Structural confirmation of the dihydrosphinganine and fatty acid constituents of the dental pathogen *Porphyromonas gingivalis*†

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Porphyromonas gingivalis, a recognized periodontal pathogen, is a source of sphinganine bases, fatty acids, free ceramides as well as complex lipids that potentiate interleukin-1b-mediated secretory responses in gingival fibroblasts. The purpose of this study is the structural verification of the sphinganine bases and fatty acids that had been proposed as major components of the complex lipids found in *P. gingivalis*. The putative C17, C18, and C19 sphinganine bases were prepared from Garner's aldehyde (**1**) or from a protected serine Weinreb's amide (**2**). We confirmed that isobranched sphinganine bases are the major structural feature of the ceramides observed from *P. gingivalis*. We also prepared a C17 unsaturated fatty acid, along with an isobranched C17 3-hydroxy fatty acid, and determined that the major component of the active lipids was the latter.

Introduction

The anaerobic, Gram-negative organism, *Porphyromonas gingivalis*, is thought to be a major periodontal pathogen associated with inflammatory periodontal disease in adults. Since inflammatory periodontal disease in adults is initiated with the accumulation of specific bacteria in the sulcus around the teeth followed by a chronic inflammatory reaction to these organisms by the host, identification of the active components associated with *P. gingivalis* is important for understanding and treating periodontal disease. Previous work used GC-mass[1] and electrospray-mass[2] spectroscopic analysis to show that the lipid extracts of *P. gingivalis* are similar to lipid profiles in extracts of diseased tooth roots and diseased gingival tissues.[1] Lipid extracts of *P. gingivalis* are known to potentiate prostaglandin secretion from gingival fibroblasts when co-treated with interleukin-1b, and more recent work demonstrated that phosphorylated dihydroceramides are the primary biologically active lipid constituents.[3] Beyond the GC-MS data, little is known about the specific lipids responsible for this biological activity. Preliminary structural identification pointed to phosphorylated dihydroceramides with an unusual sphinganine base and fatty acid components that are proposed to contain isobranched aliphatic chains. Within each polar or nonpolar sphingolipid class of *P. gingivalis*, a mixture of lipid species exists and preliminary evidence based on analysis of the lipid mixtures suggested that the base dihydroceramide core structures comprise C17, C18 and C19 long chain dihydrosphinganine bases in amide linkage to 3-OH isoC17:0 fatty acids. Mass spectral evidence on enriched lipid fractions, along with proton NMR data of these mixtures, suggests that the C17 and C19 long chain bases are isobranched. Similar analysis led us to conclude that the aliphatic chain of the 3-OH isoC17:0 fatty acid was similarly isobranched, and that the isobranching was critical for expression of biological activity.[4] Since each bioactive fraction was a mixture, the presence of dihydroceramides as the main components, the length of the carbon chain of both the dihydrosphinganine component and the fatty acid, the presence of isobranches, and the β-hydroxyl unit on the fatty acid unit, remained tentative identifications. Before proceeding with a complete total synthesis to establish both the structure of the active dihydroceramides, along with establishment of the relative and absolute stereochemistry of these compounds, it was imperative that we synthesize both the long chain bases and the fatty acid for confirmation that these structures are present in the biologically active fractions, by direct comparison with hydrolyzed lipid extracts recovered from *P. gingivalis*. Verification of the constituent long chain bases and fatty acids will allow the preparation of synthetic *P. gingivalis* dihydroceramide lipid standards for evaluation of host cell recognition and activation of these impressive lipid products. The work described herein verifies the structures of the base and fatty acid components.

Results and discussion

Our first targets were the sphinganine bases described above as putative metabolites of *P. gingivalis*. The stereochemistry of these compounds is unknown, but we initiated a synthesis based on L-serine as a template, as this is the biological form of serine. We used Garner's aldehyde (**1**)[5,6] as the starting material in one approach, and a protected serine derivative that is activated as a Weinreb amide (**2**)[7] in a second. Both starting materials can be prepared from L-serine.[8] Subsequent synthesis of dihydrosphinganine compounds from **1** used a coupling reaction with an appropriate alkyne

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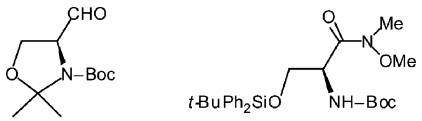
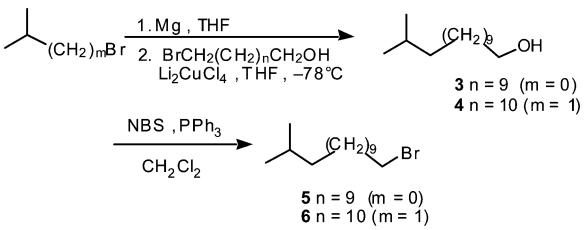
anion, whereas **2** was reacted with a long-chain organometallic, followed by reduction of the resulting ketone.



1 **2**

We examined the L-serine template-Garner's aldehyde route, and prepared the targeted C17 and C19 straight-chain components from the appropriate alkyne anions. Although this approach was successful, poor yields and isolation problems plagued key steps in the syntheses of the isobranched compounds. These problems led us to use **2** and **8** for the preparation of the latter compounds.

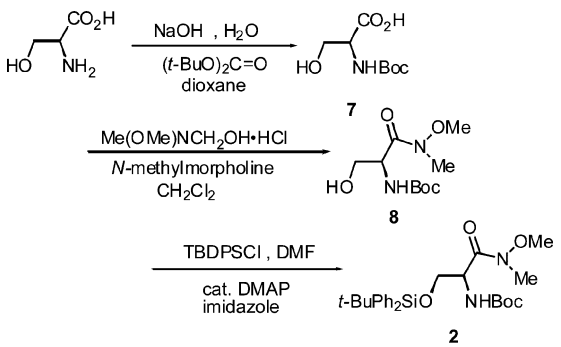
The reaction of commercially available 11-bromo-1-undecanol with the Grignard reagent of 2-bromopropane and Li_2CuCl_4 (prepared *in situ*) provided 12-methyl-1-tridecanol (**3**)[9] in 94% yield. This procedure is essentially identical to that described by Singh *et al.*[9] or Mori and co-workers.[10] Similarly, the Grignard reagent derived from 1-bromo-3-methylbutane was coupled with 11-bromo-1-undecanol to give 14-methyl-1-pentadecanol (**4**)[11] in 95% yield. Subsequent treatment of **3** with NBS and triphenylphosphine[12] led to an 81% yield of the bromide, **5**.[12] Similarly, **4** was converted to **6**[13] in 86% yield.



