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A convergent total synthesis of a new antiepileptic ceramide and its triacetyl derivative using olefin cross metathesis

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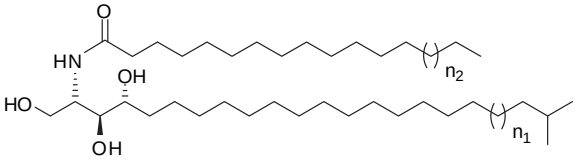
ABSTRACT

A convergent total synthesis of a new antiepileptic ceramide **1b** and its triacetyl derivative **1b′** was completed by using two important C–C bond forming reactions, Wittig methylenation and olefin cross metathesis as the key steps. The easily available 3,4,6-tri-*O*-benzyl-D-galactal was used as a chiral pool for the synthesis of highly functionalized amide **3** and the commercially available 1,12-dodecanediol for the long chain olefin counterpart **4**. The long hydrocarbon chain of the new ceramide **1b** was installed by using olefin cross metathesis between amide **3** and long chain terminal olefin **4** followed by hydrogenation.

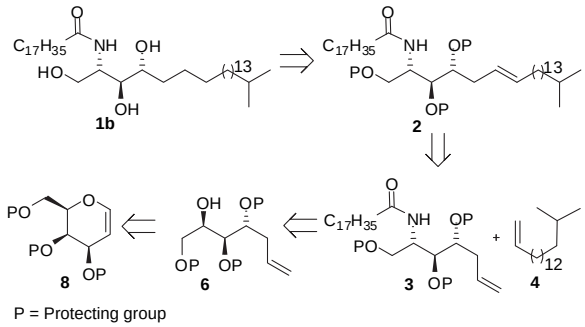
1. Introduction

Epilepsy is a seizure disorder, which affects the nervous system. The disease is characterized by 40 different types of recurrent unprovoked seizures.[1] According to WHO around 50 million population of world is affected by epilepsy out of which 90% found in developing countries.[2] Although the current marketed drugs provide ample seizure control in many patients, they have to compromise with notable adverse side effects and about 28–30% patients are not seizure free with the available antiepileptic drugs.[3] As the epilepsy cannot be totally controlled by available medicines, there is always need of safe and more effective antiepileptic agents to control epilepsy. In 2008, Ahmed et al.[4] reported isolation of ceramide mixture **1** having two ceramides **1a** and **1b** from *Negombata corticata*, a red sea sponge (Fig. 1). This mixture was found to exhibit in vivo anticonvulsant[5a] activity comparable to diazepam.[5b] The antiepileptic activity of this mixture of natural products **1a** and **1b** and, also as a result of our interest toward the total synthesis of natural products and their analogues starting from commercially available sugars[6] motivated us to take attempt toward the total synthesis of ceramide **1a** or **1b**. Herein we wish to report a chiron approach to the total synthesis of ceramide **1b** via olefin cross metathesis reaction between two terminal olefin building blocks.[5c]



1a $n_1 = 3, n_2 = 1$
1b $n_1 = 1, n_2 = 3$

Figure 1. Structure of stereo isomeric ceramide **1a** and **1b**.



P = Protecting group

Scheme 1. Retrosynthesis analysis of ceramide **1b**.

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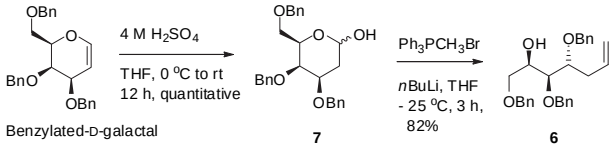
2. Results and discussion

The retrosynthetic strategy for target ceramide **1b** is depicted in Scheme 1. We envisaged that **1b** could be elaborated from protected amide **2** by hydrogenation of the double bond and removal of protecting groups. The amide **2** could in turn be prepared by utilizing the olefin cross metathesis between the amide **3** and the long chain olefin counterpart **4**. The amide part **3** could be obtained from D-galactal derived alcohol **6** by S_N2 displacement of free OH with a nitrogen nucleophile to generate amino functionality and subsequent N-acylation of the free amine.

As per retrosynthesis shown in Scheme 1, synthesis of assembly **3** was commenced from readily available 3,4,6-tri-*O*-benzyl-D-galactal. Its exposure to 4 M H_2SO_4 in THF furnished 2-deoxy-D-galactose **7** quantitatively (Scheme 2).[7] In the next step Wittig methylenation of hemiacetal **7** was essentially required to obtain alcohol **6**. Therefore, several trial experiments were carried out to optimize the condition for Wittig methylenation[8] of the hemiacetal **7** with methyltriphenylphosphonium bromide in the presence of *n*-BuLi (1.6 M solution in THF) at various temperatures (0 °C to −30 °C). Here, performing the reaction at −20–0 °C was not very clean. The column chromatographic purification of the reaction mixture led to the isolation of the desired olefin **6** in less than 30% yield along with an inseparable mixture containing **6** and by products. However, the reaction was found trouble free when it was strictly carried out below −20 °C and the best result was obtained when the reaction was performed at −25 °C using an ethanol bath cooled by an immersion cooler furnishing **6** in 82% yield (Table 1).



