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Spacer-modified, photolabile tetrasaccharides as analogues of maltopentaose are versatile probes for porcine pancreatic alpha-amylase

Jochen Lehmann* and Lothar Ziser
Institut für Organische Chemie und Biochemie der Universität Freiburg i. Br., Albertstr. 21, D-7800 Freiburg i. Br. (F.R.G.)

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ABSTRACT

The syntheses are described of methyl 4'-*O*-[4-*S*-(3-azi-4-α-D-glucopyranosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosyl]-α-maltoside (**28**), methyl 4-*O*-[4-*S*-(3-azi-4-α-maltosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranosyl]-α-D-glucopyranoside (**29**), and methyl 4-*S*-(3-azi-4-α-maltotriosyloxybutyl)-6-deoxy-4-thio-α-D-*xylo*-hex-5-enopyranoside (**30**), which are analogues of maltopentaose in which a central glucosyl unit is replaced by an acyclic, four-membered hydrocarbon spacer carrying a photolabile azi group. Only **30** was slowly cleaved by high concentrations of pancreatic alpha-amylase. The K_i values (mM) were 0.15 (**28**), 2.1 (**29**), and 2.5 (**30**). Deactivation of the enzyme by irradiation in the presence severally of **28–30** was 96.4%, 98.1%, and 40%, respectively. There is an indication for regiospecific photoaffinity labelling of the binding subsites of the enzyme.

INTRODUCTION

In order to locate the subsites in a multi-site receptor by photoaffinity labelling, spacer-modified oligosaccharides have been used[1,2]. A spacer-modified oligosaccharide has one or more monosaccharide units replaced by an acyclic[1–4] spacer of equal length. Such compounds show significant affinities for receptor sites even if the remaining carbohydrate part has been greatly reduced[4]. Ideally, a spacer-modified oligosaccharide ought to occupy the full area of a receptor site and form mainly one complex. If the receptor is an enzyme, the ligand should be stable during the photoaffinity labelling. Equally, the receptor protein ought to retain its structure and function during photoaffinity labelling. Spacer-modified di-, tri-, and tetra-saccharides, each carrying a photolabile azido group attached to the spacer, have been described for use on the five-subsite binding area of porcine pancreatic alpha-amylase[5]. Each compound had a significant affinity for the enzyme, and, as expected, the spacer-modified tetrasaccharide, which is likely to occupy the whole length of the binding area, was the best competitive inhibitor in an alpha-amylase assay. However, attempted photoaffinity labelling failed because photolysis of the azide required prolongued irradiation with u.v. light at 300 nm that denatured and precipitated the enzyme. Syntheses of analogues are now reported in

* Author for correspondence.

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which the azido group is replaced by a diazirino group for photolysis under milder conditions. A similar strategy of synthesis was followed.

RESULTS AND DISCUSSION

Three spacer-modified oligosaccharide structures are possible, which can occupy variously subsites B–D of pancreatic alpha-amylase (Fig. 1). If photoaffinity labelling could be carried out with such compounds, regioselective chemical modification of subsites would be effected (I–III in Fig. 1) and could help to determine the structure of the binding region especially if the three-dimensional structure of the protein is known.

The strategy[2] of the syntheses makes use of the reactive synthon **23**, which allows mild and facile regio- and stereo-selective introduction of functional groups[6]. Compounds **23–25** represent the reducing-end moieties of the planned spacer-modified tetrasaccharides. The non-reducing moiety must be an α-D-glucoside which is a receptor for glucosyl transfer mediated by CGTase and α-cyclodextrin (cyclomaltohexaose)[7] and carry as an aglycon the spacer with the photolabile diazirino group. Assembly of the two units involves regioselective opening of the epoxide in **23–25** by a primary thiol group.

Allyl α-D-glucopyranoside (**1**) was benzylated to give **2**, ozonolysis[8] of which yielded the aldehyde **3**. Reaction of **3** with an allyl Grignard reagent gave **4**, and ozonolysis and then reduction yielded the diol **5**. Tritylation of the primary hydroxyl group in **5** (→**6**) followed by oxidation of the secondary hydroxyl group gave the ketone **7**. Detritylation[9] of **7** gave **8**, which was converted[10] into the diazirine **9**. Tosylation of **9** (→**10**) followed by acetolytic *O*-debenzylation[11] gave **11** that was reacted with potassium thiobenzoate to give **12**. Total deblocking of **12** and immediate reaction of the resulting thiol severally with **23–25** yielded the spacer-modified di-, tri-, and tetra-

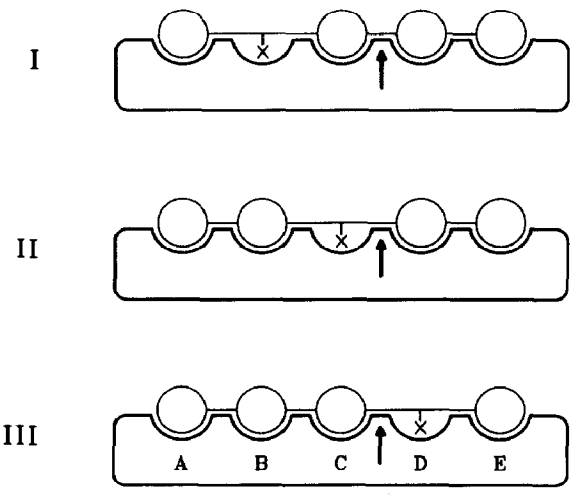
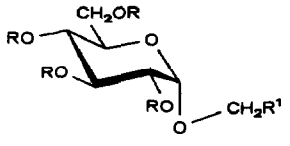
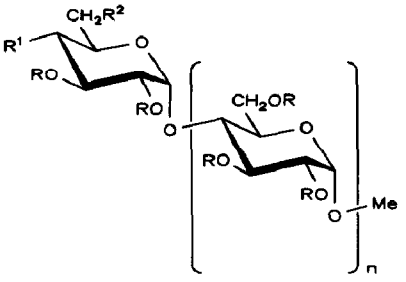


Fig. 1. Schematic presentation of isomeric spacer-modified oligosaccharides bound to the five-centered binding area of porcine pancreatic alpha-amylase.

saccharides **26–28**, respectively. Compounds **26** and **27** were each glycosylated by CGTase and α-cyclodextrin. Trimming of the mixture of the oligosaccharides, obtained from **26**, by beta-amylase gave mainly the spacer-modified tri- and tetra-saccharides, and, of those obtained from **27**, mainly the spacer-modified tetra- and penta-saccharides together with maltose. Each spacer-modified tetrasaccharide was obtained as an amorphous solid that was essentially stable towards alpha-amylase. Only with **30** could traces of maltose and **26** be detected after prolongued incubation with high concentrations of alpha-amylase.



