Synthesis of two Unique Compounds, a Ceramide and a Cerebroside, Occurring in Human Stratum Corneum

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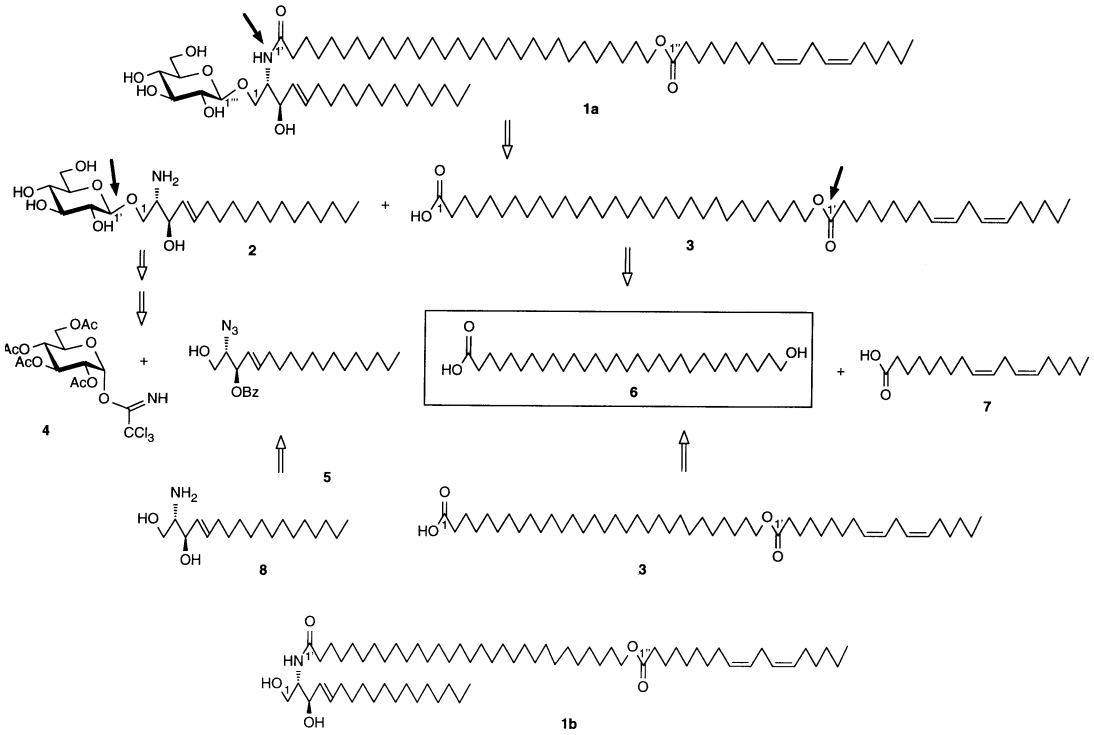
Abstract. The cerebroside **1a** and the ceramide **1b**, both playing important roles in epidermal barrier function, were synthesized by *N*-acylation of 1-*O*-glucosylated C_{18}-sphingosine **2** and C_{18}-sphingosine **8**, respectively, with *O*-acyl fatty acid **3**. The required compound **3** was obtained from *ω*-hydroxy fatty acid **6** and linoleic acid **7** by esterification. The *ω*-hydroxy C_{30}-fatty acid **6** was prepared as follows: Copper-catalyzed coupling of *ω*-hydroxy alkyl halide **11** with the Grignard reagent derived from bromo compound **13** afforded after oxidation C_{17}-aldehyde **15**. Wittig reaction with phosphonium salt **10**, derived from *ω*-bromo-tridecanoic acid **9**, and subsequent hydrogenation and *O*-deprotection furnished **6** in high yield.

