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Synthesis of oligosaccharide fragments of the glycosylinositolphospholipid of *Trypanosoma cruzi*: a new selenoglycoside glycosyl donor for the preparation of 4-thiogalactofuranosyl analogues†

Karla D. Randell, Blair D. Johnston, Ernest E. Lee and B. Mario Pinto *

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

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Abstract

A new selenoglycoside, phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-1-selenogalactofuranose, has been synthesized. This 4-thiogalactofuranosyl donor was used in the syntheses of heteroatom analogues of the di-, tri-, and tetrasaccharides corresponding to the oligosaccharide β-D-Gal*f*-(1→3)-α-D-Man*p*-(1→2)-(β-D-Gal*f*-(1→3))-α-D-Man*p*. These compounds represent fragments of the terminal end of the glycosylinositolphospholipid oligosaccharide found in the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, and are intended for use as inhibitors of the enzymes that construct the native oligosaccharides. The syntheses employed the selective activation of a phenyl 4-thio-1-selenogalactofuranoside glycosyl donor over ethyl 1-thioglycoside glycosyl acceptors with NIS/TfOH. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Carbohydrates are highly functionalized molecules that are used by Nature for diverse tasks. They play an important role in biochemical recognition pathways. They are involved in growth and development, immune responses, infection by viruses and bacteria, host–pathogen interactions, cell adhesion, tumor metastasis, and signal transduction.[1] For example, inhibition of tumor metastasis is an important area of research and this goal may be achieved through the inhibition of glycosidases used in the oligosaccharide processing pathways.[2–4] The synthesis of heteroanalogues of sugars as potential glycosidase inhibitors has been a focus of our laboratory. The syntheses of novel glycosidase inhibitors as analogues of methyl maltoside and alkyl kojibiosides in which the ring and/or the interglycosidic oxygen atoms have been

* Corresponding author. Tel: (604) 291-4327; fax: (604) 291-3765; e-mail: bpinto@sfu.ca

† Dedicated to P. Sinay in celebration of his 62nd birthday.

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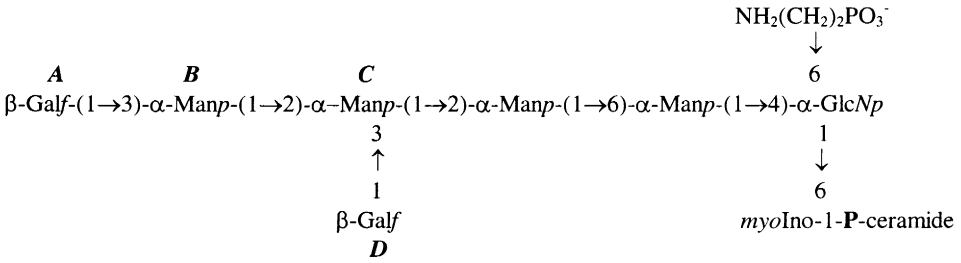
replaced with sulfur or selenium have been reported.[5–8] More recently, the syntheses of disaccharide analogues containing sulfur in the ring and nitrogen in the interglycosidic linkage have been achieved.[9–11]

Previous reports from our laboratory demonstrated the viability of phenyl 2,3,5,6-tetra-*O*-acetyl-β-D-selenogalactofuranoside as a glycosyl donor.[12,13] The synthesis of oligosaccharides containing galactofuranose is important because it has been determined that galactofuranose (Gal*f*) is present as a constituent of external cellular structures in protozoa,[14] bacteria,[15,16] and fungi.[17] These structures do not appear to be present on mammalian cells and elicit a strong antigenic response during infection.[18] It is known that Gal*f* is part of the oligosaccharide core of the glycosylinositolphospholipid (GIPL) from the protozoan *Trypanosoma cruzi*, the infectious agent of Chagas disease.[19] The GIPL structure is:[20,21]



This structure is the most abundant cell surface glycoconjugate present in the insect dwelling epimastigote stage of the *T. cruzi* life cycle.[21] 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.[18] Oligosaccharides containing 4-thio-Gal*f* may therefore be useful for understanding the role Gal*f* plays in microorganisms, for studying the biosynthesis of furanosyl-containing glycoconjugates, and may also be used as inhibitors to probe the development of infections.

In this study, we report the first synthesis of oligosaccharides containing 4-thio-Gal*f* using a new glycosyl donor, phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-D-selenogalactofuranoside **5**. This work is an extension of previous studies on the selective activation of selenoglycoside glycosyl donors in the presence of glycosyl acceptors containing a latent thioglycoside.[12,13,22–25] In the present application, these methods led to the ready preparation of heteroanalogues containing sulfur in the non-reducing (and branched) galactofuranose ring(s) of the disaccharide **1**, trisaccharide **2**, and tetrasaccharide **3** corresponding to the terminus of the glycosylinositolphospholipid oligosaccharides of *T. cruzi* (Fig. 1).

2. Results and discussion

The required monosaccharides 1,2,3,5,6-penta-*O*-acetyl-4-thio-D-galactofuranose **4**,[26] ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **6**,[27] and methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **8**,[28] and the disaccharide ethyl 2-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl)-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **12**[13] were synthesized following literature methods.

Phenyl 2,3,5,6-tetra-*O*-acetyl-1-seleno-4-thio-D-galactofuranoside **5** was prepared by reaction of peracetylated 4-thiogalactofuranose **4** with phenylselenol and BF_3:Et_2O in 94% yield. A ^1H NMR spectrum showed the presence of an α:β mixture in a ratio of 1:3 (see Scheme 1). The 2D NOESY spectrum showed an NOE between H1 and H3 for the major isomer, indicating the presence of a β-linkage; the minor isomer showed no NOE between H1 and H3, indicating the presence of an α-linkage. The J_{C1H1} values are very similar for both the isomers **5α** (163 Hz) and **5β** (160 Hz). As with furanose sugars in

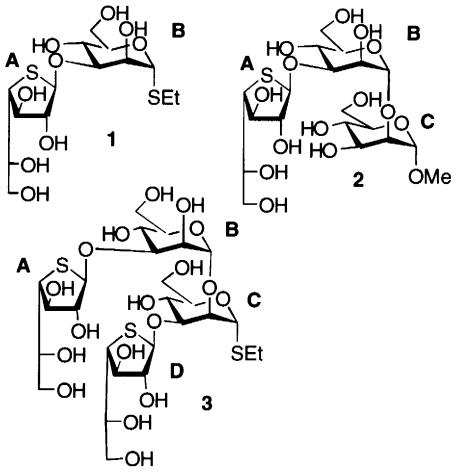
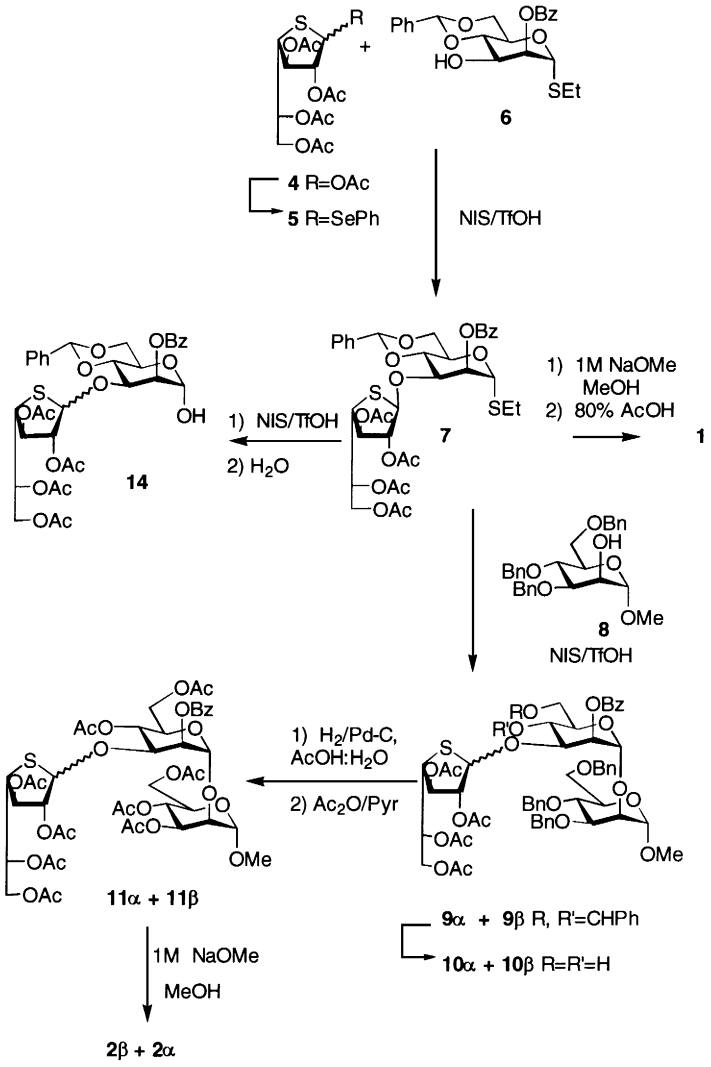


Fig. 1.

general,[29] J_{C1H1} values do not seem to be reliable indicators of α- or β-linkage in the 4-thio-furanosyl residues.

