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Influence of Side Chain Conformation and Configuration on Glycosyl Donor Reactivity and Selectivity as Illustrated by Sialic Acid Donors Epimeric at the 7-Position

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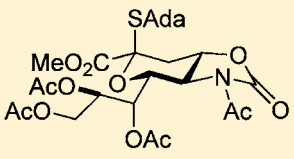
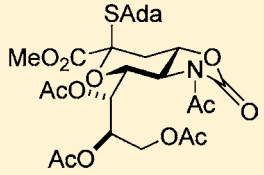
Supporting Information

ABSTRACT: Two *N*-acetyl 4*O*,5*N*-oxazolidinone-protected sialyl thioglycosides epimeric at the 7-position have been synthesized and their reactivity and stereoselectivity in glycosylation reactions have been compared. It is demonstrated that the natural 7*S*-donor is both more reactive and more α-selective than the unnatural 7*R*-isomer. The difference in reactivity is attributed to the side chain conformation and specifically to the proximity of O7 to the anomeric center. In the natural 7*S*-isomer, O7 is closer to the anomeric center than in its unnatural 7*R*-epimer and, therefore, better able to support incipient positive charge at the locus of reaction. The difference in selectivity is also attributed to the side conformation, which in the unnatural 7*R*-series is placed perpendicularly above the α-face of the donor and so shields it to a greater extent than in the 7*S*-series. These observations are consistent with earlier conclusions on the influence of the side chain conformation on reactivity and selectivity derived from conformationally locked models in the glucose and galactose series and corroborate the suggestion that those effects are predominantly stereoelectronic rather than torsional. The possible relevance of side chain conformation as a factor in the influence of glycosylation stereoselectivity by remote protecting groups and as a control element in enzymic processes for glycosidic bond formation and hydrolysis are discussed. Methods for assignment of the anomeric configuration in the sialic acid glycosides are critically surveyed.



Natural, 7*S*
more reactive,
more α-selective



Unnatural, 7*R*
less reactive,
less α-selective

INTRODUCTION

The development of efficient, stereocontrolled methods for glycosidic bond synthesis is a key element in the continued evolution of the field of chemical glycobiology.[1,2] The rational development of such methods necessarily depends on an understanding of the mechanisms of glycosylation and on the factors that influence them.[3−9] In this respect, the influence of side chain protecting groups and conformation on the reactivity and selectivity of glycosyl donors is an area of considerable current interest.[10] Interest in the role of side chain conformation stems from the discovery of Fraser-Reid and co-workers of the retarding effect of cyclic protecting groups on the reactivity of glycosyl donors, which they termed the torsionally disarming effect,[11,12] and from the critical role played by 4,6-*O*-benzylidene acetals in β-mannopyranosylation.[3,13−15] Bols and co-workers dissected the benzylidene effect into torsional and electronic components based on the relative rates of hydrolysis of a series of cyclic 6-methoxy-7-carba-glucopyranoside probes (Figure 1a),[16] reaching the conclusion that the major part of the effect is electronic and arises from the locking of the C5−C6 bond in the *tg* (*trans*−*gauche*) conformation[17,18] (Figure 2a). Subsequently, however, the Bols laboratory working in the mannopyranosyl series with related cyclic 7-carba analogs lacking the additional methoxy group suggested that the torsional component plays a larger role in the benzylidene effect than was originally thought.[19] In our laboratory, we prepared a series of *cis*-fused 6-methoxy-7-carba-galactopyranoside probes, and determined their relative rates of hydrolysis by the Bols protocol. We found the conformer in which the 6-methoxy group is antiperiplanar to the C5−O5 to be the least reactive (Figure 1b) consistent with the earlier work in the *trans*-fused gluco-system.[20] Taking into account the additional torsional interactions introduced by the replacement of a $-O-CH_2-$ moiety in a benzylidene ring by a $-CH_2-CH(OCH_3)-$ group (Figure 1a,b) or even by a simple $-CH_2CH_2-$ group,[19] we concluded that the Bols-type probes overestimate the torsional component of the disarming effect of the fused ring and, therefore, that maximization of the electron-withdrawing effect of the C6−O6 bond is of the greatest importance.[20] This conclusion is re-enforced by our earlier studies with a series of 6-fluoro-, 6,6-difluoro-, and 6,6,6-trifluororhamnopyranosyl donors in which it was found that the decomposition temperature and β-selectivity of the intermediate rhamnosyl triflates increased with increasing fluorine content, i.e., that donor reactivity is related inversely to the electron-withdrawing ability of substituents at the 6-position.[21]

Continuing our studies on the effect of side chain conformation on glycosyl donor reactivity, we have now turned our attention to a comparison of the reactivity and selectivity of sialic acid donors and their 7-epi-isomers (Figure 3). This study

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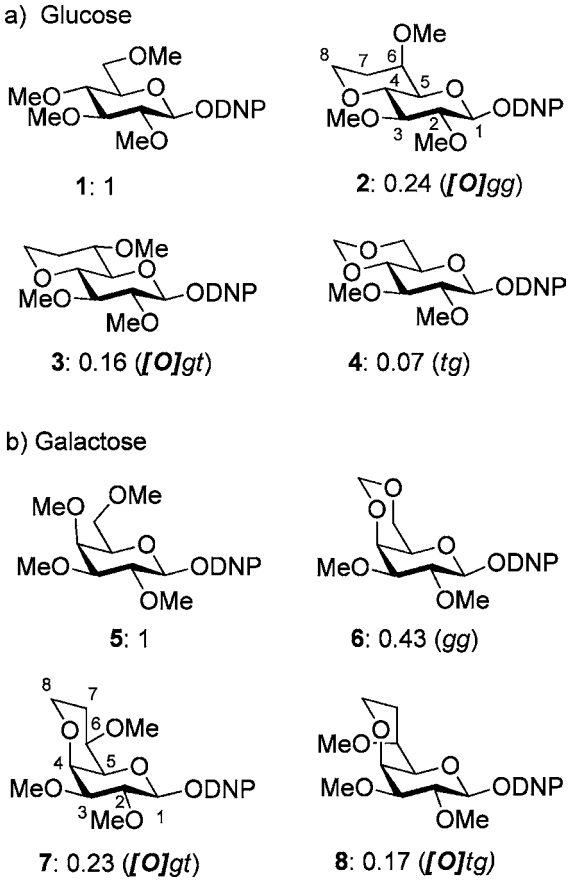

Figure 1. Relative rates of hydrolysis of 2,4-dinitrophenyl glycosides as a function of conformation of the C6−O6 bond, and numbering convention for the 7-carba-sugars.

