Two-directional, convergent synthesis of a pentasaccharide that is involved in the hyperacute rejection response in xenotransplantation from pig to man

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A novel, highly convergent, regioselective glycosylation methodology based on differences in reactivity between hydroxy groups of a glycosyl donor and an acceptor has been employed for the synthesis of a pentasaccharide that is involved in the hyperacute rejection response in xenotransplantation from pig to man.

Introduction

Nowadays, organ transplantation in clinical practice has become a common means to treat the operational failure of many vital physiological processes. Unfortunately, the feasibility of this therapy is seriously limited due to the increasing shortage of donor organs suitable for such clinical application. Although in principle non-human primates provide the least immunological barrier associated with xenotransplantation, the number of fully functional primate organs that could be obtained is also far short of the number needed. To alleviate this shortage, the xenotransplantation practice is increasingly resorting to other readily available animal sources (*i.e.* pigs' organs). However, unlike allografts, xenografts from 'discordant' species, such as pigs, are rejected hyperacutely—usually within minutes. Therefore, the suppression of xenograft hyperacute rejection (HAR), which is usually triggered by xenoreactive natural antibodies (XNAs) in human sera, is considered a key precautionary procedure in the process of xenotransplantation.

Cooper[1] and Good *et al.*[2] reported that oligosaccharides containing a non-reducing terminal α(1→3) galactose residue show by far the highest affinity with human anti-pig antibodies. This anti-Gal-α-(1→3)-Gal antibody specificity appeared to represent the most significant human anti-pig carbohydrate antibodies.[3] A major glycosphingolipid in rabbit red blood cells membrane ceramide pentahexoside, which contains a pentasaccharide residue (Fig. 1), binds specifically with human anti-gal antibody.[4] It has been proposed that the pentasaccharide of compound **I** serves as the binding site for human anti-pig antibodies.

Results and discussion

We report here a highly convergent synthesis of the methyl glycoside of the pentasaccharide of compound **I**.[5] The latter was constructed by a crucial glycosylation between partially protected glycosyl donor **8** and acceptor **9** to give the trisaccharide **10**, which immediately was glycosidated with donor **6**, containing the prerequisite α-Gal(1→3)-Gal unit bearing an anomeric thioethyl group, to give the fully protected pentasaccharide **11**.

The chemical synthesis of complex oligosaccharides requires highly convergent strategies in which well designed glycosyl donors and acceptors are assembled by involving a minimum of synthetic steps and protecting-group manipulations.[6] Recently, we reported that regioselective glycosylations between glycosyl donors and acceptors both containing a free hydroxy group may provide di- and tri-saccharides that can be used as glycosyl acceptors in subsequent glycosylations without the need to perform protecting-group manipulations.[7] In combination with the previously reported chemoselective glycosylations[8] this methodology provides a powerful method to assemble oligosaccharides in a highly convergent manner avoiding protecting-group manipulations at the oligosaccharide stage. A prerequisite of the novel regioselective glycosylations is that the hydroxy functionality of the acceptor is sufficiently more reactive than the hydroxy group of the glycosyl donor and differences in reactivity may be achieved by primary *vs.* secondary or equatorially *vs.* axial disposition of the hydroxy groups. In addition, the reactivity can be tuned further by the nature of the protecting groups present, and hydroxy groups in sugars partially protected with alkyl groups are in general more reactive than those partially acylated.

We envisaged that the reactivity of 3′-OH of partially benzylated lactoside **2**[9] is much higher than its 4′-OH and that of the 4-OH of partially benzoylated amino sugar **1**. Indeed, *N*-iodosuccinimide (NIS) and a catalytic amount of trimethylsilyl triflate (TMSOTf)-mediated glycosylation between substrates **1**[10] and **2** afforded trisaccharide **3** in 56% yield (Scheme 1). The stereochemical outcome resulted from the neighbouring-group participation of the phthalimido group at C-2 of the donor. Mass spectroscopic analysis [matrix-isolated laser-desorption time-of-flight (MALDI-TOF)] of the crude reaction mixture indicated that the self-condensation or oligomerisation of compound **1** had not occurred. Disaccharide **6** was obtained by a classical armed–disarmed chemoselective glycosylation.[8b] Thus, iodonium dicollidine perchlorate (IDCP)-mediated glycosyl-