Glycosylation of the acceptor **6** with the new glycosyl donor **5** was performed using NIS/TfOH. Activation of the phenyl 1-selenogalactofuranoside over the ethyl 1-thio mannopyranoside was achieved with good selectivity, and the protected 1→3-linked disaccharide **7** was obtained as a white foam in 80% yield (see Scheme 1). The stereochemical integrity of the disaccharide **7** was confirmed by examination of the NOE contacts for the galactofuranosyl residue and the J_{C1H1} values of the mannopyranosyl residue. The 2D NOESY spectrum showed the presence of an NOE between H1A and H3A (of the 4-thio-Gal*f* ring A) and the absence of an NOE between H1A and H4A, indicating the presence of a β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. The β-isomer was the only compound isolated even though an α:β mixture of the donor was used. The J_{C1H1} value is 168 Hz for the Man*p* (B) ring, indicating the presence of an α-configuration about C1 for the mannopyranosyl residue.[30] The disaccharide **7** was deprotected by methanolysis of the esters followed by hydrolysis of the benzylidene acetal to give compound **1** in 75% yield. Again, the 2D NOESY spectrum showed an NOE between H1A and H3A (of the 4-thio-Gal*f* ring A), indicating the preservation of the β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. The J_{C1H1} value is 167 Hz for the Man*p* (B) ring, indicating the expected α-configuration about C1 for the mannopyranosyl residue.[30]

The protected disaccharide **7** was used as a donor in the next glycosylation reaction without any further manipulation. Glycosylation of the acceptor **8** with the donor thioglycoside **7** was considerably slower using NIS/TfOH than the previous reaction with the selenoglycoside. The reaction gave a mixture of products that included compounds **9α** and **9β** (see Scheme 1) as the major components**,** which could not be separated by column chromatography. The mixture was analyzed by ^{1}H NMR and ^{13}C NMR spectroscopy. We initially considered the possibility that one of the major compounds was an orthoester because a high-field, three-proton singlet was observed at δ 1.48 ppm in the ^{1}H NMR spectrum. However, a ^{13}C DEPT experiment indicated that there was no quaternary carbon in the region around 120 ppm as would be expected for an orthoester. In a subsequent glycosylation reaction, the reaction mixture was warmed to room temperature before quenching, but the ^{1}H NMR spectrum of the crude product mixture showed that the peak at δ 1.48 ppm was present with the same intensity. Since these conditions (which should promote rearrangement of any orthoester product) made no difference, the possibility of an orthoester was discounted. The high-field singlet was dismissed as resulting from an unusually shielded acetate group. A COSY spectrum, together with a C–H correlation spectrum, permitted assignment of



Scheme 1.

the other ^{1}H and ^{13}C NMR signals, as expected for the structures **9α** and **9β**. Next, the benzylidene acetal was removed by hydrolysis to give **10α** and **10β**. The ^{1}H NMR spectrum indicated that there was no longer a singlet at 1.48 ppm, and all the acetate peaks were found between 2.01 and 1.85 ppm. A COSY spectrum, together with a C–H correlation spectrum, permitted assignment of the ^{1}H and ^{13}C NMR signals that was consistent with the presence of **10α** and **10β** (see Scheme 1). Finally, the benzyl ethers were removed by hydrogenolysis and the mixture was acetylated for purification purposes (see Scheme 1). In subsequent experiments, the deprotection sequence was simplified by first removing both the benzylidene and the benzyl ethers by hydrogenolysis, followed by acetylation to give a mixture of **11α** and **11β**. The ^{1}H NMR spectrum showed the presence of two compounds in a ratio of 2:3. A NOESY spectrum showed an NOE between H1A and H3A (of the 4-thio-Gal*f* ring A) for the major compound, indicating the presence of a β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. This confirmed that the major isomer was indeed the desired compound **11β**. No NOE was found between H1A and H3A for the minor compound, indicating the presence of an α-linkage between the 4-thio-Gal*f*

(A) and Man*p* (B) rings. A COSY and a TOCSY spectrum, together with a C–H correlation spectrum, then permitted complete assignment of the ^{1}H and ^{13}C NMR signals for **11α** as the minor product and **11β** as the major product. The J_{C1H1} values are 172 Hz for both the Man*p* (B) ring and the Man*p* (C) ring, indicating the presence of α-configurations about C1 for both mannose residues, in both the major and minor compounds.[30]

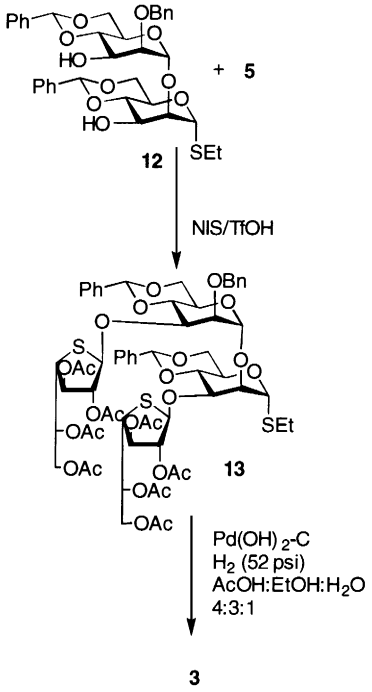
In the ^{13}C{^{1}H} NMR spectra for all three sets of compounds, **9**–**11**, the Gal*f* C4 resonances are found between 40 and 50 ppm due to shielding by the sulfur atom. The C1β and C4β signals are always downfield from the C1α and C4α signals. This can be explained by the *syn* vs. *anti* orientation of the ring substituents in the molecules.[31] In Gal*f*, the C1α and C4α are shielded because of a *syn* arrangement between the aglycon and O2 and between the aglycon and the substituent at C4, respectively. Also, a *syn* arrangement between the aglycon and O3 of the β-Gal*f* causes C4β to be deshielded. The latter effect has been observed with C4 of pento- or hexofuranosides, containing *gluco*, *manno*, and *allo* configurations.[31]

The synthesis of the trisaccharide **9** was also attempted using either methyl triflate[32] or iodonium di-*sym*-collidine perchlorate[33] as the promoter, but both reactions were unsuccessful. It was hoped that these promoters would be more selective for the ethylthio aglycon of **7**.

The trisaccharides **11α**/**β** were deprotected by methanolysis of the esters to give the target trisaccharide **2β**, plus the α-anomer in 94% yield, with an α:β ratio of 1:1.6. Again, COSY, TOCSY, and C–H correlation spectra enabled the assignment of the signals of rings A, B, and C for both the α- and the β-isomer. The 2D NOESY spectrum showed an NOE between H1A and H3A (of the 4-thio-Gal*f* ring A) for the major compound, indicating the preservation of the β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings. No NOE is found between H1A and H3A for the minor compound, indicating the preservation of the α-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings.

The tetrasaccharide **13** was synthesized by double glycosylation of the disaccharide **12** with 2.2 equivalents of phenyl 2,3,5,6-tetra-*O*-acetyl-4-thio-D-selenogalactofuranoside **5** (see Scheme 2). This reaction was immediate and resulted in tetrasaccharide products in 93% yield. The desired compound **13** containing β-linkages between both Gal*f* rings and the mannosyl residues comprised >80% of the tetrasaccharide. An isomeric tetrasaccharide containing an α-linkage between one of the Gal*f* rings and a mannosyl residue could not be separated from the desired compound. This reaction was repeated at room temperature and much less α/β selectivity was observed. The tetrasaccharide was deprotected by methanolysis of the esters, followed by hydrolysis of the benzylidene acetals and hydrogenolysis of the benzyl ether to give the target tetrasaccharide **3**, containing a minor amount of the α anomeric isomer, in 25% yield. The low overall yield for deprotection steps resulted from poisoning of the palladium catalyst by the sulfur atoms during the hydrogenolysis step. It was therefore necessary to use large amounts of Pd/C and to replace the catalyst three times in order to completely remove the benzyl group, thus leading to a reduction in overall yield because some of the compound was adsorbed on the Pd/C catalyst.

