Design, Synthesis, and Biological Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Featuring Polyamine Side Chains on the Lactam Nitrogen

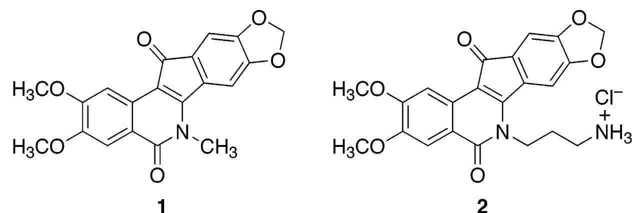
Muthukaman Nagarajan,[†] Xiangshu Xiao,[†] Smitha Antony,[‡] Glenda Kohlhagen,[‡] Yves Pommier,[‡] and Mark Cushman*[,†]

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

Received June 26, 2003

The indenoisoquinolines are a class of noncamptothecin topoisomerase I inhibitors that display significant cytotoxicity in human cancer cell cultures. They offer a number of potential advantages over the camptothecins, including greater chemical stability, formation of more persistent cleavage complexes, and induction of a unique pattern of DNA cleavage sites. Molecular modeling has suggested that substituents on the indenoisoquinoline lactam nitrogen would protrude out of the DNA duplex in the ternary cleavage complex through the major groove. This indicates that relatively large substituents in that location would be tolerated without compromising biological activity. As a strategy for increasing the potencies and potential therapeutic usefulness of the indenoisoquinolines, a series of compounds was synthesized containing polyamine side chains on the lactam nitrogen. The rationale for the synthesis of these compounds was that the positively charged ammonium cations would increase DNA affinity through electrostatic binding to the negatively charged DNA backbone, and the polyamines might also facilitate cellular uptake by utilization of polyamine transporters. The key step in the synthesis involved the condensation of Schiff bases, containing protected amine side chains, with substituted homophthalic anhydrides, to afford *cis*-3-aryl-4-carboxy-1-isoquinolones. These isoquinolones were then converted to indenoisoquinolines with thionyl chloride. Although monoamines were much more potent than the lead compound, no significant increase in potency was observed through incorporation of additional amino groups in the side chain. However, one of the monoamine analogues, which features a bis(2-hydroxyethyl)amino group in the side chain, proved to be one of the most cytotoxic indenoisoquinoline synthesized to date, with a GI50 mean-graph midpoint (MGM) of 0.07 μM in the NIH human cancer cell culture screen, and topoisomerase I inhibitory activity comparable to that of camptothecin.

The discovery of the topoisomerase I inhibitory activity of the indenoisoquinoline **1** (NSC 314622) has naturally generated interest in its potential clinical utility as an anticancer agent.[1] Comparison of the properties of **1** with those of the camptothecins, which are the only topoisomerase I inhibitors in clinical use, has revealed a number of differences that might be advantageous for the indenoisoquinolines. First, in contrast to the camptothecins, which are inactivated by lactone hydrolysis at physiological pH,[2] the indenoisoquinolines are chemically stable. Second, the enzyme–DNA cleavage complexes stabilized by indenoisoquinoline **1** are more persistent than those induced by camptothecin.[1] Long camptothecin infusion times are necessary to compensate for the reversibility of the camptothecin cleavage complexes during chemotherapy to achieve maximal activity.[3] Third, the indenoisoquinolines induce a unique pattern of DNA cleavage sites relative to the camptothecins, indicating that they may target the human genome differently and therefore potentially exhibit a different spectrum of anticancer activity from the camptothecins.[1,4,5] However, the potential clinical utility of the lead compound **1** itself is limited by its moderate activity, both as a cytotoxic agent in cancer cell cultures and as a topoisomerase I poison.



In a search for more potent and useful indenoisoquinolines based on the lead structure **1**, a series of analogues were synthesized in which the *N*-methyl group was replaced by various substituents.[4–6] One of the most cytotoxic topoisomerase I inhibitors proved to be compound **2**, having a 3-aminopropyl substituent on the indenoisoquinoline nitrogen. Compound **2** was more cytotoxic than **1** in human cancer cell cultures by a factor of 125, and it was also more potent as a topo-

* To whom correspondence should be addressed: Tel: 765-494-1465. Fax: 765-494-6790. E-mail: cushman@pharmacy.purdue.edu.
[†] Purdue University.
[‡] National Cancer Institute.

