

Chemoenzymatic Syntheses of Linear and Branched Hemithiomaltodextrins as Potential Inhibitors for Starch-Debranching Enzymes

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Abstract: Oligosaccharides embodying the *S*-maltosyl-6-thiomaltosyl structure have been readily synthesised by using convergent chemoenzymatic approaches. The key steps for the preparation of these molecules involved: 1) transglycosylation reactions of maltosyl fluorides onto suitable acceptors catalysed by the bacterial transglycosylase, cyclodextrin glycosyltransferase (CGTase), and 2) the S_N2-type displacement of a 6-halide from acetylated

acceptors by activated 1-thioglycoses. The target molecules, which were obtained in good overall yields, proved to be useful for investigating substrate binding in the active sites of several enzymes that act upon the α -1,6-linkage of pullulan and/or amylopectin. The

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compounds exhibit *K_i* values in the 2.5–1350 μ M range with the different enzymes, and the highest affinity found by using these molecules was seen for the pullulanase from *Bacillus acidopullulyticus*. Both barley-malt limit dextrinase and pullulanase type II from *Thermococcus hydrothermalis* only recognised the longest linear thiooligosaccharide, while a branched heptasaccharide was the strongest inhibitor of pullulanase from *Klebsiella planticola*.

Introduction

A large proportion of the photosynthetically assimilated carbon in plants is channelled into the biosynthesis of starch and sucrose, by far the two most widely used carbohydrate-based chemicals in food and nonfood industries. Starch consists of a mixture of two distinct polysaccharide types: amylose, ideally a linear polymer of 1,4- α -D-glucosyl units, and amylopectin, a branched polymer containing short amylose chains linked by α -1,6-branching points. Amylose is mainly hydrolysed by various amylases, usually classified as endo-acting 1,4- α -D-glucan glucanohydrolase [α -amylases; EC 3.2.1.1; GH family 13], or the exo-acting 1,4- α -D-glucan

maltohydrolase [β -amylases; EC 3.2.1.1; GH family 14] and 1,4- α -D-glucan glucohydrolase [glucoamylases; EC 3.2.1.3; GH family 15]. The α - and β -amylases attack bonds in the 1,4- α -D-glucan-backbone chains of amylopectin to generate oligosaccharides called α - and β -limit dextrins, respectively. The α -1,6-bonds in amylopectin and the limit dextrins are hydrolysed by pullulanases [EC 3.2.1.41, GH family 13], limit dextrinases [EC 3.2.1.142, GH family 13], and isoamylases [EC 3.2.1.68, GH family 13]. Moreover, some enzymes such as amylopullulanase [EC 3.2.1.1/41, GH families 13 or 57] and glucoamylase [EC 3.2.1.3, GH family 15] can hydrolyse both the α -1,4 and α -1,6-bonds.^[1, 2] As indicated above, glycoside hydrolases are grouped into different families according to a classification that is based upon amino acid sequence similarities. This classification does not coincide perfectly with the biochemical classification as defined by the Enzyme Commission based on enzyme specificity. Thus, a given glycoside hydrolase family can contain several enzymes with different EC numbers, while enzymes with the same substrate specificity can act with retention or inversion of the anomeric configuration and occur with different structural folds, and hence belong to different structural families.

Given the structural complexity of amylopectin and the difficulties associated with the isolation of pure oligomers from natural sources, there is a high demand for the development of strategies for the preparation of compounds that can be used to determine enzyme specificity. However, few

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chemical syntheses of branched oligosaccharides representing the branch point of amylopectin have been reported.^[3, 4]

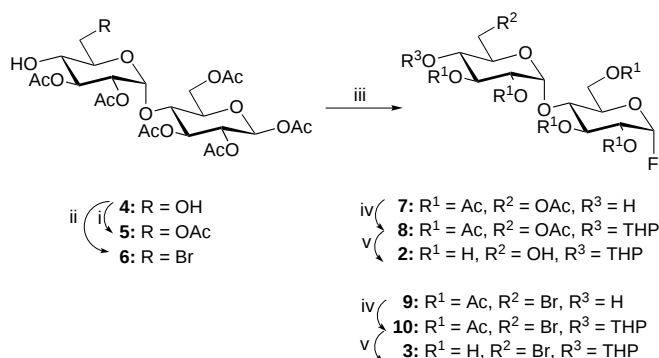
For many years, we have been involved in the syntheses of hydrolytically inert substrate analogues, the thiooligosaccharides, that can be used as tools for structural biology and in biochemical studies of a wide variety of enzymes.^[5–7] In such a molecule, only the scissile bond has to be replaced by a thio-linkage. The major challenges associated with the synthesis of potential substrates are concerned with the efficiency and the accuracy of regiospecific glycosylation. Here we report the use of chemoenzymatic approaches for the preparation of potential substrate analogues of α -1,6-degrading enzymes that enable the distinction of fine differences in enzyme–substrate recognition and catalytic mechanisms.

Results and Discussion

Although from an industrial point of view the enzymatic degradation of the branching point of amylopectin is important, comparative studies on the substrate specificity of debranching enzymes of the pullulanase type are lacking because suitable substrates or substrate analogues have not been available. Therefore, to facilitate such specificity characterisation, we embarked on the synthesis of molecules exhibiting the general structure shown in Scheme 1.

The 6-thio-isomaltosyl motif is the common disaccharidic unit of all the compounds prepared in this study. Previous work has demonstrated that *S*- α -D-glucopyranosyl-6^o-thio-maltooligosaccharides can be synthesised in high yield^[7] by the nucleophilic displacement of the halide of 6^o-iodo-maltooligosaccharides by using the activated form of fully acetylated-1-thio- α -D-glucopyranose, which itself is generated from the corresponding tritylthio derivative **1**. This motivated the present enzymatic approach to the assembly of several mono- and disaccharide building blocks around the thio unit, which resulted in the target molecules. Previously, substituted maltosyl fluorides were used as donor and acceptor molecules for active-site mapping of cyclodextrin glycosyltransferase [CGTase; EC 2.4.1.19, GH family 13] and in the enzymatic synthesis of regularly substituted cyclodextrins.^[8, 9] CGTase

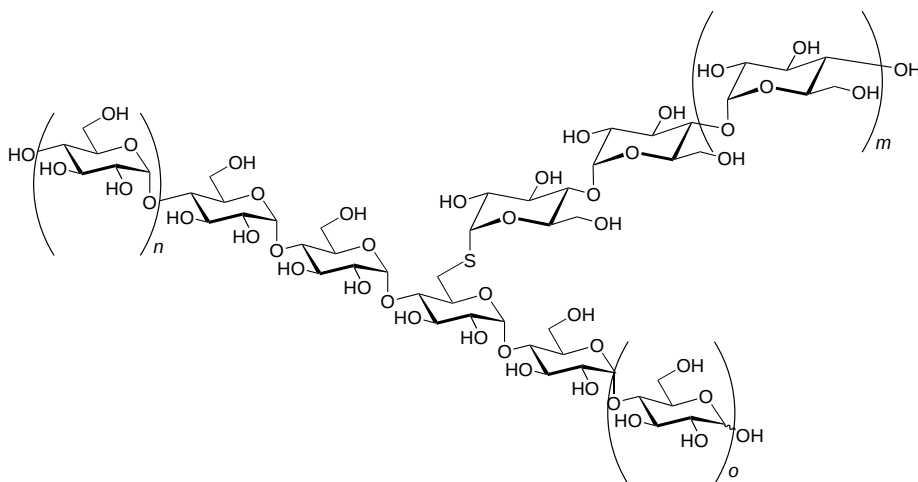
was therefore also employed in the presence of maltosyl fluorides for the synthesis of the α -1,4-bonds of the target compounds. However, in order to prevent self-condensation of the donors during synthesis, it was necessary to block the 4th-OH of the maltosyl fluorides. Since 4th-O-tetrahydropyranyl- α -cellobiosyl fluoride was previously used successfully for enzymatic syntheses of β -1,4-oligosaccharides,^[10, 11] 4th-O-tetrahydropyranyl- α -maltosyl fluoride (**2**) and 6th-bromo-4th-O-tetrahydropyranyl- α -maltosyl fluoride (**3**) were used for the present syntheses. Preferential acetylation of the known hexaacetyl maltose **4** was readily obtained in excellent yield by using the 1-acetyloxybenzotriazole procedure (Scheme 2).^[12] The fluorination of the expected compound **5**



Scheme 2. Syntheses of the maltosyl fluorides **2** and **3**. i) 1-acetyloxybenzotriazole, TEA, CH₂Cl₂, RT (80 %); ii) P(C₆H₅)₃, pyridine, CBr₄, 0 °C to 50 °C (84 %); iii) HF/pyridine, 0 °C (95 % for **7**, 90 % for **9**); iv) dihydropyran, camphorsulfonic acid, CH₂Cl₂, RT, (98 % for **8**, 95 % for **10**); v) MeONa, MeOH, 0 °C (98 %) for **2** and **3**.

was achieved in 95 % yield by using a commercially available pyridine–hydrogen fluoride reagent, as described earlier for fully acetylated maltose and cellobiose derivatives.^[13] The 4th-OH of maltosyl fluoride **7** was tetrahydropyranylated in 98 % yield and **8** was de-*O*-acetylated with sodium methoxide in methanol at room temperature to give the corresponding pure fluoride **2** in almost quantitative yield. Mild and selective bromination of the free primary hydroxyl group of **4** with carbon tetrabromide and triphenylphosphine in pyridine,^[14] gave **6** in 84 % yield. Fluorination, tetrahydropyranylation and de-*O*-acetylation, as already reported for **5**, gave **9**, **10** and **3** in 90, 95 and 98 % yield, respectively.

