EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201500606

First Total Synthesis of Ganglioside GAA-7 from Starfish *Asterias amurensis versicolor*[‡]

Hideki Tamai,[a,b] Akihiro Imamura,[a] Junya Ogawa,[a] Hiromune Ando,*[a,b] Hideharu Ishida,[a] and Makoto Kiso*[a,b]

Keywords: Total synthesis / Natural products / Glycosylation / Glycolipides / Gangliosides / Echinoderms

The first total synthesis of neuritogenic ganglioside GAA-7 was achieved using the glucosyl ceramide (Glc-Cer) cassette approach. The stereocenter triad within the ceramide moiety of the target molecule was efficiently established from D-lyxose. The assembly of the ceramide moiety was followed by glycosylation with glucosyl donors to give Glc-Cer cassettes, which underwent conjugation with the oligosaccharide moiety, followed by global deprotection. By using the most suitable Glc-Cer cassette, the target molecule was successfully synthesized. In vitro evaluation indicated that the synthesized GAA-7 and its glycan moiety both showed strong neuritogenic activity towards neuron-like rat adrenal pheochromocytoma (PC12) cells in the presence of neurite growth factor.

Introduction

In mammals, sialic-acid-containing glycosphingolipids called gangliosides are crucial in many biological processes, including cell-type-specific adhesion, the binding of bacterial toxins, and the entry of pathogens into host cells.[1] Diverse gangliosides have also been found in echinoderms (e.g., starfish and sea cucumbers), and their unique structures have been identified. It was found that echinodermatous gangliosides (EGs) show neuritogenic activity towards neuron-like rat adrenal pheocromocytoma (PC12) cells in the presence of the nerve growth factor (NGF).[2] Because the activities of EGs are comparable or superior to that of mammalian ganglioside GM1, which has been used in the development of a drug for Alzheimer's disease,[3] EGs are thought to have great potential as a source for neurotherapeutics. Most EGs contain partially modified sialic acid residues and/or repeated sequences of sialic acid, both of which are unique to EGs. In addition, the structures of the ceramide lipid moieties in EGs are composed of various pairs of fatty acids and sphingoid bases. Due to the structural diversity of EGs, it is not understood which structures are responsible for the neuritogenic activity, and neither is the mechanism by which EGs potentiate neurite outgrowth. Thus, it is very important to supply homogeneous EGs for molecular-level mechanism studies. Therefore, the synthesis of EGs and their glycan parts have been investigated intensively by many research groups, including our group.[4–8] Recently, we have been tackling the synthesis of LLG-3 and GAA-7, which are highly potent neuritogenic EGs that have 8-*O*-methyl sialic acids as the common monosaccharide residues. In 2011, we demonstrated that synthetic LLG-3 potentiates the neuritogenesis of PC-12 cells in the presence of NGF.[9]

Ganglioside GAA-7 (**1**) was found in the starfish *Asterias amurensis versicolor* Sladen in 1993.[10] GAA-7 has a unique glycan structure linked to a cerebroside ceramide moiety, which is composed of an α-hydroxy fatty acid and an unsaturated phytosphingosine base (Figure 1). Glycan moiety **2**, which features the terminal branch of the 8-*O*-Me-*N*-Gc-sialic acid (Gc = glycolyl) residue stemming from *N*-acetyl-

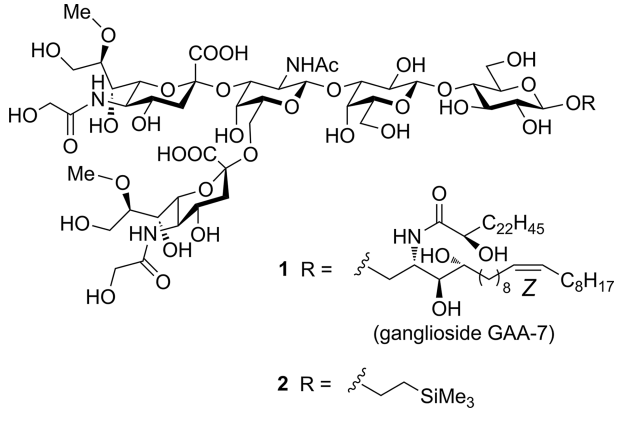


Figure 1. Structures of ganglioside GAA-7 (**1**) and its glycan moiety (**2**).

[‡] Synthetic Studies on Sialoglycoconjugates, 160. Part 159: Ref.[12f]

[a] Department of Applied Bioorganic Chemistry, Gifu University, 1-1 Yanagido, Gifu-shi, Gifu 501-1193, Japan
E-mail: hando@gifu-u.ac.jp
kiso@gifu-u.ac.jp
http://www1.gifu-u.ac.jp/~kassei1/top%20eng%20frame.html
[b] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University,
Yoshida Ushinomiya-cho, Sakyo-ku, Kyoto 606-8501, Japan

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201500606.

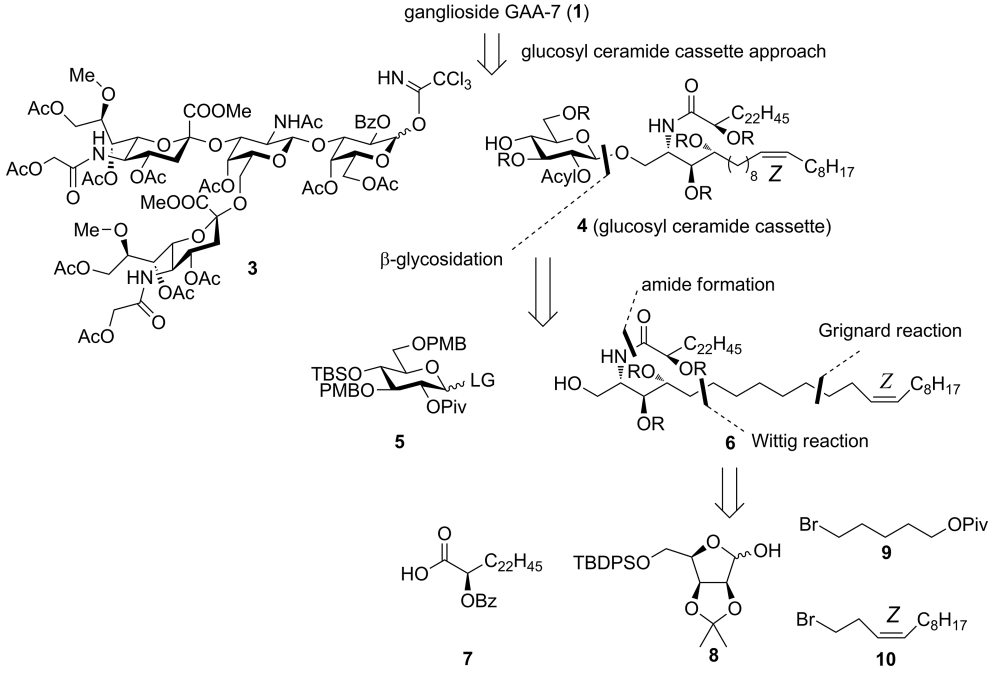
galactosamine residue, has recently been synthesized as a glycoside by our research group through the double sialylation of a GalN-Gal acceptor with an 8-*O*-Me-*N*-Troc (Troc = 2,2,2-trichloroethoxycarbonyl) sialyl donor, and coupling of the tetrasaccharyl donor with a Glc acceptor.[11] Based on the successful synthesis of the glycan part of GAA-7, in this paper we report the synthesis of the entire structure of GAA-7, and examine the neuritogenic activity of the synthetic GAA-7 and its glycan part.

Results and Discussion

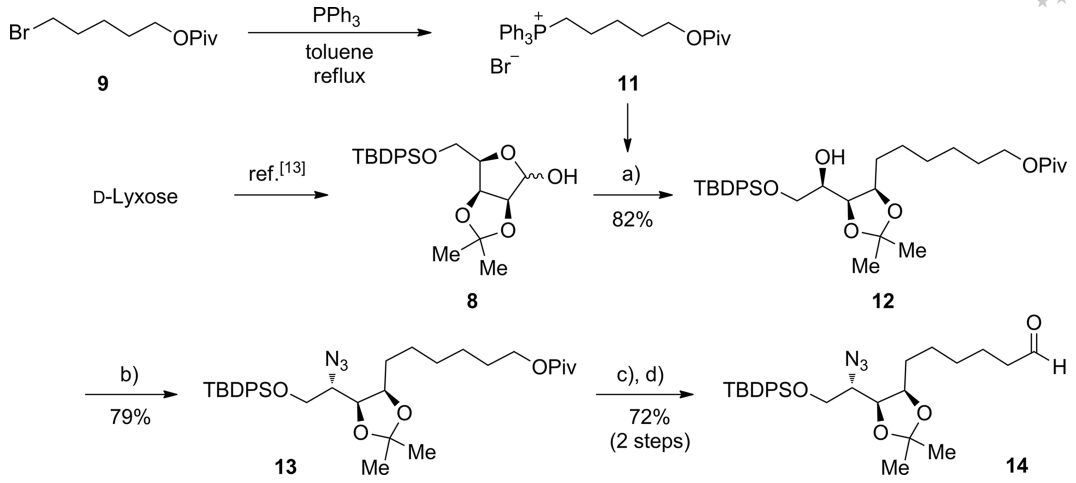
Taking advantage of the efficacy of the glucosyl ceramide (Glc-Cer) cassette approach,[12] target molecule **1** was disconnected at the Galβ(1,4)Glc linkage to give tetrasaccharyl donor **3** and Glc-Cer cassette **4** (Scheme 1). Because the glycosylation at the C-4 hydroxy group of a glucosyl acceptor with tetrasaccharyl donor **3** gave a high yield in the synthesis of the glycan moiety (i.e., **2**),[11] donor **3** was also used for this total synthesis. Glc-Cer cassette **4** was designed as a coupling partner for **3** based on our previous work on the total syntheses of complex gangliosides.[9,12] Then, **4** was disconnected at the glycoside bond to give glucosyl donor **5** and ceramide acceptor **6**. In keeping with previously reported ganglioside syntheses, protecting groups for the hydroxy groups of the Glc donor were chosen so as to achieve a high-yielding, stereoselective glycosylation of ceramide **6**, and to obtain a high yield in the final coupling between Glc-Cer cassette **4** and oligosaccharyl donor **3**. Thus, a pivaloyl group was used for 2-OH protection to impart β-selectivity, and a TBS group was used at O-4 to temporarily protect the 4-OH group, and achieve a high-yielding glycosylation. The use of PMB groups to protect the C-3 and C-6 hydroxy groups was expected to enhance the reactivity of Glc donor **5** and also the 4-OH in Glc-Cer cassette **4**. For ceramide construction, the ceramide moiety was retrosynthetically disconnected at the amide bond at C-2, the C-5–C-6 bond, and the homoallylic carbon–carbon bond at C-11, giving α-hydroxy fatty acid **7**, lactol **8**, and alkyl bromide **9** for the Wittig reaction, and alkenyl bromide **10** for Grignard coupling.

The synthesis of the ceramide moiety started with the construction of the inner segment of the phytosphingosine moiety (Scheme 2). To establish the stereocenter triad at C-2, C-3, and C-4 (2*S*,3*S*,4*R*), D-lyxose was converted into a suitably protected lactol (**8**) by following a reported procedure.[13] Next, lactol **8** underwent Wittig olefination with the phosphonium ylid obtained by treatment of **11** (prepared from **9**) with LHMDS in THF. Subsequent hydrogenation gave enantiomeric alcohol **12** in 82% yield. The hydroxy group at C-2 was then substituted with an azido group in a Mitsunobu reaction[14] using DPPA, Ph_3P, and DEAD. This gave **13**, which has the desired stereocenters. Finally, removal of the pivaloyl group with DIBAL-H at –80 °C,[15] and oxidation with Dess–Martin periodinane,[16] provided aldehyde **14** (72% over two steps). Although we examined Zemplén conditions (NaOMe in MeOH) for the removal of the pivaloyl group, the reaction was sluggish, and the TBDPS group at the C-1 position was also partially cleaved.

