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Spacer-modified disaccharide and pseudo-trisaccharide methyl glycosides that mimic maltotriose, as competitive inhibitors for pancreatic alpha-amylase: a demonstration of the "clustering effect"

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ABSTRACT

The synthesis is reported of methyl 4,4'-dithio-α-maltotrioside (**12**) and the spacer-modified disaccharide glycosides methyl 4-*S*-(4-α-D-glucopyranosylthio-2-hydroxybutyl)-4-thio-α-D-glucopyranoside (**20**) and methyl 4-*S*-[(1,5/4,6)- and (4,6/1,5)-4-α-D-glucopyranosylthio-5,6-dihydroxy-2-cyclohexen-1-yl]-4-thio-α-D-glucopyranoside (**29a/b**), which are analogues of methyl α-maltotrioside. The K_i values for alpha-amylase for these compounds were determined as were those of methyl α-maltotrioside and maltose.

INTRODUCTION

The centre D-glucopyranosyl residue in maltotriose can be replaced by an acyclic aliphatic spacer[1]. This spacer-modified disaccharide can mimic a trisaccharide. Thus, methyl 4-*O*-(4-α-D-glucopyranosyloxy-4-methoxybutyl)-α-D-glucopyranoside (**1**) may be regarded as an alkyl α-D-glucopyranoside or a methyl 4-*O*-alkyl-α-D-glucopyranoside, neither of which have any affinity for alpha-amylase[1]. However, the fact that **1** is a competitive, although weak, inhibitor of alpha-amylase action can be explained by the so-called "clustering effect"[2], which enhances the affinity for the enzyme, when two or more potential ligands of a multisite receptor are linked covalently by a flexible spacer[1,3]. Compound **1** contains a non-polar, acyclic spacer, which may be repelled from a binding subsite that normally accommodates a glucopyranosyl unit.

For purposes of comparison, we have synthesised two new types of maltotriose analogues, namely, methyl 4-*S*-(4-α-D-glucopyranosylthio-2-hydroxybutyl)-4-thio-α-D-glucopyranoside (**20**), which has an acyclic, flexible, and polar spacer, and methyl 4-*S*-[(1,5/4,6)- and (4,6/1,5)-4-α-D-glucopyranosylthio-5,6-dihydroxy-2-cyclohexen-1-yl]-4-thio-α-D-glucopyranoside (**29a/b**), which has an equally polar but rigid spacer. By definition, **20** is a spacer-modified disaccharide, and **29a/b** is a pseudo-trisaccharide.

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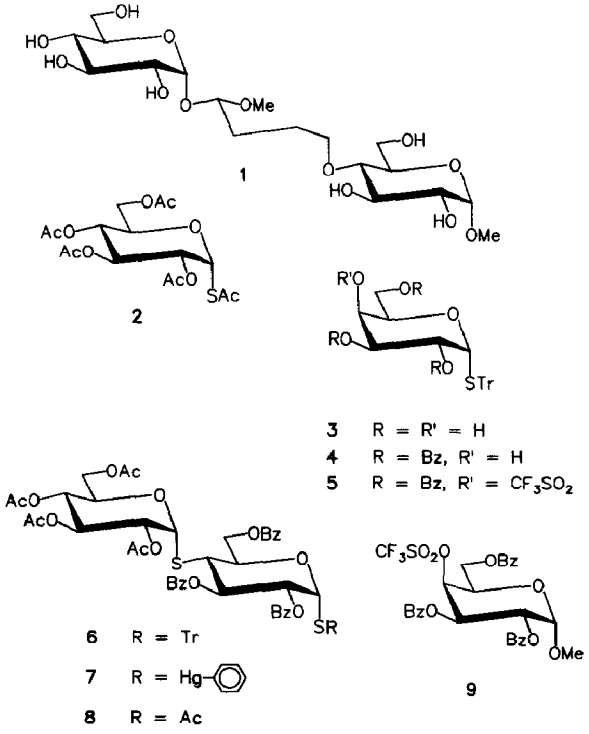
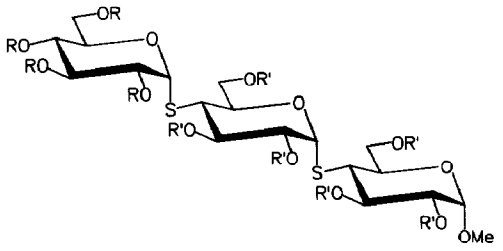
RESULTS AND DISCUSSIONS

Maltotriose is the shortest substrate for alpha-amylase and although the rate of cleavage is moderate, it is a substrate rather than a competitive inhibitor. The fact that thioglycosides are resistant to enzyme-catalysed hydrolysis makes thioglycosides and oligosaccharides with thioglycosidic bonds ideally suited as competitive inhibitors for glycoside hydrolases and glycanases. The three new analogues of maltotriose are linked via sulfur.