With regard to the preparation of the donors for the thio-glycosylation, halide displacement of acetochloro- β -maltose, generated from peracetyl- β -maltose (**11**)^[15] and triphenylmethyl mercaptan according to the methodology developed in the “gluco” and “galacto” series^[16, 17] gave the acetylated tri-

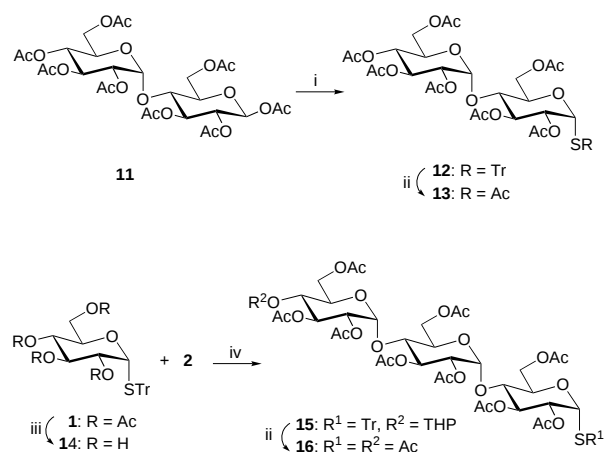


Scheme 1. Structure of the target molecules.

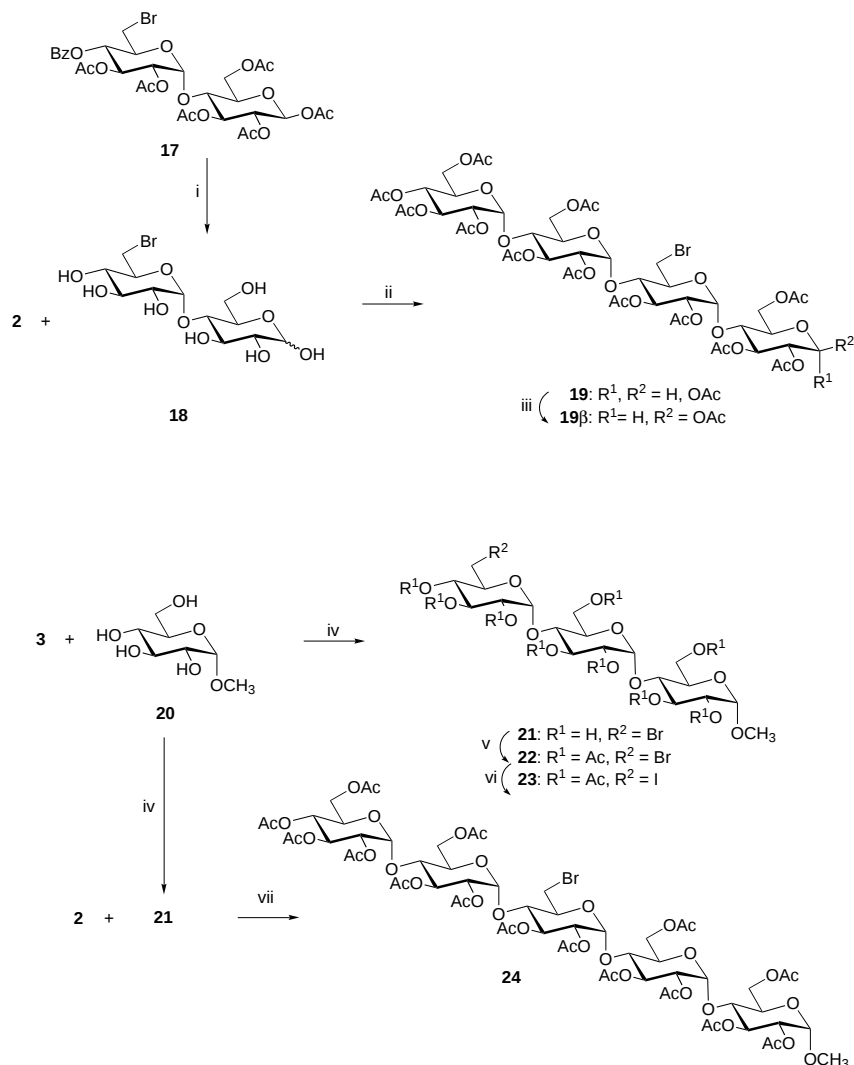
tyl-1-thio- α -maltoside (**12**) in 31% yield (Scheme 3). This compound was then transformed into the known donor **13**,^[18] by treatment with triethylsilane^[19] and trifluoroacetic acid (TFA) in dichloromethane followed by acetylation, with a yield of 95%. For the synthesis of the corresponding trisaccharide **16**, instead of optimising the previously described procedure for unknown acetochloro- β -maltotriose, we investigated an alternative enzymatic condensation of the fluoride **2** and the acceptor **14** derived from **1**.^[17] The reaction, catalysed by *Bacillus sp.* CGTase, afforded the trisaccharide **15** in 86% yield after acetylation. This compound was then transformed into the desired donor **16** as described for the preparation of **13** (overall yield 69%).

Having confirmed the usefulness of this methodology, we turned to the exploitation of fluorides **2** and **3** for the preparation of 6^o-halogenomaltooligosaccharides as acceptors for the thioglycosylation reactions. The known bromomaltose derivative **17**^[20] may be used for these reactions, but it can also act as an acceptor for enzymatic transglycosylation after de-*O*-acetylation. In fact, the condensation of **2** and the known **18**^[20] gave, after acidic removal of the ether protecting group and acetylation, the tetrasaccharide **19** in 70% yield as an anomeric mixture. For the purposes of spectral analysis, an aliquot was converted into the pure β -maltotetraosyl derivative **19 β** (Scheme 4). To avoid this additional step, we decided to employ methyl α -D-glucoside **20** as an acceptor for the transglycosylation reaction with **3**. After acidic treatment, methyl 6^{III}-bromo- α -maltotrioside **21** could be either acetylated to afford **22** in 75% overall yield, or elongated with the fluoride **2**. Following the previously described treatment (tetrahydropyranyl (THP) removal and acetylation), the methyl 6^{III}-bromomaltopentaoside **24** was obtained in 52% overall yield from the fluoride **2**.

At this point, having successfully prepared all the necessary precursors, synthesis of the linear hemithiomaltotetraose **26**, methyl pentaoside **28** and hexaoside **30** was attempted. Chemoselective deprotection and activation of 1-*S*-acetylated glucose was achieved by the action of cysteamine dithioerythritol in hexamethylphosphoramide (HMPA), and the resulting thiolate was coupled with 6^o-iodomaltooligosaccharides, result-



