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Facile Synthesis of Tumor-Associated Carbohydrate Antigen Ganglioside GM_3 from Sialic Acid, Lactose, and Serine

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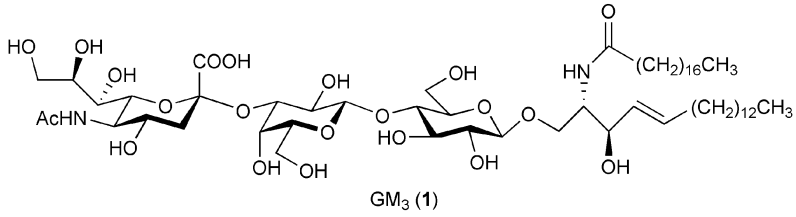
Ganglioside GM_3 [α-Neu5Ac-(2,3)-β-Gal-(1,4)-β-Glc-(1,1)-Cer; **1**] is considered as an important tumor-associated carbohydrate antigen, which can be used in the development of tumor vaccine. In this study, a facile and convergent synthetic strategy for GM_3 was developed, and the preparation of three building blocks started from the most readily available compounds sialic acid, lactose, and L-serine. Ceramide aglycon **9** was constructed from L-serine in 13 steps with 6 % overall yield, and lactosyl trichloroacetimidate **14** was synthesized from lactose in 7 steps with 25 % yield. With novel *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected *p*-toluenethiosialoside **15** as donor, which was developed by our group, the sialylation of benzoyl-protected lactosyl ceramide diol **23** was successfully accomplished in 54 % yield. Our strategy here provides a shorter linear total synthesis of GM_3 in five steps and with 26 % overall yield.

Introduction

Gangliosides, bearing neuraminic residues in their structures, comprise a big family of glycosphingolipids. It is found that gangliosides widely exist in vertebrate cells, especially in central nervous system cells, which are the essential building blocks of the plasma membrane. Many biological studies illustrate the significance of gangliosides in cell recognition, signal transduction, immunological modulation, and so on, which are involved in many aspects of cell physiological events.[1]

Ganglioside GM_3 [α-Neu5Ac-(2,3)-β-Gal-(1,4)-β-Glc-(1,1)-Cer] (**1**, Scheme 1) was first isolated from horse erythrocyte in 1952.[2] It is composed of a trisaccharide carbohydrate moiety and a long-chain ceramide tail. In recent years, GM_3 has attracted great attention as an important tumor-associated carbohydrate antigen, as it was found to be overexpressed in some tumor tissues of small-cell lung cancer, human breast cancer, melanomas, and so on, but had a more restricted distribution in normal tissues.[3] As a good target for cancer-active immunotherapy, the GM_3-based vaccine has been efficiently developed for preclinical/clinical study by several research groups.[4] In addition, GM_3 is not only the precursor to deacetyl-, lyso-, or deacetyl-lyso-GM_3 derivatives,[5] but it is also the key intermediate to further achieve more complex ganglioside analogues such as GM_1, GM_2, GD_3, and so on,[1a] which are also crucial tumor-associated carbohydrate agents.

However, one of the bottlenecks to prepare GM_3-based vaccine for biological activity assay is the limited supply of chemically pure GM_3. Although there have been reports to synthesize GM_3 by chemical or chemoenzymatic strategies[6] since the first GM_3 synthesis in 1985 by Ogawa et al.,[6i] many of these procedures suffer from drawbacks:



GM_3 (**1**)

Scheme 1. Structure of ganglioside GM_3.

(i) The chemoenzymatic preparation of GM_3 has generally been carried out on smaller scales. (ii) Despite the significant progress made in the syntheses of various sialic acid containing oligosaccharides, the chemical approaches to GM_3 are usually met with low regio- and stereoselectivity

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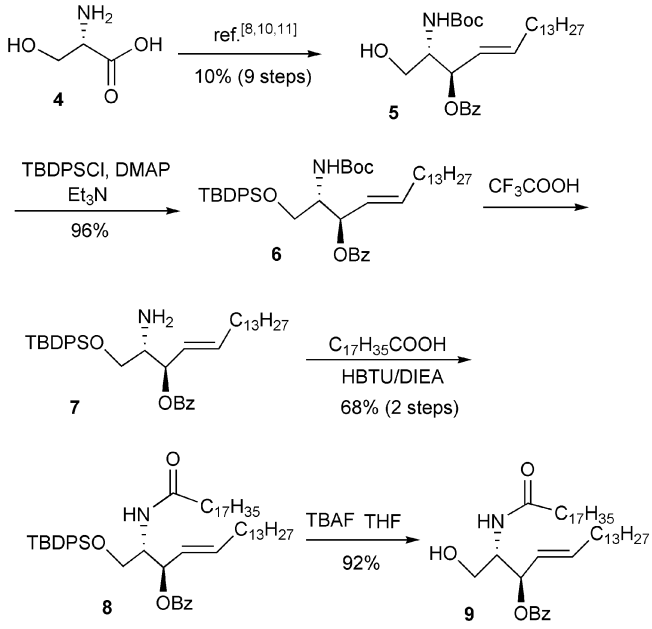
due to the harsher sialylation. (iii) The synthetic route is usually long owing to complicated protecting group manipulations. (iv) It is worth noting that the de novo synthesis of GM_3 has not yet been reported with sialic acid, lactose, and serine as the most readily available starting materials. On the basis of our previous studies on sialylation[7] and glycolipid synthesis,[8,9] and as a part of our efforts to develop novel GM_3-protein complex vaccine, we are interested in developing a general and facile strategy for the synthesis of GM_3 and its related structures.

