Published in final edited form as:

J Org Chem. 2018 December 21; 83(24): 14894–14904. doi:10.1021/acs.joc.8b01645.

Synthesis and Evaluation of 1,5-Dithia-D-laminaribiose, Triose and Tetraose as Truncated β-(1→3)-Glucan Mimetics

Xiaoxiao Liao[a], **Václav Větvička**[b], and **David Crich**[a,*]

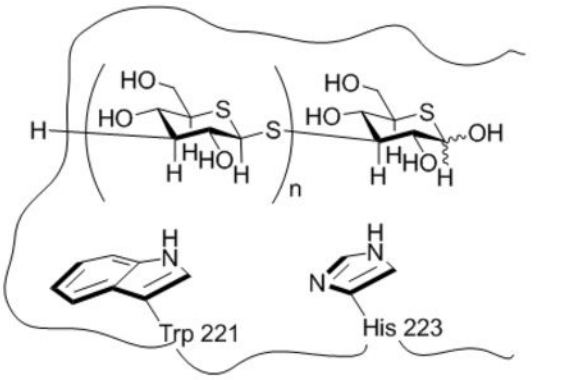
[a)]Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA

[b)]Department of Pathology, University of Louisville, 323 East Chestnut Street, Louisville, KY 40202, USA

Abstract

The preparation and characterization of a series of di-, tri- and tetrasaccharide analogs of β-(1→3)-glucans is described in which each pyranoside ring is replaced by a 5-thiopyranosyl ring and each glycosidic oxygen by a thioether. These oligomeric 1,5-dithio-D-glucopyranose derivatives were shown to inhibit the staining of human neutrophils and of mouse macrophages by fluorescent anti-CR3 and anti-dectin-1 antibodies, respectively. The compounds were also demonstrated to stimulate phagocytosis and pinocytosis indicative of binding to the carbohydrate binding domains of complement receptor 3 (CR3) and dectin-1. Activity in all three assays was optimum at the level of the trisaccharide mimic suggesting that while the replacement of ethereal oxygens by thioethers results in greater affinity for the aromatic-lined hydrophobic binding pockets, the presence of multiple longer C-S bonds eventually results in a mismatch and a loss of affinity.

Graphical Abstract



- Synthesis of 1,5-dithio-β-(1→3)-glucans (n = 0 → 2)
- Optimum β-(1→3)-glucan-like activity when n = 2

* Dcrich@chem.wayne.edu.

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b01645.

Copies of the ^{1}H and ^{13}C NMR spectra of all new compounds (PDF)

Introduction

The β-(1→3)-glucans (Figure 1) are widely–occurring natural immunomodulating agents, for which yeasts, seaweeds, fungi and grains are the most common sources.[1–9] The immunostimulating properties of the β-(1→3)-glucans have resulted in their application as agents to enhance the natural immune system and to relieve side effects associated with chemotherapy. For example, schizophyllan and lentinan, fungal β-(1→3)-glucans with different molecular weight distributions and degrees of β-(1→6)-branching, are used in the treatment of uterine, stomach, colorectal and gastro-intestinal cancers.[9–13] The β-(1→3)-glucans are also known to potentiate tumor-specific antibodies, to modulate the effects of radiation and photodynamic therapy[14–17] to mitigate allergic rhinitis,[18] regulate stress,[9] to afford protection of the liver,[19] and to protect from symptoms of Inflammatory Bowel Diesease.[20–27]

Despite their widespread availability in nature, the heterogeneity of natural isolates complicates the isolation of pure homogeneous glycoforms of β-(1→3)-glucans for biological studies and the establishment of structure activity relationships. Accordingly, much effort has been devoted to the chemical synthesis of β-(1→3)-glucans resulting in the preparation of multiple oligomers and evaluation of their immunomodulating properities.[28–33]

The immunostimulating properties of the β-(1→3)-glucans are considered to arise primarily from their affinity for the lectin regions of Complement Receptor 3 (CR3)[8,9,34–41] and Dectin-1[42–44] to which their binding triggers a cascade of effects including phagocytosis.[9] Studies with homogenous β-(1→3)-glucans obtained by controlled acidic hydrolysis and extensive purification demonstrated that the shortest β-(1→3)-glucan capable of detectable binding to recombinant murine Dectin-1 in a microarray format is the 10- or 11-mer.[45] Subsequently a surface plasmon resonance-based assay revealed the heptasaccharide to be the minimum binding unit for recombinant murine Dectin-1.[46] Earlier work with glucans isolated from yeast cell walls indicated that the interaction of pure β-glucans with monocyte glucan receptors (now recognized to be CR3) showed specificity for the linear chains of β-(1→3)-D-glucans, and that the heptasaccharide, subsequently revised to the tetrasaccharide, was the smallest β-(1→3)-glucan able to block the ability of monocytes to ingest zymosan (via CR3).[9] With a series of homogeneous synthetic β-(1→3)-glucans it has been demonstrated that even the tetramer and especially the pentamer are sufficient to show immunostimulatory effects, such as the potentiation of phagocytosis, approaching those of phycarine, a β-(1→3)-glucan isolated from brown algae.[29] Short synthetic β-(1→3)-glucans (penta- and hexamers) modified at the reducing end by the replacement of the terminal glucopyranose residue by its manno-stereoisomer, by a 4-deoxyglucopyranose moiety, and by gluco- and manno-configured glycitols (eg, **2**) retain the ability to promote phagocytosis.[30,47]

X-Ray crystallographic studies of recombinant Dectin 1 reveal a shallow carbohydrate binding groove featuring a hydrophobic pocket lined by the side chains of Trp 221 and His 223.[48] STD-NMR experiments revealed that laminarin, a natural β-(1→3)-glucan from brown algae with degree of polymerization from 18-31, binds to recombinant Dectin-1

through interaction of the α-faces of its terminal pyranose rings (at both the reducing and non-reducing ends) with the hydrophobic binding patch of the lectin domain (Figure 2).[49,50] Interestingly however, in view of the immunostimulatory effects observed with penta- and hexamers,[30,47] STD NMR experiments revealed little or no binding between a synthetic hexamer and recombinant CR3 and Dectin-1.[49] More recent STD NMR studies revealed a synthetic β-(1→3)-glucan hexadecamer, but not a hexamer to bind to the lectin domain of Dectin-1.[50] These observations are consistent with a relatively weak interaction between the lectin-binding domain and a short carbohydrate epitope, as is typical in carbohydrate-protein interactions,[51–54] that is significantly enhanced by a multivalent effect arising in this case by the repeated presentation of the epitope in the form of the polymeric glucan. Surface-plasmon resonance (SPR) experiments are consistent with the binding of multiple Dectin-1 molecules to a single polymeric β-(1→3)-glucan backbone with affinity increasing in an additive fashion, i.e., through a multivalent interaction.[46] The availability of defined synthetic β-(1→3)-glucans has also enabled their detailed conformational analysis by NMR spectroscopy.[50,55]

The identification of hydrophobic binding pockets in CR3 and Dectin-1 accepting small carbohydrate epitopes suggests that the ligand-receptor interaction may be enhanced by modification of the carbohydrate epitope. Efforts to approach the β-(1→3)-glycans in this manner were limited to the short synthetic β-(1→3)-glucans and their deoxy and 1-thia analogs **3**-**5** [56,57] before our recent description of synthetic hydroxylamine-based glycan analogs **6** and **7** which show significant affinity for CR3 and Dectin-1.[56]

We hypothesized that the enhanced binding of the oligohydroxylamines relative to that of comparable length β-(1→3)-glucans is the result of the replacement of the C2-OH group in the repeat unit by a C-H bond and of the ring oxygen by a methylene group, both of which augment the hydrophobicity of the α-face of the repeat unit, and so enhance affinity for the hydrophobic binding pocket of the lectin domain in CR3 and Dectin-1 (Figure 3).[57] The enhanced effects of thioglycosides **3**-**5** with respect to the corresponding simple glycosides, in addition to resistance to acidic and enzymatic hydrolysis,[57] might also arise from enhanced interactions with proteins due to the replacement of the glycosidic oxygen by sulfur (Figure 3)[58] as is well recognized and exploited in medicinal chemistry.[59–61] Indeed, similar observations and hypotheses have been made by others albeit for different saccharide mimics in other contexts.[62–67]

Building on these observations we designed a series of short β-(1→3)-glucan mimetics in which both the ring and glycosidic oxygens are replaced by sulfur and report here on the synthesis of the di-, tri-, and tetramers **8**-**10** (Figure 4). We also report that these short oligomeric molecules inhibit binding of fluorescent anti-CR3 and anti-Dectin-1 antibodies to human neutrophils and mouse macrophages, respectively, and stimulate phagocytosis and pinocytosis.

Results and Discussion

Synthesis.

Synthetic efforts began with the large scale preparation of pentaacetyl 5-thia-D-glucopyranose essentially following the method of Whistler.[68] Thus, 1,2;5,6-di-O-isopropylidene-D-glucofuranose was converted to 3-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose **11** by known methods[69] and then exposed to a 10% molar excess of benzoyl chloride in dichloromethane at −30 °C in the presence of pyridine and 4-dimethylaminopyridine. In situ treatment of the resulting monobenzoate with methanesulfonyl chloride then gave the fully functionalized glucofuranose derivative **12**[70] in 83% yield (Scheme 1). Treatment with sodium methoxide in methanol, neutralization with Amberlyst IR-120 resin, and subsequent heating with thiourea next afforded the known 5,6-dideoxy-5,6-episulfide **13**[71] in 65% yield. Heating with potassium acetate in a mixture of acetic anhydride and acetic acid then provided the 3,5,6-triacetyl derivative of 1,2-*O*-isopropylidene-5-thia-D-glucofuranose **14**[72] in 43% yield. Finally, isopropylidene removal with aqueous acetic acid, followed by Zemplen deesterification, and peracetylation gave pentaacetyl 5-thia-D-glucopyranose **15**[72] in 60% yield (Scheme 1).

Adapting the approaches of Ferrières to short β-(1→3)-glucans[73] and of Hindsgaul to thia-linked derivatives,[74] Zemplen desesterification of **15** gave a crude preparation of 5-thia-D-glucose that was transformed in 73% overall yield to the diisopropylidene furanose derivative **16**[75] on stirring in anhydrous acetone with *p*-toluenesulfonic acid (Scheme 2). Swern oxidation followed by sodium borohydride reduction then afforded the allo-isomer **17**[75] in 57% yield. Finally, reaction with triflic anhydride and pyridine provided the triflate **18** in 85% yield in the form of a white solid (Scheme 2). A second aliquot of pentaacetyl 5-thia-D-glucopyranose **15** was treated with hydrogen bromide in acetic acid at 0 °C to give a crude preparation of the two anomers of tetraacetyl-5-thia-D-glucopyranosyl bromide which, on stirring with potassium thioacetate in dimethylformamide at 0 °C, gave 46% of pentaacetyl 1,5-dithia-β-D-glucopyranose **19** (Scheme 2).

