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All-α-D-linked tetra- and penta-saccharide substructures of Trestatin A by block syntheses with triflic anhydride as promoter

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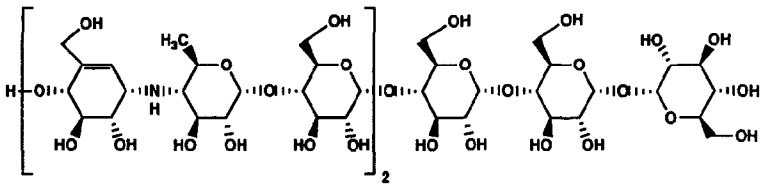
ABSTRACT

The perbenzylated maltosyl and maltotriosyl fluorides **6** and **16** were treated with 2,3,2′,3′,6′-penta-*O*-benzyl-4,6-*O*-benzylidene-α,α-trehalose (**7**) using triflic anhydride as a promoter to give all-α-D-linked tetra- and penta-saccharides which were finally deblocked to the free oligosaccharides 4-*O*-α-maltosyl- **9** and 4-*O*-α-maltotriosyl-α,α-trehaloses **18**. The ^{1}H NMR spectra of some of the compounds were fully analyzed by 1D TOCSY and ROESY experiments.

INTRODUCTION

Trestatin A (**1**) is a pseudo-nonasaccharide obtained from strains of *Streptomyces dimorphogenes* which was developed as an alpha amylase inhibitor[1] and, which after sulfation, potently inhibits the proliferation of smooth muscle cells[2]. We have recently described[3] an effective approach for the synthesis of the trehalose end trisaccharide unit of Trestatin A using trifluoromethanesulfonic (triflic) anhydride as reactive promoter[4,5] for the *cis*-glycosidation reaction. Now the extension of this work towards block syntheses of tetra- and penta-saccharide substructures of Trestatin A is reported.



Scheme 1. Trestatin A.

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RESULTS AND DISCUSSION

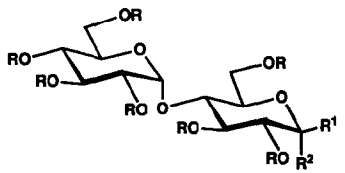
A maltosyl donor **3** without a participating group next to the anomeric center was prepared by glycosidation of hepta-*O*-acetyl-α-maltosyl bromide with allyl alcohol followed by deacetylation and benzylation[6]. The ^{1}H NMR spectrum of this compound was analyzed (see Experimental) using one-dimensional TOCSY (1D TOCSY) experiments[3,7] to serve as a comparison for the higher saccharides. Compound **3** was deallylated[8] to give **4** (ref. 6), which can also be prepared by regioselective hydrogenolysis of the corresponding benzyl maltoside[9]. Chlorination with oxalyl chloride and catalytic amounts of *N,N*-dimethylformamide furnished the known[6] α-chloride **5**, which without purification, was converted with silver fluoride in acetonitrile[10] to the stable β-fluoride **6** in 69% yield. The ^{1}H NMR spectrum of this compound was also fully analyzed with the aid of 1D TOCSY and 1D ROESY experiments.

The known[3] trehalose derivative **7** (compound **10** in ref. 3) was glycosidated with maltosyl fluoride **6** in ether, conditions which usually give preferential formation of *cis*-glycosides. Triflic anhydride as a reactive promoter furnished α-D-linked tetrasaccharide **8** in 58% yield; with titanium tetrafluoride[11], the next less-reactive promoter of the activity row developed for the synthesis of the analogous trisaccharide[4], only 26% of **8** was obtained.

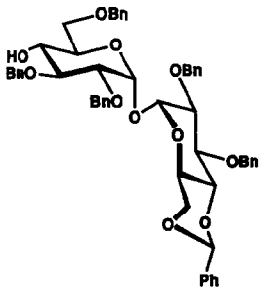
The structure of the tetrasaccharide could be proven by ^{1}H NMR spectroscopy. As was previously shown[3,7] the standard approach for analyzing the often crowded ^{1}H NMR spectra of oligosaccharides is to apply various 1D TOCSY and ROESY experiments. Starting from the frequently well separated anomeric signals, a set of 1D TOCSY experiments with increasing mixing time resulted in well-resolved subspectra of the different saccharide moieties. The assignment of successive coupled protons was based on the appearance of their signals as a function of the increasing mixing time. The assignment of the different subspectra to the corresponding rings was then achieved by a few selected 1D or a 2D ROESY experiments, since the linkage of the rings was revealed by characteristic interring nuclear Overhauser effects[3]. In the case of **8**, a 2D ROESY spectrum was acquired (see Experimental), which revealed the appropriate interring connectivities and thus allowed an unambiguous assignment of the subspectra.

Hydrogenation of **8** in ethyl acetate–ethanol–water mixtures with increasing polarity afforded the deblocked compound **9.** This tetrasaccharide, which was also isolated[12] from cultures of *Streptococcus lactis*, constitutes a substructure of Trestatin A.