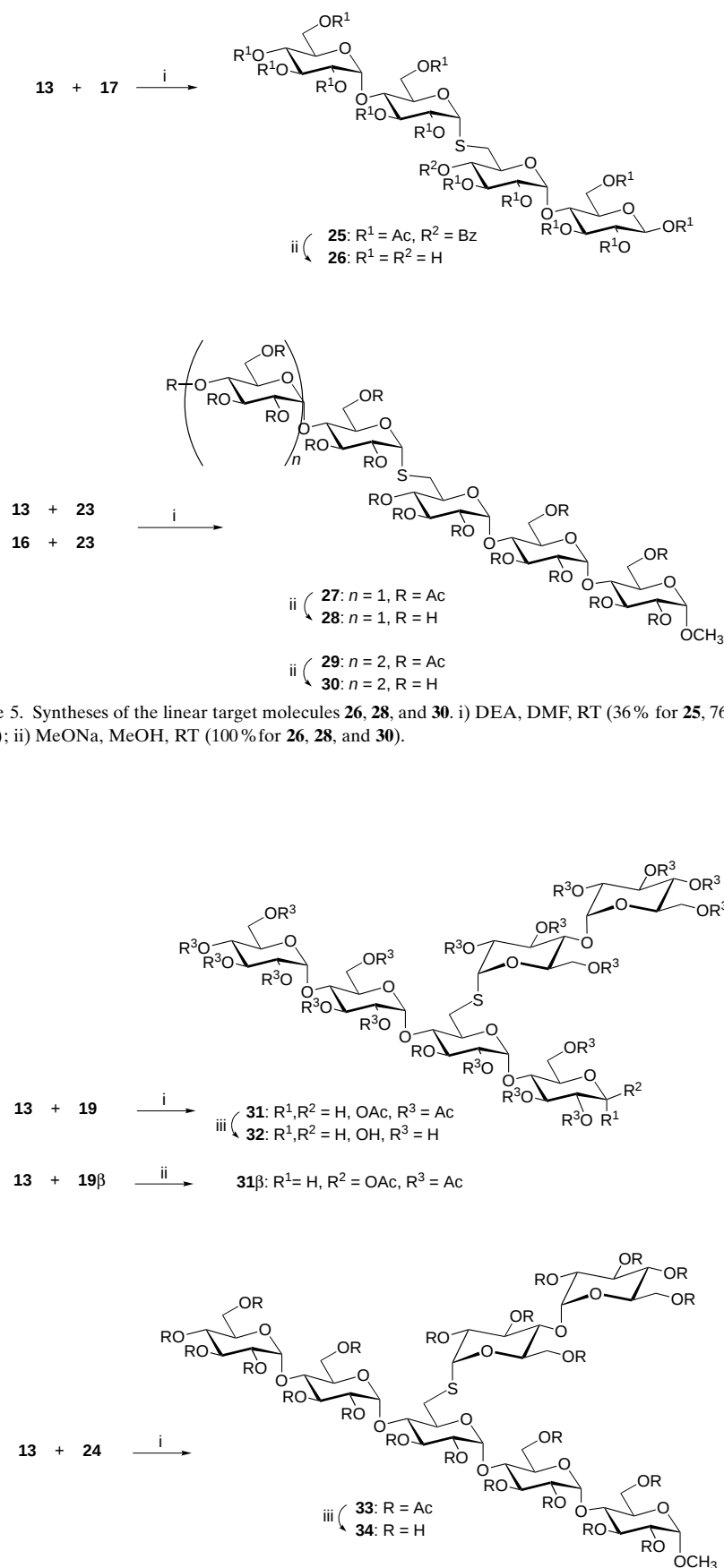
Scheme 3. Syntheses of the 1-*S*-acetyl-1-thio-glycosides **13** and **16**. i) AlCl₃, CHCl₃, 0 °C to RT, then TrSN(Bu)₄, toluene, RT (31%); ii) (Et)₃SiH, TFA, CH₂Cl₂, RT, then Ac₂O, pyridine, RT (95% for **13**, 69% for **16**); iii) MeONa, MeOH, RT (100%); iv) CGTase, phosphate buffer, 40 °C, then Ac₂O, pyridine, DMAP (86% overall). Tr = triphenylmethyl.



Scheme 4. Syntheses of the 6-halogeno-6-maltooligosaccharides **19** and **22–24**. i) MeONa, MeOH, RT (100%); ii) a) CGTase, phosphate buffer, 40 °C; b) HCl (1M), RT; c) Ac₂O, pyridine, DMAP (70% overall); iii) HBr/Ac₂O, CH₂Cl₂, 0 °C, then AgOAc, AcOH, Ac₂O, RT, (52%); iv) a) CGTase, phosphate buffer, 40 °C; b) HCl (1M), RT; v) Ac₂O, pyridine, DMAP (75%); vi) KI, DMF, 70 °C, (92%); vii) a) CGTase, phosphate buffer, 40 °C; b) HCl (1M), RT; c) Ac₂O, pyridine, DMAP (52% overall).

ing in the corresponding hemithiomaltodextrins in high yield.^[6] It was also shown that *N,N*-dimethylformamide (DMF) was a good solvent for the effective substitution of tosylate and bromide at C-6 of mono- and oligosaccharides, while the donor was *S*-deacetylated and activated in situ with diethylamine (DEA).^[21] However, for practical reasons, we preferred the latter procedure in this work. Condensation of **13** and **17** in DMF in the presence of DEA gave the expected compound **25** but in only 67% yield (Scheme 5). To overcome this drawback with the methyl maltotrioxide **22**, an exchange of halogen (Br $\rightarrow$ I) was achieved by treatment of the bromo compound with KI in DMF for 2 h at 70 °C. Likewise, the iodo derivative **23** was obtained in 92% yield. The halogen displacement of **23** under the previous conditions with **13** or **16** gave the expected penta- (**27**) or hexasaccharide (**29**) with yields of 76%. De-*O*-acylation of **25** and de-*O*-acetylation of **27** and **29** gave **26**, **28** and **30** respectively, in almost quantitative yields.

Scheme 6 outlines the syntheses of two branched hemithiomaltodextrins **32** and **34**. A one-pot procedure was adopted for the transformation of the bromo derivatives **19** and **24** into the corresponding iodo analogues, which were then engaged in coupling reactions with acetylated 1-thio- α -maltose (**13**). During thioglycosylation, some de-*O*-acetylation occurred, and an acetylation step was performed. Under these experimental conditions, the acetylated hexamer **31** and heptamer **33** were isolated with yields of 72 and 53%, respectively. To facilitate NMR analysis of this complex structure, **19 β** was engaged in the thioglycosylation reaction in place of **19**, and both halogen exchange and acetylation were omitted. Likewise, **31 β** was isolated but in only a 43% yield.



Scheme 5. Syntheses of the linear target molecules **26**, **28**, and **30**. i) DEA, DMF, RT (36% for **25**, 76% for **27** and **29**); ii) MeONa, MeOH, RT (100% for **26**, **28**, and **30**).

Scheme 6. Syntheses of the branched target molecules **32** and **34**. i) a) **19** and KI, DMF, 70 °C; b) **13**, DTE, DEA, DMF, 0 °C to RT; c) Ac₂O, pyridine, DMAP (72% overall for **31** and 53% overall for **33**); ii) DEA, DMF, RT (43%); iii) MeONa, MeOH, RT (96% for **32** and 99% for **34**).

The structures of the compounds were confirmed by ^1H NMR data analysis, the spectra are given as Supporting Information. Assignments were done on the protected compounds. These spectra were assigned by using COSY, COSY-relayed and TOCSY 2D experiments employing the Bruker library pulse sequences.

The structural characterisation is based on comparison of different types of data obtained on the different compounds. The general procedure was: identification of a characteristic signal and assignment of all the protons of the same unit by using homonuclear-correlation COSY or TOCSY. When units exhibit the same characteristics, the assignments may be reversed. The coupling constants were read from the 1D spectra or from the 2D COSY map when overlaps occurred. Assignments were verified by using a 1D simulation spectrum on WIN-DAISY 4.0 software.

^{13}C NMR data were assigned by using model compounds. For the total attribution given for some derivatives, HMQC and HMBC experiments were performed by using Bruker library pulse sequences.

Compounds **26**, **28**, **30**, **32** and **34** were tested as potential inhibitors during the hydrolysis of pullulan by pullulanases from *Bacillus acidopullulyticus* (Ba) and *Klebsiella planticola* (Kp), barley limit dextrinase (LD) and pullulanase type II from *Thermococcus hydrothermalis* (Th) (Table 1). None of the compounds was hydrolysed under our experimental conditions.

Table 1. Inhibitory capacities given as the K_i (μM) of the target compounds on pullulan hydrolysis by four different enzymes.

	LD	Kp	Pullulanase Ba	Th (type II)
26	> 2000	> 2000	≈ 300	> 2000
28	> 2000	≈ 300	2.5	> 2000
30	≈ 500	≈ 200	≈ 300	≈ 122
32	> 2000	≈ 1350	≈ 625	> 2000
34	> 2000	≈ 80	≈ 320	≈ 545

The smallest hemithiodextrin **26** only affected Ba with a relatively weak K_i value of about $300\ \mu\text{M}$. Compound **28**, which is extended in the reducing end by a methyl α -D-glucosyl unit, increased the affinity of Ba more than 100-fold to give a K_i of only $2.5\ \mu\text{M}$. Compounds **28** and **30** affected Kp with K_i values of about $300\ \mu\text{M}$ and $200\ \mu\text{M}$, respectively. In addition, the branched methyl maltoheptaoside **34** was found to be the best inhibitor for this enzyme with a K_i value of about $80\ \mu\text{M}$. Only compound **30** has significant an inhibitory effect on barley limit dextrinase with a K_i value of about $500\ \mu\text{M}$. Curiously, the differences of enzyme specificity revealed by these substrate analogues are not in accordance with their almost identical affinities measured for the corresponding natural oligosaccharide substrates (Jensen and Svensson, unpublished results). This apparent discrepancy remains to be solved.

Like LD, Th was not affected by **26** or **28**. However, **30** and **34** did inhibit Th, giving K_i values of $122\ \mu\text{M}$ and $545\ \mu\text{M}$, respectively (Table 1). Clearly, compared with Ba, which was strongly inhibited by **28**, Th must possess a larger active site in

which six glucosyl residues are readily accommodated. Moreover, the inefficiency of **28** towards Th activity underlines the extreme importance of recognition of the additional glucosyl unit at the nonreducing end of **30**.

Experimental Section

General procedures: Roman numerals in ascending order are given to the residues from the reducing end to the terminal unit of the branch. NMR spectra were recorded at 303 K on a Bruker AC300, Bruker Avance400 or Varian Unity500. Proton chemical shifts (δ) are reported in ppm downfield from TMS; carbon chemical shifts are reported with reference to internal solvent.

