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Synthesis of an Aminooxy Derivative of the GM3 Antigen and Its Application in Oxime Ligation

Kristopher A. Kleski, Mengchao Shi, Matthew Lohman, Gabrielle T. Hymel, Vinod K. Gattoji, and Peter R. Andreana*

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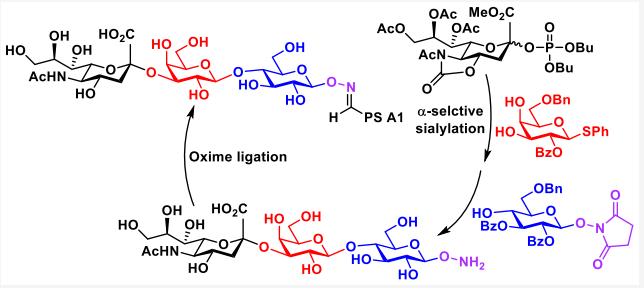
ABSTRACT: The anomeric aminooxy GM3 trisaccharide cancer antigen (Neu5Acα2,3Galβ1,4Glcβ-ONH$_2$) has been chemically synthesized using a linear glycosylation approach. The key step involves a highly α(2,3)-stereoselective sialylation to a galactose acceptor. The Neu5Acα2,3Gal intermediate was functionalized as a donor for a [2 + 1] glycosylation, including a glucose acceptor that featured an *O*-succinimidyl group on the reducing end as an aminooxy precursor. The fully deprotected anomeric aminooxy GM3 trisaccharide was then conjugated to the immunologically relevant zwitterionic polysaccharide PS A1 via an oxime link.



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Oxime ligation has emerged as an attractive conjugation method in vaccine formulation for a number of reasons: (1) oxime bonds are hydrolytically stable in physiological conditions,[1−3] (2) the oxime bond is immunologically "silent" as compared to other conjugation linkages,[4,5] and (3) the condensation of aminooxy functions are fast, quantitative, and only produce water as a byproduct.[6,7] Our group has used the oxime ligation strategy to create entirely carbohydrate immunogens capable of generating protective immune responses against tumor associated carbohydrate antigens (TACAs).[8−11] These glycoconjugates feature the unique zwitterionic polysaccharide PS A1 which is isolated from the commensal organism *Bacteroides fragilis* (ATCC 25285/NCTC 9343). The alternating charge character on the repeating unit of PS A1 provides distinct properties that permit binding to the MHC II complex on antigen presenting cells and downstream activation of CD4+ T cells through the MHC II:$\alpha\beta$TCR complex pathway.[12,13] Formation of these immunogens is materialized by the oxime condensation of a chemically derived aldehyde on PS A1 and with an aminooxy derivative of a chosen TACA (Figure 1). This vaccine platform has been successfully demonstrated with Tn-PS A1[8,14] and STn-PS A1,[10] where we observed robust IgG antibody production, cellular immunity, and tumor cell lysis.

Progress of these initial developments led us to expand our TACA toolbox to incorporate the ganglioside GM3 (Neu5Acα2,3Galβ1,4Glcβ-Cer). GM3 is overexpressed in carcinomas of the breast and skin and has implications in tumor cell proliferation and metastasis.[15,16] GM3 overexpression is also positively correlated with tumor malignancy, leading to poor prognosis.[16] Due to these characteristics, GM3 has been prioritized within the top 50 cancer antigens for immune intervention.[17]

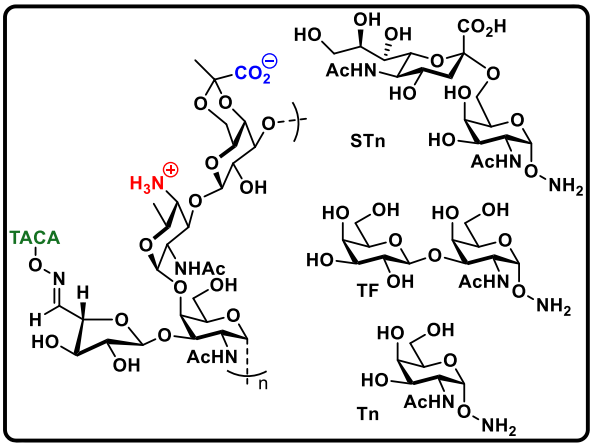

Figure 1. General structure of TACA-PS A1 glycoconjugates and aminooxy derivatives of TACAs.

The biological relevance of the GM3 antigen implicates necessity for preparative ease and availability for immunological experiments. Indeed, both chemo-enzymatic and chemical methods exist for the production of the GM3 antigen, but they have limitations.[18−25] Chemo-enzymatic methods offer excellent regio- and stereoselective control of

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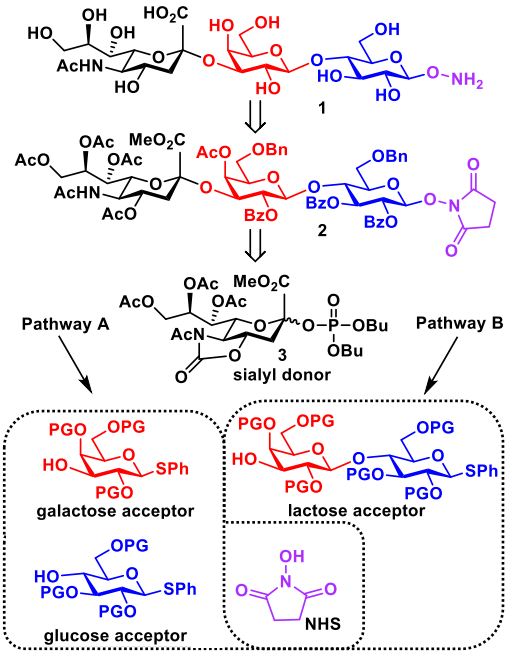
glycan production but are typically demonstrated on smaller scales.[19,26] On the other hand, synthetic reports of the GM3 glycan relay poor stereoselectivity when forming the challenging $\alpha(2 \rightarrow 3)$ glycosidic bond. Up to date, only one synthesis of the aminooxy GM3 glycan has been reported through a sialyltransferase catalyzed glycosylation.[19] Herein, we describe our chemical synthetic platform to produce the aminooxy GM3 glycan by using a linear approach that features a high α-selective sialylation in good yield. The final step involves a microwave assisted global deprotection of -OAc/-OBz protecting groups and an *O*-succinimidyl (OSu) protecting group to achieve the corresponding alcohol and aminooxy functions, respectively. The fully deprotected aminooxy GM3 glycan was then conjugated to PS A1 by oxime ligation to form the GM3-PS A1 glycoconjugate.

The synthetic route to the aminooxy GM3 glycan (**1**) must feature high α-selectivity in the Neu5Ac2,3Gal bond forming step. To achieve this, we elected to use the α-directing trans-fused 4*O*,5*N*-acetyl oxazolidinone ring system on the sialic acid donor.[10,27,28] This moiety exhibits strain on the cyclic system which destabilizes the formation of the glycosyl oxocarbenium ion intermediate, and instead, a β-triflate intermediate is more likely formed. This pushes the mechanism of glycosylation toward more of an S_N2-like associative mechanism as opposed to an unselective S_N1 dissociative mechanism.[29] Additionally, a highly reactive anomeric leaving group, such as a phosphate ester,[30,31] was desired based on our previous experiences.[10] These two features led us to adopt the sialyl phosphate donor **3** (Scheme 1, Scheme S1). The synthetic strategy can be

Scheme 1. Retrosynthetic Analysis of the Aminooxy GM3 Glycan



further broken down through pathway A or pathway B (Scheme 1). Reports that describe pathway A in the synthesis of the GM3 glycan discuss high yields and α-selectivity in the $\alpha(2 \rightarrow 3)$ bond forming step but feature a greater number of overall synthetic steps than pathway B.[24] Contemporary reports of GM3 synthesis describe pathway B with the use of lactosyl acceptors, but these reports describe low yields in the $\alpha(2 \rightarrow 3)$ bond forming step.[25] We must also account for the introduction of -OSu on the reducing end of the protected trisaccharide as the precursor for an aminooxy group.[9,10] This requires limited use of benzyl ether protecting groups, as long exposure times of hydrogenation can cause undesired cleavage of the -OSu N−O bond.[32] We proceeded to screen various glycosyl acceptors for the $\alpha(2 \rightarrow 3)$ bond forming step using sialyl phosphate **3** as the donor (Table 1).

We commenced with screening lactose acceptors **4** (Table 1, entry 1) and **5** (Table 1, entry 2, Scheme S2). Both acceptors feature a 3′,4′-diol and differ on their reducing ends with **4** containing thiophenol and **5** containing an *O*-succinimidyl. Both acceptors **4** and **5** provided the β-anomer as the major product with α:β ratios of 1:5 and 1:4, respectively. Poor selectivity is in line with previous reports and is a consequence of the disarming effect of acetyl group protections.[25,33] We then looked toward the 3,2′,3′,4′-unprotected lactoside **6** (Table 1, entry 3) which has been described to provide improved α-formation as compared to **4** and **5** due to the relatively weaker disarming effect of pivaloyl groups.[34] Acceptor **6** resulted in an improved α:β ratio at 1:1, although this ratio remained unsatisfactory. Based on these observed limitations of lactose acceptors, we investigated the use of galactose acceptor **7**[35] which featured a 3,4-diol with a 2-*O*-benzoyl-6-*O*-benzyl protection strategy (Table 1, entry 4). The resulting α:β ratio was drastically improved to provide the α-anomer in an 8:1 ratio in 89% yield. The greatly improved α:β ratio led us to explore galactose acceptor **8**[31] which contains the conformation restricting 4,6-*O*-benzylidene acetal (Table 1, entry 5). Galactose acceptor **8** provided excellent α-selectivity with an α:β ratio of 16:1 in 87% yield. It was logical at this point to proceed through pathway A using galactosyl acceptor **8**.

With disaccharide **13** in hand, we turned our attention to the D-glucose acceptor **16** as shown in Scheme 2. Here, thio-donor **14** was activated using *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of *N*-hydroxysuccinimide (NHS) to afford compound **15** in 84% yield as a pure β-anomer. Selective ring opening of the 4,6-benzylidene acetal on compound **15** was performed with triethylsilane (Et_3SiH) as the hydride source and boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) as the catalyst. Successful regioselective ring opening to afford **16** was confirmed using HMBC as the C-6 position nicely correlated with the benzylic protons on the benzyl ether.

