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How O-Substitution of Sialyl Donors Affects Their Stereoselectivity

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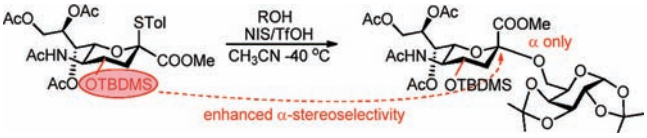
ABSTRACT



The profound effect of substituents at C-5 of glycosyl sialosides on their stereoselectivity is well-known although the exact nature of this effect is somewhat less understood. Presented herein is a comparative study of a range of novel sialyl donors with various O-substituents. It is demonstrated that O-substituents at C-4 and C-7 may also have a significant effect on the reactivity of sialyl donors and on the stereoselectivity of chemical sialylation.

Sialic acid containing glycoconjugates are natural products that, in the past two decades, have become extremely important synthetic targets, due to their direct involvement in numerous biological phenomena.[1,2] Unfortunately, the stereoselective synthesis of the glycosidic linkage between sialic acid and a glycoconjugate chain is still a major synthetic challenge. In fact, sialylation reactions are often plagued by low yields of the desired target (α-anomer) due, in part, to the low stereoselectivity of the reaction itself and the concomitant elimination reaction that is sometimes observed. Among the many sialyl donors that have been investigated, those modified at C-5 have probably been the most exploited.[3] From the first modifications as *N*-acetylacetamido and trifluoroacetamido by Boons et al.[4] to the azido modification of Wong et al.[5] and the oxazolidinone trans-fused ring and its N-acetyl derivative, introduced by Takahashi,[6,7] De Meo,[8,9] and Crich,[10,11] there has been a general belief that C-5 modifications can dramatically improve the synthesis of α-sialosides. Thus, we were extremely intrigued when we found a series of results with *N*-benzoylacetamido per-*O*-benzoylated sialyl donor **1a** reported by Ye et al.[12] that led to the unnatural *β*-anomer when coupled with glycosyl acceptors **2** and **3** (Figure 1 and Scheme 1). In our opinion, these results posed the question of whether it was the 5-*N*-benzoyl modification, or rather the presence of *O*-benzoyl groups (or a combination of both), that actually led to the unanticipated enhanced *β*-stereoselectivity (the use of *O*-benzoylated building blocks[13] is rather uncommon in sialic acid chemistry). It seems unlikely that the high *β*-stereoselectivity obtained with sialyl donor **1a** might be related to the use of CH_2Cl_2 as a replacement for the common stereocontrolling solvent acetonitrile

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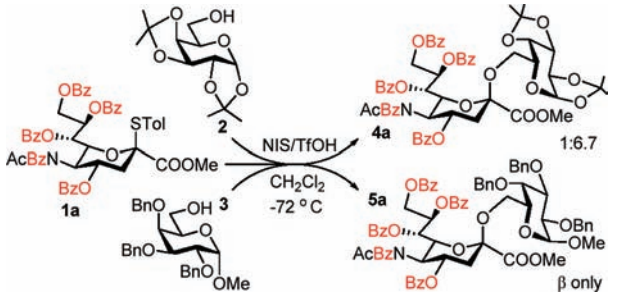
(Ye reported a lack of activation in acetonitrile). Recent sialylations have been successfully performed in several solvents, including dichloromethane.[10,14,15] To help understand the effect that N- or O-benzoyl groups may have on stereoselectivity, we synthesized two sialyl donors bearing mixed protecting group patterns: per-*O*-benzoylated sialyl donor **1b** and sialyl donor **1c** benzoylated only at N-5 (Figure 1; see the Supporting Information (SI) for the synthesis of all building blocks reported herein).

Scheme 1. Unusually High β-Stereoselectivity Obtained with Sialyl Donor **1a**, as Reported by Ye et al.[12]



The comparative testing of sialyl donors **1b**–**d** with glycosyl acceptors **2** and **3** was performed under the same experimental conditions as those reported by Ye for the glycosidation of **1a**, i.e. NIS/TfOH in dichloromethane at −72 °C (entries 1 and 2, Table 1). Per-*O*-benzoylated sialyl donor **1b** lacking the *N*-benzoyl substituent still showed a preference for β-stereoselectivity leading to the corresponding disaccharides **4b** (α/β = 1/1.4) and **5b** (α/β = 1/6.0, entries 3 and 4). On the other hand, N-5 benzoylated sialyl donor **1c** gave the corresponding disaccharides **4c** and **5c** with a shift toward α-stereoselectivity (α/β = 2.0/1 and 1.1/1, respectively, entries 5 and 6), although the overall stereoselectivity remained unimpressive. It is noteworthy that, as reported by Ye, the introduction of a benzoyl group at C-5 creates two rotamers around the C5–N bond. Hence, to achieve high resolution signals, all ^{1}H NMR spectra of *N*-benzoylated mono- and disaccharides had to be recorded at 80 °C in DMSO, whereas spectra recorded at −40 °C clearly showed two distinctive groups of signals corresponding to each rotamer.

