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Photolabile derivatives of maltose and maltotriose as ligands for the affinity labelling of the maltodextrin-binding site in porcine pancreatic alpha-amylase

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ABSTRACT

The 3-azibutyl group was linked through sulfur to the anomeric position of maltose and maltotriose to yield the photolabile thioglycosides 3-azibutyl 1-thio-α-maltoside (**11**) and 3-azibutyl 1-thio-α-maltotrioside (**12**), and to the 4′- and 6′-position of maltose to give the thioethers 4′-*S*-(3-azibutyl)-4′-thiomaltose (**8**) and 6′-*S*-(3-azibutyl)-6′-thiomaltose (**15**). All four compounds were good competitive inhibitors of the action of porcine pancreatic alpha-amylase. Compound **12** irreversibly deactivated the enzyme to ~ 100% when irradiated together with the protein. The other compounds were much less effective. It is likely that separate areas of the enzyme binding site are chemically modified by the different ligands.

INTRODUCTION

Porcine pancreatic alpha-amylase has a binding site which can accommodate five (1→4)-linked α-D-glucopyranosyl residues and which resembles a groove, open at both ends[1]. For the covalent modification of separate parts of the binding area, the reactive unit should be attached either to the reducing or to the non-reducing end of a malto-oligosaccharide molecule. As enzyme-resistant models of longer-chain photolabile probes, which may be susceptible to hydrolysis, maltose derivatives with a photolabile unit attached at either end of the molecule have been prepared for affinity labelling.

RESULTS AND DISCUSSION

The 3-azibutyl group, as a unit for photoaffinity labelling, can be attached most conveniently to a monosaccharide by preparing a thioglycoside with that group as an aglycon. 3-Azi-1-(*p*-toluenesulfonyloxy)butane[2] reacts smoothly with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose or -galactopyranose[3] to yield the photolabile β-thioglycosides[4]. For the formation of 3-azibutyl 1-thio-α-D-glucopyranoside (**10**), 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose[5] was reacted with the more reactive 3-azi-1-

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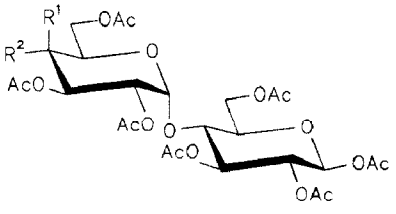
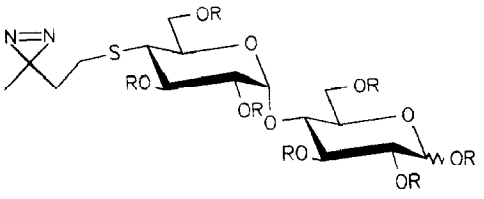
(trifluoromethanesulfonyloxy)butane[6]. Apparently, the axial sulfhydryl group is less accessible to alkylation. Compound **10** can be glycosylated using first CGT-ase and cyclomaltohexaose (α-cyclodextrin) for elongation of the chain and then with beta-amylase for trimming. The two major products of this enzymic reaction sequence are 3-azibutyl 1-thio-α-maltoside (**11**) and 3-azibutyl 1-thio-α-maltotrioside (**12**).

For the preparation of 6′-thiomaltose, 6′-*O*-tosylmaltose[7] was treated with potassium thiobenzoate. Saponification of the thioester and subsequent alkylation with 3-azi-1-(*p*-toluenesulfonyloxy)butane yielded 6′-*S*-(3-azibutyl)-6′-thiomaltose (**15**).

By a sequence of activation and displacement reactions, 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranose (**1**), obtained by selective acetylation of 1,2,3,6,2′,3′-hexa-*O*-acetyl-β-maltose[7], was converted into 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-glucopyranosyl)-β-D-glucopyranose (**5**). Compound **5** was deprotected selectively to give 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-thio-α-D-glucopyranosyl)-β-D-glucopyranose (**6**), which was *S*-alkylated with 3-azi-1-(trifluoromethanesulfonyloxy)butane to give **7**. Deprotection of **7** yielded 4′-*S*-(3-azibutyl)-4′-thiomaltose (**8**).





