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Isolation, Structural Elucidation, and Absolute Stereochemistry of Enigmazole A, a Cytotoxic Phosphomacrolide from the Papua New Guinea Marine Sponge *Cinachyrella enigmatica*

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Abstract: Enigmazole A (**1**), a novel phosphate-containing macrolide, was isolated from a Papua New Guinea collection of the marine sponge *Cinachyrella enigmatica*. The structure of **1**, including the absolute stereochemistry at all eight chiral centers, was determined by a combination of spectroscopic analyses and a series of microscale chemical derivatization studies. Compound **1** is comprised of an 18-membered phosphomacrolide that contains an embedded exomethylene-substituted tetrahydropyran ring and an acyclic portion that spans an embedded oxazole moiety. Two additional analogues, 15-*O*-methylenigmazole A and 13-hydroxy-15-*O*-methylenigmazole A, were also isolated and assigned. The enigmazoles are the first phosphomacrolides from a marine source and **1** exhibited significant cytotoxicity in the NCI 60-cell line antitumor screen, with a mean GI_{50} of 1.7 μM.

Marine organisms have proven to be an exceptionally valuable resource for bioactive natural products discovery efforts, particularly in the area of potential anticancer compounds. Metabolites isolated from marine samples often possess unique structural features and incorporate new or unusual assemblages of functional groups. They have demonstrated a broad range of potent biological activities, and there are currently at least 11 marine-derived compounds under clinical development in the U.S. and Europe.[1] In addition, there are currently two approved marine-derived drugs, the cone snail peptide Prialt[2] for chronic nerve pain and the ascidian alkaloid Yondelis[3] for soft tissue sarcoma treatment. In light of the tremendous potential of marine natural products as drug development leads, the U.S. National Cancer Institute has maintained an active marine collection program for the past 25 years that is worldwide in its scope. Extracts from a broad taxonomic range of marine organisms have been prepared and screened by the NCI for cytotoxic properties and HIV inhibition and in assays targeted toward specific cellular pathways, processes, or phenotypic responses.[4] Detailed chemical analyses of extracts identified through these screening efforts have provided a vast array of new biologically active compounds, many of which exhibit potential anticancer activities.[5] The cytotoxic properties of marine metabolites are of particular interest for the development of new anticancer agents, and there is a continuing interest in identifying such compounds that have novel structural features. These new chemotypes can serve as possible drug development candidates in their own right, or they may provide new structural scaffolds for the synthetic or semisynthetic production of lead compounds.

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(1) (a) Glaser, K. B.; Mayer, A. M. S. *Biochem. Pharmacol.* **2009**, *78*, 440–448. (b) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69–85. (c) Mayer, A. M. S.; Gustafson, K. R. *Eur. J. Cancer* **2008**, *44*, 2357–2387.

(2) Prialt (Elan Pharmaceuticals), also known as ziconotide, is a synthetic form of the cone snail toxin φ-conotoxin VIIA. Olivera, B. M.; Gray, W. R.; Zeikus, R.; McIntosh, J. M.; Varga, J.; Rivier, J.; de Santos, V.; Cruz, L. J. *Science* **1985**, *230*, 1338–1343.

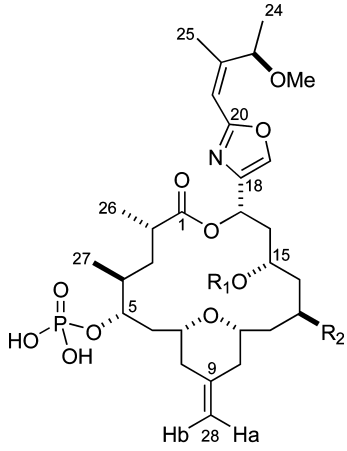
(3) Yondelis (Zeltia and Johnson & Johnson/OrthoBiotech), also known as trabectedin, ecteinascidin-743, or ET-743, was originally isolated from the ascidian *Ecteinascidia turbinata*. (a) Wright, A. E.; Forleo, D. A.; Gunawardana, G. P.; Gunasekera, S. P.; Koehn, F. E.; McConnell, O. J. *J. Org. Chem.* **1990**, *55*, 4508–4512. (b) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512–4515.

(4) See, for example: (a) Kang, M.-I.; Henrich, C. J.; Bokesch, H. R.; Gustafson, K. R.; McMahon, J. B.; Baker, A. R.; Young, M. R.; Colburn, N. H. *Mol. Cancer Ther.* **2009**, *8*, 571–581. (b) Booth, N. L.; Sayers, T. J.; Brooks, A. D.; Thomas, C. L.; Jacobsen, K.; Goncharova, E. I.; McMahon, J. B.; Henrich, C. J. *Cancer Immunol. Immunother.* **2009**, *58*, 1229–1244.

(5) Molecular Targets Laboratory. Catalog of Compounds, https://ccrod.cancer.gov/confluence/display/CCRMTDPBeu/Introduction+to+MTL.

 10.1021/ja1016766

As part of our ongoing natural products discovery program, an extract from the sponge *Cinachyrella enigmatica*,[6] collected from Papua New Guinea, showed significant cytotoxic activity in the NCI 60-cell antitumor screen[7] and was selected for more detailed bioassay-guided chemical investigation. *Cinachyrella* is a genus in the Desmosponge family Tetillidae that has not been the focus of extensive chemical study in the past. Other than a few fatty acid derivatives[8] or steroids[9] that were isolated from various *Cinachyrella* species, only a lactose-specific lectin from *Cinachyrella apion*[10] and a tricyclic alkaloid from *Cinachyrella* sp.[11] have been described. There are no prior reports in the chemical literature on metabolites from *C. enigmatica*. Both the CH_2Cl_2−MeOH (1:1) extract of *C. enigmatica* and the *n*-BuOH-soluble fraction of the aqueous extract of the sponge were combined and partitioned between 60% aqueous MeOH and CH_2Cl_2. The aqueous MeOH layer was successively fractionated first by vacuum chromatography on a C_4-wide pore support and then by size-exclusion chromatography on Sephadex LH-20 to give one principal cytotoxic fraction. Purification of this fraction by reversed-phase HPLC yielded enigmazole A (**1**) and two congeners, 15-*O*-methylenigmazole A (**2**) and 13-hydroxy-15-*O*-methylenigmazole A (**3**).



