

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

Seunguk Paik, Shmuel Carmeli,¹ Jean Cullingham, Richard E. Moore,*
Gregory M. L. Patterson, and Marcus A. Tius

Contribution from the Department of Chemistry, University of Hawaii at Manoa,
Honolulu, Hawaii 96822

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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 spongelliae*, 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 segment⁶ 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 cyanophyte⁵ was subjected to rapid reverse-phase chro-

matography 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 C₁₈H₂₆Cl₄N₂O₅ (+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 C₁₇H₂₄Cl₄NO₃ 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 ¹³C 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 CH₂ 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

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(1) Present address: School of Chemistry, Tel-Aviv University, Ramat-Aviv 69978, Israel.

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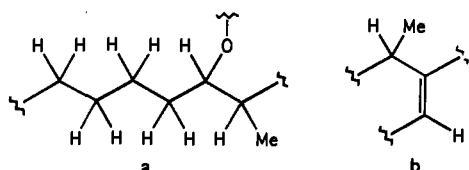
Table 1. NMR Data for Mirabimide E in Acetone- d_6 (7.5 mg/mL)^a

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 ₂ CO 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 NH₂ 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 NH₂ group, an unresolved 2H signal ($w_{1/2}$ = 30 Hz) at 2.55 ppm attributed to a CH₂ 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



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 (NH₂, OCH₃, and the CH₃ 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 NH₂ groups and a C₂Cl₄ 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

¹³C to 80% and ¹⁵N to 90%.⁹ In the ¹³C 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 (J_{CN} = 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'-H₃, 7'-H₂, 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'-H₃ 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 H₃-10' 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'-H₃ 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' (d₁), whereas in the other one, C-6' is *syn* to C-9' (d₂). The 10'-H₃ signals for these *syn* rotamers have different chemical shifts because in the d₁ rotamer (2.43 ppm) the protons on C-10' are deshielded (van der Waals type) by the protons on C-7' whereas in the d₂ 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 substituents 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 analysis¹⁰ 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 oxidation¹¹ 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*.

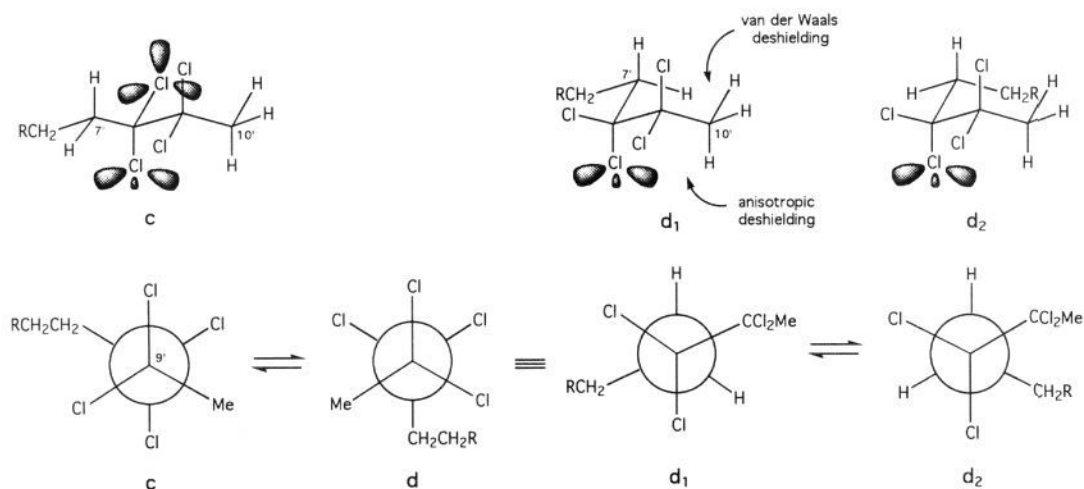
Acid hydrolysis of 1 with 5.5 N HCl produced a complex mixture of products, with some of them still bearing the carbamate group. Interestingly, one of the compounds was identified as

