Mirabimide E, an Unusual *N*-Acylpyrrolinone from the Blue-Green Alga *Scytonema mirabile*: Structure Determination and Synthesis

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Received May 9, 1994®

Abstract: Mirabimide E, a solid tumor selective cytotoxin from the terrestrial blue-green alga *Scytonema mirabile* UH strain BY-8-1, possesses an unprecedented tetrachlorinated ethylene group and has been identified as (5*S*,2′*R*,3′*R*)-*N*-(*anti*-8′,8′,9′,9′-tetrachloro-3′-(carbamoyloxy)-2′-methyldecanoyl)-4-methoxy-5-methyl-3-pyrrolin-2-one. The total structure, including absolute stereochemistry, of this novel *N*-acylpyrrolinone was concluded from a combination of spectral and chemical studies, including stereoselective syntheses of three degradation products, viz. methyl (2*R*,3*R*)-*anti*-8,8,9,9-tetrachloro-3-hydroxy-2-methyldecanoate, (5*R*,6*R*)-*trans*-5-methyl-6-(5,5′,6,6′-tetrachloroheptyl)-1-oxa-3-azacyclohexane-2,4-dione, and (5*S*)-4-methoxy-5-methyl-3-pyrrolin-2-one, and the total synthesis of mirabimide E itself. The influence of the carbamate ester group on the chemical degradation and synthesis of mirabimide E is described.

Naturally-occurring *N*-acylpyrrolinones are associated with sponges of the genus *Dysidea*[2] and the marine blue-green alga *Lyngbya majuscula*.[3] Interestingly, a symbiotic blue-green alga, *Oscillatoria spongeliae*, is present in *D. herbacea* and appears to be responsible for the production of these compounds (e.g. dysidin) in this sponge.[4] We have found cytotoxic imides of this type in a terrestrial strain (BY-8-1) of *Scytonema mirabile* (Dillwyn) Bornet,[5] of which mirabimide E (**1**) is structurally and pharmacologically the most interesting since it possesses an unprecedented tetrachlorinated ethylene segment[6] and displays murine solid tumor selective cytotoxicity in the Corbett assay.[7] We report here the total structure determination and synthesis of **1**.

S. mirabile BY-8-1 was isolated from an algal sample collected on Mt. Tantalus, Oahu, HI,[8] and mass cultured in the laboratory. The strongly cytotoxic 7:3 EtOH/water extract of the freeze-dried cyanophyte[5] was subjected to rapid reverse-phase chromatography on C-18, and the fractions that were eluted with 1:1, 3:7, and 9:1 MeOH/water were combined and subjected to HPLC on C-18 with 2:1:1 MeCN/MeOH/water to give mirabimide E in 0.16% yield.

Gross Structure Determination. The FD and FAB (thioglycerol matrix) mass spectra of mirabimide E displayed 75:100:50:10:1 M^+ and MH^+ ion clusters at *m/z* 490/492/494/496/498 and 491/493/495/497/499, respectively, which indicated that **1** possessed four chlorine atoms. A high-resolution FABMS measurement suggested that the molecular formula was $C_{18}H_{26}Cl_4N_2O_5$ (+12.0-mmu error). When glycerol was used as the matrix, however, the FABMS failed to exhibit a protonated molecular ion cluster for **1** and instead showed a cluster of ion peaks at *m/z* 430/432/434/436/438 for a protonated decomposition product having the formula $C_{17}H_{24}Cl_4NO_3$ as shown by a high-resolution FABMS (–0.5-mmu error). A small ion cluster at *m/z* 430/432/434/436/438 was also observed in the FABMS of **1** using thioglycerol as the matrix. The elements of carbamic acid appeared to have been lost (by β-elimination) from **1**.

When a normal 1–2-s recycling delay was used for data acquisition, the ^{13}C NMR spectrum (Table 1) showed only 16 signals, i.e. four nonprotonated, four methine, four methylene, and four methyl carbon peaks. One of the methylene signals (26.6 ppm) was very broad ($w_{1/2}$ = 40 Hz) and low in intensity (one-half of the height of the carbonyl signals and one-seventh of the height of the other CH_2 signals). Eighteen carbon signals could be seen only when a 5-s or longer recycling delay was used. The two additional carbon signals appeared at 95.8 and 100.3 ppm and were small and quite broad.

Although most of the proton signals were well-defined, the ^1H NMR spectrum (see the supplementary material) showed three

* Abstract published in *Advance ACS Abstracts*, August 1, 1994.

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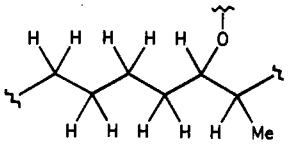
Table 1. NMR Data for Mirabimide E in Acetone-d_6 (7.5 mg/mL)[a]

carbon position	δ_C	HMQC	HMBC
	Acyl Unit		
1′	174.1 (s)		5.03, 4.18, 1.08
2′	43.2 (d)	4.18 (dq)	5.03, 1.85, 1.60, 1.08
3′	75.5 (d)	5.03 (ddd)	4.18, 1.85, 1.60, 1.52, 1.48, 1.08
4′	32.3 (t)	1.85, 1.60	4.18, 1.88, 1.80, 1.52, 1.48
5′	24.7 (t)	1.52, 1.48	5.03, 1.88, 1.85, 1.80, 1.60
6′	26.6 (t)	1.88, 1.80	1.52, 1.48
7′	42.3 (t)	2.55	1.88, 1.80, 1.52, 1.48
8′	100.3 (s)		
9′	95.8 (s)		
10′	33.7 (q)	2.47 (br s)	
Me on C2′	13.6 (q)	1.08 (d)	5.03, 4.18
NH_2CO_2 on C3′	156.9 (s)		5.03
	Pyrrolinone Unit		
2	169.8 (s)		5.17, 4.54
3	93.6 (d)	5.17 (s)	4.54
4	181.3 (s)		4.54, 3.92, 1.38
5	56.1 (d)	4.54 (q)	5.17, 1.38
OMe on C4	59.5 (q)	3.92 (s)	
Me on C5	17.0 (q)	1.38 (d)	4.54

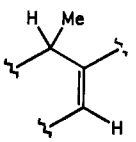
[a] No HMQC and HMBC cross peaks were observed to the NH_2 signal at 5.70 ppm.

broad signals, viz. an exchangeable 2H peak ($w_{1/2}$ = 55 Hz) at 5.70 ppm which was assigned to an NH_2 group, an unresolved 2H signal ($w_{1/2}$ = 30 Hz) at 2.55 ppm attributed to a CH_2 group, and a relatively broad 3H singlet ($w_{1/2}$ = 5 Hz) at 2.47 ppm for a methyl group.

A COSY experiment allowed us to construct two partial structures a and b which accounted for most of the protons. In



a



b

the b unit, the two methine protons showed allylic coupling, but since NOE effects were not observed between these methine protons or between the olefinic methine and methyl protons, the proton of the olefinic methine and the carbon of the allylic methine had to be *trans* to each other. The remaining three groups of protons (NH_2, OCH_3, and the CH_3 showing the broad proton signal) could not be attached to units a and b from COSY data.

The information from HMQC and HMBC experiments (Table 1), however, permitted a further expansion of these partial structures. In unit a, the methyl-bearing methine could be attached to a carbonyl (174.1 ppm) and the oxygen on the methine to a second carbonyl (156.9 ppm). In unit b, the olefinic methine (93.6 ppm) could be connected to a third carbonyl (169.8 ppm) and both methines and the methoxyl to an olefinic carbon (181.3 ppm). The HMBC experiment, however, did not allow connection of a to b. Although other correlations were observed, an unambiguous structure could not be deduced. No correlations were present to indicate how to attach the remaining methyl and NH_2 groups and a C_2Cl_4 segment unequivocally to the expanded a and b units. The chemical shift of the carbonyl at 156.9 ppm, however, did suggest that a carbamate group was attached to the methine in a.

The gross structure problem was solved by NMR analysis of a sample of mirabimide E that had been uniformly enriched with ^{13}C to 80% and ^{15}N to 90%.[9] In the ^{13}C NMR spectrum, the carbon at 156.9 ppm was found to be coupled only to nitrogen (J_{CN} = 26.1 Hz), confirming the presence of the carbamate ester group, and for the first time, the two broad nonprotonated carbon signals at 95.8 and 100.3 ppm could be clearly seen with a 1.5-s recycling delay. The results of an INADEQUATE experiment allowed us to complete the gross structure of **1**. The methylene carbon at 42.3 ppm (C-7′) could be connected first to the quaternary dichloro-bearing carbon at 100.3 ppm (C-8′), then to the quaternary dichloro-bearing carbon at 95.8 ppm (C-9′), and finally to the methyl carbon at 33.7 ppm (C-10′). Moreover, the olefinic carbon at 181.3 ppm (C-3) could be attached to the olefinic methine at 93.6 ppm (C-4) and then to the carbonyl carbon at 169.8 ppm (C-5). The carbonyl carbons at 169.8 and 174.1 ppm and the methine carbon at 56.1 ppm (C-2) were all coupled ($^1J_{C,N}$ = 3–5 Hz) to the pyrrolinone nitrogen.

Only after the gross structure of **1** had been determined were we able to explain why the proton signals at 2.55 and 2.47 ppm and the carbon signal at 26.6 ppm were broad. The signals for these nuclei sharpened dramatically when the spectrum was observed at 50 °C, indicating that the broadness was due to restricted rotation about the C–C bonds bearing the geminal dichloro groups. The nuclei that exhibited the broad signals at room temperature, viz. 10′-H_3, 7′-H_2, and C-6′, were three bonds away from chlorine, but the signals for C-10′ (32.8 ppm) and C-7′ (41.4 ppm), which were also three bonds away from chlorine, were not broad. At −50 °C, the 10′-H_3 signal split into three sharp singlets at 2.40, 2.43, and 2.55 ppm (relative intensities 1:1:2, respectively). The peak at 2.55 ppm had to be assigned to H_3-10′ for all rotamers having C-10′ *anti* to C-7′ (c); in these rotamers, the protons on C-10′ are deshielded by two *syn* chloro groups on C-8′. The peaks at 2.40 and 2.43 ppm, however, could only be attributed to 10′-H_3 in two types of rotamers where C-10′ and C-7′ are *syn* (d) and where the protons on C-10′ are deshielded by only one *syn* chloro group on C-8′. In one type of d rotamer, C-6′ is *anti* to C-9′ (d_1), whereas in the other one, C-6′ is *syn* to C-9′ (d_2). The 10′-H_3 signals for these *syn* rotamers have different chemical shifts because in the d_1 rotamer (2.43 ppm) the protons on C-10′ are deshielded (van der Waals type) by the protons on C-7′ whereas in the d_2 rotamer (2.40 ppm) they are not.

Stereochemistry. It was not possible to deduce the relative and absolute stereochemistries of the three asymmetric carbons in **1** by spectral analysis alone. The 8.7-Hz coupling between 2′-H and 3′-H in the ^1H NMR spectrum suggested that the substitutents on C-2′ and C-3′ of the *N*-acyl unit were *anti*; however, NOE experiments failed to reveal the relative stereochemistry between the *N*-acyl and pyrrolinone units. In order to solve the absolute stereochemistry of the *N*-acyl moiety, we needed to generate an unepimerized C-3′ alcohol so that Mosher analysis[10] could be performed. Unfortunately, all attempts to degrade **1** to optically active *anti*-8,8,9,9-tetrachloro-3-hydroxy-2-methyldecanoic acid and 4-methoxy-5-methyl-3-pyrrolin-2-one by simple acid or base hydrolysis were unsuccessful. Leumieux oxidation[11] of **1** prior to acid hydrolysis, however, led to alanine, which after Fischer esterification with isopropyl alcohol and acylation with trifluoroacetic anhydride could be identified as the isopropyl ester of *N*-trifluoroacetyl-L-alanine by chiral GCMS. This meant that the absolute configuration of C-5 in the pyrrolinone ring of **1** was *S*.

