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Novel Autoxidative Cleavage Reaction of 9-Fluoredenes Discovered during Synthesis of a Potential DNA-Threading Indenoisoquinoline

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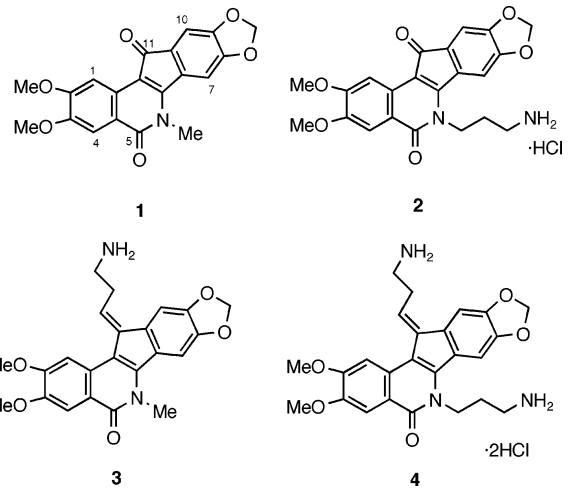
The indenoisoquinolines are a novel class of cytotoxic non-camptothecin topoisomerase I inhibitors. A potential DNA-threading agent was designed by attaching different amine side chains on the lactam nitrogen as well as on the C11 position of the indenoisoquinoline ring system. It was hypothesized that substituents on the lactam nitrogen could protrude out toward the DNA major groove while those on the C11 project out toward the DNA minor groove in the ternary "cleavage complex." Compound **4** was synthesized in order to test this DNA-threading scenario. It was found unexpectedly that an alkenyl substituent on the C11 position was autoxidatively cleaved under basic conditions to afford a ketone. A possible mechanism for this unusual oxidative cleavage was proposed on the basis of the studies of a 9-fluoredene model compound. The proposed mechanism was further supported by computational studies. Although the designed compound **4** showed potent cytotoxicities in various cancer cell lines, it was less potent than its nonthreading counterparts and was not a topoisomerase I inhibitor.

Introduction

Indenoisoquinoline **1**, first described in 1978 as a byproduct obtained during a total synthesis of nitidine chloride,[1] did not capture much attention until the late 1990s, when it was found to be a non-camptothecin topoisomerase I inhibitor with moderate cytotoxicity in cancer cell cultures.[2] Fueled by this finding, a number of indenoisoquinoline analogues have been synthesized to optimize both the cytotoxicity and topoisomerase I inhibitory potency.[3−9] Two of the most potent analogues proved to be compound **2**,[4] with an aminopropyl group at the lactam nitrogen, and **3**,[6] having an aminopropylidene side chain at the C11 position.



1 **2** **3** **4**

According to the hypothetical model of the binding of indenoisoquinolines in the DNA-topoisomerase I "cleavage complex" (Figure 1),[7,9] substituents on the lactam nitrogen project out of the duplex toward the DNA major groove while those at the C11 position protrude out of the duplex toward the DNA minor groove. The increased biological activities of compounds **2** and **3** relative to those

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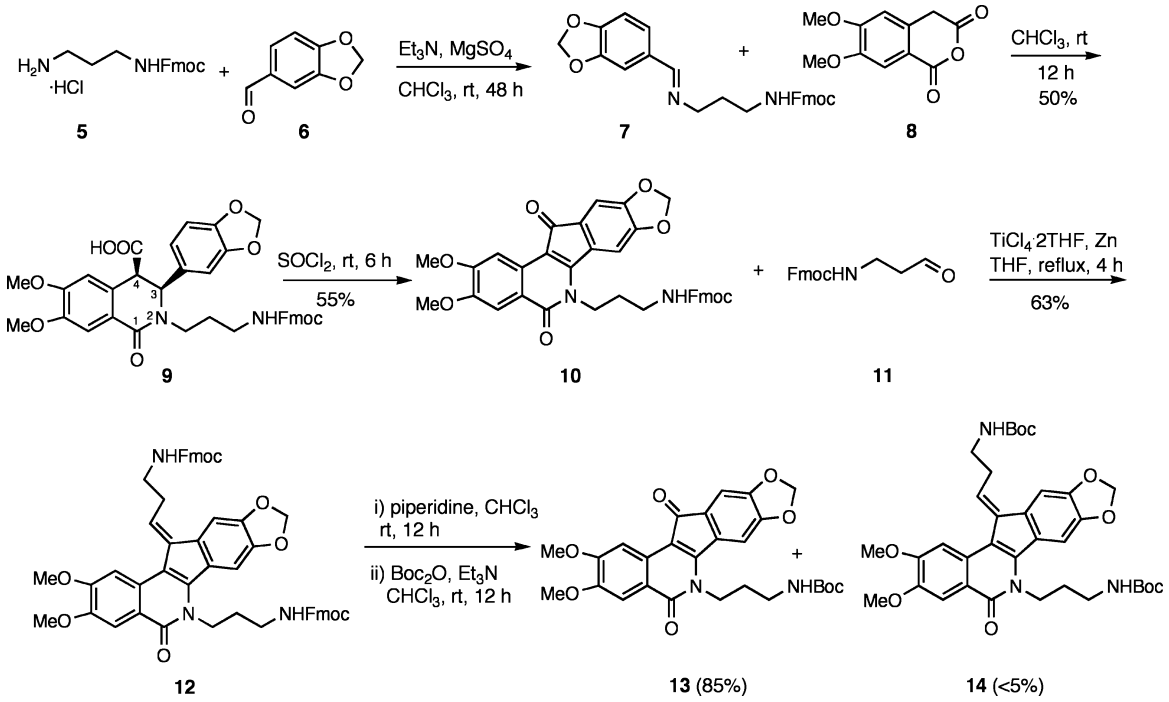
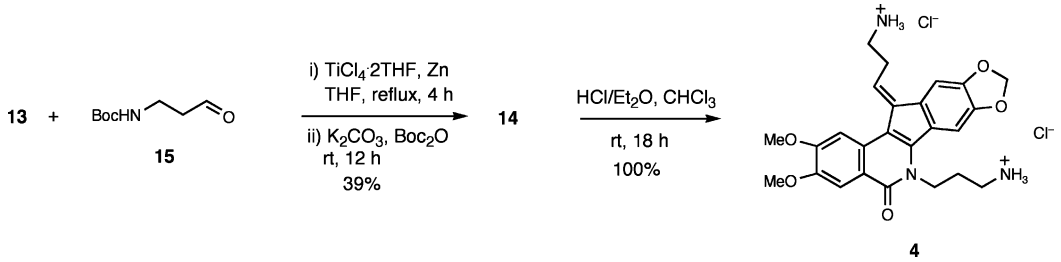
SCHEME 1



