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Tetrahedron
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Tetrahedron 62 (2006) 563–577

Synthetic sulfated glucuronosyl paragloboside (SGPG) and its use for the detection of autoimmune peripheral neuropathies

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Received 10 August 2005; revised 6 October 2005; accepted 10 October 2005

Available online 7 November 2005

Abstract—The sulfated glucuronosyl paragloboside SO_4-3-GlcA(β1-3)Gal-(β1-4)GlcNAc(β1-3)Gal-(β1-4)Glcβ(1-1′)Cer (SGPG) is a specific constituent of the peripheral nervous system involved in human dysimmune neuropathies. Although it is highly immunogenic in human pathology, it is not a major constituent of peripheral nerve in humans. Pure SGPG is very difficult to obtain from natural sources, but is needed for the design of efficient and reproducible immunoassays. We describe a new chemical synthesis of this important glycolipid with a stearic tail, including its complete characterization by mass spectrometry and its use in the detection of autoimmune neuropathies.
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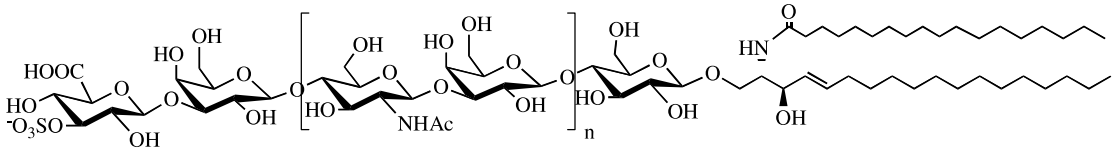
1. Introduction

Sphingoglycolipids are constituents of cellular structures of the peripheral nervous system (PNS) and the central nervous system (CNS). Several autoimmune neuropathies can be characterized, not only on clinical and electrophysiological grounds, but also on immunochemical grounds, related to the nature of the antigen recognized by antiglycolipid antibodies. The sulfated glucuronosyl paragloboside SO_4-3-GlcA(β1-3)Gal-(β1-4)GlcNAc-(β1-3)Gal-(β1-4)Glcβ(1-1′)Cer (SGPG) is a specific constituent of the peripheral nervous system. The terminal trisaccharide sequence of the SGPG (glucuronosyl sulfate-galactosyl *N*-acetylglucosaminyl) is the HNK-1 epitope, common to the SGPG lipid, and its derivatives with two lactosaminyl residues (Fig. 1), and to several adhesion molecules and myelin proteins, among which is P0, the major protein of PNS, and MAG (myelin-associated glycoprotein).[1] Clinico-biological studies in neuropathies have been performed, mainly to search for anti MAG/SGPG antibodies (reviewed in Ref. 2). Interestingly, the predominantly sensory demyelinating neuropathies with monoclonal immunoglobulins of the M type (IgMs) have antiMAG/SGPG activities, whereas axonal neuropathies, predominantly motor, or some cases of motor neuron diseases[3] with monoclonal IgMs present only anti SGPG activity.[4] Thus, there is a broad spectrum of dysimmune neuropathies characterized by anti SGPG antibodies and this has therapeutic consequences.



HNK-1 epitope

SGPG n=1
SGLPG n=2

Figure 1.

Keywords: SGPG; Sphingoglycolipid; Chemical synthesis; Peripheral nervous system; Neuropathy; Electrospray mass spectrometry.
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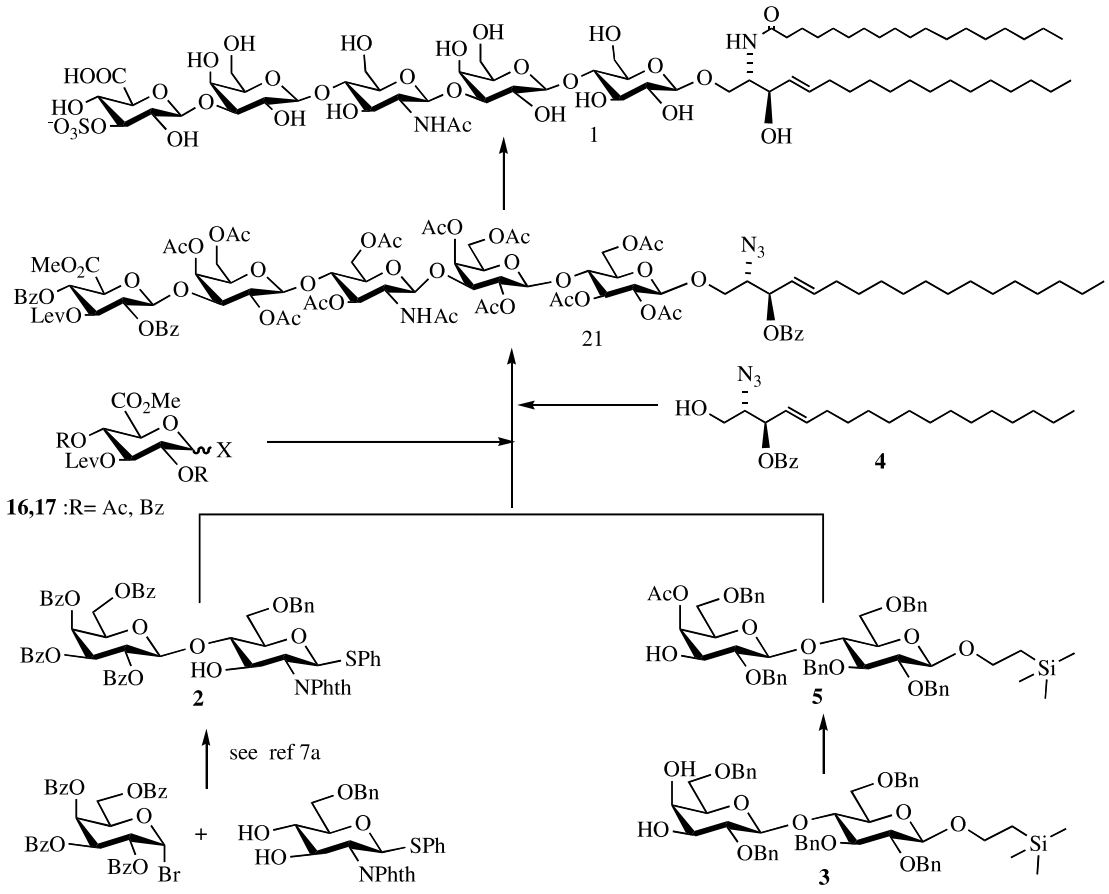
doi:10.1016/j.tet.2005.10.021



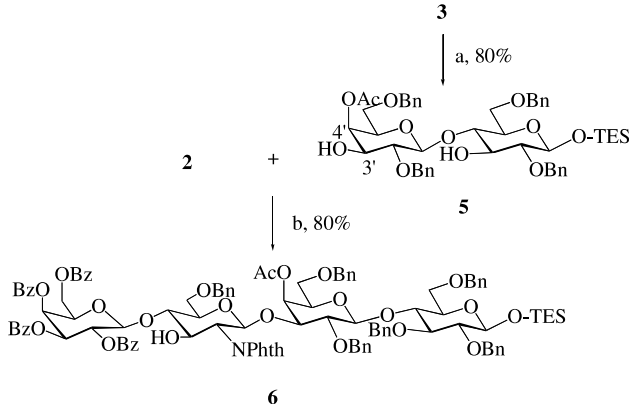
Scheme 1. Retrosynthetic analysis.

SGPG is not commercially available. Furthermore, although it is highly immunogenic in human pathology, it is not a major constituent of peripheral nerve in humans and is only a minor constituent of PNS of several animal species.[5] Thus, its purification remains a hard task. For this reason, it appears important for clinical purposes to synthesize this glycolipid.[6] In this paper, we describe a new chemical synthesis of this important glycolipid with a stearic tail, including its complete spectroscopic characterization and its use in immunodetections.

2. Chemical synthesis

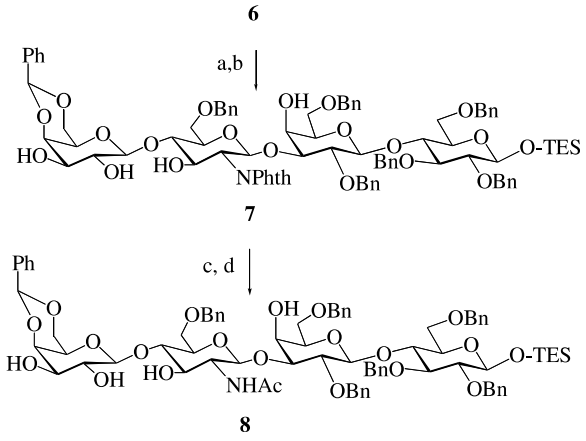
The 1+2+2 synthesis proposed (Scheme 1) is based upon our previous synthetic work[7] on lacto glycolipids. All the glycosidic linkages are 1,2 *trans*, this stereochemistry was easily assured by the use of a participating group at C2 of each glycosyl donors (acetate, benzoate and phthalimido (Phth)). The main difficulty was the expected 'placidness' of the two deactivated glycosyl donors: the acylated glucuronic unit (**16**, **17**) and the acetylated pentasaccharide imidate **20**. Three of the four key intermediates have been prepared previously: the lactose unit **3**,[8] the lactosamine unit **2**[7a] and the azidosphingosine **4**[9] and have been prepared according to a literature procedure.

We tried first the glycosylation between **2** and **3** using NIS, TfOH as a promoter in dichloromethane. The hydroxyl group at position 3 was expected to be the more reactive of the two hydroxyl groups. In contrast to previous results obtained using a Lewis X trisaccharide donor,[7a,b] a mixture of two regioisomers (the expected 3-regioisomer (55%) and its 4-regioisomer (30%)) has been obtained. In order to avoid this side reaction, we decided then to protect the position 4 of **3** by acetylation using the Lemieux method[10] yielding **5** (95%) (Scheme 2). The acetate position was checked in ^{1}H NMR (H-4′: 5.4 ppm ($J_{4,3}$=3.1 Hz) and H-3′: 3.6 ppm). The glycosylation between **2** and **5** gave, uneventfully, rise to the formation of **6** (80%) (Scheme 2). The β-stereochemistry of the newly formed glycosidic bond was checked by ^{1}H NMR ($J_{1,2}$=8.5 Hz).



Scheme 2. Reagents: (a) $MeC(OEt)_3$, CSA then AcOH 80%, 81%; (b) NIS, TfOH, CH_2Cl_2, −30 °C, 80%.

This tetrasaccharide **6** was transformed in four steps into a glycosyl acceptor **8**, ready to react with a glucuronosyl donor (Scheme 3). The hydroxyl group at position 3 of Gal IV is usually the more reactive of the four hydroxyl group present in **8**.



Scheme 3. Reagents: (a) MeONa/MeOH, CH_2Cl_2, 88%; (b) $PhCH(OMe)_2$, CSA, CH_3CN, 82%, (c) N_2H_4, EtOH, 80 °C, (d) Ac_2O, MeOH, 81% (two steps).

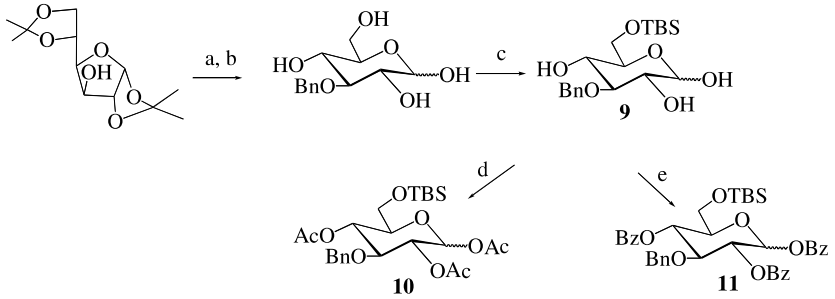
Two glucuronosyl donors have been synthesized (Schemes 4 and 5). They have a levulinoyl group at C3. This cetoester, selectively removed in presence of acetate or benzoate, is a classical and reliable choice in sulfated glycosaminoglycan chemical synthesis.[11] We introduced first a levulinate group at C3 of diacetone D-glucose and then tried without success to remove the two isopropylidene groups using hot aqueous AcOH. We moved then to a more stable temporary group, the benzyl ether. Thus, 3-*O*-benzyl glucose[12] was prepared in two steps from diacetone glucose, then silylated by terbutyldimethylsilyl chloride in pyridine[13] followed by one-pot acylation with Ac_2O to give **10** (or with BzCl to give **11**) (Scheme 4).

Jones oxidation, followed by methylation, afforded the two methyl 3-*O*-benzyl uronates in good yields. Exchange of benzyl to levulinate was at this stage uneventful. The acylated products **14** and **15** have been then transformed into glucuronosyl bromides **16** and **17** in good yield using HBr in acetic acid (Scheme 5).

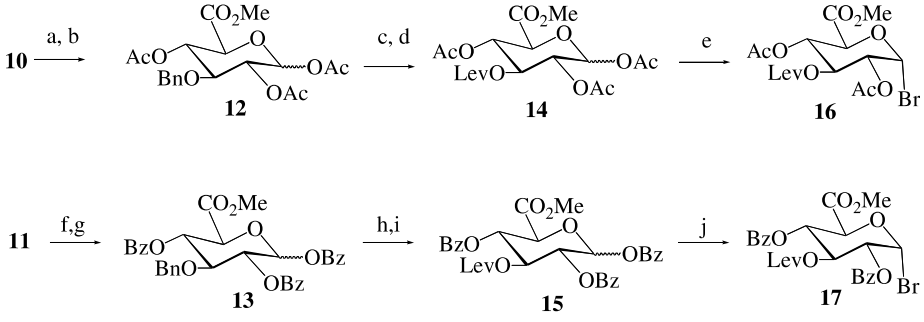
The [**8**+**16**] condensation in dichloromethane promoted by silver triflate or with Ag_2CO_3/I_2[14] at room temperature failed to give any pentasaccharide. However, the benzoylated bromide **17** (2 equiv) finally gave the pentasaccharide **18** in an acceptable yield of 50% (72% based on recovered **8**) (Scheme 6). We tried to improve this step using analogous benzoylated thiophenyl glycosides and trichloroacetimidate, without any success.

