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Exploiting an aromatic aglycone as a reporter of glycosylation stereochemistry in the synthesis of 1,6-linked maltooligosaccharides

Laurence Marmuse,[a] Sergey A. Nepogodiev[a] and Robert A. Field[a,b,*]

[a]*Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK*
[b]*Department of Biological Chemistry, John Innes Centre, Norwich NR4 7UH, UK*

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Abstract—Analysis of glycosylation stereoselectivity in the synthesis of branched maltooligosaccharides is hampered by poor spectral dispersion due to the repetitive nature of the saccharide chain and overlap of sugar H-1 signals with benzylic proton signals from the typically used benzyl ether protecting groups. A suitably protected *p*-methoxyphenyl maltoside acceptor, when coupled with benzylated maltooligosaccharide donors, gives discrete aglycone ^{1}H NMR signals that can be used to report on the stereoselectivity of 1,6-glycosylation reactions.
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1. Introduction

Starch is a ubiquitous biopolymer that is composed of two distinct α-glucans, the linear α(1,4)-linked amylose and the branched α-1,4(α-1,6)-linked amylopectin (Fig. 1).[1] The ratio of these two glucans, and other components,[2] and their propensity to self-organise into precise three dimensional, helical arrangements, are key features in the generation, structure and properties of the starch granule. The European Union starch trade has been estimated at ca. 3.5 million tonnes annually, worth ca. $1700 million, which reflects the broad range of applications for which starch and its derivatives are used (e.g., as a component of adhesives, seed coatings, talcum powder, surfactants, viscosity modifiers and glazing agents in food, absorbent personal health products, biodegradable filler).[1] To date bulk chemical modification processes have largely been used to generate novel starch-based products.[1] However, with recent advances in genetic engineering, modified crop plants are increasingly investigated as a means of producing of 'designer' starches.[3] Such approaches would benefit enormously from a better understanding of the structure, dynamics and assembly of the starch granule,[4] from a materials science perspective, and also from a more detailed picture of the biochemical events necessary for amylose, amylopectin, and hence ultimately starch granule formation.[5] Recent studies[6,7] have investigated the impact of glycan structure and solvation on amylopectin branch points and their propensity to initiate helix formation.

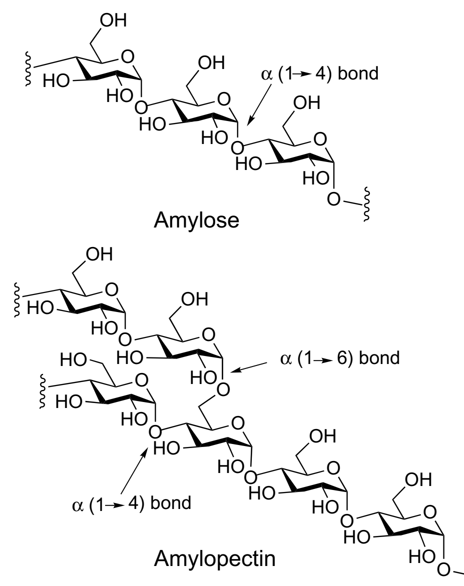

Figure 1. Representative partial structures of amylose and amylopectin.

* Corresponding author. Fax: +44 1603 592003; e-mail: r.a.field@uea.ac.uk

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These reports illustrate that the numbers of sugar units present in models under study has an impact on the conclusions reached; there is a need to move to large structures (perhaps >20 sugar residues) in order to obtain physiologically relevant information. We, therefore, set out to develop synthetic routes to complex linear and branched maltooligosaccharides that could be used to address issues relating to starch structure and biosynthesis.

Stepwise enzymatic syntheses of maltooligosaccharides using immobilised amylosucrase[8] and glycogen phosphorylase[9] have been reported, as have blockwise enzymatic syntheses of linear and branched maltodextrins and hemithiomaltodextrins using cyclodextrin glucosyltransferase in conjunction with maltose derivatives as donor substrates.[10] In terms of chemical syntheses, several effective, but technically complex, syntheses of branched maltooligosaccharides, up to a nonasaccharide, have been reported by Motawia et al.[11] In order to access even larger structures, we had in mind the use of readily available cyclodextrins as precursors of maltooligosaccharide building blocks (DP 6, 7, 8 from α-, β- and γ-CD, respectively). Similar approaches have been used before to generate such building blocks for synthetic chemistry[12,13] and enzyme studies.[9,14]

Taking β-cyclodextrin as a starting material, coupling two maltoheptasaccharide together, for instance, would generate one new stereogenic centre in a molecule that possesses no fewer than 70 stereogenic centres in total. Clearly the physical differences that one can anticipate from diastereomers differing in only 1 of 70 stereogenic centres does not leave one optimistic that chromatographic separation of mixed anomers is going to be a fruitful practice in the synthesis of increasingly large structures. Robust coupling reactions that give essentially complete control of anomeric stereochemistry are, therefore, required. We considered thioglycosides to be particularly suitable glycosyl donor building blocks since they allow for protecting group manipulation at a late stage and can be activated in a chemoselective fashion.[15] Herein we report the assessment of a model system that allows straightforward analysis of the stereoselectivity of thioglycoside-based 1,6-glycosylation reactions between maltooligosaccharide donors and a maltoside acceptor, relying on environment-induced changes in chemical shift of the acceptor aromatic aglycone protons as a read-out of α/β selectivity (Fig. 2).

2. Results and discussion

These studies required access to a suitably protected maltoside acceptor possessing both an aglycone that gives rise to distinct ^{1}H NMR signals, along with a free hydroxyl group at C-6 of the reducing terminal unit. Appropriate maltosaccharide donors were also required; these were accessed directly from commercial maltotriose (trisaccharide), and through selective acetolysis of protected β-cyclodextrin (heptasaccharide).

2.1. Synthesis of the maltoside acceptor

Commercial maltose monohydrate **1** was acetylated with acetic anhydride and iodine[16] and, on treatment with BF_3–OEt_2, was converted into the mixed α,β *p*-methoxyphenyl glycosides in a combined 69% yield (Scheme 1). Fractional crystallisation from diethyl ether gave the pure β-glycoside **2** in 41% isolated yield. Deacetylation and subsequent benzylidenation under standard conditions gave compound **3** in 65% yield over two steps.

Selective *tert*-butyldimethylsilylation of the primary alcohol in **3** gave silyl ether **4** in 85%, which was subsequently benzoylated under standard conditions to give the crystalline, fully protected maltoside **5**. Removal of the silyl ether protecting group with TBAF led to maltose derivative **6**, with the required free hydroxyl group at C-6, in 70% yield.

