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Amphimic Acids and Related Long-chain Fatty Acids as DNA Topoisomerase I Inhibitors from an Australian Sponge, *Amphimedon* sp.: Isolation, Structure, Synthesis, and Biological Evaluation

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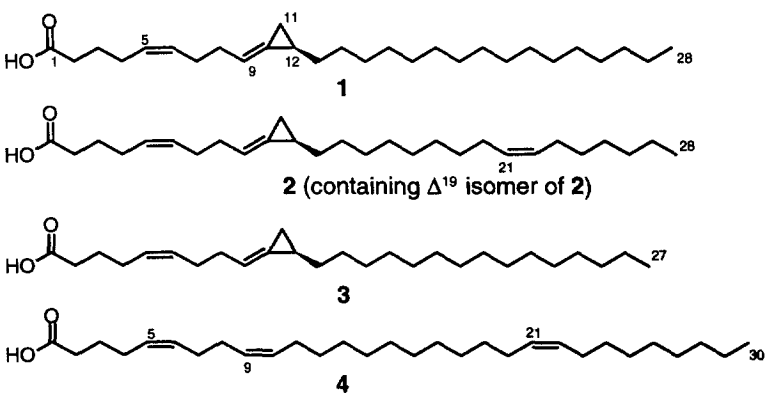
Abstract: Several C_{27}-C_{30} unsaturated fatty acids have been isolated from an Australian sponge, *Amphimedon* sp., as human DNA topoisomerase I inhibitors. Three of them, amphimic acids A (**1**)-C (**3**), are unusual fatty acids possessing a cyclopropylidene group, and one is a new C_{30} fatty acid **4**. Their structures were elucidated by spectroscopic analysis and chemical degradation. The enantioselective synthesis of **1** was carried out to determine the absolute configuration of amphimic acids. The inhibitory activity of DNA topoisomerase I was evaluated for these natural products and synthetic derivatives.

In continuation of our search for DNA topoisomerase I (topo I) inhibitors from marine invertebrates, we recently reported chemical studies on novel lipids, e.g., ceramide 1-sulfates[1] and long-chain fatty acids.[2] Amphimic acids A (**1**) and B (**2**) are C_{28} unsaturated fatty acids isolated from an Australian sponge of the genus *Amphimedon*.[2] These compounds possess a cyclopropylidene substructure and show inhibitory activity against a human topo I. Further examination of the extract of this sponge has resulted in the isolation of an additional congener amphimic acid C (**3**), a new C_{30} fatty acid **4**, and known C_{27}-C_{28} fatty acids **5-8** (structures shown in Table 1 in the last section). We now report the details of the isolation of these fatty acids, the enantioselective synthesis of **1** to determine the stereochemistry of amphimic acids, and biological evaluation of the natural compounds and related analogues synthesized.

From 3.8 kg (wet wt) of the sponge collected at the Great Barrier Reef, eight long-chain fatty acids **1-8** were isolated by standard chromatographic methods followed by HPLC separation.

Amphimic acid A (**1**) was obtained as colorless needles. The molecular formula of $C_{28}H_{50}O_2$ was determined by high-resolution negative FABMS and elementary analysis. The structural elucidation was



1

2 (containing Δ^{19} isomer of **2**)

3

4

performed by standard spectroscopic analysis.[2]

Amphimic acid B (**2**) has the molecular formula of $C_{28}H_{48}O_2$ (high-resolution negative FABMS) and is easily presumed to be a dehydro analogue of **1** by comparison of the spectral data between **1** and **2**.[2] However, the elucidation of the position of the extra double bond in **2** required MS/MS analysis and degradation experiments. It is reported that negative-mode FAB-MS/MS analysis is useful for determining the double-bond position in unsaturated fatty acids.[3] Thus, collision-induced dissociation (CID) spectra using the precursor ion $(M-H)^-$ of monounsaturated fatty acids show significant product ion peaks due to two allylic cleavages. However, the CID spectrum of **2** was complicated and less clear-cut than those of monounsaturated fatty acids (Figure 1). This could be due to overlapping of two sets of the allylic cleavages and led us to suspect the presence of a regioisomer in the sample of **2**. Thus, the major allylic cleavages at *m/z* 289/343 and the minor cleavages at *m/z* 261/315 indicate the product ions from Δ^{21} and Δ^{19} isomers, respectively. This was confirmed as follows. Methylation of **2** with diazomethane followed by ozonolysis of the methyl ester gave a mixture of aldehydes. GC-MS analysis of the mixture showed the production of heptanal and nonanal in the ratio of ca. 3:1 together with 5-oxovaleric acid methyl ester, establishing that the position of the extra double bond in **2** was at C21 and the Δ^{19} isomer existed in the sample as a minor component. The chemical shifts of the carbons [δ 27.2 (C20 and C23)] allylic to the double bond showed the *Z* geometry of C21. Therefore, **2** is the 21-dehydro derivative of **1** that exists together with the Δ^{19} isomer as an inseparable 3:1 mixture, though the NMR spectra look like those of a single compound.

Amphimic acid C (**3**) has the molecular formula of $C_{27}H_{48}O_2$ (high-resolution negative FABMS). The ^1H NMR spectra of **3** is superimposable on that of **1** and the molecular formula of **3** is less than that of **1** by CH_2, indicating that **3** is a C_{27} analogue of **1**.

The structures of other unsaturated long-chain fatty acids **4**-**8** were also determined in a similar manner by using ^1H NMR, COSY, and linked scan FAB-MS/MS analysis. Compound **4** has the molecular formula of $C_{30}H_{54}O_2$ (high resolution negative FABMS). The 1D NMR spectra of **4** showed no cyclopropylidene

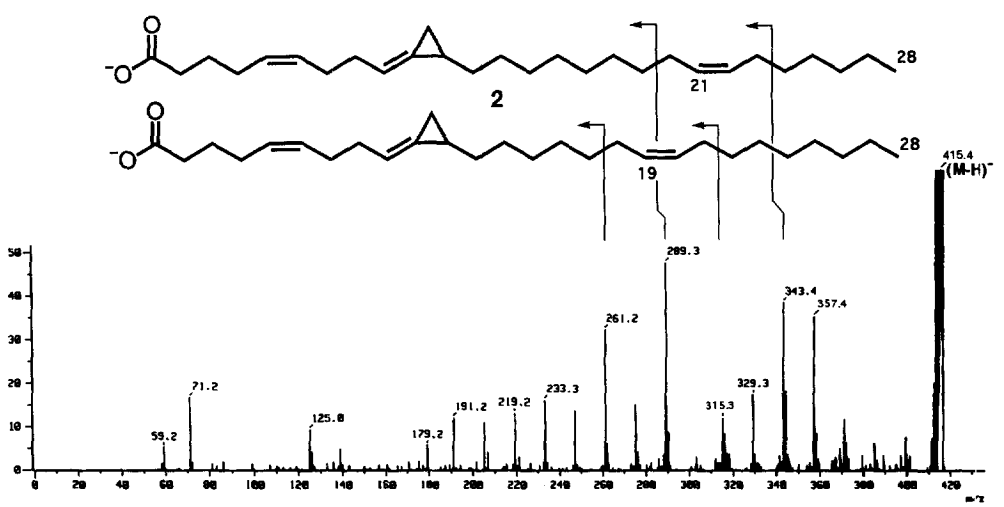


Figure 1. Linked scan FAB-MS/MS spectrum (negative) of a mixture of **2** and its Δ^{19} isomer.

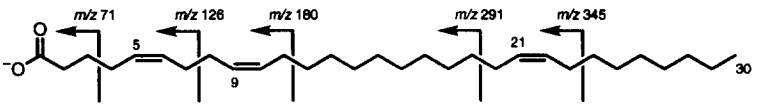
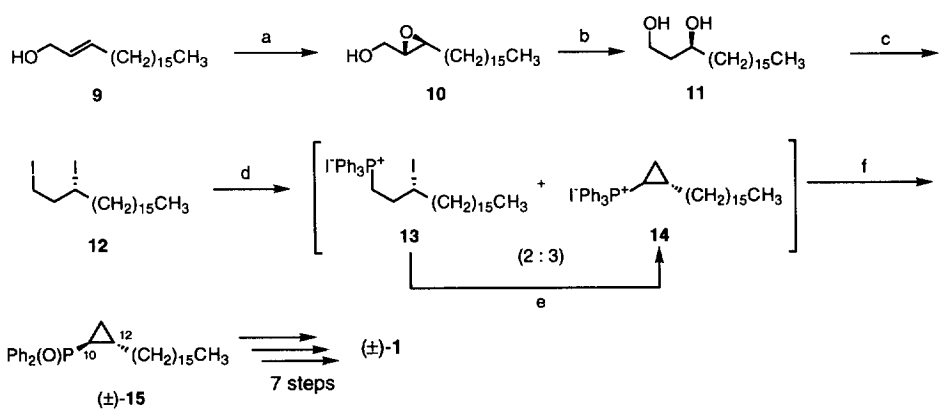


Figure 2. The major diagnostic fragmentations in FAB-MS/MS (negative) of **4**.

structure such as amphimic acids A (**1**)-C (**3**) but the presence of three disubstituted double bonds. The position of these double bonds was elucidated by MS/MS analysis, in which selective cleavage at allylic positions was observed as shown in Figure 2. The geometry of these double bonds was determined to be all *Z* from the chemical shifts of allylic carbons (C4, C7, C8, C11, C20, and C23). Thus, the compound **4** proved to be a new natural fatty acid (5Z,9Z,21Z)-5,9,21-triacontatrienoic acid. The compound **5** showed NMR spectra similar to **4** and was determined to be a mixture of (5Z,9Z,19Z)-5,9,19-octacosatrienoic acid[4] and (5Z,9Z,21Z)-5,9,21-octacosatrienoic acid[5] in the ratio of ca. 1:1 by MS/MS analysis and the degradation/GC-MS method (data not shown). The compounds **6-8** showed similar ^{1}H NMR spectra and were easily determined to be (5Z,9Z)-5,9-heptacosadienoic acid,[6] (5Z,9Z)-5,9-octacosadienoic acid,[7] and (5Z,9Z)-25-methyl-5,9-hexacosadienoic acid,[8] respectively. The compounds **5-8** are known fatty acids isolated from marine sponges.

