

## Total Synthesis and Biological Evaluation of 22-Hydroxyacuminatine

Xiangshu Xiao,<sup>†</sup> Smitha Antony,<sup>‡</sup> Yves Pommier,<sup>‡</sup> and Mark Cushman<sup>\*,†</sup>

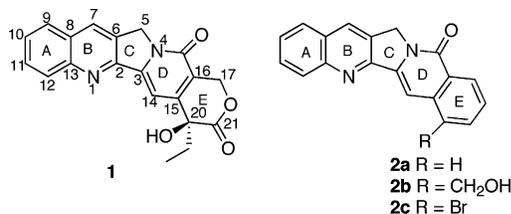
Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana 47907, and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892-4255

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A total synthesis of 22-hydroxyacuminatine, a cytotoxic alkaloid isolated from *Camptotheca acuminata*, is reported. The key step in the synthesis involves the reaction of 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline with a brominated phthalide to generate a substituted pentacyclic 12*H*-5,11*a*-diazadibenzo[*b,h*]fluoren-11-one intermediate. Despite its structural resemblance to camptothecin and luotonin A, a biological evaluation of 22-hydroxyacuminatine in a topoisomerase I-deficient cell line P388/CPT45 has confirmed that the observed cytotoxicity is not due to topoisomerase I inhibition, even though 22-hydroxyacuminatine has a hydroxyl group that can theoretically hydrogen bond to Asp533. This result is consistent with the hypothesis that  $\pi$ - $\pi$  stacking is more important than hydrogen-bonding interactions in determining topoisomerase I inhibitor binding in the ternary cleavage complex.

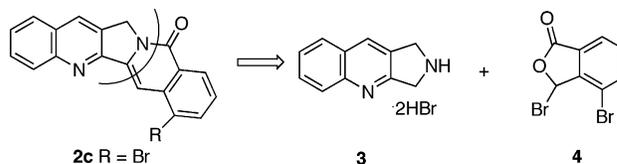
### Introduction

Camptothecin (CPT, **1**) is a potent topoisomerase I (Top1) inhibitor with significant cytotoxicity against a variety of different cancer cell lines.<sup>1</sup> Top1 is a nuclear enzyme that relaxes the DNA supercoils generated during DNA replication and transcription. The enzyme operates by carrying out a transesterification reaction involving a nucleophilic attack of the Tyr723 hydroxyl group on a DNA phosphodiester bond, thus creating a transient nick that is rapidly resealed by the reverse transesterification reaction.<sup>2</sup> CPT inhibits the reverse reaction by intercalating between the base pairs at the DNA cleavage site, thus stabilizing the ternary DNA cleavage complex consisting of the drug, DNA, and the enzyme. Collision of the DNA replication fork with the single-stranded DNA break then results in a double-stranded break and cancer cell apoptosis. Two clinically useful CPT derivatives, topotecan (Hycamtin) and irinotecan (Camptosar), are presently in clinical use as anticancer agents.<sup>3</sup> However, lactone ring hydrolysis of the CPT system produces the hydroxy carboxylate which has a high affinity to human serum albumin protein, and this limits the efficacy of CPT derivatives.<sup>4</sup> As a consequence, there is interest in developing metabolically stable non-CPT Top1 inhibitors as novel anticancer drugs.<sup>5</sup>



As a strategy to enhance stability, replacement of the E-ring present in CPT with an aromatic ring has been investigated. The resulting compounds are collectively referred to “aromathecin” as opposed to camptothecins. We have previously shown that aromathecin **2a** is a weak Top1 inhibitor with low cytotoxicity.<sup>6</sup> On the basis of the crystal structure of the CPT-

### Scheme 1. Retrosynthetic Analysis of Aromathecin **2c**



stabilized DNA–Top1 cleavage complex, which revealed that the crucial 20-OH of CPT is hydrogen bonded with Asp533,<sup>7</sup> introduction of an additional hydrogen bond donor to the E-ring of the aromathecins capable of interacting with Asp533 would be expected to increase the Top1 inhibitory potency. Therefore, aromathecin **2b** was considered. Interestingly, a review of the literature revealed that compound **2b** had been previously isolated from *Camptotheca acuminata* as a rare natural product (0.000006% yield) named 22-hydroxyacuminatine.<sup>8</sup> Although **2b** exhibited cytotoxicity toward P388 and KB cell lines, it has remained to be determined whether this cytotoxicity is due to Top1 inhibition. This question prompted us to investigate the total synthesis of 22-hydroxyacuminatine<sup>9</sup> as well as its biological properties. We also wanted to establish a flexible route that would open up a way to synthesize analogues for investigation of structure–activity relationships.

### Results and Discussion

Bromide **2c** was envisioned as a precursor to 22-hydroxyacuminatine (**2b**) in that a variety of different transformations are possible with the bromide, which is considered as an advantage in terms of potential analogue syntheses. By analogy to the synthesis of CPT<sup>10</sup> and aromathecin **2a**,<sup>6</sup> the bromide **2c** was dissected in the D-ring to give fragments **3**<sup>11</sup> and **4** (Scheme 1).