2.2. Synthesis of the maltotrioside and maltoheptaoside donors

Acetylated maltotriose was prepared by treating maltotriose with sodium acetate in acetic anhydride.[17] Without purification, the resulting undecaacetate **7** (Scheme 2) was converted into peracetylated mixed anomeric thiomethyl glycosides **8** (α:β ca. 1:1.5) in 90% yield by treatment with iodine, dimethyldisulfide and hexamethyldisilane.[18] In order to effect α-glycosylation later, participating acetate groups in **8** were replaced with

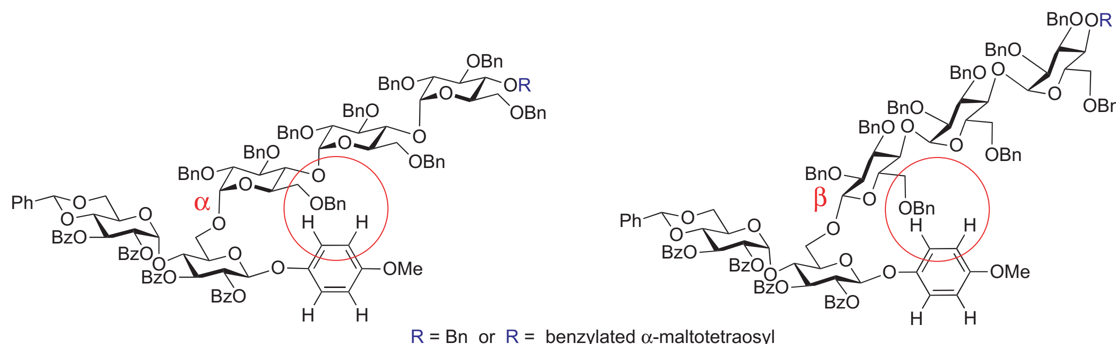
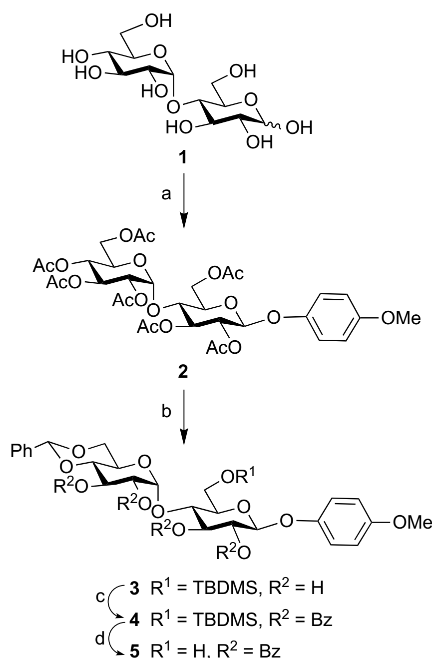
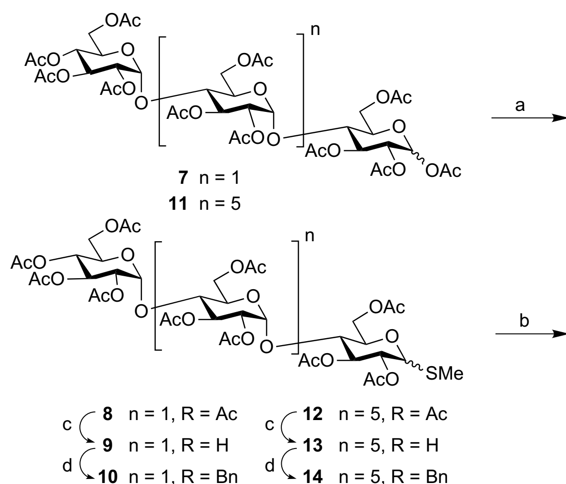


Figure 2. Potential differences in environment that might be encountered by the aromatic aglycone protons of α-1,6- and β-1,6-linked maltooligosaccharyl-maltoside, and which may give rises to differences in aglycone ^{1}H NMR signals.



Scheme 1. Reagents and conditions: (a) *p*-methoxyphenol, CH_2Cl_2, BF_3–OEt_2, 41%; (b) (i) MeOH, NaOMe, (ii) benzaldehyde dimethylacetal, DMF, *p*-toluenesulfonic acid, 65%; (c) *tert*-butyldimethylsilylchloride, pyridine, 75%; (d) BzCl, pyridine, 83%; (e) Bu_4NF, THF, 70%.



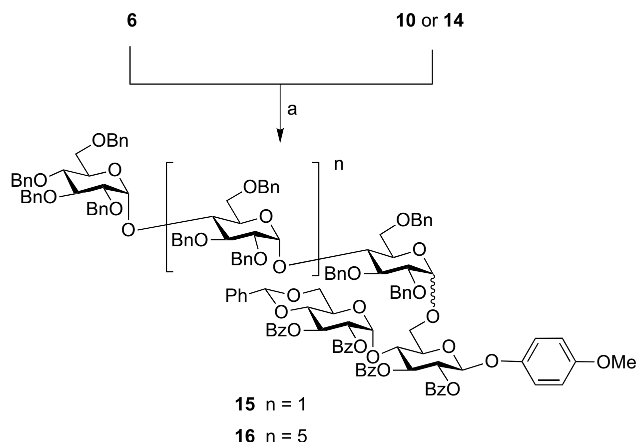
Scheme 2. Reagents and conditions: (a) Me–S–S–Me, I_2, Me_3Si–$SiMe_3$, CH_2Cl_2, **8** 90%, **12** 86%; (b) MeOH, NaOMe, **9** 92%, **13** 94%; (c) for **10**: BnBr, NaH, Et_4NI, DMF. For **14**: BnBr, NaH, Et_4NI, imidazole, DMF, **10** 85%, **14** 80%.

non-participating benzyl ether protecting groups. After removal of the acetate groups with sodium methoxide in methanol, giving thiomethyl maltotrioside **9**, treatment with benzyl bromide in the presence of sodium hydride and tetraethylammonium iodide in DMF[11d] gave trisaccharide donor **10**.

For the preparation of benzylated thioheptasaccharide donor **14**, β-cyclodextrin (cyclomaltoheptaose) was considered a suitable starting material in which one glycosidic bound can be selectively cleaved to give a linear heptasaccharide. Initial studies investigated acetolysis of per-*O*-benzylated β-cyclodextrin[19] using the method developed for per-*O*-acetylated β-cyclodextrin[13] or for thiolytic cleavage of per-*O*-methylated α/β-cyclodextrin.[20] However, in the case of per-*O*-benzylated cyclodextrin reaction conditions typically employed in such processes gave mixed products arising from benzyl ether cleavage.[21] Resorting to acetolysis of per-*O*-acetylated β-cyclodextrin with Ac_2O and H_2SO_4,[13] peracetylated maltoheptaosides **11** were obtained in 46% yield (Scheme 2). Following the procedure for the preparation of benzylated thiomaltotriosides **10**, reaction of **11** with iodine, dimethyldisulfide and hexamethyldisilane gave peracetylated heptasaccharide thioglycosides **12** (α:β ca. 1:1.5) in 86% yield. Deacetylation of **12** with sodium methoxide in methanol and subsequent benzylation with sodium hydride, imidazole and tetraethylammonium iodide in DMF[12b] gave the required heptasaccharide donors **14** (72% from **12**).

