Organic LETTERS

Letter

pubs.acs.org/OrgLett

New Duclauxamide from *Penicillium manginii* YIM PH30375 and Structure Revision of the Duclauxin Family

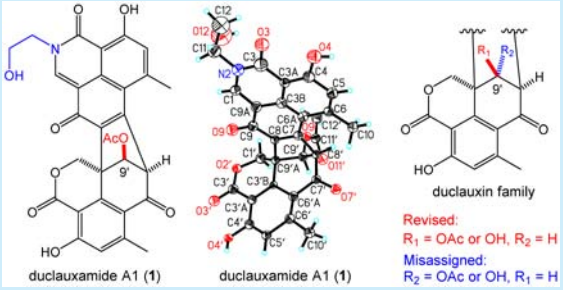
Pei Cao,[†] Jing Yang,[†] Cui-Ping Miao,[‡] Yijun Yan,[†] Ya-Tuan Ma,[†] Xiao-Nian Li,[†] Li-Xing Zhao,*[,‡] and Sheng-Xiong Huang*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[‡]Key Laboratory for Microbial Resources of the Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, China

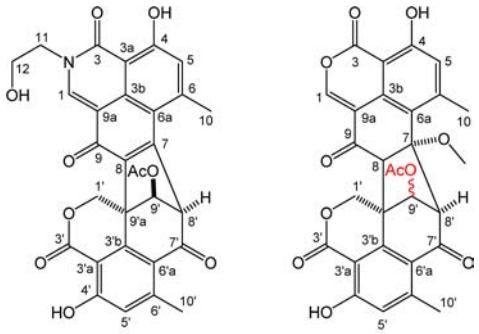
Supporting Information

ABSTRACT: Duclauxamide A1 (**1**), a new polyketide-derived heptacyclic oligophenalenone dimer with a *N*-2-hydroxyethyl moiety, was isolated from *Penicillium manginii* YIM PH30375. Spectroscopic analysis, X-ray single crystal diffraction, and ^{13}C NMR DFT calculations confirmed that compound **1** and other duclauxin analogues possess the unified *S* configuration at C-9′, which corrects a long-standing misrepresentation of duclauxins as C-9′*R* epimers. A plausible biosynthetic pathway for duclauxins is proposed on the basis of previous acetate labeling results for duclauxin and sclerodin.



It is estimated that there are more than 23 000 microbial natural products known, of which around 45% are from fungi, thus warranting them as among the most prolific producers of bioactive natural products.[1] Endophytic fungi colonize living tissues of plants without showing negative effects, and commonly held conception that they produce natural products which endow a selective advantage to their host, typically by inhibiting the growth of other organisms or by making the host unattractive to predators.[2] Many plants have an extensive ethnobotanical history of use by indigenous people, and endophytes appear to be behind traditional Chinese medicines (TCMs) for which there exists a tremendous cultural history in China.[3] The Chinese are by far the largest users of TCMs with over 5000 plants and plant products in their pharmacopeia. However, little is known about the endophytes behind TCMs. This deficiency, combined with the strong track record of TCM use in China, suggests that endophytes, particularly those associated with TCM plants, are likely to yield new natural products.[4]

The stem of *Panax notoginseng* (Burk.) F.H. Chen is mainly cultivated in the Southwest China, especially the Wenshan region of Yunnan province, and is ordinarily referred to as "*Sanqi*". This herb and its major constituents have been clinically shown to possess hypolipidemic, hepatoprotective, renoprotective, and stasis resolving activities, and have traditionally been used as tonics and hemostatic agents for the treatment of cardiovascular disease, pain, and internal bleeding.[5] Herein, we describe the isolation and structural elucidation of the new naturally occurring compound duclauxamide A1 (**1**) from *Penicillium manginii* YIM PH30375, an endophytic fungus living inside the elder root of *P. notoginseng*. In addition, we present a structural revision of the duclauxin family based upon biogenesis considerations and ^{13}C NMR calculations. Finally, a biosynthetic pathway for generating the duclauxin family scaffold is proposed on the basis of previous acetate labeling results for duclauxin (**2**) and sclerodin.[6]



