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Total Synthesis of the Repeating Unit of *Bacteroides fragilis* Zwitterionic Polysaccharide A1

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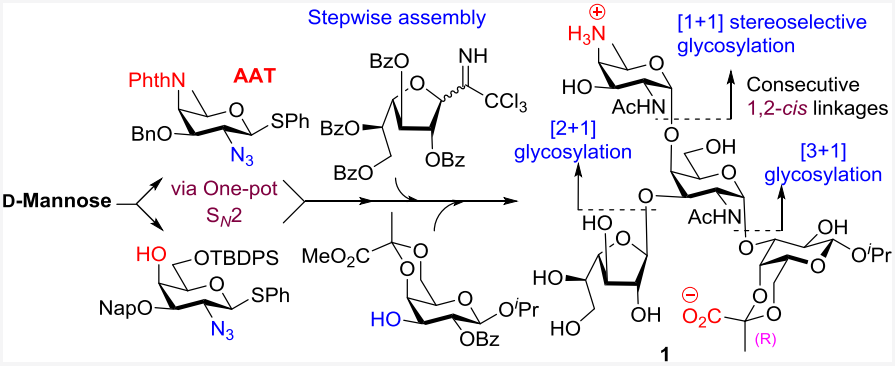

ABSTRACT: Zwitterionic polysaccharides isolated from commensal bacteria are endowed with unique immunological properties and are emerging as immunotherapeutic agents as well as vaccine carriers. Reported herein is a total synthesis of the repeating unit of *Bacteroides fragilis* zwitterionic polysaccharide A1 (PS A1). The structurally complex tetrasaccharide unit contains a rare sugar 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) and two consecutive 1,2-*cis* glycosidic linkages. The repeating unit was efficiently assembled by rapid synthesis of D-galactosamine and AAT building blocks from cheap and abundant D-mannose via a one-pot S_N2 displacement of 2,4-bistriflates and installation of all of the glycosidic bonds in a highly stereoselective manner. The total synthesis involves a longest linear sequence of 17 steps with 3.47% overall yield.

INTRODUCTION

Zwitterionic polysaccharides (ZPSs) are emerging as an important class of immunotherapeutic agents as well as potential carriers of carbohydrate-based vaccines.[1,2] ZPSs present on the surfaces of commensal bacteria possess a unique ability to activate major histocompatibility complex class II (MHC-II)-mediated T-cell-dependent immune response in the absence of proteins.[3] Over the past few years, a variety of semisynthetic polysaccharide-derived ZPSs have been shown to display potent immunostimulatory activity.[4] The promising applications of ZPSs both as adjuvants and immunostimulators have made them attractive synthetic targets, and as a result, a few highly complex ZPSs have been already synthesized.[5]

The most well studied natural ZPS is polysaccharide A1 (PS A1, Figure 1),[6] which is found on the capsule of the commensal bacterium *Bacteroides fragilis*. Numerous reports have highlighted the potent immunostimulating properties of PS A1. It displays anti-inflammatory properties and plays a key role in the development and the maintenance of a balanced mammalian immune system.[7,8] It also stimulates IL-10 secretion, modulates surgical fibrosis,[9] inhibits intestinal inflammatory disease caused by *Helicobacter hepaticus*,[10] and protects against central nervous system (CNS) demyelinating

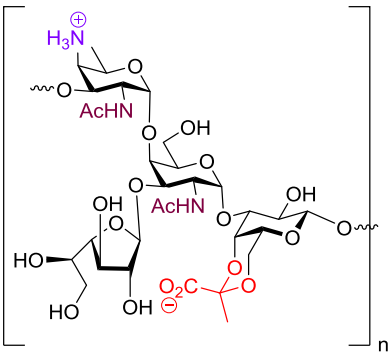

Figure 1. Structure of the zwitterionic tetrasaccharide PS A1 repeating unit.

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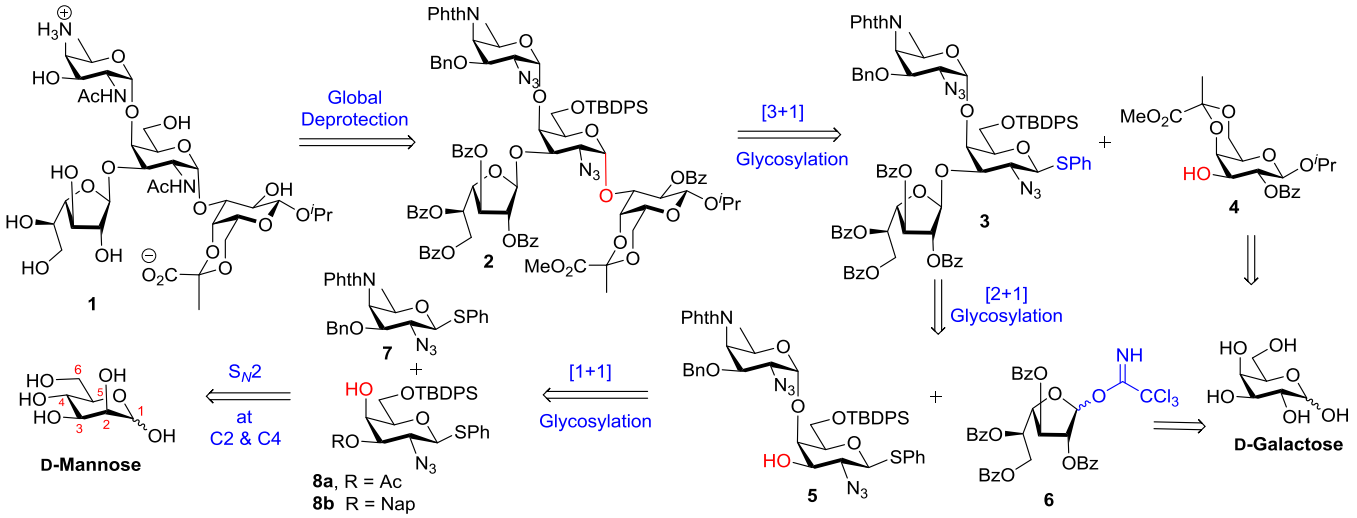
Scheme 1. Retrosynthetic Analysis



disease. In addition to immunostimulating properties, PS A1 can also bind to MHC-II and elicit a strong T-cell-dependent immune response. An entirely carbohydrate-based vaccine has been developed by employing PS A1 in place of protein carriers for conjugation with other glycans such as the tumor-associated cancer antigens Tn[11] and STn[12] as well as the repeating unit of *Streptococcus dysgalactiae* 2023 polysaccharide.[13] Given its biological importance, there is a great interest in developing novel, efficient strategies for the construction of the structurally well-defined repeating unit of PS A1 for structure−activity relationship (SAR) studies.

The tetrasaccharide repeating unit of PS A1 (Figure 1) consists of four monosaccharide units, viz., D-galactosamine, pyruvilated D-galactose, D-galactofuranoside, and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT). The positive charge on amine and negative charge on the carboxylate group are crucial for the activation of T-cell-dependent immune response, and the complete structure has nearly 120 repeating units.[14]

Synthesis of the fully protected tetrasaccharide repeating unit of PS A1 was carried out by van der Marel and co-workers in 2007.[15] They assembled the trisaccharide using the iterative glycosylation method in a one-pot manner in 62% yield. However, difficulties were encountered in the final [3 + 1] glycosylation using the AAT donor with a trisaccharide acceptor, and under dehydrative glycosylation conditions using Ph_2SO and Tf_2O, the fully protected tetrasaccharide was obtained in 17% yield. Due to the low yield of this step, global deprotection was not performed, and the authors concluded that an alternative route is desired for the assembly of PS A1. In 2011, Seeberger and co-workers systematically studied the preference of installation of glycosidic bonds and established a successful route to achieve the first total synthesis of the PS A1 tetrasaccharide in the longest linear sequence of 20 steps with 1.79% overall yield.[16] The route involved assembly of tetrasaccharide starting from the nonreducing end AAT to the reducing end pyruvilated galactopyranose unit. The rare sugar AAT was synthesized through a *de novo* approach starting from L-threonine via azidonitration of a glycal intermediate as a key step. Although the elegant synthetic route leaves very little scope for alteration, the low selectivity observed in the key azidonitration step for the procurement of the key rare sugar AAT building block (3.5:1 dr) calls for alternate strategies.[17] Also, the stereoselectivity observed in the glycosylation of the AAT donor to obtain the key disaccharide (α/β = 5:1) and the yield of final glycosylation (58%) needed further improvement. Indeed, in their subsequent study, Seeberger's group improved the selectivity and efficacy of azidonitration (4:1 dr) and glycosylations (α/β = 19:1, 77%) to synthesize a conjugation-ready thioether-linked tetrasaccharide unit of PS A1.[18] Recently, Andreana and co-workers reported a total synthesis of an alternative structure of the PS A1 repeating unit as a β-OPMP glycoside with alternating charges on adjacent monosaccharides, by keeping the pyruvilated galactose at the nonreducing end, linked to AAT and the GalNAc unit at the reducing end.[19] They followed a similar strategy for making AAT via azidonitration of a D-fucal derivative (3.5:1 dr). The synthesis of the target molecule was achieved in the longest linear sequence of 19 steps with 0.75% overall yield. Our laboratory has developed an expedient protocol to access orthogonally protected D-galactosamine and bacterial rare sugar building blocks (AAT, Bac, Fuc) via a one-pot double-displacement of D-mannose-derived 2,4-bistriflates.[20−22] Using this methodology, we also assembled the key AAT-α (1 → 4)-$GalN_3$ disaccharide unit, which was however deemed unsuitable for the total synthesis of the PS A1 tetrasaccharide due to nonorthogonality of the protecting groups.[23] Particularly, the presence of base-sensitive OAc, OBz, and phthalimide groups in the disaccharide precluded its utilization for the construction of repeating unit for which we would require their selective removal in the presence of others. Thus, we designed an alternate protecting group scheme for this purpose by replacing Bz with Bn and Ac with 2-naphthylmethyl (Nap) protecting groups. Herein, we report a total synthesis of the tetrasaccharide repeating unit of PS A1 in 17 linear steps with 3.47% overall yield and exclusive selectivity.

