Organic LETTERS

Letter

pubs.acs.org/OrgLett

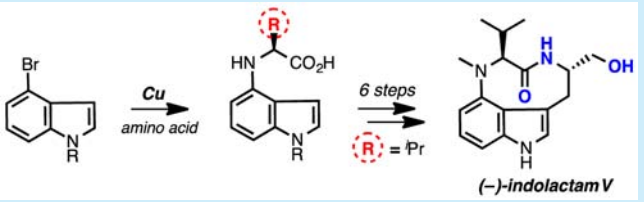
Modular Total Synthesis of Protein Kinase C Activator (−)-Indolactam V

Jeremy Haynes-Smith, Italia Diaz, and Kelvin L. Billingsley*

Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, California 94132, United States

Supporting Information

ABSTRACT: A concise, eight-step total synthesis of (−)-indolactam V, a nanomolar agonist of protein kinase C, is reported. The synthesis relies upon an efficient copper-catalyzed amino acid arylation to establish the indole C4−nitrogen bond. This cross-coupling method is applicable to a range of hydrophobic amino acids, providing a platform for further diversification of indolactam alkaloid scaffolds and studies on their potent biological activity.



Protein kinase C (PKC) is a family of serine and threonine kinases that play critical roles in cellular signal transduction,[1] and dysfunction of specific isozymes has been implicated in a range of disease states.[2] Small molecule therapeutics that regulate PKC activity have correspondingly been employed for the treatment of numerous disorders, including various cancers, Alzheimer's disease, and HIV/AIDS.[3] In addition, these molecular agents have been applied in biochemical studies to understand disease onset and progression.[4] For example, the indolactam alkaloids (Figure 1)

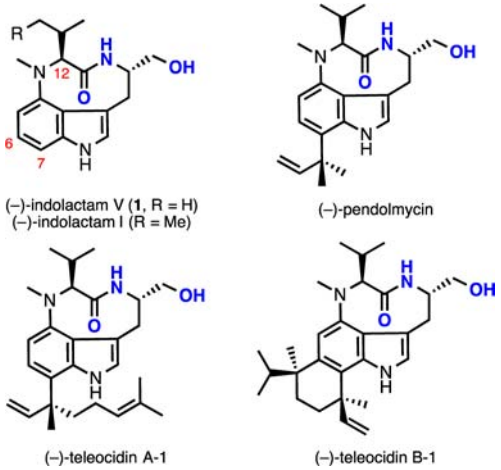


Figure 1. Representative indolactam alkaloids. Pharmacophore shown in blue.

have been used in mechanistic investigations of stem cell differentiation,[5] tumor growth,[6] and neurodegeneration.[7] These natural products regulate kinase activity through binding to the PKC regulatory C1 domain, mimicking the endogenous ligand diacylglycerol.[8] However, unlike other PKC modulators (e.g., bryostatin 1) that suppress cancer cell proliferation, indolactam alkaloids promote tumor growth.[9] The mechanistic basis for these dichotomous effects, tumor growth promotion versus suppression, for PKC ligands remains poorly understood,[10] which has to date limited the therapeutic potential of the indolactam alkaloids.

The biological significance of the indolactam alkaloids has resulted in numerous synthetic approaches for accessing these natural products. The majority of these strategies target the 9-membered lactam parent structure, (−)-indolactam V (**1**), which is an agonist of both conventional and novel PKC isoforms at nanomolar concentrations.[11] These previous syntheses have primarily relied upon an intramolecular peptide coupling to establish the macrocycle.[11] Other innovative approaches to **1** have included (1) photochemically induced 9-membered ring formation[11f] or (2) trapping of indolyne intermediates to establish the C4−nitrogen bond followed by Lewis acid mediated macrocyclization.[11k] However, while further hydrophobic substitution on the scaffold, particularly at C6, C7, or C12, has been shown to have profound effects on the potency and tumor promoting capacity of these molecules,[12] the lack of general synthetic strategies to selectively diversify at these specific positions has restricted systematic structure−activity relationship studies.[13] Furthermore, biological evaluation of tumor promotion properties for PKC modulators has relied upon a variety of assays,[14] and inconsistencies among previous reports has further complicated the preclinical analyses of indolactam alkaloids.[15] To fully appreciate the molecular features that confer potency and/or selectivity to these scaffolds and expand their therapeutic potential, a systematic structure−activity platform is required.

