The Substrate Specificity of the Enzyme Amyloglucosidase (AMG). Part I. Deoxy Derivatives

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The eight possible monodeoxy derivatives of methyl β-maltoside and two bisdeoxy derivatives have been synthesized. The unprotected glycosides have all been investigated by NMR (^{1}H and ^{13}C) spectroscopy in order to confirm their structures and to obtain supporting information about their preferred solution conformations. The compounds have all been tested as substrates toward the hydrolase, amyloglucosidase (AMG) and it has been demonstrated that three hydroxy groups (3, 4′ and 6′) are essential for the compounds to act as substrate for the enzyme. The kinetic parameters K_M (Michaelis-Menten constant) and V_M (maximum rate for the reaction) have been determined using ^{1}H NMR spectroscopy at 500 MHz.

The recognition of complex oligosaccharides by antibodies has been studied extensively by Lemieux and his coworkers.[1–4] This work has demonstrated that specific hydroxy groups are required for the binding of the carbohydrates by the protein, whereas other hydroxy groups do not play a major role in the recognition process but merely contribute to the overall strength of the binding to the protein.

In order to investigate this proposal in a model system where oligosaccharides are cleaved by hydrolytic enzymes we have synthesized a series of monodeoxy derivatives of methyl β-maltoside. Preliminary reports of this work have appeared previously.[5–6] The synthetic compounds have been investigated with respect to their substrate activity towards the enzyme amyloglocusidase [AMG (EC.3.2.1.3)]. This enzyme is produced technically in very large amounts and plays an important role in the processing of starch. The amino acid sequence of the enzyme has been determined by Svensson *et al.*,[7] but it has not been possible to crystallize it in order to obtain a detailed picture of the active site and the mechanism by which the enzyme functions when it hydrolyses maltose and higher oligomers to glucose.

Here we wish to report on the synthesis of all the mono- and two bisdeoxy derivatives of maltose and an investigation of whether these compounds are substrates for the enzyme AMG.

Results and discussion

The peracetates of the monodeoxy derivatives methyl 6-deoxy-β-maltoside (**1b**), methyl 6′-deoxy-β-maltoside (**2b**) and 4-*O*-α-D-glucopyranosyl-1,5-anhydro-D-glucitol (**3b**) have all been synthesized previously.[8–10] The unprotected compounds **1a**, **2a** and **3a** were prepared according to the published procedures, followed by deacetylation and characterization by ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and 2).

The 3-deoxy derivative **5a** was prepared by tinhydride reduction of the 3-chloro-3-deoxy *allo*-compound (**4**)[11] and isolated in high yield (75 %) as the 3-deoxy peracetate (**5b**). Deacetylation with sodium methoxide in methanol afforded **5a** in 73 % yield after purification by chromatography on a Sephadex G-15 column.

The 2′-deoxy compound was prepared by glycoside synthesis using methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**)[12] as the aglycone and tri-

Table 1. ^{1}H NMR spectral data for compounds **1–16** in D_2O[a].

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	OMe
1a a-unit	5.34 3.8	3.55 9.2	3.62	3.35	3.71	3.71	3.80	
1a b-unit	4.30 8.5	3.23 9.2	3.66	3.33	3.57 6.3	1.34		3.49
2a a-unit	5.32 3.5	3.59	3.62 9.0	3.16 9.0	3.78 6.3	1.27		
2a b-unit	4.39 8.0	3.29 9,2	3.75	3.58	3.55	3.90 5.0	3.72 5.1, 12.0	3.55
5a a-unit	5.10 3.8	3.56 9.5	3.61	3.42	3.62[b]	3.84 2.5	3.76 5.1, 12.0	
5a b-unit	4.38 7.5	3,46 2.59eq 5.0, 12.0	1.49az 12.0	3.71	3.61[b]	3.96 2.5	3.70 6.0. 12.0	3.56
9a a-unit	5.45 3.8	1.70ax 13.0, 4.0 2.21eq 13.1	3.84 12.0 5.0	3.34 10.0	3.62	3.82 2.5	3.74 5.0, 12.0	
9a b-unit	4.33 8.0	3.24 9.5	3.61	3.56	3.49	3.88 2.0	3.70 5.5, 12.1	3.53
11a a-unit	5.23 3.8	3.77 2.12eq 4.5, 11.5	1.69ax 11.5	3.55		3.79 1.5	3.66 5.5, 12.5	
11b b-unit	4.34 8.0	3.25 9.5	3.71 10.0	3.59	3.54	3.92 1.5	3.75 5.0, 12.5	3.53
14a a-unit	5.35 3.8	3.47 10.0	3.73	1.90ax 12.0 2.55eq 2.0, 5.0, 12.0	3.97	3.88 5.0	3.90 5.0, 12.0	
14 b-unit	4.35 8.0	3.26 9.5	3.72	3.55	3.54	3.91 2.0	3.63 5.0, 12.0	3.53
15a a-unit	5.27 3.5	3.57	3.57 9.0	3.13 9.0	3.59 6.3	1.26		
15a b-unit	4.32 8.0	3.25 9.5	3.66 9.5	3.28 9.5	3.80 6.3	1.32		3.52
16a a-unit	3.50ax 12.4, 12.4 3.95eq 5.2, 1.9	1.64ax 12.5 1.98eq 1.7	3.90 9.0	3.46 9.0	3.38	3.83 2.2	3.70 5.5, 12.1	
16a b-unit	5.34 3.9	3.57 9.5	3.67 9.5	3.39 9.5	3.7	3.87 2.2, 12.1	3.7	

[a]Measured at 500 MHz in D_2O at 300 K, in ppm relative to DOH = 4.75 ppm. Observed first-order coupling constants given below the chemical shifts. [b]Assignments may be reversed.

