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Rhodomyrtials A and B, Two Meroterpenoids with a Triketone-Sesquiterpene-Triketone Skeleton from *Rhodomyrtus tomentosa*: Structural Elucidation and Biomimetic Synthesis

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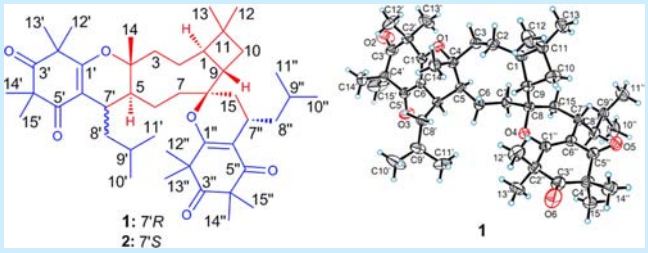
Supporting Information

ABSTRACT: Rhodomyrtials A and B (**1** and **2**), two unprecedented triketone-sesquiterpene-triketone adducts, along with five biogenetically related intermediates, rhodomentone A (**3**) and tomentodiones A–D (**4**–**7**), were isolated from the leaves of *Rhodomyrtus tomentosa*. Their structures and absolute configurations were determined by a combination of NMR spectroscopy, chemical conversion, and X-ray diffraction analysis. Compounds **1** and **2** were biomimetically synthesized via **5** and **4**, respectively, rather than **3**, revealing their key ordering of biosynthetic events and confirming their structural assignments. Compound **7** exhibited potent metastatic inhibitory activity against DLD-1 cells by suppressing the activation of matrix metalloproteinase (MMP)-2 and MMP-9.



Plants from the family Myrtaceae have yielded a number of meroterpenoids that display diverse structure and a broad spectrum of biological activities.[1] This class of meroterpenoids is characterized by a phloroglucinol moiety coupled with a terpenoid unit. Recently, their unique structures and potential therapeutic applications, especially *Psidium* meroterpenoids, have provoked considerable interests in isolation and synthetic efforts.[2,3] In continuing the search for structurally diverse meroterpenoids from natural origin and investigation of their intriguing biogenetic pathway,[4] we throw our sight to *Rhodomyrtus tomentosa* (Aiton) Hassk., an evergreen shrub, which is the only *Rhodomyrtus* species in China.[5] Its stems, leaves, and fruits are used as traditional Chinese medicines to treat chronic dysentery, rheumatism, hepatitis, and hyperlipemia.[5] Previous chemical investigations of the leaves of the title plant have led to the isolation of two hexacyclic acylphloroglucinol derivatives.[6]

In our current study, two unique meroterpenoids, rhodomyrtials A and B (**1** and **2**; Figure 1), possessing an unprecedented triketone-sesquiterpene-triketone framework constructed from the conjugation of two-1,1,3,3-tetramethyl-isopentyl cyclohexatrione and caryophyllene moieties, along with five biosynthetically related intermediates, rhodomentone A (**3**) and tomentodiones A–D (**4**–**7**), were isolated from the leaves of *R. tomentosa*. Biosynthetically, compounds **1** and **2** are hypothesized to arise from the hetero-Diels–Alder (HDA) reaction, starting from leptospermone and caryophyllene. Strikingly, the coisolated **3**–**5** inspired two plausible biosynthetic pathways to compounds **1** and **2**. Herein, we describe the structural elucidation and biomimetic synthesis of **1** and **2** to provide experimental support for their proposed biosynthesis. In addition, the potent metastatic inhibitory activity of **7** against DLD-1 cells is shown.

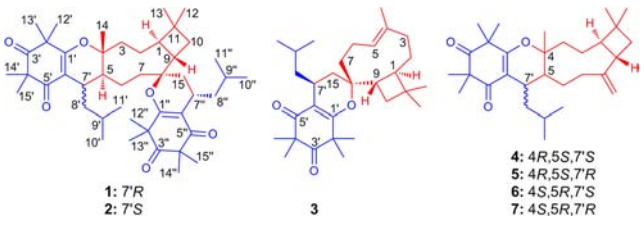


Figure 1. Structures of compounds **1**–**7**.

