Salinipyrones and Pacificanones, Mixed-Precursor Polyketides from the Marine Actinomycete *Salinispora pacifica*

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Chemical examination of a phylogenetically unique strain of the obligate marine actinomycete *Salinispora pacifica* led to the discovery of four new polyketides, salinipyrones A and B (**1**, **2**) and pacificanones A and B (**3**, **4**). These compounds appear to be derived from a mixed-precursor polyketide biosynthesis involving acetate, propionate, and butyrate building blocks. Spectral analysis, employing NMR, IR, UV, and CD methods and chemical derivatization, was used to assign the structures and absolute configurations of these new metabolites. Salinipyrones A and B displayed exactly opposite CD spectra, indicating their pseudoenantiomeric relationship. This relationship was shown to be a consequence of the geometric isomerization of one double bond. The phenomenon of polyketide module skipping is proposed to explain the unusual biosynthesis of the salinipyrones and the pacificanones.

The existence of actinomycetes indigenous to the marine environment has been debated,[1] but recent research results have shown that the ocean is indeed a rich habitat for these chemically prolific microorganisms. Although their ecological roles in the marine environment remain largely unknown, some marine actinomycetes are adapted to live in seawater, some are found in association with invertebrate hosts, while others have been recovered from the deepest ocean trenches. As an example, a salt-dependent pelagic marine actinomycete belonging to the family Nocardioidaceae was recently identified in surface sea water.[2] Marine invertebrates, especially sponges, are now well-known as hosts for diverse bacterial assemblages and represent rich sources for novel actinobacteria.[3] From marine sediments, we recently reported the first obligate marine actinomycete genus, the *Salinispora*,[4] which has proven to be a prolific source of secondary metabolites.[5] Our continuing research on *Salinispora* diversity, based upon comprehensive comparisons of 16S rDNA sequence data, has resulted in the cultivation of a third *Salinispora* species for which the name *Salinispora pacifica* has been proposed.[6] An investigation of the secondary metabolites produced by *S. pacifica* led to the isolation of the structurally novel metabolites cyanosporasides A and B, which raised intriguing questions about the genetic capacity of *Salinispora* strains to produce enediyne antibiotics.[7] Further genetic analysis of other *S. pacifica* strains collected from Palau[8] revealed a new phylotype (strain CNS-237) that differed from the cyanosporaside-producing strains at only three nucleotide positions (16S rRNA gene).[9] Chemical screening of this strain by LC-MS analysis indicated that a secondary metabolite profile was quite different from those of the other *S. pacifica* phylotypes.

Here we report the isolation and structural determination of four secondary metabolites from *S. pacifica*, strain CNS-237, cultivated in a seawater-based medium. In culture, this strain produced the related polyketides salinipyrones A and B (**1**, **2**) and pacificanones A and B (**3**, **4**).

Results and Discussion

Salinipyrone A (**1**) was obtained as a viscous oil that analyzed for the molecular formula $C_{17}H_{24}O_4$ by ESIHRMS (obsd $[M + H]^+$ at *m/z* 293.1744, calcd $[M + H]^+$ 293.1747). This molecular formula was supported by ^1H and ^{13}C NMR data (Table 1). The ^{13}C NMR spectrum of **1** showed nine sp^2 carbon resonances, one oxygenated sp^3 carbon, and seven carbon signals in the aliphatic region. An observed IR absorption at 1655 cm^{-1} demonstrated the presence of an unsaturated ester functionality, and this was confirmed by the presence of an ester carbonyl carbon at δ_C 167.5 in the ^{13}C NMR spectrum. Since four double bonds (eight olefinic carbons) and one ester carbonyl accounted for five of the six double-bond equivalents inherent in the molecular formula, salinipyrone A must be a monocyclic compound.

The ^1H NMR spectrum of salinipyrone A (**1**) displayed five distinct methyl signals. Two were aliphatic methyl groups; one was observed as a triplet [δ_H 0.95] and one as a doublet [δ_H 1.03]. Three olefinic methyls were also observed at δ_H 2.04, 1.93, and 1.87. Two mutually coupled *E* vinyl protons were observed at δ_H 7.09 (d, 15.5 Hz) and 6.37 (d, 15.5 Hz). Interpretation of gHMQC and $^1H-^1H$ gCOSY spectral data allowed the assignment of the side-chain part of the molecule (C-9 to C-13) with C-17 methyl substitution at C-10 and connectivity of C-6 and C-7. Analysis of the gHMBC spectrum allowed the connection of C-7 and C-8 by observation of correlations from H_3-16 to C-7 and C-8 and from H-7 to C-8 and C-9. The α-pyrone ring system was constructed on the basis of HMBC correlations from the olefinic methyl groups. The two-bond and three-bond heteronuclear couplings from H_3-14 to C-1, C-2, and C-3 and from H_3-15 to C-3, C-4, and C-5 established the ester linkage and closed the pyrone ring. Finally, HMBC signals from H-6 to C-5 completed the planar structure of **1** by connecting the α-pyrone ring with the side chain.

The geometries of the conjugated double bonds in **1** were determined by coupling constant and 1D NOE analyses (Figure 1a). The large coupling constant (15.5 Hz) between H-6 and H-7 allowed the C-6–C-7 *E*-geometry to be assigned. NOE correlations between H-7 and H-9 and between H-6 and H_3-16 established the C-8–C-9 *E*-olefin geometry. The relationship of the pyrone ring to the chain was determined by an observed NOE correlation between H_3-15 and H-6.