Table 2. ^{13}C NMR spectral data for compounds **1–16** in D_2O[a].

Compound	C-1	C-2	C-3	C-4	C-5	C-6	OMe
1a a-unit	100.5	72.7	73.9	70.5	73.6	61.6	
1a b-unit	104.1	74.3	77.2	83.1	71.6	18.4	58.2
2a a-unit	100.9	72.7	73.4	75.6	69.4	17.3	
2a b-unit	103.9	73.9	77.0	77.8	75.3	61.6	58.0
3a a-unit	69.8	70.3	78.2	78.9	79.7	61.5	
3a b-unit	100.7	72.7	73.9	69.6	69.6	61.9	
5a a-unit	95.7	72.1	73.7	70.6	74.1	61.6	
5a b-unit	106.5	68.8	35.2	69.0	79.1	62.1	58.2
9a a-unit	99.6	37.9	68.9	71.8	74.3	61.8	
9a b-unit	104.1	74.2	76.2	77.5	75.6	61.6	58.1
11a a-unit	99.2	67.7	35.2	65.0	74.5	61.8[b]	
11a b-unit	104.1	74.0	77.3	77.6	75.7	61.6[b]	58.1
14a a-unit	101.3	74.3	70.5[b]	34.7	67.9[b]	64.4	
14a b-unit	103.9	73.8	77.0	78.0	75.3	61.6	57.9
15a a-unit	100.5	72.7	73.5	75.6	69.3	17.2	
15a b-unit	103.8	74.0	76.9	83.4	71.4	18.0	58.0
16a a-unit	66.2	33.7	79.7	73.6	80.6	62.1	
16a b-unit	101.0	70.0	69.4	67.9	68.3	61.4	

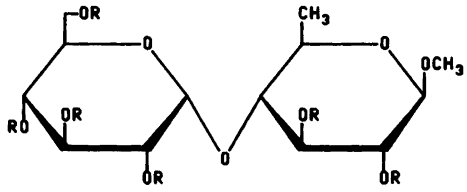
[a]Measured at 125.7 MHz in D_2O at 300 K. [b]Assignments may be reversed.

O-acetyl-D-glucal (**7**) as the glycosylating reagent in an *N*-iodosuccinimide-catalyzed reaction as described by Thiem.[13] The yield of the α-linked disaccharide (**8**) was 26 %. Removal of the iodine with Pd/C in ethyl acetate and triethylamine to the deoxy compound gave mixtures of products. Reduction with tributyltin hydride gave the desired deoxy compound **9b**, isolated in 62 % yield after catalytic debenzylation and re-acetylation. De-*O*-acetylation gave the desired 2′-deoxy compound **9a** in 80 % yield.

The 3′-deoxy derivative **11a** was obtained through a glycoside synthesis using the same aglycone (**6**) as described above. The glycosylating reagent was 2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**10**), prepared from the corresponding methyl 4,6-*O*-benzylidene-3-deoxy-α-D-glucopyranoside.[14] The α-linked disaccharide **11c** was isolated in 38 % yield together with the β-linked compound (38 %) using the halide-catalyzed glycosylation reaction conditions described by Lemieux.[15] Catalytic hydrogenation of the α-linked compound with Pd/C in methanol and acetic acid gave the desired compound **11a** in 88 % yield. The product was characterized as its peracetate (**11b**).

The 4′-deoxy derivative was synthesized from methyl 4,6-*O*-benzylidene-β-maltoside, which was protected as the 2,3,6,2′,3′ penta-*O*-benzyl derivative (**12**) and then reductively opened using sodium cyanoborohydride, as described by Hultberg and Garegg,[12] to give the methyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-maltoside (**13a**) in good yield. This compound was converted into the phenoxy thiocarbonate (**13d**),[16] and the thioester was reductively removed using tinhydride to give the 4′-deoxy compound **14c**, isolated in 67 % yield. The benzyl groups were finally removed catalytically and the unprotected 4′-deoxy compound **14a** isolated in 94 % yield as a syrup. The 6,6′-dideoxy compound **15a** and the 4-*O*-α-D-glucopyranosyl-2-deoxy-1,5-anhydro-D-*arabino*-hexitol (**16a**) were synthesized as described[9,17] and de-protected to give the free sugars.

All unprotected compounds were characterized through their ^1H and ^{13}C NMR parameters determined at 500 MHz. The data are presented



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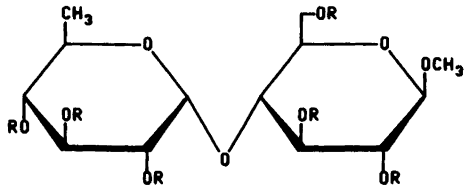

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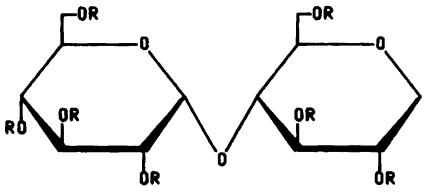

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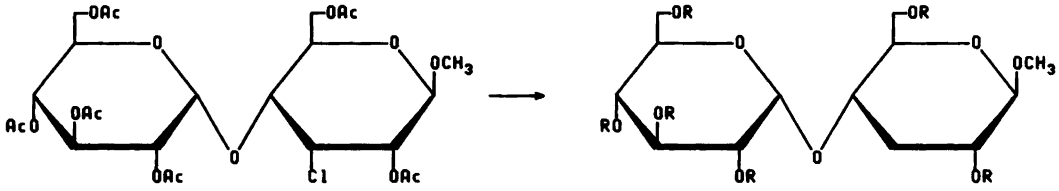