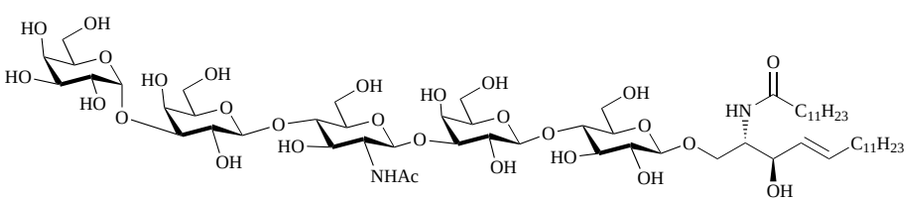
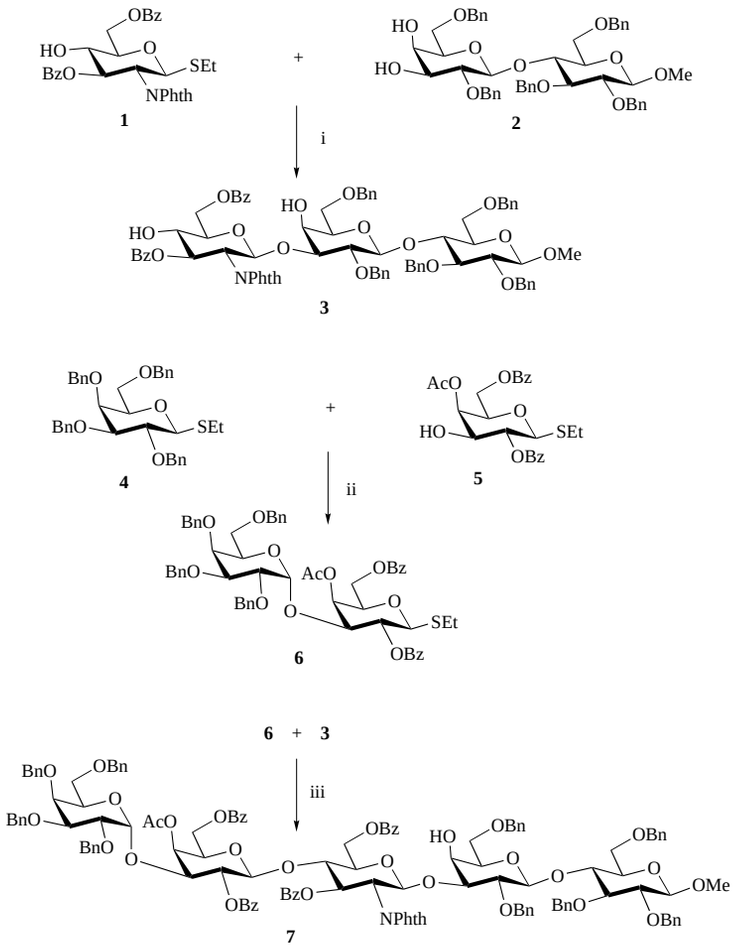


Fig. 1 Ceramide pentahexoside **I**



Scheme 1 *Reagents and conditions*: (i) NIS–TMSOTf, CH_2Cl_2, 4 Å mol. sieves, −15 °C (56%); (ii) IDCP, toluene–1,4-dioxane (1:3, v/v), 4 Å mol. sieves (67%); (iii) NIS–TMSOTf, CH_2Cl_2, 4 Å mol. sieves, 0 °C

