JOC The Journal of Organic Chemistry

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Stereoselective Synthesis of 5-*epi*-α-Sialosides Related to the Pseudaminic Acid Glycosides. Reassessment of the Stereoselectivity of the 5-Azido-5-deacetamidosialyl Thioglycosides and Use of Triflate as Nucleophile in the Zbiral Deamination of Sialic Acids

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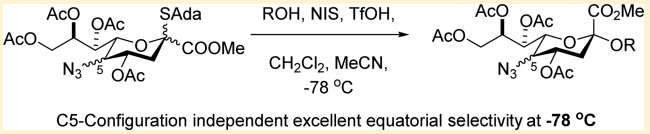
Supporting Information

ABSTRACT: With a view to the eventual synthesis of glycosyl donors for the stereocontrolled synthesis of pseudaminic acid glycosides, the stereocontrolled synthesis of a D-glycero-D-gulo sialic acid adamantanylthioglycoside carrying an axial azide at the 5-position is described. The synthesis employs levulinic acid as nucleophile in the oxidative deamination of an *N*-acetylneuraminic acid thioglycoside leading to the formation of a 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) derivative selectively protected as 5-*O*-levulinate. Replacement of the levulinate by triflate enables introduction of the axial azide and hence formation of the glycosyl donor. A shorter synthesis uses trifluoromethanesulfonate as nucleophile in the oxidative deamination step when the 5-*O*-triflyl KDN derivative is obtained directly. Glycosylation reactions conducted with the 5-azido-D-glycero-D-gulo-configured sialyl adamantanylthioglycoside at −78 °C are selective for the formation of the equatorial glycosides, suggesting that the synthesis of equatorial pseudaminic acid glycosides will be possible as suitable donors become available. A comparable *N*-acetylneuraminic acid adamantanylthioglycoside carrying an equatorial azide at the 5-position was also found to be selective for equatorial glycoside formation under the same conditions, suggesting that reinvestigation of other azide-protected NeuAc donors is merited. Glycosylation stereoselectivity in the D-glycero-D-gulo series is discussed in terms of the side-chain conformation of the donor.



C5-Configuration independent excellent equatorial selectivity at **-78 °C**

INTRODUCTION

The legionaminic (**1**, Leg) and pseudaminic (**2**, Pse) acids are the most common members of a set of deoxy acetamido analogues of *N*-acetylneuraminic acid (**3**, NeuAc) that are unique to microorganisms (Figure 1). Their glycosides are found in the lipopolysaccharides and glycoproteins of multiple Gram-negative bacteria such as the important human pathogens *Pseudomonas aeruginosa*, *Legionella pneumophilia* serogroup 1, *Camphylobacter jejuni*, and *Camphylobacter coli*.[1−3] The equatorial glycosides of NeuAc and Leg are classified as α-glycosides because of the nomenclature convention[4] that links anomeric configuration to the configuration of the stereogenic center at the bottom of the Fischer projection. The equatorial glycosides of Pse, on the other hand, are formally β-glycosides. In this paper, to avoid confusion, we will simply refer to them all as equatorial or axial glycosides.

Unlike NeuAc, which is almost exclusively found in the form of its equatorial glycosides, Leg and Pse are both found as either equatorial or axial glycosides in different pathogenic bacteria. Further complications arise because of the variability of the substituents on the two amines in Leg and Pse, acetyl in Figure 1 for simplicity, but frequently more complex hydroxyl-substituted acyl groups or amidines. For example, in *L. pneumophila*, the cause of Legionnaire's disease, Leg is found[5] in the serotype 1 O-chain lipopolysaccharide in the form of an equatorially linked (2,4)-homopolymer **4** carrying an amidine at C5, while equatorially linked Pse is found in the lipopolysaccharide repeating unit from *P. aeruginosa* O10 (Figure 2).[6]

In view of their widespread occurrence in human pathogens, there is much interest in the biology of Leg and Pse glycosides and their applications in diagnostics and even in therapeutic vaccines.[7−14] All work in this area is, however, severely constrained by the difficulties in obtaining sufficient quantities of Leg and Pse from natural sources or by biosynthesis, not to mention the very considerable difficulties presented by the need for stereoselective glycosidic bond formation. Indeed, only two simple glycosides have been prepared chemically to date, one each of Leg and Pse, both involving formation of very simple axial glycosides with primary alcohols.[13,14] Seeberger has presented a synthesis of Leg in 11 steps from D-threonine[13] with numerous subsequent steps needed to prepare a simple axial glycoside; the more difficult equatorial glycosides were not addressed.[13] Earlier syntheses by Tsvetkov and Ito involved homologation of suitable hexoses and subsequent adjustment of functionality and were longer.[14−16] NeuAc is also an attractive starting material for the synthesis of Leg, Pse, and even other higher carbon sugars, particularly given its current modest cost (~$5/g for 100 g batches). For example, we recently described

Received: September 9, 2016
Published: November 2, 2016

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

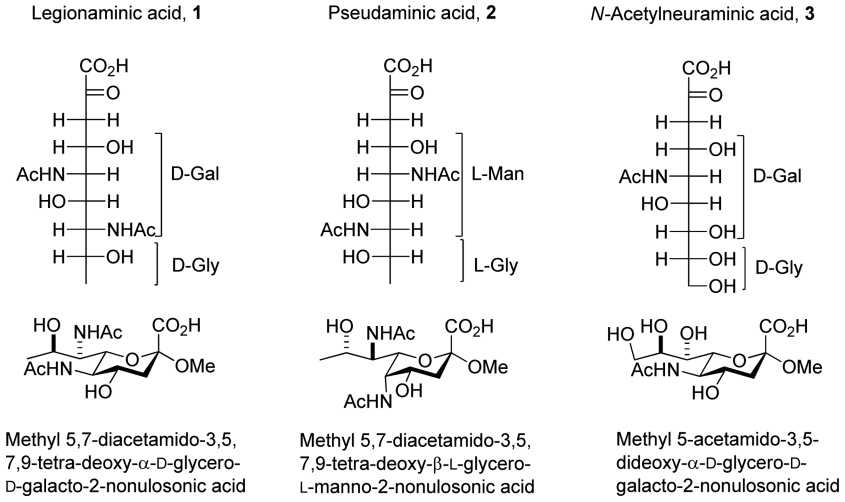


Figure 1. Fischer projections of legionaminic, pseudaminic, and *N*-acetylneuraminic acid and structures and formal names of their equatorial methyl pyranosides.

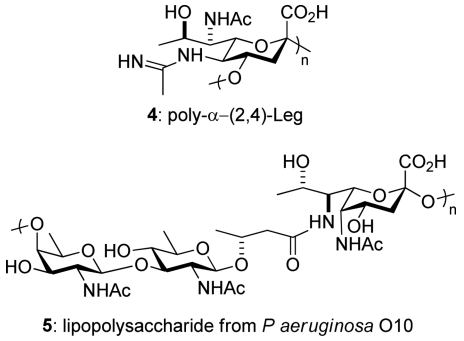
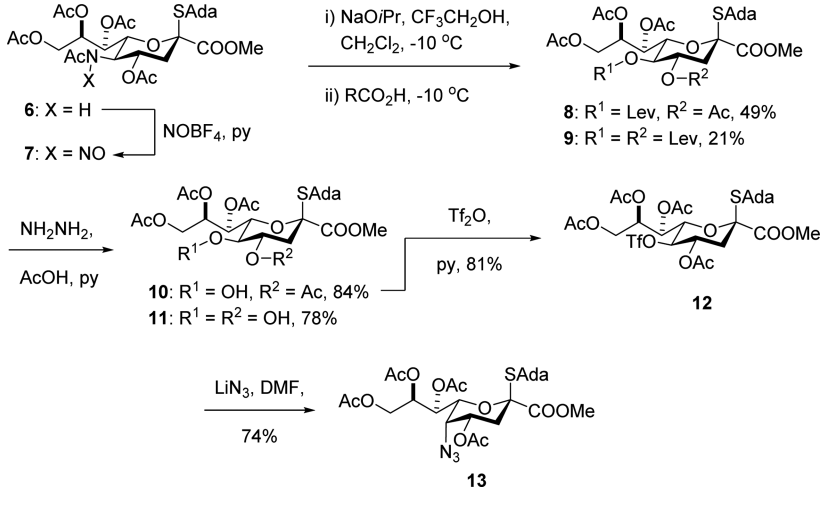


Figure 2. Structures of polylegionaminic acid and the O-specific lipopolysaccharide from *P. aeruginosa* O10.

a synthesis of the seven-carbon aglycon of the antibiotic septicidin by trimming one carbon from each end of NeuAc.[17] Even more recently, Kiefel and co-workers described the synthesis of Pse from NeuAc[18,19] exploiting an oxidative deamination reaction[20] for the stereospecific replacement of the C5−N bond by a C−O bond that was first applied to NeuAc derivatives by the Ogura and Zbiral laboratories.[21−23] Chemoenzymatic approaches to Pse based on the biosynthetic pathways have not yet been conducted on a scale sufficient to provide material for synthetic work and have only recently become available for Leg.[7,8,12,24,25]

With regard to glycosylation, and especially to the formation of the equatorial glycosides, methods for the synthesis of the NeuAc glycosides[26−31] cannot be directly transposed owing to uncertainties arising from the differences in configuration at the 5-, 7-, and 8-positions in Pse and the replacement of the C−O bonds at the 7- and 9-positions by C−N and C−H bonds, respectively, in both Leg and Pse. Indeed, we have previously demonstrated that even the simple inversion of configuration at the 7-position in NeuAc affects the reactivity and selectivity of the widely used O4,N5-oxazolidinone-type sialyl donors,[32] due to the imposed change in side chain conformation.[33] Moreover, the influence of the N5 protecting group on the stereoselectivity in the formation of NeuAc glycosides is widely reported,[34−40] and the replacement of an equatorial substituent (typically a C−O bond) by the axial equivalent typically

Scheme 1. Use of Levulinate as Nucleophile in the Deamination of NeuAc Thioglycoside 6 and Formation of the Azido Derivative 13



DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

influences reactivity and selectivity of glycopyranosyl donors.[41−50]

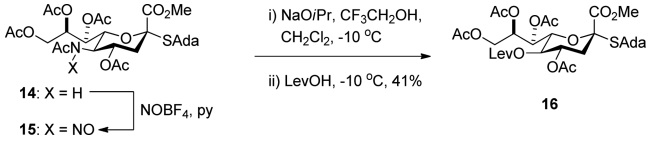
As a logical first step in a systematic investigation into the synthesis of the Pse glycosides, we describe here the synthesis of a 5-*epi*-NeuAc thioglycoside in which the native amide is protected in the form of an azide and the use of this donor in highly equatorial selective glycosylation reactions. We present improvements to the oxidative deamination of NeuAc employing levulinic acid and trifluoromethanesulfonic acid as nucleophile that considerably shorten the approach to the introduction of the axial azido group compared to the more conventional approach of Kiefel and co-workers.[18,19] Finally, we reinvestigate the azide moiety as a protecting group for N5[51] in the formation of NeuAc glycosides and report, contrary to the earlier literature,[52−54] that high equatorial selectivity with such donors is not restricted to primary acceptors.

RESULTS

We first explored the synthesis of a selectively protected derivative of a 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) derivative suitable for introduction of the nitrogen functionality at the 5-position from the readily available[37] NeuAc thioglycoside **6**. Accordingly, **6** was converted to the *N*-nitroso derivative **7** by reaction with nitrosonium tetrafluoroborate in the presence of pyridine in dichloromethane at −10 °C as described previously[23,55] before exposure to sodium isopropoxide and trifluoroethanol in dichloromethane at −10 °C and subsequent addition of levulinic acid (Scheme 1). Workup and chromatographic purification then afforded the 5-*O*-levulinyl KDN derivative **8** in 49% yield together with the 4,5-di-*O*-levulinyl product **9** in 21% yield. The exchange of the 4-*O*-acetate group for the nucleophilic carboxylate as a minor process, as in the formation of **9**, in the oxidative deamination reaction follows the pattern uncovered in our mechanistic work on the Zbiral deamination of **6** when using isotopically labeled acetic acid as nucleophile.[23] Treatment of the levulinate **8** with hydrazine hydrate in acetic acid and pyridine then gave 84% of the KDN 5-ol **10**, whereas application of the same protocol to the dilevulinate **9** gave 78% of the 4,5-diol **11**, thereby confirming the regioselectivity of the ester exchange reaction in the formation of **9**. Reaction of **10** with triflic anhydride in the presence of pyridine gave the 5-*O*-triflate **12** in 81% yield. Subsequent heating with lithium azide in DMF then afforded the D-glycero-D-gulo-configured sialic acid thioglycoside **13** in 74% yield ready for use as a glycosyl donor (Scheme 1).

