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Synthesis of an Aminooxy Derivative of the Tetrasaccharide Repeating Unit of *Streptococcus dysgalactiae* 2023 Polysaccharide for a PS A1 Conjugate Vaccine

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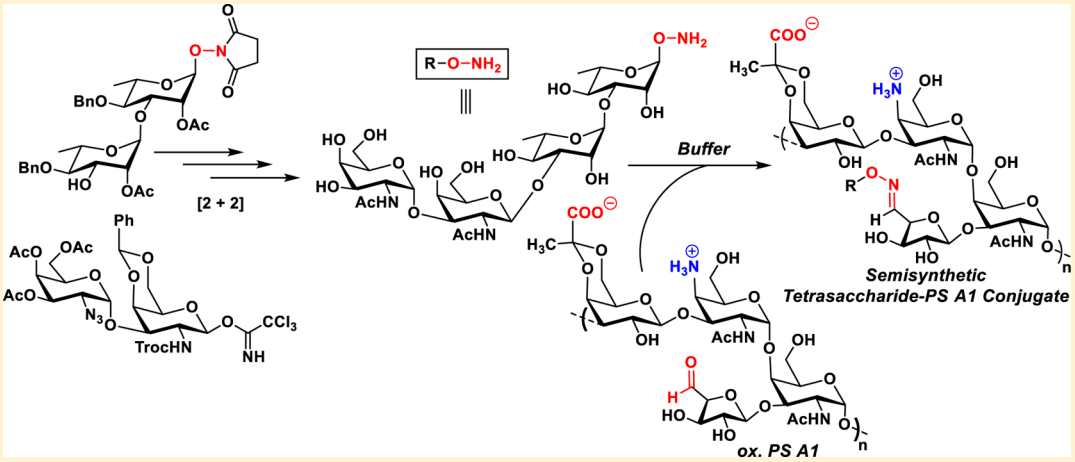
Supporting Information



ABSTRACT: A highly efficient and stereocontrolled synthesis of an aminooxy derivative of the tetrasaccharide repeating unit of a rhamnose-rich polysaccharide isolated from the cell envelop of bovine mastitis *Streptococcus dysgalactiae* 2023 is reported for the first time. The synthesis was accomplished utilizing a stereoselective and convergent [2 + 2] glycosylation strategy inclusive of a disaccharide Schmidt donor and an inclusive rhamnose disaccharide acceptor. The synthetic aminooxy tetrasaccharide was conjugated to T-cell stimulating immunogen PS A1 from *Bacteroides fragilis* ATCC 25285/NCTC 9343 via a physiologically stable oxime linkage to furnish the first semisynthetic bacterial-based immunogen construct targeting *S. dysgalactiae* 2023. The synthetic tetrasaccharide was assembled in 19 steps with a ∼5.0% overall yield.

INTRODUCTION

Bovine mastitis is an infection of the bovine mammary gland caused by a small cohort of bacteria. Among more than 135 identified causative microorganisms for mastitis, only five have been recognized as major contributing agents including *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli*.[1] As a consequence of bacterial invasion and proliferation in the udder, released toxins and enzymes from bacteria induce inflammatory responses, which result in mastitis that can be categorized as either clinical or subclinical.[1,2] Irrespective of the category of disease, the aftermath is a negative impact on the quality and quantity of milk production by infected cows resulting in ∼$2 billion economic loss each year for the dairy industry.[3]

One of the more potent mastitis agents, out of the 5 listed above, is the α-hemolytic Lancefield group C pathogen *S. dysgalactiae* also known as a contagious and environmental pathogen that can be transmitted via infected udders, manure, and other organic matter including bedding and can survive in the tonsils, mouth and vagina during nonlactating periods.[1] Other than bovine mastitis, *S. dysgalactiae* is identified to cause toxic shock like syndrome in cattle,[4] bacteremia in dogs,[5] polyarthritis in lambs,[6] severe septicemia and systemic granulomatous inflammatory disease in fish.[7]

Antibiotics for treatment of mastitis disease can lead to contaminated milk, which is considered a growing threat toward human health. In cases of subclinical mastitis, where no visible signs of infection are noticeable, the infection will still result in bacteria contaminated milk. This includes unpasteurized dairy products,[8] which represents yet another human health risk. Although Lancefield group C pathogens are mainly considered as animal pathogens,[9] several human maladies have been diagnosed to include glomerulonephritis, puerperal sepsis, bacterial endocarditis, cellulitis, bronchitis, arthritis, toxic shock like syndrome, prosthetic joint infection, pneumonia, osteomyelitis, bacterial endocarditis, and fatal neonatal and adult

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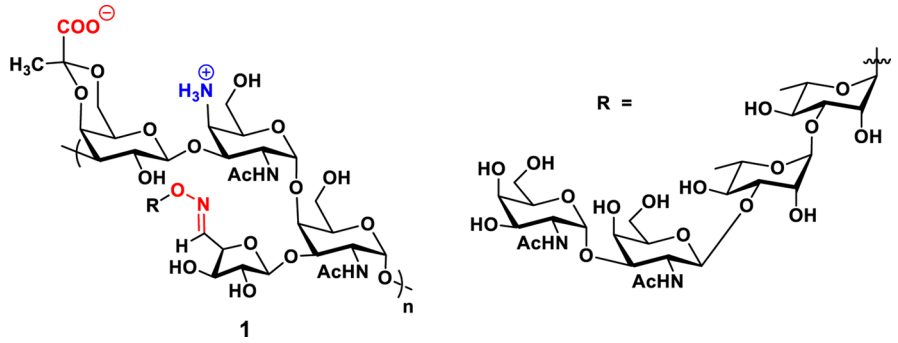

Figure 1. PS A1 conjugate **1** consisting of an oxime link (oligomer on left) and the repeating rhamnose rich tetrasaccharide moiety (oligomer on right) from *S. dysgalactiae* 2023.

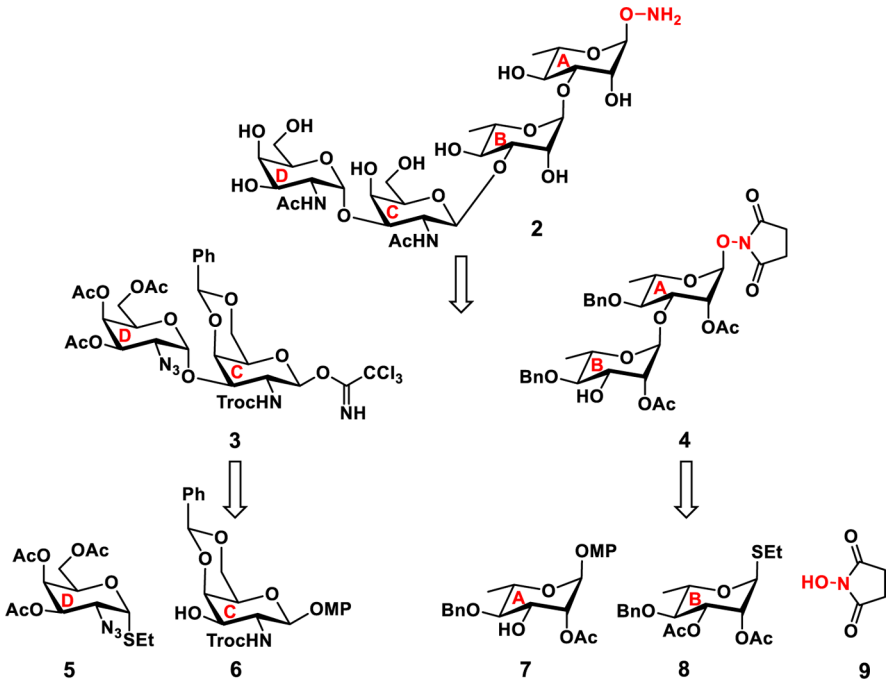

Figure 2. Retrosynthetic convergent [2 + 2] glycosylation approach.

meningitis.[10] Furthermore, it has been revealed that *S. dysgalactiae* can survive in mammary epithelial cells for extended periods without causing visible damage to the host cells resulting in persistent infection and protection of the bacteria from antimicrobial drugs and host defense mechanisms.[11] *S. dysgalactiae* is recognized as a zoonotic pathogen[12] which is generally known to infect persons handling infected animals or raw seafood[10a] and most likely individuals with poor immune systems.[10b] In a microarray study containing 220 virulence genes from bacteria *S. pyogenes*, isolates of *S. dysgalactiae* infected bovine and isolates of equisimilis from human infection were tested for the presence of these virulence genes and similarities with many bovine genes and human pathogens were observed. This observation alone suggested that *S. dysgalactiae* is a pathogen that can lead to virulence and pathogenesis in humans.[12] Therefore, considering human health risks and increased bacterial resistance to antibiotics, an effective vaccine to treat this disease would alleviate numerous health risks and concerns.

A number of strategies to develop vaccines against mastitis with diversified approaches have already been reported. To date these include capsular polysaccharide-protein conjugates,[13] lipopolysaccharide-protein conjugates,[14] total antigen,[15] attenuated bacteria,[16] recombinant forms of cell surface protein antigens,[17] capsular polysaccharide-based[18] and DNA-based vaccines.[19] Despite a varying number of attempts, very little has been done to target *S. dysgalactiae* as the actual culprit. Until now, only recombinant forms of *S. dysgalactiae* surface proteins GapC and Mig have been studied[17c] for immunogenicity and reported as potential antigens against *S. dysgalactiae* related mastitis. However, a vaccine with clinical efficacy remains elusive and to achieve this goal further studies are necessary.

