Synthesis of a Novel Ether-Bridged GM3-Lactone Analogue as a Target for an Antibody-Based Cancer Therapy

Lutz F. Tietze,* Holger Keim, Christian O. Janßen, Christoph Tappertzhofen, and Jens Olschimke[a]

Abstract: We describe herein the synthesis of a new analogue of the GM3-lactone containing a cyclic ether moiety. The ether moiety was chosen as a replacement for the regular lactone group since it shows high resemblance with the lactone and is completely stable under biological conditions. The cyclic ether moiety was formed by reduction of the corresponding lactone to give the lactol followed by formation of the *S*,*O*-hemiacetal and hydrogenation. In addition, we have prepared haptens with a hexanoic acid moiety, which can be used for the preparation of poly- and monoclonal antibodies after binding to BSA or KLH. This is the first example of an analogue of the GM3-lactone which is stable under hydrolytic conditions in vitro and probably also in vivo. Reaction of lactone **18** with a Red/Al derivative led to the lactol **19** which was transformed into the *S*,*O*-hemiacetal **20** using 2,2′-bis(pyridinium) disulfide in quantitative yield. Hydrogenation with Raney Nickel gave **21** from which after removal of the protecting group at C-1a the trichloroacetimidate **25** was prepared. Reaction with azidosphingosine to give **26** followed by reduction of the azido group with $NHEt_3^+[(PhS)_3Sn]$, acylation with stearic acid using EDC and removal of the protecting groups led to the desired ether analogue of GM3 lactone **4**. In addition the trichloroacetimidate **25** was glycosidated with 6-hydroxyhexanoic acid methyl ester, which was deprotected to give **29**. The compound will be used for the preparation of poly- and monoclonal antibodies after coupling with BSA and KLH.

Keywords: cancer · 1,4-dioxanes · gangliosides · glycosidation · immunisation

Introduction

Tumor immunology has become an important topic in the treatment of cancer with the development of therapeutic and diagnostic tools.[1] A lot of progress has been made in the identification of several glycosphingolipids[2] as tumor-associated antigens using monoclonal antibodies,[3] but it seems that all of these glycosphingolipids are also found on normal cells.[4] However, a difference in the organization of these compounds attached to the membranes of malignant compared with normal cells and the formation of clusters consisting of transducer molecules to establish a type of microdomain may be responsible for the strong immune response towards these glycosphingolipids on tumor cells;[5] this effect is believed to be coupled with a signal transduction. Gangliosides play an important role in cell–cell and cell–matrix recognition processes and are known to possess antiproliferative activity by interaction with different growth factors, for example the epidermal growth factor (EGF).[6] On the other hand, it is well known that glycosphingolipids are present on tumor cells to a much higher extent and with a different distribution relative to normal cells.[3] In addition, there is some evidence that gangliosides, glycosphingolipids which contain a neuraminic acid unit, may form a lactone moiety by condensation of the carbocylic acid group of the neuraminic acid unit and one of the hydroxyl groups of one of the adjacent sugars.[7] The increased formation of the lactone on cancer cells could be attributed to the lower pH of cancer cells due to their metabolic difference in comparison to normal cells. The lactones of several gangliosides have been used as antigens for the formation of antibodies.[8] However, due to their low hydrolytic stability they are not suitable for an immune therapy. In an attempt to overcome this problem ganglioside lactams have been prepared.[9] Antibodies raised against the GM3-[1,2]lactam **3** were found to cross-react with GM3-lactone **2** in vitro, but not with the open form of GM3-ganglioside **1**. However, the lactams show also some hydrolytic instability at pH 7.0 and in addition, may be cleaved by proteases in vivo. Other approaches to an immune therapy of cancer are based on the adenocarcinoma antigen KH-1[10] and on various glycosphingolipids of the globo series.[11, 12]

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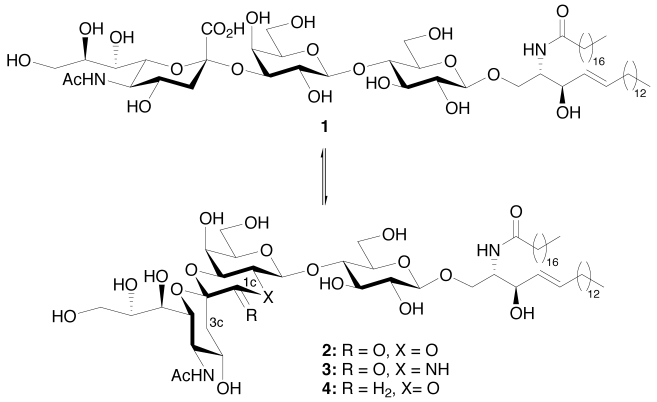
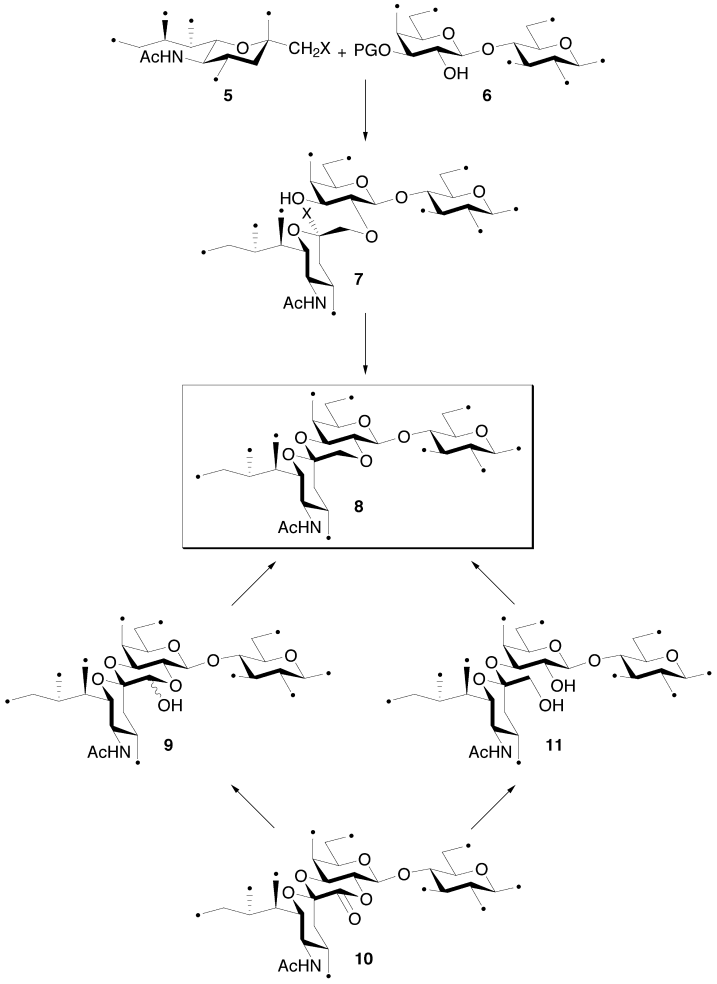


Scheme 1. Ganglioside GM3 **1**, GM3-[1,2]lactone **2** and its analogues **3** and **4.**

In this paper we describe the synthesis of the new analogue **4** of the GM3-lactone **2** containing a cyclic ether moiety. We have chosen the ether moiety as a replacement for the lactone group since it shows high resemblance with the lactone and is completely stable in a biological system. In addition, an ether moiety usually shows either none or only very little immunogenity in itself and thus prevents cross reactivity. For the formation of the ether linkage in **4** three different approaches were analysed:[13]

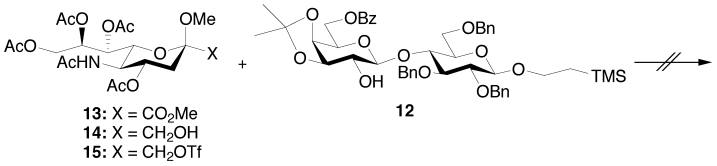
1) Alkylation of the hydroxyl group at C-2b of the lactose derivative **6** with a neuraminic acid derivative **5** containing a CH_2-X group instead of the carboxylic acid moiety followed by an intramolecular glycosidation to give **8** via **7**.
2) Reduction of the GM3-lactone derivative **10** to the corresponding lactol **9** and reductive replacement of the hydroxyl group to give **8**.
3) Reduction of the GM3-lactone derivative **10** to the corresponding diol **11** followed by formation of a sulfonate at the primary hydroxyl group at C-1c and intramolecular alkylation to give **8**.



Scheme 2. Approaches to the GM3-lactone ether analogue.