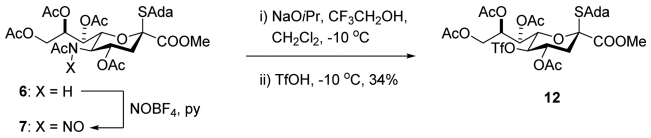
With regard to the deamination chemistry, we note that the use of levulinic acid as nucleophile in the deamination of the diastereomeric thioglycoside **14** gave 41% of the 5-*O*-levulinate **16**, via the *N*-nitroso amide **15**, and that the formation of 4,5-di-*O*-levulinate diastereomeric with **9** was not observed (Scheme 2). This confirms our previous observation using isotopically labeled acetate as nucleophile that doubly substituted products such as **9** are only formed in substrates carrying an axial thioglycoside.[56]

Scheme 2. Use of Levulinate as Nucleophile in the Deamination of the Epimeric Thioglycoside 14



A more direct entry into the key triflate **12** involved use of triflic acid as nucleophile in the Zbiral deamination of **7** when the desired product was isolated in 34% yield (Scheme 3). This reaction is clearly of interest as the triflate anion is best known as an excellent nucleofuge in substitution reactions, and this is by far its most common application.[57,58]

Scheme 3. Use of Triflate as Nucleophile in the Oxidative Deamination of NeuAc Thioglycoside 6



With a synthesis of donor **13** in hand, we turned to the study of its glycosylation reactions. Accordingly, **13** was activated in a 2:1 dichloromethane/acetonitrile mixture at −78 °C in the presence of 4 Å acid-washed molecular sieves and assorted acceptor alcohols by the action of *N*-iodosuccinimide and triflic acid. The acceptor alcohols **17**−**19** were either commercial or were prepared by standard methods, while **20**[59] was accessed by HCl-mediated sodium cyanoborohydride reduction of the corresponding 4,6-*O*-benzylidene acetal.[60] As reported in Table 1, all glycosylation reactions gave good yields of the anticipated glycosides in the form of single equatorial anomers. As each of the glycosides isolated were found, on the basis of NMR coupling constant analysis, to exist as the $^2C_5$ conformers depicted, their anomeric configuration was determined by measurement of the $^3J_{C,H}$ heteronuclear coupling constant between the C1 carboxyl carbon and the axial H3.[32,61−65]

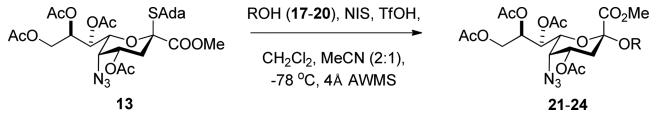
Although azide-protected NeuAc donors have been described by several groups, their use has typically been constrained to the use of primary alcohols as acceptors.[34] The excellent results obtained with donor **13** in acetonitrile dichloromethane mixtures at −78 °C (Table 1) caused us to reexamine the use of the azide moiety as a protecting group for N5 in the formation of NeuAc glycosides as they clearly raise the possibility that azide-protected NeuAc donors would also be highly selective at −78 °C. With this in mind, we again turned to the use of the tertiary thioglycosides, which are more readily activated by the *N*-iodosuccinimide triflic acid combination than aryl thioglycosides or primary and secondary alkyl thioglycosides.[37,66] Accordingly, reaction of thioglycoside **25**[37] with imidazole sulfonyl azide in the presence of copper sulfate and potassium carbonate gave 92% of the azide **26**, which was converted to the donor **27** on standard acetylation (Scheme 4). Alternatively, and following reports by Zbiral and Schmidt,[22,51] conversion of acetamide **6** to the *N*-nitrosoacetamide **7** followed by treatment with sodium isopropoxide and trifluoroethanol and then hydrazoic acid gave **27** in 51% yield (Scheme 4).

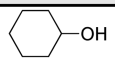
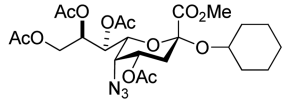
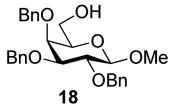
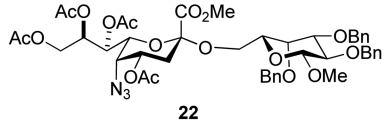
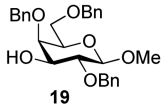
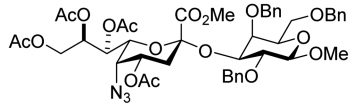
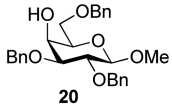
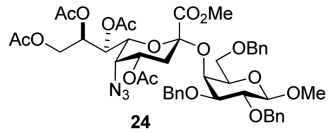
Coupling reactions of donor **27** were then conducted with the acceptors **17**−**20** in 2:1 dichloromethane/acetonitrile at −78 °C with activation by *N*-iodosuccinimide and triflic acid (Table 2). As is clear from Table 2, donor **27** is exquisitely selective in its coupling reactions to the primary alcohol **18** as well as to the three secondary alcohols **17**, **19**, and **20** in reactions conducted at −78 °C.

The deprotection of selected glycosides was initiated by hydrogenolysis over palladium−charcoal in aqueous dioxane, resulting in benzyl ether cleavage and the reduction of the azide

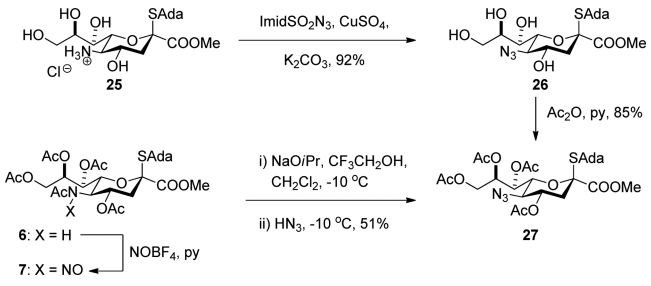
DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

Table 1. Glycosylation Reactions with D-Glycero-D-gulo Donor 13



Entry	Acceptor	Product	Yield	$^3J_{C1,H3}$ (Hz)
1	 17	 21	79%	6.9
2	 18	 22	72%	6.6
3	 19	 23	57%	7.1
4	 20	 24	44%	7.2

Scheme 4. Synthesis of the 5-Azido D-Glycero-D-galacto Donor 27



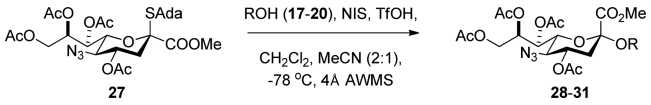
functionality to the corresponding amine. Partial O → N migration of acetyl groups[67,68] was observed in the course of the hydrogenolysis resulting in complex reaction mixtures. Consequently, the crude reaction mixtures were acetylated, thereby completing the process of acetamide formation and enabling the isolation of the peracetates in high yield (Table 3). Saponification of the esters was then achieved with barium hydroxide in water at 60 °C, followed by precipitation of barium salts with carbon dioxide and lyophilization to give the D-glycero-D-gulo- and D-glycero-D-galacto-nonulosonic acid glycosides **36**–**39** (Table 3) in high yield.

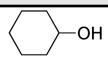
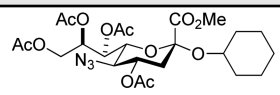
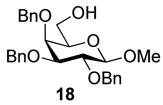
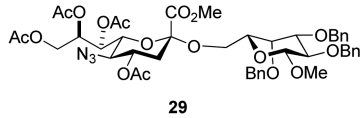
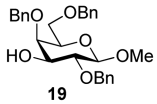
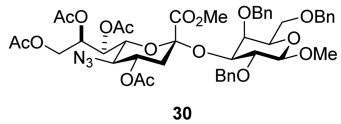
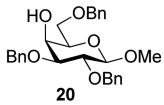
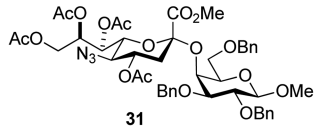
To ascertain the role, if any, of the anomeric configuration of donors **13** and **27** in the selectivity of the glycosylations reported in Tables 1 and 2, we prepared the anomer of **27**. Thus, adapting the protocol employed (Scheme 4) for the preparation of **27**, the equatorial adamantanyl thioglycoside **14**[23,37] was converted to the *N*-Boc derivative **40** by treatment with Boc_2O and DMAP in 76% yield. A three-step sequence of saponification, carbamate removal, and azide formation then gave the azido thioglycoside **43** in 86% overall yield via the intermediates **41** and **42**. Finally, acetylation afforded 84% of the desired donor **44**. Alternatively, **44** was accessed from **14**, via the *N*-nitrosoamide **15**, in 46% yield by the Zbiral deamination protocol employing hydrazoic acid as nucleophile (Scheme 5).

The equatorial thioglycoside **44** was then employed in the glycosylation of cyclohexanol in 2:1 dichloromethane/acetonitrile at −78 °C with activation by *N*-iodosuccinimide and triflic acid resulting in the formation of the equatorial glycoside **28** in 86% isolated yield as a single diastereomer (Scheme 6). As the equatorial glycoside **28** was formed with comparable yield and selectivity from either anomer of the donor, **27** or **44**, we conclude that the equatorial selectivity observed with the axial thioglycosides **13** and **27** is not the result of direct displacement of the activated thioglycoside by the incoming acceptor alcohol.

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

Table 2. Glycosylation Reactions with D-Glycero-D-galacto Donor 27



Entry	Acceptor	Product	Yield	$^3J_{C1,H3}$ (Hz)
1	 17	 28	81%	6.2
2	 18	 29	74%	6.4
3	 19	 30	66%	6.8
4	 20	 31	41%	6.9

Such S_N2-like displacement of activated adamantanyl thioglycosides has been postulated recently in the sialic acid series to account for a dependence of stereoselectivity on the anomeric configuration of the donors in a set of spirocyclic glycoside forming reactions.[69] Those results, however, were obtained with an intramolecular alcohol as nucleophile and consequently benefited from the high effective molarity of the acceptor.

DISCUSSION

Use of Azide as a Protecting Group at the 5-Position in Sialyl Donors. The excellent equatorial selectivities observed with the D-glycero-D-gulo- and D-glycero-D-galacto-configured donors **13** and **27** reported in Tables 1 and 2 clearly augur well for the stereocontrolled synthesis of equatorial Pse glycosides and of equatorial NeuAc glycosides.

The high selectivities observed with **13** find some parallel with the excellent equatorial selectivities reported by the Oscarson group with a 3-deoxy-D-manno-2-octulosonic acid (KDO) based thioglycoside carrying an axial acetoxy group at the 5-position.[70] The results observed with **27**, on the other hand, stand in contrast to the reputation of azido-protected NeuAc donors as being only useful for glycosylation of primary alcohols (Figure 3).[34] Thus, Unverzagt and co-workers first described the synthesis of the 5-azido methylthio sialoside **45** in the form of an α/β mixture and reported that it was less satisfactory than the corresponding 5-acetamido system in coupling to a complex trisaccharyl galactosyl 3-OH acceptor on activation at −40 °C in acetonitrile: only a 26% yield of the desired tetrasaccharide was obtained, and the selectivity was not discussed.[71] Wong and co-workers subsequently reported that the equatorial anomers of both **45** and the *p*-tolyl thioglycoside **46** gave good yields and selectivity on coupling to carbohydrate-based primary alcohols in acetonitrile at −40 °C.[52] Li and co-workers reported the use of **46** in couplings to primary and secondary alcohols in acetonitrile at −40 °C but with the exception of a single primary alcohol found poor selectivities.[72] Schmidt and co-workers prepared the ethyl thioglycoside **47** but did not report on its use as a glycosyl donor.[51] The more highly armed thioglycoside **48** was prepared by Lin and co-workers and was reported to give moderate to excellent equatorial selectivity on coupling to primary alcohols in acetonitrile at −40 °C, but only very poor selectivity and yield with the single example of a secondary alcohol were described.[54] The sialyl phosphite **49** was found to give good yields and equatorial selectivity with primary alcohols at −40 °C in acetonitrile, but no couplings to secondary alcohols were reported.[52] Finally, Mukaiyama and co-workers prepared the sialyl fluoride **50** and reported its coupling to primary alcohols at −10 °C in propionitrile with excellent yield and selectivity, but attempted application to a secondary acceptor gave a much reduced yield and minimal selectivity.[53] The clear difference between this body of literature results and those reported in

DOI: 10.1021/acs.joc.6b02221
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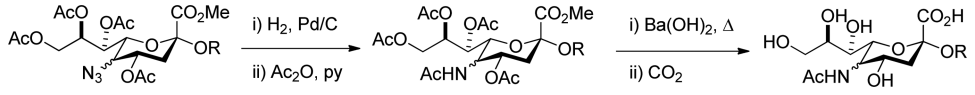
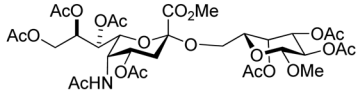
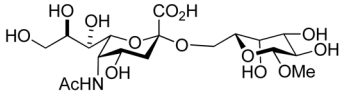
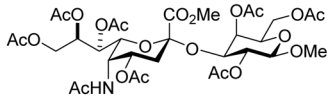
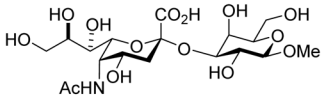
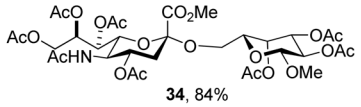
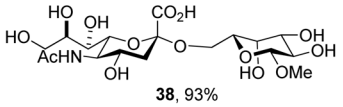
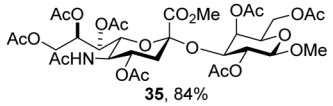
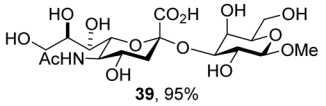
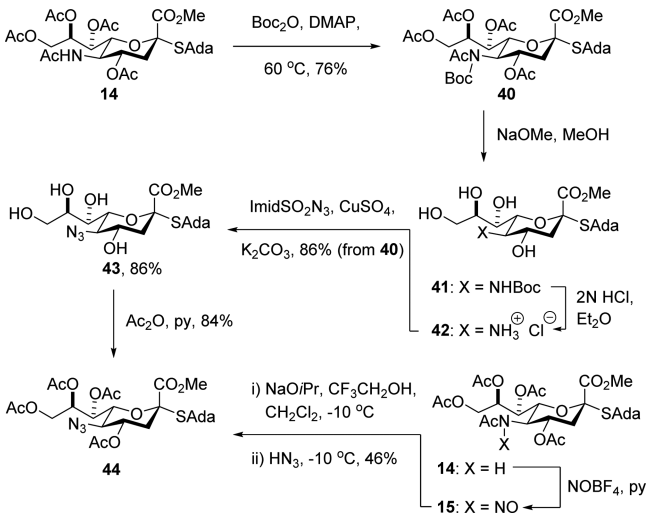
Table 3. Deprotection of Selected Examples

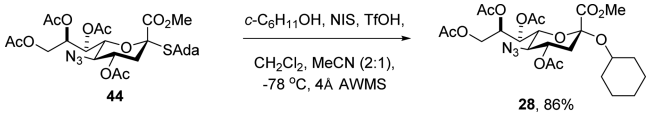


Substrate	Hydrogenolysis Product, % yield	Sialic Acid Glycoside, % yield
22	 **32**, 82%	 **36**, 92%
23	 **33**, 77%	 **37**, 93%
29	 **34**, 84%	 **38**, 93%
30	 **35**, 84%	 **39**, 95%

Scheme 5. Preparation of the Equatorial Thioglycoside 44



Scheme 6. Stereoselective Glycosylation of Cyclohexanol with Equatorial Thiosialoside 44



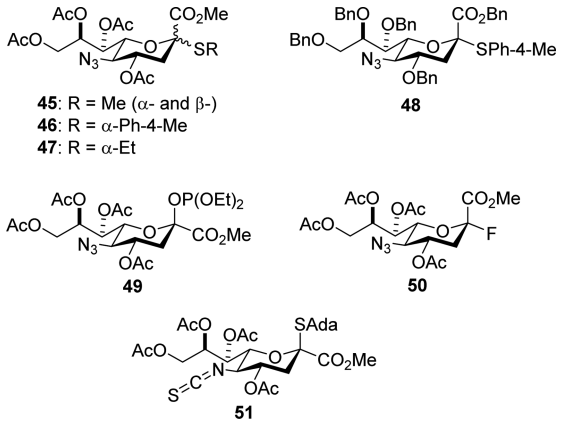


Figure 3. Literature 5-azido and 5-isothiocyanato D-glycero-D-galacto-nonulosonic acid donors.