	R	R^1
1	H	$-CH=CH_2$
2	Bn	$-CH=CH_2$
3	Bn	$-CHO$
4	Bn	$-CH(OH)-CH_2-CH=CH_2$
5	Bn	$-CH(OH)-CH_2-CH_2-OH$
6	Bn	$-CH(OH)-CH_2-CH_2-OTr$
7	Bn	$-C(=O)-CH_2-CH_2-OTr$
8	Bn	$-C(=O)-CH_2-CH_2-OH$
9	Bn	$-C(=N=N)-CH_2-CH_2-OH$
10	Bn	$-C(=N=N)-CH_2-CH_2-OTs$
11	Ac	$-C(=N=N)-CH_2-CH_2-OTs$
12	Ac	$-C(=N=N)-CH_2-CH_2-SBz$



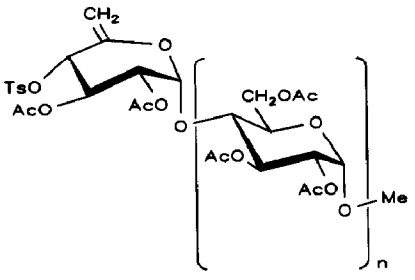
	n	R	R^1	R^2
13	1	H	OH	OH
14	2	H	OH	OH
15	1	Ac	O—CH(Ph)—O	
16	2	Ac	O—CH(Ph)—O	
17	1	Ac	OTs	OTs
18	2	Ac	OTs	OTs
19	1	Ac	OTs	I
20	2	Ac	OTs	I

Binding and deactivation studies. — Each of the spacer-modified tetrasaccharides **28–30** markedly inhibited the hydrolysis of 4-nitrophenyl α-maltotrioside by alpha-amylase. The K_i values (mM) were as follows: **28**, 0.15; **29**, 2.1; and **30**, 2.5. According to Robyt and French[5], cleavage of maltopentaose occurs between subsites C and D. With **28–30**, these subsites cannot be both occupied by (1→4)-linked α-D-glucopyranosyl

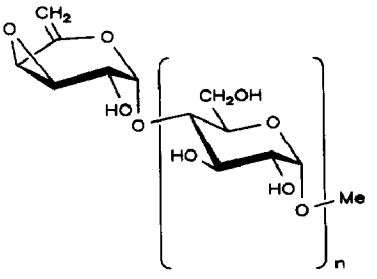
TABLE I

Irreversible inactivation of alpha-amylase with spacer-modified tetrasaccharides

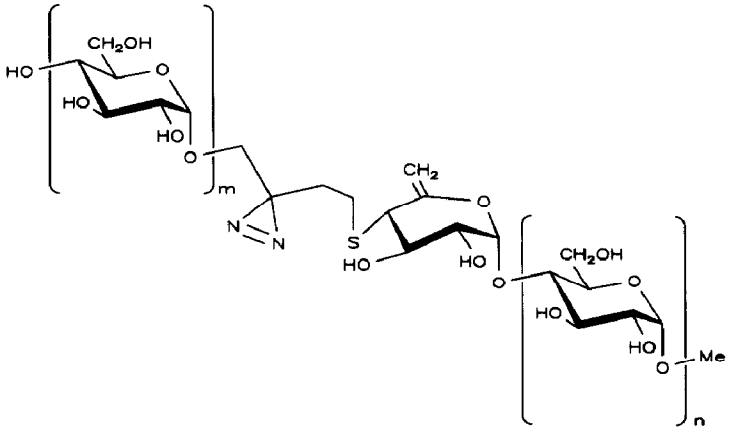
Compound	28		29		30	
Maltotriose (mM)	0	720	0	720	0	720
Remaining activity (%)	3.6	94.0	1.9	79.8	60.0	89.6



21 n = 1
22 n = 2



23 n = 0
24 n = 1
25 n = 2



	m	n
26	1	0
27	1	1
28	1	2
29	2	1
30	3	0

units (Fig. 1), which may explain their resistance to the enzyme. Each of the ligands **28–30** appears to form mainly one complex with the enzyme, and the binding modes I–III are probably correct.

On irradiating a mixture of alpha-amylase with each of **28–30**, marked differences in irreversible deactivation of the enzyme were observed (Table I). Thus, the best competitive inhibitor **28** was relatively ineffective, whereas the relatively poor inhibitor **29** was highly effective. Moreover, **30** was ineffective as an irreversible inhibitor although its inhibition constant is almost identical to that of **29**. Each irreversible deactivation was supressed by high concentrations of maltotriose.

These results indicate that, as expected, different sites in the enzyme are selectively modified chemically by the photolabile spacers of **28–30**.

EXPERIMENTAL

Methods. — All reactions were monitored by t.l.c. on Silica Gel $60F_{254}$ (Merck). Column chromatography was performed on Silica 32–63, 60 A (ICN). H.p.l.c. (Knauer) was performed with three pumps 64, a dynamic mixing chamber, an injection valve, and a variable wavelength monitor. Solvents and columns were used as indicated. I.r. spectra were obtained with a Perkin–Elmer 1320 spectrophotometer, optical rotations with a Schmidt & Haensch Polartronic I on solutions in $CHCl_3$ unless stated otherwise, 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), and ^{13}C-n.m.r. spectra with a Bruker AM 400 at 100.6 MHz for solutions in H_2O (internal MeCN, δ 1.30). Photolyses were carried out with a Rayonet RPR 100 reactor equipped with 16 RPR 3500 Å lamps. Kinetic data were obtained with an Eppendorf photometer at 405 nm connected with a transformation unit and an SE 120 recorder (BBC).

