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Comparative studies on the O-sialylation with four different α/β-oriented (*N*-acetyl)-5-*N*,4-*O*-carbonyl-protected *p*-toluenethiosialosides as donors

Xiao-tai Zhang, Zhen-yuan Gu, Guo-wen Xing *

Department of Chemistry, Beijing Normal University, Beijing 100875, China

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ABSTRACT

Four types of 5-*N*,4-*O*-carbonyl-protected *p*-toluenethiosialosides were synthesized and their couplings with different acceptors were thoroughly investigated. The results indicate that the sialyl donor structure, the amount of glycosyl acceptor, and the detailed promotion conditions have great influence on the sialylation stereoselectivties and product yields. Under the $(p\text{-Tol})_2SO/Tf_2O$ activation conditions, the glycosylations with simple alcohols provided declined α-selectivities and higher yields with increasing the amounts of acceptors from 1.1 equiv to 2.0 equiv. However, the outcome of same sialylation was independent of the relative amounts of sugar alcohol acceptors. With NIS/TfOH as promoter, the α-selectivities of the sialylations were significantly improved compared with the cases activated by $(p\text{-Tol})_2SO/Tf_2O$. In general, the difference in configuration of N-acetylated sialyl donors (**D2** and **D4**) has little effect on the sialylation yield and stereoselectivity. In contrast, the N-deacetylated α/β sialyl donors (**D1** and **D3**) show complex sialylation profiles with different acceptors.

1. Introduction

Sialic acid is a large family of natural nine-carbon sugar acids and predominantly exists at the non-reducing termini of glycoproteins and glycolipids in various biosystems like human beings and other mammalian species. Among over 50 structural derivatives of sialic acid, *N*-Acetylneuraminic acid (Neu5Ac) is the widely known member which is involved in many significant biological phenomena on the cell surface through α glycosidic linkage, including cell-cell interaction, cell-virus recognition and so on.[1] The significances of sialic acid and its derivatives have intrigued the chemist's great research interests. During the past decades, to achieve efficient sialylation with high α-selectivity, tremendous efforts have been made to design and modify the structure of sialyl donors, such as application of neighboring group participation at C-1,[2] utilization of different leaving groups at C-2,[3] introduction of auxiliary groups at C-3[4] as well as derivatization of amino group at C-5.[5]

As a novel protecting group, (*N*-acetyl)-5-*N*,4-*O*-carbamate has been introduced to sialyl donor, especially thiosialoside,[3f,6–8] by several research groups in the past few years. Both α and β anomers of *N*-acetyl-5-*N*,4-*O*-carbonyl-protected phenylthiosialoside (**1**)[6a] have been coupled to simple alcohols and sugar alcohols promoted by *N*-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH) in CH_2Cl_2 at −40 °C to provide excellent yield and stereoselectivity. *N*-Acetyl-5-*N*,4-*O*-carbonyl-protected adamantanyl thiosialoside (**2**)[6b] can also achieve efficient α-sialylations by means of the nitrile effect, but the complicated synthetic route to obtain donor **2** may confine its application. Meanwhile, 5-*N*,4-*O*-carbonyl-protected α-thiosialosides (**3**,[6c] **4**[3f]) have also been devised with thiophenyl and benzoxazolylsulfenyl (S-Box) as leaving groups. Further improvements in stereoselectivity of α-(2,6) sialylations between donor **4** and galactosyl acceptors were observed compared with the cases using *N*,*N*-diacetyl or *N*-trifluoroacetyl counterpart donors. The convincing reason why 5-*N*,4-*O*-carbamate can dramatically enhance the α-selectivity of sialylation has been recently investigated, which owed to the steric hindrance effect brought by *N*,*O*-*trans*-fused ring[7] (Fig. 1).

Although several *N*-acetyl or *N*-deacetyl 5-*N*,4-*O*-carbonyl-protected thiosialoside donors with either α or β-orientation have been reported, unfortunately, it is difficult to accurately evaluate the sialylation outcome to find the best 5-*N*,4-*O*-carbonyl-protected thiosialoside donor based on the results provided by different research groups. To clarify this issue, as a part of our parallel investigations on sialic acid glycosylation, herein we report the comparative and thorough studies on the sialylations with two couples of α/β-oriented (*N*-acetyl)-5-*N*,4-*O*-carbonyl-protected *p*-toluenethiosialosides as donors (**D1**–**D4**).

* Corresponding author.
E-mail address: gwxing@bnu.edu.cn (G. Xing).