The synthesis of methyl 4,4'-dithiomaltotrioside (**12**) is based on the selective *in situ* *S*-deacetylation and activation procedure of 1-thio-α-D-glucopyranose pentaacetate[4], and involves a more general and flexible route to higher thio-oligosaccharides, which is based on differential blocking of thiol groups[5]. Triphenylmethyl 2,3,6-tri-*O*-benzoyl-4-*O*-trifluoromethanesulfonyl-1-thio-α-D-galactopyranoside (**5**), the putative centre unit, was synthesised (89%) by selective benzoylation of triphenylmethyl 1-thio-α-D-galactopyranoside (**3**) followed by triflation. The left-hand unit, 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (**2**) was accessible readily from triphenylmethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside[6] as described for the *galacto* analogue[5]. Coupling of **2** and **5** in the presence of cysteamine and dithioerythritol led stereospecifically to triphenylmethyl 2,3,6-tri-*O*-benzoyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1,4-dithio-α-D-glucopyranoside (**6**), which was then converted into the nucleophilic coupling compound, 1-*S*-acetyl-2,3,6-tri-*O*-benzoyl-4-*S*-(2,3,4,6-tetra-





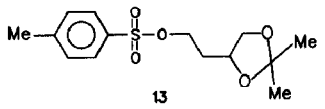
10 R = Ac, R' = Bz
11 R = R' = Ac
12 R = R' = H

O-acetyl-α-D-glucopyranosyl)-1,4-dithio-α-D-glucopyranose (**8**) as described[5], which was reacted with methyl 2,3,6-tri-*O*-benzoyl-4-*O*-trifluoromethanesulfonyl-α-D-galactopyranoside (**9**), under standard conditions, to give, after deprotection, **12**.

Compound **20** was synthesised as follows. Deacetylation of **2** gave the corresponding thiolate[7], that was coupled with (2*R,S*)-1,2-di-*O*-isopropylidene-4-*O*-*p*-toluenesulfonyl-1,2,4-butanetriol (**13**), derived from (±)-1,2,4-butanetriol, to give (3*R,S*)-3,4-isopropylidenedioxybutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside (**14**) in 84% yield. The isopropylidene group was removed from **14** under mild conditions, using aqueous tetrafluoroboric acid[8]. Attempts to introduce selectively a leaving group at the primary position failed. Therefore, (3*R,S*)-3,4-benzylidenedioxybutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside (**16**) was prepared by using α,α-dimethoxytoluene and camphorsulfonic acid[9]. The ^{13}C-n.m.r. spectrum of **16** indicated the formation of two stereoisomers associated with the chiral benzylic carbon atom. Oxidative ring opening of the benzylidene acetal gave (3*R,S*)-3-benzoyloxy-4-bromobutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside (**17**). Coupling of **17** with the sodium salt of methyl 4-thio-α-D-glucopyranoside, generated from methyl 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-glucopyranoside (**18**), gave **19**, deacylation of which gave **20**.

The preparation of **29a/b** was based on the facile opening of the activated oxirane [(1/4,5,6)- and (4,5,6/1)]-6-bromo-4,5-epoxy-2-cyclohexen-1-ol[10] (**21a/b**) by thiols[11]. Methyl 2,3,6-tri-*O*-acetyl-4-thio-α-D-glucopyranoside (**22**), obtained from **18**, reacted smoothly with **21a/b** to give the diastereomers methyl 2,3,6-tri-*O*-acetyl-4-*S*-[(1,4/5,6)- and (5,6/1,4)-5-bromo-4,6-dihydroxy-2-cyclohexen-1-yl]-4-thio-α-D-glucopyranoside (**23a/b**), isolated as the penta-acetates (**24a/b**), the absolute configurations of which were not established. Methyl 2,3,6-tri-*O*-acetyl-4-*S*-[(1,4,5/6)- and (6/1,4,5)-4,5-epoxy-6-hydroxy-2-cyclohexen-1-yl]-4-thio-α-D-glucopyranoside (**25a/b**), prepared from **23a/b** by treatment with alkali, was coupled with 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside[6] (**26**). The resulting two diastereomeric pseudo-trisaccharide derivatives (**27a/b**) were purified as their nona-acetates (**28a/b**), one of which was obtained crystalline. Deacetylation of **28a/b** gave amorphous **29a/b.**

Kinetic investigations were performed using commercial porcine pancreatic alpha-amylase, with *p*-nitrophenyl maltotrioside as substrate. All of the thio com-



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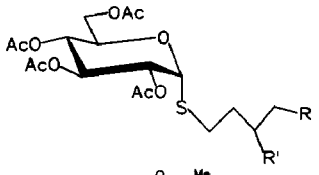
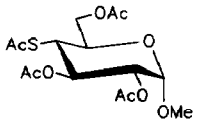


14 R, R' = 

15 R = R' = OH

16 R, R' = 

17 R = Br, R' = OBz



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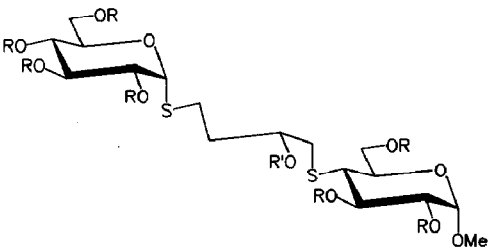
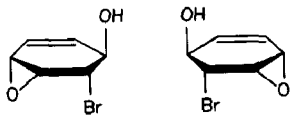


