Isolation and First Total Synthesis of PM050489 and PM060184, Two New Marine Anticancer Compounds

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · JUNE 2013

Impact Factor: 12.11 · DOI: 10.1021/ja404578u · Source: PubMed

14 AUTHORS, INCLUDING:

Fernando Reyes
Fundación MEDINA
94 PUBLICATIONS **904** CITATIONS
SEE PROFILE

María Garranzo
PharmaMar
2 PUBLICATIONS **19** CITATIONS
SEE PROFILE

Carlos de Eguilior
PharmaMar
3 PUBLICATIONS **42** CITATIONS
SEE PROFILE

Andres Francesch
Pharma Mar, S.A.
78 PUBLICATIONS **951** CITATIONS
SEE PROFILE

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

pubs.acs.org/JACS

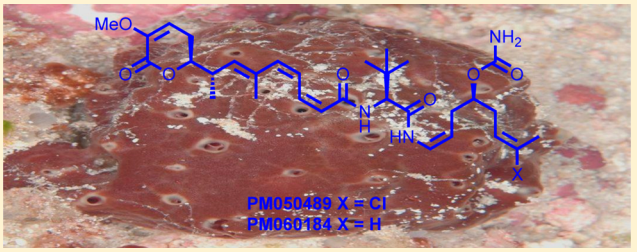
Isolation and First Total Synthesis of PM050489 and PM060184, Two New Marine Anticancer Compounds

María Jesús Martín, Laura Coello, Rogelio Fernández, Fernando Reyes,[§] Alberto Rodríguez, Carmen Murcia, María Garranzo, Cristina Mateo, Francisco Sánchez-Sancho,[⊥] Santiago Bueno, Carlos de Eguilior, Andrés Francesch, Simon Munt, and Carmen Cuevas*

Research and Development, PharmaMar, S. A., 28770 - Colmenar Viejo, Madrid, Spain

Supporting Information

ABSTRACT: Microtubules continue to be one of the most successful anticancer drug targets and a favorite hit for many naturally occurring molecules. While two of the most successful representative agents in clinical use, the taxanes and the vinca alkaloids, come from terrestrial sources, the sea has also proven to be a rich source of new tubulin-binding molecules. We describe herein the first isolation, structural elucidation and total synthesis of two totally new polyketides isolated from the Madagascan sponge *Lithoplocamia lithistoides*. Both PM050489 and PM060184 show antimitotic properties in human tumor cells lines at subnanomolar concentrations and display a distinct inhibition mechanism on microtubules. The development of an efficient synthetic procedure has solved the supply problem and, following pharmaceutical development, has allowed PM060184 to start clinical studies as a promising new drug for cancer treatment.



INTRODUCTION

The discovery and development of new drugs that can be used in the treatment of cancer remains one of the biggest challenges for the scientific community and the pharmaceutical industry. In recent decades there has been a boom in so-called targeted therapies and one of the most successful anticancer drug targets, tubulin, continues to be the focus of intense research efforts. Interestingly, nature has proven to be one of the best sources of new molecules that bind to tubulin and two of the most successful microtubule-targeting drugs, paclitaxel and the natural vinca alkaloids, were originally isolated from terrestrial sources.

The sea has also proven to be a highly productive source of compounds that bind to tubulin and some of them, such as discodermolide,[1] dolastatins and hemiasterlin, have entered clinical trials but were later discontinued due to toxicity issues. More successfully, plinabulin, a synthetic analogue of halimide, continues in clinical development (Figure 1) and eribulin (Halaven), an analogue of the marine natural macrolide halichondrin B synthesized by Kishi,[2] was approved by the FDA and European authorities for breast cancer in 2010 and 2011, respectively.

As part of our continuing research program to discover new anticancer compounds from marine sources, we describe herein the first isolation, full stereochemical structural determination, total synthesis and biological activity of two new marine natural products, PM050489 and PM060184 (**1** and **2**, Figure 2a), belonging to a new class of polyketides isolated from the sponge *Lithoplocamia lithistoides* collected in Madagascar and, to the best of our knowledge, hitherto uninvestigated.

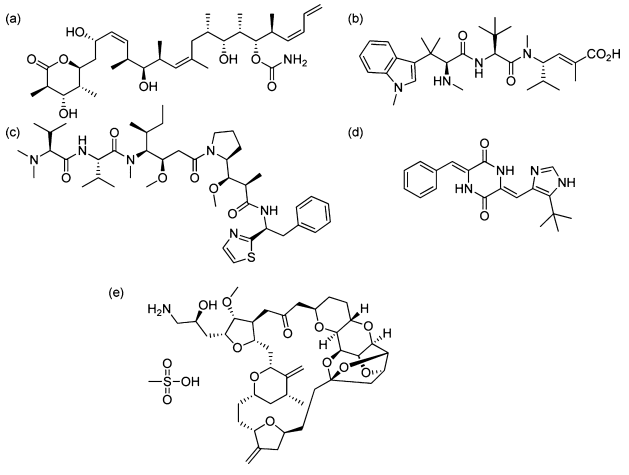


Figure 1. Chemical structures of tubulin binders. Marine natural products: (a) discordemolide, (b) hemiasterlin, (c) dolastatin 10. Synthetic compounds inspired by marine natural compounds: (d) plinabulin, (e) eribulin mesylate.

PM050489 and PM060184 have shown subnanomolar *in vitro* activity in human cancer cell lines, potent antimitotic activity, a new biochemical mechanism of interaction with tubulin[3] and potent *in vivo* activity in different animal models, thereby demonstrating that tubulin continues to be a valid target for cancer treatment. On the basis of its activity, distinct

Received: May 7, 2013
Published: June 10, 2013

dx.doi.org/10.1021/ja404578u | J. Am. Chem. Soc. 2013, 135, 10164−10171

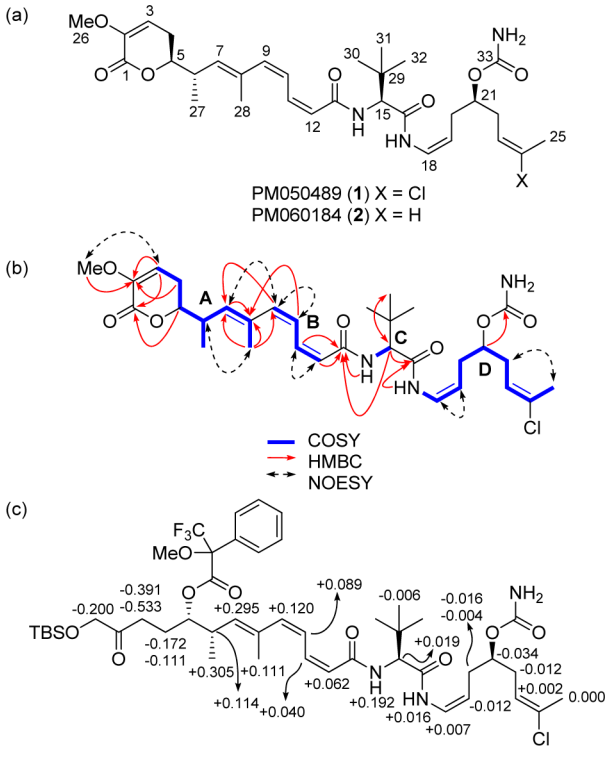


Figure 2. (a) Chemical structures of PM050489 (**1**) and PM060184 (**2**). (b) Key 2D NMR correlations observed for PM050489 (**1**). (c) $\Delta\delta(R,S)$ values for the MTPA esters **5a** and **5b**.

mechanism of action and good safety profile, PM060184 has entered clinical development, and phase I trials are currently underway in France, Spain and the United States of America.