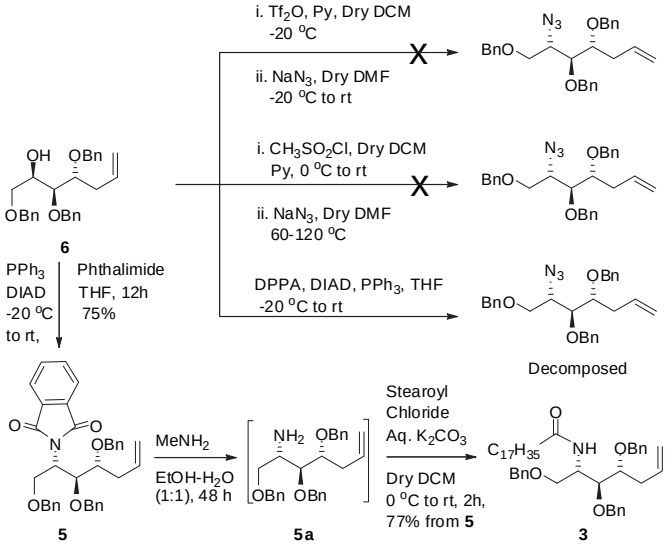
Scheme 2. Synthesis of alcohol **6**.

Table 1
Wittig reaction of hemiacetal **7** with CH_3PPh_3Br and *n*-BuLi under different conditions

Entry	Temperature (in °C)	Time (h)	Isolated yield (%) of **6**
1	0 to rt	2	<10
2	−5	2	12
3	−10	2.5	17
4	−15	2.5	20
5	−20	3	27
6	**−25**	**3**	**82**
7	−30	6	80

To complete the synthesis of compound **3**, the free hydroxyl group at C-2 in compound **6** had to be converted into amino functionality with inversion of configuration. This requires S_N2 displacement of OH with a nitrogen nucleophile. Unfortunately, the stereochemical inversion at C-2 was unsuccessful when **6** was subjected to Mitsunobu inversion in presence of triphenylphosphine (TPP), diphenylphosphoryl azide (DPPA), and diisopropyl azidocarboxylate (DIAD) in THF at −20 °C to 0 °C.[8a] To overcome this problem, the free OH in **6** was derivatized to 2-*O*-mesylate but its S_N2 displacement with sodium azide in DMF was disappointing and yielded a complex mixture of products. Similarly, S_N2 displacement of its 2-*O*-triflate derivative with sodium azide at 0 °C to room temperature was also futile (Scheme 3).

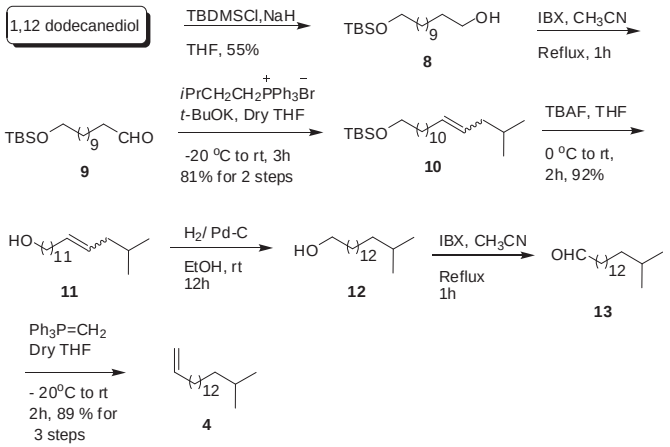
Gratifyingly, when the alcohol **6** was subjected to Mitsunobu inversion in presence of PPh_3, phthalimide, and DIAD in THF at −20 °C to room temperature, the desired inverted phthalimido compound **5** was obtained in 75% yield as a sole stereoisomer (Scheme 3).[8b,9,10] The next step was conversion of phthalimido



Scheme 3. Synthesis of amide **3**.

complex **5** to the corresponding amine and this was done smoothly by treating **5** with $MeNH_2$ in $EtOH/H_2O$ (1:1) for 48 h at room temperature.[11] The crude amine was passed through a short filter column of silica gel and then the required N-acylation[12] was accomplished by treating the column filtered amine with stearoyl chloride in presence of aqueous K_2CO_3 to obtain **3** with the natural ceramide backbone in 77% yield over two steps (Scheme 3).

