

A New and Efficient Approach to Prepare *N*-Acetyl GM₃ Ganglioside via Trisaccharide [1→4] Lactone

Abel Regalado Calderín, Miriam Mesa Hernández, Diamela Chávez Piñeiro, Rícelia González Serpa, Lázaro Andrés Monteserín Castanedo, María del Carmen Rodríguez Montero, Blanca Tolón Murgía, Roberto Carlos Veloso Pita, Raine Garrido Arteaga, Violeta Fernández Santana, Vicente Verez Bencomo, and Miguel Antonio López López*

Center of Biomolecular Chemistry (CQB), Calle 200 y 21, Atabey, Playa, 11600 La Habana, Cuba

ABSTRACT: The *N*-acetyl GM₃ ganglioside (NAcGM₃) is an important glycosphingolipid currently used to prepare a new therapeutic cancer vaccine. Some quantities of this ganglioside were obtained by using [1→4] lactone as the trisaccharide protective function. Thus, sialylation of hexabenzylactose acceptor with 5-acetyl neuraminythiophenyl donor afforded the (2→3) trisaccharide as an α/β (3:1) mixture. The α -anomer was isolated through selective [1→4] lactone formation followed by chromatography. The lactone was hydrogenolyzed, per-*O*-acetylated, and selectively deacetylated, and a trichloroacetimidate donor was synthesized from the obtained compound. Azidosphingosine glycosylation, followed by azide group reduction and acylation of the resulting amino glycoside with stearic acid provided the protected ganglioside, which was finally subjected to the Zemplén's procedure, before saponification, to obtain the NAcGM₃ in an overall yield of 11.5% at multigram scale.

INTRODUCTION

N-Acetyl GM₃ ganglioside (NAcGM₃) (Figure 1) is a ubiquitous metabolite with a variety of cellular activities that

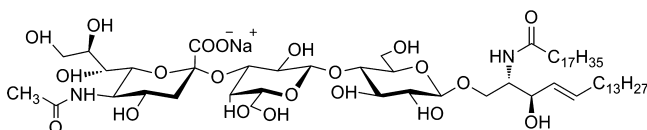


Figure 1. Chemical structure of NAcGM₃ (C-18) ganglioside.

include differentiation,¹ the modulation of several receptors and other enzymes,^{2,3} immunosuppression,⁴ and tumor association.⁵

NAcGM₃ is the major ganglioside of human melanoma,⁶ with similar expression levels both in vitro and in vivo.⁷ The finding that chemotherapy-resistant cancer cells express very high levels of NAcGM₃⁸ favors the view that this ganglioside could be a useful target for immunotherapy.

Currently, a new therapeutic cancer vaccine is in development. Since no practical synthetic method to prepare NAcGM₃ is presently available, all the initial biological studies, including some clinical trials, have been carried out with the natural ganglioside extracted and purified with a high grade of purity from canine erythrocytes. As clinical trials are ongoing, thus demanding some quantities of NAcGM₃, we are very interested in exploring alternatives for large-scale production of NAcGM₃ to be able to supply the quantities needed.

There have already been a number of previous syntheses of NAcGM₃,^{9–24} but in all the cases only research quantities of the ganglioside have been obtained. One of the problems to prepare NAcGM₃ at larger scale is that the use of the sialyl donor and the lactose acceptor that are cheaper and more readily available (Figure 2) afford an α/β mixture of the corresponding sialosides in a relatively low ratio to the desired

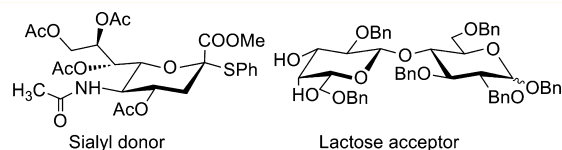


Figure 2. More readily available sialyl donor and lactose acceptor.

α -anomer (3:1 in the best). Because of the similar chromatographic properties of the two anomers, the isolation of the α -anomer represents a cumbersome problem limiting large-scale production.

In order to increase the α -stereoselectivity during sialylation, the synthetic strategies have been focused on the development of new sialyl donors and lactose acceptors with different substitution patterns.²⁵ Although in this way higher α -stereoselectivity has been obtained, the synthesis of these starting compounds requires more expensive reagents and time-consuming multistep procedures that are inappropriate for large-scale production. Considering the previous studies, the aim of the present work was to develop a synthetic route that allows the preparation of NAcGM₃ in a multigram scale from the readily available sialyl donor and lactose acceptor shown in Figure 2.

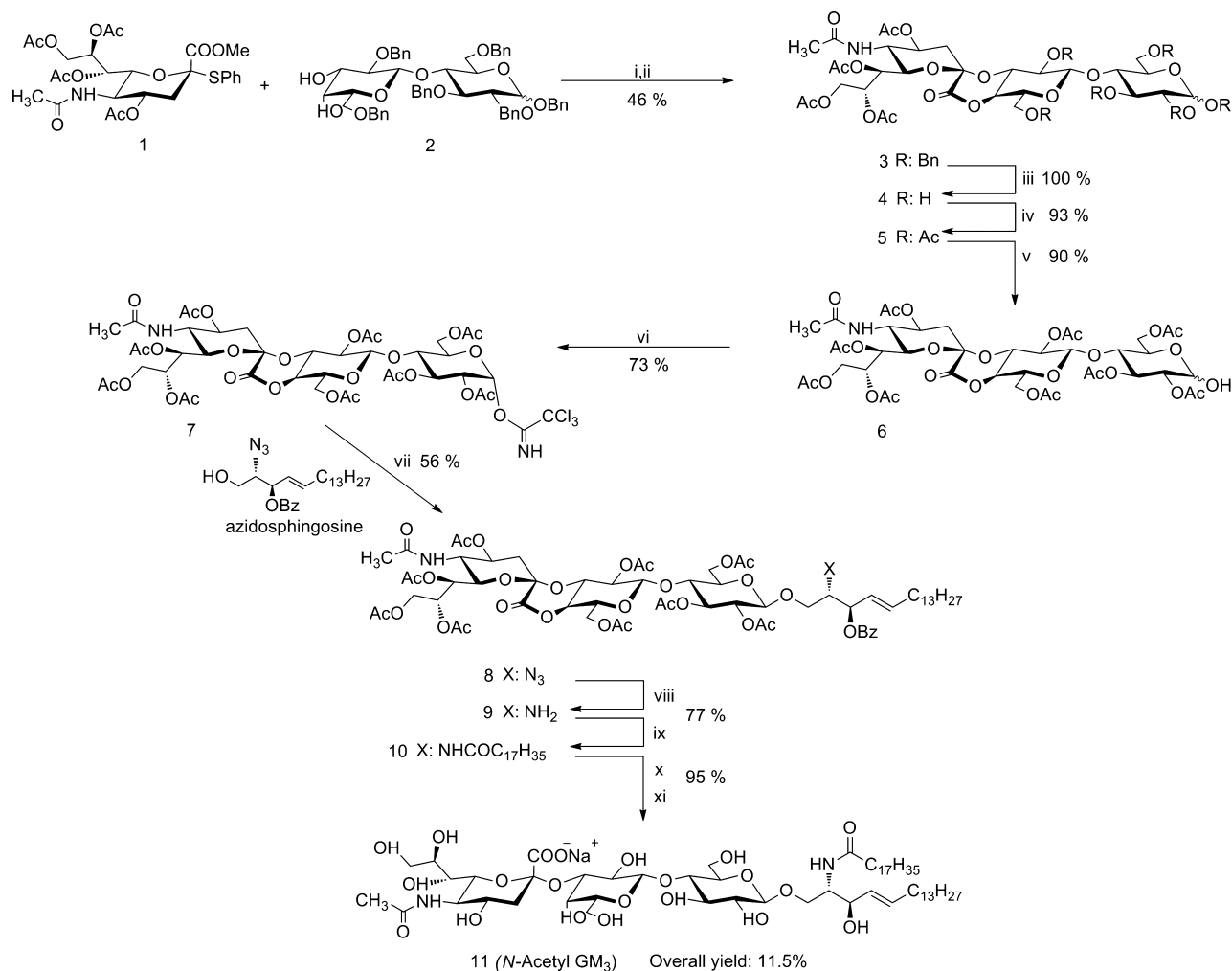
RESULTS AND DISCUSSION

The synthetic pathway used during the present work is shown in Scheme 1.