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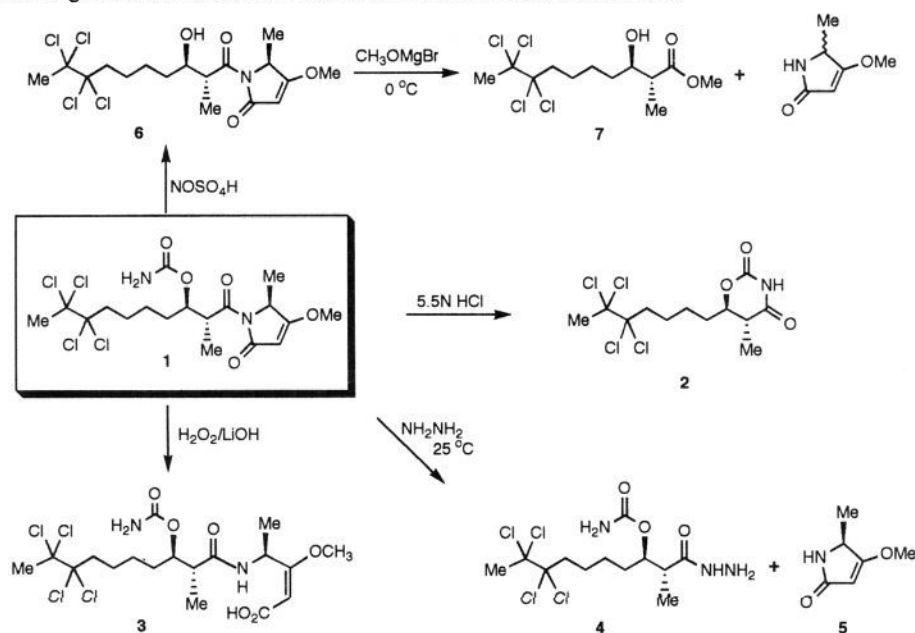
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Chart I



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 ¹H 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 substituents 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 hydroperoxide¹² 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.

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(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 oxazolindone-derived carboximides.¹²

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.

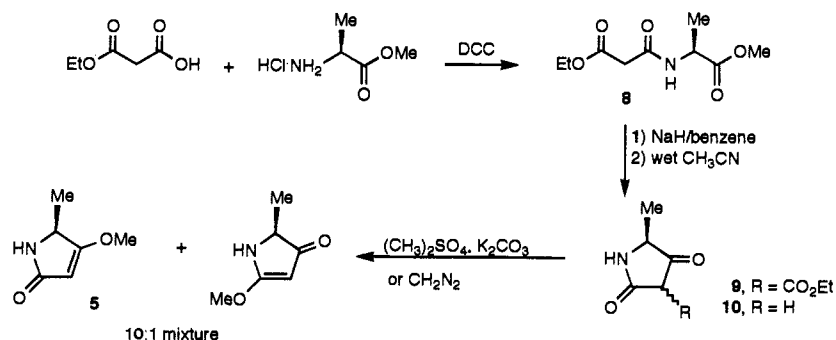
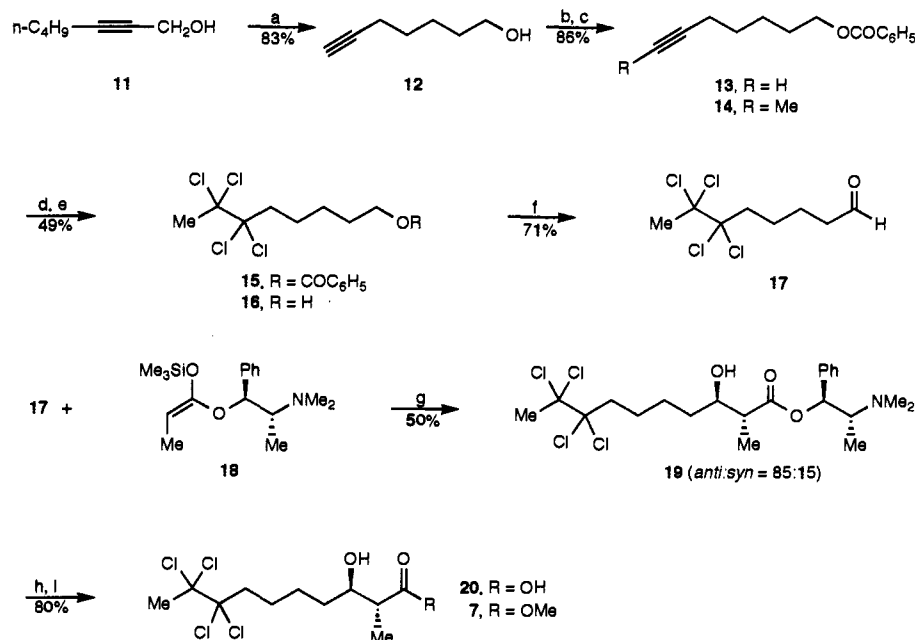
Cleavage of the *O*-carbamoyl bond in 1 and formation of alcohol 6 with retention of configuration at C-3' were accomplished by nitrosation.¹⁵ Reaction of 1 in chloroform with 23% nitrosylsulfuric acid in 1:4 sulfuric acid-*d*₂/D₂O at 0 °C led to decarbonylation and the generation of a nitrite ester. 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-

