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A building block approach to the synthesis of a family of S-linked α-1,6-oligomannosides

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ABSTRACT

The syntheses of α-1,6-S-linked methyl di-, tetra- and hexamannosides are reported. The sulfur linkages are generated through coupling of thiolates (derived from anomeric thioacetates or isothiouronium bromides) with 6-deoxy-6-iodo sugars. Two approaches are detailed that involve [2 + 2 + 2] construction from either the reducing end or the non-reducing end. In constructing from the reducing end, coupling of a disaccharide thioacetate with a 6'-iodo reducing end disaccharide, followed by activation of the resulting tetrasaccharide to a 6'''-iodide, and iterative coupling with the same disaccharide thioacetate afforded the S-linked hexasaccharide, as well as the intermediate di- and tetrasaccharides. On the other hand, construction from the non-reducing end involved coupling of the above disaccharide thioacetate with an anomeric *S*-trityl protected 6'-iodo disaccharide. The resulting *S*-trityl tetrasaccharide was converted to a tetrasaccharide thioacetate, which was coupled with the same anomeric S-trityl protected 6'-iodo disaccharide to afford the hexasaccharide, which was elaborated to the methyl thioglycoside. The developed methodology may prove useful for the construction of other S-linked oligosaccharides.

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1. Introduction

Sulfur-linked glycoconjugates have attracted significant interest owing to their presence in various natural products (eg glucosinolates,[1] lincomycin,[2] glycoproteins[3–5]), and because of the chemically- and biochemically-inert nature of a thioglycoside linkage relative to and O-glycosidic linkage.[4,6–9] Oxygen and sulfur are members of the same group, and have similar bonding and geometries; however, as sulfur is less basic than oxygen, S-linked glycoconjugates typically do not undergo enzymatic transformation (for exceptions see Refs.[10–12]). S-linked glycoconjugates have received attention as enzyme inhibitors,[7,13] ligands for affinity chromatography,[14,15] and oligosaccharide[16] and glycoprotein[5,17] mimics.

Sulfur-linked glycoconjugates have proven to be especially useful mechanistic and structural probes for the study of glycosidases.[7] In the main, glycosidases operate through mechanisms in which two acidic amino acid residues play catalytic roles as acid/base and nucleophile, or acid and base.[18] Efforts to obtain structural data of so-called Michaelis complexes formed between substrate and wild-type glycosidases have been facilitated by the development of non-hydrolyzable substrate analogs, in particular S-linked glycosides. Seminal studies of a complex of *Fusarium oxysporum* endoglucanase I with an S-linked cellopentaoside provided compelling evidence in support of substrate distortion in a Michaelis complex of a glycoside hydrolase.[19] This general approach has since been used to solve X-ray structures of Michaelis complexes for a large number of glycoside hydrolases[7,20–22] and phosphorylases,[23] providing unprecedented insight into substrate binding and distortion and allowing assignment of enzymatic conformational itineraries.

Unlike O-glycoside formation, the synthesis of S-linked glycosides can be readily achieved by S_N2 displacement of sugar halides by sugar thiolates.[4] A strategic factor is therefore whether to include the mercapto group into the glycosyl acceptor (with the leaving group on the glycosyl donor) or vice versa. For β-linked S-glycosides, it is particularly convenient to use the former approach as α-glycosyl halides are readily prepared. However, the latter approach is also viable as anomeric thiolates, once formed, are typically configurationally stable for the duration of the coupling reaction[24,25] (see Refs.[16,26] for examples using this approach). For α-linked S-glycosides, it is generally more convenient to install the sulfur into the glycosyl donor, thereby controlling the stereochemistry at the building block stage (see Refs.[24,27,28] for examples of using this approach). As part of an ongoing study of family GH76 *endo*-α-1,6-mannanases, glycoside hydrolases with the ability to hydrolyze yeast α-1,6-mannan,[29–31] we wish to report on synthetic efforts that have led to the synthesis of a family of S-linked α-1,6-oligomannosides **1**–**3**, with potential to act as substrate mimics for structural investigations. Preliminary studies with these compounds have been recently reported.[32]

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2. Results and discussion

2.1. [2 + 4] approach

We envisioned an iterative, disaccharide coupling approach, initially building from the reducing end with an early installation of the methylthio group (Fig. 1). Initial formation of a reducing end disaccharide **6** (from the coupling of iodide **4** and thioacetate **5**), elaboration to a C6'-iodide **7** (a disaccharide electrophile), and coupling to a disaccharide anomeric thiolate (derived from **8** upon treatment with Et_2NH[33]) would afford a tetrasaccharide **9**. Elaboration of the tetrasaccharide to the non-reducing-end electrophile **10** and coupling to a thiolate derived from **8** would complete the assembly of the hexasaccharide **11**.

Isothiouronium bromide **12**[34] represents a readily available, configurationally-homogeneous α-mercapto precursor. Treatment of **12** with MeI/Et_3N in MeCN, conditions developed by Ibatullin et al.[35] afforded the thiomethyl glycoside **13**. Deacetylation under Zemplén conditions, followed by iodination using I_2, Ph_3P, imidazole,[36] and acetylation afforded the iodide **4** (Fig. 2a).

In assembling the disaccharide building block **8**, we chose to first introduce the mercapto group into each monosaccharide precursor, prior to forming the disaccharide, in order to avoid poorly stereoselective anomeric activation/sulfurization processes prior to

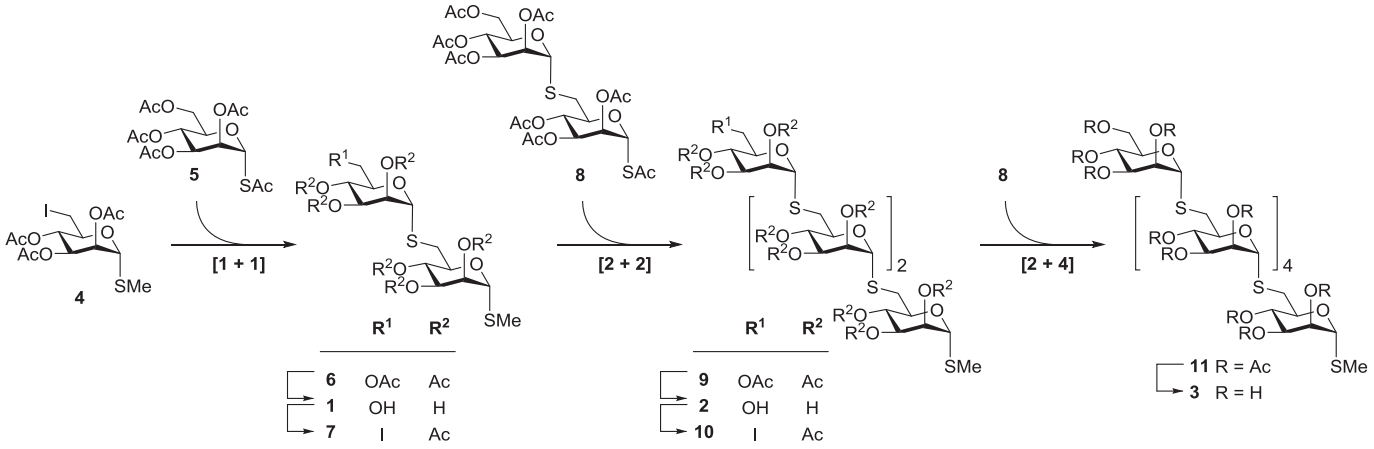

Fig. 1. Non-reducing end extension ([2 + 4]) approach to *S*-linked α-1,6-oligomannosides **1**–**3**.

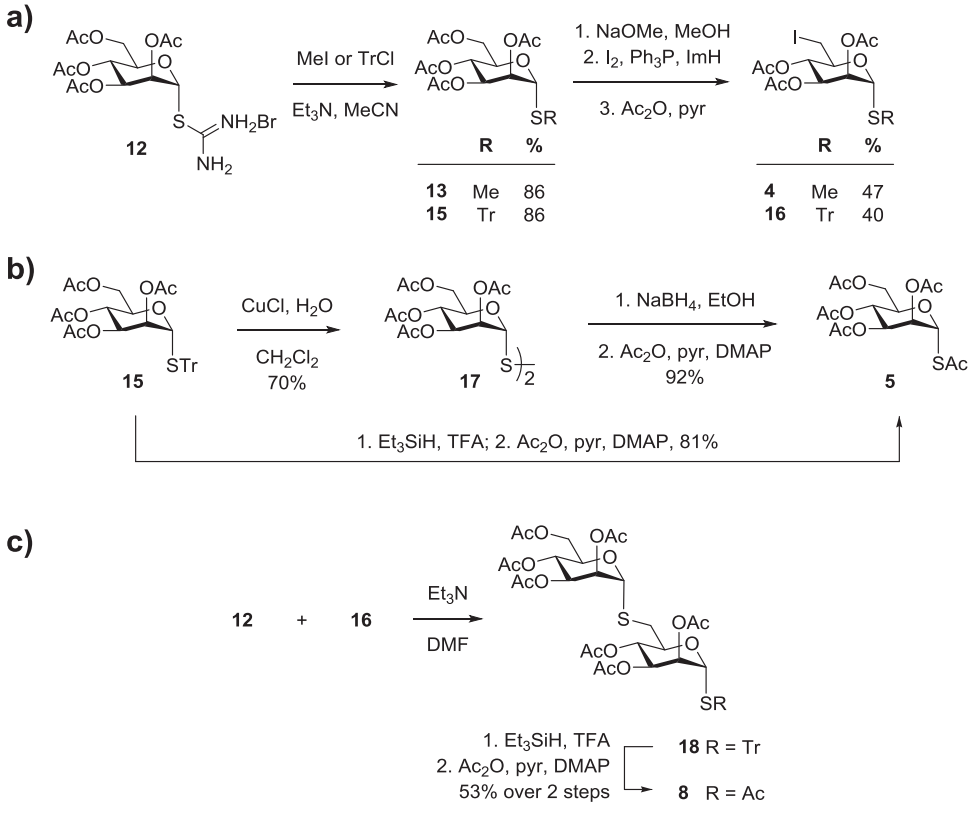

Fig. 2. (a) Synthesis of building blocks **4** and **16**. (b) Alternative approaches for removal of anomeric *S*-trityl group. (c) Synthesis of building blocks **8** and **18**.