2.3. Glycosylation reactions: preparation of the branched pentasaccharides 15 and nonasaccharides 16

Our ultimate objective was to develop a set of glycosylation reaction conditions that would give high yields and α-stereoselectivity with complex maltooligosaccharide donors and acceptors. In initial reactions between maltoside acceptor **6** and thiomaltotrioside donor **10**, diethyl ether-based solvent mixtures were employed because of the reported α-selectivity that they afford.[22] Due to the limited solubility of acceptor **6** in neat diethyl ether, glycosylation reactions were carried out in diethyl ether–DCM 4:1, with NIS/TMSOTf[23] as the promoter at −60 °C. Under such conditions, the branched pentasaccharides **15** were obtained in 57% isolated yield (Scheme 3). The ^{1}H NMR spectrum of the mixture, complicated by the presence of the benzylic protecting group proton signals, did not easily allow confirmation of the stereoselectivity of the glycosylation reaction. However, mass spectrometry did confirm that coupling



Scheme 3. Reagents and conditions: (a) NIS, TMSOTf, Et_2O–CH_2Cl_2 (1:4), **15** 57%, **16** 36%.

to give pentasaccharide products **15** had been achieved: the observed spectrum of the $[M+Na]^+$ ion ($C_{142}H_{138}O_{31}Na$, major peak *m/z* 2362.9) and the isotope pattern for $[M+Na]^+$ of this compound were an excellent match.

To allow more detailed analysis of NMR data, the pure α- and β-anomers **15α** and **15β** were isolated from the above mixture by extensive, laborious column chromatography on silica gel, giving isolated yields of 21% for the pentasaccharide **15α** and 6% for the pentasaccharide **15β**. From preliminary synthetic reactions of the type outlined, coupled with extensive chromatography, and long-winded two-dimensional NMR experiments, it was evident that anomeric ^{1}H NMR signals for internal β-linkages in maltooligosaccharides lie at ca. 4.6 ppm, enveloped by both other sugar and benzylic proton signals, whilst anomeric ^{1}H signals for α-linkages lie clear of other signals at ca. 5.5–6.0 ppm. In contrast, anomeric ^{13}C NMR signals for internal β-linkages in maltooligosaccharides (ca. 105 ppm) are distinct from C-1 signals for internal α-linked sugars (ca. 96–98 ppm). However, spectral overlap in the former case, and the insensitivity of the technique in the latter, make direct ^{1}H or ^{13}C NMR quantitation of α/β mixtures by analysis of sugar signals impractical.

On first inspection, the ^{1}H NMR spectra for pure and mixed anomers of pentasaccharide **15** look very similar (Fig. 3, **15**). On closer inspection, clear differences in the aromatic region are evident. Two sets of peaks corresponding to the protons of the *p*-methoxyphenyl aglycone can be distinguished. These protons show chemical shifts that are influenced by the stereochemistry of the newly formed 1,6-linkage; in the case of the major component of the mixture, the α-glycoside **15α**, doublets appear at 6.63 and 6.82 ppm, whereas for the minor β-linked component **15β**, doublets appear at 6.57 ppm and 6.77 ppm (Fig. 4). Hence, the anomeric composition of the mixture of pentasaccharides **15** can easily be determined by integration of the *p*-methoxyphenyl aglycone proton signals.

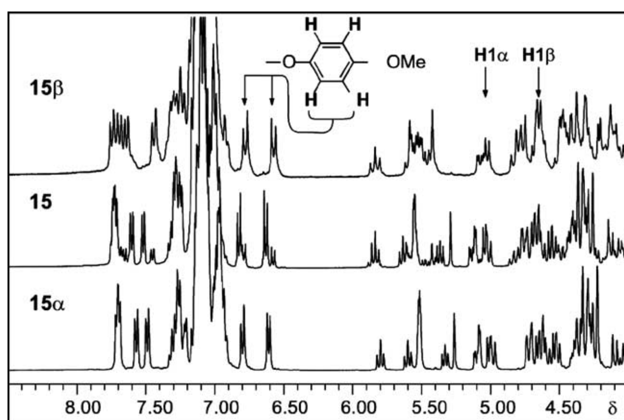


Figure 3. Extracts of ^{1}H NMR spectra (400 MHz, $CDCl_3$) of pentasaccharides **15** (α,β mixture) obtained by glycosylation of **6** with **10** and purified anomers **15α** and **15β**.

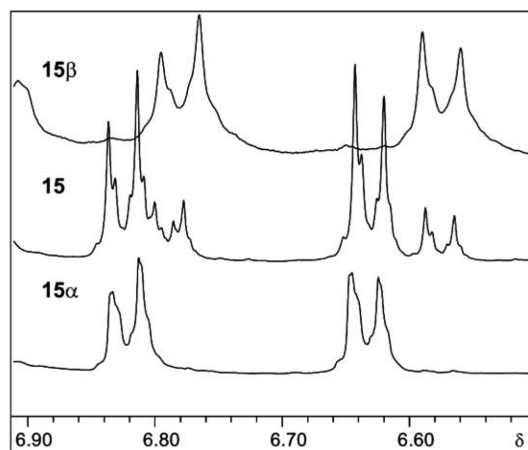


Figure 4. Expansion of the methoxyphenyl region of ^{1}H NMR spectra (400 MHz, $CDCl_3$) of pentasaccharides **15** (α,β mixture) and purified anomers **15α** and **15β**.

Coupling of maltoside acceptor **6** with maltoheptaoside donor **14** (Scheme 3) under similar conditions to those used in the pentasaccharide synthesis gave a mixture of anomeric nonasaccharides, **16**, in 36% combined yield (α:β ca. 3.7:1). Very careful chromatographic purification afforded small quantities of pure **16α** (16 mg, 4% isolated yield). Although less distinct from other aromatic signals in this instance, due to the 22 benzyl protecting groups in **16**, ^{1}H NMR analysis illustrates that aglycone ^{1}H NMR signals (6.75/6.95 ppm for **16α**; 6.68/6.88 ppm for **16β**) can again be used to report on the stereochemical composition of the mixed anomeric products (Fig. 5).