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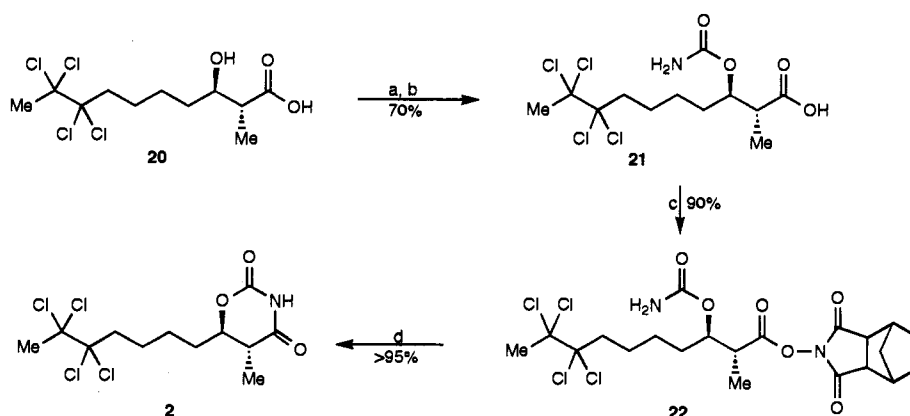
(15) Nitrosation with other reagents such as nitrous acid or nitrosium tetrafluoroborate led to either no reaction or undesired products.

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

Scheme 2

Scheme 3^a

^a (a) $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$, 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.