RESULTS AND DISCUSSION

Isolation and Structural Elucidation. PM050489 (**1**) and PM060184 (**2**) were isolated from extracts (CH_2Cl_2/MeOH, 50:50) of the sponge *Lithoplocamia lithistoides* and purified by reversed-phase RP-18 chromatography and semipreparative HPLC. Initially, 1.6 mg (0.003%) of PM050489 (**1**) was obtained from a 61 g sample of frozen *L. lithistoides*. Further isolation of **1** from a second, larger group of sponge samples yielded 160.8 mg of **1** (0.002%) and 2.6 mg of **2** (0.00003%) from 7.66 kg of frozen animal material. The positive ion high-resolution (ESI) mass spectrum of **1** displayed a pseudomolecular ion at m/z 606.2940 $[M + H]^+$ with an isotopic distribution consistent with the presence of a chlorine atom in the molecule. These MS data and the presence of 31 signals in its ^{13}C NMR spectrum (Table 1) gave a molecular formula of $C_{31}H_{44}ClN_3O_7$ for the compound (calcd for $C_{31}H_{45}{}^{35}ClN_3O_7$ 606.2946).

The analysis of the HSQC spectrum revealed the presence in the molecule of six aliphatic and one oxygenated methyl groups, three aliphatic methylene units, and 13 methines, including one aliphatic, one nitrogenated, two oxygenated, and nine olefinic. In addition, the ^{13}C spectrum displayed signals for eight quaternary carbons, attributed to the presence in the molecule of three carbonyl groups, one carbamate, three quaternary olefinic, and one quaternary aliphatic carbon. Two signals in the ^1H spectrum at δ_H 8.78 and 6.51 ppm did not display any correlations in the HSQC spectrum and were therefore assigned to two NH amide groups on the basis of COSY and HMBC correlations.

Table 1. ^1H and ^{13}C NMR ($CDCl_3$, 500 and 125 MHz) assignments for PM050489 (1) and PM060184 (2)

	PM050489 (**1**)		PM060184 (**2**)	
position	δ_C, mult.	δ_H (m, J in Hz)	δ_C, mult.	δ_H (m, J in Hz)
1	161.6 C	–	161.6 C	–
2	145.2 C	–	145.2 C	–
3	108.2 CH	5.63 (dd, 6.5, 2.6)	108.2 CH	5.63 (dd, 6.6, 2.7)
4	26.1 CH_2	2.45 (ddd, 17.3, 11.5, 2.6) 2.37 (ddd, 17.3, 6.5, 4.1)	26.1 CH_2	2.44 (m) 2.39 (m)
5	81.9 CH	4.24 (ddd, 11.5, 7.1, 4.1)	81.9 CH	4.25 (ddd, 11.3, 7.0, 4.0)
6	37.1 CH	2.85 (ddq, 9.8, 7.1, 6.7)	37.1 CH	2.85 (ddq, 9.9, 7.0, 6.7)
7	134.1 CH	5.29 (d, 9.8)	134.1 CH	5.29 (d, 9.9)
8	133.7 C	–	133.8 C	–
9	140.2 CH	6.17 (d, 11.6)	140.2 CH	6.15 (d, 11.6)
10	124.6 CH	7.30 (dd, 11.6, 11.6)	124.4 CH	7.31 (dd, 11.6, 11.6)
11	137.6 CH	6.91 (dd, 11.6, 11.6)	137.5 CH	6.90 (dd, 11.6, 11.6)
12	120.7 CH	5.70 (d, 11.6)	120.8 CH	5.72 (br d, 11.6)
13	166.3 C	–	166.3 C	–
14	–	6.51 (d, 9.5)	–	6.53 (d, 9.6)
15	60.8 CH	4.41 (d, 9.5)	60.7 CH	4.44 (d, 9.6)
16	168.2 C	–	168.2 C	–
17	–	8.78 (d, 10.8)	–	8.69 (d, 10.4)
18	124.5 CH	6.84 (br dd, 10.8, 9.7)	124.2 CH	6.82 (ddd, 10.4, 9.1, 0.9)
19	105.0 CH	4.80 (m)	105.8 CH	4.82 (m)
20	30.7 CH_2	2.46 (m) 2.09 (ddd, 14.1, 8.4, 8.1)	30.9 CH_2	2.46 (m) 2.12 (ddd, 14.1, 8.0, 8.0)
21	74.9 CH	4.41 (m)	75.6 CH	4.45 (m)
22	33.0 CH_2	2.33 (m), 2H	31.4 CH_2	2.35 (m), 2H
23	122.4 CH	5.61 (br t, 6.8)	124.9 CH	5.40 (m)
24	132.0 C	–	127.1 CH	5.60 (m)
25	21.0 CH_3	2.06 (s), 3H	13.0 CH_3	1.63 (dd, 6.8, 1.0), 3H
26	55.4 CH_3	3.66 (s), 3H	55.4 CH_3	3.66 (s), 3H
27	16.3 CH_3	1.15 (d, 6.7), 3H	16.4 CH_3	1.15 (d, 6.7), 3H
28	17.1 CH_3	1.82 (s), 3H	17.1 CH_3	1.82 (s), 3H
29	34.7 C	–	34.8 C	–
30	26.7 CH_3	1.04 (s), 3H	26.7 CH_3	1.04 (s), 3H
31	26.7 CH_3	1.04 (s), 3H	26.7 CH_3	1.04 (s), 3H
32	26.7 CH_3	1.04 (s), 3H	26.7 CH_3	1.04 (s), 3H
33	157.2 C	–	157.6 C	–

Careful study of the COSY and HMBC spectra of **1** led to the identification of four discrete spin systems (A–D) represented in Figure 2b (H_3–H_{28}, H_9–H_{12}, NH_{14}–H_{15} and NH_{17}–H_{25}). Connectivity between these different systems was

dx.doi.org/10.1021/ja404578u | J. Am. Chem. Soc. 2013, 135, 10164–10171

established by $^{1}H-^{13}C$ long-range correlations obtained from HMBC experiments. Indeed, cross peaks between Me_{28} and C_7, C_8 and C_9, H_9 and C_7, and from H_7 and H_{10} to C_8 linked segments A and B. Likewise, correlations from H_{11}, H_{12}, NH_{14} and H_{15} to C_{13} coupled segment B to C through an amide carbonyl group (C_{13}). Finally, segments C and D were linked in a similar manner based on the observation of HMBC correlations from H_{15} and NH_{17} to C_{16}.