SCHEME 2



of the parent compound **1** were partly explained as being due to the hydrogen bond formation and electrostatic interactions between the amino groups on the side chains and the surrounding DNA base residues and the enzyme amino acid residues in the ternary cleavage complexes.[7,9] A further extension of this concept would be to attach both aminated side chains in a single molecule **4**, which could conceivably occupy both the DNA major and minor grooves (Figure 1). Assuming compound **4** could in fact intercalate between the DNA base pairs in the cleavage complex, the resulting DNA-threading agent could hypothetically have increased topoisomerase I inhibitory potency as well as cancer cell cytotoxicity by stabilizing the ternary cleavage complex.[10−12]

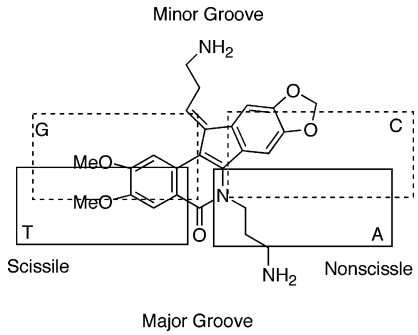


FIGURE 1. Hypothetical model of the orientation of indenoisoquinoline **4** relative to DNA in the ternary complex containing topoisomerase I, DNA, and the inhibitor **4**.

Results and Discussion

The free base of compound **4** was expected to be prepared from global deprotection of Fmoc-protected compound **12**, which in turn could be made from McMurry coupling between indenoisoquinoline derivative **10** and aldehyde **11** (Scheme 1). Thus, mono-Fmoc-protected 1,3-propanediamine **5**[13] was condensed with piperonal (**6**) to form the imine **7**. Condensation of imine **7** with 3,4-dimethoxyhomophthalic anhydride (**8**)[1] gave cis acid **9**, whose relative configuration was determined by ^{1}H NMR.[14] Treatment of the cis acid **9** with thionyl chloride provided the desired indeno[1,2-*c*]isoquinoline **10**,[1] which was then coupled with aldehyde **11**[15] under McMurry conditions[6,9] to afford the Fmoc-protected compound **12**.

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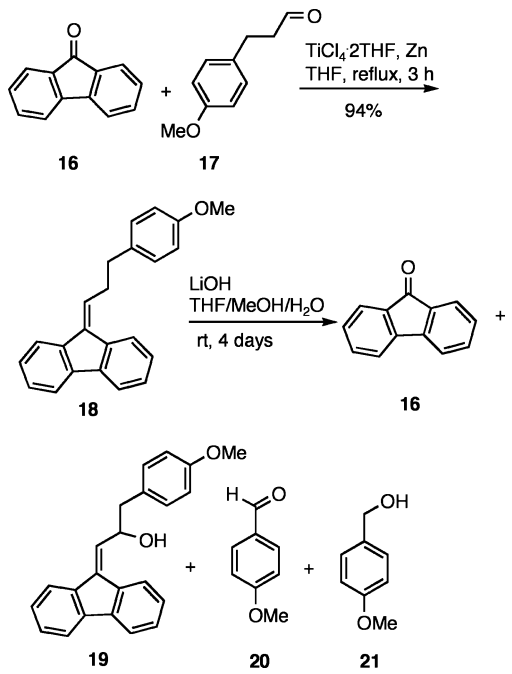
SCHEME 3