Results and Discussion

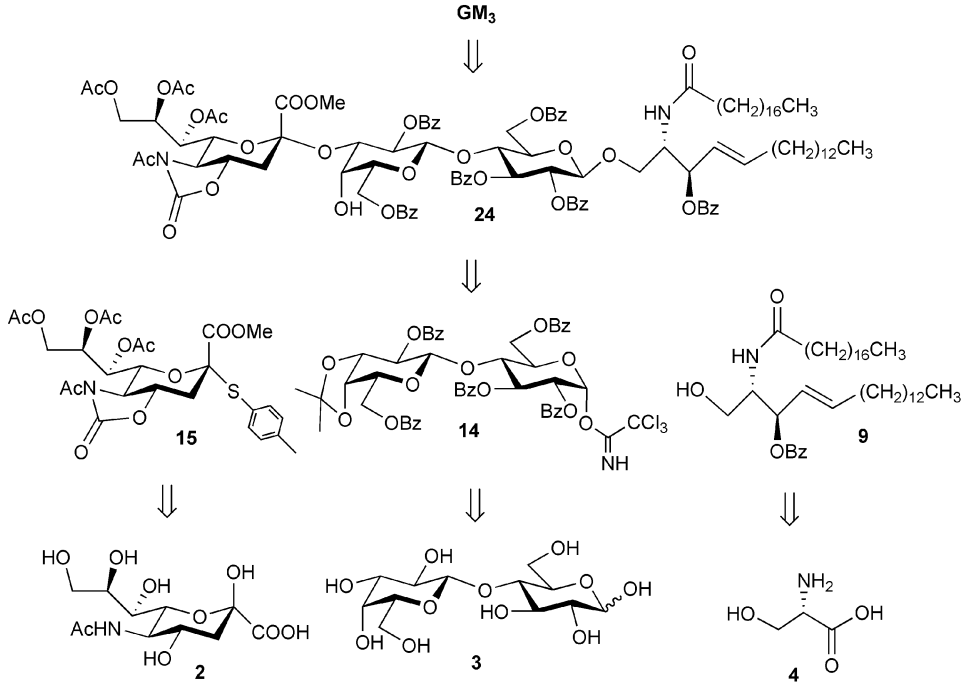
The retrosynthetic analysis of GM_3 is shown in Scheme 2. Our facile convergent synthesis starts from sialic acid, lactose, and L-serine, three relatively cheap and simple starting materials, which contain all the necessary stereochemical information located in GM_3. In order to avoid laborious and time-consuming protection–deprotection steps and to shorten the synthetic route, only acetyl or benzoyl groups were chosen for building blocks **9**, **14**, and **15**. There are two key steps for the total synthetic strategy: one involves formation of the glycosidic bond between the trisaccharide and ceramide and the other involves sialylation of lactose. To accomplish the former coupling, lactosyl trichloroacetamidate **14** was utilized in this study. For the latter glycosylation, *N*-acetyl-5-*N*,4-*O*-oxazolidinone-protected *p*-toluenethiosialoside **15**,[7] which was developed by our group, was selected as the sialyl donor.

Firstly, ceramide building block **9** was constructed from L-serine (Scheme 3). According to our previous study[8] and related works,[10,11] alcohol **5** was prepared in nine steps with 10% yield. Protection of the 1-OH in compound **5** by using TBDPS (*tert*-butyldiphenylsilyl) and cleavage of the Boc group with TFA produced amine **7**. Then, condensation of the 3-NH_2 in **7** with stearic acid in the presence of HBTU and Hünig's base, and removal of the TBDPS protection by treatment with TBAF afforded building block **9** in good yield.

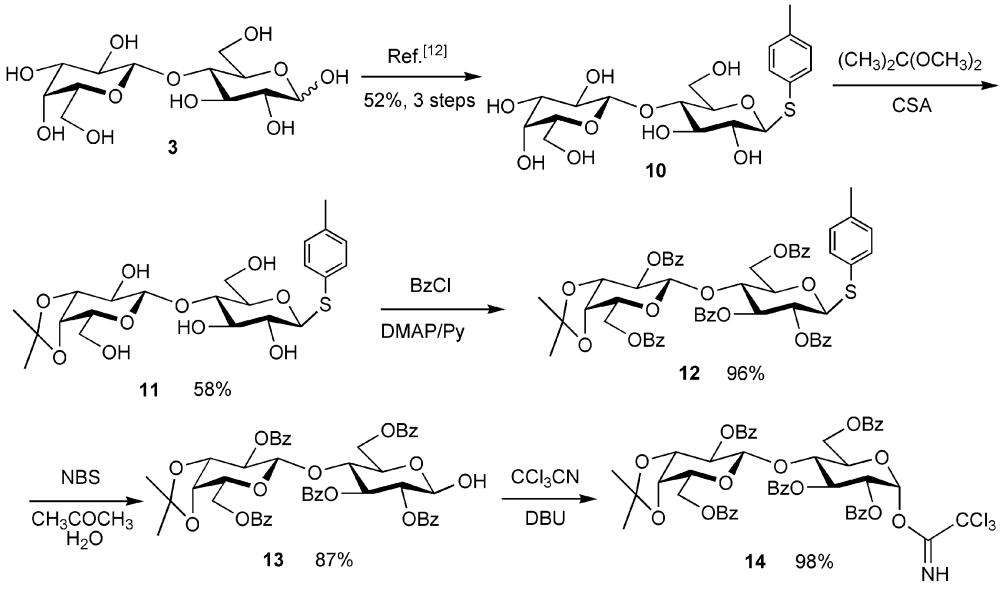


Scheme 3. Synthesis of ceramide building block **9**.

Secondly, lactose building block **14** was prepared from lactose (Scheme 4). Thioglycoside **10**[12] was treated with 2,2-dimethoxypropane containing 10-camphorsulfonic acid



Scheme 2. Retrosynthetic analysis of GM_3.



Scheme 4. Synthesis of lactose building block **14**.

(CSA) under reflux, followed by neutralization with triethylamine and concentration. The resulting mixture was dissolved in 10:1 methanol/water and then boiled for 6 h[13] to afford β-lactosyl *p*-toluenethioside **11**. Because it is possible to obtain 2,3-, 2′,3′-, or 4′,6′-acetal at the same time during the reaction, it is necessary to carefully identify the structure of **11**. In the previous study,[12] its structure was determined only by NMR spectroscopic analysis. Considering the crystal of **11** was not good enough for X-ray diffraction, herein we identified the structure of the product by X-ray crystallographic analysis of its derivative. Thus, benzoylation of **11** with the use of BzCl in pyridine, followed by removal[14] of the *p*-toluenethio group with NBS in acetone/water provided compound **13**. The solid-state structure of **13** was confirmed by single-crystal X-ray diffraction (Figure 1),[15] which illustrated that the desired 3′,4′-*O*-isopropylidene lactoside **11** had been successfully synthesized. Then, by using a routine procedure, compound **13** was easily converted into lactosyl trichloroacetamidate **14** in high yield (98%).