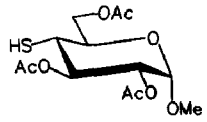
19 R = Ac, R' = Bz

20 R = R' = H

pounds tested were resistant to the enzyme and did not behave as ordinary competitive inhibitors. The K_i values decreased, to a greater or lesser extent, with increase in the time of incubation, and increased with increase in the concentration of the inhibitor. This phenomenon may be explained by depletion of cleavable substrate through transfer to the inhibitor and abnormal binding of the inhibitor. This speculation is supported by the fact that the abnormal behaviour was observed only when the inhibitor had a low d.p. and could occupy only part of the subsite. With short incubation times, however, linearity was observed and an apparent K_i value could be determined. It is remarkable that the spacer-modified disaccharide glycoside **20** as well as the two diastereomeric pseudo-trisaccharide glycosides **29a/b** have affinities for alpha-amylase that were higher than that of maltose. This situation is again a clear demonstration of the clustering effect caused by the linking of two glucosyl units by a spacer of suitable length. The added



21a/b



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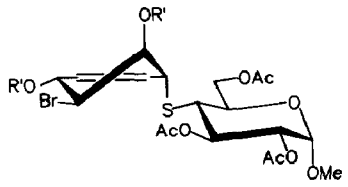
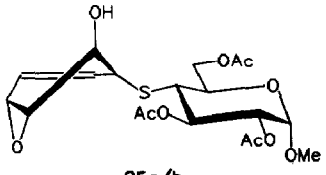
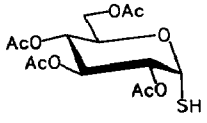


23a/b R = Ac, R' = OH

24a/b R = Ac, R' = Ac



25a/b



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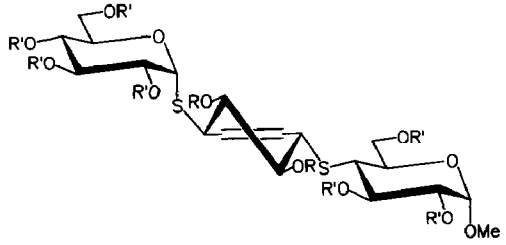

27a/b R = H, R' = Ac

28a/b R = R' = Ac

29a/b R = R' = H

polarity of the spacer may increase the binding energy. Whereas **20**, with the flexible spacer, has the same affinity regardless of the stereochemistry of the butyl moiety, the diastereomers **29a/b**, with the fairly rigid spacer, show significant differences in binding. This finding points to the importance of properly oriented hydroxyl groups. The comparably low affinity of the 4,4'-dithio analogue **12** of methyl maltotrioside can be assigned to its increased overall length by 2 Å for the more favorable conformation[12], which may prevent the optimised, "natural" fit of three (1→4)-linked α-D-glucopyranosyl units in three of the five subsites of porcine pancreatic alpha-amylase, since methyl α-maltotrioside has a K_i value[13] of 3mM.

The K_i values (mM) were: **12**, 9; **20**, 17; **29a**, 11; **29b**, 16; methyl α-maltotrioside[13], 3, and maltose[14], 20.

EXPERIMENTAL

General methods. — Melting points are uncorrected, optical rotations were measured with a Polartronic I spectrometer (Schmidt and Haensch). All reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck) with the solvents indicated. Flash-column chromatography was performed on ICN silica gel (32–63, 60A). ^{1}H-N.m.r. spectra were recorded with Bruker WM 250 and AM 400 spectrometers on solutions in $CDCl_3$ (internal Me_4Si). ^{13}C-N.m.r. spectra (75 MHz) were recorded with a Bruker AC 300 spectrometer. Kinetic data were determined with an Eppendorf photometer (405 nm) with a transformation unit and Siemens Kompensograph X-T C-1011. Usual work-up means dilution of the reaction mixture with CH_2Cl_2 (100 mL), and successive washings with ice-cold aqueous $KHSO_4$ (100 mL, 10%), saturated aqueous $NaHCO_3$ (100 mL), and ice-cold water (100 mL). Aqueous washings were extracted with CH_2Cl_2 (2 × 100 mL), the combined extracts were dried (Na_2SO_4) and filtered, and the solvent was evaporated under diminished pressure.

Enzymes. — Alpha-amylase [(1→4)-α-D-glucan glucanohydrolase, EC 3.2.1.1, 1260 U/mg] from porcine pancreas was purchased from Boehringer Mannheim.

Determination of the inhibition constants (K_i). — *p*-Nitrophenyl α-maltotrioside (Boehringer Mannheim) was used as substrate (0.17–5.3mM, K_m 2.2mM) in triethanolamine–triethanolamine · HCl buffer (pH 7.0, 10mM $CaCl_2$) at 30°. Inhibitors were used in the following concentrations (mM): **12**, 0.25–22; **20**, 1–19; **29a**, 3.0; **29b**, 3.2. Each assay involved 15 U/mL of alpha-amylase.

*Triphenylmethyl 2,3,6-tri-*O*-benzoyl-1-thio-α-D-galactopyranoside* (**4**). — Benzoyl chloride (5.0 mL, 43.6 mmol) was added dropwise to a cold solution of triphenylmethyl 1-thio-α-D-galactopyranoside[5] (**3**; 5.78 g, 13.2 mmol) in pyridine (150 mL) at −15°. The mixture was stored overnight at room temperature, then treated with MeOH (5 mL). The usual work-up and two successive column chromatographies (EtOAc–light petroleum, 1:3) gave **4** as an oil (8.84 g, 89%), $[\alpha]_D$ +123° (*c* 1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 61.8 (C-6), 67.5, 67.6, 69.3, 71.4 (C-2,3,4,5), 83.5 (C-1). For the ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{46}H_{38}O_8S$: C, 73.58; H, 5.10; S, 4.27. Found: C, 72.95; H, 5.18; S, 4.77.

