Sarinfacetamides A and B, Nitrogenous Diterpenoids with Tricyclo[6.3.1.0^{1,5}]dodecane Scaffold from the South China Sea Soft Coral *Sarcophyton infundibuliforme*

Fei Ye,[†,‡] Jing Li,[Δ] Yu Wu,[Δ] Zheng-Dan Zhu,[†,‡] Ernesto Mollo,[□] Margherita Gavagnin,[□] Yu-Cheng Gu,[‖] Wei-Liang Zhu,[†,‡,§] Xu-Wen Li, [*,†,§] and Yue-Wei Guo[*,†,‡,§]

[†] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

[‡] University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, China

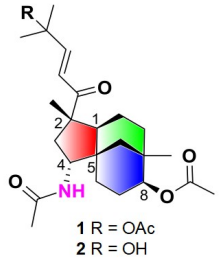
[Δ] Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, 5 Yu-Shan Road, Qingdao 266003, China

[‖] Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom

[□] Institute of Biomolecular Chemistry (ICB) of the National Research Council (CNR), Via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy

[§] Open Studio for Druggability Research of Marine Natural Products, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Aoshanwei, Jimo, Qingdao, 266237, China

Supporting Information Placeholder



ABSTRACT: Two novel nitrogenous diterpenoids, sarinfacetamides A (**1**) and B (**2**), featuring an uncommon tricyclo[6.3.1.0^{1,5}]dodecane scaffold, and a known related diterpene (**3**), were isolated from the South China Sea soft coral *Sarcophyton infundibuliforme*. Their structures, including the absolute configuration of **1**, were established by extensive spectroscopic analysis and TDDFT-ECD calculation. Compounds **1** and **3** exhibited interesting promotion effects on the ConA-induced T lymphocyte proliferation. A plausible biosynthetic pathway for **1** and **2** was also proposed.

Soft corals of the genus *Sarcophyton* (order *Alcyonacea*, family *Alcyoniidae*) comprise a wealth of secondary metabolites, including terpenoids, prostaglandins, ceramides, lipids, steroids, *etc*. These natural products exhibited a diverse range of pharmaceutical potentials, such as protein tyrosine phosphatase 1B (PTP1B) inhibitory, antibacterial, cytotoxic and immune enhancement properties.[1] A literature survey revealed that, among all the species of *Sarcophyton*, the chemical constituents of *Sarcophyton infundibuliforme* have only been previously reported by Wang *et. al*.,[2] dealing with the discovery of typical cembranoids and glycosylglycerols as its main secondary metabolites. In the course of our continuous efforts toward searching for bioactive marine natural products from Chinese soft corals,[3] especially those of the genus *Sarcophyton*,[3b-3e] the title animal was encountered off the coast of Ximao Island, Hainan Province, China, and chemically investigated resulting in the isolation and characterization of two novel nitrogenous diterpenoids, namely sarinfacetamides A (**1**) and B (**2**), and a known related one, nanolobatin B (**3**) (Figure 1). Herein, we report the isolation, structure determination, bioactivity evaluation, as well as plausible biosynthetic pathway of the new compounds.

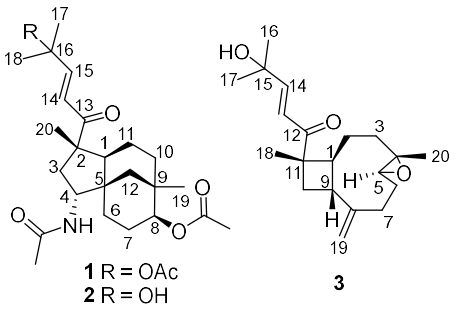


Figure 1. Structures of **1**–**3**.

The usual workup[3] of the Et_2O-soluble portion of the acetone extract of the animals of *S. infundibuliforme* yielded the pure compounds **1** (2.8 mg), **2** (1.1 mg) and **3** (2.3 mg), re-

spectively. The known compound **3** was readily identified as nanolobatin B, a xeniaphyllane-type diterpenoid previously isolated from the Taiwan soft coral *Sinularia nanolobata*,[4] by direct comparison of its NMR data and specific rotation with those reported in the literature.