The pentasaccharide **18** was then transformed into a glycosyl donor in four steps (Scheme 7): first removal of the benzylidene and benzyl groups (H_2, Pd/C) followed by acetylation to give **19**. The regioselectivity of the last glycosylation was easily checked by NMR (H-3 Gal IV δ= 3.8 ppm). The trimethylsilylethyl protecting group was then removed and the α imidate **20** was prepared using trichloroacetonitrile and DBU as a base.

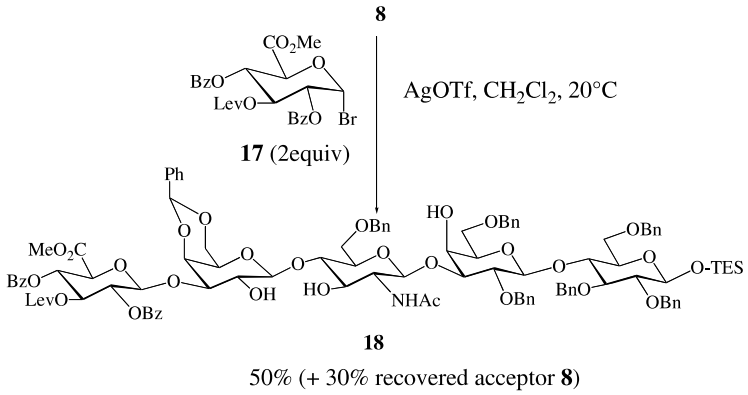
Coupling with **4** gave the glycoconjugate **21** with a good yield (75%) (Scheme 8). Compound **21** was then easily converted into **1** in a few steps (Scheme 9): reduction of azide with PPh_3[15] in aqueous THF at 50 °C, introduction of



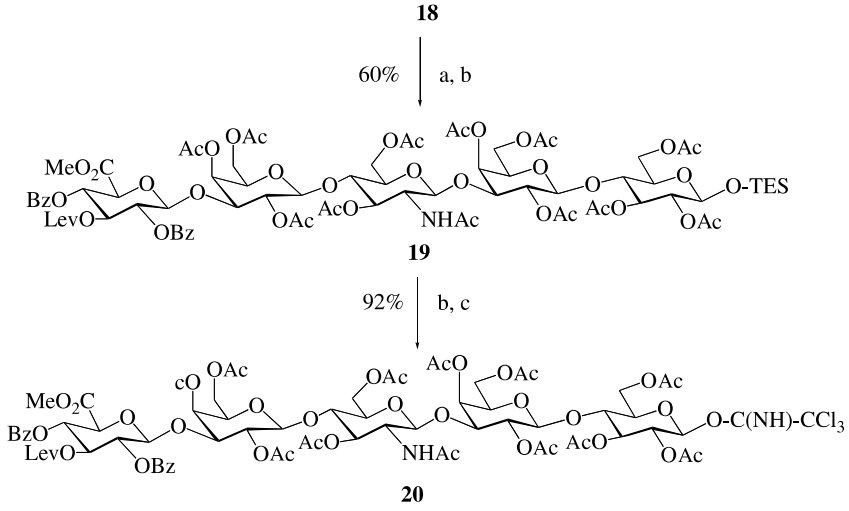
Scheme 4. Reagents: (a) BnBr, NaH, DMF; (b) H_2O, IR 120 (H^+), 85% (two steps); (c) TBS-Cl, Pyr., (d) Ac_2O, Pyr., 88% (two steps); (e) BzCl, Pyr. 90% (two steps).



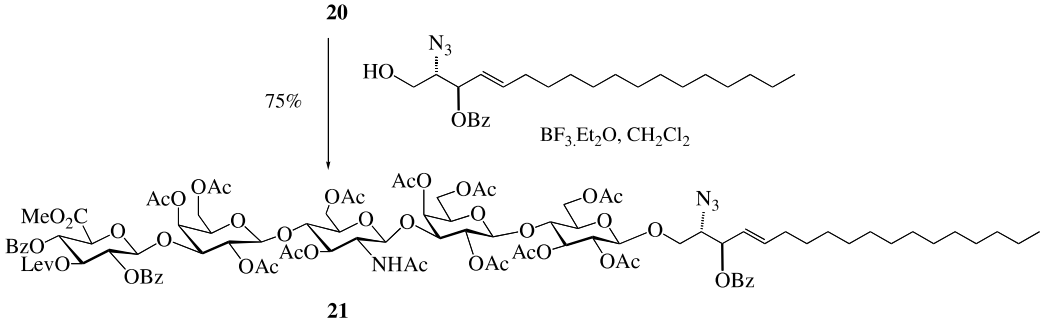
Scheme 5. Reagents (a) CrO_3, H_2SO_4, acetone; (b) MeI, $KHCO_3$, DMF 65% (two steps); (c) H_2, Pd/C, MeOH; (d) Lev_2O, Pyr., 82% (two steps); (e) HBr, AcOH, CH_2Cl_2, 90%; (f) CrO_3, H_2SO_4, acetone; (g) MeI, $KHCO_3$, DMF 68% (two steps); (h) H_2, Pd/C, MeOH; (i) Lev_2O, Pyr., 80% (two steps); (j) HBr, AcOH, CH_2Cl_2, 70%.



Scheme 6.



Scheme 7. Reagents: (a) H_2, Pd/C, MeOH; (b) Ac_2O, Pyr.; (c) CF_3COOH, CH_2Cl_2, 0–20 °C; (d) Cl_3CCN, DBU, CH_2Cl_2.

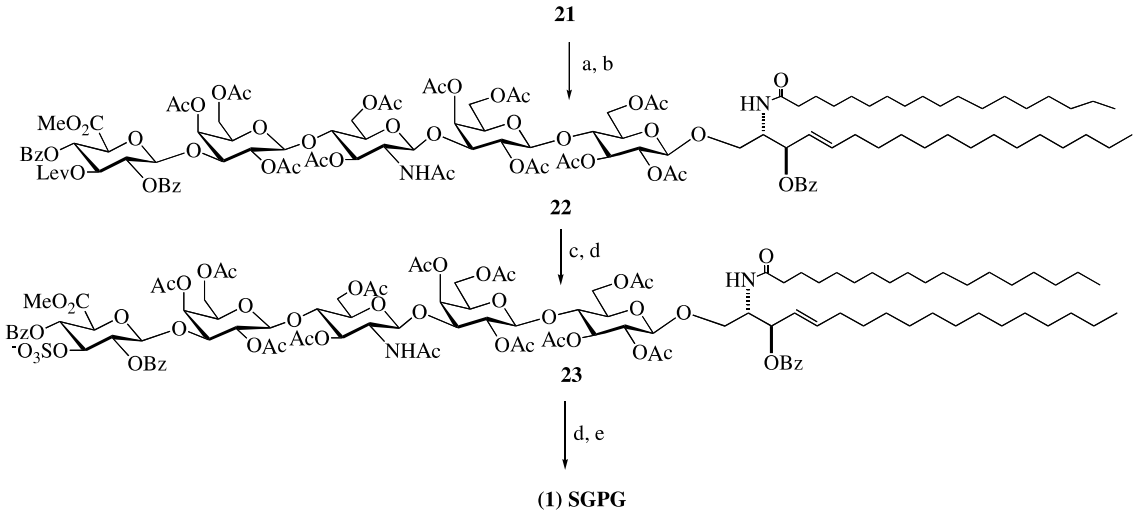


Scheme 8.

stearic acid, with DCC as a coupling agent; removal of levulinate with hydrazine monoacetate, and sulfatation of the free hydroxyl group with $SO_3 \cdot NMe_3$ in DMF at 50 °C. Final deprotection was done in two steps, that is, saponification of the methyl ester with lithium hydroxide, followed by an treatment with MeONa/MeOH/THF. This two steps sequence limits the β elimination. The ^1H NMR spectrum in DMSO-d_6 (see Fig. 2 for ethylenic and anomeric proton signals) was found in accordance with reported spectrum for natural SGPG.[6b]

3. Mass characterisation of synthetic SGPG

The negative ion ESI mass spectrum of the synthetic SGPG (Fig. 3a), recorded using the negative ion mode, displayed various singly charged ions at *m/z* 1510.1 and *m/z* 1532.1 and doubly-charged ions at *m/z* 754.5, that correspond to the, $[M-H]^-$, $[M-2H+Na]^-$ and $[M-2H]^{2-}$ quasi-molecular species, respectively. From this mass spectrum, it appears that the molecular weight of the synthetic compound was 1511.1 u consistent with the expected



Scheme 9. Reagent: (a) PPh_3, THF, H_2O, 50 °C; (b) DCC, stearic acid, CH_2Cl_2, 78% (two steps); (c) $N_2H_5 \cdot AcO$, EtOH, 81%; (d) $(Me)_3N–SO_3$, DMF, 50 °C, 80%; (e) LiOH, THF, H_2O, 0 °C; (f) MeONa, MeOH, THF, 90% (two steps).

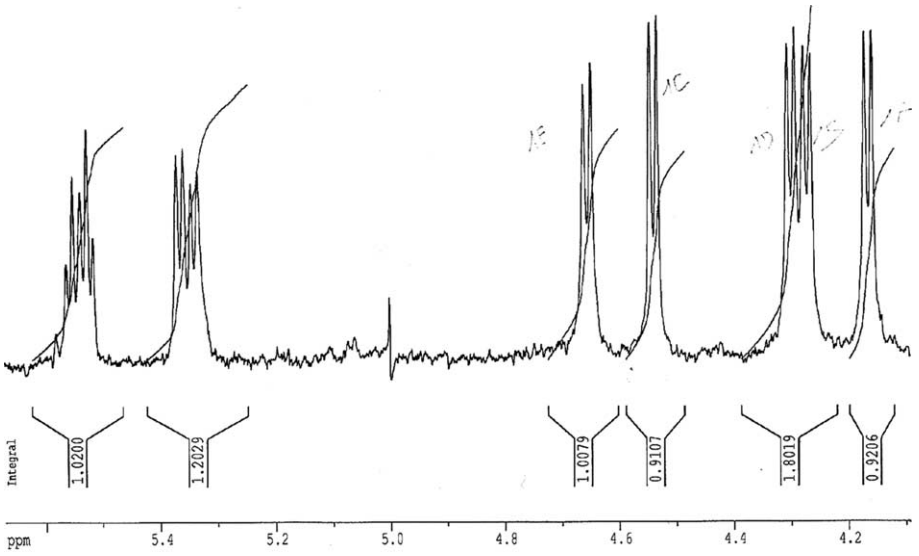


Figure 2. ^1H NMR selected data (DMSO-d_6, 400 MHz): ethylenic and anomeric protons.

monoisotopic peak as well as with to the natural isotopic cluster distribution, which is superimposable on the calculated cluster distribution (Fig. 3a). Furthermore, prompt fragmentations are limited since only ions at *m/z* 1492.1 (i.e., loss of water) and *m/z* 357.0 raised up to 10% of base peak (i.e., at *m/z* 1532.1), under these ESI source conditions. These quasi-molecular species being sufficiently abundant they can be studied by MS/MS under low energy collision excitation (CID processes).

Firstly, the collision excitation of the deprotonated molecule (*m/z* 1510.1) shows this precursor ion was very stable since only two product ions are displayed at *m/z* 1430.1 (100%) and *m/z* 1334.1 (5%) in the CID spectrum (not reported herein). These ions are promoted from the deprotonated molecule carrying out the negative charge at the sulfate site (the larger acidic group in gas phase) by releasing the SO_3 neutral (i.e., 80 u) and the desulfated glucuronic terminal unit, respectively. The latter loss is generated by sulfate migration form the C3 position of terminal residue likely to hydroxyl of the neighbouring glucosidic unit (study in progress) yielding a terminal sulfated glucose in the *m/z* 1334.1 production. This behaviour makes ambiguity on the sulfate group location.

