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Photolabile, spacer-modified oligosaccharides for probing malto-oligosaccharide binding sites in proteins*

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ABSTRACT

O-Deacylation and *S*-deacylation of the diastereomers of 2-azido-4-*S*-benzoyl-4-mercaptobutyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**9**) with methanolic sodium methoxide and coupling of the resulting thiol to methyl 3,4-anhydro-6-deoxy-β-L-*arabino*-hex-5-enopyranoside (**2**) gave the corresponding diastereomers of the spacer-modified disaccharide methyl 4-*S*-(3-azido-4-α-D-glucopyranosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranoside (**10**). Glucosylation of the diastereomers of **10** with α-cyclodextrin-CGTase and treatment of the products with beta-amylase gave the diastereomers of the spacer-modified oligosaccharides methyl 4-*S*-(3-azido-4-α-maltosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosides (**11**) and 4-*S*-(3-azido-4-α-maltotriosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosides (**12**). The diastereomers of **10** each had a good affinity for pancreatic alpha-amylase and the maltose-binding protein from *E. coli*. The affinities of the diastereomers of **11** and **12** were higher by at least one order of magnitude.

INTRODUCTION

Malto-oligosaccharides, (1→4)-α-D-gluco-oligosaccharides, are recognized by alpha- and beta-amylases, cyclodextrinases, transport proteins, and lectins. The binding sites of such proteins have subsites[1], each of which can accommodate one 4-linked α-D-glucopyranosyl unit. The specificity of these receptor proteins is high and structural changes in the 4-linked α-D-glucopyranosyl units are not tolerated. The requirements of the binding sites merit study in order to demonstrate the extent of homology between proteins with the same binding specificity but of different origin and function. The three-dimensional structure of a protein–ligand complex can be determined by X-ray analysis, and the peptide segment(s) which form the binding site can be labelled chemically and then fitted into the structure of the protein. For the covalent labelling of a malto-oligosaccharide binding site, reactive groups can be attached either to the reducing or the non-reducing end of the malto-oligosaccharide without much disturbance of the binding. However, the probability of a reactive group at the end of the chain contacting the binding site is low.

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.
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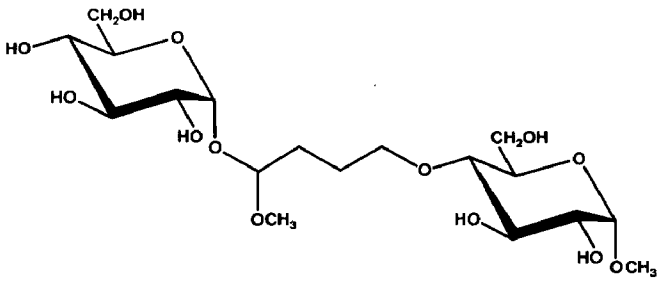
We have shown that methyl 4-*O*-(4-α-D-glucopyranosyloxy-4-methoxybutyl)-α-D-glucopyranoside (**1**), the sugar residues of which are separated by a 6-membered spacer and thereby mimic maltotriose, has a reasonable affinity for porcine pancreatic alpha-amylase[2] and inhibits the transport of malto-oligosaccharides into *E. coli* cells mediated by a maltose-binding protein (MBP)[3]. Compound **1** may be regarded as an alkyl α-D-glucopyranoside or a methyl 4-*O*-alkyl-α-D-glucopyranoside, neither of which alone would associate with alpha-amylase[2] or with MBP[4]. In such spacer-modified oligosaccharides, the flexible, acyclic spacer can accommodate a reactive or photolabile group for covalent modification of the binding site[5].



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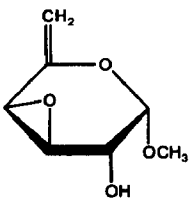

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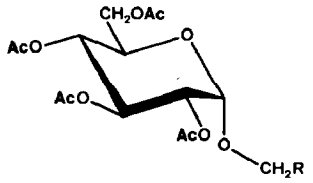
Porcine pancreatic alpha-amylase[1] has a pentasaccharide binding site, the catalytic groups being located at the glycosidic bond between the second and the third glucose residue, counting from the reducing end. Thus, maltopentaose is cleaved preferentially into maltotriose and maltose. The periplasmic maltose-binding protein from *E. coli* also binds malto-oligosaccharides[4], but the binding site can accommodate only 3–4 glucose residues including the reducing unit[6]. Therefore, spacer-modified malto-oligosaccharides for labelling these receptor proteins will require 1–2 monosaccharide residues on the reducing side and 1–3 residues on the non-reducing side of the spacer that carries the photolabile group. Thus, a spacer-modified maltose can be regarded as a minimal unit.