The sialylation of acceptor 2 with the thiophenyl donor 1 was performed according to a standard procedure, *N*-iodosuccinimide (NIS)/triflic acid (TfOH) as thiophilic promoter system and acetonitrile as participant solvent,^{26,27}

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Scheme 1. Synthetic pathway used to prepare NAcGM₃

Reagents and conditions (i) NIS, TFOH, molecular sieves 3 Å, MeCN, -35 °C, 1–2 h; (ii) DBU, CH₂Cl₂, 0–5 °C, 2 h; (iii) H₂, Pd/C, MeOH/AcOH (9:1 v/v), 25 °C, 24 h; (iv) Ac₂O, I₂, 45 °C, 2–3 h; (v) NH₄OAc, DMF, 25 °C, 24 h; (vi) trichloroacetonitrile, DBU, CH₂Cl₂, 25 °C, 2 h; (vii) BF₃·OEt₂, molecular sieves 4 Å, CH₂Cl₂, 0 °C, 1–2 h; (viii) H₂S (g), Py/H₂O/Et₃N, 0 °C, 24 h; (ix) stearic acid, EDC·HCl, CH₂Cl₂, 25 °C, 24 h; (x) NaOMe, MeOH, 25 °C, 24 h; (xi) H₂O, 25 °C, 24 h.

to obtain an α/β mixture of the corresponding sialosides in an approximately 3:1 ratio. To isolate the α -anomer it was decided to use the method reported by Castro-Palomino,²⁸ consisting in the treatment of the sialylation mixture with DBU in dichloromethane to lactonize selectively and quantitatively the α -anomer (the β -anomer remains intact). As the lactone, the α -anomer can be easily separated from the undesired products by the usual chromatographic techniques due the notable difference in polarity between this compound and the β -anomer.

Since some lactone degradation was observed by thin layer chromatography (TLC) during the reaction and final workup, some modifications of the original method were made (reaction temperature was set between 0–5 °C; a half quantity of DBU was used and neutralization of the reaction mixture was made with acetic acid instead of Amberlite resin). Under these new conditions the lactone 3 was obtained in 46% yield referred to the acceptor 2.

In order to prepare NAcGM₃, Castro-Palomino²⁸ regenerated the α -anomer methyl ester by opening the lactone ring with catalytic amounts of DBU in methanol at -20 °C. When we use this procedure as reported, no reaction was observed.

Only by adding more DBU (in order to reach a pH value between 8 and 9) did the methyl ester formation occur, but the reaction had to be stopped when approximately half of the lactone had been transformed because partial deacetylation of both (the lactone and the methyl ester) started. As a consequence, a chromatographic purification was necessary to obtain the methyl ester in only 54% yield. In order to avoid this additional step and its negative influence on the ganglioside overall yield, in the present work it was decided to prove the concept that it is possible to maintain the [1→4] lactone as a protecting group during the entire synthetic sequence to obtain NAcGM₃. Thus, the benzyl groups of the lactone 3 were removed by hydrogenolysis in the presence of Pd/C as catalyst and a mixture of methanol/acetic acid as the solvent to afford the compound 4 quantitatively.

To prepare the per-O-acetylated derivative 5, two methods were explored: (a) the traditional acetic anhydride/pyridine technique and (b) the procedure reported by Kartha et al.²⁹ for mono- and disaccharides (acetic anhydride and iodine as Lewis acid catalyst). Both methods afforded the compound 5 successfully, but the treatment of 4 with acetic anhydride in the presence of iodine at 45 °C (Kartha's method) proceeded

smoothly, and the reaction concluded in only 2–3 h. The per-*O*-acetylated trisaccharide **5** was obtained in a 93% yield with high purity, and it was unnecessary to purify the product by chromatography. Then it was concluded that the acetic anhydride/iodine method is more advantageous because it avoids the use of pyridine, is less time-consuming (2–3 h vs 24 h), and has no need for further chromatographic purification.

Selective anomeric deacetylation of **5** glucose moiety to prepare compound **6** was comparatively performed by two methods: the standard hydrazine acetate/DMF procedure³⁰ and the technique reported by Wang et al.³¹ for mono- and disaccharides by using ammonium acetate/DMF reagent at 25 °C. Although both methods gave the same results (~90% yield) and no lactone opening was detected, the use of ammonium acetate/DMF is safest because avoids the use of toxic hydrazine acetate and guarantees a better reaction control since the formation of side products was not observed (it is known that the reaction with hydrazine acetate has to be effected at 50 °C and must be carefully controlled to prevent overdeacetylation). Despite the higher reaction time required (24 h vs 1–1.5 h), it was concluded that the use of ammonium acetate/DMF is the best option because no chromatographic purification of the final product was necessary.

The α -trichloroacetimidate **7** was synthesized by treatment of compound **6** with trichloroacetonitrile and DBU as the catalyst in dichloromethane at 25 °C following a typical procedure³² to afford the trisaccharide donor in 73% yield.

Initially, and considering the results reported by some authors,^{9,10,13,14,24} we attempted the glycosylation of ceramide (C-18) by using $\text{BF}_3 \cdot \text{OEt}_2$ as the catalyst at 25 °C. However, during the present work a mixture of glycosylated products (in only 10% total yield) that was unseparable by normal column chromatography was obtained. For this reason, the ceramide moiety was constructed following the most used and best approach: azidosphingosine glycosylation, azido function reduction, and acylation of the resultant amino group to obtain the protected ganglioside.^{11,12,15,17,18,23,26}

Azidosphingosine was glycosylated with the trichloroacetimidate **7** by using $\text{BF}_3 \cdot \text{OEt}_2$ as the catalyst and dichloromethane as the solvent at 0 °C to afford a β/α (20:1) mixture of the glycosides. The β glycoside **8** was easily isolated from the reaction mixture by chromatography in 56% yield. Reduction of the azido group was performed by passing H_2S gas through a solution of the glycoside **8** in pyridine/water/triethylamine during 1 h at 0 °C, stirring the mixture for 6 h, passing again H_2S gas during 1 h, and stirring the solution at 0 °C until it was confirmed by TLC that the reaction finished (approximately 16 h). After coevaporation with toluene the obtained crude aminoglycoside (compound **9**) was used in the next step without a further purification.

In order to avoid the use of toxic H_2S gas, the Staudinger method was explored by using triphenylphosphine in a mixture of THF/water.³³ Although by TLC a spot with the same R_f value as that of the aminoglycoside **9** was observed (indicating the possible completion of the reaction), the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of the compound isolated from the reaction mixture showed the presence of only one peak corresponding to that of the molecular mass of the iminophosphorane intermediary. Increasing the reaction time (48 and 96 h) and the temperature (up to 50 °C) gave the same result. Thus, it was possible to conclude that, under the usual reaction conditions of the Staudinger method, the

iminophosphorane hydrolysis does not occur, and the desired aminoglycoside **9** is not obtained. Additionally, the TLC result suggests that the silica gel catalyzes the iminophosphorane hydrolysis during the chromatographic analysis. Since the hydrolysis of the triphenylphosphine iminophosphorane adduct is the problematic step, a possible alternative to avoid the use of H_2S is to use trimethyl or tributylphosphine in the Staudinger method because the hydrolysis of the corresponding iminophosphorane adducts is faster.

To prepare the protected ganglioside **10** two acylation methods were used: (a) the treatment of the aminoglycoside **9** with stearoyl chloride in a biphasic solvent system (THF/sodium acetate solution)³⁴ and (b) the reaction of compound **9** with stearic acid in dichloromethane as the solvent and EDC·HCl as condensing agent.^{11,19} In the first case the reaction concluded after 3 h, and by MALDI-TOF MS it was possible to detect that the lactone opening occurs to obtain the compound shown in Figure 3. Although at this stage of the process the

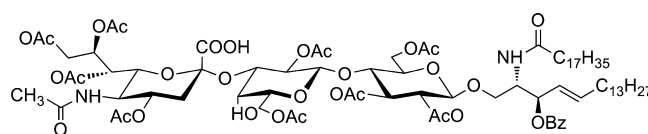


Figure 3. Product obtained during acylation with stearoyl chloride in THF/sodium acetate.

lactone opening would not affect the final synthesis of NACGM_3 , the yield obtained during the acylation was relatively low (44% calculated from **8**).

When the acylation was performed by using stearic acid and EDC·HCl, the lactone opening did not occur, and the protected ganglioside **10** was obtained with good yield (77% calculated from **8**). The excess of EDC·HCl (a known genotoxic impurity) was removed by washing with water followed by chromatographic purification after the workup. Although this acylation method is more time-consuming (24 h), it is more appropriate because it increases the overall yield of the final product.