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0008-6215/

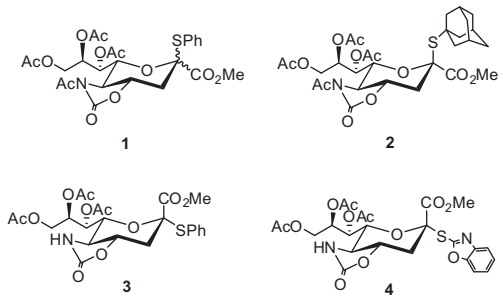
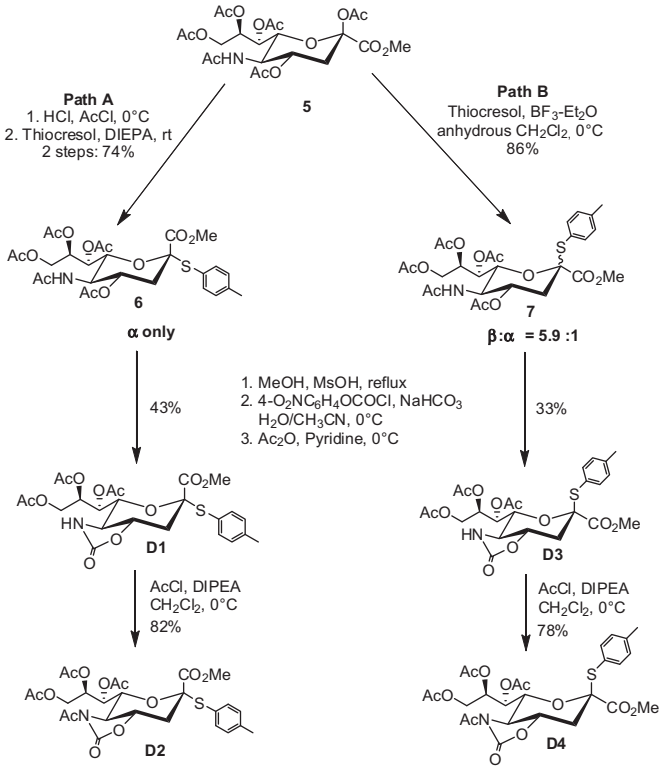


Figure 1. Several types of the known *N*-acetyl or *N*-deacetyl 5-*N*,4-*O*-carbonyl-protected thiosialoside donors.

2. Results and discussion

In our previous work,[8] α-*N*-acetyl-5-*N*,4-*O*-carbonyl-protected *p*-toluenethiosialoside (**D2**,[8a] Scheme 1) was prepared readily from sialic acid and *p*-toluenethiol, the α glycosidic linkage between sialic acid and various acceptors promoted by Ph_2SO/Tf_2O or NIS/TfOH have been successfully constructed in CH_2Cl_2 or CH_2Cl_2/CH_3CN co-solvent. Compared with the phenyl group at C-2 of **1** and **3**, the greater electron donating property of *p*-methylphenyl guarantees higher reactivity of *p*-toluenethiosialoside. Meanwhile, the utilization of *p*-toluenethiol (mp 40–44 °C, much higher than that of thiophenol) can efficiently relieve the odor problem in the preparation of sulfide sialyl donors.

In the current study, to investigate the glycosylation associated with 5-*N*,4-*O*-carbonyl-protected *p*-toluenethiosialoside systematically and deeply, we prepared other three (*N*-acetyl)-5-*N*,4-*O*-carbonyl-protected *p*-toluenethiosialosides (**D1**,[6d] **D3**,[6e] **D4**,[6f] Scheme 1). These donors can be divided into two groups, **D2** and **D4** are *N*-acetyl α/β isomers, while **D1** and **D3** are *N*-deacetyl α/β



Scheme 1. Synthetic route of four sialyl donors.

counterparts. The 2-α-tolylsulfenyl derivative of Neu5Ac (**6**) was obtained exclusively from peracetylated methyl sialylate (**5**) through **path A** including the generation of 2-chloro derivative and the introduction of tolylsulfenyl leaving group.[5c] However, if BF_3-Et_2O was used as lewis acid catalyst (**path B**), β-dominant mixture **7** (β:α = 5.9:1) was afforded in 86% yield.[9] After deacetylation with MsOH (methanesulfonic acid) in MeOH and the introduction of 5-*N*,4-*O*-carbonyl protective group, the free hydroxyl groups were acetylated afresh by acetic anhydride in pyridine to provide **D1** and **D3** successfully in 33–43% yield (3 steps). It is worth noting that α-anomer of **7** can be readily removed through flash column chromatography purification during the following acetylation procedure to obtain the single β-anomer **D3**. In addition, **D1** and **D3** can be further acetylated by acetyl chloride to give *N*-acetyl-5-*N*,4-*O*-carbonyl-protected donors **D2** and **D4** in good yields.