To acquire comparison data, traditional sialyl donor **1d** was also coupled to acceptors **2** and **3** under the same reaction conditions. In our hands, a modest α-stereoselectivity for the synthesis of **4d** (α/β = 1.5/1) and a notable shift toward β-stereoselectivity for the synthesis of **5d** (α/β = 1/4.0) were noted (entries 7 and 8). It is noteworthy that while sialylations with benzoylated compounds **1b** and **1c** were completed within 20 min, sialylations with standard acetylated donor **1d** occurred at a much slower rate (6–9 h). In our opinion, the data summarized in Table 1 suggest that, while a concomitant per-*N*,*O*-benzoylation has a strong β-directing effect (donor **1a**), the major β-directing contribution is probably due to per-*O*-benzoylation as seen in the case of donor **1b**.

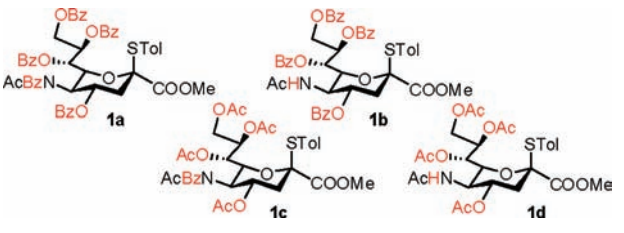
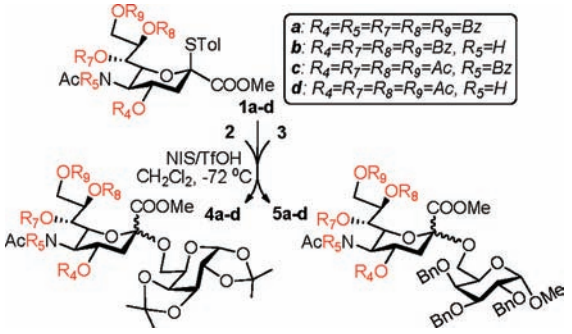


Figure 1. Sialyl donors **1a**–**d** for comparative study.

Table 1. Comparison of Sialyl Donors **1a**–**d** in Couplings with Glycosyl Acceptors **2** and **3**



entry[a]	donor	acceptor	product	yield[b]	α/β ratio[c]
1[12]	**1a**	**2**	**4a**	86%	1:6.7
2[12]	**1a**	**3**	**5a**[d]	90%	β only
3	**1b**	**2**	**4b**	63%	1:1.4
4	**1b**	**3**	**5b**	64%	1:6.0
5	**1c**	**2**	**4c**	62%	2.0:1
6	**1c**	**3**	**5c**	86%	1.1:1
7	**1d**	**2**	**4d**	96%	1.5:1
8	**1d**	**3**	**5d**	99%	1:4.0

[a] Reaction conditions: donor/acceptor ratio 1.5:1, NIS/TfOH, CH_2Cl_2, −72 ° C. [b] The anomeric stereochemistry was assigned based on the chemical shift of H-3eq. [c] The yield was determined after Sephadex LH-20 size exclusion chromatography. [d] Donor/acceptor ratio is 1:1.5.

In accordance with the initial goal of our investigation, this preliminary comparative study suggests that it is not the N-5 modification alone that is responsible for the high β-stereoselectivity observed in the coupling of **1a**. Instead, this initial study (*vide supra*) clearly shows that the impact of O-protection on the stereoselectivity of sialylations is also of very high significance. It should be noted that while the effect of O-protecting groups on the outcome of common glycosylation reactions is well-known,[16] to the best of our knowledge, no systematic study of O-modifications has yet been reported for sialyl donors. To begin a systematic study of this effect, we decided to focus on the O-4 and

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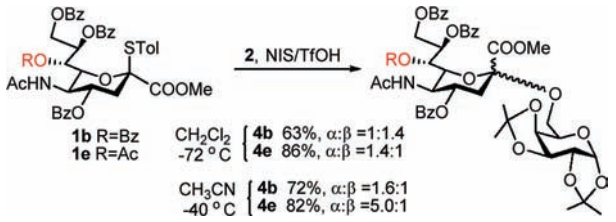
O-7 positions. Our rationale is based on the assumption of the existence of a conformational equilibrium of the oxacarbenium ion, with one all-axial conformation that places O-7 in close proximity to the anomeric center and an axial O-4 with a potentially relevant stereoelectronic effect. The existence of different conformations of the oxacarbenium ion for simpler heterocyclic systems has been described by Woerpel and others.[17−20] Although no extensive mechanistic studies have been reported for derivatives of sialic acid, the syntheses of 2,7-anhydro, 1,7-lactone, and 1,5-lactam derivatives[21−25] suggest the existence of a reaction intermediate conformation with an all-axial orientation of substituents.