RESULTS AND DISCUSSION

Our retrosynthetic strategy for the target molecule **1**, along the lines of Seeberger's synthesis, is outlined in Scheme 1. Accordingly, **1** can be synthesized from fully protected tetrasaccharide **2** by global deprotection. Tetrasaccharide **2** can be obtained via coupling of the trisaccharide donor **3** with

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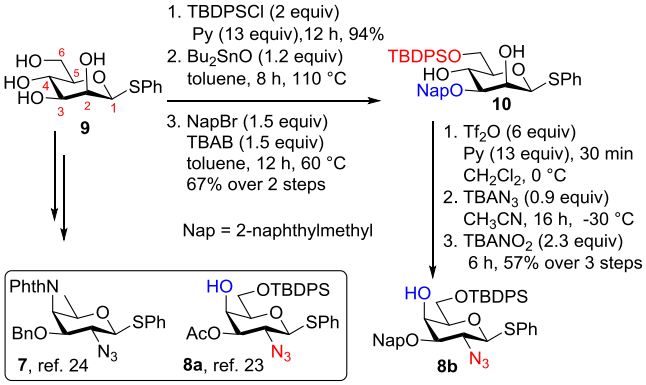
D-galactopyruvate acceptor **4**. It was envisaged that a nonparticipating azido group at C2 and a bulky TBDPS group at the C6 position on the galacto-configured sugar would guide the entry of the donor from the less hindered bottom face and offer α-selectivity, which can be further augmented by use of participating solvent such as ether. The trisaccharide **3** can be obtained by coupling of disaccharide acceptor **5** with trichloroacetimidate donor **6**. The disaccharide acceptor **5** in turn can be furnished by glycosylation of AAT donor **7** with D-galactosamine 4-OH acceptor **8a** or **8b** under orthogonal glycosylation conditions by changing the leaving group in AAT **7** to imidate or halide. Building blocks **7**,[20] **8a**,[23] and **8b** can be conveniently synthesized from D-mannose via one-pot sequential inversions of corresponding 2,4-bistriflates as a key step.[21] Galactofuranose donor **6** and acceptor **4** can be obtained from cheaply available D-galactose. With these considerations, we began the total synthesis of **1**.

As shown in Scheme 2, for the synthesis of the rare deoxyamino sugar AAT **7** and 4-OH galactosamine acceptor

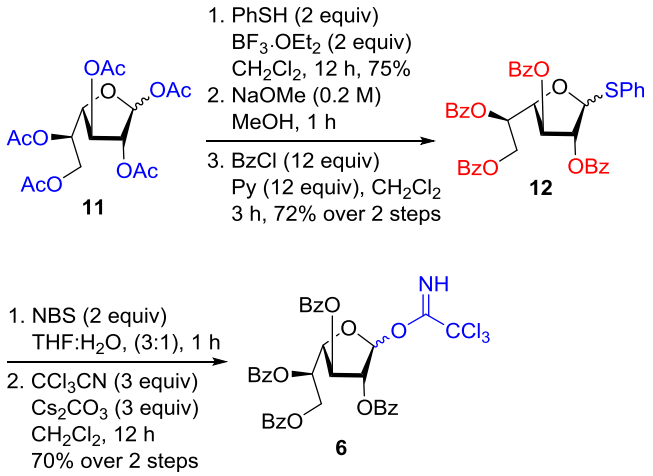
Scheme 2. Synthesis of Rare AAT and D-Galactosamine Building Blocks



8a and **8b**, we started from cheaply available D-mannose. The AAT building block **7**[24] and D-galactosamine derivative **8a**[23] were easily obtained starting from D-thiomannoside **9**[25] by following our established protocol via sequential one-pot displacement of corresponding 2,4-bistriflates, in good overall yields. Synthesis of the alternative 4-OH D-galactosamine acceptor **8b** having a 3-ONap group was also achieved from D-mannose derivative **9**. The primary hydroxyl group of **9** was protected with TBDPSCl in pyridine, followed by selective 2-naphthylmethylation of the C-3-hydroxyl group[26] using dibutyl tin oxide, tetrabutylamonnium bromide (TBAB), and 2-naphthylmethylbromide (NapBr), affording the desired 2,4-diol **10** in 67% yield. The diol **10** was subjected to triflation using triflic anhydride and pyridine in CH_2Cl_2 to afford the corresponding 2,4-bistriflate, which was as such treated with a stoichiometric amount of $TBAN_3$ in CH_3CN at −30 °C to displace the C2-O-triflate. After the completion of the reaction as monitored by thin-layer chromatography (TLC), $TBANO_2$ was added in the same pot to displace the C4-OTf to obtain the D-galactose derivative **8b** in 57% yield over three steps. It should be noted that the Lattrell−Dax reaction using an ambident nucleophile (nitrite) smoothly delivered the desired C4-OH product by attack of the oxygen center. The corresponding C4-NO_2 product, resulting by the attack of the nitrogen center was not observed. Earlier, it has been proposed that the presence of a neighboring ester functionality is essential to control the ambident nucleophilicity of the nitrite nucleophile.[27] However, in this case, the reaction proceeded selectively even in the absence of the neighboring C3-ester group.

Synthesis of D-galactofuranose donor **6** started from known per-acetylated galactofuranose derivative **11**[28] (Scheme 3).

Scheme 3. Synthesis of D-Galactofuranose Donor 6



Nucleophilic displacement of anomeric acetate in **11** with thiophenol in the presence of $BF_3{\cdot}Et_2O$ furnished the corresponding thioglycoside in 75% yield. At this stage, to circumvent the possible orthoester formation, the acetates were replaced by benzoate groups by removal of the acetate groups under Zemplén conditions followed by treatment with benzoyl chloride in CH_2Cl_2 to get perbenzoylated thioglycoside **12**. Subsequent hydrolysis of **12** using *N*-bromosuccinimide (NBS) in tetrahydrofuran (THF)/H_2O (3:1) afforded the corresponding anomeric hemiacetal, which was treated with trichloroacetonitrile and cesium carbonate in CH_2Cl_2 to afford trichloroacetimidate donor **6**. The ^1H and ^{13}C NMR spectral data of **6** matched well with its reported data.[19]

Synthesis of the appropriately protected D-galactose unit **4** is shown in Scheme 4. Regioselective protection of the 3-OH of the easily accessible 2,3-diol **13** was carried out using cat. dimethyltin dichloride and FmocCl to give the desired compound in 84% yield, and the remaining 2-OH was benzoylated to afford the fully protected D-galactose derivative **14** in good yield. Benzylidene deprotection of **14** afforded the desired 4,6-diol, which was subsequently masked with methyl pyruvate to give the corresponding pyruvate derivative **15**. The stereochemistry of the pyruvate center was confirmed from nuclear Overhauser effect (NOE) interaction between the equatorial H-4 of D-galactose and the methyl group of the ester (see Supporting Information (SI)). Glycosylation of donor **15** with isopropanol using NIS/AgOTf as a promoter system afforded the desired *O*-glycoside (71%), which upon concomitant deprotection of the Fmoc group using triethylamine furnished acceptor **4** in 80% yield.

After having all of the building blocks in our hand, first we went ahead for the synthesis of disaccharide acceptor **5** (Scheme 5). AAT donor **7** was converted to its corresponding bromide using Br_2 in CH_2Cl_2 to afford glycosyl bromide, which was further coupled with acceptor **8a** using AgOTf as a promoter to obtain disaccharide **16a** (75%), with complete α-selectivity. The characteristic NMR signals [^1H NMR δ = 5.09

