A New and Practical Procedure for the Preparation of the Glucohexatose Phytoalexin Elicitor†

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The phytoalexin elicitor β-(1→3)-branched β-(1→6)-linked glucohexatose has been regio- and stereospecifically synthesized by coupling of the 3,6-branched gluco-trisaccharide Schmidt reagent 10 with a mixture of multiol 3,6-branched gluco-trisaccharides 13 which consists of free 5,6′-OH trisaccharide, free 5,2′,6′-OH trisaccharide, free 5,3′,6′-OH trisaccharide and so on. The compounds 10 and 13 were prepared from 1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose, 2, 3, 4, 6-tetra-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate, and 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate through regio- and stereoselective manners.

Keywords oligosaccharide, phytoalexin-elicitor, regio- and stereoselective synthesis

Introduction

The term "elicitor" is introduced to describe substances that, at low concentrations, stimulate biosynthesis of plant antibiotics (phytoalexins) in quantities sufficient to inhibit growth of microbial pathogens. Some oligosaccharides such as β-(1→4)-linked oligosaccharides of chitin and chitosan, α-(1→4)-linked galacturonide oligomers, and β-(1→3) branched β-(1→6)-linked glucose oligomers can act as elicitors. Among them, the branched β-glucooligosaccharides, first isolated from mycelial walls of the fungus *Phytophthora megasperma* f. sp. *Glycinea*, have been well characterized, and the most active species is a heptamer **1**[1] (Fig. 1) which is effective in very low doses, approximately 0.1 pmol per cotyledon.[2] Biological assays of several oligosaccharides revealed that *D*-glucohexatose **2** is the minimum structural element required for high elicitor activity.[3] It is noted that, although much of this work was done with soybean cotyledons, the glucan elicitor can also elicit the synthesis of different phytoalexins in a wide range of other plant species.[4] These important discoveries stimulated the interest of scientists. Since their isolation and identification, the glucan elicitors have been prepared by different groups,[5] and various methods and strategies have been used including the very elegant solid-phase strategies.[5k,5l] However, most of the reported procedures are only suitable for the preparation of samples for the investigation of structure-bioactivity relationships. Production of these molecules on a large scale, which is very important from the point of view of both carbohydrate chemistry and its practical application, has been hampered by the expensive reagents and complex operations involved in the synthesis. Seeberger has made phytoalexin elicitor oligasaccharides in a completely automated fashion on the solid phase, but the key disaccharide glycosyl donors used in his synthesis were made in solution phase via a complex procedure.[5l] Recently, we have disclosed an efficient method for the synthesis of 3,6-branched β-linked gluco-oligosaccharides using 1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose, 2,3,4,6-tetra-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate, and 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate as the starting materials.[6] Synthesis of the β-(1→3)-branched β-(1→6)-linked glucohexatose phytoalexin elicitor on a 100 g scale was achieved in our laboratory, and higher oligosaccharides of the elicitor including

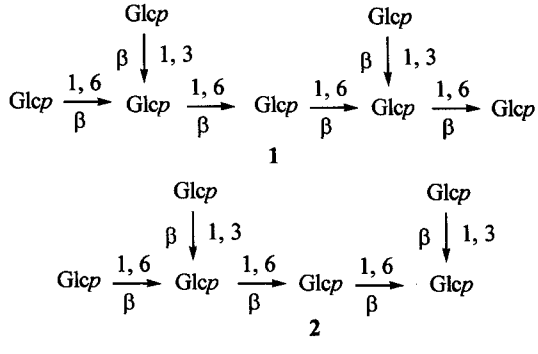

Fig. 1 Glucoheptatose and glucohexatose.

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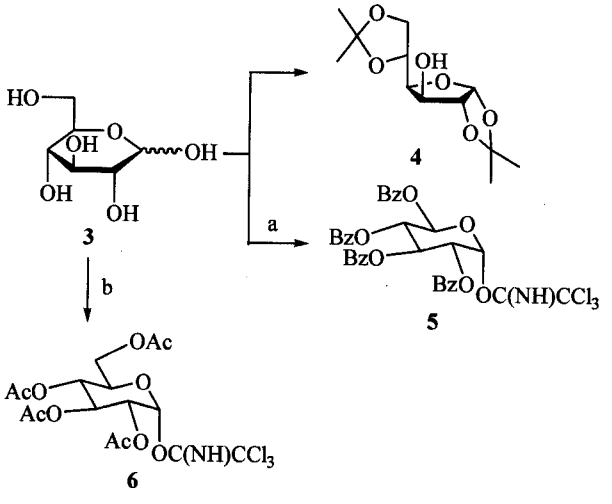
the hepta-, nona-, dodeca- and tetradecasaccharides were also readily synthesized by the developed strategy.[7] For further improving the glucohexatose preparation, in this paper, one of the glycosyl donors, 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate used in our previous synthesis,[7] is replaced by 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate, because it is more easily available.

