

# Scopariusins, A New Class of *ent*-Halimane Diterpenoids Isolated from *Isodon scoparius*, and Biomimetic Synthesis of Scopariusin A and Isoscoparin N

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## ABSTRACT



Scopariusins A–C (1–3), three novel rearranged *ent*-halimanoids with a bicycle[5.4.0]undecane ring system, two new normal *ent*-halimanoids (4 and 5), and a new *ent*-clerodanoid (6) were isolated from *Isodon scoparius*. Moreover, a biomimetic transformation from the *ent*-clerodanoid to the normal and the rearranged *ent*-halimane diterpenoids was successfully accomplished, which not only validated the biogenetic hypothesis in this plant but also confirmed the absolute configurations of 1 and 5.

The *Isodon* (Lamiaceae) genus has attracted a lot of attention as a prolific source of diterpenoids with diverse structures and biological properties.<sup>1</sup> More than 700 new diterpenoids, including *ent*-kauranes, *ent*-pimaranes, *ent*-isopimaranes, isopimaranes, *ent*-abietanes, abietanes, *ent*-atisanes, *ent*-clerodanes, *ent*-labdanes, and so on, have been isolated from this genus over the past 30 years.<sup>1</sup> Recent phytochemical studies on this genus resulted in

the discoveries of several novel diterpenoids,<sup>2</sup> such as ternifolide A with an unusual 10-membered lactone ring,<sup>2a</sup> neolaxiflorin A possessing a bicycle[3.1.0]hexane unit,<sup>2b</sup> and neoadenolide A, a new diterpene C-glycoside with a unique C<sub>26</sub> framework.<sup>2c</sup>

*Isodon scoparius* (C. Y. Wu and H. W. Li) H. Hara, a rare herb, growing in the rocky mountains of the northwest district of Yunnan Province, P. R. China, has been used as an antipyretic agent by local inhabitants.<sup>3</sup> Previous phytochemical studies of this herb revealed that the main constituents were found to be two types of bicyclic diterpenoids: *ent*-clerodane and *ent*-labdane.<sup>4</sup> Surprisingly, most of the *Isodon* species (more than 60 species) which have been studied mainly contained

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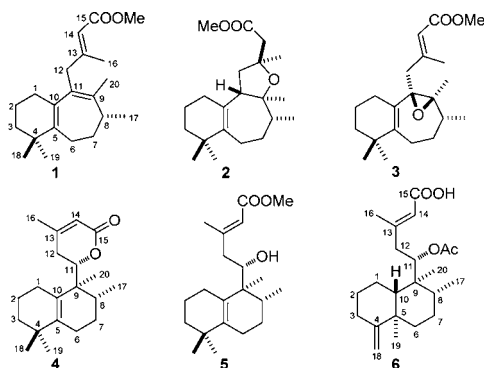
(1) (a) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673–698. (b) Sun, H. D.; Xu, Y. L.; Jiang, B. *Diterpenoids from Isodon Species*; Science Press: Beijing, 2001.

(2) (a) Zou, J.; Du, X.; Pang, G.; Shi, Y. M.; Wang, W. G.; Zhan, R.; Kong, L. M.; Li, X. N.; Li, Y.; Pu, J. X.; Sun, H. D. *Org. Lett.* **2012**, *14*, 3210–3213. (b) Wang, W. G.; Du, X.; Li, X. N.; Wu, H. Y.; Liu, X.; Shang, S. Z.; Zhan, R.; Liang, C. Q.; Kong, L. M.; Li, Y.; Pu, J. X.; Sun, H. D. *Org. Lett.* **2012**, *14*, 302–305. (c) Zhao, W.; Wang, W. G.; Li, X. N.; Du, X.; Zhan, R.; Zou, J.; Li, Y.; Zhang, H. B.; He, F.; Pu, J. X.; Sun, H. D. *Chem. Commun.* **2012**, *48*, 7723–7725.

(3) Academia Sina. *Botany of China*; Science Publishing House: Beijing, 1979; Vol. 66, p 472.