High- (HRMS) and low-resolution mass spectra were recorded on a VG ZAB and on a Nermag R-1010C spectrometer, respectively. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. Melting points were measured on a Büchi 535 apparatus. Microanalyses were performed by the “Laboratoire Central d’Analyses du CNRS” (Vernaison). Progress of synthesis was monitored by analytical thin-layer chromatography with silica gel 60 F254 precoated plates (Merck, Darmstadt). The enzyme CGTase was from *Bacillus* sp. and was a gift from Wacker Industrie S.A. (Lyon, France).

All reactions in organic medium were carried out under argon with freshly distilled solvents. After workup, organic phases were dried over anhydrous Na_2SO_4 .

Enzyme inhibition assays: The inhibition of pullulanase from *Bacillus acidopullulyticus* and *Klebsiella planticola* (Megazyme) and barley malt limit dextrinase^[22] was determined in a competitive assay with pullulan as substrate at 40°C in sodium acetate (20 mM, pH 5.0). The activity was calculated from the release of reducing sugar, as measured by the copper bicinonitate method essentially as described.^[22, 23] K_m and k_{cat} were determined in the same assay by using pullulan (0.06 to $10\ \text{mg mL}^{-1}$) and found to be $0.16\ \text{mg mL}^{-1}$ and $33\ \text{s}^{-1}$ for barley limit dextrinase, $0.24\ \text{mg mL}^{-1}$ and $120\ \text{s}^{-1}$ for Ba, and $0.09\ \text{mg mL}^{-1}$ and $81\ \text{s}^{-1}$ for Kp, respectively. K_i values were calculated by assuming competitive inhibition from $1/v = (S + K_m)/(S \cdot k_{cat}) + K_m/(k_{cat} \cdot S \cdot K_i) \times [I]$, in which v is the rate measured in the absence or presence of inhibitor, S is the substrate concentration ($0.5\ \text{mg mL}^{-1}$ for limit dextrinase and $0.25\ \text{mg mL}^{-1}$ for Ba and Kp), and $[I]$ is the concentration of inhibitor (0 – $2\ \text{mM}$).

For the inhibition of pullulanase type II from *Thermococcus hydrothermalis*,^[24] measurements were carried out at 80°C in the presence of pullulan (0.1 to $10\ \text{mg mL}^{-1}$) as substrate in CaCl_2 (5 mM), sodium acetate (50 mM), pH 5.5. Activity was determined by measuring the release of reducing sugar by using a previously described modification of the Kidby–Davidson method.^[25] The inhibitor concentration was varied over the range 0 to $1\ \text{mM}$, and the data were analysed by using SigmaPlot 2000 software. K_i values were determined on the basis of a mixed-inhibition model.

(2,3,6-Tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl- β -D-glucopyranose (5): A solution of compound **4** (6.08 g, 10.2 mmol), 1-(acetyloxy)benzotriazole (2.0 g, 1.2 equiv) and triethylamine (2.1 mL) in dichloromethane (60 mL) was stirred for 20 h at room temperature. The resulting solution was evaporated, and crystallisation in diethyl ether gave the monohydroxy compound **5** (5.2 g, 80%). M.p. 176°C , (lit: 175 – 177°C);^[12] $[\alpha]_D^{25} = +46$ ($c = 0.88$ in CHCl_3), (lit: $+46.5$);^[12] ^1H NMR (300 MHz, CDCl_3) see Supporting Information; ^{13}C NMR (75 MHz, CDCl_3) $\delta = 171.1$ – 168.7 ($7 \times \text{OCOCH}_3$), 95.75 (C-1^{H}), 91.09 (C-1^{H}), 74.94, 72.88, 72.31, 71.46, 70.84, 70.77, 70.03, 68.47 (C-2^{H} , C-3^{H} , C-4^{H} , C-5^{H}), 62.37 (C-6^{H}), 20.53–20.26 (OCOCH_3); elemental analysis calcd (%) for $\text{C}_{26}\text{H}_{36}\text{O}_{18}$: C 49.306, H 5.70; found C 48.97, H 5.85.

(2,3-Di-O-acetyl-6-bromo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl- β -D-glucopyranose (6): Triphenylphosphine (4 g, 2 equiv) and tetrabromomethane (2.57 g, 1.02 equiv) were added to a solution of compound **4** (5.0 g, 7.86 mmol) in pyridine (54 mL) at 0°C . After the solution had been stirred for 15 min at 0°C and then for 3 h at 50°C , methanol (5 mL) was added, and the reaction mixture was concentrated and co-evaporated with toluene. Purification by flash chromatography (EtOAc /petroleum ether 1:1, v/v) gave **6** (4.2 g, 84%). $[\alpha]_D^{25} = +55.3$ ($c = 0.77$ in CHCl_3); ^1H NMR (300 MHz, CDCl_3) see Supporting Information;

^{13}C NMR (75 MHz, CDCl_3) δ = 171.1–168.8 ($6 \times \text{OCOCH}_3$), 95.67 (C-1'), 91.18 (C-1''), 74.96, 73.12, 72.44, 71.73, 71.28, 70.82, 70.72, 70.14 (C-2'–11), C-3'–11, C-4'–11, C-5'–11), 62.61 (C-6'), 32.66 (C-6''), 20.75–20.38 (OCOCH_3); elemental analysis calcd (%) for $\text{C}_{24}\text{H}_{33}\text{BrO}_{16}$: C 43.85, H 5.06, Br 12.15; found C 43.75, H 5.29, Br 11.76.

(2,3,6-Tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl fluoride (7): In a plastic vessel, a solution of compound 5 (5.0 g, 7.9 mmol) in hydrogen fluoride/pyridine (30 mL, 7:3) was stirred at 0 °C for 30 min, then diluted with dichloromethane (20 mL) and poured in a plastic beaker containing an ice-cooled solution of ammonia (25 mL, 3M). The organic layer was washed with saturated aq. sodium hydrogen carbonate until neutralisation, dried (Na_2SO_4) and concentrated under reduced pressure. Flash column chromatography (petroleum ether/EtOAc 1:2, v/v) of the residue with triethylamine neutralised silica gave the α -fluoride 7 (4.45 g, 95 %). $[\alpha]_D^{25}$ = +93 (c = 0.8 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) see Supporting Information; ^{13}C NMR (75 MHz, CDCl_3) δ = 171.3–169.7 ($6 \times \text{OCOCH}_3$), 103.56 (d, $^1J_{\text{CF}}$ = 229.3 Hz, C-1'), 95.84 (C-1''), 71.78/71.66/71.58/71.21/70.72/70.30/69.99/68.69 (C-2'–11, C-3'–11, C-4'–11, C-5'–11), 62.36/62.12 (C-6', C-6''), 20.71/20.41 (OCOCH_3); elemental analysis calcd (%) for $\text{C}_{24}\text{H}_{33}\text{FO}_{16}$: C 48.32, H 5.58, F 3.18 %; found C 48.34, H 5.82, F 2.98.

(2,3,6-Tri-*O*-acetyl-4-*O*-tetrahydropyranyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl fluoride (8): Freshly distilled dihydropyran (2.3 mL) and camphorsulfonic acid (86 mg) were added to a solution of compound 7 (3.0 g, 5.0 mmol) in CH_2Cl_2 (120 mL). After 3 h at room temperature, the solution was diluted with CH_2Cl_2 (120 mL) and successively washed with H_2O and saturated aqueous NaHCO_3 . The organic layer was dried, concentrated and purified by flash chromatography (EtOAc/petroleum ether 1:1, v/v) to give compound 8 in 98 % yield. ^{13}C NMR (75 MHz, CDCl_3) δ = 170.54–169.31 ($6 \times \text{OCOCH}_3$), 103.56 (d, $^1J_{\text{CF}}$ = 229.3 Hz, C-1'), 102.05/101.62 (CH THP group), 95.72 (C-1''), 75.28/73.59/71.75/71.68/71.48/70.77/70.43/70.38/70.26/69.99/69.84/69.74 (C-2'–11, C-3'–11, C-4'–11, C-5'–11), 64.03/63.34/62.61/62.14/62.1/62.02 (C-6', C-6'') R and S, CH_2 THP group), 20.76–20.41 (OCOCH_3), 20.09/19.67 (CH_2 THP group); DCIMS: m/z : 698 [M^+ + NH_4]; elemental analysis calcd (%) for $\text{C}_{29}\text{H}_{41}\text{FO}_{17}$: C 51.18, H 6.07, F 2.79; found: C 50.98, H 6.21, F 2.73.