In order to obtain more information on the sequence (except for the sulfate position), in situ consecutive ion excitations (called 'tickle excitation') were applied: (i) to the first generation product *m/z* 1430.1 ion and (ii) in second step, to the second generation product *m/z* 1412.2 ion (formed by water release from the previous precursor of the first generation). The recorded CID spectrum (Fig. 3b) displays a series of the losses of 158 u (glucuronic unit having lost an OH group), 162 u (glucosidic unit), 203 u (GlcNAc residue) produced either by consecutive fragmentations or directly by competitive cleavages in the ion trap cell. These different neutral losses give rise to the formation of the following ions at *m/z* 1412.3, *m/z* 1254.2, *m/z* 1092.1, *m/z* 889.9, *m/z* 726.7, and *m/z* 564.6 corresponding to the Y_i series (without significant presence of the complementary B_j series)

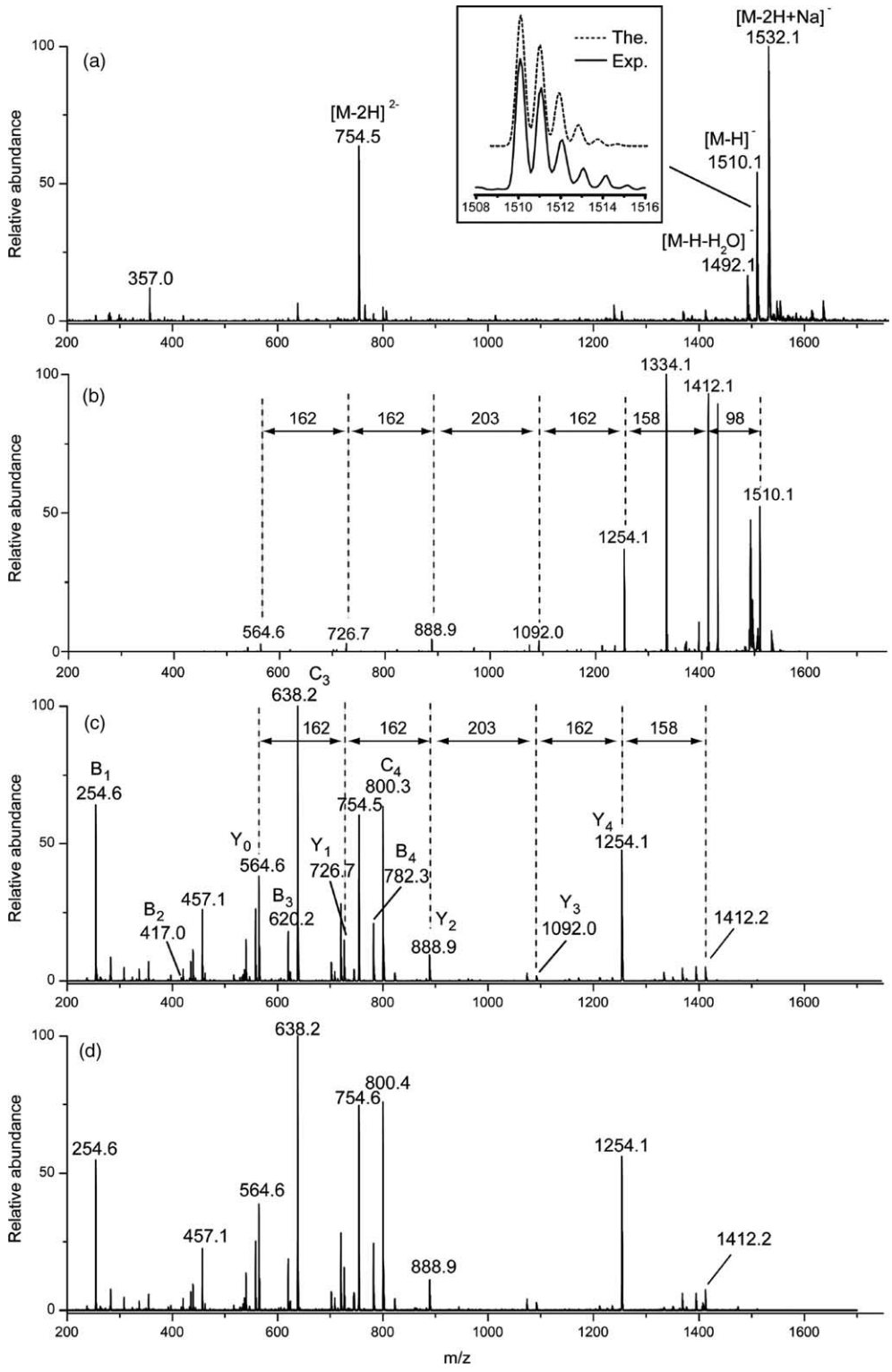


Figure 3. Negative ion ESI mass spectrum of synthetic-SGPG (a) and MS^2 experiments under CID conditions of singly-deprotonated (b) and doubly-deprotonated $[M-2H]^{2-}$ synthetic SGPG (*m/z* 754.5) (c) in comparison with CID spectrum of the doubly deprotonated natural SGPG recorded in same experimental conditions (d).

according to Domon and Costello's nomenclature.[16] They are produced after the SO_3 release by charge promoted competitive cleavages of sugar linkages and the charge is retained at the ceramide moiety. This orientation can be expected assuming that the charge location occurs at the ceramide amide group because of its particular gas phase acidity relative to other acidic sites. This observed series of product ions is consistent with the proposed sequence without assuring the sulfate position on glucuronic acid moiety.

Evidence of its location as well as the total sequence in one scanning has been achieved from the collision excitation of the doubly-deprotonated $[M-2H]^{2-}$ molecule (*m/z* 754.5).

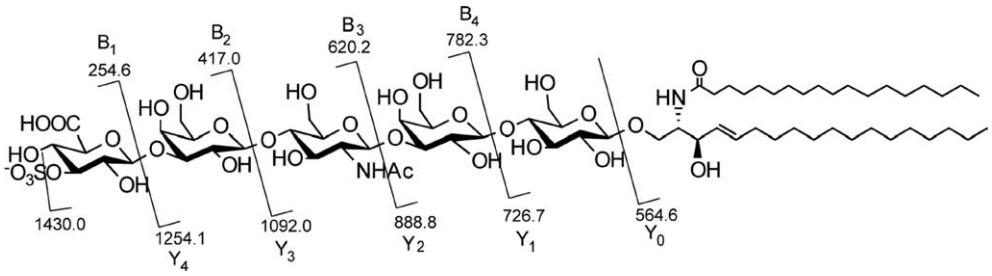


Figure 4. Main complementary product ions of the deprotonated synthetic-SGPG (C18:0; d18:1) according to Domon and Costello's nomenclature. These cleavages are observed from the doubly charged SGPG.

The CID spectrum of this precursor ion (Fig. 3c) displays different series of singly-charged product ions. These series are complementary and are noted as Y_i and B_i (Fig. 4). Interestingly, two abundant complementary product ions (more than 50% of base peak at *m/z* 638.2) appear at *m/z* 1254.1 to *m/z* 254.6 (Fig. 3c) corresponding to the Y_4 and B_1 ions (Fig. 4) give immediately the evidence on the sulfate location on the terminal glucuronidic residue. Other B_j/Y_i couples of complementary product ions are too observed at *m/z* 417.0 /*m/z* 1092.0, *m/z* 620.2/*m/z* 888.9, *m/z* 782.3/*m/z* 726.7, which confirm the SGPG whole sequence as well as the presence of sulfate on glucuronidic unit. In addition to the previous series is accompanied by other ions such as the C_4, C_3, and C_2 product ions corresponding to *m/z* 800.3, *m/z* 638.2, *m/z* 435.3, respectively, (Fig. 4). However, the complementary product Z_i ions are not observed, which indicates that they decompose into low *m/z* fragment ions beyond the product ion *m/z* ratio range (the parent ion *m/z* values must be four times lower than that of its product ions). This value is related to the low mass cut-off fixed automatically by the software of the mass spectrometer. Furthermore, from all the product B_j and C_j ions, a 80 u neutral loss is released systematically (Table 1).

Table 1. Observed consecutive loss of SO_3 (80 u) from each product B_j and C_j ions generated from the precursor doubly-deprotonated $[M-2H]^{2-}$ molecule

Series of ions	$i=4$	$i=3$	$i=2$
B_j	782.3→702.3	620.2→540.2	416.8→336.8
C_j	800.3→720.3	638.2→558.2	435.0→354.9

Finally, to obtain a definitive confidence in our interpretation, the same MS/MS experiment was performed from doubly-deprotonated natural SGPG (Fig. 3). The recorded fingerprints represented by their respective CID spectra were superimposable. Furthermore, even the peaks characterized by very weak abundances are present with similar relative abundances, which confirms the proposed SGPG structure.

4. Immunology

Elisa measurements have been done on 96-well micro plates according to the established procedure for antiglycolipid antibody Elisa[17] with two minor modifications: 100 ng of SGPG were coated per well (instead of 200 ng of glycolipid), and the enzymatic reaction was quenched 3 M HCl (instead of 3 M H_2SO_4).

We compared the sera of 49 patients with predominantly sensory peripheral neuropathies associated with the presence of a monoclonal IgM. We were able, with this synthetic SGPG (**1**), to clearly classify them into two groups: 24 had anti SGPG antibodies, 25 were completely negative (that is under the detection level). Anti SGPG antibodies titers of the 24 positives patient are shown in Figure 5 and confirm the relation between SGPG and the neuropathy, which is important for therapeutic purpose.[18] The titers were extremely variable and no correlation can be clearly established with the total amount of monoclonal IgM.

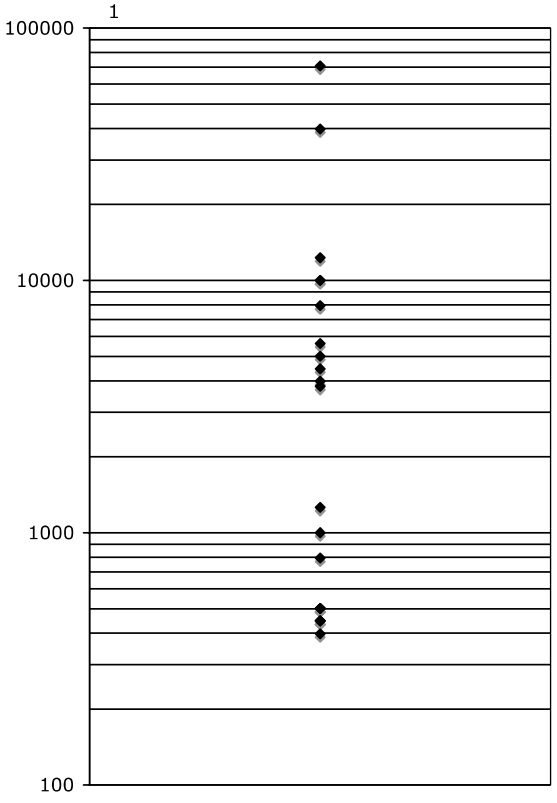


Figure 5. Anti SGPG antibodies titers, in predominantly sensory demyelinating neuropathy with monoclonal IgM. Negative sera are not shown.

This technique is much more sensitive (up to 1000 fold) than the previously used one using crude nerve extracts purified and immunodetected on a thin-layer chromatography

plate.[19] For instance, patients under 1000 in Figure 5 were not diagnosed with this technique. This improved technique is now in use in routine analysis in Salpétrière hospital for diagnosis of neuropathies.

5. Conclusion

The synthetic product, although containing a single fatty acid, has the same specificity for IgM SGPG antibodies as the natural product (data not shown). The ELISA test that we have set up for clinical purposes is considerably more sensitive than immunodetection on thin-layer chromatography, and thus will allow the detection of dysimmune neuropathies, which were undiagnosed in the past. This may have therapeutic consequences, as treatments are becoming more and more specific and designed to lower antibody concentration or to interfere with immunopathogenic mechanisms. It may be that the use of solid-phase immunoadsorbents containing a reactive oligosaccharide, will permit a more selective therapy for antibody-mediated neuropathies. Synthesis of ganglioside epitopes for oligosaccharide specific immunoadsorption therapy has been suggested for the Guillain–Barré syndrome, with the aim of removing auto-antibodies either by using soluble blocking ligands administered systematically or as immunoaffinity ligands for use as extracorporeal immunoadsorbents.[20,21] This type of approach could be used for other antigens such as SGPG.

6. Experimental

6.1. General procedures

All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. Melting points were determined in capillary tubes in a Büchi 510 apparatus, and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 digital polarimeter at 22±3 °C. Compound purity was checked by TLC on Silica gel 60 F_{254} (E. Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica gel 60 (E. Merck). ^{1}H NMR spectra were recorded with Brüker AM 250, AM 400 instruments. Elemental analyses were performed by Service d'Analyse de Université Pierre et Marie Curie. Chemical ionization and FAB mass spectrometry were recorded with Jeol MS700: CI (gas: ammonia); FAB (matrix: NBA, NaI). MALDI-TOF mass spectra were recorded with PerSpective Biosystems Voyager Elite (Framingham, MA, USA) equipped with a nitrogen laser (337 nm) using a 2,5-dihydroxybenzoic acid (2,5-DHB) matrix.

6.2. Mass spectrometry procedure

SGPG was analyzed by using an electrospray ionization (ESI)-ion-trap mass spectrometer[22] (Esquire 3000, Bruker) (Bremen, Germany). The source conditions were as follow: capillary high voltage 3500 V, capillary exit −50 V, skimmer 1 −20 V. The ion analysis occurred within a scan rate of 13,000 Th/s using a mass/charge ratio range of 3000 Th by using analytical scan mode through resonant ion ejection to the non-linear field at $\beta_z=2/3$. The injection low mass cut off (LMCO) was 70 Th. Negative ion mode detection was used. The automated ion charge control (ICC) was set to 10,000 in order to avoid space charge effect. Sequential MS^n experiments were performed under resonant excitation conditions from precursor ion selected by application of broadband frequency excitation with a notch allowed a m/z ratio selection window of 2 Th. In the case of MS^n application (with $n>2$), when chosen precursor ion was characterized by a too low abundance, it was directly excited by application of resonant frequency without to use the ion selection step. The LMCO used during the collision induced dissociation (CID) experiments (related to the excitation at particular β_z value) was set to 27% of the mass to charge ratio of the precursor ion.