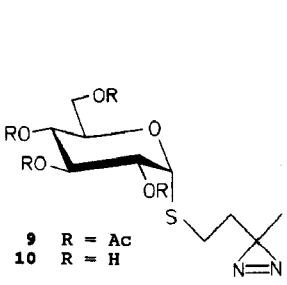
1 R^1 = H R^2 = OH
2 R^1 = H R^2 = OTf
3 R^1 = OH R^2 = H
4 R^1 = OTf R^2 = H
5 R^1 = H R^2 = SAc
6 R^1 = H R^2 = SH

7 R = Ac
8 R = H

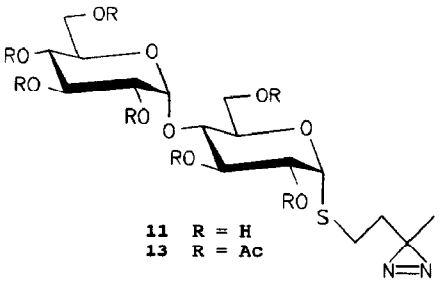
Compounds **8**, **11**, **12**, and **15** had remarkable affinities for porcine pancreatic alpha-amylase compared with those of maltose and maltotriose; K_i values (mM): **8**, 2.6; **11**, 1.8; **12**, 1.5; **15**, 2.9; maltose, 20; and maltotriose, 1.8.

Although these derivatives, except perhaps **12**, are too short to bind in one stable mode only, it may be assumed that the thioglycosides **11** and **12** may, with their photolabile diazirino group, reach the left-hand part of the binding site, and the thioethers **8** and **15** the right-hand part. That these ligands can be used to chemically modify different areas of the binding site of the enzyme was supported by the finding that the degree of irreversible inhibition of alpha-amylase by photoaffinity labelling depends on the compounds used. Although the inhibition constants of the thioglycosides **11** and **12** are similar, as are those of the thioethers **8** and **15**, the extents of irriversible inhibition are markedly different as shown in Table I.

The irriversible deactivation of alpha-amylase by **12** was almost complete. In the presence of a large excess of maltotriose as a competitive inhibitor, irreversible deactivation of the enzyme by photoaffinity labelling was prevented or greatly reduced.



9 R = Ac
10 R = H



11 R = H
13 R = Ac

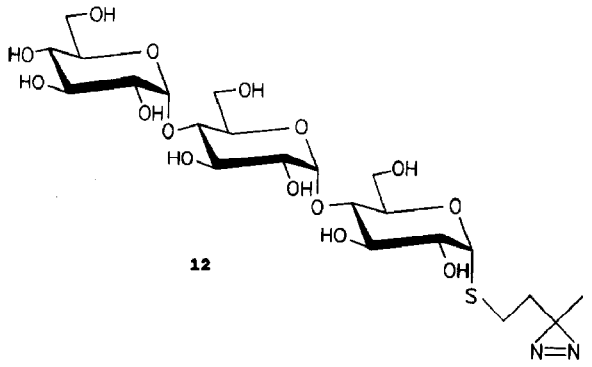


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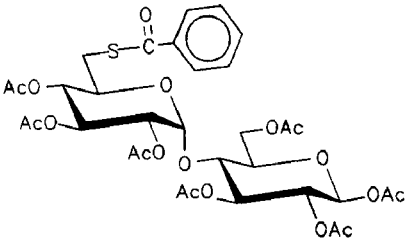
TABLE I

Deactivation of alpha-amylase by photolabile derivatives of maltose and maltotriose derivatives

Compound	*Concentration*	*Remaining activity (%) of the alpha-amylase*	
			With 0.5M maltotriose
8	10 K_i	73	100
11	10 K_i	90	100
12	5 K_i	0.1	70
15	10 K_i	66	100
—	—	100[a]	100[a]

[a] Reference for enzymic activity after irradiation and dialysis.