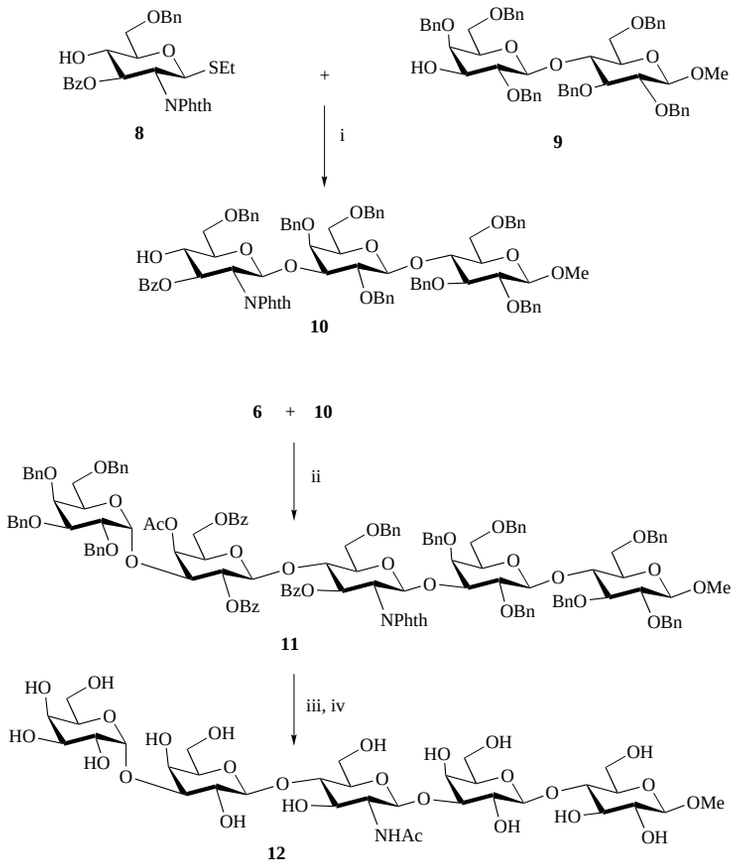
ation between 'armed' thioglycosyl donor **4** and 'disarmed' glycosyl acceptor **5**[11] in a novel solvent system (toluene–1,4-dioxane 1:3, v/v)[12] afforded compound **6** as the only anomer, in good yield (67%) (Scheme 1), whereas performing this glycosylation in dichloromethane–diethyl ether (1:5, v/v) produced an anomeric mixture of disaccharides (α:β 10:1). Coupling of compounds **6** and **3** in the presence of NIS–TMSOTf gave the desired pentasaccharide **7** in a modest 41% yield; however, the product was contaminated with small amounts of a regioisomer and a heptasaccharide which was formed by the condensation of compound **6** with both hydroxy groups of compound **3**. We rationalised that the low reactivity of 4″-OH in **3**, which was dramatically deactivated by two neighbouring benzoyl groups, resulted in the low yield of the coupling. Therefore, partially benzoylated thioglycoside **8**[13] with a benzyl protecting group at the primary position, and partially benzylated methyl lactoside **9**[14] which only has one hydroxy functionality, were selected as building blocks to solve the problems associated with glycosylation yield (Scheme 2). Thus, glycosylation between substrates **8** and **9** in the presence of NIS–TMSOTf gave trisaccharide **10** in good yield (77%). Self-condensation or oligomerisation of sulfide **8** was not observed by mass spectroscopic analysis. Finally, NIS–TMSOTf-mediated coupling between substrates **6** and **10** afforded the fully protected pentasaccharide **11** in 61% yield. Deprotection of compound **11** was accomplished as follows: the phthalimido functionality was converted into an NHAc moiety by base-mediated removal of the acetyl and benzoyl functionalities followed by treatment with hydrazine and re-acetylation with acetic anhydride in pyridine, the benzyl protecting groups were cleaved by catalytic hydrogenation over Pd, and the O-acetyl groups were removed by treatment with KO^{t}Bu in methanol to give pentasaccharide **12** in good yield (76%, based on **11**).

In conclusion, we have described the synthesis of the methyl glycoside of the pentasaccharide moiety in compound **I** which serves as a binding site for human anti-pig antibodies. The protected pentasaccharide **11** was assembled from the building blocks **4**, **5**, **8** and **9** without a single protecting-group manipulation. The assembly is two-directional and the key compound **8** served first as a glycosyl donor but after condensation could immediately be used as an acceptor. It is expected that the strategic principle used here will be an important tool for the assembly of other complex oligosaccharides.

Experimental

General methods and materials

All the 1D ^{1}H NMR, ^{13}C NMR and 2D correlated spectroscopy (^{1}H–^{1}H COSY, HOHAHA, ^{1}H–^{13}C correlation) were recorded on a Bruker DRX500 spectrometer. Chemical shifts (δ) are given in ppm relative to the internal standard signal of tetramethylsilane. *J*-Values are given in Hz. Fast-atom bombardment mass spectra were recorded using a VG Zabspec spectrometer with *m*-nitrobenzyl alcohol as matrix. Chemicals were purchased from Aldrich and Fluka and used without further purification. Molecular sieves were activated at 350 °C for 3 h *in vacuo*. All solvents were distilled from the appropriate drying agents: dichloromethane, benzene and toluene were distilled from P_2O_5 and stored over 4 Å molecular sieves. 1,4-Dioxane was distilled from CaH_2, re-distilled from $LiAlH_4$ and stored over sodium wire. Dimethylformamide (DMF) was stirred with CaH_2 for 16 h, then distilled under reduced pressure and stored over 4 Å molecular sieves. Pyridine was distilled from CaH_2 and



Scheme 2 *Reagents and conditions*: (i) NIS–TMSOTf, CH_2Cl_2, 4 Å mol. sieves, −40 °C (77%); (ii) NIS–TMSOTf, CH_2Cl_2, 4 Å mol. sieves, 0 °C (61%); (iii) KO′Bu, MeOH; then $NH_2NH_2{\cdot}H_2O$, EtOH, reflux; then Ac_2O, pyridine, DMAP; (iv) H_2, $Pd(OAc)_2$, sonication; then KO′Bu, MeOH (76% overall yield)

stored over 4 Å molecular sieves. Methanol was distilled from sodium and stored over 4 Å molecular sieves. All the reactions were performed under anhydrous conditions and were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Flash chromatography was performed on silica gel (Merck, mesh 70–230). Size-exclusion column chromatography was performed on Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) and dichloromethane–methanol (1 : 1, v/v) was used as eluent. Extracts were evaporated under reduced pressure at <40 °C (bath). Petroleum spirit refers to the fraction with distillation range 60–80 °C. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,6-di-*O*-benzyl-3-*O*-(3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 3