Table 2 is the reaction temperature, leading us to conclude that the azide group is an excellent protecting for peracetyl sialyl donors provided that the coupling reactions are conducted at −78 °C. We suspect that, if activation can be achieved at comparably low temperatures, other classes of sialyl donor carrying the azido moiety at the 5-position, such as donors **45**−**50**, will also be equatorially selective for secondary acceptors.

Further, because of the results with azide-protected donors **45**−**50**, and in particular the very limited success in couplings to secondary alcohols, we were surprised to discover recently

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

that donor **51**, with an isosteric isothiocyanate group at the 5-position, gave excellent yields and selectivities on coupling to both primary and secondary alcohols in acetonitrile dichloromethane mixtures at −78 °C.[40] The finding that the azide-protected NeuAc donor **27** is highly equatorially selective at −78 °C suggests that there is little difference between the influence of the isosteric azide and isocyanato groups provided that the reactions are conducted under the same low-temperature conditions.

Influence of the C5 Configuration on Side-Chain Conformation and Glycosylation Stereoselectivity. Typically, hexopyranosyl donors with an axial C−O bond at the 4-position give a greater proportion of axial glycosides than do their C-4 epimers. This is because the axial C−O bond has a gauche relationship to and is better able to stabilize developing positive charge at the ring oxygen during glycosylation than the corresponding antiperiplanar equatorial ring oxygen.[45,49] It is remarkable, therefore, that both the D-glycero-D-gulo- and D-glycero-D-galacto-configured donors **13** and **27** exhibit very high levels of equatorial selectivity in their glycosylation reactions at −78 °C (Tables 1 and 2) in spite of their differing configurations at the 5-position (which corresponds to the 4-position in the hexopyranosides). Indeed, a cursory analysis of the problem would have predicted donor **13** with the axial azide to afford a greater proportion of the axial glycosides than the **27** with the equatorial donor. However, inspection of the ^{1}H NMR spectra of **13** and **27** reveals them to adopt different conformations about the exocyclic bond to the side chain. Thus, the D-glycero-D-galacto donor **27** has $^3J_{6,7}$ = 2.2 Hz consistent with a predominant *gg*-conformation[73−76] of the C6−C7 bond (Figure 4) in accordance with previous analyses

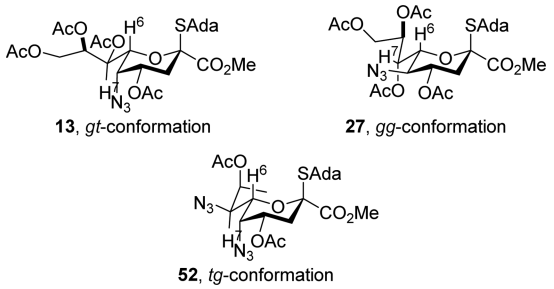


Figure 4. Predominant side-chain conformations of donors **13** and **27** and predicted side-chain conformation of the pseudaminic acid donor **52**.

of *N*-acetylneuraminic acid, its derivatives and glycosides.[32,75,77−81] On the other hand, the D-glycero-D-gulo donor **13** has $^3J_{6,7}$ = 8.8 Hz, indicating H6 and H7 to be close to antiperiplanar and the C6−C7 bond to adopt very predominantly the *gt*-conformation (Figure 4). The $^3J_{6,7}$ of 10.0 and 10.1 Hz found in **36** and **37**, respectively, indicates that the predominant *gt* conformation about the C6−C7 bond of the D-glycero-D-gulo series is also found in aqueous solution. The switch from predominant *gg*- to predominant *gt*-conformations in **13** arises from a combination of steric and electrostatic repulsions between the C−N and C7−O bonds in the *gg*-conformation (Figure 4). Indeed, the change in side conformation from **27** to **13** reflects that seen on going from glucopyranose, with its approximate 6:4:0 *gg*;*gt*:*tg* conformational mixture, to galactopyranose which displays an approximate 2:6:2 mixture of *gg*;*gt*:*tg* conformers.[73−76] Glycosyl donors on which the *gt*-conformation has been imposed are less reactive[82,83] and less prone to the formation of axial glycosides than their *gg*-isomers.[33,84] Therefore, the comparable selectivity of **13** and **27** appears to arise from the fortuitous canceling of the effect of the axial azide group in **13** by the switch to the predominant *gt*−conformation of its side chain. Extrapolating to the L-glycero-L-manno configuration found in the pseudaminic acid itself, the hypothetical donor **52** can be expected to populate the *tg*-conformation (Figure 4), which is the most electron-withdrawing and which leads to the expectation of high equatorial selectivity in glycosylation reactions.

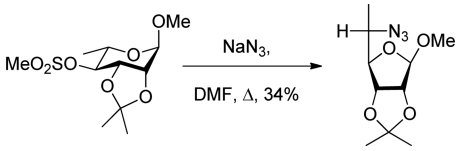
Nucleophilic Triflate in the Deamination Reaction. Zefirov and Kozmin have previously demonstrated that supernucleofuges such as triflate and perchlorate are also powerful nucleophiles toward cation-like electrophiles, when they can even out-compete more common nucleophiles such as acetate.[57,85] Indeed, in close parallel with the result described in Scheme 3, Zefirov and co-workers reported on the use of perchlorate as nucleophile in the nitrous acid-based deamination of amines.[85] The high nucleophilicity of triflate toward cation-like species also is supported by recent computational work, which attributes a higher intrinsic nucleophilicity index to triflate than to methanol.[86] Similarly, it is the high nucleophilicity of triflate that underlies the formation glycosyl triflates[87] as intermediates in glycosylation reactions, even when conducted only in the presence of catalytic quantities of the triflate ion, and of other classes of transient covalent triflates.[88−91] The ability of the triflate anion to function as a nucleophile in S_N1-like processes is no doubt related to its relatively low degree of solvation as discussed by Zefirov and Kozmin,[85] but also to the thermodynamic driving force provided by the formation of the strong C−O bond. The ability to detect, isolate, and study covalent triflates formed in such processes clearly depends on the blocking of their subsequent reactions as electrophiles, either by working with the exclusion of other nucleophiles,[87] or in systems that preclude their subsequent displacement.[56,85] In the present case, the two electron-withdrawing C−O bonds vicinal to the triflate in structure **12** retard any subsequent displacement reactions, as with a multitude of other carbohydrate-based triflates, and enable its isolation and characterization.

Nucleofuge-Dependent Stereoselectivity in Substitution at the NeuAc 5-Position. Finally, it is of interest to consider the contrasting stereoselectivities in the oxidative deamination of **6** and related substances, which proceed with exquisite levels of retention of configuration consistent with the literature from the Zbiral and other groups,[18,19,21−23,55] and the stereoinvertive displacement of the triflate from **12** by azide to give **13** and related reactions described by Kiefel and co-workers (Scheme 1).[18,19] On the basis of extensive experimentation, we have rationalized the stereoretentive nature of the deamination reaction on the basis of participation by the pyranoside ring oxygen in the displacement of molecular nitrogen via an oxabicyclo[3.1.0]hexanium ion.[23] This process is facilitated by the extreme leaving group ability of molecular nitrogen to the extent that the ring oxygen intervenes, either directly or at the level of an intermediate carbenium ion, before any intermolecular attack by external nucleophiles including azide.[22,50] Although for most purposes an outstanding leaving group, triflate is a weaker nucleofuge than molecular nitrogen and does not admit participation by the ring oxygen in competition with the observed S_N2 displacement by azide in the formation of **13**. When the displacement of the sulfonate by

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

external nucleophiles is sterically hindered, participation by the ring oxygen again becomes possible under forcing conditions. This is exemplified by a series of pyranose to furanose ring contractions described by the Stevens and Hanessian laboratories, of which an example is given in Scheme 7.[92−94] A related ring contraction has been reported, but only as a minor product, in a Zbiral-type deamination of an *N*-acetylneuraminic acid glycoside.[21]

Scheme 7. Ring Contraction of a Rhamnosyl 4-*O*-Methanesulfonate



CONCLUSION

We conclude that the presence of an azido group at the 5-position of sialyl donors, whether axial or equatorial, supports the highly stereoselective synthesis of the corresponding equatorial glycosides provided that the reactions are conducted at a sufficiently low temperature. The high selectivity observed in the D-glycero-D-gulo series is related to the *gt*-side chain conformation, which compensates for the presence of the axial azide in the pyranose ring. This suggests that the stereoselective synthesis of equatorial pseudaminic acid glycosides will be possible when suitable donors become available through either chemical or enzymatic methods and that azide-protected NeuAc donors merit reinvestigation at lower temperatures than those previously employed in the literature. We also conclude that the trifluoromethanesulfonate anion is a viable nucleophile in the Zbiral type oxidative deamination of NeuAc derivatives, consistent with the important role it plays in many glycosylation reactions, and with earlier descriptions of its exceptional nucleophilicity toward carbenium ion like electrophiles.

EXPERIMENTAL SECTION

General Experimental Methods. Commercially available starting materials were used without further purification. All solvents were dried according to standard methods. TLC was performed on precoated glass plates employing UV absorption and charring with ceric ammonium molybdate (CAM) stain for visualization. For column chromatography, silica gel 60, 230−400 mesh, 40−63 μm was used. ^{1}H and ^{13}C NMR spectra were recorded on 400 and 600 MHz instruments at 300 K. Chemical shifts were calibrated to solvent residual peaks. Stereochemical assignments of coupled sialosides are based on $^3J_{C1-H3ax}$ values.[32,61−65] Specific rotations were measured using a digital polarimeter; values are given in 10^{-1} deg·cm^2·g^{-1}. High-resolution mass spectra were recorded with an electrospray source coupled to a time-of-flight mass analyzer.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-5-O-levulinyl-3-deoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (**8**) and Methyl (1-Adamantyl 7,8,9-tri-O-acetyl-4,5-di-O-levulinyl-3-deoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (**9**).* A solution of sialoside **6**[37] (1 g, 1.56 mmol) in dry dichloromethane (14 mL) was treated with dry pyridine (1.14 mL, 14.04 mmol) under argon and cooled to −10 °C. After the solution was stirred for 15 min, crushed nitrosyl tetrafluoroborate (0.65 g, 2.62 mmol) was added in one portion. The reaction mixture was stirred at −10 °C until TLC/MS showed complete conversion and then was diluted with cold dichloromethane and washed with cold 1 N HCl, saturated aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to obtain the nitrosated sialoside 7, which was carried forward without any further purification. A solution of the crude nitrosated sialoside 7 (1.06 g, 1.59 mmol) in dry dichloromethane (16 mL) and 2,2,2-trifluoroethanol (184 μL, 2.38 mmol) under argon at −10 °C was stirred for 0.5 h and then treated with freshly prepared sodium isopropoxide in 2-propanol (0.2 N; 9.49 mL, 1.91 mmol). The resulting mixture was stirred for 2 min and then treated with a cold solution of levulinic acid (3.23 mL, 31.77 mmol) in 32 mL of dry dichloromethane. After being stirred for 5 min, the reaction mixture was warmed to 0 °C and quenched with saturated aq $NaHCO_3$. It was then diluted with dichloromethane, washed with cold brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 10% acetone in toluene to afford **8** (535 mg, 49%) and **9** (243 mg, 21%).

*Compound **8**:* $[\alpha]^{21}_D$ −36.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (t, *J* = 2.3 Hz, 1H), 5.36 (ddd, *J* = 11.9, 9.6, 4.9 Hz, 1H), 5.21 (dt, *J* = 8.7, 1.6 Hz, 1H), 4.96 (dd, *J* = 12.4, 1.6 Hz, 1H), 4.87 (t, *J* = 9.8 Hz, 1H), 4.72 (dd, *J* = 10.0, 2.6 Hz, 1H), 4.25 (dd, *J* = 12.4, 8.6 Hz, 1H), 3.82 (s, 3H), 2.82−2.69 (m, 1H), 2.65−2.52 (m, 3H), 2.49−2.37 (m, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.02−1.96 (m, 9H), 1.92−1.84 (m, 4H), 1.71−1.62 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.9, 171.7, 170.7, 170.4, 170.2, 170.1, 169.8, 86.0, 73.2, 71.2, 69.0, 68.6, 68.2, 63.2, 63.2, 52.8, 50.5, 43.8, 43.4, 39.6, 37.7, 36.1, 35.9, 29.9, 29.8, 29.6, 27.9, 21.0, 20.8, 20.6; ESI-HRMS ($C_{33}H_{46}NaO_{14}S$) [M + Na]$^+$ *m*/*z* 721.2506, found 721.2486.