1: R_1 = H, R_2 = H
2: R_1 = Me, R_2 = H
3: R_1 = Me, R_2 = OH

The molecular formula of **1** was established as $C_{29}H_{46}NO_{10}P$ by HR-FABMS measurements ($[M - H]^-$ obsd *m/z* 598.2747, calcd 598.2781, Δ −3.4 mmu) in combination with extensive NMR analyses. The presence of a phosphate group in **1** was verified by the observation of a single resonance at δ 2.17 ppm in a ^{31}P NMR spectrum.[12] Analysis of the ^1H NMR and HSQC data revealed the presence of three aliphatic methyls (δ_H 0.97, 1.10, and 1.26), an olefinic methyl (δ_H 1.89), an *O*-methyl group (δ_H 3.20/δ_C 56.4), eight methylenes, an exomethylene (δ_H 4.69, 4.70/δ_C 108.8), two aliphatic methines (δ_H 1.62, 2.98), six oxymethines (δ_H/δ_C 4.42/75.8, 3.12/77.2, 3.29/76.2, 3.62/69.8, 5.95/65.9, 5.24/77.0), an olefinic methine (δ_H 6.21/δ_C 113.9), and an isolated aromatic proton (δ_H 7.68/δ_C 136.0). On the basis of COSY and TOCSY data, these subunits were assembled into three partial structures: C26−C27 (fragment **A**), C5−C19 (**B**), and C21−C25 (**C**), as indicated in Figure 1. Placement of the C9−C28 exomethylene unit between C8 and C10 was supported by small allylic couplings between δ 1.97/4.70 (H8a/H28b) and 1.84/4.69 (H10a/H28a), while placement of the aromatic double bond (C18−C19) at the terminus of **B** was evident from a benzylic coupling between H19 (δ 7.68) and H17 (δ 5.95). Similarly, allylic COSY crosspeaks between H21 (δ 6.21) and both H_3-25 and H23 (δ 5.24) helped construct fragment **C**. No coupling was observed between the methine at δ 1.62 (H4) in **A** and the oxymethine signal at δ 4.42 (H5) in **B**, which was indicative of a 90° dihedral angle between these two protons. However, HMBC crosspeaks from H_3-27 (δ 0.97) to C5 (δ 75.8) and from H5 (δ 4.42) to C3 (δ 39.3) clearly defined the connection of **A** with **B** (Figure 1). At the terminus of substructure **B**, an HMBC correlation was observed from H19 to a quaternary carbon resonating at δ_C 161.7 (C20). HMBC correlations from H21 and H_3-25 in **C** to this same aromatic carbon indicated that fragments **B** and **C** were joined via a conjugated oxazole ring. This assignment was further supported by a characteristically large heteronuclear coupling ($^1J_{CH}$ = 209.2) between H19 and C19[13] and a UV absorption maximum

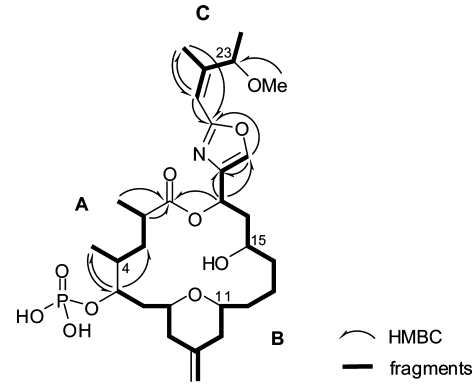


Figure 1. Fragments **A**−**C** deduced from COSY, HSQC, and selected HMBC correlations in **1**.

(6) *Cinachyrella enigmatica* (Burton, 1934) is the accepted taxonomic classification, but this sponge has also been described in the past as *Raphidotethya enigmatica*. Van Soest, R. W. M.; Rützler, K. In *Systema Porifera: A Guide to the Classification of Sponges*; Hooper, J. N. A., Van Soest, R. W. M., Eds.; Kluwer Academic/Plenum Publishers: New York, 2002; pp 85−98.

(7) Boyd, M. R. In *Current Therapy in Oncology*; Niederhuber, J. E., Ed.; B. C. Decker, Inc.: Philadelphia, PA, 1993; pp 11−22.

(8) (a) Barnathan, G.; Mirallés, J.; Gaydou, E. M.; Boury-Esnault, N.; Kornprobst, J.-M. *Lipids* **1992**, *27*, 779–784. (b) Barnathan, G.; Mirallés, J.; Kornprobst, J.-M. *Nat. Prod. Lett.* **1993**, *3*, 113–118. (c) Barnathan, G.; Doumenq, P.; Njinkour, J.-M.; Mirallés, J.; Mangoni, A.; Debitus, C.; Levi, C.; Kornprobst, J.-M. *Lipids* **1994**, *29*, 297–303. (d) Barnathan, G.; Genin, E.; Velosaotsy, N. E.; Kornprobst, J.-M.; Al-Lihaibi, S.; Al-Sofyani, A.; Nongonierma, R. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.* **2003**, *135*, 297–308. (e) Li, L. Y.; Deng, Z. W.; Li, J.; Fu, H. Z.; Lin, W. H. *Beijing Da Xue Xue Bao* **2004**, *36*, 12–17.

(9) (a) Xiao, D.-J.; Peng, X.-D.; Deng, S.-Z.; Ma, W.-J.; Wu, H.-M. *Youji Huaxue (Chin. J. Org. Chem.)* **2005**, *25*, 1606–1609. (b) Rodríguez, J.; Nuñez, L.; Peixinho, S.; Jiménez, C. *Tetrahedron Lett.* **1997**, *38*, 1833–1836. (c) Barnathan, G.; Mirallés, J.; Njinkour, J.-M.; Mangoni, A.; Fattorusso, E.; Debitus, C.; Boury-Esnault, N.; Kornprobst, J.-M. *Comp. Biochem. Physiol.* **1992**, *103B*, 1043–1047. (d) Cui, J.; Huang, L.; Fan, L.; Zhou, A. *Steroids* **2008**, *73*, 252–256.

(10) Medeiros, D. S.; Medeiros, T. L.; Riberio, J. K.; Monteiro, N. K.; Migliolo, L.; Uchoa, A. F.; Vasconcelos, I. M.; Oliveira, A. S.; de Sales, M. PP.; Santos, E. A. *Comp. Biochem. Physiol. B, Biochem. Mol. Biol.* **2010**, *155*, 211–216.

(11) (a) Shimogawa, H.; Kuribayashi, S.; Teruya, T.; Suenaga, K.; Kigoshi, H. *Tetrahedron Lett.* **2006**, *47*, 1409–1411. (b) Berlinck, R. G. S.; Burtoloso, A. C. B.; Kossuga, M. H. *Nat. Prod. Rep.* **2008**, *25*, 919–954.

(12) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 2780–2781.

(13) Hiemstra, H.; Houwing, H. A.; van Leusen, A. M. *Can. J. Chem.* **1979**, *57*, 3168–3170.

