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Stereocontrolled synthesis of sulfur-linked analogues of the branched tetrasaccharide repeating-unit of the immunostimulant polysaccharide schizophyllan and of its β-(1 → 3)-branched, β-(1 → 6)-linked isomer [1]

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Abstract

The branched, sulfur-linked tetrasaccharide *S*-(β-D-glucopyranosyl)-(1 → 3)-*S*-[(6-*S*-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1 → 3)-*S*-3-thio-D-glucopyranose (**9**) has been conveniently prepared by S_N2 displacement of the triflate group in 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose with the sodium salt of 2,4-di-*O*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,3,6-trithio-β-D-glucopyranose (**5**). Conversely, reaction of the sodium salt of **5** with 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranose afforded the positional isomer *S*-(β-D-glucopyranosyl)-(1 → 6)-*S*-[(3-*S*-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1 → 6)-*S*-6-thio-D-glucopyranose (**12**).

Keywords: Sulfur-linked thiooligosaccharides; Schizophyllan tetrasaccharide repeating-unit thio analogue; Immunostimulant polysaccharide repeating-unit thio analogue; Phytoalexin elicitor repeating-unit thio analogue

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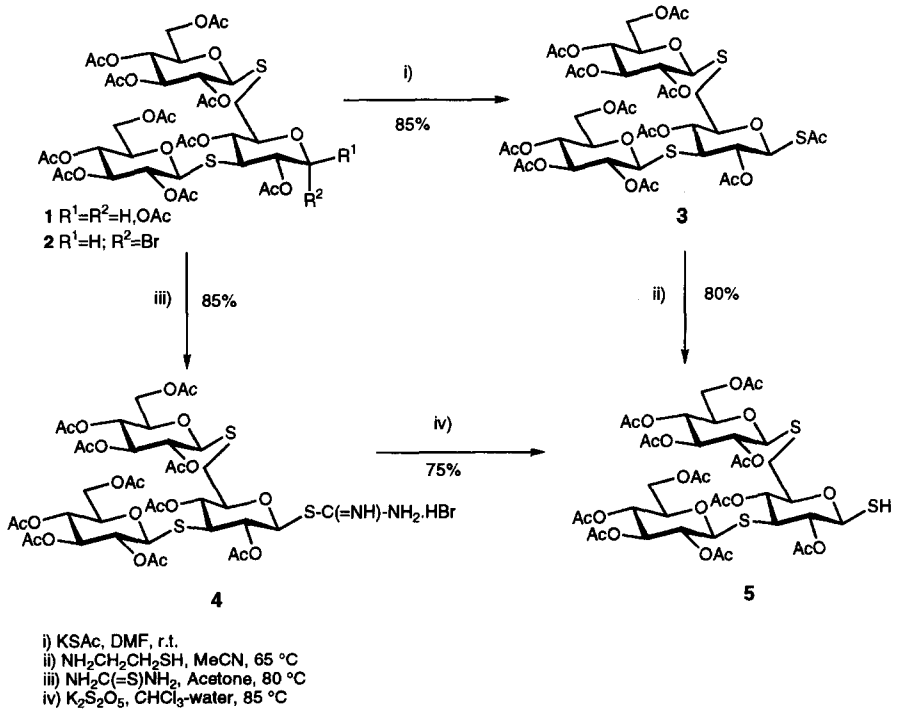
1. Introduction

Several fungal (1 → 3)-linked β-D-glucopyranans substituted at O-6 by single-unit β-D-glucopyranosyl side-chains are long known to display host-mediated antitumor activity [2]. Lentinan, from *Lentinus edodes*, is a β-(1 → 3)-D-glucan with two branches for every five D-glucopyranosyl residues [3]. Schizophyllan, from *Schizophyllum commune*, has a main chain of β-(1 → 3)-D-glucopyranosyl units with every third unit carrying a β-(1 → 6)-D-glucopyranosyl group [4]. The latter repeating-unit has been also found in scleroglucan from *Sclerotium glucanicum* [5], in tylopilan (from *Tylopilus felleus*) [6], in grifolan-7N (from *Grifola frondosa*) [7], and in HA β-glucan (from *Pleurotus ostreatus* (Fr.) Quél.) [8]. Both lentinan and schizophyllan have been registered as anticancer drugs in Japan and clinical effectiveness was observed in patients with lung cervical and gastric cancers. The effect of these antitumoral polysaccharides on tumor systems seems to be correlated with an activation of macrophages and T-lymphocytes as well as an enhancement of interferon production [9]. In addition, fungal β-glucans also modulate macrophage release of cytokines in response to bacterial lipopolysaccharide, preventing the adult respiratory distress syndrome [10]. Furthermore, isomeric branched β-(1 → 3), β-(1 → 6)-linked glucans are known as elicitors of phytoalexins in plants [11].



