S-Linked Thiomimetics of Phytoalexin-Elicitor-Active, Branched Oligosaccharides, Their Synthesis, Protein-Binding Ability and Phytoalexin-Inducing Activity

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The sulfur-linked pentathiohexasaccharide $3^{I},3^{IV}$-di-β-D-glucopyranosylthiogentiotetraose (**12**) has been prepared by a convergent approach involving the reaction of 1,2,4-tri-*O*-acetyl-6-deoxy-6-iodo-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-β-D-glucopyranose (**10**) with the sodium salt of 2,3,4-tri-*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]-1,6-dithio-β-D-glucopyranose (**4**). A further reaction, involving the sodium salt of the peracetylated β-1-thio derivative of **12** with 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranose (**26**), afforded the homologous sulfur-linked hexathioheptasaccharide $3^{II},3^{V}$-di-β-D-glucopyranosylthiogentiopentaose (**28**). Related sulfur-linked positional isomers $3^{II},3^{IV}$-di-D-β-glucopyranosylthiogentiotetraose (**34**) and $3^{III},3^{V}$-di-β-D-glucopyranosylthiogentiopentaose (**39**) have been prepared using analogous synthetic strategies. Thus, S_N2 displacement of the iodine atom in **10** by 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 afforded a tetrathiopentasaccharide, which resulted in the pentathiohexasaccharide **34** by a sequence of reactions involving the 1-thioglycose **32** in reaction with **26**. The hexathioheptasaccharide **39** was obtained conveniently by the reaction of **26** with the acetylated 1-thio-6^{I}, 3^{II}, 6^{II}, 3^{IV}, 6^{IV}-pentathio derivative **37**, followed by deacylation. The four isomeric pentathiohexa- and hexathioheptasaccharides **12**,**34** and **28**,**39**, respectively, were all found to be active in eliciting phytoalexin accumulation in soybean cotyledon tissue and in binding to a glucan-binding protein of soybean, although to a lesser extent than the corresponding *O*-oligosaccharides, the alternate thiohexa- and thioheptasaccharides **12**,**28** being more active as compared to the geminally branched isomers **34**,**39**.

Introduction

Oligosaccharides which elicit molecular defense mechanisms in plants, otherwise known as oligosaccharins, have attracted much interest in recent years[1] and among these are β-(1→6)-linked glucooligosaccharides with β-(1→3)-linked glucosyl residues. Comparison of the relative ability of several related oligosaccharides to induce phytoalexin accumulation in soybean cotyledons revealed that the hexaglucoside $3^{I},3^{IV}$-di-β-D-glucopyranosylgentiotetraose is the smallest structural component required to elicit such activity. In addition, it was suggested that the structural epitope required to trigger the signal transduction involves a branched β-(1→3),β-(1→6)-trisaccharide at the non-reducing end of the oligosaccharide structure.[1d] Oligosaccharins, however, remain difficult to obtain from natural sources, and progress in this field is highly dependent on the development of efficient methods for the synthesis of both oligosaccharins and their analogs. On the other hand, *S*-linked thio analogs of oligosaccharins might be expected to be less prone to enzymatic inactivation as compared to their *O*-linked natural counterpart.[2] In previous papers,[3][4] we have developed a synthetic methodology which allows access to small thiooligosaccharides related to the structure of phytoalexin elicitor branched glucooligosaccharides. We now report on the preparation, and a preliminary screening of the biological activity, of the *S*-linked pentathio analog **12** of the elicitor-active $3^{I},3^{IV}$-di-β-D-glucopyranosylgentiotetraose, the homologous hexathioheptasaccharide **28**, as well as the isomeric pentathiohexasaccharide **34** and hexathioheptasaccharide **39**; all bear the trisaccharide epitope at the non-reducing end and so represent potentially enzymatically stable phytoalexin-elicitor analogs.

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Results and Discussion

The general stereocontrolled method for the synthesis of thiooligosaccharides involving the S_N2 displacement of a non-anomeric leaving group (triflate or iodide) by an anomeric thiolate[2–4] was followed for the preparation of **12**, **28**, **34** and **39**.

The peracetylated *S*-linked pentathiohexasaccharide $3^{I},3^{IV}$-di-β-D-glucopyranosylthiogentiotetraose (**11**) was obtained in a yield of 83% by the reaction of the sodium 1-

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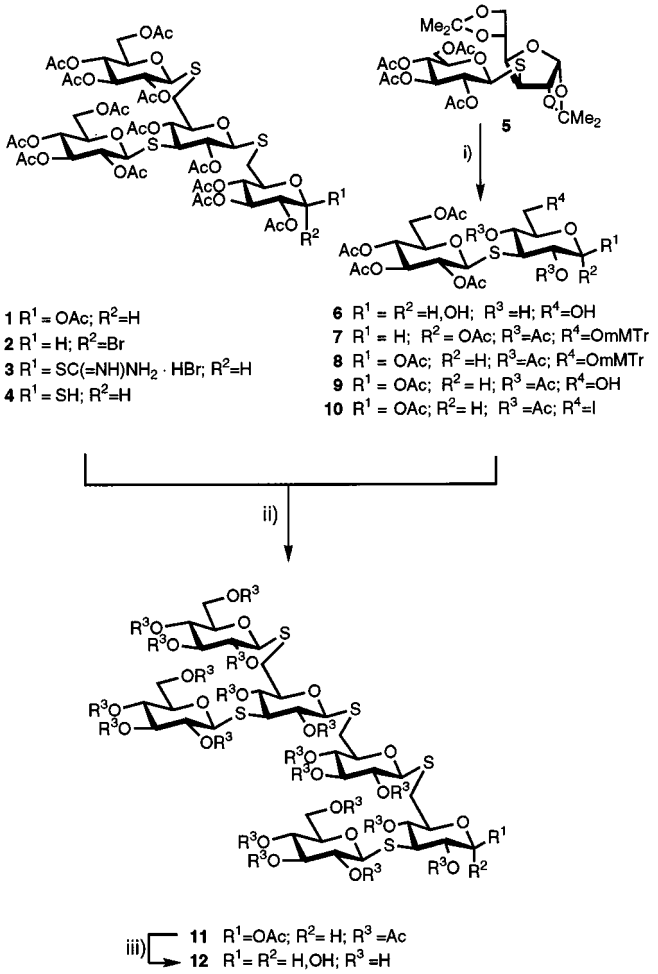
thiolate derivative of the tetrathiotetrasaccharide **4** with the 6^{I}-deoxy-6^{I}-iodo-3-thiolaminaribiose derivative **10** in *N*,*N*-dimethylformamide (see Scheme 1). Preparation of **4** started from 1,2,3,4-tetra-*O*-acetyl-6-*S*-[2,4-di-*O*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-6-thio-β-D-glucopyranose (**1**),[4] which gave the α-D-glycosyl bromide **2** by treatment with 33% hydrogen bromide in acetic acid, and was then transformed into the β-thioglycose derivative **4** by alkaline hydrolysis of the *S*-alkylisothiouronium salt intermediate **3**. The 6-deoxyiodo electrophilic counterpart **10** was prepared from 1,2:5,6-di-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-α-D-glucofuranose (**5**)[3] in four steps. Removal of the isopropylidene groups with aqueous trifluoroacetic acid, treatment with chloro(4-methoxyphenyl)-diphenylmethane and acetylation gave the 6-*O*-methoxytrityl heptaacetates **7** and **8** as a 1:1 α/β-anomeric mixture which was separated by column chromatography. Detritylation of **8** with 80% aqueous acetic acid afforded the β-acetate **9**. The latter was transformed, with a yield of 80%, into the 6-deoxyiodo derivative **10** in a one-pot procedure by treatment with trifluoromethanesulfonic anhydride, in the presence of 2,6-di-*tert*-butyl-4-methylpyridine, and subsequent displacement with tetrabutylammonium iodide of the intermediate triflate.[5] The FAB MS of **10** gave the expected quasimolecular ion at *m*/*z* 785. The presence of the iodine atom was assessed by ^{13}C-NMR spectroscopy (see Table 2) which showed a high-field chemical shift for C-6^{I} ($\delta = 2.8$). Zemplén *O*-deacetylation of **11** yielded the doubly *S*-branched trithiogentiotetraose mimetic **12**, which was submitted to LC and characterized by ^{13}C NMR and FAB MS, the latter showing distinctly the quasimolecular ion $[M + Na]^+$ at *m*/*z* 1093.

