J|A|C|S
ARTICLES
Published on Web 01/13/2006

Marinomycins A−D, Antitumor-Antibiotics of a New Structure Class from a Marine Actinomycete of the Recently Discovered Genus "*Marinispora*"

Hak Cheol Kwon, Christopher A. Kauffman, Paul R. Jensen, and William Fenical*

Contribution from the Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093-0204

Received September 8, 2005; E-mail: wfenical@ucsd.edu

Abstract: Four antitumor-antibiotics of a new structure class, the marinomycins A−D (**1**−**4**), were isolated from the saline culture of a new group of marine actinomycetes, for which we have proposed the name "*Marinispora*". The structures of the marinomycins, which are unusual macrodiolides composed of dimeric 2-hydroxy-6-alkenyl-benzoic acid lactones with conjugated tetraene-pentahydroxy polyketide chains, were assigned by combined spectral and chemical methods. In room light, marinomycin A slowly isomerizes to its geometrical isomers marinomycins B and C. Marinomycins A−D show significant antimicrobial activities against drug resistant bacterial pathogens and demonstrate impressive and selective cancer cell cytotoxicities against six of the eight melanoma cell lines in the National Cancer Institute's 60 cell line panel. The discovery of these new compounds from a new, chemically rich genus further documents that marine actinomycetes are a significant resource for drug discovery.

Introduction

The actinomycetes are Gram-positive bacteria, which for more than 50 years provided a significant source for bioactive secondary metabolites. These mainly soil-derived microorganisms have yielded more than 10 000 bioactive compounds including more than 70% of the natural antibiotics discovered.[1] Unfortunately, beginning in the late 1980s, the rate of discovery of new drug candidates from terrestrial actinomycetes began to decrease, which rendered continued exploration of this source inefficient. Ultimately, this led many of the major international pharmaceutical industries to abandon terrestrial actinomycetes in favor of alternate sources of chemical diversity such as directed and combinatorial synthesis. With the dramatic increase in the emergence of drug-resistant infectious diseases, the need to discover and develop new antibiotics has never been greater. Unfortunately, the current sources for chemical diversity do not seem to be generating new antibiotic drugs in response to this emerging challenge.

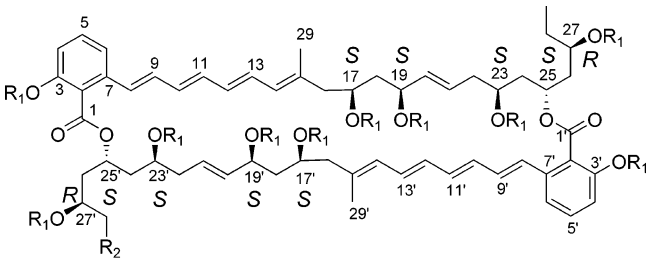
Because actinomycete bacteria are very common in soils, they are introduced into the oceans in large numbers, by runoff and river flows, leading to the hypothesis that the vast majority of strains isolated from marine sources may be of terrestrial origin.[2] With this in mind, and considering the difficulty in exploring marine ecosystems, it is no wonder that the oceans have been largely overlooked as a source for these chemically prolific bacteria. Quite recently, it has been recognized that actinomycetes adapted for life in the sea do indeed occur.[3] Using various sampling tools, including methods that provide access to deep ocean sediments, we have cultivated multiple new groups of actinomycetes from marine samples. These studies, which combine new sampling and culture methods with phylogenetic evaluation of the strains observed, have demonstrated that numerous new actinomycete taxa are present in marine habitats. This approach led to the discovery of the chemically rich genus *Salinispora*[4,5] and, more recently, a new marine actinomycete genus, originally designated as MAR2,[6] for which we now suggest the name "*Marinispora*".

The first *Marinispora* strain to be subjected to chemical study, strain CNQ-140, was isolated from a sediment sample collected at a depth of 56 m offshore of La Jolla, CA. Strain CNQ-140 was cultivated in a seawater-based medium and then extracted with the adsorbent resin XAD-7. The resin was eluted with acetone, the solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. Removal of the ethyl acetate provided an extract that demonstrated in vitro cytotoxicity against HCT-116 human colon carcinoma (IC_{50} = 1.2 μg/mL). Activity guided fractionation of the ethyl acetate extract by a diversity of chromatographic methods led to the isolation of four macrodiolides, marinomycins A−D (**1**−**4**). These interesting compounds were subsequently purified, and their structures were assigned by combined spectral and chemical methods. The marinomycins possess significant antibiotic activities, with MIC values of 0.1−0.6 μM,

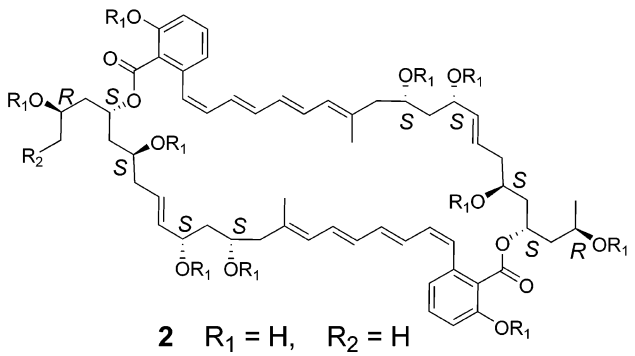
(1) Berdy, J. *J. Antibiot.* **2005**, *58*, 1−26.
(2) Goodfellow, M.; Haynes, J. A. *Biological, Biochemical, and Biomedical Aspects of Actinomycetes*; Academic Press: New York, 1984; pp 453−472.
(3) Mincer, T. J.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Appl. Environ. Microbiol.* **2002**, *68*, 5005−5011.
(4) Maldonado, L. A.; Fenical, W.; Jensen, P. R.; Kauffman, C. A.; Mincer, T. J.; Ward, A. C.; Bull, A. T.; Goodfellow, M. *Int. J. Syst. Appl. Microbiol.* **2005**, *55*, 1759−1766.
(5) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355−357.
(6) Jensen, P. R.; Mincer, T. J.; Williams, P. G.; Fenical, W. *Antonie van Leeuwenhoek* **2005**, *87*, 43−48.

10.1021/ja0558948 CCC: $33.50

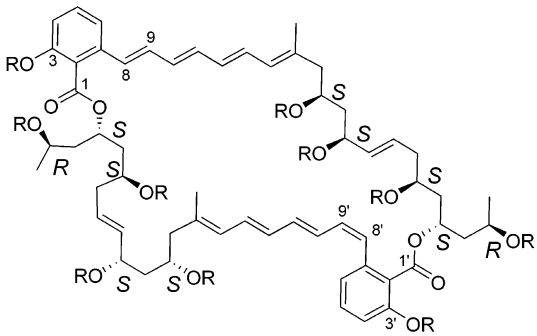
against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faceium* (VREF). In addition, the marinomycins inhibit cancer cell proliferation with average LC_{50} values of 0.2−2.7 μM against the NCI's 60 cancer cell line panel. The results of that testing further revealed that the marinomycins had potent and selective cytotoxicities against six of the eight melanoma cell lines.



1 R_1 = H, R_2 = H
1a R_1 = Ac, R_2 = H
5 R_1 = H, R_2 = CH_3



2 R_1 = H, R_2 = H
2a R_1 = Ac, R_2 = H
4 R_1 = H, R_2 = CH_3
4a R_1 = Ac, R_2 = CH_3



3 R = H,
3a R = Ac,

Results and Discussion

Marinomycin A (**1**) was obtained as a yellow powder that analyzed for the molecular formula $C_{58}H_{76}O_{14}$ by interpretation of HR-MALDI-FTMS ($[M + Na]^+$ *m/z* 1019.5190) and NMR data. The IR spectrum of **1** displayed absorption bands at 3375 and 1713 cm^{-1}, indicating the presence of hydroxyl and ester functionalities, while a UV absorption band at 359 nm was suggestive of a highly conjugated polyene moiety.[7] Proton NMR spectral data, including correlations from COSY and *J*-resolved experiments, illustrated signals attributable to a conjugated tetraene [(δ 6.35 (1H, dd, J = 11.0, 2.5 Hz), 6.49 (1H, dd, J = 14.0, 10.2 Hz), 6.52 (1H, dd, J = 14.6, 10.5 Hz), 6.63 (1H, dd, J = 14.0, 10.5 Hz), 6.74 (1H, dd, J = 14.6, 11.0 Hz), 7.11 (1H, dd, J = 15.5, 10.2 Hz), and 7.24 (1H, d, J = 15.5 Hz)] (Table 1). Two-dimensional NMR analysis, using HMBC and HMQC experiments, showed that these proton signals correlated with ^{13}C NMR bands at δ 128.7, 129.5, 131.2, 132.1, 132.4, 132.8, 135.7, and 138.3. Other characteristic features of the ^1H NMR spectrum of **1** were the presence of five oxymethine protons [δ 4.29 (1H, br m), 4.30 (1H, br m), 4.64 (1H, br m), 4.78 (1H, br m), and 6.55 (1H, m)], five methylene protons [δ 1.76−2.50], one secondary methyl group at δ 1.37 (3H, d, J = 6.3 Hz), and two additional nonconjugated trans olefinic protons [δ 5.94 (1H, dd, J = 15.0, 6.5 Hz) and 6.22 (1H, dt, J = 15.0, 7.6 Hz)]. The relative lack of branching demonstrated in these ^1H NMR signals suggested marinomycin A was composed of a linear, polyketide-type chain. Overall analysis of the NMR data indicated that marinomycin A possessed the molecular formula $C_{29}H_{38}O_7$, exactly one-half of the molecular formula, $C_{58}H_{76}O_{14}$, determined by HRMS. Thus, it became clear that marinomycin A was a symmetrical macrodiolide dimer composed of two identical C_{29} units. This was further indicated by acetylation, which yielded the deca-acetate **1a**, illustrating that marinomycin A possesses at least 10 hydroxyl groups.