Results and discussion

The 1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose (**4**), 2,3,4,6- tetra-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate (**5**) and 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate (**6**) are starting materials in our synthesis. Compound **4** is prepared from *D*-glucose (**3**) according to the standard procedure.[8] Compound **5** was prepared as fine crystals from benzoylation of *D*-glucose (**3**), followed by 1-*O*-debenzoylation with ammonia in THF-CH_3OH and trichloroacetimidation (Scheme 1). Compound **6** was obtained as good crystals from acetylation of *D*-glucose (**3**), followed by 1-*O*-deacetylation with ammonia in THF-CH_3OH and trichloroacetimidation.

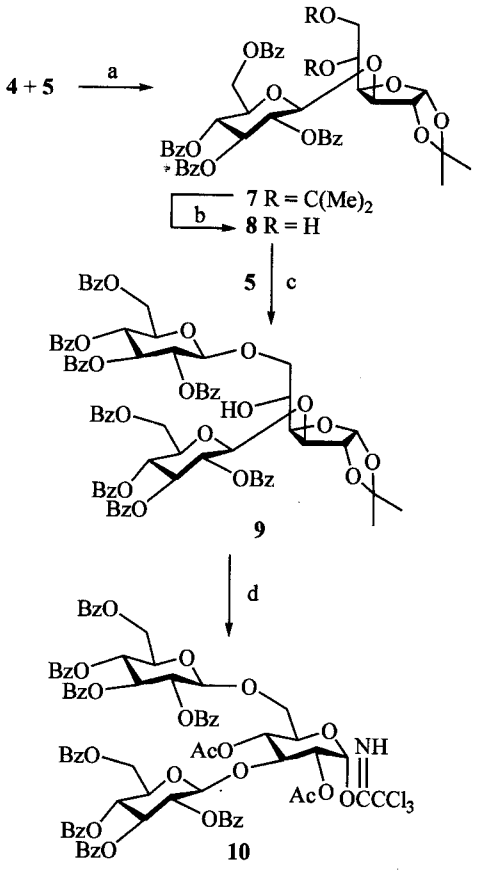
Scheme 1



Reagents and conditions: (a) (i) PhCOCl, pyridine and toluene, 70 ℃, 8 h; (ii) 3:1 (*V*/*V*) THF-CH_3OH-1.5 N NH_3, rt, 12 h; (iii) CH_2Cl_2, CCl_3CN, K_2CO_3, rt, 12 h, 56% (for three steps). (b) (i) Ac_2O, Pyridine, rt, 3 h; (ii) 3:1 (*V*/*V*) THF-CH_3OH-1.5 N NH_3, rt, 3 h; (iii) CH_2Cl_2, CCl_3CN, K_2CO_3, rt, 12 h, 54% (for three steps).

Coupling of 1,2:5,6-di-*O*-isopropylidene-α-*D*-glucofuranose (**4**) with perbenzoyl glucosyl imidate **5** in the presence of TMSOTf as catalyst, followed by selective 5,6-*O*-deacetonation afforded β-(1→3)-linked disaccharide **8** as crystals in a high yield (81% for two steps) (Scheme 2). Condensation of **8** with **5** catalyzed by TMSOTf regio- and stereoselectively gave the key 3,6-branched trisaccharide **9** in excellent yields (90%). Acid-catalyzed (80% HOAc) removal of the 1,2-*O*-isopropylidene group of **9** followed by acetylation with acetic anhydride in pyridine, selective 1-*O*-deacetylation with ammonia in THF-CH_3OH, and subsequent treatment with trichloroacetonitrile in the presence of K_2CO_3 afforded the desired trisaccharide glycosyl donor **10** in a good yield (71% for four steps). All of the trisaccharide intermediates except compound **10** involved in the synthesis can be directly subjected to the next reaction without column separation.

