Carbohydrate Research 368 (2013) 35–39

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Concise synthesis of α-galactosyl ceramide from D-galactosyl iodide and D-lyxose

Yu-Fen Yen [a], Suvarn S. Kulkarni [b], Chun-Wei Chang [a], Shun-Yuan Luo [a,*]

[a] Department of Chemistry, National Chung Hsing University, Taichung 402, Taiwan
[b] Department of Chemistry, Indian Institute of Technology Bombay, Mumbai 400076, India

ARTICLE INFO

Article history:
Received 16 October 2012
Received in revised form 12 November 2012
Accepted 14 November 2012
Available online 21 November 2012

Keywords:
α-Galactosyl ceramide
KRN7000
Glycosyl iodide
One-pot protection-glycosylation
Wittig reaction

ABSTRACT

α-Galactosyl ceramide is synthesized from D-lyxose in 32% overall yield in five steps. The short and efficient protocol involves a one-pot protection and glycosidation of D-lyxose with D-galactosyl iodide as a key step. The α-linked disaccharide obtained was subsequently transformed into α-galactosyl ceramide in four steps involving *Z*-selective Wittig olefination at C-1, stereo-inversion at C-4 using azide, one-pot reduction of azide and amidation, and global deprotection.

1. Introduction

Glycolipids play significant roles in numerous biological processes.[1] For example, α-galactosylceramide **1**, also known as KRN7000,[2] a simplified glycolipid analog of Agelasphins originally isolated from a marine sponge *Agelas mauritianus*,[3] acts as a powerful stimulator of the invariant Natural Killer T (iNKT) cells by binding to the CD1d glycoprotein. The binding complex of CD1d and **1** recognizes the T-cell receptor of iNKT cells to produce cytokines that trigger an inflammatory response called T helper 1 (Th1), which governs the antitumor and antimicrobial properties, and immunomodulatory response termed T helper 2 (Th2), which controls various autoimmune diseases.[4] Since these responses cancel out each other's effect, there is an ongoing search for glycolipid analogs, with varied lipid chain length, that selectively trigger either Th1 or Th2 type responses.[5]

Structurally, α-galactosyl ceramide **1** (Fig. 1), is composed of an α-linked D-galactopyranoside with phytosphingosine-derived ceramide. Numerous methods for the synthesis of α-glactosyl ceramide are reported in the literature, mostly involving stereoselective coupling of phytosphingosine derivatives with various glycosyl donors.[6–8] Among them, Gervay-Hague's elegant protocol of glycosyl iodide mediated stereoselective glycosylation offers excellent selectivity and yields.[7] The high α-selectivity is attributed to the in situ anomerization of the initially formed α-galactosyl iodide to a more reactive β-iodide and its S_N2-like displacement by a phytosphingosine acceptor. On the other hand, the preparation of ceramide acceptors is done mostly through chiron approach using various starting materials such as amino acids, sugars, and other chiral materials. However, some of the approaches involve multistep sequences and result in moderate to low overall yields. D-Lyxose that has all the requisite chiral centers in place is the most suitable precursor for phytosphingosine.[9] Based on this finding, Panza and co-workers[8c] reported a novel synthesis of α-galactosyl ceramide via the coupling of D-galactosyl bromide with a D-lyxose acceptor derived from D-mannose. For the development of glycolipids as vaccine adjuvants, we are interested in establishing a short and general synthesis of α-GalCer and related analogs. We envisioned that a merger of Panza's approach and Gervay-Hague's convenient protocol[7a] would offer a facile access to these glycolipids. Herein, we report an efficient five-step route for the synthesis of KRN7000 from commercially available D-lyxose, in 32% overall yields. To the best of our knowledge this is the shortest reported sequence for the synthesis of α-galactosyl ceramide till date. The key feature of this synthesis lies in controlling the regio- and stereoselectivities of glycosylation to obtain a [α-D-Gal*p*→D-lyx*f*] disaccharide, the right hand sugar unit (D-lyxofuranose) of which could be subsequently manipulated to install the required functionalities, thus avoiding any complications and difficulties usually encountered in coupling glycosyl donors with azido ceramide or ceramide acceptors. Hung and co-workers[10] have established earlier a one-pot protection-glycosylation protocol starting from 2,3-di-*O*-TMS protected D-glucopyranosides to access various

* Corresponding author. Tel.: +886 4 22840411; fax: +886 4 22862547.
E-mail address: syluo@dragon.nchu.edu.tw (S.-Y. Luo).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carres.2012.11.008

