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Synthesis of Sphingolipids with an ω-Esterified Long Acyl Chain, Ceramide Components of the Human Epidermis*

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Esterified ceramides, (2*S*,3*R*,4*E*)-2-[(30′-stearoyloxy)triacontanamido]octadec-4-ene-1,3-diol (2) and (2*S*,3*R*,4*E*,6*R*)-2-[(30′-stearoyloxy)triacontanamido]octadec-4-ene-1,3,6-triol (4), are minor components of the human stratum corneum. We synthesized these ceramides by employing olefin cross metathesis as the key reaction for constructing their ω-hydroxytriacontanoyl part (13).

Key words: ceramide; olefin cross metathesis; epidermis; sphingolipid; stratum corneum

The stratum corneum is the upper layer of the epidermis. It protects the body from loss of water and against intrusion of environmental damaging factors.[2,3] Ceramides are the major lipid components, accounting for 30–40% by weight of the mammalian stratum corneum.[2] To date, nine subclasses of free extractable ceramides have been identified from the human stratum corneum.[4–8] Among these, ceramides 1, 4, and 9 are structurally unique sphingolipids, and respectively comprise 8%, 5%, and 6% of free and extractable ceramides.[4] They have a long acyl chain possessing an ω-hydroxy group esterified with a fatty acid like linoleic acid. It has been proposed that these esterified ceramides function as molecular rivets in locking together the multiple intercellular lamellae with their alkyl chains in the stratum corneum.[9,10]

Esterified ceramides **1**, **3**, and **5** (Fig. 1) are the major respective components of ceramides 1, 4, and 9.[4–8] They have an ω-linoleoyloxy acyl group, but it has not yet been clarified why the linoleoyl group is so abundantly distributed in the epidermal ceramides. We have reported the synthesis of **1** and **3**, and confirmed their structures as shown.[11,12] Due to the fragility of the 1,4-diene structure of the linoleoyl part, ceramides **1** and **3** are unstable. Therefore Dr. S. Hamanaka, a dermatologist, asked us to synthesize ω-stearoylated ceramides **2** and **4** as stable standard samples for a dermatological study. It is known that **2** and **4** are natural minor components of ceramides 1 and 4.[4] We reported in 1992 a synthesis of ω-stearoyloxy ceramide with an icosasphingosine (C_{20}) chain.[13] However, the synthesis of its sphingosine (C_{18})-type ceramide (**2**) and 6-hydroxysphingosine-type one (**4**) have not previously been reported.

We synthesized **2** and **4** from 15-pentadecanolide (**6**) in 9 steps as shown in Schemes 1 and 2. The respective

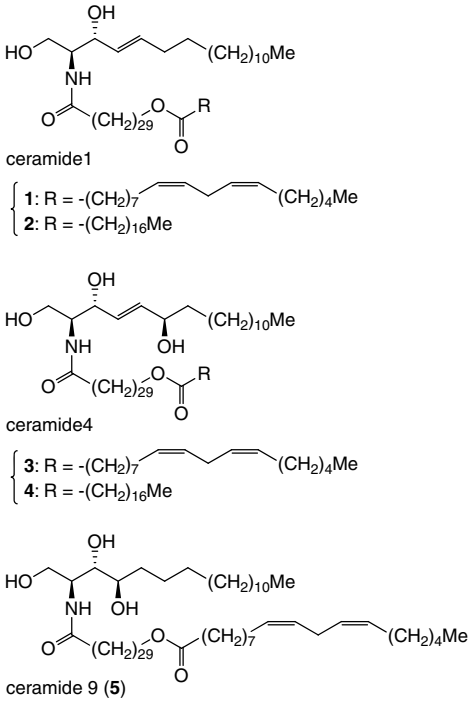

Fig. 1. Structures of Esterified Ceramides **1**–**5**, Components of the Human Stratum Corneum.

overall yields of **2** and **4** were 17% and 12%, based on **6**. We employed olefin cross metathesis with Grubbs's first-generation catalyst as the key reaction to construct their ω-hydroxytriacontanoic acid part.[14–16]

Results and Discussion

Our previous synthesis of **1** and **3**,[11,12] including icosasphingosine-type ceramide 1,[13] employed the Wittig reaction for constructing their ω-hydroxytriacontanoyl part.[17] Although the overall yield is generally very high, the Wittig reaction requires the cumbersome preparation of both an ylide and an aldehyde, and makes the synthetic pathway redundant. We chose in this present synthesis the olefin cross metathesis reaction to assemble the long acyl chain part.[14–16]

Figure 2 summarizes the retrosynthetic analysis of **2** and **4**. We considered that the synthesis of the target ceramides, including **1** and **3**, could be achieved by a two-step conversion, acylation and deprotection, from

* Synthesis of sphingosine relatives, Part 35. See Ref. 1 for Part 34.

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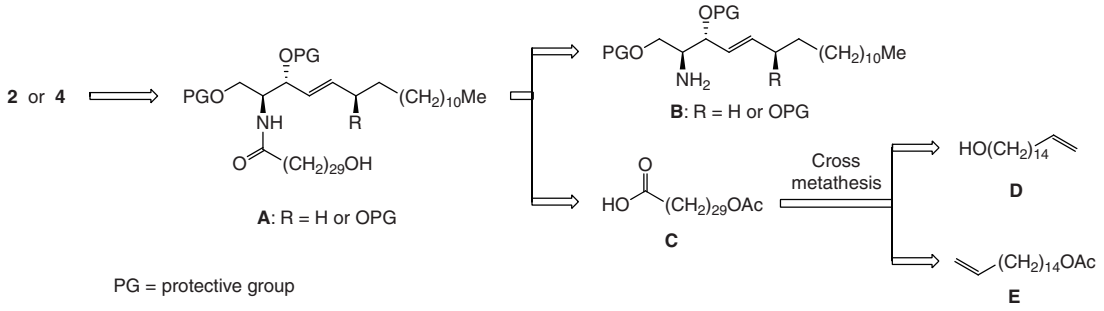
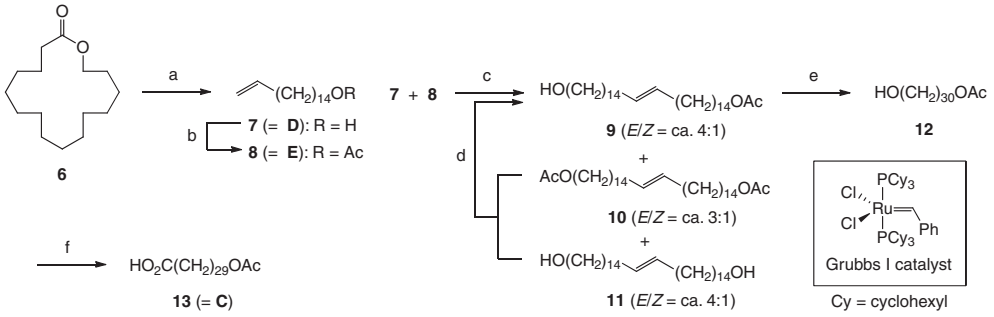