Results and Discussion

For the alkylation of the lactose derivative **12**,[14] which was prepared from glucose and galactose in 13 steps in 27% overall yield, we used neuraminic acid derivative **15**. The compound was synthesized by reduction of the methyl ester **13** with sodium borohydride in THF at $-70\,^{\circ}C$ to give the corresponding alcohol **14** in 44% yield. This alcohol was then transformed into the triflate **15** in 92% yield with triflic acid anhydride in CH_2Cl_2 in the presence of pyridine at $-50\,^{\circ}C$. To our surprise **15** was very stable and could be purified by chromatography and left at $20\,^{\circ}C$ for a week without any decomposition. We also tried to prepare the corresponding iodide and bromide by reaction of **14** with I_2, imidazole,[15] and triphenylphosphane as well as CBr_4 and triphenylphosphane.[16] However, both reactions did not lead to the formation of the desired products. The reaction of **15** with the lactose derivative **12** also was not successful applying different reaction conditions. We then performed a reaction of **15** with ethylene glycol in the presence of pyridine and also after formation of the alkoxide with sodium hydride in THF. Even in these cases a formation of the expected product was not observed, which can be explained by a strong steric hindrance of the CH_2-OTf group due to the adjacent quarternary center.



Scheme 3. Alkylation of **12** with the neuraminic acid derivative **15**.

Since the alkylation of **12** with **15** was not feasible we moved to the second approach. The necessary trisaccharide **16** containing an unprotected hydroxyl group at C-2b and C-4b was prepared from glucose, galactose, and neuraminic acid in 18 steps and an overall yield of 18% by known procedures. We did not expect a conflict of selectivity since the hydroxyl group at C-4b usually expresses a rather low nucleophilicity.[17] However, under basic conditions for example with DBU only a non-separable 1:1.7 mixture of the 1,2- and 1,4-lactone was obtained in 86% yield. Under acidic conditions for example

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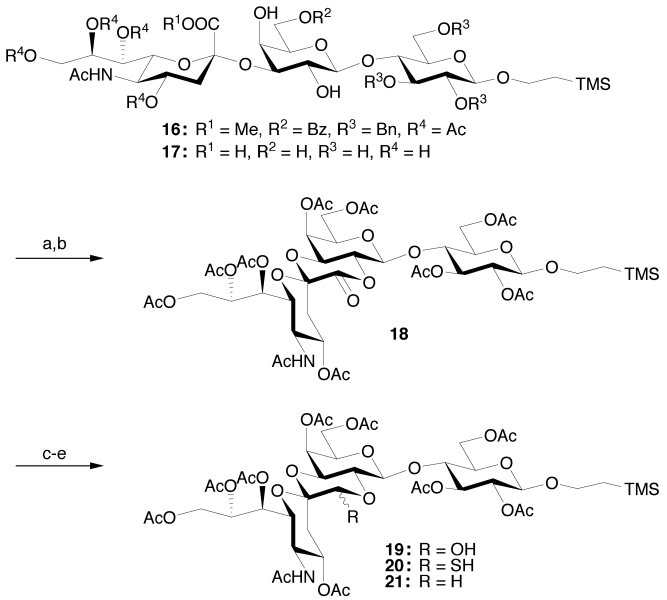
employing AcOH a reaction did not take place. In contrast, reaction of the unprotected trisaccharide **17** with acetic acid led exclusively to the desired 1,2-lactone in 60% yield.[18] Peracetylation of the unprotected lactone with acetic anhydride in pyridine in the presence of 4-(dimethylamino)pyridine[19] (DMAP) gave the GM3-ganglioside lactone derivative **18** which was then employed in the synthesis of the ether analogue **4**. The reduction of the lactone moiety in **18** to give the lactol **19** could be accomplished with the Red/Al derivative $Na[AlH(OCH_2CH_2OCH_3)_2\text{-}OEt]$[20] in toluene as solvent at $-78\,^{\circ}C$ in 70% yield. The acetyl groups were stable under these conditions.

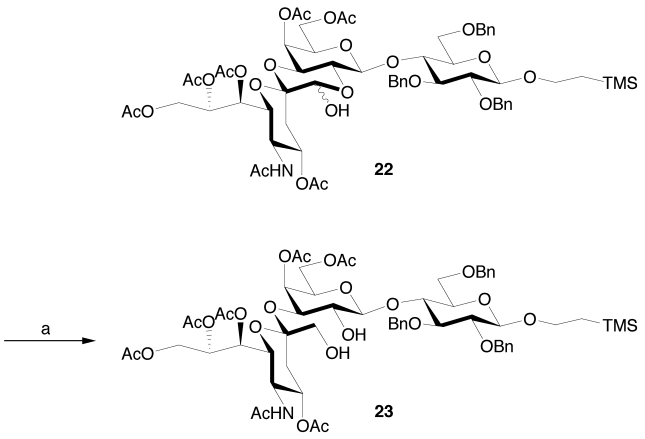


Scheme 4. Synthesis of **21**: a) HOAc, 3 d, rt, 57%; b) Ac_2O, pyridine, DMAP, 14 h, rt, 94%; c) $Na[AlH(CH_2OEt)_2OEt]$, toluene, $-78\,^{\circ}C$, 0.5 h, 70%; d) $(PyS)_2$, nBu$_3$P, CH_2Cl_2, 3 d, rt, 6 h, reflux, 100%; e) Raney nickel, EtOH, H_2 (1 atm), 30 h, rt, then Ac_2O, pyridine, DMAP, 14 h, 97%.

In contrast, the use of diisobutylaluminium hydride (DIBAL)[21] as reducting agent mostly led to partially deacetylated products while employing $LiBH_4$[22] or $NaBH_4$[23] led to the corresponding diols as the main products. The conversion of the lactol moiety in **19** into an ether functionality proved to be quite difficult. All attempts to directly remove the hydroxyl group in **19** in an one-step process for example with triethylsilane and $BF_3 \cdot OEt_2$ led to a cleavage of the glycosidic linkage at C-1a in **19**. The obvious transformation into an *S*,*O*-acetal followed by a reductive removal of sulfur also failed initially. The usual procedure using a thiol in the presence of $BF_3 \cdot OEt_2$[24] was not suitable since the formation of the thioglycoside at C-1a was observed. We therefore activated the hydroxyl group of the hemiacetal in **19** by conversion into the acetate and trichloroacetimidate, respectively, with acetic anhydride/pyridine as well as trichloroacetonitrile/DBU in high yield. But again, reaction with thiophenol in the presence of $BF_3 \cdot OEt_2$ or trimethylsilyl triflate only yielded the C-1a thiophenyl glycoside with an undesired cleavage of the protecting group at C-1a. However, reaction of **19** with five equivalents of 2,2′-bis(pyridinium) disulfide and tri-*n*-butylphosphane[25] in dichloromethane at 20 °C resulted in the formation of the thiohemiacetal **20** in quantitative yield. Surprisingly, the thiohemiacetal **20** with an SH-group and not the *S*,*O*-acetal containing an *S*-pyridyl moiety was obtained. The NMR spectra of **20** clearly show that **20** does not contain a pyridine moiety. We assume that under the reaction conditions the initially formed *S*,*O*-acetal undergoes an addition–elimination process at the pyridine group of the *S*,*O*-acetal with the 2-mercapto-pyridine produced in the reaction or the phosphane serving as a nucleophile. However, further study of this reaction was not carried out. Compound **20** could easily be transformed into the trisaccharide core **21** with the cyclic ether moiety in 97% yield by hydrogenation with H_2 and Raney nickel.[26] Acetylation in acetic anhydride and pyridine followed because the protecting groups were partly removed under the reaction conditions of hydrogenation. The presence of an ether functionality was proven by NMR spectroscopy in form of $^1H-^1H$ correlation experiments. Two doublets at $\delta = 3.44$ and 3.89 ($^2J(H,H) = 11.5$ Hz) can be assigned to the two geminal protons at C-1c. The sialic acid derivative was shown to be attached by an α-glycosidic bond following the rule $\delta[\alpha(3c\text{-}H_{eq})] > \delta[\beta(3c\text{-}H_{eq})]$ and $\delta[\alpha(3c\text{-}H_{ax})] < \delta[\beta(3c\text{-}H_{ax})]$. The α-anomer showed signals for 3c-H_{ax} and 3c-H_{eq} at $\delta = 1.63$ and $\delta = 2.70$, respectively, whereas for the corresponding β-anomer signals were found at $\delta = 1.89$ and $\delta = 2.15$ (3c-H_{ax} and 3c-H_{eq}).