RESULTS

Synthesis of the reducing moiety. — 4-Substituted glucose or glucoside derivatives can be synthesized under mild conditions by the reaction of methyl 3,4-anhydro-6-deoxy-β-L-*arabino*-hex-5-enopyranoside (**2**) with a suitable nucleophile[7]. The vinyloxirane grouping, which undergoes stereo- and regio-selective reactions, may be introduced into maltose or maltotriose in order to provide synthons for chain extension towards the reducing end. The enolic double bond can be hydrated by hydroboration to yield a 4-substituted glucoside[8] or hydrogenated to yield the corresponding quinovoside[9]. Since enolic double bonds easily undergo alkoxy mercuration[10], such a grouping may be useful for introducing mercury into a ligand already covalently attached to the protein as a heavy metal for X-ray investigations.

Synthesis of the non-reducing moiety. — This moiety must consist of an α-D-glucopyranosyl moiety that carries the 6-membered spacer with a photolabile group and functionality that will allow easy coupling to the reducing moiety.

An easily accessible starting material is allyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[11] (**3**), which, on ozonolysis[12], yielded 2-oxoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**4**). Chain extension was carried out with (triphenylphosphoranylidene)acetaldehyde[13] to yield (*E*)-4-oxobut-2-enyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**5**), which was amenable to vinylogous addition of azide[14] to give (2*R*,*S*)-2-azido-4-oxobutyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**6**). Reduction of **6** gave (2*R*,*S*)-2-azido-4-hydroxybutyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**7**), which was tosylated to give 2-azido-4-tosyloxybutyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosides (**8a*** and **8b**). The separation of the diastereomers by h.p.l.c. was most convenient at this stage. The tosylates **8a** and **8b** were each treated with potassium thiobenzoate to yield the 2-azido-4-*S*-benzoyl-4-mercaptobutyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosides (**9a** and **9b**).



3 R = $CH{=}CH_2$

4 R = CHO

5 R = $CH{=}CH_2{-}CHO$

6 R = $CH(N_3){-}CH_2{-}CHO$

7 R = $CH(N_3){-}CH_2{-}CH_2OH$

8 R = $CH(N_3){-}CH_2{-}CH_2OTs$

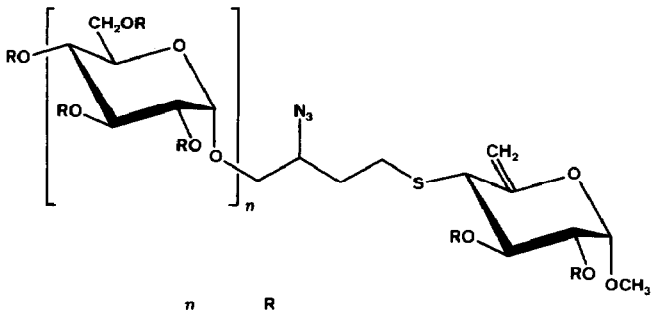
9 R = $CH(N_3){-}CH_2{-}CH_2SBz$

Coupling of the reducing and non-reducing moieties. — The free thiolate for the coupling reaction was generated *in situ* from **9** with simultaneous *O*-deacetylation by treatment with methanolic sodium methoxide. Addition of freshly prepared **2** in acetone then resulted in coupling to give methyl 4-*S*-(3-azido-4-α-D-glucopyranosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosides (**10a** and **10b**).

The use of the thiolate for the coupling procedure allows the introduction of a ^{35}S radiolabel at a late stage in the synthesis. The spacer-modified disaccharides **10a** and **10b** were crystalline and obtained in good yields.

* The slow- and fast-moving diastereomers (t.l.c.) are designated **a** and **b**; the absolute configurations have not been determined.