Enzymes. — CGTase [(1→4)-α-D-glucan 4-α-D-glucanotransferase, EC 2.4.1.19, cyclising] from *Bacillus macerans* was a donation from Boehringer Mannheim. Alpha-amylase [(1→4)-α-D-glucan glucanohydrolase, EC 3.2.1.1] from porcine pancreas (1000 U/mg) and beta-amylase [(1→4)-α-D-glucan maltohydrolase, EC 3.2.1.2] from sweet potato (500 U/mg) were purchased from Boehringer Mannheim.

Enzymic investigations. — For the determination of the inhibition constants, commercial 4-nitrophenyl α-maltotrioside was used as substrate (0.2–5.3mM, K_m 2.2mM) at 30° in 50mM Et_3N–HCl buffer (pH 7.0) containing 10mM $CaCl_2$. Inhibitor concentrations were 0–1.0mM for **28**, 0–4.4mM for **29**, and 0–4.6mM for **30**. Each assay was started by adding 22 U/mL of alpha-amylase. The irreversible inactivation of alpha-amylase by irradiation (10 min) with u.v. light at 350 nm was carried out in solutions of **28–30** (4 K_i) in the absence and presence of maltotriose (720mM). The remaining enzymic activity in the irradiated and dialysed solutions was determined by measuring K_m and V_{max} of 4-nitrophenyl α-maltotrioside.

Allyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**2**). — To a stirred solution of **1** (5.0 g, 22.7 mmol) in dry *N,N*-dimethylformamide (200 mL) was added sodium hydride

(3.0 g, 115 mmol) followed by benzyl chloride (25 mL). Stirring was continued for 15 h, the excess of hydride was destroyed by adding MeOH (20 mL), and the mixture was diluted with water (1 L) and extracted with ether (5 × 100 mL). The combined extracts were washed with water (2 × 100 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 25 cm) chromatography (1:8 EtOAc–cyclohexane) of the residue gave syrupy **2** (12.0 g, 91%), $[\alpha]_D^{22}$ +36° (*c* 1.1), R_F 0.49 (1:3 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IIA and IIB.

Anal. Calc. for $C_{37}H_{40}O_6$: C, 76.53; H, 6.94. Found: C, 76.85; H, 6.99.

*2-Oxoethyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**3**). — Ozone (2 h, ~ 10 mmol/h) was bubbled through a stirred solution of **2** in 1:1 CH_2Cl_2–MeOH (300 mL) at −75°. Excess of ozone was removed with a stream of oxygen (15 min, 30 L/h), methyl sulfide (6 mL) was added, the solution was allowed to attain room temperature, and the solvent was evaporated. Column (5.5 × 22 cm) chromatography (2:3 EtOAc–cyclohexane) of the residue gave syrupy **3** (9.5 g, 95%), $[\alpha]_D^{22}$ +45° (*c* 0.9), R_F 0.21 (1:2 EtOAc–cyclohexane); ν_{max}^{film} 1735 cm^{-1} (C=O). For the ^{1}H-n.m.r. data, see Tables IIA and IIB.

*2-Hydroxypent-4-enyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**4**). — A solution of **3** (8.2 g, 14.1 mmol) in dry ether (50 mL) was added dropwise with stirring to allylmagnesium chloride[12] [prepared from magnesium (12 g, 494 mmol) and allyl chloride (8 g, 105 mmol) in dry ether (400 mL)]. The mixture was stirred for 1 h and then poured into aq. 10% NH_4Cl (1.5 L). The aqueous layer was extracted with ether (3 ×

TABLE IIA

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

Proton	*Compound*					
	2	**3**	**4**	**5**	**7**	**8**
H-1	4.84 d	4.83 d	4.79 d	4.76 d	4.83 d	4.89 d
H-2	3.63 dd	3.62 dd	3.57 dd	3.57 dd	3.64 dd	3.61 dd
H-3	4.04 t	4.05 dd	3.98 t	3.97 t	4.03 t	4.02 t
H-4	3.66 t	3.63 dd	3.61 t	3.52–3.88 m	3.65 t	3.62 t
H-5	3.82 d	3.82 ddd	3.83 ddd	3.52–3.88 m	3.79 d	3.80 ddd
H-6a	3.58 dd	3.61 dd	3.63 dd	3.52–3.88 m	3.56 dd	3.62 dd
H-6b	3.73 dd	3.70 dd	3.69 dd		3.69 dd	3.69 dd
H-1′a	4.00 ddd	4.16 dd	3.30 dd	3.27–3.88 m	4.16–4.35 m	4.15 d
H-1′b	4.16 ddd	4.17 dd	3.72 dd			4.20 d
H-2′	5.93 dddd	9.72 t	3.88 ddt	3.92–4.08 m		
H-3′a	5.20 dd		2.24 tt	1.54–1.74 m	2.66 t	2.68 dt
H-3′b	5.31 dd					2.76 dt
H-4′			5.82 ddt	3.52–3.88 m	3.41 t	3.86 q
H-5′a			5.08 dq			
H-5′b			5.11 dq			
H-O			2.97 d	2.03 s		2.42 t
				2.84 s		
H-Bn	4.41–5.04 m	4.60–5.05 m	4.43–4.98 m	4.42–4.89 m	4.38–5.08 m	4.43–5.03 m
H-Ph	7.09–7.39 m	7.10–7.42 m	7.10–7.36 m	7.09–7.42 m	7.11–7.46 m	7.10–7.42 m

TABLE IIB

^{1}H-N.m.r. data, coupling constants

Proton	*Compound*					
$J_{H,H}$	**2**	**3**	**4**	**5**	**6**	**7**
1,2	3.8	3.7	3.8	3.6	3.9	3.6
2,3	9.5	9.8	9.8	9.8	8.9	9.6
3,4	9.0	9.8	9.3	9.8	9.5	9.0
4,5	9.6	9.8	9.8		9.7	9.2
5,6a	3.8	2.1	2.4		3.0	1.5
5,6b	3.6	3.8	3.9		3.6	3.9
6a,6b	10.5	10.8	10.5		10.8	10.8
1'a,1'b	13.2	17.7	10.5			17.2
1'a,2'	6.0	0.8	8.4			
1'a,3'a	0.8					
1'a,3'b	1.5					
1'b,2'	4.8	0.8	3.0			
1'b,3'a	0.8					
1'b,3'b	1.5					
2',3'a	10.5		6.8			
2',3'b	17.3					
2',OH			3.0			
3'a,3'b						16.0
3'a,4'			6.8		6.2	5.4
3'b,4'						5.4
3',5'a			1.0			
3',5'b			1.6			
4',5'a			10.4			
4',5'b			17.0			
4',OH						5.9

150 mL), and the combined organic layers were washed with water (4 × 100 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 25 cm) chromatography (1:3 EtOAc–cyclohexane) of the residue yielded syrupy **4** (8.0 g, 91%), R_F 0.41 (1:2 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IIA and IIB.