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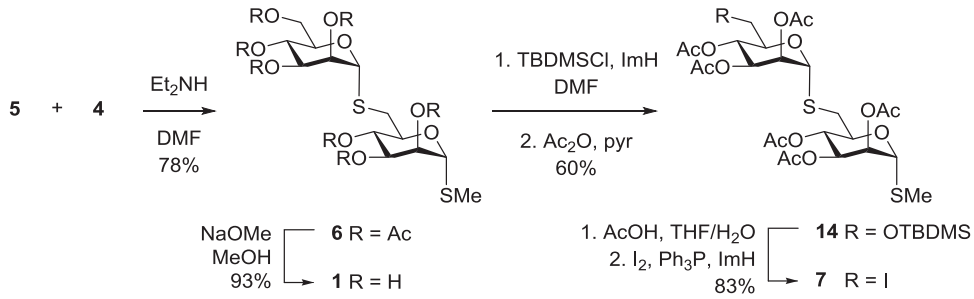

Fig. 3. Synthesis of S-linked α-1,6-dimannoside **1** and disaccharide iodide **7**.

executing the next coupling. This required the temporary protection of the anomeric sulfur to allow nucleophilic S-glycoside formation, and then unveiling of the latent thiolate precursor for the subsequent coupling reaction. Only a few protecting groups have been reported for anomeric sulfhydryl groups with sufficient sturdiness to withstand protecting group manipulations, most notably *S*-xanthenyl,[37] *S*-methoxytrityl,[27,38] and *S*-trityl.[28,39–42] Zhu and coworkers have reported difficulties with the installation and removal of anomeric *S*-trityl groups[38] and so we first investigated some of their basic transformations. An *S*-trityl group could be readily installed directly by treatment of **12** with TrCl/Et_3N in MeCN,[35] affording the thioglycoside **15** in excellent yield (Fig. 2a). Ma et al. have reported the mild deprotection of *S*-trityl groups using CuCl and H_2O in CH_2Cl_2, with the formation of disulfides.[43] When applied to **15**, this method proved applicable for the preparation of the diglycosyl disulfide **17**, which could be converted in two steps to the anomeric thioacetate **5** by sequential reduction and acetylation (Fig. 2b). More conveniently, we were able to reliably cleave the anomeric *S*-trityl group of **15** by treatment with Et_3SiH/TFA,[44] followed by acetylation, as reported by Fort et al.,[42] affording thioacetate **5** in good yield (Fig. 2b). Accordingly, building block **8** was then readily prepared from **15**. Zemplén deacetylation, iodination (I_2, Ph_3P, imidazole),[36] and acetylation afforded the iodide **16**. Condensation of **12** and **16** in the presence of Et_3N afforded the disaccharide **18** (Fig. 2c). Finally, cleavage of the *S*-trityl group with Et_3SiH/TFA, followed by acetylation, afforded the disaccharide thioacetate **8**, in 53% over 2 steps.

Returning to the preparation of the disaccharide **1**, condensation of thioacetate **5** and iodide **4** in the presence of Et_2NH in DMF[33] afforded the disaccharide **6** in 78% yield (Fig. 3). Deacetylation under Zemplén conditions afforded **1**. Towards the higher oligomers, efforts to directly iodinate **1** using I_2 and Ph_3P in various solvents including toluene, THF or DMF were disappointing. Instead, **1** was treated with TBDMSCl and imidazole in DMF,[45] followed by acetylation, affording the silyl ether **14**. Careful desilylation of **14** by treatment with AcOH in THF/H_2O afforded the primary alcohol, which was prone to acetyl migration. Nonetheless, with care iodination (I_2, Ph_3P, imidazole)[36] could be achieved in good yield, affording the disaccharide electrophile **7**.

Condensation of thioacetate **8** with disaccharide iodide **7** proceeded in 52% yield, affording the tetrasaccharide **9** (Fig. 4). Deacetylation (NaOMe/MeOH) delivered the second target, tetrasaccharide **2**. Toward the tetrasaccharide, **2** was silylated (TBDMSCl, imidazole, DMF) and acetylated (Ac_2O, pyr) affording silyl ether **19**. Removal of the silyl ether (AcOH in THF/H_2O) followed by iodination (I_2, Ph_3P, imidazole), gave the tetrasaccharide iodide **10**. Finally, condensation of **8** and **10** promoted by Et_2NH afforded the protected hexasaccharide **11**, in modest yield (27%), with the major byproduct consisting of the anomeric disulfide derived from **8**. The reaction was repeated in the presence of dithioerythritol (DTE), which has been employed previously to limit competing oxidation.[28] In this case, tlc analysis revealed reduced formation of the disulfide; however, numerous additional compounds were observed, possibly derived from alkylation of DTE, and the hexasaccharide was isolated in an even poorer 11% yield. Compound **11** was deprotected under Zemplén conditions to afford hexasaccharide **3**.

2.2. [4 + 2] approach

The [2 + 4] approach, which involves extension from the nonreducing end, was effective in providing access to the target oligosaccharides; however, the approach was cumbersome. Inefficiencies accrue owing to the need for protecting group manipulation to install the leaving group, and in particular, the failure to identify a direct means to activate the primary hydroxyl of compounds **1** and **2**, which instead each required a 4-step silylation/desilylation/iodination/acetylation protocol, via the acetylated primary alcohols that proved sensitive to intramolecular acyl group migration.

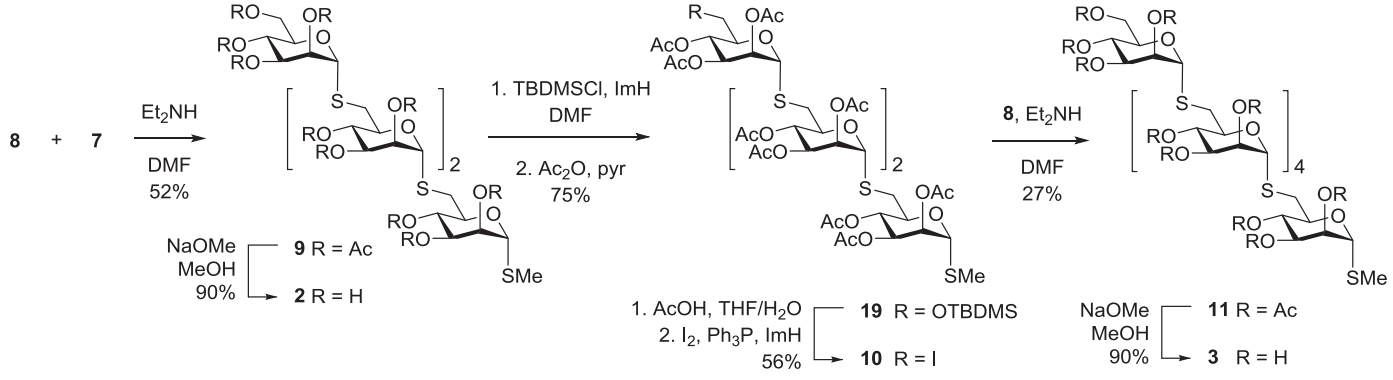

Fig. 4. Synthesis of S-linked α-1,6-tetramannoside **2** and α-1,6-hexamannoside **3**.

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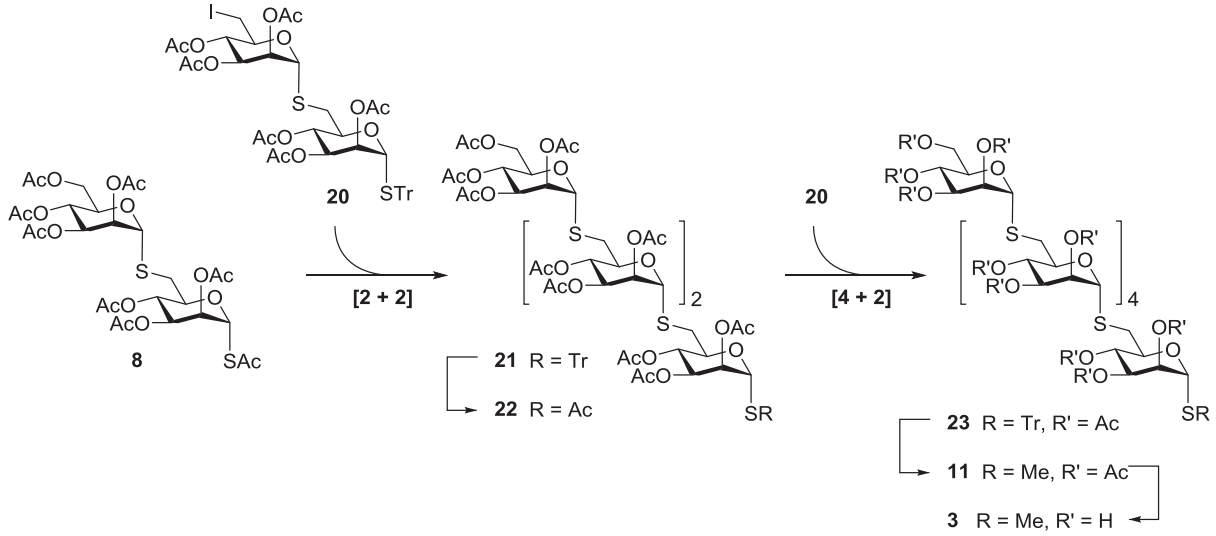

Fig. 5. Reducing end elongation ([4 + 2]) approach to S-linked α-1,6-oligomannoside **3**.

An alternative approach to the hexasaccharide involves reversing the order of assembly, commencing from non-reducing end disaccharide and coupling this to a disaccharide electrophile with a temporarily protected (as S-trityl) anomeric sulfhydryl group, followed by elaboration of the resulting tetrasaccharide to a tetrasaccharide thiolate and another coupling reaction to deliver the hexasaccharide. By this means, the inefficient and low yielding activation of the primary alcohol of the tetrasaccharide is avoided. The manipulations that had previously been involved in transforming a di- or tetrasaccharide to an electrophile would instead be replaced by manipulations involved in transforming a tetra- or hexasaccharide into a thiolate nucleophile. The general approach is shown in Fig. 5, and requires disaccharide thioacetate **8** and thioglycoside **20** as building blocks.

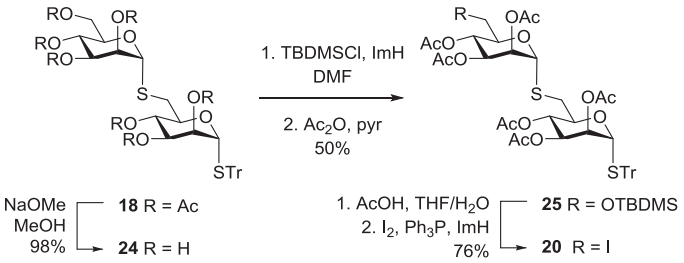

Fig. 6. Synthesis of *S*-trityl disaccharide iodide **20**.

Using the general methodology outlined above, disaccharide **20** was prepared uneventfully from thioglycoside **18** by deacetylation (**24**), silylation and acetylation (**25**), followed by desilylation and iodination (Fig. 6).

Condensation of **8** and **20** under the agency of Et_2NH afforded **21** in 47% yield (Fig. 7). Cleavage of the trityl group with Et_3SiH/TFA, followed by acetylation (Ac_2O, pyr) afforded **22**. Condensation of **22** and **20**, promoted by Et_2NH, afforded **23** in 35% yield. Again, the major byproduct was assigned as a disulfide derived from **20**. The anomeric thiotrityl group was converted to a thiomethyl group by sequential treatment with Et_3SiH/TFA, and MeI/Et_3N to afford **11**.

