The Substrate Specificity of the Enzyme Amyloglucosidase (AMG). Part II. 6-Substituted Maltose Derivatives

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The synthesis of maltose derivatives substituted in the 6-position with F, I, N_3, NH_2, NHAc, COOH or COOMe, and having a 5–6 double bond, are described together with the preparation of the known 6-Cl and 6-Br derivatives using improved synthetic procedures. Furthermore, the 6′-Br and -F derivatives have been prepared. The identities of the deprotected methyl glycosides have in all cases been established by ^1H and ^{13}C NMR spectroscopy.

The synthetic compounds have been tested as substrates for the enzyme amyloglucosidase (AMG) and it has been found that compounds with a charged group in the 6-position, such as amino or carboxylate, or with substituents in the 6′-position are not substrates for the enzyme; all the other compounds can be hydrolysed by the enzyme, although at widely differing rates. The 6-halo compounds proved in competition experiments to be potent enzyme inhibitors.

The enzyme amyloglucosidase (AMG) (EC. 3.2.1.3) catalyzes the hydrolysis of maltose and higher glucose oligomers to glucose and is produced industrially in large amounts due to its use in the processing of starch. The amino acid sequence of the enzyme, which is a glycoprotein, is known,[1] but it has not been possible to crystallize the enzyme for X-ray studies that would provide detailed information on the mode of enzyme action. We have therefore synthesized modified substrates in order to obtain information about the requirements of the enzyme active site when it is hydrolysing maltose derivatives. In a previous publication[2] we have reported the synthesis and substrate specificities of a series of deoxy derivatives of maltose. In this paper we report the synthesis of substrates modified in the 6 and 6′ position, together with their behaviour towards the enzyme AMG. Preliminary reports on this work have been presented.[3,4]

Results and discussion

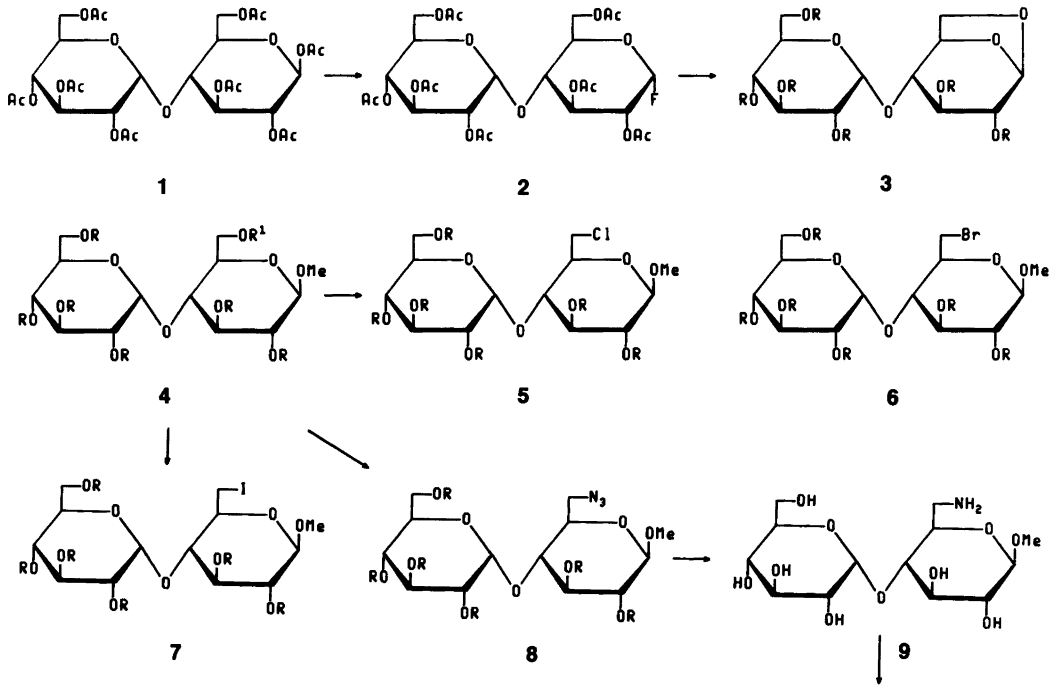
Modification at the 6-position in maltose is most conveniently accomplished through the synthesis of the selectively protected methyl 2,3,2′,3′,4′,6′-hexa-*O*-acetyl-β-maltoside, **4c**, which has a free hydroxy group in the 6-position ready for selective substitution. This compound was synthesized from maltose octaacetate, **1a**, which was converted into the glycosyl fluoride, **2a**, in high yield using anhydrous hydrogen fluoride. Treatment of the fluoride with a strong base, potassium hydroxide, gave the 1,6-anhydro compound, which after re-acetylation could be crystallized as the hexaacetate, **3a**, in 32 % overall yield based on **1a**. We find this method easier to perform on a large scale than the recently proposed methods using pentachloro-[5] or pentabromophenylglycosides.[6] The selective opening of the 1,6-anhydro compound with dichloromethyl methyl ether as originally proposed by Bognar,[7] but slightly modified, gave, after treatment with methanol and silver carbonate, the desired compound, **4c**, which could be isolated by crystallization in 53 % yield. This method is more convenient than that described by Fujimaki and Kuzuhara,[8] where the 1,6-anhydro compound is reacted with titanium tetrabromide and **4c** is isolated in 53 % yield, after a chromatographic purification.

The hexaacetate, **4c**, was converted into the

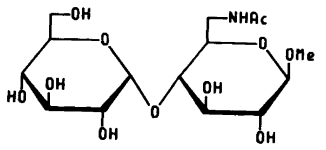
6-chloro compound **5a** by treatment with methanesulfonyl chloride in *N,N*-dimethylformamide; this gave the crystalline product, **5a**, in 63 % yield. Similarly, treatment of **4c** with methanesulfonyl bromide as described by Takeo *et al.*[9] for substitution of primary hydroxy groups in cyclodextrins and amylose gave the corresponding bromide, **6a**, which could also be isolated directly in 60 % yield by crystallization. The corresponding iodide, **7a**, was prepared by nucleophilic substitution of the 6-*O*-mesylate, **4d**, with sodium iodide in *N,N*-dimethylformamide. The mesylate has been described previously by Fujimaki and Kuzuhara,[8] but had an optical rotation 34° higher that the value reported here for the crystalline material.

Deprotection of the acetates **5a, 6a** and **7a** with sodium methoxide in methanol gave good yields of the deprotected methyl glycosides of the 6-chloro, -bromo and -iodo maltosides **5b, 6b** and **7b**, respectively. These compounds have all been prepared previously, by other methods, by Takeo.[10]

Nucleophilic substitution of the mesylate, **4c**, with sodium azide in *N,N*-dimethylformamide gave the corresponding 6-azide, **8a**, in 90 % yield as a syrup which could not be induced to crystallize. The compound was therefore de-*O*-acetylated to the crystalline methyl 6-azido-β-maltoside, **8b**, which was fully characterized. The corresponding 6-amino derivative, **9**, was prepared in good yield by reduction of the azide with hy-



a : R = Ac
b : R = H
c : R = Ac, R^1 = H
d : R = Ac, R^1 = CH_3SO_2



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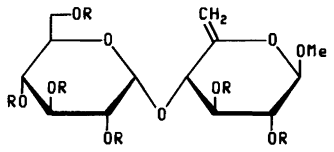
drogen sulfide in pyridine. It was not possible to crystallize the compound either as the hydrochloride or as the free base, and it was therefore characterized only by its NMR parameters.