Table 1. ^{1}H NMR (δ_H) and ^{13}C NMR (δ_C) Data for **1** and **2** in $CDCl_3$

	1		**2**	
No.	δ_H, mult (*J*, Hz)[a]	δ_C[b]	δ_H, mult (*J*, Hz)[a]	δ_C[b]
1	1.99 m	44.8	1.99 m	45.0
2	–	55.0	–	55.2
3a	1.98 dd (13.0, 7.5)	40.3	1.98 dd (13.0, 7.5)	40.3
3b	1.85 dd (13.0, 6.4)		1.85 dd (13.0, 6.4)	
4	4.15 ddd (9.0, 7.5, 6.4)	57.9	4.14 ddd (9.0, 7.5, 6.4)	58.0
5	–	45.1	–	45.2
6a	1.44 m	27.8	1.44 m	27.8
6b	1.07 ddd (12.5, 4.4, 1.9)		1.07 ddd (12.5, 4.4, 1.9)	
7a	1.96 m	24.5	1.96 m	24.5
7b	1.70 m		1.70 m	
8	4.56 t (2.7)	76.4	4.55 brt (2.7)	76.4
9	–	33.7	–	33.7
10a	1.50 dd (13.4, 10.8)	33.1	1.49 m	33.1
10b	1.29 dd (10.8, 5.3)		1.29 m	
11	2.03 m	22.0	2.03 m	22.0
12a	1.62 d (13.3)	37.2	1.62 d (13.3)	37.1
12b	1.16 d (13.3)		1.16 d (13.3)	
13	–	203.9	–	204.5
14	6.41 d (15.5)	121.6	6.62 d (15.2)	120.7
15	6.97 d (15.5)	150.9	6.99 d (15.2)	154.3
16	–	79.6	–	71.4
17	1.55 s	26.5	1.38 s	29.7
18	1.54 s	26.7	1.38 s	29.8
19	0.87 s	28.1	0.87 s	28.1
20	1.24 s	20.4	1.24 s	20.4
4-NHCO$\underline{CH_3}$	2.00 s	23.8	2.00 s	23.8
4-N$\underline{H}$COCH$_3$	5.78 d (9.0)	–	5.86 d (9.0)	
4-NH$\underline{C}$OCH$_3$		169.9[c]		169.8
8-OCO$\underline{CH_3}$	2.04 s	21.5	2.04 s	21.5
8-O$\underline{C}$OCH$_3$		171.0		171.0
16-OCO$\underline{CH_3}$	2.02 s	22.1	–	–
16-O$\underline{C}$OCH$_3$		169.9[c]	–	–

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. Assignments were deduced by analysis of 1D and 2D NMR spectra. [c] The precise ^{13}C NMR chemical shifts for these two carbons are 169.87 and 169.86 ppm, respectively, which may be interchangeable.

Sarinfacetamide A (**1**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{26}H_{39}NO_6$ from the HRESIMS at *m/z* 462.2857 ([M+H]$^+$, calculated as 462.2856), suggesting eight degrees of unsaturation. The IR spectrum of **1** displayed characteristic absorptions indicative of ester carbonyl (1737 cm^{-1}), amide group (3381 and 1655 cm^{-1}), and *α, β*-unsaturated ketone (1681 cm^{-1}). The presence of an unsaturated ketone moiety was further confirmed by the UV absorption at λ_{max} 226 nm (logε 3.71). The ^{1}H NMR spectrum (Table 1) of **1** showed signals for four singlet methyls at δ_H 0.87, 1.24, 1.54 and 1.55, three carbonyl-connected methyls at 2.00, 2.02, and 2.04, as well as two olefinic protons at δ_H 6.97 (d, *J* = 15.5) and 6.41 (d, *J* = 15.5). The ^{13}C NMR, DEPT and HSQC spectra of **1** revealed 26 carbon signals, including seven methyls, six sp^3 methylenes, three sp^3 methines (an oxygenated one at δ_C 76.4), four sp^3 quaternary carbons (an oxygenated one at δ_C 79.6), two sp^2 methines (one disubstituted double bond at δ_C 150.9 and 121.6), and four carbonyls (one ketone at δ_C 203.9 three ester or amide carbonyls at δ_C 171.0, 169.9, and 169.9). One double bond and four carbonyls accounted for five degrees of unsaturation, thus, the remaining three degrees were ascribed to a tricyclic ring system as depicted in **1**.