Anal. Calc. for $C_{39}H_{44}O_7$: C, 74.89; H, 7.10. Found: C, 74.85; H, 7.05.

2,4-Dihydroxybutyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**5**). — A solution of **4** (6.6 g, 10.6 mmol) in MeOH (250 mL) was ozonolysed (70 min, ~ 10 mmol/h) as described above. Excess of ozone was removed with a stream of oxygen (30 min, 30 L/h), sodium borohydride (3.5 g) was added, and the mixture was allowed to attain room temperature, neutralised with acetic acid (10 mL), and concentrated to dryness. The residue was partitioned in 5:1 water–$CHCl_3$ (600 mL), the aqueous layer was extracted with $CHCl_3$ (6 × 50 mL), and the combined organic layers were washed with saturated aq. NaCl (100 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 20 cm) chromatography (3:1 EtOAc–cyclohexane) of the residue yielded **5** (6.1 g, 92%), m.p. 58–67° (from ether–light petroleum), R_F 0.20 (2:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IIA and IIB.

*2-Hydroxy-4-triphenylmethoxybutyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**6**). — A solution of **5** (4.2 g, 6.68 mmol) and triphenylmethyl chloride (5.0 g, 18 mmol) in dry pyridine (40 mL) was kept at room temperature overnight. Excess of reagent was destroyed with MeOH (10 mL), and the solution was diluted with $CHCl_3$ (200 mL), washed with saturated aqueous $NaHCO_3$ (100 mL) and water (100 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 22 cm) chromatography (1:5 EtOAc–cyclohexane) of the residue gave syrupy **6** (5.0 g, 86%), R_F 0.55 (1:2 EtOAc–cyclohexane).

*2-Oxo-4-triphenylmethoxybutyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**7**). — To a stirred solution of **6** (6.5 g, 7.46 mmol) in dry 20:1 CH_2Cl_2–C_5H_5N (100 mL) was added pyridinium chlorochromate (7.0 g, 32.5 mmol), and the mixture was stirred overnight, then filtered through a column (5.5 × 10 cm) of silica gel with 1:1 EtOAc–cyclohexane, and concentrated. Column (5.5 × 22 cm) chromatography (1:5 EtOAc–cyclohexane) of the residue yielded amorphous **7** (5.8 g, 89%), $[\alpha]_D^{22}$ +43.5° (*c* 2.5), R_F 0.43 (1:3 EtOAc–cyclohexane); ν_{max}^{film} 1730 cm^{-1} (C=O). For the ^{1}H-n.m.r. data, see Tables IIA and IIB.

*4-Hydroxy-2-oxobutyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**8**). — Formic acid (15 mL) was added to a solution of **7** (4.4 g, 5.06 mmol) in ether (15 mL). The mixture was kept at room temperature for 1 h, then diluted with ether (100 mL), washed with water (4 × 50 mL), saturated aq. $NaHCO_3$ (6 × 50 mL), and water (100 mL), dried ($MgSO_4$), and concentrated. Column (2.6 × 20 cm) chromatography (1:1 EtOAc–cyclohexane) of the residue gave syrupy **8** (1.8 g, 56.8%), $[\alpha]_D^{22}$ +57.5° (*c* 1.9), R_F 0.22. For the ^{1}H-n.m.r. data, see Tables IIA and IIB.

Anal. Calc. for $C_{38}H_{42}O_8$: C, 72.82; H, 6.75. Found: C, 72.73; H, 6.72.

*2-Azi-4-hydroxybutyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**9**). — Ammonia (~100 mL) was condensed into a solution of **8** (2.4 g, 3.83 mmol) in dry MeOH (75 mL) at −50°. The mixture was kept at −20° with stirring for 4 h, then cooled to −50°, and a solution of hydroxylamine *O*-sulfonic acid (0.5 g, 4.42 mmol) in dry MeOH (10 mL) was added dropwise. Stirring was continued overnight whilst the mixture was allowed to attain room temperature. The ammonium sulfate was removed, the filtrate was concentrated *in vacuo*, and to a solution of the residue in dry 10:1 MeOH–Et_3N (80 mL) was added methanolic 10% iodine (~8 mL) dropwise with stirring at 0° until the iodine colour persisted. The solution was concentrated *in vacuo*, the residue was partitioned in 3:2 ether–water (500 mL), and the mixture was decolourised by shaking with $Na_2S_2O_3$ (0.5 g). The aqueous layer was extracted with ether (3 × 50 mL), and the combined organic layers were washed with saturated aq. NaCl (100 mL), dried ($MgSO_4$), and concentrated. Column (2.6 × 20 cm) chromatography (1:2 EtOAc–cyclohexane) of the residue gave **9** (1.45 g, 59.3%), $[\alpha]_D^{22}$ +42° (*c* 1), R_F 0.49 (1:1 EtOAc–cyclohexane); λ_{max}^{MeCN} 340 nm (ε_{mM} 0.065). For the ^{1}H-n.m.r. data, see Tables IIIA and IIIB.

Anal. Calc. for $C_{38}H_{42}N_2O_7$: C, 71.45; H, 6.68; N, 4.39. Found: C, 71.38; H, 6.53; N, 4.31.