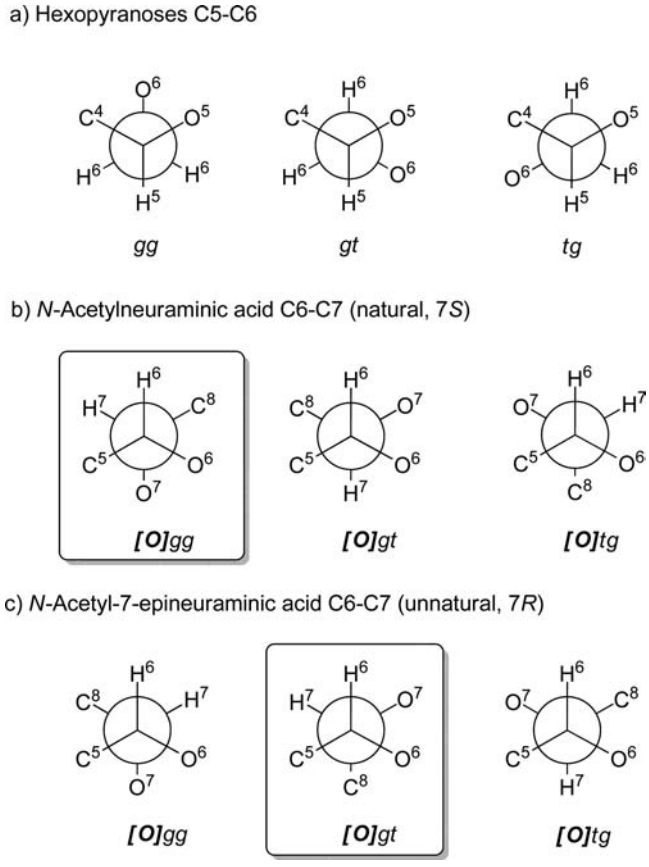

Figure 2. Staggered conformations about the exocyclic bond in the hexopyranoses (C5−C6 bond), the *N*-acetylneuraminic acids (C6−C7 bond), and the *N*-acetyl-7-epineuraminic acids (C6−C7 bond) with preferred conformations of the latter two boxed.

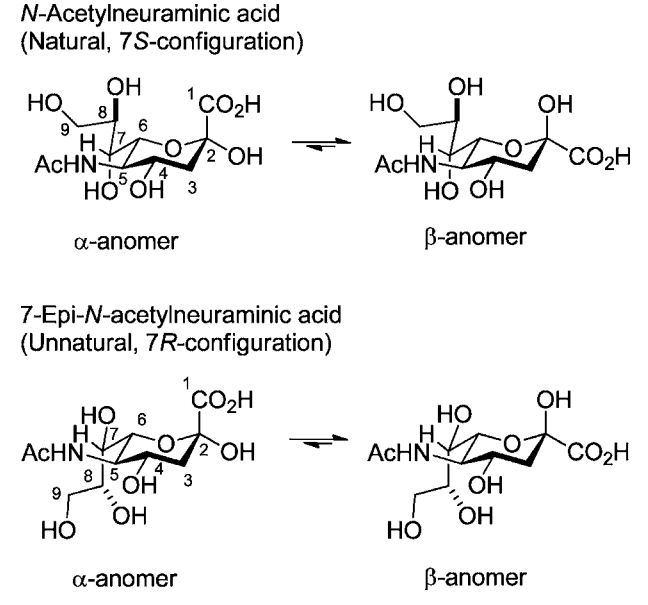

Figure 3. *N*-Acetylneuraminic acid and *N*-acetyl-7-epineuraminic acid with predominant side chain conformations.

is based on the prediction that the two isomers will exhibit different predominant side chain conformations and so different reactivities and stereoselectivities. In so far as this study does not make use of substrates with artificially restricted side chain conformations, it obviates the problem of additional torsional interaction that characterizes the Bols-type system. To exclude any possibility that donors of different side chain configuration might react via different conformations of the pyranose ring, we elected to carry out this study with the rigid *N*-acetyl-4*O*,5*N*-oxazolidinone-protected donors[22,23] which retain the $^2C_5$ conformation in the crystal and in solution[22,24] and which, along with the *N*-desacetyl analogs,[25,26] have proven to be some of the most selective sialyl donors available for the synthesis of *O*-,[27−37] *C*-,[38,39] and *S*-sialosides.[40] The sensing of the side chain conformation and configuration in the sialic acid series by substituents at the anomeric position follows from the anomeric configurational dependence in ^{1}H NMR spectra of (i) the $^3J_{H7,H8}$ coupling constant,[41,42] and (ii) the H_{9a},H_{9b} chemical shift difference,[42] both of which have been applied as methods for the determination of anomeric configuration. Knowledge of the influence of side chain conformation and configuration on anomeric reactivity will ultimately contribute to our mechanistic understanding of glycosylation and so to the design of improved systems.