The absolute stereochemistry of **1** was elucidated by the enantioselective synthesis. Although there are a few methods for the preparation of cyclopropylidenes[9], the construction of optically active cyclopropylidenes has not been reported. We introduced the asymmetric carbon at C12 of **1** by the Sharpless asymmetric epoxidation. Thus, the epoxidation of allyl alcohol **9**[10] gave epoxide **10** with 95% ee judged from ^{1}H NMR analysis of an MTPA ester (Scheme 1). Regioselective reduction of **10** with Red-Al provided 1,3-diol **11** exclusively.[11] Iodination of **11** gave diiodide **12**, which was treated with triphenylphosphine under basic conditions to yield a mixture of γ-iodoalkylphosphonium salt **13** and cyclopropylphosphonium salt **14**. The mixture was then treated with a base to converge on **14**, which was then converted to phosphine oxide **15** in one-pot, because an aqueous workup to isolate **14** yielded a mixture of **14** and **15**. The *trans* stereochemistry of **15** was determined by NOESY experiments, in which NOEs were observed at H10/H13 and H12/Ar-H. After

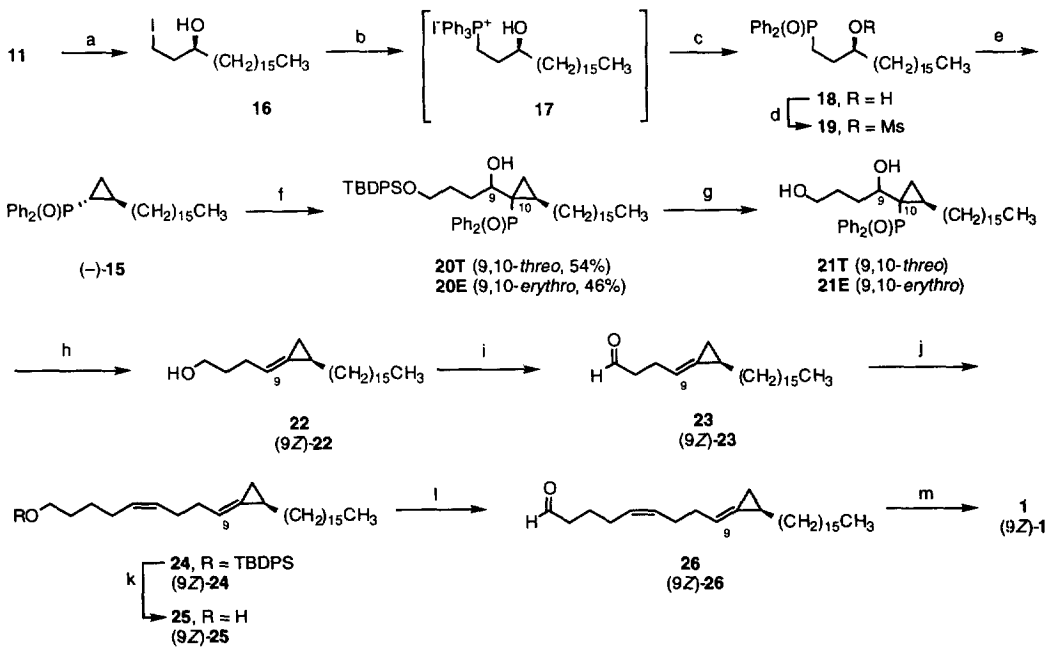


(a) *t*-BuOOH, (+)-DET, Ti(O-*i*-Pr)$_4$, CH_2Cl_2, -20 °C and then -10 °C, 76%. (b) Red-Al, THF, 0 °C, 94%. (c) I_2, PPh_3, HMPA, toluene, 50 °C, 80%. (d) PPh_3, K_2CO_3, CH_3CN-toluene (9:1), 55-60 °C. (e) NaH, THF, rt and then 50 °C. (f) NaOH, THF/H_2O, 50 °C, 79% (from **12**).

Scheme 1

completion of the total synthesis of **1** from **15** in seven steps, which are described in detail below (Scheme 2), **1** turned out to racemize completely. Since the specific rotation of **15** was zero, we assumed that the racemization occurred during the formation of the phosphonium salts **13** and **14** by repetition of the substitution reaction of the iodide ion. Therefore, we changed the synthetic route, in which monoiodide **16** was used as an intermediate instead of diiodide **12** (Scheme 2).

Iodination of diol **11** with a limited amount of iodine gave primary iodide **16**, which was converted to phosphine oxide **18** via phosphonium salt **17** (Scheme 2). Mesylation of **18** followed by cyclization of the resulting mesylate **19** provided the optically active cyclopropylphosphine oxide (–)-**15**. The Wittig-Horner reaction of (–)-**15** with 4-(*tert*-butyldiphenylsiloxy)butanal provided a diastereomeric mixture of β-hydroxy phosphine oxides in high yield.[12] The mixture was chromatographed on silica gel to afford two mixtures **20T** and **20E** in the ratio of 54:46. Each mixture consisted of two diastereomers which could not be further purified. The elimination reaction of these compounds resulted in low yields of the desired alkenes. After deprotection of the major and less polar isomers **20T**, however, the elimination reaction of the resulting diol **21T** with sodium hydride proceeded smoothly to provide cyclopropylidene compound **22** with the *E* geometry exclusively. On the other hand, **20E** gave the *Z* isomer (9*Z*)-**22** by the same reaction sequence. These olefin geometries were determined on the basis of NOEs of H8/H11 in **22** and H9/H11 in (9*Z*)-**22**. These results suggest that **20T** and **20E** are 9,10-*threo* and 9,10-*erythro* isomers, respectively. After oxidation of the hydroxyl group of **22**, the resulting aldehyde **23** was coupled with a phosphonium salt by the Wittig reaction to provide olefin **24**.



Yields were shown for the synthesis of **1**. (a) I_2, PPh_3, HMPA, toluene, rt, 72%. (b) PPh_3, $CaCO_3$, CH_3CN, 80 °C. (c) NaOH, THF/H_2O, 50 °C, 72% (2 steps). (d) MsCl, Et_3N, CH_2Cl_2, 0 °C, 100%. (e) $NaN(TMS)_2$, THF, 0 °C, 87%. (f) $TBDPSO(CH_2)_3CHO$, LDA, THF, -78 °C then 0 °C; chromatographic separation. (g) Bu_4NF, THF, rt, 71%. (h) NaH, DMF, 60-70 °C, 80%. (i) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, -78 °C then 0 °C, 91%. (j) $TBDPSO(CH_2)_5P^+Ph_3I^-NaN(TMS)_2$, toluene, rt, 100%. (k) Bu_4NF, THF, rt, 100%. (l) DMSO, $(COCl)_2$, Et_3N, -78 °C then 0 °C, 79%. (m) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, THF, rt, 97%.

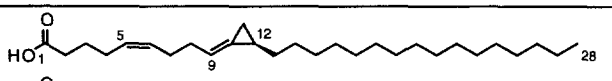
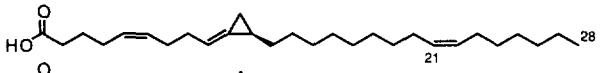
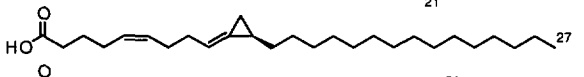
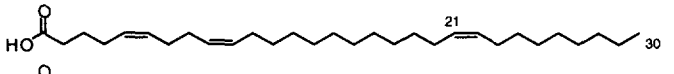
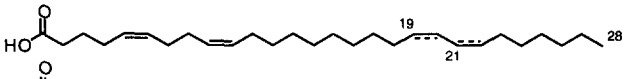
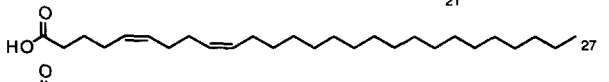
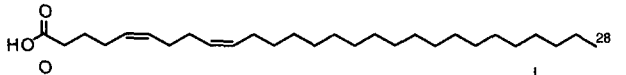
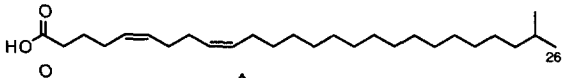
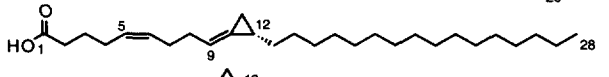
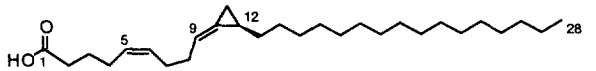
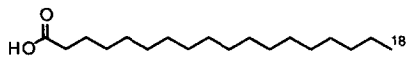
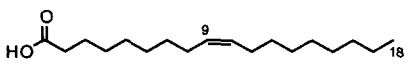
Scheme 2

Deprotection of the silyl group of **24** gave alcohol **25**, which was subjected to the Swern oxidation. The resulting aldehyde **26** was finally oxidized to give amphimic acid A (**1**), which was identical with the natural compound in all respects (mp, $[\alpha]_D$, IR, NMR, MS, and TLC). Thus, the absolute configuration of **1** was determined to be 12*R* unambiguously.

