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Synthesis of 4′-*O*-acetyl-maltose and α-D-galactopyranosyl-(1→4)-D-glucopyranose for biochemical studies of amylose biosynthesis

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Abstract

The chemical synthesis of the title compounds as maltose analogs, in which the non-reducing end is modified by acetylation of the 4′-OH group or by reversing its configuration, is reported. For synthesis of the 4′-*O*-acetylated analog, β-maltose was converted into its per-*O*-benzylated-4′,6′-*O*-benzylidene derivative followed by removal of the benzylidene acetal function and selective silylation at C-6′. Acetylation at C-4′ of the obtained silylated compound followed by removal of the benzyl ether protecting groups and subsequent desilylation afforded the desired analog. The other maltose analog was synthesized via the glycosidation reaction between the glycosyl donor, *O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)trichloroacetimidate and the glycosyl acceptor, phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside followed by removal of the phenylthio group and debenzylation to provide the desired analog.

Keywords: Maltose derivatives; Phenyl thioglycosides; Glycosyl trichloroacetamidate; Glycosidation; Hydrogenolysis; α-D-Galactopyranosyl-(1→4)-D-glucopyranose

1. Introduction

Starch consists of two D-glucose polymers, amylose and amylopectin. Amylose is an essentially linear molecule composed of a backbone of α-(1→4)-linked D-glucose residues decorated with a few short α-(1→4) glucan chains bound at their reducing end to the main chain via α-(1→6) linkages. Amylopectin is a much larger molecule built of clusters of α-(1→4)-linked D-glucose chains, each of which are bound at their reducing end via an α-(1→6) linkage to another glucan chain resulting in an uneven distribution of the α-(1→6) linkages along the molecule and in the formation of intermittent crystalline and amorphous regions with a unit length of 90 Å.[1–3] The formation of all glucosidic link-

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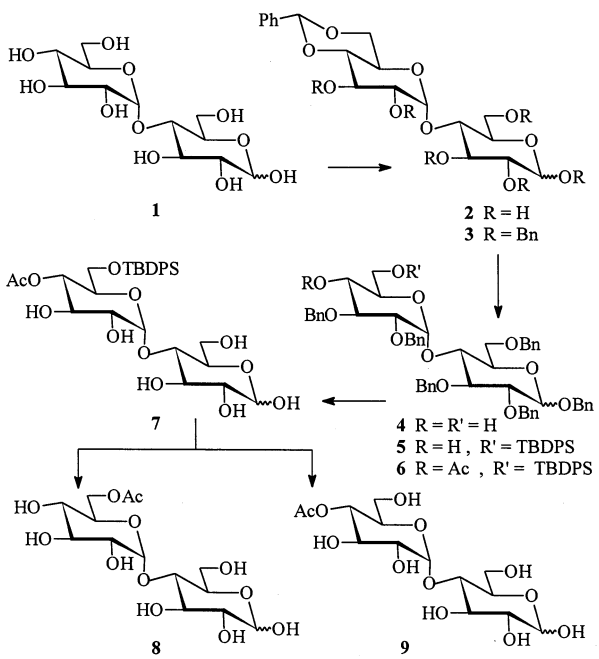
ages in amylose and amylopectin may be accounted for by the action of multiple isoforms of starch synthases (SS) and starch branching enzymes (SBE).[3–9]

Studies using mutants have demonstrated that one particular isoform of starch synthase, granule-bound starch synthase I (GBSS I), is responsible for amylose synthesis.[9] In the absence of GBSS I, other soluble and granule-bound isoforms of starch synthases are unable to synthesize amylose. Accordingly, these isoforms are assigned a role in amylopectin synthesis. Amylose biosynthesis takes place in the polysaccharide matrix and it is experimentally possible to obtain amylose synthesis in vitro using purified granules.[3,10] Two models have been proposed to explain the mechanism of amylose synthesis.[11] The first model is based on studies in pea and involves small-chain malto-oligosaccharides.[12] The second model is based on studies in *Chlamydomonas* and proposes that external α-glucans of amylopectin serve as primers for amylose synthesis. In this model, primer extension is terminated by cleavage to produce amylose.[13] No evidence for the operation of this mechanism was found in starch granules from developing pea.[14] The ability of malto-oligosaccharides to promote amylose synthesis may reflect their involvement as primers for initiation of new amylose molecules or a function as effectors–activators of GBSSI. Discrimination between these two possibilities would require the availability of malto-oligosaccharides specifically blocked or altered at their non-reducing end to prevent their functioning as primers while supposedly retaining their function as effectors–activators. In the present study, we report the synthesis of two such compounds, namely 4-*O*-acetyl-α-D-glucopyranosyl-(1→4)-D-glucopyranose (**9**) and α-D-galactopyranosyl-(1→4)-D-glucopyranose (**26**).



Scheme 1. Synthesis of 4′-*O*-acetyl maltose (**8**).

2. Results and discussion

For the synthesis of compound **9** from maltose, the following strategy for introducing specifically, an acetyl group at the 4′-OH of the non-reducing end, was devised. The strategy is also applicable to other (1→4)-linked oligomers of hexopyranoses. Starting with the free carbohydrate, a 4,6-*O*-benzylidene acetal is formed at the non-reducing end. Then all remaining free OH groups are protected by benzylation. Removal of the 4,6-*O*-benzylidene acetal now exposes the C-6 hydroxyl for selective protection with sterically demanding groups like trityl[15] or diphenyl-*tert*-butylsilyl[16], providing a specific access to the 4-OH position.