A solution of sulfide **1** (84 mg, 0.15 mmol) and disaccharide **2** (80 mg, 0.10 mmol) in dichloromethane (3 ml) was stirred in the presence of 4 Å molecular sieves (100 mg, powdered) for 30 min. The mixture was cooled (−15 °C) and NIS (36 mg, 0.16 mmol) and TMSOTf (3 μl, 0.017 mmol) were added. After 5 min, TLC analysis (ethyl acetate–petroleum spirit 1 : 2, v/v) showed that most of the sulfide **1** was consumed. The reaction mixture was diluted with dichloromethane (40 ml), filtered, and the filtrate was washed successively with aq. sodium thiosulfate (20%; 2 × 15 ml), aq. sodium hydrogen carbonate (10%; 2 × 15 ml) and water (2 × 10 ml). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. Purification of the residue by silica gel column chromatography [eluent: ethyl acetate–petroleum spirit (60–80 °C) 1 : 3, v/v] gave title compound **3** as a syrup (73 mg, 56%), δ_H(500 MHz; $CDCl_3$) 7.88–8.15 (m, 4 H, ArH in NPhth), 6.82–7.61 (m, 35 H, ArH in 5 × Bn, 2 × Bz), 5.93 (dd, $J_{3'',4''}$ 8.8, 1 H, H-3″), 5.71 (d, $J_{1'',2''}$ 8.4, 1 H, H-1″), 4.95, 4.92, 4.69 and 4.67 (AB q, J_{AB} 10.7, 2 H, OCH_2Ph), 4.84, 4.82, 4.70 and 4.68 (AB q, J_{AB} 11.5, 2 H, OCH_2Ph), 4.79 (dd, $J_{5'',6''a}$ 2.6, $J_{6''a,6''b}$ 12.1, 1 H, H^a-6″), 4.76 (dd, $J_{5'',6''b}$ 4.7, 1 H, H^b-6″), 4.59 (dd, $J_{2'',3''}$ 10.6, 1 H, H-2″), 4.49, 4.46, 4.27 and 4.24 (AB q, J_{AB} 12.3, 2 H, OCH_2Ph), 4.45, 4.43, 4.33 and 4.30 (AB q, J_{AB} 12.1, 2 H, OCH_2Ph), 4.38, 4.35, 4.29 and 4.26 (AB q, J_{AB} 12.4, 2 H, OCH_2Ph), 4.32 (d, $J_{1',2'}$ 7.0, 1 H, H-1′), 4.17 (d, $J_{1,2}$ 7.7, 1 H, H-1), 4.15 (d, $J_{3',4'}$ 2.2, 1 H, H-4′), 4.03 (ddd, 1 H, H-5″), 3.92 (t, $J_{4'',5''}$ 9.2, 1 H, H-4″), 3.88 (dd, $J_{3,4}$ 9.1, $J_{4,5}$ 9.7, 1 H, H-4), 3.61 (dd, $J_{5',6'a}$ 6.4, $J_{6'a,6'b}$ 10.1, 1 H, H^a-6′), 3.53 (dd, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 11.0, 1 H, H^a-6), 3.51 (dd, 1 H, H-3′), 3.50 (dd, $J_{5',6'b}$ 7.0, 1 H, H^b-6′), 3.49 (s, 3 H, OCH_3), 3.48 (dd, $J_{2',3'}$ 9.6, 1 H, H-2′), 3.44 (t, $J_{2,3}$ 9.1, 1 H, H-3), 3.41 (dd, $J_{5,6b}$ 1.8, 1 H, H^b-6), 3.38 (ddd, 1 H, H-5′), 3.32 (dd, 1 H, H-2) and 2.84 (s, 1 H, 4′-OH); δ_C(125 MHz; $CDCl_3$) 166.7–168.0 (4 C, –C=O), 123.4–139.1 (48 C, Ar-C), 104.7 (C-1), 102.0 (C-1′), 98.9 (C-1″), 84.1 (C-3′), 82.7 (C-3), 81.8 (C-2′), 78.0 (C-2), 75.9 (C-4), 75.3 (O*C*H$_2$Ph), 74.8 (C-5, O*C*H$_2$Ph), 74.6 (C-5″), 74.2 (C-3″, O*C*H$_2$Ph), 73.3 (O*C*H$_2$Ph), 73.1 (O*C*H$_2$Ph), 72.9 (C-5′), 70.3 (C-4″), 68.7 (C-6′), 67.9 (C-6), 67.7 (C-4′), 63.3 (C-6″), 56.9 (OCH_3) and 54.4 (C-2″); FAB-MS *m*/*z* 1329 [M + Na]$^+$ (Found: [M + Na]$^+$, 1328.483 697. $C_{76}H_{75}NNaO_{19}$ requires *m*/*z*, 1328.483 100).

Ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside 6

IDCP (84 mg, 0.18 mmol) was added to a stirred mixture of sulfides **4** (70 mg, 0.12 mmol) and **5** (47 mg, 0.10 mmol) and molecular sieves (4 Å, powdered) in toluene–1,4-dioxane (1 : 3, v/v; 4 ml). The reaction mixture was stirred in the dark at room temp. and, after 1 h, TLC analysis [ethyl acetate–petroleum spirit (60–80 °C) 1 : 3, v/v] indicated the completion of the reaction. The reaction mixture was diluted with dichloromethane

(40 ml), filtered, and the filtrate was washed successively with aq. sodium thiosulfate (20%; 2 × 15 ml) and water (2 × 10 ml). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography [eluent: ethyl acetate–petroleum spirit (60–80 °C) 1:5, v/v], to give title compound **6** as a foam (67 mg, 67%), δ_H(500 MHz, $CDCl_3$) 7.11–8.05 (m, 30 H, ArH), 5.68 (d, $J_{3,4}$ 3.19, 1 H, H-4), 5.57 (t, $J_{2,3} = J_{1,2} = 9.89$, 1 H, H-2), 5.22 (d, $J_{1',2'}$ 3.35, 1 H, H-1′), 4.77, 4.74, 4.33 and 4.30 (AB q, J_{AB} 11.79, 2 H, OCH_2Ph), 4.65 (s, 2 H, OCH_2Ph), 4.63, 4.61, 4.40 and 4.38 (AB q, J_{AB} 11.79, 2 H, OCH_2Ph), 4.55 (d, 1 H, H-1), 4.52, 4.50, 4.37 and 4.35 (AB q, J_{AB} 11.83, 2 H, OCH_2Ph), 4.49 (dd, $J_{5,6a}$ 7.01, $J_{6a,6b}$ 11.32, 1 H, H^a-6), 4.31 (dd, $J_{5,6b}$ 6.15, 1 H, H^b-6), 4.14 (dd, 1 H, H-3), 3.89–3.95 (m, 3 H, H-2′, -5, -5′), 3.56 (dd, $J_{3',4'}$ 2.76, $J_{2',3'}$ 10.14, 1 H, H-3′), 3.46 (dd, $J_{5',6'a}$ 7.13, $J_{6'a,6'b}$ 9.59, 1 H, H^a-6′), 3.23 (dd, $J_{4',5'}$ 1.4, 1 H, H-4′), 3.21 (dd, $J_{5',6'b}$ 5.15, 1 H, H^b-6′), 2.66–2.80 (m, 2 H, SCH_2), 1.91 (s, 3 H, CH_3CO) and 1.25 (t, J 7.3, 3 H, SCH_2CH_3); δ_C(125 MHz, $CDCl_3$) 170.31, 166.00 and 164.93 (3 C, CH_3CO, 2 × Ph*C*O), 126.92–138.68 (36 C, Ar-C), 93.43 (C-1′), 84.19 (C-1′), 78.81 (C-3′), 75.68 (C-2′), 74.92 (C-4′), 74.80 (C-5), 74.42 (O*C*H_2Ph), 73.38 (O*C*H_2Ph), 73.25 (O*C*H_2Ph), 73.16 (O*C*H_2Ph), 72.73 (C-3), 69.96 (C-5′), 69.57 (C-6′), 69.16 (C-2), 65.14 (C-4), 62.34 (C-6), 24.38 (SCH_2), 20.47 (*C*H_3CO) and 14.91 (SCH_2-*C*H_3); FAB-MS *m*/*z* 1019 $[M + Na]^+$ (Found: $[M + Na]^+$, 1019.367 198. $C_{58}H_{60}NaO_{13}S$ requires *m*/*z*, 1019.365 234); $[\alpha]_D^{30}$ +2.696 (*c* 1.0, CH_2Cl_2).

