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Total Synthesis of a Glycoglycerolipid from *Meiothermus taiwanensis* through a One-Pot Glycosylation Reaction and Exploration of its Immunological Properties

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Abstract: The total synthesis of a glycoglycerolipid isolate of *Meiothermus taiwanensis* and its truncated structural analogues is reported. Our synthesis employed DMF-modulated and low-concentration glycosylation reactions for the construction of α- and β-glycosidic bonds in the absence of participating protecting groups. Further simplification of the synthesis was achieved by employing a low-concentration one-pot glycosylation procedure. Preliminary immunological studies showed that one of the truncated structural analogues suppressed the cytokine production of THP-1 monocytes.

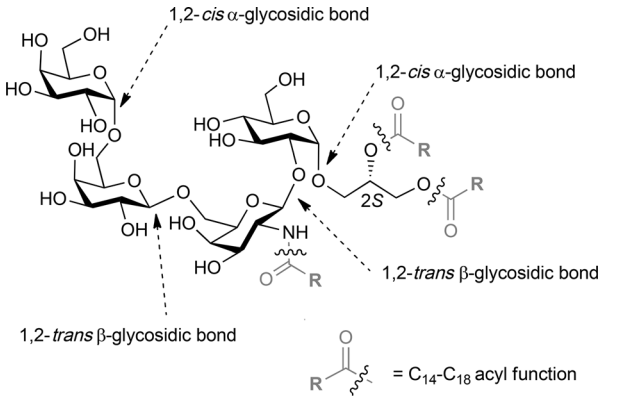
Keywords: glycosylation · immunology · lipids · natural products · total synthesis

Introduction

In nature, there is an inexhaustible pool of compounds for exploration. Studies on the physiochemical and biological properties of newly discovered compounds provide inspiration for the design of new therapeutic agents and materials. For example, investigations of natural cerebrosides that were isolated from marine sponges led to the discovery of synthetic glycolipid KRN7000, a potent immunostimulating agent that has exhibited bioactivity against a range of pathogens.[1]

Meiothermus taiwanensis ATCC BAA-400 is a Gram-negative bacteria that was isolated in Taiwan.[2] Biochemical studies revealed the presence of abundant phosphoglycolipid (PGL) and glycoglycerolipid (GL) molecules in the cell membrane of *M. taiwanesis*. Such amphiphobic molecules play an important role in maintaining the thermal stability of the bacterial membrane, in particular in harsh environments.[3,4] A few examples of phosphoglycolipids from *M. taiwanensis*[5] and other *T. thermophilus*[6] have been investigated in the induced production of proinflammatory cytokines; however, little is known of the immunological properties of glycoglycerolipids. Consequently, it is highly desirable to develop a practical synthetic route to glycoglycerolipids from *M. taiwanesis* species, as well as their relevant structural analogues, to pave the way for exploration of their biological properties.

The general structure of the glycoglycerolipids from *M. taiwanensis* comprises a α-D-Gal-(1→6)-β-D-Gal-(1→6)-β-D-GalNacyl-(1→2)-D-Glc tetrasaccharide core that is connected to the 3-hydroxy function of a *sn*-glycerol through a 1,2-*cis* α-glycosidic linkage (Scheme 1).[3] The remaining hydroxy functions of the glycerol and the amino group of the galactosamine connect to fatty acids through ester and amide bonds, respectively. The chain length of the fatty acid varies from 14 to 18 carbon atoms. In addition to variation in the chain length, different isomeric forms of the fatty



Scheme 1. Structure of the glycoglycerolipid isolate from *Meiothermus taiwanensis* ATCC BAA-400.

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acids are present. Owing to the high heterogeneity of the lipids in the glycoglycerolipids, the isolation of homogeneous samples from the natural bacterial source is almost impossible. Accordingly, chemical synthesis provides a practical entry to pure glycoglycerolipid samples for research studies.

Over the past decade, numerous publications concerning the synthesis of glycolipids have been reported.[5–9] The length of the synthetic route depended on the complexity of the carbohydrate and algycone components. If an oligosaccharide fragment was present, additional protecting-group manipulations were required for stereochemical control of the glycosidic-bond formation and post-glycosylation modification, which affected the overall yields of the syntheses.[7b,8,9] In 2007, Wu and co-workers reported a one-pot synthesis of the fully protected tetrasaccharide core of the glycoglycerolipids of *M. taiwanensis*,[10] although the stereochemical control in this work was moderate and the total synthesis of the complete glycoglycerolipid molecule was not accomplished. Herein, we report the first total synthesis of a glycoglycerolipid from *M. taiwanensis*, as well as its truncated structural analogues. Furthermore, the immunological properties of these molecules will be discussed.

Results and Discussion

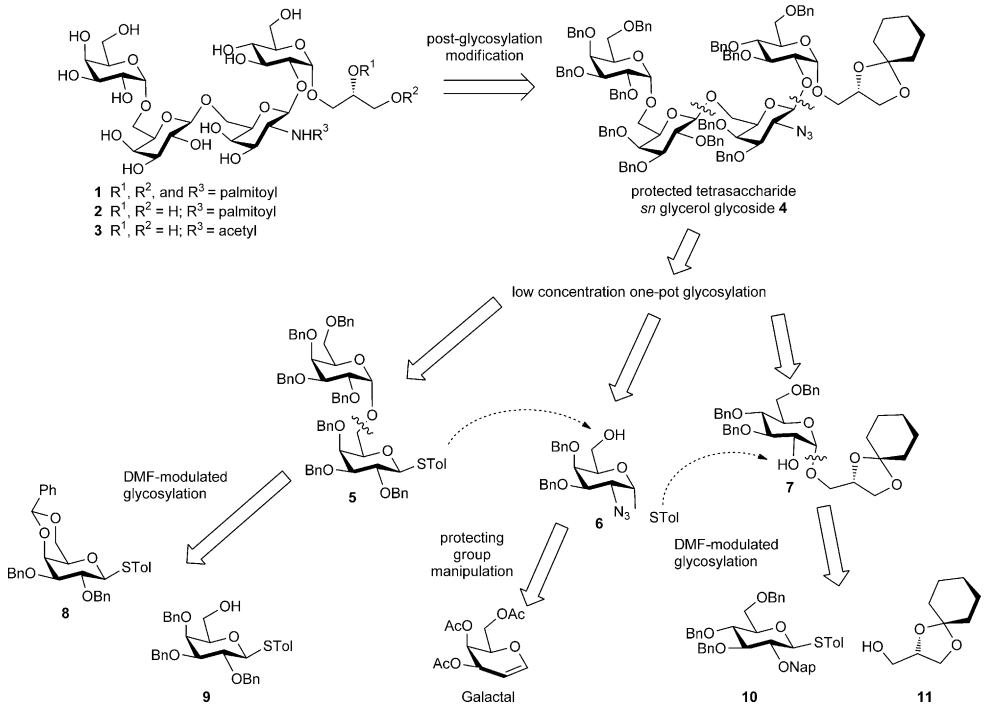
General Considerations

We chose glycoglycerolipid **1**, which contained three palmitic acid chains, as our target (Scheme 2). During the course of the synthesis, two truncated structural analogues, namely 2′-palmitamido tetrasaccharide **2** and 2′-acetamido tetrasaccharide **3**, were also prepared, which contained one and no palmitic acid chains, respectively.