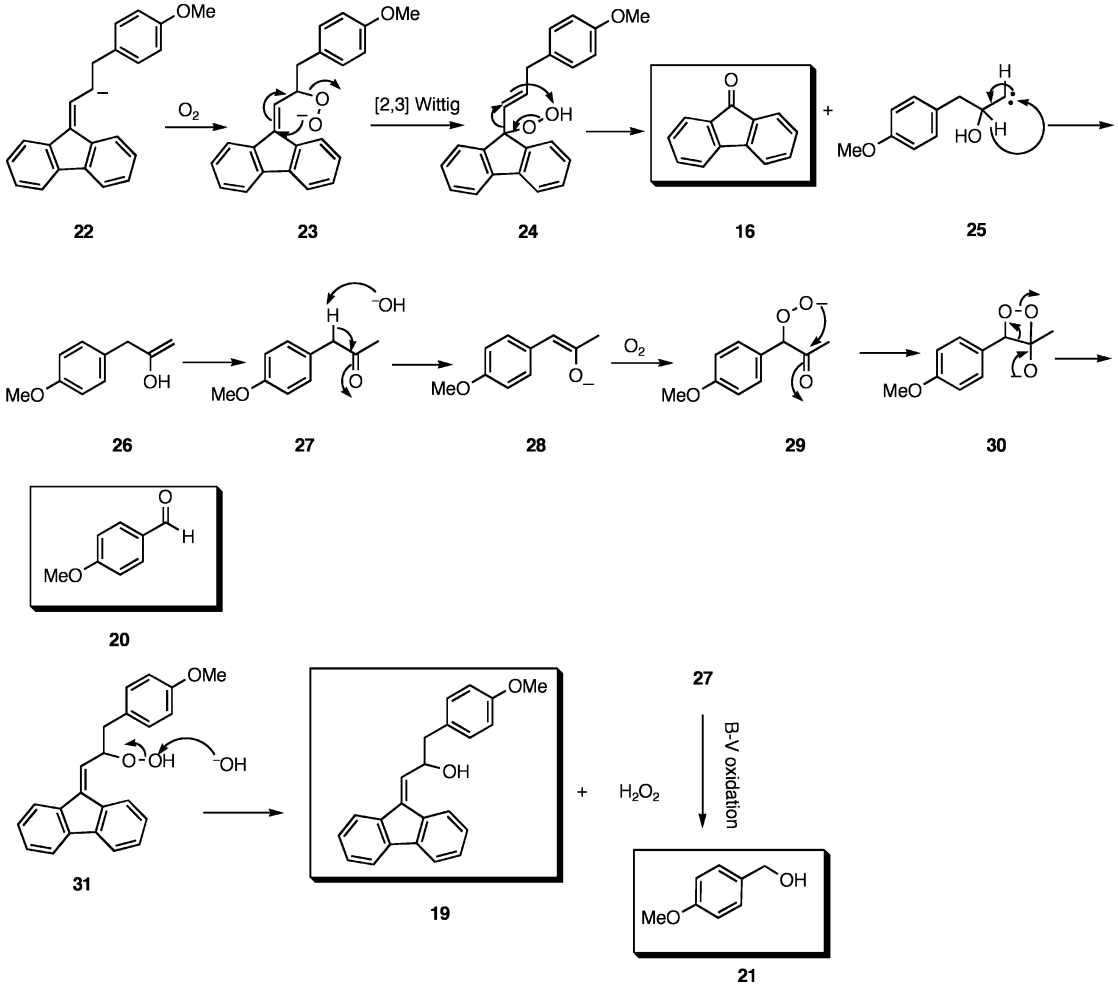


Deprotection of Fmoc groups in **12** under basic conditions, however, gave predominantly the oxidatively cleaved product **13** (85%) after Boc protection. Only a trace amount of the desired compound **14** was obtained.

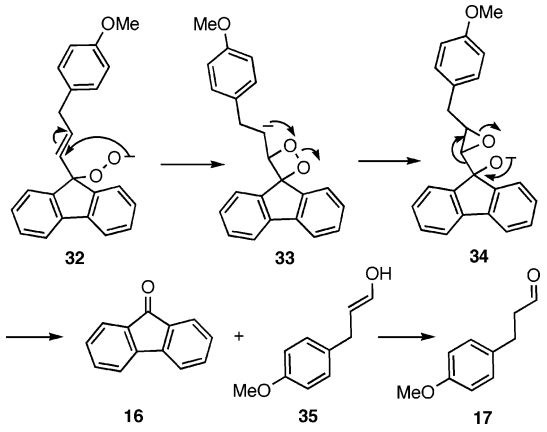
The fact that the C11 exocyclic double bond in related indenoisoquinolines was stable under acidic conditions[6,9] suggested that Boc might be an appropriate protecting group for these amino groups. Therefore, the Fmoc in **10** was exchanged for Boc to yield **13** in 93% yield, which was then again McMurry coupled with Boc-protected aldehyde **15**[6] generating **14** in 39% yield after Boc reprotection (Scheme 2).[9] The hydrochloride **4** was obtained in quantitative yield by acidic Boc deprotection.