Scheme 1.

Table 1
^{1}H NMR data (400 MHz, $CDCl_3$) for thiooligosaccharides **2**, **3**, **5**, **7**, **8**α, **8**β, and **11**

	Unit	Chemical shifts (δ)							Coupling constants (Hz)						
		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
2	I	6.56d	4.75dd	3.29t	4.87dd	4.16ddd	2.85dd	2.80dd	3.6	11.3	11.1	9.7	3.4	6.9	14.0
	II	4.74d	4.88dd	5.17t	5.04t	3.67ddd	4.10dd	4.23dd	10.2	9.0	9.2	9.7	2.1	4.3	12.4
	III	4.53d	4.93dd	5.17t	5.03t	3.72ddd	4.09dd	4.22dd	10.1	9.3	9.2	9.7	2.1	4.3	12.4
3	I	5.15d	5.08t	3.02t	4.69dd	3.78ddd	2.78dd	2.65dd	10.4	10.4	10.4	9.6	8.5	2.3	14.5
	II	4.73d	4.87dd	5.14t	5.03t	3.69ddd	4.13dd	4.26dd	10.2	9.2	9.2	9.2	2.4	4.7	12.4
	III	4.63d	4.91dd	5.11t	5.05t	3.59ddd	4.11dd	4.21dd	10.2	9.2	9.2	9.2	2.4	4.7	12.2
5	I	4.40t	4.93dd	2.92t	4.67dd	3.62ddd	2.71dd	2.79dd	9.7	10.7	10.8	9.4	3.0	8.0	14.2
	II	4.68d	4.95dd	5.16t	5.06dd	3.69ddd	4.12dd	4.20dd	10.1	9.2	9.3	5.9	2.3	4.6	12.4
	III	4.62d	4.86dd	5.14t	5.04dd	3.67ddd	4.14dd	4.24dd	10.2	9.1	9.3	5.9	2.5	4.6	12.3
7	I	5.87d	4.92d	3.41d	3.97dd	4.26ddd	4.06–4.22dd		3.6	0	4.0	8.7	2.0	5.6	[a]
	II	4.59d	4.90dd	2.97t	4.60dd	3.65ddd	2.75dd		10.1	10.9	10.7	8.2	[a]	5.4	14.0
	III	4.59d	4.94dd	5.12t	5.04dd	3.65ddd	4.06–4.22dd		10.1	9.1	9.2	5.9	2.3	4.6	12.4
	IV	4.45d	4.84dd	5.15t	5.06dd	3.65ddd	4.06–4.22dd		10.0	9.5	9.2	5.9	2.5	4.6	12.3
8α	I	6.22d	5.08dd	3.21t	4.96dd	4.01ddd	4.04dd	4.16dd	3.5	11.3	11.3	9.9	2.4	4.8	12.5
	II	4.64d	4.83dd	2.95t	4.69dd	3.63ddd	2.75dd	2.84dd	9.6	10.5	10.5	9.6	2.5	4.8	13.6
	III	4.59d	4.82dd	5.21t	5.02dd	[a]	4.07–4.23dd		10.1	9.5	9.5	[a]	[a]	[a]	[a]
	IV	4.50d	4.95dd	5.16t	4.95dd	3.67ddd	4.07–4.23dd		9.9	9.4	9.4	[a]	[a]	[a]	[a]
8β	I	5.60d	5.10dd	3.05t	4.89dd	3.84ddd	4.04dd	4.21dd	7.7	10.4	10.4	9.6	2.4	4.9	12.4
	II	4.53d	4.83dd	2.92t	4.66dd	3.61ddd	2.76dd	2.81dd	9.9	10.5	10.6	9.9	2.5	4.8	13.6
	III	4.58d	4.82dd	5.12t	5.02dd	[a]	[a]	[a]	10.1	9.5	9.5	[a]	[a]	[a]	[a]
	IV	4.50d	4.95dd	5.16t	4.95dd	[a]	[a]	[a]	9.9	9.4	9.4	[a]	[a]	[a]	[a]
11	I	5.68d	5.09dd	5.22t	4.94t	3.85ddd	2.78dd	2.81dd	8.2	9.6	9.6	9.5	3.8	9.6	10.3
	II	4.54d	4.91t	3.00t	4.66dd	3.52ddd	2.78dd	2.81dd	10.0	10.1	10.1	9.4	3.8	6.9	10.3
	III	4.65d	4.88dd	5.16t	5.05dd	3.70ddd	4.13dd	4.24dd	10.2	9.8	9.8	9.6	2.5	5.0	12.5
	IV	4.60d	4.97dd	5.19t	5.07dd	3.70ddd	4.14dd	4.25dd	9.8	9.8	9.8	9.6	2.5	5.0	12.5