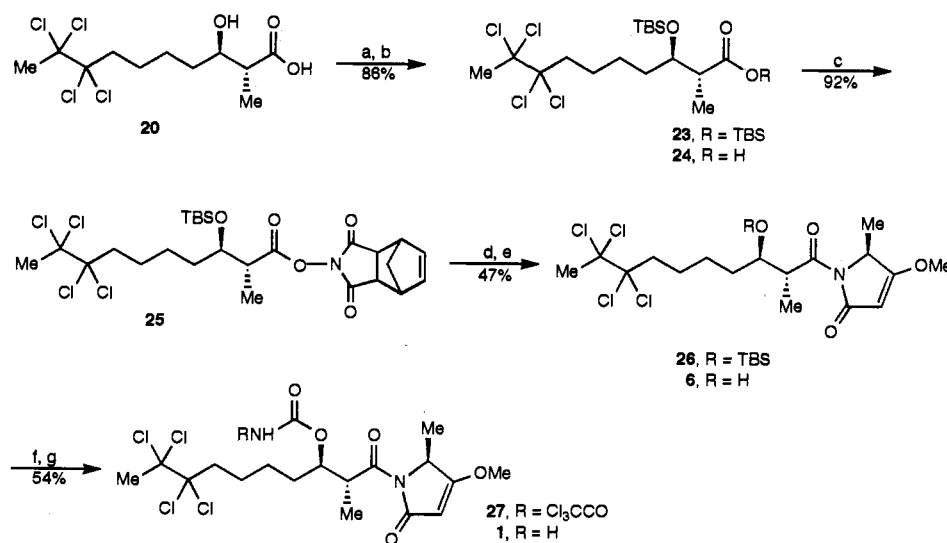
Scheme 4^a

^a (a) $\text{Cl}_3\text{CCO}-\text{N}=\text{C}=\text{O}$, 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 HONb²⁵ (*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 decarbamylation product of **1**. Finally, carbamylation was carried out by first treating **6** with trichloroacetyl isocyanate and then removing the trichloroacetyl group from the resulting imide **7** by chromatography over silica gel²⁴ 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) K₂CO₃, MeOH/THF/H₂O (3:1:1), 25 °C; KHSO₄(aq). (c) HONb, DCC, CH₂Cl₂, 25 °C. (d) 5, NaH, THF; 25, THF, 25 °C. (e) 15% HF (40%) in CH₃CN, 25 °C. (f) Cl₃CCON=C=O, CHCl₃, 25 °C. (g) CHCl₃/MeOH (9:1) over silica gel, 25 °C.

(¹H and ¹³C 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 [³⁶Cl]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 C₁ pool. This was rigorously established by two feeding experiments in which sodium [1,2-¹³C₂]acetate and [methyl-¹³C]-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-¹³C]-L-methionine, the 1 isolated from this incubation showed two strongly and equally enhanced signals (specific incorporation 4% above natural abundance) in its ¹³C 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 E²⁹ 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 malignancy 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 CDCl₃ on 11.75- and 7.05-T instruments operating at 500 and 300 MHz for ¹H and 125 and 75 MHz for ¹³C, respectively. ¹H chemical shifts are referenced to the signal for the residual acetone-*d*₅ (2.04 ppm) or CHCl₃ (7.24 ppm); ¹³C chemical shifts are referenced to the methyl-*d*₃ signal of acetone-*d*₆ (29.8 ppm) or to the CDCl₃ signal. Homonuclear ¹H NOEs were obtained by difference NOE experiments using a 3-s irradiation period. Heteronuclear ¹H-¹³C connectivities were determined by HMQC³⁰ and HMBC³¹ 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 found⁵ 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 IC₅₀s 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.

(30) Bax, A.; Subramanian, S. *J. Magn. Reson.* 1986, 67, 565–9.

(31) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* 1986, 108, 2093–4.

was flash chromatographed on a RP-18 column (40 mL of YMC-GEL, ODS 120A). The chromatogram was developed with 100 mL of each of the following solvents: 4:1, 1:1, 1:3, and 1:9 H₂O/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), [α]_D +6.5° (CHCl₃, *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 – NH₂CO₂H]⁺). High-resolution FABMS: *m/z* 491.0802 (C₁₁H₂₇Cl₁N₂O₅, Δ–12.8 mmu), 430.0505 (C₁₇H₂₄Cl₄N₃O₃, Δ0.5 mmu). IR (CHCl₃): ν_{\max} 3546, 3432, 3022, 2945, 1724, 1686, 1582, 1380, 1322 cm⁻¹. ¹H NMR (CDCl₃): 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'-OCONH₂), 1.45–1.90 (m, 4'-H₂, 5'-H₂, and 6'-H₂), 2.47–2.57 (br, 7'-H₂), 2.45 (br s, 10'-H₃). 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 NaH¹³C₃ (99 atom %) and 4.0 g of Na¹⁵N₃ (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 ¹³C NMR spectrum in acetone-*d*₆ indicated uniform enrichment in ¹³C to 82% and ¹⁵N 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, NH₂CO₂ 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 [1,2-¹³C]acetate and unlabeled acetate was fed to a culture of *S. mirabile* BY-8-1 in an 8-L bottle on day 30 after inoculation. Similarly 250-mg portions of [methyl-¹³C]-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 ¹³C NMR spectrum in acetone-*d*₆ showed roughly 0.6% ¹³C 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 6.9-g batch of dried alga from the methionine feeding experiment gave 4.9 mg of labeled 1; inspection of its proton-noise-decoupled ¹³C NMR spectrum in acetone-*d*₆ showed roughly 4% ¹³C 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 NaIO₄ solution containing a small amount of KMnO₄ 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 (CF₃CO)₂O/CH₂Cl₂ 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 CH₂Cl₂ 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 N₂. 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, [α]_D –20° (CHCl₃, *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-H₂, 8-H₂, and 9-H₂), 2.54 (br, 10-H₂), 2.46 (br s, 13-H₃). 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 – CO₂NH), 266/268/270 (M – CH₃CCl₂), 258/260 (18:6, M – 3Cl), 214/216 (18:6, M – 3Cl – CO₂), 197/199 (15:5, M – 3Cl – CO₂NH), 117 (100). HREIMS: *m/z* 266.0303 (C₁₀H₁₄Cl₂NO₃, Δ4.8 mmu), 258.0883 (C₁₂H₁₇ClNO₃, Δ1.4 mmu).