Based on this strategy, the synthesis of the target molecule **9** from β-maltose monohydrate (**1**) is outlined in Scheme 1. β-Maltose monohydrate (**1**) was treated with α,α-dimethoxytoluene in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid monohydrate[17] to give 4-*O*-(4,6-*O*-benzylidene-α-D-glucopyranosyl)-D-glucopyranose (**2**).[17] Benzylation of **2** with benzyl bromide in DMF–NaH afforded the corresponding *O*-benzylated-4,6-*O*-benzylidene derivative **3** as an anomeric mixture with an α/β ratio of 2:5 in 88% yield after chromatographic purification. Removal of the benzylidene functional group from the anomeric mixture **3** with (3:2 v/v) acetic acid–water gave benzyl 2,3,6-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-α-D-glucopyranosyl)-D-glucopyranoside (**4**) in 85% yield

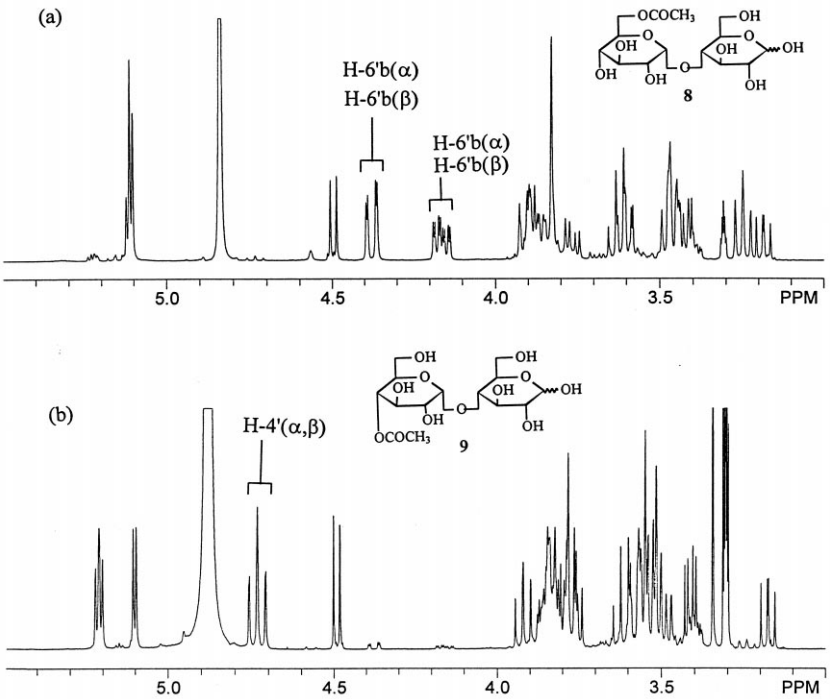


Fig. 1. ^{1}H NMR spectra at 400.13 MHz of compounds **8** and **9** in CD_3OD.

after chromatographic purification. From the anomeric mixture **4** pure β anomer could be isolated as a crystalline product by crystallization from diethyl ether. The 6′-OH group of **4** was then regioselectively *tert*-butyldiphenylsilylated by treatment with *tert*-butylchlorodiphenylsilane in DMF–imidazole at room temperature to afford compound **5** in quantitative yield after chromatographic purification. The 4′-OH group of **5** was readily acetylated with acetic anhydride in dry pyridine resulting in the corresponding 4′-*O*-acetyl derivative **6** in quantitative yield. Hydrogenolysis of **6** to remove the benzyl ether protecting groups was carried out under atmospheric pressure of hydrogen over 10% palladium on carbon with (2:1 v/v) EtOH–EtOAc as the solvent and produced compound **7** in 65% yield after chromatographic purification. Finally, removal of the *tert*-butyldiphenylsilyl protecting group from C-6′ of **7** was achieved using tetrabutylammonium fluoride in THF.[16] As expected, desilylation of **7** using TBAF in THF resulted in complete removal of the TBDPS group. However, a simultaneous migration of the acetyl group from C-6 toC-6′ was observed probably due to the presence of the fluoride ion, resulting in **8** in 95% yield instead of the expected formation of **9**. This is in contradiction with a previous report by Nicolaou et al.[18] where desilylation using exactly the same reaction conditions and a similar functional group pattern was reported not to result in acetyl migration. However, attempted desilylation of **7** using H_3PO_4 in 50% aq MeOH furnished the formation of the desired target molecule **9** in quantitative yield. Under these desilylation reaction conditions, only 8–10% acetyl migrations occurred as revealed by the ^{1}H NMR spectrum of compounds **8** and **9** shown in Fig. 1. Extensive ^{1}H and ^{13}C NMR chemical shift assignments for **8** and **9** listed in Table 1 were done using 2D spectra (COXY, TOCSY, HSQC and HMBC).

In the ^{1}H NMR spectrum of **8** (Fig. 1(a)), a one-proton doublet of doublets assignable to H-6′b (α,β anomeric mixture; $J_{6'a,6'b} = 11.8$ and $J_{5',6'b} = 2.1$ Hz) observed at δ 4.38 ppm and a one-proton multiplet (two doublet of doublets) assignable to H-6′a (α anomer; $J_{6'a,6'b} =$ 11.7 and $J_{5',6'a} = 1.2$ Hz) and H-6′a (β anomer; $J_{6'a,6'b} = 11.8$ and $J_{5',6'a} = 1.3$ Hz) observed at δ 4.18 and 4.15 ppm, respectively. The appearance of these signals at lower magnetic fields dictates that the acetyl group is located at C-6′ after its migration from C-4′. In contrast, the

^{1}H NMR spectrum of **9** (Fig. 1(b)) reveals that the H-4′ proton (α,β anomeric mixture) resonates at δ 4.73 ppm as a doublet of doublets (appearing as a triplet). Integration of the relevant ^{1}H-signals shows that 93% of the acetyl group have remained at C-4′ whereas only 7% have migrated. An unambiguous determination of the location of the acetyl group in analogs **8** and **9** was confirmed from long range ^{1}H–^{13}C COSY [^{1}heteronuclear multiple

Table 1
^{1}H and ^{13}C NMR data (400.13 MHz) for analogs **8** and **9** in CD_3OD