[a] Not assigned.

Table 2
^{13}C NMR chemical shifts for thiooligosaccharides **2**, **3**, **5**, **7**, **8**, **9**, **11**, and **12**

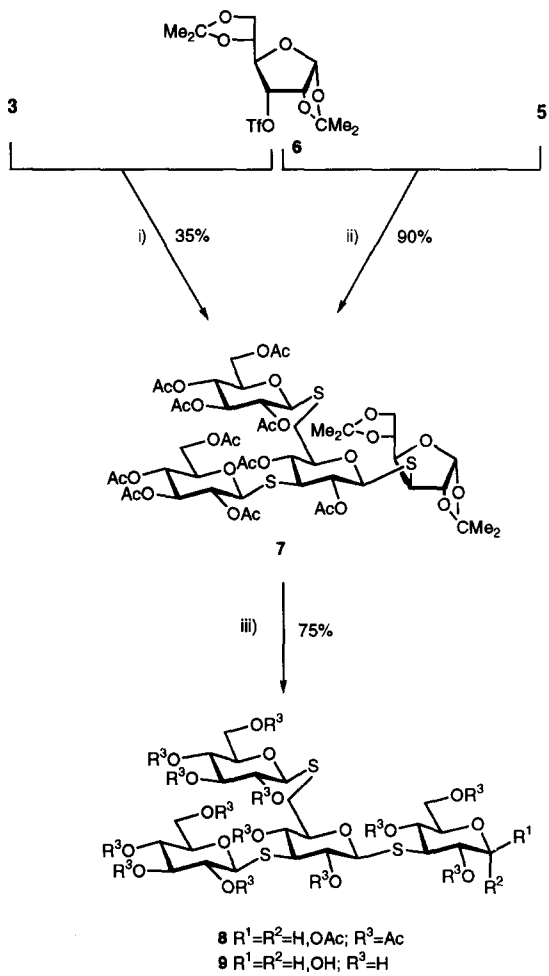
	Unit	Carbon					
		C-1	C-2	C-3	C-4	C-5	C-6
2 [a]	I	88.5	72.2	47.0	68.2	75.3	30.1
	II	83.1	70.4	73.9	68.2	75.9	62.2
	III	83.1	70.1	73.9	68.3	76.1	61.9
3 [a]	I	81.9	75.9	52.2	70.2	82.3	30.9
	II	82.8	70.2	73.8	68.4	75.8	62.0
	III	84.3	70.2	73.8	68.2	75.8	62.0
5 [a]	I	80.4	75.6	52.0	70.0	81.5	30.9
	II	82.9	70.4	73.9	68.4	76.0	61.9
	III	84.4	70.2	73.8	68.2	75.7	62.0
7 [a]	I	105.2	86.3	50.1	80.5	74.0	67.5
	II	84.6	72.1	52.2	70.0	80.2	32.7
	III	84.4	70.3	73.9	68.2	76.2	61.9
	IV	84.4	70.2	73.9	68.2	75.7	61.9
8 [a]	Iα	88.9	70.8	46.0	65.9	71.0	62.1
	Iβ	93.0	72.1	49.5	66.3	75.4	62.1
	IIα	84.2	72.2	52.0	68.4	79.5	31.5
	IIβ	85.7	72.3	52.1	68.5	80.2	31.3
	III	83.2	70.1	73.8	68.2	76.1	62.1
	IV	84.2	70.1	73.8	68.2	75.7	62.1
9 [b]	Iα	92.0	[c]	53.2	[c]	[c]	[c]
	Iβ	97.4	[c]	54.4	[c]	[c]	[c]
	II	84.5	[c]	55.9	[c]	[c]	32.7
	III	86.5	[c]	[c]	[c]	[c]	[c]
	IV	85.9	[c]	[c]	[c]	[c]	[c]
11 [a]	I	91.7	70.4	72.6	71.2	76.0	30.6
	II	84.6	71.9	51.9	69.9	80.3	31.0
	III	84.4	70.1	73.8	68.3	76.0 [d]	61.8
	IV	82.9	70.1	73.8	68.3	75.7 [d]	61.8
12 [b]	Iα	92.9	71.6	74.4	71.4	81.8	32.2
	Iβ	96.0	72.7	75.7	72.2	81.5	32.2
	II	88.2	72.7	55.8	69.8	81.5	32.2
	III	84.4	72.7	77.2	69.4	79.8	60.8
	IV	86.1	72.7	77.2	69.4	79.8	60.8