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 10

Compound **8** (82 mg, 0.15 mmol) was coupled with disaccharide **9** (90 mg, 0.10 mmol) in the presence of NIS (36 mg, 0.16 mmol) and TMSOTf (3 µl, 0.017 mmol) as described for the preparation of compound **3**. The crude product was purified by silica gel column chromatography [eluent: ethyl acetate–petroleum spirit (60–80 °C) 1:3, v/v] to give title compound **10** as a syrup (72 mg, 52%, 77% based on unrecovered acceptor); δ_H(500 MHz) 6.96–7.88 (m, 44 H, ArH in 7 × Bn, Bz, Phth), 5.98 (dd, $J_{3'',4''}$ 8.69, 1 H, H-3″), 5.67 (d, $J_{1'',2''}$ 8.28, H-1″), 5.07, 5.05, 4.58 and 4.56 (AB q, J_{AB} 11.40, 2 H, OCH_2Ph), 4.92, 4.90, 4.59 and 4.57 (AB q, J_{AB} 10.54, 2 H, OCH_2Ph), 4.83, 4.81, 4.70 and 4.68 (AB q, J_{AB} 11.04, 2 H, OCH_2Ph), 4.63, 4.61, 4.59 and 4.57 (AB q, J_{AB} 11.86, 2 H, OCH_2Ph), 4.53 (dd, $J_{2'',3''}$ 10.77, 1 H, H-2″), 4.51, 4.49, 4.28 and 4.26 (AB q, J_{AB} 11.67, 2 H, OCH_2Ph), 4.38, 4.36, 4.24 and 4.22 (AB q, J_{AB} 11.94, 2 H, OCH_2Ph), 4.35, 4.33, 4.10 and 4.07 (AB q, J_{AB} 12.13, 2 H, OCH_2Ph), 4.29 (d, $J_{1',2'}$ 8.26, 1 H, H-1′), 4.15 (d, $J_{1,2}$ 7.63, 1 H, H-1), 4.06 (d, $J_{3',4'}$ 2.94, 1 H, H-4′), 3.96 (dd, $J_{4'',5''}$ 9.06, 1 H, H-4″), 3.83–3.92 (m, 4 H, H_2-6″, H-4, -5″), 3.63 (dd, $J_{2',3'}$ 9.83, 1 H, H-3′), 3.52–3.56 (m, 2 H, H^a-6, -6′), 3.51 (dd, 1 H, H-2′), 3.48 (s, 3 H, OCH_3), 3.34–3.42 (m, 4 H, H-3, -5, H^b-6, -6′), 3.31 (dd, $J_{2,3}$ 9.68, 1 H, H-2), 3.27 (br s, 1 H, 4″-OH) and 2.98 (ddd, $J_{4,5}$ 9.80, $J_{5,6a}$ 1.82, $J_{5,6b}$ 3.90, 1 H, H-5); δ_C(125 MHz; $CDCl_3$) 167.40 and 166.93 (3 C, −C=O), 123.23–139.32 (54 C, Ar-C), 104.52 (C-1), 102.31 (C-1′), 99.32 (C-1″), 82.83 (C-3), 81.90 (C-3′), 81.62 (C-2), 78.73 (C-2′), 76.59 (C-4′), 75.77 (C-4), 75.29 (O*C*H_2Ph), 74.98 (O*C*H_2Ph), 74.79 (O*C*H_2Ph), 74.72 (C-5), 74.49 (C-5″), 74.33 (C-3″), 73.92 (O*C*H_2Ph), 73.78 (O*C*H_2Ph), 73.25 (O*C*H_2Ph), 72.99 (O*C*H_2Ph, C-5′), 71.58 (C-4″), 70.06 (C-6″), 68.23 (C-6′), 67.78 (C-6), 56.83 (OCH_3) and 54.81 (C-2″); FAB-MS *m*/*z* 1405 $[M + Na]^+$ (Found: $[M + Na]^+$, 1404.552 943. $C_{83}H_{83}NNaO_{18}$ requires *m*/*z*, 1404.550 786); $[\alpha]_D^{30}$ +0.166 (*c* 1.0, CH_2Cl_2).

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-{3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-phthalimido-4-*O*-[4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl}-β-D-galactopyranosyl)-β-D-glucopyranoside 11