(4) Xiang, W.; Li, R. T.; Song, Q. T.; Na, Z.; Sun, H. D. *Helv. Chim. Acta* **2004**, *87*, 2860–2865.

tetracyclic or tricyclic diterpenoids, while *I. scoparius* was the only exceptional case.<sup>1a</sup> This finding prompted us to systematically reinvestigate the aerial parts of this special species. As a result, scopariusers A–C (**1–3**), three bicyclic diterpenoids with a unique bicyclo[5.4.0] undecane ring system, which might be biosynthetically formed from *ent*-halimane diterpenoids by migration of the C-9–C-10 bond to the C-11 as the key step, were discovered. What's more, isoscoparins M and N (**4** and **5**), two new normal *ent*-halimane diterpenoids with an internal  $\Delta^{5,10}$  olefin, and a new *ent*-clerodanoid, isoscoparin O (**6**), were also obtained from the title herb.



Interestingly, the chemical features of the isolated diterpenoids **1–6** indicated that the normal and the rearranged *ent*-halimanes appeared to be biosynthetically related to the *ent*-clerodanes in this plant. In order to prove this point, a biomimetic synthesis was successfully accomplished, which also confirmed the absolute configurations of diterpenoids **1** and **5**. Herein, we describe the structure elucidation of compounds **1–6** and the biomimetic synthesis of scopariuserin A (**1**) and isoscoparin N (**5**) starting from isoscoparin O (**6**).

Scopariuserin A (**1**) was isolated as a colorless oil. Its molecular formula  $C_{21}H_{32}O_2$  was determined on the basis of the HREIMS at  $m/z$  316.2401  $[M]^+$  (calcd 316.2402), corresponding to 6 degrees of unsaturation. The  $^{13}C$  NMR and DEPT spectra resolved the 21 carbon signals (Table 1) as six methyls (including one oxygenated), six methylenes, two methines (of which one was  $sp^2$  methine), and seven quaternary carbons (including five  $sp^2$  carbons and one carbonyl). Among them, one carbonyl carbon and six olefinic carbons occupied four degrees of unsaturation. These data suggested that scopariuserin A was a diterpenoid with a dicyclic ring system. Analysis of the  $^1H$ – $^1H$  COSY spectrum of **1** exhibited two structural fragments [**a**:  $-CH_2-$  (1)– $CH_2$ (2)– $CH_2$ (3)–; **b**:  $-CH_2$ (6)– $CH_2$ (7)– $CH$ (8)– $CH_3$  (17)] (Figure 1). Then, the HMBC spectrum was applied to assemble the two subunits with the quaternary carbons and other functionalities. In fragment **a**, the HMBC correlations from protons of  $H_2$ -2 to an olefinic carbon at  $\delta_C$  132.6 (s, C-10) led to the C-1 and C-10 linkage. Meanwhile, the correlations from  $H_2$ -2 to an  $sp^3$  quaternary carbon at  $\delta_C$  35.1 (s, C-4) and  $H_2$ -3 to another olefinic carbon at  $\delta_C$  142.9 (s, C-5) indicated that C-3 was linked with C-5 through C-4. These data revealed that C-1, C-2, C-3, C-4, C-5, and C-10 formed a six-membered carbon ring A.

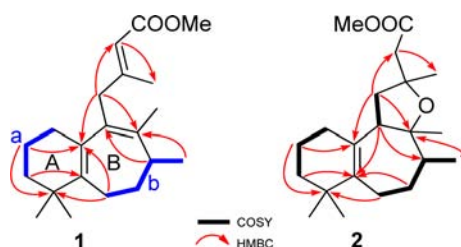
**Table 1.**  $^{13}C$  NMR Spectroscopic Data ( $\delta$  in ppm)<sup>a</sup> of **1–5** in  $CDCl_3$  and **6** in  $C_5D_5N$

