Synthesis and Biological Evaluation of Bisindenoisoquinolines as Topoisomerase I Inhibitors

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The indenoisoquinolines represent a class of non-camptothecin topoisomerase I (Top1) inhibitors that exert cytotoxicity by trapping the covalent complex formed between DNA and Top1 during relaxation of DNA supercoils. As an ongoing evaluation of Top1 inhibition and anticancer activity, indenoisoquinolines were linked via their lactam side chains to provide polyamines end-capped with intercalating motifs. The resulting bisindenoisoquinolines were evaluated for cytotoxicity in the National Cancer Institute's human cancer cell screen and for Top1 inhibition. Preliminary findings suggested that the 2−3−2 and 3−3−3 linkers, referring to the number of carbons between nitrogen atoms, were optimal for both potent Top1 inhibition and cytotoxicity. Using optimized linkers, bisindenoisoquinolines were synthesized with nitro and methoxy substituents on the aromatic rings. The biological results for substituted compounds revealed a disagreement between the structure−activity relationships of monomeric indenoisoquinolines and bisindenoisoquinolines as Top1 inhibitors, but cytotoxicity was maintained for both series of compounds.

Introduction

The topoisomerase I (Top1)[a] inhibitory activity of the indenoisoquinolines (represented by compound **1**, Figure 1) was discovered after a COMPARE analysis of the cytotoxicity profile of an indenoisoquinoline derivative revealed a strong correlation with the cytotoxicity profiles of several other known Top1 inhibitors, including camptothecin (**2**) and the clinically useful analogue topotecan.[1] The indenoisoquinolines, like the camptothecins, were found to stabilize DNA−Top1 cleavage complexes by intercalating at the DNA cleavage site, resulting in inhibition of the religation reaction.[1−3] These inhibitors are therefore classified as Top1 "poisons" as opposed to Top1 "suppressors", which inhibit the DNA cleavage reaction. Although the indenoisoquinolines and the camptothecins have a similar mechanism of action, there are differences in the biological activities that warrant further development of the indenoisoquinolines as Top1 inhibitors. Notable differences include cleavage site specificity,[1,4] ternary complex stability,[1,5] and inherent molecular stability.[6−9] Consequently, we have synthesized and evaluated a number of indenoisoquinoline analogues in an effort to develop a therapeutic alternative to the camptothecins.[10−15]

The present investigation was undertaken in order to determine the effect of indenoisoquinoline dimerization (e.g. Figure 1, compound **3**) on cytotoxicity and Top1 inhibition. For effective Top1 inhibition, a stable ternary complex consisting of DNA, Top1, and the drug molecule is required. Since the camptothecins and the indenoisoquinolines are not covalently linked to either the enzyme or DNA, drug dissociation from the ternary complex can occur. The bisindenoisoquinolines were designed under the auspice that they would act as DNA bisintercalators and increase the stability of the ternary complex by disfavoring drug dissociation relative to a monointercalator. Assuming intercalation of both indenoisoquinoline pharmacophores occurs in the presence of DNA and Top1, deintercalation of both indenoisoquinoline moieties would have to occur prior to the religation of the DNA backbone and release of competent enzyme. Deintercalation of each indenoisoquinoline moiety from a bisintercalated DNA complex would be reversible, but after deintercalation of one indenoisoquinoline ring system, the bisindenoisoquinoline would still be bound to DNA through the remaining indenoisoquinoline moiety (Scheme 1). In theory, reintercalation of the dissociated indenoisoquinoline moiety in the mono-intercalated complex could occur before de-intercalation of the remaining intercalated indenoisoquinoline. In order for the bisindenoisoquinoline to completely dissociate

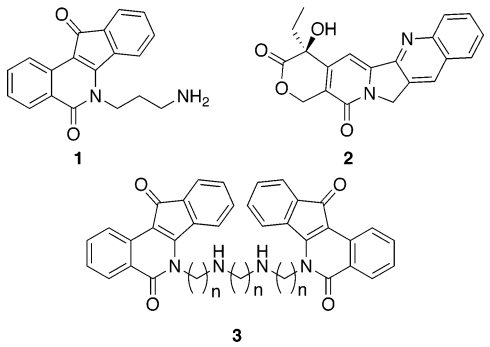

Figure 1. Representative topoisomerase I inhibitors.

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[a] Abbreviations: Top1, topoisomerase I.; Boc, *tert*-butoxycarbonyl; GI50, 50% growth inhibition; MGM, mean graph midpoint; IP, intraperitoneal; SC, subcutaneous; TOTO, 1,1′-(4,4,8,8-tetramethyl-4,8-diazaundecamethylene)bis[4-(3-methyl-2,3-dihydrobenzo-1,3-thiazolyl-2-methylidene)quinolinium] tetraiodide; SAR, structure−activity relationship; Top2, topoisomerase II.; TFA, trifluoroacetic acid.

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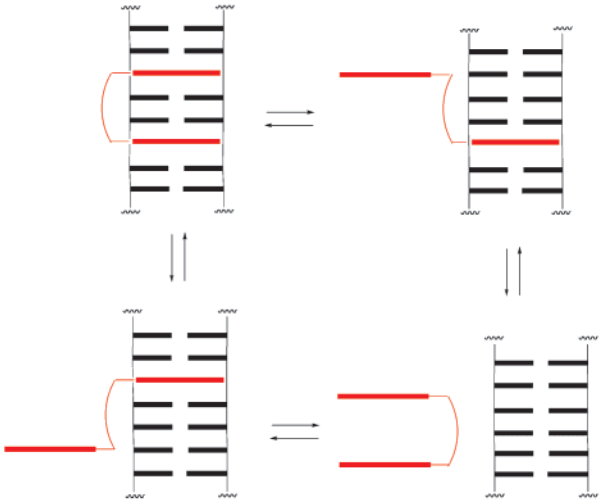
Scheme 1. Hypothetical Deintercalation of a Bisintercalator



from DNA, both intercalators would have to be deintercalated simultaneously. Increasing the number of intercalation moieties from one to two could therefore theoretically result in an increase in the stability of the ternary complex and, in the present case, enhance Top1 inhibition and cytotoxicity in cancer cell cultures. These arguments are similar to those originally proposed to explain large increases in the DNA affinities of bisintercalators vs monointercalators in the diacridine series.[16,17] In the case of substituted acridine dimers, DNA affinity constants as high as 2×10^{11} M^{-1} have been reported, which is 60 000 times larger than the corresponding monomer.[18]

There are numerous literature reports of both symmetrical and unsymmetrical drug dimers designed to interact with biological targets.[19] Rationally designed DNA bisintercalators (such as elinafide[20,21] and 9-aminoacridine[22]), DNA binding agents (such as linked berberine dimers[23]), and acetylcholinesterase inhibitors[24] represent a small handful of recent examples. Furthermore, the intentional targeting of Top1 with bisintercalators is supported in theory by successful work on the inhibition of topoisomerase II with bisacridine conjugates.[22] In the present case, it is conceivable that the indenoisoquinoline bisintercalators could exert antitumor activity by targeting DNA itself, apart from Top1, as has been observed with a wide variety of symmetrical acridine[25−31] and pyridocarbazole[32] dimers. In those cases, the cytotoxicity to cancer cells may result from inhibition of RNA synthesis[25,28,33] or from undefined effects on the cell membrane.[29]

The design of bisindenoisoquinolines has been aided by several recent developments. Crystallographic analysis has established the orientation of the indenoisoquinolines in the ternary complex, indicating that the aromatic nucleus is oriented parallel between the DNA base pairs and the lactam side chain is protruding into the major groove.[3,11,34] Moreover, a number of indenoisoquinolines that contain aminoalkyl and polyaminoalkyl substituents on the lactam nitrogen have been synthesized, and some of these compounds display potent cytotoxicity and Top1 inhibitory activity commensurate with that of camptothecin.[13,15] In addition, polyamine linker chains have previously been employed successfully in bisintercalators to increase water solubility and enhance DNA affinity through electrostatic interactions with the phosphate backbone.[16,18,25,28,31−33,35,36] With these facts in mind, we have designed bisindenoisoquinolines that are linked by polyaminoalkyl spacers between the lactam nitrogens of two indenoisoquinoline monomers. This orientation would not preclude the dimers from being bisintercalators, since the two aromatic cores would still have the potential to adopt the necessary orientation for intercalation (with the long axis of each indenoisoquinoline parallel to the long axis of the DNA base pairs) with the linker chain in the major groove. However, if the dimer failed to bisintercalate, it could potentially exert inhibitory effects by mono-intercalating between two DNA base pairs, leaving the rest of the dimer protruding from the major groove where it could interact with Top1. At the outset, the chemical synthesis proposed for the bisindenoisoquinolines appeared straightforward, relying on a variant of the chemistry already reported.[13] The immediate objectives of the present study were to establish optimal spacer length, investigate the effects of ammonium cation placement within the linker chain, and determine structural tolerances for Top1 inhibition and cytotoxicity in cancer cell cultures.

Chemistry

The synthesis of symmetrical bisindenoisoquinolines is outlined in Scheme 2. The general strategy involved the condensation of commercially available polyamines **5**−**17** with benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**4**)[37] to ultimately provide the desired targets in modest to good yield. Initially, this strategy proved to be viable, providing compounds **18**, **20**, and **21**. Bisindenoisoquinolines **19**, **22**, **23**, and **26** required conversion to their ammonium salts (**37**−**39** and **42**) in order to improve solubility for characterization and biological testing. As the polyamines increased in polarity, purification of the corresponding bisindenoisoquinolines (**24**, **25**, and **27**−**30**) proved to be more difficult, necessitating the secondary amines in the linker chains to be protected as *tert*-butyl carbamates **31**−**36** to improve the handling of the intermediates and aid in their purification. Following chromatographic separation, the Boc-protected bisindenoisoquinolines were deprotected using either trifluoroacetic acid or hydrochloric acid to provide compounds **40**, **41**, and **43**−**46**.