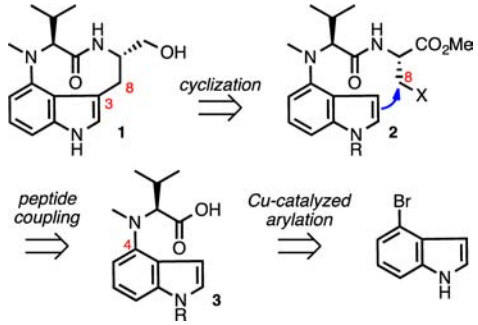
A primary objective of our research program is to develop modular strategies toward indolactam natural products and analogues. Through their biochemical assessment, we aim to determine the structural features that govern the biological profile of indolactam-based agents and, in turn, increase the

Received: March 3, 2016
Published: April 13, 2016

DOI: 10.1021/acs.orglett.6b00614
Org. Lett. 2016, 18, 2008−2011

therapeutic relevance of this family of PKC activators. The initial aim in this program was to establish a concise synthetic approach to access the core indolactam scaffold, **1**. We envisioned that indolactam macrocycle in **1** could be accessed through intramolecular indole alkylation at C3 with a C8 electrophile derived from dipeptide precursor **2** (Scheme 1).

Scheme 1. Retrosynthetic Analysis of 1



Dipeptide **2** would in turn be readily assembled from the *N*-indolated amino acid **3**. We reasoned that the amino acid moiety in **3** might be introduced through a metal-catalyzed arylation with a 4-haloindole electrophile. Critically, use of a cross-coupling method that is amenable to introduction of other hydrophobic amino acids permits further diversification of the indolactam scaffold. In this report, we describe a modular cross-coupling protocol to assemble a collection of substituted *N*-aryl amino acids and apply our synthetic strategy in the concise total synthesis of natural product indolactam V (**1**).

Establishing an effective, high-yielding method for amino acid arylation was our first key objective, as indolactam alkaloids typically incorporate a hydrophobic amino acid side chain at the C12-position of the 9-membered lactam ring. For example, while **1** contains a valine moiety and corresponding isopropyl substituent at the C12-position, other naturally occurring variants (e.g., (−)-indolactam I) integrate isoleucine, exhibiting a *sec*-butyl C12 alkyl chain (Figure 1). In addition, since the 9-membered ring exists as two stable conformers (*cis*- and *trans*-amide) at room temperature, conformationally restricted analogues possessing hydrophobic substituents at appropriate positions have been designed and displayed increased selectivity for novel PKC isozymes.[16] While C12 hydrophobicity has been observed to play a key role in the potency of these compounds, the low yielding microbial-based synthetic methods currently used to prepare indolactam scaffolds have precluded systematic analysis of hydrophobic substituents.[17] We sought to utilize a copper-catalyzed cross-coupling method that allows for the arylation of a diverse collection of hydrophobic amino acids with commercially available 4-bromoindole.

The copper-catalyzed carbon−nitrogen bond-forming reaction has been extensively researched over the past several decades, and numerous catalysts and protocols have been developed for the coupling of aryl halides with amine nucleophiles.[18] Our early studies demonstrated that both unprotected and *N*-alkyl- or *N*-benzyl-protected 4-bromoindoles underwent decomposition of the heteroaryl electrophile under the conditions for copper catalysis. Drawing on a single report by Ishikawa and co-workers describing *N*-arylation of valine with a derivatized 4-bromoindole,[19] we found that tosyl protection of the indole ring enabled successful copper-catalyzed coupling. While successful, the protocol described in this initial report afforded the cross-coupling product in modest yield (62%), employed long reaction times (52 h), and was not applicable to other hydrophobic alkyl amino , feature critical for indolactam analogue synthesis. To develop a more general protocol, we first examined the arylation of valine with 4-bromo-*N*-tosylindole (**2**). While we successfully isolated the desired *N*-arylvaline product (**3**) in a 56% yield after 52 h of reaction time, we also observed the presence of a byproduct (4-amino-*N*-tosylindole, **6**) in a ∼2:1 ratio (56% and 30% isolated yields of **5** and **6**, respectively) (Figure 2A). Through further