Two independent convergent approaches, which proved less satisfactory, were attempted for the synthesis of **12** involving trifluoroacetic acid hydrolysis of the acetal-protected pentathiohexasaccharide intermediate **15**. On one hand, nucleophilic displacement of the iodine atom in 5-*O*-acetyl-6-deoxy-6-iodo-1,2-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucofuranose (**14**)[3] with the sodium salt of the 1-*S*-acetyl-6^{I},3^{II},6^{II}-tetrathiotetrasaccharide **13** in *N*,*N*-dimethylformamide provided **15** in a yield of only 30% (see Scheme 2). The β-thioacetate precursor **13** was obtained in a yield of 65% from the α-glycosyl bromide **2** by reaction with potassium thioacetate in *N*,*N*-dimethylformamide. The FAB MS of **15**, in the presence of sodium iodide, showed the expected quasimolecular ion $[M + Na]^+$ at *m*/*z* 1890. The ^{1}H- and ^{13}C-NMR spectra, which showed the expected high-field chemical shifts for C-1^{II-VI}, C-$3^{I,IV}$, C-$6^{I,III,IV}$, and H-1^{II-VI}, H-$3^{I,IV}$, confirmed the structure.

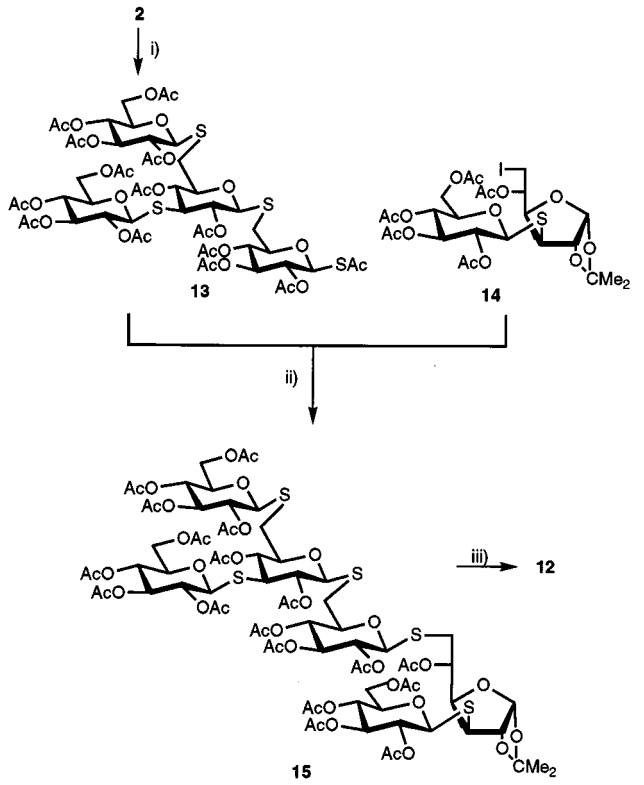
On the other hand, reaction of the sodium salt of the acetylated β-thiotrisaccharide **22**,[3] or of the corresponding hydroxy-free compound obtained from 2,4-di-*O*-acetyl-1-*S*-acetyl-3,6-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,3,6-trithio-β-D-glucopyranose (**21**)[3] by treatment with sodium methoxide in methanol, with the 6^{III}-deoxyiodo-branched trisaccharide **20** in *N*,*N*-dimethylformamide at



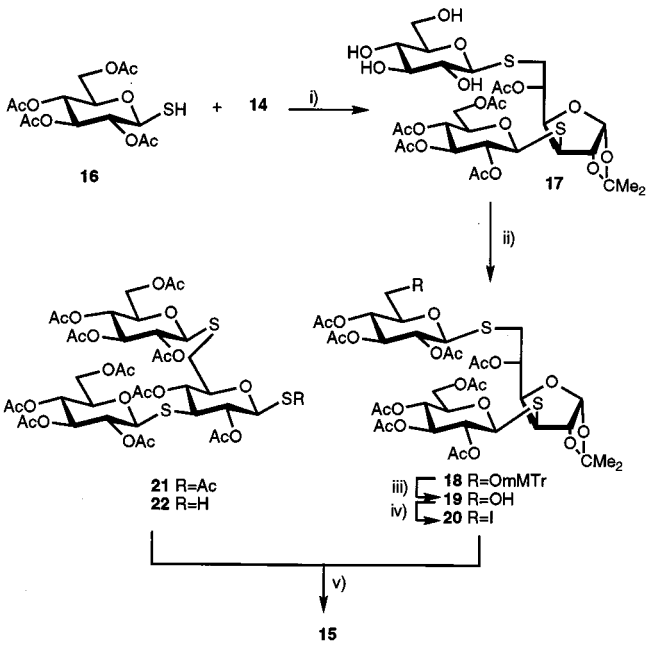
Scheme 1. Synthesis of the branched thiohexasaccharide **12**: i) TFA/H_2O, 9:1, 30°C, 40 min; ii) NaH, THF; DMF, room temp., 1 h, 83%; iii) NaOMe, MeOH, room temp., 50 h, 100%

60−70°C led to the pentathiohexasaccharide **15** in yields of 35% and 20%, respectively (see Scheme 3). The latter trisaccharide building block **20** could be obtained by reaction of the sodium salt of 1-thio-β-D-glucopyranose obtained from **16**,[6] with **14** in *N*,*N*-dimethylformamide at room temp. resulting in the hemiprotected branched dithiotrisaccharide **17** which, after treatment with chloro(4-methoxyphenyl)diphenylmethane in pyridine and acetylation (→ **18**), detritylation with aqueous acetic acid (→ **19**) and subsequent replacement of the C-6^{III} primary hydroxy group by iodine using the procedure described for the preparation of **10**, afforded **20** from **18** in an overall yield of 42%.

The β-pentathiohexasaccharide peracetate **11** was used as starting material in the preparation of the homologous heptathiosaccharide **28** (see Scheme 4). Thus, treatment of **11** with 33% hydrogen bromide in acetic acid (→ **23**) and reaction of the resulting α-glycosyl bromide with thiourea (→ **24**) followed by alkaline hydrolysis with potassium pyrosulfite afforded **25** with an overall yield of 90% based on the β-thioacetate **11**. Reaction of the sodium salt of **25** with 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranose (**26**)[7] in *N*,*N*-dimethylformamide afforded **27** in a yield of

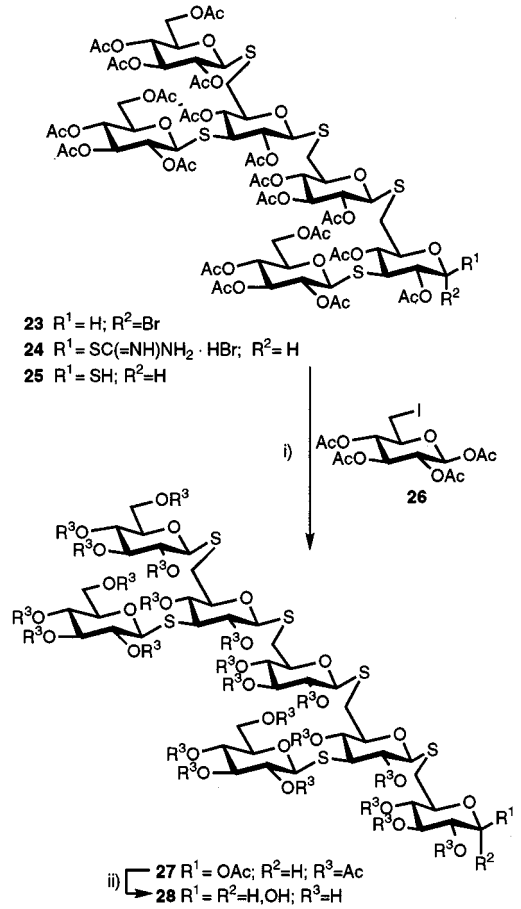


Scheme 2. Alternative synthesis of the branched thiohexasaccharide **12** involving the acetal-protected thiohexasaccharide **15**: i) KSAc, DMF, room temp.; ii) NaOMe, MeOH; DMF, 60°C, 4 h; Ac_2O/pyridine, room temp., 30%; iii) NaOMe, MeOH, room temp., 18 h; TFA/water, 9:1, 10 min, room temp., 85%



Scheme 3. Alternative synthesis of the acetal-protected branched thiohexasaccharide **15** involving thiotrisaccharide precursors **20** and **21**: i) NaOMe, MeOH; DMF, 60°C, 4 h; ii) mMTrCl/pyridine, room temp., 18 h; Ac_2O, room temp., 2 h, 73%.; iii) $MeCO_2H$/water, 4:1, room temp., 2 h; iv) $(CF_3SO_2)_2O$/*t*BuMePyr/CH_2Cl_2, room temp., 45 min; $[Me(CH_2)_3]_4NI$, room temp., 3 h, 45%; v) NaH, THF; DMF, 70°C, 4 h, 20%

