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Synthesis of Lewis X trisaccharide analogues in which glucose and rhamnose replace *N*-acetylglucosamine and fucose, respectively

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Abstract

Two analogues of the Le^x trisaccharide, α-L-Fuc*p*-(1→3)-[β-D-Gal*p*-(1→4)]-D-Glc*p* were synthesized as allyl glycosides. In these derivatives either only the *N*-acetylglucosamine is replaced by glucose or both the *N*-acetylglucosamine and the fucosyl residue are replaced by glucose and rhamnose, respectively. Our synthetic scheme used armed β-thiophenyl fuco- and rhamnoside glycosyl donors that were prepared anomerically pure from the corresponding α-glycosyl bromides. The protecting groups were chosen to allow access to the fully deprotected trisaccharides without reduction of the allyl glycosidic group. These analogues will be used as soluble antigens in binding experiments with anti-Le^x antibodies and can also be conjugated to a carrier protein and used as immunogens. In the course of this synthetic work, we also describe the use of reversed-phase HPLC to purify key protected trisaccharide intermediates prior to their deprotection.

Keywords: Lewis X analogues; Thiophenyl glycosides; Oligosaccharide synthesis; Reversed-phase HPLC

1. Introduction

Aberrant glycosylation in human cancer and the structures of tumor-associated carbohydrate antigens (TACAs) have been reviewed.[1–3] TACAs can be involved in specific immune responses during which antibodies recognizing specific carbohydrate epitopes are produced. In this context, extensive work has been performed on glycoconjugates displaying TACAs as potential vaccines against cancer, and the approach was shown to be very promising.[4–9] Monoclonal antibodies raised and selected against the TACA dimLe^x **1** (Fig. 1, dimLe^x), were shown[10–14] to bind specifically to carbohydrate epitopes that are displayed by colorectal tumour tissues. However, if used as a vaccine, the dimLe^x hexasaccharide is also likely to trigger the production of antibodies binding to the non-reducing trisaccharide Le^x (Fig. 1) largely expressed on normal tissues and cells.[15] To avoid such reactions that could lead to the destruction of these normal cells, we are studying analogues of hexasaccharide **1** in which one of the sugar units defining the Le^x epitope is replaced by other sugar residue, i.e., *N*-acetylglucosamine (A′) by glucose, fucose (B′) by rhamnose or galactose (C′) by glucose.

We are reporting here the chemical synthesis of two trisaccharide analogues of Le^x in which either only the *N*-acetylglucosamine is replaced by glucose or in which both the *N*-acetylglucosamine and the fucosyl residue are replaced by glucose and rhamnose, respectively. The synthetic work reported here had two objectives. The main purpose was to provide these trisaccharide analogues as allyl glycosides that can be used as soluble antigens in binding experiments as well as easily

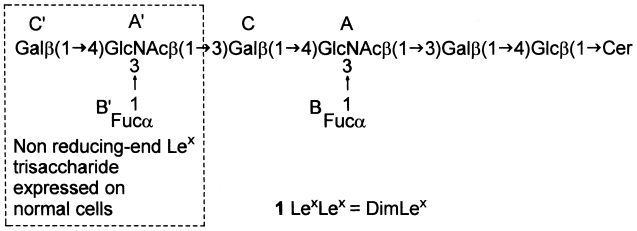

Fig. 1. Tumor-associated carbohydrate antigen dimeric Lewis X.

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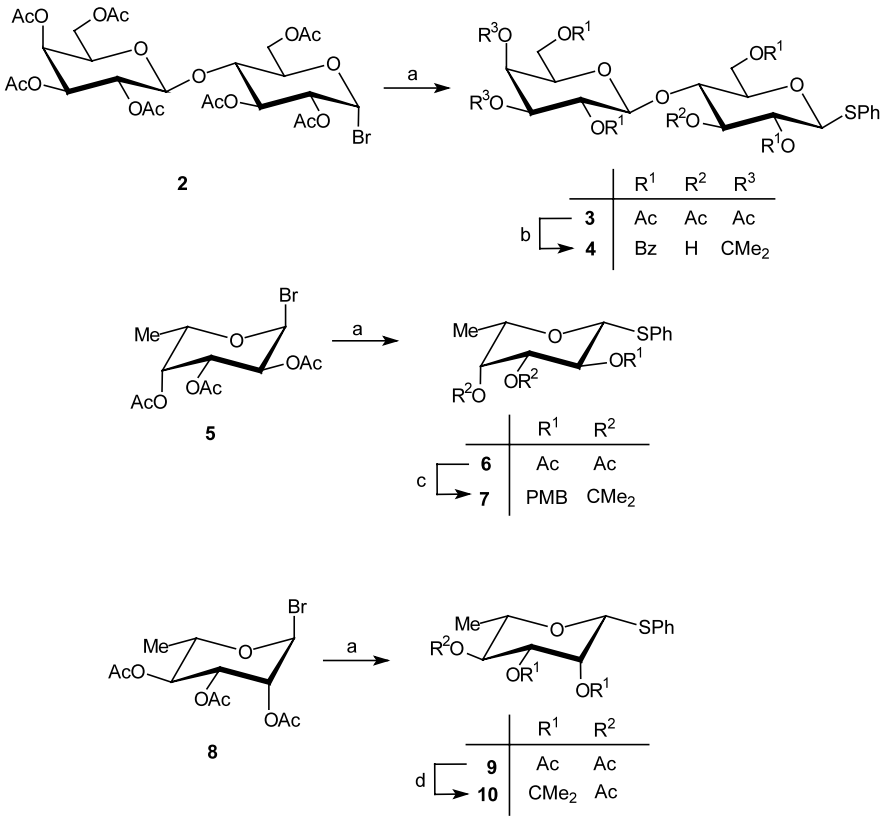
conjugated[16] to a carrier protein to be used as immunogens. The second goal was to prepare thiophenyl trisaccharide building blocks that could be used for the chemical synthesis of hexasaccharide analogues of dimLe^x. In fact, numerous syntheses of the dimLe^x antigen[17,18] have been reported. In addition, due to the importance of sialyl Le^x (SLe^x) in the inflammation process, the preparation of SLe^x analogues in which the fucosyl residue is substituted by a rhamnosyl unit[19] or in which the *N*-acetylglucosaminyl unit is replaced by a glucose residue have also been described.[20,21] Building on these reports and most particularly on the work of Tsukida and co-workers,[20] we first attempted the preparation of our analogues using thiophenyl glycoside intermediates and the armed/disarmed concept introduced by Fraser-Reid.[22] However, since the thiophenyl trisaccharides obtained could not be converted to the corresponding allyl glycosides, we describe the synthesis of the allyl trisaccharides through the glycosylation of allyl lactosides with our thiophenyl fuco- and rhamnoside glycosyl donors. As part of this synthetic scheme, we report a procedure modified from that of Fischer and Delbrück[23] to prepare β-peracetylated thiophenyl glycosides from acetylated glycosyl bromides. Finally we also describe the use of reversed-phase HPLC to separate protected trisaccharide intermediates from unreacted disaccharide glycosyl acceptors.

2. Results and discussion

Our first synthetic scheme based on the armed/disarmed concept relied on the selective activation of fucosyl and rhamnosyl thiophenyl glycosyl donors in the presence of thiophenyl lactose. This activation can be achieved when the glycosyl acceptor carries a deactivating electron-withdrawing acyl group at O-2, while the donor is substituted by an activating alkyl group on the same position.

