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 compounds exhibit K_i values in the 2.5 – 1350 μM 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*.

Keywords: active-site mapping · carbohydrates · enzymatic synthesis · enzymes · oligosaccharides

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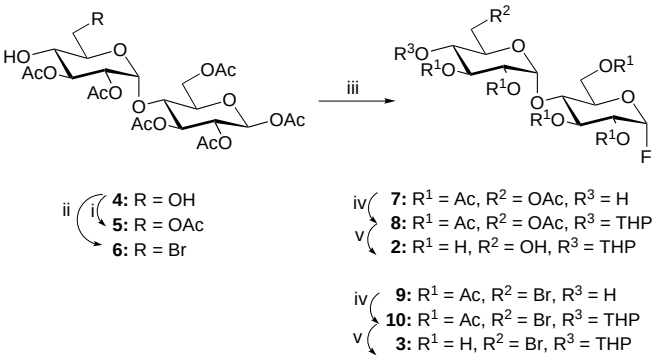
chemical syntheses of branched oligosaccharides representing the branch point of amylopectin have been reported.[3, 4]

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.

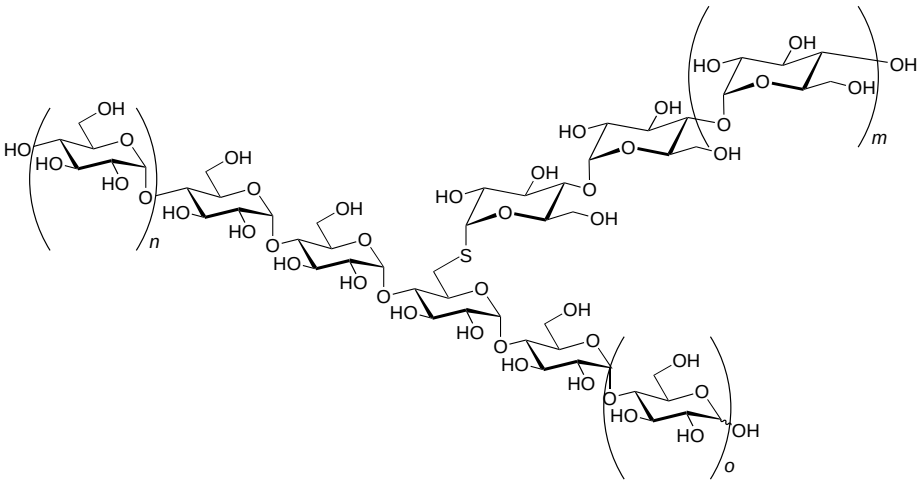
The 6-thio-isomaltosyl motif is the common disaccharidic unit of all the compounds prepared in this study. Previous work has demonstrated that *S*-α-D-glucopyranosyl-6$^{\omega}$-thiomaltooligosaccharides can be synthesised in high yield[7] by the nucleophilic displacement of the halide of 6$^{\omega}$-iodomaltooligosaccharides 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 4^{II}-OH of the maltosyl fluorides. Since 4^{II}-*O*-tetrahydropyranyl-α-cellobiosyl fluoride was previously used successfully for enzymatic syntheses of β-1,4-oligosaccharides,[10, 11] 4^{II}-*O*-tetrahydropyranyl-α-maltosyl fluoride (**2**) and 6^{II}-bromo-4^{II}-*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, CH_2Cl_2, RT (80%); ii) $P(C_6H_5)_3$, pyridine, CBr_4, 0 °C to 50 °C (84%); iii) HF/pyridine, 0 °C (95% for **7**, 90% for **9**); iv) dihydropyran, camphorsulfonic acid, CH_2Cl_2, 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 4^{II}-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 thioglycosylation, 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.