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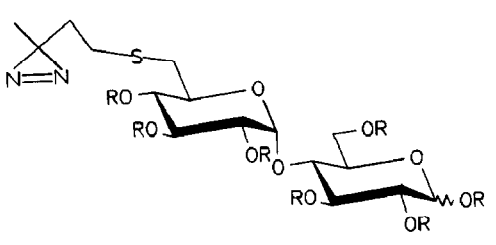

15 R = H
16 R = Ac

EXPERIMENTAL

General methods. — Silica gel 32–63, 60 A (ICN) was used for column chromatography. All reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck). Melting points were determined with a Büchi apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter, and extinction coefficients and u.v. spectra with a Zeiss PMQ II spectrophotometer. Kinetic data were obtained with an Eppendorf photometer (405 nm) with a transformation unit and an Se 120 recorder. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 spectrometer for solutions in $CDCl_3$ (internal Me_4Si), and ^{13}C-n.m.r. spectra with a Bruker AM 300 spectrometer. Photolabile compounds were irradiated with a Rayonet RPR reactor equipped with 16 lamps (RPR 3500 Å). Elemental analyses were carried out with a Perkin–Elmer 240 analyser.

Enzymes. — CGT-ase [(1→4)-α-D-glucan 4-α-D-glucanotransferase, cyclising, EC 2.4.1.19, 760 U/mL] from *Bacillus macerans* was a gift from Boehringer Mannheim. Alpha-amylase [(1→4)-α-D-glucan glucanohydrolase, EC 3.2.1.1, 1260 U/mg] from porcine pancreas and beta-amylase [(1→4)-α-D-glucan malthohydrolase, EC 3.2.1.2, 845 U/mg] from *Ipomoea batatas* were purchased from Boehringer Mannheim.

Determination of the inhibition constants (K_i). — *p*-Nitrophenyl α-maltotrioside (Boehringer Mannheim) was used as substrate (0.2–5.4mM, K_m 2.0–2.1mM) in 50mM triethanolamine–triethanolamine·HCl buffer (pH 7.0, 10mM $CaCl_2$) at 30°. Inhibitors were used in the following concentrations (mM): **11** 0.5, 1.0, 1.5, 3.0, 5.0; **12** 0.5, 1.0, 2.0, 3.0; **8** 0.5, 1.0, 2.0, 4.0; **15** 0.5, 1.0, 2.0, 4.0; maltose 5.0, 10.0, 20; maltotriose 1.0, 3.0, 5.0. Each assay involved 15 U/mL of alpha-amylase.

Irreversible inhibition of alpha-amylase. — Each assay mixture, containing alpha-amylase (1 mg) dissolved in the above buffer (1 mL), photolabile compounds as indicated, and maltotriose (0.5M) when indicated, in a plastic tube, was flushed with nitrogen (10 min), closed, and irradiated for 25 min, then dialysed against buffer (4 × 250 mL, 3 × 2 h, 1 × 10 h) at 8°. Each solution was diluted to give a specific u.v. absorbance at 280 nm and the activity was determined as described above by incubating with 50-μL aliquots of the respective enzyme solutions.

1,2,3,6-Tetra-O-acetyl-4-O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-β-D-glucopyranose (**1**). — To a stirred solution of 1,2,3,6,2′,3′-hexa-*O*-acetyl-β-maltose[7] (10.7 g, 18.0 mmol) and 1-(acetoxy)benzotriazole[8] (3.49 g, 19 mmol) in CH_2Cl_2 (200 mL) was added triethylamine (2.87 mL, 21 mmol). The mixture was stirred at room temperature for 24 h, diluted with CH_2Cl_2 (200 mL), washed successively with ice-cold saturated aqueous $NaHCO_3$ (200 mL) and water (200 mL), and dried (Na_2SO_4), and the solvent was evaporated. Column chromatography of the residue (1:1 EtOAc–light petroleum) gave **1** (8.4 g, 73%), m.p. 175–177° (from Et_2O), $[\alpha]_D^{21}$ +46.5° (*c* 1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 62.4, 62.5 (C-6,6′), 66.7, 70.0, 70.9, 71.1, 71.8, 72.4, 73.1, 75.2 (C-2,2′, 3,3′, 4,4′, 5,5′), 91.2 (C-1′), 95.8 (C-1). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.7. Found: C, 48.78; H, 5.75.