Catalytic hydrogenolysis of the 6-azido compound, **8b**, in methanol and acetic anhydride gave the 6-acetamido compound **10**, which was isolated as a syrup and characterized by its NMR data.

Preparation of the 6-fluoro derivative was first attempted by heating the 6-*O*-mesylate, **4c**, with tetrabutylammonium fluoride in toluene under refluxing, but this resulted only in formation of the elimination product, **11a**, which was isolated crystalline in 39 % yield after chromatographic separation. Sugawara and Kuzuhara[11] have previously described the synthesis of this compound in high yield by an elimination reaction with the base 1,5-diazabicyclo[5,4,0]undecene-5. It was isolated as a syrup with an optical rotation 32° higher than the value reported here for the crystalline material. The alkene, **11a**, was further converted into the deblocked compound, which was characterized by ^{1}H and ^{13}C NMR spectroscopy. The 6-fluoro compound, **12b**, was, however, obtained in low yield by treatment of the 6-OH compound, **4c**, with DAST (*N,N*-diethylaminosulfur trifluoride) but the product was contaminated with methyl hepta-*O*-acetyl-β-maltoside, presumably formed by intermolecular acylation of decomposition products. It was only possible to remove this after de-*O*-acetylation, whereupon **12b** was separated by preparative HPLC and isolated in 13 % yield. The 6-fluoro compound was finally characterized as its crystalline hexaacetate, **12a**.

Oxidation of the 6-OH compound, **4c**, with potassium permanganate in acetic acid gave the corresponding carboxylic acid, **13a**, as a crystalline compound in 74 % yield. Treatment of the acid with diazomethane in diethyl ether gave the corresponding methyl ester, **14a**.

Both **13a** and **14a** were de-*O*-acetylated with sodium methoxide in methanol to give the unprotected compounds, **13b** and **14b**, in 73 % and 81 % yield, respectively. Abbot and Weigel have previously[12] described the preparation of compounds **13** and **14** by oxidation of the methyl β-maltosides with Pt/O_2, but in low yields (5–10 %). The reported melting point and optical rotation of **14a** are, however, in poor agreement with the data we report here for the crystalline



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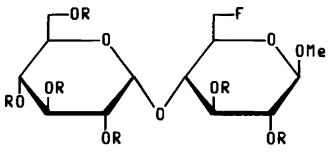

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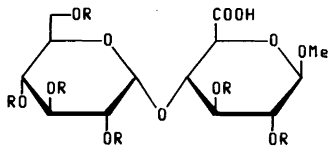

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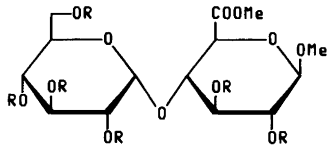

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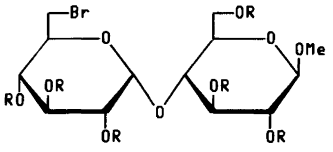

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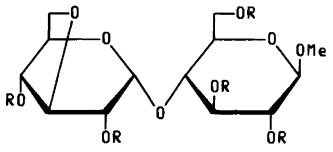

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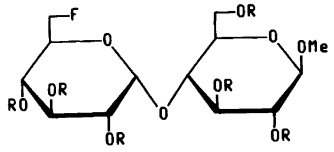

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Table 1. Substrate specificity of compounds **3** to **17** towards amyloglucosidae (AMG).[a]

Compound	Substrate	Not substrate
3b		–
5b	+	
6b	+	
7b	+	
8b	+	
9		–
10	+	
11b	+	
12b	+	
13b		–
14b	+	
15b		–
16b		–
17b		–

[a]Measured qualitatively as described in Experimental.

material. However, these compounds give NMR data which are in complete agreement with the proposed structures.

The 6′-bromide, **15b**, was prepared by de-*O*-acetylation of the known[2] acetate under mild conditions.

Treatment with stronger base gave the corresponding 3′,6′-anhydro compound, **16b**, which was characterized as its crystalline pentaacetate, **16a**.

The unprotected methyl maltosides were all tested qualitatively as substrates for the enzyme AMG, and the results are presented in Table 1. These data clearly show that the enzyme can accept a wide range of modifications in the 6-position provided that the substituents are not charged groups. If this is the case, as in the 6-amino and 6-carboxy derivatives, then the enzyme cannot hydrolyse the compounds, indicating that the active site accommodates the substrates with the 6-position in a hydrophobic environment.

Competitive experiments with the 6-chloro- or 6-fluoro compounds, using NMR spectroscopy and progress curve kinetics,[13] show (Table 2) that these compounds are bound very tightly to the active site of the enzyme since they show substantial inhibition of the reaction of the normal substrate. This is not the case for the 6′-bromo or 6′-fluoro compounds, which are neither substrates nor inhibitors of the enzyme. This observation is consistent with previously reported results[2] which have demonstrated that a 6′-OH group is required if the enzyme is to hydrolyse the substrate.

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter. NMR spectra were recorded on Bruker

Table 2. Kinetic data for the degradation of maltose and derivatives with amyloglucosidase (AMG).[a]

Substrate	Conc./mM	v_o/mM min^{-1}	$t_{1/2}$/min
Methyl 6-chloro-6-deoxy-β-maltoside	7.2	0.19	254
Methyl 6-deoxy-β-maltoside	8.3	4.07	12
Methyl β-maltoside	7.3	1.85	27
Methyl 3′-deoxy-β-maltoside	8.3	4.16	13
Methyl 6-chloro-6-deoxy-β-maltoside	3.6	0.20	215
Methyl β-maltoside	3.8	0.32	127
Methyl 3′-deoxy-β-maltoside	4.2	2.79	19
Methyl β-maltoside	3.8	1.11	42
Methyl 6-fluoro-6-deoxy-β-maltoside	4.0	0.45	106
Methyl β-maltoside	3.8	0.47	102
Methyl 3-deoxy-β-maltoside	4.2	0.00	~
Methyl β-maltoside	3.8	4.90	9

[a]At 27°C, pH 4.3, $5.5 \cdot 10^{-10}$ M AMG.

WH-90, HX-270 and AM-500 NMR instruments. The spectra of protected compounds were measured in $CDCl_3$. Proton-proton coupling constants are only given if they differ from expected values. The spectra of unprotected compounds were measured in D_2O relative to either acetone (δ 2.22) (for ^1H NMR spectra) or dioxane (67.4 ppm) (for ^{13}C NMR spectra). Microanalyses were performed by Novo Microanalytical Laboratory, Copenhagen, Denmark. TLC was performed on silica gel-coated plates (Merck F-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.