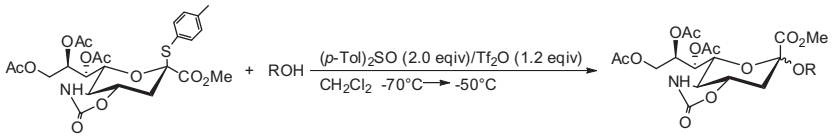
With the four sialyl donors in hand, the sialylations with different acceptors were investigated. Especially, the effect of the amount of acceptors on glycosylation was carefully explored. Initially, the seldom-used β-anomer **D3** was employed as the donor for the sialylation under $(p\text{-Tol})_2SO$ (di(*p*-tolyl) sulfoxide) and Tf_2O promotion conditions. According to the previous research in our laboratory, $(p\text{-Tol})_2SO$ was a more preferable additive compared with Ph_2SO due to its better compatibility to *p*-toluenethiosialoside.[3g,10] Donor **D3** was first coupled to 1.1 equiv of 1-octanol (**8**) in CH_2Cl_2 to obtain high yield (79%) and α-selectivity (6.2:1) (Table 1, entry 1). Similar results were observed when secondary alcohol cyclohexanol (**9**) was used as the acceptor (Table 1, entry 3). For acceptors **8** and **9**, if their amount increased from 1.1 equiv to 2.0 equiv, to our surprise, the corresponding yields increased obviously while the reaction selectivity declined to some extent (Table 1, entry 2, 4). Efficient construction of (2, 6) or (2, 3) glycosidic linkage were made successfully when 1.1 equiv of methyl 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (**10**) or methyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (**11**) were used respectively. Meanwhile, an excellent yield and moderate α-selectivity were obtained in the 6- or 3-O-sialylation (Table 1, entry 5, 7). Similarly, we increased the amount of **10** and **11**, unlike the reactions between **D3** and simple alcohols, the changes in yield and α-selectivity were quite inconspicuous (Table 1, entry 6, 8).

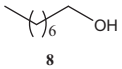
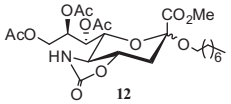
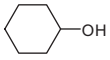
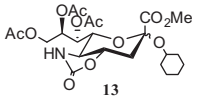
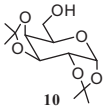
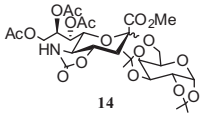
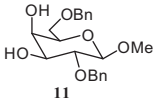
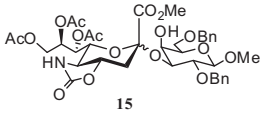
To further probe the effect of acceptor amount on the sialylation, NIS/TfOH activation system was used for the glycosylation with **D3** as donor. As Table 2 indicates, all of the coupling reactions gave excellent yields, even a quantitative product yield was obtained with cyclohexanol as acceptor (Table 2, entry 4). Except for acceptor **10** and **11**, the product α/β ratio significantly dropped down with increasing the amount of acceptor from 1.1 equiv to 2.0 equiv. The results are comparative with those using $(p\text{-Tol})_2SO/Tf_2O$ as promotion system (Table 1), indicating that the acceptor amount indeed has great influence on the sialylation α-selectivity.

Although above results show the yield and stereoselectivity of the O-sialylation will change according to the amount of acceptor added. To our delight, some other information can also be obtained when carefully comparing the experimental data in Tables 1 and 2. With regard to all of the four acceptors, the α-selectivities were remarkably improved in large degree when NIS/TfOH was chosen as the promotion system instead of $(p\text{-Tol})_2SO/Tf_2O$. For the simple alcohols **8** and **9**, higher yields were achieved under the activation of NIS/TfOH with either 1.1 equiv or 2.0 equiv acceptors employed. In contrast, the yields of oligosaccharides **14** and **15** were not highly dependent of the promotion conditions.

To explain the experimental results for donor **D3**, a hypothetical mechanism for sialylations promoted by $(p\text{-Tol})_2SO/Tf_2O$ or NIS/TfOH was proposed in Scheme 2. The primer **D3** can be activated rapidly by di(*p*-tolyl) sulfoxide bi(triflate) **16** which was derived from $(p\text{-Tol})_2SO$ and Tf_2O in situ to yield sulfonium salt

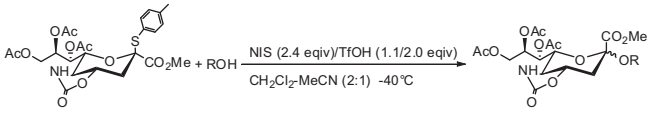
Table 1
$(p\text{-Tol})_2SO/Tf_2O$ promoted sialylations of thiosialoside donor **D3** with various alcohol acceptors



