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METHYL 4-*O*-(4-α-D-GLUCOPYRANOSYLOXY-4-METHOXYBUTYL)-α-D-GLUCOPYRANOSIDE, A MODIFIED OLIGOSACCHARIDE FOR STUDYING THE INTERACTIONS OF CARBOHYDRATES WITH MULTI-SITE PROTEINS

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ABSTRACT

Methyl 4-*O*-(4-α-D-glucopyranosyloxy-4-methoxybutyl)-α-D-glucopyranoside (**9**) was synthesised by transacetalation from methyl 2,3,6-tri-*O*-acetyl-4-*O*-(4,4-dimethoxybutyl)-α-D-glucopyranoside and trimethylsilyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside followed by removal of the blocking groups. Compound **9**, which is methyl α-maltotrioside modified by replacing the middle D-glucosyl residue with an acyclic spacer, competitively inhibits the hydrolysis of *p*-nitrophenyl α-maltotrioside by porcine alpha-amylase.

INTRODUCTION

Endoglucanases[1], oligo- or poly-saccharide-binding proteins[2], as well as oligosaccharide-transporting proteins[3] seem to possess extended binding-sites, which recognise and bind oligosaccharide sequences. The model for alpha-amylase reported by Robyt and French[4] is the first attempt to give a detailed description of such extended binding-sites. This so-called subsite model was further confirmed by numerous investigations[5], mainly by studying the complicated pattern of malto-oligosaccharide fragmentation and resynthesis catalysed by alpha-amylase. No attempt has been made so far to label the binding site, probably because of the difficulty in finding ligands which cover the whole of the binding region and which carry functional groups suitable for reaction with the binding site. These functional groups must be tolerated by the protein but be close enough to allow covalent attachment. The best location for such a functional group would be in the middle of an oligosaccharide chain where both ends bind to the protein. Thus, for malto-oligosaccharides, one of the D-glucosyl residues could either be functionalised by a suitable photolabile group or replaced by a spacer which mimics D-glucose in that it would not interfere with the binding of the flanking D-glucose residues. We now describe a maltotriose derivative modified in the latter manner.

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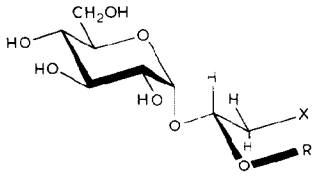
RESULTS AND DISCUSSION

Maltotriose is the smallest oligosaccharide bound by alpha-amylases of different origin and which is also slowly hydrolysed. The modified maltotriose would have to contain two terminal α-D-glucopyranose residues linked by a spacer that can adopt the conformation of the middle D-glucosyl residue, thereby retaining the overall molecular shape. It must also be possible to introduce, into the spacer, reactive functional groups, such as azido or diazirino groups, for photoaffinity labelling[6], without exceeding the bulk of a glucosyl residue. Recently, several mixed-acetal glycosides (**1**) have been synthesised and found to be substrates and inhibitors for glycosidases[7,8]. The acyclic spacer can mimic a six-membered ring and is easily variable by the attachment of different groups R and X. Since the preparation of such compounds can be effected by transacetalation[7,9,10] catalysed by proton or Lewis acids, this method was chosen to link two α-D-glucopyranose residues through positions 1 and 4, using a suitable spacer. The simplest spacer with the appropriate length is an *n*-butyl chain, and the appropriate compound was synthesised as follows. Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[11] (**2**) was alkylated with 1-bromopent-4-ene to give methyl 2,3,6-tri-*O*-benzyl-4-*O*-(pent-4-enyl)-α-D-glucopyranoside (**3**). Oxidative cleavage of the double bond in **3** with OsO_4^- and IO_4^- gave methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4-oxobutyl)-α-D-glucopyranoside (**4**), which, with methanol, gave methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,4-dimethoxybutyl)-α-D-glucopyranoside (**5**). Compound **5** was *O*-debenzylated (→**6**) and then acetylated