*2-Azi-4-*p*-toluenesulfonyloxybutyl 2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranoside* (**10**). — To a solution of **9** (2.8 g, 4.38 mmol) in dry 10:1 CH_2Cl_2–C_5H_5N (20 mL) was

TABLE IIIA

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

Proton	*Compound*					
	9	**10**	**11**	**12**	**15**	**16**
H-1	4.67 d	4.68 d	4.97 d	5.05 d	4.83 d	4.84 d
H-2	3.56 dd	3.55 dd	4.87 dd	4.89 dd	4.79 dd	4.77 dd
H-3	3.98 t	3.93 t	5.45 dd	5.48 dd	5.50–5.59 m	5.49–5.58 m
H-4	3.55–3.75 m	3.57–3.74 m	5.05 dd	5.06 t	3.93–4.06 m	3.92–4.05 m
H-5	3.55–3.75 m	3.57–3.74 m	3.96 ddd	4.00 ddd	3.93–4.06 m	3.92–4.05 m
H-6a	3.55–3.75 m	3.57–3.74 m	4.08 dd	4.09 dd	4.28 dd	4.22 dd
H-6b			4.25 dd	4.26 dd	4.52 dd	4.50 dd
H-1′a	3.29 d	3.20–3.32 m	3.39 d	3.49 d	5.38 d	5.31 d
H-1′b	3.34 d		3.41 d	3.52 d		
H-2′					4.88 dd	4.75 dd
H-3′a	1.49 ddd	1.81 t	1.70–1.91 m	1.75–1.91 m	5.47 t	5.38–5.47 m
H-3′b	1.70 ddd					
H-4′	3.55–3.75 m	3.84–4.05 m	3.89–4.02 m	2.88–3.07 m	3.63 t	3.92–4.05 m
H-5′a					3.87 dt	3.92–4.05 m
H-6′a					3.73 t	4.32 dd
H-6′b					4.25 dd	4.56 dd
H-1″						5.38 d
H-2″						4.88 dd
H-3″						5.47 t
H-4″						3.63 t
H-5″						3.85 dt
H-6″a						3.73 t
H-6″b						4.25 dd
H-O	2.89 t					
H-Bn	4.38–5.01 m	4.41–5.03 m				
H-Ph	7.08–7.42 m	7.11–7.81 m	7.40–7.85 m	7.42–7.99 m	7.31–7.47 m	7.32–7.48 m
HC-Ph					5.48 s	5.48 s
Me-Ph		2.40 s	2.48 s			
MeO					3.42 s	3.43 s
OAc			2.03 s	2.02 s	2.02 s	1.98 s
			2.05 s	2.03 s	2.04 s	2.02 s
			2.09 s	2.08 s	2.05 s	2.04 s
				2.09 s	2.08 s	2.05 s
					2.13 s	2.08 s
						2.14 s
						2.20 s

added *p*-toluenesulfonyl chloride (1.5 g, 7.9 mmol), and the mixture was kept for 3 days at room temperature. To this was added 25:1 $CHCl_3$–MeOH (50 mL), and the solution was washed with saturated aq. $NaHCO_3$ (30 mL) and with water (50 mL), dried ($MgSO_4$), and concentrated. Column (2.6 × 23 cm) chromatography (1:3 EtOAc–cyclohexane) of the residue yielded syrupy **10** (2.9 g, 83.5%), $[\alpha]_D^{22}$ + 39° (*c* 1.2), R_F 0.50 (1:2 EtOAc–cyclohexane); λ_{max}^{MeCN} 335 nm (ε_{mM} 0.060). For the ^{1}H-n.m.r. data, see Tables IIIA and IIIB.

Anal. Calc. for $C_{45}H_{48}N_2O_9S$: C, 68.16; H, 6.10; N, 3.53. Found: C, 68.17; H, 6.17; N, 3.35.

TABLE IIIB

^{1}H-N.m.r. data, coupling constants

Proton	*Compound*					
$J_{H,H}$	**9**	**10**	**11**	**12**	**15**	**16**
1,2	3.6	3.6	3.8	3.9	3.8	3.5
2,3	9.0	9.5	10.2	10.2	9.8	10.0
3,4	9.0	10.0	9.6	9.6		
4,5			10.0	10.0		
5,6a			2.4	2.3		
5,6b			4.5	4.5		
6a,6b			12.5	12.3	12.4	12.8
1'a,1'b	12.3		12.0	12.6		
1'a,2'					4.1	4.2
2',3'a					10.3	10.8
3'a,3'b	15.0					
3'a,4'	4.2/6.3	6.5			9.7	
3'b,4'	4.5/7.5					
4',5'a					9.5	
4',OH	5.3					
5',6'a					10.1	
5',6'b					4.5	
6'a,6'b					10.1	12.8
1",2"						4.2
2",3"						10.2
3",4"						9.8
4",5"						9.6
5",6"a						9.8
5",6"b						4.5
6"a,6"b						10.1

*2-Azi-4-*p*-toluenesulfonyloxybutyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**11**). — Anhydrous $FeCl_3$ (400 mg) was dissolved in a solution of **10** (2.0 g, 2.52 mmol) in acetic anhydride (15 mL), and the mixture was kept for 4 h at room temperature, then poured into stirred ice–water (200 mL), and left stirring for 1 h. The mixture was extracted with $CHCl_3$ (3 × 100 mL), and the combined extracts were neutralised with saturated aq. $NaHCO_3$ (3 × 100 mL), washed with water (200 mL), dried ($MgSO_4$), and concentrated. Column (2.6 × 20 cm) chromatography (2:3 EtOAc–cyclohexane) of the residue yielded syrupy **11** (0.55 g, 36.3%), $[\alpha]_D^{22}$ +86° (*c* 0.75), R_F 0.38 (1:1 EtOAc–cyclohexane); λ_{max}^{MeCN} 330 nm (ε_{mM} 0.095). For the ^{1}H-n.m.r. data, see Tables IIIA and IIIB. The remaining partially benzylated glycoside could be separated and recycled.

*2-Azi-4-benzoylthiobutyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**12**). — To a solution of **11** (2.5 g, 4.16 mmol) in dry acetone was added potassium thiobenzoate (2.0 g, 11.3 mmol). The mixture was stirred for 5 h at room temperature, then concentrated, the residue was partitioned in 1:1 $CHCl_3$–water (150 mL), and the aqueous layer was extracted with $CHCl_3$ (2 × 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (50 mL) and water (100 mL), dried ($MgSO_4$), and concen-

trated. Column (2.6 × 20 cm) chromatography (2:3 EtOAc–cyclohexane) of the residue yielded syrupy **12** (1.9 g, 80.5%), $[\alpha]_D^{22}$ + 95° (*c* 0.53), R_F 0.51 (1:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IIIA and IIIB.