The presence of a *tert*-butyl group in the molecule was inferred from the presence of a single signal at δ_H 1.04 ppm in the ^{1}H NMR spectrum integrating for nine protons, which correlated in both the HSQC and HMBC spectra with an intense ^{13}C signal at δ_C 26.7 ppm accounting for three of the carbons in the molecule. Other HMBC correlations observed between the methyl groups Me_{30}, Me_{31}, and Me_{32} and carbons C_{15} and C_{29}, and from H_{15} to carbons C_{29} to C_{32} showed the presence of a *tert*-leucine (*tert*-butylglycine) in the molecule and confirmed the location of this amino acid residue in the structure of **1**. Additionally, the presence of a 6-substituted 3-methoxy-5,6-dihydro-2*H*-pyran-2-one in PM050489 (**1**) was shown by cross-peaks in the HMBC spectrum from H_3, both H_4 protons, and the oxygenated methyl group Me_{26} to C_2, and from H_3 and H_5 (weak) to the carbonyl group C_1.

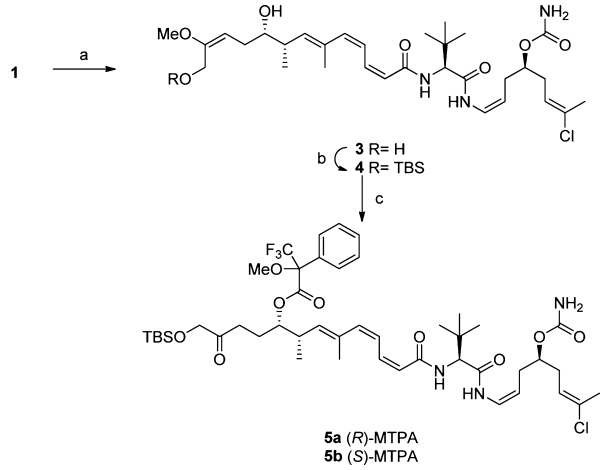
A signal in the ^{13}C NMR spectrum at δ_C 157.2 ppm accounted for the presence in the molecule of a carbamate group that was placed at C_{21} on the basis of a $^{1}H-^{13}C$ long-range correlation observed between H_{21} and C_{33} in the HMBC spectrum. The two NH_2 protons of this latter group appeared in the ^{1}H NMR spectrum as a broad signal that changed intensity and chemical shift, depending on the temperature and concentration of the sample. Finally, to complete the planar structure of **1**, the chlorine atom present in the molecule was placed at C_{24} in agreement with the substitution pattern observed for the $C_{23}-C_{24}$ double bond and the chemical shifts measured for these two olefinic carbons.[4]

The geometry of the double bonds in **1** was determined on the basis of NOESY correlations (Figure 2b) and $^{3}J_{H-H}$ coupling constant values. Values of $^{3}J_{H9-H10}$ = 11.6 Hz, $^{3}J_{H11-H12}$ = 11.6 Hz, and $^{3}J_{H18-H19}$ = 9.7 Hz, and NOESY correlations between the pairs H_9/H_{10}, H_{11}/H_{12}, and H_{18}/H_{19} gave a *Z* geometry for the double bonds at C_9-C_{10}, $C_{11}-C_{12}$, and $C_{18}-C_{19}$. On the other hand, the *E* geometry of the olefins at C_2-C_3, C_7-C_8, and $C_{23}-C_{24}$ was established through NOESY cross peaks observed between Me_{26}/H_3, H_6/Me_{28}, and H_{22}/Me_{25}, respectively. Additionally, the *E* geometry of the C_7-C_8 olefin was also supported by a NOESY correlation between H_7 and H_9.

To determine the absolute configuration at C_{15}, the Marfey's method for chiral amino acid analysis was used.[5] Compound **1** was dissolved in 6 N HCl and heated at 110 °C for 16 h in a sealed vial. After evaporation of the solvent under a N_2 stream and reaction of the residue with L-FDAA (*Nα*-(2,4-dinitro-5-fluorophenyl)-L-alanamide), the mixture was subjected to reversed-phase HPLC-MS analysis. Comparison of the retention time of the derivatized *tert*-leucine with that of the derivatized *R* and *S* standards of the amino acid established the *S* configuration for the chiral center at C_{15} in PM050489 (**1**).

The *S* absolute stereochemistry at C_5 was elucidated by Mosher analysis of the corresponding hydroxyl group,[6] obtained after reduction of the lactone and protection of the resulting primary hydroxyl group at C_1 as its TBS (*tert*-butyldimethylsilyl) ether (Scheme 1). Treatment of **1** with $NaBH_4$ (sodium borohydride) yielded 1,5-diol **3** that was

Scheme 1. Synthesis of the MTPA Esters at C_5 (5a and 5b)[a]



[a]Conditions: (a) $NaBH_4$, MeOH, THF, 23 °C, 3 h, 100%. (b) TBSCl, imidazole, CH_2Cl_2, 23 °C, 18 h, 27%. (c) MTPA-Cl, Et_3N, DMAP, CH_2Cl_2, 23 °C, 4 h, 26%.

regioselectively converted into its 1-TBS derivative **4** by reaction with TBSCl (*tert*-butyldimethylsilyl chloride). Esterification of this compound with *S*- and *R*-MTPA-Cl (*α*-methoxy-*α*-trifluoromethylphenylacetyl chloride) yielded compounds **5a** and **5b** in which elimination of the C_{26} methyl group and conversion of the resulting enol to the ketone had taken place in addition to the formation of the corresponding *R*- or *S*-MTPA ester at C_5. Analysis of the $\Delta\delta(R,S)$ values obtained for each derivative (Figure 2c) indicated the *S* absolute stereochemistry for the C_5 chiral center according to Riguera's predictive models for MTPA esters.[7]

In the case of PM060184 (**2**), the positive ion high-resolution (ESI) mass spectrum displayed a pseudomolecular ion at *m/z* 594.3152 $[M + Na]^+$ with an isotopic distribution consistent with the absence of chlorine atoms in the molecule. These MS data and the presence of 31 signals in its ^{13}C NMR spectrum (Table 1) gave a molecular formula of $C_{31}H_{45}N_3O_7$ for the compound (calcd for $C_{31}H_{45}N_3O_7Na$ 594.3150). The ^{1}H NMR spectra of **2** were identical to the corresponding spectra for **1** except for the presence of an extra olefinic proton (at 5.60 ppm), which was assigned to the C_{24} position by COSY and HMBC. The geometry of the $C_{23}-C_{24}$ olefin was assigned as *Z* by the ROESY cross-peak between H_{22}/Me_{25}. The configuration at C_5, C_6, C_{15} and C_{21}, and the geometry of the double bonds C_2-C_3, C_7-C_8, C_9-C_{10}, $C_{11}-C_{12}$ and $C_{18}-C_{19}$ was seen to be identical to those of PM050489 (**1**) by comparing the NMR data.

