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Synthetic Approach to the Core Structure of Oleandrin and Related Cardiac Glycosides with Highly Functionalized Ring D

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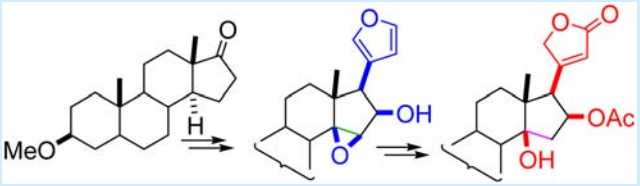
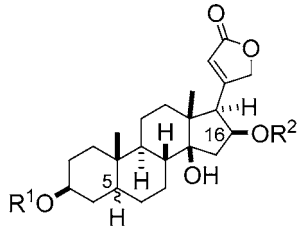
Supporting Information

ABSTRACT: The first synthetic approach to the core structure of cardiac glycoside oleandrin exhibiting a potent cytotoxic activity, starting from a common androstane derivative, has been accomplished. The synthesis is focused on stereoselective transformations in the densely substituted and sterically shielded five-membered ring (steroid ring D). The developed synthesis paves a route to the synthesis of related bufadienolides, i.e., constituents of traditional drug Ch'an Su, bufotalin, and cinobufagin.



The shrub *Nerium oleander*, widely appreciated for its beautiful flowers and evergreen lanceolate leaves, has been recognized for centuries as an important medicinal plant (and also known for deadly toxicity). Oleandrin (Figure 1, **1**)



1, 5β, R^1 = diginosyl, R^2 = Ac, oleandrin
2, 5β, R^1 = H, R^2 = Ac, oleandrigenin
3, 5α, R^1 = diginosyl, R^2 = Ac, 5α-oleandrin
4, 5β, R^1 = tri(digitoxosyl), R_2 = H, gitoxin
5, 5α, R^1 = Me, R^2 = Ac

Figure 1. Structure of oleandrin and related cardenolides bearing acetyloxy- or hydroxy group in position 16*β*.

composed of the steroid aglycone, oleandrigenin (**2**), and a sugar moiety (primarily D-diginosyl) has been identified as the major biologically active component of *N. oleander*.[1] The presence of the acetoxy group at the 16*β* position is the distinctive structural feature of oleandrigenin among the majority of other cardiac glycoside aglycones.[2] Other constituents of *N. oleander* carrying acetoxy function at C16-*β* have been recently identified,[3,4] most notably 5*α*-H oleandrin[4] (**3**) with the *trans*-fused AB rings. A few other cardiac glycosides carrying a free or substituted hydroxy group at the position 16*β* have also been isolated from other sources. These include gitoxin (**4**) (16*β*-hydroxydigitoxin), one of the major cardioactive principles of *Digitlis purpurea*.[5]

Stimulation of the cardiac muscle and moderating arrhythmia are traditional domains of therapeutic applications of oleandrin as well as other cardiac glycosides, such as digitoxin and ouabain.[6] Recently, a great deal of attention is being devoted to the anticancer properties of cardenolides.[7,8] In particular, oleandrin and related C16*β* acetoxy glycosides have been shown to have potent and specific cytotoxic activity.[4,8,9] 5*α*-Oleandrin (**3**) has been found to be highly active against multidrug-resistant human ovarian cancer 2780 AD cells with only low toxicity found toward parental cells.[4] Anti-HIV and anti-inflamatory activities of oleandrin have also been reported.[4,10]

Synthesis of oleandrigenin or other cardenolide aglycones bearing a hydroxyl function at C16 has not been reported until now.[11,12] Our synthetic approach to oleandrigenin and other cardenolides bearing hydroxy group or substituted hydroxy group at the position 16*β* starting from common steroid starting material is presented below (Scheme 1).