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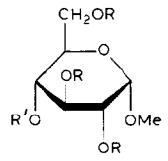
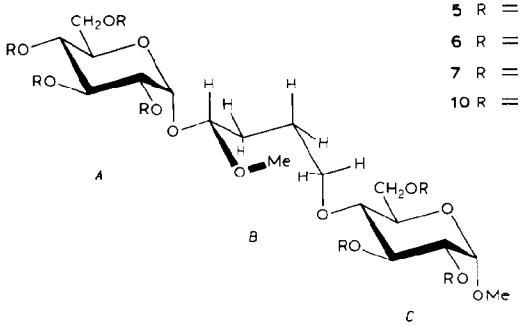


2 R = Bzl , R′ = H
3 R = Bzl , R′ = $CH_2-CH_2-CH_2-CH=CH_2$
4 R = Bzl , R′ = $CH_2-CH_2-CH_2-CH=O$
5 R = Bzl , R′ = $CH_2-CH_2-CH_2-CH(OMe)_2$
6 R = H , R′ = $CH_2-CH_2-CH_2-CH(OMe)_2$
7 R = Ac , R′ = $CH_2-CH_2-CH_2-CH(OMe)_2$
10 R = H , R′ = $CH_2-CH_2-CH_2-CH=O$



A B C

8 R = Ac
9 R = H

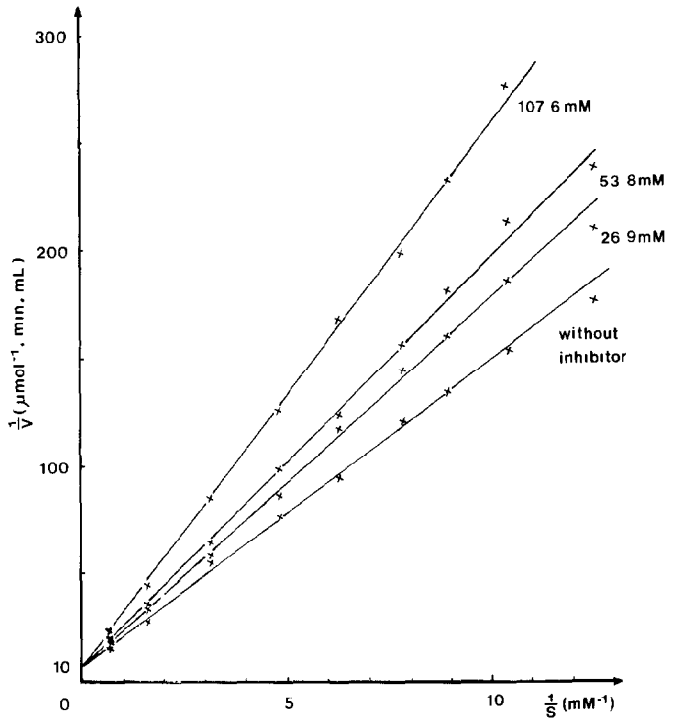


Fig. 1 Determination of the inhibition constant (K_I) for methyl 4-*O*-(4-α-D-glucopyranosyloxy-4-methoxybutyl)-α-D-glucopyranoside (**9**) in triethanolamine buffer [0.05M, pH 7.6, containing calcium chloride (10mM)] at 30°. The concentrations of substrate (*p*-nitrophenyl α-maltotrioside) and inhibitor were varied as indicated.