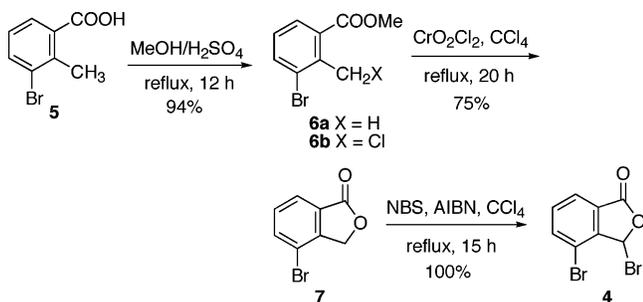
The synthesis of fragment **4** is presented in Scheme 2. Methylation of commercially available benzoic acid derivative **5** provided methyl ester **6a**. Oxidation of the methyl group in **6a** to the corresponding aldehyde proved to be problematic. No reaction was observed with CrO<sub>3</sub>/Ac<sub>2</sub>O/HOAc,<sup>12</sup> or ceric ammonium nitrate (CAN),<sup>13</sup> or selenium dioxide (SeO<sub>2</sub>),<sup>14</sup> presumably due to the steric hindrance. Consistent with this hypothesis, oxidation of **5** with CrO<sub>3</sub>/Ac<sub>2</sub>O/HOAc resulted in overoxidation of the methyl group to a carboxylic acid followed by cyclization to the anhydride. On the other hand, Étard

\* Corresponding author. Phone: 765-494-1465. Fax: 765-494-6790. E-mail: cushman@pharmacy.purdue.edu.

<sup>†</sup> Purdue University.

<sup>‡</sup> National Cancer Institute.

## Scheme 2. Synthesis of Bromide 4



reaction of methyl ester **6a** with chromyl chloride<sup>15</sup> provided lactone **7** instead of the expected aldehyde derivative.<sup>16</sup> When less than 2 equiv of chromyl chloride was used, another product **6b** was also generated from the Étard reaction. Radical bromination of lactone **7** gave the requisite bromide **4** uneventfully.

Coupling of fragments **3**<sup>11</sup> and **4** afforded bromide **2c** in 20% yield (Scheme 3). Treatment of **2c** with *n*-BuLi to undergo bromine–lithium exchange to give the corresponding anion **8**, followed by trapping with paraformaldehyde, would give 22-hydroxyacuminatine (**2b**). However, the product formed from the reaction was **11**, while **2b** was not formed at all. Employment of excess *n*-BuLi (2 equiv) for the reaction did not result in the formation of any **2b**. This result indicated that the bromine–lithium exchange occurred at a faster rate than deprotonation at C-5 (see the CPT numbering system in structure **1**). However, the resulting anion **8** evidently undergoes rapid proton transfer involving the 5 position to give benzylic anion **9**. The <sup>1</sup>H NMR spectrum of the product **11** is interesting in that the methine proton next to the hydroxymethyl side chain is presented as a doublet instead of the expected triplet. This unusual <sup>1</sup>H NMR feature is presumably due to intramolecular hydrogen bond formation between the hydroxyl group and the lactam oxygen, which results in the H<sub>1</sub>–C<sub>2</sub>–C<sub>3</sub>–H<sub>4</sub> dihedral angle being close to 90°. This dihedral angle was found to be 91.6° after the structure was energetically minimized by AM1 in Gaussian03 (Figure 1).<sup>17</sup>

The failure to transform **2c** to **2b** suggests that it might be advantageous to install the one-carbon unit before the coupling reaction. Therefore, the possible intermediate **14** with a cyano group at the desired position was considered, which would

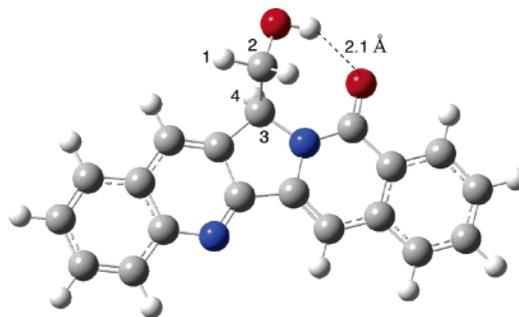
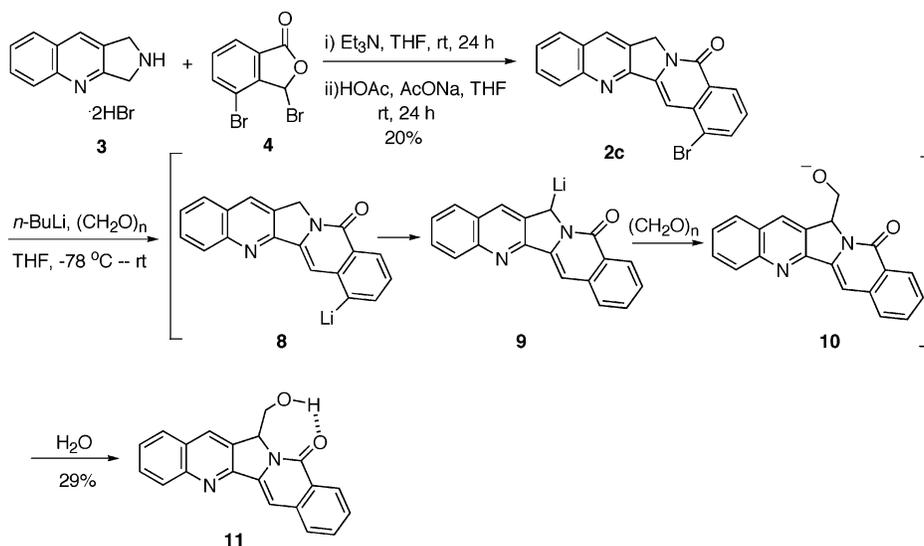
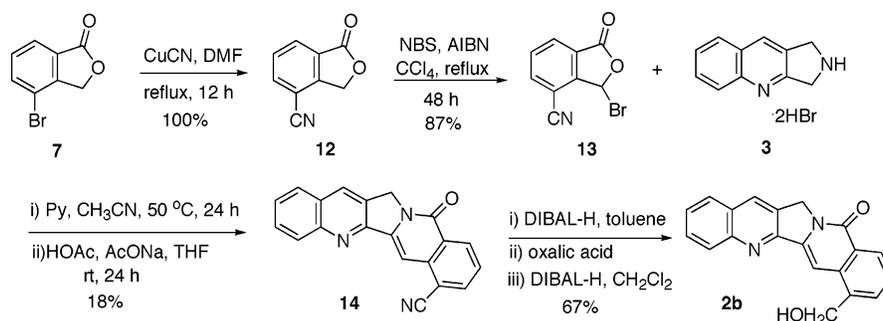


