

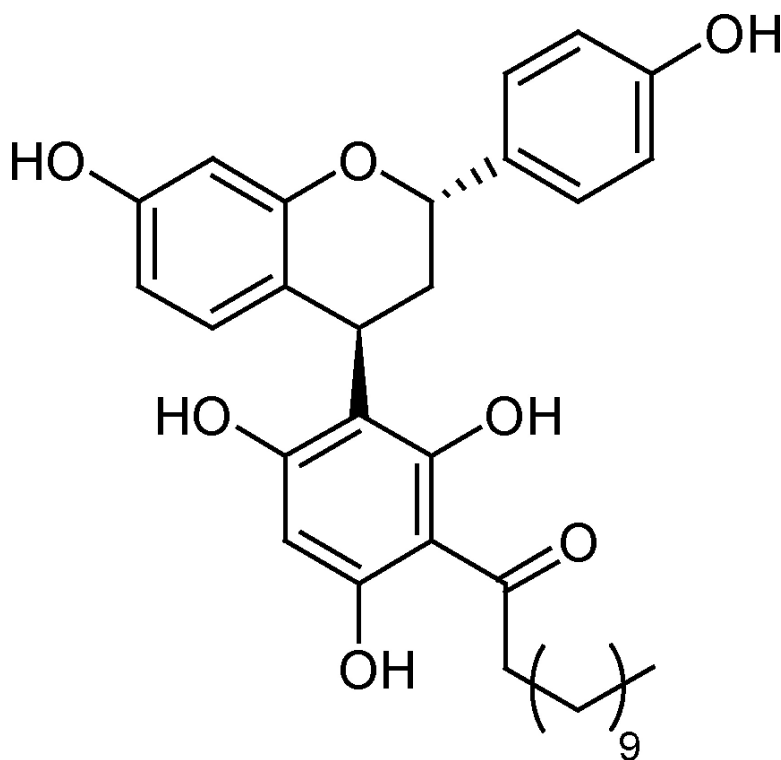
Communication

**(+)-Myristinin A, a Naturally Occurring DNA Polymerase  
 $\beta$  Inhibitor and Potent DNA-Damaging Agent**

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**(+)-Myristinin A**

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## (+)-Myristinin A, a Naturally Occurring DNA Polymerase $\beta$ Inhibitor and Potent DNA-Damaging Agent

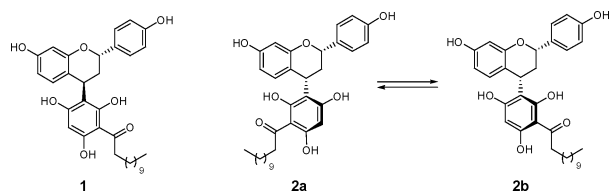
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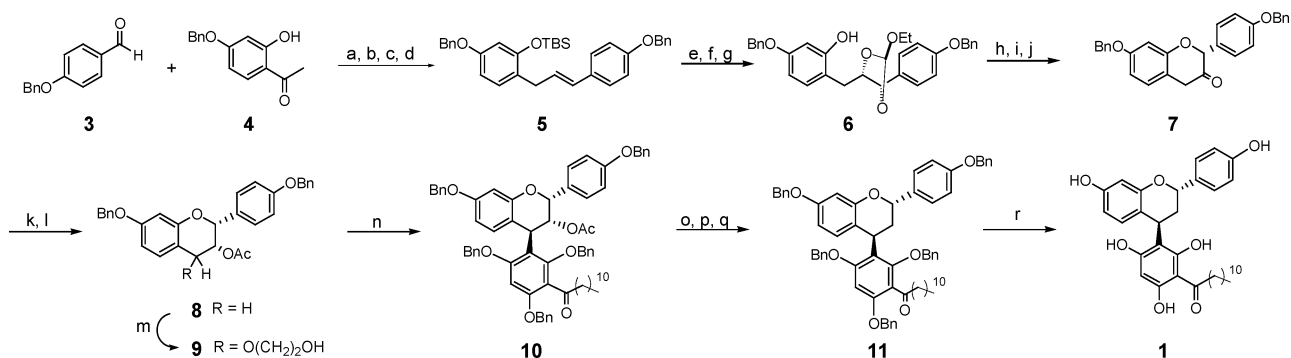
Recently, we have described the isolation from natural sources of a number of DNA polymerase  $\beta$  inhibitors that are capable of blocking the repair of DNA damage inflicted by clinically used damaging agents and thereby potentiating their cytotoxicity.<sup>1</sup> Of special interest were several 5-alkylresorcinols that also mediate Cu<sup>2+</sup>-dependent DNA damage and thus inhibit the polymerase  $\beta$  mediated repair of the DNA damage that they inflict.<sup>2</sup> Accordingly, we have sought additional classes of agents that exhibit this dual activity.