In the last step of the synthetic sequence, the protective groups of **10** were removed by Zemplen's method³⁵ followed by the lactone saponification to prepare NACGM_3 (**11**). Since the ganglioside must be obtained as the corresponding sodium salt, during the Zemplen's procedure it was necessary to add sodium methoxide not only to remove protective groups but also to hydrolyze (after the water addition) the generated esters (methyl acetate and benzoyl acetate) and to neutralize the sialyl moiety acid function. Thus, **10** was treated with a methanolic solution of sodium methoxide (approximately 11 mmol by mmol of **10**) over 24 h, and then water was added to open the lactone and to form the ganglioside sodium salt. After neutralizing the excess of base and evaporating the solvents, the residue was dialyzed to remove the sodium acetate and benzoate generated during the saponification process. The resulting solution of the ganglioside was dried by lyophilization to obtain NACGM_3 with an excellent yield (95%) and high purity. Since it is known that NACGM_3 is not stable and undergoes lactonization, the synthetic ganglioside was packed in sealed vials and stored at –20 °C. Under these conditions the NACGM_3 remains stable during 22 months. At storage temperatures between 2–8 °C (also in sealed vials) the ganglioside is stable for 1 year. In the stability studies conducted during the periods of time mentioned above, neither

the ganglioside lactonization nor the formation of other degradation products was detected.

Considering all the synthetic steps, the overall yield (calculated from acceptor **2**) was 11.5%, a better yield than that obtained by the efficient chemo-enzymatic approach reported by Danishefsky¹⁵ (10.1%) and consequently provides a very satisfactory result to prepare this ganglioside at larger scale. Although during the sialylation step the α/β (3:1) ratio obtained is similar to that reported by other procedures, the developed approach is more appropriate for scale-up because it combines the following factors: (a) the use of a sialic acid donor and lactose acceptor that are cheaper and easier to synthesize (only three synthetic steps are required for each); (b) after the sialylation, the easy isolation of the trisaccharide α -anomer as the corresponding lactone; (c) the simultaneous protection by the lactone of both the acid function and the galactose 4-hydroxy group during the complete synthetic sequence; (d) the use of the classical reactions of carbohydrate and organic chemistry during the preparation of the trisaccharide intermediates and the ceramide moiety construction; (e) low complexity of the chemical reactions involved so that, consequently, the reagents used are readily available and inexpensive; (f) significant reduction of the number of chromatographic purification steps by the methods used to carry out the per-*O*-acetylation and the selective trisaccharide deacetylation.

Considering that the requirement for ongoing clinical trials of ganglioside is 30 g per year, the developed process that allows obtaining 3.7 g per run is perfectly able to supply the quantities needed to support these trials.

CONCLUSION

The [1→4] lactone can be used as a protective function during the entire synthetic sequence to prepare NAcGM₃. This new approach allows developing an efficient procedure to prepare this ganglioside in a multigram scale from the readily available sialyl donor **1** and lactose acceptor **2**. As the ganglioside needs are modest, the developed procedure is able to provide the quantities needed for ongoing clinical trials, and no further scale up is required.

Although there are some steps that require chromatographic purification, the per-*O*-acetylation of compound **4** by the acetic anhydride/iodine method and the anomeric selective deacetylation of compound **5** by using ammonium acetate/DMF simplify the procedure for obtaining the NAcGM₃ because it is unnecessary to purify the resulting products by chromatography. Since it is the first time that [1→4] lactone has been used as a protective function during the entire synthetic sequence to prepare NAcGM₃, the compounds **4**, **5**, **6**, **7**, **8**, **9**, and **10** are new.

EXPERIMENTAL SECTION

General Methods. Reactions to obtain the compounds **3** and **8** were carried out under argon. When it was required, the organic phases were dried over anhydrous Na₂SO₄, followed by filtration through glass wool and solvent removal by rotary evaporation under diminished pressure. Silica gel (grade 60, 230–400 mesh) was used for column chromatography. Analytical TLC and HPTLC were performed on precoated plates of Silica gel 60, and the chromatograms were visualized by spraying the plates with 5% H₂SO₄/EtOH reagent followed by heating. When it was required, the product solutions were

filtered through Celite 545. Melting points (mp) were determined in a Büchi M-565 apparatus. Optical rotations were measured with an ADP 220 Bellenhgan + Stanley Ltd. polarimeter at 25 °C. NMR analysis was performed on a Bruker/Avance DPX 250-MHz instrument, operating under Topspin 1.3 software with a 5 mm QNP *z*-axis gradient probe at a basic proton frequency of 250.13 MHz. The gradient-selective homonuclear correlation (gs-COSY) experiments were run with a second excitation pulse of 45° in magnitude mode. The total correlation (TOCSY) experiments were run for complementing the proton assignments. The ¹³CNMR-edited spectra were obtained at 62.9 MHz ¹³C frequency through a polarization enhancement during attached nucleus testing (PENDANT) experiment. The two-dimensional gradient-selective heteronuclear single-quantum correlation (gs-HSQC) and gradient-selective heteronuclear multiple-quantum correlation (gs-HMQC) experiments assisted the characterization processes. In addition, the gradient-selective heteronuclear multiple-bond correlation (gs-HMBC) experiments allowed several supported assignments. The purity of synthetic NAcGM₃ and of all the intermediates was determined by quantitative ¹H NMR spectroscopy (qHNMR) using an external standard solution of 3-(trimethylsilyl)-2,2,3,3-tetra-deuteriopropionic acid sodium salt (TPS-*d*₄) in DMSO-*d*₆. A coaxial stem insert was filled with the solution of the reference compound and inserted in the NMR tube containing a solution of the compound to be analyzed in the appropriate deuterated solvent. The optimized qHNMR experiment was set without sample spinning, a relaxation delay of 60 s, a pulse angle of 90°, and ¹³C decoupling using the globally optimized alternating phase rectangular pulse (GARP) sequence. To obtain the spectra, each free induction decay (FID) was processed with a Fourier transform apodized by an exponential function (LB 0.3 Hz). To guarantee the signal-integration process, baseline adjustment was required. The MALDI-TOF MS was performed on a MALDI-TOF mass spectrometer (Axima Performance, Shimadzu, Japan), operated in reflectron positive mode. Two microliters of sample solution (CHCl₃/MeOH 2:1, 7 mg/mL) was mixed with 2 μL of 2,5-dihydroxybenzoic acid (DHB) as matrix solution (5 mg/mL) (Agilent, U.S.A.). The sample plus matrix was spotted onto the MALDI target. Spectra were obtained by accumulating 100 consecutive laser shots. Calibration was performed recording the [M + H]⁺ ions of a mixture of peptides including bradykin fragment (*m/z* 757.39), angiotensin II (*m/z* 1046.54), angiotensin I (*m/z* 1296.69), Glu fib (*m/z* 1570.68), renin substrate (*m/z* 1800.94), ACTH fragment 1–17 (*m/z* 2093.09), ACTH fragment 18–39 (*m/z* 2465.20), and C₁₆₇H₂₅₈N₄₇O₄₆ (*m/z* 3657.93). The calibrating mixture (0.5 μL) was spotted onto a MALDI target followed immediately by 0.5 μL of matrix solution containing 10 mg/mL of α -cyano-4-hydroxycinnamic acid in 0.1% (v/v) TFA and 60% (v/v) MeCN.