Before laying out a synthetic plan, we considered some potential issues in the synthesis of the target glycoglycerolipids. The targets contain two 1,2-*cis* α-glycosidic linkages, one connecting the non-reducing-end galactosyl residues and the other connecting the reducing-end glucoside unit to the *sn*-glycerol, and their formation is generally thought to be challenging. Although several α-selective glycosylation methods have been developed, most of them invoked the use of specific protecting groups, which required additional synthetic steps for their installation and removal.[11] Because we aimed to develop a shorter synthetic scheme, we considered that DMF-modulated glycosylation may be useful for the construction of the α-glycosidic bonds in this target compound because this method employs DMF as a stereodirecting agent.[12]

In general, racemization of the C2 chiral center of *sn*-glycerol occurs frequently in the synthesis of glycoglycerolipids. Such a side reaction is attributed to the migration of hydroxy protecting functions in *sn*-glycerol.[7a,10,13] We addressed this migration issue by protecting the *sn*-glycerol moiety with a cyclohexylidene acetal group, which is known to resist migration.[8a,14] The challenge facing the total synthesis of glycoglycerolipids is the long synthetic route for the assembly of the oligosaccharide component and post-glycosylation modification. To simplify the assembly of the tetrasaccharide core, the low-concentration one-pot glycosylation method is employed.[15]

Based on a consideration of these factors, a retrosynthetic analysis of the glycoglycerolipid target was proposed



Scheme 2. Retrosynthetic analysis of a selected glycoglycerolipid of *Meiothermus taiwanensis* (**1**) and its truncated structural derivatives (**2** and **3**), building blocks (**5**–**11**), and fully protected tetrasaccharide core (**4**). Bn = benzyl, Tol = tolyl, Ac = acetyl.

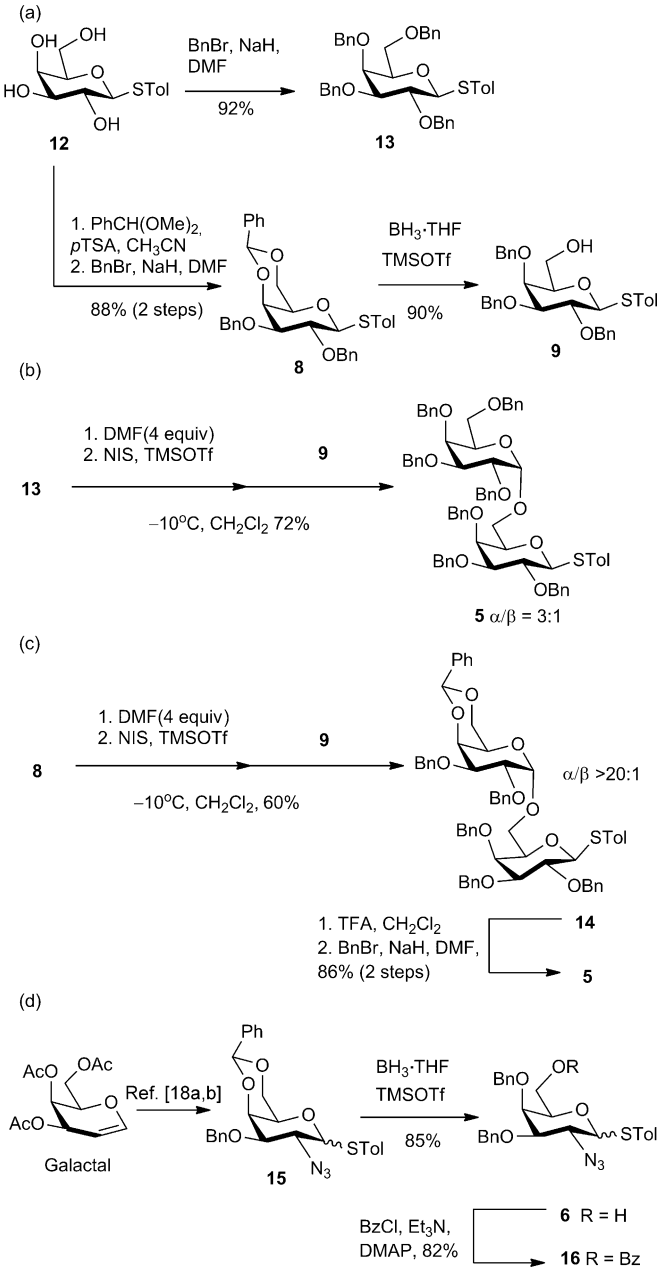
(Scheme 2). The target compounds (**1**–**3**) would be obtained from a fully protected tetrasaccharide core (**4**). Disconnection of compound **4** at the β-glycosidic junctions would afford three glycosyl building blocks, namely α-Gal-(1→6)-Gal disaccharide thioglycoside **5**, 2-azido-2-deoxy-thiogalactoside ($GalN_3$) **6**, and α-glucosyl glycerol building block **7**. Disaccharide building block **5** would be prepared from thiogalactosides **8** and **9** and glucosyl building block **7** would be prepared from thioglucoside **10** and *sn*-glycerol aglycon **11**. The $GalN_3$ thioglycoside (**6**) was proposed to be synthesized from known per-*O*-acetyl galactal.[16] Notably, no participating protecting groups would be present on these glycosyl units, which would facilitate the post-glycosylation-modification and global-deprotection steps.

Glycosyl building blocks **6** and **8**–**10** contained a thioacetal anomeric leaving group, which is known to be stable under a diverse range of reaction conditions.[17] Owing to the presence of an ester group in glycoglycerolipid **1**, the thioglycosyl building blocks for the assembly of tetrasaccharide **4** did not contain any ester-type protecting groups. If such functions had been present, they would have had to be modified before the introduction of the fatty acid component. To avoid this redundant process, non-participation thioglycosyl building blocks **5** and **6** were employed for the formation of the 1,2-*trans* β-glycosidic bond through the low-concentration glycosylation (LCG) method.[15]