An extensive literature search on capsular polysaccharides produced few reports of validated mastitis related carbohydrate-based structures[20] that could be readily accessible through synthetic means for targeting *S. dysgalactiae*. However, Neiwert et al., determined the structure of a particularly interesting tetrasaccharide repeating unit (Figure 1) of a rhamnose rich polysaccharide isolated from the cell envelop of *S. dysgalactiae* 2023. As capsular polysaccharides have proven to be strongly antigenic[21] and early efforts toward semisynthetic oligomer-protein conjugate vaccines showing promising immune responses toward bacterial antigens,[22] we became inspired to synthesize the rhamnose rich tetrasaccharide repeating unit that

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could then be coupled to an appropriate carrier for targeting *S. dysgalactiae* related diseases. An added immunogenic advantage with this repeating unit is the presence of two L-rhamnose moieties proven to be antigenic determinants on their own in numerous studies.[23]

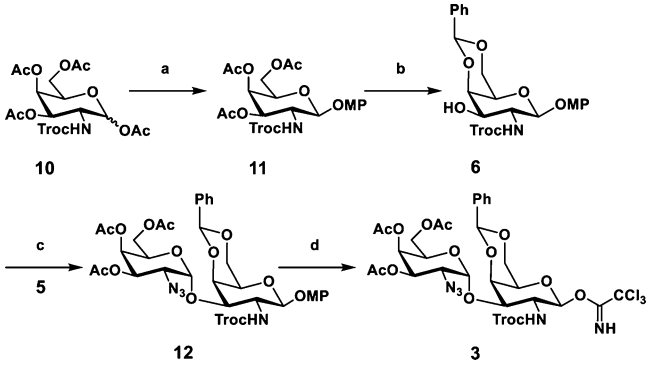
To the best of our knowledge there are no current reports documenting the total synthesis of the aforementioned tetrasaccharide repeating unit and herein we account for an efficient synthesis aimed at controlling stereochemistry. Furthermore, we envisioned the synthesis of an aminooxy derivative of this hapten as a continuance of a linker-free conjugation strategy[24] we have been developing and pursuing. As research emanating from our group has shown, employing immunogenic zwitterionic polysaccharide PS A1[25] as a carrier, isolated from commensal anaerobe *Bacteroidis fragilis* ATCC 25285/NCTC 9343, in conjunction with carbohydrate antigens, can elicit a T-cell dependent immune response and provide an alternative to carrier proteins. Accessing this entirely carbohydrate based vaccine construct with the rhamnose rich tetrasaccharide would ensure carbohydrate homogeneity and allow for specific and selective immune responses toward the antigen, which otherwise might be suppressed or even elusive when using strongly immunogenic carrier proteins.[26] With this idea in mind, we embarked on the synthesis of immunogenic construct **1** (Figure 1) composed of the rhamnose rich tetrasaccharide antigen of *S. dysgalactiae* 2023 conjugated to PS A1 via a physiologically stable oxime linkage.

RESULTS AND DISCUSSION

Retrosynthetic Analysis. We designed our synthetic strategy for aminooxy antigen target compound **2** to include a trichloroacetimidate (TCA) disaccharide donor **3** and anomeric succinimide disaccharide acceptor **4**. To ensure that the final glycosylation would be stereoselective we elected to incorporate a 2,2,2-trichloroethoxycarbonyl (Troc) protecting group at amino in C-2 for anchimeric assistance that would serve the purpose of β-selective glycosylation (Figure 2). Disaccharide Schmidt donor **3** was envisioned to come from monosaccharide **5**[27] and acceptor **6** using a thiol-donor glycoside strategy followed by 4-methoxyphenyl (OMP) deprotection and subsequent installation of the trichloroacetimidate. Acceptor **4** would be assembled from an α-selective glycosylation of acceptor **7** with thioethyl protected donor **8** followed by an α-selective glycosylation with *N*-hydroxysuccinimide (NHS).[24b] Acetate protection on the C-2 hydroxyl group of L-rhamnose sugar **8** was incorporated to ensure the above-mentioned α-selective glycosylation, as neighboring group participation would only make the β-face unavailable toward nucleophilic attack. The anomeric positions of acceptors **6** and **7** were protected with the 4-methoxyphenyl (OMP) groups for facile removal under mild oxidative conditions for further derivatization to the Schmidt's TCA donor chemistry.[28] The anomeric position of acceptor **4** was envisioned to be protected with NHS because of the inherent stability of the succimidyl group in later reaction conditions[24d,29] and to furnish the important and necessary aminooxy moiety after a Gabriel-type reaction for oxime conjugation to oxidized PS A1.

Synthesis of Disaccharide Donor 3. The synthesis of disaccharide donor **3** was initiated with monosaccharide precursors **5**[27] and **10**,[30] which were synthesized following established literature protocols. Reaction of **10** with 4-methoxyphenol in the presence of $BF_3{\cdot}Et_2O$ afforded compound **11**, which was then subjected to deacetylation with 20% triethylamine[30b] in methanol (Scheme 1). Subsequent treatment with benzaldehyde dimethylacetal in acetonitrile with camphorsulfonic acid (until solution became acidic) gave the benzylidene acetal **6**. To this end, NIS/TMSOTf promoted glycosylation[31] was accomplished using donor **5** and acceptor **6** at −20 °C for 1 h to obtain 4-methoxyphenyl protected disaccharide **12** in 85% yield and >99% α-selectivity; no detectable β-anomer was observed in the crude reaction mixture using NMR. The selectivity was confirmed by determining the H1/H2 coupling constant ($J_{1,2}$ = 2.4 Hz at δ 5.25). Treatment of compound **12** with ceric ammonium nitrate (CAN)[32] in acetonitrile and water (4:1) provided the hemiacetal, which was subsequently reacted, without purification, with trichloroacetonitrile in the presence of 1,8-diazabicycloundec-7-ene (DBU) to provide the Schmidt disaccharide donor **3**.

Scheme 1. Synthesis of Disaccharide Donor 3[a]



[a]Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, 4-methoxyphenol, anhyd. DCM, 0−5 °C, 3 h, 93%; (b) (i) 20% Et_3N, methanol, 0−5 °C, 3 h; (ii) benzaldehyde dimethylacetal, camphorsulfonic acid (until get acidic pH about 4−5), CH_3CN, rt, 2 h, 88% over two steps; (c) NIS/TMSOTf, anhyd. DCM, MS-4 Å, −20 °C, 1 h, 78%; (d) (i) CAN, CH_3CN:H_2O (4:1), rt, 2 h, 60%; (ii) trichloroacetonitrile, DBU, anhyd. DCM, MS-4 Å, −5 °C, 1 h.

Synthesis of Disaccharide Acceptor 4. The syntheses of monosaccharide building blocks **7**[32] and **8**[33] commenced from L-rhamnose and were accomplished following literature procedures (Scheme 2). Compounds **7** and **8** were dissolved in anhydrous dichloromethane (DCM) and subjected to glycosylation conditions using TMSOTf as promoter at −10 °C for 30 min and afforded disaccharide **13** in 89% yield. Due to the presence of neighboring group participation at the C-2 position of the donor with a preinstalled acetate protecting group, the reaction yielded **13** in >99% α-selectivity. The newly formed α-linkage was determined by observing the H1 proton at δ 5.07 as a doublet ($J_{1,2}$ = 1.6 Hz). Compound **13** was then subjected to Zemplén deacetylation[34] conditions using freshly prepared NaOMe/MeOH from sodium metal and methanol. After neutralization with DOWEX 50 × 8−100, the resulting reaction mixture, without further purification, was treated with 2,2-dimethoxypropane (DMP) to afford the C2′-C3′ isopropylidene acetal,[32] which was subsequently reacetylated giving rise to compound **14**. Installing the isopropylidene acetal was necessary at this point because the disaccharide was noted to be base sensitive when the succinimidyl group was present. Once compound **14** was obtained, treatment with CAN in a 4:1 acetonitrile/water solvent system was used to access the anomeric hemiacetal, which was treated with TCA to