*Compound **9**:* $[\alpha]^{21}_D$ −28.1 (*c* 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (t, *J* = 2.2, 1H), 5.36 (ddd, *J* = 11.9, 9.7, 4.9 Hz, 1H), 5.20 (dt, *J* = 8.8, 1.6 Hz, 1H), 4.96 (dd, *J* = 12.3, 1.5 Hz, 1H), 4.87 (t, *J* = 9.8 Hz, 1H), 4.71 (dd, *J* = 10.0, 2.6 Hz, 1H), 4.25 (dd, *J* = 12.4, 8.7 Hz, 1H), 3.81 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.02−1.96 (m, 9H), 1.92−1.80 (m, 4H), 1.65 (br s, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.6, 206.2, 171.7, 170.8, 170.4, 170.2, 169.8, 86.0, 73.2, 71.2, 69.0, 68.9, 68.1, 63.2, 52.8, 50.5, 43.4, 39.5, 37.7, 35.9, 29.8, 29.7, 27.9, 27.8, 21.0, 20.6; ESI-HRMS ($C_{36}H_{50}NaO_{15}S$) [M + Na]$^+$ *m*/*z* 777.2768, found 777.2766.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-3-deoxy-2-thio-D-glycero-β-D-galacto-non-2-ylopyranosid)onate (**10**).* To a solution of **8** (0.56 g, 0.80 mmol) in 30 mL of dry dichloromethane at room temperature under argon was added 1.9 mL of dry pyridine. This was followed by addition of hydrazine monohydrate (0.11 mL, 3.18 mmol) and glacial acetic acid (1.42 mL). The resulting mixture was stirred at room temperature for 1 h. Then the reaction mixture was quenched with 10 mL of acetone and further stirred for 15 min. The reaction mixture was then diluted with dichloromethane and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 45% EtOAc in hexane to afford **10** (0.40 g, 84%): $[\alpha]^{21}_D$ −54.8 (*c* 0.81, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (dd, *J* = 3.7, 2.6 Hz, 1H), 5.36−5.22 (m, 2H), 4.84 (dd, *J* = 12.5, 1.6 Hz, 1H), 4.43(dd, *J* = 9.6, 2.5 Hz, 1H), 4.33 (dd, *J* = 12.5, 7.1 Hz, 1H), 3.80 (s, 3H), 3.22 (t, *J* = 9.5 Hz, 1H), 3.10 (br s, 1H), 2.60 (dd, *J* = 13.6, 4.8 Hz, 1H), 2.18 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.98 (d, *J* = 11.2 Hz, 6H), 1.88−1.81 (m, 4H), 1.79 (dd, *J* = 13.4, 11.9 Hz, 1H), 1.65 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 170.7, 170.5, 170.3, 170.1, 85.8, 73.0, 72.1, 71.0, 70.7, 69.2, 62.7, 52.7, 50.3, 43.4, 39.4, 35.9, 29.8, 21.1, 21.0, 20.8, 20.7; ESI-HRMS ($C_{28}H_{40}NaO_{12}S$) [M + Na]$^+$ *m*/*z* 623.2138, found 623.2137.

*Methyl (1-Adamantyl 7,8,9-tri-O-acetyl-3-deoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (**11**).* To a solution of **9** (150 mg, 0.21 mmol) in 20 mL of dry dichloromethane at room temperature under argon was added 0.7 mL of dry pyridine. This was followed by addition of hydrazine monohydrate (28 μL, 0.81 mmol) and glacial acetic acid (0.5 mL). The resulting mixture was stirred at room temperature for 1 h. Then the reaction mixture was quenched with 10 mL of acetone and further stirred for 15 min. The reaction mixture was then diluted with dichloromethane and washed with water and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

chromatography on silica gel eluting with 45% EtOAc in hexane to afford the pale yellow oil **11** (87 mg, 78%): $[\alpha]^{21}_{D}$ −55 (*c* 0.82, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (dd, *J* = 3.7, 2.2 Hz, 1H), 5.30 (ddd, *J* = 6.6, 3.8, 1.5 Hz, 1H), 4.88 (dd, *J* = 12.6, 1.4 Hz, 1H), 4.36 (dd, *J* = 12.4, 6.9 Hz, 1H), 4.33 (dd, *J* = 9.5, 2.2 Hz, 1H), 4.11 (ddd, *J* = 12.0, 8.9, 4.7 Hz, 1H), 3.80 (s, 3H), 3.39 (br s, 1H), 2.96 (t, *J* = 9.3 Hz, 1H), 2.78 (br s, 1H), 2.54 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.19 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.96 (d, *J* = 12.5 Hz, 7H), 1.81 (d, *J* = 10.7 Hz, 4H), 1.75 (dd, *J* = 13.7, 12.1 Hz, 2H), 1.64 (s, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 170.5, 170.3, 170.3, 86.1, 72.7, 72.1, 71.9, 71.5, 67.8, 62.7, 52.7, 50.1, 43.4, 41.3, 35.9, 29.7, 21.1, 20.9, 20.7; ESI-HRMS ($C_{26}H_{38}NaO_{11}S$) $[M + Na]^+$ *m*/*z* 581.2033, found 581.2020.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-5-O-triflyl-3-deoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (**12**). Method 1.* A solution of sialoside **6** (50 mg, 0.08 mmol) in dry dichloromethane (1 mL) was treated with dry pyridine (63 μL, 0.78 mmol) under argon and cooled to −10 °C. After the solution was stirred for 15 min, crushed nitrosyl tetrafluoroborate (36 mg, 2.62 mmol) was added in one portion. The reaction was stirred at −10 °C until TLC/MS showed complete conversion and then was diluted with cold dichloromethane and washed with cold 1 N HCl, saturated aq $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to obtain the nitrosated sialoside **7**, which was carried forward without any further purification. 18-Crown-6 (47 mg, 0.18 mmol) and sodium 2,2,2-trifluoroethoxide (2 mg, 0.164 mmol) were dissolved in anhydrous dichloromethane (0.4 mL) under argon, cooled to −10 °C, and added to the crude nitrosyl sialoside **7** (55 mg, 0.082 mmol) in anhydrous dichloromethane (0.8 mL) at −10 °C under argon. After 2 min, triflic acid (0.37 mL, 4.1 mmol) was added to the reaction mixture. The mixture was stirred for 5 min and quenched with methanol (2 mL). The volatiles were evaporated, and the crude compound was purified by column chromatography on silica gel (eluent: 25% EtOAc in hexane) to afford **12** (19 mg, 34%).

Method 2. To a solution of **10** (100 mg: 0.156 mmol) in 5 mL of dry dichloromethane was added dry pyridine (50 μL, 0.62 mmol), and the temperature was lowered to −78 °C. At −78 °C, Tf_2O (52 μL, 0.312 mmol) was added, and the mixture was stirred for 15 min. Then the mixture was brought to 0 °C and further stirred for 2 h before it was diluted with dichloromethane and washed with 1 N HCl and water. Then the organic layer was dried (Na_2SO_4) and concentrated to give a yellowish solid, which was then subjected to column chromatography (25% EtOAc in hexane) to give **12** (98 mg, 81%): $[\alpha]^{21}_{D}$ −72.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (ddd, *J* = 11.7, 9.1, 5.1 Hz, 1H), 5.36 (dt, *J* = 8.8, 4.4 Hz, 1H), 5.32−5.25 (m, 1H), 5.01 (t, *J* = 9.4 Hz, 1H), 4.76 (dd, *J* = 9.6, 2.3 Hz, 1H), 4.68 (dd, *J* = 12.3, 2.1 Hz, 1H), 4.24 (dd, *J* = 12.4, 7.1 Hz, 1H), 3.85−3.76 (m, 2H), 2.72 (dd, *J* = 13.7, 5.1 Hz, 1H), 2.14 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.91−1.82 (m, 4H), 1.66 (s, 6H), 1.43(m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.5, 169.4, 169.3, 169.2, 85.3, 79.9, 71.7, 70.0, 69.0, 68.2, 62.8, 52.9, 50.9, 43.5, 43.3, 39.4, 35.9, 29.8, 29.7, 20.6, 20.5; ESI-HRMS ($C_{29}H_{39}F_3NaO_{14}S_2$) $[M + Na]^+$ *m*/*z* 755.1601, found 755.1592.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero-β-D-gulo-non-2-ulopyranosid)onate (**13**).* To a solution of **12** (201 mg, 0.28 mmol) in 15 mL of dry DMF was added LiN_3 (202 mg, 4.13 mmol). The resulting mixture was stirred at 0 °C for 24 h, and then the was diluted with ethyl acetate and washed with $NaHCO_3$, water, and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **13** (127 mg, 74%): $[\alpha]^{21}_{D}$ −106 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dd, *J* = 8.8, 3.3 Hz, 1H), 5.38 (ddd, *J* = 11.4, 5.4, 3.3 Hz, 1H), 5.27 (dt, *J* = 6.5, 3.8 Hz, 1H), 4.51 (dd, *J* = 8.9, 1.0 Hz, 1H), 4.43 (dd, *J* = 12.1, 4.2 Hz, 1H), 4.19 (dd, *J* = 12.1, 6.7 Hz, 1H), 4.10 (d, *J* = 1.5 Hz, 1H), 3.78 (s, 3H), 2.35−2.21 (m, 2H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 1.97 (d, *J* = 11.4 Hz, 8H), 1.82 (d, *J* = 11.1 Hz, 3H), 1.65 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.9, 169.9, 169.8, 169.3, 86.0, 70.7, 69.4, 69.0, 61.6, 58.5, 52.7, 50.4, 43.3, 36.0, 34.2, 29.8, 20.8, 20.7, 20.6, 20.6; ESI-HRMS ($C_{28}H_{39}N_3NaO_{11}S$) $[M + Na]^+$ *m*/*z* 648.2203, found 648.2199.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-5-O-levulinic-3-deoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (**16**).* The nitrosyl sialoside **15** (157 mg, 0.23 mmol) was deaminated using the general procedure with 2,2,2-trifluoroethanol (27 μL, 0.35 mmol), 0.2 N sodium isopropoxide in isopropanol (1.4 mL, 0.28 mmol), and levulinic acid (477 μL, 4.68 mmol) to afford **16** after flash chromatography over silica gel eluting with 10% acetone in toluene as a colorless oil (67 mg, 41%): $[\alpha]^{21}_{D}$ +24.5 (*c* = 0.96, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28 (s, 2H), 4.87−4.78 (m, 2H), 4.30 (d, *J* = 12.3 Hz, 1H), 4.13 (t, *J* = 10.6 Hz, 2H), 3.81 (s, 3H), 2.87−2.77 (m, 1H), 2.76−2.70 (m, 1H), 2.60 (dd, *J* = 9.3, 4.0 Hz, 1H), 2.57−2.52 (m, 1H), 2.50−2.42 (m, 1H), 2.19 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.96 (d, *J* = 12.0 Hz, 4H), 1.86 (d, *J* = 11.7 Hz, 3H), 1.66 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.1, 171.7, 170.7, 170.4, 169.9, 169.6, 84.5, 72.8, 69.3, 68.5, 67.8, 66.4, 61.5, 52.8, 51.1, 43.5, 39.6, 37.7, 36.0, 29.9, 29.7, 27.9, 21.1, 20.8, 20.7; ESI-HRMS ($C_{33}H_{46}NaO_{14}S$) $[M + Na]^+$ *m*/*z* 721.2506, found 721.2491.

*Methyl (1-Adamantyl 5-azido-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (**26**).* To a solution of **25**[40] (810 mg, 1.73 mmol) in 1:1 MeOH/H_2O (18 mL) was added imidazole-1-sulfonyl azide hydrochloride (0.72 g, 3.46 mmol), K_2CO_3 (717 mg, 5.19 mmol), and $CuSO_4.5H_2O$ (43 mg, 0.17 mmol). The reaction mixture was stirred at room temperature for 3 h. Thereafter, the solvent was evaporated, and the residue was purified by chromatography on silica gel eluting 8% MeOH in $CHCl_3$ system to afford the white sticky solid **26** (724 mg, 92%): $[\alpha]^{21}_{D}$ −48.1 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 4.84 (s, 3H), 4.22 (d, *J* = 10.4 Hz, 1H), 4.06 (ddt, *J* = 11.8, 9.8, 5.9 Hz, 1H), 3.90−3.75 (s, 4H), 3.75−3.63 (s, 3H), 3.35 (t, *J* = 11.7 Hz, 1H), 2.33 (dd, *J* = 13.6, 4.7 Hz, 1H), 2.08−1.88 (br s, 8H), 1.81−1.60 (m, 7H), 1.22(m, 1H); ^{13}C NMR (101 MHz, CD_3OD); δ 172.4, 85.9, 78.6, 70.9, 70.00, 69.8, 67.9, 63.9, 63.8, 59.8, 52.1, 49.5, 43.0, 35.7, 29.9, 19.9, 13.1; ESI-HRMS ($C_{20}H_{31}N_3NaO_7S$) $[M + Na]^+$ *m*/*z* 480.1796, found 480.1789.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (**27**). Method 1.* To a solution of **26** (724 mg, 1.58 mmol) in 15 mL of dry pyridine was added acetic anhydride (1.2 mL, 12.66 mmol). The resulting mixture was stirred under argon at room temperature for 4 h, diluted with ethyl acetate, and washed successively with saturated aq $NaHCO_3$ solution, 1 N HCl, and again with saturated aq $NaHCO_3$ solution. The organic layer was then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **27** (0.84 g, 85%).