A mixture of triflate **18** with a 20 mol% excess of the β-diathia sugar **19** was stirred with diethylamine[76,77] at 0 °C in DMF resulting in selective cleavage of the labile anomeric thioacetate, and alkylation of the resulting thiolate to give the disaccharide mimetic **20** in 86% yield (Scheme 3). As expected from the absence of mutarotation seen in other cyclic dithiahemiacetals,[78] **20** was isolated in the form of a single β-anomer about of the dithioglycosidic linkage. Zemplen deesterification then gave **21** quantitatively, and heating with Dowex-50 resin followed by acetylation gave the peracetyl laminaribiose derivative **22** in 91% yield (Scheme 3). Zemplen deesterification of **22** finally gave the target trithia-laminaribiose derivative **8** in 95 yield as a 4:1 α:β-anomeric mixture. Treatment of the peracetate **22** with hydrogen bromide in acetic acid[72] afforded the corresponding anomeric bromide **23** in 73% yield, whose exposure to potassium thioacetate gave the β-thioacetate **24** in 89% yield. Selective cleavage of the thioacetate in **24** with diethylamine in the presence of triflate **18** then afforded the crystalline trisaccharide mimetic **25** in 64% isolated yield, that was converted to the peracetyl derivative **27** in 95% yield by acidic hydrolysis of the isopropylidene derivatives and acetylation. Finally, removal of the acetates with sodium

methoxide gave the trisaccharide mimetic **9** in the form of a 4:1 α:β-mixture of anomers in quantitative fashion. Application of the sequence of hydrogen bromide in acetic acid and then potassium thioacetate to the peracetate **27** gave first the α-bromide **28** and then the β-thioacetate **29** in 43% and 90% yields, respectively. Subsequent treatment of **29** with diethylamine and triflate **18** afforded the tetrasaccahride mimetic **30** in 46% yield from which the esters were removed with sodium methoxide to give **31** quantitatively. Finally hydrolysis of the isopropylidene group with Dowex-50 resin water afforded the tetrasaccharide mimetic **10** in 85% yield as a 4:1 α:β anomeric mixture (Scheme 3).

Evaluation of Binding to CR3 and Dectin-1 Receptors.

Glycan mimetics **8**-**10** were screened for their ability to inhibit anti-CR3 or anti-Dectin-1 fluorescein isothiocyanate (FITC)-conjugated antibody staining of human neutrophils and mouse macrophages, which is indicative of their affinity for CR3 and Dectin-1 (Table 1).[37] For comparison purposes the previously reported performances of the di- and trimeric hydroxylamines **6** and **7** in the same assay are also presented in Table 1.

Evaluation of phagocytic activity.

The ability of glycan mimetics **8**-**10** to stimulate phagocytosis of synthetic polymeric 2-hydroxyethyl methacrylate particles[79] by human macrophage-like RAW 264 cells was also examined (Table 2) in comparison to the commercial highly purified yeast-derived insoluble Glucan #300.[80] Again, for comparison purposes the phagocytic activity of the di- and trimeric hydroxylamines **6** and **7** are also presented in Table 2. In a comparable assay, 47.4 ± 5.2 and 58.3 ± 4.9% stimulation of phagocytosis was observed with laminaritetraose and pentaose, the smallest β-(1→3)-glucans for which data is available.[29]

Finally, the ability of glycan mimetics **8**-**10** to stimulate pinocytosis, an important mechanism of cellular internalization, was determined in comparison to Glucan #300 by spectrophotometric measurement of neutral red dye accumulation by mouse macrophages on incubation with the dye (Table 3).[81]

Discussion.

Each of the di-, tri- and tetrameric forms of 1,5-dithia-D-glucopyranose **8**-**10** displayed the ability to inhibit staining of human neutrophils and mouse macrophages by fluorescent anti-CR3 and anti-Dectin-1 antibodies, respectively (Table 1) indicative of their binding to the carbohydrate binding domains of CR3 and Dectin-1. Notably, the greatest inhibition of staining by the fluorescent antibodies was observed with the trimer **9** and not with the longer tetramer **10**. Further, the levels of inhibition of antibody staining by the dimer **8** and the trimer **9** were comparable to those observed previously with the di- and trimeric hydroxylamines **6** and **7**, respectively. This pattern of activity is repeated in the stimulation of phagocytosis by **8**-**10** (Table 2), with the trimer **9** being the most active and the levels of activity comparable with those of the hydroxylamines of corresponding length. The most active compound, the trimer **9**, was half as effective at stimulating phagocytosis as the yeast derived β-(1→3)-glucan Glucan #300, which was selected on the basis of its superior immunostimulating activities in comparative studies,[80] and approximately one third as active

as laminaritetraose – the smallest β-(1→3)glucan for which data is available.[29] Finally, the same pattern was found for the stimulation of pinocytosis of neutral red dye by mouse macrophages (Table 3), with the trimer **9** being more active than either the dimer **8** or the tetramer **10**, and approximately one third as active as the comparator Glucan #300.

Conclusions

As already demonstrated with the di- and trimeric hydroxylamines **6** and **7**,[56] the present study demonstrates that small molecule mimetics of β-(1→3)-glucans can be designed that display significant activity in the inhibition of staining of human neutrophils and mouse macrophages by fluorescent anti-CR3 and anti-Dectin-1 antibodies suggestive of binding to the carbohydrate binding domains of the respective proteins. The affinity for the carbohydrate binding domains of CR3 and Dectin-1 is reflected in the stimulation of phagocytosis and of pinocytosis by compounds **8**-**10**. Unexpectedly, the trimer **9** is more active than either the dimer **8** or the tetramer **10** in each of the three assays conducted suggesting that, at least for the present series of glucan mimetics, there is little to be gained by preparing higher oligomers. This maximization of activity in the trimer **9** might be accounted for by a tradeoff between the greater affinity for the CR3 and Dectin-1 carbohydrate binding sites arising from the presence of the multiple thioethers on the positive side, and the accumulation of multiple long C-S bonds eventually causing a mismatch with the binding site on the negative side.

Experimental Section

General.

All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise stated. All reactions were performed under an argon atmosphere unless otherwise stated. Reactions were monitored by analytical thin-layer chromatography with pre-coated glass backed plates and visualized by UV absorption (254 nm) or by staining with a 5% solution of H_2SO_4 in MeOH or ceric ammonium molybdate solution (4.0 g ceric sulfate; 10 g ammonium molybdate; 40 mL H_2SO_4; 360 mL H_2O) followed by heating. Optical rotations were measured with an automatic polarimeter in the solvent specified at 589 nm at 23 °C with a path length of 10 cm. ^1H, ^{13}C, HSQC, HMBC, COSY, and TOCSY NMR spectra were recorded at 400 or 600 MHz. High resolution mass spectra were recorded with a Walters LC/MS with an electrospray source coupled to a time-of-flight mass analyzer. Melting points were recorded with an electrothermal melting point apparatus.

General Procedure A: Coupling Reactions.

To a 1.0 M solution of a 1-*S*-acetyl-5-thio-β-D-glucopyranose **19** and triflate **18** (1.1 eq) in DMF was added diethylamine (2.5 eq) dropwise at 0 °C. The reaction mixture was stirred at room temperature until completion. The reaction mixture was diluted with ethyl acetate and washed with water, brine, dried over $MgSO_4$ and concentrated in *vacuo*. The residue was purified by silica gel chromatography.

General Procedure B: Deacetylation.

At 0 °C, sodium methoxide (0.2 eq) was added to a 0.1 M solution of substrate in anhydrous methanol, which was then stirred until completion. Amberlyst IR120 resin was added to neutralize the reaction. When the pH was neutral (monitored by pH paper), the resin was filtered off and the solution was concentrated in vacuo. Furanosyl systems were subjected to acidic hydrolysis according to general procedure C, whereas the pyranose forms were purified by Sephadex G-25 gel chromatography eluting with water.

General procedure C: Hydrolysis and Acetylation.

A solution of the substrate in deionized water (0.05 M) was treated with DOWEX-50WX2 hydrogen form resin (400 mg per 100 mg substrate) then stirred at 90 °C until the completion. The resin was filtered off and the solution was concentrated in *vacuo*. The crude mixture was taken up in pyridine (0.5 M), acetic anhydride (2 eq per OH) and 4-(dimethylamino)pyridine (0.1 eq) were added, and the reaction mixture was stirred at room temperature until the completion. The solution was concentrated in vacuo and purified by silica gel chromatography.

General Procedure D: Bromination.

To a solution of per-*O*-acetyl-5-thio-glucopyranose in anhydrous DCM (0.5 M) was added 33% HBr in acetic acid (7.0 eq) at 0 °C. The reaction mixture was kept at 5 °C for 12 h, then was diluted with DCM and quenched with ice cold aqueous $NaHCO_3$. The organic layer was washed with water, and brine, dried with $MgSO_4$, and concentrated in *vacuo*. The residue was purified by silica gel chromatography.

General procedure E: Thioacylation.

At 0 °C, potassium thioacetate (1.5 eq) was added to a solution of the per-*O*-acetyl-5-thio-glucopyranosyl bromide in DMF (1.0 M) and the reaction mixture stirred 12 h at room temperature. After completion, the reaction mixture was diluted with ethyl acetate and washed with water, and brine, and dried with $MgSO_4$. The reaction mixture was concentrated in vacuo and purified by silica gel chromatography.

3-*O*-Acetyl-5-*O*-methanesulfonyl-6-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (12).

To a stirred solution of compound **11**[69] (21.0 g, 0.08 mol) in DCM (500 mL, 0.16 M) was added pyridine (30.0 mL, 4.66 mol) and DMAP (0.98 g, 8.0 mmol). At −30 °C benzoyl chloride (10.1 mL, 0.09 mol) was added dropwise and the reaction mixture was stirred at −30 °C for 1 h. After completion, methanesulfonyl chloride (12.2 mL, 0.16 mol) was added to the reaction mixture at −30 °C. The reaction mixture was stirred at room temperature for 10 min before it was concentrated at 40 °C to remove DCM. After DCM was removed, the reaction mixture was stirred at room temperature until completion. The reaction mixture was diluted with DCM and quenched with aqueous $NaHCO_3$. The solution was washed with water, and brine and dried over $MgSO_4$. The reaction mixture concentrated in vacuo to give product **12** (29.5 g, 83%) as a white solid, whose spectral data are identical with literature.[70]

5,6-Anhydro-5,6-epithio-1,2-*O*-isopropylidene-α-D-glucofuranose (13).

At 0 °C, to a stirred solution of compound **12** (24 g, 0.05 mol) in anhydrous methanol (500 mL, 0.1 M) was added NaOMe (3.2 g, 0.06 mol) portionwise. The reaction mixture was stirred at 0 °C until the completion. Amberlyst IR 120 was added portionwise to neutralize the reaction mixture. The resin was filtered off and thiourea (7.6 g, 0.1 mol) was added to the reaction mixture which was then stirred at 80 °C for 3 h before it was concentrated in vacuo. The residue was dissolved in DCM and washed with water, and brine and dried over $MgSO_4$ and concentrated in vacuo. The crude mixture was crystallized from ethyl acetate and hexane to give product **13** (7.1 g, 65%) as a white solid, mp 140-142 °C, with spectral data identical to the literature.[71]

3,6-Di-*O*-acetyl-5-*S*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (14).

A stirred solution of compound **13** (25.5 g, 0.12 mol) in a mixture of acetic anhydride (213 mL) and glacial acetic acid (43 mL) was treated with anhydrous potassium acetate (18.7 g, 0.18 mol) and stirred at 145 °C for 12 h. After the reaction mixture was cooled, it was poured into ice water and extracted with chloroform. The chloroform layer was washed with aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in *vacuo*. The residue was decolorized with charcoal and recrystallized from ethanol to give **14** (18.1 g, 43%) as white crystals (mp 145-146 °C), with spectal data are identical to the literature.[72]

1,2,3,4,6-Penta-*O*-acetyl-5-thio-α,β-D-glucopyranose (15).

A solution of compound **14** (17.5 g, 0.05 mol) in acetic acid (100 mL) and water (100 mL) was stirred at 90 °C for 12 h, then concentrated in *vacuo* and co-evaporated with toluene (3× 10 mL) to remove acetic acid. The residue was dissolved in anhydrous methanol (300 mL) and NaOMe (540 mg, 0.01 mol) was slowly added at 0 °C, followed by stirring at 0 °C for 4 h before Amberlyst IR120 resin was added to quench the reaction (monitored by pH paper). The resin was filtered off and the filtrate was concentrated in *vacuo*. The residue was dissolved in pyridine (100 mL) and acetic anhydride (46 mL, 0.5 mol) was added at 0 °C. The reaction mixture was stirred at room temperature for 12 h before it was concentrated in vacuo. The residue was purified by silica gel chromatography eluting ethyl acetate: hexane (1:4) to give **15** (11.5 g, 60%) as a colorless oil, with spectral data identical to the literature.[72]

1,2-*O*;5-*S*,6-*O*-Di-isopropylidene-5-thio-α-D-glucofuranose (16).