Preparation of Glycosyl Building Blocks 5–7

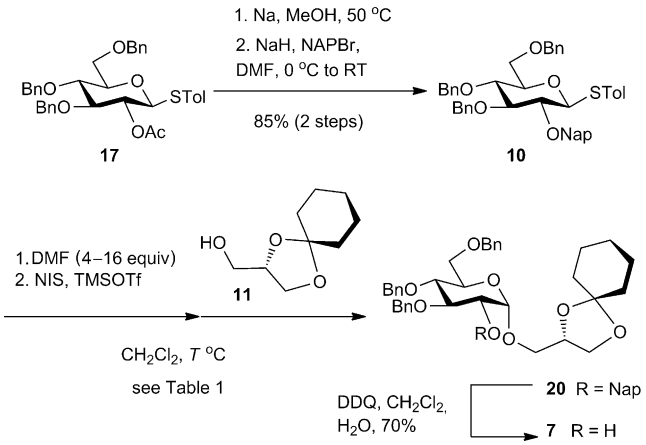
Our synthesis commenced with the preparation of the α-Gal-(1→6)-Gal disaccharide building block (**5**). Two synthetic routes were examined from thiogalactoside building blocks **8**, **9**, and **13**; these building blocks were readily prepared from a known thiogalactoside (**12**) by employing literature procedures (Scheme 3a).[15b] In the first attempt, per-*O*-benzyl-protected thiogalactoside donor **13** was coupled with thiogalactoside **9** through a DMF-modulated glycosylation method.[12] The expected disaccharide (**5**) was obtained in 72% yield, but only in a moderate 3:1 α/β anomeric ratio (Scheme 3b). In the second attempt, 4,6-*O*-benzylidene-acetal-protected thiogalactoside **8** was employed as the donor because it has been used to give higher α-selectivities for glycosylation reactions in a modulated-glycosylation context. Pleasingly, the desired α-anomer of disaccharide **14** was produced in excellent (>20:1) α/β ratio and in 60% yield (Scheme 3c). The α-configuration of compound **14** was confirmed by the chemical shift of the anomeric proton at $\delta = 4.77$ ppm and the ^3J(H,H) coupling constant of 2.4 Hz.[18] The subsequent transformation of compound **14** into per-*O*-benzyl-protected disaccharide **5** was readily accomplished by 1) removal of the acetal function under acidic conditions and 2) standard benzylation.

With disaccharide building block **5** in hand, we prepared 2-azido-2-deoxy thiogalactoside building block **6**. Previous studies have shown that non-participating 2-azido-2-deoxy-thioglycosides could be used for the formation of β-glycosides under LCG conditions.[19] Thus, a known galactal was converted into 2-azido-2-deoxy-thiogalactoside intermediate **15** according to literature procedures.[16,20] The treatment of compound **15** with BH_3·THF and TMSOTf at 0°C furnished the desired building block (**6**) in 85% yield (Scheme 3d).[21] In addition, the C6 hydroxy group of compound **6** was protected with a benzoyl group to give fully protected 2-azido-2-deoxy-thiogalactoside **16**, which would be used as a model donor for evaluation of the β-selectivity of the LCG method.



Scheme 3. a) Preparation of thiogalactoside building blocks **8**, **9**, and **13**. b) Preparation of disaccharide building block **5** from a glycosylation reaction between compounds **9** and **13** by using a DMF-modulated glycosylation method. c) Preparation of disaccharide building block **5** from compounds **9** and **8**. d) Preparation of 2-azido-2-deoxy-thiogalactoside building block **6** from a known galactal. Bz = benzoyl, Nap = 2-napthylmethyl.

The synthesis of 3-*O*-(α-glucosyl)-*sn*-glycerol **7** began with known thioglucoside **17**. Thus, thioglucoside **17** underwent deacetylation and alkylation with naphthylmethylene bromide (NAP-Br) to furnish 2-*O*-NAP-protected thioglucoside **10** in 85% yield (Scheme 4). Subsequently the coupling of



Scheme 4. Preparation of the reducing-end α-glucosyl building block (**7**).

compound **10** with cyclohexylidene-protected glycerol **11** produced fully protected 3-*O*-(α-glucosyl)-*sn*-glycerol **20**. The construction of the α-glycosidic bond in compound **20** deserves some discussion. Previous glycosylation reactions of the *sn*-glycerol acceptor resulted in 1:1 and 3.2:1 mixtures of the α/β-glucosides.[8,10] Our initial attempts at preparing glucoside **20** resulted in a modest (2:1) α/β anomeric mixture, even under standard DMF-modulated glycosylation conditions (Table 1, entry 1).[12] Thus, optimization of the reaction conditions was required to improve the α-selectivity of the reaction (Table 1).

Table 1. Optimization of the synthesis of α-glucosyl *sn*-glycerol (**20**).

Entry[a]	Solvent	*t* [h]	DMF [equiv]	*T* [°C]	Yield [%]	α/β ratio[a]
1	CH_2Cl_2	24	4	−10	75	2:1
2	CH_2Cl_2	42	8	−10	65	3:1
3	CH_2Cl_2	24	6	−30	58	5:1
4	CH_2Cl_2	48	6	−25/−20	68	7.3:1
5	CH_2Cl_2	36	16	−25/−20	63	8.2:1
6	Et_2O	30	0	−20	60	4:1

[a] The α/β ratio was determined by HPLC analysis of the crude product mixture.

During the optimization process, different reaction conditions were examined, including 1) various reaction temperatures, 2) various solvents, and 3) employing a stoichiometric amount of DMF (Table 1). The best result entailed the use of 16 equivalents of DMF and a lower reaction temperature of −25/−20 °C, thereby affording the target glycoside (**20**) as an 8.2:1 α/β mixture. Notably, at low reaction temperatures (< −20 °C), longer (1.5 to 2 days) reaction times were needed (Table 1, entry 5). The α-configuration of the desired anomer (**20**) was confirmed by the chemical shift of the anomeric proton at $\delta = 4.94$ ppm and by the corresponding coupling constant ($^3J(H,H) = 2.8$ Hz).[18] With the desired anomer of compound **20** in hand, the final 3-*O*-(α-glucosyl)-*sn*-glycerol acceptor (**7**) was produced by removal of the NAP protecting group with 2,3-dichloro-5,6-dicyano-quinone (DDQ).[22]