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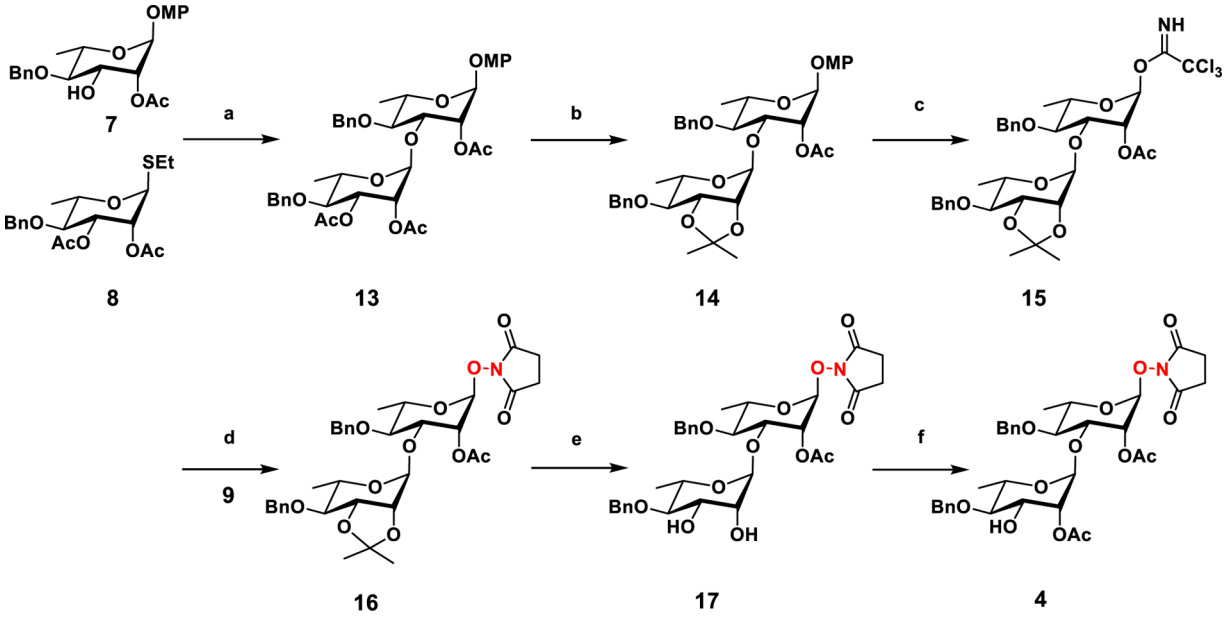
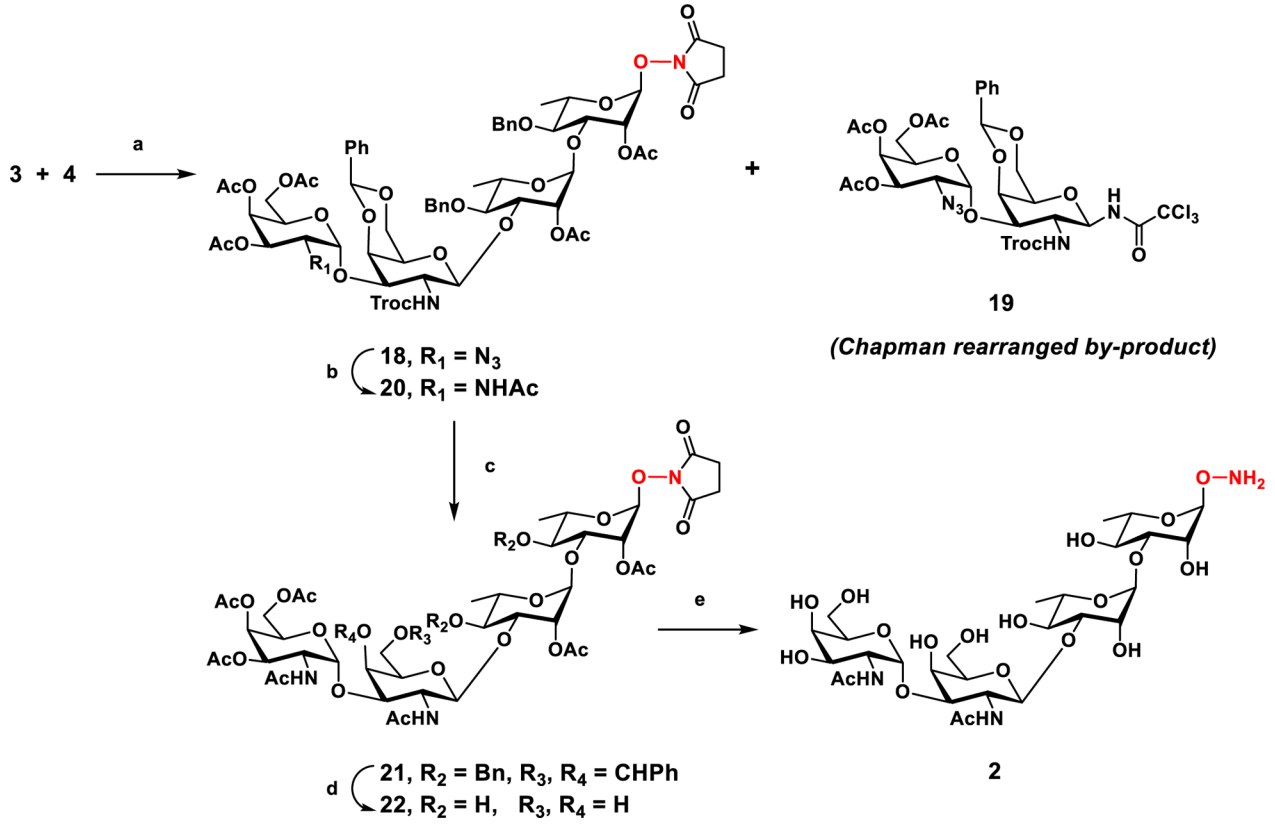
Scheme 2. Synthesis of Disaccharide Acceptor 4[a]



[a]Reagents and conditions: (a) NIS/TMSOTf, anhyd. DCM, MS-4 Å, −10 °C, 30 min, 89%; (b) (i) NaOMe/MeOH, rt, 45 min; (ii) DMP, camphorsulfonic acid, rt, 2 h; (iii) Ac_2O/pyr., rt (71% yield over three steps); (c) (i) CAN, CH_3CN:H_2O (4:1), rt, 2 h; (ii) TCA, DBU, anhyd. DCM, −5 °C, 30 min; (d) NHS, TMSOTf, anhyd. DCM, MS-4 Å, −15 °C, 4 h; (39% over three steps); (e) 80% AcOH, 80 °C, 2 h, 95%; (f) (i) triethylorthoester, camphorsulfonic acid, DMF, rt, 1.5 h; (ii) 80% AcOH, rt, 1 h (84% yield over two steps).

Scheme 3. Synthesis of Tetrasaccharide 2[a]



[a]Reagents and conditions: (a) TMSOTf, anhyd. DCM, MS-4 Å, −15 °C, 45 min, 53%; (b) thioacetic acid, pyr., DCM, rt, 18 h, 87%; (c) activated Zn, THF: AcOH: Ac_2O (3:2:1), 0 °C → rt, 72%; (d) 10% Pd−C, H_2, rt, 2 h; 64% (e) NH_2·NH_2·H_2O, MeOH, rt, 10 h (55%).

obtain trichloroacetimidate donor **15**. The concomitant use of TMSOTf promoted the clean glycosylation of **15** with *N*-hydroxysuccinimide (NHS) at −15 °C and yielded the α-selective disaccharide **16**. Treatment of compound **16** with 80%

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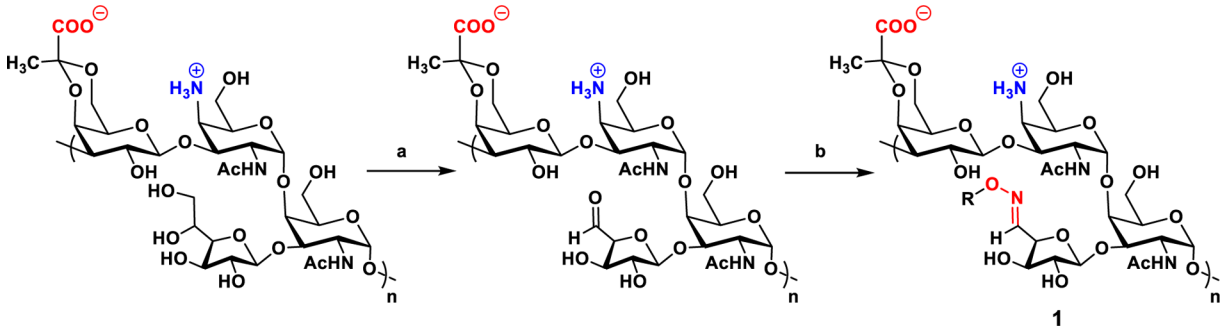
Scheme 4. Synthesis of *Tetrasaccharide*-PS A1 Conjugate 1[a]



[a]Reagents and conditions: (a) 100 mM acetate buffer (pH 5.1), 2 mM $NaIO_4$ solution, dark, rt, 90 min; (b) compound **2**, 100 mM acetate buffer, rt, overnight.

AcOH[32] at 80 °C resulted in diol **17**, which underwent orthoester mediated protection followed by successive regioselective deprotection to provide disaccharide acceptor **4**.

Synthesis of Tetrasaccharide 2. With donor **3** and acceptor **4** in hand, we elected to follow our previous approaches (Schemes 1 and 2) and perform a Schmidt glycosylation[28] using TMSOTf as the promoter in anhydrous DCM at −15 °C for 45 min. Under these reaction conditions tetrasaccharide **18** was afforded in 53% yield with exclusive β-selectivity (Scheme 3). The selectivity was confirmed by evaluating the coupling constant of the anomeric proton for the sugar donor ring of **3** at δ 4.88, which was 8.4 Hz. However, in addition to the product spot observed on the TLC plate, we also observed a TLC spot at a higher R_f value, which was initially analyzed by mass spectroscopy. Surprisingly, the ESI-MS gave the same compound mass as the TCA donor disaccharide **3**. Due to the fact that there was a large discrepancy in the R_f values of the two compounds, we assumed that a plausible Chapman rearrangement[35] might have occurred because of the acidic media used in the reaction. Our assumption was confirmed by observing an anomeric triplet at δ 5.18 with a coupling constant J = 9.18 Hz (β anomer) in the ^{1}H NMR spectrum and an additional carbonyl peak at δ 162.9 in the ^{13}C NMR spectrum.

Compound **18** was then subjected to a series of deprotection procedures to garner the aminooxy derivative of the tetrasaccharide to ultimately be utilized for further conjugation with biologically active polysaccharide PS A1. The deprotection procedure commenced with the treatment of thioacetic acid in pyridine[36] and DCM to convert the N_3 group to NHAc to furnish compound **20**. Next Zn/AcOH[30b] mediated removal of the Troc group followed by acetylation using Ac_2O in a single-pot process resulted in the acetyl protected amine **21**. Since Zn/AcOH is a very common reagent for N_3 reduction, we purposefully designed our strategy so that both N_3 and Troc were influenced by this single set of reagents. Unfortunately the reaction did not go to expected completion, even after using excess Zn/AcOH and purification of the desired product proved difficult resulting in a poor isolated yield. In order to overcome this shortcoming, we elected to exploit the thioacetic acid/pyridine reaction first. To this end, the purified product was subjected to hydrogenolysis using 10% Pd−C at room temperature under a balloon filled with H_2 to remove the benzylidene and benzyl groups. After complete hydrogenolysis, as noted by TLC, compound **22** was then treated with hydrazine hydrate[24a−d] resulting in our target aminooxy derivative of the rhamnose rich tetrasaccharide **2**, which was readily purified using a P2 gel column with water as the eluent. We were able to obtain tetrasaccharide **2** in a ∼5.0% overall yield in just 19 steps using a TCA-based convergent [2 + 2] strategy.

Synthesis of *Tetrasaccharide*-PS A1 Conjugate 1. To garner our desired immunogen construct **1**, ultrapure aminooxy tetrasaccharide **2** was conjugated with oxidized PS A1 (Scheme 4) utilizing a similar strategy that we have previously reported.[24c,d]

To this end, the final rhamnose rich tertasaccharide-PS A1 conjugate was confirmed by monitoring oxime doublets of the *E* and *Z* isomers at δ 7.6 and δ 7.0 ppm respectively with a 600 MHz ^{1}H NMR in D_2O as the solvent. The antigen loading was calculated to be ∼35% by analyzing ^{1}H NMR spectrum considering the mole fraction of the oxime doublets with respect to the mole fraction of PS A1 methyl group present on the pyruvate ring acetal of PS A1 (see Experimental Section).