Extra- and intracellular ceramides of the epidermis seem to play important roles [1–3]. The permeability barrier of the skin, which prevents transcutaneous water loss and penetration of harmful drugs from the environment, is localized in the horny layer (stratum corneum) of the epidermis [1, 2]. The stratum corneum lipids are essentially responsible for the proper functioning of the epidermal barrier. Mostly ceramides, cholesterol and free fatty acids are found within the stratum corneum [4, 5]. These lipids, arranged in multiple intercellular lamellae, are derived from the contents of lamellar granules [6]. The lamellar granules display stacks of small disks each of which represents a flattened liposome [7]. It was found, that some unique sphingolipids play a special role in barrier function [8, 9]. Two of these important compounds (**1a** and **1b**) are shown in Scheme 1. The glucosylceramide **1a** is found within the lamellar granules [10]. It has been suggested, that this compound



Scheme 1 Building blocks for the synthesis of the target molecules **1a**,**b**

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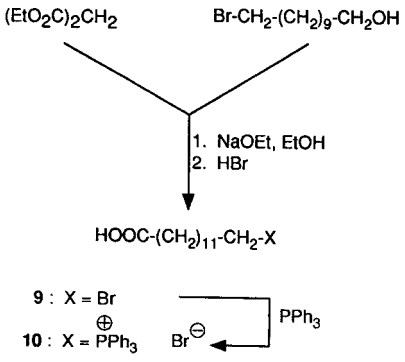
may provide the driving force for assembly of the stacks of flattened disks [8]. In the stratum corneum itself no glycolipids are found [5], but in a similar role the unusual acylcermide **1b** serves as a molecular rivet to hold together the multiple broad bilayers found within the stratum corneum [8, 11]. On account of their importance in barrier function, the cerbroside (**1a**) and the ceramide (**1b**) should be synthesized on gram scale for further investigations in dermatology and cosmetics. In this paper, we present an efficient synthesis of **1a** and **1b**, respectively [12, 13]. The synthesis of structurally related compounds containing unsaturated long chain ω-hydroxy fatty acids has been already reported [14, 15].

Results and Discussion

Scheme 1 shows our synthetic plan towards the synthesis of cerebroside **1a** and ceramide **1b**. Retrosynthetic bond disconnection of **1a** leads to the glucosylated C_{18}-sphingosine **2** and the *O*-acyl fatty acid **3** as necessary building blocks. The glycosylated C_{18}-sphingosine **2** can be prepared by azidosphingosine glycosylation [16, 17] *via* the trichloroacetimidate **4** and the 3-*O*-protected azidosphingosine **5**. For the synthesis of the *O*-acyl fatty acid **3**, 30-hydroxytriacontanoic acid **6** and linoleic acid **7** are required. Linoleic acid is a readily available natural product, so the remaining problems are the synthesis of the ω-hydroxy fatty acid and the connection with linoleic acid. Retrosynthetic bond disconnection of **1b** leads to the C_{18}-sphingosine **8** and the *O*-acyl fatty acid **3**. The C_{18}-sphingosine **8** can be prepared from **5** as described previously [18].

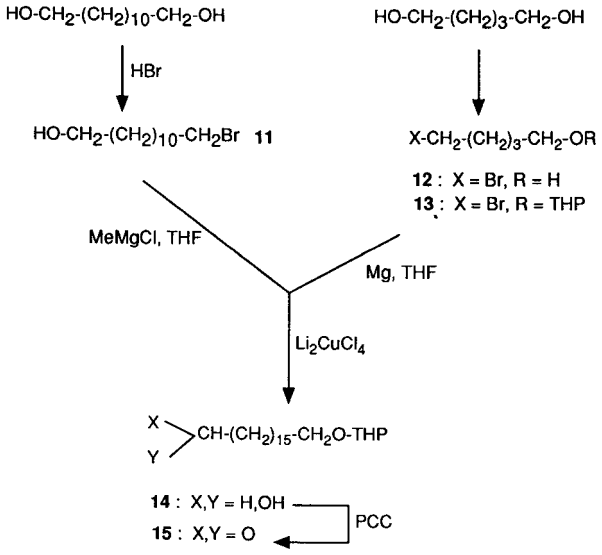
The main task in the synthesis of the epidermal compounds **1a** and **1b** was the preparation of ω-hydroxy fatty acid **3**. Three different coupling reactions were involved to obtain full chain length (Scheme 2–4): coupling with diethyl malonate to introduce the carboxy function, coupling of a Grignard compound with an alkyl



Scheme 2 Synthesis of ω-carboxy Wittig reagent **10**

halide for chain elongation, and coupling in a Wittig reaction to connect the building blocks of longer chain length. All methods worked in very high yields.