Flavanoids have been of considerable interest for their antibacterial,<sup>3</sup> anticancer,<sup>4</sup> and antiviral<sup>5</sup> properties. Flavanoids **1** and **2**, known as myristinin A (**1**) and myristinin B/C (**2a/2b**), have been isolated by Sawadajoon and co-workers from *Myristica cinnamomea*,<sup>6</sup> and more recently in our laboratory from *Knema elegans* using bioassay-guided fractionation involving polymerase  $\beta$  inhibition. The myristinins also cleave DNA. The unusual biochemical activities and limited availability of these compounds from natural sources prompted us to investigate their synthesis.



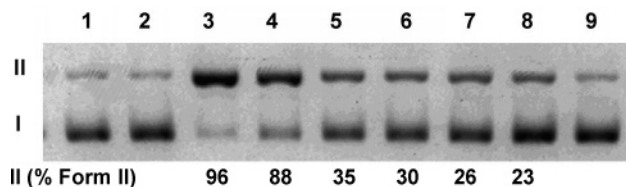
Initial biochemical assays revealed that **1** exhibited more potent copper-dependent DNA-damaging activity and polymerase  $\beta$  inhibition than the inseparable mixture of atropisomers **2a** and **2b**, thus making **1** the more appealing synthetic target. Presently, we describe the stereoselective synthesis, structure confirmation, and determination of the absolute stereochemistry of **1**. Also described is its remarkable potency of DNA cleavage.

### Scheme 1. Synthesis of (+)-Myristinin A (**1**)<sup>a</sup>



<sup>a</sup> Conditions: (a) 40% (w/v) KOH in MeOH, MeOH, reflux, (97%); (b) ethyl chloroformate, NEt<sub>3</sub>, THF, 0 °C; (c) NaBH<sub>4</sub>, H<sub>2</sub>O, (74%, 2 steps); (d) TBDMSCl, imidazole, DMF, (100%); (e) ADMIX- $\alpha$ , methanesulfonamide, *t*-BuOH/H<sub>2</sub>O (1:1), (84%); (f) CH(OEt)<sub>3</sub>, cat. PPTS, CH<sub>2</sub>Cl<sub>2</sub>, (92%); (g) TBAF, THF, (100%); (h) CH(OEt)<sub>3</sub>, PPTS, Cl(CH<sub>2</sub>)<sub>2</sub>Cl, 60 °C, (89%); (i) K<sub>2</sub>CO<sub>3</sub>, THF/MeOH (1:1), (98%); (j) Dess–Martin periodinane, CH<sub>2</sub>Cl<sub>2</sub>, (94%); (k) L-selectride, LiBr, THF, -78 °C, (78%); (l) Ac<sub>2</sub>O, cat. DMAP, CH<sub>2</sub>Cl<sub>2</sub>, (93%); (m) DDQ, ethylene glycol, CH<sub>2</sub>Cl<sub>2</sub>, (79%); (n) TMSOTf, 1-(2,4,6-tris(benzyloxy)phenyl)dodecan-1-one, THF, -35 to -5 °C, (77%); (o) K<sub>2</sub>CO<sub>3</sub>, THF/MeOH (1:1), (100%); (p) PhOC(S)Cl, DMAP, MeCN, 50 °C; (q) SnBu<sub>3</sub>H, cat. AIBN, toluene, 100 °C, (65%, 2 steps); (r) Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, THF/MeOH (1:1), (75%).

The synthesis of (+)-myristinin A (**1**) is outlined in Scheme 1. Condensation of 4-benzyloxybenzaldehyde (**3**)<sup>7</sup> and 4-benzyloxy-2-hydroxyacetophenone (**4**)<sup>8</sup> was achieved via a Claisen–Schmidt reaction<sup>9</sup> to give the chalcone in 97% yield. Decarbonylation using lithium aluminum hydride and AlCl<sub>3</sub> proved overly harsh and resulted in removal of the benzyl protecting groups. Instead, a mild method was employed involving ethyl chloroformate and NaBH<sub>4</sub> in a two-step sequence.<sup>10</sup> Sharpless asymmetric dihydroxylation<sup>11</sup> was attempted in the presence of the free phenol and proved unsuccessful. Therefore, the phenol was protected as the TBDMS ether, providing the substituted 1,3-diphenylpropene **5** in 74% yield. With the TBDMS group in place, asymmetric dihydroxylation of **5** with AD-MIX  $\alpha$ <sup>11</sup> proceeded smoothly. Treatment of the resulting diol with triethyl orthoformate in the presence of catalytic pyridinium *p*-toluenesulfonate (PPTS) gave the ortho ester in 77% yield over two steps. Deprotection of the silyl ether was accomplished using TBAF in THF to give phenol **6** quantitatively. Treatment of **6** with triethyl orthoformate and PPTS in dichloroethane at 60 °C gave the intermediate 2,3-*trans*-pyran formate ester (having <sup>3</sup>J<sub>2,3</sub> of 6.0 Hz, typical of a 2,3-*trans*-flavan-3-ol) in good yield (89%).<sup>12</sup> Removal of the formate ester was accomplished using K<sub>2</sub>CO<sub>3</sub> in methanol, and oxidation of the resulting alcohol using Dess–Martin periodinane<sup>13</sup> afforded ketone **7**. Diastereoselective reduction with L-selectride in the presence of LiBr gave exclusive formation of the desired product with the *cis* configuration (<sup>3</sup>J<sub>2,3</sub> < 1.0 Hz).<sup>14</sup> Acetylation of the resulting hydroxyl group using Ac<sub>2</sub>O and NEt<sub>3</sub> afforded **8** in good yield (73% from **7**). Several groups have investigated the oxidation of C-4 in similar systems and reported that 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave the best yields.<sup>14,15</sup> Therefore, **8** was treated with DDQ and ethylene glycol in CH<sub>2</sub>Cl<sub>2</sub> to afford 4-O-alkylated **9** in 79% yield. Lewis acid promoted condensation<sup>15,16</sup> of 1-(2,4,6-tris(benzyloxy)phenyl)-