Compound **6** (100 mg, 0.10 mmol) was coupled with compound **10** (69 mg, 0.05 mmol) in the presence of NIS (25 mg, 0.11 mmol) and TMSOTf (2 µl, 0.011 mmol) as described for the preparation of compound **3**. The crude mixture was subjected to silica gel flash column chromatography (eluent: ethyl acetate–toluene 1:5, v/v), followed by purification by LH-20 size-exclusion chromatography (eluent: dichloromethane–methanol 1:1, v/v) to afford title compound **11** as a syrup (71 mg, 61%), δ_H(500 MHz) 6.92–8.03 (m, 74 H, ArH in 11 × Bn, 3 × Bz, Phth), 6.15 (dd, $J_{2'',3''}$ 10.8, $J_{3'',4''}$ 8.7, 1 H, H-3″), 5.62 (d, $J_{1'',2''}$ 8.2, 1 H, H-1″), 5.41 (dd, $J_{1''',2'''}$ 7.8, $J_{2''',3'''}$ 9.9, 1 H, H-2‴), 5.37 (d, $J_{3''',4'''}$ 3.3, 1 H, H-4‴), 5.07 (d, $J_{1'''',2''''}$ 3.3, 1 H, H-1‴), 5.06, 5.04, 4.49 and 4.47 (AB q, 2 H, J_{AB} 10.9, OCH_2Ph), 4.89, 4.87, 4.57 and 4.55 (AB q, J_{AB} 10.5, 2 H, OCH_2Ph), 4.81, 4.79, 4.68 and 4.66 (AB q, J_{AB} 11.3, 2 H, OCH_2Ph), 4.74, 4.72, 4.26 and 4.24 (AB q, J_{AB} 11.4, 2 H, OCH_2Ph), 4.70 (d, 1 H, H-1‴), 4.65, 4.62, 4.43 and 4.41 (AB q, J_{AB} 11.8, 2 H, OCH_2Ph), 4.61, 4.59, 4.58 and 4.56 (AB q, J_{AB} 11.5, 2 H, OCH_2Ph), 4.50 (dd, 1 H, H-2″), 4.48, 4.45, 4.28 and 4.26 (AB q, J_{AB} 11.7, 2 H, OCH_2Ph), 4.45, 4.43, 4.29 and 4.27 (AB q, J_{AB} 11.8, 2 H, OCH_2Ph), 4.43, 4.41, 4.29 and 4.27 (AB q, J_{AB} 11.8, 2 H, OCH_2Ph), 4.33, 4.31, 4.19 and 4.17 (AB q, J_{AB} 11.9, 2 H, OCH_2Ph), 4.30, 4.28, 4.08 and 4.06 (AB q, J_{AB} 12.1, 2 H, OCH_2Ph), 4.25 (d, $J_{1',2'}$ 7.5, 1 H, H-1′), 4.19 (dd, $J_{4'',5''}$ 9.8, 1 H, H-4″), 4.12 (d, $J_{1,2}$ 7.6, 1 H, H-1), 3.98 (d, $J_{3',4'}$ 2.9, 1 H, H-4′), 3.91 (dd, $J_{2''',3'''}$ 9.9, 1 H, H-3‴), 3.87 (dd, $J_{2'''',3''''}$ 10.2, 1 H, H-2‴), 3.85 (dd, $J_{3,4}$ 9.0, $J_{4,5}$ 9.8, 1 H, H-4), 3.79 (t, $J_{5'''',6''''a}$ 5.8, 1 H, H-5‴), 3.73 (dd, $J_{5'',6''a}$ 3.2, $J_{6''a,6''b}$ 10.6, 1 H, H^a-6″), 3.68–3.72 (m, 1 H, H-5″), 3.69 (dd, $J_{5'''',6''''a}$ 7.3, $J_{6''''a,6''''b}$ 10.9, 1 H, H^a-6‴), 3.64 (d, $J_{5'',6''b} \approx 0$, 1 H, H^b-6″), 3.61 (dd, $J_{5'''',6''''b}$ 6.3, 1 H, H^b-6‴), 3.57 (dd, 1 H, H-3‴), 3.54 (dd, $J_{2',3'}$ 9.8, 1 H, H-3′), 3.52 (dd, $J_{5,6a}$ 3.9, $J_{6a,6b}$ 11.3, 1 H, H^a-6), 3.49 (dd, $J_{2',3'}$ 10.3, 1 H, H-2′), 3.48 (dd, $J_{5',6'a}$ 5.3, $J_{6'a,6'b}$ 10.9, 1 H, H^a-6′), 3.47 (s, 3 H, OCH_3), 3.41 (dd, 1 H, H-5‴), 3.32–3.37 (m, 5 H, H-3, H^a-6‴, H-5′, H^b-6′, -6), 3.28 (dd, $J_{2,3}$ 9.3, 1 H, H-2), 3.19 (dd, $J_{3'''',4''''}$ 2.8, $J_{4'''',5''''}$ 1.4, 1 H, H-4‴), 3.17 (dd, $J_{5'''',6''''b}$ 5.8, $J_{6''''a,6''''b}$ 9.2, 1 H, H^b-6‴), 2.95 (ddd, 1 H, H-5) and 1.68 (s, 3 H, CH_3CO); δ_C(125 MHz, $CDCl_3$) 170.10, 167.42, 167.33, 165.56, 165.04 and 164.38 (CH_3CO, 3 × Ph*C*O, Phth), 139.30–137.94, 131.17 and 130.97 (Cq, Ar-C, 16 C), 133.75–132.91 and 129.80–123.04 (74 C, Ar-C), 104.56 (C-1), 102.31 (C-1′), 100.68 (C-1‴), 99.39 (C-1″), 93.97 (C-1‴), 82.84 (C-3), 82.09 (C-3′), 81.65 (C-2), 78.69 (C-2′, -3‴), 76.59 (C-4′), 75.80 (C-4, -4″), 75.52 (C-2‴), 75.32 (O*C*H_2Ph), 75.09 (O*C*H_2Ph), 74.95 (C-4‴), 74.81 (O*C*H_2Ph), 74.71 (C-5), 74.48 (O*C*H_2Ph), 74.29 (C-5″), 74.04 (O*C*H_2Ph), 73.45 (O*C*H_2Ph), 73.40 (O*C*H_2Ph), 73.24 (2 × O*C*H_2Ph), 73.16 (O*C*H_2Ph), 72.96 (O*C*H_2Ph, C-5′), 72.20 (C-3‴), 71.19 (C-2‴), 71.08 (C-3″), 70.87 (C-5‴), 69.81 (C-5‴), 69.27 (C-6‴), 68.21 (C-6″), 68.00 (C-6″), 67.67 (C-6), 64.44 (C-4‴), 60.97 (C-6‴), 58.67 (OCH_3), 55.45 (C-2″) and 20.26 (*C*H_3CO); FAB-MS *m*/*z* 2339 $[M + Na]^+$ (Found: $[M + Na]^+$, 2338.904 961. $C_{139}H_{137}NNaO_{31}$ requires *m*/*z*, 2338.907 228); $[\alpha]_D^{30}$ +0.726 (*c* 1.0, CH_2Cl_2).