*Triphenylmethyl 2,3,6-tri-*O*-benzoyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-1,4-dithio-α-D-glucopyranoside* (**6**). — To an ice-cold solution of **4** (1.92 g, 2.56 mmol) in CH_2Cl_2–pyridine (30 mL, 14:1) was added trifluoromethanesulfonic anhydride (850 μL, 5.12 mmol), and the mixture was stored for 30 min at 0° and for 1 h at room temperature. The usual work-up gave the amorphous 4-triflate **5** (2.26 g, 2.54 mmol), to a solution of which in hexamethylphosphoric triamide (8 mL) were added successively 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose[7] (**2**; 865 mg, 2.13 mmol), 1,4-dithioerythritol (325 mg, 2.13 mmol), and cysteamine (162 mg, 2.3 mmol)[4]. The mixture was kept for 30 min at room temperature, more cysteamine (81 mg 1.15 mmol) was added, and the mixture was stirred at room temperature for 2 h, then poured into ice–water. The solid was collected on Celite and washed with water, and a

TABLE I

^{1}H-N.m.r. data for **4, 6, 8, 11**

Atom	*Chemical shifts (δ, p.p.m.)*			
	4	**6**	**8**	**11**
H-1	5.26 d	5.21 d	5.44 d	4.86 d
H-2	5.87 dd	5.27 dd	5.46 dd	4.75 dd
H-3	5.57 dd	5.86 t	5.70 t	5.49 t
H-4	4.32 m	3.31 t	3.32 t	2.93 t
H-5	4.67 o	4.50 dt	4.24 m	3.83 o
H-6a	4.50 dd	4.57 dd	4.80 dd	4.65 dd
H-6b	4.10 dd	4.36 dd	4.55 dd	4.54 dd
H-1′		5.77 d	5.80 d	5.67 d
H-2′		4.87 dd	4.92 dd	4.83 dd
H-3′		5.13 dd	5.15 dd	5.31 t
H-4′		4.95 t	4.27 m	2.99 t
H-5′		4.23 m	4.27 m	4.30 m
H-6′a		4.19 dd	4.27 w	4.30 m
H-6′b		3.83 dd	4.00 dd	4.30 m
H-1″				5.84 d
H-2″				4.94 dd
H-3″				5.23 t
H-4″				5.03 t
H-5″				4.15 m
H-6″a				4.20 m
H-6″b				4.10 m
	J values (Hz)			
	4	**6**	**8**	**11**
$J_{1,2}$	5.5	6.0	5.5	3.5
$J_{2,3}$	11.0	10.5	10.0	10.0
$J_{3,4}$	3.0	10.5	10.0	10.0
$J_{4,5}$	2.0	10.5	10.5	10.5
$J_{5,6a}$	9.0	3.0	2.5	–
$J_{5,6b}$	5.0	2.5	2.5	–
$J_{6a,6b}$	11.0	12.0	12.0	–
$J_{1',2'}$		6.0	6.0	6.0
$J_{2',3'}$		10.5	10.5	10.0
$J_{3',4'}$		9.5	9.5	10.0
$J_{4',5'}$		9.5	9.5	10.5
$J_{5',6'a}$		3.5	–	–
$J_{5',6'b}$		2.5	4.0	–
$J_{6'a,6'b}$		13.0	14.0	–
$J_{1'',2''}$				6.0
$J_{2'',3''}$				10.5
$J_{3'',4''}$				9.5
$J_{4'',5''}$				9.5
$J_{5'',6''a}$				–
$J_{5'',6''b}$				–
$J_{6''a,6''b}$				–

solution in CH_2Cl_2 (100 mL) was extracted with ice–water (100 mL) and then concentrated. Column chromatography (EtOAc–light petroleum, 1:2) of the product gave **6** (1.74 g, 75%), $[\alpha]_D$ +216° (*c* 0.7, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 45.4 (C-4), 61.3 (C-6'), 63.9 (C-6), 67.8, 68.6, 69.5, 70.0, 70.5, 72.1, 73.4 (C-2,3,4,5 and C-2',3',4',5'), 82.5 (C-1), 83.3 (C-1'). For the ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{60}H_{56}O_{16}S_2$: C, 65.68, H, 5.14; S, 5.84. Found: C, 65.46; H, 4.99; S, 5.91.