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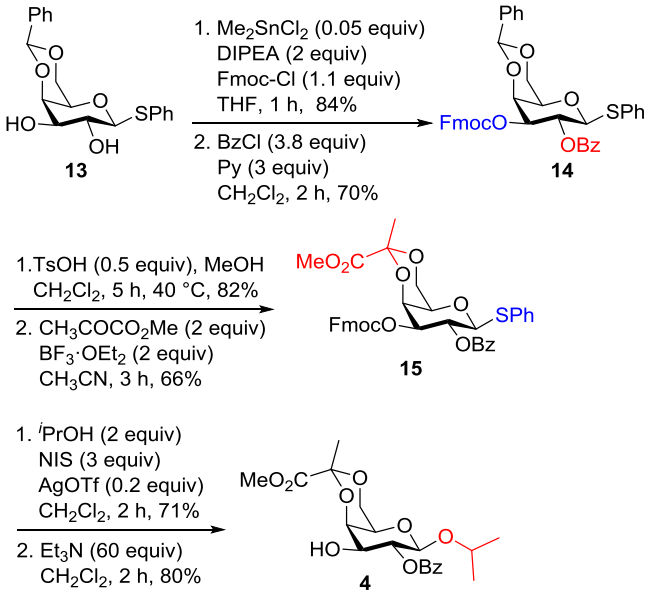
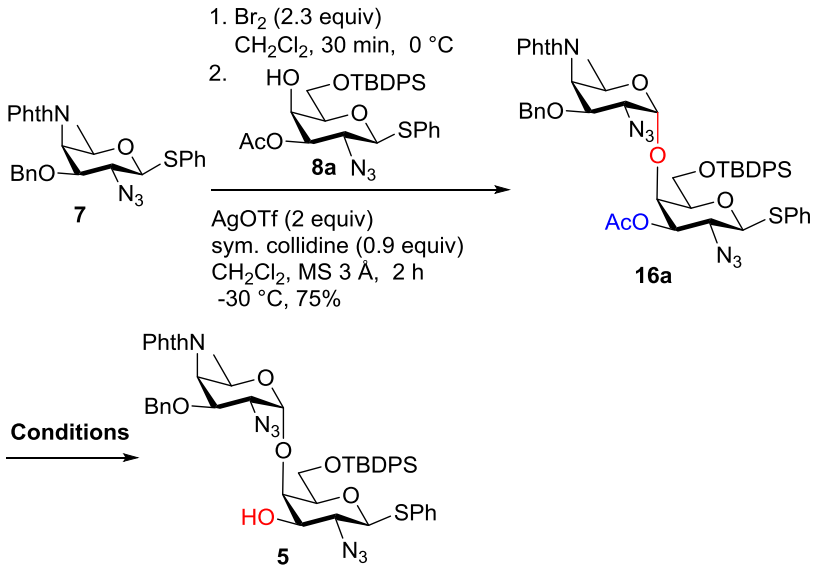
Scheme 4. Synthesis of Pyruvilated D-Galactose 3-OH Acceptor 4



ppm (d, 1H, J = 3.6 Hz, H-1), ^{13}C NMR δ = 98.9 ppm] confirmed the α-linkage of the newly formed glycosidic bond in **16a**. The observed α-stereoselectivity in this case can be perhaps attributed to (i) the presence of the nonparticipating azido group at the C2 position of the glycosyl donor, (ii) the presence of the bulky phthalimide group at the C4 position in axial orientation, which may hinder the top face attack or completely block it by neighboring group participation,[18] and (iii) the possible formation of a more reactive β-glycosyl triflate using AgOTf and its concomitant S_N2-type displacement by the nucleophilic acceptor **8a**. Removal of the acetate group in disaccharide **16a** turned out be a difficult task. We tried several conditions to cleave the *O*-acetate group, none of which gave satisfactory results (Scheme 5). For instance, use of acetyl chloride and methanol resulted in simultaneous loss of acetate and TBDPS groups. A similar result was obtained when we used potassium carbonate and methanol conditions. Alternatively, Zemplén deacetylation conditions cleaved the acetate and phthalimide groups. Likewise, the reaction with guanidium nitrate[29] was not clean, as indicated by multiple spots on TLC. Use of a milder condition with triethylamine and methanol at 50 °C after 2 days gave only 50% product **5** and 20% recovered starting material. The reaction did not go to completion even after keeping it for a longer time. As the deacetylation of compound **16a** did not give satisfactory yield, we decided to use the alternate building block **8b** (Scheme 2) having a 2-naphthylmethyl (Nap) ether protecting group at O3 in place of Ac.

Accordingly, as shown in Scheme 6, AAT donor **7** was treated with Br_2 in CH_2Cl_2 to generate the corresponding glycosyl bromide, which was further coupled with acceptor **8b** using AgOTf as a promoter to afford disaccharide **16b** (78%), with complete α-selectivity. The characteristic NMR signals [^{1}H NMR δ = 5.25 ppm (d, 1H, J = 3.5 Hz, H-1), ^{13}C NMR δ = 99.2 ppm] confirmed the α-linkage of the newly formed glycosidic bond in **16b**. Removal of the Nap group from

Scheme 5. Synthesis of Disaccharide Acceptor 5



Conditions	Results
AcCl, MeOH, 10 min to 3 h	OAc and OTBDPS deprotected
NaOMe, MeOH,15 min	OAc and phthalimide deprotected
Guanidium nitrate, 10 min	Multiple spots on TLC
K_2CO_3, MeOH, 30 min to 1 h	OAc and OTBDPS deprotected
Et_3N, MeOH, 2 d, 50 °C	**5** (50%) and **16a** (20%)

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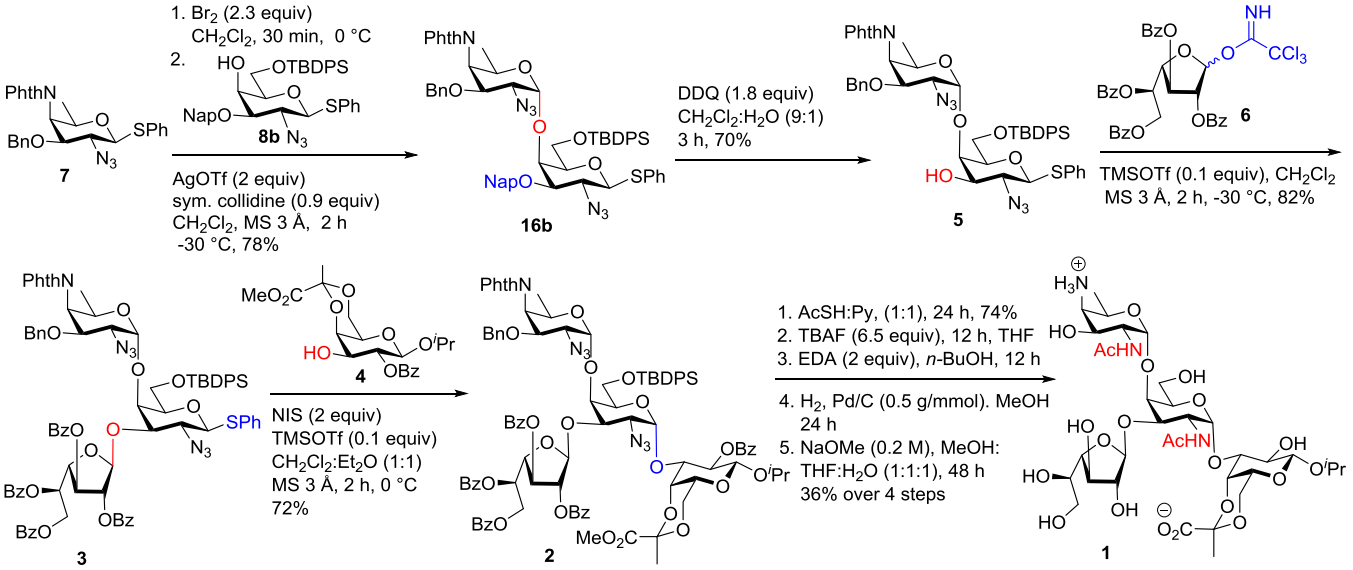
Scheme 6. Assembly of PS A1 Tetrasaccharide and Global Deprotection



disaccharide **16** using DDQ, CH_2Cl_2/H_2O (9:1) afforded the disaccharide acceptor **5** in 70% yield. At this stage, the D-Gal*f* imidate **6** (perbenzoylated trichloroacetimidate) was glycosylated with acceptor **5** in the presence of the TMSOTf promoter at −30 °C to furnish trisaccharide **3** in 82% yield. The characteristic NMR signals (^{1}H NMR δ = 5.72 ppm, ^{13}C NMR δ = 105.7 ppm) confirmed the β-linkage of the newly formed glycosidic bond in **3**. Glycosylation of trisaccharide donor **3** with acceptor **4** in the presence of the NIS/TMSOTf promoter and ether as a participating cosolvent afforded the desired tetrasaccharide **2** in good yield (72%), exclusively. The characteristic NMR signals (^{1}H NMR δ = 5.64 ppm, ^{13}C NMR δ = 92.1 ppm) confirmed the α-linkage of the newly formed glycosidic bond in **2**. All of the synthetic intermediates were thoroughly characterized by two-dimensional (2D) NMR spectroscopy (see SI).

Global deprotection of tetrasaccharide was done in five steps. First, conversion of azides to NHAc using AcSH in pyridine (74% yield), followed by TBDPS deprotection using TBAF/AcOH, removal of the phthalimide group using ethylenediamine (EDA)/*n*-BuOH, removal of benzyl groups using H_2/Pd/C in methanol, and finally cleavage of esters using NaOMe, in MeOH/THF/H_2O (1:1:1) furnished tetrasaccharide **1** in good overall yield. The final purification was done using high-performance liquid chromatography (HPLC) on a prep-C18 column using a 10% methanol/H_2O eluent system to obtain the zwitterionic tetrasaccharide **1**. The ^{1}H NMR data of compound **1** matched well with the data reported by Seeberger and co-workers.[16] The compound also gave satisfactory ^{13}C NMR and high-resolution mass spectrometry (HRMS) data (see SI).

CONCLUSIONS

In conclusion, an expedient access to rare sugar AAT and D-galactosamine building blocks from D-mannose via a one-pot S_N2 displacement of 2,4-bistriflates enabled an efficient total synthesis of PS A1 tetrasaccharide repeating unit **1**. In this synthesis, all of the glycosylations proceeded in high stereoselectivity and yields. The repeating unit was efficiently assembled from known and easily available β-D-thiomannoside **9** in 17 linear steps with 3.47% overall yield. Efforts are underway to construct larger fragments of PS A1.