Hepta-O-acetyl-α-maltosyl fluoride (**2**). Octa-*O*-acetyl-β-maltose (**1**)[14] (50 g, 73.7 mmol) was cooled to −70 °C in a polyethylene flask. Dry HF (50 ml) was added and the solution was left at 0 °C for 5 min. Et_2O (75 ml) was added at −70 °C and the mixture was poured into a mixture of CH_2Cl_2 (200 ml) and ice (100 ml). The organic phase was washed once with ice-water (100 ml), twice with cold saturated $NaHCO_3$ (150 ml), dried ($MgSO_4$) and evaporated to dryness yielding foamy **2** (46.7 g, 73.2 mmol, 99 %) which was sufficiently pure for further reaction. Crystallization from EtOH gave **2** with m.p. 166–169 °C, $[\alpha]_D^{23}$ + 110.3° (*c*. 0.9, $CHCl_3$). Lit.[15] m.p. 172–174 °C, $[\alpha]_D^{20}$ + 110.2° ($CHCl_3$). ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.7 ppm (C-1′), 70.1 (C-2′), 69.3 (C-3′), 68.0 (C-4′), 68.6 (C-5′), 61.4 (C-6′), 103.6 (C-1), 70.7 (C-2), 70.3 (C-3), 71.7, 71.8 (C-4, C-5), 62.1 (C-6). J_{13C1F} 229, J_{13C2F} 24, J_{13C3F} 4.4 Hz. ^1H NMR (270 MHz, $CDCl_3$): δ 5.40 (H-1′), 4.83 (H-2′), 5.31 (H-3′), 5.03 (H-4′), 4.14 (H-5′), 3.95 (H-6′a), 4.22 (H-6′b), 5.61 (H-1), 4.79 (H-2), 5.51 (H-3), 4.05 (H-4), 3.91 (H-5), 4.51 (H-6a), 4.22 (H-6b). J_{1F} 52.0, J_{2F} 23.0 Hz.

Hexa-O-acetyl-1,6-anhydro-β-maltose (**3**). The fluoride (**2**) (46.7 g, 73.2 mmol) was dissolved in a mixture of EtOH (150 ml) and H_2O (200 ml). Potassium hydroxide (50 g) was added, and the temperature was kept at 80 °C for 30 min. The mixture was then neutralized with concentrated HCl and evaporated. Toluene (50 ml) was added and evaporated twice. Acetic anhydride (100 ml) and pyridine (100 ml) were added, and the mixture was boiled under reflux for 2 h. Conventional work-up yielded a dark syrup (27.3 g), from which 15.26 g of **3a** (24.0 mmol, 32 %) could be crystallized from EtOH. M.p. 175–180 °C, $[\alpha]_D^{23}$ + 47.7° (*c* 0.8, $CHCl_3$). Lit.[16] m.p. 182–183 °C, $[\alpha]_D$+ 48.0° (*c* 2.0, $CHCl_3$). ^{13}C NMR (67.89 MHz, $CDCl_3$): 96.9 ppm (C-1′), 70.3 (C-2′), 70.1 (C-3′), 67.8, 68.2, 68.5, (C-4′, C-5′, C-3), 61.8 (C-6′), 98.8 (C-1), 69.5 (C-2), 76.5 (C-4), 74.1 (C-5), 64.7 (C-6).

1,6-Anhydro-β-maltose (**3b**). De-*O*-acetylation of **3a** (1.33 g, 2.3 mmol) in 20 ml of 0.1 % NaOMe in MeOH yielded **3b** (0.704 g, 2.2 mmol, 96 %) as an amorphous powder. Crystallization from 80 % EtOH yielded **3b** (0.310 g, 0.96 mmol, 42 %) with m.p. 130–145 °C. Recrystallization from 80 % EtOH gave **3b** with m.p. 132–137 °C, $[\alpha]_D^{23}$ + 75.1° (*c* 1.1, H_2O). Lit.[17] m.p. 132–137 °C, $[\alpha]_D$ + 76.6° (H_2O). ^{13}C NMR (67.89 MHz, D_2O): 98.7 ppm (C-1′), 72.4 (C-2′), 73.8, 73.2 (C-3′, C-5′), 70.5 (C-4′), 61.5 (C-6′), 102.0 (C-1), 70.6 (C-2), 70.7 (C-3), 76.8 (C-4), 76.2 (C-5), 66.1 (C-6). ^1H NMR (270 MHz, D_2O): δ 5.14 (H-1′), 3.57 (H-2′), 3.81 (H-3′), 3.44 (H-4′), 3.79 (H-5′), 3.87 (H-6′a), 3.79 (H-6′b), 5.48 (H-1), 3.58 (H-2), 3.86 (H-3), 3.73 (H-4), 4.79 (H-5), 4.14 (H-6), 3.76 (H-6a).

Methyl 2,3,2′,3′,4′,6′-hexa-O-acetyl-β-maltose (**4**). A solution of **3a** (6.0 g, 10.4 mmol) in CH_2Cl_2 (40 ml) was treated with anhydrous $ZnBr_2$ (0.5 g, 2 mmol) and $CHCl_2OCH_3$ (10 ml, 100 mmol). After stirring for 2 h the mixture was concentrated, and toluene (10 ml) was added and evaporated. The residue was dissolved in CH_2Cl_2 (20 ml), and MeOH (20 ml) and $AgCO_3$ (7.0 g, 25.4 mmol) were added; the mixture was then stirred for 16 h. The mixture was filtered through activated carbon and the filter was washed with CH_2Cl_2 (50 ml). The combined organic phases were evaporated, dissolved in CH_2Cl_2 (75 ml), washed 3 times with 4 M HCl (25 ml), twice with saturated $NaHCO_3$, dried ($MgSO_4$) and concentrated, yielding 5.98 g of a syrup from which **4c** (3.29 g, 5.41 mmol, 52 %) was crystallized from EtOH; m.p. 140–149 °C. Recrystallization from EtOH gave a product with m.p. 149–152 °C, $[\alpha]_D^{20}$ + 50.1° (*c* 1.2, $CHCl_3$). Lit.[18] m.p. 154–155 °C, $[\alpha]_D^{20}$ + 55° (*c* 0.5, $CHCl_3$). ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.0 ppm (C-1′), 70.1 (C-2′), 69.3 (C-3′), 68.1 (C-4, C-5), 61.7, 61.1 (C-6′, C-6), 101.5 (C-1), 70.4 (C-2), 72.1 (C-3), 74.3 (C-4), 75.3 (C-5), 57.0 (OMe).