The relative configurations of the two consecutive stereogenic centers (C-10 and C-11) were first proposed by comparison of the ^{13}C NMR chemical shifts of **1** with published values obtained from synthetic diastereomers[10] sharing common partial structures from C-8 to C-13 with C-17 substitution (Figure 2). In this analysis of ^{13}C NMR chemical shifts, the shift of the C-17 methyl group is the most significantly distinguishable, as it is reported to be dependent on the relative configuration of the methyl-hydroxy-substituted stereogenic centers.[11] The ^{13}C NMR chemical shifts of **1** suggested that the relative configurations were 10*S** and 11*R**,

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Table 1. NMR Data for Salinipyrone A (**1**) in CD_3OD

C/H	δ_H[a]	mult (*J* in Hz)	δ_C[b]	#H	COSY	HMBC	NOE
1			167.5	C			
2			100.0	C			
3			167.7	C			
4			110.0	C			
5			154.3	C			
6	6.37	d (15.5)	115.0	CH	7	5, 7, 8	15, 16
7	7.09	d (15.5)	140.8	CH	6	5, 6, 8, 9	9
8			134.9	C			
9	5.80	br. d (10.0)	142.1	CH	10, 16	7, 10, 16, 17	7, 10, 11, 12, 17
10	2.67	dqd (10.5, 7.0, 5.0)[c]	40.0	CH	9, 11, 17	9, 17	9, 11, 12, 13, 17
11	3.38	ddd (8.5, 5.0, 4.0)[c]	78.0	CH	10, 12	16, 13	9, 10, 12, 13, 17
12a	1.47	dqd (14.0, 7.5, 4.0)[c]	28.6	CH_2	11, 13	11	9, 10, 11, 13
12b	1.38	ddq (14.0, 8.5, 7.5)[c]			11, 13	11	9, 10, 11, 13
13	0.95	t (7.5)	10.8	CH_3	12	11, 12	10, 11, 12
14	1.93	s	9.1	CH_3		1, 2, 3	
15	2.04	s	9.6	CH_3		3, 4, 5	6
16	1.87	d (1.0)	12.7	CH_3	9	7, 8, 9	6
17	1.03	d (7.0)	17.5	CH_3	10	9, 10, 11	9, 10, 11

[a] 500 MHz. [b] 75 MHz. [c] Acquired by homodecoupling experiments.

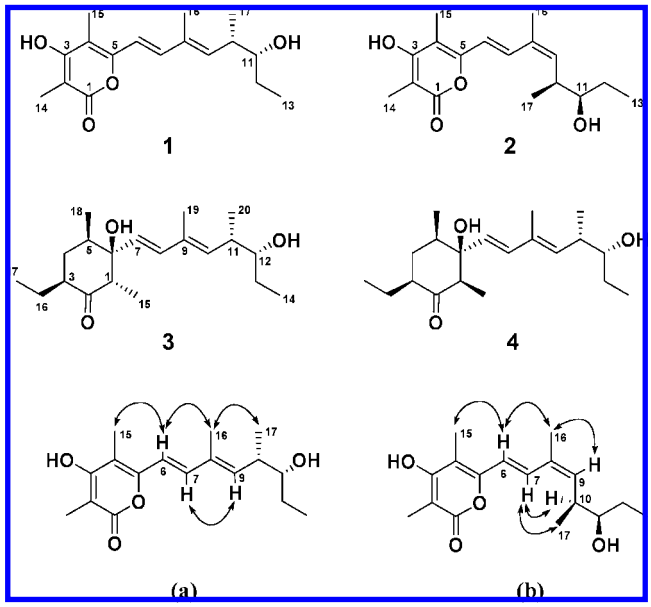

Figure 1. 1D NOE correlations used to assign the double-bond geometries in **1** (a) and **2** (b).

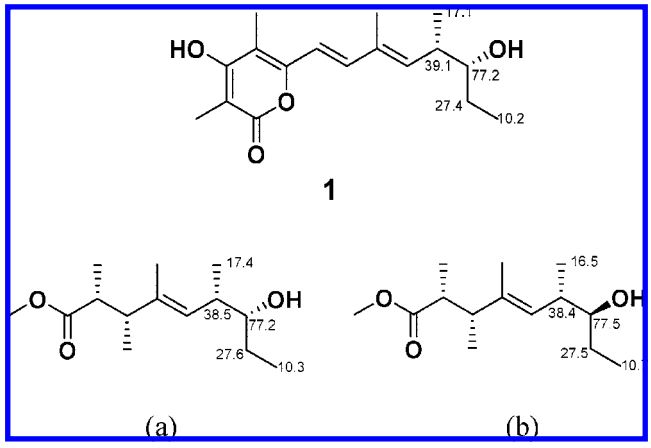

Figure 2. Comparison of ^{13}C NMR chemical shifts (in $CDCl_3$) of salinipyrone A (**1**) with two synthetic model compounds (a and b) to establish the relative configurations at C-10 and C-11 in **1**.

respectively, although the differences in chemical shifts were small. To confirm the relative configuration indicated by chemical shift

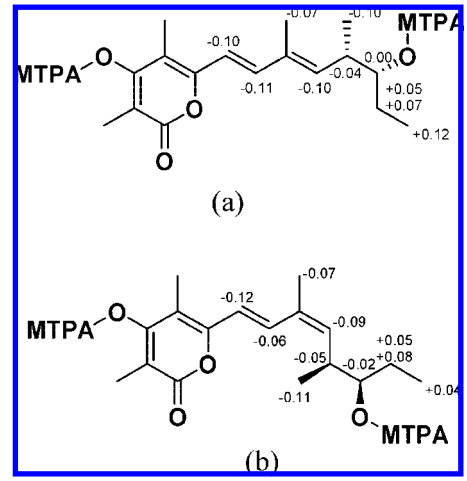

Figure 3. ^1H NMR $\Delta\delta_{S-R}$ values in ppm for the *S*- and *R*-MTPA esters of **1** (a) and **2** (b) in CD_3OD.

comparisons, we undertook a comprehensive examination of the 1D NOE data for the side-chain protons in **1**. Careful consideration of the expected NOE correlations from every possible rotamer (see the Supporting Information) also indicated that the relative configurations of C-10 and C-11 were *S** and *R**, respectively.

The overall absolute configuration of **1** was determined by application of the modified Mosher method.[12] Acylation of **1** with *R*-(−) and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride (MTPA-Cl) furnished bis-*S*- and *R*-MTPA esters (**6a** and **6b**), respectively. Analysis of ^1H NMR data for these MTPA esters allowed the assignment of the $\Delta\delta_{S-R}$ values, which were positive for H_2-12 and H_3-13, while those of H-6, H-7, H-9, H-10, H_3-16, and H_3-17 were negative. This is sufficiently consistent to establish the absolute configuration of C-11 as *R* (Figure 3a). On the basis of the relative configuration of C-10 and C-11, the absolute configuration of C-10 was assigned as *S*.