Methyl 2,3,6,2',3'-penta-O-*acetyl-4',6'*-O-*benzylidene-α-maltoside* (**15**). — A mixture of methyl α-maltoside (34.5 g, 96.8 mmol), benzaldehyde dimethylacetal (16 g, 105 mmol), and *p*-toluenesulfonic acid (~ 70 mg) in dry *N,N*-dimethylformamide (100 mL) was stirred for 4 h at 60°/15 Torr, then concentrated. A solution of the residue in 3:2 C_5H_5N–Ac_2O (250 mL) was kept overnight at 50°, then diluted with water (1 L), and extracted with $CHCl_3$ (4 × 100 mL). The combined extracts were washed with saturated aq. $NaHCO_3$ (2 × 100 mL) and water (200 mL), dried ($MgSO_4$), and concentrated. Column (9.5 × 17 cm) chromatography (2:3 EtOAc–cyclohexane) of the residue yielded syrupy **15** (31.6 g, 49.9%), $[\alpha]_D^{22}$ + 99.5° (*c* 1.1), R_F 0.31 (1:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IIIA and IIIB.

Methyl 2,3,6,2',3',6',2'',3''-octa-O-*acetyl-4'',6''*-O-*benzylidene-α-maltotrioside* (**16**). — A mixture of methyl α-maltotrioside (8.2 g, 15.8 mmol), benzaldehyde dimethylacetal (3.0 g, 19.7 mmol), and *p*-toluenesulfonic acid (~ 25 mg) was stirred for 10 h at 60°/15 Torr, then concentrated. The residue was acetylated as described for **15**. Column (5.5 × 20 cm) chromatography (1:1 EtOAc–cyclohexane) of the product gave amorphous **16** (6.7 g, 45%), $[\alpha]_D^{22}$ + 119.5° (*c* 1.3), R_F 0.21. For the ^{1}H-n.m.r. data, see Tables IIIA and IIIB.

Anal. Calc. for $C_{42}H_{54}O_{24}$: C, 53.50; H, 5.77. Found: C, 53.50; H, 5.66.

Methyl 2,3,6,2',3'-penta-O-*acetyl-4',6'-di*-O-*toluenesulfonyl-α-maltoside* (**17**). — A solution of **15** (4.5 g, 6.87 mmol) in 4:1 EtOH–EtOAc (100 mL) was hydrogenated (1 atm) over 10% Pd/C (250 mg) with stirring, then filtered, and concentrated *in vacuo*. To a solution of the residue in dry C_5H_5N (50 mL) was added *p*-toluenesulfonyl chloride (8 g, 42 mmol), and the mixture was stored for 3 days at room temperature. Ice (5 g) and water (1 L) were added, the mixture was extracted with $CHCl_3$ (4 × 50 mL), and the combined extracts were washed with saturated aq. $NaHCO_3$ (2 × 100 mL) and water (200 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 12 cm) chromatography (1:1 EtOAc–cyclohexane) of the residue gave syrupy **17** (4.5 g, 74.8%), $[\alpha]_D^{22}$ + 92.5° (*c* 1), R_F 0.45 (2:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IVA and IVB.

Anal. Calc. for $C_{37}H_{46}O_{20}S_2$: C, 50.68; H, 5.52. Found: C, 50.72; H, 5.28.

Methyl 2,3,6,2',3',6',2'',3''-octa-O-*acetyl-4'',6''-di*-O-*toluenesulfonyl-α-maltotrioside* (**18**). — A solution of **16** (6.9 g, 7.32 mmol) in 4:3 EtOH–EtOAc (350 mL) was hydrogenolysed over 10% Pd/C (1 g) as described for **17**, then filtered, and concentrated *in vacuo*. The residue was treated with *p*-toluenesulfonyl chloride (10 g, 52.5 mmol) in C_5H_5N (50 mL). After 6 days, the product was isolated as described for **17**. Column (5.5 × 20 cm) chromatography (3:2 EtOAc–cyclohexane) of the product gave amorphous **18** (7.0 g, 82%), $[\alpha]_D^{22}$ + 110° (*c* 1.5), R_F 0.45 (2:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IVA and IVB.

Anal. Calc. for $C_{49}H_{62}O_{28}S_2$: C, 50.60; H, 5.37. Found: C, 50.31; H, 5.24.

Methyl 2,3,6,2',3'-penta-O-*acetyl-6'-deoxy-6'-iodo-4'*-O-p-*toluenesulfonyl-α-maltoside* (**19**). — To a solution of **17** (9.0 g, 10.3 mmol) in Ac_2O (60 mL) was added sodium