Fig. 2. Synthetic Plan.



Scheme 1. Synthesis of ω-Acetoxytriacontanoic Acid (**13**).

Reagents and conditions: (a) $(i\text{-Bu})_2$AlH, toluene, $-78\,^\circ$C, 30 min; then $Ph_3P{=}CH_2$ [prepared from Ph_3PMeBr and $LiN(SiMe_3)_2$], THF, $-78\,^\circ$C to room temperature (64%); (b) Ac_2O, pyridine, room temperature (99%); (c) 5 mol % Grubbs I catalyst [$(Cy_3P)_2Ru(=CHPh)Cl_2$], CH_2Cl_2, reflux, 3 h (47% for **9**, 22% for **10**, 23% for **11**); (d) 5 mol % Grubbs I catalyst, CH_2Cl_2, reflux, 3 h (45% for **9**, 23% for **10**, 20% for **11**); (e) H_2, 10% Pd–C, THF, room temperature, 15 h (93%); (f) Jones CrO_3 reagent, acetone, 60 °C (96%).

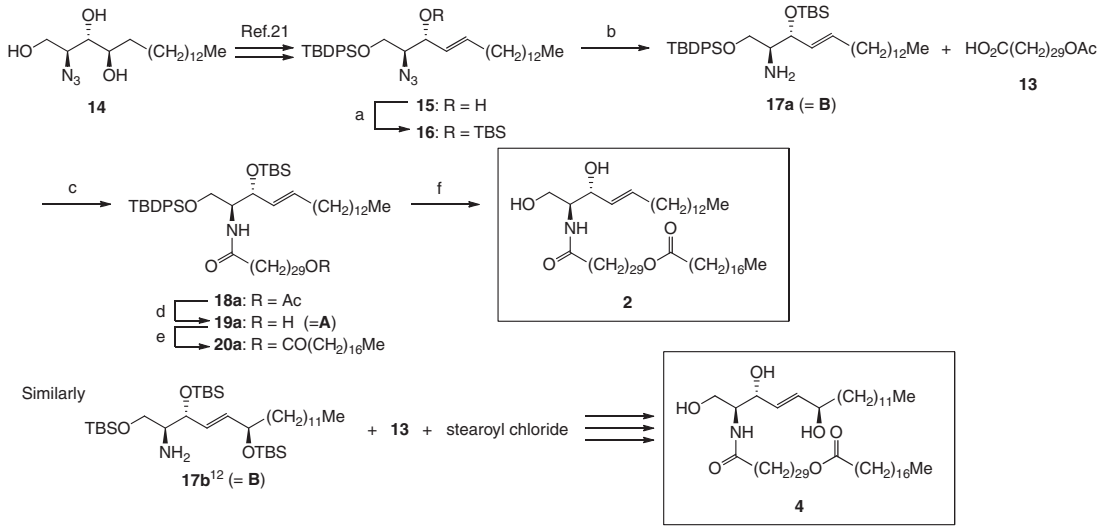
intermediate **A**. Alcohol **A** could be prepared by condensing amine **B** with acid **C**. To construct **C**, we planned to employ the olefin cross metathesis reaction with Grubbs's first-generation catalyst between **D** and **E** which could be readily prepared from commercially available 15-pentadecanolide (**6**).

Scheme 1 shows how ω-acetoxytriacontanoic acid **13** (= **C**) was synthesized from **6**. Lactone **6** was converted to alken-1-ol **7** (= **D**) *via* the corresponding hemiacetal by reduction with diisobutylaluminum hydride and subsequent treatment with the Wittig reagent in a one-pot procedure (64%). Acetylation of **7** gave **8** (= **E**) in a 99% yield. The olefin cross metathesis reaction between **7** and **8** (a 1:1 mixture) in the presence of 5 mol % of Grubbs's first-generation catalyst gave desired olefin **9** (47%, $E/Z = ca.$ 4:1, determined by a 500 MHz ^{1}H-NMR analysis) together with diacetate **10** (22%) and diol **11** (23%).[14–16] These compounds were separated by column chromatography, and the olefin cross metathesis reaction of a 1:1 mixture of separated **10** and **11** provided three kinds of olefin, **9** (45%), **10** (23%), and **11** (20%). The reaction mixture needed to be loaded into a chromatography column while it was hot after stirring under reflux for 3 h to achieve equilibrium of the metathesis reaction. Purification after cooling to room temperature resulted in respective isolated yields for **9**, **10** and **11** of 39% ($E/Z = ca.$ 4:1), 23% ($E/Z = ca.$ 4:1) and 29% ($E/Z = ca.$ 9:1). The solubility of **11** in dichloromethane was lower than that of **9** and **10**, and it separated out from the solution when it was cooled to room temperature. Therefore, **11** was regenerated from **9** through the metathesis reaction in the solution. This decreased the yield of **9**, and **11** was obtained in more than the theoretical yield in this case. Hydrogenation of **9** yielded **12** (93%) which was then oxidized with the Jones chromic acid reagent to give acid **13** in a 96% yield.