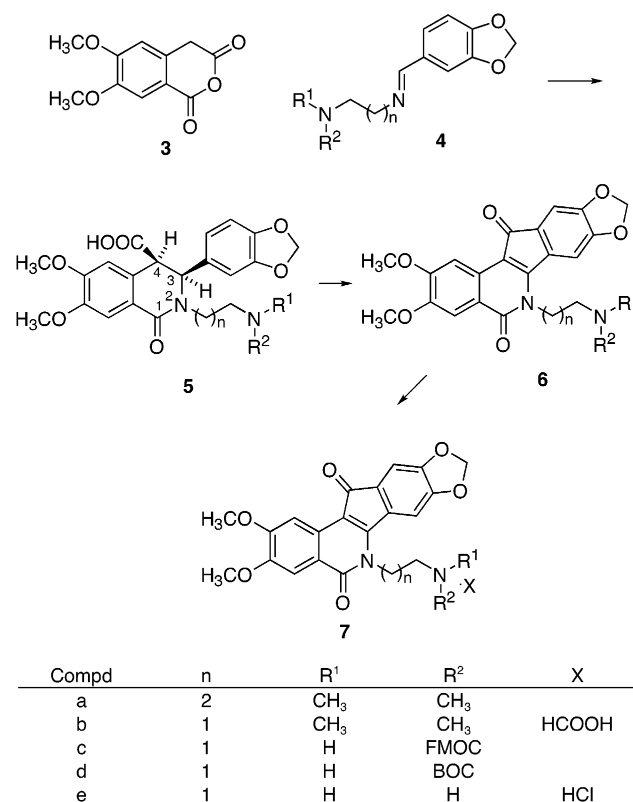
10.1021/jm030313f CCC: $25.00 © 2003 American Chemical Society
Published on Web 11/21/2003

isomerase I inhibitor.[5] The reasons for the enhanced potency of the 3-aminopropyl analogue **2** remain to be established. One possibility is that the positively charged ammonium cation could possibly increase the affinity of **2** for DNA by an electrostatic attraction to the negatively charged phosphodiester linkages of the DNA. This would be similar to the situation established for the polyamines and polyamine derivatives related to spermine and spermidine.[7−10] Another possible reason is that the 3-aminopropyl analogue **2** could be actively transported into cells similarly to the polyamines.[9−14] However, in general, the affinities of polyamines for the "polyamine transporter" are in the order tetraamines > triamines > diamines > monoamines, so monoamines such as the 3-aminopropyl analogue **2** are the least likely to utilize the polyamine transport system efficiently.[11,15,16] Since both DNA affinity and transport of polyamines are facilitated by an increase in the number of basic nitrogens in the polyamine structure, the cytotoxicity and topoisomerase I inhibitory activity of the 3-aminopropyl analogue **2** might be enhanced further by the incorporation of additional basic nitrogens in the side chain.[7,11,17] In addition, since polyamines have a high affinity for DNA but are "motionally free" so that they can move rapidly along the DNA backbone, the attachment of a polyamine to an intercalator could allow the conjugate to slide along the DNA until it finds an ideal location for formation of the cleavage complex.[18] Accordingly, the goal of the present study was to synthesize indenoisoquinolines with polyamine side chains on the indenoisoquinoline nitrogen and to test them for topoisomerase I inhibitory activity as well as for cytotoxicity in human cancer cell cultures.