Methyl hexa-O-acetyl-6-chloro-6-deoxy-β-maltoside (**5a**). A solution of **4c** (100 mg, 0.165 mmol) in DMF (2 ml) was treated with methanesulfonyl chloride (0.15 ml, 1.98 mmol) at 70 °C for 16 h. The mixture was evaporated at 1 mmHg, and purification of the residue by preparative TLC, eluting with Et_2O, followed by crystallization from EtOH gave **5a** (65 mg, 0.10 mmol, 63 %) with m.p. 122–124 °C. Recrystallization from EtOH gave **5a** with m.p. 128–129 °C, $[\alpha]_D^{23}$ + 44.9° (*c* 1.4, $CHCl_3$). Lit.[10] m.p. 126–127 °C, $[\alpha]_D^{24}$ + 43.8°. ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.2 ppm (C-1′), 69.9 (C-2′), 69.2 (C-3′), 68.0 (C-4′), 68.5 (C-5′), 61.6 (C-6′), 100.9 (C-1), 72.0 (C-2, C-3), 75.2 (C-4), 72.8 (C-5), 44.0 (C-6), 56.8 (OMe).

Methyl 6-chloro-6-deoxy-β-maltoside (**5b**). De-*O*-acetylation of **5a** (200 mg, 0.320 mmol) in 0.1 % NaOMe in MeOH (5 ml) yielded **5b** (115 mg, 0.308 mmol, 96 %) as a syrup, which crystallized spontaneously to give a product with m.p. 164–170 °C. Recrystallization from EtOH gave **5b** with m.p. 168–170 °C, $[\alpha]_D^{23}$ + 70.3° (*c* 0.5, H_2O). Lit.[10] m.p. 182–183 °C, $[\alpha]_D^{24}$ + 72.6°. ^{13}C NMR (67.89 MHz, D_2O): 100.3 ppm (C-1′), 72.4 (C-2′), 73.8 (C-3′), 70.1 (C-4′), 73.5 (C-5′), 61.5 (C-6′), 104.0 (C-1), 73.7 (C-2), 76.7 (C-3), 77.9 (C-4), 73.8 (C-5), 45.0 (C-6), 58.7 (OMe). ^1H NMR (220 MHz, D_2O): δ 5.46 (H-1′), 3.59 (H-2′), 3.70 (H-3′), 3.44 (H-4′), 3.85 (H-5′), 3.87 (H-6a′), 3.79 (H-6b′), 4.45 (H-1), 3.33 (H-2), 3.79 (H-3), 3.78 (H-4), 3.74 (H-5), 4.00 (H-6a), 3.92 (H-6b), 3.58 (OMe).

Methyl hexa-O-acetyl-6-bromo-6-deoxy-β-maltoside (**6a**). A solution of **4c** (1.40 g, 2.31 mmol) in DMF (6 ml) was treated with methanesulfonyl bromide[18] (2 ml, 15 mmol) at 65 °C for 24 h. Pyridine (2 ml) and H_2O (5 ml) were added, and the mixture was washed 3 times with 4 M HCl (25 ml), twice with saturated $NaHCO_3$ (25 ml), dried ($MgSO_4$), filtered through activated carbon and concentrated, yielding 1.43 g of a syrup. Crystallization from EtOH yielded **6a** (0.927 g, 1.38 mmol, 60 %) with m.p. 115–120 °C. Purification at 100 mg by preparative TLC using Et_2O as eluent gave **6a** with m.p. 126–128 °C, $[\alpha]_D^{23}$ + 44.4° (*c* 1.2, $CHCl_3$). Lit.[10] m.p. 128–129.5 °C, $[\alpha]_D^{24}$ + 45.1° ($CHCl_3$). ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.3 ppm (C-1′), 69.8 (C-2′), 69.2 (C-3′), 68.0 (C-4′), 68.5 (C-5′), 61.7 (C-6′), 100.8 (C-1), 72.0 (C-2), 72.3 (C-3), 75.1 (C-4), 73.7 (C-5), 32.4 (C-6), 56.8 (OMe).

Methyl 6-bromo-6-deoxy-β-maltoside (**6b**). De-*O*-acetylation of **6a** (687 mg, 1.03 mmol) in 0.1 % NaOMe in MeOH (10 ml) yielded **6b** (409 mg, 0.978 mmol, 95 %), which crystallized spontaneously with m.p. 160–164 °C. Purification by chromatography on Sephadex G-15 eluted with $MeOH/H_2O$ (1:1) gave **6b** with m.p. 171–172 °C, $[\alpha]_D^{23}$ 72.8° (*c* 0.7, H_2O). Lit.[10] m.p. 166–167 °C, $[\alpha]_D^{24}$ + 69.1° (H_2O). ^{13}C NMR (67.89 MHz, D_2O): 100.3 ppm (C-1′), 72.4 (C-2′), 73.7 (C-3′), 70.1 (C-4′), 73.6 (C-5′), 61.3 (C-6′), 103.9 (C-1), 73.7 (C-2), 76.6 (C-3), 79.5 (C-4), 73.7 (C-5), 34.0 (C-6), 58.5 (OMe). ^1H NMR (270 MHz, D_2O): δ 5.46 (H-1′), 3.60 (H-2′), 3.71 (H-3′), 3.45 (H-4′), 3.78 (H-5′), 3.89 (H-6a′), 3.79 (H-6b′), 4.46 (H-1), 3.33 (H-2), 3.80 (H-3), 3.71 (H-4), 3.75 (H-5), 3.88 (H-6a), 3.76 (H-6b), 3.59 (OMe).

Methyl hexa-O-acetyl-6-O-methanesulfonyl-β-maltoside (**4d**). A solution of **4c** (2.0 g, 3.3 mmol) in pyridine (5 ml) was cooled to 0 °C and methanesulfonyl chloride (1 ml) was added. After stirring for 2 h at 0 °C, H_2O (20 ml) was added and the mixture was left for 4 h at room temperature. Conventional work-up yielded 2.2 g of a syrup, from which **4d** (1.80 g, 2.62 mmol, 80 %) with m.p. 110–115 °C could be crystallized from EtOH. Recrystallization from EtOH yielded **4d** with m.p. 141–143 °C, $[\alpha]_D^{23}$ + 43.8 (*c* 2.1 MeOH). Lit.[8] amorphous, $[\alpha]_D^{24}$ + 74° (*c* 0.5, MeOH). Anal. $C_{26}H_{38}O_{19}S$: C, H. ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.1 ppm (C-1′), 71.1 (C-2′), 69.7 (C-3′), 68.3 (C-4′), 68.5 (C-5′), 61.2 (C-6′), 100.9 (C-1), 71.6, 71.8 (C-2, C-3), 74.7 (C-4), 68.9 (C-5), 67.7 (C-6), 56.8 (OMe), 37.6 (OMe).

