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An Efficient Synthesis of Methyl Tetra-*O*-hexyl Gentiooctaoside, an Octaosyl Analogue of ANP Receptor Antagonist HS-142-1†

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Abstract—Methyl *O*-(3-*O*-hexyl-β-D-glucopyranosyl)-(1→6)-[*O*-(β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-hexyl-β-D-glucopyranosyl)-(1→6)]$_3$-β-D-glucopyranoside, a 3-*O* hexyl analogue of the octaosyl component of fungal lipooligosaccharide HS-142-1, was stereo- and regioselectively synthesized as a potent antagonist for the tetrameric atrial natriuretic peptide (ANP) receptors.

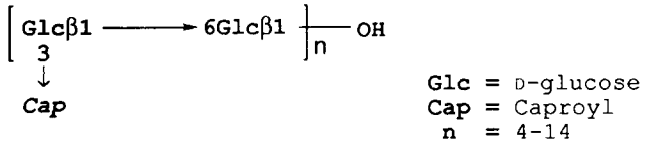

Figure 1. Putative structure of HS-142-1.

Introduction

During the screening for novel atrial natriuretic peptide (ANP) receptor ligands from microbial sources, HS-142-1 was discovered in the culture broth of *Aureobasidium pullulas* var. *melanigenum* and deduced to be a mixture of β 1→6 oligoglucosides (decasaccharides-triacontasaccharide) which are partly acylated with caproyl groups.[1] Based on the mass spectral analyses, it was suggested that the major components of HS-142-1 were esterified at the O-3 position of the every other glucose residue in the linear gentiooligosaccharide frameworks as shown in Figure 1.[2] This unique lipooligosaccharide has been demonstrated to be a specific antagonist for the tetramer-type receptors (NPR-A, NPR-B, and NPR-D) of natriuretic peptide,[3] a family of circulating hormones involved in the central and peripheral control of body fluid volume and blood pressure. Since isolation of a single homogeneous active constituent from the mixture has not been achieved, chemically synthesized homogeneous oligosaccharide-ligands would be desirable to use in the studies on molecular level mechanism in binding between HS-142-1 and ANP receptors. Furthermore, due to the lack of cytotoxicity, HS-142-1 would serve as a useful lead for developing novel drugs in the treatment of diseases derived from the excessive actions of the natriuretic peptides.[4]

We have previously reported[5] a synthesis of three gentiohexaosyl derivatives of HS-142-1 (**i**–**iii**) depicted in Figure 2. Those compounds were designed to elucidate the minimum structural requirements for the active oligosaccharides, and were synthesized via a common intermediate **iv**. Owing to the unstable property of 3-*O*-caproyl groups in the presence of the vicinal hydroxyl groups, compound **i** was obtained only as a mixture of tri-*O*-caproyl, di-*O*-caproyl, and mono-*O*-caproyl gentiohexaose derivatives, and exhibited no

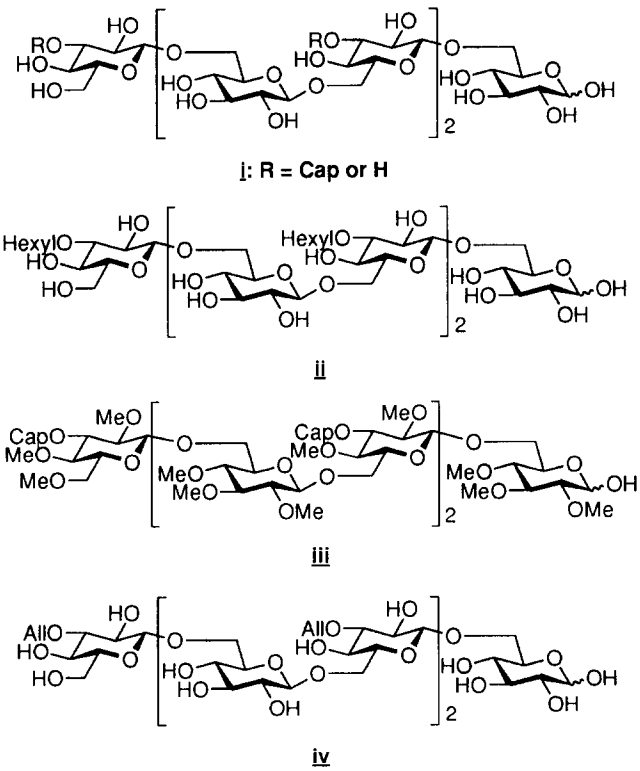

Figure 2. Synthetic gentiohexaosyl derivatives related to HS-142-1.

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†Synthetic studies on the polysaccharide HS-142-1, a novel nonpeptide antagonist for the atrial natriuretic peptide receptor, Part 3.

appreciable antagonist-activity to the ANP receptors. In contrast, its alkyl ether-type congener, tri-*O*-hexyl gentiohexaose (**ii**) was prepared as a stable single compound and demonstrated weak but certain antagonist-activity. Another stable gentiohexaoside (**iii**) masked with 16 methyl groups, however, did not show any activity. In this connection, it is to be noted that the *O*-methylated pentasaccharide analogues of heparin have been shown to possess an affinity for antithrombin III in intensity comparable to the non-methylated pentasaccharides.[6]

Observation of the biological activity of the synthetic glucoside **ii** prompted us to synthesize larger oligomers with 3-*O* hexyl groups, which might carry enhanced bioactivity. In this paper, we describe synthesis of a gentiooctaose with four 3-*O* hexyl substituents aiming at a mimic of the active HS-142-1 constituents.

Results and Discussion

Synthesis of the target compound **1** was first designed according to the synthetic route established previously, so that hexyl groups would be introduced after selective deallylation of compound **3** which would be obtainable by coupling of properly protected tetrasaccharide donor **6** and acceptor **5** as shown in Figure 3.