RESULTS

We begin with a definition of the staggered conformations about the exocyclic side bonds employed in this Article. With respect to the hydroxymethyl groups of the hexopyranoses, the conformation is described by the stereochemical relationship (*gauche*, or *trans*) of O6 to (i) the ring oxygen, and (ii) to C4 (Figure 2a).[17,18] Thus, for example, the methylidene protected

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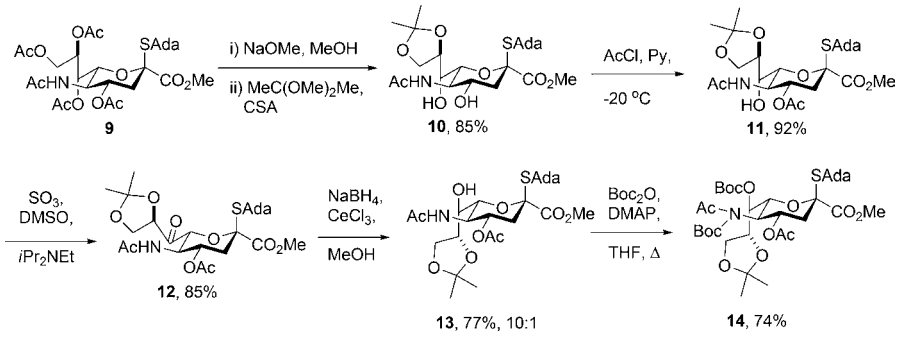
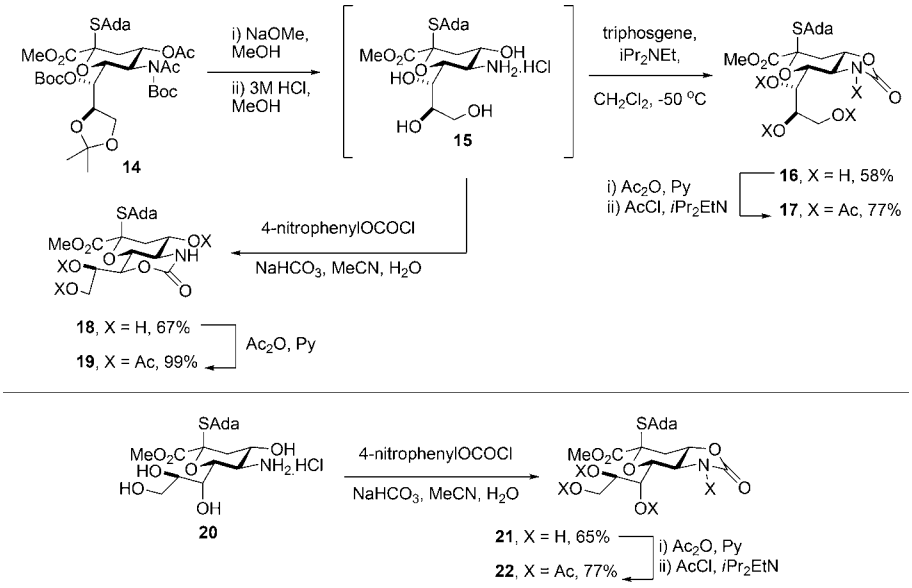
Scheme 1. Synthesis of an Advanced 7-Epineuraminic Acid 1-Adamantanyl Thioglycoside



Scheme 2. Formation of Oxazolidinone and Oxazinone Derivatives



glucose derivative **4** is described as having the *tg* conformer of its "hydroxymethyl" group (Figure 2a). In the case of the higher carbon sugars, as noted by Grindley for the sialic acids,[18] formally the definition of the conformation of the exocyclic bond is based on the stereochemical relationship of the next carbon along the chain to (i) the ring oxygen, and (ii) to C4 (C5 in the sialic acids). According to this definition, compounds **2** and **3** (Figure 2a) are formally both *tg* conformers. For consistency, and to highlight the importance of the C−O bond at the exocyclic position, all conformations in this paper are defined by the relationship of the C−O bond at the exocyclic position to (i) the ring oxygen, and (ii) to the ring carbon (C4 in the hexoses and C5 in the neuraminic acids) as was done in the earlier papers by Bols and ourselves.[16,19,20] To indicate structures in which a side chain oxygen atom has been used as reference atom rather than a formally more correct side chain carbon, the conformational descriptors have been prefaced by the term *[O]*.

Synthesis of a 7-episialyl donor began with the known adamantanyl thiosialoside **9**[23] and proceeded through Zemplen deacetylation, and standard installation of an 8,9-*O*-acetonide giving **10**.[43] Adapting literature methods,[43−45] reaction of diol **10** with acetyl chloride at −20 °C enabled the selective esterification of the less hindered 4-OH giving **11**, of which Parikh-Doering oxidation[46] then afforded the 7-keto sialyl donor **12**. Selective reduction of **12** with Luche's reagent[47] provided the 7-epi sialyl donor **13** in 77% yield and 10:1 selectivity, and this was followed by treatment with Boc_2O in the presence of DMAP in hot THF to give the *N*,*O*-di-Boc-derivative **14** (Scheme 1).

Zemplen deacetylation of **14** followed by treatment with 3 M HCl in methanol afforded the 7-epi neuraminate **15**, which was immediately converted into the 4*O*,5*N*-oxazolidinone **16** by treatment with triphosgene in the presence of Hünig's base at −50 °C. We note in passing that the more conventional use of trifluoroactic acid/water,[23] or 2 M HCl in ether,[48] or more dilute solutions of HCl in methanol for the removal of the Boc groups from **14** were complicated by formation of the oxazinone **18** alongside the desired **15**, which we interpret as being due to the rapid cleavage of the *N*-Boc group with subsequent cyclization onto the remaining *O*-Boc moiety. Acetylation of **16** with acetic anhydride in pyridine and then with acetyl chloride and Hünig's base finally gave the requisite donor **17** (Scheme 2). With the reaction of **15** with 4-nitrophenyl chloroformate and sodium bicarbonate in aqueous acetonitrile, the regioisomeric 5*N*,7*O*-oxazinone **18** was obtained in 67% yield (Scheme 2); subsequent acetylation afforded the donor **19**. The formation of the oxazinone **18** provides a first indication of the influence of stereochemistry at C7 on general reactivity in this series, as application of the same protocol to the analogue **20** with the natural stereochemistry reliably affords the 4*O*,5*N*-oxazolidinone **21**, two stage peracetylation of which gives the donor **22** as described previously (Scheme 2).[23]