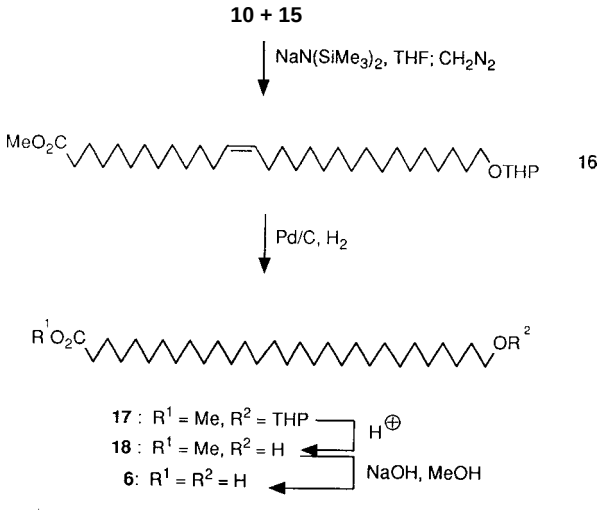
13-Bromotridecanoic acid **9** (Scheme 2) was prepared from diethyl malonate and 11-bromoundecanol [19] (first coupling step), which was heated with triphenylphosphine to give the phosphonium salt **10** in 80% yield, one of the building blocks in the Wittig reaction. The second building block, C_{17}-aldehyde **15** (Scheme 3) was prepared in the following manner: Treatment of 1,12-dodecanediol with hydrobromic acid gave the monobromo compound **11** in 86% yield [20].



Scheme 3 Synthesis of THP-protected ω-hydroxy aldehyde **15**

The same procedure was used to prepare 5-bromopentanol **12** from 1,5-pentanediol. Protection of the hydroxy group of **12** as its tetrahydropyranyl (THP) ether **13** was followed by treatment of **13** with magnesium to give a Grignard reagent. Coupling of the Grignard reagent with the chloromagnesium salt of **11** in the presence of dilithium tetrachlorocuprate (Li_2CuCl_4) [21] yielded **14** in 95% yield. Pyridinium chlorochromate (PCC) oxidation of **14** furnished the desired C_{17}-aldehyde **15**, required for the Wittig reaction, in 77% yield.

The coupling reaction of **10** and **15** under salt free conditions [22] (Scheme 4) afforded the methyl ester **16** in 88% yield after esterification of the crude reaction product. Esterification was necessary to provide the required solubility after removal of the double bond (see below). The olefinic ester **16** was hydrogenated over palladium on carbon to give **17** in 89% yield. After cleavage of the THP-ether to **18**, the ester was hydrolized to provide the acid **6** in 95% yield.



Scheme 4 Synthesis of ω-hydroxy fatty acid **6**

The esterification of linoleic acid **7** with the hydroxy fatty acid **6** proved to be difficult, because the saturated ω-hydroxy fatty acid **6** was almost insoluble in common solvents. However, activation of linoleic acid with 2-chloro-1-methylpyridinium iodide [23] afforded the desired *O*-acyl fatty acid **3** in 40% yield. *N*-acylation of the C_{18}-sphingosine **8** or of the 1-*O*-glucosylated sphingosine **2** with **3** in the presence of EEDQ gave the cerebroside **1a** and the ceramide **1b**, respectively, in 60% yields. The ^{1}H NMR data of the cerbroside are in agreement with those of material isolated from the epidermis [24] and with a similar compound (containing C_{20}-sphingosine) employing a different synthetic strategy [13].

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Experimental

Solvents were purified in the usual way; boiling range of petroleum ether: 36–65 °C. – Column chromatography: Kieselgel 60 (Fa. Merck, 0.063–0.200 mm). – Flash chromatography: silica gel (Fa. J. T. Baker, particle size 40 µm). – Thin layer chromatography (TLC): DC-Plastikfolien Kieselgel 60 F_{254} (Fa. Merck, layer thickness 0.2 mm). – Melting points are uncorrected. – ^{1}H NMR: Bruker WM 250 Cryospec, Bruker AC 250 Cryospec; internal standard tetramethyl silane (TMS). – Optical rotations: Perkin-Elmer polarimeter 241 MC; 1 dm cell.