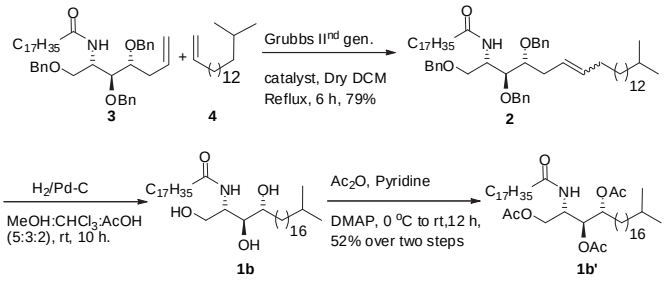
With the assembly **3** in hand, our next attempt was focused toward the synthesis of long chain olefin counterpart **4**. To accomplish its synthesis, the commercially available 1,12-dodecanediol was protected with TBSCl to give the monoprotected 12-(*tert*-butyldimethylsilyloxy)dodecan-1-ol **8** in 55% yield.[13] Oxidation of the long chain primary alcohol **8** with IBX in acetonitrile and Wittig olefination[6c] of the resulting aldehyde **9** furnished olefin **10** in 81% yield over two steps. The cleavage of silyl ether in **10** with TBAF in THF yielded alcohol **11** in excellent yield (92%). The internal double bond of alcohol **11** was then hydrogenated with H_2 in presence of Pd/C to obtain the saturated alcohol **12**, which on oxidation with IBX in acetonitrile followed by Wittig olefination of the resulting aldehyde **13** furnished the desired long chain olefin **4** in 89% yield over three steps (Scheme 4).



Scheme 4. Synthesis of alkene **4**.

Having synthesized two building blocks **3** and **4**, our next target was to couple them together to obtain **2**, a synthetic precursor of ceramide **1b**. Removal of double bond and benzyl ether protection by hydrogenation of **2** should then furnish the target molecule **1b**. Thus, the cross metathesis between **3** and **4** in presence of Grubbs'

second generation catalyst (5 mol %) generated compound **2** (Scheme 5).[14] It was observed that by increasing the equivalents of long chain alkene **4** the yield of cross metathesis product was enhanced[6a,6b] and therefore, when 3 equiv of alkene **4** were used the cross metathesis product **2** was obtained in 79% yield (Table 2, entry 3).



Scheme 5. Synthesis of ceramide **1b** and **1b′**.

Table 2
Optimization of cross metathesis reaction of **3** with long chain alkene **4** in DCM in presence of Grubbs' second generation catalyst (5 mol %)

Entry	Equivalent of alkene **4**	Time (h)	Isolated yield (%) of **2**
1	1	6	62
2	2	6	70
3	**3**	**6**	**79**
4	5	6	81

Unfortunately our efforts to cleave three benzyl groups and reduction of the internal double bond in **2** under an atmosphere of H_2 in presence of catalytic amount of 10% Pd/C or $Pd(OH)_2$ by dissolving it in different solvent systems ended with very low yield of the desired product **1b** and it was presumably due to the problem associated with poor solubility of the corresponding intermediates and the target ceramide formed during hydrogenation. However, the hydrogenation of **2** in MeOH/$CHCl_3$/AcOH (5:3:2) by H_2 in the presence of 10% Pd/C by adopting the method reported by Lee et al. was proceeded smoothly to provide the target natural product **1b** (Table 3).[15] The chromatographic purification of the reaction mixture furnished **1b** in 28% yield. Here, it is presumed that most of the hydrogenated product got stuck in the column during its column chromatography (silica gel) owing to the highly polar nature of the ceramide **1b** and thus reduces its isolated yield.

Table 3
Optimization of solvent system and catalyst for hydrogenation reaction of compound **2**

Entry	Solvent system	Catalyst	Isolated yield[a] (%)
1	MeOH+$CHCl_3$ (1:1)	$Pd(OH)_2$	16
2	MeOH+$CHCl_3$ (2:1)	$Pd(OH)_2$	19
3	EtOAc+$CHCl_3$ (1:1)	Pd/C	12
4	MeOH+$CHCl_3$ (1:1)	Pd/C	10
5	MeOH+$CHCl_3$ (2:1)	Pd/C	17
6	**MeOH/$CHCl_3$/AcOH (5:3:2)**	**Pd/C**	**52**

[a] Isolated yield of the acetylated ceramide **1b′** from **2** (over two steps, hydrogenation and acetylation).