To a stirred solution of compound **15** (3.6 g, 8.8 mmol) in anhydrous methanol was added NaOMe (75 mg, 1.4 mmol). The reaction mixture was stirred at room temperature for 10 min before Amberlyst IR 120 resin was added to quench the reaction (monitored by pH paper). The resin was filtered off and the filtrate was concentrated in vacuo then was dissolved in anhydrous acetone (100 mL) and treated with *p*-toluenesulfonic acid (3.0 g, 17.4 mmol). The reaction mixture was stirred at room temperature for 12 h before aqueous $NaHCO_3$ was added. The reaction mixture was diluted with ethyl acetate and washed with water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography with eluting with hexane: ethyl acetate (3:1) to give **16** (1.8 g, 74%) as a colorless oil with spectra identical to the literature.[75]

1,2-*O*;5-*S*,6-*O*-Di-isopropylidene-5-thio-α-D-allofuranose (17).

To a solution of dimethyl sulfoxide (5.6 mL, 0.08 mol) in DCM (22 mL) at −78 °C was added oxalyl chloride (3.4 mL, 0.04 mol) dropwise. The reaction mixture was stirred at −78 °C for 10 min before a solution of **16** (3.5 g, 12.6 mmol) in DCM (20 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 1 h before triethylamine (12.6 mL, 0.09 mol) was added. Stirring was continued at −78 °C for 10 min before the reaction mixture was poured into ice water, extracted with ethyl acetate, washed with aqueous $NaHCO_3$, and brine, dried over $MgSO_4$ and concentrated in *vacuo*. The residue was dissolved in ethanol (70 mL), cooled to 0 °C, and treated with sodium borohydride (715 mg, 0.02 mol). After stirring at 0 °C for 30 min, the reaction mixture was quenched with acetone, diluted with DCM and washed with aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. The reaction mixture was concentrated in vacuo and purified by silica gel chromatography (eluent: hexane:ethyl acetate 3:1) to give **17** as a white solid (2.0 g, 57%) with spectral data identical to the literature.[75]

3-*O*-Trifluoromethanesulfonyl-1,2-*O*;5-*S*,6-*O*-di-isopropylidene-5-thio-α-D-allofuranose (18).

To a stirred solution of compound **17** (2.0 g, 7.2 mmol) and pyridine (5.8 mL, 72 mmol) at 0 °C was added trifluoromethanesulfonic anhydride (1.8 mL, 10.8 mmol) dropwise. The reaction mixture was stirred at 0 °C for 0.5 h before ice cold aqueous $NaHCO_3$ was added. The reaction mixture was diluted in DCM, washed by water, and brine and dried over $MgSO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography (eluent: hexane:ethyl acetate 10:1) to give product **18** as a white solid (2.5 g, 85%). Mp 79-80 °C. $[\alpha]^{23}_D$ = +15.0° (*c* 0.6, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.78 (d, $J_{1,2}$ = 3.9 Hz, 1H, H_1), 4.87 (dd, $J_{3,4}$ = 7.6 Hz, $J_{3,2}$ = 5.3 Hz, 1H, H_3), 4.73 (t, J = 4.6 Hz, 1H, H_2), 4.32 (t, J = 7.2 Hz, 1H, H_4), 4.25 (dd, $J_{6,6'}$ = 10.3 Hz, $J_{6,5}$ = 2.5 Hz, 1H, H_6), 4.13 (dd, $J_{6,6'}$ = 10.3 Hz, $J_{6',5}$ = 5.8 Hz, 1H, $H_{6'}$), 3.79 (m, 1H, H_5), 1.69 (s, 3H), 1.60 (s, 3H), 1.56 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 118.3 (CF_3), 114.2 (isopropylidene C), 103.8 (C_1), 93.2 (isopropylidene C), 83.2 (C_3), 79.0 (C_4), 77.6 (C_2), 70.6 (C_6), 51.7 (C_5), 30.7, 30.4, 26.8, 26.5 (isopropylidene CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{13}H_{19}O_7F_3NaS_2$ 431.0422, found 431.0424.

1-*S*-Acetyl-2,3,4,6-tetra-*O*-acetyl-1,5-dithio-β-D-glucopyranose (19).

Compound **15** (4.95 g, 12.2 mmol) was subjected to bromination according to general procedure D to give an α,β mixture of anomeric bromides. The crude product was subjected to thioacylation according to general procedure E to give the α,β mixture of anomeric thioacetates from which the β-isomer was isolated by silica gel chromatography eluting with hexane:ethyl acetate (3:1) to give **19** (2.36 g, 46%) as a colorless solid. Mp 121-122 °C. $[\alpha]^{23}_D$ = +66.1° (*c* 0.6, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.26 (dd, $J_{4,5}$ = 10.7 Hz, $J_{4,3}$ = 9.6 Hz, 1H, H_4), 5.22 (dd, $J_{2,1}$ = 11.0 Hz, $J_{2,3}$ = 9.4 Hz, 1H, H_2), 5.08 (t, J = 9.5 Hz, 1H, H_3), 4.66 (d, $J_{1,2}$ = 11.0 Hz, 1H, H_1), 4.25 (dd, $J_{6,6'}$ = 12.1 Hz, $J_{6,5}$ = 5.4 Hz, 1H, H_6), 4.09 (dd, $J_{6',6}$ = 12.1 Hz, $J_{6',5}$ = 3.2 Hz, 1H, $H_{6'}$), 3.41 – 3.35 (m, 1H, H_5), 2.35 (s, 3H, $SCOCH_3$), 2.05 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H).^{13}C NMR (151 MHz, $CDCl_3$) δ 191.4 ($SCOCH_3$), 170.5, 169.5, 169.34, 169.29 ($OCOCH_3$), 74.4 (C_3), 72.4 (C_2),

71.5 (C_4), 61.0 (C_6), 44.9 (C_1), 44.0 (C_5), 30.5 ($SCOCH_3$), 20.6, 20.5, 20.4, 20.4 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{16}H_{22}O_9NaS_2$ 445.0603, found 445.0605.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-1,2-*O*;5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (20).

Compounds **18** (0.66 g, 1.5 mmol) and **19** (0.73 g, 1.8 mmol) were coupled according to general procedure A. The crude reaction mixture was subjected to silica gel chromatography (eluent: ethyl acetate:hexane 1:4) to give the product **20** (0.75 g, 78%) as a colorless solid. Mp 161-162 °C. $[\alpha]^{23}_D$ = +20.0° (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, $J_{1b,\ 2b}$ = 3.6 Hz, 1H, H_{1b}), 5.29 (dd, $J_{4a,\ 5a}$ = 10.7 Hz, $J_{4a,\ 3a}$ = 9.3 Hz, 1H, H_{4a}), 5.13 (dd, $J_{2a,\ 1a}$ = 10.6 Hz, $J_{2a,\ 3a}$ = 9.4 Hz, 1H, H_{2a}), 5.05 (t, *J* = 9.4 Hz, 1H, H_{3a}), 4.69 (d, $J_{2b,\ 1b}$ = 3.6 Hz, 1H, H_{2b}), 4.39 (dd, $J_{4b,\ 5b}$ = 10.5 Hz, $J_{4b,\ 3b}$ = 4.0 Hz, 1H, H_{4b}), 4.34 – 4.26 (m, 2H, $H_{6a\&6b}$), 4.15 – 4.06 (m, 2H, $H_{6'a\&6'b}$), 3.98 (d, $J_{1a,\ 2a}$ = 10.6 Hz, 1H, H_{1a}), 3.65 – 3.60 (m, 1H, H_{5b}), 3.57 (d, $J_{3b,\ 4b}$ = 4.0 Hz, 1H, H_{3b}), 3.23 – 3.18 (m, 1H, H_{5a}), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 1.53 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 169.7, 169.3, 169.2 ($OCOCH_3$), 112.3 (isopropylidene C), 105.3 (C_{1b}), 92.7 (isopropylidene C), 86.0 (C_{2b}), 81.4 (C_{4b}), 74.5 (C_{3a}), 73.7 (C_{2a}), 72.0 (C_{6b}), 71.6 (C_{4a}), 61.0 (C_{6a}), 53.4 (C_{3b}), 50.6 (C_{5b}), 47.9 (C_{1a}), 44.6 (C_{5a}), 31.6, 30.7, 26.7, 26.4 (isopropylidene CH_3), 20.6, 20.6, 20.5, 20.4 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{26}H_{38}O_{12}NaS_3$ 661.1423, found 661.1423.

5-Thio-β-D-glucopyranosyl-(1→3)-1,2-*O*;5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (21).

Compound **20** (800 mg, 1.2 mmol) was subjected to deacylation according to general procedure B to give the product **21** (590 mg, quantitative) as a colorless solid. $[\alpha]^{23}_D$ = +43.7° (*c* 1.4, MeOH).^{1}H NMR (600 MHz, CD_3OD) δ 5.87 (d, $J_{1b,\ 2b}$ = 3.6 Hz, 1H, H_{1b}), 4.89 (d, $J_{2b,\ 1b}$ = 3.6 Hz, 1H, H_{2b}), 4.36 (dd, $J_{4b,\ 5b}$ = 10.5 Hz, $J_{4b,\ 3b}$ = 3.9 Hz, 1H, H_{4b}), 4.25 (dd, $J_{6b,\ 6'b}$ = 9.9 Hz, $J_{6b,\ 5b}$ = 2.2 Hz, 1H, H_{6b}), 4.09 (dd, $J_{6'b,\ 6b}$ = 9.9 Hz, $J_{6'b,\ 5b}$ = 4.8 Hz, 1H, $H_{6'b}$), 3.89 (dd, $J_{6a,6'a}$ = 11.4 Hz, $J_{6a,\ 5a}$ = 4.3 Hz, 1H, H_{6a}), 3.82 (d, $J_{1a,\ 2a}$ = 10.2 Hz, 1H, H_{1a}), 3.75 (dd, $J_{6'a,\ 6a}$ = 11.4 Hz, $J_{6'a,\ 5a}$ = 6.0 Hz, 1H, $H_{6'a}$), 3.63 (d, $J_{3b,\ 2b}$ = 3.9 Hz, 1H, H_{3b}), 3.62 – 3.60 (m, 1H, H_{5b}) 3.53 (t, *J* = 9.5 Hz, 1H, H_{4a}), 3.35 (dd, $J_{2a,\ 1a}$ = 10.2 Hz, $J_{2a,\ 3a}$ = 8.7 Hz, 1H, 3H, H_{2a}), 3.16 (t, *J* = 8.8 Hz, 1H, H_{3a}) 2.90 – 2.86 (m, 1H, H_{5a}) 1.62 (s, 3H), 1.55 (s, 3H), 1.46 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 111.6 (isopropylidene C), 105.4 (C_{1b}), 91.9 (isopropylidene C), 85.6 (C_{2b}), 81.1 (C_{4b}), 79.4 (C_{3a}), 76.3 (C_{2a}), 74.8 (C_{4a}), 71.6 (C_{6b}), 61.5 (C_{6a}), 52.2 (C_{3b}), 50.5 (C_{5b}), 49.6 (C_{5a}), 49.2 (C_{1a}), 30.7, 29.6, 25.5, 25.1 (isopropylidene CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{18}H_{30}O_8S_3Na$ 493.1000, found 493.0999.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (22).