Disaccharide **13** (Table 1, entry 5) was subjected to Zemplén sodium methoxide conditions to remove the oxazolidinone, and the resulting free hydroxyls were acetylated to provide compound **17**, as shown in Scheme 3. Interestingly, the C-2 *O*-benzoylated protection on compound **13** was not removed during the Zemplén method. This is likely due to the steric hindrance of this position, especially within disaccharide systems.[21] With disaccharide donor **17** and glucose acceptor **16** in hand, we sought to perform this [2 + 1] glycosylation under typical NIS/TMSOTf activation conditions. TLC analysis of the reaction mixture revealed partial consumption of the donor (R_f = 0.4) accompanied by the presence of a new spot (R_f = 0.2). Investigation of the reaction revealed the new spot to be the glycosyl succinimide product (**S6**). Indeed, this activation system produces the weakly nucleophilic succinimide as a byproduct, which can outcompete weakly nucleophilic or sterically hindered alcohols on glycosyl acceptors. This phenomenon has been described for donor/

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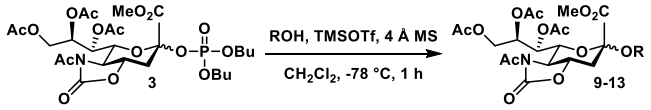
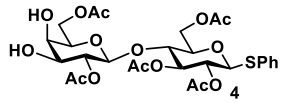
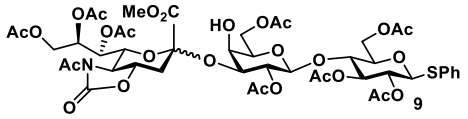
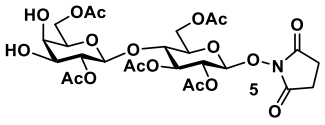
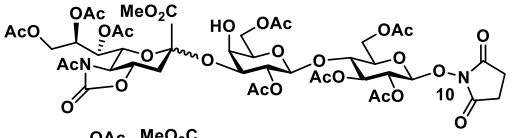
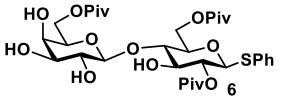
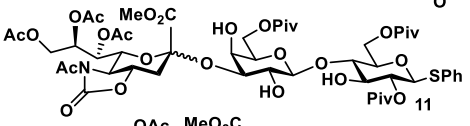
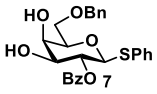
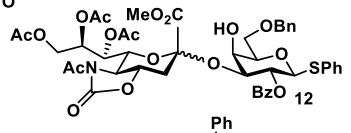
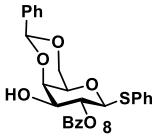
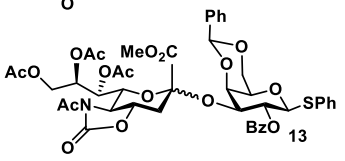
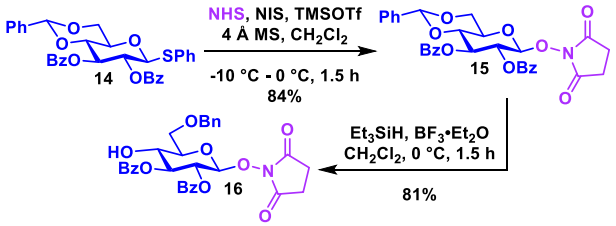
Table 1. Optimization of $\alpha(2,3)$-Glycosylation[a]



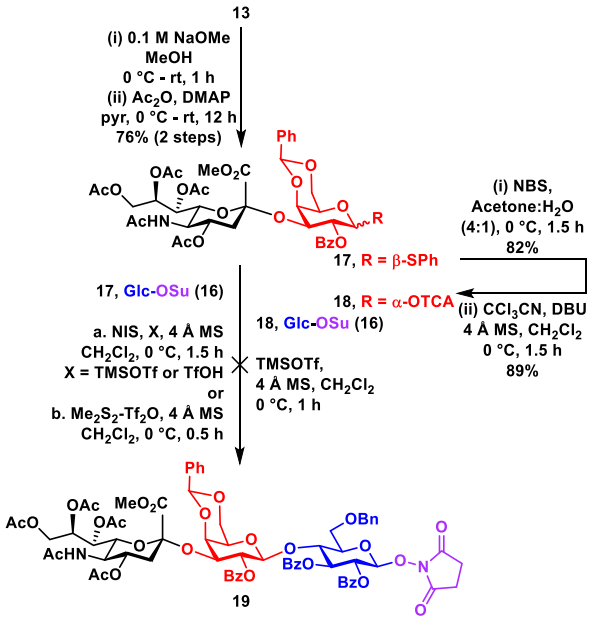
entry	acceptor	product	yield[b]	α:β ratio[c]
1			68%	1:5
2			81%	1:4
3			52%	1:1
4			89%	8:1
5			87%	16:1

[a]Typical conditions: 1.5 equiv of donor, 1.0 equiv of acceptor, 1.5 equiv of TMSOTf, in dry CH_2Cl_2 at −78 °C for 1 h. [b]Isolated yield. [c]Determined by ^1H NMR spectroscopic analysis of the unpurified reaction mixture.

Scheme 2. Synthesis of Glc-OSu Acceptor 16



Scheme 3. Attempted 2 + 1 Glycosylation Through Intermediate 13



acceptor pairs with mismatched reactivities.[36] Notably, we also discovered partial proton exchange with trimethyl silane on acceptor **16**. We attempted the [2 + 1] coupling using different activation systems such as NIS/trifluoromethanesulfonic acid (TfOH), dimethyl disulfide-triflic anhydride (Me_2S_2-Tf_2O), and a trichloroacetamidate (TCA) donor (**18**). The results led to a glycosyl succinimide byproduct, a complex mixture, and the Chapman rearrangement,[37] respectively (Scheme 3). These experiments offer further evidence of the torsional disarming effect from 4,6-*O*-acetal bicyclic donors.[38]

We sought to deviate the synthetic route through the more armed disaccharide donor compound **12** (Table 1, entry 4). As illustrated in Scheme 4, preparation of the disaccharide donor **20** mirrored the preparation of donor **17**, with the C-2-*O*-benzoyl protection remaining intact. Donor **20** was then activated with the NIS/TfOH system in the presence of acceptor **16** and provided the desired, fully protected trisaccharide **2** in 87% yield as the β-anomer. No evidence of the glycosyl succinimide was present.

Deprotection of key intermediate **2** began with the cleavage of the benzyl ethers using hydrogen gas at 1 atm and a suspension of Pearlman's catalyst. TLC indicated full consumption of starting material after 1 h of mixing, and the reaction was purged with argon gas to remove dissolved hydrogen gas. The reaction was filtered, concentrated, and passed through a short silica column to afford the debenzylated product **S7** in 89% yield with no evidence of -OSu N−O cleavage. Deprotection of the Neu5Ac C-1 methyl ester was

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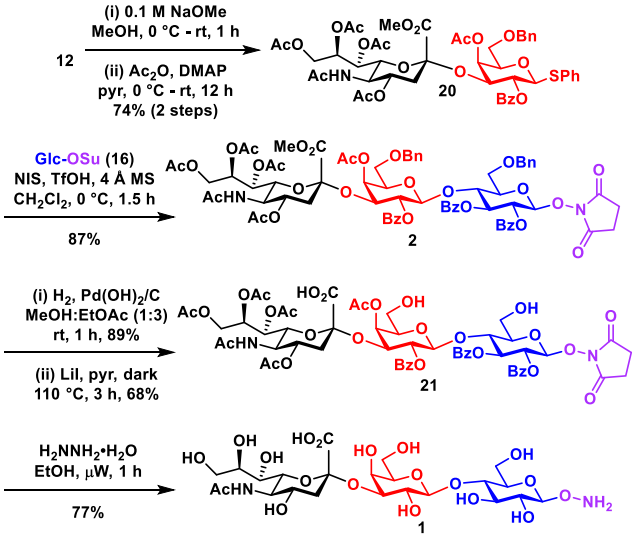
Scheme 4. Synthesis of the Aminooxy GM3 Glycan

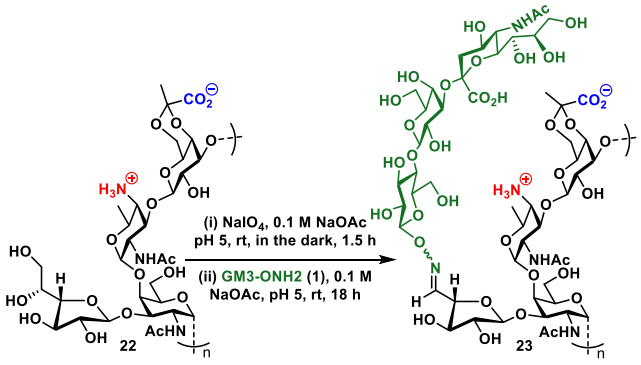


accomplished using the Krapcho-like demethylation by lithium iodide in pyridine.[10] This mixture was refluxed at 110 °C for 3 h in the absence of light. Pyridine was then removed *in vacuo* and the crude material was purified by silica flash chromatography to afford compound **21** in 68% yield.

In the final deprotection step, we utilized excess hydrazine-hydrate to remove the remaining -OAc/-OBz protecting groups and to remove -OSu to expose the aminooxy functionality. However, based on the results of Zemplén sodium methoxide conditions as described for **12** and **13**, we did not expect deprotection of the C-2-*O*-benzoylatd positions using hydrazine hydrate at room temperature. Additionally, literature precedent suggested long reaction times[24] and elevated temperatures[25] in hydroxide or methoxide conditions for C-2-*O*-benzoyl removal, which would then cause an undesirable ring opening of the installed -OSu group and failure of aminooxy deprotection. To overcome this limitation, we turned to microwave assistance in the final deprotection step. Compound **22** was dissolved in ethanol followed by the addition of excess hydrazine hydrate. The mixture was then stirred for 1 h at room temperature and MS analysis did not detect any formation of product. The reaction was then subjected to microwave irradiation at 200 W with constant temperature of 40 °C for 20 min. Once again, MS analysis indicated little-to-no deprotection of compound **21**. We then increased the temperature to a constant 90 °C for 30 min with no change in maximum Watts. Following this procedure, MS analysis of the reaction mixture revealed partial deprotection of **21**. Repetitive cycling (flow-chemistry techniques) of the aforementioned conditions led to MS analysis clearly depicting fully deprotected **21**. The reaction mixture was then concentrated, resuspended in water, and passed through a P2 BioGel column using water as the eluent. Fractions identified to contain compound **1** by TLC staining were collected, frozen, and lyophilized. The resulting white solid was characterized and compound **1** was definitively confirmed by NMR and HRMS (See Supporting Information).

With the aminooxy GM3 glycan complete, we pursued oxime ligation with PS A1 (Scheme 5, **22**). The repeating unit of PS A1 contains the side chain D-galactofuranose with a 1° and 2° vicinal diol that can be chemo- and regioselectively oxidized by sodium periodate to afford an aldehyde.[8−11] After

Scheme 5. Synthesis of GM3-PS A1



PS A1 oxidation, the aldehyde moiety was then subjected to 4 equiv of **1** and subsequently stirred for 18 h in a 0.1 M sodium acetate buffer at a pH of 5. The reaction mixture was then filtered and rinsed through a 10 kDa molecular weight cutoff filter. The filtered material was then resuspended in water, frozen, and lyophilized to produce **23** as a white (cotton-like) substance. NMR analysis of this material at 60 °C provided the evidence we needed to note successful conjugation with PS A1. The defining peaks included an oxime proton peak (δ = 7.99−8.00), an additional anomeric proton peak (δ = 5.37), an additional *N*-acetyl peak (δ = 2.31), and the presence of the Neu5Ac H-3eq (δ = 3.03−3.06) and H-3ax peaks (δ = 2.04−2.08). Based on integration analysis of the Neu5Ac H-3eq and PS A1 AAT methyl peak, we determined the relative ratio of GM3:PS A1 to be 1:2.33, which translates into approximately 51.5 GM3 conjugation sites per PS A1.

