ELSEVIER

Carbohydrate Research 268 (1995) 93–106

CARBOHYDRATE RESEARCH

Syntheses of 4′-deoxy-α-maltosyl fluoride and 4″-deoxy-α-maltotriosyl fluoride as probes of α-glucanotransferase mechanisms

Thisbe K. Lindhorst, Curtis Braun, Stephen G. Withers *

Department of Chemistry, 2036 Main Mall, University of British Columbia, Vancouver, BC, V6T 1Z1, Canada

Received 20 June 1994; accepted 3 October 1994

Abstract

4′-Deoxy-α-maltosyl fluoride and 4″-deoxy-α-maltotriosyl fluoride were synthesized to serve as mechanistic probes for α-glucanotransferases such as the glycogen debranching enzyme. The deoxy derivatives were prepared starting from maltose and maltotriose, respectively. Deoxygenation of the terminal 4-hydroxyl group was accomplished by radical reduction of the iodo sugar formed after iodide displacement of the corresponding protected triflate. The anomeric fluoride was introduced by treatment of the protected hemiacetal with diethylaminosulfur trifluoride, predominantly giving the β-anomeric product. Conversion to the α anomer was achieved using HF · pyridine.

Keywords: 4′-Deoxy-α-maltosyl fluoride; 4″-Deoxy-α-maltotriosyl fluoride; Enzyme probe; α-Glucanotransferase

1. Introduction

α-Glucanotransferases catalyse the rearrangement of α-linked glucans to yield products of different chain lengths. Such activities are important, *inter alia*, in starch metabolism and in the branching and debranching of glycogen [1,2]. Better knowledge of the active sites of these enzymes could lead to the development of improved inhibitors which could be useful in control of cell wall growth, or of digestion. Many of these transferases belong to a large sequence-related family of enzymes. Although the overall sequence homology in these families is often weak, significant local sequence

* Corresponding author.

SSDI 0008-6215(94)00317-3

homology exists around residues which are highly conserved. Indeed recently determined 3-dimensional structures of several enzymes from this family [3–5] confirm the active site location of these key conserved residues.

The overall reaction involves a glycosyl transfer with net retention of anomeric configuration, thus a double displacement is likely. Such a mechanism involves initial formation of a glycosyl–enzyme intermediate via enzymatic attack on the polysaccharide, followed by decomposition of the intermediate via transglycosylation to the nonreducing terminus of another polysaccharide chain. The specific roles of the amino acids identified crystallographically in this mechanism are not yet known. In particular, the identification of the catalytic nucleophile is of interest, and one possible approach to that with rabbit muscle glycogen debranching enzyme (EC 3.2.1.33 + EC 2.4.1.25) is as follows. The compounds α-maltosyl fluoride and α-maltotriosyl fluoride [6] recently have been found [7] to function as substrates for the transferase activity of the debranching enzyme, yielding longer oligosaccharide fluorides as products via a transglycosylation reaction. Addition of 4″-deoxy-α-maltotriosyl fluoride to the enzyme should result in the formation of a glycosyl–enzyme intermediate which cannot then transfer, since the necessary nucleophilic acceptor (another molecule of itself) is not available. This derivatised amino acid could then be identified using known strategies [8].

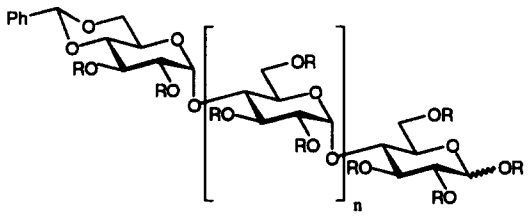
Herein we describe the syntheses of the deoxygenated analogues **11** and **22** necessary for such a study.

2. Results and discussion

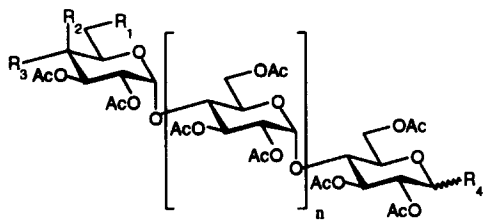
For the syntheses of the deoxygenated di- and tri-saccharide derivatives **11** and **22**, analogous strategies were chosen, starting from maltose in one case and from maltotriose in the other. The synthetic avenues involved the synthesis of analogues in which all except the 4′- and 4″-hydroxy groups, respectively, were protected, deoxygenation of the 4′ or 4″ position and finally the introduction of an α-fluoro substituent at C-1.

To obtain a favourable protection pattern, maltose was first benzylidenated to **1**, which was purified and acetylated to give **2** in ca. 40% overall yield. These first two standard steps of the synthesis occur with the most modest yields of all procedures described. Simultaneous 1,2:4′,6′-di-*O*-benzylidenation in the first step complicates the product mixture and results in tedious purification problems. In the disaccharide case column chromatography was supplemented by suitable extraction steps (see Experimental). Pure **2** was then submitted to opening of the 4′,6′-dioxolane ring following Garegg's method using sodium cyanoborohydride and HCl-saturated ether [9,10]. The reaction proceeds regioselectively to give only the 4′-unprotected derivative **3** in excellent yield. For the deoxygenation of **3** the most convenient method involved an activation–inversion procedure. Since equatorial sulfonyloxy groups are known to be displaced less easily than axial ones, the trifluoromethanesulfonyloxy group was chosen for the activation of **3**. The resulting 4′-triflyloxy ester was readily converted into the axial 4′-deoxy-4′-iodo derivative **4** without prior purification. The high-field shift (36.70 ppm for C-4′) in the ^{13}C NMR spectrum proved the 4′-iodo substitution. In addition, inversion of the D-*gluco* to the D-*galacto* configuration of the terminal part of the

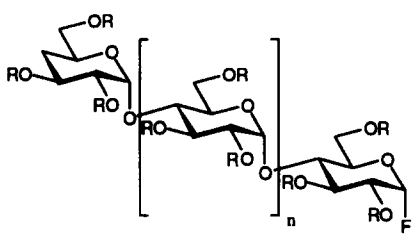
disaccharide could be seen from the vicinal proton coupling constants. Free-radical reduction through the *O*-(imidazolylthiocarbonyl) derivative [11] was not successful.



	n	R
1	0	H
2	0	Ac
12	1	H
13	1	Ac



	n	R_1	R_2	R_3	R_4
3	0	OBn	H	OH	OAc
4	0	OBn	I	H	OAc
5	0	OBn	H	H	OAc
6	0	OH	H	H	OAc
7	0	OAc	H	H	OAc
8	0	OAc	H	H	OH
9	0	OAc	H	H	F
14	1	OBn	H	OH	OAc
15	1	OBn	I	H	OAc
16	1	OBn	H	H	OAc
17	1	OH	H	H	OAc
18	1	OAc	H	H	OAc
19	1	OAc	H	H	OH
20	1	OAc	H	H	F



	n	R
10	0	Ac
11	0	H
21	1	Ac
22	1	H

Surprisingly, it was not possible to simultaneously reduce the 4′-iodo function and the 6′-benzyl ether group in **4** by catalytic hydrogenation with palladium-on-charcoal (10%, 1 bar) in good yield. The 4′-iodo function was therefore reduced in a radical reaction using tributyltin hydride yielding **5**, followed by hydrogenolysis of the 6′-benzyl ether group and reacetylation of the resulting **6** to give **7** in almost 90% yield over three steps. Routinely **6** was not isolated, but was acetylated without purification. Zemplén deprotection of **7** yielded free 4′-deoxy maltose.

For an unequivocal NMR assignment, a sample of the two diastereomers of **7** was separated into the anomers. Pure α and pure β derivatives gave rise to very clear NMR spectra with the typical signal pattern for deoxy saccharides. The 4′-deoxy protons appear at high field, the axial H-4′(β) displaying a quartet at 1.59 ppm and the equatorial H-4′(α) appearing as a ddd-system at 2.13 ppm. Typically H-3′ and H-5′ are complicated, H-3′(β) appearing as a sextet at 5.15 ppm. The presence of an additional methylene group was also confirmed in an attached proton test (APT) ^{13}C NMR spectrum.