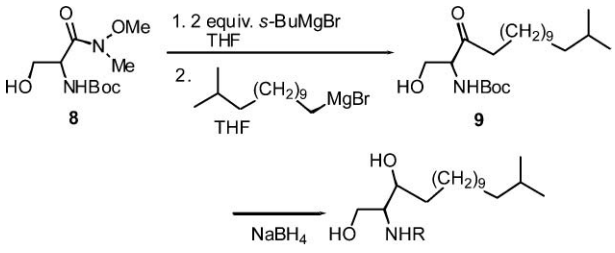
3 n = 9 (m = 0)
4 n = 10 (m = 1)

5 n = 9 (m = 0)
6 n = 10 (m = 1)

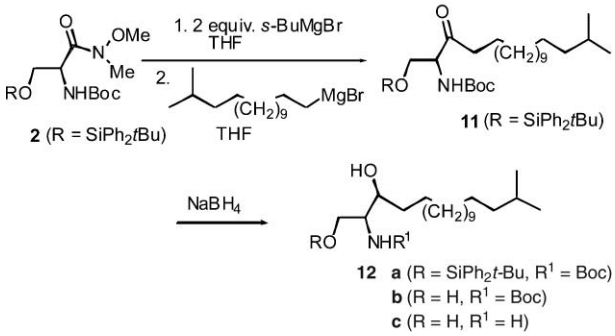
Protection of L-serine as the *N*-Boc derivative (**7**)[14] by a literature procedure[15] was followed by conversion to the corresponding Weinreb amide[16] **8**, in 81% yield, by reaction with *N*,*O*-dimethyhydroxylamine hydrochloride and *N*-methylmorpholine. When **8** reacted with *tert*-butyldiphenylsilyl chloride in DMF, in the presence of imidazole and a catalytic amount of DMAP,[7] the silyl-protected derivative **2** was obtained in 93% yield.



The targeted dihydrosphinganine compounds were prepared by reaction of **8** or **2** with the appropriate Grignard reagent, followed by reduction of the resulting ketone. The reaction of **8** with 2 equivalents of a sacrificial base (*sec*-butylmagnesium bromide) in THF, followed by addition of the Grignard reagent prepared from **5** and magnesium in THF,[7] gave ketone **9** in 21% yield.



10 **a** (R = Boc)
b (R = H)



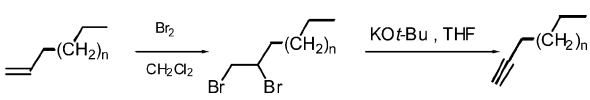
2 (R = $SiPh_2tBu$)

11 (R = $SiPh_2tBu$)

12 **a** (R = $SiPh_2t$-Bu, R^1 = Boc)
b (R = H, R^1 = Boc)
c (R = H, R^1 = H)

Subsequent reaction of **9** with $NaBH_4$ in methanol[17,18] gave **10a** in 89% yield as a 3 : 1 mixture of diastereomers favoring the *anti*-diastereomer. This procedure failed to give good yields of coupling product using **6**, and we used **2** to address problems with reactivity. When **8** was treated with one equivalent of *sec*-butylmagnesium bromide in THF, and then with the Grignard reagent formed from **6**, we obtained a 34% yield of ketone **11**. Reduction with $NaBH_4$ as before gave a 56% yield of **12a**, again as a 3 : 1 mixture of diastereomers favoring *anti*-**12a**. Deprotection with aqueous trifluoroacetic acid provided **10b** in 57% yield. The presence of the silyl protecting group in **12** forced us to use two steps to give **12c** from **12a**. Deprotection of **12a** with tetrabutylammonium fluoride (TBAF) gave **12b** in 93% yield, and final deprotection of **12b** provided **12c** in 83% yield.

The straight-chain sphinganine compounds were prepared from the appropriate alkyne by coupling with Garner's aldehyde (**1**). Garner's aldehyde was prepared from serine by literature methods.[5] The alkynes were prepared from commercially available 1-hexadecene or 1-pentadecene. 1-Hexadecene (**13**) was converted to dibromide **15** by reaction with bromine in dichloromethane, in 95% yield. Reaction of **15** with potassium *tert*-butoxide in THF gave 1-hexadecyne **17** in 65% yield. Similarly, 1-pentadecene (**14**) gave the corresponding dibromide **16** in 95% yield, and elimination provided 1-pentadecyne **18** in 65% yield. If the temperature of the reaction was not well-controlled, the 2-alkyne was obtained as the major product, occasionally the only product. Under the conditions described, we obtained the desired alkynes in poor-to-moderate yield.



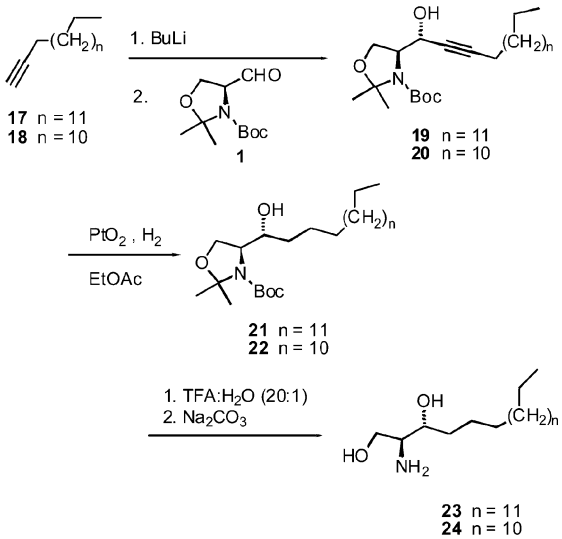
13 n = 11
14 n = 10

15 n = 11
16 n = 10

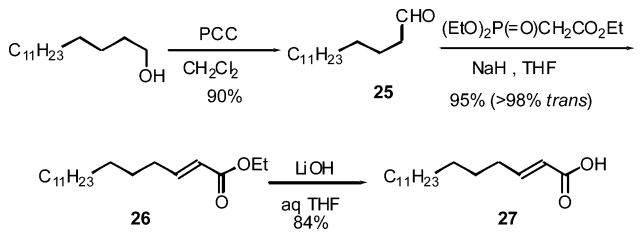
17 n = 11
18 n = 10

The C19 and C18 straight-chain sphinganine compounds were synthesized according to the procedures developed by Garner

