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Chemical synthesis of 6‴-α-maltotriosyl-maltohexaose as substrate for enzymes in starch biosynthesis and degradation

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Abstract

A branched nonasaccharide 6‴-α-maltotriosyl-maltohexaose was synthesised in 40 steps from D-glucose and maltose. Phenyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-1-thio-β-D-glucopyranoside and *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α,β-D-glucopyranosyl trichloroacetimidate were coupled by a general condensation reaction to form the per-*O*-benzylated branched hexasaccharide phenyl thioglycoside. The phenylthio group of this compound was converted into a trichloroacetimidate, which was coupled with phenyl *O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside to afford the per-*O*-benzylated branched nonasaccharide phenyl thioglycoside. Replacement of the phenylthio group with a free OH-group followed by hydrogenolysis gave the desired product. The synthons reported for this synthesis constitute a versatile tool for the chemical synthesis of other complex carbohydrates.

Keywords: Carbohydrates; Branched oligosaccharide; Nonasaccharide; 6‴-α-Maltotriosyl-maltohexaose

1. Introduction

Starch can be distinguished from glycogen by the presence of highly ordered and densely packed glucan chains permitting packaging of the starch as large insoluble granules in the plastids of plants. Starch is composed of amylose, which is a predominantly linear α-(1→4)-glucan, and of amylopectin, which is a higher-molecular-mass α-(1→4)-glucan with frequent α-(1→6)-glucan branch points. Although studies within biochemistry, molecular biology and genetics have resulted in isolation, cloning and heterologous expression of a large number of enzymes in starch metabolism, the precise nature and sequential action of the enzymatic reactions involved in starch biosynthesis [1–3] and starch degradation [4,5] have

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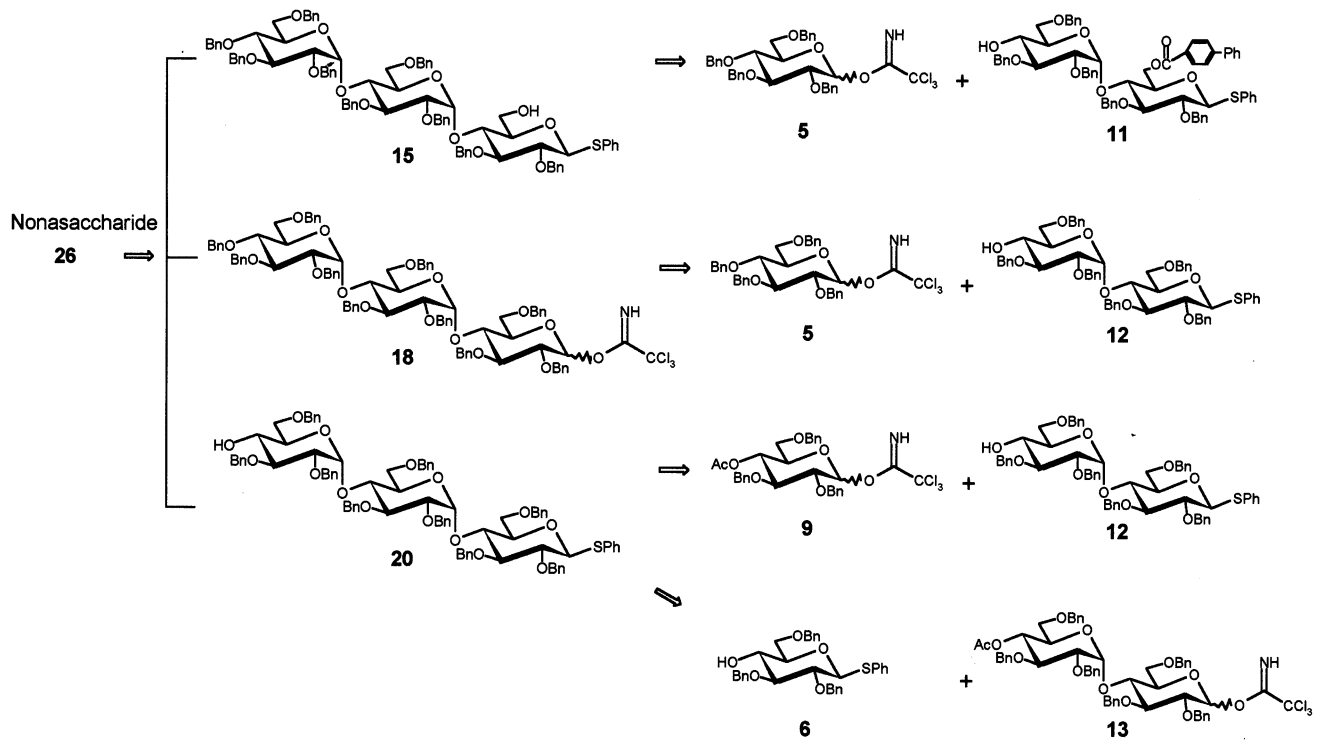
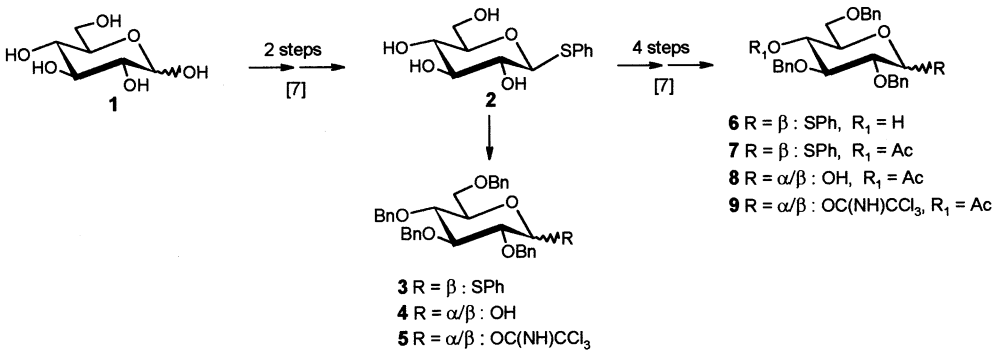
proven difficult to elucidate. First, starch deposition and mobilisation involves the complex interplay of a large number of enzymes. Secondly, most of these enzymes occur as multiple isoforms. Thirdly, the structure of the α-(1→4)/α-(1→6)-glucan molecules that serve as substrates for these enzymes are poorly defined. Attempts to resolve the function of the individual enzymes and isoforms by production of plants in which expression of the particular gene is blocked using anti-sense technology have been greatly hampered by the simultaneous presence of a multitude of enzymes or isoforms that exhibit partly overlapping catalytic activities. The bottle neck in defining the exact substrate specificity and catalytic actions of the different enzymes and differences in the properties of specific isoforms thus remains the availability of structurally well-defined α-(1→4)/α-(1→6)-glucan substrates. Accordingly, we have embarked on a research programme to chemically synthesise a range of complex oligosaccharides to be used as substrates or primers for enzymes in starch biosynthesis and as substrates for starch-degrading enzymes. The approach is based on a limited number of synthons. In this context, we have previously reported the synthesis of a protected branched tetrasaccharide [6] and its use in the synthesis of 6′-α-maltosyl-maltotriose [7]. Here, we report the synthesis of 6‴-α-maltotriosyl-maltohexaose.

