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Kulokekahilide-2, a Cytotoxic Depsipeptide from a Cephalaspidean Mollusk *Philinopsis speciosa*[†]

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A cytotoxic depsipeptide, kulokekahilide-2 (**1**), was isolated from a cephalaspidean mollusk, *Philinopsis speciosa*. The structure elucidation of kulokekahilide-2 was carried out by spectroscopic analysis and chemical degradation. Kulokekahilide-2 showed potent cytotoxicity against several cell lines (P388, SK-OV-3, MDA-MB-435, and A-10 with IC_{50} values ranging from 4.2 to 59.1 nM) indicating cancer cell selectivity.

The marine carnivorous mollusk *Philinopsis speciosa* is a bountiful source of structurally and biologically unique compounds.[1] Among them, the most characteristic constituents are depsipeptides,[1c−f] which are reminiscent of those from other marine mollusks such as *Dolabella auricularia*[2] and *Onchidium* sp.[3] The *Philinopsis* compounds are thought to be sequestered by predation of smaller sized mollusks such as the sea hare *Stylocheilus longicaudus*, which feeds on cyanobacteria.[1d] Further investigation of the cytotoxic fractions of *P. speciosa* led to the isolation of a new depsipeptide, kulokekahilide-2 (**1**),[1f] which is closely related to aurilide (**2**) isolated from *D. auricularia*.[2a]

The organic extract of *P. speciosa* was evaporated and separated by the modified Kupchan procedure[4] to yield *n*-hexane, CH_2Cl_2, and aqueous MeOH extracts. The CH_2Cl_2 extract was purified by a two-step ODS flash chromatography process, followed by gel filtration, and amino column chromatography. The fraction containing peptides was further separated by sequential ODS HPLC to give kulokekahilide-2 (**1**; 3.4 mg; 3.8×10^{-5} % yield based on wet weight).

The molecular formula of kulokekahilide-2 (**1**) was established as $C_{44}H_{67}N_5O_{10}$ on the basis of HRFABMS [*m/z* 826.4942 (M + H)$^+$ (Δ −2.4 mmu)]. In the ^1H NMR spectrum (CD_2Cl_2), **1** exhibited two sets of signals in a 1:1 ratio, which was later assigned to two conformers, **1*cis*** and **1*trans***, derived from the *cis−trans* isomerism at the amide bond between *N*-methylphenylalanine (MePhe) and *N*-methylglycine (Sar).

Detailed analysis of the 2D NMR data enabled us to assign all signals for both **1*cis*** and **1*trans*** and revealed a structural framework consisting of peptidal and polyketidal moieties (substructures **a** and **b**, respectively). Substructure **a** was composed of five amino acids, Ile, Sar, MePhe, and two Ala, and 2-hydroxyisocaproic acid (Hica). The sequence of these residues was deduced from HMBC correlations between H-21/C-14, H_3-32/C-20, H_3-35/C-23, H-37 and NH-37/C-33, and H-43 and NH-43/C-36 to make substructure **a**.

Substructure **b** was elucidated as follows: COSY analysis connected proton signals from the olefinic proton H-3, via the allyllic methylene protons H_2-4 and H-5 oxymethine signal, to the methine proton H-6, which showed correlations to the methyl at C-12 and the oxymethine proton H-7. The other spin system could be traced from CH_3-10, via an olefinic proton H-9, to the other methyl (CH_3-13) through an allyllic coupling ($J = 1.1$ Hz). These two units were connected by HMBC[5] cross-peaks observed between H-7/C-13, H_3-13/C-7, and H-7/C-8. Further analysis of the HMBC spectrum connected C-3 and C-2 (cross-peaks between H_2-4/C-2), which was also bearing a methyl group (CH_3-11) and a carbonyl carbon C-1 (cross-peaks between H-3/C-11, H_3-11/C-3, H-3/C-1, and H_3-11/C-1) to furnish the partial structure **b**.

Substructures **a** and **b** were connected on the basis of HMBC analysis. The α-proton (H-15) of Hica showed a cross-peak to the C-1 carbonyl carbon of substructure **b**, and H-7 of **b** correlated with the C-42 carbonyl carbon of the C-terminal Ala-2 residue of substructure **a** to make a 26-membered ring.

NOESY analysis supported the sequence of substructure **a** for both **1*cis*** and **1*trans***; however, differences in NOE signals for these conformers were observed between the MePhe and Sar residues. In **1*trans***, NOEs were observed between H-24 and H-27/Me-35; on the other hand, in **1*cis***, no NOE was seen among these protons, but instead an NOE was observed between H-24/Ha-34. These NOE patterns suggested a *trans*-amide linkage between MePhe/Sar for **1*trans*** and *cis* for **1*cis***.

Further NOE analysis enabled us to predict the relative stereochemistry at three successive methines, C-5 to C-7. Although rotation between C-3 and C-4 seemed different between **1*cis*** and **1*trans***, NOEs around C-5 to C-7 were well preserved. Diagnostic NOEs from Me-12 to H-5 and

Chart 1. Structures of Kulokekahilide-2 (**1**) and Aurilide (**2**)

	R
kulokekahilide-2 (**1**)	H
1a	*S*-MTPA
1b	*R*-MTPA

aurilide (**2**)

Table 1. NMR Data of Kulokekahilide-2 (**1**) in CD_2Cl_2

	1*trans*			**1*cis***		
atom no.	^{13}C	^1H (ppm, mult., Hz)	HMBC	^{13}C	^1H (ppm, mult., Hz)	HMBC
1	166.9			168.9		
2	128.2			128.9		
3	141.9	6.97 dt 8.4, 1.1	1, 11	143.0	6.92 ddd 9.0, 4.9, 1.3	1, 11
4a	32.5	2.37 dd 14.3, 8.4	2, 3	31.4	2.14 m	2, 3, 5
4b		2.14 m	2, 3, 5		2.30 ddd 16.1, 9.0, 8.5	3
5	72.1	3.51 bdd 8.7, 5.6		72.1	3.67 m	
6	41.4	2.11 m	5, 7	40.3	2.05 m	5, 7
7	83.5	5.22 d 9.8	5, 6, 8, 13, 42	83.4	4.93 d 10.7	5, 6, 8, 9, 12, 13, 42
8	133.2			132.4		
9	125.9	5.55 qd 6.6, 1.1	10, 13	126.4	5.56 bqd 6.7, 1.1	7, 10, 13
10	13.1	1.61 dq 6.6, 0.9	8, 9	13.1	1.60 dq 6.6, 1.1	8, 9
11	12.7	1.83 bs	1, 2, 3	12.6	1.86 bs	1, 2, 3
12	11.6	0.79 d 6.9	5, 6, 7	10.7	0.70 d 7.1	5, 6, 7
13	11.2	1.64 bs	7, 8, 9	11.0	1.54 bs	7, 8, 9
14	170.4			171.2		
15	72.6	5.14 dd 10.3, 5.8	1, 14, 16	73.5	4.83 dd 8.5, 3.3	1, 14, 16, 17
16a	40.9	1.77 m	14, 15, 17, 18, 19	40.6	1.83 m	14, 15, 17, 18, 19
16b		1.71 m	15, 17, 18, 19		1.54 m	14, 15, 17, 18, 19
17	24.9	1.69 m	16, 18, 19	25.0	1.76 m	18, 19
18	21.9	0.91 d 6.3	16, 17, 19	21.9	0.91 d 6.3	16, 17, 19
19	23.3	0.91 d 6.3	16, 17, 18	23.3	0.92 d 6.7	16, 17, 18
20	173.0			173.6		
21	45.5	4.71 dq 7.8, 6.7	14, 20, 22	45.1	4.56 dq 7.6, 7.1	14, 20, 22
22	17.5	0.87 d 6.7	20, 21	16.5	0.78 d 7.1	20, 21
NH		7.10 d 7.8			6.53 d 7.6	14
23	172.5			170.2		
24	56.3	5.62 dd 9.1, 7.0	23, 25, 32	54.3	5.40 dd 10.3, 5.8	20, 25, 32
25a	35.3	3.25 dd 14.1, 7.0	23, 24, 26, 27, 31	35.3	3.05 dd 14.5, 10.3	23, 24, 26, 27, 31
25b		3.07 dd 14.1, 9.1	23, 24, 26, 27, 31		2.98 dd 14.5, 5.8	24, 26, 27, 31
26	136.9			137.4		
27	129.9	7.32 bd 8.0	25, 29, 31	129.8	7.14 bd 7.7	25, 29, 31
28	128.6	7.25 dd 8.0, 7.1	26, 30	128.4	7.21 dd 7.7, 7.0	26, 30
29	127.2	7.21 t 7.1	27, 31	126.8	7.16 t 7.0	27, 31
30	128.6	7.25 dd 8.0, 7.1	26, 28	128.4	7.21 dd 7.7, 7.0	26, 28
31	129.9	7.32 bd 8.0	25, 27, 29	129.8	7.14 bd 7.7	25, 27, 29
32	31.2	2.95 s	20, 24	30.4	2.97 s	20, 24
33	169.7			169.5		
34a	52.9	4.28 bd 15.4	33	51.5	3.99 d 17.9	33, 35
34b		3.72 bd 15.4	23, 33, 35		3.35 d 17.9	23, 33, 35
35	36.3	2.71 s	23, 34	36.7	2.91 s	23, 34
36	170.8			171.7		
37	58.8	4.09 dd 8.2, 7.1	33, 36, 38, 39, 41	57.7	4.38 dd 9.2, 8.5	33, 36, 38, 39, 41
38	35.8	1.96 m		37.8	1.89 m	
39a	25.1	1.48 m		25.0	1.37 m	38, 40, 41
39b		1.12 m	38, 40		1.37 m	38, 40, 41
40	11.3	0.88 t 7.5	38, 39	10.9	0.94 t 6.7	38, 39
41	16.0	0.92 d 5.7	37, 38	15.60	0.97 d 7.1	37, 38
NH		6.95 d 8.2	33		7.51 d 9.2	33
42	171.6			170.6		
43	48.7	4.44 dq 7.6, 7.1	36, 42, 44	50.1	4.25 dq 6.7, 7.1	36, 42
44	18.5	1.35 d 7.1	42, 43	17.4	1.38 d 7.1	42
NH		6.32 d 7.6	36		6.36 d 6.7	36

H-7 were indicative of the relative stereochemistry as 5*S**,6*S**,7*S**.