At this point, the small amounts of each molecule isolated from the natural source had served to show their promise not only as potent anticancer agents but also for initial structural elucidation as described above. Nevertheless, the absolute configuration of the stereocenters at C_6 and C_{21} remained unknown, and we turned our attention to the design of a synthetic process that not only would allow the configuration of the remaining two stereocenters to be determined but would also solve the supply problem and open the way to the possible future clinical development of these compounds. The supply issue is often one of the biggest barriers to the successful development of marine natural products.

Total Synthesis. PM050489 (**1**) and PM060184 (**2**) are formed by an *α,β*-unsaturated *δ*-lactone, a conjugated triene

dx.doi.org/10.1021/ja404578u | J. Am. Chem. Soc. 2013, 135, 10164−10171

system and a small natural amino acid (L-*tert*-leucine) that are linked via a (*Z*)-enamide to a diene system containing a carbamate subunit. Of the different possible synthetic approaches, we chose to adopt a convergent strategy, disconnecting the $C_{10}-C_{11}$ bond to give two subunits, fragments **A** and **B** (Figure 3). While fragment **A** is common

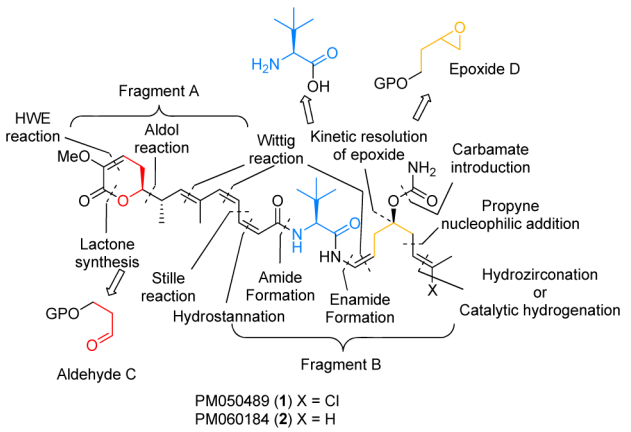


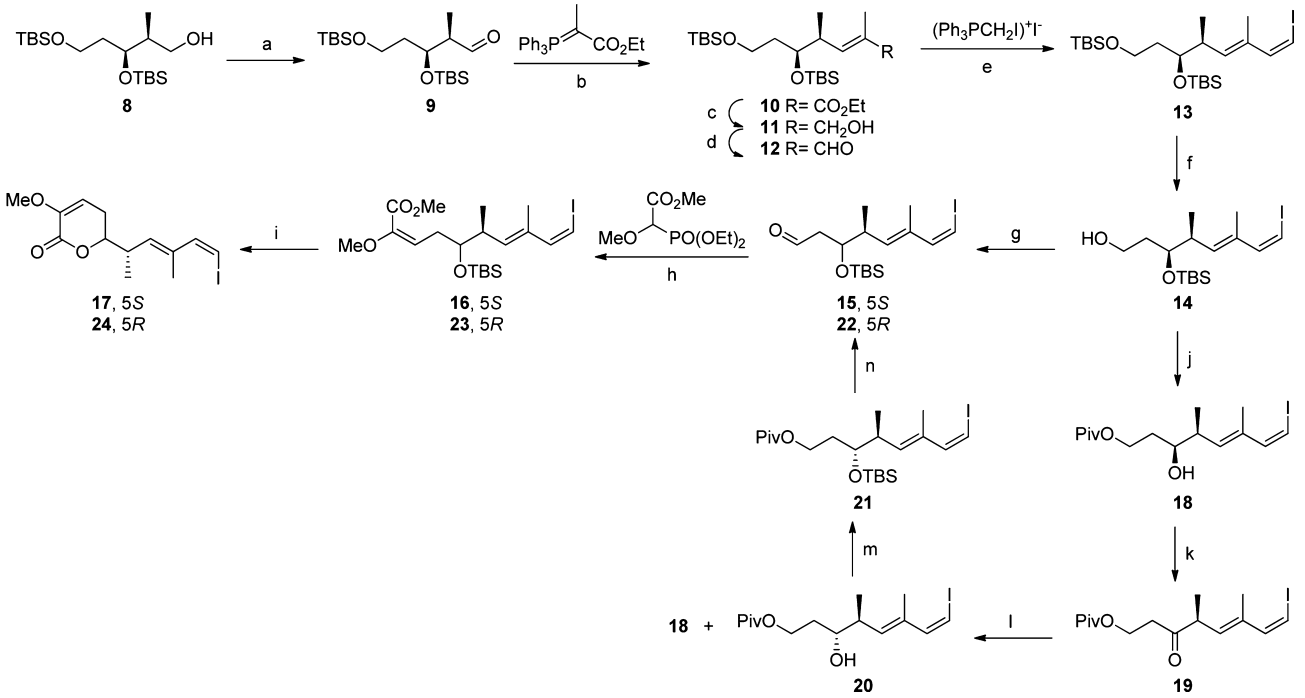
Figure 3. Retrosynthetic analysis of PM050489 (**1**) and PM060184 (**2**).

to both compounds and crucial for elucidating the absolute stereochemistry at C_6, fragment **B** needed to be prepared both with and without chlorine at C_{24} to obtain **1** and **2**, respectively. A metal-promoted coupling[8] between the vinylic fragment **A** and the organometallic fragment **B** was expected to afford the main skeleton of each molecule.