to give methyl 2,3,6-tri-*O*-acetyl-4-*O*-(4,4-dimethoxybutyl)-α-D-glucopyranoside (**7**), which underwent transacetalation when treated with trimethylsilyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[12] in the presence of trimethylsilyl triflate as catalyst[10]. Trimethylsilyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside could not be obtained in reasonable yield and free from the β anomer as described by Tietze *et al.*[13], but was prepared from 2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose by trimethylsilylation followed by flash chromatography[14]. The deblocked product of transacetalation was identified as methyl 4-*O*-(4-α-D-glucopyranosyloxy-4-methoxybutyl)-α-D-glucopyranoside (**9**), the ^{1}H-n.m.r. data for which revealed two diastereomers (ratio 3:1) due to the new asymmetric center at C-4′. Since such asymmetry did not significantly influence the kinetic parameters when mixed-acetal glycosides were used as enzyme inhibitors or substrates[8], isolation of the diastereomers was not attempted. The transacetalation reaction normally yields more of the *R* isomer and hence it is assumed that the major diastereomer of **9** had the *R* configuration at C-4′.

Compound **9** was hydrolysed smoothly when incubated with α-D-glucosidase, to give D-glucopyranose and methyl 4-*O*-(4-oxobutyl)-α-D-glucopyranoside (**10**),

but was not affected by porcine pancreas alpha-amylase. When **9** was added to incubation mixtures containing *p*-nitrophenyl α-maltotrioside and porcine pancreas alpha-amylase, it acted as a competitive inhibitor. A K_i value of 140mM was found by a Lineweaver–Burk plot (Fig. 1) (*cf.* K_i values of 980mM for methyl α-D-glucopyranoside and 1.8mM for maltotriose[15]). This finding can be interpreted by the assumption that both the glucopyranose residues in **9** are bound at separate subsites, with the spacer bridging the central subsite. It is unlikely that the spacer contributes to the binding of **9** and alpha-amylase.

Work is in progress on higher homologues of **9** with photolabile groups on the spacer.

EXPERIMENTAL

Methods. — All reactions were monitored by t.l.c. on silica gel 60 F_{254} (Merck), using the solvents indicated. G.l.c. was performed with a Pye–Unicam

TABLE I

^{1}H-N M R DATA (250 MHz) FOR **3–5, 7, 8** ($CDCl_3$, INTERNAL Me_4Si), AND **9** (D_2O, INTERNAL DSS)

Proton	*Compound*					
	3	**4**	**5**	**7**	**8**	**9**
Chemical shifts (δ)						
H-1	4.60 d	4.61 d	4.59 d	4.85 d	4.86 d	4.80 d
H-2	3.50 dd	3.49 dd	3.50 dd	4.83 dd	4.82 dd	3.56 dd
H-3	3.40 dd	3.36 dd	3.41 dd	5.47 dd	5.46 dd	3.64–3.90 m
H-4	3.88 dd	3.83 dd	3.87 dd	3.42 dd	3.39 dd	3.28 dd
H-5	3.68 m	3.62 m	3.66 m	3.85 ddd	3.87 ddd	3.64–3.90 m
H-6	3.66 m	3.60 m	3.66 m	3.27 dd	4.02–4.13 m	3.64–3.90 m
H*-6	3.66 m	3.60 m	3.66 m	3.35 dd	4.02–4.13 m	3.64–3.90 m
H-1′	3.78 dt	3.76 m	3.78 m	3.50–3.56 m	3.41–3.58 m	3.64–3.90 m
H*-1′	3.43 dt	3.36 m	3.42 m	3.50–3.56 m	3.41–3.58 m	3.64–3.90 m
H-2′,H*-2′	1.53 p	1.70 m	1.51 m	1.52–1.63 m	1.53–1.73 m	1.61–1.83 m
H-3′,H*-3′	1.98 dt	2.27 dt	1.51 m	1.52–1.63 m	1.52–1.73 m	1.61–1.83 m
H-4′	5.73 ddt	9.61 t	4.28 t	4.33 t	4.50 t, 4.64 t	3.64–3.90 m
H-5′*trans*	4.95 m	—	—	—	—	—
H-5′*cis*	4.92 m	—	—	—	—	—
H-1″	—	—	—	—	5.28 d, 5.32 d	5.10 d, 5.19 d
H-2″	—	—	—	—	4.94 dd, 4.93 dd	3.64 dd
H-3″	—	—	—	—	5.49 dd	3.64–3.90 m
H-4″	—	—	—	—	5.08 dd	3.42 dd
H-5″	—	—	—	—	4.23 m	3.64–3.90 m
H-6″	—	—	—	—	4.27 m	3.64–3.90 m
H*-6″	—	—	—	—	4.34 m	3.64–3.90 m
MeO-1	3.38 s	3.36 s	3.38 s	3.38 s	3.39 s	3.40 s
OCH_2Ph	4.49–4.93 m	4.47–4.96 m	4.48–4.94 m	—	—	—
Ph	7.32 m	7.32 m	7.31 m	—	—	—
MeO-4	—	—	3.27 s	3.31 s	3.28 s, 3.39 s	3.42 s, 3.47 s
Ac	—	—	—	2.07 s, 2.13 s	2.01–2.14 s	—