3-*O*-Methyl 5*α*-oleandrigenin **5** has been chosen as the model target compound. 3*β*-Methoxy-5*α*-androstan-17-one (**6**, Scheme 1) was transformed into vinyl iodide **7**, via respective hydrazone, following Barton's protocol.[13] The Suzuki–Miyaura cross-coupling reaction[14] of **7** and 3-furyl boronic acid in the presence of *tetrakis*-(triphenylphosphine)palladium(0) afforded **8** in a 72% yield with 10 mol % of the catalyst (at 0.1 g scale; 62% yield with 5% of the catalyst at 5–10 g scale).[12,15]

Hydroboration of **8** using borane–THF complex in THF affected selectively the aliphatic double bond[16] and occurred on the less shielded *α*-face of the ring D.[17] The product was, without isolation, oxidized with alkaline hydrogen peroxide to afford the 17*β*-(3-furyl)-16*α*-hydroxy derivative **9** as the single product (84% yield). The hydroxy group in **9** was oxidized with the Dess–Martin periodinane[18] to provide 16-one **10**. Confirmation of the *β*-orientation of the furyl substituent in ketone **10** as well as in the subsequently prepared enone **13** was accomplished by comparison of their experimental and calculated CD spectra.

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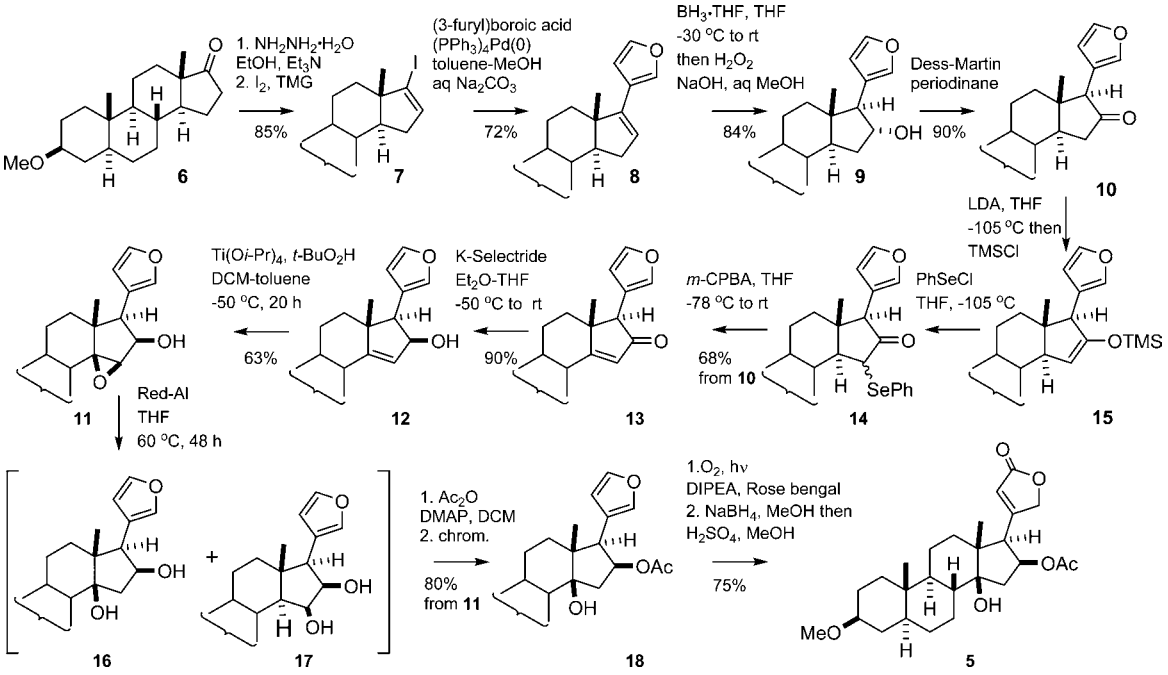
Scheme 1. Synthesis of Oleandrigenin Derivative 5