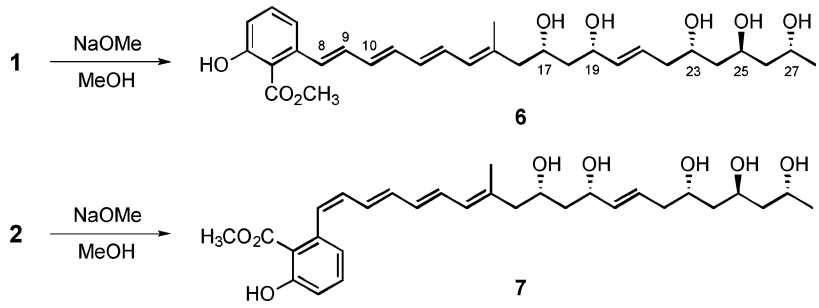
Table 1. ^1H, ^{13}C, and HMBC NMR Data for Marinomycin A (**1**)

position	δH mult (J,[g] Hz)[a]	δC[b]	HMBC[a]
1, 1′		169.7	
2, 2′		122.7	
3, 3′		156.7	
4, 4′	7.04 dd (8.2, 1.6)	115.6	1 (1′), 3 (3′), 2 (2′), 6 (6′), 5 (5′)
5, 5′	7.27 t (8.2)	131.0	3 (3′), 7 (7′), 6 (6′)
6, 6′	7.31 dd (8.2, 1.6)	116.2	7 (7′), 2 (2′), 4 (4′), 8 (8′), 5 (5′)
7, 7′		137.0	
8, 8′	7.24 d (15.5)	129.5	7 (7′), 9 (9′), 10 (10′), 6 (6′)
9, 9′	7.11 dd (15.5, 10.2)	132.4	7 (7′), 11 (11′), 10 (10′), 8 (8′)
10, 10′	6.49 dd (14.0, 10.2)[f]	132.8	11 (11′), 12 (12′), 8 (8′)
11, 11′	6.63 dd (14.0, 10.5)[f]	135.7	12 (12′), 13 (13′)
12, 12′	6.52 dd (14.6, 10.5)[f]	132.1	11 (11′), 10 (10′), 13 (13′), 14 (14′)
13, 13′	6.74 dd (14.6, 11.0)	131.2	15 (15′), 11 (11′), 12 (12′), 14 (14′)
14, 14′	6.35 dd (11.0, 2.5)	128.7	12 (12′), 13 (13′), 16 (16′), 29 (29′)
15, 15′		138.3	
16, 16′	2.43[c,f]	49.9	17 (17′), 18 (18′), 29 (29′), 15 (15′), 14 (14′)
	2.32 br d (12.2)[f]		
17, 17′	4.29 br m[d,f]	70.2	
18, 18′	2.03 m[e]	45.6	19 (19′), 17 (17′), 20 (20′)
	1.76 br d (14.2)		
19, 19′	4.78 br m	73.1	
20, 20′	5.94 dd (15.0, 6.5)	136.8	21 (21′), 19 (19′), 22 (22′)
21, 21′	6.22 dt (15.0, 7.6)	128.3	20 (20′), 19 (19′), 22 (22′), 23 (23′)
22, 22′	2.50 m[c,f]	42.6	23 (23′), 20 (20′), 21 (21′)
	2.38 m[c,f]		
23, 23′	4.30 br m[d,f]	67.8	
24, 24′	2.10 m[e,f]	44.5	23 (23′), 25 (25′), 26 (26′), 22 (22′)
25, 25′	6.55 m	71.7	1 (1′)
26, 26′	2.18 br ddd (14.2, 6.5, 3.0)	46.1	25 (25′), 24 (24′), 27 (27′), 28 (28′)
	2.00 m[e]		
27, 27′	4.64 br m	64.0	
28, 28′	1.37 d (6.3)	24.9	27 (27′), 26 (26′)
29, 29′	1.88 s	17.8	16 (16′), 15 (15′), 13 (13′), 14 (14′)

[a] 300 MHz, pyridine-d_5. [b] 75 MHz, pyridine-d_5. [c−e] Overlapping signals. [f] Chemical shifts were assigned using $^1H-^1H$ COSY and homo-*J*-resolved ^1H NMR spectral data. [g] Coupling constants derived from analysis of homo-*J*-resolved ^1H NMR spectral data.

(7) Bruno, T. J.; Svoronos, P. D. N. *Handbook of basic tables for chemical analysis*; CRC press: Florida, 2000; p 222.

Scheme 1. Methanolysis of Marinomycin A (**1**) and Marinomycin B (**2**) To Yield Esters **6** and **7**, Respectively



The ^{13}C NMR and HMQC spectra of **1** allowed all protons to be assigned to their respective carbons. The oxymethine protons correlated with oxygenated carbons carbons at δ 64.0, 67.8, 70.2, 71.7, and 73.1, while the methylene protons correlated with five methylene carbons at δ 42.6, 44.5, 45.6, 46.1, and 49.9. The aliphatic methyl group protons correlated with a carbon signal at δ 24.9, and the two nonconjugated olefinic protons to carbon signals at δ 128.3 and 136.8. In addition, the ^{1}H NMR spectrum of **1** showed additional signals attributed to a phenyl group [δ 7.04 (1H, dd, J = 8.2, 1.6 Hz), 7.27 (1H, t, J = 8.2 Hz), and 7.31 (1H, dd, J = 8.2, 1.6 Hz)], and a vinyl methyl group [δ 1.88 (3H, s)]. The HMQC NMR spectrum showed that the phenyl protons correlated to carbons at δ 115.6, 116.2, and 131.0, while the olefinic methyl group was observed at δ 17.8. As expected, the ester carbon signal was observed at δ 169.7.

Comprehensive collation of 2D NMR data from ^{1}H−^{1}H COSY, TOCSY, ROESY, HMQC, and HMBC experiments led to the construction of a 2-hydroxy-6-alkenyl-benzoic acid ester with the tetraene chain in the 6 position. The chemical shifts of the aromatic ring in this specific constellation were comparable to those from distantly related compounds available in the literature.[8] Key HMBC correlations (Table 1) allowed the vinyl methyl group to be positioned at C-15, while the full carbon chain from C-16 to C-28, including the presence of oxygen at C-17, -19, -23, -25, and -27, was readily assigned by these combined data. An HMBC correlation from the carbinol proton at C-25 to the ester carbon (C-1) indicated the site of lactonization was at C-25. The geometries of three of the double bonds in the tetraene chain (C-8−C-13) were determined to be *trans* (*E*) on the basis of their characteristic coupling constants ($J \geq$ 14.0 Hz) observed in the homo *J*-resolved ^{1}H NMR spectrum. The $\Delta^{14,15}$ trisubstituted olefin was assigned as *E* on the basis of a prominent ROESY NMR correlation between the vinyl methyl protons (H_3-29) and H-13.

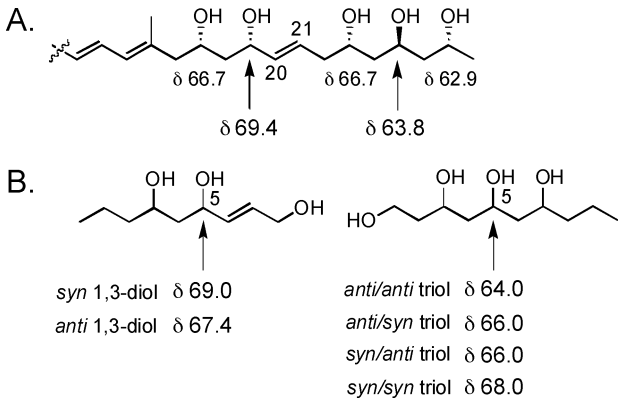


Figure 1. ^{13}C NMR chemical shift comparisons of the carbinol carbons in methyl ester **6** (A) with those of model compounds (B).

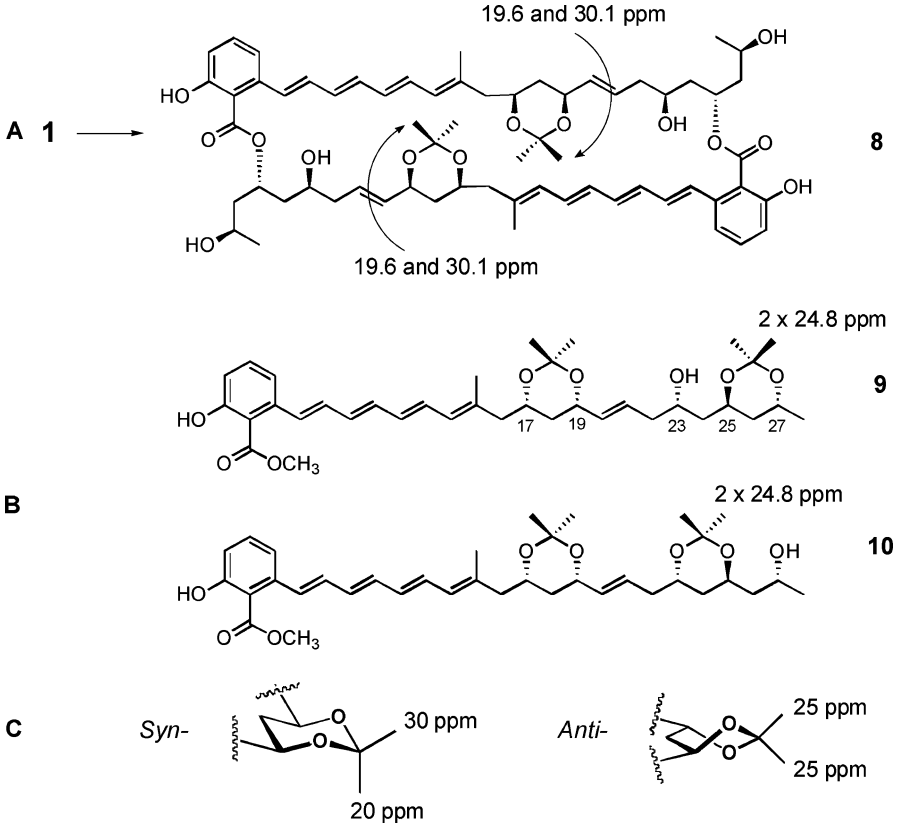
Methanolysis of marinomycin A with NaOMe in MeOH cleaved both lactone linkages to yield the monomer methyl ester **6** (Scheme 1), which was fully characterized by LC-MS, and 1D and 2D NMR methods (see Experimental Section). This experiment, and the complete NMR analysis described above, defined marinomycin A as a dimeric macrodiolide possessing an unprecedented 44-membered ring.

The relative stereochemistry of marinomycin A (**1**) was assigned on the basis of spectral analysis and chemical modification. The relative stereochemistries of the polyol functionalities (C-17, C-19, C-23, C-25, and C-27) were initially assigned by application of Kishi's Universal NMR Database,[9] and by conversion to two different acetone ketals. Kobayashi et al. showed that the relative stereochemistry of 1-ene-3,5-diols and 1,3,5-triols can be predicted by comparison of ^{13}C NMR chemical shifts at C-4 and C-5 of the stereoisomers with the model compounds non-2-en-1,4,6-triol and decan-1,3,5,7-tetraol, respectively.[9] Comparison of the ^{13}C NMR data derived from the methanolysis product **6** with those from the above model compounds (Figure 1) allowed the relative stereochemistries at several centers to be assigned. The ^{13}C NMR chemical shift of C-19 (δ 69.4) in the spectrum of **6** (DMSO-d_6) was very close to the C-4 value (δ 69.0) of the *syn*-diol in non-2-en-1,4,6-triol. Similarly, the ^{13}C NMR chemical shift of C-25 (δ 63.8) of **6** was very close to that of the C-5 carbon (δ 64.0) of *anti*,*anti*-decan-1,3,5,7-tetraol.