Methyl hexa-O-acetyl-6-deoxy-6-iodo-β-maltoside (**7a**). A solution of **4d** (137 mg, 0.20 mmol) in DMF was treated with sodium iodide (300 mg, 2.0 mmol) at 100 °C for 16 h. After cooling to room temperature, CH_2Cl_2 (20 ml) was added. The mixture was washed twice with 10 % sodium thiosulfate solution (10 ml), 3 times with H_2O (10 ml), dried ($MgSO_4$) and concentrated to yield crystalline **7a** (120 mg, 0.17 mmol, 84 %) with m.p. 115–119 °C. Purification by preparative TLC, using Et_2O as eluent, yielded **7a** (90 mg,

0.13 mmol, 63 %) with m.p. 125–128 °C, $[\alpha]_D^{23}$ + 47.2° (*c* 1.1, $CHCl_3$). Lit.[10] m.p. 129–130 °C, $[\alpha]_D^{24}$ + 47.7° ($CHCl_3$). ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.2 ppm (C-1′), 69.7 (C-2′), 69.1 (C-3′), 68.8 (C-4′), 68.5 (C-5′), 61.8 (C-6′), 100.6 (C-1), 72.1 (C-2, C-3), 75.8 (C-4), 74.8 (C-5), 5.6 (C-6), 56.8 (OMe).

Methyl 6-deoxy-6-iodo-β-maltoside (**7b**). De-*O*-acetylation of **7a** (222 mg, 0.309 mmol) in 0.1 % NaOMe in MeOH yielded **7b**, which was crystallized from EtOH as the monohydrate (102 mg, 0.211 mmol, 68 %) with m.p. 167–173 °C. Recrystallization from EtOH gave **7b** with m.p. 172–174 °C, $[\alpha]_D^{23}$ + 62.8° (*c* 0.6, H_2O). Anal. $C_{13}H_{23}IO_{10} \cdot H_2O$: C, H. ^{13}C NMR (67.89 MHz, D_2O): 100.6 ppm (C-1′), 73.9 (C-2′), 72.7 (C-3′), 70.4 (C-4′), 73.6 (C-5′), 61.6 (C-6′), 104.0 (C-1), 73.9 (C-2), 76.7 (C-3), 81.9 (C-4), 74.0 (C-5), 7.7 (C-6), 58.4 (OMe). ^1H NMR (270 MHz, D_2O): δ 5.44 (H-1′), 3.57 (H-2′), 3.59 (H-3′), 3.42 (H-4′), 3.74 (H-5′), 3.89 (H-6a′), 3.78 (H-6b′), 4.45 (H-1), 3.31 (H-2), 3.80 (H-3), 3.56 (H-4), 3.43 (H-5), 3.72 (H-6a), 3.43 (H-6b), 3.58 (OMe).

Methyl hexa-O-acetyl-6-azido-6-deoxy-β-maltoside (**8a**). A solution of **4d** (1.0 g, 1.46 mmol) in DMF (10 ml) was treated with sodium azide (1.0 g, 15 mmol) at 100 °C for 16 h. The mixture was poured onto ice (75 ml), followed by stirring for 1 h. An amorphous solid was filtered off and dissolved in CH_2Cl_2 (50 ml), and the solution was dried ($MgSO_4$) and concentrated, yielding crude **8a** (0.84 g, 1.33 mmol, 90 %) which could not be crystallized as described.[10] However, the product was pure enough for further reactions, as seen from a ^{13}C NMR spectrum. ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.0 ppm (C-1′), 69.8 (C-2′), 69.0 (C-3′), 68.0 (C-4′), 68.3 (C-5′), 61.6 (C-6′), 100.9 (C-1), 72.0, 71.8 (C-2, C-3), 75.0 (C-4), 73.7 (C-5), 50.9 (C-6), 56.5 (OM3).

Methyl 6-azido-6-deoxy-β-maltoside (**8b**). De-*O*-acetylation of **8a** (205 mg, 0.32 mmol) in 0.1 % NaOMe in MeOH (10 ml) yielded **8b** (90 mg, 0.23 mmol, 74 %) as a syrup, which crystallized from EtOH yielding **8b** as the monohydrate (45 mg 0.11 mmol, 35 %) with m.p. 143–146 °C. Recrystallization from EtOH gave **8b** with m.p. 150–153 °C, $[\alpha]_D^{23}$ + 70.0° (*c* 1.7, H_2O). Anal. $C_{13}H_{23}N_3O_{10} \cdot H_2O$: C, H. ^{13}C NMR (67.89 MHz, D_2O): 100.8 ppm (C-1′), 72.6 (C-2′), 73.8 (C-3′), 70.4 (C-4′), 73.8 (C-5′), 61.6 (C-6′), 104.0 (C-1), 73.8 (C-2), 76.8 (C-3), 79.2 (C-4), 74.3 (C-5), 52.0 (C-6), 57.9 (OMe). ^1H NMR (270 MHz, D_2O): δ 5.38 (H-1′), 3.55 (H-2′), 3.65 (H-3′), 3.39 (H-4′), 3.66 (H-5′), 3.85 (H-6a′), 3.75 (H-6b′), 4.41 (H-1), 3.29 (H-2), 3.75 (H-3), 3.60 (H-4), 3.74 (H-5), 3.71 (H-6a), 3.59 (H-6b), 3.56 (OMe).

Methyl 6-amino-6-deoxy-β-maltoside (**9**). Hydrogen sulfide was bubbled through a solution of **8b** (46 mg, 0.12 mmol) in pyridine (2 ml) and H_2O (1 ml) for 7 h. The mixture was concentrated, dissolved in H_2O (10 ml), and filtered through celite. Evaporation to dryness yielded **9** (40 mg, 0.11 mmol, 94 %) as a syrup which could not be crystallized, neither as the free amine nor as the hydrochloride. The product was homogeneous by TLC, using EtOAc/MeOH/CH_3COOH/H_2O (3:2:1:1) as eluent. ^{13}C NMR (67.89 MHz, D_2O, pH~1): 100.6 ppm (C-1′), 72.3 (C-2′), 73.6 (C-3′), 70.3 (C-4′), 73.6 (C-5′), 61.5 (C-6′), 103.9 (C-1), 73.8 (C-2), 76.5 (C-3), 79.9 (C-4), 71.8 (C-5), 41.7 (C-6), 58.1 (OMe). ^1H NMR (270 MHz, D_2O, pH~1): δ 5.38 (H-1′), 3.56 (H-2′), 3.65 (H-3′), 3.38 (H-4′), 3.65 (H-5′), 3.86 (H-6a′), 3.72 (H-6b′), 4.42 (H-1), 3.32 (H-2), 3.78 (H-3), 3.57 (H-4), 3.83 (H-5), 3.53 (H-6a), 3.17 (H-6b), 3.57 (OMe).