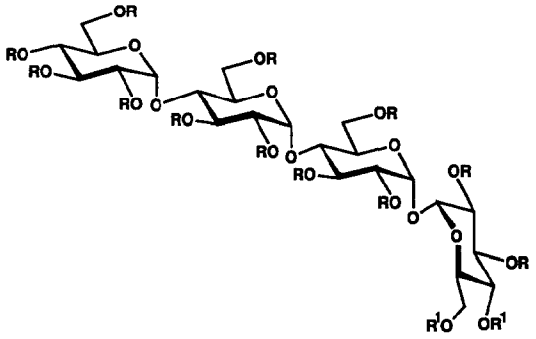
For the block synthesis of a pentasaccharide subunit, a trisaccharide glycosyl donor was prepared in analogy to the synthesis of the disaccharide donor. Thus, undeca-*O*-acetyl-α-D-glucotriosyl bromide[13] (**10**) was converted into the allyl glycoside **11** by treatment with allyl alcohol in the presence of mercuric cyanide. Its ^{1}H NMR spectrum was analyzed by comparison with the analogous hepta-*O*-acetyl-β-maltoside, the synthetic precursor of **2.**



2 R = H, R^1 = OAll, R^2 = H
3 R = Bn, R^1 = OAll, R^2 = H
4 R = Bn; R^1, R^2 = H, OH
5 R = Bn, R^1 = H, R^2 = Cl
6 R = Bn, R^1 = F, R^2 = H



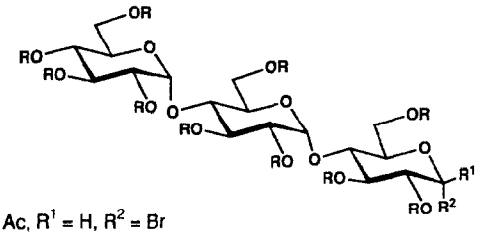
7



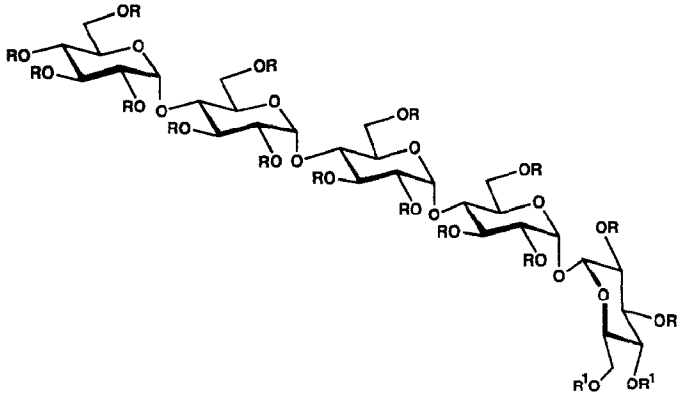
8 R = Bn, R^1 => CHPh
9 R = R^1 = H

Scheme 2.

Trisaccharide **11** was then deacylated to furnish **12**, which was converted into **13** by standard benzylation. The ^{1}H NMR spectrum of the latter compound was analyzed by 1D TOCSY and 1D ROESY experiments in the same way as already described. The ^{1}H NMR data of the outer pyranose rings are in good agreement with those of the respective disaccharide **3** ($\Delta\delta \pm 0.04$ ppm). The anomeric protective group of **13** was cleaved using Ogawa's reaction conditions[14] (palladium chloride–sodium acetate in aqueous acetic acid), together with sonication, to give **14** in excellent yield (90% over two steps) as a mixture of anomers ($\alpha/\beta \approx 5:3$ by ^{1}H NMR); interestingly, the anomeric signal at low field, usually attributable to H-1″, is split, whereas the anomeric signal at 5.51 ppm is not. The anomeric mixture **14** was cleanly converted into the α-chloride **15** by treatment with the Vilsmeier salt prepared in situ with catalytic amounts[15] of *N,N*-dimethylformamide. Nucleophilic substitution using silver fluoride afforded exclusively the



10 R = Ac, R^1 = H, R^2 = Br
11 R = Ac, R^1 = OAll, R^2 = H
12 R = R^2 = H, R^1 = OAll
13 R = Bn, R^1 = OAll, R^2 = H
14 R = Bn; R^1, R^2 = OH,H
15 R = Bn, R^1 = H, R^2 = Cl
16 R = Bn, R^1 = F, R^2 = H



17 R = Bn, R^1 = >CHPh
18 R = R^1 = H

Scheme 3.