Chemistry

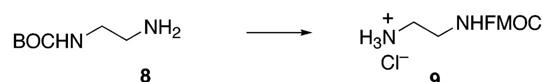
Scheme 1 outlines our general strategy for the synthesis of indenoisoquinolines. A key reaction in this sequence is the condensation of Schiff bases with homophthalic anhydrides to afford substituted isoquinolones.[19] Thus, the reaction of 4,5-dimethoxyhomophthalic anhydride (**3**)[20] with Schiff bases **4a**−**c** in chloroform at room temperature afforded the expected racemic mixtures of the isoquinolones **5a**−**c** in moderate yields. The 5−6 Hz coupling constant observed for the two methine protons in the ^{1}H NMR spectra of the products **5a**−**c** is consistent with the assigned cis stereochemistry.[21] In contrast, these two protons usually appear as broad singlets in the ^{1}H NMR spectra of the trans diastereomers in closely related systems.[21] The (methylenedioxy)phenyl ring and the carboxyl group are both pseudoaxial in the trans diastereomers, and one pseudoaxial proton and one pseudoequatorial proton (most likely at C-3) are present in the cis diastereomers. Treatment of the cis diastereomers **5a**−**c** with thionyl chloride afforded the corresponding indeno[1,2-*c*]isoquinolones **6a**−**c**.[22] The BOC-protected compound **6d** was prepared by removal of the FMOC group in a 1:1 mixture of chloroform and piperidine to provide the corresponding primary amine, which was converted in situ to the BOC-protected intermediate **6d**. To increase aqueous solubility, the formate salt **7b** was made by treatment of **6b** with formic acid, while the hydrochloride salt **7e** was obtained by subjection of the BOC-protected compound **6d** to hydrochloric acid. In this

Scheme 1



Compd	n	R[1]	R[2]	X
a	2	CH_3	CH_3	
b	1	CH_3	CH_3	HCOOH
c	1	H	FMOC	
d	1	H	BOC	
e	1	H	H	HCl

Scheme 2

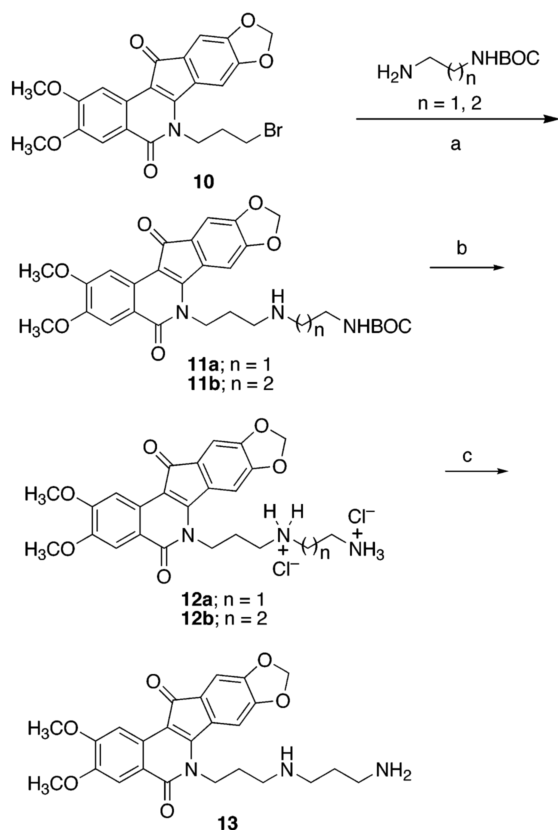


sequence of reactions, it was necessary to employ tertiary amines or protected amines **4** to avoid simple aminolysis of the anhydride at the expense of condensation with the Schiff base moiety. The FMOC protecting group was chosen since it was stable to the hydrochloric acid generated during the treatment of the isoquinolones with thionyl chloride to produce indenoisoquinoline **6**. The FMOC-protected amine necessary to prepare **4c** was obtained from **9**, which in turn was made from the BOC-protected amine **8** (Scheme 2).[23,24]

It was anticipated that side chains containing two, three, or four basic nitrogens could be attached to the isoquinolone nitrogen by reaction of appropriately protected polyamines with the *N*-(3-bromopropyl) intermediate **10**[5] (Scheme 3). This strategy proved to be successful in the synthesis of the BOC-protected intermediates **11a** and **11b** (Scheme 3), as well as **18a**−**d** (Scheme 5) and **22** (Scheme 7). The BOC protecting groups were then removed under acidic conditions to afford the hydrochloride salts **12a** and **12b** (Scheme 3), **19a**−**d** (Scheme 5), and **23** (Scheme 7). In the case of the bis(hydrochloride) salt **12b**, the diamine **13** was obtained after neutralization with sodium hydroxide (Scheme 3).