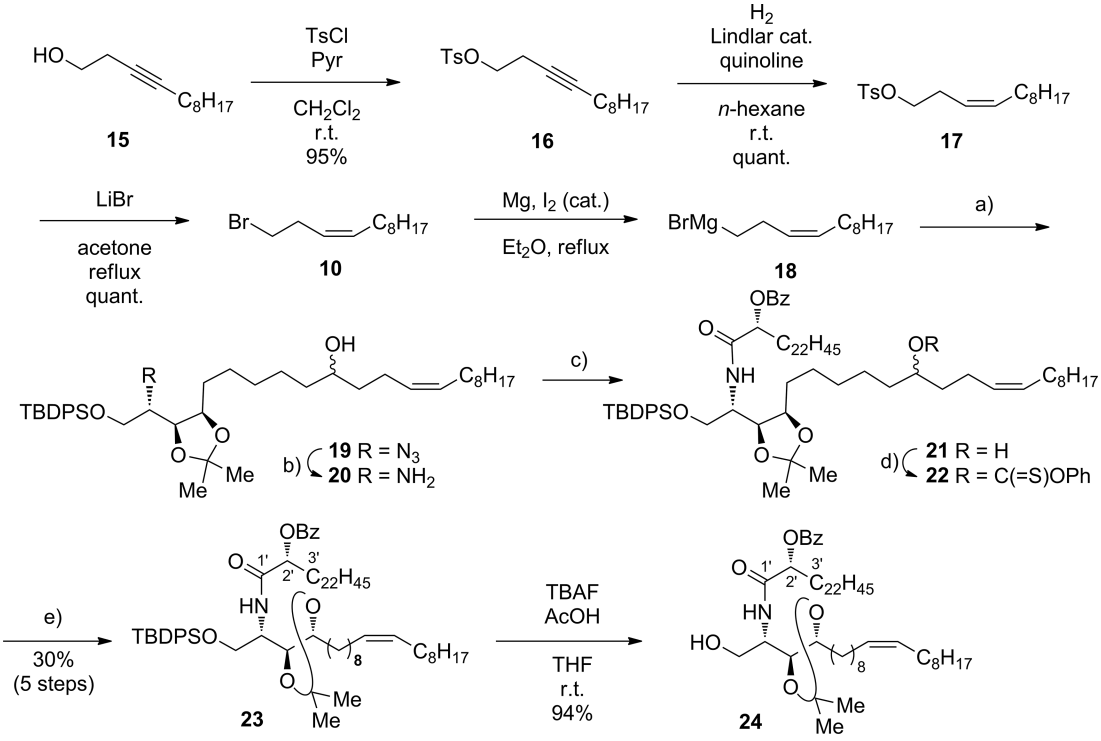
To construct the counterpart of aldehyde **14**, alkynyl tosylate **16** was prepared from known alkynyl alcohol **15**[17] in 95% yield, and was transformed quantitatively into *Z*-olefin **17** by hydrogenation with Lindlar catalyst (0.1 equiv.) and quinoline (1.0 equiv.) (Scheme 3). Subsequently, alkenyl tosylate **17** was treated with LiBr (3.0 equiv.) in acetone un-



Scheme 1. Retrosynthetic analysis of ganglioside GAA-7; LG = leaving group, TBS = *tert*-butyldimethylsilyl, PMB = *p*-methoxybenzyl, TBDPS = *tert*-butyldiphenylsilyl, Piv = pivaloyl.



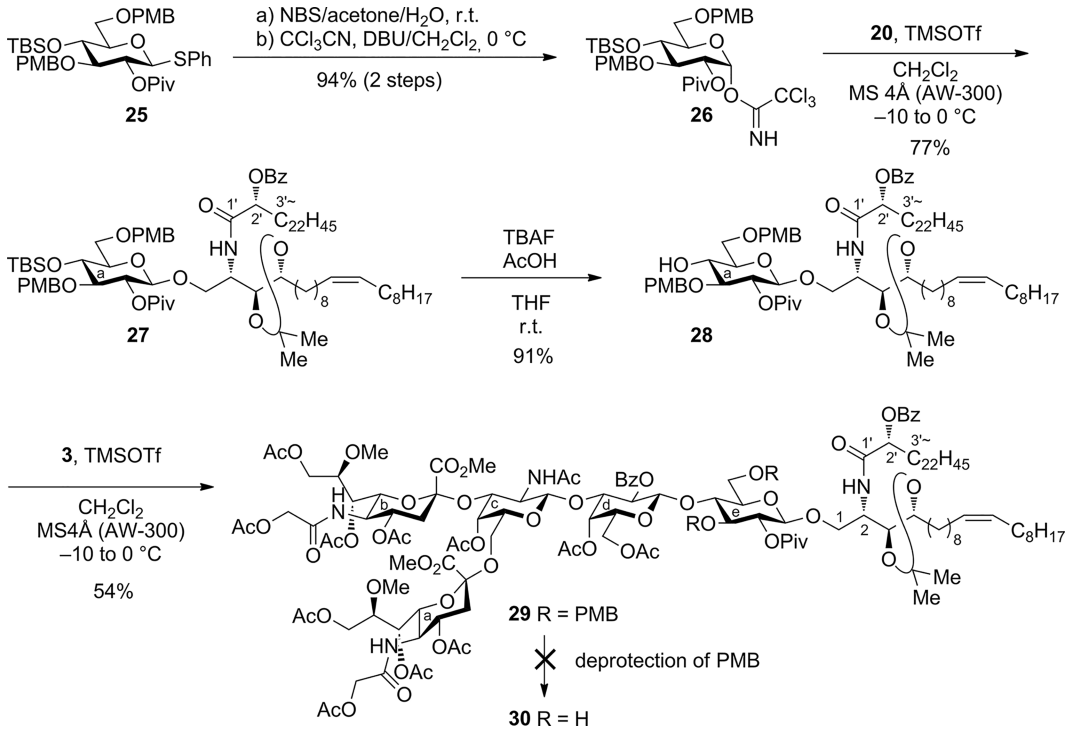
Scheme 2. Preparation of phytosphingosine derivative **14**. Reagents and conditions: a) (i) **11**, LHMDS, THF, 0 °C to room temp., (ii) H_2, Pd/C, EtOAc, room temp., 82% (two steps); b) DPPA, DEAD, PPh_3, THF, 0 °C to room temp., 79%; c) DIBAL-H, toluene, CH_2Cl_2, –80 °C; d) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, room temp., 72% (two steps). LHMDS = lithium bis(trimethylsilyl)amide, DPPA = diphenylphosphoryl azide, DEAD = diethyl azodicarboxylate, DIBAL-H = diisobutylaluminum hydride.



Scheme 3. Synthesis of GAA-7 ceramide acceptor **24**. Reagents and conditions: a) **14**, Et_2O, 0 °C to r.t.; b) PPh_3, THF, H_2O, room temp.; c) **7**, EDC·HCl, CH_2Cl_2, room temp.; d) phenyl chlorothionocarbonate, DMAP, pyridine, 40 °C; e) nBu_3SnH, AIBN, toluene, 100 °C, 30% (five steps); Ts = *p*-tolylsulfonyl, AIBN = 2,2′-azobis(isobutyronitrile), EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, TBAF = tetra-*n*-butylammonium fluoride, DMAP = 4-(dimethylamino)pyridine.

der reflux to give alkenyl bromide **10**[18] quantitatively. Upon treatment with Mg and a catalytic amount of iodine, this bromide was then converted into Grignard reagent **18**, the coupling partner for aldehyde **14**. We then carried out the coupling reaction with aldehyde **14** (1.0 equiv.). This was followed by amide formation with α-hydroxy fatty acid derivative **7**,[6] thiocarbonylation, and radical reduction with nBu_3SnH (10.0 equiv.) and AIBN (0.05 equiv.) to give phytoceramide **23** in 30% yield over five steps. Finally, the C-1 hydroxy group was selectively deprotected with TBAF (1.5 equiv.) in the presence of AcOH (2.0 equiv.) to give ceramide acceptor **24** in 94% yield.

With phytoceramide **24** in hand, we synthesized the glucosyl ceramide cassette for the final conjugation with the oligosaccharyl donor (Scheme 4). Glucosyl imidate donor **26** was prepared from known phenylthioglycoside **25**.[9] Treatment of **25** with NBS in acetone/H_2O[19] at room temperature, and subsequent imidoylation of the resulting 1-



Scheme 4. Preparation of Glc-Cer acceptor **28** and examination of the cassette approach. PMB = *p*-methoxybenzyl, NBS = *N*-bromosuccinimide, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, MS = molecular sieves, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

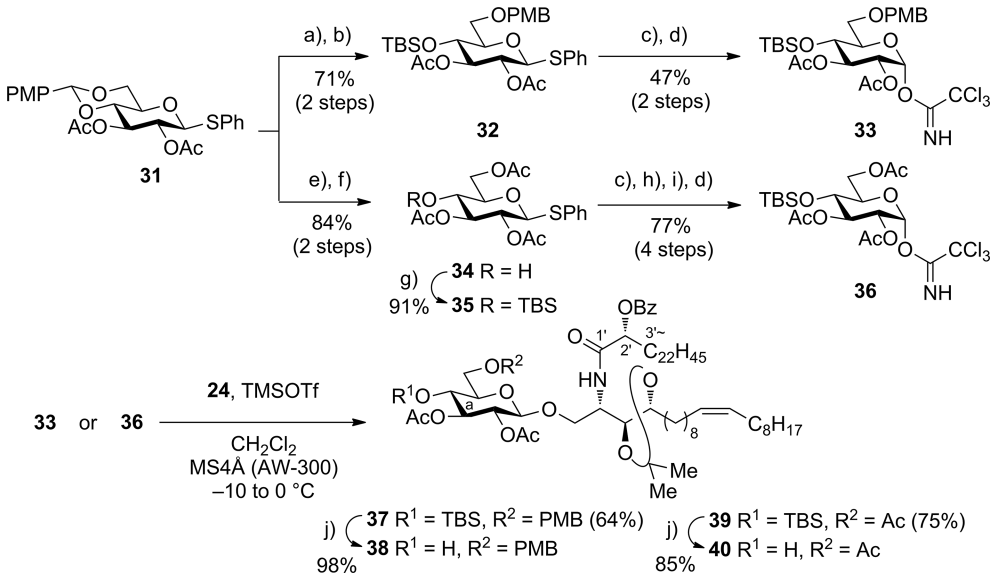
OH with CCl_3CN and DBU[20] in CH_2Cl_2 at 0 °C gave **26** in 90% yield over two steps. Then, the glycosylation of acceptor **24** with donor **26** was carried out under mild conditions to give glucosyl ceramide **27** in high yield. Subsequent removal of the TBS group at O-4 yielded glucosyl ceramide cassette **28** in high yield, and this was then subjected to the final glycosylation. Similarly to previously reported ganglioside syntheses based on the Glc-Cer cassette approach, tetrasaccharyl donor **3** and cassette **28** were coupled with TMSOTf (0.1 equiv.) at –10 °C, and the reaction was continued at 0 °C for 1 h. This gave the protected GAA-7 (i.e., **29**) in 54% yield in a stereoselective manner, with none of the orthoester analog formed. Considering that the coupling yield with the glucosyl acceptor [2-(trimethylsilyl)ethyl glycoside analog of **28**] in the synthesis of the GAA-7 glycan moiety (i.e., **2**) was 73%,[11] ganglioside framework **29** was generated in an acceptable yield, which suggested that this glucosyl cassette approach would be a viable synthetic route. However, cleavage of the PMB groups failed unexpectedly in the next step. We examined various reaction conditions for the removal of the PMB groups [(i) TFA (trifluoroacetic acid), CH_2Cl_2; (ii) TFA, anisole, CH_2Cl_2; (iii) DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), CH_3CN, H_2O;[21] (iv) $SnCl_4$, PhSH, CH_2Cl_2[22]]. Under all the reaction conditions tested, an unknown by-product was generated along with the desired product (i.e., **30**), giving an inseparable mixture of reaction products.

To solve the deprotection problem, we reconsidered the design of the glucosyl donor. Because the electron-donating properties of the PMB group are crucial for increasing the reactivity of the C-4 hydroxy group in the Glc-Cer cassette, the PMB group was retained at C-6 in glucosyl donor **33**, in which the C-2 and C-3 hydroxy groups were protected with acetyl groups (Scheme 5).

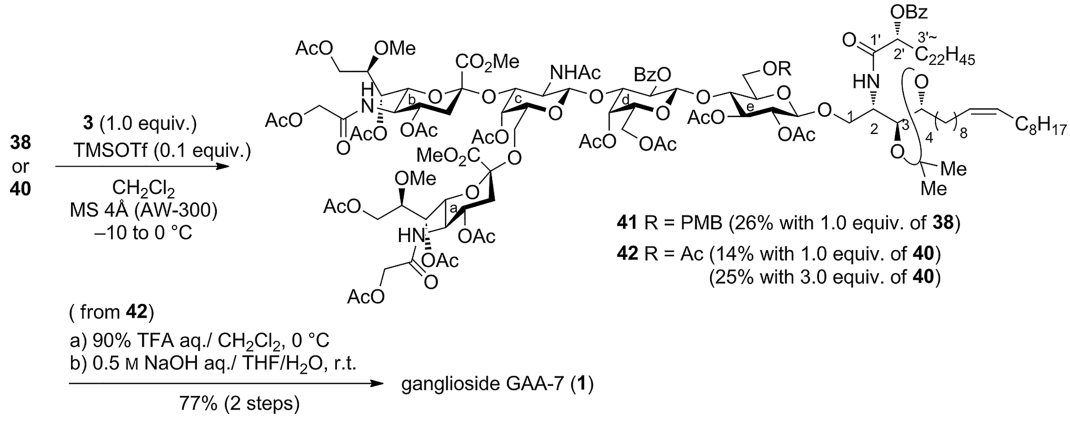
An analogous triacetylated glucosyl donor **36** was also used for comparison with **33**. Donors **33** and **36** were derived from **31**[23] in a straightforward manner. For donor **33**, the PMB ether at C-6 was established by a reductive ring-opening of the 4,6-anisylidene group,[24] which was followed by silylation to give **32** in high yield. Then, **32** was converted into glucosyl imidate **33**. For donor **36**, compound **31** was transformed into known intermediate **34**,[25] which was then silylated to give triacetylated thioglycoside **35**. Unlike **32**, subsequent hydroxylation of **35** generated a mixture of 1-OH and 2-OH derivatives. Therefore, in the next step, the mixture was acetylated, and the product was treated with hydrazine acetate to give the hemiacetal, which was then converted into trichloroacetimidate **36**. Next, phytoceramide acceptor **24** was treated with glucosyl donors **33** and **36**, and this was followed by deprotection of the C-4 hydroxy group to give Glc-Cer cassettes **38** and **40**, respectively.

Consistent with our initial speculation, reducing the number of PMB groups in the Glc-Cer cassette decreased the yield of the glycosylation with tetrasaccharyl donor **3** (Scheme 6). Taking the results for **28** (glycosylation yield: 50%) together with the results for **38** and **40**, a decrease by one in the number of PMB groups caused a 50% decrease in the glycosylation yields.