Ahmed et al. reported ^1H and ^{13}C NMR spectra of ceramide mixture **1** (**1a** and **1b**) in $CHCl_3$ as well as in C_5D_5N.[4] Our synthesized chromatographic pure ceramide **1b** was insoluble in $CHCl_3$ and also in MeOH but was sparingly soluble in $CHCl_3$/MeOH mixture (2:1). Here, the ^1H NMR of **1b** was recorded in $CDCl_3$ and CD_3OD mixture but due to the solubility problem, we were not able to record its ^{13}C NMR spectrum. In order to further confirm its structure, the precursor **2** was hydrogenated with H_2 in presence of 10% Pd/C and the resulting worked up crude product mixture was treated with Ac_2O and pyridine in the presence of catalytic amount of DMAP to obtain its corresponding triacetyl derivative **1b′** in 52% yield over two steps from **2** (Scheme 5 and Table 3, entry 6). The identification of the triacetyl derivative **1b′** was completed by analyzing its detailed spectral data, which further led to confirm the formation of the target trihydroxy natural ceramide **1b** (see Supplementary data).

3. Conclusion

In summary, herein we achieved the first total synthesis of ceramide **1b** and its triacetyl derivative **1b′** by utilizing olefin cross metathesis reaction between long chain alkene **4** and amide **3**. The highly functionalized amide **3** with a terminal double bond could also serve as a versatile building block for synthesis of various kinds of ceramides. Since the reported natural ceramide mixture **1** (**1a** and **1b**) was found to exhibit in vivo anticonvulsant activity comparable to diazepam,[4] this class of compounds and their analogues thus may deserve interest for their development as more potent and safe antiepileptic agents. Therefore, by using carbohydrate based amide building block **3** one can install different cross olefin counterparts and this flexibility will facilitate the synthesis of various analogues of this ceramide for more improved anticonvulsant activity.

4. Experimental section

4.1. General

All the organic solvents were dried by standard methods. NMR spectra of the synthesized compounds were recorded on Bruker Avance DPX 200FT, Bruker Robotics, and Bruker DRX 300 Spectrometers at 200, 300 MHz (^1H) and 50, 75 MHz (^{13}C), respectively. Experiments were recorded in $CDCl_3$ and CD_3OD at 25 °C. Chemical shifts are given on the δ scale and are referenced to the TMS at 0.00 ppm for proton and 0.00 ppm for carbon. For ^{13}C NMR reference $CDCl_3$ appeared at 77.4 ppm. Mass spectra were recorded on a JEOLJMS-600H high resolution spectrometer using EI and DART mode at 70 eV and IR spectra on Perkin–Elmer 881 and FTIR-8210 PC Shimadzu Spectrophotometers. Analytical TLC was performed using 2.5×5 cm plates coated with a 0.25 mm thickness of silica gel (60 F-254), and visualization was accomplished with $CeSO_4$ (1% in 2 N H_2SO_4) followed by charring over hot plate. Silica gel (100–200 and 230–400 mesh) was used for column chromatography. All the products were characterized by ^1H, ^{13}C, IR, ESI-MS spectroscopy. Optical rotations were determined on an Autopol III polarimeter using a 1 dm cell at 28 °C in chloroform as the solvents; concentrations mentioned are in g/100 mL. Low-temperature reactions were performed by using immersion cooler with ethanol as the cooling agent. Grubbs' second generation catalyst was purchased from Sigma–Aldrich Co.

4.2. Compound 6

Methyltriphenylphosphonium bromide (2.9 g, 8.1 mmol) was taken in dry THF (25 mL) and the mixture was cooled to −78 °C. *n*-BuLi (3.62 mL, 5.8 mmol, 1.6 M in THF) was added to the mixture dropwise under N_2 atmosphere and the resulting mixture was stirred for 1 h at −30 °C. Hemiacetal **7** (0.504 g, 1.16 mmol) in THF (3 mL) was added to the reaction mixture in dropwise manner. The reaction mixture was then stirred at −25 °C for 3 h. After completion of the reaction it was quenched with saturated aqueous NH_4Cl, and the mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed twice with brine, dried over Na_2SO_4, and evaporated under reduced pressure to give a residue.

Column chromatography purification of the residue yielded compound **6** as an oil (0.411 g, 0.95 mmol, 82%).

Eluent for column chromatography: EtOAc/hexane (1/15, v/v); R_f=0.34 (1/6, EtOAc/hexane); $[\alpha]_D^{28}$ −60.82 (*c* 1.43, $CHCl_3$); IR (neat, cm^{-1}): 3459, 3021, 2926, 2360, 1549, 1216; ^1H NMR (200 MHz, $CDCl_3$) δ 2.41–2.46 (m, 2H), 2.84 (br s, 1H, OH), 3.49 (m, 2H), 3.61 (q, *J*=2.8 Hz, 1H), 3.68–3.77 (m, 1H), 4.00 (m, 1H), 4.40–4.69 (m, 6H), 5.04–5.13 (m, 2H), 5.75–5.90 (m, 1H), 7.22–7.33 (m, 15H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4) δ 35.8 (CH_2), 70.0 (CH), 71.6 (CH_2), 72.9 (CH_2), 73.8 (CH_2), 74.1 (CH_2), 79.3 (CH), 79.7 (CH), 117.9 (=CH_2), 128.1–128.8 (15×ArC), 135.3 (=CH), 138.5–138.6 (3×Ar–qC); DART–HRMS: *m*/*z* $[M+H]^+$, calcd for $C_{28}H_{33}O_4$ 433.2379, found 433.2364.