The maltose derivative **7** is a key precursor molecule which already bears the desired 4′-deoxy function and which has now to be equipped with the anomeric α-fluoride functionality. Since it proved not to be possible to use the HF · pyridine complex alone to effect anomeric fluorination in good yield in this case, although this has been shown to work well on many other glycosyl peracetates [12], an alternative procedure was chosen. This involved the selective deprotection of the anomeric acetate using hydrazine acetate [13]. This mild method gave rise to the reducing sugar **8**, which was then converted with diethylaminosulfur trifluoride (DAST) to the maltosyl fluoride **9** in almost quantitative yield. Unfortunately, **9** was obtained as a mixture of both the anomeric disaccharide fluorides, the β fluoride predominating by ca. 9:1 (by ^{1}H NMR spectroscopy). This mixture was readily seen in the ^{1}H decoupled ^{19}F NMR spectra, with the α anomer exhibiting a singlet at −149.21 ppm and the β anomer at −133.27 ppm.

For the reaction with α-glucanotransferases, the α anomer is required. Further, since β-glycosyl fluorides are significantly less stable than α-glycosyl fluorides, significant contamination by the "wrong" anomer could seriously complicate kinetic analysis. An anomerization step was therefore necessary, but this proved problematic particularly since reaction progress could not be detected by TLC, necessitating careful NMR analysis. Of all the methods tested, treatment of the β fluoride with an excess of the neat HF · pyridine complex gave the best results, producing **10** as a crystalline solid obtained in typical yields of ca. 60%. Under the conditions required for complete anomerization, two major byproducts, 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*xylo*-hexopyranosyl fluoride and 2,3,6-tri-*O*-acetyl-α-D-glucosyl fluoride, were formed. These two side products presumably arise from attack of fluoride upon the glycosidic bond and subsequent anomerization to the monomeric α fluorides. The deprotection of **10** under standard conditions gave the target molecule **11** in high yield, which was readily freeze-dried and obtained as a white foam.

The synthesis of 4″-deoxy-α-maltotriosyl fluoride (**22**) was accomplished by similar procedures to those worked out for the preparation of the disaccharide derivative, 4′-deoxy-α-maltosyl fluoride (**11**), but starting from maltotriose instead of maltose. In order to obtain the selectively protected trisaccharide derivative **14**, maltotriose was benzylidenated to **12** and after purification acetylated to **13**. Tedious column purifications were necessary after each step and contributed to the low overall yield of ca. 30%. Regioselective cleavage of the dioxolane ring with sodium cyanoborohydride led to **14** in high yield. Activation of the 4″-hydroxy group as its triflyloxy ester and inversion with sodium iodide gave the iodo-*galacto*-derivative **15**. Once again the reduction of the 4″-iodo function and the 6″-*O*-benzyl protection group was best carried out in two separate steps, firstly a free-radical reduction procedure to give **16**, followed by hydrogenolysis of the benzyl group to yield **17**. After reacetylation the 4″-deoxy key intermediate **18** was obtained. ^{1}H NMR analysis revealed that the equatorial H-4″ was hidden under the acetate signals, but the axial H-4″ could clearly be seen at 1.58 ppm. Deprotection under Zemplén conditions gave the unblocked 4″-deoxy-maltotriose, which was characterized by ^{1}H NMR spectroscopy. The anomeric functionalization of **18** was carried out as in the disaccharide synthesis. Selective cleavage of the anomeric acetate with hydrazine acetate gave the reducing trisaccharide derivative **19**, which was almost

quantitatively converted to the anomeric mixture of glycosyl fluorides **20** by reaction with DAST. The ^{1}H-decoupled ^{19}F NMR spectrum of the α anomer shows a singlet at -149.23 ppm, whereas the β anomer appears at -132.03 ppm. The $\alpha:\beta$ ratio of both anomers was determined from the ^{1}H NMR spectrum as $\sim 8:1$. The anomerization step converting the β glycosyl fluoride of **20** into the α fluoride **21** was again the major hurdle in the synthesis, use of neat HF · pyridine never giving more than a 60% yield. Progress of the reaction was again followed by ^{1}H and ^{19}F NMR spectroscopy, and once again fluorolysis of the glycosidic bond was a side reaction under the conditions necessary for complete anomerization. In this case the less stable glycosidic bond was cleaved, that between the 4″-deoxy moiety and the remaining disaccharide, releasing 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*xylo*-hexopyranosyl fluoride and 2,3,6,2′,3′,6′-hexa-*O*-acetyl-α-maltosyl fluoride. These were removed by column chromatography after acetylation. After deprotection of **21** with catalytic amounts of sodium methoxide, the target trisaccharide **22** was obtained in good yield.

3. Experimental

General methods.—Melting points (mp) were determined on a Laboratory Devices Mel-Temp II melting-point apparatus, and are uncorrected. Solvents and reagents used were either reagent grade, certified, or spectral grade. HF · pyridine (70%) was obtained from Aldrich Chemical Company. The reactions were monitored by thin-layer chromatography (TLC), which was performed on E. Merck Kieselgel 60 F_{254} analytical plates. Product spots were either detected with UV-light and/or with charring after dipping into a 10% H_2SO_4 solution in MeOH. Column chromatography was performed under elevated pressure using Kieselgel 60 (230–400 mesh). NMR spectra were recorded on a 200 MHz Bruker AC 200, a 400 MHz Bruker WH 400, a 300 MHz Varian XL 300, or a 500 MHz Varian (Unity 500) instrument. Where required, the interpretation was supported by COSY or APT NMR experiments. Chemical shifts (δ) are given relative to internal solvent standards with $CDCl_3$ (7.24 ppm), D_2O (4.80 ppm), and MeOD (3.33 ppm, q). ^{19}F NMR spectra were recorded on a 200 MHz Bruker AC 200 spectrometer and are proton decoupled except where otherwise indicated. Chemical shifts (δ) are reported in ppm relative to external trifluoroacetic acid (-76.53 relative to Freon). Desorption chemical-ionization mass spectra (DCIMS) generated with ammonia as the reactive gas were recorded on a Delsi Nermag R10-10C mass spectrometer. Microanalyses were performed by Mr. Peter Borda in the Microanalytical Laboratory of the University of British Columbia.

1,2,3,6,2′,3′-Hexa-O-*acetyl-4′,6′*-O-*benzylidenemaltose* (**2**).—Maltose (40.00 g, 111.0 mmol) was dissolved in dry DMF (100 mL), benzaldehyde dimethylacetal (18.0 mL, 120.0 mmol) and *p*-toluenesulfonic acid (~ 100 mg) were added, and the mixture was rotated at 60–70°C on a rotary evaporator under a weak vacuum to remove MeOH that formed. The reaction was continued for 6 h, with occasional replenishment of DMF. TLC analysis (7:2:1 EtOAc–MeOH–water) showed one major and several other minor products. The mixture was neutralized with a basic ion-exchange resin (Dowex-1, OH^- form), filtered, and coevaporated with toluene. The remaining syrup was subjected to