The disarmed lactose acceptor **4** was synthesized in three steps from the corresponding acetylated glycosyl bromide **2**. Conversion of the bromide **2** to thiophenyl lactose **3** has been reported in homogeneous conditions by Fischer and Delbrück,[23] and subsequently by Purves.[24] More recently, to avoid unwanted deacetylation, Roy and co-workers reported a modified procedure that used phase-transfer conditions.[25] In Purves' method, acetylated lactosyl bromide was dissolved in chloroform and reacted 30 min on a water bath with a slight excess of thiophenol solubilized in 95% ethanol containing an equimolar amount of potassium hydroxide. We further simplified these conditions: lactosyl bromide **2** was dissolved in acetone and treated with thiophenol (1.15 equiv) and a solution (1.14 equiv) of KOH in 95% ethanol at room temperature. As seen by TLC the reaction proceeded virtually instantaneously, and the β-thiophenyl lactoside **3** was isolated in 80% yield. The peracetylated lactose **3** was deacetylated, treated with 2,2-dimethoxypropane as described by Hasegawa and co-workers[26] to introduce an isopropylidene group at O-3/O-4 of the galactose residue. The resulting polyol was selectively benzoylated as described by Tsukida and co-workers[20] to yield the known disarmed acceptor **4**.

Since the armed/disarmed strategy requires that the glycosyl donors carry non-participating groups at O-2, we decided to prepare anomerically pure β-thiophenyl fucosyl and rhamnosyl donors to favor the formation of α glycosidic bonds to the acceptor **4** through S_N2 displacement. The preparation of peracetylated thiophenyl fucose[27] and rhamnose[28,29] have been described using the method of Ferrier and Furneaux.[30] However, these Lewis acid catalyzed reactions were shown to give anomeric mixtures of the corresponding thiophenyl glycosides or at best only the pure α anomer of peracetylated rhamnose.[29] Relying on our success in preparing anomerically pure β-thiophenyl lactoside **3** from the corresponding α-bromide **2**, we applied this method to the synthesis of thiophenyl fucoside and rhamnoside **6** and **9**, respectively. Thus, fucosyl bromide **5** was prepared in two steps from fucose (Ac_2O–pyridine; HBr–AcOH), then dissolved in acetone and treated with thiophenol and potassium hydroxide in 95% ethanol as describe above. Once again TLC showed that the reaction proceeded virtually instantaneously, and the β-thiophenyl fucoside **6** was isolated in 80% yield over the three steps. Similarly, rhamnosyl bromide **8** was prepared from rhamnose (Ac_2O–pyridine; HBr–AcOH) and treated with thiophenol–KOH to give as the only thiophenyl glycoside the β rhamnoside **9** in 31% over the three steps. The configuration of the thiophenyl group in **9** was deduced from the $J_{C,H}$ coupling constant at the anomeric centre (153 Hz), which, as expected for the β anomer,[31] was more than 10 Hz smaller than that reported by Pozgay and co-workers[29] for the corresponding α-thiophenyl glycoside (169 Hz). When compared to the preparation of **3** and **6**, this reaction appeared to be much slower and led to extensive hydrolysis of the anomeric bromide. Despite the participating effect of the acetyl group at C-2 in **8**, no β-thiophenyl rhamnoside could be isolated during this reaction. Thus, we conclude that the displacement of the anomeric bromide in **2**, **5** or **8** by thiophenoxide ions proceeded mostly through an S_N2-type mechanism. It appears that the axial acetyl group at C-2 of the α-rhamnosyl bromide **8** stabilizes the bromide towards this displacement resulting in a lower yield of the wanted β-thiophenyl rhamnoside. However, since it allowed the preparation of β-thiophenyl fuco- and rhamnosides **6** and **9** as only and major products, respectively, this strategy to prepare β-thiophenyl glycosides constitutes an interesting alternative to the

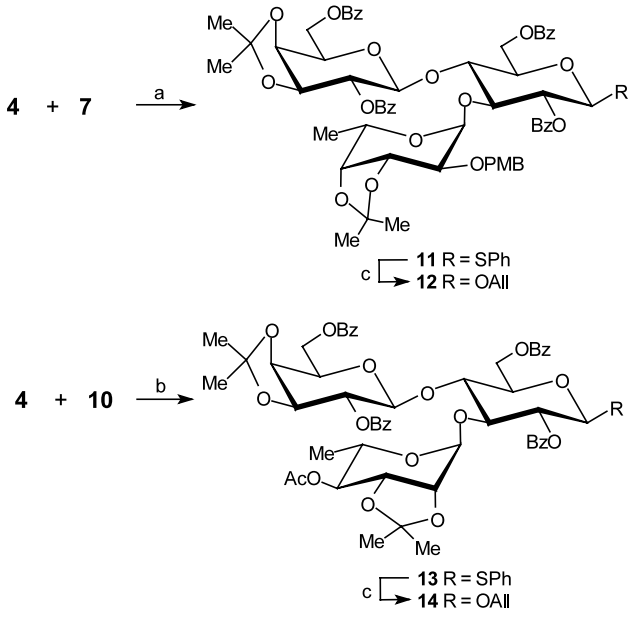


Scheme 1. (a) PhSH, KOH in 95% EtOH, acetone, room temperature; (b) (i) MeONa–MeOH, (ii) 2,2-dimethoxypropane, *p*TsOH, DMF, 80 °C as described by Hasegawa and co-workers,[26] (iii) BzCl, $PhCH_3$–C_6H_5N, 0 °C as described by Tsukida and co-workers;[20] (c) (i) MeONa–MeOH, (ii) 2-methoxypropene, *p*TsOH, CH_3CN, (iii) NaH, *p*MeOBnCl, DMF (87% from **6**); (d) (i) MeONa–MeOH, (ii) 2-methoxypropene, *p*TsOH, CH_3CN, (iii) Ac_2O–C_6H_5N (69% from **9**).

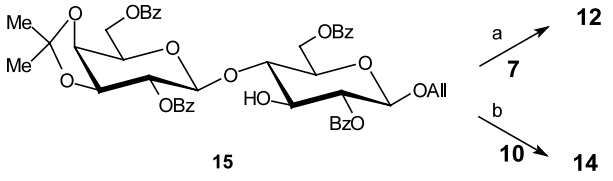
Lewis acid catalyzed reaction of peracetylated fucose or rhamnose with thiophenol.[27,28] Peracetylated thioglycosides **6** and **9** were converted to the armed glycosyl donors **7** and **10**. Using a reaction sequence analogous to that described by Khane and co-workers on the anomeric mixture,[27] the β-thiophenyl fucoside **6** was submitted to Zemplén deacetylation and protected successively with an isopropylidene group on O-3/O-4 and a non-participating *p*-methoxylbenzyl group at O-2 (Scheme 1). The resulting thiofucosyl donor **7** was isolated in 87% from the peracetate **6**. Peracetylated rhamnose **9** was also deacetylated, an isopropylidene group was introduced at O-2/O-3, and the remaining hydroxyl group at C-4 was acetylated to yield **10** (69% yield from **9**, Scheme 1).