trisaccharide β-fluoride **16**, as shown by ^{1}H NMR spectroscopy. Here, the assignments of ring protons were again based on various 1D TOCSY and 1D ROESY experiments. Reaction of the glycosyl donor **16** with the glycosyl acceptor **7**, employing triflic anhydride as a promotor, afforded pentasaccharide **17** in a yield of 30%. In contrast, use of titanium tetrafluoride as a promoter led to the isolation of only 3% of **17**. This again demonstrates the need for a strong promoter in block syntheses with components of relatively low reactivity, and underlines the utility of triflic anhydride in such demanding α-D-glycosidation reactions. The all α-D-linkages in **17** are seen in the ^{1}H NMR spectrum of the compound from the typical $J_{H\text{-}1,H\text{-}2}$ coupling constants of 3.5–3.7 Hz. Standard deblocking of **17** by hydrogenation furnished the free glucoside **18** which is a 6″″-oxygenated pentasaccharide substructure of Trestatin A.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Perkin–Elmer polarimeter 241. Mass spectra were recorded with MS 902 (FAB) with data system DS 2050 (VG) and MS 9 (EI) with DS 200 data system (Finnigan MAT). ^{1}H NMR spectra were recorded on a Bruker AM-400 (400MHz) spectrometer with an Aspect 3000 process controller and 160 MB disk. Chemical shifts are given in ppm relative to Me_4Si or sodium 4,4-dimethyl-4-silapentanoate as internal standard in $CDCl_3$ or D_2O as specified below. All 1D TOCSY and 1D ROESY spectra were measured as described previously[3]. The 2D ROESY spectrum of **8** was accumulated in the phase-sensitive mode using a chopped spin-lock for mixing[16]; experimental conditions: coaddition of the fid's with spin-lock durations of 0.4, 0.6, and 0.8 s, an effective spin-lock field of 1.8 kHz, 4K × 346 experiments, zero-filling to 2K in F_1, spectral widths in F_2 and F_1 4000 Hz, digital resolution 1.9 and 3.9 Hz per point, cosine-square filter in both directions, 1.5 s relaxation delay, 48 scans per t_1 increment, total acquisition time ~ 14 h. Column chromatography was performed on columns of silica gel (E. Merck, 63–200 μm), medium pressure liquid chromatography (MPLC) on Lobar columns, Lichroprep Si 60 (E. Merck, 40–63 μm) at 2–5 bar (Labomatic MD80/100 pump). TLC was performed on precoated Silica Gel 60F 254 plates (E. Merck), detection by UV light (254 nm) and spraying with 10% solution of concd H_2SO_4 followed by heating. Sonication was performed with TEC-15, 33 kHz.

*Allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**3**). — $[\alpha]_D^{20}$ + 29.2° (*c* 0.5, $CHCl_3$), lit.[8] $[\alpha]_D^{26}$ + 28° (*c* 3, $CHCl_3$); ^{1}H NMR data ($CDCl_3$; 1D TOCSY): δ 7.28–7.10 (m, 35 H, aromatic), 5.97, 5.34, 5.21, 4.43 and 4.14 (5 H, allyl), 4.96, 4.92, 4.87, 4.78, 4.77, 4.75, 4.61, 4.57, 4.54 (2 H), 4.52, 4.45, 4.43 and 4.32 (14 d, 14 H, 7 CH_2Ph), 5.67 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 4.47 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.05 (dd ~ t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.89 (dd ~ t, 1 H, $J_{3',4'}$ ~ 8.8 Hz, H-3'), 3.80 (dd, 1 H, $J_{5,6a}$ ~ 2, $J_{6a,6b}$ ~ 11.8 Hz, H-6a), 3.78 (dd ~ t, 1 H, $J_{3,4}$ ~ 8.5 Hz, H-3), 3.75 (ddd ~ br d, 2 H, H-5'; dd, H-6b), 3.63 (dd ~ t, 1 H, $J_{4',5'}$ ~ 10.2 Hz, H-4'), 3.55 (2 H, ddd, H-5; dd, H-6a'), 3.54 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2), 3.48 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-2'), and 3.43 (dd, 1 H, $J_{5',6b'}$ 1.8, $J_{6a',6b'}$ 10.6 Hz, H-6b').

*2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-β-*D*-glucopyranosyl fluoride* (**6**). — A solution of oxalyl chloride (0.42 mL, 4.92 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise at 4°C to a solution of **4** (4.35 g, 4.47 mmol) in $CHCl_2$ containing DMF (0.1 mL). After 3 h at room temperature the solution was concentrated and dried to afford 4.43 g of crude **5**, which was dissolved in dry MeCN (10 mL) and stirred for 1 h in the presence of AgF (1.87 g, 14.75 mmol). The mixture was poured into aq NaCl and extracted three times with ether, the organic phases were washed twice with water, dried (Na_2SO_4), and evaporated. MPLC with 9:1 hexane–EtOAc gave **6** (3.00 g, 69%) as a colourless syrup; $[\alpha]_D^{20}$ + 60.7° (*c* 0.15, dioxane); FABMS: *m/z* 976 ± 1 (0.2%, M^+); ^{1}H NMR data

($CDCl_3$; 1D TOCSY and 1D ROESY): δ 7.28–7.09 (m, 35 H, aromatic) 5.58 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 5.35 (dd, 1 H, $J_{1,2}$ 6.1, $J_{1,F}$ 54.0 Hz, H-1), 4.87 and 4.78 (2d, 2 H, J 10.4 Hz, CH_2Ph), 4.85 and 4.70 (2d, 2 H, J 11.6 Hz, CH_2Ph), 4.78 and 4.42 (2d, 2 H, J 10.8 Hz, CH_2Ph), 4.77 and 4.60 (2d, 2 H, J 11.2 Hz, CH_2Ph), 4.59 and 4.52 (2d, 2 H, J 11.9 Hz, CH_2Ph), 4.53 (s, 2 H, CH_2Ph), 4.52 and 4.28 (2d, 2 H, J 12.0 Hz, CH_2Ph), 4.16 (dd ~ t, 1 H, $J_{4,5}$ 8.6 Hz, H-4), 3.88 (dd ~ t, 1 H, $J_{3',4'}$ 9.0 Hz, H-3'), 3.83–3.79 (m, 2 H, H-5, 6a), 3.81 (dd ~ t, 1 H, $J_{3,4}$ 8.0 Hz, H-3), 3.73 (~ dd, 1 H, H-6b), 3.72 (ddd, 1 H, H-5'), 3.65 (ddd, 1 H, $J_{2,3}$ 8.0, $J_{2,F}$ 11.6 Hz, H-2), 3.63 (dd ~ t, 1 H, $J_{4',5}$ 10.2 Hz, H-4'), 3.52 (dd, 1 H, $J_{5',6a'}$ 3.0 Hz, H-6a'), 3.50 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-2'), and 3.38 (dd, 1 H, $J_{5',6b'}$ 1.9, $J_{5a',6b'}$ 11.0 Hz, H-6b'). Anal. Calcd for C_{61} H_{63} F O_{10}: C, 75.13; H, 6.51. Found: C, 74.97; H, 6.58.