To confirm the relative stereochemistry predicted above, the four possible diastereoisomers of triol, **3a**, **3b**, **3c**, and
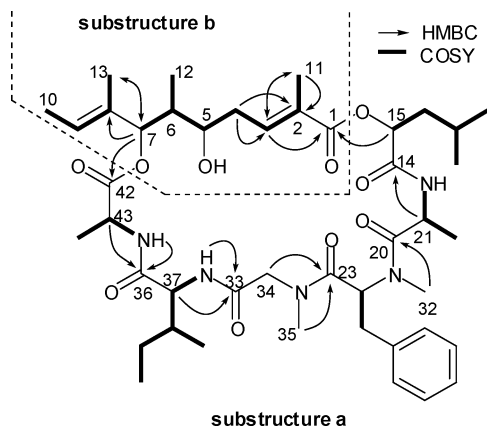
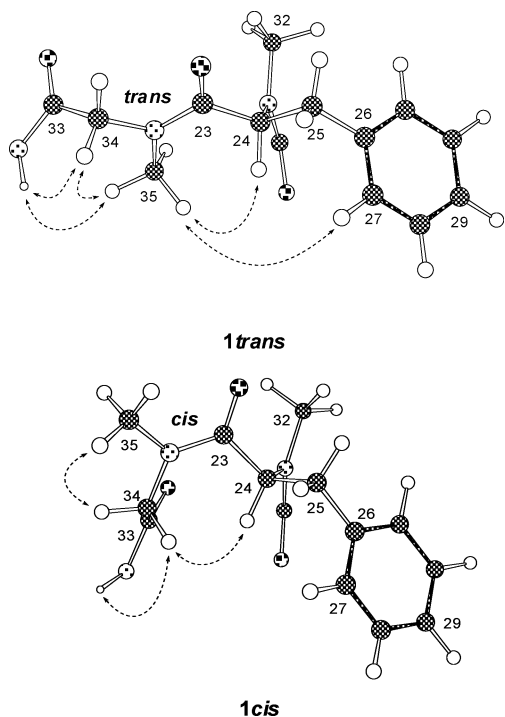**Figure 1.** Key COSY and HMBC correlations for **1**.

Figure 2. NOE patterns of substructure **a** for **1***cis* and **1***trans*.

Figure 3. NOE patterns of substructure **b** for **1***cis* and **1***trans*.

3d, were prepared by diastereoselective synthesis. These triols were prepared basically following the method employed for aurilide from *Dolabella auricularia*.[2a] The synthetic strategy for **3a** and **3c** applied the *syn*-selective aldol reaction by Evans,[6] whereas the *anti*-selective aldol reaction by Heathcock[7] was used for **3b** and **3d**.

The *syn*-selective aldol reaction via a closed transition state of *N*-propionyl oxazolidinone **5a** with *trans*-2-methyl-2-butenal, which used 1 equiv of Lewis acid, Bu$_2$BOTf, gave the aldol **6a** with the 2*R*,3*R* configuration. Conversely, the *anti*-selective aldol reaction via an open transition state of **5a** with the same aldehyde, which used 2 equiv of Bu$_2$BOTf, provided the aldol **6b** with the 2*S*,3*R* configuration. Likewise, the stereoselective aldol reaction of oxazolidinone **5b** with *trans*-2-methyl-2-butenal provided aldols **6c** (2*S*,3*S*) and **6d** (2*R*,3*S*), respectively. Subsequent treatment of **6a**−**d** with the aluminum amide reagent derived from *N*,*O*-dimethylhydroxylamine hydrochloride and AlMe$_3$, according to the procedure of Weinreb,[8] gave the desired transamination product, the *N*-methoxy-*N*-methylamides **7a**−**d**. Protection of **7a**−**d** with *tert*-butyldimethylsilyl (TBS) chloride and imidazole afforded the corresponding amides
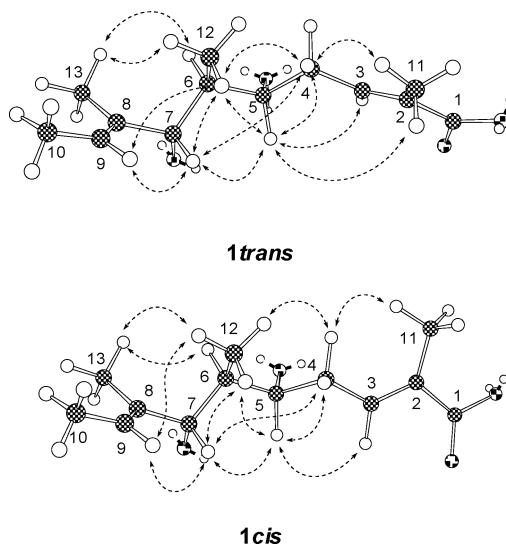
8a−**d**.[9] Reduction of amides **8a**−**d** to the aldehydes **9a**−**d** proceeded with diisobutylaluminum hydride (DIBAL) in THF.[10]

To establish the C-5 stereocenter by the second coupling reaction, the vinylogous Mukaiyama aldol reaction[11] was applied to aldehydes **9a**−**d** and 1-methoxy-2-methyl-1-trimethylsiloxy-1,3-butadiene,[12] affording conjugated methyl esters **10a**−**d**, respectively. The relative stereochemistry at C-5 through C-7 in **10a**−**d** was assigned on the basis of NMR analysis after the diols were derivatized to the corresponding acetonides.[13] All attempts to employ the modified Mitsunobu reaction[14] to prepare the desired 5*S* configuration for both **3a** and **3d** by inverting the 5-OH in both **10a** and **10d** were not successful. Therefore, **10a** and **10d** were subjected to Moffatt oxidation,[15] which yielded corresponding ketoesters **11a** and **11d**. Stereoselective reduction of **11a** with LiAlH$_4$[16] and **11d** with NaBH$_4$[17] afforded the desired protected triol **13a** and methyl ester **12d**, respectively.

DIBAL reduction of methyl esters **10b**, **10c**, and **12d** afforded deprotected triol **3b** as well as protected triols **13c** and **13d**. Removal of TBS groups in **13a**, **13c**, and **13d** afforded **3a**, **3c**, and **3d,** respectively. Thus, all four possible diastereoisomers of 2,6,8-trimethyl-2,8-decadiene-1,5,7-triol were successfully prepared.

Comparison of ^{1}H NMR spectra of synthetic triols **3a**−**d** with that obtained from natural **1** clearly indicated the relative stereochemistry as 5*S**,6*S**,7*S** (Figure 4).

To deduce the absolute stereochemistry of C-5 through C-7, *S*- and *R*-MTPA esters (**1a** and **1b**) were introduced to the C-5 hydroxyl group of **1**, respectively.[18] Although values for H-11 and H-12 (underlined) did not show the expected sign, the $\Delta\delta_{(S-R)}$ values for H-3, -4, -7, -8, -9, -10, and -13 were suggestive of 5*S*,6*S*,7*S*, which is identical to that of aurilide (**2**). MM2 calculation suggested that introduction of MTPA esters at C-5 causes a dramatic change in ring conformation for both **1a** and **1b**. For both models, rings were bent at C-4 and C-6 to make the bulky MTPA ester groups protrude from the ring. As a result of these conformational changes, methyl groups 11 and 12 might be located outside of the shielding area by phenyl rings of the MTPA esters (Figure 5). Proof of the stereochemistry obtained above by total synthesis of **1** is in progress.

To assign the absolute configuration of Hica and the amino acids, **1** was converted to fragment **4** (Scheme 2).

Scheme 1. Synthetic Route to Triols **3a−d**
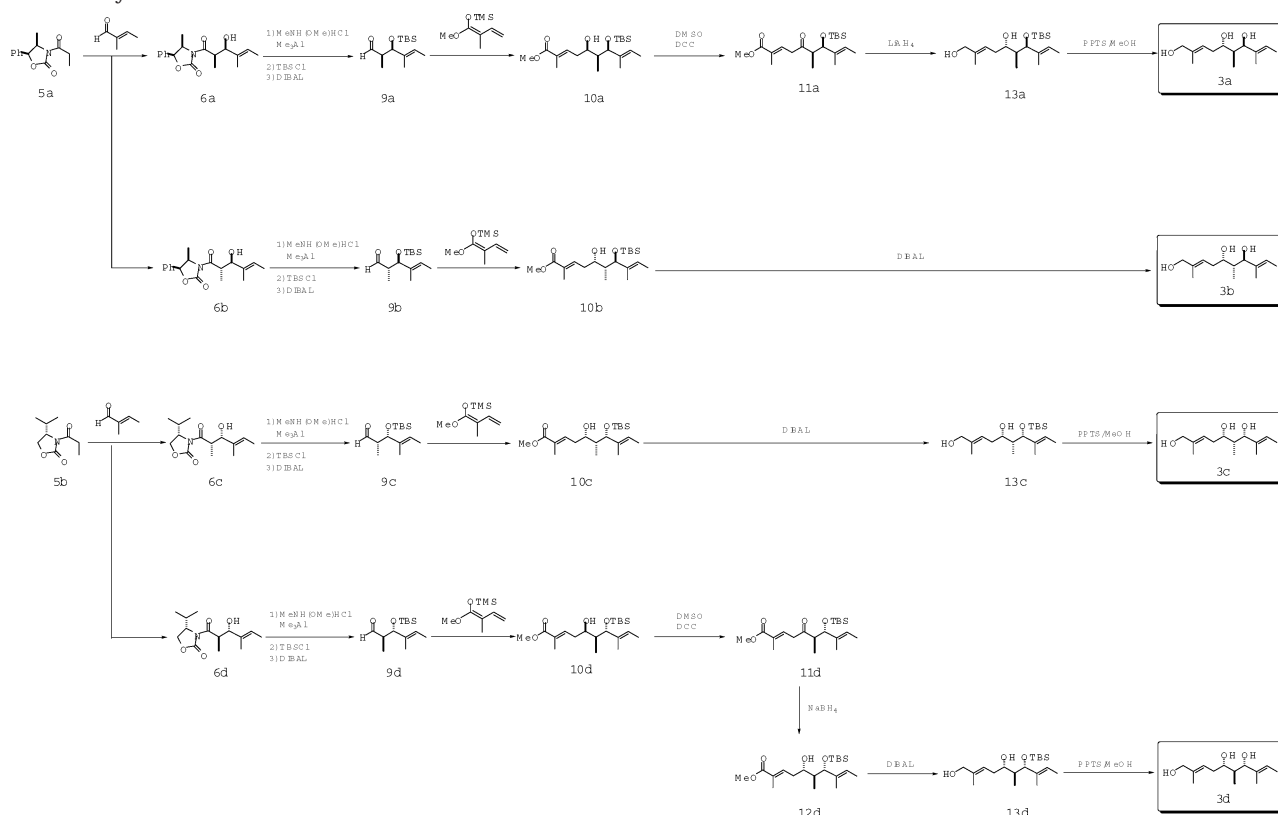
One-half the quantity of **4** was acid hydrolyzed and separated by ODS HPLC to yield Hica, Ala, Ile, and MePhe. The absolute stereochemistry of Hica was determined as D by chiral HPLC analysis. Marfey analysis[19] of each amino acid indicated L-Ile, L-MePhe, and both D- and L-Ala residues were present in **1**. To differentiate between the configurations for Ala-1 and -2, the remaining quantity of **4** was subjected to hydrazinolysis,[20] which yielded only Ala-2 as an intact amino acid. Marfey analysis disclosed

the L-stereochemistry for Ala-2; therefore Ala-1 was deduced as having D-stereochemistry.