In conclusion, we have discussed the first fully chemical synthetic method to provide the aminooxy GM3 glycan. This synthesis features highly α-selective chemistries in the formation of the Neu5Ac(2 → 3)Gal bond. In these experiments, we showed the benefit of using the armed galactose acceptors as opposed to the disarmed lactose acceptors, which gave undesirable α:β ratios. This synthesis also features a three-step deprotection strategy of the fully protected trisaccharide. Notably, the final step utilized microwave assisted chemistry to deprotect the sterically hindered C-2 *O*-benzoylations in excellent yield, after reiterations. Lastly, we highlighted the utility of the aminooxy functionality by conjugating the GM3 glycan to the immunologically relevant PS A1 to generate the GM3-PS A1 glycoconjugate. The GM3-PS A1 vaccine candidate will be investigated for its immunological characteristics in due time.

EXPERIMENTAL SECTION

General Information. Relevant reagents and solvents were purchased from commercial sources and used without further purification. Molecular sieves (4 Å) were dried overnight in a vacuum oven set to 140 °C, further dried under high vacuum using a heat gun for 5 min, and cooled to room temperature under high vacuum. Synthesized compounds were purified using flash chromatography with SiliCycle Inc. 60 Å 230−400 mesh silica gel or size exclusion chromatography with Bio-Gel P-2 (Bio-Rad Laboratories, Inc.). Thin layer chromatography (TLC) was performed with SiliCycle Inc. silica gel TLC 250 μm w/h F-254. Proton and carbon NMR spectra were recorded using a Bruker Avance III 600 Ultrafield Cryoprobe spectrometer. $CDCl_3$, CD_3OD, and D_2O were used as solvents, and the chemical shifts were referenced relative to residual solvent. Low resolution mass spectrometry data were taken on an LCQ Deca ESI-

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MS machine. High resolution mass spectrometry data were collected using an Orbitrap Fusion Tribrid Mass Spectrometer.

Experimental Procedures. *Methyl (Phenyl-5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-2-thio-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (**S2**).* Compound **S1**[28] (5.20 g, 8.91 mmol, 1.0 equiv) was suspended in 75 mL of MeOH with vigorous stirring. To the suspension was added $MeSO_3H$ (2.90 mL, 44.6 mmol, 5.0 equiv) at room temperature, and the suspension was refluxed for 24 h. The mixture was then cooled to room temperature, and the reaction quenched with excess TEA. The solution was then concentrated under reduced pressure and dried under vacuum. The resulting crude concentrate was then dissolved in 50 mL (the bars over the zeroes represent standard notation for significant figures) of MeCN and 100 mL of H_2O followed by the addition of $NaHCO_3$ (3.74 g, 44.6 mmol, 5.0 equiv). The mixture was stirred vigorously to a uniform suspension and cooled to 0 °C. A separate solution of triphosgene (7.93 g, 26.7 mmol, 3.0 equiv) dissolved in 50 mL of MeCN was then added to the vigorously stirred suspension via dropping funnel at a slow rate of ∼1 drop/5 s. The reaction was maintained at 0 °C for 5 h. EtOAc (100 mL) was then added to the mixture and vigorously stirred for 10 min. The organic and aqueous layers were separated, and the aqueous layer was extracted with EtOAc (100 mL, 2×). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was passed through a short, thick bed of silica eluting with EtOAc:MeOH (9:1). Fractions containing the intermediate were pooled and concentrated under reduced pressure and then set under high vacuum overnight to give the crude intermediate (3.02 g, 7.56 mmol, 85% yield of crude). The crude material was then dissolved in pyridine (35 mL) followed by the addition of DMAP (0.18 g, 1.5 mmol, 0.2 equiv). Gas was exchanged with Ar and the reaction mixture was cooled to 0 °C with vigorous stirring. Ac_2O (14.3 mL, 151 mmol, 20 equiv) was then injected into the reaction mixture and the reaction was stirred for 12 h while the reaction was allowed to slowly warm to room temperature. The reaction was then quenched with MeOH at 0 °C and then concentrated under reduced pressure followed by codistillation with toluene (3×). The crude material was then dissolved in CH_2Cl_2 (50 mL) and washed with saturated $NaHCO_3$ (50 mL, 3×). The resulting organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was then purified by silica flash chromatography eluting with hexanes:EtOAc (1:1) to give compound **S2** as a white solid (4.00 g, 7.05 mmol, 79% yield over 3 steps). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.96 (3H, s), 2.09 (3H, s), 2.15 (3H, s), 2.34 (1H, t, J = 13.0 Hz), 2.53 (3H, s), 2.91 (1H, dd, J = 3.7, 13.1 Hz), 3.63 (3H, s), 3.76 (1H, dd, J = 9.2, 11.3 Hz), 3.90 (1H, dd, J = 8.2, 12.1 Hz), 4.37 (1H, dd, J = 2.5, 12.0 Hz), 4.78 (1H, td, J = 4.3, 15.0 Hz), 4.89 (1H, dd, J = 11.7, 6.6 Hz), 5.00 (1H, td, J = 2.2, 8.2 Hz), 5.56 (1H, t, J = 2.3 Hz), 7.36 (2H, m), 7.43 (1H, m), 7.50 (2H, m). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.7, 20.8, 21.1, 24.7, 36.0, 52.8, 59.6, 62.9, 72.7, 73.9, 75.1, 75.7, 88.4, 128.2, 129.2, 130.2, 136.8, 153.6, 167.8, 169.7, 170.4, 171.2, 172.5. LRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{25}H_{29}NO_{12}SNa$ 590.1; Found 590.2.

*Methyl (5-Acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-2-(dibutylphosphoryl)-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulopyranoside)onate (**3**).* Compound **S2** (1.00 g, 1.76 mmol, 1.0 equiv) was dried by codistillation with toluene (3×) followed by high vacuum for 3 h. The dry compound was then dissolved in 20 mL of dry CH_2Cl_2 followed by the addition of 4 Å molecular sieves (4 g) and dibutyl phosphate (1.75 mL, 8.80 mmol, 5.0 equiv). Gas in the reaction vessel was exchanged with Ar and this solution was then stirred for 30 min at room temperature, followed by the addition of NIS (1.19 g, 5.28 mmol, 3.0 equiv). Gas was then exchanged with Ar again, and the reaction was stirred at room temperature for 12 h. After 12 h, the reaction was diluted with CH_2Cl_2 (30 mL) and quenched with a saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ (30 mL). The quenched reaction was then filtered, and the collected organic layer was washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ (30 mL, 2×). The resulting organic layer was washed with brine, dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude compound was then purified via flash chromatography using hexanes:EtOAc (1:1) to give sialyl donor **3** as an anomeric mixture and as a yellow oil (1.05 g, 1.57 mmol, 89% yield). (**3α**) ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.96 (6H, t, J = 7.4 Hz), 1.43 (4H, m), 1.68 (4H, m), 2.06 (3H, s), 2.13 (3H, s), 2.17 (3H, s), 2.52 (3H, s), 2.71 (1H, t, J = 12.7 Hz), 3.03 (1H, dd, J = 4.0, 12.2 Hz), 3.86 (3H, s), 3.89 (1H, t, J = 10.1 Hz), 4.14 (6H, m), 4.43 (1H, dd, J = 2.8, 12.3 Hz), 4.78 (1H, dd, J = 1.4, 9.5 Hz), 5.34 (1H, m), 5.69 (1H, dd, J = 1.4, 7.5 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 13.6, 18.6, 18.7, 20.8, 20.8, 21.0, 24.7, 32.1, 32.1, 32.1, 35.9, 35.9, 53.5, 58.3, 62.5, 68.0, 68.1, 68.1, 68.2, 69.8, 71.5, 74.2, 98.2, 98.2, 153.6, 167.3, 167.3, 169.9, 170.0, 170.7, 171.9. LRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{27}H_{42}NO_{16}PNa$ 690.2; Found 690.4. (**3β**) ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 0.96 (6H, dt, J = 4.2, 7.4 Hz), 1.42 (4H, m), 1.68 (4H, m), 2.05 (3H, s), 2.12 (3H, s), 2.15 (3H, s), 2.32 (1H, dt, J = 2.8, 12.7 Hz), 2.52 (3H, s), 2.91 (1H, dd, J = 3.7, 12.8 Hz), 3.79 (1H, dd, J = 9.5, 11.3 Hz), 3.88 (3H, s), 4.13 (5H, m), 4.56 (1H, dd, J = 2.6, 12.2 Hz), 4.60 (1H, td, J = 3.7, 11.4, 12.6 Hz), 4.75 (1H, dd, J = 2.0, 9.5 Hz), 5.28 (1H, m), 5.64 (1H, dd, J = 2.0, 4.0 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 13.6, 18.6, 18.6, 20.8, 21.0, 24.7, 32.1, 32.1, 32.1, 32.2, 36.1, 36.1, 53.5, 58.9, 62.9, 68.4, 68.4, 68.5, 68.6, 71.8, 72.6, 74.0, 76.7, 98.8, 98.9, 153.5, 165.6, 169.8, 170.6, 170.7, 172.2. LRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{27}H_{42}NO_{16}PNa$ 690.2; Found 690.4.

*Phenyl (2,6-Di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside (**4**).* Acceptor **4** was prepared as previously described.[39] Acceptor **4** was purified by silica flash chromatography using 2% MeOH in CH_2Cl_2 as the eluent to provide **4** as a white solid (0.50 g, 0.78 mmol). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 2.03 (3H, s), 2.08 (3H, s), 2.09 (3H, s), 2.10 (3H, s), 2.11 (3H, s), 3.59 (1H, dd, J = 3.5, 9.8 Hz), 3.62 (1H, t, J = 6.5 Hz), 3.69 (2H, m), 3.86 (1H, d, J = 3.0 Hz), 4.16 (1H, dd, J = 5.5, 11.8 Hz), 4.22 (1H, dd, J = 6.6, 11.4 Hz), 4.32 (2H, m), 4.51 (1H, dd, J = 1.9, 12.0 Hz), 4.69 (1H, d, J = 10.1 Hz), 4.89 (1H, dd, J = 8.0, 9.8 Hz), 4.93 (1H, t, J = 9.7 Hz), 5.19 (1H, t, J = 8.9 Hz), 7.31 (3H, m), 7.47 (2H, m). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 21.1, 21.1, 21.2, 21.2, 62.7, 68.8, 70.4, 72.6, 73.0, 73.7, 74.1, 76.5, 77.1, 85.9, 101.2, 128.5, 129.2, 133.0, 169.9, 170.8, 170.8, 171.3, 171.7. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{28}H_{36}O_{15}SNa$ 667.1667; Found 667.1769.