Acid hydrolysis of **1** with 5.5 N HCl produced a complex mixture of products, with some of them still bearing the carbamate group. Interestingly, one of the compounds was identified as

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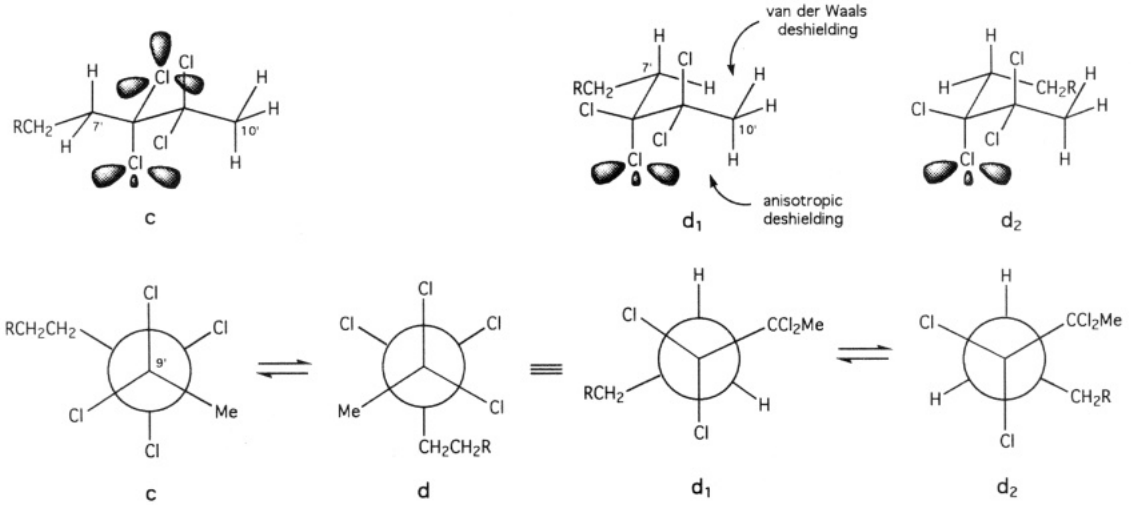
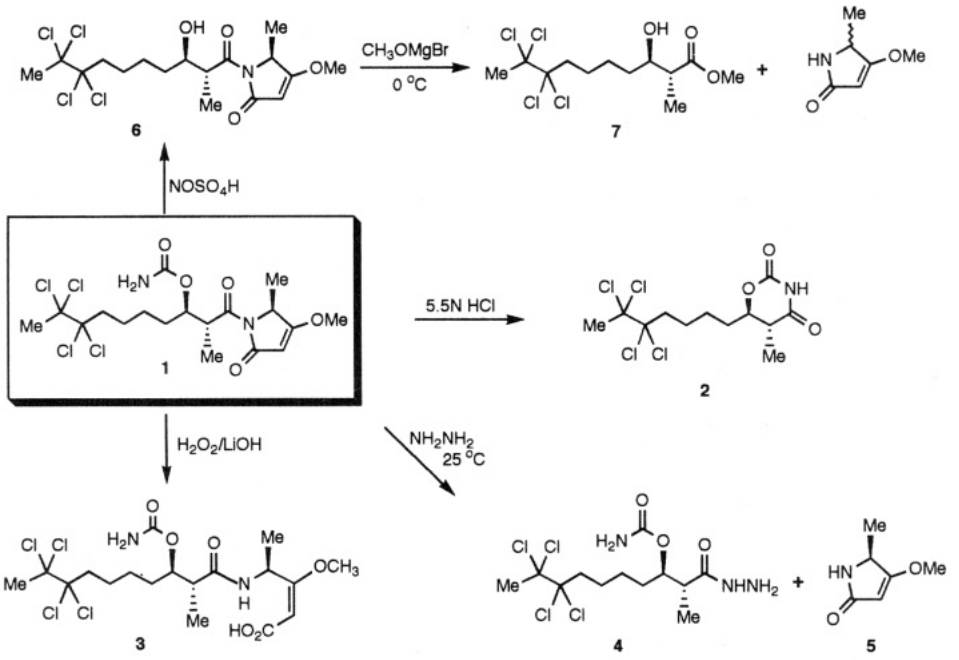
(11) Leumieux, R. U.; von Rudloff, E. *Can. J. Chem.* **1955**, *33*, 1701–9.

Chart I



Scheme 1. Chemical Degradation of Mirabimide E under Several Reaction Conditions



trans-5-methyl-6-(5,5′,6,6′-tetrachloroheptyl)-1-oxa-3-azacyclohexane-2,4-dione (**2**) (Scheme 1). We had concluded from ^{1}H NMR data that 5-H and 6-H in **2** were pseudoaxial and *trans* to each other, as $J_{5,6}$ was 10.5 Hz. This again indicated that the substitutents on C-2′ and C-3′ of **1** were *anti*; however, we were not able to deduce its absolute stereochemistry.

Treatment of **1** with lithium hydroperoxide[12] also did not result in cleavage of the *N*-acyl bond. To our surprise, the N1–C2 bond of the pyrrolinone ring ruptured instead, resulting in the acyclic compound **3**.[13] On prolonged contact with lithium hydroperoxide (25 °C, 1 week), a small amount of 8,8,9,9-tetrachloro-2-methyldec-2-enoic acid could be detected in the reaction mixture, probably due to the presence of LiOH in the reaction mixture. The *O*-carbamoyl bond was not hydrolyzed under these conditions.

Hydrazinolysis of **1** with hydrazine in methanol at room temperature afforded acyl hydrazide **4** and optically pure pyrrolinone **5** in >90% yield. No epimerization was noted in **4** or **5** (*vide infra*) during the reaction as shown by NMR analysis. Unfortunately, all attempts to degrade the acyl hydrazide, e.g. via the corresponding acyl azide or by hydrolysis with aqueous $Cu(OH)_2$,[14] to the corresponding carboxylic acid failed.

Cleavage of the *O*-carbamoyl bond in **1** and formation of alcohol **6** with retention of configuration at C-3′ were accomplished by nitrosation.[15] Reaction of **1** in chloroform with 23% nitrosylsulfuric acid in 1:4 sulfuric acid-d_2/D_2O at 0 °C led to decarbamoylation and the generation of a nitrite ester. Methanolysis of the nitrite ester with 10% MeOH/chloroform in the presence of silica gel gave **6** in an overall yield of 74%. No epimerization occurred at either C-2′ or C-3′ during this degradation. Presumably nitrosation of the carbamate amino group had led to a diazonium intermediate which had then successively decomposed to a monoester of carbonic acid and decarboxylated to alcohol **6**. In the presence of the excess nitrosylsulfuric acid, however, **6** was converted to a nitrite ester.[16] Unfortunately, we were not able to solve the absolute stereo-

(12) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141–4.

(13) It is noteworthy that LiOOH displays endocyclic N–CO cleavage regioselectivity with pyrrolinone-derived imides (e.g. **1** and **3**) compared to exocyclic N–CO cleavage regioselectivity with oxazolidone-derived carboximides.[12]

(14) Tsuji, J.; Nagashima, T.; Qui, N. T.; Takayanagi, H. *Tetrahedron* **1980**, *36*, 1311–5.

(15) Nitrosation with other reagents such as nitrous acid or nitrosonium tetrafluoroborate led to either no reaction or undesired products.

(16) The same nitrite ester was formed when **3** was treated with nitrosylsulfuric acid under identical reaction conditions.

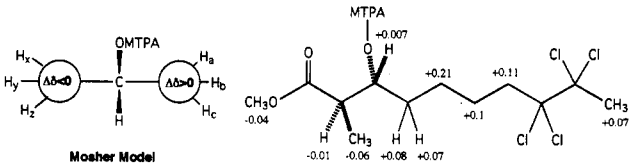


Figure 1. $\Delta\delta$ values ($\delta_S - \delta_R$) in ppm obtained at 500 MHz.

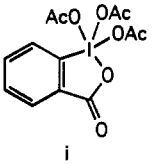
chemistry at C-3′ in alcohol **6** by Mosher analysis because the MTPA esters could not be formed in satisfactory yield.

Treatment of imide **6** with lithium hydroperoxide (25 °C, 36 h) again led to undesired endocyclic cleavage, similar to that noted above for the conversion of **1** to **3**.[13] Alternatively, methanolysis of **6** with 2 equiv of 0.08 M magnesium methoxide solution in methanol[17] at 0 °C for 10 h under a nitrogen atmosphere gave the desired exocyclic cleavage product, methyl *anti*-8,8,9,9-tetrachloro-3-hydroxy-2-methyldecanoate (**7**), in 94% yield. 4-Methoxy-5-methyl-3-pyrrolin-2-one was also formed in high yield; however, under the stronger basic conditions of the methanolysis, the pyrrolinone had completely racemized. The ^{1}H NMR spectrum of **7** showed a coupling of 7.0 Hz between 2-H and 3-H, which was consistent with $J_{2,3}$ values observed for other methyl *anti*-β-hydroxy-α-methylalkanoates.[18] Moreover, **7** was levorotatory in chloroform solution, $[\alpha]_D$ −2.4°, strongly suggesting that its absolute stereochemistry was 2*R*,3*R*.[18] The absolute stereochemistry of **7** was rigorously confirmed by the modified Mosher method.[10b] The *O*-(*R*)- and *O*-(*S*)-MTPA (2-methoxy-2-(trifluoromethyl)-2-phenylacetyl) derivatives of **7** were prepared, and $\Delta\delta$ values (δ_S–δ_R) were determined at 500 MHz for all of the protons. Negative $\Delta\delta$ values were found for protons on the C1–C2 side of the MTPA plane, whereas positive values were found for protons on the C4–C10 side (Figure 1). This meant that C-3 did indeed have the *R* configuration and, furthermore, that the absolute stereochemistry of **7** was therefore 2*R*,3*R*, and by extension, the *N*-acyl group in mirabimide E was 2′*R*,3′*R* as shown in **1**.

Synthesis. Total synthesis of mirabimide E was carried out to unambiguously establish the absolute stereochemistry of **1** but, more importantly, to procure adequate amounts of the natural product for *in vivo* antitumor evaluation. This endeavor also allowed us to synthesize degradation products **2**, **5**, and **7**, since an N-acylation coupling of the pyrrolinone and tetrachlorodecanoic acid segments appeared to be the most obvious route to **1**, with the carbamate group being introduced prior to or after the N-acylation.