Chemical shifts δ (ppm)	**8**		**9**	
(J in Hz)	α anomer	β anomer	α anomer	β anomer
H-1	5.11 d	4.50 d	5.10 d	4.49 d
($J_{1,2}$)	(3.8)	(7.6)	(3.7)	(7.8)
C-1	93.8	98.2	93.8	98.2
H-2	3.42 dd	3.18 dd	3.41 dd	3.18 dd
($J_{2,3}$)	(9.5)	(9.5)	(9.8)	(9.2)
C-2	73.4	75.8	73.5	75.8
H-3	3.90 t	3.60 t	3.92 t	3.62 t
($J_{3,4}$)	(9.4)	(9.4)	(9.5)	(9.5)
C-3	74.7	77.9	74.6	77.8
H-4	3.47 t	3.47 t	3.55 t	3.55 t
($J_{4,5}$)	(9.5)	(9.5)	(9.5)	(9.5)
C-4	82.3 [a]	82.5 [a]	81.6	81.8
H-5 C-5	3.36–3.50 m	3.36–3.50 m	3.46–3.57 m	3.37–3.42 m
	76.7	76.8	71.6	76.6
H-6a	3.91 dd	3.80–3.88 m	3.77 dd	3.77 dd
($J_{6a,6b}$) ($J_{5,6a,}$)	(11.9) (1.8)		(11.9) (2.1)	(11.9) (2.1)
H-6b	3.76 dd	3.80–3.88 m	3.80–3.88 m	3.80–3.88 m
($J_{5,6b}$)	(5.2)			
C-6	62.5	62.3	62.2	62.4
H-1′	5.11 d [b]	5.12 d [b]	5.21 d	5.22 d
($J_{1',2'}$)	(3.8)	(4.1)	(3.7)	(3.7)
C-1′	103.1	103.1	102.5	102.5
H-2′	3.44–3.48 m	3.44–3.48 m	3.52 dd	3.52 dd
($J_{2',3'}$)			(9.5)	(9.5)
C-2′	74.1	74.4	74.1	74.2
H-3′	3.61 t [c]	3.63 t [c]	3.76 t	3.80–3.88 m
($J_{3',4'}$)	(9.1)	(9.1)	(9.5)	
C-3′	75.1 [c]	75.0 [c]	73.5 [d]	72.7 [d]
H-4′	3.25 t	3.25 t	4.73 t	4.73 t
($J_{4',5'}$)	(9.5)	(9.5)	(9.5)	(9.5)
C-4′	71.7	71.4	72.6	72.6
H-5′	3.80–3.88 m	3.80–3.88 m	3.80–3.88 m	3.80–3.88 m
C-5′	72.2	72.2	72.9	72.9
H-6′a	4.38 dd	4.38 dd	3.58 dd	3.58 dd
($J_{6'a,6'b}$)	(12)	(11.9)	(12)	(12)
($J_{5',6'a,}$)	(1.9)	(1.7)	(2.5)	(2.5)
H-6′b	4.17 dd	4.16 dd	3.49 dd	3.49 dd
($J_{5',6'b}$)	(2.2)	(2.3)	(6.4)	(6.4)
C-6′	65.2	65.2	62.4	62.4
CH_3CO	2.08	2.08	2.09	2.08
	20.8, 172.9	20.8, 172.9	20.9, 172.2	20.9, 172.2

[a] Assignments may be reversed.
[b] Assignments may be reversed.
[c] Assignments may be reversed.
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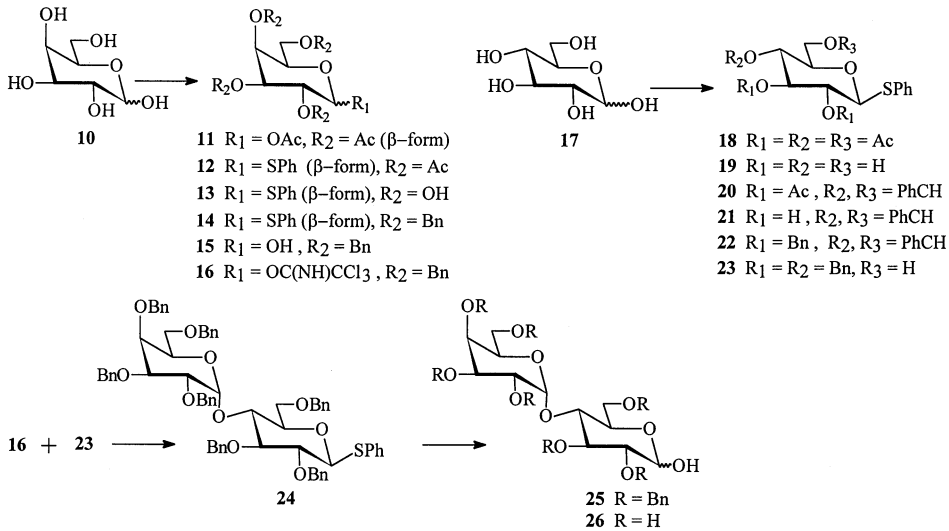

Scheme 2. Synthesis of α-D-galactopyranosyl-(1→4)-glucopyranose (**25**).

bond connectivity (HMBC)] spectra of **8** and **9**. Thus, the HMBC spectrum of **8** showed cross-peaks (4.38/172.2 and 4.18/172.9) which connect the carbonyl carbon of the acetyl group to the H-6′ indicating that the acetyl group is located at C-6′. Correspondingly, the HMBC spectrum of **9** showed a cross-peak (4.73/172.2) which connects the carbonyl carbon of the acetyl group to H-4′ indicating that the acetyl group is located at C-4′-position. The target compound **9** was obtained in 96% yield after chromatographic purification.