All glycosylations were carried out under conditions similar to those used successfully by Tsukida and co-workers[20] for the coupling of perbenzylated thiophenyl fucose with acceptor **4**. These reactions were carried out at low temperature (-30 or -20 °C) in anhydrous chloroform containing activated powdered 4 Å molecular sieves, and using *N*-iodosuccinimide (2.3 equiv) and triflic acid (0.8 equiv) as promoters. Based on the armed–disarmed concept, the acceptor **4** was glycosylated with an excess (1.7 equiv) of thiophenyl fucoside **7** and gave, as estimated by ^1H NMR spectroscopy, 29% yield of the wanted trisaccharide **11** (Scheme 2) that co-migrated with the unreacted glycosyl acceptor (see Fig. 2A). While **4** and **11** could not be separated by normal-phase flash chromatography or HPLC (Fig. 2B), the trisaccharide **11** was obtained pure by reversed-phase HPLC using a Prep Nova Pak® HR C18 column (Waters) and a mixture of 9:1 acetonitrile–water as eluant. In contrast to the fucosylation, coupling of the acceptor **4** with the rhamnosyl donor **10** gave the pure trisaccharide **13** in excellent yield (79%). The configuration of the rhamnosidic linkage was again deduced from $J_{C,H}$ coupling constant at C-1 of rhamnose (172 Hz), which was in perfect agreement with that of an α linkage.[29,31,32] Although the fucosylated trisaccharide **11** was obtained in poor yield, thus limiting its application as a building block to prepare larger oligosaccharides, it was isolated in sufficient quantities to be converted to the wanted allyl trisaccharide **12**. Thus, the conversion of thiophenyl trisaccharides **11** and **13** to the corresponding allyl trisaccharides **12** and **14**, respectively, was then attempted. Since **11** and **13** are disarmed glycosyl donors, excess NIS (up to 4.5 equiv) and triflic acid (up to 1.5 equiv) were needed to promote glycosylation. Unfortunately, these conditions led mostly to

the degradation of the fucosylated trisaccharide **11**, and only a minute amount of impure fucosylated trisaccharide **12** (less than 10%) was obtained. Similarly, reaction of **13** with allyl alcohol, gave only a low yield (16%) of the pure rhamnosylated trisaccharide **14**, while some unreacted thiophenyl trisaccharide (35%) was also recovered. These results showed that the strategy based on the armed/disarmed concept and thiophenyl trisaccharides **11** and **13** as building block intermediates was not applicable to the preparation of the allyl trisaccharides **16** and **17**. In addition, our objective to prepare the thiophenyl trisaccharides **11** and **13** as intermediates that can be used in further elongation reactions is currently being revised. As an alternative route to **16** and **17**, the allyl lactoside **15** was prepared as described previously by Nashed and Musser.[33] Coupling of **15** and **4** gave the fucosylated trisaccharide **12** in moderate yield (29%) after separation from the unreacted acceptor by RP-HPLC. In contrast, the reaction of **15** with **7** gave trisaccharide **14** in excellent yield (89%). Again, the $J_{C,H}$ coupling constant (172 Hz) measured for the anomeric carbon of rhamnose in trisaccharide **14** was in perfect agreement with that of an α-rhamnosidic linkage.[29,31,32] Thus, while glycosylations of **4** and **15** with thiophenyl rhamnoside **10** gave the wanted trisaccharides in good yields, their fucosylation with glycosyl donor **7** gave at best only poor yields of the trisaccharides. We observed that activation of thiophenyl glycoside **7** towards its coupling with the poorly reactive glycosyl acceptors **4** and **15** required a relatively large quantity of triflic acid that led competitively to its degradation. Therefore, we are investigating other fucose glycosyl donors and lactose glycosyl acceptors to prepare the larger dimLe^x hexasaccharide analogue (Scheme 3).



Scheme 2. (a) Donor **7** (1.7 equiv), NIS (2.3 equiv), TfOH (0.8 equiv), $CHCl_3$, 4 Å MS, −30 °C (29% as estimated from the ^{1}H NMR spectrum); (b) donor **10** (1.9 equiv), NIS (2.3 equiv), TfOH (0.8 equiv), $CHCl_3$, 4 Å MS, −30 °C (79%); (c) AllOH (5 equiv), NIS (up to 3.3 equiv), TfOH (up to 1.5 equiv), $CHCl_3$, 4 Å MS, −20 °C (11% impure for **12**, 16% for **14** with 35% of recovered **13**).



Scheme 3. (a) Donor **7** (1.7 equiv), NIS (2.3 equiv), TfOH (0.8 equiv), $CHCl_3$, 4 Å MS, −30 °C (29%); (b) donor **10** (1.7 equiv), NIS (2.3 equiv), TfOH (0.8 equiv), $CHCl_3$, 4 Å MS, −30 °C (85%).

Despite the low glycosylation yield of **15** with **7**, the allyl trisaccharide **12** was isolated in sufficient amount to allow access to the wanted trisaccharide **16**. Deprotection of trisaccharide **12** started with the selective removal of the *p*-methoxybenzyl group using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. In order to stabilize the fucosidic linkage during the acid-catalyzed hydrolysis of the isopropylidene groups,[34] the resulting alcohol was acetylated with 10% acetic anhydride in pyridine. The isopropylidene groups were then removed quantitatively in two hours using 90% aqueous acetic acid at 80 °C. Finally, the acyl groups were transesterified with methanolic (0.2 M) sodium methoxide and trisaccharide **16** was obtained pure in 73% yield from the protected trisaccharide **12**. Since the rhamnosidic linkage is more stable towards acid treatment than the fucosidic linkage, trisaccharide **14** could be first deacylated by methanolic sodium methoxide and, in turn, submitted to acid hydrolysis using 90% acetic acid at 80 °C to remove the *O*-isopropylidene groups. The fully deprotected trisaccharide **17** was purified by chromatography and obtained pure in 87% yield from trisaccharide **14**.

The trisaccharides **16** and **17** were characterized by NMR spectroscopy and high-resolution mass spectrometry. In a first study, they will be used as soluble antigens in competitive binding experiments with anti-Le^x polyclonal antibodies. If they do not react with these antibodies, they will be, in turn, conjugated to a carrier protein through their allyl glycosidic group[16] and used as immunogens to raise polyclonal antibodies that will be tested for their reactivity with the natural antigen Le^x. If there is no cross-reactivity between Le^x and either analogue **16** or **17**, the corresponding hexasaccharide analogues of dimLe^x will be prepared, their cross-reactivity with dimLe^x studied, and their potential application as vaccine candidates further investigated.