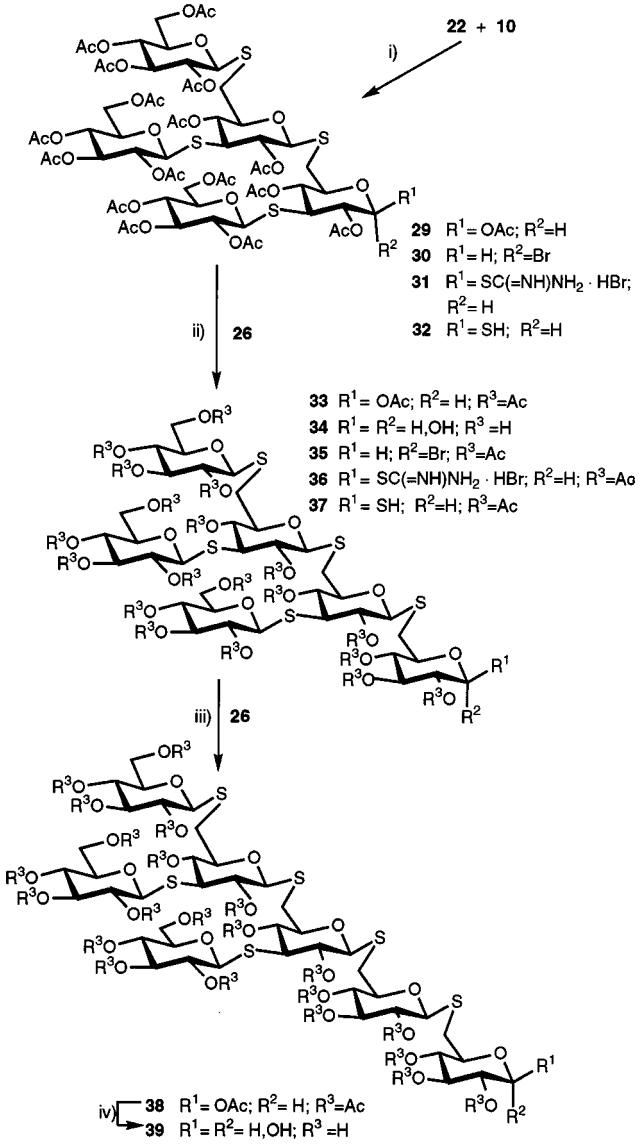
73%, which was quantitatively *O*-deacetylated by the Zemplén procedure to **28**, and unambiguously characterized by ^{13}C-NMR spectroscopy and FAB MS.



Scheme 4. Synthesis of the branched thioheptasaccharide **28**: i) NaH, THF; DMF, room temp., 1 h, 73%; ii) NaOMe, MeOH, room temp., 60 h, 100%

Following this successful approach, which allowed high yielding preparation of branched thiooligosaccharides by stepwise incorporation of 1-thio-β-D-glucopyranosyl residues into block sequences, the isomeric *S*-linked $3^{II},3^{IV}$-di-β-D-glucopyranosylpentathiogentiotetraose **34** and $3^{III},3^{V}$-di-β-D-glucopyranosylhexathiogentiopentaose **39** have been synthesized (see Scheme 5). Reaction of the sodium salt of **22**[3] with the deoxyiodo derivative **10** in *N*,*N*-dimethylformamide at room temp. afforded **29** (88% yield) which was transformed into the corresponding β-1-thio derivative **32**, in an overall yield of 85%, based on **29**. The latter sequence involved conversion of **29** into the α-bromide **30** by reaction with hydrogen bromide in acetic acid, followed by treatment with thiourea and alkaline hydrolysis of the resulting isothiouronium salt **31** to **32**. Further reaction of the sodium salt of **32** with **26** resulted in **33** in a yield of 80%. A similar sequence from **33** to give the β-1-thio derivative **37** followed by reaction with **26** yielded **38** in a yield of 71%. Respective Zemplén *O*-deacetylation of **33** and **38** gave the target thiooligosaccharides **34** and **39**, which were submitted to LC for biological assessment. FAB MS for both molecules, measured in the presence of sodium iodide, showed the ex-

pected intense cationized quasimolecular ion peaks at *m/z* 1093 and 1271, respectively.



Scheme 5. Synthesis of the branched thiohexasaccharide **34**: i) NaH, THF; DMF, room temp., 2 h, 88%; ii) NaH, THF; DMF, room temp., 1 h, 80% and of the thioheptasaccharide **39**; iii) NaH, THF; DMF, room temp., 2 h, 71%; iv) NaOMe, MeOH, room temp., 50 h, 100%

The ^{1}H- and ^{13}C-NMR spectra of the fully unprotected thiooligosaccharides **12**, **28**, **34** and **39** in D_2O could not be unequivocally assigned due to extensive overlapping and the presence of both α- and β-pyranose forms at the reducing end in the solution. In order to further confirm the proposed structures, extensive homonuclear and heteronuclear 1D- and 2D-NMR experiments were performed on the β-peracetate precursors **11**, **27**, **33** and **38** (see Table 1 and 2). The low-field chemical shifts of the carbon atoms bearing the *S*-linked glucopyranosyl substituents as well as those of the directly attached protons were consistent with the corresponding branching pattern. The $J_{1,2}$ coupling constant values supported the picture of complete stereocontrol in the formation of the thioglycosidic linkages by this synthetic methodology, even in the case of these rather intricate structures, and confirmed the value of the synthetic scheme for the preparation in high yield and selectivity of thioanalogs of complex oligosaccharides.

The ability of soybean β-glucan-binding sites to bind the *S*-linked thioanalogs of branched hexa- and hepta-glucosides was analyzed in competition experiments with the ^{125}I-labeled hepta-β-glucoside (HG-APEA). An hexa-β-glucoside is the biologically active motif in the hepta-β-glucoside elicitor described by Cheong et al.[1d] Affinity measurements at the β-glucan-binding sites gave apparent K_d values of about 1 to 3 nM for the hepta-β-glucoside.[1c,8] Competition for binding of HG-APEA by increasing concentrations of the *S*-linked thioanalogs **12**, **28**, **34** and **39** of branched hexa- and heptaglucosides demonstrated progressive inhibition of binding of radioiodinated HG-APEA. The concentrations of the different thioanalogs required to inhibit binding of the radioligand at the 50% level (IC_{50} values) are shown in Table 3. Thio analogs **12** and **28** were about ten times more active in competing for HG-APEA binding than the positional isomers **34** and **39**. Compounds **12** and **28** were, however, about three orders of magnitude less active as competitors than the natural hepta-*O*-glucoside.[1c,8]

The ability of each of the thioglucosides to induce phytoalexin accumulation in soybean cotyledon tissue was determined. The results of these bioassays, shown in Table 3, demonstrated that these compounds were differentially effective at inducing phytoalexin accumulation, thiohexa- and thioheptaglucosides **12** and **28** being more active than their respective positional isomers **34** and **39**. Again, the concentrations resulting in phytoalexin production at the 50% level (EC_{50} value) were much higher than those of the *O*-glucosides giving a similar response in the bioassay.[1c,8] Thiooligosaccharides having a higher elicitor activity were also more efficient competitors of binding of the radiolabeled HG-APEA to the membrane-localized β-glucan-binding sites of soybean (Table 3). These results contrast with recently published data involving an amide-linked heptaglucoside mimetic, which did not display any phytoalexin-elicitor activity,[9] and are further support for the growing interest in this class of glycomimetics in glycobiology.[2,14]

Experimental Section

General Methods: Melting points: Capillary tubes, Büchi 535 apparatus, uncorrected values. − Optical rotations: Jobin-Yvon (Paris) Digital Micropolarimeter. − ^{1}H (200, 300, 400 and 500 MHz) and ^{13}C NMR (50.3, 75.5 and 125.7 MHz): Bruker AC 200, MLS 300, AM 400 and DRX 500, reference signals at δ = 7.34 (^{1}H) and the central line of the $CDCl_3$ triplet (δ = 76.9 for ^{13}C) for solutions in $CDCl_3$, or signals at δ = 29.2 (^{13}C) and δ = 2.17 (^{1}H) when [D_6]acetone was used as internal reference for solutions in D_2O. Assignments of ^{1}H and ^{13}C signals were assisted by 1D-TOCSY, 2D ^{1}H-COSY, 2D ^{1}H-TOCSY and 2D ^{1}H-^{13}C CORR experiments. − FAB MS (Xe, accelerating potential 8 kV): ZAB-SEQ (VG), sodium iodide was usually added as cationizing agent. − Reactions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) and detection was accomplished by charring with H_2SO_4.

