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Carbohydrate Research 341 (2006) 1533–1542
Carbohydrate RESEARCH

Synthesis of monodeoxy analogues of the trisaccharide α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*OMe recognised by Calreticulin/Calnexin

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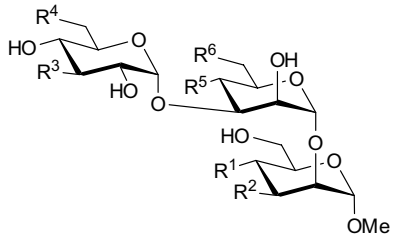
Received 1 February 2006; received in revised form 7 March 2006; accepted 12 March 2006
Available online 17 April 2006

Abstract—Six (3,4,4′,6′,3″ or 6″)-monodeoxy analogues of the title trisaccharide (**1**–**6**) have been prepared utilising monodeoxy monosaccharide precursors. The reducing end deoxy derivatives were synthesised by *N*-iodosuccinimide/silver trifluoromethanesulfonate (NIS/AgOTf)-promoted couplings of a common disaccharide thioglycoside donor **10** to suitably protected monodeoxy acceptors **9** and **12**, affording trisaccharides, which after deprotection yielded target structures **1** and **2**. The non-reducing end deoxy derivatives could similarly be produced by halide-assisted glycosylations of a common disaccharide acceptor **17** with monodeoxy glycosyl bromide donors (obtained from thioglycosides **18** and **20**) to yield, after removal of protecting groups, target trisaccharides **3** and **4**. The analogues with the deoxy function in the middle mannose residue, were obtained through orthogonal halide-assisted coupling of tetrabenzyl-glucopyranosyl bromide to monodeoxy thioglycoside acceptors to give thioglycoside disaccharides, which subsequently were used as donors in NIS/AgOTf-promoted couplings to a common 2-hydroxy-mannose acceptor **15** to afford trisaccharides; deprotection yielded the final target compounds **5** and **6**.

Keywords: Calreticulin substrates; Monodeoxy derivatives; Oligosaccharide synthesis

1. Introduction

The control of the folding process of all proteins glycosylated with N-glycan chains is highly dependent on the recognition of the high-mannose-type glycan $Glc_1Man_9(GlcNAc)_2$ by the chaperone proteins Calreticulin/Calnexin.[1–4] The binding to these lectins is mediated mainly through the glucose-containing branch, that is, the tetrasaccharide α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*. Isothermal titration calorimetry studies of the interaction between Calreticulin and synthesised truncated oligosaccharides of the glucosylated branch showed a 25-fold stronger binding for the trisaccharide unit, that is, α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-OMe, compared to the disaccharide unit (α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-OMe).[5,6] Deoxy and deoxyfluoro analogues have frequently been shown to be valuable and complementary



1 R^1 = H, R^2 = R^3 = R^4 = R^5 = R^6 = OH;
2 R^2 = H, R^1 = R^3 = R^4 = R^5 = R^6 = OH;
3 R^3 = H, R^1 = R^2 = R^4 = R^5 = R^6 = OH;
4 R^4 = H, R^1 = R^2 = R^3 = R^5 = R^6 = OH;
5 R^5 = H, R^1 = R^2 = R^3 = R^4 = R^6 = OH;
6 R^6 = H, R^1 = R^2 = R^3 = R^4 = R^5 = OH

Figure 1. The target structures.

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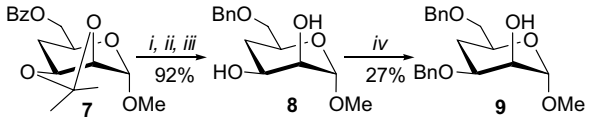
doi:10.1016/j.carres.2006.03.015

tools to determine the role of each hydroxyl group in binding and 2-deoxy-2-fluoro analogues of the title trisaccharide have already been synthesised and investigated.[7] To investigate in further detail binding interactions to Calreticulin, a series of deoxy analogues of the glucose-containing trisaccharide were desired. Herein we present the synthesis of six monodeoxy derivatives of this trisaccharide (**1**–**6**, Fig. 1). Similar analogues of the branched trimannoside core structure of N-glycans have earlier been synthesised by us and proven to be most valuable tools in binding studies with various lectins.[8–11]

2. Results and discussion

Because all the target structures are monodeoxy analogues of the same trisaccharide, a possible pathway would be to synthesise a precursor trisaccharide structure protected with a plethora of orthogonal protecting groups, which then could be removed selectively to expose a single hydroxyl group for deoxygenation. However, our experiences with this approach have been disappointing.[12] Not only is the synthesis of such variably protected derivatives difficult, but selective deoxygenation at the oligosaccharide level is also frequently problematic. Hence, all the target structures were eventually synthesised starting from monodeoxy monosaccharide intermediates (**9**, **12**, **18**, **20**, **23** and **29**).

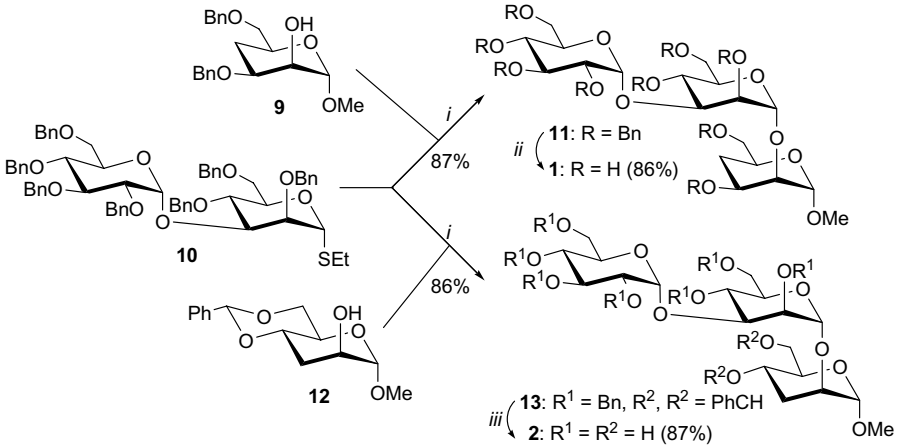
In the assembly of the reducing end deoxy derivatives **1** and **2**, a known common disaccharide donor **10**[13] was used for the preparation of both target compounds (Scheme 2). For the synthesis of the 4-deoxy derivative **1**, a suitable 2-hydroxy acceptor **9** was obtained in a four-step sequence from the published 4-deoxy compound **7**[14] (Scheme 1). Exchange of the 6-*O*-benzoyl group for a benzyl group followed by removal of the 2,3-*O*-isopropylidene acetal gave diol **8**, which was converted into **9** by regioselective tin-mediated benzylation of the equatorial 3-hydroxyl group.