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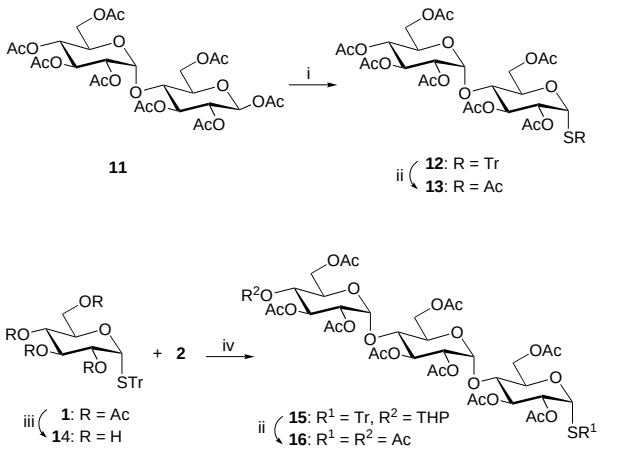
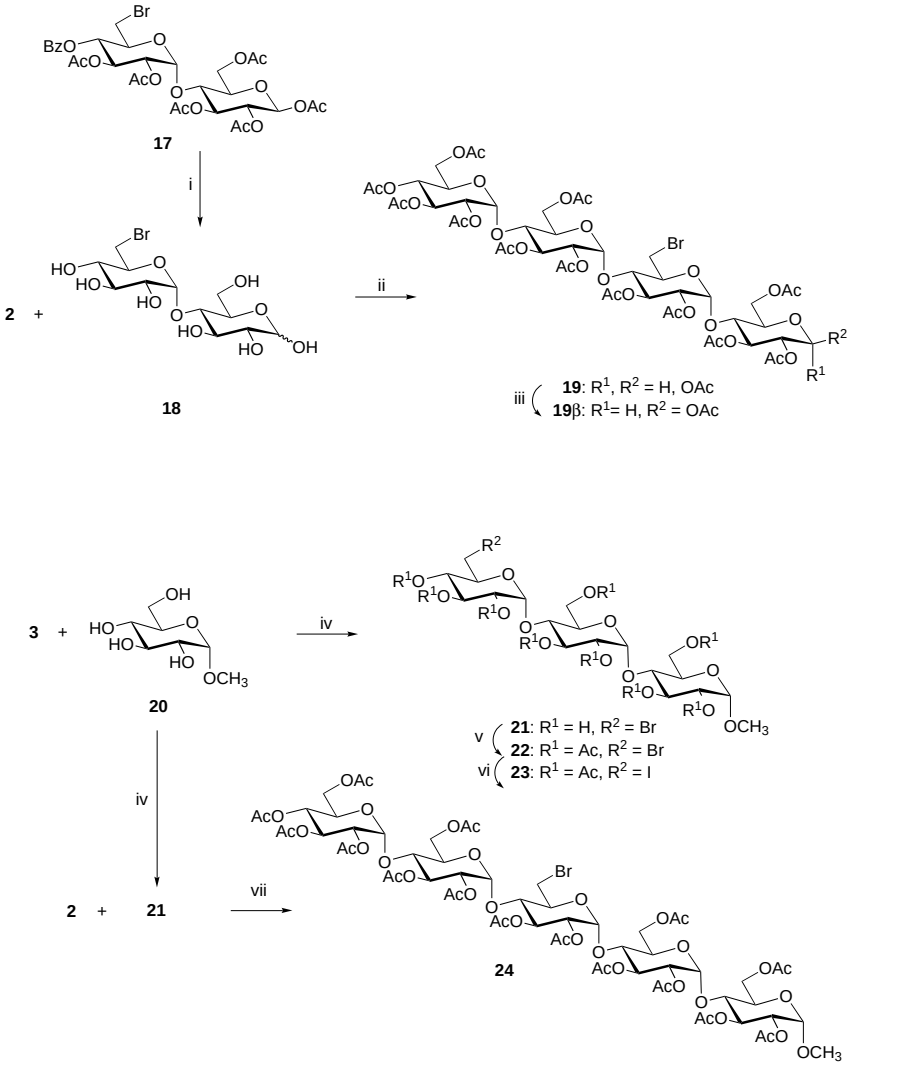
tyl-1-thio-α-maltoside (**12**) in 31% yield (Scheme 3). This compound was then transformed into the known donor **13**,[18] by treatment with triethylsilane[19] and trifluoroacetic acid (TFA) in dichloromethane followed by acetylation, with a yield of 95%. For the synthesis of the corresponding trisaccharide **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$^{\omega}$-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*-acylation. 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$^{\omega}$-iodomaltooligosaccharides, result-



Scheme 3. Syntheses of the 1-*S*-acetyl-1-thio-glycoses **13** and **16**. i) $AlCl_3$, $CHCl_3$, 0 °C to RT, then $TrSN(Bu)_4$, toluene, RT (31%); ii) $(Et)_3SiH$, TFA, CH_2Cl_2, RT, then Ac_2O, pyridine, RT (95% for **13**, 69% for **16**); iii) MeONa, MeOH, RT (100%); iv) CGTase, phosphate buffer, 40 °C, then Ac_2O, 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) Ac_2O, pyridine, DMAP (70% overall); iii) HBr/Ac_2O, CH_2Cl_2, 0 °C, then AgOAc, AcOH, Ac_2O, RT, (52%); iv) a) CGTase, phosphate buffer, 40 °C; b) HCl (1M), RT; v) Ac_2O, pyridine, DMAP (75%); vi) KI, DMF, 70 °C, (92%); vii) a) CGTase, phosphate buffer, 40 °C; b) HCl (1M), RT; c) Ac_2O, pyridine, DMAP (52% overall).