Compound **21** (590 mg, 1.2 mmol) was deprotected according to general procedure C to give the product **22** (830 mg, 91%) as a 3:1 α:β mixture in the form of a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) α isomer: δ 6.02 (d, *J* = 3.1 Hz, 1H, $H_{1b\alpha}$), 3.54–3.49 (m, 1H, $H_{5b\alpha}$), 2.20 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.97

(s, 3H), β isomer: 5.84 (d, J = 8.4Hz, 1H, $H_{1b\beta}$), 5.37 (dd, $J_{1,2}$ = 9.6 Hz, $J_{2,3}$ = 8.4 Hz, 1H, $H_{2b\beta}$), 3.20 – 3.17 (m, 1H, $H_{5b\beta}$), 3.05 (t, J = 10.1 Hz, 1H, $H_{3b\beta}$), 2.14 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H); common and overlapping signals: 5.31 – 5.21 (m, H_{4a} & $H_{2b\alpha}$), 5.15 – 4.94 (m, H_{2a} & H_{3a} & $H_{4b\beta}$ & $H_{4b\alpha}$), 4.32 – 4.22 (m, H_{6a} & $H_{6b\alpha}$ & $H_{6'b\beta}$), 4.18 – 4.13 (m, $H_{6'a}$), 4.09 – 4.02 (m, $H_{6'b\alpha}$ & H_{1a} & $H_{6b\beta}$), 3.36 – 3.22 (m, H_{5a} & $H_{3b\alpha}$), 2.01 – 2.00 (m, Acetyl CH_3) . ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.7, 169.2, 169.1, 168.9 (Carbonyl C), 75.8 (C_{2b}), 74.2 (C_{3a}), 73.2 (C_{2a}), 71.8 (C_{4a}), 70.5 (C_{4b}& C_{1b}), 61.5 (C_{6b}), 61.1 (C_{6a}), 50.3 (C_{1a}), 49.9 (C_{3b}), 44.5 (C_{5a}), 40.6 (C_{5b}), 21.1, 20.8, 20.6, 20.5, 20.4, 20.3 (Acetyl CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{38}O_{16}S_3Na$ 749.1220, found 749.1223.

5-Thio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (8).

Compound **22** (20 mg, 0.03 mmol) was subjected to deacetylation according to general procedure B to give **8** (10 mg, 95%) as a 4:1 α:β mixture in the form of a white solid. ^{1}H NMR (600 MHz, D_2O) α isomer: δ 4.86 (d, J = 3.0 Hz, 1H, $H_{1b\alpha}$), 3.12 – 3.09 (m, 1H, $H_{5b\alpha}$), 3.00 (t, J = 10.7 Hz, 1H, $H_{3b\alpha}$), β isomer: 4.60 (d, J = 8.9Hz, 1H, $H_{1b\beta}$), 2.94 – 2.91 (m, 1H, $H_{5b\beta}$), 2.67 (t, J = 10.4 Hz, 1H, $H_{3b\beta}$); common and overlapping signals: 3.82 – 3.62 (m, $H_{2b\alpha,2b\beta}$ & $H_{6a,6'a}$ & $H_{6b,6'b}$), 3.95 (d, J = 10.4 Hz, H_{1a}), 3.54 – 3.41 (m, H_{4a} & $H_{4b\alpha}$ & $H_{4b\beta}$), 3.34 (dd, $J_{2,1}$ = 10.4 Hz, $J_{2,3}$ = 9.8 Hz, H_{2a}), 3.17 (t, J = 9.8Hz, H_{3a}), 2.90–2.84 (m, H_{5a}). ^{13}C NMR (151 MHz, D_2O) δ 77.8 (C_{3a}), 77.2 ($C_{2b\beta}$) 76.2 (C_{2a}), 75.0 ($C_{1b\beta}$) 74.6 ($C_{2b\alpha}$), 72.9 (C_{4a}), 72.4 ($C_{1b\alpha}$), 70.8 (C_{4b}), 60.7 ($C_{6b\beta}$), 60.5 ($C_{6b\alpha}$), 60.0 (C_{6a}), 57.5 ($C_{3b\beta}$) 54.4 ($C_{3b\alpha}$), 48.59 (C_{1a}), 48.56 (C_{5a}), 48.1 ($C_{5b\beta}$), 43.4 ($C_{5b\alpha}$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{12}H_{22}O_8NaS_3$ 413.0374, found 413.0374.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-α-D-glucopyranosyl bromide (23).

Compound **22** (246 mg, 0.34 mmol) was subjected to bromination according to general procedure D to give product **23** (184 mg, 73%) as a colorless oil. $[\alpha]^{23}_D$ = +112.0° (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.44 (d, $J_{1b,2b}$ = 3.4 Hz, 1H, H_{1b}), 5.26 (dd, $J_{4a,3a}$ = 10.7 Hz, $J_{4a,5a}$ = 9.6 Hz, 1H, H_{4a}), 5.09 (t, J = 10.7 Hz, 1H, H_{3a}), 5.05 (t, J = 10.9 Hz, 1H, H_{2a}), 5.00 (t, J = 9.5 Hz, 1H, H_{4a}), 4.92 (dd, $J_{2b,3b}$ = 10.9 Hz, $J_{2b,1b}$ = 3.4 Hz, 1H, H_{2b}), 4.36 (dd, $J_{6b,6'b}$ = 12.2 Hz, $J_{6b,5b}$ = 4.9 Hz, 1H, H_{6b}), 4.25 (dd, $J_{6a,6'a}$ = 12.0 Hz, $J_{6a,5a}$ = 5.0 Hz, 1H, H_{6a}), 4.13 (dd, $J_{6a,6'a}$ = 12.1 Hz, $J_{6b,5b}$ = 3.4 Hz, 1H, $H_{6'a}$), 4.10 – 4.07 (m, 2H, $H_{6'a}$ & H_{1a}), 3.62 – 3.59 (m, 1H, H_{5b}), 3.37 (t, J = 10.9 Hz, 1H, H_{3b}), 3.27– 3.34 (m, 1H, H_{5a}), 2.20 (s, 3H), 2.15 (s, 3H), 2.06 (s, 4H), 2.03 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.7, 169.3, 169.2, 169.0, 76.7 (C_{2b}), 74.3(C_{3a}), 73.3 (C_{2a}), 71.6 (C_{4a}), 70.1 (C_{4b}), 61.1 (C_{6a}), 61.0 (C_{6b}), 54.8 (C_{3b}), 50.7 (C_{1a}), 49.9 (C_{1b}), 44.5 (C_{5a}), 42.4 (C_{5b}), 20.9, 20.8, 20.6, 20.5, 20.4, 20.3 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{26}H_{35}O_{14}NaS_3Br$ 769.0270, found 769.0269.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-1-*S*-acetyl-2,4,6-tri-*O*-acetyl-1,3,5-trideoxy-1,3,5-trithio-β-D-glucopyranose (24).

Compound **23** (67 mg, 0.09 mmol) was used according to general procedure E to give **24** (60 mg, 89%) as a colorless solid. $[\alpha]^{23}_D$ = +43.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.25 (dd, J = 10.7, 9.5 Hz, 1H, H_{4a}), 5.21 (t, J = 10.7 Hz, 1H, H_{2b}), 5.06 (dd, J = 11.0, 9.5

Hz, 1H, H_{2a}), 4.95 (t, J = 10.7 Hz, 1H, H_{4b}), 4.93 (t, J = 9.5 Hz, 1H, H_{3a}), 4.57 (d, J = 10.7 Hz, 1H, H_{1b}), 4.23 (dd, J = 12.0, 5.3 Hz, 1H, H_{6a}), 4.18 (dd, J = 12.0, 5.6 Hz, 1H, H_{6b}), 4.13 (dd, J = 12.0, 3.3 Hz, 1H, $H_{6'a}$), 4.09 (dd, J = 12.0, 3.2 Hz, 1H, $H_{6'b}$), 3.99 (d, J = 11.0 Hz, 1H, H_{1a}), 3.34 (m, 1H, H_{5b}), 3.16 (m, 1H, H_{5a}), 3.01 (t, J = 10.8 Hz, 1H, H_{3b}), 2.35 (s, 3H, $SCOCH_3$), 2.12 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.9 ($SCOCH_3$), 170.5, 170.4, 169.7, 169.3, 169.2, 169.1, 168.9 ($OCOCH_3$), 75.2 (C_{2b}), 74.4 (C_{3a}), 72.9 (C_{2a}), 71.6 (C_{4a}), 70.2 (C_{4b}), 61.6 (C_{6a}), 61.0 (C_{6b}), 55.1 (C_{3b}), 50.5 (C_{1a}), 47.2 (C_{5b}), 45.8 (C_{1b}), 44.6 (C_{5a}), 30.5 ($SCOCH_3$), 20.8, 20.8, 20.6, 20.6, 20.4, 20.4, 20.2 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{38}O_{15}NaS_4$ 765.0991, found 765.0989.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-1,2-*O*;5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (25).

Compounds **24** (615 mg, 0.83 mmol) and **18** (507 mg, 1.24 mmol) were subjected to coupling according to general procedure A to give **25** (510 mg, 64%) as white crystals. Mp 163–164 °C. $[\alpha]^{23}_D$ = +26.5° (*c* 0.4, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.87 (d, $J_{1c,2c}$ = 3.6 Hz, 1H, H_{1c}), 5.26 (dd, $J_{4a,5a}$ = 10.7 Hz, $J_{4a,3a}$ = 9.6 Hz, 1H, H_{4a}), 5.16 (t, J = 10.6 Hz, 1H, H_{2b}), 5.08 (dd, $J_{1a,2a}$ = 11.0 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, H_{2a}), 5.02 – 4.94 (m, 2H, H_{4b} & H_{3a}), 4.71 (d, $J_{1c,2c}$ = 3.6 Hz, 1H, H_{2c}), 4.39 (dd, $J_{4c,5c}$ = 10.5 Hz, $J_{3c,4c}$ = 4.0 Hz, 1H, H_{4c}), 4.32 (dd, $J_{6c,5c}$ = 10.0 Hz, $J_{6c,6'c}$ = 2.5 Hz, 1H, H_{6c}), 4.25 (dd, $J_{6a,6'a}$ = 12.0 Hz, $J_{6a,5a}$ = 5.1 Hz, 1H, H_{6a}), 4.22 (dd, $J_{6b,6'b}$ = 12.0 Hz, $J_{6b,5b}$ = 5.4 Hz, 1H, H_{6b}), 4.15 – 4.11 (m, 2H, $H_{6'a}$ & $H_{6'c}$), 4.09 (dd, $J_{6b,6'b}$ = 12.0 Hz, $J_{6'b,5b}$ = 3.3 Hz, 1H, $H_{6'b}$), 4.00 (d, $J_{1a,2a}$ = 11.0 Hz, 1H, H_{1a}), 3.83 (d, $J_{1b,2b}$ = 10.6 Hz, 1H, H_{1b}), 3.64 (m, 1H, H_{5c}), 3.55 (d, $J_{3c,4c}$ = 4.0 Hz, 1H, H_{3c}), 3.25 – 3.14 (m, 2H, H_{5a} & H_{5b}), 2.93 (t, J = 10.8 Hz, 1H, H_{3b}), 2.20 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.53 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.5, 169.8, 169.3, 169.2, 169.1, 168.8, 112.3 (isopropylidene C), 105.4 (C-1c), 92.7 (isopropylidene C), 86.1 (C_{2c}), 81.4 (C_{4c}), 76.2 (C_{2b}), 74.3 (C_{3a}), 72.6 (C_{2a}), 72.0 (C_{6c}), 71.9 (C_{4a}), 70.3 (C_{4b}), 61.7 (C_{6b}), 61.2 (C_{6a}), 55.3 (C_{3b}), 53.6 (C_{3c}), 50.7 (C_{1a}), 50.6 (C_{5c}), 50.1 (C_{1b}), 47.0 (C_{5b}), 44.4 (C_{5a}), 31.6, 30.7, 26.7, 26.5 (Isopropylidene CH_3), 21.0, 20.9, 20.6, 20.5, 20.4, 20.2 (Acetyl CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{54}O_{18}NaS_5$ 981.1811, found 981.1810.

5-Thio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-1,2-*O*;5-*S*,6-*O*-diisopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (26).