Kulokekahilide-2 (**1**) showed potent cytotoxicity against the cell lines P388, SK-OV-3, MDA-MB-435, and A-10 with IC_{50} values of 4.2, 7.5, 14.6, and 59.1 nM, respectively, and it showed cancer cell selectivity, as the A-10 cell line is not transformed. Kulokekahilide-2 was also tested for its effects on microtubules, intermediate filaments, and actin filaments, but it showed no effects on these cytoskeleton networks. Recently, the combinatorial syntheses of aurilide analogues were achieved.[21] Further study with these analogues will disclose the detailed structure−activity relationship and their mode of action.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a digital spectropolarimeter. UV spectra were measured with a diode array spectrophotometer. NMR spectra were recorded at 500.115 MHz for ^{1}H and 125.766 MHz for ^{13}C. Glycerol was used as a matrix for FABMS measurements. Poly(ethylene glycol) was used as a marker for HR-FABMS.

Isolation. *Philinopsis speciosa* (300 animals, 9.0 kg wet weight) collected on midsummer nights in 1994 at Shark's Cove, Pupukea, O'ahu, were extracted with EtOH (3 × 3 L) and CHCl$_3$/MeOH (1:1, 3 L). The combined extracts were concentrated and extracted with CHCl$_3$. The aqueous layer was further extracted with *n*-BuOH, and the *n*-BuOH extract was combined with the CHCl$_3$ layer. The combined organic layers were evaporated to dryness and separated by the modified Kupchan procedure to yield *n*-hexane, CH$_2$Cl$_2$, and aqueous MeOH extracts. The CH$_2$Cl$_2$ extract was evaporated to dryness and purified by a two-step ODS flash chromatography process (first with aqueous MeOH as solvent, second with aqueous MeCN), followed by gel filtration (Sephadex LH-20, MeOH) and amino column chromatography [1.5 × 3.5 cm, CHCl$_3$, CHCl$_3$/MeOH (9:1), CHCl$_3$/MeOH/H$_2$O (7:3:0.5), and MeOH]. The CHCl$_3$/MeOH (9:1) fraction was separated by ODS HPLC
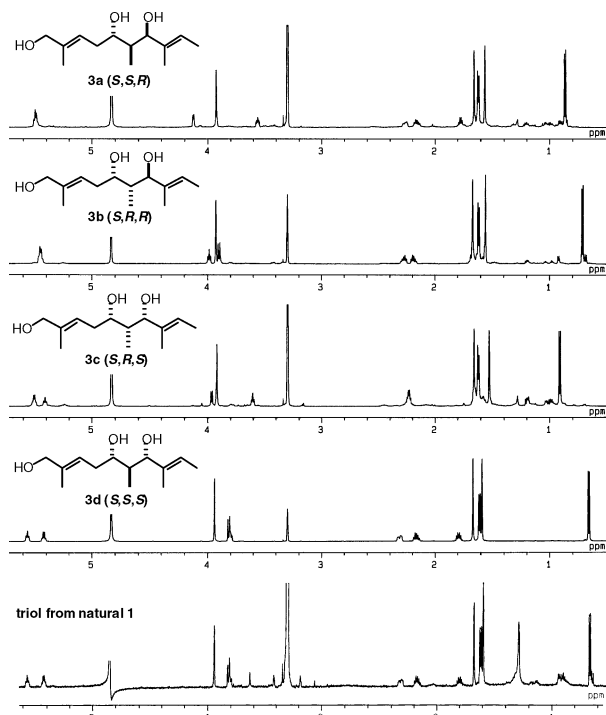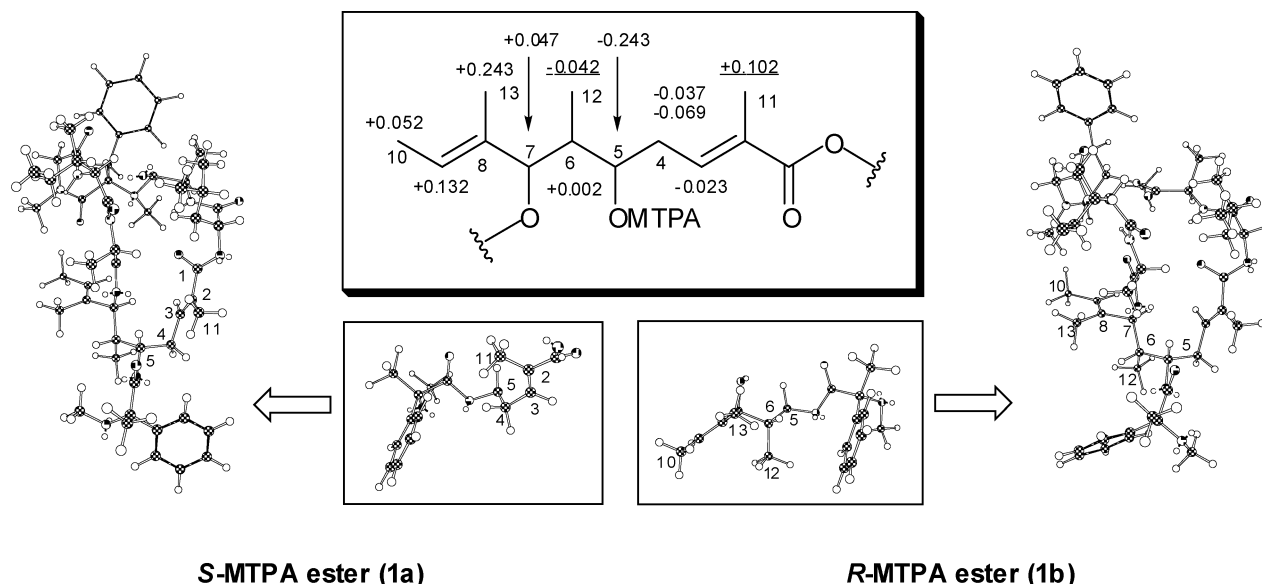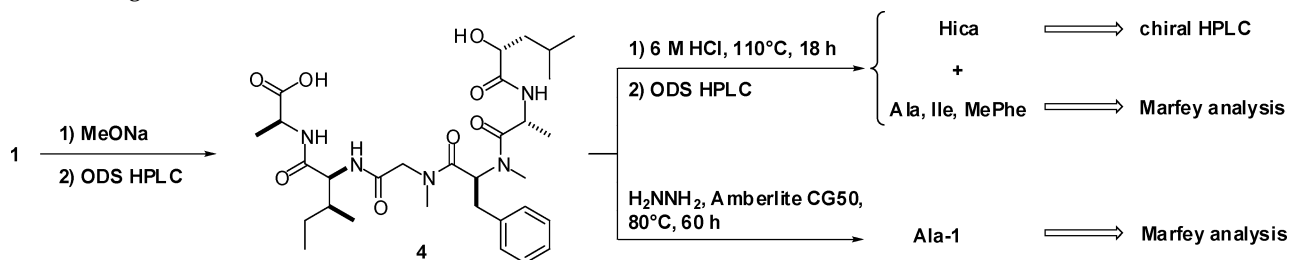

Figure 4. Comparison of ^{1}H NMR spectra of triols.

S-MTPA ester (1a)   **R-MTPA ester (1b)**

Figure 5. $\Delta\delta_{(S-R)}$ values and models of MTPA esters (**1a**,**b**).

Scheme 2. Degradation Scheme of **1**

[COSMOSIL 5C$_{18}$-AR, MeCN/H$_2$O (7:3)], giving nine fractions (1−9). Fraction 2 was separated by sequential ODS HPLC [COSMOSIL 5C$_{18}$-AR, MeCN/H$_2$O (1:1); 2-PrOH/H$_2$O (1:1); MeCN/H$_2$O (55:45); 2-PrOH/H$_2$O (47.5:52.5)] and finally purified again on an ODS column [COSMOSIL 5C$_{18}$-MS, MeCN/H$_2$O (1:1)] to give kulokekahilide-2 (**1**; 3.4 mg; 3.8 × 10^{-5} % yield based on wet weight).

Kulokekahilide-2 (1): colorless amorphous solid; [α]$_D$ −15° (*c* 0.04, MeOH); UV (MeOH) 205 nm (ε 15 000); see Table 1 for ^{1}H and ^{13}C NMR data; HRFABMS *m/z* 826.4942 (M + H)$^+$ (for C$_{44}$H$_{68}$N$_5$O$_{10}$, Δ −2.4 mmu).

(4*R***,5***S***,2′***R***,3′***R***,4′***E***)-3-(2′,4′-Dimethyl-3′-hydroxy-1′-oxo-4′-hexenyl)-4-methyl-5-phenyl-2-oxazolidinone (6a).** A stirred solution of *N*-propionyl oxazolidinone **5a** (1.0 mL, 5.0 mmol) in dry CH$_2$Cl$_2$ (15 mL) under argon was treated with 1 M dibutylboron triflate in CH$_2$Cl$_2$ (5.5 mL, 5.5 mmol) and diisopropylethylamine (1.1 mL, 6.0 mmol) at 0 °C. After 30 min, the reaction mixture was cooled to −78 °C and *trans*-2-methyl-2-butenal (530 μL, 5.5 mmol) was added dropwise. The resulting mixture was stirred at −78 °C for 30 min and then 90 min at room temperature. The reaction was quenched by addition of pH 7 aqueous phosphate buffer (10 mL) and oxidized with 30% hydrogen peroxide/methanol (1:1, 20 mL). The resulting solution was stirred at 0 °C for 1 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water (30 mL) and extracted with EtOAc (25 mL × 3). The combined organic layer was washed with 5% NaHCO$_3$ (25 mL) and brine (25 mL), dried over MgSO$_4$, and concentrated under reduced pressure, yielding a viscous yellow oil. The crude oil was purified by preparative thin-layer chromatography (PTLC) (EtOAc/*n*-hexane, 25:75), and the aldol **6a** was obtained as a colorless oil (1.48 g, 4.7 mmol, 94%): [α]$_D$ +27° (*c* 0.25, CHCl$_3$); IR (KBr) 3449, 1780, 1699, 1363, 1195, 767, 700 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.30−7.44 (m, 5H, Ar-H), 5.67 (d, 1H, *J* = 7.3 Hz, H-5), 5.63 (q, 1H, *J* = 6.9 Hz, H-5′), 4.77 (dq, 1H, *J* = 6.9, 6.4 Hz, H-4), 4.37 (brs, 1H, H-3′), 3.98 (dq, 1H, *J* = 6.9, 3.7 Hz, H-2′), 2.74 (brs, 1H, OH),

1.65 (d, 3H, *J* = 6.9 Hz, H-6′), 1.63 (s, 3H, H-7′), 1.16 (d, 3H, *J* = 6.9 Hz, H-8′), 0.89 (d, 3H, *J* = 6.4 Hz, H-6); ^{13}C NMR (CDCl$_3$) δ 176.9, 152.6, 134.3, 133.1, 128.8, 128.7 (2C), 125.6 (2C), 120.5, 78.9, 75.5, 54.9, 40.6, 14.3, 13.1, 13.0, 10.4.