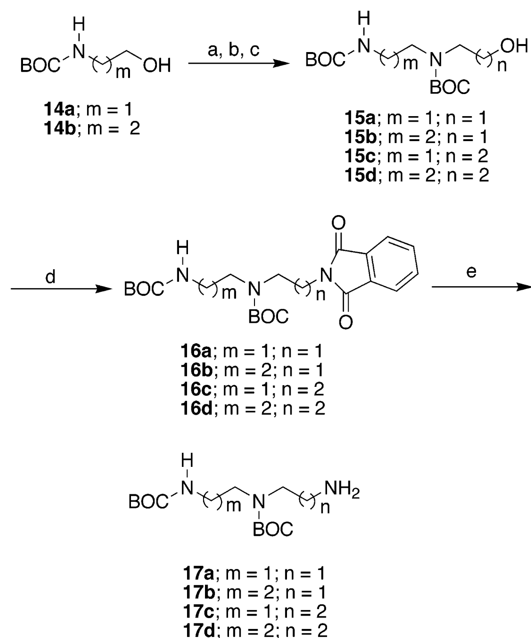
The BOC-protected polyamines **17a**−**d** necessary for the synthesis of the triamine salts **19a**−**d** were synthesized as outlined in Scheme 4. The two BOC-protected amino alcohols **14a** and **14b** were converted to their mesylates, reacted with ethanolamine or propanolamine, and the resulting secondary amines were then

Scheme 3[a]



[a] Reagents: (a) K_2CO_3, 1,4-dioxane, 100 °C, 4 h; (b) 2 M HCl in ether, RT, 8 h; (c) 2 M NaOH, $CHCl_3$, RT.

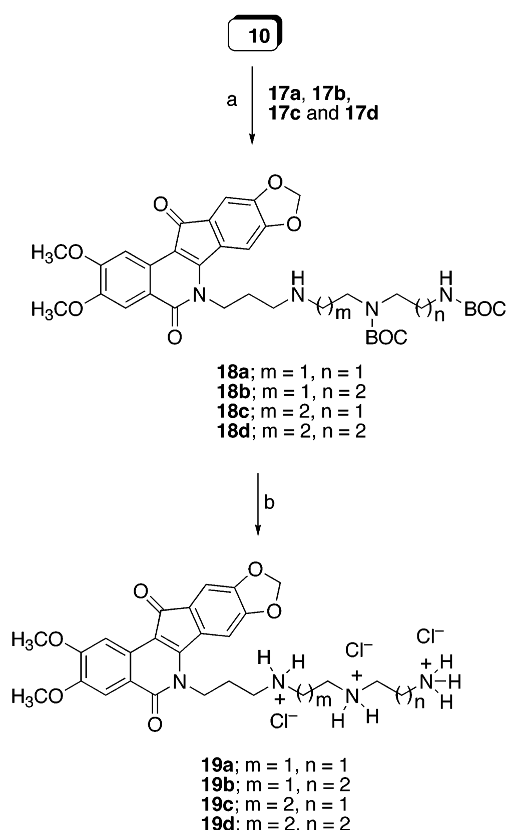
Scheme 4[a]



[a] Reagents: (a) CH_3SO_2Cl, Et_3N, $CHCl_3$, 0 °C, 4 h; (b) ethanolamine or propanolamine (neat), 80 °C, 8 h; (c) BOC_2O, Et_3N, $CHCl_3$, 0 °C–RT, 8 h; (d) DEAD, PPh_3, phthalimide, dry THF, 0 °C–RT, 5 h; (e) $NH_2NH_2\cdot nH_2O$, CH_3OH, reflux, 12 h.

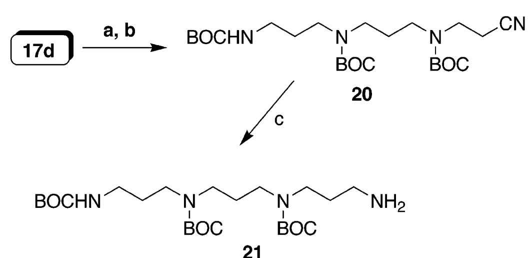
BOC-protected to afford intermediates **15a–d**. Mitsunobu reaction of the primary alcohols **15a–d** with phthalimide afforded intermediates **16a–d**, which were then converted to the primary amines **17a–d** by hydrazinolysis.