Entry	Acceptor		Product	Yield[a] (%)	α:β[b]
1		1.1 Equiv		79	6.2:1
2		2.0 Equiv		94	2.9:1
	8		**12**		
3		1.1 Equiv		78	9.1:1
4		2.0 Equiv		91	7.8:1
	9		**13**		
5[c]		1.1 Equiv		97	4.6:1
6[c]		2.0 Equiv		97	3.0:1
	10		**14**		
7		1.1 Equiv		90	1.6:1
8		2.0 Equiv		88	1.8:1
	11		**15**		

[a] Isolated yields after column chromatography.
[b] Determined by ^1H NMR analysis of the crude products.
[c] The ^1H NMR spectroscopic data are consistent with those reported in Ref. 6g.

Table 2
NIS/TfOH promoted sialylations of thiosialoside donor **D3** with various alcohol acceptors

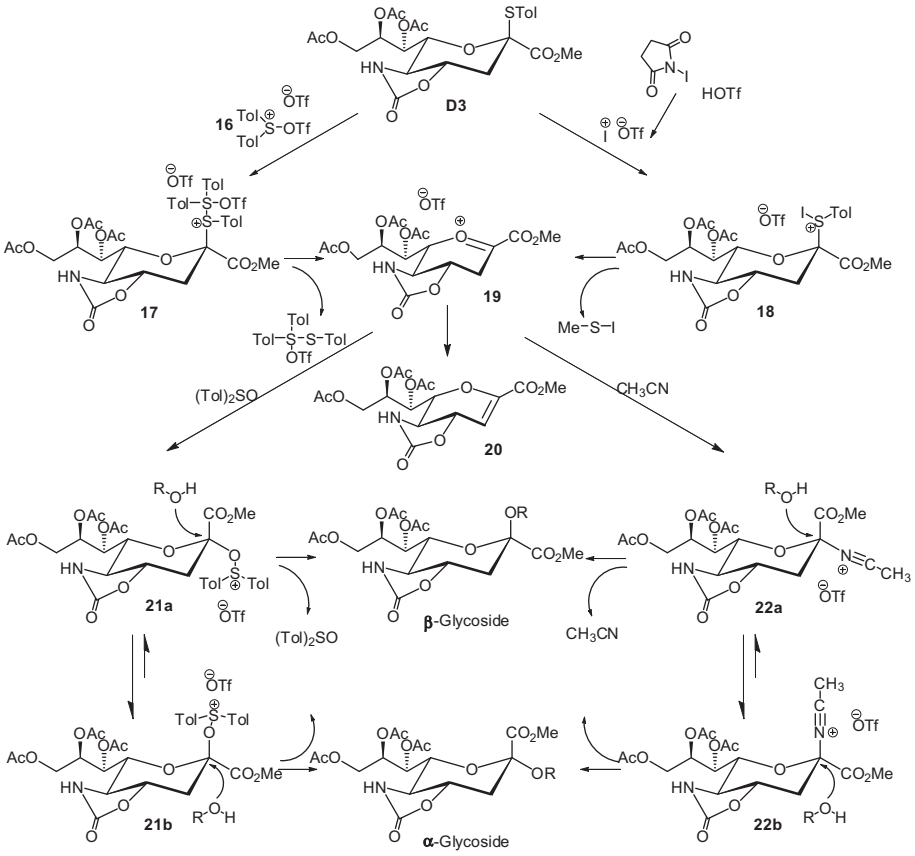


Entry	Acceptor[a]		Product	Yield[b] (%)	α:β[c]
1	**8**	1.1 Equiv	**12**	98	23.1:1
2		2.0 Equiv		99	12.5:1
3	**9**	1.1 Equiv	**13**	99	α Only
4		2.0 Equiv		Quant.	12.5:1
5[d]	**10**	1.1 Equiv	**14**	96	20.0:1
6[d]		2.0 Equiv		98	20.0:1
7	**11**	1.1 Equiv	**15**	85	4.2:1
8		2.0 Equiv		83	2.4:1

[a] Equal amounts of acceptors and TfOH were employed.
[b] Isolated yields after column chromatography.
[c] Determined by ^1H NMR analysis of the crude products.
[d] The ^1H NMR spectroscopic data are consistent with those reported in Ref. 6g.