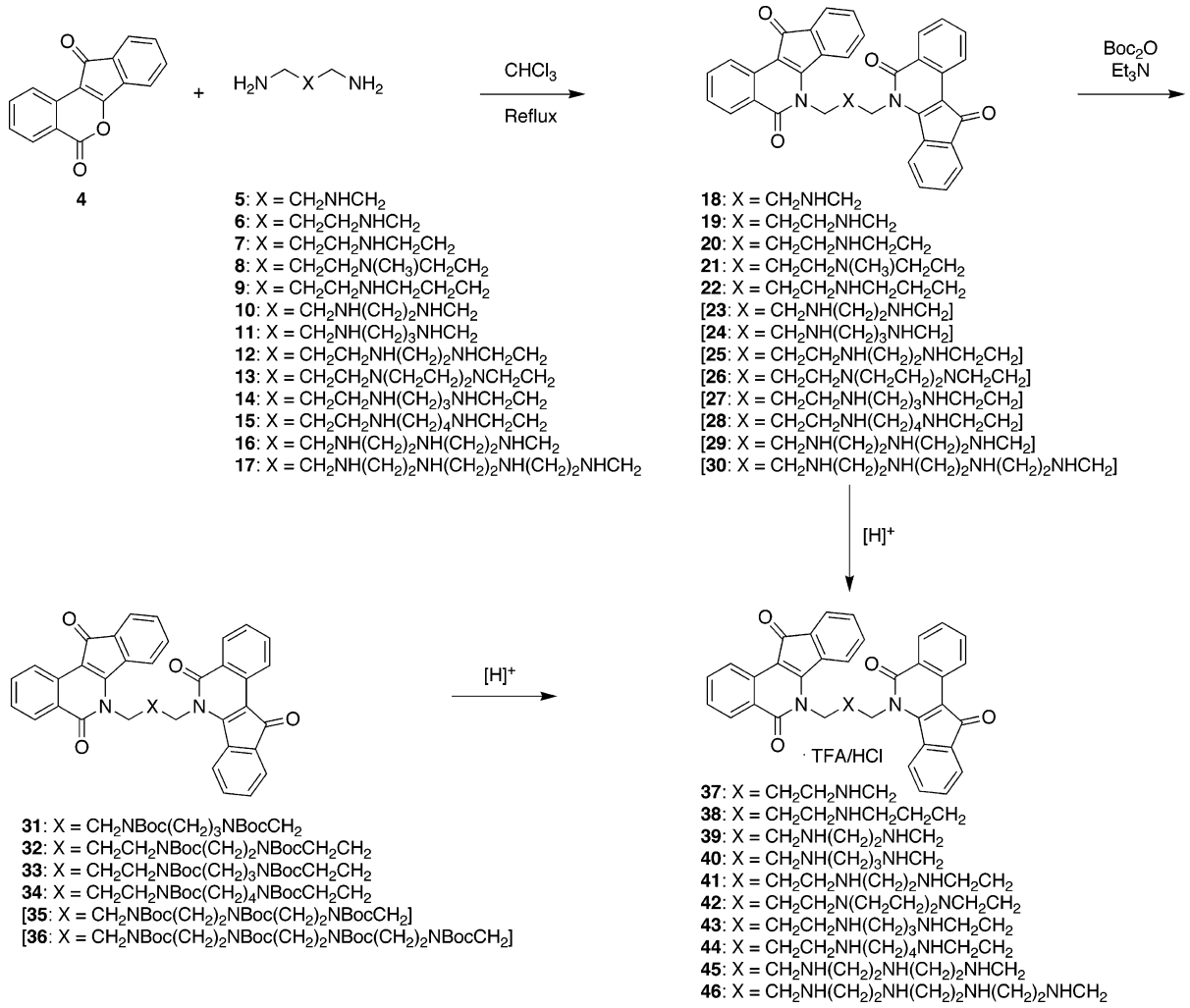
After investigation of the use of various polyamines during the work performed in Scheme 2, substituted bisindenoisoquinolines were synthesized using optimized linkers as outlined in Scheme 3. Benz[*d*]indeno[1,2-*b*]pyran-5,11-diones **47**[37] and **48**[37] were each condensed with polyamines **11** and **14** to provide substituted, symmetrical bisindenoisoquinolines **49**−**52**. Analogous to the chemistry performed in Scheme 2, carbamate protection greatly simplified purification of the crude products and facilitated the isolation of pure bisindenoisoquinolines as their trifluoroacetic acid salts.

Last, the synthesis of a substituted, unsymmetrical bisindenoisoquinoline was devised (Scheme 4). Unsymmetrical bisintercalators in the acridine and pyridocarbazole series have been previously prepared and found to bisintercalate into DNA with high affinities, although a loss in antitumor potency was observed.[35] In the present case, condensation of polyaminoindenoisoquinoline **53**[15] with compound **4**, followed by carbamate protection of the secondary amines in the linker chain, provided bisindenoisoquinoline **54**. This molecule was subsequently treated with trifluoroacetic acid to cleave the carbamate protecting groups and provide compound **55**.

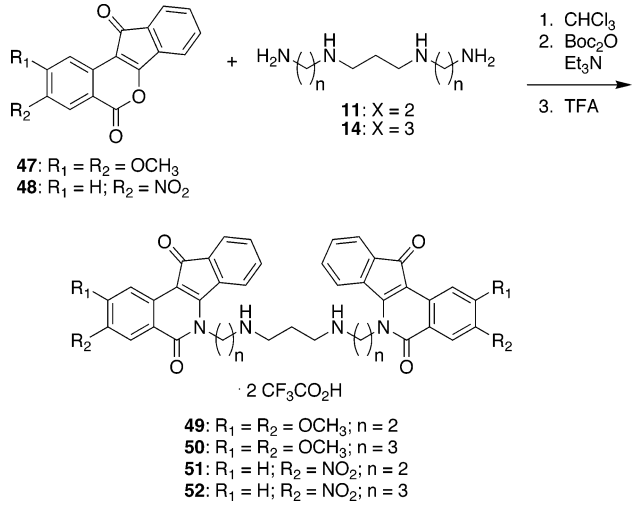
Biological Results and Discussion

The bisindenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated in approximately 55 different cancer cell lines of diverse tumor origins. The GI50 values obtained with selected

Scheme 2. Synthesis of Unsubstituted Bisindenoisoquinolines



Scheme 3. Synthesis of Substituted, Symmetrical Bisindenoisoquinolines



cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a calculation of the average GI50 for all of the cell lines tested (approximately 55) in which GI50 values below and above the test range (10^{-8} to 10^{-4} molar) are taken as the minimum (10^{-8} molar) and maximum (10^{-4} molar) drug concentrations used in the screening test. For comparison purposes, the activities of the previously reported compound **1**,[13] camptothecin (**2**), and previously reported compound **53**[15] are also included in the table. The relative potencies of the compounds in the production of Top1-mediated DNA cleavage are also listed in Table 1, with the ability of the compounds to produce Top1-mediated DNA cleavage expressed semiquantitatively as follows: + and ++: weak activity; +++: similar activity as compound **1**; ++++: similar activity as 1 μM camptothecin.

First generation analogues **18**−**46** were synthesized according to the methods outlined in Scheme 2, providing symmetrical compounds with unsubstituted intercalating pharmacophores for an investigation of optimal length and ammonium cation placement within the linker region. A shorthand notation for the linker region will be referred to throughout the rest of the article in which the number of carbon atoms between nitrogen atoms will be listed as numbers and separated by hyphens, which indicate the placement of a nitrogen atom. For example, polyamine **5** would be represented as 2−2, and the corresponding bisindenoisoquinoline (**18**) would be referred to as having a 2−2 linker. Regarding the Top1 inhibition for the unsubstituted, symmetrical compounds (**18**−**46**), several generalities are evident. First, Top1 inhibition tends to increase with a concomitant increase in the length of the linker. Compounds **18**, **20**, **21**, and **37**−**39**, all of which have linker spacings of 5−8 atoms, have a poor ability to inhibit Top1 and are all less potent than parent compound **1** (Top1 +++) in this respect. Compounds **40** (Top1 ++++), **41** (Top1 ++++), and **43**-**45** (all Top1 +++) demonstrated the greatest ability to inhibit Top1,

Scheme 4. Synthesis of Substituted, Unsymmetrical Bisindenoisoquinolines

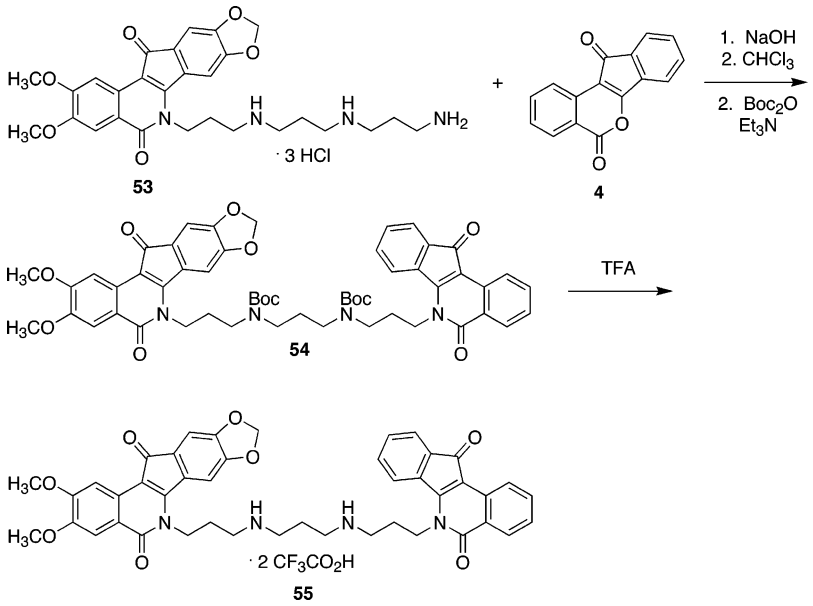


Table 1. Cytotoxicities and Topoisomerase I Inhibitory Activities of Indenoisoquinoline Analogues