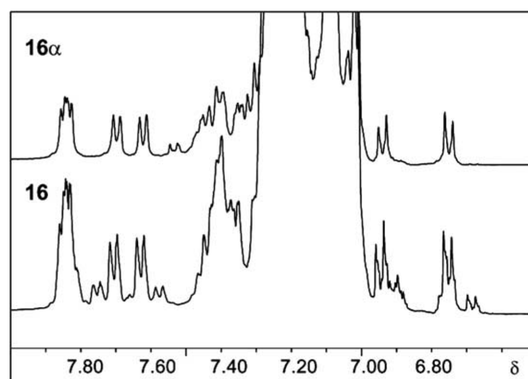


Figure 5. Extracts of the aromatic region of ^{1}H NMR spectra (400 MHz, $CDCl_3$) of nonasaccharides **16** (α,β mixture) and purified anomer **16α**.

3. Conclusion

In summary, judicious choice of a *p*-methoxyphenyl acceptor aglycone provides, through ^{1}H NMR spectroscopy, direct access to information about glycosylation stereoselectivity for 1,6-maltooligosaccharide couplings that would otherwise be tortuous to analyse. Optimisation of maltooligosaccharide glycosylation reaction stereochemistry using this reporter system is ongoing.

4. Experimental

4.1. General methods

Reactions were carried out in dry solvents using septa and syringes for addition of reagents. Anhydrous DMF and Et_2O were purchased from Aldrich, CH_2Cl_2 was distilled from CaH_2, MeOH was distilled from $Mg(OMe)_2$, pyridine was distilled from P_2O_5 and stored over 4 Å molecular sieves. Cation-exchange resins were washed with water and dry MeOH before use. TLC was performed on precoated aluminium plates (Silica Gel 60 F_{254}, Merck). Spots were visualised by exposure to UV light or by immersion into 5% ethanolic H_2SO_4 followed by heating to 150 °C. Solutions of reaction products were dried over $MgSO_4$ and solvents were evaporated under reduced pressure at 25–40 °C. Column chromatography was performed on silica gel (40–70 μm, BDH–Merck). Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured at 25 °C using a Perkin–Elmer 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded at 24 °C with a Varian Gemini 2000 spectrometer at 300 and 75 MHz, respectively, or with a Varian Unity Plus spectrometer at 400 and 100 MHz, respectively, using TMS (for solution in $CDCl_3$) or MeOH (δ 49.9, for solutions in D_2O) as internal standards. Resonance assignments were made with the aid of COSY and HSQC experiments. In cases where spectral dispersion was poor, only selected diagnostic NMR data are given; other spectral features were in accord with the proposed structures. Due to the repetitive sequence of the saccharides being analysed, in places attributing a given signal to an individual sugar residue is not possible and assignments given should be taken as tentative. Sugar residues are referred to alphabetically from the reducing terminus as a, b etc.; the second chain introduced through a 1,6-linkage, is referred to as a′, b′, etc. Accurate electrospray ionisation mass spectra (ESI-MS) were obtained using positive ionisation mode on Finnigan MAT 900 XLT mass spectrometer. For compounds with molecular masses >1000 low resolution ESI-MS were obtained on the same instrument or low resolution MALDI-TOF MS were obtained on Applied Biosystems Voyager mass spectrometer and experimental data were matched to theoretical isotope patterns.

4.2. *p*-Methoxyphenyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside, 2

Maltose octaacetate **1** (13 g, 19 mmol) was prepared by reaction of maltose monohydrate (6.9 g, 19 mmol) with acetic anhydride (70 mL) in the presence of iodine (500 mg) using a literature procedure.[16] The peracetate obtained in this way was dissolved in dry CH_2Cl_2 (100 mL), *p*-methoxyphenol (10.85 g, 87 mmol) was added, the mixture was cooled on an ice bath and BF_3–OEt_2 (13 mL, 54.6 mmol) was added dropwise. The mixture was stirred for 12 h at room temperature, diluted with CH_2Cl_2 (150 mL), washed successively with H_2O (2 × 80 mL), saturated $NaHCO_3$ solution (2 × 80 mL) and H_2O (2 × 80 mL) and dried over $MgSO_4$. Concentration in vacuo and crystallisation (diethyl ether) gave the title β-aryl glycoside (5.8 g, 41%); mp 130–132 °C (diethyl ether); $[\alpha]_D$ = +49.2 (*c* 1.0, $CHCl_3$); δ_H ($CDCl_3$): 2.01, 2.03, 2.04, 2.05, 2.06, 2.11 (2) (21H, 7 × s, 7 × Ac), 3.78 (4H, m, OMe, H-5a), 3.97 (1H, m, H-5b), 4.01 (2H, m, H-4b, H-6), 4.26 (2H, m, H-6,6′), 4.49 (1H, dd, $J_{5,6}$ 2.9 Hz, $J_{6,6'}$ 12.2 Hz, H-6′), 4.87 (1H, dd, $J_{1,2}$ 3.9 Hz, H-2b), 4.99 (1H, d, $J_{1,2}$ 7.6 Hz, H-1a), 5.06 (2H, m, H-2a, H-4a), 5.32 (1H, t, $J_{2,3} \sim J_{3,4}$ 8.8 Hz, H-3a), 5.37 (1H, t, $J_{2,3} \sim J_{3,4}$ 10.2 Hz, H-3b), 5.44 (1H, d, $J_{1,2}$ 3.9 Hz, H-1b), 6.82 (2H, J_{AB} 8.0 Hz, C_6H_4–OMe), 6.94 (2H, J_{AB} 8.0 Hz, C_6H_4–OMe); δ_C ($CDCl_3$): 20.7 (2), 20.8 (2), 20.9, 21.0, 21.1 (7 × CH_3C=O), 55.8 (OMe), 61.7, 63.0 (C-6a, C-6b), 68.2, 68.7, 69.5, 70.2, 72.2, 72.4, 72.8, 75.5 (C-2a,b to C-5a,b), 95.7 (C-1b), 99.8 (C-1a), 114.7, 114.9, 116.1, 118.9 (Ar), 150.9, 155.9 (Ar quat.), 169.7, 169.9, 170.2, 170.5, 170.6, 170.7, 170.8 (CH_3C=O); *m*/*z* (ES) 765.3 (70% $[M+Na]^+$), (ES) (Found: $[M+NH_4]^+$, 760.2659. $C_{33}H_{46}O_{19}N$ requires 760.2659).