Conclusion. We have synthesized a tetrasaccharide repeating unit of *S. dysgalactiae* 2023 polysaccharide. The entire synthesis was accomplished utilizing a designed strategy inclusive of stereoselective glycosylations, which consequently led to facile purification procedures and provided the desire compounds in good overall isolated yields. In addition, the incorporation of an aminooxy moiety on our rhamnose rich aminooxy tetrasaccharide eliminates the use of any additional artificial linkers in our conjugation strategy; artificial linkers are known to generate immune responses on their own. Overall, and including conjugation of the aminooxy hapten to oxidized PS A1, we have successfully obtained the first semisynthetic entirely carbohydrate immunogen targeting *S. dysgalactiae* 2023, which will be biologically examined in the near future for potential to combat bovine mastitis as the most economically debilitating disease in the dairy industry. Finally, we anticipate that further immunological studies of this *tetrasaccharide*-PS A1 construct will yield promise as a potential therapeutic because it contains two highly antigenic rhamnose moieties known to be antigenic in humans.

EXPERIMENTAL SECTION

General Experimental Methods. All chemicals and solvents were of commercial grade and purchased from commercial sources and used without further purification unless otherwise stated. Molecular sieves (MS-4 Å) were activated by heating over 150 °C overnight under a vacuum in a high temperature vacuum oven equipped with an inert gas line. All reactions progress was monitored by thin layer chromatography over silica gel-coated TLC plates. Silica gel was used to perform normal phase column chromatography. Size exclusion column was

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performed using Biorad P2 gel. ^{1}H, ^{13}C, DEPT 135, H−H COSY and HMQC NMR spectra were recorded on a 600 MHz NMR spectrometer with $CDCl_3$, MeOD, D_2O as solvents. The residual $CHCl_3$ was referenced to δ 7.26 and δ 77.26 ppm in proton and carbon spectra respectively, the residual HDO was referenced to δ 4.79 with spectra taken in D_2O. Chemical shifts are reported in δ ppm. Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, m = multiplet) and coupling constants in Hertz (Hz). Low resolution and high resolution mass spectra were obtained using electrospray ionization (ESI) and time-of-flight (TOF) techniques. Yields refer to chromatographically and spectroscopically pure material unless otherwise noted. PS A1 was isolated and purified according to a published protocol.[24c]

*4-Methoxyphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside (**11**).* NH-Troc galactosamine tetraacetate **10** (2 g, 3.8 mmol) and 4-methoxyphenol (1.42 g, 11.4 mmol) were dissolved in anhydrous dichloromethane (25 mL) under an inert atmosphere. Then the mixture was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (14.2 mL, 115 mmol) was slowly added to it. The mixture was warmed to room temperature and stirred for 6 h. The reaction mixture was quenched with saturated $NaHCO_3$. The organic layer was washed three times with aqueous $NaHCO_3$ and water in succession, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified over silica gel column using hexane−EtOAc (5:1) as the eluent to furnish pure α anomeric product **11** (2.08 g, 93%) as a white solid: mp 146−148 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.97−6.79 (m, 5 H, Ar−H), 5.42 (d, J = 3.1 Hz, 1 H, 4_C), 5.33 (d, J = 10.0 Hz, 1 H, 3_C), 5.24 (d, J = 8.4 Hz, 1 H, N*H*), 5.09 (d, J = 8.0 Hz, 1 H, 1_C), 4.76−4.70 (m, 2 H, CH_2 of NHTroc), 4.23−4.13 (m, 2 H, 6_C), 4.06−4.00 (m, 2 H, 2_C, 5_C), 3.77 (s, 3 H, OCH_3), 2.18 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 2.02 (s, 3 H, $COCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 ($COCH_3$), 170.7 ($COCH_3$), 170.6 ($COCH_3$), 156.0 (*C*O of NHTroc), 154.4−114.8 (Ar−C), 100.8 (1_C), 95.8 (CCl_3), 74.7 (CH_2 of NHTroc), 71.2 (5_C), 69.7 (3_C), 66.9 (4_C), 61.8 (6_C), 56.0 (OCH_3), 53.2 (2_C), 21.0 (3 C, $COCH_3$). ESI-MS [(M + Na)$^+$] calcd for $C_{22}H_{26}Cl_3NNaO_{11}$ is 608.04, found 609.00.

*4-Methoxyphenyl 4,6-O-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside (**6**).* A solution of compound **11** (2.08 g, 3.56 mmol) in 20% triethylamine (Et_3N) in methanol (10 mL) was stirred at 0 °C for 3 h and then neutralized with DOWEX 50WX8−100 ion exchange (H^+) resin. After neutralization, the reaction mixture was filtered and the filtrate was evaporated to dryness. Anhydrous toluene was added to azeotrope any remaining water in the reaction mixture by evaporation at reduced pressure and this process was repeated 3 times. Subsequently the mixture was left under a high vacuum for 2 h. To a solution of the crude mass in CH_3CN (30 mL) were added benzaldehyde dimethyl acetal (0.92 mL, 1.57 mmol) and camphorsulfonic acid (100 mg, 0.43 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with Et_3N (2 mL) and the solvents were removed under reduced pressure and the crude product was purified over silica gel column using hexane−EtOAc (3:1) as eluent to give pure compound **6** (1.71 g, 3.12 mmol, 88%) as a fluffy white solid: mp 167−169 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56−6.81 (m, 9 H, Ar−H), 5.62 (s, 1 H, PhC*H*), 5.27 (br s, 1 H, N*H*), 5.14 (d, J = 6.7 Hz, 1 H, 1_C), 4.78−4.71 (m, 2 H, CH_2 of NHTroc), 4.40−4.38 (m, 1 H, 6_{Ca}), 4.29 (d, J = 3.6 Hz, 1 H, 4_C), 4.13−4.11 (m, 2 H, 3_C, 6_{Cb}), 3.87 (br s, 1 H, 2_C), 3.79 (s, 3 H, OCH_3), 3.61 (s, 1 H, 5_C). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.9 (*C*O of NHTroc), 154.8−114.8 (Ar−C), 101.7 (PhCH), 100.4 (1_C), 95.7 (CCl_3), 75.2 (4_C), 74.9 (CH_2 of NHTroc), 70.1 (3_C), 69.3 (6_C), 67.0 (5_C), 56.1 (2_C), 55.9 (OCH_3). HRMS:TOF [(M + Na)$^+$] calcd for $C_{23}H_{24}Cl_3NNaO_8$ is 570.0465, found 570.0464.

*4-Methoxyphenyl (2-deoxy-2-azido-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside (**12**).* To a solution of compound **5** (0.82 g, 2.18 mmol) and **6** (1.0 g, 1.82 mmol) in anhydrous CH_2Cl_2 (30 mL) preactivated molecular sieves (MS-4 Å, 0.5 g) were added and the reaction mixture was stirred at room temperature for 30 min under argon atmosphere. NIS (*N*-iodosuccinimide) (0.59 g, 2.62 mmol) was added and the reaction mixture cooled to −20 °C. After 10 min at the same temperature, TMSOTf (20 μL) was added and the reaction mixture was stirred until completion of the reaction (1 h) was noted by TLC. The reaction mixture was quenched with saturated $NaHCO_3$ and sodium thiosulfate, filtered through a pad of Celite-545, and finally washed with DCM (100 mL). The organic layer was then washed three times with aqueous $NaHCO_3$ and water in succession, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified over silica gel column using hexane−EtOAc (5:1) as the eluent to furnish pure α anomeric product **12** (1.22 g, 1.41 mmol, 78%) as an off-white solid: mp 151−153 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59−6.78 (m, 9 H, Ar−H), 5.80 (d, J = 6.9 Hz, 1 H, N*H*), 5.63 (s, 1 H, PhC*H*), 5.45 (br s, 1 H, 4_D), 5.42 (d, J = 8.3 Hz, 1 H, 1_C), 5.40−5.37 (m, 1 H, 3_D), 5.25 (d, J = 2.4 Hz, 1 H, 1_D), 4.87 (d, J = 12.0 Hz, 1 H, CH_2 of NHTroc), 4.67 (d, J = 9.6 Hz, 1 H, 3_C), 4.57 (d, J = 12.0 Hz, 1 H, CH_2 of NHTroc), 4.45 (br s, 1 H, 4_C), 4.42 (d, J = 12.4 Hz, 1 H, 6_{Ca}), 4.30 (t, J = 6.4 Hz, 1 H, 5_D), 4.15−4.08 (m, 3 H, 6_{Cb}, 6_{Da}, 6_{Db}), 3.89−3.86 (m, 1 H, 2_C), 3.78 (s, 3 H, OCH_3), 3.63−3.61 (m, 2 H, 5_C, 2_D), 2.16 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.06 (s, 3 H, $COCH_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 ($COCH_3$), 170.2 ($COCH_3$) 169.9 ($COCH_3$) 155.7 (*C*O of NHTroc), 154.2−114.6 (Ar−C), 101.0 (PhCH), 99.3 (1_C), 95.7 (CCl_3), 95.5 (1_D), 74.5 (CH_2 of NHTroc), 73.0 (3_C), 71.8 (4_C), 69.4 (6_C), 67.8 (3_D), 67.5 (4_D), 67.2 (5_D), 66.6 (5_C), 61.5 (6_D), 57.1 (2_D), 55.8 (OCH_3), 53.9 (2_C), 21.0 ($COCH_3$), 20.8 (2 C, $COCH_3$). ESI-MS [(M + Na)$^+$] calcd for $C_{35}H_{39}Cl_3N_4NaO_{15}$ is 883.2, found 883.5.