Pyrrolinone **5** was produced as shown in Scheme 2. The methyl ester of *N*-(carbethoxyacetyl)-L-alanine (**8**) was cyclized to (5*S*)-3-carbethoxy-5-methylpyrrolidine-2,4-dione (**9**) in the presence of sodium hydride.[19] Hydrolysis and decarboxylation gave (5*S*)-5-methylpyrrolidine-2,4-dione[19] (**10**), which could be methylated with diazomethane (or dimethyl sulfate) to a 10:1 mixture of **5** and (5*S*)-2-methoxy-5-methylpyrrolin-4-one. Synthetic **5** exhibited a dextrorotatory optical rotation and a negative Cotton effect at 217 nm in its CD spectrum, as did the hydrazinolysis product of **1**; however, significant racemization occurred during the conversion of **8** to **5**. Whereas the optical purity (enantiomeric excess) of the degradation product was >95%, the optical purity of the synthetic **5** was only 52% as determined from NMR analysis (integration) of the methoxy signal using the chiral shift reagent [Eu(hfc)$_3$].[20]

To synthesize **7** (Scheme 3), 2-heptyn-1-ol (**11**) was first converted to 6-heptyn-1-ol (**12**) using a procedure developed by Abrams[21] for the base-catalyzed isomerization of internal alkynes to terminal alkynes with potassium (2-aminoethyl)amide. Alcohol **12** was protected as the benzoate ester **13**, and this alkyne was then treated with LDA in THF and methylated with iodomethane to 6-octyn-1-yl benzoate (**14**). Introduction of the four vicinal chlorines onto the alkyne proved to be quite difficult and required prolonged reaction times. Exposure of **14** to sulfuryl chloride in refluxing benzene led rapidly to a 6,7-dichlorooct-6-en-1-yl benzoate intermediate which was slowly chlorinated to the desired 6,6,7,7-tetrachloro compound **15** over the next 4 days, but only with frequent replenishment of the chlorinating reagent. Base hydrolysis of **15** removed the protecting group from **15** to give 6,6,7,7-tetrachloro-1-octanol (**16**). Oxidation of **16** with Dess–Martin reagent[22] (i, periodinane 12-I-5) produced aldehyde **17**.



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Using an enantioselective synthesis developed by Gennari,[23] an aldol adduct was obtained by condensing **17** with the (*E*)-silylketene acetal **18**, derived from (1*S*,2*R*)-*N*-methylephedrinyl propionate, in the presence of $TiCl_4$. NMR analysis indicated that the product was an 85:15 mixture of *anti*/*syn* diastereomers. Chromatographic separation and subsequent saponification of the major *anti* diastereomer (**19**) afforded (2*R*,3*R*)-*anti*-8,8,9,9-tetrachloro-3-hydroxy-2-methyldecanoic acid (**20**) as a white solid, mp 125–127 °C and $[\alpha]_D$ +2.2° (*c* 0.91, $CHCl_3$), after recrystallization from dichloromethane and petroleum ether. Esterification with diazomethane gave the methyl ester **7**, which was identical in all respects, including levorotatory optical rotation[18] ($[\alpha]_D$ −2.2°, 92% ee based on NMR analysis of the Mosher esters[10]), with the degradation product (*vide supra*). The overall yield of **20** from 2-heptyn-1-ol was 11%.

The coupling of the carbamoylated decanoic acid segment with pyrrolinone **5** was attempted first (Scheme 4). Treatment of **20** with trichloroacetyl isocyanate followed by mild base hydrolysis[24] ($NaHCO_3$/50% MeOH) of the imide intermediate led to carbamate **21**. The carboxylic acid in **21** was then activated by converting it to an ester (**22**) with *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (HONb)[25] in the presence of dicyclohexylcarbodiimide (DCC). Attempts to form mirabimide E by N-acylation of the sodium salt of (5*S*)-4-methoxy-5-methyl-3-pyrrolin-2-one (**5**) with **22**, however, failed. Under the reaction conditions, **22** underwent a faster intramolecular base-catalyzed cyclization to **2**, a lactim which proved to be identical with the minor acid hydrolysis product obtained from **1**.

Since the presence of the carbamate group had prevented the desired imide formation between the decanoic acid and the pyrrolinone from taking place, this functionality was introduced after imide formation (Scheme 5). The hydroxyl group was first protected by treating **20** with *tert*-butyldimethylsilyl chloride ((TBS)Cl) in the presence of imidazole in dimethyl formamide, a reaction which also resulted in derivatization of the carboxylic acid group (**23**).[26] Selective hydrolysis[27] of **23** with potassium carbonate in aqueous methanolic THF, however, yielded car-

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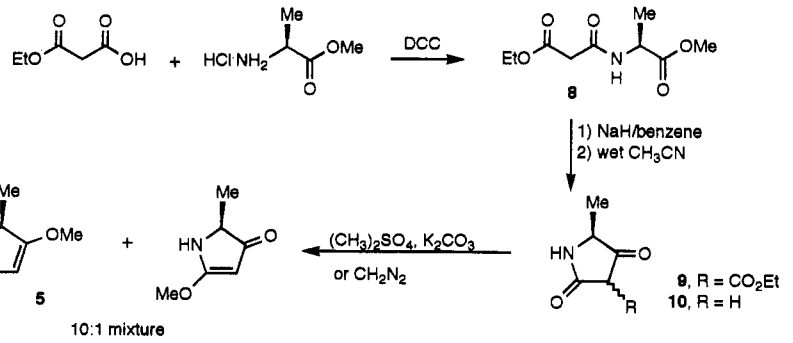
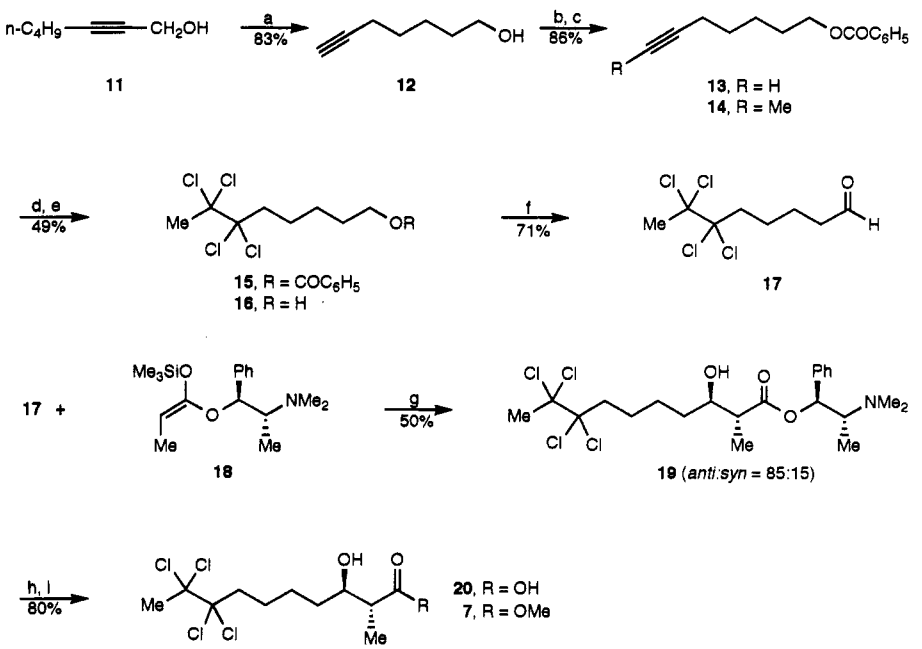
Scheme 2

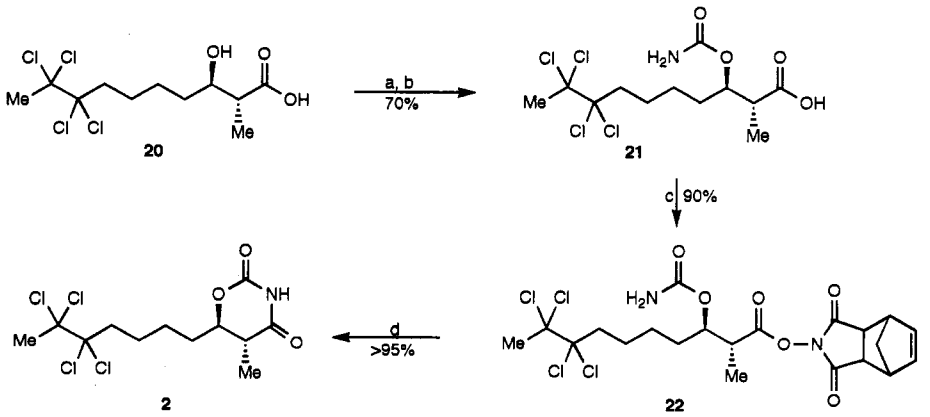


Scheme 3[a]



[a] (a) $H_2NCH_2CH_2NH_2$, Li, *t*-BuOK, 25 °C; 1 N HCl. (b) Benzoyl chloride, CH_2Cl_2, 0 °C. (c) LDA, CH_3I, THF, −78 °C. (d) SO_2Cl_2, benzene, 80 °C. (e) NaOH, 75% MeOH, 25 °C. (f) Periodinane (i), CH_2Cl_2, 25 °C. (g) $TiCl_4$, CH_2Cl_2, −78 °C. (h) NaOH, 75% MeOH, 25 °C. (i) CH_2N_2, Et_2O, 25 °C.

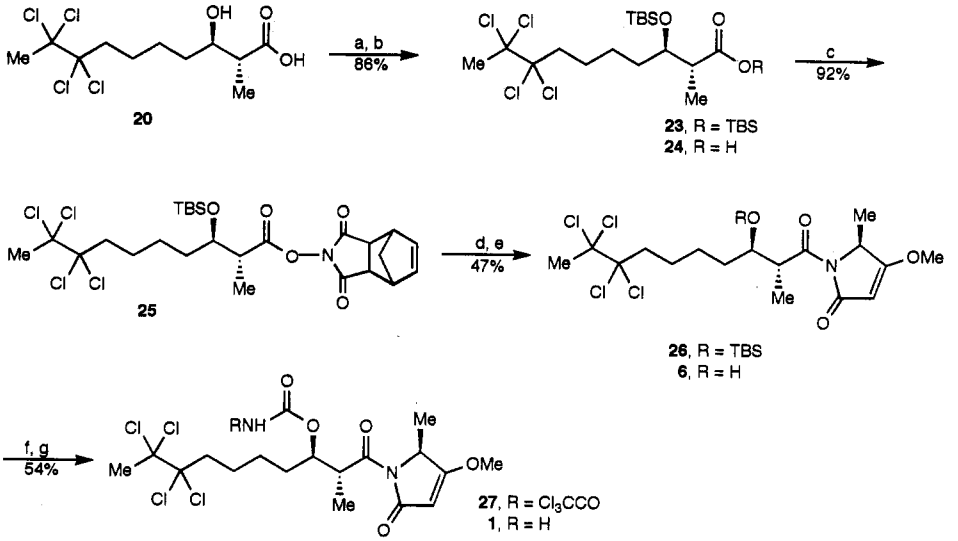
Scheme 4[a]



[a] (a) $Cl_3CCO—N=C=O$, $CHCl_3$, 0–25 °C. (b) $NaHCO_3$, 50% MeOH or silica gel, 10% MeOH/$CHCl_3$. (c) HONb, DCC, THF, 25 °C. (d) **5**, NaH, THF, 25 °C; **22**.

boxylic acid **24**, which was activated with HONb[25] (*vide supra*) to give ester **25**. Subsequent condensation of **25** and the sodium amide of **5**, the latter having an optical purity of 52% ee, led to (5*S*,2′*R*,3′*R*)-imide **26** in 49% yield. The undesired (5*R*,2′*R*,3′*R*)-imide, which was not fully characterized, could be separated from **26** by silica gel chromatography. Further racemization of **5** during this coupling reaction appeared to be negligible. The silyl protecting group was removed by treating **26** with a 15% solution of 40% hydrofluoric acid in CH_3CN (25 °C, 45 min, 95%), resulting in compound **6**, which was identical (IR, NMR, and TLC) with the decarbamoylation product of **1**. Finally, carbamoylation was carried out by first treating **6** with trichloroacetyl isocyanate and then removing the trichloroacetyl group from the resulting imide **27** by chromatography over silica gel[24] to give **1** in 54% yield after HPLC separation (20% overall yield from **20**). Synthetic mirabimide E was found to be identical in all respects

Scheme 5



[a] (a) (TBS)Cl, imidazole, DMF, 25 °C. (b) K_2CO_3, MeOH/THF/H_2O (3:1:1), 25 °C; $KHSO_4$(aq). (c) HONb, DCC, CH_2Cl_2, 25 °C. (d) **5**, NaH, THF; **25**, THF, 25 °C. (e) 15% HF (40%) in CH_3CN, 25 °C. (f) $Cl_3CCON{=}C{=}O$, $CHCl_3$, 25 °C. (g) $CHCl_3$/MeOH (9:1) over silica gel, 25 °C.