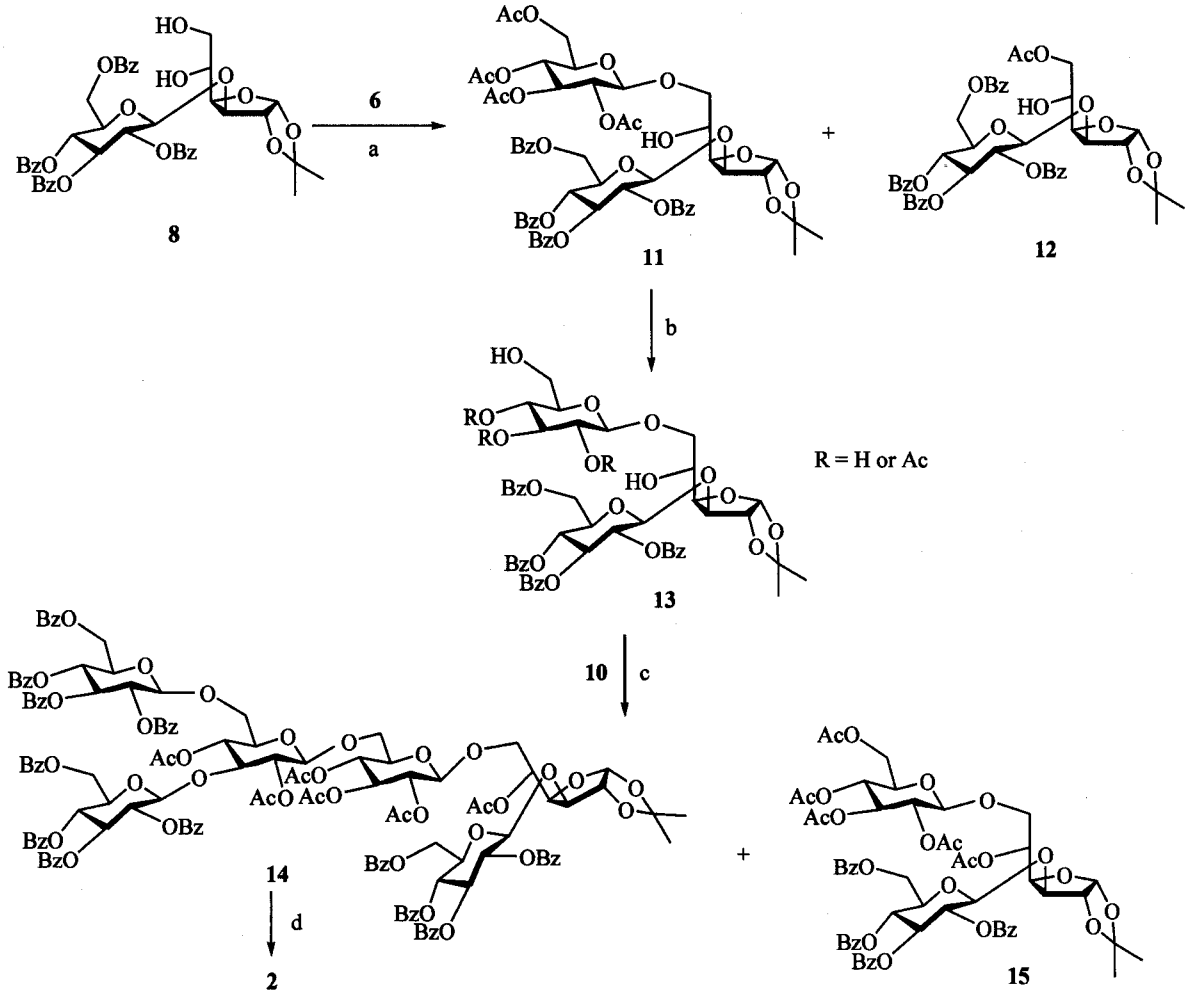
Scheme 2



Reagents and conditions: (a) TMSOTf, MS 4 Å, CH_2Cl_2, rt, 3 h. (b) 90% HOAc, 40 ℃, 24 h, 81% (for 2 steps). (c) **5**, TMSOTf, MS 4 Å, CH_2Cl_2, rt, 3 h, 90%. (d) (i) 80% HOAc, reflux, 5 h; (ii) Ac_2O-Pyridine, rt, 3 h; (iii) THF-CH_3OH-1.5 N NH_3, rt, 3 h; (iv) CH_2Cl_2, CCl_3CN, K_2CO_3, rt, 12 h, 71% (for four steps).