Salinipyrone B (**2**) was isolated as a viscous oil, which analyzed for the same molecular formula, $C_{17}H_{24}O_4$, as **1** (six degrees of unsaturation) by ESI high-resolution mass spectrometry (obsd $[M + H]^+$ at *m/z* 293.1747, calcd $[M + H]^+$ 293.1747) in combination with interpretation of ^1H and ^{13}C NMR data (Table 2). The ^1H NMR coupling patterns observed for salinipyrone B were identical to those of **1**, but the chemical shifts of the protons were slightly different. ^{13}C NMR and gHMQC spectra recorded for **2** also displayed a high degree of similarity to those of **1** except for one allylic methyl signal [δ_C 20.5], which was relatively deshielded. As in **1**, the gross structure of salinipyrone B was constructed by analysis of gCOSY,

Table 2. NMR Data for Salinipyrone B (**2**) in CD_3OD

C/H	δ_H[a]	mult (*J* in Hz)	δ_C[b]	#H	COSY	HMBC	NOE
1			167.7	C			
2			100.2	C			
3			168.5	C			
4			110.7	C			
5			154.0	C			
6	6.47	d (15.5)	117.5	CH	7	5, 7, 8	15, 16
7	7.50	d (15.5)	132.4	CH	6	5, 6, 8, 9	10, 17
8			133.0	C			
9	5.63	d (10.0)	139.6	CH	10	7, 10, 16, 17	10, 16, 17
10	2.90	dqd (10.0, 7.0, 4.5)[c]	38.9	CH	9, 11, 17	9, 17	7, 9, 11, 12, 13, 17
11	3.38	ddd (8.5, 4.5, 4.0)[c]	78.2	CH	10, 12	16, 13	10, 12, 13, 17
12a	1.46	dqd (14.0, 7.5, 4.0)[c]	28.6	CH_2	11, 13	11	10, 11, 13
12b	1.36	ddq (14.0, 8.5, 7.5)[c]			11, 13	11	10, 11, 13
13	0.94	t (7.5)	10.9	CH_3	12	11, 12	10, 11, 12
14	1.94	s	9.1	CH_3		1, 2, 3	
15	2.05	s	9.6	CH_3		3, 4, 5	6
16	1.94	s	20.5	CH_3		7, 8, 9	6, 9
17	1.05	d (7.0)	18.2	CH_3	10	9, 10, 11	9, 10, 11

[a] 500 MHz. [b] 75 MHz. [c] Acquired by homodecoupling experiments.

gHMQC, and gHMBC data. The geometries of the double bonds were determined by coupling constant and 1D NOE analysis (Figure 1b). The only difference between salinipyrone B (**2**) and **1** is the geometry of the C-8−C-9 double bond. NOE correlations between H-9 and H_3-16 and between H-7 and H-10 as well as H_3-17 clearly established the C-8−C-9 *Z*-geometry. This geometry is consistent with the relatively deshielded chemical shift of the olefinic methyl (C-16 δ_C 20.5) due to less steric compression compared to that of the C-16 methyl for the C-8−C-9 *E*-geometry in **1** (C-16 δ_C in **1**: 12.7).

The relative configurations at C-10 and C-11 in **2** were proposed as *S** and *R** (identical to the relative configurations in **1** based on the high degree of similarity of the $^1H-^1H$ coupling constants and NOE correlations around the C-10 and C-11 stereogenic centers to those in **1**). The absolute configuration of C-11 in **2** was determined as *R* by application of the modified Mosher method, which involved conversion to the corresponding *S*- and *R*-MTPA esters (**7a** and **7b**) with *R*- and *S*-MTPA chloride, respectively. In these experiments, the configuration at C-10 was assigned as *S* (Figure 3b).

Interestingly, the specific rotation values ($[\alpha]_D$) of **1** and **2** were unequal and showed opposite signs (−87 and +146 for **1** and **2**, respectively). This led us to acquire CD spectra to examine their chiroptical properties. Even though these two compounds possess identical stereogenic centers and are not enantiomers, their CD spectra displayed characteristics similar to those of enantiomeric structures (Figure 4). This is a clear example of the effects of double-bond geometry on the sign of the optical rotation and upon circular dichroic behavior. Inversions of optical rotation and CD spectra have been previously reported in polyene amides[13] and norsesquiterpenes,[14] respectively.

Pacificanone A (**3**) was obtained as a viscous oil, the molecular formula of which was assigned as $C_{20}H_{34}O_3$ by interpretation of ESIHRMS (obsd $[M + Na]^+ = m/z$ 345.2392, calcd $[M + Na]^+$ 345.2400) combined with ^1H and ^{13}C NMR features (Table 3). The proton NMR spectrum of **3** displayed *E*-coupled olefinic protons [δ_H 6.30 (d, 16.0 Hz); 5.67 (d, 16.0 Hz)], one olefinic proton [δ_H 5.47 (d, 10.0 Hz)], one oxygenated methine signal [δ_H 3.35 (ddd, 8.5, 5.0, 4.0 Hz)], and five methyl groups including an olefinic methyl group at δ_H 1.78. The ^{13}C NMR spectrum showed one ketone [δ_C 218.0], four olefinic carbons from δ_C 131 to 136, two oxygenated carbons [δ_C 81.0; 78.1], and 13 aliphatic signals. Analysis of 2D gCOSY, gHMQC, and gHMBC NMR spectra allowed all protons and carbons to be assigned. This analysis led to the conclusion that the side chain from C-7 to C-14, including the C-19 and C-20 methyl groups, was identical to the side chain of salinipyrone A (**1**). COSY correlations established the connectivity of C-17−C-16−C-3−C-4−C-5−C-18 and C-1−C-15, respectively. Long-range heteronuclear couplings from H-1, H-5, H_3-15, and H_3-18 to C-6 allowed the oxygen-bearing quaternary carbon to be placed between C-1 and C-5. Further, HMBC correlations to the ketone carbon established the 1,3,5,6-tetra-alkyl-substituted cyclohexanone moiety. The linkage between the side chain and the cyclohexanone ring was secured by HMBC correlations from H-7 to C-6 and C-1, which allowed the planar structure of **3** to be assigned.