We used the protected GAA-7 (i.e., **41**) to examine global deprotection. However, we again failed to obtain ganglioside GAA-7 because of the generation of an unknown by-product during the cleavage of the PMB groups that could



Scheme 5. Synthesis of Glc-Cer acceptors **38** and **40**. Reagents and conditions: a) $NaBH_3CN$, HCl (2 M in Et_2O), THF, MS (3 Å), 0 °C; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to room temp.; c) NBS, acetone, H_2O, room temp.; d) CCl_3CN, DBU, CH_2Cl_2, 0 °C; e) $TsOH{\cdot}H_2O$, MeOH, 0 °C, 89%; f) AcCl, pyridine, CH_2Cl_2, –40 °C to room temp., 88%; g) TBSCl, pyridine, 40 °C, 91%; h) Ac_2O, pyridine, room temp.; i) hydrazine acetate, THF, room temp.; j) TBAF, AcOH, THF, room temp., PMP = *p*-methoxyphenyl.



Scheme 6. Final coupling and global deprotection to give the target molecule.

not be separated from the desired product. As a result, triacetyl Glc-Cer **40** was used for the synthesis of the target compound. To minimize the loss of valuable oligosaccharyl donor **3**, 3.0 equiv. of **40** was used in the reaction to give **42**, and this resulted in an increase in the glycosylation yield to 25%. Finally, **42** was treated with TFA at 0 °C, followed by basic hydrolysis of ester groups, to give ganglioside GAA-7 (**1**) in 77% yield over two steps (Scheme 6).

The synthesized GAA-7 (i.e., **1**) and its glycan moiety (**2**) were evaluated in terms of neuritogenic activity towards PC-12 cells. The results are summarized in Figure 2. Consistent with the results obtained with natural GAA-7,[2] the synthesized GAA-7 (**1**) potentiated the neurite outgrowth in the presence of NGF (5.0 ng/mL). GAA-7 (**1**) at 10 nM increased the total length of neurites by around 1.5 times, compared to the length without added GAA-7 (Figure 2, a). In particular, the number of neurites with a length of

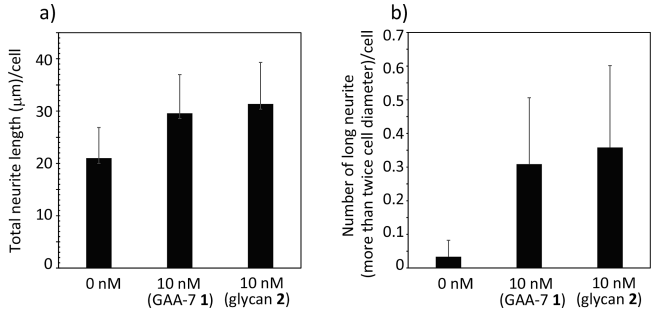


Figure 2. Neurite outgrowth evaluation. PC-12 cells were incubated with or without the synthesized GAA-7 (**1**) or the glycan moiety (**2**) (10 nM each) in the presence of NGF (5 ng/mL) for 5 d. (a) Neurites were counted, and the total length per cell was calculated. (b) The number of neurites that were longer than twice the cell-body diameter was counted per cell. Results are shown as average total length or number of neurites per cell ± the standard error.

more than twice the cell-body diameter was significantly increased by exogenous GAA-7 (**1**) (Figure 2, b). More interestingly, the glycan moiety (**2**) showed activity similar to that of GAA-7, which indicates that the glycan moiety is crucial for neurite outgrowth activation.

Conclusions

The first total synthesis of ganglioside GAA-7 (**1**) was achieved, using tetrasaccharyl donor **3** and Glc-Cer cassette **38** as the key building blocks to construct the glycolipid framework of the target molecule. Furthermore, evaluation of the neuritogenic activity of the synthesized molecules **1** and **2** revealed that the glycan moiety of GAA-7 is essential for the potentiation of neurite outgrowth in the presence of NGF.

Experimental Section

General Remarks: All reactions were carried out under a positive pressure of argon, unless otherwise noted. All chemicals were purchased from commercial suppliers, and were used without further purification, unless otherwise noted. Molecular sieves were purchased from Wako Chemicals Inc. (Miyazaki, Japan) and dried at 300 °C for 2 h in a muffle furnace before use. Reaction solvents were dried with molecular sieves, and used without purification. TLC analysis was carried out on Merck TLC plates (silica gel 60F254 on glass plate). Compounds were detected either by exposure to UV light (253.6 nm) or by soaking in H_2SO_4 (10% solution in ethanol) followed by heating. Silica gel (80 mesh and 300 mesh; Fuji Silysia Co., Aichi, Japan) was used for flash column chromatography. The quantity of silica gel was usually 100 to 200 times the weight of the crude sample. Solvent systems for chromatography are specified as v/v ratios. Evaporation and concentration were carried out in vacuo. ^{1}H and ^{13}C NMR spectra were recorded with 500 and 800 MHz spectrometers (Biospin AVANCE III, Bruker, Billerica, MA, USA). Chemical shifts in ^{1}H NMR spectra are expressed in ppm (δ) relative to the Me_4Si signal, adjusted to δ = 0.00 ppm. Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, m = multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz), and position of the corresponding proton. COSY methods were used to confirm the NMR peak assignments. High-resolution mass spectra (ESI-TOF) were obtained with a mass spectrometer (micrOTOF, Bruker). Optical rotations were measured with a high-sensitivity polarimeter [SEPA-300, Horiba (Kyoto, Japan)].

(2*R*,3*S*,4*R*)-1-*O*-*tert*-Butyldiphenylsilyl-3,4-*O*-isopropylidene-10-*O*-pivaloyl-1,2,3,4,10-decanepentol (12): Triphenylphosphine (45 g, 171 mmol) was added to a solution of 5-bromopentyl pivaloylate (**9**; 14.2 g, 56.7 mmol) in toluene (200 mL). The mixture was stirred for 2 d under reflux, then it was diluted with toluene, and the supernatant was decanted. The resulting residue was dried in vacuo for 6 h.

The residue was then suspended in THF (60 mL), the mixture was cooled to 0 °C, and LHMDS (1.0 M solution in THF; 57 mL, 57 mmol) was added. The mixture was stirred for 90 min, then a solution of lactol **8** (8.10 g, 18.9 mmol) in THF (60 mL) was added to the stirred mixture at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, and then for a further 6 h at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 2:1). After the consumption of starting material was confirmed, the reaction was quenched with satd. aq. NH_4Cl, and the mixture was extracted with EtOAc. The organic layer was successively washed with H_2O, satd. aq. $NaHCO_3$, and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 8:1) to give an *E*/*Z* mixture as a colorless syrup.

Next, Pd/C (5% on activated carbon; 1.4 g) was added to a solution of the *E*/*Z* mixture in EtOAc (190 mL). The mixture was stirred for 12 h under a hydrogen atmosphere, and the progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). Next, the reaction mixture was diluted with EtOAc, and filtered through a pad of Celite. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 8:1) to give compound **12** (7.70 g, 82%) as a colorless syrup. $[\alpha]_D$ = –6.8 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.69–7.36 (m, 10 H, Ar), 4.17 (dd, $J_{2,3}$ = 2.7, $J_{3,4}$ = 6.4 Hz, 1 H, 3-H), 4.12 (m, 1 H, 4-H), 4.04 (t, $J_{9,10}$ = 6.6 Hz, 2 H, 10-H), 3.72–3.65 (m, 3 H, 1a-H, 1b-H, 2-H), 2.35 (d, 1 H, OH), 1.74–1.21 (m, 16 H, 5 CH_2, 2 Me), 1.19 (s, 9 H, *t*Bu), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.6, 135.6, 135.6, 133.3, 129.8, 129.7, 127.7, 127.7, 107.7, 77.4, 76.4, 69.8, 65.2, 64.4, 38.7, 29.8, 29.7, 29.5, 29.4, 29.2, 28.6, 27.3, 27.2, 26.8, 26.7, 25.9, 25.1, 19.2 ppm. HRMS (ESI): calcd. for $C_{34}H_{52}O_6Si$ [M + Na]$^+$ 607.3425; found 607.3426.

(2*S*,3*S*,4*R*)-2-Azido-1-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-10-*O*-pivaloyl-1,3,4,10-decanetetrol (13): DEAD (40% solution in toluene; 421 μL, 1.07 mmol) and PPh_3 (281 mg, 1.07 mmol) were added to a solution of compound **12** (569 mg, 0.972 mmol) in THF. The mixture was stirred for 10 min at 0 °C, then DPPA (231 μL, 1.07 mmol) was added. The mixture was then stirred for 1 h at 0 °C, and the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 8:1) to give compound **13** (471 mg, 79%) as a colorless syrup. $[\alpha]_D$ = +8.3 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.73–7.34 (m, 10 H, Ar), 4.12 (m, 1 H, 4-H), 4.06 (t, $J_{9,10}$ = 6.6 Hz, 2 H, 10-H), 4.03 (dd, J_{gem} = 10.9, $J_{1a,2}$ = 2.6 Hz, 1 H, 1a-H), 3.95 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 5.5 Hz, 1 H, 3-H), 3.42 (td, 1 H, 2-H), 1.66–1.39 (m, 10 H, 5 CH_2), 1.27 (2 s, 6 H, 2 Me), 1.20 and 1.08 (2 s, 18 H, 2 *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.6, 135.7, 135.6, 133.0, 132.9, 129.8, 129.7, 129.4, 128.9, 128.6, 127.7, 127.7 127.5, 108.2, 77.7, 75.2, 65.2, 64.4, 61.7, 38.7, 29.3, 29.2, 28.6, 28.1, 27.2, 26.9, 26.7, 26.3, 25.9, 25.7, 25.1, 19.1 ppm. HRMS (ESI): calcd. for $C_{34}H_{51}N_3O_5Si$ [M + Na]$^+$ 632.3490; found 632.3491.

(4*R*,5*S*)-4-{2,2-Dimethyl-5-[(1*S*)-1-azido-2-*tert*-butyldiphenylsilyloxyethyl]-1,3-dioxolan-4-yl}hexanal (14): DIBAL-H (1 M solution in toluene; 5.9 mL, 5.84 mmol) was added to a solution of compound **13** (1.62 g, 2.66 mmol) in CH_2Cl_2 (27 mL) at –80 °C. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). The mixture was stirred for 30 min at –80 °C, then the reaction was quenched with satd. aq. NH_4Cl, and satd. aq. sodium potassium tartrate was added. The mixture was stirred vigorously for 30 min, then it was extracted with $CHCl_3$. The organic layer was successively washed with H_2O and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 6:1) to give a hydroxy derivative.

This hydroxy derivative was exposed to high vacuum for 10 h, then it was dissolved in CH_2Cl_2 (27 mL). Dess–Martin periodinane

(1.74 g, 4.11 mmol) and $NaHCO_3$ (345 mg, 4.11 mmol) were added to the solution at ambient temperature, and the reaction mixture was stirred for 25 min at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 4:1). Then, the reaction mixture was extracted with Et_2O. The organic layer was successively washed with H_2O and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 6:1) to give compound **14** (1.04 g, 72% over two steps) as a colorless syrup. $[\alpha]_D$ = +31.6 (*c* = 0.6, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 9.77 (t, $J_{9,CHO}$ = 1.8 Hz, 1 H, CHO), 7.73–7.37 (m, 10 H, Ar), 4.11 (m, 1 H, 4-H), 4.03 (dd, J_{gem} = 10.8, $J_{1a,2}$ = 2.6 Hz, 1 H, 1a-H), 3.95 (dd, $J_{2,3}$ = 9.8, $J_{3,4}$ = 5.5 Hz, 1 H, 3-H), 3.85 (dd, $J_{1b,2}$ = 6.9 Hz, 1 H, 1b-H), 3.40 (td, 1 H, 2-H), 2.45 (td, $J_{8,9}$ = 7.3 Hz, 2 H, 9-H), 1.70–1.37 (m, 8 H, 4 CH_2), 1.27 (2 s, 6 H, 2 Me), 1.08 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 202.6, 135.7, 135.6, 133.1, 132.9, 129.8, 129.7, 127.8, 127.7, 108.2, 77.6, 75.2, 65.2, 61.6, 43.8, 29.3, 29.1, 28.1, 26.7, 26.2, 25.7, 22.0, 19.1 ppm. HRMS (ESI): calcd. for $C_{29}H_{41}N_3O_4Si$ $[M + Na]^+$ 546.2759; found 546.2757.

1-*O*-(*p*-Tolylsulfonyl)dodec-3-yn-1-ol (16): *p*-Toluenesulfonyl chloride (2.40 g, 11.8 mmol) was added to a solution of dodec-3yn-1-ol **15** (1.80 g, 9.87 mmol) in pyridine/CH_2Cl_2 (1:4; 33 mL) at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 7:1). The mixture was stirred for 4 h at ambient temperature, then it was coevaporated with toluene, and extracted with EtOAc. The organic layer was successively washed with HCl (2 M), H_2O, and brine, dried with Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/Et_2O, 10:1) to give compound **16** (3.15 g, 95%) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.80 and 7.35 (2 d, 4 H, Ar), 4.06 (t, *J* = 7.3 Hz, 2 H, 1-H), 2.52 (m, 2 H, 2-H), 2.45 (s, 3 H, Me), 2.07 (m, 2 H, 5-H), 1.43–1.26 (m, 12 H, 6 CH_2), 0.88 (s, 3 H, Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 144.8, 133.2, 129.8, 127.9, 83.0, 73.8, 68.3, 31.8, 29.2, 29.1, 28.8, 28.7, 22.6, 21.6, 19.8, 18.6, 14.1 ppm. HRMS (ESI): calcd. for $C_{19}H_{28}O_3S$ $[M + Na]^+$ 359.1651; found 359.1651.