TABLE II

^{1}H-N.m.r. data

Chemical shifts (δ, p.p.m.)								
Atom	**1**	**5**	**6**	**7**	**9**	**13**	**14**	**16**
H-1	5.74 d	5.75 d	5.72 d	5.75 d	5.63 d	5.51 d	5.73 d	6.25 d
H-2	4.98 dd	4.83 dd	4.79 dd	4.99 dd	5.00 dd	4.91 dd	5.00 dd	4.98 dd
H-3	5.28 t	5.21 t	5.14 t	5.30 dd	5.37 t	5.36 dd	5.35 dd	5.51 dd
H-4	4.08 t	4.01 t	3.87 dd	3.99 dd	5.02 t	3.91 dd	4.03 dd	4.03 dd
H-5	3.82 m	3.83 dq	3.73 m	3.83 ddd	4.42 ddd	4.03 m	4.09 m	3.97 ddd
H-6a	4.48 m	4.49 dd	4.17 dd	4.15 dd	4.08 dd	4.07 dd	4.25 dd	4.27 dd
H-6b	4.48 m	4.59 dd	4.36 m	4.45 dd	4.31 dd	4.25 dd	4.45 dd	4.49 dd
H-1′	5.33 d	5.40 d	5.28 d	5.36 d		5.39 d	5.37 d	5.40 d
H-2′	4.81 dd	4.70 dd	4.56 dd	4.80 dd		4.92 dd	4.83 dd	4.84 dd
H-3′	5.21 dd	5.36 dd	5.04 t	5.27 t		5.39 t	5.29 dd	5.37 dd
H-4′	3.52 m	3.66 t	2.85 q	2.69 t		5.01 t	4.97 dd	5.04 t
H-5′	3.82 m	4.17 m	3.69 m	3.93 m		4.35 m	3.81 m	4.13 m
H-6a′	4.21 dd	4.17 m	4.47 dd	4.43 m		4.28 dd	3.35 m	2.58 dd
H-6b′	4.21 dd	4.30 dd	4.52 m	4.43 m		4.43 dd	3.35 m	2.68 dd
H-1″				2.40 m	2.41 m	2.43 m		2.43 m
H-2″				1.57 m	1.63 m	1.68 m		1.63 m
H-4″				1.04 s	1.05 s	1.07 s		1.06 s
H-Ph							7.46 m 7.60 m 7.96 m	
OAc	2.00– 2.10 5 s	1.99– 2.10 6 s	2.00– 2.11 5 s	2.02– 2.14 7 s	2.01– 2.08 4 s	2.01– 2.17 7 s	1.99– 2.14 7 s	2.01– 2.23 7 s
SAc		2.26 s						
Coupling constants $J_{H,H}$ *(Hz)*								
1,2	8.0	8.0	8.0	8.1	6.0	6.0	8.3	3.6
2,3	9.0	9.0	9.0	9.2	9.8	9.9	9.0	10.4
3,4	9.0	9.0	9.0	8.9	9.8	8.3	8.1	8.3
4,5	9.0	9.0	9.5	9.2	9.8	8.3	8.7	9.9
5,6a	4.0	4.5	4.5	2.0	2.3	2.3	3.0	2.6
5,6b	—	3.0	2.0	4.5	5.2	3.8	4.5	3.8
6a,6b	12.0	12.5	12.0	12.2	12.0	12.5	12.0	12.5
1′,2′	4.0	4.0	3.5	4.2		3.9	4.2	3.9
2′,3′	10.0	10.0	10.5	10.4		9.8	10.5	10.7
3′,4′	9.5	11.0	10.5	10.5		9.8	9.0	9.8
4′,5′	—	11.0	10.5	10.7		9.8	9.8	9.8
5′,6a′	2.4	—	2.5	—		2.3	1.5	3.3
5′,6b′	5.0	4.5	4.0	—		3.8	4.5	6.2
6a′,6b′	12.0	12.5	12.0	—		12.5	0.5	14.3