To efficiently build an α-Neu5Ac-(2,3)-Gal glycosidic bond is the critical point of the total synthesis of GM_3. Among many contributions to this issue, the galactose acceptor was commonly protected by a benzyl group. With various leaving groups such as halides,[6j,16] phosphates,[6d,6e,6h,17,18] xanthates,[6l,17] and so on, the corresponding sialylation was furnished in different yield (25–61%) and α-selectivity (α/β = 1:1.2 to >9.0:1) depending on different research groups. However, it is worth noting that there are only few publications detailing the use of an acyl protecting group for the galactose acceptors. The sialylation efficiency is altered with a change in the acyl type. In the case of a benzoyl protecting group, a lower glycosylation yield (16%) was obtained with diethyl sialyl phosphate.[6h] Although a higher sialylation yield (72%) was achieved with 2β-chloro-3β-phenylthio sialylate coupled

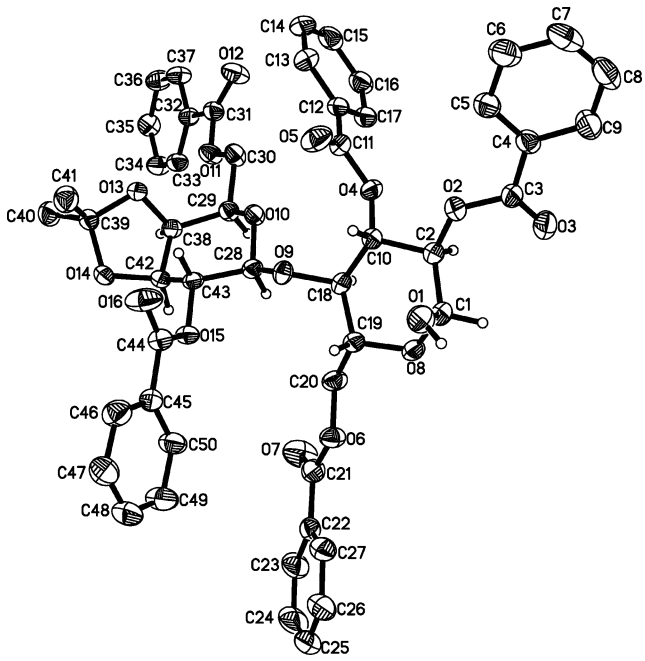


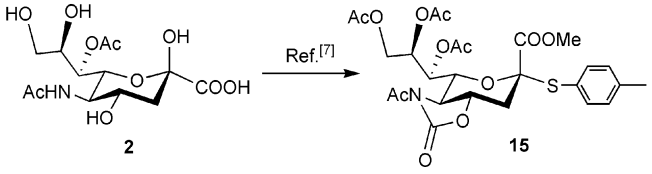
Figure 1. X-ray crystallographic structure of **13**.

with 2,6,6′-tri-*O*-pivaloylthiolactoside and no β-product was observed,[6a] the reaction temperature is higher (60 °C), which is seldom used in glycosylation reactions.

Generally, if acyl (benzoyl) was used for the protection of galactose/lactose, followed by sialylation, the whole synthetic route for GM_3 could become shorter than if benzyl was used as a protecting group. Because there is a double bond in the ceramide aglycon of GM_3, it is unfeasible to use hydrogenation conditions to remove the benzyl protecting groups in the galactose/lactose unit during the final deprotection step. Considering the benzoyl lactose derivative has

a relatively low reactivity for glycosylation, it is therefore important to find new suitable sialyl donors to overcome this problem.

Recently, we successfully designed and synthesized a novel sialic acid donor *N*-acetyl-5-*N*,4-*O*-oxazolidinone-protected *p*-toluenethiosialoside **15**,[7] which can be readily prepared from sialic acid and *p*-toluenethiol (Scheme 5). It was demonstrated that **15** could be successfully applied to various sialylations with good yields and stereoselectivities at –40 °C with CH_2Cl_2/MeCN (2:1) as reaction media. To further broaden its substrate adaptability, we found donor **15** could be efficiently coupled not only with benzyl-protected methyl galactoside diol **16**, but also with benzoyl-protected methyl galactoside diol **17** with α/β = 1.6–2.0, and the corresponding sialylation products **19** and **20** were afforded in high yields (72–89%).[7,12] On the basis of these results, a methyl glycoside of ganglioside GM_3 trisaccharide **21** was successfully synthesized from compound **15** as donor and methyl 2,3,6,2′,6′-penta-*O*-benzoyl-β-lactoside (**18**) as acceptor in a 68% yield and α/β = 1.6:1 (Figure 2).[12]



Scheme 5. Synthesis of sialyl donor **15**.

Inspired by the above results, and with building blocks **9**, **14**, and **15** in hand, a facile linear synthesis of GM_3 was carried out (Scheme 6). Condensation of 3-*O*-benzyl ceramide **9** with 3′,4′-*O*-isopropylidene-2,3,6,2′,6′-penta-*O*-benzoyl-1-*O*-α-lactosyl trichloroacetimidate (**14**) by using the promoter TMSOTf in CH_2Cl_2, afforded glycolipid **22** in 60% yield.[19] With the participation of the neighboring group (OBz) during the glycosylation step, the newly formed glycosidic bond of **22** was shown to be 1,2-*trans* on the basis of its ^{1}H NMR spectra ($J_{1'\text{-H},2'\text{-H}}$ = 7.6 Hz). Removal of the isopropylidene group of **22** led to 3′,4′-*O*-unprotected lactosyl ceramide acceptor **23** in 84% yield.