(3*Z*)-1-*O*-(*p*-Tolylsulfonyl)dodec-3-en-1-ol (17): Lindlar catalyst (0.502 g, 0.237 mmol) and quinoline (0.56 mL, 4.75 mmol) were added to a solution of compound **16** (1.60 g, 4.75 mmol) in *n*-hexane (47 mL). The flask containing the mixture was evacuated and filled with H_2 gas, and this process was repeated three times. The mixture was then stirred at ambient temperature under a hydrogen atmosphere, and the progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 12:1). The mixture was stirred for 2 h, then it was filtered through a pad of Celite, and the filter residue was washed with EtOAc. The filtrate was successively washed with HCl (2 M), H_2O, and brine. The organic layer was dried with Na_2SO_4, and concentrated to give compound **17** (1.61 g, quant.) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 7.79 and 7.34 (2 d, 4 H, Ar), 5.48 (m, $J_{3,4}$ = 10.8, $J_{4,5}$ = 6.9 Hz, 1 H, 4-H), 5.21 (m, $J_{2,3}$ = 6.9 Hz, 1 H, 3-H), 4.01 (t, $J_{1,2}$ = 7.0 Hz, 2 H, 1-H), 2.45 (s, 3 H, Me), 2.39 (near q, 2 H, 2-H), 1.95 (q, $J_{5,6}$ = 6.9 Hz, 2 H, 5-H), 1.30–1.19 (m, 12 H, 6 CH_2), 0.88 (s, 3 H, Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 144.6, 134.0, 129.8, 127.9, 122.5, 69.8, 31.9, 29.4, 29.2, 27.3, 27.1, 22.6, 21.6, 14.1 ppm. HRMS (ESI): calcd. for $C_{19}H_{30}O_3S$ $[M + Na]^+$ 361.1808; found 361.1807.

(3*Z*)-1-Bromododec-3-ene (10): Lithium bromide (0.604 g, 14.3 mmol) was added to a solution of compound **17** (1.61 g, 4.75 mmol) in acetone (47 mL). The mixture was then stirred for 3 h under reflux, and the progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 12:1). The reaction mixture was filtered through paper. The filtrate was then concentrated, and diluted with EtOAc. The organic phase was successively washed with H_2O and brine, and dried with Na_2SO_4. The solution was concentrated to give compound **10**[18] (1.17 g, quant.) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 5.53 (m, 1 H, 4-H), 5.35 (m, 1 H, 3-H), 3.36 (t, $J_{1,2}$ = 7.2 Hz, 2 H, 1-H), 2.63 (near q, $J_{2,3}$ = 7.4 Hz, 2 H, 2-H), 2.03 (q, $J_{3,4}$ = 7.2 Hz, 2 H, 5-H), 1.37–1.27 (m, 12 H, 6 CH_2), 0.88 (t, 3 H, Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 133.2, 125.7, 32.5, 31.9, 30.8, 29.5, 29.4, 29.3, 27.4, 22.7, 14.1 ppm.

(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(Benzoyloxy)tetracosanoylamino]-1-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (23): Magnesium foil (78 mg, 3.21 mmol) and I_2 (10 mg, 0.040 mmol) were suspended in Et_2O (10 mL). The mixture was stirred for 30 min under reflux, and then a solution of bromoalkene **10** (594 mg, 2.41 mmol) in Et_2O (10 mL) was added to the suspension dropwise at 35 °C. The reaction mixture was stirred for a further 60 min under reflux. The mixture was then added dropwise to a solution of aldehyde **14** (267 mg, 0.510 mmol) in Et_2O (10 mL) at 0 °C. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 5:1). The mixture was stirred for 2 h at ambient temperature, then the reaction was quenched with satd. aq. NH_4Cl, and the mixture was filtered through a pad of Celite. The mixture was extracted with Et_2O, and the organic phase was successively washed with H_2O and brine, dried with Na_2SO_4, and concentrated.

The resulting crude material (compound **19**) was dried in vacuo for 6 h, and then it was dissolved in a mixture of THF (16 mL) and H_2O (0.8 mL). Next, PPh_3 (820 mg, 3.20 mmol) was added, and the mixture was stirred for 18 h at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 5:1, developed twice). Then, the reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 7:1) to give amino derivative **20**.

This compound was dissolved in CH_2Cl_2 (16 mL), and EDC·HCl (154 mg, 0.802 mmol) and compound **7** (392 mg, 0.802 mmol) were added to the solution at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 8:1). The mixture was stirred for 2 h, and then it was diluted and extracted with Et_2O. The organic layer was successively washed with H_2O and brine, dried with Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 15:1→8:1→5:1) to give amide derivative **21** (326 mg, 0.287 mmol), which was dried under high vacuum.

The dried compound was dissolved in pyridine (6 mL). PhOC(=S)Cl (115 μL, 0.860 mmol) and DMAP (7.0 mg, 57.4 μmol) were added to the solution, and the mixture was stirred for 2 h at 40 °C. The reaction was monitored by TLC (*n*-hexane/EtOAc, 8:1). The reaction mixture was coevaporated with toluene, and the residue was roughly purified by silica gel chromatography (*n*-hexane/EtOAc, 100:1→20:1). The resulting material (compound **22**) was dried in vacuo for 6 h.

This compound was then dissolved in toluene (29 mL), and *n*Bu_3SnH (0.386 mL, 1.43 mmol) and AIBN (4.7 mg, 0.028 mmol) were added to the resulting solution at ambient temperature. The mixture was stirred for 2 h at 100 °C, then it was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 100:1→40:1→25:1→15:1) to give compound **23** (269 mg, 30% over five steps) as a colorless syrup. $[\alpha]_D$ = +25.8 (*c* = 0.3, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.04–7.29 (m, 15 H, Ar), 6.52 (d, $J_{2,NH}$ = 9.8 Hz, 1 H, NH), 5.48 (t, $J_{2,3}$ = 5.9 Hz, 1 H, 2′-H), 5.36 (m, 2 H, 13-H, 14-H), 4.35 (dd, $J_{2,3}$ = 9.5, $J_{3,4}$ = 5.4 Hz, 1 H, 3-H), 4.21 (near t, 1 H, 2-H), 4.13 (m, 1 H, 4-H), 3.95 (dd, J_{gem} = 10.1, $J_{1a,2}$ = 2.3 Hz, 1 H, 1a-H), 3.62 (dd, $J_{1b,2}$ = 2.4 Hz, 1 H, 1b-H), 2.07–1.96 (m, 6 H, 12-H, 15-H, 3′-H), 1.58–1.23 (m,

72 H, 33 CH_2, 2 Me), 0.88 (2 t, 6 H, 2 Me), 0.81 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.0, 165.2, 135.6, 135.4, 133.5, 132.8, 132.8, 129.9, 129.9, 129.8, 129.7, 129.6, 129.1, 128.6, 127.7, 127.5, 108.1, 77.9, 75.5, 74.5, 63.7, 49.0, 32.1, 31.9, 31.9, 29.8, 29.8, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.3, 28.3, 27.2, 27.2, 27.1, 26.5, 26.3, 26.0, 24.9, 22.7, 19.0, 14.1 ppm. HRMS (ESI): calcd. for $C_{72}H_{117}NO_6Si$ $[M + Na]^+$ 1142.8542; found 1142.8544.

(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(Benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (24): Tetrabutylammonium fluoride (1.0 M solution in THF; 233 μL, 0.233 mmol) was added to a solution of compound **23** (131 mg, 0.117 mmol) and AcOH (10 μL, 0.175 mmol) in THF (1.2 mL) at ambient temperature. The mixture was stirred for 6 h, and the progress of the reaction was monitored by TLC (*n*-hexane/Et_2O, 5:2). The mixture was evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/Et_2O, 4:1→5:2) to give **24** (96 mg, 94%) as a colorless syrup. $[\alpha]_D$ = +2.2 (*c* = 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.08–7.47 (m, 5 H, Ar), 6.60 (d, $J_{2,NH}$ = 8.4 Hz, 1 H, NH), 5.39–5.32 (m, 3 H, 13-H, 14-H, 2′-H), 4.19–4.15 (m, 2 H, 3-H, 4-H), 4.09 (m, 1 H, 2-H), 3.87 (br. dt, 1 H, 1a-H), 3.65 (m, 1 H, 1b-H), 2.45 (br. s, 1 H, OH), 2.03–1.94 (m, 6 H, 12-H, 15-H, 3′-H), 1.61–1.13 (m, 72 H, 33 CH_2, 2 Me), 0.88 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.9, 165.5, 133.6, 129.9, 129.8, 129.8, 129.2, 128.6, 108.2, 77.9, 77.7, 77.6, 74.8, 63.1, 50.2, 32.6, 31.9, 31.9, 31.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.3, 29.2, 27.5, 27.2, 26.8, 25.2, 25.0, 22.7, 14.1 ppm. HRMS (ESI): calcd. for $C_{56}H_{99}NO_6$ $[M + Na]^+$ 904.7365; found 904.7367.

4-*O*-*tert*-Butyldimethylsilyl-3,6-di-*O*-*p*-methoxybenzyl-2-*O*-pivaloyl-α-D-glucopyranosyl Trichloroacetimidate (26): NBS (98 mg, 546 μmol) was added to a solution of compound **25** (259 mg, 364 μmol) in acetone/H_2O (95:5; 3.8 mL) at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 5:1). After the consumption of starting material was confirmed, the reaction mixture was extracted with EtOAc. The organic layer was successively washed with satd. aq. $Na_2S_2O_3$ and brine, dried with Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/acetone, 3:2→2:3) to give a hemiacetal intermediate, which was then dried in vacuo.

This compound was dissolved in CH_2Cl_2 (4.2 mL), and trichloroacetonitrile (549 μL, 5.47 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (55 μL, 365 μmol) were added to the solution at 0 °C. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 5:1) to give **26** (44 mg, 94%) as an amorphous powder. $[\alpha]_D$ = +64.6 (*c* = 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.57 (s, 1 H, C=NH), 7.25–6.80 (4 d, 8 H, 2 MeO$C_6H_4CH_2$), 6.52 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.12 (dd, $J_{2,3}$ = 9.7 Hz, 1 H, 2-H), 4.77–4.41 (4 d, 4 H, 4 ArCH_2O), 3.95 (td, $J_{4,5}$ = 9.5, $J_{5,6a}$ = 2.9 Hz, $J_{5,6b}$ = 2.5 Hz, 1 H, 5-H), 3.89 (t, $J_{3,4}$ = 9.2 Hz, 1 H, 3-H), 3.83 (t, 1 H, 4-H), 3.78 and 3.77 (2 s, 6 H, OMe), 3.67–3.62 (m, 2 H, 6a-H, 6b-H), 1.08 (s, 9 H, *t*Bu), 0.83 (s, 9 H, *t*Bu), 0.0 and –0.1 (2 s, 6 H, SiMe_2*t*Bu) ppm. ^{13}C NMR (125 Hz, $CDCl_3$): δ = 177.5, 160.8, 160.1, 158.8, 130.4, 130.2, 129.1, 129.0, 128.1, 128.1, 125.3, 113.8, 113.7, 113.5, 93.7, 91.2, 79.5, 74.9, 74.3, 72.8, 72.8, 70.2, 68.1, 60.3, 55.2, 55.1, 38.7, 27.0, 25.9, 25.8, 21.4, 21.0, 18.0, 14.2, 14.1, –3.7, –4.9 ppm. HRMS (ESI): calcd. for $C_{35}H_{50}Cl_3NO_9Si$ $[M + Na]^+$ 784.2213; found 784.2213.

4-*O*-*tert*-Butyldimethylsilyl-3,6-di-*O*-*p*-methoxybenzyl-2-*O*-pivaloyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (27): Molecular sieves (4 Å, MS AW300; 0.28 g), compound **26** (83 mg, 109 μmol), and compound **24** (96 mg, 109 μmol) were suspended in CH_2Cl_2 (17.5 mL) at ambient temperature. The mixture was stirred for 30 min, then it was cooled to –10 °C. Then, TMSOTf (1.0 μL, 5.5 μmol) was added to the suspension, which was then stirred for 1 h at 0 °C. The progress of the reaction was monitored by TLC (toluene/EtOAc, 6:1). Next, the reaction was quenched with satd. aq. $NaHCO_3$, the mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (toluene/EtOAc, 15:1) to give **27** (124 mg, 77%) as a colorless syrup. $[\alpha]_D$ = –16.5 (*c* = 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.10–6.81 (m, 13 H, Ar), 6.60 (d, $J_{2,NH}$ = 8.6 Hz, 1 H, NH), 5.37–5.32 (m, 2 H, 13-H, 14-H), 5.22 (t, $J_{2,3}$ = 6.3 Hz, 1 H, 2′-H), 4.84 (t, $J_{1,2}$ = $J_{2,3}$ = 8.2 Hz, 1 H, 2^a-H), 4.56–4.34 (5 d, 5 H, 4 ArCH_2O, 1^a-H), 4.33 (dd, $J_{2,3}$ = 8.9, $J_{3,4}$ = 5.6 Hz, 1 H, 3-H), 4.06–3.97 (m, 3 H, 1a-H, 2-H, 4-H), 3.79–3.76 (m, 7 H, 2 OMe, 1b-H), 3.62 (dd, J_{gem} = 10.3, $J_{5,6a}$ = 1.7 Hz, 1 H, $6a^a$-H), 3.44–3.32 (m, 4 H, 3^a-H, 4^a-H, 5^a-H, $6b^a$-H), 2.03–1.90 (m, 6 H, 12-H, 15-H, 3′-H), 1.62–1.15 (m, 72 H, 33 CH_2, 2 Me), 1.11 (s, 9 H, *t*Bu), 0.88 (2 t, 6 H, 2 Me), 0.79 (s, 9 H, *t*Bu), –0.09 and –0.12 (2 s, 6 H, SiMe_2*t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.6, 169.6, 165.6, 159.3, 158.8, 133.3, 130.3, 130.3, 130.0, 129.9, 129.9, 129.9, 129.7, 129.3, 128.5, 128.4, 113.9, 113.5, 107.9, 101.2, 82.8, 77.9, 76.1, 75.4, 75.0, 73.9, 73.6, 73.0, 71.0, 69.3, 69.0, 55.2, 55.2, 49.2, 38.7, 32.6, 31.9, 31.9, 31.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.2, 28.1, 27.3, 27.2, 27.0, 26.7, 25.9, 25.8, 25.2, 22.7, 17.9, 14.1, –3.8, –4.8 ppm. HRMS (ESI): calcd. for $C_{89}H_{147}NO_{14}Si$ $[M + Na]^+$ 1505.0483; found 1505.0483.