3. Conclusion

We report two approaches to the preparation of S-linked α-1,6-oligomannosides. The first approach provides access to di-, tetra- and hexasaccharide **1**–**3** through the iterative extension of a reducing-end thioglycoside, in which the reducing-end thiomethyl group is already installed, through sequential activation and nucleophilic substitution by an anomeric thiolate. The approach suffers from inefficient protecting group manipulations to gain access to the primary hydroxyl on a disaccharide and tetrasaccharide for activation as an iodide (compounds **7** and **10**). The second approach involves the iterative extension of a non-reducing end disaccharide, and utilizes an *S*-trityl group as a temporary protecting group for sulfur. While the method still requires a series of protecting group

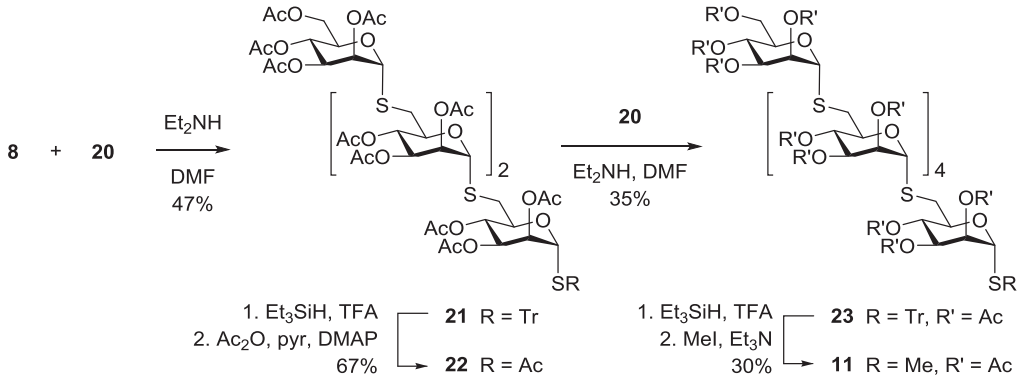

Fig. 7. Synthesis of protected S-linked α-1,6-hexamannoside **11**.

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manipulations to gain access to activated iodide (compound **20**), this reversed approach conducts this transformation on a disaccharide building block and does not require it to be repeated on a tetrasaccharide. We note a consistent reduction in yield of coupling reactions performed using anomeric thioacetates and 6-iodo sugars, with yields in the range 71–78% for monosaccharide ([1 + 1]) couplings; 47–52% for disaccharide ([2 + 2]) couplings; and 16–27% for couplings of disaccharides and tetrasaccharides (both [2 + 4] and [4 + 2] approaches); in addition, alkylation of hexasaccharide **23** with methyl iodide occurred in a similarly low 30% yield. The decline in yield may be associated with the small scale upon which these reactions were performed and the sensitivity to competing oxidation, and provide a limitation to extending the present methodology to higher oligomers. Nonetheless, this work outlines an effective approach to the construction of α-linked oligosaccharides through a building block approach, and are complementary to a recently reported approach for the assembly of S-linked α-1,6-oligoglucosides.[27]

4. Experimental

4.1. General

Proton nuclear magnetic resonance spectra (^{1}H NMR, 400 or 500 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 100 or 125 MHz) were obtained in deuterochloroform or methanol-d_4 (CD_3OD) with residual protonated solvent or solvent carbon signals as internal standards. Abbreviations for multiplicity are s, singlet; d, doublet; t, triplet; q, quartet. For oligosaccharides, rings are labeled A,B,C. . . from the reducing end. Flash chromatography was carried out on silica gel 60 according to the procedure of Still et al.[46] Analytical thin layer chromatography (t.l.c.) was conducted on aluminum-backed 2 mm thick silica gel 60 F_{254} and chromatograms were visualized with ceric ammonium molybdate (Hanessian's stain), potassium permanganate or 5% H_2SO_4/MeOH, with charring as necessary. Melting points were obtained using a hot-stage or capillary apparatus and are corrected. High resolution mass spectra (HRMS) were obtained using an ESI-TOF-MS; all samples were run using 0.1% formic acid. Dry CH_2Cl_2, THF, and Et_2O were obtained from a dry solvent apparatus (Glass Contour of SG Water, Nashua, USA) as per the procedure of Pangborn et al.[47] Dry DMF was dried over 4 Å molecular sieves. Pet. spirits refers to petroleum ether, boiling range 40–60 °C.

4.2. Methyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (13)

A solution of tetra-*O*-acetyl-α-D-mannopyranosyl isothiouronium bromide **12**[34] (0.350 g, 0.720 mmol) in acetone (2.0 ml) was treated with methyl iodide (0.461 ml, 7.40 mmol) and Et_3N (1.17 ml, 8.40 mmol), and the mixture was stirred at rt for 2 h. The solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc and washed with water, then dried ($MgSO_4$), and the solvent evaporated. The residue was subjected to flash chromatography (EtOAc/pet. spirits) to give compound **13** (0.241 g, 86%); mp 115–118 °C (lit.[48] mp 121–122 °C). The physical and spectroscopic data were consistent with literature values.[48]

4.3. Methyl 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-1-thio-α-D-mannopyranoside (4)

4.3.1. Methyl 1-thio-α-D-mannopyranoside

A solution of mannoside **13** (0.290 g, 0.777 mmol) was treated with 1 M NaOMe in methanol (0.25 ml) at rt with stirring for 2 h. The solution was adjusted to pH 5–6 with Amberlite IR-120 resin (H^+ form), filtered, and the filtrate evaporated to dryness under reduced pressure to give the title tetraol (0.130 g, 79%).

4.3.2. Methyl 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-1-thio-α-D-mannopyranoside (4)

A solution of triphenylphosphine (187 mg, 0.714 mmol), imidazole (65 mg, 0.95 mmol), iodine (181 mg, 0.713 mmol) and tetraol (0.100 g, 0.476 mmol) in THF (14 ml) was heated at reflux with stirring for 2 h. The solvent was evaporated to dryness under reduced pressure. The residue was dissolved in acetic anhydride (0.23 ml, 2.4 mmol) and pyridine (6 ml) and the solution was stirred at rt for 1.5 h. The solvent was removed under reduced pressure with acetic anhydride removed azeotropically by co-evaporation with toluene. The resulting residue was purified by flash chromatography using EtOAc/pet. spirits (5% Et_3N in 20% EtOAc/pet. spirits) to give iodide **4** (0.124 g, 59%); mp 155–158 °C; $[\alpha]_D^{23}$ +65.1 (c 1.0 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.34 (1 H, dd, $J_{1,2}$ 1.4, $J_{2,3}$ 3.3 Hz, H2), 5.26 (1 H, dd, $J_{2,3}$ 3.4, $J_{3,4}$ 9.9 Hz, H3), 5.21–5.11 (2 H, m, H1, 4), 4.21 (1 H, ddd, $J_{4,5}$ 9.2, $J_{5,6}$ 2.5 Hz, H5), 3.32 (1 H, dd, $J_{5,6}$ 2.5, $J_{6,6}$ 10.9 Hz, H6), 3.21 (1 H, dd, $J_{5,6}$ 2.5, $J_{6,6}$ 10.9 Hz, H6), 2.25, 2.16, 2.08, 1.99 (12 H, s, 4 × CH_3); δ_C (100 MHz, $CDCl_3$) 169.8 (3 × C = O), 83.5 (C1), 70.8, 70.5, 70.2 (C3,4,5), 69.1 (C2), 20.85, 20.75, 20.6 (3 × CH_3, CO), 14.0 (SCH_3), 3.6 (C6); HRMS (ESI$^+$) calcd for $C_{13}H_{19}IO_7SNa$ $(M + Na)^+$ 468.9788. Found 468.9790.

4.4. Triphenylmethyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (15)

A mixture of tetra-*O*-acetyl-α-D-mannopyranosyl isothiouronium bromide **12**[34] (0.300 g, 0.617 mmol), triphenylmethyl chloride (0.26 g, 0.93 mmol) and triethylamine (0.22 ml, 1.54 mmol) in dry MeCN (5 ml) was stirred at rt for 3 h. The solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc and washed with water, then dried ($MgSO_4$), and the solvent evaporated. The residue was purified by flash chromatography (3:7 EtOAc/pet. spirits) to give compound **15** (0.321 g, 86%); mp 155–158 °C, $[\alpha]_D^{26}$ +83.0 (c 0.5 in $CHCl_3$; lit.[41] 19.9). δ_H (400 MHz, $CDCl_3$) 7.56–6.93 (15 H m, Ph), 5.26 (3 H, m, H2,3,4), 4.82 (1 H, d, $J_{1,2}$ 1.6 Hz, H1), 4.28 (2 H, m, H5,6), 3.90 (1 H, dd, $J_{5,6}$ 3.3, $J_{6,6}$ 13.3 Hz, H6), 2.10, 2.02, 2.02, 1.97 (12 H, 4 × s, Ac); δ_C (100 MHz, $CDCl_3$) 169.8, 169.8, 169.7, 169.5 (4 × C = O), 144.1, 129.9, 128.1, 127.2 (Ph), 83.0 (C5), 72.3 (C1), 71.0 (C6), 69.2 (C2), 66.0 (C4), 62.4 (C3), 60.4 (**C**Ph_3), 20.8, 20.7, 20.6, 20.6 (4 C, CH_3).

4.5. Triphenylmethyl 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-1-thio-α-D-mannopyranoside (16)

4.5.1. Triphenylmethyl 1-thio-α-D-mannopyranoside

A solution of mannoside **15** (0.161 g, 0.262 mmol) was treated with 1 M NaOMe (0.09 ml) in methanol at rt with stirring for 4 h. The solution was adjusted to pH 5–6 with Amberlite IR-120 resin (H^+ form), filtered, and the filtrate evaporated to dryness under reduced pressure to give the title tetraol (0.0920 g, 75%), which was used without purification in the next step.

4.5.2. Triphenylmethyl 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-1-thio-α-D-mannopyranoside (16)

A solution of triphenylphosphine (86.0 mg, 0.328 mmol), imidazole (37.0 mg, 0.544 mmol), iodine (841 mg, 0.544 mmol) and tetraol (100 mg, 0.234 mmol) in THF (4 ml) was heated at reflux with stirring for 1 h. The solvent was evaporated to dryness under reduced pressure and pyridine (6.33 ml) was added to dissolve the residue. The solution was treated with acetic anhydride (0.23 ml, 2.4 mmol) at rt with stirring for 1 h. The solvent was removed under reduced pressure with acetic anhydride removed azeotropically by co-evaporation with toluene. The resulting residue was purified by flash

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chromatography (2% Et_3N in 20% EtOAc/hexane) to give iodide **16** (84.0 mg, 54%), mp 53–56 °C, $[\alpha]_D^{25}$ +76.2 (c 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) δ 7.39–7.20 (15 H, m, Ph), 5.26 (1 H, dd, $J_{2,3}$ 3.0, $J_{3,4}$ 9.8 Hz, H3), 5.13–5.06 (2 H, m, H2,4), 4.76 (1 H, d, $J_{1,2}$ 1.9 Hz, H1), 4.01 (1 H, ddd, $J_{4,5}$ 2.9, $J_{5,6}$ 6.5, 9.5 Hz, H5), 3.21 (1 H, dd, $J_{5,6}$ 3.0, $J_{6,6}$ 11.0 Hz, H6), 3.12 (1 H, dd, $J_{5,6}$ 6.6, $J_{6,6}$ 11.1 Hz, H6), 2.05, 1.99, 1.96 (9 H, 3s, 3 × Ac); δ_C (100 MHz, $CDCl_3$) 169.69, 169.66, 169.3 (C = O), 144.0 (Ph), 129.9, 128.1, 127.1 (Ph), 82.5 (C1), 72.4, 72.3, 70.21, 70.17 (C2,3,4,5), 68.9 (CPh), 20.8, 20.7, 20.6 (CH_3); HRMS (ESI^+) calcd for $C_{31}H_{31}IO_7SNa$ $(M + Na)^+$ 697.0727. Found 697.0728.