Duclauxamide A1 (**1**) was isolated as pale yellow needles (from acetone and pyridine) with the molecular formula $C_{30}H_{23}NO_{10}$ (20 double bond equivalents) as determined from HR-ESI-MS ($[M + Na]^+$ at m/z 580.1259). The IR spectrum indicated the presence of hydroxyl (3425 cm^{-1}) and carbonyl (1753 and 1668 cm^{-1}) groups. In the ^1H NMR spectrum, two downfield singlets at δ_H 12.88 (4-OH) and 12.24 (4′-OH) were assigned to two chelated phenolic OH groups that were

Received: January 9, 2015
Published: February 19, 2015

DOI: 10.1021/acs.orglett.5b00081
Org. Lett. 2015, 17, 1146−1149

hydrogen bonded to the carbonyl oxygens of their nearby aldehyde or ketone groups. Two aromatic protons were shown at δ_H 6.84 (br.s, H-5′) and 6.82 (br.s, H-5). A broad singlet at δ_H 5.82 (H-9′) showing HMBC correlation with the carbonyl carbon at δ_C 170.7 suggested a proton attached to an oxygenated carbon bearing an acetoxyl group. Its neighboring proton was found at δ_H 5.05 (br.s, 1H, H-8′) by the correlation in a COSY experiment. The appearance of two doublet protons as one AB quartet (at δ_H 5.06, 2H, J = 12.4 Hz, H-1′) suggested the presence of one $-CH_2O-$ group. The connection of $HO-CH_2-CH_2-N-$ was built from the COSY correlations for signals at δ_H 4.26 (m, 2H, H-11) and 3.87 (br.s, 2H, H-12). Two aromatic methyl groups were seen as singlets at δ_H 2.99 (H-10) and 2.58 (H-10′). The presence of one OAc group was indicated by the appearance of a signal at δ_H 2.08 (3H, s). The ^{13}C NMR and DEPT data indicated the presence of three primary, five secondary, three tertiary, and 19 quaternary carbons. The signal of one quaternary carbon was observed at δ_C 49.0 ppm and the others appeared in the range above 100 ppm. The appearance of many quaternary (aromatic) carbons indicated a condensed polyaromatic system. The comparison of NMR spectra of compound **1** with previously isolated duclauxin (**2**) and bacillisporins[7] revealed that the displacement of the O atom with a N-containing chain occurred without the modification of their original carbon skeleton (Figure 1). The

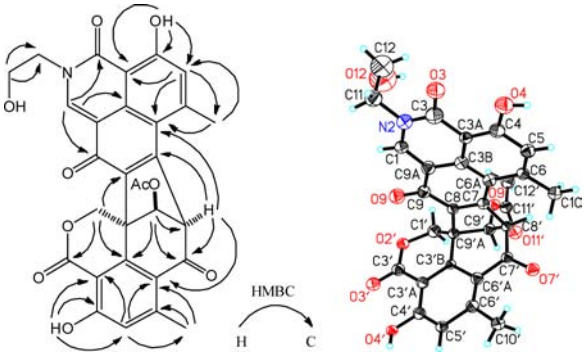


Figure 1. Key HMBC correlations and X-ray structure of **1**.

complete interpretation of the NMR spectroscopic data of **1** (Table 1) was rationalized as a result of detailed DEPT, COSY, HSQC, and HMBC experiments. The HMBC technique confirmed the planimetric map of **1** from the main correlations as shown in Figure 1. Consequently, the structure of **1** was determined to be a new duclauxamide.