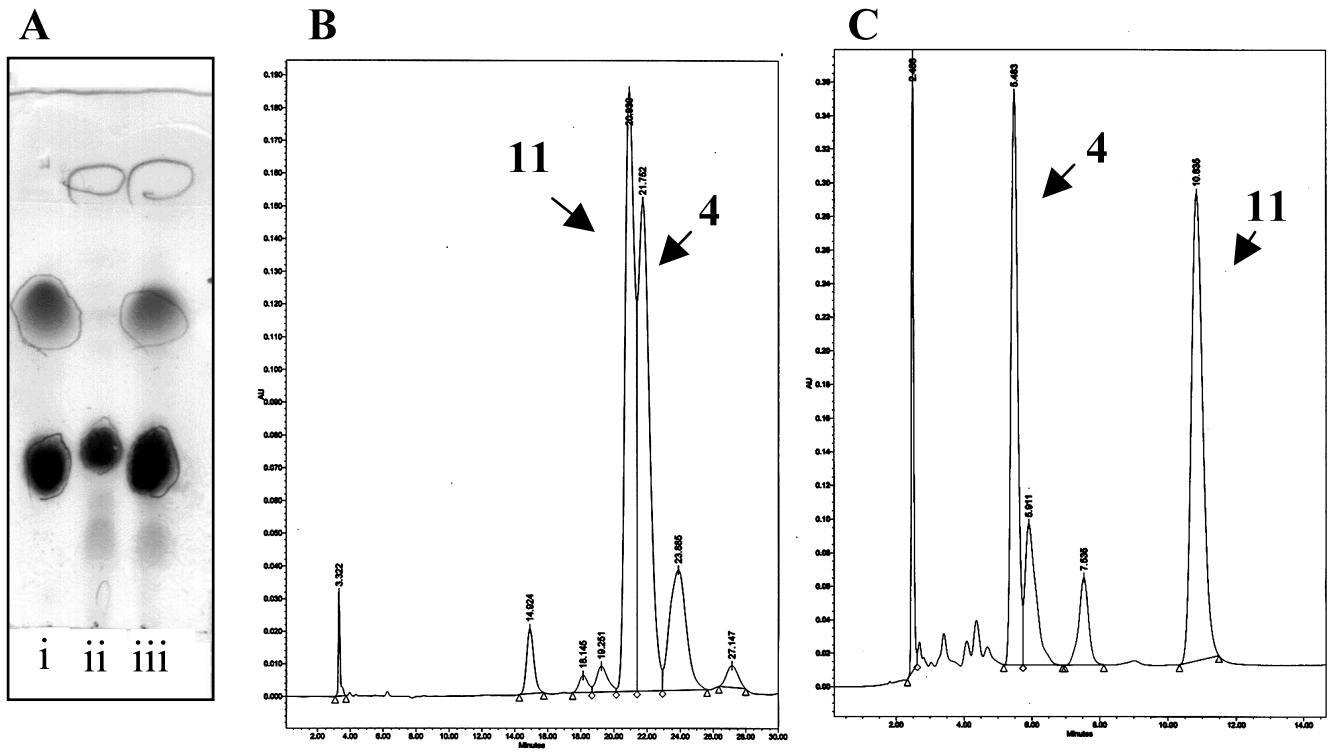
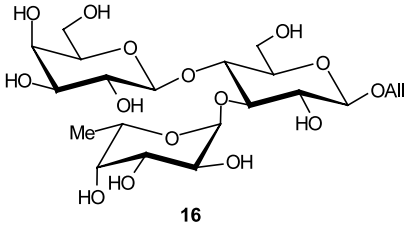


Fig. 2. (A) TLC 7:3 hexane–EtOAc, UV detection as indicated with pencil lines and charring with 10% ethanolic sulfuric acid, (i) mixture of **4** and **7**, (ii) reaction mixture, (iii) mixture of **4**, **7** and the reaction mixture; (B) normal-phase analytical HPLC on the pre-purified mixture of **4** and **11**, Radial-Pack, silica 6 μm, 60 Å, 8 × 100 mm, 1 mL/min, 2:8 EtOAc–hexane; (C) reversed-phase analytical HPLC on the pre-purified mixture of **4** and **11**, Nova-Pack, C-18, 4 μm, 60 Å, 3.9 × 150 mm, 0.5 mL/min, 9:1 CH_3CN–H_2O.



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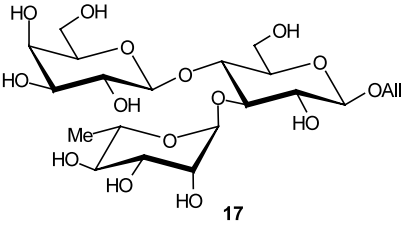

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3. Experimental

3.1. General methods

^1H (400.13 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on a Bruker Avance-400 NMR spectrometer for solutions in $CDCl_3$ (internal standard, for ^1H residual $CHCl_3$ δ 7.27; for ^{13}C: $CDCl_3$ δ 77.0) or D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of one-dimensional spectra. Assignments of proton and carbon resonances were based on COSY and ^{13}C–^1H heteronuclear correlated experiments. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. TLC were performed on precoated aluminum plates with Silica Gel 60 F_{254} (E. Merck) and detected with UV light and/or charred with a solution of 10% H_2SO_4 in EtOH. Compounds were purified by flash chromatography with Silica Gel 60 (230–400 mesh) unless otherwise stated. Solvents were distilled and dried according to standard procedures,[35] and organic solutions were dried over Na_2SO_4 and concentrated below 40 °C, under reduced pressure. Reversed-phase HPLC purifications were carried out on a Prep Nova Pak® HR C18, 6 μm 60 Å (25 × 100 mm) column using mixtures of MeCN and water as eluant. Elemental analyses were performed at the Canadian Microanalytical Service Ltd. (Delta, B.C.) or at M-H-W Laboratories (Phoenix, Arizona). High-resolution electrospray-ionization mass spectra (HRESIMS) were recorded on a Micro-Mass ZabSpec Hydroid Sector-TOF or Applied Biosystems Mariner Biospectrometry Workstation by the analytical services of the department of Chemistry at the University of Alberta. Melting points were measured on a Electrothermal digital melting point apparatus and are uncorrected.

3.2. Preparation of phenyl 2,4,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (3)

Thiphenol (2 mL, 19.5 mmol) and a solution of 1.78 M KOH in 95% EtOH (8 mL, 14.2 mmol) were added to a solution of peracetylated lactosyl bromide[36] **2** (9.11 g, 13 mmol) in acetone (50 mL). The mixture was stirred at room temperature (rt) for 1 h, diluted with CH_2Cl_2 (150 mL), washed with 1 M aq NaOH and satd aq NaCl. The aqueous phases were re-extracted twice with CH_2Cl_2, and the combined organic solutions were dried and concentrated. The solvents were evaporated, and the thiophenyl lactoside **3** (7.6 g, 80%) crystallized upon addition of Et_2O: mp 163–166 °C; $[\alpha]_D$ −15° (*c* 1.2, $CHCl_3$); lit.[23] mp 164 °C, $[\alpha]_D$ −18° ($CHCl_3$); lit.[24] mp 156 °C, $[\alpha]_D$ −20° ($CHCl_3$); lit.[25] mp 165–167 °C, $[\alpha]_D$ −18° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.48, 7.31 (2 m, 5 H, Ar), 5.34 (bd, 1 H, $J_{3',4'}$ 3 Hz, H-4′), 5.21 (t, 1 H, $J_{2,3+3,4}$ 18 Hz, H-3), 5.10 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2′), 4.94 (dd, 1 H, H-3′), 4.90 (t, 1 H, $J_{1,2+2,3}$ 19.5 Hz, H-2), 4.67 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.53 (dd, 1 H, $J_{5,6}$ 1.5, $J_{6a,6b}$ 12 Hz, H-6a), 4.47 (d, 1 H, H-1′), 4.10 (m, 3 H, H-6b, H-6′a, H-6′b), 3.87 (bt, 1 H, $J_{5',6'a+5',6'b}$ 13.5 Hz, H-5′), 3.74 (t, 1 H, $J_{3,4+4,5}$ 19 Hz, H-4), 3.64 (m, 1 H, H-5), 2.15, 2.11, 2.09, 2.08, 2.045, 2.04, 2.03, 1.96 (7 s, 21 H, 7 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.0, 169.6, 169.5, 169.0 (CO), 133.0, 128.8, 128.2 (Ar), 131.7 (Ar quat), 100.95 (C-1′), 85.4 (C-1), 76.6 (C-5), 76.05 (C-4), 73.75 (C-3), 70.9 (C-3′), 70.6 (C-5′), 70.2 (C-2), 69.0 (C-2′), 66.5 (C-4′), 62.0 (C-6), 60.7 (C-6′), 20.7, 20.5, 20.4 (*C*H$_3$CO).