Many methods have been published to construct the sphingosine chain.[18,19] We prepared it in the present synthesis from commercially available and inexpensive phytosphingosine **14**[20] *via* Kim's azide **15** (Scheme 2).[21] Protection of the 3-hydroxy group of **15** as a *tert*-butyldimethylsilyl (TBS) ether provided **16**. Reduction of **16** with trimethylphosphine and subsequent alkaline treatment gave amine **17a** (= **B**) in an 88% yield. Condensation of **17a** with acid **13** afforded **18a** (98%), and deacetylation of **18a** yielded the key intermediate, alcohol **19a** (= **A**), in a 90% yield. Acylation of the ω-hydroxyl group of **19a** with stearoyl chloride gave **20a** (91%) which was then desilylated with tetrabutylammonium fluoride (TBAF) to furnish one of the desired ω-stearoyloxy ceramides, **2**. The overall yield was 17% in 9 steps from **6**.

Synthesis of a 6-hydroxysphingosine chain like **17b** has been reported by three groups.[12,22–24] Another target ceramide (**4**) was synthesized from amine **17b**, which had been prepared according to the method in our previous report,[12] in the same manner as that just described. The overall yield of **4** was 12% based on **6** in 9 steps.

Synthesized **2** and **4** were hardly soluble in $CHCl_3$, although it has been reported that **1** and **3** were soluble in $CHCl_3$ even at room temperature.[11,12] The physical properties of these esterified ceramides might be influenced by the nature of their ω-acyloxy groups. The respective melting points of the synthesized ω-stearoyloxy ceramides (**2** and **4**) were 94–95 °C and 109–111 °C. The bioactivity of these ceramides is now



Scheme 2. Synthesis of Esterified Ceramides **2** and **4**.

Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C (quant.); (b) PMe_3, THF, room temperature, 17 h; then 1.0 M NaOH aq., room temperature, 2 h (88%); (c) **13**, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, $(i\text{-Pr})_2$NEt, room temperature, 19 h (98%); (d) K_2CO_3, MeOH, CH_2Cl_2, room temperature, 18 h (90%); (e) stearoyl chloride, pyridine, $CHCl_3$, room temperature, 45 min (91%); (f) TBAF, THF, room temperature, 24 h (80%).

under investigation, and will be reported in due course by Dr. S. Hamanaka.

Conclusion

The minor ceramide components (**2** and **4**) of the human stratum corneum were synthesized from sphingosine bases (**17a** and **17b**) and ω-acetoxytriacontanoic acid (**13**). The latter was prepared by olefin cross metathesis between hexadec-15-en-1-ol (**7**) and its acetate (**8**), employing Grubbs's first-generation catalyst. The preparation of **7** was accomplished by a one-pot reduction/Wittig reaction procedure from 15-pentadecanolide (**6**).

Experimental

Refractive indices (n_D) were measured with an Atago 1T refractometer. Melting point (mp) data were recorded with Yanaco MP-S3 melting point measuring apparatus and are uncorrected. Optical rotation values were measured with a Jasco P-1010 polarimeter, and IR spectra were measured with a Jasco FT/IR-460 plus spectrometer. ^{1}H-NMR spectra (TMS at $\delta_H = 0.00$ or pyridine at $\delta_H = 7.55$ as the internal standards) and ^{13}C-NMR spectra (pyridine at 135.5 as the internal standard) were recorded with a Varian VNMRS-500 spectrometer. High-resolution mass spectrometry (HRMS) was performed with a Jeol JMS-700 [electron ionization (EI)] or Waters MALDI SYNAPT™ G2 HDMS [electrospray ionization (ESI)] mass spectrometer. Column chromatography was performed with Merck Kieselgel 60 Art 1.07734.

*Hexadec-15-en-1-ol (**7**).* To a stirred suspension of methyltriphenylphosphonium bromide (4.47 g, 12.5 mmol) in dry THF (20 mL) was added a solution of lithium bis(trimethylsilyl)amide (LHMDS, 1.0 M in THF, 12.5 mL, 12.5 mmol) at 0 °C. The mixture was stirred for 2 h at 0 °C to prepare a solution of methylenetriphenylphosphorane.

To a stirred solution of **6** (1.44 g, 5.99 mmol) in dry toluene (30 mL) at −78 °C, a solution of diisobutylaluminum hydride (DIBAL-H, 1.02 M in toluene, 6.5 mL, 6.6 mmol) was slowly added over 30 min. After stirring at −78 °C for 30 min, a yellow solution of the phosphorous ylide just prepared was slowly added over 10 min. The mixture was gradually warmed to room temperature while stirring for 17 h. The reaction was then quenched with 1 M hydrochloric acid, and the mixture was stirred for 30 min. The mixture was extracted with EtOAc, and the combined organic phase was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (50 g, hexane/EtOAc = 10:1) to give **7** (928 mg, 64%) as colorless needles. IR and ^{1}H-NMR spectra were in accordance with those reported in ref. 25. Mp 33–35 °C (ref. 25, oil); IR ν_{max} (KBr) cm^{-1}: 3280 (br m, OH), 3080 (w), 1645 (m, C=C), 990 (m), 915 (s), 760 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 1.20 (1H, br s), 1.23–1.34 (20H, m), 1.36 (2H, quint., $J = 7.0$ Hz), 1.57 (2H, quint., $J = 7.0$ Hz), 2.04 (2H, dddt, $J = 1.0$, 1.5, 7.0, 7.0 Hz), 3.64 (2H, t, $J = 6.5$ Hz), 4.93 (1H, ddt, $J = 2.0$, 10, 1.0 Hz), 4.99 (1H, ddt, $J = 2.0$, 17, 1.5 Hz), 5.82 (1H, ddt, $J = 10$, 17, 7.0 Hz). HRMS (EI+): calcd. for $C_{16}H_{30}$ [$(M - H_2O)^+$], 222.2348; found, 222.2345.