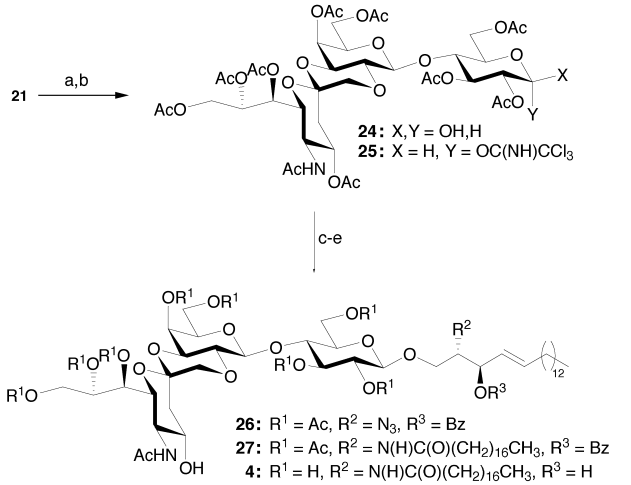
Though the desired trisaccharide **21** with the cyclic ether moiety was now in our hands we also tried to prepare **21** by an intramolecular alkylation according to our third approach. For this purpose we reduced the lactol **22**[14] prepared in a similar way as **19** with $NaBH_4$ to give the diol **23**. However, neither a Mitsunobu reaction[27] with DEAD and triphenylphosphane nor the formation of the corresponding bromide with CBr_4 and triphenylphosphane[15] was successful. In the first process a transformation was not observed and in the second reaction a cleavage of the glycosidic bond to the neuraminic acid derivative was found. In contrast, treatment of **23** with mesyl chloride led to the corresponding monomethanesulfonate at the primary hydroxyl group, but the formation of the ether moiety could not be achieved using even rather harsh conditions. These results are in line with the experiments on the intermolecular alkylation with **12** and ethylene glycol clearly showing that a nucleophilic attack at the CH_2-X group of the transformed neuraminic acid moiety seems to be highly sterically hindered.

For the synthesis of the complete ether analogue of GM3-lactone **4** we coupled the trisaccharide moiety **21** with ceramide. First, the trimethylsilyl ethyl protecting group at C-1a was removed with trifluoroacetic acid in dichloromethane,[28] which, however, led to a partial epimerisation at C-2c to give the thermodynamically more stable β-anomer to an extent of about 17%. Using stronger acids such as $BF_3 \cdot OEt_2$[29] or $ZnCl_2$[30] a nearly quantitative epimerisation at C-2c was observed, whereas under milder conditions including the use of tetrabutyl ammonium fluoride[31] a cleavage of the CH_2CH_2TMS group at C-1a could not be accomplished. The isomerisation could be confirmed by ^1H-NMR spectroscopy. Thus, the equatorial 3c-H of the 2c-α-epimer **24** resonates at $\delta = 2.50$, whereas for the 2c-β-epimer a signal at



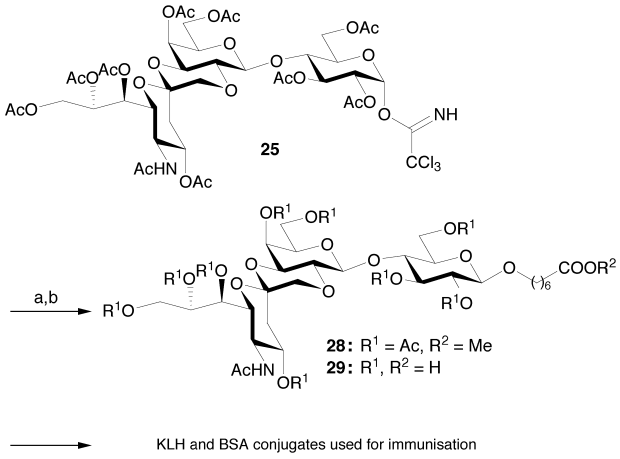
Scheme 5. Synthesis of **23**: a) $NaBH_4$, THF, 0 °C, 4 h, 62%.

$\delta = 1.96$ was observed for this hydrogen atom. A separation of the two isomers using different chromatographic techniques was not possible. However, at a later stage namely after the attachment of the sphingosine moiety a purification could be performed. For the glycosidation **24** was transformed into the corresponding trichloroacetimidate **25** in 93% yield based on **21** using trichloroacetonitrile and DBU. $BF_3 \cdot OEt_2$-mediated glycosidation with azido sphingosine at -40 °C in dichloromethane yielded the sphingosine derivative **26** in 66% yield.[32] Reduction of the azido group in **26** to give the corresponding amine was effected in good yields by use of $NHEt_3^+[(PhS)_3Sn]$.[33] Many other reagents tested including triphenylphosphane,[34] 1,3-propanedithiol,[35] and H_2S[36] led to lower yields. The crude amine was transformed into the ceramide moiety with stearic acid upon addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to afford **27**. As the final step, **27** was deprotected using Zemplén[37] conditions to provide the fully functionalized ganglioside lactone analogue **4** in 37% over three steps.



Scheme 6. Synthesis of **4**: a) CF_3COOH/CH_2Cl_2 2:1, 30 min, rt; b) CCl_3CN (30 equiv), DBU, CH_2Cl_2, 1 h, 0 °C, 93% (two steps); c) azido sphingosine (3 equiv), $BF_3 \cdot OEt_2$ (3 equiv), CH_2Cl_2, molecular sieves 4 Å, 1 h, -40 °C, 66%; d) NEt_3, PhSH (5 equiv), $(PhS)_2Sn$ (5 equiv), CH_2Cl_2, 4 h, rt; stearic acid (5 equiv), EDC (5 equiv), CH_2Cl_2, 2 h, rt, 73% (two steps); e) NaOMe, MeOH, 20 h, rt, 77%.

Our intention is to use the trisaccharide core **21** for the production of polyclonal as well as monoclonal antibodies which recognize with the GM3-lactone **2** but not GM3 **1**. For this purpose the trisaccharide was linked to a suitable spacer moiety for coupling to keyhole-limpet hemocyanin (KLH) and to bovine serum albumin (BSA). We used hexanoic acid as a spacer moiety. Reaction of the trichloroacetimidate **25** with 6-hydroxyhexanoic acid methyl ester in the presence of $BF_3 \cdot OEt_2$ gave the glycoside **28** in 70% yield which was contaminated with 17% of the 2c-epimer. Cleavage of all acetyl groups with sodium methoxide followed by deprotection of the methyl ester with sodium hydroxide in methanol yielded **29** in 92% yield.



Scheme 7. Synthesis of **29**: a) 6-hydroxyhexanoic acid methyl ester, $BF_3 \cdot OEt_2$, molecular sieves 4 Å, CH_2Cl_2, -50 °C $\rightarrow -20$ °C, 90 min; b) NaOMe, MeOH, 24 h, rt then H_2O, 1 h, rt, 92% (from **28**).

In summary, for a potential vaccination against cancer haptens with a defined epitope of tumor-associated antigens must be designed. Since there is strong evidence that the GM3-lactone **2** which is not stable under physiological conditions is selectively expressed on the cell membrane of cancer cells we prepared the stable analogue **4**. The cyclic ether moiety in **4** was formed by reduction of the corresponding lactone to give the lactol followed by formation of the *S*,*O*-hemiacetal and hydrogenation. In addition, we have prepared haptens with a hexanoic acid moiety, which can be used for the preparation of poly- and monoclonal antibodies after binding to BSA or KLH. Compound **4** is the first example of an analogue of the GM3-lactone which is stable under hydrolytic conditions in vitro and probably also in vivo.

Experimental Section

Melting points were taken on a Mettler FP61 apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on SIL G/UV_{254} Macherey, Nagel silica gel precoated aluminum plates. IR spectra were recorded on a Bruker IFS 25. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian VXR 500S (500 and 125.7 MHz). Chemical shifts are given in ppm relative to TMS (^{1}H: $\delta = 0.0$, ^{13}C: $\delta = 0.00$).