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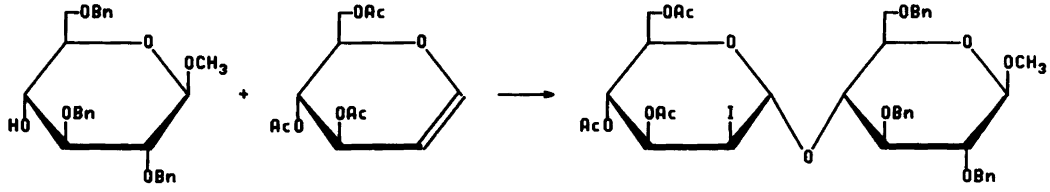

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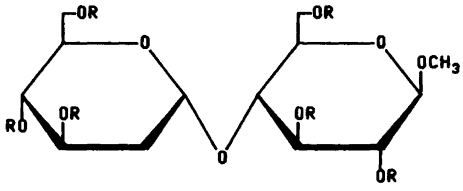
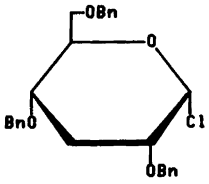


9

a: R = H
b: R = Ac
c: R = Bn



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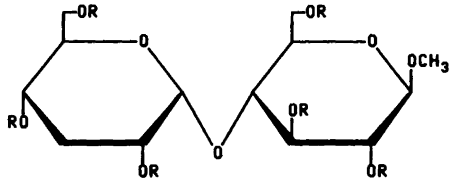

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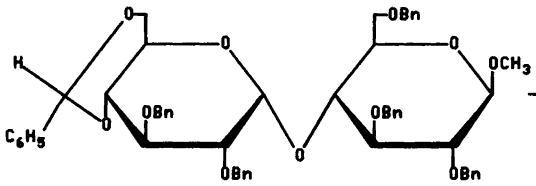

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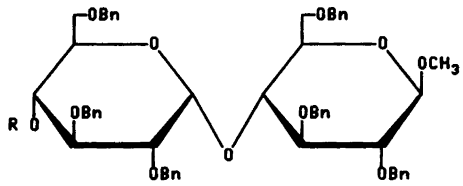

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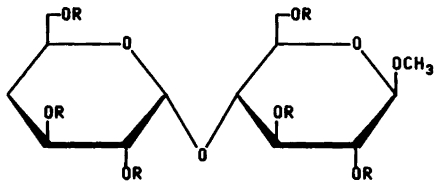

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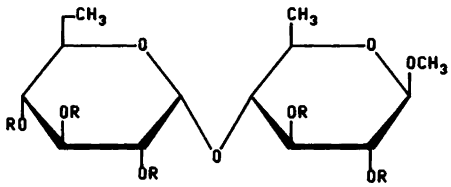

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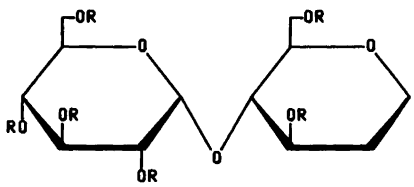

16

a: R = H

b: R = Ac

c: R = Bn

d: R = C_6H_5OCS

Table 3. Substrate specificity of deoxy-maltose derivatives towards amyloglucosidase.[a]

Compound	Substrate	Not substrate
1a	+	
2a		−
3a	+	
5a		−
9a	+	
11a	+	
14a		−
15a		(−)[b]
16a	+	

[a]Qualitative experiments as described in the Experimental. [b]The compound is a very poor substrate on prolonged exposure to AMG.

in Tables 1 and 2 and are in all cases in full agreement with the proposed structures.

The deoxy compounds described above were all tested qualitatively as potential substrates for the enzyme AMG in a 0.1 M acetate buffer at pH 4.5 and 27 °C. The results presented in Table 3 clearly demonstrate that key-polar groups[4] are important for the enzymatic activity since the 3-, 4′ and 6′-deoxy compounds were not substrates for the enzyme. All the other deoxy derivatives are hydrolyzed by the enzyme, however, at different rates relative to the reference compound methyl β-maltoside.

Four of the substrates, viz. **1a, 3a, 9a** and **16a,** were investigated in more detail using ^{1}H NMR spectroscopy and progress curve kinetics[18] to determine the Michaelis-Menten constant (K_M) and maximum rate (V_M) of the reactions. These results were compared with data for methyl α- and β-maltoside (Table 4) and it is clearly seen that even though the deoxy derivatives are hydrolysed faster (smaller V_M) than the corresponding hydroxy compounds, the K_2/K_M values are lower. This indicates[18] that the binding of a deoxy derivative to the enzyme is weaker than that of the corresponding hydroxy compound, but that the transition states of the two reactions are of comparable energy; consequently, the hydrolysis of the deoxy compound is faster than that of the normal natural substrate, maltose.

Conclusion

The monodeoxy derivatives of maltose have been synthesized and fully characterized. It has been shown that the presence of each of three specific hydroxy groups (3, 4′ and 6) in maltose is essential for the compounds to act as substrate for the hydrolytic enzyme amyloglucosidase. On the other hand, removal of other hydroxy groups generally gives substrates which are hydrolysed faster than methyl maltoside, due to weaker association to the enzyme, but which have transition states of comparable energy.