(^{1}H and ^{13}C NMR, IR, HPLC t_R, and optical rotation) with the natural product.

Biosynthesis. The tetrachloroethylene segment in mirabimide E is very unusual and hitherto has not been reported in a natural product.[6] When one speculates about the possible origin of such a unit, the chlorination of an alkyne precursor by a haloperoxidase is an attractive possibility. Curiously no other lipophilic chlorine-containing compounds could be found in the alga, even as trace constituents. When *S. mirabile* was grown in a medium containing [^{36}Cl]chloride, mirabimide E was the only radiochlorine-containing organic compound detected in the lipophilic extract of the alga by HPLC using a continuous radioactivity detector.

The structure and oxygenation pattern of the *N*-acyl subunit in **1** suggested a normal polyketide origin for the carbon skeleton derived from five acetate units and a methyl group from the C_1 pool. This was rigorously established by two feeding experiments in which sodium [1,2-$^{13}C_2$]acetate and [*methyl*-^{13}C]-L-methionine were administered independently to cultures of *S. mirabile* BY-8-1. The **1** isolated from the culture to which the labeled acetate had been administered showed enrichment in the signals assigned to carbons C-1′ to C-10′. The specific incorporation was approximately 0.6% above natural abundance. Satellites were readily discernible in the signals due to carbons C-1′ to C-7′ and C-10′, and the sizes of the $^1J_{C,C}$ coupling constants were in agreement with the incorporation of five intact acetate units into the decanoic acid. Satellites were also observed in the signals assigned to C-4 and C-5 of the pyrrolinone unit, strongly suggesting that the biosynthesis of this moiety involves the condensation of an acetate with L-alanine. In the feeding experiment with [*methyl*-^{13}C]-L-methionine, the **1** isolated from this incubation showed two strongly and equally enhanced signals (specific incorporation 4% above natural abundance) in its ^{13}C NMR spectrum for the carbons of the methyl group on C-3′ and the methoxy group on C-4.

Studies on the biosynthesis of the tetrachloroethylene segment are planned, if and when the cyanophyte can be induced to produce **1** again.[28]

Biological Activity. Of the 32 cytotoxins, viz. mirabimides A-D[5a] and E, tolytoxin and three other scytophycin-type compounds,[5b] tantazoles A, B, F, and I and ten other analogs,[5c,e,f] didehydromirabazole A and mirabazoles B and C,[5d,e] and mirabilenes A-F,[5g] that have been isolated from *S. mirabile* BY-8-1, mirabimide E[29] was one of only five compounds showing solid tumor selectivity in the Corbett assay,[7b] a soft agar disk diffusion assay modeled after the one commonly used in antifungal and antibacterial testing. In this assay, **1** was tested against four different cell types, viz. a murine leukemia (L1210), a murine solid tumor (colon adenocarcinoma 38), a human solid tumor (colon CX-1), and a low malignacy fibroblast (LML), and showed zones of inhibition of 300–430, 800, 330, and 400 zone units (200 zone units = 6 mm), respectively, at 5 μg/disk. Since **1** showed a zone of inhibition against the murine solid tumor cell line which was 370 zone units, i.e. 11 mm, larger than the zone of inhibition for the murine lymphocytic leukemia cell line, mirabimide E was concluded to possess solid tumor selective cytotoxicity and therefore became a high priority candidate for *in vivo* evaluation against solid tumors. In the Corbett assay, agents that exhibit a 250 or greater zone unit differential against one or more solid tumor cell lines versus the leukemia are considered to be solid tumor selective.[7]

Since we were not able to produce adequate amounts of **1** from the cultured alga for the *in vivo* studies,[28] a minimum of 1 g of **1** is being synthesized using the procedure described in this paper. The details of the *in vivo* antitumor activity and the mode of action will be published elsewhere.

Experimental Section

Spectral Analysis. NMR spectra were determined in acetone-d_6 or $CDCl_3$ on 11.75- and 7.05-T instruments operating at 500 and 300 MHz for ^{1}H and 125 and 75 MHz for ^{13}C, respectively. ^{1}H chemical shifts are referenced to the signal for the residual acetone-d_5 (2.04 ppm) or $CHCl_3$ (7.24 ppm); ^{13}C chemical shifts are referenced to the methyl-d_3 signal of acetone-d_6 (29.8 ppm) or to the $CDCl_3$ signal. Homonuclear ^{1}H NOEs were obtained by difference NOE experiments using a 3-s irradiation period. Heteronuclear ^{1}H–^{13}C connectivities were determined by HMQC[30] and HMBC[31] experiments.

Culture Conditions. *Scytonema mirabile* (Dillwyn) Bornet, designated strain number BY-8-1, was isolated from an algal sample collected from a shingled roof of an abandoned home on the slopes of Mt. Tantalus, Oahu, HI, and cultured as described elsewhere.[8]

Isolation of Mirabimide E (1). The freeze-dried alga (1.7 g) was extracted with 0.5 L of 7:3 EtOH/water for 24 h. The extract (388 mg)

(28) After many passages (>20) of the *S. mirabile* BY-8-1 culture used in the studies described in this paper, the strain no longer elaborated **1**, nor did it produce the other cytotoxins that have been found[5] in this cyanophyte. Since the summer of 1991, we have not been able to recultivate the alga from frozen stocks and produce adequate amounts of **1** for testing.

(29) Cytotoxicity IC_{50}s against KB (a human nasopharyngeal carcinoma cell line) and LoVo (a human colon adenocarcinoma cell line) are approximately 0.5 and 1 μg/mL, respectively.

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was flash chromatographed on a RP-18 column (40 mL of YMC-GEL, ODS 120A). The chromatogram was developed with 100 mL each of the following solvents: 4:1, 1:1, 1:3, and 1:9 H_2O/MeOH mixtures, MeOH, and EtOAc. Six fractions (100 mL) were collected. Fractions 2–4 were combined and evaporated to give 65 mg of a gummy residue. This material in 250 mL of MeOH was subjected to HPLC on a 30-cm × 20-mm RP-18 column (YMC AM-343-5 ODS, 120A) with 2:1:1 MeCN/MeOH/water at a flow rate of 6 mL/min to give 2.8 mg (0.16% yield) of mirabimide E (t_R 33.8 min), $[\alpha]_D$ +6.5° ($CHCl_3$, *c* 0.9). FDMS: *m/z* 490/492/494/496/498 (75:100:50:10:1 M^+ ion cluster). FABMS (thioglycerol): *m/z* 491/493/495/497/499 (75:100:50:10:1 MH^+ ion cluster), 430/432/434/436/438 (75:100:50:10:1 ion cluster for $[MH - NH_2CO_2H]^+$). High-resolution FABMS: *m/z* 491.0802 ($C_{18}H_{27}Cl_4N_2O_5$, Δ–12.8 mmu), 430.0505 ($C_{17}H_{24}Cl_4NO_3$, Δ0.5 mmu). IR ($CHCl_3$): ν_{max} 3546, 3432, 3022, 2945, 1724, 1686, 1582, 1380, 1322 cm^{-1}. ^1H NMR ($CDCl_3$): pyrrolinone unit δ 5.02 (s, 3-H), 3.85 (s, 4-OMe), 4.59 (q, *J* = 6.6 Hz, 5-H), 1.44 (d, *J* = 6.6 Hz, 5-Me); *N*-acyl unit 4.18 (dq, *J* = 8.1 and 6.9 Hz, 2′-H), 1.17 (d, *J* = 6.9 Hz, 2′-Me), 5.11 (td, *J* = 8.1 and 3.3 Hz, 3′-H), 4.52 (br, 3′-$OCONH_2$), 1.45–1.90 (m, 4′-H_2, 5′-H_2, and 6′-H_2), 2.47–2.57 (br m, 7′-H_2), 2.45 (br s, 10′-H_3). See Table 1 for NMR data in acetone-d_6.

Uniform Enrichment of 1. *S. mirabile* BY-8-1 was grown in a 10-L glass bottle on 5.3 g of $NaH^{13}CO_3$ (99 atom %) and 4.0 g of $Na^{15}NO_3$ (99 atom %) as previously described,[8,9] except that the aeration rate was 0.1 L/min. After 38 days, the 8-L culture was harvested by filtration and the alga lyophilized to give 1.30 g of dried cells. Workup resulted in the isolation of 2 mg of labeled **1**; inspection of its proton-noise-decoupled ^{13}C NMR spectrum in acetone-d_6 indicated uniform enrichment in ^{13}C to 82% and ^{15}N to >90%: δ 181.3 (ddd, *J* = 74.4, 47.2, and 7.5 Hz, C-4), 174.1 (ddm, J_{CC} = 69.7 and J_{CN} = 7 Hz, C-1′), 169.8 (ddm, J_{CC} = 62 and J_{CN} = 7 Hz, C-2), 156.9 (d, J_{CN} = 26.1 Hz, NH_2CO_2 on C-3′), 100.3 (br m, C-8′), 95.8 (br m, C-9′), 93.6 (t, *J* = 72.2 Hz, C-3), 75.5 (t, *J* = 39.2 Hz, C-3′), 59.5 (s, OMe), 56.1 (br ddm, C-5), 43.2 (m, C-2′), 42.3 (td, *J* = 37.2 and 2.2 Hz, C-7′), 33.7 (d, *J* = 41.4 Hz, C-10′), 32.3 (t, *J* = 37.0 Hz, C-4′), 26.6 (br m, C-6′), 24.7 (t, *J* = 34.5 Hz, C-5′), 17.0 (d, *J* = 36.2 Hz, Me on C-5), 13.6 (d, *J* = 34.2 Hz, Me on C-2′).

Biosynthetic Feeding Experiments. A 1-g amount of a 2:3 mixture of sodium [1,2-^{13}C]acetate and unlabeled acetate was fed to a culture of *S. mirabile* BY-8-1 in an 8-L bottle on day 30 after innoculation. Similarly 250-mg portions of [*methyl*-^{13}C]-L-methionine were fed to a second culture in a 20-L bottle on days 23, 25, 27, and 29. The algal cultures were harvested by filtration on days 42 and 40, respectively, and immediately freeze-dried. The 1.7-g batch of dried alga from the acetate feeding experiment gave 2.8 mg of labeled **1**; inspection of its proton-noise-decoupled ^{13}C NMR spectrum in acetone-d_6 showed roughly 0.6% ^{13}C enrichment in the signals for C-1′ to C-10′, C-2, and C-3 and satellite peaks (doublets) for the following carbon signals: δ 174.1 ($J_{1',2'}$ = 49.1 Hz, C-1′), 169.8 ($J_{2,3}$ = 70.4 Hz, C-2), 93.6 ($J_{2,3}$ = 70.4 Hz, C-3), 75.5 ($J_{3',4'}$ = 39.2 Hz, C-3′), 43.2 ($J_{1',2'}$ = 49.1 Hz, C-2′), 42.3 ($J_{7',8'}$ = 38.9 Hz, C-7′), 33.7 ($J_{9',10'}$ = 40.9 Hz, C-10′), 32.3 ($J_{3',4'}$ = 39.2 Hz, C-4′), 24.7 ($J_{5',6'}$ = 35.1 Hz, C-5′). The 6.94-g batch of dried alga from the methionine feeding experiment gave 4.9 mg of labeled **1**; inspection of its proton-noise-decoupled ^{13}C NMR spectrum in acetone-d_6 showed roughly 4% ^{13}C enrichment in the following carbon signals: δ 59.5 (OMe), 13.6 (Me on C-2′).