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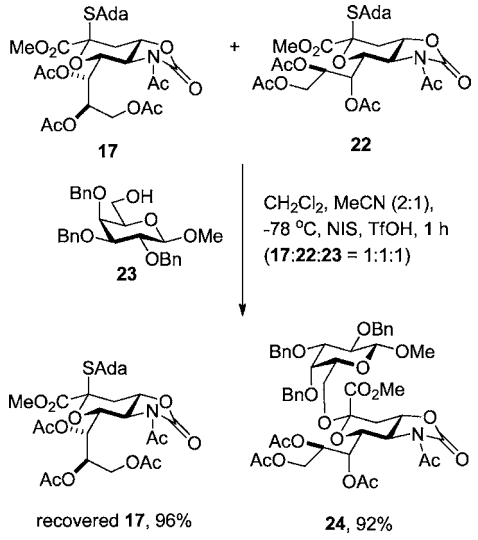
With the stereoisomeric donors **17** and **22** in hand, we began by comparing their reactivity toward activation with the *N*-iodosuccinimide/triflic anhydride combination. To this end, a 1:1 mixture of **17** and **22** was activated with NIS/TfOH at −78 °C in a 2:1 mixture of dichloromethane and acetonitrile and 3 Å acid-washed molecular sieves in the presence of the acceptor **23** with quenching at −78 °C after 1 h by the addition of triethylamine (Scheme 3). Examination of the crude reaction

Scheme 3. Competitive Reaction of Epimeric Donors 17 and 22 at −78 °C



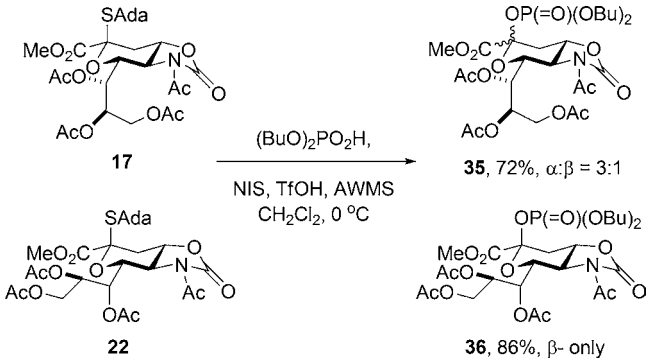
mixture by HPLC and ^{1}H NMR spectroscopy revealed clean preferential activation of the natural 7*S*-isomer **22** over the inverted 7*R*-epimer **17**. Consistent with this observation, unreacted **17** was recovered from the reaction mixture in 96% yield, while the glycoside **24** was isolated in 92% yield as a single α-anomer in agreement with the original report on the coupling of **17** and **23** under comparable conditions.[23] This experiment clearly demonstrates the influence of side chain configuration (and conformation) on anomeric reactivity.

Subsequent experimentation revealed a temperature of around −60 °C to be the lowest at which donor **17** was activated in a reasonable time frame by the NIS/TfOH combination in the 2:1 CH_2Cl_2/acetonitrile solvent mixture in the presence of acid washed molecular sieves. Accordingly, with a view to comparing the stereoselectivity of the epimers **17** and **22**, the two donors were coupled separately with acceptors **23**, **25**, **26**, and **27** under a standard set of conditions giving rise to the products **24** and **28**−**34** presented in Table 1.

With donor **22** bearing the natural 7*S*-configuration, all couplings were α-selective (Table 1) albeit and expectedly less so than for comparable reactions[23] previously conducted at −78 °C. With the epimeric 7*R*-isomer **17**, coupling to the relatively unhindered primary carbohydrate acceptor **23** and to 1-octanol **27** (Table 1, entries 1 and 4) was α-selective, albeit to smaller extent than with the natural 7*S*-configured donor **22**. With the two secondary alcohol acceptors studied, couplings to the unnatural 7*R*-configured donor were moderately β-selective (Table 1, entries 2 and 3). Therefore, the 7*R*-configured donor **17** is less α-selective than its 7*S*-isomer **22**, and with the less reactive alcohols, this selectivity is even inverted to a modest preference for β-selectivity. When the galactose-3,4-diol **25** was employed as acceptor, both couplings took place through the less sterically hindered 3-OH, as is well-known in the field,[49−51] giving rise to the α,β-(2→3)-configured disaccharides **28** and **32**.

Returning to the question of relative reactivity, we prepared and isolated the anomeric sialyl phosphates **35** and **36** from **17** and **22**, respectively, following the method of Wong and co-workers (Scheme 4).[28] Again, a difference in selectivity was observed with the unnatural 7*R*-isomer **22** affording a 3:1 α:β-mixture of phosphates **35**, while the 7*S*-epimer gave a single β-product **36**.

Scheme 4. Preparation of Sialyl Phosphates 35 and 36



Phosphates **35** and **36** were then examined by ESI mass spectrometry when, using the standard cone voltage of 40 V, they both showed clean sodiated molecular ions and the absence of fragmentation. The ESI mass spectra were then recorded at increasing cone voltages until the onset of fragmentation as determined by the observation of a daughter ion resulting from the loss of dibutylphosphoric acid. With the natural isomer **36**, fragmentation was detectable at a cone voltage of 85 V, consistent with the literature,[52] whereas the unnatural required isomer **35** required a cone voltage of 98 V before fragmentation was observed. While it is the case that the energies required for mass spectral fragmentation of glycosides are dependent on anomeric configuration,[53] this experiment clearly demonstrates that both anomers of **35** are considerably less susceptible to decomposition in this manner than the β-isomer of **36**, which is considered to be the most stable anomer in view of previous observations on the equilibration of sialyl glycosides.