To shed some light on the unusual oxidative cleavage of **12** under alkaline conditions, a model compound **18** was prepared to study the mechanism. McMurry coupling between 9-fluorenone (**16**) and aldehyde **17**, derived from Swern oxidation of the corresponding alcohol, provided the alkene **18** in 94% yield (Scheme 3). The near quantitative yield of this cross-coupling reaction suggested a titanocarbenoid intermediate.[16] When the model compound **18** was treated with piperidine in $CHCl_3$ in the presence of air at room temperature, in striking contrast to compound **12**, no decomposition was observed even after stirring for 7 days. However, when the stronger base LiOH was employed, appreciable oxidative cleavage was observed after 4 days. In addition to the recovered starting material **18** (73% conversion), four products **16** (62%), **19** (32%), **20** (14%), and **21** (22%) were

SCHEME 4



SCHEME 5



isolated from the reaction mixture (the yields are based on recovered starting material) after careful silica gel chromatography. When oxygen was stringently excluded from the reaction mixture, no cleavage reaction was observed even after stirring for 7 days.

A plausible mechanism for this unusual oxidative cleavage is outlined in Scheme 4. Deprotonation of **18** generates an allylic anion **22**. Autoxidation[17,18] of anion **22** gives rise to peroxide **23**, which undergoes a [2,3] sigmatropic rearrangement (oxygen analogue of the [2,3] Wittig rearrangement) and protonation yielding hydroperoxide **24**. The carbon−oxygen bond formation that occurs in the conversion of **22** to **23** is thought to be initiated by a one-electron transfer from the carbanion to triplet oxygen to form a carbon radical and the radical anion of molecular oxygen, followed by short radical chain reactions and/or radical pair cage-type reactions.[19] Fragmentation of **24** produces 9-fluorenone (**16**) and an α-hydroxy carbene species **25**, rearrangement of which provides ketone **27**.[20] A similar autoxidation and fragmentation of ketone **27** gives *p*-anisaldehyde (**20**).[17,18] The hydroperoxide **31** derived from protonation of peroxide **23** undergoes an oxygen−oxygen bond cleavage in the presence of hydroxide, affording alcohol **19** and hydrogen peroxide, the latter of which Baeyer−Villiger oxidizes the ketone **27**, resulting in the formation of *p*-methoxybenzyl alcohol (**21**). It is possible, but not likely in this case, that *p*-methoxybenzyl alcohol (**21**) is derived from Cannizzaro reaction of *p*-anisaldehyde (**20**), because *p*-methoxybenzoic acid was not observed in the reaction mixture. The intermediacy of ketone **27** instead of aldehyde **17** during the course of oxidative cleavage is consistent with the observation that only ketone **27** could produce a trace amount (2%, isolated yield) of *p*-anisaldehyde (**20**), among other products, when treated with LiOH in the presence of air. Upon similar treatment, no *p*-anisaldehyde (**20**) was generated from aldehyde **17**. This also rules out such mechanisms as shown in Scheme 5[21] involving the formation of aldehyde **17** as the intermediate. Also in agreement with this mechanism, compound **36**, without any allylic protons, did not show any decomposition under the same conditions.

To gain further insight into the mechanism, the relative acidities of the protons indicated in structures **27**, **37**, and **38** were compared by calculating their respective heterolytic bond dissociation enthalpies (BDE) derived from the isodesmic reaction shown in eq 1. An

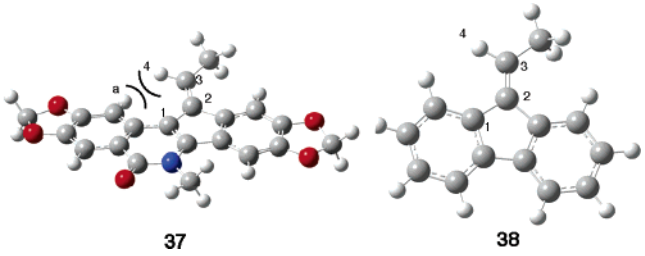
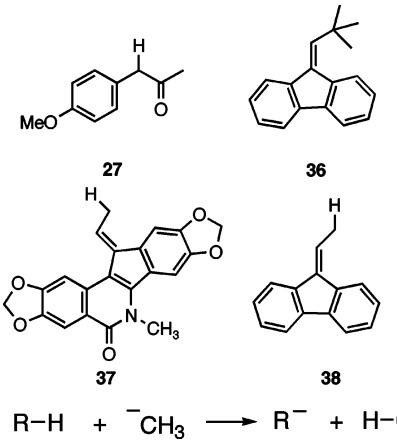


FIGURE 2. B3LYP/6-31G(d,p) optimized geometries of compounds **37** and **38**.

experimental BDE of 416.8 kcal/mol for methane was employed for calculations.[22] All calculations were performed in Gaussian03[23] at the B3LYP/6-31G(d,p) level of theory. The calculated BDE (298 K) is 318.7 kcal/mol for **37**, 327.5 kcal/mol for **38**, and 330.0 kcal/mol for **27** after inclusion of thermal and zero-point corrections. On the basis of these calculations, the proton indicated in structure **37** is even more acidic than that in **27**, which is adjacent to a carbonyl and phenyl ring. The weaker acidity of the proton indicated in **38** in comparison to that in **37** may explain why compound **18** was left intact when treated with a weak base such as piperidine while compound **12** was reactive under the same conditions.



$$R{-}H + {}^{-}CH_3 \longrightarrow R^{-} + H{-}CH_3 \quad (1)$$

The final optimized structures of compounds **37** and **38** are shown in Figure 2. The exo-cyclic double bond in **37** is not coplanar with the aromatic ring system (the dihedral angle of C1−C2−C3−H4 is 1.8° vs 0.0° in **38**), presumably as a result of the steric clash between the olefinic proton H4 and the proton labeled "a" in Figure 2. The unusual geometry may contribute to the driving force for the oxidative cleavage reaction to go to comple-

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tion to give the indenoisoquinoline compound in which the whole system is highly conjugated and planar.