Scheme 5[a]



[a] Reagents: (a) K_2CO_3, 1,4-dioxane, 100 °C, 4 h; (b) 2 M HCl in ether, RT, 8 h.

Scheme 6[a]

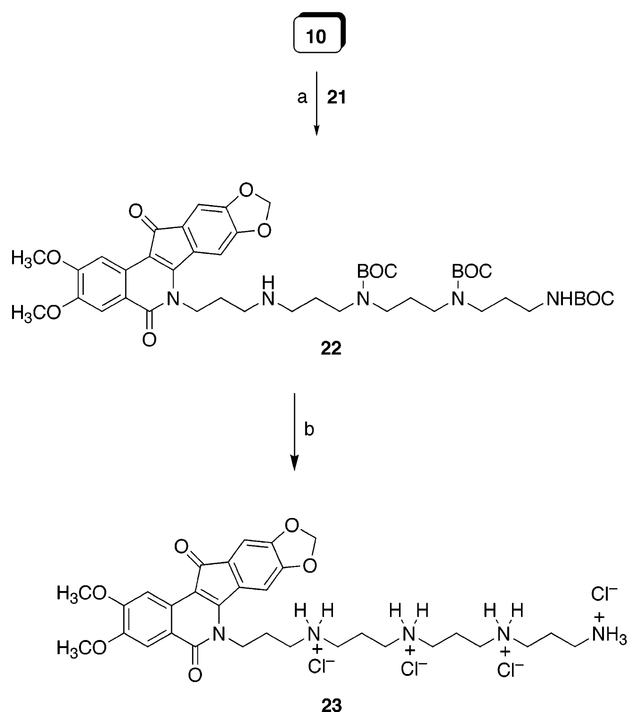


[a] Reagents: (a) Acrylonitrile, CH_3OH, RT, 16 h; (b) BOC_2O, Et_3N, $CHCl_3$, 0 °C–RT, 12 h; (c) H_2, Raney Ni, EtOH–2 M NaOH, RT, 16 h.

The BOC-protected amine **21** necessary for the preparation of the tetraamine salt **23** was obtained by addition of the primary amino group of **17d** to acrylonitrile, followed by BOC protection of the resulting secondary amine (Scheme 6). Catalytic hydrogenation of the nitrile **20** using Raney nickel then afforded the primary amine **21**. Alternative approaches to the synthesis of BOC-protected polyamines that are structurally related to **17a–d** and **21** have also been reported.[25–27]

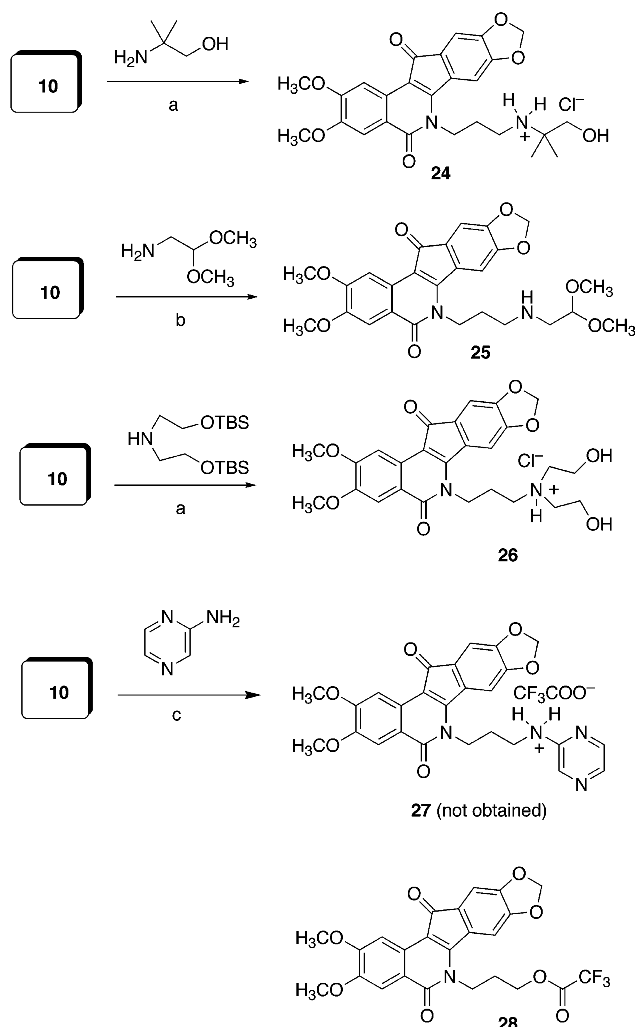
Finally, an assortment of additional analogues **24–26** were obtained by displacement of bromide from **10** using various amines. Two of the amines were isolated as their hydrochloride salts **24** and **26**, while amine **25** was obtained as the free base. In the case of the reaction with aminopyrazine, the trifluoroacetate **28** was obtained in low yield instead of the expected trifluoroacetate salt **27**. Evidently, the aniline-type nitrogen of aminopyrazine is not nucleophilic enough to displace the