[a] At 50 MHz, in $CDCl_3$.
[b] At 50 MHz, in D_2O.
[c] Not assigned.
[d] Assignments may have to be reversed.

In order to obtain structural analogues for both types of repeating-units, which may be of interest in eliciting such cell-mediated immune response and simultaneously present an enhanced resistance to enzymatic inactivation [12], the two isomeric sulfur-linked thiotetraoligosaccharides **9** and **12** have been prepared.

2. Results and discussion

The general strategy for the synthesis of thiooligosaccharides, which involves the S_N2 displacement of a non-anomeric leaving-group (sulfonate or halide) by an activated



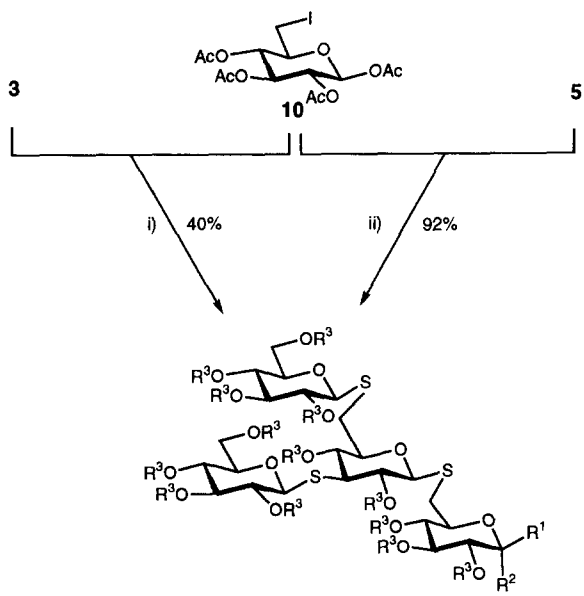
i) Na, MeOH/ DMF, r.t.
ii) NaH, THF/ DMF, r.t.
iii) 90% TFA-H_2O/ 1:1 Ac_2O-pyridine

Scheme 2.

1-thioglycosyl group [1,12], was followed for the preparation of both thiotetraoligosaccharides **9** and **12**.

The preparation of the 1-thio-3^1,6^1-dithiotrisaccharide precursor **5** from 1,2,4-tri-*O*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-α,β-D-glucopyranose (**1**) [1] was accomplished by two alternative approaches (Scheme 1). Reaction of the α-glycosyl bromide **2** with potassium thioacetate in *N*,*N*-dimethylformamide resulted in the crystalline β-thioacetate **3** in 85% yield, which was selectively *S*-deacetylated with 2-aminoethanethiol in acetonitrile [13] to afford **5**. Alternatively, the thiol **5** was obtained by alkaline hydrolysis of the *S*-alkylthiouronium salt **4**, resulting from the treatment of the α-glycosyl bromide **2** with thiourea in acetone. Both procedures afforded the trisaccharide thiol **5** in an overall 65% yield.