Figure 1. AM1-optimized structure of **11**.

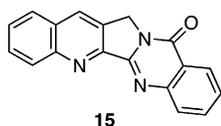
require the preparation of nitrile **13** as one of the building blocks. To this end, Rosenmund–von Braun reaction<sup>18</sup> of bromide **7** with CuCN in refluxing DMF gave cyanide **12** in quantitative yield leaving the lactone intact (Scheme 4). No reaction was observed at a temperature of 120 °C or lower. Radical bromination of the new lactone **12** delivered bromide **13**, which reacted with the tricyclic amine hydrobromide **3** to yield cyanide **14**. DIBAL-H reduction followed by hydrolysis and further DIBAL-H reduction of the resulting aldehyde afforded 22-hydroxyacuminatine (**2b**) in 67% yield.

Due to the structural similarity of 22-hydroxyacuminatine (**2b**) with CPT (**1**) and luotonin A (**15**), both of which are Top1 inhibitors exhibiting Top1-dependent growth inhibition in yeast,<sup>19</sup> a Top1-mediated DNA cleavage assay<sup>20</sup> was performed to examine the Top1 inhibitory potency. In this assay, only marginal DNA cleavage was observed with **2b**, indicating that it is a weak Top1 inhibitor. To further verify whether the reported cytotoxicity of **2b**<sup>8</sup> is due to the weak Top1 inhibition, the cytotoxicity of **2b** in a P388 leukemia cell line and its Top1-deficient subclone P388/CPT45<sup>21</sup> was investigated.<sup>22</sup> The IC<sub>50</sub> observed for 22-hydroxyacuminatine (**2b**) in P388 cells is 35.5 ± 0.5 μM, while in P388/CPT45 cells (a Top1-deficient cell line) it is 43.0 ± 1.0 μM. The IC<sub>50</sub> in P388 cells is roughly an order of magnitude higher than the originally reported value.<sup>8</sup> More importantly, these values demonstrate that the cytotoxicity of **2b** is not due to Top1 inhibition. It seems unlikely that differences between the camptothecins and 22-hydroxyacuminatine (**2b**) in their hydrogen bonding to the protein are responsible for the observed difference in Top1 inhibitory activities, since docking and energy minimization of **2b** in the ternary complex indicates that, like the lactone form of the

Scheme 3. Synthesis of 2c and Its Reaction with *n*-BuLi

**Scheme 4.** Synthesis of 22-Hydroxyacuminatine (**2b**)

camptothecins,<sup>7,23</sup> the hydroxyl group of **2b** can form a direct hydrogen bond with the Asp533 side chain carboxylate of Top1



(Figure 2). On the other hand, the biological evaluation results are consistent with the hypothesis that  $\pi$ - $\pi$  stacking is more important than hydrogen-bonding interactions in determining inhibitor binding in the ternary cleavage complex,<sup>24,25</sup> and the results are also in agreement with an earlier report that compound **2a** does not exhibit its cytotoxicity by poisoning Top1 in yeast.<sup>26</sup> The inefficient  $\pi$ - $\pi$  stacking between 22-hydroxyacuminatine (**2b**) and the neighboring base pairs is most likely due to the nonpolar nature of the E-ring, where no heteroatoms are included or are directly attached, resulting in an electrostatically neutral potential surface (Figure 3) that does not complement the electrostatics of the adjacent DNA base pairs as well as the E-ring of camptothecin does. According to the energy-minimized hypothetical model, the E-ring of 22-hydroxyacuminatine is not well positioned to overlap with the adjacent base pairs (see Figure 2). This further demonstrates that the electronic properties of the E-ring of the aromathecins and camptothecins are critical determinants of enzyme inhibitory activity.<sup>27</sup>

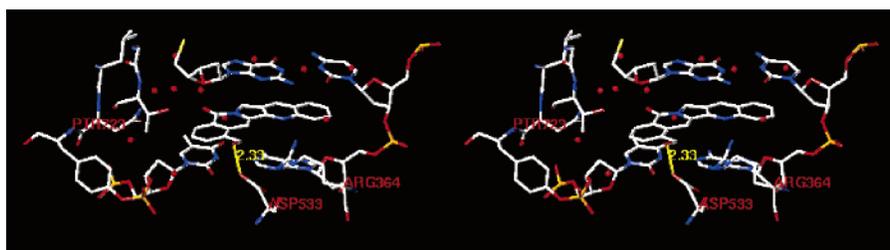
In conclusion, a highly convergent total synthesis of 22-hydroxyacuminatine (**2b**) has been achieved. The biological properties of 22-hydroxyacuminatine were characterized, demonstrating that Top1 inhibition is not a significant factor in its cytotoxicity despite its structural resemblance to CPT.