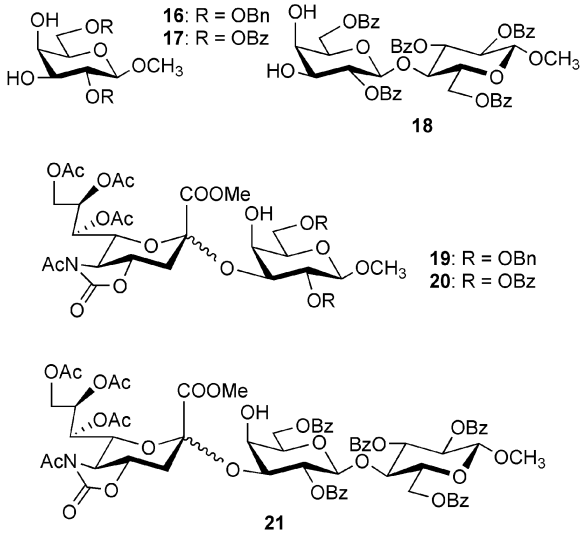
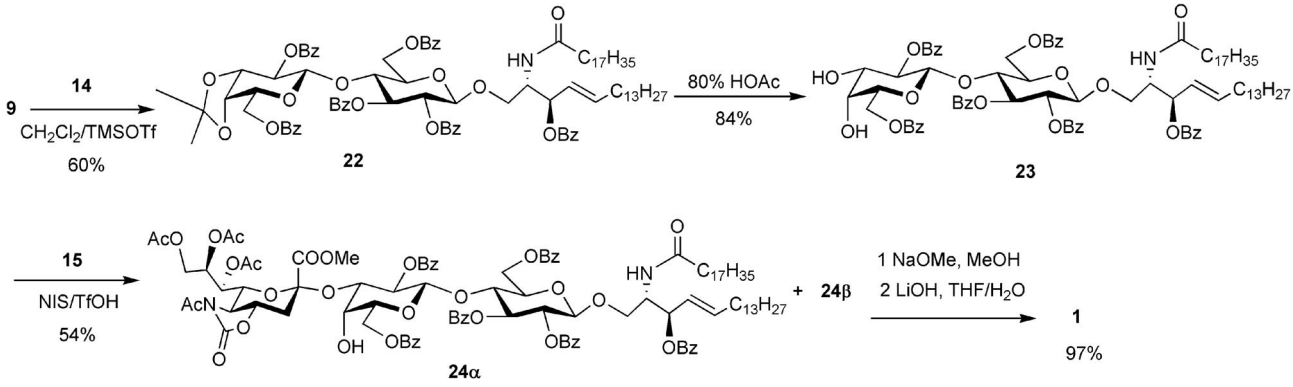


Figure 2. Structures of galactose/lactose acceptors and their sialylation products.

As mentioned above, ganglioside GM_3 trisaccharide derivative **21** was prepared from *p*-toluenethiosialoside **15** as donor. The sialylation occurred at the more reactive 3-OH of acceptor **18** and no glycosylation product with 1→4 linkage was observed. The anomeric stereochemistry of **21** was assigned on the basis of the chemical shifts of the sialic acid 3eq-H[21] and the $^3J_{\text{C-1,3ax-H}}$ coupling constant.[22] For anomer **21α**, $^3J_{\text{C-1,3ax-H}}$ = 6.0 Hz, 3eq-H = 2.74 ppm compared with that of **21β** (3eq-H = 2.62 ppm).[12] On the basis of the study of the model reaction, we finally performed the key sialylation between donor **15** and acceptor **23**. Similarly, GM_3 precursor **24** was successfully produced in 54% yield and α/β = 4:1. For anomer **24α**, 3eq-H = 2.74 ppm and for **24β** 3eq-H = 2.58 ppm. Global deprotection of all the acyl groups with sodium methoxide and saponification of the methyl ester with LiOH in THF/H_2O generated the ganglioside GM_3 in 97% yield. The physical data of synthetic **1** were in good agreement with those reported for the natural product.



Scheme 6. Linear synthesis of GM_3 **1**.

Conclusions

In summary, our strategy provides the facile and convergent total synthesis of GM_3 in a shorter linear sequence of five steps and 26% overall yield. With *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected *p*-toluenethiosialoside **15** as donor, the sialylation on benzoyl protected lactosyl ceramide diol **23** was successfully accomplished. The results of this study should be value to synthesize more complex sialylated glycolipids. The preparation of GM_3-protein conjugate for developing tumor vaccine is currently being investigated and will be reported in due course.

Experimental Section

General: All chemicals were purchased as reagent grade and used without further purification. All glycosylation reactions were performed in flame-dried glassware under an inert argon atmosphere. Dichloromethane (DCM) or acetonitrile (MeCN) were distilled from calcium hydride, and tetrahydrofuran (THF) was distilled from sodium/benzophenone. Reactions were monitored with analytical thin-layer chromatography (TLC) on silica gel F_{254} glass plates and visualized under UV light (254 nm) and/or by staining with acidic ceric ammonium molybdate. Flash column chromatography was performed on silica gel (200–300 mesh). ^{1}H NMR spectra were recorded with a 400 or 500 MHz NMR spectrometer at 20 °C. Chemical shifts (in ppm) were determined relative to tetramethylsilane (δ = 0 ppm) in deuterated solvents. Coupling constant(s) in Hertz (Hz) were measured from 1D spectra. ^{13}C Attached Proton Test (C-APT) spectra were obtained with at either 100 or 125 MHz and are calibrated with $CDCl_3$ (δ = 77.23 ppm). ESI and high-resolution mass spectra were recorded with a Bruker Apex IV FTMS or Waters LCT Premier XE mass spectrometer. Optical rotations were measured at 589 nm with a Perkin–Elmer 343 polarimeter by using a 10-cm microcell.