4.6. 2,3,4,6-Tetra-O-acetyl-1-S-acetyl-1-thio-α-D-mannopyranose (5)

4.6.1. Bis-(tetra-O-acetyl-α-D-mannopyranosyl) disulfide (17)

A mixture of compound **15** (0.101 g, 0.171 mmol), CuCl (66.0 mg, 0.66 mmol) and water (12 μL, 0.66 mmol) in dichloroethane (1.5 ml) was heated to reflux with stirring for 1 h. The solvent was evaporated to dryness under reduced pressure and EtOAc (5 ml) was added. The EtOAc solution was washed with water (2 × 5 ml), dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (3:7 EtOAc/pet. spirits) to give disulfide **17** (84.1 mg, 70%), mp 55–58 °C; $[\alpha]_D^{24}$ +116.8 (c 0.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) δ 5.33 (1 H, dd, $J_{1,2}$ 1.5, $J_{2,3}$ 3.4 Hz, H2), 5.26 (1 H, dd, $J_{2,3}$ 9.9, $J_{3,4}$ 3.4 Hz, H3), 5.18 (1 H, d, $J_{1,2}$ 1.4 Hz, H1), 5.15 (1 H, t, $J_{3,4}$ 9.7 Hz, H4), 4.20 (1 H, m, H5), 3.32 (1 H, dd, $J_{5,6}$ 2.6, $J_{6,6}$ 10.9 Hz, H6), 3.21 (1 H, dd, $J_{5,6}$ 8.8, $J_{6,6}$ 10.9 Hz, H6), 2.25, 2.15, 2.08, 1.99 (12 H, 4 × s, Ac); δ_C (100 MHz, $CDCl_3$) 170.6, 169.7, 169.7, 169.6 (C = O), 87.3 (C1), 70.9, 69.6, 68.9, 65.8 (C2,3,4,5), 62.0 (C6), 20.8, 20.65, 20.56 (4 × CH_3). The spectroscopic data were consistent with literature values.[49]

4.6.2. 2,3,4,6-Tetra-O-acetyl-1-S-acetyl-1-thio-α-D-mannopyranose (5)

(a) Disulfide **17** (81.2 mg, 0.112 mmol) in ethanol (1 ml) was treated with $NaBH_4$ (8.5 mg, 0.22 mmol) for 30 min. The solvent was evaporated under reduced pressure and the thiolate residue was treated with acetic anhydride (500 μL) and pyridine (500 μL) for 15 min. The solvent was co-evaporated using toluene and EtOAc (5 ml) added. The organic layer was washed with water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and purified by flash chromatography using EtOAc: pet. spirits (3:7) to give compound **5** (61.7 mg, 92%); mp 49–53 °C; $[\alpha]_D^{23}$ +72.5 (c 0.25 in $CHCl_3$; lit.[50] +73) δ_H (400 MHz, $CDCl_3$) 5.94 (1 H, d, $J_{1,2}$ 1.9 Hz, H1), 5.38–5.24 (2 H, m, H2, 4), 5.08 (1 H, dd, $J_{3,4}$ 3.3, $J_{2,3}$ 10.0 Hz, H3), 4.27 (1 H, dd, $J_{5,6}$ 4.8, $J_{6,6}$ 12.4 Hz, H6), 4.04 (1 H, m, H6), 3.91 (1 H, ddd, $J_{4,5}$ 10.0, $J_{5,6}$ 4.8, $J_{5,6}$ 2.5 Hz, H5), 2.41 (3 H, s, SAc), 2.17, 2.07, 2.03, 1.98 (12 H, 4s, 4 × Ac). The ^{13}C NMR spectrum was identical to that reported.[51]

(b) A solution of trityl thiomannoside **15** (150 mg, 0.250 mmol) and triethylsilane (379 μL, 2.35 mmol) in dry CH_2Cl_2 (4 ml) was treated with TFA (2.6 ml, 0.93 mmol) for 45 min. The solvent was evaporated and the intermediate thiol treated with acetic anhydride (2.5 ml) and pyridine (2.5 ml) for 12 h. The reaction solvent was evaporated to dryness under reduced pressure. The residue was diluted with CH_2Cl_2 (5 ml), washed with water (5 ml) and sat. aq $NaHCO_3$ (5 ml). The organic layer was evaporated to dryness and purified by flash chromatograpy (3:7 EtOAc/pet. spirits), to give thioacetate **5** (81.4 mg, 81%). The physical and chemical data was identical to that of the previous procedure.

4.7. Methyl S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (6)

A solution of the iodide **4** (39.3 mg, 0.0686 mmol) and thioacetate **5** (32.1 mg, 0.0789 mmol) in DMF (0.1 ml) was treated with diethylamine (0.020 ml, 0.137 mmol) and the solution stirred at rt for 1 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The solution was washed with 0.1 N HCl, water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and purified by flash chromatography (40% EtOAc/pet. spirits) to give the corresponding thiomannoside **6** (36 mg, 78%), mp 56–59 °C, $[\alpha]_D^{26}$ +145.1 (c 1.0 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.23–5.34 (7 H, m, $H(2,3,4)^A$,$(1,2,3,4)^B$), 5.12 (1 H, s, $H1^A$), 4.28–4.37 (3 H, m, $H(5,6)^A$,5^B), 4.08 (1 H, dd, $J_{5,6}$ 1.6, $J_{6,6}$ 12.0 Hz, $H6^A$), 2.85 (1 H, dd, $J_{5,6}$ 2.7, $J_{6,6}$ 14.0 Hz, $H6^B$), 2.73 (1 H, dd, $J_{5,6}$ 7.5 Hz, $H6^B$), 2.17 (3 H, SCH_3), 2.16, 2.15, 2.11, 2.05, 2.04 (15 H, 5s, 5 × Ac), 1.98 (6 H, s, 2 × Ac); δ_C (100 MHz, $CDCl_3$) 170.6, 170.0, 169.9, 169.82, 169.75, 169.72, 169.67 (7 C, 7 × C = O), 83.4, 82.3 ($C1^{A,B}$), 70.8, 70.7, 69.7, 69.32, 69.29, 69.2, 68.9, 66.2 (7 C, 7 × CH, $C(2,3,4,5)^A$,$(2,3,4,5)^B$), 62.2 ($C6^B$), 31.7 ($C6^A$), 20.9, 20.8, 20.73, 20.69, 20.67, 20.61, 20.59 (7 C, 7 × CH_3CO), 13.8 (SCH_3); HRMS (ESI^+) calcd for $C_{27}H_{38}O_{16}S_2Na$ $(M + Na)^+$ 705.1494. Found 705.1492.

4.8. Methyl S-(α-D-mannopyranosyl)-(1→6)-(1,6-dithio-α-D-mannopyranoside) (1)

A solution of thiomannoside **6** (3.09 g, 4.53 mmol) was treated with 1 M NaOMe in methanol (1.8 ml) at rt with stirring for 5 h. The solution was adjusted to pH 5–6 with Amberlite IR-120 resin (H^+ form), filtered, and the filtrate evaporated to dryness under reduced pressure. The residue crystallized upon standing to give disaccharide (**1**) (1.63 g, 93%), mp 53–56 °C, $[\alpha]_D^{24}$ +122.5 (c 1.0 in MeOH). δ_H (400 MHz, CD_3OD) 5.29 (1 H, s, $H1^B$), 5.09 (1 H, s, $H1^A$), 4.01 (1 H, m, $J_{5,6}$ 8.1 Hz, $H5^A$), 3.90 (3 H, m, $H2^A$,$(2,5)^B$), 3.84 (1 H, dd, $J_{5,6}$ 2.1, $J_{6,6}$ 11.8 Hz, $H6^B$), 3.73 (1 H, dd, $J_{5,6}$ 5.9, $J_{6,6}$ 11.8 Hz, $H6^B$), 3.69–3.59 (4 H, m, $H(3,4)^{A,B}$), 3.16 (1 H, dd, $J_{5,6}$ 8.2, $J_{6,6}$ 13.9 Hz, $H6^A$), 2.74 (1 H, dd, $J_{5,6}$ 8.2, $J_{6,6}$ 13.9 Hz, $H6^A$), 2.15 (3 H, s, SCH_3); δ_C (100 MHz, CD_3OD) 86.1, 84.8 ($C1^{A,B}$), 73.6, 72.2, 71.9, 71.8, 71.7, 70.0, 67.4 (8 C, $C(2,3,4,5)^{A,B}$), 61.4 ($C6^B$), 31.5 ($C6^A$), 12.2 (SCH_3); HRMS (ESI^+) calcd for $C_{13}H_{24}O_9S_2Na$ $(M + Na)^+$ 411.0754. Found 411.0746.

4.9. S-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-α-D-mannopyranose) (8)

4.9.1. Triphenylmethyl S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (18)

A solution of iodide **16** (139 mg, 0.206 mmol) and tetra-*O*-acetyl-α-D-mannopyranosyl isothiouronium bromide **12**[34] (138 mg, 0.308 mmol) in DMF (1.1 ml) was treated with triethylamine (0.34 ml, 2.4 mmol) for 2 h. The solvent was evaporated under reduced pressure and EtOAc was added to dissolve the residue. The EtOAc solution was washed with water, dried ($MgSO_4$), evaporated to dryness and the residue was purified by flash chromatography (30% EtOAc/pet. spirits) to give impure trityl thiomannoside **18** (134 mg).