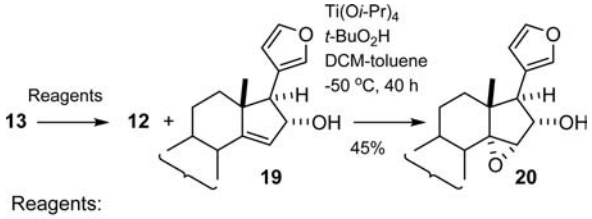


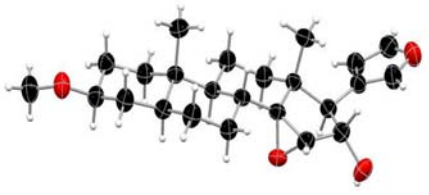
Treatment of **10** with LDA in THF at −78 °C, followed by trimethylsilyl chloride afforded silyl enol ether **15**. However, considerable amount of side products were also generated, presumably through silylation of the furyl moiety. The best results were obtained when **10** was deprotonated with LDA in THF at −105 °C, and the enolate was quenched with trimethylsilyl chloride at the same temperature. Thus, prepared derivative **15**, showing in its ^{1}H NMR only traces of a "foreign" trimethylsilyl group signal from side products, was used for the next step.

Oxidation of silyl enol ether **15** with palladium(II) acetate[19] was sluggish. Oxidation of **15** with freshly prepared IBX and 4-methoxypyridine *N*-oxide in anhydrous DMSO[20] afforded a mixture of enone **13** and the parent ketone **10**. Although the required product **13** could be isolated by column chromatography, its yield did not exceed 30%. The best yield of transformation of silyl enol ether **15** into α,β-unsaturated ketone **13** was realized following the classic route via phenylselenyl oxide elimination.[21] Thus, **15** in THF solution at −105 °C was treated with phenylselenyl chloride, and the product was isolated by a flash chromatography on silica gel. A mixture of two phenylselenyl epimers **14** was obtained in a 95% yield from **10** with the epimer ratio 10:3 (by ^{1}H NMR, presumed 15α- and 15β-isomers, respectively). The phenylselenyl derivatives **14**, without separation, were dissolved in THF and treated at −78 °C with one mol equiv of *m*-CPBA (77%). The mixture was allowed to warm to room temperature, and the product was isolated by chromatography. The enone **13** was obtained in 68% yield from ketone **10**.

The selective reduction of enone **13** to 16β-hydroxy derivative **12** was of prime importance for the efficiency of synthesis. After extensive experimentation it was found that treatment of **13** with K-Selectride in THF at −50 to −40 °C afforded alcohol **12** as the only isolated product in 90% yield. Some results of preliminary experiments on reduction of **13** are also worth mentioning. The Luche reagent[22] ($NaBH_4$−$CeCl_3$·$7H_2O$, MeOH, −20 °C) gave a mixture of **12** and **19** in a ratio of 13:87 (Scheme 2). The isomers were well resolved on TLC plates and easily separated by column chromatography. $LiAlH_4$

Scheme 2. Reduction of Enone 13 and Preparation and ORTEP Projection of the X-ray Structure of Hydroxy Epoxide 20





in THF at −78 °C afforded **19** in 74% yield (after chromatography). L-Selectride in THF at −78 °C gave **12** in a 45% yield along with the starting ketone **13** and a side product, presumably the 17-epimer of **13** (not separated on TLC), 15% combined yield. Ketone **13** and its presumed epimer were recovered from the reaction mixture even when a large excess of L-Selectride was used, which may be explained by an enolate formation occurring in parallel with the reduction.

Structures of alcohols **12** and **19** were tentatively assigned on the grounds of their ^{1}H NMR spectra (C16−H, C17−H coupling constants). It was assumed that the larger of the $J_{16,17}$

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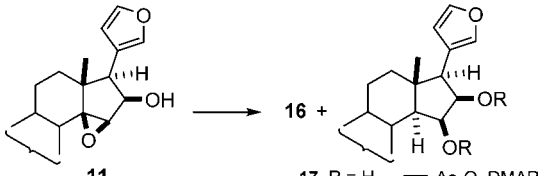
values, 5.7 and 8.2 Hz, corresponds to the *trans*-protons relation (**19**).

For the selective oxidation of double bond in allylic alcohols in the presence of the furan moiety, the Sharpless epoxidation[23] using *tert*-butylhydroperoxide and Ti(O*i*-Pr)$_4$ was employed.[24] Oxidation of 16α-hydroxy derivative **19** was examined first. Reaction at −50 °C with one equiv of the oxidant for 40 h (Scheme 2) afforded epoxide **20** as the major product (45% yield). An epoxidation of the synthetic intermediate **12** under similar conditions was faster and more efficient providing **11** (Scheme 1) in 63% yield along with unchanged **12**, 7% (20 h). Structure of hydroxy-epoxide **20** has been confirmed by single crystal X-ray analysis (Scheme 2).