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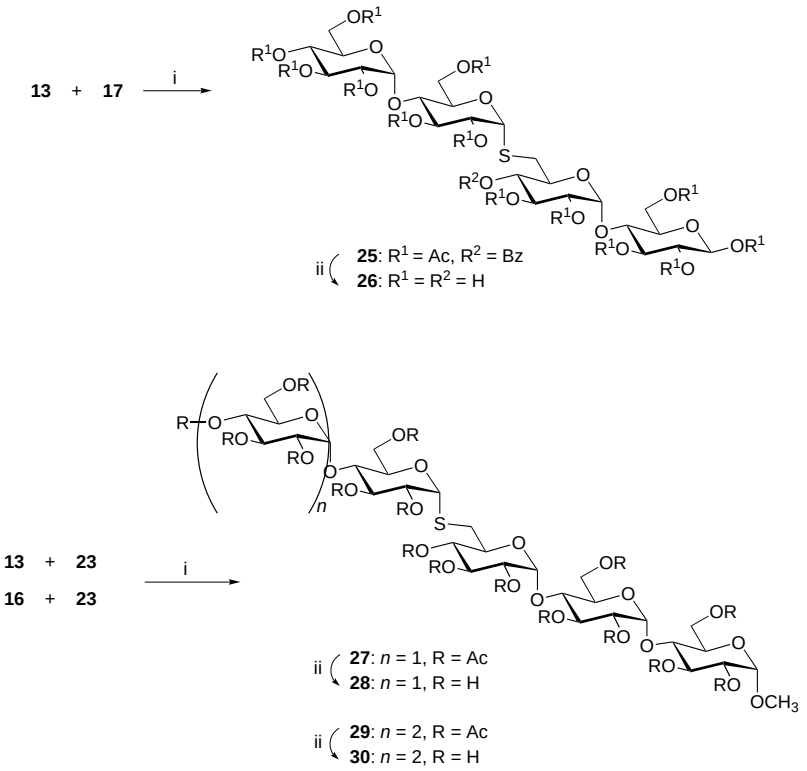
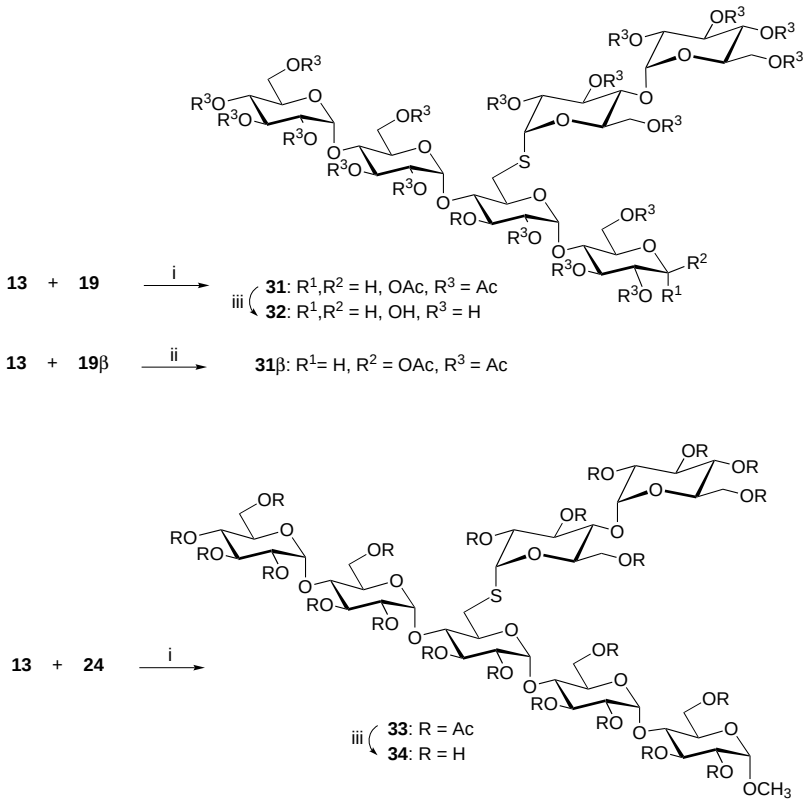
ing in the corresponding hemithiomaltodextrins in high yield.[6] It was also shown that *N*,*N*-dimethylformamide (DMF) was a good solvent for the effective substitution of tosylate and bromide at C-6 of mono- and oligosaccharides, while the donor was *S*-deacetylated and activated in situ with diethylamine (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 maltotrioside **22**, an exchange of halogen (Br→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) Ac_2O, 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**).

