The final publication is available at

https://doi.org/10.1055/s-0036-1588353

Additional Information

A Short and Improved Synthesis of the Antiprotozoal Abietane Diterpenoid (–)-Sugikurojin A

Miguel A. González-Cardenete*[a]
Ramón J. Zaragozá*[b]

[a] Instituto de Tecnología Química (UPV-CSIC), Universitat Politècnica de Valencia-Consejo Superior de Investigaciones Científicas, Avda de los Naranjos s/n, 46022 Valencia, Spain

[b] Departamento de Química Orgánica, Universidad de Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain

migoncar@itq.upv.es; Ramon.J.Zaragoza@uv.es

Click here to insert a dedication.
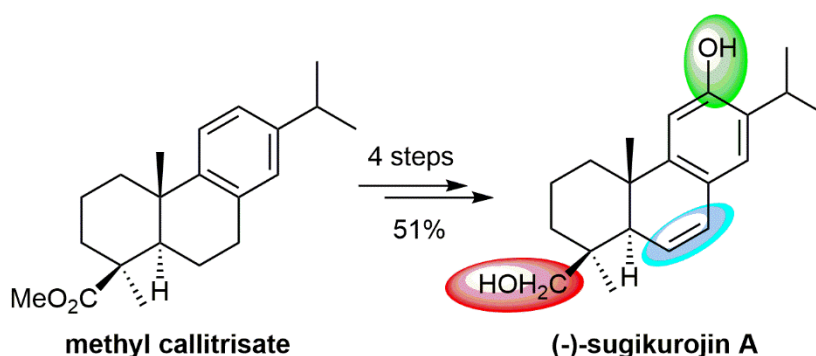
Abstract An efficient and straightforward semisynthesis of the antiprotozoal abietane diterpenoid (–)-sugikurojin A, starting from the readily available methyl ester of callitrisic acid (4-epidehydroabietic acid) isolated from sandarac resin, is reported. This optimized semisynthetic route provides a convenient source of the antiprotozoal compound in four steps with 50% overall yield from methyl callitrisate for further biological studies.

Key words Terpenoids, abietane, semisynthesis, acylation, callitrisic acid

Aromatic abietane diterpenoids are well-known natural products found in the plant kingdom, which have shown a wide range of biological activities.[1] Their synthetic derivatives have been extensively studied as potential chemotherapeutics.[2] In recent years, an interesting subgroup of abietanes bearing an unusual oxygenated group at the axial C-19 position, rather than the equatorial C-18 position, have been isolated and subjected to biological studies. For example, sugikurojin A (**1**) (Figure 1) was isolated from the Japanese cedar *Cryptomeria japonica*, which is called "sugi" in Japanese.[3] The synthetic levorotatory enantiomer of this phenolic diterpenoid has shown significant antileishmanial and trypanocidal activities.[4] The isolation of jiadifenoic acids C (**2**) and B (**3**) (Figure 1) and related metabolites has led to some biological studies aimed at their antiviral properties.[5] Other examples of bioactive C19-functionalized abietanes are the anti-inflammatory[6] majusanic acid B (**4**) and the cytotoxic[7] and immunosuppressive[8] 19-hydroxyferruginol (**5**) (Figure 1).

Recently, we reported the semisynthesis of jiadifenoic acid C (**2**) from the methyl ester (**6**) of callitrisic acid (4-epidehydroabietic acid) isolated from Moroccan sandarac resin.[9] Within this work, a new protocol for the isolation of methyl callitrisate (**6**) without the use of AgNO₃-based chromatography was developed. The accessibility to this material prompted further semisynthetic studies. Sugikurojin A (**1**) was considered an interesting synthetic target due to the interesting antiparasitic properties of the synthetic levorotatory enantiomer and limited access