Benzyl O-[5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy- α -D-erythro-L-glucopyranosyl-1→4-lactone]-O-(2,6-di-*O*-benzyl- β -D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl- α,β -D-glucopyranoside (3**).** A solution of thiophenyl donor **1** (30 g, 46.8 mmol), hexabenzyl lactose acceptor **2** (24 g, 27.2 mmol), and dried 3 Å molecular sieves (36 g) in dry MeCN (230 mL) was stirred for 1 h. After cooling to –35 °C, NIS (14.4 g, 64 mmol) was added followed by TfOH (0.56 mL, 6.28 mmol), and the mixture was stirred for 1–2 h at the same temperature. The reaction was monitored by TLC and when finished, CH₂Cl₂ (240 mL)

was added, and the mixture was filtered through Celite. The filtrate was washed with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution, the organic layer was neutralized by addition of triethylamine (Et_3N), and the solvents were removed by evaporation under reduced pressure. The residue was dissolved in dry CH_2Cl_2 (250 mL), the obtained solution was cooled to 0–5 °C, DBU (6.8 mL, 45.6 mmol) was added, and the mixture was stirred for 2 h at the same temperature. After neutralization with AcOH, the solvent was evaporated, and the residue was chromatographed (8:1 toluene/acetone) to recover the unreacted **2**, followed by the pure α -[1→4] lactone **3** (Yield: 16.6 g, 46%. Purity: 98–99%). Amorphous white solid that was homogeneous by TLC (2:1 toluene/acetone, R_f : 0.49). **Mp**: 74–76 °C. $[\alpha]_D$: –28.4° (c 0.5, CH_2Cl_2). ^1H NMR (CDCl_3): 2.14, 2.03, 2.00, 1.92, 1.87 [(3 H, s, CH_3) × 5]; 7.17–7.45 (30 H, m, Ar); benzylic, 4.33–4.86 [12 H (d × 11)]; **Glc**: 4.49 (1 H, d, $^3J_{1,2}$ 8.2 Hz, 1), 3.46 (1 H, dd, $^3J_{2,3}$ 7.5 Hz, 2), 3.56 (1 H, dd, $^3J_{3,4}$ 8.6 Hz, 3), 3.74 (1 H, m, 4), 3.35 (1 H, m, 5), 3.76 (1 H, m, 6a), 3.72 (1 H, m, 6b); **Gal**: 4.42 (1 H, d, $^3J_{1,2}$ 7.9 Hz, 1), 3.25 (1 H, dd, $^3J_{2,3}$ 9.5 Hz, 2), 4.14 (1 H, dd, $^3J_{3,4}$ 4.1 Hz, 3), 4.97 (1 H, dd, 4), 3.54 (1 H, m, 5), 3.70 (1 H, m, 6a), 3.40 (1 H, m, 6b); **Neu**: 1.77 (1 H, dd, $^3J_{3\text{ax},3\text{ec}}$ 12.9 Hz, $^3J_{3\text{ax},4}$ 12.0 Hz, 3ax), 2.06 (1 H, m, 3ec), 5.46 (1 H, m, 4), 4.08 (1 H, dd, 5), 3.69 (1 H, m, 6), 5.20–5.35 (2 H, m, 7, 8), 4.33 (1 H, m, 9a), 3.94 (1 H, m, 9b), 6.24 (1 H, d, $^3J_{5,5\text{-NH}}$ 10.3 Hz, 5-NH). ^{13}C NMR (CDCl_3): 170.7, 170.5, 170.3, 170.1, 170.0, 164.2 [C=O × 6]; 23.0, 20.9, 20.7 (3C) [CH_3 × 5]; 138.78, 138.39, 138.06, 138.02, 137.74, 137.38, 128.5–127 (30 C); [Ar × 36]; 75.3, 75.1, 74.9, 73.6, 73.2, 70.9 [benzylic × 6]; **Glc**: 101.2 (1), 82.7 (2), 81.7 (3), 73.3 (4), 74.9 (5), 68.1 (6); **Gal**: 101.9 (1), 78.7 (2), 73.1 (3), 71.6 (4), 71.9 (5), 67.4 (6); **Neu**: 95.1 (2), 37.8 (3), 70.0 (4), 49.2 (5), 73.8 (6), 68.3 (7), 68.1 (8), 62.1 (9). **MALDI-TOF MS**: $[\text{M} + \text{Na}]^+$ m/z 1346.62 (calcd 1346.51).

(5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1→4-lactone)-(β -D-galactopyranosyl)-(1→4)- α , β -D-glucopyranose (4**).** The α -[1→4] lactone **3** (16.6 g, 12.5 mmol) was dissolved in 680 mL of MeOH/AcOH (9:1 v/v), 2.82 g of 10% Pd/C was added, H_2 was injected, and the mixture was stirred at 25 °C for 24 h. The catalyst was removed by filtration through Celite, and the solvents were evaporated to give a quantitative yield of **4** (Yield: 9.82 g. Purity: 95–97%) that was used in the next step without further purification. Purification of the crude product by column chromatography provided an analytical sample as an amorphous white solid that was homogeneous by TLC (64:25:4 $\text{CH}_3\text{Cl}/\text{MeOH}/\text{H}_2\text{O}$, R_f : 0.46). **Mp**: 125–127 °C. $[\alpha]_D$: +8.1° (c 1.0, MeOH). ^1H NMR (CDCl_3): 1.98, 1.97, 1.94, 1.90, 1.88 [(3 H, s, CH_3) × 5]; **Glc**: 4.34 (1 H, d, $^3J_{1,2}$ 7.8 Hz, 1), 2.99 (1 H, dd, $^3J_{2,3}$ 8.0 Hz, 2), 3.38 (1 H, dd, $^3J_{3,4}$ 7.7 Hz, 3), 3.64 (1 H, m, 4), 3.97 (1 H, m, 5), 4.23–4.10 (2 H, m, 6a, 6b); **Gal**: 4.39 (1 H, d, $^3J_{1,2}$ 7.8 Hz, 1), 3.22–3.16 (2 H, m, 2, 5), 3.50 (1 H, dd, $^3J_{2,3}$ 5.8 Hz, $^3J_{3,4}$ 3.5 Hz, 3), 4.92 (1 H, dd, $^3J_{3,4}$ 5.8 Hz, 4), 3.62–3.54 (2 H, m, 6a, 6b); **Neu**: 1.69 (1 H, dd, $^3J_{3\text{ax},3\text{ec}}$ 15.4 Hz, $^3J_{3\text{ax},4}$ 9.1 Hz, 3ax), 2.48 (1 H, dd, $^3J_{3\text{ec},4}$ 5.2 Hz, 3ec), 5.29 (1 H, m, 4), 3.96–3.84 (2 H, m, 5, 9b), 3.71 (1 H, m, 6), 5.18–5.02 (2 H, m, 7, 8), 4.23–4.10 (1 H, m, 9a), 6.16 (1 H, br s, 5-NH). ^{13}C NMR (CDCl_3): 170.2, 169.8, 169.6, 169.3, 164.0, 161.1 [C=O × 6]; 22.1 (2), 20.5 (2), 20.2 [CH_3 × 5]; **Glc**: 102.0 (1 β), 96.3 (1 α), 74.2 (2), 79.8 (3), 69.5 (4), 48.4 (5), 61.3 (6); **Gal**: 102.1 (1), 69.0 (2), 66.4 (3), 90.9 (4), 71.1 (5), 59.6 (6); **Neu**: 94.7 (2), 28.9 (3), 69.4 (4), 47.4 (5), 72.1 (6), 67.8 (7), 66.0 (8), 61.0 (9). **MALDI-TOF MS**: $[\text{M} + \text{Na}]^+$ m/z 806.20 (calcd 806.23).