(2S,3R,4E)-1-O-(β-D-Glucopyranosyl)-2-[30-linoleoyloxy)-triacontanoyl]-amino-4-octadecene-3-ol (**1a**)

A suspension of 109 mg (0.236 mmol) of (2*S*,3*R*,4*E*)-2-amino-1-(β-D-glucopyranosyloxy)-4-octadecene-3-ol (**2**) [17], 173 mg (0.236 mmol) of **3** and 70 mg (0.28 mmol) of EEDQ in dry ethanol (10 ml) was stirred at 50 °C for 25 h. After evaporation of the solvent *in vacuo*, the residue was chromatographed over silica gel with chloroform/methanol (9:1) to afford 166 mg (60%) **1a** as a colourless solid; *m.p.* 128–129 °C, $[\alpha]_D^{22} = -24.7$ (*c* = 1, pyridine). – TLC [chloroform/methanol (9:1)]: R_f = 0.28. – ^{1}H NMR [250 MHz, $CDCl_3$/methanol-d_4 (2:1)]: δ/ppm = 0.89 (t, $J_{17'',18''}$ = 6.8 Hz, 3H, CH_3) 0.9 (t, $J_{17,18}$ = 6.9 Hz, 3H, CH_3), 1.27, 1.32 (bs, 86H, 43 CH_2), 1.61–1.63 (m, 6H, 3 CH_2), 1.98–2.07 (m, 6H, 3 CH_2–C=C), 2.17 (t, $J_{2'',3''}$ = 7.5 Hz, 2H, CH_2COO), 2.32 (t, $J_{2',3'}$ = 7.5 Hz, 2H, CH_2CON), 2.79 (t, $J_{10'',11''} = J_{11'',12''}$ = 5.6 Hz, 2H, C=C–CH_2–C=C), 3.22–3.42 (m, 4H, 2'''-H, 3'''-H, 4'''-H, 5'''-H), 3.58 (dd, $J_{1A,2}$ = 2.9 Hz, $J_{1A,1B}$ = 10.2 Hz, 1H, 1A-H), 3.72 (dd, $J_{5''',6'''A}$ = 5 Hz, $J_{6'''A,6'''B}$ = 12 Hz, 1H, 6'''A-H), 3.87 (dd, $J_{5''',6'''B}$ = 2.4 Hz, 1H, 6'''B-H), 4.01 (m, 1H, 2-H), 4.07 (t, $J_{29',30'}$ = 6.6 Hz, 2H, CH_2OCO), 4.10 (dd, $J_{3,4}$ = 7.2 Hz, 1H, 3-H), 4.17 (dd, $J_{1B,2}$ = 4.4 Hz, 1H, 1B-H), 4.26 (d, $J_{1''',2''''}$ = 7.7 Hz, 1H, 1'''-H), 5.30–5.39 (m, 4H, *cis* CH=CH), 5.45 (dd, $J_{4,5}$ = 15.3 Hz, 1H, 4-H), 5.64–5.73 (m, 1H, 5-H).

$C_{72}H_{135}NO_{10}$	Calcd.:	C 73.61	H 11.58	N 1.19
(1174.9)	Found:	C 73.45	H 11.52	N 0.98.

(2S,3R,4E)-2-[30-(Linoleoyloxy)-triacontanoyl]amino-4-octadecene-3-ol (**1b**)