Methyl 6-acetamido-6-deoxy-β-maltoside (**10**). To a solution of **8b** (100 mg, 0.27 mmol) in MeOH (30 ml) was added acetic anhydride (20 ml) and palladium-on-carbon (5 %, 50 mg). The mixture was hydrogenated at 1 atm. hydrogen pressure for 2 h. The catalyst was filtered off, the filtrate was evaporated, and EtOH (20 ml) was added and evaporated twice, leaving 100 mg of a syrup. The 90 MHz ^1H NMR spectrum in D_2O showed several signals at about 2 ppm, indicating that some *O*-acetylation had occurred. The product was therefore dissolved in 0.1 % NaOMe in MeOH (20 ml) and the solution was left for 30 min. The solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin and filtered. The resin was washed with MeOH (50 ml), and the filtrate and washing liquid were combined and concentrated, yielding **10** (90 mg, 0.22 mmol, 81 %) as a syrup which was pure according to the 90 MHz ^1H NMR spectrum; the latter showed a

singlet at 2.0 ppm (3H, *N*-acetyl). ^{13}C NMR (67.89 MHz, D_2O): 100.8 ppm (C-1'), 72.8 (C-2'), 73.9, 73.8 (C-3', C-5'), 70.4 (C-4'), 61.6 (C-6'), 104.1 (C-1), 73.8 (C-2), 77.2 (C-3), 79.6 (C-4), 74.1 (C-5), 41.7 (C-6), 58.2 (OMe), 22.9 (NCH_3). ^1H NMR (270 MHz, D_2O): δ 5.35 (H-1'), 3.36 (H-4'), 3.80 (H-6a'), 3.72 (H-6b'), 4.31 (H-1), 3.24 (H-2), 3.96 (H-6a), 3.22 (H-6b), 3.49 (OMe), 2.00 (NCH_3).

*Methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-6-deoxy-β-D-*xylo*-hex-5-enopyranoside* (**11a**). A solution of tetrabutylammonium fluoride (100 mg, 0.38 mmol) in toluene (2 ml) dried by azeotropic distillation was added to a solution of **4d** (60 mg, 0.087 mmol) in DMF (2 ml). The mixture was heated to 90 °C for 2 h. CH_2Cl_2 (10 ml) was added, and the mixture was washed 4 times with H_2O (10 ml), dried ($MgSO_4$) and concentrated, yielding a syrup (70 mg). The product was purified by preparative TLC, using Et_2O as eluent. Crystallization from EtOH gave **11a** (20 mg, 0.034 mmol, 39 %) with m.p. 85–92 °C. Recrystallization from EtOH gave **11a** with m.p. 92–93 °C, $[\alpha]_D^{23}$ + 37.2° (*c* 0.5, EtOH). Lit.[11] amorphous $[\alpha]_D^{22}$ + 50° (*c* 0.1, EtOH). Anal. $C_{25}H_{34}O_{16}$: C, H. ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.4 ppm (C-1'), 76.6 (C-2'), 69.5 (C-3'), 68.1 (C-4', C-5'), 61.5 (C-6'), 101.0 (C-1), 71.6, 72.0 (C-2, C-3), 73.5 (C-4), 151.7 (C-5), 96.5 (C-6), 56.3 (OMe). Lit.[11]: 151 ppm (C-5), 101 (C-1), 96 (C-6), 95 (C-1').

*Methyl 4-*O*-α-D-glucopyranosyl-6-deoxy-β-D-*xylo*-hex-5-enopyranoside* (**11b**). De-*O*-acetylation of **11a** (25 mg, 0.042 mmol) in 0.1 % NaOMe in MeOH (5 ml) yielded **11b** (10 mg, 0.030 mmol, 71 %) as a syrup. ^{13}C NMR (67.89 MHz, D_2O): 99.8 ppm (C-1'), 72.4 (C-2'), 73.9, 73.6 (C-3', C-5'), 70.3 (C-4'), 61.4 (C-6'), 105.2 (C-1), 73.9 (C-2), 75.3, 76.1 (C-3, C-4), 153.6 (C-5), 98.9 (C-6), 58.0 (OMe). ^1H NMR (270 MHz, D_2O): δ 5.37 (H-1'), 3.59 (H-2'), 3.80 (H-3'), 3.42 (H-4'), 3.84 (H-5'), 3.69 (H-6a', H-6b'), 4.53 (H-1), 3.50 (H-2), 3.73 (H-3), 4.23 (H-4), 4.88, 4.91 (H-6a, H-6b), 3.52 (OMe).

*Methyl hexa-*O*-acetyl-6-deoxy-6-fluoro-β-maltoside* (**12a**) *and Methyl-6-deoxy-6-fluoro-β-maltoside* (**12b**). A solution of **4c** (1.0 g, 1.64 mmol) in CH_2Cl_2 (5 ml) was cooled to −70 °C, thereupon *N,N*-diethylaminosulfur trifluoride (1.2 ml, 6.2 mmol) was added and the mixture was allowed to reach room temperature. The mixture was again cooled to −70 °C, MeOH (10 ml) was added, and the mixture was allowed to reach room temperature. After evaporation, the residue was dissolved in CH_2Cl_2 (50 ml), washed 3 times with 4 M HCl (25 ml), twice with saturated $NaHCO_3$ (25 ml), dried ($MgSO_4$) and concentrated to yield 550 mg of crude product. Purification by preparative TLC, using Et_2O as eluent, yielded a crystalline product (290 mg) which was a mixture of **12a** (80 %) and methyl hepta-*O*-acetyl-β-maltoside (20 %). Further purification of **12a** by recrystallization or chromatography was not possible. The product was de-*O*-acetylated in 0.1 % NaOMe in MeOH (10 ml), and after removal of sodium ions the product (139 mg) was passed through a Merck silica gel 60 column using EtOAc/MeOH/CH_2O (6:1:1) as eluent. Pure, syrupy **12b** (78 mg, 0.22 mmol, 13 % based on **4c**) was obtained first [as seen from the 500 MHz ^1H NMR spectrum in D_2O: δ 5.45 (H-1'), 3.56 (H-2'), 3.67 (H-3'), 3.40 (H-4'), 4.01 (H-6a'), 3.77 (H-6b'), 4.49 (H-1), 3.29 (H-2), 3.75 (H-3), 4.75 (H-6a, H-6b)], followed by methyl β-maltoside.

Acetylation of **12b** (50 mg, 0.14 mmol) with pyridine (2 ml) and acetic anhydride (2 ml) for 4 h gave, after evaporation to dryness and column chromatography using ethyl acetate/ether (1:1), compound **12a**, which could be crystallized from EtOH (65 mg, 0.11 mmol, 76 %) with m.p. 115–118 °C. Further recrystallization gave **12a** with m.p. 118–119 °C, $[\alpha]_D^{23}$ + 41.5° (*c* 1.3, $CHCl_3$). Anal. $C_{25}H_{35}FO_{16}$: C, H. ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.1 ppm (C-1'), 70.0 (C-2'), 69.2 (C-3'), 68.1 (C-4'), 68.4 (C-5'), 61.5 (C-6'), 101.2 (C-1), 70.0 (C-2), 72.0 (C-3), 75.3 (C-4), 73.0 (C-5), 81.1 (C-6), 56.9 (OMe). J_{13C6F} 176, J_{13C5F} 19 Hz.