EXPERIMENTAL PROCEDURES

General Methods. All reactions were conducted under a dry nitrogen atmosphere. Solvents (CH_2Cl_2 > 99%, THF 99.5%, acetonitrile 99.8%, dimethylformamide (DMF) 99.5%) were purchased in capped bottles and dried under sodium or CaH_2. All other solvents and reagents were used without further purification. All glassware used was oven-dried before use. All reactions that required elevated temperatures were carried out under traditional oil bath heating. TLC was performed on precoated aluminum plates of silica gel 60 F254 (0.25 mm, E. Merck). Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in ammonium molybdate/cerium(IV) sulfate solution. Silica gel column chromatography was performed using silica gel (100−200 mesh) and employed a solvent polarity correlated with TLC mobility. NMR experiments were conducted on 600, 500, and 400 MHz instruments using $CDCl_3$ (*D*, 99.8%) and D_2O (*D*, 99.9%) as solvents. Chemical shifts are relative to the deuterated solvent peaks and are in parts per million (ppm). Structural assignments were made with additional information from gCOSY, gHSQC, and gHMBC experiments. Mass spectra were acquired in the SI mode using a Q-TOF analyzer. Specific rotation experiments were carried out at 589 nm (Na) and 25 °C.

*Phenyl 6-O-tert-Butyldiphenylsilyl-3-O-(2-naphthylmethyl)-1-thio-β-D-mannopyanoside (**10**).* *tert*-Butyldiphenylsilyl chloride (2.86 mL, 11.02 mmol) was added to a stirred solution of tetraol **9** (1.5 g, 5.51 mmol) in pyridine (18.6 mL). After 12 h, the reaction mixture was concentrated, and the residue was dissolved in $CHCl_3$ and washed with 1 N HCl and water sequentially. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, and the crude product was purified by silica gel column chromatography (40:60 to 80:20 ethyl acetate/petroleum ether) to yield C6 OTBDPS triol (2.2 g, 94% yield) as a white foam. Triol (2.0 g, 3.92 mmol) was dissolved in toluene (28 mL), and to this clear solution, Bu_2SnO (1.17 g, 4.70 mmol) was added, and the reaction mixture was kept for stirring at 110 °C for 8 h. After complete consumption of the starting material, the solvent was removed under reduced pressure and the crude product was dried under high vacuum. The crude product was dissolved in toluene (12 mL), then TBAB (1.89 g, 5.88 mmol) and 2-naphthylmethylbromide (1.30 g, 5.88 mmol) were added, and the solution was stirred at 60 °C for 12 h. After conversion of the starting material into product, the reaction mixture was diluted with EtOAc and washed with brine. The separated organic layer was dried over

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Na_2SO_4, concentrated, and purified by column chromatography (30:70 ethyl acetate/petroleum ether) to give **10** (1.69 g, 67% over two steps) as a pale yellow viscous liquid. $[\alpha]_D^{25}$ = −13.15 (*c* = 2.3, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3455, 3050, 2959, 2929, 2857, 1112, 1067, 951, 858, 822, 758, 741, 705, 690, 635, 615, 504; ^1H NMR (500 MHz, $CDCl_3$): δ 7.87−7.81 (m, 4H, Ar*H*), 7.72−7.70 (m, 3H, Ar*H*), 7.52−7.49 (m, 5H, Ar*H*), 7.43−7.35 (m, 7H, Ar*H*), 7.26−7.21 (m, 3H, Ar*H*), 4.95 (d, *J* = 13.4 Hz, 1H, C*H*HNap 1H), 4.85 (d, *J* = 14.5 Hz, 2H, H-1, CH*H*Nap), 4.30 (s, 1H, H-2), 4.01 (t, *J* = 10 Hz, 1H, H-4), 3.96−3.94 (m, 2H, H-6a & H-6b), 3.51 (dd, *J* = 7.2, 3.0 Hz, 1H, H-3), 3.45−3.43 (m, 1H, H-5), 2.82 (bs, 1H, O*H*), 2.59 (bs, 1H, O*H*), 1.07 (s, 9H), ((CH_3)$_3$); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 135.7 (Ar*C*), 135.6 (Ar*C*), 135.1 (Ar*C*), 134.9 (Ar*C*), 133.2 (Ar*C*), 133.2 (Ar*C*), 133.0 (Ar*C*), 132.8 (Ar*C*), 130.6 (Ar*C*), 129.8 (Ar*C*), 129.8 (Ar*C*), 129.0 (Ar*C*), 128.7 (Ar*C*), 128.0 (Ar*C*), 127.8 (Ar*C*), 127.2 (Ar*C*), 127.0 (Ar*C*), 126.4 (Ar*C*), 126.3 (Ar*C*), 125.7 (Ar*C*), 86.9 (C-1), 81.7, 79.4, 72.0, 69.7, 68.3, 64.9, 26.8 (TBDPS CH_3), 19.2 (TBDPS quaternary *C*); HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{39}H_{42}O_5NaSSi$ 673.2413; found, 673.2414.

*Phenyl 2-Azido-6-O-tert-butyldiphenylsilyl-2-deoxy-3-O-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (**8b**).* Pyridine (2.01 mL, 24.96 mmol) and trifluoromethanesulfonic anhydride (1.96 mL, 11.52 mmol) were added sequentially at 0 °C to a stirred solution of compound **10** (1.25 g, 1.92 mmol) in CH_2Cl_2 (25 mL). After 30 min, the reaction mixture was diluted with CH_2Cl_2 and washed successively with 1 N HCl, aq $NaHCO_3$, and brine. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was dissolved in acetonitrile (26 mL); to this, $TBAN_3$ (0.49 g, 1.72 mmol) was added at −30 °C, and the reaction was stirred at the same temperature for 16 h. After 16 h, $TBANO_2$ (1. 27 g, 4.41 mmol) was added and the reaction mixture was stirred at rt for 6 h. The reaction mixture was diluted with EtOAc and washed with brine. The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (15:85 ethyl acetate/petroleum ether) to obtain **8b** as a pale yellow viscous liquid (0.74 g, 57%, over three steps). $[\alpha]_D^{25}$ = −24.66 (*c* = 0.56, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3468, 3064, 2929, 2854, 2113, 1590, 1474, 1366, 1282, 1112, 822, 745, 703, 670, 505; ^1H NMR (400 MHz, $CDCl_3$): δ 7.87−7.81 (m, 4H, Ar*H*), 7.72−7.66 (m, 5H, Ar*H*), 7.60−7.58 (m, 2H, Ar*H*), 7.54−7.48 (m, 3H, Ar*H*), 7.43−7.26 (m, 8H, Ar*H*), 4.92−4.85 (m, 2H, C*H*$_2$Nap), 4.37 (d, *J* = 10.0 Hz, 1H, H-1), 4.12 (s, 1H, H-4), 3.99−3.89 (m, 2H, H-6a & H-6b), 3.75 (t, *J* = 9.6 Hz, 1H, H-2), 3.43−3.41 (m, 1H, H-5), 3.40−3.38 (m, 1H, H-3), 2.77 (bs, 1H, O*H*), 1.05 (s, 9H, (CH_3)$_3$); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 135.7 (Ar*C*), 135.6 (Ar*C*), 134.6 (Ar*C*), 133.2 (Ar*C*), 133.0 (Ar*C*), 132.9 (Ar*C*), 132.8 (Ar*C*), 131.8 (Ar*C*), 129.9 (Ar*C*), 129.0 (Ar*C*), 128.6 (Ar*C*), 128.1 (Ar*C*), 128.0 (Ar*C*), 127.8 (Ar*C*), 127.8 (Ar*C*), 127.0 (Ar*C*), 126.3 (Ar*C*), 126.2 (Ar*C*), 125.8 (Ar*C*), 86.4 (C-1), 81.0, 78.0, 72.0, 66.0, 68.4, 63.7, 61.0, 26.8 (TBDPS CH_3), 19.2 (TBDPS quaternary *C*); HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{39}H_{41}N_3O_4NaSSi$ 698.2477; found, 698.2479.

*1,2,3,5,6-Penta-O-acetyl-D-galactofuranose (**11**).* Compound **11** was prepared from per-*O*-*tert*-butyldimethylsilylgalactofuranoside (7.0 g, 9.31 mmol) using acetic anhydride (45 mL, 475.0 mmol) and *p*-TsOH (35.4 g, 186.3 mmol) in CH_2Cl_2 (94 mL). After complete consumption of the starting material (72 h, monitored by TLC), the reaction mixture was diluted with CH_2Cl_2 and washed with aq $NaHCO_3$ and brine. The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (15:85 ethyl acetate/pet ether) to obtain per-*O*-acetylated galactofuranoside as an anomeric mixture (α/β 1:5) as a colorless liquid (35:65 ethyl acetate/petroleum ether, 2.5 g, 64%). $[\alpha]_D^{25}$ = +6.637 (*c* = 1.8, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 2942, 2441, 2110, 1748, 1435, 1372, 1235, 1049, 965, 883, 820, 757, 604, 520; ^1H NMR ($CDCl_3$, 400 MHz): δ 6.25−6.06 (m, 2H), 5.44−4.95 (m, 6H), 4.26−3.98 (m, 6H), 2.05−1.95 (m, 30H, carbonyl CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ 170.3 ($COCH_3$), 170.2 ($COCH_3$), 169.9 ($COCH_3$), 169.8 ($COCH_3$), 169.7 ($COCH_3$), 169.3 ($COCH_3$), 169.1 ($COCH_3$), 168.9 ($COCH_3$), 98.9 (C-1), 92.9 (C-1), 89.5, 82.1, 80.4, 78.9, 76.2, 75.1, 73.2, 70.1, 69.1, 68.6, 67.3, 67.2, 66.3, 62.3, 61.9, 61.1, 20.8 ($COCH_3$), 20.6 ($COCH_3$), 20.5 ($COCH_3$), 20.5 ($COCH_3$), 20.4 ($COCH_3$), 20.3 ($COCH_3$); HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{16}H_{22}NaO_{11}$ 413.1061; found, 413.1054.