water–EtOAc extraction, the organic phase being extracted twice with water, and the combined aqueous phases then washed three times with EtOAc. This procedure removes the more nonpolar byproducts from the aqueous phase, thus facilitating the purification procedure. The aqueous phase was concentrated by evaporation and purified by flash chromatography (8:1:1 EtOAc–MeOH–water). The 4′,6′-*O*-benzylidene compound **1** (20.40 g, 47.40 mmol, 43%) obtained was then dissolved in pyridine (300 mL) and acetylated with Ac_2O (200 mL). After the acetylation reaction was complete, the mixture was diluted with CH_2Cl_2 and neutralized with satd aq $NaHCO_3$. The aqueous phase was extracted three times with CH_2Cl_2, and the organic phases were washed twice with water. Coevaporation with toluene yielded **2** (31.8 g, 46.6 mmol, 42% from maltose) as an almost colourless syrup; ^{1}H NMR ($CDCl_3$, 200 MHz), the β anomer only: δ 7.45–7.30 (m, 5 H, Ar), 5.72 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.46 (s, 1 H, PhC*H*), 5.44 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 9.8 Hz, H-3′), 5.34 (d, 1 H, $J_{1',2'}$ 4.2 Hz, H-1′), 5.29 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 8.6 Hz, H-3), 4.96 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 9.6 Hz, H-2), 4.86 (dd, 1 H, $J_{1',2'}$ 4.2, $J_{2',3'}$ 10.3 Hz, H-2′), 4.48 (dd, 1 H, $J_{5',6'a}$ 2.3, $J_{6'a,6'b}$ 12.5 Hz, H-6′a), 4.30–4.17 (m, 2 H, H-6′b, H-6a), 4.03 (dd, 1 H, $J_{3,4}$ 8.6, $J_{4,5}$ 9.6 Hz, H-4), 3.89–3.55 (m, 4 H, H-6b, H-5, H-5′, H-4′), 2.10, 2.09, 2.05, 2.03, 2.01, 2.00 (6 s, 18 H, 6 OAc); DCIMS: Calcd for $C_{31}H_{38}O_{17}$: $(M + NH_4^+)$, 700.66. Found: $(M + NH_4^+)$, 701.

1,2,3,6,2′,3′-Hexa-O-*acetyl-6′*-O-*benzylmaltose* (**3**).—To a mixture of **2** (18.70 g, 27.39 mmol) in dry THF (200 mL), $Na(CN)BH_3$ (15.00 g, 238.7 mmol) was added. The mixture was stirred at room temperature, and a saturated solution of HCl gas in dry ether was added in small portions until gas development ceased and the mixture remained acidic. The reaction was complete after another 30 min of stirring (TLC, 1:1 EtOAc–hexanes). The mixture was concentrated by evaporation to ~ 20 mL, CH_2Cl_2 (200 mL) was added, and the mixture was neutralized with satd aq $NaHCO_3$. The aqueous phase was extracted three times with CH_2Cl_2, and the combined organic phases were washed with water, dried ($MgSO_4$), filtered, and evaporated. Column chromatography (1:1 EtOAc–hexanes) yielded **3** (18.20 g, 26.58 mmol, 97%) as a white foam; ^{1}H NMR ($CDCl_3$, 500 MHz), the β anomer only: δ 7.38–7.28 (m, 5 H, Ar), 5.71 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.34 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.25 (dd, 1 H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.1 Hz, H-3), 5.18 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 9.2 Hz, H-3′), 4.94 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 9.0 Hz, H-2), 4.80 (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.4 Hz, H-2′), 4.57, 4.55 (2 d, 2 H, PhCH_2), 4.44 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.16 (dd, 1 H, $J_{5,6b}$ 4.3, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.01 (t, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.2 Hz, H-4), 3.79 (m, 1 H, H-4′), 3.72 (m, 3 H, H-6′a, H-5, H-5′), 3.60 (dd, 1 H, $J_{5,6'b}$ 3.3 $J_{6'a,6'b}$ 10.3 Hz, H-6′b), 2.73 (s, 1 H, 4′-OH), 2.08, 2.06, 2.03, 2.01, 1.99, 1.98 (6 s, 18 H, 6 OAc); DCIMS: Calcd for $C_{31}H_{40}O_{17}$: $(M + NH_4^+)$, 702.68; found: $(M + NH_4^+)$, 703.

1,2,3,6-Tetra-O-*acetyl-4*-O-*(2,3-di*-O-*acetyl-6*-O-*benzyl-4-deoxy-4-iodo-α*-D-*galactosyl)*-D-*glucopyranose* (**4**).—A solution of **3** (3.00 g, 4.38 mmol) in dry CH_2Cl_2 (30 mL) and dry pyridine (10 mL) was cooled to −20°C under N_2, and triflic anhydride (2.00 mL, 11.9 mmol) was slowly added through a septum. The mixture was allowed to warm to room temperature and was stirred until the reaction was complete (TLC, 1:1 EtOAc–hexanes). Occasionally, in addition to the main product, a slightly slower moving byproduct, which was not further characterized, was detected on TLC in minor amounts. When the reaction was complete, the volume of the mixture was doubled with

CH_2Cl_2, and the solution was poured into satd aq $NaHCO_3$. The aqueous phase was extracted twice with CH_2Cl_2, and the combined organic phases were washed with water. The organic phase was coevaporated with toluene or acetonitrile at low temperature. The resulting syrup was dissolved in DMF (50 mL), treated with NaI (3.00 g, 20.0 mmol), and stirred at room temperature overnight. After addition of CH_2Cl_2 (50 mL), the solution was poured into water, the aqueous phase was extracted twice with CH_2Cl_2, and the combined organic phases were washed twice with water. The organic phase was concentrated by evaporation and purified by flash chromatography (4:6 EtOAc–hexanes). The reaction yielded **4** (2.74 g, 3.45 mmol, 79%) as a colourless foam; ^{1}H NMR ($CDCl_3$, 500 MHz), the β anomer only: δ 7.38–7.26 (m, 5 H, Ar), 5.70 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.33 (d, 1 H, $J_{1',2'}$ 4.2 Hz, H-1′), 5.23 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 8.8 Hz, H-3), 5.18 (dd, 1 H, $J_{1,2}$ 4.2, $J_{2,3}$ 10.7 Hz, H-2′), 4.94 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.2 Hz, H-2), 4.74 (d, 1 H, $J_{3',4'}$ 4.1, $J_{4',5'} < 1$ Hz, H-4′), 4.51 (m, 3 H, PhCH_2, H-6a), 4.42 (dd, 1 H, $J_{2',3'}$ 10.7, $J_{3',4'}$ 4.1 Hz, H-3′), 4.14 (dd, 1 H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.97 (dd, 1 H, $J_{3,4}$ 8.8, $J_{4,5}$ 9.6 Hz, H-4), 3.78 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6a}$ 2.6, $J_{5,6b}$ 5.1 Hz, H-5), 3.53 (m, 1 H, $J_{4',5'} < 1$, $J_{5',6'a}$ 3.5, $J_{5',6'b}$ 6.0 Hz, H-5′), 3.41 (m, 2 H, H-6′a, H-6′b), 2.08, 2.07, 2.04, 2.03, 2.00, 1.98 (6 s, 18 H, 6 OAc); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 170.62, 170.30, 170.14, 169.67, 169.61, 168.80 (6 s, 6 CH_3CO), 137.46, 128.44, 127.85 (Ar C), 96.24 (C-1), 91.18 (C-1′), 75.33, 73.69, 73.22, 72.96, 72.41, 70.98, 70.03, 67.95, 67.62 (C-2, C-3, C-4, C-5, C-6, C-2′, C-3′, C-5′, C-6′), 62.73 (PhCH_2), 36.70 (C-4′), 20.91, 20.82, 20.75, 20.60, 20.55 (6 CH_3CO); DCIMS: Calcd for $C_{31}H_{39}IO_{16}$: ($M^+ + NH_3$), 811.57; found: ($M^+ + NH_3$), 812.