Rhodomyrtial A (**1**) was obtained as colorless needle crystals. Its molecular formula, $C_{45}H_{68}O_6$, was determined by high-resolution ESIMS (*m*/*z* 705.5084 $[M + H]^+$; calcd: 705.5089). The ^{13}C NMR and DEPT spectra of **1** revealed the presence of 45 carbon signals, including four ketonic, 11 quaternary, seven methinic, eight methylenic, and 15 methylic carbons. Comprehensive analysis of the 1D- and 2D-NMR data of **1** revealed the presence of two identical 1,1,3,3-tetramethylcyclohexatrione units (Table S1), two isopentyl groups, and three additional singlet methyls. The aforementioned spectroscopic data implied that **1** might be a double triketone-coupled sesquiterpenoid.

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Further detailed 2D NMR spectroscopic analysis enabled the construction of the structure of **1** (Figure 2A). The ^{1}H−^{1}H

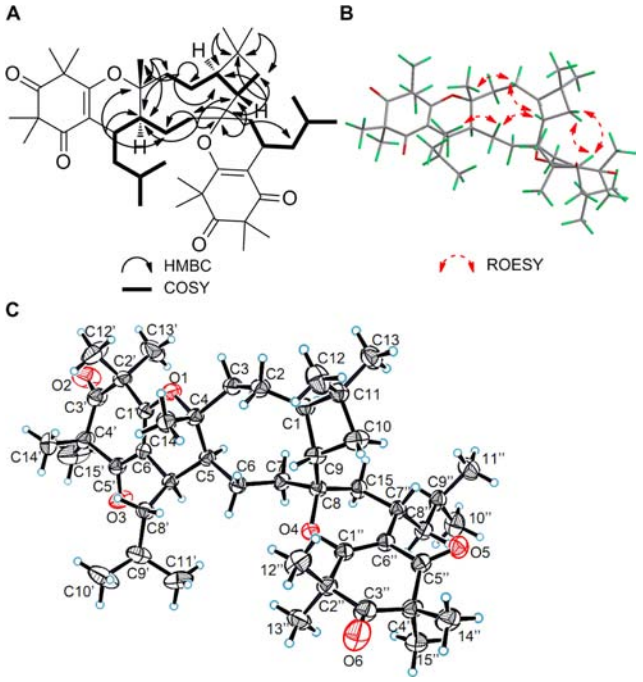


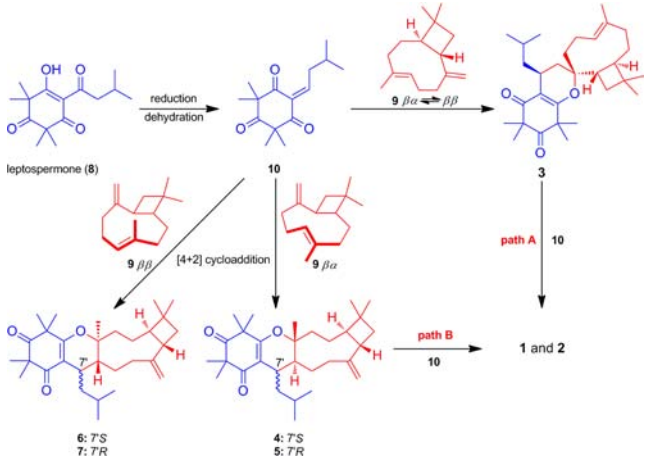
Figure 2. (A) Key ^{1}H−^{1}H COSY and HMBC correlations, (B) key ROESY correlations, and (C) X-ray single-crystal structure of **1**.

COSY correlations of H-1/H-9/H-10 and the HMBC correlations from H_3-12 to C-11, C-1, and C-10, from H_3-13 to C-11, C-1, and C-10, from H-9 to C-1, C-10, and C-11 suggested the presence of a cyclobutane ring characteristic for caryophyllene. The caryophyllene moiety was further supported by the ^{1}H−^{1}H COSY correlations of H-1/H-2/H-3 and H-5/H-6/H-7, and the HMBC correlations from H_3-14 to C-3, C-4, and C-5, from H-3 to C-1, C-2, C-4, C-5, and C-14, and from H-7 to C-5, C-6, C-8, and C-9. Furthermore, the HMBC correlations from H-7′ to C-4, C-5, and C-6 indicated that one triketone unit was fused to the caryophyllene moiety at C-4 and C-5 forming a dihydropyran ring, while the HMBC correlations from H-15 to C-7″, C-8″, C-7, C-8, and C-9, and from C-8 to H-7″, H-7, H-9, and H-15 revealed that another triketone unit was fused to the caryophyllene moiety at C-8 forming an oxaspiro ring. Finally, the relative and absolute configurations of **1** were unambiguously determined by ROESY (Figure 2B) and X-ray crystallographic experiments (Figure 2C, CCDC 1479660). Thus, the full structure of this novel meroterpenoid named rhodomyrtial A was elucidated. This is the first example of triketone-sesquiterpene-triketone featuring a unique 1-oxaspiro[5,8]tridecane motif.