Compound **4** was examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of the compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. Judged by the mean graph midpoint (MGM) value (1.38 μM), this compound was cytotoxic, although it was less potent than either **2** or **3** (see Supporting Information). Interestingly, compound **4** has no capability to inhibit the topoisomerase I as seen in the topoisomerase I-mediated DNA cleavage assay (data not shown).[2] The cytotoxicity exerted by compound **4** therefore has nothing to do with topoisomerase I inhibition. As a consequence of these results, it can be concluded that the hypothetical ternary complex involving compound **4** does not form, although the evidence indicates quite clearly that similar complexes result with both of the topoisomerase I inhibitors **2** and **3**. Since a reasonable model of a ternary complex involving compound **4** can be derived by molecular modeling, it seems likely that the additional side chain present in **4** inhibits the process of complex formation rather than destabilizing the complex itself.

In conclusion, a potential DNA-threading agent was designed and synthesized on the basis of the hypothetical binding model of indenoisoquinolines in the DNA-topoisomerase I "cleavage complex". Although the designed compound **4** showed cytotoxicity across different cancer cell lines, it had no topoisomerase I inhibitory activity. The overall outcome of the oxidative alkene cleavage reaction of **18** to afford **16** and intermediate **27** is similar to that observed in the photochemically induced 2 + 2 addition of alkenes with singlet oxygen to form dioxetanes that cleave to two carbonyl compounds.[24−31] However, the reactions clearly proceed by different mechanisms, since the reactions of **12** and **18** involve triplet oxygen instead of singlet oxygen. The presently reported alkaline autoxidative cleavage reaction of the C11 alkenyl side chain in the indenoisoqinoline **12** is a novel transformation. A possible mechanism for this transformation has been proposed on the basis of the experimental results and computational details of a model study on the 9-fluoredene compound **18**.

Experimental Section

11-(3′-Aminopropylidene)-6-(3′-aminopropyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5-oxo-11*H*-indeno-[1,2-*c*]isoquinoline Dihydrochloride (4). HCl (2.0 M in ether, 1 mL, 2 mmol) was added to a stirred solution of **14** (17 mg, 0.026 mmol) in $CHCl_3$ (2 mL) at room temperature. The resulting mixture was stirred at room temperature for an additional 18 h, then filtered, and washed with $CHCl_3$, yielding a light yellow powder (13.5 mg, 100%): mp > 244 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.11 (brs, 2 H), 7.89 (brs, 2 H), 7.70 (s, 1 H), 7.58 (s, 1 H), 7.52 (s, 1 H), 7.50 (s, 1 H), 6.98 (brt, 1 H), 6.16 (s, 2 H), 4.60 (t, J = 5.7 Hz, 2 H), 4.02 (s, 3 H), 3.88 (s, 3 H), 3.35−3.44 (m, 2 H), 3.12−3.22 (m, 2 H), 2.85−2.96 (m, 2 H), 2.05−2.15 (m, 2 H); ESIMS m/z (rel intensity) 450 (MH^+, 100). Anal. Calcd for $C_{25}H_{29}N_3O_5Cl_2$·HCl·0.95$CHCl_3$: C, 46.36; H, 4.64; N, 6.25. Found: C, 46.07; H, 5.23; N, 6.30.