4.3. *p*-Methoxyphenyl 4-*O*-(4,6-*O*-benzylidene-α-D-glucopyranosyl)-β-D-glucopyranoside, 3

β-Aryl glycoside **2** (2.94 g, 3.88 mmol) was dissolved in dry MeOH (30 mL), sodium methoxide solution (8 mL of 0.2 M solution in methanol) was added and the mixture was stirred at room temperature until the reaction was complete, as judged by TLC. Amberlite IR-120 (H^+) ion exchange resin was added to neutralise the mixture, followed by concentration in vacuo. The resulting residue was dissolved in DMF (20 mL) and benzaldehyde dimethyl acetal (1.16 mL, 7.76 mmol) and *p*-toluenesulfonic acid monohydrate (50 mg) were added. The mixture was stirred at room temperature for 12 h, neutralised with triethylamine, concentrated in vacuo and the resulting residue was subjected to column chromatography (acetone–CH_2Cl_2, 7:3) to give the title compound (1.3 g, 65%); $[\alpha]_D^{25}$ = +27.0 (*c* 0.9, MeOH); δ_H (CD_3OD): 3.91–4.22 (15H, m, H-2a,b, H-3a,b, H-4a,b, H-5a,b, H-6a,b, OMe), 4.79 (1H, d, $J_{1,2}$ 7.7 Hz, H-1a), 4.86 (1H, s, C*H*–Ph), 5.22 (1H, d, $J_{1,2}$ 3.7 Hz, H-1b), 6.83 (2H, d, J_{AB} 9.2 Hz, C_6H_4–OMe), 7.05 (2H, d, J_{AB} 9.2 Hz, C_6H_4–OMe), 7.31–7.50 (9H, m, Ar); δ_C ($CDCl_3$): 55.7 (OMe), 61.2, 63.8 (C-6a, C-6b), 68.7, 70.9, 73.1, 73.3, 74.8, 76.0, 79.8, 80.7 (C-2a,b to C-5a,b), 101.8, 102.0 (C-1a, C-1b), 102.2 (CH_2Ph), 114.8–155.7 (Ar); *m*/*z* (ES) 559.1 (100% $[M+Na]^+$), (ES) (Found: $[M+NH_4]^+$, 554.2230. $C_{26}H_{36}O_{12}N$ requires 554.2232).

4.4. *p*-Methoxyphenyl 4-*O*-(4,6-*O*-benzylidene-α-D-glucopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside, 4

To a solution of benzylidene-protected maltoside **3** (1.33 g, 2.47 mmol) in dry pyridine (5 mL) was added *tert*-butyldimethylsilyl chloride (632 mg, 4.19 mmol). After stirring overnight, the mixture was concentrated in vacuo and co-evaporated with toluene (three times).

Chromatography of the resulting residue (CH_2Cl_2–MeOH, 12:1) gave the title compound (1.39 g, 85%); $[\alpha]_D^{25} = +30.7$ (*c* 1.1, $CHCl_3$); δ_H ($CDCl_3$): 0.02 (6H, s, $SiMe_2$), 0.87 (9H, s, *t*-Bu), 3.29–4.18 (12H, m, H-2 to H-6), 3.70 (3H, s, OMe), 4.65 (1H, d, $J_{1,2}$ 7.7 Hz, H-1a), 4.99 (1H, d, $J_{1,2}$ 3.5 Hz, H-1b), 5.42 (1H, s, C*H*–Ph), 6.70 (2H, d, J_{AB} 9.1 Hz, C_6H_4–OMe), 6.98 (2H, d, J_{AB} 9.1 Hz, C_6H_4–OMe), 7.25–7.48 (5H, m, Ar); δ_C ($CDCl_3$): −5.4, −5.3 (*Me*$_2$CSiCMe$_3$) 18.2 (Me$_2$*C*SiCMe$_3$), 25.8 (Me$_2$CSi*t-Bu*), 55.4 (OMe), 62.5, 63.6 (C-6a, C-6b), 68.7, 70.8, 73.2, 73.4, 75.5, 76.1, 80.52, 80.7 (C-2a,b to C-5a,b), 101.6 (C-1a), 101.8 (*C*H–Ph), 102.1 (C-1b), 114.3–137.8 (Ar), 151.2, 155.2 (Ar quat. from C_6H_4–OMe); *m*/*z* (CI) 668.4 (45% [M+NH$_3$]), 544.3 (100, [M−124.1]), (ES) (Found: $[M+NH_4]^+$, 668.3101. $C_{32}H_{50}O_{12}SiN$ requires 668.3097).

4.5. *p*-Methoxyphenyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-β-D-glucopyranoside, 5

Silylated benzylidene maltoside **4** (1.40 g, 2.15 mmol) was dissolved in dry pyridine (20 mL), cooled on an ice bath and benzoyl chloride (2 mL, 17.2 mmol) was added. After 2 days the mixture was diluted with DCM (200 mL), washed successively with 1 M HCl (3 × 60 mL) and saturated $NaHCO_3$ solution (3 × 60 mL) and dried over $MgSO_4$. Concentration in vacuo gave a residue which was subjected to column chromatography (toluene–ethyl acetate, 10:1) and crystallised from ethanol to give small crystals of the title compound (1.72 g, 75%); mp 176–178 °C (ethanol); $[\alpha]_D^{25} = +60.1$ (*c* 1.0, $CHCl_3$); δ_H ($CDCl_3$): 0.12 (3H, s, $SiMe_2$), 0.16 (3H, s, $SiMe_2$), 0.96 (9H, s, *t*-Bu), 3.73–3.87 (6H, m, OMe, H-4b, H-5b, H-6a), 4.11–4.15 (3H, m, H-5a, H-6b, H-6′b), 4.39–4.45 (2H, m, H-4a, H-6′a), 5.15 (1H, d, $J_{1,2}$ 7.7 Hz, H-1a), 5.19 (1H, dd, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 9.9 Hz, H-2b), 5.46 (1H, dd, $J_{1,2}J_{2,3}$ 9.4 Hz, H-2a), 5.52 (1H, s, C*H*-Ph), 5.64 (1H, d, $J_{1,2}$ H-1b), 5.72 (1H, t, $J_{2,3}$, $J_{3,4}$ 9.4 Hz H-3a), 5.92 (1H, t, $J_{2,3}$, $J_{3,4}$ 9.9 Hz, H-3b), 6.75 (2H, d, J_{AB} 8.9 Hz, C_6H_4–OMe), 6.92 (2H, d, J_{AB} 8.9 Hz, C_6H_4–OMe), 7.24–7.85 (25H, m, Ar); δ_C ($CDCl_3$): −5.05, −5.0 (*Me*$_2$CSi*t*-Bu), 18.5 (Me$_2$*C*Si*t*-Bu), 26.0 (Me$_2$CSi*t-Bu*), 55.5 (OMe), 62.2, 63.8 (C-6a, C-6b), 68.6, 69.3, 71.4, 71.5, 72.1, 75.1, 75.5, 79.2 (C-2a,b to C-5a,b), 96.7 (C-1b), 100.3 (C-1a), 101.6 (*C*H–Ph), 114.4–136.8 (Ar), 151.2, 155.2 (Ar quat. from C_6H_4–OMe), 164.9, 165.2, 165.4, 165.5 (*C*=O); *m*/*z* (ES) 1089.6 $[M+Na]^+$.