Coupling of **8** with **6** catalyzed by TMSOTf gave the desired trisaccharide **11** in 66% yield, together with the 6-*O*-acetylated disaccharide **12** (Scheme 3). In the glycosylation, some of the acetyl groups of the glycosyl donor **6** were transfered to the free 6-position of glycosyl acceptor **8**. Similar phenomenon was not found in the coupling reaction of **8** with **5**. Treatment of **11** with CH_2Cl_2-CH_3OH containing 0.3% HCl gave a mixture of the trisaccharides **13** containing 6-*O*-deacetylated trisaccharide, 2,6-*O*-deacetylated trisaccharide, 3,6-*O*-deacetylated trisaccharide, 4,6-*O*-deacetylated trisaccharide and so on in 84% yield (calculated on the hypothesis that all of the trisaccharides are the 6-*O*-deacetylated trisaccharide). Coupling

Scheme 3



Reagents and conditions: (a) **6**, TMSOTf, MS 4 Å, CH_2Cl_2, rt, 3 h, 66%. (b) 0.3% HCl in CH_2Cl_2-CH_3OH, rt, 20 h, 84%. (c) (i) **10**, TMSOTf, MS 4 Å, CH_2Cl_2, rt, 3 h; (ii) Ac_2O-Pyridine, rt, 3 h, 81% (for two steps). (d) (i) 80% HOAc, reflux, 4 h; (ii) CH_2Cl_2-CH_3OH saturated with ammonia, rt, 24 h, 95% (for two steps).

of the trisaccharide mixture **13** with **10** using TMSOTf as the catalyst, followed by acetylation, afforded the blocked hexasaccharide **14** in 81% yield (based on **10**) and the recovered trisaccharide **15**. Acid-catalyzed (80% HOAc) deisopropylidenation of **14**, followed by deacetylation in CH_2Cl_2-CH_3OH saturated with ammonia, furnished the free hexasaccharide **2** as an amorphous white solid in 95% yield (for two steps).

In all of the synthesis, very easily accessible materials and cheap reagents were used and the reactions were carried out smoothly in high yields and on large scales. Using 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate as glycosyl donor instead of 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate simplified the preparation of β-(1→3) branched β-(1→6)-linked hexasaccharide **2**.

Experimental

Melting points were determined with a "Mel-Temp" apparatus. Optical rotations were determined at 25 ℃ with digital polarimeter. The NMR spectra were recorded with Bruker ARX 400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C) for solutions in $CDCl_3$ or D_2O as indicated. Mass spectra were recorded on an autospec mass spectrometer using ESI technique to introduce the sample. Elemental analyses were done on elemental analyzer model 1108 EA. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (*V*/*V*) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted on a column (10 × 240 mm, or 18 × 300 mm, or 35 × 400 mm) of silica gel (100—200 mesh) with EtOAc-petroleum ether (60—90 ℃) as eluent. Solutions were concentrated at < 60 ℃ under reduced pressure. Dry solvents were distilled over CaH_2 and stored over molecular sieves.

2,3,4,6-Tetra-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**5**)

BzCl (680 mL, 5.83 mol) was added to a solution of *D*-glucose (**3**) (200 g, 1.11 mol) in toluene (2500 mL)

and pyridine (473 mL, 5.85 mol) over 1 h to keep the temperature under 70 ℃, and then the mixture was stirred at 70 ℃ for further 7 h. Filtration of pyridine-hydrogen chloride salt and concentration of the filtrate gave a residue which was directly dissolved in a 1.5 N NH_3 solution of 3:1 THF-CH_3OH (5000 mL). The solution was kept at room temperature for 12 h, at the end of which time TLC (3:1 petroleum ether-EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (1000 mL) and CCl_3CN (120 mL, 1.2 mol) containing K_2CO_3 (300 g, 2.17 mol). The reaction mixture was stirred for 12 h at room temperature, at the end of which time TLC (3:1 petroleum ether-EtOAc) indicated that the reaction was complete. The mixture was filtered, the solution was concentrated under reduced pressure, and the residue was decolorized by passing through a short silica-gel column with 3:1 petroleum ether-EtOAc as eluent, and the 2,3,4,6-tetra-*O*-benzoyl-*α*-*D*-glucopyranosyl trichloroacetimidate (**5**) (460 g, 56% for three steps) was crystallized from 3:1 petroleum ether-EtOAc as pretty good crystals.[9]