1,2,3,6-Tetra-O-*acetyl*-4-O-*(2,3,6-tri*-O-*acetyl*-4-S-*acetyl*-4-*thio*-α-D-*glucopyranosyl)*-β-D-*glucopyranose* (**5**). — To an ice-cold solution of **1** (2.0 g, 3.2 mmol in CH_2Cl_2–pyridine (30:1, 31 mL) was added trifluoromethanesulfonic anhydride (1.15 mL). The mixture was stirred for 30 min at 0° and then for 1 h at room temperature. The crude triflate **2** was extracted and treated with sodium nitrite (663 mg, 9.6 mmol) as described[9]. Column chromatography (1:1 EtOAc–light petroleum) of the product gave **3** (1.3 g, 65%). ^{13}C-N.m.r. data ($CDCl_3$): δ 62.5, 62.6 (C-6,6'), 67.0, 67.1, 68.7, 69.2, 70.8, 72.1, 73.1, 75.1 (C-2,2', 3,3', 4,4', 5,5'), 91.1 (C-1'), 96.2 (C-1).

Compound **3** was triflated as described for **1**, and a solution of the product (**4**; 1.5 g, 1.9 mmol) in hexamethylphosphoric triamide (10 mL) was treated with potassium thioacetate (605 mg, 5.3 mmol) at room temperature for 3 h. The mixture was diluted with ether (200 mL), then washed with ice-cold water (100 mL), and the solvent was evaporated. Column chromatography (1:1 EtOAc–light petroleum) of the residue gave **5** (955 mg, 65%), $[\alpha]_D^{21}$ +66° (*c* 1.1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 30.6 (CH_3COS), 43.7 (C-4'), 62.4, 62.7 (C-6,6'), 67.3, 69.5, 70.9, 71.5, 72.3, 73.1, 75.2 (C-2,2', 3,3',4,5,5'), 91.3 (C-1'), 95.9 (C-1). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{28}H_{38}O_{18}S$: C, 48.40; H, 5.51; S, 4.61. Found: C, 48.23; H, 5.63; S, 4.81.

1,2,3,6-Tetra-O-*acetyl*-4-O-*(2,3,6-tri*-O-*acetyl*-4-*thio*-α-D-*glucopyranosyl)*-β-D-*glucopyranose* (**6**). — A solution of **5** (490 mg, 0.7 mmol) in hexamethylphosphoric triamide (4 mL) was treated with cysteamine (68 mg, 0.84 mmol) in the presence of 1,4-dithioerythritol (112 mg, 0.7 mmol). The mixture was kept for 2 h at room temperature, ice and water (10 mL) were added, and the solid was collected on Celite and washed with water (10 mL). A solution of the product in CH_2Cl_2 (50 mL) was washed with ice-cold water (30 mL), then dried (Na_2SO_4), and the solvent was evaporated. Column chromatography (1:2 EtOAc–light petroleum) of the oily residue yielded **6** (300 mg, 66%), $[\alpha]_D^{21}$ +47° (*c* 1.3, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 40.4 (C-4'), 62.4, 63.0 (C-6,6'), 70.8, 70.9, 71.2, 72.3, 72.9, 73.1, 75.0 (C-2,2',3,3',4,5,5'), 91.2 (C-1'), 96.0 (C-1). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{26}H_{36}O_{17}S$: C, 47.85; H, 5.56; S, 4.91. Found: C, 47.83; H, 5.71; S, 4.89.

1,2,3,6-Tetra-O-*acetyl*-4-O-*[2,3,6-tri*-O-*acetyl*-4-S-*(3-azibutyl)*-4-*thio*-α-D-*glucopyranosyl]*-β-D-*glucopyranose* (**7**). — To a solution of **6** (500 mg, 0.77 mmol) and 3-azi-1-(trifluoromethanesulfonyloxy)butane (5 mL, 10% solution in CH_2Cl_2) in dry CH_2Cl_2 (10 mL) was added K_2CO_3 (276 mg, 2 mmol). The mixture was stirred vigorously at reflux overnight, then diluted with CH_2Cl_2 (100 mL), and the organic layer was washed with water (50 mL), dried, and concentrated. Flash chromatography (3:2 cyclohexane–EtOAc) of the residual yellow oil gave **7**, isolated as a colourless syrup (408 mg, 72%), R_F 0.4 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{21}$ +47° (*c* 1, chloroform); λ_{max} 348 nm (ε 65). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{30}H_{42}N_2O_{17}S$: C, 49.04; H, 5.76; N, 3.81; S, 4.36. Found: C, 48.75; H, 5.66; N, 4.16; S, 4.09.