*Phenyl 2,3,5,6-tetra-O-Benzoyl-1-thio-α,β-D-galactofuranoside (**12**).* $BF_3{\cdot}Et_2O$ (2.6 mL, 20.5 mmol) was added dropwise to the stirred solution of compound **11** (4.0 g, 10.25 mmol) in CH_2Cl_2 and thiophenol (2.1 mL, 20.5 mmol) at 0 °C, and the reaction was continued for 12 h at rt. After 12 h, the reaction mixture was diluted with sat. $NaHCO_3$ and the separated organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography on silica gel (20:80 ethyl acetate/petroleum) to obtain per-*O*-acetylated thiogalactofuranoside (3.4 g, 75%) as a colorless liquid. NaOMe (0.2 M) was added to a stirred solution of per-*O*-acetylated thiogalactofuranoside (2.1 g, 4.77 mmol) in MeOH (21 mL). After 1 h, the reaction mixture was neutralized with Amberlite-IR 120 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure to afford the desired tetraol as a white foam. Benzoyl chloride (7.7 mL, 66.08 mL) was added to the stirred solution of tetraol (1.5 g, 5.50 mmol) in CH_2Cl_2 (15 mL) and pyridine (5.32 mL, 66.08 mmol) at 0 °C. Next, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine. The separated organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and purified by column chromatography on silica gel (25:75 ethyl acetate/petroleum) to afford the desired compound **12** (2.7 g, 72%, over two steps) as a white foam. $[\alpha]_D^{25}$ = −1.788 (*c* = 0.56, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3064, 2924, 2857, 1728, 1601, 1586, 1432, 1266, 1178, 1109, 1069, 1027, 757, 687; ^1H NMR ($CDCl_3$, 500 MHz): δ 8.09−7.88 (m, 5H, Ar*H*), 7.59−7.43 (m, 10H, Ar*H*), 7.38−7.27 (m, 10H, Ar*H*), 6.12−6.09 (q, *J* = 2.8 Hz, 1H, H-5), 5.84 (s, 1H, H-3), 5.72−5.70 (d, *J* = 4.8 Hz, 1H, H-1), 5.67 (s, 1H, H-2), 4.96−4.94 (t, *J* = 4.4 Hz, 1H, H-4), 4.77−4.69 (m, 2H, H-6); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): δ 166.1 (COPh), 165.7 (COPh), 165.5 (COPh), 165.4 (COPh), 133.7 (Ar*C*), 133.5 (Ar*C*), 133.3 (Ar*C*), 133.1 (Ar*C*), 132.1 (Ar*C*), 132.5 (Ar*C*), 130.1 (Ar*C*), 130.0 (Ar*C*), 129.9 (Ar*C*), 129.8 (Ar*C*), 129.59 (Ar*C*), 129.50 (Ar*C*), 129.1 (Ar*C*), 128.9 (Ar*C*), 128.6 (Ar*C*), 128.5 (Ar*C*), 128.4 (Ar*C*), 128.0 (Ar*C*), 91.4 (C-1), 82.4, 81.6, 77.9, 70.3, 63.4; HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{40}H_{32}NaO_9S$, 711.1659; found, 711.1659.

*2,3,5,6-tetra-O-Benzoyl-α,β-D-galactofuranoside-N-trichloroacetimidate (**6**).* NBS (0.21 g, 1.16 mmol) was added to the stirred solution of compound **12** (0.4 g, 0.58 mmol) in THF/H_2O (3:1). After 1 h, the reaction mixture was diluted with EtOAc and washed with $Na_2S_2O_3$ and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude hemiacetal was dissolved in CH_2Cl_2 (13 mL). CCl_3CN (0.14 mL, 1.74 mmol) and Cs_2CO_3 (0.57 g 1.74 mmol) were added to it at 0 °C. The mixture was stirred for 12 h at rt. The reaction mixture was diluted with CH_2Cl_2, filtered over celite, and concentrated *in vacuo*. The crude compound was purified by silica gel column chromatography (20:80 ethyl acetate/petroleum) to obtain compound **6** (0.3 g, 70%, over two steps). $[\alpha]_D^{25}$ = +25.02 (*c* = 0.79, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3443, 3062, 3021, 2924, 2854, 1727, 1602, 1585, 1452, 1316, 1267, 1217, 1178, 1111, 1070, 1027, 758, 711, 686, 668; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.74 (s, 1H, NH imidate), 8.11−7.88 (m, 7H, Ar*H*), 7.61−7.25 (m, 13H, Ar*H*), 6.71 (s, 1H, H-1), 6.15−6.14 (m, 1H, H-5), 5.80 (d, *J* = 4 Hz, 1H, H-3), 5.77 (s, 1H, H-2), 4.88 (t, *J* = 4 Hz, 1H, H-4), 4.79−4.77 (m, 2H, H-6); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ 166.0 (COPh), 165.7 (COPh), 165.5 (COPh), 165.1 (COPh), 160.2 (COPh), 133.7 (Ar*C*), 133.6 (Ar*C*), 133.3 (Ar*C*), 133.1 (Ar*C*), 130.0 (Ar*C*), 130.0 (Ar*C*), 129.9 (Ar*C*), 129.7 (Ar*C*), 128.5 (Ar*C*), 128.4 (Ar*C*), 128.3 (Ar*C*), 102.9 (C-1), 90.9, 84.6, 80.7, 77.0, 70.1, 63.4; HR-ESI-MS (*m*/*z*): [M + K]$^+$ calcd. for $C_{36}H_{28}Cl_3KNO_{10}$ 778.0515; found, 778.0410.

*Phenyl 2-O-Benzoyl-4,6-O-benzylidene-3-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside (**14**).* Diol **13** (1.3 g, 3.61 mmol) was dissolved in THF (30 mL). After complete dissolution of compound **13**, *N*,*N*-diisopropylethylamine (DIPEA; 1.25 mL, 7.22 mmol), dimethyltin dichloride (0.04 g 0.18 mmol), and FmocCl (1.02

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g, 3.97 mmol) were added sequentially at rt and stirred for 1 h. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl and brine, and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (20:80 to 40:60 ethyl acetate/petroleum) to give the C3-Fmoc-protected compound as a white foam (1.76 g, 84% yield).

The above obtained compound (1.2 g, 2.06 mmol) was dissolved in pyridine (0.49 mL, 6.09 mmol) and CH_2Cl_2 (12 mL) and cooled to 0 °C. To this solution, BzCl (0.48 mL, 4.12 mmol) was added dropwise, and the reaction was stirred for 2 h at rt. The reaction mixture was diluted with CH_2Cl_2 and washed with 1 N HCl and brine. The organic portion was concentrated under reduced pressure. The crude product was purified by silica gel chromatography (20:80 to 40:60 ethyl acetate/petroleum) to yield compound **14** as a white foam (0.98 g, 70%). $[\alpha]_D^{25}$ = −19.05 (*c* = 0.86, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3020, 2925, 2857, 1723, 1451, 1403, 1366, 1268, 1216, 1124, 1099, 815, 758, 710; ^1H NMR (500 MHz, $CDCl_3$): δ 8.15−8.14 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.73−7.68 (m, 4H, Ar*H*), 7.59−7.44 (m, 10 H, Ar*H*), 7.44−7.05 (m, 7H, Ar*H*), 5.80 (d, *J* = 10.0 Hz, 1H, H-3), 5.56 (s, 1H, C*H*Ph), 5.1 (dd, *J* = 3 Hz, 1H, H-3), 4.97 (d, *J* = 10 Hz, 1H, H-1), 4.54 (d, *J* = 2.5 Hz, 1H, H-4), 4.44 (d, *J* = 12.2 Hz 1H, −CH Fmoc), 4.31−4.29 (m, 2H, CH_2 Fmoc), 3.64 (s, 1H, H-5), 4.14 (dd, 2H, H-6a, *J* = 12.5 Hz, 8 Hz, H-6b); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$): 164.8 (COPh), 154.4 (Fmoc CO), 143.1 (ArC), 143.0 (ArC), 141.1 (ArC), 137.5 (ArC), 133.7 (ArC), 133.3 (ArC), 131.3 (ArC), 129.9 (ArC), 129.6 (ArC), 129.1 (ArC), 128.8 (ArC), 128.5 (ArC), 128.3 (ArC), 128.2 (ArC), 128.2 (ArC), 127.8 (ArC), 127.8 (ArC), 127.1 (ArC), 126.5 (ArC), 125.1 (ArC), 125.1 (ArC), 119.9 (ArC), 101.0 (CHPh), 85.2, 76.8, 73.3, 70.3, 69.3, 69.0, 67.4, 46.4 (Fmoc CH); HR-ESI-MS (*m*/*z*): $[M + Na]^+$ calcd. for $C_{41}H_{34}NaO_8S$, 709.1866; found 709.1867.