Table 1. ^{1}H and ^{13}C NMR Data for Enigmazole A (**1**) in CD_3OD

position	^{13}C[a]	^{1}H, m, *J* (Hz)	HMBC ($^{1}H \rightarrow ^{13}C$)[b]	NOESY[c]
1	176.4			
2	39.6	2.98[a]	1, 26	(3a), 3b, 5, 26
3 (a)	39.3	1.38 t 10.8	4, 5	(2), (15), 26, 27
(b)		1.88 m	1, 4	2, 26, 27
4	36.2	1.62[a]	27	5, 7, (15), 27
5	75.8	4.42 m	3, 27	2, 4, (6a), 6b, 7
6 (a)	40.1	1.87 m	5, 7	(5), (7), 28
(b)		2.10 m	4, 5, 7, 8	5, (7)
7	77.2	3.12 dd 9.8, 10.3		4, 5, (6a), (6b), (8a), 8b, 11
8 (a)	43.0	1.97 dd 12.3, 12.8	7, 9, 28	(7)
(b)		2.21 d 12.8	9, 28, 10	7, 28b
9	146.3			
10 (a)	42.4	1.84[a]	9, 28	
(b)		2.13 d 12.8	8, 9, 28	28a, 11, 12a, (12b)
11	76.2	3.29[a]		7, 10b, 12a, (12b), 14b
12 (a)	36.2	1.37 t 11.3	10, 13, 14	10b, 11, 13b, (14a)
(b)		1.64[a]	10, 14	(10b), (11)
13 (a)	21.8	1.54 q 12.4		14b, 15
(b)		1.72[a]		12a
14 (a)	33.6	1.02 dt 3.4, 12.0		(12a), 16a
(b)		1.76[a]		11, 13a, 15
15	69.8	3.62 dt 4.3, 11.1		(3a), (4), 13a, 14b, 16a, 16b, (27)
16 (a)	42.6	1.77[a]	15	14a, 15, 17
(b)		2.50 dt 13.2, 3.4	14, 15, 17, 18	15, 17
17	65.9	5.95 dd 12.8, 2.5	1, 15, 16, 18, 19	16a, 16b
18	142.3			
19	136.0	7.68 s	18, 20	
20	161.7			
21	113.9	6.21 s	20, 22, 23, 25	25
22	152.7			
23	77.0	5.24 q 6.5	21, 24, 25, 23-OMe	24, 23-OMe
24	19.4	1.26 d 6.4 (3H)	22, 23	25, 23
25	17.6	1.89 s (3H)	20, 21, 22	21
26	18.2	1.10 d 6.4 (3H)	1, 2, 3, 4	2, 3a, 3b
27	14.7	0.97 d 6.4 (3H)	3, 4, 5	3a, 4, (15), 6a
28 (a)	108.8	4.69 d 1.5	8	10b
(b)		4.70 d 1.5	10	8b
23-OMe	56.8	3.20 s (3H)	23	23

[a] Signals assigned from HSQC data. [b] Data obtained with mixing time 60 and 100 ms. [c] Data obtained with mixing time 300 ms. Geminal correlations omitted. Weak NOE interactions are in parentheses.

at 260.5 nm (ϵ 12000). HMBC correlations from δ 2.98 (H2) and 1.10 (H_3-26) at the terminus of **A** to a carboxylic resonance at δ_C 176.4 (C1), and from H17 in **B** to the same carbonyl resonance, confirmed the final connectivity of **A** with **B** via an ester bridge, thus revealing the macrocyclic nature of **1**. Of the five remaining oxymethines, the location of the methoxyl group was provided by an HMBC correlation between the -OMe resonance (δ 3.20) and C23 (δ 77.0). The presence of an ether bridge linking C7 with C11 was revealed by NOESY crosspeaks between H7 (δ 3.12) and H11 (δ 3.29), which also established their *syn*-1,3-diaxial relationship in the resulting tetrahydropyran (THP) ring. Although diagnostic correlations across this ether linkage were lacking in the HMBC spectrum, this assignment was secured by the observation of large vicinal (diaxial) couplings between H7 and H8a (8.2−11.7 Hz in DMSO-d_6, Supporting Information) and between H10a and H11 (9.2 Hz in DMSO-d_6, Supporting Information). These large couplings suggested that, even with the constraint of the exomethylene substituent at C9, the embedded THP ring in **1** adopts a conformation similar to a typical chair conformation. Attachment of a phosphate group at C5 was deduced from splitting of this carbon resonance (δ 71.5) by $^{2}J_{CP} = 4.7$ Hz in the ^{13}C NMR spectrum in DMSO-d_6 (Supporting Information).[14] With this assignment secured, the final oxymethine at C15 had to be substituted with a hydroxyl group to satisfy the molecular formula. Finally, the *Z*-geometry of the $\Delta^{21,22}$ double bond was deduced from a NOESY correlation between H21 and H_3-25. This completed the assignment of the planar structure of enigmazole A (**1**) based on spectral analysis of the parent compound. ^{1}H and ^{13}C NMR data for **1** in CD_3OD are summarized in Table 1.

Definition of the absolute configuration of the eight stereogenic centers in **1** required a series of microscale derivatization reactions and spectroscopic analysis of their products. The absolute configuration at C15 was determined by applying a modified Mosher's ester method to the secondary hydroxyl group.[15] The phosphate group in **1** required protection by permethylation with tetramethylsilyldiazomethane (TMS-diazomethane) before it could be efficiently reacted with the (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chlorides to give MTPA esters **4a** and **4b**, respectively. A consistent distribution of positive and negative $\Delta\delta$ values around C15 (Figure 2) allowed the assignment of *S*-configuration for C15.

This result was correlated to the configuration at C17 by forming an acetonide linkage between C15 and C17. To

(14) In addition, splitting of δ 32.8 (C4) by $^{3}J_{CP} = 5.3$ Hz and slight broadening of δ 38.7 (C6) were observed.

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

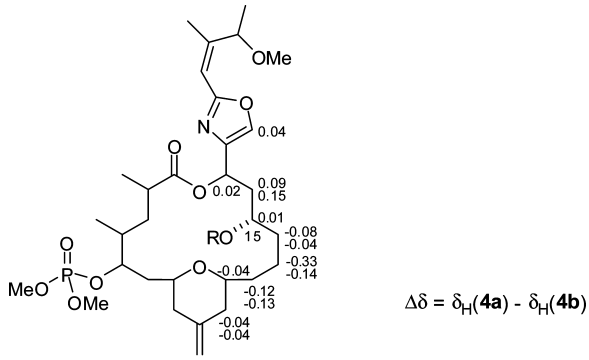


Figure 2. $\Delta\delta$ values calculated for the MTPA esters of **1**.

accomplish this, **1** was reacted with methylamine in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) to convert the phosphate group to an *N*,*N*′-dimethylphosphorodiamidate. The macrolide ring was then opened with KOH/MeOH, and the resulting carboxylic acid was converted to the methyl ester derivative **5** by treatment with TMS−diazomethane (described below). Methylation was necessary to facilitate separation of the ring-opened derivative from a reaction byproduct, tripyrrolidinophosphine oxide, which originated from PyBOP. Compound **5** was then reacted with pyridinium *p*-toluenesulfonate (PPTS) and 2,2-dimethoxypropane to give acetonide **6** (Scheme 1). A NOESY experiment with **6** showed that both of the oxymethine protons H15 (δ 4.05) and H17 (δ 4.99) had nuclear Overhauser effect (NOE) interactions with the same acetonide methyl group (δ 1.56) but not with the other one (δ 1.39), thus revealing a *syn* relationship for this 1,3-diol system. This assignment was also supported by characteristic ^{13}C NMR chemical shifts measured for the geminal acetonide methyl groups (δ_C 19.8 and 30.2).[16] Thus, the absolute configuration at C17 was established to be *S*.