Table 1. ^{1}H-NMR data (500 MHz, $CDCl_3$) for thiooligosaccharides **11**, **27**, **33** and **38**

	Unit	Chemical shifts (δ)						
		1-H	2-H	3-H	4-H	5-H	6a-H	6b-H
11	I	5.61 d	5.10 dd	3.01 t	4.70 t	3.78 ddd	2.80 dd	2.75 dd
	II	4.66 d	5.03 t	5.13 t	4.86 t	3.70 ddd	4.25 dd	4.09 dd
	III	4.64 d	4.89 dd	5.13 t	4.86 t	3.61 ddd	2.84 dd	2.74 dd
	IV	4.41 d	4.92 dd	2.94 t	4.66 t	3.95 ddd	2.79 dd	2.75 dd
	V	4.60 d	4.85 t	5.14 t	5.03 t	3.68 ddd	4.24 dd	4.11 dd
	VI	4.55 d	4.94 t	5.15 t	5.05 t	3.70 ddd	4.22 dd	4.14 dd
27	I	5.69 d	5.09 dd	5.22 t	4.88 t	3.82 dt	<—2.72 d—>	
	II	4.55 d	4.92 dd	2.99 t	4.66 t	3.55 ddd	2.87 dd	2.76 dd
	III	4.69 d	4.84 t	5.12 t	5.02 t	3.68 ddd	4.25 dd	4.08 dd
	IV	4.68 d	4.91 t	5.14 t	4.89 t	3.68 ddd	2.84 dd	2.75 dd
	V	4.43 d	4.95 dd	2.97 t	4.62 t	3.62 td	<—2.74 m—>	
	VI	4.62 d	4.87 t	5.13 t	5.06 t	3.67 ddd	4.23 dd	4.10 dd
	VII	4.55 d	4.97 t	5.17 t	5.06 t	3.69 ddd	4.21 dd	4.13 dd
33	I	5.62 d	4.99 dd	5.14 t	4.85 t	3.76 ddd	2.72 dd	2.71 dd
	II	4.51 d	4.82 t	2.91 t	4.60 t	3.42 td	2.78 dd	2.65 dd
	III	4.64 d	4.79 t	5.08 t	4.97 t	3.66 ddd	4.21 dd	4.04 dd
	IV	4.38 d	4.86 t	2.86 t	4.59 t	3.54 ddd	<−2.72 m−>	
	V	4.55 d	4.78 t	5.08 t	4.96 t	3.63 ddd	4.16 dd	4.04 dd
	VI	4.53 d	4.86 t	5.11 t	4.97 t	3.64 ddd	4.16 dd	4.06 dd
38	I	5.88 d	5.07 t	5.26 t	4.92 t	3.89 ddd	2.83 dd	2.74 dd
	II		<—4.90 m—>	5.15 t	4.90 m	3.71 td	2.80 dd	2.73 dd
	III	4.45 d	4.95 t	2.99 t	4.73 t	3.67 td	2.84 dd	2.75 dd
	IV	4.76 d	4.88 t	5.17 t	5.06 t	3.80 ddd	4.35 dd	4.13 dd
	V	4.41 d	4.97 t	3.00 t	4.74 t	3.66 dd	2.93 ddd	2.79 dd
	VI	4.62 d	4.86 t	5.15 t	5.05 t	3.68 ddd	4.27 dd	4.12 dd
	VII	4.56 d	4.95 t	5.19 t	5.05 t	3.70 ddd	4.28 dd	4.11 dd

	Unit	Coupling constants [Hz]						
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
11	I	8.1	10.5	10.5	10.5	3.6	7.9	14.1
	II	10.1	10.1	10.1	10.1	4.4	2.6	12.4
	III	10.0	9.3	9.3	9.3	2.8	8.5	13.2
	IV	9.8	10.5	10.5	10.5	3.6	8.2	14.0
	V	10.2	10.2	10.2	10.2	4.2	2.7	12.3
	VI	10.0	10.0	10.0	10.0	4.1	2.6	12.3
27	I	8.2	9.4	9.4	9.4	4.9	4.9	–
	II	10.0	10.5	10.5	10.5	3.6	8.7	13.3
	III	10.3	10.3	10.3	10.5	3.4	2.0	12.5
	IV	10.0	10.0	10.0	10.0	2.8	9.0	14.0
	V	9.7	10.5	10.5	9.6	2.8	9.6	–
	VI	9.6	9.9	9.9	9.9	3.3	2.4	12.6
	VII	9.6	10.0	10.0	10.0	3.4	2.0	12.6
33	I	8.0	9.5	9.5	9.7	7.5	3.5	14.5
	II	9.5	10.0	10.0	10.0	3.0	9.5	14.0
	III	9.8	9.5	9.5	9.5	4.0	2.0	12.5
	IV	10.0	10.1	10.1	10.0	4.5	5.1	–
	V	10.5	10.0	10.0	10.0	4.0	2.0	12.5
	VI	10.0	9.5	9.5	9.5	4.5	2.0	12.4
38	I	8.3	9.5	9.5	9.7	3.4	8.1	14.3
	II	–	9.6	9.6	9.2	3.1	9.2	13.6
	III	9.9	10.2	10.2	10.2	3.0	9.0	14.1
	IV	9.9	9.4	9.4	9.4	5.1	2.8	12.8
	V	9.9	10.1	10.1	10.1	9.4	–	13.8
	VI	10.5	9.9	9.3	9.3	3.6	2.0	12.6
	VII	10.1	9.8	9.3	10.1	3.9	2.0	12.4

– FC: Silica Gel 60 (230–400 mesh, E. Merck), Büchi 680 fitted with a Knauer refractometric detector 188.00. – HPLC (4×10^3 kPa): Purification of unprotected thiooligosaccharides was carried out with a Perkin–Elmer chromatograph, fitted with an LC 250 isocratic pump, an LC 30 refractometric detector, and a 1020S integrator, on a LiChrosorb NH_2 (7 mm) column (250 × 10 cm, eluent MeCN/water). – Elemental analyses: Service Central d′Analyse du CNRS, Vernaison.

Plant Material and Chemicals: Soybean (*Glycine* max L. cv. Canton) seeds were obtained from Asgrow (Bruchsal, Germany). The hepta-(1→3)-(1→6)-β-glucoside was from Biocarb (Lund, Sweden).

Table 2. ^{13}C-NMR chemical shifts (50.3 MHz, $CDCl_3$) for thiooligosaccharides **2**, **4**, **7**−**11**, **13**, **18**−**20**, **27**, **33** and **38**

	Unit	C-1	C-2	C-3	C-4	C-5	C-6
2	I	86.4	70.8	70.2	70.6	74.6	30.4
	II	85.1	72.1	52.1	70.1	80.7	31.2
	III	84.6	70.2	73.8	68.3	75.7	62.0
	IV	83.3	70.2	73.8	68.3	76.1	62.0
4	I	82.8	71.9	73.5	71.1	78.2	30.8
	II	80.2	73.1	51.7	69.7	78.7	30.8
	III	84.6	69.7	73.5	67.9	75.7	61.6
	IV	84.0	69.7	73.5	67.9	75.7	61.6
7	I	89.2	70.9	46.8	66.6	72.7	62.3
	II	82.6	70.3	74.0	68.4	75.8	62.0
8	I	93.6	72.1	50.3	66.6	77.0	62.3
	II	83.5	70.3	74.0	68.4	75.8	62.0
9	I	93.3	71.8	50.1	66.3	77.8	61.3
	II	83.9	70.3	73.9	68.1	75.7	61.9
10	I	93.0	71.8	49.8	69.8	76.5	2.8
	II	83.9	70.3	73.8	68.3	75.9	62.0
11[a]	I	93.0	71.8	50.2	69.6	78.4	31.4
	II	84.1	70.1	73.7	68.2	76.0	61.9
	III	82.9	71.6	73.8	70.1	77.5	31.1
	IV	84.8	72.0	52.1	70.2	80.2	30.8
	V	84.7	70.0	73.6	68.2	75.5	61.9
	VI	83.1	69.8	73.7	68.1	75.5	61.9
13	I	80.4	71.4	73.9	69.3	79.5	31.1
	II	84.5	72.1	52.1	70.2	80.2	31.1
	III	84.5	70.2	73.9	68.4	75.8	62.0
	IV	83.1	70.2	73.9	68.4	76.1	62.0
18	I	105.0	86.4	49.5	76.9	70.5	30.3
	II	82.8	70.7	74.4	68.2	75.7	61.9
	III	82.3	70.1	73.9	68.8	76.5	62.0
19	I	105.0	86.5	49.5	76.6	70.6	31.1
	II	82.9	70.8	74.0	68.3	77.8	62.2
	III	82.4	70.2	73.9	68.9	76.5	61.7
20	I	104.9	86.5	49.5	77.0	70.4	30.7
	II	82.1	70.1	73.8	68.2	76.3	62.0
	III	82.3	71.1	73.4	72.4	77.3	2.9
27[a]	I	91.7	70.5	72.7	70.4	75.8	30.3
	II	84.4	72.1	52.2	70.3	80.5	31.6
	III	84.5	70.0	73.6	68.3	75.5[b]	61.9
	IV	83.0	71.5	73.9	71.0	75.4[b]	31.8
	V	85.1	71.9	52.4	70.3	80.0	31.2
	VI	84.9	70.0	74.8	68.3	77.6	62.0
	VII	83.2	69.8	73.9	68.3	76.0	62.0
33[a]	I	91.7	70.2	72.6	71.1	75.9	30.3
	II	84.4	72.1	52.1	70.2	79.8	31.7
	III	84.3	71.9	73.8	70.3	75.4	61.9
	IV	84.9	70.1	52.2	70.2	80.5	31.3
	V	84.7	70.2	73.8	68.3	76.1	61.9
	VI	83.1	70.2	73.6	68.3	75.5	62.1
38[a]	I	91.6	70.7	72.5	70.4	75.1	31.2
	II	83.2	71.4	73.8	70.0	77.7	32.4
	III	85.9	72.2	52.4	70.5	79.3	33.5
	IV	84.5	70.3	73.7	68.1	75.1	62.0
	V	86.6	71.7	52.1	70.5	80.5	31.6
	VI	85.0	70.0	73.7	68.4	75.7	61.9
	VII	83.5	70.1	73.9	68.0	76.0	61.8

[a] At 125.7 MHz. − [b] Assignments may be reversed.