(2,3-Di-*O*-acetyl-6-bromo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl fluoride (9): In a plastic vessel, a solution of compound 6 (2.04 g, 3.11 mmol) in hydrogen fluoride/pyridine (20 mL, 7:3) stirred at 0 °C for 30 min, was worked up and purified as already described for the preparation of 7. The α -fluoride 9 was obtained (1.71 g, 90 %). $[\alpha]_D^{25}$ = +95.7 (c = 0.67 in CHCl_3); ^1H NMR (300 MHz, CDCl_3) see Supporting Information; ^{13}C NMR (75 MHz, CDCl_3) δ = 171.33/169.76 ($5 \times \text{OCOCH}_3$), 103.54 (d, $^1J_{\text{CF}}$ = 227.8 Hz, C-1'), 95.72 (C-1''), 71.80/71.31/70.7/70.35/70.06 (C-2'–11, C-3'–11, C-4'–11, C-5'–11), 62.17 (C-6'), 32.71 (C-6''), 20.9–20.36 (OCOCH_3); ES^+ HRMS: calcd for $\text{C}_{22}\text{H}_{30}\text{BrFO}_{14}$ [M^+ +Na]: 639.0701, found: 639.0705.

(2,3,6-Tri-*O*-acetyl-6-bromo-6-deoxy-4-*O*-tetrahydropyranyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl fluoride (10): Compound 9 (1.72 g, 2.79 mmol) was treated as described for its analogue 14. The expected fluoride 10 was obtained as a mixture of diastereoisomers (1.8 g, 95 %). ES^+ HRMS: calcd for $\text{C}_{27}\text{H}_{38}\text{BrFO}_{15}$ [M^+ +Na]: 723.1276, found: 723.1281.

(4-*O*-Tetrahydropyranyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)- α -D-glucopyranosyl fluoride (2): Fluoride 8 (1.55 g, 2.28 mmol) in methanol (50 mL) was treated with sodium methoxide (1M, 2.5 mL) for 30 min at room temperature. The mixture was then cooled to 0 °C and neutralised with Amberlite IRN 120 (H^+) resin, the resin was removed by filtration, and the filtrate was concentrated. The free fluoride 2 was dissolved in water and freeze dried (956 mg, 98 %). In this form, compound 2 was stable for several weeks at –18 °C. ES^+ HRMS: calcd for $\text{C}_{17}\text{H}_{29}\text{FO}_{11}$ [M^+ +Na]: 451.1592, found: 451.1596.

(6-Bromo-6-deoxy-4-*O*-tetrahydropyranyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)- α -D-glucopyranosyl fluoride (3): The fluoride 10, de-*O*-acetylated and treated as described for 8, afforded 3 in 98 % yield (683 mg). ES^+ HRMS: calcd for $\text{C}_{17}\text{H}_{28}\text{BrFO}_{10}$ [M^+ +Na]: 513.0748, found: 513.0747.

Triphenylmethyl (2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetylthio- α -D-glucopyranoside (12): AlCl_3 (2 g) was added to a solution of octaacetyl- β -maltose 11 (10 g, 14.7 mmol) in anhydrous chloroform (42 mL) at 0 °C. After being stirred for 15 min, the solution was allowed to warm up to room temperature. After 30 min, Celite filtration

and evaporation of solvent and co-evaporation with toluene (3×20 mL) gave crude acetochloro- β -maltose, which was used in the next step without further purification and characterisation. A solution of this compound in toluene (23 mL) was added to a slurry of tetrabutylammonium triphenylmethanethiolate, prepared as already described^[16] with triphenylmethylthiol (4 g, 14.4 mmol). The mixture was stirred at room temperature for 3 h, then evaporated. The residue, after purification by flash chromatography (1: CH_2Cl_2 , 2: EtOAc/petroleum ether 1:1, v/v), gave compound 12 (3.99 g, 31 %). $[\alpha]_D^{25}$ = +147 (c = 0.56 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) see Supporting Information; ^{13}C NMR (100 MHz, CDCl_3) δ = 170.62–169.39 ($7 \times \text{OCOCH}_3$), 144.32 (CC_3H_5), 129.82/127.88/127.06 (CC_3H_5), 95.70 (C-1''), 81.84 (C-1'), 73.42 (C-4'), 72.96 (C-3'), 70.26 (C-2'), 69.95 (C-2''), 69.79 (C-5'), 69.32 (C-3''), CC_3H_5), 68.33 (C-5''), 67.95 (C-4''), 62.77 (C-6'), 61.31 (C-6''), 20.95–20.56 (OCOCH_3); ES^+ HRMS: calcd for $\text{C}_{45}\text{H}_{50}\text{O}_{17}\text{S}$ [M^+ +Na]: 917.2666, found: 917.2667.

(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio- α -D-glucopyranoside (13): Triethylsilane (645 μL , 4.0 mmol) and trifluoroacetic acid (18 mL) were added to a stirred solution of derivative 12 (1.2 g, 1.7 mmol) in CH_2Cl_2 (28 mL) at room temperature. The solution was stirred for 45 min, then evaporated, and the residue was acetylated in a mixture of acetic anhydride and pyridine (1:1, v/v, 18 mL). After 12 h at room temperature, the reaction mixture was cooled to 0 °C, and methanol was added (10 mL). Evaporation of the solution, usual workup and flash chromatography (EtOAc/petroleum ether 1:1, v/v) gave 13 (1.08 g, 95 %). $[\alpha]_D^{25}$ = +68 (c = 0.72 in CHCl_3) (lit: +68);^[18] ^{13}C NMR (75 MHz, CDCl_3) δ = 191.55 (SCOCH_3), 170.28–169.22 ($7 \times \text{OCOCH}_3$), 95.77 (C-1''), 79.74 (C-1'), 72.88/72.76/71.80/69.84/69.33/69.45/67.88 (C-2'–11, C-3'–11, C-4'–11, C-5'–11), 62.61/61.34 (C-6'–11), 31.24 (SCOCH_3), 20.41 (OCOCH_3); DCIMS: m/z = 712 [M^+ + NH_4].

Triphenylmethyl-1-thio- α -D-glucopyranoside (14): This compound was obtained in quantitative yield by Zemplén de-*O*-acetylation of known compound 1 (200 mg, 0.33 mmol) with sodium methoxide (1M 1 % v/v) in MeOH (20 mL). $[\alpha]_D^{25}$ = +216.4 (c = 0.83 in MeOH); ^1H NMR (300 MHz, CDCl_3) see Supporting Information; ^{13}C NMR (100 MHz, CD_3OD) δ = 146.25 (CC_3H_5), 131.32/128.69/127.84 (CC_3H_5), 87.42 (C-1), 76.06 (C-3), 75.11 (C-5), 73.38 (C-2), 71.28 (C-4), 69.99 (CC_3H_5), 62.10 (C-6); ES^+ HRMS: calcd for $\text{C}_{25}\text{H}_{26}\text{O}_5\text{S}$ [M^+ +Na]: 461.1399, found: 461.1402; calcd for $\text{C}_{25}\text{H}_{26}\text{O}_5\text{S}$ [M^+ +K]: 477.1138, found: 477.1155.

Triphenylmethyl (2,3,6-Tri-*O*-acetyl-4-*O*-tetrahydropyranyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl-1-thio- α -D-glucopyranoside (15): CGTase (207 μL) was added to a solution of THP-fluoride 2 (50 mg, 0.12 mmol) and compound 14 (1.2 equiv) in a sodium phosphate buffer (3 mL, 0.1M, pH 7.0). The reaction mixture was gently shaken in an oven at 40 °C for 2 h, freeze-dried and acetylated (acetic anhydride/pyridine 1:1, v/v, 10 mL) in the presence of a trace of dimethylaminopyridine. After 12 h at 70 °C, the reaction mixture was cooled to 0 °C, quenched by adding MeOH (5 mL) and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and washed with water and saturated aq. NaHCO_3 . The organic layers were concentrated, coevaporated with toluene, and purified by flash chromatography (EtOAc/petroleum ether 1:1, v/v) to generate the title compound 15 (127 mg, 86 %). ^{13}C NMR (75 MHz, CDCl_3) δ = 170.69–169.20 (OCOCH_3), 144.35 (CC_3H_5), 129.79/127.80/126.94 (CH CPh_3), 101.14/100.67 (CH THP group), 95.77/95.65 (C-1'–11), 81.66 (C-1'), 76.58/75.28/73.93/73.57/72.59/72.42/71.78/71.56/70.36/70.26/69.79/69.60/69.25/68.76 (C-2'–11, C-3'–11, C-4'–11, C-5'–11), CC_3H_5), 63.91/63.20/63.00/62.54/62.24/62.00 (C-6'–11, C-6''), CH_2 THP group), 20.85–20.46 (OCOCH_3), 20.16/19.53 (CH_2 THP group); ES^+ HRMS: calcd for $\text{C}_{60}\text{H}_{72}\text{O}_{25}\text{S}$ [M^+ +Na]: 1247.3981, found: 1247.3989.