The route for the second target molecule **26** is summarized in Scheme 2. D-Galactose (**10**) was acetylated[19] to produce **11** which upon treatment with phenylthiotrimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate as catalyst afforded **12** which was subsequently deacetylated to phenyl 1-thio-β-D-galactopyranoside (**13**) as previously described.[20] Standard benzylation of **13** using benzyl bromide in DMF–NaH afforded the known[21] per-*O*-benzylated thioglycoside (**14**) in 72% yield. By treating the thioglycoside **14** with *N*-bromosuccinimide–acetone–water[22] for 5 min at room temperature, the phenylthio group was replaced with a free OH group to afford 2,3,4,6-*O*-benzyl-D-galactopyranose[21,23] (**15**) in 99% yield. Compound **15** was converted into its trichloroacetimidate[24] by the action of trichloroacetonitrile with anhydrous potassium carbonate as a catalyst in dry dichloromethane to obtain the glycosyl donor **16**. The potential of *O*-glycosyl-trichloroacetimidates as strong glycosylation donors under mild acidic catalytic conditions has previously been documented.[25] We have previously reported[25] synthesis of the glycosyl acceptor **23** in six steps starting from D-glucose (**17**). The glycosidation reaction between **16** and **23** was performed in dry diethyl ether using trimethylsilyl triflate as a catalyst and provided the desired disaccharide derivative **24** in 79% yield after chromatographic purification. Minor amounts of the β anomer were removed during the chromatographic step. The replacement of the phenylthio group of **24** with a free OH group was achieved using the *N*-bromosuccinimide method[22] and provided 2,3,6-tri-*O*-benzyl -4-*O*- (2,3,4,6-tetra-*O*-benzyl α-D-galactopyranosyl)-D-glucopyranose (**25**) in quantitative yield after chromatographic purification. Hydrogenolysis of **25** for removal the benzyl ether protecting groups was performed under hydrogen in the presence of 10% palladium on carbon, and produced the target compound **26** in 80% yield after chromatographic purification.

Biosynthetic experiments were carried out using specific malto-oligosaccharides as well as the two chemically synthesized maltose analogs **9** and **26** in which the non-reducing end was modified to exclude their function as

primers in amylose synthesis. De novo synthesis of amylose was obtained upon incubation of GBSS I bound to starch granules in the presence of malto-oligosaccharides. Neither of the maltose analogs **9** and **26** were able to support amylose synthesis.[14] This demonstrates that the ability of malto-oligosaccharides to stimulate amylose synthesis reflects that GBSSI use these malto-oligosaccharides as direct primers for amylose synthesis and not as effectors–activators of GBSS I.[14]

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 21 ± 2 °C with an optical activity Ltd AA-1000 polarimeter.

All reactions were monitored by TLC on aluminum sheets coated with silica Gel $60F_{254}$ (0.2 mm thickness, E. Merck, Darmstadt, Germany) and the components present were detected by charring with 10% H_2SO_4 in MeOH. Column chromatography was carried out using Silica Gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM, E. Merck). Solvent extracts were dried with anhyd $MgSO_4$ unless otherwise specified. Microanalysis was performed at DB Lab Danish Bioprotein A/S, Stenhuggervej 22, PO Box 829, DK-5230 Odense M, Denmark.

The ^1H and ^{13}C NMR spectra were recorded on a Bruker AC250P spectrometer at 250 and 63 MHz, respectively. In water, dioxane was used as an internal reference [δ_H (dioxane) = 3.75; δ_C (dioxane) = 67.4]. In other solvents, δ_H values are relative to internal Me_4Si and δ_C values are referenced to the solvent [δ_C ($CDCl_3$) = 77.0; δ_C (Me_2SO-d_6) = 39.4].

4-O-(4,6-O-benzylidene-α-D-glucopyranosyl)-D-glucopyranose (**2**).—Compound **2** was synthesized as reported previously;[17] $[\alpha]_D$ +79.5° (*c* 1.06; D_2O), lit. $[\alpha]_D$ +81.1° (*c* 1.40; D_2O)[14]; ^1H NMR (D_2O): δ 7.58–7.47 (m, 5 H, ArH), 5.72 (s, 1 H, benzylic-H), 5.45 (d, 0.4 H, $J_{1',2'}$ 4.0 Hz, H-1′, α anomer), 5.44 (d, 0.6 H, $J_{1',2'}$ 4.0 Hz, H-1′, β anomer), 5.24 (d, 0.4 H, $J_{1,2}$ 3.8 Hz, H-1α), 4.64 (d, 0.6 H, $J_{1,2}$ 8.2 H, H-1β); ^{13}C NMR (D_2O): δ 137.0, 130.7, 129.6, 129.6, 127.1, 127.1 (Ph), 102.6 (2 C, C-1′, α and β anomers), 101.3 (Ph*C*H, α anomer), 101.2 (Ph*C*H, β anomer), 96.6 (C-1β), 92.8 (C-1α), 81.0, 77.9, 75.2, 74.1, 73.2, 72.2, 70.6, 64.1 (C-2,3,4,5,2′,3′,4′,5′ α anomer), 81.0, 77.8, 77.0, 74.8, 73.1, 70.9, 70.9, 64.1 (C-2,3,4,5,2′,3′,4′,5′ β anomer), 68.8 (2 C, C-6′, α and β anomers), 61.4 (C-6, β anomer), 61.3 (C-6, α anomer).