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)-2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (**8**). — *A*. To a stirred mixture of well-dried fluoride **6** (8.49 g, 8.7 mmol), glucosyl acceptor **7** (3.8 g, 4.32 mmol), and 4A molecular sieves (3 g) in diethylether (30 mL) was added TiF_4 (268 mg, 2.18 mmol) at 0°C. After 3 days at room temperature the mixture was filtered over a pad of silica gel, the filtrate concentrated, and the product immediately chromatographed on silica gel (14:1 toluene–EtOAc). Product fractions were rechromatographed (99:1 toluene–acetone) to give pure **8** (2.07 g, 26%) as a colourless syrup.

B. To a stirred mixture of well-dried fluoride **6** (500 mg, 0.51 mmol), glycosyl acceptor **7** (212 mg, 0.26 mmol), and 4A molecular sieves (0.5 g) in dry diethyl ether (10 mL) was added dropwise triflic anhydride (43 μL, 0.26 mmol) in diethyl ether (10 mL) at −20°C. After 50 h at this temperature, Et_3N was added and the mixture was filtered over a pad of Celite and evaporated. Chromatography on silica gel (29:1 toluene–EtOAc, then 4:1 hexane–acetone) furnished **8** (276 mg, 58%); $[\alpha]_D^{20}$ + 70.0° (*c* 0.2, $CHCl_3$); ^1H NMR data ($CDCl_3$; 1D TOCSY and 2D ROESY): δ 7.52–7.49 (m, 2 H, aromatic), 7.44–7.36 (m, ~5 H, aromatic), 7.33–7.08 (m, ~53 H, aromatic), 5.71 (d, 1 H, $J_{1''',2'''}$ 3.6 Hz, H-1'''), 5.56 (d, 1 H, $J_{1'',2''}$ ~ 3.6 Hz, H-1''), 5.55 (s, 1 H, CHPh), 5.18 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 5.13 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.01 and 4.89 (2d, 2 H, J 11.9 Hz, CH_2Ph), 5.00 and 4.94 (2d, 2 H, J 11.2 Hz, CH_2Ph), 4.94 and 4.76 (2d, 2 H, J 11.8 Hz, CH_2Ph), 4.82 and 4.71 (2d, 2 H, J 11.0 Hz, CH_2Ph), 4.78 and 4.42 (2d, 2 H, J 10.9 Hz, CH_2PH), 4.77 and 4.70 (2d, 2 H, J 12.6 Hz, CH_2Ph), 4.67 and 4.63 (2d, 2 H, J 12.0 Hz, CH_2Ph), 4.55 and 4.50 (2d, 2 H, J 11.5 Hz, CH_2Ph), 4.51 and 4.25 (2d, 2 H, J 12.5 Hz, CH_2Ph), 4.49 and 4.39 (2d, 2 H, J 12.5 Hz, CH_2Ph), 4.46 and 4.39 (2d, 2 H, J 12.5 Hz, CH_2Ph), 4.40 and 4.37 (2d, 2 H, J 12 Hz, CH_2Ph), 4.27 (ddd ~ dt, 1 H, H-5), 4.21 (ddd, 1 H, $J_{5',6b'}$ ~ 1.5 Hz, H-5'), 4.20 (dd ~ t, 1 H, $J_{3,4}$ ~ 9 Hz, H-3), 4.11 (dd ~ t, 1 H, H-3'), 4.10 (dd, 1 H, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 10.0 Hz, H-6a), 4.08 (dd ~ t, 1 H, H-4''), 4.05 (dd ~ t, 1 H, H-3''), 4.04 (dd ~ t, 1 H, H-4'), 3.90 (dd ~ t, $J_{3''',4'''}$ 8.6 Hz, H-3'''), 3.89 (~ br d, 1 H, H-5''), 3.75 (dd, 1 H, $J_{5',6a'}$ 3.1, $J_{6a',6b'}$ 11.0 Hz, H-6a'), 3.71 (br d, 1 H, H-5'''), 3.69 (dd, 1 H, $J_{5'',6a''}$ ~ 3 Hz, H-6a''), 3.66 (2dd, 2 H, $J_{4''',5'''}$ ~ 10.8 Hz, H-4'''; H-2'), 3.65 (dd ~ t, 1 H, H-6b), 3.64 (dd ~ t, 1 H, $J_{4,5}$ ~ 10.5

Hz, H-4), 3.58 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 3.52 (dd and br d, 2 H, $J_{2'',3''} \sim 9.5$ Hz, H-2''; $J_{5''',6a'''} \leq 2$ Hz, H-6a'''), 3.49 (dd, 1 H, $J_{2''',3'''}$ 9.5 Hz, H-2'''), 3.48 (dd, 1 H, $J_{5'',6b''} < 2$ Hz, H-6b''), and 3.37 (dd, 1 H, $J_{5''',6b'''}$ 1.5, $J_{6a''',6b'''}$ 10.8 Hz, H-6b'''). Anal. Calcd for C_{115} H_{118} O_{21}: C, 75.22; H, 6.48. Found: C, 75.06; H, 6.60.