4.3. Compound 5

A solution of phthalimide (342 mg, 2.32 mmol), triphenylphosphine (609 mg, 2.32 mmol), and the alcohol **6** (500 mg, 1.16 mmol) in dry THF (10 mL) was cooled to −20 °C under argon atmosphere. An ice cooled solution of DIAD (0.46 mL, 2.32 mmol) in dry THF (2 mL) was added dropwise to the solution and then the reaction mixture was stirred at the same temperature for 2 h and then at room temperature. After overnight stirring, the reaction mixture was evaporated under reduced pressure to give a residue, which on column chromatographic purification provided compound **5** (490 mg, 0.87 mmol, 75%).

Eluent for column chromatography: EtOAc/hexane (1/15, v/v); R_f=0.44 (1/6, EtOAc/hexane); $[\alpha]_D^{28}$ −103.3 (*c* 1.27, $CHCl_3$); IR (neat, cm^{-1}): 3855, 3750, 3679, 3455, 3022, 2364, 1708, 1650, 1519, 1216; ^1H NMR (300 MHz, $CDCl_3$) δ 2.35–2.41 (m, 1H), 2.47–2.52 (m, 1H), 3.40–3.43 (m, 1H), 3.89 (dd, *J*=4.1, 10.2 Hz, 1H), 4.13 (t, *J*=10.3 Hz, 1H), 4.35–4.50 (m, 4H), 4.59–4.67 (m, 3H), 4.90 (d, *J*=11.2 Hz, 1H), 4.96 (d, *J*=10.2 Hz, 1H), 5.04 (dd, *J*=1.3, 17.2 Hz, 1H), 5.77–5.84 (m, 1H), 7.13–7.32 (m, 15H, ArH), 7.69 (m, 2H, ArH), 7.79 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.2 (CH_2), 52.1 (CH), 67.6 (CH_2), 71.9 (CH_2), 73.0 (CH_2), 74.5 (CH_2), 76.8 (CH), 80.4 (CH), 117.2 (=CH_2), 123.7 (2×Ar–C), 127.8–128.7 (15×Ar–C), 132.2 (2×Ar–qC), 134.3 (2×Ar–C), 135.9 (=CH), 138.4 (Ar–qC), 138.5 (Ar–qC), 138.6 (Ar–qC), 168.7 (2×C=O); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{36}H_{36}NO_5$ 562.2594, found 562.2594.

4.4. Compound 3

A solution of compound **5** (170 mg, 0.30 mmol) in EtOH/H_2O (1:1, 10 mL) was treated with a 40% aqueous solution of methyl amine (20 equiv) and stirred at room temperature for 48 h. The reaction mixture was then concentrated under reduced pressure, dissolved in water (15 mL), and extracted with ethyl acetate (3×10 mL). The combined organic extracts were washed twice with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude amine was passed through a filter column for purification.

To a solution of amine **5a** in DCM (7 mL) was added an aqueous potassium carbonate solution (3 mL, 50%) at 0 °C and at the same temperature after 10 min under vigorous stirring to this reaction mixture was added portion wise stearoyl chloride (0.11 mL, 0.33 mmol). The mixture was further vigorously stirred for 2 h at room temperature. After completion of the reaction, the reaction mixture was poured into brine and extracted with DCM (3×10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure to give a residue. Flash chromatography of this residue yielded compound **3** as an oil (162 mg, 0.23 mmol, 77% for two steps).

Eluent for column chromatography: EtOAc/hexane (1/20, v/v); R_f=0.50 (1/4, EtOAc/hexane); $[\alpha]_D^{28}$ −27.92 (*c* 2.43, $CHCl_3$); IR (neat, cm^{-1}): 3439, 2927, 2363, 1637, 1219; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J*=6.1 Hz, 3H), 1.25 (br m, 28H), 1.51 (t, *J*=5.9 Hz, 2H), 1.97–2.02 (m, 2H), 2.49–2.51(m, 2H), 3.50 (dd, *J*=4.0, 9.5 Hz, 1H), 3.55–3.60 (m, 1H), 3.76–3.80 (m, 2H), 4.23–4.31 (m, 1H), 4.39–4.84 (m, 6H), 5.04–5.16 (m, 2H), 5.75 (d, *J*=9.0 Hz, 1H), 5.85–5.98 (m, 1H), 7.26–7.35 (m, 15H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5 (CH_3), 23.1 (CH_2), 26.1 (CH_2), 29.7–30.1 (12 CH_2), 32.3 (CH_2), 34.7 (CH_2), 37.2 (CH_2), 49.8 (CH), 69.4 (CH_2), 72.2 (CH_2), 73.5 (CH_2), 74.0 (CH_2), 79.4 (CH), 80.4 (CH), 117.3 (=CH_2), 128.0–128.8 (15×Ar–C), 136.0 (=CH), 138.4 (Ar–qC), 138.9 (Ar–qC), 139.0 (Ar–qC), 173.0 (C=O); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{46}H_{68}NO_4$: 698.5148, found 698.5127.

