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Synthesis of galactofuranosyl-containing oligosaccharides corresponding to the glycosylinositolphospholipid of *Trypanosoma cruzi*

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Abstract

The oligosaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→2)-[β-D-Gal*f*-(1→3)]-α-D-Man*p*-(1→2)-α-D-Man*p* corresponds to the terminal end of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease. Syntheses of methyl or ethylthio glycosides of the terminal disaccharide, trisaccharide, tetrasaccharide, and pentasaccharide corresponding to this structure are described. These syntheses employ the selective activation of a phenyl 1-selenogalactofuranoside or a phenyl 1-selenomannopyranoside donor over ethyl 1-thioglycoside acceptors with NIS–TfOH.

Keywords: Glycosylation; Glycosides; Galactofuranose; *Trypanosoma cruzi*

1. Introduction

Galactofuranose (Gal*f*) is present as a constituent of external cellular structures in protozoa [1], bacteria [2,3], and fungi [4]. These structures do not appear to be present on mammalian cells and elicit a strong antigenic response during infection [5]. Many glycoconjugates are anchored to the plasma membrane via glycosylphosphatidylinositol (GPI) anchors. The GPI anchors of plasma membrane proteins have been detected in organisms ranging from yeast to man, but occur with a much higher frequency in lower eukaryotes such as protozoa [6,7]. All of the GPI anchors that have been characterized to date contain an identical ethanolamine phosphate–α-Man*p*-(1→2)-α-Man*p*-(1→6)-α-Man*p*-(1→4)-α-Glc*Np*-(1→6)-*myo*inositol backbone, suggesting that this sequence is likely to be conserved in all GPI anchors. In protozoa, GPI anchors have been widely studied in their role as anchors for cell-surface proteins. Several protozoa also synthesize unique GPI derivatives that are not covalently linked to protein or modified by additional glycoconjugates. These low-molecular-weight structures, referred to as glycosylinositolphospholipids (GIPLs), are included as members of the GPI family by virtue of the core sequence α-Man*p*-(1→4)-α-Glc*Np*-(1→6)-*myo*inositol [6]. It is known that Gal*f* is part of the oligosaccharide core of the glycosylinositolphospholipid from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas' disease [8]. The GIPL structure of *T. cruzi* contains the same tetrasaccharide core sequence as the protein-bound GPI anchors, but diverges from the

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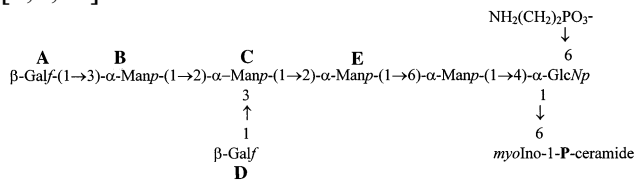
protein anchors beyond this sequence. The GIPL contains up to two additional β-Gal*f* residues and there is a 2-aminoethylphosphonic acid group located at the C-6 position of the glucosamine residue. The lipid moiety is a ceramide containing sphinganine and N-linked lignoceric ($C_{24:0}$) acid instead of the alkylglycerol found in the protein anchors [6,9,10]:



This structure is the most abundant cell-surface glycoconjugate present in the insect-dwelling epimastigote stage of the *T. cruzi* life cycle [1]. The glycoconjugates on the cell surface during the infectious stage of *T. cruzi* are not modified with galactofuranose; however, it has been shown that the β-D-Gal*f* moiety is recognized by antibodies that inhibit *T. cruzi* internalization into mammalian cells [5]. Recently, it was demonstrated that *T. cruzi* GIPLs are able to block T-lymphocyte activation [11]. Thus, interaction between host cellular defense mechanisms and the GIPLs of *T. cruzi* may play a role in establishment and maintenance of chronic infection [12].

Galactofuranose has also been isolated from another class of GIPLs found in *Leishmania* [13]. This GIPL differs from *T. cruzi* by having a glycerolipid instead of a ceramide and the β-D-Gal*f*-(1→3)-α-Man*p* moiety as an internal unit in the oligosaccharide core. Glycoconjugates containing galactofuranose have also been found in the GIPLs of *Leptomonas samueli* [14] and *Endotrypanum schaudinni* [15], both of which contain a ceramide lipid. The Gal*f* moiety is the terminal non-reducing sugar in *L. samueli*, and it is internal in *E. schaudinni*. In all of the above examples, the β-D-galactofuranose is linked (1→3) to α-Man*p*. This specificity suggests that a β-(1→3)-galactofuranosyltransferase might be involved in the biosynthesis, although a sugar donor has not been identified [10].

The specific capsular polysaccharide produced by *Streptococcus pneumoniae* type 20 contains both an internal Gal*f* and a branched Gal*f* moiety [16]. The acidic glycolipid, purified from *Paracoccidioides brasiliensis*, containing a terminal Gal*f*, has been shown to be reactive with sera of patients infected with paracoccidioidomycosis [17]. The synthesis of oligosaccharides containing Gal*f* may therefore be useful for understanding the role Gal*f* plays in microorganisms and for studying the biosynthesis of furanosyl-containing glycoconjugates. Compounds containing Gal*f* may also be used as inhibitors to probe the development of infections, to develop diagnostic methods, or as vaccines.

Oligosaccharide synthesis involving furanosyl glycosyl donors has not been studied to the same extent as with pyranosyl donors, but methods have been developed that employ thioglycosides [18], *n*-pentenyl glycosides [19,20], anomeric benzoates [21], anomeric xanthates [22], trichloroacetimidates [23,24], and selenoglycosides [25,26]. Anomeric benzoates [21] and *n*-pentenyl glycosides [19] give stereoselective syntheses of the β anomers, while activation of anomeric xanthates leads to α:β mixtures. In addition, an indirect approach to galactofuranosyl-containing disaccharides involving acyclic glycosyl donors has recently been reported [27]. The syntheses of α-D-galactofuranose-containing oligosaccharides have not been as widely investigated as those of the β anomers, but have been achieved in high yields, using ethyl 2,3,5,6-tetra-*O*-benzyl-α-D-thiogalactofuranoside and *N*-bromosuccinimide [18].

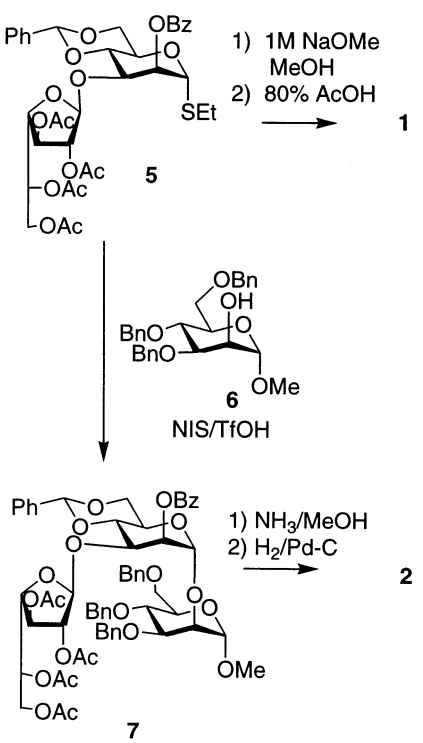
A previous report from our laboratory described the viability of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside (**16**) as a glycosyl donor [26]. As an extension of this and other studies of the selective activation of selenoglycoside donors in the presence of thioglycoside acceptors [28,29], we now report the syntheses of the terminal disaccharide **1**, trisaccharide **2**, tetrasaccharide **3**, and pentasaccharide **4** corresponding to the terminus of the glycosylinositolphospholipid oligosaccharide of *T. cruzi*, which employ **16** as a glycosyl donor.