Treatment of 1 with Lithium Hydroperoxide. Using Evans' procedure,¹² H₂O₂ (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/H₂O (0.5 mL). The resulting mixture was stirred at 5–10 °C for 20 h, quenched with Na₂SO₃, 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 (CDCl₃): *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-H₃); *N*-acyl unit 2.61 (m, 2'-H), 1.21 (d, 2'-Me), 4.91 (br q, 3'-H), 4.79 (br s, 3'-OCONH₂), 1.45–1.95 (m, 4'-H₂, 5'-H₂, and 6'-H₂), 2.55 (br m, 7'-H₂), 2.48 (br s, 10'-H₃).

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, [α]_D +4.5° (MeOH, *c* 0.5); ¹H NMR (CDCl₃): 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*-(carboxyoxycetyl)-L-alanine methyl ester (8, 1 g, 4.6 mmol) was cyclized to (5*S*)-3-carboxy-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/CHCl₃), mp 103.5–105 °C. IR (KBr): ν_{\max} 3194, 1768, 1686, 1267 cm⁻¹. ¹H NMR (CDCl₃): 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). [α]_D –5.28° (CHCl₃, *c* 4.62). To a solution of 10 (320 mg, 2.8 mmol) in acetone (20 mL) was added K₂CO₃ (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; [α]_D +2.5° (MeOH, *c* 4.0). IR (KBr): ν_{\max} 3204, 1678, 1618, 1359, 1234 cm⁻¹. Anal. Calcd for C₆H₉NO₂: 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 Et₂O (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.

Decarbamyoylation of 1. A solution of 1 (6.0 mg, 12 × 10⁻³ mmol) in CDCl₃ (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 D₂O (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/CH₂Cl₂ 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/CHCl₃ to give 4 mg (74%) of **6**, $R_f = 0.78$ (silica gel, 1:10 MeOH/CHCl₃). IR (CHCl₃): ν_{\max} 3550, 2933, 1722, 1659, 1628 1320 cm⁻¹. ¹H NMR (CDCl₃): 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'-H₂, 5'-H₂, and 6'-H₂), 2.55 (m, 7'-H₂), 2.48 (br s, 10'-H₃). ¹³C NMR (CDCl₃): 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⁺ – H₂O 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 (C₁₇H₂₆Cl₄NO₄, MH⁺, $\Delta = -1.1$).

(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 (CDCl₃): δ 7.29–7.19 (m, Ph-H₃), 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'-H₂, 5'-H₂, and 6'-H₂), 2.42 (m, 7'-H₂), 2.46 (s, 3H, 10'-H₃).

Conversion of 6 to Methyl (2S,3S)-8,8,9,9-Tetrachloro-3-hydroxy-2-methyldecanoate (7). Compound **6** (3.1 mg, 6.9×10^{-3} mmol) was dissolved in 170 μ L (13.8 $\times 10^{-3}$ 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 CHCl₃/MeOH), $[\alpha]_D^{20}$ (CHCl₃, c 0.6) and 2.3 mg (94%) of methyl ester **7** as a viscous oil ($R_f = 0.68$ (silica gel, 20:1 CHCl₃/MeOH), $[\alpha]_D^{20} -2.4^\circ$ (CHCl₃, c 0.6)). IR (CHCl₃): ν_{\max} 3540, 2915, 1722, 1191 cm⁻¹. ¹H NMR (CDCl₃): 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'-H₂, 5'-H₂, and 6'-H₂), 2.52–2.60 (m, 7'-H₂), 2.47 (br s, 10-H₃). ¹³C NMR (CDCl₃): 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 C₁₁H₁₇O₃Cl₄, –1.2-mmu error).