4.6. *p*-Methoxyphenyl 2,3-di-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-β-D-glucopyranoside, 6

To a solution of fully protected silyl maltoside **5** (1.5 g, 1.40 mmol) in dry THF (10 mL) was added dropwise Bu_4NF (2.8 mmol, 2.8 mL of a 1 M solution in THF). The mixture was stirred at room temperature for 1 h and the solvent was removed in vacuo. The resulting residue was dissolved in DCM (100 mL), washed with H_2O (30 mL), saturated $NaHCO_3$ solution (3 × 30 mL) and H_2O (3 × 30 mL). Concentration in vacuo and column chromatography (CH_2Cl_2–ethyl acetate, 1:0–95:5) gave the title compound (936 mg, 70%) as white foam; $[\alpha]_D^{25} = +77$ (*c* 1.0, $CHCl_3$); δ_H ($CDCl_3$): 3.73 (3H, s, OMe), 3.80–3.88 (3H, m, H-4b, H-5a/b, H-6), 4.04–4.14 (3H, m, H-5a/b, 2 × H-6), 4.46 (1H, dd, $J_{5,6}$ 4.7 Hz, $J_{6,6'}$ 10.1 Hz, H-6), 4.53 (1H, t, $J_{3,4} \sim J_{4,5}$ 9.3 Hz, H-4a), 5.19–5.23 (2H, m, H-1a, H-2b), 5.49 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.5 Hz, H-2a), 5.53 (1H, s, C*H*–Ph), 5.67 (1H, d, $J_{1,2}$ 4.2 Hz, H-1b), 5.75 (1H, t, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3a), 5.92 (1H, t, $J_{2,3} \sim J_{3,4}$ 9.5 Hz, H-3b), 6.77 (2H, d, J_{AB} 9.1 Hz, C_6H_4–OMe), 6.89 (2H, d, J_{AB} 9.1 Hz, C_6H_4–OMe), 7.25–7.86 (25H, m, Ar); δ_C ($CDCl_3$): 55.5 (OMe), 61.5, 63.8 (C-6a, C-6b), 68.5, 69.4, 71.2 (2), 72.2, 74.9, 75.0, 78.9 (C-2a,b to C-5a,b), 97.1 (C-1b), 100.2 (C-1a), 101.4 (*C*H–Ph), 114.6–136.8 (Ar), 151.0, 155.7 (Ar quat. from C_6H_4–OMe), 165.0, 165.3, 165.6, 165.7 (*C*=O); *m*/*z* (ES) 975.4 (100%, [M+Na$^+$]), (ES) (Found: $[M+NH_4]^+$, 970.3286 $C_{54}H_{52}O_{16}N$ requires 970.3281).

4.7. Methyl (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-1-thio-2,3,6-tri-*O*-acetyl-α,β-D-glucopyranoside 8

Maltotriose undecaacetate **7** was prepared according to the procedure described by Wolfrom and Thompson.[17] Acetic anhydride (20 mL) and NaOAc (2 g) were heated to reflux and maltotriose (2.05 g, 4.06 mmol) was carefully added and the mixture was refluxed for a further hour. The reaction mixture was cooled and the solvent was co-evaporated with toluene. The resulting residue was dissolved in CH_2Cl_2 (150 mL) and successively washed with H_2O (3 × 50 mL), saturated $NaHCO_3$ solution (3 × 50 mL) and H_2O (3 × 50 mL) and dried over $MgSO_4$. This material was used directly in the next step without characterisation. The title compound was prepared according to the procedure described by Kartha and Field.[18] Crude maltotriose undecaacetate **7** (3.71 g, 3.83 mmol) was dissolved in dry CH_2Cl_2 (40 mL), dimethyldisulfide (190 μL, 2.10 mmol) and hexamethyldisilane (430 μL, 2.10 mmol) were added, followed by I_2 (970 mg, 3.83 mmol). The mixture was stirred at room temperature for 5 h, diluted with CH_2Cl_2 (200 mL), washed with NaS_2O_3 solution (3 × 70 mL) and H_2O (2 × 80 mL) and dried over $MgSO_4$. Concentration in vacuo and column chromatography (toluene–ethyl acetate, 9:1–1:1) gave the title compound as a white foam (3.50 g, 86%); α:β ca. 1:1.5; δ_H ($CDCl_3$): 4.26 (d, $J_{1,2}$ 10.9 Hz, H-1aβ), 5.28 (d, $J_{1,2}$ 3.9 Hz, H-1b or H-1c), 5.41 (d, $J_{1,2}$ 4.1 Hz, H-1b or H-1c), 5.43 (d, $J_{1,2}$ 5.5 Hz, H-1aα); δ_C ($CDCl_3$): 11.3, 12.3 (SMe, α/β), 82.5 (2, C-1aα, C-1aβ), 95.6, 95.8 (C-1b, C-1c).

4.8. Methyl α-D-glucopyranosyl-(1→4)-glucopyranosyl-(1→4)-1-thio-α,β-D-glucopyranoside, 9

Fully acetylated methyl thiomaltotrioside **8** (3.43 g, 3.37 mmol) was dissolved in dry MeOH (30 mL) containing a catalytic amount of sodium methoxide and the mixture was stirred at room temperature until the reaction was complete, as judged by TLC. Amberlite IR-120 (H^+) ion exchange resin was added to neutralise the mixture, followed by concentration in vacuo to give

the title compound (1.66 g, 92%); α:β ca. 1:1.5; $[\alpha]_D^{25}$ = +149.0 (*c* 0.6, MeOH); δ_H (CD_3OD): 2.05, 2.17 (SMe, α/β), 4.27 (d, $J_{1,2}$ 9.7 Hz, H-1aβ), 5.14–5.17 (m, H-1b/c,α/β), 5.20 (d, $J_{1,2}$ 4.5 Hz, H-1aα); δ_C (CD_3OD): 12.0, 12.7 (SMe, α/β), 87.1, 87.9 (C-1aα, C-1aβ), 102.5, 102.6, 102.7, 102.8 (C-1b/c,α/β); *m*/*z* (ES) 557.3 (50%, $[M+Na]^+$), (ES) (Found: $[M+NH_4]^+$, 552.1957 $C_{19}H_{38}O_{15}N$ requires 552.1957).