The enantiomer of amphimic acid A, *ent*-**1**, was synthesized from *ent*-**10** in the same manner. The 9Z isomer of amphimic acid A, (9Z)-**1**, was also synthesized from 9,10-*erythro* isomers **20E** by the same reaction sequence (Scheme 2). These synthetic compounds were evaluated for topo I inhibitory activity.

Topo I inhibitory activity of natural long-chain fatty acids **1-8**, synthetic analogues *ent*-**1** and (9Z)-**1**, and usual fatty acids was determined using the camptothecin resistant human topo I[13] and the results were summarized in Table 1. Amphimic acid A (**1**) is the most potent inhibitor of topo I among the natural long-chain fatty acids isolated. The synthetic material of **1** showed an activity similar to natural **1**, while the enantiomer *ent*-**1** and the geometrical isomer (9Z)-**1** were both less active, confirming the structure of **1** biologically. Interestingly, amphimic acid B (**2**), which possesses only one extra double bond at C21 (or C19), is about 10-fold less active than **1**. Trienes **4** and **5**, which also possess a double bond at C21 (or C19), are 6 to 7-fold less active than **1**. It is noteworthy that these long-chain fatty acids are on the average 100-fold more active than

Table 1. Structures and topo I inhibitory activity of **1-8** and related fatty acids.

Structure		Topo I inhibition (IC_{50}, μM)
	**1**	0.47 0.72[a]
	**2** (+Δ^{19})	6.7
	**3**	1.2
	**4**	3.1
	**5**	3.2
	**6**	0.86
	**7**	1.3
	**8**	1.1
	*ent*-**1**	1.7
	(9Z)-**1**	3.0
	stearic acid	290
	oleic acid	100

[a]The value of the synthetic **1**.

typical C18 fatty acids, e.g., stearic and oleic acids. These findings suggest that not only the linearity of the molecule but also carbon-chain length, rather than the presence of the cyclopropylidene moiety, plays important roles in topo I inhibitory activity. Since **1** shows also cytotoxicity against P388 leukemia cells with an IC_{50} value (1.8 μM) similar to that in topo I inhibitory activity, the respective activities may be correlated to each other. An inhibition experiment was conducted to see how **1** inhibited the DNA relaxation activity of topo I. The enzymic activity was not restored on increasing the concentration of DNA, but the inhibition was released by increasing the amount of the enzyme (data not shown). Consequently, the inhibitory effect of **1** (and other related long-chain fatty acids) may well be explained by the interaction with the enzyme rather than with the substrate DNA. The well-known topo I inhibitor camptothecin stabilizes an intermediate cleavable complex to interfere with the single-strand cleavage-resealing equilibrium by topo I.[14] On the other hand, acidic phospholipids are reported to interact with topo I directly to inhibit DNA binding, presumably due to masking of DNA binding site(s) of the enzyme.[15] The mechanism of action of these long-chain fatty acids seems to be same as that of such phospholipids in consideration of the inhibition mode and the structural similarity between these lipids. Although a number of long-chain fatty acids were isolated from marine sponges,[4-8,16] cyclopropylidene-containing fatty acids such as **1-3** have not been reported as natural compounds.

EXPERIMENTAL

General methods. Melting points were uncorrected. HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Infrared (IR) spectra were recorded on a JASCO FT/IR-7000S. Ultraviolet (UV) spectra were recorded on a JASCO Ubest-50 UV/VIS spectrophotometer. NMR spectra were recorded on a Bruker-ARX400 (400 MHz) or a JEOL EX-270 (270 MHz). NMR chemical shifts were referenced to the solvent peak of δ_H 7.26 (residual $CHCl_3$) or δ_C 77.0 ppm for $CDCl_3$. Mass spectra were recorded on a JEOL JMA-2000 (EIMS), a JMS DX-705L (GC-MS, FABMS), or a Mstation JMS-700 (high-resolution FABMS and linked scan MS/MS) mass spectrometer. The matrix used in FABMS experiments was *m*-nitrobenzyl alcohol.

Topo I inhibition assay. The reaction mixture consisted of 20 μl of 11 mM Tris-HCl, pH 7.9, containing 185 mM NaCl, 1 mM EDTA, 1 mg/ml bovine serum albumin, 10% glycerol, 1% DMSO, 0.25 μg of supercoiled plasmid pBR322 DNA (Boehringer Mannheim GmbH, 0.25 μg/μl), inhibitors (or extracts), and 1 unit of camptothecin-resistant human recombinant topo I.[13] The mixture was incubated at 37 °C for 30 min, and the reaction was stopped by addition of 3 μl of a solution of 6% SDS, 0.15% bromophenol blue, and 30% glycerol in water. The reaction mixtures were then analyzed by electrophoresis on a 1% agarose gel at 80 V for 3 h. The DNA bands on the gel were visualized by staining with 0.5 μg/ml ethidium bromide. The IC_{50} is defined as the concentration of an inhibitor giving 50% inhibition of 1 unit of the enzyme.

Collection, extraction, and isolation. The greenish brown-colored sponge *Amphimedon* sp. was collected at a depth of eight metres in the Great Barrier Reef, Australia. The sponge (3.8 kg wet weight) was homogenized in MeOH (8 litres) and the mixture was filtered. The filtrate was concentrated to 300 ml and the resulting aqueous mixture was extracted with EtOAc (3 x 300 ml). The EtOAc-soluble portion (2.05 g) was dissolved in 90% MeOH (50 ml) and extracted with hexane (2 x 25 ml) to give the hexane portion (1.6 g) and aqueous MeOH portion (0.45 g). The hexane portion was chromatographed on silica gel (32 g), eluted with benzene-EtOAc (9:1, 8:1, and then 4:1), EtOAc, and MeOH, successively. The least polar fraction (0.81 g) eluted with benzene-EtOAc (9:1) was subjected to chromatography on silica gel (50 g) with hexane-EtOAc (9:1). The fraction containing several long-chain fatty acids [168 mg, R_f = 0.39 on TLC developed with hexane-EtOAc-AcOH (80:20:0.5)] was separated from a mixture of usual fatty acids (R_f = 0.32 under the same conditions) and subjected to reversed-phase HPLC [column Develosil ODS-10 (20 i.d. x 250 mm, Nomura Chemical), 0.01 M NH_4OAc in 95% MeOH, flow rate 5.0 ml/min) to afford **2** (16.0 mg, t_R = 44 min), crude **5** (21.1 mg, t_R = 49 min), a mixture of **3** and **8** (9.8 mg, t_R = 54 min), a mixture of **1** and **6** (36.8 mg, t_R = 61 min), and a mixture of **4** and **7** (19.0 mg, t_R = 67 min). Each mixture was further separated by reversed-phase HPLC [same column, 0.01 M NH_4OAc in CH_3CN-MeOH-H_2O (50:48:2)] to afford **1** (15.6 mg, t_R = 73 min),

3 (1.8 mg, t_R = 63 min), **4** (6.0 mg, t_R = 80 min), **5** (5.8 mg, t_R = 62 min), **6** (7.0 mg, t_R = 70 min), **7** (5.2 mg, t_R = 87 min), and **8** (2.0 mg, t_R = 66 min).

Amphimic acid A (1). Colorless needles; mp 39-39.5 °C (CH_3CN-Et_2O); $[\alpha]^{22}_D$ +7.7 (*c* 0.49, MeOH); IR ($CHCl_3$) 3400-2500, 1710, 1460, 1420, 1300, 1130, and 950 cm^{-1}; UV (MeOH) λ_{max} 196 nm (ε 9210); for ^1H (400 MHz) and ^{13}C NMR (100 MHz) data see ref. 2; HRMS (FAB) calcd for $C_{28}H_{49}O_2$ (M-H) *m/z* 417.3732, found 417.3711. Anal. Calcd for $C_{28}H_{50}O_2$: C, 80.38; H, 11.96. Found: C, 80.10; H, 12.25.