A suspension of 2.55 g (8.5 mmol) of (2*S*,3*R*,4*E*)-2-amino-4-octadecene-1,3-diol **8** [18], 6.23 g (8.5 mmol) of **3** and 2.52 g (10.2 mmol) of EEDQ in dry ethanol (250 ml) was stirred at 50 °C for 25 h. After evaporation of the solvent *in vacuo*, the residue was purified first by flash chromatography over silica gel with chloroform/methanol/dioxane (50:0.5:1 and 15:0.5:1) and then by flash chromatography with chloroform/methanol (95:5). In a final step the product was suspended in ether, and after acetonitrile was added, the precipitated product was collected and washed with acetonitrile. This procedure gave 5.16 g (60%) **1b** as a white solid; *m.p.* 86 °C, $[\alpha]_D^{20}$ = 7.4 (*c* = 1, pyridine). – TLC [chloroform/methanol (95:5)]: R_f = 0.24. – ^{1}H NMR [250 MHz, $CDCl_3$/methanol-d_4 (2:1)]: δ/ppm = 0.89 (t, $J_{17'',18''}$ = 6.8 Hz, 3H, CH_3), 0.9 (t, $J_{17,18}$ = 6.5 Hz, 3H, CH_3), 1.27, 1.31, 1.32 (bs, 86H, 43 CH_2), 1.58–1.63 (m, 6H, 3 CH_2), 2.03–2.07 (m, 6H, 3 CH_2-C=C), 2.21 (t, $J_{2'',3''}$ = 7.6 Hz, 2H, CH_2COO), 2.31 (t, $J_{2',3'}$ = 7.5 Hz, 2H, CH_2CON), 2.78 (t, $J_{10'',11''} = J_{11'',12''}$ = 5.6 Hz, 2H, C=C–CH_2–C=C), 3.65 (ddd, $J_{1A,2}$ = 4.5 Hz, $J_{1B,2}$ = 4.6 Hz, $J_{2,3}$ = 7.4 Hz, 1H, 2-H), 3.83 (2 dd, $J_{1A,1B}$ = 8.3 Hz, 2H, 2 1-H), 4.07 (t, $J_{29',30'}$ = 6.6 Hz, 2H, CH_2OCO), 4.14 (dd, $J_{3,4}$ = 6.8 Hz, 1H, 3-H), 5.28–5.41 (m, 4H, *cis* CH=CH), 5.47 (dd, $J_{4,5}$ = 15.3 Hz, 1H, 4-H), 5.68–5.77 (m, 1 H, 5-H).

$C_{66}H_{125}NO_5 \cdot 0.5\ H_2O$	Calcd.:	C 77.59	H 12.43	N 1.37
(1021.7)	Found:	C 77.69	H 12.36	N 1.16.

30-(Linoleoyloxy)-triacontanoic Acid (**3**)

A mixture of 10.13 g (39.65 mmol) of 2-chloro-1-methyl pyridinium iodide, 10.3 ml (33 mmol) of linoleic acid and 11.41 ml (47.83 mmol) of tributylamine in dry pyridine (300 ml) was stirred at 110 °C under nitrogen for 2 h. Then 13.13 g (28 mmol) of **6** were added and heating was continued for 14 h. After cooling, the mixture was concentrated *in*

vacuo and codistilled three times with toluene to remove all of the pyridine. The residue was suspended in ether. Acetonitrile was added, and the flocky precipitate was collected and washed several times with acetonitrile to remove excess linoleic acid. The precipitate was chromatographed over silica gel. Elution with chloroform/methanol (95:5) gave 8.2 g (40%) of **3** as light yellow crystals; *m.p.* 78–79 °C. – TLC [chloroform/methanol (95:5)]: R_f = 0.33. – ^{1}H NMR (250 MHz, $CDCl_3$): δ/ppm = 0.89 (t, $J_{17',18'}$ = 6.8 Hz, 3H, CH_3), 1.25, 1.31 (bs, 64H, 32 CH_2), 1.58–1.64 (m, 6H, 3 CH_2), 2.00–2.06 (m, 4H, 2CH_2–C=C), 2.29 (t, $J_{2',3'}$ = 7.7 Hz, 2H, CH_2COO), 2.35 (t, $J_{2,3}$ = 7.4 Hz, 2H, CH_2COO), 2.77 (t, $J_{10',11'}$ = $J_{11',12'}$ = 5.7 Hz, 2H, C=C–CH_2–C=C), 4.03 (t, $J_{29,30}$ = 6.7 Hz, 2H, CH_2OCO), 5.32–5.39 (m, 4H, 2CH=CH).

$C_{48}H_{90}O_4$	Calcd.:	C 78.84 H 12.41
(731.3)	Found:	C 78.87 H 12.45.

O-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)-trichloroacetimidate (**4**)

The imidate **4** was prepared as described previously [25].
(2S,3R,4E)-2-Azido-3-benzoyloxy-4-octadecene-1-ol (**5**)

This compound was prepared as described previously [18].
30-Hydroxytriacontanoic Acid (**6**)

A suspension of 29 g (60 mmol) of 18 and 28.8 g (720 mmol) of sodium hydroxide in methanol (500 ml) was heated to reflux for 24 h. After cooling with ice, the precipitate was collected, suspended in water and acidified with concentrated hydrochloric acid (pH 1). The product was filtered off and recrystallized from glacial acetic acid to give 26.7 g (95%) of **6** as colourless crystals; *m.p.* 125 °C. – TLC [chloroform/methanol (9:1)]: R_f = 0.50.