2, 3, 4, 6-*Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate* (**6**)

D-Glucose (**3**) (100 g, 0.56 mol) was treated with acetic anhydride (500 mL) and pyridine (500 mL) for 3 h at room temperature. The acetylated sugar was dissolved in a 1.5 N NH_3 solution of 3:1 THF-CH_3OH (3000 mL), and the solution was kept at room temperature for 3 h, at the end of which time TLC (3:1 petroleum ether-EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in a solution of CH_2Cl_2 (500 mL) and CCl_3CN (60 mL, 0.6 mol) containing K_2CO_3 (150 g, 1.08 mol). The reaction mixture was stirred for 12 h at room temperature. After the mixture was filtered, the combined filtrate and washings were concentrated, and the residue was subjected to column chromatography with 3:1 petroleum ether-EtOAc as eluent to give the 2,3,4,6-tetra-*O*-acetyl-*α*-*D*-glucopyranosyl trichloroacetimidate (**6**) (149 g, 54% for three steps) as pretty good crystals.[9]

2,3,4,6-*Tetra-O-benzoyl-α-D-glucopyranosyl*-(1→3)-1,2-*O-isopropylidene-α-D-glucofuranose* (**8**)

To a stirred solution of 1,2:5,6-di-*O*-isopropylidene-*α*-*D*-glucofuranose (**4**) (100 g, 0.38 mol) and 2,3,4,6-tetra-*O*-benzoyl-*α*-*D*-glucopyranosyl trichloroacetimidate (**5**) (260 g, 0.35 mol) in dry dichloromethane (3000 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 700 μL, 35 mmol) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the resulting residue was directly dissolved in 90% aqueous acetic acid solution (5000 mL). The mixture was kept at 40 ℃ for 24 h, and then concentrated to a residue under reduced pressure. The resulting residue was subjected to a short silica-gel column to give compound **8** (226 g, 81% yield for two steps) as white crystals; m.p. 121—123 ℃; $[\alpha]_D$ + 34 (*c* 2.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.11—7.28 (m, 20 H, 4 × BzH), 5.94 (t, $J_{2',3'} = J_{3',4'} = 9.7$ Hz, 1H, H-3'), 5.72 (t, $J_{3',4'} = J_{4',5'} = 9.7$ Hz, 1H, H-4'), 5.54 (dd, $J_{1',2'} = 7.9$ Hz, $J_{2',3'} = 9.7$ Hz, 1H, H-2'), 5.53 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1), 5.03 (d, $J_{1',2'} = 7.9$ Hz, 1H, H-1'), 4.84 (dd, $J_{5',6a'} = 3.6$ Hz, $J_{6a',6b'} = 11.9$ Hz, 1H, H-6a'), 4.42 (dd, $J_{5',6b'} = 4.3$ Hz, $J_{6a',6b'} = 11.9$ Hz, 1H, H-6b'), 4.41 (d, $J_{3,4} = 2.6$ Hz, 1H, H-3), 4.24—4.23 (m, 2H, H-2, 5'), 4.16 (dd, $J_{3,4} = 2.6$ Hz, $J_{4,5} = 8.8$ Hz, 1H, H-4), 4.02 (m, 1H, H-5), 3.83 (dd, $J_{5,6a} = 3.2$ Hz, $J_{6a,6b} = 11.4$ Hz, 1H, H-6a), 3.67 (dd, $J_{5,6b} = 6.0$ Hz, $J_{6a,6b} = 11.4$ Hz, 1H, H-6b), 1.44, 1.09 (2 s, 6H, 2 × CCH_3). Anal. calcd for $C_{43}H_{42}O_{15}$: C 64.66, H 5.30; found C 64.79, H 5.25.