Tetrasaccharide **7**[5] was treated with trifluoroacetic acid[7] and the resulting hemiacetal was converted [Cl_3CCN, DBU(Cat.)] into a trichloroacetimidate **6** quantita-

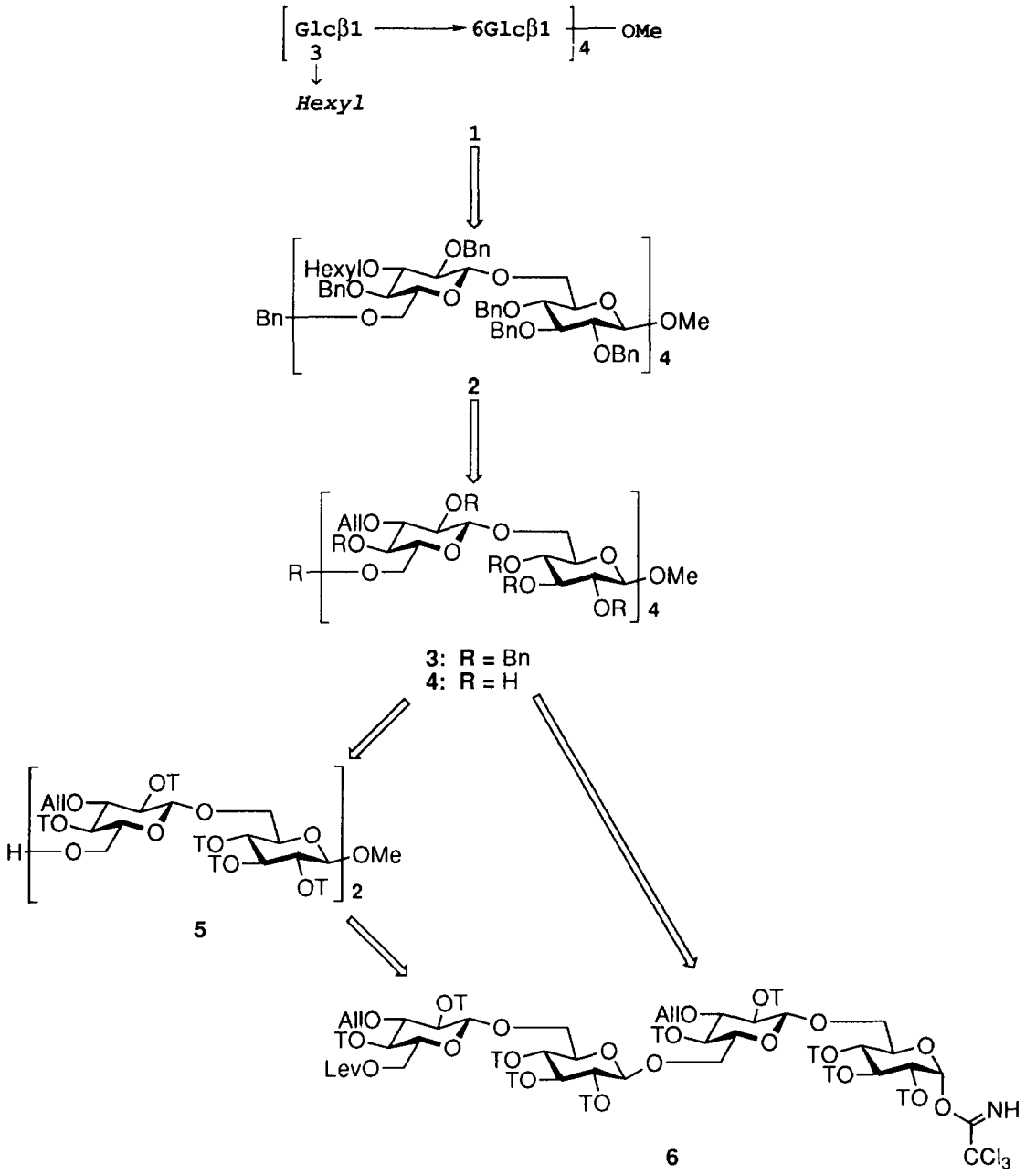


Figure 3. Synthetic strategy.

tively. Glycosylation with methanol was promoted by silver trifluoromethanesulfonate to afford **9** (82%), from which levulinoyl group was selectively removed by stirring at room temperature with methylhydrazine acetate[8] in the presence of acetic acid to give **5** (92%). No migration of toluoyl group was observed during this transformation.

Glycosylation of **5** with **6** (2 equivalents) was done with silver trifluoromethanesulfonate[9] to afford the gentiooctaosyl derivative **14** in 58% yield. Deacylation of **14** with a catalytic amount of sodium methoxide in THF:MeOH (1:1) followed by purificaion on Sephadex LH-20 afforded a henicosaol **4** in 75% yield. Benzylation of **4**, however, was not completed under the usual condition (NaH, BnBr, DMF), but gave a mixture of partly benzylated compounds even after the repeated benzylation.

This unexpected result compelled us to modify the synthetic route. We next examined the use of the pre-benzylated tetraose **13** as a glycosyl acceptor which was readily prepared in four steps from **5** [1. deacylation, 2. selective tritylation (TrCl, DMAP, Et_3N, DMF),[10] 3. benzylation (NaH, BnBr, DMF), 4. detritylation ($ZnBr_2$, $MeOH/CHCl_3$)[11]] in 46% yield.