The absolute stereochemistry at C2 was determined by applying a chiral anisotropy method to the ring-opened, phosphate-protected product of **1** using 2-phenylglycine methyl ester (PGME).[17] To accomplish this, the methyl ester in compound **5** was hydrolyzed with KOH and the carboxylic product condensed with (*S*)-(+)- or (*R*)-(−)-PGME to give the PGME amides **7a** and **7b**, respectively (Scheme 1). The $\Delta\delta$ value for each proton was calculated in an analogous manner with Mosher's method ($\Delta\delta = \delta_{(S)} - \delta_{(R)}$). In this case, the C26 methyl protons had a negative $\Delta\delta$ value while the other neighboring protons had positive values. This allowed assignment of *S*-configuration for C2.

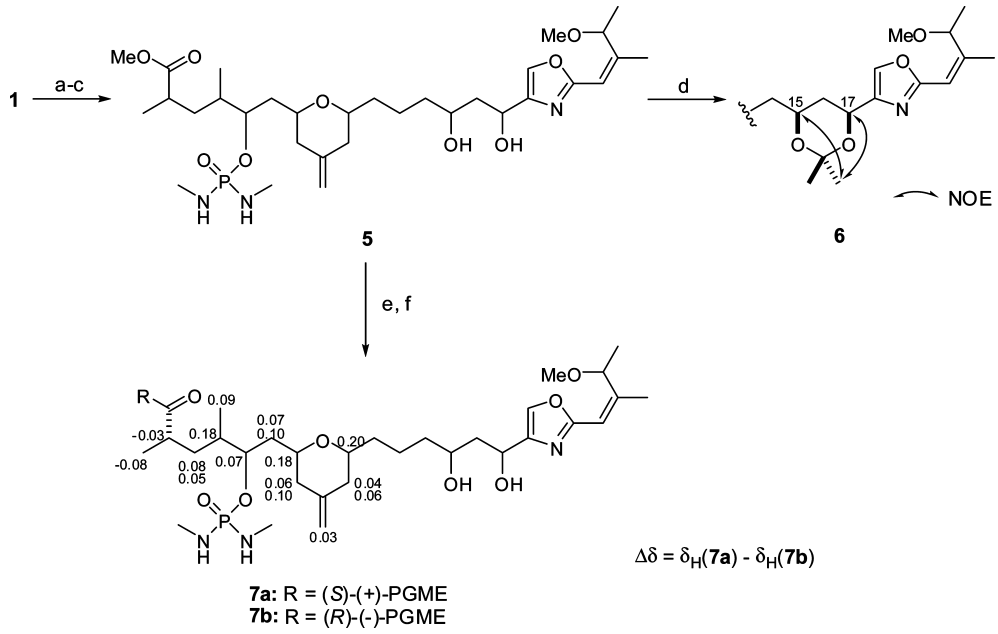
To deduce the absolute configurations at C4 and C5, we first attempted to apply *J*-based configurational analysis[18] to the C2−C5 aliphatic chain using the linear derivative **5**. However, measurement of the necessary long-range C−H couplings by HETLOC and HSQMBC experiments[19] was problematic. The protons in question (H2, 3a, 3b, 4, and 5) are highly coupled with multiple neighboring protons that severely decrease the intensity of the crosspeaks and complicate the phasing and interpretation of the extracted 1D spectral slices. In addition, spectral overlap and close proximity of key resonances in both the proton and carbon dimensions contribute to ambiguity in the assignment of diagnostic crosspeaks and measurement of the $^{2,3}J_{H,C}$ couplings. Therefore, a new strategy was pursued to remove the phosphate group, open the macrolide ring, and recyclize the product to form a δ-lactone ring at C5. This would allow conformational analysis of the δ-lactone ring substituents by standard NMR experiments. Initial attempts at enzymatic cleavage of the phosphate ester linkage in **1** by either alkaline (bovine) or acid (potato) phosphatase[20] were unsuccessful and only gave starting material. Consequently, a mild hydrolysis at an elevated temperature was attempted.[21] Enigmazole A (**1**) was dissolved in a small amount of DMSO and refluxed in an acetate buffer (pH ~4) for 20 h,[22] which successfully gave the desired product **8** with an acceptable yield (~20%). Conformational analysis of the δ-lactone moiety of **8** by a NOESY experiment revealed that the ring adopts a half-chair conformation,[23] as depicted in Scheme 2. This was evident from the observation of NOE correlations between H2/H$_3$-27, H4/H5, and H6a/H$_3$-27 but not between H5/H$_3$-27. Since the absolute configuration at C2 was already established as *S*, this required that the absolute configurations at C4 and C5 were both *S*.[24]

The absolute configurations of the remaining two chiral centers at C7 and C11 were deduced from ^{1}H−^{1}H coupling constant analyses and NOE interactions observed in CD_3OD and DMSO-d_6, in conjunction with molecular modeling studies. Key NOEs included those between H2/H5, H4/H5, H4/H7, H5/H7, H7/H11, and H11/H14b that indicated all of these protons are on the same face of the macrolide ring, in addition to the cross-ring NOEs between H15 and H3a, H4, and the C28 methyl group. Molecular modeling showed that this set of NOE interactions could only be possible if C7 and C11 each had *R*-configurations. Molecular modeling also supported the full set of stereochemistry assignments around the macrolide ring. Beginning with the planar structure of enigmazole A and the full set of observed NOE interactions, we used a series of restrained conformational searches (described in the Supporting Information) to arrive independently at the same relative chirality for the seven stereocenters in the macrolide ring that was deduced from spectroscopic studies. An unrestrained global energy minimum conformation in solution for the C2 *S*, C4 *S*, C5 *S*, C7 *R*, C11 *R*, C15 *S*, C17 *S* stereoisomer was then calculated and is shown in Figure 3. This structure is fully consistent with all of the measured coupling constants and NOEs, including key transannular NOE interactions.