4.9. Methyl (2,3,4,6)-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-1-thio-2,3,6-tri-*O*-benzyl-α/β-D-glucopyranoside

Deprotected trisaccharide **9** (1.6 g, 2.95 mmol) was dissolved in dry DMF (35 mL), NaH (88.8 mmol, 4.0 g of a 60% w/v suspension in mineral oil) was added slowly to the reaction and stirred at room temperature for half an hour. The mixture was then cooled on an ice bath and BnBr (10.5 mL, 88.8 mmol) was added, followed by Et_4NI (50 mg). The stirred mixture was allowed to slowly warm to room temperature over 5 h and excess NaH was decomposed by dropwise addition of MeOH (50 mL). The resulting solution was concentrated in vacuo, diluted with CH_2Cl_2 (200 mL), washed with H_2O (3 × 70 mL) and dried with $MgSO_4$. The crude product was purified by column chromatography (hexane–ethyl acetate, 9:1–7:3) to give the title compound (3.63 g, 85%) as white solid; $[\alpha]_D^{25}$ = +67.2 (*c* 1.06, $CHCl_3$); δ_H ($CDCl_3$): 5.34 (d, $J_{1,2}$ 5.3 Hz, H-1aα), 5.54 (d, $J_{1,2}$ 3.5 Hz, H-1b/c,α/β), 5.56 (d, $J_{1,2}$ 3.5 Hz, H-1b/c,α/β), 5.65 (d, $J_{1,2}$ 3.5 Hz, H-1b/c,α/β), 5.66 (d, $J_{1,2}$ 3.8 Hz, H-1b/c,α/β), 5.68 (d, $J_{1,2}$ 3.5 Hz, H-1b/c,α/β); δ_C ($CDCl_3$): 12.2, 12.6 (SMe, α/β), 84.2 (C-1aα), 85.0 (C-1aβ), 96.5, 96.8, 96.9, 97.0 (C-1b/c,α/β); *m*/*z* (ES) 1453.0 (20% $[M+NH_4]^+$), 180.9 (100, [M−1272.1]).

4.10. Tricosa-*O*-acetylmaltoheptaose, 11

The title compound was prepared according to the procedure described by Sakairi et al.[13] Peracetylated β-cyclodextrin (20 g, 9.91 mmol) was dissolved in solution of Ac_2O–H_2SO_4, (50:1, 120 mL). The mixture was heated at ∼60 °C for 20 h, poured into ice water (200 mL) containing sodium acetate (12 g) and the mixture was stirred overnight. Organic material was extracted with CH_2Cl_2 (3 × 100 mL), washed with brine (2 × 100 mL), saturated $NaHCO_3$ solution (2 × 100 mL), and water (1 × 100 mL) and dried over $MgSO_4$. Concentration in vacuo and purified by column chromatography (ethyl acetate–hexane, 9:1–1:0) gave the title compound as white solid (9.76 g, 46%); α:β ca. 8:1; $[\alpha]_D^{25}$ = +162.0 (*c* 1.15, $CHCl_3$); δ_H ($CDCl_3$): 4.85 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4.95 (1H, dd, $J_{1,2}$ 3.1 Hz, $J_{2,3}$ 10.1 Hz, H-2aα), 5.08 (1H, t, $J_{3,4} \sim J_{4,5}$ 9.5 Hz, H-4g), 5.52 (1H, t, $J_{2,3} \sim J_{3,4}$ 9.3 Hz, H-3aα), 5.76 (0.11H, $J_{1,2} \sim 9$ Hz, H-1αβ), 6.24 (1H, d, $J_{1,2}$ 3.6 Hz, H-1aα) δ_C ($CDCl_3$): 20.2–20.8 (CH_3C=O), 61.3, 62.08, 62.3, 67.8, 68.3, 68.8, 69.0, 69.2, 69.7, 69.9, 70.1, 70.3, 70.4, 71.5, 71.6, 72.1, 72.3, 73.2, 76.7 (C-2 to C-6), 88.8 (C-1aα), 95.6, 95.6, 95.8 (C-1), 169.0, 169.5, 169.6, 169.8, 169.9, 170.4, 170.5 (2), 170.6, 170.7, 170.8 (CH_3*C*=O); *m*/*z* (ES) 2141 (33%, $[M+Na]^+$).

4.11. Methyl (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-{(2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-(1→4)}$_5$-2,3,6-tri-*O*-acetyl-1-thio-α,β-D-glucopyranoside 12

Per-*O*-acetylmaltoheptaose **11** (1.00 g, 0.47 mmol) was dissolved in dry CH_2Cl_2 (5 mL), dimethyldisulfide (23 μL, 0.26 mmol) and hexamethyldisilane (52 μL, 0.26 mmol) were added, followed by I_2 (0.47 mmol, 120 mg) and the mixed was stirred at room temperature for 8 h. The reaction was then diluted with CH_2Cl_2 (150 mL) and washed with NaS_2O_3 solution (3 × 50 mL) and H_2O (2 × 50 mL) and dried over $MgSO_4$. Concentration in vacuo and column chromatography (hexane–ethyl acetate, 4:1) gave the title compound as a yellowish solid (850 mg, 86%); $[\alpha]_D^{25}$ = +145.0 (*c* 1.1, $CHCl_3$); δ_H ($CDCl_3$): 5.28–5.42 (7 × H-1); δ_C ($CDCl_3$): 11.2, 12.2 (SMe, α/β), 82.4 (C-1aα,β), 95.7 (6) (C-1b-g); *m*/*z* (MALDI-TOF) 2113.5 (100% $[M+Li]^+$).

4.12. Methyl α-D-glucopyranosyl-(1→4)-{α-D-glucopyranosyl-(1→4)}$_5$-1-thio-α,β-D-glucopyranoside 13

Acetylated methyl thiomaltoheptoside **12** (850 mg, 0.40 mmol) was dissolved in dry MeOH (10 mL) containing a catalytic amount of sodium methoxide and the mixture was stirred at room temperature until the reaction was complete, as judged by TLC. H_2O (5 mL) was added, followed by Dowex AG-50 (H^+) resin to neutralise the mixture. Concentration in vacuo gave the title compound (446 mg, 94%) as a solid; α:β ca. 1:2; δ_H (D_2O): 1.93, 2.02 (3H, s, SMe α/β), 4.27 (d, $J_{1,2}$ 9.9 Hz, H-1β), 5.14 (d, $J_{1,2}$ 4.1 Hz, H-1α), 5.21 (m, H-1b-g); δ_C (D_2O): 11.4, 12.2 (SMe α/β), 85.5 (C-1aα), 86.3 (C-1aβ), 99.6–99.8 (C-1b-g); *m*/*z* (ES) 1205.5 (100% $[M+Na]^+$), (ES) (Found: $[M+NH_4]^+$, 1200.4081. $C_{43}H_{78}O_{35}SN$ requires 1200.4070).