Amphimic acid B (2). A colorless oil; $[\alpha]^{27}_D$ +6.2 (*c* 0.98, MeOH); IR ($CHCl_3$) 3400-2500, 1710, 1460, 1410, 1260, and 1100 cm^{-1}; UV (MeOH) λ_{max} 199 nm (ε 9940); for ^1H (400 MHz) and ^{13}C NMR (100 MHz) data see ref. 2; HRMS (FAB) calcd for $C_{28}H_{47}O_2$ (M-H) *m/z* 415.3576, found 415.3551.

Amphimic acid C (3). A colorless oil; $[\alpha]^{27}_D$ +6.3 (*c* 0.11, MeOH); IR ($CHCl_3$) 3400-2500 (br), 1710, 1460, 1420, 1130 cm^{-1}; UV (MeOH) λ_{max} 199 nm (ε 8210); ^1H NMR (400 MHz, $CDCl_3$) δ 0.64 (m, 1 H, H11), 0.88 (t, *J* = 6.8 Hz, 3 H, H27), 1.14 (dd, *J* = 7.8 and 7.8 Hz, 1 H, H11), 1.2-1.4 (m, 28 H, H13-26), 1.35 (m, 1 H, H12), 1.71 (tt, *J* = 7.5 and 7.4 Hz, 2 H, H3), 2.11 (dt, *J* = 7.2 and 7.4 Hz, 2 H, H4), 2.16 (m, 2 H, H7), 2.18 (m, 2 H, H8), 2.36 (t, *J* = 7.5 Hz, 2 H, H2), 5.34 (dt, *J* = 10.6 and 7.2 Hz, 1 H, H5), 5.45 (dt, *J* = 10.6 and 6.7 Hz, 1 H, H6), 5.76 (m, 1 H, H9); ^{13}C NMR (100 MHz, $CDCl_3$) 8.5 (t, C11), 14.1 (q, C27), 15.3 (d, C12), 22.7 (t, C26), 29.4, 29.5, 29.7, and 32.9 (t, 13 C, C13-25), 24.6 (t, C3), 26.5 (t, C4), 27.2 (t, C7), 31.9 (t, C8), 33.4 (t, C2), 116.7 (d, C9), 128.0 (s, C10), 128.4 (d, C5), and 130.9 (d, C6), no carbonyl signal observed; HRMS (FAB) calcd for $C_{27}H_{47}O_2$ (M-H) *m/z* 403.3576, found 403.3564.

(5Z,9Z,21Z)-5,9,21-Triacontatrienoic acid (4). A colorless oil; IR ($CHCl_3$) 3400-2500 (br), 1710, 1460, 1410, 1260, 1090, and 1020 cm^{-1}; UV (MeOH) λ_{max} 199 nm (ε 7210); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3 H, H30), 1.2-1.4 (m, 28 H, H12-19 and H24-29), 1.71 (tt, *J* = 7.5 and 7.5 Hz, 2 H, H3), 2.02 (m, 6 H, H11, 20, and 23), 2.08 (m, 4 H, H7, and 8), 2.11 (dt, *J* = 7.5 and 7.5 Hz, 2 H, H4), 2.36 (t, *J* = 7.5 Hz, 2 H, H2), 5.2-5.4 (m, 5 H, H5, 9, 10, 21, and 22), and 5.44 (m, 1 H, H6); ^{13}C NMR (100 MHz, $CDCl_3$) 14.1 (q, C30), 22.7 (t, C29), 24.6 (t, C3), 26.5 (t, C4), 27.2 (t, 2 C, C18 and 21), 27.3 (t, 2 C, C7 and 8), 27.4 (t, C11), 29.3, 29.5, 29.6, 29.7, 29.8 and 31.9 (t, 13 C, C12-17 and C22-28), 33.1 (t, C2), 128.6 (d, C5), 128.9 (d, C9), 129.9 (d, 2 C, C19 and 20), 130.6 (d, 2 C, C6 and 10), and 178.3 (s, C1); HRMS (FAB) calcd for $C_{30}H_{53}O_2$ (M-H) *m/z* 445.4046, found 445.4028.

Degradation of amphimic acis B (2). Treatment of **2** (2.8 mg) in Et_2O with diazomethane gave methyl ester (3.0 mg) as a colorless oil. Ozonolysis of the methyl ester (1.0 mg) was performed in MeOH (1 ml) at -78 °C for 15 min. After a usual workup with Me_2S, the mixture was analyzed by GC-MS under the following conditions: capillary column, DB-17 (J&W) 15 m x 0.25 mm i.d.; time program, initial temp. 50 °C, rate 40 °C/min. Retention times of heptanal, 5-oxovaleric acid methyl ester, and nonanal were 2'16", 2'45", and 3'16", respectively. Aldehydes were detected as the $(M\text{-}CH_3CHO)^+$ ion.

Epoxide 10. (*E*)-2-Nonadecen-1-ol (**9**)[10] (1.00 g, 3.55 mmol) was subjected to the Sharpless asymmetric epoxidation by the reported procedure using L-(+)-diethyl tartrate (102 mg, 0.497 mmol), titanium (IV) isopropoxide (0.10 ml, 0.36 mmol), and *tert*-butyl hydroperoxide (2.15 ml, 7.10 mmol, 3.3 M in toluene).[17] The crude product was purified by column chromatography on silica gel [$CHCl_3$-EtOAc (50:1, 20:1)] followed by recrystallization from benzene-hexane to give **10** as colorless needles (800 mg, 76% yield, 95% ee by ^1H NMR analysis of the (-)-MTPA ester): mp 80.5-81 °C; $[\alpha]^{23}_D$ -21 (*c* 0.40, $CHCl_3$); IR ($CHCl_3$) 3600, 3500-3100 (br), 1470, and 1080 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.83 (t, *J* = 6.8 Hz, 3 H), 1.2-1.4 (m, 26 H), 1.45 (m, 2 H), 1.58 (m, 2 H), 2.96 (ddd, *J* = 2.5, 5.4, and 5.4 Hz, 1 H), 2.94 (ddd, *J* = 2.5, 2.5, and 7.0 Hz, 1 H), 3.63 (ddd, *J* = 4.2, 7.0, and 12.6 Hz, 1 H), 3.91 (ddd, *J* = 2.5, 5.3, and 12.6 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1 (q), 22.7 (t), 25.9 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 31.6 (t), 31.9 (t), 56.0 (d), 58.4 (d), 61.7 (t); MS (EI) *m/z* (relative intensity) 280 (M^+-H_2O, 2), 267 (16), 236 (5), 208 (6), 111 (62), 97 (99), 83 (100), and 69 (90). Anal. Calcd for $C_{19}H_{38}O_2$: C, 76.51; H, 12.75. Found: C, 76.54; 12.65.

1,3-Diol 11. To a solution of **10** (800 mg, 2.68 mmol) in THF (10 ml) at 0 °C was added Red-Al (1.80 ml, 6.12 mmol, 3.4 M in toluene). After being stirred at 0 °C for 5 h, the reaction was quenched with MeOH (2 ml). Ether (20 ml), saturated brine (2 ml), and $MgSO_4$ (5 g) were then added successively. The mixture was stirred at room temperature for 30 min and filtered. The filtrate was concentrated and the residual oil was purified by column chromatography on silica gel [hexane-EtOAc (3:2)] to give **11** (759 mg, 94%) as a colorless oil: $[\alpha]^{23}_D$ +2.9 (*c* 0.19, MeOH); IR ($CHCl_3$) 3610, 3600-3150 (br), and 1060 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3 H), 1.2-1.4 (m, 28 H), 1.47 (m, 2 H), 1.70 (m, 2 H), 2.40 (br s, 2 H), and 3.8-3.9 (m, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.1 (q), 22.7 (t), 25.5 (t), 29.4 (t), 29.6 (t), 29.7 (t),

31.9 (t), 37.8 (t), 38.1 (t), 61.9 (t), 72.5 (d); MS (EI) *m/z* (relative intensity) 300 (M^+, 1), 282 (2), 264 (9), 253 (13), 236 (10), and 75 (100). Anal. Calcd for $C_{19}H_{40}O_2$: C, 76.00; H, 13.30. Found: C, 76.00; H, 13.39.

Diiodide 12. To a solution of **11** (270 mg, 0.899 mmol) in toluene (5 ml) were added $(Me_2N)_3PO$ (1.29 ml, 7.20 mmol), Ph_3P (943 mg, 3.60 mmol), and iodine (914 mg, 3.60 mmol). The mixture was stirred at 50 °C overnight and then diluted with hexane (25 ml) and 90% aqueous MeOH (25 ml). The hexane layer was separated, washed with 90% aqueous MeOH (2 x 25 ml), concentrated, and purified by column chromatography on silica gel (hexane) to give **12** (372 mg, 80%) as a colorless oil: $[\alpha]^{23}_D$ -6.6 (*c* 2.00, hexane); IR ($CHCl_3$) 1470 and 1170 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3 H), 1.2-1.6 (m, 28 H), 1.73 (dddd, *J* = 5.0, 5.3, 9.6, and 14.2 Hz, 1 H), 1.90 (dddd *J* = 5.0, 8.5, 9.9, and 14.2 Hz, 1 H), 2.12 (dddd, *J* = 3.9, 7.4, 8.5, and 15.6 Hz, 1 H), 2.30 (dddd *J* = 5.0, 7.1, 9.6, and 15.6 Hz, 1 H), 3.27 (ddd, *J* = 7.1, 8.5, and 9.9 Hz, 1 H), 3.40 (ddd, *J* = 5.0, 7.4, and 9.9 Hz, 1 H), 4.16 (dddd, *J* = 3.9, 5.0, 8.5, and 9.6 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 6.1 (t), 14.1 (q), 22.7 (t), 28.8 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 31.9 (t), 38.9 (t), 40.2 (d), 43.3 (t); MS (EI) *m/z* (relative intensity) 520 (M^+, 71), 393 (86), 155 (24), 111 (16), 97 (36), 85 (53), 71 (64), and 55 (100). Anal. Calcd for $C_{19}H_{38}I_2$: C, 43.84; H, 7.31. Found: C, 43.66; H, 7.47.