*Phenyl 2-O-Benzoyl-3-O-fluorenylmethoxycarbonyl-4,6-O-[1-(R)-(methoxycarbonyl)ethylidene]-1-thio-β-D-galactopyranoside (**15**).* *p*-TsOH (0.114 g, 0.66 mmol) was added to the stirred solution of compound **14** (0.91 g, 1.32) in MeOH (7.9 mL) and CH_2Cl_2 (3 mL), and the reaction mixture was stirred for 5 h at 40 °C. The reaction was quenched by addition of pyridine (1 mL), and the solution was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with (30:70 to 50:50 ethyl acetate/pet ether) to give 4,6 diol (0.65 g, 82% yield). The 4,6 diol (0.87 g, 1.45 mmol) was dissolved in acetonitrile (20 mL). The resulting suspension at 0 °C was treated with methyl pyruvate (0.26 mL, 2.91 mmol) and $BF_3{\cdot}Et_2O$ (0.36 mL, 2.91 mmol). After stirring for 3 h, the reaction was quenched with pyridine (1 mL). The solution was diluted with EtOAc, washed with aq $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting crude material was purified with silica gel column chromatography (20:80 to 30:70 ethyl acetate/petroleum) to yield pyruvilated galactose **15** as a white foam (0.66 g, 66%). $[\alpha]_D^{25}$ = +6.329 (*c* = 0.43, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 2928, 2864, 1744, 1647, 1449, 1274, 1115, 10895, 1026, 824, 758, 712; ^1H NMR (400 MHz, $CDCl_3$): δ 8.10−8.09 (m, 2H, Ar*H*), 7.22−7.70 (m, 2H, Ar*H*), 7.62−7.43 (m, 8H, Ar*H*), 7.36−7.11 (m, 6H, Ar*H*), 5.72 (t, *J* = 10 Hz, 1H, H-2), 5.02 (dd, *J* = 10.3, 3.6 Hz, 1H, H-3), 4.91 (d, *J* = 10.0 Hz, 1H, H-1), 4.57 (d, *J* = 2.8 Hz, 1H, H-4), 4.34−4.29 (m, 1H, C*H* Fmoc), 4.26−4.24 (m, 1H, H-6a), 4.22−4.17 (m, 2H, CH_2 Fmoc), 4.01 (d, *J* = 12.1 Hz, 1H, H-6b), 3.63 (s, 3H, Pyr. CO_2CH_3), 3.5 (s, 1H, H-5), 1.6 (s, 3H, Pyr. CH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 170.0 (CO_2Me), 164.9 (COPh), 154.2 (Fmoc CO), 143.2 (ArC), 143.1 (ArC), 141.1 (ArC), 141.1 (ArC), 133.6 (ArC), 133.3 (ArC), 131.5 (ArC), 129.9 (ArC), 129.5 (ArC), 128.8 (ArC), 128.5 (ArC), 128.3 (ArC), 127.8 (ArC), 127.8 (ArC), 127.1 (ArC), 125.3 (ArC), 125.2 (ArC), 119.9 (ArC), 98.6 (pyruvate quaternary C), 85.5 (C-1), 70.5, 68.8, 68.8, 67.1, 65.3, 52.5 (OCH_3), 46.3 (CH of Fmoc), 25.6 (pyruvate CH_3); HR-ESI-MS (*m*/*z*): $[M + Na]^+$ calcd. for $C_{38}H_{34}NaO_{10}S$, 705.1764; found, 705.1765.

*Isopropyl 2-O-Benzoyl-4,6-O-[1-(R)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranoside (**4**).* *i*-PrOH (0.05 mL, 0.67 mmol) was added to the stirred solution of compound **15** (0.23 g, 0.336 mmol) in CH_2Cl_2 (4 mL), and the flask was cooled to −40 °C. To this solution, NIS (0.151 g, 0.673 mmol) and AgOTf (0.017 g, 0.067 mmol) were added sequentially. The solution was slowly warmed to rt over 2 h. The solution was diluted with EtOAc (50 mL), washed with aq $NaHCO_3$ (3 × 10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The resulting crude compound was purified by silica gel chromatography (40:60, ethyl acetate/pet ether) to give the linker coupled product (0.151 g, 71%) as a yellow foam. The linker coupled product (0.151 g, 0.238 mmol) was dissolved in CH_2Cl_2 (1.6 mL) and treated with NEt_3 (1.5 mL, 10.43 mmol) at rt. After the solution was stirred for 2 h, it was concentrated under reduced pressure to give a crude oil. The crude oil was purified by silica gel column chromatography (60:40 ethyl acetate/petroleum ether) to yield alcohol **4** as a white foam (0.078 g, 80%). $[\alpha]_D^{25}$ = −26.10 (*c* = 0.58, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3456, 3018, 2926, 2857, 1732, 1452, 1373, 1270, 1216, 1122, 1086, 982, 758, 711, 668; ^1H NMR (400 MHz, $CDCl_3$): δ 8.06−8.04 (m, 2H, Ar*H*), 7.58−7.54 (m, 1H, Ar*H*), 7.46−7.42 (m, 2H), 5.30 (t, *J* = 8 Hz, 1H, H-2), 4.59 (d, *J* = 8 Hz, 1H, H-1), 4.17 (d, *J* = 13.2 Hz, 1H, H-4), 4.12−4.02 (m, 2H, H-6), 3.95 (m, 1H, C*H* isopropanol), 3.83 (s, 3H, Pyr. CO_2CH_3), 3.82−3.79 (m, 1H, H-3), 3.42 (s, 1H, H-5), 2.70 (d, *J* = 10.4 Hz, 1H, O*H*), 1.63 (s, 3H, CH_3), 1.21 (d, *J* = 6.2 Hz, 3H, CH_3), 1.07 (d, *J* = 6.2 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 170.4 (CO_2Me), 166.4 (COPh), 133.1 (ArC), 130.3 (ArC), 129.9 (ArC), 128.4 (ArC), 99.6 (C-1), 98.9 (pyruvate quaternary C), 73.0, 72.5, 71.9, 71.4, 65.9, 65.3, 52.9 (OCH_3), 25.9 (pyruvate CH_3), 23.4 (isopropanol C), 22.1 (isopropanol C); HR-ESI-MS (*m*/*z*): $[M + Na]^+$ calcd. for $C_{20}H_{26}NaO_9$, 433.1465; found, 433.1469.

*Phenyl 2-Azido-3-O-benzyl-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranosyl-(1 → 4)-3-O-acetyl-2-azido-2-deoxy-6-O-tert-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (**16a**).* Bromine (22 μL, 0.44 mmol) was added to a solution of **7** (0.10 g, 0.19 mmol) in CH_2Cl_2 (3 mL) at 0 °C, and after 30 min stirring, the mixture was concentrated and the residue was coevaporated twice with toluene. A solution of acceptor **8a** (55 mg, 0.0951 mmol) in CH_2Cl_2 (1.3 mL) was added to a suspension of glycosyl bromide, 3 Å MS (0.25 g), and sym. collidine (22 μL, 0.18 mmol) in CH_2Cl_2 (1.3 mL) and kept stirring at −30 °C for 30 min. Then, silver triflate (0.10 g, 0.38 mmol) was added and stirring was continued at the same temperature. After 2 h, triethylamine was added and the reaction mixture was diluted with CH_2Cl_2, filtered through celite, and concentrated. The residue was purified by column chromatography on silica gel (20:80 ethyl acetate/petroleum ether) to obtain the desired product **16a** as a foam (69 mg, 75%): $[\alpha]_D^{25}$ +121.21 (*c* = 0.05, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 2900, 2830, 2109, 1734, 1320, 1250, 1218, 1106, 1065, 1002, 950, 745, 710, 610, 543; ^1H NMR (400 MHz, $CDCl_3$): δ 7.86−7.84 (m, 2H, Ar*H*), 7.76−7.66 (m, 9H, Ar*H*), 7.47−7.42 (m, 7H, Ar*H*), 7.29−7.20 (m, 6H, Ar*H*), 5.09 (d, *J* = 3.6 Hz, 1H, H-1′), 4.89−4.84 (m, 2H, H-3, H-4′), 4.85 (d, *J* = 2.4 Hz, 1H, H-3), 4.62−4.52 (m, 3H, H-2′ & CH_2Bn), 4.45 (d, *J* = 9.8, 1H, H-1), 4.31 (dd, *J* = 4 Hz, 1H, H-5′), 4.25 (d, *J* = 2 Hz, 1H, H-4), 4.15 (dq, *J* = 7.6 Hz, 1H, H-6b), 4.02−3.97 (m, 2H, H-3′ & H6a), 3.68−3.63 (m, 2H, H-2 & H-5), 2.13 (s, 3H, CH_3), 1.1 (s, 9H, $(CH_3)_3CSi$), 1.02 (d, *J* = 6.4 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 170.0 ($COCH_3$), 137.1 (ArC), 135.8 (ArC), 135.7 (ArC), 134.3 (ArC), 134.1 (ArC), 133.4 (ArC), 133.2 (ArC), 133.0 (ArC), 130.9 (ArC), 130.1 (ArC), 130.0 (ArC), 129.1 (ArC), 128.9 (ArC), 128.5 (ArC), 128.4 (ArC), 128.3 (ArC), 128.2 (ArC), 128.0 (ArC), 128.0 (ArC), 127.9 (ArC), 127.9 (ArC), 123.6 (ArC), 98.9 (C-1), 85.7 (C-1′), 79.3, 75.0, 74.8, 73.3, 72.0, 64.0, 61.7, 60.8, 59.7, 51.6, 27.0 (TBDPS CH_3), 21.2 ($COCH_3$), 19.3 (quaternary C of TBDPS), 17.0 (CH_3 of AAT); HR-ESI-MS (*m*/*z*): $[M + Na]^+$ calcd. for $C_{51}H_{53}N_7O_9NaSSi$, 990.3287; found, 990.3291.