Compound **25** (568 mg, 0.59 mmol) was deacetylated according to general procedure B to give **26** (394 mg, quantitative) as a colorless solid. $[\alpha]^{23}_D$ = +17.2° (*c* 2.6, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 5.90 (d, $J_{1c,2c}$ = 3.5 Hz, 1H, H_{1c}), 4.92 (d, $J_{2c,1c}$ = 3.5 Hz, 1H, H_{2c}), 4.36 (dd, $J_{4c,5c}$ = 10.5 Hz, $J_{4c,3c}$ = 3.9 Hz, 1H, H_{4c}), 4.25 (dd, $J_{6c,6'c}$ = 10.0 Hz, $J_{6c,5c}$ = 2.1 Hz, 1H, H_{6c}), 4.10 (dd, $J_{6c,6'c}$ = 9.9 Hz, $J_{6'c,5c}$ = 4.8 Hz, 1H, $H_{6'c}$), 3.92 (d, J = 10.1 Hz, 1H, H_{1a}), 3.91 – 3.85 (m, 3H, H_{1b}, H_{6a}, H_{6b}), 3.82 (dd, $J_{6b,6'b}$ = 11.5 Hz, $J_{6'b,5b}$ = 5.6 Hz, 1H, $H_{6'b}$), 3.73 (dd, $J_{6'a,6a}$ = 11.4 Hz, $J_{6'a,5a}$ = 6.0 Hz, 1H, $H_{6'a}$), 3.69 (d, $J_{3c,4c}$ = 3.9 Hz, 1H, H_{3c}), 3.64 – 3.61 (m, 1H, H_{5c}), 3.55 – 3.47 (m, 2H, H_{4a} & H_{4b}), 3.47 – 3.39 (m, 2H, H_{2a} & H_{2b}), 3.19 (t, J = 8.9 Hz, 1H, H_{3a}), 2.98 – 2.95 (m, 1H, H_{5a}), 2.93 – 2.90 (m, 1H, H_{5b}), 2.74 (t, J = 10.1 Hz, 1H, H_{3b}), 1.62 (s, 3H), 1.56 (s, 3H), 1.46 (s, 3H), 1.32 (s, 3H),

(isopropylidene CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 111.6 (isopropylidene C), 105.5 (C_{1c}), 91.9 (isopropylidene C), 85.5 (C_{2c}), 81.2 (C_{4c}), 79.0 (C_{3a}), 77.9 (C_{2a}), 76.2 (C_{2b}), 74.4 (C_{4a}), 73.0 (C_{4b}), 71.6(C_{6c}), 62.4 (C_{3b}), 61.8 (C_{6b}), 61.5 (C_{6a}), 52.1(C_{3c}), 51.6 (C_{5b}), 51.4 (C_{1b}), 50.5 (C_{5c}), 49.9 (C_{5a}), 49.5 (C_{1a}), 30.7, 29.7, 25.6, 25.3 (CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{24}H_{40}O_{11}NaS_5$ 687.1072, found 687.1073.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-1,2,4,6-tetra-*O*-acetyl-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (27).

Compound **26** (394 mg, 0.59 mmol) was deprotected according to general procedure C to give **27** (586 mg, 95%) as a 3:1 α:β mixture in the form of a colorless solid. ^1H NMR (600 MHz, $CDCl_3$) δ α isomer: 6.03 (d, $J_{1c\alpha,\ 2c\alpha}$ = 3.1 Hz, 1H, $H_{1c\alpha}$), 3.56 – 3.48 (m, 1H, $H_{5c\alpha}$), 3.28 (t, *J* = 11.5 Hz, 1H, $H_{3c\alpha}$), 2.20 (s, 3H), 2.14 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), β isomer: 5.87 (d, $J_{1c\beta,\ 2c\beta}$ = 7.1 Hz, 1H, $H_{1c\beta}$), 5.36 (t, *J* = 7.1Hz, 1H, $H_{2c\beta}$), 3.37 – 3.33 (m, 1H, $H_{5c\beta}$), 2.09 (s, 3H); common and overlapping signals: 5.28 – 5.23 (m, H_{4a} & $H_{2c\alpha}$), 5.14 – 5.04 (m, H_{2b} & H_{2a} & $H_{4c\alpha}$ & $H_{4c\beta}$), 4.99 – 4.93 (m, H_{4b} & H_{3a}), 4.30 – 4.08 (m, $H_{6a,6'a}$ & $H_{6b,6'b}$ & $H_{6c\alpha}$ & $H_{6c\beta}$), 4.05 (dd, $J_{6c\beta,\ 6'c\beta}$ = 12.1 Hz, $J_{6'c\beta,\ 5c\beta}$ = 3.3 Hz, $H_{6'c\beta}$), 4.03 – 3.91 (m, H_{1b} & H_{1a} & $H_{6'c\alpha}$), , 3.25 – 3.21 (m, H_{5b}), 3.17– 3.05 (m, H_{5a} & $H_{3c\beta}$), 2.93 – 2.86 (m, H_{3b}), 2.17 – 2.11 (m, Acetyl CH_3), 2.08 – 2.04 (m, Acetyl CH_3), . ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.57, 170.54, 170.42, 169.67, 169.23, 169.17, 169.14, 169.10, 168.99, 168.86, 168.72 (Carbonyl C), 76.2 (C_{2b}), 75.5 ($C_{2c\alpha}$), 74.8 ($C_{2c\beta}$), 74.2 (C_{3a}), 72.7 ($C_{1c\beta}$), 72.6 (C_{2a}), 71.9 (C_{4a}), 70.8 ($C_{4c\alpha}$), 70.5 ($C_{1c\alpha}$ & C_{4b}), 70.2 ($C_{4c\beta}$), 62.8 ($C_{6c\beta}$), 61.8 ($C_{6c\alpha}$), 61.5 (C_{6b}), 61.1 (C_{6a}), 51.6 (C_{1b}), 50.8 (C_{1a}), 50.0 ($C_{3c\alpha}$), 46.6 (C_{5b}), 44.5 (C_{5a}), 42.9 ($C_{5c\beta}$), 40.7 ($C_{5c\alpha}$), 21.1, 21.0, 20.9, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4, 20.2 (Acetyl CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{40}H_{54}O_{22}NaS_5$ 1069.1608, found 1069.1613.

5-Thio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (9).

Compound **27** (20 mg, 0.02 mmol) was deacetylated according to general procedure B to give **9** (11 mg, quantitative), 4:1 α:β mixture as a colorless solid. ^1H NMR (600 MHz, D_2O) δ α isomer: 4.85 (d, *J* = 2.9 Hz, 1H, $H_{1c\alpha}$), β isomer: 4.59 (d, *J* = 12.0 Hz, 1H, $H_{1c\beta}$); common and overlapping signals: 3.98 (t, *J* = 10.9 Hz, H_{1a}&H_{1b}), 3.83 – 3.64 (m, $H_{6a,6'a}$&$H_{6b,6'b}$&$H_{6c,6'c}$), 3.58 – 3.42 (m, H_{2b}&H_{4a}&H_{4b}&H_{4c}), 3.35 (t, *J* = 9.0 Hz, H_{2a}), 3.17 (t, *J* = 9.0 Hz, H_{3a}), 3.14 – 3.10 (m, H_{5c}), 3.05 (t, *J* = 10.7 Hz, H_{3c}), 2.95– 2.91 (m, H_{5b}), 2.90–2.85 (m, H_{5a}), 2.73 (t, *J* = 10.4 Hz, H_{3b}). ^{13}C NMR (151 MHz, D_2O) δ 77.9 (C_{3a}), 77.3 ($C_{2c\beta}$), 76.1 (C_{2a}&C_{2b}), 75.1 ($C_{1c\beta}$), 74.7 ($C_{2c\alpha}$), 72.8 (C_{4a}), 72.5 ($C_{1c\alpha}$), 70.8 (C_{4b}), 70.7 ($C_{4c\beta}$), 70.6 ($C_{4c\alpha}$), 60.7 ($C_{6c\beta}$), 60.5 ($C_{6c\alpha}$), 60.4 (C_{6b}), 60.0 (C_{6a}), 59.2 (C_{3b}), 57.6 ($C_{3c\beta}$), 54.5 ($C_{3c\alpha}$), 50.6 (C_{5b}), 50.4 (C_{1b}), 48.8 (C_{1a}), 48.9 (C_{5a}), 48.1 ($C_{5c\beta}$), 43.5 ($C_{5c\alpha}$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{18}H_{32}O_{11}NaS_5$ 607.0446, found 607.0447

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-trideoxy-3,5-dithio-α-D-glucopyranosyl bromide (28).

Bromination was performed on compound **27** (53 mg, 0.05 mmol) according to general procedure D to give **28** (23 mg, 43%) as a colorless oil. $[\alpha]^{23}_{D}$ = +90.0° (*c* 0.6, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.47 (d, *J* = 3.4 Hz, 1H, H_{1c}), 5.17 – 5.02 (m, 1H, H_{4a}), 5.17 – 5.02 (m, 3H, H_{2a}&H_{2b}& H_{3a}), 5.01 – 4.89 (m, 3H, H_{2c}&H_{4b}&H_{4c}), 4.34 (dd, $J_{6a,6'a}$ = 12.2 Hz, $J_{6a,5a}$ = 5.1 Hz, 1H, H_{6a}), 4.29 – 4.06 (m, 5H, $H_{6'a}$&H_{6b}&$H_{6'b}$&H_{6c}&$H_{6'c}$), 4.00 (d, *J* = 10.7 Hz, 1H, H_{1b}), 3.95 (d, *J* = 10.9 Hz, 1H, H_{1a}), 3.67 – 3.58 (m, 1H, H_{5c}), 3.42 (t, *J* = 10.9 Hz, 1H, H_{3c}), 3.29 – 3.20 (m, 1H, H_{5b}), 3.15 – 3.07 (m, 1H, H_{5a}), 2.97 – 2.85 (m, 1H, H_{3b}). 2.23 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.5, 170.4, 169.7, 169.3, 169.2, 169.1, 169.1, 168.9, 168.6 (Carbonyl C), 76.3 (C_{2c}), 76.2 (C_{2b}), 74.3 (C_{3a}), 72.6 (C_{2a}), 71.9 (C_{4a}), 70.6 (C_{4c}), 70.4 (C_{4b}), 61.7 (C_{6c}), 61.1 (C_{6b}&C_{6a}), 55.3 (C_{3b}), 55.0 (C_{3c}), 51.6 (C_{1b}), 50.8 (C_{1a}), 50.6 (C_{1c}), 46.8 (C_{5b}), 44.5 (C_{5a}), 42.6 (C_{5c}), 20.9, 20.8, 20.6, 20.5, 20.4, 20.2 (Acetyl CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{37}H_{47}O_{25}NaS_3Br$ 1089.0650, found 1089.0653.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-1-*S*-acetyl-2,4,6-tri-*O*-acetyl-1,3,5-trideoxy-1,3,5-trithio-β-D-glucopyranose (29).