Along with **1**, another triketone-caryophyllene-triketone adduct, rhodomyrtial B (**2**), as well as five triketone-coupled caryophyllenes (**3**−**7**), were isolated from the title plant. The planar structure and relative configuration of **2** were determined by spectroscopic data, while the absolute configuration tentatively remained unknown due to the absence of a qualified crystal. Fortunately, the structures with absolute configurations of **3**−**7** were completely elucidated by spectroscopic data, chemical conversion, and X-ray crystallography (for details of the structural elucidations of **2**−**7**, see Supporting Information). Coincidentally, **3**−**5** were also isolated from the title plant by Qiu et al. during the preparation and submission of this letter.[7]

The plausible biosynthetic pathway for these compounds is depicted in Scheme 1. Leptospermone (**8**) and caryophyllene

Scheme 1. Plausible Biogenetic Pathway for 1−7



(**9**) were considered as the biosynthetic precursors of compounds **1**−**7**. The co-occurrence of **3**−**5** inspired us to propose two possible pathways for constructing **1** and **2**. In pathway A, compounds **1** and **2** would be formed through HDA cycloaddition using **3** as a dienophile precursor. Alternatively, in pathway B, compounds **1** and **2** would be constructed through HDA cycloaddition using **5** and **4** as a dienophile precursor, respectively. Due to the higher reactivity of the endocyclic double bond in **3**,[8] pathway A might be more feasible than pathway B. However, compounds **4**−**7** were proposed to be four stereoisomers derived from a [4 + 2] cycloaddition between **10** and **9**, while **3** was proposed to be a structural isomer formed in this HDA process, biosynthetically. Furthermore, **4** and **5** were proposed to be derived from the *βα* conformer of **9**, while **6** and **7** from the *ββ* conformer by comparing the spatial structures of **4**−**7** (Figure S6).[3b] For the rotation of two conformers of **3** in solvent (Figure S3), **3** was proposed to be derived from both *βα* and *ββ* conformers of **9**. To clear their biogenetic pathways and further confirm their stereochemistry, the biomimetic synthesis of **1** and **2** was subsequently conducted.

The biomimetic synthesis of **1** and **2** was conducted with commercially available phloroglucinol and caryophyllene (**9**) as substrates. Prior to synthesizing **1** and **2**, the synthesis of their biosynthetically related intermediates **3**−**7** was performed. According to the literature,[9] the requisite diene **10** can be derived from a simple Knoevenagel condensation between syncarpic acid (**11**) and isovaleraldehyde (**12**). Due to the propensity of **10** toward isomerization,[4a,10] a domino three-component one-pot reaction between **11**, **12**, and **9** would be an effective and concise synthetic strategy for **3**−**7**. With syncarpic acid (**11**) in hand,[4a] our initial attempt to synthesize **3**−**7** was made by using the condition reported by Bharate and Singh.[11] However, compounds **3**−**7** were obtained in very poor yield under this condition. Lewis and Brønsted acids have been proven to be a powerful tool for promoting Diels−Alder reactions in synthetic organic chemistry.[12] As shown in Table S5, several Lewis and Brønsted acids were investigated. To our delight, the use of 60 mol % ZnI_2 accelerated the reaction more readily, and the reaction proceeded to completion within 1 h at 110 °C with a good combined yield (Table 1, entry 5, 64%). Accordingly, this

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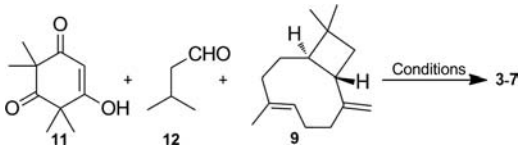
strategy is effective to synthesize **3**–**7** by the Knoevenagel condensation/HDA domino reaction.