2,3,4,6-*Tetra-O-benzoyl-α-D-glucopyranosyl*-(1→3)-[2,3,4,6-*tetra-O-benzoyl-β-D-glucopyranosyl*-(1→6)]-1,2-*O-isopropylidene-α-D-glucofuranose* (**9**)

To a stirred solution of **8** (80 g, 0.1 mol) and **5** (80 g, 0.108 mol) in dry dichloromethane (400 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 200 μL, 1.0 mmol) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1.5:1 petroleum ether-ethyl acetate as eluent to give the trisaccharide **9** (124 g, 90%) as a white amorphous solid; $[\alpha]_D$ + 25.3 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.06—7.28 (m, 40H, 8 × BzH), 5.88 (t, $J = 9.7$ Hz, 1H, H-3), 5.87 (t, $J = 9.7$ Hz, 1H, H-3), 5.69 (t, $J = 9.7$ Hz, 1H, H-4), 5.64 (t, $J = 9.7$ Hz, 1H, H-4), 5.53 (dd, $J = 7.9$, 9.7 Hz, 1H, H-2), 5.43 (dd, $J = 7.9$, 9.7 Hz, 1H, H-2), 5.41 (d, $J = 3.5$ Hz, 1H, H-1), 4.96 (d, $J = 7.9$ Hz, 1H, H-1), 4.93 (d, $J = 7.9$ Hz, 1H, H-1), 4.68 (dd, $J = 3.4$, 12.3 Hz, 1H, H-6), 4.48 (dd, $J = 4.9$, 12.2 Hz, 1H, H-6), 4.67 (dd, $J = 3.4$, 12.2 Hz, 1H, H-6), 4.35 (dd, $J = 4.9$, 12.2 Hz, 1H, H-6), 4.34—3.65 (m, 8H), 1.26, 1.03 (2s, 6H, 2 × CCH_3). Anal. calcd for $C_{77}H_{68}O_{24}$: C 67.15, H 4.98; found C 67.29, H 5.02.

2,3,4,6-*Tetra-O-benzoyl-β-D-glucopyranosyl*-(1→3)-[2,3,4,6-*tetra-O-benzoyl-α-D-glucopyranosyl*-(1→6)]-2,4-*di-O-acetyl-α-D-glucopyranosyl trichloroacetimidate* (**10**)

Compound **9** (50 g, 0.036 mol) was added to 80% aqueous acetic acid solution (500 mL) and the mixture was heated under reflux for 5 h. The mixture was concentrated and the residue was acetylated with acetic anhydride (250 mL) in pyridine (280 mL) for 3 h at room tempera-

ture. The resultant trisaccharide was dissolved in a 1.5 N NH_3 solution of 3:1 THF-CH_3OH (500 mL) and the solution was stirred at room temperature for 3 h. The solution was concentrated, and the residue was dissolved in dichloromethane (200 mL). To the solution was added K_2CO_3 (10 g, 0.072 mol), CCl_3CN (8 mL, 0.072 mol), and the mixture was stirred at room termperature for 12 h. After the mixture was filtered, the combined filtrate and washings were concentrated, and the residue was subjected to column chromatography giving the trisaccharide donor **10** as a white amorphous solid (40.0 g, 71% for four steps); $[\alpha]_D$ + 23.3 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.33 (s, 1H, NH), 8.07—7.19 (m, 40H, 8 × BzH), 6.19 (d, J = 3.6 Hz, 1H, H-1), 5.91 (t, J = 9.6 Hz, 1H, H-4), 5.85 (t, J = 9.6 Hz, 1H, H-4), 5.62 (t, J = 9.6 Hz, 1H, H-3), 5.61 (t, J = 9.6 Hz, 1H, H-3), 5.46 (dd, J = 7.9, 9.6 Hz, 1H, H-2), 5.42 (dd, J = 7.9, 9.6 Hz, 1H, H-2), 4.97 (d, J = 7.9 Hz, 1H, H-1), 4.96 (d, J = 7.9 Hz, 1H, H-1), 4.85 (t, J = 9.6 Hz, 1H, H-4), 4.67—4.59 (m, 3H), 4.50—4.37 (m, 2H), 4.19—4.02 (m, 4H), 3.91 (dd, J = 3.2, 12.0 Hz, 1H), 3.69 (dd, J = 4.4, 12.0 Hz, 1H), 1.94, 1.78 (2s, 6H, 2 × CH_3CO). Anal. calcd for $C_{80}H_{68}NO_{26}Cl_3$: C 61.37, H 4.38; found C 61.53, H 4.41.