TABLE IVA

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

Proton	*Compound*					
	17	**18**	**19**	**20**	**21**	**22**
H-1	4.82 d	4.83 d	4.83 d	4.83 d	4.81 d	4.82 d
H-2	4.76 dd	4.76 dd	4.77 dd	4.76 dd	4.77 dd	4.76 dd
H-3	5.47 dd	5.52 dd	5.51 dd	5.52 dd	5.49 dd	5.52 dd
H-4	3.80–3.92 m	3.81–4.03 m	3.89–4.01 m	3.84–4.03 m	4.03 dd	3.78–4.44 m
H-5	3.80–3.92 m	3.81–4.03 m	3.89–4.01 m	3.84–4.03 m	3.89 ddd	3.78–4.44 m
H-6a	4.16 dd	4.04–4.33 m	4.30 dd	4.30 dd	4.07 dd	3.78–4.44 m
H-6b	4.31 dd	4.33–4.66 m	4.38 dd	4.43 dd	4.40 dd	
H-1'a	5.28 d	5.25–5.29 m	5.41 d	5.29 d	5.47 d	5.26 d
H-2'	4.69 dd	4.66–4.76 m	4.81 dd	4.73 dd	4.87 dd	4.72 dd
H-3'a	5.40 dd	5.36 dd	5.48 dd	5.39 dd	5.42 dd	5.37 dd
H-3'b						
H-4'	4.69 t	3.81–4.03 m	4.63 t	3.84–4.03 m	5.09 dt	3.78–4.44 m
H-5'a	3.92 ddd	3.81–4.03 m	3.60 ddd	3.84–4.03 m		3.78–4.44 m
H-6'a	4.00 dd	4.04–4.33 m	3.13 dd	4.24 dd	4.67 t	3.78–4.44 m
H-6'b	4.23 dd	4.33–4.66 m	3.40 dd	4.43 dd	4.78 t	
H-1''		5.25–5.29 m		5.38 d		5.46 d
H-2''		4.66–4.76 m		4.79 dd		4.88 dd
H-3''		5.39 dd		5.45 dd		5.40 t
H-4''		4.71 t		4.62 t		5.10 dt
H-5''		3.81–4.03 m		3.58 ddd		
H-6''a		3.99 dd		3.11 dd		4.66 t
H-6''b		4.24 dd		3.37 dd		4.79 t
H-Ph	7.30–7.85 m	7.30–7.85 m	7.35–7.82 m	7.35–7.82 m	7.34–7.81 m	7.35–7.81 m
HC-Ph						
Me-Ph	2.45 s	2.45 s	2.47 s	2.46 s	2.46 s	2.46 s
	2.48 s	2.48 s				
MeO	3.40 s	3.42 s	3.42 s	3.43 s	3.40 s	3.43 s
OAc	1.95 s	1.96 s	1.96 s	1.96 s	1.90 s	1.91 s
	2.01 s	1.98 s	2.02 s	1.98 s	2.01 s	1.98 s
	2.03 s	2.01 s	2.05 s	2.03 s	2.04 s	2.01 s
	2.05 s	2.03 s	2.06 s	2.04 s	2.05 s	2.03 s
	2.07 s	2.04 s	2.08 s	2.05 s	2.09 s	2.04 s
		2.05 s		2.06 s		2.06 s
		2.08 s		2.10 s		2.11 s
		2.15 s		2.16 s		2.16 s

iodide (2.5 g, 16.7 mmol), and the mixture was stirred at 120° for 45 min, then cooled, poured into stirred acetone (250 mL), filtered, and concentrated. The residue was partitioned in 1:4 $CHCl_3$–water (300 mL), the aqueous layer was extracted with $CHCl_3$ (3 × 50 mL), and the organic layers were combined, neutralised with saturated aq. $NaHCO_3$, decolourised with aq. 1% $Na_2S_2O_3$ (100 mL), washed with water (200 mL), dried ($MgSO_4$), and concentrated. Column (5.5 × 20 cm) chromatography (2:3 EtOAc–cyclohexane) of the residue yielded **19** (6.5 g, 76%), $[\alpha]_D^{22}$ + 102° (*c* 1.1), R_F 0.33 (1:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IVA and IVB.

Anal. Calc. for $C_{30}H_{39}IO_{17}S$: C, 43.38; H, 4.73. Found: C, 43.59; H, 4.59.

TABLE IVB

^{1}H-N.m.r. data, coupling constants

Proton	*Compound*					
$J_{H,H}$	**17**	**18**	**19**	**20**	**21**	**22**
1,2	3.6	3.6	3.5	3.5	3.6	3.3
2,3	10.2	10.1	10.2	10.2	10.0	9.9
3,4	8.7	8.4	8.9	8.6	8.7	8.1
4,5					9.9	
5,6a					5.0	
5,6b					2.6	
6a,6b	12.3		12.0	12.0	12.0	
1'a,2'	3.9		3.9	4.2	3.5	4.2
2',3'a	10.5		10.5	10.2	10.4	10.5
3'a,4'	9.3	8.3	9.2	8.4	9.3	8.1
4',5'a	9.9		9.2			
4',6'a					2.1	
4',6'b					2.1	
5',6'a	4.2		5.7			
5',6'b	1.8		2.9			
6'a,6'b	11.3		11.3	12.3	2.1	
1",2"				3.9		3.8
2",3"				10.5		10.5
3",4"		9.3		9.2		9.5
4",5"		9.3		9.3		
4",6"a						2.2
4",6"b						2.2
5",6"a				5.6		
5",6"b				3.0		
6"a,6"b		12.0		11.4		2.2

*Methyl 2,3,6,2',3',6',2",3"-octa-*O*-acetyl-6"-deoxy-6"-iodo-4"-*O*-*p*-toluenesulfonyl-α-maltotrioside* (**20**). — A mixture of **18** (6.2 g, 5.33 mmol), Ac_2O (30 mL), and sodium iodide (1.5 g, 10 mmol) was treated as described for **19**. Column (5.5 × 20 cm) chromatography (3:2 EtOAc–cyclohexane) of the product yielded amorphous **20** (5.8 g, 98%), $[\alpha]_D^{22}$ + 114° (*c* 1), R_F 0.48 (2:1 EtOAc–cyclohexane). For the ^{1}H-n.m.r. data, see Tables IVA and IVB.

Anal. Calc. for $C_{42}H_{55}IO_{25}S$: C, 45.09; H, 4.95. Found: C, 44.84; H, 4.85.

*Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-6-deoxy-4-*O*-p-toluenesulfonyl-α-*D*-xylo-hex-5-enopyranosyl)-α-*D*-glucopyranoside* (**21**). — A mixture of **19** (2.5 g, 3.01 mmol) and silver fluoride (2.0 g, 15.8 mmol) in dry C_5H_5N (15 mL) was stirred at room temperature overnight, then filtered through a column (2.6 × 30 cm, EtOAc) of silica gel and concentrated. Column (2.6 × 20 cm) chromatography (1:1 EtOAc–cyclohexane) of the residue yielded **21** (1.7 g, 80.4%) as an unstable, amorphous solid, $[\alpha]_D^{22}$ + 64° (*c* 4.1), R_F 0.26. For the ^{1}H-n.m.r. data, see Tables IVA and IVB.

*Methyl 2,3,6,2',3',6'-hexa-*O*-acetyl-4'-*O*-(2,3-di-*O*-acetyl-6-deoxy-4-*O*-p-toluenesulfonyl-α-*D*-xylo-hex-5-enopyranosyl)-α-maltoside* (**22**). — A mixture of **20** (1.6 g,

1.43 mmol) and silver fluoride (1.1 g, 8.7 mmol) in dry C_5H_5N was treated as described for **21**. Column (2.6 × 30 cm) chromatography (3:2 EtOAc–cyclohexane) of the product yielded amorphous, unstable **22** (0.9 g, 63.5%), $[\alpha]_D^{22}$ +75° (*c* 3.9). For the ^{1}H-n.m.r. data, see Tables IVA and IVB.