*1,2,3,6,2′,3′-Hexa-*O*-acetyl-6′-*O*-benzyl-4′-deoxymaltose* (**5**).—A solution of **4** (650 mg, 0.818 mmol) in benzene (10 mL) was added to a hot mixture of tributyltin hydride (1.00 mL, 3.72 mmol) and a catalytic amount of AIBN (2,2′-azobisisobutyronitrile) in benzene (40 mL), and the mixture was heated under reflux. TLC analysis (1:1 EtOAc–hexanes) showed the formation of a new spot which was slightly slower moving than the starting material. The reaction was stopped as soon as all starting material had disappeared (0.5–4 h), concentrated by evaporation, dissolved in acetonitrile, and it was then washed with hexanes three times. The acetonitrile phase was concentrated and purified by flash column chromatography (elution first with hexanes, then with 1:1 EtOAc–hexanes), yielding **5** (500 mg, 0.75 mmol, 91%) as a colourless syrup; ^{1}H NMR ($CDCl_3$, 500 MHz), the β anomer only: δ 7.37–7.25 (m, 5 H, Ar), 5.70 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.38 (d, 1 H, $J_{1',2'}$ 4.1 Hz, H-1′), 5.25 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 9.1 Hz, H-3), 5.15 (ddd, 1 H, $J_{2',3'}$ 10.6, $J_{3',4'e}$ 5.0, $J_{3',4'a}$ 11.3 Hz, H-3′), 4.94 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.2 Hz, H-2), 4.79 (dd, 1 H, $J_{1',2'}$ 4.1, $J_{2',3'}$ 10.6 Hz, H-2′), 4.54, 4.52 (2 d, 2 H, PhCH_2), 4.43 (dd, 1 H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.17 (dd, 1 H, $J_{5,6b}$ 4.7, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.02 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.5 Hz, H-4), 3.95 (m, 1 H, H-5′), 3.77 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 2.3, $J_{5,6b}$ 4.7 Hz, H-5), 3.42 (m, 2 H, H-6′a, H-6′b), 2.11 (ddd, 1 H, $J_{3',4'e}$ 5.0, $J_{4'e,5}$ 2.2, $J_{4'e,4'a}$ 12.8 Hz, H-4′e), 2.07, 2.03, 2.01, 1.99, (4 s, 12 H, 4 OAc), 1.98 (s, 6 H, 2 OAc), 1.68 (q, 1 H, H-4a′) ppm; DCIMS: Calcd for $C_{31}H_{40}O_{16}$: ($M + NH_4^+$), 686.68; found: ($M + NH_4^+$), 687.

*1,2,3,6,2′,3′-Hexa-*O*-acetyl-4′-deoxymaltose* (**6**).—To a solution of **5** (400 mg, 0.598 mmol) in EtOH (30 mL) a catalytic amount of Pd–C (10%) catalyst was added, and the mixture was hydrogenated until the reaction was complete (TLC, 1:1 EtOAc–hexanes).

The mixture was filtered, evaporated, and purified over a short column (2:1 EtOAc–hexanes) to yield **6** (340 mg, 0.587 mmol, 98%) as a colourless syrup; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.22 (d, $J_{1,2}$ 3.6 Hz, H-1α), 5.72 (d, $J_{1,2}$ 8.2 Hz, H-1β), 5.49 (dd, $J_{2,3}$ 10.2, $J_{3,4}$ 8.4 Hz, H-3α), 5.39 (d, $J_{1',2'}$ 4.0 Hz, H-1'α), 5.36 (d, $J_{1',2'}$ 4.0 Hz, H-1'β), 5.26 (dd, $J_{2,3}$ 9.4, $J_{3,4}$ 8.8 Hz, H-3β), 5.19 (m, H-3'α), 5.16 (ddd, H-3'β), 4.95 (dd, $J_{1,2}$ 8.2, $J_{2,3}$ 9.4 Hz, H-2β), 4.94 (dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.2 Hz, H-2α,), 4.78 (dd, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.6 Hz, H-2'α), 4.77 (dd, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.4 Hz, H-2'β), 4.44 (dd, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.3 Hz, H-6bβ), 4.42 (dd, H-6bα), 4.20 (dd, H-6aα), 4.19 (dd, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 12.3 Hz, H-6aβ), 4.04 (m, H-5α), 4.01 (dd, $J_{3,4}$ 8.4, $J_{4,5}$ 10.0 Hz, H-4α), 4.00 (dd, $J_{3,4}$ 8.8 $J_{4,5}$ 9.8 Hz, H-4β), 3.87 (m, H-5'α), 3.86 (m, H-5'β), 3.79 (ddd, $J_{4,5}$ 9.8, $J_{5,6a}$ 4.5, $J_{5,6b}$ 2.2 Hz, H-5β), 3.60 (dd, $J_{5',6'b}$ 3.6, $J_{6'a,6'b}$ 12.0 Hz, H-6'bβ), 3.59 (dd, H-6'bα), 3.52 (dd, H-6'aα), 3.51 (dd, $J_{5',6'a}$ 5.8, $J_{6'a,6'b}$ 12.0 Hz, H-6'aβ), 2.11, 2.08, 2.04, 2.02, 2.01, 2.00 (6 s, 18 H, 6 OAcβ), 1.57 (ddd, H-4'aα), 1.56 (ddd, H-4'aβ). The compound was further characterized after acetylation.

1,2,3,6,2',3',6'-Hepta-O-acetyl-4'-deoxymaltose (**7**).—A solution of **6** (870 mg, 1.50 mmol) in pyridine (50 mL) was acetylated with Ac_2O (5 mL) at room temperature overnight. CH_2Cl_2 (50 mL) was added, the mixture was poured into water and extracted twice with CH_2Cl_2, and then the combined organic phases were washed with water. Coevaporation with acetonitrile yielded **7** (920 mg, 1.48 mmol, 99%) as a white foam; ^{1}H NMR ($CDCl_3$, 500 MHz), the β anomer only: δ 5.72 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.35 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1'), 5.25 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 9.1 Hz, H-3), 5.15 (ddd, 1 H, H-3'), 4.95 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 8.9 Hz, H-2), 4.79 (dd, 1 H, $J_{1',2'}$ 4.1, $J_{2',3'}$ 10.5 Hz, H-2'), 4.42 (dd, 1 H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.18 (dd, 1 H, $J_{5,6b}$ 5.0, $J_{6a,6b}$ 12.2 Hz, H-6b), 4.11–3.95 (m, 4 H, H-4, H-5', H-6'a, H-6'b), 3.89 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6a}$ 2.2, $J_{5,6b}$ 5.0 Hz, H-5), 2.13 (ddd, 1 H, $J_{3',4'e}$ 4.6, $J_{4'e,5'}$ 1.8, $J_{4'e,4'a}$ 12.6 Hz, H-4'e), 2.09, 2.04, 2.00, 1.99, 1.98 (5 s, 15 H, 5 OAc), 2.07 (s, 6 H, 2 OAc), 1.59 (q, 1 H, H-4'a); DCIMS: Calcd for $C_{26}H_{36}O_{17}$: ($M^+ + NH_3$), 637.59; found: ($M^+ + NH_3$), 638.

4'-Deoxymaltose.—A solution of the peracetate **7** (50 mg, 0.081 mmol) in dry MeOH (10 mL) was treated with a catalytic amount of solid NaOMe at room temperature. The mixture was stirred until the deprotection reaction was complete (TLC in 7:2:1 EtOAc–MeOH–water), then neutralized with an acidic ion-exchange resin (Bio-Rad, AG 50W-X 12, 50–100 mesh, H^+ form), filtered, and the solvent removed in vacuo to yield 25.0 mg (0.077 mmol, 95%) of a colourless syrup. ^{1}H NMR (D_2O, 400 MHz): δ 5.37 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1'), 5.19 (d, $J_{1,2}$ 3.9 Hz, H-1α), 4.61 (d, $J_{1,2}$ 8.0 Hz, H-1β), 3.47 (dd, $J_{1,2}$ 3.9, $J_{2,3}$ 9.8 Hz, H-2α, H-2'), 3.24 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2β), 1.96 (dd, 1 H, H-4'e), 1.41 (q, 1 H, H-4'a); DCIMS: Calcd for $C_{12}H_{22}O_{10}$: ($M + NH_4^+$), 344.63; found: ($M + NH_4^+$), 345.