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-methyl-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl-1-ol (14): Sodium borohydride (10 mg, 264 mmol) was added to a solution of **13** (48.0 mg, 95.0 μmol) in THF

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(5 mL). After stirring for 40 h at rt the solution was refluxed for 5 h. The reaction was quenched with half saturated NH_4Cl solution (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3×8 mL). After drying over $MgSO_4$ the organic phases were concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to yield **14** (20.0 mg, 44 %): $[\alpha]_D^{20} = -39.0^\circ$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.18$ (silca gel, EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.82$ (t, $J = 13.5$ Hz, 1H, 3-H_{ax}), 1.89, 2.02, 2.04, 2.09, 2.14 (s, 15H, 5CH_3CO), 2.23 (dd, $J = 5.0$, 13.5 Hz, 1H, 3-H_{eq}), 3.27 (s, 3H, OMe), 3.63 (d, $J = 12.0$ Hz, 1H, 1-H), 3.71 (d, $J = 12.0$ Hz, 1H, 1-H′), 3.87–4.18 (m, 3H, 5-H, 6-H, 9-H), 4.60 (dd, $J = 2.5$, 12.5 Hz, 1H, 9-H′), 5.14–5.40 (m, 4H, 4-H, 7-H, 8-H, NH); ^{13}C NMR (50.31 MHz, $CDCl_3$): $\delta = 20.78$, 20.95, 21.04, 23.15 (5*C*H_3CO), 35.88 (C-3), 49.03, 49.62 (C-5, OMe), 62.42 (C-9), 63.76 (C-1), 68.29, 69.51, 71.01, 71.08 (C-4, C-6, C-7, C-8), 100.4 (C-2), 170.1, 170.3, 170.7, 170.8, 171.2 (5Me*C*O); IR (KBr): $\tilde{\nu} = 3380$ (OH), 1746, 1548, 1438, 1372, 1316, 1224, 1156, 1124, 1074, 1038, 602 cm^{-1}; MS (DCI): *m*/*z* (%): 495.4 (100) $[M+NH_3+H]^+$; $C_{20}H_{31}O_{12}N$ (477.47) · 0.5 H_2O: calcd for C 49.38, H 6.63; found: C 49.38, H 6.42.

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-methyl-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl-1-trifluoromethanesulfonic acid ester (15): Trifluoromethanesulfonic acid anhydride (116 μL, 0.69 mmol) was added at −50 °C to a solution of **14** (110 mg, 226 mmol) and pyridine (0.10 mL) in CH_2Cl_2 (10 mL) and the solution was stirred for 2 h at −50 °C. The solution was allowed to warm up to −30 °C within 0.5 h and was stirred for another 1 h at this temperature. The reaction was quenched with water (2 mL) and 2N HCl (10 mL) and the layers were separated. The organic layer was washed with saturated $NaHCO_3$, water, dried over Na_2SO_4 and concentrated in vacuo to yield **15** (129 mg, 92 %): M.p.: 126.6 °C (dec.); $[\alpha]_D^{20} = -24.0^\circ$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.40$ (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.85$ (t, $J = 13.5$ Hz, 1H, 3-H_{ax}), 1.90, 2.04, 2.09, 2.14 (s, 15H, 5CH_3CO), 2.31 (dd, $J = 5.0$, 13.5 Hz, 1H, 3-H_{eq}), 3.39 (s, 3H, OMe), 3.19 (dd, $J = 2.0$, 10.5 Hz, 1H, 6-H), 3.96 (dd, $J = 7.0$, 12.5 Hz, 1H, 9-H), 4.17 (q, $J = 10.5$ Hz, 1H, 5-H), 4.46 (d, $J = 11.0$ Hz, 1H, 1-H), 4.58 (d, $J = 11.0$ Hz, 1H, 1-H′), 4.60 (dd, $J = 2.5$, 12.5 Hz, 1H, 9′-H), 5.13 (m, 1H, 8-H), 5.26 (m, 1H, 4-H), 5.32 (d, $J = 9.5$ Hz, 1H, NH), 5.34 (dd, $J = 2.0$, 5.5 Hz, 1H, 7-H); ^{13}C NMR (50.31 MHz, $CDCl_3$): $\delta = 20.68$, 20.75, 20.90, 21.04, 23.15 (5*C*H_3CO), 36.57 (C-3), 48.93, 49.46 (C-5, OMe), 62.15 (C-9), 68.02, 68.58, 71.03, 71.54 (C-4, C-6, C-7, C-8), 73.03 (C-1), 98.00 (C-2), 118.6 (q, $J = 260$ Hz, CF_3), 170.0, 170.3, 170.5, 170.6, 171.1 (5Me*C*O); MS (DCI): *m*/z (%): 627.5 (100) $[M+NH_3+H]^+$; $C_{20}H_{31}O_{12}$ (609.53): calcd for C 41.38, H 4.96; found: C 42.63, H 5.11.

2′-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-lacton)-β-D-galactopyranosyl]-β-D-glucopyranoside (18): K_2CO_3 (3.00 g, 21.7 mmol) was added to a solution of **16** (4.32 g, 3.35 mmol) in methanol (300 mL). After stirring for 22 h at rt acetic acid (5.0 mL, 87 mmol) was added and the resulting mixture was concentrated in vacuo. The residue was purified by flash chromatography (EtOAc → EtOAc/MeOH 1:1, TLC: EtOAc/MeOH 1:1, $R_f = 0.54$) to yield a white solid (2.95 g). The substance was dissolved in methanol (5 mL) and added dropwise to a suspension of Pd/C (10 %, 2.0 g) in methanol (250 mL). After addition of acetic acid (1.0 mL, 17 mmol) the solution was stirred under hydrogen atmosphere for 24 h at rt. The mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was dissolved in toluene (10 mL) and concentrated in vacuo to give **17** (2.20 g) as a single product. Compound **17** was dissolved in acetic acid (350 mL) and stirred for 24 h at rt. The mixture was concentrated in vacuo. The residue was dissolved in toluene (30 mL) and concentrated in vacuo. The residue was dissolved in dry CH_2Cl_2 (50 mL) and pyridine (20 mL), then Ac_2O (10 mL) and DMAP (20 mg, 0.16 mmol) were added. After stirring for 20 h at rt the mixture was concentrated in vacuo and the residue was purified by flash chromatography to yield **18** (2.05 g, 56 %): $[\alpha]_D^{20} = -41.4^\circ$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.36$ (EtOAc); m.p: 115.6 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.01$ (s, 9H), 0.91 (m, 2H), 1.89, 2.01, 2.03, 2.05, 2.06, 2.07, 2.09, 2.11 (s, 30H, 10Ac), 2.44 (dd, $J = 5.5$, 13.5 Hz, 1H, 3c-H_{eq}), 3.55 (m, 1H), 3.66 (dd, $J = 2.0$, 10.5 Hz, 1H, 3b-H), 3.69 (m, 1H, 5a-H), 3.89–4.19 (m, 8H), 4.28 (dd, $J = 3.0$, 12.5 Hz, 1H, 9c-H_a), 4.44 (dd, $J = 4.0$, 12.5 Hz, 1H, 9c-H_b), 4.50 (dd, $J = 1.5$, 12.0 Hz, 1H), 4.51, 4.56 (d, $J = 8.0$ Hz, 2H, 1a-H, 1b-H), 4.73 (dd, $J = 7.5$, 8.0 Hz, 1H), 7.88 (dd, $J = 8.0$, 9.0 Hz, 1H), 5.12 (m, 1H), 5.24 (t, $J = 9.5$ Hz, 1H), 5.27 (dd, $J = 2.0$, 6.5 Hz, 1H), 5.37–5.46 (m, 3H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = -1.41$ ($SiMe_3$), 17.89, 20.45, 20.61, 20.66, 20.75, 20.87, 20.97, 23.12 (10*C*H_3CO), 37.57 (C-3c), 49.15 (C-5c), 61.02, 62.28, 67.54 (C-6a, C-6b, C-6c), 65.81, 67.44, 69.39, 69.87, 71.08, 72.11, 72.40, 72.80, 72.97, 73.49, 73.70, 76.70 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-4c, C-6c, C-7c, C-8), 97.07 (C-2c), 99.92, 99.95 (C-1a, C-1b), 163.2 (C-1c), 169.6, 169.8, 169.9, 170.4, 170.5, 170.6, 170.8, 170.91 (10CH_3*C*O); IR (KBr) $\tilde{\nu} =$ 1754, 1690, 1370, 1292, 1234, 1172, 1120, 1054 cm^{-1}; MS (DCI): *m*/*z* (%): 1111.9 $[M+NH_3+H]^+$; $C_{46}H_{67}NO_{27}Si$ (1094.11): calcd C 50.50, H 6.17; found: C 51.00, H 6.23.