Upon examination of the NMR data for **1**, we noticed abnormal coupling constants in the H-8′/H-9′ system. Both the pseudorotating model and Chem3D energy-minimized structures for the five-membered ring of **1**[8] revealed an approximately 30° dihedral angle for H-8′/H-9′ with a preferred *R* configuration for C-9′, indicating that one medium $^3J_{H,H}$ value should be observed (Figure 2A). In contrast, a simulation with the C-9′*S* configuration generated a dihedral angle of ∼90° for H-8′/H-9′, which should produce the smaller $^3J_{H,H}$ value that was consistent with the broad singlet peaks (H-8′ and H-9′) we observed experimentally (Figure 2B). Thus, the relative configuration of C-8′/C-9′ is opposite to the previously proposed C-9′*R* stereochemistry.[9] Surprisingly, in the pertinent references, br.s peaks of H-8′ were presented for all the structurally related duclauxin analogues.[7] Because duclauxin (**2**) could convert to semisynthetic duclauxamide or

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Data of 1 (δ in ppm, J in Hz, Recorded in Acetone-d_6)

no.	δ_H	δ_C	no.	δ_H	δ_C
1	8.58 (br.s, 1H)	145.5	**1′**	5.06 (q, 2H, 12.4)	69.3
3		166.0	**3′**		168.7
3a		108.9	**3′a**		105.2
3b		135.9	**3′b**		147.5
4		165.1	**4′**		164.9
5	6.82 (br.s, 1H)	118.3	**5′**	6.84 (br.s, 1H)	120.9
6		149.8	**6′**		154.0
6a		115.0	**6′a**		117.9
7		152.1	**7′**		191.0
8		140.6	**8′**	5.05 (br.s, 1H)	64.2
9		177.8	**9′**	5.82 (br.s, 1H)	85.3
9a		114.2	**9′a**		49.0
10	2.99 (s, 3H)	25.0	**10′**	2.58 (s, 3H)	23.7
11	4.26 (m, 2H)	53.3	OAc		170.7
12	3.87 (br.s, 2H)	59.6	OAc	2.08 (s, 3H)	20.8
4-OH	12.88 (s, 1H)		4′-OH	12.24 (s, 1H)	

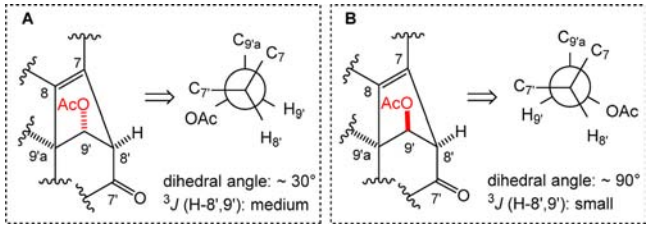


Figure 2. Determination of relative stereochemistry of C-9′ in **1**. (A) The conformer with C-9′*R* configuration; (B) the conformer with C-9′*S* configuration.

its *N*-alkylated derivatives in mild conditions,[7b] compound **1** and duclauxins presumably share the similar biosynthetic machinery and should have the same configuration at C-9′. Our analysis has therefore implicated a possible error in previous absolute configuration assignment at C-9′ for all duclauxin analogues.

Although three X-ray structures including heavy atom incorporated monobromoduclauxin[9a,b] and duclauxin[9c] have been reported without a CIF file, all structures were depicted in the C-9′*R* configuration. Moreover, this stereochemistry was either cited directly in notable biological studies[10] or used to deduce its derivatives; even recently, solely through direct comparison of NMR spectroscopic data.[11] We therefore consider a structural revision necessary due to inconsistencies in these reports. We then obtained eutectics of **1** with pyridine for X-ray analysis after various trials. The structure and the relative configuration of the bis-oligophenalenone system was unequivocally corroborated using Cu K-alfa single crystal diffraction with much improved precision (Figure 1). The refined Hooft parameter value 0.28(16) for 9861 Bijvoet pairs[12] with a probability of 1.000 and the previous X-ray results of monobromoduclauxin[9a,b] demonstrated that duclauxamide A1 (**1**) presumably has the C-9′*S* configuration (Supporting Information). Since it is not biosynthetically logical that compound **1** belongs to a different stereochemical series than the other members of the family, we may tentatively conclude that the other duclauxin analogues possess a common C-9′*S* absolute configuration as depicted in Figure S1 (Supporting Information).