Scheme 2. Comparison of Stereoselectivity Provided by Sialyl Donors **1b** and **1e**



To obtain a general and very preliminary working hypothesis on the nature of the effect of the O-7 position, we designed sialyl donor **1e** that has only one structural variant, the 7-*O*-acetyl group, in comparison to that of per-benzoylated sialyl donor **1b** (Scheme 2). Sialylation of **1e** with acceptor **2** in dichloromethane gave the corresponding disaccharide **4e** in 15 min. The anomeric ratio observed (α/β = 1.4/1) was a modest shift toward the α-anomer formation in comparison to the stereoselectivity obtained with sialyl donor **1b** (α/β = 1/1.4). However, a more dramatic enhancement in α-stereoselectivity was obtained in acetonitrile, in which disaccharide **4e** was obtained as a α/β = 5/1 anomeric ratio vs α/β = 1.6/1 for **4b**.

In our opinion, this set of results clearly indicates that the 7-O-protecting group alone may play a significant role in providing enhanced β-stereoselectivity obtained with per-*O*-benzoylated donors **1a** and **1b**. However, whether the nature of this effect shall be attributed to the increased steric bulk of benzoyl vs acetyl or the change in the electronic profile with the benzoyl substituent being marginally more electron-withdrawing than acetyl[26] remained unclear. To expand upon these findings we synthesized a range of new donors **1f**–**h** (Scheme 3), where we also included protection at O-4 with a bulky *tert*-butyldimethylsilyl (TBDMS) group. Strategically, we chose to introduce the 4-*O*-TBDMS group, taking into account the experimental convenience, to eliminate the possible effect of an electron-withdrawing substituent at O-4. Finally, since all sialyl donors of the new series are equipped with a 4-*O*-TBDMS group it would still allow for direct side-by-side comparison. However, direct comparison with the previously investigated series can be only made between **1d** and **1f**, which only differ by the pretecting group at O-4. The latter comparison would then allow insight into the effect of the substituent at O-4.

The initial set of coupling reactions of new sialyl donors **1f**–**h** with acceptor **2** was performed in dichloromethane at −72 °C. For the coupling of 7-*O*-acetylated **1f**, we observed a relatively long reaction time (3 h) along with the preference toward the formation of the α-anomer of **4f** (α/β = 2.8/1, Table 2, entry 1). Interestingly, 7-*O*-benzoylated donor **1g** showed a dramatic increase in the reactivity toward sialylation and also provided a further shift toward α-sialylation. Thus, disaccharide **4g** was obtained in only 15 min (α/β = 4.3/1, entry 2). Upon activation of 7-*O*-TBDMS protected sialyl donor **1h**, the slowest reaction time (5 h) within this series was recorded along with the bias toward β-linked disaccharide **4h** (α/β = 1/2.2, entry 3). Thus, upon activation of sialyl donor **1g**, mainly α-disaccharide **5g** was obtained in 10 min (α/β = 2.5/1, entry 5). Conversely, activation of donor **1h** resulted in the slow formation of **5h** with the predominance of the β-anomer (α/β = 1/2.3, entry 6). Interestingly, no reaction of **1f** and acceptor **3** took place (entry 4). This unusual behavior of **1f** may be related to the existence of a mismatched donor−acceptor pair[27] or a double stereodifferentiation effect.[28] This observation cannot be explained by the currently accepted monomolecular mechanism of sialylation[29] (the activation of a leaving group should not be influenced by the nature of the nucleophile) and clearly raises questions for future studies.

As mentioned earlier, the solvent effect in sialylations is known to be the most significant factor influencing the stereoselectivity (and often reactivity) of sialyl donors. Since it is commonly accepted that CH_3CN helps to obtain high α-stereoselectivity, a vast majority of chemical sialylations are performed using this solvent.[30,31] As reported by Ye et al., per-*N*,*O*-benzoylated sialyl donor **1a** cannot be activated in acetonitrile. Our further experimentation