Method 2. A solution of sialoside **6** (300 mg, 0.468 mmol) in dry dichloromethane (4.6 mL) was treated with dry pyridine (0.38 mL, 4.68 mmol) under argon and cooled to −10 °C. After the solution was stireed for 15 min, crushed nitrosyl tetrafluoroborate (218 mg, 1.87 mmol) was added in one portion. The reaction was stirred at −10 °C until TLC/MS showed complete conversion and then was diluted with cold dichloromethane (25 mL) and washed with cold 1 N HCl, saturated aq $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to obtain the nitrosated sialoside **7**, which was carried forward without any further purification. A solution of the crude nitrosated sialoside **7** (320 mg, 0.48 mmol) in dry dichloromethane (4.8 mL) and 2,2,2-trifluoroethanol (55 μL, 0.72 mmol) under argon at −10 °C was stirred for 30 min and treated with freshly prepared sodium isopropoxide in 2-propanol (0.2 N; 9.49 mL, 1.91 mmol). After being stirred for 3 min, the reaction mixture was treated with 1.7 N hydrazoic acid in chloroform[95] (5.61 mL, 9.54 mmol). The reaction mixture was further stirred at −10 °C for 10 min, warmed to 0 °C, and quenched with saturated aq $NaHCO_3$. It was then diluted with dichloromethane, washed with cold brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **27** (148 mg, 51%): $[\alpha]^{21}_{D}$ −106 (*c* 1.0, $CHCl_3$); ^{1}H

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

NMR (400 MHz, $CDCl_3$): δ 5.59 (dd, J = 3.6, 2.2 Hz, 1H), 5.32 (ddd, J = 11.7, 9.8, 4.8 Hz, 1H), 5.25−5.17 (m, 1H), 4.82 (dd, J = 12.5, 1.6 Hz, 1H), 4.29 (ddd, J = 15.8, 10.6, 4.7 Hz, 2H), 3.79 (s, 3H), 3.19 (t, J = 10.0 Hz, 1H), 2.67 (dd, J = 13.5, 4.8 Hz, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.03 (s, 1H), 2.02 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.85−1.72 (m, 4H), 1.62 (s, 6H), 1.25 (t, J = 7.1 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 169.8, 169.7, 169.4, 85.7, 72.3, 71.0, 70.5, 70.1, 62.6, 60.4, 52.8, 50.6, 43.3, 39.3, 35.9, 29.7, 21.1, 20.9, 20.7; ESI-HRMS ($C_{28}H_{39}N_3NaO_{11}S$) $[M + Na]^+$ m/z 648.2203, found 648.2187.

Acid-Washed Molecular Sieves. Molecular sieves (4 Å, 30 g) were soaked in 2 N HCl (80 mL) for 12 h. The mixture was concentrated under reduced pressure and then slurried with water (100 mL). The slurry was filtered and washed with water (200 mL). The resulting solid was dried at 254 °C for 24 h to give acid-washed molecular sieves (28 g), which were used directly for glycosylation.

General Coupling Protocol with Donors 13 or 27. A mixture of donor **13** or **27** (0.15 mmol), acceptor (0.18 mmol), and activated 4 Å acid-washed powdered molecular sieves (300 mg, 2 g/mmol of donor) in CH_2Cl_2/CH_3CN (2:1, 2 mL) was stirred for 2 h at room temperature, was cooled to −78 °C, and treated with NIS (42 mg, 0.18 mmol) and TfOH (2 μL, 0.02 mmol). The reaction mixture was stirred at −78 °C for 5 h and then quenched with triethylamine (7 μL). The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with 20% aqueous $Na_2S_2O_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with mixtures of EtOAc and hexane to afford the desired coupled products.

*Methyl (Cyclohexyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-gulo-non-2-ulopyranosid)onate (**21**).* Compound **21** was prepared according to the general glycosylation procedure using donor **13** (25 mg, 0.04 mmol) and compound **17** as acceptor (5 μL, 0.05 mmol) in CH_2Cl_2/CH_3CN (1.0 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), **21** (17.6 mg, 79%) was obtained as a white foam: $[\alpha]^{21}_D$ −11.6 (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.53−5.40 (dd, J = 6.0, 4.4 Hz, 1H), 5.26 (td, J = 5.8, 3.0 Hz, 1H), 4.92−4.85 (m, 1H), 4.36 (dd, J = 12.4, 2.9 Hz, 1H), 4.21 (dd, J = 12.4, 5.5 Hz, 1H), 3.96 (dd, J = 4.3, 0.9 Hz, 1H), 3.91 (d, J = 2.5 Hz, 1H), 3.75 (t, J = 3.5 Hz, 1H), 3.70−3.61 (m, 1H), 2.38 (dd, J = 12.8, 4.5 Hz, 1H), 2.20 (dd, J = 12.1, 6.5, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.87 (t, J = 7.3 Hz, 2H), 1.74−1.64 (m, 2H), 1.60 (d, J = 11.8 Hz, 2H), 1.47 (dd, J = 8.8, 4.0 Hz, 2H), 1.41−1.02 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.6, 170.1, 170.0, 169.9, 169.0 ($^3J_{C-H}$ = 6.9 Hz), 99.0, 74.2, 70.3, 70.1, 70.0, 69.1, 61.6, 58.9, 52.6, 34.5, 33.2, 32.8, 25.4, 24.4, 24.3, 21.0, 20.8, 20.7, 20.6; ESI-HRMS ($C_{24}H_{35}N_3NaO_{12}$) $[M + Na]^+$ m/z 580.2118, found 580.2109.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-gulo-non-2-ulopyranosid)onate]-(2 → 6)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**22**).* Compound **22** was prepared according to the general glycosylation procedure using donor **13** (45 mg, 0.07 mmol) and acceptor **18** (40.07 mg, 0.08 mmol) in CH_2Cl_2/CH_3CN (2.0 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **22** (47.5 mg, 72%) was obtained as a white foam: $[\alpha]^{21}_D$ −18.4 (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37−7.30 (m, 15H), 5.43 (dd, J = 6.3, 4.6 Hz, 1H), 5.26 (dd, J = 5.6, 3.0 Hz, 1H), 4.99−4.90 (m, 2H), 4.86 (d, J = 11.0 Hz, 1H), 4.78−4.67 (m, 3H), 4.63 (t, J = 7.8 Hz, 1H), 4.34 (d, J = 3.0 Hz, 1H), 4.32 (d, J = 3.0 Hz, 1H), 4.28 (d, J = 7.7 Hz, 1H), 4.20−4.14 (m, 1H), 3.95 (dd, J = 7.1 Hz, 3.7 Hz, 2H), 3.89 (d, J = 2.7 Hz, 1H), 3.80 (dt, J = 7.1, 3.7 Hz, 1H), 3.79−3.74 (m, 2H), 3.64−3.59 (m, 3H), 3.53 (d, J = 4.9 Hz, 3H), 2.38 (dd, J = 12.8, 4.6 Hz, 1H), 2.21 (t, J = 12.8 Hz, 1H), 2.16 (t, J = 4.5 Hz, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.0, 169.9, 169.8, 168.0 ($^3J_{C-H}$ = 6.6 Hz), 138.9, 138.8, 138.5, 128.3, 128.2, 128.1, 128.0, 127.6, 127.4, 127.4, 127.2, 105.0, 98.8, 82.5, 79.6, 75.1, 74.3, 73.2, 72.9, 72.6, 70.5, 70.3, 69.9, 68.9, 62.3, 61.5, 58.8, 57.0, 52.8, 32.2, 29.7, 20.9, 20.8, 20.7, 20.6; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$) $[M + Na]^+$ m/z 944.3429, found 944.3409.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-gulo-non-2-ulopyranosid)onate]-(2 → 3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**23**).* Compound **23** was prepared according to the general glycosylation procedure using donor **13** (40 mg, 0.06 mmol) and acceptor **19** (35.63 mg, 0.07 mmol) in CH_2Cl_2/CH_3CN (1.8 mL, 2:1) at −78 C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **23** (33.6 mg, 57%) was obtained as a white foam: $[\alpha]^{21}_D$ −11.6 (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43−7.15 (m, 15H), 5.39 (dd, J = 6.9, 3.4 Hz, 1H), 5.29−5.22 (m, 1H), 5.07−5.01 (m, 1H), 4.95 (d, J = 11.6 Hz, 1H), 4.83 (d, J = 11.1 Hz, 1H), 4.65 (d, J = 11.1 Hz, 1H), 4.53 (d, J = 11.7 Hz, 1H), 4.47 (d, J = 11.7 Hz, 1H), 4.41 (d, J = 11.6 Hz, 1H), 4.36−4.30 (m, 2H), 4.04 (dd, J = 12.4, 5.1 Hz, 1H), 3.98 (dd, J = 10.0, 2.9 Hz, 1H), 3.92 (s, 1H), 3.87 (d, J = 1.9 Hz, 1H), 3.83−3.80 (m, 1H), 3.78 (d, J = 2.8 Hz, 1H), 3.69 (s, 3H), 3.63 (m, 2H), 3.55 (s, 3H), 2.46 (t, J = 13.0 Hz, 1H), 2.21 (dd, J = 13.5, 4.9 Hz, 1H), 2.16 (d, J = 5.9 Hz, 1H), 2.08 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.9, 169.8, 169.6, 168.2 ($^3J_{C-H}$ = 7.1 Hz), 139.3, 138.5, 138.1, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.1, 104.9, 100.0, 77.5, 76.2, 75.1, 74.7, 73.5, 73.4, 70.2, 69.9, 69.7, 69.0, 68.9, 61.3, 59.3, 57.0, 52.8, 29.6, 20.9, 20.7, 20.6; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$) $[M + Na]^+$ m/z 944.3429, found 944.3412.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-gulo-non-2-ulopyranosid)onate]-(2 → 4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**24**).* Compound **24** was prepared according to the general glycosylation procedure using donor **13** (30 mg, 0.04 mmol) and acceptor **20** (30 mg, 0.05 mmol) in CH_2Cl_2/CH_3CN (1.2 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **24** (19 mg, 44%) was obtained as a colorless oil: $[\alpha]^{21}_D$ −10.2 (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53−7.06 (m, 15H), 5.54 (t, J = 5.6 Hz, 1H), 5.40−5.30 (m, 2H), 5.10 (s, 1H), 4.77 (d, J = 10.9 Hz, 1H), 5.40−5.30 (m, 2H), 4.73−4.66 (m, 1H), 4.60 (d, J = 11.0 Hz, 1H), 4.55 (d, J = 12.2 Hz, 1H), 4.42−4.36 (m, 1H), 4.31−4.25 (m, 1H), 4.22 (d, J = 7.3 Hz, 1H), 4.12 (s, 1H), 4.06 (dd, J = 12.3, 6.4 Hz, 1H), 3.98 (s, 3H), 3.92−3.80 (m, 2H), 3.68 (s, 3H), 3.58 (dd, J = 18.0, 10.9 Hz, 1H), 3.50 (s, 2H), 2.42−2.30 (m, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.1, 169.8, 169.8, 167.7 ($^3J_{C-H}$ = 7.2 Hz), 138.6, 137.8, 137.6, 128.4, 128.2, 128.1, 128.0, 127.6, 127.5, 104.9, 98.7, 78.3, 75.1, 73.5, 73.4, 72.6, 71.1, 69.4, 69.3, 68.9, 68.1, 67.3, 61.6, 60.0, 58.9, 57.1, 52.1, 21.0, 20.9, 20.7, 20.6; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$) $[M + Na]^+$ m/z 944.3429, found 944.3408.

*Methyl (Cyclohexyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (**28**). Method 1.* Compound **28** was prepared according to the general glycosylation procedure using donor **27** (40 mg, 0.06 mmol) and acceptor **17** (8 μL, 0.07 mmol) in CH_2Cl_2/CH_3CN (1.8 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **28** (28.4 mg, 81%) was obtained as a white foam.

Method 2. Compound **28** was also prepared according to the general glycosylation procedure using donor **44** (75 mg, 0.12 mmol) and acceptor **17** (15 μL, 0.15 mmol) in CH_2Cl_2/CH_3CN (3.0 mL, 2:1) at −78 °C. After chromatographic purification over silica gel (gradient elution of 2−30% EtOAc in hexanes), **28** (57.2 mg, 86%) was obtained as a white foam: $[\alpha]^{21}_D$ −9.2; (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.49 (d, J = 12.6 Hz, 1H), 4.21 (dd, J = 12.5, 4.4 Hz, 1H), 3.78 (t, J = 5.1 Hz, 3H), 3.69−3.60 (m, 1H), 3.19 (t, J = 10.2 Hz, 1H), 2.71 (dd, J = 12.7, 4.6 Hz, 1H), 2.17 (s, 3H), 2.14 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.88 (s, 1H), 1.71 (dd, J = 13.1 Hz, 8.7 Hz, 2H), 1.66 (d, J = 14.3 Hz, 2H), 1.55 (d, J = 10.1 Hz, 2H), 1.48 (d, J = 12.3 Hz, 2H), 1.33 (dd, J = 10.3, 6.3 Hz, 2H), 1.26−1.18 (m, 2H), 1.11 (d, J = 9.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 169.8, 169.7, 168.4 ($^3J_{C-H}$ = 6.2 Hz), 98.4, 74.3, 71.4, 71.1, 68.1, 67.9, 61.9, 60.1, 52.5, 38.0, 34.7, 33.0, 25.4, 24.2, 24.1, 21.0, 20.9, 20.8, 20.7; ESI-HRMS ($C_{24}H_{35}N_3NaO_{12}$) $[M + Na]^+$ m/z 580.2118, found 580.2109.

Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-galacto-non-2-ylopyranosid)onate]-(2 → 6)-2,3,4-tri-

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

*O-benzyl-β-D-galactopyranoside (**29**).* Compound **29** was prepared according to the general glycosylation procedure using donor **27** (45 mg, 0.07 mmol) and acceptor **18** (40.1 mg, 0.09 mmol) in CH_2Cl_2/CH_3CN (2.0 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **29** (48.8 mg, 74%) was obtained as a white foam: $[\alpha]^{21}_D$ −15.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43−7.14 (m, 15H), 5.48 (d, *J* = 9.1 Hz, 1H), 5.34 (dd, *J* = 9.0, 3.2 Hz, 1H), 4.96−4.92 (m, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.81(dd, *J* = 12.0, 4.9 Hz, 1H), 4.76−4.68 (m, 2H), 4.65 (t, *J* = 9.9 Hz, 1H), 4.30 (d, *J* = 2.1 Hz, 1H), 4.27 (dd, *J* = 5.1, 2.6 Hz, 1H), 4.18 (d, *J* = 4.3 Hz, 1H), 4.16 (d, *J* = 4.4 Hz, 1H), 3.88 (dd, *J* = 8.8, 5.7 Hz, 1H), 3.84 (d, *J* = 3.2 Hz, 1H), 3.80−3.75 (m, 2H), 3.61 (s, 3H), 3.55 (s, 3H), 3.50 (m, 2H), 3.28−3.18 (m, 1H), 2.70 (dd, *J* = 12.9 Hz, 4.8 Hz, 1H), 2.23 (d, *J* = 4.4 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.75 (t, *J* = 12.4 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 169.7, 169.7, 169.6, 167.4 ($^3J_{C-H}$ = 6.4 Hz), 138.8, 138.7, 138.5, 128.3, 128.2, 128.1, 128.0, 127.6, 127.5, 127.4, 127.2, 104.9, 99.0, 82.0, 79.5, 75.1, 74.2, 73.3, 72.9, 72.6, 71.6, 71.1, 68.0, 67.8, 52.9, 61.8, 59.9, 57.0, 52.8, 37.3, 21.0, 20.9, 20.8, 20.7; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$) $[M + Na]^+$ *m*/*z* 944.3429, found 944.3401.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-galacto-non-2-ulopyranosid)onate]-(2 → 3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**30**).* Compound **30** was prepared according to the general glycosylation procedure using donor **27** (35 mg, 0.05 mmol) and acceptor **19** (31.2 mg, 0.06 mmol) in CH_2Cl_2/CH_3CN (1.5 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **30** (33.9 mg, 66%) was obtained as a white foam: $[\alpha]^{21}_D$ −17.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50−6.99 (m, 15H), 5.45 (dd, *J* = 9.2, 1.1 Hz, 1H), 5.40 (ddd, *J* = 9.3, 3.9, 2.2 Hz, 1H), 4.90−4.84 (m, 2H), 4.80 (d, *J* = 11.2 Hz, 1H), 4.66 (d, *J* = 11.3 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.38 (d, *J* = 11.6 Hz, 1H), 4.30 (dd, *J* = 4.9, 2.7 Hz, 1H), 4.28 (d, *J* = 2.1 Hz, 1H), 4.02 (d, *J* = 4.0 Hz, 1H), 4.00 (d, *J* = 4.0 Hz, 1H), 3.96 (d, *J* = 2.9 Hz, 1H), 3.94 (d, *J* = 3.0 Hz, 1H), 3.70 (s, 3H), 3.64−3.59 (m, 4H), 3.54 (s, 3H), 3.51 (d, *J* = 3.9 Hz, 1H), 3.15 (t, *J* = 10.2 Hz, 1H), 2.61 (dd, *J* = 13.3, 4.8 Hz, 1H), 2.11 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.5, 169.4, 167.6 ($^3J_{C-H}$ = 6.8 Hz), 139.1, 138.8, 138.0, 128.3, 128.1, 128.0, 127.8, 127.7, 127.7, 127.4, 127.1, 104.9, 98.8, 77.5, 76.3, 76.2, 74.9, 74.8, 73.5, 73.1, 71.4, 71.2, 68.5, 68.2, 67.7, 61.6, 59.8, 57.1, 52.9, 41.7, 35.9, 35.4, 29.4, 28.5, 21.0, 20.8, 20.7, 20.4; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$) $[M + Na]^+$ *m*/*z* 944.3429, found 944.3403.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-azido-D-glycero-α-D-galacto-non-2-ulopyranosid)onate]-(2 → 4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**31**).* Compound **31** was prepared according to the general glycosylation procedure using donor **27** (40 mg, 0.064 mmol) and acceptor **15** (35.63 mg, 0.077 mmol) in CH_2Cl_2/CH_3CN (1.8 mL, 2:1) at −78 °C. After chromatographic purification on silica gel (gradient elution of EtOAc/hexanes 2−30%), compound **30** (23.2 mg, 41%) was obtained as a colorless oil: $[\alpha]^{21}_D$ −21.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.14 (m, 15H), 5.57 (s, 2H), 5.50−5.35 (m, 1H), 4.86−4.72 (m, 3H), 4.59 (d, *J* = 10.9 Hz, 1H), 4.47 (dd, *J* = 18.1, 12.2 Hz, 3H), 4.34−4.24 (m, 1H), 4.19 (d, *J* = 7.7 Hz, 1H), 4.13−4.02 (m, 2H), 3.89−3.81 (m, 1H), 3.81−3.72 (m, 1H), 3.62−3.54 (m, 1H), 3.49 (s, 3H), 3.44 (s, 3H), 3.31 (dd, *J* = 16.2, 10.9 Hz, 2H), 2.65 (dd, *J* = 12.9, 4.9 Hz, 1H), 2.23 (s, 3H), 2.12 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.4, 170.1, 169.4, 167.7 ($^3J_{C-H}$ = 6.9 Hz), 138.1, 137.3, 128.6, 128.6, 128.4, 128.2, 128.0, 127.9, 127.4, 127.4, 105.2, 98.4, 82.0, 78.5, 75.1, 73.4, 73.3, 72.5, 70.7, 69.6, 69.1, 68.3, 66.8, 62.0, 60.9, 57.1, 51.9, 38.7, 21.2, 20.9; ESI-HRMS ($C_{46}H_{55}N_3NaO_{17}$) $[M + Na]^+$ *m*/*z* 944.3429, found 944.3407.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-acetamido-D-glycero-α-D-gulo-non-2-ulopyranosid)onate]-(2 → 6)-2,3,4-tri-O-acetyl-β-D-galactopyranoside (**32**).* To a solution of **22** (50 mg, 0.06 mmol) in 10 mL of 1,4-dioxane/water (1:1) was added 50 mg of 5% Pd/C (100 wt %) followed by 0.18 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the solution was filtered off, and the solvents were evaporated. To the residue were added 5 mL of Ac_2O and 5 mL of pyridine, and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated, and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford the colorless oil **32** (35 mg, 82%): $[\alpha]^{21}_D$ −13.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.16 (d, *J* = 9.6 Hz, 1H), 5.61 (d, *J* = 3.3 Hz, 1H), 5.46 (dd, *J* = 7.0, 3.8 Hz, 1H), 5.17 (dd, *J* = 10.5, 7.9 Hz, 1H), 5.11−5.03 (m, 2H), 4.81−4.73 (m, 1H), 4.69−4.62 (m, 1H), 4.41 (t, *J* = 9.4 Hz, 1H), 4.21 (dt, *J* = 12.7, 6.4 Hz, 1H), 4.16 (dd, *J* = 11.8, 6.1 Hz, 1H), 3.97 (dd, *J* = 7.0, 1.2 Hz, 1H), 3.91−3.86 (m, 1H), 3.79 (s, 3H), 3.78−3.74 (m, 1H), 3.57−3.50 (m, 1H), 3.48 (s, 3H), 2.35 (dd, *J* = 13.4, 4.7 Hz, 1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1, 170.8, 170.4, 170.2, 170.1, 170.0, 169.6, 169.5, 167.5, 102.0, 99.3, 73.4, 71.5, 71.3, 71.1, 68.8, 68.6, 67.3, 67.1, 62.9, 61.0, 57.0, 53.0, 45.5, 32.8, 23.0, 23.0, 20.9, 20.8, 20.8, 20.7, 20.6, 20.6; ESI-HRMS ($C_{33}H_{47}NNaO_{21}$) $[M + Na]^+$ *m*/*z* 816.2538, found 816.2487.

*Methyl [Methyl (4,7,8,9-tetro-O-acetyl-3,5-dideoxy-5-acetamido-D-glycero-α-D-gulo-non-2-ulopyranosid)onate]-(2 → 3)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (**33**).* To a solution of **23** (30 mg, 0.03 mmol) in 6 mL of 1,4-dioxane/water (1:1) was added 30 mg of 5% Pd/C (100 wt %) followed by 0.1 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the solution was filtered off, and the solvents were evaporated. To the residue were added 3 mL of Ac_2O and 3 mL of pyridine, and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated, and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford the colorless oil **33** (19 mg, 77%): $[\alpha]^{21}_D$ −16.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.88 (d, *J* = 9.9 Hz, 1H), 5.41 (d, *J* = 7.1 Hz, 1H), 5.04 (d, *J* = 3.7 Hz, 1H), 5.02 (td, *J* = 5.9, 3.4 Hz, 1H), 4.89−4.81 (m, 2H), 4.63 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.40 (dd, *J* = 9.4, 3.9 Hz, 1H), 4.33 (d, *J* = 7.9 Hz, 1H), 4.22 (dd, *J* = 11.8, 5.5 Hz, 1H), 4.17−4.13 (m, 1H), 4.12−4.07 (m, 2H), 3.85 (s, 3H), 3.79−3.72 (m, 1H), 3.50 (s, 3H), 3.49 (d, *J* = 7.0 Hz, 1H), 2.40 (dd, *J* = 13.3, 4.9 Hz, 1H), 2.22 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 170.4, 170.3, 170.3, 170.2, 169.9, 169.8, 169.7, 167.9, 101.9, 96.8, 73.1, 72.0, 71.7, 71.2, 70.5, 69.3, 67.5, 67.5, 61.9, 60.9, 57.0, 53.1, 45.4, 32.4, 23.1, 21.3, 20.7, 20.7, 20.6, 20.6, 20.5, 20.5; ESI-HRMS ($C_{33}H_{47}NNaO_{21}$) $[M + Na]^+$ *m*/*z* 816.2538, found 816.2483.

*Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-acetamido-D-glycero-α-D-galacto-non-2-ulopyranosid)onate]-(2 → 6)-2,3,4-tri-O-acetyl-β-D-galactopyranoside (**34**).* To a solution of **29** (100 mg, 0.12 mmol) in 14 mL of 1,4-dioxane/water (1:1) was added 100 mg of 5% Pd/C (100 wt %) followed by 0.36 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the palladium catalyst was filtered off, and the solvents were evaporated. To the residue were added 7 mL of Ac_2O and 7 mL of pyridine, and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated, and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford **34** (72 mg, 84%): $[\alpha]^{21}_D$ −11.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.42 (d, *J* = 3.3 Hz, 1H), 5.36−5.31 (m, 1H), 5.26 (dd, *J* = 8.9, 1.8 Hz, 1H), 5.15 (dd, *J* = 10.2, 8.0 Hz, 1H), 5.05−5.00 (m, 1H), 4.86−4.79 (m, 1H), 4.43 (dd, *J* = 7.9, 1.1 Hz, 1H), 4.34−4.25 (m, 1H), 4.10−3.97 (m, 4H), 3.90 (t, *J* = 6.8 Hz, 1H), 3.77 (m, 1H), 3.76 (s, 3H), 3.51 (s, 3H), 3.42−3.35 (m, 1H), 2.50 (dd, *J* = 12.9, 4.6 Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H) 2.11 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.86 (s, 3H) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 170.6, 170.3, 170.2, 170.1, 170.00, 169.7, 169.6, 167.9, 101.9, 98.9, 72.6, 71.4, 71.1, 69.1, 68.7, 68.1, 67.2, 67.1, 62.7, 62.6, 57.0, 52.9, 49.3, 37.9, 23.2, 21.0, 20.8, 20.8, 20.7, 20.7, 20.6, 20.5; ESI-HRMS ($C_{33}H_{47}NNaO_{21}$) $[M + Na]^+$ *m*/*z* 816.2538, found 816.2474.

Methyl [Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-acetamido-D-glycero-α-D-galacto-non-2-ulopyranosid)onate]-(2 → 3)-2,4,6-tri-

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

*O-acetyl-β-D-galactopyranoside (**35**).* To a solution of **30** (90 mg, 0.11 mmol) in 12 mL of 1,4-dioxane/water (1:1) was added 90 mg of 5% Pd/C (100 wt %) followed by 0.29 mL of glacial acetic acid. The resulting mixture was stirred at room temperature under hydrogen gas (1 atm) for 16 h. Then the palladium catalyst was filtered off, and the solvents were evaporated. To the residue were added 6 mL of Ac_2O and 6 mL of pyridine, and the resulting mixture was stirred at room temperature for 10 h. Then the solvents were evaporated, and the residue was subjected to column chromatography on silica gel (5% MeOH in CH_2Cl_2) to afford **35** (64 mg, 84%): $[\alpha]^{21}_D$ −12.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.56−5.52 (m, 1H), 5.37 (dd, *J* = 9.2, 2.7 Hz, 1H), 5.08 (d, *J* = 10.3 Hz, 1H), 5.01 (dd, *J* = 10.1, 8.0 Hz, 1H), 4.91 (d, *J* = 3.3 Hz, 1H), 4.89−4.84 (m, 1H), 4.54 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.34 (dd, *J* = 12.5, 2.7 Hz, 1H), 4.09−3.97 (m, 4H), 3.85 (d, *J* = 6.6 Hz, 1H), 3.83 (s, 3H), 3.63 (dd, *J* = 10.7, 2.7 Hz, 1H), 3.51 (s, 3H), 2.57 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.21 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.84 (s, 3H) ; ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 170.6, 170.5, 170.4, 170.3, 170.3, 169.7, 169.6, 167.9, 101.7, 96.0, 72.0, 71.4, 70.5, 69.6, 69.3, 67.8, 67.7, 67.0, 62.3, 62.0, 56.9, 53.2, 49.1, 37.5, 23.2, 21.5, 21.0, 20.8, 20.8, 20.7, 20.7, 20.6; ESI-HRMS ($C_{33}H_{47}NNaO_{21}$) $[M + Na]^+$ *m*/*z* 816.2538, found 816.2502.