2′-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-lactol)-β-D-galactopyranosyl]-β-D-glucopyranoside (19): A solution of **18** (344 mg, 0.315 mmol) in dry toluene (7 mL) was cooled to −78 °C and a freshly prepared Red/Al solution (1.5 mL), which was prepared by adding ethanol (0.2 mL) to a solution of 3.4M Red/Al (1 mL) in dry toluene (2 mL), was added dropwise. After stirring for 0.5 h at −78 °C the reaction was quenched with acetic acid (0.2 mL). The solution was diluted with CH_2Cl_2 (30 mL) and successively washed with 0.2N HCl and saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to give **19** (241 mg, 70 %): $[\alpha]_D^{20} = -12.0^\circ$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.31$ (EtOAc); m.p.: 121.3 °C; ^{1} HNMR (500 MHz, $CDCl_3$): $\delta = 0.01$ (s, 9H, $SiMe_3$), 0.93 (m, 2H, CH_2SiMe_3), 1.87 (2s, 3H, CH_3CON), 2.01–2.15 (13s, 27H, 9CH_3CO), 2.71 (dd, $J = 5.5$, 13.0 Hz, 1H, 3c-H_{eq}), 3.52–4.54 (m, 15H), 4.69 (d, $J = 11.5$ Hz, 1H), 4.83–5.43 (m, 9H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = -1.48$ ($SiMe_3$), 17.88, 20.39, 20.51, 20.58, 20.64, 20.68, 20.73, 20.82, 20.91, 20.93, 20.95, 21.08, 23.10, 23.15 (10*C*H_3CO), 33.61, 35.05 (C-3c), 49.12, 49.53 (C-5c), 61.35, 61.42, 62.52, 62.99, 63.04, 64.01, 67.54, 67.61 (C-6a, C-6b, C-9c, O*C*$H_2CH_2SiMe_3$), 66.28, 66.33, 66.45, 66.52, 67.54, 67.61, 68.05, 68.56, 69.38, 69.57, 70.75, 71.62, 71.73, 71.79, 71.88, 72.32, 72.55, 72.60, 72.70, 72.87, 73.04, 73.59, 73.69, 77.99, 78.49 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-4c, C-6c, C-7c, C-8c), 90.90, 94.35 (C-1c), 97.15, 98.24 (C-2c), 100.0 (C-1b), 103.0, 103.4 (C-1a), 169.6, 169.7, 170.0, 170.2, 170.3, 170.4, 170.6, 170.8, 170.9, 171.1, 171.3, 172.8; IR (KBr): $\tilde{\nu} = 3442$, 3406, 1748, 1372, 1236, 1170, 1060 cm^{-1}; MS (DCI): *m*/*z* (%): 1114.0 $[M+NH_3+H]^+$; $C_{46}H_{69}NO_{27}Si$ (1096.13): calcd for C 50.41, H 6.34; found: C 51.08, H 6.24.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-thiolactol)-β-D-galactopyranosyl]-β-D-glucopyranoside (20): 2,2′-Bis(pyridinium) disulfide (1.58 g, 7.15 mmol) and tributylphosphane (1.78 mL, 7.15 mmol) were added to a solution of **19** (757 mg, 0.691 mmol) in dry CH_2Cl_2 (30 mL). The reaction was stirred for 3 d at rt and for 6 h at reflux. The solution was diluted with CH_2Cl_2 (50 mL) and successively washed with 2N HCl (20 mL) and saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to yield **20** (789 mg, 100 %): $[\alpha]_D^{20} = -30.6^\circ$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.34$ (EtOAc); m.p.: 120.3 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = -0.01$ (s, 9H, $SiMe_3$), 0.91 (m, 2H, C*H*$_2$SiMe$_3$), 1.90 (s, 3H, CH_3CON), 2.01–2.15 (8s, 27H, 9CH_3CO), 2.81 (dd, $J = 5.5$, 13.0 Hz, 1H, 3c-H_{eq}), 3.51–4.43 (m, 15H), 4.45, 4.48 (d, $J = 8.0$ Hz, 2H, 1a-H 1b-H), 4.54 (dd, $J = 2.5$, 12.5 Hz, 1H, 9c-H_a), 4.87 (dd, $J = 8.0$, 9.5 Hz, 1H, 2a-H), 5.14–5.26 (m, 3H, 3a-H, 4c-H, 8c-H), 5.30 (dd, $J = 2.0$, 4.5 Hz, 1H, 7c-H), 5.33 (dd, $J = 1.5$, 3.0 Hz, 1H, 4b-H), 5.52 (d, $J = 9.5$ Hz, 1H, NH), 6.02 (s, 1H, 1c-H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = -1.43$ ($SiMe_3$), 17.90 (*C*H_2SiMe_3), 20.40, 20.61, 20.70, 20.73, 20.78, 20.84, 20.89, 21.08, 23.24 (10*C*H_3CO), 34.43 (C-3c), 49.73 (C-5c), 61.27, 62.14, 62.34, 67.58 (C-6a, C-6b, C-9c, O*C*$H_2CH_2SiMe_3$), 66.58, 67.51, 68.17, 68.53, 71.48, 71.82, 71.86, 72.08, 72.09, 72.41, 73.24, 77.40 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-4c, C-6c, C-7c, C-8c), 90.34 (C-1c), 95.85 (C-2c), 100.1, 101.3 (C-1a, C-1b), 169.6, 169.8, 170.0, 170.3, 170.4, 170.5, 170.7 (10CH_3*C*O); IR (KBr):$\tilde{\nu} = 2958$, 1750, 1436, 1372, 1234, 1156, 1044, 840 cm^{-1}; MS (DCI): *m*/*z* (%): 1095.8 $[M-SH+NH_3+H]^+$; $C_{46}H_{69}NO_{26}SSi$ (1112.20).

2′-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-1,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (21): Raney nickel (5 g) was washed with water (2×25 mL) and ethanol (2×25 mL). Afterwards the Raney nickel was suspended in ethanol (30 mL). The suspension was evaporated and ventilated with hydrogen ($3 \times$). A solution of **20** (300 mg, 0.270 mmol) in ethanol (3 mL) was added dropwise. After stirring under H_2 atmosphere for 1 h at rt the suspension was filtered through a pad of Celite and concentrated in vacuo.

The residue was dissolved in Ac_2O (0.3 mL), pyridine (0.6 mL), and CH_2Cl_2 (5 mL) and DMAP (10 mg) was added. After stirring for 14 h the solution was concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to yield **21** (282 mg, 97 %): $[\alpha]_D^{20} = -6.6^\circ$ ($c = 0.5$ in $CHCl_3$); $R_f = 0.19$ (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.01$ (s, 9H, $SiMe_3$), 0.93 (m, 2H, CH_2SiMe_3), 1.87 (dd, $J = 10.5$, 13.5 Hz, 1H, 3c-H_{ax}), 1.90 (s, 3H, CH_3CON), 2.03, 2.06, 2.08, 2.09, 2.13, 2.15 (s, 27H, 9 CH_3CO), 2.60 (dd, $J = 5.5$, 13.5 Hz, 1H, 3c-H_{eq}), 3.44 (d, $J = 11.5$ Hz, 1H, 1c-H_{ax}), 3.59 (dd, $J =$ 7.5, 10.5 Hz, 1H, 2b-H), 3.52 – 3.58 (m, 1H, $OCH_2CH_2SiMe_3$), 3.64 (dt, $J =$ 3.5, 10.0 Hz, 1H, 5a-H), 3.71 (dd, $J = 3.0$, 10.5 Hz, 1H, 3b-H), 3.81 (t, $J =$ 9.5 Hz, 1H, 4-H), 3.80 – 3.91 (m, 4H, 5b-H, 5c-H, 6b-H_a, 6c-H), 3.89 (d, $J =$ 11.5 Hz, 1H, 1c-H_{eq}), 3.92 – 3.99 (m, 1H, $OCH_2CH_2SiMe_3$), 4.02 – 4.13 (m, 2H, 6a-H_a, 6b-H_b), 4.10 (q, $J = 6.0$ Hz, 1H, 9c-H_a), 4.35 ($J = 7.5$ Hz, 1H, 1b-H), 4.38 – 4.43 (m, 2H, 6a-H_b, 9c-H_b), 4.49 (d, $J = 8.0$ Hz, 1H, 1a-H), 4.88 (dd, $J = 8.0$, 9.5 Hz, 1H, 2-H), 5.21 (t, $J = 9.5$ Hz, 1H, 3a-H), 5.15 – 5.23 (m, 2H, 4c-H, 8c-H), 5.25 (dd, $J = 2.0$, 6.5 Hz, 1H, 7c-H), 5.29 (dd, $J = 1.5$, 3.0 Hz, 1H, 4b-H), 5.32 (d, $J = 9.0$ Hz, 1H, NH); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = -1.45$ ($SiMe_3$), 17.89 (CH_2SiMe_3), 20.56, 20.61, 20.73, 20.76, 20.88, 20.91, 20.93, 23.28 (10 CH_3CO), 34.18 (C-3c), 50.39 (C-5c), 61.43, 62.31, 62.43, 62.52, 68.11 (C-1c, C-6a, C-6b, C-9c, $OCH_2CH_2SiMe_3$), 66.75, 68.00, 68.90, 70.49, 71.49, 71.66, 71.84, 72.59, 72.66, 73.19, 73.58, 77.19 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-4c, C-6c, C-7c, C-8c), 96.74 (C-2c), 100.1 (C-1b), 102.0 (C-1a), 169.6, 169.9, 167.0, 170.4, 170.6, 170.7 (10 CH_3CO); IR (KBr): $\tilde{\nu} = 1750$, 1372, 1234, 1160, 1048, 840 cm^{-1}; MS (DCI): m/z (%): 1098.3 $[M+NH_3+H]^+$; $C_{46}H_{69}NO_{26}Si$ (1080.13): calcd C 51.15, H 6.44; found: C 51.10, H 6.11.