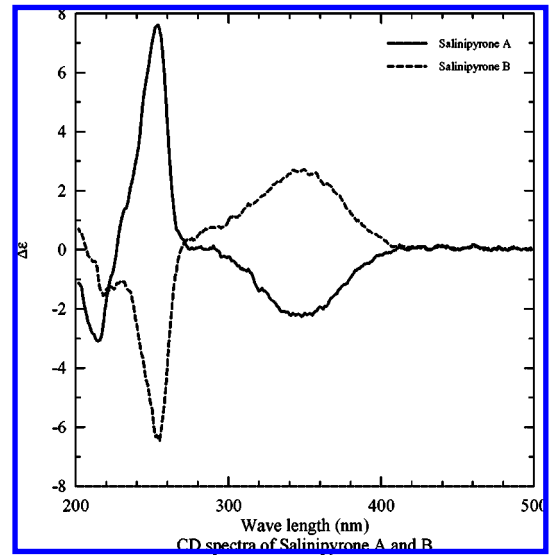

Figure 4. CD spectra of salinipyrones A and B (**1** and **2**).

The relative configuration of the 1,3,5,6-tetra-alkyl-substituted cyclohexanone was established by 1D NOE experiments (Figure 5a). NOE enhancements of H-3 and H-5 were observed upon irradiation of H_3-15, indicating that these two protons are in axial positions relative to the axial C-15 methyl group. As a result, the C-18 methyl group and the ethyl group [C-16−C-17] were assigned at equatorial positions. NOE enhancements upon irradiation of both H-7 and H-8 to H-1, H_3-15, H-5, and H_3-18 established the equatorial position of the chain. Overall, these data defined a substituted cyclohexanone ring in a rigid chair conformation with 1*S**, 3*S**, 5*R**, and 6*R** relative configurations.

The assignment of 11*S** and 12*R** configurations in **3**, which are identical to the relative configurations of C-10 and C-11 in **1**, was based on the high degree of identity of carbon chemical shifts and proton coupling constants with those of the identical side chain in **1**.

Table 3. NMR Data for Pacificanone A (**3**) in CD_3OD

C/H	δ_H[a]	mult (*J* in Hz)	δ_C[b]	#H	COSY	HMBC	NOE
1	2.28	m[c]	59.2	CH	15	2, 3, 6, 15	7, 8, 15
2			218.0	C			
3	2.52	m	47.5	CH	4a, 4b, 16	2, 4, 16, 17	4α, 5, 15, 16a, 16b, 17
4α	1.87	ddd (13.0, 6.5, 4.5)	37.0	CH_2	3, 4b, 5	2, 3, 5, 6, 16, 18	3, 5, 16a, 16b, 17, 18
4*β*	1.48	m[d]			3, 4a, 5	2, 3, 5, 6, 16, 18	16a, 16b, 17, 18
5	2.26	m[c]	34.9	CH	4a, 4b, 18	4, 6, 18	3, 4α, 7, 8, 15, 18
6			81.0	C			
7	5.67	d (16.0)	131.8	CH	8	1, 5, 6, 8, 9	1, 5, 15, 18, 19
8	6.30	d (16.0)	135.2	CH	7	1, 6, 7, 9, 10, 19	1, 5, 10, 15, 18
9			134.3	C			
10	5.47	d (10.0)	136.0	CH	11	8, 11, 12, 19, 20	8, 11, 12, 20
11	2.61	dqd (10.5, 6.5, 5.0)[e]	39.4	CH	10, 12, 20	9, 10, 11, 13, 20	10, 12, 13a, 13b, 14, 20
12	3.35	ddd (8.5, 5.0, 4.0)[e]	78.1	CH_2	11, 13a, 13b	10, 11, 13, 14, 20	11, 13a, 13b, 14. 20
13a	1.46	m[d]	28.5	CH_2	12, 13b, 14	11, 12, 14	11, 12, 14
13b	1.36	m			12, 13a, 14	11, 12, 14	11, 12, 14
14	0.93	t (7.5)	10.9	CH_3	13a, 13b	12, 13	11, 12, 13a, 13b
15	1.15	d (7.5)	15.4	CH_3	1	1, 2, 6	1, 3, 5, 7, 8
16a	1.74	m	23.0	CH_2	3, 16b, 17	2, 3, 4, 17	3, 4α, 4*β*, 17
16b	1.20	m			3, 16a, 17	2, 3, 4, 17	3, 4α, 4*β*, 17
17	0.90	t (7.5)	11.9	CH_3	16a, 16b	2, 16	3, 4α, 4*β*, 16a, 16b
18	0.87	d (6.5)	15.2	CH_3	5	4, 5, 6	4α, 4*β*, 7, 8
19	1.78	s	13.1	CH_3		8, 9, 10	7, 11
20	1.00	d (7.0)	17.7	CH_3	11	10, 11, 12	10, 11, 12

[a] 500 MHz. [b] 75 MHz. [c] Overlapping signals. [d] Overlapping signals. [e] Acquired by homodecoupling experiments.

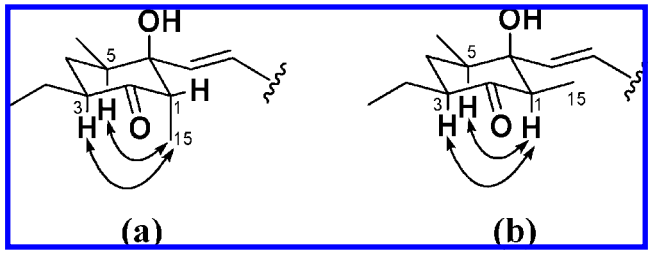

Figure 5. Key NOE correlations that defined the relative configurations for substituents on the chair cyclohexanone rings in **3** (a) and **4** (b).

Pacificanone B (**4**), also isolated as a viscous oil, was analyzed for the molecular formula $C_{20}H_{34}O_3$ by ESI high-resolution mass spectrometry (obsd $[M + Na]^+$ $m/z = 345.2392$, calcd $[M + Na]^+$ 345.2400). The 1D and 2D NMR data (Table 4) for **4** were virtually identical to those observed from **3**, suggesting that this compound is a stereoisomer of **3** with the same planar structure. Proton 1D NOE experiments and ^1H coupling constant analysis revealed an equatorial C-15 methyl group rather than the axial C-15 methyl found in **3** (Figure 5b). The large vicinal coupling constant (12.0 Hz) for H-5 showed it was in an axial position. NOE correlations from H-5 to H-1 and H-3 established their axial relationships, thus also indicating an equatorial position for C-15. The relatively upfield carbon chemical shift for C-15 [δ_C 8.6] in **4** compared to C-15 [δ_C 15.4] in **3** also supported this assignment.