3.3. Phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (6)

Fucose (2.23 g, 13.6 mmol) was stirred overnight at rt in a mixture of C_6H_5N (10 mL) and Ac_2O (10 mL). Solvents were co-evaporated with toluene, and the oily residue dissolved in CH_2Cl_2 was washed successively with 1 M HCl, satd aq $NaHCO_3$, and H_2O. The aqueous phases were re-extracted twice with CH_2Cl_2, and the combined organic phases were dried, and concentrated. The peracetate obtained was dissolved in anhyd CH_2Cl_2 (40 mL) containing Ac_2O (1 mL), and a 33% solution of HBr in AcOH (10 mL) was added. The reaction mixture was stirred at rt for 4 h and diluted with toluene (50 mL), and the solvents were evaporated. Residual acid was co-evaporated with toluene, and the residue dissolved in CH_2Cl_2 was washed with cold satd aq $NaHCO_3$. The aqueous washing was re-extracted twice with CH_2Cl_2, and the organic phases were combined, dried, and concentrated. The crude glycosyl bromide **5** was obtained as a yellow oil and dissolved in acetone (40 mL). Ethylacetate (2 mL), thiophenol (1.6 mL, 15.6 mmol) and a solution of KOH in 95% EtOH (1.82 M, 8.55 mL, 15.5 mmol) were successively added to the bromide solution, and the reaction mixture was stirred at rt for 2 h. It was diluted with CH_2Cl_2 (100 mL) and washed successively with 0.5 M aq NaOH and satd aq NaCl. The aqueous phases were re-extracted twice with CH_2Cl_2, and the combined organic solutions were dried and concentrated. Flash chromatography (8:2 hexane–EtOAc) of the residue gave the pure thiophenyl fucoside **6** (4.19 g, 80% calcd from fucose) as a colorless oil: $[\alpha]_D$ −7° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.50, 7.32 (2 m, 5 H, Ar), 5.27 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1 Hz, H-4), 5.23 (t, 1 H, $J_{1,2+2,3}$ 20 Hz, H-2), 5.06 (dd, 1 H, $J_{2,3}$ 10 Hz, H-3), 4.72 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 3.84 (m, 1 H, H-5), 2.15, 2.10, 1.98 (3 s, 9 H, 3 × CH_3O), 1.25 (d, 1 H, $J_{5,6}$ 6.5 Hz, H-6). Anal. Calcd for $C_{18}H_{22}O_7S$: C, 56.53; H, 5.80. Found: C, 56.60; H, 5.48.

3.4. Phenyl 3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside (7)

A methanolic solution of sodium methoxide (1 M, 2.5 mL) was added to a suspension of **6** (8.1 g, 21.2 mmol) in MeOH (50 mL). The mixture was stirred at rt for 2 h, diluted with MeOH (30 mL) and deionized with Dowex 50 (H^+) resin. Once the pH was neutral, the resin was filtered off and rinsed with MeOH. The filtrate and washings were combined and concentrated. *p*TsOH (56 mg) and 2-methoxypropene (2.23 mL, 23.3 mmol) were added to a solution of the triol obtained in CH_3CN (50 mL). The reaction was stirred at rt for 10 h, quenched with Et_3N (100 μL) and concentrated. The residue was dissolved in $CHCl_3$ and washed successively with satd aq $NaHCO_3$ and brine. The aqueous phases were re-extracted twice with $CHCl_3$, and the combined organic solutions were dried and concentrated to give 6.13 g of the isopropylidene derivative: ^{1}H NMR ($CDCl_3$): δ 7.57, 7.32 (2 m, 5 H, Ar), 4.43 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 4.06 (m, 1 H, H-3), 4.05 (m, 1 H, H-4), 3.89 (m, 1 H, H-5), 3.55 (m, 1 H, H-2), 1.45 (d, 3 H, $J_{5,6}$ 6 Hz, H-6), 1.44, 1.36 (2 s, 6 H, 2 × CH_3C); ^{13}C NMR ($CDCl_3$): δ 132.7, 128.9, 128.0 (Ar), 132.1 (Ar quat), 109.9 (*C*(CH$_3$)$_2$), 87.9 (C-1), 79.0, 76.3 (C-3, C-4), 72.8 (C-5), 71.3 (C-2), 28.1, 26.3 (C(*C*H$_3$)$_2$), 16.9 (C-6). A portion of the above-mentioned isopropylidene derivative (200 mg, 0.67 mmol) was dissolved in DMF (2 mL), and sodium hydride (60% in oil, 116.6 mg, 2.9 mmol) was added. The reaction mixture was stirred at rt for 1 h, then cooled to 0 °C, and 4-methoxybenzyl chloride (0.18 mL, 1.35 mmol) was added. The reaction mixture was allowed to warm up to rt, and stirring was continued for another 2 h. After completion of the reaction, MeOH was added to decompose excess NaH. The reaction mixture was co-concentrated with toluene, and the residue dissolved in $CHCl_3$ (15 mL) was washed with water. The aqueous phase was re-extracted with

$CHCl_3$, and the combined organic solutions were dried and concentrated. Flash chromatography (8:2 hexane–EtOAc) of the residue gave the pure donor **7** (254 mg, 87% calcd from **6**) as a colorless oil; $[\alpha]_D$ −1° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.54, 7.35, 7.29, 6.87 (4 m, 9 H, Ar), 4.76, 4.61 (2 d, 2 H, *J* 11 Hz, CH_2Ph), 4.59 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.22 (bt, 1 H, $J_{2,3+3,4}$ 12 Hz, H-3), 4.04 (bd, 1 H, $J_{3,4}$ 5.5 Hz, H-4), 3.82 (m, 1 H, H-5), 3.80 (s, 3 H, CH_3O), 3.50 (bdd, 1 H, $J_{2,3}$ 7 Hz, H-2), 1.43 (s, 3 H, CH_3C), 1.41 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6), 1.37 (s, 3 H, CH_3C); ^{13}C NMR ($CDCl_3$): δ 159.2, 133.8, 131.0 (Ar quat), 132.0, 129.8, 128.7, 127.2, 113.6 (Ar), 109.6 (*C*(CH_3)$_2$), 86.1 (C-1), 79.8 (C-3), 76.6 (C-2), 76.3 (C-4), 73.0 (OCH_2), 72.3 (C-5), 55.2 (CH_3O), 27.8, 26.3 (C(*C*H_3)$_2$), 16.8 (C-6). Anal. Calcd for $C_{23}H_{28}O_5S$: C, 66.32; H, 6.78. Found: C, 65.85; H, 6.63.

3.5. Phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside (9)

Rhamnose monohydrate (2.51 g, 13.8 mmol) was converted to the peracetylated rhamnosyl bromide **8** as described above for the fucosyl bromide **5** and was dissolved in acetone (40 mL). Thiophenol (1.7 mL, 16.5 mmol) and a solution of KOH in 95% EtOH (1.81 M, 8.38 mL, 15.16 mmol) were added, and the reaction mixture was stirred overnight at rt. It was diluted with CH_2Cl_2 (100 mL) and washed successively with 0.5 M aq NaOH and brine. The aqueous phases were re-extracted with CH_2Cl_2, and the combined organic solutions were dried and concentrated. Flash chromatography (8:2 hexane–EtOAc, 500 mL then 7:3, 500 mL) gave the pure thiophenyl glycoside **9** (1.61 g, 31% calcd from rhamnose) that crystallized on standing: mp 87–88 °C; $[\alpha]_D$ +73° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.51, 7.28 (2 m, 5 H, Ar), 5.67 (d, 1 H, $J_{2,3}$ 3 Hz, H-2), 5.11 (t, 1 H, $J_{3,4+4,5}$ 19.5 Hz, H-4), 5.00 (dd, 1 H, $J_{3,4}$ 10 Hz, H-3), 4.91 (s, 1 H, H-1), 3.57 (m, 1 H, H-5), 2.21, 2.05, 1.99 (3s, 9 H, 3 × CH_3CO), 1.33 (d, 3 H, $J_{5,6}$ 6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 170.2, 170.1, 169.8 (CO), 133.2 (Ar quat), 132.0, 129.0, 128.0 (Ar), 85.3 ($J_{C,H}$ 153 Hz, C-1), 74.9 (C-5), 71.8 (C-3), 71.0 (C-2), 70.1 (C-4), 20.7, 20.6, 20.5 (*C*H_3CO), 17.7 (C-6). Anal. Calcd for $C_{18}H_{22}O_7S$: C, 56.53; H, 5.80. Found: C, 56.76; H, 5.87.