no.	1	2	3	4	5	6
1	28.0 t	29.6 t	25.5 t	28.4 t	25.8 t	24.1 t
2	20.0 t	19.9 t	19.4 t	20.0 t	19.9 t	28.5 t
3	39.7 t	39.4 t	39.4 t	40.0 t	39.7 t	33.7 t
4	35.1 s	35.3 s	34.7 s	34.9 s	34.9 s	160.3 s
5	142.9 s	141.9 s	138.3 s	139.6 s	141.7 s	40.8 s
6	26.5 t	28.1 t	24.5 t	23.7 t	21.6 t	37.5 t
7	45.8 t	32.7 t	30.5 t	26.6 t	26.6 t	28.8 t
8	35.4 d	49.1 d	38.0 d	33.8 d	31.9 d	37.9 d
9	138.4 s	85.3 s	65.7 s	44.2 s	45.6 s	44.6 s
10	132.6 s	129.7 s	128.7 s	129.3 s	128.3 s	48.8 d
11	131.4 s	50.3 d	67.2 s	81.8 d	75.1 d	76.9 d
12	41.2 t	39.9 t	42.8 t	30.8 t	42.7 t	42.1 t
13	159.6 s	79.0 s	156.4 s	158.2 s	159.2 s	155.7 s
14	115.1 d	47.0 t	118.9 d	116.4 d	117.1 d	120.8 d
15	167.8 s	171.9 s	170.0 s	165.8 s	167.3 s	169.1 s
16	19.4 q	29.3 q	20.6 q	23.4 q	19.3 q	19.0 q
17	18.0 q	17.8 q	16.1 q	16.7 q	17.5 q	17.1 q
18	28.4 q	27.4 q	27.0 q	28.0 q	28.7 q	103.8 t
19	30.2 q	28.1 q	28.4 q	29.4 q	28.9 q	21.0 q
20	14.7 q	16.0 q	12.4 q	16.6 q	20.4 q	13.7 q
OMe	51.0 q	51.7 q	51.1 q	—	51.0 q	—
OAc	—	—	—	—	—	171.2 s
	—	—	—	—	—	21.1 q

<sup>a</sup>Data of compounds **1–6** were recorded at 125 MHz.

In fragment **b**, the correlations from  $H_2$ -6 to C-4 and C-10 and from  $H_2$ -7 to C-5 established the linkage from C-5 to C-6. In turn,  $H_2$ -7 and a methyl (q, C-17) showed HMBC correlations to an  $sp^2$  quaternary carbon at  $\delta_C$  138.4 (s, C-9), and H-8 showed a critical correlation to another  $sp^2$  quaternary carbon at  $\delta_C$  131.4 (s, C-11), which suggested the connection between C-8 from fragment **b** and C-11 by C-9. Furthermore, the correlations from  $H_2$ -12 to C-9 and C-11 revealed that C-5 to C-11 constructed a seven-membered carbon ring B fused to ring A at C-5 and C-10. Similarly, the HMBC correlations from  $H_2$ -12 to C-13 and from H-14 to C-13, C-15, and C-16 easily established the connection of the side chain (C-12–C-16). Therefore, compound **1** should have a novel 10(9→11) *abeo-ent*-halimane skeleton with an unusual bicyclo[5.4.0] undecane ring system. However, we failed to obtain the single crystal of **1** for determining its absolute configuration. Finally, the transformation from **6** to **1** was successfully mimicked to confirm the absolute configuration of **1** as 8*R*. Thus, compound **1** was elucidated as methyl 10(9→11) *abeo-ent*-halima-5(10),9(11), 13*E*-triene-15-oate and named as scopariuserin A.

Scopariuserin B (**2**), obtained as a colorless oil, gave the molecular formula  $C_{21}H_{34}O_3$  from HREIMS, indicating 5 degrees of unsaturation. The HMQC spectrum resolved the 21 carbon signals, which was consistent with the carbon skeleton of compound **1**. Analyses of its 1D and 2D NMR spectra revealed that **2** was similar to **1**, except for the presence of a tetrahydrofuran moiety and the absence of two double bonds in **2**. This implied that **2** might be



**Figure 1.** Key COSY and HMBC correlations of **1** and **2**.

biosynthetically related with **1** and most likely via hydroxylations on C-9 and C-13 of **1**, followed by an etherification between the two hydroxyls to form **2** (Scheme 1). The relative stereochemistry of **2** was assigned from ROESY experiment, in which the cross peaks of H-8/H-11, H-11/H-12 $\beta$ , and H<sub>2</sub>-14/H-8, H-12 $\beta$  indicated that H-8, H-11, H-12 $\beta$ , and H<sub>2</sub>-14 possessed the same orientation, while H-12 $\alpha$ , Me-16, Me-17, and Me-20 were in the opposite orientation (Figure S2). According to the absolute configuration of **1**, we supposed that **2** might have the absolute configuration of 8*R*, 9*S*, 11*S*, and 13*S* on biogenetic grounds.