The final stereogenic center at C23 was remote from the other chiral centers, and it was not directly amenable to stereochemical

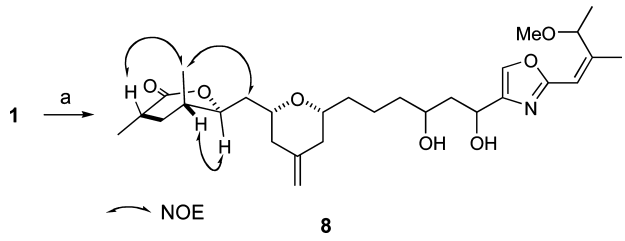
(16) Rychnovsky, S. D.; Richardson, T. I.; Rogers, B. N. *J. Org. Chem.* **1997**, *62*, 2925–2934.
(17) Nagai, Y.; Kusumi, T. *Tetrahedron Lett.* **1995**, *36*, 1853–1856.
(18) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
(19) Williamson, R. T.; Márquez, B. L.; Gerwick, W. L.; Kövér, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265–273.
(20) Boger, D. L.; Hikota, M.; Lewis, B. M. *J. Org. Chem.* **1997**, *62*, 1748–1753.
(21) A recent study has verified that hydrolysis of alkyl phosphate monoesters proceeds by a unimolecular mechanism. See: Grzyska, P. K.; Czyryca, P. G.; Purcell, J.; Hengge, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 13106–13111.
(22) Grzyska, P. K.; Czyryca, P. G.; Golightly, J.; Small, K.; Larsen, P.; Hoff, R. H.; Hengge, A. C. *J. Org. Chem.* **2002**, *67*, 1214–1220.
(23) Brandänge, S.; Färnbäck, M.; Leijonmarck, H.; Sundin, A. *J. Am. Chem. Soc.* **2003**, *125*, 11942–11955.
(24) In a separate experiment to assess possible epimerization at C2 during δ-lactone formation, the macrolide was opened by saponification ($NaOD-D_2O/CD_3OD$) and then recyclized to the δ-lactone **8** by prolonged treatment with $CD_3CN/CF_3COOD-D_2O$ (see Supporting Information). No epimerization was observed since H2 of **8** integrated for one full proton in the ^{1}H NMR spectrum and the mass spectrum showed no evidence of deuterium incorporation.

Scheme 1. Preparation of Acetonide **6** and PGME Amides **7a** and **7b**[a]



[a] Reagents and conditions: (a) $MeNH_2$−THF, PyBOP, HOBt, DMF, rt, 25 h. (b) 1 N KOH/MeOH (1:2), 57 °C, 12 h. (c) $TMSCHN_2$−*n*-hexane, MeOH, rt, 30 min. (d) PPTS, 2,2-dimethoxypropane, dichloromethane. (e) 0.5 N KOH/MeOH (1:1), rt, 2 h. (f) PGME, PyBOP, HOBt, TEA, DMF, rt, 85 min.

Scheme 2. Preparation of δ-Lactone **8**[a]



[a] Reagent and conditions: (a) DMSO, 0.2 M potassium acetate buffer (pH 4.1), reflux, 20 h.

analysis by NMR experiments. The strategy to define its absolute configuration involved oxidative cleavage of the $\Delta^{21,22}$ double bond in **1** to generate 3-methoxy-2-butanone and comparison of the CD spectrum of the reaction product to spectra of synthetically prepared standards. Due to the relatively high volatility of 3-methoxy-2-butanone, and to aid in its final purification, the reaction product needed to be trapped as its 2,4-dinitrophenylhydrazone derivative[25] before subjecting it to chiroptic comparisons. Thus, **1** was treated with $RuCl_3$−$NaIO_4$ to produce 3-methoxy-2-butanone, which was then condensed with 2,4-dinitrophenylhydrazine under weakly acidic conditions to yield hydrazone **9**. Only a single hydrozone derivative with the energetically favored *E* geometry of the hydrozone double bond was observed under these conditions. The synthesis of authentic (*R*)-3-methoxy-2-butanone (*E*)-2,4-dinitrophenylhydrazone (*R*-**9**) is shown in Scheme 3. The starting material, (*R*)-acetoin (3-hydroxy-2-butanone), was synthesized as an aqueous solution according to Crout's procedure.[26] A key step in the methylation of acetoin was the use of MeI−Ag_2O, since optically active acetoin is known to racemize when taken to dryness, and this combination of reagents allowed the coexistence of water in the reaction system. Racemic hydrazone (*rac*-**9**) was prepared in the same manner starting from racemic acetoin. The CD spectra of synthetic *R*-**9** and racemic *rac*-**9** were recorded and compared to the CD spectrum of hydrozone **9** derived from enigmazole A (**1**). The CD spectrum of *R*-**9** revealed a pronounced negative Cotton effect, with minima observed at 239.5 and 348.4 nm, and this closely matched the CD spectrum of **9** that was generated from enigmazole A (**1**) (Figure 4). Thus, the configuration at C23 was unambiguously proved to be *R*, and this completed the structural assignment of enigmazole A (**1**).

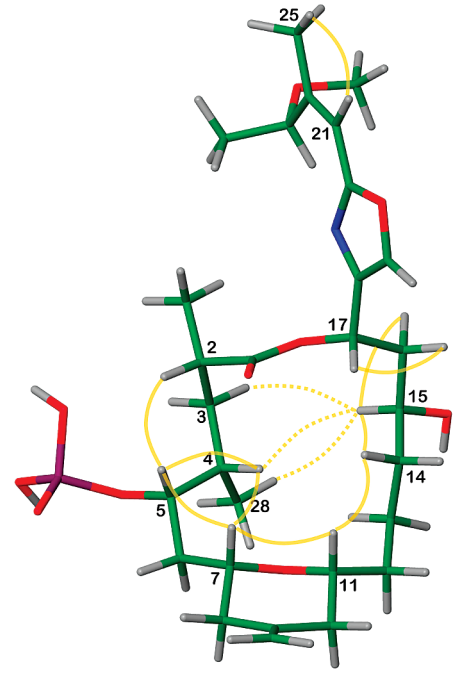
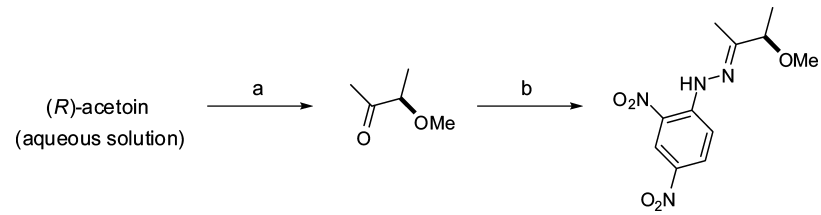


Figure 3. Global energy minimum conformation of **1** with selected NOE interactions (in yellow).

(25) Behforouz, M.; Bolan, J. L.; Flynt, M. S. *J. Org. Chem.* **1985**, *50*, 1186–1189.
(26) Crout, D. H. G.; Morrey, S. M. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2435–2440.