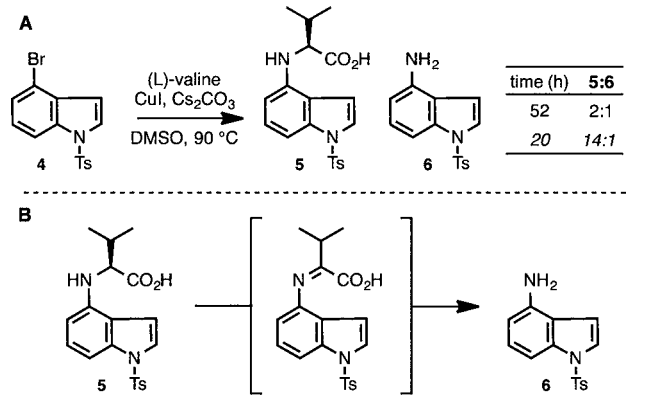


Figure 2. (A) Copper-catalyzed arylation of valine with 4-bromo-*N*-tosylindole. (B) In situ oxidation of **5** results in *N*-arylimino acid intermediate, which yields **6** upon hydrolysis.

investigation, we discovered that desired product **5** was prone to oxidation under the reaction conditions,[20] affording an *N*-arylimino acid intermediate, which then yielded byproduct **6** upon hydrolysis (Figure 2B). Since C−N bond formation occurred at a faster rate than the undesired oxidation, we hypothesized that adjusting the reaction time as well as concentration would prevent in situ degradation of **5** and increase the isolated yield of the desired product. Accordingly, shortening the reaction to 20 h provided **5**:**6** in a 14:1 ratio and 85% isolated yield of **5**, with no epimerization of the α-carbon (Figure 2A).

The substrate scope of this copper-catalyzed process was examined with a range of hydrophobic amino acids. Similar to valine, the reaction of **4** with isoleucine was effective, resulting in a greater than 90% yield of the desired product **7** (Figure 3). Less sterically hindered amino acids leucine and norvaline also cleanly allowed for C−N bond formation in 83% and 88% isolated yields, respectively (Figure 3). Further, a conformationally restricted amino acid, proline, was a suitable nucleophile for this cross-coupling process. However, it should be noted that the product of this transformation readily degrades upon purification. Lastly, amino acids such as phenylalanine and methionine, which possess hydrophobic functional groups, were coupled to **4** in high yield. Importantly, this initial sequence for installation of an amino acid-derived subunit addresses a key practical limitation of previous synthetic routes, which are typically low yielding and/or require numerous steps to prepare suitable partners to construct the hydrophobic amino acid motif.[11] Therefore, this copper-catalyzed arylation of diverse hydrophobic amino acids circumvents lengthy manipulations and provides a general strategy to introduce the desired substituents at the C12-position.