Binding Assays: The 4-(2-aminophenyl)ethylamine conjugate of the hepta-β-glucoside (HG-APEA) was prepared and radioiodinated as described previously.[10][11] The average specific radioactivity of the radioligand was 10 TBq mmol^{-1}. Binding assays were carried out using a standardized glucan-binding assay.[1c] Inflection points (IC_{50} values) were obtained from ligand competition experiments using increasing concentrations of various thio analogs of branched hexa- and heptaglucosides as competitors. Protein content was measured according to Bradford.[12]

Table 3. Binding and phytoalexin elicitor activity of *S*-linked thio analogs of branched hexa- and heptaglucosides

Compound	Ligand competition (IC_{50}) [mM]	Biological activity (EC_{50}) [mM]
12	10	40
28	8	20
34	> 100	>> 100
39	100	200

Biological Activity Assays: Detached cotyledons from 5-d-old greenhouse seedlings of the soybean cultivar Canton were cut and aliquots of β-glucooligosaccharide solutions (60 mL) were placed on wounded areas.[13] The cotyledons were incubated for 22 h at 27°C on moist filter paper in Petri dishes in the dark. Phytoalexin accumulation in the wound-droplet solutions was determined by measuring the absorbance (A) at 285 nm.

2,3,4-Tri-*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-α-D-glucopyranosyl Bromide (2): 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 (**1**)[4] (850 mg, 0.65 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and treated at 0°C with a solution of 33% HBr in AcOH (2 mL). After 4 h at room temp., TLC (EtOAc/petroleum ether, 2:1) showed complete conversion of the starting peracetate. Toluene was added (80 mL), and the solution concentrated to give **2** as a foam which was used in the next step without further purification. − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2.

2,3,4-Tri-*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]-1,6-dithio-β-D-glucopyranosylisothiouronium Bromide (3): Thiourea (260 mg, 3.3 mmol) was added to a solution of **2** (850 mg, 0.65 mmol) in dried acetone (20 mL) and the reaction mixture was stirred at 85°C with TLC monitoring (EtOAc/petroleum ether, 3:1) until complete conversion of the starting bromide occurred (4 h). Evaporation of the solvent gave **3** as an amorphous solid, which was dried and used in the following step without further characterization.

2,3,4-Tri-*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]-1,6-dithio-β-D-glucopyranose (4): A mixture of the *S*-glycosylthiouronium salt **3** (850 mg, 0.65 mmol) and potassium pyrosulfite (720 mg, 3.24 mmol) in $CHCl_3/H_2O$, 1:1 (25 mL) was stirred at 85°C for 30 min. The two phases were then separated and the aqueous layer washed with $CHCl_3$ (3 × 10 mL). The combined organic extracts were dried ($MgSO_4$), concentrated and the resulting residue purified by column chromatography (EtOAc/petroleum ether, 2:1) to give **4** (700 mg, 85%) as a solid. − $[\alpha]_D$ = + 15.38 (*c* = 0.22, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%):1299 (100) $[M + Na]^+$. − $C_{50}H_{68}O_{30}S_4$ (1277.3): calcd. C 47.02, H 5.31, S 10.03; found C 47.30, H 5.61; S 9.88.

3-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-α,β-D-glucopyranose (6): A solution of 1,2:5,6-di-*O*-isopropylidene-3-*S*-

(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-α-D-glucofuranose (**5**, 400 mg, 0.66 mmol) in TFA/H_2O, 9:1 (5 mL) was stirred under vacuum (water pump) for 40 min at 30°C until no more acetone distilled. Subsequent freeze-drying of the solution gave **6** which was used without further purification.

1,2,4-Tri-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*O*-methoxytrityl-3-thio-α and β-D-glucopyranose (7 and 8): Chloro(4-methoxyphenyl)diphenylmethane (1 g, 1.4 equiv.) was added to a solution of **6** (1.25 g, 2.3 mmol) in pyridine (18 mL) at 0°C. The mixture was stirred for 18 h at room temp. and then acetylated by addition of Ac_2O (18 mL) and further stirring for 2 h. Compounds **7** and **8** (2.0 g, 90%) were obtained as a 1:1 mixture which could be separated by column chromatography (EtOAc/petroleum ether, 1:3). − **7**: − M.p. 165−166°C (ethanol). − $[\alpha]_D$ = + 13.3 (*c* = 0.42, $CHCl_3$). − **8**: − M.p. 203−204°C (ethanol). − $[\alpha]_D$ = + 10.8 (*c* = 0.37, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%): 947 (15) $[M + Na]^+$, 273 (100) $[C(C_6H_5)_2C_6H_4OMe]^+$. − $C_{46}H_{52}O_{18}S$ (924.9): calcd. C 59.74, H 5.63, S 3.46; found C 59.17, H 5.73, S 3.25.

1,2,4-Tri-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-β-D-glucopyranose (9): A solution of **8** (1.82 g, 1.97 mmol) in aqueous 80% AcOH (80 mL) was stirred for 8 h at room temp. (TLC; hexane/acetone, 1:1), then the mixture was concentrated under reduced pressure and the volatiles were coevaporated with toluene. Column chromatography of the residue (hexane/acetone, 2:1) gave **9** (1.15 g, 89.5%): − M.p. 196−197°C (hexane/acetone, 1:1). − $[\alpha]_D$ = + 7 (*c* = 0.2, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%): 675 (20) $[M + Na]^+$, 331 (70). − C26H36O17S (652.3): calcd. C 47.85, H 5.52, S 4.91; found C 47.66, H 5.46, S 4.82.

1,2,4-Tri-*O*-acetyl-6-deoxy-6-iodo-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-β-D-glucopyranose (10): Trifluoromethanesulfonic anhydride (0.075 mL, 0.43 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (90 mg, 0.43 mmol) were dissolved in CH_2Cl_2 (5 mL) and **9** (200 mg, 0.31 mmol) in CH_2Cl_2 (10 mL) was added to this solution. After stirring at room temp. for 1 h, tetrabutylammonium iodide (342 mg, 0.93 mmol) was added. The mixture was kept for 1 h, neutralized with satd. aqueous $NaHCO_3$ and extracted with chloroform. The extract was dried ($MgSO_4$), and concentrated. Recrystallization from ethanol afforded **10** (190 mg, 80%). − M.p. 222−223°C (ethanol). − $[\alpha]_D$ = + 25.0 (*c* = 0.4, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%): 785 (20) $[M + Na]^+$, 703 (70) $[M - OAc]^+$, 331 (100). − $C_{26}H_{35}IO_{16}S$ (762.5): calcd. C 40.94, H 4.59, S 4.20; found C 40.87, H 4.64, S 4.02.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[1,2,4-tri-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)]-3,6-dithio-β-D-glucopyranose (11):** Sodium hydride (5 mg, 0.21 mmol) was added under N_2 to a solution of the thiol **4** (170 mg, 0.13 mmol) in THF (10 mL) at room temp. The suspension was stirred until hydrogen evolution had ceased. The resulting solution was then concentrated under reduced pressure, and the amorphous residue was dissolved in DMF (3 mL). **10** (107 mg, 0.14 mmol) in DMF (5 mL) was then added to this stirred solution. After 1 h at room temp., conventional work up and column chromatography (petroleum ether/EtOAc/acetone, 4:2:1) gave **11** (210 mg, 83%) as an amorphous solid. − $[\alpha]_D$ = −19.92 (*c* = 0.50, $CHCl_3$). − ^1H NMR (500 MHz, $CDCl_3$): Table 1. − ^{13}C NMR (125.7 MHz, $CDCl_3$): Table 2. − $C_{76}H_{102}O_{46}S_5$ (1911.9): calcd. C 47.75, H 5.34, S 8.37; found C 47.34, H 5.64, S 8.81.