6.2.1. 2-(Trimethylsilyl)ethyl (2,6-di-*O*-benzyl-4-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). To a stirred solution of **3** (9 g, 10 mmol) in triethyl orthoacetate (30 mL) was added, at room temperature, CSA (200 mg). After 30 min, aqueous acetic acid 80% (26 mL) was added dropwise. The mixture was stirred (15 min) and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 20% EtOAc in cyclohexane) to afford **5** (9.3 g, 95%) as a colourless oil. $[\alpha]_D$ −10 (c 1 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.30 (m, 25H, arom.), 5.40 (dd, 1H, $J_{4-3}=3.4$ Hz, $J_{4-5}=0.6$ Hz, H-4B), 5.03 (d, 1H, $J_{gem}=10.5$ Hz, CHPh), 4.97 (d, 1H, $J_{gem}=11.0$ Hz, CHPh), 4.87 (d, 1H, $J_{gem}=11.4$ Hz, CHPh), 4.82 (d, 1H, $J_{gem}=10.5$ Hz, CHPh), 4.79 (d, 1H, $J_{gem}=11.0$ Hz, CHPh), 4.73 (d, 1H, $J_{gem}=11.4$ Hz, CHPh), 4.65 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.53 (d, 1H, $J_{1-2}=7.7$ Hz, H-1B), 4.51 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.49 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.45 (d, 1H, $J_{1-2}=7.7$ Hz, H-1A), 4.29 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.11–4.08 (m, 1H, O–C*H*–$CH_2Si(CH_3)_3$), 4.04 (dd, 1H, $J_{4-3}=9.0$ Hz, $J_{4-5}=9.5$ Hz, H-4A), 3.97–3.80 (m, 2H, H-6A), 3.72–3.67 (m, 1H, H-3B), 3.69–3.65 (m, 1H, O–C*H*–$CH_2Si(CH_3)_3$), 3.63 (t, 1H, $J_{3-2}=9.0$ Hz, H-3A), 3.57 (dt, 1H, $J_{5-6}=6.6$ Hz, H-5B), 3.48–3.43 (m, 3H, H-2B, H-2A and H-5A), 3.38 (d, 2H, H-6B), 2.40 (d, 1H, $J_{OH-3}=2.4$ Hz, OH), 2.09 (s, 3H, O–C=O–CH_3), 1.10 (m, 2H, O–CH_2–C*H*$_2$Si$(CH_3)_3$), 0.09 (s, 9H, Si$(CH_3)_3$); ^{13}C NMR (100 MHz): δ 170.9 (O–*C*=O–CH_3), 139.0, 138.7, 138.2, 138.1 and 137.9 (5C arom.), 128.4–127.3 (25CH arom.), 103.1 (C-1A), 102.3 (C-1B), 82.7 (C-3A), 81.8 (C-2B), 80.0 (C-2A), 76.5 (C-4A), 75.2 (CH_2Ph), 75.0 (C-5A), 74.9 (CH_2Ph), 74.9 (CH_2Ph), 73.3 (CH_2Ph), 73.1 (CH_2Ph), 72.4 (C-3B), 71.9 (C-5B), 69.5 (C-4B), 68.2 (C-6A), 67.4 (O–*C*H_2–CH_2Si–), 67.1 (C-6B), 20.8 (–O–C=O–*C*H_3), 18.4 (O–CH_2–*C*H_2Si–), −1.4 (Si(*C*$H_3)_3$); MS m/z (CI, NH_3): 952.7 $(M+NH_4)^+$; Anal. Calcd for $C_{54}H_{66}O_{12}Si$ (935.204): C 69.35, H 7.11; found C 69.30, H 7.17.

6.2.2. 2-(Trimethylsilyl)ethyl(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). To a stirred mixture of **2** (6.64 g, 6.2 mmol), **5** (5.8 g, 6.2 mmol) and MS-4 Å (15 g) in anhydrous CH_2Cl_2 (150 mL) were successively added, at −30 °C and under argon, NIS (2.8 g, 12.4 mmol) and TfOH (0.165 mL, 1.86 mmol). The mixture was stirred

for 30 min at −30 °C, neutralized by saturated sodium hydrogencarbonate and filtered through Celite. The filtrate was washed with saturated sodium thiosulfate, brine, dried ($MgSO_4$) and evaporated in vacuum. The residue was purified by flash chromatography (eluent gradient, 20% EtOAc in cyclohexane) and precipitated (EtOAc/hexanes) to afford **6** (9.4 g, 80%) as a white powder. $[\alpha]_D$ +48 (*c* 1 in chloroform); mp: 153–154 °C (EtOAc/hexanes); ^{1}H NMR (400 MHz, $CD_3C{=}OCD_3$): δ 8.24–7.15 (m, 54H, arom.), 6.21 (dd, 1H, $J_{4-3}=2.8$ Hz, $J_{4-5}=0.7$ Hz, H-4D), 6.03 (m, 2H, H-2D and H-3D), 5.65 (de, 1H, $J_{4-3}=3$, 6 Hz, H-4B), 5.59 (d, 1H, $J_{1-2}=8.5$ Hz, H-1C), 5.57 (d, 1H, $J_{1-2}=7.0$ Hz, H-1D), 5.10 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 5.00 (d, 1H, $J_{gem}=11.5$ Hz, CHPh), 5.00–4.90 (m, 1H, H-5D), 4.90 (dd, 1H, $J_{6b-6a}=11.5$ Hz, $J_{6b-5}=4.0$ Hz, H-6Db), 4.87 (d, 1H, $J_{OH-3C}=2.1$ Hz, OH), 4.85 (d, 1H, $J_{gem}=11.5$ Hz, CHPh), 4.76 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 4.79–4.73 (m, 1H, H-3C), 4.63 (d, 1H, $J_{gem}=12.2$ Hz, CHPh), 4.59 (d, 1H, $J_{gem}=12.2$ Hz, CHPh), 4.58 (d, 1H, $J_{1-2}=7.8$ Hz, H-1B), 4.57 (d, 1H, $J_{gem}=12.4$ Hz, CHPh), 4.58–4.53 (m, 1H, H-6Da), 4.53 (d, 1H, $J_{gem}=12.2$ Hz, CHPh), 4.50 (d, 1H, $J_{1-2}=7.7$ Hz, H-1A), 4.40 (d, 2H, $J_{gem}=12.2$ Hz, 2CHPh), 4.38 (d, 1H, $J_{gem}=12.2$ Hz, CHPh), 4.35 (d, 1H, $J_{gem}=12.4$ Hz, CHPh), 4.28 (dd, 1H, $J_{2-3}=10.8$ Hz, H-2C), 4.12–4.06 (m, 2H, O–C*H*–CH_2Si– and H-4C), 4.04 (dd, 1H, $J_{4-3}=9.2$ Hz, $J_{4-5}=9.8$ Hz, H-4A), 3.95 (dd, 1H, $J_{3-2}=9.6$ Hz, H-3B), 3.83–3.79 (m, 1H, H-5C), 3.77–3.70 (m, 5H, H-5B, H-6C, H-6Ab and O–C*H*–CH_2Si), 3.60–3.56 (m, 3H, H-3A, H-6Bb and H-6Aa), 3.50 (dd, 1H, H-2B), 3.46 (dd, 1H, $J_{6a-6b}=10.3$ Hz, $J_{6a-5}=6.6$ Hz, H-6Ba), 3.39 (ddd, 1H, $J_{5-6a}=3.5$ Hz, $J_{5-6b}=1.5$ Hz, H-5A), 3.30 (dd, 1H, $J_{2-3}=9.2$ Hz, H-2A), 2.14 (s, 3H, O–C=O–C*H*$_3$), 1.15–1.12 (m, 2H, O–CH_2–C*H*$_2$Si), 0.15 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz): δ 169.4, 168.9, 168.4, 164.7, 164.5, 164.0 and 163.9 (5O–*C*=O–CH_3/Ph and 2C=O Phth), 138.7, 138.5, 138.2, 138.0, 137.7, 137.6, 2×130.5, 128.5, 128.4 and 128.0 (11C arom.), 128.8–126.0 (54CH arom.), 101.9 (C-1A), 100.8 (C-1B), 100.7 (C-1D), 97.9 (C-1C), 81.6 (C-3A), 81.4 (C-4C), 81.1 (C-2A), 78.2 (C-2B), 77.8 (C-3B), 74.7 (C-4A), 73.9 (CH_2Ph), 73.6 (C-5A), 73.4 (CH_2Ph), 73.3 (C-5C), 73.3 (CH_2Ph), 72.1 (CH_2Ph), 72.0 (C-5B), 71.8 (CH_2Ph), 71.7 (CH_2Ph), 71.1 (C-5D), 70.9 (C-3D), 69.6 (C-4B), 69.0 (C-2D), 68.3 (C-3C), 67.9 (C-4D), 67.8, 67.4, 66.9 and 61.9 (4C-6), 65.5 (O–*C*H$_2$–CH_2Si–), 55.7 (C-2C), 19.2 (–O–C=O–*C*H$_3$), 17.1 (O–CH_2–*C*H$_2$Si–), −3.0 ($Si(CH_3)_3$); MS *m*/*z* (CI, NH_3): 1912.9 $(M+NH_4)^+$. Anal. Calcd for $C_{109}H_{111}NO_{27}Si$ (1895.17): C 69.08, H 5.90, N 0.74; found C 68.98, H 5.92, N 0.71.

6.2.3. 2-(Trimethylsilyl)ethyl (4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7). Sodium (40 mg, 0.02 M) was added by portions to a stirred solution of **6** (7.11 g, 3.75 mmol) in MeOH/CH_2Cl_2 (80 mL/40 mL). The mixture was stirred for 2 h, neutralized by Amberlite-IR 120 (H^+), filtered and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 10% MeOH in DCM) to afford a white powder (4.98 g, 92%), which was directly engaged in the next step. Benzaldehyde dimethylacetal (1.04 mL, 6.93 mmol) and *p*TsOH (190 mg, 1 mmol) were added to a stirred solution of the previous powder in CH_3CN (100 mL). The mixture was stirred at room temperature (15 min), neutralized by solid K_2CO_3, filtered through Celite and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 65% EtOAc in cyclohexane) to afford **7** (4.8 g, 91%) as a white powder. $[\alpha]_D$ −18 (*c* 1 in chloroform); mp: 95–96 °C (EtOAc/cyclohexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.20 (m, 39H, arom.), 5.51 (d, 1H, $J_{1-2}=8.4$ Hz, H-1C), 5.47 (s, 1H, benzylidène), 1.08–1.04 (m, 2H, O–CH_2–C*H*$_2$Si), 0.06 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz): δ 168.2, 167.9 (2C=O Phth), 139.0, 138.7, 138.4, 138.3, 138.2, 137.7, 137.2 and 131.1 (8C arom.), 133.8–126.2 (CH arom.), 103.6, 102.9, 101.9, 101.1 and 98.7 (benzylidène, C-1A, C-1B, C1-C and C1-D); MS *m*/*z* (CI, NH_3): 1541 $(M+NH_4)^+$. Anal. Calcd for $C_{86}H_{97}NO_{22}Si$ (1524.80): C 67.74, H 6.41, N 0.92; found C 67.62, H 6.52, N 0.93.

6.2.4. 2-(Trimethylsilyl)ethyl (4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8). $NH_2NH_2{\cdot}H_2O$ (45 mL) and H_2O (25 mL) were added to a stirred solution of **7** (4.8 g, 3.15 mmol) in ethanol (200 mL). The mixture was stirred overnight under reflux and then evaporated in vacuum. The residue was diluted in a mixture of DCM–MeOH (v/v=1/1, 20 mL). The precipitate was filtered and acetic anhydride (5 mL, excess) was added to the filtrate at room temperature. After 2 h, solvents were evaporated in vacuum. The residue was purified by flash chromatography (eluent, 5% MeOH in DCM) and precipitated in a mixture of EtOAc/hexanes to afford **8** (3.74 g, 83%) as a white powder. $[\alpha]_D$ −11 (*c* 1 in chloroform); mp: 98–102 °C (EtOAc/hexanes); ^{1}H NMR (400 MHz, C_6D_6/CD_3OD: 1:1): δ 7.80–7.40 (m, 35H, arom.), 5.59 (s, 1H, benzylidene), 5.43 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 5.17 (d, 1H, $J_{gem}=11.3$ Hz, CHPh), 5.03 (s, 2H, CH_2Ph), 5.00 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 4.98 (d, 1H, $J_{gem}=11.3$ Hz, CHPh), 4.92 (d, 1H, $J_{1-2}=8.4$ Hz, H-1C), 4.81 (d, 1H, $J_{1-2}=8.8$ Hz, H-1B), 4.80 (d, 1H, $J_{gem}=11.8$ Hz, CHPh), 4.77 (d, 1H, $J_{gem}=11.8$ Hz, CHPh), 4.67 (2d, 2H, $J_{gem}=12.0$ Hz, 2CHPh), 4.60 (d, 1H, $J_{1-2}=7.8$ Hz, H-1D), 4.56 (d, 1H, $J_{1-2}=7.8$ Hz, H-1A), 4.50 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.49 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.47 (de, 1H, $J_{4-3}=3.3$ Hz, H-4B), 4.32 (t, 1H, $J_{4-3}=J_{4-5}=9.8$ Hz, H-4A), 4.30 (dd, 1H, $J_{2-3}=10.0$ Hz, H-2C), 4.26–4.14 (m, 4H, H-6Db, –O–C*H*–CH_2–Si and H-6C), 4.13 (de, 1H, $J_{4-3}=3.7$ Hz, H-4D), 4.07–4.02 (m, 4H, H-2D, H-2B, H-6Bb and H-6Ab), 4.02 (t, 1H, $J_{3-4}=10.0$ Hz, H-3C), 3.96 (t, 1H, $J_{4-5}=10.0$ Hz, H-4C), 3.94–3.89 (m, 2H, H-6Da and H-6Ba), 3.86 (dd, 1H, $J_{3-2}=9.3$ Hz, H-3B), 3.86–3.78 (m, 5H, H-5C, H-3A, H-5B, H-6Aa and –O–C*H*–CH_2–Si), 3.77 (dd, 1H, $J_{3-2}=9.9$ Hz, H-3D), 3.65 (dd, 1H, $J_{2-3}=9.1$ Hz, H-2A), 3.50–3.48 (m, 1H, H-5A), 3.30 (se, 1H, H-5D), 1.96 (S, 3H, –NH–C=O–C*H*$_3$), 1.23–1.16 (m, 2H, O–CH_2–CH_2–Si), 0.18 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz): δ 173.7 (–NH–*C*=O–CH_3), 140.6, 140.4, 140.2, 139.9, 139.6, 139.6 and 139.3 (7C arom.), 129.9–127.4 (35CH arom.), 105.0 (C-1D), 104.3 (C-1A), 104.0 (C-1C), 103.7 (C-1B), 102.0 (benzylidène), 84.0 (C-3A), 83.9 (C-3B), 83.2 (C-2A), 81.4 (C-4C), 80.2 (C-2B), 77.6 (C-4A), 76.9 (C-4D), 76.4 (CH_2Ph), 76.2 (C-5A), 76.0 (CH_2Ph), 75.9 (CH_2Ph), 75.6 (C-5C), 75.0 (C-5B), 74.6 (CH_2Ph), 74.5 (CH_2Ph), 74.1 (CH_2Ph), 73.6

(C-3D), 73.5 (C-3C), 71.9 (C-2D), 70.5 (C-6B), 70.1 (C-4B), 69.9 (C-6C), 69.9 (C-6D), 69.3 (C-6A), 68.2 (O–*C*H_2–CH_2–Si–), 68.0 (C-5D), 57.0 (C-2C), 23.3 (–NH–C=O–*C*H_3), 19.4 (O–CH_2–*C*H_2–Si–), −0.8 (Si(CH_3)$_3$); MS *m*/*z* (CI): 1436.8 MH^+. Anal. Calcd for $C_{80}H_{97}NO_{21}Si$ (1436.7): C 66.87, H 6.80, N 0.97; found C 66.57, H 6.81, N 0.98.