**Experimental Section**

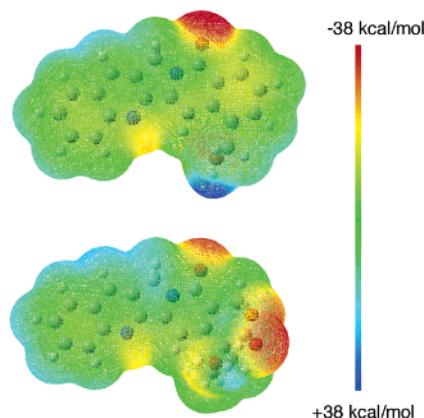
**22-Hydroxyacuminatine (2b).** DIBAL-H (1.0 M in  $\text{CH}_2\text{Cl}_2$ , 57  $\mu\text{L}$ , 57  $\mu\text{mol}$ ) was added to a stirred solution of nitrile **14** (11.7 mg, 38  $\mu\text{mol}$ ) in toluene (3 mL) at  $-78^\circ\text{C}$ . The resulting reaction mixture was stirred at  $-78^\circ\text{C}$  for 3 h, when MeOH (0.1 mL) was added to quench the reaction. The reaction mixture was poured into a 1.0 M solution of oxalic acid (5 mL) and stirred at room temperature for 2 h. Solid  $\text{Na}_2\text{CO}_3$  was added to neutralize the oxalic

acid at  $0^\circ\text{C}$  and the reaction mixture was extracted with  $\text{CHCl}_3$  ( $3 \times 30$  mL). The combined organic layers were washed with  $\text{H}_2\text{O}$  ( $2 \times 5$  mL) and brine ( $2 \times 5$  mL). The organic solution was dried over  $\text{Na}_2\text{SO}_4$ , filtered, concentrated, and the residue was subjected to a flash column chromatography, eluting with  $\text{CHCl}_3$ , yielding a light yellow solid 9.0 mg (76%):  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  10.52 (s, 1 H), 8.97 (s, 1 H), 8.81 (d,  $J = 8.4$  Hz, 1 H), 8.38 (s, 1 H), 8.29 (t,  $J = 8.4$  Hz, 1 H), 8.22 (d,  $J = 8.4$  Hz, 1 H), 7.91 (d,  $J = 7.8$  Hz, 1 H), 7.82 (t,  $J = 7.8$  Hz, 1 H), 7.71 (t,  $J = 7.8$  Hz, 1 H), 7.64 (t,  $J = 7.8$  Hz, 1 H), 5.39 (s, 2 H). DIBAL-H (1.0 M in  $\text{CH}_2\text{Cl}_2$ , 58  $\mu\text{L}$ , 58  $\mu\text{mol}$ ) was added to a stirred solution of aldehyde obtained as above (9.0 mg, 29  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (2 mL) at  $-78^\circ\text{C}$ . The reaction mixture was stirred at  $-78^\circ\text{C}$  for 2 h, when MeOH (0.1 mL) was added to quench the reaction at  $-78^\circ\text{C}$ . A saturated aqueous solution of potassium sodium tartrate (5 mL) was added, and the solution was stirred at room temperature for 1 h.  $\text{CHCl}_3$  ( $3 \times 20$  mL) was used to extract the product. The combined organic layers were washed with  $\text{H}_2\text{O}$  ( $2 \times 5$  mL) and brine ( $2 \times 5$  mL). The organic solution was dried over  $\text{Na}_2\text{SO}_4$ , filtered, concentrated, and the residue was subjected to a flash column chromatography, eluting with  $\text{CHCl}_3$  and  $\text{CHCl}_3$ -MeOH (10:1), yielding a light yellow solid 6.0 mg (67%): mp  $>255^\circ\text{C}$  (dec) [lit.,<sup>8</sup> mp  $258$ – $260^\circ\text{C}$  (dec)].  $^1\text{H NMR}$  (500 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.66 (s, 1 H), 8.29 (d,  $J = 7.5$  Hz, 1 H), 8.19 (d,  $J = 8.5$  Hz, 1 H), 8.11 (d,  $J = 7.5$  Hz, 1 H), 7.83 (td,  $J = 8.5, 1.5$  Hz, 1 H), 7.81 (d,  $J = 7.0$  Hz, 1 H), 7.73 (s, 1 H), 7.69 (td,  $J = 8.0, 1.5$  Hz, 1 H), 7.57 (t,  $J = 7.5$  Hz, 1 H), 5.51 (t,  $J = 5.0$  Hz, 1 H), 5.36 (s, 2 H), 4.95 (d,  $J = 5.0$  Hz, 2 H); ESIMS  $m/z$  (rel intensity) 315 ( $\text{MH}^+$ , 100); HRESIMS  $m/z$  Calcd for  $\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}_2 + \text{H}$  315.1134, found 315.1136. Anal. ( $\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}_2 \cdot 1.0\text{H}_2\text{O}$ ) C, H, N.