Treatment of **1** with 2,2-dimethoxypropane and pyridinium-*p*-toluenesulfonate in methanol afforded the bis-acetone ketal **8**, resulting from ketal formation at the C-17, C-19 and C-17′, C-19′ hydroxyl groups (Scheme 2). The chemical shifts of the acetonide methyl groups were observed at δ 19.6 and δ 30.1 (HSQC spectral data), indicating the six-membered 1,3-dioxane ring was in a chair conformation. In the NMR method described by Rychnofsky,[10] the ^{13}C NMR chemical shifts of methyl groups in a *syn*-acetonide (chair form) are commonly at δ 20 and δ 30 (axial and equatorial methyls), while the methyl signals of an *anti*-acetonide (the skew form yields identical methyl groups) are both observed at δ 25. On the basis of the observed acetonide methyl chemical shifts at δ 19.6 and 30.1, the hydroxyl groups at C-17, 19 (and C-17′, 19′) were assigned syn configurations, respectively.

(8) (a) Kim, J. W.; Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Org. Chem.* **1999**, *64*, 153−155. (b) Hedge, V. R.; Puar, M. S.; Dai, P.; Patel, M.; Gullo, V. P.; Das, P. R.; Bond, R. W.; McPhail, A. T. *Tetrahedron Lett.* **2000**, *41*, 1351−1354.
(9) Kobayashi, Y.; Czechtizky, W.; Kishi, Y. *Org. Lett.* **2003**, *5*, 93−96.
(10) Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9−17.

Scheme 2. (A) Formation of Acetonide **8** Illustrating the ^{13}C NMR Chemical Shifts of the Acetonide Methyl Carbons; (B) Acetonides **9** and **10** Illustrating the ^{13}C NMR Chemical Shifts of the Acetonide Methyl Carbons; (C) The Conformations of *syn*- and *anti*-1,3 Diol Acetonides Illustrating the Predicted ^{13}C NMR Chemical Shifts of the Acetonide Methyl Groups



In a similar fashion, treatment of the methanolysis product **6** with 2,2-dimethoxypropane and pyridinium-*p*-toluenesulfonate in methanol afforded two diacetonides, **9** and **10**, which were readily separated by HPLC methods (Scheme 2). NMR data for both bis-ketals showed acetonide methyl group signals at δ 19.6, 24.8 ($\times$2), and 30.1, consistent with the presence of both syn and anti acetonides, and consistent with the assignment predicted by Kishi's Universal Database analysis. On the basis of these NMR experiments, the relative stereochemistry of the three 1,3-diols in **1** were assigned as 17,19-*syn* (17′,19′-*syn*), 23,25-*anti* (23′,25′-*anti*), and 25,27-*anti* (25′,27′-*anti*).

The absolute stereochemistry of marinomycin A (**1**) was determined by application of the modified Mosher ester NMR method using the acetonides **9** and **10**. Treatment of acetonide **9**, in separate experiments, with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (*R*-MTPA-Cl) and (*S*)-(+)-MTPA-Cl, yielded the *S*-Mosher ester **11a** and *R*-Mosher ester **11b**, respectively. Analysis of ^1H NMR chemical shift differences ($\Delta\delta_{S-R}$) between **11a** and **11b** revealed that the absolute stereochemistry of C-23 is *S* (Figure 2).[11] Similarly, preparation of the *S*-MTPA ester (**12a**) and *R*-MTPA ester (**12b**) from **10**, followed by NMR analysis, revealed the absolute stereochemistry at C-27 is *R* (Figure 2).

The absolute stereochemistry of the allylic alcohol at C-19 in **1** was difficult to assign by the Mosher method because the hydroxyl group tended to eliminate on attempted acylation under a variety of mild reaction conditions. To approach establishing the absolute stereochemistry at this center, a series of derivatives were prepared involving hydrogenation of the olefinic bonds in **1**. Catalytic hydrogenation of **1** (10% Pd/C) yielded three HPLC-resolvable stereoisomers of perhydro-marinomycin A in a 1/2/1 ratio. The isomer mixture was fractionated [C-18 reverse phase, acetonitrile−water (9:1)] to yield **13**, the major stereoisomer of unknown configuration at C-15 (Scheme 3). Successive acetonide formation, acetylation, and acetonide deprotection

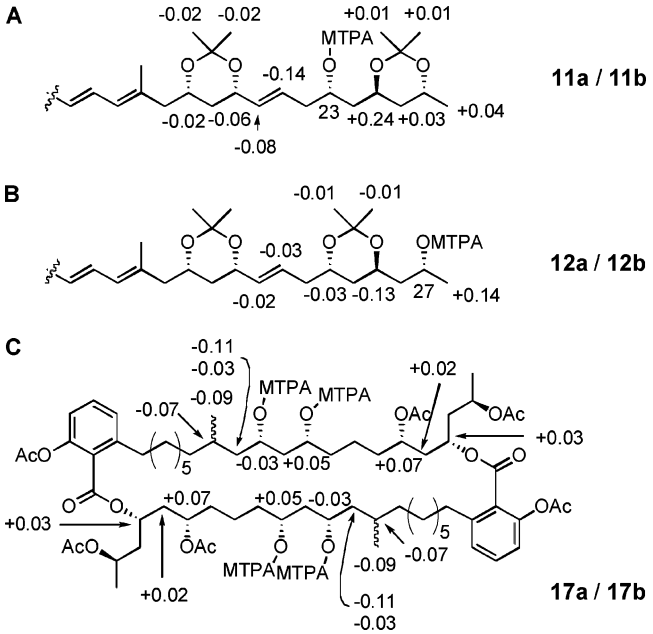
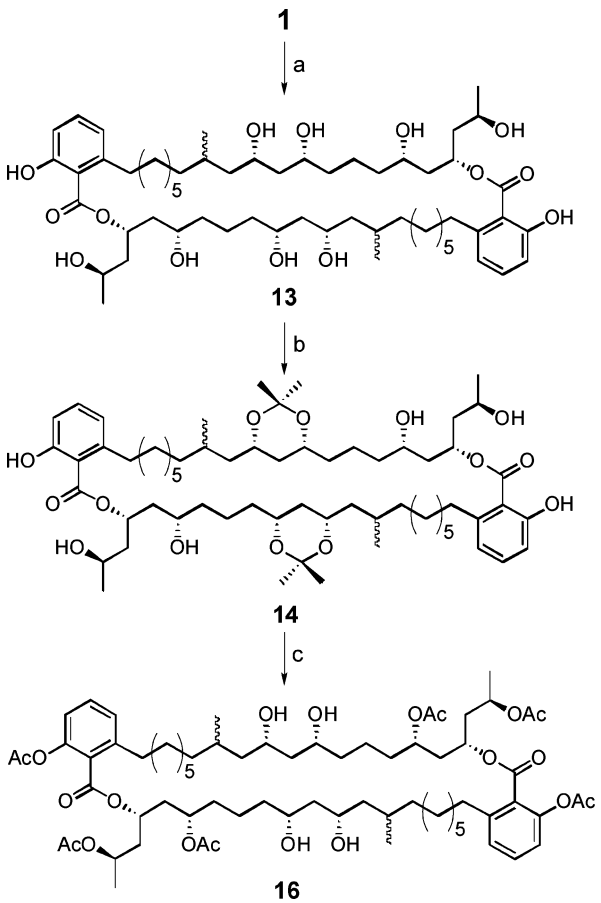


Figure 2. $\Delta\delta_{S-R}$ values for the Mosher esters **11a**/**11b**, **12a**/**12b**, and **17a**/**17b**.

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

Scheme 3. Preparation of Compound **16**[a]



[a] Reagents and conditions: (a) (i) H_2 gas, 10% Pd/C, ethanol, (ii) C-18 RP HPLC using MeCN/H_2O (9:1); (b) MeOH, 2,2-dimethoxypropane, PPTS, 0 °C; (c) (i) acetic anhydride, pyridine, (ii) MeOH, PPTS, room temperature, (iii) C-18 RP HPLC using MeCN/H_2O (9:1).

of **13** yielded the 3,3′,23,23′,27,27′-hexa-acetate derivative **16** (Scheme 3). Treatment of **16** with *R*-MTPA-Cl and *S*-MTPA-Cl, under standard acylation conditions, afforded tetra-*S*-MTPA-ester (**17a**) and tetra-*R*-MTPA-ester (**17b**), respectively. Application of both the classic Mosher ester ^{19}F NMR method and the modified Mosher ester analysis[12] allowed assignment of the absolute stereochemistry at C-17, C-17′, C-19, and C-19′. NMR data, showing negative Δ_{S-R} values for the four fluorine signals in the ^{19}F NMR spectrum of **17a** and **17b** [**17a** δ 5.38, 5.39, 5.46, and 5.55; **17b** δ 5.56 (×3), 5.68; TFA δ 0.00], were obtained (Figure 2).[13] These results supported the assignment of the absolute stereochemistry at C-17, C-17′, C-19, C-19′, C-23, C-23′, C-25, and C-25′ as *S*, while C-27 and C-27′ were assigned as *R*. While it is not always feasible to correctly interpret the NMR shift data derived from multiple Mosher esters, Riguera and co-workers have shown[14] that the MTPA esters of *syn*-1,3-diols can be confidently used to assign absolute stereochemistry.

Marinomycin B (**2**) was isolated as a yellow powder that analyzed for the molecular formula $C_{58}H_{76}O_{14}$ by HRESI-MS data ([obsd M + Na]$^+$ at *m/z* 1019.5110), and by comprehensive analysis of NMR data (Table 2). The UV absorption spectrum of **2** showed bands at 315 and 340 nm, which were suggestive of the presence of the conjugated polyene as in **1**. However, the UV absorption spectrum of **2** differed in fine structure, indicating that **2** is the geometric isomer of **1**.[7] The configurations of the double bonds in **2** were assigned on the basis of *J*-resolved and 1D proton−proton NMR coupling constant data and upon analysis of ROESY NMR information. The geometries of the $\Delta^{8,9}$ and $\Delta^{12,13}$ olefins were assigned as *Z* and *E*, respectively, based upon proton coupling constants of $J_{H-8,H-9}$ = 11.0 Hz and $J_{H-12,H-13}$ =14.6 Hz. ROESY NMR correlations between the vinyl methyl protons (H_3-29) and the proton at C-13 established the configuration of the $\Delta^{14,15}$ olefin as *E*. Unfortunately, overlapping NMR signals for the protons at C-10 and C-11 made assignment of the $\Delta^{10,11}$ olefin geometry by coupling constant analysis impossible. However, ROESY NMR correlations between H-9 (δ 6.43) and H-12 (δ 6.19) with the overlapping H-10/H-11 overlapping pair (δ 6.60), coupled with a lack of correlation between H-9 and H-12 (1D ROE experiment), indicated that the configuration of the $\Delta^{10,11}$ double bond was *E*.