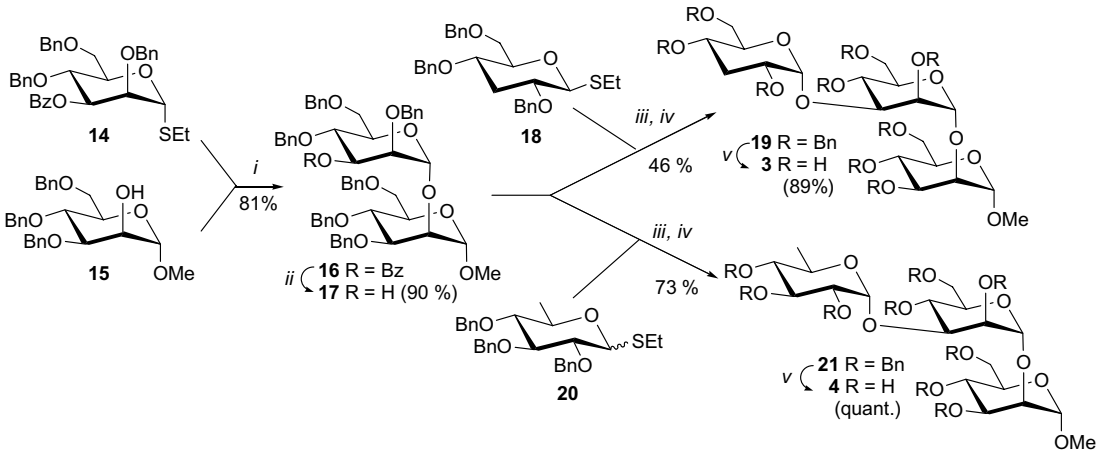
Scheme 1. Synthesis of the 4-monodeoxy acceptor **9**. Key: (i) $NaOCH_3$, CH_3OH; (ii) NaH, BnBr, DMF; (iii) 80% aq TFA; (iv) 1. Bu_2SnO, CH_3OH; 2. BnBr, DMF.

Coupling of acceptor **9** with the common donor ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**10**), in a NIS/AgOTf-promoted reaction gave the fully protected trisaccharide **11** (87%). One-step deprotection by catalytic hydrogenolysis afforded trisaccharide **1** in 86% yield. The 3-deoxy derivative **2** was obtained from a NIS/AgOTf-mediated glycosylation of the known 4,6-*O*-benzylidene 3-deoxy derivative **12**[15] with donor **10**, affording trisaccharide **13** in high yield (86%) followed by catalytic hydrogenolysis (87%).

For the synthesis of the non-reducing end deoxy derivatives **3** and **4**, a common disaccharide precursor was also used (Scheme 3). This disaccharide, **17**, prepared from the known donor **14**[16] and acceptor **15**[17] in a NIS/AgOTf-promoted coupling followed by removal of the temporary 3′-benzoate protecting group, was chosen because it avoids the use of a deactivating 4′,6′-*O*-benzylidene acetal and contains a 2′-*O*-benzyl group thus maximising the acceptor efficiency of the 3′-hydroxyl group. Coupling of the 3-monodeoxy glucosyl bromide, prepared in situ from the corresponding ethyl thioglycoside **18**,[18] under halide-assisted conditions afforded the pure α-anomer **19** in acceptable yields (46%). Target compound **3** was obtained after removal of the benzyl ethers by catalytic hydrogenolysis (89%). A similar sequence, starting with the 6-deoxy glucosyl



Scheme 2. Synthesis of the 4 and 3-monodeoxy analogues **1** and **2**. Key: (i) NIS/AgOTf, CH_2Cl_2; (ii) H_2, Pd/C, CH_3OH/EtOAc 5:1; (iii) H_2, Pd/C, CH_3OH/1 M aq HCl.

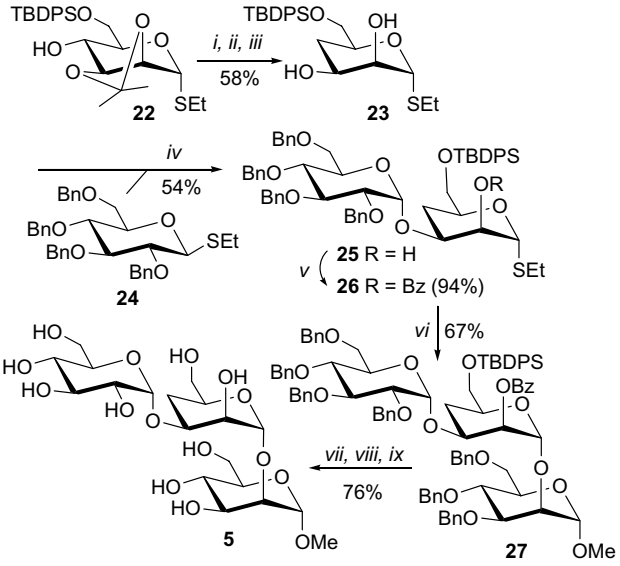


Scheme 3. Synthesis of the 3″- and 6″-monodeoxy analogues **3** and **4**. Key: (i) NIS/AgOTf, CH_2Cl_2; (ii) $NaOCH_3$, CH_3OH; (iii) Br_2, CH_2Cl_2; (iv) Et_4NBr, CH_2Cl_2; (v) H_2, Pd/C, CH_3OH/THF 1:1.

donor **20**,[19] produced the 6″-deoxy trisaccharide **21** in good yield (73%), which afforded target compound **4** after deprotection.

Preparation of the 4′-deoxy trisaccharide **5** and 6′-deoxy analogue **6**, both having the deoxy function in the non-reducing mannose moiety, turned out to be more difficult.[12] Attempts to stereoselectively introduce an α-glucopyranoside residue at the 3 position of a 2,6 protected 4-deoxy mannoside under in situ anomerisation conditions afforded only low amounts of the desired product, even after prolonged reaction times (1–2 weeks). Earlier experiments had shown that a free 2-hydroxyl increases the reactivity of the 3-hydroxyl group and that regioselective 3-*O*-glycosylation of 2,3-unprotected mannose acceptors is possible.[8] Hence, the 4-deoxy 2,3-diol **23** (Scheme 4) was prepared according to standard procedures from the 2,3-*O*-isopropylidene derivative **22**.[20] Deoxygenation under Barton–McCombie conditions[21] followed by hydrolysis of the isopropylidene acetal with 90% aqueous TFA provided the acceptor **23** (58%). Glycosylation with 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl bromide, prepared in situ from ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside **24**,[22] under halide-assisted conditions produced disaccharide **25** in acceptable isolated yield (54%). The coupling reaction occurred with good regioselectivity (3.4:1 for 3-*O*-glycosylation) and with complete α-stereoselectivity. After 2-*O*-benzoylation (94%) yielding donor **26**, the fully protected trisaccharide **27** was assembled in good yield (76%) using acceptor **15** and NIS/AgOTf as promoter. A three-step deprotection sequence then afforded target compound **5** (76%).

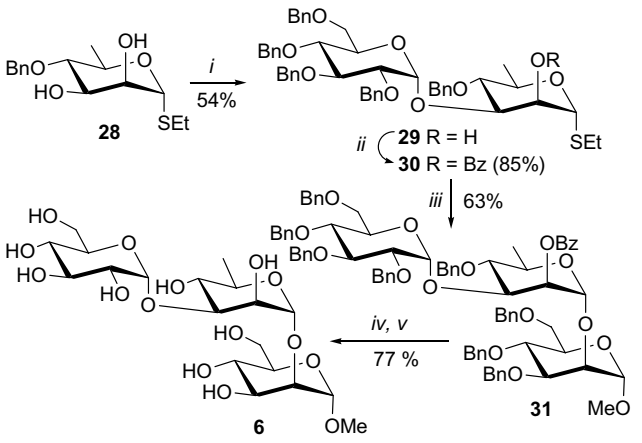
The last target compound in this series, derivative **6**, was prepared similarly starting from the known ethyl 6-deoxy thiomannoside **28**[23] (Scheme 5). Regioselective (2.6:1 for 3-*O*-glycosylation) halide-assisted coupling between diol acceptor **28** and 2,3,4,6-tetra-*O*-benzyl-D-



Scheme 4. Synthesis of the 4′-monodeoxy analogue **5**. Key: (i) Im_2CS, DCE; (ii) *n*-Bu_3SnH, AIBN, benzene; (iii) AcOH/H_2O/TFA 10:2:1 (v/v/v); (iv) 1. Br_2, CH_2Cl_2; 2. Et_4NBr, CH_2Cl_2; (v) BzCl, pyridine; (vi) **15**, NIS, AgOTf, CH_2Cl_2; (vii) $NaOCH_3$, CH_3OH; (viii) TBAF, THF; (ix) H_2, Pd/C, CH_3OH/THF 1:1.

glucopyranosyl bromide gave disaccharide **29** (54%) with complete α-selectivity. Again, 2-*O*-benzoylation (85%) furnished a donor **30**, which was coupled in a NIS/AgOTf-mediated reaction with the same acceptor **15** as above to produce trisaccharide **31** (63%). Removal of the 2′-*O*-benzoate with sodium methoxide, followed by hydrogenolysis of benzyl groups then afforded the 6′-deoxy analogue **6** (77%).