4.5. Compound 10

The mono TBS protected alcohol **8** (500 mg, 1.58 mmol) and IBX (1.7 g, 6.32 mmol) was taken in a 100 mL round bottom flask in acetonitrile (15 mL) and the resulting reaction mixture was refluxed for 1 h. The reaction mixture was then cooled to room temperature and diluted with ether and cooled to 0 °C. After 1 h the reaction mixture was filtered through a Celite bed and the filtrate was concentrated under reduced pressure to obtain an oil **9** (490 mg), which was immediately used for the next step without further purification.

3-Methyl-1-butane triphenylphosphonium bromide (1.96 g, 4.74 mmol) and *t*-BuOK (354 mg, 3.16 mmol) were taken in a flame dried two neck round bottom flask and cooled to −20 °C using an ethanol bath cooled by an immersion cooler. Dry THF (25 mL) was added to the reaction mixture under nitrogen atmosphere and it was stirred for 1 h without further cooling. After 1 h, the reaction mixture was again cooled to −20 °C. The solution of above obtained aldehyde **9** in THF (3 mL) was added to the mixture dropwise at −20 °C. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. After completion of the reaction, saturated aqueous NH_4Cl was added to the reaction mixture. The reaction mixture was extracted with EtOAc (3×20 mL). The combined organic layers were washed twice with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a residue. Column Chromatography of the residue yielded compound **10** as oil (472 mg, 1.28 mmol, 81% for two steps).

Eluent for column chromatography: EtOAc/hexane (1/30, v/v); R_f=0.59 (1/49, EtOAc/hexane); IR (neat, cm^{-1}): 2928, 2857, 2363, 2106, 1218, 1096; ^1H NMR (300 MHz, $CDCl_3$) δ 0.05 (m, 6H), 0.88–0.91 (m, 15H), 1.27 (m, 16H), 1.47–1.67 (m, 3H), 1.89–2.04 (m, 4H), 3.60 (t, *J*=6.6 Hz, 2H), 5.32–5.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9 (2×CH_3), 18.8 (qC), 22.8 (2×CH_3), 26.2 (CH_2), 26.4 (3×CH_3), 27.7 (CH_2), 29.1 ($CH(CH_3)_2$), 29.8–30.2 (7×CH_2), 33.3 (CH_2), 36.8 (CH_2), 63.7 (CH_2), 128.9 (=CH), 131.0 (=CH); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{23}H_{49}OSi$ 369.3553, found 369.3531.

4.6. Compound 11

To a stirred solution of **10** (450 mg, 1.22 mmol) in THF (10 mL) was added TBAF (1.35 mL, 1.0 M solution in THF) at 0 °C and left for stirring at room temperature. After 2 h, saturated aqueous solution of NH_4Cl (20 mL) was added to the reaction mixture and it was extracted with EtOAc (3×15 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give a residue, which on column chromatographic purification provided compound **11** (285 mg, 1.12 mmol, 92%) as a colorless oil.

Eluent for column chromatography: EtOAc/hexane (1/15, v/v); R_f=0.44 (1/4, EtOAc/hexane); IR (neat, cm^{-1}): 3414, 2364, 1637, 1440, 1219; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87–0.89 (m, 6H), 1.27 (m, 16H), 1.51–1.63 (m, 4H), 1.89–2.03 (m, 4H), 3.62 (t, *J*=6.6 Hz, 2H), 5.31–5.43 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7 (2×CH_3), 26.1 (CH_2), 27.7 (CH_2), 29.1 (CH), 29.7–30.1 (7×CH_2), 33.2 (CH_2), 36.7

(CH_2), 63.4 (CH_2), 128.9 (=CH), 131.0 (=CH); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{17}H_{35}O$ 255.2688, found 255.2714.

4.7. Compound 12

Catalytic amount of 10% Pd/C (20 mg) was added to a solution of **11** (250 mg, 0.982 mmol) in ethanol (10 mL). A vacuum was created in a round bottom flask containing the above reaction mixture with the help of pump and left for stirring under a positive pressure of H_2 in a balloon. After the completion of the reaction (TLC control, 12 h) catalyst was removed by filtration, washed with methanol twice and the combined filtrate was concentrated to afford compound **12** (260 mg) as a semi-solid residue, which was immediately used for the next step without further purification.