***cis*-4-Carboxy-2-(9*H*-fluoren-9-yl-methoxycarbonylaminopropyl)-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (9).** Et_3N (1.35 mL, 9.72 mmol) and $MgSO_4$ (5.0 g) were added to a stirred solution of **5** (2.3 g, 7.13 mmol) in $CHCl_3$ (250 mL) at room temperature. Piperonal **(6)** (972 mg, 6.48 mmol) was added, and the resulting mixture was stirred for an additional 48 h. The suspension was filtered and washed with $CHCl_3$. The organic solution was washed with water (2 × 30 mL) and brine (2 × 30 mL), dried over Na_2SO_4, filtered, and concentrated, yielding a colorless oil (2.97 g) containing 20% of piperonal based on ^{1}H NMR. The mixture was used for next operation without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1 H), 7.69 (d, J = 7.5 Hz, 2 H), 7.56 (d, J = 7.5 Hz, 2 H), 7.39 (t, J = 7.5 Hz, 2 H), 7.34 (s, 1 H), 7.28 (t, J = 7.5 Hz, 2 H), 7.10 (d, J = 8.4 Hz, 1 H), 6.80 (d, J = 8.4 Hz, 1 H), 5.94 (s, 2 H), 5.47 (brs, 1 H), 4.34 (d, J = 6.9 Hz, 2 H), 4.19 (t, J = 6.9 Hz, 1 H), 3.64 (t, J = 6.3 Hz, 2 H), 3.36 (q, J = 6.0 Hz, 2 H), 1.88 (quin, J = 6.3 Hz, 2 H). Homophthalic anhydride derivative **8** (1.42 g, 6.38 mmol) was added to a stirred solution of imine **7** (2.97 g, containing 20% piperonal, 6.38 mmol) in $CHCl_3$ (20 mL) at room temperature. The resulting mixture was further stirred at room temperature for 12 h. Et_2O (15 mL) was added, and the precipitate was collected by filtration. The residue was washed with $CHCl_3$, yielding a light yellow solid (2.07 g, 50%): mp 212−214 °C; ^{1}H NMR (300 MHz, DMSO−d_6) δ 7.87 (d, J = 7.5 Hz, 2 H), 7.66 (d, J = 7.5 Hz, 2 H), 7.55 (s, 1 H), 7.40 (t, J = 7.5 Hz, 2 H), 7.31 (t, J = 7.5 Hz, 2 H), 7.14 (s, 1 H), 6.74 (d, J = 8.4 Hz, 1 H), 6.52 (d, J = 8.4 Hz, 1 H), 6.45 (s, 1 H), 5.92 (s, 2 H), 4.99 (d, J = 6.0 Hz, 1 H), 4.56 (d, J = 6.0 Hz, 1 H), 4.32 (d, J = 6.6 Hz, 2 H), 4.19 (t, J = 6.6 Hz, 1 H), 3.81 (s, 3 H), 3.74 (s, 3 H), 2.99 (brs, 2 H), 2.75 (brs, 2 H), 1.64 (brs, 2 H); ESIMS (rel intensity) m/z 651 (MH^+, 100). Anal. Calcd for $C_{37}H_{34}N_2O_9$·0.45$CHCl_3$: C, 63.86; H, 4.93; N, 3.98. Found: C, 63.99; H, 5.17; N, 3.72.

6-(9*H*-Fluoren-9-yl-methoxycarbonylaminopropyl)-2,3-dimethoxy-5,11-dioxo-8,9-methylenedioxy-11*H*-indeno-[1,2-*c*]isoquinoline (10). Thionyl chloride (5 mL) was slowly added to the acid **9** (300 mg, 0.46 mmol) with stirring. The resulting mixture was then stirred for an additional 6 h. Excess thionyl chloride was removed under reduced pressure. Benzene (2 × 5 mL) was added, and the mixture was concentrated in vacuo. The residue was then subjected to silica gel (40 g) flash column chromatography, eluting with $CHCl_3$ to obtain a dark red solid (160 mg, 55%): mp 175−177 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, J = 7.5 Hz, 2 H), 7.84 (s, 1 H), 7.67 (d, J = 7.5 Hz, 2 H), 7.46 (s, 1 H), 7.38 (t, J = 7.5 Hz, 2 H), 7.30 (t, J = 7.5 Hz, 2 H), 7.23 (s, 1 H), 7.05 (s, 1 H), 6.14 (s, 2 H), 4.38 (brs, 2 H), 4.30 (d, J = 6.6 Hz, 2 H), 4.20 (t, J = 6.6 Hz, 1 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.14 (brs, 2 H), 1.98 (brs, 2 H);

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ESIMS (rel intensity) *m*/*z* 653 (MNa^+,100). Anal. Calcd for $C_{37}H_{30}N_2O_8 \cdot 1.4H_2O$: C, 67.76; H, 5.04; N, 4.27. Found: C, 67.78; H, 5.15; N, 3.93.

11-[3′-(9*H*-Fluorenylmethoxycarbonyl)aminopropylidene]-6-[3′-(9*H*-fluorenylmethoxycarbonyl)aminopropyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (12). $TiCl_4$−THF (1:2) complex (160 mg, 0.48 mmol) and zinc dust (63 mg, 0.96 mmol) were put in a two-necked round-bottomed flask. THF (7 mL) was added. The resulting suspension was heated under reflux for 4 h. At this point, a mixture of aldehyde **11**[15] (56 mg, 0.19 mmol) and indenoisoquinoline **10** (100 mg, 0.16 mmol) in THF (7 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 4 h, and then 4 N HCl (5 mL) was added to the reaction mixture at 0 °C. The resulting mixture was stirred at 0 °C for 1 h. $CHCl_3$ (3 × 60 mL) was used to extract the product, and the combined organic layers were washed with H_2O (2 × 10 mL) and brine (2 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was subjected to silica gel (80 g) flash column chromatography, eluting with $CHCl_3$, giving a yellow powder (89 mg, 63%): mp 184−186 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19−7.68 (m, 20 H), 6.89 (t, J = 6.0 Hz, 1 H), 6.14 (t, J = 6.9 Hz, 1 H, exchangeable with D_2O), 5.98 (s, 2 H), 5.12 (brs, 1 H), 4.57 (brs, 2 H), 4.37 (d, J = 6.9 Hz, 4 H), 4.22 (t, J = 6.9 Hz, 2 H), 4.00 (s, 3 H), 3.95 (s, 3 H), 3.58 (brs, 2 H), 3.25 (brs, 2 H), 3.04 (brs, 2 H), 2.03 (brs, 2 H); ESIMS *m*/*z* (rel intensity) 894 (MH^+, 100). Anal. Calcd for $C_{55}H_{47}N_3O_9 \cdot 0.85H_2O$: C, 72.65; H, 5.40; N, 4.62. Found: C, 73.02; H, 6.17; N, 4.60.