In conclusion, six monodeoxy trisaccharides analogues, **1**–**6**, of the Glc-containing branch of the high-mannose-type glycan $Glc_1Man_9(GlcNAc)_2$, needed for detailed binding studies with the Calreticulin/Calnexin



Scheme 5. Preparation of the 6′-monodeoxy analogue **6**. Key: (i) 1. **24**, Br_2, CH_2Cl_2; 2. Et_4NBr, CH_2Cl_2; (ii) BzCl, pyridine; (iii) **15**, NIS, AgOTf, CH_2Cl_2; (iv) $NaOCH_3$, CH_3OH; (v) H_2, Pd/C, CH_3OH/THF 1:1.

chaperone proteins, have been efficiently synthesised. The approach involved the use of monodeoxy monosaccharide building blocks and a number of common di- and monosaccharide acceptors and donors.

3. Experimental

3.1. General methods

CH_2Cl_2 was distilled from calcium hydride. Organic solutions were concentrated under reduced pressure at <45 °C (bath temperature). NMR spectra were recorded at 400 MHz for ^1H and at 100 MHz for ^{13}C. Chemical shifts are reported relative to chloroform [δ_H 7.26, δ_C (central of triplet) 77.0] or to HOD (δ_H 4.80). TLC was performed on Silica Gel 60 F254 with detection by charring with 8% sulfuric acid. Silica gel (0.040–0.063 mm) was used for column chromatography.

3.2. Methyl 3,6-di-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (9)

3.2.1. Methyl 6-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (8). Methyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-4-deoxy-α-D-*lyxo*-hexopyranoside **7** (300 mg, 0.93 mmol) was dissolved in CH_3OH (10 mL) and a catalytic amount of 1 M $NaOCH_3$ in CH_3OH was added. After being stirred overnight, the reaction was neutralised with Dowex 50 (H^+) ion exchange resin. The resin was filtered off and the filtrate concentrated and dried in vacuo. The crude residue was dissolved in DMF (4 mL), and NaH (60%, 74 mg, 1.86 mmol) was added at 0 °C. After 10 min, benzyl bromide (167 μL, 1.39 mmol) was added and the ice-bath removed. After 2 h, ice was added to the reaction, and the mixture extracted with EtOAc. The organic phase was washed with H_2O, dried with Na_2SO_4 and concentrated. The residue was dissolved in 80% aqueous TFA (10 mL) and stirred at rt for 2 h. Toluene was added and the mixture subsequently concentrated and co-evaporated with toluene several times. The resulting residue was purified by silica gel chromatography (30:1, CH_2Cl_2/CH_3OH) to give **8** (230 mg) in 92% yield. $[\alpha]_D$ +56 (*c* 1.0, $CHCl_3$), Lit.[24] +18; ^1H NMR ($CDCl_3$): δ 1.63 (q, 1H, H-4b, $J = 12$ Hz), 1.75 (m, 1H, H-4a), 2.11 (d, 1H, O*H*, $J = 8.5$ Hz), 2.15 (d, 1H, O*H*, $J = 7$ Hz), 3.38 (s, 3H, OCH_3), 3.51 (dd, 1H, H-6b, $J_{6b,6a} = 10$ Hz, $J_{6b,5} = 4$ Hz), 3.55 (dd, 1H, H-6a, $J_{6a,5} = 6$ Hz), 3.73 (m, 1H, H-2), 3.88–4.01 (m, 2H, H-3, H-5), 4.58 (2d, 2H, CH_2Ph, $J = 12$ Hz), 4.78 (d, 1H, H-1, $J_{1,2} = 1.1$ Hz), 7.25–7.34 (m, 5H, H-Aromatic).

3.2.2. Methyl 3,6-di-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (9). The obtained diol **8** (225 mg, 0.84 mmol) was dissolved in dry CH_3OH (5 mL) containing dibutyltin oxide (245 mg, 0.98 mmol) and heated at 50 °C for 3 h. The solvent was removed under reduced pressure and the residue dissolved in DMF (5 mL). Benzyl bromide (235 μL, 1.96 mmol) was added and the solution heated at 110 °C for 12 h. Satd aq $NaHCO_3$ was poured into the mixture, which was extracted with EtOAc. The organic phase was washed with 1 N HCl and H_2O and dried over $MgSO_4$. After concentration, silica gel chromatography (6:1, toluene/EtOAc) afforded **9** (80 mg, 27%). $[\alpha]_D$ +31 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.77 (m, 2H, H-4b, H-4a), 2.40 (br s, 1H, O*H*), 3.37 (s, 3H, OCH_3), 3.50 (dd, 1H, H-6b, $J_{6b,6a} = 10$ Hz, $J_{6b,5} = 4$ Hz), 3.60 (dd, 1H, H-6a, $J_{6a,5} = 6$ Hz), 3.83 (m, 1H, H-5), 3.91 (m, 2H, H-2, H-3), 4.44–4.69 (m, 4H, CH_2Ph), 4.81 (d, 1H, H-1, $J_{1,2} = 1.5$ Hz), 7.28–7.35 (m, 10H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 28.5 (C-4), 54.9 (O*C*H_3), 66.7, 67.4, 70.1, 72.9, 73.0, 73.5 (C-2, C-3, C-5, C-6, *C*H_2Ph), 101.1 (C-1), 127–129, 138.1, 138.3 (C-Aromatic). Anal. Calcd for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.27; H, 7.26.

3.3. Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (11)

Acceptor **9** (60 mg, 0.17 mmol) and ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**10**, 145 mg, 0.14 mmol) were dissolved in CH_2Cl_2 (2 mL) and the solution stirred under N_2 for 10 min in the presence of powdered 4 Å molecular sieves. *N*-iodosuccinimide (44 mg, 0.2 mmol) was added and thereafter a catalytic amount of silver trifluoromethanesulfonate. After 45 min the reaction was neutralised with Et_3N, and the mixture filtered through a Celite pad and concentrated. Silica gel chromatography of the residue (15:1, toluene/EtOAc) gave **11** (160 mg, 87%). $[\alpha]_D$ +30 (*c* 1.0, $CHCl_3$); ^1H NMR

($CDCl_3$): δ 1.81 (m, 2H, H-4b, H-4a), 3.32 (s, 3H, OCH_3), 3.52 (m, 3H), 3.71 (m, 4H), 3.99 (m, 8H), 4.21 (m, 1H), 4.35 (m, 2H), 4.44–4.68 (m, 13H), 4.78–4.97 (m, 4H), 5.15 (2br s, 2H), 5.34 (d, 1H, $J = 2$ Hz), 7.05–7.40 (m, 45H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 29.3 (C-4), 54.8 (O*C*H_3), 67.9, 68.3, 69.7, 70.3, 70.9, 72.2, 72.8, 73.3, 73.5, 73.9, 74.6, 74.8, 75.6, 77.1, 77.7, 79.7, 79.8, 81.9 (C-2-3, C-5-6, C-2′-6′, C-2″-6″, *C*H_2Ph), 98.4, 99.1, 101.1 (C-1, C-1′, C-1″), 127-129, 138.0, 138.4, 138.5, 138.6, 138.7, 138.9 (C-Aromatic). Anal. Calcd for $C_{82}H_{88}O_{15}$: C, 74.98; H, 6.75. Found: C, 75.10; H, 6.71.