2. Results and discussion

The required disaccharide, ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-α-D-thiomannopyranoside (**5**) [26] and the monosaccharides methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**6**) [30] and phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside (**16**) [26] were synthesized following literature methods. The disaccharide **5** was deprotected by methanolysis of the esters, followed by hydrolysis of the benzylidene acetal to give compound **1** in 70% yield.

The reaction of the glycosyl donor, disaccharide **5**, with an excess of the acceptor **6**, mediated by NIS–TfOH, resulted in the protected trisaccharide **7** as a colorless foam in 68% yield (see Scheme 1). The trisaccharide was deprotected by aminolysis of the esters, followed by hydrolysis of the benzylidene acetal and hydrogenolysis of the benzyl ethers, to give compound **2** in 85% yield.

The stereochemical integrity of the oligosaccharides **1** and **2** was confirmed by examination of the J_{C1H1} values of the mannopyranosyl residues and NOE contacts for the galactofuranosyl residues. The J_{C1H1} values

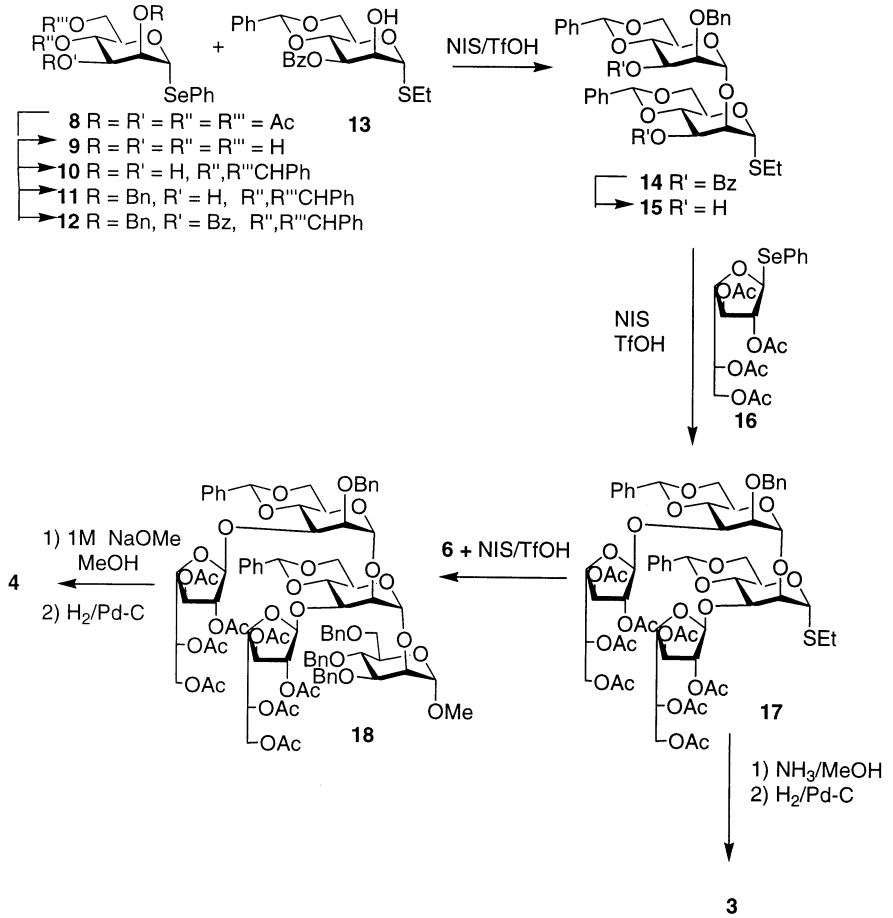


Scheme 1.

cannot be used as a reliable indicator of the α or β configuration for the galactofuranosyl residues [31]. For the trisaccharide **2**, the 2D NOESY spectrum showed an NOE between H-1A and H-3A (of the Gal*f* ring A) and no NOE between H-1A and H-4A, indicating the presence of a β linkage between the Gal*f* (A) and Man*p* (B) rings. The J_{C1H1} values are 171 Hz for the Man*p* (B) ring and 172 Hz for the Man*p* (C) ring, indicating the presence of α configurations about C-1 for both mannopyranosyl residues [32].

Synthesis of the monosaccharide acceptor phenyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-selenomannopyranoside (**12**) was carried out from phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-selenomannopyranoside (**8**). Compound **8** was deacetylated using NaOMe–MeOH, and the crude product **9** was selectively protected as a 4,6-*O*-benzylidene acetal, in a manner analogous to that reported by Franzyk et al. [33], to give **10** (see Scheme 2). Phase-transfer-catalyzed benzylation gave the 2-*O*-benzyl compound **11** that was subsequently benzoylated to give **12** [34]. Ethyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-thiomannopyranoside (**13**) was synthesized in an analogous manner to that reported by Seymour [35] for the methyl glycoside.

Glycosylation of the acceptor **13** with the donor **12** was again performed using NIS–TfOH, with selective activation of the phenyl selenomannoside over the ethyl thiomannoside; the protected disaccharide **14** was obtained as a white foam in 60% yield (see Scheme 2). The benzoate esters at the 3-positions of both mannose residues were removed using NaOMe–MeOH to give the disaccharide **15**, which was subsequently used as an acceptor. The tetrasaccharide **17** was synthesized in one pot using the disaccharide **15** and 2.4 equivalents of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside (**16**). Again, selective activation of the phenyl selenogalactofuranoside over the ethyl thiomannopyranoside yielded the desired tetrasaccharide **17** as white crystals in 85% yield. Compound **17** was then deprotected by aminolysis of the esters followed by hydrolysis of the benzylidene acetals and hydrogenolysis of the benzyl ether to give compound **3** in 71% yield.



Scheme 2.

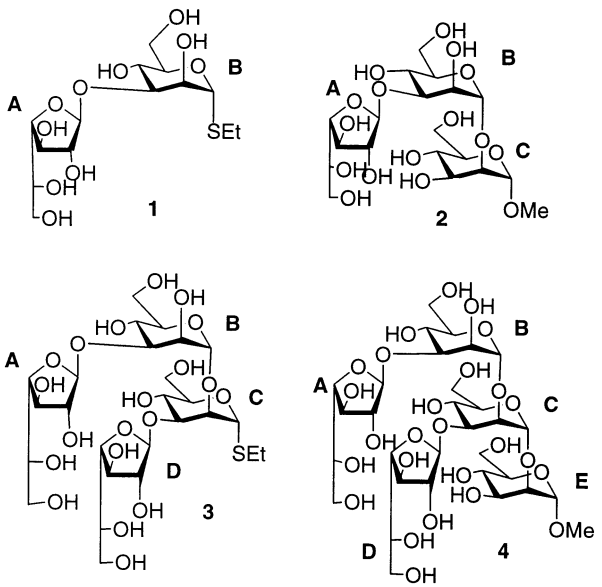
Assignment of the NMR signals of the Man*p* (C) ring of the tetrasaccharide **3** was based on the fact that C-1 for this residue has a characteristic upfield chemical shift due to the ethyl thioglycoside. A C–H correlation spectrum, together with a COSY and a TOCSY spectrum, then permitted complete assignment of the ^{1}H and ^{13}C NMR signals. The assignment of signals for the Man*p* (B) ring was based on the presence of a NOE contact across the glycosidic linkage between H-1B and H-2C, and COSY and TOCSY transfer between ^{1}H NMR signals of the B ring. Assignment of signals of the Gal*f* rings A and D was based on NOE contacts across the glycosidic linkages between H-1A and H-3B, and H-1D and H-3C, respectively. The 2D NOESY spectrum also showed an NOE between H-1A and H-3A (of the Gal*f* ring A), and another NOE between H-1D and H-3D (of the Gal*f* ring D), indicating the presence of a β linkage between the Gal*f* (A) and Man*p* (B) rings, and also between the Gal*f* (D) and Man*p* (C) rings. The J_{C1H1} values are 170 Hz for the Man*p* (B) ring and 167 Hz for the Man*p* (C) ring, indicating the presence of α configurations about C-1 for both mannopyranosyl residues [32].