6-(3′-*tert*-Butoxycarbonylaminopropyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (13). Fmoc-protected indenoisoquinoline derivative **10** (400 mg, 0.64 mmol) was dissolved in $CHCl_3$−piperidine (1:1, 60 mL) and stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the residue was subjected to a short silica gel column, eluting with $CHCl_3$, to remove the side product derived from the Fmoc moiety. The product was eluted by $CHCl_3$−MeOH−$NH_3 \cdot H_2O$ (100:30:5). The crude product (260 mg) was dissolved in $CHCl_3$ (20 mL), and then Et_3N (0.18 mL, 1.28 mmol) and Boc_2O (209 mg, 0.96 mmol) were added sequentially to the stirred solution at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred at room temperature for 20 h. $CHCl_3$ (150 mL) was added to dilute the reaction mixture, which was then washed with 1 N HCl (2 × 10 mL), water (2 × 10 mL), and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered, and concentrated, and the residue was subjected to silica gel (40 g) flash column chromatography, eluting with $CHCl_3$, yielding a purple powder (301 mg, 93%): mp 215−217 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1 H), 7.63 (s, 1 H), 7.07 (s, 1 H), 7.00 (s, 1 H), 6.08 (s, 2 H), 4.52 (t, J = 7.2 Hz, 2 H), 4.03 (s, 3 H), 3.98 (s, 3 H), 3.20−3.30 (m, 2 H), 1.97−2.08 (m, 2 H), 1.44 (s, 9 H); CIMS *m*/*z* (rel intensity) 508 (M^+,100). Anal. Calcd for $C_{27}H_{28}N_2O_8 \cdot 0.55H_2O$: C, 62.55; H, 5.66; N, 5.40. Found: C, 62.44; H, 5.61; N, 5.45.

11-[3′-*tert*-Butoxycarbonylaminopropylidene]-6-(3′-*tert*-butoxycarbonylaminopropyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (14). $TiCl_4$−THF (1:2) complex (200 mg, 0.60 mmol) and zinc dust (78 mg, 1.2 mmol) were put in a three-necked round-bottomed flask. THF (7 mL) was added. The resulting suspension was heated under reflux for 4 h. At this point, a mixture of Boc-protected β-alaninal (**15**)[6] (42 mg, 0.24 mmol) and indenoisoquinoline **13** (100 mg, 0.20 mmol) in THF (7 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 4 h. HCl (4 N, 5 mL) was added to the reaction mixture at 0 °C, and the reaction mixture was further stirred at 0 °C for 1 h. Anhydrous K_2CO_3 was added to neutralize HCl at 0 °C until pH > 7. Boc_2O (536 mg, 2 mmol) was added to the reaction mixture, and the reaction mixture was stirred overnight. $CHCl_3$ (200 mL) was added to dilute the reaction mixture, which was then washed with 1 N HCl (2 × 15 mL), H_2O (2 × 15 mL), and brine (2 × 15 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered, and concentrated, and the residue was subjected to silica gel (40 g) flash column chromatography, eluting with $CHCl_3$, yielding a yellow powder (50 mg, 39%): mp 193−195 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1 H), 7.50 (s, 1 H), 7.32 (s, 1 H), 7.22 (s, 1 H), 6.93 (t, J = 6.9 Hz, 1 H), 6.07 (s, 2 H), 5.72 (brs, 1 H, exchangeable with D_2O), 4.70 (brs, 1 H, exchangeable with D_2O), 4.62 (t, J = 7.2 Hz, 2 H), 4.07 (s, 3 H), 4.01 (s, 3 H), 3.51 (q, J = 5.7 Hz, 2 H), 3.18 (q, J = 5.7 Hz, 2 H), 3.02 (q, J = 6.6 Hz, 2 H), 2.04 (quin, J = 5.7 Hz, 2 H), 1.44 (s, 9 H), 1.38 (s, 9 H); ESIMS *m*/*z* (rel intensity) 650 (MH^+, 100). Anal. Calcd for $C_{35}H_{43}N_3O_9$: C, 64.70; H, 6.67; N, 6.47. Found: C, 64.53; H, 6.45; N, 6.22.