With the 7*R*-oxazinone **19** in hand, we briefly examined conditions for its activation and its selectivity in a glycosylation reaction. Consistent with earlier work from our laboratory with the analogous 7*S*-configured oxazinone **37**,[54] this donor was substantially less reactive than the oxazolidinones. For this reason, as in the previous work with **37**, we employed the potent diphenyl sulfoxide/triflic anhydride combination[55] for activation. With the use of 1-adamantanol as acceptor, the glycoside **38** was obtained in 27% yield as a single α-anomer along with 70% of the recovered donor (Scheme 5). This selectivity of this coupling is the opposite of that observed previously with **37** when the β-glycoside **39** was the sole product (Scheme 5), and indeed with that observed by Tanaka and co-workers with the closer 7*S*-analog **40**.[56]

The side chain conformation of thioglycosides **17** and **22**, as well as of a number of *O*-glycosides in both the natural and 7-epi series, was examined by NMR spectroscopy. The NOE contacts and the $^3J_{6,7}$ coupling employed to this end along with the predominant conformation about the exocyclic C6−C7 bond for each substance studied are listed in Table 2. In the unnatural 7*R*-configured oxazolidinone-protected series, strong NOE inter-

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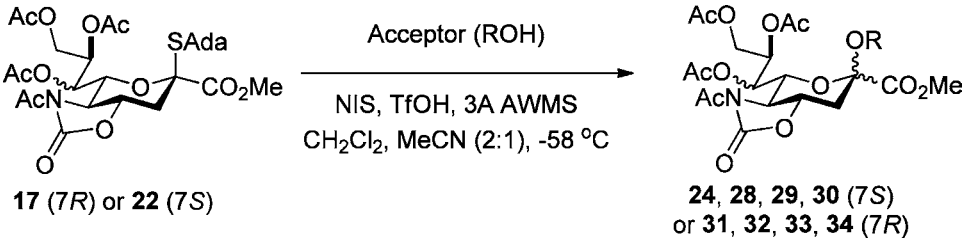
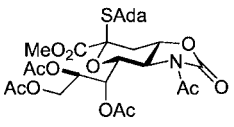
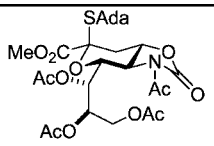
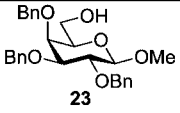
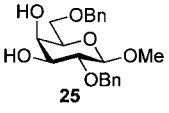
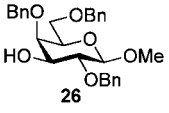
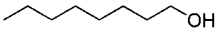
Table 1. Glycosylation Stereoselectivity as a Function of Donor Side Chain Configuration

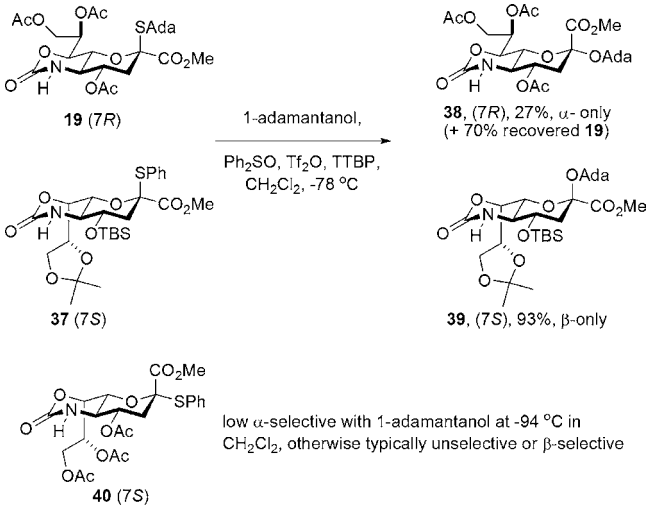



22, 7S, natural


17, 7R, unnatural

Entry	ROH	Cmpd, yield	α:β ratio	Cmpd, yield	α:β ratio
1	 23	**24**, 89%	α- only	**31**, 74%	4:1
2	 25	**28**, 82%	2.5:1	**32**, 67%	1:1.4
3	 26	**29**, 76%	1.6:1	**33**, 83%	1:3
4	 27	**30**, 85%	α-only	**34**, 76%	10:1

Scheme 5. Reactivity and Selectivity of the Oxazinone Protected Donors 19, 37,[54] and 40[56]



actions between the axial ring hydrogen H5 and the side chain hydrogen H8, together with $^3J_{6,7}$ coupling constants between 2.4 and 3.6 Hz, consistent with earlier observations by Bandgar and Zbiral on simple peracetylated sialosides,[44] indicate that, with two exceptions, an approximate *[O]gt* conformation about the C6−C7 bond predominates in the unnatural series for both the *α*- and *β*-glycosides (Table 2). Furthermore, the assignment of the *[O]gt* side chain conformation to the protected 7-episialosides is consistent with the observations of Zbiral and co-workers on the side chain conformation of 7-epi-*N*-acetyl neuraminic acid itself.[57] Accordingly, we represent the side of these substances in the *[O]gt* conformation in this Article. The two exceptions to this rule are the *α*-glycosides **32** and **33** of secondary alcohols, when the H8−H5 NOE interaction, while still present, is noticeably weaker and the $^3J_{6,7}$ coupling constants much larger at 7.2 Hz, suggesting an important contribution from the *[O]tg* conformation. In the natural 7*S*-series, all oxazolidinone protected glycosides examined (Table 2), including the *α*-glycoside of a secondary alcohol **29*α***, showed a strong NOE correlation between the axial ring hydrogen H6 and the side chain hydrogen H8. Together with their observed $^3J_{6,7}$ coupling constants of 1.5−2.4 Hz, this suggests a predominant *[O]gg* conformation as is typically found in the *N*-acetyl neuraminic acid series.[57−62]