Glucosylation of **10a** *and* **10b**. — Any D-glucopyranosyl moiety, unmodified except at position 1, or in some instances at position 6, can be α-D-glucopyranosylated at position 4 through CGTase acting on cyclomaltohexaose (α-cyclodextrin)[15]. In such a way, a homologous series of malto-oligosaccharides can be prepared. If such a mixture is treated with beta-amylase, the oligosaccharide chains are shortened to 1–3 glucose residues. This procedure was applied to **10a** and **10b**, and yielded diastereomeric pairs of homologous spacer-modified tri- and tetra-saccharides, namely, methyl 4-*S*-(3-azido-4-α-maltosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosides (**11a** and **11b**) and 4-*S*-(3-azido-4-α-maltotriosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosides (**12a** and **12b**).



	n	R
10	1	H
11	2	H
12	3	H
13	1	Ac

Interaction of the spacer-modified malto-oligosaccharides with alpha-amylase and maltose-binding protein. — The affinity of **10–12** for alpha-amylase, as determined by competitive inhibition of the enzymic hydrolysis of *p*-nitrophenyl maltotrioside[15], increased, as expected, with increasing chain length (see Table I). The K_i values of **10a** and **10b** are remarkable because the compounds can be regarded as simple alkyl α-D-glucopyranosides. Thus, it is clear that the reducing and non-reducing moieties, at least of **10**, bind to the receptor subsites, thus bridging a third subsite with the spacer. All compounds except the tetrasaccharides **12a** and **12b** were stable towards alpha-amylase during incubation for 12 h. The diastereomers of **12** were slowly hydrolysed by the enzyme into maltose and the corresponding disaccharides **10**. From former investigations[16], it can be deduced that good hydrolytic activity needs good binding of the reducing residue at the catalytic site. This may be the reason why maltotriose is not formed from **12**, which mimics maltopentaose. Since the pairs of diastereomers **10–12a** and **10–12b** differ to some extent in K_i, it can be assumed that the spacer does not interfere strongly with the binding but is in contact with the binding site.

In studies of the interaction of malto-oligosaccharides with MBP[6], it was demonstrated that recognition of the oligosaccharide chain involves the reducing-end glucose residue so that structural alteration at that site can be critical. The affinities of the

TABLE I

K_i values of the spacer-modified oligosaccharides with alpha-amylase

Compound	**10a**	**10b**	**11a**	**11b**	**12a**	**12b**
K_i (mM)	39	37	5.1	6.3	0.92	1.75

diastereomers of **10** were not high and could not be measured accurately. Lengthening the chain of the non-reducing moiety by one glucosyl unit increases the affinity significantly. Another added glucosyl unit as in **12** does not enhance the binding greatly, which accords with earlier findings.

EXPERIMENTAL

Methods. — All reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck). Column chromatography was carried out with Silica 32-63, 60 A (ICN). H.p.l.c. was performed with an LKB 2152 h.p.l.c. controller, two LKB 2150 pumps, a Rheodyne 7126 injector, an LKB variable wavelength monitor, and a Shimadzu C-R2Ax integrator. Preparative h.p.l.c. (Knauer) involved three pumps 64, a dynamic mixing chamber, an injection valve, and a variable wavelength monitor. Columns (Bischoff) were used as indicated. Fluorescence measurements were obtained with a Perkin–Elmer 165/10s spectrometer, i.r. spectra with a Perkin–Elmer 1320 spectrophotometer, optical rotations with a Perkin–Elmer 141 polarimeter, and u.v. spectra and extinction coefficients with a Zeiss PMQ II spectrophotometer. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 spectrometer at 250 MHz for solutions in $CDCl_3$ (internal Me_4Si). Melting points are uncorrected. Ozonolyses were carried out with a Fischer ozone generator 500 M. Photolysis of **10a** was performed with a Rayonet RPR 100 reactor equipped with 16 RPR 3000 Å lamps. Kinetic data were obtained with an Eppendorf photometer at 405 nm connected with a transformation unit and a SE 120 recorder (BBC).

Enzymes. — Periplasmic maltose-binding protein (MBP) from *E. coli* was a gift of Professor W. Boos (Konstanz). CGTase [(1→4)-α-D-glucan 4-α-D-glucanotransferase, EC 2.4.1.19, cyclizing] from *Bacillus macerans* (760 U/mL) was a donation from Boehringer Mannheim. Alpha-amylase [(1→4)-α-D-glucan glucanohydrolase, EC 3.2.1.1] from porcine pancreas (1260 U/mg) and beta-amylase [(1→4)-α-D-glucan maltohydrolase, EC 3.2.1.2] from sweet potato (845 U/mg) were purchased from Sigma. α-D-Glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) from yeast (10 U/mg) was obtained from Boehringer Mannheim.

