviates the need for extensive adjustment of armed or disarmed protective groups and significantly reduces the amount of time needed for preparing building blocks. However, since the reactivity of thiosialoside is 10^5 times less than that of the corresponding thioglycosides of the other sugars,[17] up to now, chemistry has not progressed to the level at which thiosialosides are contemplated as components of pre-activations based iterative one-pot glycan syntheses. In order to assemble α-sialoside-based oligosaccharides by a one-pot method, usually the sialylated disaccharides have been used as building blocks.[18] However, application of this strategy is limited by the lack of an efficient and α-selective sialic acid donor that possesses a leaving group orthogonal to the thioglycoside. Based on the reactivity based one-pot method, Crich successfully developed an adamantanyl sialyl thioglycoside which could be promoted with NIS/TfOH and reacted with phenyl thioglycoside acceptor.[19] Up to now, there was no report about the pre-activation based sialylation. Herein, we report our preliminary observations of pre-activation based and highly α-selective sialylation by using *p*-tolyl-*N*-acetyl-5-*N*,4-*O*-carbonyl thiosialoside as donor.



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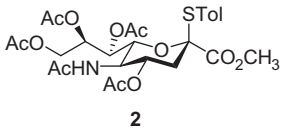

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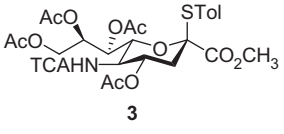

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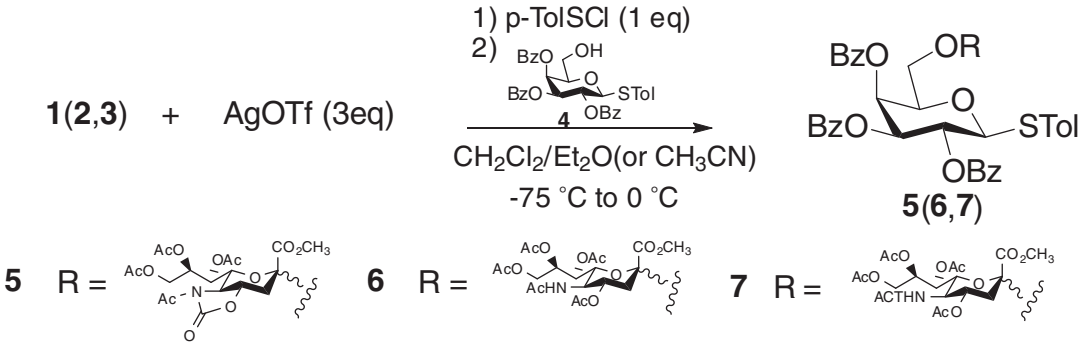
Table 1
Sialylation results of different sialyl thioglycosides



Entry	Donor	Condition	Acceptor	Product	Yield (%)	α/β
1	**1** (0.1 mmol)	A	**4** (0.075 mmol)	**5**	87	5:1[a]
2	**1** (0.1 mmol)	B	**4** (0.075 mmol)	**5**	91	10:1[a]
3	**1** (0.1 mmol)	C	**4** (0.075 mmol)	**5**	91	α Only
4	**2** (0.1 mmol)	C	**4** (0.075 mmol)	**6**	83	1:1[b]
5	**3** (0.1 mmol)	C	**4** (0.075 mmol)	**7**	33	α Only

A: Compound **1** was dissolved in CH_2Cl_2 (5 mL), AgOTf was dissolved in Et_2O (1 mL), **4** was dissolved in CH_2Cl_2 (2 mL). B: Compound **1** was dissolved in CH_2Cl_2 (5 mL), AgOTf was dissolved in CH_3CN (0.1 mL), **4** was dissolved in CH_2Cl_2 (2 mL). C: Compound **1** was dissolved in CH_2Cl_2 (5 mL), AgOTf was dissolved in CH_3CN (1 mL), **4** was dissolved in CH_2Cl_2 (2 mL).