***S*-(β-D-Glucopyranosyl)-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)]-3,6-dithio-D-glucopyranose (12):** (a) Methanolic NaOMe (1 M, 1 mL) was added to a solution of the acetylated hexathiosaccharide **11** (80 mg, 0.04 mmol) in MeOH (10 mL), and the mixture was stirred for 50 h at room temp. The solution was then demineralized with Amberlite ion-exchange resin IRN 77(H^+). Concentration of the solution gave a syrup which was subjected to reverse-phase LC (Lichrosorb NH_2, 7 μm, MeCN/water, 78:22) and freeze-dried to give **12** as a foam (45 mg, 100%). − $[\alpha]_D$ = −16.69 (*c* = 0.59, H_2O). − ^{13}C NMR (50.3 MHz, D_2O): δ = 97.3 (C-1^{Ib}), 91.7 (C-1^{Ia}), 88.3, 86.3, 84.6, 81.6, 80.5 (C-1^{II-VI}), 57.6 (C-3^{Ib}), 56.1 (C-3^{IV}), 54.7 (C-3^{Ia}), 32.9, 32.4 (C-$6^{I,III,IV}$). − FAB MS; *m/z* (%):1093 (43) $[M + Na]^+$. − (b) Conventional *O*-deacetylation of **15** (130 mg, 0.07 mmol) in MeOH (10 mL) with methanolic NaOMe (1 M, 0.15 mL) for 18 h at room temp., followed by concentration and treatment of the residue with TFA/water, 9:1 (1.4 mL) for 10 min at room temp., afforded **12** (63 mg, 85%), identical in all respects to the product obtained in (a).

2,3,4-Tri-*O*-acetyl-1-*S*-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]-1,6-dithio-β-D-glucopyranose (13): A mixture of the bromide **2** (240 mg, 0.18 mmol) and potassium thioacetate (66 mg, 0.58 mmol) in DMF (3 mL) was stirred overnight at room temp. and concentrated under reduced pressure. The resulting residue was treated with CH_2Cl_2 (10 mL), washed with water (2 × 8 mL), dried ($MgSO_4$), and concentrated. Purification by column chromatography (EtOAc/petroleum ether, 2:1) yielded **13** (155 mg, 65%) as a syrup. − $[\alpha]_D$ = −18.0 (*c* = 1.0, $CHCl_3$). − ^1H NMR (400 MHz, $CDCl_3$): δ = 5.21 (d, 1 H, $J_{1,2}$ = 9.3 Hz, H-1^I), 5.20 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3^I), 5.19 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3^{III}), 5.15 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3^{IV}), 5.13 (t, 1 H, $J_{2,3}$ = 9.3 Hz, H-2^I), 5.06 (t, 1 H, $J_{4,5}$ = 9.8 Hz, H-4^{III}), 5.04 (t, 1 H, $J_{4,5}$ = 9.3 Hz, H-4^{IV}), 5.02 (t, 1 H, $J_{1,2}$ = 9.8 Hz, H-2^{III}), 4.94 (dd, 1 H, $J_{4,5}$ = 10.1 Hz, H-4^I), 4.90 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 10.7 Hz, H-2^{II}), 4.85 (dd, 1 H, $J_{1,2}$ = 10.0 Hz, H-2^{IV}), 4.61 (d, 1 H, H-1^{II}), 4.58 (d, 1 H, H-1^{IV}), 4.64 (dd, 1 H, $J_{3,4}$ = 10.7, $J_{4,5}$ = 9.4 Hz, H-4^{II}), 4.21 (dd, 1 H, $J_{6a,6b}$ = 12.4, $J_{5,6b}$ = 4.5 Hz, H-$6b^{IV}$), 4.10 (dd, 2 H, H-$6a^{III,IV}$), 3.81 (ddd, 1 H, $J_{5,6b}$ = 6.1, $J_{5,6a}$ = 4.5 Hz, H-5^I), 3.67 (ddd, 2 H, $J_{5,6a}$ = 2.1 Hz, H-$5^{III,IV}$), 3.48 (ddd, 1 H, $J_{5,6b}$ = 6.6, $J_{5,6a}$ = 4.8 Hz, H-5^{II}) , 2.75 (m, 4 H, H-$6a^{I,II}$, H-$6b^{I,II}$). − ^{13}C NMR (50.3 MHz, $CDCl_3$), Table 2. − FAB MS; *m/z* (%): 1341 $[M + Na]^+$.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[5-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-gluco-pyranosyl)-1,2-*O*-isopropylidene]-3,6-dithio-α-D-glucofuranose (15):** (a) Sodium hydride (2.6 mg, 0.11 mmol) was added under N_2 to a solution of the thiol **22**[2] (100 mg, 0.1 mmol) in dry THF (10 mL). The suspension was stirred until hydrogen evolution had ceased. The resulting solution was then concentrated under reduced pressure and the residue dissolved in DMF (5 mL). **20** (102 mg, 0.1 mmol) was added to this solution and the mixture stirred for 4 h at 70°C, then concentrated. A solution of the residue in CH_2Cl_2 (10 mL), was washed with water (8 mL), dried ($MgSO_4$), and concentrated to a syrup which was purified by column chromatography (EtOAc/petroleum ether, 2:1) yielding **15** as an amorphous solid (38 mg, 20%). − $[\alpha]_D$ = −30 (*c* = 1.0, $CHCl_3$). − ^1H NMR (400 MHz, $CDCl_3$): δ = 5.95 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1^I), 4.78 (d, 1 H, $J_{1,2}$ = 10.1 Hz, H-1^{II}), 4.68 (d, 2 H, $J_{1,2}$ = 10.1 Hz, H-$1^{V,VI}$), 4.61 (d, 2 H, $J_{1,2}$ = 10.1 Hz, H-$1^{III,IV}$), 3.53 (d, 1 H, $J_{3,4}$ = 4.2 Hz, H-3^I), 2.96 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 10.1 Hz, H-3^{III}). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 104.9 (C-1^I), 85.3, 84.7, 83.2, 82.3,

82.2 (C-1^{II-VI}), 52.4 (C-3^{IV}), 49.3 (C-3^{I}), 31.3 (C-$6^{III,IV}$), and 30.7 (C-6^{I}). − FAB MS; *m/z* (%): 1890 (100) $[M + Na]^+$, 1848 (40) $[M - OAc]^+$. − $C_{75}H_{102}O_{44}S_5$ (1867.9): calcd. C 48.22, H 5.50, S 8.58; found C 47.97, H 5.21, S 8.30. − (b) Methanolic NaOMe (1 M, 0.27 mL) was added to a solution of **21**[2] (250 mg, 0.25 mmol) in MeOH (5 mL). After being stirred at room temp. for 18 h, the solution was concentrated under reduced pressure. A solution of **20** (210 mg, 0.21 mmol) in DMF (10 mL) was added to the resulting residue in DMF (10 mL), under nitrogen, and the mixture was stirred for 4 h at 60°C. The workup as described in (a), followed by conventional acetylation with Ac_2O/pyridine, 1:1 (14 mL) and column chromatography (EtOAc/petroleum ether, 2:1) afforded **15** (130 mg, 35%), identical in all respects to the product obtained in (a). − (c) The same protocol as in (b) was followed starting from **13** (110 mg, 0.08 mmol), methanolic NaOMe (1 M, 0.09 mL) and **14**[2] (75 mg, 0.1 mmol) yielding **15** (36 mg, 30%), identical in all respects to the product obtained in (a).

5-*O*-Acetyl-1,2-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*S*-(β-D-glucopyranosyl)-3,6-dithio-α-D-glucofuranose (17): 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**16**) (300 mg, 0.82 mmol) was dissolved in methanol (3 mL) containing sodium methoxide (1 M, 2 mL). After stirring for 15 min at room temp., the resulting sodium salt was filtered, dried, and added to a solution of 5-*O*-acetyl-6-deoxy-6-iodo-1,2-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-thio-α-D-glucofuranose (**14**, 300 mg, 0.4 mmol) in DMF (10 mL). After being stirred for 20 h at room temp., evaporation of the solvent led to an oil which was used without further purification in the next step. − FAB MS; *m/z* (%): 809 (100) $[M + Na]^+$, 331 (95).