The tetrasaccharide **17** contains the ethyl thioglycoside at C-1 of the Man*p* (C) ring, and without any manipulation, could be used as a donor to make the pentasaccharide. Thus, glycosylation of the acceptor **6** with the donor tetrasaccharide **17** gave the desired compound **18** as a clear syrup. Both the donor **17** and the product of the reaction **18** had similar R_f values: a ^{1}H NMR spectrum showed the presence of **18**:**17** in a ratio of 3:1, with an estimated 35% yield of the desired pentasaccharide **18**. Extensive chromatography gave an analytically pure sample of **18**. In subsequent experiments, the mixture of **17** and **18** was subjected to methanolysis, hydrolysis, and hydrogenolysis; the deprotected pentasaccharide **4** could be isolated as a pure compound (see below).

In a recent paper from Zhang et al., the synthesis of oligosaccharides in one pot by sequential addition of acceptors and promoters was reported [36]. Using this approach, a second attempt at the synthesis of **18** was carried out in one pot starting with disaccharide **15**. The tetrasaccharide **17** was first synthesized by reacting the disaccharide **15** with 2.1 equivalents of the galactofuranosyl donor **16** at 0 °C; then, without any workup, the acceptor **6** was added together with an additional 1.2 equivalents of NIS and activated 4 Å molecular sieves. Again, a mixture of the tetrasaccharide **17** and the product **18** was obtained, but **18** was formed in a slightly better yield (28% for two steps). The pentasaccharide **18** was deprotected by methanolysis of the esters followed by hydrolysis of the benzylidene acetals and hydrogenolysis of the benzyl ethers, to give the target pentasaccharide **4** in 60% yield.

The assignment of the NMR signals of the Man*p* (E) ring of the pentasaccharide **4** was based on the ^{1}H NMR spectrum in which this H-1 signal was the most upfield. The 2D NOESY spectrum also showed a contact between H-1E and the methyl aglycon. The assignment of the remaining signals of the Man*p* (E) ring then followed from a COSY experiment. The signals of the Man*p* rings B and C were assigned based on NOE contacts across the glycosidic linkages between H-1C and H-2E, and H-1B and H-2C, respectively. Assignment of signals of the Gal*f* rings A and D was based on NOE contacts across the glycosidic linkages between H-1A and H-3B, and H-1D and H-3C, respectively. The 2D NOESY spectrum also showed an NOE between H-1A and H-3A (of the Gal*f* ring A), and another NOE between H-1D and H-3D (of the Gal*f* ring D), indicating the presence of β linkages between the Gal*f* (A) and Man*p* (B) rings, and between the Gal*f* (D) and Man*p* (C) rings. The J_{C1H1} values were 171 Hz for the Man*p* (B) ring, 172 Hz for the Man*p* (C) ring, and 173 Hz for the Man*p* (E) ring, indicating the presence of α configurations about C-1 for all three mannopyranosyl residues [32].

In summary, di- up to pentasaccharides **1**–**4** corresponding to the terminal end of the glycosylinositolphospholipid oligosaccharide of the protozoan, *T. cruzi*, the causative agent of Chagas' disease, have been synthesized by selective activation of selenoglycoside donors in the presence of thioglycoside acceptors. The selenoglycoside **16** is a versatile furanosyl donor that gives oligosaccharides with β selectivity. The four target compounds **1**–**4** will be tested as inhibitors against *T. cruzi* proliferation and also in the inhibition of proliferation of B lymphocytes.



3. Experimental

General methods.—Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were measured at 21 °C with a Rudolph Research Autopol II automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer at 400.13 and 100.6 MHz, for proton and carbon, respectively. Chemical shifts are given in ppm downfield from TMS for those measured in $CDCl_3$ or CD_2Cl_2 and from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in D_2O. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra. All assignments were confirmed with the aid of two-dimensional ^{1}H–^{1}H (COSYDFTP), ^{1}H–^{13}C (INVBTP), ^{1}H (NOESYTP), and ^{1}H (MLEVTP) experiments using standard

Bruker pulse programs and an inverse detection, ^1H–X double-resonance probe. Sugar rings are denoted **A**, **B**, **C**, **D**, and **E**, respectively, as shown in the diagrams for compounds **1**–**4**; intermediates are labeled correspondingly. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with E. Merck Silica Gel 60 F254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with a solution containing 1% $Ce(SO_4)_2$ and 1.5% molybdic acid in 10% aq H_2SO_4 and heated. Compounds were purified by flash column chromatography on Kieselgel 60 (230–400 mesh). Solvents were distilled before use and were dried as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40 °C.

General procedure for glycosylation reactions.—A mixture of the glycosyl donor, the acceptor, and activated 4 Å molecular sieves was stirred in dry CH_2Cl_2 at room temperature (rt) under an N_2 atmosphere. The reaction mixture was cooled in an ice bath and NIS (1.2–1.3 equiv relative to the donor) was added, followed by addition of TfOH (0.05 equiv). The reaction mixture was stirred at 0 °C, under an N_2 atmosphere, until TLC showed that the reaction was complete. The mixture was quenched by addition of Et_3N, diluted with CH_2Cl_2 and filtered through a pad of Celite. The mixture was washed with 10% $Na_2S_2O_3$, followed by satd aq $NaHCO_3$. The organic layer was dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by column chromatography.

General procedure for deprotection.—The protected oligosaccharide was dissolved in freshly distilled MeOH, and NH_3(g) was bubbled through the solution, while stirring under an N_2 atmosphere, until TLC indicated that no further change was occurring. The reaction mixture was concentrated by rotary evaporation, then placed under high vacuum ($\sim$0.05 torr), at 50 °C overnight to remove NH_4OAc. The residue was purified by column chromatography to give the desired partially deprotected oligosaccharide. The ^1H NMR spectrum confirmed that the acyl groups had been removed. The partially deprotected oligosaccharide was dissolved in 4:1 HOAc–H_2O (10 mL) and stirred with Pd–C (100 mg) under H_2 (52 psi). After 20 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with water. The combined filtrates were evaporated to dryness, and the residue was co-evaporated several times with distilled water to remove any traces of HOAc. The target oligosaccharide was purified by column chromatography.

Ethyl β-D-galactofuranosyl-(1→3)-1-thio-α-D-mannopyranoside (**1**).—To a solution of the disaccharide **5** (45 mg, 0.06 mmol) in freshly distilled MeOH (5 mL) was added 1 M NaOMe–MeOH (0.5 mL). The reaction mixture was stirred at rt, overnight, under an N_2 atmosphere. The reaction mixture was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The crude product was then dissolved in 80% AcOH (5 mL), and the solution was stirred at rt overnight. The solvent was removed, and the crude product was purified by column chromatography using 7:2:1 EtOAc–MeOH–H_2O as the eluant to yield the desired disaccharide **1** as a clear glass (13 mg, 70%): $[\alpha]_D$ +35° (*c* 0.028, H_2O); ^1H NMR (D_2O): δ 5.31 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1B), 5.09 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1A), 4.21 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2B), 4.11 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2A), 4.04 (dd, 1 H, $J_{3,4}$ 6.6 Hz, H-3A), 4.01 (m, 1 H, H-5A), 4.00 (dd, 1 H, $J_{4,5}$ 3.8 Hz, H-4A), 3.85 (dd, 1 H, $J_{5,6}$ 2.3, $J_{6,6'}$ 12.4 Hz, H-6A), 3.81 (m, 2 H, H-3B, H-5B), 3.74 (dd, 1 H, $J_{5,6}$ 6.0 Hz, H-6A′), 3.70 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.7 Hz, H-4B), 3.67 (dd, 1 H, $J_{5,6}$ 4.5, $J_{6,6'}$ 11.7 Hz, H-6B), 3.61 (dd, 1 H, $J_{5,6}$ 7.4 Hz, H-6B′), 2.64 (m, 2 H, SCH_2CH_3), 1.24 (t, 3 H, *J* 7.4 Hz, SCH_2CH_3), ^{13}C NMR (D_2O): δ 106.90 (C-1A), 86.60 (C-1B), 85.51 (C-4A), 83.97 (C-2A), 79.62 (C-3A), 78.31 (C-3B), 75.65 (C-5A), 73.28 (C-5B), 71.06 (C-2B), 67.99 (C-4B), 65.41 (C-6B), 63.47 (C-6A), 27.45 (SCH_2CH_3), 16.67 (SCH_2CH_3). Anal. Calcd for $C_{14}H_{26}O_{10}S$: C, 43.52; H, 6.78. Found: C, 43.85; H, 6.78.