Many cross-coupling type reactions could be employed to form the $C_{10}-C_{11}$ bond, such as the Stille, Suzuki or Hiyama reactions, with the halide (or triflate) and the organometallic moiety being on either fragment **A** or **B**. The Sonogashira coupling reaction between a terminal alkyne and a vinyl halide could also be a good option. Retrosynthetic analysis of fragment **A** (Figure 3) includes double bond formation at C_2-C_3 through Horner–Wadsworth–Emmons olefination, double bond formation at both C_7-C_8 and C_9-C_{10} via Wittig olefination, and Evans oxazolidinone-mediated aldol condensation between aldehyde **C** and (*R*)-4-benzyl-3-propionyl-2-oxazolidinone to control the C_5 and C_6 carbon centers. For fragment **B**, the C_{21} stereocenter is introduced through resolution of racemic epoxide **D**, addition of propyne, and regioselective hydrozirconation followed by Cl substitution with *N*-chlorosuccinimide or by catalytic hydrogenation.

Synthesis of Fragment A. The starting point for the implementation of this strategy was the alcohol **8** (Scheme 2) obtained in six steps from 1,3-propanodiol and (*R*)-4-benzyl-3-propionyl-2-oxazolidinone.[9] Alcohol **8** was oxidized to aldehyde **9** in 90% yield using sulfur trioxide pyridine complex. Wittig reaction of aldehyde **9** with the stabilized ylide (1-ethoxycarbonylethylidene)triphenylphosphorane afforded stereoselectively (*E*:*Z* > 95:5) the corresponding ester **10**, which was reduced with DIBAL (diisobutylaluminum hydride) to allylic alcohol **11** in 77% yield. To introduce the diene moiety, alcohol **11** was oxidized with manganese(IV) oxide to aldehyde

Scheme 2. Synthesis of Fragment A[a]



[a]Conditions: (a) SO_3Py, Et_3N, CH_2Cl_2/DMSO (2.2:1), 0 °C, 2 h, 90%. (b) (1-ethoxycarbonylethylidene)triphenylphosphorane, toluene, 60 °C, 17 h, 96%. (c) DIBAL, THF, −78 °C, 4 h, 77%. (d) MnO_2, Et_2O, 23 °C, 2 h, 94%. (e) iodomethyl triphenylphosphonium iodide, NaHMDS, DMPU, THF, −78 °C, 2 h, 84%. (f) PPTS, EtOH, 23 °C, 25 h, 93%. (g) SO_3Py, Et_3N, CH_2Cl_2/DMSO (2.2:1), 0 °C, 2 h, 80%. (h) diethyl(methoxy(methoxycarbonyl)methyl)phosphonate, KHMDS, 18-crown-6, THF, −78 °C, 1.5 h, 59% (**16**) and 85% (diastereomer **23**). (i) HCl 37%, MeOH, 23 °C, 6 h, 94% (**17**) and 57% (diastereomer **24**). (j) (i) pivaloyl chloride, Et_3N, THF, 23 °C, 17 h; (ii) HCl 37%, MeOH, 23 °C, 3 h, 33%. (k) Dess–Martin periodinane, CH_2Cl_2, 23 °C, 45 min, 95%. (l) $NaBH_4$, MeOH, −78 °C, 40 min, 80%. (m) TBSOTf, 2,6-lutidine, CH_2Cl_2, 23 °C, 30 min, 95%. (n) (i) NaOH, MeOH, 40 °C, 4 h, 91%; (ii) Dess–Martin periodinane, CH_2Cl_2, 23 °C, 1 h, 51%.

dx.doi.org/10.1021/ja404578u | J. Am. Chem. Soc. 2013, 135, 10164–10171

12, which was then converted to vinyl iodide **13** by olefination with (iodomethyl)triphenylphosphonium iodide[10] in 84% yield with a 95:5 ratio of *Z*:*E* isomers.[11] Homologation of **13** began with regioselective deprotection of the primary TBS ether using PPTS (pyridinium *p*-toluenesulfonate) in EtOH to give alcohol **14** in excellent yield (93%) before oxidation to aldehyde **15** with sulfur trioxide pyridine complex in 80% yield. Horner–Wadsworth–Emmons olefination of aldehyde **15** with diethyl (methoxy(methoxycarbonyl)methyl)phosphonate[12] provided ester **16** as a 90:10 mixture of (*E*:*Z*) isomers. On treatment of the isomers with HCl in MeOH, only the major *E*-isomer was able to cyclize, giving the desired lactone **17** (5*S*,6*S*, relative *anti* configuration) in a yield of 94%. Lactone **17** serves as fragment **A** for the synthesis of both **1** and **2**.

In order to complete the full stereochemical elucidation of **1** and **2**, we also synthesized the (5*R*,6*S*)-lactone (relative *syn* configuration), from intermediate **14** following the strategy of oxidation/reduction of the alcohol at C_5. Orthogonal protection of alcohol **14** with pivaloyl chloride and removal of the TBS ether gave intermediate **18**. Oxidation of **18** with DMP (Dess–Martin periodinane)[13] afforded ketone **19** which was nonselectively reduced with $NaBH_4$ to obtain a mixture of diastereoisomers (*R*,*S*)-**20** and (*S*,*S*)-**18** which were separated by column chromatography. Protection of the secondary alcohol of **20** as a TBS ether with TBSOTf (*tert*-butyldimethylsilyl trifluoromethanesulfonate) afforded compound **21**. Finally, deprotection of the pivaloyl ether with NaOH and oxidation of the resulting primary alcohol with DMP yielded aldehyde **22** which was transformed into lactone **24** (5*R*,6*S*, relative *syn* configuration) via ester **23** as described previously. Instead of using *J*-based configurational[14] analysis to determine the configuration at C_6, we compared the NMR spectroscopic data of lactones **17** (5*S*,6*S*) and **24** (5*R*,6*S*) and observed multiplicity patterns for protons H_5 and H_6 of the *anti*-lactone **17** that were very similar to those of **1**, while great differences were observed in the case of the *syn*-lactone **24** (Figure 4). As such, the *syn* configurations (5*R*,6*S*) and (5*S*,6*R*)

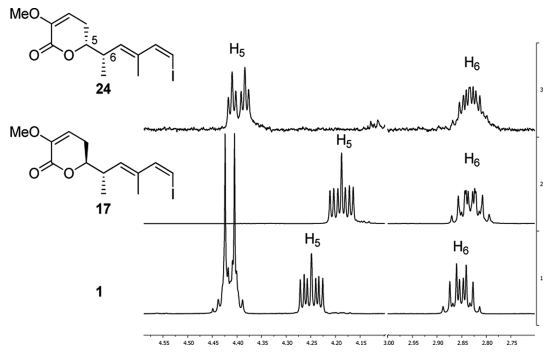


Figure 4. Partial ^{1}H NMR Spectra of Lactones **17** and **24**.

could be eliminated leaving only the two *anti* configurations, (5*S*,6*S*) and (5*R*,6*R*), as remaining possibilities. Then, since the absolute configuration at C_5 had already been unambiguously assigned as *S* (see above), the C_6 stereochemistry could also now be unambiguously assigned as *S*.