TABLE I *(continued)*

Proton	*Compound*					
	3	**4**	**5**	**7**	**8**	**9**
$J_{H,H}$ *(Hz)*						
1,2	3.6	3.8	3.6	3.8	3.3	4.0
2,3	9.6	9.6	9.6	9.7	10.4	10.5
3,4	9.3	9.2	9.0	9.3	9.3	10.5
4,5	9.3	9.2	9.0	9.8	9.3	9.0
5,6	—	—	—	2.4	2.7	—
5,6*	—	—	—	4.5	4.2	—
6,6*	—	—	—	12.0	12.9	—
1′,1′*	9.0	—	8.7	—	—	—
1′,2′	7.0	6.7	5.1	5.3	—	—
1′*,2′	6.7	6.7	5.1	5.3	—	—
1′,2′*	6.3	6.7	5.1	5.3	—	—
1′*,2′*	6.7	6.7	5.1	5.3	—	—
2′,3′	6.9	7.5	5.1	5.3	—	—
2′*,3′	6.9	7.5	5.1	5.3	—	—
2′,3′*	6.9	7.5	5.1	5.3	—	—
2′*,3′*	6.9	7.5	5.1	5.3	—	—
3′,4′	6.5	1.5	5.3	5.0	5.2	—
3′*,4′	6.5	1.5	5.3	5.0	5.2	—
4′,5′*cis*	10.4	—	—	—	—	—
4′,5′*trans*	17.0	—	—	—	—	—
5′,5′*	3.6	—	—	—	—	—
3′,5′*cis*	1.5	—	—	—	—	—
3′,5′*trans*	2.1	—	—	—	—	—
1″,2″	—	—	—	—	3.9	3.7
2″,3″	—	—	—	—	10.5	10.6
3″,4″	—	—	—	—	9.0	10.4
4″,5″	—	—	—	—	9.0	9.2
5″,6″	—	—	—	—	2.4	—
5″,6″*	—	—	—	—	4.5	—
6″,6″*	—	—	—	—	12.0	—

[a]For **3**–**5** and **7**, H-1′ *etc.* refer to the aglycon; for **8** and **9**, H-1, H-1′, and H-1″ refer to units *A*–*C*, respectively, in the depicted formulae.

GCD chromatograph, with glass columns and SE-52 (3%) on Chromosorb G, AW-DMCS. H.p.l.c. was performed with a system consisting of an LKB 2152 h.p.l.c. controller, two LKB 2150 pumps, a Rheodyne 7126 injector Lichrosorb RP-18 column (4 × 250 mm, 5 μ), an LKB variable wavelength monitor, an LKB 2211 Super Rac fraction collector, and a Shimadzu C-R2Ax integrator. Flash column chromatography[14] was performed on silica gel (230–400 mesh, Merck). Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 (250 MHz) spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate). Light petroleum refers to the fraction b.p. 60–70°.