2,3,6,2',3',6'-Hexa-O-acetyl-4'-deoxymaltose (**8**).—A solution of the peracetate **7** (310 mg, 0.500 mmol) in DMF (4 mL) was reacted with hydrazine acetate (100 mg, 1.08 mmol) at 50°C. The reaction was carefully monitored by TLC (1:1 EtOAc–hexanes). After 30 min all starting material had disappeared. EtOAc (10 mL) and water (10 mL) were added, and the aqueous phase was extracted twice with EtOAc. The combined EtOAc phases were washed with water, evaporated, and purified by flash chromatography (1:1 EtOAc–hexanes, later 2:1) to yield **8** (200 mg, 0.345 mmol, 69%) as a

colourless syrup. Analysis of the anomeric protons: the ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) indicated selective deprotection of the anomeric center. Detailed assignment was not possible. The reducing sugar **8** was further characterized after the next step.

2,3,6,2′,3′,6′-Hexa-O-*acetyl-4′-deoxy-α- and β-maltosyl fluoride* (**9**).—To a solution of **8** (180 mg, 0.311 mmol) in dry CH_2Cl_2 (10 mL) DAST (0.05 mL, 0.378 mmol) was added at 0°C. The mixture was stirred at room temperature for 30 min until the reaction was complete (TLC, 1:1 EtOAc–hexanes). The mixture was quenched with water, extracted twice with CH_2Cl_2, and the combined organic phases were washed once with water. After evaporation of the solvent flash chromatography (1:1 EtOAc–hexanes) yielded **9** (175 mg, 0.301 mmol, 97%) as a colourless syrup which mainly comprised the β anomer (β:α ~ 9:1 by ^{1}H NMR); ^{1}H NMR ($CDCl_3$, 500 MHz): β anomer only: δ 5.38 (dd, 1 H, $J_{1,F}$ 52.6, $J_{1,2}$ 5.3 Hz, H-1), 5.36 (d, 1 H, $J_{1',2'}$ 4.1 Hz, H-1′), 5.18 (ddd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'e}$ 4.7, $J_{3',4'a}$ 11.1 Hz, H-3′), 5.12 (dd, 1 H, $J_{2,3}$ 6.7, $J_{3,4}$ 7.5 Hz, H-3), 4.92 (ddd, 1 H, $J_{1,2}$ 5.3, $J_{2,3}$ 6.7, $J_{2,F}$ 9.2 Hz, H-2), 4.78 (dd, 1 H, $J_{1',2'}$ 4.1, $J_{2',3'}$ 10.4 Hz, H-2′), 4.52 (dd, 1 H, $J_{5,6a}$ 2.8, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.18 (dd, 1 H, $J_{5,6b}$ 5.2, $J_{6a,6b}$ 12.2 Hz, H-6b), 4.08 (m, 4 H, H-4, H-5′, H-6′a, H-6′b), 3.92 (m, 1 H, H-5), 2.16 (ddd, 1 H, $J_{3',4'e}$ 4.7, $J_{4'e,4'a}$ 12.8, $J_{4'e,5'}$ 2.0 Hz, H-4′e), 2.11, 2.08, 2.07, 2.03, 2.02, 2.00 (6 s, 18 H, 6 OAc), 1.59 (q, 1 H, H-4′a); ^{19}F NMR ($CDCl_3$, 188 MHz): δ −149.21 (α), −133.27 (β); the compound was further characterized after anomerization.

2,3,6,2′,3′,6′-Hexa-O-*acetyl-4′-deoxy-α-maltosyl fluoride* (**10**).—(*a*) The β fluoride **9** (130 mg, 0.224 mmol) was treated with HF · pyridine (0.50 mL) in a plastic vial for 2 h at −50°C. Then the reaction was quenched by adding CH_2Cl_2 and neutralizing with satd aq $NaHCO_3$. The aqueous phase was extracted twice with CH_2Cl_2, and the combined organic phases were washed with water. The solvent was removed in vacuo and the success of the anomerization was determined by ^{19}F NMR spectroscopy. The ^{19}F NMR spectrum displayed three singlets (δ −148.64, −149.24, −149.31 ppm), with no peak for the β anomer. The mixture was purified by column chromatography (1:1 EtOAc–hexanes) which led to the α-maltosyl fluoride **10** and 2,3,6-tri-*O*-acetyl-α-D-glucosyl fluoride (−149.31 ppm). For further purification this mixture was acetylated with Ac_2O in pyridine, subjected to aqueous extraction and passed over a column (1:1 EtOAc–hexanes) to yield pure **10** (83.0 mg, 0.143 mmol, 64%) as a white amorphous solid.

(*b*) Compound **10** could also be obtained from **7** without intermediate purification steps. A solution of the peracetate **7** (850 mg, 1.37 mmol) in DMF (50 mL) was treated with hydrazine acetate (300 mg, 3.26 mmol) for 30 min when TLC showed only one product. After workup, as above, the resulting syrup was dissolved in dry CH_2Cl_2 and fluorinated with DAST (0.40 mL, 3.02 mmol). Following the usual workup, the product was treated with HF · pyridine (1.00 mL) at −50°C for 1 h, then purified as described in (*a*) to give **10** (370 mg, 0.637 mmol, 47% overall) as colourless crystals after recrystallisation; mp (ether–petroleum ether) 153°C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.60 (dd, 1 H, $J_{1,2}$ 2.7, $J_{1,F}$ 53.3 Hz, H-1), 5.48 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 9.2 Hz, H-3), 5.36 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.13 (ddd, 1 H, $J_{2',3'}$ 9.0, $J_{3',4'e}$ 4.9, $J_{3',4'a}$ 11.2 Hz, H-3′), 4.79 (ddd, 1 H, $J_{1,2}$ 2.8, $J_{2,F}$ 24.2, $J_{2,3}$ 10.1 Hz, H-2), 4.76 (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.4 Hz, H-2′), 4.47 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.18 (dd, 1 H, $J_{5,6b}$

4.3, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.10 (ddd, 1 H, $J_{4,5}$ 10.0, $J_{5,6a}$ 2.2, $J_{5,6b}$ 4.1 Hz, H-5), 4.1–3.95 (m, 4 H, H-4, H-5′, H-6′a, H-6′b), 2.12 (ddd, 1 H, $J_{3',4'e}$ 4.9, $J_{4'e,4'a}$ 12.7, $J_{4'e,5'}$ 1.9 Hz, H-4′e), 2.08, 2.05, 2.03, 2.01 (4 s, 12 H, 4 OAc), 1.97 (s, 6 H, 2 OAc), 1.69 (q, 1 H, H-4′a); ^{19}F NMR ($CDCl_3$, 188 MHz): δ −149.22; ^{19}F NMR ($CDCl_3$, 188 MHz, coupled): −149.24 (dd, $J_{1,F}$ 53.0, $J_{2,F}$ 23.9 Hz); DCIMS: Calcd for $C_{24}H_{33}FO_{15}$: $(M + NH_4^+)$, 598.54; found: $(M + NH_4^+)$, 598. Anal. Calcd for $C_{24}H_{33}FO_{15}$: C, 49.65; H, 5.73. Found: C, 49.81; H, 5.54.

4′-Deoxy-α-maltosyl fluoride (**11**).—The protected α fluoride **10** (83 mg, 0.143 mmol) was dissolved in dry MeOH (10 mL) and stirred with a catalytic amount of solid NaOMe at room temperature. TLC analysis (7:2:1 EtOAc–MeOH–water) showed three spots after 25 min, of which only the most polar remained when the reaction was complete. The mixture was neutralized with acidic ion-exchange resin (Bio-Rad, AG 50W-X 12, 50–100 mesh, H^+ form), filtered, and evaporated to yield **11** (46 mg, 0.140 mmol, 98%) as a colourless glass; ^{1}H NMR (D_2O, 500 MHz), selected data: δ 5.64 (dd, 1 H, $J_{1,F}$ 53.6, $J_{1,2}$ 2.8 Hz, H-1), 5.37 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 3.68 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 9.5 Hz, H-3), 3.62 (dd, 1 H, H-6), 3.60 (ddd, 1 H, $J_{1,2}$ 2.8, $J_{2,3}$ 9.8, $J_{2,F}$ 26.4 Hz, H-2), 3.54 (dd, 1 H, H-6), 3.46 (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 9.7 Hz, H-2′), 1.93 (ddd, 1 H, $J_{3',4'e}$ 4.8, $J_{4'e,4'a}$ 12.6, $J_{4'e,5'}$ 1.9 Hz, H-4′e), 1.39 (q, 1 H, H-4′a); ^{19}F NMR (D_2O, 188 MHz): δ −149.82. Anal. Calcd. for $C_{12}H_{21}FO_9 \cdot 2H_2O$: C, 39.56; H, 6.86. Found: C, 39.81; H, 6.75.