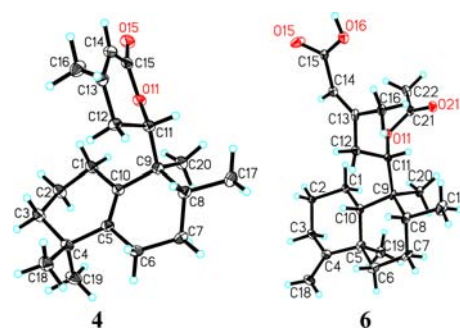
The 1D and 2D NMR spectra of **3**, together with its molecular formula information, indicated that compound **3** resembled **1**, but with the presence of an epoxy ring between C-9 and C-11 instead of a double bond at the same position. The relative configuration of **3** was assigned by the ROESY correlations. The cross peaks of H<sub>3</sub>-17/H<sub>3</sub>-20 and H<sub>3</sub>-20/H<sub>2</sub>-12 indicated that Me-17, Me-20, and H<sub>2</sub>-12 possessed the same orientation (Figure S2). The absolute configuration of **3** was also established as 8*R*, 9*S*, and 11*R* on the basis of the biogenetic pathway.

The <sup>13</sup>C NMR and DEPT spectra of **4** displayed 20 carbon signals corresponding to five methyls, six methylenes, three methines (including one oxygenated and one olefinic carbon), and six quaternary carbons (of which one was carbonyl). This was consistent with a skeleton of a halimane diterpenoid. The presence of a six-membered  $\beta$ -methyl- $\alpha,\beta$ -unsaturated lactone ring was deduced by detailed analysis of HMBC correlations of **4**. Its absolute configuration was determined by single crystal X-ray diffraction analysis (Figure 2).

The <sup>1</sup>H and <sup>13</sup>C NMR spectra showed that isoscoparin N (**5**) was similar to **4**, except for the presence of a carbomethoxy group at the C-15 in **5**. This indicated that **4** underwent opening of the  $\alpha,\beta$ -unsaturated lactone ring and then a methyl esterification of C-15 to generate **5**. The synthesis of **5** has been achieved in three steps from its potential precursor, isoscoparin O (**6**), which accessed unambiguously its absolute configuration.

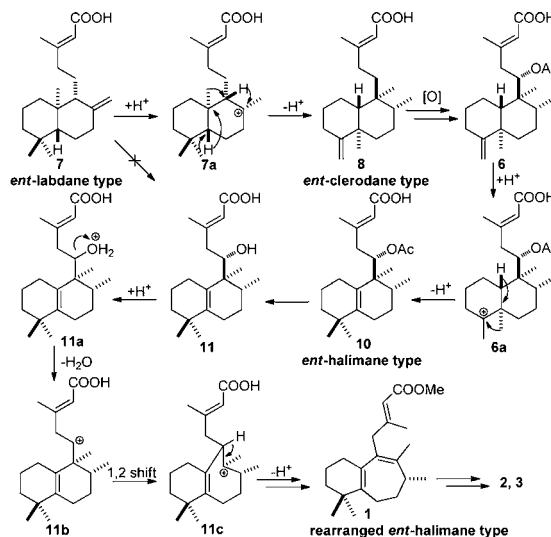
The 1D and 2D NMR spectra showed that isoscoparin O (**6**) had the same planar structure with isoscoparin A<sup>4</sup>. Its absolute configuration was determined by single crystal X-ray diffraction analysis (Figure 2).

Generally speaking, *ent*-halimanoids are very rare in nature, which appear to arise from an intermediate in



**Figure 2.** X-ray crystal structures of **4** and **6**.

**Scheme 1.** Plausible Biogenetic Pathway of the *ent*-Halimane and 10(9→11)*abeo*-*ent*-Halimane Diterpenoids in This Plant



the biosynthetic transformation of *ent*-labdanes into *ent*-clerodanes.<sup>5</sup> However, an in vitro biomimetic synthesis of them has never been achieved.<sup>6</sup> As far as we know, *ent*-clerodanes and *ent*-halimanes were often isolated from the same plants in the absence of *ent*-labdanes,<sup>7</sup> which suggested that many *ent*-halimanes might be directly derived from *ent*-clerodanes rather than from *ent*-labdanes in some cases. In this study, we found that the isolated *ent*-halimanes (**4** and **5**) possessing the 8*R*, 9*S*, and 11*S* configuration had the same configuration as the C-8-nonoxygenated and C-11-oxygenated *ent*-clerodanes (like **6**, accounting for 5% of the total crude extract) which were isolated as the major constituents from this plant. Meanwhile, we did not