Scheme 3. Preparation of (*R*)-3-Methoxy-2-butanone (*E*)-2,4-Dinitrophenylhydrazone (*R*-**9**)[a]



[a] Reagents and conditions: (a) MeI, Ag_2O, DCM, rt, 16 h. (b) 2,4-Dinitrophenylhydrazine, 0.25 M NaH_2PO_4 in H_2O−EtOH−DCM, rt, 16 h.

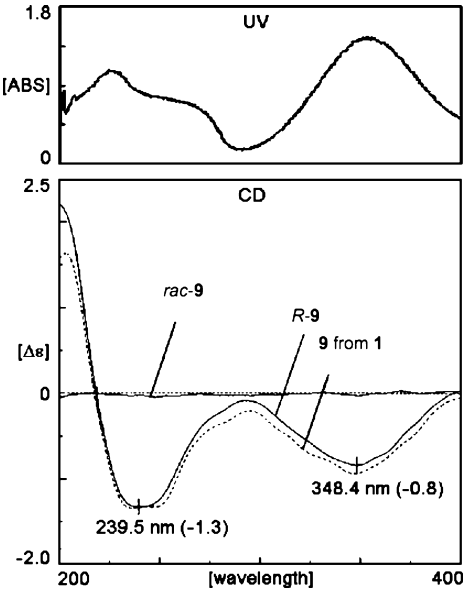


Figure 4. Overlaid CD spectra for authentic 3-methoxy-2-butanone (*E*)-2,4-dinitrophenylhydrazone (*rac*-**9** and *R*-**9**) and **9** derived from the natural product **1**. The reference UV spectrum is of *rac*-**9**.

The molecular formula of compound **2** was determined to be $C_{30}H_{48}NO_{10}P$ by NMR analyses and HR-ESIMS measurements (obsd *m*/*z* 612.2943 $[M - H]^-$, calcd for $C_{30}H_{47}NO_{10}P$ 612.2954, Δ −1.1 mmu). This differed from the molecular formula of **1** by the addition of CH_2. The ^1H and ^{13}C NMR spectra of **2** were very similar to those recorded for **1**, except a new *O*-methyl resonance (δ_H 3.38/δ_C 57.2) was evident (see Supporting Information for tabulated NMR data). Extensive 2D NMR analyses showed that **2** was simply the 15-*O*-methyl derivative of enigmazole A (**1**). To confirm this structure, the hydroxyl and phosphate groups in **1** were methylated with $Me_3O^+BF_4^-$ in the presence of Proton Sponge to afford 15-*O*-methylenigmazole A methyl phosphodiester (compound **10**, Supporting Information). The ^1H NMR and HSQC data of **10** were virtually identical to those of the corresponding methyl phosphodiester derivative prepared from **2** (Supporting Information). This result confirmed the structure and absolute stereochemistry of compound **2** as 15-*O*-methylenigmazole A.

NMR spectra recorded for compound **3** corresponded closely to those of **2**, except for the presence of an additional oxymethine proton at δ 4.06 (see Supporting Information for tabulated NMR data). Diagnostic $^1H-^1H$ COSY, HSQC, and HMBC correlations revealed that **3** was the hydroxylated analogue 13-hydroxy-15-*O*-methylenigmazole A. This assignment was supported by HR-ESIMS measurements that established a molecular formula of $C_{30}H_{48}NO_{11}P$ (obsd *m*/*z* 628.2881 $[M - H]^-$, calcd for $C_{30}H_{47}NO_{11}P$ 628.2887, Δ −0.6 mmu). Structure confirmation and assignment of the absolute stereochemistry of **3** were accomplished by preparation and comparison of suitable derivatives. Compound **3** was successfully dephosphorylated using Antarctic phosphatase (Supporting Information), and then the two hydroxyl groups were derivatized with (*R*)-(+)- and *S*-(−)-MTPA chloride for Mosher's analysis. NMR comparison of the resulting bis(*S*)-(+)- and (*R*)-(−)-MTPA esters (Supporting Information) indicated that the absolute stereochemistry at C5 and C13 was *S* and *R*, respectively. Further support for the structural assignment of **3** was provided by comparison of the spectral properties of dephospho **3** with those of the dephosphorylated analogue of **2** (Supporting Information). The ^1H and ^{13}C chemical shift values in dephospho **3** were fully consistent with those of dephospho **2** ($\Delta\delta_C < 0.3$ ppm, $\Delta\delta_H < 0.02$ ppm), except in the region from C12 to C16. This established that compounds **2** and **3** had identical stereochemistries, with the exception of the additional hydroxyl group in **3**. An NOE comparison between the dephospho derivatives of **2** and **3** (Supporting Information) also supported the assignment of 13-hydroxy-15-*O*-methylenigmazole A (**3**).

Enigmazole A (**1**) and its congeners represent a new structural family of marine phosphomacrolides with an unusual array of structural motifs and functional groups. Compounds **1**−**3** each contain an 18-membered macrocyclic ring with an embedded 2,6-disubstituted 4-methylenetetrahydropyran moiety[27] and a $C_9H_{12}NO_2$ appendage to the macrolide that includes a disubstituted oxazole. The methylation and hydroxylation patterns observed in the macrolide portion of enigmazoles **1**−**3** are clearly indicative of polyketide-based biosynthesis. The oxazole-containing appendage, at least in part, is suggestive of a non-ribosomal peptide origin. The substitution of a phosphate group on the macrolide ring of the enigmazoles is unprecedented for marine macrolides. While a number of phosphorylated lipids, nucleotides, sterols, and saccharides have been reported from marine sources, the occurrence of phosphate substituents in complex secondary metabolites is quite rare, and there have been no prior reports of phosphorylated marine macrolides. However, several phosphate-substituted macrolides have been described from two terrestrial fungal sources[28] and an isolate of a *Bacillus* bacterium.[29] The calyculins[30] and a series of calyculin-like variants[31] represent the largest and most extensively studied group of phosphate-containing marine metabolites. These compounds were all isolated from sponges, and they comprise a family of linear polyketides that contain both phosphate and

(27) Tanaka, J.; Higa, T. *Tetrahedron Lett.* **1996**, *37*, 5535–5538.
(28) (a) Hosoe, T.; Fukushima, K.; Yakizawa, K.; Itabashi, T.; Kawahara, N.; Vidotto, V.; Kawai, K. *J. Antibiot.* **2006**, *59*, 597–600. (b) Kozoe, I.; Ueda, J.; Watanabe, M.; Nogami, S.; Nagai, A.; Inaba, S.; Ohya, Y.; Takagi, M.; Shin-ya, K. *J. Antibiot.* **2009**, *62*, 159–162.
(29) (a) Zimmerman, S. B.; Schwartz, C. D.; Monaghan, R. L.; Pelak, B. A.; Weisserger, B.; Gilfillan, E. C.; Mochales, S.; Hernandez, S.; Currie, S. A.; Tejera, E.; Stapley, E. O. *J. Antibiot.* **1987**, *40*, 1677–1681. (b) Wilson, K. E.; Flor, J. E.; Schwartz, R. E.; Joshua, H.; Smith, J. L.; Pelak, B. A.; Liesch, J. M.; Hensens, O. D. *J. Antibiot.* **1987**, *40*, 1682–1691. (c) Zweerink, M. M.; Edison, A. *J. Antibiot.* **1987**, *40*, 1692–1697.