*Hexadec-15-enyl acetate (**8**).* To a stirred solution of **7** (819 mg, 3.41 mmol) in pyridine (15 mL), Ac_2O (644 µL, 6.81 mmol) was added at 0 °C. After stirring at room temperature for 15 h, the reaction was quenched with water. The mixture was extracted with EtOAc, and the separated organic phase was successively washed with 1 M hydrochloric acid, water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (20 g, hexane/EtOAc = 20:1) to give **8** (952 mg, 99%) as a colorless oil, n_D 1.4477. IR ν_{max} (film) cm^{-1}: 3080 (w), 1740 (s, C=O), 1640 (m, C=C), 1240 (br s, C–O), 1040 (br m, C–O), 990 (w), 910 (m); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 1.22–1.35 (20H, m), 1.36 (2H, quint., $J = 7.0$ Hz), 1.62 (2H, quint., $J = 7.0$ Hz), 2.03 (2H, br q, $J = 7.0$ Hz), 2.05 (3H, s), 4.05 (2H, t, $J = 7.0$ Hz), 4.93 (1H, ddt, $J = 2.0$, 10, 1.0 Hz), 4.99 (1H, ddt, $J = 2.0$, 17, 1.5 Hz), 5.82 (1H, ddt, $J = 10$, 17, 7.0 Hz). HRMS (EI+): calcd. for $C_{18}H_{34}O$ [M^+], 282.2559; found, 282.2551.

*(15EZ)-30-Hydroxytriacont-15-enyl acetate (**9**).*

Method A: A solution of **7** (524 mg, 2.18 mmol), **8** (615 mg, 2.18 mmol), and $(Cy_3P)_2Ru(=CHPh)Cl_2$ (Aldrich, Grubbs's first-generation catalyst, 106 mg, 129 mmol, 5.6 mol % to **7**) in dry and degassed CH_2Cl_2 (20 mL) was stirred under reflux for 3 h in an argon atmosphere. The reaction mixture was then diluted with hot $CHCl_3$ and loaded into a chromatographic column of silica gel (80 g) while the mixture was hot. Purification by column chromatography was then performed at room temperature.

(15*EZ*)-Diacetoxytriacont-15-ene (**10**, 263 mg, 22%) was eluted with $CHCl_3$/MeOH=150:1 as a pale brown solid (*E*:*Z* = *ca.* 3:1). Mp 56–60 °C; IR ν_{max} (KBr) cm^{-1}: 1740 (br s, C=O), 1250 (br s), 1060 (m, C–O), 1040 (m, C–O), 965 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 1.20–1.37 (44H, m), 1.61 (4H, quint., $J = 7.0$ Hz), 1.96 (3H, m), 2.01

(1H, m), 2.05 (6H, s), 4.05 (4H, t, $J = 7.0$ Hz), 5.35 (0.5H, tt, $J = 1.0$, 4.5 Hz), 5.38 (1.5H, tt, $J = 1.5$, 3.5 Hz). HRMS (EI+): calcd. for $C_{34}H_{64}O_4$ [M^+], 536.4805; found, 536.4805.

(15*EZ*)-30-Hydroxytriacont-15-enyl acetate (**9**, 510 mg, 47%) was eluted with $CHCl_3$/MeOH=150:2 as pale brown powder (*E*:*Z* = *ca.* 4:1). Mp 66–69 °C; IR ν_{max} (KBr) cm^{-1}: 3280 (br m, OH), 1740 (br s, C=O), 1255 (br s), 1060 (m, C–O), 1045 (m, C–O), 960 (m); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 1.21–1.37 (45H, m), 1.56 (2H, quint., $J = 7.0$ Hz), 1.62 (2H, quint., $J = 7.0$ Hz), 1.96 (3.2H, m), 2.01 (0.8H, m), 2.05 (3H, s), 3.64 (2H, t, $J = 7.0$ Hz), 4.05 (2H, t, $J = 7.0$ Hz), 5.35 (0.4H, tt, $J = 1.0$, 4.5 Hz), 5.38 (1.6H, tt, $J = 1.5$, 4.0 Hz). HRMS (EI+): calcd. for $C_{32}H_{62}O_3$ [M^+], 494.4699; found, 494.4697.

(15*EZ*)-Triacont-15-ene-1,30-diol (**11**, 223 mg, 23%) was eluted with $CHCl_3$/MeOH=150:3 as pale brown powder (*E*:*Z* = *ca.* 4:1). Mp 95–98 °C; IR ν_{max} (KBr) cm^{-1}: 3280 (br m, OH), 1060 (m, C–O), 960 (m); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$, 40 °C): 1.19 (2H, br s), 1.20–1.38 (44H, m), 1.56 (4H, quint., $J = 6.5$ Hz), 1.96 (3.2H, m), 2.01 (0.8H, m), 3.63 (4H, t, $J = 6.5$ Hz), 5.35 (0.4H, br t, $J = 4.5$ Hz), 5.38 (1.6H, tt, $J = 1.5$, 4.0 Hz). HRMS (EI+): calcd. for $C_{30}H_{60}O_2$ [M^+], 452.4593; found, 452.4589.

Method B: A suspension of **10** (277 mg, 0.516 mmol), **11** (234 mg, 0.517 mmol), and Grubbs's first-generation catalyst (Aldrich, 22 mg, 0.027 mmol, 5.2 mol % to **10**) in dry and degassed CH_2Cl_2 (20 mL) was stirred under reflux for 3 h in an argon atmosphere. The mixture was then diluted with hot $CHCl_3$ and purified by column chromatography on silica gel (30 g, $CHCl_3$/MeOH = 100:1, 100:2, and 100:3 for **10**, **9**, and **11**, respectively) with the same procedure as that just described to give **10** (129 mg, 23%, *E*/*Z* = *ca.* 4:1), **9** (288 mg, 45%, *E*/*Z*=*ca.* 3:1) and **11** (95 mg, 20%, *E*/*Z* = *ca.* 5:1).