The planned reductive opening of the epoxide ring in densely substituted and sterically shielded five-membered ring in **11** turned out frustrating. Treatment of **11** with LiAlH$_4$ in diethyl ether at room temperature afforded a mixture of the tertiary-secondary diol **16** along with disecondary diol to which structure **17** was assigned, the later predominating (a ratio of 3:4, respectively). A hydride ion addition at a tertiary position in an epoxide opening is rather unusual. In principle, a rearrangement of **11** into the respective 14α-H, 15-one could have occurred prior to reduction. However, selective generation of 15β-hydroxy group in **17** makes unlikely the occurrence of the 15-one as an integrated intermediate. Reduction of **11** with LiAlH$_4$ in THF at 50 °C afforded **16** and **17** in 50 and 30% yield, respectively. The ratio **16**/**17** has been improved by adding isopropanol (1.2 equiv) to a solution of LiAlH$_4$ in THF (carefully!) and using this in situ prepared reagent for the reduction of **11** (see Supporting Information). However, the procedure was arduous, and no adequate reproducibility of results could be reached. Eventually, it was found that reduction of **11** with Red-Al in THF at 60 °C led to a mixture of diols **16** and **17** in a ratio of 83:17 (by ^{1}H NMR). Since the isomers were poorly separated on a silica gel column, the crude reduction product mixture was acetylated with acetic anhydride in the presence of DMAP in DCM, and the obtained mixture of monoacetate **18** and diacetate **21** (Scheme 3) was separated by chromatography. In this way **18** was obtained in 80% yield from **11**. Its structure has been confirmed by X-ray analysis (Figure 2).

Scheme 3. Reduction of Hydroxyl-epoxide 11 and Transformation of Diol 17



For the transformation of furan moiety in **17** into butenolide the oxidation with singlet oxygen in the presence of a base developed by Kernan and Faulkner[25] was employed.[12] Thus, a solution of **17** in dichloromethane containing rose bengal as a sensitizer and diisopropylethylamine (DIPEA) under an oxygen atmosphere, at −78 °C, was stirred while irradiated with a tungsten lamp. After the starting material was consumed the mixture was worked up, and crude product (presumably containing γ-hydroxybutanolides) was treated with NaBH$_4$ followed by acidification.[26] The product was isolated by column chromatography to afford 3-*O*-methyl oleandrigenin **5** in 75% yield.

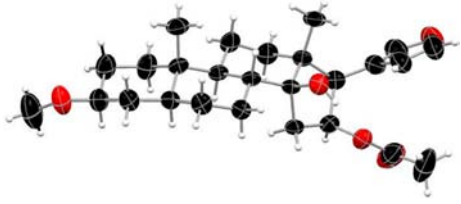


Figure 2. ORTEP projection of the X-ray structure of compound **18**.

The presented above preparation of 3-*O*-methyl 5α-oleandrigenin **5** constitutes also a new approach to the synthesis of the related group of compounds, bufadienolides, bearing α-pyrone ring at the C17β position and acetoxy group in the position 16β, such as constituents of the traditional Chinese toad-derived drug, Chan'Su, bufotalin, and cinobufagin.[27] The method for transforming the furyl moiety in intermediates such as **9** (Scheme 1, devoid of the C16 hydroxy group) into the respective α-pyrone derivative has been previously reported.[28] A significant amount of data has been accumulated, indicating that the 16β-substituted bufadienolides of Ch'an Su and relevant compounds of plant origin show useful therapeutic activities, including specific cytotoxic activity.[29]

In conclusion, a synthetic approach to 16β-acyloxy (hydroxy-, alkoxy-) cardenolides from common steroid starting materials was achieved. A sequence of selective transformations in a densely substituted and sterically shielded five-membered ring was developed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03157.