Eluent for column chromatography: EtOAc/hexane (1/15, v/v); R_f=0.44 (1/4, EtOAc/hexane); IR (neat, cm^{-1}): 3423, 3022, 2929, 2368, 1217; ^1H NMR (200 MHz, $CDCl_3$) δ 0.84–0.87 (m, 6H), 1.25 (m, 24H), 1.45–1.54 (m, 3H), 3.61 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.0 (2×CH_3), 26.1 (CH_2), 27.8 (CH_2), 28.4 (CH), 29.8–30.3 (9×CH_2), 33.2 (CH_2), 39.5 (CH_2), 63.5 (CH_2); DART–HRMS: *m*/*z* $[M-H]^+$ calcd for $C_{17}H_{35}O$ 255.2688, found 255.2689.

4.8. Compound 4

To a solution of compound **12** (260 mg) in acetonitrile (6 mL) was added IBX (825 mg, 2.95 mmol) and the reaction mixture was allowed to stir under reflux for 1 h. The resulting mixture was then cooled to room temperature, diluted with anhydrous ether and cooled to 0 °C. After 1 h the resulting reaction mixture was filtered through a Celite bed and the filtrate obtained was concentrated under reduced pressure to give the corresponding aldehyde **13** (241 mg), which was immediately used for Wittig olefination without further purification.

To a precooled (−20 °C, using an ethanol bath cooled by immersion cooler) mixture of methyltriphenylphosphonium bromide (1.753 g, 4.91 mmol) and *t*-BuOK (330 mg, 2.95 mmol), dry THF (20 ml) was added and the reaction mixture was stirred without further cooling. After 1 h, the reaction mixture was again cooled to −20 °C and the solution of crude aldehyde **13** in dry THF (3 mL) was added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 2 h. After completion of the reaction, it was quenched with saturated aqueous NH_4Cl, and the resulting mixture was extracted with EtOAc (3×15 mL). The combined organic layers were washed twice with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Flash chromatography of the resulting residue yielded compound **4** as an oil (220 mg, 0.87 mmol, 89% for three steps).

Eluent for column chromatography: hexane; R_f=0.92 (hexane); IR (neat, cm^{-1}) 3022, 2926, 2855, 2362, 1647, 1521, 1217; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87–0.89 (m, 6H), 1.16–1.18 (m, 2H), 1.27 (m, 20H), 1.37–1.41 (m, 2H), 1.47–1.60 (m, 1H), 2.05 (dd, *J*=6.8, 12.5 Hz, 2H), 4.92–5.03 (m, 2H), 5.75–5.89 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1 (2×CH_3), 27.9 (CH_2), 28.4 (*C*H(CH_3)), 29.4 (CH_2), 29.6 (CH_2), 30.0 (CH_2), 30.1–30.2 (6×CH_2), 30.4 (CH_2), 34.3 (CH_2), 39.5 (CH_2), 114.5 (=CH_2), 139.6 (=CH); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{18}H_{37}$ 253.2895, found 253.2877.

4.9. Compound 2

To a 50 mL two necked oven dried round bottom flask fitted with a reflux condenser and septum was added Grubbs' second generation catalyst (12 mg, 0.014 mmol) under argon atmosphere. Dry degassed CH_2Cl_2 (2 mL) was then added to the above round bottom flask through a syringe and the solution was kept for stirring. Compounds **3** (200 mg, 0.287 mmol) and long chain alkene **4** (220 mg, 0.86 mmol) in DCM (2 mL each) were added in succession through a syringe to the stirring reaction mixture. The septum was replaced with a glass stopper while the stirring was continued. The solution was refluxed for 6 h. The temperature of the reaction mixture was brought slowly to room temperature. The organic solvent was evaporated under reduced pressure to give a black residue, which was purified by column chromatography to give compound **2** as a semi-solid compound (210 mg, 0.228 mmol, 79%).

Eluent for column chromatography: EtOAc/hexane (1/20, v/v); R_f=0.61 (1/4, EtOAc/hexane); $[\alpha]_D^{28}$ −20.98 (*c* 0.33, $CHCl_3$); IR (neat, cm^{-1}) 3421, 2927, 2855, 2364, 1641, 1217; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85–0.87 (m, 9H), 1.16–1.18 (m, 2H), 1.25 (m, 50H), 1.49–1.56 (m, 3H), 1.95–1.98 (m, 4H), 2.34–2.43 (m, 2H), 3.50–3.54 (m, 2H), 3.73–3.78 (m, 2H), 4.32 (br s, 1H), 4.43–4.81 (m, 6H), 5.48–5.52 (m, 2H), 5.71 (d, *J*=9.1 Hz, NH), 7.29–7.30 (br m, 15H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.46 (CH_3), 23.0 (2×CH_3), 23.1 (CH_2), 26.1 (CH_2), 27.8 (CH_2), 28.4 (CH), 29.7–29.8 (4×CH_2), 29.9 (2×CH_2), 30.0 (CH_2), 30.1 (15×CH_2), 32.3 (CH_2), 33.1 (CH_2), 33.5 (CH_2), 37.2 (CH_2), 39.5 (CH_2), 49.8 (CH), 69.5 (CH_2), 72.2 (CH_2), 73.4 (CH_2), 73.9 (CH_2), 79.6 (CH), 80.7 (CH), 126.9 (=CH), 127.9–128.8 (15×ArC), 133.6 (=CH), 138.5 (Ar–qC), 139.0 (Ar–qC), 139.1 (Ar–qC), 172.9 (HN*C*=O); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{62}H_{100}N_1O_4$ 922.7652, found 922.7644.