Conversion of 1 to Isopropyl (*S*)-2-(Trifluoroacetamido)propionate. Mirabimide E (0.5 mg) in acetone (0.1 mL) was reacted with excess aqueous $NaIO_4$ solution containing a small amount of $KMnO_4$ for 15 h at 25 °C. Methanol was added, and the mixture was allowed to stand until the permanganate color had discharged. The solvent was removed with a nitrogen stream, and the residue was dissolved in 6 N HCl (0.3 mL) and heated at 100 °C for a few hours. 2-Propanol (0.7 mL) was added, and the mixture was heated for 1 h and evaporated. The residue, which contained the isopropyl ester of alanine, was treated with 0.5 mL of 1:1 $(CF_3CO)_2O/CH_2Cl_2$ at 100 °C for 5 min, the excess reagent was then evaporated with a stream of nitrogen, and the resulting mixture, which contained the isopropyl ester of *N*-trifluoroacetyl alanine was dissolved in 0.5 mL of CH_2Cl_2 for GCMS analysis on a 25-m × 0.25-mm Chirasil-Val column (Alltech). Using the following conditions, viz. column temperature of 60–110 °C at 2 deg/min and a 12 psi head pressure (flow rate estimated to be about 0.6 mL), the retention times for the isopropyl ester *N*-trifluoroacetate derivatives of D-(*R*)- and L-(*S*)-alanine are 2.60 and 3.03 min, respectively. The retention time for the derivative from degradation of **1** was found to be 3.02 min.

Acid Hydrolysis of 1 to 2. Mirabimide E (3.5 mg) dissolved in a limited amount of alcohol was added to 1 mL of 5.5 N HCl, and the solution was allowed to stand overnight at room temperature under N_2. The material that deposited on the walls of the container was redissolved in 1 N methanolic HCl, and this solution was heated to 50 °C for 4 h. The mixture of products was separated by HPLC on a Phenomenex RP-18 column (250 × 10 mm, Ultracarb ODS 30, 10 μm) using a MeCN/water gradient (7:3 MeCN/water for 2 min followed by 7:3 MeCN/water to MeCN over 30 min; flow rate 2.5 mL/min). Compound **2**, $[\alpha]_D$ –20° ($CHCl_3$, *c* 0.2), eluted from the column at 9.5 min. ^1H NMR (acetone-d_6): δ 7.46 (br, NH), 2.56 (dq, *J* = 10.5 and 7.0 Hz, 5-H), 1.27 (d, *J* = 7.0 Hz, 5-Me), 4.23 (ddd, *J* = 10.5, 8.2, and 2.5 Hz, 6-H), 1.50–1.94 (multiplets for 7-H_2, 8-H_2, and 9-H_2), 2.54 (br, 10-H_2), 2.46 (br s, 13-H_3). EIMS (relative intensity and assignment): *m/z* 363/365/367/369/371 (0.75:1:0.5:0.1:0.01 M^+ ion cluster), 302/304/306/308/310 (1.5:2:1:0.2:0.02, $M-CO_2NH$), 266/268/270 ($M-CH_3CCl_2$), 258/260 (18:6, M – 3Cl), 214/216 (18:6, M – 3Cl – CO_2), 197/199 (15:5, $M-3Cl-CO_2NH$), 117 (100). HREIMS: *m/z* 266.0303 ($C_{10}H_{14}Cl_2NO_3$, Δ4.8 mmu), 258.0883 ($C_{12}H_{17}ClNO_3$, Δ1.4 mmu).

Treatment of 1 with Lithium Hydroperoxide. Using Evans' procedure,[12] H_2O_2 (3.4 μL) and LiOH (0.8 mg, 32 μmol) were added to a solution of **1** (2.0 mg, 4.1 μmol) in 3:1 THF/H_2O (0.5 mL). The resulting mixture was stirred at 5–10 °C for 20 h, quenched with Na_2SO_3, acidified to pH 2, and extracted with ethyl acetate. The crude extract was subjected to preparative TLC on silica gel using 1:9 MeOH/chloroform to give **3** (1.5 mg) as an oil. ^1H NMR ($CDCl_3$): *cis*-2-pentenoic acid unit δ 3.76 (s, 1H, 2-H), 3.79 (s, 3-OMe), 4.61 (quintet, 4-H), 6.42 (br d, 4-NH), 1.42 (d, 5-H_3); *N*-acyl unit 2.61 (m, 2′-H), 1.21 (d, 2′-Me), 4.91 (br q, 3′-H), 4.79 (br s, 3′-$OCONH_2$), 1.45–1.95 (m, 4′-H_2, 5′-H_2, and 6′-H_2), 2.55 (br m, 7′-H_2), 2.48 (br s, 10′-H_3).

Hydrazinolysis of 1. To a NMR tube containing **1** (4 mg, 8 μmol) in MeOH-d_4 (0.6 mL) was added hydrazine monohydrate (4 μL, 82 μmol) at 25 °C. The course of the reaction was monitored by NMR analysis, and after 120 h, the hydrazinolysis was 95% complete. (5*S*)-4-Methoxy-5-methylpyrrolin-2-one (**5**, 0.95 μg) was separated from the acyl hydrazide **4** [δ 2.56 (quint, 1H, *J* = 7.0 Hz, C-2), 2.45 (s, 3H, C-10), 1.12 (d, 3H, *J* = 7.0 Hz, Me)] by successive preparative TLC on silica gel using 10:1 chloroform/methanol and HPLC on silica gel (Econosil 10 μm, 250- × 10-mm column, flow rate 2 mL/min) using 5% methanol/chloroform where **5** had a t_R of 13.5 min. Compound **5**. $[\alpha]_D$ +4.5° (MeOH, *c* 0.5); ^1H NMR ($CDCl_3$): δ 5.62 (1H, NH), 5.00 (s, 1H, 3-H), 4.08 (q, 1H, *J* = 6.9 Hz, 5-H), 3.81 (s, 3H, OMe), 1.33 (d, 3H, *J* = 6.9 Hz, 5-Me).

Synthesis of 5. Using the procedure of Lowe and Yeung,[19] *N*-(carbethoxyacetyl)-L-alanine methyl ester (**8**, 1 g, 4.6 mmol) was cyclized to (5*S*)-3-carbethoxy-5-methylpyrrolidine-2,4-dione (**9**) in benzene (20 mL, reflux 18 h) containing 60% NaH (184 mg, 4.6 mmol) and the product then hydrolyzed and decarboxylated in wet acetonitrile (25 mL, reflux 6 h) to give 0.2 g (40%) of (5*S*)-5-methylpyrrolidine-2,4-dione (**10**) as a pale yellow solid after flash chromatography on silica gel (5% MeOH/$CHCl_3$), mp 103.5–105 °C. IR (KBr): ν_{max} 3194, 1768, 1686, 1267 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.43 (br s, 1H, NH), 4.12 (q, 1H, *J* = 6.6 Hz, C-5), 3.04 (s, 2H, C-3), 1.38 (d, 3H, *J* = 6.6 Hz). $[\alpha]_D$ –5.28° ($CHCl_3$, *c* 4.62). To a solution of **10** (320 mg, 2.8 mmol) in acetone (20 mL) was added K_2CO_3 (390 mg, 2.8 mmol) and dimethyl sulfate (270 μL, 2.8 mmol). The mixture was allowed to react at 23 °C for 12 h. After filtration, the filtrate was concentrated to dryness *in vacuo* and the residue was subjected to chromatography on silica gel using 20:1 chloroform/methanol to afford (5*S*)-4-methoxy-5-methylpyrrolin-2-one (**5**, 120 mg, 34%) as a light yellow solid [52% ee as determined by NMR analysis (integration) of the methoxy signal in the presence of the chiral shift reagent Eu(hfc)$_3$],[20] mp 112.5–117.3 °C; $[\alpha]_D$ +2.5° (MeOH, *c* 4.0). IR (KBr): ν_{max} 3204, 1678, 1618, 1359, 1234 cm^{-1}. Anal. Calcd for $C_6H_9NO_2$: C, 56.68; H, 7.13. Found: C, 56.54; H, 7.11.

Treatment of (5*S*)-5-methylpyrrolidine-2,4-dione (100 mg, 0.88 mmol) in acetonitrile (10 mL) with excess diazomethane in Et_2O (dark, 25 °C, 24 h) led to a 10:1 mixture of **5** and (5*S*)-2-methoxy-5-methylpyrrolin-4-one, which was separated by flash chromatography on silica gel (5% chloroform/methanol) to give 79 mg (70%) of **5** (52% ee, *vide supra*), which was recrystallized from ether.

Decarbamoylation of 1. A solution of **1** (6.0 mg, 12 × 10^{-3} mmol) in $CDCl_3$ (0.7 mL) was added to a mixture of nitrosylsulfuric acid (42.4 mg, 0.33 mmol) and sulfuric acid-d_2 (22.9 μL, 0.43 mmol) in D_2O (80 μL) at 0 °C. After the mixture was shaken for 1 min, NMR analysis indicated that the 3′-H signal (a doublet of triplets) at 5.08 ppm for **1** had shifted downfield to 5.81 ppm. No epimerization at C-2′ occurred. The mixture was washed with water, and the organic phase was dissolved in 10 mL of 10% MeOH/CH_2Cl_2 and treated with stirring with silica

gel (0.5 g) for 2 h. After filtration and evaporation, the residue was subjected to preparative TLC on silica gel with 1:20 MeOH/$CHCl_3$ to give 4 mg (74%) of **6**, R_f = 0.78 (silica gel, 1:10 MeOH/$CHCl_3$). IR ($CHCl_3$): ν_{max} 3550, 2933, 1722, 1659, 1628 1320 cm^{-1}. ^1H NMR ($CDCl_3$): pyrrolinone unit δ 5.07 (s, 3-H), 3.88 (s, 4-OMe), 4.58 (q, J = 6.6 Hz, 5-H), 1.51 (d, J = 6.6 Hz, 5-Me); acyl unit 3.92 (dq, J = 6.0 and 6.9 Hz, 2′-H), 1.25 (d, J = 6.9 Hz, 2′-Me), 3.72 (m, 3′-H), 1.50–1.97 (m, 4′-H_2, 5′-H_2, and 6′-H_2), 2.55 (m, 7′-H_2), 2.48 (br s, 10′-H_3). ^{13}C NMR ($CDCl_3$): δ 180.8 (C-4), 176.4 (C-1′), 170.9 (C-2), 99.4 (C-8′), 94.7 (C-9′), 93.0 (C-3), 70.4 (C-3′), 58.8 (OMe), 55.9 (C-5), 44.2 (C-2′), 42.2 (C-7′), 35.1 (C-10′), 33.4 (C-4′), 29.7 (C-6′), 25.2 (C-5′), 17.3 (Me on C-5), 14.5 (Me on C-2′). EIMS: m/z 429/431/433/435/437 (75:100:50:10:1 $M^+ - H_2O$ ion cluster), 212, 183, 127 (100). FABMS: m/z 448/450/452/454/456 (75:100:50:10:1 MH^+ ion cluster), 430/432/434/436/438 (75:100:50:10:1 ion cluster for MH^+). High-resolution FABMS: m/z 448.0627 ($C_{17}H_{26}Cl_4NO_4$, MH^+, Δ–1.1).