*Methyl [(3,5-Dideoxy-5-acetamido-D-glycero-α-D-gulo-non-2-ulopyranosid)onic acid]-(2 → 6)-β-D-galactopyranoside (**36**).* To a solution of **32** (30 mg, 0.06 mmol) in 3 mL of H_2O was added 0.5 mL of saturated aq $Ba(OH)_2$. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO_2, followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice−acetone bath and lyophilized to obtain the white foam **36** (16.8 mg, 92%): $[\alpha]^{21}_D$ −2.3 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.15 (dd, *J* = 9.7, 5.6 Hz, 1H), 3.87 (dd, *J* = 10.1, 8.3 Hz, 1H), 3.79 (d, *J* = 2.2 Hz, 1H), 3.76 (d, *J* = 3.0 Hz, 1H), 3.72 (t, *J* = 4.2 Hz, 1H), 3.68 (t, *J* = 3.5 Hz, 1H), 3.67 (m, 2H), 3.63 (d, *J* = 3.8 Hz, 1H), 3.53 (dd, *J* = 7.6, 2.5 Hz, 1H), 3.50 (d, *J* = 3.3 Hz, 1H), 3.48−3.45 (m, 1H), 3.43 (s, 3H), 3.37−3.30 (m, 1H), 2.36 (dd, *J* = 12.8, 4.6 Hz, 1H), 1.88 (s, 3H), 1.54 (t, *J* = 12.8 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.7, 173.6, 103.9, 100.8, 73.5, 72.5, 72.2, 72.1, 70.5, 70.4, 68.8, 66.7, 63.9, 62.1, 57.4, 50.4, 35.5, 22.1; ESI-HRMS ($C_{18}H_{30}NO_{14}$) $[M - H]^-$ *m*/*z* 484.1666, found 484.1683.

*Methyl [(3,5-Dideoxy-5-acetamido-D-glycero-α-D-gulo-non-2-ulopyranosid)onic acid]-(2 → 3)-β-D-galactopyranoside (**37**).* To a solution of **33** (10 mg, 0.02 mmol) in 2 mL of H_2O was added 0.2 mL of saturated aq $Ba(OH)_2$. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO_2, followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice−acetone bath and lyophilized to obtain the white foam **37** (5.7 mg, 93%): $[\alpha]^{21}_D$ −1.6 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.21 (d, *J* = 8.0 Hz, 1H), 4.13 (d, *J* = 3.6 Hz, 1H), 4.00 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.78 (d, *J* = 9.6 Hz, 1H), 3.68 (m, 4H), 3.57 (m, 3H), 3.53−3.46 (m, 2H), 3.41 (s, 3H), 2.42 (dd, *J* = 12.5, 4.4 Hz, 1H), 1.89 (s, 3H), 1.56 (t, *J* = 12.5 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.7, 173.8, 103.6, 99.6, 75.3, 74.8, 72.2, 71.8, 70.9, 69.3, 66.8, 66.6, 61.9, 60.9, 60.0, 57.1, 50.2, 35.6, 22.0; ESI-HRMS ($C_{18}H_{30}NO_{14}$) $[M - H]^-$ *m*/*z* 484.1666, found 484.1697.

*Methyl [(3,5-Dideoxy-5-acetamido-D-glycero-α-D-gulo-non-2-ulopyranosid)onic acid]-(2 → 3)-β-D-galactopyranoside (**38**).* To a solution of **34** (40 mg, 0.08 mmol) in 4 mL of H_2O was added 0.7 mL of saturated aq $Ba(OH)_2$. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO_2 followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice-acetone bath and lyophilized to obtain the white foam **38** (22.1 mg, 93%): $[\alpha]^{21}_D$ −1.4 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.14 (d, *J* = 6.8 Hz, 1H), 3.77 (dd, *J* = 6.7, 2.1 Hz, 1H), 3.71 (d, *J* = 10.5 Hz, 1H), 3.65 (t, *J* = 9.9 Hz, 1H), 3.61 (t, *J* = 4.3 Hz, 1H), 3.53 (m, 2H), 3.49−3.47 (m, 3H), 3.47−3.43 (m, 1H), 3.40 (s, 3H), 3.32 (t, *J* = 8.4 Hz, 1H), 2.56 (dd, *J* = 12.4, 3.5 Hz, 1H), 1.86 (s, 3H), 1.52 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.0, 173.4, 103.8, 100.4, 73.3, 72.6, 72.5, 71.7, 70.6, 68.6, 68.2, 68.1, 63.3, 62.6, 57.3, 51.8, 40.1, 22.0); ESI-HRMS ($C_{18}H_{30}NO_{14}$) $[M - H]^-$ *m*/*z* 484.1666, found 484.1692.

*Methyl [(3,5-Dideoxy-5-acetamido-D-glycero-α-D-galacto-non-2-ulopyranosid)onic acid]-(2 → 3)-β-D-galactopyranoside (**39**).* To a solution of **35** (50.0 mg, 0.1 mmol) in 5 mL of H_2O was added 0.8 mL of saturated aq $Ba(OH)_2$. The resulting solution was stirred at 60 °C for 2 h. Then the reaction mixture was brought to room temperature and bubbled with CO_2 followed by filtering off the precipitate formed. The filtrate was then frozen using a dry ice−acetone bath and lyophilized to obtain the white foam **39** (29.0 mg, 95%): $[\alpha]^{21}_D$ +0.3 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.21 (d, *J* = 7.4 Hz, 1H), 3.91 (dd, *J* = 7.3 Hz, 2.4 Hz, 1H), 3.77 (d, *J* = 6.5 Hz, 1H), 3.67 (m, 3H), 3.59−3.54 (m, 2H), 3.53−3.42 (m, 4H), 3.41 (s, 3H), 2.57 (d, *J* = 12.1 Hz, 1H), 1.85 (s, 3H), 1.62 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.9, 173.8, 103.4, 99.8, 75.8, 74.8, 72.8, 71.7, 69.1, 68.2, 68.0, 67.5, 62.5, 60.9, 57.00, 51.6, 39.5, 22.0; ESI-HRMS ($C_{18}H_{30}NO_{14}$) $[M - H]^-$ *m*/*z* 484.1666, found 484.1678.

*Methyl (1-Adamantyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-N-(1,1-dimethylethoxy)carbonyl-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (**40**).* To a stirred solution of **14** (1.5 g, 2.35 mmol) in anhydrous THF (10 mL) were added di-*tert*-butyl dicarbonate (5.11 g, 23.52 mmol) and DMAP (114 mg, 0.95 mmol) at room temperature. The mixture was stirred for 10 h at 60 °C under argon before it was cooled to room temperature and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with 35% EtOAc in hexanes to give **40** (1.3 g, 76%): $[\alpha]^{21}_D$ +42.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.32−5.22 (m, 2H), 5.19−5.10 (m, 1H), 4.86 (t, *J* = 10.5 Hz, 1H), 4.69 (d, *J* = 10.3 Hz, 1H), 4.32 (m, 1H), 4.04 (dd, *J* = 12.6, 3.7 Hz, 1H), 3.77 (s, 3H), 2.76 (m, 1H), 2.35 (s, 3H), 2.17 (s, 3H), 2.05 (s, 3H), 1.99 (m, 6H), 1.93 (s, 3H), 1.88 (s, 3H), 1.65 (m, 6H), 1.54 (s, 9H), 1.49 (m, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.9, 170.6, 170.1, 170.1, 169.9, 169.6, 151.8, 84.7, 84.3, 73.0, 69.0, 67.2, 66.5, 61.4, 52.6, 52.4, 51.2, 43.5, 41.2, 36.0, 27.9, 27.8, 26.7, 21.2, 20.1, 20.7, 20.6; ESI-HRMS ($C_{35}H_{51}NNaO_{14}S$) $[M + Na]^+$ *m*/*z* 764.2928, found 764.2936.

*Methyl (1-Adamantyl 5-azido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (**43**).* To a stirred solution of **40** (1.25 g, 1.68 mmol) in dry methanol (7 mL) was added a catalytic amount of sodium methoxide. The solution was stirred for 1 h at room temperature and then quenched with Amberlyst 15 ion-exchange resin. The mixture was filtered through Celite and concentrated under reduced pressure to give a crude preparation of **41** (0.9 g), which was dissolved in 8 mL of dry THF and was treated with 2 N HCl in diethyl ether (6 mL) at 0 °C. The resulting mixture was brought to room temperature and stirred for 5 h, and then the mixture was concentrated under reduced pressure to give a crude preparation of **42** (0.75 g, 1.6 mmol), which was dissolved in 1:1 MeOH/H_2O (16 mL) followed by addition of imidazole-1-sulfonyl azide hydrochloride (0.66 g, 3.2 mmol), K_2CO_3 (0.66 g, 4.8 mmol), and $CuSO_4.5H_2O$ (40 mg, 0.16 mmol). The reaction mixture was stirred at room temperature for 3 h. Thereafter, the solvent was evaporated, and the residue was purified by flash chromatography on silica gel eluting with 8% MeOH in $CHCl_3$ to afford the white sticky solid **43** (0.66 g, 86% for three steps): $[\alpha]^{21}_D$ +20.6 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 3.84 (s, 3H), 3.65 (m, 3H), 3.56−3.40 (m, 3H), 3.31 (m, 1H), 2.64 (dd, *J* = 8.3, 4.2 Hz, 1H), 2.08 (m, 9H), 1.72 (m, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 171.9, 84.6, 75.1, 71.8, 69.5, 68.9, 63.3, 62.8, 59.1, 52.2, 50.1, 43.4, 42.1, 41.7, 39.5, 35.7, 31.5, 29.9; ESI-HRMS ($C_{20}H_{31}N_3NaO_7S$) $[M + Na]^+$ *m*/*z* 480.1796, found 480.1775.

*Methyl (1-Adamantyl 4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (**44**). Method 1.* To a solution of **43** (420 mg, 1.30 mmol) in 8 mL of dry pyridine was added acetic anhydride (0.99 mL, 10.44 mmol). The resulting mixture was stirred under argon at room temperature for 4 h and then was diluted with ethyl acetate and washed successively with saturated aq $NaHCO_3$ solution, 1 N HCl, and again with saturated aq $NaHCO_3$ solution. The organic layer was then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by

DOI: 10.1021/acs.joc.6b02221
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column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **44** (480 mg, 84%).

Method 2. A solution of sialoside **14** (300 mg, 0.47 mmol) in dry dichloromethane (4.6 mL) was treated with dry pyridine (0.38 mL, 4.68 mmol) under argon and cooled to −10 °C. After the solution was stirred for 15 min, crushed nitrosyl tetrafluoroborate (218 mg, 1.87 mmol) was added in one portion. The reaction was stirred at −10 °C until TLC and MS showed complete conversion and then diluted with cold dichloromethane (25 mL) and washed with cold 1 N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under 10 °C to give the nitrosated sialoside **15**, which was carried forward without any further purification. A solution of the crude nitrosated sialoside **15** (320 mg, 0.48 mmol) in dry dichloromethane (4.8 mL) and 2,2,2-trifluoroethanol (55 μL, 0.72 mmol) under argon at −10 °C was stirred for 30 min and treated with freshly prepared sodium isopropoxide in 2-propanol (0.2 N; 9.49 mL, 1.91 mmol). After being stirred for 3 min, the reaction mixture was treated with 1.7 N hydrazoic acid in chloroform[95] (5.61 mL, 9.54 mmol). The reaction mixture was further stirred at −10 °C for 10 min, warmed to 0 °C, and quenched with saturated aq $NaHCO_3$. It was then diluted with dichloromethane, washed with cold brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 30% EtOAc in hexane to afford **44** (135 mg, 46%): $[\alpha]^{21}_D$ + 63.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 (d, *J* = 9.8 Hz, 1H), 5.28 (d, *J* = 9.8 Hz, 1H), 4.69 (t, *J* = 9.5 Hz, 1H), 4.25 (dd, *J* = 11.4, 4.6 Hz, 2H), 3.79 (s, 3H), 3.16 (t, *J* = 10.1 Hz, 1H), 2.75 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 1.86 (m, 9H), 1.65 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.0, 169.9, 169.6. 169.6, 84.1, 72.8, 71.2, 68.4, 67.6, 61.5, 60.1, 52.8, 51.2, 43.5, 39.3, 35.9, 29.9, 21.0, 20.9, 20.8; ESI-HRMS ($C_{28}H_{39}N_3NaO_{11}S$) $[M + Na]^+$ *m*/*z* 648.2203, found 648.2180.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02221.

^{1}H and ^{13}C spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the NIH (GM62160) for support of this work.

■ REFERENCES

(1) Knirel, Y. A.; Shashkov, A. S.; Tsvetkov, Y. E.; Jansson, P.-E.; Zaehringer, U. *Adv. Carbohydr. Chem. Biochem.* **2003**, *58*, 371−417.

(2) Zunk, M.; Kiefel, M. J. *RSC Adv.* **2014**, *4*, 3413−3421.

(3) Miyagi, T.; Yamaguchi, K. In *Comprehensive Glycoscience*; Kamerling, J. P., Ed.; Elsevier: Amsterdam, 2007; Vol. 3, pp 297−323.

(4) McNaught, A. D. *Carbohydr. Res.* **1997**, *297*, 1−92.