4.9.2. S-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-α-D-mannopyranose) (8)

A solution of impure trityl thiomannoside **18** (50.0 mg, 0.055 mmol) and triethylsilane (20.8 μL, 0.129 mmol) in CH_2Cl_2 (1 ml) was treated with TFA (1 ml) for 45 min to give the intermediate thiol, which was treated with acetic anhydride (0.6 ml, 6.3 mmol) and pyridine (0.6 ml) for 12 h. At completion, the solvent was evaporated under reduced pressure. The resulting residue was diluted with CH_2Cl_2 (5 ml), and the solution was washed with water (5 ml) and sat. aq $NaHCO_3$ (5 ml). The organic layer was dried ($MgSO_4$) and filtered and evaporated to dryness. The residue was purified by flash chromatography (3:7 → 1:1 EtOAc/pet. spirits) to give thioacetate **8** (29.4 mg, 53%), mp 52–55 °C, $[\alpha]_D^{24}$ +79.9 (c 1.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.88 (1 H, d, $J_{1,2}$ 1.9 Hz, $H1^A$), 5.39–5.20 (6 H, m, $H(2,4)^A$,$(1,2,3,4)^B$), 5.06 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.1 Hz, $H3^A$), 4.34–4.25 (2 H, m, $H5^A$,6^B), 4.08 (1 H, m, $H6^B$), 3.89 (1 H, ddd, $J_{4,5}$ 9.3,

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$J_{5,6}$ 6.1, $J_{5,6}$ 3.1 Hz, H5^{B}), 2.87 (1 H, dd, $J_{5,6}$ 3.1, $J_{6,6}$ 14.3 Hz, H6^{A}), 2.67 (1 H, dd, $J_{5,6}$ 6.1, $J_{6,6}$ 14.3 Hz, H6^{A}), 2.43 (3 H, s, SAc), 2.16, 2.09, 2.04, 2.03, 1.98, 1.97 (21 H, 6 s, 7 × OAc); δ_H (100 MHz, $CDCl_3$) 190.2 (SC = O), 171.1, 170.6, 169.92, 169.85, 169.8, 169.67, 169.67 (OC = O), 82.8 (C1^{B}), 79.9 (C1^{A}), 73.6, 70.82, 70.75, 69.68, 69.3, 69.2, 68.0, 66.1 (C(2,3,4)^{A,B}), 62.2 (C6^{B}), 31.7 (C6^{A}), 31.2 (SCO**C**H$_3$), 20.9, 20.8, 20.70, 20.67, 20.6 (**C**H$_3$CO$_2$); HRMS (ESI$^+$) calcd for $C_{28}H_{38}O_{17}S_3$ (M + Na)$^+$ 733.1443. Found 733.1442.

4.10. Methyl S-(2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (14)

Disaccharide **1** (0.630 g, 1.62 mmol) in dry DMF (1.2 ml), was treated with TBDMSCl (0.342 g, 2.27 mmol) and imidazole (0.276 g, 4.05 mmol) at 35 °C with stirring for 1 h. The reaction mixture was cooled to rt and acetic anhydride, pyridine and DMAP were added and allowed to stir at room temperature for 1 h. The solvent was removed under reduced pressure and acetic anhydride removed azeotropically by co-evaporation with toluene. The resulting residue was diluted with CH_2Cl_2 and washed sequentially with 0.1 M aq HCl, brine, and water, dried ($MgSO_4$), evaporated to dryness under reduced pressure. The resulting residue was purified by flash chromatography (3:7 → 1:1 EtOAc/pet. spirits), to give silyl thiomannoside **14** as clear oil (0.739 g, 60%), $[\alpha]_D^{25}$ +160.0 (c 0.25 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.38–5.20 (7 H, m, H(2,3,4)^{A},(1,2,3,4)^{B}), 5.12 (1 H, d, $J_{1,2}$ 1.3 Hz, H1^{A}), 4.35–4.26 (1 H, m, H5^{A}), 4.13 (1 H, ddd, $J_{4,5}$ 9.7, $J_{5,6}$ 4.5, $J_{5,6}$ 2.4 Hz, H5^{B}), 3.73 (1 H, dd, $J_{5,6}$ 4.6, $J_{6,6}$ 11.6 Hz, H6^{B}), 3.67 (1 H, dd, $J_{5,6}$ 2.4, $J_{6,6}$ 11.6 Hz, H6^{B}), 2.85 (1 H, dd, $J_{5,6}$ 2.9, $J_{6,6}$ 14.0 Hz, H6^{A}), 2.72 (1 H, dd, $J_{5,6}$ 7.5, $J_{6,6}$ 14.0 Hz, H6^{A}), 2.18, 2.15, 2.13, 2.05, 2.02 (18 H, 5s, 6 × COCH$_3$), 1.98 (3 H, s, SCH$_3$), 0.9 (9 H, s, C(CH$_3$)$_3$), 0.05, 0.03 (6 H, 2 s, Si(CH$_3$)$_2$); δ_C (100 MHz, $CDCl_3$) 170.0, 169.94, 169.91, 169.8, 169.5 (6 C, C = O), 83.3, 82.2 (C1^{A,B}), 71.8, 71.1, 70.1, 69.7, 69.4, 69.0, 66.49 (8 C, C(2,3,4,5)^{A,B}), 62.0 (C6^{B}), 31.6 (C6^{A}), 25.8 (C(**C**H$_3$)$_3$), 20.9, 20.85, 20.76, 20.75, 20.66, 20.62 (6 C, 6 × CH$_3$CO), 18.2 (**C**(CH$_3$)$_3$), 13.7 (SCH$_3$); HRMS (ESI$^+$) calcd for $C_{31}H_{50}O_{15}S_2SiNa$ (M + Na)$^+$ 777.2253. Found 777.2252.

4.11. Methyl S-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (7)

4.11.1. Methyl S-(2,3,4-tri-O-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside)

Silyl thiomannoside **14** (30.0 mg, 0.0397 mmol) was treated with AcOH, H_2O, THF (3:1:1, 2.5 ml) at 40 °C with stirring overnight. The solvent was co-evaporated with water to remove excess acid and the residue was purified by flash chromatography (7:3 EtOAc/pet. spirits) to give the title alcohol (25.2 g, 99%), mp 63–66 °C, $[\alpha]_D^{26}$ +138.8 (c 1.0 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.38–5.21 (7 H, m, H(1,2,3,4)^{A},(2,3,4)^{B}), 5.12 (1 H, d, $J_{1,2}$ 1.4 Hz, H1^{B}), 4.36–4.27 (1 H, m, H5^{A}), 4.12 (1 H, m, H5^{B}), 3.73–3.58 (2 H, m, H6^{B}), 2.89 (1 H, dd, $J_{5,6}$ 3.0, $J_{6,6}$ 14.0 Hz, H6^{A}), 2.73 (1 H, dd, $J_{5,6}$ 7.1, $J_{6,6}$ 14.0 Hz, H6^{A}), 2.47 (1 H, dd, *J* 8.1, *J* 6.1 Hz, OH), 2.18, 2.15, 2.15, 2.08, 2.06, 2.00 (18 H, 6 s, 6 × Ac), 1.98 (3 H, s, SCH$_3$); δ_C (100 MHz, $CDCl_3$) 170.9, 170.1, 170.0, 169.9, 169.8, 169.7 (6 C, 6 × C = O), 83.4, 82.4 (C1^{A,B}), 71.4, 70.9, 70.7, 69.6, 69.3, 69.03, 69.01, 66.6 (C(2,3,4,5)^{A,B}), 61.3 (C6^{B}), 32.0 (C6^{A}), 20.87, 20.86, 20.8, 20.7, 20.6 (6 C, 6 × CO**C**H$_3$), 13.8 (SCH$_3$); HRMS (ESI$^+$) calcd for $C_{25}H_{36}O_{15}S_2Na$ (M + Na)$^+$ 663.1388. Found 663.1387.

4.11.2. Methyl S-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (7)

A solution of triphenylphosphine (18.4 mg, 0.053 mmol), imidazole (9.6 mg, 0.14 mmol), iodine (16.5 mg, 0.065 mmol) and primary alcohol (30.0 mg, 46.8 mmol) in toluene (1 ml) was heated at reflux with stirring for 90 min. The solvent was evaporated to dryness under reduced pressure and the residue was purified by flash chromatography (30% EtOAc/pet. spirits) to give iodide **7** (29.3 mg, 84%). δ_H (400 MHz, $CDCl_3$) 5.34–5.13 (8 H, m, H(1,2,3,4)^{A,B}), 4.34 (1 H, m, H5^{B}), 4.20 (1 H, dd, $J_{4,5}$ 9.2, $J_{5,6}$ 2.6 Hz, H5^{A}), 3.30 (1 H, dd, $J_{5,6}$ 2.5, $J_{6,6}$ 10.9 Hz, H6^{B}), 3.19 (1 H, dd, $J_{5,6}$ 8.6, $J_{6,6}$ 10.9 Hz, H6^{B}), 3.04 (1 H, dd, $J_{5,6}$ 2.7, $J_{6,6}$ 14.3 Hz, H6^{A}), 2.77 (1 H, dd, $J_{5,6}$ 2.7, $J_{6,6}$ 14.3 Hz, H6^{A}), 2.19, 2.16, 2.16. 2.08, 2.07, 1.99 (21 H, 6s, 6 × Ac,SMe); δ_C (100 MHz, $CDCl_3$) 170.0, 169.80, 169.77, 169.7 (6 C, C = O), 83.3, 81.8 (C1^{A,B}), 70.80, 70.78, 70.5, 70.1, 69.43, 69.38, 69.0, 68.9 (C(2,3,4,5)^{A,B}), 31.5 (C6^{A}), 21.0, 20.9, 20.8, 20.7, 20.61, 20.56 (6 × **C**H$_3$CO), 13.7 (SCH$_3$), 3.7 (C6^{B}); HRMS (ESI$^+$) calcd for $C_{25}H_{35}IO_{14}S_2Na$ (M + Na)$^+$ 773.0405. Found 773.0405.

4.12. Methyl S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (9)

A solution of iodide **7** (56.9 mg, 0.0834 mmol) and thioacetate **8** (68.2 mg, 0.0959 mmol) in DMF (0.2 ml) was treated with diethylamine (24 μL) with stirring at rt for 30 min. The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic layer was washed with aq 0.1 N HCl, water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and the residue was purified by flash chromatography (4:6 →6:4→8:2 EtOAc/pet. spirits) to give thiomannoside **9** (56.0 mg, 52%), mp 50–53 °C, $[\alpha]_D^{27}$ +169.1 (c 0.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.34–5.20 (15 H, m, H(2,3,4)^{A},(1,2,3,4)^{B,C,D}), 5.12 (1 H, d, $J_{1,2}$ 1.2 Hz, H1^{A}), 4.34–4.21 (5 H, m, H(5^{A,B,C},(5,6)^{D}), 4.15–4.03 (1 H, m, H6^{D}), 3.04–2.60 (6 H, m, H(6,6)^{A,B,C}), 2.17, 2.16, 2.14, 2.14, 2.13, 2.09, 2.07, 2.04, 1.98, 1.98 (42 H, 10s, 13 × Ac, SCH$_3$); δ_C (100 MHz, $CDCl_3$) 170.5, 170.1, 169.94, 169.92, 169.78, 169.77, 169.62, 169.62, 169.61 (13 × C = O), 83.2, 81.6, 81.4, 81.0 (C1^{A,B,C,D}), 77.2, 70.89, 70.88, 70.80, 70.77, 69.4, 69.3, 69.17, 69.15, 69.02, 68.99, 68.9, 68.8, 66.25, 62.24 (C(2,3,4,5)^{A,B,C},(2,3,4,5,6)^{D}), 31.6, 31.2, 30.7 (C6^{A,B,C}), 20.90, 20.87, 20.84, 20.82, 20.69, 20.66, 20.61, 20.60, 20.59 (13 × **C**H$_3$CO), 13.6 (SCH$_3$); HRMS (ESI$^+$) calcd for $C_{51}H_{70}O_{30}S_4Na$ (M + Na)$^+$ 1313.2727. Found 1313.2750.