oxazole moieties. Calyculin A, the lead compound in this class, is highly cytotoxic and has been shown to be a potent inhibitor of protein phosphatase 1 (PP1) and PP2A.[32] Additional phosphorylated marine natural products that have been reported include the phosphodiester pokepola ester,[33] two deoxynojirimycin derivatives,[34] two haplosamate steroids,[35] and two celebeside cyclic depsipeptides.[36] All of these compounds were isolated from sponge extracts, but their structural features and diverse taxonomic distribution suggest that the true biosynthetic origins are likely sponge-associated microorganisms.[37]

Marine macrolides constitute an important class of bioactive marine natural products, and they have been the focus of numerous studies and developmental efforts as potential antitumor agents.[38] Macrolides have recently been reported from at least six different sponge genera[39] and an array of marine microorganisms, including sediment-derived actinomycetes,[40a] plant-associated Gram-positive bacteria,[40b] benthic dinoflagellates,[40c] sponge-associated fungi,[40d,e] and cyanobacteria.[40f] In addition to clearly recognizable polyketide motifs, these macrolides exhibit diverse structural features including glycosylation, halogenation, and nitrogen or sulfur incorporation. Many of these recently isolated compounds were reported to exhibit significant antitumor and cytotoxic properties. Enigmazole A (**1**) was the primary cytotoxic constituent of the *C. enigmatica* extract, and it was tested for antiproliferative activity in the NCI 60-cell antitumor assay. It was shown to be active against the 60 human tumor cell lines, with a mean GI_{50} value of 1.7 μM, but there was no particular pattern of tumor panel specificity or differential activity among the cell lines. Thus, application of the COMPARE algorithm to identify possible molecular targets or pathways associated with its cytotoxic activity was not feasible.[41] Since enigmazole A (**1**) shared several functional groups and structural features with calyculin A, it was tested for activity against a panel of 16 different protein phosphatase enzymes including PPT1 and PPT2A, but it was inactive at a high test concentration of 40 μg/mL. This indicated that the cytotoxic activity of **1** was not due to inhibition of a phosphatase, but it left open the possibility that **1** interfered with cellular phosphorylation dynamics by blocking the addition of phosphate groups to proteins. To explore this possibility, **1** was also tested against a panel of 70 different protein kinases, and again it was inactive at 40 μg/mL. The phosphorylation state of key signaling proteins controls the function of cellular signal transduction pathways, and disruption of these pathways often results in growth inhibition or cell death. The presence of a phosphate functionality on enigmazole A (**1**) suggested that it might affect protein phosphorylation processes, but extensive testing failed to substantiate this hypothesis.

Subsequent to the structural studies with enigmazole A (**1**), compounds **1**−**3** and several advanced chromatographic fractions from the *C. enigmatica* extract were tested in an assay for inhibitors of the receptor tyrosine kinase c-Kit. Mutations causing constitutive activation of c-Kit have been implicated in a variety of cancers, including acute myelogenous leukemia and gastrointestinal stromal tumors.[42] The assay screened for compounds that were differentially cytotoxic to cells expressing constitutively active kinase domain mutants of c-Kit relative to those with wild-type c-Kit. While compounds **1**−**3** were equally cytotoxic to cells with either wild-type or mutant c-Kit, several of the side fractions that were tested selectively inhibited cells expressing mutant c-Kit. This was a very rare phenotypic effect, as only 32 natural product extracts out of 134 631 extracts that were tested produced the desired differential response.[43] Spectroscopic analysis of the differentially cytotoxic fractions revealed the presence of additional enigmazole analogues that were responsible for activity in the c-Kit assay. Structural and biological characterization of these compounds will be communicated in the future. Thus, the core enigmazole structure provides a potential scaffold for the discovery and development of compounds that can selectively target cells with aberrant c-Kit signaling. Enigmazoles **1**−**3** are structurally unique, as they constitute the first family of marine phosphomacrolides, but like