Trisaccharide derivatives **2** and **3** were characterized by their quasimolecular ion $[M + Na]^+$ at m/z 1042 and m/z 1037 in FABMS. The values of $J_{1,2}$ (Table 1) for H-1^1 (3.6 and 10.4 Hz, respectively) confirmed the respective α- and β-anomeric configuration for **2** and **3**, while the presence of the C-1 thioacetate in **3** was confirmed by the signal at 2.40 ppm in ^{1}H NMR and the expected shielding for the signal of C-1^1 at 81.9 ppm as compared with C-1^1 of **2** (δ 88.5) in ^{13}C NMR (Table 2). Compound **5** showed the expected $[M + Na]^+$ quasimolecular ion at m/z 995. The ^{1}H NMR spectrum (Table 1) showed a doublet at δ 2.12, attributable to the SH group and a



11 R^1=OAc; R^2=H; R^3=Ac
12 R^1=R^2=H,OH; R^3=H

i) Na, MeOH/ DMF, r.t.
ii) NaH, THF/ DMF, r.t.

Scheme 3.

triplet at δ 4.4 for H-1 with $J_{1,2}$ 9.7 Hz, in agreement with a β-anomeric configuration. In the ^{13}C NMR spectrum (Table 2), a high-field signal for C-1^{I} (δ 80.4) confirmed the presence of the sulfur atom at the anomeric position.

The protected trithiotetrasaccharide **7** has been obtained by two pathways which differ in the activation procedure of the corresponding 1-thio derivative precursor (Scheme 2). The most successful approach involved the reaction of the sodium salt of **5**, prepared by treatment with sodium hydride in dry tetrahydrofuran, with 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose (**6**) [14] in *N,N*-dimethylformamide, which afforded **7** in 90% yield. Compound **7** was also obtained when the 1-*S*-acetate **3** was treated with sodium methoxide and the resulting 1-thiolate allowed to react with the triflate derivative **6** in *N,N*-dimethylformamide giving, after acetylation, **7** in 35% yield. The FABMS spectrum of **7** gave the expected $[M + Na]^+$ quasimolecular ion at *m/z* 1237. The structure of the thiooligosaccharide was also confirmed by its ^1H and ^{13}C NMR spectra (Tables 1 and 2) which showed high-field chemical shifts for C-3^{I}, C-3^{II}, C-6^{II}, and H-3^{I}, H-3^{II}, H-6^{II}. The $J_{1,2}$ value for H-1^{II} was in agreement with the expected β-D configuration.

Deacetonation of **7** with 90% aq trifluoroacetic acid and subsequent acetylation afforded **8** (75%) as an 1:1 anomeric mixture. Zemplén *O*-deacetylation of **8** resulted in **9**, which was purified by LC. Its mass spectrum showed the expected cationized quasimolecular ion at *m/z* 737 in the presence of sodium iodide.

Displacement of the iodine atom in 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranose (**10**) [15] by the sodium salt of **5** in *N,N*-dimethylformamide afforded the protected thiotetrasaccharide **11** in 92% yield. Alternatively, compound **11** was obtained in lower yield (40%) by reaction of the sodium salt resulting from the sodium methoxide treatment of **3** with **10** in *N,N*-dimethylformamide (Scheme 3).

The β configuration at H-1^{II} in **11** was clearly assessed from the large value of $J_{1,2}$ (10.0 Hz). Key ^1H-resonances were assigned by NOE experiments. Zemplén deacetylation of **11** afforded **12**, which was purified by LC and characterized by its ^{13}C NMR and FABMS data (Table 2).

3. Experimental

General methods.—The methods described in ref. [1] were followed.