13-Bromotridecanoic Acid (**9**)

The procedure of preparation of compound **9** is known in the literature [19]. Modification of the described procedure gives a higher yield (80%) than in ref. [19] (61%). 11-Bromoundecanol (50 g, 0.2 mol) in dry ethanol was added to diethyl sodiomalonate, prepared from diethyl malonate (60.4 ml, 0.4 mol) and sodium (9.2 g, 0.4 mol) in dry ethanol (500 ml). The mixture was heated under reflux for 21 h, cooled and evaporated *in vacuo*. Dilute sulfuric acid was added to the residue and the mixture extracted several times with ether. The combined extracts were dried and concentrated *in vacuo*. To the remaining oil 100 ml of glacial acetic acid and hydrobromic acid (100 ml, 47%) were added and the resulting alcohol was slowly distilled through a Vigreux column. The bath temperature was raised slowly to 130–140 °C. Sulfuric acid (10 ml, 20N) was added to the residue, and the mixture was refluxed for 2 h to complete decarboxylation. Hydrobromic acid (100 ml, 47%) and concentrated sulfuric acid (30 ml) were added and the solution was heated under reflux for 15 h, then poured into ice-water and the precipitate was filtered off. The precipitate was dissolved in hydrobromic acid (200 ml, 30%) and concentrated sulfuric acid (25 ml) and heated under reflux for 6 h, poured into ice-water and the precipitate was filtered off. Recrystallization from petroleum ether gave 46.9 g (80%) of **9** as light yellow crystals; *m.p.* 60 °C (ref. [19] 59–61 °C).

12-Carboxydodecyltriphenylphosphonium Bromide (**10**)

A mixture of 46 g (0.156 mol) of **9** and 40.9 g (0.156 mol) of triphenylphosphine was heated to 140 °C for 15 h. The resulting substance was dissolved in chloroform, as less as possible. The solution was added dropwise to ether to precipitate the phosphonium salt. The precipitate was filtered off and for purification the salt was dissolved in chloroform and precipitated in ether twice. After drying at 80 °C *in vacuo* over night 87.9 g (100%) of **10** were obtained as light yellow crystals.

5-Bromopentanol (**12**)

A mixture of 1,5-pentanediol (30 g, 0.29 mol) and hydrobromic acid (150 ml, 47%) was kept at 80 °C and extracted continuously with petroleum ether (*b.p.* 100–140 °C) to remove the monobrominated product from the reaction mixture. After 22 h the organic extract was washed with saturated sodium hydrogen carbonate solution, dried with magnesium sulfate, and concentrated *in vacuo*. Chromatography with petroleum ether/ethyl acetate (6:4) gave 17.1 g (36%) of **12** as a light yellow oil; *b.p.* 76 °C, 0.5 Torr (ref. [26] *b.p.* 75–76 °C). – TLC [petroleum ether/ethyl acetate (6:4)]: R_f = 0.52.

5-Bromo-1-(tetrahydro-2-pyranyloxy)-pentane (**13**)

To a mixture of 11.3 ml (0.124 mol) of 4,5-dihydro-(2*H*)pyrane and 17.1 g (0.1 mol) of **12** 4 drops of concentrated hydrochloric acid were added. After 2.5 h at room temp., the mixture was diluted with a small amount of petroleum ether, washed with saturated sodium hydrogen carbonate solution, and dried over magnesium sulfate. The solvent was evaporated and the residue was chromatographed over silica gel. Elution with petroleum ether/ethyl acetate (9:1) gave 24.6 g (96%) of **13** as a colourless oil; *b.p.* 83 °C, 0.4 Torr (ref. [26] *b.p.* 84 °C). – TLC [petroleum ether/ethyl acetate (9:1)]: R_f = 0.57.