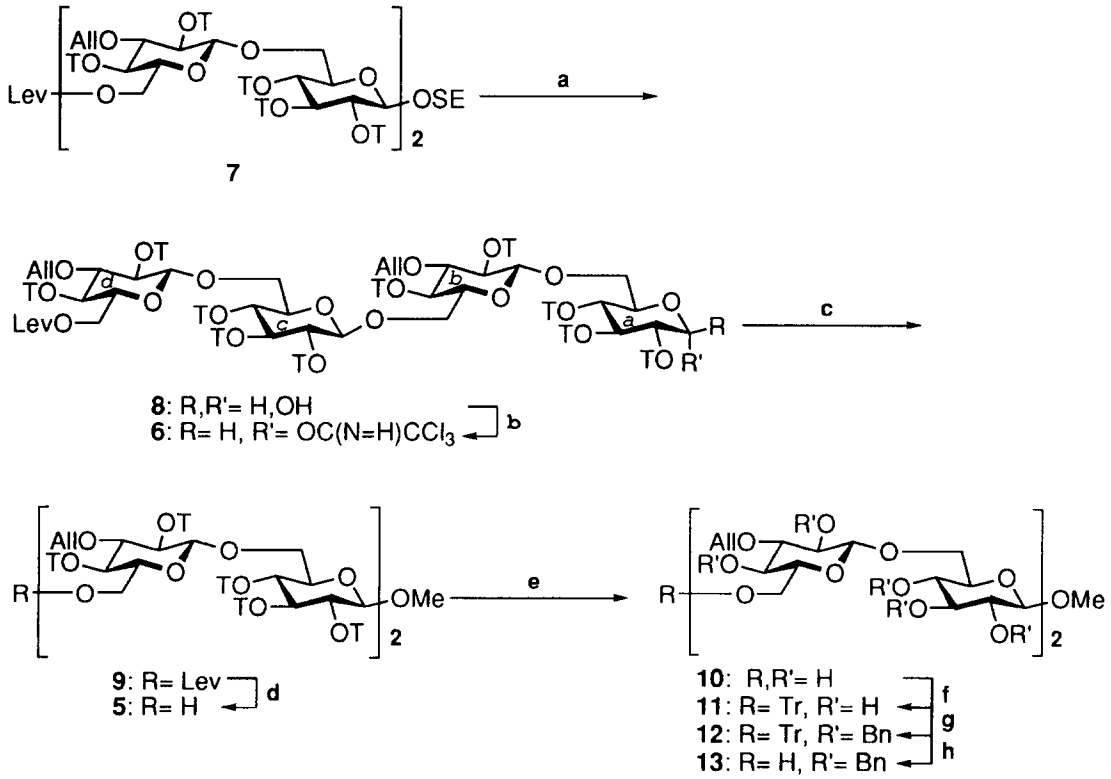
Glycosylation of **13** with **6** (2.4 equivalents) was promoted by a catalytic amount of trimethylsilyl trifluoromethanesulfonate[12] to afford a gentiooctaose **15** in 85% yield. Deacylation of **15** followed by benzylation proceeded smoothly to give **3** in 76% yield (2 steps).

Deallylation involving iridium complex-catalysed isomerization[13] and hydrolysis with mercury salt[14] in aqueous acetone gave a tetraol **17** (82%, 2 steps). Alkylation with hexyl bromide was performed with sodium hydride in DMF to give compound **2** in 91% yield.

Finally, catalytic hydrogenolysis of **2** with palladium hydroxide on carbon[15] and then purification by passing through a short reverse-phase column (Bond-elut C8) with MeOH, gave the target compound **1** in 77% yield. NMR and mass spectra of the synthetic sample were in good accordance with the postulated structure of **1**.

The activity of **1** was assayed by competitive inhibition of the radio-labeled rat ANP binding to rabbit kidney cortex membrane.[1,2] As a preliminary result, it turned out that compound **1** exhibited the inhibitory activity at a concentration approximately 30-fold higher than HS-142-1. The moderate increase in activity compared with tri-*O*-hexyl-gentiohexaose derivative synthesized previously was also observed. The detail of the bioassay will be described elsewhere.

In conclusion, methyl tetra-*O*-hexyl gentiooctaoside, an analogue for HS-142-1, was synthesized by a regio- and stereo-controlled sequence. Preliminary bioassay of the synthetic sample showed the antagonist-activity to the tetrameric ANP receptors. Further work is in progress



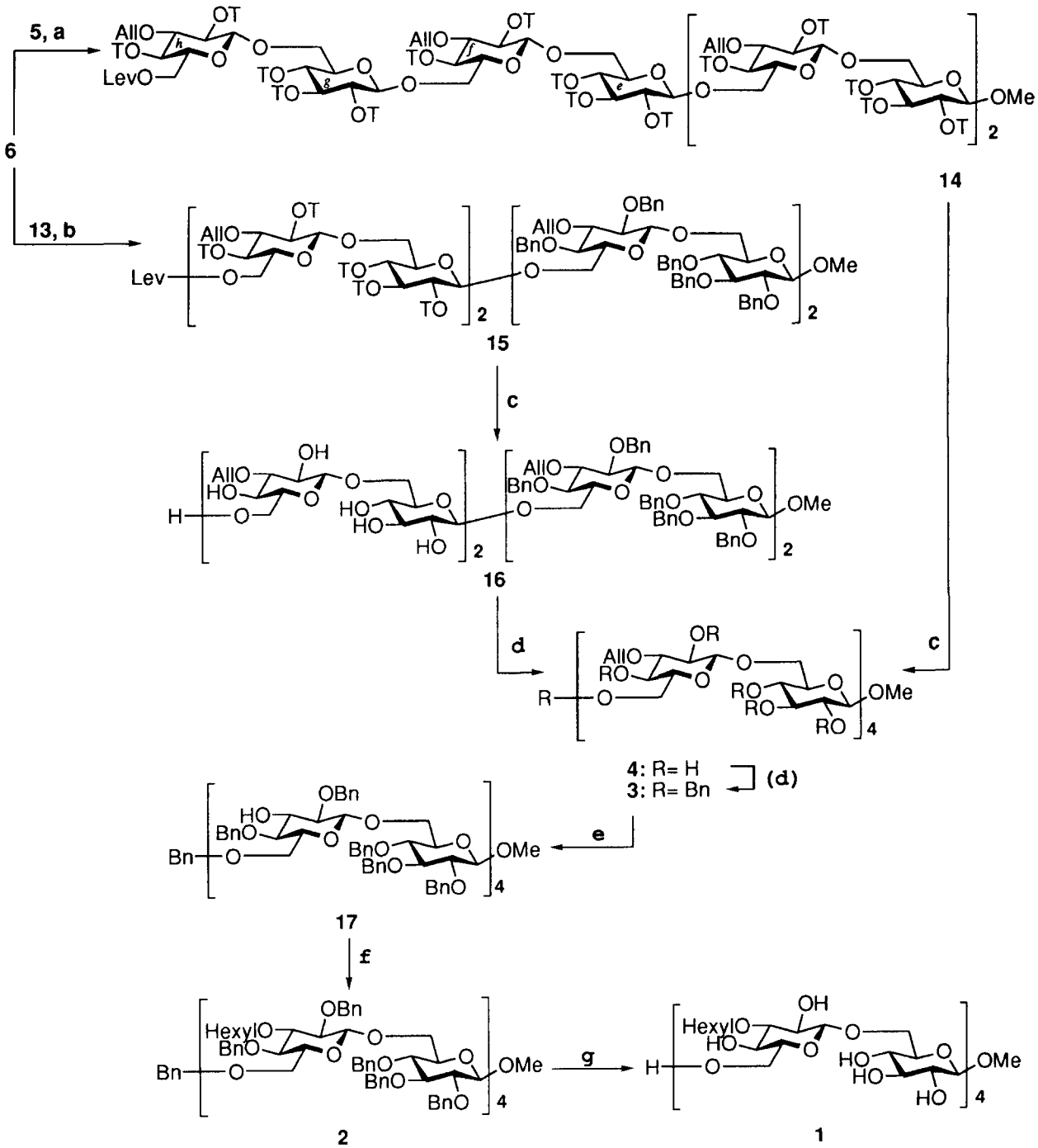
Scheme 1. Preparation of gentiotetraose synthons.

to synthesize larger size oligomers with high inhibitory potency.