3,6-Di-*O*-*p*-methoxybenzyl-2-*O*-pivaloyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (28): Tetrabutylammonium fluoride (1.0 M solution in THF; 269 μL, 0.269 mmol) was added to a solution of compound **27** (133 mg, 0.0896 mmol) and AcOH (7.7 μL, 134 μmol) in THF (2.0 mL) at ambient temperature. The mixture was stirred for 6 h, and the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). The mixture was evaporated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 3:1) to give **28** (111 mg, 91%) as a colorless syrup. $[\alpha]_D$ = +4.3 (*c* = 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.08–6.84 (m, 13 H, Ar), 6.50 (d, $J_{2,NH}$ = 8.6 Hz, 1 H, NH), 5.34 (m, 2 H, 13-H, 14-H), 5.25 (t, $J_{2',3'}$ = 5.7 Hz, 1 H, 2′-H), 4.83 (dd, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.1 Hz, 1 H, 2^a-H), 4.61–4.44 (4 d, 4 H, 4 Ar-CH_2O), 4.40 (d, 1 H, 1^a-H), 4.27 (dd, $J_{2,3}$ = 8.7, $J_{3,4}$ = 5.6 Hz, 1 H, 3-H), 4.07–3.98 (m, 3 H, 1a-H, 2-H, 4-H), 3.79 and 3.78 (2 s, 6 H, 2 OMe), 3.73 (dd, J_{gem} = 10.7, $J_{1b,2}$ = 3.4 Hz, 1 H, 1b-H), 3.64 (dd, J_{gem} = 10.1, $J_{5,6a}$ = 4.1 Hz, 1 H, $6a^a$-H), 3.57 (dd, $J_{5,6b}$ = 5.6 Hz, 1 H, $6b^a$-H), 3.52–3.40 (m, 3 H, 3^a-H, 4^a-H, 5^a-H), 2.55 (s, 1 H, OH), 2.04–1.89 (m, 6 H, 12-H, 15-H, 3′-H), 1.69–1.11 (m, 72 H, 33 CH_2, 2 Me), 0.88 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.6, 169.5, 165.5, 159.5, 159.3, 133.4, 129.9, 129.9, 129.8, 129.6, 129.5, 129.3, 129.2, 128.5, 113.9, 113.9, 107.9, 101.2, 82.2, 77.7, 75.5, 74.8, 73.9, 73.8, 73.4, 72.7, 71.6, 70.1, 68.8, 55.2, 55.2, 49.2, 38.7, 31.9, 31.9, 31.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.3, 29.2, 28.0, 27.2, 27.2, 27.1, 26.6, 25.8, 25.1, 22.7, 14.1 ppm. HRMS (ESI): calcd. for $C_{83}H_{133}NO_{14}$ $[M + Na]^+$ 1390.9624; found 1390.9624.

[Methyl 5-Acetoxyacetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)-{[methyl 5-Acetoxyacetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-8-*O*-

methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)}-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-*p*-methoxybenzyl-2-*O*-pivaloyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (29): Molecular sieves (4 Å, MS AW300; 0.20 g), compound **3** (54.6 mg, 30.9 μmol), and compound **28** (55.9 mg, 40.8 μmol) were suspended in CH_2Cl_2 (1 mL) at ambient temperature. The mixture was stirred for 30 min, then the mixture was cooled to –10 °C. TMSOTf (0.3 μL, 1.55 μmol) was added to the suspension, which was then stirred for 1 h at 0 °C. The reaction was monitored by TLC ($CHCl_3$/acetone, 1:1). Next, the reaction was quenched with satd. aq. $NaHCO_3$, the mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by gel filtration using Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), and silica gel column chromatography ($CHCl_3$/acetone, 5:1→5:2→1:1) to give **29** (49.9 mg, 54%) as a white solid. $[\alpha]_D$ = –7.2 (*c* = 1.0, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.00–6.80 (m, 18 H, Ar), 6.35 (d, $J_{2,NH}$ = 8.8 Hz, 1 H, NH^{Cer}), 5.84 and 5.82 (2 d, 2 H, NH^a, NH^b), 5.66 (d, $J_{2,NH}$ = 8.0 Hz, 1 H, NH^c), 5.38 (dd, $J_{1,2}$ = 8.2, $J_{2,3}$ = 9.8 Hz, 1 H, 2^d-H), 5.37–5.32 (m, 3 H, 4^d-H, 13^{Cer}-H, 14^{Cer}-H), 5.18 (t, $J_{2',3'}$ = 6.2 Hz, 1 H, $2'^{Cer}$-H), 5.11 (dd, $J_{6,7}$ = 1.6, $J_{7,8}$ = 9.3 Hz, 1 H, 7^{Neu}-H), 5.04–4.97 (m, 4 H, 4^a-H, 4^b-H, 7^{Neu}-H, 4^c-H), 4.82–4.78 (m, 3 H, 3^c-H, 2^e-H, ArCH_2O), 4.65 (d, 1 H, 1^d-H), 4.59–4.52 (m, 4 H, 1^c-H, ArCH_2O, 2 AcOCH_2CO), 4.35 (d, 1 H, ArCH_2O), 4.31–4.23 (m, 4 H, 3^{Cer}-H, 2 AcOCH_2CO, ArCH_2O), 4.18 (d, 1 H, 1^e-H), 4.15–3.77 (m, 27 H, $1a^{Cer}$-H, 2^{Cer}-H, 4^{Cer}-H, 6^a-H, 6^b-H, $9a^a$-H, $9b^a$-H, 4^e-H, $6a^d$-H, 5^a-H, 5^b-H, 8^a-H, 8^b-H, 3^d-H, $6a^c$-H, 2 OMe, 2 COOMe), 3.72–3.62 (m, 5 H, $9b^a$-H, $9b^b$-H, $1b^{Cer}$-H, $6b^c$-H, 5^d-H), 3.58–3.47 (m, 6 H, $6a^e$-H, 2^c-H, $6b^d$-H, OMe), 3.43–3.26 (m, 6 H, 3^e-H, $6b^e$-H, 5^c-H, OMe), 3.07 (near td, 1 H, 5^e-H), 2.57 and 2.52 (2 dd, 2 H, $3eq^a$-H, $3eq^b$-H), 2.17–1.95 (12 s, 36 H, 12 Ac), 1.88–1.78 (m, 7 H, $3ax^{Neu}$-H, 12^{Cer}-H, 15^{Cer}-H), 1.66 (t, 1 H, $3ax^{Neu}$-H), 1.49–1.12 (m, 72 H, 33 CH_2, 2 Me), 1.09 (s, 9 H, *t*Bu), 0.88 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.4, 171.0, 170.9, 170.4, 169.9, 169.7, 169.6, 169.5, 169.5, 169.3, 167.9, 167.7, 167.6, 167.5, 165.5, 164.5, 159.4, 158.9, 133.6, 133.5, 130.8, 130.1, 129.9, 129.8, 129.7, 129.5, 129.3, 128.6, 113.9, 113.4, 107.9, 101.1, 99.8, 98.7, 97.6, 79.9, 77.6, 77.5, 75.7, 75.5, 75.2, 75.0, 74.8, 73.3, 73.1, 72.6, 72.2, 71.8, 71.5, 71.1, 70.4, 69.4, 68.5, 68.4, 68.0, 67.4, 66.7, 62.7, 62.7, 62.6, 61.4, 61.2, 60.9, 58.5, 58.1, 55.3, 55.2, 53.1, 52.7, 49.4, 49.3, 48.8, 38.6, 37.5, 37.2, 32.6, 31.9, 31.9, 31.7, 29.9, 29.8, 29.7, 29.7 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 29.3, 29.2, 28.1, 27.2, 27.2, 27.0, 26.5, 25.7, 25.0, 22.7, 22.6, 20.9, 20.8, 20.8, 20.7, 20.7, 20.7, 20.6, 20.5, 14.1 ppm. HRMS (ESI): calcd. for $C_{151}H_{222}N_4O_{54}$ $[M + 2Na]^{2+}$ 1507.7345; found 1507.7346.

Phenyl 2,3-Di-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (32): HCl (2 M in Et_2O; 3.2 mL, 6.40 mmol) was added to a suspension of compound **31** (498 mg, 0.842 mmol), $NaBH_3CN$ (529 mg, 8.42 mmol), and molecular sieves (3 Å; 850 mg) in THF (8.5 mL) under an argon atmosphere. The mixture was stirred for 30 min, then it was diluted with EtOAc, and filtered through a pad of Celite. The filtrate was extracted with EtOAc, and the organic phase was washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated.

The resulting residue was dissolved in CH_2Cl_2 (8.5 mL), then 2,6-lutidine (390 mL, 3.37 mmol) and TBSOTf (290 mL, 1.26 mmol) were added at 0 °C under an argon atmosphere. The reaction was monitored by TLC (*n*-hexane/EtOAc, 4:1), and when the reaction was complete, it was quenched with MeOH. The mixture was extracted with $CHCl_3$, and washed with H_2O and brine. The organic layer was dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 7:1) to give **32** (427 mg, 71%, over two steps) as a colorless syrup. $[\alpha]_D$ = –32.5 (*c* = 0.6, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.51–6.86 (m, 9 H, Ar), 5.09 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1 H, 3-H), 4.84 (near t, $J_{1,2}$ = 10.1 Hz, 1 H, 2-H), 4.72 (d, 1 H, 1-H), 4.54 and 4.46 (2 d, J_{gem} = 11.5 Hz, 2 H, ArCH_2O), 3.81 (s, 3 H, OMe), 3.78 (t, $J_{4,5}$ = 9.2 Hz, 1 H, 4-H), 3.73 (dd, J_{gem} = 10.9, $J_{5,6a}$ = 1.8 Hz, 1 H, 6a-H), 3.63 (dd, $J_{5,6b}$ = 5.4 Hz, 1 H, 6b-H), 3.50 (m, 1 H, 5-H), 2.05 and 2.01 (2 s, 6 H, 2 Ac), 0.80 (s, 9 H, *t*Bu), 0.03 and 0.00 (2 s, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.0, 169.6, 159.1, 132.6, 132.2, 131.6, 130.3, 129.4, 129.1, 128.8, 113.7, 85.2, 80.4, 77.1, 72.9, 70.9, 68.8, 68.4, 55.2, 31.5, 25.4, 22.5, 21.1, 20.7, 17.8, –4.3, –4.8 ppm. HRMS (ESI): calcd. for $C_{30}H_{42}O_8SSi$ $[M + Na]^+$ 613.2262; found 613.2262.

2,3-Di-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl Trichloroacetimidate (33): NBS (192 mg, 1.08 mmol) was added to a solution of compound **32** (426 mg, 721 μmol) in acetone/H_2O (10:1; 7.2 mL) at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 5:1). After the starting material had been consumed, the reaction mixture was extracted with EtOAc. The organic layer was successively washed with satd. aq. $Na_2S_2O_3$ and brine, dried with Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/acetone, 3:1→3:2).

Then, the resulting compound was dissolved in CH_2Cl_2 (7.2 mL). Trichloroacetonitrile (549 μL, 5.47 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (55 μL, 365 μmol) were added to the solution at 0 °C. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). When the reaction was complete, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 5:1) to give **33** (218 mg, 47%) as an amorphous powder. $[\alpha]_D$ = +57.9 (*c* = 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.59 (s, 1 H, C=NH), 7.26–6.86 (2 d, 4 H, Ar), 6.49 (d, $J_{1,2}$ = 3.7 Hz, 1 H, 1-H), 5.45 (dd, $J_{2,3}$ = 9.9, $J_{3,4}$ = 8.5 Hz, 1 H, 3-H), 4.99 (dd, 1 H, 2-H), 4.51 and 4.44 (2 d, J_{gem} = 11.6 Hz, 2 H, ArCH_2O), 3.99–3.93 (m, 2 H, 4-H, 5-H), 3.79 (s, 3 H, OMe), 3.70 (dd, J_{gem} = 10.8, $J_{5,6a}$ = 3.4 Hz, 1 H, 6a-H), 3.63 (dd, $J_{5,6b}$ = 1.4 Hz, 1 H, 6b-H), 2.04 and 1.97 (2 s, 6 H, 2 Ac), 0.82 (s, 9 H, *t*Bu), 0.05 and 0.03 (2 s, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.1, 169.6, 161.1, 159.1, 130.0, 129.2, 129.1, 113.8, 113.7, 93.5, 90.9, 74.5, 72.9, 70.6, 68.3, 67.6, 55.2, 25.7, 25.6, 21.2, 20.4, 17.9, –4.3, –4.9 ppm. HRMS (ESI): calcd. for $C_{26}H_{38}Cl_3NO_9Si$ $[M + Na]^+$ 664.1274; found 664.1272.