2. Results and discussion

The strategy was to synthesise the nonasaccharide 6‴-α-maltotriosyl-maltohexaose (**26**) from three trisaccharide building blocks (**15**, **18** and **20**), which were meant to be retrosynthesised from four building blocks – two monosaccharide glycosyl donors (**5** and **9**) and two disaccharide glycosyl acceptors (**11** and **12**) (Scheme 1). Chemical synthesis of **20** from **9** and **12** was accomplished. However, difficulties encountered in separating intermediary products as well as **20** from reactants and products formed prompted us to synthesise **20** from the monosaccharide glycosyl acceptor **6**, which is an intermediate in the synthesis of **9**, and the disaccharide **13** (Scheme 1).



Scheme 1. Retrosynthesis of the nonasaccharide 6‴-α-maltotriosyl-maltohexaose (**26**) (Scheme 4) via three trisaccharide building blocks synthesised from derivatives of glucose and maltose.

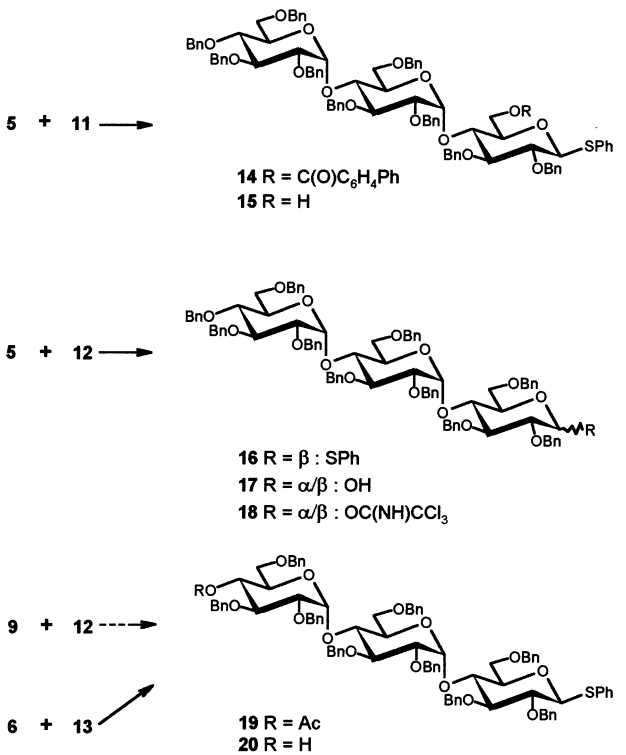


Scheme 2. Synthesis of the monosaccharide building blocks.

Synthesis of the monosaccharide building blocks (Scheme 2).—The synthesis of the desired glycosyl donors **5** and **9** and of the glycosyl acceptor **6** from D-glucose **1** is shown in Scheme 2. D-Glucose **1** was converted into phenyl 1-thio-β-D-glucopyranoside **2** in two steps [7]. Benzylation of **2** with NaH and benzyl bromide gave the known phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3**) in 60% yield [8–10]. By treating the thioglycoside **3** with *N*-bromosuccinimide in aqueous acetone [11], the phenylthio group was replaced with a free OH-group affording an anomeric mixture of 2,3,4,6-tetra-*O*-benzyl-α,β-D-glycopyranose (**4**) in 92% yield after chromatographic purification. Based on measurements of optical rotation and melting point, **4** has previously been reported as the α anomer [12–16]. In the present study, **4** was identified as an α,β mixture by NMR spectroscopy. Reaction of **4** with trichloroacetonitrile in the presence of anhydrous potassium carbonate afforded the desired glycosyl donor *O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl) trichloroacetimidate (**5**), which has been described previously [17–19], in quantitative yield. In four known steps, **2** was converted into the glycosyl acceptor phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**6**) [7], which was acetylated using acetic anhydride in pyridine to give phenyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**7**) in 98% yield. Treatment of **7** with *N*-bromosuccinimide in aqueous acetone to replace the phenylthio group with a free OH-group gave 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-α,β-D-glucopyranose (**8**) in 91% yield [11]. Compound **8** has been prepared previously by different routes [20,21]. The glycosyl trichloroacetimidate derivative **9** (to be used as glycosyl donor) was obtained in 98% yield by treating **8** with trichloroacetonitrile in the presence of anhydrous potassium carbonate.

Synthesis of the disaccharide building blocks (Scheme 1).—The three disaccharides **11**, **12** and **13** were synthesised from maltose (**10**) (to be published elsewhere).

Synthesis of the trisaccharide building blocks (Scheme 3).—All three trisaccharide building blocks were synthesised by coupling the appropriate monosaccharide derivative with the corresponding disaccharide derivative. All