from natural sources and synthetic procedures, which require lengthy multi-step syntheses. Two semisyntheses of this diterpenoid by Alvarez-Manzaneda and co-workers have been reported. One route starts from trans-communic acid and the other from abietic acid, with thirteen and nineteen steps, respectively.[10] Both semisyntheses delivered the compound with opposite specific rotation to the natural product. These lengthy synthetic methods encouraged us to develop a shorter semisynthetic route from methyl callitrisate (**6**), in order to provide (−)-sugikurojin A (**1**) efficiently for further biological studies.
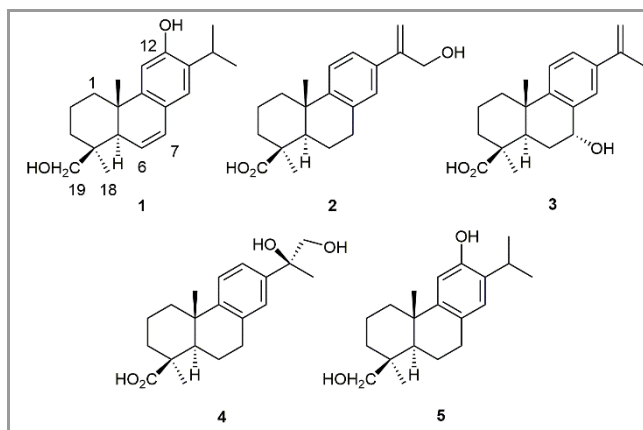
Figure 1 Examples of bioactive C19-functionalized abietane diterpenoids.

In this paper, a straightforward semisynthetic sequence to (−)-sugikurojin A (**1**) from the available methyl callitrisate (**6**), which is obtained by esterification of callitrisic acid (4-epidehydroabietic acid) isolated from Moroccan sandarac resin, is reported.

Methyl callitrisate (**6**) was selected as starting material for the semisynthesis of sugikurojin A (**1**), since it contains the aromatic ring and a C-19 oxygenated function convertible into the target molecule. Methyl callitrisate **6** was obtained from Moroccan sandarac resin following the reported protocol (Scheme 1).[9]
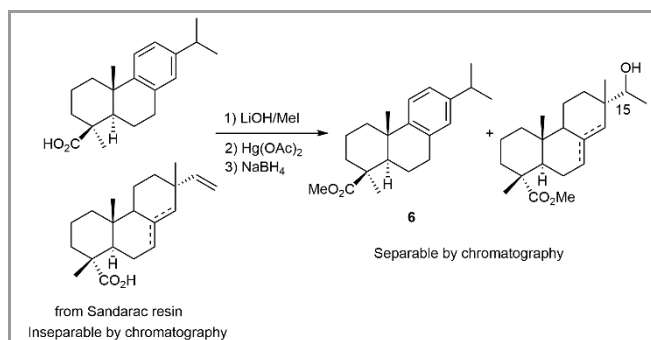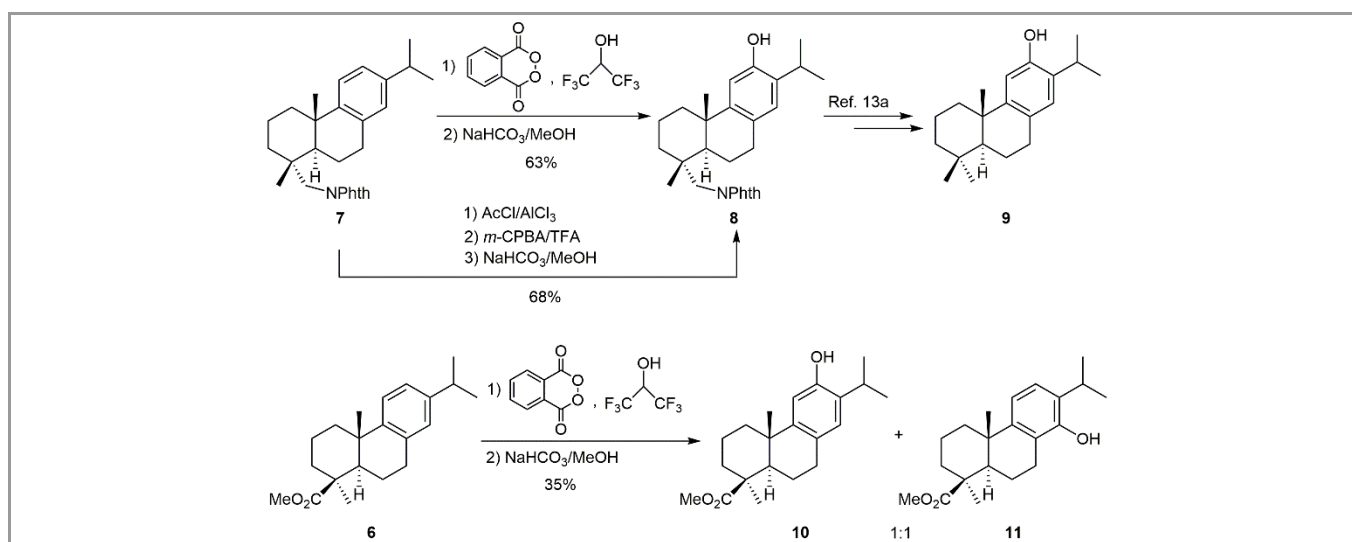
**Scheme 1** Isolation of methyl callitrisate (**6**) from Sandarac resin.