1-S-Acetyl-2,3,6-tri-O-benzoyl-4-S-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-1,4-dithio-α-D-glucopyranose (**8**). — A solution of **6** (929 mg, 0.87 mmol) in CH_2Cl_2–MeOH (36 mL, 1:2) containing phenylmercury(II) acetate (292 mg, 0.87 mmol) was kept for 2 h at room temperature, then co-concentrated twice with toluene. H_2S was bubbled for 15 min through a solution of the residue in CH_2Cl_2 (20 mL) containing pyridine (1 mL) and Ac_2O (0.5 mL), and the mixture was then stored for 1 h at room temperature. Co-concentration with toluene and column chromatography (EtOAc–light petroleum, 1:2) gave **8** as an oil (721 mg, 95%, $[\alpha]_D$ +194° (*c* 1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 45.2 (C-4), 61.6 (C-6'), 64.1 (C-6), 67.9, 68.9, 69.5, 70.0, 71.3, 72.3, 73.7 (C-2,3,5 and C-2',3',4',5'), 80.7 (C-1), 83.5 (C-1'). For the ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{43}H_{44}O_{17}S_2$: C, 57.58; H, 4.94; S, 7.15. Found: C, 57.13; H, 5.14; S, 6.97.

Methyl 2,3,6,2',3',6',2'',3'',4'',6''-deca-O-acetyl-4,4'-dithio-α-maltotrioside (**11**). — 1,4-Dithioerythritol (53 mg, 0.41 mmol) and cysteamine (27 mg, 0.47 mmol) were added successively to a solution of **8** (310 mg, 0.34 mmol) and crude methyl 2,3,6-tri-*O*-benzoyl-4-*O*-trifluoromethanesulfonyl-α-D-galactopyranoside[7] (**9**; 265 mg, 0.41 mmol) in hexamethylphosphoric triamide (4 mL). The mixture was stored for 1 h at room temperature, more cysteamine (27 mg, 0.47 mmol) was added, and, after 1 h, the mixture was poured into ice–water. The usual work-up and column chromatography (EtOAc–light petroleum, 2:3) of the product gave **10** (272 mg 58%). ^{13}C-N.m.r. data ($CDCl_3$): δ 45.2, 45.6 (C-4,4'), 55.4 (CH_3), 61.3 (C-6''), 63.8, 64.5 (C-6,6'), 67.8, 68.4, 68.8, 69.4, 70.0 (2), 71.4, 72.3, 72.6, 73.2 (C-2,3,5, C-2',3',5', and C-2'',3'',4'',5''), 83.4, 83.5 (C-1',1''), 97.0 (C-1).

Deprotection of **10** (500 mg, 0.37 mmol) was carried out conventionally in MeOH (50 mL) and methanolic M sodium methoxide (0.5 mL). The solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness, and the residue was acetylated with pyridine–Ac_2O (5 mL, 1:1). After the usual work-up, column chromatography (EtOAc–light petroleum, 1:1) of the product gave **11** as an oil (326 mg, 90%), $[\alpha]_D$ +275° (*c* 0.7, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 43.7 (C-4'), 44.2 (C-4), 55.3 (CH_3), 61.5 (C-6''), 63.3, 63.8 (C-6,6'), 67.9, 68.1, 68.7, 69.4, 69.8, 70.3, 71.7, 72.0, 72.1, 72.5 (C-2,3,5, C-2',3',5', and C-2'',3'', 4'',5''), 82.4 (C-1''), 82.5 (C-1'), 96.9 (C-1). For the ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{39}H_{54}O_{24}S_2$: C, 48.24; H, 5.60; S, 6.60. Found: C, 48.26; H, 5.52; S, 6.75.

Methyl 4,4'-dithio-α-maltotrioside (**12**). — A solution of **11** (276 mg, 0.28 mmol) in

MeOH (50 mL) was treated conventionally with methanolic M sodium methoxide (50 μL). The mixture was neutralised, the solvent was evaporated, and a solution of the residue in water was freeze-dried to give **12** (156 mg), $[\alpha]_D$ + 370° (*c* 1, methanol). ^{13}C-N.m.r. data (D_2O): δ 46.6, 46.7 (C-4,4'), 55.0 (CH_3), 60.4 (C-6''), 61.4, 61.5 (C-6,6'), 69.4, 70.6, 71.0, 72.0, 72.2 (2), 72.6, 72.9 (2), 73.4 (C-2,3,5,C-2',3',5', and C-2'',3'',4'',5''), 85.6, 85.7 (C-1',1''), 99.3 (C-1).

Anal. Calc. for $C_{19}H_{34}O_{14}S_2 \cdot 2H_2O$: C, 38.90; H, 6.53; S, 10.93. Found: C, 39.23; H, 6.67; S, 11.60.

*(3R,S)-3,4-Isopropylidenedioxybutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**14**). — A solution of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-α-D-glucopyranose[7] (2.2 g, 4.9 mmol) in MeOH (50 mL) containing methanolic M sodium methoxide (5.5 mL) was kept at room temperature overnight, then concentrated. The residue was dried *in vacuo* over phosphorus pentaoxide and to a solution in hexamethylphosphoric triamide (6 mL) was added the tosylate **13**[15] (1.44 g, 4.9 mmol). The mixture was stirred overnight at room temperature, Ac_2O–pyridine (1:1, 24 mL) was added, and the mixture was kept at 60° for 10 h. The mixture was diluted with EtOAc (100 mL), and the organic layer was washed successively with ice-cold aqueous 10% $KHSO_4$, saturated aqueous $NaHCO_3$, and ice–water. The aqueous washings were back-extracted with EtOAc, and the combined extracts were concentrated. Column chromatography (EtOAc–light petroleum, 1:3) of the residue gave **14** (2.0 g, 84%), $[\alpha]_D$ + 148° (*c* 0.97, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 26.0 (C-2'), 33.4 (C-1'), 61.6 (C-6), 67.6, 68.6, 69.0, 70.5, 70.7 (C-2,3,4,5 and C-3',4'), 81.7 (C-1).