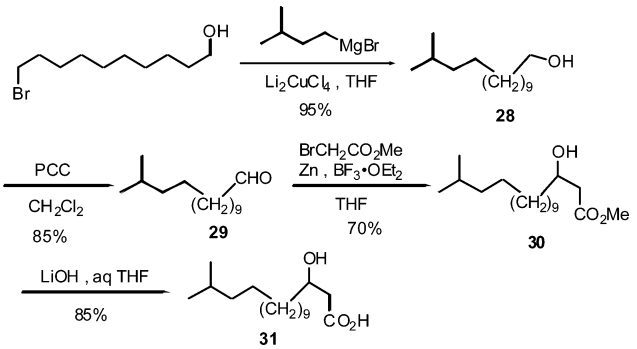
et al.[8b] and by Liotta *et al.*[5b] The addition of *n*-butyllithium to 1-hexadecyne (**17**) generated the alkyne anion, and subsequent reaction with **1** gave alcohol **19** in 62% yield. Similar reaction of 1-pentadecyne led to **20**, also in 62%. Catalytic hydrogenation with Adams catalyst in ethyl acetate provided the saturated alcohols **21** in 91% yield and **22** in 92% yield. Deprotection with a 20:1 mixture of trifluoroacetic acid and water, followed by neutralization with sodium carbonate, gave the targeted sphinganine **23** in 86% yield and **24** in 89% yield.



Analysis of the lipid fractions from *P. gingivalis* suggested two major 17-carbon fatty acids were present, heptadec-3-enoic acid (**27**) and 15-methyl-3-hydroxyhexadecanoic acid (**31**). We first targeted the major component of the extracts, **27**. Commercially available 1-pentadecanol was oxidized to pentadecanal **25**[19] with PCC, in 90% yield. Subsequent Horner–Wadsworth–Emmons olefination with ethyl diethylphosphonoacetate and sodium hydride gave a 95% yield of the expected conjugated ester, **26**.[20] Analysis by NMR and GC-MS showed that the alkene unit in **26** was >98% *trans*. Saponification with aqueous lithium hydroxide provided **27**[21] in 84% yield.



The 3-hydroxy fatty acid **31** was prepared from commercially available 10-bromo-1-decanol. Initial coupling with the Grignard reagent of 1-bromo-3-methylbutane, in the presence of Li_2CuCl_4 according to the procedure reported by Singh *et al.*[9] and by Mori *et al.*,[10] gave 13-methyl-1-tetradecanol (**28**) in 95% yield, analogous to the preparation of **3** and **4** described above. Oxidation with PCC gave aldehyde **28** in 85% yield. Reformatsky reaction with methyl bromoacetate gave the requisite 3-hydroxy ester **30** in 70% yield. Saponification gave **31** in 85% yield.



Comparison of synthetic standards with lipid extracts from *P. gingivalis*

Analysis of esterified fatty acids was accomplished by treating a sample of the lipid fraction with 0.5 N sodium methoxide in anhydrous methanol (0.5 mL, 40 °C for 20 min). The reaction was stopped with the addition of 100 mL of glacial acetic acid and 1 mL of water. The sample was then extracted twice with hexane and the contents dried under nitrogen. The sample was reconstituted in hexane for GC-MS analysis. For long chain base and amide linked fatty acid analyses, samples of HPLC fractions were hydrolyzed in 4 N KOH (0.5 mL, 100 °C for 4 h). After cooling, the sample was extracted twice with 2 mL of $CHCl_3$ and dried under nitrogen. Trimethylsilyl (TMS) derivatives of long chain bases were prepared by treating with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (40 mL, room temperature overnight). TMS derivatives of bacterial fatty acids and long chain bases were analyzed using gas chromatography combined with electron impact mass spectrometry. For analyses, selected HPLC fractions were treated to form TMS derivatives and were applied to an SPB-1 column (15 m × 0.25 mm, 0.1 mm film thickness, Supelco, Inc., State College, PA). Complex lipid samples were injected with the inlet block at 310 °C using the splitless mode with a temperature program of 10 °C min^{-1} from 200 °C to 300 °C followed by 5 °C min^{-1} to 310 °C and 4 min at 310 °C. The mass spectrometer was used in the electron impact ionization mode with the ion source temperature of 150 °C, an electron energy of 70 eV and an emission current of approximately 300 mA. The injector block and transfer tube were held at 310 °C. For positive ion chemical ionization analyses, methane was used as the reagent gas and was maintained at 0.5 torr and the ion source temperature was held at 150 °C. ESI-MS analysis was accomplished using a Micromass Quattro II mass spectrometer system. Ceramide lipid fractions were dissolved in hexane–isopropanol (6 : 8, v/v, elution solvent) and the samples injected at a maximum concentration of 100 mg mL^{-1}. Lipid samples (10 mL) were infused at a flow rate of 20 mL min^{-1}. The solvation and inlet block temperatures were 80 °C and 120 °C, respectively. The cone voltage was usually 30 volts, the electrospray potential was 3500 volts and the mass range analyzed was 0–2000 amu for initial electrospray MS analyses. MS-MS analysis used a collision energy of between 28 and 30 volts, and argon was introduced at a pressure of 10^{-2} to 10^{-4} torr. The gas and collision energies were adjusted to minimize the parent ion recoveries and maximal daughter ion recoveries. These conditions were used for both positive and negative ion electrospray MS-MS analyses.

Direct comparison of the lipids isolated from *P. gingivalis* with sphinganines **10b**, **12c**, **23**, **24**, and **27** as well as fatty acids **21** and **26** confirms that *P. gingivalis* synthesizes phosphoceramides with a core lipid structure consisting of a straight chain 18-carbon and isobranched 17 and 19 carbon long chain bases, in an amide linkage to a 3-OH isoC17:0 fatty acid. Although **27** was confirmed as the major fatty acid constituent of the *P. gingivalis* lipid extracts, it does not correlate as a constituent of the bioactive compound(s). The free ceramides of *P. gingivalis* detected in HPLC fractions 7–8 (data not shown) and described in previous reports,[1–4] likely serve as the substrate molecules for the synthesis of the phosphoceramides described here.

An earlier report concluded that the unsaturated ceramides are synthesized by *P. gingivalis* because the α,β-unsaturated C17:0 fatty acid is recovered in alkaline hydrolyzates of *P. gingivalis* lipids. However, this monounsaturated C17:0 fatty acid is not recovered in HPLC fraction 20 lipids (data not shown), which suggested that it cannot be a constituent of this bioactive ceramide fraction. The synthesis of **27** and direct comparison with the lipid fractions confirmed this hypothesis.