17.[3g,8b,10,11] Analogously, sulfonium ion **18** was produced due to the activation of NIS/TfOH.[12] After the loss of a neutral molecule (thio derivative), either **17** or **18** collapsed to form 5-*N*,4-*O*-carbonyl protected oxacarbenium cation **19** which was considered as a crucial intermediate in the whole reaction system. According to the postulation of Crich[13] and our studies,[3g,8b,10] intermediate **19** would rapidly transform to major by-product glycal **20** in the absence of proper nucleophiles. Under the $(p\text{-Tol})_2SO/Tf_2O$ promotion conditions, the overwhelming majority of **19** was trapped by excessive $(p\text{-Tol})_2SO$ to provide two species differing in configuration at C-2, namely 5-*N*,4-*O*-carbonyl-protected C-2-sialyloxosulfonium salts **21a**/**21b**. As a part of reaction medium, acetonitrile can also attack intermediate **19** through the lone pair electrons on the nitrogen atom to bring out the nitrilium ion pair **22a**/**22b**.[5c,14] Nucleophilic substitutions of **21b**/**22b** which adopted stereo-preferred β-configuration gave the α-sialoside as the major product in the glycosylation reaction, while the β-sialoside resulting from α-oriented intermediates **21a**/**22a** was obtained as the minor product.

During the process of sialylations, the equilibration of intermediates **21a**/**21b** or **22a**/**22b** plays significant roles in controlling the O-sialylation stereoselectivities. We suggested that increased amounts of glycosyl acceptors might weaken the advantage of β-configuration intermediate (**21b**/**22b**), which are more favorable to being approached by ROH to give α-sialoside, that is to say, the equilibrations mentioned above could be modulated by excessive acceptors to a great extent. As a consequence, lower selectivities were obtained if 2.0 equiv of acceptors were used, especially for simple alcohols such as **8** and **9** (Table 1, entries 1–4 and Table 2, entries 1–4). Surprisingly, small changes were observed for the glycosylation α/β ratios when different amounts of sugar alcohols (**10** and **11**) were utilized as acceptors (Table 1, entries 5–8 and Table 2,



Scheme 2. Hypothetical mechanism for the sialylations of **D3** promoted by $(p\text{-Tol})_2SO/Tf_2O$ or NIS/TfOH.

entries 5–8). The results could be attributed to 'effective concentration' which means that the actual concentration around intermediate molecules. The effective concentration of **10** or **11** would not change much owing to their large molecule volume in spite of increasing their absolute equivalence from 1.1 to 2.0.

In addition, higher yields were obtained in the sialylations promoted by NIS/TfOH than $(p\text{-Tol})_2SO/Tf_2O$. The results can be explained by the relative reactivities of the key intermediate **21a**/**21b** and **22a**/**22b**. Actually, **22a**/**22b** possess a better leaving group (CH_3CN) than that ($(p\text{-Tol})_2SO$) of **21a**/**21b** due to the stronger lewis acidity of nitrilium ion than sulfonium ion. Consequently, CH_3CN can break away from the glycosyl moiety readily than $(p\text{-Tol})_2SO$ to provide better product yield.

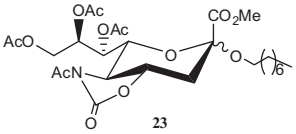
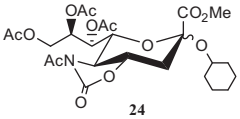
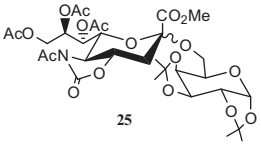
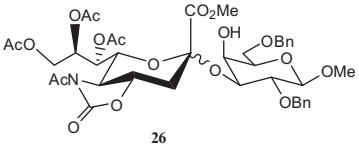
Encouraged by above results, the combination of other three donors (**D1**, **D2**, and **D4**) and four acceptors were performed to comprehensively evaluate the reactivities of different sialyl donors (Table 3). As for **D3**, the amount of acceptors has been determined to be 1.1 equiv to achieve higher α-selectivities with both $(p\text{-Tol})_2SO/Tf_2O$ and NIS/TfOH as promoters (Tables 1 and 2). Therefore, the same coupling conditions were employed for the reactions with donors **D1**, **D2**, or **D4**. We compared the differences in stereoselectivity and yield caused by different promotion systems. In general, the results were similar with those using **D3** as donor. Much more improved α-selectivities and product yields were obtained under the activation of NIS/TfOH than $(p\text{-Tol})_2SO/Tf_2O$. The anometric stereochemistry of the new sialylation compounds (**12**, **13**, **15**, and **24**) was assigned on the basis of the $^3J_{C\text{-}1,3ax\text{-}H}$ coupling constant.[15] The α anomer has the $^3J_{C\text{-}1,3ax\text{-}H}$ value varying from 5.3 to 5.7 Hz, which has good consistence with the data reported in literature.[6a]