With methyl callitrisate (**6**) in hand, the focus was shifted to the synthetic target, compound **1** (sugikurojin A). This aromatic diterpenoid shows characteristic structural features such as a phenolic group, a double bond between C-6 and C-7, as well as a C-19 hydroxymethyl group. Firstly, the introduction of the phenolic moiety at C-12 was investigated. A method developed recently by Siegel and co-workers, based on arene to phenol transformation with phthaloyl peroxide, was firstly studied.[11] This new method had been successfully used with compound **7** to give phenol **8** in two steps in 63% overall yield (Scheme 2), recovering unreacted **7** in 7% yield. When we used these conditions with methyl callitrisate **6**, a poor yield of phenol **10** was obtained along with unreacted **6** (19%). Phenol **10** was accompanied by regioisomer **11** as a ca. 1:1 (35% yield) inseparable mixture as consequence of a non-regioselective reaction.

Another option involves a Friedel-Crafts acylation of the aromatic compound followed by Baeyer-Villiger oxidation.[12] In our group, there was previous experience with this methodology, which was used during our synthesis of the abietane diterpenoid (+)-ferruginol (**9**) from phthaloyl-protected dehydroabietylamine **7** (Scheme 2).[13] After some optimization changing the amount of solvent, the treatment with AcCl (3.5 equiv.) and AlCl$_3$ (3.0 equiv.) of compound **6** in 1,2-dichloroethane gave the corresponding acetyl derivative **12** in 76% yield (Scheme 3). Attempts to improve this reaction with other conditions used with the C-4 epimer (methyl dehydroabietate) of **6** were unsuccessful.[14]

Next, the Baeyer-Villiger oxidation of compound **12** with m-CPBA (2.6 equiv.) and CF$_3$CO$_2$H (1.0 equiv.) in DCM afforded the acetate **13** in almost quantitative yield. Thus, the phenolic moiety introduction was solved and then it was needed to set up the double bond between C-6 and C-7. To this end, benzylic oxidation at C-7 followed by reduction and elimination would give the required double bond. Standard benzylic oxidation conditions with the oxidant CrO$_3$ in HOAc gave ketone **14** in 80% yield. This intermediate had ^{1}H and ^{13}C NMR spectra in agreement with the reported data, including the specific rotation data ([α]$_D^{20}$= +66.0, c 0.8, CHCl$_3$; lit.,[10a] +53.3, c 0.9, CHCl$_3$). The reduction of **14** with excess LiAlH$_4$ in THF at room temperature was known to furnish directly the target sugikurojin A (**1**), as a result of acetate, ketone, and methyl ester reduction to the corresponding alcohol moieties with concomitant benzylic alcohol elimination.[10a] Initially, we suspected that the elimination of the benzylic alcohol intermediates, in hands of Alvarez-Manzaneda and co-workers, occurred probably after acidic workup.[10b] However, the reaction under similar reduction conditions (LiAlH$_4$ in THF at room temperature)[10a] and both acidic and basic workup protocols gave sugikurojin A (**1**) (ca. 80% yield) as well, without isolation of the corresponding benzylic alcohol intermediates. The ^{1}H and ^{13}C NMR spectra, together with HRMS data (calcd for C$_{20}$H$_{29}$O$_2$ [M+ H]: 301.2168; found: 301.2175) corresponded to those reported for the natural product.[3] The specific rotation value of freshly synthesized sugikurojin A ([α]$_D^{25}$= -35.0, c 0.4, CHCl$_3$) was of opposite sign to that reported for the natural compound (naturally derived sugikurojin A had [α]$_D^{25}$= +32.8 (c 0.39, CHCl$_3$)). It is worthy to mention that synthetic (−)-sugikurojin A (**1**) was quite unstable to air and/or light in a chloroform solution prepared for measuring the specific rotation. This resulted in evident decomposition by ^{1}H NMR and noticed by the yellowing of the solution. Interestingly, a three-day aged sample of sugikurojin A (**1**) gave a specific rotation value of +20º.