Assignment of the NMR signals of the Man*p* (C) ring of the tetrasaccharide **3** was based on the fact that C1 for this residue has a characteristic upfield chemical shift due to the ethyl thioglycoside. A C–H correlation spectrum, together with a COSY and TOCSY spectrum, then permitted complete assignment of the ^{1}H and ^{13}C NMR signals of this ring. The assignment of signals for the Man*p* (B) ring was based on the presence of an NOE contact across the glycosidic linkage between H1B and H2C, and COSY and TOCSY transfer between ^{1}H NMR signals of the B ring. Assignment of signals of the 4-thio-Gal*f* rings A and D was based on NOE contacts across the glycosidic linkages between H1A and H3B, and H1D and H3C, respectively. The 2D NOESY spectrum showed an NOE between H1A and H3A (of the 4-thio-Gal*f* ring A), and another NOE between H1D and H3D (of the 4-thio-Gal*f* ring D), indicating the presence of a β-linkage between the 4-thio-Gal*f* (A) and Man*p* (B) rings, and also between the 4-thio-Gal*f* (D) and



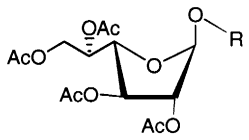
Scheme 2.

Man*p* (C) rings. The J_{C1H1} values are ~172 Hz for the Man*p* (B) ring and 167 Hz for the Man*p* (C) ring, indicating the presence of α-configurations about C1 for both mannopyranosyl residues.[30]

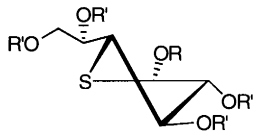
The coupling constants observed in the ^{1}H NMR spectrum for the 4-thio-galactofuranosyl residues are of interest. The *J* values for the 4-thio-Gal*f* residue in **11α** and **2α** suggest that the ring is in a $^2T_3$ (D) conformation (see Scheme 3).[34] In this conformation, the anomeric linkage from 4-thio-Gal*f* (A) to Man*p* (B) is quasi-axially oriented and the other substituents are quasi-equatorial. The large values of $J_{1,2}$ (4.2 Hz), $J_{2,3}$ (9.8 Hz), and $J_{3,4}$ (8.2 Hz) indicate that H-2/H-3 and also H-3/H-4 must have dihedral angles near 180°. This was also observed by Varela et al.[26] for 1,2,3,5,6-penta-*O*-acetyl-4-thio-α-D-galactofuranose **4α**.

The coupling constants $J_{1,2}$ (2.4–2.6 Hz), $J_{2,3}$ (4.0–4.6 Hz), and $J_{3,4}$ (6.6–7.6 Hz) for **7**, **11β**, and **13** indicate that the 4-thio-galactofuranosyl residue in these compounds exists as a mixture of several conformations. This is very different from the E_O (D) conformation observed for the β-D-galactofuranosides (i.e. the *O*-series) [$J_{1,2}$ (<1 Hz), $J_{2,3}$ (1.5 Hz), and $J_{3,4}$ (5.6 Hz)], as seen in Scheme 3.[13,35] The coupling constants that have been reported for **4β** [$J_{1,2}$ (3.1 Hz), $J_{2,3}$ (5.4 Hz), $J_{3,4}$ (6.4 Hz)], methyl 2,3,5,6-tetra-*O*-acetyl-4-thio-β-D-galactofuranoside [$J_{1,2}$ (2.5 Hz), $J_{2,3}$ (5.0 Hz), $J_{3,4}$ (5.3 Hz)], and 6-deoxy-2,3,5-tri-*O*-acetyl-4-thio-β-D-galactofuranose [$J_{1,2}$ (3.3 Hz), $J_{2,3}$ (6.0 Hz), $J_{3,4}$ (7.4 Hz)] were attributed to result from an equilibrium mixture of several conformations, including $^4T_3$ (D) (see Scheme 3).[34,36] In a study of 4-thiofuranoside derivatives of D-galactosamine, it was observed that all the β-isomers had coupling constants, $J_{1,2}$ (3.3–5.7 Hz), $J_{2,3}$ (6.1–7.6 Hz), and $J_{3,4}$ (6.7–7.7 Hz), that indicated the presence of a mixture of conformations.[37] Upon deprotection, the β-linked Gal*f* residue(s) in compounds **1**, **2**, and **3** also adopt a conformation similar to the $^4T_3$ (D) conformation (see Scheme 3), with $J_{1,2}$ (5.5–5.9 Hz), $J_{2,3}$ (8.3 Hz), and $J_{3,4}$ (8.8 Hz). In an attempt to determine whether hydrogen-bonding networks were responsible for the conformational changes, the ^{1}H NMR spectrum of **1** in

α-S-series $^{2}T_{3}$ (D)
11α / 2α



β-O-series E_{o} (D)



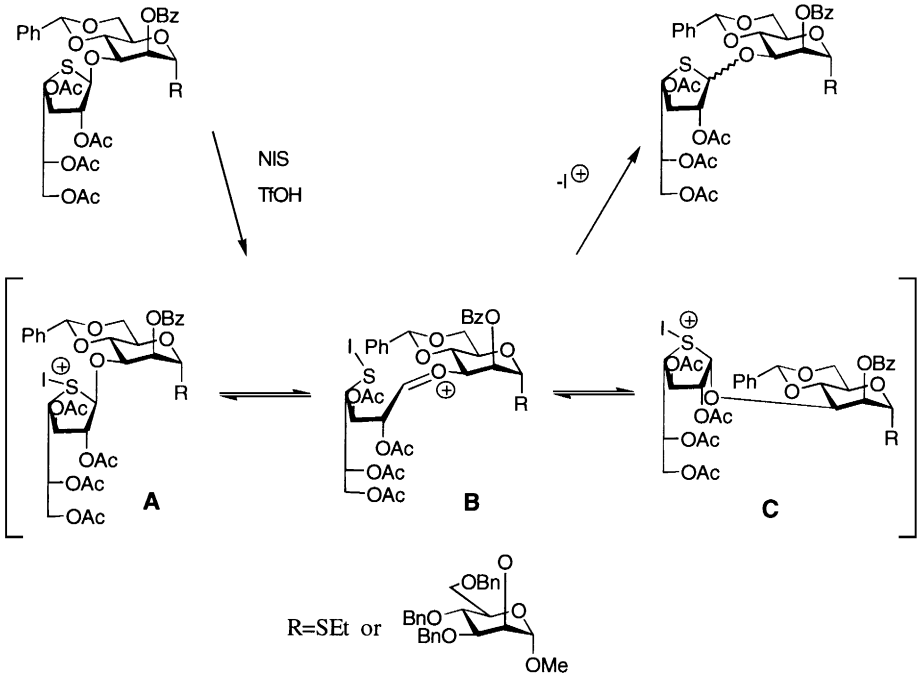
β-S-series $^{4}T_{3}$ (D)
1, 2β, 3, 7, 11β, 13

Scheme 3.

DMSO-d_6 was obtained, but addition of D_2O showed no significant changes in the resonances of the OH groups.[38]

We propose that the isomerization of the Gal*f* unit during the glycosylation to give the trisaccharide **9** may occur either in the disaccharide donor **7** or in the trisaccharide product by way of open-chain intermediates (Scheme 4). If there is a competition between the sulfur atom of the aglycon and the sulfur atom of the Gal*f* ring in the donor **7** for the electrophilic iodine generated from the NIS/TfOH reagent, then isomerization may occur before glycosylation. In a control experiment, the disaccharide **7** was reacted with NIS/TfOH in the absence of the acceptor. The ^{1}H NMR spectrum indicated that isomerization occurred at the glycosidic linkage between the Gal*f* ring and the mannosyl residue to give compound **14α/β** (Scheme 1). If the ring sulfur atom of the Gal*f* moiety reacts preferentially, the β sulfonium iodide intermediate **A** (Scheme 4) may be in equilibrium with an open-chain oxonium ion **B** which can reclose to regenerate **A**. Alternatively, **B** may, by rotation about single bonds, attain a conformation in which attack of the sulfenyl iodide occurs from the opposite face of the oxonium ion to give the α sulfonium ion intermediate **C**. Loss of I^{+} from either **A** or **C** would then lead to a mixture of α- and β-isomers in the donor **7** and, hence, to the observed anomeric mixture in trisaccharide **9** (Scheme 1). On the other hand, there still remains the possibility that the Gal*f* in the trisaccharide **9** may isomerize by a similar open-chain mechanism catalyzed by excess NIS. A glycosylation reaction performed with only 1 equiv. of NIS showed that a noticeable amount of isomerization still occurred, albeit to a lesser extent. We conclude that oligosaccharide thioglycoside donors that also contain 4-thio-Gal*f* units are likely to be of only limited use in NIS/TfOH-promoted glycosylation reactions.