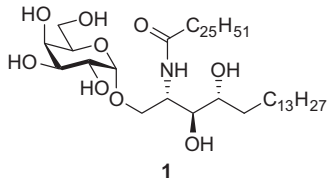
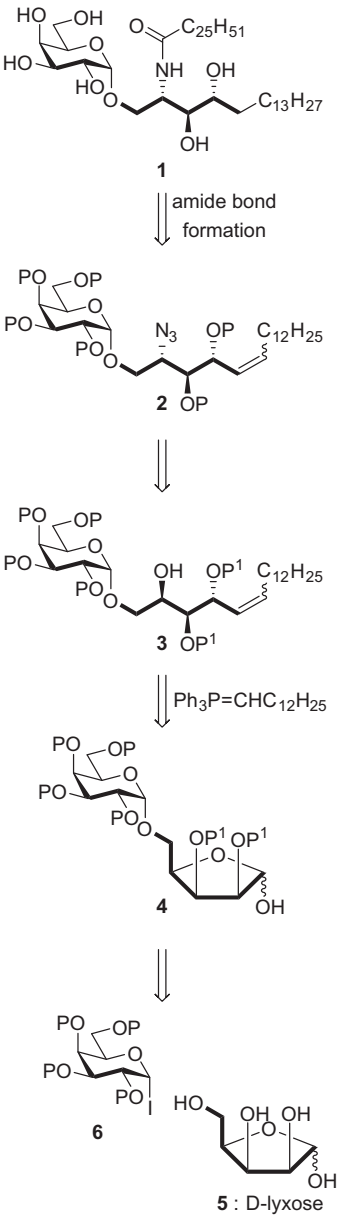


Figure 1. Structure of KRN7000 **1**.

3-O-arylmethylated 1→2 linked disaccharides. However, a tandem regioselective protection and glycosylation of free sugars is unprecedented in the literature.

2. Results and discussion

Our retrosynthetic plan for the synthesis of α-GalCer **1** entails commercially available D-lyxose **5** as a precursor (Scheme 1). The stereochemical resemblance between the two is indicated by the boldfaced carbon framework. The azido group in precursor **2** could be reduced to amine and the obtained galactosyl phytosphingosine can be coupled with suitable fatty acids to give α-galactosyl ceramide **1**. The D-galactosyl phytosphingosine derivative **2** is a key intermediate which could be in turn obtained by a Wittig olefination with the galactosyl-lyxose disaccharide **4**, followed by S_N2 displacement of the sulfonate activated hydroxyl group by an azide. It was envisaged that, the disaccharide **4** can be obtained directly from unprotected D-lyxose **5** by coupling with an appropriate galactosyl donor **6** via a one-pot protection–glycosylation involving in situ anomerization of glycosyl iodides.

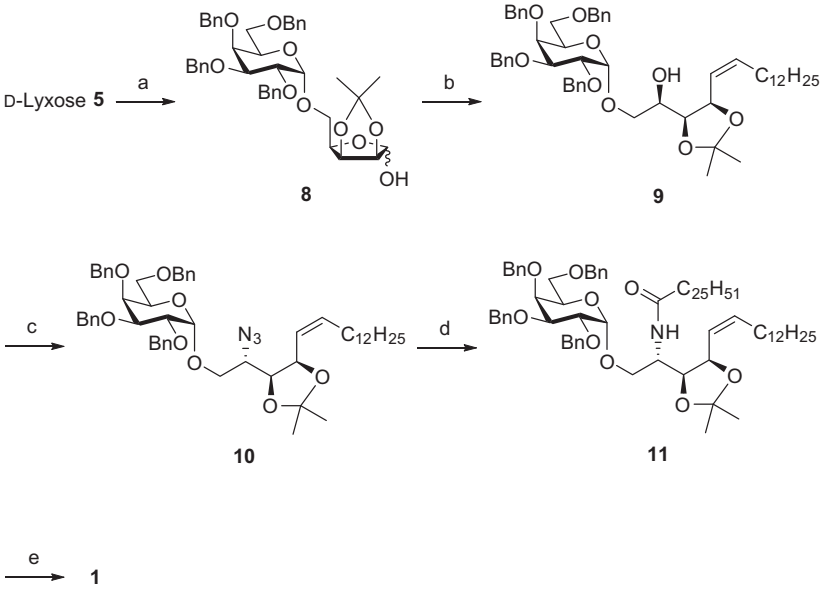


Scheme 1. Retrosynthetic analysis of α-galactosyl ceramide **1**.