0947-6539/02/0823-5450 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 23

The structures of the compounds were confirmed by ^{1}H 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	Pullulanase		
		Kp	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 μM. 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 μM. Compounds **28** and **30** affected Kp with K_i values of about 300 μM and 200 μM, 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 μM. Only compound **30** has significant a inhibitory effect on barley limit dextrinase with a K_i value of about 500 μM. 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 μM and 545 μM, 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 AC 300, Bruker Avance 400 or Varian Unity 500. 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 bicinconitate method essentially as described.[22, 23] K_m and k_{cat} were determined in the same assay by using pullulan (0.06 to 10 mg mL^{-1}) and found to be 0.16 mg mL^{-1} and 33 s^{-1} for barley limit dextrinase, 0.24 mg mL^{-1} and 120 s^{-1} for Ba, and 0.09 mg mL^{-1} and 81 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 mg mL^{-1} for limit dextrinase and 0.25 mg mL^{-1} for Ba and Kp), and [I] is the concentration of inhibitor (0–2 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 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 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→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] ^{1}H NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 171.1 - 168.7$ ($7 \times OCOCH_3$), 95.75 (C-1^{II}), 91.09 (C-1^{I}), 74.94, 72.88, 72.31, 71.46, 70.84, 70.77, 70.03, 68.47 (C-2^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}), 62.37 (C-6^{I,II}), 20.53–20.26 ($OCOCH_3$); elemental analysis calcd (%) for $C_{26}H_{36}O_{18}$: 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→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 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) see Supporting Information;

^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 171.1-168.8$ ($6\times OCOCH_3$), 95.67 (C-1^{I}), 91.18 (C-1^{II}), 74.96, 73.12, 72.44, 71.73, 71.28, 70.82, 70.72, 70.14 (C-2^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}), 62.61 (C-6^{I}), 32.66 (C-6^{II}), 20.75–20.38 ($OCOCH_3$); elemental analysis calcd (%) for $C_{24}H_{33}BrO_{16}$: 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→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, 3 M). 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 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 171.3-169.7$ ($6\times OCOCH_3$), 103.56 (d, $^1J_{CF} = 229.3$ Hz, C-1^{I}), 95.84 (C-1^{II}), 71.78/71.66/71.58/71.21/70.72/70.30/69.99/68.69 (C-2^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}), 62.36/62.12 (C-6^{I}, C-6^{II}), 20.71/20.41 ($OCOCH_3$); elemental analysis calcd (%) for $C_{24}H_{33}FO_{16}$: 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→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, $CDCl_3$) $\delta = 170.54-169.31$ ($6\times OCOCH_3$), 103.56 (d,$^1J_{CF} = 229.3$ Hz, C-1^{I}), 102.05/101.62 (*C*H THP group), 95.72 (C-1^{II}), 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^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}), 64.03/63.34/62.61/62.14/62.1/62.02 (C-6^{I}, C-6^{II} R and S, *C*H_2 THP group), 20.76–20.41 ($OCOCH_3$), 20.09/19.67 (*C*H_2 THP group); DCIMS: *m*/*z*: 698 [M^++NH_4]; elemental analysis calcd (%) for $C_{29}H_{41}FO_{17}$: 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→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 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 171.33/169.76$ ($5\times OCOCH_3$), 103.54 (d, $^1J_{CF} = 227.8$ Hz, C-1^{I}), 95.72 (C-1^{II}), 71.80/71.31/70.7/70.35/70.06 (C-2^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}), 62.17 (C-6^{I}), 32.71 (C-6^{II}), 20.9–20.36 ($OCOCH_3$); ES$^+$ HRMS: calcd for $C_{22}H_{30}BrFO_{14}$ [M^++Na]: 639.0701, found: 639.0705.

(2,3,6-Tri-*O*-acetyl-6-bromo-6-deoxy-4-*O*-tetrahydropyranyl-α-D-glucopyranosyl)-(1→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 $C_{27}H_{38}BrFO_{15}$ [M^++Na]: 723.1276, found: 723.1281.

(4-*O*-Tetrahydropyranyl-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranosyl fluoride (2): Fluoride **8** (1.55 g, 2.28 mmol) in methanol (50 mL) was treated with sodium methoxide (1 M, 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 $C_{17}H_{29}FO_{11}$ [M^++Na]: 451.1592, found: 451.1596.

(6-Bromo-6-deoxy-4-*O*-tetrahydropyranyl-α-D-glucopyranosyl)-(1→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 $C_{17}H_{28}BrFO_{10}$ [M^++Na]: 513.0748, found: 513.0747.