*30-Hydroxytriacontyl acetate (**12**).* To a stirred solution of **9** (498 mg, 1.01 mmol) in THF (40 mL), 10% Pd–C (Kawaken, 84 mg) was added. After stirring at room temperature for 15 h in a hydrogen atmosphere, the mixture was filtered and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 10:1) to give **12** (467 mg, 93%) as colorless powder. Mp 84–86 °C; IR ν_{max} (KBr) cm^{-1}: 3280 (br m, OH), 1735 (s, C=O), 1260 (s), 1055 (m, C–O); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 1.20–1.38 (53H, m), 1.57 (2H, quint., $J = 7.0$ Hz), 1.62 (2H, quint., $J = 7.0$ Hz), 2.05 (3H, s), 3.64 (2H, t, $J = 7.0$ Hz), 4.05 (2H, t, $J = 7.0$ Hz). HRMS (EI+): calcd. for $C_{32}H_{64}O_3$ [M^+], 496.4855; found, 496.4847.

*30-Acetoxytriacontanoic acid (**13**).* To a stirred suspension of **12** (422 mg, 0.849 mmol) in dry acetone (30 mL), a solution of Jones CrO_3 (2.67 M, 953 µL, 2.54 mmol) was added in one portion at room temperature. After stirring at 60 °C for 30 min, the mixture was diluted with water. The precipitate was collected by filtration and then washed with water. After air-drying, 415 mg (96%) of **13** was obtained as colorless powder and was used in the next step without further purification. The analytical sample was obtained by column chromatography on silica gel ($CHCl_3$/MeOH = 50:2). Mp 90–93 °C; IR ν_{max} (KBr) cm^{-1}: 3000 (br s, OH), 1740 (s, C=O), 1710 (br s, C=O), 1250 (br s), 1045 (br m); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 1.22–1.37 (50H, m), 1.57 (1H, br s), 1.58–1.67 (4H, m), 2.05 (3H, s), 2.35 (2H, t, $J = 7.0$ Hz), 4.05 (2H, t, $J = 7.0$ Hz). HRMS (ESI+): calcd. for $C_{32}H_{62}O_4Na$ [$(M + Na)^+$], 533.4546; found, 533.4546.

*(2S,3R,4E)-2-Azido-3-*O*-(*tert*-butyldimethylsilyl)-1-*O*-(*tert*-butyldiphenylsilyl)octadec-4-ene-1,3-diol (**16**).* To a stirred solution of **15**[21] (574 mg, 1.02 mmol) and 2,6-lutidine (355 µL, 3.05 mmol) in dry CH_2Cl_2 (10 mL), *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf, 351 µL, 1.53 mmol) was added at 0 °C. After stirring at 0 °C for 15 min, the reaction was quenched with MeOH (0.5 mL), and the mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, and successively washed with a saturated aqueous $NaHCO_3$ solution, water and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 50:1) to give **16** (711 mg, quant.) as a colorless oil, n_D^{23} 1.5040; $[\alpha]_D^{23}$ −11.8 (*c* 1.03, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 2110 (s, N_3), 1670 (w, C=C), 1590 (w), 1255 (br m, *t*-Bu, Si-Me), 1015 (br s, C–O), 1070 (br s, C–O), 970 (m), 840 (br s), 780 (m), 700 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 0.01 (3H, s), 0.03 (3H, s), 0.83 (9H, s), 0.89 (3H, t, $J = 7.0$ Hz), 1.08 (9H, s), 1.23–1.36 (22H, m), 2.00 (2H, dr q, $J = 7.0$ Hz), 3.50 (1H, dt, $J = 5.5$, 7.0 Hz), 3.64 (1H, dd, $J = 7.0$, 11 Hz), 3.71 (1H, dd, $J = 5.5$, 11 Hz), 4.20 (1H, dd, $J = 5.5$, 7.0 Hz), 5.38 (1H, ddt, $J = 1.5$, 7.0, 15 Hz), 5.60 (1H, dt, $J = 7.0$, 15 Hz), 7.37–7.46 (6H, m), 7.67 (4H, t, $J = 7.0$ Hz). HRMS (ESI+): calcd. for $C_{40}H_{67}N_3O_2Si_2Na$ [$(M + Na)^+$], 700.4670; found, 700.4658.

*(2S,3R,4E)-2-Amino-3-*O*-(*tert*-butyldimethylsilyl)-1-*O*-(*tert*-butyldiphenylsilyl)octadec-4-ene-1,3-diol (**17a**).* To a stirred solution of **16** (447 mg, 0.659 mmol) in dry THF (15 mL), a solution of trimethylphosphine (1.0 M in THF, 3.3 mL, 3.3 mmol) was added at room temperature. After stirring at room temperature for 17 h, an aqueous solution of NaOH (1.0 M, 6.6 mL, 6.6 mmol) was added to the mixture. The resulting mixture was stirred at room temperature for 2 h. The mixture was then poured into water and extracted with EtOAc. The separated organic phase was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with K_2CO_3, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 9:1) to give **17a** (378 mg, 88%) as a colorless oil, n_D^{23} 1.5062; $[\alpha]_D^{23}$ −3.36 (*c* 1.00, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3390 (w, NH), 1660 (w, C=C), 1590 (w), 1255 (s, *t*-Bu, Si-Me), 1115 (s, C–O), 1080 (br s, C–O), 970 (m), 835 (br s), 775 (m), 740 (m), 700 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): −0.04 (3H, s), −0.03 (3H, s), 0.80 (9H, s), 0.88 (3H, t, $J = 7.0$ Hz), 1.06 (9H, s), 1.22–1.36 (22H, m), 1.50 (2H, br s), 2.00 (2H, dr q, $J = 7.0$ Hz), 2.89 (1H, dt, $J = 7.0$, 5.0 Hz), 3.57 (1H, dd, $J = 7.0$, 10 Hz), 3.73 (1H, dd, $J = 5.0$, 10 Hz), 4.07 (1H, t, $J = 7.0$ Hz), 5.34 (1H, br dd, $J = 7.0$, 15 Hz), 5.60 (1H, dt, $J = 15$, 7.0 Hz), 7.34–7.45 (6H, m), 7.63–7.67 (4H, m). HRMS (ESI+): calcd. for $C_{40}H_{70}NO_2Si_2$ [$(M + H)^+$], 652.4945; found, 652.4926.