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter. NMR spectra were obtained on Bruker WH-90 and AM-500 NMR instruments. The spectra of protected compounds were measured in $CDCl_3$, while those of unprotected compounds were measured in D_2O relative to the internal references acetone (δ 2.22, ^{1}H NMR spectra) or dioxane (67.4 ppm, ^{13}C NMR spectra). Microanalyses were performed by Novo Microanalytical Laboratory, Copenhagen, Denmark. TLC was performed on silica gel-coated plates (Merck F-

Table 4. Kinetic parameters determined from dynamic NMR experiments.[18]

Compound	S_o[a]/μmol ml^{-1}	k_M/ μmol ml^{-1}	V_M/μmol min^{-1}	K_2/K_M[b]/M s^{-1}
Methyl β-maltoside	5.98	0.33	0.13	$9.7 \cdot 10^3$
Methyl β-maltoside	31.2	0.52	0.30	$1.4 \cdot 10^4$
1a	7.23	0.36	0.14	$9.0 \cdot 10^3$
3a	17.8	5.2	0.89	$4.2 \cdot 10^3$
9a	7.27	3.6	0.39	$2.5 \cdot 10^3$
16a	22.3	9.1	0.47	$1.2 \cdot 10^3$

[a]Initial concentration. [b]Constant enzyme amount in each experiment 50 μg (MW = 71 000 g mol^{-1}).

254). Preparative TLC was performed on 20×40 cm plates coated with 1 mm of silica gel. The enzyme amyloglucosidase [AMG (EC. 3.2.1.3)] was a gift from Novo A/S, Denmark.

*Methyl 2,3,6,2',3',4'-hexa-*O*-acetyl-6'-bromo-6'-deoxy-β-maltoside.* A mixture of methyl 2,3,6,2',3'-penta-*O*-acetyl-4',6'-*O*-benzylidene-β-maltoside[19] (2.0 g, 3.06 mmol) and barium carbonate (1 g, 5.08 mmol) in tetrachloromethane (150 ml) was dried by distilling off tetrachloromethane (75 ml). *N*-Bromosuccinimide (600 mg, 3.37 mmol) was added, and the mixture was heated under reflux with magnetic stirring for 30 min. The mixture was filtered while still hot, and the filter cake was washed with dichloromethane (50 ml). The combined filtrate and washings were washed twice with 10 % sodium thiosulfate solution (50 ml), three times with 4 M hydrochloric acid (50 ml), and three times with saturated sodium hydrogen carbonate solution (50 ml). Drying of the organic phase over magnesium sulfate and evaporation to dryness yielded 2.10 g (2.86 mmol, 94 %). The 90 MHz ^{1}H NMR spectrum showed two complex spin systems at 7.47 ppm and 7.98 ppm (4'-*O*-benzoyl). The 6'-bromide (2.10 g, 2.86 mmol) was treated with 0.1 % sodium methoxide in methanol (25 ml) for 16 h. Solid carbon dioxide (2 g) was added, and the mixture was evaporated. Water (10 ml) was added and evaporated three times to remove methyl benzoate. Toluene (10 ml) was then added and evaporated twice to remove water, and the residue was treated with acetic anhydride (10 ml) and pyridine (10 ml) for 4 h. Work-up in the conventional manner gave a syrup (1.80 g), from which the title compound (830 mg, 1.24 mmol, 43 %) with m.p. 111–113 °C could be crystallized from ethanol. Recrystallization from ethanol gave m.p. 118–120 °C, $[\alpha]_D^{23}$ + 60.1° (*c* 2.9, chloroform). Anal. $C_{25}H_{35}BrO_{16}$: C, H. ^{13}C NMR ($CDCl_3$, 22.63 MHz): 95.4 (C-1), 70.1 (C-2), 69.2, 69.1 (C-3, C-4), 70.7 (C-5), 31.2 (C-6), 101.2 (C-1'), 72.3, 72.1 (C-2', C-3'), 75.4 (C-4'), 73.1 (C-5'), 63.1 (C-6') and 57.0 ppm. (OMe).

*Methyl 2,3,6,2',3',4'-hexa-*O*-acetyl-6'-deoxy-β-maltoside* (**2b**). To a solution of the above 6'-bromide (600 mg, 0.89 mmol) in ethyl acetate (50 ml) was added triethylamine (0.1 ml) and Raney nickel (1 g). The mixture was shaken under 2.5 atm hydrogen pressure for 3 d. The catalyst was filtered off and washed with ethyl acetate, and the filtrate and washings were combined and evaporated to dryness. The residue was dissolved in dichloromethane (50 ml), and the organic phase was washed three times with water (25 ml). Drying over magnesium sulfate and evaporation to dryness gave a syrup (520 mg), from which **2b** (400 mg, 0.68 mmol, 76 %) with m.p. 164–172 °C was obtained. Recrystallization from ethanol gave **2b** with m.p. 173–175 °C, $[\alpha]_D^{23}$ + 49.5° (*c* 1.1, chloroform). Lit.[9] m.p. 176–177 °C. ^{13}C NMR ($CDCl_3$, 22.63 MHz): 95.4 (C-1), 70.4 (C-2), 69.4 (C-3), 77.3 (C-4), 66.4 (C-5), 17.3 (C-6), 101.2 (C-1') 72.1, 72.2 (C-2', C-3'), 75.7 (C-4'), 73.4 (C-5'), 62.7 (C-6') and 57.0 ppm. (OMe).