Extensive analysis of the ^{1}H–^{1}H COSY spectrum of **1** disclosed the proton connectivity for four structural fragments **a**–**d** (Figure 2), by clear correlations of H-1 (δ_H 1.99)/H$_2$-11 (δ_H 2.03)/H$_2$-10 (δ_H 1.50, 1.29) (**a**); H$_2$-3 (δ_H 1.98, 1.85)/H-4 (δ_H 4.15)/NH (δ_H 5.78) (**b**); H$_2$-6 (δ_H 1.44, 1.07)/H$_2$-7 (δ_H 1.96, 1.70)/H-8 (δ_H 4.56) (**c**); and H-14 (δ_H 6.41)/H-15 (δ_H 6.97) (**d**), respectively. The subunits **a**–**c** were connected, bearing in mind two methyls at δ_C 20.4 and 28.1, three quaternary carbons at δ_C 33.7, 45.1 and 55.0, and an isolated methylene at δ_C 37.2, by detailed interpretation of the well resolved HMBC correlations (Figure 2) from H-4 to C-1 (δ_C 44.8)/C-2 (δ_C 55.0)/C-5 (δ_C 45.1)/C-6 (δ_C 27.8)/C-12 (δ_C 37.2), from H-8 to C-6/C-12, from H$_2$-10 to C-1/C-8 (δ_C 76.4)/C-9 (δ_C 33.7)/C-12, from H$_3$-19 (δ_H 0.87) to C-8/C-9/C-10 (δ_C 33.1)/C-12, and from H$_3$-20 (δ_H 1.24) to C-1/C-2/C-3 (δ_C 40.3), leading the construction of a partial structure **X**, an uncommon tricyclo[6.3.1.0^{1,5}]dodecane skeleton.

Subtraction of the above identified **X** moiety from the molecular formula of **1** indicated that, besides the partial structure **d**, there are still two acetoxyl groups, one acetamide, one ketone and two tertiary methyls remaining unassigned. The ketone (C-13) conjugated with the disubstituted olefin **d** is very clear based on the typical IR and UV absorptions of **1**, which was confirmed by the HMBC cross peak from H-15 to C-13. Further, the other side of the double bond was substituted by

an oxygenated quaternary carbon bearing one acetoxyl and two methyls as evidenced by the typical downfield ^{13}C chemical shift of C-16 at δ_C 79.6, and the HMBC correlations from H_3-17 (δ_H 1.55)/H_3-18 (δ_H 1.54) to C-16 and C-15 (δ_C 150.9), respectively. Finally, the above identified partial structure **Y** was located at the C-2 position of the **X** fragment by the obvious HMBC correlation from H_3-20 to C-13 (Figure 2). In addition, the typical ^{13}C chemical shifts of δ_C at 57.9 (C-4) and 76.4 (C-8) clearly indicated that these two carbons bear one acetamide and one acetoxyl, respectively, which were further confirmed by the clear HMBC correlations from H-4 to 4-NHAc (CO, δ_C 169.9) and from H-8 to 8-OAc (CO, δ_C 171.0). In light of these observations, the planar structure of **1** was established as shown in Figure 2.

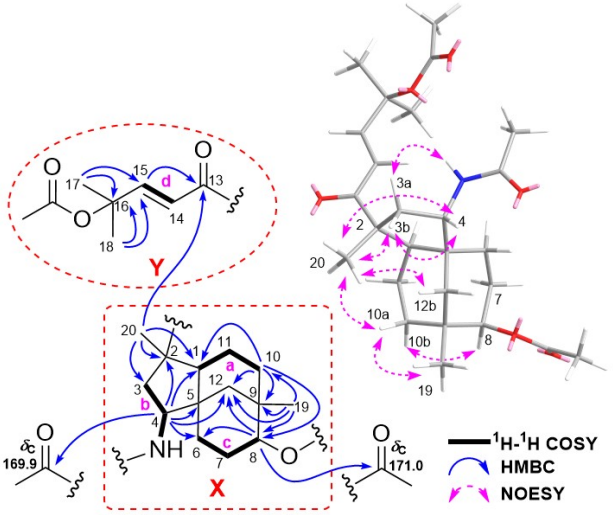


Figure 2. ^{1}H–^{1}H COSY, key HMBC and NOESY correlations of **1**.