3.4. Methyl α-D-glucopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-4-deoxy-α-D-*lyxo*-hexopyranoside (1)

Compound **11** (70 mg, 0.053 mmol) was dissolved in CH_3OH/EtOAc (5:1 v/v, 10 mL). A catalytic amount of Pd/C was then added and the reaction stirred under 1 atm H_2. After two days, the catalyst was filtered off and the solution concentrated. The residue, dissolved in distilled H_2O, was applied to a 600 mg C_{18} MAXI-CLEAN cartridge (purchased from Alltech) and eluted with H_2O, to give pure **1** (23 mg, 86%). $[\alpha]_D$ +117 (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 1.67 (m, 2H, H-4b, H-4a), 3.36 (s, 3H, OCH_3), 3.53 (dd, 1H, $J = 10$ Hz, $J = 4$ Hz), 3.59–3.89 (m, 13H), 3.95 (m, 1H), 4.07 (m, 1H), 4.20 (br s, 1H), 4.98 (d, 1H, $J = 1.4$ Hz), 5.00 (d, 1H, $J = 1.5$ Hz), 5.23 (d, 1H, $J = 4$ Hz); ^{13}C NMR (D_2O): δ 29.6 (C-4), 54.8 (O*C*H_3), 60.8, 61.1, 64.2, 65.2, 66.4, 69.3, 69.8, 70.0, 71.9, 72.5, 73.0, 73.5, 76.4, 78.4 (C-2-3, C-5-6, C-2′-6′, C-2″-6″), 100.2, 100.5, 102.1 (C-1, C-1′, C-1″); MALDI-TOFMS calcd for $C_{19}H_{34}O_{15}$ $[M+Na]^+$: 525.1795, $[M+K]^+$: 541.1535. Found 525.49, 541.48.

3.5. Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (13)

Donor **10** (145 mg, 0.14 mmol) and methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside **12** (50 mg, 0.19 mmol) were dissolved in CH_2Cl_2 (1 mL) and the solution stirred for 15 min in the presence of powdered 4 Å molecular sieves under N_2. *N*-iodosuccinimide (48 mg, 0.21 mmol) was added and thereafter a catalytic amount of silver trifluormethanesulfonate. The reaction was neutralised with Et_3N after 30 min and filtered through a Celite pad. The filtrate was washed with aqueous $Na_2S_2O_3$ and H_2O and concentrated. The crude material was purified on a silica gel column (30:1→15:1, toluene–EtOAc) affording trisaccharide **13** (150 mg, 86%). $[\alpha]_D$ +41 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.05 (m, 2H, H-3b, H-3a), 3.32 (s, 3H, OCH_3), 3.56 (m, 3H), 3.64–3.96 (m, 9H), 4.12 (m, 3H), 4.24 (m, 1H), 4.42–4.68 (m, 11H), 4.78–4.97 (m, 3H), 5.04 (d, 1H, $J = 1.8$ Hz), 5.12 (m, 2H), 5.56 (s, 1H, C*H*Ph), 7.12–7.52 (m, 40H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 29.8 (C-3), 54.8 (O*C*H_3), 68.8, 69.5, 69.6, 71.1, 71.2, 72.4, 73.1, 73.4, 73.6, 74.1, 74.6, 74.8, 74.9, 75.7, 77.9, 78.2, 79.9, 81.9 (C-2, C-4-6, C-2′-6′, C-2″-6″, *C*H_2Ph), 99–100 (C-1, C-1′, C-1″), 102.1 (*C*HPh), 126–129, 137.7, 137.8, 138.3, 138.4, 138.4, 138.6, 138.8, 138.8 (C-Aromatic). Anal. Calcd for $C_{75}H_{80}O_{15}$: C, 73.75; H, 6.60. Found: C, 73.64; H, 6.53.

3.6. Methyl α-D-glucopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-3-deoxy-α-D-*arabino*-hexopyranoside (2)

Compound **13** (140 mg, 0.11 mmol) was dissolved in CH_3OH (5 mL), a catalytic amount of Pd/C and 1 N HCl (20 μL) were added, and the mixture was stirred under H_2 (1 atm). After 3 h the catalyst was removed by filtration and the solvent evaporated. Purification of the residue on a 600 mg C_{18} MAXI-CLEAN cartridge (purchased from Alltech) afforded **2** (50 mg, 87%). $[\alpha]_D$ +113 (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 1.74 (m, 1H, H-3b), 2.21 (dt, 1H, H-3a, $J = 13$ Hz, $J = 4$ Hz), 3.39 (t, 1H, $J = 9$ Hz), 3.42 (s, 3H, OCH_3), 3.55 (dd, 1H, $J = 10$ Hz, $J = 4$ Hz), 3.63 (m, 1H), 3.69–3.98 (m, 13H), 4.11 (br s, 1H), 4.78 (br s, 1H), 4.99 (d, 1H, $J = 1.4$ Hz), 5.24 (d, 1H, $J = 4$ Hz); ^{13}C NMR (D_2O): δ 30.1 (C-3), 54.7 (O*C*H_3), 60.9, 61.1, 61.3, 61.8, 66.3, 69.9, 70.4, 71.9, 72.5, 73.0, 73.1, 73.4, 73.7, 78.4 (C-2, C-4-6, C-2′-6′, C-2″-6″), 98.4, 98.5, 100.5 (C-1, C-1′, C-1″); MALDI-TOFMS calcd for $C_{19}H_{34}O_{15}$ $[M+Na]^+$: 525.1795, $[M+K]^+$: 541.1535. Found 525.49, 541.52.