**7-Bromo-12*H*-5,11a-diazadibenzo[*b,h*]fluoren-11-one (2c).** A solution of bromide **4** (193 mg, 0.66 mmol) in THF (7 mL) was added to a stirred suspension of tricyclic amine hydrobromide **3** (220 mg, 0.66 mmol) and  $\text{Et}_3\text{N}$  (3.0 mL) in THF (7 mL). The resulting mixture was stirred at room temperature for 24 h.  $\text{CHCl}_3$  (100 mL) was added to dilute the reaction mixture, which was then washed with water ( $2 \times 10$  mL) and brine ( $2 \times 10$  mL). The organic solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated, yielding a brown residue, which was used for the next operation without further purification. The product obtained as above was dissolved in acetic acid (7 mL), and sodium acetate (0.7 g) was added to the reaction mixture, which was then stirred at room temperature for 36 h. Then acetic acid was removed under



**Figure 2.** Hypothetical model of the binding of 22-hydroxyacuminatine (**2b**) in the Top1-DNA-inhibitor ternary complex. The cleaved DNA strand is on the left, while the uncleaved strand is on the right. The DNA minor groove side is facing forward, while the major groove is on the back. The model is programmed for walled (relaxed) viewing.



**Figure 3.** Electrostatic potential surfaces of 22-hydroxyacuminatine (top) and camptothecin (bottom) mapped on their total electron densities calculated at the HF/6-31G\*\* level of theory. Both electrostatic potential surface maps are scaled to a range of  $-38$  to  $+38$  kcal/mol.

reduced pressure, and the residue was dissolved in  $\text{CHCl}_3$  (100 mL), and washed with saturated  $\text{NaHCO}_3$  ( $2 \times 10$  mL), water ( $2 \times 10$  mL), and brine ( $2 \times 10$  mL). The organic solution was dried over  $\text{Na}_2\text{SO}_4$ , filtered, concentrated, and the residue was subjected to silica gel (40 g) flash column chromatography, eluting with  $\text{CHCl}_3$ , yielding a light yellow solid (49 mg, 20%): mp  $>270$  °C (dec).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.50 (d,  $J = 8.4$  Hz, 1 H), 8.37 (s, 1 H), 8.31 (d,  $J = 8.4$  Hz, 1 H), 8.10 (s, 1 H), 7.97 (d,  $J = 7.5$  Hz, 1 H), 7.91 (d,  $J = 7.8$  Hz, 1 H), 7.82 (t,  $J = 7.2$  Hz, 1 H), 7.64 (t,  $J = 6.9$  Hz, 1 H), 7.40 (t,  $J = 7.8$  Hz, 1 H), 5.37 (s, 2 H); ESIMS  $m/z$  (rel intensity) 365 ( $^{81}\text{BrMH}^+$ , 100), 363 ( $^{79}\text{BrMH}^+$ , 100). Anal. ( $\text{C}_{19}\text{H}_{11}\text{N}_2\text{OBr}$ ) C, H, N.

**3,4-Dibromo-3H-isobenzofuran-1-one (4).** A mixture of lactone **7** (906 mg, 4.27 mmol), NBS (818 mg, 4.7 mmol), and AIBN (71 mg, 0.43 mmol) in  $\text{CCl}_4$  (40 mL) was heated at reflux for 15 h.  $\text{CCl}_4$  was removed under reduced pressure. The residue was extracted with hot *n*-hexane ( $3 \times 100$  mL). Removal of hexane yielded a white powder (1.25 g, 100%): mp  $89$ – $90$  °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.89 (d,  $J = 7.8$  Hz, 1 H), 7.87 (d,  $J = 7.8$  Hz, 1 H), 7.50 (t,  $J = 7.8$  Hz, 1 H), 7.22 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.2, 147.3, 138.7, 132.6, 126.3, 125.0, 117.8, 74.5; CIMS  $m/z$  (rel intensity) 295 ( $^{81}\text{Br}^{81}\text{BrMH}^+$ , 29), 293 ( $^{81}\text{Br}^{79}\text{BrMH}^+$ , 63), 291 ( $^{79}\text{Br}^{79}\text{BrMH}^+$ , 31), 100 (100). Anal. ( $\text{C}_8\text{H}_4\text{-Br}_2\text{O}_2$ ) C, H, N.

**Methyl 3-Bromo-2-methylbenzoate (6a).** Concentrated  $\text{H}_2\text{SO}_4$  (0.5 mL) was added to a stirred solution of 3-bromo-2-methylbenzoic acid (**5**) (2.15 g, 10 mmol) in MeOH (20 mL) at room temperature. The resulting mixture was then heated under reflux for 12 h. Methanol was partially removed under reduced pressure, and  $\text{Et}_2\text{O}$  (150 mL) was added to dilute the residue, which was then washed with a saturated aqueous solution of  $\text{NaHCO}_3$  ( $2 \times 10$  mL),  $\text{H}_2\text{O}$  ( $2 \times 10$  mL), and brine ( $2 \times 10$  mL). The organic solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated, yielding a colorless liquid oil (2.15 g, 94%):  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.64 (d,  $J = 7.8$  Hz, 1 H), 7.60 (d,  $J = 7.8$  Hz, 1 H), 7.00 (t,  $J = 7.8$  Hz, 1 H), 3.98 (s, 3 H), 2.55 (s, 3 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.8, 138.7, 135.9, 132.6, 129.1, 127.1, 126.9, 52.2, 20.6; IR (film) 2997, 2951, 1725, 1434, 1285, 1255, 1096, 1010,  $753\text{ cm}^{-1}$ ; EIMS  $m/z$  (rel intensity) 230 ( $^{81}\text{BrM}^+$ , 19), 228 ( $^{79}\text{BrM}^+$ , 19), 89 (78), 63 (100). Anal. ( $\text{C}_9\text{H}_6\text{BrO}_2$ ) C, H, Br.