Enzymic investigations. — For the determination of the inhibition constants, commercial *p*-nitrophenyl α-maltotrioside was used as substrate (0.17–5.3mM, K_m 2.0mM) at 30° in 50mM triethanolamine buffer (pH 7.0) containing 10mM $CaCl_2$.

Inhibitor concentrations were 0–63.6mM for **10a**, 0–66.8mM for **10b**, 0–9.5mM for **11a**, 0–6.8mM for **11b**, 0–7.0mM for **12a**, and 0–5.0mM for **12b**. The concentration of alpha-amylase in the assay was 68 U/mL. For the determination of the K_d values (Table II) of the oligosaccharides with MBP, 50mM Tris/HCl buffer (pH 7.4) was used at room temperature. Concentrations of ligands were 0.1–1000mM in twelve steps. The wavelength of activation was 280 nm and the decrease of fluorescence at 330 nm with increasing protein-ligand-binding was measured. The oligosaccharides **10–12** (1% in buffer) were treated with α-D-glucosidase (25 U/mL) and separately with alpha-amylase (1200 U/mL). The incubation mixtures were examined by t.l.c. (4:2:1 EtOAc–MeOH–water) after 1, 2, and 12 h. Compounds **10–12** were cleaved by α-D-glucosidase to glucose (R_F 0.44) and a product with R_F 0.75. The diastereomers of **10** and **11** were not affected by incubation with alpha-amylase for 12 h. The diastereomers of **12** were cleaved by alpha-amylase quantitatively after 12 h into the diastereomers of **10** and maltose (R_F 0.31).

TABLE II

K_d values of the spacer-modified oligosaccharides with maltose-binding protein (MBP)

Compound	**10a/10b**	**11a/11b**	**12a/12b**	Maltose
K_d (μM)	>5000	100–300	50–100	3.7

2-Oxoethyl 2,3,4,6-tetra-O-*acetyl*-α-D-*glucopyranoside* (**4**). — Ozone (30 L/h O_2, 10 mmol O_3/h) was bubbled through a solution of allyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (3.9 g, 10 mmol) in 1:1 methanol–dichloromethane (150 mL) at −78°. After 75 min, the excess of ozone was removed with a stream of oxygen. Methyl sulfide (10 mL) was added, the mixture was allowed to attain room temperature, then concentrated. Column chromatography (1:1 cyclohexane–EtOAc) of the residue yielded syrupy **4** (4.0 g) almost quantitatively; R_F 0.22 (1:2 cyclohexane–EtOAc). For the ^{1}H-n.m.r. data, see Table III.

(E)-*4-Oxobut-2-enyl 2,3,4,6-tetra*-O-*acetyl*-α-D-*glucopyranoside* (**5**). — A solution of **4** (3.2 g, 8.2 mmol) in dry benzene (40 mL) was stirred with (triphenylphosphoranylidene)acetaldehyde (2.5 g, 8.2 mmol) at room temperature overnight, then concentrated to dryness. Column chromatography (1:1 cyclohexane–EtOAc) of the residue gave **5**, isolated as a colourless syrup (3.0 g, 88%), $[\alpha]_D^{22}$ +133° (*c* 1, chloroform), R_F 0.38 (1:2 cyclohexane–EtOAc); ν_{max}^{film} 1700 cm^{-1} (C=O). For the ^{1}H-n.m.r. data, see Table III.

(2R,S)-*2-Azido-4-oxobutyl 2,3,4,6-tetra*-O-*acetyl*-α-D-*glucopyranoside* (**6**). — A solution of **5** (2.6 g, 6.25 mmol) in acetic acid (40 mL) was stirred whilst a solution of sodium azide (2.6 g, 40 mmol) in water (15 mL) was added dropwise during 30 min. After 3 h, the solution was poured into water (500 mL), and extracted with chloroform (4 × 50 mL), and the combined extracts were neutralized with saturated aqueous

$NaHCO_3$ (2 × 200 mL), washed with water (2 × 200 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography (1:1 cyclohexane–EtOAc) of the residue gave **6** (1.9 g, 66%), isolated as a syrup, R_F 0.41 (1:2 cyclohexane–EtOAc); ν_{max}^{film} 2100 cm^{-1} (N_3). For the ^{1}H-n.m.r. data, see Table III.