Synthesis of Fragment B. Having established efficient access to the common lactone-diene framework of **1** and **2**, the next challenge consisted in the synthesis of fragment **B**. Our starting material was but-3-en-1-yloxy-*tert*-butyl-dimethylsilane (Scheme 3). The required chiral center at C_{21} was obtained from racemic butene oxide (±)-**25** by way of Jacobsen's hydrolytic kinetic resolution.[15] Addition of a lithium anion derived from propyne to (*R*)-**26** in THF then yielded alcohol **27**, and subsequent conversion to the PMB (*p*-methoxybenzyl) ether provided **28**. Gratifyingly, when chiral alkyne **28** was reacted with 3 equiv of Schwartz's reagent (Cp_2Zr·HCl, bis(cyclopentadienyl)zirconium(IV) chloride hydride) in toluene at 50 °C, followed by addition of *N*-chlorosuccinimide, **29** was obtained in high yield and with excellent control of the olefin geometry (99% yield, 100% regioselectivity). PMB proved to be a key group in the hydrozirconation of alkyne **28**[16] with the TBS or TBDPS (*tert*-butyldiphenylsilyl) ether analogues of **28** being found to give low levels of regioselectivity with Schwartz's reagent and mixtures of the (*E*)-2-choroalkene and (*E*)-3-chloroalkene isomers. Cleavage of the TBS group of **29** with TBAF (tetra-*n*-butylammonium fluoride) solution in THF afforded alcohol **30** which was converted to the vinyl iodide **32** using methodology similar to that employed in fragment **A**. The most favorable oxidation process for alcohol **30** was Piancatelli conditions[17] employing BAIB ((diacetoxyiodo)benzene) and a catalytic amount of TEMPO (2,2,6,6-tetramethylpiperidinin-1-yl)oxyl.

After several attempts to use the PMB ether throughout the synthetic sequence, problems with the final deprotection of the PMB ether forced us to abandon this intermediate in favor of the TBS analogue **34**. The PMB protecting group of **32** was removed with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) in aqueous CH_2Cl_2[18] and purification of **33** simplified by treating the mixture with $NaBH_4$ to reduce the *p*-methoxybenzaldehyde byproduct to the alcohol. Protection of secondary alcohol **33** using TBSOTf and 2,6-lutidine was straightforward and gave the key vinyl iodide **34** required for the synthesis of **1**.

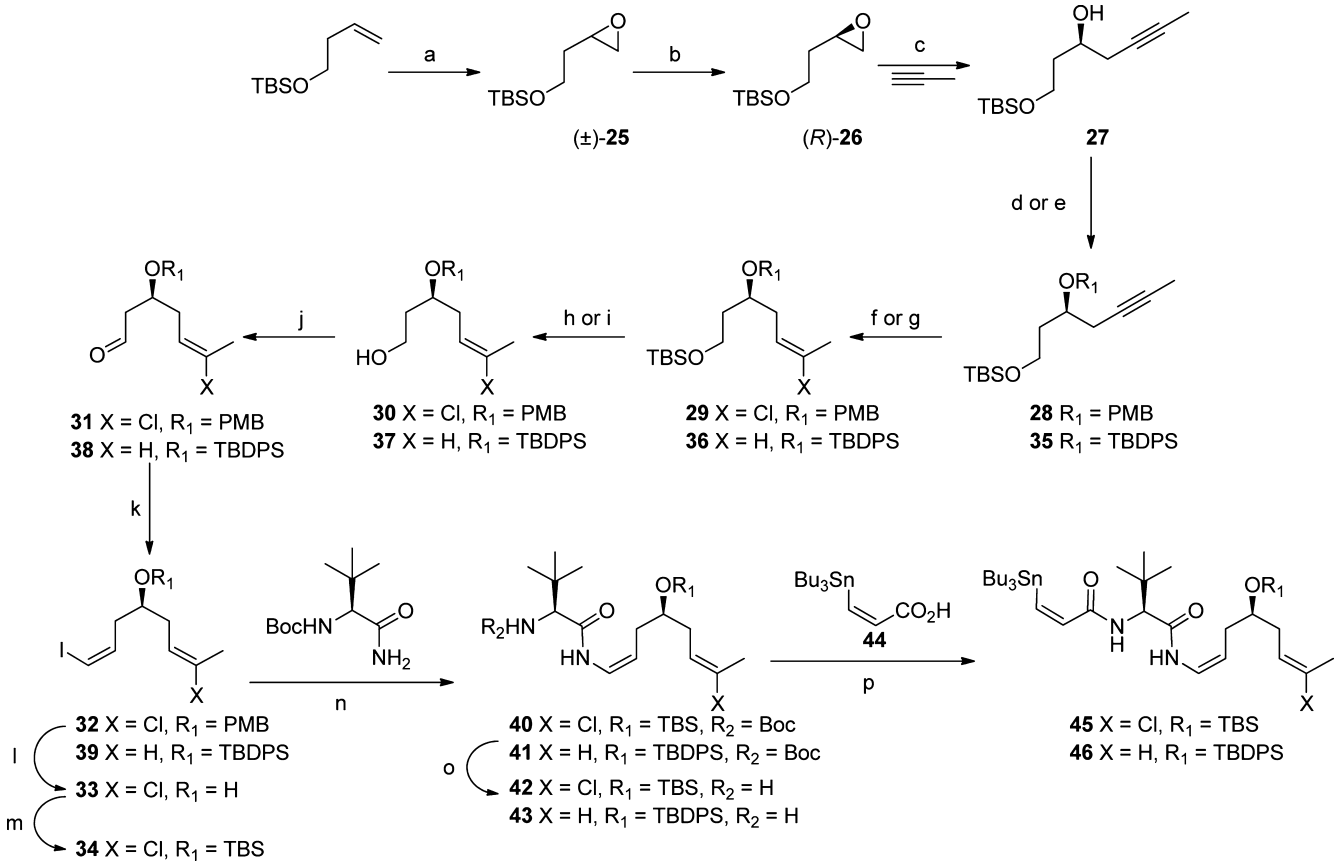
For the synthesis of **2**, we used alcohol **27** as starting material to prepare vinyl iodide **39** which is an analogue of **34** but without chlorine. In this case, we decided to use TBDPS as protecting group and reaction of **27** with TBDPSCl (*tert*-butyldiphenylsilyl chloride) in the presence of DMAP (4-dimethylaminopyridine) afforded intermediate **35** which was subjected to Lindlar catalytic hydrogenation[19] at atmospheric pressure to obtain stereoselectively *Z*-alkene **36**. Finally, vinyl iodide **39** was obtained via intermediates **37** and **38** using the same synthetic strategy as used for **32**.