Triphenylmethyl (2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→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 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.62-169.39$ ($7\times OCOCH_3$), 144.32 (*C*C_5H_5), 129.82/127.88/127.06 (C*C*$_5H_5$), 95.70 (C-1^{II}), 81.84 (C-1^{I}), 73.42 (C-4^{I}), 72.96 (C-3^{I}), 70.26 (C2^{I}), 69.95 (C-2^{II}), 69.79 (C-5^{I}), 69.32 (C-3^{II}, C*C*$_5H_5$), 68.33 (C-5^{II}), 67.95 (C-4^{II}), 62.77 (C-6^{I}), 61.31 (C-6^{II}), 20.95–20.56 ($OCOCH_3$); ES$^+$ HRMS: calcd for $C_{45}H_{50}O_{17}S$ [M^++Na]: 917.2666, found: 917.2667.

(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (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, $CDCl_3$) $\delta = 191.55$ (S*C*OCH_3), 170.28–169.22 ($7\times OCOCH_3$), 95.77 (C-1^{II}), 79.74 (C-1^{I}), 72.88/72.76/71.80/69.84/69.33/69.45/67.88 (C-2^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}), 62.61/61.34 (C-6^{I,II}), 31.24 (SCO*C*H_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 Zemplèn de-*O*-acetylation of known compound **1** (200 mg, 0.33 mmol) with sodium methoxide (1 M 1 % *v*/*v*) in MeOH (20 mL). $[\alpha]_D^{25} = +216.4$ ($c = 0.83$ in MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, CD_3OD) $\delta =$ 146.25 (*C*C_5H_5), 131.32/128.69/127.84 (C*C*$_5H_5$), 87.42 (C-1), 76.06 (C-3), 75.11 (C-5), 73.38 (C-2), 71.28 (C-4), 69.99 (C*C*$_5H_5$), 62.10 (C-6); ES$^+$ HRMS: calcd for $C_{25}H_{26}O_5S$ [M^++Na]: 461.1399, found: 461.1402; calcd for $C_{25}H_{26}O_5S$ [M^++K]: 477.1138, found: 477.1155.

Triphenylmethyl (2,3,6-Tri-*O*-acetyl-4-*O*-tetrahydropyranyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→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, 0.1 M, 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, $CDCl_3$) $\delta = 170.69-169.20$ ($OCOCH_3$), 144.35 (*C*C_5H_5), 129.79/127.80/126.94 (CH CPh_3), 101.14/100.67 (*C*H THP group), 95.77/95.65 (C-1^{II,III}), 81.66 (C-1^{I}), 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^{I,II,III}, C-3^{I,II,III}, C-4^{I,II,III}, C-5^{I,II,III}, C*C*$_5H_5$), 63.91/63.20/63.00/62.54/62.24/62.00 (C-6^{I,II}, C-6^{III}, *C*H_2 THP group), 20.85–20.46 ($OCOCH_3$), 20.16/19.53 (*C*H_2 THP group); ES$^+$ HRMS: calcd for $C_{60}H_{72}O_{25}S$ [M^++Na]: 1247.3981, found: 1247.3989.

(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (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 $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 191.51$ (S*C*OCH_3), 170.3–169.2 ($OCOCH_3$), 96.02 (C-1^{II}), 95.65 (C-1^{III}), 79.75 (C-1^{I}), 73.84 (C-5^{III}), 72.71

0947-6539/02/0823-5452 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 23

(C-3^{I}), 72.47 (C-5^{II}), 71.76 (C-3^{II}, C-4^{I}), 70.34 (C-2^{II}), 70.04 (C-2^{III}), 69.41 (C-2^{I}), 69.35 (C-3^{III}), 68.94 (C-5^{I}), 68.47 (C-4^{II}), 67.86 (C-4^{III}), 62.88 (C-6^{I}), 62.23 (C-6^{II}), 61.32 (C-6^{II}), 31.24 ($SCOCH_3$), 20.41 ($OCOCH_3$); ES^+ HRMS: calcd for $C_{40}H_{54}O_{26}S$ [M^++Na]: 1005.2522, found: 1005.2519.