4′-O-(4,6-O-Benzylidene-α-D-glucopyranosyl)maltose (**12**).—Maltotriose (5.00 g, 9.91 mmol) was dissolved in DMF (100 mL), then half of the volume was distilled off in order to remove traces of water. *p*-Toluenesulfonic acid (~ 20 mg) and benzaldehyde dimethylacetal (1.6 mL, 10.65 mmol) were added, and the mixture was kept rotating for 8 h at 60–70°C under a slight vacuum, to remove the MeOH formed (TLC, 7:2:1 EtOAc–MeOH–water). The mixture was then neutralized with a basic ion-exchange resin (Dowex-1, OH^- form), filtered, evaporated, and purified by column chromatography (7:2:1 EtOAc–MeOH–water) to yield pure **12** (1.9 g, 3.2 mmol, 32%) as a colourless syrup; the compound was characterized after acetylation.

1,2,3,6,2′,3′,6′-Hepta-O-acetyl-4′-O-(2,3-di-O-acetyl-4,6-O-benzylidene-α-D-glucopyranosyl)maltose (**13**).—The hemiacetal **12** (1.90 g, 3.21 mmol) was dissolved in pyridine (50 mL) and acetylated with Ac_2O (20 mL) overnight at 50°C. CH_2Cl_2 (70 ml) was added, the mixture poured into satd aq $NaHCO_3$, then the aqueous phase was extracted three times with CH_2Cl_2, and the combined organic phases were washed with water. Coevaporation with toluene yielded **13** (3.10 g, 3.19 mmol, 99.8%) as an almost colourless syrup which was not further purified; ^{1}H NMR ($CDCl_3$, 200 MHz), selected data α and β anomer only: δ 7.38–7.28 (m, 5 H, Ar), 6.18 (d, $J_{1,2}$ 4.0 Hz, H-1α), 5.70 (d, $J_{1,2}$ 8.0 Hz, H-1β), 5.50–5.10 (m, 6 H, H-1′, H-1″, H-3, H-3′, H-3″, PhC*H*); DCIMS: Calcd for $C_{43}H_{54}O_{25}$: $(M + NH_4^+)$, 984.19; found: $(M + NH_4^+)$, 984.

1,2,3,6,2′,3′,6′-Hepta-O-acetyl-4-O-(2,3-di-O-acetyl-6-O-benzyl-α-D-glucopyranosyl)maltose (**14**).—Compound **13** (2.90 g, 2.98 mmol) was dissolved in dry THF (50 mL) and $Na(CN)BH_3$ (2.00 g, 31.8 mmol) was added. Under a constant flow of N_2, a saturated solution of HCl in dry ether was added until the gas development ceased and the pH of the solution remained acidic. After 30 min of stirring the reaction was complete (TLC, 2:1 EtOAc–hexanes). After concentration of the mixture to ~ 10

mL, CH_2Cl_2 (100 mL) was added, and the was mixture washed with satd aq $NaHCO_3$. The aqueous phase was extracted three times with CH_2Cl_2 and the combined organic phases were washed with water, dried over $MgSO_4$, evaporated, and purified by column chromatography (2:1 EtOAc–hexanes) yielding **14** as a colourless syrup (2.40 g, 2.46 mmol, 83%); ^{1}H NMR ($CDCl_3$, 200 MHz), β anomer only: δ 7.35 (m, 5 H, Ar), 5.72 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.40–5.10 (m, 5 H, H-1′, H-1″, H-3, H-3′, H-3″), 4.95 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.0 Hz, H-2), 4.77 (dd, 1 H, $J_{1'',2''}$ 4.0, $J_{2'',3''}$ 10.0 Hz, H-2″), 4.70 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.0 Hz, H-2′), 4.56 (s, 2 H, PhCH_2), 2.70 (s, 1 H, 4″-OH), 2.12–1.90 (9 s, 27 H, 9 OAc); DCIMS: Calcd for $C_{43}H_{56}O_{25}$: ($M^+ + NH_3$), 989.93; found: ($M^+ + NH_3$), 990.

1,2,3,6,2′,3′,6′-Hepta-O-*acetyl-4′*-O-*(2,3-di*-O-*acetyl-6*-O-*benzyl-4-deoxy-4-iodo-α*-D-*galactopyranosyl)maltose* (**15**).—Compound **14** (2.40 g, 2.46 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and pyridine (10 mL) and cooled to 0°C under a constant flow of N_2. Triflic anhydride (2.0 mL, 11.89 mmol) was slowly added, and stirring was continued at room temperature until the reaction was complete (20 min) according to TLC analysis (2:1 EtOAc–hexanes). Water was added, and the aqueous phase was extracted two times with CH_2Cl_2. The combined organic phases were then washed with water and coevaporated with acetonitrile. The resulting syrup was dissolved in DMF (50 mL) and stirred with NaI (2.50 g, 16.69 mmol) overnight. The 4″-iodo compound could not be distinguished from the 4″-triflate on the TLC. After addition of CH_2Cl_2 (50 mL) the mixture was poured into water, and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$), evaporated, and purified by flash column chromatography (1:1 EtOAc–hexanes) to yield **15** (1.90 g, 1.75 mmol, 71%) as a white foam; ^{1}H NMR ($CDCl_3$, 500 MHz), β anomer only: δ 7.37–7.25 (m, 5 H, Ar), 5.69 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 5.32 (d, 1 H, $J_{1'',2''}$ 4.3 Hz, H-1″), 5.32 (m, 1 H, $J_{2',3'}$ 10.3 Hz, H-3′), 5.26 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 9.1 Hz, H-3), 5.21 (d, 1 H, $J_{1',2'}$ 3.9 Hz, H-1′), 5.16 (dd, 1 H, $J_{1'',2''}$ 4.3, $J_{2'',3''}$ 10.7 Hz, H-2″), 4.93 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 8.9 Hz, H-2), 4.75 (bd, 1 H, $J_{3'',4''}$ 3.3, $J_{4'',5''} < 1$ Hz, H-4″), 4.67 (dd, 1 H, $J_{1',2'}$ 3.9, $J_{2',3'}$ 10.3 Hz, H-2′), 4.49 (m, 3 H, PhCH_2, H-6a′), 4.41 (m, 2 H, H-3″, H-6a), 4.25 (dd, 1 H, $J_{5,6b}$ 4.3, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.09 (dd, 1 H, $J_{5',6'b}$ 2.1, $J_{6'a,6'b}$ 12.2 Hz, H-6′b), 3.96 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.2 Hz, H-4), 3.87 (m, 2 H, H-4′, H-5′), 3.82 (m, 1 H, H-5), 3.52 (m, 1 H, H-5″), 3.37 (m, 2 H, H-6″a, H-6″b), 2.12, 2.06, 2.05, 2.04, 2.00, 1.98 (6 s, 18 H, 6 OAc), 1.97 (s, 9 H, 3 OAc) ppm; DCIMS: Calcd for $C_{43}H_{55}IO_{24}$: ($M + NH_4^+$), 1100.83; found: ($M + NH_4^+$), 1101.