Methyl 2,3,5,6-tetra-O-acetyl-β-D-galactofuranosyl-(1→3)-2-O-benzoyl-4,6-O-benzylidene-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**7**).—The methyl mannopyranoside acceptor **6** (308 mg,

0.66 mmol) was glycosylated with the thioglycoside donor **5** (379 mg, 0.51 mmol) following the general procedure. An immediate reaction to produce a dark purple–brown color ensued; reaction time was 3.5 h at rt. The crude product was purified by column chromatography using 3:1 toluene–EtOAc as the eluant. The desired trisaccharide **7** was obtained as a colorless foam (393 mg, 68%): $[\alpha]_D$ −36° (*c* 0.25, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 8.12–7.09 (m, 25 H, aromatic), 5.70 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.6 Hz, H-2B), 5.64 (s, 1 H, PhC*H*), 5.29 (bs, 1 H, H-1A), 5.25 (ddd, 1 H, $J_{4,5}$ 3.4, $J_{5,6}$ 7.5, $J_{5,6'}$ 4.1 Hz, H-5A), 5.24 (d, 1 H, H-1B), 4.91 (d, 1 H, $J_{2,3}$ 1.5 Hz, H-2A), 4.85, 4.57 (2 d, 2 H, J_{gem} 11.1 Hz, OCH_2Ph), 4.82 (dd, 1 H, $J_{3,4}$ 5.6 Hz, H-3A), 4.80 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1C), 4.70, 4.66 (2 d, 2 H, J_{gem} 11.8 Hz, OCH_2Ph), 4.64, 4.58 (2 d, 2 H, J_{gem} 12.2 Hz, OCH_2Ph), 4.41 (m, 1 H, H-3B), 4.32 (dd, 1 H, $J_{5,6}$ 4.1, $J_{6,6'}$ 10.2 Hz, H-6B), 4.24 (dd, 1 H, H-4A), 4.11–4.01 (m, 4 H, H-2C, H-4B, H-5B, H-6A), 3.96 (dd, 1 H, $J_{6,6'}$ 11.8 Hz, H-6A′), 3.92–3.83 (m, 3 H, H-3C, H-6B′, H-4C), 3.77–3.72 (m, 3 H, H-5C, H-6C, H-6C′), 3.37 (s, 3 H, –OC*H*$_3$), 2.11, 2.08, 1.89, 1.82 (4 s, 3 H each, –C(O)C*H*$_3$). ^{13}C NMR (CD_2Cl_2): δ 170.55, 170.28, 170.20, 169.45 (*C*OCH$_3$), 165.62 (*C*OPh), 139.13–126.46 (30 C, aromatic), 103.14 (C-1A), 102.21 (CHPh), 100.64 (C-1B), 100.24 (C-1C), 81.18 (C-2A), 81.05 (C-4A), 80.41 (C-3C), 77.69 (C-4B), 77.25 (C-3A), 75.37 (2 C, C-4C *C*H$_2$Ph), 75.05 (C-2C), 73.60 (*C*H$_2$Ph), 72.76 (*C*H$_2$Ph), 72.25 (C-5C), 70.47 (C-3B), 69.98 (C-6C), 69.64 (2 C, C-5A and C-2B), 69.17 (C-6B), 64.76 (C-5B), 62.96 (C-6A), 55.01 (–O*C*H$_3$), 20.97, 20.94, 20.78, 20.54 (CO*C*H$_3$). Anal. Calcd for $C_{62}H_{68}O_{21}$: C, 64.80; H, 5.96. Found: C, 64.95; H, 5.91.

*Methyl β-*D*-galactofuranosyl-(1→3)-α-*D*-mannopyranosyl-(1→2)-α-*D*-mannopyranoside* (**2**).—The trisaccharide **7** (129 mg, 0.11 mmol) was deprotected following the general procedure. After deacylation, the partially deprotected trisaccharide was purified by column chromatography using 5:1 CH_2Cl_2–MeOH as the eluant. This product was further deprotected to yield the target trisaccharide **2** as a colorless foam, (43 mg, 96%): $[\alpha]_D$ −54.5° (*c* 0.16, H_2O); ^{1}H NMR (D_2O): δ 5.13 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1A), 5.03 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B), 4.96 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1C), 4.24 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2B), 4.20 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2A), 4.04 (dd, 1 H, $J_{3,4}$ 6.5 Hz, H-3A), 4.01 (dd, 1 H, $J_{4,5}$ 3.8 Hz, H-4A), 3.93 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2C), 3.89 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3B), 3.83 (dd, 1 H, $J_{3,4}$ 9.7 Hz, H-3C), 3.83–3.36 (m, 11 H, H-4C, H-4B, H-5C, H-5B, H-5A, H-6C, H-6C′, H-6B, H-6B′, H-6A, H-6A′), 3.37 (s, 3 H, –OC*H*$_3$). ^{13}C NMR (D_2O): δ 107.02 (C-1A), 104.73 (C-1B), 101.93 (C-1C), 85.50 (C-4A), 83.98 (C-2A), 81.27 (C-2C), 79.63 (C-3A), 77.87 (C-3B), 75.82, 75.20, 75.35, 72.81, 69.60 (C-4B, C-4C, C-5A, C-5B, C-5C), 69.31 (C-2B), 67.80, 65.48, 63.58 (C-6A, C-6B, C-6C), 63.76 (C-3C), 57.46 (–O*C*H$_3$). Anal. Calcd for $C_{19}H_{34}O_{16}$: C, 44.02; H, 6.61. Found: C, 43.68; H, 6.30.

*Phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-α-*D*-mannopyranoside* (**8**).—To a solution of 50% H_3PO_2 (90 mL) was added diphenyl diselenide (9.1 g, 29 mmol), and the mixture was rapidly stirred at reflux, under an N_2 atmosphere until the yellow color disappeared. The reaction mixture was cooled to 0 °C and extracted with CH_2Cl_2 and washed with cold water. The combined extracts were washed with half-saturated NaCl and dried over $MgSO_4$. The solution was filtered into a flask containing peracetylated mannose (15 g, 39 mmol) and $BF_3{\cdot}Et_2O$ (9.9 mL, 78 mmol) was added. The reaction was stirred overnight under an N_2 atmosphere. The reaction mixture was cooled in an ice bath and quenched with Et_3N (4 mL) and satd aq $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2. The extracts were washed with satd aq $NaHCO_3$, followed by cold water and dried over Na_2SO_4. The crude product was purified by column chromatography using 2:1 hexanes–EtOAc as the eluant. The white solid was recrystallized from hexanes–EtOAc to yield white crystals of compound **8** (9.2 g, 49%): mp 89–92 °C; $[\alpha]_D$ +138° (*c* 0.33, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.60–7.20 (m, 5 H, aromatic), 5.75 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 5.56 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 5.34 (dd, 1 H, H-4), 5.28 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 4.46 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6}$ 5.8, $J_{5,6'}$ 2.3 Hz, H-5), 4.31 (dd, 1 H, $J_{6,6'}$ 12.3 Hz, H-6), 4.09 (dd, 1 H, H-6′), 2.13, 2.07, 2.06, 2.01 (4 s, 3 H each,

–C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.40, 169.76, 169.71, 169.61 (*C*OCH$_3$), 134.37–128.30 (6 C, aromatic), 82.51 (C-1), 71.57 (C-2), 71.31 (C-5), 69.82 (C-3), 66.41 (C-4), 62.41 (C-6), 20.76, 20.60, 20.52 (4 C, CO*C*H$_3$). Anal. Calcd for $C_{20}H_{24}O_9Se$: C, 49.29; H, 4.96. Found: C, 49.38; H, 5.03.

*Phenyl 4,6-*O*-benzylidene-1-seleno-α-*D*-mannopyranoside* (**10**).—To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-α-D-mannopyranoside (**8**, 2.0 g, 4.1 mmol) in freshly distilled MeOH (60 mL) was added 1 M NaOMe–MeOH (2 mL). The reaction mixture was stirred overnight, under an N_2 atmosphere. The solution was neutralized with Rexyn 101 (H^+). The resin was filtered, the filtrate was concentrated in vacuo and placed under high vacuum overnight. To a solution of the crude product **9** in DMF (6 mL) was added *p*-toluenesulfonic acid (7.6 mg, 0.04 mmol) and benzaldehyde dimethyl acetal (0.72 mL, 4.8 mmol). The reaction mixture was heated at 45–50 °C for 1.5 h. An excess of K_2CO_3 was added, and the solvent was removed by rotary evaporation under a high vacuum. The white solid was dissolved in EtOAc, washed with H_2O, dried over Na_2SO_4, and the solvent was removed in vacuo. The white solid was recrystallized from hexanes–EtOAc to yield white powdery crystals of compound **10** (1.20 g, 72%). This compound was very insoluble in both EtOAc and CH_2Cl_2, and in subsequent repetitions of this reaction it was not purified; mp softens at ~70 °C, melts 214–216 °C; $[\alpha]_D$ +237° (*c* 0.54, DMSO); ^{1}H NMR (DMSO-d_6): δ 7.60–7.30 (m, 10 H, aromatic), 5.71 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.62 (s, 1 H, C*H*Ph), 5.54 (d, 1 H, $J_{2,OH}$ 4.0 Hz, OH-2), 5.20 (d, 1 H, $J_{3,OH}$ 6.2 Hz, OH-3), 4.07 (m, 2 H, H-2, H-6), 3.95 (m, 2 H, H-4, H-5), 3.79 (m, 1 H, H-6′), 3.73 (m, 1 H, H-3). ^{13}C NMR (DMSO-d_6): δ 137.75–126.34 (12 C, aromatic), 101.13 (*C*HPh), 87.79 (C-1), 78.32 (C-4), 73.02 (C-2), 68.32 (C-3), 67.36 (C-6), 66.93 (C-5). Anal. Calcd for $C_{19}H_{20}O_5Se$: C, 56.03; H, 4.95. Found: C, 56.18; H, 4.91.

*Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-seleno-α-*D*-mannopyranoside* (**11**).—To a solution of **10** (1.63 g, 4.0 mmol) in CH_2Cl_2 (75 mL) was added $(Bu)_4NHSO_4$ (272 mg, 0.8 mmol), benzyl bromide (0.83 mL, 7.0 mmol) and 5% aq NaOH (8 mL). The reaction mixture was stirred at reflux, under an N_2 atmosphere, for 40 h. The reaction mixture was cooled to rt and diluted with CH_2Cl_2, washed with water and dried over Na_2SO_4. The solvent was removed in vacuo and the white solid was purified by column chromatography using 4:1 hexanes–EtOAc as the eluant. The product **11** was recrystallized from hexanes–EtOAc to give white needle-like crystals, (1.35 g, 68%): mp 149–150 °C; $[\alpha]_D$ +142° (*c* 0.76, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.56–7.25 (m, 15 H, aromatic), 5.85 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.59 (s, 1 H, C*H*Ph), 4.73, 4.59 (2 d, 2 H, J_{gem} 11.6 Hz, OCH$_2$Ph) 4.25–4.15 (m, 2 H, H-5, H-6), 4.17 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 4.11, (m, 1 H, H-3), 3.99 (bt, 1 H, $J_{3,4}$, $J_{4,5}$ 9.3 Hz, H-4), 3.84 (m, 1 H, H-6′), 2.42 (bd, 1 H, O*H*). ^{13}C NMR ($CDCl_3$): δ 137.33–126.34 (18 C, aromatic), 102.19 (*C*HPh), 83.96 (C-1), 80.72 (C-2), 79.56 (C-4), 73.04 (*C*H$_2$Ph), 69.34 (C-3), 68.34 (C-6), 66.59 (C-5). Anal. Calcd for $C_{26}H_{26}O_5Se$: C, 62.78; H, 5.27. Found: C, 63.01; H, 5.26.

*Phenyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-1-seleno-α-*D*-mannopyranoside* (**12**).—To a solution of **11** (620 mg, 1.25 mmol) in pyridine (10 mL) at 0 °C; was added benzoyl chloride (0.26 mL, 2.3 mmol) and DMAP (catalytic). The reaction mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with MeOH, and the pyridine was removed by rotary evaporation under a high vacuum. The crude product was purified by column chromatography using 4:1 hexanes–EtOAc as the eluant. The desired product **12** was obtained as a white foam, (650 mg, 86%): $[\alpha]_D$ +67° (*c* 0.60, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 8.20–7.10 (m, 20 H, aromatic), 5.83 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.64 (s, 1 H, C*H*Ph), 5.54 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.3 Hz, H-3), 4.63, 4.50 (2 d, 2 H, J_{gem} 12.0 Hz, OCH$_2$Ph), 4.43 (dd, 1 H, H-4), 4.41, (dd, 1 H, H-2), 4.36 (m, 1 H, $J_{5,6}$ 4.9, $J_{4,5}$, $J_{5,6'}$ 10.0 Hz, H-5), 4.28 (dd, 1 H, $J_{6,6'}$ 10.2 Hz, H-6), 3.93 (dd, 1 H, H-6′). ^{13}C NMR ($CDCl_3$): δ 165.82 (*C*OPh), 137.28–126.20 (24 C, aromatic), 101.81 (*C*HPh), 83.91 (C-1), 78.44 (C-2), 76.34 (C-4), 73.04 (*C*H$_2$Ph), 71.44 (C-3), 68.42 (C-6), 67.22 (C-5). Anal. Calcd for $C_{33}H_{30}O_6Se$: C, 65.89; H, 5.03. Found: C, 65.75; H, 5.01.