6.2.5. 1,2,4-Tri-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-(α/β)-D-glucopyranose (10α/β). Sodium hydride (9.2 g, 230 mmol, 60% in mineral oil) was added to a stirred solution of diacetone glucose (30 g, 115 mmol) in anhydrous DMF (300 mL) and benzyl bromide (27.4 mL, 230 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h, cooled to 0 °C and anhydrous methanol (10 mL) was added dropwise. The solvent was removed in vacuum and the resulting residue was partitioned between Et_2O and water. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuum. Amberlite-IR 120 (H^+) (60 g) was added to a suspension of the previous residue in H_2O (300 mL). After 15 h under reflux, the mixture was filtered and evaporated in vacuum. The crude compound was purified by precipitation (acetone/cyclohexane) to afford **9** (24.2 g, 85%) as a white powder. TBSCl (6.42 g, 42.58 mmol) was added to a stirred solution of **9** (10.95 g, 40.55 mmol) in pyridine (100 mL) at 0 °C under argon. The mixture was immediately warmed to room temperature and stirred for 2 h. Then acetic anhydride (37 mL, 365 mmol) was added dropwise to the mixture. After one night at room temperature, solvent was removed in vacuum. The residue was diluted with DCM, washed successively with HCl (1 M), saturated sodium hydrogencarbonate, brine, dried ($MgSO_4$) and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 15% EtOAc in cyclohexane) to afford a mixture of **10**α/β (α/β=1/1, 18.61 g, 90%) as a white powder.

Compound **10**β. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.28 (m, 5H, arom.), 5.70 (d, 1H, J_{1-2}=8.2 Hz, H-1), 5.16 (dd, 1H, J_{2-3}=9.3 Hz, H-2), 5.13 (t, 1H, J_{4-3}=J_{4-5}=9.5 Hz, H-4), 4.65 (s, 2H, CH_2Ph), 3.78 (t, 1H, H-3), 3.60 (ddd, 1H, J_{5-6a}=2.9 Hz, J_{5-6b}=5.2 Hz, H-5), 3.75–3.65 (m, 2H, H-6), 2.10, 2.01 and 2.00 (3s, 9H, 3–O–C=O–CH_3), 0.90 (s, 9H, SiC(CH_3)$_3$), 0.00 (2s, 6H, Si(CH_3)$_2$).

Compound **10**α. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.28 (m, 5H, arom.), 6.35 (d, 1H, J_{1-2}=3.7 Hz, H-1), 5.15 (t, 1H, J_{4-3}=J_{4-5}=10.0 Hz, H-4), 5.06 (dd, 1H, J_{2-3}=10.0 Hz, H-2), 4.68 (d, 1H, J_{gem}=11.8 Hz, CHPh), 4.65 (d, 1H, J_{gem}=11.8 Hz, CHPh), 4.00 (t, 1H, H-3), 3.90 (ddd, 1H, J_{5-6a}=2.7 Hz, J_{5-6b}=4.1 Hz, H-5), 3.75–3.65 (m, 2H, H-6), 2.20, 2.03 and 2.00 (3s, 9H, 3–O–C=O–CH_3), 0.90 (s, 9H, SiC(CH_3)$_3$), −0.03 (2s, 6H, Si(CH_3)$_2$).

MS *m*/*z* (CI, NH_3): 528 $(M+NH_4)^+$. Anal. Calcd for $C_{40}H_{44}O_9Si$ (510.23): C 58.80, H 7.50; found C 58.63, H 7.52.

6.2.6. 1,2,4-Tri-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-(α/β)-D-glucopyranose (11α/β). Sodium hydride (9.2 g, 230 mmol, 60% in mineral oil) was added to a stirred solution of diacetone glucose (30 g, 115 mmol) in anhydrous DMF (300 mL) and benzyl bromide (27.4 mL, 230 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h, cooled to 0 °C and anhydrous methanol (10 mL) was added dropwise. The solvent was removed in vacuum and the resulting residue was partitioned between Et_2O and water. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuum. Amberlite-IR 120 (H^+) (60 g) was added to a suspension of the previous residue in H_2O (300 mL). After one night under reflux, the mixture was filtered and evaporated in vacuum. The crude compound was purified by precipitation (acetone/cyclohexane) to afford **9** (24.2 g, 85%) as a white powder. TBDMSCl (6.42 g, 42.58 mmol) was added to a stirred solution of **9** (10.95 g, 40.55 mmol) in pyridine (100 mL) at 0 °C under argon. The mixture was immediately warmed to room temperature and stirred for 2 h. Then benzoyl chloride (27.86 mL, 240 mmol) was added dropwise to the mixture. After one night at room temperature, solvent was removed in vacuum. The residue was diluted with DCM, washed successively with HCl (1 M), saturated sodium hydrogencarbonate, brine, dried ($MgSO_4$) and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 20% EtOAc in cyclohexane) to afford a mixture of **11**α/β (α/β=1/3, 25.4 g, 90%) as a white powder. A small amount of this mixture was selectively recrystallized from hexanes and gave pure **11**β as a white powder.

Compound **11**β. $[\alpha]_D$ −31 (*c* 1 in chloroform); mp: 129–130 °C (hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.04 (m, 20H, arom.), 6.17 (d, 1H, J_{1-2}=7.8 Hz, H-1), 5.71 (dd, 1H, J_{2-3}=8.8 Hz, H-2), 5.58 (t, 1H, J_{4-3}=J_{4-5}=8.8 Hz, H-4), 4.68 (d, 1H, J_{gem}=11.5 Hz, CHPh), 4.64 (d, 1H, J_{gem}=11.5 Hz, CHPh), 4.20 (t, 1H, H-3), 3.98 (ddd, 1H, J_{5-6a}=3.2 Hz, J_{5-6b}=5.3 Hz, H-5), 3.91 (dd, 1H, J_{6b-6a}=11.4 Hz, H-6b), 3.82 (dd, 1H, H-6a), 0.85 (s, 9H, SiC(CH_3)$_3$), −0.02 and −0.01 (2s, 6H, Si(CH_3)$_2$); ^{13}C NMR (100 MHz): δ 164.9, 164.9 and 164.8 (3O–*C*=O), 137.1, 129.5, 129.3 and 128.7 (4C arom.), 133.6–127.6 (20CH arom.), 92.5 (C-1), 79.3 (C-3), 76.0 (C-5), 73.9 (CH_2Ph), 72.1 (C-2), 70.4 (C-4), 62.7 (C-6), 25.7 (SiC(*C*H_3)$_3$), 18.2 (Si*C*(CH_3)$_3$), −5.5 (SiCH_3), −5.4 (SiCH_3); MS *m*/*z* (CI, NH_3): 714.4 $(M+NH_4)^+$. Anal. Calcd for $C_{40}H_{44}O_9Si$ (696.87): C 68.94, H 6.36; found C 68.98, H 6.38.

Compound **11**α. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.04 (m, 20H, arom.), 6.80 (d, 1H, J_{1-2}=3.7 Hz, H-1), 5.62 (t, 1H, J_{4-3}=J_{4-5}=9.7 Hz, H-4), 5.56 (dd, 1H, J_{2-3}=9.7 Hz, H-2), 4.72 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.64 (d, 1H, J_{gem}=11.5 Hz, CHPh), 4.50 (t, 1H, H-3), 4.28–4.23 (m, 1H, H-5), 3.85–3.82 (m, 2H, H-6), 0.83 (s, 9H, SiC(CH_3)$_3$), −0.03 (2s, 6H, Si(CH_3)$_2$).

6.2.7. Methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-(α/β)-D-glucopyranosuronate (12α/β). A solution of the Jones reagent (44.9 mL of H_2SO_4 (3.5 M) and 9.7 g of CrO_3) was dropwise added to a stirred solution of **10**α/β (12.5 g, 24.5 mmol) in acetone (100 mL) at 0 °C. This mixture was immediately warmed to room temperature, stirred for 5 h and then diluted with CH_2Cl_2 and H_2O. The aqueous layer was extracted three times with CH_2Cl_2 and the combined extracts were dried ($MgSO_4$), filtered and the solvent was removed in vacuum. The residue was dissolved in DMF (100 mL) and stirred at room temperature. CH_3I

(8.2 mL, 122.5 mmol) and $KHCO_3$ (15.3 g, 147 mmol) were added to the mixture. After one night of stirring, the suspension was filtered through Celite, the filtrate was diluted with H_2O, extracted three times with Et_2O, dried ($MgSO_4$) and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 20% EtOAc in cyclohexane) to afford a mixture of **12**α/β (α/β=1/1, 6.75 g, 65%) as a white powder.

Compound **12**β. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.17 (m, 5H, arom.), 5.63 (d, 1H, J_{1-2}=7.5 Hz, H-1), 5.17 (dd, 1H, J_{4-3}=9.0 Hz, J_{4-5}=9.4 Hz, H-4), 5.10 (dd, 1H, J_{2-3}=8.9 Hz, H-2), 4.64 (d, 1H, J_{gem}=12.0 Hz, CHPh), 4.58 (d, 1H, J_{gem}=12.0 Hz, CHPh), 4.00 (d, 1H, H-5), 3.73 (t, 1H, H-3), 3.65 (s, 3H, –CO_2CH_3), 1.94 and 2×1.93 (2s, 9H, 3 –O–C=O–CH_3).

Compound **12**α. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.17 (m, 5H, arom.), 6.32 (d, 1H, J_{1-2}=3.6 Hz, H-1), 5.18 (dd, 1H, J_{4-3}=9.4 Hz, J_{4-5}=9.9 Hz, H-4), 5.02 (dd, 1H, J_{2-3}=9.4 Hz, H-2), 4.58 (s, 2H, CH_2Ph), 4.26 (d, 1H, H-5), 3.93 (t, 1H, H-3), 3.64 (s, 3H, –CO_2CH_3), 2.09, 2.03 and 1.91 (3s, 9H, 3 –O–C=O–CH_3); MS *m/z* (CI, NH_3): 442 (M+NH_4)$^+$; l. Calcd for $C_{20}H_{24}O_{10}$ (424.407): C 56.60, H 5.70; found C 56.52, H 5.72.

6.2.8. Methyl 1,2,4-tri-*O*-benzoyl-3-*O*-benzyl-(α/β)-D-glucopyranosuronate (13α/β). Oxidation and esterification of **11**α/β (12.33 g, 17.70 mmol), as described for **12**α/β, yielded **13**α/β (7.35 g, 68%) as a white powder.

Compound **13**β. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.16 (m, 20H, arom.), 6.29 (d, 1H, J_{1-2}=6.2 Hz, H-1), 5.86 (t, 1H, J_{4-3}=J_{4-5}=7.4 Hz, H-4), 5.70 (dd, 1H, J_{2-3}=7.4 Hz, H-2), 4.82 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.76 (d, 1H, J_{gem}=11.4 Hz, CHPh), 4.57 (d, 1H, H-5), 4.29 (t, 1H, H-3), 3.55 (s, 3H, –CO_2CH_3); ^{13}C NMR (100 MHz): δ 167.6, 165.2, 164.9, and164.6 (4O–C=O), 136.9, 2×129.0 and 128.9 (4C arom.), 136.9–127.8 (20CH arom.), 91.5 (C-1), 76.5 (C-3), 73.9 (CH_2Ph), 73.1 (C-5), 70.6 (C-2), 70.0 (C-4), 52.8 (–CO_2CH_3).

Compound **13**α. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.16 (m, 20H, arom.), 6.87 (d, 1H, J_{1-2}=3.5 Hz, H-1), 5.86 (dd, 1H, J_{4-3}=9.0 Hz, J_{4-5}=9.3 Hz, H-4), 5.70 (dd, 1H, J_{2-3}=9.1 Hz, H-2), 4.78 (s, 2H, CH_2Ph), 4.70 (d, 1H, H-5), 4.29 (t, 1H, H-3), 3.71 (s, 3H, –CO_2CH_3); ^{13}C NMR (100 MHz): δ 167.6, 165.2, 165.1 and 164.0 (4O–C=O), 136.9, 2×129.0 and 128.9 (4C arom.), 136.9–127.8 (20CH arom.), 89.8 (C-1), 75.6 (C-3), 74.6 (CH_2Ph), 71.5 (C-5), 71.0 (C-2), 70.9 (C-4), 52.9 (–CO_2CH_3); MS *m/z* (CI, NH_3): 628.1 (M+NH_4)$^+$. Anal. Calcd for $C_{35}H_{30}O_{10}$ (610.62): C 68.84, H 4.95; found C 68.76, H 4.91.

6.2.9. Methyl 1,2,4-tri-*O*-acetyl-3-*O*-levulinyl-(α/β)-D-glucopyranosuronate (14α/β). A solution of **12**α/β (3 g, 7.07 mmol) in MeOH (10 mL) and EtOAc (10 mL) was hydrogenated in the presence of 10% Pd/C (100 mg) for 1 h at room temperature, filtered through Celite and concentrated. The residue was dissolved in pyridine (20 mL) and stirred at room temperature. Levulinic anhydride (5.25 g, 21.21 mmol) was added and the mixture was stirred overnight. Solvent was removed in vacuum. The residue was diluted with DCM, washed with HCl (1 M), saturated sodium hydrogen carbonate and brine. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 20% EtOAc in cyclohexane) to afford a mixture of **14**α/β (2.44 g, 82%) as a white powder.