Comprehensive NMR analysis, utilizing data from COSY, HMQC, and HMBC experiments, allowed the complete assignment of the proton and carbon signals for **2**, leading to the

Table 2. ^1H and ^{13}C NMR Data of Marinomycin B (**2**) in Pyridine-d_5 and $CDCl_3$

position	δH mult (*J*, Hz[n]) 300 MHz, pyridine-d_5	δH mult (*J*, Hz[n]) 400 MHz, $CDCl_3$	δC (pyridine-d_5)	δC ($CDCl_3$)
1, 1′			170.5	171.6
2, 2′			122.2	110.8
3, 3′			160.0	163.4
4, 4′	7.06 d (8.2)	6.91 d (8.2)	116.1	117.2
5, 5′	7.32 t (8.2)	7.34 t (8.2)	132.0	134.5
6, 6′	6.94 d (8.2)	6.70 d (8.2)	121.6	123.1
7, 7′			139.2	140.8
8, 8′	6.90 d (11.0)	6.66 d (11.0)	129.7	132.8
9, 9′	6.43 m	6.13 m[f]	130.2	129.8
10, 10′	6.60 m[a]	6.23 m[g]	128.0	128.2[l]
11, 11′	6.60 m[a]	6.23 m[g]	136.5	135.1
12, 12′	6.19 m (14.6, 10.3)[b,m]	6.13 m[f]	128.3[j]	128.4[l]
13, 13′	6.71 dd (14.6, 11.0)	6.35 dd (14.6, 11.0)	130.8	131.8
14, 14′	6.22 d br d (11.0, 2.5)[b,m]	5.86 d br d (11.0, 2.5)	128.3[j]	128.5[l]
15, 15′			139.0	135.7
16, 16′	2.42 m[c,m]	1.81 m[h,m]	49.1	49.1
17, 17′	4.35 br m[d,m]	3.57 br tt (10.0, 4.0)	68.8	70.4
18, 18′	2.0−2.2 μ[e] 1.83 br dt (15.5, 3.5)	1.34 br d (15.5) 1.08 m	44.6[k]	44.2
19, 19′	4.71 br m	3.91 br d (9.5)	71.6	71.9
20, 20′	5.91 dd (15.2, 5.9)	5.29 br s[i]	136.5	135.1
21, 21′	6.13 dt (15.2, 7.5)[b]	5.29 br s[i]	127.4	128.6[l]
22, 22′	2.50 m[c,m] 2.53 m[c,m]	1.90 m	42.0	41.1
23, 23′	4.17 br m	3.41 br tt (9.0, 4.5)	67.0	66.7
24, 24′	2.0−2.2 μ[e]	1.71 m[h,m]	44.6[k]	43.0
25, 25′	6.30 m	5.42 m	72.2	73.5
26, 26′	2.0−2.2 μ[e]	1.66 m[h,m] 1.79 m[h,m]	44.6[k]	46.1
27, 27′	4.38 br m[d,m]	3.99 m	64.0	65.0
28, 28′	1.37 d (6.4)	1.17 d (6.4)	24.2	23.5
29, 29′	1.92 s	1.68 s	17.8	17.3
OH		4.20 (4H, br s) 4.40 (2H, br s)		
3-OH		11.68 s		

[a−k] Overlapping signals. [l] Interchangeable signals. [m] Chemical shifts were assigned by interpretation of $^1H-^1H$ COSY NMR data. [n] Coupling constants determined by analysis of homo-*J*-resolved ^1H NMR spectral data.

(12) Seco, J. M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 12−117.
(13) Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203−10213.
(14) Freire, F.; Seco, J. M.; Quiñoa, E.; Riguera, R. *J. Org. Chem.* **2005**, *70*, 3778−3790.

Table 3. ^{1}H and ^{13}C NMR Data for Marinomycin C (**3**)

position	δH mult (*J*, Hz)[a]	δC[b]	position	δH mult (*J*, Hz)[a]	δC[b]
1		170.7	1′		171.7
2		116.3	2′		110.4
3		158.7	3′		163.2
4	6.81 d (8.2)	116.3	4′	6.92 d (8.1)	117.1
5	7.24 t (8.2)	132.8	5′	7.36 t (8.1)	134.3
6	7.01 d (8.2)	118.0	6′	6.71 d (8.1)	123.0
7		138.7	7′		140.3
8	6.89 d (14.7)	130.8	8′	6.69 d (10.6)	132.4[l]
9	6.66 dd (14.7, 10.0)	132.5[l]	9′	6.16 m[i]	129.8
10	6.30−6.50 m[c]	129.6	10′	6.30−6.50 μ[c]	128.2[p]
11	6.30−6.50 m[c]	134.6[m]	11′	6.30−6.50 μ[c]	134.7[m]
12	6.31 m[c,q]	131.4	12′	6.16 m[i]	128.4[p]
13	6.40 m[c,q]	131.8[n]	13′	6.40 m[c,q]	131.6[n]
14	5.97 d (10.4)	127.9	14′	5.88 d (11.0)	128.5[p]
15		136.3[o]	15′		136.1[o]
16	2.00−2.10 m[d]	49.6	16′	1.80−2.10 μ[d]	48.3
17	3.80 m[e,q]	69.4	17′	3.55 br m[n,q]	69.3
18	1.50−1.70 m[f]	43.7	18′	1.40 br d (15.0)	44.4
				0.90 m[q]	
19	4.25 br m[g]	73.5	19′	4.02 br m[j,q]	72.2
20	5.51 br s[h]	135.6	20′	5.35 br s[k]	135.1
21	5.51 br s[h]	127.9	21′	5.35 br s[k]	128.6[p]
22	2.15 m[d,q]	41.4	22′	1.95 m[d,q]	41.0
23	3.75 br m[e,q]	66.7	23′	3.46 br m[n,q]	66.7
24	1.65−1.85 m[f]	42.5	24′	1.60−1.75 μ[f]	42.8
25	5.65 br m	71.2	25′	5.55 br m	72.2
26	1.65−1.85 μ[f]	44.5	26′	1.60−1.75 μ[f]	45.7
27	3.85 br m[e,q]	64.1	27′	3.95 br m[j,q]	64.3
28	1.18 d (6.5)	23.2	28′	1.16 d (6.5)	23.2
29	1.72 s	17.7	29′	1.68 s	16.4
OH	4.25 br s[g]		OH	11.55 s/9.82 br s	
	4.61 br s, 4.49 br s				

[a] 300 MHz, $CDCl_3$. [b] 75 MHz, $CDCl_3$. [c−k] Overlapping signals. [l−p] Interchangeable signals. [q] Chemical shifts were assigned by analysis of ^{1}H−^{1}H COSY NMR data.

assignment of a planar structure for this compound. As in **1**, methanolysis of marinomycin B (**2**) with NaOMe in MeOH yielded only one product, the methyl ester **7**, which confirmed that **2** was also a symmetrical dimeric macrodiolide (Scheme 1).

Marinomycin C (**3**) was also obtained as yellow powder that analyzed for the formula $C_{58}H_{76}O_{14}$ by HRESI-TOF MS data ([obsd M + Na]$^+$ at *m*/*z* 1019.5110) and comprehensive NMR data. As in marinomycins A and B, UV absorption bands at 319, 345, and 358 nm confirmed that **3** also possessed the conjugated phenyl-tetraene functionality. The ^{1}H and ^{13}C NMR spectra of **3** showed features similar to those of **1** and **2**, but in this case the NMR data were more complex, indicating that **3** was an unsymmetrical dimer (Table 3). Methanolysis of **3** with NaOMe in MeOH yielded **6** and **7** in an approximately 1/1 ratio by NMR and LC-MS analyses. Given this information, marinomycin C (**3**) was assigned as an unsymmetrical dimer possessing the all *E* tetraene functionality in one half and the $\Delta^{8,9} = Z$ olefin in the second half.

The assignment of the absolute stereochemistries of the polyol carbons in marinomycins B and C (**2** and **3**) were not approached using the classic methods applied to marinomycin A. This proved to be unnecessary, because it was observed that marinomycin A could be photochemically converted to a mixture of B and C under ordinary room light ($T^{1/2}$ = ca. 1 h). The NMR data, HPLC retention times, and optical rotations derived from marinomycins B and C, derived by photoconversion from A, were identical to those data derived from B and C originally isolated from the fermentation broth.

Marinomycin D (**4**) was isolated as a yellow powder that analyzed for the molecular formula $C_{59}H_{78}O_{14}$, by HR ESI-TOF MS (*m*/*z* 1033.5267 [M + Na]$^+$) and NMR methods. This formula showed the addition of CH_2 to the molecular formulas of the other marinomycins, suggesting an additional methylene or methyl group had been added to the polyketide-like chain. The UV absorption spectrum of **4** was in good agreement with that of marinomycin B. The ^{1}H and ^{13}C NMR spectra for **4** were almost identical to those of the *Z*,*E*,*E*,*E*-tetraene in **2** (Table 4.). The major difference between the spectra for **2** and **4** was the presence of a new triplet methyl group (C-29′, δ 0.93). HMBC NMR spectral data showed a strong correlation between the methyl triplet (H-29′), and C-28′, and C-27′, thus indicating that the linear chain in one half of marinomycin D had been extended by one carbon. Because one half of marinomycin D possessed a different carbon skeleton, we were unable to chemically correlate the stereochemistry of **4** to the fully defined stereochemistry of marinomycin A. However, based upon the optical properties of **4**, and the excellent comparison of NMR data to those from **2**, we reasonably assume that the stereochemistry is identical to **2** at comparable centers. Having rigorously defined the absolute stereochemistries of marinomycins A−C at all oxymethine centers, we were surprised to find that marinomycin A showed $[\alpha]_D = +180°$, while marinomycins B−C showed negative $[\alpha]_D$ values of −245° and −151°, respectively. The rotation of marinomycin D was $[\alpha]_D = -233°$, a value similar to marinomycin B, its methylene lower homologue. Because it seemed unreasonable that these simple olefin geometrical isomers would show opposite rotations, we examined the CD spectra of **1**−**4** (Figure 3). Unusual and complex results were obtained that indicated that marinomycin A (**1**) was more optically complex than B−D. The CD spectrum of marinomycin A (**1**) showed a positive peak at 380 nm and a negative peak at 360 nm, as well as a broad negative band between 325 and 365 nm (Figure 3). Because the UV spectrum of **1** showed λ_{max} at 325 (sh), 345 (sh), 359, and 378 nm, the peaks at 380 and 360 nm seem to be due to a positive Cotton effect by exciton coupling between the polyene chromophores of the monomeric units. While the CD spectra of **2** and **3** did not indicate an exciton coupling pattern, they did show a broad negative band between 320 and 410 nm, which corresponds to the UV absorption bands of **2** and **3**. In the end, we concluded that these data were too complex to interpret, but that they suggested differences in ring conformations, and perhaps that the chirality was influenced by, if not derived from, interaction of the polyene chains in marinomycin A.