	cytotoxicity (GI50 in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	Top 1 cleavage[c]
1	0.20	0.18	0.25	0.26	1.38	0.16	0.22	0.78	0.32 ± 0.23	+++
2	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.04	0.0405 ± 0.0187	++++
18	>25.1	>25.1	>25.1	>25.1	>25.1	>25.1	>25.1	>25.1	18.2	+
20	0.794	0.550	3.63	6.61	2.95	1.55	1.00	8.91	4.28 ± 1.89	+
21	NT	NT	1.12	2.00	1.20	0.589	NT	1.55	0.934 ± 0.476	++
31	22.4	22.9	>50.1	>50.1	21.4	>50.1	>50.1	>50.1	33.9	0
32	20.0	14.1	>50.1	45.7	13.2	39.8	>50.1	>50.1	28.2	0
34	11.0	1.91	8.13	93.3	69.2	36.3	47.9	69.2	35.5	++
37	0.977	1.05	14.5	5.01	8.91	11.0	1.91	2.24	5.25	+
38	0.028	0.056	NT	0.513	0.372	0.132	0.288	0.562	0.357 ± 0.087	+
39	0.032	0.029	NT	0.331	1.66	0.178	0.182	1.66	0.427 ± 0.01	+
40	0.339	<0.005	0.155	0.182	0.093	<0.005	0.079	0.024	0.122 ± 0.064	++++
41	<0.010	<0.010	NT	0.052	1.02	<0.010	<0.010	0.933	0.152 ± 0.062	++++
42	12.9	35.5	>100	>100	>100	15.5	24.0	>100	44.8 ± 2.05	0
43	<0.010	<0.010	0.011	0.042	0.074	<0.010	NT	0.107	0.394 ± 0.33	++++
44	0.525	<0.005	0.251	0.562	0.135	<0.005	0.234	0.676	0.225 ± 0.084	+++
45	0.048	0.112	0.275	0.269	1.15	0.017	0.331	1.00	0.474 ± 0.143	+++
46	0.977	0.200	0.012	NT	0.032	NT	0.085	0.126	0.262 ± 0.100	++
49	0.068	0.045	0.170	1.23	0.269	0.028	0.209	0.813	0.562	++
50	1.51	0.331	4.17	4.27	9.55	0.240	19.5	3.98	6.03	++
51	0.631	0.044	0.324	0.603	0.245	0.123	0.813	0.437	0.354 ± 0.184	++
52	3.02	1.45	1.17	1.78	2.29	1.17	0.912	3.89	1.50 ± 0.24	0
53	NT	43	>100	44	0.88	33	>100	68	58.9	++
54	>100	>100	NT	>100	NT	>100	NT	>100	68.0	0
55	0.191	0.022	<0.010	NT	<0.010	NT	<0.010	0.155	0.046 ± 0.010	+

[a] The cytotoxicity GI50 values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c] The compounds were tested at concentrations ranging up to 10 μM. The activity of the compounds to produce Top1-mediated DNA cleavage was expressed semiquantitatively as follows: + and ++: weak activity; +++: similar activity as compound **1**; ++++: similar activity as 1 μM camptothecin; NT: not tested.

with compounds **40** and **41** being equally as potent as camptothecin (**2**, Top1 ++++) in this assay. This indicates that with respect to the linker length, a 9−12 atom spacing appears to be optimal. Increasing the spacing to 14 atoms (i.e. compound **46**) resulted in a loss in Top1 inhibition relative to compounds within the 9−12 atom window. Therefore, it appears that for Top1 inhibition, there is a limit to the linker length for optimal activity.

Comparing the cytotoxicities of the unsubstituted compounds, it seems that longer may just indeed be better, but at a sacrifice to the selective inhibition of Top1. Compounds **38**−**41** and **43**−**46**, whose linker regions roughly increase in chain length, all demonstrated submicromolar cytotoxicity, with compounds **40**, **41**, and **44** displaying more potent activity than the corresponding indenoisoquinoline **1**. As mentioned previously, Top1 inhibition began to decline upon extending beyond the optimal window of intercalator spacing but the cytotoxicity remained approximately the same magnitude. This could imply that although the longer linkers (such as the 2−2−2−2−2 linker in compound **46**) may not be as effective at inhibiting Top1 in the enzyme assay, molecules such as **46** that possess multiple ammonium cations may exhibit improved cellular penetration (via a polyamine transporter)[38] or more effective localization in the nucleus (as is the case for the polyamines spermine and spermidine).[39] Alternatively, compounds with longer linkers may

Table 2. Hollow Fiber Activities of Indenoisoquinoline Analogues

compound	IP score[a]	SC score[a]	total score	cell kill[b]
40	2	4	6	N
43	26	6	32	N
44	12	4	16	N
45	10	6	16	N
Paclitaxel[41]	24	8	32	Y

[a] The IP and SC scores listed are the sums of all the IP and SC scores for each compound. [b] A net cell kill at one or more implant sites is indicated with a Y.

interact with targets other than Top1 and exert additional cytotoxic effects despite diminished Top1 inhibition.

Information regarding the placement of ammonium cations can be inferred from the data in Table 1. To attain similar or better Top1 inhibition than indenoisoquinoline **1**, the bisindenoisoquinolines were required to possess multiple ammonium cations. Similar activity as **1** (Top1 +++) was attained with linkers spaced as 3−3−3, 3−4−3, and 2−2−2−2 as in compounds **43**, **44**, and **45**. Top1 inhibition was improved by the use of linkers spaced as 2−3−2 and 3−2−3 as in compounds **40** (Top1 ++++) and **41** (Top1 ++++). Carbamate-protected analogues **31** (Top1 0), **32** (Top1 0), and **33** (Top1 ++) exhibited poor Top1 inhibition and cytotoxicity. After evaluation of the preliminary carbamate-protected compounds, the isolation and biological testing of the subsequent carbamate intermediates was entirely skipped. As to the necessity for the ammonium cations for potent biological activity, this could imply that the bisindenosioquinolines are substrates for a cationic transporter (such as the polyamine transporter).[38] It could also imply that the cations are involved in targeting the molecules to DNA (as is the case for polyamines)[39] or that they are involved in critical contacts with either DNA, Top1, or both. Flexibility of the ammonium cations within the linker was also found to be important for biological activity. Replacing the 2−2−2 linker present in **39** (MGM 0.427 μM) with one containing a piperazine ring provided **42** and resulted in a 100-fold loss in cytotoxicity and complete loss in Top1 inhibitory activity. The inactivity of **42** is noteworthy in view of the fact that pyridocarbazole[32] and acridine[32] dimers with conformationally restricted linker chains displayed significant antitumor activity.

Four of the most active bisindenoisoquinoline analogues (**40**, **43**, **44**, and **45**) were evaluated as anticancer agents in an in vivo animal model in which polyvinylidene fluoride hollow fibers containing various cancer cell cultures were implanted intraperitoneally (IP) and subcutaneously (SC) into athymic nude mice and compounds were administered by the IP route. The effects of the compounds on the reduction of viable cancer cell mass compared to those of controls were determined. Each compound was tested in the hollow fiber assay against a panel of twelve human tumor cell lines as described previously.[40] The compounds were solubilized in 10% DMSO in saline/Tween-80 and administered intraperitoneally once daily for a total of four doses at each of two dose levels. The two doses were selected based on single dose toxicity studies for each derivative. A score of 2 was assigned each time the compound produced a 50% or greater reduction in viable cell mass compared to vehicle-treated controls. The score for each compound was summed for the intraperitoneal fibers and the subcutaneous fibers to provide the total score for each derivative (Table 2). For comparative purposes, the score for the clinically used anticancer drug paclitaxel is provided. For the evaluated derivatives, compound **43**, with its 3−3−3 linker, was clearly the most active compound tested in the hollow fiber assay with a total score of 32. This suggested that **43** was as active as paclitaxel in this assay,[41] with the only striking difference being that **43** did not result in a net cell kill. Compounds with a combined IP + SC score of 20, an SC score of 8, or a net cell kill of one or more cell lines are considered to be significantly active. As a result, compound **43** is currently undergoing advanced xenograft testing.

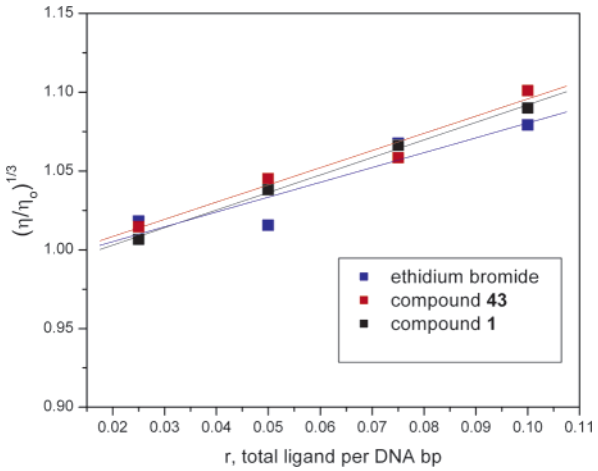


Figure 2. Viscometry studies of the interaction of indenoisoquinoline **1** and bisindenoisoquinoline **43** with sonicated calf thymus DNA. Intrinsic viscosities were measured in the presence of increasing concentrations of **1**, **43**, and ethidium bromide. Experiments were conducted at pH 7 in 0.2 M NaCl.

In an attempt to understand the requirement for a linker chain of 9−12 atoms in the bisindenoisoquinolines for potent Top1 inhibitory activity and cytotoxicity, molecular models of the binary **43**−DNA complex were constructed. The models were derived by overlap of the two intercalating moieties of **43** with those of the bisintercalator TOTO in the TOTO−DNA complex, removal of TOTO, and energy minimization of the **43**−DNA complex.[42] The molecular modeling results indicated that **43** could potentially bisintercalate in two different orientations, one with the polyamine linker extending into the major groove and the other with the linker extending into the minor groove (see Supporting Information).

To confirm this hypothesis, DNA viscometry experiments were performed. In the absence of high-resolution structural data, viscometry provides the most reliable method for establishing DNA binding mode.[43] It has been established that the intrinsic viscosity increases of sonicated DNA solutions as a function of ligand concentration are approximately twice as large for a bisintercalator as they are for a monointercalator.[16,32] The viscosity increases of DNA solutions observed for ethidium bromide (a standard monointercalator), the indenoisoquinoline **1**, and the bisindenoisoquinoline **43** as their concentrations increase are all parallel, with a slope near 1, indicating clearly that the bisindenoisoquinoline is a DNA monointercalator (Figure 2).