4.10. Compound 1b

Catalytic amount of 10% Pd/C (20 mg) was added to a solution of **2** (40 mg, 0.043 mmol) in MeOH/$CHCl_3$/AcOH (5:3:2, 5 mL). A vacuum was created in a round bottom flask containing the above reaction mixture with the help of pump and left for stirring under H_2 in a balloon with a positive pressure. After the completion of the reaction (TLC control, 10 h), the catalyst was removed by filtration, washed with MeOH/$CHCl_3$ (1:2, 2×10 mL) and the combined filtrate was concentrated under reduced pressure to afford a solid residue, which was purified by column chromatography to give **1b** (8 mg, 0.012 mmol, 28%) as a white powder.

Eluent for column chromatography: MeOH/$CHCl_3$ (1/5, v/v); R_f=0.55 (1/9, MeOH/$CHCl_3$); $[\alpha]_D^{28}$ +41.23 (*c* 0.11, $CHCl_3$); IR (neat, cm^{-1}) 3424, 2921, 2853, 2359, 1644, 1219; ^1H NMR (300 MHz, $CDCl_3$+CD_3OD) δ 0.85–0.87 (m, 9H), 1.16 (m, 2H), 1.26 (br m, 56H), 1.47–1.68 (m, 5H), 2.21 (t, *J*=7.1 Hz, 2H), 3.38 (m, 2H), 3.54 (m, 1H), 3.78–3.82 (m, 1H), 4.08 (m,1H), 6.93 (d, 1H, *J*=8.9 Hz, NH); DART–HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{41}H_{84}N_1O_4$ 654.6400, found 654.6404.

4.11. Compound 1b′

The crude trihydroxy ceramide (35 mg) obtained after hydrogenation of compound **2** (50 mg, 0.054 mmol) was taken in pyridine (1 mL), cooled to 0 °C and after five min Ac_2O (0.5 mL) and catalytic amount of DMAP was added to it. The reaction mixture was allowed to stir for overnight. After completion of the reaction, the reaction mixture was concentrated and purified by column chromatography to furnish acetylated ceramide **1b′** (22 mg, 0.028 mmol, 52% over two steps).

Eluent for column chromatography: EtOAc/hexane (1/7, v/v); R_f=0.55 (1/3, EtOAc/hexane); $[\alpha]_D^{28}$ +30.74 (*c* 0.21, $CHCl_3$); IR (neat, cm^{-1}) 3427, 3022, 2924, 2362, 1713, 1654, 1218; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H), 0.87 (m, 6H), 1.16 (m, 2H), 1.26 (m, 56H), 1.41–1.58 (m, 5H), 2.04 (s, 6H), 2.07 (s, 3H), 2.18–2.23 (m, 2H), 3.99 (dd, *J*=2.9, 11.7 Hz, 1H), 4.26–4.31 (m, 1H), 4.44–4.51 (br m, 1H), 4.91–4.95 (m, 1H), 5.09–5.12 (m, 1H), 5.94 (d, *J*=9.4 Hz, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5 (CH_3), 21.1 (CH_3), 21.2 (CH_3), 21.4 (CO*C*H_3), 23.0 (2×CO*C*H_3), 23.1 (CH_2), 25.9 (CH_2), 26.0 (CH_2), 27.8 (CH_2), 28.4 (CH), 28.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (2×CH_2), 29.9 (2×CH_2), 30.1 (17×CH_2), 30.4 (CH_2), 32.3 (CH_2), 37.2 (CH_2), 39.5 (CH_2), 47.8 (CH), 63.3 (CH_2), 72.4 (CH), 73.4 (CH), 170.5 (CH_3C=*O*),

171.3 ($CH_3C{=}O$), 171.5 ($CH_3C{=}O$), 173.2 ($NHC{=}O$); DART–HRMS: m/z $[M+H]^+$ calcd for $C_{47}H_{90}N_1O_7$ 780.6717, found 780.6708.

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.07.049. These data include MOL files and InChIKeys of the most important compounds described in this article.

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