(R)-MTPA Ester of 7. Using the procedure of Kakisawa et al.,^{10b} treatment of **7** (2 mg, 5.6 μ mol) with (S)-(+)-MTPA chloride (4 mg, 15.8 μ mol) 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 (CDCl₃): 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-H₂), 1.17 (m, 5-H₂), 1.73 (m, 6-H₂), 2.39 (m, 7-H₂), 2.44 (br s, 10-H₃).

(S)-MTPA Ester of 7. This ester was produced as described above from **7** and (R)-(-)-MTPA chloride. ¹H NMR (CDCl₃): 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, $J = 6$ Hz, 3-H), 1.76 and 1.73 (m, 4-H₂), 1.38 (m, 5-H₂), 1.83 (m, 6-H₂), 2.50 (br m, 7-H₂), 2.50 (br s, 10-H₃).

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 N₂ 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 CH₂Cl₂, and the extract was washed with 1 N HCl (aqueous wash back-extracted twice with CH₂Cl₂). The organic layers were combined, dried over MgSO₄, 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 (CDCl₃): δ 3.66 (t, $J = 6.0$ Hz, 1-H₂), 2.21 (dd, $J = 2.7$ and 6.6 Hz, H₂-5), 1.94 (t, $J = 2.7$ Hz, 7-H), 1.62–1.44 (m, 2-H₂, 3-H₂, and 4-H₂). ¹³C NMR (CDCl₃): 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 (C₇H₁₂O, $\Delta = -1.9$ mmu).

6-Octyn-1-yl Benzoate (14). To a solution of **12** (100 mg) in 5 mL of CH₂Cl₂ 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 CH₂Cl₂, and the organic layers were combined and dried over MgSO₄. 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 (CHCl₃): 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-H₂), 2.22 (m, 5-H₂), 1.95 (t, $J = 2.7$ Hz, 7-H), 1.79 (m, 2-H₂), 1.59 (m, 3-H₂ and 4-H₂). ¹³C NMR (CDCl₃): 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 (C₁₄H₁₆O₂, $\Delta = -2.8$ 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 (MgSO₄) 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 (CDCl₃): 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-H₂), 2.16 (m, 5-H₂), 1.77 (t, $J = 2.7$ Hz, 8-H₃), 1.54–1.64 (m, 2-H₂, 3-H₂, and 4-H₂). ¹³C NMR (CDCl₃): 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 (C₁₅H₁₈O₂, $\Delta = -5.5$ 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 CH₂Cl₂ (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 CH₂Cl₂. The extract was dried over MgSO₄ 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 (CDCl₃): 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-H₂), 2.59 (m, 5-H₂), 2.48 (br s, 8-H₃), 1.82–1.95 (m, 2-H₂ and 4-H₂), 1.50–1.68 (m, 3-H₂). ¹³C 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 (C₁₅H₁₈Cl₄O₂, $\Delta 4.7$ 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 \times 5 mL). The combined extracts were washed with saturated aqueous NaCl, dried (MgSO₄), 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 (CDCl₃): δ 3.69 (t, $J = 6.3$ Hz, 1-H₂), 2.56 (m, 5-H₂), 2.48 (br s, 8-H₃), 1.92 (m, 4-H₂), 1.50–1.69 (m, 2-H₂ and 3-H₂). ¹H NMR (acetone-*d*₆): δ 3.56 (t, $J = 6.0$ Hz, 1-H₂), 2.53 (m, 5-H₂), 2.46 (bs, 8-H₃), 1.90–1.79 (m, 4-H₂), 1.45–1.62 (m, 2-H₂ and 3-H₂). ¹³C 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 – H₂O ion cluster), 220/222/224/226/228 (75:100:50:10:1), 123, 109 (100), 89. HREIMS: m/z 219.9426 (C₆H₈Cl₄, $\Delta = -4.6$ mmu).