Phenyl 2,3,6-Tri-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (35): TBSCl (2.30 g, 15.2 mmol) was added to a solution of compound **34** (604 mg, 1.52 mmol) in pyridine (15 mL) under an argon atmosphere. The reaction was monitored by TLC (*n*-hexane/EtOAc, 2:1). The mixture was stirred for 12 h at 40 °C, then the reaction mixture was coevaporated with toluene. The residue was extracted with EtOAc, and the organic layer was washed with HCl (2 M aq.), $NaHCO_3$, and brine, then it was dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 6:1) to give **35** (708 mg, 91%) as a colorless syrup. $[\alpha]_D$ = –25.8 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.48–7.28 (m, 5 H, Ph), 5.11 (t, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, 1 H, 3-H), 4.85 (near t, $J_{1,2}$ = 10.1 Hz, 1 H, 2-H), 4.72 (d, 1 H, 1-H), 4.48 (dd, J_{gem} = 12.0, $J_{5,6a}$ = 2.1 Hz, 1 H, 6a-H), 4.09 (dd, $J_{5,6b}$ = 5.4 Hz, 1 H, 6b-H), 3.77 (t, $J_{4,5}$ = 9.3 Hz, 1 H, 4-H), 3.56 (m, 1 H, 5-H), 2.09–2.04 (3 s, 9 H, 3 Ac), 0.83 (s, 9 H, *t*Bu), 0.05 and 0.03 (2 s, 6 H, 2 Me) ppm. ^{13}C NMR

(125 MHz, $CDCl_3$): δ = 170.5, 170.0, 169.7, 132.8, 132.0, 128.8, 128.1, 85.4, 78.3, 70.7, 68.9, 62.8, 25.6, 21.2, 20.8, 20.7, 17.8, –4.1, –4.9 ppm. HRMS (ESI): calcd. for $C_{24}H_{36}O_8SSi$ $[M + Na]^+$ 535.1792; found 535.1792.

2,3,6-Tri-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranosyl Trichloroacetimidate (36): NBS (708 mg, 4.00 mmol) was added to a solution of compound **35** (680 mg, 1.33 mmol) in acetone/H_2O (10:1; 14 mL) at ambient temperature. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 5:1). After the starting material had been consumed, the reaction mixture was extracted with EtOAc. Then, the organic layer was successively washed with satd. aq. $Na_2S_2O_3$ and brine, and dried with Na_2SO_4. The solution was concentrated to give a crude mixture including a hemiacetal and 2-OH derivatives.

This residue was dried in vacuo, then it was dissolved in pyridine (14 mL), and Ac_2O (7 mL) was added to the solution at 0 °C under an argon atmosphere. The mixture was stirred for 10 h, then it was coevaporated with toluene. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 4:1) to give the tetra-acetylated derivative.

This compound was dissolved in DMF (14 mL), and hydrazine acetate (180 mg, 2.00 mmol) was added to the resulting solution at ambient temperature. After TLC confirmed that the reaction was complete, the reaction mixture was extracted with EtOAc, and the organic phase was washed with H_2O and brine. The organic layer was dried with Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 2:1) to give a hemiacetal, which was dried under high vacuum.

The hemiacetal was dissolved in in CH_2Cl_2 (20 mL), and the solution was cooled to 0 °C. Trichloroacetonitrile (2.6 mL, 26 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (219 μL, 1.47 mmol) were added, and the mixture was stirred for 30 min. The progress of the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). Then, the reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 5:1) to give **36** (576 mg, 77%, over four steps) as an amorphous powder. $[\alpha]_D$ = +75.0 (c = 2.3, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.62 (s, 1 H, C=NH), 6.42 (d, $J_{1,2}$ = 3.7 Hz, 1 H, 1-H), 5.43 (t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 4.98 (dd, 1 H, 2-H), 4.41 (dd, J_{gem} = 12.1, $J_{5,6a}$ = 1.7 Hz, 1 H, 6a-H), 4.08 (dd, $J_{5,6b}$ = 4.2 Hz, 1 H, 6b-H), 3.99 (m, 1 H, 5-H), 3.89 (t, $J_{4,5}$ = 9.4 Hz, 1 H, 4-H), 2.05–1.96 (3 s, 9 H, 3 Ac), 0.83 (s, 9 H, *t*Bu), 0.04 (2 s, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4, 170.1, 169.5, 163.7, 161.0, 93.2, 72.6, 72.3, 70.4, 68.6, 62.1, 25.6, 25.5, 21.1, 20.7, 20.4, 17.9, 14.0, –4.2, –5.1 ppm. HRMS (ESI): calcd. for $C_{20}H_{32}Cl_3NO_9Si$ $[M + Na]^+$ 586.0804; found 586.0805.

2,3-Di-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (37): Molecular sieves (4 Å, MS AW300, 0.22 g), compound **33** (83 mg, 129 μmol), and compound **24** (95 mg, 109 μmol) were suspended in CH_2Cl_2 (2.2 mL) at ambient temperature. The mixture was stirred for 30 min, then it was cooled to –10 °C. TMSOTf (0.23 μL, 1.3 μmol) was added to the suspension, and the mixture was stirred for 1 h at 0 °C. The reaction was monitored by TLC (toluene/EtOAc, 6:1). The reaction mixture was quenched with satd. aq. $NaHCO_3$. The mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (toluene/EtOAc, 15:1) to give **37** (93 mg, 64%) as a white solid. $[\alpha]_D$ = +8.4 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.08–6.85 (m, 9 H, Ar), 6.38 (d, $J_{2,NH}$ = 8.0 Hz, 1 H, NH), 5.35–5.32 (m, 2 H, 13-H, 14-H), 5.25 (t, $J_{2',3'}$ = 6.2 Hz, 1 H, 2′-H), 4.96 (t, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, 1 H, 3^a-H), 4.53–4.42 (3 d, J_{gem} = 11.7, $J_{1,2}$ = 8.0 Hz, 3 H, ArCH_2O-, 1^a-H), 4.19 (dd, J = 5.3, J = 9.0 Hz, 1 H, 3-H), 4.13 (m, 1 H, 2-H), 4.05–4.00 (m, J_{gem} = 10.8, $J_{1a,2}$ = 3.9 Hz, 2 H, 1a-H, 4-H), 3.80 (s, 3 H, OMe), 3.72 (dd, $J_{1b,2}$ = 3.5 Hz, 1 H, 1b-H), 3.61 (dd, J_{gem} = 11.6, $J_{5,6a}$ = 1.7 Hz, 1 H, 6a^a-H), 3.57 (t, $J_{4,5}$ = 9.1 Hz, 1 H, 4^a-H), 3.45 (dd, $J_{5,6b}$ = 5.9 Hz, 1 H, 6b^a-H), 3.38 (m, 1 H, 5^a-H), 2.03–1.91 (m, 12 H, 3 Ac, 12-H, 15-H, 3′-H), 1.50–1.13 (m, 74 H, 34 CH_2, 2 Me), 0.88 (2 t, 6 H, 2 Me), 0.78 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.1, 169.6, 169.4, 159.3, 133.4, 130.0, 129.9, 129.8, 129.5, 129.2, 128.6, 113.8, 108.0, 100.4, 75.9, 74.8, 73.1, 72.3, 69.2, 68.6, 68.3, 68.2, 55.2, 48.5, 31.92, 31.90, 31.7, 29.8, 29.78, 29.71, 29.70, 29.6, 29.56, 29.53, 29.51, 29.41, 29.38, 29.35, 29.33, 29.31, 29.2, 29.1, 28.9, 28.1, 27.24, 27.22, 26.7, 25.8, 25.6, 25.1, 22.7, 21.2, 29.6, 17.9, 14.1, 14.0, –4.3, –4.7 ppm. HRMS (ESI): calcd. for $C_{80}H_{135}NO_{14}Si$ $[M + Na]^+$ 1384.9544; found 1384.9544.

2,3-Di-*O*-aceyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (38): Tetrabutylammonium fluoride (1.0 M solution in THF; 116 μL, 0.116 mmol) was added to a solution of compound **37** (106 mg, 0.077 mmol) and AcOH (9.0 μL, 154 μmol) in THF (1.5 mL) at ambient temperature. The mixture was stirred for 6 h, and the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). The mixture was evaporated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 3:1) to give **38** (96 mg, 98%) as a colorless syrup. $[\alpha]_D$ = +24.4 (c = 0.2, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07–6.86 (m, 9 H, Ar), 6.28 (m, 1 H, NH), 5.38–5.30 (m, 2 H, 13-H, 14-H), 5.28 (t, $J_{2',3'}$ = 6.1 Hz, 1 H, 2′-H), 4.96 (t, $J_{2,3}$ =, $J_{3,4}$ = 9.4 Hz, 1 H, 3^a-H), 4.79 (dd, $J_{1,2}$ = 8.0 Hz, 1 H, 2^a-H), 4.51–4.43 (m, 3 H, 1^a-H, ArCH_2O), 4.15–4.12 (m, 2 H, 2-H, 3-H), 4.03–4.02 (m, 2 H, 1a-H, 4-H), 3.79 (s, 3 H, OMe), 3.70–3.63 (m, 3 H, 1b-H, 6a^a-H, 6b^a-H), 3.59 (t, $J_{4,5}$ = 9.1 Hz, 1 H, 4^a-H), 3.47 (m, 1 H, 5^a-H), 2.99 (br. s, 1 H, OH), 2.16–1.89 (m, 12 H, 2 Ac, 12-H, 15-H, 3′-H), 1.52–1.23 (m, 74 H, 34 CH_2, 2 Me), 0.88 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.2, 169.34, 169.31, 165.5, 159.4, 133.5, 129.9, 129.83, 129.80, 129.42, 129.39, 129.34, 128.5, 113.9, 108.0, 100.5, 77.7, 75.6, 74.7, 73.9, 73.4, 71.1, 70.9, 69.8, 68.5, 55.2, 48.6, 31.89, 31.87, 29.8, 29.74, 29.67, 29.64, 29.62, 29.60, 29.52. 29.50, 29.45, 29.34, 29.32, 29.29, 29.28, 29.1, 28.0, 27.2, 27.19, 26.6, 25.7, 25.0, 22.6, 20.8, 20.5, 14.1 ppm. HRMS (ESI): calcd. for $C_{74}H_{121}NO_{14}$ $[M + Na]^+$ 1270.8679; found 1270.8679.

2,3,6-Tri-*O*-acetyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(Benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (39): Molecular sieves (4 Å, MS AW300; 0.410 g), compound **36** (232 mg, 411 μmol), and compound **24** (184 mg, 206 μmol) were suspended in CH_2Cl_2 (4.1 mL) at ambient temperature. The mixture was stirred for 30 min, then the mixture was cooled to –10 °C. TMSOTf (3.6 μL, 20.6 μmol) was added to the suspension. The mixture was stirred for 1 h at 0 °C, and the reaction was monitored by TLC (toluene/EtOAc, 6:1). The reaction mixture was quenched with satd. aq. $NaHCO_3$. The mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by silica gel column chromatography (toluene/EtOAc, 15:1) to give **39** (202 mg, 75%) as a colorless syrup. $[\alpha]_D$ = –40 9 (c = 0.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.05–7.44 (m, 5 H, Ph), 6.20

($J_{2,NH}$ = 8.8 Hz, 1 H, NH), 5.35–5.30 (m, 2 H, 13-H, 14-H), 5.25 (t, $J_{2',3'}$ = 6.2 Hz, 1 H, 2′-H), 4.96 (t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1 H, 3^a-H), 4.67 (dd, $J_{1,2}$ = 7.9 Hz, 1 H, 2^a-H), 4.44 (d, 1 H, 1^a-H), 4.36 (dd, J_{gem} = 11.9, $J_{5,6a}$ = 2.1 Hz, 1 H, 6a^a-H), 4.14–4.01 (m, 3 H, 2-H, 3-H, 4-H), 3.98 (dd, $J_{5,6b}$ = 5.3 Hz, 1 H, 6b^a-H), 3.95 (dd, J_{gem} = 10.7, $J_{1a,2}$ = 4.1 Hz, 1 H, 1a-H), 3.67 (dd, $J_{1b,2}$ = 3.3 Hz, 1 H, 1b-H), 3.64 (t, $J_{4,5}$ = 9.1 Hz, 1 H, 4^a-H), 3.41 (m, 1 H, 5^a-H), 2.05–1.86 (m, 15 H, 3 Ac, 12-H, 15-H, 3′-H), 1.47–1.18 (m, 74 H, 34 CH_2, 2 Me), 0.86–0.78 (m, 15 H, 2 Me, *t*Bu), 0.02 and 0.00 (2 s, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.5, 169.9, 169.6, 165.6, 133.5, 130.3, 130.2, 129.9, 129.8, 129.4, 128.6, 108.3, 108.1, 100.4, 77.8, 75.9, 75.6, 74.8, 74.4, 72.2, 69.1, 68.7, 62.7, 48.4, 32.6, 31.9, 31.8, 31.6, 29.8, 29.7, 29.67, 29.62, 29.59, 29.57, 29.50, 29.46, 29.35, 29.32, 29.30, 29.28, 29.18, 29.17, 29.10, 28.0, 27.5, 27.21, 27.20, 26.6, 25.7, 25.6, 25.1, 22.6, 21.1, 20.9, 20.7, 20.5, 17.9, 17.8, 14.1, –4.2, –4.9 ppm. HRMS (ESI): calcd. for $C_{74}H_{129}NO_{14}Si$ $[M + Na]^+$ 1306.9075; found 1306.9077.