(4*R***,5***S***,2′***S***,3′***R***,4′***E***)-3-(2′,4′-Dimethyl-3′-hydroxy-1′-oxo-4′-hexenyl)-4-methyl-5-phenyl-2-oxazolidinone (6b).** A stirred solution of *N*-propionyl oxazolidinone **5a** (466 mg, 2.0 mmol) in dry CH$_2$Cl$_2$ (6 mL) under argon was treated with 1 M dibutylboron triflate in CH$_2$Cl$_2$ (4.0 mL, 4.0 mmol) and diisopropylethylamine (440 μL, 2.4 mmol) at 0 °C. After 30 min, the reaction mixture was cooled to −78 °C and *trans*-2-methyl-2-butenal (250 μL, 250 mmol) was added dropwise. After 2 h at −78 °C, the reaction was quenched by addition of pH 7 aqueous phosphate buffer (4 mL) and oxidized with 30% hydrogen peroxide/methanol (1:1, 8 mL). The resulting solution was allowed to slowly warm from −78 °C to 0 °C over a period of 1 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water (10 mL) and extracted with EtOAc (10 mL × 3). The combined organic layer was washed with 5% NaHCO$_3$ (10 mL) and brine (10 mL), dried over MgSO$_4$, and concentrated under reduced pressure, yielding a viscous yellow oil. The crude oil was purified by PTLC (EtOAc/*n*-hexane, 25:75), and the aldol **6b** was obtained as a colorless oil (383 mg, 1.2 mmol, 60%): [α]$_D$ +40° (*c* 0.18, CHCl$_3$); HREIMS *m/z* 300.1575 (M − OH) $^+$ (for C$_{18}$H$_{22}$NO$_3$, Δ −2.4 mmu); IR (KBr) 3448, 1780, 1699, 1346, 1197, 767, 700 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.29−7.43 (m, 5H, Ar-H), 5.66 (d, 1H, *J* = 7.1 Hz, H-5), 5.52 (q, 1H, *J* = 6.6 Hz, H-5′), 4.78 (dq, 1H, *J* = 7.1, 6.6 Hz, H-4), 4.13 (overlapping dq and d, 2H, H-2′, H-3′), 2.55 (brs, 1H, OH), 1.67 (s, 3H, H-7′), 1.62 (d, 3H, *J* = 6.6 Hz, H-6′), 1.06 (d, 3H, *J* = 6.4 Hz, H-8′), 0.90 (d, 3H, *J* = 6.6 Hz, H-6); ^{13}C NMR (CDCl$_3$) δ 176.6, 153.4, 135.2, 133.2, 128.7 (3C), 125.6 (2C), 123.6, 81.2, 78.9, 55.2, 40.7, 14.8, 14.3, 13.1, 10.6.

(4*S***,2′***S***,3′***S***,4′***E***)-3-(2′,4′-Dimethyl-3′-hydroxy-1′-oxo-4′-hexenyl)-4-isopropyl-2-oxazolidinone (6c).** Using the method described for the preparation of **6a**, *N*-propionyl-oxazolidinone **5b** (500 mg, 2.7 mmol) was treated with 1 M

dibutylboron triflate in CH$_2$Cl$_2$ (3.0 mL, 3.0 mmol) and diisopropylethylamine (600 μL, 3.2 mmol). The resulting enol borinate was allowed to react with *trans*-2-methyl-2-butenal (300 μL, 3.0 mmol). After workup and purification, aldol **6c** was obtained as a colorless oil (686 mg, 2.6 mmol, 94%): [α]$_D$ +70° (*c* 0.99, CHCl$_3$); IR (KBr) 3449, 2926, 1778, 1703, 1384, 1205 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.60 (q, 1H, *J* = 6.9 Hz, H-5′), 4.44 (ddd, 1H, *J* = 9.2, 4.1, 2.8 Hz, H-4), 4.32 (brs, 1H, H-3′), 4.27 (dd, 1H, *J* = 9.2, 9.2 Hz, H-5b), 4.21 (dd, 1H, *J* = 9.2, 2.8 Hz, H-5a), 3.97 (dq, 1H, *J* = 6.9, 3.7 Hz, H-2′), 2.90 (brs, 1H, OH), 2.34 (dsept, 1H, *J* = 6.9, 4.1 Hz, H-6), 1.62 (d, 3H, *J* = 6.9 Hz, H-6′), 1.58 (s, 3H, H-7′), 1.16 (d, 3H, *J* = 7.3 Hz, H-8′), 0.91 (d, 3H, *J* = 6.9 Hz, H-8), 0.87 (d, 3H, *J* = 6.9 Hz, H-7); ^{13}C NMR (CDCl$_3$) δ 177.4, 153.5, 134.0, 120.5, 75.1, 63.3, 58.3, 40.4, 28.3, 17.9, 14.7, 13.1, 13.0, 11.0.

(4*S*,2′*R*,3′*S*,4′*E*)-3-(2′,4′-Dimethyl-3′-hydroxy-1′-oxo-4′-hexenyl)-4-isopropyl-2-oxazolidinone (6d). Using the method described for the preparation of **6b**, *N*-propionyl-oxazolidinone **5b** (185 mL, 1.0 mmol) was treated with 1 M dibutylboron triflate in CH$_2$Cl$_2$ (2.0 mL, 2.0 mmol) and diisopropylethylamine (220 μL, 1.2 mmol). The resulting enol borinate was allowed to react with *trans*-2-methyl-2-butenal (125 μL, 1.25 mmol). After workup and purification, aldol **6d** was obtained as a colorless oil (219 mg, 0.8 mmol, 81%): [α]$_D$ +55° (*c* 0.18, CHCl$_3$); HREIMS *m/z* 252.1604 (M$^+$ − OH)$^+$ (for C$_{14}$H$_{22}$NO$_3$, Δ 0.5 mmu); IR (KBr) 3449, 2966, 1774, 1699, 1386, 1205, 748, 706 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.52 (q, 1H, *J* = 6.9 Hz, H-5′), 4.45 (dt, 1H, *J* = 7.3, 3.2 Hz, H-4), 4.27 (dd, 1H, *J* = 9.2, 7.3 Hz, H-5b), 4.21 (dd, 1H, *J* = 9.2, 3.2 Hz, H-5a), 4.14 (dq, 1H, *J* = 8.7, 6.4 Hz, H-2′), 4.06 (d, 1H, *J* = 8.7 Hz, H-3′), 2.63 (brs, 1H, OH), 2.39 (m, 1H, H-6), 1.65 (s, 3H, H-7′), 1.62 (d, 3H, *J* = 6.9 Hz, H-6′), 1.03 (d, 3H, *J* = 6.4 Hz, H-8′), 0.91 (d, 3H, *J* = 6.9 Hz, H-8), 0.89 (d, 3H, *J* = 6.9 Hz, H-7); ^{13}C NMR (CDCl$_3$) δ 176.7, 154.5, 135.3, 123.4, 81.3, 63.3, 58.9, 40.3, 28.4, 17.9, 14.7, 14.6, 13.1, 10.7.

(2*R*,3*R*,4*E*)-3-Hydroxy-*N*-methoxy-*N*,2,4-trimethyl-4-hexenamide (7a). To a stirred suspension of *N*,*O*-dimethyl-hydroxylamine hydrochloride (317.5 mg, 3.25 mmol) in CH$_2$Cl$_2$ (6 mL) at 0 °C under argon was slowly added 15% trimethyl-aluminum in toluene (1.6 mL, 3.2 mmol) with concomitant evolution of gas. The resulting homogeneous solution was stirred for 40 min at room temperature and then recooled to 0 °C, and a solution of aldol **6a** (516 mg, 1.6 mmol) in CH$_2$Cl$_2$ (5 mL) was added over a period of 5 min. The solution was stirred for 1.5 h at 0 °C, and then ice-cooled 0.5 M aqueous HCl (30 mL) and CH$_2$Cl$_2$ (10 mL) were added. The layers were separated, and the aqueous layer was extracted with CH$_2$Cl$_2$ (10 mL × 3). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by PTLC (EtOAc/*n*-hexane, 50:50), and amide **7a** was obtained as a colorless solid (265 mg, 1.32 mmol, 81%): [α]$_D$ −8° (*c* 0.16, CHCl$_3$); IR (KBr) 3430, 2935, 1635, 1456, 1384, 991 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.65 (q, 1H, *J* = 6.9 Hz, H-5), 4.26 (brs, 1H, H-3), 3.71 (s, 3H, Me-O), 3.20 (brs, 3H, Me-N), 3.07 (m, 1H, H-2), 1.63 (d, 3H, *J* = 6.9 Hz, H-6), 1.59 (s, 3H, H-7), 1.09 (d, 3H, *J* = 7.3 Hz, H-8); ^{13}C NMR (CDCl$_3$) δ 178.0, 133.6, 120.4, 75.4, 61.5, 36.9, 32.0, 13.3, 13.0, 10.4.

(2*S*,3*S*,4*E*)-3-Hydroxy-*N*-methoxy-*N*,2,4-trimethyl-4-hex-enamide (7c). Using the method described for the preparation of **7a**, *N*,*O*-dimethylhydroxylamine hydrochloride (254 mg, 2.6 mmol) was treated with 15% trimethylaluminum in toluene (1.3 mL, 2.6 mmol). To the resulting solution was added a solution of aldol **6c** (350 mg, 1.3 mmol). After workup and purification, amide **7c** was obtained as a colorless solid (90.6 mg 0.45 mmol, 35%): [α]$_D$ +11° (*c* 0.31, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 5.65 (q, 1H, *J* = 6.9 Hz, H-5), 4.26 (brs, 1H, H-3), 3.71 (s, 3H, Me-O), 3.20 (brs, 3H, Me-N), 3.08 (m, 1H, H-2), 1.64 (d, 3H, *J* = 6.9 Hz, H-6), 1.59 (s, 3H, H-7), 1.09 (d, 3H, *J* = 6.9 Hz, H-8); ^{13}C NMR (CDCl$_3$) δ 178.0, 133.7, 120.3, 75.4, 61.5, 36.9, 31.9, 13.2, 12.9, 10.4.