The synthesis started with a regio- and stereoselective one-pot preparation of the key disaccharide **8**[8c] (Scheme 2). First, a selective acetonide protection of 2,3-dihydroxyl groups of D-lyxose **5** was achieved using catalytic sulfuric acid and anhydrous acetone.[11] Upon completion of the reaction, as indicated by TLC (4 h), DIPEA (1 equiv), TBAI (3 equiv) and 4 Å MS in toluene were sequentially added to the reaction and the temperature was raised to 65 °C. A preformed and well azeotroped solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl iodide **7**[12] (1 equiv) was added dropwise in the same pot, and the reaction was continued for an hour. This one-pot operation afforded exclusively α-linked disaccharide **8** in 63% overall yield. Thus, the coupling between glycosyl iodide and ispropylidene D-lyxofuranose 1,5-diol took place regio- and stereoselectively at the primary 5-OH group leaving the hemiacetal OH free for further reaction. The selectivity observed in this reaction saved two steps which would have been otherwise necessary for blocking the anomeric OH and removing it post-glycosylation. Wittig olefination of hemiacetal **8** with $Ph_3P{=}CHC_{12}H_{25}$ at 0 °C gave the *Z*-olefin **9** as a single isomer in 92% yield. It should be noted that, of the several conditions attempted for Wittig reaction, the in situ generation of the phosphorane[13] via a slow addition of the base to the well-stirred suspension of **8** and the phosphonium salt at 0 °C worked the best to afford the desired product **9**, stereoselectively. Compound **9** was sequentially treated with trifluoromethanesulfonic anhydride (Tf_2O) and tetramethylguanidinium azide (TMGA)[14] to produce the azido compound **10** (82% yield) with the inversion of the reacting carbon center.[15] After the reduction of the azido group in **10** with PPh_3 and pyridine in ACS grade quality of THF critical, hexacosanoic acid, EDC, HOBt and triethylamine were directly added into reaction mixture to obtain the corresponding amide **11** in 72% yield in a one-pot manner. After meticulous experimentation, we found the appropriate reaction conditions to remove the acetonide and simultaneously reduce the double bond and remove benzyl groups of **11** in a one-pot manner. Thus, a solution of **11** in 0.8 N HCl (aq) in 3:1 MeOH/$CHCl_3$ solution was stirred for 1 d at room temperature until the starting material was consumed completely and then treated with a catalytic amount of $Pd(OH)_2$ under an atmosphere of hydrogen for 2 d at room temperature to obtain KRN7000 **1** (94%).

3. Conclusion

In conclusion, we successfully synthesized biologically potent α-galactosyl ceramide **1** from the commercially available D-lyxose in 32% yield over five steps. The similarity between our approach and the synthesis reported by Panza and co-workers is evident. The key difference however lies in the regioselective one-pot protection and glycosylation reaction on the unprotected D-lyxose. Also, a highly *Z*-stereoselective Wittig reaction circumvents isomer separation or hydrogenation at an early stage. Along with this, one-pot global deprotection saves several steps. In Panza's synthesis, which although in itself is a marked improvement over the existing routes, the protected lyxofuranose 5-OH acceptor was prepared in four steps from D-mannose and the low yields encountered during the azide displacement of the *E*/*Z* olefin, possibly due to the con-



Scheme 2. Concise synthesis of α-galactosyl ceramide **1**. Reagent and conditions: (a) cat. H_2SO_4, acetone, room temp, 4 h; then TBAI, DIPEA, toluene, 65 °C, 10 min; then 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl iodide **7**, 1 h, 63%; (b) LHMDS, $C_{13}H_{27}PPh_3Br$, THF, 0 °C, 6 h, 92%; (c) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 2 h; then TMGA, CH_2Cl_2, 0 °C, overnight, 82%; (d) PPh_3, pyridine, wet THF, 60 °C, 12 h; then EDC, HOBt, $C_{25}H_{51}COOH$, Et_3N, room temp, 12 h, 72%; (e) 0.8 N HCl, MeOH, $CHCl_3$, room temp, 1 d; then $Pd(OH)_2$/C, H_2, room temp, 2 d, 94%.

current reaction of olefin with the azido group, necessitated the extra step for double bond reduction. We employed TMGA instead of sodium azide and observed that it does not react with the double bond of the compound **9**. The short and efficient route described here for the synthesis of α-galactosyl ceramide is expected to provide access to other structurally related glycolipids for exploring their immunostimulating activities and other biological properties. The phytosphingolipid chains can be easily modified by using a different Wittig salt, and the analogs can be altered by the use of different starting iodoglycosides.