Scheme 7[a]



[a] Reagents: (a) K_2CO_3, 1,4-dioxane, 100 °C, 4 h; (b) 2 M HCl in ether, RT, 8 h.

Scheme 8[a]



[a] Reagents: (a) (i) K_2CO_3, 1,4-dioxane, 100 °C, 4 h; (ii) 2 M HCl in ether, RT, 8 h; (b) K_2CO_3, 1,4-dioxane, 100 °C, 4 h; (c) (i) K_2CO_3, 1,4-dioxane, 100 °C, 4 h; (ii) CF_3COOH (neat), RT, 30 min.

bromide from **10** even after prolonged heating at 100 °C in 1,4-dioxane, and the trifluoroacetate **28** subsequently results from the reaction of **10** with the trifluoroacetic acid that was intended to form the salt **27**.

Biological Results and Discussion. The indenoisoquinolines 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 with approximately 55 different cancer cell lines of diverse tumor origins. The GI50 values obtained with selected 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} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. Therefore, the MGM value represents an overall assessment of toxicity of the compound across numerous cell lines. For comparison purposes, the activities of the previously reported lead compound **1**[1] and its more potent *N*-3′-aminoalkyl derivative **2**[5] are also included in the table. The relative potencies of the compounds in the production of topoisomerase I-mediated DNA cleavage are also listed in the table.

During the design of the present series of indenoisoquinolines, the ring system and its appendages were held constant except for the substituent on the lactam nitrogen. This was done to focus on the biological effects of side chain variation through the incorporation of amino groups.

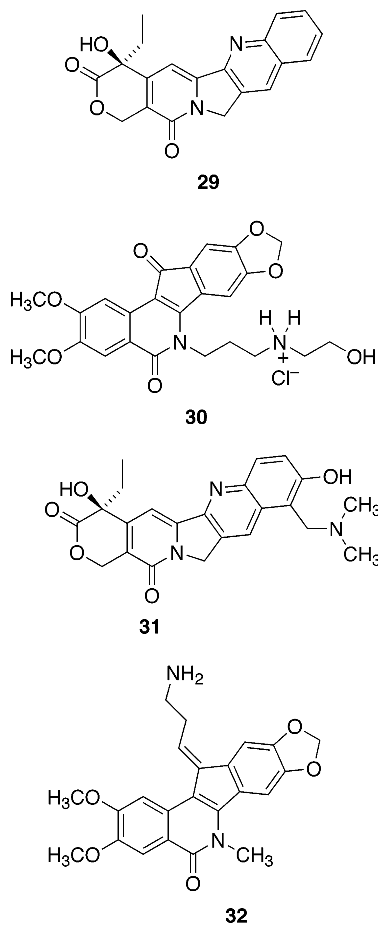
It is apparent from the MGM values listed in Table 1 that most of the indenoisoquinolines containing amine side chains were more cytotoxic than the lead compound **1**. They were also generally more potent as topoisomerase I inhibitors, although **19b** and **19d** did not display increased potency, and the tetraamine **23** was inactive. However, in contrast to prior literature reports correlating the cytotoxicities of polyamine derivatives to the number of basic nitrogens in the polyamine chain,[11,12,17] the incorporation of additional amino groups in the polyamine side chain beyond the one amino group in **2** did not result in increased cytotoxicity in the present series of compounds. It therefore seems likely that these polyamines are not actively transported by the polyamine uptake system. Although the present series of polyamine derivatives is not based on the exact structures of spermine and spermidine, the selectivity of the polyamine transport systems is not strictly limited to the naturally occurring polyamines, as a wide variety of synthetic polyamines are also transported.[12] This would argue that the failure to observe increased cytotoxicity with an increase in the number of basic nitrogens in the side chain in the present series of compounds should not be strictly attributed to the use of synthetic polyamines instead of spermine or spermidine. On the other hand, at least in the series of N^1-anthracenylmethyl−polyamine conjugates studied by Phanstiel et al., the architecture of the polyamine vector presented to the