1,2,3,6,2′,3′,6′-Hepta-O-*acetyl-4*-O-*(2,3-di*-O-*acetyl-6*-O-*benzyl-4-deoxy-α*-D-xylo-*hexopyranosyl)maltose* (**16**).—A solution of **17** (1.90 g, 1.75 mmol) in benzene (10 mL) was added to a hot solution of tributyl tinhydride (2.60 mL, 9.66 mmol) and a catalytic amount of AIBN in benzene (20 mL). The mixture was refluxed for 45 min when a small amount of starting material could still be detected by TLC (2:1 EtOAc–hexanes) and a slightly slower moving byproduct had started to form in addition to the desired product. Heating was stopped after 50 min, then the mixture was evaporated, dissolved in acetonitrile, and washed several times with hexanes. After evaporation of the solvent, the product was purified over a silica gel column which had been packed with hexanes and was eluted with 1:1 EtOAc–hexanes, yielding **16** as a colourless syrup (1.58 g, 1.65 mmol, 94%); ^{1}H NMR ($CDCl_3$, 500 MHz), β anomer only: δ 5.72 (d, 1 H, $J_{1,2}$ 8.1 Hz,

H-1), 5.38 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1''), 5.36 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 9.3 Hz, H-3'), 5.27 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 8.8 Hz, H-3), 5.24 (d, 1 H, $J_{1',2'}$ 4.1 Hz, H-1'), 5.16 (ddd, 1 H, $J_{3'',4''e}$ 4.9 Hz, H-3''), 4.94 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 8.9 Hz, H-2), 4.79 (dd, 1 H, $J_{1'',2''}$ 3.8, $J_{2'',3''}$ 10.4 Hz, H-2''), 4.71 (dd, 1 H, $J_{1',2'}$ 4.1, $J_{2',3'}$ 10.3 Hz, H-2'), 4.54 (d, 1 H, PhC*H*), 4.52 (d, 1 H, PhC*H*), 4.45–4.11 (m, 4 H, H-6a, H-6b, H-6'a, H-6'b), 4.00–3.81 (m, 5 H, H-4, H-4', H-5, H-5', H-5''), 3.44 (m, H-6''a, H-6''b), 2.12 (ddd, 1 H, H-4''e), 2.13, 2.08, 2.04, 2.03, 2.00, 1.98, 1.97 (7 s, 21 H, 7 OAc), 1.99 (s, 6 H, 2 OAc), 1.70 (ddd, 1 H, H-4''a); DCIMS: Calcd for $C_{43}H_{56}O_{24}$: $(M + NH_4^+)$, 974.93; found: 975.

1,2,3,6,2',3',6'-Hepta-O-*acetyl-4'*-O-*(2,3-di*-O-*acetyl-4-deoxy-α*-D-xylo-*hexopyranosyl)maltose* (**17**).—To a solution of **16** (1.58 g, 1.65 mmol) in EtOAc (10 mL) and EtOH (50 mL) Pd catalyst (10% on charcoal, 100 mg) was added, and the mixture was hydrogenated. After the reaction was complete (TLC, 2:1 EtOAc–hexanes), the mixture was filtered through a Celite bed, concentrated by evaporation, and purified by column chromatography (2:1 EtOAc–hexanes) to yield **17** (1.35 g, 1.56 mmol, 94%) as a colourless glass; the compound was characterized after acetylation; DCIMS: Calcd for $C_{36}H_{50}O_{24}$: $(M + NH_4^+)$, 884.81; found: $(M + NH_4^+)$, 885.

1,2,3,6,2',3',6'-Hepta-O-*acetyl-4'*-O-*(2,3,6-tri*-O-*acetyl-4-deoxy-α*-D-xylo-*hexopyranosyl)maltose* (**18**).—(*a*) A solution of **17** (1.30 g, 1.50 mmol) in pyridine (10 mL) was stirred with Ac_2O (0.5 mL) overnight at room temperature, CH_2Cl_2 was added, and the mixture was poured into water and extracted with CH_2Cl_2. The combined organic phases were washed with water and coevaporated with toluene. Evaporation from ether–hexanes gave **18** (1.35 g, 1.48 mmol, 99%) as a white foam.

(*b*) Compound **18** could also be obtained directly from **15** by catalytic hydrogenation and subsequent acetylation. The iodide **19** (240 mg, 0.22 mmol) was dissolved in EtOH (20 mL), one drop of Et_3N was added, and the mixture was hydrogenated with Pd (10% on charcoal, 100 mg) for 3 days. TLC analysis (2:1 EtOAc–hexanes) showed that the 4''-iodo substituent was reduced first. After the reaction was complete, the mixture was filtered, evaporated, and acetylated using pyridine (10 mL) with Ac_2O (1 mL) overnight. After addition of CH_2Cl_2 (10 mL), the mixture was poured into satd aq $NaHCO_3$, the aqueous phase extracted twice with CH_2Cl_2, and the combined organic phases were washed with water and coevaporated with toluene. The resulting syrup was purified by column chromatography (1:1 EtOAc–hexanes) to yield **18** (180 mg, 0.198 mmol, 90%) as a colourless syrup; ^{1}H NMR ($CDCl_3$, 500 MHz), β anomer only: δ 5.37 (d, 1 H, $J_{1'',2''}$ 3.9, H-1''), 5.36 (dd, 1 H, H-3'), 5.35 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.27 (dd, 1 H, $J_{2,3}$ 8.9, $J_{3,4}$ 8.8 Hz, H-3), 5.23 (d, 1 H, $J_{1',2'}$ 4.1 Hz, H-1'), 5.15 (ddd, 1 H, $J_{3'',4''e}$ 4.9 Hz, H-3''), 4.93 (dd, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 4.78 (dd, 1 H, $J_{1'',2''}$ 3.9, $J_{2'',3''}$ 10.4 Hz, H-2''), 4.71 (dd, 1 H, $J_{1',2'}$ 4.1, $J_{2',3'}$ 10.3 Hz, H-2'), 2.20–1.83 (m, 31 H, 10 OAc, H-4''e), 1.58 (ddd, 1 H, H-4''a); DCIMS: Calcd for $C_{38}H_{52}O_{25}$: $(M + NH_4^+)$, 926.84; found: $(M + NH_4^+)$, 927.

4'-O-*(4-Deoxy-α*-D-xylo-*hexopyranosyl)maltose*.—The peracetate **18** (40 mg, 44.01 mmol) was dissolved in dry MeOH (10 mL) and stirred with a catalytic amount of NaOMe overnight. After neutralization with ion-exchange resin (Bio-Rad, AG 50W-X 12, 50–100 mesh, H^+ form), the mixture was filtered and evaporated to yield a colourless syrup (20 mg, 40.95 mmol, 93%); ^{1}H NMR (D_2O, 400 MHz): δ 5.36 (2 d, 2

H, H-1′, H-1″), 5.19 (d, $J_{1,2}$ 3.7 Hz, H-1α,), 4.62 (d, $J_{1,2}$ 7.9 Hz, H-1β), 3.47 (dd, $J_{1,2}$ 3.9, $J_{2,3}$ 9.8 Hz, H-2α), 3.24 (dd, $J_{1,2}$ 7.9, $J_{2,3}$ 9.4 Hz, H-2β), 1.96 (ddd, 1 H, H-4″e), 1.42 (ddd, 1 H, H-4″a).

2,3,6,2′,3′,6′-Hexa-O-*acetyl-4′*-O-*(2,3,6-tri*-O-*acetyl-4-deoxy*-α-D-xylo-*hexopyranosyl)maltose* (**19**).—The peracetate **18** (1.25 g, 1.38 mmol) was dissolved in DMF (10 mL) and reacted with hydrazine acetate (250 mg, 2.71 mmol) at 50°C for 25 min. EtOAc (20 mL) was then added and the solution was washed with water. The aqueous phase was extracted twice with EtOAc, and the combined organic phases were washed with water, and then concentrated by evaporation and purified by flash chromatography (2:1 EtOAc–hexanes) to yield the hemiacetal **19** (1.12 g, 1.29 mmol, 94%).