4. Experimental section

4.1. General remarks

Dichloromethane was purified and dried from a safe purification system containing activated Al_2O_3. Anhydrous pyridine, and methanol were used as purchased. All reagents obtained from commercial sources were used without purification, unless otherwise mentioned. Flash column chromatography was carried out on Silica Gel 60 (230–400 mesh, E. Merck). TLC was performed on pre-coated glass plates of Silica Gel 60 F254 (0.25 mm, E. Merck); detection was executed by spraying with a solution of $Ce(NH_4)_2(NO_3)_6$ (0.5 g), $(NH_4)_6Mo_7O_{24}$ (24 g) and H_2SO_4 (28 mL) in water (500 mL) and subsequently heating on a hot plate. Melting points were determined with a capillary apparatus and are uncorrected. Optical rotations were measured at 589 nm (Na) at ~25 °C. ^1H, ^{13}C NMR, DEPT, ^1H–^1H COSY, ^1H–^{13}C COSY, and NOESY spectra were recorded with 400 and 600 MHz instruments. Chemical shifts are in ppm from Me_4Si, generated from the $CDCl_3$ lock signal at δ 7.24. IR spectra were taken with a FT-IR spectrometer using KBr plates. Mass spectra were analyzed on a Finnigan LTQ-Orbitrap XL instrument with an ESI source.

4.2. 2,3-*O*-isopropylidene-5-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-D-lyxofuranose (8)

To a solution of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (704 mg, 1.209 mmol) in anhydrous dichloromethane (7.0 mL) was added iodotrimethylsilane (224 μL, 1.511 mmol) at 0 °C under nitrogen. After 30 min, the mixture was evaporated in vacuo. Toluene (10 mL) was added to the residue and evaporated in vacuo three times to afford **7** as a pale yellowish residue. This residue was dissolved in toluene and kept under dry nitrogen atmosphere. To a solution of D-lyxose **5** (200 mg, 1.332 mmol) in anhydrous acetone (2 mL), prepared in a separate flask, was added sulfuric acid (5.7 μL, 0.106 mmol) and stirred for 4 h at rt. After the complete disappearance of the starting material on TLC, diisopropylethylamine (210 μL, 1.209 mmol), tetrabutylammonium iodide (1.33 g, 3.627 mmol) and 4 Å molecular sieves in anhydrous toluene (3 mL) were added to it and the mixture was stirred for 10 min at 65 °C under nitrogen. A solution of iodide **7** in toluene was added into the reaction flask at this stage and the mixture was kept stirring for 1 h. The reaction was stopped by adding ethyl acetate and the suspension was filtered through celite to separate the white precipitate and molecular sieves. The resulting solution was washed with aqueous $Na_2S_2O_3$ and brine, and the organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel to afford disaccharide **8** (552 mg, 63%) as a colorless oil: $[\alpha]_D^{28}$ +34.5 (*c* 1.3, $CHCl_3$); IR (thin film): ν 3436, 3030, 1454, 1096 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.40–7.28 (m, 20H, ArH), 5.32 (d, *J* = 2.8 Hz, 1H, H-1), 4.94 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.90 (d, *J* = 3.6 Hz, 1H, H-1′), 4.84 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.82 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.76–4.74 (m, 1H, H-2), 4.74 (d, *J* = 11.2 Hz, 1H, CH_2Ph), 4.68 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.57 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.56 (d, *J* = 5.6 Hz, 1H, H-3), 4.45 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.41–4.38 (m, 1H, H-4), 4.38 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.06–3.96 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.85–3.83 (m, 2H, H-5a, H-5b), 3.52–3.51 (m, 2H, H-6a′, H-6b′), 2.5 (s, 1H, OH), 1.40 (s, 3H, CH_3), 1.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 138.8 (C), 138.6 (C), 138.5 (C), 137.8 (C), 128.33 (CH × 2), 128.28 (CH × 2), 128.25 (CH × 2), 128.2 (CH), 128.16 (CH × 2), 128.1 (CH × 2), 127.9 (CH), 127.8 (CH × 2), 127.66 (CH), 127.61 (CH), 127.5 (CH), 127.37 (CH), 127.35 (CH × 2), 112.4 (C), 101.0 (CH), 97.9 (CH), 85.4 (CH), 79.9 (CH), 78.9 (CH), 78.6 (CH), 76.4 (CH), 74.9 (CH), 74.7 (CH_2), 73.3 (CH_2), 73.2 (CH_2), 72.9 (CH_2), 69.2 (CH), 68.9 (CH_2), 66.3 (CH_2), 26.0 (CH_3), 24.8 (CH_3);

HRMS (FAB): *m*/*z* Calcd for $C_{42}H_{48}O_{10}Na$ ([M+Ma]$^+$): 735.3145. Found: 735.3129.