Compound **14**β. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (d, 1H, J_{1-2}=7.9 Hz, H-1), 5.35 (t, 1H, J_{3-2}=J_{3-4}=9.4 Hz, H-3), 5.24 (t, 1H, J_{4-5}=9.7 Hz, H-4), 5.17 (dd, 1H, H-2), 4.20 (d, 1H, H-5), 3.76 (s, 3H, –CO_2CH_3), 2.75 and 2.51 (m, 4H, CH_3C=O–CH_2–CH_2–), 2.17, 2.13, 2.09 and 2.08 (4s, 12H, 3 –C=O–CH_3 and CH_3C=O–CH_2).

Compound **14**α. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.40 (d, 1H, J_{1-2}=3.7 Hz, H-1), 5.57 (t, 1H, J_{3-2}=J_{3-4}=9.9 Hz, H-3), 5.24 (t, 1H, J_{4-5}=9.7 Hz, H-4), 5.16 (dd, 1H, H-2), 4.40 (d, 1H, H-5), 3.77 (s, 3H, –CO_2CH_3), 2.75 and 2.51 (m, 4H, CH_3C=O–CH_2–CH_2–), 2.20, 2.18, 2.10 and 2.07 (4s, 12H, 3 –C=O–CH_3 and CH_3C=O–CH_2); MS *m/z* (CI, NH_3): 450 (M+NH_4)$^+$. Anal. Calcd for $C_{18}H_{24}O_{12}$ (432.38): C 50.00, H 5.59; found C 49.91, H 5.61.

6.2.10. Methyl 1,2,4-tri-*O*-benzoyl-3-*O*-levulinyl-(α/β)-D-glucopyranosuronate (15α/β). Hydrogenation and protection with levulinic anhydride of **15**α/β (2.96 g, 4.85 mmol), as described for **15**α/β, yielded **15**α/β (2.4 g, 80%) as a white powder. A small amount of this mixture was selectively crystallized from Et_2O and gave pure **15**β as a white powder.

Compound **15**β. $[\alpha]_D$ −9 (*c* 1 in chloroform); mp: 153–155 °C (Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.43 (m, 15H, arom.), 6.26 (d, 1H, J_{1-2}=7.5 Hz, H-1), 5.84 (t, 1H, J_{3-2}=J_{3-4}=8.9 Hz, H-3), 5.72 (dd, 1H, H-2), 5.67 (t, 1H, J_{4-5}=9.0 Hz, H-4), 4.54 (d, 1H, H-5), 3.66 (s, 3H, –CO_2CH_3), 2.58 and 2.47 (2t, 4H, CH_3C=O–CH_2–CH_2–), 1.97 (s, 3H, CH_3C=O–CH_2); ^{13}C NMR (100 MHz): δ 205.3 (C=O), 171.6, 166.9, 165.2, 164.9 and 164.4 (5O–C=O), 2×128.7 and 128.2 (3C arom.), 133.9–128.5 (15CH arom.), 92.1 (C-1), 73.2 (C-5), 71.1 (C-3), 70.0 (C-2), 69.4 (C-4), 52.9 (–CO_2CH_3), 37.7 and 27.9 (CH_3C=O–CH_2–CH_2–), 29.3 (CH_3C=O–CH_2); MS *m/z* (CI, NH_3): 636.3 (M+NH_4)$^+$. Anal. Calcd for $C_{33}H_{30}O_{12}$ (618.59): C 64.07, H 4.88; found C 63.98, H 4.90.

Compound **15**α. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.40 (m, 15H, arom.), 6.85 (d, 1H, J_{1-2}=3.7 Hz, H-1), 6.06 (t, 1H, J_{3-2}=J_{3-4}=10.0 Hz, H-3), 5.59 (t, 1H, J_{4-5}=10.0 Hz, H-4), 5.23 (dd, 1H, H-2), 4.66 (d, 1H, H-5), 3.67 (s, 3H, –CO_2CH_3), 2.53 and 2.42 (2t, 4H, CH_3C=O–CH_2–CH_2–), 1.92 (s, 3H, CH_3C=O–CH_2); ^{13}C NMR (100 MHz): δ 205.3 (C=O), 171.8, 167.2, 165.2, 165.1 and 164.0 (5O–C=O), 2×128.7 and 128.2 (3C arom.), 133.9–128.5 (15CH arom.), 89.6 (C-1), 70.8 (C-5), 2×69.7 (C-2 and C-4), 69.2 (C-3), 53.0 (–CO_2CH_3), 37.7 and 27.9 (CH_3C=O–CH_2–CH_2–), 29.3 (CH_3C=O–CH_2).

6.2.11. Methyl 2,4-di-*O*-acetyl-1-bromo-1-deoxy-3-*O*-levulinyl-α-D-glucopyranosyluronate (16). Hydrobromic acid 33% in AcOH (9 mL, excess) was added to a stirred solution of **14**α/β (1.4 g, 3.23 mmol) in CH_2Cl_2 (8 mL) at room temperature. The mixture was stirred for 2 h and

neutralized with saturated sodium hydrogencarbonate. The organic layer was washed with H_2O, dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 25% EtOAc in cyclohexane) to afford **16** (1.31 g, 90%) as a white unstable foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.54 (d, 1H, $J_{1-2}=4.0$ Hz, H-1), 5.57 (t, 1H, $J_{3-2}=J_{3-4}=10.0$ Hz, H-3), 5.17 (dd, 1H, $J_{4-5}=10.3$ Hz, H-4), 4.80 (dd, 1H, H-2), 4.50 (d, 1H, H-5), 3.70 (s, 3H, $-CO_2CH_3$), 2.70 and 2.45 (m, 4H, $CH_3C{=}O{-}CH_2{-}CH_2{-}$), 2.09, 2.06 and 2.03 (3s, 9H, 3 $-C{=}O{-}CH_3$ and $CH_3C{=}O{-}CH_2$).

6.2.12. Methyl 2,4-di-*O*-benzoyl-1-bromo-1-deoxy-3-*O*-levulinyl-α-D-glucopyranosyluronate (17). Bromination of **15**α/β (2.3 g, 3.72 mmol), as described for **14**α/β, yielded **17** (1.7 g, 80%) as a white unstable foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00–7.40 (m, 10H, arom.), 6.77 (d, 1H, $J_{1-2}=4.0$ Hz, H-1), 5.94 (t, 1H, $J_{3-2}=J_{3-4}=9.8$ Hz, H-3), 5.48 (dd, 1H, $J_{4-5}=10.2$ Hz, H-4), 5.12 (dd, 1H, H-2), 4.70 (d, 1H, H-5), 3.61 (s, 3H, $-CO_2CH_3$), 2.46 and 2.35 (2t, 4H, $CH_3C{=}O{-}CH_2{-}CH_2{-}$), 1.85 (s, 3H, $CH_3C{=}O{-}CH_2$); ^{13}C NMR (100 MHz): δ 205.2 (C=O), 171.6, 166.7, 165.2 and 165.1 (4O–C=O), 133.9 and 133.7 (2C arom.), 129.9–128.2 (10CH arom.), 85.5 (C-1), 72.2 (C-5), 70.6 (C-2), 69.2 (C-3), 68.6 (C-4), 52.9 ($-CO_2CH_3$), 37.7 and 27.9 ($CH_3C{=}O{-}CH_2{-}CH_2{-}$), 29.3 ($CH_3C{=}O{-}CH_2$).

6.2.13. 2-(Trimethylsilyl)ethyl(methyl 2,4-di-*O*-benzoyl-3-*O*-levulinyl-β-D-glucopyranosyluronate)-(1→3)-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (18). To a stirred mixture of **8** (1.8 g, 1.25 mmol), **17** (0.73 g, 1.25 mmol) and MS-4 Å (2.5 g) in anhydrous CH_2Cl_2 (15 mL) was added, at 0 °C and under argon, AgOTf (0.386 g, 1.5 mmol). After 1 h at 0 °C, one more equivalent of **17** and 1.2 equiv of AgOTf were added to the mixture, which was stirred for 2 h at room temperature, neutralized by Et_3N and filtered through Celite. The filtrate was successively washed with saturated sodium thiosulfate, brine, dried ($MgSO_4$) and evaporated in vacuum. The residue was purified by flash chromatography (eluent gradient, 2% MeOH in DCM) to afford first **18** (1.2 g, 50%) as a white powder and then **8** (0.54 g, 30%). $[\alpha]_D$ +2 (*c* 0.15 in chloroform); mp: 183–185 °C (DCM/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03–7.25 (m, 45H, arom.), 5.68 (t, 1H, $J_{3-2}=J_{3-4}=9.5$ Hz, H-3E), 5.60 (t, 1H, $J_{4-5}=9.5$ Hz, H-4E), 5.48 (s, 1H, benzylidène), 5.43 (dd, 1H, $J_{2-1}=7.4$ Hz, H-2E), 5.42 (d, 1H, H-1E), 5.12 (m, 1H, N*H*Ac), 5.03 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 4.93 (d, 1H, $J_{gem}=11.1$ Hz, CHPh), 4.90 (d, 1H, $J_{gem}=12.4$ Hz, CHPh), 4.78 (d, 1H, $J_{gem}=10.7$ Hz, CHPh), 4.76 (d, 1H, $J_{gem}=11.1$ Hz, CHPh), 4.74 (d, 1H, $J_{1-2}=7.8$ Hz, H-1C), 4.62 (d, 1H, $J_{gem}=12.4$ Hz, CHPh), 4.61 (d, 1H, $J_{gem}=12.1$ Hz, CHPh), 4.55 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.50 (d, 1H, $J_{gem}=12.0$ Hz, CHPh), 4.48 (d, 1H, $J_{1-2}=8.0$ Hz, H-1B), 4.47 (d, 1H, $J_{gem}=11.8$ Hz, CHPh), 4.43 (d, 1H, $J_{gem}=12.1$ Hz, CHPh), 4.38 (d, 1H, $J_{1-2}=7.9$ Hz, H-1A), 4.36 (d, 1H, $J_{1-2}=7.8$ Hz, H-1D), 4.35 (d, 1H, $J_{gem}=11.8$ Hz, CHPh), 4.31 (d, 1H, H-5E), 4.30 (d, 1H, $J_{4-3}=3.3$ Hz, H-4D), 4.22 (de, 1H, $J_{6b-6a}=12.1$ Hz, H-6Db), 4.06 (de, 1H, $J_{4-3}=3.6$ Hz, H-4B), 4.04–4.01 (m, 1H, H-6Da), 4.01–3.99 (m, 1H, $-CH{-}CH_2Si$), 3.98 (t, 1H, $J_{4-3}=J_{4-5}=9.1$ Hz, H-4A), 3.88 (dd, 1H, $J_{2-3}=9.9$ Hz, H-2D), 3.87–3.84 (m, 1H, H-6Bb), 3.81 (dd, 1H, H-3D), 3.78–3.74 (m, 3H, H-6A and H-6Ba), 3.70 (t, 1H, $J_{4-3}=J_{4-5}=10.0$ Hz, H-4C), 3.68 (s, 3H, CO_2CH_3), 3.67–3.62 (m, 2H, H-2C and H-2B), 3.62–3.58 (m, 1H, $-CH{-}CH_2Si$), 3.72 (t, 1H, $J_{3-2}=9.1$ Hz, H-3A), 3.58–3.52 (m, 3H, H-3C, H-3B and H-5B), 3.53–3.50 (m, 2H, H-6C), 3.49–3.44 (m, 2H, H-5D and H-5C), 3.42 (dd, 1H, H-2A), 3.40–3.37 (m, 1H, H-5A), 2.53 and 2.42 (2t, 4H, $J_{CH_2-CH_2}=6.7$ Hz, $CH_3C{=}O{-}CH_2{-}CH_2{-}$), 1.90 (s, 3H, $CH_3C{=}O{-}CH_2$), 1.59 (s, 3H, NH*Ac*), 1.06 (m, 2H, $-CH_2{-}CH_2Si$), 0.05 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz): δ 206.0 (C=O), 172.0, 171.4, 167.8, 165.7 and 165.5 (3O–C=O, C=O–O and NHC=O), 139.5, 139.3, 139.2, 138.8, 138.7, 138.2, 138.0, 129.7 and 129.1 (9C arom.), 134.5–126.5 (45CH arom.), 103.9 (C-1D), 103.5 (C-1A), 102.8 (C-1B), 101.8 (C-1C), 100.8 (CH, benzylidene), 100.7 (C-1E), 83.2, 2×82.3, 81.5, 79.8, 78.7, 77.0, 75.7, 75.5, 74.1, 73.4, 73.2, 72.8, 72.2, 72.1, 70.2, 69.8, 68.5, 67.3 and 56.8 (20 CH cycle), 75.8, 75.3, 74.9, 2×73.8 and 73.7 (6CH_2Ph), 69.8, 2×69.0 and 68.7 (4C-6), 67.7 ($-CH_2{-}CH_2Si$), 53.3 ($-CO_2CH_3$), 38.2 and 28.4 ($CH_3C{=}O{-}CH_2{-}CH_2{-}$), 29.7 ($CH_3C{=}O{-}CH_2$), 23.4 (NH–C=O–$CH_3$), 18.8 ($-CH_2{-}CH_2Si$), −1.0 ($Si(CH_3)_3$); MS *m/z* (FAB): 1955 $(M+Na)^+$. Anal. Calcd for $C_{106}H_{121}NO_{31}Si$ (1933.21): C 65.85, H 6.30, N 0.72; found C 65.80, H 6.31, N 0.69.