Low-Concentration One-Pot Synthesis of the Tetrasaccharide Core

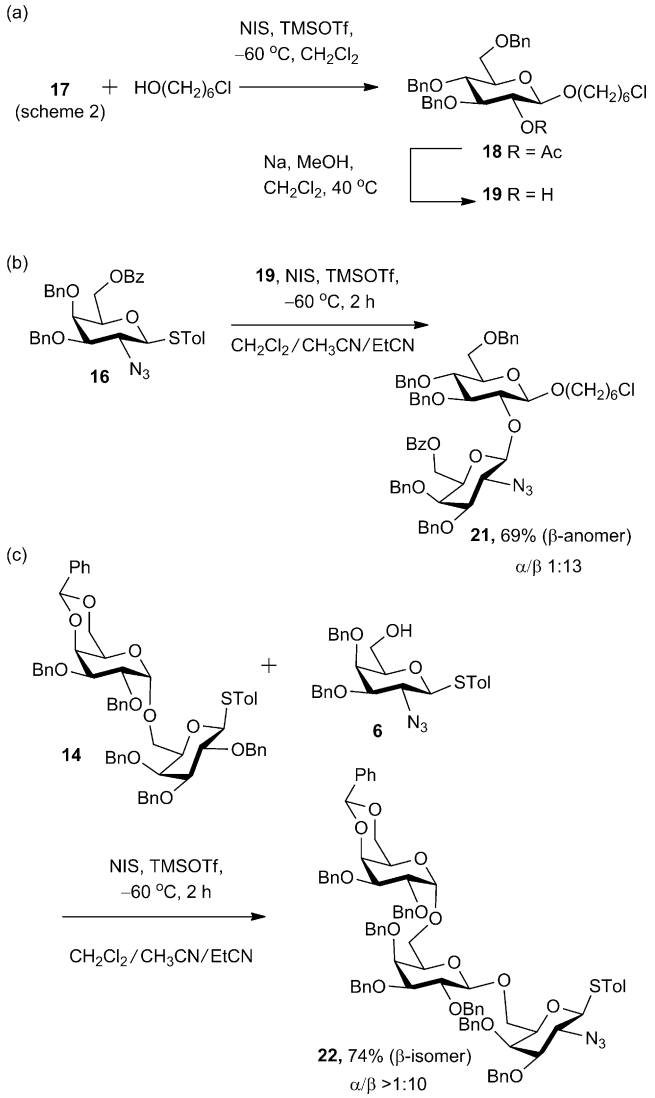
With glycosyl building blocks **5**–**7** in hand, we attempted the assembly of the protected tetrasaccharide (**4**). Because we applied the one-pot glycosylation method under low-concentration conditions, it was necessary to assess the selectivity for each of the β-glycosidic-bond-formation steps.[15b]

For the formation of the reducing-end β-GalN_3-(1→2)-Glc disaccharide, model 2-azido-2-deoxy-thiogalactosyl donor **16** and glucoside acceptor **19** were employed. Acceptor **19** was obtained from protected glucoside **18**, which, in turn, was prepared from chlorohexanol and thioglucoside **17** (Scheme 5a). The glycosylation of glucoside acceptor **19** with thioglycoside donor **16** furnished the desired disaccharide (**21**) in 69% yield with an excellent α/β anomeric ratio (1:13, Scheme 5b).[15a] The β-configuration of the desired anomer of compound **21** was supported by the chemical shift of the anomeric carbon atom at $\delta = 101.3$ ppm and by the coupling constant of $^1J(C,H) = 161$ Hz.[18]

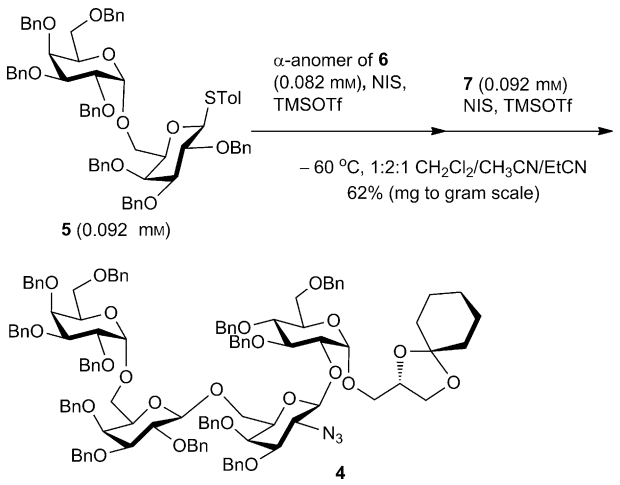
To assess the selectivity in the construction of the β-glycosidic linkage in the β-Gal-(1→6)-GalN_3 unit, model disaccharide thioglycoside **14** was coupled with 2-azido-2-deoxy β-thiogalactoside **6** under the LCG conditions. To our delight, the desired β-anomer of trisaccharide thioglycoside **22** was produced in a high yield (74%) with a >1:10 α/β ratio (Scheme 5b).

Having confirmed the β-selectivity of the glycosylation reaction under the LCG conditions, we attempted the one-pot synthesis of tetrasaccharide **4** (Scheme 6). Thus, α-Gal-(1→6)-Gal disaccharide thioglycoside **5** (0.092 mM) was coupled with the 2-azido-2-deoxy-α-thiogalactoside **6** (0.082 mM) in CH_2Cl_2/MeCN/EtCN (1:2:1v/v/v) at −60 °C to afford the desired α-Gal-(1→6)-Gal-β-(1→6)-GalN_3 trisaccharide thioglycoside. Without isolation, the trisaccharide thioglycoside was activated by using NIS and TMSOTf and reacted with 3-*O*-(α-glucosyl)-*sn*-glycerol **7** to furnish the desired tetrasaccharide (**4**). After standard chromatographic purification, compound **4** was isolated as a single anomer in high 62% yield. To prepare sufficient material for post-glycosylation modification and global deprotection, the one-pot synthesis of compound **4** was repeated on the gram scale.

Notably, the β-anomer of 2-azido-2-deoxy thiogalactoside **6** was initially employed in the low-concentration one-pot glycosylation reaction, but the second glycosylation step did not go to completion, even after reacting overnight. Thus, it appeared that the trisaccharide intermediate that was derived from the β-anomer of compound **6** was less reactive than that derived from its α-anomer counterpart. Accordingly, we used the α-anomer of compound **6** for the one-pot reaction.



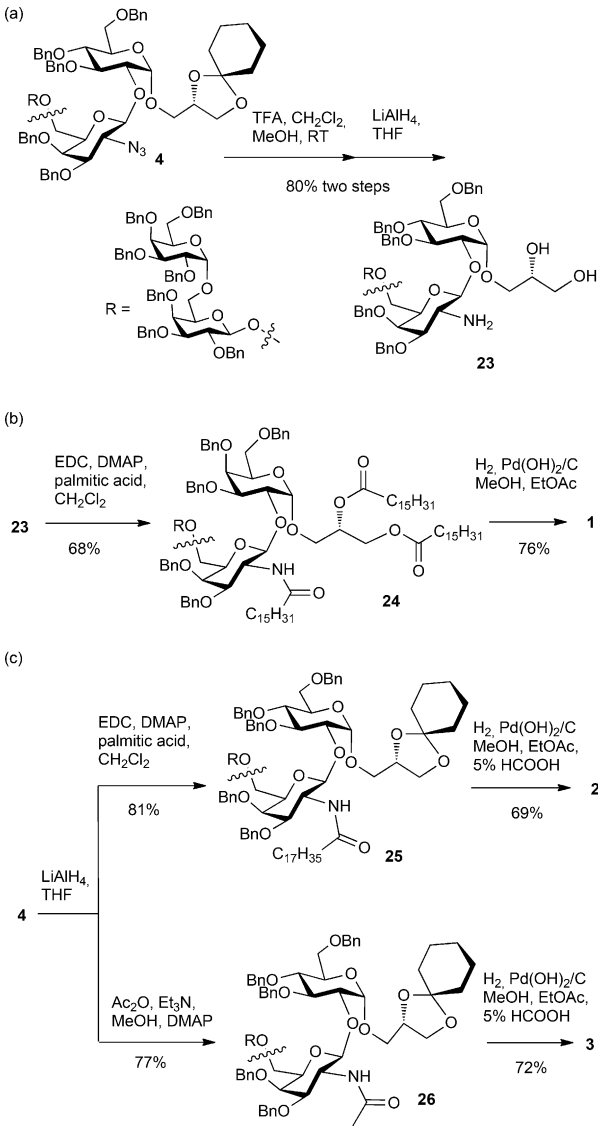
Scheme 5. Evaluation of the low-concentration glycosylation method for the construction of the β-glycosidic linkages in the target tetrasaccharide (**4**).