In addition, the difference in configuration of N-acetylated donors (**D2** and **D4**) has little effect on yields and stereoselectivities. The results are well consistent with previous work reported by Crich[6a] and our groups.[10] In contrast, the donors (**D1** and **D3**) which bear a hydrogen atom instead of acetyl on 5-*N* afforded varied stereoselectivity with different acceptors. Sialylation of **D1** led to higher α-selectivities with primary alcohol as acceptors (Table 3, entries 1, 9), while **D3** was in favor of stereocontrol in the sialylations of secondary alcohols (Table 3, entries 6, 14). The results can be rationalized by the complicated intermolecular hydrogen-bonding network formed between NH and the C=O groups of **D1**/**D3** or MeCN in solutions (Scheme 3), which has been reported by Kononov and co-workers[16] in other similar sialylations. Moreover, the structures of acceptors should also interfere and influence the hydrogen-bonding networks to bring about the unexpected outcome of sialylations.

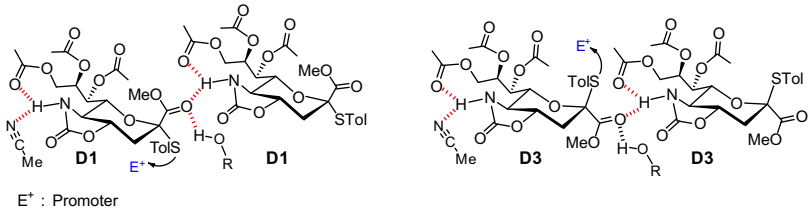
3. Conclusion

In conclusion, four types of 5-*N*,4-*O*-carbonyl protected *p*-toluenethiosialoside were synthesized conveniently, and all of them were used for the preparation of sialylconjugates. For sialyl donor **D3**, we have demonstrated that the amounts of glycosyl acceptors significantly influence glycosylation α-selectivities, especially with simple alcohols (**8** and **9**) as acceptors. NIS/TfOH was considered to be a more efficient activator system compared with $(p\text{-Tol})_2SO/Tf_2O$ to afford better α-selectivities and higher reaction yields. Generally, the stereoselectivity of the glycosylation of **D3** varied due to the equilibration of the crucial intermediates 5-*N*,4-*O*-carbonyl protected C-2-sialyloxosulfonium salts **21a**/**21b** or nitrilium ion pair **22a**/**22b** in the sialylation. In addition, N-deacetylated donors (**D1** and **D3**) were more sensitive to the structures of acceptors to provide complex sialylation profiles, while similar sialylation

Table 3
Sialylations of thiosialoside donors with various alcohol acceptors

Entry	Acceptor[a]	Donor	Product	Yield[b] (%) (p-Tol)$_2$SO-Tf$_2$O/NIS-TfOH	α:β [c] (p-Tol)$_2$SO-Tf$_2$O/NIS-TfOH
1	**8**	**D1**	**12**	86/86	14.1:1/28.3:1
2		**D3**		79/98	6.2:1/23.1:1
3[d]		**D2**		70/Quant.	8.1:1/20.7:1
4[d]		**D4**		65/Quant.	12.5:1/25.2:1
			 **23**		
5	**9**	**D1**	**13**	85/99	8.1:1/23.6:1
6		**D3**		78/99	9.1:1/α Only
7		**D2**		61/90	11.5:1/21.3:1
8		**D4**		67/96	12.5:1/21.6:1
			 **24**		
9[d]	**10**	**D1**	**14**	92/96	8.3:1/24.1:1
10[d]		**D3**		97/96	4.6:1/20.0:1
11[d]		**D2**		90/Quant.	6.1:1/15.0:1
12[d]		**D4**		Quant./Quant.	7.1:1/12.5:1
			 **25**		
13	**11**	**D1**	**15**	76/77	1.7:1/2.7:1
14		**D3**		90/85	1.6:1/4.2:1
15[d]		**D2**		73/64	1:1/1.7:1
16[d]		**D4**		86/82	1.5:1/1.5:1
			 **26**		

[a] Equal amounts of acceptors and TfOH were employed under the promotion of NIS/TfOH.
[b] Isolated yields.
[c] Determined by ^{1}H NMR analysis of the crude products.
[d] The ^{1}H NMR spectroscopic data are consistent with those reported in Refs. 6a,g.



Scheme 3. Proposed H-bonding network of **D1** or **D3** in solutions. Dimers are shown for clarity.

outcomes were obtained for N-acetylated donors (**D2** and **D4**). Current study in this paper should enable a deeper insight into the methodology of sialylation to synthesize complex glycoconjugates.