**3-Bromo-phthalide (7).** A solution of methyl ester **6a** (510 mg, 2.23 mmol) in  $\text{CCl}_4$  (3 mL) was placed in a round-bottomed flask immersed in an ice-cold bath. A solution of chromyl chloride (0.36 mL, 4.45 mmol) in  $\text{CCl}_4$  (3 mL) was added over a period of 1 h. The resulting mixture was stirred at room temperature for 2 h and then slowly heated to reflux for 20 h. The reaction mixture was cooled to room temperature and poured into an ice-cold saturated solution of  $\text{Na}_2\text{SO}_3$  (5 mL) at 0 °C.  $\text{EtOAc}$  ( $3 \times 30$  mL) was used to extract the product. The combined organic layers were washed with  $\text{H}_2\text{O}$  ( $2 \times 10$  mL) and brine ( $2 \times 10$  mL). The organic solution

was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated, yielding a colorless powder (356 mg, 75%): mp  $88$ – $89$  °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.67 (d,  $J = 7.8$  Hz, 1 H), 7.63 (d,  $J = 7.8$  Hz, 1 H), 7.31 (t,  $J = 7.8$  Hz, 1 H), 5.03 (s, 2 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  169.4, 146.2, 136.3, 130.5, 127.4, 124.0, 116.1, 69.4; EIMS  $m/z$  (rel intensity) 214 ( $^{81}\text{BrM}^+$ , 31), 212 ( $^{79}\text{BrM}^+$ , 30), 185 (100), 183 (98), 157 (27), 155 (29), 75(63). Anal. ( $\text{C}_8\text{H}_5\text{BrO}_2$ ) C, H, Br.

**12-Hydroxymethyl-12H-5,11a-diaza-dibenzo[*b,h*]fluoren-11-one (11).** *n*-BuLi (16  $\mu\text{L}$ , 2.5 M in hexane, 0.04 mmol) was added to a stirred solution of bromide **2c** (10 mg, 0.027 mmol) in THF (2 mL) at  $-78$  °C. The resulting dark red solution was stirred at  $-78$  °C for 15 min, when a suspension of paraformaldehyde (4 mg, 0.135 mmol) in THF (0.5 mL) was added. The reaction mixture was then allowed to warm to room temperature and stirred at room temperature for 1 h.  $\text{H}_2\text{O}$  (2 mL) was added to quench the reaction.  $\text{CHCl}_3$  (50 mL) was added to dilute the reaction mixture, which was then washed with  $\text{H}_2\text{O}$  ( $2 \times 5$  mL) and brine ( $2 \times 5$  mL). The organic solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The residue was subjected to silica gel flash column chromatography, eluting with  $\text{CHCl}_3$ -MeOH (20:1), yielding a light yellow solid (2.5 mg, 29%): mp  $>250$  °C (dec).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.53 (d,  $J = 8.1$  Hz, 1 H), 8.32 (s, 1 H), 8.25 (d,  $J = 8.7$  Hz, 1 H), 7.92 (d,  $J = 7.8$  Hz, 1 H), 7.73–7.83 (m, 4 H), 7.58–7.66 (m, 2 H), 5.95 (d,  $J = 7.5$  Hz, 1 H), 4.38 (d,  $J = 12.3$  Hz, 1 H), 4.05 (dd,  $J = 12.6$ , 7.5 Hz, 1 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  163.0, 149.1, 139.6, 137.4, 133.1, 131.2, 130.7, 129.9, 129.5, 128.8, 128.5, 128.2, 127.9 (2 C), 127.6 (2 C), 127.1, 102.3, 66.8, 66.0; ESIMS  $m/z$  (rel intensity) 315 ( $\text{MH}^+$ , 100); HREIMS  $m/z$  Calcd for  $\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}_2$  314.1055, found 314.1046.

**3-Cyano-phthalide (12).** A mixture of bromide **7** (1.0 g, 4.72 mmol) and CuCN (634 mg, 7.1 mmol) in anhydrous DMF (15 mL) was heated under reflux for 12 h. The reaction mixture was cooled to room temperature and quenched by slow addition of brine (20 mL).  $\text{EtOAc}$  ( $3 \times 60$  mL) was used to extract the product. The combined organic layers were washed with  $\text{H}_2\text{O}$  ( $2 \times 20$  mL) and brine ( $2 \times 20$  mL). The organic solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The residue was subjected to silica gel flash column chromatography, eluting with hexanes– $\text{EtOAc}$  (4:1), yielding a white powder (750 mg, 100%): mp  $173$ – $174$  °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.13 (d,  $J = 7.5$  Hz, 1 H), 7.97 (d,  $J = 7.8$  Hz, 1 H), 7.69 (t,  $J = 7.5$  Hz, 1 H), 5.45 (s, 2 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  168.8, 149.3, 137.3, 130.1 (2 C), 127.4, 114.9, 107.4, 68.6; IR (film) 3077, 2235, 1770, 1267, 1020,  $754\text{ cm}^{-1}$ ; CIMS  $m/z$  (rel intensity) 160 ( $\text{MH}^+$ , 100). Anal. ( $\text{C}_9\text{H}_5\text{-NO}_2$ ) C, H, N.