Conclusions

The results of this study demonstrate that a major periodontal pathogen, *P. gingivalis*, synthesizes a group of unusual complex lipids, specifically phosphoglycerol ceramides, that appear to account for the ability of *P. gingivalis* lipid extract to potentiate interleukin-1β-mediated prostaglandin secretion from fibroblasts. The key lipid bases are now confirmed as **10a**, **12c** and **24**, and the key fatty acid is **31**. The stereochemistry of all stereogenic centers has not yet been correlated with the biological activity of the dihydroceramides, but all sphinganine bases in this study are derived from L-serine. For purposes of comparison with hydrozylates from *P. gingivalis* in this study, the C3 hydroxyl unit is a 3 : 1 mixture of *anti* : *syn* diastereomers, and the β-hydroxyl fatty acid is racemic at that stereocenter. Therefore, our analysis is based on a mixture of stereoisomeric compounds that confirms the constitutional identity of the components, but not the specific stereochemistry. That work will follow as we prepare the ceramides and a bioassay that will allow us to verify the stereochemical identity of the bioactive compound(s).

Experimental

All glassware was flame-dried under nitrogen, and all reactions were performed under a nitrogen atmosphere. All chemicals were purchased from the Aldrich Chemical Co. and used without further purification. The tetrahydrofuran was dried over sodium–benzophenone and distilled immediately prior to its use. The ^{1}H NMR (400 MHz) and the ^{13}C NMR (100.65 MHz) spectra were recorded on a Brücker DRX-400 instrument, in $CDCl_3$ unless otherwise noted, and all chemical shifts are reported relative to tetramethylsilane (TMS) as an internal standard. Infrared spectra were recorded on a JASCO FT/IR-410 instrument, and reported in cm^{-1}. Mass spectra were recorded on a Hewlett-Packard 5970 GC-MS instrument. High resolution mass spectrometry of new compounds was performed on a Micromass VB-QTOF tandem mass spectrometer.

[2-Hydroxy-1-(methoxymethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester, 8

A solution of *N*-(*tert*-butoxycarbonyl)-L-serine in 75 mL dry CH_2Cl_2, cooled to −15 °C, was treated with *N*,*O*-dimethylhydroxylamine hydrochloride (1.91 g, 19.60 mmol), followed by *N*-methylmorpholine (2.15 mL, 19.60 mmol). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (3.76 g, 19.60 mmol) was then added portionwise as a solid over a period of 30 minutes. The reaction mixture was stirred for 1.5 h at the same temperature and then ice cold 1 M HCl was added (15 mL). The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated to yield a white solid, **8**[15] (3.80 g, 15.30 mmol, 81% over 2 steps), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.59 (bs, 1H), 4.81 (bs, 1H), 3.84–3.80 (m, 5H), 3.25 (s, 3H), 2.58 (bs, 1H), and 1.47 ppm (s, 9H); MS (EI) *m*/*z* 188 [*M* − N(OMe)Me], 175, 132, 104, 57.

(2*S*)-2-(*N*-*t*-Butoxycarbonyl)amino-1-hydroxy-15-methylhexadecane-3-one, 9

[2-Hydroxy-1-(methoxymethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (**8**, 0.82 g, 3.3 mmol) was dissolved in dry THF (7 mL) under N_2.[15] The resulting solution was cooled to −15 °C and *sec*-BuMgBr [prepared from 2-bromobutane (0.73 mL, 6.6 mmol) and Mg (0.19 g, 7.92 mmol) in 8 mL dry THF] was added dropwise at −15 °C. After 10 minutes, 12-methyltridecylmagnesium bromide [prepared from **5** (1.83 g, 6.6 mmol) and Mg (0.19 g, 7.92 mmol) in 8 mL dry THF] was added at −15 °C. The resulting solution was allowed to warm to room temperature overnight. The mixture was again cooled to −15 °C and HCl (9 mL) was added, followed by ethyl acetate (10 mL). The two layers were separated and the aqueous layer extracted further with CH_2Cl_2. The combined organic layers were washed with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (hexane–EtOAc 75 : 25) provided the product as a white solid. **9**[15] (0.27 g, 0.69 mmol, 21%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.67 (d, J = 5.8 Hz, 1H), 4.34 (bs, 1H), 3.92 (m, 1H), 3.66–3.62 (t, J = 6.7 Hz, 2H), 2.82 (bs, 1H), 2.66–2.48 (m, 2H), 1.67–1.46 (m, 10H), 1.29–1.14 (m, 19H), and 0.88–0.85 ppm (d, J = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.9, 156.0, 80.3, 63.2, 61.6, 60.4, 39.9, 39.0, 29.9, 29.7, 29.6, 29.5, 29.4, 29.1, 28.3, 28.0, 27.4, 23.4, 22.6, 21.0, and 14.2 ppm; LCMS (ES+) *m*/*z* 410.2 (Na adduct); HRMS (ESI+): *m*/*z* (*M* + H)$^+$, calcd for $C_{22}H_{43}O_4N$: 386.3265; found: 386.3279.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1,3-dihydroxy-15-methylhexadecane, 10a

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-hydroxy-15-methylhexadecane-3-one (0.27 g, 0.7 mmol) in methanol (34 mL) was treated with a solution of $NaBH_4$ (0.05 g, 1.32 mmol) in H_2O (2 mL) stabilized with 1 drop of a 1 M NaOH solution. The resulting mixture was stirred for 2 h at room temperature and then poured into cold water (68 mL). The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed *in vacuo* to yield a white solid **10a**[18] as a 3 : 1 mixture of diastereomers (0.24 g,

0.62 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$ with methanol-d_4): δ 3.88–3.84 (dd, $J = 3.9$, 11.8 Hz, 1H), 3.67–3.61 (d, $J = 7.8$ Hz, 2H), 3.43 (bs, 1H), 1.50–1.38 (m, 12H), 1.30–1.06 (m, 20H), and 0.87–0.84 ppm (d, $J = 6.7$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ with methanol-d_4): δ 79.7, 76.7, 73.6, 62.1, 39.0, 34.2, 29.9, 29.6, 28.3, 27.9, 27.4, 25.9, and 22.6 ppm; LCMS (ESI+) m/z 408.4 (Na adduct); HRMS (ESI+): m/z $(M + H)^+$, calcd for $C_{22}H_{45}O_4N$: 388.3421; found: 388.3413.