*(2*R,S*)-2-Azido-4-hydroxybutyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**7**). — To a stirred solution of **6** (8 g, 17.4 mmol) in dry ethanol (40 mL) containing acetic acid (3.6 mL), was added a solution of sodium cyanoborohydride (1.3 g, 20.7 mmol) in dry ethanol (10 mL) during 15 min. The mixture was kept at room temperature for 3 h, then concentrated *in vacuo*, and a solution of the residue in water (500 mL) was extracted with chloroform (4 × 100 mL). The combined extracts were neutralized with saturated aqueous $NaHCO_3$ (2 × 200 mL), washed with water (2 × 200 mL), dried ($MgSO_4$), and concentrated. Column chromatography (1:2 cyclohexane–EtOAc) of the residue gave **7** (4.5 g, 56%), isolated as a colourless syrup, R_F 0.24 (1:2 cyclohexane–EtOAc). For the ^{1}H-n.m.r. data, see Table III.

*(2*R,S*)-2-Azido-4-tosyloxybutyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**8a** *and* **8b**). — To a solution of **7** (4.5 g, 9.8 mmol) in pyridine (30 mL) was added *p*-toluenesulfonyl chloride (6 g, 31.6 mmol), and the mixture was kept at room temperature for 3 h. Excess of reagent was then hydrolyzed with ice (10 g), the mixture was poured into water (1 L) and extracted with chloroform (4 × 50 mL), and the combined extracts were neutralized with saturated aqueous $NaHCO_3$ (2 × 100 mL), washed with water (2 × 200 mL), dried ($MgSO_4$), and concentrated. Column chromatography (2:1 cyclohexane–EtOAc) of the residue gave **8** (3.44 g, 57%), isolated as a slightly yellow syrup. H.p.l.c. (Hypersil 5 μm, 250 × 20 mm; 1:2 cyclohexane–EtOAc, 20 mL/min) of the mixture gave, first, **8b**, R_F 0.33 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{22}$ +79.5° (*c* 0.5, chloroform); ν_{max}^{film} 2120 cm^{-1} (N_3). Eluted second was **8a**, R_F 0.32 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{22}$ + 66.5° (*c* 0.5, chloroform); ν_{max}^{film} 2120 cm^{-1} (N_3). For the ^{1}H-n.m.r. data, see Table III.

*2-Azido-4-*S*-benzoyl-4-mercaptobutyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**9a** *and* **9b**). — To separate solutions of **8a** (1.1 g, 1.79 mmol) and **8b** (1.1 g, 1.79 mmol) in dry acetone (100 mL) was added potassium thiobenzoate (1 g, 5.7 mmol). Each mixture was stirred at room temperature for 2 h, then concentrated, and the residue was partitioned in 1:1 chloroform–water (40 mL). Each aqueous layer was extracted with chloroform (2 × 25 mL), and the combined extracts were washed with water (2 × 100 mL), dried ($MgSO_4$), and concentrated. Column chromatography (2:1 cyclohexane–EtOAc) of the residues gave syrupy **9a** (1.0 g, 96%), R_F 0.48 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{22}$ +54° (*c* 0.6, chloroform), and **9b** (1.0 g, 96%), R_F 0.49 (1:1 cyclohexane–EtOAc), $[\alpha]_D^{22}$ +112° (*c* 0.8, chloroform). For the ^{1}H-n.m.r. data, see Table III.