4. Experimental section

4.1. General

All chemicals were purchased as reagent grade and used without further purification. All sialylation reactions were performed in flame-dried glassware under an inert argon atmosphere. Dichloromethane and acetonitrile were distilled over calcium hydride (CaH_2). Reactions were monitored by analytical thin-layer chromatography on silica gel F_{254} glass plates. Spots were detected under UV (254 nm) directly, or by dipping in the solution of EtOH/H_2SO_4 followed by cauterization. Flash column chromatography was performed on silica gel (200–300 mesh). ^{1}H NMR spectra were recorded on a 400 MHz NMR spectrometer at 20 °C. Chemical shifts (in ppm) were referenced to tetramethylsilane (δ = 0 ppm) in deuteriated chloroform. ^{13}C NMR spectra were recorded with a 400 MHz NMR spectrometer (100 MHz) and calibrated with $CDCl_3$ (δ = 77.23 ppm). High-resolution mass spectra were recorded using electrospray ionization (ESI).

4.2. General sialylation procedure with (p-Tol)$_2$SO/Tf$_2$O as promotion system

A solution of sialyl donors (**D1**–**D4**, 37.2/40.1 mg, 0.069 mmol, 1.0 equiv), (p-Tol)$_2$SO (31.8 mg, 0.138 mmol, 2.0 equiv) and activated 4 Å powdered sieves in anhydrous CH_2Cl_2 (2 mL) was stirred for 15 min at −70 °C under Ar, followed by the addition of Tf_2O (13.6 μL, 0.083 mmol, 1.2 equiv). The mixture was stirred at −70 °C for another 30 min, then a solution of acceptor (1.1/2.0 equiv) in anhydrous CH_2Cl_2 (1 mL) was added. The reaction was stirred for 2.0 h at −70 °C then warmed to −50 °C for another 2.0 h. After quenching with Et_3N (0.1 mL), the mixture was diluted

with CH_2Cl_2 (about 50 mL), filtered through Celite, washed with saturated brine (3 × 10 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluting with a hexanes/EtOAc system to give the coupling products.

4.3. General sialylation procedure with NIS/TfOH as promoter

A solution of sialyl donors (**D1**–**D4**, 37.2/40.1 mg, 0.069 mmol, 1.0 equiv), acceptor (1.1–2.0 equiv), NIS (37.3 mg, 0.1656 mmol, 2.4 equiv) and activated 4 Å powdered sieves in anhydrous CH_2Cl_2–CH_3CN (2:1, 2.67/2.40 mL) was stirred for 10 min at room temperature under Ar. Then the mixture was stirred at −40 °C for 20 min, followed by the addition of CH_2Cl_2–CH_3CN (2:1) solution of TfOH (0.33/0.60 mL, 1.1/2.0 equiv). After stirred for another 1.0 h at −40 °C, the reaction was quenched with Et_3N (0.1 mL). The mixture was diluted with CH_2Cl_2 (about 50 mL), filtered through Celite, washed with 20% aq $Na_2S_2O_3$ solution (2 × 10 mL) and saturated brine (2 × 10 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluting with a hexanes/EtOAc system to give the coupling products.

4.4. Methyl (octyl 7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (12α)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.47 (d, J = 9.7 Hz, 1H), 5.34 (s, 1H), 5.13 (d, J = 9.7 Hz, 1H), 4.36–4.25 (m, 3H), 3.97–3.90 (m, 1H), 3.79 (s, 3H, COOCH3), 3.76–3.70 (m, 1H), 3.22–3.16 (m, 1H), 3.05 (t, J = 10.3 Hz, 1H), 2.89 (dd, J = 12.1, 3.2 Hz, 1H, H-3eq), 2.19 (s, 6H), 2.07 (s, 3H), 1.63 (br s, 1H), 1.55-1.50 (m, 2H), 1.27 (br s, 9H), 0.88 (t, J = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.5, 170.5, 169.8, 168.7(C1, $^3J_{C1,H3ax}$ = 5.3 Hz), 159.3, 100.2, 76.8, 73.4, 68.9, 67.0, 65.6, 61.7, 58.0, 52.8, 37.6, 31.8, 29.5, 29.2, 25.8, 22.6, 21.0, 20.8, 20.7, 14.1 ppm; HRMS (ESI-TOF): m/z calcd for $C_{25}H_{39}NO_{12}Na$ $[M+Na]^+$ 568.2370, found: 568.2352.