The absolute configuration of pacificanone B (**4**) was determined by application of the modified Mosher method on the alcohol obtained by reduction of the ketone functionality. Treatment of pacificanone B with sodium borohydride in MeOH for 30 min at 0 °C generated the alcohol **5**, which was purified and structurally defined by NMR methods. The ring hydroxy group in **5** was found to be in an axial position on the basis of interpretation of chemical shift and coupling constant data from the H-5 carbinol proton [δ_H 3.71 (br s, the width of the peak ≈ 10 Hz)].[15] Mosher acylation of **5** with *R*- and *S*-MTPA-Cl yielded the bis-*S*- and *R*-MTPA esters (**8a** and **8b**). Analysis of ^1H chemical shifts in the 1D ^1H NMR and gCOSY spectra of the esters allowed calculation of $\Delta\delta_{S-R}$ values and established the absolute configurations of C-2 as *S* and C-12 as *R* (Figure 6). These assignments, coupled with the established relative configurations, allowed 1*R*, 3*S*, 5*R*, 6*R*, and 11*S* absolute configurations to be assigned. The identical positive Cotton effect at 240 nm in the CD spectrum of **4**, which is derived from the *E*,*E*-diene and hydroxy group interaction,[16] led us to propose the same absolute configuration for pacificanone A (**3**) at relevant centers, except for the C-1 position. The C-1 configuration in **3** is assumed to be *S* since the relative configuration is opposite of that in **4**.

Salinipyrones A and B appear to be α-pyrone polyketides that are derived from incorporation of acetate and propionate precursors. Propionate polyketide α-pyrones are well-known from marine invertebrates such as sacoglossans[17] and pulmonates,[18] and on occasion from actinomycetes.[19] The pacificanones bear a unique 1,3,5,6-tetra-alkyl-substituted cyclohexanone ring, which is a structural feature not previously reported in natural products. The biosynthetic origin of the salinipyrones and the pacificanones seems most likely via a type I polyketide biosynthetic pathway. The carbon backbone of the pacificanones differs from that of the salinipyrones in that an additional ethylmalonyl-CoA building block appears to have been incorporated. This skeletal variation can be explained by evoking a PKS module skipping mechanism, an event reported in the biosynthesis of methymycin and pikromycin by *Streptomyces venezulae*.[20] However, the possibility that the salinipyrones and the pacificanones are derived from two separate PKS I modules, while unlikely, cannot be excluded without further experimental information.

The biological activity of compounds **1**−**4** is currently being examined in diverse bioassays. In initial screening, the salinipyrones and the pacificanones displayed no significant activity in a cancer cell cytotoxicity assay (HCT-116 human colon cancer), nor did these compounds show appreciable antibiotic activities against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and amphotericin-resistant *Candida albicans*. In an isolated mouse splenocyte model of allergic inflammation, salinipyrone A displayed moderate inhibition of interleukin-5 production by 50% at 10 μg/mL without measurable human cell cytotoxicity (HCT-116).

The discovery of the salinipyrones and the pacificanones is the result of a comprehensive phylogenetic analysis approach. Fine-scale phylogenetic analyses of the 16S rDNA sequences of *Salinispora* strains revealed subtle diversity within the *S. pacifica* clade, which prompted detailed chemical analyses of strain CNS-237. This diversity-based approach led to the discovery of the salinipyrones with unusual chiroptical properties and the pacificanones, which appear to constitute a new example of module

Table 4. NMR Data for Pacificanone B (**4**) in CD_3OD

C/H	δ_H[a]	mult (*J* in Hz)	δ_C[b]	#H	COSY	HMBC	NOE
1	2.64	q (6.5)	54.2	CH	15	2, 3, 6, 15	3, 5, 7, 8, 15
2			214.6	C			
3	2.40	m	52.6	CH	4a, 4b, 16	2, 4, 16, 17	1, 4α, 5, 16a, 16b, 17
4α	1.94	ddd (13.0, 6.0, 3.5)	38.4	CH_2	3, 4b, 5	2, 3, 5, 6, 16, 18	3, 5, 16a, 16b, 17, 18
4*β*	1.48	m[c]			3, 4a, 5	2, 3, 5, 6, 16, 18	16a, 16b, 17, 18
5	2.12	dqd (12.0, 6.5, 3.5)[d]	41.6	CH	4a, 4b, 18	4, 6, 18	1, 3, 4α, 7, 8, 18
6			83.3	C			
7	5.52	d (16.0)	134.9	CH	8	1, 5, 6, 8, 9	1, 5, 15, 18, 19
8	6.22	d (16.0)	135.1	CH	7	1, 6, 7, 9, 10, 19	1, 5, 10, 15, 18
9			134.1	C			
10	5.45	d (10.0)	135.4	CH	11	8, 11, 12, 19, 20	8, 11, 12, 20
11	2.61	dqd (10.5, 6.5, 5.0)[d]	39.4	CH	10, 12, 20	9, 10, 11, 13, 20	10, 12, 13a, 13b, 14, 20
12	3.35	ddd (8.5, 5.0, 4.0)[d]	78.2	CH_2	11, 13a, 13b	10, 11, 13, 14, 20	11, 13a, 13b, 14. 20
13a	1.46	m[c]	28.5	CH_2	12, 13b, 14	11, 12, 14	11, 12, 14
13b	1.36	m			12, 13a, 14	11, 12, 14	11, 12, 14
14	0.93	t (7.5)	10.9	CH_3	13a, 13b	12, 13	11, 12, 13a, 13b
15	0.89	d (6.5)	8.6	CH_3	1	1, 2, 6	1, 3, 5, 7, 8
16a	1.74	m	23.2	CH_2	3, 16b, 17	2, 3, 4, 17	3, 4α, 4*β*, 17
16b	1.20	m			3, 16a, 17	2, 3, 4, 17	3, 4α, 4*β*, 17
17	0.90	t (7.5)	12.2	CH_3	16a, 16b	2, 16	3, 4α, 4*β*, 16a, 16b
18	0.87	d (6.5)	15.5	CH_3	5	4, 5, 6	4α, 4*β*, 7, 8
19	1.78	s	13.1	CH_3		8, 9, 10	7, 11
20	1.01	d (6.5)	17.7	CH_3	11	10, 11, 12	10, 11, 12

[a] 500 MHz. [b] 75 MHz. [c] Overlapping signals. [d] Acquired by homodecoupling experiments.