(2*R*,3*R*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-*N*-methoxy-*N*,2,4-trimethyl-4-hexenamide (8a). A mixture of **7a** (265 mg, 1.3 mmol), TBSCl (600 mg, 4.0 mmol), and imidazole (540 mg, 7.9 mmol) in DMF (6 mL) was stirred overnight at room

temperature. The reaction mixture was quenched with water and extracted with EtOAc (15 mL × 3). The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated under reduced pressure, yielding a colorless oil. The resulting oil was purified by column chromatography (EtOAc/*n*-hexane, 50:50), and protected amide **8a** was obtained as a colorless oil (415 mg, 1.3 mmol, 100%): [α]$_D$ −5° (*c* 0.27, CHCl$_3$); IR (KBr) 2958, 2932, 2858, 1666, 1381, 1256, 1057, 876, 837, 775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.36 (q, 1H, *J* = 6.9 Hz, H-5), 4.11 (d, 1H, *J* = 9.2 Hz, H-3), 3.63 (s, 3H, Me-O), 3.11 (overlapping brs and m, 4H, Me-N, H-2), 1.56 (s, 3H, H-7), 1.52 (d, 3H, *J* = 7.3 Hz, H-8), 1.17 (d, 3H, *J* = 6.9 Hz, H-6), 0.88 (s, 9H, (Me)$_3$CSi), 0.04 (s, 3H, Me-Si), −0.04 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 176.2, 136.4, 121.6, 80.2, 61.5, 40.4, 32.1, 25.8(3C), 18.2, 14.8, 13.0, 11.0, −4.8, −5.1.

(2*S*,3*R*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-*N*-methoxy-*N*,2,4-trimethyl-4-hexenamide (8b). To a stirred suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (216 mg, 2.2 mmol) in THF (2.2 mL) at 0 °C under argon was slowly added 1.0 M trimethylaluminum in *n*-hexane (2.2 mL, 2.2 mmol) with concomitant evolution of gas. The resulting homogeneous solution was stirred for 30 min at room temperature and then recooled to 0 °C, and a solution of aldol **6b** (351 mg 1.1 mmol) in THF (2.2 mL) was added over a period of 5 min. The solution was stirred for 2.5 h at 50 °C, and then ice-cooled 0.5 M aqueous HCl (30 mL) and EtOAc (10 mL) were added. The layers were separated, and the aqueous layer was extracted with EtOAc (10 mL × 3). The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated under reduced pressure. To the residue were added TBSCl (500 mg, 3.3 mmol) and imidazole (450 mg, 6.6 mmol) in DMF (6 mL) and stirred overnight at room temperature. The reaction mixture was quenched with water and extracted with EtOAc (15 mL × 3). The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated under reduced pressure, yielding a yellow oil. The resulting oil was purified by PTLC (EtOAc/*n*-hexane, 15:85), and a protected amide **8b** was obtained as a colorless solid (317 mg, 1.0 mmol, 92%): [α]$_D$ +30° (*c* 0.25, CHCl$_3$); IR (KBr) 2929, 2856, 1663, 1387, 1250, 1057, 862, 837, 777 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.43 (q, 1H, *J* = 6.4 Hz, H-5), 4.14 (d, 1H, *J* = 10.1 Hz, H-3), 3.73 (s, 3H, Me-O), 3.14 (overlapping m and brs, 4H, H-2, Me-N), 1.60 (d, 3H, *J* = 6.4 Hz, H-6), 1.56 (s, 3H, H-7), 0.83 (d, 3H, *J* = 6.9 Hz, H-8), 0.79 (s, 9H, (Me)$_3$CSi), −0.02 (s, 3H, Me-Si), −0.06 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 176.5, 135.6, 123.3, 81.7, 61.3, 38.8, 31.8, 25.6 (3C), 18.0, 14.2, 13.0, 10.0, −5.0, −5.3.

(2*S*,3*S*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-*N*-methoxy-*N*,2,4-trimethyl-4-hexenamide (8c). Using the method described for the preparation of **8a**, amide **7c** (265 mg, 1.3 mmol) was silylated with TBSCl (600 mg, 4.0 mmol) and imidazole (540 mg, 7.9 mmol) to give protected amide **8c** as a colorless oil (167 mg, 0.53 mmol, 84%): [α]$_D$ +3° (*c* 0.50, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 5.35 (q, 1H, *J* = 6.4 Hz, H-5), 4.09 (d, 1H, *J* = 9.1 Hz, H-3), 3.61 (s, 3H, Me-O), 3.07 (overlapping brs and m, 4H, Me-N, H-2), 1.55 (s, 3H, H-7), 1.50 (d, 3H, *J* = 6.4 Hz, H-6), 1.15 (d, 3H, *J* = 6.9 Hz, H-8), 0.86 (s, 9H, (Me)$_3$CSi), 0.02 (s, 3H, Me-Si), −0.06 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 176.2, 136.3, 121.5, 80.2, 61.4, 40.4, 32.0, 25.8 (3C), 18.2, 14.7, 12.9, 10.9, −4.8, −5.1.

(2*R*,3*S*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-*N*-methoxy-*N*,2,4-trimethyl-4-hexenamide (8d). To a stirred suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (302 mg, 3.1 mmol) in THF (3.0 mL) at 0 °C under argon was slowly added 1.0 M trimethylaluminum in *n*-hexane (3.0 mL, 3.0 mmol) with concomitant evolution of gas. The resulting homogeneous solution was stirred for 30 min at room temperature and then recooled to 0 °C, and a solution of aldol **6d** (166 mg, 0.62 mmol) in THF (1.0 mL) was added over a period of 5 min. The solution was stirred overnight at room temperature, and then ice-cooled 0.5 M aqueous HCl (30 mL) and EtOAc (10 mL) were added. The layers were separated, and the aqueous layer was extracted with EtOAc (10 mL × 3). The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated under reduced pressure. To the residue were added TBSCl (272 mg, 1.8 mmol) and imidazole (245 mg, 3.6 mmol) in DMF (3

mL) and stirred overnight at room temperature. The reaction mixture was quenched with water and extracted with EtOAc (10 mL × 3). The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated under reduced pressure, yielding a viscous yellow oil. The resulting oil was purified by PTLC (EtOAc/*n*-hexane, 15:85), and protected **8d** was obtained as a colorless solid (127 mg, 0.4 mmol, 65%): [α]$_D$ −31° (*c* 0.23, CHCl$_3$); IR (KBr) 2930, 2858, 1662, 1386, 1250, 1057, 862, 837, 777 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.41 (q, 1H, *J* = 6.9 Hz, H-5), 4.12 (d, 1H, *J* = 10.1 Hz, H-3), 3.71 (s, 3H, Me-O), 3.14 (overlapping brs and m, 4H, Me-N, H-2), 1.58 (d, 3H, *J* = 6.9 Hz, H-6), 1.54 (s, 3H, H-7), 0.81 (d, 3H, *J* = 6.9 Hz, H-8), 0.78 (s, 9H, (Me)$_3$CSi), −0.04 (s, 3H, Me-Si), −0.08 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 176.4, 135.5, 123.3, 81.6, 61.3, 38.7, 31.8, 25.6 (3C), 18.0, 14.1, 12.9, 10.0, −5.0, −5.4.

(2*R*,3*R*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-4-hexenal (9a). To a solution of amide **8a** (407 mg, 1.29 mmol) in THF (5 mL) at −78 °C was added 1 M DIBAL in THF (3.9 mL, 3.9 mmol) under argon. After 1.5 h the reaction mixture was quenched with saturated aqueous Na$_2$SO$_4$ (10 mL) and EtOAc (10 mL) and the solution stirred vigorously. After 10 min, anhydrous Na$_2$SO$_4$ (ca. 5 g) was added and the reaction mixture stirred vigorously for a further 30 min. The mixture was filtered through a pad of anhydrous Na$_2$SO$_4$ in a funnel. The solvents were removed under reduced pressure. The residue was purified by column chromatography (EtOAc/*n*-hexane, 2.5:97.5), and aldehyde **9a** was obtained as a colorless oil (262 mg, 1.02 mmol, 79%): [α]$_D$ −1.9° (*c* 0.16, CHCl$_3$); IR (KBr) 3440, 2929, 2858, 1728, 1251, 1058, 837, 775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 9.60 (d, 1H, *J* = 1.8 Hz, H-1), 5.41 (q, 1H, *J* = 6.9 Hz, H-5), 4.21 (d, 1H, *J* = 6.4 Hz, H-3), 2.46 (ddq, 1H, *J* = 6.9, 6.4, 1.8 Hz, H-2), 1.54 (d, 3H, *J* = 6.9 Hz, H-6), 1.52 (s, 3H, H-7), 0.98 (d, 3H, *J* = 6.9 Hz, H-8), 0.83 (s, 9H, (Me)$_3$CSi), −0.02 (s, 3H, Me-Si), −0.07 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 204.2, 135.5, 121.7, 77.9, 51.0, 25.7 (3C), 18.0, 12.8, 11.9, 9.2, −4.7, −5.4.

(2*S*,3*R*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-4-hexenal (9b). To a solution of LiAlH$_4$ (190 mg, 4.0 mmol) in THF (4 mL) was added amide **8b** (317 mg, 1.0 mmol) in THF (10 mL) at −40 °C, and the mixture was stirred for 1 h. The reaction mixture was quenched with 1 M aqueous HCl (10 mL) and then extracted with EtOAc (10 mL × 3). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/*n*-hexane, 2.5:97.5), and aldehyde **9b** was obtained as a colorless oil (124 mg, 0.48 mmol, 48%): [α]$_D$ +28° (*c* 0.45, CHCl$_3$); IR (KBr) 3440, 2930, 2858, 1730, 1251, 1053, 858, 837, 775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 9.74 (d, 1H, *J* = 2.8 Hz, H-1), 5.44 (q, 1H, *J* = 6.4 Hz, H-5), 4.06 (d, 1H, *J* = 9.2 Hz, H-3), 2.55 (m, 1H, H-2), 1.62 (d, 3H, *J* = 6.4 Hz, H-6), 1.56 (s, 3H, H-7), 0.84 (overlapping s and d, 12H, (Me)$_3$CSi, H-8), 0.01 (s, 3H, Me-Si), −0.04 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 205.4, 135.3, 123.1, 80.6, 50.2, 25.7 (3C), 18.0, 13.0, 10.9, 10.5, −4.6, −5.4.

(2*S*,3*S*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-4-hexenal (9c). Using the method described for the preparation of **9a**, amide **8c** (132 mg, 0.42 mmol) in THF (2 mL) was treated with 1 M DIBAL in THF (1.25 mL, 1.25 mmol) and yielded aldehyde **9c** as a colorless oil (86.5 mg, 0.34 mmol, 81%): [α]$_D$ +2.8° (*c* 0.54, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 9.63 (d, 1H, *J* = 1.4 Hz, H-1), 5.44 (q, 1H, *J* = 6.4 Hz, H-5), 4.23 (d, 1H, *J* = 6.4 Hz, H-3), 2.50 (ddq, 1H, *J* = 6.9, 6.4, 1.4 Hz, H-2), 1.58 (d, 3H, *J* = 6.4 Hz, H-6), 1.55 (s, 3H, H-7), 1.01 (d, 3H, *J* = 6.9 Hz, H-8), 0.86 (s, 9H, (Me)$_3$CSi), 0.02 (s, 3H, Me-Si), −0.04 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 204.6, 135.6, 121.8, 78.0, 51.1, 25.7 (3C), 18.1, 12.9, 11.9, 9.3, −4.6, −5.3.