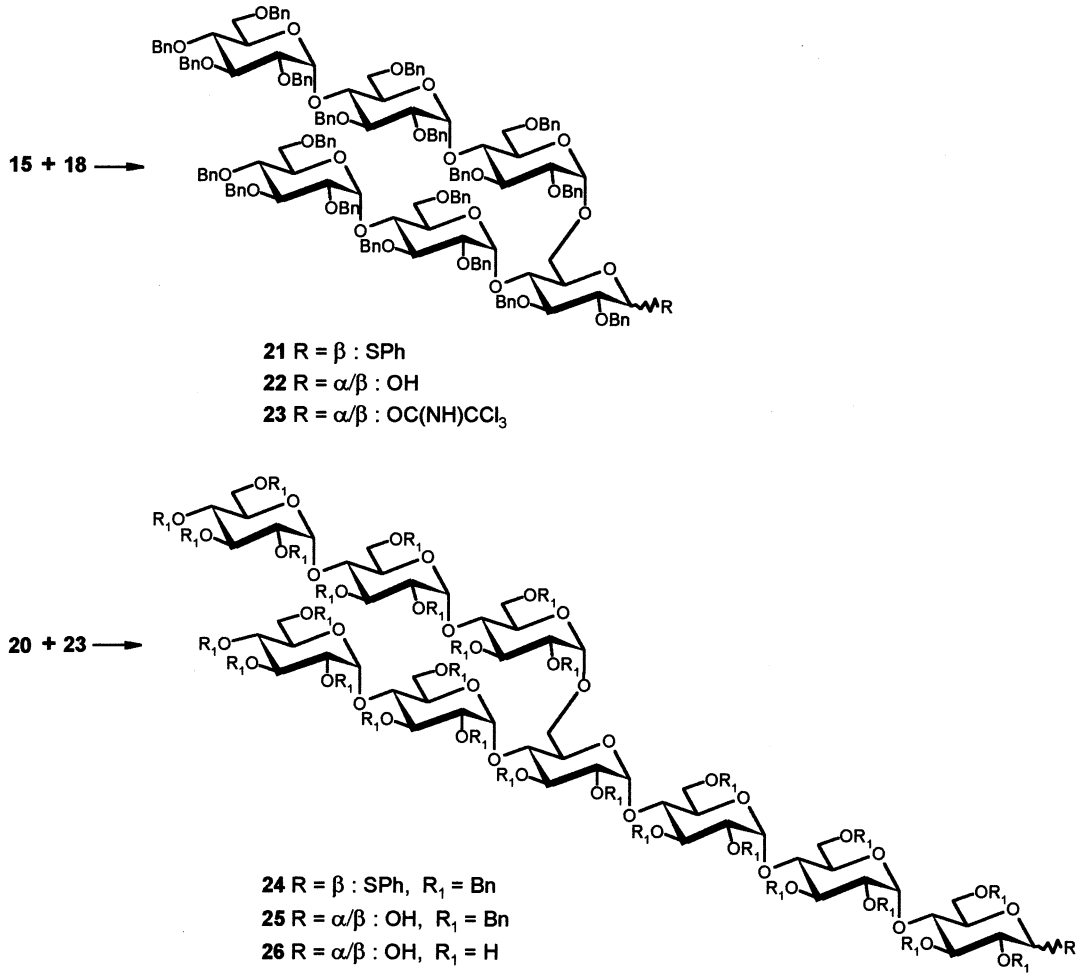


Scheme 3. Synthesis of the three trisaccharide building blocks.

synthetic schemes are based on the possibility of quantitative removal of the phenylthio group protecting the anomeric centre by treatment with *N*-bromosuccinimide in aqueous acetone [11]. Diethyl ether favouring the formation of 1,2-*cis*-glycosides [22,23] was the choice of solvent in all glycosidation reactions. Coupling the glycosyl donor **5** with the glycosyl acceptor **11** in dry diethyl ether, using trimethylsilyl triflate as catalyst, provided the 4-phenylbenzoyl derivative **14** in 48% yield after chromatographic purification. The coupling reaction resulted in the expected formation of an α-linkage as verified from the diagnostic signal for C-1″ at 97.2 ppm in the ^{13}C NMR spectrum. The introduction of a β-linkage would have given rise to a signal with a value higher than 102 ppm [24,25]. The signal at 86.9 ppm reflects C-1 and the signal at 101.3 ppm reflects C-1′ as also seen in **11** (unpublished data). In the ^{1}H NMR spectrum, the presence of a doublet at δ 5.61 with $J_{1,2}$ 3.5 Hz reflects the newly formed α-linkage. The two other anomeric protons are overlapped by the methylene protons of the benzyl groups. The desired phenyl thioglycoside building block **15**, having a free OH-6 at the reducing terminal D-glucopyranoside unit, was obtained in 80% yield after chromatographic purification by treating **14** with sodium methoxide in methanol. The phenyl thioglycoside **16** was obtained in 80% yield after chromatographic purification by coupling the glycosyl donor **5** and the glycosyl acceptor **12** in diethyl ether with trimethylsilyl triflate as catalyst. Less than 3% of formed β isomer as well as other impurities were removed by chromatography. In the ^{1}H NMR spectrum, a doublet at δ 5.50 with $J_{1,2}$ 3.6 Hz and a doublet at δ 5.62 with $J_{1,2}$ 3.6 Hz reflect the two α-linkages. In the ^{13}C NMR spectrum a signal at 87.2 ppm reflects C-1 and two signals at 96.8 and 96.9 ppm reflect C-1′ and C-1″α-linkages. By treatment of the thioglycoside **16** with *N*-bromosuccinimide in aqueous acetone [11], the phenylthio group was replaced with a free OH-group to give **17** [26,27] in 91% yield after chromatographic purification. Reaction of **17** with trichloroacetonitrile in the presence of anhydrous potassium carbonate afforded the desired building block **18** in quantitative yield. Coupling of the glycosyl donor **9** and the glycosyl acceptor **12** in diethyl ether, using trimethylsilyl triflate as catalyst, produced a mixture of **19** and **12**. Attempts to separate the product **19** from the reactant **12** by chromatography were unsuccessful. Subsequent deacetylation of this mixture with sodium methoxide in methanol produced a mixture of **20** and **12**, which could not be separated either. To overcome these problems, **19** was synthesised from the monosaccharide glycosyl acceptor **6** and the disaccharide glycosyl donor **13** in 55% yield after chromatographic purification. Exclusive introduction of an α-linkage by the coupling reaction was verified by ^{1}H and ^{13}C NMR spectroscopy. In the ^{1}H NMR spectrum, a doublet at δ 5.50 with $J_{1,2}$ 3.7 Hz and a doublet at δ 5.58 with $J_{1,2}$ 3.7 Hz reflect the two α-linkages. In the ^{13}C NMR spectrum a signal at 87.3 ppm reflects C-1 and two signals at 96.7 and 96.8 ppm reflect the α-linkage configuration of C-1′ and C-1″. Deacetylation of **19** with sodium methoxide in methanol gave the desired building block **20** in quantitative yield after chromatographic purification.

Synthesis of the hexasaccharides (Scheme 4).—The branched hexasaccharide **21** was synthesised from the glycosyl acceptor **15** and the glycosyl donor **18** in 64% yield using dry diethyl ether and trimethylsilyl triflate as catalyst. The coupling reaction resulted in the specific introduction of an α-linkage as verified by ^{13}C NMR spectroscopy. A signal at 87.2 ppm reflects C-1 and five signals at 96.7, 96.8, 97.0, 97.3, 100.3 reflect the five internal anomeric carbons. In the ^{1}H NMR spectrum, only three anomeric protons are identified. The signals of the remaining anomeric protons are overlapped with signals from the methylene protons of the benzyl groups. Treatment of the thioglycoside **21** with *N*-bromosuccinimide in aqueous acetone afforded **22** in 87% yield after chromatographic purification. Reaction of **22** with trichloroacetonitrile in the presence of anhydrous potassium carbonate provided the desired building block **23** in quantitative yield.