Methyl 6'-Deoxy-β-maltoside (**2a**). De-*O*-acetylation of **2b** (100 mg, 0.17 mmol) in 0.1 % sodium methoxide in methanol (10 ml) yielded **2a** (49 mg, 0.14 mmol, 85 %)) as a syrup. NMR data are given in Tables 1 and 2.

*Methyl 2,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-3-deoxy-β-D-ribo-hexopyranoside* (**5b**). Methyl 2,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-3-chloro-3-deoxy-β-D-allopyranoside[11] (**4**) (700 mg, 1.12 mmol) was dissolved in toluene. α,α'-Azobisisobutyronitrile (50 mg, 0.33 mmol) and tributyltin hydride (2 g, 3.1 mmol) were added, and the mixture was heated under reflux under nitrogen for 3 h. After evaporation the product was purified by preparative TLC using ether as eluent (4 plates), yielding a syrup from which **4** (495 mg, 0.84 mmol, 75 %) could be crystallized from ethanol. M.p. 85–90 °C. Recrystallization from ethanol gave **5b** with m.p. 102–104 °C, $[\alpha]_D^{23}$ + 56.3° (*c* 0.6, chloroform). Anal. $C_{25}H_{36}O_{16}$: C, H.

*Methyl 3-deoxy-4-*O*-(α-D-glucopyranosyl)-β-D-ribo-hexopyranoside* (**5a**). De-*O*-acetylation of **5b** (100 mg, 0.17 mmol) in 0.1 % sodium methoxide in methanol (10 ml) yielded **5a** (42 mg, 0.12 mmol, 73 %) as a syrup. NMR data are given in Tables 1 and 2.

*Methyl 4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-iodo-α-D-mannopyranosyl)-2,3-6-tri-*O*-benzyl-β-D-*

glucopyranoside (**8**). A mixture of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-l-enitol (**7**)[20] (250 mg, 0.92 mmol), methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**)[12] (200 mg, 0.43 mmol), *N*-iodosuccinimide[21] (35: mg, 1.56 mmol) and 3 Å molecular sieves in acetonitrile (2 ml) was stirred in the dark under nitrogen for 3 d. The reaction mixture was diluted with dichloromethane (20 ml), filtered and washed three times with 4 M hydrochloric acid and three times with saturated sodium hydrogen carbonate solution. Drying over magnesium sulfate and evaporation to dryness yielded a syrup (433 mg), which was applied to a column of silica gel. Elution with ethyl acetate/light petroleum (1:3) yielded **8** as a syrup (95 mg, 0.11 mmol, 26 %). ^{13}C NMR ($CDCl_3$, 22.3 MHz): 104.6 (C-1, $J_{C\text{-}1,\,H\text{-}1}$ 154 Hz), 102.9 (C-1′, $J_{C\text{-}1',\,H\text{-}1}$ 180 Hz), 57.1 (*O*-methyl) and 29.7 ppm (C-2′). Other signals: 83.8, 82.3, 77.5, 75.1, 74.4, 74.3, 73.6, 70.0, 69.4, 68.7, 67.7 and 62.2 ppm. From the crude product, unreacted **6** (54 mg, 0.12 mmol) could be isolated.

*Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranosyl)-β-D-glucopyranoside* (**9b**). To a solution of **8** (95 mg, 0.11 mmol) in toluene (5 ml) was added tributyltin hydride[22] (100 mg, 0.34 mmol) and the mixture was stirred under nitrogen for 2 h. Evaporation left a syrup which was purified by chromatography on a column packed with silica gel using ethyl acetate/light petroleum (1:3) as eluent. The syrupy product (63 mg) was dissolved in methanol (20 ml) and acetic acid (4 ml), and 5 % palladium on activated carbon (20 mg) was added. The mixture was stirred under 1 atm hydrogen pressure for 16 h. The catalyst was filtered off, washed with methanol, and the combined organic phases were evaporated to dryness, yielding a syrup (35 mg) which was treated with acetic anhydride (5 ml) and pyridine (5 ml) for 16 h. Work-up by evaporation and chromatography on a column of silica gel with ethyl acetate/light petroleum (1:1) as eluent yielded **9b** as a syrup (40 mg, 0.068 mmol, 62 %). Crystallization from ethanol gave **9b** with m.p. 168–169 °C, $[\alpha]_D^{23}$ + 37.6° (c 1.8, chloroform). Anal. $C_{25}H_{36}O_{16}$: C, H.

*Methyl 4-*O*-(2-deoxy-α-D-*arabino*-hexopyranosyl)-β-D-glucopyranoside* (**9a**). De-*O*-acetylation of **9b** (30 mg, 0.05 mmol) in 0.1 % sodium methoxide in methanol (5 ml) yielded **9a** (13 mg, 0.04 mmol, 80 %) as a syrup. NMR data are given in Tables 1 and 2.

*2,4,6-Tri-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranosyl chloride* (**10**). A solution of methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside[14] (1.0 g, 3.76 mmol) in acetic acid (40 ml) and water (10 ml) was heated on a steam bath for 30 min. The mixture was evaporated to dryness, water (10 ml) was added and evaporated three times, and the residue was dissolved in *N,N*-dimethylformamide (15 ml). A slurry of sodium hydride (0.5 g, 20 mmol) in *N,N*-dimethylformamide (10 ml) was added and the mixture was stirred for 1 h. Benzyl bromide (1.8 ml, 15 mmol) was added and the mixture was stirred for 16 h. Methanol (10 ml) was added in small portions with cooling, and the mixture was stirred for 30 min. The mixture was then poured into water (50 ml) and the pH was adjusted to 7 with acetic acid. The mixture was extracted three times with dichloromethane, and the combined organic phases were washed three times with 4 m hydrochloric acid and three times with saturated sodium hydrogen carbonate solution. Drying over magnesium sulfate and evaporation yielded a syrup, which was applied to a column of silica gel. Elution with ethyl acetate/light petroleum (6:1) yielded the glycoside (1.01 g, 2.26 mmol, 60 %). $[\alpha]_D^{23}$ + 63.5° (*c* 1.8, chloroform). ^{13}C NMR ($CDCl_3$, 22.63 MHz): 97.2 (C-1), 54.7 (*O*-methyl) and 30.1 ppm (C-3). Other signals at 73.7, 73.5, 71.9, 71.1, 70.8, 70.6 and 68.8 ppm.