Experimental

General methods

Melting points (mp) were measured with a Yanagimoto micro-melting-point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP 370 polarimeter at 25 ± 5 °C for solution in $CHCl_3$, unless otherwise stated. NMR spectra were recorded with a JEOL JNM-EX270 spectrometer or a Bruker AM400 spectrometer, and chemical shifts are given in ppm relative to internal standards. The following signals were used as the references: $(CH_3)_4Si$, δ 0.00 (^1H in $CDCl_3$); $CHCl_3$, δ 7.26 (^1H in $CDCl_3$) and δ 77.0 (^{13}C in $CDCl_3$); $(CH_3)_2CO$, δ 2.0 (^1H in $CDCl_3$); CD_3OD, δ 49.8 (^{13}C in CD_3OD); HDO, δ 4.7 (^1H in D_2O). Assignment of signals was performed based on 2D H-H, C-H COSY and DEPT. FAB mass spectra were measured with a JEOL JMS-HX110 mass spectrometer. TLC was performed on silica gel F_{254}



Scheme 2. Preparation of gentiotetraoside derivatives.

(Merck, Darmstadt, Germany) with detection by UV light and/or by charring with 10% sulfuric acid in ethanol. Flash chromatography was performed on silica gel [silica gel C-60 (230–400 mesh)]. Reverse-phase chromatography was performed on Bond Elut-C8 (Varian, Harbor City, CA, U.S.A.). Powdered molecular sieves were desiccated at 180 °C under vacuum overnight immediately prior to their use.

***O*-(3-*O*-Allyl-6-*O*-levulinoyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-toluoyl-α-D-glucopyranosyl trichloroacetimidate (6).** To a stirred soln of **7** (1.7 g, 0.79 mmol) in 1,2-dichloroethane (15 ml) was added TFA (10 ml) at room temperature. The mixture was stirred for 0.5 h and concd in vacuo to give hemiacetal **8** [R_f 0.21 (EtOAc:toluene, 1:4)], which was used for the next reaction without further purification. A catalytic amount of DBU (0.2 ml, 1.2 mmol) was added at 0 °C to a mixture of **8** and trichloroacetonitrile (2.4 ml, 24 mmol) in dichloromethane (25 ml), and the mixture was stirred at room temperature for 24 h. The mixture, without concn, was chromatographed on silica gel with EtOAc:toluene (1:4) afforded **6** (1.7 g, 100%, 2 steps) as a white solid. R_f 0.44 (EtOAc:toluene, 1/4). Mp 105–106 °C, $[\alpha]_D$ −21° (C 1.1).^{1}H NMR ($CDCl_3$): δ 8.25 (s, 1H, NH), 8.00–7.61 and 7.35–6.90 (2m, 40H, aromatic), 6.72 (d, 1H, J=3.6 Hz, H-1a), 6.16 and 5.77 (2t, 2H, J=9.9 Hz, H-3a, 3c), 5.61–5.41 and 5.35–4.88 (m, 4H, 11H, H-1, 2, 4, CH_2=CH), 4.63 (d, 1H, J=7.9 Hz, H-1), 4.56 (d, 1H, J=7.6 Hz, H-1), 4.23–3.74 [m, 15H, H-3b, 3d, 5a, 6, OCH_2 (All)], 3.62–3.53 (m, 3H, H-5b, 5c, 5d), 2.74–2.70 and 2.59–2.55 [2m, 4H, CH_2 (Lev)], 2.47, 2.40, 2.39, 2.37, 2.35, 2.33, 2.29, 2.26, 2.24 and 2.22 [10s, 30H, CH_3 (T)], 2.15 [s, 3H, CH_3 (Lev)].^{13}C NMR ($CDCl_3$): δ 206.4 [C=O (Lev)], 172.3 [OC=O (Lev)], 165.5–164.8 [OC=O (T)], 160.2 (C=NH), 144.1–143.5, 129.9–125.2 (aromatic), 134.3 [=CH (All)], 117.3, 117.2 [CH_2= (All)], 101.1, 100.5 (C-1b, 1c, 1d), 93.0 (Cl_3C), 90.7 (C-1a), 79.5, 79.4 (C-3b, 3d), 74.9–67.3 [C-2, 3a, 3c, 4, 5, 6, OCH_2 (All)], 63.0 (C-6d), 37.9, 28.0 [CH_2 (Lev)], 29.7 [CH_3 (Lev)], 21.6–21.5 [CH_3 (T)]. Anal. calcd for $C_{117}H_{116}O_{33}NCl_3 \cdot 3.5H_2O$: C, 62.91; H, 5.55; N, 0.63%. Found: C, 62.82; H, 5.27; N, 0.85.