Synthesis of the nonasaccharides (Scheme 4).—The trisaccharide **20** (glycosyl acceptor)



Scheme 4. Synthesis of hexasaccharides and nonasaccharides.

and the hexasaccharide **23** (glycosyl donor) were coupled in diethyl ether and trimethylsilyl triflate to afford the protected branched nonasaccharide thioglycoside **24**, in 56% yield, after chromatographic purification. The coupling resulted in formation of the expected α-linkage as verified by ^{13}C and ^{1}H NMR spectroscopy. In the ^{13}C NMR spectrum, a signal at 87.2 ppm reflects the C-1 and eight signals at 95.9, 96.1, 96.6, 96.7, 96.8, 97.0, 97.4, 98.7 reflect the eight internal anomeric carbon atoms. In the ^{1}H NMR spectrum, four doublets at δ 5.18 with $J_{1,2}$ 3.6 Hz, δ 5.32 with $J_{1,2}$ 3.3 Hz, δ 5.50 with $J_{1,2}$ 3.5 Hz, δ 5.54 with $J_{1,2}$ 3.5 Hz and a multiplet containing four protons reflect the eight internal anomeric protons. The H-1_β is overlapped by the signals from the methylene protons of the benzyl groups. The phenylthio group in **24** was replaced with a free OH-group by using *N*-bromosuccinimide in aqueous acetone to afford **25** in 86% yield. 6‴-α-Maltotriosyl-maltohexaose (**26**) was obtained in 80% yield after chromatographic purification by hydrogenolysis of the benzyl ether protecting groups with 10% Pd–C under hydrogen (Fig. 1).

Conclusions.—The availability of the synthons used for chemical synthesis of **26** combined with those previously used for chemical synthesis of 6′-α-maltosyl-maltotriose [7] offers excellent possibilities for chemical synthesis of an infinite number of other complex carbohydrates, which may be either linear oligosaccharides with a defined degree of polymerisation, or branched oligosaccharides encompassing one or more branch points with defined position and spacing. The use of *N*-bromosuccinimide for removal of the thiophenyl group at the anomeric position to generate a reducing sugar with the original

protecting groups still present [11] constitutes a key reaction in all synthetic schemes. The outlined strategy for chemical synthesis renders it possible to chemically synthesise desired partial structures of the starch polymers, amylopectin and amylose and to test these as substrates for starch-synthesising or -degrading enzymes.

3. Experimental

General methods.—Melting points were determined using a Mettler FP81 MBC Cell connected to a Mettler FP80 Central Processor unit. Optical rotations were measured at 27 ± 1 °C with an Optical Activity Ltd AA-1000 Polarimeter. All reactions were moni-

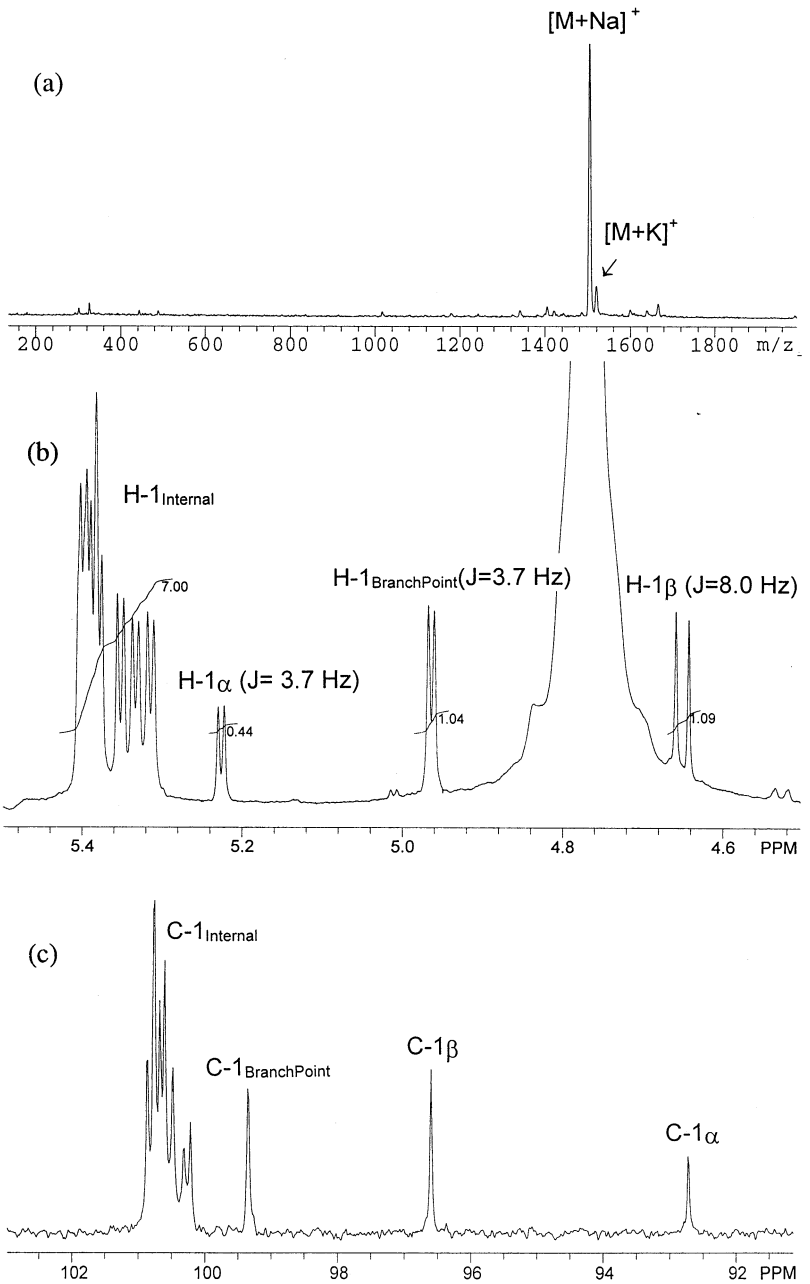


Fig. 1. (a) MALDI spectrum of **26**; (b) 500 MHz ^{1}H NMR spectrum of **26** in D_2O; (c) 126 MHz ^{13}C NMR spectrum of **26** in D_2O.

tored by thin layer chromatography (TLC) on aluminium sheets coated with Silica Gel $60F_{254}$ (0.2 mm thickness, E. Merck, Darmstadt, Germany) and the components were visualised by charring with 10% H_2SO_4 in MeOH. Column chromatography was carried out using Silica Gel (particle size 0.040–0.063 mm, 230–400 mesh ASTM, E. Merck) and stepwise elution with the solvent mixtures, known from TLC chromatography, to permit separation of the individual components. Solvent extracts were dried with anhyd $MgSO_4$ unless otherwise specified. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AC250P spectrometer or a Bruker AM500 spectrometer. In D_2O, dioxane was used as internal reference (δ_H(dioxane) $\pm$ 3.75; δ_C(dioxane) $\pm$ 67.4). In $CDCl_3$, the δ_H-values are relative to internal Me_4Si and the δ_C-values are referenced to the solvent (δ_C($CDCl_3$) $\pm$ 77.0). MALDI spectra were recorded on a Tofspec E spectrometer (Micromass).