Ethyl 3-O-benzoyl-4,6-O-benzylidene-1-thio-α-D-mannopyranoside (**13**).—To a solution of ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside [34] (770 mg, 2.47 mmol) in pyridine (15 mL) at −25 °C; was added benzoyl chloride (0.34 mL, 2.9 mmol). The reaction mixture was stirred at −25 °C for 4 h. The pyridine was removed by rotary evaporation under a high vacuum. The residue was dissolved in $CHCl_3$, washed with 0.1 M HCl, followed by satd aq $NaHCO_3$ and then water. The solution was dried over Na_2SO_4, and the solvent was removed in vacuo to give a white solid. The crude product was purified by column chromatography using 3:1 hexanes–EtOAc as the eluant. The desired product **13** was obtained as a white foam, (711 mg, 69%): $[\alpha]_D$ +80° (*c* 0.28, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 8.10–7.30 (m, 10 H, aromatic), 5.60 (s, 1 H, C*H*Ph), 5.48 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.1 Hz, H-3), 5.38 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.42 (ddd, 1 H, $J_{5,6}$ 4.8, $J_{5,6'}$ $J_{4,5}$ 9.9 Hz, H-5), 4.36 (m, 1 H, H-2), 4.31, (dd, 1 H, H-4), 4.28 (dd, 1 H, $J_{6,6'}$ 10.3 Hz, H-6), 3.91 (dd, 1 H, H-6′), 2.70 (m, 2 H, SCH_2CH$_3$), 2.49 (d, 1 H, $J_{OH,\ 2}$ 4.0 Hz, OH), 1.32 (t, 3 H, *J* 7.5 Hz, SCH$_2$CH_3). ^{13}C NMR (CD_2Cl_2): δ 165.78 (*C*OPh), 137.93–126.56 (12 C, aromatic), 102.27 (*C*HPh), 85.55 (C-1), 76.86 (C-4), 72.03 (C-3), 71.84 (C-2), 69.04 (C-6), 64.91 (C-5), 25.61 (S*C*H$_2$CH$_3$), 15.1 (SCH$_2$*C*H$_3$). Anal. Calcd for $C_{22}H_{24}O_6S$: C, 63.45; H, 5.81. Found: C, 63.19; H, 5.91.

Ethyl 3-O-benzoyl-2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranosyl-(1→2)-3-O-benzoyl-4,6-O-benzylidene-1-thio-α-D-mannopyranoside (**14**).—The thioglycoside acceptor **13** (375 mg, 0.900 mmol) was glycosylated with the selenoglycoside donor **12** (650 mg, 1.08 mmol) following the general procedure. The reaction time was 1.5 h at 0 °C. The disaccharide was purified by column chromatography using 4:1 hexanes–EtOAc as the eluant. The desired product **14** was obtained as a white foam (464 mg, 60%): $[\alpha]_D$ −49° (*c* 0.60, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 8.12–7.00 (m, 25 H, aromatic), 5.70 (s, 1 H, C*H*Ph), 5.67 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.4 Hz, H-3B), 5.61 (s, 1 H, C*H*Ph), 5.51 (dd, 1 H, $J_{2,3}$ 3.6, $J_{3,4}$ 9.9 Hz, H-3C), 5.50 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1C), 4.96 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B), 4.46–4.38 (m, 3 H, H-2C, H-4C, H-5C), 4.34, 4.21 (2 d, 2 H, J_{gem} 11.6 Hz, OCH_2Ph), 4.31–4.23 (m, 3 H, H-6B, H-6C, H-4B), 4.18–4.10 (m, 2 H, H-2B, H-5B), 4.01 (dd, 1 H, $J_{5,6}$, $J_{6,6'}$ 10.0 Hz, H-6C′), 3.86 (dd, 1 H, $J_{5,6}$, $J_{6,6'}$ 10.3 Hz, H-6B′), 2.72 (m, 2 H, SCH_2CH$_3$), 1.38 (t, 3 H, *J* 7.5 Hz, SCH$_2$CH_3). ^{13}C NMR (CD_2Cl_2): δ 166.18, 166.04 (*C*OPh), 137.97–126.52 (30 C aromatic), 102.21 (*C*HPh), 102.12 (*C*HPh), 101.85 (C-1B), 84.94 (C-1C), 79.68 (C-2C), 77.56 (C-2B), 76.88 (C-4C), 76.69 (C-4B), 74.28 (*C*H$_2$Ph), 71.45 (C-3C), 71.26 (C-3B), 68.42 (2 C, C-6B, C-6C), 65.36 (C-5B), 65.04 (C-5C), 26.01 (S*C*H$_2$CH$_3$), 15.19 (SCH$_2$*C*H$_3$). Anal. Calcd for $C_{49}H_{48}O_{12}S$: C, 68.36; H, 5.62. Found: C, 68.35; H, 5.66.

Ethyl 2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranosyl-(1→2)-4,6-O-benzylidene-1-thio-α-D-mannopyranoside (**15**).—To a solution of the disaccharide **14** (110 mg, 0.128 mmol) in freshly distilled MeOH (10 mL) was added 1 M NaOMe–MeOH (0.5 mL). The reaction mixture was stirred at rt, overnight, under an N_2 atmosphere. The solution was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The partially deprotected disaccharide was purified by column chromatography using 2:1 hexanes–EtOAc as the eluant. The desired product **15** was obtained as a white foam (81 mg, 96%): $[\alpha]_D$ +69° (*c* 0.67, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.60–7.30 (m, 15 H, aromatic), 5.60 (s, 1 H, C*H*Ph), 5.59 (s, 1 H, C*H*Ph), 5.33 (bs, 1 H, H-1C), 5.21 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1B), 4.76, 4.66 (2 d, 2 H, J_{gem} 11.7 Hz, OCH_2Ph), 4.25–4.15 (m, 4 H, H-6B, H-6C, H-5C, H-3B), 4.11 (m, 2 H, H-2C, H-3C), 3.98 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2B), 3.96–3.87 (m, 5 H, H-6B′, H-6C′, H-4B, H-4C, H-5B), 2.65 (m, 2 H, SCH_2CH$_3$), 1.35 (t, 3 H, *J* 7.4 Hz, SCH$_2$CH_3). ^{13}C NMR ($CDCl_3$): δ 137.61–126.17 (18 C aromatic), 102.29 (*C*HPh), 102.07 (*C*HPh), 100.71 (C-1B), 84.73 (C-1C), 79.93 (C-2C), 79.48 (2 C, C-4B, C-4C), 78.39 (C-2B), 73.55 (*C*H$_2$Ph), 69.55 (C-3C), 68.74 (C-6C), 68.64, 68.60 (C-3B, C-6B), 64.34 (C-5B), 64.04 (C-5C), 25.62 (S*C*H$_2$CH$_3$), 14.94 (SCH$_2$*C*H$_3$). Anal. Calcd for $C_{35}H_{40}O_{10}S$: C, 64.40; H, 6.18. Found: C, 64.60; H, 6.16.