Scheme 2 Attempted introduction of C-12 oxygenation on methyl callitrisate (**6**).
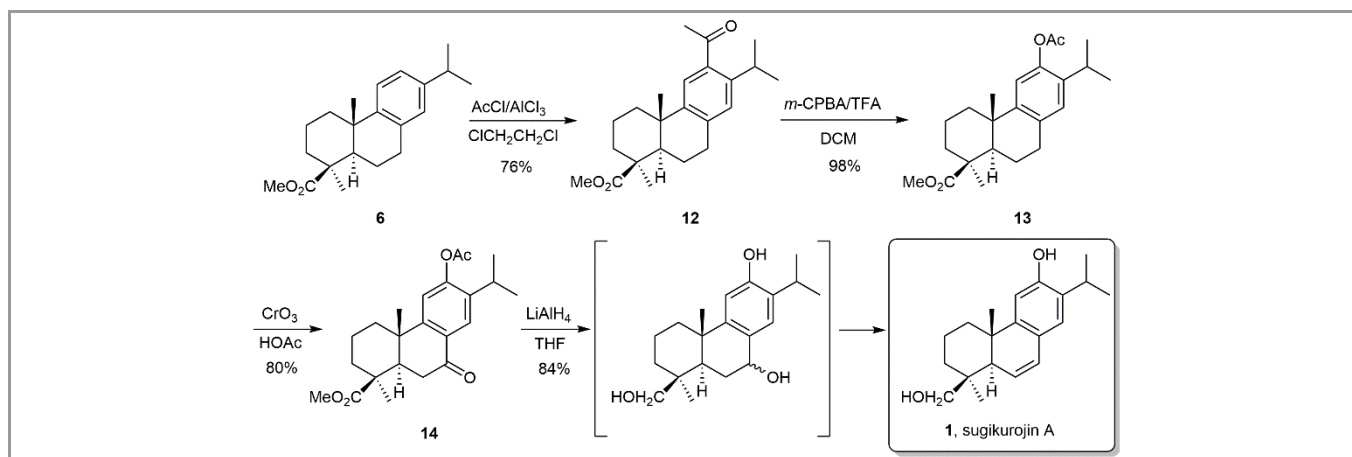
Scheme 3 Semisynthesis of sugikurojin A (**1**) from methyl callitrisate (**6**).

In summary, (−)-sugikurojin A (**1**), an antiprotozoal agent, was synthesized from available methyl ester of callitrisic acid **6** isolated from sandarac resin, in 50% overall yield. Therefore, the synthetic strategy provides an efficient and improved alternative to obtain (−)-sugikurojin A (**1**) for additional studies. Our results suggests that the natural sugikurojin A may belong actually to the enantiomeric series of that assumed during isolation, unless there was either a mistake in the reported sign of the specific rotation or the sample decomposed before measuring the same. However, to the best of our knowledge, *ent*-abietanes have not been found in *Cryptomeria japonica* so far.

The experimental section has no title; please leave this line here.