2′-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl-1c-ol)-β-D-galactopyranosyl]-β-D-glucopyranoside (23): Sodium borohydride (25.0 mg, 389 mmol) was added at 0 °C to a solution of **22** (100 mg, 82.2 μmol) in THF (5 mL). After stirring at 0 °C for 4 h the reaction was quenched with saturated NH_4Cl (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). After drying over $MgSO_4$ the organic layer was concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to give **23** (62.0 mg, 62 %): $R_f = 0.22$ (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.01$ (s, 9H, $SiMe_3$), 1.02 (m, 2H, CH_2SiMe_3), 1.85 (dd, $J = 11$, 13 Hz, 1H, 3c-H_{ax}), 1.98 – 2.09 (7s, 21H, CH_3CO), 2.31 (dd, $J = 5.0$, 13.0 Hz, 1H, 3c-H_{eq}), 3.40 (dd, $J = 8.0$, 9.0 Hz, 1H), 3.49 – 3.67 (m, 8H), 3.73 (dd, $J = 6.0$, 11.0 Hz, 1H), 3.80 (dd, $J = 1.5$, 10.5 Hz, 1H), 3.85 (t, $J = 10.0$ Hz, 1H), 3.91 – 4.05 (m, 5H), 4.11 (dd, $J = 3.5$, 9.5 Hz, 1H), 4.30 (dd, $J = 2.5$, 12.5 Hz, 1H), 4.38 (d, $J = 8.0$ Hz, 1H, 1b-H), 4.58 (d, $J = 12.0$ Hz, 1H, OCH_2Bn), 4.66 (d, $J = 11.5$ Hz, 1H, OCH_2Bn), 4.73 (d, $J = 7.5$ Hz, 1H, 1-H), 4.87 – 4.95 (m, 3H, 3 OCH_2Bn), 5.05 (dd, $J =$ 1.5, 3.0 Hz, 1H, 4b-H), 5.09 (m, 1H, 8c-H), 5.22 (dd, $J = 1.5$, 8.0 Hz, 1H, 7c-H), 5.31 (m, 1H, 4b-H), 5.34 (d, $J = 9.5$ Hz, 1H, NH), 7.2 – 7.36 (m, 15H, Ph); MS (DCI): m/z (%): 1260.2 (100) $[M+NH_3+H]^+$.

2,3,6-Tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-1,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl trichloroacetimidate (25): Trifluoroacetic acid (2 mL) was added to a solution of **21** (110 mg, 0.102 mmol) in dry CH_2Cl_2 (1 mL). After stirring for 0.5 h at rt the solution was concentrated in vacuo, diluted with toluene (5 mL), and concentrated in vacuo again. The residue was purified by column chromatography (EtOAc/MeOH 10:1). The filtrate was concentrated and dried in vacuo for 3 h and then dissolved in CH_2Cl_2 (5 mL). The solution was cooled to 0 °C and trichloroacetonitrile (0.34 mL) and DBU (13 μL) were added. After stirring for 1 h at 0 °C the solution was concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/MeOH 10:1) to yield **25** (106.5 mg, 93 %): $R_f = 0.55$ (EtOAc/MeOH 10:1); ^{1}H NMR (500 MHz, $CDCl_3$, both anomers (C-2c)): $\delta = 1.91 - 2.16$ (s, 30H, 10 CH_3CO), 2.51 (dd, $J = 5.5$, 13.5, 1H, 3c-H_{eq} (β-anomer)), 2.57 (dd, $J =$ 5.5, 13.5 Hz, 1H, 3c-H_{eq} (α-anomer)), 3.43 (d, $J = 11.5$ Hz, 1H, 1c-H_{ax}), 4.39 (d, $J = 8.0$ Hz, 1H, 1b-H (β-anomer)), 4.64 (d, $J = 8.0$ Hz, 1H, 1b-H (α-anomer)), 6.49 (d, $J = 3.5$ Hz, 1H, 1a-H (β-anomer)), 6,50 (d, $J = 3.5$ Hz, 1H, 1b-H (α-anomer)), 8.66 (s, 1H), 8.70 (s, 1H, C=NH (β-anomer)); $C_{43}H_{57}N_2O_{26}Si_3$ (1024.28).