*2,4-Di-*O*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-α-D-glucopyranosyl bromide* (**2**).—To a solution of 1,2,4-tri-*O*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-α,β-D-glucopyranose (**1**) [1] (0.2 g, 0.19 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added commercial 33% HBr in AcOH (0.28 mL, 12 eq). After 2 h at room temperature, TLC (4:1 EtOAc–hexane) showed complete conversion of the starting peracetate **1**. Toluene was added (50 mL), and the solution was concentrated under reduced pressure to give **2** (0.185 g, 91%), mp 173–174 °C (from ether), $[\alpha]_D^{20}$ +22° (*c* 0.45, $CHCl_3$); NMR: ^1H (400 MHz, $CDCl_3$): Table 1; ^{13}C (50 MHz, $CDCl_3$): Table 2; FABMS: *m/z* 1042 (20, $[M + Na]^+$) and 331 (100, acetylated glucopyranosyl cation). Anal. Calcd for $C_{38}H_{51}O_{23}S_2Br$: C, 44.75; H, 5.00; S, 6.28. Found: C, 44.80; H, 4.82; S, 6.60.

*2,4-Di-*O*-acetyl-1-*S*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,3,6-trithio-β-D-glucopyranose* (**3**).—Potassium thioacetate (35 mg, 0.3 mmol) was

added to a solution of **2** (0.1 g, 0.1 mmol) in dry DMF (3 mL) and the mixture was stirred at room temperature for 3 h, and then concentrated. The resulting residue was dissolved in CH_2Cl_2 (5 mL), washed with water (2 × 5 mL), dried ($MgSO_4$), and evaporated to yield **3** (86 mg, 85%) which crystallized from aq EtOH, mp 233–234 °C, $[\alpha]_D^{20}$ −40° (*c* 0.17, $CHCl_3$); NMR: ^1H (400 MHz, $CDCl_3$): Table 1 and δ 2.40 (s, 3 H, SAc); ^{13}C (50 MHz, $CDCl_3$): Table 2 and δ 30.4 (SAc); FABMS: *m/z* 1037 (30, $[M + Na]^+$) and 331 (30, acetylated glucopyranosyl cation). Anal. Calcd for $C_{40}H_{54}O_{24}S_3 \cdot 3H_2O$: C, 44.94; H, 5.62; S, 8.99. Found: C, 44.81; H, 5.39; S, 8.71.

2,4-Di-O-*acetyl-3,6-di*-S-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl)-1,3,6-trithio*-β-D-*glucopyranosylisothiouronium bromide* (**4**).—Thiourea (8 mg, 1.1 equiv) was added to a solution of **2** (0.1 g, 0.1 mmol) in acetone (10 mL) and the mixture was stirred at 80 °C for 1 h under N_2. Evaporation of the solvent gave **4** (90 mg, 85%) as an amorphous hygroscopic powder, which was dried and used in the following step without further characterization.

2,4-Di-O-*acetyl-3,6-di*-S-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl)-1,3,6-trithio*-β-D-*glucopyranose* (**5**).—(a) A mixture of the *S*-glycosylthiouronium salt **4** (100 mg, 0.09 mmol) and potassium pyrosulfite (22 mg, 0.1 mmol) in $CHCl_3$–water (3:2, 25 mL) was stirred at 85 °C for 15 min. The two phases were then separated and the organic layer washed with water (3 × 10 mL), dried ($MgSO_4$), and concentrated to afford **5** (68 mg, 75%) as a semicrystalline powder, mp 233–234 °C, $[\alpha]_D^{20}$ −24.2° (*c* 0.2, $CHCl_3$); NMR: ^1H (400 MHz, $CDCl_3$): Table 1 and δ 2.12 (d, 1 H, $J_{1,SH}$ 9.8 Hz, SH); ^{13}C (50 MHz, $CDCl_3$): Table 2; FABMS: *m/z* 995 (20, $[M + Na]^+$) and 331 (20, acetylated glucopyranosyl cation). Anal. Calcd for $C_{38}H_{52}O_{23}S_3$: C, 46.91; H, 5.35; S, 9.88. Found: C, 46.83; H, 5.29; S, 9.35.