Table 1. Biomimetic Synthesis of Compounds 3–7 Under Different Conditions



entry	catalyst	mol %	solvent	temp (°C)	time (h)	yield[a] (%)
1	NaOAc	10	CH_3COOH	80	24	6
2	NaOAc	10	CH_3COOH	80	72	31
3	$ZnCl_2$	40	toluene	110	24	30
4	$ZnCl_2$	40	toluene	110	72	56
5	ZnI_2	60	toluene	110	1	64[b]

[a]Combined isolated yield. [b]**3**/**4**/**5**/**6**/**7** = 2:3:14:12:1.

After successfully synthesizing compounds **3**–**7**, the synthesis of rhodomyrtials A and B (**1** and **2**) was subsequently conducted. Considering the higher reactivity of rhodomentone A (**3**) for cycloaddition, pathway A to access **1** and **2** was preferred to be performed. Applying the above established strategy for **3**–**7**, a one-pot procedure was used for the synthesis of **1** and **2**. Unexpectedly, none of the cycloadduct was detected in the presence of ZnI_2 though the heating time was prolonged to 7 days. After many tries, a small quantity of cycloadduct was detected when the reaction was conducted in the presence of $TiCl_4$ under N_2. However, the reaction always resulted in a low yield even though the amount of catalyst and the temperature were increased. We speculated that a molecule of H_2O eliminated from the Knoevenagel condensation step might have an influence on this reaction. To solve this problem, we decided to perform a stepwise protocol. The requisite diene **10** was obtained through the Knoevenagel condensation between syncarpic acid (**11**) with isovaleraldehyde (**12**) according to the previous literature.[9b] Subsequent Diels–Alder cycloaddition was immediately conducted since **10** was prone to isomerization. After careful optimization, two cycloadducts were obtained in a 71% combined yield in the presence of $TiCl_4$ (3 equiv) in toluene at room temperature. To our surprise, the reaction generated a new stereoisomer **13** instead of **2** with a high stereoselectivity (ca. 7:1), while **1** was obtained in only a 9% yield (Figure 3). The low selectivity to afford **1** in this [4 + 2] cycloaddition might be caused by the steric repulsion of the methyl group in the more stable conformer **3α**. However, we did not obtain any **13** from the leaves of *R. tomentosa*. In addition, the LC–MS analysis of the extraction (Figure S9) also suggested that **13** did not exist in the title plant. Consequently, pathway A is less likely to occur in nature.

Accordingly, our attention was turned to the alternative pathway B to synthesize compounds **1** and **2**. Because **5** was more accessible than **4** (Table 1), the synthesis began with **5** to afford rhodomyrtial A (**1**). The [4 + 2] cycloaddition between **5** and **10** was first performed in the presence of $TiCl_4$. Surprisingly, a novel rearranged product **14** was obtained, while the desired cycloadduct was not obviously detected (Figure 4). Subsequently, despite an extensive screening of different Lewis and Brønsted acids, reaction temperatures, and solvents in a one-pot or stepwise protocol, the cycloaddition also failed (Table S7). After many failures, compound **1** was finally obtained as a

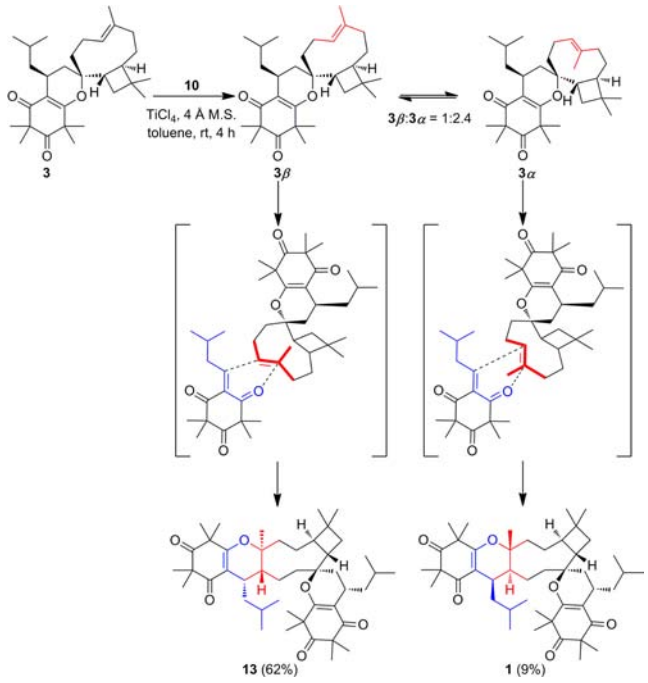


Figure 3. Biomimetic synthesis of **1** from path A.