Cyclopropylphosphine oxide (±)-15. To a solution of **12** (383 mg, 0.737 mmol) in MeCN-toluene (9:1) (15 ml) were added Ph_3P (965 mg, 3.68 mmol) and K_2CO_3 (203 mg, 1.47 mmol). The mixture was stirred at 55-60 °C for 68 h. The supernatant was separated by centrifugation, and the precipitates were washed with MeCN (2 x 15 ml). The supernatant and washings were combined and concentrated. The residual oil was dissolved in hexane (20 ml) and extracted with 90% aqueous MeOH (3 x 20 ml). The combined aqueous MeOH layers were concentrated and the residual oil was purified by column chromatography on silica gel [$CHCl_3$-MeOH (30:1)] to give a mixture of phosphonium salts **13** and **14** (335 mg). To a solution of the mixture in THF (10 ml) were added NaH (47.1 mg, 0.120 mmol, 60% dispersion in mineral oil) and one drop of EtOH, and the mixture was stirred at room temperature for 1 h and at 50 °C for 1 h. After being cooled to 0 °C the mixture was treated with 20% (w/w) aqueous NaOH (1 ml) and stirred at 50 °C for 1 h. The solution was diluted with saturated NH_4Cl (15 ml) and water (15 ml), and the mixture was extracted with EtOAc (3 x 30 ml). The combined extracts were washed with saturated brine, dried, and concentrated. The residual oil was purified by column chromatography on silica gel [hexane-EtOAc (1:1)] to give (±)-**15** (199 mg, 79% from **12**) as colorless needles: mp 92.5-93 °C (hexane); $[\alpha]^{23}_D$ ±0 (*c* 0.65, $CHCl_3$); IR ($CHCl_3$) 1460, 1140, 1170, 1120, 720, 700, and 540 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80 (dddd, *J* = 4.6, 4.6, 9.0, and 13.8 Hz, 1 H), 0.87 (t, *J* = 6.8 Hz, 3 H), 1.02 (dddd, *J* = 5.7, 5.7, 9.0, and 13.8 Hz, 1 H), 1.1-1.4 (m, 31 H), 1.45 (ddddt, *J* = 4.6, 5.7, 9.2, 13.8, and 7.5 Hz, 1 H), 7.4-7.6 (m, 6 H), and 7.6-7.8 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 10.4 (dt, J_{CP} = 5 Hz), 14.1 (q), 14.5 (dd, J_{CP} = 104 Hz), 17.1 (dd, J_{CP} = 4 Hz), 29.2 (t), 29.3 (t), 29.5 (t), 29.7 (t), 33.7 (dt, J_{CP} = 2.4 Hz), 128.4 (dd, J_{CP} = 12 Hz), 131.0 (dd, J_{CP} = 10 Hz), 131.1 (dd, J_{CP} = 10 Hz), 131.6 (d), and 133.7 (d, J_{CP} = 108 Hz); MS (EI) *m/z* (relative intensity) 466 (M^+, 100), 255 (18), 202 (63), and 201 (67). Anal. Calcd for $C_{31}H_{47}OP$: C, 79.83; H, 10.09. Found: C, 79.83 H, 10.28.

Primary iodide 16. To a solution of **11** (759 mg, 2.53 mmol) in toluene (15 ml) were added $(Me_2N)_3PO$ (1.00 ml, 5.59 mmol), Ph_3P (729 mg, 2.78 mmol), and iodine (707 mg, 2.78 mmol). After being stirred at room temperature for 90 min, the reaction mixture was diluted with hexane (50 ml) and 90% aqueous MeOH (50 ml). The hexane layer was separated, washed with 90% aqueous MeOH (2 x 50 ml), concentrated, and purified by column chromatography on silica gel [hexane, then hexane-EtOAc (10:1)] to give **16** (750 mg, 72%) as a colorless solid: needles, mp 55-55.5 °C (hexane); $[\alpha]^{23}_D$ +16.3 (*c* 0.405, $CHCl_3$); IR ($CHCl_3$) 3630, 3600-3200 (br), and 1460 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85 (t, *J* = 6.8 Hz, 3 H), 1.2-1.6 (m, 30 H), 1.88 (dddd, *J* = 5.7, 6.0, 8.5, and 14.5 Hz, 1 H), 1.99 (dddd *J* = 3.4, 8.2, 8.2, and 14.5 Hz, 1 H), 3.29 (ddd, *J* = 5.7, 8.2, and 14.5 Hz, 1 H), 3.33 (ddd, *J* = 8.2, 8.5, and 14.5 Hz, 1 H), 3.71 (dddd, *J* = 3.4, 6.0, 6.0, and 9.2 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.2 (t), 14.1 (q), 22.7 (t), 25.5 (t), 29.4 (t), 29.6 (t), 29.7 (t), 31.9 (t), 37.3 (t), 40.7 (t), 71.8 (d); MS (EI) *m/z* (relative intensity) 410 (M^+, 2), 283 (3), 255 (16), 185 (24), 155 (18), and 57 (100);. Anal. Calcd for $C_{19}H_{39}IO$: C, 55.60; H, 9.50. Found: C, 55.67; H, 9.48.

Phosphine oxide 18. A mixture of **16** (876 mg, 2.14 mmol), Ph_3P (1.12 g, 4.28 mmol), and $CaCO_3$ (64.2 mg, 0.642 mmol) in MeCN (10 ml) was stirred at 80 °C overnight. The workup was accomplished by the procedure same as that for the synthesis of (±)-**15** to give crude phosphonium salt **17**. To a solution of crude **17** in THF (15 ml) was added 20% (w/w) aqueous NaOH (5 ml), and the mixture was stirred at 50 °C for 2 h. The reaction mixture was diluted with saturated NH_4Cl (20 ml) and water (5 ml) and extracted with EtOAc (3 x 25 ml). The combined extracts were washed with saturated brine, dried, and concentrated. The residual

solid was purified by recrystallization from hexane to give **18** (744 mg, 72%) as colorless needles: mp 94-94.5 °C; $[\alpha]^{23}_D$ +3.5 (*c* 0.19, MeOH); IR ($CHCl_3$) 3550-3050 (br), 1470, 1440, 1170, 1120, 700, and 540 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3 H), 1.2-1.5 (m, 30 H), 1.66 (m, 1 H), 1.88 (m, 1 H), 2.42 (dddd, *J* = 6.7, 6.7, 13.4, and 13.4 Hz, 1 H), 2.47 (dddd, *J* = 6.7, 6.7, 13.4, and 13.4 Hz, 1 H), 3.68 (m, 1 H), 7.4-7.6 (m, 6 H), and 7.6-7.8 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1 (q), 22.7 (t), 25.8 (t), 26.3 (dt, J_{CP} = 72 Hz), 29.3 (t), 29.6 (t), 29.7 (t), 31.9 (t), 37.3 (t), 71.4 (dd, J_{CP} = 9 Hz), 128.5 (dd, J_{CP} = 12 Hz), 128.7 (dd, J_{CP} = 12 Hz), 130.8 (dd, J_{CP} = 9 Hz), 131.8 (d), 131.9 (d), 132.0 (dd, J_{CP} = 10 Hz), and 133.5 (d, J_{CP} = 108 Hz). Anal. Calcd for $C_{31}H_{49}O_2P$: C, 76.86; H, 10.12. Found: C, 76.83; H, 10.06.