(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,di-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-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 (0.1M, pH 7.0, 25 mL) were incubated with CGTase (750 μL)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‰M 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%). ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 6.62$ (d, $^3J = 3.7$ Hz, 1H; H$^{1\alpha}$-1), 5.69 (d, 1H, $^3J = 8.0$ Hz H$^{1\beta}$-1; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta =$ 170.56–169.88 ($OCOCH_3$), 96.01–95.64 (C-1^{II,III,IV}), 91.21 (C-1$^{I\beta}$), 88.81 (C-1$^{I\alpha}$), 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^{I,II,III,IV}, C-3^{I,II,III,IV}, C-4^{I,II,III,IV}, C-5^{I,II,III,IV}), 62.72/61.38 (C-6^{I,III,IV}), 33.38 (C-6^{II}), 20.77–20.48 ($OCOCH_3$); elemental analysis calcd (%) for $C_{24}H_{33}BrO_{16}$: 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→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,di-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl)-(1→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 CH_2Cl_2 (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 CH_2Cl_2 and washed with ice-cold H_2O and ice-cold saturated aq. $NaHCO_3$. The resulting crude bromide (500 mg) and AgOAc (707 mg, equiv.) in Ac_2O/AcOH (10 mL, 1:1 *v*/*v*) were stirred in the dark overnight at room temperature. The mixture was diluted with CH_2Cl_2 and filtered trough a Celite bed, and the filtrate was washed with saturated aqueous $NaHCO_3$, dried and concentrated. Flash chromatography (EtOAc/petroleum ether 1.5:1, *v*/*v*) gave **19*β*** (271 mg, 52%). $[\alpha]_D^{25} = +86$ (*c* = 0.25 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information, ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 170.42-168.7$ ($OCOCH_3$), 96.01/95.55 (C-1^{II,III,IV}), 91.21 (C-1$^{I\beta}$), 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^{I,II,III,IV}, C-3^{I,II,III,IV}, C-4^{I,II,III,IV}, C-5^{I,II,III,IV}), 62.72/61.38 (C-6^{I,III,IV}), 33.35 (C-6^{II}), 20.77–20.51 ($OCOCH_3$); FABMS: *m*/*z* = 1299 [M^++Na].

Methyl (2,3,6-Tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (22): CGTase (207 μL) 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, 0.1M, 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 (Et_2O). $[\alpha]_D^{25} = +112.3$ (*c* = 0.44 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 171.2-168.7$ ($OCOCH_3$), 96.58 (C-1^{I}), 95.57/95.42 (C-1^{II,III}), 73.72/72.86 (C-4^{I,II}), 72.56 (C-3^{I}), 71.58 (C-3^{II}), 71.23 (C-2^{I}), 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^{I,II,III}), 62.96/62.53 (C-6^{I,II}), 55.27 (OCH_3), 31.15 (C-6^{III}), 21.00–20.38($OCOCH_3$); ES^+ HRMS: calcd for $C_{37}H_{51}BrO_{24}$ [M^++Na]: 981.1851, found: 981.1843.

Methyl (2,3,6-Tri-*O*-acetyl-6-iodo-6-deoxy-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→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%). $[\alpha]_D^{25} = +52$ (*c* = 0.6 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.55-169.75$ ($OCOCH_3$), 96.57/95.57/95.39 (C-1^{I,II,III}), 73.65/73.02/72.59/72.22/71.48/71.26/70.33/69.02/68.81/67.50 (C-2^{I,II,III}, C-3^{I,II,III}, C-4^{I,II,III}, C-5^{I,II,III}), 62.99/62.67 (C-6^{I,II}), 55.36 (OCH_3), 20.90–20.58 ($OCOCH_3$), 4.06 (C-6^{III}); ES^+ HRMS: calcd for $C_{37}H_{51}IO_{24}$ [M^++Na]: 1029.1713, found: 1029.1694.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,di-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→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 (0.1M, pH 7.0, 3.7 mL) were incubated with CGTase (130 μL)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‰M) and stirred at room temperature for 20 min, then neutralised with aqueous ammonia. Fluoride **2** (112 mg, 1.3 equiv) and CGTase (130 μL) in phosphate buffer (0.1M, 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: Et_2O, 2: Et_2O/acetone 10:1, *v*/*v*) gave **19** (92 mg, 36%) and the expected compound **24** (161 mg, 52%). $[\alpha]_D^{25} = +105$ (*c* = 0.25 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta =$ 170.45–169.56 ($OCOCH_3$), 96.58/95.87/95.65/95.53 (C-1^{I,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^{I,II,III,IV,V}, C-3^{I,II,III,IV,V}, C-4^{I,II,III,IV,V}, C-5^{I,II,III,IV,V}), 63.00/62.71/62.56/61.36 (C-6^{I,II,IV,V}), 55.33 (OCH_3), 33.45 (C-6^{III}), 20.83–20.51 ($OCOCH_3$); ES^+ HRMS: calcd for $C_{61}H_{83}BrO_{40}$ [M^++Na]: 1557.3542, found: 1557.3542.