*Trichloroacetimidatyl (2,6-Di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**S4**).* Compound **S3**[39] (0.67 g, 0.98 mmol, 1.0 equiv) was dissolved in an acetone:H_2O (4:1, 10 mL) mixture and then cooled to 0 °C. While stirring the mixture, NBS (0.52 g, 2.9 mmol, 3.0 equiv) was added to the reaction gradually over the duration of 30 min. The reaction was then stirred for an additional 30 min at room temperature. The solution was then concentrated under reduced pressure until the solution became turbid. The turbid mixture was diluted with CH_2Cl_2 (20 mL) and then quenched and washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ (15 mL, 3×). The organic layer was then washed with brine, dried with Na_2SO_4, filtered, and then concentrated under reduced pressure to give the crude hemiacetal intermediate as a white solid (0.51 g, 0.86 mmol, 88% yield of crude). The crude hemiacetal was then codistilled with toluene under reduced pressure (3×) and set under high vacuum for 3 h. The crude hemiacetal was then dissolved in dry CH_2Cl_2 (10 mL) followed by the addition of 4 Å molecular sieves (2 g) and trichloroacetonitrile (345 μL, 3.44 mmol, 4.0 equiv), and then gas was exchanged with Ar. The solution was stirred at room temperature for 30 min and was then cooled to 0 °C followed by the addition of DBU (26 μL, 0.17 mmol, 0.2 equiv). The reaction was then stirred for 1 h at 0 °C. The reaction was filtered and then concentrated under reduced pressure to provide the crude lactosyl donor. The crude material was then purified by silica flash chromatography using toluene:acetone (3:2) as the eluent to provide lactosyl donor **S4** as a white solid (0.44 g, 0.60 mmol, 62% yield over 2 steps). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.31 (3H, s), 1.54 (3H, s), 2.01 (3H, s), 2.07 (3H, s), 2.08 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 3.83 (1H, t, J = 9.8 Hz), 3.93 (1H, m), 4.14 (3H, m), 4.19 (1H, dd, J = 4.5, 12.2 Hz), 4.31 (2H, m), 4.41 (1H, d, J = 7.3 Hz), 4.43 (1H, dd, J = 2.0, 12.2 Hz), 4.85 (1H, dd, J = 6.1, 7.3 Hz),

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5.06 (1H, dd, J = 3.8, 10.1 Hz), 5.55 (1H, t, J = 9.8 Hz), 6.48 (1H, d, J = 3.8 Hz), 8.64 (1H, s). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.7, 21.0, 21.0, 21.0, 21.1, 26.2, 27.4, 61.9, 63.3, 69.3, 70.1, 71.0, 71.2, 72.9, 73.1, 75.8, 77.0, 90.8, 93.1, 100.8, 111.0, 161.1, 169.4, 169.8, 170.3, 170.5, 170.9. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{27}H_{36}Cl_3NO_{16}Na$ 758.1; Found 758.3.

*Succinimidyl (2,6-Di-O-acetyl-3,4-O-isopropylidene-β-D-galacto-pyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**S5**).* Lactosyl donor **S4** (80 mg, 0.11 mmol, 1.0 equiv) and NHS (63 mg, 0.55 mmol, 5.0 equiv) were codistilled with toluene under reduced pressure (3×) followed by high vacuum overnight. The mixture was then dissolved in 5.0 mL of dry CH_2Cl_2 and then 4 Å molecular sieves (0.2 g) were added to the solution. Gas was then exchanged with Ar and the solution was stirred at room temperature for 30 min. TMSOTf (4.0 μL, 0.022 mmol, 0.2 equiv) was then introduced into the mixture at room temperature, and the reaction was stirred for 30 min. The reaction was then quenched with TEA, filtered, and then concentrated under reduced pressure. The crude material was then purified by silica flash chromatography using 1% MeOH in CH_2Cl_2 to provide compound **S5** as a white solid (69 mg, 0.10 mmol, 90% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.31 (3H, s), 1.53 (3H, s), 2.08 (3H, s), 2.08 (3H, s), 2.10 (3H, s), 2.12 (3H, s), 2.12 (3H, s), 2.12 (3H, s), 2.73 (4H, s), 3.74 (1H, m), 3.94 (1H, m), 4.10 (1H, dd, J = 8.5, 10.0 Hz), 4.14 (2H, m), 4.19 (1H, dd, J = 5.8, 12.0 Hz), 4.27 (1H, dd, J = 7.5, 11.7 Hz), 4.33 (1H, dd, J = 4.7, 11.7 Hz), 4.39 (1H, d, J = 7.7 Hz), 4.41 (1H, dd, J = 2.3, 12.0 Hz), 4.86 (1H, dd, J = 6.4, 7.6 Hz), 5.13 (1H, d, J = 6.3 Hz), 5.17 (1H, t, J = 6.8 Hz), 5.20 (1H, t, J = 7.9 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.8, 20.9, 21.0, 21.0, 25.5, 26.3, 27.5, 62.1, 63.3, 70.2, 71.1, 72.5, 72.8, 73.1, 73.2, 75.5, 77.0, 100.7, 102.7, 111.0, 169.4, 169.6, 169.9, 170.3, 170.6, 171.0. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{29}H_{39}NO_{18}Na$ 712.2; Found 712.1.

*Succinimidyl (2,6-Di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**5**).* Compound **S5** (69 mg, 0.10 mmol, 1.0 equiv) was dissolved in 5 mL of an AcOH:H_2O (4:1) mixture and was heated at 80 °C for 1 h with a condenser attached to the reaction flask. The reaction was then concentrated under reduced pressure followed by codistillation with toluene under reduced pressure (3×). The crude was then purified by silica flash chromatography using 2% MeOH in CH_2Cl_2 as the eluent to provide lactosyl acceptor **5** as a white solid (55 mg, 0.084 mmol, 84% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.08 (3H, s), 2.11 (3H, s), 2.11 (3H, s), 2.12 (3H, s), 2.13 (3H, s), 2.73 (4H, s), 3.00 (1H, br), 3.28 (1H, br), 3.61 (1H, dd, J = 2.7, 9.4 Hz), 3.64 (1H, t, J = 6.5 Hz), 3.75 (1H, m), 3.84 (1H, br), 4.10 (1H, t, J = 9.4 Hz), 4.21 (2H, m), 4.36 (1H, dd, J = 6.4, 11.5 Hz), 4.40 (1H, d, J = 7.9 Hz), 4.47 (1H, dd, J = 2.3, 12.0 Hz), 4.86 (1H, dd, J = 8.0, 9.6 Hz), 5.13 (1H, d, J = 6.4 Hz), 5.19 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.8, 21.0, 21.0, 21.0, 21.0, 25.5, 62.1, 62.4, 68.4, 70.1, 72.3, 72.7, 72.9, 73.1, 73.9, 75.7, 101.0, 102.7, 169.6, 170.2, 170.4, 170.7, 171.2, 171.8. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{26}H_{35}NO_{18}Na$ 672.1746; Found 672.1750.

*Phenyl (6-O-Pivaloyl-β-D-galactopyranosyl)-(1 → 4)-2,6-di-O-pivaloyl-1-thio-β-D-glucopyranoside (**6**).* Acceptor **6** was prepared as previously described.[34] Acceptor **6** was purified by silica flash chromatography using toluene:acetone (1:1) as the eluent to provide **6** as a white solid (0.31 g, 0.45 mmol). ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.19 (9H, s), 1.21 (9H, s), 1.26 (9H, s), 3.46 (1H, t, J = 9.2 Hz), 3.60 (1H, m), 3.66 (1H, m), 3.77 (4H, m), 3.91 (1H, t, J = 3.3 Hz), 4.16 (2H, m), 4.27 (3H, m), 4.36 (1H, dd, J = 4.1, 11.9 Hz), 4.50 (1H, d, J = 3.8 Hz), 4.69 (1H, d, J = 10.9 Hz), 4.72 (1H, d, J = 10.2 Hz), 4.90 (1H, t, J = 9.7 Hz), 7.28 (3H, m), 7.50 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 27.1, 27.1, 27.2, 38.7, 38.8, 38.9, 63.4, 63.7, 68.6, 70.7, 70.9, 73.2, 73.3, 74.4, 76.8, 81.5, 86.2, 104.2, 127.8, 128.9, 132.2, 133.3, 176.8, 178.8, 179.2. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{33}H_{50}O_{13}SNa$ 709.3; Found 709.2.

*Phenyl (6-O-Benzyl-2-O-benzoyl-1-thio-β-D-galactopyranoside (**7**).* Acceptor 7 was prepared as previously described.[35] Acceptor 7 was purified by silica flash chromatography using hexanes:EtOAc (1:1) as the eluent to provide **7** as a white solid (0.80 g, 1.7 mmol). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.95 (1H, d, J = 3.6 Hz), 3.21 (1H, d, J = 7.6 Hz), 3.78 (1H, t, J = 4.8 Hz), 3.83 (1H, m), 3.88 (2H, d, J = 5.10 Hz), 4.14 (1H, t, J = 3.5 Hz), 4.64 (2H, br), 4.84 (1H, d, J = 10.0 Hz), 5.23 (1H, t, J = 9.6 Hz), 7.28 (3H, m), 7.37 (5H, m), 7.50 (4H, m), 7.63 (1H, m), 8.11 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 69.7, 69.8, 72.2, 73.8, 74.1, 77.2, 86.0, 127.8, 127.9, 128.0, 128.5, 128.5, 128.9, 129.5, 130.1, 132.6, 132.6, 133.5, 137.7, 167.0. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{26}H_{26}O_6SNa$ 489.1; Found 489.0.

*Phenyl (4,6-O-Benzylidene-2-O-benzoyl-1-thio-β-D-galactopyranoside) (**8**).* Acceptor **8** was prepared as previously described.[31] Acceptor **8** was purified by silica flash chromatography using hexanes:EtOAc (1:1) as the eluent to provide **8** as a white solid (1.70 g, 3.66 mmol). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.65 (1H, d, J = 10.8 Hz), 3.61 (1H, s), 3.91 (1H, td, J = 10.1, 3.6 Hz), 4.06 (1H, d, J = 12.3 Hz), 4.26 (1H, d, J = 3.5 Hz), 4.43 (1H, d, J = 12.3 Hz), 4.84 (1H, d, J = 9.8 Hz), 5.28 (1H, t, J = 9.7 Hz), 5.55 (1H, s), 7.28 (2H, m), 7.34 (1H, m), 7.45 (7H, m), 7.60 (3H, m), 8.09 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 69.2, 70.1, 70.7, 73.0, 75.7, 84.9, 101.5, 126.6, 128.3, 128.3, 128.5, 128.9, 129.5, 129.9, 130.0, 131.2, 133.3, 134.0, 137.5, 166.0. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{26}H_{24}O_6SNa$ 487.1; Found 487.1.