3.7. Methyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (16)

Ethyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**14**, 255 mg, 0.43 mmol) and acceptor **15** (180 mg, 0.39 mmol) were dissolved in CH_2Cl_2 (2 mL) and stirred for 10 min in the presence of 4 Å powdered molecular sieves under N_2. *N*-Iodosuccinimide (144 mg, 0.64 mmol) and a catalytic amount of silver trifluoromethanesulfonate were added. The reaction was stirred for 30 min and then neutralised by dropwise addition of Et_3N. The mixture was directly applied onto a silica gel column, eluted with toluene/EtOAc (15:1). Disaccharide **16** (315 mg, 81%) was thus obtained. $[\alpha]_D$ −6 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.28 (s, 3H, OCH_3), 3.73–3.94 (m, 7H), 4.09–4.14 (m, 4H), 4.35 (d, 1H, CH_2Ph, $J = 12$ Hz), 4.47–4.72 (m, 10H), 4.83 (br s, 1H, H-1), 4.86 (d, 1H, CH_2Ph, $J = 12$ Hz), 5.24 (br s, 1H, H-1′), 5.56 (dd, 1H, H-3′, $J_{3',4'} = 8.5$ Hz, $J_{3',2'} = 3.5$ Hz), 7.05–7.40 (m, 30H, H-Aromatic), 7.45 (t, 2H, H-Aromatic), 7.58 (t, 1H, H-Aromatic), 8.05 (d, 2H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 54.7 (O*C*H_3), 69.3, 69.6, 71.8, 72.0, 72.6, 72.7, 73.4, 73.5,

73.9, 74.2, 74.6, 75.0, 75.3, 76.1, 80.3 (C-2-6, C-2′-6′, CH_2Ph), 99.7 (C-1, C-1′), 127.5–128.8, 129.9, 130.1, 132.9, 138.0, 138.1, 138.3, 138.4, 138.5, 138.7 (C-Aromatic), 165.6 (Ph*C*=O). Anal. Calcd for $C_{62}H_{64}O_{12}$: C, 74.38; H, 6.44. Found: C, 74.49; H, 6.35.

3.8. Methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (17)

Compound **16** (310 mg, 0.31 mmol) was dissolved in CH_3OH (10 mL) and a catalytic amount of 1 M sodium methoxide in CH_3OH was added. After 3 days, the reaction was neutralised with Dowex 50 (H^+) ion exchange resin and the solvent removed under reduced pressure. Silica gel column chromatography (6:1, toluene/EtOAc) of the residue afforded **17** (250 mg, 90%). $[\alpha]_D$ +9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.29 (d, 1H, O*H*, $J = 9$ Hz), 3.26 (s, 3H, OCH_3), 3.65–3.85 (m, 8H), 3.89–3.94 (m, 2H), 4.03 (dt, 1H, $J = 9$ Hz, $J = 3.5$ Hz), 4.09 (br s, 1H), 4.27 (d, 1H, CH_2Ph, $J = 12$ Hz), 4.47–4.57 (m, 5H, CH_2Ph), 4.62–4.73 (m, 4H, CH_2Ph), 4.78 (d, 1H, $J = 1.8$ Hz), 4.86 (d, 2H, CH_2Ph, $J = 12$ Hz), 5.24 (br s, 1H), 7.19–7.38 (m, 30H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 54.7 (O*C*H$_3$), 69.3, 69.5, 71.4, 71.6, 71.8, 72.4, 72.7, 73.4, 74.7, 75.1, 75.2, 78.2, 80.2 (C-2-6, C-2′-6′, CH_2Ph), 98.7, 99.9 (C-1, C-1′), 127.5-128.5, 137.9, 138.4, 138.4, 138.5, 138.6, 138.6 (C-Aromatic). Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.72; H, 6.84.

3.9. Methyl 2,4,6-tri-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (19)

Ethyl 2,4,6-tri-*O*-benzyl-3-deoxy-1-thio-α-D-*ribo*-hexopyranoside (**18**, 120 mg, 0.25 mmol) was dissolved in CH_2Cl_2, and bromine (0.38 mmol, 20 μL) added at 0 °C. The reaction mixture was stirred under N_2 for 1 h and then concentrated. The obtained crude glucosyl bromide was dissolved in CH_2Cl_2 and added to a solution of acceptor **17** (120 mg, 0.13 mmol) and Et_4NBr (60 mg, 0.38 mmol) in CH_2Cl_2 (1 mL) and DMF (100 μL). After two weeks of stirring at rt in the presence of powdered 4 Å molecular sieves, the reaction mixture was filtered through a Celite pad and concentrated. Silica gel column chromatography (20:1, toluene/EtOAc) of the residue afforded **19** (80 mg, 46%). $[\alpha]_D$ −25 (*c* 2.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.96 (m, 1H, H-3b″), 2.34 (m, 1H, H-3a″), 3.25 (s, 3H, OCH_3), 3.41–3.64 (m, 3H), 3.71–3.81 (m, 6H), 3.89–4.10 (m, 6H), 4.26–4.64 (m, 19H), 4.84 (m, 2H), 5.21 (br s, 2H), 7.15–7.36 (m, 45H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 31.0 (C-3″), 54.7 (O*C*H$_3$), 68.4, 69.4, 69.7, 70.5, 70.8, 71.3, 71.7, 71.8, 71.9, 72.3, 73.3, 73.3, 73.5, 73.7, 74.4, 75.1, 75.2, 75.3, 77.8, 80.1 (C-2-6, C-2′-6′, C-2″, C-4″-6″, CH_2Ph), 97.9, 99.3, 100.0 (C-1, C-1′, C-1″), 127.0–128.4, 138.2, 138.3, 138.4, 138.5, 138.5, 138.6, 138.9 (C-Aromatic). Anal. Calcd for $C_{82}H_{88}O_{15}$: C, 74.98; H, 6.75. Found: C, 75.06; H, 6.67.

3.10. Methyl 3-deoxy-α-D-*ribo*-hexopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (3)

Compound **19** (50 mg, 0.038 mmol) was dissolved in CH_3OH/THF (1:1 v/v, 3 mL) and a catalytic amount of Pd/C (5 mg, 10%) was added. The reaction mixture was set under hydrogen atmosphere at atmospheric pressure and stirred at room temperature. After 5 h, the slurry was filtered through a sandwich of filters (10 μm on top of a 5 μm filter pellet) and concentrated. The residue was dissolved in distilled H_2O, applied on a C_{18} column (10 g) and eluted with H_2O to give **3** (17 mg, 0.034 mmol, 89%). $[\alpha]_D$ +126 (*c* 1.4, H_2O); ^{1}H NMR (D_2O): δ 1.82 (q, 1H, H-3b″, $J = 12$ Hz), 2.16 (m, 1H, H-3a″), 3.39 (s, 3H, OCH_3), 3.56–3.98 (m, 16H), 4.26 (m, 1H), 4.99 and 5.02 (br s, 1H, H-1′ and br s, 1H, H-1), 5.14 (d, 1H, H-1″, $J_{1'',2''} = 3.6$ Hz); ^{13}C NMR (D_2O): δ 34.5 (C-3″), 55.0 (O*C*H$_3$), 60.9, 61.1, 61.2, 64.5, 66.4, 67.0, 67.1, 70.0, 70.4, 72.7, 73.3, 73.5, 78.1, 78.7 (C-2-6, C-2′-6′, C-2″, C-4″-6″), 99.3, 99.5, 102.4 (C-1, C-1′, C-1″); MALDI-TOFMS calcd for $C_{19}H_{34}O_{15}$ $[M+Na]^+$: 525.1795, $[M+K]^+$: 541.1535. Found 525.49, 541.47.