DOI: 10.1021/acs.orglett.6b00614
Org. Lett. 2016, 18, 2008−2011

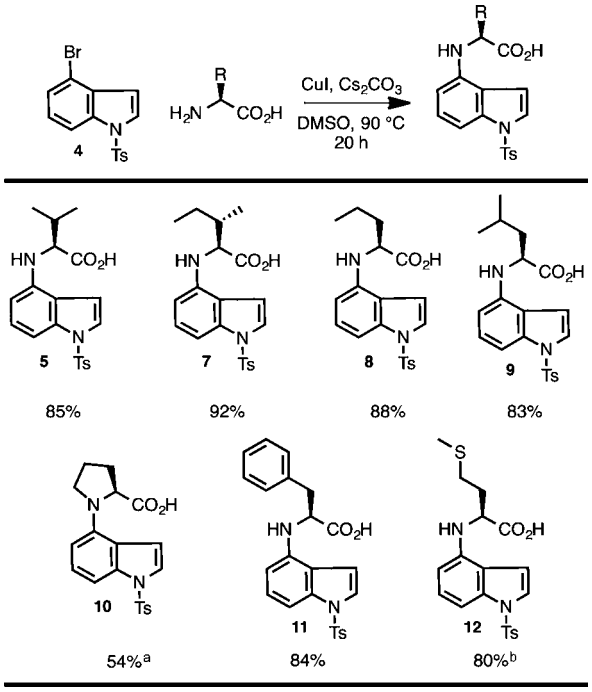
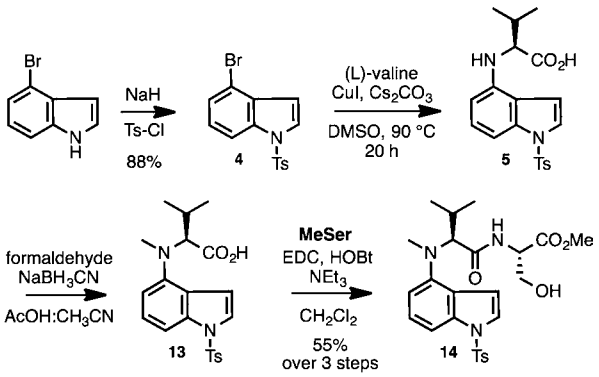


Figure 3. Copper-catalyzed arylation of hydrophobic amino acids with 4-bromo-*N*-tosylindole. Reaction conditions: 0.50 mmol of **4**, 0.60 mmol of amino acid, 0.05 mmol of CuI, and 0.75 mmol of Cs_2CO_3 in 0.50 mL of DMSO under argon atmosphere for 20 h. Isolated yield is average of two experiments. (a) Product decomposes during isolation; reported yield is based upon ^{1}H NMR analysis of crude product mixture with internal standard. (b) 0.10 mmol CuI used.

We next sought to establish conditions for the concise assembly of macrocyclic indolactam structures, commencing with synthesis of indolactam V (**1**) (Scheme 2). Upon tosyl

Scheme 2. Preparation of Intermediate 8[a]

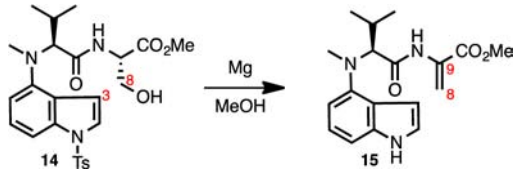


[a]MeSer = L-serine methyl ester HCl.

protection of 4-bromoindole, **4** was subjected to three consecutive steps: (1) C−N bond formation with valine to furnish **5**, (2) reductive amination of **5** with formaldehyde and $NaBH_3CN$ to provide **13**, and (3) EDC- promoted acid coupling between **13** and serine methyl ester to afford **14**. Importantly, this sequence yielded **14** in a 55% yield over 3 steps and required only a single purification (column chromatography purification of **14**) to be performed.

Our macrolactamization strategy relied on electrophilic activation of a C8-alcohol and ring closure via nucleophilic addition from the 3-position of the free N−H indole. Numerous protocols for *N*-tosyl protecting group removal, including mildly basic conditions,[21] fluoride reagents,[22] and single-electron-transfer processes,[23] resulted only in decomposition of **14**. However, when **14** was subjected magnesium in methanol, detosylation occurred with concurrent dehydration of the C8-alcohol to provide a 12% yield of alkene **15** (Table 1). Importantly, **15** possesses the necessary structural proper-

Table 1. Deprotection−Dehydration of 14[a]