4.13. Methyl S-(α-D-mannopyranosyl)-(1→6)-S-(6-thio-α-D-mannopyranosyl)-(1→6)-S-(6-thio-α-D-mannopyranosyl)-(1→6)-(1,6-dithio-α-D-mannopyranoside) (2)

A solution of thiomannoside **9** (50.0 mg, 0.0387 mmol) was treated with 1 M NaOMe in methanol (50.0 μL) at rt with stirring for 4 h. The solution was adjusted to pH 5–6 with Amberlite IR-120 resin (H$^+$ form), filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was purified by reverse phase chromatography (1→19:1→9:1 H_2O/MeCN, on C18 silica) to give tetrasaccharide **2** (26.0 mg, 90%), mp 169–172 °C, $[\alpha]_D^{24}$ +302.3 (c 1.0 in MeOH). δ_H (400 MHz, CD_3OD) 5.32–5.27 (3 H, m, H1^{B,C,D}), 5.10 (1 H, d, $J_{1,2}$ 1.2 Hz, H1^{A}), 4.08–3.56 (15 H, m, H(2,3,4)^{A,B,C},(2,3,4,5,6,6)^{D}), 3.24–3.12 (3 H, m, H6^{A,B,C}), 2.74 (3 H, m, H6^{A,B,C}), 2.14 (3 H, s, SCH$_3$); δ_C (100 MHz, CD_3OD) 85.9, 87.5, 84.5, 84.3 (C1^{A,B,C,D}), 73.6, 72.1, 72.0, 71.9, 71.8, 71.7, 71.6, 71.5, 70.34, 70.29, 70.1, 67.5 (C(2,3,4,5)^{A,B,C},(2,3,4,5,6)^{D}), 30.9 (C6^{A,B,C}), 13.9 (SCH$_3$); HRMS (ESI$^+$) calcd for $C_{25}H_{44}O_{17}S_4Na$ (M + Na)$^+$ 767.1354. Found 767.1353.

4.14. Methyl S-(2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (19)

Tetrasaccharide **2** (20.0 mg, 26.9 μmol) in dry DMF (0.1 ml) at 35 °C, was treated with TBDMSCl (5.67 mg, 37.6 μmol) and imidazole

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(4.58 mg, 67.3 μmol) at 35 °C with stirring for 1 h. The reaction mixture was cooled to rt and acetic anhydride (1 ml) and pyridine (0.5 ml) were added and the solution was stirred at rt for 1 h. The solvent was evaporated under reduced pressure with azeotropic removal by co-evaporation with toluene. The resulting residue was diluted with CH_2Cl_2 and washed sequentially with aq 0.1 M HCl, brine, and water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and the residue purified by flash chromatography (4:6 → 6:4 EtOAc/pet. spirits) to give silyl thiomannoside **19** (27.4 mg, 75%), mp 91–94 °C, $[\alpha]_D^{23}$ +146.3 (c 1.0 in $CHCl_3$). δ_H (700 MHz, $CDCl_3$) 5.41–5.27 (13 H, m, H(2,3,4)^A,(1,2,3,4)^B,(2,3,4)^{C,D}), 5.16 (1 H, s, $J_{1,2}$ 1.3 Hz, H1^A), 4.34–4.30 (3 H, m, H5^{B,C,D}), 4.13 (1 H, ddd, $J_{4,5}$ 9.7, $J_{5,6}$ 4.2, $J_{5,6}$ 2.1 Hz, H5^A), 3.75 (1 H, dd, $J_{5,6}$ 4.3, $J_{6,6}$ 11.6 Hz, H6^D), 3.69 (1 H, dd, $J_{5,6}$ 1.9, $J_{6,6}$ 11.6 Hz, H6^D), 3.05 (1 H, dd, $J_{5,6}$ 2.4, $J_{6,6}$ 14.8 Hz, H6^C), 2.99 (1 H, dd, $J_{5,6}$ 2.2, $J_{6,6}$ 14.6 Hz, H6^B), 2.84 (1 H, dd, $J_{5,6}$ 2.4, $J_{6,6}$ 14.2 Hz, H6^A), 2.80–2.74 (2 H, m, H6^{B,C}), 2.70 (1 H, dd, $J_{5,6}$ 8.2, $J_{6,6}$ 14.2 Hz, H6^A), 2.22, 2.18, 2.18, 2.18, 2.17, 2.11, 2.07, 2.05, 2.02, 2.00 (39 H, 10s, 12 × Ac, SCH_3), 0.93 (9 H, s, $C(CH_3)_3$), 0.07, 0.06 (6 H, 2 s, $Si(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 170.1, 170.0, 169.93, 169.88, 169.85, 169.84, 169.73, 169.69, 169.5 (12 × C = O), 83.2, 81.7, 81.3, 80.9 (C1^{A,B,C,D}), 71.9, 71.3, 71.0, 70.85, 70.83, 69.6, 69.43, 69.40, 69.3, 69.25, 69.20, 69.1, 69.0, 68.89, 68.88, 66.6, 62.0 (C(2,3,4,5)^{A,B,C},(2,3,4,5,6)^D), 31.4, 30.59, 30.57 (C6^{A,B,C}), 25.8 ((**C**H_3)$_3$C), 20.92, 20.87, 20.8, 20.7, 20.65, 20.63 (12 × **C**H_3CO), 18.4 ((CH$_3$)$_3$**C**), 13.6 (SCH_3), –5.30, –5.62 ($SiMe_2$); HRMS (ESI$^+$) calcd for $C_{55}H_{82}O_{29}S_4SiNa$ $(M + Na)^+$ 1385.3486. Found 1385.3515.

4.15. Methyl S-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (10)

4.15.1. Methyl S-(2,3,4-tri-O-acetyl-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside)

Silyl thiomannoside **19** (0.140 g, 0.104 mmol) was treated with AcOH, H_2O, THF (3:1:1, 30 ml) with stirring at 40 °C overnight. The solvent was evaporated under reduced pressure with co-evaporated with additional water and the residue was purified by flash chromatography (7:3→9:1 EtOAc/pet. spirits) to give the title alcohol (90.3 mg, 70%), mp 88–91 °C, $[\alpha]_D^{23}$ +113.9 (c 0.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.35–5.21 (13 H, m, H(2,3,4)^A,(1,2,3,4)^B,(2,3,4)^{C,D}), 5.12 (1 H, d, $J_{1,2}$ 1.3, H1^A), 4.32–4.27 (1 H, m, H5^C), 4.12–4.06 (1 H, m, H5^D), 3.64 (2 H, m, H(6,6)^D), 2.98 (1 H, dd, $J_{5,6}$ 3.0, $J_{6,6}$ 14.0 Hz, H6^C), 2.91 (1 H, dd, $J_{5,6}$ 3.0, $J_{6,6}$ 14.0 Hz, H6^B), 2.86 (1 H, dd, $J_{5,6}$ 3.0, $J_{6,6}$ 14.0 Hz, H6^A), 2.78–2.65 (3 H, m, H6^{A,B,C}), 2.29–2.31 (1 H, m, OH), 2.17, 2.15, 2.14, 2.14, 2.08, 2.08, 2.07, 2.04, 1.99, 1.98 (36 H, 10s, 12 × Ac), 1.96 (3 H, SCH_3); δ_C (100 MHz, $CDCl_3$) 171.4, 170.9, 170.5, 170.3, 170.1, 170.0, 169.9, 169.82, 169.76, 169.7 (C = O), 83.3, 82.0, 81.4, 81.1 (C1^{A,B,C,D}), 71.64, 71.57, 71.2, 71.0, 70.8, 69.4, 69.4, 69.3, 69.2, 69.1, 69.0, 68.9, 66.1, 63.2 (C(2,3,4,5)^{A,B,C},(2,3,4,5,6)^D), 31.5, 30.81, 30.79 (C6^{A,B,C}), 20.9, 20.85, 20.82, 20.75, 20.7, 20.6 (12 × **C**H_3CO), 13.7 (SCH_3); HRMS (ESI$^+$) calcd for $C_{49}H_{68}O_{29}S_4Na$ $(M + Na)^+$ 1271.2621. Found 1271.2639.

4.15.2. Methyl S-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (10)

A solution of triphenylphosphine (16.1 mg, 0.0614 mmol), imidazole (7.3 mg, 0.11 mmol), iodine (11.0 mg, 0.043 mmol) and alcohol (51.4 mg 37.8 μmol) in toluene (4 ml) was heated at 70 °C with stirring for 1 h. The solvent was evaporated to dryness under reduced pressure. The residue was purified by flash chromatography (1:1→6:4 EtOAc/pet. spirits) to give iodide **10** (44.7 mg, 80%), mp 194–197 °C, $[\alpha]_D^{23}$ +191.0 (c 0.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.36–5.09 (16 H, m, H(1,2,3,4)^{A,B,C,D}), 4.34–4.31 (3 H, m, H5^{B,C,D}), 4.17 (1 H, dd, $J_{4,5}$ 9.1, $J_{5,6}$ 2.2 Hz, H5^A), 3.27 (1 H, dd, $J_{5,6}$ 2.3, $J_{6,6}$ 10.9 Hz, H6^D), 3.15 (1 H, dd, $J_{5,6}$ 9.0, $J_{6,6}$ 10.9 Hz, H6^D), 3.06–2.94 (3 H, m, H6^{A,B,C}), 2.76–2.68 (3 H, m, H6^{A,B,C}), 2.17, 2.15, 2.13, 2.13, 2.13, 2.08, 2.07, 2.06, 1.98, 1.96, 1.95 (39 H, 11s, 12 × Ac, SCH_3); δ_C (100 MHz, $CDCl_3$) 170.1, 170.0, 169.94, 169.92, 169.83, 169.81, 169.78, 169.77, 169.7, 169.64, 169.55 (12 × C = O), 83.2, 81.3, 81.1, 80.6 (C1^{A,B,C,D}), 71.03, 70.95, 70.81, 70.77, 70.7, 70.2, 69.4, 69.3, 69.19, 69.15, 69.1, 69.0, 68.94, 68.88, 68.8, 68.6 (C(2,3,4,5)^{A,B,C,D}), 31.1, 30.5, 30.3 (C6^{A,B,C}), 21.1, 20.93, 20.90, 20.87, 20.8, 20.7, 20.62, 20.58 (36 H, 8s, 12 × Ac), 13.7 (SCH_3), 3.6 (C6^D); HRMS (ESI$^+$) calcd for $C_{49}H_{67}O_{28}S_4Na$ $(M + Na)^+$ 1381.1639. Found 1381.1664.