Benzyl 2,3,6-tri-O-benzyl-4-O-(4,6-O-benzylidene-2,3-di-O-benzyl-α-D-glucopyranosyl)-D-glucopyranoside (**3**).—A solution of **2** (3 g, 7 mmol) in dry DMF (100 mL) was stirred with NaH (3.4 g, 60% dispersion in mineral oil) for 2 h at rt and the mixture cooled to 0 °C. Benzyl bromide (10.5 mL) was added dropwise to the mixture and stirring was continued overnight at rt. The mixture was cooled to 0 °C and excess NaH decomposed by dropwise addition of MeOH. The solution was concentrated in vacuo and diluted with EtOAc (250 mL) and water (100 mL). The organic phase was separated, washed with water (4 × 50 mL) and brine (50 mL). After drying, the solvent was evaporated and the residue chromatographed on silica gel (210 g) with (7:3 v/v) *n*-pentane–Et_2O as eluent to give **3** (6 g, colorless syrup, 88%); $[\alpha]_D$ +9.1° (*c* 0.55; $CHCl_3$); ^1H NMR ($CDCl_3$):δ 7.52–7.14 (m, 35 H, ArH), 5.75 (d, 0.3 H, $J_{1',2'}$ 3.8 Hz, H-1′, α anomer), 5.70 (d, 0.7 H, $J_{1',2'}$ 3.9 Hz, H-1′, β anomer), 5.53 (s, 0.7 H, Ph*C*H, β anomer), 5.52 (s, 0.3 H, Ph*C*H, α anomer), 5.08–4.44 (m, 13 H, PhCH_2 and anomeric protons), 4.20–3.47 (m, 12 H, skeleton protons); ^{13}C NMR ($CDCl_3$): δ 102.3 (C-1β), 101.1 (2 C, Ph*C*H, α and β anomers), 97.2 (2 C, C-1′, α and β anomers), 95.0 (C-1α), 84.9, 82.3, 82.3, 78.7, 78.7, 74.3, 71.7, 63.2 (C-2,3,4,5,2′,3′,4′,5′ β anomer), 82.2, 82.2, 82.0, 80.2, 78.8, 71.9, 69.6, 63.2 (C-2,3,4,5,2′,3′,4′,5′ α anomer), 75.2, 75.2, 74.6, 73.6, 73.4, 70.9 (6 × Ph*C*H_2, β anomer), 74.1, 73.8, 73.8, 73.3, 72.8, 72.8 (6 × Ph*C*H_2, α anomer), 69.1 (C-6, α anomer), 68.9 (C-6, β anomer), 68.8 (C-6′, β anomer), 68.6 (C-6′, α anomer); FAB$^+$MS: *m*/*z* 993 [M + Na]$^+$.

Benzyl 2,3,6-tri-O-*benzyl-4*-O-*(2,3-di*-O-*benzyl-α*-D-*glucopyranosyl)*-D-*glucopyranoside* (**4**).—A stirred solution of **3** (5.8 g, 5.97 mmol) in AcOH (60 mL) was heated to 95 °C, water (40 mL) was added dropwise, and the stirring was continued for 30 min at 95 °C. The mixture was cooled, evaporated in vacuo and the residue dissolved in EtOAc (200 mL) and water (100 mL). The organic phase was separated, washed with 1 M aq NaOH (3 × 50 mL), water (4 × 100 mL) and brine (50 mL). After drying, the solvent was evaporated and the residue chromatographed on silica gel (210 g) using a gradient of EtOAc in *n*-pentane (20, 30, and 40%) as eluent to obtain **4** (4.6 g, colorless syrup, 85%); $[\alpha]_D$ +22.5° (*c* 0.69; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.44–7.16 (m, 30 H, ArH), 5.72 (d, 0.4 H, $J_{1',2'}$ 3.6 Hz, H-1′ α anomer), 5.67 (d, 0.6 H, $J_{1',2'}$ 3.6 Hz, H-1′ β anomer), 5.11–4.44 (m, 13 H, PhCH_2 and anomeric protons), 4.12–3.36 (m, 12 H, skeleton protons); ^{13}C NMR ($CDCl_3$): β anomer: δ 102.3 (C-1), 96.5 (C-1′), 84.8, 82.2, 81.2, 78.9, 74.6, 72.6, 71.4, 70.6 (C-2,3,4,5,2′,3′,4′,5′), 75.2, 75,2, 74.6, 73.3, 73.0, 70.9 (6 × PhCH_2), 69.3 (C-6), 62.3 (C-6′); α anomer: δ 96.5 (C-1′), 95.0 (C-1), 82.0, 81.1, 80.2, 79.0, 71.5, 71.4, 70.5, 68.9 (C-2,3,4,5,2′,3′,4′,5′), 74.2, 73.8, 73.8, 73.5, 73.2, 72.8 (6 × PhCH_2), 69.1 (C-6), 62.2 (C-6′).

Benzyl 2,3,6-tri-O-*benzyl-4*-O-*(2,3-di*-O-*benzyl-6*-O-tert-*butyldiphenylsilyl-α*-D-*glucopyranosyl)*-D-*glucopyranoside* (**5**).—A stirred solution of **4** (4.2 g, 4.76 mmol) in dry DMF (100 mL) and imidazol (1.3 g, 19.1 mmol) was cooled to 0 °C and *tert*-butylchloro-diphenylsilane (2.5 mL, 9.55 mmol) was added dropwise during a period of 5 min. The cooling bath was removed and the mixture was stirred for 1 h at rt. The mixture was diluted with EtOAc (300 mL) and water (100 mL). The organic phase was washed successively with water (3 × 100 mL), satd aq $NaHCO_3$ (3 × 50 mL), water (3 × 100 mL), and brine (50 mL). After drying, the solvent was evaporated and the residue chromatographed on silica gel (210 g) with (7:3, v/v) *n*-pentane–Et_2O to afford **5** (5.12 g, colorless syrup, 96%, α/β ratio ≈ 2:3); $[\alpha]_D$ +23.6° (*c* 0.84; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.68–7.19 (m, 40 H, ArH), 5.66 (d, 0.4 H, $J_{1',2'}$ 3.6 Hz, H-1′ α anomer), 5.63 (d, 0.6 H, $J_{1',2'}$ 3.6 Hz, H-1′ β anomer), 5.08–4.40 (m, 13 H, PhCH_2 and anomeric protons), 4.14–3.36 (m, 12 H, skeleton protons), 1.05, 1.03 (2s, 9 H, SiC(CH_3)$_3$; ^{13}C NMR ($CDCl_3$): β anomer: δ 102.2 (C-1), 96.1 (C-1′), 84.8, 82.0, 81.1, 79.1, 77.2, 72.6, 71.9, 71.1 (C-2,3,4,5,2′,3′,4′,5′), 75.3, 75.3, 74.6, 73.3, 72.8, 70.1 (6 × PhCH_2), 69.3 (C-6), 63.9 (C-6′), 26.9 [(SiC(CH_3)$_3$], 19.3 [(SiC(CH_3)$_3$]; α anomer: δ 96.1 (C-1′), 94.9 (C-1), 81.9, 81.0, 80.0, 79.3, 72.5, 71.9, 71.0 (C-2,3,4,5,2′,3′,4′,5′), 74.6, 74.1, 73.7, 73.2, 72.9, 72.9 (6 × PhCH_2), 69.1 (C-6), 63.7 (C-6′), 26.9 [(SiC(CH_3)$_3$], 19.3 [(SiC(CH_3)$_3$]; FAB$^+$MS: m/z 1143 $[M + Na]^+$.