**3-Bromo-4-cyano-3H-isobenzofuran-1-one (13).** A mixture of lactone **12** (705 mg, 4.43 mmol), NBS (868 mg, 4.88 mmol), and AIBN (73 mg, 0.443 mmol) in  $\text{CCl}_4$  (30 mL) was heated under reflux for 60 h.  $\text{CCl}_4$  was removed under reduced pressure, and the residue was extracted with  $\text{Et}_2\text{O}$ . Evaporation of  $\text{Et}_2\text{O}$  resulted in a residue that was subjected to silica gel flash column chromatography, eluting with hexanes– $\text{EtOAc}$  (4:1), yielding a white powder 922 mg (87%): mp  $118$ – $119$  °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.16 (d,  $J = 7.8$  Hz, 1 H), 8.05 (d,  $J = 7.8$  Hz, 1 H), 7.78 (t,  $J = 7.8$  Hz, 1 H), 7.50 (s, 1 H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  177.7, 150.5, 138.8, 131.8, 130.2, 125.6, 113.8, 108.3, 71.9; CIMS  $m/z$  (rel intensity) 240 ( $^{81}\text{BrMH}^+$ , 18), 238 ( $^{79}\text{BrMH}^+$ , 20), 158 (100).

**7-Cyano-12H-5,11a-diazadibenzo[*b,h*]fluoren-11-one (14).** A solution of bromide **13** (150 mg, 0.63 mmol) in  $\text{CH}_3\text{CN}$  (2 mL) was added to a stirred suspension of tricyclic amine hydrobromide **3** (105 mg, 0.31 mmol) and pyridine (1.5 mL) in  $\text{CH}_3\text{CN}$  (2 mL). The resulting mixture was stirred at room temperature for 24 h.  $\text{CHCl}_3$  (100 mL) was added to dilute the reaction mixture, which was then washed with water ( $2 \times 10$  mL) and brine ( $2 \times 10$  mL). The organic solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated, yielding a brown residue, which was used for the next operation without further purification. The product obtained as above was dissolved in acetic acid (5 mL), and sodium acetate (0.5 g) was added to the reaction mixture, which was then heated

at 50 °C for 24 h. Then acetic acid was removed under reduced pressure, and the residue was dissolved in CHCl<sub>3</sub> (100 mL) and washed with saturated NaHCO<sub>3</sub> (2 × 10 mL), water (2 × 10 mL), and brine (2 × 10 mL). The organic solution was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, concentrated, and the residue was subjected to silica gel (40 g) flash column chromatography, eluting with CHCl<sub>3</sub>, yielding a light yellow solid (18 mg, 18%): mp >262 °C (dec). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.73 (d, *J* = 8.4 Hz, 1 H), 8.36 (s, 1 H), 8.25 (d, *J* = 8.4 Hz, 1 H), 8.05 (dd, *J* = 8.4, 1.2 Hz, 1 H), 7.98 (s, 1 H), 7.90 (d, *J* = 7.5 Hz, 1 H), 7.81 (td, *J* = 7.2, 1.2 Hz, 1 H), 7.63 (td, *J* = 7.2, 1.2 Hz, 1 H), 7.59 (t, *J* = 7.8 Hz, 1 H), 5.37 (s, 2 H); ESIMS *m/z* (rel intensity) 310 (MH<sup>+</sup>, 100). Anal. (C<sub>20</sub>H<sub>11</sub>N<sub>3</sub>O·0.75H<sub>2</sub>O) C, H, N.

**Molecular Modeling Procedure.** The structure of the ternary complex, containing topoisomerase I, DNA, and topotecan, was downloaded from the Protein Data Bank (PDB code 1K4T). One molecule of PEG and the topotecan carboxylate form were deleted. All of the atoms were then fixed according to the Sybyl atom types. Hydrogens were added and minimized using MMFF94s force field and MMFF94 charges. The structure of the 22-hydroxyacuminatine, constructed in Sybyl and energy minimized with the Tripos force field and Gasteiger–Hückel charges, was overlapped with the structure of topotecan using the A-, B-, C-rings, and the structure of topotecan was then deleted. The new whole complex was subsequently subjected to energy minimization using MMFF94s force field with MMFF94 charges. During the energy minimization, the structure of the 22-hydroxyacuminatine was allowed to move, while the structures of the protein, nucleic acids, and the surrounding water molecules were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/mol Å energy gradient convergence criterion and a distance-dependent dielectric function.