(30) (a) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780–2781. (b) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K. *J. Org. Chem.* **1988**, *53*, 3930–3932. (c) Matsunaga, S.; Fujiki, H.; Sakata, D.; Fusetani, N. *Tetrahedron* **1991**, *47*, 2999–3006. (d) Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1991**, *32*, 5605–5606.
(31) (a) Fu, X.; Schmitz, F. J.; Kelly-Borges, M.; McCready, T. L.; Holmes, C. F. B. *J. Org. Chem.* **1998**, *63*, 7957–7963. (b) Dumdei, E. J.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *J. Org. Chem.* **1997**, *62*, 2636–2639. (c) Matsunaga, S.; Wakimoto, T.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 2640–2642. (d) Edrada, R. A.; Ebel, R.; Supriyono, A.; Wray, V.; Schupp, P.; Steube, K.; van Soest, R.; Proksch, P. *J. Nat. Prod.* **2002**, *65*, 1168–1172.
(32) (a) Holmes, C. F. B.; Maynes, J. T.; Perreault, K. R.; Dawson, J. F.; James, M. N. G. *Curr. Med. Chem.* **2002**, *9*, 1981–1989. (b) Wakimoto, T.; Matsunaga, S.; Takai, A.; Fusetani, N. *Chem. Biol.* **2002**, *9*, 309–319.
(33) Kalidindi, R. S.; Yoshida, W. Y.; Palermo, J. A.; Scheur, P. J. *Tetreahedron Lett.* **1994**, *35*, 5579–5582.
(34) Sakai, R.; Kamiya, H. *J. Antibiot.* **2006**, *59*, 507–511.
(35) Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; van Soest, R. W. M.; Heubes, M.; Faulkner, D. J.; Fusetani, N. *Tetrahedron* **2001**, *57*, 3885–3890.
(36) Plaza, A.; Bifulco, G.; Keffer, J. L.; Lloyd, J. R.; Baker, H. L.; Bewley, C. A. *J. Org. Chem.* **2009**, *74*, 504–512.
(37) Simmons, T. L.; Coates, R. C.; Clark, B. R.; Engene, N.; Gonzalez, D.; Esquenazi, E.; Dorrestein, P. C.; Gerwick, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4587–4594.
(38) Napolitano, J. G.; Daranas, A. H.; Norte, M.; Fernández, J. J. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 122–137.
(39) (a) Dalisay, D. S.; Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. *J. Am. Chem. Soc.* **2009**, *131*, 7552–7553. (b) Field, J. J.; Singh, A. J.; Kanakkanthara, A.; Halafihi, T.; Northcote, P. T.; Miller, J. H. *J. Med. Chem.* **2009**, *52*, 7328–7332. (c) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 153–156. (d) Plaza, A.; Bifulco, G.; Keffer, J. L.; Lloyd, J. R.; Baker, H. L.; Bewley, C. A. *J. Org. Chem.* **2009**, *74*, 504–512. (e) Oguchi, K.; Tsuda, M.; Iwamoto, R.; Okamoto, Y.; Kobayashi, J.; Fukushi, E.; Kawabata, J.; Ozawa, T.; Masuda, A.; Kitaya, Y.; Omasa, K. *J. Org. Chem.* **2008**, *73*, 1567–1570. (f) Greve, H.; Kehraus, S.; Kcrick, A.; Kelter, G.; Maier, A.; Fiebig, H.-H.; Wright, A. D.; König, G. M. *J. Nat. Prod.* **2008**, *71*, 309–312. (g) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Tetrahedron Lett.* **2008**, *49*, 4355–4358. (h) Plaza, A.; Baker, H. L.; Bewley, C. A. *J. Nat. Prod.* **2008**, *71*, 473–477. (i) MacMillan, Xiong-Zhou, G.; Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 3699–3706. (j) Amagata, T.; Johnson, T. A.; Cichewicz, R. H.; Tenney, K.; Mooberry, S. L.; Media, J.; Edelstein, M.; Valeriote, F. A.; Crews, P. *J. Med. Chem.* **2008**, *51*, 7234–7242.
(40) (a) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2009**, *74*, 675–684. (b) Xue, C.; Tian, L.; Xu, M.; Deng, Z.; Lin, W. *J. Antibiot.* **2008**, *61*, 668–674. (c) Oguchi, K.; Tsuda, M.; Iwamoto, R.; Okamoto, Y.; Kobayashi, J.; Fukushi, E.; Kawabata, J.; Ozawa, T.; Masuda, A.; Kitaya, Y.; Omasa, K. *J. Org. Chem.* **2008**, *73*, 1567–1570. (d) Kito, K.; Ookura, R.; Yoshida, S.; Namikoshi, M.; Ooi, T.; Kusumi, T. *Org. Lett.* **2008**, *10*, 225–228. (e) Greve, H.; Schupp, P. J.; Eguereva, E.; Kehraus, S.; Kelter, G.; Maier, A.; Fiebig, H.-H.; König, G. M. *Eur. J. Org. Chem.* **2008**, 5085–5092. (f) Teruya, T.; Sasaki, H.; Kitamura, K.; Nakayama, T.; Suenaga, K. *Org. Lett.* **2009**, *11*, 2421–2424.
(41) Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91–109.
(42) (a) Lennartsson, J.; Jelacic, T.; Linnekin, D.; Shivakrupa, R. *Stem Cells* **2005**, *23*, 16–43. (b) Lennartson, J.; Ronnstrand, L. *Curr. Cancer Drug Targets* **2006**, *6*, 65–75.
(43) Henrich, C. J.; Goncharova, E. I.; Wilson, J. A.; Gardella, R. S.; Jonhson, T. R.; McMahon, Takada, K.; Bokesch, H. R.; Gustafson, K. R. *Chem. Biol. Drug Des.* **2007**, *69*, 321–330.

many other cytotoxic natural products, the actual cellular target of these bioactive compounds is not known at this time.

Experimental Section

Collection, Extraction, and Isolation. Samples of *Cinachyrella enigmatica* were collected along the south coast of Papua New Guinea in 1998. The voucher number for this collection is 0CDN5936, and a voucher sample is maintained at the Smithsonian Institution in Washington, DC. The frozen sponge (202 g, wet weight) was ground in dry ice to a fine powder and extracted with H_2O at 4 °C to give 46.6 g of the aqueous extract. The animal residue was then successively soaked in MeOH and MeOH/CH_2Cl_2 (1:1) to give 4.6 g of a combined organic solvent extract. A 16.5 g aliquot of the aqueous extract was partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble fraction was combined with a 3.3 g aliquot of the organic extract and partitioned between 60% aqueous MeOH and CH_2Cl_2. The aqueous MeOH fraction was desalted on a C_4-wide pore reversed-phase support by washing with H_2O. A fraction recovered by eluting with 60% aqueous CH_3CN was subjected to size-exclusion chromatography using LH-20 (solvent: 70% aqueous CH_3CN containing 0.05% TFA), and aliquots of each fraction were directly tested for cytotoxicity against the IC-2 murine mast cell line. The combined active fraction (188.4 mg) was passed through a cotton plug and then purified by reversed-phase HPLC (Varian Dynamax C_{18}, 2 × 25 cm) with a gradient elution from 28% to 53% aqueous CH_3CN containing 0.1 M $NaClO_4$ over 30 min to give enigmazole A (**1**, 21.2 mg, 3.0×10^{-2}% yield based on wet weightt), 15-*O*-methylenigmazole A (**2**, 13.5 mg), and 13-hydroxy-15-*O*-methylenigmazole A (**3**, 2.6 mg).

Enigmazole A (1). Pale yellow solid; $[\alpha]^{25}_D$ −2.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 260.5 nm (4.08); HRFABMS $(M - H)^-$ *m/z* 598.2747 (calcd for $C_{29}H_{45}NO_{10}P$ 598.2781, Δ −3.4 mmu); ^1H and ^{13}C NMR data, see Table 1.

15-*O*-Methylenigmazole A (2). Pale yellow solid; $[\alpha]^{25}_D$ −13.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 261 nm (4.00); HRESIMS $(M - H)^-$ *m/z* 612.2943 (calcd for $C_{30}H_{47}NO_{10}P$ 612.2954, Δ −1.1 mmu); ^1H and ^{13}C NMR data, see Table S3 (Supporting Information).

13-Hydroxy-15-*O*-methylenigmazole A (3). Pale yellow solid; $[\alpha]^{25}_D$ −7.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 261 nm (3.90); HRESIMS $(M - H)^-$ *m/z* 628.2881 (calcd for $C_{30}H_{47}NO_{11}P$ 628.2887, Δ −0.6 mmu); ^1H and ^{13}C NMR data, see Table S3 (Supporting Information).

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Supporting Information Available: Experimental procedures and molecular modeling; NMR data tables for **1** in DMSO-d_6 and for **2** and **3** in CD_3OD; ^1H NMR, ^{31}P NMR, COSY, TOCSY, HSQC, HMBC, and NOESY spectra of **1**; ^1H NMR, COSY, HSQC, HMBC spectra of **2** and **3**; ^1H NMR and NOESY spectra of **8**, **11**, and **14**; and ^1H NMR spectra of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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