3.11. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (21)

Ethyl 2,3,4-tri-*O*-benzyl-6-deoxy-1-thio-D-glucopyranoside (**20**, 133 mg, 0.28 mmol) was dissolved in CH_2Cl_2 and bromine (0.42 mmol, 22 μL) added at 0 °C. The reaction mixture was stirred under N_2 for 1 h and then concentrated. The crude glucosyl bromide was added to a solution of acceptor **17** (130 mg, 0.15 mmol) and Et_4NBr (65 mg, 0.41 mmol) in CH_2Cl_2 (1 mL) and DMF (100 μL). After two weeks of stirring at rt in the presence of powdered 4 Å molecular sieves, the reaction mixture was filtered through a Celite pad and concentrated. Silica gel column chromatography (20:1, toluene/EtOAc) of the residue afforded **21** (140 mg, 73%). $[\alpha]_D$ +51 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.17 (d, 3H, H-6″, $J = 6$ Hz), 3.13 (t, 1H, $J = 9$ Hz), 3.28 (s, 3H, OCH_3), 3.49 (dd, 1H, $J = 10$ Hz, $J = 3.5$ Hz), 3.71–3.77 (m, 5H), 3.81–4.06 (m, 6H), 4.12 (br s, 1H), 4.19 (m, 1H), 4.44–4.70 (m, 14H), 4.76–4.93 (m, 5H), 5.08 (m, 2H), 5.23 (s, 1H), 7.11–7.39 (m, 45H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 18.2 (C-6″), 54.8 (O*C*H$_3$), 67.2, 67.4, 69.4, 69.7, 71.7, 72.0, 72.5, 72.7, 73.3, 73.4, 73.9, 74.5, 74.9, 75.1, 75.2, 75.6, 78.1, 80.2, 80.2, 81.7, 84.2 (C-2-6, C-2′-6′, C-2″-5″, CH_2Ph), 98.6, 99.1, 100.1 (C-1, C-1′, C-1″), 127.1–129.1, 138.3, 138.4, 138.5, 138.5, 138.7, 138.8, 138.9 (C-Aromatic). Anal. Calcd for $C_{88}H_{82}O_{15}$: C, 74.98; H, 6.75. Found: C, 74.90; H, 6.79.

3.12. Methyl 6-deoxy-α-D-glucopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (4)

Compound **21** (109 mg, 0.089 mmol) was dissolved in CH_3OH/THF (1:1 v/v, 5 mL) and a catalytic amount of Pd/C (5 mg, 10%) was added. The reaction mixture was set under an hydrogen atmosphere at atmospheric pressure and stirred at room temperature. After 5 h, the slurry was filtered through a sandwich of filters (10 μm on top of a 5 μm filter pellet) and concentrated. The residue was dissolved in distilled H_2O, applied on a C_{18} column (10 g) and eluted with H_2O, to give pure **4** (42 mg, 0.089 mmol, quant.). $[\alpha]_D$ +116 (*c* 0.7, H_2O); ^{1}H NMR (D_2O): δ 1.26 (d, 3H, H-6″, $J = 6$ Hz), 3.16 (t, 1H, $J = 9$ Hz), 3.40 (s, 3H, OCH_3), 3.54–3.91 (m, 14H), 3.96 (br s, 1H), 4.17 (br s, 1H), 4.98 and 5.02 (br s, 1H, H-1 and br s, 1H, H-1′), 5.17 (d, 1H, H-1″, $J_{1'',2''} = 3$ Hz); ^{13}C NMR (D_2O): δ 16.7 (C-6″), 55.0 (O*C*H_3), 61.1, 61.2, 66.2, 67.1, 68.5, 70.1, 70.4, 72.2, 72.7, 72.8, 73.5, 75.1, 78.5, 78.9 (C-2-6, C-2′-6′, C-2″-5″), 99.5, 100.7, 102.2 (C-1, C-1′, C-1″); MALDI-TOFMS calcd for $C_{19}H_{34}O_{15}$ $[M+Na]^+$: 525.1795, $[M+K]^+$: 541.1535. Found 525.51, 541.50.

3.13. Ethyl 4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-*lyxo*-hexopyranoside (23)

Compound **22** (270 mg, 0.54 mmol) was dissolved in CH_2Cl_2 (10 mL) together with 1,1′-thiocarbonyldiimidazole (191 mg, 1.07 mmol) and the reaction was heated at reflux for 10 h. The solvent was removed under reduced pressure and the brown residue was passed through a silica gel column (6:1, toluene/EtOAc). The crude product was dissolved in benzene (5 mL) and *n*-Bu_3SnH (2.60 mmol, 700 μL) was added. The mixture was degassed and put under N_2 atmosphere. AIBN (~20 mg) was added, the solution was heated at reflux for 10 min and then concentrated. The residue was partitioned between pentane–CH_3CN and the CH_3CN layer washed twice with pentane. After concentration of the CH_3CN layer, the crude product was dissolved in AcOH/H_2O/TFA (10:2:1 v/v, 13 mL) and the solution kept at 0 °C for 2 h. The reaction mixture was then co-evaporated with toluene several times and the resulting residue purified by chromatography on a silica gel column (5:1, toluene/EtOAc) affording **23** (140 mg, 58%). $[\alpha]_D$ +87 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H, C(CH_3)$_3$), 1.26 (t, 3H, SCH$_2$CH_3), 1.59 (q, 1H, H-4b, $J = 12$ Hz), 1.80 (m, 1H, H-4a), 2.06 (d, 1H, O*H*, $J = 8.5$ Hz), 2.24 (d, 1H, O*H*, $J = 6.5$ Hz), 2.52–2.67 (m, 2H, SCH_2CH$_3$), 3.63 (dd, 1H, H-6b, $J_{6b,6a} = 10.6$ Hz, $J_{6b,5} = 5$ Hz), 3.73 (dd, 1H, H-6a, $J_{6a,5} = 6$ Hz), 3.83 (m, 1H), 3.96 (m, 1H), 4.23 (m, 1H), 5.33 (d, 1H, H-1, $J_{1,2} = 1.1$ Hz), 7.34–7.45 (m, 6H, H-Aromatic), 7.65–7.67 (m, 4H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 14.8 (SCH$_2$*C*H$_3$), 19.3 (*C*(CH$_3$)$_3$), 24.7 (S*C*H$_2$CH$_3$), 26.9 (C(*C*H$_3$)$_3$), 31.5 (C-4), 66.3, 66.4, 69.1, 70.7 (C-2-3, C-5-6), 84.1 (C-1), 127.8, 129.8, 133.4, 135.7 (C-Aromatic). Anal. Calcd for $C_{24}H_{34}O_4SSi$: C, 64.53; H, 7.67. Found: C, 64.42; H, 7.61.

3.14. Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α-D-*lyxo*-hexopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (27)

3.14.1. Ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-*lyxo*-hexopyranoside (26). Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**24**, 264 mg, 0.45 mmol) was dissolved in CH_2Cl_2 (1 mL), and bromine (0.68 mmol, 35 μL) added at 0 °C. The reaction mixture was stirred under N_2 for 1 h and concentrated. The crude glucosyl bromide was added to a solution of acceptor **23** (90 mg, 0.20 mmol) and Et_4NBr (104 mg, 0.49 mmol) in CH_2Cl_2 (1 mL) and DMF (100 μL). The reaction mixture was stirred at rt in the presence of 4 Å powdered molecular sieves for 36 h. This was then directly applied on a silica gel column and eluted with toluene/EtOAc (15:1→10:1) to yield **25** (105 mg, 54%). Compound **25** was dissolved in pyridine (5 mL) and benzoyl chloride (18 μL, 0.15 mmol) added. The reaction mixture was stirred overnight, concentrated and co-evaporated with toluene several times. The residue was purified by silica gel chromatography (30:1, toluene/EtOAc), affording disaccharide **26** (110 mg, 94%). $[\alpha]_D$ +35 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.07 (s, 9H, C(CH_3)$_3$), 1.29 (t, 3H, SCH$_2$CH_3), 1.92 (d, 1H, H-4b, $J = 12$ Hz), 2.08 (q, 1H, H-4a, $J = 12$ Hz), 2.60–2.69 (m, 2H, SCH_2CH$_3$), 3.45–3.87 (m, 8H), 4.08 (m, 1H), 4.26 (m, 1H), 4.38 (t, 2H, CH_2Ph, $J = 12$ Hz), 4.57 (t, 2H, CH_2Ph, $J = 12$ Hz), 4.65–4.82 (m, 4H, CH_2Ph), 4.96 (d, 1H, H-1′, $J_{1',2'} = 4$ Hz), 5.35 (br s, 1H, H-2), 5.52 (s, 1H, H-1), 7.03 (m, 2H, H-Aromatic), 7.22–7.45 (m, 23H, H-Aromatic), 7.54 (t, 2H, H-Aromatic), 7.67–7.71 (m, 4H, H-Aromatic), 8.06 (d, 3H, H-Aromatic), 8.18 (d, 1H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 15.0 (SCH$_2$*C*H$_3$), 19.4 (*C*(CH$_3$)$_3$), 25.4 (S*C*H$_2$CH$_3$), 26.9 (C(*C*H$_3$)$_3$), 29.1 (C-4), 66.6, 68.2, 69.6, 70.9, 72.3, 73.0, 73.5, 74.6, 75.6, 80.2, 81.6 (C-2-3, C-5-6, C-2′-6′, *C*H$_2$Ph), 82.4 (C-1), 96.4 (C-1′), 127.5–128.9, 129.7, 129.9, 130.3, 130.7, 133.1, 133.4, 133.6, 134.6, 135.7, 135.8, 138.0, 138.5, 138.9 (C-Aromatic), 165.7 (*C*=O).