(*R*)-MTPA Ester of 6. Using the procedure of Kakisawa et al.,[10b] **6** was esterified with (S)-(+)-MTPA chloride to give the (*R*)-MTPA ester in 10–15% yield. ^1H NMR ($CDCl_3$): δ 7.29–7.19 (m, Ph-H_5), 3.86 (s, 4-OMe), 4.55 (q, 1H, J = 6.5 Hz, 5-H), 1.38 (d, 3H, J = 6.5 Hz, 5-Me), 4.23 (quintet, J = 7.0 Hz, 2′-H), 1.17 (d, J = 7.0 Hz, 2′-Me), 5.55 (m, 3′-H), 1.45–1.85 (m, 4′-H_2, 5′-H_2, and 6′-H_2), 2.42 (m, 7′-H_2), 2.46 (s, 3H, 10′-H_3).

Conversion of 6 to Methyl (2*S*,3*S*)-8,8,9,9-Tetrachloro-3-hydroxy-2-methyldecanoate (7). Compound **6** (3.1 mg, 6.9 × 10^{-3} mmol) was dissolved in 170 μL (13.8 × 10^{-3} mmol) of 0.08 M MgOMeBr in MeOH under a nitrogen atmosphere, and the mixture was allowed to stand at 0 °C for 10 h. The solution was quenched with 1 N HCl to pH 3 at –78 °C and then evaporated to dryness *in vacuo*. The residue was subjected to preparative TLC on silica gel with ether to give 0.8 mg (92%) of racemic 4-methoxy-5-methyl-3-pyrrolin-2-one (R_f = 0.52 (silica gel, 9:1 $CHCl_3$/MeOH), $[\alpha]_D$ 0° ($CHCl_3$, *c* 0.6)) and 2.3 mg (94%) of methyl ester **7** as a viscous oil (R_f = 0.68 (silica gel, 20:1 $CHCl_3$/MeOH), $[\alpha]_D$ –2.4° ($CHCl_3$, *c* 0.6)). IR ($CHCl_3$): ν_{max} 3540, 2915, 1722, 1191 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.73 (s, 1-OMe), 2.55 (quintet, J = 7.0 Hz, 2-H), 1.23 (d, J = 7.0 Hz, 2-Me), 3.69 (m, 3-H), 1.45–1.9 (m, 4′-H_2, 5′-H_2, and 6′-H_2), 2.52–2.60 (m, 7′-H_2), 2.47 (br s, 10-H_3). ^{13}C NMR ($CDCl_3$): δ 169.8 (C-1), 99.2 (C-8), 94.5 (C-9), 71.8 (C-3), 45.2 (C-2), 41.5 (C-7), 34.6 (C-10), 33.4 (C-4), 25.5 (C-6), 25.0 (C-5), 14.4 (Me on C-2). EIMS: m/z 337 (M^+ – Me), 321 (M^+ – OMe), 279, 207, 117, 88 (100), 57. HREIMS: m/z (calcd for $C_{11}H_{17}O_3Cl_4$, –1.2-mmu error).

(*R*)-MTPA Ester of 7. Using the procedure of Kakisawa et al.,[10b] treatment of **7** (2 mg, 5.6 μmol) with (S)-(+)-MTPA chloride (4 mg, 15.8 μmol) at 25 °C for 15 h afforded pure (*R*)-MTPA ester (3 mg, 93%) after preparative TLC (silica gel, 2:1 hexane/EtOAc). ^1H NMR ($CDCl_3$): δ 7.39–7.55 (m, Ph of MTPA), 3.55 (br, OMe of MTPA), 3.65 (s, 1-OMe), 2.86 (quintet, J = 7.0 Hz, 2-H), 1.18 (d, J = 7.0 Hz, 2-Me), 5.38 (m, 3-H), 1.69 and 1.65 (m, 4-H_2), 1.17 (m, 5-H_2), 1.73 (m, 6-H_2), 2.39 (m, 7-H_2), 2.44 (br s, 10-H_3).

(*S*)-MTPA Ester of 7. This ester was produced as described above from **7** and (*R*)-(–)-MTPA chloride. ^1H NMR ($CDCl_3$): δ 7.41–7.54 (m, Ph of MTPA), 3.51 (br m, OMe of MTPA), 3.61 (s, 1-OMe), 2.85 (quintet, J = 7 Hz, C-2), 1.12 (d, J = 7 Hz, Me on C-2), 5.39 (q, J = 6Hz, 3-H), 1.76 and 1.73 (m, 4-H_2), 1.38 (m, 5-H_2), 1.83 (m, 6-H_2), 2.50 (br m, 7-H_2), 2.50 (br s, 10-H_3).

6-Heptyn-1-ol (12). The isomerization of 2-heptyn-1-ol (**11**) to **12** was carried out using the procedure developed by Abrams.[21] 1,2-Diaminoethane (42 mL, distilled from BaO and stored over molecular sieves) and Li wire (0.73 g, prewashed with hexane followed by 5% ethanol in hexane and dried *in vacuo*) were heated under N_2 for 1 h at 70 °C until the blue color had discharged and a white suspension of the Li salt had formed. The mixture was cooled to room temperature and freshly sublimed potassium *tert*-butoxide (7.2 g) was then added all at once. The purple–brown solution was stirred for 15 min, and **11** (1.8 g) was added in one portion. The resulting brown mixture was stirred for 3 h and then poured into ice slowly. The mixture was extracted three times with CH_2Cl_2, and the extract was washed with 1 N HCl (aqueous wash back-extracted twice with CH_2Cl_2). The organic layers were combined, dried over $MgSO_4$, and evaporated to give 1.5 g (83%) of **12** after silica gel chromatography with 20% EtOAc/hexane, R_f = 0.22 (silica gel, 3:7 EtOAc/hexane). IR (neat) ν_{max} 3370, 3295, 2925, 2110 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.66 (t, J = 6.0 Hz, 1-H_2), 2.21 (dd, J = 2.7 and 6.6 Hz, H_2-5), 1.94 (t, J = 2.7 Hz, 7-H), 1.62–1.44 (m, 2-H_2, 3-H_2, and 4-H_2). ^{13}C NMR ($CDCl_3$): δ 84.3 (s, C-6), 68.2 (d, C-7), 62.3 (t, C-1), 32.0 (t, C-2), 28.1 (t, C-4), 24.8 (t, C-3), 18.2 (t, C-5). EIMS (relative intensity): m/z 112, 111, 79 (100). High-resolution EIMS: m/z 112.0907 ($C_7H_{12}O$, Δ–1.9 mmu).

6-Octyn-1-yl Benzoate (14). To a solution of **12** (100 mg) in 5 mL of CH_2Cl_2 was added triethylamine (0.37 mL). The solution was stirred for 10 min, and benzoyl chloride (0.21 mL) was added dropwise. Stirring was continued for 1 h, and water was added. The mixture was extracted three times with CH_2Cl_2, and the organic layers were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the product was purified by silica gel chromatography with 1:9 EtOAc/hexane to give 176 mg (91%) of 6-heptyn-1-yl benzoate (**13**): R_f = 0.74 (silica gel, 3:17 EtOAc/hexane). IR (neat): ν_{max} 3295, 2925, 2110, 1725, 1275 cm^{-1}. ^1H NMR ($CHCl_3$): δ 8.04 (dd, J = 1.2 and 7.5 Hz, benzoate 2-H and 6-H), 7.55 (dt, J = 1.2 and 7.5 Hz, benzoate 4-H), 7.43 (t, J = 7.2 Hz, benzoate 3-H and 5-H), 4.33 (t, J = 6.6 Hz, 1-H_2), 2.22 (m, 5-H_2), 1.95 (t, J = 2.7 Hz, 7-H), 1.79 (m, 2-H_2), 1.59 (m, 3-H_2 and 4-H_2). ^{13}C NMR ($CDCl_3$): 166.5, 132.7, 130.3, 129.4, 128.2, 84.1, 68.4, 64.7, 28.1, 28.0, 25.1, 18.2. EIMS: m/z 216 (M^+), 215, 188, 181, 169, 123, 105 (100), 94, 79. HREIMS: m/z 216.1178 ($C_{14}H_{16}O_2$, Δ–2.8 mmu).

To a solution of LDA (generated at 0 °C from 0.22 mL of diisopropylamine and 0.60 mL of *n*-BuLi) in 10 mL of THF at –78 °C was added **13** (230 mg) in 2 mL of THF. After 0.5 h, MeI (0.10 mL) was added and the solution was stirred overnight. The reaction was quenched with water, and the solution was extracted three times with ether. The extract was dried ($MgSO_4$) and evaporated. The residue was purified by silica gel chromatography with 1:9 EtOAc/hexane to give 232 mg (95%) of **14**, R_f = 0.74 (silica gel, 3:17 EtOAc/hexane). IR (neat): ν_{max} 2925, 1720, 1270 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.04 (d, J = 7.5 Hz, benzoate 2-H and 6-H), 7.56 (t, J = 7.5 Hz, benzoate 4-H), 7.44 (t, J = 7.5 Hz, benzoate 3-H and 5-H), 4.22 (t, J = 6.6 Hz, 1-H_2), 2.16 (m, 5-H_2), 1.77 (t, J = 2.7 Hz, 8-H_3), 1.54–1.64 (m, 2-H_2, 3-H_2, and 4-H_2). ^{13}C NMR ($CDCl_3$): δ 166.5, 132.7, 130.4, 129.4, 128.2, 78.7, 75.56, 64.7, 28.5, 28.2, 27.8, 25.1, 18.5. EIMS: m/z 230 (M^+), 215, 181, 169, 160, 145, 123, 105 (100), 93, 77. HREIMS: m/z 230.1362 ($C_{15}H_{18}O_2$, Δ–5.5 mmu).

6,6,7,7-Tetrachloro-1-octanol (16). To a solution of **14** (100 mg) in benzene (5 mL) was added 1 M sulfuryl chloride in CH_2Cl_2 (1.8 mL). The mixture was heated to reflux for 4 days, during which time additional 1 M sulfuryl chloride (0.9 mL) was added four times a day. It was then washed with aqueous sodium bicarbonate solution and extracted three times with CH_2Cl_2. The extract was dried over $MgSO_4$ and evaporated under reduced pressure. The residual oil was purified on a column of silica gel using 5% EtOAc/hexane to give 6,6,7,7-tetrachloro-1-octyl benzoate (**15**) (97 mg, 60%), R_f = 0.74 (silica gel, 3:17 EtOAc/hexane). IR (neat): ν_{max} 2940, 1725, 1450, 1270, 1110, 710 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.06 (d, J = 7.5 Hz, benzoate 2-H and 6-H), 7.56 (t, J = 7.5 Hz, benzoate 4-H), 7.44 (t, J = 7.5 Hz, benzoate 3-H and 5-H), 4.37 (t, J = 6.6 Hz, 1-H_2), 2.59 (m, 5-H_2), 2.48 (br s, 8-H_3), 1.82–1.95 (m, 2-H_2 and 4-H_2), 1.50–1.68 (m, 3-H_2). ^{13}C NMR (acetone-d_6): δ 166.7, 133.7, 131.3, 130.1, 129.3, 100.5 (C-6), 95.8 (C-7), 65.3 (C-1), 42.2 (C-5), 33.9, 33.7, 26.3, 26.2. EIMS: m/z 370/372/374/376/378 (75:100:50:10:1 MH^+ ion cluster), 123, 105 (100), 77. HREIMS: m/z 370.0014 ($C_{15}H_{18}Cl_4O_2$, Δ4.7 mmu).