17-(Tetrahydro-2-pyranyloxy)-heptadecan-1-ol (**14**)

A solution of 21.23 g (80 mmol) 12-bromododecanol (**11**) [20] in dry THF (160 ml) under nitrogen was cooled to –20 °C. A methyl magnesium chloride solution in THF (27 ml, 3 M) was added. After the cessation of gas evolution, Li_2CuCl_4 (8 ml, 0.2 M in THF) was added followed by the addition of the Grignard reagent, prepared from 40.10 g (160 mmol) **13** and 6.22 g (256 mmol) magnesium in dry THF (320 ml), over a 30-minute period. Stirring at –20 °C was continued for 1 h, then the temperature was allowed to rise to room temp. After 15 h the mixture was poured into aqueous ammonium acetate solution and extracted several times with ether. The combined organic extracts were washed with water, dried with sodium sulfate and concentrated *in vacuo*. Chromatography on silica gel with petroleum ether/ethyl acetate (8:2 and 7:3) yielded 27.1 g (95%) **14** as colourless crystals; *m.p.* 39 °C. – TLC [petroleum ether/ethyl acetate (7:3)]: R_f = 0.39. – ^{1}H NMR (250 MHz, $CDCl_3$): δ/ppm = 1.25 (bs, 26H, 13 CH_2), 1.48–1.83 (m, 10 H, 2 3'-H, 2 4'-H, 2CH_2, CH_2O), 3.38 (td, $J_{4',5'}$ = 6.9 Hz, $J_{5'A,5'B}$ = 9.6 Hz, 1H, 5'-H), 3.47–3.52 (m, 1H, 2'-H), 3.64 (t, $J_{1,2}$ = 6.6 Hz, 2H, CH_2OH), 3.73 (td, 1H, 5'-H), 3.83–3.87 (m, 1H, 2'-H), 4.58 (t, $J_{1',2'}$ = 3.5 Hz, 1H, 1'-H).

$C_{22}H_{44}O_3$ Calcd.: C 74.10 H 12.44
(356.6) Found: C 74.18 H 12.56.

17-(Tetrahydro-2-pyranyloxy)-heptadecanal (**15**)

A solution of 41 g (0.115 mol) of **14** in dry dichloromethane (30 ml) was added to a suspension of 37.2 g (0.173 mol) of PCC in dry dichloromethane (240 ml) under nitrogen. After 2.5 h at room temp. the black mixture was diluted with about 900 ml of dry ether. After decantation the black residue was washed twice with dry ether (100 ml). After evaporation of the combined solution *in vacuo*, chromatography over silica gel with petroleum ether/ethyl acetate (7:1) gave 31.4 g (77 %) of **15** as a colourless oil. – TLC [petroleum ether/ethyl acetate (7:1)]: R_f = 0.47. – ^{1}H NMR (250 MHz, $CDCl_3$): δ/ppm = 1.25 (bs, 24 H, 12 CH_2), 1.53–1.82 (m, 10H, 2 3'-H, 2 4'-H, 2 CH_2, CH_2O), 2.41 (td, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 7.3 Hz, 2H, C$\underline{H}_2$CHO), 3.38 (td, $J_{4',5'}$ = 6.9 Hz, $J_{5'A,5'B}$ = 9.6 Hz, 1H, 5'-H), 3.46–3.52 (m, 1H, 2'-H), 3.73 (td, 1H, 5'-H), 3.83–3.87 (m, 1H, 2'-H), 4.58 (t, $J_{1',2'}$ = 3.5 Hz, 1H, 1'-H), 9.76 (t, 1H, CHO).
$C_{22}H_{42}O_3$ Calcd.: C 74.52 H 11.94
(354.8) Found: C 74.15 H 11.85.

Methyl (Z)-30-(Tetrahydro-2-pyranyloxy)-13-triacontenoate (**16**)