Acetyl O-[5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1→4-lactone]-O-(2,4,6-tri-O-acetyl- β -D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl- α , β -D-glucopyranoside (5**).** To a stirred solution of **4** (9.82 g, 12.5 mmol) in Ac_2O (46 mL) was added I_2 (0.98 g, 3.88 mmol) and the mixture was heated at 45 °C for 2–3 h. The obtained solution was cooled to 25 °C, diluted with CH_2Cl_2 (100 mL), and washed with an aqueous, saturated $\text{Na}_2\text{S}_2\text{O}_3$ solution. The organic layer was separated, the solvent was removed, the residue was poured into cool water (0–5 °C), and the mixture was stirred for 2 h. After being neutralized to pH 7 with saturated Na_2CO_3 solution, the mixture was extracted with CH_2Cl_2 (3 × 140 mL), the organic extracts were combined and dried, and the solvent was evaporated to obtain **5** (Yield: 12 g, 93%. Purity: 96–97%) as an amorphous white solid that was homogeneous by TLC (1:1 toluene/acetone, R_f : 0.73). **Mp**: 135–137 °C. $[\alpha]_D$: –24.2° (c 0.5, CH_2Cl_2). ^1H NMR (CDCl_3): 2.15, 2.14, 2.09 (3 CH_3), 2.08 (2 CH_3), 2.02, 1.97, 1.96 (2 CH_3) [(3 H, s, CH_3) × 11]; **Glc**: 6.26 (1 H, d, $^3J_{1,2}$ 8.2 Hz, 1), 3.46 (1 H, dd, $^3J_{2,3}$ 7.5 Hz, 2), 3.56 (1 H, dd, $^3J_{3,4}$ 8.6 Hz, 3), 3.74 (1 H, m, 4), 3.35 (1 H, m, 5), 3.76 (1 H, m, 6a), 3.72 (1 H, m, 6b); **Gal**: 4.42 (1 H, d, $^3J_{1,2}$ 7.9 Hz, 1), 3.25 (1 H, dd, $^3J_{2,3}$ 9.5 Hz, 2), 4.14 (1 H, dd, $^3J_{3,4}$ 4.1 Hz, 3), 4.97 (1 H, m, 4), 3.54 (1 H, m, 5), 3.70 (1 H, m, 6a), 3.40 (1 H, m, 6b); **Neu**: 1.77 (1 H, dd, $^3J_{3\text{ax},3\text{ec}}$ 12.9 Hz, $^3J_{3\text{ax},4}$ 12.0 Hz, 3ax), 2.06 (1 H, m, 3ec), 5.46 (1 H, m, 4), 4.08 (1 H, m, 5), 3.69 (1 H, m, 6), 5.20–5.35 (2 H, m, 7, 8), 4.33 (1 H, m, 9a), 3.94 (1 H, m, 9b), 6.24 (1 H, d, $^3J_{5,5\text{-NH}}$ 10.3 Hz, 5-NH). ^{13}C NMR (CDCl_3): 170.6, 170.5, 170.4, 170.2 (2C), 169.8 (2C), 169.6, 169.3, 168.9, 168.6, 162.8 [C=O × 12]; 23.2 (2C), 21.8, 20.5 (8C) [CH_3 × 11]; **Glc**: 88.8 (1), 69.0 (2), 69.0 (3), 75.3 (4), 71.7 (5), 61.4 (6); **Gal**: 100.0 (1), 71.7 (2), 62.3 (3), 61.3 (4), 69.2 (5), 70.8 (6); **Neu**: 95.0 (2), 37.6 (3), 68.6 (4), 49.0 (5), 67.7 (6), 70.8 (7), 71.2 (8), 61.5 (9). **MALDI-TOF MS**: $[\text{M} + \text{Na}]^+$ m/z 1058.42 (calcd 1058.3).

(5-Acetamido-4,7,8,9-tetra-O-Acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1→4-lactone)-O-(2,4,6-tri-O-acetyl- β -D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl- α , β -D-glucopyranose (6**).** To a stirred solution of **5** (12 g, 11.6 mmol) in dry DMF (60 mL) was added NH_4OAc (1.8 g, 24 mmol), and the mixture was stirred at 25 °C for 24 h. After ethyl acetate (300 mL) was added, the organic phase was washed with saturated NaCl aqueous solution (5 × 60 mL) and dried, and the solvent was removed to give **6** (Yield: 10.4 g, 90%. Purity: 95–96%) as an amorphous white solid that was homogeneous by TLC (1:1 toluene/acetone, R_f : 0.59). **Mp**: 133–135 °C. $[\alpha]_D$: –20.0° (c 0.5, CH_2Cl_2). ^1H NMR (CDCl_3): 2.13, 2.08, 2.07 (2 CH_3), 2.05 (2 CH_3), 2.02, 2.00, 1.97, 1.95 [(3 H, s, CH_3) × 10]; **Glc**: 5.36 (1 H, d, $^3J_{1,2}$ 3.4 Hz, 1 α), 4.90–4.70 (2 H, m, 1 β , 2), 5.3–5.1 (2 H, m, 3, 4), 4.32–4.00 (3 H, m, 5, 6a, 6b); **Gal**: 4.25 (1 H, d, $^3J_{1,2}$ 7.6 Hz, 1), 4.32–4.00 (2 H, m, 2, 6b), 3.80–3.60 (1 H, m, 3), 5.3–5.1 (1 H, m, 4), 3.95 (1 H, m, 5), 4.62 (1 H, m, 6a); **Neu**: 1.76 (1 H, dd, $^3J_{3\text{ax},3\text{ec}}$ 13.9 Hz, $^3J_{3\text{ax},4}$ 12.5 Hz, 3ax), 2.46 (1 H, dd, $^3J_{3\text{ec},4}$ 4.9 Hz, 3ec), 5.60–5.40 (2 H, m, 4, 7), 4.32–4.00 (2 H, m, 5, 9b), 3.80–3.60 (1 H, m, 6), 4.90–4.70 (1 H, m, 8), 4.46 (1 H, m, 9a), 6.28 (1 H, d, $^3J_{5,5\text{-NH}}$ 10.2 Hz, 5-NH). ^{13}C NMR (CDCl_3): 170.9, 170.6, 170.5, 170.2 (2C), 170.0, 169.7 (2C), 169.1, 167.9, 162.9 [C=O × 11]; 20.8 (2C), 20.3 (8C) [CH_3 × 10]; **Glc**: 99.7 (1 β), 89.7 (1 α), 67.8 (2), 76.3 (3), 66.9 (4), 72.0 (5), 62.2 (6); **Gal**: 99.5 (1), 71.4 (2), 68.3 (3), 66.9 (4), 70.8 (5), 62.2 (6); **Neu**: 95.0 (2), 37.9 (3), 69.2 (4), 48.9 (5),

72.3 (6), 69.2 (7), 70.0 (8), 62.2 (9). MALDI-TOF MS: $[M + Na]^+$ m/z 1016.09 (calcd 1016.28).

2,2,2-trichloroethanimidoyl O-[5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1 \rightarrow 4-lactone]-O-(2,4,6-tri-O-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-O-acetyl- α -D-glucopyranoside (7). To a stirred solution of **6** (10.4 g, 10.42 mmol) and trichloroacetonitrile (30 mL, 220 mmol) in dry CH_2Cl_2 (120 mL) was added DBU (1.6 mL, 10.4 mmol) dropwise over 5 min. After the mixture stirred for 2 h at 25 °C, the solvent was evaporated, and the residue was chromatographed (6:1 CH_2Cl_2 /acetone) to give trichloroacetimidate **7** (Yield: 8.66 g, 73%. Purity: 96–98%) as an amorphous white solid that was homogeneous by TLC (1:1 toluene/acetone, R_f : 0.73). **mp**: 110–112 °C. $[\alpha]_D$: -48.2° (c 0.5, CH_2Cl_2). 1H NMR ($CDCl_3$): 2.11 (2 CH_3), 2.09, 2.08 (2 CH_3), 2.04, 1.99 (2 CH_3), 1.98, 1.86 [(3 H, s, CH_3) \times 10]; **Glc**: 6.52 (1 H, d, $^3J_{1,2}$ 3.8 Hz, 1), 5.08 (1 H, dd, $^3J_{2,3}$ 10.2 Hz, 2), 5.60–5.40 (1 H, m, 3), 3.86 (1 H, dd, $^3J_{3,4}$ 9.6 Hz, $^3J_{4,5}$ 9.7 Hz, 4), 4.25–4.05 (3 H, m, 5, 6a, 6b), 8.72 (1 H, s, $Cl_3C=NH$); **Gal**: 4.48 (1 H, d, $^3J_{1,2}$ 8.0 Hz, 1), 4.25–4.05 (1 H, m, 2), 3.65 (1 H, dd, $^3J_{2,3}$ 10.6 Hz, $^3J_{3,4}$ 1.5 Hz, 3), 5.35–5.15 (2 H, m, 4, 5), 4.32 (1 H, dd, $^3J_{5,6a}$ 2.5 Hz, $^3J_{6a,6b}$ 4.7 Hz, 6a), 3.75 (1 H, m, 6b); **Neu**: 1.78 (1 H, dd, $^3J_{3ax,3ec}$ 13.7 Hz, $^3J_{3ax,4}$ 11.7 Hz, 3ax), 2.47 (1 H, dd, $^3J_{3ec,4}$ 5.5 Hz, 3ec), 5.60–5.40 (1 H, m, 4), 4.25–4.05 (1 H, m, 5), 4.00–3.75 (3 H, m, 6, 9a, 9b), 5.35–5.15 (2 H, m, 7, 8), 6.48 (1 H, d, $^3J_{5,5-NH}$ 3.8 Hz, 5-NH). ^{13}C NMR ($CDCl_3$): 170.7 (2C), 170.6, 170.5, 170.3 (2C), 169.9 (2C), 169.5, 169.1, 165.2 [$C=O \times 11$]; 162.8 [$C=N$]; 23.1 (2C), 20.8 (3C), 20.7, 20.6 (2C), 20.5, 20.4 [$CH_3 \times 10$]; **Glc**: 92.8 (1 α), 69.9 (2), 69.3 (3), 75.9 (4), 75.1 (5), 62.3 (6); **Gal**: 93.3 (1), 72.0 (2), 72.7 (3), 66.9 (4), 68.3 (5), 62.3 (6); **Neu**: 94.8 (2), 38.0 (3), 62.3 (4), 49.1 (5), 65.7 (6), 60.1 (7), 61.4 (8), 61.6 (9). MALDI-TOF MS: this product decomposes during the analysis and only one peak corresponding to the starting compound **6** ($[M + Na]^+$ m/z 1016.09) was observed.