The absence of this isomerization in the glycosylation reaction with the selenoglycoside donor **5** to give disaccharide **7**, and the much smaller extent of isomerization observed during the formation of the tetrasaccharide **13**, is a reflection of the almost instantaneous glycosylation reactions with the selenoglycoside donor. This results in exposure of the reactants and products to the isomerizing NIS/TfOH reagent for much less time. The selectivity for reaction of NIS/TfOH at the selenium atom of **5** over reaction with the sulfur atom of the Gal*f* ring or with the sulfur atom of ethyl thioglycoside acceptors



Scheme 4.

such as **12** appears to be excellent. Acceptable α/β ratios in the products, even for such challenging reactions as the double-glycosylation of **12** to give tetrasaccharide **13**, are therefore attainable.

In summary, di-, tri-, and tetrasaccharide heteroanalogues **1**–**3** corresponding to the terminal end of the glycosylinositolphospholipid oligosaccharide of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease, have been synthesized by selective activation of selenoglycoside donors in the presence of thioglycoside acceptors. The selenoglycoside **5** is a versatile furanosyl donor that gives oligosaccharides with β-selectivity. The three target compounds **1**–**3** will be tested as inhibitors against *T. cruzi* proliferation and also in the inhibition of proliferation of B-lymphocytes.

3. Experimental

3.1. General methods

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, $^{1}H/X$ double-resonance probe. Sugar rings are denoted A, B, C, and D, respectively, as shown in the diagrams for compounds **1**–**3**. High resolution liquid secondary ion mass spectra (FAB) were recorded on a Kratos Concept H instrument using glycerine–thioglycerine as the matrix. Analytical thin-layer chromatography (TLC) was performed on

aluminum plates precoated with Merck silica gel 60F-254 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.

3.2. *Phenyl 2,3,5,6-tetra*-O-*acetyl-1-seleno-4-thio-α/β*-D-*galactofuranoside* **5**

To a solution of 50% H_3PO_2 (50 mL) was added diphenyldiselenide (1.0 g, 3.2 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 (2×30 mL). The combined extracts were washed with ice cold water (20 mL) and dried over $MgSO_4$. The solution was filtered into a round-bottomed flask and cooled to 0°C. Peracetylated 4-thiogalactofuranose **4** (1.29 g, 3.17 mmol) and $Et_2O{:}BF_3$ (0.60 mL, 4.7 mmol) were added. The reaction mixture was stirred at 0°C for 40 min. The mixture was quenched with cold satd. $NaHCO_{3(aq)}$ and extracted with CH_2Cl_2 (2×30 mL). The combined extracts were washed with additional satd. $NaHCO_{3(aq)}$ and dried over $MgSO_4$. The solvent was removed in vacuo and the crude product was purified by column chromatography using hexanes:EtOAc (2:1) as the eluant. The desired monosaccharide donor **5** was obtained as a colorless syrup (1.5 g, 94%, α:β=1:3).

Compound **5α**: ^{1}H NMR ($CDCl_3$): δ 7.65–7.30 (5H, m, aromatic), 5.64 (1H, dd, $J_{2,3}$=7.3, $J_{3,4}$=6.3 Hz, H3), 5.38 (1H, dd, $J_{1,2}$=5.2 Hz, H2), 5.24 (1H, m, H5), 4.96 (1H, d, H1), 4.36 (1H, dd, $J_{5,6}$=4.0, $J_{6,\ 6'}$=12.1 Hz, H6), 4.06 (1H, dd, $J_{5,6'}$=5.7 Hz, H6′), 3.52 (1H, dd, $J_{4,5}$=6.4 Hz, H4), 2.16, 2.06, 2.05, 1.85 (4s, 3H each, C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.33–169.54 (4C, 4×*C*(O)CH_3), 135.74–127.69 (6 C, aromatic), 78.15 (C2), 74.94 (C3), 70.41 (C5), 63.43 (C6), 47.56 (C4), 45.56 (C1, J_{C1H1}=163 Hz), 20.86–20.32 (4C, 4×C(O)*C*H_3).

Compound **5β**: ^{1}H NMR ($CDCl_3$): δ 7.65–7.30 (5H, m, aromatic), 5.46 (1H, dd, $J_{3,4}$=6.4 Hz, H3β), 5.26 (1H, dd, $J_{2,3}$=6.6 Hz, H2), 5.23 (1H, m, H5), 4.59 (1H, d, $J_{1,2}$=6.5 Hz, H1), 4.27 (1H, dd, $J_{5,6}$=3.9, $J_{6,\ 6'}$=12.1 Hz, H6), 4.04 (1H, dd, $J_{5,6'}$=5.9 Hz, H6′), 3.66 (1H, dd, $J_{4,5}$=6.7 Hz, H4), 2.10, 2.047, 2.045, 1.99 (4s, 3H each, C(O)CH_3). ^{13}C NMR ($CDCl_3$): δ 170.33–169.54 (4C, 4×*C*(O)CH_3), 135.74–127.69 (6 C, aromatic), 81.31 (C3), 76.45 (C2), 69.80 (C5), 63.43 (C6), 49.01 (C4), 45.14 (C1, J_{C1H1}=160 Hz), 20.86–20.32 (4C, 4×C(O)*C*H_3). Anal. calcd for $C_{20}H_{24}O_8SeS$: C, 47.72; H, 4.81. Found: C, 47.99; H, 4.81 (for the α/β mixture).

3.3. *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 (25–30 mM of acceptor) at room temperature 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 with 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. $NaHCO_{3(aq)}$. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by column chromatography.

3.4. *Ethyl 3-O-(2,3,5,6-tetra-O-acetyl-4-thio-β-D-galactofuranosyl)-2-O-benzoyl-4,6-O-benzylidene-1-thio-α-D-mannopyranoside* **7**

The thioglycoside acceptor **6** (99 mg, 0.24 mmol) was glycosylated with the selenoglycoside donor **5** (143 mg, 0.28 mmol) following the general procedure. The reaction time was 5 min at 0°C. The disaccharide was purified by column chromatography using hexanes:EtOAc (2:1) as the eluant. The desired disaccharide **7** was obtained as a white foam (143 mg, 80%): $[\alpha]_D$ −69 (*c* 0.18, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 8.13–7.32 (10H, m, aromatic), 5.66 (1H, s, C*H*Ph), 5.58 (1H, dd, $J_{1,2}$=1.4, $J_{2,3}$=3.4 Hz, H2B), 5.41 (1H, d, H1B), 5.22 (1H, d, $J_{1,2}$=2.6 Hz, H1A), 5.19 (1H, dd, $J_{2,3}$=4.6 Hz, H2A), 5.17 (1H, ddd, $J_{5,6}$=3.8 Hz, H5A), 5.11 (1H, dd, $J_{3,4}$=7.4 Hz, H3A), 4.33 (1H, ddd, H5B), 4.28 (1H, dd, $J_{3,4}$=9.7 Hz, H3B), 4.27 (1H, dd, $J_{5,6}$=4.8, $J_{6,6'}$=10.0 Hz, H6B), 4.17 (1H, dd, $J_{4,5}$=9.5 Hz, H4B), 4.05 (1H, dd, $J_{6,6'}$=12.0 Hz, H6A), 3.91 (1H, dd, $J_{5,6'}$=9.8 Hz, H6B′), 3.89 (1H, dd, $J_{5,6'}$=6.5 Hz, H6A′), 3.75 (1H, dd, $J_{4,5}$=5.4 Hz, H4A), 2.76–2.60 (2H, m, SC*H*$_2$CH$_3$), 2.04, 1.95, 1.94, 1.84 (4s, 3H each, C(O)C*H*$_3$), 1.31 (3H, t, *J*=7.4 Hz, SCH$_2$C*H*$_3$). ^{13}C NMR (CD_2Cl_2): δ 170.54, 170.17, 170.11, 169.62 (*C*(O)CH$_3$), 165.77 (*C*(O)Ph), 137.99–126.60 (12C, aromatic), 102.13 (*C*HPh), 87.58 (C1A, J_{C1H1}=167 Hz), 83.80 (C1B, J_{C1H1}=168 Hz), 82.90 (C2A), 78.33 (C4B), 77.50 (C3A), 74.14 (C3B), 71.75 (C2B), 69.49 (C5A), 68.99 (C6B), 64.96 (C5B), 64.07 (C6A), 50.0 (C4A), 26.01 (S*C*H$_2$CH$_3$), 20.89, 20.84, 20.77, 20.67 (C(O)*C*H$_3$), 15.11 (SCH$_2$*C*H$_3$). Anal. calcd for $C_{36}H_{42}O_{14}S_2$: C, 56.68; H, 5.55. Found: C, 56.44; H, 5.55.