The benzylated glycoside (400 mg, 0.89 mmol) was dissolved in ether (50 ml) saturated with hydrogen chloride at 0 °C. The solution was evaporated at room temperature, and toluene (10 ml) was added and evaporated twice. The resulting syrupy **10** (405 mg, 0.89 mmol, 100 %) was used immediately. The 90 MHz ^{1}H NMR spectrum showed a doublet (*J* 3.3 Hz) at 6.1 ppm, typical for H-1 in a glycosyl chloride.

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranosyl)-β-D-glucopyranoside* (**11c**) *and methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-*ribo*-hexopyranosyl)-β-D-glycopyranoside.* To a mixture of **6** (180 mg, 0.39 mmol), silver trifluoromethanesulfonate (50 mg, 0.97 mmol) and 4 Å molecular sieves cooled to −70 °C was

added a solution of **10** (405 mg, 0.89 mmol) in toluene (2 ml), nitromethane (2 ml) and tetramethylurea (0.2 ml). The mixture was stirred under nitrogen at −23 °C for 16 h, and was then allowed to reach room temperature. The mixture was diluted with dichloromethane (25 ml) and filtered through celite. The organic phase was washed three times with 4 M hydrochloric acid (10 ml) and three times with saturated sodium hydrogen carbonate solution (10 ml). Drying over magnesium sulfate and evaporation to dryness yielded a syrup (420 mg), which was purified by preparative TLC (2 plates) by elution with ethyl acetate/light petroleum (1:3). The slower moving fraction gave **11c** (132 mg, 0.15 mmol, 38 %, for which the 90 MHz ^{1}H NMR spectrum showed a doublet (*J* 3.8 Hz) at 5.6 ppm (αH-1). The faster moving fraction yielded material (130 mg, 0.16 mmol, 38 %) which on catalytic hydrogenation gave a product for which the 90 MHz ^{1}H NMR spectrum in deuterium oxide showed two doublets (*J* 7.5 Hz) at about 4.4 ppm (βH-1 and βH-1′).

*Methyl 4-O-(3-deoxy-α-D-*ribo*-hexopyranosyl)-β-D-glucopyranoside* (**11a**). To a solution of **11c** (132 mg, 0.15 mmol) in methanol (20 ml) and acetic acid (5 ml) was added 5 % palladium on activated carbon (30 mg). The mixture was stirred for 16 h under 1 atm hydrogen pressure. The catalyst was filtered off and washed with methanol. The filtrate and washings were combined and evaporated. Water (5 ml) was added and evaporated twice to remove traces of acetic acid. This procedure left **11a** (45 mg, 0.13 mmol, 88 %). NMR data are given in Tables 1 and 2.

*Methyl 2,3,6-tri-*O*-acetyl-4-O-(2,4,6-tri-*O*-acetyl-3-deoxy-α-D-ribo-hexopyranosyl)-β-D-glucopyranoside* (**11b**). Compound **11a** (20 mg, 0.059 mmol) was treated with pyridine (2 ml) and acetic anhydride (2 ml) for 16 h. The reaction mixture was concentrated, and toluene (10 ml) was added and evaporated twice. The resulting syrup was applied to a column of silica gel and eluted with ethyl acetate/light petroleum (1:1), yielding **11b** as a syrup (30 mg, 0.051 mmol) with $[\alpha]_D^{23}$ + 51.2° (*c* 0.4, chloroform). Anal. $C_{25}H_{36}O_{16}$: C, H.

*Methyl 2,3,6,2′,3′-penta-*O*-benzyl-4′,6′-O-benzylidene-β-maltoside* (**12**). To a solution of methyl 4′,6′-*O*-benzylidene -β-maltoside[19] (680 mg, 1.53 mmol) in *N,N*-dimethylformamide (10 ml) was added a slurry of sodium hydride (350 mg, 14.6 mmol) in *N,N*-dimethylformamide (10 ml). The mixture was stirred for 1 h, and benzyl bromide (1.0 ml, 8.4 mmol) was added and the mixture was stirred for 16 h. Methanol (10 ml) was added in portions with cooling. The mixture was stirred for 30 min and then poured into water (50 ml), and the pH was adjusted to 7 with acetic acid. The mixture was extracted three times with dichloromethane (25 ml), and the combined organic phases were washed three times with 4 M hydrochloric acid and three times with saturated hydrogen carbonate solution. Drying and evaporation of the organic phase yielded a syrup, which was applied to a column of silica gel. Elution with ethyl acetate/light petroleum (1:4) yielded **12** as a syrup (983 mg, 1.10 mmol, 72 %) with $[\alpha]_D^{23}$ + 17.7° (*c* 1.9, chloroform). Anal. $C_{55}H_{58}O_{11}$: C, H. ^{13}C NMR ($CDCl_3$, 22.63 MHz): 104.4 (C-1) 101.0 (benzylidenic), 97.1 (C-1′) and 56.8 ppm (O-methyl). Other signals at 84.7, 82.0 (2 carbons), 78.6 (2 carbons), 75.1 74.4, 74.2, 73.6, 73.3, 72,0 (2 carbons), 68.8 (2 carbons) and 63.2 ppm.

*Methyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-β-maltoside* (**13a**). To a solution of **12** (900 mg, 1.0 mmol) and sodium cyanoborohydride (800 mg, 12 mmol) in tetrahydrofuran (30 ml, freshly distilled from lithium aluminium hydride) was added ether saturated with hydrogen chloride until the evolution of gas had ceased. After 5 min the mixture was diluted with dichloromethane (50 ml), and washed twice with water (20 ml) and three times with saturated sodium hydrogen carbonate solution (20 ml). Drying over magnesium sulfate and evaporation of the organic phase yielded a syrup, which was applied to a column of silica gel. Elution with ethyl acetate/light petroleum (1:4) yielded **13a** as a syrup (566 mg, 0.63 mmol, 63 %) with $[\alpha]_D^{23}$ + 23.0° (*c* 0.7, chloroform). Anal. $C_{55}H_{60}O_{11}$: C, H. ^{13}C NMR ($CDCl_3$, 22.63 MHz): 104.6 (C-1), 96.6 (C-1′) and 56.9 ppm (*O*-methyl). Other signals at 84.8, 82.3, 81.3, 78.9, 75.3, 74.6 (2 carbons), 73.8, 73.6, 73.3, 73.0, 72.6, 71.4, 70.7, 69.7 and 69.1 ppm.

*Methyl 2,3,6,2′,3′,6′-hexa-*O*-benzyl-4′-O-phenoxythiocarbonyl-β-maltoside* (**13d**). To a so-

lution of **13a** (856 mg, 0.90 mmol) in dichloromethane (30 ml) and pyridine (5 ml) was added phenoxythiocarbonyl chloride[23] (200 mg, 1.16 mmol). The mixture was stirred for 16 h and then ice (5 ml) was added. After stirring for 1 h, the mixture was washed three times with 4 M hydrochloric acid (15 ml) and three times with saturated sodium hydrogen carbonate solution. Drying over magnesium sulfate and evaporation of the organic phase yielded a syrup (1.02 g), which was purified by preparative TLC (4 plates) with ethyl acetate/light petroleum (1:3) as eluent. Yield of **13d**, 296 mg (0.261 mmol, 30 %). The 90 MHz ^{1}H NMR spectrum showed a triplet (J 9.7 Hz) at 5.65 ppm (H-4'). ^{13}C NMR ($CDCl_3$, 22.63 MHz): 104.7 (C-1), 96.6 (C-1') and 57.0 ppm (*O*-methyl). Other signals at 84.7, 82.4, 80.7, 79.5, 79.0, 75.5, 74.6, 74.0, 73.8, 73.7, 73.6, 73.4, 69.4, 69.3 and 68.9 ppm.

Methyl 2,3,6-tri-O-*benzyl-4*-O-*(2,3,6-tri*-O-*benzyl-4-deoxy-α-D*-xylo-*hexopyranosyl)*-β-*D-glucopyranoside* (**14c**). To a solution of **13d** (250 mg, 0.24 mmol) in toluene was added tributyltin hydride[22] (200 mg, 0.69 mmol) and α,α'azobisisobutyronitrile (10 mg, 0.06 mmol). The mixture was heated under reflux under nitrogen for 1 h, and was then left for 16 h at room temperature under nitrogen. The reaction mixture was evaporated, leaving a syrup which was purified by preparative TLC (2 plates) with dichloromethane/methanol (95:5) as eluent. This yielded **14c** as a syrup (141 mg, 0.16 mmol, 67 %) with $[\alpha]_D^{23}$ + 30.4° (*c* 0.9 chloroform). Anal. $C_{55}H_{60}O_{10}$: C, H. ^{13}C NMR ($CDCl_3$, 22.63 MHz): 104.5 (c-1), 97.6 (C-1'), 57.0 (*O*-methyl) and 33.8 ppm (C-4'). Other signals at 84.8, 82.4, 80.0, 75.5, 74.6 (2 carbons), 73.9, 73.4 (2 carbons), 72.8 (2 carbons), 72.3, 72.0, 69.7 and 67.6 ppm.

Methyl 4-O-*(4-deoxy-α-D*-xylo-*hexopyranosyl)-β-D-glucopyranoside* (**14a**). To a solution of **14c** (140 mg, 0.16 mmol) in methanol (10 ml) and acetic acid (2 ml) was added 5 % palladium on activated carbon (50 mg). The mixture was stirred under 1 atm hydrogen pressure for 16 h. The catalyst was filtered off and washed with methanol, and the combined filtrate and washings were evaporated. Water (5 ml) was added and evaporated three times to remove traces of acetic acid. This yielded **14a** as a syrup (50 mg, 0.15 mmol, 95 %). NMR data are given in Tables 1 and 2.