General Procedure for Sialylations in Table 1. Acceptors (1.0 equiv) were codistilled with toluene under reduced pressure (3×) and then were combined with freshly prepared sialyl phosphate donor **3** (1.5 equiv) followed by high vacuum for 3 h. The donor−acceptor pair was then dissolved in dry CH_2Cl_2 (volume calculated to make 20 mM solution of acceptor), 4 Å molecular sieves were added (mass = 4 × acceptor mass), gas was exchanged with Ar, and the resulting solution was stirred at room temperature for 1 h. The reaction mixture was cooled to −78 °C, and TMSOTf (1.5 equiv) was added to initiate the reaction. The reaction was stirred at −78 °C for 1 h, and the reaction was quenched with saturated $NaHCO_3$ with vigorous stirring as the reaction slowly warmed to room temperature. The quenched reaction mixture was then diluted with CH_2Cl_2 (10 mL) and the organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was then analyzed by ^1H NMR to determine α:β ratios based on the integrals of the Neu5Ac C3 equatorial protons. Crudes were then purified by silica flash chromatography using appropriate solvent mixtures to isolate α:β mixtures of the products to obtain the isolated yield. Isolated and pure α anomers were then characterized.

*Phenyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside (**9**).* Compound **9** was purified by silica flash chromatography using toluene:acetone (1:1) as the eluent to provide **9** as a white solid (0.29 g, 0.26 mmol, 68% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.01 (3H, s), 2.02 (3H, s), 2.03 (3H, s), 2.07 (6H, s), 2.08 (3H, s), 2.10 (3H, s), 2.12 (1H, m), 2.16 (3H, s), 2.49 (3H, s), 2.70 (1H, d, J = 5.0 Hz), 2.83 (1H, dd, J = 3.5, 12.1 Hz), 3.67 (3H, m), 3.77 (2H, m), 3.81 (4H, m), 4.01 (2H, m), 4.09 (1H, t, J = 3.8 Hz), 4.14 (1H, dd, J = 6.3, 11.91 Hz), 4.40 (1H, dd, J = 2.8, 12.2 Hz), 4.51 (2H, m), 4.59 (1H, dd, J = 1.5, 9.4 Hz), 4.68 (1H, d, J = 10.1 Hz), 4.87 (2H, m), 5.16 (1H, dd, J = 7.9, 10.2 Hz), 5.20 (1H, t, J = 9.2 Hz), 5.36 (1H, dt, J = 2.8, 7.6 Hz), 5.56 (1H, dd, J = 1.6, 7.7 Hz), 7.29 (3H, m), 7.46 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.8, 20.9, 20.9, 21.0, 21.0, 21.0, 21.2, 21.6, 24.8, 35.8, 53.5, 53.6, 59.0, 62.5, 63.0, 63.5, 66.4, 69.3, 69.7, 70.4, 71.9, 72.8, 73.5, 73.9, 74.8, 76.0, 76.1, 85.4, 99.3, 100.9, 128.3, 129.0, 132.0, 133.0, 153.6, 168.4, 169.4, 169.8, 169.9, 170.2, 170.3, 170.4, 170.5, 171.2, 172.3. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{47}H_{59}NO_{27}SNa$ 1124.2887; Found 1124.2898.

*Succinimidyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-(2,6-di-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**10**).* Compound **10** was purified by silica flash chromatography using toluene:acetone (1:1) as the eluent to provide **10** as a white solid (0.13 g, 0.12 mmol, 81% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.00 (1H, t, J = 12.8 Hz), 2.07 (3H, s), 2.09 (3H, s), 2.11 (3H, s), 2.11 (3H, s), 2.14 (9H, s),

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2.23 (3H, s), 2.50 (3H, s), 2.74 (4H, s), 2.97 (1H, dd, J = 3.2, 12.0 Hz), 3.47 (1H, br), 3.73 (3H, m), 3.81 (3H, s), 3.89 (2H, m), 3.98 (1H, dd, J = 7.1, 12.3 Hz), 4.18 (1H, t, J = 9.3 Hz), 4.23 (1H, dd, J = 6.3, 11.9 Hz), 4.27 (2H, d, J = 6.2 Hz), 4.36 (1H, dd, J = 3.0, 10.0 Hz), 4.42 (1H, dd, J = 2.0, 11.8 Hz), 4.48 (1H, dd, J = 2.4, 12.2 Hz), 4.53 (1H, dd, J = 1.7, 9.4 Hz), 4.64 (1H, d, J = 7.9 Hz), 5.05 (1H, dd, J = 8.1, 9.9 Hz), 5.12 (1H, t, J = 6.6 Hz), 5.17 (1H, t, J = 7.2 Hz), 5.24 (1H, t, J = 8.1 Hz), 5.60 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.8, 20.8, 20.9, 20.9, 20.9, 21.0, 21.2, 24.9, 25.5, 36.8, 53.1, 59.3, 62.1, 63.2, 68.9, 69.8, 69.8, 71.0, 72.2, 72.5, 72.9, 73.7, 74.5, 74.9, 75.9, 76.4, 79.3, 99.8, 101.6, 103.3, 154.5, 166.7, 168.9, 169.5, 170.0, 170.2, 170.7, 170.7, 170.8, 171.0, 171.3, 173.0. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{45}H_{58}N_2O_{30}Na$ 1129.2967; Found 1129.2948.

*Phenyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-(6-O-pivaloyl-β-D-galactopyranosyl)-(1 → 4)-2,6-di-O-pivaloyl-1-thio-β-D-glucopyranoside (**11**).* Compound **11** was purified by silica flash chromatography using toluene:acetone (1:1) as the eluent to provide **11** as a white solid (0.16 g, 0.14 mmol, 52% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.20 (9H, s), 1.21 (9H, s), 1.26 (9H, s), 2.05 (3H, s), 2.13 (3H, s), 2.16 (3H, s), 2.21 (1H, t, J = 13.0 Hz), 2.49 (1H, d, J = 2.64 Hz), 2.52 (3H, s), 3.00 (1H, dd, J = 3. 5, 12.3 Hz), 3.20 (1H, d, J = 1.7 Hz), 3.52 (1H, t, J = 9.2 Hz), 3.70 (2H, m), 3.78 (4H, m), 3.84 (3H, s), 4.04 (2H, m), 4.09 (1H, dd, J = 7.9, 12.1 Hz), 4.15 (1H, dd, J = 3.3, 9.6 Hz), 4.23 (1H, dd, J = 8.1, 11.9 Hz), 4.31 (1H, d, J = 1.0 Hz), 4.40 (1H, dd, J = 4.2, 11.9 Hz), 4.47 (1H, dd, J = 2.6, 12.3 Hz), 4.51 (1H, d, J = 7.9 Hz), 4.65 (1H, dd, J = 1.1, 9.5 Hz), 4.71 (1H, d, J = 10.3 Hz), 4.88 (1H, dd, J = 1.5, 12.1 Hz), 4.94 (1H, dd, J = 9.4, 10.1 Hz), 5.55 (1H, td, J = 4.1, 10.7 Hz), 5.70 (1H, dd, J = 1.1, 8.5 Hz), 7.28 (3H, m), 7.51 (2H, m). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.7, 20.9, 21.2, 24.7, 27.1, 27.1, 27.2, 36.6, 38.7, 38.8, 53.5, 58.8, 63.2, 63.3, 63.6, 67.9, 68.8, 68.9, 70.7, 71.2, 72.6, 74.4, 74.7, 76.7, 76.9, 77.2, 82.2, 86.6, 97.9, 104.2, 127.6, 128.9, 131.8, 134.0, 153.3, 168.3, 169.8, 170.4, 170.7, 172.0, 176.7, 177.9, 178.6. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{52}H_{73}NO_{25}SNa$ 1166.4085; Found 1166.4072.

*Phenyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-2-O-benzoyl-6-O-benzyl-1-thio-β-D-galactopyranoside (**12**).* Compound **12** was purified by silica flash chromatography using hexanes:EtOAc (1:1) as the eluent to provide **12** as a white solid (1.30 g, 1.41 mmol, 89% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.48 (3H, s), 2.02 (3H, s), 2.07 (1H, t, J = 6.3 Hz), 2.13 (3H, s), 2.44 (3H, s), 2.74 (1H, br), 2.86 (1H, dd, J = 3.4, 12.0 Hz), 3.55 (1H, dd, J = 9.4, 11.2 Hz), 3.73 (3H, s), 3.85 (5H, m), 3.95 (1H, dd, J = 7.4, 12.2 Hz), 4.43 (1H, dd, J = 2.5, 12.3 Hz), 4.49 (1H, dd, J = 1.8, 9.4 Hz), 4.52 (1H, dd, J = 3.1, 9.5 Hz), 4.61 (2H, m), 4.96 (1H, d, J = 10.0 Hz), 5.43 (1H, t, J = 9.7 Hz), 5.49 (1H, dd, J = 1.9, 8.7 Hz), 5.55 (1H, td, J = 2.4, 8.7 Hz), 7.25 (3H, m), 7.35 (5H, m), 7.49 (4H, m), 7.61 (1H, t, J = 7.4 Hz), 8.18 (2H, dd, J = 1.0, 8.1 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.2, 20.8, 21.3, 24.6, 35.9, 53.3, 58.8, 63.4, 67.5, 68.5, 68.9, 69.1, 71.5, 73.6, 74.9, 75.2, 75.5, 76.5, 86.7, 97.5, 127.7, 127.7, 127.8, 128.4, 128.5, 128.8, 130.1, 130.2, 132.6, 132.9, 133.3, 138.1, 153.4, 165.3, 168.5, 170.0, 170.5, 170.8, 171.8. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{45}H_{49}NO_{18}SNa$ 946.3; Found 946.2.

*Phenyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-5-N,4-O-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-2-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**13**).* Compound **13** was purified by silica flash chromatography using hexanes:EtOAc (1:1) as the eluent to provide **13** as a white solid (0.96 g, 1.0 mmol, 87% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.76 (1H, dd, J = 12.3, 13.5 Hz), 1.82 (3H, s), 2.05 (3H, s), 2.21 (3H, s), 2.46 (3H, s), 2.92 (1H, dd, J = 3.2, 12.1 Hz), 3.48 (3H, s), 3.53 (1H, dd, J = 9.5, 11.2 Hz), 3.73 (1H, br), 3.75 (1H, m), 4.01 (1H, m), 4.12 (1H, d, J = 3.2 Hz), 4.17 (1H, dd, J = 1.4, 12.0 Hz), 4.39 (1H, dd, J = 1.4, 12.1 Hz), 4.47 (2H, m), 4.62 (1H, dd, J = 3.5, 9.7 Hz), 5.00 (1H, d, J = 9.8 Hz), 5.38 (1H, s), 5.44 (1H, t, J = 9.7 Hz), 5.57 (2H, m), 7.25 (2H, m), 7.31 (1H, m), 7.38 (3H, m), 7.43 (2H, m), 7.50 (2H, t, J = 7.8 Hz), 7.61 (3H, m), 8.16 (2H, d, J = 7.1 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 20.7, 21.0, 21.6, 24.8, 37.2, 53.0, 58.9, 63.9, 68.1, 68.3, 69.3, 69.6, 71.6, 73.0, 73.9, 75.0, 75.1, 85.2, 96.9, 101.0, 126.7, 128.1, 128.3, 128.6, 128.8, 129.2, 130.1, 130.5, 131.4, 133.3, 134.1, 137.9, 153.5, 165.0, 168.8, 170.3, 170.6, 171.1, 172.1. LRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{45}H_{47}NO_{18}SNa$ 944.2; Found 944.1.

