

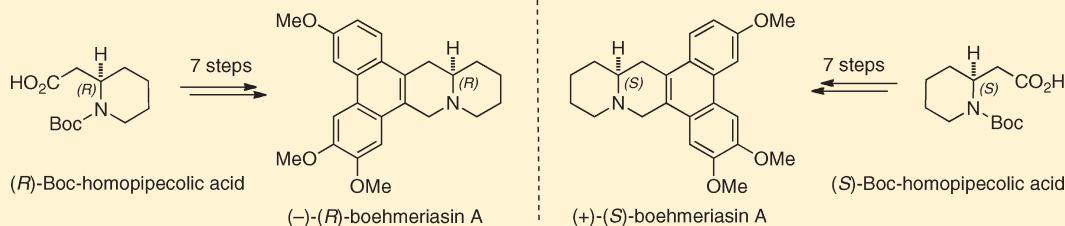
Total Syntheses and Cytotoxicity of (*R*)- and (*S*)-Boehmeriasin A

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Supporting Information

ABSTRACT:



Both enantiomers of boehmeriasin A were synthesized in seven steps each using a chiral pool approach. Key steps in the syntheses are a one-flask, two-step protocol to generate the quinolizidine core and a C–H functionalization reaction between tetrahydroquinolizinones and an aryltrifluoroborate. The natural product (*R*)-boehmeriasin A demonstrated potent cytotoxicity against several cancer cell lines, whereas the unnatural (+)-(*S*)-isomer was significantly less potent.

KEYWORDS: Natural product synthesis, phenanthroquinolizidine, boehmeriasin A, cytotoxicity, drug resistance

Boehmeriasin A and B were recently isolated from the aqueous ethanolic extract of *Boehmeria siamensis* via bioassay-guided fractionation (Figure 1).¹ The alkaloids were evaluated against a panel of cancer cell lines that included leukemia and cancers of the lung, colon, breast, prostate, and kidney. Boehmeriasin A was found to be more potent than paclitaxel and boehmeriasin B in most cell lines evaluated, with GI_{50} values ranging from 0.80 to 265 nM. In addition, boehmeriasin A potently inhibits the proliferation of the breast cancer cell line MDA-MB-231 through G1 cell cycle arrest and differentiation induction by altering the expression levels of several genes involved with cell proliferation, cell cycle regulation, and apoptosis.^{2,3} The synthesis of this natural product was first reported in racemic form, leaving the absolute stereochemistry of the natural product initially unknown.^{4,5}

While our work on the enantiospecific synthesis of (*R*)- and (*S*)-boehmeriasin A was ongoing, an asymmetric synthesis of (*R*)-boehmeriasin A was reported.⁶ The synthesis of the natural product was achieved in 13 steps and involved the SAMP-hydrazone method to introduce asymmetry, ring-closing metathesis to form the piperidine ring system, an aldol reaction to prepare the quinolizidine core structure, and a radical reaction to furnish the phenanthrene moiety.

We had become interested in this natural product because of the promising anticancer activity reported and our interest in this class of compounds.^{7,8} We now report the total synthesis of both boehmeriasin A enantiomers and data concerning their *in vitro* anticancer activity. In a retrosynthetic sense (Scheme 1), boehmeriasin A (**1**) was to be derived from an intramolecular biaryl coupling of intermediate **2**, which itself was to be accessed from a

palladium-mediated cross-coupling reaction. The coupling partner **3** for the cross-coupling was to be obtained by conversion of cyclic enaminone **4** to the triflate **3**. This advanced intermediate **4** was envisioned to arise from a novel, palladium(II)-catalyzed C–H functionalization utilizing organotrifluoroborates.⁹ The coupling partner for this transformation would be obtained from Weinreb amide **6** through methods developed in our laboratories.^{10–12}

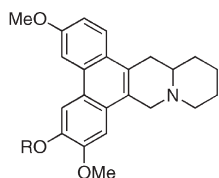
The commercially available acids **7** were converted into the corresponding Weinreb amides **6** under standard conditions and subsequently treated with ethynylmagnesium bromide to afford the corresponding ynones **8** in excellent yields (Scheme 2).^{13,14} These intermediates were subjected to a one-flask, two-step protocol for the cyclization of Boc-ynones to enaminones to afford the desired enaminones **5** in good yields.^{10–12}