3.5. *Ethyl 3-O-(4-thio-β-D-galactofuranosyl)-1-thio-α-D-mannopyranoside* **1**

To a solution of the disaccharide **7** (40 mg, 0.052 mmol) in freshly distilled MeOH (5 mL) was added 1 M NaOMe/MeOH (1 mL). The reaction mixture was stirred under an N_2 atmosphere for 2.5 h and then neutralized by the addition of Rexyn 101 (H^+) ion exchange resin. The resin was removed by filtration and the filtrate was concentrated in vacuo. The crude product was dissolved in 80% $AcOH_{(aq)}$ and stirred overnight at room temperature. The reaction mixture was concentrated and co-concentrated with distilled water to remove traces of AcOH. The crude product was purified by column chromatography using $CHCl_3$:MeOH (3:1) as the eluant. The disaccharide **1** was obtained as a clear syrup (15 mg, 71%): $[\alpha]_D$ −34 (*c* 0.029, H_2O); ^{1}H NMR (D_2O): δ 5.31 (1H, d, $J_{1,2}$=1.0 Hz, H1B), 5.13 (1H, d, $J_{1,2}$=5.5 Hz, H1A), 4.20 (1H, dd, $J_{2,3}$=~3.0 Hz, H2B), 4.06 (1H, dd, $J_{2,3}$=8.3 Hz, H2A), 3.99 (1H, m, H5B), 3.92 (1H, m, H5A), 3.90 (1H, dd, H3A), 3.85 (1H, dd, $J_{5,6}$=2.2, $J_{6,6'}$=12.4 Hz, H6B), 3.74 (1H, dd, $J_{5,6'}$=6.1 Hz, H6B′), 3.70 (1H, dd, $J_{3,4}$=$J_{4,5}$=9.4 Hz, H4B), 3.67 (1H, dd, H3B), 3.55 (1H, dd, $J_{5,6}$=4.7, $J_{6,6'}$=11.7 Hz, H6A), 3.50 (1H, dd, $J_{3,4}$=8.8, $J_{4,5}$=3.9 Hz, H4A), 3.48 (1H, dd, $J_{5,6'}$=7.0 Hz, H6A′), 2.72–2.55 (2H, m, SC*H*$_2$CH$_3$), 1.23 (3H, t, *J*=7.3 Hz, SCH$_2$C*H*$_3$). ^{13}C NMR (D_2O): δ 88.46 (C1A, J_{C1H1}=165 Hz), 86.66 (C1B, J_{C1H1}=167 Hz), 84.29 (C2A), 81.87 (C3B), 77.77 (C3A), 75.78 (C5B), 72.53 (C5A), 71.34 (C2B), 68.17 (C4B), 67.02 (C6A), 63.46 (C6B), 52.89 (C4A), 27.50 (S*C*H$_2$CH$_3$), 16.00 (SCH$_2$*C*H$_3$). Anal. calcd for $C_{14}H_{26}O_9S_2$: C, 41.78; H, 6.51. Found: C, 41.65; H, 6.63.

3.6. *Methyl 2-O-(3-O-(2,3,5,6-tetra-O-acetyl-4-thio-α/β-D-galactofuranosyl)-4,6-di-O-acetyl-2-O-benzoyl-α-D-mannopyranosyl)-3,4,6-tri-O-acetyl-α-D-mannopyranoside* **11**

The methyl glycoside acceptor **8** (121 mg, 0.26 mmol) was glycosylated with the thioglycoside donor **7** (238 mg, 0.31 mmol) following the general procedure. The reaction time was 2.5 h at 0°C. The trisaccharide **9** was obtained as a mixture of compounds (α:β, 1.3:1). The mixture could not be purified by column chromatography.

Compound **9α**: ^{1}H NMR (CD_2Cl_2): δ 8.20–7.10 (25H, m, aromatic), 5.67 (1H, dd, $J_{1,2}$=1.8 Hz, H2B), 5.66–5.62 (3H, m, H3A, H1A, CHPh), 5.25 (1H, ddd, H5A), 5.21 (1H, d, H1B), 5.00 (1H, dd, $J_{1,2}$=4.1, $J_{2,3}$=9.5 Hz, H2A), 4.89–4.53 (6H, CH_2Ph), 4.79 (1H, d, $J_{1,2}$=1.8 Hz, H1C), 4.34 (1H, dd, $J_{2,3}$=3.7, $J_{3,4}$=9.3 Hz, H3B), 4.31 (1H, dd, $J_{5,6}$=4.7, $J_{6,6'}$=10.1 Hz, H6B), 4.25 (1H, dd, $J_{5,6}$=3.9, $J_{6,6'}$=12.1 Hz, H6A), 4.12–4.01 (4H, m, H6A′, H2C, H5B, H4B), 3.93–3.84 (3H, m, H4C, H3C, H6B′), 3.78–3.71 (3H, m, H5C, H6C, H6C′), 3.50 (1H, dd, $J_{3,4}$=7.4, $J_{4,5}$=6.1 Hz, H4A), 3.37 (3H, OCH_3), 2.0–1.46 (4s, 3H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 172.52–169.74 (4C, *C*(O)CH_3), 165.53 (*C*(O)Ph), 139.06–125.61 (30C, aromatic), 102.00 (*C*HPh), 100.60 (C1B), 100.48 (C1C), 82.35 (C1A), 80.24 (C3C), 78.40 (C2A), 78.00 (C4B), 75.58 (C2C), 75.38–75.18 (2C, C4C, *C*H_2Ph), 74.36 (C3A), 73.84 (C3B), 73.59 (*C*H_2Ph), 72.80 (*C*H_2Ph), 72.23 (C5C), 71.14 (C5A), 69.92 (C6C), 69.47 (C2B), 69.07 (C6B), 64.07 (C5B), 63.95 (C6A), 55.01 (-O*C*H_3), 44.58 (C4A), 21.51–20.05 (4C, C(O)*C*H_3).

Compound **9β**: ^{1}H NMR (CD_2Cl_2): δ 8.20–7.10 (25H, m, aromatic), 5.76 (1H, dd, $J_{1,2}$=1.6, $J_{2,3}$=3.7 Hz, H2B), 5.66–5.62 (1H, s, CHPh), 5.27 (1H, d, $J_{1,2}$=2.7 Hz, H1A), 5.22 (1H, dd, $J_{2,3}$=4.8 Hz, H2A), 5.20 (1H, d, H1B), 5.17 (1H, ddd, $J_{5,6}$=4.0, $J_{5,6'}$=9.3, $J_{4,5}$=5.2 Hz, H5A), 5.12 (1H, dd, $J_{3,4}$=7.5 Hz, H3A), 4.89–4.53 (6H, CH_2Ph), 4.79 (1H, d, $J_{1,2}$=1.8 Hz, H1C), 4.47 (1H, dd, $J_{3,4}$=9.6 Hz, H3B), 4.31 (1H, dd, $J_{5,6}$=4.7, $J_{6,6'}$=10.1 Hz, H6B), 4.15 (1H, dd, $J_{4,5}$=9.4 Hz, H4B), 4.12–4.01 (3H, m, H6A, H2C, H5B), 3.93–3.84 (4H, m, H3C, H4C, H6A′, H6B′), 3.78–3.71 (4H, m, H4A, H6C, H6C′, H5C), 3.37 (3H, s, OCH_3), 2.00–1.84 (4s, 3H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.52–169.74 (4C, *C*(O)CH_3), 165.46 (*C*(O)Ph), 139.06–125.61 (30C, aromatic), 102.09 (*C*HPh), 100.60 (C1B), 100.18 (C1C), 87.64 (C1A), 82.76 (C2A), 80.41 (C4B), 80.10 (C3C), 77.43 (C3A), 75.43 (C2C), 75.38–75.18 (2C, C4C, *C*H_2Ph), 73.56 (*C*H_2Ph), 72.71 (*C*H_2Ph), 72.16 (C5C), 71.77, 71.73 (C2B, C3B), 69.69 (C6C), 69.47 (C5A), 69.03 (C6B), 64.66 (C5B), 63.32 (C6A), 55.01 (-O*C*H_3), 49.70 (C4A), 21.51–20.05 (4C, C(O)*C*H_3).