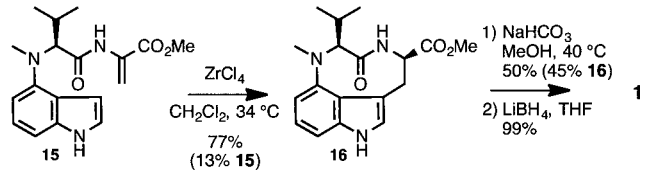
entry	Mg (equiv)	temp (°C)	time (h)	yield[a] (%)
1	5	rt	7	12
2	10	rt	7	27
3	**20**	**rt**	**7**	**50**
4	20	rt	24	22
5	20	50	7	<5
6	40	rt	7	26
7	80	rt	7	<5

[a]Isolated yields.

ties for cyclization: nucleophilic C3-position (deprotected indole) and electrophilic C8-position (2-aminoacrylate moiety). To capitalize on this result, we undertook further optimization of the deprotection−dehydration reaction. Increasing the equivalents of magnesium from 5 to 10 resulted in a higher yield (27%), and 20 equiv proved to be ideal, providing a 50% yield of **15**. Additional magnesium beyond 20 equiv resulted in reduction of the C8−C9 alkene of **15** and reduced overall yields. Longer reaction times or higher temperatures were likewise deleterious.

To close the indolactam V macrocycle we envisioned activation of the C8−C9 enoate alkene with an oxophilic Lewis acid and C3-alkylation of the pendant indole (Scheme 3). Garg and co-workers have previously studied the cyclization

Scheme 3. Total Synthesis of (−)-Indolactam V (1)



properties of **15**, which they prepared in 15 steps from commercially available 5-benzyloxyindole.[11k] Accordingly, application of the modified conditions for activation with $ZrCl_4$ reported by the Garg effectively promoted cyclization of **15** to the desired tricycle **16** in a 77% yield (13% recovered **15**). Epimerization at C9 to the natural stereoisomer with $NaHCO_3$ (50% yield with 45% **16** recovered)[11d] followed by reduction of the ester with $LiBH_4$ (99% yield) provided indolactam V (**1**) with an optical rotation identical to that of the reported value.[24]

In summary, we have developed a concise, eight-step synthetic route of indolactam V that utilizes our modular cross-coupling protocol. The development of an effective

DOI: 10.1021/acs.orglett.6b00614
Org. Lett. 2016, 18, 2008−2011

magnesium-promoted one-pot deprotection−dehydration reaction further allows rapid access to an advanced precursor and facile cyclization to the indolactam tricycle. A key advantage of our strategy is the application of our optimized copper-catalyzed amino acid arylation, which provides a high yielding method for introduction of diverse hydrophobic subunits. We anticipate that application of this protocol to acidic, basic, and unnatural amino acids will serve as a versatile platform for diversification of the indolactam scaffold. Ongoing studies are focused on the synthesis and biological evaluation these analogues as well as more elaborate members of the indolactam alkaloid family.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00614.

Experimental procedures and characterization of the described compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: klbillin@sfsu.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by generous startup funds from the San Francisco State University College of Science and Engineering.

■ REFERENCES

(1) Newton, A. C. *J. Biol. Chem.* **1995**, *270*, 28495.

(2) (a) Churchill, E.; Budas, G.; Vallentin, A.; Koyanagi, T.; Mochly-Rosen, D. *Annu. Rev. Pharmacol. Toxicol.* **2008**, *48*, 569. (b) Masliah, E.; Cole, G.; Shimohama, S.; Hansen, L.; DeTeresa, R.; Terry, R. D.; Saitoh, T. *J. Neurosci.* **1990**, *10*, 2113. (c) Dempsey, E. C.; Newton, A. C.; Mochly-Rosen, D.; Fields, A. P.; Reyland, M. E.; Insel, P. A.; Messing, R. O. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2000**, *279*, L429.

(3) (a) Mochly-Rosen, D.; Das, K.; Grimes, K. V. *Nat. Rev. Drug Discovery* **2012**, *11*, 937. (b) Mackay, H. J.; Twelves, C. J. *Nat. Rev. Cancer* **2007**, *7*, 554.