(b) To a solution of **3** (0.11 g, 0.1 mmol) in MeCN (5 mL) was added 2-aminoethanethiol (8.3 mg, 0.11 mmol). The mixture was stirred at 65 °C for 10 min under N_2, then concentrated and extracted with Et_2O (3 × 5 mL). Concentration of the ethereal phase and column chromatography (2:3 petroleum ether–EtOAc) of the resulting syrupy residue yielded **5** (78 mg, 80%), identical in all respects with the compound prepared by method (a).

1,2:5,6-Di-O-*isopropylidene-3*-S-*[2,4-di*-O-*acetyl-3,6-di*-S-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl)-3,6-dithio*-β-D-*glucopyranosyl]-3-thio*-α-D-*glucofuranose* (**7**).—(a) Sodium hydride (15 mg, 0.5 mmol) was added to a solution of the thiol **5** (400 mg, 0.4 mmol) in dry THF (10 mL) at 0 °C. The suspension was stirred under N_2 until hydrogen evolution had ceased. The resulting solution was then concentrated under reduced pressure, and the amorphous residue dissolved in DMF (5 mL). To this stirred solution, 1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluoromethylsulfonyl-α-D-allofuranose (**6**) [14] (300 mg, 0.65 mmol) in DMF (5 mL) was added dropwise. After 2 h at room temperature under N_2, the mixture was concentrated. A solution of the residue in CH_2Cl_2 was washed with water, dried (Na_2SO_4), and concentrated. The crude product was purified by column chromatography (1:1 petroleum ether–EtOAc) giving **7** (450 mg, 90%), mp 124–130 °C (from EtOH), $[\alpha]_D^{20}$ −40.0° (*c* 0.15, $CHCl_3$); NMR: ^1H (400 MHz, $CDCl_3$): Table 1; ^{13}C (50 MHz, $CDCl_3$): Table 2; FABMS: *m/z* 1237 (50, $[M + Na]^+$), 939 (10) and 331 (100, acetylated glucopyranosyl cation). Anal. Calcd for $C_{50}H_{70}O_{28}S_3$: C, 49.42; H, 5.81; S, 7.91. Found: C, 49.13; H, 5.97; S, 7.77.

(b) To a solution of **3** (100 mg, 0.1 mmol) in MeOH (8 mL) was added methanolic NaOMe (M, 0.1 mL). The mixture was stirred for 18 h at room temperature, evaporated, and the resulting sodium thiolate dissolved in DMF (5 mL). To this solution was added dropwise **6** (75 mg, 0.2 mmol) in DMF (5 mL). After 18 h at room temperature under N_2, the mixture was concentrated under reduced pressure. A solution of the residue in CH_2Cl_2 was washed with water, dried (Na_2SO_4), and concentrated. The crude product was chromatographed (1:2 petroleum ether–EtOAc) giving **7** (40 mg, 35%), identical in all respects with the compound prepared in (a).

1,2,4,6-Tetra-O-*acetyl-3*-S-*[2,4-di*-O-*acetyl-3,6-di*-S-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)-3,6-dithio-β*-D-*glucopyranosyl]-3-thio-α,β*-D-*glucopyranose* (**8**).—To a solution of the acylated thiosaccharide **7** (100 mg, 0.08 mmol) in MeOH (5 mL) was added methanolic NaOMe (M, 0.1 mL). The mixture was stirred for 2 h at room temperature, then demineralized with Amberlite IRN-77 (H^+) cation-exchange resin and filtered. A solution of the residue in 90% TFA–water (10 mL) was kept at 30 °C under reduced pressure (15 mmHg) until the distillation of acetone ceased (10 min). The solvent was then eliminated under vacuum and the resulting syrupy residue was acetylated (1:1 Ac_2O–pyridine, 10 mL) yielding **8** (80 mg, 75%), which crystallized from EtOH; $\alpha:\beta$ ratio 1:1 (from NMR integration of H-1 signals), mp 123–128 °C, $[\alpha]_D^{20}$ 0° (*c* 0.2, $CHCl_3$); NMR: ^1H (400 MHz, $CDCl_3$): Table 1; ^{13}C (50 MHz, $CDCl_3$): Table 2; FABMS: *m/z* 1325 (20, $[M + Na]^+$) and 331 (50, acetylated glucopyranosyl cation). Anal. Calcd for $C_{52}H_{70}O_{32}S_3$: C, 47.93; H, 5.38; S, 7.37. Found: C, 47.76; H, 5.46; S, 7.21.