[a] Determined by ^1H NMR on the crude reaction mixture.

[b] Isolated product.

Crich and his co-workers found that *N*-acetyl-5-*N*,4-*O*-carbonyl thiosialoside donors can lead to highly α-selective sialylation and that the oxazolidinone group can be easily cleaved under mild conditions leaving the acetamide intact.[14] Thus, initially we examined the pre-activation of *p*-tolyl thiosialoside donor **1** by AgOTf/*p*-TolSCl in CH_2Cl_2/CH_3CN (or CH_2Cl_2/Et_2O) at −75 °C, followed by coupling with *p*-Tol-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galacopyranoside (**4**) and were able to achieve a sialylation product in good yield and with high α-selectivity (Table 1).

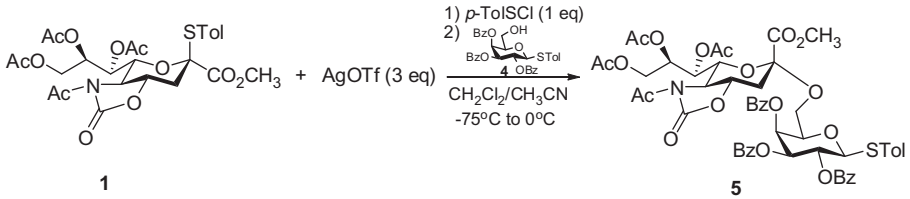
The solvent to dissolve AgOTf directly affects the α-selectivity of sialylation. When using 1 mL of Et_2O to dissolve AgOTf, the ratio of α:β isomer of the product is 5:1, and when using 1 mL of acetonitrile only α isomer was obtained. The reason for this result was mostly due to the fact that acetonitrile reacts with activated sialic donor **1** (oxonium ion) to form nitrilium cations and to stabilize oxonium ion against collapse to eliminated glycal byproducts before adding the acceptor.[4g,20] On the other hand, nitrilium cations also can explain the stereoselectivities of sialylation in acetonitrile solvents.[4g,21] In addition, we also investigated the reactivity of donor **2** and donor **3** and found that donor **2** provided good sialylation yields (83%), but no selectivity (α/β = 1/1) and donor **3** led to good α-selectivity (α only), but compared to donor **1** and **2**, the yield was very low (33%), most of donor **3** being converted to the eliminated product.[9b]

In Table 1, the ratio of donor:acceptor is 1:0.75, donor **1** gave an excellent α-sialylation result, but TLC and column chromatography indicated that a small amount of donor **1** was converted to glycal. In order to improve the sialylation efficiency, the ratio of donor to acceptor was changed with the results shown in Table 2.

The results of Table 2 indicate that when the ratio of donor to acceptor was increased from 0.75 to 0.9, the sialylation yield (based on the quantity of **4**) was not changed (ca. 91%), and when the ratio was increased to 0.95, the sialylation yield decreased to 87%, TLC also showed that there was a small amount of acceptor left unreacted. Hence, we prefer 0.9 as the best ratio for donor **1** to acceptor **4**.

Encouraged by the results of donor **1** coupling with acceptor **4**, under the reaction conditions (3 equiv of AgOTf, 1 equiv of *p*-TolSCl, ratio of donor to acceptor 1:0.9, CH_2Cl_2/CH_3CN, under N_2, at −75 °C

Table 2
The relationship of elimination product and the ratio of donor and acceptor



Entry	**1** (mmol)	**4** (mmol)	Yield[d] (%)	α/β	Glycal
1	0.1	0.075	91	α Only	Yes[a]
2	0.1	0.08	92	α Only	Yes[a]
3	0.1	0.085	90	α Only	Yes[b]
4	0.1	0.09	91	α Only	No
5	0.1	0.095	87	α Only	No[c]

[a] Isolated product.

[b] TLC indicated.

[c] TLC indicated small part of **4** left.