Methyl *O*-(3-*O*-allyl-6-*O*-levulinoyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4-tri-*O*-toluoyl-β-D-glucopyranoside (9). Silver trifluoromethanesulfonate (100 mg, 0.40 mmol) was added to a stirred mixture of **6** (790 mg, 0.36 mmol) and methanol (0.1 ml) in dichloromethane (12 ml) at room temperature under an argon atmosphere, and the mixture was stirred in the dark for 48 h. Without work-up, the reaction mixture was chromatographed on silica gel with EtOAc:toluene (1:4) to afford **9** (236 mg, 82%) as a syrup. R_f 0.41 (EtOAc:toluene, 1:4). $[\alpha]_D$ −41° (*c* 1.0). ^{1}H NMR ($CDCl_3$): δ 8.04–7.62 and 7.40–6.94 (2m, 40H, aromatic), 5.75, 5.63 (2t, 2H, J=9.6 Hz, H-3a, 3c), 5.66–5.50 [m, 2H, =CH (All)], 5.37–5.20 (m, 6H, H-2, 4), 5.07–4.91 [m, 6H, H-1, 2, CH_2= (All)], 4.75 (t, 1H, J=9.2 Hz, H-4), 4.66, 4.47, 4.45 (3d, 3H, J=7.9 Hz, H-1), 4.24 (d, 2H, J=4.0 Hz, H-6d), 4.14–3.42 [m, 17H, H-3b, 3d, 5, 6, OCH_2 (All)], 3.17 (s, 3H, OCH_3), 2.77–2.70 and 2.61–2.56 [2m, 4H, CH_2 (Lev)], 2.49, 2.41, 2.40, 2.38, 2.35, 2.35, 2.32, 2.29, 2.27 and 2.23 [10s, 30H, CH_3 (T)], 2.15 [s, 3H, CH_3 (Lev)]. ^{13}C NMR ($CDCl_3$): δ 206.5 [C=O (Lev)], 172.4 [OC=O (Lev)], 165.7–165.0 [C=O (T)], 144.5–143.7, 130.1–125.9 (aromatic), 134.4 [=CH (All)], 117.4, 117.3 [CH_2= (All)], 101.7, 101.5, 101.3, 100.3 (C-1), 79.4, 79.3 (C-3b, 3d), 77.2–69.0 (C-2, 3a, 3c, 4, 5), 73.5, 73.2 [OCH_2 (All)], 68.3, 67.8 (C-6), 63.1 (C-6d), 56.5 (OCH_3), 38.0, 28.0 [CH_2 (Lev)], 29.8 [CH_3 (Lev)], 21.8–21.5 [CH_3 (T)]. Anal. calcd for $C_{116}H_{118}O_{33} \cdot 0.5H_2O$: C, 67.99; H, 5.85. Found: C, 67.84; H, 5.79.

Methyl *O*-(3-*O*-allyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4-tri-*O*-toluoyl-β-D-glucopyranoside (5). Methylhydrazine acetate (57 mg, 0.54 mmol) in AcOH (0.5 ml) was added to a soln of **9** (560 mg, 0.27 mmol) in toluene (5 ml). After stirring at room temperature for 2 h, the mixture was diluted with EtOAc, washed with water, satd aq $NaHCO_3$, and brine, dried and concd. Purification of the crude product by flash chromatography on silica gel with EtOAc:$CHCl_3$ (1:9) gave **5** (489 mg, 92%) as a white solid. R_f 0.23 (EtOAc:$CHCl_3$, 1:9). Mp 134–135 °C. $[\alpha]_D$ −52° (*c* 1.0). ^{1}H NMR ($CDCl_3$): δ 8.00–7.62 and 7.37–6.93 (2m, 40H, aromatic), 5.75 and 5.68 (2t, 2H, J=9.57 Hz, H-3a, 3c), 5.62–5.49 [m, 2H, =CH (All)], 5.38–4.82 [m, 13H, H-1, 2, 4, CH_2= (All)], 4.72 and 4.48 (2d, 2H, J=7.9 Hz, H-1), 4.52 (d, 1H, J=7.6 Hz, H-1), 4.13–3.48 [m, 18H, H-3b, 3d, 5, 6, OCH_2 (All)], 3.18 (s, 3H, OCH_3), 2.91 (q, 1H, J=5.3, 9.2 Hz, OH), 2.48, 2.45, 2.41, 2.40, 2.39, 2.35, 2.35, 2.34, 2.28 and 2.23 [10s, 30H, CH_3 (T)]. ^{13}C NMR ($CDCl_3$): δ 166.0, 165.7, 165.6, 165.5, 165.46, 165.4, 165.2, 165.1, 165.0, 164.9 [C=O (T)], 144.4–143.7, 130.1–125.3 (aromatic), 134.4, 134.3 [=CH (All)], 117.3 [CH_2= (All)], 101.7, 101.4, 100.8, 100.3 (C-1), 79.5, 79.4 (C-3b, 3d), 77.2–67.5 [C-2, 3a, 3c, 4, 5, 6, OCH_2 (All)], 61.5 (C-6d), 56.5 (OCH_3), 21.7–21.4 [CH_3 (T)]. Anal. calcd for $C_{111}H_{112}O_{31} \cdot H_2O$: C, 68.01; H, 5.86. Found: C, 68.06; H, 5.76.

Methyl *O*-(3-*O*-allyl-2,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2, 4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (13). To a soln of **5** (219 mg, 0.11 mmol) in MeOH:THF (2 ml:2 ml) was added 0.1 N methanoic sodium methoxide (2 ml). The mixture was stirred at room temperature for 24 h, neutralized with Amberlyst 15, and concd. Purification of the crude product by gel filtration chromatography on Sephadex LH-20 with MeOH afforded **10** [R_f 0.23 (MeOH:$CHCl_3$=2:3)]. To a mixture of **10**, DMAP (15 mg, 0.12 mmol) and triethylamine (0.24 ml, 1.7 mmol) in

DMF (2 ml) was added trityl chloride (190 mg, 0.67 mmol) at room temperature and the mixture was stirred for 24 h. The mixture was chromatographed on Sephadex LH-20 with MeOH to afford **11** [R_f 0.36 (MeOH:$CHCl_3$, 1:3)]. To a soln of **11** in DMF (2 ml) was added portionwise NaH (200 mg, 5 mmol, 60% in mineral oil) at 0 °C under an argon atmosphere and the mixture was stirred for 0.5 h. Benzyl bromide (0.4 ml, 3.3 mmol) was added at 0 °C and the reaction mixture was stirred at room temperature for 24 h. Excess reagents were destroyed by carful addition of ice water, and the mixture was concd in vacuo. The residure was purified by gel permeation chromatography on Bio-beads S-X1 with toluene to afford **12**. To a soln of **12** in MeOH:$CHCl_3$ (2.5 ml:2.5 ml) was added zinc bromide (500 mg, 2.2 mmol) at room temperature for 2.5 h, and concd. Purification of the crude product by flash chromatography on silica gel with EtOAc:toluene (1:4) afforded **13** (87 mg, 46%, 4 steps) as a colorless syrup.