(2*S*,3*R*,*E*)-3-*O*-Benzoyl-2-(*tert*-butoxycarbonyl)amido-1-(*tert*-butyldiphenylsilyloxy)-4-octadecene-1,3-diol (6): To a solution of **5** (370 mg, 0.73 mmol) in DCM was added TBDPSCl (305 mg, 0.29 mL), Et_3N (0.13 mL), and DMAP (27 mg). The reaction mixture was stirred at room temperature for 6 h. The reaction was quenched by adding MeOH (0.2 mL) at 0 °C. After stirring for 10 min, the mixture was then diluted with DCM (150 mL), washed with saturated sodium hydrogen carbonate solution (3 × 30 mL) and brine (3 × 30 mL), and dried with sodium sulfate. After removal of the solvent, the mixture was purified by column chromatography (hexanes/EtOAc, 40:1) to give **6** (523 mg, 0.70 mmol, 96%) as a thick oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 3 H, CH_3), 1.05 [s, 9 H, $SiC(CH_3)_3$], 1.23–1.25 (m, 22 H, CH_2), 1.45 [s, 9 H, $OC(CH_3)_3$], 1.98–2.03 (m, 2 H, 6-H), 3.73 (dd, *J* = 10.1, 3.6 Hz, 1 H, 1a-H), 3.80 (dd, *J* = 10.2, 3.5 Hz, 1 H, 1b-H), 4.07–4.11 (m, 1 H, 2-H), 4.78 (d, *J* = 9.7 Hz, 1 H, NH), 5.47 (dd, *J* = 15.6, 7.6 Hz, 1 H, 4-H), 5.62 (t, *J* = 7.2 Hz, 1 H, 3-H), 5.88 (dt, *J* = 15.4, 7.1 Hz, 1 H, 5-H), 7.20–7.43 (m, 8 H, ArH), 7.54–7.65 (m, 5 H, ArH), 7.97–7.98 (m, 2 H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3 (OC=O), 155.3 (NC=O), 137.2 (C-5), 135.6 (2 C), 134.8, 133.1, 132.8 (2 C), 130.5, 129.8, 129.7 (2 C), 129.6 (2 C), 128.3, 127.8, 127.7, 124.7 (C-4), 79.4 [O*C*$(CH_3)_3$], 74.5 (C-3), 62.7 (C-1), 54.2 (C-2), 32.4, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 28.4, 28.3 (2 C), 26.9, 26.8, 26.6, 22.7, 19.3, 14.2 (CH_3) ppm. HRMS (ESI): calcd. for $C_{46}H_{67}NO_5SiNa$ $[M + Na]^+$ 764.4686; found 764.4663.

(2*S*,3*R*,*E*)-2-Amino-3-*O*-benzoyl-1-(*tert*-butyldiphenylsilyloxy)-4-octadecene-1,3-diol (7): Anisole (1 mL) was added to a solution of compound **6** (470 mg,0.63 mmol) in anhydrous DCM (2 mL). TFA was slowly dropped into the reaction mixture at 0 °C. The reaction mixture was stirred at 0 °C for 10 min, warmed to room temperature, and stirred for an additional 0.5 h. After complete consumption of the starting material (TLC monitoring), anhydrous ether (20 mL) was added and the solvent was evaporated, which was repeated for three times. Then, toluene (20 mL) was added and coevaporated to completely remove the remaining TFA. Crude product **7** was directly used in the next step without further purification.

(2*S*,3*R*,*E*)-3-*O*-Benzoyl-1-(*tert*-butyldiphenylsilyloxy)-2-octadecanamido-4-octadecene-1,3-diol (8): To a solution of stearic acid (272 mg,0.96 mmol) in DCM/1,4-dioxane (2:1, 4 mL) was added *O*-benzotriazole *N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU; 315 mg,083 mmol) and *N*-ethyldiisopropylamine (DIEA; 165 mg,0.22 mL,1.28 mmol). After stirring for 10 min, above crude amine was added to the mixture. The reaction was allowed to stir overnight. After complete consumption of the starting material (TLC monitoring), the solution was diluted with EtOAc (100 mL), washed with saturated sodium hydrogen carbonate solution (3 × 20 mL) and brine (2 × 30 mL), and dried with sodium sulfate. After removal of the solvent, the residue was purified by column chromatography (hexanes/EtOAc, 35:1) to give **8** (394 mg, 0.43 mmol, 68%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.91 (t, *J* = 6.9 Hz, 6 H, CH_3), 1.08 [s, 9 H, $SiC(CH_3)_3$], 1.26–1.33 (m, 50 H, CH_2), 1.60–1.63 (m, 2 H, CH_2), 2.02–2.04 (m, 2 H, 6-H), 2.14–2.18 (m, 2 H, CH_2), 3.74 (dd, *J* = 10.5, 3.9 Hz, 1 H, 1a-H), 3.92 (dd, *J* = 10.5, 3.3 Hz, 1 H, 1b-H), 4.45–4.46 (m, 1 H, 2-H), 5.52 (dd, *J* = 15.4, 7.6 Hz, 1 H, 4-H), 5.69 (t, *J* = 7.5 Hz, 1 H, 3-H), 5.77 (d, *J* = 9.4 Hz, 1 H, NH), 5.91 (dt, *J* = 15.4, 7.3 Hz, 1 H, 5-H), 7.21–8.02 (m, 15 H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.5 (OC=O), 165.3 (NC=O), 137.2 (C-5), 135.5, 135.4, 133.0, 132.9, 132.6, 130.4, 129.9, 129.8, 129.7, 128.4, 127.8, 127.7, 125.1 (C-4), 74.1 (C-3), 62.4 (C-1), 52.2 (C-2), 37.0, 32.4, 31.9, 29.7 (2 C), 29.6, 29.5 (2 C), 29.4, 29.3, 29.2, 29.0, 26.9 (3 C), 25.8, 22.7, 19.3, 14.1 (CH_3) ppm. HRMS (ESI): calcd. for $C_{59}H_{93}NO_4NaSi$ $[M + Na]^+$ 930.6772; found 930.6792.