5-*O*-Acetyl-1,2-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*S*-(2,3,4-tri-*O*-acetyl-6-*O*-methoxytrityl-β-D-glucopyranosyl)-3,6-dithio-α-D-glucofuranose (18): Chloro(4-methoxyphenyl)diphenylmethane (284 mg, 1.2 equiv) was added to a solution of **17** (315 mg, 0.4 mmol) in pyridine (5 mL) at 0°C, the mixture was stirred for 18 h at room temp. and then acetylated with Ac_2O (3 mL) for 2 h. Extraction with CH_2Cl_2 followed by the usual workup led, after concentration, to a syrup which was purified by column chromatography (EtOAc/petroleum ether, 1:1) to give **18** (360 mg, 73%). − M.p. 153−154°C (ethanol). − $[\alpha]_D$ = −86.9 (*c* = 0.3, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%): 1207 (70) $[M + Na]^+$. − $C_{57}H_{68}O_{23}S_2$ (1185.3): calcd. C 57.77, H 5.74, S 5.40; found C 57.18, H 5.90, S 5.90.

5-*O*-Acetyl-1,2-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*S*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-α-D-glucofuranose (19): A solution of **18** (200 mg, 0.17 mmol) in aqueous 80% acetic acid (5 mL) was kept for 2 h at room temp. Then, the mixture was neutralized with satd. aqueous $NaHCO_3$, extracted with chloroform, dried ($MgSO_4$), and concentrated. Column chromatography of the residue (EtOAc/petroleum ether, 3:2) gave **19** (85 mg, 93%) as a foam. − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2.

5-*O*-Acetyl-1,2-*O*-isopropylidene-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-6-*S*-(2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranosyl)-3,6-dithio-α-D-glucofuranose (20): Trifluoromethanesulfonic anhydride (0.16 mL, 1.44 equiv) and 2,6-di-*tert*-butyl-4-methylpyridine (90 mg, 0.98 mmol) were added to a solution of **19** (600 mg, 0.66 mmol) in CH_2Cl_2 (10 mL). After being stirred at room temp. for 45 min, tetrabutylammonium iodide (730 mg, 1.98 mmol) was added. The mixture was kept for 3 h, neutralized with satd. aqueous $NaHCO_3$ and extracted with chloroform. The extract was dried ($MgSO_4$), concentrated, and purified by column chromatography (EtOAc/petroleum ether, 1:1) leading to the deoxyiodo derivative **20** as a foam (300 mg, 45%), $[\alpha]_D$ = −42.1 (*c* = 0.2, $CHCl_3$). − ^1H NMR (400 MHz, $CDCl_3$): δ = 5.82 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1^{I}), 5.11 (t, 1 H, $J_{2,3} = J_{3,4}$ 10.0 Hz, H-3^{II}), 5.09 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3^{III}), 5.05 (dt, 1 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6a} = J_{5,6b}$ = 3.0 Hz, H-5^{I}), 4.96 (t, 1 H, $J_{4,5}$ = 10.0 Hz, H-4^{II}), 4.87 (t, 1 H, $J_{1,2}$ = 10.0 Hz, H-2^{II}), 4.87 (t, 1 H, $J_{4,5}$ = 9.3 Hz, H-4^{III}), 4.85 (d, 1 H, $J_{2,3}$ = 0 Hz, H-2^{I}), 4.81 (t, 1 H, $J_{1,2}$ = 9.5 Hz, H-2^{III}), 4.77 (d, 1 H, H-1^{III}), 4.63 (d, 1 H, H-1^{II}), 4.56 (dd, 1 H, $J_{3,4}$ = 4.0 Hz, H-4^{I}), 4.14 (dd, 1 H, $J_{6a,6b}$ = 12.0, $J_{5,6a}$ = 2.2 Hz, H-$6a^{II}$), 4.05 (dd, 1 H, $J_{5,6b}$ = 4.5 Hz, H-$6b^{II}$), 3.57 (ddd, 1 H, H-5^{II}), 3.47 (ddd, 1 H, $J_{5,6b}$ = 8.6, $J_{5,6a}$ = 2.8 Hz, H-5^{III}), 3.39 (d, 1 H, H-3^{I}), 3.26 (dd, 1 H, $J_{6a,6b}$ = 15.0 Hz, H-$6a^{I}$), 3.17 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, H-$6a^{III}$), 3.07 (dd, 1 H, H-$6b^{I}$), 3.04 (dd, 1 H, H-$6b^{III}$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%): 1045 (60) $[M + Na]^+$, 895 (15) $[M - I]^+$, 399 (40), 331 (40).

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)]-3,6-dithio-α-D-glucopyranosyl Bromide (23):** HBr in AcOH (33%, 1 mL) was added to a solution of the hexathiooligosaccharide **11** (400 mg, 0.21 mmol) in CH_2Cl_2 (15 mL) at −10°C. The mixture was stirred at room temp. for 2 h. The mixture was concentrated under reduced pressure, the volatiles were coevaporated with toluene and the residue was used without purification in the next step.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)]-1,3,6-trithio-β-D-glucopyranosylisothiouronium Bromide (24):** The procedure described for the preparation of **3** was followed starting from **23** (400 mg, 0.21 mmol), acetone (15 mL), and thiourea (81 mg, 1 mmol). TLC (petroleum ether/EtOAc/acetone, 2:1:1) of the reaction showed complete conversion of the bromide **23** to **24**. Concentration resulted in a solid residue which was used in the following step without further characterization.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)]-1,3,6-trithio-β-D-glucopyranose (25):** A mixture of the *S*-glycosylthiouronium salt **24** (420 mg, 0.21 mmol) and potassium pyrosulfite (240 mg, 1 mmol) in $CHCl_3/H_2O$, 1:1 (30 mL) was stirred at 85°C for 30 min. The two phases were then separated and the aqueous layer washed with $CHCl_3$ (3 × 10 mL). The combined organic extracts were dried ($MgSO_4$), concentrated and the resulting residue purified by column chromatography (petroleum ether/EtOAc/acetone, 2:1:1) to give **25** as a solid (360 mg, 91%). − $[\alpha]_D$ = −29.07 (*c* = 0.34, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 84.8, 84.7, 84.4, 83.1, 82.3, 81.7 (C-1^{I-VI}), 52.1 (C-$3^{I,IV}$), 31.5, 31.0 (C-$6^{I,III,IV}$), 61.9 (C-$6^{II,V,VI}$). − FAB MS; *m/z* (%): 2017 (4) $[M + Cs]^+$, 331 (63), 169 (100). − $C_{74}H_{100}O_{44}S_6$ (1885.9): calcd. C 47.13, H 5.30, S 10.19; found C 46.49, H 5.37, S 10.60.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-1,2,3,4-tetra-*O*-acetyl-6-thio-β-D-glucopyranose (27):** The procedure described above for the preparation of **11** was followed starting from

25 (360 mg,0.19 mmol); sodium hydride (7.5 mg, 0.31 mmol) and 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranose (**26**)[7] (184 mg, 0.39 mmol) in DMF (5 mL). After being stirred for 1 h at room temp., purification by column chromatography (petroleum ether/EtOAc/acetone, 2:1:1) afforded **27** as an amorphous solid (310 mg, 73%). − $[\alpha]_D$ = −14.63 (*c* = 0.82, $CHCl_3$). − ^{1}H NMR (500 MHz, $CDCl_3$): Table 1. − ^{13}C NMR (125.7 MHz, $CDCl_3$): Table 2. − FAB MS; *m*/*z* (%): 2237 (7) $[M + Na]^+$, 939 (4), 331 (100). − $C_{88}H_{118}O_{53}S_6$ (2216.2): calcd. C 47.69, H 5.33, S 8.67; found C 48.02, H 5.61, S 8.47.