Experimental procedures, characterization data, and copies of NMR, IR, and CD spectra for new compounds (PDF)
Compound **18** (CIF)
Compound **20** (CIF)

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Author Contributions

M.M. is responsible for the X-ray analyses.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Neumann, W. *Ber. Dtsch. Chem. Ges. B* **1937**, *70*, 1547. (b) Tschesche, R. *Ber. Dtsch. Chem. Ges. B* **1937**, *70*, 1554.
(2) Fieser, L. F.; Fieser, M. *Steroids*; Reinhold: New York, 1959.
(3) (a) Huq, M. M.; Jabbar, A.; Rashid, M. A.; Hasan, C. M.; Ito, C.; Furukawa, H. *J. Nat. Prod.* **1999**, *62*, 1065. (b) Begum, S.; Siddiqui, B. S.; Sultana, R.; Zia, A.; Suria, A. *Phytochemistry* **1999**, *50*, 435. (c) Bai, L.; Hasegawa, R.; Hirose, K. i.; Ando, M.; Sakai, J.-i.; Andoe, M. *Heterocycles* **2009**, *78*, 2361. (d) Bai, L.; Zhao, M.; Toki, A.; Sakai, J.; Yang, X.; Bai, Y.; Ando, M.; Hirose, K.; Ando, M. *Chem. Pharm. Bull.* **2010**, *58*, 1088. (e) Sharma, P.; Gupta, Y. K.; Sharma, M. C.; Dobhal, M. P. *Indian J. Chem. Sec. B* **2010**, *49*, 374. (f) Zhao, M.; Bai, L. M.; Toki, A.; Hasegawa, R.; Sakai, J.; Hasegawa, T.; Ogura, H.; Kataoka, T.; Bai, Y. H.; Ando, M.; Hirose, K.; Ando, M. *Chem. Pharm. Bull.* **2011**, *59*, 371.
(4) Zhao, M.; Bai, L.; Wang, L.; Toki, A.; Hasegawa, T.; Kikuchi, M.; Abe, M.; Sakai, J.-i.; Hasegawa, R.; Bai, Y.; Mitsui, T.; Ogura, H.; Kataoka, T.; Oka, S.; Tsushima, H.; Kiuchi, M.; Hirose, K.; Tomida, A.; Tsuruo, T.; Ando, M. *J. Nat. Prod.* **2007**, *70*, 1098.
(5) Jacobs, W. A.; Gustus, E. L. *Science (Washington, DC, U. S.)* **1929**, *70*, 639.
(6) (a) Chugh, S. S.; Havmoeller, R.; Narayanan, K.; Singh, D.; Rienstra, M.; Benjamin, E. J.; Gillum, R. F.; Kim, Y.-H.; McAnulty, J. H.; Zheng, Z.-J.; Forouzanfar, M. H.; Naghavi, M.; Mensah, G. A.; Ezzati, M.; Murray, C. J. L. *Circulation* **2014**, *129*, 837. (b) Ewy, G. A. *Am. J. Med.* **2015**, *128*, 1272.
(7) (a) Winnicka, K.; Bielawski, K.; Bielawska, A. *Acta Polym. Pharm. Drug Res.* **2006**, *63*, 109. (b) Mijatovic, T.; Kiss, R. *Planta Med.* **2013**, *79*, 189.
(8) Newman, R. A.; Yang, P. Y.; Pawlus, A. D.; Block, K. I. *Mol. Interventions* **2008**, *8*, 36.
(9) (a) Pathak, S.; Multani, A. S.; Narayan, S.; Kumar, V.; Newman, R. A. *Anti-Cancer Drugs* **2000**, *11*, 455. (b) Yang, P.; Menter, D. G.; Cartwright, C.; Chan, D.; Dixon, S.; Suraokar, M.; Mendoza, G.; Llansa, N.; Newman, R. A. *Mol. Cancer Ther.* **2009**, *8*, 2319. (c) Turan, N.; Akgun-Dar, K.; Kuruca, S. E.; Kilicaslan-Ayna, T.; Seyhan, V. G.; Atasever, B.; Mericli, F.; Carin, M. *J. Exp. Ther. Oncol.* **2006**, *6*, 31. (d) Rashan, L. J.; Franke, K.; Khine, M. M.; Kelter, G.; Fiebig, H. H.; Neumann, J.; Wessjohann, L. A. *J. Ethnopharmacol.* **2011**, *134*, 781. (e) Kumar, A.; De, T.; Mishra, A.; Mishra, A. K. *Pharmacogn. Rev.* **2013**, *7*, 131. (f) Hong, D. S.; Henary, H.; Falchook, G. S.; Naing, A.; Fu, S.; Moulder, S.; Wheler, J. J.; Tsimberidou, A.; Durand, J. B.; Khan, R.; Yang, P.; Johansen, M.; Newman, R. A.; Kurzrock, R. *Invest. New Drugs* **2014**, *32*, 1204.