Scheme 6. One-pot synthesis of protected tetrasaccharide glycoside **4** under low-concentration glycosylation conditions.

Post-Glycosylation Modification

With sufficient quantities of protected tetrasaccharide **4** in hand, the stage was set to conclude the synthesis of glycoglycerolipid **1**. Thus, the treatment of compound **4** with trifluoroacetic acid (TFA), followed by reduction with $LiAlH_4$, produced tetrasaccharide intermediate **23** (Scheme 7 a). Notably, the removal of the acetal group in compound **4** with TFA was carefully monitored to prevent cleavage of the glycosidic linkage. Upon chromatographic purification, compound **23** was functionalized by coupling with palmitic acid by using ethyl-(dimethylaminopropyl) carbodiimide (EDC) and dimethylaminopyridine (DMAP) to obtain fully protected glycoglycerolipid **24** in good yield (68%, Scheme 7 b).[23] Subsequent hydrogenolysis of compound **24** with 10% $Pd(OH)_2$ (Degussa-type) completed the total synthesis of



Scheme 7. Post-glycosylation modification of protected tetrasaccharide core **4** to afford target compounds **1**–**3**.

the target glycoglycerolipid (**1**). In summary, natural glycoglycerolipid **1** was prepared in ten linear steps from monosaccharide building blocks **6**, **8**, **9**, and **10**, *sn*-glycerol **11**, and palmitic acid in an overall 6% yield.

The synthesis of truncated analogues 2′-palmitamido tetrasaccharide **2** and 2′-acetamido tetrasaccharide **3** required the reduction of the azide group in protected tetrasaccharide **4** to afford an amine moiety, which was subsequently either coupled with 1) palmitic acid to give protected 2′-palmitamido tetrasaccharide **25** or 2) acetic anhydride (Ac_2O) to give protected 2′-acetamido tetrasaccharide **26** (Scheme 7c). Removal of the benzyl ether and cyclohexylidene acetal protecting groups in compounds **25** and **26** was achieved in one pot by hydrogenolysis in formic-acid/MeOH (5% v/v) with $Pd(OH)_2$ as a catalyst. Truncated analogues **2** and **3** were obtained in 69% and 72% yield, respectively.

Because the *N*-acetyl derivative of the natural glycoglycerolipid from *M. taiwanensis* had been reported, the ^{1}H NMR spectrum of 2′-acetamido tetrasaccharide **3** was compared with the literature data. The chemical shifts of the anomeric protons of compound **3** at $\delta = 5.04$ (H), 4.85 (H‴), 4.46 (H′), and 4.33 ppm (H″) fully matched with those reported in the literature (Figure 1).[3] From the assignment of the ^{1}H NMR spectrum of compound **3**, assignment of the anomeric protons in the respective ^{1}H NMR spectra of glycoglycerolipid **1** and 2′-palmitamido tetrasaccharide **2** was performed.

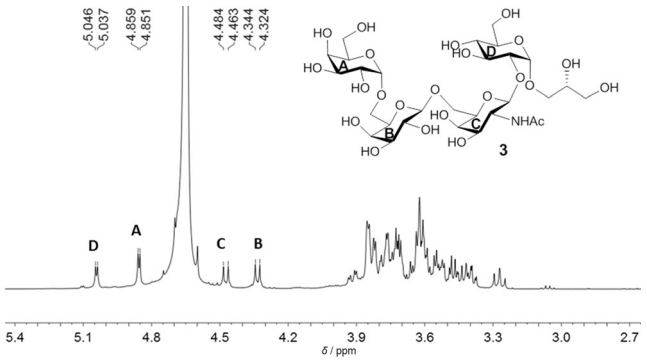


Figure 1. Selected ^{1}H NMR spectrum of 2′-acetamido tetrasaccharide **3**.

Preliminary Immunological Studies

Tetrasaccharide compounds **1**–**3** contained different numbers of palmitic acids; thus, we were intrigued to investigate how the number of the acyl chains affected the inflammatory activity of immune cells.[24] To this end, a series of immunological experiments were performed to examine the immunomodulating activities of these molecules. THP-1 cells were treated with compound **1**, **2**, or **3** in the presence or absence of lipopolysaccharides (LPS, 1.0 μg mL^{-1}) and incubated for 24 h. The levels of IL-6, IL-1β, and TNF-α production in the cell-free supernatant were measured by an enzyme-linked immunosorbent assay (ELISA; R&D systems, Minneapolis, MN).[25,26]

THP-1 cells that were treated with the individual tetrasaccharide compounds (**1**, **2**, or **3**) did not induce any obvious change in the secretion levels of IL-6, IL-1β, or TNF-α cytokines, thus suggesting that these compounds had no immunostimulation capacity (Figure 2a–c, left). In terms of the suppression of LPS-induced cytokine production, 2′-palmitamido tetrasaccharide **2** exhibited a higher capacity for the suppression of the production of IL-6, IL-1β, and TNF-α cytokines, as compared to THP-1 cells that were treated with LPS alone (Figure 2a–c, right).

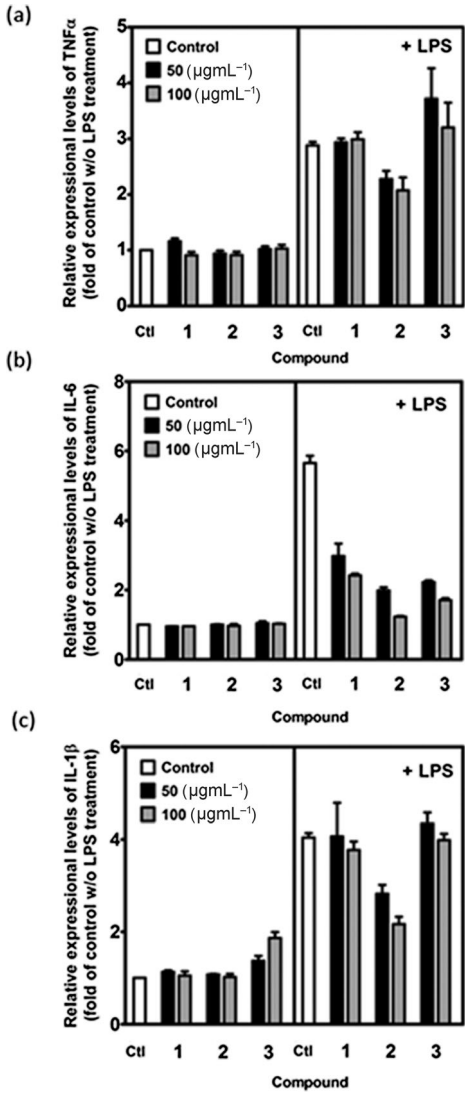


Figure 2. Tetrasaccharides **1**–**3** exhibited negative regulation on the production of inflammatory cytokines IL-6. THP-1 cells were stimulated with or without LPS, followed by incubation with tripalmitoyl glycoglycerolipid **1**, 2′-palmitamido tetrasaccharide **2**, or 2′-acetamido tetrasaccharide **3** (50 or 100 μg mL^{-1}) for 24 h. The culture media were collected and subjected to an ELISA assay to detect the expression level of IL-6. The level of IL 6 in the THP-1 cells without treatment of any compound (control) is referred to as one aliquot of relative expression. Quantitative results are presented as the mean ± SEM of triplicate measurements from three independent experiments and were analyzed by Student′s t-tests.