(2*R*,3*S*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-4-hexenal (9d). Using the method described for the preparation of **9a**, amide **8d** (109 mg, 0.35 mmol) in THF (1 mL) was treated with 0.93 M DIBAL in *n*-hexane (560 μL, 0.52 mmol) and yielded aldehyde **9d** as a colorless oil (80.9 mg, 0.32 mmol, 90%): [α]$_D$ −28° (*c* 0.49, CHCl$_3$); IR (KBr) 3440, 2956, 2929, 2858, 1728, 1251, 1053, 860, 837, 775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 9.74 (d, 1H, *J* = 3.2 Hz, H-1), 5.44 (q, 1H, *J* = 6.9 Hz, H-5), 4.06 (d, 1H, *J* = 8.7 Hz, H-3), 2.54 (m, 1H, H-2),

1.61 (d, 3H, *J* = 6.4 Hz, H-6), 1.56 (s, 3H, H-7), 0.84 (overlapping s and d, 12H, (Me)$_3$CSi, H-8), 0.01 (s, 3H, Me-Si), −0.05 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 205.4, 135.3, 123.1, 80.6, 50.2, 25.7 (3C), 18.0, 12.9, 10.9, 10.5, −4.6, −5.4.

(5*R*,6*S*,7*R*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethyl-2,8-decadienoic Acid Methyl Ester (10a). Boron trifluoride etherate (128 μL, 1.02 mmol) was added dropwise to a solution of aldehyde **9a** (262 mg, 1.02 mmol) and 1-methoxy-2-methyl-1-trimethylsiloxy-1,3-butadiene (209 mg, 1.12 mmol) in CH$_2$Cl$_2$ (10 mL) at −78 °C under argon. The reaction mixture was stirred for 1 h at −78 °C and quenched by addition of 5% NaHCO$_3$ (10 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (10 mL × 3). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The residue was purified by PTLC (EtOAc/*n*-hexane, 20:80), and methyl ester **10a** was obtained as a colorless oil (304 mg, 0.82 mmol, 81%): [α]$_D$ +23° (*c* 0.27, CHCl$_3$); IR (KBr) 3440, 2929, 2858, 1718, 1256, 1055, 869, 837, 773 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 6.76 (t, 1H, *J* = 6.9 Hz, H-3), 5.44 (q, 1H, *J* = 6.4 Hz, H-9), 3.98 (d, 1H, *J* = 6.9 Hz, H-7), 3.75 (m, 1H, H-5), 3.73 (s, 3H, Me-O), 2.42 (m, 1H, H-4b), 2.25 (m, 1H, H-4a), 1.85 (s, 3H, H-13), 1.60 (overlapping d and m, 4H, H-10, H-6), 1.52 (s, 3H, H-11), 0.91 (d, 3H, *J* = 6.9 Hz, H-12), 0.89 (s, 9H, (Me)$_3$CSi), 0.05 (s, 3H, Me-Si), −0.04 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 168.5, 138.8, 136.7, 129.2, 121.4, 81.8, 72.2, 51.7, 41.0, 34.6, 25.9 (3C), 18.1, 12.9, 12.7, 12.0, 7.5, −4.5, −5.2; *anal.* calcd for C$_{20}$H$_{38}$O$_4$Si, C, 64.82; H, 10.34; found, C, 64.51; H, 10.23.

(5*S*,6*S*,7*R*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethyl-2,8-decadienoic Acid Methyl Ester (10b). Using the method described for the preparation of **10a**, aldehyde **9b** (262 mg, 1.02 mmol) and 1-methoxy-2-methyl-1-trimethylsiloxy-1,3-butadiene (209 mg, 1.12 mmol) were treated with boron trifluoride etherate (128 μL, 1.02 mmol) and yielded methyl ester **10b** as a colorless oil (111 mg, 0.3 mmol, 63%): [α]$_D$ −10° (*c* 0.14, CHCl$_3$); IR (KBr) 3505, 2929, 2858, 1716, 1258, 1049, 862, 837, 775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 6.78 (t, 1H, *J* = 7.3 Hz, H-3), 5.51 (q, 1H, *J* = 6.4 Hz, H-9), 4.00 (overlapping m and d, 2H, H-5, H-7), 3.70 (s, 3H, Me-O), 2.38 (m, 1H, H-4b), 2.19 (m, 1H, H-4a), 1.83 (s, 3H, H-13), 1.68 (m, 1H, H-6), 1.61, (d, 3H, *J* = 6.4 Hz, H-10), 1.51 (s, 3H, H-11), 0.89 (overlapping s and d, 12H, (Me)$_3$CSi, H-12), 0.06 (s, 3H, Me-Si), −0.03 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 168.4, 139.4, 135.4, 128.8, 121.2, 82.4, 70.6, 51.6, 39.1, 33.7, 25.8 (3C), 18.0, 12.9, 12.6, 12.5, 11.4, −4.6, −5.3; *anal.* calcd for C$_{20}$H$_{38}$O$_4$Si, C, 64.82; H, 10.34; found, C, 64.88; H, 10.24.

(5*S*,6*R*,7*S*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethyl-2,8-decadienoic Acid Methyl Ester (10c). Using the method described for the preparation of **10a**, aldehyde **9c** (70.6 mg, 0.28 mmol) and 1-methoxy-2-methyl-1-trimethylsiloxy-1,3-butadiene (65 mg, 0.33 mmol) were treated with boron trifluoride etherate (35 μL, 0.28 mmol) and yielded methyl ester **10c** as a colorless oil (47.8 mg, 0.13 mmol, 49%): [α]$_D$ −21° (*c* 0.30, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 6.75 (t, 1H, *J* = 7.3 Hz, H-3), 5.43 (q, 1H, *J* = 6.9 Hz, H-9), 3.97 (d, 1H, *J* = 6.9 Hz, H-7), 3.73 (s, 3H, Me-O), 2.41 (m, 1H, H-4b), 2.25 (m, 1H, H-4a), 1.85 (s, 3H, H-13), 1.60 (overlapping d and m, 4H, H-10, H-6), 1.52 (s, 3H, H-11), 0.91 (d, 3H, *J* = 6.9 Hz, H-12), 0.88 (s, 9H, (Me)$_3$CSi), 0.05 (s, 3H, Me-Si), −0.05 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 168.5, 138.8, 136.7, 129.2, 121.4, 81.8, 72.3, 51.7, 41.0, 34.6, 25.9 (3C), 18.1, 12.9, 12.7, 12.0, 7.5, −4.5, −5.2; *anal.* calcd for C$_{20}$H$_{38}$O$_4$Si, C, 64.82; H, 10.34; found, C, 64.63; H, 10.28.

(5*R*,6*S*,7*S*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethyl-2,8-decadienoic Acid Methyl Ester (10d). Using the method described for the preparation of **10a**, aldehyde **9d** (80.9 mg, 0.31 mmol) and 1-methoxy-2-methyl-1-trimethylsiloxy-1,3-butadiene (70 mg, 0.37 mmol) were treated with boron trifluoride etherate (40 μL, 0.34 mmol) and yielded methyl ester **10d** as a colorless oil (72.6 mg, 0.2 mmol, 63%): [α]$_D$ +10° (*c* 0.15, CHCl$_3$); IR (KBr) 3440, 2929, 2858, 1717, 1256, 1049, 862, 837, 777 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 6.78 (t, 1H, *J* = 7.3 Hz, H-3), 5.50 (q, 1H, *J* = 6.9 Hz, H-9), 4.00 (overlapping m and d, 2H, H-5, H-7), 3.70 (s, 3H, Me-O), 2.37 (m, 1H, H-4b), 2.19 (m, 1H, H-4a), 1.83 (s, 3H, H-13), 1.67 (m,

1H, H-6), 1.60 (d, 3H, $J = 6.9$ Hz, H-10), 1.50 (s, 3H, H-11), 0.88 (overlapping s and d, 12H, (Me)$_3$CSi, H-12), 0.05 (s, 3H, Me-Si), −0.04 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 168.4, 139.4, 135.4, 128.8, 121.2, 82.3, 70.6, 51.6, 39.1, 33.7, 25.8 (3C), 18.0, 12.9, 12.6, 12.5, 11.4, −4.6, −5.3; *anal.* calcd for C$_{20}$H$_{38}$O$_4$Si, C, 64.82; H, 10.34; found, C, 64.22; H, 10.24.

(6*R*,7*R*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-oxo-2,6,8-trimethyl-2,6,8-decadienoic Acid Methyl Ester (11a). To a solution of **10a** (80.0 mg, 0.22 mmol) in DMSO/diethyl ether (1:1, 3 mL) were added pyridinium trifluoroacetate (21 mg, 0.11 mmol) and DCC (134 mg, 0.65 mmol). After stirring at room temperature for 1.5 h, the reaction mixture was diluted with EtOAc (10 mL), and the insolubles were removed by filtration. The filtrate was washed with 0.5 M aqueous HCl (10 mL) and saturated NaHCO$_3$ (10 mL). The organic layer was dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by PTLC (EtOAc/*n*-hexane, 10:90), and keto ester **11a** was obtained as a colorless oil (54.2 mg, 0.15 mmol, 68%): ^{1}H NMR (CDCl$_3$) δ 6.85 (t, 1H, $J = 6.9$ Hz, H-3), 5.32 (q, 1H, $J = 6.4$ Hz, H-9), 4.04 (d, 1H, $J = 8.3$ Hz, H-7), 3.71 (s, 3H, Me-O), 3.24 (m, 2H, H-4), 2.80 (dq, 1H, $J = 8.3$, 6.9 Hz, H-6), 1.79 (s, 3H, H-13), 1.55 (s, 3H, H-11), 1.51 (d, 3H, $J = 6.4$ Hz, H-10), 1.08 (d, 3H, $J = 6.9$ Hz, H-12), 0.85 (s, 9H, (Me)$_3$CSi), 0.01 (s, 3H, Me-Si), −0.06 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 209.2, 167.9, 135.9, 133.2, 130.3, 122.5, 80.1, 51.8, 51.5, 42.6, 25.8 (3C), 18.1, 13.6, 12.9, 12.8, 11.1, −4.7, −5.2.

(6*R*,7*S*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-oxo-2,6,8-trimethyl-2,8-decadienoic Acid Methyl Ester (11d). Using the method described for the preparation of **11a**, methyl ester **10d** (160.8 mg, 0.43 mmol) was treated with pyridinium trifluoroacetate (83 mg, 0.43 mmol) and DCC (266 mg, 0.51 mmol) in DMSO/Et$_2$O (1:1, 6 mL) and yielded keto ester **11d** as a colorless oil (126 mg, 0.34 mmol, 80%): ^{1}H NMR (CDCl$_3$) δ 6.98 (t, 1H, $J = 6.9$ Hz, H-3), 5.40 (q, 1H, $J = 6.4$ Hz, H-9), 4.04 (d, 1H, $J = 9.6$ Hz, H-7), 3.71 (s, 3H, Me-O), 3.39 (m, 2H, H-4), 2.80 (m, 1H, H-6), 1.82 (s, 3H, H-13), 1.57 (d, 3H, $J = 6.9$ Hz, H-10), 1.52 (s, 3H, H-11), 0.86 (d, 3H, $J = 13.3$ Hz, H-12), 0.77 (s, 9H, (Me)$_3$CSi), −0.08 (s, 3H, Me-Si), −0.10 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 210.3, 168.0, 135.2, 133.3, 130.0, 123.6, 82.3, 51.7, 49.4, 44.3, 25.7 (3C), 17.9, 13.7, 12.9 (2C), 10.0, −4.8, −5.5.