(2R,3S,4E)-2-azido-3-(benzoyloxy)octadec-4-en-1-yl O-[5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1 \rightarrow 4-lactone]-O-(2,4,6-tri-O-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-O-acetyl- β -D-glucopyranoside (8). A solution of **7** (8.66 g, 7.6 mmol), azidosphingosine (6.24 g, 14.5 mmol) and dried 4 Å molecular sieves (10 g) in dry CH_2Cl_2 (70 mL) was stirred for 1 h at 25 °C. The mixture was cooled to 0 °C and $BF_3 \cdot OEt_2$ (2 mL, 15.8 mmol) was added over 5 min. After 2 h, the reaction mixture was neutralized with Et_3N , filtered through Celite, the solvent was removed and the residue was chromatographed (8:1 CH_2Cl_2 /acetone) to give the pure glycoside **8** (Yield: 6 g, 56%. Purity: 96–98%) as an amorphous white solid that was homogeneous by TLC (2:1 toluene/acetone, R_f : 0.51). **mp**: 73–75 °C. $[\alpha]_D$: -21.7° (c 1, CH_2Cl_2). 1H NMR ($CDCl_3$): 2.13 (2 CH_3), 2.09 (2 CH_3), 2.07, 2.04, 2.02, 2.01 (2 CH_3), 1.89 [(3 H, s, CH_3) \times 10]; 7.40–7.63 (5 H, m, Ar); **Glc**: 4.53 (1 H, d, $^3J_{1,2}$ 7.7 Hz, 1), 4.94 (1 H, dd, $^3J_{2,3}$ 9.7 Hz, 2), 5.15 (1 H, dd, $^3J_{3,4}$ 9.4 Hz, 3), 3.79 (1 H, m, 4), 3.62 (1 H, m, 5), 4.46 (1 H, m, 6a), 4.11 (1 H, m, 6b); **Gal**: 4.42 (1 H, d, $^3J_{1,2}$ 7.9 Hz, 1), 4.83 (1 H, dd, $^3J_{2,3}$ 8.1 Hz, 2), 4.16 (1 H, m, 3), 5.04 (1 H, dd, $^3J_{3,4}$ 3.0 Hz, $^3J_{4,5}$ 3.9 Hz, 4), 3.96 (1 H, m, 5), 3.86 (1 H, m, 6a), 3.59 (1 H, m, 6b); **Neu**: 1.73 (1 H, dd, $^3J_{3ax,3ec}$ 13.8 Hz, $^3J_{3ax,4}$ 11.8 Hz, 3ax), 2.45 (1 H, dd, $^3J_{3ec,4}$ 5.7 Hz, 3ec), 5.44 (1 H, m, 4), 4.12 (1 H, m, 5), 3.72 (1 H, m, 6), 5.20–5.25 (2 H, m, 7, 8), 4.62 (1 H, m, 9a), 4.26 (1 H, m, 9b),

8.10 (1 H, d, $^3J_{5,5-NH}$ 3.8 Hz, 5-NH); **Sph**: 3.86 (1 H, m, 1a), 3.62 (1 H, m, 1b), 3.93 (1 H, m, 2), 5.59 (1 H, m, 3), 5.53 (1 H, dd, $^3J_{4,3}$ 7.3 Hz, $^3J_{4,5}$ 14.7 Hz, 4), 5.92 (1 H, dt, $^3J_{5,6}$ 6.3 Hz, 5), 1.22–1.42 (24 H, m, 6–17), 0.88 (3 H, t, $^3J_{17,18}$ 6.9 Hz, 18). ^{13}C NMR ($CDCl_3$): 170.8, 170.7, 170.6 (2C), 170.4, 170.3, 170.0 (2C), 169.4, 169.2, 165.1, 162.9 [$C=O \times 12$]; 22.9, 20.8, 20.7 (2C), 20.6 (3C), 20.5 (2C), 20.4 [$acetyl\ CH_3 \times 10$]; **Glc**: 100.3 (1), 71.7 (2), 72.7 (3), 76.2 (4), 72.7 (5), 62.3 (6); **Gal**: 99.9 (1), 70.0 (2), 71.7 (3), 72.3 (4), 70.9 (5), 61.9 (6); **Neu**: 95.0 (2), 38.0 (3), 69.3 (4), 48.7 (5), 72.3 (6), 67.0 (7), 70.9 (8), 68.2 (9), 21.9 (5-NAc); **Sph**: 62.3 (1), 63.4 (2), 74.5 (3), 122.6 (4), 138.8 (5), 32.2, 31.8, 29.5 (5C) 29.2 (2C), 29.0, 28.6, 22.5, 13.6 (6–18). MALDI-TOF MS: $[M + Na]^+$ m/z 1427.63 (calcd 1427.57).

(2R,3S,4E)-2-amino-3-(benzoyloxy)octadec-4-en-1-yl O-[5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1 \rightarrow 4-lactone]-O-(2,4,6-tri-O-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-O-acetyl- β -D-glucopyranoside (9). This reaction was conducted in a well-ventilated hood by using a jacketed glass reactor (magnetically stirred), equipped with an inlet tube connected to a H_2S cylinder and an outlet tube connected to a trap system suitable for trapping the excess of gas. Through a stirred solution of **8** (6 g, 4.26 mmol) in 145 mL of $Py/H_2O/Et_3N$ (10:1:0.3), a stream of H_2S gas (CAUTION: H_2S is toxic. Use only with appropriate safety procedures!) was passed at 0 °C for 1 h with the inlet and outlet tubes closed; the mixture was stirred for 6 h at the same temperature. After this time, an additional treatment with H_2S gas was effected under the same conditions, the inlet and outlet tubes were closed, and the mixture was stirred for 16 h at 0 °C. The reaction was monitored by TLC, and when the reaction was finished, the solvents were removed by coevaporation with toluene to give **9** (Purity: 95–97%) that was used in the next step without a further purification. Purification of the crude product provided an analytical sample as an amorphous white solid that was homogeneous by TLC (2:1 CH_2Cl_2 /acetone, R_f : 0.63). **mp**: 92–94 °C. $[\alpha]_D$: -22.8° (c 2.2, CH_2Cl_2). 1H NMR ($CDCl_3$): 2.18, 2.14 (2 CH_3), 2.08, 2.05, 2.04, 2.03, 2.02, 2.01, 1.89 [(3 H, s, CH_3) \times 10]; **Glc**: 4.53 (1 H, d, $^3J_{1,2}$ 7.9 Hz, 1), 4.94 (1 H, dd, $^3J_{2,3}$ 9.7 Hz, 2), 5.15 (1 H, dd, $^3J_{3,4}$ 9.4 Hz, 3), 3.79 (1 H, m, 4), 3.62 (1 H, m, 5), 4.46 (1 H, m, 6a), 4.11 (1 H, m, 6b); **Gal**: 4.42 (1 H, d, $^3J_{1,2}$ 8.3 Hz, 1), 4.83 (1 H, dd, $^3J_{2,3}$ 8.1 Hz, 2), 4.16 (1 H, m, 3), 5.04 (1 H, dd, $^3J_{3,4}$ 3.0 Hz, $^3J_{4,5}$ 3.9 Hz, 4), 3.96 (1 H, m, 5), 3.86 (1 H, m, 6a), 3.59 (1 H, m, 6b); **Neu**: 1.73 (1 H, dd, $^3J_{3ax,3ec}$ 13.8 Hz, $^3J_{3ax,4}$ 11.8 Hz, 3ax), 2.45 (1 H, dd, $^3J_{3ec,4}$ 5.7 Hz, 3ec), 5.44 (1 H, m, 4), 4.12 (1 H, m, 5), 3.72 (1 H, m, 6), 5.20–5.25 (2 H, m, 7, 8), 4.62 (1 H, m, 9a), 4.26 (1 H, m, 9b), 8.06 (1 H, d, $^3J_{5,5-NH}$ 7.14 Hz, 5-NH); **Sph**: 3.97 (1 H, m, 1a), 3.30 (1 H, m, 1b), 3.68 (1 H, m, 2), 8.01 (1 H, d, $^3J_{2,2-NH}$ 6.5 Hz, 2-NH), 6.25 (1 H, m, 3), 5.54 (1 H, dd, $^3J_{4,3}$ 7.3 Hz, $^3J_{4,5}$ 14.7 Hz, 4), 5.94 (1 H, dt, $^3J_{5,6}$ 6.3 Hz, 5), 1.76 (2 H, m, 6), 1.00–1.50 (24 H, m, 7–17), 0.87 (3 H, t, $^3J_{17,18}$ 6.9 Hz, 18). ^{13}C NMR ($CDCl_3$): 170.5 (3C), 170.4, 170.2 (2C), 169.8 (2C), 169.4, 169.0, 165.1, 162.8 [$C=O \times 12$]; 23.0 (2C), 20.8 (2C), 20.7 (2C), 20.6 (2C), 20.5 (3C) [$CH_3 \times 11$]; **Glc**: 100.5 (1), 71.9 (2), 72.4 (3), 76.2 (4), 72.7 (5), 62.3 (6); **Gal**: 99.9 (1), 70.0 (2), 71.7 (3), 72.3 (4), 70.9 (5), 61.9 (6); **Neu**: 94.9 (2), 38.0 (3), 62.3 (4), 48.8 (5), 72.3 (6), 67.0 (7), 70.9 (8), 62.0 (9); **Sph**: 62.3 (1), 53.4 (2), 75.5 (3), 122.6 (4), 138.8 (5), 25.1 (6), 13.9 (18). MALDI-TOF MS: $[M + Na]^+$ m/z 1401.42 (calcd 1401.58).