*4-Methoxyphenyl (2,3,-di-O-acetyl-4-benzyl-α-L-rhamnopyranosyl)-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**13**).* Glycosyl donor **8** (1.14 g, 2.98 mmol) and glycosyl acceptor **7** (1 g, 2.48 mmol) were dissolved in anhydrous DCM (30 mL). Preactivated molecular sieves (MS-4 Å, 0.5 g) were added to the reaction mixture and it was stirred at room temperature for 30 min under an atmosphere of argon. NIS (0.80 g, 3.33 mmol) was the added and the reaction mixture cooled to −10 °C. TMSOTf (25 μL) was added to the mixture at the same temperature and 30 min later completion of the reaction was observed by TLC. The reaction mixture was quenched with saturated $NaHCO_3$ and sodium thiosulfate, filtered through a pad of Celite-545 and finally washed with DCM (100 mL). The organic layer was washed three times with aqueous $NaHCO_3$ and water in succession, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was purified using a silica gel column with hexane−EtOAc (5:1) as the eluent to furnish pure α anomeric product **13** (1.60 g, 2.21 mmol, 89%) as a fluffy white solid: mp 91−93 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38−6.82 (m, 14 H, Ar−H), 5.40−5.39 (m, 1 H, 2_B), 5.37 (d, J = 1.6 Hz, 1 H, 1_A), 5.36−5.33 (m, 2 H, 2_A, 3_B), 5.07 (d, J = 1.6 Hz, 1 H, 1_B), 4.91 (d, J = 10.8 Hz, 1 H, PhCH_2), 4.73−4.67 (m, 3 H, PhCH_2), 4.32 (dd, $J_{2/3,\,3/4}$ = 3.4, 9.5 Hz, 1 H, 3_A), 3.95−3.88 (m, 2 H, 5_A, 5_B), 3.78 (s, 1 H, OCH_3), 3.58 (*t*, J = 9.5 Hz, 1 H, 4_A), 3.53 (t, J = 9.5 Hz, 1 H, 4_B), 2.23 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 2.00 (s, 3 H, $COCH_3$), 1.35 (d, J = 6.2 Hz, 3 H, CCH_3), 1.32 (d, J = 6.2 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7 ($COCH_3$), 170.2 ($COCH_3$) 170.0 ($COCH_3$) 155.2−114.8 (Ar−C), 99.8 (1_A), 96.2 (1_B), 80.3 (4_A), 78.6 (4_B), 77.8 (3_A), 75.9 (PhCH_2), 74.9 (PhCH_2), 72.2 (3_B), 71.6 (2_A), 70.6 (2_B), 68.8 (5_A), 68.6 (5_B), 55.8 (OCH_3), 21.3 ($COCH_3$), 21.1 ($COCH_3$), 21.0 ($COCH_3$), 18.1 (CCH_3), 18.0 (CCH_3). ESI-MS [(M + Na)$^+$] calcd for $C_{35}H_{39}Cl_3N_4NaO_{15}$ is 745.3, found 745.3.

*4-Methoxyphenyl (3,4-O-isopropylidene-4-benzyl-α-L-rhamnopyranosyl)-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**14**).* A solution of compound **13** (1.60 g, 2.21 mmol) in 0.1 M NaOMe in methanol (5 mL) was stirred at room temperature for 1 h and then the base was neutralized with DOWEX 50 × 8−100 ion exchange (H^+) resin. After neutralization, the reaction mixture was filtered and the filtrate was evaporated to dryness. Anhydrous toluene was added to azeotrope any remaining water in the reaction mixture by evaporation at reduced pressure and this process was repeated 3 times. Subsequently the mixture was left under a high vacuum for 2 h. To a

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solution of the crude mass in CH_3CN (30 mL) were added 2,2-dimethoxypropane (0.49 mL, 4.7 mmol) and camphorsulfonic acid (80 mg, 0.34 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with Et_3N (2 mL) and the solvents were removed under reduced pressure. To the crude reaction mixture was added acetic anhydride (2 equiv) and pyridine (excess) and it was stirred at the room temperature for 2 h. After completion of the reaction, the solvents were removed under reduced pressure and the crude product was purified over silica gel column using hexane−EtOAc (3:1) as eluent to give pure compound **14** (1.06 g, 1.56 mmol, 71%) as a colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.30−6.81 (m, 14 H, Ar−H), 5.39 (d, J = 1.7 Hz, 1 H, 1_A), 5.30 (s, 1 H, 1_B), 5.28−5.27 (m, 1 H, 2_A), 4.91 (d, J = 11.6 Hz, 1 H, PhCH_2), 4.84 (d, J = 11.2 Hz, 1 H, PhCH_2), 4.70−4.66 (m, 2 H, PhCH_2), 4.32 (dd, $J_{2/3,\ 3/4}$ = 3.4, 9.5 Hz, 1 H, 3_A), 4.29−4.27 (m, 1 H, 3_B), 4.21 (d, J = 5.9 Hz, 1 H, 2_B), 3.93−3.3.90 (m, 1 H, 5_A), 3.76 (s, 1 H, OCH_3), 3.74−3.70 (m, 1 H, 5_B), 3.55 (t, J = 9.5 Hz, 1 H, 4_A), 3.24 (dd, $J_{3/4,\ 4/5}$ = 7.2, 9.8 Hz, 1 H, 4_B), 2.19 (s, 3 H, COCH_3), 1.52 (s, 3 H, isopropyl CH_3), 1.35 (s, 3 H, isopropyl CH_3), 1.34 (d, J = 6.2 Hz, 3 H, CCH_3), 1.29 (d, J = 6.3 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7 ($COCH_3$), 155.2−109.3 (Ar−C), 100.3 (1_B), 96.2 (1_A), 81.1 (4_B), 80.6 (4_A), 78.8 (3_B), 77.7 (3_A), 76.6 (2_B), 75.9 (PhCH_2), 73.3 (PhCH_2), 72.7 (2_A), 68.7 (5_A), 65.7 (5_B), 55.8 (OCH_3), 28.2 (isopropyl CH_3), 26.5 (isopropyl CH_3), 21.3 ($COCH_3$), 18.2 (CCH_3), 17.8 (CCH_3). ESI-MS [(M + Na)$^+$] calcd for $C_{38}H_{46}O_{11}$ is 701.8, found 701.7. HRMS:TOF [(M + Na)$^+$] calcd for $C_{38}H_{46}NaO_{11}$ is 701.2938, found 701.2914.

*Succinimidyl (3,4-O-isopropylidene-4-benzyl-α-L-rhamnopyranosyl)-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**16**).* To a solution of compound **14** (1.06 g, 1.56 mmol) in CH_3CN−H_2O (25 mL; 4:1, v/v) was added ceric ammonium nitrate (CAN; 2.57 g, 4.68 mmol) and the reaction mixture was stirred at room temperature for 2 h. After that the reaction mixture was diluted with DCM (80 mL) and the organic layer was washed with saturated $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and evaporated to dryness to give disaccharide hemiacetal. To a solution of the hemiacetal in anhydrous DCM (15 mL) was added trichloroacetonitrile (16 mL, 1.11 mmol) and the reaction mixture was cooled to 0 °C. DBU (0.1 mL, 0.65 mmol) was added to the cooled reaction mixture and it was stirred at 0 °C for 30 min. The reaction mixture was evaporated to dryness and the crude product was passed through quickly a short pad of silica to furnish (3,4-*O*-isopropylidene-4-benzyl-α-L-rhamnopyranosyl-(1−3)-2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl trichloroacetimidate (**15**) (0.54 g, 0.75 mmol), which was used immediately for the next step. To a solution of compound **15** (0.54 g, 0.75 mmol) and *N*-hydroxysuccinimide (**9**) (0.26 g, 2.26 mmol) in anhydrous DCM (20 mL) preactivated molecular sieves (MS-4 Å) were added and the reaction mixture was stirred at room temperature for 30 min under argon atmosphere. Then the reaction mixture was cooled to −15 °C. To the cooled reaction mixture TMSOTf (30 μL) was added and it was stirred at −15 °C for 4 h. The reaction mixture was diluted with DCM (50 mL) and the organic layer was washed with saturated $NaHCO_3$ and water in succession, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified over silica gel column using hexane−EtOAc (5:1) as an eluent to give pure compound **16** (0.41 g, 0.60 mmol, 39% overall yield for 3 steps) as a yellowish solid: mp 87−89 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39−7.28 (m, 10 H, Ar−H), 5.46−5.45 (m, 1 H, 2_A), 5.28 (d, J = 1.8 Hz, 1 H, 1_A), 5.25 (s, 1 H, 1_B), 4.90 (d, J = 11.7 Hz, 1 H, PhCH_2), 4.80 (d, J = 10.9 Hz, 1 H, PhCH_2), 4.67−4.64 (m, 2 H, PhCH_2), 4.52−4.49 (m, 1 H, 5_A), 4.26−4.24 (m, 1 H, 3_B), 4.21 (dd, $J_{2/3,\ 3/4}$ = 3.4, 8.8 Hz, 1 H, 3_A), 4.15 (d, J = 5.8 Hz, 1 H, 3_B), 3.76−3.73 (m, 1 H, 5_B), 3.51 (t, J = 9.2 Hz, 1 H, 4_A), 3.23−3.20 (m, 1 H, 4_B), 2.72 (s, 4 H, CH_2 of succinimide), 2.17 (s, 3 H, COCH_3), 1.50 (s, 3 H, isopropyl CH_3), 1.33 (s, 3 H, isopropyl CH_3), 1.31 (d, J = 6.2 Hz, 3 H, CCH_3), 1.29 (d, J = 6.2 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7 (CO of succinimide), 169.7 ($COCH_3$), 138.5−127.8 (Ar−C),109.2 (C of isopropyl), 101.7 (1A), 99.4 (1B), 80.9 (4_B), 79.9 (4_A), 78.7 (3_B), 76.4 (2_B), 76.1 (3_A), 75.3 (PhCH_2), 73.2 (PhCH_2), 70.6 (5_A), 70.2 (2_A), 65.7 (5_B), 28.1 (isopropyl CH_3), 26.5 (isopropyl CH_3), 25.6 (CH_2 of succinimide) 21.1 ($COCH_3$), 17.9 (CCH_3), 17.8 (CCH_3). HRMS:TOF [(M + Na)$^+$] calcd for $C_{35}H_{43}NNaO_{12}$ is 692.2683, found 692.2672.