(5*S*,6*S*,7*S*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethyl-2,8-decadienoic Acid Methyl Ester (12d). To a solution of **11d** (108 mg, 0.29 mmol) in MeOH (1.5 mL) was added NaBH$_4$ (37 mg, 0.88 mmol) at −40 °C. After 2 h, the reaction mixture was quenched with saturated NaHCO$_3$ (10 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were dried over MgSO$_4$ and concentrated reduced pressure. The residue was purified by PTLC (EtOAc/*n*-hexane, 20:80), and ester **12d** was obtained as a colorless oil (53.5 mg, 0.15 mmol, 85%): [α]$_D$ −31° (*c* 0.30, CHCl$_3$); IR (KBr) 3440, 2954, 2929, 2858, 1717, 1256, 1047, 860, 837, 775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 6.94 (t, 1H, $J = 6.4$ Hz, H-3), 5.36 (q, 1H, $J = 6.9$ Hz, H-9), 3.82 (d, 1H, $J = 9.1$ Hz, H-7), 3.79 (dt, 1H, $J = 7.3$, 3.7 Hz, H-5), 3.70 (s, 3H, Me-O), 2.40 (m, 1H, H-4b), 2.30 (m, 1H, H-4a), 1.83 (s, 3H, H-13), 1.75 (m, 1H, H-6), 1.57 (d, 3H, $J = 6.9$ Hz, H-10), 1.53 (s, 3H, H-11), 0.86 (s, 9H, (Me)$_3$CSi), 0.62 (d, 3H, $J = 6.9$ Hz, H-12), 0.06 (s, 3H, Me-Si), −0.03 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 168.4, 139.2, 136.3, 128.6, 123.3, 86.2, 74.1, 51.6, 41.0, 33.5, 25.8 (3C), 18.0, 13.0, 12.9, 12.6, 10.6, −4.4, −5.3.

(5*S*,6*S*,7*R*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-2,6,8-trimethyl-2,8-decadien-1,5-diol (13a). To a solution of LiAlH$_4$ (28.5 mg, 0.75 mmol) in THF (750 μL) at −78 °C was added ester **11a** (27.6 mg, 0.075 mmol) in THF (500 μL). The solution was stirred for 1 h at −78 °C and then warmed to 0 °C for 1 h. The reaction mixture was quenched with 1 M aqueous HCl (10 mL) and extracted with EtOAc (15 mL × 3). The combined organic layers were washed with saturated NaHCO$_3$ (10 mL) and dried over MgSO$_4$. Removal of the solvent gave a residue, which was purified by SIL-HPLC (EtOAc/*n*-hexane, 45:55), and protected triol **13a** was obtained as a colorless oil (2.2 mg, 0.0064 mmol, 8.6%): [α]$_D$ +14° (*c* 0.21, CHCl$_3$); IR (KBr) 3422, 2927, 1385, 1249, 1049, 870, 837,

775 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.53 (dt, 1H, $J = 6.4$, 1.4 Hz, H-3), 5.41 (q, 1H, $J = 6.9$ Hz, H-9), 4.12 (d, 1H, $J = 4.6$ Hz, H-7), 4.04 (s, 2H, H-1), 3.58 (dt, 1H, $J = 8.2$, 3.2 Hz, H-5), 2.25 (m, 1H, H-4b), 2.15 (m, 1H, H-4a), 1.76 (m, 1H, H-6), 1.69 (s, 3H, H-13), 1.61 (overlapping s and d, 6H, H-11, H-10), 0.89 (s, 9H, (Me)$_3$CSi), 0.80 (d, 3H, $J = 6.9$ Hz, H-12), 0.05 (s, 3H, Me-Si), −0.03 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 137.1, 126.9, 122.3, 121.5, 80.4, 73.2, 68.9, 42.7, 32.7, 25.9 (3C), 18.1, 14.0, 13.0, 12.9, 11.8, −4.6, −5.2.

(5*S*,6*R*,7*S*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-2,6,8-trimethyl-2,8-decadien-1,5-diol (13c). To a solution of **10c** (35.0 mg, 0.095 mmol) in THF (2.0 mL) at −78 °C was added 0.93 M DIBAL in *n*-hexane (300 μL, 0.28 mmol). After the reaction mixture was stirred for 1 h and warmed to 0 °C for 1 h, saturated aqueous Na$_2$SO$_4$ (10 mL) and EtOAc (10 mL) were added and the solution was stirred vigorously. After 10 min, anhydrous Na$_2$SO$_4$ (ca. 5 g) was added and the reaction mixture stirred vigorously for 30 min. The mixture was filtered through a pad of anhydrous Na$_2$SO$_4$ by vacuum filtration. The solvents were removed under reduced pressure. The residue was purified by SIL-HPLC (EtOAc/*n*-hexane, 45:55), and protected triol **13c** was obtained as a colorless oil (13.1 mg, 0.057 mmol, 60%): [α]$_D$ −17° (*c* 0.21, CHCl$_3$); IR (KBr) 3421, 2927, 1385, 1256, 1057, 870, 835, 773 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.43 (overlapping t and q, 2H, H-3, H-9), 4.02 (s, 2H, H-1), 3.97 (d, 1H, $J = 6.9$ Hz, H-7), 3.64 (ddd, 1H, $J = 6.4$, 6.4, 1.8 Hz, H-5), 2.30 (m, 1H, H-4b), 2.13 (m, 1H, H-4a), 1.68 (s, 3H, H-13), 1.62 (overlapping d and m, 4H, H-10, H-6), 1.52 (s, 6H, H-11), 0.91 (d, 3H, $J = 6.9$ Hz, H-12), 0.89 (s, 9H, (Me)$_3$CSi), 0.06 (s, 3H, Me-Si), −0.04 (s, 3H, Me-Si); ^{13}C NMR (CDCl$_3$) δ 137.0, 136.8, 122.2, 121.3, 81.9, 72.8, 68.7, 40.7, 33.6, 25.9 (3C), 18.1, 13.9, 12.9, 11.9, 7.7, −4.5, −5.1.

(5*S*,6*S*,7*S*,2*E*,8*E*)-7-(*tert*-Butyldimethylsilyloxy)-2,6,8-trimethyl-2,8-decadien-1,5-diol (13d). Using the method described for the preparation of **13c**, ester **12d** (42.5 mg, 0.12 mmol) in THF (2.0 mL) was treated with 1 M DIBAL in THF (570 μL, 0.57 mmol) and yielded protected triol **13d** as a colorless oil (35.5 mg). The resultant oil was used without purification in the subsequent reaction.

(5*S*,6*S*,7*R*,2*E*,8*E*)-2,6,8-Trimethyl-2,8-decadien-1,5,7-triol (3a). To a solution of **13a** (2.1 mg, 0.006 mmol) in MeOH (500 μL) was added pyridinium *p*-toluene sulfonate (PPTS) (9.5 mg, 0.038 mmol) overnight at room temperature. After concentration, removal of the salt by Waters Sep-Pak Vac 12 cm^3 Silica-2g gave a residue, which was purified by ODS-HPLC (MeCN/H$_2$O, 40:60). Triol **3a** was obtained as a colorless oil (1.3 mg, 0.0057 mmol, 95%): [α]$_D$ +3° (*c* 0.14, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 5.53 (overlapping t and q, 2H, H-3, H-9), 4.36 (brs, 1H, H-7), 4.05 (s, 2H, H-1), 3.70 (m, 1H, H-5), 2.35 (m, 2H, H-4), 1.77 (m, 1H, H-6), 1.71 (s, 3H, H-13), 1.65 (d, 3H, $J = 6.9$ Hz, H-10), 1.56 (s, 3H, H-11), 0.88 (d, 3H, $J = 7.1$ Hz, H-12); ^{13}C NMR (CDCl$_3$) δ 137.7, 136.1, 121.9, 118.8, 75.7, 74.9, 68.6, 39.6, 33.5, 14.0, 13.6, 13.0, 10.6.

(5*S*,6*S*,7*R*,2*E*,8*E*)-2,6,8-Trimethyl-2,8-decadien-1,5,7-triol (3b). To a solution of **10b** (35.8 mg, 0.097 mmol) in THF (1.0 mL) at −78 °C was added 1 M DIBAL in THF (2.4 mL, 2.4 mmol). After the reaction mixture was stirred for 30 min and warmed to 0 °C for 1 h, saturated aqueous Na$_2$SO$_4$ (10 mL) and EtOAc (10 mL) were added and the solution was stirred vigorously. After 10 min, anhydrous Na$_2$SO$_4$ (ca. 5 g) was added and the reaction mixture stirred vigorously for 30 min. The mixture was filtered through a pad of anhydrous Na$_2$SO$_4$ in a funnel. The solvents were removed under reduced pressure. The residue was purified by ODS-HPLC (MeCN/H$_2$O, 40:60), and triol **3b** was obtained as a colorless oil (11.0 mg, 0.048 mmol, 50%): [α]$_D$ −21° (*c* 0.31, CHCl$_3$); HRFABMS *m/z* 457.3521 (2M + H)$^+$ (for C$_{26}$H$_{49}$O$_6$, Δ −0.8 mmu); IR (KBr) 3390, 2918, 1418, 1383, 1009 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.49 (overlapping t and q, 2H, H-3, H-9), 3.98 (overlapping s and d, 3H, H-1, H-7), 3.87 (m, 1H, H-5), 2.33 (m, 1H, H-4b), 2.12 (m, 1H, H-4a), 1.85 (m, 1H, H-6), 1.68 (s, 3H, H-13), 1.62 (d, 3H, $J = 6.4$ Hz, H-10), 1.57 (s, 3H, H-11), 0.80 (d, 3H, $J = 6.9$ Hz, H-12),; ^{13}C NMR (CDCl$_3$) δ 137.3, 136.5, 122.6, 121.9, 80.9, 72.8, 68.6, 39.0, 31.7, 14.0, 13.0, 11.7, 11.1.