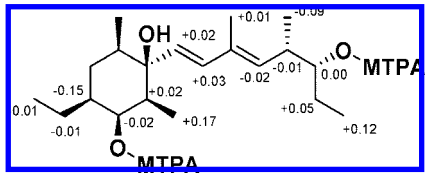

Figure 6. ^1H NMR $\Delta\delta_{S-R}$ values in ppm for the bis-*S*- and *R*-MTPA esters of alcohol **5** in CD_3OD.

skipping in a PKS I biosynthetic pathway. Application of comprehensive 16S rDNA phylogenetic analysis appears to be an effective method to define diverse actinomycete taxa from marine ecosystems, thus enhancing the rate of discovery of novel secondary metabolites.

Experimental Section

General Experimental Procedures. The optical rotations were measured using a Rudolph Research Autopol III polarimeter with a 10 cm cell. UV spectra were recorded in a Varian Cary UV–visible spectrophotometer with a path length of 1 cm. CD spectra were collected in an AVIV model 215 CD spectrometer with a 0.5 cm long cell. IR spectra were acquired in a Nicolet Magna 550 FT-IR series II FT-IR spectrometer. ^1H, ^{13}C, and 2D NMR data were obtained on Varian Inova 500 and 600 MHz spectrometers. High-resolution mass spectra (HRESITOFMS) were recorded at The Scripps Research Institute, La Jolla, CA. Low-resolution LC/MS data were acquired using a Hewlett-Packard series 1100 LC/MS system with a reversed-phase C_{18} column (Phenomenex Luna, 4.6 mm × 100 mm, 5 μm) at a flow rate of 0.7 mL/min.

Collection and Phylogenetic Analysis of Strain CNS-237. The marine actinomycete strain CNS-237 was isolated from a sediment sample collected on the island of Palau in 2004. The strain appears to represent a new *Salinispora* species (proposed as *Salinispora pacifica*) based on 16S rDNA analysis and DNA−DNA hybridization studies. The full 16S rDNA sequence data have been deposited in GenBank under the acquisition number DQ3128246.

Fermentation and Extraction. *Salinispora* strain CNS-237 was cultured at 27 °C with shaking at 250 rpm in twelve 2.8 L Fernbach flasks each containing 1 L of the medium A1BFe+C (10 g of starch, 4 g of yeast extract, 2 g of peptone, 1 g of $CaCO_3$, 40 mg of $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg of KBr, and 1 L of sea water). After 6 days, the whole culture (12 L) was extracted twice with EtOAc, and the resulting extract was concentrated by rotary evaporation.

Isolation of Salinipyrones A and B (1, 2) and Pacificanones A and B (3, 4). The crude extract (1.9 g) was fractionated by C_{18} vacuum column chromatography eluting with a step gradient from 10 to 100% MeOH in H_2O. The 60% MeOH/H_2O fraction (219 mg) was subjected to reversed-phase HPLC with 75% aqueous MeOH (Waters Prep LC 4000 system, Waters preparative column C_{18} 25 mm × 200 mm, 10 mL/min, UV detection at 254 nm). Pacificanones A and B were isolated, as pure metabolites, with retention times of 21.7 and 23 min. Salinipyrones A and B were subsequently purified from a complex fraction that eluted at 14.7 and 13.5 min by normal-phase HPLC (Lichrospher semipreparative column Si gel, 10 mm × 250 mm, 2 mL/min, refractive index detection) with a solvent composed of EtOAc/isooctane/MeOH, 47.5:47.5:5.

Salinipyrone A (1): yellow oil; $[\alpha]_D$ −87 (*c* 0.33, CH_3OH); IR (neat) ν_{max} 3397, 1672, 1545, 1222 cm^{-1}; UV (CH_3OH) λ_{max} (log ϵ) 255 (4.5), 348 (4.0) nm; CD (CH_3OH) ($\Delta\epsilon$) 252 (+7.9), 350 (−2.4) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD), see Table 1; HRESITOFMS $[M + H]^+$ *m*/*z* 293.1744 ($C_{17}H_{25}O_4$, calcd $[M + H]^+$ 293.1747).

Salinipyrone B (2): yellow oil; $[\alpha]_D$ +146 (*c* 0.21, CH_3OH); IR (neat) ν_{max} 3380, 1664, 1548, 1220 cm^{-1}; UV (CH_3OH) λ_{max} (log ϵ) 256 (4.5), 351 (3.9) nm; CD (CH_3OH) ($\Delta\epsilon$) 253 (–7.6), 349 (+2.9) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD), see Table 2; HRESITOFMS $[M + H]^+$ *m*/*z* 293.1747 ($C_{17}H_{25}O_4$, calcd $[M + H]^+$ 293.1747).

Pacificanone A (3): colorless oil; $[\alpha]_D$ −10 (*c* 0.16, CH_3OH); IR (neat) ν_{max} 3414, 2960, 1698, 1519 cm^{-1}; UV (CH_3CN) λ_{max} (log ϵ) 239 (4.3) nm; CD (CH_3CN) ($\Delta\epsilon$) 240 (+4.5), 290 (–1.7) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD), see Table 3; HRESITOFMS $[M + Na]^+$ *m*/*z* 345.2392 ($C_{20}H_{34}O_3Na$, calcd $[M + Na]^+$ 345.2400).

Pacificanone B (4): colorless oil; $[\alpha]_D$ −3 (*c* 0.20, CH_3OH); IR (neat) ν_{max} 3439, 2960, 1706, 1519 cm^{-1}; UV (CH_3CN) λ_{max} (log ϵ) 237 (4.3) nm; CD (CH_3CN) ($\Delta\epsilon$) 220 (+3.6), 240 (+3.2), 290 (–2.1) nm; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD), see Table 4; HRESITOFMS $[M + Na]^+$ *m*/*z* 345.2392 ($C_{20}H_{34}O_3Na$, calcd $[M + Na]^+$ 345.2400).

Reduction of 4 to Yield Alcohol 5. Pacificanone B (**4**, 2 mg) was dissolved in 1 mL of dry MeOH. Two milligrams of $NaBH_4$ was added to the solution, and the reaction mixture was stirred for 30 min. Ten milliliters of DCM was added, and the mixture was partitioned with 2.5% aqueous NH_4Cl solution. The DCM/MeOH portion was then washed again with brine. The organic layer was dried with $MgSO_4$, and the major product, alcohol **5**, was purified by reversed-phase HPLC (Alltech semipreparative column C_{18}, 10 mm × 250 mm, 2 mL/min, refractive index detection) with the isocratic solvent mixture 70% CH_3CN in H_2O. Alcohol **5** (2 mg) was eluted at 31 min. The molecular

formula of the reduction product was confirmed as $C_{20}H_{36}O_3$ by ESIHRMS analysis (obsd $[M + Na]^+$ *m/z* 347.2557, calc *m/z* 347.2562).