4.5. Methyl (cyclohexyl 7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (13α)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (d, J = 9.7 Hz, 1H), 5.34 (s, 1H), 5.30 (s, 1H), 5.12 (dd, J = 9.8, 1.6 Hz, 1H), 4.35–4.26 (m, 2H), 4.21 (dd, J = 9.9, 1.6 Hz, 1H), 3.94–3.87 (m, 1H), 3.77 (s, 3H, COOCH3), 3.57 (br s, 1H), 3.04 (t, J = 10.4 Hz, 1H), 2.90 (dd, J = 12.0, 3.4 Hz, 1H, H-3eq), 2.19 (s, 3H), 2.18 (s, 3H), 2.06 (s, 3H), 1.89 (br s, 1H), 1.71–1.49 (m, 5H), 1.37–1.15 (m, 5H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.5, 170.5, 169.7, 169.0(C1, $^3J_{C1,H3ax}$ = 5.5 Hz), 159.4, 100.3, 76.9, 75.3, 73.4, 68.9, 67.2, 61.8, 57.9, 52.6, 37.9, 34.9, 33.0, 25.3, 24.3, 24.1, 21.0, 20.8, 20.6 ppm; HRMS (ESI-TOF): m/z calcd for $C_{23}H_{33}NO_{12}Na$ $[M+Na]^+$ 538.1901, found: 538.1876.

4.6. Methyl 7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate-(2 → 3)-methyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (15α)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.28 (m, 10 H), 5.52 (ddd, J = 9.6, 3.2, 2.1 Hz, 1H), 5.31 (s, 1H), 5.09 (dd, J = 9.6, 1.8 Hz, 1H), 4.81 (d, J = 11.9 Hz, 1H), 4.70 (d, J = 11.9 Hz, 1H), 4.58 (s, 2H), 4.35 (d, J = 7.7 Hz, 1H), 4.24 (dd, J = 12.7, 1.9 Hz, 1H), 4.19–4.15 (m, 2H), 4.06 (dd, J = 9.6, 3.2 Hz, 1H), 3.96–3.89 (m, 1H), 3.81 (dd, J = 9.9, 6.2 Hz, 1H), 3.76 (s, 3H, COOCH3), 3.74 (d, J = 5.6 Hz, 1H), 3.72 (d, J = 3.8 Hz, 1H), 3.61 (t, J = 5.9 Hz, 1H), 3.56 (s, 3H), 3.53 (dd, J = 9.6, 7.8 Hz, 1H), 3.02 (t, J = 10.4 Hz, 1H), 2.89 (dd, J = 12.2, 3.3 Hz, 1H, H-3 eq), 2.13 (s, 3H), 2.12 (t, J = 12.7 Hz, 1H), 2.05 (s, 3H), 1.92 (s, 3H), 1.25 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.4, 170.4, 169.7, 168.6(C1, $^3J_{C1,H3ax}$ = 5.7 Hz), 159.1, 139.0, 138.1, 128.4, 128.1, 127.7, 127.6, 127.5, 104.7, 99.1, 76.6, 76.3, 74.7, 73.8, 73.6, 72.5, 69.1, 69.0, 68.0, 67.2, 61.8, 57.9, 56.9, 52.1, 36.5, 29.7, 21.0, 20.6, 20.4 ppm; HRMS (ESI-TOF): m/z calcd for $C_{38}H_{48}NO_{17}$ $[M+H]^+$ 790.2922, found: 790.2939.

4.7. Methyl (cyclohexyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*, 4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside) onate (24α)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.58 (d, J = 8.0 Hz, 1H), 5.40–5.42 (m, 1H), 4.56 (d, J = 9.2 Hz, 1H), 4.37 (dd, J = 12.2, 2.4 Hz, 1H), 4.06 (dd, J = 12.2, 6.8 Hz, 1H), 3.92–3.99 (m, 1H), 3.80 (s, 3H), 3.67–3.74 (m, 2H), 2.89 (dd, J = 11.9, 3.3 Hz, 1H, H-3eq), 2.50 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H), 1.94 (s, 1H), 1.16–1.70 (m, 10H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.0, 170.6, 170.1, 169.3 (C1, $J_{C1,H3ax}$ = 5.7 Hz), 153.8, 99.2, 75.24, 75.18, 71.9, 69.3, 63.1, 59.2, 52.7, 37.0, 34.9, 33.1, 25.4, 24.7, 24.3, 24.1, 21.2, 20.9, 20.8 ppm; HRMS (ESI-TOF): m/z calcd for $C_{25}H_{35}NO_{13}$ $[M+H]^+$ 558.2187, found: 558.2184.

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Supplementary data

Supplementary data (^{1}H NMR spectra of sialyl donors **D1**–**D4**, ^{1}H and ^{13}C NMR spectra of new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2014.02.006.

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