Benzyl 2,3,6-tri-O-*benzyl-4*-O-*(4*-O-*acetyl-2,3-di*-O-*benzyl-6*-O-tert-*butyldiphenylsilyl-α*-D-*glucopyranosyl)*-D-*glucopyranoside* (**6**).—To a stirred solution of **5** (2.24 g, 2.0 mmol) in dry pyridine (30 mL) was added Ac_2O (2.0 mL) at rt and stirring was continued overnight. The solvents were removed in vacuo and the last trace solvents were removed by repeated co-evaporation with toluene. The residue was dissolved in EtOAc (100 mL) and washed with satd aq $NaHCO_3$ (3 × 25 mL), water (3 × 25 mL), and brine (25 mL). After drying, the solvent was evaporated and the residue chromatographed on silica gel (210 g) with (4:1, v/v) *n*-pentane–Et_2O to obtain **6** (2.2 g, colorless syrup, 95%); $[\alpha]_D$ +35.8° (*c* 1.1; $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.68–7.15 (m, 40 H, ArH), 5.60 (d, 0.7 H, $J_{1',2'}$ 3.6 Hz, H-1′ β anomer), 5.59 (d, 0.3 H, $J_{1',2'}$ 3.6 Hz, H-1′ α anomer), 5.11–4.42 (m, 13 H, PhCH_2 and anomeric protons), 4.18–3.43 (m, 12 H, skeleton protons), 1.82, 1.81 (2s, 3 H, COCH_3), 1.03, 1.02 [(2s, 9 H, SiC(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$): β anomer: δ 169.3 (COCH$_3$), 102.2 (C-1), 96.1 (C-1′), 84.7, 82.0, 79.5, 79.1, 74.7, 73.4, 71.2, 68.9 (C-2,3,4,5,2′,3′,4′,5′), 75.0, 75.0, 74.6, 73.3, 73.1, 70.9 (6 × PhCH_2), 69.6 (C-6), 62.6 (C-6′), 26.8 [SiC(CH_3)$_3$], 20.8 (COCH_3), 19.2 [SiC(CH_3)$_3$]; α anomer: δ 169.3 (COCH$_3$), 96.3 (C-1′), 95.0 (C-1), 81.8, 79.8, 79.6, 79.0, 73.6, 73.2, 71.1, 70.0 (C-2,3,4,5,2′,3′,4′,5′), 74.3, 73.8, 73.8, 73.2, 73.1, 72.8 (6 × PhCH_2), 69.4 (C-6), 62.6 (C-6′), 26.8 [SiC(CH_3)$_3$], 20.8 (COCH_3), 19.2 [SiC(CH_3)$_3$]; FAB$^+$MS: m/z 1185 $[M + Na]^+$.

4-O-*(4*-O-*Acetyl*-*6*-O-tert-*butyldiphenylsilyl*-α-D-*glucopyranosyl)*-D-*glucopyranose* (**7**).—A solution of **6** (1.8 g) in (1:2, v/v) EtOAc–EtOH (60 mL) was hydrogenated in the presence of 10% Pd–C (0.5 g) at normal pressure for 3 days at rt. The catalyst was removed by filtration through a Celite pad and a silica gel layer, and thoroughly washed with EtOH (6 × 30 mL). The combined filtrates were evaporated in vacuo and the residue chromatographed on silica gel (50 g) with (4:1 v/v) CH_2Cl_2–MeOH as eluent to obtain **7** (0.63, white powder, 65%); mp 98–99 °C; $[\alpha]_D$ + 73.9° (*c* 0.38; CH_3OH); ^1H NMR (CD_3OD): δ 7.78–7.30 (m, 10 H, ArH), 5.24 (d, 0.5 H, $J_{1',2'}$ 3.8 Hz, H-1′ β anomer), 5.22 (d, 0.5 H, $J_{1',2'}$ 3.8 Hz, H-1′ α anomer), 5.11 (d, 0.5 H, $J_{1,2}$ 3.8 Hz, H-1 α anomer), 4.99 (t, 0.5 H, $J_{3',4'}$ 9.6, $J_{4',5'}$ 10 Hz, H-4′, α anomer), 4.96 (t, 0.5 H, $J_{3',4'} = 9.4$, $J_{4',5'} = 10.2$ Hz, H-4′, β anomer), 4.49 (d, 0.5 H, $J_{1,2}$ 7.9 Hz, H-1 β anomer), 3.98–3.17 (m, 11 H, skeleton protons), 1.99 (2s, 3 H, CH_3CO), 1.03 (s, 9 H, [SiC(CH_3)$_3$]); ^{13}C NMR (CD_3OD): δ 171.8, 171.7 (2 × $COCH_3$ α and β anomers), 102.8 (C-1′ β anomer), 102.7 (C-1′ α anomer), 98.1 (C-1 β anomer), 93.8 (C-1 α anomer), 82.0, 81.6, 77.7, 76.8, 75.9, 74.5, 74.3, 74.1, 73.5, 73.2, 73.1, 72.9, 72.8, 72.1, 72.1, 71.8 (C-2,3,4,5,2′,3′,4′,5′ α and β anomers), 64.1 (C-6, β anomer), 64.0 (C-6, α anomer), 62.6 (C-6′ β anomer), 62.4 (C-6′, α anomer), 27.1 ([Si*C*-(CH_3)$_3$]), 21.0 ($CO*CH*_3$), 20.0 ([Si*C*(CH_3)$_3$]). Anal. Calcd for $C_{30}H_{42}O_{12}Si{\cdot}H_2O$: C, 56.9; H, 6.9. Found: C, 56.6; H, 7.1.