Phenyl 2,3,4,6-tetra-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**3**).—A soln of **2** (12.26 g, 45.02 mmol) in dry DMF (200 mL) was stirred with NaH (14.53 g, 60% in mineral oil) for 3 h at room temperature (rt). The mixture was cooled to 0 °C and benzyl bromide (43.2 mL) was added dropwise and stirring was continued at rt for 45 h. After cooling to 0 °C and addition of MeOH to decompose excess NaH, the soln was concd in vacuo and diluted with EtOAc (150 mL) and water (50 mL). The organic phase was washed with water (4 × 25 mL) and brine, dried and evaporated. Compound **3** was obtained by crystallisation from EtOH as white needles (17.03 g, 60%): mp 93 °C, lit. 92–93 °C [8]; $[\alpha]_D \pm 0.01°$ (*c* 0.31, $CHCl_3$), lit. $[\alpha]_D \pm 1°$ ($CHCl_3$) [8]; ^1H NMR ($CDCl_3$): δ 3.47–3.54 (m, 2 H, H-2 and H-5), 3.61–3.75 (m, 3 H, H-3, H-4 and H-6b), 3.79 (dd, 1 H, $J_{6a,6b}$ 10.9 Hz, $J_{6a,5}$ 2.0 Hz, H-6a), 4.67 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 4.50–4.92 (m, 8 H, 4 CH_2Ph), 7.17–7.60 (m, 25 H, H-arom, SPh and $PhCH_2$); ^{13}C NMR ($CDCl_3$): δ 69.0 (C-6), 73.4, 75.0, 75.4, 75.8 (4 *C*H_2Ph), 77.8, 79.1, 80.8 (C-2, C-4, C-5), 86.7 (C-3), 87.4 (C-1), 127.4–138.4 (C-arom, SPh and $PhCH_2$). Anal. Calcd for $C_{40}H_{40}O_5S$: C, 75.92; H, 6.37; S, 5.07. Found: C, 75.77; H, 6.66; S, 5.52.

2,3,4,6-Tetra-O-*benzyl-α,β*-D-*glucopyranose* (**4**).—Acetone–water (9:1 v/v, 276 mL) was added to a soln of **3** (17.00 g, 26.86 mmol) in acetone (138 mL). NBS (9.56 g, 53.72 mmol) was added to the soln in one portion and after 5 min the reaction was quenched by adding solid $NaHCO_3$ (20 g). The mixture was stirred for 10 min, evaporated in vacuo at rt and then diluted with EtOAc (300 mL). The organic phase was washed with water until pH neutral, then with brine, dried and evaporated to dryness. The residue was chromatographed on Silica Gel (220 g) with CH_2Cl_2–MeOH (19:1 v/v) as eluent to give **4** as white solid (13.29 g, 92%) as an α,β mixture (2:1): mp 151–152 °C, lit. 151–152 °C [16]; $[\alpha]_D \pm 19.3°$ (*c* 0.99, $CHCl_3$), lit. $\pm 21.7°$ (*c* 2.19, $CHCl_3$) [16]; ^1H NMR ($CDCl_3$): δ 3.36–4.07 (m, 6 H, H-2, H-3, H-4, H-5, 2 H-6), 4.44–4.97 (m, 8.3 H, 4 CH_2Ph and H-1_β), 5.22 (d, 0.7 H, $J_{1,2}$ 3.5 Hz, H-1_α), 7.10–7.36 (m, 20 H, H-arom, 4 $PhCH_2$; ^{13}C NMR ($CDCl_3$): For the α anomer: δ 68.6 (C-6), 73.1, 73.4, 74.9, 75.6 (4 *C*H_2Ph), 70.2, 77.7, 80.0, 81.7 (C-2, C-3, C-4, C-5), 91.2 (C-1), 127.6–138.7 (C-arom, $PhCH_2$). For the β anomer: δ 68.9 (C-6), 74.6, 75.6, 74.6, 75.6 (4 *C*H_2Ph), 74.7, 77.8, 83.1, 84.5 (C-2, C-3, C-4, C-5), 97.4 (C-1), 127.6–138.7 (C-arom, $PhCH_2$). Anal. Calcd for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.33; H, 6.85.

O-*(2,3,4,6-Tetra*-O-*benzyl-α,β*-D-*glucopyranosyl) trichloroacetimidate* (**5**).—A soln of **4** (12.50 g, 23.12 mmol) in dry CH_2Cl_2 (150 mL) was stirred vigorously with CCl_3CN (30 mL) and K_2CO_3 (15 g) for 23 h at rt under N_2. The reaction mixture was filtered through a sea sand pad and a layer of Silica Gel. The filtrate was evaporated to dryness to give **5** as colourless syrup (15.79 g, quantitative) as an α,β mixture (1:10), which was used directly for the next step without further purification. The ^1H NMR spectral data were identical to the literature data [17–19]; ^{13}C NMR ($CDCl_3$): For the α anomer: δ 68.0 (C-6), 72.8, 73.4, 75.1, 75.3 (4 *C*H_2Ph), 73.1, 76.8, 79.4, 81.4 (C-2, C-3, C-4, C-5), 91.3 (*C*Cl_3), 94.4 (C-1), 127.6–138.4 (C-arom, $PhCH_2$), 161.3 (*C*=NH). For the β anomer: δ 68.2 (C-6), 73.3, 74.8, 74.9, 75.6 (4 *C*H_2Ph), 75.9, 77.3, 80.9, 84.6 (C-2, C-3, C-4, C-5), 91.0 (*C*Cl_3), 98.3 (C-1), 127.6–138.4 (C-arom, $PhCH_2$), 161.1 (*C*=NH).

Phenyl 4-O-acetyl-2,3,6-tri-O-benzyl-1-thio-β-D-glucopyranoside (**7**).—Acetic anhydride (50 mL) was added to a soln of **6** (5.13 g, 9.45 mmol) in dry pyridine (100 mL) and stirred for 24 h at rt. The reaction mixture was concd, coevaporated with toluene (3 × 50 mL) and then dissolved in EtOAc (250 mL). The organic phase was successively washed with satd aq $NaHCO_3$, water until pH neutral, then with brine and dried before evaporation to dryness. Compound **7** was obtained by crystallisation from EtOH as white fibres (5.43 g, 98%): mp 81–84 °C; $[\alpha]_D$-21.1° (*c* 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.84 (s, 3 H, $COCH_3$), 3.50–3.70 (m, 5 H, H-2, 2 H-6, H-3, H-5), 4.50 (s, 2 H, CH_2Ph), 4.63, 4.81 (2 d, 2 H, *J* 11.4 Hz, CH_2Ph), 4.69 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.70, 4.88 (2 d, 2 H, *J* 10.4 Hz, CH_2Ph), 5.00 (m, 1 H, H-4), 7.20–7.59 (m, 20 H, H-arom, $PhCH_2$ and SPh); ^{13}C NMR ($CDCl_3$): δ 20.8 ($COCH_3$), 69.7 (C-6), 73.5, 75.4, 75.5 (3 CH_2Ph), 70.8 (C-4), 77.6 (C-5), 80.6 (C-2), 84.0 (C-3), 87.5 (C-1), 127.5–138.1 (C-arom, SPh and $PhCH_2$), 169.7 (C=O). Anal. Calcd for $C_{35}H_{36}O_6S$: C, 71.89; H, 6.21; S, 5.48. Found: C, 71.34; H, 6.30; S, 5.55.