*Succinimidyl (4-benzyl-α-L-rhamnopyranosyl)-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**17**).* A solution of compound **16** (0.41 g, 0.60 mmol) in 80% aq acetic acid (25 mL) was stirred at 80 °C for 2 h. Then the solvents were evaporated and coevaporated with toluene to remove residual AcOH. The residue was purified by flash chromatography using hexane−EtOAc (1:3) as eluent to give pure disaccharide diol (**17**) (0.36 g, 0.57 mmol) as a white solid: mp 105−107 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36−7.26 (m, 10 H, Ar−H), 5.46−5.45 (m, 1 H, 2_A), 5.25 (d, J = 1.8 Hz, 1 H, 1_A), 5.03 (s, 1 H, 1_B), 4.76−4.63 (m, 4 H, PhCH_2), 4.48−4.46 (m, 1 H, 5_A), 4.17 (dd, $J_{2/3,\ 3/4}$ = 3.4, 9.0 Hz, 1 H, 3_A), 3.85−3.84 (m, 1 H, 2_B), 3.81−3.79 (m, 2 H, 3_B, 5_B), 3.46 (t, J = 9.3 Hz, 1 H, 4_A), 3.32 (t, J = 9.4 Hz, 1 H, 4_B), 2.69 (s, 4 H, CH_2 of succinimide), 2.13 (s, 3 H, COCH_3), 1.32 (d, J = 6.2 Hz, 3 H, CCH_3), 1.27 (d, J = 6.2 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 (CO of Succinimide), 169.7 ($COCH_3$), 138.5−128.0 (Ar−C), 101.7 (1_A), 101.2 (1_B), 81.4 (4_B), 80.1 (4_A), 75.7 (3_A), 75.4 (PhCH_2), 74.8 (PhCH_2), 71.4 (2_B), 71.1 (3_B), 70.7 (5_A), 70.0 (2_A), 68.3 (5_B), 25.6 (CH_2 of Succinimide), 21.1 ($COCH_3$), 18.1 (CCH_3), 17.9 (CCH_3). ESI-MS [(M + Na)$^+$] calcd for $C_{34}H_{41}NNaO_{13}$ is 652.2, found 652.3.

*Succinimidyl 2-O-acetyl-4-benzyl-α-L-rhamnopyranosyl-(1−3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**4**).* To a solution of **17** (0.36 g, 0.57 mmol) in anhydrous DMF (10 mL), trimethyl orthoacetate (0.13 mL, 0.80 mmol) was added followed by camphorsulfonic acid (CSA) (20 mg). The mixture was stirred at room temperature until complete conversion of the starting material to a faster moving spot on TLC (1.5 h) was noted. The solvent was evaporated under reduced pressure and the residue was dissolved in AcOH−H_2O (4:1, 25 mL) and stirred at room temperature for 1 h. After that solvent was evaporated in vacuo and the residue was purified by flash chromatography using hexane−EtOAc (1:2) as the eluent to give pure compound **4** (0.34 g, 0.51 mmol, 84% overall for 2 steps) as a white solid: mp 97−99 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38−7.28 (m, 10 H, Ar−H), 5.47 (s, 1 H, 2_A), 5.32 (s, 1 H, 1_A), 5.20 (s, 1 H, 2_B), 5.01 (s, 1 H, 1_B), 4.82−4.73 (m, 3 H, PhCH_2), 4.63 (d, J = 11.04 Hz, 1 H, PhCH_2), 4.49−4.47 (m, 1 H, 5_A), 4.21−4.19 (m, 1 H, 3_A), 4.04−4.02 (m, 1 H, 3_B), 3.86−3.84 (m, 1 H, 5_B), 3.50 (t, J = 9.3 Hz, 1 H, 4_A), 3.36 (t, J = 9.4 Hz, 1 H, 4_B), 2.72 (s, 4 H, CH_2 of succinimide), 2.17 (s, 3 H, COCH_3), 2.10 (s, 3 H, COCH_3), 1.35 (d, J = 6.24 Hz, 3 H, CCH_3), 1.27 (d, J = 6.06 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8 (2 C, $COCH_3$, CO of succinimide), 169.8 ($COCH_3$), 138.3−128.0 (Ar−C), 101.7 (1_A), 99.4 (1_B), 81.4 (4_B), 80.1 (4_A), 75.3 (PhCH_2), 75.0 (3_A), 74.9 (PhCH_2), 72.6 (2_B), 70.7 (5_A), 69.8 (3_B), 69.7 (2_A), 68.5 (5_B), 25.6 (CH_2 of succinimide) 21.1 (2 C, $COCH_3$), 18.1 (CCH_3), 17.8 (CCH_3). HRMS:TOF [(M + Na)$^+$] calcd for $C_{34}H_{41}NNaO_{13}$ is 694.2476, found 694.2469.

*Succinimidyl (2-deoxy-2-azido-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside-(1 → 3)-2-O-acetyl-4-benzyl-α-L-rhamnopyranosyl-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**18**).* To a solution of compound **12** (1.22 g, 1.41 mmol) in CH_3CN−H_2O (25 mL; 4:1, v/v) ceric ammonium nitrate (CAN; 2.33 g, 4.25 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with DCM (80 mL) and the organic layer was washed with saturated $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and evaporated to dryness to give disaccharide hemiacetal. To a solution of the hemiacetal in anhydrous DCM (15 mL) was added trichloroacetonitrile (0.14 mL, 0.97 mmol) and the reaction mixture was cooled to 0 °C. DBU (0.1 mL, 0.65 mmol) was added to the cooled reaction mixture and it was stirred at 0 °C for 1 h. The reaction mixture was evaporated to dryness and the crude product was passed quickly through a short pad of silica to furnish (2-deoxy-2-azido-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl)-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranosyl trichloroacetimidate (**3**) (0.57 g, 0.64 mmol), which was used immediately for the next step. To a solution of compound **3** (0.57 g, 0.64 mmol) and compound **4** (0.34 g, 0.51 mmol) in anhydrous DCM (15 mL)

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preactivated molecular sieves (MS-4 Å) were added and the reaction mixture was stirred at room temperature for 30 min under argon atmosphere. Then the reaction mixture was cooled to −15 °C. To the cooled reaction mixture was added TMSOTf (30 μL) and it was stirred at −15 °C for 45 min. The reaction mixture was diluted with DCM (50 mL) and the organic layer was washed with saturated $NaHCO_3$ and water in succession, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified over silica gel column using hexane−EtOAc (2:1) as eluent to give pure compound **18** (0.38 g, 0.27 mmol, 53%) as a white solid: mp 139−141 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54−7.28 (m, 15 H, Ar−H), 5.52 (s, 1 H, PhC*H*), 5.49−5.48 (m, 1H, 2_A), 5.41 (br s, 1H, 4_D), 5.34−5.332 (m, 2 H, 3D, N*H*$_C$), 5.28 (d, *J* = 1.9 Hz, 1 H, 1_A), 5.20 (br s, 1 H, 2_B), 5.19 (d, *J* = 3.1 Hz, 1 H, 1_D), 5.05 (d, *J* = 1.6 Hz, 1 H, 1_B), 4.98 (d, *J* = 11.2 Hz, 1 H, PhC*H*$_2$), 4.90 (d, *J* = 11.9 Hz, 1 H, PhC*H*$_2$), 4.88 (d, *J* = 8.0 Hz, 1 H, 1_C), 4.69 (d, *J* = 12.0 Hz, 1 H, PhC*H*$_2$), 4.63−4.60 (m, 2 H, PhC*H*$_2$, C*H*$_2$ of NHTroc), 4.49−4.46 (m, 2 H, 5_A, C*H*$_2$ of NHTroc), 4.34 (d, *J* = 6.5 Hz, 1 H, 3_C), 4.23−4.17 (m, 4 H, 3_A, 4_C, 6_{Ca}, 5_D), 4.10−4.07 (m, 2 H, 6_{Dab}), 4.05−4.03 (m, 1 H, 3_B), 3.88−3.86 (m, 1 H, 6_{Cb}), 3.76−3.74 (m, 1 H, 5_B), 3.57−3.49 (m, 4 H, 4_A, 4_B, 2_C, 2_D), 3.01 (br s, 1 H, 5_C), 2.72 (s, 4 H, C*H*$_2$ of succinimide), 2.21 (s, 3 H, COC*H*$_3$), 2.15 (s, 1 3 H, COC*H*$_3$), 2.13 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 2.04 (s, 3 H, COC*H*$_3$), 1.28 (d, *J* = 6.1 Hz, 3 H, CC*H*$_3$), 1.25 (d, *J* = 6.1 Hz, 3 H, CC*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1 (CO of succinimide), 170.8 ($COCH_3$), 170.7 (2 C, $COCH_3$), 170.1 ($COCH_3$), 169.8 (2 C, $COCH_3$), 154.1 (CO of NHTroc), 139.0−125.5 (Ar−C), 101.6 (1_A), 100.8 (PhCH), 99.4 (1_C), 99.0 (1_B), 95.5 (CCl_3), 95.4 (1_D), 79.9 (4_B), 79.4 (4_A), 79.1 (3_B), 75.2 (C*H*$_2$ of NHTroc), 75.0 (PhC*H*$_2$), 74.4 (PhC*H*$_2$), 72.9 (3_C), 71.3 (2_B), 71.1 (3_A), 70.5 (5_A), 69.9 (2_A), 69.2 (6_C), 68.7 (5_B), 67.5(4_D), 67.4 (3_D), 67.1 (2 C, 4_C, 5_D), 66.1 (5_C), 61.4 (6_D), 57.0 (2_C), 54.1 (2_D), 25.6 (C*H*$_2$ of succinimide), 21.3 ($COCH_3$), 21.2 ($COCH_3$), 21.0 ($COCH_3$), 20.8 (2 C, $COCH_3$), 18.0 (CCH_3), 17.8 (CCH_3). ESI-MS [(M + Na)$^+$] calcd for $C_{62}H_{72}Cl_3N_5NaO_{26}$ is 1430.3, found 1430.7.