4.3. (2*R*,3*S*,4*R*)-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopy-ranosyl)-octadec-5-en-1,2,3,4-tetraol (9)

A mixture of disaccharide **8** (147.6 mg, 0.207 mmol) and tridecanyltriphenylphosphonium bromide (490 mg, 0.932 mmol) in anhydrous tetrahydrofuran (1.5 mL) was cooled down to 0 °C under nitrogen. A 1.0 M solution of lithium hexamethyldisilylamide in THF (932 μL, 0.932 mmol) was added to the mixture and the reaction solution was kept stirring for another 6 h at 0 °C. Water (1.5 mL) was added to quench the reaction and the mixture was extracted with EtOAc (3 × 2 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give a residue. The residue was purified by column chromatography to give the olefin **9** (167.2 mg, 92%) as a colorless oil: R_f 0.50 (EtOAc/Hex = 1:3); $[\alpha]_D^{29}$ +9.5 (*c* 1.3, $CHCl_3$); IR (thin film): *v* 3492, 2925, 1605, 1454, 1099 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.39–7.24 (m, 20H, ArH), 5.69–5.62 (m, 2H, H-5, H-6), 4.95 (t, *J* = 7.8 Hz, 1H, H-4), 4.92 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.82 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.81 (d, *J* = 3.6 Hz, 1H, H-1′), 4.80 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.73 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.55 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.47 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.39 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.12 (dd, *J* = 6.6, 3.0 Hz, 1H, H-3), 4.05–4.02 (m, 2H, H-2′, H-5′), 3.96–3.93 (m, 2H, H-3′, H-4′), 3.77 (br s, 1H, H-2), 3.65 (dd, *J* = 10.8, 4.2 Hz, 1H, H-1a), 3.53–3.48 (m, 3H, H-1b, H-6a′, H-6b′), 2.73 (d, *J* = 5.4 Hz, 1H, 2-OH), 2.13–1.98 (m, 2H, H-7a, H-7b), 1.50 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.30–1.24 (m, 20H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 138.7 (C), 138.5 (C), 138.4 (C), 137.9 (C), 135.4 (CH), 128.33 (CH × 3), 128.31 (CH × 2), 128.2 (CH × 3), 127.9 (CH × 2), 127.72 (CH × 2), 127.65 (CH × 2), 127.54 (CH), 127.45 (CH), 127.4 (CH × 2), 125.0 (CH), 108.4 (C), 98.0 (CH), 79.0 (CH), 77.4 (CH), 76.3 (CH), 74.8 (CH), 74.7 (CH_2), 73.42 (CH_2), 73.37 (CH_2), 73.0 (CH), 72.9 (CH_2), 70.0 (CH_2), 69.4 (CH), 68.9 (CH_2), 68.6 (CH), 31.9 (CH_2), 29.64 (CH_2 × 3), 29.62 (CH_2 × 2), 29.58 (CH_2), 29.49 (CH_2), 29.47 (CH_2), 29.32 (CH_2), 29.25 (CH_2), 27.7 (CH_2), 27.1 (CH_3), 25.0 (CH_3), 22.7 (CH_2), 14.1 (CH_3); HRMS (FAB): *m*/*z* Calcd for $C_{55}H_{74}O_9Na$ ([M+Na]$^+$): 901.5231. Found: 901.5220.

4.4. (2*S*,3*S*,4*R*)-2-azido-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-octadec-5-en-1,3,4-triol (10)

A mixture of compound **9** (239 mg, 0.27 mmol) and pyridine (55 μL, 0.68 mmol) was dissolved in anhydrous dichloromethane (2.5 mL) under nitrogen, the reaction flask was immersed in an ice bath, and trifluoromethanesulfonic anhydride (126 μL, 0.41 mmol) was added dropwise to the solution. After stirring for 2 h at 0 °C, the solution of TMGA (171 mg, 1.08 mmol) in dichloromethane (1 mL) was injected into the reaction mixture and the reaction was stirred overnight at the same temperature. Water (5 mL) was added to the solution, the mixture was extracted with dichloromethane (3 × 5 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to provide a residue, which was purified by column chromatography to give azido-compound **10** (201 mg, 82%) as a colorless oil: R_f 0.60 (EtOAc/Hex = 1:6); $[\alpha]_D^{29}$ +1.80 (*c* 1.1, $CHCl_3$); IR (thin film): *v* 2924, 2854, 2100, 1455, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.39–7.23 (m, 20H, ArH), 5.75–5.70 (m, 1H, H-6), 5.46–5.42 (m, 1H, H-5), 4.98–4.96 (m, 1H, H-4), 4.94 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.92 (d, *J* = 3.6 Hz, 1H, H-1′), 4.84 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.80 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.72 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.71 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.56 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.48 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.40 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.09–4.04 (m, 2H, H-3, H-2′), 4.02 (dd, *J* = 10.8, 3.0 Hz, 1H, H-1a), 3.99–3.97 (m, 2H, H-3′, H-4′), 3.94 (m, 1H, H-5′), 3.65 (dd, *J* = 10.8, 7.2 Hz, 1H, H-1b), 3.53–3.48 (m, 3H, H-2, H-6a′, H-6b′), 2.18–2.06 (m, 2H, H-7a, H-7b), 1.42 (s, 3H, CH_3), 1.41–1.36 (m, 2H, CH_2), 1.23 (s, 3H, CH_3), 1.29–1.24 (m, 18H, CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 138.8 (C × 2), 138.6 (C), 137.9 (C), 135.8 (CH), 128.33 (CH × 2), 128.27 (CH × 2), 128.24 (CH × 2), 128.18 (CH × 2), 128.17 (CH × 2), 127.7 (CH × 2), 127.64 (CH), 127.57 (CH × 2), 127.53 (CH), 127.49 (CH × 2), 127.38 (CH × 2), 123.9 (CH), 108.9 (C), 98.7 (CH), 78.6 (CH), 76.5 (CH), 76.1 (CH), 75.1 (CH), 74.7 (CH_2), 73.4 (CH_2), 73.3 (CH), 73.2 (CH_2), 72.8 (CH_2), 69.7 (CH), 69.5 (CH_2), 69.0 (CH_2), 60.5 (CH), 31.9 (CH_2), 29.7 (CH_2), 29.64 (CH_2), 29.62 (CH_2), 29.57 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.28 (CH_2), 27.9 (CH_3), 27.7 (CH_2), 25.4 (CH_3), 22.7 (CH_2), 14.1 (CH_3); HRMS (ESI): *m*/*z* Calcd for $C_{55}H_{73}O_8N_3Na$ ([M+Na]$^+$): 926.5290. Found: 926.5275.