*Methyl 4-*S*-(3-azido-4-α-*D*-glucopyranosyloxybutyl)-6-deoxy-4-thio-α-*D*-xylo-hex-5-enopyranoside* (**10a** *and* **10b**). — To separate solutions of **9a** (1.1 g, 1.89 mmol) and **9b** (1.2 g, 2.06 mmol) in dry methanol (10 mL) was added methanolic M sodium methoxide (4.5 mL). After 10 min, a solution of freshly prepared **2** (~ 760 mg, 4.8 mmol) in acetone (20 mL) was added to each mixture. Reaction was complete within 10 min (t.l.c.) and each solution was desalted by elution from a short column of silica gel with

TABLE III

^{1}H-N.m.r. data (250 MHz)

Proton	*Compound*									
	4	**5**	**6**	**7**	**8a**	**8b**	**9a**	**9b**	**13a**	**13b**
H-1	5.13	5.12	5.12	5.13	5.09	5.08	5.12	5.13	4.96	4.95
H-2	4.90	4.94	4.90	4.90	4.90	4.89	4.91	4.91	4.98	4.98
H-3	5.53	5.51	5.48	5.50	5.47	5.47	5.51	5.50	5.38	5.39
H-4	5.08	5.10	5.07	5.08	5.07	5.07	5.09	5.08	3.32	3.32
H-5	4.05–4.30	4.02	4.04	4.04	3.99	4.01	4.07	4.04		
H-6a	4.05–4.30	4.11	4.12	4.11/4.13	4.11	4.11	4.13	4.11	4.92	4.93
H-6b	4.05–4.30	4.27	4.25/4.27	4.27	4.26	4.27	4.28	4.27	5.19	5.20
H-1′a	4.05–4.30	4.31	3.52/3.59	3.51/3.62	3.57	3.41	3.61	3.48	2.65–2.83	2.64–2.85
H-1′b	4.05–4.30	4.52	3.83/3.86	3.75–3.92	3.73	3.83	3.81	3.89		
H-2′	9.69	6.83	4.05–4.20	3.75–3.92	3.71	3.75	3.72	3.77	1.68–1.80	1.66–1.77
H-3′a		6.41	2.71/2.76	1.63–1.98	1.57–2.00	1.66	1.88	1.83	3.61–3.74	3.75
H-3′b						1.80–1.96				
H-4′a		9.62	9.80/9.82	3.75–3.92	4.15	4.14	3.20	3.19	3.58	3.45
H-4′b									3.75	3.85
H-1″									5.11	5.13
H-2″									4.90	4.92
H-3″									5.50	5.49
H-4″									5.08	5.09
H-5″									4.04	4.05
H-6″a									4.12	4.12
H-6″b									4.28	4.28
H-O				3.75–3.92						
MeO									3.45	3.45
H-Bn					2.47	2.47				
H-Ph					7.32	7.40	7.47	7.47		
					7.82	7.82	7.60	7.60		
							7.97	7.97		

OAc	2.02	2.03	2.03	2.02	2.02	2.02	2.02	2.02	2.02	2.02
	2.03	2.04	2.05	2.04	2.04	2.05	2.04	2.04	2.05	2.05
	2.09	2.11	2.08	2.08	2.07	2.06	2.07	2.07	2.09	2.08
	2.11	2.11	2.11	2.11	2.10	2.10	2.10	2.10	2.10	2.10
									2.11	2.11
									2.12	2.12
$J_{H,H}$										
1,2	3.8	3.5	3.8	3.8	3.8	3.8	3.8	3.8	3.2	3.0
2,3	10.5	10.2	10.4	10.3	10.2	10.2	10.3	10.2	8.8	10.0
3,4	9.5	9.5	9.3	9.8	9.6	9.6	9.6	9.6	10.2	10.2
4,5	9.5	10.1	10.2	9.8	10.2	10.2	9.9	10.0		
4,6									2.0	2.1
5,6a		2.6	2.3	2.3	2.3	2.3	2.4	2.0		
5,6b		4.5	4.5	4.5	4.5	4.5	4.5	4.5		
6a,6b		12.3	12.3	12.3	12.3	12.3	12.4	12.6		
1′a,1′b		16.7	10.5	12.8/13.5	13.5	10.2	9.6	10.4		
1′a,2′		4.1	7.4/4.2	6.5/6.9	6.8	7.4	3.0	7.7		
1′b,2′		4.1	6.2/3.6		6.8	3.2	6.8	3.0		
1′a,3′										
1′b,3′										
2′,3′		15.5	6.5		4.2	6.4	6.8			
3′,4′a		7.7	1.0		1.5		6.8		3.0	7.5
3′,4′b									6.9	3.0
4′a,4′b									9.3	10.2
1″,2″									3.8	3.8
2″,3″									10.2	10.1
3″,4″									9.8	9.6
4″,5″									9.9	10.1
5″,6″a									3.8	2.3
5″,6″b									4.5	4.5
6″a,6″b									12.3	12.3