(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio- α -D-glucopyranoside (16): Triethylsilane (35.8 μL , 0.22 mmol) and trifluoroacetic acid (1.2 mL) were added to a stirred solution of the THP derivative 15 (81 mg, 0.66 mmol) in CH_2Cl_2 (2.0 mL) at room temperature. The solution was stirred for 45 min, then evaporated, and the residue was acetylated in a mixture of acetic anhydride/pyridine (1.5:2, v/v, 3.5 mL). After 12 h at room temperature and workup as already described for 13, flash chromatography (EtOAc/petroleum ether 1.5:1, v/v) gave 16 (45 mg, 69 %). $[\alpha]_D^{25}$ = +183 (c = 0.27 in CHCl_3); ^1H NMR (400 MHz, CDCl_3) see Supporting Information; ^{13}C NMR (100 MHz, CDCl_3) δ = 191.51 (SCOCH_3), 170.3–169.2 (OCOCH_3), 96.02 (C-1''), 95.65 (C-1''), 79.75 (C-1'), 73.84 (C-5''), 72.71

(C-3'), 72.47 (C-5^{II}), 71.76 (C-3^{II}, C-4'), 70.34 (C-2^{II}), 70.04 (C-2^{III}), 69.41 (C-2'), 69.35 (C-3^{III}), 68.94 (C-5'), 68.47 (C-4^{II}), 67.86 (C-4^{III}), 62.88 (C-6'), 62.23 (C-6^{II}), 61.32 (C-6^{III}), 31.24 (SCOCH₃), 20.41 (OCOCH₃); ES⁺ HRMS: calcd for C₄₀H₅₄O₂₆S [*M*⁺+Na]: 1005.2522, found: 1005.2519.

(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,di-*O*-acetyl-6-bromo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-*O*-acetyl- β -D-glucopyranose (19): Maltosyl fluoride **2** (250 mg, 0.58 mmol) and the known 6^{II}-bromomaltose^[20] **18** (235 mg, 1 equiv) in phosphate buffer (0.1 M, pH 7.0, 25 mL) were incubated with CGTase (750 μ L) at 40 °C for 1 h, then the mixture was boiled for 5 min and filtered through a cotton plug. The filtrate was acidified down to pH 2.0 with 1% HCl and stirred at room temperature for 20 min. The solution was then neutralised with aqueous ammonia, lyophilised and acetylated under standard conditions. Usual workup and flash chromatography (EtOAc/petroleum ether 2:1.5, *v/v*) gave the acetylated derivative **19** (521 mg, 70 %). ¹H NMR (300 MHz, CDCl₃) δ = 6.62 (d, ³*J* = 3.7 Hz, 1H; H^{1a}-1), 5.69 (d, 1H, ³*J* = 8.0 Hz H^{1b}-1; ¹³C NMR (75 MHz, CDCl₃) δ = 170.56–169.88 (OCOCH₃), 96.01–95.64 (C-1^{II,III,IV}), 91.21 (C-1^{Ib}), 88.81 (C-1^{Ia}), 75.14/74.56/73.63/73.51/72.89/72.40/72.15/71.73/70.99/70.40/70.15/69.97/69.71/69.39/69.18/68.96/68.47/68.01 (C-2^{II,III,IV}, C-3^{II,III,IV}, C-4^{II,III,IV}, C-5^{II,III,IV}), 62.72/61.38 (C-6^{II,III,IV}), 33.38 (C-6^{II}), 20.77–20.48 (OCOCH₃); elemental analysis calcd (%) for C₂₄H₃₃BrO₁₆: C 47.07, H 5.29, Br 6.26; found: C 47.25, H 5.52, Br 6.34.

(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,di-*O*-acetyl-6-bromo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-*O*-acetyl- β -D-glucopyranose (19 β): An aliquot of the anomeric mixture **19** (520 mg, 0.4 mmol) was dissolved in dry CH₂Cl₂ (20 mL) and cooled to 0 °C. HBr (30 % *w/v* in AcOH, 10 mL) was added. After being stirred for 1.5 h, the mixture was diluted in CH₂Cl₂ and washed with ice-cold H₂O and ice-cold saturated aq. NaHCO₃. The resulting crude bromide (500 mg) and AgOAc (707 mg, equiv.) in Ac₂O/AcOH (10 mL, 1:1 *v/v*) were stirred in the dark overnight at room temperature. The mixture was diluted with CH₂Cl₂ and filtered through a Celite bed, and the filtrate was washed with saturated aqueous NaHCO₃, dried and concentrated. Flash chromatography (EtOAc/petroleum ether 1.5:1, *v/v*) gave **19 β** (271 mg, 52 %). α _D²⁵ = +86 (*c* = 0.25 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) see Supporting Information, ¹³C NMR (75 MHz, CDCl₃) δ = 170.42–168.7 (OCOCH₃), 96.01/95.55 (C-1^{II,III,IV}), 91.21 (C-1^{Ib}), 75.14/74.45/73.62/72.91/72.32/71.73/71.07/70.97/70.38/70.31/69.97/69.40/69.16/68.91/68.44/67.98 (C-2^{II,III,IV}, C-3^{II,III,IV}, C-4^{II,III,IV}, C-5^{II,III,IV}), 62.72/61.38 (C-6^{II,III,IV}), 33.35 (C-6^{II}), 20.77–20.51 (OCOCH₃); FABMS: *m/z* = 1299 [*M*⁺+Na].

Methyl (2,3,6-Tri-*O*-acetyl-6-bromo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (22): CGTase (207 μ L) was added to a solution of fluoride **3** (80 mg, 0.163 mmol) and methyl α -D-glucopyranoside **20** (95 mg, 3 equiv) in sodium phosphate buffer (7 mL, 0.1 M, pH 7.0). The solution was incubated for 14 h at 40 °C, and workup was performed as described for compound **19**. Compound **22** (118 mg, 75 %) was isolated after flash chromatography (Et₂O). [α]_D²⁵ = +112.3 (*c* = 0.44 in CHCl₃); ¹H NMR (300 MHz, CDCl₃) see Supporting Information; ¹³C NMR (75 MHz, CDCl₃) δ = 171.2–168.7 (OCOCH₃), 96.58 (C-1'), 95.57/95.42 (C-1^{II,III}), 73.72/72.86 (C-4^{II,III}), 72.56 (C-3'), 71.58 (C-3^{II}), 71.23 (C-2'), 70.53 (C-4^{III}), 70.34/70.12 (C-2^{II,III}), 69.09/69.04/68.98/67.52 (C-3^{III}, C-5^{II,III,III}), 62.96/62.53 (C-6^{II,III}), 55.27 (OCH₃), 31.15 (C-6^{III}), 21.00–20.38 (OCOCH₃); ES⁺ HRMS: calcd for C₃₇H₅₁BrO₂₄ [*M*⁺+Na]: 981.1851, found: 981.1843.

Methyl (2,3,6-Tri-*O*-acetyl-6-iodo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (23): A mixture of bromo derivative **22** (200 mg, 0.21 mmol) and KI (41.54 mg, 1.2 equiv) in DMF (2 mL) was stirred at 70 °C for 2 h. After being cooled to room temperature, the solution was diluted with water and extracted with EtOAc, dried and concentrated under reduced pressure. Flash chromatography (EtOAc/petroleum ether 1:1, *v/v*) gave the expected compound **23** (193 mg, 92 %). [α]_D²⁵ = +52 (*c* = 0.6 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) see Supporting Information; ¹³C NMR (100 MHz, CDCl₃) δ = 170.55–169.75 (OCOCH₃), 96.57/95.57/95.39 (C-1^{II,III,III}), 73.65/73.02/72.59/72.22/71.48/71.26/70.33/69.02/68.81/67.50 (C-2^{II,III,III}, C-3^{II,III,III}, C-4^{II,III,III}, C-5^{II,III,III}), 62.99/62.67 (C-6^{II,III}), 55.36 (OCH₃), 20.90–20.58 (OCOCH₃), 4.06 (C-6^{III}); ES⁺ HRMS: calcd for C₃₇H₅₁IO₂₄ [*M*⁺+Na]: 1029.1713, found: 1029.1694.

Methyl (2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,di-*O*-acetyl-6-bromo-6-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (24): 6^{II}-Bromomaltosyl fluoride **3** (100 mg, 0.20 mmol) and methyl α -D-glucopyranoside **20** (43.5 mg, 1.1 equiv) in phosphate buffer (0.1 M, pH 7.0, 3.7 mL) were incubated with CGTase (130 μ L) at 40 °C for 1 h, then the mixture was boiled for 5 min and filtered through a cotton plug. The filtrate was acidified (pH 2.0) with HCl (1% *v/v*) and stirred at room temperature for 20 min, then neutralised with aqueous ammonia. Fluoride **2** (112 mg, 1.3 equiv) and CGTase (130 μ L) in phosphate buffer (0.1 M, pH 7.0, 1 mL) were added to this solution of crude **21**. After 2 h of incubation, the boiling and the neutralisation steps were repeated, and were followed by acetylation and workup of the mixture as described for the preparation of **19**. Flash chromatography (1: Et₂O, 2: Et₂O/acetone 10:1, *v/v*) gave **19** (92 mg, 36 %) and the expected compound **24** (161 mg, 52 %). [α]_D²⁵ = +105 (*c* = 0.25 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) see Supporting Information; ¹³C NMR (100 MHz, CDCl₃) δ = 170.45–169.56 (OCOCH₃), 96.58/95.87/95.65/95.53 (C-1^{II,III,IV,V}), 76.79/74.18/73.67/72.64/72.34/71.73/71.26/70.36/69.97/69.40/69.11/68.94/68.77/68.45/67.98/67.54 (C-2^{II,III,IV,V}, C-3^{II,III,IV,V}, C-4^{II,III,IV,V}, C-5^{II,III,IV,V}), 63.00/62.71/62.56/61.36 (C-6^{II,III,IV,V}), 55.33 (OCH₃), 33.45 (C-6^{III}), 20.83–20.51 (OCOCH₃); ES⁺ HRMS: calcd for C₆₁H₈₃BrO₄₀ [*M*⁺+Na]: 1557.3542, found: 1557.3542.