4.5. (2*S*,3*S*,4*R*)-2-Hexacosanoylamino-3,4-*O*-isopropylidene-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-octadec-5-en-1,3,4-triol (11)

To a solution of compound **10** (106 mg, 0.12 mmol) and triphenylphosphine (62 mg, 0.24 mmol) in ACS grade THF (1.5 mL) was added pyridine (0.5 mL). The reaction flask was warmed up to 60 °C, and the mixture was kept stirring for 12 h. The reaction was gradually cooled down to room temperature, hexaeicosanoic acid (60 mg, 0.15 mmol), HOBt (29 mg, 0.21 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbo-diimide hydro-chloride (EDC, 41 mg, 0.21 mmol), and triethylamine (17 μL, 0.12 mmol) were sequentially added to the solution, and the mixture was continuously stirred for 12 h. The reaction solution was diluted with EtOAc, and the resulting mixture was washed with water. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Purification of this residue via column chromatography afforded amide **11** (105 mg, 72%): R_f 0.43 (EtOAc/Hex = 1:4); $[\alpha]_D^{29}$ +19.6 (*c* 1.6, $CHCl_3$); IR (thin film) *v* 3316, 2919, 2850, 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.39–7.23 (m, 20H, ArH), 6.06 (d, *J* = 9.0 Hz, 1H, NH), 5.58–5.53 (m, 1H, H-6), 5.43–5.39 (m, 1H, H-5), 4.93 (d, *J* = 4.2 Hz, 1H, H-1′), 4.91 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.83 (dd, *J* = 9.0, 5.5 Hz, 1H, H-4), 4.81 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.79 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.73 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.66 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.56 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.49 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.37 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.23 (dd, *J* = 9.0, 6.0 Hz, 1H, H-3), 4.09–4.02 (m, 2H, H-2, H-2′), 3.98–3.88 (m, 4H, H-1a, H-3′, H-4′, H-5′), 3.65 (dd, *J* = 11.5, 2.5 Hz, 1H, H-1b), 3.50 (dd, *J* = 9.0, 6.5 Hz, 1H, H-6a′), 3.41 (dd, *J* = 9.5, 6.5 Hz, 1H, H-6b′), 2.08–1.84 (m, 4H, CH_2), 1.56–1.47 (m, 2H), 1.44 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.30–1.23 (m, 64H, CH_2), 0.87 (t, *J* = 7.0 Hz, 6H, CH_3 × 2); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 172.3 (C), 138.7 (C), 138.5 (C), 138.4 (C), 137.6 (C), 134.9 (CH), 128.4 (CH × 2), 128.36 (CH × 2), 128.35 (CH × 2), 128.3 (CH × 2), 128.2 (CH × 2), 127.9 (CH × 2), 127.8 (CH × 3), 127.76 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH × 2), 124.3 (CH), 108.3 (C), 99.3 (CH), 78.9 (CH), 76.8 (CH), 76.1 (CH), 74.7 (CH_2), 74.67 (CH), 73.5 (CH_2), 73.4 (CH_2), 73.1 (CH), 72.9 (CH_2), 69.7 (CH), 69.6 (CH_2), 69.1 (CH_2), 49.1 (CH), 36.7 (CH_2), 31.9 (CH_2 × 2), 29.7 (CH_2 × 20), 29.6 (CH_2 × 2), 29.5 (CH_2), 29.46 (CH_2 × 2), 29.3 (CH_2 × 3), 27.9 (CH_3), 26.7 (CH_2), 25.6 (CH_3), 25.4 (CH_2), 22.7 (CH_2 × 2), 14.1 (CH_3 × 2); HRMS (ESI): *m*/*z* Calcd for $C_{81}H_{126}O_9N$ ([M+H]$^+$): 1256.9427. Found: 1256.9472.