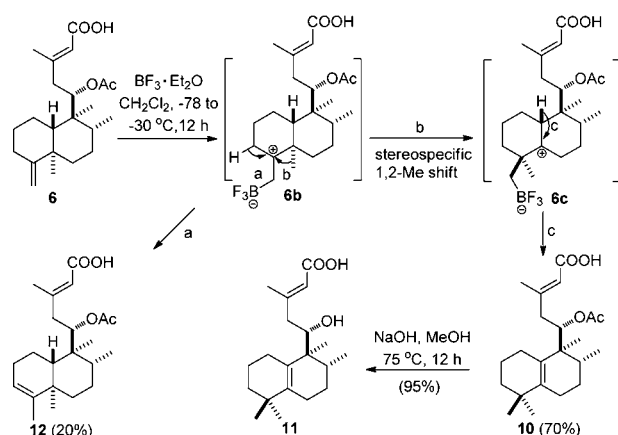
(5) (a) Merritt, A. T.; Ley, S. V. *Nat. Prod. Rep.* **1992**, *9*, 243–287. (b) Peters, R. J. *Nat. Prod. Rep.* **2010**, *27*, 1521–1530.

(6) George, J. H.; McArdle, M.; Baldwin, J. E.; Adlington, R. M. *Tetrahedron* **2010**, *66*, 6321–6330.

(7) (a) Nagashima, F.; Tanaka, H.; Kan, Y.; Huneck, S.; Asakawa, Y. *Phytochemistry* **1995**, *40*, 209–212. (b) Chen, C. Y.; Chang, F. R.; Shih, Y. C.; Hsieh, T. J.; Chia, Y. C.; Tseng, H. Y.; Chen, H. C.; Chen, S. J.; Hsu, M. C.; Wu, C. Y. *J. Nat. Prod.* **2000**, *63*, 1475–1478.

obtain the C-11-oxygenated *ent*-labdanes. Furthermore, the 10(9→11)*abeo-ent*-halimanes were biosynthesized through acid-catalyzed rearrangement of the C-11-oxygenated *ent*-halimane derivatives, in which the oxygenated substituents at C-11 were indispensable for the transformation (Scheme 1). It might indicate that the *ent*-halimanes as well as the 10(9→11)*abeo-ent*-halimanes had a closer biogenetic relationship with *ent*-clerodanes than *ent*-labdanes in this plant. If the biomimetic synthesis of the *ent*-halimanes and the 10(9→11)*abeo-ent*-halimanes could be achieved from *ent*-clerodanes in the laboratory, it could not only confirm the biogenetic hypothesis but also provide evidence for determining the absolute configurations of compounds **1**–**5**. Thus, we turned our attention to the bioinspired synthesis of scopariusin A (**1**) and isoscoparin N (**5**).

**Scheme 2.** Synthesis of *ent*-Halimane Diterpenoid **11** from **6**



The absolute configuration of the possible precursor **6**, a main *ent*-clerodane derivative in this species, was established by X-ray analysis (Figure 2), and it was converted by treatment with  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  in  $\text{CH}_2\text{Cl}_2$  cooled at  $-78^\circ\text{C}$  and further stirring at  $-30^\circ\text{C}$  for 12 h to produce **10** and **12**.<sup>6,8</sup> The rearranged product **10** was treated with concentrated aq NaOH solution in MeOH at  $75^\circ\text{C}$  for 12 h to give **11** (Scheme 2).<sup>9</sup> And then the deacetylated product **11** was methylated with  $\text{CH}_3\text{I}$  in  $\text{K}_2\text{CO}_3$ –acetone to afford **5** (Scheme 3).<sup>10</sup>

From a biosynthetic viewpoint, the 10(9→11)*abeo-ent*-halimanes appeared to be related with the C-11-oxygenated normal *ent*-halimanes which underwent a Wagner–Meerwein rearrangement via selective migration of the more electron-rich double bond between C-9 and C-10 instead of the C-8–C-9 bond.<sup>11</sup> With **11** in hand, the key biomimetic ring-expansion reaction could be studied.