(2*S*,3*R*,4*E*)-2-Azido-3-(benzoyloxy)octadec-4-enyl 2,3,6-tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-1,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (26): A solution of **25** (106 mg, 94,3 μmol), 2-azido-3-*O*-benzoylsphingosine (125 mg, 0.291 mmol), and molecular sieves (4 Å) in CH_2Cl_2 (5 mL) was stirred for 2 h at rt. The solution was cooled to −40 °C and $BF_3 \cdot OEt_2$ (30 mL) was added. The mixture was allowed to warm up to −20 °C within 1 h and the reaction was quenched by addition of triethylamine (0.2 mL). After filtration the solution was concentrated in vacuo and the residue purified by flash chromatography (EtOAc) to yield **26** (86 mg, 66 %): $[\alpha]_D^{20} = -7.4^\circ$ ($c = 0.5$ in $CHCl_3$, β-anomer (C-2c)); $R_f = 0.33$ (α-anomer (C-2c)), 0.29 (β-anomer (C-2c)) (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, α-anomer (C-2c)): $\delta = 0.88$ (t, $J = 7.0$ Hz, 3H, CH'_3), 1.20 – 1.40 (m, 22H, CH'_2), 1.90 – 2.15 (10s, 30H, 10 CH_3CO), 2.50 (dd, $J = 5.5$, 13.5 Hz, 1H, 3c-H_{eq}), 3.45 (d, $J = 11.5$ Hz, 1H, 1c-H_{ax}), 3.56 – 3.61 (m, 2H, 2b-H, 1′-H′a), 3.65 (m, 1H, 5a-H), 3.71 (dd, $J =$ 3.0, 10.5 Hz, 1H, 3b-H), 3.83 (t, $J = 10.0$ Hz, 1H, 4a-H), 3.89 (d, $J = 11.5$ Hz, 1H, 1c-H_{eq}), 3.80 – 3.96 (m, 4H, 5b-H, 6b-H_a, 1′-H_b, 2′-H_a), 4.02 – 4.09 (m, 2H, 6a-H_a, 6b-H_b), 4.10 (q, $J = 6.5$ Hz, 1H, 9c-H_a), 4.34 (d, $J = 8.0$ Hz, 1H, 1b-H), 4.35 – 4.42 (m, 2H, 6a-H_b, 9c-H_b), 4.52 (d, $J = 8.0$ Hz, 1H, 1a-H), 4.94 (dd, $J = 8.0$, 10.0 Hz, 1H, 2a-H), 5.16 – 5.23 (m, 3H, 3a-H, 4c-H, 8c-H), 5.25 (dd, $J = 2.0$, 6.5 Hz, 1H, 7c-H), 5.29 (dd, $J = 1.5$, 3.0 Hz, 1H, 4b-H), 5.31 (d, $J = 9.0$ Hz, 1H, NH), 5.53 (ddt, $J = 1.5$, 8.0, 15.0 Hz, 1H, 4′-H), 5.59 (dd, $J = 4.0$, 8.0 Hz, 1H, 3′-H), 5.92 (dt, $J = 6.5$, 15.0 Hz, 1H, 5′-H), 7.44 (m, 2H, OBz), 7.56 (m, 1H, OBz), 8.03 (m, 2H, OBz); ^{1}H NMR (500 MHz, $CDCl_3$, β-anomer (C-2c)): $\delta = 0.88$ (t, $J = 7.0$ Hz, 3H, CH'_3), 1.20 – 1.42 (m, 22H, CH'_2), 1.90 – 2.16 (10s, 30H, 10 CH_3CO), 1.96 (dd, $J = 5.5$, 12.5 Hz, 1H, 3c-H_{eq}), 3.39 (dd, $J = 8.0$, 10.0 Hz, 1H, 2b-H), 3.42 (d, $J = 12.0$ Hz, 1H, 1c-H_{ax}), 3.58 (dd, $J = 6.0$, 10.5 Hz, 1H, 1′-H_a), 3.64 (ddd, $J = 2$, 5, 10.0 Hz, 1H, 5a-H), 3.76 – 4.10 (m, 11H), 4.23 (dd, $J = 5.5$, 12.0 Hz, 1H), 4.39 (dd, $J = 2.0$, 12.0 Hz, 1H), 4.50 (d, $J = 8.0$ Hz, 1H, 1b-H), 4.64 (d, $J = 7.5$ Hz, 1H, 1a-H), 4.73 (dd, $J = 3.0$, 12.5 Hz, 1H), 4.97 (dd, $J = 8.0$, 9.5 Hz, 1H), 5.10 (m, 1H), 5.20 – 5.26 (m, 2H), 5.31 (d, $J = 10.5$ Hz, 1H, NH), 5.36 – 5.43 (m, 2H), 5.56 (ddt, $J = 1.5$, 7.5, 15.0 Hz, 1H, 4′-H), 5.62 (dd, $J = 4.0$, 8.5 Hz, 1H, 3′-H), 5.92 (dt, $J = 6.5$, 15.0 Hz, 1H, 5′-H), 7.45 (m, 2H, OBz), 7.57 (m, 1H, OBz), 8.04 (m, 2H, OBz); ^{13}C NMR (125.7 MHz, $CDCl_3$, α-anomer (C-2c)): $\delta = 14.12$ ($C'H_3$), 20.65, 20.76, 20.91, 20.96, 23.33 (10 CH_3CO), 22.86, 28.73, 29.16, 29.35, 29.39, 29.59, 29.65, 31.92, 32.38 (12 CH_2), 50.46 (C-5c), 61.45, 62.30, 63.50, 66.75, 67.99, 68.12, 68.31, 68.87, 70.45, 71.51, 71.71, 72.65, 72.82, 72.98, 73.57, 74.66, 76.49, 96.75 (C-2c), 100.4 (C-1b), 102.1 (C-1a), 122.6 (C=), 128.5, 129.7 (*o*-, *m*-Ph-C), 129.9 (*i*-Ph), 133.2 (*p*-Ph), 139.0 (C=), 165.1, 170.3, 170.4 (10 CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$, β-anomer (C-2c)): $\delta = 14.12$ ($C'H_3$), 20.69, 20.71, 20.74, 20.78, 20.86, 20.90, 21.03, 23.19 (10 CH_3CO), 22.70, 28.75, 29.17, 29.36, 29.41, 29.60, 29.66, 29.67, 29.69, 31.93, 32.39 (12 CH_2), 36.40 (C-3c), 49.48 (C-5c), 61.21, 62.17, 62.43, 68.34, 71.14 (C-1c, C-6a, C-6b, C-9c, C-1′), 63.54, 66.91, 67.78, 68.57, 68.71, 70.36, 70.44, 71.26, 71.46, 72.90, 73.20, 74.48, 74.70, 96.07 (C-2c), 100.5, 101.2 (C-1a, C-1b), 122.7 (C=), 128.5, 129.7 (*o*-, *m*-Ph-C), 130.0 (*i*-Ph-C), 133.2 (*p*-Ph-C), 139.0 (C=), 165.1, 169.6, 169.9, 170.0, 170.1, 170.3, 170.6, 170.7, 170.8, 171.0 (10 CH_3CO); IR (KBr, β-anomer (C-2c)): $\tilde{\nu} = 2928$, 2108, 1748, 1690, 1372, 1232, 1176, 1110, 1068, 1042 cm^{-1}; MS (DCI): m/z (%): 1408.9 $[M+NH_3+H]^+$; $C_{66}H_{94}N_4O_{28}$ (1391.48).

(2*S*,3*R*,4*E*)-3-Benzoyloxy-2-(octadecanamido)octadec-4-enyl 2,3,6-tri-*O*-acetyl-4-*O*-[4,6-di-*O*-acetyl-3-*O*-(4,7,8,9-tetra-*O*-acetyl-5-acetamido-1,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (27): Di(thiophenyl)tin(II) (28 μL, 270 mmol) and triethylamine (37 μL, 270 mmol) were successively added at 0 °C to a solution of **26** (75 mg, 54 mmol) in CH_2Cl_2 (5 mL). After stirring for 4 h at rt ethyl acetate (30 mL) was added and the solution was washed with 0.1N NaOH (3 × 20 mL) and water (20 mL). The combined aqueous layers were extracted with CH_2Cl_2 (2 × 5 mL) and the combined organic layers were washed with water (5 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. After drying in vacuo for 1 h the residue was dissolved in CH_2Cl_2 (5 mL) and stearic acid (77 mg, 270 μmol) and EDC (52 mg, 270 mmol) were added. The reaction was stirred for 2 h at rt and diluted with CH_2Cl_2 (20 mL). The solution was washed successively with 8N HCl (8 mL) and saturated $NaHCO_3$ solution. The solution was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to give **27** (64.5 mg, 73 %): $[\alpha]_D^{20} = +4.8^\circ$ ($c = 0.5$ in $CHCl_3$, β-anomer (C-2c)); $R_f = 0.27$ (EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, α-anomer (C-2c)): $\delta = 0.88$ (t, $J = 7.0$ Hz, 6H, CH'_3), 1.20 – 1.40 (m, 52H, CH'_2), 1.65 (m, 2H, 6′-$H_{a,b}$), 1.90 – 2.15 (10s, 30H, 10 CH_3CO), 2.49 (dd, $J = 5.5$, 13.5 Hz, 1H, 3c-H_{eq}), 3.43 (d, $J = 11.5$ Hz, 1H, 1c-H_{ax}), 3.56 – 3.61 (m, 2H, 2b-H, 1′-H), 3.64 (m, 1H, 5a-H), 3.70 (dd, $J =$ 3.0, 10.5 Hz, 1H, 3b-H), 3.78 (t, $J = 9.5$ Hz, 1H, 4a-H), 3.80 – 3.93 (m, 3H), 4.00 – 4.12 (m, 5H), 4.23 (dd, $J = 2.0$, 12.0 Hz, 1H), 4.28 (dd, $J = 4.0$,