6.2.14. 2-(Trimethylsilyl)ethyl (methyl 2,4-di-*O*-benzoyl-3-*O*-levulinyl-β-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (19). A solution of **18** (1.2 g, 0.620 mmol) in MeOH (20 mL) was hydrogenated in the presence of 10% Pd/C (200 mg) for 5 h at room temperature, filtered through Celite and concentrated. The residue was acetylated with acetic anhydride (15 mL)-pyridine (30 mL) for 20 h at room temperature. Solvent was then removed in vacuum. The residue was diluted with DCM, washed with HCl (1 M), saturated sodium hydrogencarbonate and brine. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 2.5% MeOH in DCM) to afford **19** (0.70 g, 60%) as a white powder. $[\alpha]_D$ +6 (*c* 0.4 in chloroform); mp: 168–172 °C (DCM/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00–7.47 (m, 10H, arom.), 5.62 (dd, 1H, $J_{3-2}=8.5$ Hz, $J_{3-4}=9.2$ Hz, H-3E), 5.54 (dd, 1H, $J_{4-5}=9.7$ Hz, H-4E), 5.50 (d, 1H, $J_{4-3}=3.1$ Hz, H-4D or B), 5.40 (d, 1H, $J_{NHAc-2}=8.8$ Hz, N*H*Ac), 5.30 (d, 1H, $J_{4-3}=3.4$ Hz, H-4D or B), 5.26 (dd, 1H, $J_{2-1}=7.3$ Hz, H-2E), 5.18 (t, 1H, $J_{3-2}=J_{3-4}=9.3$ Hz, H-3A), 5.09 (t, 1H, $J_{3-2}=J_{3-4}=9.3$ Hz, H-3C), 5.07 (dd, 1H, $J_{2-3}=10.5$ Hz, $J_{2-1}=8.0$ Hz, H-2D or B), 5.00 (dd, 1H, $J_{2-3}=9.2$ Hz, $J_{2-1}=8.0$ Hz, H-2D or B), 4.90 (dd, 1H, $J_{2-1}=8.0$ Hz, H-2A), 4.87 (d, 1H, H-1E), 4.68 (de, 1H, $J_{6b-6a}=10.5$ Hz, H-6Cb), 4.59 (d, 1H, $J_{1-2}=7.7$ Hz, H-1C), 4.49 (d, 1H, H-1A), 4.46 (de, 1H, $J_{6b-6a}=11.9$ Hz, H-6Ab), 4.40 (d, 1H, H-1D or B), 4.35 (d, 1H, H-1D or B), 4.24 (d, 1H, H-5E), 4.16–4.11 (m, 2H, H-6Aa and H-6Db or H-6Bb), 4.07–4.05 (m, 2H, H-6Db or H-6Bb and H-6Da or H-6Ba), 4.03–3.99 (m, 1H, H-6Da or H-6Ba), 3.99–3.95 (m, 1H, $-CH{-}CH_2Si$), 3.96–3.94 (m, 1H, H-6Ca), 3.90 (dd, 1H, H-3D or B), 3.82 (t, 1H, $J_{5-6}=6.5$ Hz, H-5D or B), 3.78 (t, 1H, $J_{5-6}=6.5$ Hz, H-5D

or B), 3.78–3.71 (m, 3H, H-4C, H-4A and H-3D or H-3B), 3.73 (s, 3H, CO_2CH_3), 3.65–3.63 (m, 1H, H-5A), 3.59 (dd, 1H, H-2C), 3.56–3.54 (m, 1H, –C*H*–CH_2Si), 3.46–3.43 (m, 1H, H-5C), 2.49 and 2.37 (2t, 4H, $J_{CH_2-CH_2}=6.7$ Hz, CH_3–C=O–CH_2–C*H*2–), 2.19–1.91 (12s, 36H, 11 –O–C=O–CH_3 and CH_3C=O–CH_2), 1.68 (s, 3H, NH*Ac*), 1.00–0.90 (m, 2H, –CH_2–CH_2Si), 0.03 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz): δ 205.3 (C=O), 171.6, 170.5, 170.5, 2×170.4, 170.3, 170.1, 169.8, 2×169.7, 169.5, 168.8, 168.3, 166.7, 164.9 and 164.5 (15O–C=O), 129.0 and 128.8 (2C arom.), 133.5–128.4 (10CH arom.), 100.7 (C-1D or B), 100.6 (C-1E and C-1D or B), 100.4 (C-1C), 99.9 (C-1A), 76.8 (C-3D or B), 75.8 (C-4A), 75.7 (C-3D or B), 75.0 (C-4C), 72.7 (C-5E and C-3A), 72.6 (C-5C), 72.5 (C-5A), 71.6 (C-3C and C-2A), 71.5 (C-3E), 71.4 (C-2E), 71.1 (C-5D or B), 71.0 (C-5D or B), 70.9 (C-2D or B), 70.5 (C-2D or B), 69.5 (C-4E), 68.7 (C-4D or B), 68.1 (C-4D or B), 67.4 (–CH_2–CH_2Si), 62.2 (C-6A), 61.7 and 61.5 (C-6D and C-6B), 60.4 (C-6C), 54.4 (C-2C), 52.8 (–CO_2CH_3), 37.6 and 27.9 (CH_3C=O–CH_2–CH_2–), 29.2 (CH_3C=O–CH_2), 23.0–20.1 (11 –O–C=O–CH_3 and 1 NH–C=O–CH_3), 17.8 (–CH_2–CH_2Si), −1.5 ($Si(CH_3)_3$); MS *m*/*z* (CI, NH_3): 1783.0 $(M+NH_4)^+$. Anal. Calcd for $C_{79}H_{103}NO_{42}Si.1H_2O$: C 53.16, H 5.93, N 0.78; found C 52.73, H 5.93, N 0.61.

6.2.15. (Methyl 2,4-di-*O*-benzoyl-3-*O*-levulinyl-β-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl) trichloroacetimidate (20). To a stirred solution of **19** (500 mg, 0.283 mmol) in CH_2Cl_2 (15 mL) was added, at 0 °C, trifluoroacetic acid (2.4 mL, 31.3 mmol). After 4 h at room temperature, 1 mL of trifluoroacetic acid was added. The mixture, after stirring for 3 h, was diluted with CH_2Cl_2, neutralized with saturated sodium hydrogencarbonate. The organic layer was dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was dissolved in anhydrous CH_2Cl_2 (7 mL) and stirred at 0 °C under argon. Trichloroacetonitrile (0.426 mL, 4.25 mmol) and DBU (9 μL, 0.056 mmol) were successively added and, after stirring for 30 min, the mixture was directly purified by flash chromatography (eluent, 4% MeOH in DCM) to afford **20** (476 mg, 92%) as a white unstable foam; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H, –O–C=NH–CCl_3), 6.45 (d, 1H, $J_{1-2}=3.7$ Hz, H-1A).

6.2.16. (Methyl 2,4-di-*O*-benzoyl-3-*O*-levulinyl-β-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (21). To a stirred mixture of **20** (300 mg, 0.165 mmol), acceptor (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (180 mg, 0.414 mmol) and MS-4 Å (500 mg) in anhydrous CH_2Cl_2 (5 mL) was added, at 0 °C and under argon, $BF_3\cdot Et_2O$ (0.063 mL, 0.495 mmol). After stirring for 3 h at 0 °C, the mixture was neutralized with saturated sodium hydrogencarbonate and filtered through Celite. The organic layer was washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 2% MeOH in DCM) to afford **21** (258 mg, 75%) as a white foam. $[\alpha]_D$ −10 (*c* 0.5 in chloroform); mp: 114.5–115 °C (DCM/MeOH); IR (film): C–N_3 2109.3 cm^{-1}; C=O 1758.5 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.46 (m, 15H, arom.), 5.95 (dt, 1H, $J_{5-4}=15.0$ Hz, $J_{5\text{-}CH_2}=6.7$ Hz, H-5 sphingosine), 5.63 (dd, 1H, $J_{3-2}=9.1$ Hz, $J_{3-4}=9.5$ Hz, H-3E), 5.63–5.61 (m, 1H, H-3 sphingosine), 5.61–5.60 (m, 1H, H-4 sphingosine), 5.54 (dd, 1H, $J_{4-5}=9.7$ Hz, H-4E), 5.50 (de, 1H, $J_{4-3}=3.4$ Hz, H-4D or B), 5.38 (d, 1H, $J_{NHAc-2}=8.7$ Hz, N*H*Ac), 5.30 (d, 1H, $J_{4-3}=3.8$ Hz, H-4D or B), 5.27 (dd, 1H, $J_{2-1}=7.3$ Hz, H-2E), 5.18 (t, 1H, $J_{3-2}=J_{3-4}=9.2$ Hz, H-3A), 5.09 (dd, 1H, $J_{3-2}=10.0$ Hz, $J_{3-4}=9.3$ Hz, H-3C), 5.07 (dd, 1H, $J_{2-3}=10.0$ Hz, $J_{2-1}=8.0$ Hz, H-2D or B), 5.00 (dd, 1H, $J_{2-3}=10.0$ Hz, $J_{2-1}=8.0$ Hz, H-2D or B), 4.90 (dd, 1H, $J_{2-1}=7.8$ Hz, H-2A), 4.87 (d, 1H, H-1E), 4.69 (dd, 1H, $J_{6b-6a}=12.0$ Hz, $J_{6b-5}=2.2$ Hz, H-6Cb), 4.59 (d, 1H, $J_{1-2}=7.6$ Hz, H-1C), 4.53 (d, 1H, H-1A), 4.46 (dd, 1H, $J_{6b-6a}=11.3$ Hz, $J_{6b-5}=1.6$ Hz, H-6Ab), 4.40 (d, 1H, H-1D or B), 4.36 (d, 1H, H-1D or B), 4.25 (d, 1H, H-5E), 4.13 (dd, 1H, $J_{6b-6a}=11.5$ Hz, $J_{6b-5}=6.2$ Hz, H-6Bb), 4.09–3.99 (m, 4H, H-6Aa, H-6D and H-6Ba), 3.98–3.94 (m, 1H, H-2 sphingosine), 3.92 (dd, 1H, $J_{6a-5}=4.0$ Hz, H-6Ca), 3.90 (dd, 1H, H-3D or H-3B), 3.91–3.86 (m, 1H, H-1b sphingosine), 3.83–3.76 (m, 2H, H-5D and H-5B), 3.79–3.73 (m, 3H, H-4C, H-4A and H-3D or H-3B), 3.73 (s, 3H, CO_2CH_3), 3.64–3.57 (m, 3H, H-5A, H-2C and H-1a sphingosine), 3.44 (dt, 1H, $J_{5-4}=9.3$ Hz, H-5C), 2.50 and 2.37 (2t, 4H, $J_{CH_2-CH_2}=6.4$ Hz, CH_3–C=O–CH_2–CH_2–), 2.19–1.91 (12s, 36H, 11 –O–C=O–CH_3 and CH_3C=O–CH_2), 1.70 (s, 3H, NH*Ac*), 1.47–1.21 (m, 24H, CH_2 sphingosine), 0.91 (t, 3H, $J_{CH_3-CH_2}=7.0$ Hz, CH_3 sphingosine); ^{13}C NMR (100 MHz): δ 205.3 (C=O), 171.6, 170.6, 170.5, 2×170.4, 170.3, 170.2, 2×169.8, 169.7, 169.5, 168.8, 168.4, 166.7, 165.0, 164.9 and 164.5 (17O–C=O), 129.8, 129.0 and 128.7 (3C arom.), 133.5–128.4 (15CH arom.), 100.7 (C-1D and C-1B), 100.6 (C-1E), 100.4 (C-1C), 100.3 (C-1A), 76.8 (C-3D or C-3B), 75.7 (C-4A or C-4C), 75.6 (C-4C or C-4A), 75.0 (C-3D or C-3B), 74.6 (C-3 sphingosine), 72.7 (C-5E and C-5A), 72.6 (C-5C), 72.5 (C-3A), 71.6 (C-2D or C-2B), 71.5 (C-2A), 71.4 (C-3E), 71.3 (C-2E), 71.2 (C-5D or C-5B), 71.0 (C-5D or C-5B), 70.9 (C-2D or C-2B), 70.5 (C-3C), 69.5 (C-4E), 68.7 (C-4D or C-4B), 68.3 (C-1 sphingosine), 68.1 (C-4D or C-4B), 63.4 (C-2 sphingosine), 61.9 (C-6A), 61.7 and 61.5 (C-6D and C-6B), 60.4 (C-6C), 54.4 (C-2C), 52.8 (–CO_2CH_3), 37.6 and 27.8 (CH_3C=O–CH_2–CH_2–), 29.2 (CH_3C=O–CH_2), 32.9, 31.8, 29.6–28.6 and 22.6 (CH_2 sphingosine), 23.0–20.1 (11 –O–C=O–CH_3 and 1 NH–C=O–CH_3), 14.0 (CH_3 sphingosine); MS *m*/*z* (FAB): 2099.8 $(M+Na)^+$. Anal. Calcd for $C_{99}H_{128}N_4O_{44}$ (2078.159): C 57.21, H 6.20, N 2.69; found C 56.73, H 6.20, N 2.47.