(2*S*)-2-Amino-1,3-dihydroxy-15-methylhexadecane, 10b

A solution of trifluoroacetic acid (2.42 mL) and water (0.11 mL) was added to (2*S*)-2-(*N*-*tert*-butoxycarbonyl)-amino-1,3-dihydroxy-15-methylhexadecane **10a** (182.1 mg, 0.47 mmol) at ambient temperature. After 1 h, aqueous saturated sodium bicarbonate was added to the reaction mixture until a white solid formed as a suspension (about 50 mL). The solid was filtered and washed with water, giving **10b** as a tan solid (76.7 mg, 0.27 mmol, 57%). ^{1}H NMR (400 MHz, pyridine-d_5)[22] δ 5.92 (bs, 1H), 4.89 (bs, 3H), 4.28–4.24 (dd, $J = 4.1$, 10.2 Hz, 1H), 4.10–4.06 (dd, $J = 7.5$, 10.4 Hz, 1H), 4.00 (m, 1H), 3.30–3.28 (m, 1H), 1.83–1.12 (m, 23H), and 0.86–0.84 ppm (t, $J = 6.7$ Hz, 6H); ^{13}C NMR (100 MHz, pyridine-d_5): δ 73.6, 64.4, 58.3, 39.0, 34.1, 30.0, 29.8, 29.7, 28.0, 27.5, 26.4, and 22.5 ppm; LCMS (ESI+): m/z 288.2; HRMS (ESI+): m/z $(M + H)^+$, calcd for $C_{17}H_{37}NO_2$: 288.2902. Found, 288.2876.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)-amino-1-*tert*-butyldiphenylsilanyloxy-17-methyloctadecane-3-one, 11

[2-(*tert*)-Butyldiphenylsilanyloxy)-1-(methoxymethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester (**2**, 1.70 g, 3.49 mmol) was dissolved in dry THF (8 mL) under N_2. The resulting solution was cooled to −15 °C and *sec*-BuMgBr [2.0 M solution in diethyl ether (1.66 mL, 3.32 mmol)] was added dropwise at −15 °C. After 10 minutes, 14-methylpentadecylmagnesium bromide [prepared from **6** (1.70 g, 5.57 mmol) and Mg (0.16 g, 6.68 mmol) in 15 mL dry THF] was added at −15 °C. The resulting solution was allowed to warm to room temperature overnight. The mixture was again cooled to −15 °C and HCl (10 mL) was added, followed by EtOAc (10 mL). The two layers were separated and the aqueous layer extracted further with CH_2Cl_2. The combined organic layers were washed with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (CH_2Cl_2–EtOAc 100 : 0 to 0 : 100) provided a white solid, **11** (0.77 g, 1.19 mmol, 34%): ^{1}H NMR: δ 7.64–7.61 (m, 4H), 7.47–7.41 (m, 6H), 5.57–5.55 (d, $J = 7.5$ Hz, 1H), 4.37–4.35 (ddd, $J = 2.9$, 3.9, 7.5 Hz, 1H), 4.08–4.05 (dd, $J = 10.4$, 2.9 Hz, 1H), 3.94–3.91 (dd, $J = 10.6$, 3.9 Hz, 1H), 2.56–2.48 (m, 2H), 1.64–1.01 (m, 43H), and 0.91–0.89 ppm (d, $J = 6.7$ Hz, 6H); ^{13}C NMR: δ 207.6, 155.3, 135.6, 132.8, 130.0, 129.9, 127.8, 79.7, 79.5, 64.3, 61.1, 40.1, 39.1, 30.3, 30.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 28.4, 28.0, 27.6, 26.8, and 26.5 ppm; HRMS (ESI+): m/z $(M + H)^+$, calcd for $C_{40}H_{65}O_4NSi$: 652.4756; found: 652.4755.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)-amino-1-*tert*-butyldiphenylsilanyloxy-17-methyloctadecane-3-ol, 12a

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)-amino-1-*tert*-butyldiphenylsilanyloxy-17-methyloctadecane-3-one (**11**, 2.72 g, 4.17 mmol) in methanol (160 mL) was treated with a solution of $NaBH_4$ (0.32 g, 8.53 mmol) in H_2O (12 mL) stabilized with 4 drops of a 1 M NaOH solution. The resulting mixture was stirred for 2 h at room temperature and then poured into cold water (120 mL). The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and the solvents were removed *in vacuo* to yield a white solid, **12a** as a 3 : 1 mixture of diastereomers (1.52 g, 2.34 mmol, 56%). ^{1}H NMR: δ 7.75–7.66 (m, 4H), 7.48–7.42 (m, 6H), 5.31 (bs, 1H), 3.97–3.93 (dd, $J = 11.1$, 3.2 Hz, 1H), 3.86–3.84 (bs, 1H), 3.70 (bs, 1H), 3.60 (bs, 1H), 2.86–2.84 (d, $J = 6.7$ Hz, 1H), 1.58–1.48 (m, 13H), 1.29–1.05 (m, 32H), and 0.94–0.88 ppm (d, $J = 6.8$ Hz, 6H); ^{13}C NMR: δ 155.8, 135.6, 134.8, 132.7, 132.6, 130.0, 129.5, 129.9, 127.9, 127.6, 79.4, 73.8, 64.2, 54.6, 39.1, 34.5, 33.9, 30.0, 29.8, 29.7, 29.6, 28.5, 28.0, 27.5, 26.9, 26.6, 25.9, 25.6, 22.7, 19.2, and 19.0 ppm; HRMS (ESI+): m/z $(M + H)^+$, calcd for $C_{40}H_{67}O_4NSi$: 654.4912; found: 654.4882.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)-amino-1,3-dihydroxy-17-methyloctadecane, 12b

A 1 M solution of tetrabutylammonium fluoride (1.29 mL, 1.29 mmol) was added to a stirred solution of **12a** [(2*S*)-2-(*N*-*tert*-butoxycarbonyl)-amino-1-*tert*-butyldiphenylsilanyloxy-17-methyloctadecan-3-ol] (0.56 g, 0.86 mmol) in dry THF (5 mL) at ambient temperature. The reaction was allowed to stir overnight, and quenched with aqueous saturated ammonium chloride. The aqueous layer was extracted twice with ethyl acetate, and then twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column flash chromatography on silica gel (CH_2Cl_2–EtOAc, 100 : 0 to 50 : 50) gave a white solid, **12b** (0.33 g, 0.81 mmol, 94%). ^{1}H NMR (400 MHz, pyridine-d_5):[23] δ 4.96–4.89 (bs, 3H), 4.47–4.45 (m, 1H), 4.33–4.30 (m, 3H), 1.94–1.16 (m, 36H), and 0.89–0.88 ppm (d, $J = 6.5$ Hz, 6H); ^{13}C (100 MHz; 150 MHz, pyridine-d_5): δ 72.7, 62.8, 58.3, 39.7, 35.4, 30.6, 30.4, 29.0, 28.6, 28.1, and 23.2 ppm; HRMS (ESI+): m/z $(M + H)^+$, calcd for $C_{24}H_{49}NO_2$: 416.3740. Found, 416.3748.