4.6. α-Galactosyl ceramide (1)

To a solution of compound **11** (104 mg) in a mixed solvent of MeOH/$CHCl_3$ (3:1 ratio, 1 mL) was added 8 N HCl (100 μL) and the reaction was stirred for 1 d. $Pd(OH)_2$/C (200 mg, Degussa type)

was added to the same flask and nitrogen was bubbled through the solution for 5 min. The reaction flask was equipped with a hydrogen balloon and the suspension was kept stirring for 2 d at room temperature. After restoring nitrogen, the mixture was filtered through celite and the filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography on sephadex G-10 to afford the target molecule **1** (67 mg, 94%): R_f 0.60 (MeOH/$CHCl_3$ = 1:4); $[\alpha]_D^{29}$ +38.2 (*c* 0.05, $CHCl_3$/MeOH); mp 182–185 °C; IR (KBr) ν 3426, 2918, 2851, 1645 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz): δ 8.55 (d, *J* = 8.4 Hz, 1H), 5.54 (d, *J* = 3.6 Hz, 1H), 5.23 (m, 1H), 4.65–4.25 (m, 10H), 2.42 (t, 2H), 2.32–2.21 (m, 1H), 1.92–1.57 (m,5H), 1.29–1.22 (m, 66H), 0.84 (t, 6H); ^{13}C NMR (C_5D_5N, 150 MHz): δ 173.3 (C), 101.4 (CH), 76.5 (CH), 73.0 (CH), 72.4 (CH), 71.5 (CH), 70.9 (CH), 70.2 (CH), 68.5 (CH_2), 62.6 (CH_2), 51.4 (CH), 36.8 (CH_2), 34.2 (CH_2), 32.1 (CH_2), 30.3–29.3 (CH_2), 26.5 (CH_2), 26.4 (CH_2), 22.9 (CH_2), 14.3 (CH_3); HRMS (ESI) Calcd for $C_{50}H_{100}O_9N$ ($[M+H]^+$) 858.7398. Found: 858.7408. Our NMR data of compound **1** matched well with the reported one.[8c]

Acknowledgments

The authors thank the National Science Council in Taiwan (NSC 99–2113-M-005–013-MY2 and NSC 101–2113-M-005–006-MY2) and National Chung Hsing University for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.11.008.