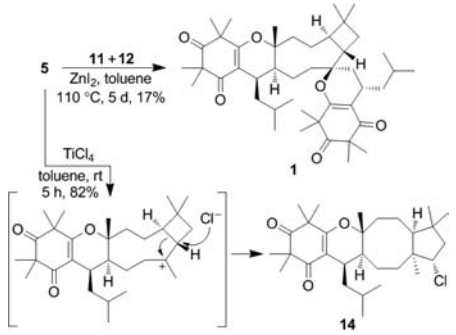


Figure 4. Biomimetic synthesis of **1** from path B.

predominant cycloadduct in a 17% yield by a one-pot manner in the presence of ZnI_2, heating at 110 °C for 5 days (Figure 4). Compound **2** was also successfully obtained from **4** in a 12% yield under similar conditions. Since the absolute configuration of **4** had been determined by single-crystal X-ray analysis, the absolute configuration of **2** was finally assigned as 1*R*, 4*R*, 5*S*, 8*R*, 9*S*, 7′*S*, and 7″*S* by combination of its successful synthesis and the key ROESY correlations from H-7″ to H_2-10 (Figure S1). Hence, compounds **1** and **2** are prone to be biosynthetically derived via pathway B.

Compounds **1**–**7** were screened for their inhibitory effects on tumor metastasis through the transwell migration assay with DLD-1 cells. Among all of the tested compounds (Figure S10), **7** exhibited preferential activity to inhibit cell migration in a dose-dependent manner (Figures S11 and S12). To better understand the potential mechanism, Western blot analysis was performed to investigate the effect of **7** on MMP-2 and MMP-9, which play crucial roles in tumor metastasis.[13] As a result, compound **7** substantially reduced 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced MMP-2 and MMP-9 activation (Figure S13). Further investigations (Figure S14) showed that the induction of Akt and ERK phosphorylation induced by TPA was blocked in the presence of **7**, but not p38 and JNK. These results suggested

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that the antimetastatic effect of **7** on DLD-1 cells may result from reduced activation of Akt and ERK induced by TPA, ultimately leading to the downregulation of MMP-2 and MMP-9 expressions.

In summary, two unique triketone-sesquiterpene-triketone adducts, rhodomyrtials A (**1**) and B (**2**), were isolated from the leaves of *R. tomentosa*, along with five biosynthetically related intermediates (**3**−**7**). Their successful biomimetic synthesis not only enabled unambiguous structural assignments but also provided experimental support for their optimal biosynthesis. Moreover, the preliminary biological studies indicated that compound **7** possessed potent tumor metastatic inhibitory activity. Our concise synthesis provides convenient access to these meroterpenoids and allows for an intensive biological exploration of these novel natural products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01944.

Details of isolation, synthesis and biological experimental procedures, detailed structure elucidation of **2**−**7**, and NMR spectra for new compounds (PDF)
Crystallographic data for **1** (CIF)
Crystallographic data for **3** (CIF)
Crystallographic data for **4** (CIF)
Crystallographic data for **5** (CIF)
Crystallographic data for **6** (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558−591. (b) Ghisalberti, E. L. *Phytochemistry* **1995**, *41*, 7−22. (c) Yin, S.; Xue, J. J.; Fan, C. Q.; Miao, Z. H.; Ding, J.; Yue, J. M. *Org. Lett.* **2007**, *9*, 5549−5552. (d) Yang, S. P.; Zhang, X. W.; Ai, J.; Gan, L. S.; Xu, J. B.; Wang, Y.; Su, Z. S.; Wang, L.; Ding, J.; Geng, M. Y.; Yue, J. M. *J. Med. Chem.* **2012**, *55*, 8183−8187.