4.16. Methyl S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (11)

(a) A solution of iodide **10** (45.0 mg, 32.9 μmol) and thioacetate **8** (35.0 mg, 49.4μmol) in DMF (0.25 ml) was treated with and diethylamine (21 μL) with stirring at rt for 1 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic layer was washed with 0.1 N HCl, water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and the residue was purified by recrystallisation from EtOAc to give thiomannoside **11** (16 mg, 27%), mp 250–253 °C, $[\alpha]_D^{23}$ +135.3 (c 0.5 in $CHCl_3$). δ_H (700 MHz, $CDCl_3$) 5.38–5.23 (15 H, m, H(1,2,3,4)^A,(2,3,4)^{B,C,D,E,F}), 5.16 (1 H, s, H1^A), 4.36–4.25 (7 H, m, H5^{A,B,C,D,E},(5,6)^F), 4.13 (1 H, dd, $J_{5,6}$ 2.1, $J_{6,6}$ 12.3 Hz, H6^F), 3.10–3.01 (3 H, m, H6^{C,D,E}), 2.97 (1 H, m, H6^B), 2.82 (1 H, dd, $J_{5,6}$ 2.3, $J_{6,6}$ 14.3 Hz, H6^A), 2.80–2.66 (5 H, m, H6^{A,B,C,D,E}), 2.21, 2.20, 2.19, 2.18, 2.17, 2.13, 2.12, 2.08, 2.08, 2.03, 2.02, 2.01, 2.00, 1.99 (60 H, 14s, 19 × Ac, SCH_3); δ_C (100 MHz, $CDCl_3$) 170.6, 170.2, 170.05, 170.03, 170.00, 169.90, 169.89, 169.88, 169.86, 169.8, 169.73, 169.72, 169.69, 169.69, 169.67, 169.6 (19 × C = O), 83.2, 81.5, 81.0, 80.39, 80.36, 80.2 (C1^{A,B,C,D,E,F}), 71.05, 71.00, 70.8, 69.4, 69.3, 69.24, 69.22, 69.12, 69.10, 69.00, 68.97, 68.92, 68.90, 68.87, 68.7, 66.32, 62.28 (C(2,3,4,5)^{A,B,C,D,E},(2,3,4,5,6)^F), 31.1, 30.32, 30.28, 30.0, 29.9 (C6^{A,B,C,D,E}), 21.0, 20.92, 20.90, 20.87, 20.73, 20.69, 20.66, 20.6 (19 × **C**H_3CO), 13.6 (SCH_3); HRMS (ESI$^+$) calcd for $C_{75}H_{102}O_{44}S_6Na$ $(M + Na)^+$ 1921.3960. Found 1921.4060.

(b) A solution of thiomannoside **23** and triethylsilane (5.2 ml, 0.033 μmol) in DMF (0.5 ml) was treated with TFA (0.5 ml) and stirred at rt for 90 min. The TFA was evaporated under a stream of nitrogen. Et_3N (18 μl, 0.13 mmol) and MeI (2.61 μl, 0.042 mmol) were added and the mixture stirred for 2 h. The solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc. The organic solution was washed with 0.1 N Hcl, H_2O, dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography (90% Et_2O/acetone) to give **11** (5.6 mg, 30%).

4.17. Methyl S-(α-D-mannopyranosyl)-(1→6)-S-(6-thio-α-D-mannopyranosyl)-(1→6)-S-(6-thio-α-D-mannopyranosyl)-(1→6)-S-(6-thio-α-D-mannopyranosyl)-(1→6)-S-(6-thio-α-D-mannopyranosyl)-(1→6)-(1,6-dithio-α-D-mannopyranoside) (3)

A solution of thiomannoside **11** (50.0 mg, 0.0387 mmol) was treated with 1 M aq NaOMe in methanol (50 μL) at rt with stirring for 4 h. The solution was adjusted to pH 5–6 with Amberlite IR-120 resin (H^+ form), filtered, and the filtrate evaporated to dryness

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under reduced pressure. The residue was purified by reverse phase chromatography (1→19:1→9:1 H_2O/MeCN) to give hexasaccharide **3** (26.0 mg, 90%), mp 78–81 °C, $[\alpha]_D^{23}$ +170.3 (c 0.25 in MeOH). δ_H (700 MHz, CD_3OD) 5.32 (3 H, s, $H1^{D,E,F}$), 5.30 (2 H, s, $H1^{B,C}$), 5.11 (1 H, d, $J_{1,2}$ 1.1 Hz, $H1^A$), 4.08–3.56 (21 H, m, $H(2,3,4)^{A,B,C,D,E}$,$(2,3,4,5,6,6)^F$), 3.23–3.11(5 H, m, $H6^{A,B,C,D,E}$), 2.83–2.70 (5 H, m, $H6^{A,B,C,D,E}$), 2.15 (3 H, s, SCH_3); δ_C (100 MHz, CD_3OD) 86.0, 84.6, 84.5, 84.3, 84.2 ($C1^{A,B,C,D,E,F}$), 73.6, 72.2, 72.1, 72.0, 71.82, 71.79, 71.76, 71.74, 71.70, 71.64, 71.58, 70.40, 70.38, 70.35, 70.1, 67.5 ($C(2,3,4,5)^{A,B,C,D,E}$,$(2,3,4,5,6)^F$), 31.4, 31.3, 31.22, 31.15, 31.0 ($C6^{A,B,C,D,E}$), 12.3 (SCH_3); HRMS (ESI^+) calcd for $C_{37}H_{64}O_{25}S_6Na$ $(M+Na)^+$ 1123.1953. Found 1123.1967.

4.18. Triphenylmethyl S-(2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (25)

4.18.1. Triphenylmethyl S-(α-D-mannopyranosyl)-(1→6)-(1,6-dithio-α-D-mannopyranoside) (24)

A solution of thiomannoside **18** (0.100 g, 0.110 mmol) was treated with 1 M NaOMe in methanol (0.044 µl) at rt with stirring for 4 h. The solution was adjusted to pH 5–6 with Amberlite IR-120 resin (H^+ form), filtered, and the filtrate evaporated to dryness under reduced pressure to give the title compound (66.5 mg, 98%) as a crystalline solid, which was used without purification for further reactions, mp 124–127 °C, $[\alpha]_D^{24}$ +311.2 (c 1.0 in MeOH); HRMS (ESI^+) calcd for $C_{31}H_{36}O_9S_2Na$ $(M+Na)^+$ 639.1693. Found 639.1684.

4.18.2. Triphenylmethyl S-(2,3,4-tri-O-acetyl-6-O-(tert-butyldimethylsilyl)-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (25)

A solution of heptaol **24** (0.596 g, 0.967 mmol) and imidazole (0.165 g, 2.42 mmol) in dry DMF (3.0 ml), was treated with TBDMSCl (0.204 g, 1.35 mmol) at 35 °C with stirring for 1 h. The solution was cooled to rt and acetic anhydride (20 ml) and pyridine (10 ml) were added and the solution was allowed to stir at rt for 1 h. The solvent was removed under reduced pressure with residual acetic anhydride removed azeotropically by co-evaporation with toluene. The residue was diluted with CH_2Cl_2 and the solution was washed sequentially with 0.1 M aq HCl, brine, and water, dried ($MgSO_4$), the solvent evaporated under reduced pressure and the residue was purified by flash chromatography (3:7 → 1:1 EtOAc/pet. spirits), to give silyl thiomannoside **25** as clear oil (0.773 g, 50%), $[\alpha]_D^{25}$ +122.8 (c 0.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 7.38–7.19 (15 H, m, Ph), 5.42–5.18 (6 H, m, $H(2,3,4)^{A,B}$), 5.09 (1 H, s, $H1^B$), 4.77 (1 H, d, $J_{1,2}$ 1.7 Hz, $H1^A$), 4.25–4.20 (1 H, m, $H5^A$), 4.16–4.07 (1 H, m, $H5^B$), 3.75 (1 H, dd, $J_{5,6}$ 4.3, $J_{6,6}$ 11.6 Hz, $H6^B$), 3.69 (1 H, dd, $J_{5,6}$ 2.2, $J_{6,6}$ 11.5 Hz, $H6^B$), 2.72 (1 H, dd, $J_{5,6}$ 3.3, $J_{6,6}$ 14.2 Hz, $H6^A$), 2.59 (1 H, dd, $J_{5,6}$ 5.5, $J_{6,6}$ 14.2 Hz, $H6^A$), 2.05, 2.03, 2.02, 2.00, 1.97 (18 H, 5s, 6 × CH_3CO), 0.90 (9 H, s, $C(CH_3)_3$), 0.05, 0.03 (6 H, 2 s, $Si(CH_3)_2$); δ_C (100 MHz, $CDCl_3$) 169.79, 169.78, 169.70, 169.68, 169.6, 169.5 (C = O), 144.0, 129.8, 128.0, 127.2 (Ph), 82.8, 82.4 ($C1^{A,B}$), 72.1, 71.9, 71.8, 71.1, 69.8, 69.3, 66.4, 62.0 ($C(2,3,4,5)^A$,$(2,3,4,5,6)^B$), 32.0 ($C6^A$), 25.8 ($C(\mathbf{C}H_3)_3$), 20.78, 20.76, 20.72, 20.69, 20.6 (6 C, 6 × CH_3CO), 18.23 ($\mathbf{C}(CH_3)_3$), –5.37, –5.46 ($SiMe_2$); HRMS (ESI^+) calcd for $C_{49}H_{62}O_{15}S_2SiNa$ $(M+Na)^+$ 1005.3192. Found 1005.3172.

4.19. Triphenylmethyl S-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (20)

4.19.1. Triphenylmethyl S-(2,3,4-tri-O-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside)

Silyl thiomannoside **25** (0.457 g, 0.465 mmol) was treated with AcOH, H_2O, THF (3:1:1, 90 ml) with stirring at 40 °C for 6 h. The solvent was co-evaporated with water to remove excess acid and the residue was purified by flash chromatography (50% EtOAc/pet. spirits) to give the title compound (0.315 g, 78%), mp 84–87 °C, $[\alpha]_D^{24}$ +109.5 (c 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.37–7.22 (15 H, m, Ph), 5.32 (1 H, dd, $J_{1,2}$ 1.4, $J_{2,3}$ 3.3 Hz, $H2^B$), 5.30 (1 H, s, $H1^B$), 5.24–5.21 (3 H, m, $H(2,3)^A$, 3^B), 5.15–5.12 (2 H, m, $H4^{A,B}$), 4.74 (1 H, d, $J_{1,2}$ 1.4 Hz, $H1^A$), 4.29–4.19 (1 H, m, $H5^A$), 4.07–3.96 (1 H, m, $H5^B$), 3.89–3.84 (2 H, m, $H6^B$), 2.77 (1 H, dd, $J_{5,6}$ 3.4, $J_{6,6}$ 14.1 Hz, $H6^A$), 2.61 (1 H, dd, $J_{5,6}$ 5.7, $J_{6,6}$ 14.0 Hz, $H6^A$), 2.38 (1 H, bs, OH), 2.06, 2.05, 2.04, 2.03, 2.01, 1.97 (18 H, 6 s, 6 × Ac); δ_C (100 MHz, $CDCl_3$) 170.7, 170.0, 169.8, 169.6, 169.5, 169.4 (6 × C = O), 144.0, 129.8, 128.0, 127.2 (Ph), 82.7, 82.5 ($C1^{A,B}$), 72.9, 72.3, 72.0, 71.6, 71.0, 70.0, 69.2, 68.6, 66.8, 62.5, 60.4 ($C(2,3,4,5)^A$,$(2,3,4,5,6)^B$), 32.2 ($C6^A$), 21.0, 20.78, 20.78, 20.75, 20.7, 20.6 (6 C, 6 × CH_3); HRMS (ESI^+) calcd for $C_{49}H_{62}O_{15}S_2SiNa$ $(M+Na)^+$ 891.2327. Found 891.2308.