Some dose-dependence was observed for the suppression of IL-6 and IL-1β production. In particular, treatment with 2′-palmitamido tetrasaccharide **2** (100 μg mL^{-1}) inhibited the secretion of IL-6 to an almost-basal level. A similar suppression pattern of cytokine production was observed when a second batch of the tetrasaccharide compound (**2**) was examined, thus validating the suppression capacity of compound **2**. In contrast, the treatment of THP-1 cells with intact glycoglycerolipid **1** and 2′-acetamido tetrasaccharide **3** decreased the LPS-induced production of IL-6, but had no observable effect on the secretions of IL-1β or TNF-α. Taken together, these preliminary results suggest that the tetrasaccharide with a single palmitoyl chain (**2**) produces the best anti-inflammatory effect on LPS-induced cytokine production.

Conclusions

We have reported the first total synthesis of a glyceroglycolipid isolate from *M. taiwanensis* and its truncated structural analogues. A short synthetic scheme was developed, which included a low-concentration one-pot glycosylation reaction. Our synthesis employed the "nitrile solvent effect" and the modulating properties of DMF to control the stereochemistry in the formation of the β- and α-glycosidic bonds. Such tactics simplified the post-glycosylation-modification and global-deprotection steps because no specialized protecting groups were involved. Preliminary immunological studies of these compounds revealed that a truncated glyceroglycolipid analogue with a single palmitoyl chain suppressed LPS-stimulated cytokine production.

Experimental Section

*Synthesis of p-Methylphenyl 6-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-2,3,4-tri-O-benzyl-1-thio-β-D-galactopyranoside (**5**) from Compounds **8** and **9***

A solution of galactoside (Gal) thioglycosyl donor **8** (626 mg, 1.13 mmol), DMF (0.35 mL, 4.50 mmol), and 4 Å molecular sieves (AW300, 1.5 g) in CH_2Cl_2 (15 mL) was stirred at RT for 10 min, then cooled to −10 °C for an additional 30 min. *N*-Iodosuccinimide (NIS, 250 mg, 1.13 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 136 μL, 0.75 mmol) were added and the mixture was stirred for 30 min. When the activation of compound **8** was complete (by TLC), Gal acceptor **9** (417 mg, 0.75 mmol) was added and the mixture was stirred for 2 h. After completion of the glycosylation reaction (by TLC), the mixture was quenched with NEt_3 (0.3 mL) at −10 °C. A small volume of a saturated aqueous solution of $NaHCO_3$ and a small lump of solid $Na_2S_2O_3$ were added and the mixture was stirred vigorously at RT until the deep-red color of the solution changed to pale yellow. The resultant mixture was dried over $MgSO_4$, filtered, and concentrated for flash chromatography on silica gel (*n*-hexane/EtOAc, 5:1 to 4:1). The desired α-anomer product (**14**, 420 mg, 0.36 mmol) was isolated in 60 % yield as a white foam.

R_f = 0.47 (*n*-hexane/EtOAc, 7:3); $[\alpha]_D^{27}$ = +42.6 (c = 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50–7.48 (m, 2H; Ar*H*), 7.42–7.22 (m, 30H; Ar*H*), 6.97 (d, J = 8.0 Hz, 2H; Ar*H*), 5.41 (s, 1H; benzylidene-C*H*), 4.92 (d, J = 11.6 Hz, 1H), 4.84 (d, J = 11.6 Hz, 1H), 4.80–4.57 (m, 10H), 4.15 (d, J = 12.8 Hz, 1H), 4.07 (d, J = 2.8 Hz, 1H), 4.03 (dd, J = 13.2, 2.8 Hz, 1H), 3.95–3.87 (m, 3H), 3.83–3.77 (m, 2H), 3.69 (s, 1H), 3.65 (m, 1H), 3.59 (dd, J = 9.2, 2 Hz, 1H), 3.34 (dd, J = 12.0 Hz, 4.0 Hz, 1H), 2.25 ppm (s, 3H; C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.2, 139.0, 138.8, 138.7, 138.6, 138.3, 137.1, 131.3, 131.0, 130.0, 129.2, 128.9, 128.79, 128.74, 128.70, 128.5, 128.4, 128.24, 128.23, 128.1, 127.9, 127.8, 126.7, 101.4 (benzylidene-*C*H), 98.5 (J(C,H) = 171 Hz; C-1′), 87.4 (J(C,H) = 152 Hz; C-1), 84.6, 77.8, 77.6, 76.8, 76.1, 75.8, 74.8, 74.7, 74.4, 74.3, 73.5, 72.2, 69.9, 68.2, 62.9, 21.5 ppm (*C*H$_3$); HRMS (ESI): m/z calcd for $C_{61}H_{62}O_{10}SNa$: 1009.3956; found: 1009.3928 $[M+Na]^+$.

TFA (12 mL, 105 mmol) was added to a solution of disaccharide **14** (3.2 g, 3.2 mmol) in MeCN (10 mL) at 0 °C and the reaction was stirred for 5 h at RT. After completion of the reaction (by TLC), the mixture was quenched with $NaHCO_3$, extracted with EtOAc (3 × 20 mL), and washed with water (3 × 15 mL). The organic phase was concentrated as a yellow oil (2.9 g, quantitative yield) and then treated with dry DMF (30 mL) under a N_2 atmosphere and NaH (60 % in oil suspension, 439 mg, 10.9 mmol) was added cautiously under an ice bath. Subsequently, benzyl bromide (0.92 mL, 7.7 mmol) was added and the mixture was stirred for 2 h. The desired product was extracted with EtOAc (3 × 30 mL), washed with H_2O (3 × 20 mL), dried (over $MgSO_4$), filtered, and, concentrated for flash chromatography on silica gel (*n*-hexane/EtOAc, 4:1). The desired product (**5**, 3.6 g) was isolated in 86 % yield as a white foam.