*Phenyl 2-Azido-3-O-benzyl-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranosyl-(1 → 4)-2-azido-2-deoxy-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (**16b**).* Bromine (79 μL, 1.53 mmol) was added to a solution of **7** (0.34 g, 0.679 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the reaction was continued for 30 min. After complete consumption of the starting material (monitored by TLC), the reaction mixture was concentrated under reduced pressure and the residue was coevaporated twice with

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toluene. A solution of acceptor **8b** (0.21 g, 0.31 mmol) in CH_2Cl_2 (4 mL) was added to a suspension of glycosyl bromide, 3 Å MS (0.5 g), and sym. collidine (79 μL, 0.62 mmol) in CH_2Cl_2 (4 mL) and kept stirring at −30 °C for 30 min. Silver triflate (0.341 g, 1.33 mmol) was added, and stirring was continued at the same temperature. After 2 h, triethylamine was added and the reaction mixture was diluted with CH_2Cl_2, filtered through celite, and concentrate under reduced pressure. The residue was purified by silica gel column chromatography (20:80 ethyl acetate/petroleum ether) to obtain the desired product **16b** as a foam (0.26 g, 78%). $[\alpha]_D^{25}$ = +107.29 (*c* = 0.05, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 2926, 2857, 2112, 1717, 1468, 1365, 1332, 1273, 1216, 1114, 1078, 760, 702; ^1H NMR (400 MHz, $CDCl_3$): δ 7.84−7.79 (m, 6H, Ar*H*), 7.71−7.55 (m, 9H, Ar*H*), 7.48−7.30 (m, 12H, Ar*H*), 7.22−7.19 (m, 4H, Ar*H*), 5.25 (d, *J* = 3.5 Hz, 1H, H-1′), 5.01−4.84 (m, 3H, H-4′ & CH_2Ph), 4.60−4.54 (m, 1H, H-5′), 4.63−4.54 (m, 3H, H-2′ & CH_2Nap), 4.27−4.18 (m, 3H, H-4, H-3, H-6′), 4.08 (dd, *J* = 8 Hz, 1H, H-3), 3.92 (dd, *J* = 7.5 Hz, 1H, H-6), 3.71 (t, *J* = 12.5 Hz, 1H, H-2), 3.31−3.27 (m, 2H, H-5), 1.09 (s, 9H, (CH_3)$_3$CSi), 0.87 (d, *J* = 6.5 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$): δ 137.2 (ArC), 135.7 (ArC), 135.6 (ArC), 134.7 (ArC), 134.4 (ArC), 134.0 (ArC), 133.5 (ArC), 133.4 (ArC), 133.2 (ArC), 133.3 (ArC), 133.0 (ArC), 131.5 (ArC), 130.1 (ArC), 130.0 (ArC), 129.0 (ArC), 128.9 (ArC), 128.6 (ArC), 128.4 (ArC), 128.4 (ArC), 128.2 (ArC), 128.0 (ArC), 128.0 (ArC), 127.9 (ArC), 127.8 (ArC), 126.8 (ArC), 126.4 (ArC), 126.2 (ArC), 125.6 (ArC), 123.6 (ArC), 123.3 (ArC), 99.2 (C-1), 86.0 (C-1′), 80.0, 79.1, 75.2, 72.2, 71.9, 71.8, 64.1, 61.1, 61.2, 60.8, 51.8, 27.1 (CH_3 of TBDPS), 19.3 (quaternary of TBDPS), 16.8 (CH_3 of AAT); HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{60}H_{59}N_7O_8NaSSi$, 1088.3807; found, 1088.3807.

*Phenyl 2-Azido-3-O-benzyl-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranosyl-(1 → 4)-2-azido-2-deoxy-6-O-tert-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (**5**).* DDQ (0.182 g, 0.806 mmol) was added to the stirred solution of **16b** (0.43 g, 0.403 mmol) in CH_2Cl_2/H_2O (10 mL, 9:1) at rt. After 3 h, solvents were evaporated and purified by silica gel chromatography (20:80 ethyl acetate/petroleum ether) to obtain acceptor **5** (0.261g, 70%). $[\alpha]_D^{25}$ = +51.52 (*c* = 0.435, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3417, 2924, 2857, 2111, 1645, 1363, 1215, 1093, 1046, 762; ^1H NMR (400 MHz, $CDCl_3$): δ 7.88−7.87 (m, 2H, Ar*H*), 7.80−7.75 (m, 7H, Ar*H*), 7.66 (d, *J* = 6.4 Hz, 2H, Ar*H*), 7.49−7.43 (m, 8H, Ar*H*), 7.29−7.24 (m, 5H, Ar*H*), 5.07 (d, *J* = 4 Hz, 1H, H-1′), 4.72 (dd, *J* = 6.4, 3.6 Hz, 1H, H-4′), 4.67−4.57 (m, 1H, H-2′ & 2H CH_2Ph), 4.38 (d, *J* = 10 Hz, 1H, H-1), 4.19 (m, 1H, H-5), 4.03−3.99 (m, 3H, H-4 & H-3′), 3.93 (dd, *J* = 6.4, 3.2 Hz, 1H, H-5′), 3.65−3.57 (m, 3H, H6a, H6b, & H-3), 3.10 (t, *J* = 10 Hz, 1H, H-2), 1.14 (s, 9H, (CH_3)$_3$CSi), 0.92 (d, *J* = 6.8 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 136.8 (ArC), 135.7 (ArC), 135.5 (ArC), 134.5 (ArC), 133.6 (ArC), 133.5 (ArC), 130.4 (ArC), 130.1 (ArC), 130.0 (ArC), 129.9 (ArC), 128.8 (ArC), 128.6 (ArC), 128.2 (ArC), 128.1 (ArC), 127.9 (ArC), 127.9 (ArC), 123.7 (ArC), 100.1 (C-1), 84.9 (C-1′), 79.7, 79.6, 74.5, 74.0, 72.1, 65.1, 63.8, 62.8, 61.0, 51.3, 26.9 (CH_3 of TBDPS), 19.4 (quaternary *C* of TBDPS), 16.4 (CH_3 of AAT); HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{49}H_{51}N_7O_8NaSSi$, 948.3180; found, 948.3181.

*Phenyl 2-Azido-3-O-benzyl-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranosyl-(1 → 4)-[2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1 → 3)]-2-azido-6-O-tert-butyldimethylsilyl-2-deoxy-β-D-galactopyranoside (**3**).* Disaccharide acceptor **5** (0.094 g, 0.1015 mmol) and galactofuranose *N*- trichloroacetimidate **6** (0.21 g, 0.283 mmol) were coevaporated with toluene (3 × 20 mL) and subjected to high vacuum for 1 h. The solution was then dissolved in CH_2Cl_2 (2.2 mL) and was cooled to −30 °C. The cooled solution was then treated with a CH_2Cl_2 solution. After 10 min, TMSOTf (0.051 mL, 0.028 mmol) was added. After 1 h, the reaction was quenched with a NEt_3 filter with a celite bed and concentrated under reduced pressure. The crude product was purified by flash silica gel chromatography (30:70, ethyl acetate/petroleum ether) to furnish trisaccharide **3** (0.125 g) in 82% yield. $[\alpha]_D^{25}$ = +33.42 (*c* = 0.93, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 2952, 2926, 2855, 2112, 1721, 1602, 1452, 1363, 1312, 1266, 1178, 1110, 1068, 1029, 759, 711; ^1H NMR (500 MHz, $CDCl_3$): δ 8.13−8.01 (m, 8H, Ar*H*), 7.89−7.70 (m, 11H, Ar*H*), 7.57−7.53 (m, 6H, Ar*H*), 7.48−7.37 (m, 14H, Ar*H*), 7.33−7.30 (m, 5H, Ar*H*), 6.08 (q, *J* = 4, 1H), 5.72 (s, 1H), 5.7 (d, *J* = 2 Hz, 1H), 5.62 (s, 1H), 5.19 (d, *J* = 12.0, 4.0 Hz, 1H), 4.90 (dd, *J* = 3.5, 6 Hz, 1H), 4.84−4.78 (m, 2H), 4.75 (dd, *J* = 4.5, Hz, 1H), 4.61−4.59 (m, 1H), 4.56−4.50 (m, 2H), 4.43 (d, *J* =10 Hz, 1H), 4.36 (m, 1H), 4.24 (d, *J* = 2 Hz, 1H), 4.19 (dd, *J* = 6.5, 3.2 Hz, 1H), 4.07−4.04 (m, 1H), 4.00−3.98 (dd, *J* = 6, 4.5 Hz, 1H), 3.92 (dd, *J* = 6.1, 2.0 Hz, 1H), 3.72−3.67 (m, 2H) (s, 9H, (CH_3)$_3$CSi), 1.06 (d, *J* = 6.5 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$): δ 166.1 (COPh), 165.9 (COPh), 165.6 (COPh), 137.1 (ArC), 135.7 (ArC), 135.7 (ArC), 134.0 (ArC), 133.6 (ArC), 133.5 (ArC), 133.4 (ArC), 133.3 (ArC), 133.2 (ArC), 133.2 (ArC), 131.0 (ArC), 130.0 (ArC), 129.9 (ArC), 129.9 (ArC), 129.8 (ArC), 129.8 (ArC), 129.6 (ArC), 129.4 (ArC), 128.8 (ArC), 128.7 (ArC), 128.6 (ArC), 128.5 (ArC), 128.4 (ArC), 105.6 (C-1 Galf), 99.0 (C-1 of AAT), 85.8 (C-1 of GalN), 81.9, 81.8, 79.8, 78.4, 77.8, 75.1, 74.1, 71.9, 70.3, 64.2, 63.3, 62.4, 61.4, 61.0, 51.7, 51.7, 29.7, 26.9 (CH_3 of TBDPS), 19.2 (quaternary *C* of TBDPS), 16.8 (CH_3 of AAT); HR-ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{83}H_{77}N_7O_{17}NaSSi$, 1526.4760; found, 1526.4758.