(2*S*,3*R*,*E*)-3-*O*-Benzoyl-2-octadecanamido-4-octadecene-1,3-diol (9): To a solution of compound **8** (340 mg,0.37 mmol) in THF (6 mL) cooled to 0 °C was added a solution of TBAF (1.13 M in THF, 1 mL) within 5 min. After stirring for 10 min, the mixture was warmed to room temperature and stirred for an additional 30 min. After complete consumption of the starting material (TLC monitoring), the reaction was quenched with saturated sodium hydrogen carbonate solution (10 mL) and diluted with DCM (150 mL), washed with saturated sodium hydrogen carbonate solution (3 × 50 mL) and brine (3 × 50 mL), and dried with sodium sulfate. After removal of the solvent, the remainder was purified by column chromatography (hexanes/EtOAc, 6:1) to yield **9** (226 mg, 0.34 mmol, 92%) as a white solid. ^{1}H NMR (400 MHz $CDCl_3$): δ = 0.88 (t, *J* = 7.0 Hz, 6 H, CH_3), 1.25 (m, 50 H, CH_2), 1.58–1.65 (m, 2 H, CH_2), 2.02–2.07 (m, 2 H, 6-H), 2.13–2.23 (m, 2 H, CH_2), 3.68 (dd, *J* = 12.0, 2.9 Hz, 1 H, 1a-H), 3.74 (dd, *J* = 12.0, 3.5 Hz, 1 H, 1b-H), 4.24–4.31 (m, 1 H, 2-H), 5.52 (t, *J* = 7.6 Hz, 1 H, 3-H), 5.61 (dd, *J* = 15.2, 7.6 Hz, 1 H, 4-H), 5.85 (dt, *J* = 14.9, 6.8 Hz, 1 H, 5-H), 6.07 (d, *J* = 8.4 Hz, 1 H, NH), 7.45–8.05 (m, 5 H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.4 (NC=O), 166.6 (OC=O), 137.5 (C-5), 133.5, 129.8, 129.7, 128.9, 124.9, 124.7 (C-4), 74.7 (C-3), 61.9 (C-1), 53.5 (C-2), 36.9, 32.3, 32.0, 29.7 (3C), 29.6, 29.5 (2C), 29.4 (2C), 29.3 (2C), 28.9, 25.8, 22.7, 14.1 (CH_3) ppm. HRMS (ESI): calcd. for $C_{43}H_{76}NO_4$ $[M + 1]^+$ 670.5774; found 670.5757.

2,3,6,2′,6′-Penta-*O*-benzoyl-3′,4′-*O*-isopropylidene-1-*O*-β-lactose (13): To a solution of **12**[12] (1.0 g, 0.99 mmol) in 90% acetone aqueous solution (20 mL) was added *N*-bromosuccinimide (0.72 g, 4.0 mmol), and the solution was stirred for 1 h at room temperature. After complete consumption of the starting material (TLC monitoring), the reaction was quenched with saturated sodium hydrogen carbonate solution (2 mL) and diluted with DCM (150 mL), washed with saturated sodium bisulfite (3 × 30 mL), sodium hydrogen carbonate solution (3 × 30 mL), and brine (3 × 30 mL), and dried with sodium sulfate. After removal of the solvent, the crude product was recrystallized (hexanes/EtOAc, 6:1) to afford **13** (0.78 g, 0.86 mmol, 87%) as granular-crystalline. M.p. 188–190 °C. $[\alpha]_D^{20}$ = +80.5 (*c* = 0.6, CH_2Cl_2). ^{1}H NMR (400 MHz $CDCl_3$): δ = 1.26 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.74 (s, 1 H, OH), 3.85–3.91 (m, 2 H, 6a′-H, 6b′-H), 4.12 (d, *J* = 5.6 Hz, 1 H, 5′-H), 4.20 (t, *J* = 9.7 Hz, 1 H, 4-H), 4.27 (t, *J* = 6.9 Hz, 1 H, 3′-H), 4.33 (m, 1 H, 5-H), 4.39 (d, *J* = 9.7 Hz, 1 H, 1-H), 4.52 (d, *J* = 12.2 Hz, 1 H, 6a-H), 4.61 (d, *J* = 12.3 Hz, 1 H, 6b-H), 4.70 (d, *J* = 7.6 Hz, 1 H, 1′-H), 5.15–5.20 (m, 2 H, 2-H, 2′-H), 5.58 (br. s, 1 H, 4′-H), 6.08 (t, *J* = 9.8 Hz, 1 H, 3-H), 7.30–7.62 (m, 15 H, ArH), 7.96–8.09 (m, 10 H, ArH) ppm. ^{13}C NMR (100 MHz $CDCl_3$): δ = 166.1, 166.0, 165.9, 165.6, 165.0 (C=O × 5), 133.4, 133.3, 133.2, 133.1, 132.9, 130.2, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.1, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 110.9 [$C(Me)_2$], 100.1 (C-1′), 90.4 (C-1), 77.2, 75.5, 73.7, 73.2, 72.3, 71.4, 69.6, 68.7, 63.1 (C-6), 62.6 (C-6′), 27.4 (CH_3), 26.2 (CH_3) ppm. HRMS (ESI): calcd. for $C_{50}H_{47}O_{16}$ $[M + 1]^+$ 903.2864; found 903.2874.

2,3,6,2′,6′-Penta-*O*-benzoyl-3′,4′-*O*-isopropylidene-1-*O*-α-lactosyl trichloroacetimidate (14): To a solution of **13** (0.70 g, 0.77 mmol) dissolved in anhydrous DCM (6 mL) was added CCl_3CN (0.8 mL, 7.7 mmol) and DBU (56 μL, 0.39 mmol). After 1 h at room temperature, the dark solution was concentrated and then purified by flash chromatography (hexanes/EtOAc/DCM, 6:1:1, containing 1% triethylamine) to yield **14** (0.80 g, 0.76 mmol, 98%) as a white solid. ^{1}H NMR (400 MHz $CDCl_3$): δ = 1.25 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 3.77–3.82 (m, 2 H, 6a′-H, 6b′-H), 4.10–4.15 (m, 1 H, 5′-H), 4.23–4.34 (m, 4 H, 2-H, 4-H, 5-H, 3′-H), 4.51 (dd, *J* = 12.3, 1.5 Hz, 1 H, 6a-H), 4.58 (d, *J* = 12.7 Hz, 1 H, 6b-H), 4.69 (d, *J* = 7.3 Hz, 1 H, 1′-H), 5.16 (t, *J* = 6.8 Hz, 1 H, 2′-H), 5.49 (dd, *J* = 10.0, 3.4 Hz, 1 H, 4′-H), 6.09 (t, *J* = 8.7 Hz, 1 H, 3-H), 6.69 (d, *J* = 3.1 Hz, 1 H, 1′-H), 7.30–7.65 (m, 15 H, ArH), 7.92–8.08 (m, 10 H, ArH), 8.54 (s, 1 H, Cl_3C-C=NH) ppm. ^{13}C NMR (100 MHz $CDCl_3$): δ = 165.9, 165.7, 165.5, 165.3, 165.0 (C=O × 5), 160.7 (C=N), 133.6, 133.5, 133.4, 133.3 (2 C), 133.1, 133.0, 129.9, 129.8, 129.7, 129.5 (2 C), 129.3, 128.6 (2 C), 128.5, 128.4 (2 C), 110.8 [$C(Me)_2$], 100.7 (C-1′), 93.1 (C-1), 77.0, 75.1, 73.7, 73.0, 71.4, 71.3, 70.6, 70.1, 62.8 (C-6), 62.1 (C-6′), 27.3 (CH_3), 26.1 (CH_3) ppm.