(2*S*)-2-Amino-1,3-dihydroxy-17-methyloctadecane, 12c

A solution of trifluoroacetic acid (2.40 mL) and water (0.12 mL) was added to (2*S*)-2-(*N*-*tert*-butoxycarbonyl)-amino-1,3-dihydroxy-17-methyloctadecane **12b** (218.5 mg, 0.53 mmol) at ambient temperature. After 1 h, aqueous saturated sodium bicarbonate was added to the reaction mixture until a white solid formed as a suspension (about 50 mL). The solid was filtered and washed with water, giving **12c** as a white solid (139.3 mg, 0.44 mmol, 83%). ^{1}H NMR (400 MHz, pyridine-d_5): δ 5.87 (bs, 1H), 4.87 (bs, 3H), 4.27–4.23 (dd, $J = 4.1$, 10.2 Hz, 1H), 4.11–4.04 (m, 1H), 4.01–3.96 (m, 2H), 3.28–3.24 (m, 1H), 1.84–1.12 (m, 26H), and 0.88–0.86 ppm (t, $J = 6.57$ Hz, 6H); ^{13}C NMR (125 MHz, pyridine-d_5): δ 74.5, 65.3, 58.9, 39.7, 34.7, 30.6, 30.5, 30.4, 28.6, 28.1, 27.1, and 23.2 ppm; HRMS (ESI+): m/z $(M + H)^+$, calcd for $C_{19}H_{41}NO_2$: 316.3216. Found, 316.3225.

1,2-Dibromohexadecane, 15

1-Hexadecene **13**, (6.9 mL, 22.3 mmol) was dissolved in CH_2Cl_2 (49 mL), and cooled in an ice bath. After slow addition of

bromine (1.26 mL, 24.5 mmol), the reaction mixture was stirred for 30 minutes at room temperature. Saturated aqueous sodium thiosulfate (4 mL) was added to the reaction mixture. After stirring for 5 minutes, water (50 mL) and CH_2Cl_2 (50 mL) were added to the reaction mixture. The organic layer was separated, washed with water (30 mL) and brine (30 mL), dried with $MgSO_4$ and concentrated *in vacuo*. Vacuum distillation gave 1,2-dibromohexadecane (**15**)[10] as a colorless oil (8.1 g, 21.2 mmol, 95%). IR (neat): 2925(s), and 1465(m) cm^{-1}; ^1H NMR: δ 4.2–4.1 (m, 1H), 3.86–3.83 (dd, 1H), 3.65–3.60 (t, 1H), 2.17–2.09 (m, 1H), 1.87–1.73 (m, 1H), 1.6–1.26 (m, 24), and 0.89–0.86 ppm (t, 3H) ppm; ^{13}C NMR: δ 56, 39–26, and 17 ppm; MS (*m*/*z*): 57 (100), 71, 85, 97, 177, 191, 233, and 305.

1,2-Dibromopentadecane, 16

1-Pentadecene (**14**, 6.0 mL, 22.3 mmol) was dissolved in CH_2Cl_2 (49 mL), and cooled in an ice bath. After slow addition of bromine (1.26 mL, 24.5 mmol), the reaction mixture was stirred for 30 minutes at room temperature. Saturated aqueous sodium thiosulfate (4 mL) was added to the reaction mixture. After stirring for 5 minutes, water (50 mL) and CH_2Cl_2 (50 mL) were added to the reaction mixture. The organic layer was separated, washed with water (30 mL) and brine (30 mL), dried with $MgSO_4$ and concentrated *in vacuo*. Vacuum distillation gave 1,2-dibromopentadecane (**16**)[24] as colorless oil (7.84 g, 21.2 mmol, 95%). IR (neat): 2925(s), and 1465(m) cm^{-1}; ^1H NMR: δ 4.2–4.1 (m, 1H), 3.86–3.83 (dd, 1H), 3.65–3.60 (t, 1H), 2.17–2.09 (m, 1H), 1.87–1.73 (m, 1H), 1.6–1.26 (m, 22), and 0.89–0.86 ppm (t, 3H) ppm; ^{13}C NMR: δ 56, 39–26, and 17 ppm; MS (*m*/*z*): 57 (100), 71, 85, 97, 177, 235, and 291.

1-Hexadecyne, 17

1,2-Dibromohexadecane (**15**, 2.784 g, 7.25 mmol) was dissolved in THF (16 mL) and potassium *tert*-butoxide (2.44 g, 21.7 mmol) was added to the reaction mixture. After refluxing overnight, saturated aqueous NH_4Cl (5 mL) was added. After stirring for 5 minutes, water (30 mL) and diethyl ether (30 mL) were added. The organic layer was extracted with ether, washed with brine (20 mL) and dried over $MgSO_4$, and filtered. After passage through a short pad of silica gel and concentration *in vacuo*, vacuum distillation gave pure 1-hexadecyne (**17**)[25] as a colorless oil (1.05 g, 65%). IR (neat): 3308 (s), 2926 (s), 2117 (w), 1467 (s) cm^{-1}; ^1H NMR: δ 2.2–2.16 (td, 2H), 1.98–1.93 (t, 1H), 1.56–1.26 (m, 27H), and 0.89–0.86 ppm (t, 3H) ppm; ^{13}C NMR: δ 87, 71, 35, 32.6–31.4, 25.6, 21, and 17 ppm; MS (*m*/*z*): 81 (100), 95, 109, and 151.

1-Pentadecyne, 18

1,2-Dibromopentadecane (**16**, 2.68 g, 7.25 mmol) was dissolved in THF (16 mL) and potassium *tert*-butoxide (2.44 g, 21.7 mmol) was added to the reaction mixture. After refluxing overnight, saturated aqueous NH_4Cl (5 mL) was added. After stirring for 5 minutes, water (30 mL) and diethyl ether (30 mL) were added. The organic layer was extracted with ether, washed with brine (20 mL) and dried over $MgSO_4$, and filtered. After passage through a short pad of silica gel and concentration *in vacuo*, vacuum distillation gave pure 1-pentadecyne (**18**)[8] as a colorless oil (0.98 g, 4.71 mmol, 65%). IR (neat): 3308 (s), 2926 (s), 2117 (w), and 1467 (s) cm^{-1}; ^1H NMR: δ 2.2–2.16 (td, 2H), 1.98–1.93 (t, 1H), 1.56–1.26 (m, 25H), and 0.89–0.86 ppm (t, 3H) ppm; ^{13}C NMR: δ 87, 71, 35, 32.6–31.4, 25.6, 21, and 17 ppm; MS (*m*/*z*): 81 (100), 95, 109, and 151.