(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*S*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-(2,3-di-*O*-acetyl-4-*O*-benzoyl-6-thio-α-D-glucopyranosyl)-(1→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 μL, 5.7 mmol). The reaction mixture was stirred under argon for 12 h at room temperature, then evaporated and coevaporated (×3) with toluene. The residue was purified by flash chromatography (EtOAc/petroleum ether 2:1, *v*/*v*), and **25** was obtained (303 mg, 67%). $[\alpha]_D^{25} = +121$ (*c* = 0.48 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.72-168.84$ ($OCOCH_3$), 165.39 (COC_6H_5), 133.79 ($COCC_5H_5$), 130.02/128.65/128.52 ($COCC_5H_5$), 95.64/95.53 (C-1^{II,IV}), 91.30 (C-1^{I}), 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^{I,II,III,IV}, C-3^{I,II,III,IV}, C-4^{I,II,III,IV}, C-5^{I,II,III,IV}), 62.76/61.43 (C-6^{I,III,IV}), 30.03 (C-6^{II}), 20.88–20.58 ($OCOCH_3$); ES^+ HRMS: calcd for $C_{57}H_{72}O_{34}S$ [M^++Na]: 1355.3523, found: 1355.3516.

(α-D-Glucopyranosyl)-(1→4)-*S*-(α-D-glucopyranosyl)-(1→6)-(6-thio-α-D-glucopyranosyl)-(1→4)-D-glucopyranose (26): Acylated **25** (200 mg, 0.15 mmol) was de-*O*-acylated by treatment with sodium methoxide (1M, 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 Et_2O. Freeze-dried compound **26** was obtained (97.3 mg, 95%). ^{13}C NMR (75 MHz, D_2O) $\delta = 100.13$ (C-1^{II,IV}), 96.11 (C-1$^{I\beta}$), 92.19 (C-1$^{I\alpha}$), 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^{I,II,III,IV}, C-3^{I,II,III,IV}, C-4^{I,II,III,IV}, C-5^{I,II,III,IV}), 61.51/60.87 (C-6^{I,III,IV}), 30.98 (C-6^{II}); ES^+ HRMS: calcd for $C_{24}H_{42}O_{20}S$ [M^++Na]: 705.1888, found: 705.1874.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*S*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-6-thio-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→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 μL, 0.15 mmol). The mixture was treated as already described for the preparation of **25**. Flash chromatography (Et_2O/acetone 10:1, *v*/*v*) afforded the expected compound **27** (116 mg, 76%). $[\alpha]_D^{25} = +156$ (*c* = 0.42 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) see Supporting Information; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 170.61-169.41$ ($OCOCH_3$), 95.64/96.54/95.62/95.32 (C-1^{I,II,III,V}), 82.3 (C-1^{IV}), 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^{I,II,III,IV,V}, C-3^{I,II,III,IV,V}, C-4^{I,II,III,IV,V}, C-5^{I,II,III,IV,V}), 63.07/62.71/62.69/61.41 (C-6^{I,II,IV,V}), 55.30 (OCH_3), 29.93 (C-6^{III}), 20.78–20.48 ($OCOCH_3$); ES^+ HRMS: calcd for $C_{63}H_{86}O_{41}S$ [M^++Na]: 1553.4263, found: 1553.4260; calcd for $C_{63}H_{86}O_{41}S$ [M^++K]: 1569.4002, found: 1569.3976.

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

with sodium methoxide (1M, 500 μL) 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) δ = 99.99/99.66/99.30 (C-$1^{I,II,III,V}$), 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-$2^{I,II,III,IV,V}$, C-$3^{I,II,III,IV,V}$, C-$4^{I,II,III,IV,V}$, C-$5^{I,II,III,IV,V}$), 61.09/60.71 (C-$6^{I,II,IV,V}$), 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+2 Na]: 903.2392, found: 903.2393.