*(2S,3R,4E)-2-[(30′-Acetoxy)triacontanamido]-3-*O*-(*tert*-butyldimethylsilyl)-1-*O*-(*tert*-butyldiphenylsilyl)octadec-4-ene-1,3-diol (**18a**).* To a stirred mixture of **17a** (302 mg, 0.463 mmol) and **13** (235 mg, 0.460 mmol) in dry CH_2Cl_2 (10 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 180 mg, 0.939 mmol), 1-hydroxybenzotriazole (HOBt, 64 mg, 0.47 mmol), and (*i*-Pr)$_2$NEt (162 µL, 0.930 mmol) were added at room temperature. After stirring at room temperature for 19 h, the mixture was poured into water and extracted with EtOAc. The separated organic phase was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 9:1) to give **18a** (522 mg, 98%) as a colorless solid. Mp 52–54 °C; $[\alpha]_D^{23}$ −3.74 (*c* 1.00, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 1735 (br m, C=O), 1680 (s, C=O), 1660 (w, C=C), 1525 (m), 1260 (br m, *t*-Bu, Si-Me), 1120 (s, C–O), 1080 (br s, C–O), 1045 (br s, C–O), 970 (m), 860 (m), 840 (m), 760 (s), 700 (m); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): −0.02 (3H, s), −0.01 (3H, s), 0.82 (9H, s), 0.88 (3H, t, $J = 7.0$ Hz), 1.07 (9H, s), 1.21–1.36 (74H, m), 1.62 (2H, quint., $J = 7.0$ Hz), 1.95 (2H, q, $J = 7.0$ Hz), 2.00 (2H, dt, $J = 5.0$, 7.0 Hz), 2.05 (3H, s), 3.70 (1H, dd, $J = 5.0$, 11 Hz), 3.88 (1H, dd, $J = 5.0$, 11 Hz), 4.01 (1H, m), 4.05 (2H, t, $J = 7.0$ Hz), 4.30 (1H, br t, $J = 7.0$ Hz), 5.34 (1H, br dd, $J = 7.0$, 15 Hz), 5.43 (1H, d, $J = 8.5$ Hz), 5.58 (1H, br dt, $J = 15$, 7.0 Hz), 7.35–7.46 (6H, m), 7.63 (4H, t, $J = 8.0$ Hz). HRMS (ESI+): calcd. for $C_{72}H_{130}NO_5Si_2$ [$(M + H)^+$], 1144.9488; found, 1144.9508.

*(2S,3R,4E,6R)-2-[(30′-Acetoxy)triacontanamido]-1,3,6-tris-*O*-(*tert*-butyldimethylsilyl)octadec-4-ene-1,3,6-triol (**18b**).* In the same manner as that just described, **17b**[12] (152 mg, 0.231 mmol) was converted to **18b** (265 mg, quant.) as colorless wax. Mp 40–43 °C; $[\alpha]_D^{24}$ −1.23 (*c* 1.02, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3440 (w, NH), 3360 (br m, NH), 1745 (s, C=O), 1675 (w, C=C), 1660 (br s, C=O), 1510 (br m), 1255 (br s, *t*-Bu, Si-Me), 1085 (br s, C–O), 970 (w), 840 (br s), 775 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 0.010 (3H, s), 0.012 (3H, s), 0.03 (6H, s), 0.04 (3H, s), 0.05 (3H, s), 0.877 (3H, t, $J = 7.0$ Hz), 0.881 (9H, s), 0.884 (9H, s), 0.89 (9H, s), 1.20–1.38 (70H, m), 1.40–1.52 (2H, m), 1.52–1.64 (4H, m), 2.05 (3H, s), 2.12 (2H, dt, $J = 2.0$, 7.5 Hz), 3.54 (1H, dd, $J = 6.0$, 10 Hz), 3.77 (1H, dd, $J = 5.5$, 10 Hz), 3.98 (1H, m), 4.05 (2H, t, $J = 7.0$ Hz), 4.12 (1H, q, $J = 6.0$ Hz), 4.42 (1H, t, $J = 6.0$ Hz), 5.47 (1H, d, $J = 7.5$ Hz), 5.60 (1H, ddd,

J = 1.0, 6.0, 16 Hz), 5.70 (1H, ddd, J = 1.0, 6.0, 16 Hz). HRMS (ESI+): calcd. for $C_{68}H_{139}NO_6Si_3Na$ [$(M + Na)^+$], 1172.9808; found, 1172.9775.

*(2S,3R,4E)-3-*O-(tert-*Butyldimethylsilyl)-1-*O-(tert-*butyldiphenylsilyl)-2-[(30′-hydroxy)triacontanamido]octadec-4-ene-1,3-diol (**19a**).* To a stirred solution of **18a** (202 mg, 0.176 mmol) in MeOH–CH_2Cl_2 (1:1, 20 mL), K_2CO_3 (50 mg, 0.36 mmol) was added at room temperature. After stirring at room temperature for 18 h, the mixture was concentrated *in vacuo*. The residue was poured into water and extracted with EtOAc. The separated organic phase was successively washed with water and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g, hexane/EtOAc = 17:3) to give **19a** (175 mg, 90%) as a colorless solid. Mp 44–45 °C; $[\alpha]_D^{23}$ −6.30 (*c* 1.01, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 3440 (w, NH), 3320 (br m, OH), 1680 (w, C=C), 1650 (br s, C=O),1590 (w), 1545 (br m), 1250 (m, *t*-Bu, Si-Me), 1115 (s, C–O), 1070 (br s, C–O), 970 (w), 840 (br s), 780 (s), 760 (s), 700 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): −0.02 (3H, s), −0.01 (3H, s), 0.82 (9H, s), 0.88 (3H, t, J = 7.0 Hz), 1.07 (9H, s), 1.21–1.39 (72H, m), 1.50–1.62 (5H, m), 1.95 (2H, quint., J = 7.0 Hz), 1.96–2.04 (2H, m), 3.64 (2H, t, J = 7.0 Hz), 3.70 (1H, dd, J = 4.5, 11 Hz), 3.88 (1H, dd, J = 5.0, 11 Hz), 4.01 (1H, m), 4.30 (1H, t, J = 6.5 Hz), 5.34 (1H, dd, J = 6.5, 15 Hz), 5.44 (1H, d, J = 9.0 Hz), 5.58 (1H, dt, J = 15, 7.0 Hz), 7.35–7.44 (6H, m), 7.63 (4H, t, J = 8.0 Hz). HRMS (ESI+): calcd. for $C_{70}H_{128}NO_4Si_2$ [$(M + H)^+$], 1102.9382; found, 1102.9384.