(2,6-Di-*O*-Benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (22): A solution of trichloroacetimidate **14** (160 mg, 0.15 mmol) and ceramide derivative **9** (67 mg, 0.10 mmol) in anhydrous DCM (8 mL) was added over freshly dried powdered 4 Å molecular sieves and cooled to –20 °C. TMSOTf (7.2 μL, 0.04 mmol) was slowly added to the solution, and the mixture was stirred at –20 °C for 1.5 h. The reaction was quenched by addition of Et_3N (0.1 mL), and the mixture was diluted with DCM and filtered through Celite. The organic layer was washed with brine (3 × 30 mL) and dried with sodium sulfate and concentrated. The residue was purified by column chromatography on silica gel (hexanes/EtOAc, 6:1) to furnish **22** (80 mg, 0.051 mmol, 60% based on consumed acceptor **9**) as a white solid, and recovered **9** (10 mg). ^{1}H NMR (400 MHz $CDCl_3$): δ = 0.88 (t, *J* = 6.9 Hz, 6 H, CH_3), 1.20–1.25 (m, 50 H), 1.60–1.82 (m, 4 H, CH_2), 1.91–1.94 (m, 2 H, 6-H), 3.52 (dd, *J* = 9.7, 3.4 Hz, 1 H, 1a-H), 3.71–3.76 (m, 3 H, 1b-H, 6a′′-H, 6b′′-H), 4.06–4.41 (m, 8 H, 2-H, 2′-H, 4′-H, 5′-H, 6a′-H, 6b′-H, 3′′-H, 5′′-H), 4.55 (d, *J* = 7.6 Hz, 1 H, 1′-H), 4.59 (d, *J* = 7.8 Hz, 1 H, 1′′-H), 5.12 (t, *J* = 7.2 Hz, 1 H, 2′′-H), 5.36–5.43 (m, 2 H, 4-H, 4′′-H), 5.47 (t, *J* = 7.5 Hz, 1 H, 3-H), 5.61 (d, *J* = 9.1 Hz, 1 H, NH), 5.70–5.80 (m, 2 H, 5-H, 3′-H), 7.28–7.64 (m, 18 H, ArH), 7.88–8.08 (m, 12 H, ArH) ppm. ^{13}C NMR (100 MHz $CDCl_3$): δ = 172.6 (NC=O), 165.9, 165.8, 165.5, 165.3, 165.1, 164.9 (6 × C=O), 137.3 (C-5), 133.3, 133.2, 133.1, 133.0, 132.9, 132.8, 130.9, 130.2, 129.9, 129.8, 129.3, 129.1, 128.8, 128.6, 124.9 (C-4), 110.8 [$C(Me)_2$], 101.0 (C-1′′), 100.2 (C-1′), 77.0, 75.2, 74.1, 73.5, 72.3, 67.7 (C-1), 62.9 (C-6′), 62.5 (C-6′′), 50.5 (C-2), 36.5, 32.3, 32.0, 29.7, 29.6 (2 C), 29.5 (2 C), 29.4, 29.2, 29.1, 28.9, 27.9, 26.8, 25.5, 22.7, 14.3 (CH_3) ppm. HRMS (ESI): calcd. for $C_{93}H_{119}NO_{19}Na$ $[M + Na]^+$ 1576.8274; found 1576.8259.

(2,6-Di-*O*-Benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (23): A solution of **22** (80 mg, 0.051 mmol) in AcOH/H_2O (80%, 2 mL) was heated to 70 °C for 5 h. After complete consumption of the starting material (TLC monitoring), the solvent was evaporated, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc, 2:1) to provide **23** (65 mg, 0.043 mmol, 84%) as a white solid. ^{1}H NMR (400 MHz $CDCl_3$): δ = 0.88 (t, *J* = 6.7 Hz, 6 H, CH_3), 1.20–1.25 (m, 52 H), 1.75–1.78 (m, 2 H, CH_2), 1.91–1.96 (m, 2 H, 6-H), 3.45 (t, *J* = 6.2 Hz, 1 H, 3′′-H), 3.54 (dd, *J* = 9.7, 3.2 Hz, 1 H, 1a-H), 3.58–3.63 (m, 1 H, 5′-H), 3.66 (dd, *J* = 9.4, 1.9 Hz, 1 H, 1b-H), 3.72–3.78 (m, 2 H, 6a′′-H, 6b′′-H), 3.97–4.01 (m, 1 H, 5′′-H), 4.07–4.12 (m, 2 H, 6a′-H, 6b′-H), 4.32–4.42 (m, 3 H, 2-H, 1′-H, 1′′-H), 4.54–4.58 (m, 2 H, 2′-H, 4′-H), 5.21–5.26 (m, 1 H, 2′′-H), 5.37–5.42 (m, 2 H, 4-H, 4′′-H), 5.47 (t, *J* = 7.4 Hz, 1 H, 3-H), 5.61–5.68 (m, 2 H, 3′-H, NH), 5.77 (dt, *J* = 15.2, 7.1 Hz, 1 H, 5-H), 7.28–7.62 (m, 18 H, ArH), 7.88–8.05 (m, 12 H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6 (NC=O), 166.3, 166.0, 165.8, 165.7, 165.3, 165.1 (6 × C=O), 137.4 (C-5), 133.5, 133.4, 133.3, 133.2, 132.8, 130.1, 129.9, 129.8 (2 C), 129.7, 129.6 (2 C), 129.5 (2 C), 129.4, 129.1, 129.0, 128.6, 128.5 (2 C), 128.4, 128.2, 124.7 (C-4), 100.8 (C-1′′), 100.7 (C-1′), 75.8, 74.0, 73.5, 73.0, 72.6 (2 C), 72.0, 68.6, 67.6 (C-1), 62.5 (C-6′), 61.9 (C-6′′), 50.4 (C-2), 36.5, 32.3, 31.9, 29.7 (2 C), 29.6 (2 C), 29.5, 29.4, 29.3, 29.2, 28.9, 25.5, 22.7, 14.2 (CH_3) ppm. HRMS (ESI): calcd. for $C_{90}H_{115}NO_{19}Na$ $[M + Na]^+$ 1536.7961; found 1536.7893.