O-α-D-*Glucopyranosyl-(1 → 4)*-O-α-D-*glucopyranosyl-(1 → 4)*-O-α-D-*glucopyranosyl*-α-D-*glucopyranoside* (**9**). — A solution of **8** (1.1 g, 0.6 mmol) in EtOH (7.5 mL) and EtOAc (5 mL) was hydrogenated in the presence of 10% Pd–C (600 mg) at room temperature. After 16 h, EtOH (5 mL), water (5 mL), and Pd–C (200 mg) were added, and the hydrogenation was continued. After 1 day water (5 mL) and Pd–C were added and the hydrogenation was continued for another day. The mixture was filtered over a pad of Celite, which was washed with EtOH–water. Evaporation and chromatography on a Sephadex® LH 20 column (1:1 MeOH–water) and on silica gel (4:1 acetone–water containing 0.1% of Et_3N) gave amorphous **9** (390 mg, 98%); $[\alpha]_D^{20}$ + 180.0° (*c* 0.2, H_2O), lit. [12] $[\alpha]_D^{20}$ + 213° (*c* 0.65, H_2O); MS (thermospray): *m/z* 684 (4%, M + NH_4) and 689 (5%, M + Na); ^1H NMR data (D_2O): δ 5.43, 5.41 (2d, 2 H, *J* 4.1, *J* 4.0 Hz, H-1''', 1''), 5.21–5.19 (2d ~ t, 2 H, H-1', 1), 4.12 (dd ~ t, 1 H), 4.00–3.57 (m, 21 H), 3.45 (dd ~ t, 1 H), and 3.43 (dd ~ t, 1 H). Anal. Calcd for C_{24} H_{42} O_{21}: C, 43.25; H, 6.35. Found: C, 43.12; H, 6.49.

Allyl O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranoside* (**11**). — A solution of **10** (ref. 13; 32.7 g, 33.1 mmol) in allyl alcohol (85 mL) was stirred in the presence of $Hg(CN)_2$ (8.5 g, 33.8 mmol) at a bath temperature of 50–60°C. After 90 min the solution was concentrated. Then ether was added, and the solution was washed with aq 1 M KI solution, aq $NaHCO_3$, and twice with water. The organic phase was dried over $MgSO_4$ and evaporated. The residual material was purified by column chromatography (1:1 EtOAc–hexane) to give the pure trisaccharide **11** (25.3 g, 80%) as a colourless foam; $[\alpha]_D^{20}$ + 75.0° (*c* 0.5, dioxane); FABMS: *m/z* 1003 (65%, M + K^+) and 987 (100%, M + Na^+); ^1H NMR ($CDCl_3$): δ 5.85 (dddd, *J* 5.0, 6.0, 10.5, 17.5 Hz, allyl), 5.41 (d, 1 H, H-1''), 5.40 (dd, 1 H, $J_{3',4'}$ 8.7 Hz, H-3'), 5.36 (dd, 1 H, $J_{3'',4''}$ 9.9 Hz, H-3''), 5.28 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1'), 5.275 (dddd ~ dq, 1 H, *J* 1.6, 17.5 Hz, allyl), 5.25 (dd ~ t, 1 H, H-3), 5.21 (dddd ~ dq, 1 H, allyl), 5.08 (dd ~ t, 1 H, $J_{4'',5''}$ 9.3 Hz, H-4''), 4.86 (dd, 1 H, $J_{1'',2''}$ 4.0, $J_{2'',3''}$ 10.5 Hz, H-2''), 4.85 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 4.74 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2'), 4.59 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.47 (dd, 1 H, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.46 (dd,1 H, $J_{5',6a'}$ 2.2, $J_{6a',6b'}$ 12.5 Hz, H-6a'), 4.32 (dd, 1 H, $J_{5,6b}$ 4.0 Hz, H-6b; dddd ~ ddt, 1 H allyl), 4.25 (dd, 1 H, $J_{5'',6a''}$ 3.5, $J_{6a'',6b''}$ 12.5 Hz, H-6a''), 4.19 (dd, 1 H, $J_{5',6b'}$ 3.5 Hz, H-6b'), 4.10 (dddd ~ ddt, 1 H, allyl), 4.05 (dd,1 H, $J_{5'',6b''}$ 2.3 Hz, H-6b''), 3.99 (dd ~ t, 1 H, $J_{3,4}$ 9.0 Hz, H-4; m, 1 H, H-5''); 3.94 (dd ~ t, 1 H, $J_{4',5'} \sim 10.3$ Hz, H-4'), 3.93 (ddd ~ dt, 1 H, H-5'), 3.71 (ddd ~ dt, 1 H, $J_{4,5}$ 9.7 Hz, H-5), 2.18 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.024 (s, 3 H, OAc), 2.020 (s, 3 H, OAc), 2.00 (s, 6 H, OAc), 1.99 (s, 3 H, OAc). Anal. Calcd for C_{41} H_{56} O_{26}: C, 51.04; H, 5.85. Found: C, 50.79; H, 5.66.