4-O-Acetyl-2,3,6-tri-O-benzyl-α,β-D-glycopyranose (**8**).—The procedure was the same as described for **4** using acetone–water (9:1 v/v, 50 mL), **7** (3.00 g, 5.13 mmol) in acetone (25 mL) and NBS (1.83 g, 10.26 mmol). Reaction time: 3.5 min. The residue was chromatographed on Silica Gel (220 g) with EtOAc–*n*-pentane (3:7 v/v) as eluent to give **8** as white fibers (2.30 g, 91%) as an α,β mixture (2:5): mp 115–116 °C, lit. 112–113° [21]; $[\alpha]_D \pm 9.8°$ (*c* 0.43, $CHCl_3$), lit. ± 10.9° (*c* 1.0, $CHCl_3$) [21]. The NMR data were identical to the literature data [20,21]. Anal. Calcd for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55. Found: C, 70.55; H, 6.90.

4-O-Acetyl-2,3,6-tri-O-benzyl-α,β-D-glucopyranosyl trichloroacetimidate (**9**).—The procedure was the same as described for **5** using **8** (2.97 g, 6.03 mmol) in dry CH_2Cl_2 (75 mL), CCl_3CN (9 mL) and K_2CO_3 (4.5 g) to give **9** as colourless syrup (3.78 g, 98%) as an α,β mixture (2:1), which was used directly for the next step without further purification: ^{1}H NMR ($CDCl_3$): δ 1.80, 1.84 (2s, 3 H, $COCH_3$, β and α anomer), 3.43–4.09 (m, 6 H, H-2, H-3, H-4, H-5, 2 H-6), 4.41–5.23 (m, 6 H, CH_2Ph), 5.85 (d, 0.3 H, $J_{1,2}$ 7.6 Hz, H-1$_\beta$), 6.50 (d, 0.7, $J_{1,2}$ 3.5 Hz, H-1$_\alpha$), 7.17–7.36 (m, 15 H, H-arom, $PhCH_2$), 8.63, 8.75 (2s, 1 H, NH, α and β anomer); ^{13}C NMR ($CDCl_3$): For the α anomer: δ 20.6 ($COCH_3$), 68.3 (C-6), 72.8, 73.3, 74.8 (3 CH_2Ph), 69.5, 71.5, 78.1, 78.8 (C-2, C-3, C-4, C-5), 91.0 (CCl_3), 93.9 (C-1), 127.4–138.4 (C-arom, $PhCH_2$), 160.8 (C=NH). For the β anomer: δ 20.6 ($COCH_3$), 68.8 (C-6), 73.2, 74.9, 75.0 (3 CH_2Ph), 70.2, 74.2, 80.4, 81.4 (C-2, C-3, C-4, C-5), 90.6 (CCl_3), 97.8 (C-1), 127.6–138.4 (C-arom, $PhCH_2$), 160.9 (C=NH). Anal. Calcd for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55. Found: C, 70.55; H, 6.90.

Phenyl O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→4)-2,3-di-O-benzyl-6-O-(4-phenylbenzoyl)-1-thio-β-D-glucopyranoside (**14**).—A soln of **5** (6.40 g, 9.34 mmol) and **11** (6.60 g, 6.20 mmol) in dry diethyl ether (200 mL) was stirred for 1 h at rt under Ar in the presence of 4 Å molecular sieves (2.5 g, activated powder). After cooling to −20 °C and addition of trimethylsilyl trifluoromethanesulfonate (290 μL, 1.57 mmol), stirring was continued for 1.5 h; during this period the temperature was raised to rt. After addition of solid $NaHCO_3$ (9 g), the reaction mixture was stirred for 10 min, diluted with EtOAc (150 mL) and filtered through a sea sand pad and a layer of Silica Gel. The filtrate was successively washed with satd aq $NaHCO_3$, water until pH neutral, brine, dried and evaporated to dryness. The residue was chromatographed on Silica Gel (210 g) with diethyl ether–*n*-pentane (1:4 and 3:7 v/v) as eluent to give **14** as gum (4.68 g, 48%): $[\alpha]_D \pm 29.7°$ (*c* 0.34, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.61 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric). Two anomeric protons were overlapped by the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 64.0 (C-6), 68.1, 68.6 (C-6′, C-6″), 73.3, 73.4, 73.5, 74.5, 74.9, 74.9, 74.9, 75.0, 75.5 (9 CH_2Ph), 71.1, 71.5, 72.9, 76.3, 77.6, 78.6, 79.3, 79.4, 80.0, 82.0, 82.1, 82.7 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 86.9 (C-1), 97.3 (C-1″), 101.3 (C-1′), 126.4–145.7 (C-arom, SPh, $PhCH_2$ and C_6H_4Ph), 165.8 (C=O).

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3-di*-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**15**).—NaOMe (4.4 mL, 30% in MeOH) was added to a soln of **14** (2.41 g, 1.52 mmol) in MeOH–EtOAc (30 mL, 1:1 v/v) at 0 °C followed by stirring at rt for 32 h. Acetic acid was added to obtain neutral pH. The reaction mixture was concd to dryness and the residue was dissolved in $CHCl_3$ (50 mL) and successively washed with 1 M aq NaOH (25 mL), water until pH neutral, brine, dried and concd. The residue was chromatographed on Silica Gel (60 g) with diethyl ether–*n*-pentane (1:4 and 3:7 v/v) as eluent to give **15** as colourless syrup (1.72 g, 80%): $[\alpha]_D \pm 32.3°$ (*c* 0.23, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.96 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 5.58 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric). H-1_β was overlapped by the signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 61.9 (C-6), 68.2, 69.0 (C-6′, C-6″), 73.3, 73.4, 73.5, 73.5, 74.4, 74.6, 75.0, 75.0, 75.5 (9 CH_2Ph), 71.0, 71.3, 73.3, 77.7, 78.5, 78.7, 79.2, 79.7, 79.9, 81.4, 81.9, 81.9 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 87.0 (C-1), 97.3 (C-1″), 100.6 (C-1′), 126.3–138.6 (C-arom, SPh and $PhCH_2$).