3.6. Phenyl 4-*O*-acetyl-2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside (10)

The peracetate **9** (910 mg, 3.54 mmol) was deacetylated and treated with 2-methoxypropene as described for compound **6**. The crude *O*-isopropylidene derivative was submitted to flash chromatography (6:4 hexane–EtOAc) and obtained pure (774 mg, 75%) as a colorless oil. The *O*-isopropylidene derivative (674 mg, 2.27 mmol) was dissolved in pyridine (4 mL) and Ac_2O (4 mL). The reaction mixture was stirred overnight at rt and then co-concentrated with toluene. The residue was dissolved in CH_2Cl_2 and washed successively with satd aq $NaHCO_3$ and brine. The aqueous phases were re-extracted with CH_2Cl_2, and the combined organic solutions were dried and concentrated to give donor **10** (0.71 g, 92% from the intermediate alcohol) pure as a colorless glass: $[\alpha]_D$ +113° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.54, 7.31 (2 m, 5 H, Ar), 5.02 (bs, 1 H, H-1), 4.97 (bt, 1 H, $J_{3,4+4,5}$ 15 Hz, H-4), 4.46 (bd, 1 H, $J_{2,3}$ 5.5 Hz, H-2), 4.15 (bt, 1 H, $J_{2,3+3,4}$ 12.5 Hz, H-3), 3.44 (m, 1 H, H-5), 2.10 (s, 3 H, CH_3CO), 1.64, 1.40 (2 s, 6 H, (C(CH_3)$_2$), 1.29 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 169.8 (CO), 134.9 (Ar quat), 130.9, 128.9, 127.4 (Ar), 110.9 (*C*(CH_3)$_2$), 84.0 (C-1), 77.1 (C-3), 76.0 (C-2), 73.7 (C-4, C-5), 27.5, 26.3 (C(*C*H_3)$_2$), 20.9 (*C*H_3CO), 17.9 (C-6). Anal. Calcd for $C_{17}H_{22}O_5S$: C, 60.34; H, 6.55. Found: C, 60.33; H, 6.74.

3.7. Phenyl 2,6-di-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-3-*O*-[3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-α-L-fucopyranosyl]-1-thio-β-D-glucopyranoside (11)

A mixture of donor **7** (79.3 mg, 0.19 mmol) and the acceptor **4**[20] (100 mg, 0.11 mmol) in anhyd $CHCl_3$ (1.5 mL) containing 4 Å powdered activated molecular sieves (240 mg) was stirred under N_2 for 3 h at rt and cooled to −30 °C. *N*-Iodosuccinimide (57 mg, 0.25 mmol) and TfOH (8 μL, 0.09 mmol) were added to the reaction mixture that was stirred under N_2 at −30 °C for an additional 15 min. Triethylamine (26 μL) was added to quench the reaction, and the solids were filtered off and washed with CH_2Cl_2. The combined filtrates were washed successively with satd aq $NaHCO_3$, satd aq $Na_2S_2O_3$ and H_2O. The aqueous washings were re-extracted with CH_2Cl_2, and the combined organic solutions were dried and concentrated. Flash chromatography (7:3 hexane–EtOAc) of the residue gave trisaccharide **11** (29% yield estimated by ^{1}H NMR spectroscopy) contaminated with the acceptor **4** (total 74.3 mg, 48% molar ratio of **11**). The trisaccharide **11** was obtained pure (25 mg, 19%) by reversed-phase HPLC (15 mL/min, 9:1 CH_3CN–H_2O): $[\alpha]_D$ −18° (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.26, 8.15, 8.08, 7.84, 7.68, 7.55, 7.36, 7.11, 7.07, 6.79, 6.53 (11 m, 29 H, Ar), 5.42 (t, 1 H, $J_{1,2+2,3}$ 19 Hz, H-2), 5.32 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′), 5.25 (t, 1 H, $J_{1'',2''+2'',3''}$ 16 Hz, H-2″), 5.09 (m, 1 H, H-5′), 4.99 (dd, 1 H, *J* 4, *J* 12 Hz, H-6a or H-6″a), 4.74 (m, 1 H, H-3′), 4.73 (d, 1 H, $J_{1,2}$ 10 Hz, H-1), 4.58 (bd, 1 H, *J* 11 Hz, H-6a or H-6″a), 4.48 (d, 1 H, $J_{1'',2''}$ 9 Hz, H-1″), 4.43 (d, 1 H, *J* 12.5 Hz, CH_2O), 4.40–4.20 (m, 6 H, H-3, H-4 or H-4″, H-6b,

H-4′, H-3″, H6″b), 4.18 (d, 1 H, CH_2O), 3.96 (m, 1 H, H-5 or H-5″), 3.92 (t, 1 H, *J* 10 Hz, H-4 or H-4″), 3.71 (s, 3 H, CH_3O), 3.59 (m, 1 H, H-5 or H-5″), 3.26 (dd, 1 H, $J_{2',3'}$ 8.5 Hz, H-2′), 1.63 (s, 3 H, CCH_3), 1.47 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6′), 1.35, 1.34, 1.21 (3 s, 9 H, 3 × CCH_3); ^{13}C NMR ($CDCl_3$): δ 166.4, 164.75, 164.7 (CO), 133.5–127.7, 113.2 (Ar), 111.0, 107.9 (*C*$(CH_3)_2$), 100.4 (C-1″), 96.4 (C-1′), 86.8 (C-1), 76.5 (C-5 or C-5″), 76.5, 75.0, 74.3, 73.3 (C-3, C-4 or C-4″, C-4′, C-3″), 75.1 (C-3′), 74.3 (C-2′), 73.6 (C-2), 73.1 (C-2″), 71.5 (C-5 or C-5″), 70.3 (O*C*H_2), 63.2 (C-5′), 62.6 (C-6, C-6″), 55.1 (*C*H_3O), 28.0, 27.2, 26.3, 26.2 (C*C*H_3), 16.3 (C-6′). HRESIMS Calcd for $C_{66}H_{68}NaO_{19}S$ 1219.3973. Found 1219.3970.