3.14.2. Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α-D-*lyxo*-hexopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (27). Compounds **15** (100 mg, 0.21 mmol) and **26** (110 mg, 0.10 mmol) were dissolved in CH_2Cl_2 (4 mL) and the solution was stirred for

30 min under N_2 in the presence of 4 Å powdered molecular sieves. *N*-Iodosuccinimide (35 mg, 0.15 mmol) was then added and thereafter a catalytic amount of silver trifluormethanesulfonate. After 2 h, the reaction was neutralised by dropwise addition of Et_3N and the mixture directly applied onto a silica gel column and eluted with toluene/EtOAc (20:1) to afford **27** (101 mg, 67%). $[\alpha]_D$ +49 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.08 (s, 9H, C(CH_3)$_3$), 1.78 (d, 1H, H-4b′, $J = 12$ Hz), 2.05 (q, 1H, H-4a′, $J = 12$ Hz), 3.29 (s, 3H, OCH_3), 3.51 (m, 3H), 3.66–3.94 (m, 10H), 4.08 (m, 2H), 4.26 (m, 2H), 4.40 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.51–4.80 (m, 12H), 4.89 (d, 1H, CH_2Ph, $J = 11$ Hz), 5.04 (d, 1H, H-1″, $J_{1'',2''} = 4$ Hz), 5.38 (d, 1H, H-1′, $J_{1',2'} = 1.1$ Hz), 5.50 (br s, 1H, H-2′), 7.05 (m, 2H, H-Aromatic), 7.14–7.43 (m, 42H, H-Aromatic), 7.54 (t, 1H, H-Aromatic), 7.71 (m, 4H, H-Aromatic), 8.08 (d, 1H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 19.4 (*C*(CH_3)$_3$), 26.9 (C(*C*H_3)$_3$), 28.7 (C-4′), 54.7 (O*C*H_3), 66.8, 68.1, 69.6, 69.7, 70.2, 70.9, 71.4, 71.6, 71.9, 72.7, 73.3, 73.4, 73.8, 74.6, 74.9, 75.2, 75.5, 79.9, 80.0, 81.6 (C-2,6, C-2′-3′, C-5′-6′, C-2″-6″, *C*H_2Ph), 96.1, 99.6, 100.0 (C-1, C-1′, C-1″), 127.4–128.5, 129.7, 129.9, 130.4, 132.9, 133.5, 133.6, 135.7, 135.8, 138.1, 138.5, 138.5, 138.7, 139.0 (C-Aromatic), 165.8 (Ph*C*=O). Anal. Calcd for $C_{91}H_{98}O_{16}Si$: C, 74.06; H, 6.69. Found: C, 74.14; H, 6.60.

3.15. Methyl α-D-glucopyranosyl-(1→3)-4-deoxy-α-D-*lyxo*-hexopyranosyl-(1→2)-α-D-mannopyranoside (5)

Compound **27** (93 mg, 0.063 mmol) was dissolved in CH_3OH/THF (5:1 v/v, 6 mL) and $NaOCH_3$ (1 M in CH_3OH) was added. The mixture was left stirring overnight and then neutralised with H^+ ion exchange resin. After removal of the resin by filtration and concentration of the filtrate, the residue was dissolved in a solution of *n*-BuN_4F in THF (5 mL, approx. 0.1 M) and stirred until removal of the silyl ether was complete (TLC; 1:1, toluene/EtOAc). The solution was concentrated and the residue was purified by silica gel chromatography (4:1→1:1, toluene/EtOAc) to give methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-4-deoxy-α-D-*lyxo*-hexopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (60 mg, 84%). This compound (60 mg, 0.053 mmol) was dissolved in CH_3OH/THF (1:1 v/v, 5 mL) and a catalytic amount of Pd/C (5 mg, 10%) added. The reaction mixture was set under an hydrogen atmosphere at atmospheric pressure and stirred at room temperature. After 3 h, the slurry was filtered through a sandwich of filters (10 μm on top of a 5 μm filter pellet) and concentrated. The obtained residue was dissolved in distilled H_2O, applied on a C_{18} column (10 g) and eluted with H_2O to give **5** (24 mg, 0.048 mmol, 91%). $[\alpha]_D$ +86 (*c* 0.7, H_2O); ^1H NMR (D_2O): δ 1.62 (q, 1H, H-4b′, $J = 12$ Hz), 1.85 (d, 1H, H-4a′, $J = 12$ Hz), 3.38 (s, 3H, OCH_3), 3.51–4.20 (m, 17H), 4.97 and 5.04 (br s, 1H, H-1′ and br s, 1H, H-1), 5.10 (d, 1H, H″-1′, $J_{1',2'} = 3.9$ Hz), 5.50 (br s, 1H, H-2′); ^{13}C NMR (D_2O): δ 26.1 (C-4′), 55.0 (O*C*H_3), 60.8, 61.1, 64.3, 67.1, 68.0, 69.8, 70.0, 70.4, 71.1, 71.5, 72.4, 72.7, 73.1, 78.5 (C-2,6, C-2′-3′, C-5′-6′, C-2″-6″), 96.6, 99.6, 103.0 (C-1, C-1′, C-1″); MALDI-TOF MS calcd for $C_{19}H_{34}O_{15}$ $[M+Na]^+$: 525.1795, $[M+K]^+$: 541.1535. Found 525.48, 541.50.