*Chapman Product (**19**).* Chapman rearranged product is a byproduct from the reaction of the synthesis of compound **18**. Silica gel column using hexane−EtOAc (7:1) as eluent afforded compound **19** (62.7 mg, 0.07 mmol, 11%) as a white solid: mp 118−120 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.7 Hz, 1 H, N*H*), 7.60−7.38 (m, 5 H, Ar−H), 5.67 (s, 1H, PhC*H*), 5.66 (d, *J* = 8.5 Hz, 1 H, N*H*), 5.45 (d, *J* = 2.7 Hz, 1 H, 4_D), 5.31−5.29 (m, 1 H, 3_D), 5.22 (d, *J* = 3.7 Hz, 1 H, 1_D), 5.18 (t, *J* = 9.2 Hz, 1 H, 1_C), 4.87 (d, *J* = 12.0 Hz, 1 H, C*H*$_{2a}$ of NHTroc), 4.66 (d, *J* = 12.0 Hz, 1 H, C*H*$_{2b}$ of NHTroc), 4.46−4.43 (m, 2 H, 5_D, 6_{Da}), 4.41 (br s, 1 H, 4_C), 4.35−4.30 (m, 1 H, 2_C), 4.16−4.11 (m, 3 H, 6_{Cab}, 6_{Db}), 4.04−4.01 (m, 1 H, 3_C), 3.73 (dd, $J_{1/2,\ 2/3}$ = 3.7, 11.3 Hz, 1 H, 2_D), 3.68 (s, 1 H, 5_C), 2.18 (s, 3 H, COC*H*$_3$), 2.12 (s, 3 H, COC*H*$_3$), 2.06 (s, 3 H, COC*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9 ($COCH_3$), 170.2 ($COCH_3$), 169.8 ($COCH_3$), 162.9 ($NHCOCCl_3$), 155.5 (CO of NHTroc), 137.2−126.2 (Ar−C), 101.0 (PhCH), 96.1 (1_D), 95.2 (CCl_3), 92.0 (CCl_3), 81.8 (1_C), 75.6 (3_C), 75.0 (C*H*$_2$ of NHTroc), 71.8 (4_C), 69.3 (6_D), 68.6 (5_C), 67.9 (3_D), 67.5 (5_D), 67.4 (4_D), 62.7 (6_C), 56.9 (2_D), 51.9 (2_C), 21.1 ($COCH_3$), 20.8 (2 C, $COCH_3$). ESI-MS [(M + Na)$^+$] calcd for $C_{30}H_{33}Cl_6N_5NaO_{14}$ is 920.0, found 920.5.

*Succinimidyl (2-deoxy-2-acetamido-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-β-D-galactopyranoside-(1 → 3)-2-O-acetyl-4-benzyl-α-L-rhamnopyranosyl-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**20**).* Compound **18** (0.38 g, 0.27 mmol) was dissolved in pyridine (2 mL) and thioacetic acid (1 mL) was added to it at 0 °C. After that the reaction mixture was stirred at room temperature for 18 h, evaporated and coevaporated with toluene to get rid of pyridine, then diluted with ethyl acetate and sequentially washed with saturated $NaHCO_3$, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to get a residue, which was purified by flash column chromatography using hexane−EtOAc (1:3) to afford compound **20** (0.34 g, 0.24 mmol, 87%) as a white solid: mp 131−133 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49−7.19 (m, 15 H, Ar−H), 5.86 (d, *J* = 8.7 Hz, 1 H, NH_D), 5.51−5.50 (m, 1 H, 2_A), 5.41 (s, 1 H, PhC*H*), 5.30 (br s, 1 H, 4_D), 5.28 (d, *J* = 1.9 Hz, 1 H, 1_A), 5.25 (br s, 1 H, 2_B), 5.06 (br s, 1 H, 1_B), 5.04 (d, *J* = 3.6 Hz, 1 H, 1_D), 4.98−4.94 (m, 3 H, 3_D, C*H*$_2$ of NHTroc), 4.74 (d, *J* = 12.0 Hz, 1 H, PhC*H*$_2$), 4.68 (d, *J* = 12.1 Hz, 1 H, PhC*H*$_2$), 4.63−4.56 (m, 3 H, 1_C, 2_D, PhC*H*$_2$), 4.50−4.47 (m, 1 H, 5_A), 4.24−4.22 (m, 1 H, 3_A), 4.17−4.12 (m, 5 H, 5_C, 6_{Ca}, 5_D, 6_{Da}, PhC*H*$_2$), 4.10−4.07 (m, 2 H, 3_B, 6_{Cb}), 3.97−3.93 (m, 1 H, 2_C), 3.87−3.83 (m, 2 H, 3_C, 6_{Db}), 3.80−3.78 (m, 1 H, 5_B), 3.55 (t, *J* = 9.4 Hz, 1 H, 4_B), 3.51 (t, *J* = 9.2 Hz, 1 H, 4_A), 2.99 (br s, 1 H, 4_C), 2.74 (s, 4 H, C*H*$_2$ of succinimide), 2.20 (s, 3 H, COC*H*$_3$), 2.17 (s, 3 H, COC*H*$_3$), 2.12 (s, 3 H, COC*H*$_3$), 2.07 (s, 3 H, COC*H*$_3$), 1.94 (s, 3 H, COC*H*$_3$), 1.35 (s, 3 H, NHCOC*H*$_3$), 1.28 (d, *J* = 6.2 Hz, 3 H, CC*H*$_3$), 1.25 (d, *J* = 6.2 Hz, 3 H, CC*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2 ($NHCOCH_3$), 170.9 (CO of succinimide), 170.8 ($COCH_3$), 170.7 ($COCH_3$), 170.6 ($COCH_3$), 170.4 ($COCH_3$), 169.8 ($COCH_3$), 154.6 (CO of NHTroc), 138.9−125.5 (Ar−C), 101.7 (PhCH), 101.6 (1_A), 100.7 (1_C), 98.7 (1_B), 95.4 (CCl_3), 93.4 (1_D), 79.7 (4_B), 79.5 (4_A), 78.9 (3_B), 75.9 (3_A), 75.1 (CH_2 of NHTroc), 74.9 ($PhCH_2$), 74.6 ($PhCH_2$), 72.7 (3_C), 71.3 (2_B), 70.5 (5_A), 70.3 (5_D), 69.8 (2_A), 69.2 (6_D), 68.7 (5_B), 68.6 (3_D), 67.1 (4_D), 67.0 (5_C), 66.0 (4_C), 61.9 (6_C), 53.2 (2_C), 47.0 (2_D), 25.6 (CH_2 of succinimide), 22.6 ($NHCOCH_3$), 21.2 (2 C, $COCH_3$), 21.1 ($COCH_3$), 20.9 (2 C, $COCH_3$), 18.0 (CCH_3), 17.8 (CCH_3). ESI-MS [(M + Na)$^+$] calcd for $C_{64}H_{76}Cl_3N_3NaO_{27}$ is 1446.4, found 1446.9.

*Succinimidyl (2-deoxy-2-acetamido-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-(1 → 3)-4,6-O-benzylidene-2-deoxy-2-(2-acetamido)-β-D-galactopyranoside-(1 → 3)-2-O-acetyl-4-benzyl-α-L-rhamnopyranosyl-(1 → 3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranoside (**21**).* To a solution of compound **20** (0.34 g, 0.24 mmol, 1 equiv), in anhydrous THF at 0 °C, zinc dust (3 w/w equiv), acetic acid (3 mL/g), and acetic anhydride (2 mL/g) were added. After addition of the reagents the reaction was stirred at room temperature and the reaction progress was monitored by TLC. Upon consumption of the starting material (3 h) the reaction mixture was immediately filtered through a pad of Celite-545. The acid was quenched with saturated $NaHCO_3$. The reaction mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash chromatography purification using hexane−EtOAc (1:4) afforded pure compound **21** (0.21 g, 0.16 mmol, 72%) as a white solid: mp 146−148 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49−7.18 (m, 15 H, Ar−H), 6.10 (d, *J* = 9.5 Hz, 1 H, NH_D), 5,78 (d, *J* = 7.7 Hz, 1 H, NH_C), 5.52−5.51 (m, 1H, 2_A), 5.41 (s, 1 H, PhC*H*), 5.31 (d, *J* = 2.8 Hz, 1 H, 4_D), 5.28 (d, *J* = 1.4 Hz, 1 H, 1_A), 5.23 (br s, 1 H, 2_B), 5.06 (br s, 1 H, 1_B), 5.03 (d, *J* = 3.8 Hz, 1 H, 1_D), 5.02−5.00 (m, 1 H, 3_D), 4.97−4.92 (m, 2 H, PhC*H*$_2$), 4.88−4.87 (d, *J* = 8.2 Hz, 1 H, 1_C), 4.74 (d, *J* = 11.8 Hz, 1 H, PhC*H*$_2$), 4.60 (d, *J* = 11.1 Hz, 1 H, PhC*H*$_2$), 4.58−4.55 (m, 1 H, 2_D), 4.50−4.47 (m, 1 H, 5_A), 4.24−4.20 (m, 2 H, 3_A, 3_C), 4.15−4.06 (m, 4 H, 4_C, 6_{Ca}, 5_D, 6_{Da}), 4.04−4.01 (m, 2 H, 3_B, 6_{Db}), 3.85−3.81 (m, 3 H, 5_B, 2_C, 6_{Cb}), 3.56−3.48 (m, 2 H, 4_A, 4_B), 3.03 (br s, 1 H, 5_C), 2.74 (s, 4 H, C*H*$_2$ of succinimide), 2.21 (s, 3 H, COC*H*$_3$), 2.16 (s, 3 H, COC*H*$_3$), 2.11 (s, 3 H, COC*H*$_3$), 2.05 (s, 3 H, COC*H*$_3$), 1.95 (s, 3 H, COC*H*$_3$), 1.57 (s, 3 H, NHCOC*H*$_3$), 1.35 (s, 3 H, NHCOC*H*$_3$), 1.30 (d, *J* = 6.2 Hz, 3 H, CC*H*$_3$), 1.27 (d, *J* = 5.8 Hz, 3 H, CC*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5 (CO of succinimide), 171.0 (2 C, $NHCOCH_3$), 171.0 (2 C, $COCH_3$), 170.7 ($COCH_3$), 170.6 ($COCH_3$), 170.0 ($COCH_3$), 138.9−125.5 (Ar−C), 101.7 (1_A), 101.6 (PhCH), 99.7 (1_C), 98.8 (1_B), 93.6 (1_D), 79.7 (4_B), 79.5 (2 C, 4_A, 3_B), 75.6 (3_A), 75.0 (2 C, $PhCH_2$), 72.0 (3_C), 71.0 (2_B), 70.6 (2 C, 5_A, 5_D), 69.8 (2_A), 69.4 (6_C), 68.8 (5_B), 68.6 (3_D), 67.0 (2 C, 4_C, 4_D), 66.1 (5_C), 61.6 (6_D), 53.1 (2_C), 47.0 (2_D), 25.6 (CH_2 of succinimide), 23.0 ($NHCOCH_3$), 22.6 ($NHCOCH_3$), 21.2 ($COCH_3$), 21.1 ($COCH_3$), 21.0 ($COCH_3$), 20.9 (2 C, $COCH_3$), 18.0 (CCH_3), 17.8 (CCH_3). ESI-MS [(M + Na)$^+$] calcd for $C_{63}H_{77}Cl_3N_3NaO_{26}$ is 1314.5, found 1314.5.