*(2S,3R,4E,6R)-1,3,6-Tris-*O-(tert-*butyldimethylsilyl)-2-[(30′-hydroxy)triacontanamido]octadec-4-ene-1,3,6-triol (**19b**).* In the same manner as that just described, **18b** (240 mg, 0.208 mmol) was converted to **19b** (211 mg, 91%) as a colorless solid. Mp 33–35 °C; $[\alpha]_D^{24}$ −1.24 (*c* 1.04, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 3420 (w, NH), 3280 (br m, OH), 1675 (w, C=C), 1650 (br s, C=O), 1545 (w), 1255 (s, *t*-Bu, Si-Me), 1080 (br s, C–O), 975 (m), 840 (s), 775 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 0.01 (3H, s), 0.02 (3H, s), 0.036 (6H, s), 0.043 (3H, s), 0.05 (3H, s), 0.880 (3H, t, J = 7.0 Hz), 0.884 (9H, s), 0.887 (9H, s), 0.891 (9H, s), 1.21–1.38 (70H, m), 1.40–1.52 (2H, m), 1.54–1.63 (4H, m), 2.13 (2H, dt, J = 2.0, 7.5 Hz), 3.54 (1H, dd, J = 5.5, 11 Hz), 3.64 (2H, t, J = 6.5 Hz), 3.78 (1H, dd, J = 5.5, 11 Hz), 3.98 (1H, m), 4.12 (1H, dt, J = 5.5, 6.0 Hz), 4.42 (1H, t, J = 5.5 Hz), 5.48 (1H, d, J = 8.0 Hz), 5.60 (1H, ddd, J = 1.0, 5.5, 16 Hz), 5.71 (1H, ddd, J = 1.0, 5.5, 16 Hz). HRMS (ESI+): calcd. for $C_{66}H_{137}NO_5Si_3Na$ [$(M + Na)^+$], 1130.9702; found, 1130.9691.

*(2S,3R,4E)-3-*O-(tert-*Butyldimethylsilyl)-1-*O-(tert-*butyldiphenylsilyl)-2-[(30′-stearoyloxy)triacontanamido]octadec-4-ene-1,3-diol (**20a**).* To a stirred solution of **19a** (71 mg, 0.064 mmol) and pyridine (26 µL, 0.32 mmol) in $CHCl_3$ (5 mL), stearoyl chloride (50 mg, 0.165 mmol) was added at 0 °C. After stirring at room temperature for 45 min, the reaction was quenched with water, and the mixture was extracted with EtOAc. The organic phase was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (10 g, hexane/EtOAc = 10:1) to give **20a** (79 mg, 91%) as a colorless solid. Mp 44–45 °C; $[\alpha]_D^{23}$ −4.40 (*c* 1.26, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 3310 (br w, NH), 1740 (s, C=O), 1650 (br m, C=O), 1260 (m, *t*-Bu, Si-Me), 1175 (s, C–O), 1100 (br s, C–O), 970 (w), 840 (br m), 780 (s), 700 (m); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): −0.02 (3H, s), −0.01 (3H, s), 0.83 (9H, s), 0.88 (6H, t, J = 7.0 Hz), 1.07 (9H, s), 1.22–1.36 (100H, m), 1.53 (2H, quint., J = 7.0 Hz), 1.61 (4H, quint., J = 7.0 Hz), 1.95 (2H, q, J = 7.0 Hz), 1.98–2.04 (2H, m), 2.29 (2H, t, J = 7.0 Hz), 3.70 (1H, dd, J = 5.0, 11 Hz), 3.88 (1H, dd, J = 5.5, 11 Hz), 4.01 (1H, m), 4.05 (2H, t, J = 7.0 Hz), 4.30 (1H, br t, J = 7.0 Hz), 5.34 (1H, br dd, J = 7.0, 15 Hz), 5.43 (1H, d, J = 9.0 Hz), 5.58 (1H, br dt, J = 15, 7.0 Hz), 7.35–7.46 (6H, m), 7.64 (4H, t, J = 8.0 Hz). HRMS (ESI+): calcd. for $C_{88}H_{162}NO_5Si_2$ [$(M + H)^+$], 1369.1992; found, 1369.1991.