Mesylate 19. To a solution of **18** (989 mg, 2.05 mmol) in CH_2Cl_2 (15 ml) at 0 °C were added Et_3N (1.70 ml, 12.3 mmol) and mesyl chloride (0.48 ml, 6.15 mmol). After being stirred at 0 °C for 15 min , ice tips (3 g) were added. The mixture was stirred at room temperature for 10 min, diluted with H_2O (17 ml), and extracted with EtOAc (2 x 50 ml). The combined extracts were washed with 0.2 M HCl, H_2O, saturated $NaHCO_3$, and saturated brine, successively, dried, and concentrated. The residual oil was purified by column chromatography on silica gel [hexane-EtOAc (1:3)] to give **19** (1.34 g, quantitative yield) as a colorless oil: $[\alpha]^{23}_D$ -5.2 (*c* 0.40, $CHCl_3$); IR ($CHCl_3$) 1460, 1440, 1330, 1170, 1120, 910, and 540 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3 H), 1.2-1.5 (m, 28 H), 1.64 (m, 1 H), 1.70 (m, 1 H), 1.93 (m, 1 H), 2.04 (m, 1 H), 2.35 (dddd, *J* = 4.3, 11.7, 11.7, and 15.2 Hz, 1 H), 2.49 (dddd, *J* = 5.3, 11.7, 11.7, and 15.2 Hz, 1 H), 2.99 (s, 3 H), 4.76 (ddt, *J* = 3.7, 6.7, and 6.7 Hz, 1 H), 7.4-7.6 (m, 6 H), and 7.6-7.8 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1 (q), 22.6 (t), 25.0 (t), 25.1 (dt, J_{CP} = 72 Hz), 26.6 (t), 29.2 (t), 29.3 (t), 29.5 (t), 29.6 (t), 29.7 (t), 31.9 (t), 34.4 (t), 38.7 (t), 83.4 (dd, J_{CP} = 15 Hz), 128.5 (dd, J_{CP} = 12 Hz), 128.8 (dd, J_{CP} = 11 Hz), 130.8 (dd, J_{CP} = 10 Hz), 132.0 (d), 132.1 (dd, J_{CP} = 10 Hz), 132.2 (d, J_{CP} = 99 Hz), and 132.3 (d, J_{CP} = 100 Hz). Anal. Calcd for $C_{32}H_{51}O_4PS$: C, 68.33; H, 9.07. Found: C, 68.24; H, 9.27.

Cyclopropylphosphine oxide (–)-15. To a solution of **19** (1.33 g, 2.37 mmol) in THF (15 ml) at 0 °C was added sodium bis(trimethylsilyl)amide (5.13 ml, 3.08 mmol, 0.6 M in toluene). After being stirred at 0 °C for 15 min, saturated aqueous NH_4Cl (20 ml) was added, and the mixture was extracted with ether (3 x 25 ml). The combined extracts were washed with saturated brine, dried, and concentrated. The residual oil was purified by column chromatography on silica gel [hexane-EtOAc (1:1, 1:3)] to give (–)-**15** (830 mg, 87%): colorless needles, mp 102.5-103 °C (hexane); $[\alpha]^{23}_D$ -3.5 (*c* 0.24, $CHCl_3$); IR ($CHCl_3$) 1460, 1140, 1170, 1120, 720, 700, and 540 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.80 (dddd, *J* = 4.6, 4.6, 9.0, and 13.8 Hz, 1 H), 0.87 (t, *J* = 6.8 Hz, 3 H), 1.02 (dddd, *J* = 5.7, 5.7, 9.0, 13.8 Hz, 1 H), 1.1-1.4 (m, 31 H), 1.45 (m, 1 H), 7.4-7.6 (m, 6 H), and 7.6-7.8 (m, 4 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 10.4 (dt, J_{CP} = 5 Hz), 14.1 (q), 14.5 (dd, J_{CP} = 104 Hz), 17.1 (dd, J_{CP} = 4 Hz), 29.2 (t), 29.3 (t), 29.5 (t), 29.7 (t), 33.7 (dt, J_{CP} = 2 Hz), 128.4 (dd, J_{CP} = 12 Hz), 131.0 (dd, J_{CP} = 10 Hz), 131.1 (dd, J_{CP} = 10 Hz), 131.6 (d), and 133.7 (d, J_{CP} = 108 Hz); MS (EI) *m/z* (relative intensity) 466 (M^+, 100), 255 (18), 202 (63), and 201 (67). Anal. Calcd for $C_{31}H_{47}OP$: C, 79.83; H, 10.09. Found: C, 79.65; H, 10.24.

β-Hydroxy phosphine oxide 20T and 20E. To a solution of diisopropylamine (0.44 ml, 3.87 mmol) in THF (5 ml) was added *n*-butyllithium (2.42 ml, 3.87 mmol, 1.6 M in hexane) at -78 °C. The solution was stirred at 0 °C for 30 min and a solution of (–)-**15** (819 mg, 1.76 mmol) in THF (3 ml + 2 x 1 ml rinse) was added. After being stirred at 0 °C for 15 min, the mixture was recooled to -78 °C, and a solution of 4-(*tert*-butyldiphenylsiloxy)butanal (1.23 g, 3.70 mmol) in THF (4 ml) was added. The mixture was stirred at -78 °C for 30 min and then at 0 °C for 30 min, diluted with saturated NH_4Cl (20 ml) and H_2O (5 ml), and extracted with CH_2Cl_2 (3 x 25 ml). The combined extracts were washed with saturated brine, dried, and concentrated. The residual oil was purified twice by column chromatography on silica gel [(i) hexane-EtOAc (20:1) and then benzene-acetone (18:1); (ii) benzene-acetone (18:1)] to give **20T** [753 mg, 54%, R_f = 0.59 on TLC developed with hexane-EtOAc (1:1)] and **20E** [641 mg, 46%, R_f = 0.45 in the same conditions] as colorless oils. **20T**: ^1H NMR (270 MHz, $CDCl_3$) δ 0.69 (m, 1 H), 0.88 (t, *J* = 6.7 Hz, 3 H), 1.00 (s, 9 H), 0.8-2.0 (m, 37 H), 3.3-3.7 (m, 3 H), 7.3-7.5 (m, 12 H), 7.5-7.7 (m, 6 H), and 7.9-8.0 (m, 2 H); HRMS (FAB) calcd for $C_{51}H_{74}O_3PSi$ (M+H) 793.5145, found 793.5116. **20E**: ^1H NMR (270 MHz, $CDCl_3$) δ 0.72 (m, 2 H), 0.88 (t, *J* = 6.7 Hz, 3 H), 1.00 (s, 9 H), 0.8-2.0 (m, 36 H), 3.3-3.6 (m, 3 H), 7.3-7.5 (m, 12 H), 7.5-7.7 (m, 6 H), and 7.9-8.0 (m, 2 H); HRMS (FAB) calcd for $C_{51}H_{74}O_3PSi$ (M+H) 793.5145, found 793.5135.

9,10-threo-Diol 21T. To a solution of **20T** (66.8 mg, 0.084 mmol) in THF (1.0 ml) was added Bu_4NF (0.184 ml, 0.184 mmol, 1.0 M in THF). The mixture was stirred at room temperature for 2 h, diluted with saturated brine (10 ml), and extracted with ether (3 x 10 ml). The combined extracts were dried, and

concentrated. The residual oil was purified by column chromatography on silica gel [benzene-acetone (3:2)] to give **21T** (33.2 mg, 71%) as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.69 (m, 1 H), 0.87 (t, J = 6.7 Hz, 3 H), 0.8-1.5 (m, 32 H), 1.5-1.9 (m, 4 H), 3.3-3.7 (m, 5 H), 7.3-7.6 (m, 6 H), 7.6-7.7 (m, 2 H), and 7.8-8.0 (m, 2 H). Anal. Calcd for $C_{35}H_{55}O_3P$: C, 75.81; H, 9.93. Found: C, 75.55; H, 10.11.

Cyclopropylidene 22. To a solution of **21** (33.2 mg, 0.060 mmol) in DMF (1.0 ml) was added sodium hydride (5.0 mg, 0.12 mmol, 60% dispersion in mineral oil). After being stirred at 60 °C for 1 h, at 65 °C for 2 h , and then at 70 °C for 2 h, the reaction mixture was diluted with saturated aqueous NH_4Cl (4 ml) and extracted with ether (3 x 5 ml). The combined extracts were washed with saturated brine, dried, and concentrated. The residual oil was purified by column chromatography on silica gel (benzene) to yield **22** (16.0 mg, 80%) as a colorless oil: $[\alpha]^{23}_D$ +6.5 (*c* 0.43, $CHCl_3$); IR ($CHCl_3$) 3630, 3500-3000 (br), 1470, and 1050 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.66 (m, 1 H), 0.88 (t, J = 6.7 Hz, 3 H), 1.16 (dd, J = 7.4 and 7.4 Hz, 1 H), 1.2-1.45 (m, 31 H), 1.73 (tt, J = 6.7 and 6.7 Hz, 2 H), 2.24 (dt, J = 6.7 and 6.7 Hz, 2 H), 3.66 (t, J = 6.7 Hz, 2 H), and 5.78 (t, J = 6.7 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.5 (t), 14.1 (q), 15.3 (d), 22.7 (t), 28.1 (t), 29.4 (t), 29.7 (t), 31.9 (t), 32.3 (t), 33.4 (t), 62.8 (t), 116.6 (d), and 128.3 (s); MS (EI) *m/z* (relative intensity) 336 (M^+, 50), 318 (9), 292 (12), 263 (23), 153 (11), 139 (37), 121 (48), 111 (100), 95 (78), 81 (70), 67 (51), and 55 (52). Anal. Calcd for $C_{23}H_{44}O$: C, 82.14; H, 13.10. Found: C, 82.17; H, 13.58.