To 181 ml of sodium bis(trimethylsilyl)amide (1M in THF) in freshly distilled, dry THF (500 ml) were slowly added 47.9 g (86.22 mmol) of **10**. After 30 min at room temp. the mixture was heated to reflux for 1 h. Cooling to –20 °C was followed by dropwise addition of 30.57 g (86.21 mmol) of **15** in dry THF. Cooling was continued for 1 h. After 15 h at room temp., the mixture was poured into satd. ammonium chloride solution, acidified with concentrated hydrochloric acid and extracted several times with ether. The combined organic extracts were washed with water, dried over magnesium sulfate and concentrated *in vacuo*. The residue was dissolved in ether and esterified with diazomethane in the usual manner. After evaporation of the solvent flash chromatography over silica gel with petroleum ether/ethyl acetate (95:5 and 9:1) gave 42.86 g (88%) of **16** as colourless crystals; *m.p.* 37–39 °C. – TLC [petroleum ether/ethyl acetate (9:1)]: R_f = 0.53. – ^{1}H NMR (250 MHz, $CDCl_3$): δ/ppm = 1.25 (bs, 42H, 21 CH_2), 1.48–1.85 (m, 10H, 2 3'-H, 2 4'-H, 2 CH_2, CH_2O), 1.97–2.02 (m, 4H, CH_2–C=C–CH_2), 2.30 (t, $J_{2,3}$ = 7.5 Hz, 2H, CH_2COO), 3.38 (td, $J_{4',5'}$ = 6.9 Hz, $J_{5'A,5'B}$ = 9.6 Hz, 1H, 5'-H), 3.48–3.54 (m, 1H, 2'-H), 3.67 (s, 3H, CH_3), 3.73 (td, 1H, 5'-H), 3.83–3.88 (m, 1H, 2'-H), 4.58 (t, $J_{1',2'}$ = 3.5 Hz, 1H, 1'-H), 5.33–5.38 (m, 2H, CH=CH).
$C_{36}H_{68}O_4$ Calcd.: C 76.54 H 12.13
(564.9) Found: C 76.79 H 11.92.

Methyl 30-(Tetrahydro-2-pyranyloxy)-triacontanoate (**17**)

To a solution of 38.3 g (67.8 mmol) **16** in ethyl acetate (420 ml) was added 1.9 g of 10% palladium on carbon. The mixture was hydrogenated until the uptake of hydrogen had stopped, then filtered through Celite® to remove the catalyst. It was necessary to wash several times with chloroform to dissolve precipitated product. The filtrate was concentrated, and the crude product **17** (34.2 g, 89%) was used in the next step without further purification. For identification the crude product was chromatographed over silica gel with petroleum ether/ethyl acetate (9:1) to afford colourless crystals; *m.p.* 74–75 °C. – TLC [petroleum ether/ethyl acetate (9:1)]: R_f = 0.49. – ^{1}H NMR (250 MHz, $CDCl_3$): δ/ppm = 1.25 (bs, 50H, 25 CH_2), 1.54–1.85 (m, 10H, 2 3'-H, 2 4'-H, 2 CH_2, CH_2O), 2.30 (t, $J_{2,3}$ = 7.5 Hz, 2H, CH_2COO), 3.38 (td, $J_{4',5'}$ = 6.9 Hz, $J_{5'A,5'B}$ = 9.5 Hz, 1H, 5'-H), 3.46–3.54 (m, 1H, 2'-H), 3.67 (s, 3H, CH_3), 3.73 (td, 1H, 5'-H), 3.83–3.91 (m, 1H, 2'-H), 4.58 (t, $J_{1',2'}$ = 3.5 Hz, 1H, 1'-H).
$C_{36}H_{70}O_4$ Calcd.: C 76.26 H 12.47
(567.0) Found: C 76.24 H 12.37.

Methyl 30-Hydroxytriacontanoate (**18**)

To a suspension of 33.3 g (58.7 mmol) of **17** in methanol (350 ml) 22.3 g (117.3 mmol) of *p*-toluenesulfonic acid was added. The mixture was stirred at room temp. for complete reaction (TLC). After evaporation of the solvent, the residue was dissolved in chloroform and washed with satd. sodium hydrogen carbonate solution. The chloroform layer was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product **18** (28.3 g, 100%) was used in the next step without further purification. Recrystallization (petroleum ether/ethyl acetate) gave colourless crystals; *m.p.* 88 °C. – TLC [petroleum ether/ethyl acetate (8:2)]: R_f = 0.29. – ^{1}H NMR (250 MHz, $CDCl_3$): δ/ppm = 1.25 (bs, 50H, 25 CH_2), 1.54–1.64 (m, 4H, 2 CH_2), 2.30 (t, $J_{2,3}$ = 7.5 Hz, 2H, CH_2COO), 3.64 (t, $J_{29,30}$ = 6.4 Hz, 2H, C$\underline{H}_2$OH), 3.67 (s, 3H, CH_3).
$C_{31}H_{62}O_3$ Calcd.: C 77.12 H 12.94
(482.8) Found: C 76.68 H 12.65.

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