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**16**).—The procedure was the same as described for **14** using **5** (2.75 g, 4.01 mmol) and **12** (2.60 g, 2.67 mmol) in dry diethyl ether (200 mL), 4 Å molecular sieves (2.5 g, activated powder) and trimethylsilyl trifluoromethanesulfonate (123 μL, 0.66 mmol). The residue was chromatographed on Silica Gel (175 g) with diethyl ether–*n*-pentane (1:4 and 3:7 v/v) as eluent to give **16** (3.19 g, 80%, syrup): $[\alpha]_D \pm 47°$ (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.50 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 5.62 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric). H-1_β was overlapped by the signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 68.2, 69.0, 69.1 (C-6, C-6′, C-6″), 72.9, 73.1, 73.2, 73.2, 73.4, 74.0, 74.4, 74.9, 75.2, 75.4 (10 CH_2Ph), 70.9, 70.9, 71.0, 73.7, 77.6, 78.8, 79.3, 79.5, 80.8, 81.5, 82.0 (C-2, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 86.5 (C-3), 87.2 (C-1), 96.8, 96.9 (C-1′, C-1″), 126.5–138.8 (C-arom, SPh and $PhCH_2$).

O-*(2,3,4,6-Tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-α,β*-D-*glycopyranose* (**17**).—The same procedure as described for **4** using acetone–water (9:1 v/v, 60 mL), **16** (4.62 g, 3.08 mmol) in acetone (30 mL) and NBS (1.10 g, 6.16 mmol). Reaction time: 5 min. The residue was chromatographed on Silica Gel (95 g) with EtOAc–*n*-pentane (1:4 and 100:0 v/v) as eluent to give **17** as colourless syrup (3.94 g, 91%) as an α,β mixture (2:1): $[\alpha]_D \pm 48°$ (*c* 0.1, $CHCl_3$). The ^1H NMR spectral data were identical to the literature data [26]; ^{13}C NMR ($CDCl_3$): δ 90.8 (C-1_α), 97.3 (C-1_β), 96.5, 96.7 (C-1′, C-1″), 126.5–138.9 (C-arom, $PhCH_2$) [26].

O-*(2,3,4,6-Tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-α,β*-D-*glucopyranosyl trichloroacetimidate* (**18**).—The same procedure as described for **5** using **17** (3.15 g, 2.24 mmol) in dry CH_2Cl_2 (60 mL), CCl_3CN (6 mL) and K_2CO_3 (3 g) to give **18** as colourless syrup (3.47 g, quantitative) as an α,β mixture (1:2), which was used directly for the next step without further purification: ^1H NMR ($CDCl_3$): for the α anomer: δ 5.58 (d, 0.3 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 5.68 (d, 0.3 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 6.54 (d, 0.3 H, $J_{1,2}$ 3.4 Hz, H-1), 8.59 (s, 0.3 H, NH). For the β anomer: δ 5.51 (d, 0.7 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 5.64 (d, 0.7 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 5.91 (d, 0.7 H, $J_{1,2}$ 7.0 Hz, H-1), 8.69 (s, 0.7 H, NH); ^{13}C NMR ($CDCl_3$): for the α anomer: δ 91.3 (CCl_3), 94.1 (C-1), 96.5, 96.7 (C-1′, C-1″), 161.4 (*C*=NH), 126.5–138.8 (C-arom, $PhCH_2$). For the β anomer: δ 91.0 (CCl_3), 96.6, 96.9 (C-1′, C-1″), 98.1 (C-1), 161.0 (*C*=NH), 126.5–138.8 (C-arom, $PhCH_2$).

Phenyl O-*(4*-O-*acetyl-2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**19**).—The same procedure as described for **14** using **6** (1.21 g, 2.23 mmol) and **13**

(2.00 g, 1.87 mmol) in dry diethyl ether (100 mL), 4 Å molecular sieves (1.5 g, activated powder) and a soln of trimethylsilyl trifluoromethanesulfonate (67 μL, 0.37 mmol) in dry diethyl ether (10 mL), which was added dropwise. The residue was chromatographed on Silica Gel (120 g) with diethyl ether–*n*-pentane (1:9, 1:4 and 3:7 v/v) as eluent to give **19** as colourless syrup (1.5 g, 55%): $[\alpha]_D \pm$ 40.7° (*c* 0.36, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.50 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1, internal anomeric), 5.58 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1, internal anomeric). H-1_β was overlapped by the signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 20.8 ($COCH_3$), 87.3 (C-1), 96.7, 96.8, (C-1′, C-1″), 126.5–138.7 (C-arom, SPh and $PhCH_2$), 169.4 (C=O).

Phenyl O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-*2,3,6-tri*-O-*benzyl*-*1-thio*-β-D-*glucopyranoside* (**20**).—NaOMe (0.5 mL, 30% in MeOH) was added to a soln of **19** (1.15 g, 0.80 mmol) in MeOH (100 mL) at 0 °C followed by stirring at rt for 32 h. The reaction mixture was worked up as described for **15**. The residue was chromatographed on Silica Gel (50 g) with diethyl ether–*n*-pentane (3:7 v/v) as eluent to give **20** as colourless syrup in quantitative yield (1.12 g): $[\alpha]_D \pm$ 38.4° (*c* 0.11, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.50 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1, internal anomeric), 5.63 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric). H-1_β was overlapped by the signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 87.3 (C-1), 96.7, 96.8, (C-1′, C-1″), 126.5–138.7 (C-arom, SPh and $PhCH_2$).

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)]*-*2,3-di*-O-*benzyl*-*1-thio*-β-D-*glucopyranoside* (**21**).—The same procedure as described for **14** using **15** (2.44 g, 1.73 mmol) and **18** (3.23 g, 2.08 mmol) in dry diethyl ether (200 mL), 4 Å molecular sieves (2.5 g, activated powder) and trimethylsilyl trifluoromethanesulfonate (80 μL, 0.43 mmol). The residue was chromatographed on Silica Gel (180 g) with diethyl ether–*n*-pentane (3:7 and 1:1 v/v) as eluent to give **21** as white foam (3.09 g, 64%): $[\alpha]_D \pm 64.7°$ (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.55 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1, internal anomeric), 5.65 (d, 2 H, $J_{1,2}$ 3.6 Hz, 2 H-1, internal anomeric). The rest of the anomeric protons were overlapped with signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 87.2 (C-1), 96.7, 96.8, 97.0, 97.3, 100.3 (5 internal anomeric C), 125.5–138.9 (C-arom, SPh and $PhCH_2$).