(2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-*O*-acetyl- β -D-glucopyranose (25): Compound **13** (251 mg, 0.361 mmol) was added to a solution of bromide **17** (260 mg, 0.34 mmol) in DMF (2 mL) with diethylamine (594 μ L, 5.7 mmol). The reaction mixture was stirred under argon for 12 h at room temperature, then evaporated and coevaporated ($\times$ 3) with toluene. The residue was purified by flash chromatography (EtOAc/petroleum ether 2:1, *v/v*), and **25** was obtained (303 mg, 67 %). [α]_D²⁵ = +121 (*c* = 0.48 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) see Supporting Information; ¹³C NMR (100 MHz, CDCl₃) δ = 170.72–168.84 (OCOCH₃), 165.39 (COC₆H₅), 133.79 (COC₆H₅), 130.02/128.65/128.52 (COC₆H₅), 95.64/95.53 (C-1^{II,IV}), 91.30 (C-1'), 82.29 (C-1^{III}), 75.29/73.02/73.01/71.12/71.00/70.67/70.28/70.14/69.95/69.40/68.46/68.45/67.99 (C-2^{II,III,IV,V}, C-3^{II,III,IV,V}, C-4^{II,III,IV,V}, C-5^{II,III,IV,V}), 62.76/61.43 (C-6^{II,III,IV}), 30.03 (C-6^{II}), 20.88–20.58 (OCOCH₃); ES⁺ HRMS: calcd for C₅₇H₇₂O₃₄S [*M*⁺+Na]: 1355.3523, found: 1355.3516.

(α -D-Glucopyranosyl)-(1 $\rightarrow$ 4)-S-(α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-D-glucopyranose (26): Acylated **25** (200 mg, 0.15 mmol) was de-*O*-acylated by treatment with sodium methoxide (1 M, 5 mL) in methanol (50 mL) for 1 h at room temperature. The mixture was neutralised with Amberlite IR 12(H⁺) resin, the resin was removed by filtration, and the filtrate was concentrated. The residue was dissolved in water and extracted with Et₂O. Freeze-dried compound **26** was obtained (97.3 mg, 95 %). ¹³C NMR (75 MHz, D₂O) δ = 100.13 (C-1^{II,IV}), 96.11 (C-1^{Ib}), 92.19 (C-1^{Ia}), 85.29 (C-1^{III}), 77.55/76.57/75.01/74.29/73.58/73.21/73.05/72.97/72.45/72.10/71.81/71.62/71.19/70.39/69.71 (C-2^{II,III,IV}, C-3^{II,III,IV}, C-4^{II,III,IV}, C-5^{II,III,IV}), 61.51/60.87 (C-6^{II,III,IV}), 30.98 (C-6^{II}); ES⁺ HRMS: calcd for C₂₄H₄₂O₂₆S [*M*⁺+Na]: 705.1888, found: 705.1874.

Methyl (2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(2,3,4-tri-*O*-acetyl-6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-acetyl- α -D-glucopyranoside (27): Compound **13** (83.3 mg, 0.12 mmol) was added to a solution of iodide **23** (101 mg, 0.10 mmol) in DMF (2 mL) with diethylamine (16 μ L, 0.15 mmol). The mixture was treated as already described for the preparation of **25**. Flash chromatography (Et₂O/acetone 10:1, *v/v*) afforded the expected compound **27** (116 mg, 76 %). [α]_D²⁵ = +156 (*c* = 0.42 in CHCl₃); ¹H NMR (400 MHz, CDCl₃) see Supporting Information; ¹³C NMR (75 MHz, CDCl₃) δ = 170.61–169.41 (OCOCH₃), 95.64/96.54/95.62/95.32 (C-1^{II,III,IV,V}), 82.3 (C-1^V), 73.92/73.03/72.49/72.34/72.24/71.90/71.21/70.92/70.43/70.13/69.95/69.88/69.82/69.34/68.94/68.72/68.41/67.99/67.49 (C-2^{II,III,IV,V}, C-3^{II,III,IV,V}, C-4^{II,III,IV,V}, C-5^{II,III,IV,V}), 63.07/62.71/62.69/61.41 (C-6^{II,III,IV,V}), 55.30 (OCH₃), 29.93 (C-6^{III}), 20.78–20.48 (OCOCH₃); ES⁺ HRMS: calcd for C₆₃H₈₆O₄₁S [*M*⁺+Na]: 1553.4263, found: 1553.4260; calcd for C₆₃H₈₆O₄₁S [*M*⁺+K]: 1569.4002, found: 1569.3976.

Methyl (α -D-Glucopyranosyl)-(1 $\rightarrow$ 4)-S-(α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)- α -D-glucopyranoside (28): De-*O*-acetylation of **27** (54.5 mg, 35.6 μ mol) by treatment

with sodium methoxide (1M, 500 μ L) in methanol (20 mL), then neutralisation with resin (H^+) and lyophilisation gave **28** in quantitative yield (31 mg). $[\alpha]_D^{25} = +206$ ($c = 0.37$ in water); ^{13}C NMR (125 MHz, D_2O) $\delta = 99.99/99.66/99.30$ ($C-1^{III,IV,V}$), 85.12 ($C-1^{IV}$), 77.35/77.25/76.69/74.14/73.53/73.06/72.91/72.81/72.28/71.95/71.70/71.46/71.23/71.05/70.28/69.52 ($C-2^{II,III,IV,V}$, $C-3^{II,III,IV,V}$, $C-4^{II,III,IV,V}$, $C-5^{II,III,IV,V}$), 61.09/60.71 ($C-6^{II,IV,V}$), 55.30 (OCH_3), 30.78 ($C-6^{III}$); ES $^+$ HRMS: calcd for $C_{31}H_{54}O_{25}S$ [$M^+ + Na$]: 881.2773, found: 881.2576; calcd for $C_{31}H_{54}O_{25}S$ [$M^+ - H + 2Na$]: 903.2392, found: 903.2393.

Methyl (2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(2,3,4-tri-O-acetyl-6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-acetyl- α -D-glucopyranoside (29): A solution of **23** (40 mg, 39.7 mmol) was treated with **16** (46.2 mg, 1.2 equiv) as described for the preparation of **25**, but in the presence of 1,4-dithioerythritol (250 mg). After being stirred for 12 h at room temperature under argon, the reaction mixture was acetylated (pyridine/ Ac_2O , 2 mL, 1:1, v/v). Workup as described for **13** and flash chromatography (EtOAc/petroleum ether 3:1, v/v) gave the expected compound **29** (52 mg, 76%). $[\alpha]_D^{25} = +158$ ($c = 0.44$ in $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.66 - 169.54$ ($OCOCH_3$), 96.57/95.78/95.65/95.61/95.32 ($C-1^{II,III,IV,V}$), 82.26 ($C-1^{IV}$), 73.94/73.86/72.54/72.43/72.21/72.17/71.97/71.74/71.25/70.92/70.46/70.36/70.13/70.02/69.92/69.88/69.37/68.97/68.89/68.73/68.43/68.40/67.91/67.50 ($C-2^{II,III,IV,V,VI}$, $C-3^{II,III,IV,V,VI}$, $C-4^{II,III,IV,V,VI}$, $C-5^{II,III,IV,V,VI}$), 63.05/62.88/62.72/62.23 ($C-6^{II,IV,V,VI}$), 55.33 (OCH_3), 29.89 ($C-6^{III}$), 20.83 - 20.36 ($OCOCH_3$); ES $^+$ HRMS: calcd for $C_{75}H_{102}O_{49}S$ [$M^+ + Na$]: 1841.5108, found: 1841.5106