Alcohol 5: colorless oil; $[\alpha]_D$ +35 (*c* 0.02, CH_3OH); IR (neat) ν_{max} 3430, 2960, 1517 cm^{-1}; UV (CH_3CN) λ_{max} (log ϵ) 237 (4.3) nm; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD), see Table S1 for the full assignment; HRESITOFMS $[M + Na]^+$ *m/z* 347.2557 ($C_{20}H_{36}O_3Na$, calcd $[M + Na]^+$ 347.2562); ^1H NMR (600 MHz, CD_3OD) δ 0.82 (3H, d, $J = 6.5$ Hz), 0.93 (3H, t, $J = 7.5$ Hz), 0.94 (3H, t, $J = 7.5$ Hz), 1.00 (3H, d, $J = 6.5$ Hz), 1.01 (3H, d, $J = 6.5$ Hz), 1.31 (1H, m), 1.36 (1H, m), 1.37 (2H, m), 1.42 (1H, m), 1.45 (1H, m), 1.50 (1H, m), 1.55 (1H, m), 1.75 (3H, s), 2.61 (1H, m), 3.34 (1H, m), 3.71 (1H, br. s), 5.32 (1H, d, $J = 16.0$ Hz), 5.40 (1H, d, $J = 10.0$ Hz), 6.19 (1H, d, $J = 16.0$ Hz).

MTPA Esters of 1, 2, and 5 (6a/6b, 7a/7b, and 8a/8b). Duplicate (1.2 mg, 4.1 μmol) samples of dry salinipyrone A (**1**) were prepared for both *R*- and *S*-MTPA acylation reactions. In separate vials, the samples were dissolved in 1.5 mL of dry pyridine, a catalytic amount of dry DMAP was added, and after stirring for 30 min, 20 μL (107 μmol) of *R*-MTPA chloride and *S*-MTPA chloride were added. The reactions were monitored by LC/MS, which showed the clean conversion to the *S*- and *R*-bis-MTPA esters, respectively. The acylation products were purified by reversed-phase HPLC (Alltech C_{18} semipreparative column, 10 mm × 250 mm, 2 mL/min, UV 254 nm detection, 20%–100% H_2O/MeOH gradient over 40 min, 100% CH_3OH isocratic after 40 min). The bis-*S*-MTPA ester (**6a**) and bis-*R*-MTPA ester (**6b**) of **1** were eluted at 58 min. The molecular formulas for **6a** and **6b** were confirmed as $C_{37}H_{38}F_6O_8$ by ESI-LC/MS analysis ($[M + H]^+$ *m/z* 725 and $[M + Na]^+$ *m/z* 747). For salinipyrone B, the same procedure above was carried out to obtain the corresponding *S*- and *R*-MTPA esters (**7a** and **7b**).

Bis-*S*-MTPA ester of 1 (6a): ^1H NMR (500 MHz, CD_3OD) δ 0.94 (3H, d, $J = 7.0$ Hz), 0.95 (3H, t, $J = 7.5$ Hz), 1.67 (1H, m), 1.72 (1H, m), 1.79 (3H, d, $J = 1.0$ Hz), 1.80 (3H, s), 1.82 (3H, s), 2.95 (1H, m), 3.53 (3H, s), 3.66 (3H, s), 5.01 (1H, m), 5.59 (1H, d, $J = 10.0$ Hz), 6.28 (1H, d, $J = 15.5$ Hz), 6.97 (1H, d, $J = 15.5$ Hz), 7.34–7.41 (3H, m), 7.50–7.54 (5H, m), 7.66–7.68 (2H, m).

Bis-*R*-MTPA ester of 1 (6b): ^1H NMR (500 MHz, CD_3OD) δ 0.83 (3H, t, $J = 7.5$ Hz), 1.03 (3H, d, $J = 7.0$ Hz), 1.60 (1H, m), 1.67 (1H, m), 1.80 (3H, s), 1.84 (3H, s), 1.86 (3H, d, $J = 1.0$ Hz), 3.00 (1H, m), 3.50 (3H, s), 3.66 (3H, s), 5.01 (1H, m), 5.69 (1H, d, $J = 10.0$ Hz), 6.38 (1H, d, $J = 15.5$ Hz), 7.08 (1H, d, $J = 15.5$ Hz), 7.37–7.42 (3H, m), 7.48–7.55 (5H, m), 7.65–7.70 (2H, m).

Bis-*S*-MTPA ester of 2 (7a): ^1H NMR (500 MHz, CD_3OD) δ 0.94 (3H, t, $J = 7.5$ Hz), 0.94 (3H, d, $J = 7.0$ Hz), 1.66 (1H, m), 1.77 (1H, m), 1.79 (3H, s), 1.80 (3H, s), 1.82 (3H, d, $J = 1.0$ Hz), 3.12 (1H, m), 3.53 (3H, s), 3.66 (3H, s), 5.01 (1H, m), 5.46 (1H, d, $J = 10.5$ Hz), 6.35 (1H, d, $J = 15.5$ Hz), 7.33–7.45 (3H, m), 7.43 (1H, m), 7.48–7.55 (5H, m), 7.64–7.71 (2H, m).

Bis-*R*-MTPA ester of 2 (7b): ^1H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, $J = 7.5$ Hz), 1.05 (3H, d, $J = 7.0$ Hz), 1.60 (1H, m), 1.69 (1H, m), 1.81 (3H, s), 1.86 (3H, s), 1.89 (3H, d, $J = 1.0$ Hz), 3.17 (1H, m), 3.48 (3H, s), 3.66 (3H, s), 5.03 (1H, m), 5.54 (1H, d, $J = 11.0$ Hz), 6.46 (1H, d, $J = 16.0$ Hz), 7.37–7.43 (3H, m), 7.47–7.56 (5H, m), 7.49 (1H, d, m), 7.65–7.71 (2H, m).