S-*(β*-D-*Glucopyranosyl)-(1 → 3)*-S-*[(6*-S-*β*-D-*glucopyranosyl)-3,6-dithio-β*-D-*glucopyranosyl]-(1 → 3)*-S-*3-thio-α,β*-D-*glucopyranose* (**9**).—Zemplén *O*-deacetylation of **8** (100 mg, 0.08 mmol) with methanolic NaOMe (1 M, 0.1 mL), followed by preparative LC (65:35 MeCN–water) and freeze-drying from an aq solution gave **9** as a white hygroscopic powder (51 mg, 93%); $[\alpha]_D^{20}$ −18.7° (*c* 2.1, water); ^{13}C NMR (50 MHz, D_2O); Table 2; FABMS: *m/z* 737 (60, $[M + Na]^+$).

1,2,3,4-Tetra-O-*acetyl-6*-S-*[2,4-di*-O-*acetyl-3,6-di*-S-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)-3,6-dithio-β*-D-*glucopyranosyl]-6-thio-β*-D-*glucopyranose* (**11**).—(a) Sodium hydride (18 mg, 0.6 mmol) was added to a solution of **5** (486 mg, 0.5 mmol) in dry THF (5 mL) at room temperature. The suspension was stirred under N_2 until hydrogen evolution had ceased. The resulting solution was then concentrated under reduced pressure, and the residue dissolved in DMF (5 mL). To this solution, 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-*β*-D-glucopyranose (**10**) [15] (230 mg, 0.5 mmol) was added and the mixture was stirred for 2 h at room temperature under N_2, then concentrated under reduced pressure. A solution of the residue in CH_2Cl_2 (30 mL) was washed with water (2 × 10 mL), dried (Na_2SO_4), and concentrated. The crude product was chromatographed (1:1 petroleum ether–EtOAc) and crystallized from EtOH giving **11** (360 mg, 92%), mp 152–153 °C, $[\alpha]_D^{20}$ −36.0° (*c* 0.2, $CHCl_3$); NMR: ^1H (400 MHz, $CDCl_3$): Table 1; ^{13}C (50 MHz, $CDCl_3$): Table 2; FABMS: *m/z* 1325 (60, $[M + Na]^+$), 939 (25), and 331 (100, acylated glucopyranosyl cation). Anal. Calcd for $C_{52}H_{70}O_{32}S_3$: C, 47.93; H, 5.38; S, 7.37. Found: C, 47.65; H, 5.45; S, 7.30.

(b) To a solution of **3** (300 mg, 0.36 mmol) in dry MeOH (7 mL) was added methanolic NaOMe (M, 0.39 mL). After being stirred at room temperature for 18 h, the

solution was concentrated under reduced pressure. To the resulting residue in DMF (5 mL) was added **10** [15] (185 mg, 0.39 mmol), and the mixture was stirred for 18 h at room temperature, then evaporated. The dried (P_2O_5) syrupy residue was acetylated (1:1 Ac_2O–pyridine, 15 mL) and purified by column chromatography (1:2 petroleum ether–EtOAc) to give **7** (190 mg, 40%) identical in all respects with the compound prepared in (a).

S-(β-D-Glucopyranosyl)-(1 → 6)-S-[(3-S-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1 → 6)-S-6-thio-α,β-D-glucopyranose (**12**).—Compound **11** (100 mg, 0.08 mmol) in MeOH (5 mL) was stirred with 1 M methanolic NaOMe (0.1 mL) for 2 h at room temperature. The solution was then demineralized with Amberlite IRN-77 (H^+) cation-exchange resin and filtered. Evaporation of the solvent gave an amorphous powder which was purified by preparative LC (65:35 MeCN–water) and freeze-dried from an aq solution to give **12** as a white hygroscopic powder (53 mg, 97%), $[\alpha]_D^{20}$ −15.2° (*c* 3.5, water); ^{13}C NMR (50 MHz, D_2O): Table 2; FABMS: *m/z* 737 (90, $[M + Na]^+$) and 715 (50, $[M + H]^+$).

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