Aldehyde 23. To a solution of oxalyl chloride (0.045 ml, 0.52 mmol) in CH_2Cl_2 (3.0 ml) at -78 °C was added DMSO (0.049 ml, 0.69 mmol). The mixture was stirred at -78 °C for 15 min and a solution of **22** (116 mg, 0.344 mmol) in CH_2Cl_2 (1 ml + 2 x 0.5 ml rinse) was added. The mixture was stirred at -78 °C for 15 min and triethylamine (0.24 ml, 1.7 mmol) was added. The resulting mixture was warmed to 0 °C and stirred for 10 min. A usual work-up gave crude product, which was purified by column chromatography on silica gel [hexane-EtOAc (30:1)] to give **23** (105 mg, 91%) as a colorless oil: $[\alpha]^{23}_D$ +7.2 (*c* 0.22, $CHCl_3$); IR ($CHCl_3$) 2720, 1720, and 1470 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.66 (m, 1 H), 0.87 (t, J = 6.7 Hz, 3 H), 1.16 (dd, J = 7.8 and 7.8 Hz, 1 H), 1.2-1.5 (m, 31 H), 2.51 (dt, J = 6.7 and 6.7 Hz, 2H), 2.59 (dt, J = 1.7 and 6.7 Hz, 2 H), 5.78 (t, J = 6.7 Hz, 1 H), and 9.77 (t, J = 1.7 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.8 (t), 14.1 (q), 15.0 (d), 22.7 (t), 24.5 (t), 29.3 (t), 29.6 (t), 29.7 (t), 31.9 (t), 33.2 (t), 43.0 (t), 115.0 (d), 129.0 (s), and 202.7 (d); MS (EI) *m/z* (relative intensity) 334 (M^+, 18), 316 (9), 290 (9), 151 (17), 137 (29), 95 (93), 81 (85), 67 (39), and 55 (100); HRMS (FAB) calcd for $C_{23}H_{41}O$ (M-H) 333.3157, found 333.3152.

Diene 24. To a solution of [5-(*tert*-butyldiphenylsiloxy)-1-pentyl]triphenylphosphonium iodide (440 mg, 0.603 mmol) in toluene (0.5 ml) was added sodium bis(trimethylsilyl)amide (0.77 ml, 0.46 mmol, 0.6 M in toluene). The mixture was stirred at room temperature for 30 min, at 55-60 °C for 10 min, and then cooled to 0 °C. To the resulting solution was added a solution of **23** (95.8 mg, 0.287 mmol) in toluene (0.8 ml). The mixture was stirred at room temperature for 30 min, diluted with H_2O (20 ml), and extracted with EtOAc (3 x 20 ml). The combined extracts were concentrated, dissolved in 90% aqueous MeOH (20 ml), and extracted with hexane (3 x 20 ml). The combined hexane layers were concentrated and purified by column chromatography on silica gel [hexane and then hexane-EtOAc (30:1)] to give **24** (190 mg, quantitative yield) as a colorless oil: $[\alpha]^{23}_D$ +5.6 (*c* 0.18, $CHCl_3$); IR ($CHCl_3$) 1470, 1110, and 820 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.64 (m, 1 H), 0.88 (t, J = 6.7 Hz, 3 H), 1.05 (s, 9 H), 1.14 (dd, J = 7.8 and 7.8 Hz, 1 H), 1.2-1.4 (m, 31 H), 1.43 (tt, J = 6.4 and 6.7 Hz, 2 H), 1.56 (tt, J = 6.4 and 6.7 Hz, 2 H), 2.04 (dt, J = 6.7 and 6.4 Hz, 2 H), 2.17 (m, 4 H), 3.66 (t, J = 6.4 Hz, 2 H), 5.35 (dt, J = 10.6 and 6.4 Hz, 1 H), 5.39 (dt, J = 10.6 and 5.7 Hz, 1 H), 5.76 (m, 1 H), 7.4-7.6 (m, 6 H), and 7.6-7.8 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.5 (t), 14.1 (q), 15.3 (d), 19.2 (s), 22.7 (t), 25.8 (t), 25.9 (t), 26.9 (q), 27.0 (t), 27.2 (t), 29.4 (t), 29.5 (t), 29.7 (t), 31.9 (t), 32.2 (t), 33.4 (t), 63.8 (t), 116.9 (d), 127.6 (d), 127.8 (s), 129.5 (d), 129.6 (d), 129.9 (d), 134.2 (s), and 135.5 (d); MS (EI) *m/z* (relative intensity) 642 (M^+, 1), 585 (100), 507 (16), 385 (9), 251 (10), and 199 (40). Anal. Calcd for $C_{44}H_{70}OSi$: C, 82.24; H, 10.90. Found: C, 82.25; H, 10.97.

Alcohol 25. To a solution of **24** (178 mg, 0.277 mmol) in THF (3.0 ml) was added Bu_4NF (0.55 ml, 0.55 mmol, 1.0 M in THF). After being stirred at room temperature for 2 h, the reaction mixture was diluted with saturated brine (20 ml) and extracted with ether (3 x 20 ml). The combined extracts were dried, concentrated, and purified by column chromatography on silica gel [CCl_4-ether (15:1)] to give **25** (128 mg, quantitative yield) as a colorless oil: $[\alpha]^{23}_D$ +7.9 (*c* 0.10, $CHCl_3$); IR ($CHCl_3$) 3610, 3600-3450 (br), 1470, and 1060 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.64 (m, 1 H), 0.88 (t, J = 6.7 Hz, 3 H), 1.14 (dd, J = 7.8 and 7.8 Hz, 1 H), 1.2-1.4 (m, 31 H), 1.42 (tt, J = 6.4 and 6.7 Hz, 2 H), 1.57 (tt, J = 6.4 and 6.7 Hz, 2H), 2.07 (dt, J = 7.1 and 6.4 Hz, 2H), 2.18 (m, 4 H), 3.64 (t, J = 6.4 Hz, 2 H), 5.36 (dt, J = 10.6 and 6.4 Hz, 1 H),

5.40 (dt, J = 10.6 and 5.7 Hz, 1 H), and 5.76 (m, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.5 (t), 14.1 (q), 15.3 (d), 22.7 (t), 25.8 (t), 26.5 (t), 27.2 (t), 29.4 (t), 29.5 (t), 29.7 (t), 31.9 (t), 32.4 (t), 33.4 (t), 62.9 (t), 116.8 (d), 127.9 (s), 129.6 (d), and 129.9 (d); MS (EI) *m/z* (relative intensity) 404 (M^+, 41), 291 (32), 193 (26), 180 (39), and 81 (100). Anal. Calcd for $C_{28}H_{52}O$: C, 83.17; H, 12.87. Found: C, 83.00; H, 13.12.

Aldehyde 26. Alcohol **25** (10 mg, 0.025 mmol) was oxidized by Swern oxidation in a usual manner. The crude product was purified by column chromatography on silica gel [hexane and then hexane-EtOAc (30:1)] to give **26** (7.9 mg, 79%) as a colorless oil: $[\alpha]^{23}_D$ +4.49 (*c* 0.775, $CHCl_3$); IR ($CHCl_3$) 2720, 1720, 1470, and 960 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.64 (m, 1 H), 0.88 (t, J = 6.6 Hz, 3 H), 1.14 (dd, J = 7.8 and 7.8 Hz, 1 H), 1.2-1.4 (m, 31 H), 1.69 (tt, J = 7.4 and 7.4 Hz, 2 H), 2.09 (dt, J = 7.4 and 7.1 Hz, 2 H), 2.16 (m, 2 H), 2.18 (m, 2 H), 2.43 (dt, J = 1.8 and 7.4 Hz, 2 H), 5.34 (dt, J = 10.6 and 7.1 Hz, 1 H), 5.44 (dt, J = 10.6 and 6.7 Hz, 1 H), 5.75 (m, 1 H), and 9.77 (t, J = 1.8 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 8.5 (t), 14.1 (q), 15.3 (d), 22.0 (t), 22.7 (t), 26.5 (t), 27.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.7 (t), 31.8 (t), 31.9 (t), 33.4 (t), 43.3 (t), 116.7 (d), 128.0 (s), 128.4 (d), 130.9 (d), and 202.6 (d); MS (EI) *m/z* (relative intensity) 402 (M^+, 49), 291 (48), 135 (43), 95 (73), 81 (100), and 67 (76); HRMS (FAB) calcd for $C_{29}H_{49}O$ (M-H) 401.3783, found 401.3784.

Synthesis of 1. To a solution of **26** (7.9 mg, 0.097 mmol) and 2-methyl-2-butene (0.11 ml, 0.99 mmol) in *tert*-butyl alcohol (0.4 ml) was added a solution of $NaClO_2$ (18 mg, 0.20 mmol) and NaH_2PO_4 (17 mg, 0.14 mmol) in water (0.20 ml) over 10 min.[18] The pale yellow solution was stirred at room temperature overnight. The mixture was diluted with H_2O (5 ml) and extracted with ether (3 x 5 ml). The combined extracts were washed with saturated brine, dried, and concentrated. The residual oil was purified by column chromatography on silica gel [hexane-EtOAc (3:1)] to give **1** (8.0 mg, 97%): colorless needles, mp 39.5-40 °C (MeCN-ether); $[\alpha]^{23}_D$ +8.8 (*c* 0.40, MeOH); UV, IR, ^{1}H, and ^{13}C NMR spectra were superimposable on natural **1**. Anal. Calcd for $C_{28}H_{50}O_2$: C, 80.38; H, 11.96. Found: C, 80.21; H, 12.22.