(5S,6R,7S,2E,8E)-2,6,8-Trimethyl-2,8-decadien-1,5,7-triol (3c). Using the method described for the preparation of **3a**, protected alcohol **13c** (9.1 mg, 0.027 mmol) in MeOH (1.0 mL) was treated with PPTS (20.0 mg 0.08 mmol) and yielded triol **3c** as a colorless oil (4.1 mg, 0.018 mmol, 68%): $[\alpha]_D$ −6° (*c* 0.40, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 5.54 (q, 1H, J = 6.9 Hz, H-9), 5.46 (t, 1H, J = 6.9 Hz, H-3), 4.18 (brs, 1H, H-7), 4.03 (s, 2H, H-1), 3.87 (ddd, 1H, J = 8.2, 5.5, 1.8 Hz, H-5), 2.35 (m, 1H, H-4b), 2.15 (m, 1H, H-4a), 1.69 (overlapping s and m, 4H, H-13, H-6), 1.64 (d, 3H, J = 6.9 Hz, H-10), 1.55 (s, 3H, H-11), 0.85 (d, 3H, J = 6.9 Hz, H-12); ^{13}C NMR (CDCl$_3$) δ 137.5, 136.1, 121.8, 119.1, 80.6, 75.4, 68.7, 38.8, 33.7, 14.0, 13.3, 13.0, 5.1; *anal.* calcd for C$_{13}$H$_{24}$O$_3$, C, 68.38; H, 10.59; found, C, 68.01; H, 10.44.

(5S,6S,7S,2E,8E)-2,6,8-Trimethyl-2,8-decadien-1,5,7-triol (3d). Using the method described for the preparation of **3a**, a mixture of **13d** (35.5 mg) in MeOH (2.0 mL) was treated with PPTS (75 mg 0.3 mmol) and yielded triol **3d** as a white solid (14.3 mg, 0.063 mmol, 55% from **12d**): mp 91−92 °C; $[\alpha]_D$ −23° (*c* 0.18, CHCl$_3$); HRFABMS m/z 457.3513 (2M + H)$^+$ (for C$_{26}$H$_{49}$O$_6$, Δ −1.7 mmu); IR (KBr) 3275, 2918, 1418, 1386, 1014 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.52 (t, 1H, J = 6.4 Hz, H-3), 5.43 (q, 1H, J = 6.0 Hz, H-9), 3.99 (s, 1H, H-1), 3.88 (d, 1H, J = 9.1 Hz, H-7), 3.67 (dt, 1H, J = 8.7, 3.2 Hz, H-5), 2.34 (m, 1H, H-4b), 2.18 (m, 1H, H-4a), 1.73 (m, 1H, H-6), 1.67 (s, 3H, H-13), 1.60 (overlapping s and d, 6H, H-11, H-10), 0.64 (d, 3H, J = 6.9 Hz, H-12); ^{13}C NMR (CDCl$_3$) δ 137.7, 136.6, 123.5, 121.5, 85.0, 76.8, 68.6, 40.2, 33.1, 14.0, 13.4, 13.0, 10.3.

Preparation of 3d from 1. To the solution of Kulokekahilide-2 (**1**, 0.3 mg in 1 mL of Et$_2$O) was added 25 μL of 1 M LiAlH$_4$ in Et$_2$O. After stirring for 30 min at room temperature, the reaction mixture was partitioned between H$_2$O and Et$_2$O. The organic layer was concentrated and then separated by ODS HPLC with 60% MeCN to yield **3d**: ^{1}H NMR (CD$_3$OD), see Figure 4.

MTPA Esters of 1. Kulokekahilide-2 (**1**, 0.3 mg each) was reacted with *R*- or *S*-MTPACl (10 μL) in 300 μL of CH$_2$Cl$_2$ containing 10 mg of DMAP. The reaction mixtures were partitioned with EtOAc/0.1 M NaHCO$_3$, and the EtOAc layers were washed with 0.1 M HCl and H$_2$O. The obtained EtOAc layers were evaporated and then separated by ODS HPLC [COSMOSIL 5C$_{18}$-AR II, MeCN/H$_2$O (7:3 and 8:2)] to yield *S*- and *R*-MTPA esters (**1a** and **1b**, respectively).

1a: ^{1}H NMR (CD$_3$CN) δ 6.585 (H-3), 2.521 (H-4a), 2.2076 (H-4b), 4.582 (H-5), 2.300 (H-6), 5.316 (H-7), 5.703 (H-9), 1.627 (H-10), 1.892 (H$_3$-11), 0.734 (H$_3$-12), 1.580 (H-13); FABMS m/z 1042 (M + H)$^+$.

1b: ^{1}H NMR (CD$_3$CN) δ 6.608 (H-3), 2.558 (H-4a), 2.276 (H-4b), 4.825 (H-5), 2.298 (H-6), 5.269 (H-7), 5.571 (H-9), 1.574 (H-10), 1.790 (H$_3$-11), 0.776 (H$_3$-12), 1.337 (H-13); FABMS m/z 1042 (M + H)$^+$.

Methanolysis of 1. Kulokekahilide-2 (**1**, 0.3 mg) was treated with 0.1 M MeONa (0.5 mL) overnight, then partitioned between H$_2$O and CHCl$_3$. The organic layer was concentrated and separated by ODS HPLC [COSMOSIL 5C$_{18}$-MS, MeOH/H$_2$O (8:2 and 19:1)] to yield fragment **4**.

Absolute Stereochemistry of Amino and Hydroxyl Acid Residues. A half portion of fragment **4** was hydrolyzed (6 M HCl, 105 °C, 18 h) and dried under N$_2$. The residue was dissolved in MeOH and separated on reversed-phase HPLC (Inertsil prep-ODS) using a gradient of MeCN/H$_2$O/TFA from 1:99:0.05 to 23:77:0.05 to yield three amino acids and Hica. Hica was analyzed by chiral HPLC [Chiralpak MA(+), MeCN/H$_2$O (15:85) with 2 mM CuSO$_4$], confirming D-Hica.

To each of the isolated amino acids were added 50 μL of 2.9 mM FDAA solution in acetone and 100 μL of 1 M NaHCO$_3$, followed by heating at 80 °C for 3 min. After being cooled to room temperature, the reaction mixtures were neutralized with 50 μL of 2 M HCl and diluted with 100 μL of MeCN/H$_2$O/TFA (50:50:0.05). These solutions were analyzed by reversed-phase HPLC [Inertsil ODS-2, MeCN/H$_2$O/TFA (25:75:0.05)] to furnish D- and L-Ala, *N*-Me-L-Phe, and L-Ile.

Hydrazinolysis of Fragment 4 from Kulokekahilide-2. The remaining half of **4** was added with 10 mg of dry Amberlite GC50, followed by 400 μL of anhydrous hydrazine. The reaction mixture was heated under argon for 60 h at 80 °C. After cooling to room temperature the reaction mixture was frozen and lyophilized, suspended in water (1.2 mL), filtered, and again frozen and freeze-dried. To the residue was added 50 μL of 2.9 mM FDAA solution in acetone and 100 μL of 1 M NaHCO$_3$, followed by heating at 80 °C for 3 min. After being cooled to room temperature, the reaction mixture was neutralized with 50 μL of 2 M HCl and diluted with 100 μL of MeCN/H$_2$O/TFA (50:50:0.05). This solution was analyzed by reversed-phase HPLC [Inertsil ODS-2, MeCN/H$_2$O/TFA (25:75:0.05)]. The only unmodified residue, Ala-2, was analyzed to show L-Ala.

Supporting Information Available: NMR spectra of kulokekahilide-2 (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Coval, S. J.; Schulte, G. R.; Matsumoto, G. K.; Roll, D. M.; Scheuer, P. J. *Tetrahedron Lett.* **1985**, *26*, 5359−5362. (b) Coval, S. J.; Scheuer, P. J. *J. Org. Chem.* **1985**, *50*, 3024−3025. (c) Reese, M. T.; Gulavita, N. K.; Nakao, Y.; Hamann, M. T.; Yoshida, W. Y.; Coval, S. J.; Scheuer, P. J. *J. Am. Chem. Soc.* **1996**, *118*, 11081−11084. (d) Nakao, Y.; Yoshida, W. Y.; Szabo, C. M.; Baker, B. J.; Scheuer, P. J. *J. Org. Chem.* **1998**, *63*, 3272−3280. (e) Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J. *Tetrahedron Lett.* **1996**, *37*, 8993−8996. (f) For the origin of the name "kulokekahilide-1", refer to: Kimura, J.; Takada, Y.; Inayoshi, T.; Nakao, Y.; Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem.* **2002**, *67*, 1760−1767.

(2) (a) Suenaga, K.; Mutou, T.; Shibata, T.; Itoh, T.; Kigoshi, H.; Yamada, K. *Tetrahedron Lett.* **1996**, *37*, 6771−6774. (b) Pettit, G. R.; Xu, J.-P.; Hogan, F.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 752−754. (c) Pettit, G. R.; Xu, J.-P.; Hogan, F.; Cerny, R. L. *Heterocycles* **1998**, *47*, 491−496.

(3) (a) Rodríguez, J.; Fernández, R.; Quiñoá, E.; Riguera, R.; Debitus, C.; Borchet, P. *Tetrahedron Lett.* **1994**, *35*, 9239−9242. (b) Fernández, R.; Rodríguez, J.; Quiñoá, E.; Riguera, R.; Muñoz, L.; Fernández-Suárez, M.; Debitus, C. *J. Am. Chem. Soc.* **1996**, *118*, 11635−11643.

(4) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.

(5) Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

(6) (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127−2129. (b) Pettit, G. R.; Burkett, D. D.; Barkóczy, J.; Breneman, G. L.; Pettit, W. E. *Synthesis* **1996**, 719−725.

(7) (a) Danda, H.; Hansen, M. M.; Heathcock, C. H. *J. Org. Chem.* **1990**, *55*, 173−181. (b) Raimundo B. C.; Heathcock, C. H. *Synlett* **1995**, 1213−1214.

(8) Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *22*, 3815−3818.

(9) Corey, E. J.; Trimble, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

(10) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815−3818.

(11) (a) Mukaiyama, T.; Banno, K.; Narasaka, K. *J. Am. Chem. Soc.* **1974**, *96*, 7503. (b) Evans, D. A.; Dart, M. J.; Duffy, J. L.; Yang, M. G. *J. Am. Chem. Soc.* **1996**, *118*, 4322−4343.

(12) Hoffman R. V.; Kim, H. *J. Org. Chem.* **1991**, *56*, 1014−1019.

(13) (a) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511−3515. (b) Rychnovsky S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945−948. (c) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, *31*, 7099−7100.

(14) Mitsunobu, O. *Synthesis* **1981**, 1−28.

(15) Pfitzinger K. E.; Moffat, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661−5670.

(16) Bloch, R.; Gilbert, L.; Girard, C. *Tetrahedron Lett.* **1988**, *29*, 1021−1024.

(17) Mutou, T.; Suenaga, K.; Fujita, T.; Itoh, T.; Takada, N.; Hayamizu, K.; Kigoshi, H.; Yamada, K. *Synlett* **1977**, 199−201.

(18) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(19) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(20) (a) Goetz, G.; Nakao, Y.; Scheuer, P. J. *J. Nat. Prod.* **1997**, *60*, 562−567. (b) Schroeder, W. A. *Methods Enzymol.* **1972**, *25*, 138−143.

(21) Takahashi, T.; Nagamiya, H.; Doi, T.; Griffiths, P. G.; Bray, A. M. *J. Comb. Chem.* **2003**, *5*, 414−428.