Methyl (α -D-Glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-(α -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)- α -D-glucopyranoside (30): De-O-acetylation of **29** (40.0 mg, 21.2 μ mol) by treatment with sodium methoxide (1M, 200 μ L) in methanol (20 mL), and treatment as described for the preparation of **28** gave **30** in quantitative yield (22 mg). $[\alpha]_D^{25} = +186$ ($c = 0.41$ in water); ^{13}C NMR (100 MHz, D_2O) $\delta = 100.18/100.14/100.00/99.84/99.48$ ($C-1^{II,III,IV,V}$), 85.31 ($C-1^{IV}$), 77.64/77.61/77.44/77.12/74.28/73.88/73.71/73.24/73.09/72.99/72.44/72.19/72.12/71.94/71.879/71.65/71.60/71.40/71.23/71.16/70.46/69.69 ($C-2^{II,III,IV,V,VI}$, $C-3^{II,III,IV,V,VI}$, $C-4^{II,III,IV,V,VI}$, $C-5^{II,III,IV,V,VI}$), 61.28/60.85 ($C-6^{II,IV,V,VI}$), 55.45 (OCH_3), 30.97 ($C-6^{III}$); ES $^+$ HRMS: calcd for $C_{37}H_{64}O_{30}S$ [$M^+ + Na$]: 1043.3101, found: 1043.3001

(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-[(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)]-(2,3,di-O-acetyl-6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl- β -D-glucopyranose (31): A mixture of bromo derivative **19** (472 mg, 0.37 mmol) and KI (95 mg, 1.5 equiv) in DMF (2.5 mL) was stirred at 70 $^{\circ}C$ for 2 h under argon. After the mixture had been cooled to 0 $^{\circ}C$, acetylated 1-thio- α -maltose **13** (550 mg, 7.9 mmol), 1,4-dithioerythritol (62 mg) and diethylamine (125 μ L, 1.2 mmol) were added. After it had been stirred at room temperature for 12 h, the solution was concentrated, and the residue was acetylated (pyridine/ Ac_2O , 17 mL, 10:7, v/v) in the presence of a catalytic amount of DMAP at 70 $^{\circ}C$ for 2 h. Following incubation, the reaction mixture was cooled (0 $^{\circ}C$), and MeOH (7 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 and washed with water, saturated aq. sodium hydrogen carbonate and aq. $KHSO_4$ (10%). The organic phase was dried and concentrated under reduced pressure. Flash chromatography (1: Et $_2$ O, 2: Et $_2$ O/acetone 10:1, v/v) gave the expected compound **31** (490 mg, 72%). 1H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.67 - 168.48$ ($OCOCH_3$), 95.94/95.64 ($C-1^{II,III,IV,V}$), 91.22 ($C-1^{IV}$), 88.87 ($C-1^{IV}$), 83.29/83.20 ($C-1^{IV,V,VI}$), 75.34/74.76/72.85/72.62/72.32/72.10/71.80/71.19/71.02/70.31/70.01/69.87/69.72/69.34/69.17/68.63/68.42/67.93 ($C-2^{II,III,IV,V,VI}$, $C-3^{II,III,IV,V,VI}$, $C-4^{II,III,IV,V,VI}$, $C-5^{II,III,IV,V,VI}$), 62.68/61.35 ($C-6^{II,IV,V,VI}$), 30.25/30.22 ($C-6^{IIa,IIb}$), 20.86 - 20.54 ($OCOCH_3$); ES $^+$ HRMS: calcd for $C_{76}H_{102}O_{30}S$ [$M^+ + Na$]: 1869.5057, found: 1869.5057.

(2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-[(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)]-(2,3,di-O-acetyl-6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl- β -D-glucopyranose (31 β): Diethylamine (6.4 μ L) was added to a solution of **19 β** (44 mg, 34.5 μ mol) and **13** (26.2 mg, 37.7 μ mol) in DMF (473 μ L). After being stirred under argon at room temperature for 4 h, the reaction mixture was

precipitated by the addition of ice-cold water. The precipitate was filtered on Celite, dissolved in CH_2Cl_2 and dried. Flash chromatography as described for **31** gave **31 β** (28 mg, 43%). $[\alpha]_D^{25} = +374$ ($c = 0.75$ in $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 170.63 - 168.72$ ($OCOCH_3$), 95.98/95.70/95.64 ($C-1^{II,III,IV,VI}$), 91.22 ($C-1^{IV}$), 83.16 ($C-1^{IV}$), 75.32/74.90/72.98/72.87/72.65/72.10/71.80/71.02/70.30/69.99/69.35/69.17/68.64/68.45/67.97 ($C-2^{II,III,IV,V,VI}$, $C-3^{II,III,IV,V,VI}$, $C-4^{II,III,IV,V,VI}$, $C-5^{II,III,IV,V,VI}$), 62.69/61.36 ($C-6^{II,IV,V,VI}$), 30.26 ($C-6^{IIa,IIb}$), 20.83 - 20.51 ($OCOCH_3$); FABMS: $m/z = 1870$ [$M^+ + Na$].

(α -D-Glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-[(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 6)]-(6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-D-glucopyranose (32): An aliquot of sodium methoxide (1M, 1 mL) was added to a solution of **31** (300 mg, 0.16 mmol) in methanol (30 mL). The mixture was stirred for 2 h at room temperature, neutralised with Amberlite IRN 120 (H^+), concentrated, diluted with water and freeze dried. The residue, diluted with water, was filtered through a C18 cartridge Sep-pak Plus (Waters) and pure **32** was obtained in 96% yield (157 mg). ^{13}C NMR (100 MHz, D_2O) $\delta = 99.24/99.22/99.18/98.87/98.71$ ($C-1^{II,III,IV,VI}$), 95.22 ($C-1^{IV}$), 91.31 ($C-1^{IV}$), 84.76 ($C-1^{IV}$), 79.57/76.77/76.47/76.45/76.15/75.66/74.09/73.44/73.37/72.74/72.67/72.46/71.20/71.18/71.06/70.96/70.81/70.72/70.34/70.26/69.56/69.46/68.79/68.72 ($C-2^{II,III,IV,V,VI}$, $C-3^{II,III,IV,V,VI}$, $C-4^{II,III,IV,V,VI}$, $C-5^{II,III,IV,V,VI}$), 60.52/60.38/59.94 ($C-6^{II,III,IV,V,VI}$), 30.13 ($C-6^{II}$); ES $^+$ HRMS: calcd for $C_{36}H_{62}O_{30}S$ [$M^+ + Na$]: 1029.2944, found: 1029.2946; calcd for $C_{36}H_{62}O_{30}S$ [$M^+ + K$]: 1045.2684, found: 1045.2773.

Methyl (2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-[(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 6)]-(2,3,di-O-acetyl-6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-acetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-acetyl- α -D-glucopyranoside (33): Methyl 6-bromo-maltopentaoside **24** (50 mg, 32.5 mmol) and then thio maltose **13** (34 mg, 48.7 μ mol) were treated as already described for **19** and **13** during the preparation of **31**. The expected compound **33** was obtained (36 mg, 53%). $[\alpha]_D^{25} = +188$ ($c = 0.32$ in $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.66 - 169.50$ ($OCOCH_3$), 96.58/95.92/95.68/95.60/95.40 ($C-1^{II,III,IV,V,VI}$), 83.47 ($C-1^{IV}$), 74.49/73.72/72.99/72.82/72.64/72.07/71.95/71.82/71.31/71.21/70.88/70.43/70.32/70.23/70.03/69.87/69.35/69.15/68.75/68.66/68.43/67.92/67.53 ($C-2^{II,III,IV,V,VI,VI}$, $C-3^{II,III,IV,V,VI,VI}$, $C-4^{II,III,IV,V,VI,VI}$, $C-5^{II,III,IV,V,VI,VI}$), 62.99/62.83/62.71/62.61/61.35 ($C-6^{II,IV,V,VI,VI}$), 55.37 (OCH_3), 29.36 ($C-6^{III}$), 20.90 - 20.59 ($OCOCH_3$); ES $^+$ HRMS: calcd for $C_{87}H_{118}O_{57}S$ [$M^+ + Na$]: 2129.5953, found: 2129.5977.

Methyl (α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-S-[(α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(α -D-glucopyranosyl)-(1 $\rightarrow$ 6)]-(6-thio- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-D-glucopyranosyl)-(1 $\rightarrow$ 4)-D-glucopyranoside (34): Compound **33** (20 mg, 0.94 μ mol) was de-O-acetylated as already described for the preparation of **32**. The expected compound **34** was obtained (11 mg, 99%). $[\alpha]_D^{25} = +174$ ($c = 0.15$ in water); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 100.12/99.77/99.45$ ($C-1^{II,III,IV,V,VI}$), 85.65 ($C-1^{IV}$), 80.4/77.37/77.22/77.01/74.27/73.86/73.65/73.37/73.21/73.06/72.08/71.86/71.70/71.57/71.39/71.25/71.15/70.51/70.40/69.65 ($C-2^{II,III,IV,V,VI,VI}$, $C-3^{II,III,IV,V,VI,VI}$, $C-4^{II,III,IV,V,VI,VI}$, $C-5^{II,III,IV,V,VI,VI}$), 61.16 - 60.81 ($C-6^{II,IV,V,VI,VI}$), 55.42 (OCH_3), 30.00 ($C-6^{III}$); ES $^+$ HRMS: calcd for $C_{43}H_{74}O_{35}S$ [$M^+ + Na$]: 1205.3629, found: 1205.3635; calcd for $C_{43}H_{74}O_{35}S$ [$M^+ + K$]: 1227.3449, found: 1227.3460.

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