Allyl O-*(α-D-glucopyranosyl)-(1 → 4)*-O-*(α-D-glucopyranosyl)-(1 → 4)-α-D-glucopyranoside* (**12**). — A solution of **11** (24.83 g, 25.7 mmol) in MeOH (150 mL) was stirred at room temperature in the presence of a catalytic amount of anhyd Na_2CO_3. After 18 h the mixture was filtered and made neutral with Amberlite IR 120 (H^+). Evaporation of solvents afforded crude **12** (13.5 g, 96.4%) as a colourless foam; $[\alpha]_D^{20} + 111.5°$ (*c* 0.2, water); EIMS: *m/z* 545 (10%, M + H^+); ^{1}H NMR (D_2O): δ 5.03 (d, 2 H, *J* 3.5 Hz, H-1′, H-1″), 4.35 (d, 1 H, H-1). The compound was further characterized as benzylated derivative **13**.

Allyl 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)-2,3,4-tri*-O-*benzyl-β-D-glucopyranoside* (**13**). — To a solution of **12** (13.2 g, 24.2 mmol) in dry DMF (350 mL) was added NaH (80% in refined oil; 8.0 g, 266.7 mmol) in portions at 1°C. After stirring for 1.5 h, benzyl bromide (32 mL, 266.7 mmol) was added dropwise during 45 min at 0–10°C. Stirring was continued for 30 min at 0°C and for 1 h at room temperature. Then methanol (100 mL) was added, and the mixture was evaporated. The residue was taken up in EtOAc and washed with aq $NaHCO_3$, and twice with water. The organic phases were combined, dried over Na_2SO_4, and evaporated. An analytical sample (0.6 g) of the residue (38.5 g) was purified by MPLC (1:6 EtOAc–hexane) to furnish pure **13** (390 mg, 71%) as a colourless syrup; $[\alpha]_D^{20} + 47.7°$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 1D TOCSY and 1D ROESY): δ 7.31–7.07 (m, ~ 50 H, aromatic), 5.97 (dddd, 1 H, *J* 5.2, 5.8, 10.5, 17.6 Hz, allyl), 5.66 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1″), 5.57 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.34 (dddd ~ dq, 1 H, *J* 17.6, 1.7 Hz, allyl), 5.21 (dddd ~ dq, 1 H, *J* 10.5, 1.5 Hz, allyl), 4.95 (d, 1 H, *J* 11.5 Hz, C*H*Ph), 4.93 (d, 1 H, *J* 10.8 Hz, C*H*Ph), 4.89 (d, 1 H, *J* 11.9 Hz, C*H*Ph), 4.84 (d, 1 H, *J* 11.3 Hz, C*H*Ph), 4.79 (d, 1 H, *J* 11.0 Hz, C*H*Ph), 4.77 (d, 2 H, 2 C*H*Ph), 4.74 (d, 1 H, C*H*Ph), 4.63 (d, 1 H, *J* 11.0 Hz, C*H*Ph), 4.56 (d, 1 H, *J* 12.5 Hz, C*H*Ph), 4.54, 4.48 (2d, 2 H, *J* 12.0 Hz, CH_2Ph), 4.51 (d, 2 H, *J* 12.2 Hz, 2 C*H*Ph), 4.48 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.42 (d, 5 H, 5 C*H*Ph; dddd ~ ddt, 1 H, allyl), 4.27 (d, 1 H, *J* 12.0 Hz, C*H*Ph), 4.15 (dddd ~ ddt, 1 H, *J* 13.0, 1.5, 5.8 Hz, allyl), 4.05 (dd ~ t, 1 H, H-4′), 4.04 (dd ~ t, 1 H, H-4), 4.00 (dd ~ t, 1 H, $J_{3',4'}$ 8.9 Hz, H-3′), 3.91 (dd, 1 H, $J_{3'',4''}$ 8.8 Hz, H-3″), 3.89 (ddd ~ br dt, 1 H, $J_{4',5'}$ 9.0 Hz, H-5′), 3.83 (dd, 1 H, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 11.0 Hz, H-6a), 3.78 (dd ~ t, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 3.77 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 3.73 (ddd, 1 H, H-5″), 3.72 (dd, 1 H, $J_{5',6a'}$ 3, $J_{6a',6b'}$ 11.0 Hz, H-6a′), 3.66 (dd, 1 H, $J_{4'',5''}$ 10.0 Hz, H-4″), 3.55 (ddd, 1 H, H-5; dd, 1 H, $J_{2,3}$ 8.8 Hz, H-2), 3.53 (dd, 1 H, $J_{5'',6a''}$ ~ 2.6 Hz, H-6a″), 3.52 (dd, 1 H, $J_{5',6b'}$ ~ 1.5 Hz, H-6b′), 3.515 (dd, 1 H, $J_{2',3'}$ 8.6 Hz, H-2′), 3.48 (dd, 1 H, $J_{2'',3''}$ 9.8 Hz, H-2″), 3.39 (dd, 1 H, $J_{5'',6b''}$ 1.9, $J_{6a'',6b''}$ 10.5 Hz, H-6b″). Anal. Calcd for $C_{91}H_{96}O_{16}$: C, 75.60; H, 6.69. Found: C, 75.13; H, 6.51.