(10) Singh, S.; Shenoy, S.; Nehete, P. N.; Yang, P.; Nehete, B.; Fontenot, D.; Yang, G.; Newman, R. A.; Sastry, K. J. *Fitoterapia* **2013**, *84*, 32.
(11) For recent cardenolide syntheses, see: (a) Kaplan, W.; Khatri, H. R.; Nagorny, P. *J. Am. Chem. Soc.* **2016**, *138*, 7194. (b) Mukai, K.; Kasuya, S.; Nakagawa, Y.; Urabe, D.; Inoue, M. *Chem. Sci.* **2015**, *6*, 3383. (c) Renata, H.; Zhou, Q. H.; Dunstl, G.; Felding, J.; Merchant, R. R.; Yeh, C. H.; Baran, P. S. *J. Am. Chem. Soc.* **2015**, *137*, 1330. (d) Sridhar Reddy, M.; Zhang, H. B.; Phoenix, S.; Deslongchamps, P. *Chem. - Asian J.* **2009**, *4*, 725. (e) For a review, see: Heasley, B. *Chem. - Eur. J.* **2012**, *18*, 3092.
(12) Honma, M.; Nakada, M. *Tetrahedron Lett.* **2007**, *48*, 1541.
(13) Barton, D. H. R.; Bashiardes, G.; Fourrey, J. L. *Tetrahedron* **1988**, *44*, 147.
(14) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
(15) (a) Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. *J. Med. Chem.* **1995**, *38*, 2463. (b) Chao, J. P.; Ling, Y. Z.; Liu, X. F.; Luo, X. D.; Brodie, A. M. H. *Steroids* **2006**, *71*, 585.
(16) Brown, H. C.; Gupta, A. K.; Vara Prasad, J. V. N. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 93.
(17) Izzo, I.; Di Filippo, M.; Napolitano, R.; De Riccardis, F. *Eur. J. Org. Chem.* **1999**, *1999*, 3505.
(18) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
(19) Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011.
(20) Nicolaou, K. C.; Gray, D. L. F.; Montagnon, T.; Harrison, S. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 996.
(21) Reich, H. J.; Renga, J. M.; Reich, I. L. *J. Am. Chem. Soc.* **1975**, *97*, 5434.
(22) Luche, J.-L.; Rodriguez-Hahn, L.; Crabbe, P. *J. Chem. Soc., Chem. Commun.* **1978**, *0*, 601.
(23) Sharpless, K. B.; Michaelson, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6136.
(24) (a) Enev, V. S.; Petrov, S. O.; Neh, H.; Nickisch, K. *Tetrahedron* **1997**, *53*, 13709. (b) Chochrek, P.; Kurek-Tyrlik, A.; Michalak, K.; Wicha, J. *Tetrahedron Lett.* **2006**, *47*, 6017.
(25) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.* **1988**, *53*, 2773.
(26) Tsai, T. Y. R.; Minta, A.; Wiesner, K. *Heterocycles* **1979**, *12*, 1397.
(27) Nogawa, T.; Kamano, Y.; Yamashita, A.; Pettit, G. R. *J. Nat. Prod.* **2001**, *64*, 1148.
(28) Jäggi, F. J.; Tsai, T. Y. R.; Wiesner, K. *Heterocycles* **1982**, *19*, 647.
(29) Gao, H.; Popescu, R.; Kopp, B.; Wang, Z. *Nat. Prod. Rep.* **2011**, *28*, 953.

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