4.13. Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-{2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)}$_5$-2,3,6-tri-*O*-benzyl-1-thio-α,β-D-glucopyranoside 14

Methyl thiomaltoheptaoside **13** (446 mg, 0.37 mmol) was dissolved in dry DMF (25 mL), NaH (33 mmol, 1.32 g of a 60% w/v suspension in mineral oil) was added slowly to the reaction and mixed at room temperature for half an hour. The mixture was then cooled on an ice bath and BnBr (2.95 mL, 24.8 mmol) was added, followed by Et_4NI (12 mg) and imidazole (5 mg). The stirred mixture was allowed to slowly warm to room temperature over 20 h and excess NaH was decomposed by dropwise addition of MeOH (10 mL). The resulting solution was concentrated in vacuo, diluted with CH_2Cl_2 (100 mL), washed with H_2O (2 × 50 mL) and dried with $MgSO_4$. The crude product was purified by column chromatography (toluene–ethyl acetate, 10:0–9:1) to give the title compound as gum (943 mg, 80%); α:β ca. 1:1.5; $[\alpha]_D^{25}$ = +69 (*c* 1.00, $CHCl_3$); δ_H ($CDCl_3$): 2.04 (s, SMeα), 2.23 (s, SMeβ), 5.33 (d, $J_{1,2}$ 5.3 Hz, H-1aα), 5.52–5.70 (H-1a, H-1b-g); δ_C ($CDCl_3$): 12.5 (SMe), 85.0 (C-1aα), 86.4 (C-1aβ), 96.4–97.0 (C-1b-g); *m*/*z* (MALDI-TOF): 3172.4 (100%, $[M+Li]^+$).

4.14. *p*-Methoxyphenyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-D-glucopyranosyl)-(1→6)-[(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α,β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranoside 15

Glycosyl donor **10** (430 mg, 0.29 mmol) and glycosyl acceptor **6** (200 mg, 0.21 mmol) were dissolved in dry Et_2O–DCM (4:1) containing activated 4 Å molecular sieves (300 mg) and stirred for 90 min. The reaction mixture was cooled to −60 °C, and *N*-iodosuccinimide (94 mg, 0.42 mmol) and TMSOTf (7.6 μL, 0.042 mmol, 10 mol% with respect to NIS) were added to the solution. The reaction was allowed to warm to ice bath temperature and stirred for 8 h. The mixture was then filtered through Celite, the filtrate was diluted with CH_2Cl_2 (100 mL) and washed successively with NaS_2O_3 solution (3 × 30 mL), saturated $NaHCO_3$ solution (2 × 40 mL) and H_2O (2 × 40 mL) and dried over $MgSO_4$. The crude product was purified by column chromatography (hexane–ethyl acetate, 6:4) to give the title compound as a white foam (283 mg, 57%), α:β ca. 3.5:1. Further purification with (CH_2Cl_2–ethyl acetate, 99:1) afforded the pure α anomer (107 mg, 21%) along with the pure β-anomer **15β** (31 mg, 6%); Compound **15α**: $[\alpha]_D^{25}$ = +66.8 (*c* 0.96, $CHCl_3$); δ_H ($CDCl_3$): 3.26–4.17 (29H, m, OMe, sugar-H), 4.56–4.28 (19H, m, Ar, H-6), 5.04 (1H, d, J_{AB} 12 Hz, Ar), 5.07 (1H, d, $J_{1,2}$ 8.3 Hz, H-1a), 5.14–5.18 (2H, m, H-1a′, H-2a′), 5.32 (1H, s, C*H*–Ph), 5.39 (1H, t, $J_{1,2}$, $J_{2,3}$ 8.3 Hz, H-2a), 5.58 (3H, m, H-1b, H-1b′-c′), 5.66 (1H, t, $J_{2,3}$, $J_{3,4}$ 8.3 Hz, H-3a), 5.86 (1H, t, $J_{2,3} \sim J_{3,4}$ 9.9 Hz, H-3a′), 6.67 (2H, d, J_{AB} 8.3 Hz, C_6H_4–OMe), 6.86 (2H, d, J_{AB} 8.3 Hz, C_6H_4–OMe), 6.97–7.78 (75H, m, H-Ar); δ_C (75 MHz, $CDCl_3$): 55.4 (OMe), 96.3, 96.8, 97.3, 97.5 (C-1b, C-1a′-c′), 100.5 (C-1a), 101.2 (*C*H–Ph), 114.6, 118.9 (C_6H_4–OMe), 151.2, 155.7 (ArC, quat. from C_6H_4–OMe); *m/z* (MALDI-TOF) 2362.9 ($[M+Na]^+$); Compound **15β**: (31 mg, 6%); δ_H ($CDCl_3$): 6.60 (2H, d, J_{AB} 12.0 Hz, C_6H_4–OMe), 6.81 (2H, d, J_{AB} 12.0 Hz, C_6H_4–OMe), 7.03–7.79 (H-Ar); δ_C ($CDCl_3$): 104.5 (C-1a′β), 101.4 (CH–Ph), 100.1 (C-1a), 96.8 (×2), 96.3 (C-1b, C-1b′, C-1c′), 114.6, 118.7 (C_6H_4–OMe), 151.2, 155.7 (ArC, quat. from C_6H_4–OMe).

4.15. *p*-Methoxyphenyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-{(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)}$_5$-(2,3,6-tri-*O*-benzyl-D-glucopyranosyl)-(1→6)-[(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α,β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranoside 16

Glycosyl donor **14** (460 mg, 0.145 mmol) and glycosyl acceptor **6** (106 mg, 0.11 mmol) were dissolved in dry Et_2O–DCM (4:1) containing activated 4 Å molecular sieves (400 mg) and stirred for 90 min. The reaction mixture was cooled to −60 °C, and *N*-iodosuccinimide (50 mg, 0.22 mmol) and TMSOTf (8 μL, 0.044 mmol, 20 mol% with respect to NIS) were added to the solution. The reaction was allowed to warm to ice bath temperature and after 2 h the mixture was then filtered through Celite, the filtrate was diluted with CH_2Cl_2 (100 mL) and washed successively with NaS_2O_3 solution (3 × 30 mL), saturated $NaHCO_3$ solution (2 × 40 mL) and H_2O (2 × 40 mL) and dried over $MgSO_4$. The crude product was purified by two rounds of column chromatography (hexane–ethyl acetate, 7:3) to give the title compound as a white foam (162 mg, 36%); α:β ca. 3.7:1; δ_C ($CDCl_3$): 55.5 (OMe), 96.2, 96.3, 97.0, 97.5 (C-1b, C-1a′-g′), 100.6 (C-1a), 101.3 (*C*H–Ph), 114.7, 119.0 (C_6H_4–OMe) 151.3, 155.8 (ArC, quat. from C_6H_4–OMe); *m/z* (MALDI) 4092.9 (100%, $[M+Na]^+$). Further purification by column chromatography (CH_2Cl_2–ethyl acetate, 99:1) afforded the pure α anomer **16α** (16 mg, 4%) as a white solid; δ_H ($CDCl_3$): 5.14 (1H, d, $J_{1,2}$ 7.7 Hz, H-1a), 5.23 (1H, dd, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.8 Hz, H-2a′), 5.40 (1H, s, C*H*–Ph), 5.45 (1H, dd, $J_{1,2}$, $J_{2,3}$ 9.5 Hz, H-2a), 5.73 (1H, t, H-3), 5.94 (1H, t, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3a′), 6.75 (2H, d, J_{AB} 9.0 Hz, C_6H_4–OMe), 6.93 (2H, d, J_{AB}, C_6H_4–OMe).

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