Methyl (2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)(1→4)-*S*-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-6-thio-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→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-dithioerytritol (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, $CDCl_3$) δ = 170.66 – 169.54 ($OCOCH_3$), 96.57/95.78/95.65/95.61/95.32 (C-$1^{I,II,III,V,VI}$), 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-$2^{I,II,III,IV,V,VI}$, C-$3^{I,II,III,IV,V,VI}$, C-$4^{I,II,III,IV,V,VI}$, C-$5^{I,II,III,IV,V,VI}$), 63.05/62.88/62.72/62.23 (C-$6^{I,II,V,V,VI}$), 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→4)-(α-D-glucopyranosyl)(1→4)-*S*-(α-D-glucopyranosyl)-(1→6)-(6-thio-α-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-α-D-glucopyranoside (30): De-*O*-acetylation of **29** (40.0 mg, 21.2 μmol) by treatment with sodium methoxide (1M, 200 μL) 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) δ = 100.18/100.14/100.00/99.84/99.48 (C-$1^{I,II,III,V,VI}$), 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-$2^{I,II,III,IV,V,VI}$, C-$3^{I,II,III,IV,V,VI}$, C-$4^{I,II,III,IV,V,VI}$, C-$5^{I,II,III,IV,V,VI}$), 61.28/60.85 (C-$6^{I,II,IV,V,VI}$), 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→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*S*-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→6)]-(2,3,di-*O*-acetyl-6-thio-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-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 °C for 2 h under argon. After the mixture had been cooled to 0 °C, acetylated 1-thio-α-maltose **13** (550 mg, 7.9 mmol), 1,4-dithioerytritol (62 mg) and diethylamine (125 μL, 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 °C for 2 h . Following incubation, the reaction mixture was cooled (0 °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_2O, 2: Et_2O/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, $CDCl_3$) δ = 170.67 – 168.48 ($OCOCH_3$), 95.94/95.64 (C-$1^{II,III,IV,VI}$), 91.22 (C-$1^{I\beta}$), 88.87 (C-$1^{I\alpha}$), 83.29/83.20 (C-$1^{V\alpha,V\beta}$), 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-$2^{I,II,III,IV,V,VI}$, C-$3^{I,II,III,IV,V,VI}$, C-$4^{I,II,III,IV,V,VI}$, C-$5^{I,II,III,IV,V,VI}$), 62.68/61.35 (C-$6^{I,III,IV,V,VI}$), 30.25/30.22 (C-$6^{II\alpha,II\beta}$), 20.86 – 20.54 ($OCOCH_3$); ES$^+$ HRMS: calcd for $C_{76}H_{102}O_{50}S$ [M^++Na]: 1869.5057, found: 1869.5057.

(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*S*-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→6)]-(2,3,di-*O*-acetyl-6-thio-α-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose (31*β*): Diethylamine (6.4 μL) was added to a solution of **19*β*** (44 mg, 34.5 μmol) and **13** (26.2 mg, 37.7 μmol) in DMF (473 μL). 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, $CDCl_3$) δ = 170.63 – 168.72 ($OCOCH_3$), 95.98/95.70/95.64 (C-$1^{II,III,IV,VI}$), 91.22 (C-$1^{I\beta}$), 83.16 (C-1^{V}), 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-$2^{I,II,III,IV,V,VI}$, C-$3^{I,II,III,IV,V,VI}$, C-$4^{I,II,III,IV,V,VI}$, C-$5^{I,II,III,IV,V,VI}$), 62.69/61.36 (C-$6^{I,III,IV,V,VI}$), 30.26 (C-$6^{II\alpha,II\beta}$), 20.83 – 20.51 ($OCOCH_3$); FABMS: m/z = 1870 [M^++Na].

(α-D-Glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-*S*-[(α-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→6)]-(6-thio-α-D-glucopyranosyl)-(1→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) δ = 99.24/99.22/99.18/98.87/98.71 (C-$1^{II,III,IV,VI}$), 95.22 (C-$1^{I\beta}$), 91.31(C-$1^{I\alpha}$), 84.76 (C-1^{V}), 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-$2^{I,II,III,IV,V,VI}$, C-$3^{I,II,III,IV,V,VI}$, C-$4^{I,II,III,IV,V,VI}$, C-$5^{I,II,III,IV,V,VI}$), 60.52/60.38/59.94 (C-$6^{I,III,IV,V,VI}$), 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→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*S*-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→6)]-(2,3–di-*O*-acetyl-6-thio-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-D-glucopyranosyl)-(1→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 μmol) 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, $CDCl_3$) δ = 170.66 – 169.50 ($OCOCH_3$), 96.58/95.92/95.68/95.60/95.40 (C-$1^{I,II,III,IV,V,VII}$), 83.47 (C-1^{VI}), 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-$2^{I,II,III,IV,V,VI,VII}$, C-$3^{I,II,III,IV,V,VI,VII}$, C-$4^{I,II,III,IV,V,VI,VII}$, C-$5^{I,II,III,IV,V,VI,VII}$), 62.99/62.83/62.71/62.61/61.35 (C-$6^{I,II,IV,V,VI,VII}$), 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→4)-(α-D-glucopyranosyl)-(1→4)-*S*-[α-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→6)]-(6-thio-α-D-glucopyranosyl)-(1→4)-(D-glucopyranosyl)-(1→4)-α-D-glucopyranoside (34): Compound **33** (20 mg, 0.94 μmol) 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, $CDCl_3$) δ = 100.12/99.77/99.45 (C-$1^{I,II,III,IV,V,VII}$), 85.65 (C-1^{VI}), 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-$2^{I,II,III,IV,V,VI,VII}$, C-$3^{I,II,III,IV,V,VI,VII}$, C-$4^{I,II,III,IV,V,VI,VII}$, C-$5^{I,II,III,IV,V,VI,VII}$), 61.16 – 60.81 (C-$6^{I,II,IV,V,VI,VII}$), 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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