DISCUSSION

It is clear from the comparison of the reactivity of conformationally locked donors **17** and **22** under standard glycosylation conditions, as well as from the differing minimum cone voltages

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Table 2. Diagnostic NOE Contacts and Scalar Coupling Constants for Determination of the Predominant C6−C7 Conformation

cmpd	C7 config.	key NOE contacts	$^3J_{6,7}$ (Hz)	predominant C6−C7 conform.
17*β*	*R*	H8−H5; H7−H5	2.4	*[O]gt*
22*β*	*S*	H8−H6; H7−H5	2.4	*[O]gg*
27*α*	*S*	H8−H6, H7−H5, H8-gal H6	1.5	*[O]gg*
28*α*	*S*	H8−H6, H7−H5, H8-gal H4	1.5	*[O]gg*
28*β*	*S*	H8−H6, H7−H5	2.5	*[O]gg*
29*α*	*S*	H8−H6, H8-gal H4	1.5	*[O]gg*
29*β*	*S*	H8−H6, H7−H5	1.5	*[O]gg*
31*α*	*R*	H8−H5, H7−H5	3.6	*[O]gt*
31*β*	*R*	H8−H5, H7−H5	3.0	*[O]gt*
32*α*	*R*	H8−H5, H8−H6, H8-gal H3 and 4, H7−H5, H7-ArH[a]	7.2	*[O]gt* ↔ *[O]tg*
32*β*	*R*	H8−H5, H7−H5	3.0	*[O]gt*
33*α*	*R*	H8−H5, H8−H6, H8-gal H3 and 4, H7−H5, H7-ArH[a]	7.2	*[O]gt* ↔ *[O]tg*
33*β*	*R*	H8−H5, H7−H5	3.6	*[O]gt*

[a]Unassigned *o*-doublet of a benzyl group on the aglycone.

required to promote fragmentation of the isomeric glycosyl phosphates **35** and **36**, that the stereochemical configuration at C7 in the *N*-acetylneuraminic acid series affects anomeric reactivity. As the glycosylation reactions, whether they proceed through associative S_N2-like or dissociative S_N1-like pathways, and the mass spectral fragmentations, whether stepwise or concerted,[52] proceed with an initial build-up of positive charge at the anomeric position, this influence on reactivity must correlate with the ability of the donor to support nascent positive charge at the anomeric carbon. In the more reactive natural series, with the 7*S*-configuration, the *[O]gg* conformation predominates in all systems studied (Table 2), whereas in the less reactive unnatural 7*R*-configured systems (Table 2), the *[O]gt* conformation predominates with the exception of the two hindered *α*-glycosides **32*α*** and **33*α*** to which we return later. The greater reactivity of the system predominantly adopting the *[O]gg* over the isomeric system with its predominant *[O]gt* conformation is fully consistent with the findings of Bols and workers[16] and of our laboratory[20] on the relative reactivities of conformationally locked gluco- and galactopyranosyl donors (Figure 1). Making the reasonable assumption that the side chains predominantly adopt closely related conformations in any $^4H_5$ half chair-like oxocarbenium ions as they do in both the *α*- and *β*-glycosides, the greater reactivity of the natural system with its *[O]gg* conformation is best accounted for by the ability of O7 to stabilize positive charge on the anomeric carbon through space by virtue of the periplanar alignment of the C7−O7 bond and the vacant p-orbital on C2 (Figure 4a). Conversely, in the unnatural 7*R* system with its predominant *[O]gt* conformation, the C7−O7 bond is inappropriately located to stabilize the *π*-system of any anomeric oxocarbenium ion (Figure 4b). Parallel arguments apply to reactions proceeding through S_N2-like mechanisms with the *[O]gg* conformation better placed for stabilization of the partial bonds at the transition state by the C7−O7 bond.

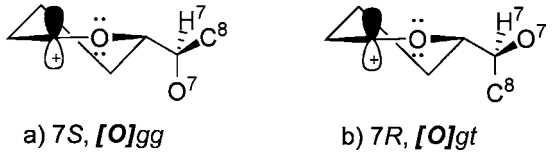

Figure 4. Relationship of the C7−O7 bond to the $^4H_5$ conformation of the sialyl oxocarbenium ion in (a) the natural 7*S* series with the *[O]gg* conformation, and (b) the unnatural 7*R*-series with the *[O]gt* conformation. All other substituents are omitted for clarity.

Alternatively, O7 is spatially closer to the point charge (or partial point charge) of the anomeric carbenium ion in the *[O]gg* conformer than that in the *[O]gt* conformer and so better able to provide electrostatic stabilization following the models of Deslongchamps,[63] Woods,[64] Woerpel,[65] and Bols.[66,67] The overall situation is consistent with the result of DFT computations by Yang and Woerpel (Figure 5) according to

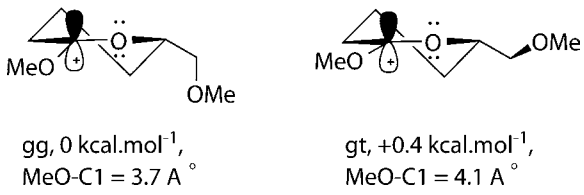

Figure 5. Relative energies (B3YLP/6-31G*) and O−C1 distances of the *gg* and *gt* conformers of the 1-methoxy-5-(methoxymethyl)-tetrahydropyranyl cation according to Yang and Woerpel.

which the *gg* conformation of the 1-methoxy-5-(methoxymethyl)tetrahydropyranosyl cation is some 0.4 kcal·mol^{-1} lower in energy than the corresponding *gt* conformation.[68] Self-evidently, this rationalization only applies to the $^4H_5$ or closely related conformation which is imposed on the system by the *trans*-fused oxazolidinone ring in the systems under study.