In light of the DNA viscosity study and the apparent lack of bisintercalation on the part of compound **43**, a hypothetical monointercalated binding model utilizing the previously reported crystal structure[34] of an indenoisoquinoline in ternary complex with DNA and Top1 was developed (Figure 3). Surprisingly, the model suggested that **43** could in fact inhibit Top1 according to a similar orientation reported for monomeric indenoisoquinolines.[34] According to the model, the ligand does not appear to be hindered in its binding by its nonintercalated portion. In fact, the lactam carbonyl of the nonintercalated ring system could potentially be involved in a bifurcated hydrogen bonding

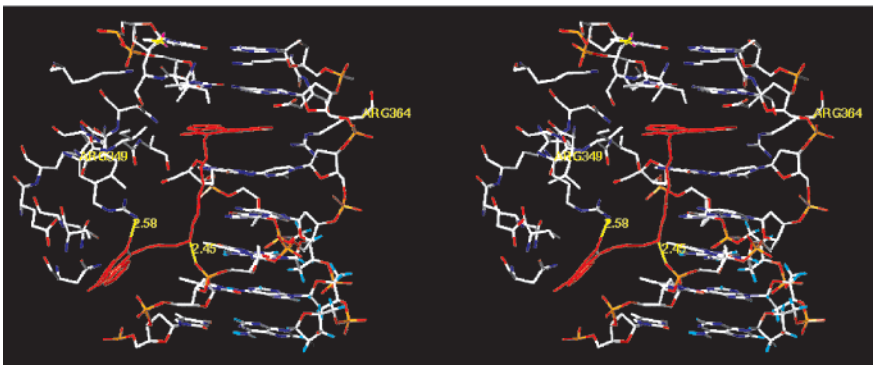


Figure 3. Hypothetical model of compound **43** (red) bound to DNA with the linker chain in the major groove. Top1 amino acid residues have been removed to improve clarity. The stereoview is programmed for wall-eyed (relaxed) viewing.

interaction with Arg349 (for clarity only one hydrogen bond in Figure 3 is shown). Furthermore, the flexible linker region may provide additional stability to the complex by interacting electrostatically with the phosphate backbone of the DNA. Thus, the hypothetical model represented in Figure 3 supports the activity of **43** as a potent Top1 inhibitor.

Collectively, the DNA viscosity study and the molecular modeling of **43** allow speculation as to the order of events leading to the poisoning of Top1. The DNA viscosity study establishes that **43** can intercalate DNA, albeit with only one ring system, in the absence of nicked DNA. Once the bisindenoisoquinoline is bound to DNA, Top1 could bind and nick the DNA at the site of monointercalation, relieving the associated strain in the DNA as a result of the intercalator being present. This would provide a ternary complex identical to the hypothetical binding model illustrated in Figure 3 and provides a reasonable explanation for how a bulky, flexible ligand such as **43** could ultimately be a good Top1 inhibitor. This seems to be a more likely sequence of events than the alternative scenario, which would involve the penetration of a very bulky bisindenoisoquinoline into the DNA−Top1 binary covalent cleavage complex. Furthermore, this ternary complex would be expected to persist since deintercalation and "escape" of the relatively large ligand from the ternary complex would be blocked by steric interactions. However, it should also be noted that some monomeric indenoisoquinolines do not intercalate with DNA in the absence of Top1, but still stabilize the ternary cleavage complex, indicating that those compounds probably intercalate into DNA at the cleavage site in the binary covalent DNA-enzyme complex.[44]

Second generation analogues (Scheme 3) were synthesized using the 2−3−2 and 3−3−3 linkers, both of which were found to be desirable linkers from the biological evaluation of the analogues synthesized in Scheme 2. The objectives of this work were 2-fold: to investigate structural tolerances relating to potential bisintercalators and to extrapolate known structure−activity relationships from the indenoisoquinolines to the bisindenoisoquinolines. To accomplish this preliminary work, the 2−3−2 and 3−3−3 polyamines (**11** and **14**) were condensed with indenopyrans **47** and **48**, which were available from a parallel synthetic endeavor.[37] This provided symmetrical bisindenoisoquinolines **49**−**52**, substituted with either di(methoxy) or nitro functionalities, both of which have been established to confer increased potency from prior work on the indenoisoquinolines.[14,37] From the activities reported in Table 1, it can be inferred that the introduction of nitro and di(methoxy) substituents on the aromatic rings of the bisindenoisoquinolines has a negative effect on their ability to inhibit Top1. In fact, for the 2−3−2-linked derivatives **49** (Top1 ++) and **51** (Top1 ++), Top1 inhibition is cut in half when compared to the parent, unsubstituted, compound **40** (Top1 ++++) regardless of the presence of either di(methoxy) or nitro substitutents. Interestingly, however, compounds **49** and **51** retained significant cytotoxicity that was comparable to that of the parent compound **40** with MGM values of 0.562, 0.354, and 0.122 μM, respectively. This suggests the cytotoxic effects for **49** and **51** could possibly involve an alternative target(s). A similar loss of Top1 inhibition was observed for the 3−3−3-linked derivatives **50** (Top1 ++) and **52** (Top1 0) when compared to the parent, unsubstituted, compound **43** (Top1 ++++). For these compounds, however, there was a concomitant decrease in cytotoxicity unlike that observed for the 2−3−2-linked derivatives. This suggests that the 3−3−3 linker in compounds **50** and **52** might be less optimal than the 2−3−2 linker for interaction with the alternative target(s). These results lead to the conclusion that SAR for indenoisoquinolines cannot be extrapolated for use with the bisindenoisoquinolines in the development of Top1 inhibitors.

Similar results were obtained in an effort to create substituted, unsymmetrical bisindenoisoquinoline Top1 inhibitors (Scheme 4). Comparing the results for compounds **53**, **54**, and **55** with parent compound **43** (all of which possess the 3−3−3 linker), the same effects seen with symmetrical substitution are apparent for the unsymmetrical compound. Polyamine indenoisoquinoline **53** (Top1 ++), with its isoquinoline and indenone rings substituted with di(methoxy) and methylenedioxy groups, respectively, was found to have its ability to inhibit Top1 halved when converted into bisindenoisoquinoline **55** (Top1 +). However, the cytotoxicity of **53** (MGM 58.9 μM) was found to improve 10 000-fold upon its conversion to **55** (MGM 0.046 μM). Once again, comparison of the activities of **43** and **55** reveals that substitution of the bisindenoisoquinolines has enhanced cytotoxicity but sacrificed Top1 inhibition, suggesting an additional target(s) could be involved.

The DNA cleavage patterns produced by camptothecin (**2**, lane 3 of each gel**)**, the monomeric indenoisoquinoline **1**, and the bisindenoisoquinolines **40**, **41**, **43**, **44**, **45**, and **55** are displayed in Figure 4. The following points are apparent from inspection of the gels: (1) The potencies of the indenoisoquinolines as Top1 inhibitors are reflected in the intensities of the DNA cleavage bands. The bands produced by the bisindenoisoquinoline **55** are relatively weak in comparison with the other bisindenoisoquinolines, as well as the monomeric indenoisoquinoline **1**. (2) Top1 inhibitors can be classified as Top1 suppressors, which inhibit DNA cleavage, and Top1 poisons, which inhibit the religation reaction after DNA cleavage. Many of the Top1-mediated DNA cleavages are trapped at lower bisindenoisoquinoline concentrations and suppressed at higher

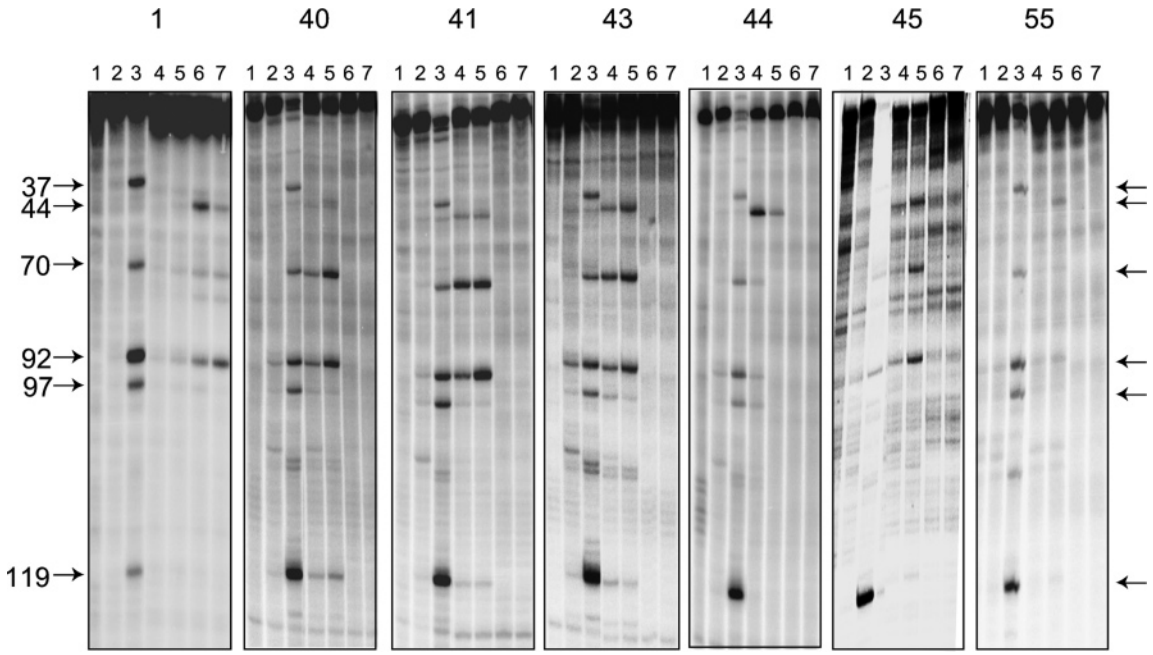


Figure 4. Comparison of the Top1-mediated DNA cleavages at different concentrations of compounds **1**, **40**, **41**, **43**, **44**, **45**, and **55**. The DNA used corresponds to the 3′-end-labeled Pvu/HindIII fragment of pBluescript SK (−) phagemid DNA. Lane 1: DNA; lane 2: DNA + Top1; lane 3: DNA + Top1 + 1 μM camptothecin; lanes 4−7: DNA + Top1 + 0.1, 1, 10, and 100 μM indenoisoquinoline Top1 inhibitor. Reactions were performed at room temperature for 30 min and stopped by adding 0.5% SDS. DNA fragments were separated on 16% polyacrylamide gels. Top1 was present in all reaction mixtures except the control lane (lane 1). Control (lane 1): DNA with neither Top1 nor drug. The seven gels were obtained separately and then placed side by side to facilitate comparison.

bisindenoisoquinoline concentrations, and therefore the bisindenoisoquinolines act as Top1 poisons at lower concentrations and Top1 suppressors at higher concentrations. The suppression could result from binding of the drug to the DNA rendering it a poorer enzyme substrate at high drug concentration, or from a direct effect on the enzyme to suppress its ability to cleave DNA. (3) There are differences in the cleavage pattern of camptothecin vs the indenoisoquinolines. For example, the cleavage at base pair 37 seen with camptothecin is absent with the indenoisoquinolines, and the cleavage at base pair 44 seen with the indenoisoquinolines is absent with camptothecin. (4) There are differences in the cleavage pattern of indenoisoquinoline **1** vs the bisindenoisoquinolines. The cleavage seen at base pair 70 is apparent with the bisindenoisoquinolines and camptothecin, but is weak with the monomeric indenoisoquinoline **1**. (5) There are apparent differences in the Top1 cleavage site selectivities of the bisindenoisoquinolines vs the monomer **1**, and within the bisindenoisoquinoline series, which may indicate that the bisindenoisoquinolines might display antitumor spectra different from monomeric indenoisoquinolines or camptothecin.