O-*(2,3,4,6-Tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)]*-*2,3-di*-O-*benzyl*-α,β-D-*glycopyranose* (**22**).—The same procedure as described for **4** using acetone–water (9:1 v/v, 36 mL), **21** (2.77 g, 0.99 mmol) in acetone (18 mL) and NBS (705 mg, 3.96 mmol). Reaction time: 6.5 min. The residue was chromatographed on Silica Gel (95 g) with EtOAc–*n*-pentane (1:4 and 3:7 v/v) as eluent to give **22** as colourless syrup (2.17 g, 87%) as an α,β mixture (1:2): $[\alpha]_D \pm$ 71.4° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.15 (d, 0.3 H, $J_{1,2}$ 3.6 Hz, H-1_α), 5.60–5.69 (m, 3 H, 3 H-1, internal anomeric). The rest of the anomeric protons were overlapped with signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): For the α anomer: δ 91.1 (C-1), 95.5, 96.0, 96.3, 96.8, 100.0 (5 internal anomeric C), 126.3–139.0 (C-arom, $PhCH_2$). For the β anomer: δ 97.2 (C-1), 96.8, 96.9, 97.0, 97.2, 100.4 (5 internal anomeric C), 126.3–139.0 (C-arom, $PhCH_2$).

O-*(2,3,4,6-Tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)]*-*2,3-di*-O-*benzyl*-α,β-D-*trichloroacetimidate* (**23**).—The procedure was the same as described for **5** using **22** (1.19 g 0.44 mmol) in dry CH_2Cl_2 (20 mL), CCl_3CN (2.25 mL) and K_2CO_3 (1.13 g) to give **23** as colourless syrup (1.25 g, quantitative) as an α,β mixture (1:1), which was used directly for the next step without further purification. ^1H NMR ($CDCl_3$): δ 5.60–5.75 (m, 5 H, 5 H-1, internal anomeric), 5.73 (d,

0.5 H, $J_{1,2}$ 7.9 Hz, , H-1$_\beta$), 6.40 (d, 0.5 H, $J_{1,2}$ 3.6 Hz, H-1$_\alpha$), 8.79 (s, 0.5 H, NH, α anomer), 8.82 (s, 0.5 H, NH, β anomer); ^{13}C NMR ($CDCl_3$): δ 90.8 (*C*Cl_3, β anomer), 91.2 (*C*Cl_3, α anomer), 93.7 (C-1$_\alpha$), 97.6 (C-1$_\beta$), 95.5, 96.1, 96.5, 96.6, 96.7, 96.8, 97.1, 97.3, 100.0, 100.2 (internal anomeric C), 160.3 (*C*=NH, β anomer), 161.0 (*C*=NH, α anomer).

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)]*-O-*(2,3-di*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**24**).—The procedure was the same as described for **14** using **20** (570 mg, 0.405 mmol) and **23** (1.07 g, 0.376 mmol) in dry diethyl ether (80 mL), 4 Å molecular sieves (1 g, activated powder) and trimethylsilyl trifluoromethanesulfonate (17.4 μL, 0.094 mmol). The residue was chromatographed on Silica Gel (55 g) with diethyl ether–*n*-pentane (2:3 v/v) as eluent to give **24** as white foam (867 mg, 56%): mp 96–99 °C; $[\alpha]_D \pm 71.6°$ (*c* 0.22, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.18 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1, internal anomeric), 5.32 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1, internal anomeric), 5.50 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1, internal anomeric), 5.54 (d, 1 H, $J_{1,2}$ 3.5 Hz H-1, internal anomeric), 5.62–5.73 (m, 4 H, 4 H-1, internal anomeric). H-1$_\beta$ was overlapped by the signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 87.2 (C-1), 95.9, 96.1, 96.6, 96.7, 96.8, 97.0, 97.4, 98.7 (8 internal anomeric C), 125.5–138.9 (C-arom, SPh and $PhCH_2$).

O-*(2,3,4,6-Tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→6)*-O-*[(2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)]*-O-*(2,3-di*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3,6-tri*-O-*benzyl-α*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-α,β*-D-*glycopyranose* (**25**).—The procedure was the same as described for **4** using acetone–water (9:1 v/v, 16 mL), **24** (857 mg, 0.209 mmol) in acetone (8 mL) and NBS (149 mg, 0.837 mmol). Reaction time: 10 min. The residue was chromatographed on Silica Gel (45 g) with diethyl ether–*n*-pentane (1:4, 1:1 and 4:1 v/v) as eluent to give **25** as white solid (720 mg, 86%) as an α,β mixture (4:3): mp 88–90 °C; $[\alpha]_D \pm 72.9°$ (*c* 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.18 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1, internal anomeric), 5.22 (bs, 0.6 H, H-1$_\alpha$), 5.31 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1, internal anomeric), 5.52 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1, internal anomeric), 5.58 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1, internal anomeric), 5.66–5.73 (m, 4 H, 4 H-1, internal anomeric). H-1$_\beta$ was overlapped by the signals from the methylene protons of the benzyl groups; ^{13}C NMR ($CDCl_3$): δ 90.7 (C-1$_\alpha$), 97.2 (C-1$_\beta$), 95.8, 95.8, 96.5, 96.6, 96.6, 96.6, 97.3, 98.6 (8 internal anomeric C), 126.2–138.8 (C-arom, $PhCH_2$). Anal. Calcd for $C_{250}H_{260}O_{46}$: C, 75.05; H, 6.55. Found: C, 74.81; H, 6.80.

6‴-α-Maltotriosyl-maltohexaose (**26**).—To a soln of **25** (720 mg, 0.18 mmol) in THF–MeOH–H_2O (130 mL, 6:6:1 v/v) was added 10% Pd–C (500 mg) and the reaction mixture was stirred under hydrogen (1 atm) for 30 h at rt. The catalyst was removed by filtration on a layer of Silica Gel and the filtrate was concd to dryness. The residue was chromatographed on Silica Gel (60 g) with MeOH–H_2O (7:3 v/v) as eluent and **26** was collected as a white powder from EtOH (212 mg, 80%) as an α,β mixture (~ 1:2, estimated from ^{13}C NMR): $[\alpha]_D \pm 166°$ (*c* 0.09, H_2O); ^{1}H NMR (D_2O): δ 4.65 (d, 0.6 H, $J_{1,2}$ 8.0 Hz, H-1$_\beta$), 4.96 (1 H, $J_{1,2}$ 3.7 Hz, H-1, branch point), 5.22 (d, 0.4 H, $J_{1,2}$ 3.7 Hz, H-1$_\alpha$), 5.31 (1 H, $J_{1,2}$ 3.7 Hz, H-1, internal anomeric), 5.33 (1 H, $J_{1,2}$ 3.7 Hz, H-1, internal anomeric), 5.35 (1 H, $J_{1,2}$ 3.7 Hz, H-1, internal anomeric), 5.37–5.40 (4 H, 4 H-1, internal anomeric); ^{13}C NMR (D_2O): δ 92.7 (C-1$_\alpha$), 96.6 (C-1$_\beta$), 99.3 (C-1, branch point), 100.2, 100.3, 100.5, 100.6, 100.7, 100.8, 100.9 (internal anomeric C). MALDI; Calcd for $C_{54}H_{92}O_{46}$ + Na: 1500.3. Found 1500.5. Calcd for $C_{54}H_{92}O_{46}$ + K: 1516.4. Found 1516.6.

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