While we have not conducted a thorough study of the oxazinone-protected donors, we previously noted[54] the lack of reactivity of the 7*S*-configured **37** under the NIS/TfOH conditions (Scheme 5) which forced us to employ the more potent diphenyl sulfoxide/Tf_2O activating system. Parallel observations were made by Tanaka and co-workers with the 7*S*-configured donor **40** (Scheme 5).[56] As reported here, the unnatural 7*R*-configured donor **19** is relatively unreactive even with activation by diphenyl sulfoxide/Tf_2O (Scheme 5). The main difference between the oxazinone-protected systems **19**, **37**, and **38**, and the oxazolidinone-protected ones **17** and **22** is the imposition of the *[O]tg* conformer in the oxazinones which leads to a reduction in activity consistent with the earlier studies on conformationally locked gluco and galactopyranosyl donors (Figure 1).[16,20] We refrain from interpreting differences in reactivity between the oxazinones **19**, **37**, and **38** because of the inconsistencies in the thioglycosides employed, in their anomeric stereochemistry, and in the side chain protecting groups all of which influence anomeric reactivity.[3−9]

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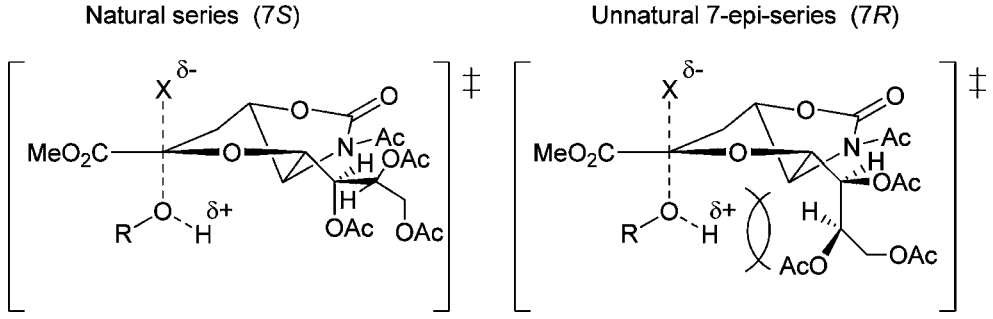

Figure 6. Differential Steric Interactions in α-face attack on the natural (7*S*) and unnatural (7*R*) donors.

Turning to the influence of side chain configuration on anomeric stereoselectivity, all glycosylation reactions with the natural 7*S*-configured donor **22** were α-selective (Table 1) consistent with the broad use of this class of oxazolidinone-protected donor in the literature.[22−36] When hindered secondary acceptors were employed, the selectivity was lower than previously reported,[23] a fact that we attribute to the higher reaction temperatures necessitated by the comparative studies. As we have discussed previously,[52] the high levels of α-selectivity observed with donor **22** are best accommodated by associative transition states proceeding with inversion of configuration at the anomeric center, even if the exact nature of the leaving group has yet to be pinpointed. Associative transition states are favored by the oxazolidinone-protected class of sialyl donors because of the strong electron-withdrawing nature of the oxazolidinone whose dipole is in the mean plane of the pyranose ring and strongly destabilizes positive charge at the anomeric center.[52] As noted above, the unnatural 7*R*-configured donor **17** is less reactive than **22**, which is a consequence of the conformation of its side chain and the reduced stabilization afforded to positive charge at the anomeric position by O7. Donor **17** is, however, less selective than donor **22** even if its relative reactivity suggests a higher degree of association in its anomeric substitution reactions. This reduced selectivity in the unnatural series is apparently a function of the additional shielding of the α-face by the predominant *[O] gt* conformation of the side chain, which retards approach of the acceptor from that face and so promotes either a competing β-selective associative mechanism or a more dissociative process. The additional shielding of the α-face in the unnatural 7*R*-series is illustrated in (Figure 6) for an associative displacement of an unspecified leaving group; similar interactions can be expected to be present in the product forming step of a dissociative substitution.

The steric origin of the reduced selectivity of donor **17** is most apparent with the secondary alcohol acceptors when the β-anomeric products **32*β*** and **33*β*** are favored (Table 1, entries 2 and 3). It is pertinent in this regard that the minor anomers **32*α*** and **33*α*** are the only compounds in the unnatural 7*R*-series to display a different side chain conformation to the otherwise predominant *[O]gt* arrangement. This change in side chain conformation presumably arises because of a more highly developed form of the steric clash between C8 in the *[O]gt* conformation and the aglycone that is the cause of the reduced selectivity in this series. Conversely, in the oxazinone series, the unnatural 7*R*-configured donor **19** is more α-selective than comparable 7*S*-configured systems **37** and **40** (Scheme 5). Consistent with the above rationale for the greater α-selectivity of **22** over **17**, this is clearly the result of the axial side chain in **37** and **40** being located directly above the α-face of the system.

As the side chain conformations of both the natural 7*S* and unnatural 7*R* *N*-acetyloxazolidinone protected sialosides found in this work correspond to those found[57−62] in simpler systems lacking the cyclic protecting group, we discount the possibility that the oxazolidinone *N*-acetyl group interferes with the side chain conformation of either epimer to any significant extent. As illustrated in Figure 6, both systems adopt conformations in which the C7−H7 bond is oriented toward the oxazolidinone *N*-acetyl group, thereby minimizing steric interactions with it.