4-O-*[4*-S-*(3-Azibutyl)*-4-*thio*-α-D-*glucopyranosyl]*-D-*glucopyranose* (**8**). — A so-

lution of **7** (320 mg, 0.44 mmol) in dry methanol (10 mL) was stirred overnight with methanolic M sodium methoxide (0.25 mL). T.l.c. then indicated the absence of **7**. The solution was neutralised with Amberlite IR-120 (H^+) resin (1 mL, in MeOH) and then concentrated. Flash chromatography (17:2:1 EtOAc–MeOH–H_2O) of the residue yielded **8**, isolated as a colourless oil, an aqueous solution of which was freeze-dried to give a white solid (147 mg, 77%), R_F 0.41 (7:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D^{21}$ +64° (*c* 0.6, water); λ_{max} 348 nm (ε 64).

Anal. Calc. for $C_{16}H_{28}N_2O_{10}S$: C, 43.63; H, 6.41; N, 6.36. Found: C, 43.32; H, 6.30; N, 6.94.

*3-Azibutyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-glucopyranoside* (**9**). — To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose[9] (1 g, 2.75 mmol) and 3-azi-1-(trifluoromethanesulfonyloxy)butane (20 mL, 10% solution in CH_2Cl_2) in dry CH_2Cl_2 (20 mL) was added K_2CO_3 (690 mg, 5 mmol), and the mixture was treated as described for compound **6**. Flash chromatography (3:1 cyclohexane–EtOAc) of the product and crystallisation from EtOH gave **9** (930 mg, 76%), m.p. 79.5°, R_F 0.47 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{21}$ +173° (*c* 1, chloroform). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{18}H_{26}N_2O_9S$: C, 48.42; H, 5.87; N, 6.27; S, 7.18. Found: C, 48.27, H, 5.83; N, 6.11; S, 6.93.

*3-Azibutyl 1-thio-α-*D*-glucopyranoside* (**10**). — A solution of **9** (800 mg, 1.8 mmol) in MeOH (10 mL) was treated as described for **7** (1 h). Flash chromatography (27:2:1 EtOAc–MeOH–H_2O) of the oily product and crystallisation from 2-propanol gave **10** (400 mg, 80%), R_F 0.53 (7:2:1 EtOAc–MeOH–H_2O), m.p. 101°, $[\alpha]_D^{21}$ +234° (*c* 1, water), λ_{max} 348 nm (ε 66).

Anal. Calc. for $C_{10}H_{18}N_2O_5S$: C, 43.15; H, 6.52; N, 10.06; S, 11.52. Found: C, 42.91; H, 6.36; N, 9.85; S, 11.30.

*3-Azibutyl 4-*O*-α-*D*-glucopyranosyl-1-thio-α-*D*-glucopyranoside* (**11**) *and 3-azibutyl 4-*O*-α-maltosyl-1-thio-α-*D*-glucopyranoside* (**12**). — A solution of **10** (300 mg, 1.08 mmol) and cyclomaltohexaose (α-cyclodextrin; 500 mg, 0.51 mmol) in distilled water (2 mL) was incubated with CGT-ase (40 U). The reaction was stopped when t.l.c. showed that equilibrium had been reached (95°, 4 min). The pH of the solution was brought to 5.5 by the addition of HOAc and the mixture was incubated with beta-amylase (25 U) until t.l.c. indicated the absence of higher homologues. The resulting mixture of **10–12** and maltose was fractionated on a column (140 × 2.5 cm) of Biogel P-2 at 40° by elution at 100 mL/h with distilled and degassed water. The appropriate fractions were freeze-dried to give **10** (130 mg, solid), **11** [R_F 0.39 (7:2:1 EtOAc–MeOH–H_2O), 160 mg, syrup], and **12** [R_F 0.24 (7:2:1 EtOAc–MeOH–H_2O), 140 mg, solid]. Separate solutions of **11** (1 mg) and **12** (1 mg) in acetate buffer (0.1 mL, 50 mM, pH 4.0) were incubated with glucoamylase (5 U) for 1 h. T.l.c. then showed that both **11** and **12** were cleaved completely, to give **10** and glucose as the only detectable products.