***S*-(β-D-Glucopyranosyl)-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(6-thio-β-D-glucopyranosyl)-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-6-thio-D-glucopyranose (28):** Zemplén deacetylation of **27** (90 mg, 0.041 mmol) with methanolic NaOMe (1 M, 1 mL) at room temp. for 60 h, followed by purification by HPLC on a LiChrosorb NH_2 7 μm column using MeCN/water, 1:1 at a flow rate of 1.5 mL/min (retention time 5.98 min), yielded **28** as a foam (51 mg, 100%). − $[\alpha]_D$ = −21.05 (*c* = 0.29, H_2O). − ^{13}C NMR (50.3 MHz, D_2O): δ = 96.0 (C-1^{Ib}), 92.0 (C-1^{Ia}), 88.4, 87.0, 84.5, 86.3, 81.5, 79.9 (C-1^{II-VII}), 56.1 (C-$3^{II,V}$), 33.2, 33.1, 32.6, 32.5 (C-$6^{I,II,IV,V}$). − FAB MS; *m*/*z* (%): 1271 (35) $[M + Na]^+$.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-1,2,4-tri-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranose (29):** Sodium hydride (18 mg, 0.75 mmol) was added to a solution of the thiol **22** (500 mg, 0.51 mmol) in THF (15 mL) at room temp.. When the hydrogen evolution ceased, the solution was concentrated, the residue dissolved in DMF (15 mL) and **10** (468 mg, 0.6 mmol) in DMF (10 mL) added to this solution. After being stirred for 2 h at room temp., work-up as described for **11**, and column chromatography (petroleum ether/EtOAc, 1:2) yielded **29** (743 mg, 88%) as an amorphous solid. − $[\alpha]_D$ = −9.45 (*c* = 1.69, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 92.5 (C-1^{I}), 84.9, 83.8, 83.3, 82.5 (C-1^{II-V}), 51.4, 49.7 (C-$3^{I,III}$), 30.8, 30.3 (C-$6^{I,III}$). − $C_{64}H_{86}O_{39}S_4$ (1607.6): calcd. C 47.82, H 5.35, S 7.97; found C 47.41, H 5.39, S 7.51.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-α-D-glucopyranosyl Bromide (30):** A solution of **29** (400 mg, 0.25 mmol) in dry CH_2Cl_2 (15 mL) at 0°C was treated with commercial 33% HBr in AcOH (1 mL) for 3 h at room temp., then the volatiles were coevaporated with toluene. The crude bromide **30** was used in the next step without purification.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,3,6-trithio-β-D-glucopyranosylisothiouronium Bromide (31):** Thiourea (95 mg, 1.2 mmol) was added to a solution of **30** (400 mg, 0.25 mmol) in acetone (15 mL) and the reaction mixture stirred at 85°C for 4 h. TLC monitoring (petroleum ether/EtOAc, 1:2) showed complete conversion of the bromide **30**. Evaporation of the solvent gave **31** which was used without further purification.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,3,6-trithio-β-D-glucopyranose (32):** A mixture of the *S*-glycosylthiouronium salt **31** (420 mg, 0.25 mmol) and potassium pyrosulfite (222 mg, 1 mmol) in $CHCl_3/H_2O$, 1:1 (20 mL) was stirred at 85°C for 40 min. Work-up as described for **4** and column chromatography (EtOAc/petroleum ether, 2:1) gave **32** (330 mg, 84%) as a syrup. − $[\alpha]_D$ = −45.56 (*c* = 0.44, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 84.9, 84.5, 84.3, 83.4, 81.5 (C-1^{I-V}), 52.2 (2 C, C-$3^{I,III}$), 31.6 (2 C, C-$6^{I,III}$). − FAB MS; *m*/*z* (%): 1604 (3) $[M + H + Na]^+$, 939 (2), 331 (100). − $C_{62}H_{84}O_{37}S_5$ (1581.6): calcd. C 47.08, H 5.31, S 10.12; found C 46.92, H 5.32, S 9.87.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-1,2,3,4-tetra-*O*-acetyl-6-thio-β-D-glucopyranose (33):** Sodium hydride (12 mg, 0.5 mmol) was added to a solution of the thiol **32** (500 mg, 0.3 mmol) in THF (10 mL) at room temp. When hydrogen evolution had ceased, the solution was concentrated, the residue dissolved in DMF (15 mL) and the deoxyiodo derivative **26** (184 mg, 0.4 mmol) in DMF (5 mL) added to this solution. After being stirred for 1 h at room temp., work-up as described for **11**, and column chromatography (petroleum ether/EtOAc/acetone, 4:2:1) yielded **33** (450 mg, 80%) as an amorphous solid. − $[\alpha]_D$ = −21.08 (*c* = 0.66, $CHCl_3$). − ^{1}H NMR (500 MHz, $CDCl_3$): Table 1. − ^{13}C NMR (125.7 MHz, $CDCl_3$): Table 2. − FAB MS; *m*/*z* (%): 1933 (8) $[M + Na]^+$, 939 (2), 331 (90). − $C_{76}H_{102}O_{46}S_5$ (1911.9): calcd. C 47.75, H 5.34, S 8.32; found C 47.33, H 5.54, S 7.91.

***S*-(β-D-Glucopyranosyl)-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-6-thio-D-glucopyranose (34):** Zemplén *O*-deacetylation of **33** (90 mg, 0.047 mmol) with methanolic NaOMe (1 M, 1 mL) and purification by HPLC on a LiChrosorb NH_2 7-μm column using MeCN/water, 65:35 at a flow rate of 3 mL/min (retention time 5.1 min) afforded **34** (50 mg, 100%). − $[\alpha]_D$ = −36.36 (*c* = 0.22, H_2O). − ^{13}C NMR (50.3 MHz, D_2O): δ = 96.2 (C-1^{Ib}), 92.3 (C-1^{Ia}), 86.2, 85.7, 84.6, 81.7 (C-1^{II-VI}), 53.8 (C-$3^{II,IV}$), 32.2 (C-$6^{I,II,IV}$). − FAB MS; *m*/*z* (%): 1093 (98) $[M + Na]^+$.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-2,3,4-tri-*O*-acetyl-6-thio-α-D-glucopyranosyl Bromide (35):** The procedure described for the preparation of **2** but starting from **33** (400 mg, 0.2 mmol), CH_2Cl_2 (15 mL) and HBr in AcOH (33%, 1 mL) was followed. The crude product was used in the next step without purification.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-2,3,4-tri-*O*-acetyl-1,6-dithio-β-D-glucopyranosylisothiouronium Bromide (36):** Thiourea (72 mg, 0.9 mmol) was added to a solution of **35** (360 mg, 0.19 mmol) in acetone (20 mL) and the mixture stirred at 85°C for 4 h. Evaporation of the solvent gave **36** which was used in the following step without further characterization.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-2,3,4-tri-*O*-acetyl-1,6-dithio-β-D-glucopyranose (37):** A mixture of the *S*-glycosylthiouronium salt **36** (380 mg, 0.19 mmol) and potassium pyrosulfite (208 mg, 0.94 mmol) in $CHCl_3/H_2O$, 1:1 (20 mL)

was stirred at 85°C for 40 min. The two phases were then separated and the aqueous layer washed with $CHCl_3$ (3 × 10 mL). The combined organic extracts were dried ($MgSO_4$), concentrated and the resulting residue was purified by column chromatography (petroleum ether/EtOAc/acetone, 4:4:1) to give **37** as an amorphous solid (260 mg, 73%). − $[\alpha]_D$ = −21.05 (*c* = 0.19, $CHCl_3$). − ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 84.9, 84.4, 84.0, 83.1, 83.0, 80.2 (C-1^{I-VI}), 52.0 (C-$3^{II,IV}$), 31.9, 31.3, 30.7 (C-$6^{I,II,IV}$). − FAB MS; *m/z* (%): 1909 (5) $[M + Na]^+$, 331 (60). − $C_{74}H_{100}O_{44}S_6$ (1886.0): calcd. C 47.13, H 5.30, S 10.19; found C 46.47, H 5.49, S 9.63.

***S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[2,4-di-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-(2,3,4-tri-*O*-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-*S*-1,2,3,4-tetra-*O*-acetyl-6-thio-β-D-glucopyranose (38):** The procedure described above for the preparation of **11** was followed starting from **37** (180 mg,0.095 mmol), sodium hydride (3 mg, 0.12 mmol) and **26** (60 mg, 0.125 mmol). After being stirred for 2 h at room temp., work-up as described for **11** and purification by column chromatography (petroleum ether/EtOAc/acetone, 4:4:1) afforded **38** (148 mg, 71%) as a white amorphous solid. − $[\alpha]_D$ = −12.9 (*c* = 0.31, $CHCl_3$). − ^{1}H NMR (500 MHz, $CDCl_3$): Table 1. − ^{13}C NMR (125.7 MHz, $CDCl_3$): Table 2. − FAB MS; *m/z* (%): 2237 (5) $[M + Na]^+$, 939 (3), 331 (100). − $C_{88}H_{118}O_{53}S_6$ (2216.2): calcd. C 47.69, H 5.33, S 8.67; found C 47.80, H 5.55, S 8.37.

***S*-(β-D-Glucopyranosyl)-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-[3-*S*-(β-D-glucopyranosyl)-3,6-dithio-β-D-glucopyranosyl]-(1→6)-*S*-6-thio-β-D-glucopyranosyl-(1→6)-*S*-6-thio-D-glucopyranose (39):** Zemplén *O*-deacetylation of **38** (75 mg, 0.034 mmol) with methanolic NaOMe (1 M, 1 mL) at room temp. for 50 h, followed by purification by HPLC on a LiChrosorb NH_2 7-μm column using MeCN/water, 1:1 at a flow rate of 1.5 mL/min (retention time 6.08 min) yielded **39** as a foam (42 mg, 100%). − $[\alpha]_D$ = −46.15 (*c* = 0.13, H_2O). − ^{13}C NMR (50.3 MHz, D_2O): δ = 96.1 (C-1^{Ib}), 92.3 (C-1^{Ia}), 88.3, 86.2, 84.6, 81.5, 80.0, 79.9 (C-1^{II-VII}), 56.1 (C-$3^{III,V}$), 33.2, 32.4 (C-$6^{I,II,III,V}$). − FAB MS; *m/z* (%): 1271 (90) $[M + Na]^+$.

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