As a method to help elucidate the mechanism of action of **55**, a COMPARE analysis[45] was performed in the National Cancer Institute's database using the GI50 values of compound **55** as a seed. Only six compounds were identified with Pearson correlation coefficients greater than 0.5. Of these six compounds, five were members of the anthracycline family of natural products which interact with DNA, either as intercalating agents, minor groove binders, or inhibitors of topoisomerase II. Thus, this COMPARE analysis indicated that compound **55** and potentially other bisindenoisoquinolines that exerted potent cytotoxicity but diminished Top1 inhibition may be mechanistically similar to the anthracyclines. Preliminary studies have shown that the bisindenoisoquinoline **43** is able to trap both Top1 and Top2 cleavage complexes, and that resistance is only partial in the Top1-deficient cell line P388/CPT45.[46] Other biological targets besides Top1 are clearly involved in the activities of the bisindenoisoquinolines.

As for the effectiveness of bisindenoisoquinolines as potential therapeutic agents, several conclusions can be drawn. For Top1 inhibition by unsubstituted compounds, the optimal spacing of the linker connecting the two intercalation motifs appears to be a strict window of 9−12 atoms and the presence of multiple ammonium cations appears to be an additional requirement. Regarding the cytotoxicity of the unsubstituted bisindenoisoquinolines, the results indicate that increasing both the linker length and the number of ammonium cations generally improves cytotoxicity, with the placement of cations within the linker being less important than their presence. However, substitution of the intercalating pharmacophore(s) has a detrimental effect on Top1 inhibition, regardless of functionality and placement, despite the fact that similar substitution of monomeric indenoisoquinolines increases Top1 inhibitory activity and cytotoxicity.[15] These results indicate that unsubstituted and substituted bisindenoisoquinolines may preferentially interact with different biological targets and this could lead to the development of two new classes of anticancer compounds effective against Top1 and a yet to be elucidated target.

This is the first report of any Top1 inhibitor having two intercalation moieties.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. Infrared spectra were obtained using $CHCl_3$ as the solvent unless otherwise specified. ^{1}H NMR spectra were determined at 300 MHz. Microanalyses were performed at the Purdue University Microanalysis Laboratory. Analytical thin-layer chromatography was carried out on Baker-flex silica gel IB2-F plates. Compounds were visualized with short wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel. Benz[*d*]indeno[1,2-*b*]pyran-5,11-dione **4** was prepared in our laboratory.[37]

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-ethyl}amine (18). Triamine **5** (0.3 g, 2.91 mmol) was added to a stirred solution of lactone **4** (2.17 g, 8.72 mmol) in $CHCl_3$ (200 mL), and the mixture was stirred under reflux for 48 h. The reaction mixture was then cooled, and the resultant orange solid was filtered through a sintered glass funnel and washed with chloroform (30 mL) to provide pure bisindenoisoquinoline **18** (0.75 g, 46%) as an orange solid: mp 240−242 °C. ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 8.9 Hz, 2 H), 8.11 (d, J = 7.7 Hz, 2 H), 7.76 (bs, 4 H), 7.47 (bs, 4 H), 7.36 (bs, 4 H), 4.51 (bs, 4 H), 3.03 (bs, 4 H); ESIMS m/z (rel intensity) (MH^+, 100). Anal. ($C_{36}H_{25}N_3O_4$) C, H, N.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-ethyl,6′-propyl)amine (19). Triamine **6** (0.2 g, 1.71 mmol) was added to a stirred solution of lactone **4** (1.06 g, 4.27 mmol) in $CHCl_3$ (200 mL), and the reaction mixture was stirred under reflux for 48 h. The reaction mixture was then cooled, and the resultant orange solid was filtered through a sintered glass funnel and washed with chloroform−methanol mixture (2:8, 50 mL) to provide pure bisindenoisoquinoline **19** (0.72 g, 73%) as an orange solid: mp 250−252 °C. ^{1}H NMR ($CDCl_3$) δ 8.69 (d, J = 8.5 Hz, 2 H), 8.29 (t, J = 7.4 Hz, 2 H), 7.70 (t, J = 7.4 Hz, 2 H), 7.62 (m, 2 H), 7.46−7.37 (m, 8 H), 4.69 (t, J = 7.3 Hz, 2 H), 4.61 (t, J = 7.5 Hz, 2 H), 3.16 (t, J = 7.3 Hz, 2 H), 2.89 (t, J = 6.0 Hz, 2 H), 2.05 (m, 2 H); ESIMS m/z (rel intensity) 578 (MH^+, 100); HRESIMS calcd for ($C_{37}H_{27}N_3O_4$)H^+: 578.2079. Found: 578.2087.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propyl}amine (20). Triamine **7** (0.3 g, 2.29 mmol) was added to a stirred solution of lactone **4** (1.7 g, 6.86 mmol) in $CHCl_3$ (200 mL), and the mixture was stirred under reflux for 48 h. The bright orange reaction mixture was purified by flash column chromatography (SiO_2/$CHCl_3$ to 3% MeOH in $CHCl_3$) to afford pure bisindenoisoquinoline **20** (0.54 g) in 40% yield as a dark orange solid: mp 223−225 °C. ^{1}H NMR ($CDCl_3$) δ 8.65 (d, J = 8.1 Hz, 2 H), 8.27 (d, J = 8.1 Hz, 2 H), 7.67 (t, J = 7.1 Hz, 4 H), 7.57 (d, J = 7.0 Hz, 2 H), 7.41 (t, J = 7.1 Hz, 4 H), 7.33 (t, J = 7.1 Hz, 2 H), 4.62 (t, J = 7.3 Hz, 4 H), 2.84 (t, J = 6.4 Hz, 4 H), 2.08 (m, 4 H); ^{1}H NMR (DMSO-d_6) δ 8.53 (d, J = 7.8 Hz, 2 H), 8.18 (d, J = 7.9 Hz, 2 H), 7.87 (d, J = 7.4 Hz, 2 H), 7.79 (t, J = 7.6 Hz, 2 H), 7.54 (t, J = 5.8 Hz, 4 H), 7.46 (m, 4 H), 4.53 (t, J = 6.9 Hz, 4 H), 2.80 (bs, 4 H), 1.99 (m, 4 H); ESIMS m/z (rel intensity) 592 (MH^+, 100). Anal. ($C_{38}H_{29}N_3O_4$·1.6 H_2O) C, H, N.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propyl}methylamine (21). 3,3′-Diamino-*N*-methyl dipropylamine (**8**) (0.10 g, 0.69 mmol) was added to a stirred solution of lactone **4** (0.38 g, 1.52 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 48 h. The reaction mixture was cooled to room temperature and purified by flash column chromatography (SiO_2/$CHCl_3$ to 5% MeOH in $CHCl_3$) to provide bisindenoisoquinoline **21** (340 mg, 82%) as a red solid: mp 230−232 °C. ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 8.1 Hz, 2 H), 8.30 (d, J = 7.6 Hz, 2 H), 7.72−7.66 (dt, J = 8.3 and 2.8 Hz, 4 H), 7.59 (d, J = 7.1 Hz, 2 H), 7.48−7.40 (q, J = 7.5 Hz, 4 H), 7.33 (t, J = 7.3 Hz, 2 H), 4.63 (t, J = 8.0 Hz, 4 H), 2.66 (t, J = 6.5 Hz, 4 H), 2.37 (s, 3 H), 2.13−2.04 (m, 4 H); ESIMS m/z (rel intensity) 606 (MH^+, 100). Anal. ($C_{39}H_{31}N_3O_4$·0.4 $CHCl_3$) C, H, N.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propyl,6′-butyl)amine (22). Triamine **9** (0.2 g, 1.38 mmol) was added to a stirred solution of lactone **4** (0.75 g, 3.03 mmol) in $CHCl_3$ (200 mL), and the reaction mixture was stirred under reflux for 48 h. The reaction mixture was then cooled, and the resultant orange solid was filtered through a sintered glass funnel and washed with chloroform−methanol mixture (5:1, 50 mL) to provide pure bisindenoisoquinoline **22** (0.63 g, 76%) as an orange solid: mp 228−230 °C. ^{1}H NMR ($CDCl_3$) δ 8.67 (d, J = 8.1 Hz, 2 H), 8.28 (d, J = 8.1 Hz, 2 H), 7.70−7.65 (m, 2 H), 7.59 (d, J = 6.8 Hz, 2 H), 7.52 (d, J = 7.4 Hz, 2 H), 7.45−7.24 (m, 6 H), 4.61 (t, J = 7.3 Hz, 2 H), 4.55 (t, J = 7.9 Hz, 2 H), 2.84 (t, J = 6.5 Hz, 2 H), 2.80 (t, J = 6.8 Hz, 2 H), 2.15−2.10 (m, 2 H), 2.00−1.95 (m, 2 H), 1.84−1.77 (m, 2 H); ESIMS m/z (rel intensity) 606 (MH^+, 100); HRESIMS calcd for ($C_{39}H_{31}N_3O_4$)H^+: 606.2393. Found: 606.2402.

Bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)−(6-ethyl-*tert*-BOCamino)}propane (31). Tetramine **11** (0.10 g, 0.62 mmol) was added to a stirred solution of lactone **4** (0.34 g, 1.37 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 72 h, providing bisindenoisoquinoline **24** as a crude intermediate. After allowing the reaction mixture to cool to room temperature, Et_3N (0.35 mL, 2.50 mmol) and Boc_2O (0.34 g, 1.56 mmol) were added, and the reaction mixture was stirred at room temperature for 8 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane and then 1−5% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **31** (380 mg, 74%) as an orange solid: mp 238−240 °C. ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J = 8.0 Hz, 2 H), 8.16 (d, J = 7.4 Hz, 2 H), 7.65−7.58 (m, 5 H), 7.53−7.45 (m, 2 H), 7.38−7.29 (m, 5 H), 4.63 (bs, 4 H), 3.62 (bs, 4 H), 3.32 (bs, 4 H), 1.90 (bs, 2 H), 1.41 (s, 18 H); ESIMS m/z (rel intensity) 821 (MH^+, 10), 721 (MH^+ − Boc, 100). Anal. ($C_{49}H_{48}N_4O_8$) C, H, N.

Bis-1,2-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)−(6-propyl-*tert*-BOCamino)}ethane (32). Tetraamine **12** (0.16 g, 0.85 mmol) was added to a stirred solution of lactone **4** (0.46 g, 1.87 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 72 h, providing bisindenoisoquinoline **25** as a crude intermediate. Upon allowing the reaction mixture to cool to room temperature, Et_3N (0.6 mL, 4.24 mmol) and Boc_2O (0.56 g, 2.60 mmol) were added to the reaction mixture and the mixture was allowed to stir at room temperature for 8 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane, then 1−5% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **32** (550 mg, 76%) as an orange solid: mp 106−108 °C. ^{1}H NMR ($CDCl_3$) δ 8.60 (bs, 2 H), 8.23 (bs, 2 H), 7.65 (bs, 2 H), 7.55 (d, J = 6.7 Hz, 2 H), 7.40−7.32 (m, 8 H), 4.48 (bs, 4 H), 3.45 (bs, 8 H), 2.12 (bs, 4 H), 1.44 (s, 9 H), 1.39 (s, 9 H); ESIMS m/z (rel intensity) 835 (MH^+, 22), 735 (MH^+ − Boc, 100). Anal. ($C_{50}H_{50}N_4O_8$·0.3 H_2O) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-(6-propyl-*tert*-BOCamino)}propane (33). Tetramine **14** (0.15 g, 0.74 mmol) was added to a stirred solution of lactone **4** (0.40 g, 1.63 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 72 h, providing bisindenoisoquinoline **27** as a crude intermediate. Upon allowing the reaction mixture to cool to room temperature, Et_3N (0.53 mL, 3.78 mmol) and Boc_2O (0.49 g, 2.27 mmol) were added to the reaction mixture and the mixture was allowed to stir at room temperature for 8 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane, then 1−5% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **33** (450 mg, 70%) as an orange solid: mp 86−88 °C. ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J = 8.1 Hz, 2 H), 8.24 (d, J = 7.6 Hz, 2 H), 7.65 (t, J = 7.3 Hz, 2 H), 7.55 (d, J = 6.7 Hz, 2 H), 7.40−7.31 (m, 8 H), 4.49 (bs, 4 H), 3.44 (bs, 4 H), 3.27 (apparent t, J = 6.2 Hz, 4 H), 2.08 (bs, 4 H), 1.86 (bs, 2 H), 1.41 (bs, 18 H); ESIMS m/z (rel intensity) 849 (MH^+, 3), 749 (MH^+ − Boc, 37), 649 (MH^+ − 2 × Boc, 100). Anal. ($C_{51}H_{52}N_4O_8$·0.5 H_2O) C, H, N.

Bis-1,4-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-(6-propyl-*tert*-BOCamino)}butane (34). Tetramine **15** (0.10 g, 0.50 mmol) was added to a stirred solution of lactone **4** (0.27 g, 1.09 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 72 h, providing bisindenoisoquinoline **28** as a crude intermediate. Upon allowing the reaction mixture to cool to room temperature, Et_3N (0.28 mL, 2.00 mmol) and Boc_2O (0.27 g, 1.25 mmol) were added to the reaction mixture and the mixture was allowed to stir at room temperature for 8 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane and then 1−5% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **34** (350 mg, 82%) as an orange solid: mp 92−94 °C. ^{1}H NMR ($CDCl_3$) δ 8.66 (d, J = 8.1 Hz, 2 H), 8.28 (d, J = 8.0 Hz, 2 H), 7.68 (t, J = 7.7 Hz, 2 H), 7.60 (d, J = 7.1 Hz, 2 H), 7.43−7.34 (m, 8 H), 4.51 (t, J = 8.3 Hz, 4 H), 3.44 (bs, 4 H), 3.28 (bs, 4 H), 2.11 (m, 4 H), 1.50 (bs, 4 H), 1.41 (s, 18 H); ESIMS m/z (rel intensity) 863 (MH^+, 13), 763 (MH^+ − Boc, 100). Anal. ($C_{52}H_{54}N_4O_8$·0.9 H_2O) C, H, N.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)}-(6-ethyl,6′-propyl)ammonium Trifluoroacetate (37). Bisindenoisoquinoline **19** (0.5 g, 0.87 mmol) was dissolved in neat CF_3COOH (30 mL), and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated and diluted with chloroform (50 mL), and the resultant solid was filtered through a sintered glass funnel and further washed with methanol (50 mL) to give bisindenoisoquinoline **37** (0.48 g, 80%) as a red solid: mp 240−242 °C. ^{1}H NMR (DMSO-d_6) δ 8.71 (bs, 1 H, −NH-), 8.56 (d, J = 7.8 Hz, 2 H), 8.17 (d, J = 8.6 Hz, 2 H), 7.83−7.75 (m, 4 H), 7.57−7.50 (m, 8 H), 4.79 (bs, 2 H), 4.57 (bs, 2 H), 3.46 (bs, 2 H), 3.17 (bs, 2 H), 2.18 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 578 ($MH^+ - CF_3COOH$, 100). Anal. ($C_{39}H_{28}N_3O_6F_3 \cdot 0.3\ H_2O$) C, H, N.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)}-(6-propyl,6′-butyl)amine Hydrochloride (38). 2 M HCl in ether (6.2 mL, 2.4 mmol) was added to a stirred solution of bisindenoisoquinoline **22** (0.5 g, 0.83 mmol) in chloroform (100 mL), and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through a sintered glass funnel, and the solid was washed with chloroform (50 mL) and methanol (50 mL) to give bisindenoisoquinoline hydrochloride **38** (0.44 g, 83%) as an orange solid: mp 280−282 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.66 (bs, 1 H), 8.54 (d, J = 7.9 Hz, 2 H), 8.18 (d, J = 8.6 Hz, 2 H), 7.83−7.70 (m, 4 H), 7.58−7.40 (m, 8 H), 4.54−4.42 (m, 4 H), 3.06 (bs, 2 H), 2.96 (bs, 2 H), 2.16 (bs, 2 H), 1.84 (bs, 2 H), 1.76 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 606 (MH^+, 100). Anal. ($C_{39}H_{32}N_3O_4Cl \cdot 1.1\ H_2O$) C, H, N.

Bis-1,2-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-ethylamino}ethane Bis(trifluoroacetate) (39). Tetraamine **10** (0.4 g, 2.74 mmol) was added to a stirred solution of lactone **4** (1.49 g, 6.02 mmol) in $CHCl_3$ (200 mL), and the reaction mixture was stirred under reflux for 48 h. The reaction mixture was then cooled, and the resultant orange solid was filtered through a sintered glass funnel and washed with chloroform (50 mL) to provide bisindenoisoquinoline **23** (0.57 g, 69%) as an insoluble orange solid. Intermediate **23** (0.5 g, 0.83 mmol) was dissolved in neat CF_3COOH (30 mL) and stirred at room temperature for 30 min. The reaction mixture was concentrated, diluted with chloroform (50 mL), and filtered through a sintered glass funnel to provide bisindenoisoquinoline **24** (0.57 g, 83%) as an orange solid: mp 230−232 °C. ^{1}H NMR (DMSO-d_6) δ 8.97 (bs, 2 H), 8.59 (d, J = 8.1 Hz, 2 H), 8.23 (d, J = 8.0 Hz, 2 H), 7.89−7.83 (td, J = 1.2 and 8.3 Hz, 2 H), 7.76 (d, J = 6.8 Hz, 2 H), 7.63−7.50 (m, 8 H), 4.83 (bs, 4 H), 3.52 (bs, 4 H), 3.32 (bs, 4 H); ESIMS *m*/*z* (rel intensity) 607 (MH^+, 100). Anal. ($C_{42}H_{32}N_4O_8F_6 \cdot 0.4\ H_2O$) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-ethylamino}propane Bis(trifluoroacetate) (40). Boc-protected bisindenoisoquinoline **31** (0.3 g, 0.36 mmol) was dissolved in neat CF_3COOH (30 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, and the resultant solid was diluted with chloroform (50 mL) and filtered through a sintered glass funnel to provide bisindenoisoquinoline **40** (0.28 g, 92%) as an orange solid: mp 244−246 °C. ^{1}H NMR (DMSO-d_6) δ 8.93 (bs, 2 H), 8.60 (d, J = 8.4 Hz, 2 H), 8.23 (d, J = 7.9 Hz, 2 H), 7.87 (t, J = 7.5 Hz, 2 H), 7.79 (d, J = 7.8 Hz, 2 H), 7.60−7.52 (m, 8 H), 4.82 (bs, 4 H), 3.47 (bs, 4 H), 3.07 (bs, 4 H), 1.95 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 621 (MH^+, 100), 274 (7). Anal. ($C_{43}H_{34}F_6N_4O_8 \cdot 1.7\ H_2O$): C, H, N.