Enzymic reactions. — Alpha-amylase (α-1,4-glucanase, 4-glucanohydrolase, EC 3.2.1.1) from porcine pancreas, as a suspension in aqueous ammonium sulphate

(10 mg/mL), was purchased from Boehringer. The inhibition constants were determined spectrometrically at 405 nm by using *p*-nitrophenyl α-maltotrioside. Tests were carried out at 30° in triethanolamine buffer (0.05M, pH 7.6, containing 10mM calcium chloride) with various substrate concentrations (0.08–1.6mM). The solutions for all tests also contained inhibitor (0–107.6 and 0–400mM).

A Lineweaver–Burk plot of the results is shown in Fig. 1. Calculation of the competitive inhibition was based on the extinction coefficient of 13.95 $cm^2/\mu mol$.

Cleavage of methyl 4-O-(4-α-D-glucopyranosyloxy-4-methoxybutyl)-α-D-glucopyranoside (**9**). — A suspension of α-D-glucosidase (maltase, α-D-glucoside glucohydrolase, EC 3.2.1.20) from yeast in aqueous ammonium sulphate (5 mg/mL, Boehringer) was used. The test was carried out at 25° with **9** (5.2 mg, 0.011 mmol) in sodium potassium phosphate buffer (500 μL; 0.5M, pH 6.8) containing magnesium chloride (mM) and α-D-glucosidase suspension (10 μL). The cleavage was monitored by t.l.c. (7:2:1 ethyl acetate–methanol–water). After 6 h, the sole products were D-glucose (R_F 0.23) and methyl 4-*O*-(4-oxobutyl)-α-D-glucopyranoside (**10**, R_F 0.44).

Methyl 2,3,6-tri-O-benzyl-4-O-(pent-4-enyl)-α-D-glucopyranoside (**3**). — To a solution of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**2**; 21 g, 45.2 mmol) in *N,N*-dimethylformamide (135 mL) at room temperature was added sodium hydride (9.8 g of a 60% suspension in oil washed with light petroleum) portionwise with stirring, the reaction mixture being cooled to 0°. 1-Bromopent-4-ene (25 mL) was then added dropwise. After 1 h, the excess of sodium hydride was decomposed with methanol, the clear solution was diluted with water (250 mL) and extracted with dichloromethane (250 mL), and the extract was dried ($MgSO_4$) and concentrated to dryness *in vacuo*. Flash column chromatography (1:4 ethyl acetate–light petroleum) yielded **3** (22 g, 91.4%) as a colourless syrup, $[\alpha]_{578}^{25}$ +23° (*c* 1.4, chloroform), R_F 0.54 (4:1 ether–light petroleum). See Table I for ^{1}H-n.m.r. data.

Methyl 2,3,6-tri-O-benzyl-4-O-(4-oxobutyl)-α-D-glucopyranoside (**4**). — To a solution of **3** (22 g, 41.3 mmol) in ether (200 mL) was added water (200 mL) followed by osmium tetraoxide (30 mg), and then sodium metaperiodate (51.6 g) was added portionwise with vigorous stirring throughout. The oxidation was monitored by t.l.c. (1:1 ethyl acetate–light petroleum). After 10 h, the ether layer was extracted with water (3 × 200 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash column chromatography (1:2.5 ethyl acetate–light petroleum) to yield **4** (20.4 g, 92.4%) as a colourless syrup, $[\alpha]_{578}^{25}$ +18° (*c* 1.6, chloroform), R_F 0.63 (1:1 ethyl acetate–light petroleum). See Table I for ^{1}H-n.m.r. data.

Methyl 2,3,6-tri-O-benzyl-4-O-(4,4-dimethoxybutyl)-α-D-glucopyranoside (**5**). — A solution of **4** (20.4 g, 38.2 mmol) in dry methanol (800 mL) was treated with conc. sulphuric acid (2 mL) and then stirred with anhydrous copper sulphate (15 g). After 15 min, the mixture was neutralised with methanolic 0.1M sodium methoxide, filtered, and concentrated to dryness *in vacuo*. A solution of the residue in dichloromethane (250 mL) was washed with water (3 × 250 mL), dried ($MgSO_4$),

and concentrated to dryness under diminished pressure. The residue was purified by flash column chromatography (1:3 ethyl acetate–light petroleum) to yield **5** (20.0 g, 90.2%) as a colourless syrup, $[\alpha]_{578}^{25}$ +14° (*c* 0.8, chloroform), R_F 0.51 (4:1 ether–light petroleum). See Table I for ^{1}H-n.m.r. data.

*Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(4,4-dimethoxybutyl)-α-*D*-glucopyranoside* (**7**). — A solution of **5** (16.2 g, 27.9 mmol) in methanol (250 mL) was hydrogenated in the presence of 10% Pd/C (780 mg). The resulting methyl 4-*O*-(4,4-dimethoxybutyl)-α-D-glucopyranoside (**6**; R_F 0.44, 7:2:1 ethyl acetate–methanol–water) was treated with pyridine (45 mL) and acetic anhydride (35 mL) for 6 h. The mixture was worked up in the usual manner to yield **7** as a colourless syrup (11.6 g, 95.3%), $[\alpha]_{578}^{25}$ +43° (*c* 0.9, chloroform), R_F 0.40 (1:1 ethyl acetate–light petroleum). See Table I for ^{1}H-n.m.r. data.

*Methyl 2,3,6-tri-*O*-acetyl-4-*O*-[4-methoxy-4-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyloxy)butyl]-α-*D*-glucopyranoside* (**8**). — A solution of trimethylsilyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (6.75 g, 16.05 mmol) in dry dichloromethane (20 mL) was added under anhydrous conditions to a mixture of **7** (9.0 g, 20.62 mmol) in dichloromethane (120 mL). Trimethylsilyl triflate (0.1M in CH_2Cl_2) was added after cooling to −80°. After 4, 24, 48, and 72 h, more catalyst (5 mL) was added. Equilibrium was reached after 93 h (t.l.c., 1:1 ethyl acetate–light petroleum). The catalyst was decomposed with triethylamine (25 mL), the mixture was warmed to 0°, washed with saturated aqueous sodium hydrogencarbonate (150 mL) and water (3 × 150 mL), dried ($MgSO_4$), and concentrated *in vacuo*, and the residue was purified by flash chromatography (1:3 ethyl acetate–light petroleum) to yield **8** (3.1 g, 25.6%; starting material could almost quantitatively be recovered and recycled) as a colourless syrup, $[\alpha]_{578}^{25}$ +74.5° (*c* 0.9, chloroform), R_F 0.27 (1:1 ethyl acetate–light petroleum). See Table I for ^{1}H-n.m.r. data.

*Methyl 4-*O*-(4-α-*D*-glucopyranosyloxy-4-methoxybutyl)-α-*D*-glucopyranoside* (**9**). — Compound **8** (2.0 g, 2.66 mmol) was deacetylated (Zemplén), and the product was purified by flash column chromatography (17:2:1 ethyl acetate–methanol–water) and by elution from Sephadex LH20 (1:3 methanol–water) to yield amorphous **9** (1.07 g, 87.7%), $[\alpha]_{578}^{25}$ +150° (*c* 1.7, methanol), R_F 0.20 (7:2:1 ethyl acetate–methanol–water). According to h.p.l.c. [20mM phosphate buffer (pH 6.8), 1.0 mL/min, u.v. 195 nm] and g.l.c. of the *O*-trimethylsilylated compound, the product **9** was homogeneous (see Table I for ^{1}H-n.m.r. data). When **9** was dried (P_2O_5, 60°, high vacuum), it was hygroscopic and rapidly (10–20 s) absorbed 1.5 mol of water when exposed to the atmosphere. Thereafter, no significant weight increase was observed.

Anal. Calc. for $C_{13}H_{34}O_{13} \cdot 1.5\ H_2O$: C, 44.48; H, 7.67. Found: C, 44.51; H, 7.69.

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