To explore a possible conformational explanation, ROESY NMR experiments were conducted with marinomycins A and B. For marinomycin A, six strong NOE correlations, which included H-6(6′) to H-9(9′), H-8(8′) to H-25(25′), H-12(12′) to H-19(19′), H-14(14′) to H-17(17′), and H-29(29′) to H-13(13′), were observed (Figure 4). The strong transannular NOEs observed between H-12(12′) across the ring to H-19(19′), and from H-14(14′) to H-17(17′), provided strong evidence that the side chains in **1** were parallel and in very close proximity. In contrast, marinomycin B showed only two NOE correlations, H-8(8′) to H-23(23′) and H-10(10′) to H-6(6′), which demonstrated that **2** existed in an entirely different ring conformation. Simple molecular models of marinomycins A and B (see Supporting Information), which were constructed based upon

Table 4. ^{1}H and ^{13}C NMR Data for Marinomycin D (**4**)

position	δH mult (*J*, Hz)[a]	δC[b]	position	δH mult (*J*, Hz)	δC
1, 1′		171.7	18, 18′	1.34 br d (14.6)/1.11 m	44.2
2, 2′		110.8	19, 19′	3.90 br d (9.0)	71.8
3, 3′		163.5	20, 20′	5.28 br s[e]	135.1
4, 4′	6.91 d (8.1)	117.2	21, 21′	5.28 br s[e]	128.6[f]
5, 5′	7.35 t (8.1)	134.5	22, 22′	1.91 m	41.1
6, 6′	6.70 d (8.1)	123.1	23, 23′	3.40 br t (9.0)	66.8
7, 7′		140.8	24, 24′	1.72 m	43.0/43.9
8, 8′	6.66 d (11.0)	132.8	25, 25′	5.43 m	73.4/73.7
9, 9′	6.13 m[c]	129.9	26, 26′	1.62 m/1.72 m	46.1
10, 10′	6.26 m[d]	128.2[f]	27, 27′	3.98 br m/3.65 br m	64.9/70.0
11, 11′	6.26 m[d]	135.1	28	1.17 d (6.6)	23.5
12, 12′	6.13 m[c]	128.3[f]	28′	1.46 m	30.4
13, 13′	6.36 dd (14.6, 11.0)	131.8	29, 30′	1.68 s	17.3
14, 14′	5.86 br d (11.0)	128.4[f]	29′	0.93 t (7.3)	10.5
15, 15′		135.7			
16, 16′	1.82 m	49.1	OH	4.20 (4H, br m)/4.40 (2H, br s)	
17, 17′	3.57 br m	70.4	3- and 3′-OH	11.68 s/11.70 s	

[a] 300 MHz, $CDCl_3$. [b] 75 MHz, $CDCl_3$. [c–e] Overlapping signals. [f] Interchangeable signals.

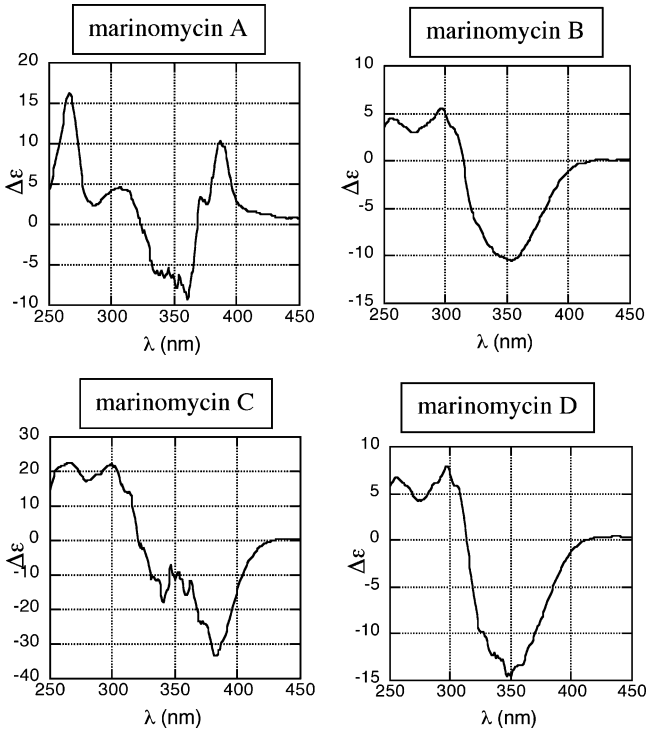


Figure 3. CD spectra of marinomycins A–D (**1**–**4**) in methanol.

these NOE data and the $\Delta^{8,9} = E$ and *Z* olefin geometries, demonstrated that in marinomycin considerable transannular interaction was indeed feasible, while in marinomycin B the ring must expand to accommodate the *Z*-olefin. In marinomycin A, the polyene-polyol chains adopt an opposing, coplanar, but adjacent array that places the polyene chains within very close proximity. In this configuration, the polyene chromophores are in proximity and, conceivably, able to interact.

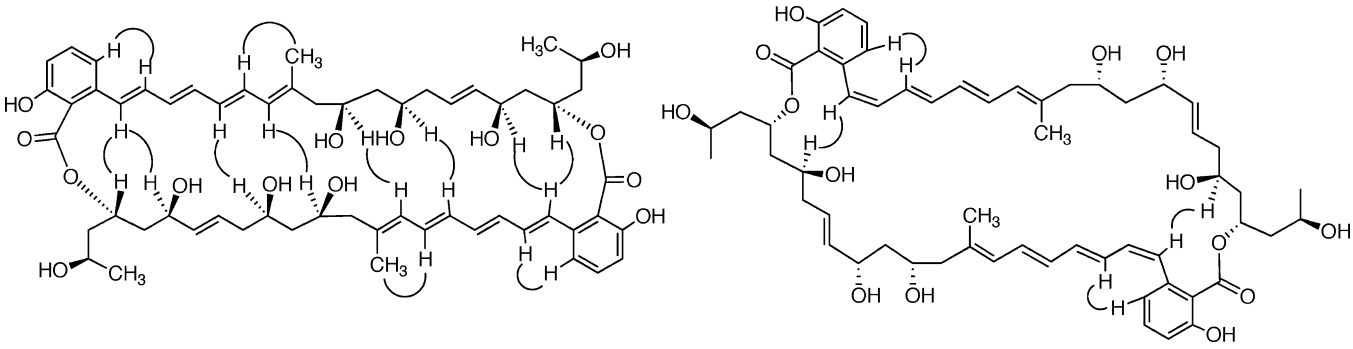
Interactions of polyolefinic systems are well known to generate chirality in the absence of chiral carbons. In the polyene carotenoid pigments, which lack chiral carbons, dimeric structures are produced by aggregation in polar solvents. This results in defined interactions of their polyolefinic backbones creating tortionally defined exciton coupling, which generate Cotton effects in their CD spectra.[15] An intramolecular exciton coupling within marinomycin A (**1**), generated by conformational interactions of the polyenes, rather than molecular aggregation, could explain the differences in the rotation and CD data for **1** and **2**–**4**.

To explore this further, we recorded the CD spectrum of the monomeric polyene **6** under identical conditions. The spectrum was essentially featureless with very poor to no absorptions in the 350–400 nm range. Hence, we hypothesize that in marinomycin A (**1**), the all-*E* geometry of the tetraene functionality leads to transannular hydrogen bonding, thus creating a configuration which may facilitate olefinic exciton coupling. In contrast, in marinomycins B–D, NOE evidence suggests that these rings are expanded to a circular configuration by virtue of their $\Delta^{8,9} = Z$ olefinic bonds, resulting in few transannular interactions.

It seem reasonable that the interactions described above could explain the positive and negative $[\alpha]_D$ values for **1** and for **2**–**4**. Although perhaps not the best models, reversals in the sign of $[\alpha]_D$ values have been observed in the olefin geometrical isomers of the secondary metabolites clathrynamide and hanliangicin.[15]

Bioactivity of the Marinomycins. The marinomycins showed significant antibacterial and in vitro cancer cell cytotoxicities. Marinomycin A (**1**) was the most potent antibacterial agent showing in vitro minimum inhibitory concentrations (MIC_{90}) of 0.13 μM against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Streptococcus faecium* (VREF) (Table 5). Given the presence of a polyene chain, and recognizing the apparent mechanism of action of the polyene antifungal agents,[17] we assumed that the marinomycins would be potent antifungal agents. This turned out to be incorrect, as only marinomycin A showed very weak activity below 10 μM against *Candida albicans*. We also evaluated the antibacterial activity of compounds **6**, **7**, **8**, the hexa-acetate derivative of **8** (**8a**), and the 3,3′,23,23′,27,27′-hexa-acetate of **1** (**8b**) (Table 5).

(15) Simonyi, M.; Bakadi, Z.; Zsila, F.; Deli, J. *Chirality* **2003**, *15*, 680–698.
(16) (a) Ojika, M.; Itou, Y.; Sakagami, Y. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1568–1573. (b) Kundim, B. A.; Itou, Y.; Sakagami, Y.; Fudou, R.; Iizuka, T.; Yamanaka, S.; Ojika, M. *J. Antibiot.* **2003**, *56*, 630–638.
(17) (a) Bolard, J. *Biochim. Biophys. Acta* **1986**, *864*, 257–304. (b) De Kruijff, B.; Demel, R. A. *Biochim. Biophys. Acta* **1974**, *339*, 57–70. (c) Finkelstein, A.; Holz, R. In *Membranes 2: Lipid Bilayers and Antibiotics*; Eisenman, G., Ed.; Marcel Dekker: New York, 1973; Chapter 5. (d) Matsumori, N.; Yamaji, N.; Oishi, T.; Murata, M. *J. Am. Chem. Soc.* **2002**, *124*, 4180–4181. (e) Matsuoka, S.; Murata, M. *Biochim. Biophys. Acta* **2002**, *1654*, 429–434.



1 **2**

Figure 4. Planar views of the 44-membered rings in marinomycins A (**1**) and B (**2**) based upon transannular nuclear Overhauser enhancements observed in ROESY NMR experiments.

Table 5. Antimicrobial Bioassay Results for Marinomycins A−D (**1**−**4**) and Synthetic Derivatives[a]

compound	*Staphylococcus aureus* methicillin-resistant (MRSA) MIC_{90} μM[b]	*Enterococcus faecium* vancomycin-resistant MIC_{90} μM[b]	*Candida albicans* wild type MIC_{90} μM[c]
1	0.13	0.13	7.8
2	0.25	NSA	NSA
3	0.25	NSA	NSA
4	0.25	NSA	NSA
6	NSA	NSA	N/T
7	NSA	NSA	N/T
8	2.3	NSA	N/T
8a	1.8	NSA	N/T
8b	NSA	NSA	N/T

[a] NSA = not significantly active (MIC_{90} values above 10.0 μM), N/T = not tested. **8a** = the hexa-acetate derivative of **8**; **8b** = 3,3′,23,23′,27,27′-hexa-acetate of **1**. [b] The optical density (OD) was measured at 600 nm using a Molecular Devices Emax microplate reader, and the MIC_{90} was determined by the analysis program SOFTmax PRO. The MIC_{90} of vancomycin is 0.195−0.391 μg/mL, and that of penicillin G is 6.25−12.5 μg/mL. [c] Alamar Blue was used as an indicator to measure cell proliferation. The dye yields a colorimetric change that enables the MIC to be confidently estimated by visual means. The MIC of amphotericin B is 1.56−0.78 μg/mL.