In another experiment, the methyl glycoside acceptor **8** (121 mg, 0.26 mmol) was glycosylated with the thioglycoside donor **7** (238 mg, 0.31 mmol) following the general procedure, except that 1 equiv. of NIS was used. The reaction time was 2.5 h at 0°C–rt. The trisaccharide **9** was obtained as a mixture of compounds (α:β, 0.47:1). The mixture could not be purified by column chromatography.

The mixture of trisaccharides **9α** and **9β** was dissolved in 80% $AcOH_{(aq)}$ and stirred overnight at room temperature. The reaction mixture was concentrated and co-concentrated with distilled toluene to remove traces of AcOH. Compounds **10α** and **10β** were obtained as a clear glass (α:β, 1:1.3).

Compound **10α**: ^{1}H NMR (CD_2Cl_2): δ 8.10–7.10 (20H, m, aromatic), 5.62 (1H, dd, $J_{2,3}$=9.3, $J_{3,4}$=7.8 Hz, H3A), 5.57 (1H, dd, $J_{1,2}$=1.8, $J_{2,3}$=3.2 Hz, H2B), 5.49 (1H, d, $J_{1,2}$=4.4 Hz, H1A), 5.18 (1H, m, H1B), 5.11 (1H, ddd, H5A), 5.07 (1H, dd, H2A), 4.90–4.54 (6H, CH_2Ph), 4.79 (1H, d, $J_{1,2}$=1.9 Hz, H1C), 4.18 (1H, dd, $J_{5,6}$=3.9, $J_{6,6'}$=12.0 Hz, H6A), 4.10–3.93 (4H, m, H6A′, H2C, H3B, H4B*), 3.92–3.79 (6H, m, H6C, H6C′, H6B, H6B′, H3C, H4C*), 3.78–3.67 (2H, m, H5C*, H5B*), 3.52 (1H, dd, $J_{4,5}$=5.9 Hz, H4A), 3.34 (3H, OCH_3), 2.01–1.85 (4s, 3H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 171.18–170.13 (4C, *C*(O)CH_3), 165.54 (*C*(O)Ph), 139.04–127.88 (24C, aromatic), 100.04 (C1C), 99.50 (C1B), 82.34 (C1A), 80.16 (C3C), 78.40 (C2A), 77.29 (C2C), 75.44–75.21 (2C, C3B, *C*H_2Ph), 75.11–66.75 (4C, C4C, C4B, C5B, C5C), 74.25 (C3A), 73.52 (*C*H_2Ph), 72.87 (*C*H_2Ph), 72.03 (C5A), 71.99 (C2B) 63.09 (C6A), 62.72, 62.68 (C6B, C6C), 55.05 (-O*C*H_3), 44.59 (C4A), 21.15–20.75 (4C, C(O)*C*H_3). *Assignments may be interchanged. Compound **10β**: ^{1}H NMR (CD_2Cl_2): δ 8.10–7.10 (20H, m, aromatic), 5.55 (1H, dd, $J_{1,2}$=1.8, $J_{2,3}$=3.1 Hz, H2B), 5.33 (1H, d, H1A), 5.28 (1H, dd, $J_{1,2}$=2.7, $J_{2,3}$=4.8 Hz, H2A), 5.25–5.19 (2H, m, H3A, H5A), 5.18 (1H, m, H1B), 4.90–4.54 (6H, CH_2Ph), 4.81 (1H, d, $J_{1,2}$=1.9 Hz, H1C), 4.24 (1H, dd, $J_{5,6}$=4.0, $J_{6,6'}$=12.1 Hz, H6A), 4.10–3.93 (4H, m, H6A′, H3B, H2C, H4B*), 3.92–3.79 (7H, m, H3C, H4C*, H4A, H6B, H6B′, H6C, H6C′), 3.78–3.67 (2H, m, H5C*, H5B*), 3.35 (3H, s, OCH_3), 2.01–1.85 (4s, 3H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 171.18–170.13 (4C, *C*(O)CH_3), 165.54

(*C*(O)Ph), 139.04–127.88 (24C, aromatic), 100.04 (C1C), 99.80 (C1B), 87.33 (C1A), 82.18 (C2A), 80.16 (C3C), 78.54 (C2C), 76.95 (C3A), 75.44–75.21 (2C, C3B, *C*H_2Ph), 75.11–66.75 (4C, C4C, C4B, C5B, C5C), 73.52 (*C*H_2Ph), 72.64 (*C*H_2Ph), 70.83 (C5A), 69.77 (C2B), 63.92 (C6A), 62.72, 62.68 (C6B, C6C), 55.05 (-O*C*H_3), 50.45 (C4A), 21.15–20.75 (4C, C(O)*C*H_3). *Assignments may be interchanged.

In subsequent experiments the mixture of compounds 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 then washed with water. The combined filtrates were evaporated to dryness and the residue was co-evaporated several times with distilled H_2O to remove any traces of AcOH. The mixture was acetylated using acetic anhydride (5 mL) and pyridine (10 mL). After 15 h the solvent was removed by rotary evaporation under high vacuum. The crude product was dissolved in CH_2Cl_2 (50 mL), washed with H_2O, dried over Na_2SO_4, and the solvent was removed in vacuo. The resulting crude product was purified by column chromatography using hexanes:EtOAc (1:1.5) as the eluant. The ^{1}H NMR spectrum showed a 2:3 ratio of an α:β mixture of the desired trisaccharide **11β** and its isomer **11α**. The products (**11α**/**β**) were obtained as a white foam (160 mg, 60% α:β, 2:3).

Compound **11α**: ^{1}H NMR ($CDCl_3$): δ 8.10–7.42 (5H, m, aromatic), 5.69 (1H, dd, $J_{2,3}$=9.8, $J_{3,4}$=8.2 Hz, H3A), 5.55 (1H, dd, $J_{1,2}$=2.0, $J_{2,3}$=3.0 Hz, H2B), 5.41 (1H, dd, $J_{3,4}$=$J_{4,5}$=9.8 Hz, H4B), 5.36 (1H, d, $J_{1,2}$=4.2 Hz, H1A), 5.33–5.27 (3H, m, H3C, H4C, H5A), 5.07 (1H, d, H1B), 4.92 (1H, dd, H2A), 4.87 (1H, d, H1C), 4.28–4.04 (10H, m, H2C, H3B, H5B, H5C, H6A, H6A′, H6B, H6B′, H6C, H6C′), 3.58 (1H, dd, $J_{4,5}$=6.6 Hz, H4A), 3.40 (3H, s, OC*H*$_3$), 2.14, 2.12, 2.10, 2.08, 2.03, 2.02, 2.01, 2.00, 1.88 (9s, 3H, C(O)C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 170.59–169.39 (9C, *C*(O)CH_3), 165.33 (*C*(O)Ph), 133.53–128.41 (6C, aromatic), 99.56, 99.32 (C1B, C1C, both J_{C1H1}=~172 Hz), 81.59 (C1A, J_{C1H1}=165 Hz), 77.72 (C2A), 77.24 (C2C), 73.08 (2C, C3A, C3B), 71.51 (C2B), 70.49, 70.12 (C3C, C5A), 69.39 (C5B), 68.41 (C5C), 67.92 (C4B), 66.21 (C4C), 62.72, 62.57 (3C, C6A, C6B, C6C), 55.21 (-O*C*H_3), 43.69 (C4A), 20.96–20.27 (9C, C(O)*C*H_3).