2,3,4,6-*Tetra-O-benzoyl-α-D-glucopyranosyl-*(1→3)-[2, 3,4, 6-*tetra-O-acetyl-β-D-glucopyranosyl-*(1→6)]-1, 2-*O-isopropylidene-α-D-glucofuranose* (**11**) *and* 2, 3, 4, 6-*Tetra-O-benzoyl-α-D-glucopyranosyl-*(1→3)-6-*O-acetyl*-1, 2-*O-isopropylidene-α-D-glucofuranose* (**12**)

To a stirred solution of **8** (18 g, 22.5 mmol) and **6** (10.8 g, 22.0 mmol) in dry dichloromethane (200 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 60 μL, 0.3 mmol) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography with 1.5 : 1 petroleum ether-ethyl acetate as eluent to give the trisaccharide **11** (16.4 g, 66%) and the disaccharide **12** (2.8 g). For **11**: $[\alpha]_D$ + 38.6 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.97—7.20 (m, 20H, 4 × BzH), 5.82 (t, J = 9.6 Hz, 1H, H-4), 5.67 (d, J = 3.6 Hz, 1H, H-1), 5.62 (t, J = 9.6 Hz, 1H, H-3), 5.47 (dd, J = 7.9, 9.6 Hz, 1H, H-2), 5.10 (t, J = 9.6 Hz, 1H, H-4), 4.98 (t, J = 9.6 Hz, 1H, H-3), 4.92 (d, J = 7.9 Hz, 1H, H-1), 4.81 (dd, J = 7.9, 9.6 Hz, 1H, H-2), 4.62 (dd, J = 3.8, 11.7 Hz, 1H, H-6), 4.57 (d, J = 7.9 Hz, 1H, H-1), 4.40 (dd, J = 4.6, 11.7 Hz, 1H, H-6), 4.28—3.69 (m, 10 H), 1.97, 1.96, 1.95, 1.93 (4s, 12H, 4 × CH_3CO), 1.25, 1.18 (2s, 6H, 2 × CCH_3). Anal. calcd for $C_{57}H_{60}O_{24}$: C 60.64, H 5.36; found C 60.97, H 5.23. For **12**: $[\alpha]_D$ + 24 (*c* 2.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.07—7.26 (m, 20H, 4 × BzH), 5.90 (t, $J_{2',3'} = J_{3',4'}$ = 9.7 Hz, 1H, H-3′), 5.70 (t, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, 1H, H-4′), 5.51 (dd, $J_{1',2'}$ = 7.9 Hz, $J_{2',3'}$ = 9.7 Hz, 1H, H-2′), 5.50 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.01 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1′), 4.78 (dd, $J_{5',6a'}$ = 3.7 Hz, $J_{6a',6b'}$ = 11.6 Hz, 1H, H-6a′), 4.42 (dd, $J_{5',6b'}$ = 4.1 Hz, $J_{6a',6b'}$ = 11.6 Hz, 1H, H-6b′), 4.40—3.78 (m, 8H), 2.07 (s, 3H, CH_3CO), 1.42, 1.09 (2s, 2 × CCH_3). Anal. calcd for $C_{45}H_{44}O_{16}$: C 64.28, H 5.27; found C 64.74, H 5.15.

2,3,4,6-*Tetra-O-benzoyl-β-D-glucopyranosyl-*(1→3)-[2, 3, 4-*tri-O-R-α-D-glucopyranosyl-*(1 → 6)]-1, 2-*O-isopropylidene-α-D-glucofuranose* (*R* = *Ac or/and H*) (**13**)

Acetyl chloride (0.4 mL) was added to a solution of compound **11** (20 g, 17.7 mmol) in CH_3OH (100 mL) and CH_2Cl_2(100 mL), and the reaction was carried out at room temperature for 20 h. After neutralization and concentration, the residue was subjected to column chromatography to afford compound **13** (16.2 g, 84%, calculated on the hypothesis that all of the trisaccharides are the 6-*O*-deacetylated trisaccharide).

2,3,4,6-*Tetra-O-benzoyl-β-D-glucopyranosyl-*(1→6)-[2, 3,4,6-*tetra-O-benzoyl-β-D-glucopyranosyl-*(1→3)]-2,4-*di-O-acetyl-β-D-glucopyranosyl-*(1 → 6)-2, 3, 4-*tri-O-acetyl-β-D-glucopyranosyl-*(1→6)-[2,3,4,6-*tetra-O-benzoyl-β-D-glucopyranosyl-*(1→3)]-5-*O-acetyl*-1, 2-*O-isopropylidine-β-D-glucopyranoside* (**14**) *and* 2,3,4,6-*Tetra-O-benzoyl-β-D-glucopyranosyl-*(1→3)-[2,3,4,6-*tetra-O-acetyl-β-D-glucopyranosyl-*(1→6)]-5-*O-acetyl*-1,2-*O-isopropylidene-α-D-glucofuranose* (**15**)