3-(4-Methoxyphenyl)-propanal (17). A solution of DMSO (1.4 mL, 20 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of oxalyl chloride (872 μL, 10 mmol) in CH_2Cl_2 (20 mL) at −78 °C over 30 min. Upon completion of the addition, the mixture was stirred at −78 °C for 5 min, followed by addition of a solution of 3-(4-methoxyphenyl)-1-propanol (1.48 g, 5 mmol) in CH_2Cl_2 (10 mL) over 30 min at −78 °C. The resulting mixture was stirred at −78 °C for 40 min. Then, Et_3N (4.2 mL, 30 mmol) was added dropwise over 10 min. The resulting mixture was allowed to warm to 0 °C and stirred at 0 °C for 1 h. Water (10 mL) was added to quench the reaction. $CHCl_3$ (200 mL) was added to dilute the reaction mixture. The organic layer was then separated from the water layer, which was further washed with water (2 × 15 mL) and brine (2 × 15 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to silica gel (80 g) flash chromatography, eluting with *n*-hexanes−EtOAc (10:1), yielding a colorless oil (800 mg, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (brs, 1 H), 7.09 (d, J = 8.4 Hz, 2 H), 6.82 (d, J = 8.4 Hz, 2 H), 3.75 (s, 3 H), 2.88 (t, J = 7.2 Hz, 2 H), 2.71 (t, J = 6.9 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.7, 158.0, 132.2, 129.1 (2 C), 113.9 (2 C), 55.1, 45.4, 27.1; EIMS (rel intensity) *m*/*z* 164 (M^+, 49), 121 (100). Anal. Calcd for $C_{10}H_{12}O_2$: C, 73.15; H, 7.37. Found: C, 73.26; H, 7.35.

9-[3′-(4-Methoxyphenyl)-propylidene]-fluorenone (18). $TiCl_4$−THF (1:2) complex (1.0 g, 3.0 mmol) and zinc dust (390 mg, 6.0 mmol) were put in a two-necked round-bottomed flask. THF (15 mL) was added. The resulting suspension was heated under reflux for 4 h. A mixture of aldehyde **17** (197 mg, 1.2 mmol) and 9-fluorenone (**16**) (180 mg, 1.0 mmol) in THF (10 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 3 h. Then, 4 N HCl (15 mL) was added to the reaction mixture at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h. EtOAc (150 mL) was added to dilute the reaction mixture, which was then washed with water (2 × 15 mL) and brine (2 × 15 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered, and concentrated, and the residue was subjected to silica gel (40 g) flash column chromatography, eluting with *n*-hexanes−EtOAc (20:1), yielding a solid as colorless needles (292 mg, 94%): mp 69−70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, J = 7.2 Hz, 1 H), 7.68 (d, J = 7.2 Hz, 1 H), 7.63 (d, J = 7.5 Hz, 1 H), 7.57 (d, J = 7.5 Hz, 1 H), 7.18−7.32 (m, 4 H), 7.15 (d, J = 8.1 Hz, 2 H), 6.82 (d, J = 8.1 Hz, 2 H), 6.69 (t, J = 7.2 Hz, 1 H), 3.72 (s, 3 H), 3.07 (q, J = 7.5 Hz, 2 H), 2.87 (t, J = 7.5 Hz, 2 H); ESIMS (rel intensity) *m*/*z* 313 (MH^+, 100). Anal. Calcd for $C_{23}H_{20}O \cdot 0.15H_2O$: C, 87.67; H, 6.49. Found: C, 87.58; H, 6.54.

Oxidative Cleavage of 9-[3′-(4-Methoxyphenyl)-propylidene]-fluorenone (18). $LiOH \cdot H_2O$ (107 mg, 2.6 mmol) was added to a stirred solution of **18** (80 mg, 0.26 mmol) in THF−MeOH−H_2O (2:2:1, 12.5 mL) at room temperature. The reaction mixture was stirred at room temperature for 4 days. During the course of the reaction, air was allowed to enter the reaction system. EtOAc (100 mL) was added to dilute the reaction mixture, which was washed with HCl (1 N, 2 × 10 mL), H_2O (2 × 10 mL), and brine (2 × 10 mL). The organic solution was dried over anhydous Na_2SO_4, filtered, and concentrated, and the residue was subjected to silica gel (40

g) flash column chromatography, gradiently eluting with *n*-hexane to *n*-hexanes−EtOAc (3:1), yielding starting material **18** (21.5 mg, 27%), **16** (21 mg, 62% brsm), **19** (20 mg, 32% brsm), **20** (3.7 mg, 14% brsm), and **21** (5.7 mg, 22% brsm). Compound **19** was obtained as a light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.5 Hz, 1 H), 7.69 (d, J = 7.5 Hz, 1 H), 7.63 (t, J = 7.5 Hz, 2 H), 7.27−7.36 (m, 4 H), 7.21 (d, J = 8.1 Hz, 2 H), 6.85 (d, J = 8.1 Hz, 2 H), 6.66 (d, J = 8.1 Hz, 1 H), 5.41 (ddd, J = 8.4, 8.1, 4.2 Hz, 1 H), 3.75 (s, 3 H), 3.03−3.09 (m, 1 H), 2.89−2.97 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.6, 141.3, 139.2, 138.9, 136.3, 136.1, 130.8, 130.6, 129.1, 128.5, 128.3, 127.2, 125.2, 120.3, 120.0, 119.6, 114.2, 69.5, 55.3, 42.5; HRCIMS *m*/*z* calcd for $C_{23}H_{20}O_2$ + H 329.1542, found 329.1549.

DTF Calculations. All the DFT studies were carried out in Gaussian03.[23] Frequency calculations were included to confirm the true minimum and correct thermal and zero-point energies. Compounds **27**, **37**, **38**, and their corresponding anions were optimized at the B3LYP/6-31G(d,p) level.

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Supporting Information Available: Cytotoxicity data of compound **4** in all of the tested cell lines, experimental procedure for compound **36**, and all B3LYP/6-31G(d,p) optimized structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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