4-O-*(6*-O-*Acetyl*-α-D-*glucopyranosyl)*-D-*glucopyranose* (**8**).—Tetrabutylammonium fluoride (0.5 mL, 1.1 M in THF) was added to a stirred solution of **7** (0.24 g) in THF (5 mL) at rt and stirring was continued for 2 h after which the solvent was evaporated and the residue chromatographed on silica gel (26 g) with (4:1, v/v) EtOAc–MeOH to give **8** (0.14 g, white powder, 95%); $[\alpha]_D$ + 102.9° (*c* 0.28; CH_3OH); FAB$^+$MS: *m*/*z* 407 $[M + Na]^+$.

4-O-*(4*-O-*Acetyl*-α-D-*glucopyranosyl)*-D-*glucopyranose* (**9**).—A clear solution of **7** (154 mg) in 50% aq MeOH (10 mL) was treated with H_3PO_4 (291.3 μL, 2.68 M) at rt and the mixture was let stand for 40 h. ^1H NMR experiments revealed complete desilylation as monitored by complete disappearance of the starting material. After neutralization with $NaHCO_3$, the mixture was taken to dryness on a rotavapor at rt. The residue was chromatographed on silica gel (15 g) with (4:1, v/v) EtOAc–MeOH to obtain pure **9** (91 mg, white powder, 96%); ESIMS positive mode: *m*/*z* 407 $[M + Na]^+$.

Phenyl 2,3,4,6-tetra-O-*benzyl*-*1*-*thio*-α-D-*galactopyranoside* (**14**).—Compound **13**[19] (3.70 g, 13.59 mmol) was benzylated as reported previously[20] to obtain **14** (6.21 g, 72%); mp 88–89 °C (Et_2O–*n*-pentane), lit.[20] mp 88–89 °C; $[\alpha]_D$ + 1.3° (*c* 0.50; $CHCl_3$), lit.[20] $[\alpha]_D$ + 1.0° (*c* 1.0; $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.58–7.16 (m, 25 H, ArH), 4.96, 4.59 (2d, 2 H, *J* 11.5 Hz, PhCH_2), 4.79, 4.73 (2d, 2 H, *J* 11.3 Hz, PhCH_2), 4.71 (s, 2 H, PhCH_2), 4.64 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1β), 4.47, 4.41 (2d, 2 H, *J* 10.5 Hz, PhCH_2), 3.98–3.57 (m, 12 H, skeleton protons); ^{13}C NMR ($CDCl_3$): δ 138.7–127.0 (C arom.), 87.7 (C-1), 84.2, 77.3, 77.3, 73.6 (C-2,3,4,5), 75.6, 74.4, 73.5, 72.7 (4 × $PhCH_2$), 68.7 (C-6).

2,3,4,6-*tetra*-O-*Benzyl*-D-*galactopyranose* (**15**).—*N*-Bromosuccinimide (1 g, 6.74 mmol) was added to a stirred solution of **14** (2 g, 3.16 mmol) in (9:1 v/v) acetone–water (20 mL) and stirred for 5 min at rt, at which time all thioglycoside had disappeared. Solid $NaHCO_3$ (5 g) was added and the solvents evaporated in vacuo. The residue was dissolved in EtOAc (150 mL) and water (50 mL) and the organic phase was separated and successively washed with satd aq $NaHCO_3$ (3 × 50 mL), water (3 × 50 mL), and brine (25 mL). After drying, the solvent was evaporated in vacuo and the residue chromatographed on silica gel (60 g) using (9:1 v/v) *n*-pentane–EtOAc as the initial eluent to remove all impurities and (7:3 v/v) *n*-pentane–EtOAc to elute **15** (1.7 g, colorless syrup, quantitative) as an anomeric mixture (α/β ratio ≈ 5:2 as determined by ^{13}C NMR; $[\alpha]_D$ + 11.5° (*c* 0.8; $CHCl_3$); ^1H NMR ($CDCl_3$):δ 7.39–7.22 (m, 20 H, ArH), 5.26 (d, 0.7 H, $J_{1,2}$ 3.5 Hz, H-1 α anomer), 4.95–4.35 (m, 8.3 H, PhCH_2 and H-1β), 4.18–3.42 (m, 6 H, skeleton protons); ^{13}C NMR ($CDCl_3$): β anomer: δ 97.7 (C-1), 82.1, 80.7, 76.5, 73.6 (C-2,3,4,5), 75.0, 74.5, 73.5, 73.5 (4 × PhCH_2),

68.9 (C-6); α anomer: δ 91.8 (C-1), 78.8, 76.5, 74.7, 69.4 (C-2,3,4,5), 74.6, 73.4, 73.4, 72.9 (4 × Ph*CH*$_2$), 69.0 (C-6).