*[Methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside]uronic Acid* (**13a**). A solution of **4c** (1.0 g, 1.64 mmol) in acetic acid (10 ml) was treated with potassium permanganate (0.78 g, 4.9 mmol) for 3 h at room temperature. Sodium oxalate (1 g, 7.5 mmol) and H_2O (20 ml) were added, and the mixture was left at 5 °C for 16 h. The mixture was extracted 3 times with $CHCl_3$ (25 ml), washed twice with H_2O (50 ml), dried ($MgSO_4$) and concentrated, yielding crude crystalline **13a** (750 mg, 121 mmol, 74 %). Recrystallization from ether

yielded **13a** (550 mg, 0.88 mmol, 54 %) with m.p. 144–147 °C $[\alpha]_D^{23}$ + 30.3° (*c* 0.8, $CHCl_3$). Anal. $C_{25}H_{34}O_{18}$: C, H. ^{13}C NMR (67.89 MHz, $CDCl_3$): 95.9 ppm (C-1'), 70.1 (C-2'), 69.4 (C-3'), 67.5 (C-4'), 68.9 (C-5'), 62.7 (C-6'), 101.4 (C-1), 72.0, 73.9 (C-2, C-3), 74.7, 74.1 (C-4, C-5), 172.7 (C-6), 57.4 (OMe).

[Methyl 4-O-(α-D-glucopyranosyl-β-D-glucopyranoside]uronic Acid (**13b**). De-*O*-acetylation of **13a** (46 mg, 0.074 mmol) in 0.1 % NaOMe in MeOH (10 ml, 0.23 mmol sodium) yielded, after removal of sodium ions with Amberlite IR C50 (H^+) ion-exchange resin and chromatography on Sephadex G-15 with MeOH/H_2O (1:1) as eluent, **13b** (20 mg, 0.054 mmol, 73 %) as a syrup. ^{13}C NMR (67.89 MHz, D_2O): 98.9 ppm (C-1'), 72.4 (C-2'), 73.6 (C-3'), 69.9 (C-4'), 73.4 (C-5'), 60.7 (C-6'), 103.8 (C-1), 72.3 (C-2), 77.0, 77.4, 77.1 (C-3, C-4, C-5), 175.7 (C-6), 57.8 (OMe). ^1H NMR (270 MHz, D_2O): δ 5.46 (H-1'), 3.51 (H-2'), 3.68 (H-3'), 3.37 (H-4'), 4.38 (H-1), 3.25 (H-2), 3.55 (H-5), 3.54 (OMe).

Methyl [methyl 2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside]uronate (**14a**). To a solution of **13a** (30 mg, 0.048 mmol) in MeOH (10 ml) was added a solution of diazomethane in Et_2O until a persistent yellow colour was obtained in the reaction mixture. Excess diazomethane was destroyed by addition of acetic acid. Evaporation and crystallization from Et_2O yielded **14a** (19 mg, 0.030 mmol, 62 %) with m.p. 115–120 °C. Recrystallization from Et_2O gave **14a** with m.p. 124–125 °C, $[\alpha]_D^{23}$ + 35.6° (*c* 0.5, $CHCl_3$). Lit.[12] m.p. 173–177 °C, $[\alpha]_D$ + 80° ($CHCl_3$). Anal. $C_{26}H_{36}O_{18}$: C, H. ^1H NMR (270 MHz, $CDCl_3$): δ 5.40 (H-1'), 4.82 (H-2'), 5.34 (H-3'), 5.03 (H-4'), 3.74 (H-5'), 4.13 (H-6a'), 4.25 (H-6b'), 4.52 (H-1), 4.88 (H-2), 5.28 (H-3), 4.35 (H-4), 4.10 (H-5), 3.50 (OMe), 3.83 (OMe-ester).

Methyl [methyl-4-O-(α-D-glucopyranosyl)-β-D-glucopyranoside]uronate (**14**). De-*O*-acetylation of **14a** (10 mg, 0.016 mmol) with 0.1 % NaOMe in MeOH (5 ml) yielded **14b** (5 mg, 0.013 mmol, 81 %) as a syrup. ^{13}C NMR (67.89 MHz, D_2O): 99.6 ppm (C-1'), 72.7 (C-2'), 73.1, 73.0 (C-3', C-5'), 69.4 (C-4'), 60.4 (C-6'), 104.0 (C-1), 71.9 (C-2), 77.4, 76.1, 74.5 (C-3, C-4, C-5), 170.9 (C-6), 58.0 (OMe), 53.9 (OMe-ester).

Methyl 6'-bromo-6'-deoxy-β-maltoside (**15b**). De-*O*-acetylation of **15a**[2] (100 mg, 0.15 mmol) in 0.1 % NaOMe in MeOH (10 ml) yielded **15b** (45 mg, 0.11 mmol, 72 %) as a syrup. ^{13}C NMR (67.89 MHz, D_2O): 100.2 ppm (C-1'), 72.2 (C-2'), 73.0 (C-3'), 71.9, 72.0 (C-4', C-5'), 33.7 (C-6'), 103.8 (C-1), 73.6 (C-2), 76.7 (C-3), 77.6 (C-4), 75.1 (C-5), 61.6 (C-6), 57.8 (OMe), ^1H NMR (270 MHz, D_2O): δ 5.43 (H-1'), 3.61 (H-2'), 3.71 (H-3'), 3.45 (H-4'), 3.87 (H-5'), 3.81 (H-6a'), 3.67 (H-6b'), 4.40 (H-1), 3.30 (H-2), 3.78 (H-3), 3.36 (H-4), 3.60 (H-5), 3.98 (H-6a), 3.78 (H-6b), 3.57 (OMe).

Methyl 3',6'-anhydro-β-maltoside (**16b**). A solution of **15a**[2] (200 mg, 0.30 mmol) in EtOH (10 ml) was heated under reflux with 1 M sodium hydroxide in H_2O (20 ml) for 30 min. After cooling to room temperature the mixture was neutralized with solid carbon dioxide (10 g) and concentrated. The residue was applied to a column of Sephadex G-15. Elution with MeOH/H_2O (1:1) yielded **16b** (85 mg, 0.25 mmol, 84 %) as a syrup. ^1H NMR (270 MHz, D_2O): δ 5.31 (H-1'), 3.99 (H-2'), 4.29 (H-4'), 4.20 (H-5'), 4.17 (H-6), 4.35 (H-1), 3.25 (H-2), 3.64 (H-4), 3.55 (H-5), 3.95 (H-6a), 3.80 (H-6b), 3.54 (OMe).

Methyl 2,3,6,2',4'-penta-O-acetyl-3',6'-anhydro-β-maltoside (**16a**). Compound **15a**[2] (200 mg, 0.30 mmol) was treated as described for the preparation of **16b**, but the salt mixture containing crude **16b** was treated directly with acetic anhydride (10 ml) and pyridine (10 ml) for 4 h. Conventional work-up gave a syrup (180 mg), from which **16a** (105 mg, 0.19 mmol, 64 %) with m.p. 159–167 °C could be recrystallized. Recrystallization from EtOH gave **16a** with m.p. 168–169 °C, $[\alpha]_D^{23}$ + 12.9° (*c* 3.2, $CHCl_3$). Anal. $C_{23}H_{32}O_{15}$: C, H. ^{13}C NMR (67.89 MHz, $CDCl_3$): 96.4 ppm (C-1'), 70.7, 69,8, 68,4 (C-2', C-4', C-5'), 74.4 (C-4'), 67.9 (C-6'), 101.4 (C-1), 72.0, 72.7 (C-2, C-3), 76.0 (C-4), 73.5 (C-5), 63.0 (C-6), 57.1 (OMe).