6.2.17. (Methyl 2,4-di-*O*-benzoyl-3-*O*-levulinyl-β-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-3-*O*-benzoyl-4-octadecene-1,3-diol (22). Compound **21** (165 mg, 79.4 μmol) and PPh_3 (62.4 mg, 238.2 μmol) were dissolved in a mixture of THF/H_2O (3 mL/0.6 mL) and stirred overnight at 50 °C. Solvents were evaporated in

vacuum. The residue was purified by flash chromatography to afford a foam, which was directly engaged in the next step. To a stirred solution of this foam in CH_2Cl_2 (4 mL) were successively added stearic acid (42 mg, 198.5 μmol) and DCC (45 mg, 158.8 μmol) at room temperature. After stirring overnight, solvent was evaporated in vacuum. The residue was purified by flash chromatography (eluent, 3% MeOH in CH_2Cl_2) to afford **22** (143 mg, 78%) as a white foam. $[\alpha]_D$ +12 (*c* 0.26 in chloroform); mp: 172.5–173 °C (DCM/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00–7.45 (m, 15H, arom.), 5.89 (dt, 1H, $J_{5-4}=15.0$ Hz, $J_{5-CH_2}=6.8$ Hz, H-5 ceramide), 5.76 (d, 1H, $J_{NH\text{-}2}=9.3$ Hz, NH ceramide), 5.63 (t, 1H, $J_{3-4}=J_{3-2}=9.4$ Hz, H-3E), 5.58–5.55 (m, 1H, H-3 ceramide), 5.55 (t, 1H, $J_{4-5}=9.5$ Hz, H-4E), 5.50 (d, 1H, $J_{4-3}=3.4$ Hz, H-4D or H-4B), 5.52–5.47 (m, 1H, H-4 ceramide), 5.35 (d, 1H, $J_{NHAc\text{-}2}=8.7$ Hz, N*H*Ac), 5.30 (d, 1H, $J_{4-3}=3.4$ Hz, H-4D or H-4B), 5.26 (dd, 1H, $J_{2-1}=7.3$ Hz, H-2E), 5.18 (t, 1H, $J_{3-2}=J_{3-4}=9.5$ Hz, H-3A), 5.08 (dd, 1H, $J_{2-3}=10.2$ Hz, $J_{2-1}=8.0$ Hz, H-2D or H-2B), 5.08 (t, 1H, $J_{3-2}=J_{3-4}=9.2$ Hz, H-3C), 5.00 (dd, 1H, $J_{2-3}=10.0$ Hz, $J_{2-1}=8.1$ Hz, H-2D or H-2B), 4.90 (dd, 1H, $J_{2-3}=9.5$ Hz, $J_{2-1}=7.8$ Hz, H-2A), 4.88 (d, 1H, H-1E), 4.69 (dd, 1H, $J_{6b-6a}=10.7$ Hz, $J_{6b-5}=3.2$ Hz, H-6Cb), 4.57 (d, 1H, $J_{1-2}=7.8$ Hz, H-1C), 4.55–4.46 (m, 1H, H-2 ceramide), 4.46 (d, 1H, H-1A), 4.40 (d, 1H, H-1D or H-1B), 4.33 (de, 1H, $J_{6b-6a}=11.8$ Hz, H-6Ab), 4.32 (d, 1H, H-1D or H-1B), 4.24 (d, 1H, H-5E), 4.13 and 4.00 (2dd, 2H, $J_{6b-6a}=11.5$ Hz, $J_{6b-5}=J_{6a-5}=6.2$ Hz, H-6D or H-6B), 4.07–4.05 (m, 2H, H-6D or H-6B), 4.05–4.02 (m, 1H, H-1b ceramide), 3.98 (dd, 1H, $J_{6a-5}=3.4$ Hz, H-6Aa), 3.92 (dd, 1H, $J_{6a-5}=3.2$ Hz, H-6Ca), 3.90 (dd, 1H, H-3D or H-3B), 3.82 (t, 1H, $J_{5-6}=6.2$ Hz, H-5D or H-5B), 3.78–3.76 (m, 1H, H-5D or H-5B), 3.76–3.70 (m, 3H, H-4C, H-4A and H-3D or H-3B), 3.73 (s, 3H, CO_2CH_3), 3.68–3.62 (m, 1H, H-1a ceramide), 3.62–3.60 (m, 1H, H-2C), 3.61–3.58 (m, 1H, H-5A), 3.44 (dt, 1H, $J_{5-4}=9.2$ Hz, H-5C), 2.50 and 2.37 (2t, 4H, $J_{CH_2-CH_2}=7.0$ Hz, CH_3–C=O–CH_2–CH_2–), 2.19–1.91 (12s, 36H, 11 –O–C=O–CH_3 and CH_3C=O–CH_2), 1.70 (s, 3H, NH*Ac*), 1.70–1.68 and 1.29–1.25 (m, 56H, CH_2 ceramide), 0.91 (2t, 6H, $J_{CH_3-CH_2}=7.0$ Hz, CH_3 ceramide); ^{13}C NMR (100 MHz): δ 205.3 (C=O), 172.6, 171.6, 170.6, 170.5, 2×170.4, 170.3, 170.2, 2×169.8, 169.7, 169.6, 168.8, 168.4, 166.7, 165.1, 164.9 and 164.6 (17O–C=O and 1 N–C=O), 137.6 (C-5 ceramide), 133.5–128.4 (15CH arom.), 130.2, 129.1 and 128.8 (3C arom.), 124.6 (C-4 ceramide), 100.7 (C-1D or C-1B), 100.6 (C-1E and C-1D or C-1B), 100.4 (C-1C), 100.2 (C-1A), 76.8 (C-3D or C-3B), 75.7 (C-4A), 75.6 (C-4C), 75.0 (C-3D or C-3B), 73.9 (C-3 ceramide), 72.7 (C-5E), 72.6 (C-5A), 72.5 (C-5C), 72.2 (C-3A), 2×71.6 (C-2D or C-2B and C-2A), 71.5 (C-3E), 71.4 (C-2E), 71.2 and 71.0 (C-5D and C-5B), 70.9 (C-2D or C-2B), 70.5 (C-3C), 69.5 (C-4E), 68.7 (C-4D or C-4B), 68.1 (C-4D or C-4B), 67.3 (C-1 ceramide), 61.9 (C-6A), 61.7 and 61.5 (C-6D and C-6B), 60.4 (C-6C), 54.4 (C-2C), 52.8 (–CO_2CH_3), 50.5 (C-2 ceramide), 37.6 and 27.9 (CH_3C=O–CH_2–CH_2–), 23.0–20.1 (11 –O–C=O–CH_3 and 1 NH–C=O–CH_3 and CH_3C=O–), 36.8, 33.3, 31.9, 29.7–28.9, 25.7 and 22.6 (CH_2 ceramide), 14.1 (CH_3 ceramide); MS *m*/*z* (FAB): 2340.0 $(M+Na)^+$. Anal. Calcd for $C_{117}H_{164}N_2O_{45}$ (2318.15): C 60.61, H 7.12, N 1.20; found C 60.68, H 7.31, N 1.11.

6.2.18. (Methyl 2,4-di-*O*-benzoyl-3-*O*-sulfo-β-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-3-*O*-benzoyl-4-octadecene-1,3-diol sodium salt (23). Hydrazine monoacetate (20 mg, 215 μmol) was added to a stirred solution of **22** (100 mg, 43 μmol) in EtOH (4 mL) at room temperature. After stirring for 1 h, the mixture was neutralized with saturated sodium hydrogen carbonate, washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was purified by flash chromatography (eluent, 2% MeOH in DCM) to afford the 3-OH compound (77 mg, 81%) as a white foam. ^{1}H RMN (400 MHz, $CDCl_3$): δ 4.15–4.1 (m, 1H, H-3E). $SO_3.Me_3N$ (72 mg, 520.5 μmol) was added to a stirred solution of the previous foam (77 mg, 34.7 μmol) in DMF (3 mL). The mixture was stirred at 40 °C for 20 h. MeOH (0.2 mL) and CH_2Cl_2 (0.2 mL) were added, and the solution was applied to a column of Sephadex LH-20 with 1:1 DCM/MeOH eluent. Glycolipid containing fractions were concentrated. Column chromatography (MeOH) of the residue on Dowex-50*2 (Na^+) resin gave **23** (65 mg, 81%) as an amorphous powder. $[\alpha]_D$ +10 (*c* 0.2 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$): δ 7.70–7.05 (m, 15H, arom.), 5.50 (dt, 1H, $J_{5-4}=13.4$ Hz, $J_{5-CH_2}=6.8$ Hz, H-5 ceramide), 5.18–5.12 (m, 1H, H-3 ceramide), 5.10 (d, 1H, $J_{4-3}=3.4$ Hz, H-4D or H-4B), 5.12–5.07 (m, 1H, H-4 ceramide), 5.00 (t, 1H, $J_{4-3}=J_{4-5}=9.7$ Hz, H-4E), 4.94 (d, 1H, $J_{4-3}=3.5$ Hz, H-4D or H-4B), 4.84 (dd, 1H, $J_{2-1}=7.8$ Hz, $J_{2-3}=9.7$ Hz, H-2E), 4.74 (t, 1H, $J_{3-2}=J_{3-4}=9.4$ Hz, H-3A), 4.70 (dd, 1H, $J_{3-2}=9.3$ Hz, $J_{3-4}=9.5$ Hz, H-3C), 4.61 (t, 1H, H-3E), 4.59 (dd, 1H, $J_{2-3}=9.5$ Hz, $J_{2-1}=8.0$ Hz, H-2D or H-2B), 4.58 (d, 1H, H-1E), 4.52 (dd, 1H, $J_{2-3}=9.9$ Hz, $J_{2-1}=8.0$ Hz, H-2D or H-2B), 4.37 (dd, 1H, $J_{2-1}=8.0$ Hz, H-2A), 4.24 (d, 1H, $J_{1-2}=8.2$ Hz, H-1C), 4.08 (de, 1H, $J_{6b-6a}=9.7$ Hz, H-6Cb), 4.17 (d, 1H, H-1A), 4.09–4.04 (m, 1H, H-2 ceramide), 4.06 (d, 1H, H-1D or H-1B), 4.00 (d, 1H, H-1D or H-1B), 3.83 (d, 1H, $J_{6b-6a}=10.0$ Hz, H-6Ab), 3.83 (d, 1H, H-5E), 3.73–3.55 (m, 7H, H-6Aa, H-5D, H-5B, H-6D and H-6B), 3.63 (dd, 1H, H-3D or H-3B), 3.58–3.55 (m, 1H, H-1b ceramide), 3.54–3.50 (m, 1H, H-6Ca), 3.39 (dd, 1H, H-3D or H-3B), 3.38 (t, 1H, H-5D or H-5B), 3.22 (t, 1H, $J_{4-5}=9.5$ Hz, H-4C), 3.28–3.25 (m, 1H, H-1a ceramide), 3.27 (s, 3H, CO_2CH_3), 3.25–3.22 (m, 1H, H-5A), 3.10 (dd, 1H, H-2C), 2.99 (dt, 1H, H-5C), 1.75–1.58 (11s, 33H, –O–C=O–CH_3), 1.50 (s, 3H, NH*Ac*), 1.82–1.70 and 0.94–0.87 (m, 56H, CH_2 ceramide), 0.49 (2t, 6H, $J_{CH_3-CH_2}=7.1$ Hz, CH_3 ceramide); ^{13}C NMR (100 MHz): δ 174.0, 171.2, 170.4, 2×170.3, 170.2, 170.0, 169.9, 169.7, 169.5, 169.4, 168.8, 168.7, 167.2, 165.3 and 2×164.9 (16O–C=O and 1 N–C=O), 132.4 (C-5 ceramide), 132.2–127.3 (15CH arom.), 129.5, 129.2 and 128.8 (3C arom.), 123.5 (C-4 ceramide), 100.0 (C-1D and C-1B), 99.8 (C-1E), 99.7 (C-1C), 99.3 (C-1A), 76.9, 76.4, 75.9, 2×75.0, 73.4, 72.2, 72, 71.7, 71.6, 71.5, 71.4, 71.0, 70.3, 70.2, 69.9, 69.8, 69.7, 68.8 and 68.1 (19 CH cycle and C-3 ceramide), 66.7 (C-1 ceramide), 61.9 (C-6C), 61.3 and 61.0 (C-6D and C-6B), 60.2 (C-6A), 53.9 (C-2C), 51.6 (–CO_2CH_3), 50.2 (C-2 ceramide), 35.5, 31.5, 31.1, 28.9–28.1, 25.3 and 21.8 (CH_2 ceramide), 21.3–18.9 (11 –O–C=O–CH_3 and 1 NH–C=O–CH_3), 12.8 (CH_3 ceramide). MS *m*/*z* (FAB):

2307.9 MH^+. Anal. Calcd for $C_{112}H_{157}N_2NaO_{46}S$ (2308.5): C 57.92, H 6.81, N 1.21; found C 59.13, H 6.96, N 1.10.

6.2.19. (3-*O*-Sulfo-β-D-glucopyranosyluronic acid)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-3-*O*-benzoyl-4-octadecene-1,3-diol disodium salt (1). To a stirred solution of **23** (60 mg, 26 μmol) in a mixture of THF/H_2O (3.8 mL/0.2 mL) was added at 0 °C a solution of 1.25 N LiOH (0.26 mL, 1040 μmol). The mixture was stirred 3 h between 0 and 5 °C. Solvents were evaporated in vacuum. The residue was dissolved in a mixture of THF/MeOH (2 mL/2 mL) and sodium (cat., 0.15 M) was added at 0 °C. The solution was stirred for 3 h at room temperature and purified on a column of Sephadex LH-20 with $CHCl_3$/MeOH/H_2O=6:4:0.8 to afford SGPG (35 mg, 90%) as a white foam; ^1H NMR (600 MHz, $CD_3S{=}OCD_3$): δ 5.54 (dt, 1H, J_{5-4}=15.2 Hz, J_{5-CH_2}=6.7 Hz, H-5 ceramide), 5.35 (dd, 1H, J_{4-5}=15.2 Hz, J_{4-3}=7.1 Hz, H-4 ceramide), 4.65, 4.54, 4.30, 4.27 and 4.16 (5d, 5H, 5H-1), 3.99 (t, 1H, $J_{3-2}=J_{3-4}$=9.0 Hz, H-3E), 1.82 (s, 3H, NH*Ac*), 0.85 (t, 6H, $J_{CH_3-CH_2}$=7.1 Hz, CH_3 ceramide); MS (MALDI-TOF): Calcd $(M+Na)^+$=1532.74; found=1532.7; Calcd $(M-H+2Na)^+$=1510.76; found=1510.7.

Acknowledgements

The authors thank Prof. Jacques Aubry (Nantes) for initiating this project.

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