Duplicate (1 mg) aliquots of alcohol **5**, from pacificanone B (**4**), were treated with *R*- and *S*-MTPA chloride in the same manner as shown above. Bis-*S*- and *R*-MTPA esters (**8a** and **8b**) of **5** (0.3 and 0.2 mg, respectively) were purified by reversed-phase HPLC (Alltech C_{18} semipreparative column, 10 mm × 250 mm, 2 mL/min, UV 230 nm detection, 20% aqueous CH_3CN gradient from 0 to 10 min, 20–100% CH_3CN from 10 to 50 min, 100% CH_3CN isocratic after 50 min) at the retention times at 63.9 and 62.9 min, respectively. ESI-LC/MS analysis resolved the bis-MTPA esters ($C_{40}H_{50}F_6O_7$) at $[M + Na]^+$ *m/z* 779.

Bis-*S*-MTPA ester of 5 (8a): ^1H NMR (500 MHz, CD_3OD) δ 0.79 (3H, t, $J = 6.5$ Hz), 0.87 (3H, d, $J = 7.5$ Hz), 0.88 (3H, d, $J = 7.5$ Hz), 0.89 (3H, t, $J = 6.5$ Hz), 0.90 (3H, d, $J = 6.5$ Hz), 1.60 (1H, m), 1.61 (1H, m), 1.62 (2H, m), 1.64 (2H, m), 1.74 (3H, s), 1.85 (1H, m), 2.02 (1H, m), 2.89 (1H, m), 3.51 (3H, s), 3.57 (3H, s), 5.00 (1H, m), 5.26 (1H, d, $J = 9.5$ Hz), 5.34 (1H, d, $J = 15.5$ Hz), 5.47 (1H, br. s), 6.18 (1H, d, $J = 15.5$ Hz), 7.38−7.64 (10H, m).

Bis-*R*-MTPA ester of 5 (8b): ^1H NMR (500 MHz, CD_3OD) δ 0.70 (3H, d, $J = 7.0$ Hz), 0.77 (3H, t, $J = 7.0$ Hz), 0.80 (3H, t, $J = 7.0$ Hz), 0.93 (3H, d, $J = 7.0$ Hz), 0.97 (3H, d, $J = 6.5$ Hz), 1.59 (3H, m), 1.60 (1H, m), 1.62 (2H, m), 1.73 (3H, s), 1.83 (1H, m), 2.17 (1H, m), 2.90 (1H, m), 3.54 (3H, s), 3.57 (3H, s), 5.00 (1H, m), 5.28 (1H, d, $J = 9.5$ Hz), 5.32 (1H, d, $J = 15.5$ Hz), 5.45 (1H, br s), 6.14 (1H, d, $J = 15.5$ Hz), 7.27−7.66 (10H, m).

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Supporting Information Available: ^1H, ^{13}C, and 2D NMR spectra of **1**–**5**, ^1H NMR data for the MTPA esters **6a** and **6b**, LC/MS traces of CNS-237, and additional discussion about the relative configurations of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Goodfellow, M.; Haynes, J. A. Actinomycetes in marine sediments. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes*; Ortiz-Ortiz, Bojalil L., Yakoleff, V., Eds.; Academic Press: London:, 1984; pp 453–472..
(2) Bruns, A.; Philipp, H.; Cypionka, H.; Brinkhoff, T. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 1917–1923.
(3) Montalvo, N.; Mohamed, N. M.; Enticknap, J. J.; Hill, R. T. *Antonie van Leeuwenhoek* **2005**, *87*, 29–36.
(4) (a) Mincer, T. J.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Appl. Environ. Microbiol.* **2002**, *68*, 5005–5011. (b) Jensen, P. R.; Gontang, E.; Mafnas, C.; Mincer, T. J.; Fenical, W. *Environ. Microbiol.* **2005**, *7*, 1039–1048. (c) 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. Evol. Microbiol.* **2005**, *55*, 1759–1766.
(5) (a) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673. (b) 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. (c) Buchanan, G. O.; Williams, P. G.; Feling, R. H.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2005**, *7*, 2731–2734.
(6) Jensen, P. R.; Mafnas, C. *Environ. Microbiol.* **2006**, *8*, 1881–1888.
(7) (a) Oh, D.-C.; Williams, P. G.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2006**, *8*, 1021–1024. (b) Udwary, D. W.; Zeigler, L.; Asolkar, R.; Singan, V.; Lapidus, A.; Fenical, W.; Jensen, P. R.; Moore, B. S. *Proc. Natl. Acad. Sci.* **2007**, *104*, 10376–10381.
(8) Gontang, E. A.; Fenical, W.; Jensen, P. R. *Appl. Environ. Microbiol.* **2007**, *73*, 3272–3282.
(9) Jensen, P. R.; Williams, P. G.; Oh, D.-C.; Zeigler, L.; Fenical, W. *Appl. Environ. Microbiol.* **2007**, *73*, 1146–1152.
(10) Cane, D. E.; Kudo, F.; Kinoshita, K.; Khosla, C. *Chem. Biol.* **2002**, *9*, 131–142.
(11) Heathcock, C. H.; Pirrung, M. C.; Sohn, J. E. *J. Org. Chem.* **1979**, *44*, 4294–4299.
(12) Séco, J. M.; Quiñoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915–2925.
(13) Ojika, M.; Itou, Y.; Sakagami, Y. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1568–1573.
(14) Song, F.; Xu, X.; Li, S.; Wang, S.; Zhao, J.; Cao, P.; Yang, Y.; Fan; X.; Shi, J.; He, L.; Lü, Y. *J. Nat. Prod.* **2005**, *68*, 1309–1313.
(15) Mihovilovic, M. D.; Rudroff, F.; Grötzl, B.; Stanetty, P. *Eur. J. Org. Chem.* **2005**, 809–816.
(16) Gawronski, J.; Gawronska, K. *J. Chem. Soc., Chem. Commun.* **1980**, *8*, 346–347.
(17) Cutignano, A.; Fontana, A.; Renszulli, L.; Cimino, G. *J. Nat. Prod.* **2003**, *66*, 1399–1401.
(18) Hochlowski, J. E.; Faulkner, D. J. *Tetrahedron Lett.* **1983**, *24*, 1917–1920.
(19) Lindel, T.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1996**, *37*, 1327–1330.
(20) (a) Xue, Y.; Sherman, D. H. *Nature* **2000**, *403*, 571–575. (b) Xue, Y.; Zhao, H.; Liu, H.-W.; Sherman, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12111–12116.

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