With rapid access to the desired enaminones established, our recently reported palladium(II)-catalyzed C–H functionalization using enaminones and potassium organotrifluoroborates was employed.⁹ This method represents an efficient means of accessing these products, as previous methods required an initial prefunctionalization of the enaminone to the appropriate α -halogenated derivative followed by a Suzuki coupling.¹⁵ This protocol eliminates this requirement and thus allows for a more streamlined approach to access these compounds. Utilizing potassium 3,4-dimethoxyphenyltrifluoroborate (**9**), prepared according to a known procedure,¹⁶ in the Pd(II)-catalyzed

Received: December 22, 2010

Accepted: February 13, 2011

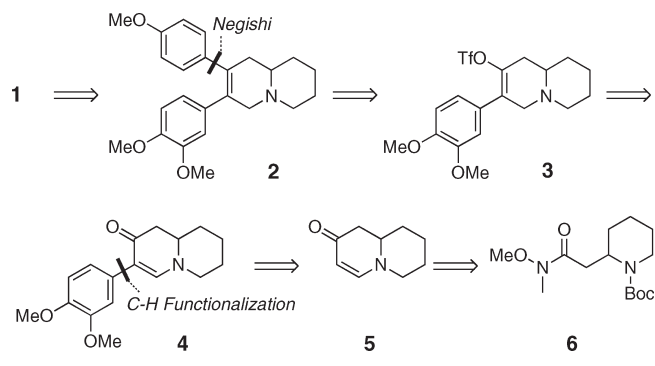
Published: February 23, 2011



R = Me; boehmeriasin A (1)
R = H; boehmeriasin B

Figure 1. Structures of boehmeriasin A and B.

Scheme 1. Retrosynthesis for Boehmeriasin A

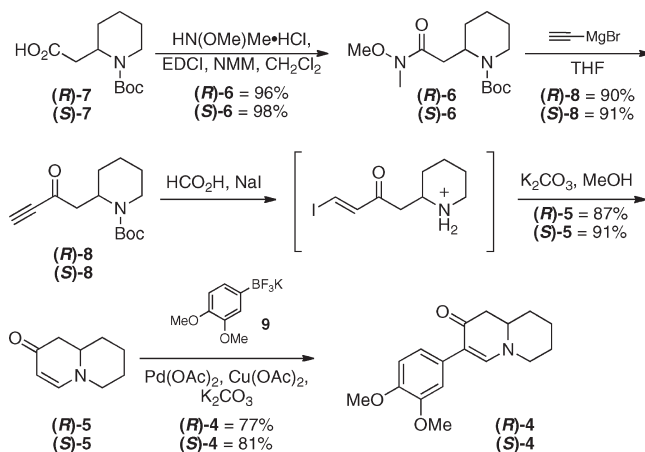


reactions with enaminones **5** furnished the desired arylated products **4** in good yields (Scheme 2).

With a route to the α -arylated intermediates established, the final synthetic sequence was undertaken as shown in Scheme 3. Enaminones **4** were treated with *L*-Selectride, and the resultant enolates were trapped with Comins' reagent (*N*-(5-chloropyridin-2-yl)-1,1,1-trifluoro-*N*-(trifluoromethylsulfonyl)methanesulfonamide) to arrive at the desired triflates **3** in good yields.^{17,18} A Negishi cross-coupling was then employed to furnish the desired intermediates **2** in near quantitative yields.¹⁹ The synthesis was completed utilizing an oxidative biaryl ring closure mediated by VOF₃ to afford (*R*)- and (*S*)-boehmeriasin A in good yields.²⁰ The final products were crystallized from CH₂Cl₂/MeOH, and the crystal structure for the (*R*)-antipode was determined.²¹ It is important to note that little racemization occurred throughout the course of the synthesis to afford (*R*)- and (*S*)-boehmeriasin A in 97.5:2.5 *er* and 98:2 *er*, as determined by chiral HPLC.^{22–24} In addition, the specific rotation of the (*R*)-enantiomer gave a matching sign and magnitude with those found in the literature (lit. -80 , MeOH, $c = 0.10$; obs -86 , MeOH, $c = 0.10$).^{1,6}