To a solution of **15** (0.81 g) in 7.5 mL of MeOH was added 1.5 N NaOH (1.8 mL), and the mixture was stirred at room temperature for 10 h. The solution was concentrated to remove MeOH and then extracted with ether (3 × 5 mL). The combined extracts were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give 0.6 g of an oily residue. The residue was subjected to silica gel chromatography with 1:10 EtOAc/hexane to give 0.47 g (81%) of **16**, R_f = 0.10 (silica gel, 3:17 EtOAc/hexane). IR (neat): ν_{max} 3320, 2940, 1450, 1380, 1070, 730, 685, 665 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.69 (t, J = 6.3 Hz, 1-H_2), 2.56 (m, 5-H_2), 2.48 (br s, 8-H_3), 1.92 (m, 4-H_2), 1.50–1.69 (m, 2-H_2 and 3-H_2). ^1H NMR (acetone-d_6): δ 3.56 (t, J = 6.0 Hz, 1-H_2), 2.53 (m, 5-H_2), 2.46 (bs, 8-H_3), 1.90–1.79 (m, 4-H_2), 1.45–1.62 (m, 2-H_2 and 3-H_2). ^{13}C NMR (acetone-d_6): δ 100.4 (C-6), 95.6 (C-7), 61.9 (C-1), 42.2 (C-5), 33.6 (C-8), 33.2 (C-2), 26.2 (C-4), 25.8 (C-3). EIMS: m/z 248/250/252/254/256 (75:100:50:10:1 M – H_2O ion cluster), 220/222/224/226/228 (75:100:50:10:1), 123, 109 (100), 89. HREIMS: m/z 219.9426 ($C_6H_8Cl_4$, Δ–4.6 mmu).

6,6,7,7-Tetrachlorooctanal (17). To a 5-mL solution of **16** (10 mg) in CH_2Cl_2 was added 2 equiv of Dess–Martin reagent[22] (periodinane) over a 1-h period. The mixture was stirred for 4 h. The solution was filtered over Celite, and the solvent was removed under reduced pressure. The residue was subjected to silica gel chromatography with 1:19 EtOAc/hexane to give 7 mg (71%) of aldehyde **17**, R_f = 0.57 (silica gel, 3:7

EtOAc/hexane). IR (neat): ν_{max} 2940, 2720, 1725, 685, 665 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 9.82 (s, 1-H), 2.56 (m, 5-H_2), 2.48 (bs, 8-H_3), 2.05 (m, 4-H_2), 1.55–1.78 (m, 2-H_2 and 3-H_2). ^{1}H NMR (acetone-d_6): δ 9.76 (bs, 1-H), 2.62-2.50 (m, 2H), 2.56 (t, 2H), 2.46 (bs, 8-H_3), 1.90–1.81 (m, 2H), 1.77–1.70 (m, 2H). ^{13}C NMR (acetone-d_6): δ 201.9 (d, C-1), 99.0 (C-6), 94.5 (C-7), 43.7, 41.3, 33.4, 25.2 (C-3), 21.4 (C-8). EIMS: *m/z* 236/238/240/242/244 (75:100:50:10:1 M – CO ion cluster), 221/223/225/227/229 (75:100:50:10:1), 193, 149, 123, 75, 67 (100). HREIMS: *m/z* 220.9446 ($C_6H_9Cl_4$, Δ–1.2 mmu).

(2*R*,3*R*)-8,8,9,9-Tetrachloro-3-hydroxy-2-methyldecanoic Acid (20). A 0.8 M solution of (*E*)-silylketene acetal (**18**) generated from (1*S*,2*R*)-*N*-methylephedrinyl *O*-propionate in CH_2Cl_2 was prepared as described by Gennari et al.[23c] To a solution of **17** (60 mg) in 5 mL of CH_2Cl_2 cooled to –78 °C was added 0.23 mL of 1 M $TiCl_4$ in CH_2Cl_2 and 0.31 mL of the 0.8 M **18** in CH_2Cl_2. The mixture was stirred at –78 °C for 2 h and then quenched with cold aqueous $NaHCO_3$ solution. The mixture was filtered with the aid of Celite, washed three times with ether (5 mL), and extracted with ether (3 × 5 mL). The combined ethereal extracts were washed with brine, dried ($MgSO_4$), filtered, and evaporated to give 100 mg (90%) of a yellow 85:15 mixture (by NMR analysis) of *anti/syn* aldol condensation products. Separation was achieved by flash chromatography on silica gel with 5:6 EtOAc/hexane to give 55 mg (50%) of the desired *anti* diastereomer **19**, R_f = 0.52 (silica gel, 1:9 MeOH/$CHCl_3$). IR (neat): ν_{max} 3600, 1740 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.30 (m, 5H), 6.33 (d, *J* = 2.7 Hz, CHPh), 3.75 (m, 3-H), 2.72–2.5 (m, 7-H_2, 2-H and CHN), 2.48 (br s, 10-H_3), 2.38 (s, NMe_2), 1.87–1.40 (m, 4-H_2, 5-H_2, 6-H_2), 1.31 (d, *J* = 7.2 Hz, ephedrine CH_3), 1.01 (d, *J* = 6.6 Hz, 2-CH_3).

To a cooled (0 °C) solution of **19** (40 mg, 0.08 mmol) in 1.6 mL of MeOH was slowly added 0.4 mL of 1 N NaOH at room temperature. After stirring for 15 h at 25 °C under an inert atmosphere, the mixture was washed with ether to remove (1*S*,2*R*)-*N*-methylephedrine. The aqueous phase was then acidified to pH 2 with 1 N HCl and extracted with ether (3 × 5 mL). The combined extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, and filtered. Removal of solvent *in vacuo* gave an essentially pure viscous oil (26 mg, 92%) which was further recrystallized from petroleum ether and CH_2Cl_2 to give **20** as a white solid (23 mg, 80%), mp 125–127 °C, R_f = 0.75 (silica gel, 1:9 MeOH/$CHCl_3$). $[\alpha]_D$ +2.2° ($CHCl_3$, *c* 0.91). IR (neat): ν_{max} 3200–2400, 1708, 1297, 1276 cm^{-1}. The ethereal extract was washed with saturated aqueous NaCl, dried, filtered, and evaporated to give essentially pure **20** (26 mg, 92%), R_f = 0.75 (silica gel, 1:9 MeOH/$CHCl_3$). IR (neat): ν_{max} 3200–2400, 1708 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.78 (m, 3-H_2), 2.61 (quintet, 2-H_2), 2.62–2.53 (br m, 7-H_2), 2.49 (br s, 10-H_3), 1.93–1.82 and 1.63–1.48 (multiplets, 4-H_2, 5-H_2, and 6-H_2), 1.30 (d, *J* = 7.2 Hz, 2-CH_3). ^{13}C NMR ($CDCl_3$): δ 180.6 (C-1), 99.3 (C-8), 94.6 (C-9), 72.9 (C-3), 45.7 (C-2), 41.5 (C-7), 34.4 (C-10), 33.4 (C-4), 25.5 (C-6), 24.9 (C-5), 14.3 (Me on C-2). EIMS: *m/z* 323/325/327/329/331 (75:100:50:10:1 M – Me ion cluster), 263/265/267/269/271 (75:100:50:10:1), 232, 215, 136, 103, 85, 74 (100). HREIMS: *m/z* 322.9778 ($C_{10}H_{15}Cl_4O_3$, Δ–0.3 mmu). Anal. Calcd for $C_{11}H_{18}Cl_4O_3$: C, 38.85; H, 5.33. Found: C, 38.89; H, 5.50.

Methyl (2*R*,3*R*)-8,8,9,9-Tetrachloro-3-hydroxy-2-methyldecanoate (7). An ethereal solution of **20** (20 mg) was treated with excess CH_2N_2 at 0 °C for 5 min. Flash chromatography on silica gel with 50:1 $CHCl_3$/MeOH gave pure **7**, which showed the same R_f value on TLC (0.68, silica gel, 20:1 MeOH/$CHCl_3$), optical rotation ($[\alpha]_D$ –2.2° ($CHCl_3$, *c* 0.8)), and NMR spectra as the degradation product.

Attempted Synthesis of Mirabimide E via Carbamate 21. Synthesis of 2. To a NMR tube containing a cooled (0 °C) solution of **20** (10 mg, 0.03 mmol) in $CDCl_3$ (0.5 mL) was added trichloroacetyl isocyanate (14 mg, 0.07 mmol). After the reaction was completed by NMR analysis (1 min), the mixture was poured into a cold solution of saturated aqueous $NaHCO_3$ (1 mL) and MeOH (1 mL). The solution was stirred at 25 °C for 2 h and then concentrated to one-half of the volume to remove methanol. The aqueous phase was washed with ether, acidified to pH 2 with 1 N HCl, and extracted with ether (3 × 5 mL). The combined extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was chromatographed on silica gel (15% MeOH/CH_2Cl_2) to yield carbamate **21** (8 mg, 70%) as a white solid, R_f = 0.45 (silica gel, 10% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.02 (m, 3-H), 4.85 (s, NH_2), 2.81 (quintet, *J* = 7.0 Hz, 2-H), 2.62–2.45 (m, 7-H_2), 2.49 (s, 10-H_3), 1.9–1.45 (m, 4-H_2/5-H_2/6-H_2), 1.21 (d, *J* = 7.0 Hz, Me on C-2).

To a cooled (0 °C), stirred solution of **21** (8 mg) in THF (5 mL) was added *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (HONb,[25] 6 mg) and DCC (5 mg). The mixture was stirred at 25 °C for 10 h, diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$, and evaporated *in vacuo*. The residue was chromatographed on silica gel (10% MeOH/$CHCl_3$) to give **22** (9 mg), R_f = 0.68 (silica gel, 10% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 6.21(s, 2CH=), 4.95 (m, 3-H), 4.80 (br s, NH_2), 3.45 (s, 2CH), 3.3 (s, 2CH), 3.02 (quintet, *J* = 7.0 Hz, 2-H), 2.65–2.45 (m, 7-H_2), 2.48 (s, 10-H_3), 1.97–1.4 (m, CH_2 and 4-H_2/5-H_2/6-H_2), 1.21 (d, *J* = 7.0 Hz, Me on C-2).

To a stirred solution of **5** (5 mg) and 60% NaH (1.3 mg) in THF (1 mL) was added active ester **22** (5 mg) in THF (3 mL). After stirring at 25 °C for 30 min, the mixture was diluted with ethyl acetate, washed successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the NMR spectrum of the crude mixture indicated that lactam **2** had been formed in almost quantitative yield. The crude product was chromatographed on silica gel (5% MeOH/$CHCl_3$) and shown to be identical (NMR and TLC R_f = 0.65 (10% MeOH/$CHCl_3$)) with **2** obtained from acid hydrolysis of mirabimide E.