4.19.2. Triphenylmethyl S-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (20)

A solution of triphenylphosphine (0.123 g, 0.470 mmol), imidazole (63.7 mg, 0.935 mmol), iodine (78.5 mg, 0.619 mmol) and alcohol (0.272 g, 0.278 mmol) in toluene (8.5 ml) was heated at reflux with stirring for 2 h. The solvent was evaporated to dryness under reduced pressure and the residue was purified by flash chromatography (4:6 EtOAc/pet. spirits) to give iodide **20** (0.263 g, 97%), mp 62–65 °C, $[\alpha]_D^{23}$ +112.9 (c 0.5 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 7.41–7.24 (15 H, m, Ph), 5.56–5.07 (7 H, m, $H(2,3,4)^A$,$(1,2,3,4)^B$), 4.78 (1 H, d, $J_{1,2}$ 1.8 Hz, $H1^A$), 4.23 (1 H, m, $J_{4,5}$ 8.7, $J_{5,6}$ 5.2, $J_{5,6}$ 3.6 Hz, $H5^B$), 4.19–4.08 (1 H, m, $H5^A$), 3.26–3.14 (2 H, m, $H(6,6^B)$, 2.79–2.52 (2 H, m, $H(6,6)^A$), 2.10, 2.07, 2.03, 2.01, 1.98, 1.97 (18 H, 6s, 6 × Ac); δ_C (100 MHz, $CDCl_3$) 169.8, 169.78, 169.76, 169.75, 169.71, 169.70 (C = O), 144.0, 129.8, 128.0, 127.2 (Ph), 82.4, 82.3 ($C1^{A,B}$), 72.1, 71.6, 70.8, 70.3, 70.2, 69.8, 69.3, 69.0, 68.3 ($C(2,3,4,5)^A$,$(2,3,4,5,6)^B$), 31.6 ($\mathbf{C}Ph_3$), 21.9, 20.80, 20.76, 20.7, 20.64, 20.56 (6 × CH_3), 4.1 ($C6^A$); HRMS (ESI^+) calcd for $C_{43}H_{47}IO_{14}S_2Na$ $(M+Na)^+$ 1001.1344. Found 1001.1336.

4.20. Triphenylmethyl S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (21)

A solution of the iodide **20** (0.146 g, 0.207 mmol) and thioacetate **8** (0.220 g, 0.310 mmol) in DMF (1.3 ml) was treated with diethylamine (64.3 µl, 0.621 mmol) with stirring at rt for 1 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic layer was washed with 0.1 N HCl, water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and purified by flash chromatography (4:6 → 1:1 EtOAc/pet. spirits) to give trityl thiomannoside **21** (0.147 g, 47%), mp 112–115 °C, $[\alpha]_D^{24}$ +151.8 (c 1.0 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.44–7.08 (15 H, m, Ph), 5.36–5.19 (14 H, m, $H(2,3,4)^A$,$(1,2,3,4)^{B,C}$,$(2,3,4)^D$), 5.02 (1 H, s, $H1^D$), 4.80 (1 H, s, $H1^A$), 4.35–4.09 (6 H, m, $H5^{A,B,C}$,$(5,6)^D$), 3.06–2.59 (6 H, m, $H(6,6)^{A,B,C}$), 2.18, 2.12, 2.11, 2.09, 2.07, 2.06, 2.03, 2.01, 1.99, 1.99, 1.98, 1.95 (39 H, 12s, 13 × Ac); δ_C (100 MHz, $CDCl_3$) 170.6, 170.04, 169.98, 169.9 (C = O), 169.8 (4 × C = O), 169.73, 169.67, 169.65, 169.62, 169.5 (C = O), 144.0, 129.8, 128.0, 127.2 (Ph), 82.4, 82.3, 81.8, 81.0 ($C1^{A,B,C,D}$), 72.1, 71.6, 70.91, 70.86, 70.8, 69.3, 69.21, 69.18, 69.13, 69.12, 68.0, 66.2, 62.2 ($C(2,3,4,5)^{A,B,C}$,$(2,3,4,5,6)^D$), 31.3, 31.1, 30.6 ($C6^{A,B,C}$), 20.93, 20.86, 20.80, 20.78, 20.72, 20.68, 20.62, 20.61, 20.59 (13 × CH_3); HRMS (ESI^+) calcd for $C_{69}H_{82}O_{30}S_4Na$ $(M+Na)^+$ 1541.3666. Found 1541.3667.

4.21. S-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-α-D-mannopyranose) (22)

A solution of trityl thiomannoside **21** (0.147 g, 0.097 mmol) and triethylsilane (36.6 µl, 0.227 mmol) in CH_2Cl_2 (2 ml) was treated with

TFA (1.0 ml) for 45 min. The solvent was evaporated and the residue was treated with a mixture of acetic anhydride:pyridine (1:1, 5 ml) for 12 h. The solvent was evaporated under reduced pressure, and the residue was diluted with CH_2Cl_2 (50 ml), washed with water (50 ml) and sat. aq $NaHCO_3$ solution (50 ml). The solvent was evaporated and the residue was purified by flash chromatography (40% EtOAc/pet. spirits) to give thioacetate **22** (86.1 mg, 67%), mp 118–121 °C, $[\alpha]_D^{25}$ +146.8 (c 1.0 in $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 5.91 (1 H, d, $J_{1,2}$ 1.6 Hz, $H1^A$), 5.42–5.23 (14 H, m, $H(2,4)^A$,$(1,2,3,4)^{B,C,D}$), 5.10 (1 H, dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.2 Hz, $H3^A$), 4.34–4.25 (6 H, m, $H5^{A,B,C}$,6^D), 4.10 (1 H, dd, $J_{5,6}$ 2.0, $J_{6,6}$ 12.3 Hz, $H6^D$), 3.95–3.90 (1 H, m, $H5^D$), 3.02 (1 H, dd, $J_{5,6}$ 2.9, $J_{6,6}$ 15.0 Hz, $H6^C$), 2.94 (1 H, dd, $J_{5,6}$ 2.3, $J_{6,6}$ 14.6 Hz, $H6^B$), 2.82 (1 H, dd, $J_{5,6}$ 2.5, $J_{6,6}$ 14.2 Hz, $H6^A$), 2.74–2.66 (6 H, m, $H6^{A,B,C,D}$), 2.44 (3 H, s, SAc), 2.18, 2.17, 2.17, 2.15, 2.11, 2.08, 2.07, 2.06, 2.05, 1.99, 1.99, 1.99, 1.97 (39 H, 13s, 13 × OAc); δ_C (100 MHz, $CDCl_3$) 190.4 (SC = O), 170.6, 170.1, 170.01, 169.97, 169.94, 169.86, 169.81, 169.75, 169.72, 169.70, 169.68, 169.67 (OC = O), 82.1, 81.7, 81.0, 79.9 ($C1^{A,B,C,D}$), 73.5, 70.91, 70.91, 70.8, 70.7, 69.8, 69.4, 69.21, 69.19, 69.18, 69.16, 69.1, 69.0, 68.9, 67.8, 66.3, 62.3 ($C(2,3,4,5)^{A,B,C}$,$(2,3,4,5,6)^D$), 31.3 ($SCO\mathbf{C}H_3$), 30.8, 30.7, 29.7 ($C6^{A,B,C}$), 20.91, 20.89, 20.87, 20.84, 20.81, 20.79, 20.72, 20.68, 20.6 ($13 \times CO_2\mathbf{C}H_3$); HRMS ($ESI^+$) calcd for $C_{52}H_{70}O_{31}S_4Na$ $(M + Na)^+$ 1341.2676. Found 1341.2635.

4.22. Triphenylmethyl S-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-O-acetyl-1,6-dithio-α-D-mannopyranoside) (23)

A solution of iodide **20** (15.7 mg, 0.016 mmol) and thioacetate **22** (24.3 mg, 0.018 mmol) in DMF (0.2 ml) was treated with diethylamine (2.3 µl, 0.032 mmol) with stirring at rt for 1 h. The solvent was evaporated and CH_2Cl_2 was added. The organic solution was washed with 0.1 N HCl, water, dried ($MgSO_4$), evaporated to dryness under reduced pressure and purified by flash chromatography (1 → 9:1 diethyl ether/acetone) to give trityl thiomannoside **23** (12.0 mg, 35%), mp 123–126 °C, $[\alpha]_D^{24}$ +127.1 (c 0.165 in $CHCl_3$). δ_H (700 MHz, $CDCl_3$) 7.36–7.23 (15 H, m, Ph), 5.35–5.19 (22 H, m, $H(2,3,4)^A$,$(1,2,3,4)^{B,C,D,E}$,$(2,3,4)^F$), 5.02 (1 H, s, $H1^F$), 4.79 (1 H, s, $H1^A$), 4.34–4.22 (6 H, m, $H5^{A,B,C,D,E}$,6^F), 4.21–4.16 (1 H, m, $H5^F$), 4.10 (1 H, dd, $J_{5,6}$ 2.0, $J_{6,6}$ 12.3 Hz, $H6^F$), 3.09–2.52 (10 H, m, $H6^{A,B,C,D,E}$), 2.18, 2.17, 2.17, 2.10, 2.10, 2.09, 2.06, 2.06, 2.05, 2.04, 2.01, 2.00, 1.99, 1.98, 1.97, 1.94 (57 H, 16s, 19 × Ac); δ_C (100 MHz, $CDCl_3$) 170.6, 170.2, 170.1, 170.04, 170.01, 169.97, 169.93, 169.90, 169.85, 169.77, 169.70, 169.67, 169.65, 169.63, 169.59, 169.5 (C = O), 83.3, 82.1, 81.5, 80.5, 80.4, 80.3 ($C1^{A,B,C,D,E,F}$), 72.1, 71.6, 71.03, 70.98, 70.88, 70.86, 69.4, 69.3, 69.20, 69.18, 69.1, 69.01, 68.99, 68.9, 68.73, 68.70, 68.67, 68.0, 66.3, 62.3 $(C(2,3,4,5)^{A,B,C,D,E}$,$(2,3,4,5,6)^F)$, 31.1, 30.8, 30.3, 30.00, 29.98 ($C6^{A,B,C,D,E}$), 21.0, 20.93, 20.89, 20.85, 20.81, 20.74, 20.71, 20.67, 20.63, 20.62, 20.59 (CH_3); HRMS (ESI^+) calcd for $C_{93}H_{114}O_{44}S_6Na$ $(M + Na)^+$ 2149.48452. Found 2149.48994.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.04.015.

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