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12.0 Hz, 1H), 4.32 (d, J = 7.5 Hz, 1H, 1b-H), 4.40 (dd, J = 2.5, 12.5 Hz, 1H), 4.45 (d, J = 8.0 Hz, 1H, 1a-H), 4.47–4.51 (m, 1H), 4.89 (dd, J = 8.0, 9.5 Hz, 1H, 2a-H), 5.16–5.23 (m, 3H, 3a-H, 4c-H, 8c-H), 5.25 (dd, J = 1.5, 6.5 Hz, 1H, 7c-H), 5.29 (dd, J = 1.5, 3.0 Hz, 1H, 4b-H), 5.38 (d, J = 9.0 Hz, 1H, NH), 5.46 (ddt, J = 1.5, 7.5, 15 Hz, 1H, 4′-H), 5.52 (t, J = 7.0 Hz, 1H, 3′-H), 5.74 (d, J = 9.0 Hz, 1H, NH), 5.87 (dt, J = 6.5, 15.0 Hz, 1H, 5′-H), 7.44 (m, 2H, OBz), 7.56 (m, 1H, OBz), 8.00 (m, 2H, OBz); ^{1}H NMR (500 MHz, $CDCl_3$, β-anomer (C-2c)): δ = 0.88 (t, J = 7.0 Hz, 6H, CH'_3), 1.20–1.40 (m, 52H, CH'_2), 1.63 (m, 2H, 6′-$H_{a,b}$), 1.90–2.15 (10s, 30H, 10 CH_3CO), 3.41 (d, J = 12.0 Hz, 1H, 1c-H_{ax}), 3.58 (ddd, J = 2.0, 5.0, 10.0 Hz, 1H), 3.63 (dd, J = 4.5, 10.0 Hz, 1H), 3.75–3.82 (m, 3H), 3.97–4.11 (m, 6H), 4.14 (dd, J = 5.0, 12.0 Hz, 1H), 4.24 (dd, J = 2.0, 10.0 Hz, 1H), 4.44 (d, J = 8.0 Hz, 1H, 1b-H), 4.47 (m, 1H), 4.61 (d, J = 7.5 Hz, 1H, 1a-H), 4.72 (dd, J = 2.5, 12.5 Hz, 1H), 4.90 (dd, J = 8.0, 10.0 Hz, 1H), 5.07 (m, 1H), 5.17–5.22 (m, 2H), 5.32 (d, J = 11.0 Hz, 1H, NH), 5.34 (dd, J = 1.5, 5.5 Hz, 1H, 7c-H), 5.38 (m, 1H), 5.46 (ddt, J = 1.5, 7.5, 15.0 Hz, 1H, 4′-H), 5.52 (t, J = 7.0 Hz, 1H, 3′-H), 5.74 (d, J = 9.0 Hz, 1H, NH), 5.87 (dt, J = 6.5, 15.0 Hz, 1H, 5′-H), 7.43 (m, 2H, OBz), 7.56 (m, 1H, OBz), 8.00 (m, 2H, OBz); ^{13}C NMR (125.7 MHz, $CDCl_3$, β-anomer (C-2c)): δ = 14.12 ($2C'H_3$), 20.61, 20.68, 20.69, 20.74, 20.75, 20.79, 20.90, 21.02, 23.18 (10 CH_3CO), 22.70, 25.75, 28.96, 29.26, 29.37, 29.40, 29.44, 29.49, 29.54, 29.57, 29.63, 29.67, 29.70, 29.72, 31.93, 32.35, 36.39, 36.87, 49.46, 50.71 (C-2′, C-5c), 61.20, 61.20, 62.16, 62.38, 66.89, 67.44, 67.75, 68.54, 68.71, 70.32, 70.43, 71.13, 71.22, 71.75, 72.82, 72.95, 74.12, 74.45, 76.67, 96.06 (C-2c), 100.5 (C-1b), 101.2 (C-1a), 124.7 (C=), 128.4, 129.6 (*o*-, *m*-Ph-C), 130.3 (*i*-Ph-C), 133.0 (*p*-Ph-C), 137.6 (C=), 165.2, 169.7, 169.9, 170.1, 170.3, 170.5, 170.7, 170.9, 171.0, 172.7 (10 CH_3CO); IR (KBr): $\tilde{\nu}$ = 3402, 2926, 2854, 1750, 1666, 1372, 1234, 1112, 1042 cm^{-1}; MS (FAB): m/z (%): 1785.0 (100) $[M+m\text{-NBA}]^+$, 1630.2 (35) $[M]^+$; MS (FAB): m/z (%): 1510.2 (100) $[M-\text{OBz}]^+$; $C_{66}H_{94}N_4O_{28}$ (1631.95).

(2*S*,3*R*,4*E*)-3-Hydroxy-2-(octadecanamido)octadec-4-enyl 4-*O*-[3-*O*-(5-acetamido-1,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (4): A solution of sodium methoxide in methanol (2.3 M, 60 μL) was added to a solution of **27** (64.5 mg, 39.6 μmol) in dry methanol (4 mL). After stirring for 20 h at rt the reaction was neutralized by addition of ion-exchange resin Levatit 100S (H^+ form). After filtration the solution was concentrated in vacuo and purified by flash chromatography (EtOAc/MeOH 2:1) to yield **4** (22.7 mg, 50%) and C-2b-epimer (12.2 mg, 27%): R_f = 0.38 (β-anomer (C-2c)), 0.35 (α-anomer (C-2c)) (EtOAc/MeOH 2:1); ^{1}H NMR (500 MHz, MeOD, α-anomer (C-2c)): δ = 0.89 (t, J = 7.0 Hz, 6H, CH'_3), 1.22–1.41 (m, 50H, CH'_2), 1.56 (m, 2H, 3″-H), 1.63 (dd, J = 11.0, 13.0 Hz, 1H, 3c-H_{ax}), 2.01 (s, 3H, CH_3CO), 2.16 (t, J = 7.5 Hz, 2H, 2′-H), 2.70 (dd, J = 5.0, 13.0 Hz, 1H, 3c-H_{eq}), 3.37 (m, 1H), 3.42 (dd, J = 1.5, 9.5 Hz, 1H), 3.45–3.58 (m, 4H), 3.61 (dd, J = 5.5, 11.5 Hz, 1H), 3.64–3.90 (m, 13H), 3.92–3.96 (m, 1H, 4c-H), 4.06 (t, J = 8 Hz, 1H, 1′-H), 4.04 (d, J = 12 Hz, 1H, 1c-H_{eq}), 4.18 (dd, J = 4.5, 10.0 Hz, 1H), 4.30 (d, J = 8.0 Hz, 1H, 1a-H), 4.57 (d, J = 8.0 Hz, 1H, 1b-H), 5.44 (dd, J = 7.5, 15.0 Hz, 1H, 4′-H), 5.68 (dt, J = 6.5, 15.0 Hz, 1H, 5′-H); ^{1}H NMR (500 MHz, MeOD, β-anomer (C-2c)): δ = 0.89 (t, J = 7.0 Hz, 6H, CH'_3), 1.22–1.41 (m, 50H, CH'_2), 1.62 (m, 2H, 3″-H), 2.00 (s, 3H, CH_3CO), 2.08 (dd, J = 5.5, 13.0 Hz, 1H, 3c-H_{eq}), 2.16 (t, J = 7.5 Hz, 2H, 2′-H), 3.27 (dd, J = 2.0, 11.0 Hz, 1H), 3.37 (m, 1H), 3.48–3.88 (m, 18H), 3.94–3.98 (m, 1H, 4c-H), 4.03–4.09 (m, 3H), 4.17 (dd, J = 4.5, 10.0 Hz, 1H), 4.29 (d, J = 8.0 Hz, 1H, 1a-H), 4.56 (d, J = 7.5 Hz, 1H, 1b-H), 5.44 (dd, J = 8.0, 15.0 Hz, 1H, 4′-H), 5.67 (dt, J = 7.0, 15.0 Hz, 1H, 5′-H); HR-MS (FAB^+): calcd for $C_{59}H_{108}CsN_2O_{19}$ 1281.6601; found: 1281.6652.

5-Methoxycarbonylpentenyl 4-*O*-[3-*O*-(5-acetamido-1,3,5-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyloyl-1c → 2b-pyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (29): A solution of **25** (82 mg, 73 μmol), 6-hydroxyhexanoic acid methyl ester (85 mg, 584 μmol), and molecular sieves (4 Å, 100 mg) in CH_2Cl_2 (3 mL) was stirred for 2 h. The solution was cooled to −50 °C and $BF_3 \cdot OEt_2$ (30 μL) was added. The reaction was allowed to warm up to −20 °C within 1.5 h and triethylamine (0.2 mL) was added at −20 °C. The reaction mixture was filtered and concentrated in vacuo. The residue was purified by flash chromatography to give **28** (56 mg, 70%).

Acetate **28** (10.0 mg, 9.03 μmol) was dissolved in dry methanol (5 mL) and a solution of sodium methoxide in methanol (5.4 M, 30 μL) was added. The reaction was stirred for 24 h at rt and water (0.1 mL) was added. After stirring for 1 h at rt the reaction was quenched by addition of ion-exchange resin Levatit 100S (H^+ form), the mixture was filtrated and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography to give **29** (6.0 mg, 92% from **28**): R_f = 0.13 (EtOAc/MeOH 2:1); ^{1}H NMR (500 MHz, MeOD, both anomers (C-2c)): δ = 1.36–1.67 (m, 6H, 2′-H, 3′-H, 4′-H), 2.00 (s, 1.29H, CH_3CO (β-anomer)), 2.01 (s, 1.71H, CH_3CO (α-anomer)), 2.15 (dd, J = 5.0, 13.0 Hz, 0.43H, 3c-H (β-anomer)), 2.33 (t, J = 7.5 Hz, 2H, 5′-H), 2.70 (dd, J = 5.0, 13.0 Hz, 0.57H, 3c-H_{eq} (α-anomer)), 3.20–4.18 (m, 23H), 3.65, 3.76 (2s, 3H, OMe (α-, β-anomer)), 4.27 (d, J = 8.0 Hz, 0.43H, 1a-H (β-anomer)), 4.28 (d, J = 8.0 Hz, 0.57H, 1a-H (α-anomer)), 4.56 (d, J = 7.5 Hz, 0.43H, 1b-H (β-anomer)), 4.58 (d, J = 8.0 Hz, 0.57H, 1b-H (α-anomer)); HR-MS (FAB^+) calcd for $C_{30}H_{51}CsNO_{19}$ 862.2110, found: 862.2142.

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