R_f = 0.55 (*n*-hexane/EtOAc, 4:1); $[\alpha]_D^{20}$ = +22.6 (c = 3.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (d, J = 8.0 Hz, 2H; Ar*H*), 7.42–7.25 (m, 35H; Ar*H*), 6.99–6.97 (d, J = 8.0 Hz, 2H; Ar*H*), 4.93 (dd, J = 11.6, 4.8 Hz, 2H), 4.80–4.69 (m, 6H), 4.65–4.57 (m, 5H), 4.47 (d, J = 12.0 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 4.04 (dd, J = 8.0, 4.0 Hz, 1H), 3.99–3.84 (m, 7H), 3.67 (t, J = 6 Hz, 1H), 3.60 (dd, J = 9.2, 2.8 Hz, 1H), 3.58–3.54 (m, 3H), 2.26 ppm (s, 3H; C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.79, 138.74, 138.69, 138.6, 138.6, 138.4, 138.3, 138.0, 137.1, 132.2, 130.1, 129.5, 128.36, 128.31, 128.2, 128.16, 128.13, 127.9, 127.8, 127.7, 127.67, 127.61, 127.59, 127.53, 127.5, 127.35, 127.31, 98.2 (C-1′), 87.7 (C-1), 84.1, 79.1, 77.2, 76.8, 76.4, 75.6, 74.8, 74.3, 73.9, 73.6, 73.5, 72.79, 72.75, 69.2, 68.7, 67.1, 21.1 ppm (*C*H$_3$); HRMS (ESI): m/z calcd for $C_{68}H_{70}O_{10}SNa$: 1101.4582; found: 1101.4571 $[M+Na]^+$.

*Synthesis of 3-O-[3,4,6-Tri-O-benzyl-2-O-(2-naphthylmethyl)-α-D-gluco pyranosyl]-1,2-O-cyclohexylidene-sn-glycerol (**20**)*

To a solution of thioglucoside donor **10** (100 mg, 0.14 mmol), DMF (182 μL, 2.3 mmol), and 4 Å molecular sieves (AW300, 300 mg) in CH_2Cl_2 (2.6 mL) were added NIS (38 mg, 0.17 mmol) and TMSOTf (30 μL, 1.13 mmol) and the mixture was stirred for 45 min, followed by the addition of 1,2-*O*-cyclohexylidene-protected *sn*-glycerol **11** (36 mg, 0.21 mmol). The reaction mixture was stirred at −25 °C for 12 h and at −20 °C for 24 h. After completion of the reaction, a few drops of Et_3N, a saturated aqueous solution of $NaHCO_3$, and a few pieces of $Na_2S_2O_3$(s) were added to the mixture, followed by vigorous stirring at RT until the color of the reacting solution turned pale yellow. Then, the mixture was filtered (through celite) to obtain the organic fraction, which was concentrated for flash chromatography on silica gel (*n*-hexane/EtOAc, 4:1). The α-anomer of glucoside **20** (80 mg, 0.08 mmol) was isolated as a colorless syrup in 63 % yield.

R_f = 0.35 (*n*-hexane/EtOAc, 4:1); $[\alpha]_D^{20}$ = +33.10 (c = 0.58, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85–7.73 (m, 4H; ArH), 7.46–7.44 (m, 3H; ArH), 7.33–7.13 (m, 15H; ArH), 5.01 (d, J = 10.8 Hz, 1H), 4.91–4.81 (m, 5H), 4.58 (d, J = 12.4 Hz, 1H), 4.46 (t, J = 10.8 Hz, 2H), 4.37 (t, J = 5.6 Hz, 1H), 4.06 (t, J = 8.0 Hz, 1H), 3.98 (t, J = 9.2 Hz, 1H), 3.77–3.58 (m, 8H), 1.62–1.58 (m, 8H; 4 × C*H*$_2$), 1.26 ppm (br s, 2H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.3, 138.7, 138.4, 136.1, 133.6, 133.5, 128.81, 128.77, 128.70, 128.35, 128.31, 128.26, 128.1, 128.0, 127.3, 126.6, 126.41, 126.39, 110.5 (quaternary-C), 97.7 (C-1), 82.3, 80.3, 78.1, 76.1, 75.5, 74.8, 73.9, 73.5, 70.8, 69.5, 68.9, 67.1, 36.9 (CH_2), 35.4 (CH_2), 25.6 (CH_2), 24.5 (CH_2), 24.3 ppm (CH_2); HRMS (ESI): m/z calcd for $C_{47}H_{52}O_8Na$: 767.3554; found: 767.3560 $[M+Na]^+$.

*Synthesis of 3-O-[2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranosyl-(1→6)-2-azido-3,4-di-O-benzyl-2-deoxy-β-D-galactopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-glucopyranosyl]-1,2-O-cyclohexylidene-sn-glycerol (**4**)*

A suspension of α-Gal-(1→6)-Gal disaccharide thioglycoside **5** (900 mg, 0.912 mmol), the α-anomer of $GalN_3$ thioglycoside **6** (402 mg, 0.820 mmol), and 4 Å molecular sieves (AW300, 12 g) in CH_2Cl_2/MeCN/EtCN (44 mL, 1:2:1 v/v) was treated with NIS (206 mg, 0.912 mmol) and TMSOTf (82 μL, 0.456 mmol). Then, the reaction mixture was stirred for 1.5 h. Glucoside acceptor **7** (445 mg, 0.912 mmol), NIS (206 mg, 0.912 mmol), and TMSOTf (82 μL, 0.456 mmol) were added to the reaction mixture. After completion of the one-pot reactions, a few drops of Et_3N, a saturated aqueous solution of $NaHCO_3$, and a few pieces of $Na_2S_2O_3$(s) were added to the mixture, followed by stirring at RT until the color of the reaction mixture turned from red to pale yellow. The resulting solution mixture was filtered (through celite) to obtain the organic fraction, which was concentrated and purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 17:3 to 4:1). Compound **4** (980 mg) was afforded in 62% yield as a pale-yellow syrup.

R_f=0.3 (*n*-hexane/EtOAc, 2:1); $[\alpha]_D^{20}$=+30.40 (c=0.28, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.22–6.94 (m, 60H; ArH), 4.92 (d, J=10.4 Hz, 1H), 4.86–4.79 (m, 3H), 4.73–4.64 (m, 9H), 4.60–4.45 (m, 8H), 4.41–4.33 (m, 5H), 4.28–4.23 (m, 2H), 4.15 (dd, J=6, 4 Hz, 1H), 3.96–3.88 (m, 5H), 3.83 (dd, J=10, 2.8 Hz, 2H), 3.78–3.72 (m, 4H), 3.70–3.59 (m, 5H), 3.57–3.39 (m, 9H), 3.23 (t, J=6.0 Hz, 1H), 3.08 (dd, J=10.4, 2.8 Hz, 1H), 1.57–1.46 ppm (m, 10H; 5×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ=139.35, 139.33, 139.2, 139.07, 139.05, 139.00, 138.9, 138.7, 138.4, 138.2, 138.0, 128.87, 128.84, 128.79, 128.76, 128.73, 128.70, 128.69, 128.66, 128.65, 128.60, 128.59, 128.57, 128.55, 128.4, 128.28, 128.23, 128.21, 128.18, 128.12, 128.06, 128.03, 128.0, 127.98, 127.94, 127.88, 127.85, 127.80, 127.7, 110.1 (quaternary-C), 104.0, 103.2, 99.4, 99.3, 82.5, 81.9, 81.1, 79.8, 79.5, 79.1, 78.7, 76.6, 75.8, 75.3,75.27, 75.21, 75.07, 75.05, 74.9, 74.1, 74.08, 74.04, 74.02, 73.8, 73.7, 73.1, 73.0, 72.6, 71.9, 70.7, 70.0, 69.2, 68.9, 68.8, 67.2, 67.0, 66.9, 63.6, 36.6 (CH_2), 35.5 (CH_2), 25.6 (CH_2), 24.5 (CH_2), 24.3 ppm (CH_2); HRMS (ESI): m/z calcd for $C_{117}H_{127}N_3O_{22}H$: 1926.8984; found: 1926.8937 $[M+H]^+$.