*(2S,3R,4E,6R)-1,3,6-Tris-*O-(tert-*butyldimethylsilyl)-2-[(30′-stearoyloxy)triacontanamido]octadec-4-ene-1,3,6-triol (**20b**).* In the same manner as that just described, **19b** (192 mg, 0.173 mmol) was converted to **20b** (233 mg, 98%) as a colorless solid. Mp 43–44 °C; $[\alpha]_D^{23}$ −0.96 (*c* 1.17, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 3440 (w, NH), 3360 (m, NH), 1740 (s, C=O), 1675 (w, C=C), 1655 (br s, C=O), 1540 (m), 1255 (s, *t*-Bu, Si-Me), 1180 (s, C–O), 1100 (br s, C–O), 975 (w), 840 (br s), 780 (s); ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 0.012 (3H, s), 0.014 (3H, s), 0.036 (6H, s), 0.043 (3H, s), 0.05 (3H, s), 0.880 (6H, t, J = 7.5 Hz), 0.884 (9H, s), 0.887 (9H, s), 0.890 (9H, s), 1.20–1.38 (100H, m), 1.40–1.52 (2H, m), 1.58–1.64 (4H, m), 2.13 (2H, dt, J = 2.0, 7.5 Hz), 2.29 (2H, t, J = 7.0 Hz), 3.54 (1H, dd, J = 6.0, 11 Hz), 3.78 (1H, dd, J = 5.5, 11 Hz), 3.98 (1H, m), 4.05 (2H, t, J = 7.0 Hz), 4.12 (1H, q, J = 5.5 Hz), 4.42 (1H, t, J = 5.5 Hz), 5.47 (1H, d, J = 8.0 Hz), 5.60 (1H, ddd, J = 1.0, 5.5, 16 Hz), 5.71 (1H, ddd, J = 1.0, 5.5, 16 Hz). HRMS (ESI+): calcd. for $C_{84}H_{171}NO_6Si_3Na$ [$(M + Na)^+$], 1397.2312; found, 1397.2297.

*(2S,3R,4E)-2-[(30′-Stearoyloxy)triacontanamido]octadec-4-ene-1,3-diol (**2**).* To a stirred solution of **20a** (185 mg, 0.135 mmol) in THF (5 mL), a solution of tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 405 µL, 0.405 mmol) was added at room temperature. After stirring at room temperature for 24 h, the mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (6 g, $CHCl_3$/MeOH = 25:1) to give a colorless solid. The obtained solid was washed with Et_2O, and then collected by filtration to give **2** (110 mg, 80%) as colorless powder. Mp 94–95 °C; $[\alpha]_D^{25}$ −25.6 (*c* 0.21, pyridine). IR ν_{max} (KBr) cm^{-1}: 3360 (br m, NH, OH), 1740 (s, C=O), 1620 (br s, C=O), 1540 (br m), 1070 (m, C–O); ^{1}H-NMR δ_H (500 MHz, pyridine-d_5, 50 °C): 0.880 (3H, t, J = 7.0 Hz), 0.884 (3H, t, J = 7.0 Hz), 1.22–1.44 (100H, m), 1.66 (2H, quint., J = 7.0 Hz), 1.71 (2H, quint., J = 7.0 Hz), 1.78–1.87 (2H, m), 2.10 (2H, dt, J = 6.0, 7.0 Hz), 2.40 (2H, t, J = 7.0 Hz), 2.43 (2H, t, J = 7.0 Hz), 4.20 (2H, t, J = 7.0 Hz), 4.24 (1H, dd, J = 5.0, 11 Hz), 4.40 (1H, dd, J = 5.5, 11 Hz), 4.66 (1H, dddd, J = 5.0, 5.5, 7.0, 8.0 Hz), 4.80 (1H, br s), 5.96 (1H, dt, J = 6.0, 15 Hz), 6.01 (1H, dd, J = 6.0, 15 Hz), 6.04 (1H, br s), 6.40 (1H, br s), 7.97 (1H, d, J = 8.0 Hz); ^{13}C-NMR δ_C (126 MHz, pyridine-d_5, 50 °C): 14.3, 23.0, 25.5, 26.3, 26.5, 29.2, 29.5, 29.57, 29.62, 29.63, 29.7, 29.79, 29.81, 29.87, 29.90, 29.93, 29.96, 29.99, 30.03, 30.05, 30.07, 32.2, 32.8, 34.6, 37.0, 57.0, 62.4, 64.5, 73.6, 132.3, 132.4, 173.56, 173.60. HRMS (ESI+): calcd. for $C_{66}H_{130}NO_5$ [$(M + H)^+$], 1016.9949; found, 1016.9948.

*(2S,3R,4E,6R)-2-[(30′-Stearoyloxy)triacontanamido]octadec-4-ene-1,3,6-triol (**4**).* In the same manner as that just described, **20b** (217 mg, 0.158 mmol) was converted to **4** (85 mg, 52%) as colorless powder. Mp 109–111 °C; $[\alpha]_D^{25}$ +45.7 (*c* 0.20, pyridine). IR ν_{max} (KBr) cm^{-1}: 3480 (w, NH), 3320 (br m, NH, OH), 1740 (s, C=O), 1625 (s, C=O), 1550 (br m), 1170 (br m, C–O), 1060 (m, C–O), 960 (w), 760 (m); ^{1}H-NMR δ_H (500 MHz, pyridine-d_5, 50 °C): 0.88 (6H, t, J = 7.0 Hz), 1.20–1.43 (98H, m), 1.58 (1H, m), 1.62–1.69 (3H, m), 1.69–1.76 (2H, m), 1.76–1.85 (2H, m), 2.40 (2H, t, J = 7.0 Hz), 2.41 (2H, t, J = 7.0 Hz), 4.20 (2H, t, J = 7.0 Hz), 4.24 (1H, dd, J = 5.0, 11 Hz), 4.39 (1H, dd, J = 5.0, 11 Hz), 4.46 (1H, dt, J = 5.5, 6.0 Hz), 4.68 (1H, m),4.93 (1H, t, J = 5.5 Hz), 6.31 (1H, ddd, J = 1.0, 5.5, 16 Hz), 6.38 (1H, ddd, J = 1.0, 5.5, 16 Hz), 8.01 (1H, d, J = 8.0 Hz); ^{13}C-NMR δ_C (126 MHz, pyridine-d_5, 50 °C): 14.3, 23.0, 25.5, 26.2, 26.36, 26.40, 29.2, 29.5, 29.59, 29.63, 29.65, 29.83, 29.84, 29.86, 29.88, 29.92, 29.94, 29.97, 29.98, 30.00, 30.04, 30.05, 30.07, 30.08, 30.11, 30.2, 32.2, 34.6, 37.0, 38.7, 57.1, 62.3, 64.5, 71.8, 73.3, 131.1, 136.7, 173.6, 173.7. HRMS (ESI+): calcd. for $C_{66}H_{130}NO_6$ [$(M + H)^+$], 1032.9898; found, 1032.9880.

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