(5) Knirel, Y. A.; Rietschel, E. T.; Marre, R.; Zähringer, U. *Eur. J. Biochem.* **1994**, *221*, 239−245.

(6) Knirel, Y. A.; Vinogradov, E. V.; Shashkov, A. S.; Dmitriev, B. A.; Kochetkov, N. K.; Stanislavsky, E. V.; Mashilova, G. M. *Eur. J. Biochem.* **1986**, *157*, 129−138.

(7) Schoenhofen, I. C.; McNally, D. J.; Brisson, J.-R.; Logan, S. M. *Glycobiology* **2006**, *16*, 8C−14C.

(8) Liu, F.; Aubry, A. J.; Schoenhofen, I. C.; Logan, S. M.; Tanner, M. E. *ChemBioChem* **2009**, *10*, 1317−1320.

(9) Schoenhofen, I. C.; McNally, D. J.; Vinogradov, E.; Whitfield, D.; Young, N. M.; Dick, S.; Wakarchuk, W. W.; Brisson, J.-R.; Logan, S. M. *J. Biol. Chem.* **2006**, *281*, 723−732.

(10) Gulati, S.; Schoenhofen, I. C.; Whitfield, D. M.; Cox, A. D.; Li, J.; Michael, F.; Vinogradov, E. V.; Stupak, J.; Zheng, B.; Ohnishi, M.; Unemo, M.; Lewis, L. A.; Taylor, R. E.; Landig, C. S.; Diaz, S.; Reed, G. W.; Varki, A.; Rice, P. A.; Ram, S. *PLoS Pathog.* **2015**, *11*, No. e1005290.

(11) Watson, D. C.; Wakarchuk, W. W.; Gervais, C.; Durocher, Y.; Robotham, A.; Fernanades, S. M.; Schnaar, R. L.; Young, N. M.; Gilbert, M. *Glycoconjugate J.* **2015**, *32*, 729−734.

(12) Watson, D. C.; Leclerc, S.; Wakarchuk, W. W.; Young, N. M. *Glycobiology* **2011**, *21*, 99−108.

(13) Matthies, S.; Stallforth, P.; Seeberger, P. H. *J. Am. Chem. Soc.* **2015**, *137*, 2848−2851.

(14) Lee, Y. J.; Kubota, A.; Ishiwata, A.; Ito, Y. *Tetrahedron Lett.* **2011**, *52*, 418−421.

(15) Tsvetkov, Y. E.; Shashkov, A. S.; Knirel, Y. A.; Zähringer, U. *Carbohydr. Res.* **2001**, *331*, 233−237.

(16) Tsvetkov, Y. E.; Shashkov, A. S.; Knirel, Y. A.; Zähringer, U. *Carbohydr. Res.* **2001**, *335*, 221−243.

(17) Price, N. P. J.; Furukawa, T.; Cheng, F.; Qi, J.; Chen, W.; Crich, D. *J. Antibiot.* **2014**, *67*, 405−414.

(18) Zunk, M.; Williams, J.; Carter, J.; Kiefel, M. J. *Org. Biomol. Chem.* **2014**, *12*, 2918−2925.

(19) Williams, J. T.; Corcilius, L.; Kiefel, M. J.; Payne, R. J. *J. Org. Chem.* **2016**, *81*, 2607−2611.

(20) Williams, J. M. *Adv. Carbohydr. Chem. Biochem.* **1975**, *31*, 9−79.

(21) Shirai, R.; Nakamura, M.; Hara, S.; Takayanagi, H.; Ogura, H. *Tetrahedron Lett.* **1988**, *29*, 4449−4452.

(22) Schreiner, E.; Zbiral, E. *Liebigs Ann. Chem.* **1990**, *1990*, 581−586.

(23) Buda, S.; Crich, D. *J. Am. Chem. Soc.* **2016**, *138*, 1084−1092.

(24) Chou, W. K.; Dick, S.; Wakarchuk, W. W.; Tanner, M. E. *J. Biol. Chem.* **2005**, *280*, 35922−35928.

(25) Hassan, M. I.; Lundgren, B. R.; Chaumun, M.; Whitfield, D. M.; Clark, B.; Schoenhofen, I. C.; Boddy, C. N. *Angew. Chem., Int. Ed.* **2016**, *55*, 12018−12021.

(26) Hasegawa, A. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Niell, R. A., Eds.; Harwood Academic Publishers: Amsterdam, 1996; pp 277−300.

(27) Hasegawa, A.; Kiso, M. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 357−379.

(28) Boons, G.-J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539−4565.

(29) Kiso, M.; Ishida, H.; Ito, H. In *Carbohydrates in Chemistry and Biology: Chemistry of Saccharides*; Ernst, B., Hart, G. W., Sinaÿ, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. *1*, p 345−365.

(30) Magnusson, G.; Nilsson, U. J. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Kuniaki, T., Thiem, J., Eds.; Springer-Verlag: Berlin, 2001; Vol. *2*, p 1543−1587.

(31) De Meo, C.; Boons, G. J.; Demchenko, A. V. In *Comprehensive Glycoscience*; Kamerling, J., Ed.; Elsevier: Amsterdam, 2007; Vol. *1*, p 583−604.

(32) Kancharla, P. K.; Crich, D. *J. Am. Chem. Soc.* **2013**, *135*, 18999−19007.

(33) Dharuman, S.; Crich, D. *Chem. - Eur. J.* **2016**, *22*, 4535−4542.

(34) De Meo, C.; Priyadarshani, U. *Carbohydr. Res.* **2008**, *343*, 1540−1552.

(35) Tanaka, H.; Nishiura, Y.; Takahashi, T. *J. Am. Chem. Soc.* **2006**, *128*, 7124−7125.

(36) Crich, D.; Li, W. *J. Org. Chem.* **2007**, *72*, 2387−2391.

(37) Crich, D.; Li, W. *J. Org. Chem.* **2007**, *72*, 7794−7797.

(38) Farris, M. D.; De Meo, C. *Tetrahedron Lett.* **2007**, *48*, 1225−1227.

(39) Hsu, C.-H.; Chu, K.-C.; Lin, Y.-S.; Han, J.-L.; Peng, Y.-S.; Ren, C.-T.; Wu, C.-Y.; Wong, C.-H. *Chem. - Eur. J.* **2010**, *16*, 1754−1760.

(40) Mandhapati, A. R.; Rajender, S.; Shaw, J.; Crich, D. *Angew. Chem., Int. Ed.* **2015**, *54*, 1275−1278.

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630

(41) Edward, J. T. *Chem. Ind.* **1955**, 1102−1104.
(42) Foster, A. B.; Overend, W. G. *Chem. Ind.* **1955**, 566−567.
(43) Overend, W. G.; Rees, C. W.; Sequeira, J. S. *J. Chem. Soc.* **1962**, 3429−3440.
(44) Bülow, A.; Meyer, T.; Olszewski, T. K.; Bols, M. *Eur. J. Org. Chem.* **2004**, *2004*, 323−329.
(45) Jensen, H. H.; Bols, M. *Acc. Chem. Res.* **2006**, *39*, 259−265.
(46) Okada, Y.; Mukae, T.; Okajima, K.; Taira, M.; Fujita, M.; Yamada, H. *Org. Lett.* **2007**, *9*, 1573−1576.
(47) Walvoort, M. T. C.; Dinkelaar, J.; van den Bos, L. J.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *Carbohydr. Res.* **2010**, *345*, 1252−1263.
(48) Heuckendorff, M.; Pedersen, C. M.; Bols, M. *Chem. - Eur. J.* **2010**, *16*, 13982−13994.
(49) Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195−1201.
(50) Pedersen, C. M.; Marinescu, L. G.; Bols, M. *C. R. Chim.* **2011**, *14*, 17−43.
(51) Schneider, R.; Freyhardt, C. C.; Schmidt, R. R. *Eur. J. Org. Chem.* **2001**, *2001*, 1655−1661.
(52) Yu, C.-S.; Niikura, K.; Lin, C.-C.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2001**, *40*, 2900−2903.
(53) Mukaiyama, T.; Mandai, H.; Jona, H. *Chem. Lett.* **2002**, *31*, 1182−1183.
(54) Lu, K.-C.; Tseng, S.-Y.; Lin, C.-C. *Carbohydr. Res.* **2002**, *337*, 755−760.
(55) Navuluri, C.; Crich, D. *Angew. Chem.* **2013**, *125*, 11549−11552.
(56) Pinto, O.; Sardinha, J.; Vaz, P. D.; Piedade, F.; Calhorda, M. J.; Abramovitch, R.; Nazareth, N.; Pinto, M.; Nascimento, M. S. J.; Rauter, A. P. *J. Med. Chem.* **2011**, *54*, 3175−3187.
(57) Howells, R. D.; McCown, J. D. *Chem. Rev.* **1977**, *77*, 69−92.
(58) Stang, P. J.; Hanack, M.; Subramanian, L. R. *Synthesis* **1982**, *1982*, 85−126.
(59) Attolino, E.; Catelani, G.; D'Andrea, F. *Eur. J. Org. Chem.* **2006**, *2006*, 5279−5292.
(60) Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97−101.
(61) Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8273−8279.
(62) Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317−6320.
(63) Haverkamp, J.; Spoormaker, T.; Dorland, L.; Vliegenthart, J. F. G.; Schauer, R. *J. Am. Chem. Soc.* **1979**, *101*, 4851−4853.
(64) Prytulla, S.; Lauterwein, J.; Klessinger, M.; Thiem, J. *Carbohydr. Res.* **1991**, *215*, 345−349.
(65) Duus, J. O.; Gotfredsen, C. H.; Bock, K. *Chem. Rev.* **2000**, *100*, 4589−4614.
(66) Lahmann, M.; Oscarson, S. *Can. J. Chem.* **2002**, *80*, 889−893.
(67) Ando, H.; Koike, Y.; Ishida, H.; Kiso, M. *Tetrahedron Lett.* **2003**, *44*, 6883−6886.
(68) Tamai, H.; Ando, H.; Tanaka, H.-N.; Hosoda-Yabe, R.; Yabe; Ishida, H.; Kiso, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 2330−2333.
(69) Amarasekara, H.; Crich, D. *Carbohydr. Res.* **2016**, *435*, 113−120.
(70) Mannerstedt, K.; Ekelöf, K.; Oscarson, S. *Carbohydr. Res.* **2007**, *342*, 631−637.
(71) Kuznik, G.; Hörsch, B.; Kretzschmar, G.; Unverzagt, C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 577−580.
(72) Gong, J.; Liu, H.; Nicholls, J. M.; Li, X. *Carbohydr. Res.* **2012**, *361*, 91−99.
(73) Bock, K.; Duus, J. O. *J. Carbohydr. Chem.* **1994**, *13*, 513−543.
(74) Grindley, T. B. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer: Berlin, 2001; Vol. *1*, pp 3−51.
(75) Rao, V. S. R.; Qasba, P. K.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood Academic Publishers: Amsterdam, 1998.
(76) Tvaroska, I.; Taravel, F. R.; Utille, J. P.; Carver, J. P. *Carbohydr. Res.* **2002**, *337*, 353−367.
(77) Christian, R.; Schulz, G.; Brandstetter, H. H.; Zbiral, E. *Carbohydr. Res.* **1987**, *162*, 1−11.
(78) Brown, E. B.; Brey, W. S., Jr.; Weltner, W., Jr. *Biochim. Biophys. Acta, Gen. Subj.* **1975**, *399*, 124−130.
(79) Flippen, J. L. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1973**, *B29*, 1881−1886.
(80) Veluraja, K.; Rao, V. S. R. *Biochim. Biophys. Acta, Gen. Subj.* **1980**, *630*, 442−446.
(81) Sabesan, S.; Bock, K.; Lemieux, R. U. *Can. J. Chem.* **1984**, *62*, 1034−1045.
(82) Jensen, H. H.; Nordstrom, M.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205−9213.
(83) Moumé-Pymbock, M.; Furukawa, T.; Mondal, S.; Crich, D. *J. Am. Chem. Soc.* **2013**, *135*, 14249−14255.
(84) Balmond, E. I.; Benito-Alifonso, D.; Coe, D. M.; Alder, R. W.; McGarrigle, E. M.; Galan, M. C. *Angew. Chem., Int. Ed.* **2014**, *53*, 8190−8194.
(85) Zefirov, N. S.; Koz'min, A. S. *Acc. Chem. Res.* **1985**, *18*, 154−158.
(86) Kiyooka, S.-i.; Kaneno, D.; Fujiyama, R. *Tetrahedron* **2013**, *69*, 4247−4258.
(87) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217−11223.
(88) Weingart, R.; Schmidt, R. R. *Tetrahedron Lett.* **2000**, *41*, 8753−8758.
(89) Heuckendorff, M.; Bols, P. S.; Barry, C.; Frihed, T. G.; Pedersen, C. M.; Bols, M. *Chem. Commun.* **2015**, *51*, 13283−13285.
(90) Bohé, L.; Crich, D. *Carbohydr. Res.* **2015**, *403*, 48−59.
(91) Krenske, E. H.; Lam, S.; Ng, J. P. L.; Lo, B.; Lam, S. K.; Chiu, P.; Houk, K. N. *Angew. Chem., Int. Ed.* **2015**, *54*, 7422−7425.
(92) Stevens, C. L.; Glinski, R. P.; Taylor, K. G.; Blumbergs, P.; Sirokman, F. *J. Am. Chem. Soc.* **1966**, *88*, 2073−2074.
(93) Hanessian, S. *Chem. Commun.* **1966**, 796−798.
(94) Stevens, C. L.; Glinski, R. P.; Taylor, K. G.; Sirokman, F. *J. Org. Chem.* **1970**, *35*, 592−596.
(95) Hassner, A.; Fibiger, R.; Andisik, D. *J. Org. Chem.* **1984**, *49*, 4237−4244.

DOI: 10.1021/acs.joc.6b02221
J. Org. Chem. 2016, 81, 10617−10630