3.8. Allyl 2,6-di-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-3-*O*-[3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-α-L-fucopyranosyl]-β-D-glucopyranoside (12)

Glycosylation of the known[33] acceptor **15** (300 mg, 0.358 mmol) with the donor **7** (253.2 mg, 0.608 mmol), as well as the workup of the reaction, were performed as described for the preparation of **11**. The pure trisaccharide **12** (117 mg, 29%) was obtained by reversed-phase HPLC (20 mL/min, 9:1 CH_3CN–H_2O): $[\alpha]_D$ −7° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.27, 8.11, 7.85, 7.52, 7.45, 7.36, 7.27, 6.79, 6.52 (9 m, 24 H, Ar), 5.62 (m, 1 H, CH_2CH=), 5.41 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9 Hz, H-2), 5.33 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1'), 5.25 (t, 1 H, $J_{2'',3''+1'',3''}$ 16 Hz, H-2″), 5.09 (m, 2 H, H-5′, CH_2=), 4.98 (m, 2 H, H-6″a, CH_2=), 4.75 (dd, 1 H, $J_{2',3'}$ 8.5, $J_{3',4'}$ 5 Hz, H-3′), 4.55 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.51 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.49 (d, 1 H, $J_{1'',2''}$ 9 Hz, H-1″), 4.44 (d, 1 H, *J* 12.5 Hz, OCH_2Ph), 4.37 (dd, 1 H, $J_{5,6b}$ 4 Hz, H-6b), 4.33 (dd, 1 H, $J_{5'',6''b}$ 3, $J_{6''a,6''b}$ 12 Hz, H-6″b), 4.30–4.14 (m, 6 H, H-3, H-4′, H-3″, H-4″, OCH_2Ph, OCH_2C=), 4.01 (t, 1 H, $J_{3,4+4,5}$ 19 Hz, H-4), 3.92 (m, 1 H, OCH_2C=), 3.89 (m, 1 H, H-5″), 3.71 (s, 3 H, CH_3O), 3.51 (m, 1 H, H-5), 3.27 (dd, 1 H, H-2′), 1.61 (s, 3 H, CCH_3), 1.48 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6′), 1.36, 1.34, 1.24 (3 s, 9 H, 3 × CCH_3); ^{13}C NMR ($CDCl_3$): δ 166.4, 165.9, 164.8, 164.4 (CO), 133.5–128.5, 117.5 (Ar, *C*H_2=, O*C*H=), 110.9, 107.9 (*C*$(CH_3)_2$), 100.2 (C-1″), 99.6 (C-1), 96.2 (C-1′), 76.5 (2 of C-3, C-4′, C-3″, C-4″), 75.1, 74.8, 74.7, 74.4 (C-2, C-4, C-2′, C-3′), 73.3, 73.2, 73.0 (C-5, C-2″, 2 of C-3, C-4′, C-3″, C-4″), 71.5 (C-5″), 70.2 (O*C*H_2Ph), 69.7 (O*C*H_2=), 63.1 (C-5′), 62.1 (C-6, C-6″), 55.1 (*C*H_3O), 28.0, 27.7, 26.3, 26.2 (C*C*H_3), 16.3 (C-6′). HRESIMS Calcd for $C_{63}H_{68}NaO_{20}$ 1167.4202. Found 1167.4200. Anal. Calcd for $C_{63}H_{68}O_{20}$: C, 66.07; H, 5.98. Found: C, 65.91; H, 5.92.

3.9. Phenyl 3-*O*-(4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-2,6-di-*O*-benzoyl-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (13)

Glycosylation of the acceptor **4**[20] (452 mg, 0.507 mmol) with the donor **10** (333 mg, 0.985 mmol), as well as the workup of the reaction, were performed as described for the preparation of **11**. Flash chromatography (7:3 hexane–EtOAc) of the residue gave the pure trisaccharide **13** (450 mg, 79%) as colorless glass: $[\alpha]_D$ +11° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.07, 7.91, 7.67, 7.62, 7.48, 7.38, 7.19, 7.07 (8 m, 25 H, Ar), 5.29 (t, 2 H, H-2, H-2″), 5.19 (s, 1 H, H-1′), 4.89 (dd, 1 H, $J_{6''a,6''b}$ 12, $J_{5'',6''a}$ 3.5 Hz, H-6″a), 4.74 (m, 3 H, H-1, H-4′, H-5′), 4.62 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 1.5 Hz, H-6a), 4.52 (m, 2 H, H-6b, H-6″b), 4.47 (d, 1 H, $J_{1'',2''}$ 8.5 Hz, H-1″), 4.30 (dd, 1 H, $J_{2'',3''}$ 8, $J_{3'',4''}$ 5 Hz, H-3″), 4.20 (dd, 1 H, $J_{4'',5''}$ 2 Hz, H-4″), 4.18 (t, 1 H, $J_{2,3+3,4}$ 18.5 Hz, H-3), 4.04 (bt, 1 H, $J_{2,3+3,4}$ 12.5, Hz, H-3′), 3.98 (m, 1 H, H-5″), 3.87 (m, 2 H, H-4, H-2′), 3.61 (m, 1 H, H-5), 2.11 (s, 3 H, CH_3CO), 1.59, 1.34 (2 s, 3 H and 6 H, 3 × CCH_3), 1.27 (d, 1 H, $J_{5',6'}$ 5.5 Hz, H-6′), 0.90 (s, 3 H, CCH_3); ^{13}C NMR ($CDCl_3$): δ 166.2, 165.9, 165.2, 164.7 (CO), 133.5–128.0 (Ar), 110.8, 109.1 (C$(CH_3)_2$), 100.5 (C-1″), 97.45 ($J_{C\text{-}1',\ H\text{-}1'}$ 172 Hz, C-1′), 85.6 (C-1), 77.5 (C-3), 77.3 (C-3″), 75.9 (C-5), 75.7, 75.3 (C-4, C-2′), 74.6 (C-3′), 73.5 (C-4′ or C-5′), 73.1 (C-4″), 72.8 (C-2, C-2″), 71.6 (C-5″), 64.0 (C-4′ or C-5′), 62.8, 62.6 (C-6, C-6″), 27.8, 27.32, 25.7 (C*C*H_3), 20.9 (*C*H_3CO), 16.8 (C-6′). HRESIMS Calcd for $C_{60}H_{62}NaO_{19}S$ 1141.3504. Found 1141.3498.

3.10. Allyl 3-*O*-(4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-2,6-di-*O*-benzoyl-4-*O*-(2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (14)

3.10.1. Method A. A mixture of trisaccharide **13** (100 mg, 0.089 mmol) and allyl alcohol (30 μL, 0.441 mmol) in anhyd $CHCl_3$ (1.5 mL) containing 4 Å powdered activated molecular sieves (160 mg) was stirred under N_2 for 3 h at rt and cooled to −20 °C. NIS (45.2 mg, 0.20 mmol) and TfOH (8 μL, 0.09 mmol) were added, and the mixture was stirred under N_2 at −20 °C for 15 min. More NIS (22.5 mg, 0.1 mmol) and TfOH (4 μL, 0.45 mmol) were added, and after an additional 15 min at −20 °C, the reaction was quenched by adding Et_3N (26 μL). The solids were filtered off and washed with CH_2Cl_2. The combined filtrates were washed successively with satd aq $NaHCO_3$, satd aq $Na_2S_2O_3$ and H_2O. The aqueous washings were re-extracted with CH_2Cl_2, and the combined organic solution was dried and concentrated. Flash chromatography (7:3 hexanes–

EtOAc), followed by RP HPLC (20 mL/min, 85:15 CH_3CN–H_2O) gave the pure trisaccharide **14** (15 mg, 16%) and some unreacted trisaccharide **13** (35 mg, 35%).