(2R,3S,4E)-3-(Benzoyloxy)-2-(stearoylamino)octadec-4-en-1-yl O-[5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy- α -D-erythro-L-glucopyranosyl-1 \rightarrow 4-lactone]-O-(2,4,6-tri-O-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-O-acetyl- β -D-glucopyranoside (**10**). To a stirred solution of the crude **9** (~6 g) in dry CH₂Cl₂ (180 mL) was added stearic acid (2.42 g, 8.54 mmol) followed by EDC·HCl (2.44 g, 12.8 mmol). After stirring at 25 °C for 24 h, the mixture was washed with water (5 \times 30 mL), the organic layer was dried, the solvent was evaporated, and the residue was chromatographed (10:1 CH₂Cl₂/acetone) to give the pure acylated glycoside **10** (Yield calculated from **8**: 5.42 g, 77%. Purity: 97–99%) as an amorphous white solid that was homogeneous by TLC (2:1 CH₂Cl₂/acetone, *R_f*: 0.87). **Mp**: 86–88 °C. [α]_D: –7.9° (c 2.2, CH₂Cl₂). ¹H NMR (CDCl₃): 2.14 (2 CH₃), 2.06, 2.04, 2.03 (2CH₃), 2.02, 1.95, 1.88 (2 CH₃), [CH₃ \times 10]; **Glc**: 4.43 (1 H, d, ³J_{1,2} 7.8 Hz, 1), 4.90 (1 H, dd, ³J_{2,3} 9.6 Hz, 2), 5.14 (1 H, dd, ³J_{3,4} 9.3 Hz, 3), 3.74 (1 H, dd, ³J_{4,5} 9.3 Hz, 4), 3.60 (1 H, m, 5), 4.35 (1 H, m, 6a), 4.09 (1 H, m, 6b); **Gal**: 4.34 (1 H, d, ³J_{1,2} 8.0 Hz, 1), 4.83 (1 H, dd, ³J_{2,3} 9.7 Hz, 2), 4.14 (1 H, dd, ³J_{3,4} 5.6 Hz, 3), 4.96 (1 H, dd, 4), 3.93 (1 H, m, 5), 4.62 (1 H, m, 6a), 4.26 (1 H, m, 6b); **Neu**: 1.77 (1 H, dd, ³J_{3ax,3ec} 13.5 Hz, ³J_{3ax,4} 12.0 Hz, 3ax), 2.73 (1 H, dd, ³J_{3ec,4} 5.5 Hz, 3ec), 5.46 (1 H, m, 4), 4.18 (1 H, dd, 5), 3.68 (1 H, m, 6), 5.20–5.25 (2 H, m, 7, 8), 4.33 (1 H, m, 9a), 3.94 (1 H, m, 9b), 7.99 (1 H, br s, 5-NH); **Sph**: 2.29 (2 H, m, 1a, 1b), 4.43 (1 H, m, 2), 8.01 (1 H, d, ³J_{2,2-NH} 8.0 Hz, 2-NH), 4.49 (1 H, m, 3), 5.54 (1 H, dd, ³J_{4,5} 7.5 Hz, ³J_{4,5} 14.7 Hz, 4), 5.80 (1 H, dt, ³J_{5,6} 7.0 Hz, 5), 1.59 (2 H, m, 6), 1.10–1.45 (24 H, m, 7–17), 0.88 (3 H, t, ³J_{17,18} 6.2 Hz, 18). ¹³C NMR (CDCl₃): 171.3 (3C), 171.1, 170.9 (2C), 170.7, 170.3, 170.0, 169.5, 168.9, 165.2, 162.9 [C=O \times 12]; 23.0 (2C), 21.9, 20.9, 20.7 (3C), 20.6 (3C), 20.5 (3C) [CH₃ \times 13]; **Glc**: 99.7 (1), 71.5 (2), 66.2 (3), 75.5 (4), 72.2 (5), 61.7 (6); **Gal**: 100.2 (1), 70.5 (2), 71.0 (3), 71.5 (4), 61.4 (5), 61.2 (6); **Neu**: 95.0 (2), 37.2 (3), 73.4 (4), 49.1 (5), 75.5 (6), 67.6 (7), 66.2 (8), 61.7 (9); **Sph**: 66.3 (1), 49.5 (2), 71.3 (3), 124.3 (4), 137.3 (5), 25.1 (6), 13.6 (18). **MALDI-TOF MS**: [M + Na]⁺ *m/z* 1667.52 (calcd 1667.84).

(2R,3S,4E)-3-hydroxy-2-(stearoylamino)octadec-4-en-1-yl-O-[5-acetamido-3,5-dideoxy- α -D-erythro-L-glucopyranosyl]-O- β -D-galactopyranosyl)-(1 \rightarrow 4)- β -D-glucopyranoside (**11**). To a stirred suspension of **10** (5.42 g, 3.28 mmol) in MeOH (240 mL) was added a 1.3 mol/L solution of NaOMe in MeOH (27.2 mL, 35.4 mmol). After stirring 24 h at 25 °C, H₂O (60 mL) was added, and the stirring was continued for an additional 24 h. The reaction mixture was neutralized with Amberlite IRA 120 (H⁺), the resin was removed by filtration and washed with MeOH, and the combined filtrates were evaporated to dryness. The residue was dissolved in H₂O (200 mL), and the resultant solution was dialyzed at 4 °C for 24 h against H₂O, using a 3.5 kDa cutoff membrane. The dialyzed solution of the product was dried by lyophilization at a constant temperature of –40 °C during 24 h, to give the pure NAcGM₃ (**11**) (Yield: 3.76 g, 95%. Purity: 98–99%) as an amorphous white solid that was homogeneous by HPTLC (25:20:5 CH₃Cl/MeOH/0.25% aqueous KCl, *R_f*: 0.56). **Mp**: >230 °C dec. [α]_D: +1.7° (c 1.5, 1:1 CHCl₃–MeOH), {lit. [α]_D: +1.5° (c 1.2, 1:1 CHCl₃–MeOH)}.¹⁹ ¹H NMR (DMSO-*d*₆): **Glc**: 4.15 (1 H, d, ³J_{1,2} 7.8 Hz, 1), 3.03 (1 H, dd, ³J_{2,3} 8.2 Hz, 2), 3.20–3.40 (3 H, m, 3, 4, 5), 3.76 (1 H, m, 6a), 3.63 (1 H, m, 6b); **Gal**: 4.21 (1 H, d, ³J_{1,2} 7.4 Hz, 1), 3.20–3.40 (2 H, m, 2, 5), 3.96 (1 H, dd, ³J_{2,3} 9.3 Hz, ³J_{3,4} 3.0