(2′*R*,3′*R*,5*S*)-1-(3′-Hydroxy-2′-methyl-8′,8′,9′,9′-tetrachlorodecanoyl)-4-methoxy-5-methyl-3-pyrrolin-2-one (6). To a stirred solution of **20** (125 mg, 0.38 mmol) and *tert*-butyldimethylsilyl chloride ((TBS)Cl) (122 mg, 0.81 mmol) in anhydrous DMF (2 mL) was added imidazole (105 mg, 1.55 mmol). After 24 h at 25 °C, the reaction mixture was poured into ice–water (5 mL) and extracted with petroleum ether (3 × 10 mL). The combined extracts were washed with 5% $NaHCO_3$, dried over $MgSO_4$, and filtered. Removal of the solvent *in vacuo* afforded 199 mg (95%) of *tert*-butyldimethylsilyl (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)-oxy)-2-methyl-8,8,9,9-tetrachlorodecanonate (**23**) as a colorless oil (>95% pure by NMR), which was used directly in the next step without further purification, R_f = 0.83 (silica gel, 1:5 EtOAc/hexane). IR ($CHCl_3$): ν_{max} 2953, 1710, 1225, 1205 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.08 (m, 3-H), 2.67 (m, 2-H), 2.56 (m, m, 7-H_2), 2.47 (s, 10-H_3), 1.94–1.32 (m, 4-H_2/5-H_2/6-H_2), 1.09 (d, *J* = 7.2 Hz, Me on C-2), 0.94 (s, 9H), 0.89 (s, 9H), 0.26 (s, 6H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 174.5 (C-1), 98.5 (C-8), 94.5 (C-9), 72.8 (C-3), 47.0 (C-2), 41.5 (C-7), 33.4 (C-10), 32.6 (C-4), 25.8 (C*Me*$_3$), 25.7 (C-6), 25.5 (C*Me*$_3$), 25.0 (C-5), 18.1 (*C*Me$_3$), 17.5 (*C*Me$_3$), 10.6 (C-2-Me), –4.89 (SiMe), –4.83 (SiMe), –4.53 (SiMe), –4.67 (SiMe). EIMS: *m/z* 509/511/513/515/517 (75:100:50:10:1 M – CMe_3 ion cluster), 3811, 309, 189 147 73 (100). HREIMS: *m/z* 556.0609 ($C_{19}H_{37}Cl_4O_3Si_2$, Δ+2.1 mmu).

A solution of K_2CO_3 (66 mg, 0.47 mmol) in water (0.6 mL) was added to a stirred solution of TBS ester **23** (90 mg, 0.16 mmol) in MeOH (1.8 mL) and THF (0.6 mL). The reaction mixture was stirred at 25 °C for 1 h, concentrated *in vacuo* to one-fourth of the volume, and diluted with saturated aqueous NaCl (2 mL). The aqueous solution was acidified to pH 4 with 1 M $KHSO_4$ at 0 °C and extracted with ether (3 × 5 mL). The combined extracts were dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Flash chromatography on silica gel (12% EtOAc/hexane) afforded 65 mg (91%) of (2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-methyl-8,8,9,9-tetrachlorodecanoic acid (**24**) as a colorless oil, R_f = 0.19 (silica gel, 1:5 EtOAc/hexane). IR ($CHCl_3$): ν_{max} 3400–2400, 1753, 1735, 1258 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.89 (q, *J* = 4.2 Hz, 3-H), 2.69 (m, 2-H), 2.52 (m, 7-H_2), 2.47 (s, 10-H_3), 1.87–1.35 (m, 4-H_2/5-H_2/6-H_2), 1.24 (d, *J* = 7.0 Hz, Me on C-2), 0.92 (s, 9H), 0.14 (s, 6H), 0.13 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 176.4 (C-1), 99.1 (C-8), 94.6 (C-9), 74.0 (C-3), 44.4 (C-2), 41.4 (C-7), 34.6 (C-10), 33.4 (C-4), 25.7 (C*Me*$_3$), 25.5 (C-6), 24.3 (C-5), 17.9 (*C*Me$_3$), 14.1 (C2-Me), –4.34 (SiMe), –4.90 (SiMe). EIMS: *m/z* 395/397/399/302/401 (75:100:50:10:1 M – CMe_3 ion cluster), 327, 309, 141, 115, 75 (100). HREIMS: *m/z* 395.0180 ($C_{13}H_{23}Cl_4O_3Si$, Δ–0.9 mmu).

endo-*N*-Hydroxy-5-norbornene-2,3-dicarboximide (HONb)[25] (33 mg, 0.18 mmol) and DCC (30 mg, 0.15 mmol) were added to a cooled (0 °C), stirred solution of **24** (55 mg, 0.12 mmol) in CH_2Cl_2. The reaction mixture was warmed to 25 °C, maintained at this temperature for 15 h, and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered again, and evaporated *in vacuo*. Flash chromatography on silica gel (15% EtOAc/hexane) afforded 68 mg (92%) of the *endo*-5-norbornene-2,3-dicarboximidyl ester **25** as a viscous oil, R_f = 0.68 (silica gel, 1:2 EtOAc/hexane). IR ($CHCl_3$): ν_{max} 2950, 1779, 1735 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.20 (s, 2CH=), 4.11 (m, 3′-H), 3.44 (s, 1-H/4-H), 3.32 (s, 2-H/3-H), 2.94 (m, 2′-H), 2.59 (m, 7′-H_2), 2.48 (s, 10′-H_3), 1.87–1.41 (m, 4′-H_2/5′-H_2/6′-H_2), 1.78 (d, *J* = 7.8 Hz, 7-H (NOE with vinyl), 1.52 (d, *J* = 7.8 Hz, 7-H), 1.23 (d, *J* = 7.0 Hz, Me on C′-2), 0.89 (s, 9H), 0.09 (s, 3H), 0.07(s, 3H). ^{13}C NMR ($CDCl_3$): δ 169.8 (CO-N), 168.5 (C′-1), 134.7 (C-5/C-6), 99.37 (C′-8), 94.6 (C′-9), 72.3 (C′-3), 51.1 (C-7), 44.6 (C-1/C-4), 43.5 (C-2/C-3), 43.1 (C′-2), 41.4 (C′-7), 32.3 (C′-10), 29.6 (C′-4), 25.6 (C*Me*$_3$), 24.6 (C-6), 22.6

(C-5), 18.0 (CMe_3), –4.8 (SiMe), –4.6 (SiMe). EIMS: *m/z* 556/558/560/562/564 (75:100:50:10:1 M – CMe_3 ion cluster), 486, 437, 381, 236, (100), 170. HREIMS: *m/z* 556.0609 ($C_{22}H_{30}Cl_4NO_5Si$, Δ+3.8 mmu).

To a stirred solution of **5** (13 mg, 0.10 mmol, 52% ee) in anhydrous THF (3 mL) was added 60% NaH (3.2 mg, 0.08 mmol), and the mixture was heated under reflux for 10 min. Active ester **25** (42 mg, 0.07 mmol) in THF (1 mL) was then added, and the mixture was stirred at 25 °C for 30 min. The mixture was diluted with ethyl ether (5 mL), washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give an oily diastereomeric mixture. Flash chromatography (silica gel, benzene) afforded 19 mg (49%) of the major diastereomer (2′*R*,3′*R*,5*S*)-1-(3′-((*tert*-butyldimethylsilyl)oxy)-2′-methyl-8′,8′,9′,9′-tetrachlorodecanoyl)-4-methoxy-5-methyl-3-pyrrolin-2-one (**26**) as an oil, R_f = 0.16 (silica gel, benzene). IR ($CHCl_3$): ν_{max} 2943, 1718, 1684, 1631, 1247 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.03 (s, 3-H), 4.61 (q, *J* = 6.6 Hz, 5-H), 4.17 (dt, *J* = 7.5 and 6.6 Hz, 3′-H), 4.02 (quintet, *J* = 6.6 Hz, 2′-H), 3.86 (s, OMe), 2.55 (m, 7′-H_2), 2.47 (s, 10′-H_3), 1.85–1.50 (m, 4′-H_2/5′-H_2/6′-H_2), 1.44 (d, *J* = 6.6 Hz, Me on C-5), 1.10 (d, *J* = 6.6 Hz, Me on C-2′), 0.89 (s, 9H), 0.15 (s, 3H), 0.10 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 180.28 (C-4), 174.19 (C-1′), 169.28 (C-2), 99.39 (C-8′), 94.71 (C-9′), 92.94 (C-3), 71.89 (C-3′), 58.79 (OMe), 55.67 (C-5), 45.44 (C-2′), 41.56 (C-7′), 33.41 (C-10′), 32.24 (C-4′), 25.92 (CMe_3), 25.74 (C-6′), 24.35 (C-5′), 18.11 (CMe_3), 17.08 (Me on C-5), 10.45 (Me on C-2′), –4.9 (SiMe), –4.7 (SiMe). EIMS: *m/z* 504/506/508/510/512 (75:100:50:10:1 M – CMe_3 ion cluster), 434, 307, 240 (100), 184. HREIMS: *m/z* 504.0701 ($C_{19}H_{30}O_4NSiCl_4$, Δ+3.8 mmu).

A solution of 14.5 mg (25.7 μmol) of **26** in 1 mL of 85:15 (v/v) acetonitrile/40% aqueous HF was stirred at 25 °C for 45 min. Saturated aqueous NaCl (2 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated *in vacuo*. Column chromatography on silica gel (25% EtOAc/hexane) afforded 10.5 mg (91%) of **6** as a colorless oil which was identical in all respects with the decarbamoylation product of **1**.

Mirabimide E (1). To a cooled (0 °C) solution of **6** (7.2 mg, 16.0 μmol) in $CDCl_3$ (0.7 mL) was added trichloroacetyl isocyanate (3.6 mg, 19.2 μmol). After 5 min at 25 °C, the formation of **27** was complete by NMR analysis. ^1H NMR ($CDCl_3$): δ 8.63 (br s, 1H, NH), 5.35 (m, 3′-H), 5.05 (s, 3-H), 4.55 (q, 5-H), 4.25 (m, 2′-H), 3.81 (s, OMe), 2.65 (m, 7′-H_2), 2.49 (s, 10′-H_3), 1.95–1.45 (m, 4′-H_2/5′-H_2/6′-H_2), 1.45 (d, Me on C-5), 1.25 (d, Me on C′-2). The mixture was stirred with silica gel (0.5 g) in 10% MeOH/$CHCl_3$ at 25 °C for 15 h. After filtration and removal of the solvent, the **27** was further decomposed[24] during preparative TLC on silica gel (10% MeOH/$CHCl_3$) to give a white solid (5.9 mg, 75%). The compound was further purified by reversed-phase HPLC (YMC AM-343-5 ODS, 120A, 20 × 300 mm) using 65% H_2O/CH_3CN as the eluant (5 mL/min) to give 4.3 mg (54%) of **1**, t_R 48 min. The synthetic compound was identical in all respects, including optical rotation ($[\alpha]_D$ +6.3° ($CHCl_3$, *c* 0.25)), to natural mirabimide E.

Acknowledgment. Studies on the isolation, structure determination, and cytotoxicity evaluation of **1** were supported by Grant CA12623 (R.E.M.) from the National Cancer Institute, Department of Health and Human Services. Research on the biosynthesis of **1** was supported by Grant CHE-9024748 from the National Science Foundation. The authors thank Bradley S. Moore for producing the ^{13}C- and ^{15}N-enriched algae and Faith Caplan and Linda K. Larsen for determining the KB and LoVo cytotoxicities.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of **1**, **12-17**, and **20** and INADEQUATE ^{13}C NMR spectra of uniformly ^{13}C- and ^{15}N-enriched **1** (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.