*Isopropyl 2-Azido-3-O-benzyl-2,4,6-trideoxy-4-phthalimido-α-D-galactopyranosyl-(1 → 4)-[2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl-(1 → 3)]-2-azido-6-O-tert-butyldimethylsilyl-2-deoxy-α-D-galactopyranosyl-(1 → 3)-2-O-benzoyl-4,6-O-[1-(R)-(methoxycarbonyl)-ethylidene]-β-D-galactopyranoside (**2**).* Thioglycoside **3** (70 mg, 0.046 mmol) and acceptor **4** (17 mg, 0.04 mmol) were coevaporated with toluene (3 × 15 mL). The crude mixture was dried under vacuum for 2 h. Then, the mixture was dissolved in CH_2Cl_2/Et_2O (1:1, 2 mL) and treated with preactivated 3 Å MS (0.1 g) and NIS (0.02 g, 0.09 mmol). After 30 min, the mixture was cooled to 0 °C and TMSOTf (0.9 μL, 0.0046 mmol) was added to it dropwise. After the addition was completed, the reaction mixture was monitored by TLC. Upon completion (2 h), the reaction mixture was quenched with triethylamine, diluted with CH_2Cl_2, and filtered on a celite bed. The organic layer was washed with aq $Na_2S_2O_3$ and brine and purified by silica gel chromatography (30:70 ethyl acetate/petroleum ether) to obtain tetrasaccharide **2** (53 mg, 72%). $[\alpha]_D^{25}$ = +17.69 (*c* = 0.87, $CHCl_3$); IR (cm^{-1}, $CHCl_3$) 3023, 2927, 2855, 2111, 1721, 1453, 1373, 1266, 1216, 1111, 1093, 1068, 760, 711, 667; ^1H NMR (500 MHz, $CDCl_3$): δ 8.21−8.18 (m, 4H, Ar*H*), 8.09−8.04 (m, 5H, Ar*H*), 7.87−7.78 (m, 12H, Ar*H*), 7.58−7.48 (m, 12H, Ar*H*), 7.45−7.44 (m, 6H, Ar*H*), 7.37−7.28 (m, 5H, Ar*H*), 6.02 (m, 1H), 5.74−5.64 (m, 5H), 5.04 (s, 1H), 4.88−4.77 (m, 3H), 4.68−4.62 (m, 3H), 4.58−4.52 (m, 2H), 4.46 (m, 1H), 4.33−4.30 (m, 3H), 4.27−4.21 (m, 3H), 4.10−4.09 (m, 2H), 4.02−3.99 (m, 2H), 3.96−3.93 (m, 4H), 3.88−3.87 (m, 2H), 1.34 (s, 3H, Pyr. CH_3), 1.29 (d, *J* = 6.1 Hz, 3H, CH_3), 1.23 (s, 9H, (CH_3)$_3$CSi), 1.18 (d, *J* = 6.5 Hz, 3H, CH_3, isopropanol), 1.13 (d, *J* = 6.5 Hz, 3H, CH_3, isopropanol); ^{13}C NMR $\{^1H\}$ (126 MHz, $CDCl_3$): δ 170.7 (CO_2Me), 165.9 (COPh), 165.7 (COPh), 165.6 (COPh), 165.3 (COPh), 164.8 (COPh), 137.0 (ArC), 135.6 (ArC), 135.5 (ArC), 133.3 (ArC), 133.3 (ArC), 133.2 (ArC), 133.1 (ArC), 133.1 (ArC), 130.1 (ArC), 130.0 (ArC), 129.9 (ArC), 129.9 (ArC), 129.8 (ArC), 129.7 (ArC), 129.5 (ArC), 129.4 (ArC), 129.3 (ArC), 128.8 (ArC), 128.6 (ArC), 128.5 (ArC), 128.4 (ArC), 128.4 (ArC), 128.3 (ArC), 128.2 (ArC), 128.1 (ArC), 127.9 (ArC), 127.7 (ArC), 127.7 (ArC), 107.0 (C-1 of Galf), 99.7 (C-1 Pyr. Gal), 99.2 (Cq Pyr.), 98.9 (C-1 AAT), 92.1 (C-1 of GalN), 81.2, 81.0, 74.4, 73.0, 72.2, 72.1, 72.0, 70.4, 70.3, 64.9, 65.5, 64.4, 63.0, 60.3, 59.0, 52.7, 51.8 (OCH_3), 29.7, 27.0, 25.6, 23.2 (CH_3 of isopropanol), 21.9 (CH_3 of isopropanol), 19.4 (quaternary *C* of TBDPS), 16.8 (CH_3 of AAT); HR-ESI-MS (*m*/*z*): [M + K]$^+$ calcd. for $C_{97}H_{97}N_7O_{26}KSi$, 1842.5880; found, 1842.5884.

*Isopropyl 2-Acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranosyl-(1 → 4)-[β-D-galactofuranosyl-(1 → 3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1 → 3)-4,6-O-[1-(R)-(carboxy)-ethylidene]-β-D-galactopyranoside (**1**).* Global deprotection of tetrasaccharide **2** (100 mg, 0.056 mmol) was carried out in five steps. Compound **2** was dissolved in pyridine (0.3 mL) and treated with thioacetic acid (0.3 mL, 0.05 mmol) at rt for 24 h. The solvents were evaporated, and the crude product was coevaporated with toluene (5 mL × 3). Purification of the crude product by silica chromatography

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(70:30, ethyl acetate/petroleum ether) afforded the *N*-acetate derivative (75 mg, 74%). The NHAc intermediate was treated with TBAF/AcOH, (0.15 mL) in 0.6 mL of THF at rt overnight to give the alcohol. The phthalimide group from this intermediate was removed using EDA/*n*-BuOH (0.15 mL/1 mL). Benzyl groups were removed using H_2/Pd/C (0.5/mmol, 30 mg) in methanol (1.2 mL). Finally, esters were deprotected using NaOMe (0.2 M) in MeOH/THF/H_2O (1:1:1) (4:4:4 mL) to obtain a crude product, which was purified by HPLC (30:70 water/acetonitrile on a C18 column) to furnish tetrasaccharide **1** (12.4 mg) in 36% yield over four steps. $[\alpha]_D^{25}$ = +27.5. ^{1}H NMR (600 MHz, D_2O): δ 5.21 (d, J = 3.6 Hz, 1H), 4.95 (d, J = 3.0 Hz, 1H), 4.90 (d, J = 3.6 Hz, 1H), 4.47 (d, J = 3.6 Hz, 1H), 4.54−4.44 (m, 1H), 4.32 (d, J = 3.6 Hz, 1H), 4.26 (dd, J = 12.0, 4.2 Hz, 1H), 4.08 (t, J = 6.0 Hz, 1H), 4.05−4.05 (m, 1H), 4.02−3.97 (m, 2H), 3.95−3.91 (m, 1H), 3.90−3.79 (m, 5H), 3.69−3.64 (m, 2H), 3.63−3.59 (m, 1H), 3.58−3.48 (m, 6H), 3.44 (s, 1H), 1.95 (s, 3H), 1.93 (s, 3H), 1.33 (s, 3H), 1.18 (d, J = 6.6 Hz, 3H), 1.14 (d, J = 6.6 Hz, 3H), 1.08 (d, J = 6.6 Hz, 3H) ^{13}C{^{1}H} NMR (126 MHz, D_2O): δ 174.6 ($NHCOCH_3$), 174.0 ($NHCOCH_3$), 171.1 (CO_2H), 108.8 (C-1 of Galf), 100.1 (C-1 of Pyr. Gal), 98.9 (quaternary *C* of pyruvate), 97.7 (C-1 of AAT), 92.8 (C-1 of GalN), 81.8, 80.5, 76.9, 75.6, 75.0, 73.8, 72.9, 72.1, 70.5, 68.6, 66.9, 65.3, 65.2, 63.5, 62.7, 60.4, 58.9, 54.6, 49.9, 48.6, 25.1, 23.3 (CH_3 of isopropanol), 22.3 (CH_3 of isopropanol), 21.9 ($NHCOCH_3$), 20.9 ($NHCOCH_3$), 16.2 (CH_3 of AAT); HR-ESI-MS (m/z): $[M + Na]^+$ calcd. for $C_{34}H_{57}N_3O_{21}Na$, 866.3376; found, 866.3377.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02935.

^{1}H NMR; ^{13}C NMR; DEPT; and ^{1}H−^{1}H COSY spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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