With vinyl iodides **34** and **39** in hand, we now needed to introduce the final chiral part of fragment **B**. Boc-*t*Bu-Gly-NH_2 (*N*-(*tert*-butoxycarbonyl)-L-*tert*-leucine-amide), prepared following the Pozdnev procedure,[20] was reacted with **34** and **39** under Buchwald conditions,[21] affording enamides **40** and **41** in moderate yield (44–53%). The propensity of both the TBS and TBDPS protecting groups to cleavage under acidic conditions forced us to remove the Boc group by pyrolysis. Dissolution of **40** and **41** in ethyleneglycol and heating at 200 °C for not more than 20 min afforded amines **42** and **43** with longer reaction times giving degradation.

The reagents HOAt (1-hydroxy-7-azabenzotriazole) and HATU (*N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)-uronium hexafluorophosphate) are widely used in peptide synthesis to give rapid couplings with minimal loss of chiral integrity.[22] As such, these reagents were used to couple amines **42** and **43** to the carboxylic acid **44** affording stannanes **45** and **46** in 66–77% yield. Carboxylic acid **44** was prepared in two steps by hydrostannation of ethyl propiolate with tributyltin hydride followed by hydrolysis with aqueous LiOH in THF.

dx.doi.org/10.1021/ja404578u | J. Am. Chem. Soc. 2013, 135, 10164–10171

Scheme 3. Synthesis of Fragment B[a]

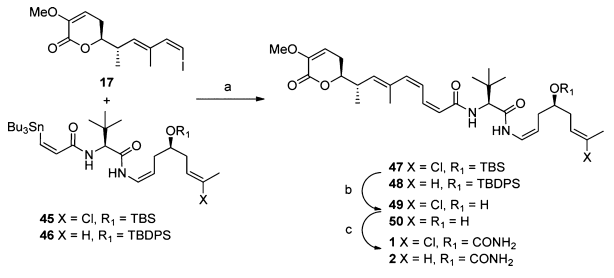


[a]Conditions: (a) *m*CPBA, CH_2Cl_2, 23 °C, 18 h, 82%. (b) (*R*,*R*)-(−)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II), AcOH, H_2O, THF, 23 °C, 18 h, 46%. (c) propyne, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 1 h, 100%. (d) *p*-methoxybenzyltrichloracetimidate, $Sc(OTf)_3$, CH_2Cl_2, 23 °C, 2 h, 55%. (e) TBDPSCl, DMAP, DMF, 23 °C, 18 h, 93%. (f) (i) Cp_2ZrHCl, toluene, 50 °C, 2.5 h; (ii) NCS, 23 °C, 30 min, 99%. (g) H_2, quinoline, Lindlar catalyst, EtOAc, 23 °C, 2 h, 90%. (h) TBAF, THF, 23 °C, 2 h, 73%. (i) PPTS, EtOH, 23 °C, 7 h, 69%. (j) BAIB, TEMPO, CH_2Cl_2, 23 °C, 20 h, 70% (**31**) and 79% (**38**). (k) iodomethyltriphenylphosphonium iodide, NaHMDS, THF, −78 °C, 2 h, 62% (**32**) and 89% (**39**). (l) DDQ, CH_2Cl_2, H_2O, 23 °C, 1.5 h, 80%. (m) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 2 h, 98%. (n) Boc-*t*Bu-Gly-NH_2, CuI, K_2CO_3, *N*,*N*′-dimethylethylenediamine, DMF, 90 °C, 18 h, 53% (**40**) and 44% (**41**). (o) ethyleneglycol, 200 °C, 15 min, 95% (**42**) and 62% (**43**). (p) (*Z*)-3-tributylstannylpropenoic acid **44**, DIPEA, HOAt, HATU, CH_2Cl_2, DMF, 23 °C, 2 h, 66% (**45**) and 77% (**46**).

Final Steps. At this point, the fragment **A** (lactone **17**) and fragment **B** (vinyl stannnes **45** and **46**) intermediates required to make **1** and **2** were now all available. For the final coupling, use of CuTc (copper(I) thiophene-2-carboxylate) in NMP (1-methyl-2-pyrrolidinone) under Liebeskind conditions[23] provided trienes **47** and **48** in 66% yield (Scheme 4). Cleavage of the TBS or TBDPS groups with TBAF in THF generated compounds **49** and **50** in adequate yields. Introduction of the C_{21} carbamate moiety was then achieved by reaction of **49** and **50** with trichloroacetyl isocyanate, and subsequent treatment with neutral alumina finally yielded the desired compounds **1** and **2** in 81−96% yield.[24] All the spectral data (^1H and ^{13}C NMR, optical rotation, IR, etc.), HPLC retention times and biological activities of the synthetic samples exactly matched those of the isolated natural products.

Following completion of the total synthesis of **1** as described above, the *R* absolute configuration could finally be assigned to C_{21}. This was achieved by using racemic epoxide (±)-**25** to prepare intermediate **34** as a mixture of epimers and subsequent downstream conversion to obtain **49** as a mixture of the two alcohol epimers at C_{21} with (5*S*,6*S*,15*S*,21*R*) and (5*S*,6*S*,15*S*,21*S*) absolute configurations. The two epimers were separated by HPLC, and the stereochemistry of each was assigned by preparing the Mosher derivatives,[6] before the introduction in each derivative the carbamate moiety. The NMR spectra of the natural product **1** clearly matched those of the **21**-*R* epimer, whereas major differences were observed for the ^1H and ^{13}C resonances in the region around the C_{21} chiral center in the **21**-*S* stereoisomer thereby confirming the *R*

Scheme 4. Final Steps of the Synthesis of PM050489 (1) and PM060184 (2)[a]



[a]Conditions: (a) copper(I) thiophene-2-carboxylate, NMP, 0 °C, 45 min, 66% (**47**) and 66% (**48**). (b) TBAF, THF, 23 °C, 18 h, 76% (**49**) and 80% (**50**). (c) (i) trichloroacetyl isocyanate, CH_2Cl_2, 0 °C, 30 min; (ii) Al_2O_3, 0 °C, 30 min, 81% (**1**) and 96% (**2**).

dx.doi.org/10.1021/ja404578u | J. Am. Chem. Soc. 2013, 135, 10164−10171

configuration for C_{21} in **1**. In the case of **2**, the lack of chlorine at C_{22} means that, although there is no change in the spatial disposition of the different groups around C_{21}, the descriptor of the C_{21} configuration is now *S* and that of the double link C_{23}–C_{24} is now *E* (not *Z* as in **1**) due to the different assignment of priorities according to the CIP (Cahn–Ingold–Prelog) priority rules.