DOI: 10.1021/acs.orglett.5b00081
Org. Lett. 2015, 17, 1146−1149

Increasing computational precision makes NMR attractive for determination of relative and absolute configurations of organic compounds.[13] To complement the above findings we utilized ab initio density functional theory (DFT) GIAO calculations of NMR chemical shifts to predict a likely correct structure for duclauxin (**2**). First, we demonstrated the reliability of the measurement for a wide variety of NMR calculations by choosing the definitively characterized compound **1** as a verified model. ^{13}C NMR data showed better agreement with the theoretical values for the C-9′*S* epimer than those for the C-9′*R* epimer of **1** (Figure 3A). Therefore, the

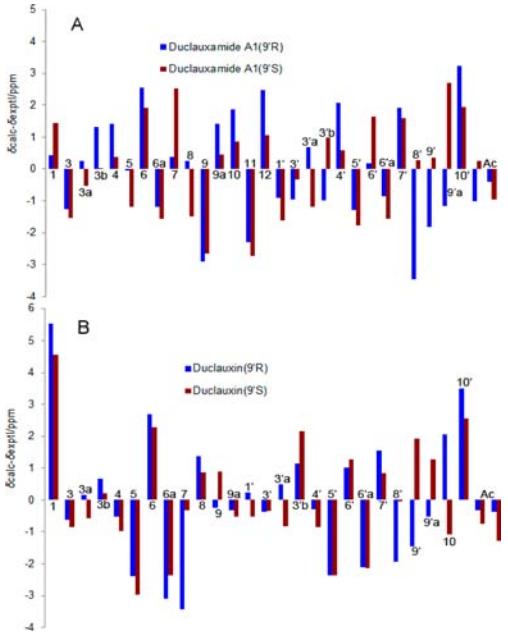


Figure 3. Differences between experimental NMR chemical shifts of studied compounds and theoretical NMR chemical shifts for the C-9′*R* epimer (blue bar) and C-9′*S* epimer (purple bar). $\Delta\delta = \delta_{calcd} - \delta_{exptl}$. (A) duclauxamide A1 [$|\Delta\delta|_{max}$ = 3.45^R, 2.73^S; $|\Delta\delta|_{mean}$ = 1.368^R, 1.268^S] (B) duclauxin [$|\Delta\delta|_{max}$ = 5.52^R, 4.55^S; $|\Delta\delta|_{mean}$ = 1.457^R, 1.342^S] (for calculation data see Supporting Information).

same quantum mechanical model together with the density functional principles was applied to **2** and its corresponding C-9′ epimer, respectively. The experimental ^{13}C NMR spectra certified a good match with those calculated for the C-9′*S* absolute configuration (Figure 3B). From both cases mimicked, the maximum absolute deviation and the mean average error are smaller for C-9′*S* epimer. In contrast to other quaternary carbons in the proximity surrounding C-9′, the chemical shift at C-8′ is exquisitely sensitive to the shielding effect caused by the adjacent C-9′ substituent in both **1** and **2** (Figure 3). The corresponding absolute deviations of C-8′ are only 0.27 ppm for duclauxamide A1(9′*S*) and 0.04 ppm for duclauxin (9′*S*), while the deviations reach to 3.45 ppm for duclauxamide A1(9′*R*) and 1.93 ppm for duclauxin (9′*R*). In summary, the stereochemical revision of C-9′ of **2** was further corroborated by computational methods.

Duclauxin is a heptacyclic oligophenalenone dimer consisting of an isocoumarin and a dihydroisocoumarin unit. These two tricyclic moieties are joined by a cyclopentane ring to form a unique hinge- or castanets-like structure. There are about 11 reported natural analogues mainly produced by several *Penicillium* species (*P. duclauxi*,[7a,b] *P. stipitatum*,[7c] and *P. herquei*[7d]). It has also been isolated from *Talaromyces bacillisporus* along with bacillisporins A−E,[7e,f] of which bacillisporin A was highly active against MCF-7 and NCl-H460.[7f] Duclauxin is effective against numerous tumor cell lines becuase it prevents ATP synthesis by inhibiting mitochondrial respiration and therefore exhibits potential as an anticancer drug lead.[7d,10b] Until now, very few examples were reported for discovering such structurally unique dimers.[7] To the best of our knowledge, no successful total synthesis has been achieved to construct these delicate architectures; furthermore, the pioneering biogenesis insights were only preliminary.[6a] We were therefore motivated to identify a probable biosynthetic pathway to inform further studies.