The melting points were measured with a Büchi 535 apparatus and are uncorrected. Optical rotations were measured using a 5 cm cell in a Schmidt-Haensch Polartronic-D polarimeter. NMR spectra were recorded on a 300 MHz Bruker spectrometer. All spectra were recorded in CDCl₃ as solvent unless otherwise stated. Complete assignments of ^{13}C NMR multiplicities were made on the basis of DEPT experiments. *J* values are given in Hz. MS data were acquired on a QTOF spectrometer. Reactions were monitored by TLC using Merck silica gel 60 F-254 in 0.25 mm-thick plates. Compounds on TLC plates were detected under UV light at 254 nm and visualized by immersion in a 10% sulfuric acid solution and heating with a heat gun. Purifications were performed by flash chromatography on Merck silica gel (230-400 mesh). Commercial reagent grade solvents and chemicals were used as purchased unless otherwise noted. Combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The starting material methyl callitrisate (**6**) was obtained following the reported protocol from Moroccan sandarac resin.[9]

Methyl 12-acetylabieta-8,11,13-trien-19-oate (12, methyl 12-acetylcallitrisate).

To a stirred solution of ester **6** (600 mg, 1.90 mmol) in 1,2-dichloroethane (20 mL) at 0 ºC was added AcCl (0.5 mL, 6.7 mmol) followed by AlCl₃ (800 mg, 6.0 mmol) in portions. The mixture was allowed to warm to rt and stirred for 24 h. Then, the resulting brown reaction mixture was poured into ice-cold aqueous 6N HCl (60 mL) and extracted with Et₂O (3 × 20 mL). The combined extracts were washed with water (15 mL), and brine (15 mL), dried, and concentrated. The resulting brown residue was chromatographed on silica eluting with *n*-hexane-EtOAc (9:1) to give 520 mg (76%) of methyl 12-acetylcallitrisate (**12**) as a yellowish solid: mp 124-126 ºC; [α]²⁰_D +96 (c 1.0, CHCl₃).

^{1}H NMR (300 MHz) δ 7.41 (1H, s), 7.04 (1H, s), 3.66 (3H, s), 3.46 (1H, m), 2.54 (3H, s), 1.28 (3H, s), 1.21 (3H, d, *J* = 6), 1.19 (3H, d, *J* = 6), 1.03 (3H, s).

^{13}C NMR (75 MHz) δ_C 203.3 (s), 177.8 (s), 145.1 (s), 144.8 (s), 139.1 (s), 136.4 (s), 127.0 (d), 125.7 (d), 52.6 (d), 51.3 (q), 43.9 (s), 39.3 (t), 38.1 (s), 37.6 (t), 32.0 (t), 30.5 (q), 28.6 (d), 28.5 (q), 24.2 (q), 24.1 (q), 23.0 (q), 20.8 (t), 19.9 (t).

HRMS (ESI) *m/z* 357.2441 [M+ H]⁺, calcd for C₂₃H₃₃O₃: 357.2430.

Methyl 12-acetoxyabieta-8,11,13-trien-19-oate (13, methyl 12-acetoxycallitrisate).

To a stirred solution of methyl ketone **12** (585 mg, 1.64 mmol) in DCM (6 mL) at 0 ºC were added m-CPBA (1.06 g, 4.3 mmol) and trifluoroacetic acid (130 μL, 1.75 mmol) dropwise. The reaction mixture was allowed to warm to rt and stirred for 22 h. Then, it was diluted with DCM (10 mL), washed with 10% Na₂SO₃ (10 mL), H₂O (10 mL), saturated NaHCO₃ (10 mL), and H₂O (10 mL), dried, and concentrated. The resulting oily residue was chromatographed on silica eluting with *n*-hexane-EtOAc (9:1) to give 600 mg (98%) of acetate **13** as a yellowish solid: mp 126-128 ºC; [α]²⁰_D +92 (c 0.8, CHCl₃).

^{1}H NMR (300 MHz) δ 6.95 (1H, s), 6.84 (1H, s), 3.65 (3H, s), 2.29 (3H, s), 1.26 (3H, s), 1.19 (3H, d, *J* = 6), 1.17 (3H, d, *J* = 6), 1.01 (3H, s).

^{13}C NMR (75 MHz) δ_C 177.8 (s), 169.9 (s), 146.7 (s), 146.3 (s), 137.0 (s), 133.2 (s), 127.0 (d), 119.1 (d), 52.5 (d), 51.2 (q), 43.9 (s), 39.3 (t), 38.2 (s), 37.6 (t), 31.6 (t), 28.5 (q), 27.1 (d), 23.0 (q), 22.9 (q), 22.9 (q), 21.0 (t), 20.9 (q), 19.8 (t).