3.10.2. Method B. Glycosylation of the known[33] acceptor **15** (300 mg, 0.358 mmol) with donor **10** (205.7 mg, 0.608 mmol), as well as workup of the reaction, were performed as described for the preparation of **11**. Flash chromatography (7:3 hexane–EtOAc), followed by RP HPLC (20 mL/min, 85:15 CH_3CN–H_2O), gave the pure trisaccharide **14** (322 mg, 85%) as a colorless glass: $[\alpha]_D$ +27° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.13, 8.05, 7.92, 7.67–7.33 (4 m, 20 H, Ar), 5.67 (m, 1 H, CH_2CH=), 5.34 (t, 1 H, $J_{1,2+2,3}$ 16 Hz, H-2), 5.29 (t, 1 H, $J_{1'',2''+2'',3''}$ 17 Hz, H-2″), 5.22 (s, 1 H, H-1′), 5.09, 5.06 (2 m, 2 H, CH_2=), 4.88″ (dd, 1 H, $J_{6a,6b}$ 11.5, $J_{5,6a}$ 3 Hz, H-6a), 4.75 (m, 2 H, H-4′, H-5′), 4.60–4.45 (m, 5 H, H-1, H-6b, H-1″, H-6″a, H-6″b), 4.26 (dd, 1 H, $J_{2'',3''}$ 8, $J_{3'',4''}$ 5 Hz, H-3″), 4.23–4.12 (m, 3 H, H-3, H-4″, CH_2CH=), 4.06 (t, 1 H, $J_{2',3'+3',4'}$ 13 Hz, H-3′), 4.03–3.88 (m, 4 H, H-4, H-2′, H-5″, CH_2CH=), 3.55 (m, 1 H, H-5), 2.12 (s, 3 H, CH_3CO), 1.59, 1.35, 1.33 (3 s, 9 H, 3 × CCH_3), 1.29 (d, 1 H, $J_{5',6'}$ 5.5 Hz, H-6′), 0.90 (s, 3 H, CCH_3); ^{13}C NMR ($CDCl_3$): δ 170.1, 166.2, 165.9, 165.2, 164.7 (CO), 133.6–133.2, 129.8–128.7, 117.6, (Ar, CH_2=, OCH=), 110.8, 109.1 ($C(CH_3)_2$), 100.3 (C-1″), 99.0 (C-1), 97.2 ($J_{C\text{-}1',\ H\text{-}1'}$ 172 Hz, C-1′), 77.3 (C-3″), 76.0 (C-3 or C-4″), 75.8, 75.7 (2 of C-4, C-2′, C-5″), 75.3 (C-3′), 74.6 (C-2), 74.5 (C-4′ or C-5′), 73.5 (C-3 or C-4″), 73.1 (C-5), 72.8 (C-2″), 71.6 (C-4 or C-2′ or C-5″), 69.8 (CH_2CH=), 63.9 (C-4′ or C-5′), 62.8 (C-6), 62.5 (C-6″), 27.7, 27.3, 26.2, 25.7 (CCH_3), 20.9 (CH_3CO), 16.8 (C-6′). HRESIMS Calcd for $C_{57}H_{62}NaO_{20}$ 1089.3732. Found 1089.3724. Anal. Calcd for $C_{57}H_{62}O_{20}$: C, 64.16; H, 5.86. Found: C, 63.98; H, 6.04.

3.11. Allyl 3-*O*-α-L-fucopyranosyl-4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (16)

DDQ (24.2 mg, 0.106 mmol) was added to a suspension of the trisaccharide **12** (60.7 mg, 0.053 mmol) in a mixture of 18:1 CH_2Cl_2–H_2O (15 mL). The reaction mixture was stirred at rt for 7 h; 20% aq $Na_2S_2O_3$ (4 mL) was added, and it was then extracted three times with EtOAc. The organic phases were combined, dried, and concentrated. The residue was purified with flash chromatography (7:3 hexane–EtOAc) and the alcohol obtained (49.4 mg, 91%) was acetylated with 10% Ac_2O in pyridine (60 mL). After stirring overnight at rt, the reaction mixture was co-concentrated with toluene, and the extract was purified by flash chromatography (7:3 hexane–EtOAc). The acetylated derivative (45.5 mg, 88%) was suspended in 90% aq AcOH (20 ml), stirred at 80 °C for 2 h, co-concentrated with toluene, and the residue was purified by flash chromatography (15:1 $CHCl_3$–MeOH). The product obtained was suspended in MeOH (4 mL), and a solution of NaOMe in MeOH (1 M, 1 mL) was added. The mixture was stirred overnight at rt and deionized with Dowex 50 (H^+) resin. The resin was filtered off, rinsed with MeOH, and the combined filtrate and washings were concentrated. Flash chromatography (6.5:3:0.5 EtOAc–MeOH–H_2O) of the residue gave trisaccharide **16** (20.4 mg, 73% calcd from **12**), which was isolated as an amorphous powder after freeze-drying: $[\alpha]_D$ −73° (*c* 0.8, MeOH); ^{1}H NMR (303 K, D_2O): δ 5.94 (m, 1 H, CH_2CH=), 5.40 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′), 5.35, 5.24 (m, 2 H, CH_2=), 4.75 (m, 1 H, H-5′), 4.49 (d, 1 H, *J* 8 Hz, H-1 or H-1″), 4.40 (d, 1 H, *J* 8 Hz, H-1 or H-1″), 4.36, 4.20 (2 m, 2 H, CH_2CH=), 4.00–3.47 (m, 13 H, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-3″, H-4″, H-5″, H-6″a, H-6″b), 3.45 (m, 2 H, H-2, H-2″), 1.12 (d, 1 H, $J_{5',6'}$ 6.5 Hz, H-6′); ^{13}C NMR (D_2O): δ 133.1 (CH_2CH=), 118.6 (CH_2=), 101.6, 101.0 (C-1, C-1″), 98.3 (C-1′), 76.9, 75.2, 74.8, 74.4, 72.6, 72.3, 71.8, 71.0, 69.1, 68.2, 67.9, 66.4 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 70.6 (CH_2CH=), 61.4, 59.6 (C-6, C-6″), 15.1 (C-6′). HRESIMS Calcd for $C_{21}H_{36}O_{15}Na$: 551.1946. Found: 551.1949.

3.12. Allyl 4-*O*-β-D-galactopyranosyl-3-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (17)

Treatment of the trisaccharide **14** (60.0 mg, 0.056 mmol) with MeONa in MeOH, as well as workup of the reaction, was carried out as described above for the deprotection of **12**. The intermediate was purified by flash chromatography (8:2 $CHCl_3$–MeOH) and dissolved in 90% aq AcOH (6 mL). The solution was stirred at 80 °C for 1 h and co-concentrated with toluene. Flash chromatography (6.5:3:0.5 EtOAc–MeOH–H_2O) of the residue gave the pure trisaccharide **17** (26.0 mg, 87%), which was isolated as an amorphous powder upon freeze-drying: $[\alpha]_D$ −38° (*c* 0.6, MeOH); ^{1}H NMR (D_2O): δ 5.95 (m, 1 H, CH_2CH=), 5.36 (m, 1 H, CH_2=), 5.32 (bs, 1 H, H-1′), 5.27 (m, 1 H, CH_2=), 4.51 (d, 1 H, *J* 8.5 Hz, H-1 or H-1″), 4.46 (m, 1 H, H-5′), 4.43 (d, 1 H, *J* 7.5 Hz, H-1 or H-1″), 4.37, 4.21 (2 m, 2 H, CH_2CH=), 4.02–3.28 (3 m, 15 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-2″, H-3″, H-4″, H-5″, H-6″a, H-6″b), 1.26 (d, 1 H, $J_{5',6'}$ 6.5 Hz, H-6′); ^{13}C NMR (D_2O) δ 133.1 (CH_2CH=), 118.6 (CH_2=), 102.1, 100.9 (C-1, C-1″), 99.6 (C-1′), 76.2, 75.1, 75.1, 74.3, 72.8, 72.4, 72.2, 70.6, 69.9, 69.8, 68.7, 68.1 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 71.2 (CH_2CH=), 61.0, 59.4 (C-6, C-6″), 16.2 (C-6′). HRESIMS Calcd for $C_{21}H_{36}NaO_{15}$: 551.1946. Found: 551.1949.

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