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)-2,3,4-tri*-O-*benzyl-D-glucopyranose* (**14**). — To a suspension of crude **13** (5.0 g) in 9:1 AcOH–water (40 mL) was added $PdCl_2$ (2.46 g, 13.8 mmol) and NaOAc (2.46 g, 30 mmol), and the mixture was treated for 3 h in an ultrasonic bath, which during that time warmed up to 50°C. Thereafter, the

mixture was filtered over a pad of Speedex, which was washed with 90% aq AcOH and EtOAc. The filtrate was evaporated, the residue taken up in EtOAc, and successively washed with aq $NaHCO_3$. The organic phases were dried over Na_2SO_4, evaporated, and purified by column chromatography on silica gel (7:1 toluene–EtOAc) to furnish pure **14** (3.99 g, 90%) as an oil; ^{1}H NMR ($CDCl_3$): δ 5.67 (d, 0.63 H, $J_{1'',2''}$ 3.6 Hz, H-1'' α), 5.65 (d, 0.37 H, $J_{1'',2''}$ 3.6 Hz, H-1'' β), 5.51 (d, 1 H, $J_{1',2'} \sim 3.5$ Hz, H-1'), 5.22 (dd ~ t, 0.63 H, $J_{1,2}$ 3.6 Hz, H-1a), 3.22 (d, 0.37 H, $J_{1,1\beta\text{-OH}}$ 5.6 Hz, 1β-OH), and 2.98 (d, 0.63 H, $J_{1,1\alpha\text{-OH}}$ 2.8 Hz, 1α-OH). Anal. Calcd for C_{88} H_{92} O_{16}: C, 75.19; H, 6.60. Found: C, 75.30; H, 6.80.

*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)-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl chloride* (**15**). — A solution of oxalyl chloride (245 μL, 2.9 mmol) in dry CH_2Cl_2 (7 mL) was added dropwise during 45 min to a solution of **14** (3.69 g, 2.6 mmol) in anhyd CH_2Cl_2 (25 mL) and abs DMF (0.1 mL) at 3°C. After stirring at room temperature for 6 h, the mixture was evaporated and dried to give crude **15**; ^{1} H NMR ($CDCl_3$): δ 7.29–7.08 (m, 50 H, aromatic), 6.07 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.68 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 5.53 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.99, 4.84 (2d, 2 H, J 11.5 Hz, CH_2Ph), 4.92 (d,1 H, J 12.0 Hz, C*H*Ph), 4.84 (d, 1 H, J 11.5 Hz, C*H*Ph), 4.82 (d, 1 H, J 11.2 Hz, C*H*Ph), 4.81 (d, 1 H, J 11.0 Hz, C*H*Ph), 4.78 (d, 1 H, J 12.5 Hz, C*H*Ph), 4.72 (d, 1 H, J 11.0 Hz, C*H*Ph), 4.64 (s, 2 H, CH_2Ph), 4.56 (d, 1 H, J 12.2 Hz, C*H*Ph), 4.515 (d, 1 H, J 12.5 Hz, C*H*Ph), 4.51 (d, 1 H, J 12.5 Hz, C*H*Ph), 4.50 (d, 1 H, J 12.0 Hz, C*H*Ph), 4.46 (s, 2 H, CH_2Ph), 4.42 (d, 1 H, J 11.0 Hz, C*H*Ph), 4.41 (d, 1 H, C*H*Ph), 4.39 (s, 2 H CH_2Ph), 4.27 (d, 1 H, J 12.5 Hz, C*H*Ph), 4.16–4.10 (m, 3 H, H-3,4,5), 4.07 (dd ~ t, 1 H, $J_{4',5'} \sim 8.8$ Hz, H-4'), 4.03 (dd ~ t, 1 H, $J_{3',4'}$ 9.0 Hz, H-3'), 3.94 (dd, 1 H, $J_{5,6a}$ 2.5 Hz, H-6a), 3.90 (dd, 1 H, $J_{3'',4''}$ 8.9 Hz, H-3''), 3.88 (ddd ~ br d, 1 H, H-5'), 3.75 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 3.72 (ddd ~ br d, 1 H, H-5''), 3.71 (dd, 1 H, $J_{5',6a'}$ 3.4, $J_{6a',6b}$ 11.0 Hz, H-6a'), 3.66 (dd ~ t, 1 H, $J_{4'',5''}$ 10.5 Hz, H-4''), 3.62 (dd, 1 H, $J_{5,6b}$ 1.5, $J_{6a,6b}$ 11.5 Hz, H-6b), 3.530 (dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2'), 3.525 (dd, 1 H, H-6a''), 3.49 (dd, 1 H, $J_{2'',3''}$ 10.0 Hz, H-2''), and 3.39 (dd, 1 H, $J_{5'',6b''}$ 1.5, $J_{6a'',6b''}$ 10.8 Hz, H-6b'').