Methyl 2,3,6-tri-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-deoxy-6-fluoro-α-D-glucopyranosyl)-β-D-glucopyranoside (**17a**). Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside[19] (213 mg, 0.4 mmol) was dissolved in CH_2Cl_2 (2 ml) and the solution was cooled to −70 °C in acetone/dry ice; *N,N*-diethyl-

aminosulfur trifluoride (DAST) (300 µl, 1.6 mmol) was added and the temperature was allowed to reach 20–25 °C over a period of 1 h. The reaction mixture was again cooled to −70 °C followed by addition of MeOH (5 ml) to destroy excess DAST; it was then allowed to reach room temperature and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (40 ml), and the solution was washed twice with H_2O and once with $NaHCO_3$, followed by drying ($MgSO_4$) and concentration. The crude product (284 mg) was separated into two fractions by preparative TLC, using ethyl acetate/pentane (1:1) as eluent. The fastest-moving fraction (119 mg) was shown by ^1H NMR spectroscopy to be a mixture of **17a** and another unknown 6′-substituted derivative. Repeated preparative TLC using pentane/Et_2O (1:6) as eluent gave complete separation of the two products, and the slowest-moving fraction (52 mg) was shown by NMR to be **17a**. The product crystallized from EtOH with m.p. 160–161 °C, $[\alpha]_D^{20}$ + 51.6° (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 4.46 (H-1), 4.82 (H-2), 5.25 (H-3), 4.09 (H-4), 3.69 (H-5), 4.50 (H-6a), 4.22 (H-6b), 5.44 (H-1′), 4.83 (H-2′), 5.39 (H-3′), 5.01 (H-4′), 3.93 (H-5′), 4.41 (H-6a′), 4.41 (H-6b′), 3.50 (OMe). J_{5F} = 22.8, J_{6F} = 40.7 Hz. ^{13}C NMR (125.7 MHz, $CDCl_3$): 101.2 ppm (C-1), 72.1 (C-2), 72.2 (C-3), 75.6 (C-4), 72.3 (C-5), 62.5 (C-6), 95.4 (C-1′), 70.0 (C-2′), 68.0 (C-3′), 67.8 (C-4′), 69.3 (C-5′), 81.2 (C-6′), 57.0 (OMe). J_{C6F} 175.8, J_{C5F} 21.1 Hz.

Methyl 4-O-(6-deoxy-6-fluoro-α-D-glucopyranosul)-β-D-glucopyranoside (**17b**). Compound **17a** (36 mg) was dissolved in MeOH (1 ml) and NaOMe/MeOH (0.5 ml, 1 M); the solution was left at room temperature for 3 h, followed by neutralization with ion-exchange resin and evaporation, leaving **17b** as a syrup (23 mg) which was characterized by its NMR data. ^1H NMR (500 MHz, D_2O): δ 4.37 (H-1), 3.28 (H-2), 3.76 (H-3), 3.62 (H-4), 3.86 (H-5), 3.91 (H-6a), 3.73 (H-6b), 5.43 (H-1′), 3.58 (H-2′), 3.69 (H-3′), 3.49 (H-4′), 3.58 (H-5′), 4.72 (H-6a′), 4.67 (H-6b′), 3.57 (OMe). J_{6F} 47, J_{5F} 23 Hz. ^{13}C NMR (125.7 MHz, D_2O): 104.4 ppm (C-1), 74.2 (C-2), 77.5 (C-3), 78.2 (C-4), 75.9 (C-5), 61.9 (C-6), 100.9 (C-1′), 72.8 (C-2′), 73.9 (C-3′), 72.7 (C-4′), 69.6 (C-5′), 83.5 (C-6′), 58.4 (OMe). J_{6F} 167.8, J_{5F} 69 Hz.

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 containing 10 mg of dry AMG per ml buffer, (10 µl) 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 references were used. Negative results were confirmed by addition of more enzyme and longer 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 solution, and it was thermostatted to the desired temperature (27 or 37 °C). AMG solution (10 µl; 10 mg AMG per ml buffer) in the deuterium buffer was added, and the sample was transferred to a 5 mm NMR tube and ^1H NMR spectra were recorded at suitable time intervals at 500 MHz.

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References

1. Svensson, B., Larsen, K., Svendsen, I. and Boel, E. *Carlsberg Res. Commun. 48* (1983) 529.
2. Bock, K. and Pedersen, H. *Acta Chem. Scand., Ser. B 41* (1987) 617.
3. Bock, K. *Pure Appl. Chem. 59* (1987) 1447.
4. Bock, K. and Pedersen, H. *The Substrate Specificity of Amyloglycosidase (EC. 3.2.1.3).* In: Lark, D., Ed., *Molecular Biology of Microbial Pathogenecity,* Academic Press, London 1986, p. 173.
5. Fujimaka, I., Ichikawa, Y. and Kuzuhara, H. *Carbohydr. Res. 101* (1982) 148.
6. Kloosterman, M., Dees, M. J., van der Marel, G. A. and van Boom, J. H. *Recl. Trav. Chim. Pays-Bas 104* (1985) 116.

7. Bognar, R., Farkas, I., Manyhart, M., Gross, H. and Paulsen H. *Carbohydr. Res. 6* (1968) 104.
8. Fujimaki, I. and Kuzahara, H. *Agric. Biol. Chem. 45* (1980) 2055.
9. Takeo, K., Suminoto, T. and Kuge, T. *Stärke 26* (1974) 111.
10. Takeo, K. *Carbohydr. Res. 69* (1979) 272.
11. Sugawara, F. and Kuzuhara, H. *Agric. Biol. Chem. 45* (1981) 301.
12. Abbott, D. and Weigel, H. *J. Chem. Soc.* (1965) 5157.
13. Bock, K. and Sigurskjold, B.W. *Biochem. J.* (1987) *Submitted for publication.*
14. Wolfrom, M.S. and Thompson, A. *Methods Carbohydr. Chem. 1* (1962) 334.
15. Genghof, D.S., Brewer, C.F. and Hehre, E.J. *Carbohydr. Res. 61* (1978) 291.
16. Asp, L. and Lindberg, B. *Acta Chem. Scand. 6* (1952) 941.
17. Mori, M., Haga, M. and Tejima, S. *Chem. Pharm. Bull. 23* (1975) 1480.
18. Sieber, G.J. *Liebigs Ann. Chem. 631* (1960) 180.
19. Durette, P.L., Hough, L. and Richardson, A.C. *J. Chem. Soc., Perkin Trans. 1* (1974) 97.

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