Compound **8** showed a roughly 10-fold decrease in MRSA activity as compared to marinomycin A.

More impressive potency and selectivity was observed when the marinomycins were examined in the NCI's panel of 60 cancer cell lines. Marinomycin A (**1**) showed significant tissue type selectivity, with the most sensitive cancer types being six of the eight melanoma cell lines (LOX IMVI, M14, SK-MEL-2, SK-MEL-5, UACC-257, and UACC-62). The most sensitive strain was SK-MEL-5 melanoma (LC_{50} = 5.0 nM), which was approximately 500 times more sensitive than the average LC_{50} value of 2.7 μM. Marinomycins B (**2**) and C (**3**) also showed potent activities with average LC_{50} values of 0.9 and 0.2 μM, respectively. Significantly, the compounds were only weakly active against all six leukemia cell lines in the panel with LC_{50} values of approximately 50 μM.

As mentioned earlier, throughout this study we were confronted with the tendency of all of the marinomycins to photoisomerize in room light. A photochemical $\Delta^{8,9}$ olefin isomerization of **1** was observed when it was exposed to ambient light, in glass (methanol) at room temperature. The time course of this interconversion, monitored by LC-MS, resulted in an equilibrium mixture of marinomycins A (**1**), B (**2**), and C (**3**) within 1 h of exposure. Knowing this in advance greatly facilitated the isolation and purification of the isomeric marinomycins under low light conditions. Cultivation in the dark resulted in the production of mainly marinomycin A (**1**) and much smaller amounts of **2** and **3**. While these observations suggest that marinomycin A (**1**) could be the true natural product, we could not rigorously prove this assumption. In addition, given that marinomycin D has one $\Delta^{8,9} = Z$ olefin, and the propensity of the olefin at this position in **1** to photoisomerize, it is conceivable that an all-*E* isomer of **4** (structure **5**) could also be the true natural product. By careful LC-MS examination, we did observe a trace component of the fermentation mixture with the correct molecular weight for **5**. However, this compound was in very small amounts, was highly photochemically reactive, and could not be fully purified.

The marinomycins are the first examples of this new macrodiolide class; however, the dimeric 2-hydroxy-6-alkylbenzoic acid lactone functionality has been observed in the microbial metabolite (+)-SCH 351448, an activator of low-density lipoprotein receptor (LDL-R) promoter.[8b] The marinomycins possess unique polyene-polyol structures, and have unique photoreactivities and chiroptical properties. Their potent and selective anticancer properties in the NCI panel suggest these metabolites may inhibit tumor proliferation by a specific, but as yet unknown, mechanism of action. The fact that these polyenes lack substantive antifungal activity suggests they are not membrane active like the well-known polyene antibiotics.[17]

Last, the marinomycins were isolated from the fermentation broth of a marine actinomycete belonging to a new genus, for which we suggest the name "*Marinispora*". Based upon phylogenetic analyses using 16s rRNA gene sequence data, this genus clusters within the family Streptomycetaceae, but clearly lies outside of all known terrestrial genera including the genus *Streptomyces*.[6] More than 20 "*Marinispora*" isolates have been recovered from a diversity of marine habitats, suggesting that this new genus is widespread in the marine environment. Chemical analysis of other "*Marinispora*" isolates has shown the production of a significant number of diverse secondary metabolites that are under current investigation.

Experimental Section

Isolation of CNQ-140 Strain, Cultivation, Extraction. *Marinispora* strain CNQ-140 was isolated on medium A1+C (10 g of starch, 4 g of peptone, 2 g of yeast extract, 1 g of calcium carbonate, 18 g of agar, 1 L of seawater) from a marine sediment collected at a depth of 56 m 1 mile Northwest of the Scripps Institution of Oceanography Pier (La Jolla, CA.) The strain was cultured in 20 × 1 L volumes of medium

TCG (3 g of tryptone, 5 g of casitone, 4 g of glucose, 1 L of seawater) while shaking at 230 rpm for 7 days. At the end of the fermentation period, 20 g/L XAD-7 adsorbent resin were added to each flask, and they were allowed to shake at a reduced speed for 2 additional hours. The resin was then collected by filtration through cheesecloth, washed with deionized water, and eluted twice with acetone. Evaporation of the extraction solvent in vacuo left a wet residue that was taken up in EtOAc, providing approximately 125 mg of dry extract per 1 L of culture after removal of solvent.

Isolation and Purification of Marinomycins A−D (1−4). The combined EtOAc extract from two 20 × 1 L fermentations (5.0 g) was subjected to silica gel column chromatography purification eluting with solvent mixtures of *n*-hexanes−EtOAc (1:1), EtOAc, EtOAc−MeOH (10:1), EtOAc−MeOH (5:1), EtOAc−MeOH (2:1), and 100% MeOH, successively. The EtOAc−MeOH (10:1) eluting fraction (Q140−3) showed the most potent HCT-116 cytotoxicity and by LC-MS contained the marinomycins. These compounds were conspicuous on silica gel and C-18 TLC because of their characteristic UV absorbance (yellow fluorescence at 365 nm) and brown color development when the plates were heated after spraying with a vanillin-H_2SO_4 reagent. Fraction Q140−3 was refractionated by C-18 reversed-phase HPLC with MeCN−H_2O (65:35) to obtain impure marinomycins A−D as major components [retention times: Q140−3-A, 26.33−35.35 min; Q140−3-B, 36.40−43.00 min; Q140−3-C, 43.50−51.65 min; Q140−3-D, 51.65−58.00 min]. Final purification of marinomycins A−D (**1**−**4**) was achieved by repeated C-18 RP HPLC with MeCN−H_2O (65:35). Typical recoveries of the marinomycins A−D from a 1 L culture were 2, 1.8, 2.4, and 0.5 mg, respectively.

Marinomycin A (1). Yellow powder, $[\alpha]_D$ +180° (*c* 0.11, EtOH). UV (EtOH): 325 sh (ϵ = 29 000), 345 sh (ϵ = 41 600), 359 (ϵ = 52 300), 378 nm (ϵ = 44 000). IR (neat): 3375, 2938, 2863, 1713, 1656, 1581, 1463, 1414, 1384, 1288, 1253, 1213, 1175, 1125, 1074, 1000, 969 cm^{-1}. HR MALDI-FT MS: obsd *m*/*z* 1019.5190 $[M + Na]^+$, $C_{58}H_{76}O_{14}Na$ requires 1019.5127. See Table 1 for NMR data.

Marinomycin B (2). Yellow powder, $[\alpha]_D$ −245° (*c* 0.15, EtOH). UV (EtOH): 315 (ϵ = 55 300), 340 (ϵ = 50 300) nm. IR (neat): 3375, 2938, 1719, 1660, 1600, 1453, 1375, 1287, 1256, 1225, 1175, 1125, 1073, 1000, 978 cm^{-1}. HR ESI-TOF MS: obsd *m*/*z* 1019.5110 $[M + Na]^+$, $C_{58}H_{76}O_{14}Na$ requires *m*/*z* 1019.5127. See Table 2 for NMR data.

Marinomycin C (3). Yellow powder, $[\alpha]_D$ −161° (*c* 0.13, EtOH). UV (EtOH): 319 sh (ϵ = 40 800), 345 sh (ϵ = 48 300), 358 (ϵ = 53 800), 375 (ϵ = 41 400) nm. IR (neat): 3375, 2925, 1719, 1656, 1588, 1450, 1375, 1338, 1288, 1256, 1225, 1125, 1069, 1000, 975 cm^{-1}. HR ESI-TOF MS: obsd *m*/*z* 1019.5110 $[M + Na]^+$, $C_{58}H_{76}O_{14}Na$ requires 1019.5127. See Table 3 for NMR data.

Marinomycin D (4). Yellow powder, $[\alpha]_D$ −233° (*c* 0.03, EtOH). UV (EtOH): 315 (ϵ = 58 200), 340 (ϵ = 50 600) nm. IR (neat): 3375, 2925, 1781, 1737, 1650, 1600, 1463, 1429, 1376, 1199, 1119, 1063, 1025, 978 cm^{-1}. HR ESI-TOF MS: obsd *m*/*z* 1033.5267 $[M + Na]^+$, $C_{59}H_{78}O_{14}Na$ requires 1033.5289. See Table 4 for NMR data.

Marinomycin A Deca-acetate (1a). Marinomycin A (**1**, 1 mg) was treated with acetic anhydride and pyridine (500 μL each) for 12 h at room temperature. Removal of the reactants under reduced pressure provided the deca-acetate derivative **1a** (1 mg). ^1H NMR (500 MHz, $CDCl_3$): δ 1.21−1.31 (br m), 1.78 (6H, br s), 1.80−1.90 (10H, m), 1.92−2.10 (30H, br m), 2.24−2.33 (10H, br m), 4.97 (6H, m), 5.19 (4H, m), 5.42 (2H, dd, *J* = 15.0, 7.0 Hz), 5.59 (2H, dt, *J* = 15.0, 7.5 Hz), 5.90 (2H, d, *J* = 11.0 Hz), 6.26 (2H, dd, *J* = 14.5, 11.0 Hz), 6.41 (2H, dd, *J* = 15.0, 10.5 Hz), 6.44 (2H, dd, *J* = 15.0, 10.5 Hz), 6.52 (2H, dd, *J* = 14.5, 10.0 Hz), 6.71 (2H, d, *J* = 15.0 Hz), 6.77 (2H, dd, *J* = 15.0, 10.0 Hz), 6.97 (2H, d, *J* = 8.0 Hz), 7.32 (2H, t, *J* = 8.0 Hz), 7.43 (2H, d, *J* = 8.0 Hz). ESI-MS: *m*/*z* 1439 $[M + Na]^+$.

Marinomycin B Deca-acetate (2a). Marinomycin B (**2**, 1 mg) was treated with acetic anhydride and pyridine at room temperature for 12 h. Removal of all reactants under reduced pressure provided marinomycin B deca-acetate (**2a**, 1 mg). ^1H NMR (300 MHz, $CDCl_3$): δ 1.24 (6H, d, *J* = 6.0 Hz), 1.62 (2H, m), 1.68 (6H, s), 1.75−1.90 (12H, m), 1.93 (6H, s), 1.97 (6H, s), 2.01 (12H, s), 2.14 (4H, m), 2.32 (3H, s), 2.35 (2H, m), 4.96 (6H, m), 5.17 (4H, m), 5.36 (2H, dd, *J* = 15.5, 6.8 Hz), 5.54 (2H, dt, *J* = 15.5, 6.8 Hz), 5.78 (2H, d, *J* = 10.5 Hz), 6.14 (2H, dd, *J* = 14.6, 9.5 Hz), 6.50 (8H, m), 6.79 (2H, dd, *J* = 15.0, 10.0 Hz), 7.03 (2H, d, *J* = 8.0 Hz), 7.37 (2H, t, *J* = 8.0 Hz), 7.39 (2H, d, *J* = 8.0 Hz). LCMS: *m*/*z* 1439 $[M + Na]^+$.