Previous feeding experiments demonstrated that duclauxin (**2**) might originate from a heptaketide, which cyclizes to a tricyclic aromatic ring system.[6a] However, the exact origin of the uniquely labeled tricyclic skeleton of duclauxins is not apparent. A hint for our attempts to solve the puzzle comes from the natural product sclerodin,[6b] whose carbon skeleton maps onto one-half of duclauxin. Since the labeling pattern of sclerodin was the same as that of **2**,[6a,b] we may reasonably deduce a similar biosynthetic logic for the monomeric partner of duclauxin. From the phenalenone (i), a contracted ring C is formed by oxidative loss of one carbon from the triketone (ii), the precursor requisite for dione (iii) and naphthalic anhydride (iv), which are obtained in turn from the decarboxylation and regioselective oxygen insertion induced by air[6b] or enzyme. Finally, we propose the selective reduction of cyclic anhydride to generate the intermediate lactone (v). The origin of the individual carbons in this lactone is consistent with the previously reported labeling results[6a] (Figure 4).

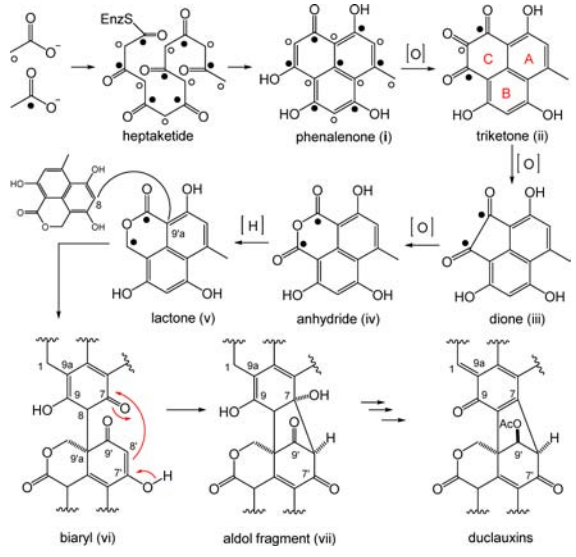


Figure 4. Plausible biosynthetic route for duclauxins.

After disconnection of the juncture sites around the middle cyclopentane ring, the duclauxins were hypothesized to arise from the dimerization of two lactone building blocks, perhaps through oxidative radical coupling between C-8 and C-9′a catalyzed by oxidative enzymes.[14] The biaryl (vi) then undergoes an intramolecular aldol condensation between C-8′ and C-7 ketone group to furnish the characteristic architecture. The aldol fragment (vii) could then experience a set of successive tailoring (methylation, reduction, dehydration, and

DOI: 10.1021/acs.orglett.5b00081
Org. Lett. 2015, 17, 1146−1149

acetylation) modifications. For the biogenetic route to the title compound **1**, we assume that one serine[15] as nitrogen donor participated in ammonolysis, and further decarboxylation of the serine moiety provided **1**. The intrinsic correlations and conversion among duclauxins and bacillosporins were also inferred (Figure S2, Supporting Information).

Duclauxamide A1 (**1**) showed moderate cytotoxicity against HL-60, SMML-7721, A-549, MCF-7, and SW480 cancer cell lines with IC_{50} values in the range of 11−32 μM.