Hz, 3), 3.72 (1 H, m, 4), 3.57 (1 H, m, 6a), 3.43 (1 H, m, 6b); **Neu**: 1.42 (1 H, dd, ³J_{3ax,3ec} 11.9 Hz, ³J_{3ax,4} 11.5 Hz, 3ax), 2.73 (1 H, dd, ³J_{3ec,4} 4.8 Hz, 3ec), 3.55–3.65 (3 H, m, 4, 8, 9a), 3.20–3.40 (4 H, m, 5, 6, 7, 9b), 8.25 (1 H, br s, 5-NH), 1.88 (3 H, s, 5-Nac); **Sph**: 3.20–3.40 (2 H, m, 1a, 1b), 3.78 (1 H, m, 2), 7.05 (1 H, d, ³J_{2,2-NH} 8.9 Hz, 2-NH), 3.88 (1 H, dd, ³J_{2,3} 7.8 Hz, ³J_{3,4} 6.7 Hz, 3), 5.37 (1 H, dd, ³J_{4,5} 15.6 Hz, 4), 5.53 (1 H, dt, ³J_{5,6} 6.7 Hz, 5), 1.93 (2 H, m, 6), 0.81 (3 H, t, ³J_{17,18} 6.3 Hz, 18). ¹³C NMR (DMSO-*d*₆): **Glc**: 103.1 (1), 72.7 (2), 74.2 (3), 80.1 (4), 74.8 (5), 59.7 (6); **Gal**: 103.5 (1), 68.1 (2), 75.2 (3), 66.1 (4), 75.1 (5), 60.0 (6); **Neu**: 172.7 (1-C=O), 99.3 (2), 41.3 (3), 66.4 (4), 52.4 (5), 72.8 (6), 68.2 (7), 70.7 (8), 62.7 (9), 170.7 (5-NC=O), 21.9 (5-Nac); **Sph**: 68.4 (1), 52.5 (2), 70.3 (3), 131.0 (4), 130.8 (5), 31.3 (6), 13.4 (18). **MALDI-TOF MS**: [M + Na]⁺ *m/z* 1202.96 (calcd 1202.73).

AUTHOR INFORMATION

Corresponding Author

*Fax: (537) 2736471. E-mail: miguel.lopez@cqb.cu.

Notes

The authors declare no competing financial interest.

In the NMR data the abbreviations **Glc**, **Gal**, **Neu** and **Sph** refer to glucose, galactose, sialic acid, and sphingosine fragments of molecules, respectively.

ABBREVIATIONS

NAcGM₃, N-acetyl GM₃ ganglioside; NIS, N-iodosuccinimide; TfOH, triflic acid; DBU, 1,8-diazabicyclo (5.4.0) undec-7-ene; DMF, dimethylformamide; BF₃·OEt₂, boron trifluoride-diethyl etherate; EDC·HCl, N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride; MeCN, acetonitrile; MeOH, methanol; AcOH, acetic acid; Ac₂O, acetic anhydride; NH₄OAc, ammonium acetate; Py, pyridine; Et₃N, triethylamine; NaOMe, sodium methoxide; TLC, thin layer chromatography; HPTLC, high performance thin layer chromatography; THF, tetrahydrofuran; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; EtOH, ethanol; mp, melting point; gs-COSY, gradient-selective homonuclear correlation spectroscopy; TOCSY, total correlation spectroscopy; PENDANT, polarization enhancement during attached nucleus testing; gs-HSQC, gradient-selective heteronuclear single-quantum correlation spectroscopy; gs-HMQC, gradient-selective heteronuclear multiple-quantum correlation spectroscopy; gs-HMBC, gradient-selective heteronuclear multiple-bond correlation spectroscopy; qHNMR, quantitative ¹H NMR spectroscopy; TPS-*d*₄, 3-(trimethylsilyl) 2,2,3,3-tetra-deuteriopropionic acid sodium salt; GARP, globally optimized alternating phase rectangular pulse; FID, free induction decay; DHB, 2,5-dihydroxybenzoic acid; TFA, trifluoroacetic acid; CDCl₃, deuterated chloroform; DMSO-*d*₆, deuterated dimethyl sulphoxide

REFERENCES

- (1) Saito, M. *Adv. Lipid Res.* **1993**, 25, 303.
- (2) Ledeen, R. W.; Wu, G. *Trends Glycosci. Glycotechnol.* **1992**, 4, 174.
- (3) Hakomori, S. I.; Igarashi, Y. *Adv. Lipid Res.* **1993**, 25, 147.
- (4) Ladisch, S.; Hasegawa, A.; Li, R.; Kiso, M. *Biochemistry* **1995**, 34, 1197.
- (5) Fredman, P. *Adv. Lipid Res.* **1993**, 25, 213.
- (6) Hamilton, W. B.; Helling, F.; Lloyd, K. O.; Livingston, P. O. *Int. J. Cancer* **1993**, 53, 566.
- (7) Tsuchida, T.; Saxton, R. E.; Morton, D. L.; Irie, R. F. *J. Natl. Cancer Inst.* **1987**, 78, 45.

- (8) Kiura, K.; Watarai, S.; Ueoka, H.; Tabata, M.; Gemba, K.; Aoe, K.; Yamane, H.; Yasuda, T.; Harada, M. *Anticancer Res.* **1998**, *18*, 2957.
- (9) Sugimoto, M.; Ogawa, T. *Glycoconjugate J.* **1985**, *2*, 5.
- (10) Numata, M.; Sugimoto, M.; Shibayama, S.; Ogawa, T. *Carbohydr. Res.* **1988**, *174*, 73.
- (11) Murase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1989**, *188*, 71.
- (12) Hasegawa, A.; Murase, T.; Morita, M.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1990**, *9*, 201.
- (13) Numata, M.; Sugimoto, M.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *203*, 205.
- (14) Ito, Y.; Paulson, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 1603.
- (15) Liu, K. K.-C.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 4933.
- (16) Aubin, Y.; Ito, Y.; Paulson, J. C.; Prestegard, J. H. *Biochemistry* **1993**, *32*, 13405.
- (17) Kiso, M.; Hasegawa, A. *Methods Enzymol.* **1994**, *242*, 173.
- (18) Tomoo, T.; Kondo, T.; Abe, H.; Tsukamoto, S.; Isobe, M.; Goto, T. *Carbohydr. Res.* **1996**, *284*, 207.
- (19) Hasegawa, A.; Suzuki, N.; Kozawa, F.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1996**, *15*, 639.
- (20) Nishimura, S. I.; Yamada, K. *J. Am. Chem. Soc.* **1997**, *119*, 10555.
- (21) Yamada, K.; Fujita, E.; Nishimura, S. I. *Carbohydr. Res.* **1998**, *305*, 443.
- (22) Zehavi, U.; Tuchinsky, A. *Glycoconjugate J.* **1998**, *15*, 657.
- (23) Tietze, L. F.; Gretzke, D. *Eur. J. Org. Chem.* **1998**, 1895.
- (24) Duclos, R. I., Jr. *Carbohydr. Res.* **2000**, *328*, 489.
- (25) Ress, D. K.; Linhardt, R. J. *Curr. Org. Synth.* **2004**, *1*, 31.
- (26) Demchenko, A. V.; Boons, G. J. *Chem.—Eur. J.* **1999**, *5*, 1278.
- (27) DeMeo, C.; Demchenko, A. V.; Boons, G. J. *J. Org. Chem.* **2001**, *66*, 5490.
- (28) Castro-Palomino, J. C. Dissertation, University of Konstanz, Konstanz, Germany; Hartung-Gorre Verlag: Konstanz, 1998; Vol 533, p 118.
- (29) Kartha, K. P. R.; Field, R. A. *Tetrahedron* **1997**, *53*, 11753.
- (30) Excoffier, G.; Gagnaire, D.; Utille, J. P. *Carbohydr. Res.* **1975**, *39*, 368.
- (31) Chittaboina, S.; Hodges, B.; Wang, Q. *Lett. Org. Chem.* **2006**, *3*, 35.
- (32) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731.
- (33) Liu, Y.; Ruan, X.; Li, X.; Li, Y. *J. Org. Chem.* **2008**, *73*, 4287.
- (34) Duclos, R. I., Jr. *Chem. Phys. Lipids.* **2001**, *111*, 111.
- (35) Zemplén, G.; Kunz, A. *Ber. Dtsch. Chem. Ges.* **1923**, *56*, 1705.