Marinomycin C Deca-acetate (3a). Marinomycin C (**3**, 1 mg) was acetylated by the same method as above to afford marinomycin C deca-acetate (**3a**, 1 mg). ^1H NMR (300 MHz, $CDCl_3$): δ 1.23 (3H, d, *J* = 6.0 Hz), 1.24 (3H, d, *J* = 6.0 Hz), 1.69 (1H, m), 1.74 (3H, s), 1.76 (3H, s), 1.77−1.89 (12H, m), 1.92 (3H, s), 1.95 (9H, s), 1.99 (3H, s), 2.01 (9H, s), 2.15−2.25 (5H, m), 2.28 (3H, s), 2.30 (3H, s), 2.35 (2H, m), 4.96 (6H, m), 5.17 (4H, m), 5.39 (2H, dd, *J* = 15.5, 6.8 Hz), 5.55 (1H, dt, *J* = 15.5, 6.8 Hz), 5.60 (1H, dt, *J* = 15.5, 6.8 Hz), 5.84 (2H, d, *J* = 10.5 Hz), 6.14 (1H, dd, *J* = 14.6, 10.0 Hz), 6.23 (1H, dd, *J* = 14.6, 10.5 Hz), 6.35−6.55 (8H, m), 6.69 (1H, d, *J* = 15.5 Hz), 6.77 (1H, dd, *J* = 15.5, 10.0 Hz), 6.96 (1H, d, *J* = 8.0 Hz), 7.02 (1H, d, *J* = 8.0 Hz), 7.24 (1H, ovlp with solvent), 7.33 (1H, t, *J* = 8.0 Hz), 7.37 (1H, t, *J* = 8.0 Hz), 7.43 (1H, d, *J* = 8.0 Hz). LCMS: *m*/*z* 1439 $[M + Na]^+$.

Marinomycin D Deca-acetate (4a). Marinomycin D (**4**, 1 mg) was acetylated using the same method used for marinomycin A to give the deca-acetate **4a** (1 mg) in pure form. ^1H NMR (500 MHz, $CDCl_3$): δ 0.92 (3H, t, *J* = 7.5 Hz), 1.21−1.31 (br m, overlap with impurity), 1.68 (6H, s), 1.75−1.90 (12H, m), 1.93 (6H, s), 1.97 (6H, s), 2.01 (12H, s), 2.14 (4H, m), 2.28 (2H, br m), 2.31 and 2.32 (6H, each s), 4.86−5.0 (6H, m), 5.11−5.20 (4H, m), 5.36 (2H, dd, *J* = 15.0, 7.0 Hz), 5.53 (2H, dt, *J* = 15.0, 7.0 Hz), 5.77 (2H, d, *J* = 11.0 Hz), 6.13 (2H, dd, *J* = 14.6, 9.8 Hz), 6.37−6.60 (10H, m), 7.02 (2H, d, *J* = 8.0 Hz), 7.24 (d, overlap with solvent peak), 7.37 (2H, t, *J* = 8.0 Hz). ESI-MS: *m*/*z* 1453 $[M + Na]^+$.

Methanolysis of 1 To Yield Ester 6. Marinomycin A (**1**, 10 mg) was dissolved in 5% NaOMe in MeOH and stirred for 10 h at 40 °C. The reaction mixture was neutralized with 1 N aqueous HCl, the aqueous phase was extracted with EtOAc, and the residue after solvent removal was purified by C18 HPLC using MeCN−H_2O (4:6) to yield compound **6** (9 mg): $[\alpha]_D$ −18.2° (*c* 0.16, EtOH). ^1H NMR (400 MHz, DMSO-d_6): δ 1.03 (3H, d, *J* = 5.9 Hz, H-28), 1.24 (2H, m, H-24), 1.30 (2H, m, H-26), 1.37 (1H, br ddd, *J* = 14.0, 6.6, 3.7 Hz, H-18), 1.47 (1H, ddd, *J* = 14.0, 8.1, 6.6 Hz, H-18), 1.75 (3H, s, H-29), 2.04 (2H, m, H-22), 2.13 (2H, br d, *J* = 5.9 Hz, H-16), 3.66 (1H, m, H-23), 3.68 (1H, m, H-17), 3.79 (3H, s, OMe), 3.78 (1H, m, H-27), 3.84 (1H, m, H-25), 4.07 (1H, m, H-19), 4.34 (3H, br s, OH), 4.50 (1H, br s, OH), 4.69 (1H, br s, OH), 5.36 (1H, dd, *J* = 15.0, 6.6 Hz, H-20), 5.54 (1H, dt, *J* = 15.0, 7.3 Hz, H-21), 5.91 (1H, d, *J* = 11.0 Hz, H-14), 6.22 (1H, dd, *J* = 14.6, 11.0 Hz, H-12), 6.35 (1H, d, *J* = 15.4 Hz, H-8), 6.39 (1H, dd, *J* = 14.6, 10.3 Hz, H-10), 6.50 (1H, dd, *J* = 14.6, 11.0 Hz, H-11), 6.55 (1H, dd, *J* = 14.6, 11.0 Hz, H-13), 6.77 (1H, d, *J* = 8.1 Hz, H-4), 6.88 (1H, dd, *J* = 15.4, 10.3 Hz, H-9), 7.07 (1H, d, *J* = 8.1 Hz, H-6), 7.16 (1H, t, *J* = 8.1 Hz, H-5), 8.50 (s, 3-OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.3 (C-29), 24.4 (C-28), 41.0 (C-22), 44.7 (C-26, C-18), 47.4 (C-24), 48.4 (C-16), 51.9 (OMe), 62.9 (C-27), 63.8 (C-25), 66.7 (C-17, C-23), 69.4 (C-19), 114.4 (C-6), 114.6 (C-4), 120.6 (C-2), 126.5 (C-21), 126.8 (C-14), 127.7 (C-8), 129.9 (C-5), 130.3 (C-13), 130.4 (C-12), 131.2 (C-9), 131.6 (C-10), 134.9 (C-7), 135.0 (C-11), 135.3 (C-20), 138.2 (C-15), 155.2 (C-3), 168.1 (C-1). ESI-LC-MS: C-18 column, rt 12.2 min (10% MeCN/H_2O−100% MeCN/H_2O, 20 min). UV (DAD): 358, 374 (sh) nm. LRMS: $[M + Na]^+$ *m*/*z* 553 amu.

Photoisomerization of Marinomycin A (1) To yield Marinomycins B and C (2, 3). A solution of marinomycin A (**1**, 7.0 mg) in methanol (5 mL) was exposed to intense room light for 2 h. The solution was analyzed by reversed-phase HPLC, which showed the presence of three major compounds. The products were isolated by semipreparative

C-18 RP HPLC using 65% MeCN/H_2O to obtain **1** (3.2 mg), **2** (0.4 mg), and **3** (1.8 mg). The ^{1}H NMR spectrum of each isolated compound (**1**−**3**), as well as their UV spectra, were identical to the compounds isolated from the original fermentation extract. The optical rotation values were **1**, $[\alpha]_D$ +168° (*c* 0.16, EtOH); **2**, $[\alpha]_D$ −310° (*c* 0.02, EtOH); **3**, $[\alpha]_D$ −151° (*c* 0.09, EtOH), which were similar to those of the compounds isolated from the fermentation mixture.

Methanolysis of 2 To Yield Ester 7. Marinomycin B (**2**, 2 mg) in 5% NaOMe in methanol was stirred for 5 h at 40 °C, and the reaction mixture was processed as above for **1**. The crude product was analyzed by LC-MS, which showed compound **7** as the major product, rt 12.8 min (10% MeCN/H_2O−100% MeCN/H_2O, 20 min). $[\alpha]_D$ −12° (*c* 0.05, EtOH). UV (DAD): 315, 340 nm. LR-ESI-MS: $[M + Na]^+$ *m*/*z* 553. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.03 (3H, d, J = 6.0 Hz, H-28), 1.22−1.37 (5H, m), 1.46 (1H, ddd, J = 14.0, 7.0, 6.5, H-18), 1.75 (3H, s, H-29), 2.05 (2H, m, H-22), 2.12 (2H, br d, J = 6.0 Hz, H-16), 3.67 (2H, m, H-23, H-17), 3.74 (3H, s, OCH_3), 3.77 (1H, m, H-27), 3.84 (1H, m, H-25), 4.06 (1H, m, H-19), 4.20 (1H, br d, J = 4.5 Hz, OH), 4.29 (2H, br s, OH), 4.45 (1H, br d, J = 5.0 Hz, OH), 4.65 (1H, br d, J = 3.5 Hz, OH), 5.35 (1H, dd, J = 15.5, 6.5 Hz, H-20), 5.53 (1H, dt, J = 15.5, 7.0 Hz, H-21), 5.87 (1H, d, J = 11.5 Hz, H-14), 6.20 (1H, m), 6.24 (1H, d, J = 11.5 Hz), 6.31 (1H, m), 6.51 (2H, m, H-10, H-11), 6.55 (1H, dd, J = 14.5, 11.5 Hz, H-13), 6.80 (1H, d, J = 8.0 Hz, H-4), 6.82 (1H, d, J = 8.0 Hz, H-6), 7.26 (1H, t, J = 8.0 Hz, H-5), 10.05 (1H, br s, OH).

Methanolysis of 3 To Yield Esters 6 and 7. Marinomycin C (**3**, 2 mg) was also treated with 5% NaOMe in methanol for 5 h at 40 °C, and the product mixture was analyzed by LC-MS as above. The LC-MS chromatogram illustrated the presence of compounds **6**, rt 12.2 min (*m*/*z* 553 amu $[M + Na]^+$), and **7**, rt 12.8 min (*m*/*z* 553 amu $[M + Na]^+$), as the major products.

Bisacetonide 8. Marinomycin A (**1**, 1.0 mg) was dissolved in 2,2-dimethoxypropane (1 mL) and methanol (0.2 mL), and pyridinium-*p*-toluenesulfonate (2 mg) was added. The reaction was allowed to stir for 6 h at 0 °C. The reaction was then quenched with 5% aqueous $NaHCO_3$, and the aqueous phase was extracted thrice with CH_2Cl_2. The CH_2Cl_2 solution was dried (anhyd. $MgSO_4$), the solvent was removed under reduced pressure, and the residue was purified by C-18 RP HPLC (82:18: MeCN−H_2O) to provided compound **8** (0.6 mg). ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.27−1.33 (8H, m), 1.36 (6H, d, J = 6.0 Hz), 1.46−1.55 (8H, m), 1.56 (6H, s), 1.60 (6H, s), 1.85 (6H, s), 2.00−2.20 (14H, m), 2.26 (2H, br d), 2.40−2.53 (8H, m), 4.22 (2H, br m), 4.32 (2H, br m), 4.57 (4H, br m), 5.82 (2H, dd, J = 15.5, 6.2 Hz), 6.20 (2H, dt, J = 15.5, 7.3 Hz), 6.23 (2H, d, J = 11.0 Hz), 6.36 (2H, dd, J = 14.6, 10.2 Hz), 6.45 (2H, m), 6.49 (2H, dd, J = 14.6, 10.2 Hz), 6.62 (2H, dd, J = 15.0, 11.0 Hz), 6.72 (2H, dd, J = 14.6, 11.0 Hz), 7.04 (2H, d, 8.0 Hz), 7.10 (2H, dd, 15.5, 10.2 Hz), 7.25 (2H, d, J = 15.5 Hz), 7.28 (2H, t, J = 8.0 Hz), 12.13 (2H, br s). ESI LCMS (C-18, 80% MeCN/H_2O): retention time 6.6 min, *m*/*z* 1099 $[M + Na]^+$.