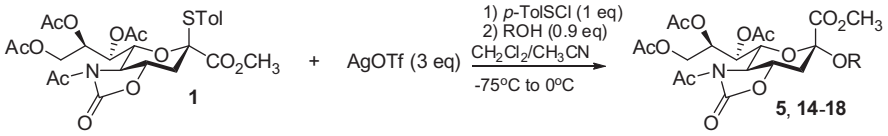
[d] Based on the quantity of acceptor.

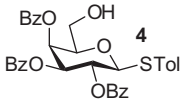
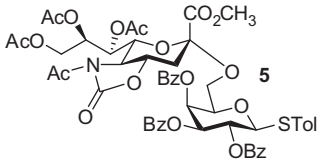
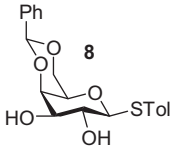
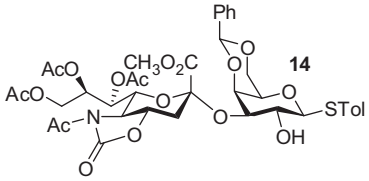
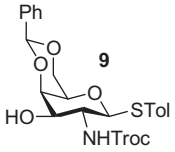
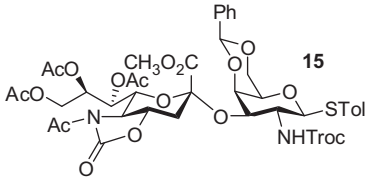
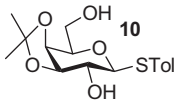
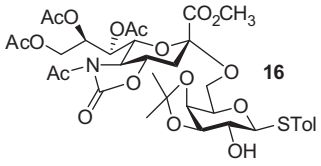
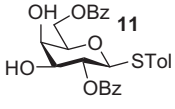
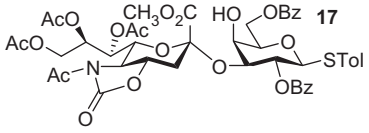
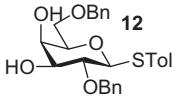
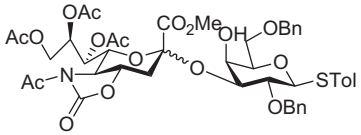
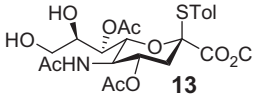
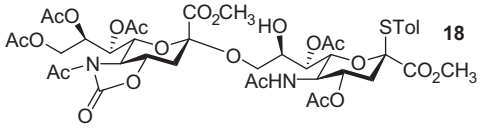
to 0 °C, ethyl acetate/hexane for column chromatography), we focused our attention on donor **1** reacting with other thioglycoside acceptors (**8**–**13**). In all cases, except **12**, isolated yields of the sialylation products ranged from good to excellent. In the case of acceptor **12**, the mass spectrum of the reaction mixture indicated a peak for sialylated disaccharide but the product could not be isolated purely. The stereoselectivity in all cases was very good, only α-linkage products were found. Regioselective sialylation of galactopyranoside 2,3-diols, 3,4-diols, and 2,6-diols analogous **8**, **11**, **10,** and **14** was observed with the formation of α-2,3 and α-2,6 linkage products, and with sialic acid 8,9-diol analog **13**, α-2,9 linked product was obtained.

The structures of sialylated disaccharides **5**, **6**, **7,** and **14**–**18** were confirmed by ^{1}H and gCOSY NMR experiments, and the anomeric configurations were assigned using empirical rules.[22] The most useful characteristic parameters to unequivocally assign α configuration were the chemical shift of H-3$_{eq}$ (δ = 2.72–2.98 ppm), $J_{H\text{-}7,\ H\text{-}8}$ (7.0–7.5 Hz), and $\Delta\delta${H-9a–H-9b} (0.1–0.45 ppm). On the other hand, the α configuration was also assigned by ^3J (C-1, H-3$_{eq}$) coupling constants[17] (Table 3). The 2→3, 2→6, and 2→9 linkages were determined by the correlation of gHMBC between the signal of H-3, H-6 of galactose unit or H-9 of sialic acid acceptor, and the signal of C-2 of the sialic acid donor.