*Succinimidyl (4,6-O-Benzylidene-2,3-di-O-benzoyl-β-D-glucopyranoside (**15**).* Compound **14**[40] (1.58 g, 2.78 mmol, 1.0 equiv) and NHS (1.60 g, 13.9 mmol, 5.0 equiv) were codistilled with toluene under reduced pressure (3×) followed by high vacuum for 3 h. The mixture was then dissolved in 10 mL of dry CH_2Cl_2 and 4 Å molecular sieves (5 g) were added to the solution. Gas was then exchanged with Ar, and the solution was stirred at room temperature for 30 min. The mixture was then cooled to −10 °C using a salted ice bath, and then NIS (1.88 g, 8.34 mmol, 3.0 equiv) was quickly added to the reaction vessel. Gas was exchanged with Ar again; TMSOTf (0.10 mL, 0.56 mmol, 0.2 equiv) was then introduced into the mixture, and the reaction was stirred at −10 °C for 30 min and then at 0 °C for 1 h. The reaction was then diluted with CH_2Cl_2 (10 mL) and quenched with a saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ (20 mL). The quenched reaction was then filtered, and the resulting organic layer was washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ (20 mL, 2×). The collected organic layer was then washed with brine, dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude material was then purified by silica flash chromatography using hexanes:EtOAc (4:1) as the eluent with 10% CH_2Cl_2 to ensure compound solubility on the column. Compound **15** was isolated as a white solid (1.34 g, 2.34 mmol, 84% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.72 (4H, s), 3.91 (1H, m), 4.02 (1H, t, J = 10.3 Hz), 4.41 (1H, dd, J = 4.9, 10.7 Hz), 4.53 (1H, t, J = 9.3 Hz), 5.49 (1H, d, J = 5.9 Hz), 5.65 (1H, s), 5.76 (2H, m), 7.35 (3H, m), 7.44 (6H, m), 7.56 (2H, m), 8.04 (2H, d, J = 7.1 Hz), 8.10 (2H, d, J = 7.1 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 25.4, 66.6, 68.3, 70.9, 72.3, 77.3, 101.5, 103.1, 126.2, 128.3, 128.4, 128.5, 128.9, 129.2, 129.2, 130.0, 130.1, 133.4, 133.6, 136.7, 165.0, 165.5, 170.4. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{31}H_{27}NO_{10}Na$ 596.1527; Found 596.1529.

*Succinimidyl (2,3-Di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside (**16**).* Compound **15** (0.80 g, 1.4 mmol, 1.0 equiv) was codistilled with toluene under reduced pressure (3×), set under high vacuum for 3 h, and was then dissolved in 10 mL of dry CH_2Cl_2. Gas was then exchanged with Ar, and the solution was cooled to 0 °C followed by the addition of Et_3SiH (0.67 mL, 4.2 mmol, 3.0 equiv) into the reaction vessel. This mixture was then stirred for 10 min at 0 °C, and then BF_3 Et_2O (0.69 mL, 5.6 mmol, 4.0 equiv) was introduced into the reaction dropwise. The reaction was then stirred for 1.5 h at 0 °C. The reaction was then quenched with saturated $NaHCO_3$ (20 mL). The aqueous layer was extracted with CH_2Cl_2 (20 mL, 3×), and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was then purified by silica flash chromatography using toluene:EtOAc (4:1) as the eluent with 10% CH_2Cl_2 to ensure compound solubility. Compound **16** was isolated as a white solid (0.65 g, 1.1 mmol, 81% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ = 2.67 (4H, m), 3.41 (1H, br), 3.80 (1H, m), 3.85 (1H, m), 3.95 (1H, dd, J = 3.9, 10.6 Hz), 4.06 (1H, t, J = 9.2 Hz), 4.65 (2H, q, J = 11.5 Hz), 5.33 (1H, d, J = 7.8 Hz), 5.5 (1H, t, J = 9.1 Hz), 5.70 (1H, dd, J = 7.9, 9.1 Hz), 7.33 (1H, m), 7.40 (8H, m), 7.55 (2H, m), 8.02 (2H, d, J = 7.1 Hz), 8.08 (2H, d, J = 7.1 Hz). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ = 25.4, 69.7, 69.9, 70.6, 73.8, 75.5, 76.6, 103.5, 127.7, 127.9, 128.4, 128.5, 128.5, 128.8, 129.1, 130.0, 130.1, 133.4, 133.7, 137.8, 165.4, 167.2, 170.1. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{31}H_{29}NO_{10}Na$ 598.1684; Found 598.1680.

*Phenyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-2-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**17**).* Compound **13** (0.66 g, 0.72 mmol, 1.0 equiv) was dissolved in MeOH (9 mL) and cooled to 0 °C. Freshly prepared 1.0 M NaOMe in MeOH (1 mL) was then introduced into the reaction mixture to generate a 0.1 M NaOMe solution with the substrate and was stirred

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for 15 min at 0 °C. The reaction was stirred at room temperature for 1 h and then quenched with Amberlite IR120 acidic resin, filtered, and concentrated under reduced pressure to reveal a white solid in quantitative yield. The solid was dried by codistillation with toluene under reduced pressure (3×) followed by high vacuum for 3 h. Dry intermediate was then dissolved in pyridine (10 mL) followed by the addition of DMAP (18 mg, 0.14 mmol, 0.2 equiv). Gas was exchanged with Ar, and then the mixture was cooled to 0 °C. Once cool, Ac_2O (1.4 mL, 14 mmol, 20 equiv) was introduced, and the reaction was stirred for 12 h while gradually warming to room temperature. The reaction was then cooled to 0 °C again and quenched with excess MeOH and stirred for 10 min. The crude was concentrated under reduced pressure, codistilled with toluene under reduced pressure (3×) and was reconstituted in CH_2Cl_2 (20 mL). The crude was then washed with saturated $NaHCO_3$ (20 mL, 3×), and the resulting organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was then purified by silica flash chromatography using toluene:acetone (3:1) as the eluent to reveal **17** as a white solid (0.51 g, 0.55 mmol, 76% yield over 2 steps). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.69 (1H, t, J = 12.7 Hz), 1.82 (3H, s), 1.83 (3H, s), 1.95 (3H, s), 2.07 (3H, s), 2.23 (3H, s), 2.56 (1H, dd, J = 4.5, 12.9 Hz), 3.56 (3H, s), 3.67 (1H, br), 3.90 (2H, m), 4.00 (1H, dd, J = 6.2, 12.2 Hz), 4.05 (1H, d, J = 3.4 Hz), 4.13 (1H, d, J = 11.2 Hz), 4.35 (1H, dd, J = 2.5, 12.4 Hz), 4.39 (1H, d, J = 12.0 Hz), 4.61 (1H, dd, J = 3.4, 9.7 Hz), 4.71 (1H, m), 4.99 (1H, d, J = 9.8 Hz), 5.06 (1H, br), 5.25 (1H, d, J = 9.5 Hz), 5.38 (1H, s), 5.43 (1H, t, J = 9.8 Hz), 5.52 (1H, m), 7.25 (3H, m), 7.38 (3H, m), 7.44 (2H, m), 7.51 (2H, t, J = 7.7 Hz), 7.60 (3H, m), 8.16 (2H, d, J = 7.1 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.6, 20.8, 20.9, 21.5, 23.2, 38.2, 49.1, 52.8, 62.6, 66.9, 67.6, 68.1, 68.8, 69.3, 69.6, 72.3, 73.3, 73.6, 85.3, 96.7, 101.0, 126.6, 127.9, 128.1, 128.4, 128.7, 129.1, 130.0, 130.4, 131.6, 133.1, 133.7, 137.8, 164.9, 168.8, 170.1, 170.2, 170.3, 170.8, 170.9. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{46}H_{51}NO_{18}SNa$ 960.2719; Found 960.2715.

*Succinimidyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-2-O-benzoyl-4,6-O-benzylidene-1-amino-β-D-galactopyranoside (**S6**).* Donor **17** (50 mg, 0.053 mmol, 1.5 equiv) and acceptor **16** (21 mg, 0.036 mmol, 1.0 equiv) were combined and codistilled with toluene under reduced pressure (3×) and set under high vacuum for 3 h. The donor−acceptor pair was dissolved in dry CH_2Cl_2 (5 mL) followed by the addition of 4 Å molecular sieves (0.2 g) and gas was exchanged with Ar. The mixture was stirred at room temperature for 1 h before cooling the mixture to 0 °C. NIS (36 mg, 0.16 mmol, 4.5 equiv) was then introduced into the reaction mixture and gas was exchanged with Ar again followed by the addition of TMSOTf (2.0 μL, 0.011 mmol, 0.3 equiv). The reaction was then stirred at 0 °C for 1.5 h. The reaction was then diluted with CH_2Cl_2 (5 mL) and quenched with a saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ (10 mL). The quenched reaction was then filtered, and the resulting organic layer was washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ (10 mL, 2×). The resulting organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude solid was then purified by silica flash chromatography using toluene:acetone (3:1) as the eluent to reveal compound **S6** and not the desired compound. This procedure was repeated using TfOH as the acid catalyst and resulted in the same product. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.68 (1H, t, J = 12.8 Hz), 1.84 (3H, s), 1.93 (3H, s), 1.97 (3H, s), 2.09 (3H, s), 2.24 (3H, s), 2.53 (1H, br), 2.56 (1H, dd, J = 4.4, 12.9 Hz), 2.65 (3H, m), 3.49 (3H, s), 3.72 (1H, s), 3.97 (2H, m), 4.04 (1H, dd, J = 6.2, 12.4 Hz), 4.15 (2H, m), 4.24 (1H, dd, J = 1.4, 12.3 Hz), 4.34 (1H, dd, J = 2.6, 12.4 Hz), 4.65 (1H, dd, J = 3.3, 9.9 Hz), 4.70 (1H, m), 5.06 (1H, br), 5.28 (1H, d, J = 10.2 Hz), 5.37 (1H, s), 5.50 (1H, td, J = 3.0, 12.2 Hz), 5.53 (1H, d, J = 9.2 Hz), 6.36 (1H, t, J = 9.6 Hz), 7.34 (1H, m), 7.39 (2H, t, J = 7.3 Hz), 7.47 (2H, t, J = 7.8 Hz), 7.58 (1H, t, J = 7.4 Hz), 7.66 (2H, d, J = 7.0 Hz), 8.02 (2H, d, J = 7.1 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.7, 20.8, 20.9, 21.5, 23.2, 27.9, 38.6, 49.3, 52.8, 62.6, 66.8, 67.0, 67.6, 68.2, 68.7, 68.7, 72.5, 72.7, 72.9, 78.7, 96.7, 101.7, 127.1, 128.3, 128.6, 129.2, 129.6, 129.9, 133.4, 137.9, 165.4, 168.8, 170.1, 170.3, 170.8, 171.0, 174.8, 176.2. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{44}H_{50}N_2O_{20}Na$ 949.2849; Found 949.2838.

*Trichloroacetimidatyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**18**).* Compound **17** (0.41 g, 0.44 mmol, 1.0 equiv) was dissolved in an acetone:H_2O (4:1) mixture (10 mL) and then cooled to 0 °C. While stirring, NBS (94 mg, 0.53 mmol, 1.2 equiv) was added gradually to the reaction and the mixture was stirred for 30 min. After 30 min of mixing, NBS (78 mg, 0.44 mmol, 1.0 equiv) was added and the mixture was stirred at 0 °C for another 1 h. The reaction was then concentrated under reduced pressure until the solution became turbid. The turbid mixture was diluted with CH_2Cl_2 (20 mL), quenched, and then washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ (15 mL, 3×). The organic layer was then washed with brine, dried with Na_2SO_4, filtered, and then concentrated under reduced pressure. The crude hemiacetal was passed through a short bed of silica using toluene:acetone (2:1) as the eluent to give the crude hemiacetal intermediate as a white solid (0.31 g, 0.36 mmol, 82% crude). The crude hemiacetal was then codistilled with toluene under reduced pressure (3×) and set under high vacuum for 3 h. The crude hemiacetal was then dissolved in dry CH_2Cl_2 (5 mL) followed by the addition of 4 Å molecular sieves (1 g), trichloroacetonitrile (144 μL, 1.44 mmol, 4.0 equiv), and the gas was exchanged with Ar. The solution was stirred at room temperature for 30 min. The mixture was then cooled to 0 °C followed by the addition of DBU (10 μL, 0.072 mmol, 0.2 equiv), and the reaction was then stirred for 1.5 h at 0 °C. The reaction was filtered and then concentrated under reduced pressure to provide the crude donor. The crude material was then purified by silica flash chromatography using toluene:acetone (3:1) as the eluent to provide donor **18** as a white solid (0.32 g, 0.32 mmol, 73% yield over 2 steps). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.79 (1H, t, J = 12.7 Hz), 1.90 (3H, s), 1.96 (3H, s), 2.06 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 2.59 (1H, dd, J = 4.4, 12.9 Hz), 3.52 (3H, s), 4.08 (3H, m), 4.14 (1H, br), 4.21 (1H, d, J = 12.5 Hz), 4.26 (1H, dd, J = 2.7, 12.5 Hz), 4.32 (1H, d, J = 12.6 Hz), 4.42 (1H, d, J = 3.1 Hz), 4.78 (1H, m), 5.11 (1H, d, J = 9.4 Hz), 5.18 (1H, dd, J = 3.3, 10.7 Hz), 5.37 (1H, dd, J = 1.5, 9.8 Hz), 5.47 (1H, s), 5.49 (1H, m), 5.70 (1H, dd, J = 3.3, 10.7 Hz), 6.79 (1H, d, J = 3.3 Hz), 7.37 (3H, m), 7.47 (2H, t, J = 7.8 Hz), 7.54 (2H, d, J = 6.7 Hz), 7.59 (1H, t, J = 7.4 Hz), 8.07 (2H, d, J = 7.1 Hz), 8.49 (1H, s). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.8, 20.9, 21.4, 23.2, 38.7, 49.5, 52.9, 62.5, 65.2, 67.0, 67.4, 68.0, 68.6, 69.0, 72.7, 73.5, 91.2, 95.1, 96.6, 100.6, 126.3, 128.2, 128.3, 128.5, 129.0, 129.1, 129.6, 129.8, 133.4, 137.7, 160.4, 165.5, 169.0, 169.8, 170.0, 170.3, 170.7, 171.0. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{42}H_{47}Cl_3N_2O_{19}Na$ 1011.2; Found 1011.1.

*Phenyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-4-O-acetyl-2-O-benzoyl-6-O-benzyl-1-thio-β-D-galactopyranoside (**20**).* Compound **12** (1.3 g, 1.4 mmol, 1.0 equiv) was dissolved in MeOH (18 mL) and cooled to 0 °C. Freshly prepared 1.0 M NaOMe in MeOH (2.0 mL) was then introduced to the reaction mixture to generate a 0.1 M NaOMe solution with the substrate and was stirred for 15 min at 0 °C. The reaction was then stirred at room temperature for 1 h, quenched with Amberlite IR120 acidic resin, filtered, and concentrated under reduced pressure to reveal a white solid in quantitative yield. The solid was dried by codistillation with toluene under reduced pressure (3×) followed by high vacuum for 3 h. Dry intermediate was then dissolved in pyridine (20 mL) followed by the addition of DMAP (35 mg, 0.28 mmol, 0.2 equiv). Gas was exchanged with Ar, and the mixture was cooled to 0 °C. Once cool, Ac_2O (3.3 mL, 35 mmol, 25 equiv) was introduced, and the reaction was stirred for 12 h while gradually warming to room temperature. The reaction was then cooled to 0 °C again and quenched with excess MeOH and stirred for 10 min. The crude was concentrated under reduced pressure, codistilled with toluene under reduced pressure (3×), and reconstituted in CH_2Cl_2 (20 mL). The crude was then washed with saturated $NaHCO_3$ (20 mL, 3×), and the resulting organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was then purified by silica flash

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chromatography using toluene:acetone (3:1) as the eluent to reveal **20** as a white solid (1.02 g, 1.04 mmol, 74% yield over 2 steps). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.43 (3H, s), 1.74 (1H, t, J = 12.4 Hz), 1.80 (3H, s), 1.99 (3H, s), 2.02 (3H, s), 2.08 (3H, s), 2.17 (3H, s), 2.56 (1H, dd, J = 4.6, 12.6 Hz), 3.51 (1H, dd, J = 6.1, 10.0 Hz), 3.61 (2H, m), 3.82 (1H, m), 3.86 (3H, s), 3.90 (1H, dd, J = 5.7, 12.5 Hz), 3.98 (1H, t, J = 6.2 Hz), 4.27 (1H, dd, J = 2.4, 12.5 Hz), 4.48 (1H, d, J = 11.6 Hz), 4.56 (1H, d, J = 11.6 Hz), 4.76 (1H, dd, J = 3.2, 9.6 Hz), 4.85 (1H, m), 4.96 (1H, d, J = 10.3 Hz), 5.10 (2H, m), 5.19 (1H, dd, J = 2.8, 9.5 Hz), 5.32 (1H, t, J = 9.8 Hz), 5.57 (1H, m), 7.25 (3H, m), 7.35 (5H, m), 7.50 (4H, m), 7.59 (1H, t, J = 7.4 Hz), 8.19 (2H, d, J = 7.1 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.2, 20.8, 20.8, 21.5, 23.2, 37.4, 48.8, 53.2, 62.3, 66.5, 67.4, 68.4, 68.5, 69.3, 69.4, 71.8, 72.4, 73.5, 75.8, 86.5, 96.8, 127.7, 127.7, 127.7, 128.3, 128.4, 128.8, 130.2, 130.4, 132.3, 133.0, 133.2, 138.0, 165.3, 168.0, 170.1, 170.3, 170.3, 170.5, 170.7, 170.9. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{48}H_{55}NO_{19}SNa$ 1004.2981; Found 1004.2967.

*Succinimidyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-(4-O-acetyl-2-O-benzoyl-6-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-6-O-benzyl-β-D-glucopyranoside (**2**).* Donor **20** (0.36 g, 0.37 mmol, 1.5 equiv) and acceptor **16** (0.14 g, 0.25 mmol, 1.0 equiv) were combined and codistilled with toluene under reduced pressure (3×) and set under high vacuum for 3 h. The donor−acceptor pair was dissolved in dry CH_2Cl_2 (5 mL) followed by the addition of 4 Å molecular sieves (1 g), and gas was exchanged with Ar. The mixture was stirred at room temperature for 1 h before cooling the mixture to 0 °C. NIS (0.25 g, 1.1 mmol, 4.5 equiv) was then introduced into the reaction mixture, and gas was exchanged with Ar again followed by the addition of TfOH (6 μL, 0.07 mmol, 0.3 equiv). The reaction was then stirred at 0 °C for 1.5 h, and the reaction was diluted with CH_2Cl_2 (15 mL) and then quenched with saturated $Na_2S_2O_3$ and $NaHCO_3$ (20 mL). The organic layer was separated, filtered, and washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ (20 mL, 2×). The resulting organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude solid was then purified by silica flash chromatography using toluene:acetone (3:1) as the eluent to afford compound **2** as a white solid (0.32 g, 0.22 mmol, 87% yield). Only β anomer was observed due to anchimeric assistance. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.41 (3H, s), 1.71 (1H, t, J = 12.4 Hz), 1.78 (3H, s), 1.94 (3H, s), 1.97 (3H, s), 2.09 (3H, s), 2.13 (3H, s), 2.56 (5H, m), 2.80 (1H, t, J = 9.2 Hz), 2.91 (1H, dd, J = 4.8, 9.5 Hz), 3.46 (2H, m), 3.55 (1H, dd, J = 2.8, 10.7 Hz), 3.65 (1H, d, J = 9.2 Hz), 3.71 (1H, t, J = 7.6 Hz), 3.78 (3H, s), 3.81 (1H, q, J = 10.5 Hz), 3.96 (1H, dd, J = 6.1, 12.5 Hz), 4.02 (1H, d, J = 11.7 Hz), 4.10 (1H, d, J = 11.8 Hz), 4.24 (2H, br), 4.34 (1H, t, J = 9.3 Hz), 4.38 (1H, dd, J = 2.28, 12.42 Hz), 4.58 (1H, dd, J = 3.2, 10.1 Hz), 4.83 (1H, td, J = 4.6, 11.9 Hz), 4.94 (1H, d, J = 3.0 Hz), 4.98 (2H, t, J = 8.9 Hz), 5.19 (2H, dd, J = 7.6, 10.0 Hz), 5.24 (1H, d, J = 7.4 Hz), 5.56 (1H, t, J = 7.6 Hz), 5.67 (2H, t, J = 8.6 Hz), 7.18 (2H, t, J = 7.3 Hz), 7.27 (3H, m), 7.32 (7H, m), 7.39 (2H, t, J = 7.8 Hz), 7.50 (4H, m), 7.57 (1H, t, J = 7.3 Hz), 8.02 (4H, t, J = 6.7 Hz), 8.26 (2H, d, J = 7.2 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.3, 20.7, 20.8, 20.9, 21.5, 23.1, 25.3, 37.2, 48.7, 53.2, 62.6, 66.4, 66.6, 67.2, 67.3, 68.7, 69.5, 70.4, 71.0, 71.7, 71.7, 72.8, 72.9, 74.0, 75.0, 75.7, 96.9, 101.3, 102.9, 127.4, 127.4, 127.4, 127.5, 128.2, 128.3, 128.3, 128.3, 128.6, 129.1, 129.2, 129.8, 129.9, 130.0, 130.1, 130.5, 133.0, 133.2, 138.0, 138.4, 164.9, 165.0, 165.2, 167.9, 170.0, 170.0, 170.2, 170.3, 170.4, 170.8, 170.8. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{73}H_{78}N_2O_{29}Na$ 1469.4582; Found 1469.4562.

*Succinimidyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-(2 → 3)-(4-O-acetyl-2-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranoside (**S7**).* Compound **2** (0.32 g, 0.22 mmol, 1.0 equiv) was dissolved in a mixture of MeOH:EtOAc (1:3, 10 mL) followed by the addition of palladium hydroxide on carbon (55 mg, 0.25 g/mmol substrate) at room temperature. The reaction was then stirred for 1 h under an atmosphere of H_2 gas (balloon, 1 atm). After 1 h, the reaction was diluted with 10 mL of the same MeOH:EtOAc mixture followed by bubbling Ar gas into the reaction solution. The reaction was then filtered over a bed a Celite followed by a short bed of silica using 1% MeOH in CH_2Cl_2 as the eluent. Compound **S7** was then concentrated under reduced pressure to a white solid (0.25 g, 0.20 mmol, 89% yield). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.46 (3H, s), 1.74 (1H, t, J = 12.5 Hz), 1.80 (3H, s), 1.98 (3H, s), 2.02 (3H, s), 2.13 (3H, s), 2.18 (3H, s), 2.52 (1H, dd, J = 4.5, 12.6 Hz), 2.68 (4H, s), 2.73 (1H, dd, J = 6.8, 12.0 Hz), 2.97 (1H, dd, J = 6.4, 12.0 Hz), 3.50 (1H, t, J = 6.7 Hz), 3.56 (1H, td, J = 3.1, 9.3 Hz), 3.69 (1H, dd, J = 2.7, 10.7 Hz), 3.76 (5H, m), 3.83 (1H, q, J = 10.4 Hz), 3.90 (1H, dd, J = 7.9, 12.2 Hz), 4.37 (1H, t, J = 8.8 Hz), 4.46 (1H, dd, J = 2.5, 12.2 Hz), 4.55 (1H, dd, J = 3.4, 10.1 Hz), 4.69 (1H, d, J = 3.2 Hz), 4.73 (1H, td, J = 6.1, 14.9 Hz), 4.91 (1H, d, J = 7.9 Hz), 4.96 (1H, d, J = 10.3 Hz), 5.13 (1H, dd, J = 2.6, 9.6 Hz), 5.22 (2H, m), 5.62 (2H, m), 5.70 (1H, td, J = 3.9, 12.1 Hz), 7.41 (4H, q, J = 8.2 Hz), 7.53 (4H, m), 7.60 (1H, t, J = 7.3 Hz), 8.02 (4H, d, J = 8.3 Hz), 8.26 (2H, d, J = 7.1 Hz). ^{13}C{^{1}H} NMR ($CDCl_3$, 150 MHz): δ = 20.3, 20.6, 20.8, 20.8, 21.4, 23.1, 25.3, 37.4, 48.7, 53.3, 59.9, 60.5, 63.3, 67.0, 67.1, 68.1, 69.2, 70.1, 71.1, 71.1, 71.9, 73.0, 74.5, 76.3, 96.8, 100.9, 104.1, 128.2, 128.3, 128.4, 128.5, 129.1, 129.1, 129.8, 129.9, 130.0, 130.4, 133.2, 133.2, 133.3, 164.9, 165.2, 165.2, 168.1, 170.2, 170.3, 170.4, 170.6, 170.8, 171.7, 171.9. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{59}H_{66}N_2O_{29}Na$ 1289.3643; Found 1289.3627.

*Succinimidyl (5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2 → 3)-(4-O-acetyl-2-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranoside (**21**).* Compound **S7** (0.25 g, 0.20 mmol, 1.0 equiv) was dissolved in 10 mL of pyridine followed by the addition of LiI (0.54 g, 4.0 mmol, 20 equiv) in the dark. This solution was then refluxed in the dark for 3 h, after which pyridine was removed under reduced pressure followed by codistillation with toluene under reduced pressure (3×). This crude was then purified by silica flash chromatography using 5% MeOH in CH_2Cl_2 as the eluent to afford compound **21** as a white solid (0.18 g, 0.14 mmol, 68% yield). Note: The white solid pertains to the protonated form of the compound. A yellow oily material may result as the lithiated species. This species has identical NMR characterization as the protonated form but exhibits the lithiated form in MS analysis i.e. [M → Li + Na]$^+$. ^{1}H NMR (CD_3OD, 600 MHz): δ = 1.58 (1H, br), 1.70 (3H, br), 1.78 (3H, s), 1.92 (3H, s), 2.04 (3H, s), 2.08 (3H, s), 2.10 (3H, s), 2.51 (1H, br), 2.66 (4H, s), 3.52 (1H, br, J = 9.6 Hz), 3.62 (2H, m), 3.69 (4H, m), 3.90 (1H, br), 3.94 (1H, dd, J = 5.6, 11.0 Hz), 4.04 (1H, dd, J = 6.2, 12.3 Hz), 4.21 (1H, br), 4.32 (1H, t, J = 9.4 Hz), 4.39 (1H, dd, J = 2.3, 12.4 Hz), 4.46 (1H, br, J = 9.1 Hz), 4.94 (1H, d, J = 8.00 Hz), 5.23 (2H, br), 5.33 (1H, d, J = 8.00 Hz), 5.44 (1H, t, J = 8.6 Hz), 5.54 (1H, br), 5.66 (1H, t, J = 9.2 Hz), 7.43 (4H, m), 7.56 (4H, m), 7.65 (1H, t, J = 7.4 Hz), 7.99 (4H, m), 8.17 (2H, d, J = 7.5 Hz). ^{13}C{^{1}H} NMR (CD_3OD, 150 MHz): δ = 19.4, 19.5, 20.1, 21.2, 25.0, 48.2, 49.1, 59.7, 62.4, 62.8, 67.5, 68.1, 70.7, 71.0, 71.2, 72.3, 73.0, 73.5, 74.5, 76.1, 101.0, 103.3, 128.0, 128.1, 128.4, 129.3, 129.5, 129.5, 129.5, 129.7, 129.8, 130.0, 133.0, 133.0, 165.5, 165.8, 170.3, 170.4, 170.5, 170.9, 170.9, 171.3, 171.9, 172.0. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{58}H_{63}N_2O_{29}LiNa$ 1281.3569; Found 1281.3561. LRMS (ESI): [M]$^-$ calcd for $C_{58}H_{64}N_2O_{29}$ 1252.4; Found 1252.1.

*Aminooxy (5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2 → 3)-(β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (**1**).* Compound **21** (20 mg, 0.016 mmol, 1.0 equiv) was dissolved in EtOH (1 mL) in a microwave reaction tube. Hydrazine hydrate (30 μL, 0.96 mmol, 60 equiv) was then introduced into the vessel which was then capped and stirred at room temperature for 1 h. Compound **21** was then subjected to microwave conditions (200 W, 90 °C) for 1 h total in two 30 min repetitive cycles. MS analysis revealed a single peak at 687.6 [M]$^-$. This peak is the acetone adduct of the aminooxy GM3 which spontaneously forms with residual acetone in the injector of the MS instrument. The reaction mixture was then concentrated under reduced pressure and then passed through a P2 biogel column using H_2O as the eluent. Fractions were found to contain final compound by staining silica gel plates. These fractions were frozen and lyophilized to reveal compound **1** as a white solid (8.0 mg, 0.012 mmol, 77% yield). ^{1}H

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NMR (D_2O, 600 MHz): δ = 1.70 (1H, t, J = 12.1 Hz), 1.94 (3H, s), 2.66 (1H, dd, J = 4.6, 12.4 Hz), 3.26 (1H, m), 3.57 (12H, m), 3.77 (4H, m), 3.86 (1H, d, J = 3.1 Hz), 3.92 (1H, dd, J = 2.1, 12.2 Hz), 4.02 (1H, dd, J = 3.2, 9.9 Hz), 4.43 (1H, d, J = 7.9 Hz), 4.51 (1H, d, J = 8.3 Hz). $^{13}C\{^1H\}$ NMR (D_2O, 150 MHz): δ = 22.0, 39.6, 51.6, 59.9, 61.0, 62.5, 67.4, 68.1, 68.3, 69.3, 71.3, 71.7, 72.8, 74.3, 74.7, 75.1, 75.4, 78.0, 99.8, 102.6, 104.8, 173.9, 175.0. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{23}H_{40}N_2O_{19}Na$ 671.2117; Found 671.2106.

*GM3-PS A1 (**23**).* Polysaccharide PS A1 (**22**) was isolated and purified as previously described.[8,41] PS A1 (1.0 mg, 9.1×10^{-9} mol) was dissolved in NaOAc buffer (1.0 mL, 0.1 M, pH 5). To this solution was added 55 μL of 10 mM $NaIO_4$ solution (5.5×10^{-7} mol). The reaction was allowed to shake gently for 90 min in the dark at room temperature. Excess $NaIO_4$ was quenched by adding ethylene glycol and the mixture was continually shaken for another 20 min in the dark. The oxidized PS A1 was purified with a centrifugal filter (Vivaspin, MWCO = 30 kDa). PS A1 was redissolved in NaOAc buffer (1.0 mL, 0.1 M, pH 5). Subsequently, 2.9 mg (4.4×10^{-6} mol) of β-aminooxy GM3 (**1**) was added, and the reaction was gently shaken for 18 h in the dark at room temperature. Unreacted β-aminooxy GM3 (**1**) was removed using a 30 kDa centrifugal filter, and purified GM3-PS A1 (**23**) conjugate was obtained as a lyophilized white foam (1.0−1.1 mg).

GM3-PS A1 NMR Analysis. NMR analysis was performed using a Bruker Avance III 600 MHz spectrometer and data processed with Bruker TopSpin 4.0.3. GM3-PS A1 ^1H spectra were obtained with a probe temperature of 60 °C using the *zg30* pulse program (D1 = 3.0 s, AQ = 2.7 s, NS = 128). Integrations of the key "diagnostic" peaks were obtained using an automated baseline correction. These integrations suggested a 1:2.33 ratio between GM3:PSA1 repeating unit. On average, PS A1 has at least 120 repeating units[12] which translates to approximately 51.5 GM3 conjugation sites per PS A1 molecule.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c00320.

NMR spectra for new or appropriate compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Peter R. Andreana − *Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, Ohio 43606, United States;* orcid.org/0000-0002-5300-6700; Email: peter.andreana@utoledo.edu

Authors

Kristopher A. Kleski − *Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, Ohio 43606, United States;* orcid.org/0000-0003-2418-5321

Mengchao Shi − *Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, Ohio 43606, United States;* orcid.org/0000-0002-8174-3302

Matthew Lohman − *Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, Ohio 43606, United States;* orcid.org/0000-0003-2823-6819

Gabrielle T. Hymel − *Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, Ohio 43606, United States*

Vinod K. Gattoji − *Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, Ohio 43606, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.0c00320

Notes

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