The anomeric configuration of all compounds prepared in this study was determined primarily by the measurement of the $^3J_{C,H}$ coupling constant between the anomeric carboxylate carbon and the axial H3 (Tables 3 and 4, Supporting Information), which provided the $^2C_5$ chair conformation pertains is diagnostic,[69−72] and are supported by consistent NOE patterns such as those laid out in Table 2. In the natural 7*S*-series, $^3J_{C1,H3ax}$ for the α-glycosides was consistently found in the range 5.0−6.3 Hz, while the corresponding β-glycosides showed $^3J_{C1,H3ax}$ = 0, in full agreement with the literature.[69−72] On the other hand, in the unnatural 7*R*-series of compounds, while the α-glycosides displayed a similar range of $^3J_{C1,H3ax}$ (4.7−6.5 Hz) to the natural series, the β-isomers deviated significantly from the norm and were found in the range 0−3.8 Hz (Tables 1 and 2, Supporting Information). This deviation from the 0 Hz for the $^3J_{C1,H3ax}$ heteronuclear coupling constant in the 7-epi-β-sialosides is not a consequence of a significant distortion of the $MeO_2C-C2-C3-H3ax$ dihedral angle from the ideal 60° (i.e., of a change in ring conformation) as the $^3J_{H3ax,H4}$, $^3J_{H4,H5}$, and $^3J_{H5,H6}$ homonuclear coupling constants indicate the antiperiplanar nature of those hydrogens in all cases. Rather, we consider this deviation from the standard[69−72] pattern in the case of the 7-epi-β-glycosides to be a consequence of the influence of the *[O]gt* side chain conformation, either directly on the electron density of the $MeO_2C-C2-C3-H3ax$ spin system or indirectly by its influence on the glycosidic torsion angle, which in turn modulates electron density on the $MeO_2C-C2-C3-H3ax$ spin system. Generally, we are of the opinion that other systems[73] for the assignment of configuration in the sialic acid glycosides based on chemical shifts of specific resonances and/or chemicals shift differences of pairs of resonances (δH3eq,[74] δH4,[41,42] δH7,[41,75] and δH8,[41,75]) are unreliable even in the natural series, particularly when only one anomer is available, as they are somewhat dependent on the nature of the aglycone (Tables 1 and 2, Supporting Information). For example, compounds **28*α*** and **28*β*** (in the natural series for which the rules are formulated) display δH4 of 3.95 and 4.55, respectively, while glycosides **29*α*** and **29*β*** (also in the natural series) have δH4 of 4.11 and 3.45, respectively, that is, with the inverted order. As the only difference between glycosides **28*α*,*β*** and **29*α*,*β*** is the presence (**28**) or absence (**29**) of a benzyl ether on O4 of the acceptor, this

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reversal of chemical shift order underlines the influence of the aglycone and even its protecting groups on chemical shift patterns within the glycoside. Methods for the assignment of anomeric configuration based on side chain coupling constants ($^3J_{7,8}$ coupling constant[41,42]) and chemical shift differences between pairs of side chain resonances ($\Delta\delta$ H9a and H9b[42,75]) cannot be transposed from the natural (7*S*) to the unnatural (7*R*) series (Tables 1 and 2, Supporting Information) because of the change in side chain conformation.

CONCLUSION

The stereochemical configuration at C7 in the oxazolidinone-protected sialyl donors influences both reactivity and stereoselectivity, as a consequence of the differing conformations of the side chains. In the natural 7*S*-series, the C6−C7 bond predominantly adopts the *[O]gg* conformation, which confers a greater reactivity on the donor than the predominant *[O]gt* conformation found in the unnatural 7*R*-series. This is a consequence of the increased through space stabilization of positive charge at the anomeric center in the *[O]gg* conformation. The *[O]gt* side chain conformation found in the unnatural series affords greater steric shielding to the α-face of the donor resulting in lower stereoselectivity in its coupling reactions. These observations are consistent with earlier conclusions on the influence of the side chain conformation of reactivity and selectivity derived from conformationally locked models in the glucose[16] and galactose[20] series and corroborate the suggestion that those effects are predominantly stereoelectronic rather than torsional.[16,20] It follows from these arguments that the use of atypical protecting group systems in sialyl donors, such as the recent use of silyl ethers at O4 and/or O7,[76] may influence anomeric selectivity through the modulation of side chain conformation as well as more directly through their arming or disarming nature. It also follows, however, that care must be taken in the assignment of anomeric configuration in such systems because standard empirical rules do not necessarily transpose to the new system;[41,42,73−75] the only reliable method in our opinion being the magnitude of the $^3J_{C,H}$ heteronuclear coupling constant between the anomeric carboxylate carbon and the axial H3 for compounds with established chair conformations.

Although the results presented in this Article have been developed through the use of *N*-acetyl neuraminic acid derivatives and their 7-epimers, the conclusions on the influence of the side chain configuration and conformation on glycosyl donor reactivity and selectivity are likely to have broader implications, not the least of which is in the formation of the legionaminic and pseudaminic acid (Figure 7) glycosides found in various pathogenic bacteria.[77−81] Furthermore, insofar as the side chain conformation of glycosyl donors might be influenced by protecting groups at other positions around the pyranose ring, for steric and or stereoelectronic reasons, it appears likely that side conformation has a role to play in the continuing debate on the influence of remote protecting groups on glycosylation reactions.[10,82−85] Finally, it is interesting to speculate that Nature may modulate the activity of enzymes involved in glycosidic bond formation and/or hydrolysis through the evolution of binding sites tailored to lock the side chain in suitable conformations.

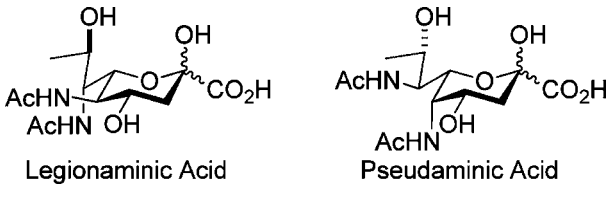

Figure 7. Structures of legionaminic and pseudaminic acid.

ASSOCIATED CONTENT

Supporting Information

Full experimental details and copies of ^{1}H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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