12: R_f 0.27 (EtOAc:toluene, 1:19)]. ^{1}H NMR ($CDCl_3$): δ 7.54–6.89 [m, 65H, aromatic (Bn, Tr), 5.95–5.86 [m, 2H, =C<u>H</u> (All)], 5.27–5.09 [m, 4H, C<u>H</u>$_2$= (All)], 5.03–4.58 [m, 18H, OC<u>H</u>$_2$ (Bn)], 4.53 (d, 1H, J=7.3Hz, H-1), 4.43–4.15 [m, 12H, H-1, 6, OC<u>H</u>$_2$ (All, Bn)], 3.80–3.19 (m, 24H, H-2, 3, 4, 5, 6, OC<u>H</u>$_3$).^{13}C NMR ($CDCl_3$): δ 143.9–137.9, 128.8–126.7 (aromatic), 135.0 [=<u>C</u>H (All)], 116.8, 116.4 [<u>C</u>H$_2$= (All)], 104.5–103.6 (C-1), 86.3 (Ph_3C), 84.7–74.3 [C-2, 3, 4, 5, O<u>C</u>H$_2$ (All, Bn)], 68.4–68.3 (C-6a, 6b, 6c), 62.3 (C-6d), 57.0 (O<u>C</u>H$_3$).

13: R_f 0.17 (EtOAc:toluene, 3:17)]. [α]$_D$+7° (c 0.9).^{1}H NMR ($CDCl_3$): δ 7.31–7.13 (m, 50H, aromatic), 5.96–5.86 [m, 2H, =C<u>H</u> (All)], 5.26–5.10 [m, 4H, C<u>H</u>$_2$= (All)], 4.98–4.03 [m, 31H, H-1, 6, OC<u>H</u>$_2$ (All, Bn)], 3.82–3.27 (m, 21H, H-2, 3, 4, 5, 6), 3.43 (s, 3H, OC<u>H</u>$_3$).^{13}C NMR ($CDCl_3$): δ 138.6–138.1, 128.4–127.4 (aromatic), 135.1 [=<u>C</u>H (All)], 116.6, 116.5 [<u>C</u>H$_2$= (All)], 104.5, 104.0, 103.6 (C-1), 84.7–74.3 [C-2, 3, 4, 5, O<u>C</u>H$_2$ (All, Bn)], 69.1, 68.7, 68.4 (C-6a, 6b, 6c), 61.9 (C-6d), 57.1 (O<u>C</u>H$_3$). Positive FAB-MS [matrix=NBA] *m/z* 1684.1 [M+Na]$^+$.

Methyl *O*-(3-*O*-allyl-6-*O*-levulinoyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-[(2,3,4-tri-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)]$_3$-*O*-2,3,4-tri-*O*-toluoyl-β-D-glucopyranoside (14). Silver trifluoromethanesulfonate (103 mg, 0.40 mmol) was added to a stirred mixture of **6** (803 mg, 0.37 mmol) and **5** (326 mg, 0.17 mmol) in dichloromethane (10 ml) at room temperature under an argon atmosphere, and stirred in the dark for 48 h. The mixture, without concentration, was chromatographed on silica gel with EtOAc:$CHCl_3$ (1:3) and gel permeation chromatography on Bio-beads S-X1 with toluene afforded **14** (380 mg, 58%) as white crystals. R_f 0.22 (acetone:n-hexane, 2:3). Mp. 226–227 °C. [α]$_D$−43° (*c* 1.1).^{1}H NMR ($CDCl_3$): δ 8.09–7.61 and 7.39–6.86 (2m, 80H, aromatic), 5.82–4.64 (m, 36H, H-1, 2, 3a, 3c, 3e, 3g, 4, C<u>H</u>$_2$=C<u>H</u>), 4.55, 4.50 and 4.49 (3d, 3H, J=7.9 Hz, H-1), 4.42 (d, 1H, J=7.6 Hz, H-1), 4.24–3.47 [m, 36H, H-3b, 3d, 3f, 3h, 5, 6, OC<u>H</u>$_2$ (All)], 3.19 (s, 3H, OC<u>H</u>$_3$), 2.74–2.71 and 2.62–2.60 [2m, 4H, C<u>H</u>$_2$ (Lev)], 2.49–2.19 [singles, 60H, C<u>H</u>$_3$ (T)], 2.15 [s, 3H, C<u>H</u>$_3$ (Lev)].^{13}C NMR ($CDCl_3$): δ 206.3 [<u>C</u>=O (Lev)], 172.2 [O<u>C</u>=O (Lev)], 165.5–164.6 [<u>C</u>=O (T)], 144.2–143.4, 130.3–125.1 (aromatic), 134.4, 134.3, 134.2, 134.1 [=<u>C</u>H (All)], 117.2, 117.1, 117.0, 116.9 [<u>C</u>H$_2$= (All)], 101.5–100.1 (C-1), 79.3–67.6 [C-2, 3, 4, 5, 6, O<u>C</u>H$_2$ (All)], 62.9 (C-6h), 56.1 (O<u>C</u>H$_3$), 37.8, 27.9 [<u>C</u>H$_2$ (Lev)], 29.6 [<u>C</u>H$_3$ (Lev)], 21.5–21.3 [<u>C</u>H$_3$ (T)]. Anal. calcd for $C_{226}H_{226}O_{63}$: C, 68.72; H, 5.77. Found: C, 68.99; H, 5.75.