**Figure 1.** Relaxation of supercoiled plasmid DNA by **1** in the presence of 20  $\mu\text{M}$   $\text{Cu}^{2+}$ : (lane 1) DNA + 20  $\mu\text{M}$   $\text{Cu}^{2+}$ ; (lane 2) DNA + 50  $\mu\text{M}$  **1**, (lanes 3–8) DNA + 20  $\mu\text{M}$   $\text{Cu}^{2+}$  + 50, 10, 2, 0.5, 0.1 and 0.05  $\mu\text{M}$  **1**, respectively; (lane 9) DNA alone.

dodecan-1-one<sup>17</sup> with **9** in THF gave the desired 2,4-*trans* isomer **10** in good yield (77%) and excellent diastereoselectivity ( $\geq 95:5$ ). Acetate deprotection using methanolic  $\text{K}_2\text{CO}_3$ , followed by thioacylation with phenyl chlorothionoformate in MeCN at 60  $^\circ\text{C}$ , gave the desired deoxygenation precursor. The thioester was treated immediately with  $\text{SnBu}_3\text{H}$  in the presence of catalytic AIBN in toluene at reflux to furnish the deoxygenation product **11** (65%, three steps).<sup>18</sup> Complete deprotection proceeded smoothly using Pearlman's catalyst ( $\text{Pd}(\text{OH})_2/\text{C}$ ) in 1:1 THF/MeOH to afford (+)-myristinin A (**1**) in 75% yield.<sup>19</sup>

Single-strand breakage of supercoiled (Form I) pSP64 plasmid DNA by **1** was observed in the presence of  $\text{Cu}^{2+}$  in a dose-dependent manner (Figure 1). The concentration of  $\text{Cu}^{2+}$  was kept constant at 20  $\mu\text{M}$  while **1** was tested at 50  $\mu\text{M}$ –50 nM concentrations. Concentration-dependent plasmid relaxation was readily apparent within 2.5 h (Figure 1). Investigations at lower concentrations of **1** revealed the accumulation of DNA cleavage in a steady fashion over a period of a few days. Under these conditions, cleavage was readily apparent at 10 pM concentration. The potency of DNA cleavage by  $\text{Cu}^{2+}$  + **1** in replicate experiments over an extended time period argues that **1** must produce a concentration of DNA breaks that greatly exceeds its own molar concentration, i.e., that it cleaves DNA "catalytically". This property is the subject of ongoing study.

In addition to its potent DNA-damaging ability, **1** was also shown to inhibit DNA polymerase  $\beta$  ( $\text{IC}_{50}$  2.8  $\mu\text{M}$ ), a DNA repair enzyme important in base excision repair. Inhibition of this enzyme has been shown to result in potentiation of the cytotoxic activity of bleomycin (BLM), indicating the potential for polymerase inhibitors to allow lower doses of DNA-damaging antitumor agents to be administered.<sup>1</sup> In fact, in cultured A549 cells, a sublethal concentration of **1** strongly potentiated the cytotoxicity of BLM.<sup>20</sup> Moreover, polymerase  $\beta$  was shown to be overexpressed in cells exposed to DNA-damaging agents, further validating the enzyme as a potential target for cancer chemotherapy.<sup>21</sup> Typically DNA polymerase  $\beta$  inhibitors only prevent DNA repair, thereby limiting their potential use to that of adjuvants with known DNA-damaging agents. In contrast, the unique dual biological activity of **1** offers the additional prospect of use as a single agent capable of mediating DNA damage and blocking repair of the induced lesions.

In summary, the first stereoselective synthesis, structure confirmation, and absolute stereochemistry of (+)-myristinin A are described. Also described are the remarkable properties of this compound as a potent DNA-damaging agent and polymerase  $\beta$  inhibitor. Further chemical and biological evaluation of **1** is underway and will be reported in due course.

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**Supporting Information Available:** Experimental procedure and characterization data for **1** and **3–11**, along with any intermediates. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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