(4) Griner, E. M.; Kazanietz, M. G. *Nat. Rev. Cancer* **2007**, *7*, 281.

(5) (a) Chen, S.; Borowiak, M.; Fox, J. L.; Maehr, R.; Osafune, K.; Davidow, L.; Lam, K.; Peng, L. F.; Schreiber, S. L.; Rubin, L. L.; Melton, D. *Nat. Chem. Biol.* **2009**, *5*, 258. (b) Thatava, T.; Nelson, T. J.; Edukulla, R.; Sakuma, T.; Ohmine, S.; Tonne, J. M.; Yamada, S.; Kudva, Y.; Terzic, A.; Ikeda, Y. *Gene Ther.* **2011**, *18*, 283.

(6) (a) Basu, A. *Mol. Pharmacol.* **1998**, *53*, 105. (b) Troll, W.; Wiesner, R. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 509.

(7) (a) Vaughan, R. A.; Huff, R. A.; Uhl, G. R.; Kuhar, M. J. *J. Biol. Chem.* **1997**, *272*, 15541. (b) Nelson, T. J.; Alkon, D. L. *Trends Biochem. Sci.* **2009**, *34*, 136.

(8) Wang, S.; Liu, M.; Lewin, N. E.; Lorenzo, P. S.; Bhattacharrya, D.; Qiao, L.; Kozikowski, A. P.; Blumberg, P. M. *J. Med. Chem.* **1999**, *42*, 3436.

(9) Fujiki, H.; Sugimura, T.; Moore, R. E. *Environ. Health Perspect.* **1983**, *50*, 85.

(10) Loy, B. A.; Lesser, A. B.; Staveness, D.; Billingsley, K. L.; Cegelski, L.; Wender, P. A. *J. Am. Chem. Soc.* **2015**, *137*, 3678.

(11) (a) Endo, Y.; Shudo, K.; Itai, A.; Hasegawa, M.; Sakai, S.-I. *Tetrahedron* **1986**, *42*, 5905. (b) de Laszlo, S. E.; Ley, S. V.; Porter, R. A. *J. Chem. Soc., Chem. Commun.* **1986**, 344. (c) Nakatsuka, S.-I.; Masuda, T.; Sakai, K.; Goto, T. *Tetrahedron Lett.* **1986**, *27*, 5735. (d) Masuda, T.; Nakatsuka, S.-I.; Goto, T. *Agric. Biol. Chem.* **1989**, *53*, 2257. (e) Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623. (f) Mascal, M.; Moody, C. J.; Slawin, A. M. Z.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* **1992**, 823. (g) Semmelhack, M. F.; Rhee, H. *Tetrahedron Lett.* **1993**, *34*, 1395. (h) Quick, J.; Saha, B.; Driedger, P. E. *Tetrahedron Lett.* **1994**, *35*, 8549. (i) Súarez, A. I.; Garćia, M. C.; Compagnone, R. S. *Synth. Commun.* **2004**, *34*, 523. (j) Xu, Z.; Zhang, F.; Zhang, L.; Jia, Y. *Org. Biomol. Chem.* **2011**, *9*, 2512. (k) Bronner, S. M.; Goetz, A. E.; Garg, N. K. *J. Am. Chem. Soc.* **2011**, *133*, 3832. (l) Mari, M.; Bartoccini, F.; Piersanti, G. *J. Org. Chem.* **2013**, *78*, 7727. (m) Fine Nathel, N. F.; Shah, T. K.; Bronner, S. M.; Garg, N. K. *Chem. Sci.* **2014**, *5*, 2184. (n) Noji, T.; Okano, K.; Tokuyama, H. *Tetrahedron* **2015**, *71*, 3833.

(12) (a) Irie, K.; Nakagawa, Y.; Ohigashi, H. *Curr. Pharm. Des.* **2004**, *10*, 1371. (b) Irie, K.; Yanagita, R. C.; Torii, K.; Nakagawa, Y. *Heterocycles* **2007**, *73*, 289.