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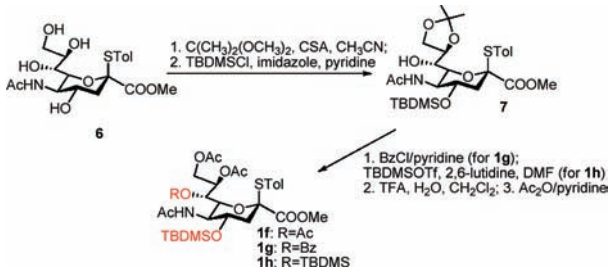
Scheme 3. Synthesis of a Series of 4-*O*-TBDMS-Protected Sialyl Donors **1f**–**h**



showed that per-*O*-benzoylated donor **1b** and *N*-benzoylated donor **1c** show similar reaction times and yields in either acetonitrile or CH_2Cl_2. In terms of α-stereoselectivity, significant enhancement was recorded for donor **1b** in acetonitrile, whereas almost no changes were detected in the case of donor **1c** (see the SI for additional experimental data). Hence, the investigation of whether CH_3CN can further impact the stereoselectivity of sialylation with novel sialyl donors **1f**–**h** appealed to us as an important subsequent step.

A very dramatic change in the sialylation outcome was observed in couplings of sialyl donor **1f** with acceptors **2** and **3** in CH_3CN vs CH_2Cl_2. Previously, disaccharide **4f** was obtained as an α/β = 2.8/1 mixture of anomers in CH_2Cl_2 (entry 1); herein, the α-anomer of **4f** was formed exclusively in CH_3CN (entry 7). The coupling of **1f** with glycosyl acceptor **3** now proceeded rapidly (recall no reaction in CH_2Cl_2, entry 4), and the resultant disaccharide **5f** was obtained in 10 min as a pure α-anomer (entry 11). These reactions stood out due to the exceptionally high stereoselectivity, even in comparison with the standard donor **1d** leading to disaccharide **4d** (α/β = 4.4/1, entry 8). This was a very clear indication that the substituents on O-4 have a very profound effect on sialylation. Results obtained with 7-*O*-benzoyl donor **1g** varied dramatically depending on the nature of the glycosyl acceptor. For the coupling of 7-*O*-benzoyl donor **1g**, while a negligible decrease in α-stereoselectivity was detected with acceptor **2** (compare entries 2 and 9), a 5-fold increase was noticed with acceptor **3** (compare entry 5 vs 12). In all cases of sialylation with 7-*O*-TBDMS donor **1h**, lower α-stereoselectivity, in comparison to that of the standard per-acetylated donor **1d**, was recorded (entries 10 and 13).

In conclusion, a range of novel sialyl donors with various O-substituents have been investigated. Our preliminary data suggests the following: (1) a bulky 7-*O*-benzoyl group alone (e.g., donor **1g**) cannot lead to preferential

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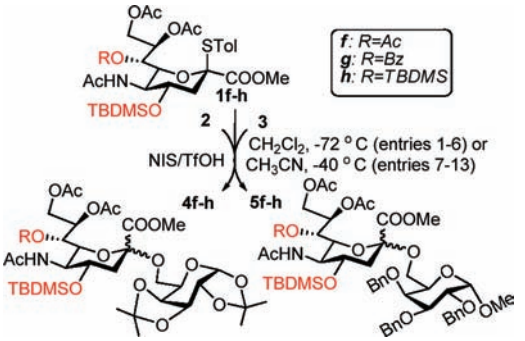
Table 2. Comparison of Sialyl Donors **1f**–**h** in Couplings with Acceptors **2** and **3** in CH_2Cl_2 and CH_3CN



entry	donor	acceptor	product	yield	α/β ratio
1	**1f**	**2**	**4f**	94%	2.8:1
2	**1g**	**2**	**4g**	94%	4.3:1
3	**1h**	**2**	**4h**	85%	1:2.2
4	**1f**	**3**	**5f**	-	-
5	**1g**	**3**	**5g**	68%	2.5:1
6	**1h**	**3**	**5h**	77%	1:2.3
7	**1f**	**2**	**4f**	79%	α only
8	**1d**	**2**	**4d**	84%	4.4:1
9	**1g**	**2**	**4g**	93%	3.1:1
10	**1h**	**2**	**4h**	85%	1.0:1
11	**1f**	**3**	**5f**	84%	α only
12	**1g**	**3**	**5g**	77%	12.9:1
13	**1h**	**3**	**5h**	75%	2.2:1

β-sialylation; (2) the influence of acetonitrile seems to be in direct correlation with the nature of the O-protecting groups, ranging from the inversion of the anomeric configuration for donors **1b** and **1h** and marginal effect on donor **1c** to complete α-stereoselectivity for donor **1g**; (3) the presence of a bulky substituent at C-4 can also exert a strong influence on the stereoselectivity of sialylations. In fact, the dramatic enhancement in α-stereoselectivity observed with sialyl donor **1f** is of high significance and may lead to the development of new methodologies for α-sialylation. To expand upon these findings and conduct a further search of activating and/or α-directing groups at C-4 and C-7, other studies are currently underway in our laboratories.

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Supporting Information Available. Experimental procedures, extended experimental data, ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.