HRMS (ESI) *m/z* 373.2391 [M+ H]⁺, calcd for C₂₃H₃₃O₄: 373.2379.

Methyl 12-acetoxy-7-oxoabieta-8,11,13-trien-19-oate (14, methyl 12-acetoxy-7-oxocallitrisate).

The acetate **13** (927 mg, 2.49 mmol) was dissolved in HOAc (25 mL) and cooled to 0-5 ºC before CrO₃ (500 mg, 5.0 mmol) was added. The reaction mixture was allowed to warm to rt and stirred for 17 h. Then, it was diluted with ice-H₂O (75 mL) and extracted with diethyl ether (4 × 30 mL). The combined organic extracts were washed with H₂O (2 × 20 mL), saturated NaHCO₃ (2 × 30 mL)(carefully, gas evolution) and brine (20 mL), dried, and concentrated. The resulting residue was chromatographed on silica eluting with *n*-hexane-EtOAc (8:2) to give 768 mg (80%) of ketone **14** as a yellowish oil that solidified upon standing: mp 135-137 ºC; [α]²⁰_D +66.0 (c 0.8, CHCl₃)(lit.,[10a] +53.3 (c 0.9, CHCl₃)).

^{1}H NMR (300 MHz) δ 8.02 (1H, s), 7.03 (1H, s), 3.70 (3H, s), 3.21 (1H, dd, *J* = 12, 12), 2.98 (2H, m), 2.34 (3H, s), 1.25 (3H, s), 1.23 (3H, d, *J* = 6), 1.21 (3H, d, *J* = 6), 1.11 (3H, s).

^{13}C NMR (75 MHz) δ_C 198.1 (s), 177.0 (s), 169.1 (s), 153.4 (s), 152.8 (s), 138.8 (s), 128.7 (s), 126.2 (d), 118.9 (d), 51.6 (q), 50.1 (d), 43.9 (s), 38.4 (s), 38.3 (t), 37.5 (t), 37.4 (t), 27.9 (q), 27.3 (d), 22.8 (q), 22.7 (q), 21.3 (q), 21.0 (q), 19.5 (t).

HRMS (ESI) *m/z* 387.2183 [M+ H]⁺, calcd for C₂₃H₃₁O₅: 387.2171.

19-hydroxy-6,7-dehydroferruginol (1, sugikurojin A).

LiAlH$_4$ (142 mg, 3.73 mmol) was added to a stirred solution of **14** (120 mg, 0.31 mmol) in anhydrous THF (10 mL) at 0 ºC. This mixture was allowed to warm to rt and stirred under an argon atmosphere for 16 h. After this time, the reaction mixture was quenched by pouring it into ice followed by addition of 2 N HCl (6 mL), stirring was continued for 1 h. Next, the mixture was extracted with diethyl ether (3 X 20 mL). The combined organic layers were washed with brine (10 mL), dried and concentrated. The resulting residue was chromatographed on silica eluting with diethyl ether to give 78 mg (84%) of **1** as a white solid, after concentrating the ether fractions with a bit of *n*-hexane (otherwise a semisolid is obtained): mp 112-114 ºC; [α]$^{25}_D$ -35.0 (c 0.4, CHCl$_3$)(lit.,[10] -29.2 (c 0.92, CHCl$_3$)).

^{1}H NMR (300 MHz) δ 6.89 (1H, s), 6.59 (1H, s), 6.49 (1H, dd, *J* = 10, 3), 5.97 (1H, dd, *J* = 10, 3), 3.86 (1H, d, *J* = 10), 3.74 (1H, d, *J* = 10), 3.15 (1H, m), 2.25 (1H, dd, *J* = 3, 3), 2.10 (1H, m), 1.88 (1H, m), 1.26 (3H, d, *J* = 6), 1.23 (3H, d, *J* = 6), 1.07 (3H, s), 0.99 (3H, s).