The relative configuration of **1** was established via analysis of its ^{1}H-^{1}H coupling constants and NOESY spectrum (Figure 2). The large coupling constant of 15.5 Hz between the two olefinic protons H-14 and H-15 indicated the *E* configuration of $\Delta^{14/15}$. The obvious correlations between H_3-20 and H-3b (δ_H 1.85)/H-4/H-10a (δ_H 1.50)/H-12b (δ_H 1.16), between H-3b and H-4, and between H-10a and H_3-19, revealed the CH_3-20, H-3b, H-4, H-10a, H-12b, and CH_3-19 are of the same orientation, arbitrarily assigned as β configuration (Figure 2, 3D structure). The NOE correlations between H-3a (δ_H 1.98) and –NHAc (δ_H 5.78) disclosed the α-orientation of –NHAc group. The clear cross peaks between H-10b (δ_H 1.29) and H-8 indicated that 8-OAc is also β-oriented. Finally, the relative configurations of all the chiral centers of **1** was established as *1R*, 2R*, 4S*, 5S*, 8R*, 9R**, respectively.

The absolute configuration of **1** was determined by the time-dependent density functional theory-electronic circular dichroism (TDDFT-ECD) calculation.[5] As shown in Figure 3, the ECD spectra (CH_3CN) of compound **1** displayed a negative π-π* Cotton effect (CE) at 225 nm (Δε −2.18). The initial torsional sampling (MCMM) and OPLS_2005 force field conformational searches of (1*R*, 2*R*, 4*S*, 5*S*, 8*R*, 9*R*)-**1** afforded 30 conformers within the 21 kJ/mol energy window.[6] The Boltzmann populations of the conformers were obtained based on the potential energy provided by the OPLS_2005 force field, leading to 5 conformers of compound **1** above 1% population for further re-optimization (SI, Figure S3). The resulting geometries were re-optimized at the B3LYP/6-311G (d, p) level with IEFPCM solvent model for CH_3CN, and frequency analysis was performed as well to confirm that the re-optimized geometries were at the energy minima. Finally, the Boltzmann-averaged ECD spectra of (1*R*, 2*R*, 4*S*, 5*S*, 8*R*, 9*R*)-**1** displayed opposite curves to the experimental one, whereas its enantiomer showed curves which highly matched to the experimental one. Consequently, the absolute configuration of all chiral carbons of **1** was determined to be 1*S*, 2*S*, 4*R*, 5*R*, 8*S*, 9*S*, and its structure was drawn as shown in Figure 1.