2,3,6,2′,3′,6′-Hexa-O-*acetyl-4′*-O-*(2,3,6-tri*-O-*acetyl-4-deoxy*-α-D-xylo-*hexopyranosyl)*-α- *and* β-*maltosyl fluoride* (**20**).—The hemiacetal **19** (130 mg, 0.143 mmol) was dissolved in dry CH_2Cl_2 (5 mL), and DAST (0.05 mL, 0.378 mmol) was added at 0°C. After stirring for 30 min at room temperature, the reaction was complete (TLC, 2:1 EtOAc–hexanes). The mixture was quenched with MeOH, concentrated by evaporation, and purified by column chromatography (2:3 EtOAc–hexanes). The maltotriosyl fluoride **20** was obtained as an amorphous white solid (anomeric mixture with $\beta:\alpha \sim 8:1$ by ^{1}H NMR, 120 mg, 0.139 mmol, 97%); ^{1}H NMR ($CDCl_3$, 400 MHz), selected data: δ 5.63 (dd, $J_{1,F}$ 52.8, $J_{1,2}$ 2.8 Hz, H-1α), 5.57 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 9.2 Hz, H-3α), 5.16 (dd, $J_{1,F}$ 51.6, $J_{1,2}$ 4.9 Hz, H-1β), 5.25 (d, $J_{1',2'}$ 4.0 Hz, H-1′), 5.15 (ddd, H-3″), 4.92 (ddd, $J_{1,2}$ 4.9, $J_{2,3}$ 8.4, $J_{2,F}$ 6.2 Hz, H-2β), 4.79 (dd, $J_{1'',2''}$ 4.0, $J_{2'',3''}$ 10.6 Hz, H-2″β), 4.70 (dd, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.2 Hz, H-2′β), 2.14, 2.11, 2.08, 2.07, 2.03, 2.01, 1.99, 1.98, 1.97 (9 s, 9 β-OAc and H-4″eβ), 1.60 (q, H-4″aβ); ^{19}F NMR ($CDCl_3$, 188 MHz): δ −131.99 (β), −149.20 (α); the compound was further characterized after anomerization.

2,3,6,2′,3′,6′-Hexa-O-*acetyl-4′*-O-*(2,3,6-tri*-O-*acetyl-4-deoxy*-α-D-xylo-*hexopyranosyl)*-α-*maltosyl fluoride* (**21**).—To the β fluoride **20** (600 mg, 0.691 mmol) contained in a plastic vial was added 2 mL of HF · pyridine. This was kept at −70°C for 10 min and at 0°C for another 50 min. The mixture was diluted with CH_2Cl_2 and quenched with satd aq $NaHCO_3$. The aqueous phase was extracted twice with CH_2Cl_2, the combined organic phases were washed with water, then concentrated by evaporation and investigated by ^{19}F NMR spectroscopy. TLC analysis (2:1 EtOAc–hexanes) showed the formation of four products with R_f values of 0.68, 0.53, 0.50, and 0.25, with that at 0.53 corresponding to the main product. Column chromatography (2:1 EtOAc–hexanes) yielded 69 mg (0.24 mmol) of the least polar material; 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*xylo*-hexopyranosyl fluoride (crystalline, ^{19}F NMR: δ −148.64), 20 mg of the most polar, which arises from hydrolysis and 510 mg of a mixture of the two middle spots. This product mixture was acetylated overnight in pyridine (10 mL) and Ac_2O (0.5 mL), diluted with CH_2Cl_2, washed with water, and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were washed with water, coevaporated with acetonitrile, and purified by column chromatography (2:3 EtOAc–hexanes, later 1:1) yielding 360 mg of **21** (0.41 mmol, 60%) as a white amorphous solid; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.63 (dd, 1 H, $J_{1,F}$ 53.6, $J_{1,2}$ 2.6 Hz, H-1), 5.53 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.2 Hz, H-3), 5.37 (m, 2 H, $J_{1'',2''}$ 4.0 Hz, H-1″, H-3′), 5.28 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1′), 5.16 (ddd, 1 H, H-3″), 4.81 (ddd, 1 H, $J_{2,F}$ 23.8, $J_{1,2}$ 2.6, $J_{2,3}$ 10.0 Hz, H-2), 4.79 (dd,

1 H, $J_{1'',2''}$ 4.0, $J_{2'',3''}$ 10.4 Hz, H-2''), 4.72 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 10.4 Hz, H-2'), 2.12, 2.08, 2.06, 2.05, 2.01, 2.00, 1.99, 1.98 (8 s, 28 H, 9 OAc and H-4''e), 1.59 (q, 1 H, H-4''a); ^{19}F NMR ($CDCl_3$, 188 MHz): δ −149.22; DCIMS calcd for $C_{36}H_{49}FO_{23}$: $(M + NH_4^+)$, 886.73; found: $(M + NH_4^+)$, 887.

*4'-O-(4-Deoxy-α-*D*-xylo-hexopyranosyl)-α-maltosyl fluoride* (**22**).—A solution of **21** (170 mg, 0.195 mmol) in dry MeOH (20 mL) was stirred with a catalytic amount of solid NaOMe at room temperature overnight. After 17 h, TLC analysis (7:2:1 EtOAc–MeOH–water) revealed a single polar product. The mixture was neutralized with ion-exchange resin (Bio-Rad, AG 50W-X 12, 50–100 mesh, H^+ form), filtered, and evaporated. Freeze-drying yielded **22** (86 mg, 90%) as an amorphous white solid; ^{1}H NMR (D_2O, 400 MHz): δ 5.75 (dd, 1 H, $J_{1,2}$ 2.8, $J_{1,F}$ 53.6 Hz, H-1), 5.47–5.45 (2d, 2 H, $J_{1',2'}$ 4.0, $J_{1'',2''}$ 4.0 Hz, H-1', H-1''), 3.57 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 9.8 Hz, H-2'), 2.04 (ddd, 1 H, $J_{3'',4''e}$ 4.8, $J_{4''e,4''a}$ 12.6, $J_{4''e,5''}$ 2.0 Hz, H-4''e), 1.50 (q, 1 H, $J_{3'',4''a}$ $J_{4''a,4''e}$ $J_{4''a,5''}$ ~ 12.5 Hz, H-4''a); ^{19}F NMR (D_2O, 188 MHz): δ −150.35; ^{19}F NMR (D_2O, 188 MHz, coupled): δ −150.40 (dd, $J_{1,F}$ 54.2, $J_{2,F}$ 26.7 Hz). Anal. Calcd for $C_{18}H_{31}FO_{14} \cdot 2H_2O$: C, 41.06; H, 6.70. Found: C, 41.25; H, 6.61.

Acknowledgements

We thank Reneé Mosi for assistance, the Deutsche Forschungsgemeinschaft for a fellowship (TKL), and the Natural Sciences and Engineering Research Council of Canada for financial support.

References

[1] G. Mooser, *Enzymes*, 20 (1992) 187–233.
[2] M.L. Sinnott, *Chem. Rev.*, 90 (1990) 1171–1202.
[3] M. Qian, R. Haser, and F. Payan, *J. Mol. Biol.*, 231 (1993) 785–799.
[4] S.B. Larson, A. Greenwood, D. Cascio, J. Day, and A. McPherson, *J. Mol. Biol.*, 235 (1994) 1560–1584.
[5] C. Klein and G. Schulz, *J. Mol. Biol.*, 217 (1991) 737–750.
[6] J. Jünnemann, J. Thiem, and C. Pedersen, *Carbohydr. Res.*, 249 (1993) 91–94.
[7] C. Braun and S.G. Withers, *Carbohydr. Res.*, (1994) submitted.
[8] S. Miao, J.D. McCarter, M.E. Grace, G.A. Grabowski, R. Aebersold, and S.G. Withers, *J. Biol. Chem.*, 269 (1994) 10975–10978.
[9] P.J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, 108 (1982) 97–101.
[10] P.J. Garegg and H. Hultberg, *Carbohydr. Res.*, 93 (1981) C10–C11.
[11] J.R. Rasmussen, C.J. Slinger, R.J. Kordish, and D. Newmans-Evans, *J. Org. Chem.*, 46 (1981) 4843–4846.
[12] J. Jünnemann, I. Lundt, and J. Thiem, *Acta Chem. Scand.*, 45 (1991) 494–498.
[13] G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.