*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)-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl fluoride* (**16**). — A solution of crude chloride **15** (2.6 mmol) in abs MeCN (10 mL) was stirred at room temperature in the presence of dry AgF (0.97 g, 7.6 mmol). After 3 h the mixture was filtered, and the precipitate was washed with ether. The organic solution was treated with aq satd NaCl solution and stirred vigorously for 15 min. After filtration, the filtrate was concentrated, diluted with ether, and washed successively with NaCl solution and water. The etheral solution was evaporated and chromatographed (1:6 EtOAc–hexane) to afford pure β-fluoride **16** (2.19 g, 60%) as a colourless syrup; $[\alpha]_D^{20}$ + 58.9° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 1D TOCSY and 1D ROESY): δ 7.28–7.07 (m, 50 H, aromatic), 5.65 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 5.48 (d,1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 5.35 (dd, 1 H, $J_{1,2}$ 6.2, $J_{1,F}$ 54.0 Hz, H-1), 4.90 (d, 1 H, J 11.8 Hz, C*H*Ph), 4.86–4.74 (m, 6 H, 6 C*H*Ph), 4.73 (d, 1 H, J 11.8 Hz,

CHPh), 4.62 (d, 1 H, *J* 11.0 Hz, CHPh), 4.55 and 4.49 (2d, 2 H, *J* 12.0 Hz, CH_2Ph), 4.53 and 4.50 (2d, 2 H, *J* 12.0 Hz, CH_2Ph), 4.52 (d, 1 H, CHPh), 4.45 (s, 2 H, CH_2Ph), 4.42 (d, 1 H, *J* 12.5 Hz, CHPh), 4.39 (s, 2 H, CH_2Ph), 4.27 (d, 1 H, *J* 12.5 Hz, CHPh), 4.15 (dd ~ t, 1 H, $J_{4,5}$ 8.3 Hz, H-4), 4.04 (dd ~ t, 1 H, $J_{4',5'}$ 8.9 Hz, H-4'), 3.99 (dd ~ t, 1 H, $J_{3',4'}$ 9.2 Hz, H-3'), 3.92 (dd, 1 H, $J_{3'',4''}$ 8.9 Hz, H-3''), 3.87 (ddd ~ br d, 1 H, H-5'), 3.86 (dd, 1 H, $J_{5,6a}$ 4.2 Hz, H-6a), 3.80 (dd ~ t, 1 H, $J_{3,4}$ 8.7 Hz, H-3), 3.795 (ddd ~ t, 1 H, H-5), 3.74 (dd, 1 H, $J_{5,6b}$ 2.1, $J_{6a,6b}$ 10.8 Hz, H-6b), 3.72 (ddd ~ br d, 1 H, H-5''), 3.70 (dd, 1 H, $J_{5',6a'}$ 2.4, $J_{6a',6b'}$ 11.0 Hz, H-6a'), 3.66 (dd, 1 H, $J_{4'',5''}$ 9,7 Hz, H-4''), 3.65 (ddd, 1 H, $J_{2,3}$ 8.2, $J_{2,F}$ 11.8 Hz, H-2), 3.52 (2 H, dd, $J_{2',3'}$ 8.8 Hz, H-2'; dd ~ br d, H-6a''), 3.49 (2 H, dd, $J_{2'',3''}$ 9.9 Hz, H-2''; dd ~ br d, H-6b'), and 3.39 (dd, 1 H, $J_{5'',6b''}$ 1.5, $J_{6a'',6b''}$ 10.9 Hz, H-6b''). Anal. Calcd for C_{88} H_{91} F O_{15}: C 75.09; H, 6.52; F, 1.35. Found: C, 74.99; H, 6.62; F, 1.50.

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 → 4)-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (**17**). — To a well-stirred mixture of fluoride **16** (1.69 g, 1.2 mmol), glycosyl acceptor **7** (538 mg, 0.61 mmol) and activated 4A molecular sieves (~ 2 g) in abs ether (20 mL) was added a solution of triflic anhydride (110 μL, 0.6 mmol) in ether (5 mL) at −20°C. The mixture was allowed to warm up to room temperature and was stirred for 48 h. After filtration over Speedex and washing with ether, the filtrate was evaporated and chromatographed (14:1 toluene–EtOAc) to furnish pentasaccharide **17** (414 mg, 30%) as a colourless syrup; $[\alpha]_D^{20}$ + 43.7°, $[\alpha]_{365}^{20}$ + 183.3° (*c* 0.08, EtOH); ^{1}H NMR ($CDCl_3$): δ 5.68 (d, 1 H, $J_{1'''',2''''}$ 3.6 Hz, H-1'''') 5.63 (d, 1 H, $J_{1''',2'''}$ 3.6 Hz, H-1'''), 5.61 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.55 (s, 1 H, O_2CHPh), 5.19 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'), 5.12 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1). Anal. Calcd for C_{142} H_{146} O_{26}: C, 75.18; H, 6.49. Found: C, 74.92; H, 6.60.

O-(α-D-glucopyranosyl)-(1 → 4)-O-(α-D-glucopyranosyl)-(1 → 4)-O-(α-D-glucopyranosyl)-(1 → 4)-α-D-glucopyranosyl-α-D-glucopyranoside (**18**). — A solution of **17** (295 mg, 0.13 mmol) in 2:1 EtOH–water (15 mL) was hydrogenated in the presence of 10% Pd–C (300 mg) for 2h at room temperature. The mixture was filtered over Speedex and washed with EtOH–water. The filtrate was evaporated and purified over Sephadex® LH 20 using water as eluent to give the unprotected pentasaccharide **18** (65 mg, 60%) as a colourless glass; $[\alpha]_D^{20}$ + 179.5° (*c* 0.2, H_2O); ^{1}H NMR (D_2O): δ 5.43–5.40 (m, 3 H, H-1'''',1''',1''), 5.20 (br s, 2 H, H-1', H-1). Anal. Calcd for C_{30} H_{52} O_{26}: C, 43.48; H, 6.32. Found: C, 43.32; H, 6.45:

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