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**Supporting Information Available:** Elemental analysis data and <sup>1</sup>H NMR spectra of compounds **2b**, **2c**, **11**, and **39**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- Thomas, C. J.; Rahier, N. J.; Hecht, S. M. Camptothecin: Current Perspectives. *Bioorg. Med. Chem.* **2004**, *12*, 1585–1604.
- Wang, J. C. Cellular Roles of DNA Topoisomerases: A Molecular Perspective. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430–440.
- Garcia-Carbonero, R.; Supko, J. G. Current Perspectives on the Clinical Experience, Pharmacology, and Continued Development of the Camptothecins. *Clin. Cancer Res.* **2002**, *8*, 641–661.
- Burke, T. G.; Mi, Z. H. The Structural Basis of Camptothecin Interactions with Human Serum-Albumin—Impact on Drug Stability. *J. Med. Chem.* **1994**, *37*, 40–46.
- Meng, L. H.; Liao, A. Y.; Pommier, Y. Non-Camptothecin DNA Topoisomerase I Inhibitors in Cancer Therapy. *Curr. Top. Med. Chem.* **2003**, *3*, 305–320.
- Fox, B. M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline–Camptothecin Hybrids. *J. Med. Chem.* **2003**, *46*, 3275–3282.
- Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. Structures of Three Classes of Anticancer Agents bound to the Human Topoisomerase I–DNA Covalent Complex. *J. Med. Chem.* **2005**, *48*, 2336–2345.
- Lin, L. Z.; Cordell, G. A. Quinoline Alkaloids from *Camptotheca acuminata*. *Phytochemistry* **1989**, *28*, 1295–1297.
- Ma, Z.; Lee, D. Y. W. Total Synthesis of the Cytotoxic Alkaloid 22-Hydroxyacuminatine. *Tetrahedron Lett.* **2004**, *45*, 6721–6723.
- Corey, E. J.; Crouse, D. N.; Anderson, J. E. Total Synthesis of Natural 20(S)-Camptothecin. *J. Org. Chem.* **1975**, *40*, 2140–2141.
- Zalkow, L. H.; Nabors, J. B.; French, K.; Bisarya, S. C. Studies in Synthesis of Camptothecin—Efficient Synthesis of 2,3-Dihydro-1*H*-Pyrrolo 3,4-*B* Quinoline. *J. Chem. Soc. (C)* **1971**, 3551–3554.
- Lieberman, S. C.; Connor, R. *p*-Nitrobenzaldehyde. *Org. Synth. Collect. Vol. II* **1943**, 441–443.
- Trahanovsky, W. S.; Young, L. B. Controlled Oxidation of Organic Compounds with Cerium(IV). II. The Oxidation of Toluenes. *J. Org. Chem.* **1966**, *31*, 2033–2035.
- Plobeck, N.; Delorme, D.; Wei, Z. Y.; Yang, H.; Zhou, F.; Schwarz, P.; Gawell, L.; Gagnon, H.; Pelcman, B.; Schmidt, R.; Yue, S. Y.; Walpole, C.; Brown, W.; Zhou, E.; Labarre, M.; Payza, K.; St-Onge, S.; Kamassah, A.; Morin, P. E.; Projean, D.; Ducharme, J.; Roberts, E. New Diarylmethylpiperazines as Potent and Selective Nonpeptidic Delta Opioid Receptor Agonists with Increased In Vitro Metabolic Stability. *J. Med. Chem.* **2000**, *43*, 3878–3894.
- Hartford, W. H.; Darrin, M. The Chemistry of Chromyl Compounds. *Chem. Rev.* **1958**, *58*, 1–61.
- Wheeler, O. H. Etard Reaction. I. Its Scope and Limitation with Substituted Toluenes. *Can. J. Chem.* **1958**, *36*, 667–671.
- Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision B.05; Gaussian, Inc.: Pittsburgh, PA, 2003.
- Ellis, G. P.; Romney-Alexander, T. M. Cyanation of Aromatic Halides. *Chem. Rev.* **1987**, *87*, 779–794.
- Cagir, A.; Jones, S. H.; Gao, R.; Eisenhauer, B. M.; Hecht, S. M. Luotonin A. A Naturally Occurring Human DNA Topoisomerase I Poison. *J. Am. Chem. Soc.* **2003**, *125*, 13628–13629.
- Kohlhagen, G.; Paull, K.; Cushman, M.; Nagafuji, P.; Pommier, Y. Protein-Linked DNA Strand Breaks Induced by NSC 314622, a Novel Noncamptothecin Topoisomerase I Poison. *Mol. Pharmacol.* **1998**, *54*, 50–58.
- Antony, S.; Kohlhagen, G.; Agama, K.; Jayaraman, M.; Cao, S.; Durrani, F. A.; Rustum, Y. M.; Cushman, M.; Pommier, Y. Cellular Topoisomerase I Inhibition and Antiproliferative Activity by MJ-III-65 (NSC 706744), an Indenoisoquinoline Topoisomerase I Poison. *Mol. Pharmacol.* **2005**, *67*, 523–530.
- Xiao, X.; Miao, Z.-H.; Antony, S.; Pommier, Y.; Cushman, M. Dihydroindenoisoquinolines Function as Prodrugs of Indenoisoquinolines. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2795–2798.
- Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analogue. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387–15392.
- Xiao, X.; Cushman, M. An Ab Initio Quantum Mechanics Calculation that Correlates with Ligand Orientation and DNA Cleavage Site Selectivity in Camptothecin–DNA–Topoisomerase I Ternary Cleavage Complexes. *J. Am. Chem. Soc.* **2005**, *127*, 9960–9961.
- Xiao, X.; Antony, S.; Pommier, Y.; Cushman, M. On the Binding of Indeno[1,2-*c*]isoquinolines in the DNA–Topoisomerase I Cleavage Complex. *J. Med. Chem.* **2005**, *48*, 3231–3238.
- Cheng, K. J.; Rahier, N. J.; Eisenhauer, B. M.; Gao, R.; Thomas, S. J.; Hecht, S. M. 14-Azacamptothecin: A Potent Water-Soluble Topoisomerase I Poison. *J. Am. Chem. Soc.* **2005**, *127*, 838–839.
- Xiao, X.; Cushman, M. The Effect of E-Ring Modifications in Camptothecin on Topoisomerase I Inhibition: A Quantum Mechanics Treatment. *J. Org. Chem.* **2005**, *70*, 9584–9587.