(13) (a) Nakagawa, Y. *Biosci., Biotechnol., Biochem.* **2012**, *76*, 1262. (b) Irie, K.; Nakagawa, Y.; Ohigashi, H. *Curr. Pharm. Des.* **2004**, *10*, 1371.

(14) (a) Bögi, K.; Lorenzo, P.; Szallasi, Z.; Acs, P.; Wagner, G. S.; Blumber, P. M. *Cancer Res.* **1998**, *58*, 1423. (b) Yotti, L. P.; Chang, C. C.; Trosko, J. E. *Science* **1979**, *206*, 1089. (c) Setterblad, N.; Onyango, I.; Pihlgren, U.; Rask, L.; Andersson, G. *J. Immunol.* **1998**, *161*, 4819. (d) Rohrschneider, L. R.; Boutwell, R. K. *Cancer Res.* **1973**, *33*, 1945.

(15) (a) Irie, K.; Yanagita, R. C.; Nakagawa, Y. *Med. Res. Rev.* **2012**, *32*, 518. (b) Kedei, N.; Lubart, E.; Lewin, N. E.; Telek, A.; Lim, L.; Mannan, P.; Garfield, S. H.; Kraft, M. B.; Keck, G. E.; Kolusheva, S.; Jelinek, R.; Blumberg, P. M. *ChemBioChem* **2011**, *12*, 1242.

(16) Nakagawa, Y.; Irie, K.; Komiya, Y.; Ohigashi, H.; Tsuda, K.-I. *Tetrahedron* **2004**, *60*, 7077.

(17) (a) Kajiyama, S.-I.; Irie, K.; Kido, T.; Koshimizu, K.; Hayashi, H.; Arai, M. *Tetrahedron* **1991**, *47*, 5453. (b) Hirota, M.; Suganuma, M.; Yoshizawa, S.; Horiuchi, T.; Nakayasu, M.; Hasegawa, M.; Endo, Y.; Shudo, K.; Fujiki, H. *Jpn. J. Cancer Res.* **1987**, *78*, 577.

(18) (a) Sambiagio, C.; Marsden, S. P.; Blacker, A. J.; McGowan, P. C. *Chem. Soc. Rev.* **2014**, *43*, 3525. (b) Ma, D.; Zhang, Y.; Yao, J.; Wu, S.; Tao, F. *J. Am. Chem. Soc.* **1998**, *120*, 12459. (c) Okano, K.; Tokuyama, H.; Fukuyama, T. *Org. Lett.* **2003**, *5*, 4987. (d) Kubo, T.; Katoh, C.; Yamada, K.; Okano, K.; Tokuyama, H.; Fukuyama, T. *Tetrahedron* **2008**, *64*, 11230.

(19) Ishikawa, T.; Kurokawa, M.; Nakanishi, W. *Heterocycles* **2007**, *71*, 847.

(20) Thomas, C.; Wu, M.; Billingsley, K. L. *J. Org. Chem.* **2016**, *81*, 330.

(21) (a) Bajwa, J. S.; Chen, G.-P.; Prasad, K.; Repič, O.; Blacklock, T. K. *Tetrahedron Lett.* **2006**, *47*, 6425. (b) Haskins, C. M.; Knight, D. W. *Tetrahedron Lett.* **2004**, *45*, 599.

(22) Carato, P.; Yous, S.; Sellier, D.; Poupaert, J. H.; Lebegue, N.; Berthelot, P. *Tetrahedron* **2004**, *60*, 10321.

(23) Ankner, T.; Hilmersson, G. *Org. Lett.* **2009**, *11*, 503.

(24) Irie, K.; Hirota, M.; Hagiwara, N.; Koshimizu, K.; Hayashi, H.; Murao, S.; Tokuda, H.; Ito, Y. *Agric. Biol. Chem.* **1984**, *48*, 1269.

DOI: 10.1021/acs.orglett.6b00614
Org. Lett. 2016, 18, 2008−2011