2,3,6-Tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (40): Tetrabutylammonium fluoride (1.0 M solution in THF; 233 μL, 0.233 mmol) was added to a solution of compound **39** (202 mg, 0.156 mmol) and AcOH (13.7 μL, 233 μmol) in THF (3.1 mL) at ambient temperature. The mixture was stirred for 6 h, and the reaction was monitored by TLC (*n*-hexane/EtOAc, 3:1). The mixture was evaporated. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 3:1) to give **40** (155 mg, 85%) as a colorless syrup. $[\alpha]_D$ = –3.7 (*c* = 0.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.08–7.47 (m, 5 H, Ph), 6.23 (d, $J_{2,NH}$ = 9.0 Hz, 1 H, NH), 5.39–5.33 (m, 2 H, 13-H, 14-H), 5.31 (t, $J_{2',3'}$ = 6.1 Hz, 1 H, 2′-H), 4.95 (t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1 H, 3^a-H), 4.81 (dd, $J_{1,2}$ = 7.8 Hz, 1 H, 2^a-H), 4.39 (dd, J_{gem} = 11.9, $J_{5,6a}$ = 4.0 Hz, 1 H, 6a^a-H), 4.27 (near d, 1 H, 6b^a-H), 4.17 (m, 1 H, 2-H), 4.11–4.04 (m, 2 H, 3-H, 4-H), 4.01 (dd, J_{gem} = 10.5, $J_{1a,2}$ = 4.1 Hz, 1 H, 1a-H), 3.70 (dd, $J_{1b,2}$ = 3.2 Hz, 1 H, 1b-H), 3.50–3.44 (m, 2 H, 4^a-H, 5^a-H), 3.03 (d, $J_{4,OH}$ = 3.2 Hz, 1 H, OH), 2.10–1.89 (m, 15 H, 3 Ac, 12-H, 15-H, 3′-H), 1.52–1.14 (m, 74 H, 34 CH_2, 2 Me), 0.88 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6, 171.3, 169.3, 169.2, 165.6, 133.6, 130.3, 130.2, 129.9, 129.8, 128.7, 128.6, 108.1, 100.6, 77.7, 75.7, 75.4, 74.7, 74.2, 71.0, 68.9, 68.5, 62.8, 48.4, 32.6, 31.89, 31.88, 31.7, 29.80, 29.75, 29.68, 29.63, 29.59, 29.51, 29.45, 29.33, 29.29, 29.20, 29.17, 29.12, 28.0, 27.21, 27.20, 26.6, 25.7, 25.0, 22.7, 20.8, 20.4, 14.1 ppm. HRMS (ESI): calcd. for $C_{68}H_{115}NO_{14}$ $[M + Na]^+$ 1192.8210; found 1192.8210.

[Methyl 5-Acetoxyacetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)-{[methyl 5-Acetoxyacetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)}-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (41): Molecular sieves (4 Å, MS AW300, 0.075 g), compound **3** (45 mg, 25.5 μmol), and compound **38** (32 mg, 25.5 μmol) were suspended in CH_2Cl_2 (0.75 mL) at ambient temperature. The mixture was stirred for 30 min, then it was cooled to –10 °C. TMSOTf (0.46 μL, 2.55 μmol) was added, and the mixture was stirred for 1 h at 0 °C. The reaction was monitored by TLC ($CHCl_3$/acetone, 1:1). Next, the reaction mixture was quenched with satd. aq. $NaHCO_3$. The mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by gel filtration using Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), followed by silica gel column chromatography ($CHCl_3$/MeOH, 40:1→35:1→30:1) to give **41** (19.7 mg, 26%) as a white solid. $[\alpha]_D$ = +5.8 (*c* = 0.4, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.02–6.92 (m, 14 H, Ar), 6.22 (d, $J_{2,NH}$ = 9.0 Hz, 1 H, NH^{Cer}), 5.81 and 5.77 (2 d, $J_{5,NH}$ = 10.0 Hz, 2 H, NH^a, NH^b), 5.59 (d, $J_{2,NH}$ = 7.8 Hz, 1 H, NH^c), 5.38–5.33 (m, 3 H, 13^f-H, 14^f-H, 4^d-H), 5.27–5.23 (m, 2 H, 2^d-H, 1′^{f}-H), 5.11 (dd, $J_{6,7}$ = 1.7, $J_{7,8}$ = 9.3 Hz, 1 H, 7^{Neu}-H), 5.05–4.98 (m, 5 H, 7^{Neu}-H, 4^a-H, 4^b-H, 3^e-H), 4.91 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1^c-H), 4.78 (dd, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.7 Hz, 1 H, 2^e-H), 4.64 (br. d, $J_{2,3}$ = 10.8 Hz, 1 H, 3^c-H), 4.59–44.52 (m, 3 H, 2 AcOCH_2CO, 1^d-H), 4.31–4.23 (m, 5 H, ArCH_2O-, 2 AcOCH_2CO-, 1^e-H, 3^d-H), 4.16 (dd, J_{gem} = 11.7, $J_{8.9a}$ = 4.3 Hz, 1 H, 9a^{Neu}-H), 4.13–3.80 (m, 23 H, ArCH_2O-, 2^{Cer}-H, 4^{Cer}-H, 9a^b-H, 9b^a-H, 6^a-H, 6^b-H, 1a^{Cer}-H, 5^a-H, 5^b-H, 4^e-H, 8^a-H, 8^b-H, 2 COOMe, OMe), 3.78–3.66 (m, 2 H, 6a^c-H, 1b^{Cer}-H), 3.65–3.63 (m, 2 H, 6b^c-H, 3^{Cer}-H), 3.57–3.35 (m, 10 H, 9b^b-H, 6a^d-H, 6b^d-H, 5^e-H, 2 OMe), 3.26 (t, $J_{5,6}$ = 8.6 Hz, 1 H, 5^c-H), 3.15 (near d, J_{gem} = 9.6 Hz, 1 H, 6b^e-H), 2.58 and 2.50 (2 dd, J_{gem} = 12.6, $J_{3eq,4}$ = 4.7 Hz, 2 H, 3eq^a-H, 3eq^b-H), 2.19–1.82 (m, 36 H, 3ax^{Neu}-H, 11 Ac, 2 CH_2), 1.67–1.49 (m, 12 H, 3ax^{Neu}-H, 3 Ac, CH_2), 1.47–1.19 (m, 72 H, 33 CH_2, 2 Me), 0.88 and 0.87 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0, 170.9, 170.8, 170.6, 170.5, 169.9, 169.8, 169.7, 169.6, 169.5, 169.4, 169.2, 167.9, 167.7, 167.6, 167.5, 165.5, 164.3, 159.4, 133.6, 133.5, 130.3, 130.1, 129.9, 129.9, 129.83, 129.80, 129.7, 129.5, 129.2, 128.6, 113.9, 108.0, 100.7, 100.4, 100.1, 98.7, 97.7, 77.7, 77.6, 75.6, 75.5, 74.7, 74.3, 73.1, 72.7, 72.6, 72.3, 71.5, 71.4, 71.2, 70.2, 69.4, 68.6, 68.2, 68.0, 67.9, 66.9, 62.8, 62.7, 61.2, 61.1, 58.3, 58.1, 55.3, 53.1, 52.18, 49.4, 48.3, 37.5, 37.2, 32.6, 31.92, 31.91, 31.7, 29.8, 29.78, 29.71, 29.66, 29.61, 29.54, 29.46, 29.36, 29.33, 29.32, 29.2, 29.1, 28.1, 27.24, 27.23, 26.6, 25.8, 25.0, 22.72, 22.68. 20.98, 20.87, 20.86, 20.84, 20.79, 20.77, 20.71, 20.69, 20.58, 20.47, 14.1 ppm. HRMS (ESI): calcd. for $C_{143}H_{212}N_4O_{54}$ $[M + Na]^+$ 2872.3858; found 2872.3858.

[Methyl 5-Acetoxyacetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→6)-{[methyl 5-Acetoxyacetamido-4,7,9-tri-*O*-acetyl-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)}-(2-acetamido-4-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*S*,4*R*,13*Z*)-2-[(*R*)-2-(benzoyloxy)tetracosanoylamino]-3,4-*O*-isopropylidenedocos-13-ene-1,3,4-triol (42): Molecular sieves (4 Å, MS AW300; 0.060 g), compound **3** (100 mg, 56.6 μmol), and compound **40** (22.0 mg, 18.9 μmol) were suspended in CH_2Cl_2 (0.6 mL) at ambient temperature. The mixture was stirred for 30 min, and then it was cooled to –10 °C. TMSOTf (0.34 μL, 1.89 μmol) was added, and the mixture was stirred for 1 h at 0 °C. The reaction was monitored by TLC ($CHCl_3$/acetone, 1:1). Next, the reaction mixture was quenched with satd. aq. $NaHCO_3$. The mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd. aq. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by gel filtration using Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), followed by silica gel column chromatography ($CHCl_3$/MeOH, 40:1→35:1→30:1) to give **42** (13.2 mg, 25%) as a white solid. $[\alpha]_D$ = –7.7 (*c* = 1.5, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07–7.43 (m, 10 H, 2 Ph), 6.15 (d, $J_{2,NH}$ = 9.1 Hz, 1 H, NH^{Cer}), 5.78 (d, $J_{5,NH}$ = 9.4 Hz, 1 H, NH^{Neu}), 5.74 (d, $J_{5,NH}$ = 10.0 Hz, 1 H, NH^{Neu}), 5.66 (d, $J_{2,NH}$ = 8.0 Hz, 1 H, NH^c), 5.37–5.31 (m, 4 H, 13^{Cer}-H, 14^{Cer}-H, 2^d-H, 4^d-H), 5.26 (t, $J_{1,2}$ = 6.2 Hz, 1 H, 1′^{Cer}-H), 5.11 (dd, $J_{6,7}$ = 1.6, $J_{7,8}$ = 9.3 Hz, 1 H, 7^{Neu}-H), 5.05 (t, $J_{2,3}$ =, $J_{3,4}$ = 9.4 Hz, 1 H, 3^e-H), 5.03–4.97 (m, 4 H, 4^a-H, 4^b-H,

7^{Neu}-H, 4^{c}-H), 4.87 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1^{c}-H), 4.76 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.6 Hz, 1 H, 2^{e}-H), 4.60–4.53 (m, 3 H, 3^{c}-H, 2 AcOCH_2), 4.48 (d, $J_{1,2}$ = 7.9 Hz, 1 H, 1^{d}-H), 4.32 (d, 1 H, 1^{e}-H), 4.31–4.23 (m, 4 H, $1a^{f}$-H, $3'^{Cer}$-H, 2 AcOCH_2), 4.17 (dd, J_{gem} = 11.7, $J_{8,9a}$ = 5.6 Hz, 1 H, $9a^{Neu}$-H), 4.13–3.91 (m, 13 H, 5^{a}-H, 5^{b}-H, 2^{Cer}-H, 3^{Cer}-H, 4^{Cer}-H, $1b^{Cer}$-H, 6^{a}-H, 6^{b}-H, $9a^{Neu}$-H, 3^{d}-H, 4^{d}-H, $6a^{e}$-H, $6b^{e}$-H), 3.85–3.80 (m, 9 H, 8^{a}-H, 8^{b}-H, 5^{c}-H, 2 COOMe), 3.67–3.52 (m, 9 H, $6a^{c}$-H, $6b^{c}$-H, $6a^{d}$-H, $6b^{d}$-H, $9b^{a}$-H, $9b^{b}$-H, OMe), 3.38–3.33 (m, 4 H, 5^{d}-H, OMe), 3.21 (t, $J_{4,5}$ = $J_{5,6a}$ =, $J_{5,6b}$ = 8.5 Hz, 1 H, 5^{e}-H), 2.54 and 2.52 (2 dd, J_{gem} = 12.5, $J_{3eq,4}$ = 4.6 Hz, $3eq^{a}$-H, $3eq^{b}$-H), 2.17–1.87 (m, 47 H, 13 Ac, 4 CH_2), 1.81 (t, $J_{3ax,4}$ = 12.5 Hz, 1 H, $3ax^{Neu}$-H), 1.79–1.64 (m, 4 H, $3ax^{Neu}$-H, Ac), 1.50–1.19 (m, 75 H, Ac, 33 CH_2, 2 Me), 0.88 and 0.87 (2 t, 6 H, 2 Me) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.93, 170.91, 170.87, 170.84, 170.6, 170.5, 170.4, 169.91, 169.89, 169.74, 169.67, 169.54, 169.50, 169.48, 169.40, 169.2, 167.8, 167.7, 167.6, 167.5, 165.6, 164.3, 133.6, 133.5, 130.4, 130.3, 130.0, 129.9, 129.8, 129.5, 129.3, 128.6, 108.1, 100.9, 100.7, 100.3, 98.7, 97.6, 77.7, 77.6, 75.6, 75.4, 74.7, 72.7, 72.5, 72.3, 71.7, 71.4, 71.3, 70.6, 69.1, 68.6, 68.5, 68.0, 66.7, 62.8, 62.7, 62.6, 62.1, 61.4, 61.0, 60.9, 58.6, 58.0, 53.1, 52.7, 49.4, 49.3, 48.3, 37.6, 37.1, 32.6, 31.9, 31.8, 31.6, 30.0, 29.81, 29.76, 29.69, 29.64, 29.59, 29.52, 29.48, 29.44, 29.34, 29.32, 29.30, 29.2, 29.1, 28.0, 27.23, 27.21, 27.1, 26.6, 25.7, 25.0, 22.8, 22.7, 21.0, 20.9, 20.85, 20.82, 20.80, 20.75, 20.68, 20.65, 20.63, 20.59, 20.42, 14.1 ppm. HRMS (ESI): calcd. for $C_{137}H_{206}N_4O_{54}$ $[M + Na]^+$ 2794.3389; found 2794.3387.