Ethyl 2,3,5,6-tetra-O-*acetyl*-β-D-*galacto*-*furanosyl*-(1→3)-*2*-O-*benzyl*-*4,6*-O-*benzylidene*-α-D-*mannopyranosyl*-(1→2)-[*2,3,5,6*-*tetra*-O-*acetyl*-β-D-*galactofuranosyl*-(1→3)]-*4,6*-O-*benzylidene*-*1*-*thio*-α-D-*mannopyranoside* (**17**).—The thioglycoside acceptor **15** (156 mg, 0.239 mmol) was glycosylated with the selenoglycoside donor **16** (280 mg, 0.574 mmol) following the general procedure. The reaction time was 40 min at 0 °C. Pure **17** (265 mg, 85%) was crystallized from the crude product mixture using hexanes–EtOAc. An analytical sample was obtained as colorless needles by column chromatography using 2:1 hexanes–EtOAc as the eluant and recrystallization from hexanes–EtOAc: mp 137–140 °C; $[\alpha]_D$ −5° (*c* 0.60, CH_2Cl_2); ^1H NMR (CD_2Cl_2): δ 7.50–7.25 (m, 15 H, aromatic), 5.62 (s, 1 H, C*H*Ph), 5.60 (s, 1 H, C*H*Ph), 5.40 (bs, 1 H, H-1C), 5.34 (m, 1 H, H5A), 5.29 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1B), 5.25 (m, 1 H, $J_{4,5}$ 4.0, $J_{5,6}$ 7.6 Hz, H-5D), 5.21 (s, 1 H, H-1A), 5.13 (s, 1 H, H-1D), 5.06 (d, 1 H, $J_{2,3}$ 1.1 Hz, H-2A), 4.96 (dd, 1 H, $J_{3,4}$ 5.8 Hz, H-3D), 4.93 (d, 1 H, $J_{2,3}$ 1.6 Hz, H-2D), 4.91 (bdd, 1 H, $J_{3,4}$ 5.4 Hz, H-3A), 4.79, 4.75 (2 d, 2 H, J_{gem} 11.1 Hz, OCH_2Ph), 4.37 (dd, 1 H, $J_{4,5}$ 3.2 Hz, H-4A), 4.30 (dd, 1 H, H-4D), 4.28 (dd, 1 H, $J_{2,3}$ 2.3, $J_{3,4}$ 10.1 Hz, H-3B), 4.25–4.18 (m, 3 H, H-5C, H-6C, H-6B), 4.18 (m, 1 H, H-2C), 4.17 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3C), 4.09 (dd, 1 H, $J_{5,6}$ 7.4 Hz, H-6A), 4.10–4.05 (m, 4 H, H-6D, H-4B, H-4C, H-2B), 3.95 (dd, 1 H, $J_{5,6'}$ 4.6, $J_{6,6'}$ 11.7 Hz, H-6A′), 3.93 (m, 1 H, H-5B), 3.92 (dd, 1 H, $J_{5,6'}$, $J_{6,6'}$ 11.6 Hz, H-6C′), 3.85 (dd, 1 H, $J_{5,6'}$ 4.1, $J_{6,6'}$ 12.0 Hz, H-6D′), 3.84 (dd, 1 H, $J_{5,6'}$, $J_{6,6'}$ 10.0 Hz, H-6B′), 2.65 (m, 2 H, SCH_2CH_3), 2.10, 2.08 (2 s, 3 H each, –C(O)CH_3), 2.06 (s, 6 H, –C(O)CH_3), 1.93, 1.92, 1.91, 1.90 (4 s, 3 H each, C(O)CH_3), 1.30 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.62, 170.34 (8 C, $COCH_3$), 138.9–126.5 (18 C, aromatic), 103.22 (C-1D), 103.04 (C-1A), 102.13 (3 C, C-1B, 2 × *C*HPh), 85.33 (C-1C), 82.58 (C-2D), 82.04 (C-2A), 81.16 (2 C, C-4A, C-4D), 77.81 (C-2C), 77.47 (2 C, C-4B and C-4C), 77.35 (C-3A), 76.98 (C-3D), 76.35 (C-2B), 74.53 (CH_2Ph), 72.42 (2 C, C-3B, C-3C), 69.85 (C-5A), 69.75 (C-5D), 69.14 (C-6C), 68.99 (C-6B), 65.58 (C-5B), 65.44 (C-5C), 63.08 (C-6D), 62.84 (C-6A), 26.14 (SCH_2CH_3), 21.0, 20.84, 20.74 (8 C, $COCH_3$), 15.29 (SCH_2CH_3). Anal. Calcd for $C_{63}H_{76}O_{28}S$: C, 57.62; H, 5.83. Found: C, 57.80; H, 5.99.

Ethyl β-D-*galactofuranosyl*-(1→3)-α-D-*mannopyranosyl*-(1→2)-[β-D-*galactofuranosyl*-(1→3)]-*1*-*thio*-α-D-*mannopyranoside* (**3**).—The tetrasaccharide **17** (50 mg, 0.038 mmol) was deprotected as described in the general procedure. The intermediate deacylated product was purified by column chromatography using 12:1 CH_2Cl_2–MeOH as the eluant to give a clear glass. The compound was then subjected to hydrogenolysis and purified by column chromatography using 5:2:1 EtOAc–MeOH–H_2O as the eluant. The tetrasaccharide **3** was obtained as a syrup (19 mg, 71%): $[\alpha]_D$ −10° (*c* 0.079, H_2O); ^1H NMR (D_2O): δ 5.55 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1C), 5.13 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1A), 5.11 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1D), 5.08 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.29 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2C), 4.21 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2B), 4.12 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2A), 4.08 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2D) 4.07–3.96 (m, 5 H, H-3A, H-3D, H-4A, H-4D, H-5C), 3.92–3.74 (m, 9 H, H-3B, H-3C, H-5A, H-5B, H-5D, H-6B, H-6C, H-6C′, H-4C), 3.71 (dd, 1 H, $J_{5,6'}$ 4.2, $J_{6,6'}$ 10.9 Hz, H-6B′), 3.69 (m, 2 H, H-6A, H-6D), 3.65 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.9 Hz, H-4B), 3.62 (dd, 1 H, $J_{5,6'}$ 7.4, $J_{6,6'}$ 11.6 Hz, H-6A′), 3.61 (dd, 1 H, $J_{5,6'}$ 7.4, $J_{6,6'}$ 11.6 Hz, H-6D′), 2.65 (m, 2 H, SCH_2CH_3), 1.30 (t, 3 H, *J* 7.5 Hz, SCH_2CH_3). ^{13}C NMR (D_2O): δ 107.30 (C-1D), 107.18 (C-1A), 104.47 (C-1B), 85.53 (2 C, C-4A, C-4D), 85.46 (C-1C), 83.98 (2 C, C-2A, C-2D), 79.68 (C-2C), 79.60 (C-3A), 79.45 (C-3D), 78.51 (C-3B), 78.05 (C-3C), 76.06 (C-5D), 78.85 (C-5C), 73.35 (C-5A), 73.26 (C-5B), 69.56 (C-2B), 68.29 (C-4C), 67.92 (C-4B), 65.50, 65.45 (2 C, C-6A, C-6D), 63.78 (C-6B), 63.54 (C-6C), 27.84 (SCH_2CH_3), 16.91 (SCH_2CH_3). Anal. Calcd for $C_{26}H_{46}O_{20}S$: C, 43.94; H, 6.52. Found: C, 43.71; H, 6.39.

Methyl 2,3,5,6-tetra-O-*acetyl*-β-D-*galacto*-*furanosyl*-(1→3)-*2*-O-*benzyl*-*4,6*-O-*benzylidene*-α-D-*mannopyranosyl*-(1→2)-[*2,3,5,6*-*tetra*-O-*acetyl*-β-D-*galactofuranosyl*-(1→3)]-*4,6*-O-*benzylidene*-α-D-*mannopyranosyl*-(1→2)-*3,4,6-tri*-O-*benzyl*-α-D-*mannopyranoside* (**18**).