6,6,7,7-Tetrachlorooctanal (17). To a 5-mL solution of **16** (10 mg) in CH₂Cl₂ was added 2 equiv of Dess–Martin reagent²² (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} . ^1H 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). ^1H 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 ($\text{C}_6\text{H}_9\text{Cl}_4$, 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*-methylphedrinyl *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} . ^1H 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*-methylphedrine. 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). $[\alpha]_D^{25} +2.2^\circ$ (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} . ^1H 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 ($\text{C}_{10}\text{H}_{15}\text{Cl}_4\text{O}_3$, Δ –0.3 mmu). Anal. Calcd for $\text{C}_{11}\text{H}_{18}\text{Cl}_4\text{O}_3$: 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 ($[\alpha]_D^{25} -2.2^\circ$ (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). ^1H 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). ^1H 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*-butyldimethylsilyloxy)-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} . ^1H 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 (CMe_3), 25.7 (C-6), 25.5 (CMe_3), 25.0 (C-5), 18.1 (CMe_3), 17.5 (CMe_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 ($\text{C}_{19}\text{H}_{37}\text{Cl}_4\text{O}_3\text{Si}_2$, 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*-butyldimethylsilyloxy)-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} . ^1H 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 (CMe_3), 25.5 (C-6), 24.3 (C-5), 17.9 (CMe_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 ($\text{C}_{13}\text{H}_{23}\text{Cl}_4\text{O}_3\text{Si}$, 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} . ^1H 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 (CMe_3), 24.6 (C-6), 22.6

(C-5), 18.0 (CMe₃), -4.8 (SiMe), -4.6 (SiMe). EIMS: *m/z* 556/558/560/562/564 (75:100:50:10:1 M - CMe₃ ion cluster), 486, 437, 381, 236, (100), 170. HREIMS: *m/z* 556.0609 (C₂₂H₃₀Cl₄NO₅Si, 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 NaHCO₃, dried over MgSO₄, 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*-butyldimethylsilyloxy)-2'-methyl-8',9',9'-tetrachlorodecanoyl)-4-methoxy-5-methyl-3-pyrrolin-2-one (**26**) as an oil, *R_f* = 0.16 (silica gel, benzene). IR (CHCl₃): ν_{max} 2943, 1718, 1684, 1631, 1247 cm⁻¹. ¹H NMR (CDCl₃): 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'-H₂), 2.47 (s, 10'-H₃), 1.85–1.50 (m, 4'-H₂/5'-H₂/6'-H₂), 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). ¹³C NMR (CDCl₃): 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 (CMe₃), 25.74 (C-6'), 24.35 (C-5'), 18.11 (CMe₃), 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 - CMe₃ ion cluster), 434, 307, 240 (100), 184. HREIMS: *m/z* 504.0701 (C₁₉H₃₀O₄NSiCl₄, 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 CH₂Cl₂ (3 × 5 mL). The combined extracts were dried over anhydrous Na₂SO₄, 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 decarbamylation product of **1**.

Mirabimide E (1). To a cooled (0 °C) solution of **6** (7.2 mg, 16.0 μmol) in CDCl₃ (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 (CDCl₃): 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'-H₂), 2.49 (s, 10'-H₃), 1.95–1.45 (m, 4'-H₂/5'-H₂/6'-H₂), 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/CHCl₃ at 25 °C for 15 h. After filtration and removal of the solvent, the **27** was further decomposed²⁴ during preparative TLC on silica gel (10% MeOH/CHCl₃) 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% H₂O/CH₃CN 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 ([α]_D +6.3° (CHCl₃, *c* 0.25)), to natural mirabimide E.

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Supplementary Material Available: ¹H and ¹³C NMR spectra of **1**, **12-17**, and **20** and INADEQUATE ¹³C NMR spectra of uniformly ¹³C- and ¹⁵N-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.