The bromide **28** (34 mg, 0.03mmol) was substituted by potassium thioacetate (5mg, 1.5 equiv) according to general procedure E to give **29** (30mg, 90%) as a colorless solid. $[\alpha]^{23}_{D}$ = +99.2° (*c* 0.6, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.25 (t, *J* = 10.9 Hz, 1H, H_{4a}), 5.22 (t, *J* =10.6 Hz, 1H, H_{2c}), 5.16 – 5.07 (m, 1H, H_{2b}), 5.05 (dd, $J_{2a,1a}$ = 11.0 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, H_{2a}), 5.00 – 4.93 (m, 4H, H_{3a}&H_{4b}&H_{4c}), 4.60 (d, $J_{1c,2c}$ = 10.6 Hz, 1H, H_{1c}), 4.28 – 4.09 (m, 6H, $H_{6a,6'a}$ & $H_{6b,6'b}$& $H_{6c,\ 6'c}$), 3.93 (d, $J_{1a,\ 2a}$ = 11.0 Hz, 1H, H_{1a}), 3.90 (d, $J_{1b,\ 2b}$ = 10.6 Hz, 1H, H_{1b}) , 3.37 – 3.34 (m, 1H, H_{5c}), 3.16 – 3.13 (m, 1H, H_{5b}), 3.11 – 3.07 (m, 1H, H_{5a}), 3.03 (t, *J* = 10.7 Hz, 1H, H_{3c}), 2.88 – 2.80 (m, 1H, H_{3b}), 2.38 (s, 3H), 2.13 (s, 6H), 2.12 (s, 6H), 2.06 (s, 6H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.0, 170.5, 170.4, 169.7, 169.3, 169.1, 168.5 (Carbonyl C), 76.1 (C_{2c}), 75.3 (C_{2b}), 74.3 (C_{3a}), 72.5 (C_{2a}), 71.8 (C_{4a}), 70.5 (C_{4c}), 70.4 (C_{4b}) 61.7 (C_{6c}), 61.7 (C_{6b}), 61.1 (C_{6a}), 55.3 (C_{3b}), 54.9 (C_{3c}), 52.4 (C_{1b}), 50.8 (C_{1a}), 47.2 (C_{5c}), 46.8 (C_{5b}), 45.7 (C_{1c}), 44.5 (C_{5a}), 30.5 (S-Acetyl CH_3), 20.8, 20.7, 20.60, 20.5, 20.4, 20.2 (Acetyl CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{40}H_{54}O_{21}NaS_6$ 1085.1380, found 1085.1383.

2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (30).

Compounds **29** (510 mg, 0.48 mmol) and **18** (490 mg, 0.72 mmol) were coupled together according to general procedure A to give product **30** (283 mg, 46%) as white crystals. Mp 139–140 °C. $[\alpha]^{23}_{D}$ = +31.6° (*c* 0.3, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.88 (d, $J_{1d,2d}$ = 3.6 Hz, 1H, H_{1d}), 5.27 (t, *J* = 10.1 Hz, 1H, H_{4a}), 5.17 (t, *J* = 10.6 Hz, 1H, H_{2c}), 5.13 – 5.08

(m, 1H, H_{2b}), 5.05 (t, J = 10.3 Hz, 1H, H_{2a}), 5.01 – 4.94 (m, 3H, H_{3a}&H_{4b}&H_{4c}), 4.70 (d, $J_{2d,1d}$ = 3.6 Hz, 1H, H_{2d}), 4.40 (dd, $J_{4d,5d}$ = 10.5 Hz, $J_{4d,3d}$ = 4.0 Hz, 1H, H_{4d}), 4.33 (dd, $J_{6d,6'd}$ = 10.1 Hz, $J_{6d,5d}$ = 2.4 Hz, 1H, H_{6d}), 4.29 – 4.18 (m, 3H, H_{6a}&H_{6b}&H_{6c}), 4.17 – 4.07 (m, 4H, $H_{6'a}$&$H_{6'b}$&$H_{6'c}$&$H_{6'd}$), 3.94 (d, $J_{1a,2a}$ = 11.1 Hz, 1H, H_{1a}), 3.90 (d, $J_{1b,2b}$ = 10.3 Hz, 1H, H_{1b}), 3.84 (d, $J_{1c,2c}$ = 10.6 Hz, 1H, H_{1c}), 3.67 – 3.62 (m, 1H, H_{5d}), 3.57 (d, $J_{3d,4d}$ = 4.0 Hz, 1H, H_{3d}), 3.20 –3.15 (m, 2H, H_{5b}&H_{5c}), 3.11 – 3.08 (m, 1H, H_{5a}), 2.95 (t, J = 10.7 Hz, 1H, H_{3c}), 2.88–2.82 (m, 1H, H_{3b}), 2.21 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.06 (s, 6H), 2.05 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.53 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.4, 169.7, 169.2, 169.1, 168.7, 168.3, 112.3 (isopropylidene C), 105.4 (C_{1d}), 92.7 (isopropylidene C), 86.1 (C_{2d}), 81.4 (C_{4d}), 76.2 (C_{2c}), 75.7 (C_{2b}), 74.2 (C_{3a}), 72.5 (C_{2a}), 72.0 (C_{6d}), 71.8 (C_{4a}), 70.6 (C_{4c}) 70.4 (C_{4b}), 61.8 (C_{6c}), 61.7 (C_{6b}), 61.0 (C_{6a}), 55.3 (C_{3b}), 54.9 (C_{3c}), 53.5 (C_{3d}), 52.5 (C_{1b}), 50.8 (C_{1a}), 50.6 (C_{5d}), 50.0 (C_{1c}), 47.0 (C_{5c}), 46.6 (C_{5b}), 44.5 (C_{5a}), 31.6, 30.7, 26.7, 26.5 (Isopropylidene CH_3), 21.0, 20.9, 20.8, 20.7, 20.6, 20.5, 20.4, 20.2 (Acetyl CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{50}H_{70}O_{24}NaS_7$ 1301.2200, found 1301.2205.

5-Thio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (31).

Compound **30** (150 mg, 0.12 mmol) was deacetylated according to general procedure B to give **31** (100 mg, quantitative) as a colorless solid. $[\alpha]^{23}_D$ = +18.9° (*c* 0.5, MeOH). ^{1}H NMR (600 MHz, CD_3OD) δ 5.88 (d, $J_{1d,2d}$ = 3.6 Hz, 1H, H_{1d}), 4.90 (d, $J_{2d,1d}$ = 3.6 Hz, 1H, H_{2d}), 4.37 (dd, $J_{4d,5d}$ = 10.5 Hz, $J_{4d,3d}$ = 4.0 Hz, 1H, H_{4d}), 4.25 (dd, J = 10.0, 1.8 Hz, 1H, H_{6d}), 4.10 (dd, J = 8.0, 4.9, 1.6 Hz, 1H, $H_{6'd}$), 4.00 (d, J = 10.0 Hz, 1H, H_{1b}) 3.97 (d, J = 10.4 Hz, 1H, H_{1a}), 3.94 – 3.86 (m, 4H, H_{1c}&H_{6a}&H_{6b}&H_{6c}), 3.81 – 3.72 (m, 2H, $H_{6'a}$&$H_{6'b}$), 3.72 – 3.67 (m, $H_{6'c}$1H), 3.66 (d, J = 4.0 Hz, 1H, H_{3d}), 3.65 – 3.60 (m, 1H, H_{5d}), 3.56 – 3.43 (m, 5H, H_{4a}&H_{4b}&H_{4c}&H_{2b}&H_{2c}), 3.38 (t, J = 10.4 Hz, 1H, H_{2a}), 3.17 (t, J = 8.9 Hz, 1H, H_{3a}), 3.02 – 2.88 (m, 3H, H_{5a}&H_{5b}&H_{5c}), 2.85 – 2.69 (m, 2H, H_{3b}&H_{3c}), 1.62 (s, 3H), 1.56 (s, 3H), 1.46 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 111.6 (isopropylidene C), 105.4 (C_{1d}), 92.0 (isopropylidene C), 85.6 (C_{2d}), 81.1 (C_{4d}), 78.6 (C_{3a}), 77.5 (C_{2a}), 77.0 (C_{2b}), 75.8 (C_{2c}), 73.7 (C_{4a}), 71.9 (C_{4b}), 71.8 (C_{4c}), 71.6 (C_{6d}), 61.8 (C_{3b}), 61.2 (C_{6c}), 61.1 (C_{3c}), 60.9 (C_{6a}&C_{6b}), 52.5 (C_{3d}), 51.7 (C_{5b}), 51.4 (C_{5c}), 51.3 (C_{1b}), 51.1 (C_{1c}), 50.5 (C_{5d}), 49.6 (C_{5a}&C_{1a}), 30.7, 29.6, 25.5, 25.2 (isopropylidene-CH_3). HRMS *m/z* $[M+Na]^+$ calcd for $C_{30}H_{50}O_{14}NaS_7$ 881.1143, found 881.1141.

5-Thio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1→3)-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (10).

Compound **31** (100 mg, 0.12 mmol) was deprotected according to general procedure C to give product **10** (78 mg, 85%) as 4:1 α:β mixture in the form of a white solid. ^{1}H NMR (600 MHz, D_2O) α isomer: δ 4.86 (d, J = 3.0 Hz, 1H, $H_{1d\alpha}$), β isomer: 4.58 (d, J = 8.8Hz, 1H, $H_{1d\beta}$); commona and overlapping signals: 4.02 – 3.90 (m, H_{1a}& H_{1b}& H_{1c}), 3.86 – 3.59 (m, 9.0H, $H_{6a,6'a}$ & $H_{6b,6'b}$ & $H_{6c,6'c}$ & $H_{6d,6'd}$ & H_{2d}), 3.57 – 3.37 (m, H_{4a} & H_{4b} & H_{4c} & H_{4d}, H_{2b} & H_{2c}), 3.31 (t, J = 9.0 Hz H_{2a}), 3.13 (t, J = 9.0 Hz, H_{3a}), 3.10 – 3.06 (m, H_{5d}), 3.01 (t, J = 10.6 Hz, H_{3d}), 2.96 – 2.81 (m, H_{5a} & H_{5b} & H_{5c}), 2.76 – 2.66 (m, H_{3b} & H_{3c}).

^{13}C NMR (151 MHz, D_2O) δ 77.9 (C_{3a}), 77.3 ($C_{2d\beta}$), 76.2 (C_{2a}&C_{2b}&C_{2c}), 75.1 ($C_{1d\beta}$), 74.7 ($C_{2d\alpha}$), 72.9 (C_{4a}), 72.5 ($C_{1d\alpha}$), 70.9 (C_{4b}), 70.8 ($C_{4d\beta}$), 70.6 (C_{4c}&$C_{4d\alpha}$), 60.6 (C_{6b} & C_{6c}), 60.5 (C_{6a}), 60.0 (C_{3c}), 59.3 (C_{3b}), 59.2 ($C_{3d\beta}$), 54.5 ($C_{3d\alpha}$), 50.7 (C_{1b}) 50.6 (C_{5b} & C_{5c}), 50.4 (C_{1c}), 48.8 (C_{1a}), 48.6 (C_{5a}), 48.1 ($C_{5d\beta}$), 43.5 ($C_{5d\alpha}$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{24}H_{42}O_{14}NaS_7$ 801.0517, found 801.0518.

Inhibition of anti-CR3-FITC antibody staining of human neutrophils and of anti-Dectin 1-FITC antibody staining of mouse macrophages.

For fluorescent staining, anti-CR3-FITC antibodies (MN-41 donated by Drs. Allison Eddy and Alfred Michael of the University of Minnesota, Minneapolis, MN, and rat anti Mouse Dectin-1 antibody labeled with FITC (purchased from AbD Serotec, Raleigh, NC) were employed. Either human neutrophils or mouse peritoneal macrophages were incubated with 0.1 μg.mL^{-1} of tested samples for 0.5 h on ice and washed. Subsequently, the cells were stained with antibodies on ice using standard techniques. After centrifugation of cells through a 3 mL cushion of 12% BSA in PBS, the cells were re-suspended in PBS containing 1% BSA and 10 mM sodium azide. Cell cytometry was performed with a Becton Dickinson-LSRII instrument. The inhibition of CR3 receptor and Dectin-1 receptor staining was calculated as described.[37]

Stimulation of phagocytosis.

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier.[82 8287]Human cells (cell line RAW 264) were incubated *in vitro* with 10 μg.mL^{-1} of tested samples for 24 h at 37 °C. After washing, 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8/mL) was added. The test tubes were incubated at 37 °C for 1 h, with intermittent shaking. Smears were stained with Wright stain. Cells with three or more HEMA particles were considered positive. The insoluble glucan Glucan #300 used as comparison standard was obtained from Yeast-derived insoluble Glucan #300 (>85% dry w/w basis) was purchased from Transfer Point (Columbia, SC, USA). This glucan contains 96% carbohydrates and 2.1% proteins. Neutral sugar analysis confirmed 91.3% glucose and 8% mannose.