Methyl *O*-(3-*O*-allyl-β-D-glucopyranosyl)-(1→6)-[*O*-(β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-β-D-glucopyranosyl)-(1→6)]$_3$-*O*-β-D-glucopyranoside (4). To a soln of **14** (295 mg, 74 μmol) in MeOH:THF (1 ml:1 ml) was added 0.1 N methanoic sodium methoxide (0.5 ml). The mixture was stirred at room temperature for 24 h, neutralized with Amberlyst 15, and concd. Purification of the crude product by gel filtration chromatography on Sephadex LH-20 with MeOH afforded **4** (82.3 mg, 75%) as a white powder. R_f 0.24 (MeOH:$CHCl_3$:H_2O, 4:5:1). [α]$_D$−52 ° (<u>C</u> 0.4, MeOH).^{1}H NMR (D_2O): δ 5.97–5.82 [m, 4H, =C<u>H</u> (All)], 5.29–5.14 [m, 8H, C<u>H</u>$_2$= (All)], 4.44–4.40 (m, 7H, H-1), 4.30–4.24 (m, 9H, H-1, 6), 4.14–4.09 (m, 8H, H-6), 3.84–3.72 [m, 8H, OC<u>H</u>$_2$ (All)], 3.66–3.13 (m, 32H, H-2, 3, 4, 5), 3.47 (s, 3H, OC<u>H</u>$_3$).^{13}C-NMR (D_2O): δ 134.2 [=<u>C</u>H (All)], 118.6 [<u>C</u>H$_2$= (All)], 103.4–102.9 (C-1), 83.4, 83.3 (C-3b, 3d, 3f, 3h), 75.8–68.8 [C-2, 3a, 3c, 3e, 3g, 4, 5, 6, O<u>C</u>H$_2$ (All)], 60.7 (C-6h), 57.5 (O<u>C</u>H$_3$). Positive FAB-MS [matrix=glycerol] m/z 1511.4 [M+Na]$^+$. Anal. calcd for $C_{61}H_{100}O_{41}\cdot 4H_2O$: C, 46.92; H, 6.97. Found: C, 46.93; H, 6.59.

Methyl *O*-(3-*O*-allyl-6-*O*-levulinoyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4-tri-*O*-toluoyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (15). To a stirred soln of **6** (80 mg, 37 μmol), **13** (25 mg, 15 μmol) and MS 4A (150 mg) in dichloromethane (2 ml) at −78 °C was added dropwise TMSOTf [0.1 ml (0.1 M soln in dichloromethane)]. After 1.5 h, the mixture was diluted with $CHCl_3$ (5 ml), neutralized with satd aq $NaHCO_3$ (0.5 ml), filtered through Celite, and concd. Purification of the crude product by gel permeation chromatography on Bio-beads S-X1 with THF:$CHCl_3$ (5:1) afforded **15** (46.6 mg, 85%) as a syrup. R_f 0.76 (EtOAc:$CHCl_3$, 1:9). [α]$_D$−38° (*c* 0.8).^{1}H NMR ($CDCl_3$): δ 8.04–7.60 [m, 20H, aromatic (T)], 7.40–6.95 [m, 70H, aromatic (Bn, T)], 5.90–4.84 [m, 26H, H-2e, 2f, 2g, 2h, 3e, 3g, 4c, 4f, 4g, 4h, =C<u>H</u> (All), OC<u>H</u>$_2$ (Bn)], 4.76–4.28 [m, 26H, H-1, 6h, C<u>H</u>$_2$= (All), OC<u>H</u>$_2$ (Bn)], 4.23–3.19 [m, 44H, H-2a, 2b, 2c, 2d, 3a, 3b, 3c, 3d, 3f, 3h, 4a, 4b, 4c, 4d, 5, 6, OC<u>H</u>$_2$ (All)], 3.42 (s, 3H, OC<u>H</u>$_3$), 2.75–2.70 and

2.59–2.55 [2m, 4H, $C\underline{H}_2$ (Lev)], 2.49, 2.40, 2.37, 2.35, 2.31, 2.30, 2.26, 2.24, 2.23 and 2.15 [10s, 30H, $C\underline{H}_3$ (T)], 2.14 [s, 3H, $C\underline{H}_3$ (Lev)].^{13}C-NMR ($CDCl_3$): δ 206.5 [$\underline{C}$=O (Lev)], 172.4 [O$\underline{C}$=O (Lev)], 165.7–164.8 [$\underline{C}$=O (T)], 144.5–125.0 [aromatic, =$\underline{C}$H (All)], 117.5–116.0 [$\underline{C}H_2$= (All)], 104.6, 104.1, 104.0, 103.8 (C-1a, 1b, 1c, 1d), 101,3, 101.2, 101.1, 100.1 (C-1e, 1f, 1g, 1h), 84.7–67.5 [C-2, 3, 4, 5, 6, O$\underline{C}H_2$ (All, Bn)], 63.0 (C-6h), 57.2 (O$\underline{C}H_3$), 38.0, 28.0 [$\underline{C}H_2$(Lev)], 29.8 [$\underline{C}H_3$ (Lev)], 21.7–21.5 [$\underline{C}H_3$ (T)]. Positive FAB-MS [matrix = NBA] m/z 3692.3 $[M+Na]^+$.

Methyl *O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-[*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-allyl-2,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)]$_3$-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (3). To a soln of **15** (47 mg, 13 μmol) in MeOH:THF (0.5 ml:0.5 ml) was added 0.1 N methanoic sodium methoxide (0.1 ml). The mixture was stirred at room temperature for 24 h, neutralized with Amberlyst 15, and concd. Purification of the crude product by gel filtration chromatography on Sephadex LH-20 with MeOH afforded **16** [R_f 0.20 (MeOH:$CHCl_3$, 3:17)]. To a soln of **16** in DMF (2 ml) was added portionwise NaH (40 mg, 1 mmol, 60% in mineral oil) at 0 °C under argon and the mixture was stirred for 0.5 h. Benzyl bromide (80 μl, 0.7 mmol) was added at 0 °C and the reaction mixture stirred at room temperature for 24 h. Excess reagents were destroyed by careful addition of ice water, and the mixture was concd in vacuo. The residue was purified by gel permeation chromatography on Bio-beads S-X1 with toluene to afford **3** (33.6 mg, 76%, 2 steps) as a syrup. R_f 0.26 (EtOAc:toluene, 1:9). $[\alpha]_D$ +25° (*c* 0.7).^{1}H-NMR ($CDCl_3$): δ 7.31–7.00 [m, 105H, aromatic (Bn)], 5.82–5.85 [m, 4H, =C$\underline{H}$ (All)], 5.21–5.10 [m, 8H, $C\underline{H}_2$= (All)], 5.01–3.90 [m, 65H, H-1, 6, O$C\underline{H}_2$ (All, Bn)], 3.80–3.31 (m, 41H, H-2, 3, 4, 5, 6), 3.48 (s, 3H, O$C\underline{H}_3$).^{13}C-NMR ($CDCl_3$): δ 138.9–137.9, 129.7–127.3 (aromatic), 135.3, 135.2 [=$\underline{C}$H (All)], 116.4–115.7 [$\underline{C}H_2$= (All)], 104.4–103.7 (C-1), 85.0–73.3 [C-2, 3, 4, 5, O$\underline{C}H_2$ (Bn)], 70.1–68.9 (C-6), 57.2 (O$\underline{C}H_3$). Anal. calcd for $C_{208}H_{226}O_{41}\cdot 2H_2O$: C, 73.09; H, 6.78. Found: C, 72.98; H, 6.65.