From the detailed structure elucidation for one new amide derivative (**1**) of the duclauxin family, we established that the configuration of duclauxin had not been correctly assigned at one site, and we further verified this via X-ray crystallography of **1**, biosynthetic considerations, and computational methods. Because all the X-ray fractional atomic coordinates and equivalent isotropic displacement parameters of duclauxin (**2**) were provided in one case,[9c] we carefully reprocessed that data using GaussView5.0 software to afford the three-dimensional crystal structure of **2**, which showed C-9′*S* stereochemistry unequivocally. We therefore assumed that the same configurational misassignment was also present in all other members of the series. Taking this as the starting point, we put forward plausible biosynthetic pathways for the duclauxin family to establish a foundation for future synthetic and biosynthetic studies. In particular, we took advantage of previously published carbon labeling results for **2** and structurally related natural products to propose the formation of the unique tricyclic scaffold from the commonly found phenalenone skeleton. To the best of our knowledge, the oxidative reactions from phenalenone (i) to lactone (v) and the two carbon−carbon bond formation reactions during the dimerization have not been reported. Therefore, all the enzymes involved in these two aspects are interesting targets to be investigated and will be reported in due course by our group.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and the copies of original 1D and 2D NMR spectra, X-ray crystallographic data of **1**, CIF file, and the related computational NMR chemical shifts. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: zlx70@163.com.
*E-mail: sxhuang@mail.kib.ac.cn.

Author Contributions

P.C. and J.Y. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Dr. Geoff Horsman, assistant professor in Department of Chemistry, Wilfrid Laurier University, Canada, for his generous support and helping us to edit the manuscript. This work was financially supported by High-End Science and Technology Talents Program of Yunnan Province to S.-X.H. (No. 2013HA022) and Kunming Institute of Botany startup foundation and Chinese Academy of Sciences.