(2) For recent isolation efforts, see: (a) Jian, Y. Q.; Huang, X. J.; Zhang, D. M.; Jiang, R. W.; Chen, M. F.; Zhao, B. X.; Wang, Y.; Ye, W. C. *Chem. - Eur. J.* **2015**, *21*, 9022−9027. (b) Gao, Y.; Wang, G. Q.; Wei, K.; Hai, P.; Wang, F.; Liu, J. K. *Org. Lett.* **2012**, *14*, 5936−5939. (c) Shao, M.; Wang, Y.; Jian, Y. Q.; Huang, X. J.; Zhang, D. M.; Tang, Q. F.; Jiang, R. W.; Sun, X. G.; Lv, Z. P.; Zhang, X. Q.; Ye, W. C. *Org. Lett.* **2012**, *14*, 5262−5265. (d) Fu, H. Z.; Luo, Y. M.; Li, C. J.; Yang, J. Z.; Zhang, D. M. *Org. Lett.* **2010**, *12*, 656−659.

(3) For selected examples, see: (a) Singh, I. P.; Sidana, J.; Bharate, S. B.; Foley, W. J. *Nat. Prod. Rep.* **2010**, *27*, 393−416. (b) Lawrence, A. L.; Adlington, R. M.; Baldwin, J. E.; Lee, V.; Kershaw, J. A.; Thompson, A. L. *Org. Lett.* **2010**, *12*, 1676−1679. (c) Tran, D. N.; Cramer, N. *Chem. - Eur. J.* **2014**, *20*, 10654−10660.

(4) (a) Wu, L.; Luo, J.; Zhang, Y. L.; Zhu, M. D.; Wang, X. B.; Luo, J. G.; Yang, M. H.; Yu, B. Y.; Yao, H. Q.; Dai, Y.; Guo, Q. L.; Chen, Y. J.; Sun, H. B.; Kong, L. Y. *Tetrahedron Lett.* **2015**, *56*, 229−232. (b) Wu, L.; Luo, J.; Wang, X. B.; Li, R. J.; Zhang, Y. L.; Kong, L. Y. *RSC Adv.* **2015**, *5*, 93900−93906.

(5) Chen, J. *Flora of China (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, 1984; Vol. *53*, pp 121−122.

(6) Hiranrat, A.; Mahabusarakam, W.; Carroll, A. R.; Duffy, S.; Avery, V. M. *J. Org. Chem.* **2012**, *77*, 680−683.

(7) (a) Liu, H. X.; Chen, K.; Tang, G. H.; Yuan, Y. F.; Tan, H. B.; Qiu, S. X. *RSC Adv.* **2016**, *6*, 48231−48236. (b) Liu, H. X.; Chen, K.; Yuan, Y.; Xu, Z. F.; Tan, H. B.; Qiu, S. X. *Org. Biomol. Chem.* **2016**, *14*, 7354.

(8) Collado, I. G.; Hanson, J. R.; Macías-Sánchez, A. J. *Nat. Prod. Rep.* **1998**, *15*, 187−204.

(9) (a) Müller, H.; Paul, M.; Hartmann, D.; Huch, V.; Blaesius, D.; Koeberle, A.; Werz, O.; Jauch, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 2045−2049. (b) Morkunas, M.; Dube, L.; Götz, F.; Maier, M. E. *Tetrahedron* **2013**, *69*, 8559−8563.

(10) Kalsi, P. S.; Singh, J.; Crow, W. D.; Chhabra, B. P. *Phytochemistry* **1987**, *26*, 3367−3369.

(11) Bharate, S. B.; Singh, I. P. *Tetrahedron Lett.* **2006**, *47*, 7021−7024.

(12) For selected examples, see: (a) Smith, C. D.; Gavrilyuk, J. I.; Lough, A. J.; Batey, R. A. *J. Org. Chem.* **2010**, *75*, 702−715. (b) Mal, K.; Das, S.; Maiti, N. C.; Natarajan, R.; Das, I. *J. Org. Chem.* **2015**, *80*, 2972−2988. (c) Li, C.; Yu, X. L.; Lei, X. G. *Org. Lett.* **2010**, *12*, 4284−4287. (d) Zhao, J. J.; Sun, S. B.; He, S. H.; Wu, Q.; Shi, F. *Angew. Chem., Int. Ed.* **2015**, *54*, 5460−5464.

(13) Pellikainen, J. M.; Ropponen, K. M.; Kataja, V. V.; Kellokoski, J. K.; Eskelinen, M. J.; Kosma, V. M. *Clin. Cancer Res.* **2004**, *10*, 7621−7628.

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