Methyl 2,3,2',4'-penta-O-*acetyl-6,6'-dideoxy-6,6'-diodo-β-maltoside*. Methyl β-maltoside monohydrate (2,6 g, 6.96 mmol) was dried at 20 mmHg and 120 °C for 20 h. It was then dissolved in pyridine (25 ml) and the solution was cooled to 0 °C. A cold solution of methanesulfonyl chloride (1.05 ml, 14.0 mmol) in pyridine (20 ml) was added, and the mixture was left at 0 °C for 16 h. Acetic anhydride (10 ml) was added, and the mixture was left at room temperature for 24 h. Conventional work-up gave the dimesylate as a syrup (4.96 g), which was crystallized and recrystallized from ethanol to give methyl 2,3,2',3',4'-penta-*O*-acetyl-6,6'-di-*O*-mesyl-β-maltoside (1.74 g, 2.41 mmol, 35 %) with m.p. 163–171 °C. Further recrystallizations from ethanol gave m.p. 170–172 °C, $[\alpha]_D^{23}$ + 60.3° (*c* 0.6, chloroform). Lit.[24] m.p. 175–176 °C, $[\alpha]_D^{23}$ + 56.5° (*c* 1.5, chloroform).

A solution of the mesylate (1.0 g, 1.39 mmol) in *N,N*-dimethylformamide (10 ml) was treated with sodium iodide (1.0 g, 6,7 mmol) at 100 °C for 16 h. TLC (ether) showed no starting material but several new compounds. Water (25 ml) and dichloromethane (50 ml) were added, and the phases were separated. The aqueous phase was extracted with dichloromethane (25 ml), and the combined organic phases were washed twice with 10 % sodium thiosulfate solution (25 ml) and three times with water. Drying over magnesium sulfate and evaporation of the organic phase left a syrup (1.02 g). This was treated with acetic anhydride (20 ml) and pyridine (20 ml) for 16 h. Conventional work-up gave a syrup (1.03 g), from which the title compound (830 mg, 1.06 mmol, 76 %) with m.p. 175–181 °C could be crystallized from ethanol. Recrystallization from ethanol gave m.p. 196–197 °C, $[\alpha]_D^{24}$ + 50.3° (*c* 2.3, chloroform). Lit.[9] m.p. 196–197 °C, $[\alpha]_D^{24}$ + 48.2° (*c* 7.6, chloroform).

Methyl 2,3,2',3',4'-Penta-O-*acetyl-6,6'-dideoxy-β-maltoside* (**15b**). To a solution of the diiodide (278 mg, 0.35 mmol) in ethyl acetate was added triethylamine (0.1 ml) and Raney nickel (1 g), and the mixture was shaken under 2.5 atm hydrogen pressure for 16 h. The catalyst was filtered off and washed well with ethyl acetate. The com-

bined filtrate and washings were evaporated to dryness. The residue was dissolved in dichloromethane (50 ml), and the organic phase was washed three times with water (25 ml). Drying over magnesium sulfate and evaporation of the organic phase gave a syrup (180 mg), from which **15b** (130 mg, 0.25 mmol, 70 %) with m.p. 157–159 °C could be crystallized from ethanol. Recrystallization from ethanol gave **15b** with m.p. 160–162 °C, $[\alpha]_D^{23}$ + 53.1° (*c* 0.3, chloroform). Lit.[9] m.p. 186–187 °C, $[\alpha]_D^{24}$ + 50.0° (*c* 3.0, chloroform).

Methyl 6,6'-dideoxy-β-maltoside (**15a**). De-*O*-acetylation of **15b** (100 mg, 0.19 mmol) with 0.1 % sodium methoxide in methanol (10 ml) yielded **15a** (46 mg, 0.14 mmol, 75 %) as a syrup. NMR data are given in Tables 1 and 2.

1,5-Anhydro-2-deoxy-4-O-(α-D-glucopyranosyl)-D-arabino-hexitol (**16a**). Hexa-*O*-acetylmaltal[20] (1.0 g, 1.8 mmol) was dissolved in ethyl acetate (10 ml), and Pd/C (5 %, 120 mg) was added and the reaction mixture hydrogenated at 1 atm hydrogen pressure for 24 h. Filtration and evaporation followed by crystallization from ether gave **16b** (935 mg, 93 %), m.p. 107–115 °C. Recrystallization from the same solvent gave **16b** with m.p. 117–120 °C, $[\alpha]_D^{23}$ + 84.3° (*c* 1.4, $CHCl_3$). Deacetylation of **16b** (545 mg, 97 mmol) with methanolic sodium methoxide (2 ml, 1) in methanol (10 ml) gave the unprotected compound **16a** as a syrup in 93 % yield after removal of the sodium ions with ion exchange resin (IR 120, H^+). NMR data are given in Tables 1 and 2.

Qualitative degradation experiments. General procedure. The substrate (2 mg) was dissolved in 0.1 M acetate buffer (0.5 ml, pH 4.3). AMG solution (10 µl, 3.9×10^{-8} M) was added, and the mixture was heated to 50 °C on a water bath for 1 h. The reaction mixture was analyzed by TLC using ethyl acetate/methanol/acetic acid/water (6:2:1:1) as eluent. Relevant reference compounds were used. Negative results were confirmed by addition of more enzyme and increasing the incubation times. When the results from the TLC analyses were not clear, the reaction mixtures were evaporated and analyzed by ^1H NMR spectroscopy.

Dynamic degradation experiments. General procedure. The substrates (1–10 mg) were dissolved in acetate buffer (0.7 ml, 0.1 M. pH 4.3) prepared from anhydrous sodium acetate, acetic acid and deuterium oxide. Nitrogen was bubbled through the sample, and it was thermostatted to the desired temperature (27 or 37 °C). AMG solution (10 µl, 3.9×10^{-8} M) in the deuterium buffer was added, and the sample was transferred to a 5 mm NMR tube and ^1H NMR spectra (500 MHz) were recorded at suitable time intervals.

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