In conclusion, regardless of huge difference between the reactivity of sialyl *p*-tolyl thioglycoside and other *p*-tolyl thioglycosides (except of **13**, the reactivity of other *p*-tolyl thioglycoside is 10^5 times higher than that of sialyl *p*-tolyl thioglycoside), *N*-acetyl-5-*N*-4-*O*-carbonyl protected p-tolyl thiosialoside donor can be

Table 3
Sialylation results of donor **1** coupled with different acceptors



Entry	Acceptor	Product	Yield (%)	^3J(C-1, H-3$_{eq}$) (Hz)	α/β
1	 **4**	 **5**	91	6.2	α Only
2	 **8**	 **14**	81	5.5	α Only
3	 **9**	 **15**	78	6.0	α Only
4	 **10**	 **16**	89	6.3	α Only
5	 **11**	 **17**	85	6.2	α Only
6	 **12**		/[a]	/	/[a]
7	 **13**	 **18**	82	6.0	α Only

[a] No pure product isolated, based on MS spectrum indication we draw structure of disaccharide, we cannot calculate yield and selectivity.

pre-activated by the promoter of AgOTf/*p*-TolSCl in the solvent of CH_2Cl_2/CH_3CN and coupled with other *p*-tolyl thioglycosides with good to excellent isolated yield and excellent α-selectivity. This protocol shows that the sialyl thioglycosides can directly be used as components of pre-activation based one-pot iterative sialic acid containing oligosacchrides synthesis.

Experimental section

General experimental procedures: All reactions were carried out under nitrogen with anhydrous solvents in flame-dried glassware, unless otherwise noted. All the glycosylation reactions were performed in the presence of molecular sieves, which were flame-dried right before the reaction under high vacuum. Glycosylation solvents were dried using a solvent purification system and used directly without further drying. Chemicals used were reagent grade as supplied except where noted. Analytical thin-layer chromatography was performed using Silica Gel 60 F254 glass plates; spots were visualized by UV light (254 nm) and by staining with a yellow solution containing $Ce(NH_4)2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}$ $4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (230–400 Mesh). NMR spectra were referenced using Me_4Si (0 ppm), residual $CHCl_3$ (^1H NMR 7.26 ppm, ^{13}C NMR 77.0 ppm). Peak and coupling constant assignments are based on ^1H NMR and ^1H–^1H gCOSY. High-resolution mass spectra were recorded on a Q-TOF Ultima API LC-MS instrument with Waters 2795 Separation Module (Waters Corporation, Milford, MA). All optical rotations were measured at 20 °C using the sodium D line.

The pre-activation based sialylation of Table 3 was carried out as follows under N_2: The reaction mixture of *p*-tolyl thiosialoside donor (0.1 mmol) and freshly activated molecular sieves (3 Å, 500 mg) in anhydrous CH_2Cl_2 (5 mL) was stirred for 30 min at room temperature, then cooled to −75 °C, a solution of AgOTf (0.3 mmol) in acetonitrile (1 mL) was added directly to the solution without touching the wall of reaction flask. After 5 min at −75 °C, *p*-TolSCl (0.1 mmol) was added via a syringe. The mixture was stirred for 10–15 min until the yellow color disappeared and the donor was completely consumed as judged by TLC. A solution of thioglycoside acceptor (0.09 mmol) in anhydrous CH_2Cl_2 (2 mL) was added slowly to the pre-activated donor. The reaction mixture was stirred for 90 min while the temperature was raised from −75 °C to 0 °C and was quenched with triethylamine (0.2 mL). The mixture was diluted with CH_2Cl_2 (50 mL) and filtered over Celite. The filtrate was concentrated and purified by flash column chromatography (hexane, ethyl acetate) to afford the desired product.

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