Methyl 4-*O*-(3-*O*-{2-acetamido-2-deoxy-4-*O*-[3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl}-β-D-galactopyranosyl)-β-D-glucopyranoside 12

Compound **11** (30 mg, 13 mmol) was dissolved in MeOH–(THF) (3 ml; 1:1, v/v) and a catalytic amount of KOBu^t was added (pH 11–12). After stirring of the reaction mixture overnight, it was neutralised with DOWEX 50 H^+ resin and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in EtOH (3 ml) and treated with NH_2-NH_2·H_2O (1 ml). After the mixture had been heated under reflux for 12 h, it was concentrated under reduced pressure and the residue was co-evaporated with toluene (3 × 5 ml). The crude product was dissolved in pyridine (3 ml)–acetic anhydride (1 ml) and, after stirring for 6 h, TLC analysis [ethyl acetate–petroleum spirit (60–80 °C) 1:1, v/v] indicated the completion

of this reaction. MeOH (1 ml) was added and the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in dichloromethane (10 ml) and washed successively with aq. sodium hydrogen carbonate (10%; 2 × 3 ml) and brine (3 ml). The organic phase was dried ($MgSO_4$), concentrated under reduced pressure, and the residue was purified by silica gel column chromatography [eluent: ethyl acetate–petroleum spirit (60–80 °C) 1 : 1, v/v]. After the addition of $Pd(OAc)_2$ (10 mg) and EtOH (3 ml), the reaction mixture was sonicated under hydrogen and, after 3 h, the reaction mixture was diluted with EtOH (10 ml), filtered through Celite, and the filtrate was concentrated to dryness. The residue was treated with KO′Bu and MeOH as described above. After evaporation off of the solvents, the crude product was washed with diethyl ether (2 × 3 ml) to give pentasaccharide **12** as a solid (9 mg, 76%); δ_H(500 MHz; D_2O) (*inter alia*) 5.06 (d, $J_{1''',2'''}$ 3.9, 1 H, H-1‴), 4.62 (d, $J_{1,2}$ 8.2, 1 H, H-1), 4.47 (d, $J_{1'',2''}$ 7.8, 1 H, H-1″), 4.35 (d, $J_{1',2'}$ 7.9, 1 H, H-1′), 4.32 (d, $J_{1'',2''}$ 7.9, 1 H, H-1″), 4.10 (d, $J_{3''',4'''}$ 3.1, 1 H, H-4‴), 4.07 (dd, $J_{3',4'}$ 3.2, $J_{4',5'}$ 0.5, 1 H, H-4′), 3.94 (dd, $J_{3''',4'''}$ 3.5, $J_{4''',5'''}$ 1.3, 1 H, H-4⁗), 3.90 (dd, $J_{5'',6''a}$ 1.9, $J_{6''a,6''b}$ 11.9, 1 H, H^a-6″), 3.86 (dd, $J_{2''',3'''}$ 10.3, 1 H, H-3⁗), 3.77 (dd, 1 H, H-2⁗), 3.57 (dd, $J_{3'',4''}$ 8.9, 1 H, H-3″), 3.51 (dd, $J_{2',3'}$ 10.0, 1 H, H-2′), 3.49 (s, 3 H, OCH_3), 3.22 (dd, $J_{2'',3''}$ 9.3, 1 H, H-2″) and 1.95 (s, 3 H, CH_3CO); FAB-MS *m*/*z* 906.2 $[M + Na]^+$.

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