Compound **11β**: ^{1}H NMR ($CDCl_3$): δ 8.10–7.42 (5H, m, aromatic), 5.50 (1H, dd, $J_{1,2}$=2.0, $J_{2,3}$=3.2 Hz, H2B), 5.36 (1H, dd, *J*=8.4, *J*=9.8 Hz, H4B), 5.33–5.27 (2H, m, H3C, H4C), 5.24 (1H, d, $J_{1,2}$=2.5 Hz, H1A), 5.22–5.19 (2H, m, H2A, H5A), 5.18 (1H, dd, $J_{2,3}$=4.6, $J_{3,4}$=6.6 Hz, H3A), 5.03 (1H, d, H1B), 4.87 (1H, dd, H1C), 4.30 (1H, dd, $J_{5,6}$=3.8, $J_{6,6'}$=12.1 Hz, H6A), 4.28–4.04 (6H, m, H2C, H3B, H6B, H6B′, H6C, H6C′), 4.02 (1H, dd, $J_{5,6}$=7.2 Hz, H6A′), 3.96–3.90 (2H, m, H5B, H5C), 3.74 (1H, dd, $J_{4,5}$=6.4 Hz, H4A), 3.41 (3H, s, OC*H*$_3$), 2.15, 2.13, 2.11, 2.10, 2.09, 2.04, 2.03, 1.99, 1.91 (9s, 3H each, C(O)C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 170.59–169.39 (9C, *C*(O)CH_3), 165.33 (*C*(O)Ph), 133.53–128.41 (6C, aromatic), 99.56, 99.32 (C1B, C1C, both J_{C1H1}=~172 Hz), 87.51 (C1A, J_{C1H1}=167 Hz), 82.45 (C2A), 77.24 (C2C), 76.89 (C3A), 74.48 (C3B), 70.20 (C3C), 69.59, 69.30 (C5A, C5C), 68.41 (C5B), 68.20 (C2B), 66.61, 66.28 (C4B, C4C), 63.55 (C6A), 62.72, 62.57 (C6B, C6C), 55.21 (-O*C*H_3), 49.92 (C4A), 20.96–20.27 (9C, C(O)*C*H_3). Anal. calcd for $C_{44}H_{56}O_{25}S$: C, 51.95; H, 5.55. Found: C, 51.82; H, 5.50.

*3.7. Methyl 2-*O*-(3-*O*-(4-thio-α/β-*D*-galactofuranosyl)-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* ***2***

To a solution of the trisaccharides **11α**/**β** (29 mg, 0.028 mmol) in freshly distilled MeOH (3 mL) was added 1 M NaOMe/MeOH (0.5 mL). The reaction mixture was stirred 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 crude product was purified by column chromatography using $CHCl_3$:MeOH (1:1) as the eluant. The desired oligosaccharide **2β** and its α-anomer were obtained as a clear syrup (14 mg, 94% α:β, 1:1.6).

Compound **2α**: ^{1}H NMR (D_2O): δ 5.20 (1H, d, $J_{1,2}$=4.2 Hz, H1A), 5.02 (1H, dd, $J_{1,2}$=1.7 Hz, H1B), 4.96 (1H, d, $J_{1,2}$=1.5 Hz, H1C), 4.39 (1H, dd, $J_{2,3}$=3.1 Hz, H2B), 4.21 (1H, dd, $J_{2,3}$=9.9, $J_{3,4}$=8.2 Hz, H3A), 4.08 (1H, dd, H2A), 3.97–3.81 (6H, m, H2C, H5A, H6C, H6C′, H3B, H3C), 3.80–3.61 (5H, m, H4B, H6B, H6B′, H4C, H5C), 3.60–3.46 (3H, m, H5B, H6A, H6A′), 3.37 (3H, s, -OCH_3), 3.23 (1H, dd, $J_{4,5}$=3.8 Hz, H4A). ^{13}C NMR (D_2O): δ 104.50 (C1B), 101.95 (C1C), 86.81 (C1A), 81.67 (C3B), 81.07 (2C, C2A, C2C), 77.81 (C3A), 75.73 (C4B), 75.22 (C5B), 73.44 (C5A), 72.82 (C3C), 71.29 (C2B), 69.57 (C4C), 68.81 (C5C), 66.85 (C6A), 63.69, 63.55 (C6B, C6C), 57.49 (-O*C*H_3), 51.05 (C4A).

Compound **2β**: ^{1}H NMR (D_2O): δ 5.18 (1H, d, $J_{1,2}$=5.8 Hz, H1A), 5.03 (1H, dd, $J_{1,2}$=1.7 Hz, H1B), 4.95 (1H, d, $J_{1,2}$=1.6 Hz, H1C), 4.24 (1H, dd, $J_{2,3}$=2.4 Hz, H2B), 4.08 (1H, dd, $J_{2,3}$=8.3 Hz, H2A), 3.97–3.81 (6H, m, H2C, H5A, H3A, H6C, H6C′, H3C), 3.80–3.61 (6H, m, H3B, H4B, H6B, H6B′, H4C, H5C), 3.60–3.46 (4H, m, H5B, H6A, H4A, H6A′), 3.37 (3H, s, -O*C*H_3). ^{13}C NMR (D_2O): δ 104.64 (C1B), 101.95 (C1C), 88.55 (C1A), 84.23 (C2A), 81.49 (C3B), 81.07 (C2C), 77.58 (C3A), 75.95 (C4B), 75.22 (C5B), 72.82 (C3C), 72.50 (C5A), 69.57 (C4C) 69.53 (C2B), 67.94 (C5C), 67.01 (C6A), 63.69, 63.55 (C6B, C6C), 57.49 (O*C*H_3), 52.67 (C4A). FAB HRMS: calcd for $C_{19}H_{34}O_{15}S$+Na: 557.1516. Found: M+Na: 557.1521.

*3.8. Ethyl 2-*O*-(3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-β-*D*-galactofuranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranosyl)-3-*O*-(2,3,5,6-tetra-*O*-acetyl-4-thio-β-*D*-galactofuranosyl)-4,6-*O*-benzylidene-1-thio-α-*D*-mannopyranoside **13***

The thioglycoside acceptor **12** (76 mg, 0.116 mmol) was glycosylated with the selenoglycoside donor **5** (140 mg, 0.279 mmol) following the general procedure. The reaction time was 10 min at 0°C. The crude product was purified by column chromatography using hexanes:EtOAc (1:1) as the eluant. The desired tetrasaccharide **13**, accompanied by 20% of an isomeric tetrasaccharide, was obtained as a white foam (145 mg, 74% based on **13**): ^{1}H NMR (CD_2Cl_2): δ 7.58–7.29 (15H, m, aromatic), 5.63 (1H, s, C*H*Ph), 5.62 (1H, s, C*H*Ph), 5.39 (1H, dd, $J_{1,2}$=2.4, $J_{2,3}$=4.0 Hz, H2A), 5.36 (1H, b s, H1C), 5.30 (1H, d, $J_{1,2}$=1.5 Hz, H1B), 5.29 (1H, d, H1A), 5.25 (1H, dd, $J_{1,2}$=2.6, $J_{2,3}$=4.3 Hz, H2D), 5.25–5.16 (5H, m, H3A, H3D, H5A, H5D, H1D), 4.85, 4.74 (2H, 2d, J_{gem}=11.2 Hz, OCH_2Ph), 4.26–4.19 (4H, m, H3B, H5B or H5C, H6B, H6C), 4.18–4.10 (4H, m, H2C, H3C, H4C, H4B), 4.10–4.01 (3H, m, H2B, H6A, H6D), 3.96–3.80 (6H, m, H5B or H5C, H6A′, H6B′, H6C′, H6D′, H4A), 3.77 (1H, dd, $J_{3,4}$=6.8, $J_{4,5}$=5.9 Hz, H4D), 2.67 (2H, m, SC$H_2$$CH_3$), 2.07, 2.06 (2s, 3H each, C(O)CH_3), 2.03 (6H, s, C(O)CH_3), 1.95 (6H, s, C(O)CH_3), 1.94, 1.93 (2s, 3H each, C(O)CH_3), 1.32 (3H, t, J=7.4 Hz, SCH_2CH_3). ^{13}C NMR (CD_2Cl_2): δ 170.55–169.95 (8C, *C*(O)CH_3), 138.8–126.4 (18C, aromatic), 101.96 (*C*HPhB), 101.88 (*C*HPhC), 101.58 (C1B, J_{C1H1}=175 Hz), 88.67, 88.49 (C1A, J_{C1H1}=167 Hz, C1D, J_{C1H1}=167 Hz), 85.15 (C1C, J_{C1H1}=~166Hz), 83.77 (C2A), 83.60 (C2D), 78.37, 78.12, 78.02, 77.87, 77.41 (C2C, C3A, C3D, C4B, C4C), 76.82 (C2B), 76.12 (C3C), 75.92 (C3B), 74.33 (*C*H_2Ph), 69.91, 69.84 (C5A, C5D), 68.95, 68.86 (C6B, C6C), 65.25, 64.98 (C5B, C5C), 64.11, 63.94 (C6A, C6D), 50.49 (C4A), 50.45 (C4D), 25.98 (S*C*$H_2$$CH_3$), 21.16, 21.07, 20.99, 20.88, 20.84, 20.76, 20.75 (8C, C(O)*C*H_3), 15.20 (SCH_2*C*H_3). Anal. calcd for $C_{63}H_{76}O_{26}S_3$: C, 56.23; H, 5.70. Found: C, 56.12; H, 5.76.