*Synthesis of 3-O-[2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-galactopyranosyl-(1→6)-2-palmitamido-3,4-di-O-benzyl-2-deoxy-β-D-galactopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (**24**)*

A solution of fully protected tetrasaccharide **4** (580 mg, 0.290 mmol) in CH_2Cl_2/MeOH (14 mL, 5:1 v/v) was treated with TFA (6.0 mL). The mixture was stirred from 0°C to RT for 3 h and quenched with a saturated aqueous solution of $NaHCO_3$ and CH_2Cl_2. The organic phase was collected, washed with water, dried ($MgSO_4$), and concentrated for chromatographic purification on silica gel (*n*-hexane/EtOAc, 3:2) to give the crude product (about 90% yield) for azide reduction. Then, the crude product (485 mg, 0.262 mmol) was dissolved in THF (10 mL) and lithium aluminum hydride (LAH, 100 mg, 2.62 mmol) was added at 0°C under a N_2 atmosphere. After stirring for 3 h, equal volumes of EtOAc and H_2O were sequentially added to the mixture to quench any excess LAH. The organic phase was separated and concentrated for chromatographic purification on silica gel (*n*-hexane/EtOAc 3:7, with 1% Et_3N) to afford the expected product (450 mg, >90% yield). Then, the product from the preceding step (250 mg, 0.981 mmol) was dissolved in CH_2Cl_2 (10 mL) and DMAP (11 mg, 0.09 mmol), EDC (112 mg, 0.588 mmol), and palmitic acid (112 mg, 0.441 mmol) were added. The resulting mixture was stirred for 12 h and diluted with CH_2Cl_2. The resulting organic phase was washed with a saturated aqueous solution of $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated for flash chromatography on silica gel (*n*-hexane/EtOAc, 4:1) to afford fully protected glycoglycerolipid **24** (224 mg, 68% yield).

R_f=0.3 (*n*-hexane/EtOAc, 3:1); $[\alpha]_D^{20}$=+22.2 (c=0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.37–7.18 (m, 58H; ArH), 7.01 (d, J=2.8 Hz, 2H; ArH), 5.77 (d, J=8.0 Hz, 1H; N*H*C=O), 5.19 (d, J=8.0 Hz, 2H), 4.94–4.69 (m, 12H), 4.66–4.53 (m, 8H), 4.45–4.25 (m, 9H), 4.04–3.85 (m, 8H), 3.77–3.46 (m, 18H), 2.21 (dt, J=6.0, 2.4 Hz, 4H; 2×CH_2C=O), 1.71 (m, 2H; C=OCH_2, signal partly overlapped with residue H_2O), 1.54 (br s>, 4H; 2×CH_2) 1.30–1.23 (m, 74H; 37×CH_2), 0.88 ppm (t, J=5.2 Hz, 9H; 3×terminal CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.9 (C=O), 173.6 (C=O), 172.9 (C=O), 138.9, 138.78, 138.75, 138.6, 138.57, 138.4, 138.2, 138.13, 137.9, 137.8, 128.4, 128.3, 128.24, 128.20, 128.1, 128.0, 127.9, 127.85, 127.81, 127.78, 127.71, 127.6, 127.59, 127.53, 127.4, 127.37, 127.34, 127.28, 127.20, 103.4, 100.4, 99.6, 98.9, 82.1, 81.1, 79.1, 79.0, 77.7, 76.1, 74.9, 74.89, 74.84, 74.7, 74.5, 74.2, 73.5, 73.4, 72.9, 72.7, 72.6, 72.5, 72.1, 71.9, 70.4, 69.8, 69.5, 68.7, 68.5, 67.1, 66.9, 66.5, 62.9, 55.5, 36.6, 34.3, 34.1, 31.9, 29.7, 29.5, 29.5, 29.3, 29.1, 25.4, 24.9, 24.8, 22.7, 14.1 ppm. Global deprotection of compound **24** was performed to give the target glycoglycerolipid (**1**) for mass spectrometry analysis.

*Synthesis of 3-O-[α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-2-palmitamido-2-deoxy-β-D-galactopyranosyl-(1→2)-α-D-glucopyranosyl]-1,2-di-O-palmitoyl-sn-glycerol (**1**)*

Fully protected tetrasaccharide **24** (180 mg, 0.07 mmol) was dissolved in EtOAc/MeOH (8 mL, 1:1 v/v) at RT. Pd/C (68 mg, Degussa type) was added to the solution, followed by stirring at RT for 12 h under a H_2 atmosphere (1 atm). Then, the reaction mixture was filtered (through celite) and the filtrate was concentrated for chromatography on silica gel (CH_2Cl_2/MeOH, 9:1 to 1:1) to afford target glycoglycerolipid **1** (70 mg, 76% yield) as a white amorphous powder.

R_f=0.17 (CH_2Cl_2/MeOH, 2:1); $[\alpha]_D^{20}$=+20.57 (c=0.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD 10:1): δ=5.16 (d, J=5.2 Hz, 1H; N*H*C=O), 4.90 (d, J=2.4 Hz, 1H; H-1), 4.83 (d, J=2.4 Hz, 1H; H-1‴), 4.58 (d, J=8.4 Hz, 1H; H-1′), 4.36 (br s, 1H; H-1″, signal partly covered by residual H_2O), 4.25 (br s, 1H), 4.12 (dd, J=12.0, 6.8 Hz, 1H), 3.91–3.36 (m, 25H), 3.26 (s, 2H), 2.23 (t, J=7.6 Hz, 4H; 2×CH_2), 2.17 (t, J=8 Hz, 2H; CH_2), 1.59–1.46 (m, 6H; 3×CH_2), 1.33–1.18 (m, 72H; 36×CH_2), 0.81–0.77 ppm (m, 9H; 3×CH_3); HRMS (ESI): m/z calcd for $C_{75}H_{140}NO_{25}$: 1454.9709; found: 1454.9695 $[M+H]^+$. The assignment of the anomeric protons of compound **1** was based on NMR data that were obtained for the *N*-acetyl derivative of the natural glycoglycerolipid isolate of *M. taiwanensis*.[3]

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