■ REFERENCES

(1) Bérdy, J. *J. Antibiot.* **2005**, *58*, 1−26.
(2) (a) Tan, R. X.; Zou, W. X. *Nat. Prod. Rep.* **2001**, *18*, 448−459. (b) Strobel, G.; Daisy, B. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 491−502. (c) Zhang, H. W.; Song, Y. C.; Tan, R. X. *Nat. Prod. Rep.* **2006**, *23*, 753−771.
(3) (a) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J. Nat. Prod.* **2004**, *67*, 257−268. (b) Gunatilaka, A. A. L. *J. Nat. Prod.* **2006**, *69*, 509−526.
(4) Zhang, D.; Ge, H.; Zou, J.-H.; Tao, X.; Chen, R.; Dai, J. *Org. Lett.* **2014**, *16*, 1410−1413.
(5) (a) Gao, B.; Huang, L.; Liu, H.; Wu, H.; Zhang, E.; Yang, L.; Wu, X.; Wang, Z. *Br. J. Pharmacol.* **2014**, *171*, 214−223. (b) Han, J.-B.; Hua, Y.-Q.; Chen, L.-Y.; Liu, L.-M. *J. Chin. Integr. Med.* **2012**, *10*, 256−263. (c) Ng, T. B. *J. Pharm. Pharmacol.* **2006**, *58*, 1007−1019. (d) Chan, R. Y. K.; Chen, W.-F.; Dong, A.; Guo, D.; Wong, M.-S. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 3691−3695.
(6) (a) Sankawa, U.; Taguchi, H.; Ogihara, Y.; Shibata, S. *Tetrahedron Lett.* **1966**, *7*, 2883−2886. (b) Ayer, W. A.; Pedras, M. S.; Ward, D. E. *Can. J. Chem.* **1987**, *65*, 760−764. (c) Elsebai, M. F.; Saleem, M.; Tejesvi, M. V.; Kajula, M.; Mattila, S.; Mehiri, M.; Turpeinen, A.; Pirttilä, A. M. *Nat. Prod. Rep.* **2014**, *31*, 628−645.
(7) (a) Shibata, S.; Ogihara, Y.; Tokutake, N.; Tanaka, O. *Tetrahedron Lett.* **1965**, *6*, 1287−1288. (b) Ogihara, Y.; Tanaka, O.; Shibata, S. *Tetrahedron Lett.* **1966**, *7*, 2867−2875. (c) Kuhr, I.; Fuska, J.; Sedmera, P.; Podojil, M.; Vokoun, J.; Vaněk, Z. *J. Antibiot.* **1973**, *26*, 535−536. (d) Bryant, F. O.; Cutler, H. G.; Jacyno, J. M. *J. Pharm. Sci.* **1993**, *82*, 1214−1217. (e) Yamazaki, M.; Okuyama, E. *Chem. Pharm. Bull.* **1980**, *28*, 3649−3655. (f) Dethoup, T.; Manoch, L.; Kijjoa, A.; Nascimento, M. S. J.; Puaparoj, P.; Silva, A. M. S.; Eaton, G.; Herz, W. *Planta Med.* **2006**, *72*, 957−960. (g) Guo, Z.; Shao, C.; She, Z.; Cai, X.; Liu, F.; Vrijimoed, L. L. P.; Lin, Y. *Magn. Reson. Chem.* **2007**, *45*, 439−441.
(8) Wu, A.; Cremer, D.; Auer, A. A.; Gauss, J. *J. Phys. Chem. A* **2002**, *106*, 657−667.
(9) (a) Ogihara, Y.; Iitaka, Y.; Shibata, S. *Tetrahedron Lett.* **1965**, *6*, 1289−1290. (b) Ogihara, Y.; Iitaka, Y.; Shibata, S. *Acta Crystallogr., Sect. B* **1968**, *B24*, 1037−1047. (c) Bryant, F. O.; Cutler, H. G. *Acta Crystallogr., Sect. C* **1995**, *C51*, 437−440.
(10) (a) Mori, H.; Kawai, K.; Ohbayashi, F.; Kuniyasu, T.; Yamazaki, M.; Hamasaki, T.; Williams, G. M. *Cancer Res.* **1984**, *44*, 2918−2923. (b) Kawai, K.; Shiojiri, H.; Nakamaru, T.; Nozawa, Y.; Sugie, S.; Mori, H.; Kato, T.; Ogihara, Y. *Cell Biol. Toxicol.* **1985**, *1*, 1−10.
(11) (a) Lin, Z.; Zhu, T.; Fang, Y.; Gu, Q.; Zhu, W. *Phytochemistry* **2008**, *69*, 1273−1278. (b) Wu, B.; Ohlendorf, B.; Oesker, V.; Wiese, J.; Malien, S.; Schmaljohann, R.; Imhoff, J. F. *Mar. Biotechnol.* **2015**, *17*, 110−119.
(12) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, *41*, 96−103.
(13) (a) Rychnovsky, S. D. *Org. Lett.* **2006**, *8*, 2895−2898. (b) Dong, L.-B.; Wu, Y.-N.; Jiang, S.-Z.; Wu, X.-D.; He, J.; Yang, Y.-R.; Zhao, Q.-S. *Org. Lett.* **2014**, *16*, 2700−2703.
(14) (a) Zhao, B.; Guengerich, F. P.; Bellamine, A.; Lamb, D. C.; Izumikawa, M.; Lei, L.; Podust, L. M.; Sundamoorthy, M.; Kalaitzis, J. A.; Reddy, L. M.; Kelly, S. L.; Stec, D.; Voehler, M.; Falck, J. R.; Moore, B. S.; Shimada, T.; Waterman, M. R. *J. Biol. Chem.* **2005**, *280*, 11599−11607. (b) Aldemir, H.; Richarz, R.; Gulder, T. A. M. *Angew. Chem., Int. Ed.* **2014**, *53*, 8286−8293.
(15) (a) Zmijewski, M. J., Jr.; Palaniswamy, V. A.; Gould, S. J. *J. Chem. Soc., Chem. Commun.* **1985**, *18*, 1261−1262. (b) Rontein, D.; Nishida, I.; Tashiro, G.; Yoshioka, K.; Wu, W.-I.; Voelker, D. R.; Basset, G.; Hanson, A. D. *J. Biol. Chem.* **2001**, *276*, 35523−35529.

DOI: 10.1021/acs.orglett.5b00081
Org. Lett. 2015, 17, 1146−1149