For ^{1}H-n.m.r. analysis, **11** (50 mg, 0.11 mmol) was acetylated in pyridine–Ac_2O (2:1, 1 mL) overnight at 40°. MeOH (1 mL) was added, the solvent was evaporated, and toluene (2 × 1 mL) was evaporated from the residue. Flash chromatography (3:2 cyclohexane–EtOAc) then yielded **13** (61.4 mg, 81%), R_F 0.32 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{21}$ +175° (*c* 1, chloroform). For the ^{1}H-n.m.r. data, see Table II.

1,2,3,6-Tetra-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-S-benzoyl-6-thio-α-D-glucopyranosyl)-D-glucopyranose (**14**). — A suspension of 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-α-D-glucopyranosyl)-β-D-glucopyranose[7] (3 g, 3.8 mmol) and potassium thiobenzoate (1.4 g, 7.95 mmol) in dry acetone was heated overnight at reflux, then filtered, and concentrated to dryness, and the residue was partitioned in CH_2Cl_2–water (100 mL, 1:1). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL), and the combined extracts were washed with water (100 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (3:2 cyclohexane–EtOAc) of the residue and crystallisation of the resulting yellow oil (2.5 g, 87%) from 2-propanol gave **14** (1.9 g, 76%), R_F 0.32 (1:1 cyclohexane–EtOAc), m.p. 137°, $[\alpha]_D^{21}$ + 58° (*c* 1, chloroform). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{33}H_{42}O_{20}S$: C, 52.39; H, 5.33; S, 4.23. Found: C, 52.19; H, 5.33; S, 4.18.

4-O-[6-S-(3-Azibutyl)-6-thio-α-D-glucopyranosyl]-D-glucopyranose (**15**). — To a solution of **14** (2 g, 2.65 mmol) and 3-azi-1-(*p*-toluenesulfonyloxy)butane (1 g, 3.94 mmol) in dry MeOH (20 mL) was added methanolic M sodium methoxide (3.5 mL) in small portions (0.5 mL each 0.5 h). The mixture was stirred overnight, then neutralised with Amberlite IR-120 (H^+) resin in MeOH and concentrated. Chromatography of the residue on Biogel P-2, as described for **10–12**, and freeze-drying yielded **15** (642 mg, 55%), R_F 0.39 (7:2:1 EtOAc–MeOH–H_2O), $[\alpha]_D^{21}$ + 95° (*c* 0.7, water), λ_{max} 347 nm (ε 68).

Anal. Calc. for $C_{16}H_{28}N_2O_{10}S$: C, 43.63; H, 6.41; N, 6.36; S, 7.28. Found: C, 43.86; H, 6.38; N, 6.41; S, 7.27.

1,2,3,6-Tetra-O-acetyl-4-O-[2,3,4-tri-O-acetyl-6-S-(3-azibutyl)-6-thio-α-D-glucopyranosyl]-D-glucopyranose (**16**). — Compound **15** (50 mg, 0.11 mmol) was acetylated (pyridine–Ac_2O 2:1, 1 mL) overnight at 40° and the mixture was worked-up as described above. Flash chromatography (3:2 cyclohexane–EtOAc) of the product gave **16** as a white solid (65.2 mg, 85%), R_F 0.37 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{21}$ + 31° (*c* 0.6, chloroform). For the ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{30}H_{42}N_2O_{17}S$: C, 49.04; H, 5.76; N, 3.81; S, 4.36. Found: C, 48.30; H, 5.69; N, 3.74; S, 4.10.

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