*Succinimidyl (2-deoxy-2-acetamido-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-(1 → 3)-2-deoxy-2-(2-acetamido)-β-D-galactopyranoside-(1 → 3)-2-O-acetyl-α-L-rhamnopyranosyl-(1 → 3)-2-O-acetyl-α-L-rhamnopyranoside (**22**).* To a solution of compound **21** (0.21 g, 0.16 mmol) in methanol (30 mL) was added 10% Pd−C (200 mg)

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and the mixture was stirred for 2 h at room temperature using a balloon filled with hydrogen. The reaction mixture was filtered through a Celite-545 bed and then washed with DCM/MeOH (20 mL; 2:1 v/v) and the combined filtrate was evaporated under reduced pressure to obtain the compound **22** (0.11 g, 0.11 mmol, 64%) as a colorless syrup. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.45−5.44 (m, 1 H, 2_A), 5.40 (d, J = 2.3 Hz, 1 H, 4_D), 5.31−5.30 (m, 1 H, 2_B), 5.24 (d, J = 1.2 Hz, 1 H, 1_A), 5.12 (d, J = 3.2 Hz, 1 H, 1_D), 5.11−5.10 (m, 1 H, 3_D), 5.05 (d, J = 1.3 Hz, 1 H, 1_B), 4.71 (d, J = 8.4 Hz, 1 H, 1_C), 4.50−4.48 (m, 1 H, 2_D), 4.41−4.38 (m, 1 H, 5_A), 4.28−4.26 (m, 1 H, 5_D), 4.23−4.20 (m, 1 H, 6_{Da}), 4.17−4.13 (m, 1 H, 2_C), 4.10−4.07 (m, 1 H, 6_{Db}), 4.00 (d, J = 2.3 Hz, 1 H, 4_C), 3.97 (dd, $J_{2/3,3/4}$ = 3.5, 9.5 Hz, 1 H, 3_A), 3.89 (dd, $J_{2/3,3/4}$ = 3.5, 9.5 Hz, 1 H, 3_B), 3.78−3.75 (m, 2 H, 3_C, 6_{Ca}), 3.74−3.70 (m, 2 H, 5_B, 6_{Cb}), 3.56 (t, J = 9.7 Hz, 1 H, 4_A), 3.52−3.50 (m, 1 H, 5_C), 3.48 (t, J = 9.7 Hz, 1 H, 4_B), 2.73 (s, 4 H, CH_2 of succinimide), 2.16 (s, 3 H, $COCH_3$), 2.15 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$), 2.06 (s, 3 H, $COCH_3$), 2.05 (s, 3 H, $COCH_3$), 1.97 (s, 3 H, $NHCOCH_3$), 1.95 (s, 3 H, $NHCOCH_3$), 1.28 (d, J = 6.2 Hz, 3 H, CCH_3), 1.25 (d, J = 5.8 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.0 (*C*O of succinimide), 172.3 (2 C, $NHCOCH_3$), 172.0 ($COCH_3$), 171.0 ($COCH_3$), 170.6 ($COCH_3$), 170.4 ($COCH_3$), 170.2 ($COCH_3$), 102.1 (1_C), 101.5 (1_A), 99.2 (1_B), 94.8 (1_D), 77.2 (3_B), 76.9 (3_C), 75.3 (3_A), 75.2 (5c), 71.7 (2_B), 71.5 (2 C, 4_A, 4_B), 71.1 (5_A), 69.6 (2_A), 69.2 (5_B), 68.4 (3_D), 66.8 (4_D), 66.6 (5_D), 64.2 (4_C), 60.8 (6_C), 60.6 (6_D), 50.7 (2_C), 47.2 (2_D), 25.1 (CH_2 of succinimide), 21.9 ($NHCOCH_3$), 21.3 ($NHCOCH_3$), 19.6 ($COCH_3$), 19.5 ($COCH_3$), 19.2 (3 C, $COCH_3$), 16.6 (CCH_3), 16.3 (CCH_3). ESI-MS [$(M + Na)^+$] calcd for $C_{42}H_{61}N_3NaO_{26}$ is 1046.3, found 1046.4.

*Aminooxy (2-deoxy-2-acetamido-α-D-galactopyranosyl)-(1 → 3)-(2-deoxy-2-acetamido)-β-D-galactopyranoside-(1 → 3)-α-L-rhamnopyranosyl-(1 → 3)-α-L-rhamnopyranoside (**2**).* Compound **22** (0.11 g, 0.11 mmol) was treated with hydrazine hydrate (0.17 mL, 50 equiv) in methanol and the mixture was stirred at room temperature for 10 h. After completion of the reaction (as noted by TLC solvent (R_f 0.3) [n-butanol-H_2O-AcOH (4:3:1)], the solvent was evaporated under reduced pressure to furnish compound **2**, which was purified through a P-2 Biogel column using H_2O as the eluent to give pure compound **2** (43 mg, 0.06 mmol, 55%) as a white fluffy solid. $[\alpha]_D^{25}$ = +61.78 (c 0.06, H_2O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.96 (d, J = 3.3 Hz, 1 H, 1_D), 4.90 (s, 1 H, 1_B), 4.72−4.71 (m, 1 H, 1_A), 4.22−4.21 (m, 1 H, 1_C), 4.17 (br s, 1 H, 2_B), 4.11 (dd, $J_{1/2,\ 2/3}$ = 3.3, 11.0 Hz, 1 H, 2_D), 4.00−3.97 (m, 2 H, 2_C, 5_C), 3.92 (br s, 1 H, 2_A), 3.89 (br s, 1 H, 4_D), 3.82 (dd, $J_{2/3,\ 3/4}$ = 3.5, 9.5 Hz, 1 H, 3_B), 3.76−3.74 (m, 1 H, 5_B), 3.71−3.62 (m, 8 H, 3_D, 5_D, 3_C, 6_{Cab}, 5_A, 6_{Dab}), 3.59 (dd, $J_{2/3,\ 3/4}$ = 3.1, 9.7 Hz, 1 H, 3_A), 3.54−3.52 (m, 1 H, 4_C), 3.42−3.39 (m, 2 H, 4_A, 4_B), 1.94 (s, 3 H, $NHCOCH_3$), 1.92 (s, 3 H, $NHCOCH_3$), 1.21 (d, J = 6.2 Hz, 3 H, CCH_3), 1.17 (d, J = 6.2 Hz, 3 H, CCH_3). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.8 ($NHCOCH_3$), 174.6 ($NHCOCH_3$), 102.8 (1_A), 102.6 (1_C), 101.9 (1_B), 93.5 (1_D), 79.8 (3_B), 78.0 (3_A), 74.8 (4_C), 74.6 (4_D), 71.4 (4_B), 71.3 (4_A), 70.8 (5_B), 69.8 (2_B), 69.2 (3_D), 68.8 (5_D), 68.6 (3_C), 68.2 (5_A), 67.6 (2_A), 63.5 (5_C), 61.0 (6_C), 60.8 (6_D), 50.9 (2_C), 49.3 (2_D), 22.2 ($NHCOCH_3$), 22.0 ($NHCOCH_3$), 16.6 (CCH_3), 16.5 (CCH_3). HRMS:TOF [$(M + Na)^+$] calcd for $C_{28}H_{49}N_3NaO_{19}$ is 754.2858, found 754.2844.

*Tetrasaccharide-PS A1 Conjugate (**1**).* 0.001 g (9.1×10^{-9} mol) PS A1 was dissolved in 0.4 mL of 100 mM NaOAc buffer (pH 5.0) in an amber vial. To this solution 0.1 mL of 10 mM $NaIO_4$ was added to obtain an overall 2 mM $NaIO_4$ concentration in the reaction mixture, which was stirred at room temperature in the dark for 90 min to afford oxidized PS A1. The reaction mixture was purified using a spin column loaded with 3k molecular weight cut off. Nanopure water was used to drain any residual $NaIO_4$ through the column by centrifuging it at 12 000 rpm for 30 min and the process was repeated 4 times. Pure PS A1 aldehyde was dissolved in 100 mM NaOAc buffer (pH 5.0) and 0.001 g tetrasaccharide (0.0014 mmol, 3 equiv) was added to it. This amount was determined by considering PS A1 has an average MW of 110 kg/mol and 120 repeating units per mole. The reaction was continuously stirred at 37 °C overnight in the dark followed by dialysis using distilled water. Lyophilization ultimately gave pure *tetrasaccharide*-PS A1 construct.

The percent loading was calculated based on the integration value of oxime protons on ^{1}H NMR spectrum and compared to methyl protons present on the pyruvate ring acetal of PS A1. The loading was determined to be ∼35% using the following formula: {MW of tetrasaccharide/(MW of PS A1 + MW of tetrasaccharide)} × (mole fraction of oxime H) × 100, where mole fraction of oxime H = oxime H integration/(methyl proton integration/3).

The calculated loading of this tetrasaccharide antigen to PS A1 is similar to that of Tn-PS A1[24c] construct, where loading of Tn antigen to PS A1 was obtained as 30−35% based on fluorescent labeling study. As Tn specific robust antibody response was obtained from Tn-PS A1 immunization with this loading, it can be concluded that the *tetrasaccharide*-PS A1 construct has optimum antigen loading to invoke antigen specific immune responses.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00195.

Copies of ^{1}H, ^{13}C, DEPT 135, H−H COSY, HMQC NMR spectra of all new compounds and HRMS data for compounds **2**, **4**, **6**, **14** and **16**. (PDF)

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Notes

The authors declare no competing financial interest.

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