^{13}C NMR (75 MHz) δ_C 152.6 (s), 147.0 (s), 131.4 (s), 127.6 (d), 125.8 (d), 125.7 (s), 124.7 (d), 109.4 (d), 65.9 (t), 50.9 (d), 38.2 (s), 37.4 (s), 35.9 (t), 35.1 (t), 26.6 (d), 26.0 (q), 22.8 (q), 22.4 (q), 20.7 (q), 18.7 (t).

HRMS (ESI) *m/z* 301.2175 [M+ H]$^+$, calcd for C$_{20}$H$_{29}$O$_2$: 301.2168.

Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

References

(1) González, M. A. *Nat. Prod. Rep.* **2015**, *32*, 684-704.

(2) González, M. A. *Eur. J. Med. Chem.* **2014**, *87*, 834-842.

(3) Arihara, S.; Umeyama, A.; Bando, S.; Imoto, S.; Ono, M.; Tani, M.; Yoshikawa, K. *Chem. Pharm. Bull.* **2004**, *52*, 354-358.

(4) (a) Ramírez-Macías, I.; Marín, C.; Chahboun, R.; Olmo, F.; Messouri, I.; Huertas, O.; Rosales, M. J.; Gutierrez-Sánchez, R.; Alvarez-Manzaneda, E.; Sánchez-Moreno, M. *Mem. Inst. Oswaldo Cruz* **2012**, *107*, 370-376. (b) Ramírez-Macías, I.; Marín, C.; Chahboun, R.; Messouri, I.; Olmo, F.; Rosales, M. J.; Gutierrez-Sánchez, R.; Alvarez-Manzaneda, E.; Sánchez-Moreno, M. *Am. J. Trop. Med. Hyg.* **2012**, *87*, 481-488.

(5) Zhang, G.-J.; Li, Y.-H.; Jiang, J.-D.; Yu, S.-S.; Qu, J.; Ma, S.-G.; Liu, Y.-B.; Yu, D.-Q. *Tetrahedron* **2013**, *69*, 1017-1023.

(6) Fang, Z.-F.; Zhang, G.-J.; Chen, H.; Bai, J.; Yu, S.-S.; Liu, Y.; Wang, W.-J.; Ma, S.-G.; Qu, J.; Xu, S. *Planta Med.* **2013**, *79*, 142-149.

(7) Zhang, L.-C.; Wu, X.-D.; He, J.; Li, Y.; Zhang, R.-P.; Zhao, Q.-S. *Phytochem. Lett.* **2013**, *6*, 364-367.

(8) Takei, M.; Umeyama, A.; Arihara, S. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 730-738.

(9) González, M. A.; Zaragozá, R. J. *J. Nat. Prod.* **2014**, *77*, 2114-2117.

(10) (a) Alvarez-Manzaneda, E.; Chahboun, R.; Cabrera, E.; Alvarez, E.; Alvarez-Manzaneda, R.; Lachkar, M.; Messouri, I. *Synlett.* **2007**, 2425-2429. (b) Alvarez-Manzaneda, E.; Chahboun, R.; Alvarez, E.; Ramos, J. M.; Guardia, J. J.; Messouri, I.; Chayboun, I.; Mansour, A. I.; Dahdouh, A. *Synthesis* **2010**, 3493-3503.

(11) Yuan, C.; Liang, Y.; Hernandez, T.; Berriochoa, A.; Houk, K. N.; Siegel, D. *Nature* **2013**, *499*, 192-196.

(12) González, M. A. *Tetrahedron* **2015**, *71*, 1883-1908.

(13) (a) González, M. A.; Pérez-Guaita, D. *Tetrahedron* **2012**, *68*, 9612-9615. (b) Malkowsky, I. M.; Nieger, M.; Kataeva, O.; Waldvogel, S. R. *Synthesis* **2007**, 773-778.

(14) (a) Cambie, R. C.; Franich, R. A. *Aust. J. Chem.* **1971**, *24*, 117-134. (b) Tolmacheva, I. A.; Tarantin, A. V.; Boteva, A. A.; Anikina, L. V.; Vikharev, Y. B.; Grishko, V. V.; Tolstikov, A. G. *Pharm. Chem. J.* **2006**, *40*, 489-493.