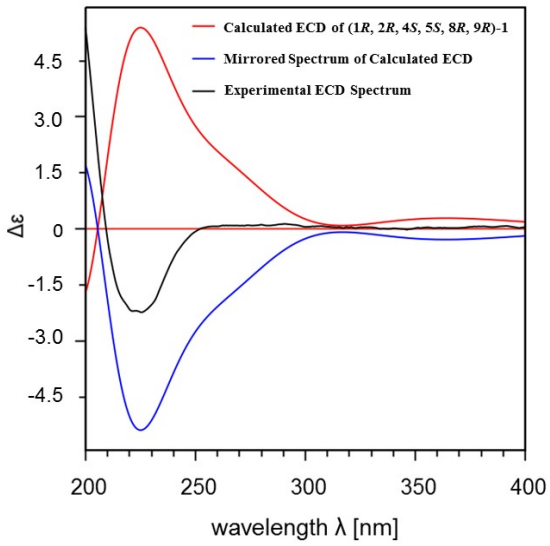


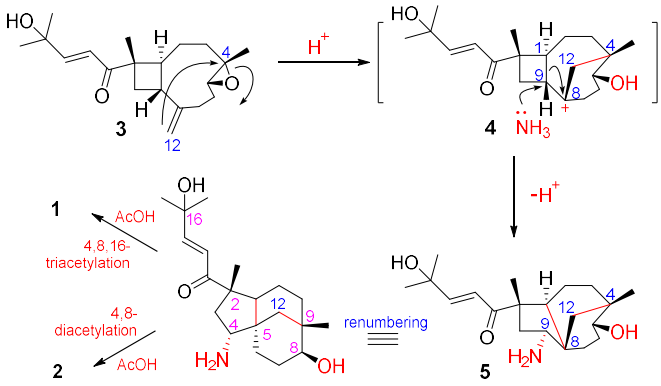
Figure 3. Experimental ECD spectrum of sarinfacetamide A (**1**) (black), the calculated ECD spectra of (1*R*, 2*R*, 4*S*, 5*S*, 8*R*, 9*R*)-**1** (red) and its enantiomer (blue), respectively.

Compound **2** displayed very similar 1D NMR data as those of **1** (Table 1). Careful comparison of the overall NMR data of **1** and **2** revealed that the differences between them mainly happened at C-16 and its neighboring carbons (*e.g.* C-15, C-17, and C-18), indicating the deacetylation at the 16-OH of **2**. In fact, due to the loss of acetyl group at C-16, the ^{13}C chemical shifts of C-16 was, as expected, apparently upfield shifted (δ_C of C-16 at 79.6 in **1** and 71.4 in **2**) whereas the C-15, C-17 and C-18 of **2** (Table 1) were all reasonably downfield shifted, according to the 42 mass units difference between the molecular weight of **1** and **2**. In addition, the presence of isoprene unit in **2**, the same as that in **3**, was further confirmed by comparing the corresponding NMR data with those of the co-occurring **3**. Thus, the structure of **2** was determined as the C-16 deacetyl derivative of **1**, named sarinfacetamide B.

The framework of sarinfacetamides A and B (**1** and **2**) are unprecedented and formally very different from the co-occurring xeniaphyllane-type diterpene nanolobatin B (**3**). But interestingly, **1**-**3** share the common carbonyl conjugated isoprene side chain, sparking our curiosity to explore the biogenetic origin of **1** and **2**, as well as the possible biosynthetic relationship between **1**–**3**. Detailed analysis of the structures of **1**–**3** allowed us to propose a plausible biosynthetic connection from **3** to **1** and **2**. As outlined in the Scheme 1, the formation of the C-12–C-4 bond accompanying the opening of the epoxide ring of **3** generated the key intermediary carbon cation **4** (Scheme 1), which, in turn, reacted with ammonia by the lone pair electrons of nitrogen atom attacking C-9, followed by the cleavage of C-1–C-9 bond and the formation of C-1–C-8 bond to give the skeleton (**5**) of sarinfacetamides, of

which acetylation at C-4/C-8/C-16 or C-4/C-8 could produce the new compounds **1** or **2**, respectively (Scheme 1).

Scheme 1. Proposed biosynthetic pathway for compounds **1** and **2**



Compounds **1** and **3** were evaluated for various biological activities. In the cytotoxic assay, both compounds were inactive, at the highest concentration of 10 μM, on the cell lines of HL-60, K562, MGC-803, BEL-7402, SH-SY5Y, HCT-116, MDA-MB-231, A549, MCF-7/ADM, HO8910, U87 and NCI-H1975. Interestingly, in the immunological assay, sarinfacetamide A (**1**) and nanolobatin B (**3**) were found to moderately promote the ConA-induced T lymphocytes at 10 μM with the proliferation rate of 36.18% and 36.32%, respectively.

In conclusion, although the Chinese soft coral *S. infundibuliforme* has been previously chemically investigated, the diterpenoids **1**–**3** are all isolated and characterized in this collection of the same species for the first time. Further, **1** and **2** are the only two members of diterpenoids with uncommon tricyclo[6.3.1.$0^{1,5}$]dodecane scaffold containing acetamide group. It is worth to point out that, actually, there are a few sesquiterpenes containing tricyclo[6.3.1.$0^{1,5}$]dodecane core which have been previously reported,[7] but their biogenetic pathways are completely different from that of the xeniaphyllane-type diterpene related compounds **1** and **2**. Therefore, the discovery of sarinfacetamides A and B not only enriched the chemical diversity and complexity of marine diterpenoids, but also would stimulate the further biomimetic or total synthetic studies, due to their intriguing and unique structural features and interesting bioactivities, so as to understand their real biogenetic origins and to deeply investigate their biological functions, as well as the ecological roles that they may play in the life cycle of the soft coral.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