Bisacetonides 9 and 10. Methyl ester **6** (5.0 mg) was dissolved in 2,2-dimethoxypropane (3 mL), and methanol (1 mL) and pyridinium-*p*-toluenesulfonate (5 mg) were added. The reaction was allowed to proceed for 12 h at room temperature and then quenched with 5% aqueous $NaHCO_3$, extracted 3 times with CH_2Cl_2, the solvent extracts were combined and dried (anhydrous $MgSO_4$), and the solvent was removed under reduced pressure. The residue obtained was fractionated by silica gel HPLC (2:1, *n*-hexanes−ethyl acetate) to provide compounds **9** (1.0 mg) and **10** (1.3 mg). For acetonide **9**, ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.15 (3H, d, J = 6.0 Hz, H-28), 1.34−1.40 (2H, m, H-18), 1.41 (3H, s, acetonide CH_3), 1.45 (3H, s, acetonide CH_3), 1.49 (3H, s, acetonide CH_3), 1.51 (3H, s, acetonide CH_3), 1.51 (1H, m, H-26), 1.65 (1H, m, H-26), 1.73 (1H, m, H-24), 1.82 (1H, m, H-24), 1.86 (3H, s, H-29), 2.23 (1H, dd, J = 13.0, 5.5 Hz, H-16), 2.45 (3H, m, H-16, H-22), 3.94 (3H, s, OCH_3), 3.98 (1H, br m, H-27), 4.14 (1H, br m, H-17), 4.23 (1H, br m, H-23), 4.45 (2H, br m, H-19, H-25), 5.74 (1H, dd, J = 15.5, 6.0 Hz, H-20), 6.11 (2H, m, H-21, H-14), 6.38 (1H, dd, J = 14.5, 11.0 Hz, H-12), 6.50 (1H, dd, J = 14.5, 10.0 Hz, H-10), 6.61 (1H, dd, J = 14.5, 11.0 Hz, H-11), 6.70 (1H, dd, J = 14.5, 11.0 Hz, H-13), 7.03 (1H, d, J = 8.0 Hz, H-8), 7.03 (1H, d, J = 8.0 Hz, H-4), 7.06 (1H, dd, J = 15.0, 10.0 Hz, H-9), 7.28 (2H, m, H-5, H-6). LCMS (C-18 RP column, 75% MeCN/H_2O), retention time 6.9 min (UV DAD λ_{max} 356, 374 (sh) nm), ESI MS *m*/*z* 633 $[M + Na]^+$. For acetonide **10**, ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.34 (3H, d, J = 6.0 Hz, H-28), 1.40 (1H, m, H-18), 1.41 (3H, s, acetonide CH_3), 1.46 (3H, s, acetonide CH_3), 1.49 (3H, s, acetonide CH_3), 1.52 (3H, s, acetonide CH_3), 1.54 (1H, m, H-18), 1.66 (2H, m, H-24, H-26), 1.73 (2H, m, H-24, H-26), 1.86 (3H, s, H-29), 2.26 (2H, m, H-16, H-22), 2.43 (1H, m, H-22), 2.44 (1H, m, H-16), 3.94 (3H, s, OCH_3), 3.98 (1H, br m, H-23), 4.15 (1H, br m, H-17), 4.26 (1H, br m, H-27), 4.41 (1H, br m, H-25), 4.48 (1H, br m, H-19), 5.72 (1H, dd, J = 15.5, 6.0 Hz, H-20), 5.92 (1H, dt, J = 15.5, 7.0 Hz, H-21), 6.14 (1H, d, J = 11.0 Hz, H-14), 6.38 (1H, dd, J = 14.5, 11.0 Hz, H-12), 6.49 (1H, dd, J = 14.5, 10.0 Hz, H-10), 6.60 (1H, dd, J = 14.5, 11.0 Hz, H-11), 6.70 (1H, dd, J = 14.5, 11.0 Hz, H-13), 7.04 (1H, d, J = 15.0 Hz, H-8), 7.04 (1H, d, J = 8.0 Hz, H-4), 7.08 (1H, dd, J = 15.0, 10.0 Hz, H-9), 7.28 (2H, m, H-5, H-6). LCMS (C-18 RP column, 75% MeCN/H_2O), retention time 6.6 min (UV DAD λ_{max} 356, 373 (sh) nm), ESI MS *m*/*z* 633 $[M + Na]^+$.

Mosher MTPA Esters 11a/11b. Acetonide **9** (1.0 mg) was divided into two portions, and each was dissolved in 500 μL of pyridine-d_5 in a 5 mm NMR tube. To each NMR tube were added 10 μL of (*R*)-MTPACl and 10 μL of (*S*)-MTPACl, respectively. After 12 h, the reaction was complete and 1D ^{1}H NMR spectra for (*S*)-mosher ester **11a** and (*R*)-mosher ester **11b** were recorded (see Supporting Information for NMR data).

Mosher MTPA Esters 12a/12b. Acetonide **10** (1.3 mg) was divided into two portions, and each one was treated with pyridine-d_5 and (*R*)- and (*S*)-MTPACl in separate 5 mm NMR tubes as above. The ^{1}H NMR spectra for the (*S*)-Mosher ester **12a** and (*R*)-Mosher ester **12b** were recorded (see Supporting Information for NMR data).

Hydrogenation of Marinomycin A (1) To Yield 13a−c. To a solution of marinomycin A (**1**, 4 mg) in ethanol (4 mL) was added 10% Pd/C (25 mg), and the mixture was stirred at room temperature, under an atmosphere of H_2 for 3 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue obtained was analyzed by C-18 LC-MS, which showed the three isomeric perhydro-marinomycin A derivatives, that were isolated by C-18 RP HPLC (9:1 MeCN−H_2O) to give compounds **13a**, **13b**, and **13c**, as viscous oils, in a 1/2/1 ratio by LC-MS analysis. LC-MS data (C-18 RP column, linear gradient elution: 10−100% MeCN/H_2O in 25 min) retention times: **13a** 23.5 min, **13b** 24.2 min, and **13c** 24.9 min. UV spectra (DAD): all peaks show λ_{max} 210, 245 nm. LRMS (ESI): **13a**, **13b**, **13c**, *m*/*z* 1017 $[M + H]^+$, *m*/*z* 1039 $[M + Na]^+$.

Conversion of Perhydro-marinomycin A to Hexa-acetate 16. To a solution of compound **13b** (1.6 mg) in methanol (0.5 mL) were added 2,2-dimethoxypropane (3 mL) and pyridinium-*p*-toluenesulfonate (5 mg), and the solution was stirred for 6 h in an ice bath. A 5% aqueous $NaHCO_3$ solution was then added, and the mixture was extracted with CH_2Cl_2 as in the preparation of acetonide **8**. The CH_2Cl_2 layer was dried (anhydrous $MgSO_4$) and concentrated under reduced pressure to obtain the bisacetonide **14** (1.5 mg). Acetylation of **14** with Ac_2O/py provided compound **15** (1.6 mg, not characterized), which was dissolved in methanol, and pyridinium-*p*-toluenesulfonate was added and then stirred for 2 h at room temperature. The reaction mixture was then quenched with 5% aqueous $NaHCO_3$, the solution was partitioned between CH_2Cl_2 and H_2O, and the organic layer was separated, dried (anhydrous $MgSO_4$), and concentrated under reduced pressure. The residue was purified by C-18 RP HPLC (MeCN−H_2O, 9:1), to provide the hexa-acetate **16** (1 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (3H, d J = 5.9 Hz), 0.86 (3H, d, J = 5.9 Hz), 1.24 (48H, br m), 1.43 (12H, m), 1.55 (br s, overlap with H_2O peak), 1.89 (8H, br s), 1.98 (12H, s),

2.28 (6H, s), 2.35 (2H, t, $J = 7.8$ Hz), 2.64 (4H, br s), 3.81 (2H, br m), 3.91 (2H, br m), 4.90 (2H, br m), 4.96 (2H, br m), 5.11 (2H, br m), 6.95 (2H, d, $J = 7.8$ Hz), 7.08 (2H, d, $J = 7.8$ Hz), 7.32 (2H, t, $J = 7.8$ Hz). LCMS (C-18 RP column, 75% MeCN/H_2O (0−5 min)−100% MeCN (−10 min) rt for **16**: 10.2 min. UV DAD spectrum: 210, 235 (sh) nm. LRMS (ESI): *m*/*z* 1269 $[M + H]^+$, *m*/*z* 1291 $[M + Na]^+$.

Tetra-MTPA Esters of 16 (17a/17b). To a solution of **16** (0.5 mg) in CH_2Cl_2 (2 mL) were added triethylamine (10 μL), (dimethylamino)-pyridine (1 mg), and (*R*)-MTPACl (10 μL) in sequence. The reaction mixture was stirred for 24 h at room temperature, and then *N*,*N*-dimethyl-1,3-propanediamine (12 μL) was added and stirring was continued for an additional 10 min. The solution was concentrated under reduced pressure, and the residue was fractionated by Sephadex LH-20 column chromatography using CH_2Cl_2−MeOH (1:1). The fractions containing the MTPA ester were combined and purified by silica gel column chromatography (*n*-hexanes−EtOAc; 2:1) to afford 0.5 mg of the (***S***)-MTPA ester **17a**. In a similar fashion, **16** was treated with (*S*)-MTPACl (10 μL) and pyridine (100 μL) to afford, after fractionation as above, 0.7 mg of the (*R*)-MTPA ester **17b** (see Supporting Information for NMR data).

Acknowledgment. This research is a result of generous financial support from the National Institutes of Health, National Cancer Institute, under grant R37 CA44848, and by the University of California Industry-University Cooperative Research Program (IUCRP, grant BioSTAR 10354). P.R.J. and W.F. are scientific advisors to and stockholders in Nereus Pharmaceuticals, the corporate sponsor of the IUCRP award. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict of interest policies.

Supporting Information Available: Spectral data sets (1D and 2D NMR, ESI-MS, UV−bis, HRMS, etc.) for **1**−**4**, ^1H and ^{13}C NMR spectra, ESI-MS data for **6**−**17**, and molecular models showing the predicted ring configurations of marinomycins A and B (**1**, **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0558948