Bis-1,2-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}ethane Bis(trifluoroacetate) (41). Boc-protected bisindenoisoquinoline **32** (0.5 g, 0.79 mmol) was dissolved in neat CF_3COOH (30 mL), and the mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated and diluted with chloroform (50 mL), and the resultant solid was filtered through a sintered glass funnel to afford bisindenoisoquinoline **41** (0.61 g, 90%) as a pale red solid: mp 220−222 °C. ^{1}H NMR (DMSO-d_6) δ 8.84 (bs, 2 H), 8.57 (d, J = 8.1 Hz, 2 H), 8.20 (d, J = 7.8 Hz, 2 H), 7.85−7.77 (m, 4 H), 7.60−7.48 (m, 8 H), 4.57 (t, J = 6.6 Hz, 4 H), 3.23 (bs, 4 H), 3.18 (bs, 4 H), 2.16 (m, 4 H); ESIMS *m*/*z* (rel intensity) 635 (MH^+, 61). Anal. ($C_{44}H_{36}N_4O_8F_6 \cdot 1.4\ H_2O$) C, H, N.

Bis-1,4-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propyl}piperazine Bis(trifluoroacetate) (42). 1,4-Bis(3-aminopropyl)piperazine **13** (0.10 g, 0.50 mmol) was added to a stirred solution lactone **4** (0.27 g, 1.10 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 60 h. The reaction mixture was then cooled, and the resultant red solid was filtered off through a sintered glass funnel, washed with chloroform (50 mL), and dried to provide intermediate **26**. This compound was further treated with CF_3COOH (40 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and diluted with chloroform (50 mL), and the resultant solid was filtered and washed with methanol−chloroform (1:9) to provide bisindenoisoquinoline **42** (430 mg, 86%) as red solid: mp 256−258 °C. ^{1}H NMR ($CDCl_3$) δ 8.57 (d, J = 8.0 Hz, 2 H), 8.21 (d, J = 8.1 Hz, 2 H), 7.85−7.77 (m, 4 H), 7.58−7.48 (m, 8 H), 4.55 (bs, 4 H), 3.34 (bs, 4 H), 3.02 (bs, 4 H), 2.72 (bs, 2 H), 2.47 (bs, 2 H, merged with DMSO-d_6 protons), 2.09 (bs, 4 H); ESIMS *m*/*z* (rel intensity) 661 (MH^+, 100). Anal. ($C_{46}H_{38}F_6N_4O_8 \cdot 0.4\ H_2O$) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}propane Bis(trifluoroacetate) (43). Boc-protected bisindenoisoquinoline **33** (0.3 g, 0.35 mmol) was dissolved in neat CF_3COOH (30 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, and the resultant solid was diluted with chloroform (50 mL) and filtered through a sintered glass funnel to provide bisindenoisoquinoline **43** (0.27 g, 89%) as an orange solid: mp 225−227 °C. ^{1}H NMR (DMSO-d_6) δ 8.66 (bs, 2 H), 8.56 (d, J = 8.1 Hz, 2 H), 8.19 (d, J = 7.9 Hz, 2 H), 7.79 (t, J = 7.9 Hz, 4 H), 7.59−7.48 (m, 8 H), 4.57 (bs, 4 H), 3.09 (bs, 4 H), 2.96 (bs, 4 H), 2.15 (bs, 4 H), 1.87 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 649 (MH^+, 100). Anal. ($C_{45}H_{38}F_6N_4O_8 \cdot 1.3\ H_2O$) C, H, N.

Bis-1,4-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}butane Bis(trifluoroacetate) (44). Boc-protected bisindenoisoquinoline **34** (0.3 g, 0.35 mmol) was dissolved in neat CF_3COOH (20 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, and the resultant solid was diluted with chloroform (50 mL) and filtered through a sintered glass funnel to provide bisindenoisoquinoline **44** (0.28 g, 90%) as an orange solid: mp 236−238 °C. ^{1}H NMR (DMSO-d_6) δ 8.58 (d, J = 8.0 Hz, 2 H), 8.52 (bs, 2 H), 8.20 (d, J = 8.1 Hz, 2 H), 7.84−7.78 (m, 4 H), 7.60−7.49 (m, 8 H), 4.57 (t, J = 6.6 Hz, 4 H), 3.08 (bs, 4 H), 2.92 (bs, 4 H), 2.16 (m, 4 H), 1.59 (bs, 4 H); ESIMS *m*/*z* (rel intensity) 663 (MH^+, 100). Anal. ($C_{46}H_{40}F_6N_4O_8 \cdot 0.4\ H_2O$) C, H, N.

Bis{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-(ethylamino)-ethyl}amine Tris(trifluoroacetate) (45). Pentaamine **16** (0.20 g, 1.06 mmol) was added to a stirred solution of lactone **4** (0.58 g, 2.32 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 4 days, providing bisindenoisoquinoline **29** as a crude intermediate. Upon allowing the reaction mixture to cool to room temperature, Et_3N (0.86 mL, 6.13 mmol) and Boc_2O (0.89 g, 4.09 mmol) were added and the mixture was allowed to stir at room temperature for 12 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane, then 1−3% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **35** (0.61 g, 61%), which was further treated with neat CF_3COOH (30 mL) and stirred at room temperature for 3 h. The reaction mixture was concentrated, and the resultant solid was diluted with chloroform (50 mL) and filtered through a sintered glass funnel to provide bisindenoisoquinoline **45** (0.42 g, 66%) as a red solid: mp 198−200 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.55 (d, J = 8.1 Hz, 2 H), 8.20 (d, J = 7.6 Hz, 2 H), 7.85−7.76 (m, 4 H), 7.59−7.48 (m, 8 H), 4.81 (bs, 4 H), 3.54 (bs, 4 H), 3.32 (bs, 8 H); ESIMS *m*/*z* (rel intensity) 650 (MH^+, 100). Anal. ($C_{46}H_{38}N_5O_{10}F_9 \cdot 0.6\ CH_2Cl_2NH_3$) C, H, N.

Bis-1,2-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-(ethylamino)-ethylamino}ethane Tetra(trifluoroacetate) (46). Hexamine **17** (0.20 g, 0.86 mmol) was added to a stirred

solution of lactone **4** (0.47 g, 1.89 mmol) in $CHCl_3$ (150 mL), and the reaction mixture was stirred under reflux for 4 days, providing bisindenoisoquinoline **30** as a crude intermediate. Upon allowing the reaction mixture to cool to room temperature, Et_3N (1.21 mL, 8.67 mmol) and Boc_2O (0.95 g, 4.34 mmol) were added to the reaction mixture and the mixture was allowed to stir at room temperature for 12 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane, then 1−3% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **36** (0.62 g, 66%), which was further treated with neat CF_3COOH (30 mL) and stirred at room temperature for 3 h. The reaction mixture was concentrated, and the resultant solid was diluted with chloroform (50 mL) and filtered through a sintered glass funnel to provide bisindenoisoquinoline **46** (0.48 g, 49%) as red solid: mp 206−208 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.55 (t, J = 8.4 Hz, 2 H), 8.19 (t, J = 8.0 Hz, 2 H), 7.82−7.61 (m, 4 H), 7.59−7.51 (m, 8 H), 4.81 (bs, 4 H), 3.52 (bs, 4 H), 3.27 (bs, 4 H), 3.19−3.13 (bs, 8 H); ESIMS *m*/*z* (rel intensity) 693 (MH^+, 100). Anal. ($C_{50}H_{44}N_6O_{12}F_{12}\cdot 0.6\ H_2O$) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-2,3-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline)-6-ethylamino}propane Bis{trifluoroacetate) (49). *N*,*N*′-Bis(3-aminoethyl)-1,3-propanediamine (**11**) (0.050 g, 0.309 mmol) was added to a solution of 2,3-dimethoxybenz[*d*]-indeno[1,2-*b*]pyran-5,11-dione (**47**) (0.200 g, 0.649 mmol) in $CHCl_3$ (50 mL). The solution was heated at reflux for 72 h and cooled to room temperature. Triethylamine (0.17 mL) and Boc_2O (0.270 g, 1.236 mmol) were added to the solution, and stirring was continued at room temperature for 16 h. The solution was washed with water (2 × 25 mL) and sat. aq NaCl (25 mL), dried over sodium sulfate, and concentrated. The crude red solid was purified by flash column chromatography (SiO_2/$CHCl_3$ to 3% MeOH in $CHCl_3$) followed by precipitation from CH_2Cl_2−hexanes to provide a pink solid. The obtained pink solid was diluted with trifluoroacetic acid (30 mL), and the mixture was stirred at room temperature for 16 h. The solution was concentrated, diluted with $CHCl_3$ (50 mL), and filtered to provide a red solid (0.257 g, 86%): mp 225−228 °C. IR (KBr) 3437, 1652, 1553, 1513, 1429, 1268, 1204, and 1021 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.96 (s, 2 H), 7.72−7.69 (bs, 2 H), 7.52−7.43 (m, 8 H), 4.71 (bs, 4 H), 3.92 (s, 6 H), 3.81 (s, 6 H), 3.06 (bs, 4 H), 1.99 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 741 (MH^+, 100). Anal. ($C_{47}H_{42}F_6N_4O_{12}\cdot 4\ H_2O$) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-2,3-dimethoxy-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}propane Bis{trifluoroacetate) (50). *N*,*N*′-Bis(3-aminopropyl)-1,3-propanediamine (**14**) (0.058 g, 0.309 mmol) was added to a solution of lactone **47** (0.200 g, 0.649 mmol) in $CHCl_3$ (50 mL). The solution was heated at reflux for 72 h and cooled to room temperature. Triethylamine (0.17 mL) and Boc_2O (0.270 g, 1.236 mmol) were added to the solution, and stirring was continued at room temperature for 16 h. The solution was washed with water (2 × 25 mL) and sat. aq NaCl (25 mL), dried over sodium sulfate, and concentrated. The crude orange solid was purified by flash column chromatography (SiO_2/$CHCl_3$ to 3% MeOH in $CHCl_3$) followed by precipitation from EtOAc to provide an orange solid. The obtained orange solid was diluted with trifluoroacetic acid (30 mL), and the mixture was stirred at room temperature for 16 h. The solution was concentrated, diluted with $CHCl_3$ (50 mL), and filtered to provide a red solid (0.221 g, 72%): mp 273−276 °C (dec). IR (KBr) 3436, 1639, 1553, 1512, 1478, 1429, 1267, 1184, and 1022 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.43 (bs, 4 H), 8.00 (s, 2 H), 7,76 (d, J = 7.58 Hz, 2 H), 7.59−7.45 (m, 8 H), 4.56 (bs, 4 H), 3.93 (s, 6 H), 3.85 (s, 6 H), 3.09 (bs, 4 H), 2.98 (bs, 4 H), 2.15 (bs, 4 H), 1.86 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 769 (MH^+, 100). Anal. ($C_{49}H_{46}F_6N_4O_{12}\cdot 6\ H_2O$) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-3-nitro-11*H*-indeno[1,2-*c*]isoquinoline)-6-ethylamino}propane Bis{trifluoroacetate) (51). *N*,*N*′-Bis(3-aminoethyl)-1,3-propanediamine (**11**) (0.056 g, 0.349 mmol) was added to a solution of lactone **48** (0.225 g, 0.767 mmol) in $CHCl_3$ (50 mL). The solution was heated at reflux for 72 h and cooled to room temperature. Triethylamine (0.19 mL) and Boc_2O (0.305 g, 1.396 mmol) were added to the solution, and stirring was continued at room temperature for 16 h. The solution was washed with water (2 × 30 mL) and sat. aq NaCl (30 mL), dried over sodium sulfate, and concentrated. The crude orange solid was purified by flash column chromatography (SiO_2/$CHCl_3$ to 3% MeOH in $CHCl_3$) to provide an orange solid. The orange solid was diluted with trifluoroacetic acid (40 mL), and the mixture was stirred at room temperature for 24 h. The solution was concentrated, diluted with $CHCl_3$ (50 mL), and filtered to provide an orange solid (0.221 g, 67%): mp 227−230 °C (dec). IR (KBr) 3433, 3087, 3022, 2819, 1679, 1615, 1560, 1505, 1429, 1138, and 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.90 (bs, 4 H), 8.79 (d, J = 9.14 Hz, 2 H), 8.66 (d, J = 9.07 Hz, 2 H), 7.93 (d, J = 6.54 Hz, 2 H), 7.74 (d, J = 7.17 Hz, 2 H), 7.67 (m, 4 H), 4.87 (bs, 4 H), 3.49 (bs, 4 H), 3.09 (bs, 4 H), 1.91 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 711 (MH^+, 100). Anal. ($C_{43}H_{32}F_6N_6O_{12}\cdot 0.5\ H_2O$) C, H, N.