Methyl *O*-(2,4,6-tri-*O*-benzyl-3-*O*-hexyl-β-D-glucopyranosyl)-(1→6)-[*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,4-di-*O*-benzyl-3-*O*-hexyl-β-D-glucopyranosyl)-(1→6)]$_3$-2,3,4-tri-O-benzyl-β-D-glucopyranoside (2). A soln of 1,5-cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (5 mg) in THF (1 ml) was degassed and actived in a hydrogen atmosphere, and then degassed. A soln of **3** (25 mg, 7 μmol) in THF (2 ml) was added to the above solution of the catalyst at room temperature under argon and the mixture was stirred for 2 h, then concd to give the 1-propenyl compound [R_f 0.38 (EtOAc:toluene, 3:4)]. The residue was dissolved in acetone:water (1 ml:0.1 ml), stirred with HgO (20 mg, 90 μmol) and $HgCl_2$ (20 mg, 50 μmol) for 24 h at room temperature, and then filtered through Celite. The filtrate was diluted with EtOAc (25 ml) and successively washed with 1 M KI and water, dried, and concd. Purification of the crude product by flash chromatography on silica gel with EtOAc:toluene (1:1) afforded **17** (18.6 mg, 82%, 2 steps) as a syrup. To a soln of **17** (6.5 mg, 2 μmol) in DMF (1 ml) was added portionwise NaH (20 mg, 0.5 mmol, 60% in mineral oil) at 0 °C under argon and stirred for 0.5 h. Hexyl bromide (0.1 ml) was added at 0 °C. and the reaction mixture was stirred at room temperature for 24 h. Excess reagents were destroyed by careful addition of ice water, and the mixture was concd in vacuo. The residue was purified by gel permeation chromatography on Bio-beads S-X1 with toluene to afford **2** (6.5 mg, 91%) as a syrup.

17: R_f 0.28 (EtOAc:toluene, 1:3). $[\alpha]_D$ +17° (*c* 0.5).^{1}H NMR ($CDCl_3$): δ 7.25–7.21 [m, 105H, aromatic (Bn)], 4.97–4.03 [m, 57H, H-1, 6, O$C\underline{H}_2$ (Bn)], 3.66–3.18 (m, 41H, H-2, 3, 4, 5, 6), 3.45 (s, 3H, O$C\underline{H}_3$).

2: R_f 0.47 (EtOAc:toluene, 3:17). $[\alpha]_D$ +20° (*c* 0.8).^{1}H NMR ($CDCl_3$): δ 7.70–7.04 [m, 105H, aromatic (Bn)], 4.98–4.10 [m, 55H, H-1, 6, O$C\underline{H}_2$ (Bn)], 3.99–3.23 [m, 51H, H-2, 3, 4, 5, 6, O$C\underline{H}_2$ (Hexyl)], 3.47 (s, 3H, O$C\underline{H}_3$), 1.55–1.52 [m, 8H, $C\underline{H}_2$ (Hexyl)], 1.25–1.22 [m, 24H, $C\underline{H}_2$ (Hexyl)], 0.83 [t, 12H, J = 3.6 Hz, $C\underline{H}_3$ (Hexyl)].^{13}C NMR ($CDCl_3$): δ 138.7–138.0, 129.1–127.7 (aromatic), 104.5, 104.4, 104.3, 104.1, 104.0, 103.9, 103.8, 103.7 (C-1), 85.8–68.6 [C-2, 3, 4, 5, 6, O$\underline{C}H_2$ (Bn, Hexyl)], 57.2 (O$\underline{C}H_3$), 31.8–22.5 [$\underline{C}H_2$ (Hexyl)], 14.1 [$\underline{C}H_3$(Hexyl)]. Anal. calcd for $C_{220}H_{258}O_{41}\cdot 3.5$ H_2O: C, 72.96; H, 7.37. Found: C, 72.82; H, 7.19.

Methyl *O*-(3-*O*-hexyl-β-D-glucopyranosyl)-(1→6)-[*O*-(β-D-glucopyranosyl)-(1→6)-*O*-(3-*O*-hexyl-β-D-glucopyranosyl)-(1→6)]$_3$-β-D-glucopyranoside (1). A soln of **2** (6.5 mg, 1.8 μmol) in EtOAc:MeOH:H_2O (0.5 ml:0.5 ml:0.5 ml) was hydrogenated with $Pd(OH)_2$:C (20%, 3 mg) at room temperature overnight. The catalyst was removed by filtration before concn. Purification of the crude product by reverse-phase chromatography on Bound Elut-C8 with water then methanol, and lyophilization afforded **1** (2.3 mg, 77%) as a powder. R_f 0.10 (H_2O:MeOH:$CHCl_3$, 1:3:6).^{1}H NMR (CD_3OD): δ 4.81–4.28 (m, 7H, H-1), 4.17–4.04 (m, 8H, H-1, 6), 3.71–3.49 [m, 17H, H-6, O$C\underline{H}_2$ (Hexyl)], 3.44 (s, 3H, O$C\underline{H}_3$), 3.42–3.07 (m, 32H, H-2, 3, 4, 5), 1.53–1.50 [m, 8H, $C\underline{H}_2$ (Hexyl)], 1.30–1.19 [m, 24H, $C\underline{H}_2$ (Hexyl)], 0.81 [t, 12H, J = 6.8 Hz, $C\underline{H}_3$ (Hexyl)]. Positive FAB-MS [matrix = glycerol] *m/z* 1687.8 $[M+Na]^+$, 1703.9 $[M+K]^+$.

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