—The methyl mannopyranoside acceptor **6** (30 mg, 0.064 mmol) was glycosylated with the thioglycoside donor **17** (100 mg, 0.076 mmol) following the general procedure. The reaction time was 4 h at rt. Purification of the pentasaccharide **18** was attempted by column chromatography, but the donor and product eluted together. The ^{1}H NMR spectrum showed a 3:1 ratio of product–donor (57 mg, 35%, corrected for presence of donor). Extensive column chromatography using 1.5:1 toluene–EtOAc as the eluant gave a small amount of pure material for characterization: $[\alpha]_D$ −6.4° (*c* 2.35, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 7.60–7.15 (m, 30 H, aromatic), 5.64 (s, 1 H, C*H*PhB), 5.59 (s, 1 H, C*H*PhC), 5.33 (d, 1 H, H-1C), 5.32 (d, 1 H, H-1B), 5.30 (m, 1 H, H-5A), 5.26 (m, 1 H, H-5D), 5.23 (s, 1 H, H-1A), 5.17 (s, 1 H, H-1D), 5.06 (d, 1 H, $J_{2,3}$ 1.2 Hz, H-2A), 4.96 (m, 2 H, H-2D, H-3D), 4.93 (m, 1 H, H-3A), 4.83, 4.54 (2 d, 2 H, J_{gem} 10.9 Hz, OCH_2PhE), 4.80, 4.75 (2 d, 2 H, J_{gem} 12.5 Hz, OCH_2PhB), 4.77 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1E), 4.73, 4.68 (2 d, 2 H, J_{gem} 12.3 Hz, OCH_2PhE), 4.63, 4.57 (2 d, 2 H, J_{gem} 12.2 Hz, OCH_2PhE), 4.36 (dd, 1 H, $J_{3,4}$ 5.3, $J_{4,5}$ 3.2 Hz, H-4A), 4.30 (dd, 1 H, $J_{3,4}$ 6.0, $J_{4,5}$ 3.2 Hz, H-4D), 4.26 (dd, 1 H, $J_{5,6}$ 3.3, $J_{6,6'}$ 9.9 Hz, H-6B), 4.26–4.22 (m, 3 H, H-2C, H-3C, H-3B), 4.17 (dd, 1 H, $J_{5,6}$ 4.6, $J_{6,6'}$ 10.0 Hz, H-6C), 4.10 (dd, 1 H, $J_{5,6}$ 7.6, $J_{6,6'}$ 11.7 Hz, H-6A), 4.08 (dd, 1 H, $J_{1,2}$, $J_{2,3}$ 1.7 Hz, H-2B), 4.07 (dd, 1 H, $J_{3,4}$ 8.1, $J_{4,5}$ 9.1 Hz, H-4B), 4.06 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4C), 4.05 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$11.9 Hz, H-6D), 4.01 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-2E), 3.99–3.85 (m, 2 H, H-5B, H-5C), 3.91 (dd, 1 H, $J_{5,6}$ 4.4 Hz, H-6A′), 3.89 (dd, 1 H, $J_{5,6'}$ 9.9 Hz, H-6B′), 3.88 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3E), 3.81 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4E), 3.80 (dd, 1 H, $J_{5,6'}$ 3.8 Hz, H-6D′), 3.79 (dd, 1 H, $J_{5,6}$ 10.3 Hz, H-6C′), 3.77–3.68 (m, 3 H, H-5E, H-6E, H-6E′), 3.35 (s, 3 H, OCH_3), 2.10, 2.08, 2.06, 2.04, 1.951, 1.950, 1.89, 1.86 (8 s, 3 H each, C(O)C*H*$_3$). ^{13}C NMR (CD_2Cl_2): δ 170.57, 170.47, 170.40, 170.31, 170.27, 170.08 (8 C, *C*OCH$_3$), 136.21–126.41 (36 C, aromatic), 103.26 (C-1D), 102.94 (C-1A), 102.06 (*C*HPhB), 101.96 (*C*HPhC), 101.68 (2 C, C-1B, C-1C), 100.24 (C-1E), 82.45 (C-2D), 81.90 (C-2A), 81.05 (C-4A), 81.00 (C-4D), 79.59 (C-3E), 77.26 (C-3A), 77.16 (2 C, C-4B, C-4C), 76.96 (C-3D), 76.22 (C-2B), 75.51 (C-4E), 75.40 (C-2C), 75.32 (*C*H$_2$PhE), 75.19 (C-2E), 74.42 (*C*H$_2$PhB), 75.57, 72.42 (2 C, *C*H$_2$PhE), 72.32 (C-5E), 72.08 (C-3C), 69.92 (C-3B), 69.73 (C-5A), 69.65 (2 C, C-5D, C-6E), 68.97 (2 C, C-6B, C-6C), 65.41 (C-5C), 65.08 (C-5B), 63.02 (C-6D), 62.77 (C-6A), 55.02 (O*C*H$_3$), 21.00, 20.75 (8 C, CO*C*H$_3$). Anal. Calcd for $C_{89}H_{102}O_{34}$: C, 62.30; H, 5.99. Found: C, 62.62; H, 5.88.

One-pot synthesis of **18**.—The thioglycoside acceptor **15** (58 mg, 0.088 mmol) was glycosylated with the selenoglycoside donor **16** (94 mg, 0.19 mmol) following the general procedure. After 40 min at 0 °C, a solution of the methyl glycoside acceptor **6** (74 mg, 0.16 mmol) in dry CH_2Cl_2 (2 mL), activated 4 Å molecular sieves (200 mg), and NIS (52 mg, 0.23 mmol) were added. The reaction mixture was stirred for 1 h at 0 °C and then warmed to rt and stirred for an additional 2 h. The color of the solution changed from a deep purple to dark rose. The reaction was worked up as described in the general procedure. The ^{1}H NMR spectrum showed a 3:1 ratio of product–donor (42 mg, 28%, corrected for presence of donor).

*Methyl β-*D*-galactofuranosyl-(1→3)-α-*D*-mannopyranosyl-(1→2)-[β-*D*-galactofuranosyl-(1→3)]-α-*D*-mannopyranosyl-(1→2)-α-*D*-mannopyranoside* (**4**).—To a solution of the impure pentasaccharide **18** (55 mg, 0.032 mmol) in freshly distilled MeOH (5 mL) was added 1 M NaOMe–MeOH (0.5 mL). The reaction mixture was stirred at rt overnight under an N_2 atmosphere. The reaction mixture was neutralized with Rexyn 101 (H^+), the resin was filtered, and the solvent was removed in vacuo. The crude product was purified by column chromatography using 10:1:1 EtOAc–MeOH–H_2O as the eluant to give a clear glass. This compound was then subjected to hydrogenolysis as described in the general procedure for deprotection. The solvent was removed in vacuo and the crude product purified by column chromatography using 5:2:1 EtOAc–MeOH–H_2O as the eluant. The pentasaccharide **4** was obtained as a clear syrup, (12 mg, 60%): $[\alpha]_D$ −30° (*c* 0.029, H_2O); ^{1}H NMR (D_2O): δ 5.24 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1C), 5.18 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1D),

5.14 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1A), 5.09 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.97 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1E), 4.31 (dd, 1 H, H-2C), 4.21 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2B), 4.12 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2A), 4.09 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2D), 4.08–4.01 (m, 4 H, H-3A, H-3D, H-4A, H-4D), 4.00 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.2 Hz, H-3C), 3.93 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2E) 3.91–3.55 (m, 20 H, H-3B, H-3E, H-4B, H-4C, H-4E, H-5A, H-5B, H-5C, H-5D, H-5E, H-6A, H-6B, H-6C, H-6D, H-6E, H-6A′, H-6B′, H-6C′, H-6D′, H-6E′), 3.39 (s, 3 H, OCH_3). ^{13}C NMR (D_2O): δ 107.47 (C-1D), 107.20 (C-1A), 104.23 (C-1B), 103.40 (C-1C), 101.94 (C-1E), 85.51, 85.43 (C-4A, C-4D), 83.99 (2 C, C-2A, C-2D), 81.40 (C-2E), 79.61, 79.46 (C-3A, C-3D), 78.05 (2 C, C-3B, C-3C), 77.18 (C-2C), 75.96, 75.89 (C-4B, C-4C), 75.25 (C-4E), 73.37, 73.32 (C-5A, C-5D), 72.83, 68.10, 67.75 (4 C, C-3E, C-5B, C-5C, C-5E), 65.51 (2 C, C-6A, C-6D), 63.61 (3 C, C-6B, C-6C, C-6E), 57.52 (OCH_3). Anal. Calcd for $C_{31}H_{54}O_{26}$: C, 44.18; H, 6.46. Found: C, 43.81; H, 6.22.

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