Stimulation of pinocytosis.

Stimulation of pinocytosis was determined spectrophotometrically as described.[81]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

We thank the NIH (GM62160) for support of this work and acknowledge the NSF (MRI-084043) for funds in support of the purchase of the 600 MHz NMR spectrometer in the Lumigen Instrument Center at Wayne State University.

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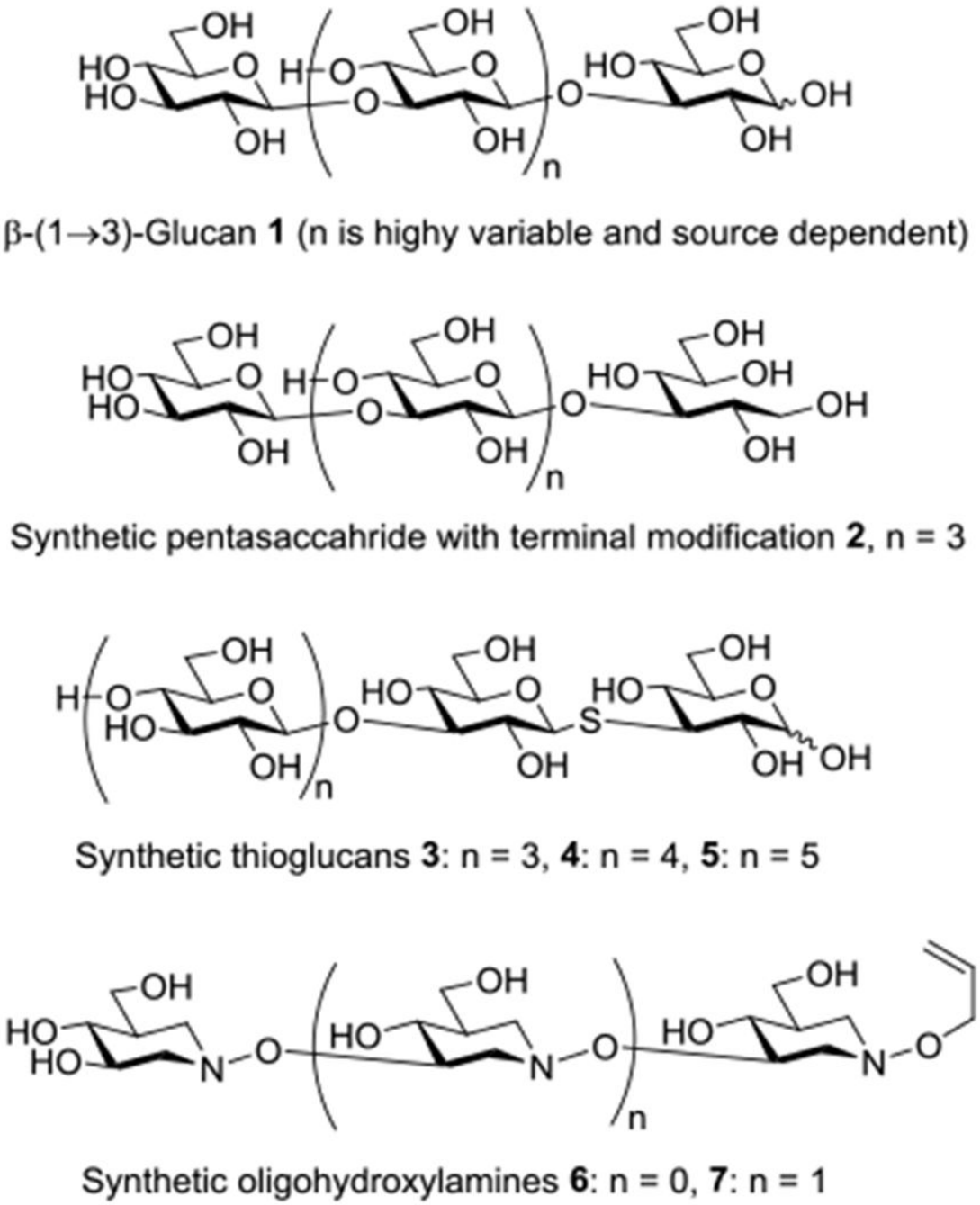


Figure 1.
Structures of β-(1→3)-glucan and synthetic analogs

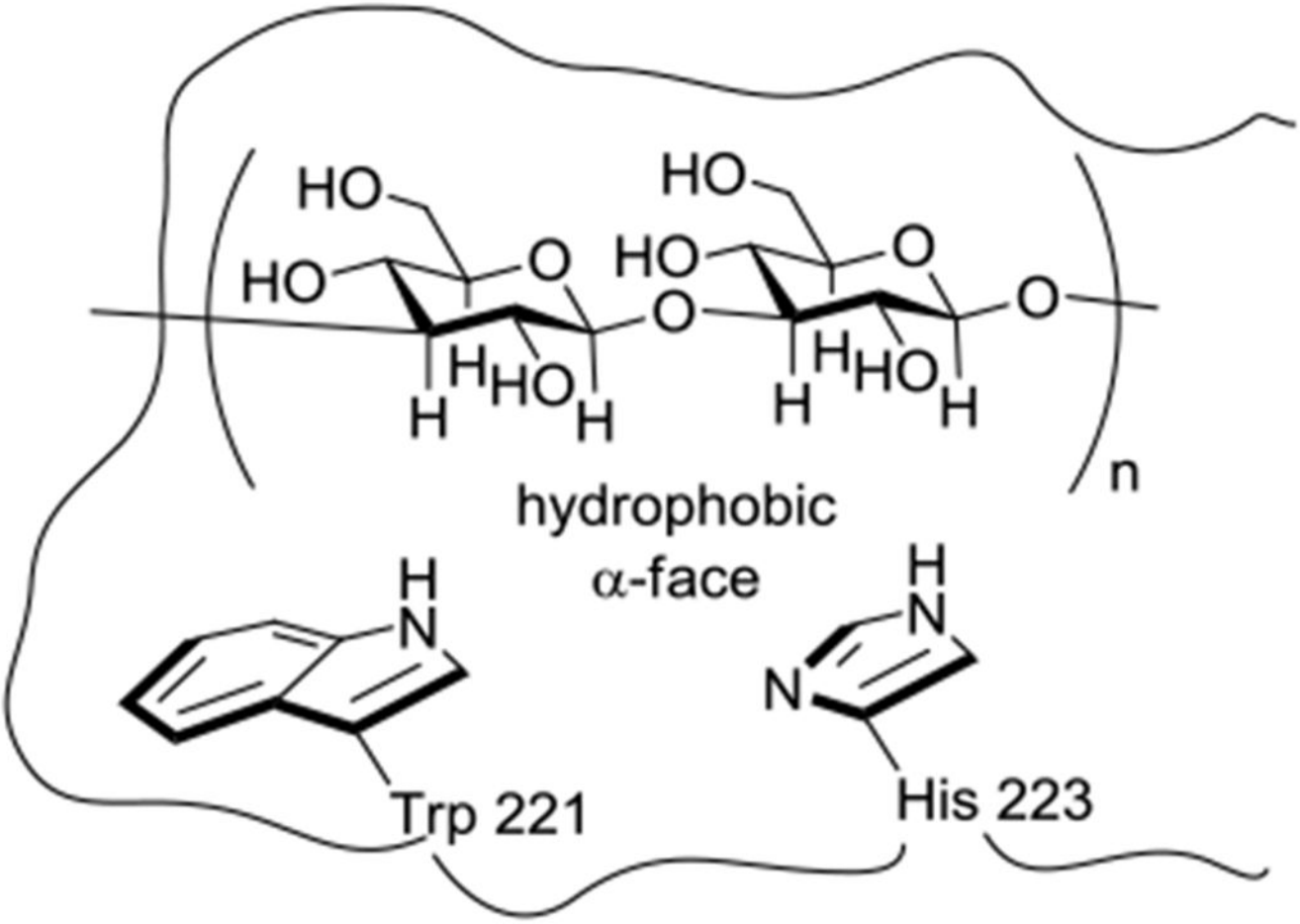


Figure 2.

Schematic representation of the hydrophobic α-face of a disaccharide unit of a β-(1→3)-glucan in complex with the hydrophobic binding pocket of the Dectin-1 lectin domain

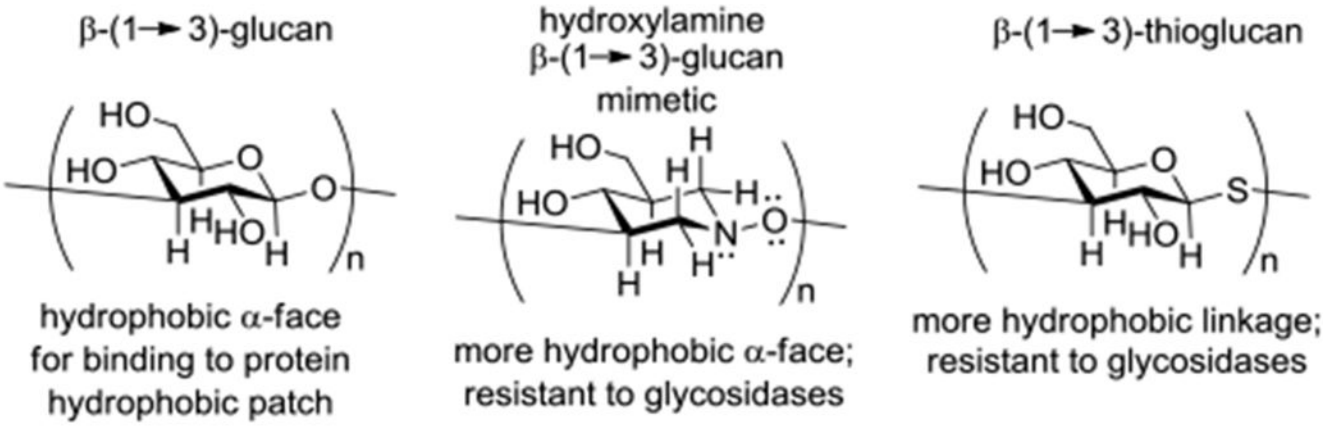


Figure 3.

Hydrophobic α-faces of the β-(1→3)-glucan, oligomeric hydroxylamines **6** and **7**, and thioglucans.