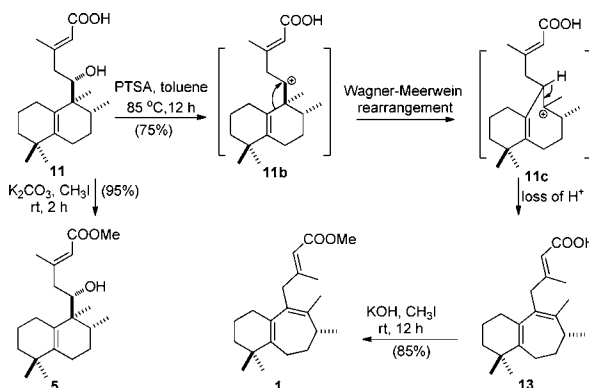
(8) Kuan, K. K. W.; Pepper, H. P.; Bloch, W. M.; George, J. H. *Org. Lett.* **2012**, *14*, 4710–4713.

(9) Kulcinski, V.; Ungur, N.; Gavagnin, M.; Carbone, M.; Cimino, G. *Eur. J. Org. Chem.* **2005**, 1816–1822.

(10) Abdel-Kader, M.; Berger, J. M.; Slebodnick, C.; Hoch, J.; Malone, S.; Wisse, J. H.; Werkhoven, M. C. M.; Mamber, S.; Kingston, D. G. I. *J. Nat. Prod.* **2002**, *65*, 11–15.

(11) (a) Mantegani, S.; Arlandini, E.; Borghi, D.; Brambilla, E.; Varasi, M. *Heterocycles* **1997**, *45*, 1493–1507. (b) Li, S. H.; Wang, J.; Niu, X. M.; Shen, Y. H.; Zhang, H. J.; Sun, H. D.; Li, M. L.; Tian, Q. E.; Lu, Y.; Cao, P.; Zheng, Q. T. *Org. Lett.* **2004**, *6*, 4327–4330.

**Scheme 3.** Synthesis of Isoscoparin N (**5**) and Scopariusin A (**1**)



Unfortunately, treatment of **11** with TFA or with  $\text{POCl}_3$  was unsuccessful.<sup>12</sup> Finally, **11** was successfully transformed into **13** in 75% yield after treatment with *p*-toluenesulfonic acid (PTSA) in toluene at  $85^\circ\text{C}$  for 12 h,<sup>13</sup> and **13** was further methylated with  $\text{CH}_3\text{I}$  in KOH–acetone to afford **1** (Scheme 3).<sup>10</sup> Spectroscopic data of **1** and **5** were identical to those isolated from *I. scoparius*. Thus, these results provided experimental validation for the biogenetic hypothesis that the precursors of *ent*-halimanes as well as rearranged *ent*-halimanes should be indeed the *ent*-clerodanes rather than *ent*-labdanes in this plant. Furthermore, the absolute configuration of C-8 did not change during the synthesis, which also allowed determination of the absolute configurations of **1** and **5**.

Using the MTT method, compounds **1**–**6** were tested for cytotoxicity in human cancer cell lines: A-549, HL-60, MCF-7, SMMC-7721, and SW-480.<sup>14</sup> Unfortunately, no activity was detected ( $\text{IC}_{50} > 40 \mu\text{M}$ ).

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**Supporting Information Available.** Experimental procedures; 1D and 2D NMR, MS, IR spectra of compounds **1**–**6** and **10**–**13**; and X-ray crystal structures of **4** and **6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

(12) (a) George, J. H.; Baldwin, J. E.; Adlington, R. M. *Org. Lett.* **2010**, *12*, 2394–2397. (b) Alvarez-Manzaneda, E.; Chahboun, R.; Alvarez, E.; Cano, M. J.; Haidour, A.; Alvarez-Manzaneda, R. *Org. Lett.* **2010**, *12*, 4450–4453.

(13) (a) Srikrishna, A.; Satyanarayana, G.; Kumar, P. R. *Tetrahedron Lett.* **2006**, *47*, 363–366. (b) Leonelli, F.; Blesi, F.; Diritto, P.; Trombetta, A.; Ceccacci, F.; Bella, A. L.; Migneco, L. M.; Bettolo, R. M. *J. Org. Chem.* **2011**, *76*, 6871–6876.

(14) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

The authors declare no competing financial interest.