*3.9. Ethyl 2-*O*-(3-*O*-(4-thio-β-*D*-galactofuranosyl)-α-*D*-mannopyranosyl)-3-*O*-(4-thio-β-*D*-galactofuranosyl)-1-thio-α-*D*-mannopyranoside **3***

To a solution of the tetrasaccharide **13** (140 mg, 0.10 mmol) in freshly distilled MeOH (10 mL) was added 1 M NaOMe/MeOH (1 mL). The reaction mixture was stirred at room temperature, 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 partially deprotected tetrasaccharide was dissolved in 80% AcOH (8 mL) and stirred overnight at room temperature. The solvent was removed in vacuo and the residue was co-concentrated with H_2O several times to remove the AcOH. The compound was then dissolved in 4:2:1 H_2O:EtOH:AcOH (10 mL) and stirred with $Pd(OH)_2$–C (200 mg) under H_2 (52 psi). After 24 h the reaction mixture was filtered through a pad of Celite, which was subsequently washed with EtOH. The combined filtrates were evaporated to dryness and the hydrogenolysis reaction was repeated twice more, using the same quantities of solvent and Pd. The residue was co-evaporated several times with H_2O to remove any traces of AcOH. The crude product was purified by column chromatography using EtOAc:MeOH:H_2O (6:2:1) as the eluant. The tetrasaccharide **3** (80% pure) was obtained as a clear glass (20 mg, 25%). ^{1}H NMR (D_2O): δ 5.52 (1H, d, $J_{1,2}$=1.2 Hz, H1C), 5.18 (1H, d, $J_{1,2}$=5.6 Hz, H1A), 5.13 (1H, d, $J_{1,2}$=5.9 Hz, H1D), 5.08 (1H, d, $J_{1,2}$=1.5 Hz, H1B), 4.22 (1H, m, H2C), 4.20 (1H, dd, $J_{2,3}$=2.6 Hz, H2B), 4.05 (1H, dd, $J_{2,3}$=8.3 Hz, H2A), 3.99 (1H, dd, $J_{2,3}$=8.5 Hz, H2D), 3.96–3.65 (13H, m, H5C, H5A, H5D, H3A, H3D, H6B, H6C, H5B, H6C′, H4C, H3C, H3B, H6B′), 3.62 (1H, dd, $J_{3,4}$=$J_{4,5}$=9.6 Hz H4B), 3.52 (2H, dd, $J_{5,6A}$= $J_{5,6D}$=4.6, $J_{6,6'A}$=$J_{6,6'D}$=11.8 Hz, H6A, H6D), 3.49 (2H, dd, $J_{3,4A}$= $J_{3,4D}$=8.9, $J_{4,5A}$= $J_{4,5D}$=3.4 Hz, H4A, H4D), 3.47 (2H, dd, $J_{5,6'A}$= $J_{5,6'D}$=7.0 Hz, H6A′, H6D′), 2.63 (2H, m, SCH_2CH$_3$), 1.22 (3H, t, J=7.0 Hz, SCH$_2$CH_3). ^{13}C NMR (D_2O): δ 104.39 (C1B, J_{C1H1}=~174 Hz), 88.43 (C1A, J_{C1H1}=164 Hz), 88.26 (C1D, J_{C1H1}=164 Hz), 85.46 (C1C, J_{C1H1}=167 Hz), 84.31 (C2D), 84.21 (C2A), 81.89, 81.35 (C3B, C3C), 79.88 (C2C), 77.63, 77.50 (C3A, C3D), 76.12 (C5B), 75.84 (C5C), 72.48 (2C, C5A, C5D), 69.50 (C2B), 68.42 (C4C), 67.99 (C4B), 66.98 (2C, C6A, C6D), 63.69 (C6B), 63.42 (C6C), 52.69, 52.69 (C4A, C4D), 27.79 (SCH$_2$CH$_3$), 16.89 (SCH$_2$$CH_3$). FAB HRMS: calcd for $C_{26}H_{46}O_{18}S_3$+Na: 765.1744. Found: M+Na: 765.1746.

3.10. 3-O-*(2,3,5,6-Tetra*-O-*acetyl-4-thio-α/β*-D-*galactofuranosyl)-2*-O-*benzoyl-4,6*-O-*benzylidene-α*-D-*mannopyranose* ***14***

The thioglycoside donor **7** (60 mg, 0.079 mmol) was reacted with NIS (21.2 mg, 0.094 mmol) and TfOH (0.4 μl, 0.039 mmol). The reaction time was 45 min at 0°C. The disaccharide was purified by column chromatography using toluene:EtOAc (1.5:1) as the eluant. The desired disaccharide **14α**/**14β** was obtained as a clear glass (32 mg, 58%).

Compound **14α**: ^{1}H NMR (CD_2Cl_2): δ 8.18–7.09 (10H, m, aromatic), 5.70 (1H, dd, $J_{1,2}$=1.6, $J_{2,3}$=3.6 Hz, H2B), 5.65 (1H, dd, H3A), 5.63 (1H, d, $J_{1,2}$=3.9 Hz, H1A), 5.61 (1H, s, CHPh), 5.32 (1H, dd, H1B), 5.29 (1H, ddd, H5A), 5.02 (1H, dd, $J_{2,3}$=9.1 Hz, H2A), 4.59 (1H, dd, $J_{3,4}$=9.4 Hz, H3B), 4.32 (1H, dd, $J_{5,6}$=3.4 Hz, H6B), 4.29 (1H, dd, H6A), 4.22–4.14 (2H, m, H5B, H6A′), 4.13 (1H, dd, $J_{4,5}$=8.7 Hz, H4B), 3.87 (1H, dd, $J_{5,6'}$=$J_{6,6'}$=10.0 Hz, H6B′), 3.51 (1H, dd, $J_{3,4}$=$J_{4,5}$=7.4 Hz, H4A), 3.48 (1H, d, $J_{1,OH}$=3.6 Hz, OH), 2.08, 2.03, 2.00, 1.45 (4s, 3H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 171.66–171.17 (4C, C(O)CH$_3$), 139.33–127.96 (12C, aromatic), 103.37 (CHPh), 95.40 (C1B), 84.64 (C1A), 82.03 (C4B), 80.73 (C2A), 76.40 (C2B), 74.08 (C5A), 73.73 (C3A), 72.59 (C3B), 70.70 (C6B), 65.82–65.19 (C5B), 64.64 (C6A), 46.43 (C4A), 22.51–21.55 (4C, C(O)CH$_3$).

Compound **14β**: ^{1}H NMR (CD_2Cl_2): δ 8.13–7.32 (10H, m, aromatic), 5.64 (1H, s, CHPh), 5.54 (1H, dd, $J_{1,2}$=1.7, $J_{2,3}$=3.5 Hz, H2B), 5.32 (1H, dd, H1B), 5.26 (1H, d, $J_{1,2}$=2.7 Hz, H1A), 5.22 (1H, dd, $J_{2,3}$=4.8 Hz, H2A), 5.18 (1H, ddd, H5A), 5.12 (1H, dd, $J_{3,4}$=7.6 Hz, H3A), 4.37 (1H, dd, $J_{3,4}$=9.6 Hz, H3B), 4.28 (1H, dd, $J_{5,6}$=4.0, $J_{6,6'}$=10.0 Hz, H6B), 4.22–4.13 (1H, m, H5B), 4.12 (1H, dd, $J_{4,5}$=9.5 Hz, H4B), 4.05 (1H, dd, $J_{5,6}$=3.8, $J_{6,6'}$=12.0 Hz, H6A), 3.90 (1H, dd, $J_{5,6'}$=6.5 Hz, H6A′), 3.87 (1H, dd, $J_{5,6'}$=9.8 Hz, H6B′), 3.77 (1H, dd, $J_{4,5}$=5.4 Hz, H4A), 3.13 (1H, d, $J_{1,OH}$=3.9 Hz, OH), 2.04, 1.95, 1.93, 1.85 (4s, 3H each, C(O)CH_3). ^{13}C NMR (CD_2Cl_2): δ 171.66–171.17 (4C, C(O)CH$_3$), 139.33–127.96 (12C, aromatic), 103.56 (CHPh), 95.40 (C1B), 88.95 (C1A), 84.31 (C2A), 79.54 (C4B),

78.95 (C3A), 74.93 (C3B), 71.53 (C2B), 70.90 (C5A), 70.75 (C6B), 65.82–65.19 (C5B, C6A), 51.30 (C4A), 22.51–21.55 (4C, C(O)CH_3).

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