3.16. Ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-D-rhamnopyranoside (30)

Compound **24** (396 mg, 0.68 mmol) was dissolved in CH_2Cl_2 and bromine (1.03 mmol, 53 μL) was added at 0 °C. The reaction mixture was stirred under N_2 for 1 h and then concentrated. The crude glucosyl bromide was added to a solution of acceptor **28**[23] (100 mg, 0.33 mmol) and Et_4NBr (156 mg, 0.73 mmol) in CH_2Cl_2 (1 mL) and DMF (100 μL). After 40 h of stirring at rt in the presence of powdered 4 Å molecular sieves, the reaction mixture was directly applied onto a silica gel column and eluted (10:1, toluene/EtOAc). The product disaccharide **29** (130 mg, 47%) was dissolved in pyridine (3 mL) and benzoyl chloride (28 μL, 0.23 mmol) was added. The mixture was stirred at rt for 1 day, then concentrated and the residue co-evaporated with toluene before being purified on a silica gel column (40:1, toluene/EtOAc) affording **30** (125 mg, 85%). $[\alpha]_D$ +38 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.26–1.35 (m, 6H, SCH_2CH_3, H-6), 2.65 (m, 2H, SCH_2CH$_3$), 3.28 (br s, 2H), 3.53 (dd, 1H, $J = 10$ Hz, $J = 4$ Hz), 3.63–3.74 (m, 3H), 3.94 (t, 1H, $J = 10$ Hz), 4.10 (dd, 1H, $J = 9.5$ Hz, $J = 3.5$ Hz), 4.16 (m, 1H), 4.27–4.37 (2d, 2H, CH_2Ph, $J = 12$ Hz), 4.51–4.59 (2d, 2H, CH_2Ph, $J = 12$ Hz), 4.66–4.74 (m, 4H, CH_2Ph), 4.87 (d, 1H, CH_2Ph, $J = 12$ Hz), 4.99 (d, 1H, H-1′, $J_{1',2'} = 3.5$ Hz), 5.23 (d, 1H, CH_2Ph, $J = 12$ Hz), 5.38 (br s, 1H, H-1), 5.48 (br s, 1H, H-2), 6.95–8.05 (m, 30H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 15.2 (SCH_2*C*H$_3$), 18.1 (C-6), 26.0 (S*C*H$_2$CH$_3$), 67.8, 68.6, 71.4, 73.2, 73.5, 74.6, 75.0, 75.4, 75.6, 77.2, 79.6, 79.9, 80.8, 81.7, 81.8 (C-1-5, C-2′-6′, *C*H_2Ph), 99.6 (C-1′), 127–130, 133.3, 137.9, 138.3, 138.6, 138.7, 138.8 (C-Aromatic), 166.0 (Ph*C*=O). Anal. Calcd for $C_{56}H_{60}O_{10}S$: C, 72.70; H, 6.54. Found: C, 72.61; H, 6.63.

3.17. Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (31)

Methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**27**, 100 mg, 0.21 mmol) and donor **30** (110 mg, 0.10 mmol) were dissolved in CH_2Cl_2 (4 mL) and the solution was stirred for 30 min under N_2 in the presence of 4 Å pow-

dered molecular sieves. *N*-iodosuccinimide (35 mg, 0.15 mmol) was added followed by a catalytic amount of silver trifluoromethanesulfonate. After 2 h, the reaction was neutralised by dropwise addition of Et_3N and the mixture directly applied onto a silica gel column and eluted (20:1, toluene/EtOAc) to yield **31** (101 mg, 67%). $[\alpha]_D$ +35 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.08 (s, 9H, C(CH_3)$_3$), 1.78 (d, 1H, H-4b′, $J = 12$ Hz), 2.05 (q, 1H, H-4a′, $J = 12$ Hz), 3.29 (s, 3H, OCH_3), 3.51 (m, 3H), 3.66–3.94 (m, 10H), 4.08 (m, 2H), 4.26 (m, 2H), 4.40 (d, 1H, CH_2Ph, $J = 11$ Hz), 4.51–4.80 (m, 12H), 4.89 (d, 1H, CH_2Ph, $J = 11$ Hz), 5.04 (d, 1H, H-1″, $J_{1'',2''} = 4$ Hz), 5.38 (d, 1H, H-1′, $J_{1',2'} = 1.1$ Hz), 5.50 (br s, 1H, H-2′), 7.05 (m, 2H, H-Aromatic), 7.14–7.43 (m, 42H, H-Aromatic), 7.54 (t, 1H, H-Aromatic), 7.71 (m, 4H, H-Aromatic), 8.08 (d, 1H, H-Aromatic); ^{13}C NMR ($CDCl_3$): δ 19.4 (C(CH$_3$)$_3$), 26.9 (C(CH$_3$)$_3$), 28.7 (C-4′), 54.7 (OCH$_3$), 66.8, 68.1, 69.6, 69.7, 70.2, 70.9, 71.4, 71.6, 71.9, 72.7, 73.3, 73.4, 73.8, 74.6, 74.9, 75.2, 75.5, 79.9, 80.0, 81.6 (C-2-6, C-2′-3′, C-5′-6′, C-2″-6″, CH$_2$Ph), 96.1, 99.6, 100.0 (C-1, C-1′, C-1″), 127.4–128.5, 129.7, 129.9, 130.4, 132.9, 133.5, 133.6, 135.7, 135.8, 138.1, 138.5, 138.5, 138.7, 139.0 (C-Aromatic), 165.8 (PhC=O). Anal. Calcd for $C_{82}H_{86}O_{16}$: C, 74.19; H, 6.53. Found: C, 74.18; H, 6.46.

3.18. Methyl α-D-glucopyranosyl-(1→3)-α-D-rhamnopyranosyl-(1→2)-α-D-mannopyranoside (6)

Compound **31** (86 mg, 0.065 mmol) was dissolved in CH_3OH/THF (3:1 v/v, 4 mL) and $NaOCH_3$ (1 M/CH_3OH) was added. The mixture was left stirring for two days and was then neutralised with H^+ ion exchange resin. The resin was removed by filtration and the filtrate concentrated. The residue was applied onto a silica gel column and eluted (toluene→5:1, toluene/EtOAc) to give methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (68 mg, 0.056 mmol, 86%). The debenzoylated material (68 mg, 0.056 mmol) was dissolved in CH_3OH/THF (1:1 v/v, 5 mL) and a catalytic amount of Pd/C (5 mg, 10%) was added. The reaction mixture was set under a hydrogen atmosphere at atmospheric pressure and stirred at rt. After 5 h, the slurry was filtered through a sandwich of filters and concentrated. The residue was dissolved in distilled H_2O, applied on a C_{18} column (10 g) and eluted with H_2O to give **6** (25 mg, 0.050 mmol, 89%). $[\alpha]_D$ +111 (*c* 0.7, H_2O); ^1H NMR (D_2O): δ 1.28 (d, 3H, H-6c′-a′, $J = 6$ Hz), 3.39 (s, 3H, OCH_3), 3.55 (dd, 1H, $J = 10$ Hz, $J = 4$ Hz), 3.57–3.94 (m, 14H), 4.23 (m, 1H), 4.86 and 4.95 (d, 1H, H-1′, $J_{1',2'} = 1.5$ Hz and d, 1H, H-1, $J_{1,2} = 1.5$ Hz), 5.23 (d, 1H, H-1″, $J_{1'',2''} = 4$ Hz); ^{13}C NMR (D_2O): δ 16.8 (C-6′), 55.0 (OCH$_3$), 60.8, 61.1, 67.0, 69.3, 69.8, 70.0, 70.4, 71.6, 71.9, 72.5, 72.9, 73.0, 78.1, 78.4 (C-2-6, C-2′-5′, C-2″-6″), 99.7, 100.5, 102.1 (C-1, C-1′, C-1″); MALDI-TOF MS calcd for $C_{19}H_{34}O_{15}$ $[M+Na]^+$: 525.1795, $[M+K]^+$: 541.1535. Found 525.52, 541.51.

Acknowledgements

We are thankful to the Swedish Research Council and EU (Glycotrain, Contract no. HPRN-CT-2000-00001) for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.03.015.

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