(Methyl 5-Acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,*E*)-3-*O*-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (24): A solution of sialyl donor **15** (21 mg, 0.036 mmol) and **23** (36 mg,0.024 mmol) in DCM (2 mL) and MeCN (1 mL) was added over freshly dried powdered 4 Å molecular sieves (100 mg). The resulting mixture was stirred for 0.5 h at room temperature under an argon atmosphere and then cooled to –50 °C and stirred for an additional 0.5 h, followed by addition of NIS (12.5 mg,0.053 mmol). After 5 min, TfOH (2 μL,0.024 mmol) was added through a syringe. The reaction was stirred at –50 °C for 2 h. After complete consumption of the starting material (TLC monitoring), the reaction was quenched by addition of Et_3N (0.1 mL), and the mixture was diluted with DCM and filtered through Celite. The organic layer was washed with 20% aqueous NaS_2O_3 solution and brine, dried with sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (hexanes/EtOAc, 2:1) to give **24** (25 mg, 0.013 mmol, 54%,

α/β = 4:1) as a glassy solid. Data for the α-anomer: ^{1}H NMR (400 MHz $CDCl_3$): δ = 0.88 (t, J = 6.6 Hz, 6 H, CH_3), 1.07–1.36 (m, 50 H, CH_2), 1.53 (s, 3 H, Ac), 1.68–1.75 (m, 4 H, CH_2), 1.91 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 2.00–2.06 (m, 3 H, CH_2, 3ax′′′-H), 2.41 (s, 3 H, Ac), 2.74 (dd, J = 12.4, 3.2 Hz, 1 H, 3eq′′′-H), 3.46–4.07 (m, 13 H, 1a-H, 1b-H, 4′-H, 5′-H, 6a′-H, 6b′-H, 2′′-H, 4′′′-H, 5′′′-H, 8′′′-H, OCH_3), 4.11–4.16 (m, 1 H, 3′′-H), 4.37–4.58 (m, 8 H, 2-H, 3′-H, 1′′-H, 6a′′-H, 6b′′-H, 6′′′-H, 9a′′′-H, 9b′′′-H), 4.74 (d, J = 7.9 Hz, 1 H, 1′-H), 5.29–5.47 (m, 5 H, 3-H, 4-H, 2′-H, 5′′-H, 7′′′-H), 5.62 (d, J = 8.8 Hz, 1 H, NH), 5.66–5.78 (m, 2 H, 5-H, 4′′-H), 7.26–7.58 (m, 18 H, ArH), 7.87–8.13 (m, 12 H, ArH) ppm. ^{13}C NMR (100 MHz $CDCl_3$): δ = 171.6 (NC=O), 170.6, 169.7, 169.0, 168.9 (4 × MeC=O), 167.5 (C=OOMe), 164.8, 164.7, 164.4, 164.3, 164.1, 163.7 (6 × PhC=O), 152.3 (NC=OO), 136.2 (C-5), 132.3, 131.9, 131.7, 129.2, 129.1, 129.0, 128.9, 128.8 (2 C), 128.7, 128.6, 128.5 (2 C), 128.4, 128.1, 127.5, 127.4, 127.3, 127.2, 123.8 (C-4), 99.8 (C-1′′), 99.6 (C-1′), 96.2 (C-2′′′), 74.3, 74.2, 73.8, 73.1, 72.9, 72.2, 71.6, 71.1, 70.8, 70.3, 69.5, 67.2, 66.5 (C-1), 65.6, 62.2 (C-9′′′), 61.5 (C-6′), 61.2 (C-6′′), 57.7, 52.1, 49.4, 35.4, 34.8, 31.2, 30.9, 28.7, 28.6, 28.5 (3 C), 28.3 (2 C), 28.2 (2 C), 27.9, 24.5, 23.5, 21.7, 19.9, 19.7, 19.2, 13.1 (CH_3) ppm. MS (ESI): m/z = 1994 $[M + Na]^+$.

(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylicacid)-(2→3)-β-galactopyranosyl-(1→4)-β-glucopyranosyl-(1→1)-(*2S*,*3R*,*E*)-2-octadecanamido-4-octadecene-1,3-diol (1): To a solution of **24α** (7 mg, 3.5 μmol) in anhydrous methanol (0.5 mL) was dropwise added a solution of sodium methoxide (5 mg NaOMe in 1 mL MeOH) in an ice bath, and this mixture was stirred at 0 °C for 2 h. Then, the reaction was warmed to room temperature and stirred overnight. Dowex (H^+) resin was added to neutralize the base. The resin was filtered off and washed several times sequentially with methanol and MeOH/DCM (1:1). The combined filtrate was concentrated, and the resultant residue was dissolved in THF/H_2O (3:1, 2 mL) at 0 °C. To this solution was added $LiOH·H_2O$ (5 mg). After stirring at 0 °C for 30 min, the mixture was neutralized with Dowex (H^+) resin. Following removal of the resin, the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:5) to obtain **1** (4 mg, 3.5 μmol, 97%) as a white solid. $[\alpha]_D^{20}$ = +2.7 (c = 0.2; $CHCl_3$/MeOH, 1:1) {ref.[6h] $[\alpha]_D^{20}$ = +1.9 (c = 0.2; $CHCl_3$/MeOH, 1:1)}. The ^{1}H NMR spectroscopic data of the compound were in agreement with those reported in Ref.[20]

Supporting Information (see also the footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of all the new compounds.

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