Synthesis of ent-1. In a similar manner *ent*-**1** was synthesized from *ent*-**10** in 5.9% overall yield: mp 37.5-38 °C (MeCN-Et_2O); $[\alpha]^{23}_D$ -7.4 (*c* 0.15, MeOH); UV, IR, ^{1}H, and ^{13}C NMR spectra were superimposable on natural **1**; HRMS (FAB) calcd for $C_{28}H_{49}O_2$ (M-H) 417.3733, found 417.3755.

Synthesis of (9Z)-amphimic acid A [(9Z)-1]. Using the procedure used for the synthesis of **1** from **20T**, (9Z)-**1** was synthesized from **20E** via the following intermediates.

9,10-erythro-Diol 21E. 378 mg (81%) from **20E** (669 mg) as a colorless oil, ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (m, 2 H), 0.87 (t, J = 6.7 Hz, 3 H), 1.1-1.4 (m, 29 H), 1.41 (m, 2 H), 1.5-2.0 (m, 4 H), 3.4-3.6 (m, 3 H), 7.3-7.5 (m, 2 H), 7.5-7.7 (m, 6 H), and 7.9-8.0 (m, 2 H). Anal. Calcd for $C_{35}H_{55}O_3P$: C, 75.81; H, 9.93. Found: C, 75.86; H, 10.26.

Cyclopropylidene (9Z)-22. 12.6 mg (41%) from **21E** (50.3 mg) as a colorless oil, $[\alpha]^{23}_D$ -49 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 3620, 3540-3340 (br), and 1470 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.68 (m, 1 H), 0.88 (t, J = 6.7 Hz, 3 H), 1.15 (ddd, J = 7.9, 6.7, and 1.7 Hz, 1 H), 1.2-1.5 (m, 31 H), 1.71 (tt, J = 6.9 and 6.4 Hz, 2 H), 2.25 (dt, J = 6.9 and 6.9 Hz, 2 H), 3.67 (t, J = 6.4 Hz, 2 H), and 5.70 (dt, J = 1.7 and 6.9 Hz, 1 H); MS (EI) *m/z* (relative intensity) 336 (M^+, 24), 318 (5), 292 (8), 263 (22), 121 (86), 111 (100), 95 (97), and 81 (99). Anal. Calcd for $C_{23}H_{44}O$: C, 82.14; H, 13.10. Found: C, 81.82; H, 13.57.

Aldehyde (9Z)-23. 56 mg (84%) from (9Z)-**22** (66.8 mg) as a colorless oil, $[\alpha]^{23}_D$ -54 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 2720, 1720, and 1470 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.68 (m, 1 H), 0.88 (t, J = 6.7 Hz, 3 H), 1.15 (ddd, J = 7.8, 7.8, and 1.7 Hz, 1 H), 1.2-1.5 (m, 31 H), 2.52 (dt, J = 6.7 and 6.7 Hz, 2 H), 2.56 (dt, J = 1.7 and 6.7 Hz, 2 H), 5.70 (dt, J = 1.7 and 6.7 Hz, 1 H), and 9.78 (t, J = 1.7 Hz, 1 H); MS (EI) *m/z* (relative intensity) 334 (M^+, 22), 316 (14), 290 (16), 263 (6), 165 (18), 151 (26), 137 (54), 119 (77), 79 (91), 67 (100), and 55 (95); HRMS (FAB) calcd for $C_{23}H_{41}O$ (M-H) 333.3157, found 333.3150.

Diene (9Z)-24. 49 mg (55%) from (9Z)-**23** (46.2 mg) as a colorless oil, $[\alpha]^{23}_D$ -19 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 1470, 1110, and 820 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.68 (m, 1 H), 0.90 (t, J = 6.7 Hz, 3 H), 1.06 (s, 9 H), 1.15 (ddd, J = 1.7, 8.2, and 8.2 Hz, 1 H), 1.2-1.4 (m, 31 H), 1.43 (m, 2 H), 1.58 (m, 2 H), 2.05 (dt, J = 6.4 and 7.0 Hz, 2 H), 2.18 (m, 4 H), 3.67 (t, J = 6.4 Hz, 2 H), 5.36 (dt, J = 10.8 and 6.4 Hz, 1 H), 5.41 (dt, J = 10.8 and 5.8 Hz, 1 H), 5.70 (dt, J = 1.7 and 6.4 Hz, 1 H), 7.3-7.5 (m, 6 H), and 7.6-7.8 (m, 4 H); MS (EI) *m/z* (relative intensity) 642 (M^+, 1), 585 (100), 507 (77), 385 (63), and 199 (99); HRMS (EI) calcd for $C_{44}H_{70}OSi$ (M) 642.5196, found 642.5214.

Alcohol (9Z)-25. 25 mg (quantitative yield) from (9Z)-**24** (39.0 mg) as a colorless oil, $[\alpha]^{23}_D$ -35 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 3660, 3600-3300 (br), and 1470 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.67 (m, 1

H), 0.88 (t, J = 6.7 Hz, 3 H), 1.14 (ddd, J = 1.7, 8.4, and 8.4 Hz, 1 H), 1.2-1.4 (m, 33 H), 1.58 (m, 2 H), 2.07 (dt, J = 6.7 and 6.4 Hz, 2 H), 2.18 (m, 4 H), 3.65 (t, J = 6.4 Hz, 2 H), 5.36 (dt, J = 10.6 and 6.4 Hz, 1 H), 5.41 (dt, J = 10.6 and 5.5 Hz, 1 H), and 5.69 (dt, J = 1.7 and 6.9 Hz, 1 H); MS (EI) *m/z* (relative intensity) 404 (M^+, 20), 386 (16), 317 (28), 291 (8), 193 (16), 161 (27), 135 (32), and 81 (100); HRMS (EI) calcd for $C_{28}H_{52}O$ (M) 404.4018, found 404.4038.

Aldehyde (9Z)-26. 7.9 mg (79%) from (9Z)-**25** (15.6 mg) as a colorless oil, $[\alpha]^{23}_D$ -26 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 2720, 1720, 1470, and 960 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.67 (m, 1 H), 0.88 (t, J = 6.7 Hz, 3 H), 1.12 (ddd, J = 1.7, 8.5, and 8.5 Hz, 1 H), 1.2-1.4 (m, 30 H), 1.49 (dddd, J = 6.0, 6.3, 6.3, and 12.8 Hz, 1 H), 1.69 (tt, J = 7.4 and 7.4 Hz, 2 H), 2.09 (dt, J = 7.7 and 7.1 Hz, 2 H), 2.17 (m, 4 H), 2.43 (dt, J = 1.7 and 6.4 Hz, 2 H), 5.35 (dt, J = 11.0 and 7.1 Hz, 1 H), 5.44 (dt, J = 11.0 and 6.8 Hz, 1 H), 5.67 (dt, J = 1.7 and 6.5 Hz, 1 H), and 9.77 (t, J = 1.7 Hz, 1 H); MS (EI) *m/z* (relative intensity) 402 (M^+, 23), 291 (21), 159 (30), 135 (32), 95 (75), 81 (99), and 67 (100); HRMS (FAB) calcd for $C_{29}H_{49}O$ (M-H) 401.3783, found 401.3799.

(9Z)-Amphimic acid A [(9Z)-1]. 9.8 mg (89%) from (9Z)-**26** (10.6 mg) as a colorless oil, $[\alpha]^{23}_D$ -28 (*c* 0.50, MeOH); IR ($CHCl_3$) 3400-2500 (br), 1710, and 1460 cm^{-1}; UV (MeOH) λ_{max} 199 nm (ε 9060); ^1H NMR (400 MHz, $CDCl_3$) δ 0.67 (m, 1H), 0.88 (t, J = 6.8 Hz, 3H), 1.14 (ddd, J = 1.7, 7.8, and 7.8 Hz, 1 H), 1.2-1.4 (m, 32 H), 1.49 (dddd, 6.0, 6.0, 6.9, and 12.8 Hz, 1 H), 2.11 (dt, J = 7.0 and 7.4 Hz, 2 H), 2.16 (dt, J = 5.2 and 6.5 Hz, 2 H), 2.20 (ddt, J = 1.0, 6.5, and 5.2 Hz, 2 H), 2.36 (t, J = 7.4 Hz, 2 H), 5.34 (dt, J = 10.9 and 7.0 Hz, 1 H), 5.44 (dt, J = 10.9 and 6.5 Hz, 1 H), and 5.66 (dt, J = 1.7 and 6.5 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.0 (t), 14.1 (q), 15.5 (d), 22.7 (t), 24.6 (t), 26.5 (t), 26.7 (t), 27.5 (t), 29.4 (t), 29.5 (t), 29.7 (t), 31.9 (t), 32.1 (t), 33.2 (t), 117.6 (d), 127.8 (s), 128.5 (d), 130.7 (d), and 178.7 (s); HRMS (FAB) calcd for $C_{28}H_{49}O_2$ (M-H) 417.3732, found 417.3702.

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