To a stirred solution of **13** (3.59 g, 3.3 mmol) and **10** (4.7 g, 3.0 mmol) in dry dichloromethane (60 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 20 μL, 0.1 mmol) at room temperature. After 3 h, triethylamine was added to the solution to quench the reaction. The solution was concentrated, and the residue was acetylated with acetic anhydride (40 mL) in pyridine (40 mL). After 3 h, the solution was concentrated to dryness. The residue was purified by column chromatography (1.5/1 petroleum ether-ethyl acetate) to afford the blocked hexasaccharide **14** (6.2 g, 81%) and the recovered trisaccharide **15** (0.4 g). For **14**: $[\alpha]_D$ + 21.8 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.08, 169.55, 169.46, 169.39, 169.09, 168.14 (6 × CH_3CO), 166.06, 166.06, 165.99, 165.80, 165.71, 165.58, 165.17, 165.12, 165.06, 165.06, 164.95, 164.41 (12 × PhCO), 112.09 ($C(CH_3)_2$), 104.90, 101.01, 101.01, 100.51, 100.44, 99.20 (6 × C-1), 82.24, 79.80, 78.22, 77.22, 76.04, 73.70, 73.31, 73.20, 72.87, 72.79, 72.71, 72.60, 72.38, 72.20, 71.92, 71.86, 71.79, 71.11, 69.65, 69.60, 69.37, 68.97, 68.79, 68.73, 68.34, 67.38, 67.05, 63.17, 63.04, 62.82 (6 × C-2, 3, 4, 5, 6), 26.55, 25.85 (2

× CCH_3), 20.73, 20.70, 20.60, 20.57, 20.54, 20.53 (6× CH_3CO). Anal calcd for $C_{135}H_{126}O_{49}$: C 64.03, H 5.01; found C 64.45, H 5.12. For **15**: $[\alpha]_D$ + 32.3 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.01—7.26 (m, 20H, 4× BzH), 5.90 (t, *J* = 9.7 Hz, 1H, H-4), 5.67 (t, *J* = 9.7 Hz, 1H, H-3), 5.44 (dd, *J* = 9.7, 8.0 Hz, 1H, H-2), 5.24 (d, *J* = 3.7 Hz, 1H, H-1), 5.13 (t, *J* = 9.7 Hz, 1H, H-4), 5.13 ~ 5.09 (m, 1H, H-5), 5.03 (t, *J* = 9.7 Hz, 1H, H-3), 4.99 (dd, *J* = 9.7, 8.0 Hz, 1H, H-2), 4.96 (d, *J* = 8.0 Hz, 1H, H-1), 4.55 (dd, *J* = 3.2, 11.5 Hz, 1H, H-6), 4.52 (d, *J* = 8.0 Hz, 1H, H-1), 4.48—4.06 (m, 8H), 3.78 (dd, *J* = 3.1, 11.6 Hz, 1H, H-6), 3.63—3.67 (m, 1H, H-5), 2.07, 2.02, 1.99, 1.97, 1.96 (5s, 15H, 5× CH_3CO), 1.46, 1.14 (2s, 6H, 2× CCH_3). Anal calcd for $C_{59}H_{62}O_{25}$: C 60.51, H 5.34; found C 60.09, H 5.41.

β-D-Glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-D-glucopyranose (**2**)

Compound **14** (5 g, 1.97 mmol) was dissolved in 80% acetic acid solution (100 mL) and the mixture was heated under reflux for 4 h. Concentration of the mixture followed by deacylation in a solution of CH_2Cl_2 (10 mL) and CH_3OH (90 mL) saturated with ammonia at room temperature for 24 h gave the target glucohexatose elicitor **2** as white powders (1.86 g, 95%); $[\alpha]_D$ − 39.1 (*c* 0.2, H_2O); ^{13}C NMR (100 MHz, D_2O) δ: 102.6 (C-1), 102.5 (C-1), 102.4 (C-1), 102.4 (C-1), 102.3 (C-1), 102.3 (C-1), 84.0 (C-3), 83.9 (C-3), 69.5 (C-6), 69.3 (C-6), 69.2 (C-6), 60.7 (C-6), 60.4 (C-6), 60.1 (C-6). ESMS for $C_{26}H_{62}O_{31}$(990.86): 989.7 $[M-1]^+$.

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