Anal. Calc. for $C_{21}H_{32}O_{11}S$: C, 51.21; H, 6.55; S, 6.50. Found: C, 50.80; H, 6.39; S, 6.24.

*(3R,S)-3,4-Dihydroxybutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**15**). — To a solution of **14** (1.02 g, 2.07 mmol) in dry MeCN (15 mL) was added aqueous tetrafluoroboric acid (35%, 0.7 mL). The mixture was kept at room temperature for 1 h, the reaction was quenched with triethylamine (1 mL), and the solution was concentrated. Column chromatography (EtOAc–light petroleum, 3:1) of the residue gave **15** (800 mg, 86%) as a syrup that was homogeneous in t.l.c. Although further column chromatography (Et_2O) did not give an analytical sample, the product had $[\alpha]_D$ + 154° (*c* 0.38, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 25.9 (C-2'), 32.3 (C-1'), 61.6 (C-6), 66.2, 67.4, 68.5, 70.3, 70.4, 70.6 (C-2,3,4,5 and C-3',4'), 81.5 (C-1).

Anal. Calc. for $C_{18}H_{28}O_{11}S$: C, 47.78; H, 6.24; S, 7.08. Found: C, 49.06; H, 6.62; S, 6.91.

*(3R,S)-3,4-benzylidenedioxybutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**16**). — To a solution of **15** (300 mg, 0.66 mmol) in dry $CHCl_3$ (30 mL) containing camphorsulfonic acid (10 mg) was added α,α-dimethoxytoluene[9] (0.7 mL). The mixture was boiled under reflux for 3 h. Solid K_2CO_3 (750 mg) was then added, and the suspension was boiled under reflux for 30 min, filtered through Celite, and concentrated. Column chromatography (EtOAc–light petroleum, 1:2) of the residue gave **16** (324 mg, 90%), $[\alpha]_D$ + 153° (*c* 0.74, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 25.6, 25.7 (C-2'), 32.6, 33.0 (C-1'), 61.6 (C-6), 67.4, 66.3, 68.3, 69.5, 70.0, 70.2, 70.4, 74.6, 75.1, 76.4 (C-2,3,4,5 and C-3',4'), 81.3 (C-1), 102.9, 103.8 (C-5').

Anal. Calc. for $C_{25}H_{32}O_{11}S$: C, 55.54; H, 5.97; S, 5.93. Found: C, 55.63; H, 6.00; S, 5.59.

*Methyl 2,3,6-tri-*O*-acetyl-4-*S*-[2-benzoyloxy-4-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosylthiobutyl)]-4-thio-α-*D*-glucopyranoside* (**19**). — To a solution of **16** (483 mg, 0.89 mmol) in dry CH_2Cl_2 (70 mL) was added $BaCO_3$ (1.78 g) and recrystallised *N*-bromosuccinimide[16] (205 mg). The mixture was boiled under reflux for 3.5 h, then filtered through Celite, washed with ice-cold water, and concentrated. Column chromatography (EtOAc–light petroleum, 1:3) of the residue afforded **17** (443 mg, 80%). ^{13}C-N.m.r. data ($CDCl_3$): δ 25.4 (C-2'), 32.2 (C-4'), 33.5 (C-1'), 61.7 (C-6), 67.6, 68.3, 70.3, 71.2 (C-2,3,5 and C-3'), 81.6 (C-1).

A solution of methyl 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-*D*-glucopyranoside[17] (**18**; 475 mg, 1.2 mmol) in MeOH (2 mL) containing methanolic M sodium methoxide (1.4 mL) was kept at room temperature overnight, and then the solvent was evaporated. The alcohol-free residue was added to a solution of **17** (400 mg, 0.64 mmol) in hexamethylphosphoric triamide (4 mL). The mixture was stirred at room temperature for 10 h, Ac_2O–pyridine (6 mL, 1:1) was added, and the mixture was kept for 6 h, then diluted with EtOAc (50 mL), washed with ice-cold aqueous $KHSO_4$ (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and water (50 mL), then concentrated. Column chromatography (EtOAc–light petroleum, 1:2) of the residue gave **19** (482 mg, 85%). Further column chromatography (Et_2O) did not give an analytical sample; **19** was characterised by ^{13}C-n.m.r. data and m.s. ^{13}C-N.m.r. data ($CDCl_3$): δ 25.5 (C-3'), 32.5, 33.6 (C-1',4'), 46.3 (C-4), 55.2 (CH_3), 61.6, 63.2 (C-6,6''), 67.5, 68.2, 68.3, 68.5, 68.8, 70.3, 70.4, 71.7, 71.9 (C-2,3,5, C-2',3', and C-2'',3'',4'',5''), 81.6 (C-1''), 96.8 (C-1). Mass spectrum (d.c.i. – NH_3/isobutane): *m/z* 892 $[M + NH_3]^+$,