In summary, the first total synthesis of PM050489 (**1**) and PM060184 (**2**) was achieved in a total of 35 and 33 steps, respectively. Eighteen steps from 1,3-propanediol is the longest linear sequence required to synthesize these marine natural products. The highly convergent synthesis we report herein has been industrialized and has also been used for the synthesis of new analogues.

Biological Evaluation. Two bioassays were conducted in parallel during fractionation of the sponge extracts: (i) cell-killing ability, evaluated against a panel of three human tumor cell lines, including colon (HT-29), lung (A-549), and breast (MDA-MB-231), and (ii) antimitotic activity, measured using a specific microplate immunoassay (ELISA). The *in vitro* antiproliferative activity was assessed using the colorimetric SRB (sulforhodamine B) method, performed as described previously.[25] The GI_{50} (nM) values (concentration that causes 50% growth inhibition) obtained for PM050489 (**1**) and PM060184 (**2**) in this assay were 0.46 and 0.42 (HT-29), 0.38 and 0.59 (A-549), and 0.45 and 0.61 (MDA-MB-231), respectively. No selectivity was observed between cell lines. The antimitotic activity was assessed in a modified cell-based immunoassay using the specific mitotic marker MPM-2, an epitope found in a set of phosphoproteins that are specifically phosphorylated during mitosis.[26] PM050489 (**1**) displayed an IC_{50} (compound concentration that produces 50% of mitotic arrest in the cell population) of 26.4 nM (0.016 μg/mL) when tested under these conditions.

CONCLUSION

We have achieved the first isolation, structural elucidation and total synthesis of PM050489 (**1**) and PM060184 (**2**), the first members of an unprecedented new class of polyketides isolated from extracts of the Madagascan sponge *Lithoplocamia lithistoides*, with subnanomolar *in vitro* activity in human tumor cell lines, potent antimitotic properties and a distinct inhibition mechanism on microtubules.

The development of an elegant and efficient total synthesis at multigram scale has provided a solution to the supply problem, and together with pharmaceutical development work, mechanism of action and preclinical studies have allowed us to initiate the clinical development of PM060184 (**2**) as a promising drug for cancer treatment.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, spectral and other characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

ccuevas@pharmamar.com

Present Addresses

[§]Fundación MEDINA, 18016 - Granada, Spain.
[⊥]Instituto de Química Médica, CSIC, 28006 - Madrid, Spain.

Notes

The authors declare the following competing financial interest(s): We are employees and shareholders of PharmaMar.

ACKNOWLEDGMENTS

We thank Dr. L. F. García-Fernández and Dr. A. Losada for the cytotoxicity and antimitotic assays, Dr. J. L. Carballo for the taxonomic identification of the sponge, and Dr. S. González for recording the NMR spectra. We thank Profs. Federico Gago, Claudio Palomo and Antonio M. Echavarren for constructive and helpful comments during preparation of the manuscript.

REFERENCES

(1) (a) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912–4915. (b) Gunasekera, S. P.; Paul, G. K.; Longley, R. E.; Isbrucker, R. A.; Pomponi, S. A. *J. Nat. Prod.* **2002**, *65*, 1643–1648.

(2) Kim, D. S.; Dong, C. G.; Kim, J. T.; Guo, H.; Huang, J.; Tiseni, P. S.; Kishi, Y. *J. Am. Chem. Soc.* **2009**, *131*, 15636–15641.

(3) Pera, B.; Barasoain, I.; Canales, A.; Matesanz, R.; Rodríguez-Salarichs, J.; García-Fernández, L. F.; Moneo, V.; Jiménez-Barbero, J.; Galmarini, C. M.; Cuevas, C.; Diaz, J. F.; Andreu, J. M. New interfacial microtubule inhibitors of marine origin with potent antitumor activity and a distinct mechanism. *ACS Chem. Biol.* Submitted for publication, **2013**.

(4) Pretsch, E. P.; Seibl, J.; Clerc, T.; Simon, W. In *Tables of Spectral Data for Structure Determination of Organic Compounds*, 2nd ed.; Springer-Verlag: New York, 1989; pp C90.

(5) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.

(6) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

(7) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.

(8) Marsden, J. A.; Haley, M. M. Cross-Coupling Reactions to sp Carbon Atoms. In *Metal-Catalyzed Cross-Coupling Reactions*, 2nd ed.; de Meijere, A., Diederich, F., Eds.; Wiley-VCH Verlag GmbH: Weinheim, Germany, 2008; pp 317–394.

(9) Phukan, P.; Sasmal, S.; Maier, M. E. *Eur. J. Org. Chem.* **2003**, 1733–1740.

(10) Seyferth, S.; Heeren, J. K.; Singh, G.; Grim, S. O.; Hughes, W. B. *J. Organomet. Chem.* **1966**, *5*, 267–274.

(11) Store, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 2173–2174.

(12) Grell, W.; Machleidt, H. *Justus Liebigs Ann. Chem.* **1966**, *699*, 53–67.

(13) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.

(14) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.

(15) (a) Tokunaga, M.; Larrow, J. F.; Kakuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936–938. (b) Schaus, S. E.; Brandes, B. D.; Larrow, J. F.; Tokunaga, M.; Hansen, K. B.; Gould, A. E.; Furrow, M. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.

(16) (a) Labinger, J. A. Hydrozirconation of C=C and C≡C and hydrometallation by other metals. In *Comprehensive Organic Synthesis*; Trost, B. M., I. Fleming, I., Eds.; Pergamon Press: Oxford, 1991; Vol. 8, pp 667–702. (b) Schwartz, J.; Labinger, J. A. *Angew. Chem., Int. Ed.* **1976**, *15*, 333–340. (c) Wipf, P.; Jahn, H. *Tetrahedron* **1996**, *52*, 12853–12910. (d) Panek, J. S.; Hu, T. J. *Org. Chem.* **1997**, *62*, 4912–4913.

(17) De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. *J. Org. Chem.* **1997**, *62*, 6974–6977.

(18) Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885–888.

(19) Lindlar, H. *Helv. Chim. Acta* **1952**, *35*, 446–450.

(20) Pozdnev, V. F. *Tetrahedron Lett.* **1995**, *36*, 7115–7118.

(21) Klapars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421–7428.

(22) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557–6602.

(23) Allred, G. D.; Liebeskind, L. S. *J. Am. Chem. Soc.* **1996**, *118*, 2748–2749.

(24) Kočovský, P. *Tetrahedron Lett.* **1986**, *27*, 5521−5524.
(25) Skehan, P.; Storeng, P. R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1980**, *82*, 1107−1112.
(26) (a) Davis, F. M.; Tsao, T. Y.; Fowler, S. K.; Rao, P. N. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2926−2930. (b) Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X.; Xu, L.; Bigg, C. M.; Kelly, M. T.; Andersen, R. J. *Cancer Res.* **2000**, *60*, 5052−5058.