General information of the experiment, experimental procedures, characterization data, biological activity assays, and NMR spectra for all the new compounds (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: xwli@simm.ac.cn; ywguo@simm.ac.cn

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Liang, L.-F.; Guo, Y.-W. *Chem. Biodivers.* **2013**, *10*, 2161–2196.

(2) (a) Wang, C.-Y.; Chen, A.-N.; Shao, C.-L.; Li, L.; Xu, Y.; Qian, P.-Y. *Biochem. Syst. Ecol.* **2011**, *39*, 853–856. (b) Sun, X.-P.; Wang, C.-Y.; Shao, C.-L.; Li, L.; Li, X.-B.; Chen, M.; Qian, P.-Y. *Nat. Prod. Commun.* **2010**, *5*, 1171–1174. (c) Li, L.; Wang, C.-Y.; Shao, C.-L.; Guo, Y.-W.; Li, G.-Q.; Sun, X.-P.; Han, L.; Huang, H.; Guan, H.-S. *Helv. Chim. Acta* **2009**, *92*, 1495–1502.

(3) (a) Ye, F.; Zhu, Z.-D.; Chen, J.-S.; Li, J.; Gu, Y.-C.; Zhu, W.-L.; Li, X.-W.; Guo, Y.-W. *Org. Lett.* **2017**, *19*, 4183–4186. (b) Liang, L.-F.; Kurtán, T.; Mándi, A.; Yao, L.-G.; Li, J.; Zhang, W.; Guo, Y.-W. *Org. Lett.* **2013**, *15*, 274–277. (c) Yan, X.-H.; Gavagnin, M.; Cimino, G.; Guo, Y.-W. *Tetrahedron Lett.* **2007**, *48*, 5313–5316. (d) Liang, L.-F.; Kurtán, T.; Mándi, A.; Gao, L.-X.; Li, J.; Zhang, W.; Guo, Y.-W. *Eur. J. Org. Chem.* **2014**, *2014*, 1841–1847. (e) Kurtán, T.; Jia, R.; Li, Y.; Pescitelli, G.; Guo, Y.-W. *Eur. J. Org. Chem.* **2012**, *2012*, 6722-6728.

(4) Ahmed, A. F.; Su, J.-H.; Shiue, R.-T.; Pan, X.-J.; Dai, C.-F.; Kuo, Y.-H.; Sheu, J.-H. *J. Nat. Prod.* **2004**, *67*, 592–597.

(5) (a) Li, X.-L.; Kurtán, T.; Hu J.-C.; Mándi, A.; Li, J.; Li, X.-W.; Guo, Y.-W. *J. Agric. Food Chem.* **2017**, *65*, 1550–1555. (b) Zhou, Z.-F.; Kurtán, T.; Yang, X.-H.; Mándi, A.; Geng M.-Y.; Ye, B.-P.; Tagliatela-Scafati, O.; Guo, Y.-W. *Org. Lett.* **2014**, *16*, 1390–1393.

(6) MacroModel, Schrödinger LLC, 2015-4; https://www.schrodinger.com/suites, accessed December 1, 2017.

(7) (a) Kuo, Y.-H.; Pu, F.-S.; Lin, Y.-T. *J. Chin. Chem. Soc.* **1977**, *24*, 141–142. (b) Chung, H.-M.; Su, J.-H.; Hwang, T.-L.; Li, J.-J.; Chen, J.-J.; Chen, Y.-H.; Chang, Y.-C.; Su, Y.-D.; Chen, Y.-H.; Fang, L.-S.; Sheu, J.-H.; Wang, W.-H.; Sung, P.-J. *Tetrahedron* **2013**, *69*, 2740–2744. (c) Meng, L.-H.; Li, X.-M.; Liu, Y.; Wang, B.-G. *Org. Lett.* **2014**, *16*, 6052–6055.