*Methyl 4-*S*-(4-α-*D*-glucopyranosylthio-2-hydroxybutyl)-4-thio-α-*D*-glucopyranoside* (**20**). — To a solution of **19** (482 mg, 0.54 mmol) in MeOH (2 mL) was added methanolic M sodium methoxide (60 μL). The solution was kept for 12 h, neutralised with Amberlite IR-120 (H^+) resin, and concentrated. Column chromatography ($CHCl_3$–MeOH, 4:1) of the residue afforded **20** (120 mg). An analytical sample, obtained by preparative h.p.l.c. on a C-18 reversed-phase column by elution with aqueous methanol (10%), had $[\alpha]_D$ +142° (*c* 0.5, water). N.m.r. data (D_2O): ^{13}C, δ 26.8 (C-3'), 36.1, 37.8 (C-1',4'), 48.9 (C-4), 55.9 (CH_3), 61.4, 62.3 (C-6,6''), 70.3, 70.6, 71.4, 72.4, 73.2, 74.6 (C-2,3,5, C-2',3', and C-2'',3'',4'',5''), 86.2 (C-1'), 100.2 (C-1); ^{1}H, δ 1.75 (m, 1 H), 2.6 (m, 4 H), 3.29 (m, 4 H, CH_3 and H-4), 3.45 (m, 2 H), 3.7 (m, 5 H), 3.9 (m, 4 H), 4.75 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.32 (d, 1 H, $J_{1',2'}$ 5 Hz, H-1').

Anal. Calc. for $C_{17}H_{32}O_{11}S_2$: C, 42.84; H, 6.77; S, 13.45. Found: C, 42.72; H, 6.89; S, 12.83.

*Methyl 2,3,6-tri-*O*-acetyl-4-thio-α-*D*-glucopyranoside* (**22**). — A solution of methyl 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-*D*-glucopyranoside[17] (584 mg, 1.5 mmol) in hexamethylphosphoric triamide (4 mL) was treated with cysteamine (145 mg, 1.8 mmol) and 1,4-dithioerythritol (240 mg, 1.5 mmol). The mixture was kept for 1 h at room temperature, diluted with EtOAc (50 mL), and washed with ice-cold, saturated aqueous $NaHCO_3$ (50 mL), and ice–water (50 mL). The aqueous layers were back-extracted with

EtOAc, and the combined organic layers were dried, and concentrated. Column chromatography (EtOAc–light petroleum, 1:2) of the residue yielded **22** (467 mg, 90%), [α][$_D$ + 89° (*c* 1.6, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 40.7 (C-4), 55.3 (CH_3), 63.3 (C-6), 71.2, 71.8, 71.9 (C-2,3,5), 97.0 (C-1).

Anal. Calc. for $C_{13}H_{20}O_8S$: C, 46.42; H, 5.99; S, 9.53. Found: C, 46.16; H, 6.05; S, 9.71.

*Methyl 2,3,6-tri-*O*-acetyl-4-*S*-[(1,4/5,6)- and (5,6/1,4)-4,6-diacetoxy-5-bromo-2-cyclohexen-1-yl]-4-thio-α-*D*-glucopyranoside* (**24a/b**). — To a solution of **22** (400 mg, 1.0 mmol) and [(1/4,5,6)- and (4,5,6/1)]-6-bromo-4,5-epoxy-2-cyclohexen-1-ol[10] (**21a/b**; 267 mg, 11.3 mmol) in MeOH (2 mL) was added water dropwise to turbidity. After 2 days, the mixture was concentrated and column chromatography (cyclohexane–EtOAc, 1:1) yielded **23a** (40 mg), **23b** (40 mg), and **23a/b** (514 mg) (combined yield, 89%), R_F **23a** 0.41, R_F **23b** 0.32 (cyclohexane–EtOAc, 1:1; run 3 times).

Acetylation of **23a** (20 mg, 38 μmol) in Ac_2O–pyridine (1:2) gave, after evaporation of the solvent, co-concentration with toluene (2 × 1 mL), and column chromatography (cyclohexane–EtOAc, 3:1) of the residue, **24a** (17 mg, 73%), m.p. 114°, [α]$_D$ + 74° (*c* 0.8, ethyl acetate). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{23}H_{31}BrO_{12}S$: C, 45.18; H, 5.11; S, 5.24. Found: C, 45.23; H, 4.90; S, 5.07.

Acetylation of **23b** (21 mg, 40 μmol) gave syrupy **24b** (18 mg, 74%), R_F 0.48 (cyclohexane–EtOAc, 1:1), [α]$_D$ + 110° (*c* 1.0, ethyl acetate).

*Methyl 2,3,6-tri-*O*-acetyl-4-*S*-[(1,5/4,6)- and (4,6/1,5)-5,6-diacetoxy-4-α-*D*-glucopyranosylthio-2-cyclohexen-1-yl]-4-thio-α-*D*-glucopyranoside* (**28a/b**). — To **23a/b** (400 mg, 0.76 mmol) and KOH (5 mg) in dry CH_2Cl_2 (12 mL) was added tetraethylammonium chloride (3 mg, 0.9 mmol). The mixture was stirred vigorously for 4 h, then filtered, and concentrated to yield amorphous **25a/b** (250 mg, 73%), R_F 0.17 (cyclohexane–EtOAc, 1:2), which was used immediately in the next step.