*Methyl 4'-O-[4-S-(3-azi-4-α-*D*-glucopyranosyloxybutyl)-6-deoxy-4-thio-α-*D*-xylo-hex-5-enopyranosyl]-α-maltoside* (**28**). — To a stirred solution of **23** (3.0 g, 3.03 mmol) in dry acetone (40 mL) was added dropwise methanolic M sodium methoxide (3.5 mL). Precipitated sodium *p*-toluenesulfonate was removed by centrifugation and a solution of **12** (0.5 g, 0.88 mmol) in dry MeOH (10 mL) was added slowly to the supernatant solution. The mixture was washed through a column (2.6 × 3 cm) of silica gel with MeOH and concentrated. H.p.l.c. (Hypersil ODS, 5 μm, column 250 × 20 mm, 35:65 MeOH–water, 15 mL/min, detection at 340 nm) of the residue gave amorphous **28** (158.1 mg, 23.1%), $[\alpha]_D^{22}$ +161° (*c* 0.7, water), R_F 0.45 (4:2:1 EtOAc–MeOH–water); λ_{max}^{water} 340 nm (ε_{mM} 0.066). ^{13}C-N.m.r. data: δ 25.38–55.52 (C-1‴,2‴,3‴,4‴ and OCH_3), 60.86–61.37 (C-6,6',6‴'), 68.67–73.85 (C-2,2',2",2‴,3,3',3",3‴,4",4‴,5,5',5‴'), 76.57–77.83 (C-4,4'), 98.42–100.51 (C-1,1',1",1‴,6"), 153.82 (C-5").

A solution of **28** (1.85 mg) in water (1 mL) was irradiated for 7 min and the decrease in extinction at 340 nm was measured every minute. A plot of *E* against the time of irradiation gave a half life of 2.4 min.

*Methyl 4-O-[4-S-(3-azi-4-α-maltosyloxybutyl)-6-deoxy-4-thio-α-*D*-xylo-hex-5-enopyranosyl]-α-*D*-glucopyranoside* (**29**). — To a stirred solution of **21** (2.0 g, 2.85 mmol) in dry acetone (20 mL) was added dropwise methanolic M sodium methoxide (3.5 mL). Precipitated sodium *p*-toluenesulfonate was removed by centrifugation and a solution of **12** (0.3 g, 0.53 mmol) in dry MeOH (5 mL) was added slowly to the supernatant solution. The solution was stored for 8 h, diluted with water (8 mL), and concentrated to ~ 10 mL. H.p.l.c. (Hypersil ODS, 5 μm, column 250 × 20 mm, 35:65 MeOH–water, 15 mL/min, detection at 340 nm) gave **27** (174 mg, 53.4%).

To a solution of **27** in water (6 mL) were added cyclomaltohexaose (340 mg, 0.35 mmol) and CGTase (50 μL, 38 U), and the solution was stored overnight at room temperature. The enzyme was inactivated by heating to 95° for 5 min, acetic acid (10 μL) and beta-amylase (20 μL, 50 U) were added, and the mixture was kept for 2 h at room temperature. The enzyme was then inactivated as described for CGTase, the solution centrifuged, and the product subjected to h.p.l.c., as described for **28**, to yield amorphous **29** (86.6 mg, 21%), $[\alpha]_D^{22}$ +139° (*c* 0.5, water), R_F 0.43 (4:2:1 EtOAc–MeOH–water); λ_{max}^{water} 338 nm (ε_{mM} 0.062). ^{13}C-N.m.r. data: δ 25.37–55.50 (C-1",2",3",4" and OCH_3), 60.86–61.55 (C-6,6‴,6‴'), 68.94–74.11 (C-2,2',2‴,2‴',3,3',3‴,3‴',4',4‴',5,5‴,5‴'), 76.42–77.58 (C-4,4‴), 98.33–100.56 (C-1,1',1‴,1‴',6'), 153.84 (C-5').

The half life of irradiated **29** (1.54 mg) in water (1 mL), determined as described for **28**, was 2.5 min.

*Methyl 4-S-(3-azi-4-α-maltotriosyloxybutyl)-6-deoxy-4-thio-α-*D*-xylo-hex-5-enopyranoside* (**30**). — A solution of **12** (0.5 g, 0.88 mmol) in dry MeOH (8 mL) was added slowly to a solution of freshly prepared **23** (1.0 g, 6.3 mmol) in dry acetone (20 mL) followed by methanolic M sodium methoxide (1 mL). The solution was kept

overnight at room temperature, then diluted with water (8 mL), and concentrated to ~10 mL. H.p.l.c. (Hypersil ODS, 5 μm, column 250 × 20 mm, 45:55 MeOH–water, 15 mL/min, detection at 340 nm) then gave amorphous **26** (345 mg, 93%), R_F 0.16 (17:2:1 EtOAc–MeOH–water). This product was dissolved in water (10 mL), cyclomaltohexaose (640 mg, 0.66 mmol) and CGTase (50 μL, 38 U) were added, and the mixture was stored for 10 h at room temperature. The enzyme was inactivated, the products were treated with beta-amylase, and the product was subjected to h.p.l.c., as described for **29**, to yield amorphous **30** (205.5 mg, 30.2%), $[\alpha]_D^{22}$ +151° (*c* 0.6, water), R_F 0.45 (4:2:1 EtOAc–MeOH–water); λ_{max}^{water} 340 nm (ε_{mM} 0.056). ^{13}C-N.m.r. data: δ 25.66–56.02 (C-1′,2′,3′,4′ and OCH_3), 60.75–60.91 (C-6″,6‴,6⁗), 69.09–73.75 (C-2,2″,2‴,2⁗,3,3″, 3‴,3⁗,4′,4⁗,5″,5‴,5⁗), 77.63–78.00 (C-4″,4‴), 98.34–101.18 (C-1,1″,1‴,1⁗,6), 153.37 (C-5).

The half life of irradiated **30** (2.16 mg) in water (1 mL), determined as described for **28**, was 2.1 min.

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