Ganglioside GAA-7 (1): Trifluoroacetic acid (90% aq.; 125 μL) was added to a solution of compound **42** (13.2 mg, 4.76 μmol) in CH_2Cl_2 (0.5 mL) at 0 °C. The progress of the reaction was monitored by TLC ($CHCl_3$/MeOH, 12:1). The mixture was stirred for 30 min at 0 °C, then it was diluted and extracted with $CHCl_3$. The organic layer was successively washed with satd. aq. $NaHCO_3$, H_2O, and brine, dried with Na_2SO_4, and concentrated. The resulting residue was purified by gel filtration (Sephadex LH-20, $CHCl_3$/MeOH, 1:1) to give a diol compound, which was dried in vacuo.

The dried residue was dissolved in THF/MeOH (2:1; 300 μL), and a solution of NaOH (0.5 M aq.; 257 μL) was then added to this solution at ambient temperature. The mixture was stirred for 36 h at ambient temperature [monitored by TLC; $CHCl_3$/MeOH/$CaCl_2$ (30 mM aq.), 5:4:1], and the mixture was then concentrated. The resulting residue was purified by column chromatography on Sephadex LH-20 ($CHCl_3$/MeOH/H_2O, 5:4:1) and subsequent column chromatography on Iatrobeads 6RS-8060 ($CHCl_3$/MeOH/H_2O, 5:4:0→5:4:0.4→5:4:1) to give compound **1** (7.0 mg, 77%). $[\alpha]_D$ = +3.3 (c = 0.5, $CHCl_3$/MeOH/H_2O, 5:4:1) ^{1}H NMR (500 MHz, CD_3OD): δ = 5.24 (td, 2 H, 13^{Cer}-H, 14^{Cer}-H), 4.51 (d, 1 H, anomeric H), 4.29 (d, 1 H, anomeric H), 4.22 (d, 1 H, anomeric H), 3.42 (s, 3 H, OMe), 3.41 (s, 3 H, OMe), 2.56 (m, 2 H, $3eq^{a}$-H, $3eq^{b}$-H), 2.00 (s, 3 H, Ac), 1.32–1.04 (m, 66 H, 33 CH_2), 0.80 (t, 6 H, 2 Me) ppm. ^{13}C NMR (200 MHz, [D_6]DMSO): δ = 177.2, 173.9, 173.8, 171.1, 159.6, 130.1, 130.0, 129.6, 129.5, 103.3, 103.2, 103.1, 100.4, 98.9, 82.5, 81.8, 81.0, 80.6, 75.2, 74.9, 74.7, 73.8, 73.4, 73.1, 72.9, 72.7, 72.3, 71.5, 70.9, 70.6, 69.8, 69.5, 69.2, 68.7, 68.2, 67.2, 67.0, 66.6, 62.9, 61.5, 61.3, 61.2, 60.9, 60.2, 59.8, 58.0, 57.8, 52.8, 50.6, 49.9, 34.4, 32.0, 31.9, 31.6, 31.3, 29.4, 29.3, 29.2, 29.1, 29.08, 29.05, 29.02, 28.9, 28.8, 26.7, 26.6, 25.5, 24.4, 23.3, 22.1, 14.0 ppm. HRMS (ESI): calcd. for $C_{90}H_{160}N_4O_{38}$ $[M - 2H]^{2-}$ 952.5355; found 952.5350.

Neurite Outgrowth Evaluation

Cell Culture: PC-12 cells were obtained from the RIKEN Cell Bank (Tsukuba, Japan). Cells were maintained in RPMI 1640 medium (Life Technologies Japan Ltd., Tokyo, Japan) supplemented with 5% heat-inactivated fetal bovine serum (FBS; PAA Laboratories GmbH, Pasching, Austria) and 10% heat-inactivated horse serum (HS; PAA Laboratories GmbH) in 5% CO_2 at 37 °C. Cells were plated onto transparent 96-well microplates precoated with poly-L-lysine (Nacalai Tesque, Kyoto, Japan).

PC-12 Differentiation with NGF: The transparent 96-well microplates were seeded with 1×10^4 cells per well, and then cells were cultured with the RPMI medium containing both sera. After 24 h, the medium was replaced by RPMI medium with low sera (0.05% heat-inactivated FBS and 0.1% heat-inactivated HS), which was supplemented with NGF (Alomone Labs Ltd., Jerusalem, Israel) at 5 ng/mL to induce differentiation. After 3 d, the culture medium was replaced by new low-serum medium with 5 ng/mL of NGF, and then the cells were cultured for a further 2 d.

Neurite Outgrowth Evaluation: Compound **1** or **2** (10 nM) was added to the low-serum culture medium with 5 ng/mL of NGF for neurite outgrowth evaluation. Cells were fixed for 30 min using paraformaldehyde (4% in PBS; phosphate-buffered saline) at room temperature, and were then stained using toluidine blue O (Sigma–Aldrich). Morphological changes were observed with an inverted microscope (IX70, Olympus, Tokyo, Japan) with a CCD camera (DP71, Olympus). For the analysis of morphological changes, four random areas per well were selected and photographed. The lengths of the neurites and cell bodies in the image were quantified by ImageJ software (National Institutes of Health, Bethesda, USA). Measurements were carried out in triplicate. Sufficient measurements were acquired for the analysis of at least 120 cells. Cells containing neurite(s) were counted. In addition, the number of neurites per well with lengths longer than two cell-body diameters was counted, and then an average number of neurites per cell was calculated.

Acknowledgments

The iCeMS is supported by the Japanese World Premier International Research Center Initiative (WPI), MEXT. This work was financially supported in part by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) [Grant-in-Aid for Scientific Research (B), grant number 22380067 to M. K., and a Grant-in-Aid for Young Scientists (A), grant number 23688014 and a Grant-in-Aid on Innovative Areas, grant number 24110505, to H. A.]. The authors deeply thank Dr. Tomio Yabe and Ritsuko Hosoda-Yabe for their technical guidance related to the examination of neurite outgrowth. Kiyoko Ito is thanked for technical assistance.

[1] a) S. Hakomori, *Annu. Rev. Biochem.* **1981**, *50*, 733–764; b) S. Ando, *Neurochem. Int.* **1983**, *5*, 507–537; c) S. Hakomori, *J. Biol. Chem.* **1990**, *265*, 18713–18716; d) L. R. Schnaar, *Glycobiology* **1991**, *1*, 477–485; e) A. Varki, *Glycobiology* **1993**, *3*, 97–130.

[2] M. Kaneko, K. Yamada, T. Miyamoto, M. Inagaki, R. Higuchi, *Chem. Pharm. Bull.* **2007**, *55*, 462–463.

[3] a) F. H. Geisler, F. C. Dorsey, W. P. Coleman, *N. Engl. J. Med.* **1991**, *324*, 1885–1887; b) L. Svennerholm, G. Brane, I. Karlsson, A. Lekman, I. Ramström, C. Wikkelsö, *Dementia Geriatr. Cognit. Disord.* **2002**, *14*, 128–136.

[4] a) S. Hanashima, Y. Yamaguchi, Y. Ito, K. Sato, *Tetrahedron Lett.* **2009**, *50*, 6150–6153; b) S. Hanashima, K. Sato, Y. Naito, H. Takematsu, Y. Kozutsumi, Y. Ito, Y. Yamaguchi, *Bioorg. Med. Chem.* **2010**, *18*, 3720–3725.

[5] a) Y.-F. Tsai, C.-H. Shih, Y.-T. Su, C.-H. Yao, J.-F. Lian, C.-C. Liao, C.-W. Hsia, H.-A. Shui, R. Rani, *Org. Biomol. Chem.*

2012, *10*, 931–934; b) J. R. Rich, S. G. Withers, *Angew. Chem. Int. Ed.* **2012**, *51*, 8640–8643; *Angew. Chem.* **2012**, *124*, 8768–8771; c) F.-F. Xu, Y. Wang, D.-C. Xiong, X.-S. Ye, *J. Org. Chem.* **2014**, *79*, 797–802.
[6] a) H. Ando, Y. Koike, S. Koizumi, H. Ishida, M. Kiso, *Angew. Chem. Int. Ed.* **2005**, *44*, 6759–6763; *Angew. Chem.* **2005**, *117*, 6917–6921; b) Y. Iwayama, H. Ando, H. Ishida, M. Kiso, *Chem. Eur. J.* **2009**, *15*, 4637–4648.
[7] Y. Iwayama, H. Ando, H.-N. Tanaka, H. Ishida, M. Kiso, *Chem. Commun.* **2011**, *47*, 9726–9728.
[8] H. Shimizu, Y. Iwayama, A. Imamura, H. Ando, H. Ishida, M. Kiso, *Biosci. Biotechnol. Biochem.* **2011**, *75*, 2079–2082.
[9] H. Tamai, H. Ando, H.-N. Tanaka, R. Hosoda-Yabe, T. Yabe, H. Ishida, M. Kiso, *Angew. Chem. Int. Ed.* **2011**, *50*, 2330–2333; *Angew. Chem.* **2011**, *123*, 2378–2381.
[10] R. Higuchi, K. Inukai, J. X. Jhou, M. Honda, T. Komori, S. Tsuji, Y. Nagai, *Liebigs Ann. Chem.* **1993**, 359–366.
[11] H. Tamai, H. Ando, H. Ishida, M. Kiso, *Org. Lett.* **2012**, *14*, 6342–6345.
[12] a) H. Sakamoto, S. Nakamura, T. Tsuda, S. Hashimoto, *Tetrahedron Lett.* **2009**, *41*, 7691–7695; b) A. Imamura, H. Ando, H. Ishida, M. Kiso, *J. Org. Chem.* **2009**, *74*, 3009–3023; c) K. Fujikawa, T. Nohara, A. Imamura, H. Ando, H. Ishida, M. Kiso, *Tetrahedron Lett.* **2010**, *51*, 1126–1130; d) K. Fujikawa, S. Nakashima, M. Konishi, T. Fuse, N. Komura, T. Ando, H. Ando, N. Yuki, H. Ishida, M. Kiso, *Chem. Eur. J.* **2011**, *17*, 5641–5651; e) S. Nakashima, H. Ando, R. Saito, H. Tamai, H. Ishida, M. Kiso, *Chem. Asian J.* **2012**, *7*, 1041–1051; f) M. Konishi, A. Imamura, K. Fujikawa, H. Ando, H. Ishida, M. Kiso, *Molecules* **2013**, *18*, 15153–15181.
[13] E. Moreno-Clavijo, T. A. Camona, A. Moreno-Vargas, M. Rodríguez-Carvajal, I. Robina, *Bioorg. Med. Chem.* **2010**, *18*, 4648–4660.
[14] S. A. Thompson, R. G. Humphrey, M. A. DeMarco, J. D. Mathre, J. J. E. Grabowski, *J. Org. Chem.* **1993**, *58*, 5886–5888.
[15] K. C. Nicolau, E. S. Webber, *Synthesis* **1986**, *6*, 453–461.
[16] D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155–4156.
[17] L. Argenti, F. Bellina, A. Carpita, E. Rossi, R. Rossi, *Synth. Commun.* **1994**, *24*, 2281–2297.
[18] C. D. Perchonock, J. A. Finkelstein, I. Uzinskas, J. G. Gleason, H. M. Sarau, L. B. Cieslinsk, *Tetrahedron Lett.* **1983**, *24*, 2457–2460.
[19] a) T. J. Martin, R. R. Schmidt, *Tetrahedron Lett.* **1992**, *33*, 6123–6126; b) H. Kondo, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 8748–8750.
[20] a) R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212–235; *Angew. Chem.* **1986**, *98*, 213–236; b) R. R. Schmidt, J. Michel, *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731–732; *Angew. Chem.* **1980**, *92*, 763–764; K. K. Sadozai, T. Nukada, Y. Ito, Y. Nakahara, T. Ogawa, A. Kobata, *Carbohydr. Res.* **1986**, *157*, 101–123.
[21] a) Y. Oikawa, T. Yoshioka, O. Yonemitsu, *Tetrahedron Lett.* **1982**, *23*, 885–888; b) K. Horita, T. Yoshioka, T. Tanaka, Y. Oikawa, O. Yonemitsu, *Tetrahedron* **1986**, *42*, 3021–3028.
[22] W. Yu, M. Su, X. Gao, Z. Yang, Z. Jin, *Tetrahedron Lett.* **2000**, *41*, 4015–4017.
[23] L. Lázár, E. Mező, M. Herczeg, A. Lipták, S. Antus, A. Borbás, *Tetrahedron* **2012**, *68*, 7386–7399.
[24] P. J. Garegg, H. Hultberg, *Carbohydr. Res.* **1981**, *93*, C10–C11.
[25] R. V. Stick, K. A. Stubbs, *Tetrahedron: Asymmetry* **2005**, *15*, 321–335.

Received: May 12, 2015
Published Online: July 8, 2015