***N*-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxy-2-heptadecynyl]-1,3-oxazolidine, 19**

Hexadecyne (3.237 g, 14.58 mmol) was dissolved in THF (80 mL) and cooled to −5 °C. After addition of *n*-BuLi (1.99 M in heptane, 8 mL, 16.04 mmol) the reaction mixture was stirred for 30 minutes at 0 °C, cooled to −78 °C, and hexamethyl phosphoramide (HMPA, 13.5 mL) was added. Garner's aldehyde (**1**, 1.67 g, 7.29 mmol) dissolved in THF (34 mL) and cooled at −78 °C was slowly added to the reaction mixture *via* cannula. After stirring for 4 h at −78 °C, saturated aqueous NH_4Cl (3 mL) was added to the reaction mixture. Water (50 mL) and diethyl ether (100 mL) were added with stirring. The organic layer was extracted with ether, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification with silica gel chromatography (1 : 7 EtOAc–hexane) gave *N*-Boc-2,2-dimethyl-(4*S*)-[(1*R*)-hydroxy-2-heptadecynyl]-1,3-oxazolidine **19**, as a colorless oil (2.04 g, 4.52 mmol, 62%). IR (neat): 3440 (m), 2926 (s), 2857 (s), and 1690 (s) cm^{-1}; ^1H NMR: δ 4.51–3.66 (m, 4H), 2.20–2.17 (t, 2H), 1.65–1.26 (m, 39H), and 0.89–0.86 ppm (t, 3H) ppm. MIMW: calcd for $C_{27}H_{49}NO_4$, *m*/*z*: 452.3740 $(M + H)^+$; found 452.3741.

***N*-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxy-2-hexadecynyl]-1,3-oxazolidine, 20**

1-Pentadecyne (3.03 g, 14.58 mmol) was dissolved in THF (80 mL) and cooled to −5 °C. After addition of *n*-BuLi (1.99 M in heptane, 8 mL, 16.04 mmol) the reaction mixture was stirred for 30 minutes at 0 °C, cooled to −78 °C and hexamethyl phosphoramide (HMPA, 13.5 mL) was added. Garner's aldehyde (**1**, 1.67 g, 7.29 mmol) dissolved in THF (34 mL) and cooled at −78 °C was slowly added to the reaction mixture *via* cannula. After stirring for 4 h at −78 °C, saturated aqueous NH_4Cl (3 mL) was added to the reaction mixture. With stirring, water (50 mL) and diethyl ether (100 mL) were added. The organic layer was extracted with ether, washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification with silica gel chromatography (1 : 7 EtOAc–hexane) gave *N*-Boc-2,2-dimethyl-(4*S*)-[(1*R*)-hydroxy-2-hexadecynyl]-1,3-oxazolidine **20**[8b] as a colorless oil (1.97 g, 4.52 mmol, 62%). IR (neat): 3440 (m), 2926 (s), 2857 (s), and 1690 (s) cm^{-1}; ^1H NMR: δ 4.51–3.66 (m, 4H), 2.20–2.17 (t, 2H), 1.65–1.26 (m, 37H), and 0.89–0.86 ppm (t, 3H); ^{13}C NMR: δ 157, 97.8, 89.5, 84, 80.8, 68, 67, 64, and 34.8–17 ppm.

***N*-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxyheptadecyl]-1,3-oxazolidine, 21**

N-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxy-2-heptadecynyl]-1,3-oxazolidine **19** (0.83 g, 1.84 mmol) was dissolved in EtOAc (6 mL). After addition of PtO_2 (0.071 g) H_2 was bubbled through the mixture, and a hydrogen balloon was placed over the reaction mixture. After stirring for 8 h, all solids were filtered through a pad of Celite and the solution was concentrated *in vacuo*. Silica gel chromatography (1 : 6 EtOAc–hexane) gave *N*-Boc-2,2-dimethyl-(4*S*)-[(1*R*)-hydroxyheptadecyl]-1,3-oxazolidine **21** as a colorless oil (0.76 g, 1.67 mmol, 91%). IR (neat): 3328 (w), 2984 (m), 2924 (s),

and 1711 (m) cm^{-1}; ^1H NMR: δ 4.05–3.75 (m, 4H), 1.65–1.25 (m, 41H), and 0.90–0.86 (t, 3H) ppm; ^{13}C NMR: δ 97, 83, 76, 68, 65, 36–25, and 17 ppm. MIMW: calcd for $C_{27}H_{53}NO_4$, *m/z*: 456.4053 $(M + H)^+$; found 456.4054.

N-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxyhexadecyl]-1,3-oxazolidine, 22

N-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxy-2-hexadecynyl]-1,3-oxazolidine **20** (0.80 g, 1.84 mmol) was dissolved in EtOAc (6 mL). After addition of PtO_2 (0.071 g) H_2 was bubbled through the mixture, and a hydrogen balloon was placed over the reaction mixture. After stirring for 8 h, all solids were filtered through Celite and the solution was concentrated *in vacuo*. Silica gel chromatography (1 : 6 EtOAc–hexane) gave *N*-Boc-2,2-dimethyl-(4*S*)-[(1*R*)-hydroxyhexadecyl]-1,3-oxazolidine **22**[26] as colorless oil (0.74 g, 1.69 mmol, 92%). IR (neat): 3328 (w), 2924 (s), 2984 (m), and 1711 (m) cm^{-1}; ^1H NMR: δ 4.05–3.75 (m, 4H), 1.65–1.25 (m, 39H), and 0.90–0.86 (t, 3H) ppm; ^{13}C NMR: δ 97, 83, 76, 68, 65, 36–25, and 17 ppm.

D-*erythro*-2-Amino-1,3-nonadecanediol, 23

N-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxyheptadecyl]-1,3-oxazolidine **21** (0.1 g, 0.22 mmol) was dissolved in a 1 : 10 mixture of water–trifluoroacetic acid (2.2 mL) and stirred for 1 h. The reaction mixture was slowly poured into 50 mL of a saturated aqueous Na_2CO_3 solution at 0 °C. The resulting suspension was extracted with hot ethyl acetate, the solvent was evaporated, and the solid was dried *in vacuo* to give D-*erythro*-2-amino-1,3-nonadecanediol **23**[27] (60 mg, 0.19 mmol, 86%). IR (KBr): 3438 (m), 2921 (s), 2853 (m), and 1680 (s) cm^{-1}; ^1H NMR: δ 3.92–3.29 (m, 4H), 1.53–1.26 (m, 29H), and 0.89–0.86 (t, 3H) ppm.