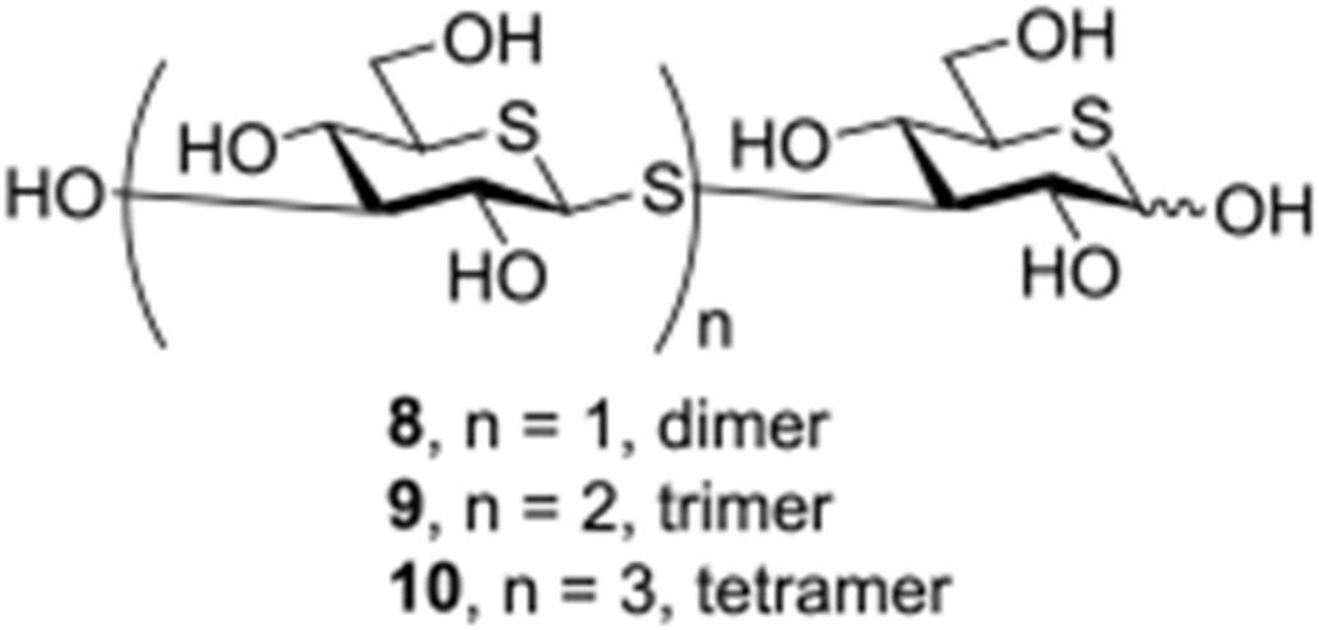
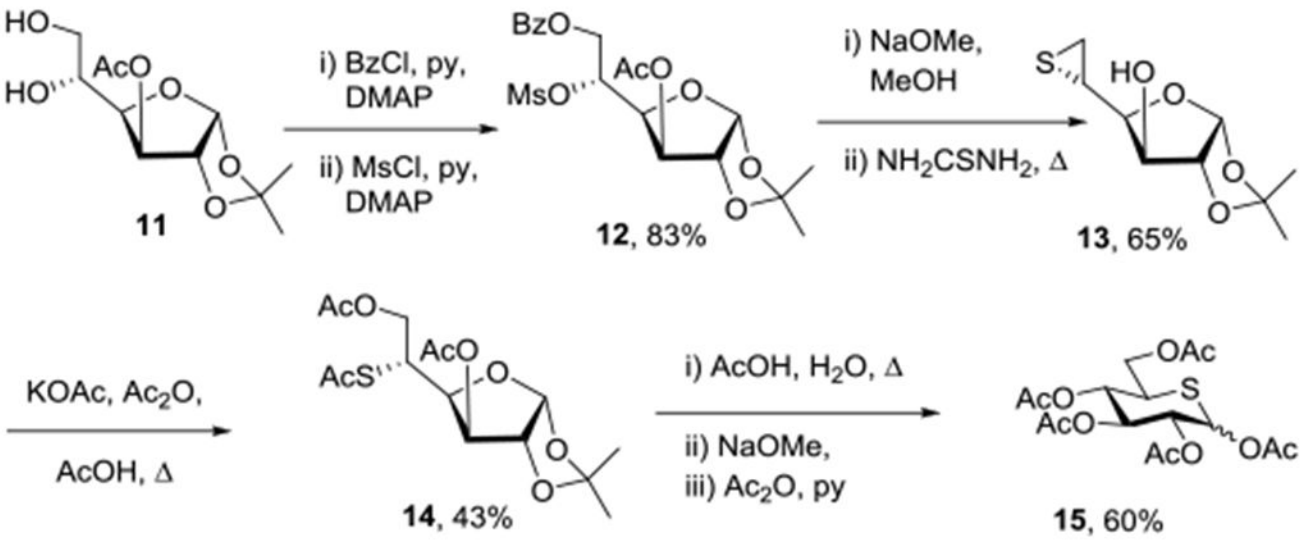
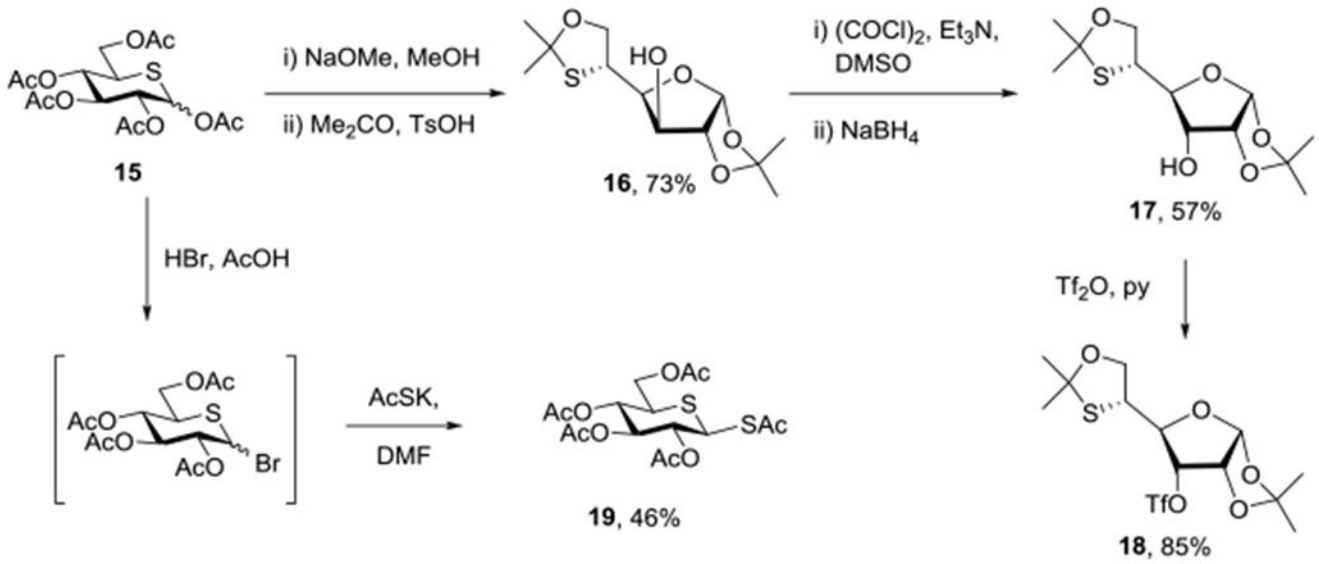


Figure 4.
Di-, tri- and tetrameric dithiaglucose mimics **8**-**10** of β-(1→3)-glucans

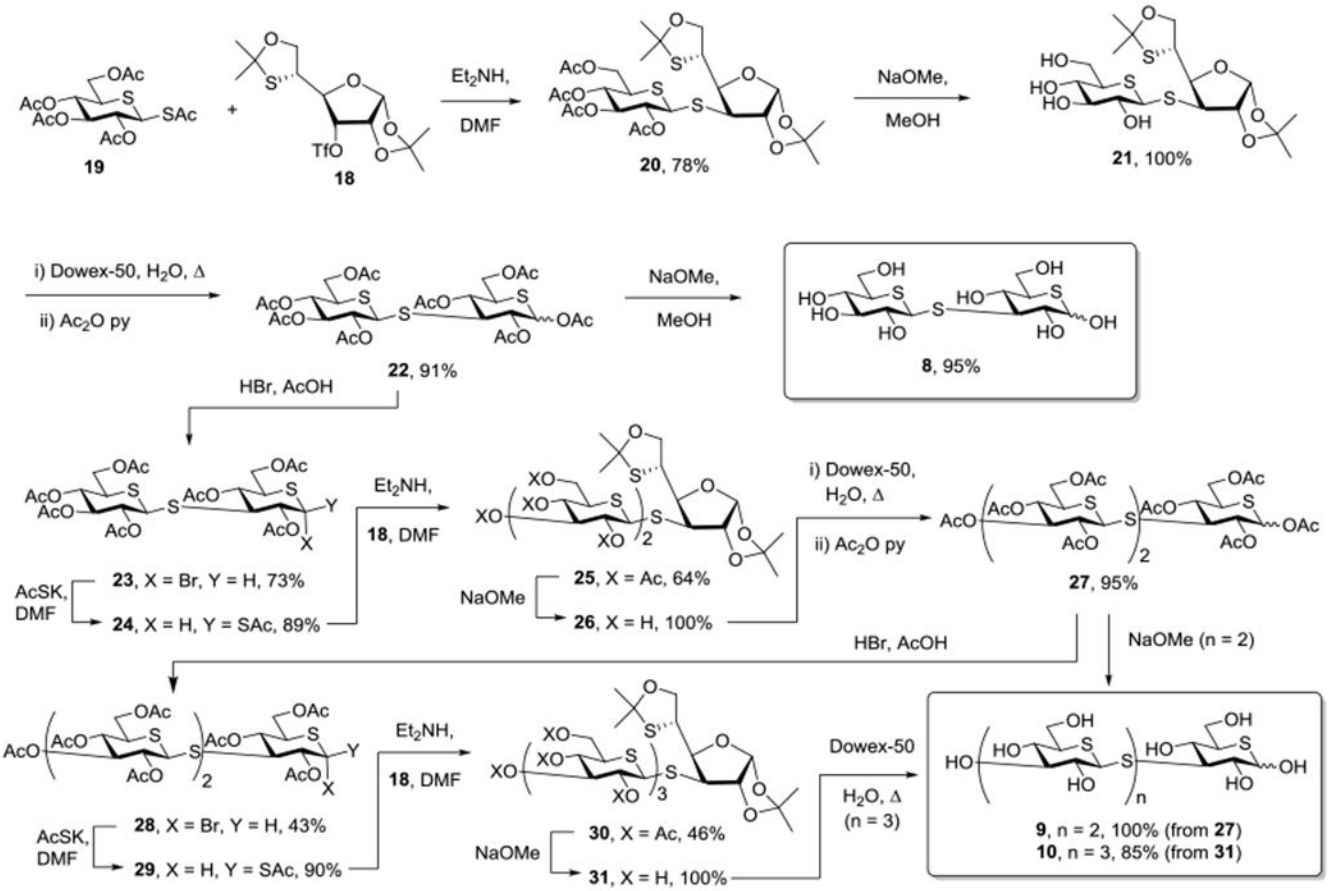


Scheme 1.
Synthesis of Pentaacetyl 5-Thia-D-glucopyranose **15**



Scheme 2.

Synthesis of the Key Building Blocks **18** and **19**



Scheme 3.

Synthesis of 1,5-Dithialaminaribose **8**, triose **9**, and tetraose **10**

Table 1.

Percentage Inhibition of anti-CR3 and anti-Dectin-1-FITC Antibody Staining of Neutrophils and Macrophages by 0.1 μg.mL^{-1} Substrate.

Cmpd	Oligomer No	% Inhibition of anti-CR3-FITC Staining of Human Neutrophils[a]	% Inhibition of anti-Dectin-1-FITC Staining of Mouse Macrophages[a]
8	dimer	20.2 ± 1.7	30.9 ± 3.4
9	trimer	37.3 ± 3.2	42.2 ± 3.8
10	tetramer	21.5 ± 1.4	33.1 ± 2.9
6	dimer	26.4 ± 2.7	28.2 ± 2.9
7	trimer	34.2 ± 3.3	43.1 ± 3.5

[a]Mean ± SD

Table 2.

Percentage Stimulation of Phagocytosis.

Cmpd	Oligomer No	% Stimulation of Phagocytosis (Raw 264 macrophages, 10 μg/mL, 24 h)[a]
8	dimer	8.7 ± 0.7
9	trimer	16.3 ± 0.9
10	tetramer	6.9 ± 0.3
6	dimer	7.8 ± 1.1
7	trimer	16.6 ± 2.0
Glucan #300	-	37.5 ± 2.5

[a]Mean ± SD; significant difference from PBS control (2.1 ± 0.2) at $P < 0.05$ level.

Table 3.

Percentage Stimulation of Pinocytosis.

Cmpd	Oligomer No	Uptake of Neutral red dye by mouse macrophages after 2 h, (ng/1 × 10^5 cells)[a]
8	dimer	7.7 ± 1.61
9	trimer	12.8 ± 1.72
10	tetramer	3.37 ± 0.36
Glucan #300	-	38.9 ± 2.81

[a]Mean ± SD; significant difference from PBS control (1.77 ± 0.11) at $P < 0.05$ level.