Bis-1,3-{(5,6-dihydro-5,11-diketo-3-nitro-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}propane Bis{trifluoroacetate) (52). *N*,*N*′-Bis(3-aminopropyl)-1,3-propanediamine (**14**) (0.064 g, 0.341 mmol) was added to a solution of lactone **48** (0.200 g, 0.682 mmol) in $CHCl_3$ (75 mL). The solution was heated at reflux for 72 h and cooled to room temperature. Triethylamine (0.19 mL) and Boc_2O (0.298 g, 1.364 mmol) were added to the solution, and stirring was continued at room temperature for 16 h. The solution was washed with water (2 × 30 mL) and sat. aq NaCl (30 mL), dried over sodium sulfate, and concentrated. The crude orange solid was purified by flash column chromatography (SiO_2/$CHCl_3$ to 3% MeOH in $CHCl_3$) to provide an orange solid. The obtained orange solid was diluted with trifluoroacetic acid (40 mL) and stirred at room temperature for 2 h. The solution was concentrated, diluted with $CHCl_3$ (50 mL), and filtered to provide an orange solid (0.206 g, 62%): mp 220−223 °C. IR (KBr) 1678, 1614, 1505, 1339, 1203, and 1132 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.88 (d, J = 2.5 Hz, 2 H), 8.75 (d, J = 9.0 Hz, 2 H), 8.63 (bs, 2 H), 8.60 (dd, J = 9.0 and 2.5 Hz, 2 H), 7.92 (d, J = 6.5 Hz, 2 H), 7.70−7.61 (m, 6 H), 4.64 (t, J = 5.9 Hz, 4 H), 3.15 (bs, 4 H), 2.98 (bs, 4 H), 2.19 (bs, 2 H); ESIMS *m*/*z* (rel intensity) 739 (MH^+, 100). Anal. ($C_{45}H_{36}F_6N_6O_{12}\cdot 3\ H_2O$) C, H, N.

1,3-{6-(3-*tert*-Butyloxycarbonylamino-1-propyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline}-{5′,6′-dihydro-6′-[(3′-*tert*-butyloxycarbonylamino)-1′-propyl]-5′,11′-dioxo-11′*H*-indeno[1,2-*c*]isoquinoline}propane (54). 5 M NaOH (aq) was added slowly to a solution of indenoisoquinoline hydrochloride **53**[15] (1.0 g, 1.58 mmol) in a water−chloroform solution (2:1, 250 mL). At a pH of 7−8, the organic layer was separated and the aqueous layer was extracted with chloroform (3 × 100 mL). The combined organic layers were washed with water (100 mL) and sat. aq NaCl (100 mL), dried over Na_2SO_4, and concentrated. Lactone **4** (0.43 g, 1.74 mmol) was added to a solution of the crude indenoisoquinoline triamine (0.70 g, 1.34 mmol) in chloroform (200 mL), and the reaction mixture was heated at reflux for 4 days. The reaction mixture was cooled to room temperature, Et_3N (0.93 mL, 6.64 mmol) and Boc_2O (0.87 g, 3.98 mmol) were added, and the solution was allowed to stir at room temperature for 12 h. The crude reaction mixture was purified by flash column chromatography (SiO_2/20% EtOAc in hexane, then 1−3% MeOH in $CHCl_3$) to provide Boc-protected bisindenoisoquinoline **54** (0.72 g, 48%) as purple solid: mp 120−122 °C. ^{1}H NMR ($CDCl_3$) δ 8.64 (d, J = 8.3 Hz, 1 H), 8.24 (d, J = 5.7 Hz, 1 H), 7.94 (s, 1 H), 7.67 (t, J = 7.4 Hz, 1 H), 7.58 (s, 1 H), 7.56 (s, 1 H), 7.42−7.34 (m, 5 H), 6.99 (s, 1 H), 6.05 (s, 2 H), 4.49 (bs, 2 H), 4.40 (bs, 2 H), 4.01 (s, 3 H), 3.92 (s, 3 H), 3.45 (bs, 4 H), 3.29 (bs, 4 H), 2.10 (bs, 4 H), 1.87 (m, 2 H), 1.42 (s, 18 H); ESIMS *m*/*z* (rel intensity) 953 (MH^+, 30), 853 (MH^+ − Boc, 100). Anal. ($C_{54}H_{56}N_4O_{12}\cdot 0.9\ CHCl_3$) C, H, N.

1,3-{6-(3-Amino-1-propyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline}-{5′,6′-Dihydro-6′-(3′-amino-1′-propyl)-5′,11′-dioxo-11′*H*-indeno[1,2-*c*]-isoquinoline}propane Bis(trifluoroacetate) (55). Boc-protected bisindenoisoquinoline **54** (0.55 g, 0.58 mmol) was dissolved in neat CF_3COOH (30 mL), and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated, and the

resultant solid was diluted with chloroform (50 mL) and filtered through a sintered glass funnel to provide bisindenoisoquinoline **55** (0.43 g, 76%) as purple solid: mp 218−220 °C. ^{1}H NMR (DMSO-d_6) δ 8.65 (bs, 2 H), 8.53 (d, J = 8.0 Hz, 1 H), 8.17 (d, J = 7.2 Hz, 1 H), 7.80−7.75 (m, 3 H), 7.55−7.50 (m, 4 H), 7.39 (s, 1 H), 7.32 (s, 1 H), 7.02 (s, 1 H), 6.18 (s, 2 H), 4.55 (bs, 2 H), 4.45 (bs, 2 H), 3.86 (s, 3 H), 3.81 (s, 3 H), 3.07−2.98 (bs, 8 H), 2.14 (bs, 4 H), 1.89 (bs, 2 H); ESIMS m/z (rel intensity) 753 (MH^+, 100). Anal. ($C_{48}H_{42}N_4O_{12}F_6 \cdot 3.1\ H_2O$) C, H, N.

Top1-Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from Baculovirus as described previously.[47] The 161 bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (50 μL reactions) for 1 h at 37 °C and separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161 bp fragment was eluted from the gel slice using the QIAEX II kit (QIAGEN Inc., Valencia, CA). Approximately 200 ng of the fragment was 3′-end labeled at the Hind III site by fill-in reaction with [alpha-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM aq NaCl) with 0.5 units of DNA polymerase I (Klenow fragment). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled 161 bp fragment was collected. Aliquots (approximately 50 000 dpm/reaction) were incubated with Top1 at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). The samples (10 μL) were mixed with 30 μL of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in denaturing gels (16% polyacrylamide, 7 M urea). Gels were dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Viscosity Studies. Viscosity studies were done exactly as described by Dedon[48] using a Cannon-Manning semi-micro viscometer (C75). Temperature was maintained using a Cannon CT-1000 Constant Temperature Bath.

Molecular Modeling. For the generation of the hypothetical bisintercalated models displayed in Supporting Information, the indenoisoquinoline moieties of structure **43** were overlapped with the two intercalation moieties in the structure of the TOTO−DNA binary complex[42] (PDB code 108D) using Sybyl 7.0. The structure of TOTO was then removed, and the energy of the **43**−DNA complex was minimized using the MMFF94s force field, MMFF94 charges, and the Powell method, with no initial optimization and a distance-dependent dielectric function, to a gradient of 0.05 kcal/mol. For the generation of a monointercalated hypothetical model, one indenoisoquinoline moiety of structure **43** was overlapped with the indenoisoquinoline in the reported ternary structure[35] (PDB code 1SC7) using Sybyl 7.0. The crystal structure ligand was then removed, and the energy of the **43**−DNA−Top1 complex was minimized using the MMFF94s force field, MMFF94 charges, and the Powell method, with no initial optimization and a distance-dependent dielectric function, to a gradient of 0.05 kcal/mol.

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Supporting Information Available: Elemental analyses for compounds **18**, **20**, **21**, **31**−**34**, and **37**−**55** and molecular models of hypothetical binary **43**−DNA complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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