D-*erythro*-2-Amino-1,3-octadecanediol, 24

N-Boc-2,2-Dimethyl-(4*S*)-[(1*R*)-hydroxyhexadecyl]-1,3-oxazolidine **22** (0.097 g, 0.221 mmol) was dissolved in a 1 : 10 mixture of water–trifluoroacetic acid (2.2 mL) and stirred for 1 h. The reaction mixture was slowly poured into 50 mL of a saturated aqueous Na_2CO_3 solution at 0 °C. The resulting suspension was extracted with hot EtOAc, the solvent was evaporated, and the solid dried *in vacuo* to give D-*erythro*-2-amino-1,3-octadecanediol **24**[28] (59 mg, 0.197 mmol, 89%). IR (KBr): 3438 (m), 2921 (s), 2853 (m), and 1680 (s) cm^{-1}; ^1H NMR: δ 3.92–3.29 (m, 4H), 1.53–1.26 (m, 27H), and 0.89–0.86 (t, 3H) ppm.

13-Methyl-1-tetradecanol, 28

A suspension of Mg turnings (1.21 g, 49.8 mmol) in THF (10 mL) was treated with 1-bromo-3-methylbutane (5 mL, 41.5 mmol) with vigorous stirring at room temperature. After the reaction had begun, an additional 25 mL of THF was added and stirred until the reaction mixture reached ambient temperature. A solution of 10-bromo-1-decanol (3 g, 12.6 mmol) dissolved in THF (27 mL) and cooled to −78 °C was treated with the Grignard reagent prepared above and Li_2CuCl_4 (0.1 M, 4 mL), at −78 °C. The reaction mixture was stirred overnight, during which time the temperature rose to room temperature, giving a dark solution. With stirring, saturated aqueous NH_4Cl (15 mL), water (150 mL) and EtOAc (150 mL) were added in that order. The organic layer was separated and washed with saturated aqueous $NaHCO_3$, brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification with silica column chromatography gave 13-methyl-1-tetradecanol **28**[10a,29] as a colorless oil (2.9 g, 47.3 mmol, 95%); IR (neat): 3335 (m), 2925 (s), 1467 (w) cm^{-1}; ^1H NMR: δ 3.70–3.67 (t, 2H), 1.64–1.17 (m, 26H), and 0.95–0.90 ppm (d, 6H).

13-Methyl-1-tetradecanal, 29

A solution of 13-methyl-1-tetradecanol (**28**, 0.215 g, 0.944 mmol) in CH_2Cl_2 (2.5 mL) was added to a stirred suspension of PCC (0.334 g, 1.55 mmol) dissolved in 4 mL of CH_2Cl_2. The reaction mixture was stirred for 4 h, at which time 4 mL of diethyl ether was added. The mixture was stirred vigorously and the supernatant liquid passed through a 2 inch pad of silica gel. The eluant was concentrated *in vacuo* and purified by silica gel chromatography (5% ether in hexane) to give 13-methyl-1-tetradecanal **29**[30] as a colorless oil (0.18 g, 0.802 mmol, 85%). IR (neat): 2924 (s), 2854 (m), 1727 (m), and 1463 (w) cm^{-1}; ^1H NMR: δ 9.76–9.75 (t, 1H), 2.43–2.39 (td, 1H), 1.64–1.49 (m, 3H), 1.29–1.13 (m, 18H), and 0.87–0.85 ppm (d, 6H); ^{13}C NMR: δ 105.4, 46.8, 41.9, 36.7–30.3, 25.5, and 25 ppm; MS (*m/z*): 57 (100), 82, 96, 180, and 208.

Methyl 3-hydroxy-15-methylhexadecanoate, 30

A well-stirred suspension of 13-methyl-1-tetradecanal (**29**, 0.49 g, 2.63 mmol), Zn dust (0.86 g, 13 mmol), and methyl bromoacetate (0.75 mL, 7.89 mmol) in THF (10 mL) was treated with $BF_3{\cdot}OEt_2$ (0.66 mL, 5.26 mmol) in portions over a period of 15 min. The mixture was stirred at room temperature for 5 h, filtered, and the residue was washed with EtOAc. The organic layer was washed with dilute aqueous HCl (5%), water, and brine and dried with $MgSO_4$. The solvent was evaporated and the crude product was purified by silica gel chromatography (20% EtOAc in hexane) to give methyl 3-hydroxy-15-methylhexadecanoate **30**[31] as a colorless oil (0.53 g, 1.84 mmol, 70%). IR (neat): 3452 (s), 2924 (s), 2853 (m), 1737 (m), and 1466 (w) cm^{-1}; ^1H NMR: δ 4.02–4.00 (m, 1H), 3.71 (s, 3H), 2.54–2.38 (ddd, 2H), 1.56–1.12 (m, 26H), and 0.87–0.85 ppm (d, 6H); ^{13}C NMR: δ 176, 70.9, 54.6, 44, 42, 39, and 32.8–25.6 ppm.

3-Hydroxy-15-methylhexadecanoic acid, 31

Aqueous LiOH (1 M, 2 mL, 2 mmol) was added to a stirred solution of methyl 3-hydroxy-15-methylhexadecanoate (**30**, 0.158 g, 0.527 mmol) in THF (0.5 mL) and MeOH (1 mL), at room temperature. The reaction mixture was stirred overnight and concentrated *in vacuo*. The residue was poured into 25 mL of EtOAc, acidified with dilute aqueous HCl to pH 3, the organic phase was separated, and the aqueous phase was extracted with EtOAc. The extract was washed with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Crystallization from hexane gave 3-hydroxy-15-methylhexadecanoic acid **31**[31b,32] as a white solid (0.128 g, 0.448 mmol, 85%). IR (KBr): 3479.9 (m), 2923.6 (s), and 1724 (m) cm^{-1}; ^1H NMR: δ 4.03 (m, 1H), 2.60–2.48 (ddd, 2H), 1.54–1.16 (m, 26H), and 0.87–0.85 ppm (d, 6H); ^{13}C NMR: δ 176.5, 68, 40.8, 39, 36, and 29.9–22.6 ppm.

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