(*R*)- and (*S*)-Boehmeriasin A and synthetic intermediates were subjected to *in vitro* cytotoxicity assays to confirm the reported biological activity and establish an initial SAR for boehmeriasin A in breast (MCF7), drug-resistant ovarian (NCI-ADR-RES), and colon (COLO-205) cancer cell lines (Table 1). Arylated enaminone **3** and *seco*-boehmeriasin A (**2**) were devoid of any cytotoxic activity in the cell lines evaluated, which indicates that a full phenanthrene ring system is required for potent cytotoxic activity. This is in accord with other studies of this class of natural products and their analogues.^{25–27} Furthermore, ($-$)-boehmeriasin A (($-$)-**1**·HCl) was more potent than its antipode, ($+$)-**1**·HCl, in all of the cell lines evaluated, indicating that the (*R*)-configuration is essential for potent cytotoxic activity.

Scheme 2. Synthesis of α -Arylated Enaminones **4**



Scheme 3. Completion of the Synthesis of (*R*)- and (*S*)-**1**

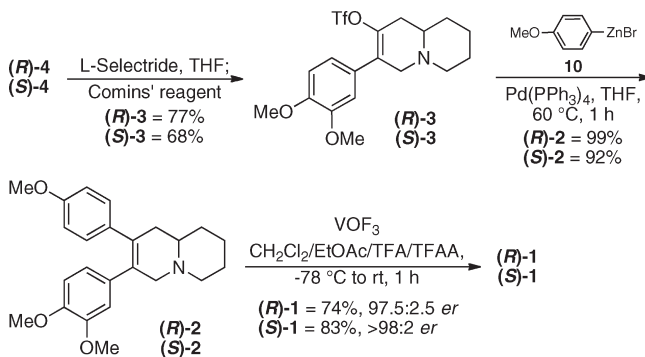


Table 1. Cytotoxicity Evaluation of ($-$)-(*R*)- and ($+$)-(*S*)-Boehmeriasin A in Comparison to Paclitaxel^a

compd	IC ₅₀ (nM)		
	COLO-205	MCF-7	NCI-ADR-RES
paclitaxel ^b	3.31	1.62	>6400
($-$)-(<i>R</i>)- 1 ·HCl ^c	4.18	43.4	36.7
($+$)-(<i>S</i>)- 1 ·HCl ^b	103	92.7	434

^a COLO-205 = human colorectal adenocarcinoma (GI₅₀ = 0.80 nM reported for ($-$)-**1**; see ref 1); MCF-7 = human breast carcinoma (GI₅₀ = 13 nM reported for ($-$)-**1**; see ref 1); NCI-ADR-RES = drug-resistant human ovarian adenocarcinoma. ^b Average of six assays each. ^c Average of three assays each.

Most significantly, the natural product showed activity in the drug resistant cancer cell line, NCI-ADR-RES, where paclitaxel is inactive.

In summary, the total syntheses of ($-$)-(*R*)- and ($+$)-(*S*)-boehmeriasin A were accomplished in seven steps from commercially available material with an overall yield of 33%, and the absolute stereochemistry of the natural product was verified to be of the (*R*)-configuration. The synthesis showcases the utility of the enaminone chemistry and the palladium(II)-mediated C–H functionalization developed in our laboratories. When evaluated

for cytotoxic activity, (–)-(R)-boehmeriasin A demonstrated potent cytotoxicity in several cancer cell lines, including a drug-resistant cancer cell line where paclitaxel is inactive. The (S)-enantiomer was significantly less potent. (R)-Boehmeriasin A will serve as a lead compound for further development, and studies in this regard are currently underway in our laboratories.

■ ASSOCIATED CONTENT

S Supporting Information. Full experimental procedures, compound characterization, and complete crystallographic data for boehmeriasin A. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ACKNOWLEDGMENT

This work was supported by National Institutes of Health Grants GM081267 and the University of Minnesota through the Vince and McKnight Endowed Chairs. We also wish to thank Dr. Victor Young for X-ray analysis, Dr. Defeng Tian for conducting the cytotoxicity assays, and Bryant Gay for the synthesis of the methyl ester of racemic **7** and (R)-**7** and their analysis by chiral HPLC.

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