O-*(2,3,4,6-tetra*-O-*Benzyl-α,β*-D-*galactopyranosyl)trichloroacetimidate*[24] (**16**).—Compound **16** was prepared from **15** (1.54 g) according to the previously published procedure.[24]

Phenyl 2,3,6-tri-O-*benzyl-4*-O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)-1-thio-β*-D-*glucopyranoside* (**24**).—A mixture of the crude product **16** (1.64 g, 2.39 mmol) and phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside[26] (0.8 g, 1.47 mmol) was co-evaporated with dry CH_2Cl_2 (3 × 25 mL) and dried for 1 h at 70 °C in vacuo. The residue was dissolved in dry (2:1 v/v) Et_2O–CH_2Cl_2 (75 mL) and stirred with 4 Å molecular sieves (3 g, activated powder) for 1 h at rt under Ar. The stirred mixture was then cooled to −50 °C and trimethylsilyl trifluoromethanesulfonate (100 μL, 0.55 mmol) was added in one portion, and stirring was continued for 1.5 h during which the temperature was gradually raised to rt. Sodium hydrogen carbonate (2 g) was added and the reaction mixture was stirred for additional 20 min and filtered through a pad of sea sand on the top of a silica gel layer. The filtrate was evaporated, EtOAc (150 mL) was added to the residue and the organic phase was successively washed with satd aq $NaHCO_3$ (2 × 25 mL), water (3 × 25 mL), and brine (25 mL). After drying, the solvent was evaporated and the residue was chromatographed on silica gel (180 g) using (4:1 v/v) *n*-pentane–Et_2O as eluent to give pure **24** (1.24 g, gum, 79%); $[\alpha]_D$ +30.7° (*c* 0.85; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.58–7.12 (m, 40 H, Ar H), 5.70 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.93–4.25 (m, 13 H, Ph*CH*$_2$ and H-1), 4.13–3.41 (m, 12 H, skeleton protons); ^{13}C NMR ($CDCl_3$): δ 97.7 (C-1′), 87.2 (C-1), 86.7, 81.0, 79.1, 78.6, 75.4, 74.6, 72.8, 69.9 (C-2,3,4,5,2′,3′,4′,5′), 75.2, 74.7, 74.2, 73.9, 73.4, 73.1, 72.6 (7 × Ph*CH*$_2$), 69.9, 68.6 (C-6,6′); FAB$^+$MS: *m*/*z* 1087 $[M + Na]^+$.

2,3,6-Tri-O-*benzyl-4*-O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*galactopyranosyl)*-D-*glucopyranose* (**25**).—Compound **24** (0.84 g, 0.79 mmol) was reacted with NBS (0.28 g, 1.58 mmol) in (9:1 v/v) acetone–water (50 mL) as described for **15** to give **25** (0.73 g, colorless syrup, 95%) after chromatographic purification on silica gel (60 g) using (3:2 v/v) Et_2O–*n*-pentane as eluent; $[\alpha]_D$ +32.5° (*c* 0.88; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.35–7.14 (m, 35 H, ArH), 5.69 (d, 0.7 H, $J_{1',2'}$ 3.6 Hz, H-1′ α anomer), 5.68 (d, 0.3 H, $J_{1',2'}$ 3.6 Hz, H-1′ β anomer), 5.18 (d, 0.7 H, $J_{1,2}$ 3.5 Hz, H-1 α anomer), 4.89–4.27 (m, 14.3 H, Ph*CH*$_2$ and H-1β), 4.19–3.35 (m, 12 H, skeleton protons); ^{13}C NMR ($CDCl_3$): α anomer: δ 97.6 (C-1′), 90.7 (C-1), 81.3, 80.0, 79.0, 75.6, 74.7, 74.1, 69.9, 69.7 (C-2,3,4,5,2′,3′,4′,5′), 74.7, 73.7, 73.4, 73.1, 73.1, 72.9, 72.7 (7 × Ph*CH*$_2$), 69.5, 68.7 (C-6,6′); β anomer: δ 97.4 (C-1), 97.2 (C-1′), 84.4, 83.1, 77.2, 75.4, 74.6, 74.2, 73.9, 70.1 (C-2,3,4,5,2′,3′,4′,5′), 74.7, 74.5, 73.5, 73.5, 73.4, 73.2, 72.9 (7 × Ph*CH*$_2$), 69.7, 68.8 (C-6,6′); FAB$^+$MS: *m*/*z* 995 $[M + Na]^+$.

α-D-*Galactopyranosyl-(1→4)*-D-*glucopyranose* (**26**).—Compound **25** (0.5 g, 0.51 mmol) was debenzylated as described for compound **7** and the crude product was chromatographed on silica gel (28 g) using (4:1 v/v) EtOAc–MeOH as eluent to give pure **26** (0.14 g, white powder, 80%); ^{1}H NMR (D_2O): δ 5.41 (d, 0.6 H, $J_{1',2'}$ 3.6 Hz, H-1′ β anomer), 5.40 (d, 0.4 H, $J_{1',2'}$ 3.6 Hz, H-1′ α anomer), 5.23 (d, 0.4 H, $J_{1,2}$ 3.8 Hz, H-1 α anomer), 4.65 (d, 0.6 H, $J_{1,2}$ 7.8 Hz, H-1 β anomer), 3.99–3.24 (m, 12 H, skeleton protons); ^{13}C NMR (D_2O): α anomer: δ 100.8 (C-1′), 92.7 (C-1), 77.8, 74.0, 72.6, 72.1, 70.8, 70.2, 70.0, 69.5 (C-2,3,4,5,2′,3′,4′,5′), 62.0, 61.4 (C-6,6′); β anomer: δ 100.7 (C-1′), 96.6 (C-1), 77.8, 77.0, 75.4, 74.8, 72.6, 70.2, 70.0, 69.4 (C-2,3,4,5,2′,3′,4′,5′), 62.0, 61.6 (C-6,6′); FAB$^+$ MS: *m*/*z* 365 $[M + Na]^+$.

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