References

1. (a) Blanken, W. M.; Van den Eijnden, D. H. *J. Biol. Chem.* **1985**, *260*, 12927–12934; (b) *Synthesis in Lipid Chemistry*; Tyman, J. H. P., Ed.; Royal Society of Chemistry, Special Publications: Cambridge, 1994.; (c) Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 1532–1568; (d) Miyagi, T.; Wada, T.; Yamaguchi, K.; Hata, K. *Glycoconjugate J.* **2004**, *20*, 189–198.
2. Kobayashi, E.; Motoki, K.; Yamaguchi, Y.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem.* **1996**, *4*, 615–619.
3. (a) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592; (b) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.
4. (a) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629; (b) Matsuda, J. L.; Kronenberg, M. J. *Curr. Opin. Immunol.* **2001**, *13*, 19–25; (c) Hong, S.; Wilson, M. T.; Serizawa, I.; Wu, L.; Nagendra, S.; Naidenko, O.; Miura, T.; Haba, T.; Scherer, D. C.; Wie, J.; Kronenberg, M.; Koezuka, Y.; van Kaer, L. *Nat. Med.* **2001**, *7*, 1052–1056; (d) Sharif, S.; Arreaza, G. A.; Zucker, P.; Mi, Q.-S.; Sondhi, J.; Naidenko, O. V.; Kronenberg, M.; Koezuka, Y.; Delovitch, T. L. *Nat. Med.* **2001**, *7*, 1057–1062; (e) Kaer, L. V. *Nat. Rev. Immunol.* **2005**, *5*, 31–42; (f) Salio, M.; Ghadbane, H.; Shepherd, D.; Cypen, J.; Aichinger, M.; Jervis, P.; Besra, G. S.; Cresswell, P.; Cerundolo, V. *Mol. Immunol.* **2012**, *51*, 38–39.
5. (a) De Libero, G.; Mori, L. *Nat. Rev. Immunol.* **2005**, *5*, 485; (b) Tsuji, M. *Cell. Mol. Life Sci.* **2006**, 1889; (c) Wu, D.; Zajonc, D. M.; Fujio, M.; Sullivan, B. A.; Kinjo, Y.; Kronenberg, M.; Wilson, I. A.; Wong, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *11*, 3972; (d) Chang, Y. J.; Huang, J. R.; Tsai, Y. C.; Hung, J. T.; Wu, D.; Fujio, M.; Wong, C.-H.; Yu, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *25*, 10299.
6. For a review on synthesis of GalCer see— Morales-Serna, J. A.; Boutureira, O.; Díaz, Y.; Matheu, M. I.; Castillón, S. *Carbohydr. Res.* **2007**, *342*, 1595–1612.
7. (a) Du, W.; Gervay-Hague, J. *Org. Lett.* **2005**, *7*, 2063–2065; (b) Du, W.; Kulkarni, S. S.; Gervay-Hague, J. *Chem. Commun.* **2007**, 2336–2338; (c) Schombs, M.; Park, F. E.; Kulkarni, S. S.; Gervay-Hague, J. *J. Org. Chem.* **2010**, *75*, 4891–4898; (d) Kulkarni, S. S.; Gervay-Hague, J. In *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*; Demchenko, A. V., Ed.; Wiley-VCH, 2008; pp 59–93. and references therein.
8. Selected recent synthesis of α-GalCer analogs: (a) Facciotti, F.; Mori, L.; De Libero, G.; Lombardi, G.; Fallarini, S.; Panza, L.; Compostella, F.; Ronchetti, F. *J. Org. Chem.* **2007**, *72*, 7757–7760; (b) Tsujimoto, T.; Ito, Y. *Tetrahedron Lett.* **2007**, *48*, 5513–5516; (c) Michieletti, M.; Bracci, A.; Compostella, F.; Libero, G. D.; Mori, L.; Fallarini, S.; Lombardi, L. G.; Panza, L. *J. Org. Chem.* **2008**, *73*, 9192–9195; (d) Park, J.; Lee, J. H.; Ghosh, S. C.; Bricard, G.; Venkataswamy, M.; Porcelli, S. A.; Chung, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3906; (e) Boututeira, O.; Morales-Serna, J. A.; Diaz, Y.; Matheu, M. I.; Castillón, S. *Eur. J. Org. Chem.* **1851**, *2008*; (f) Veerapen, N.; Brigl, M.; Garg, S.; Cerundolo, V.; Cox, L. R.; Brenner, M. B.; Besra, G. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4288; (g) Enders, D.; Terteryan, V.; Paleček, J. *Synthesis* **2010**, 2979–2984; (h) Khaja, S. D.; Kumar, V.; Ahmad, M.; Xue, J.; Matta, K. L. *Tetrahedron Lett.* **2010**, *51*, 4411–4414.
9. (a) Morita, M.; Sawa, E.; Yamaji, K.; Sakai, T.; Natori, T.; Koezuka, Y.; Fukushima, H.; Akimoto, K. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 288–292; (b) Lin, C. -C.; Fan, G. -T.; Fang, J. -M. *Tetrahedron Lett.* **2003**, *44*, 5281–5283 and references cited therein.
10. (a) Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Fan, H.-F.; Pai, C.-L.; Yang, W.-C.; Lu, L.-D.; Hung, S.-C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2360–2362; (b) Wang, C.-C.; Zulueta, M. M. L.; Hung, S.-C. *Chimia* **2011**, *65*, 54–58.
11. (a) Tsai, Y.-F.; Shih, C.-H.; Su, Y.-T.; Yao, C.-H.; Lian, J.-F.; Liao, C.-C.; Hsia, C.-W.; Shui, H.-A.; Rani, R. *Org. Biomol. Chem.* **2012**, *10*, 931–934; (b) Pearson, M. S. M.; Floquet, N.; Bello, C.; Vogel, P.; Plantier-Royon, R.; Szymoniak, J.; Bertus, P.; Behr, J.-B. *Bioorg. Med. Chem.* **2009**, *17*, 8020–8026.
12. Kulkarni, S. S.; Gervay-Hague, J. *Org. Lett.* **2006**, *8*, 5765–5768.
13. Luo, S.-Y.; Kulkarni, S. S.; Chou, C.-H.; Liao, W.-M.; Hung, S.-C. *J. Org. Chem.* **2006**, *71*, 1226–1229.
14. TMGA is prepared freshly as per the reported procedure—Wagner, R.; Tilley, J. W.; Lovey, K. *Synthesis* **1991**, 785–786.
15. Tuch, A.; Saniere, M.; Le Merrer, Y.; Depezay, J.-C. *Tetrahedron: Asymmetry* **1996**, *7*, 897–906.