To a solution of **25a/b** (250 mg, 0.56 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside[6] (**26**; 220 mg, 6 mmol) in MeOH (2 mL) were added 2 drops of water. After 3 days, the mixture was concentrated and column chromatography (cyclohexane–EtOAc, 1:2) of the residue yielded **27a/b** as a colorless syrup (190 mg, 45%), R_F 0.21 (cyclohexane–EtOAc, 1:2). The diastereomers were isolated by h.p.l.c. on a column (250 × 20 mm) of Hypersil (5 μm) with cyclohexane–EtOAc (2:3) at 20 mL/min.

A solution of **27b** (51 mg, 63 μmol) in Ac_2O–pyridine (6 mL, 1:2) was kept for 5 h, then concentrated, and co-concentrated with toluene (3 mL). Column chromatography (cyclohexane–EtOAc, 1:1) of the residue gave **28b** (50 mg, 88%), R_F 0.21 (cyclohexane–EtOAc, 1:2), m.p. 205°, [α]$_D$ + 83° (*c* 1.0, ethyl acetate).

Anal. Calc. for $C_{37}H_{50}O_{21}S$: C, 49.66; H, 5.63. Found: C, 49.58; H, 5.68.

Acetylation of **27a** (50 mg, 62 μmol) gave syrupy **28a** (41 mg, 72%), [α]$_D$ + 255° (*c* 1.1, ethyl acetate). For the ^{1}H-n.m.r. data see Table II.

*Methyl 4-*S*-[(1,5/4,6)- and (4,6/1,5)-4-α-*D*-glucopyranosylthio-5,6-dihydroxy-2-cyclohexen-1-yl]-4-thio-α-*D*-glucopyranoside* (**29a/b**). — Compounds **28a** (25 mg, 31 μmol) and **28b** (27 mg, 33 μmol) were each dissolved in dry MeOH (2 mL), and

TABLE II

^{1}H-N.m.r. data for **24a/b** and **28a/b**

Atom	*Chemical shifts (δ, p.p.m.)*			
	24a	**24b**	**28a**	**28b**
H-1	5.20 m	5.29 m	5.10 m	5.05 t
H-2	4.51 dd	4.51 dd	5.12 m	5.23 t
H-3	5.64 m	5.63 m	3.58 m	3.57 m
H-4	5.75 m	5.78 m	5.71 m	5.87 dt
H-5	5.68 m	5.69 m	5.71 m	5.53 dt
H-6	3.57 m	3.70 m	3.56 m	3.53 m
H-1′	4.95 d	4.94 d	4.90 d	4.90 d
H-2′	4.89 dd	4.87 dd	4.83 dd	4.85 dd
H-3′	5.34 dd	5.48 t	5.39 t	5.33 dd
H-4′	3.14 t	3.20 t	3.03 t	2.86 t
H-5′	4.04 ddd	3.87 ddd	3.95 ddd	3.89 ddd
H-6′a	4.45 dd	4.42 dd	4.42 dd	4.35 dd
H-6′b	4.65 dd	4.63 dd	4.56 dd	4.63 dd
H-1″			5.74 d	5.85 d
H-2″			5.02 dd	4.91 dd
H-3″			5.28 dd	5.27 t
H-4″			5.03 t	4.97 t
H-5″			4.36 ddd	3.59 ddd
H-6″a			4.10 dd	4.16 dd
H-6″b			4.29 dd	4.29 dd
OCH_3	3.42 s	3.41 s	3.39 s	3.38 s
OAc	2.08–2.18	2.08–2.18	2.01–2.13	2.01–2.15
	5 × s	5 × s	9 × s	9 × s
	J values (Hz)			
	24a	**24b**	**28a**	**28b**
$J_{1,2}$	2.3	3.0	–	10.0
$J_{2,3}$	8.9	7.5	–	9.2
$J_{3,4}$	3.8	3.8	–	2.7
$J_{4,5}$	9.0	10.5	–	9.9
$J_{5,6}$	2.2	2.9	–	2.3
$J_{6,1}$	3.3	3.8	–	10.0
$J_{1',2'}$	3.8	3.8	3.5	3.3
$J_{2',3'}$	10.2	10.2	10.5	9.3
$J_{3',4'}$	10.8	10.2	10.5	10.8
$J_{4',5'}$	10.8	10.2	10.5	10.8
$J_{5',6'a}$	2.6	1.8	2.3	1.8
$J_{5',6'b}$	4.5	3.8	3.9	4.2
$J_{6'a,6'b}$	12.0	12.3	12.0	12.0
$J_{1'',2''}$			6.0	5.7
$J_{2'',3''}$			10.5	10.2
$J_{3'',4''}$			9.75	10.2
$J_{4'',5''}$			9.75	10.2
$J_{5'',6''a}$			2.3	3.8
$J_{5'',6''b}$			4.7	4.7
$J_{6''a,6''b}$			11.25	12.8

methanolic M sodium methoxide (3 drops) was added. Filtration through a short column of silica gel, evaporation of the solvent, and lyophylisation yielded **29a** (12.7 mg, 81%) and **29b** (13 mg, 78%), R_F 0.22 for **29a/b** (EtOAc–CH_3OH–H_2O, 7:2:1).

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