methanol. Each eluate was concentrated to dryness. H.p.l.c. (Hypersil ODS, 5 μm, 250 × 20 mm; 40:60 methanol–water, 15 mL/min) of the residues gave **10a** (827 mg, 86%), m.p. 153.5° (from ^{i}PrOH), $[\alpha]_D^{22}$ +85° (*c* 1, water), R_F 0.48 (7:2:1 EtOAc–MeOH–water); ν_{max}^{KBr} 2080 and 2120 cm^{-1} (N_3); λ_{max} 282 nm (ε_{mM} 0.028). For the ^{1}H-n.m.r. data of the acetylated compound (**13a**), see Table III.

Anal. Calc. for $C_{17}H_{29}N_3O_{10}S$: C, 43.68; H, 6.25; N, 8.99; S, 6.86. Found: C, 43.71; H, 6.27; N, 8.41; S, 6.60.

Obtained likewise, **10b** (810 mg, 88%) had m.p. 84–85° (from water), $[\alpha]_D^{22}$ +120° (*c* 1, water), R_F 0.49 (7:2:1 EtOAc–MeOH–water); ν_{max}^{KBr} 2070 and 2120 cm^{-1} (N_3); λ_{max} 282 nm (ε_{mM} 0.028). For the ^{1}H-n.m.r. data of the acetylated compound (**13b**), see Table III.

Anal. Calc. for $C_{17}H_{29}N_3O_{10}S \cdot H_2O$*: C, 42.08; H, 6.44; N, 8.65. Found: C, 42.23; H, 6.48; N, 8.68.

Photolysis of **10a**. — A solution of **10a** (100 mg) in water (5 mL), placed in a quartz tube, was irradiated in a Rayonet photoreactor. Aliquots were taken after 0, 10, 20, and 30 min, and analyzed by t.l.c. (7:2:1 EtOAc–MeOH–water); spots with R_F 0.07, 0.34, and 0.60 were detected. H.p.l.c. (Hypersil ODS, 5 μm, 250 × 4 mm; 50:50 MeOH–water, 0.75 mL/min; u.v. 230 nm) revealed five products. The half-life of **10a** was 9 min.

*Methyl 4-*S*-(3-azido-4-α-maltosyloxybutyl)-*(**11ab**) *and methyl 4-*S*-(3-azido-4-α-maltotriosyloxybutyl)-6-deoxy-4-thio-*α-D-xylo-*hex-5-enopyranoside* (**12ab**). —To a solution of **10a** (600 mg, 1.24 mmol) or **10b** (420 mg, 0.90 mmol) in water (30 mL for **10a**, 20 mL for **10b**) were added α-cyclodextrin (1.1 g, 1.13 mmol for **10a**; 0.88 g, 0.90 mmol for **10b**) and CGTase (30 μL, 22 U). Each solution was kept overnight at room temperature. The enzyme was inactivated by heating to 95° for 5 min, acetic acid (0.1 mL) and beta-amylase (20 μL, 450 U) were added, and, after 30 min at room temperature, no homologues higher than **12** were detectable by t.l.c. Beta-amylase was then denaturated as for CGTase and each mixture was concentrated *in vacuo*. H.p.l.c. (Hypersil ODS, 5 μm, 250 × 20 mm; 40:60 MeOH–water, 15 mL/min) of the residues gave colourless amorphous solids after lyophilization: **11a** (195.6 mg, 0.31 mmol), $[\alpha]_D^{22}$ +117° (*c* 0.6, water), R_F 0.34; **12a** (188.3 mg, 0.24 mmol), $[\alpha]_D^{22}$ +139° (*c* 1.4, water), R_F 0.20; **11b** (143.6 mg, 0.23 mmol), $[\alpha]_D^{22}$ +137° (*c* 0.6, water), R_F 0.35; **12b** (147.1 mg, 0.19 mmol), $[\alpha]_D^{22}$ +154° (*c* 0.6, water), R_F 0.21 (7:2:1 EtOAc–MeOH–water).

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* Water content was determined by the decrease in weight on drying *in vacuo* over P_2O_5.

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