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

	cytotoxicity (GI50 in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNSSF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	topoisomerase I cleavage[c]
1	1.3	35	41	4.2	73	68	37	96	20	++
2	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21	0.16	+++
6a	0.018	0.12	0.19	0.54	1.4	0.9	0.14	0.65	0.35	++++
7b	1.1	0.66	0.90	1.2	10	4.4	1.9	6.1	3.1	+++++
7e	0.58	0.068	1.3	0.14	0.86	0.36	0.40	1.04	0.34	++++
12a	0.38	0.56	0.43	0.65	0.70	0.37	0.72	1.6	0.88	+++
13	1.6	1.3	1.2	3.4	3.1	2.3	3.1	2.5	3.2	+++
19a	2.8	0.9	0.55	2.6	1.7	2.5	1.2	2.7	2.4	++++
19b	2.9	1.3	8.6	1.9	8.1	1.7	4.6	3.4	3.0	++
19c		23	38	37	20	20	52	38	41	++++
19d		43	>100	44	0.88	33	>100	68	59	++
23	3.0	1.5		1.7	1.6	1.6	1.4	1.4	2.0	0
24	0.14	0.62	0.36	0.28	15.2	0.18	2.51	7.2	1.6	+++
25	0.45	0.18	6.7	2.86	6.9		0.54	10.8	2.1	++++
26	<0.01	<0.01	<0.01	<0.01	0.13	<0.01	<0.01	0.08	0.07	++++
28	<0.01	0.018	<0.01	<0.01	0.07	<0.01	0.66	0.03	0.15	++++
29	<0.01	0.03	<0.01	<0.01	0.22	0.02	<0.01	0.04	0.04	++++

[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 topoisomerase I-mediated DNA cleavage was expressed semiquantitatively as follows: +: weak activity; ++: similar activity as the parent compound **1**; +++ and ++++: greater activity than the parent compound **1**; ++++: similar activity as 1 μM camptothecin.

polyamine transporter was crucial for uptake.[28,29] Therefore, the optimal use of the polyamine transporter is not open to an indiscriminate array of structural motifs, and this may explain the results observed with the present series of polyamine conjugates.



29, **30**, **31**, **32**

In general, the intensities of the DNA cleavage bands produced by the amines in this study varied as a function of drug concentration, reaching a maximum intensity at 1 μM indenoisoquinoline concentration, and then falling off at higher drug concentration. This bell-shaped dose−response pattern was observed previously with the amines **2** and **30**.[5] Compound **25** was an exception, with the intensities of the cleavage bands reaching a maximum at a drug concentration of 10 μM. This biphasic pattern of response was not observed with the indenoisoqinolines **28** and **1**, which do not contain amino groups. These results indicate that the amines suppress topoisomerase I-mediated DNA cleavage at high drug concentration, which is similar to the situation observed with DNA unwinding or intercalating inhibitors.[30−32] Prior results with the amino alcohol **30** demonstrated a low affinity DNA intercalation that could be responsible for suppression of DNA cleavage at higher drug concentration.[33] Alternatively, higher concentrations of the amines may suppress topoisomerase I-mediated DNA cleavage through a direct effect on the enzyme resulting in a conformational change, as has been proposed with saintopin E.[31]

As mentioned above, the tetraamine **23** was cytotoxic even though it was inactive as a topoisomerase I inhibitor. Obviously, the cytotoxicity of this compound has nothing to do with poisoning of topoisomerase I. This raises the question of whether additional targets could be involved in the cytotoxicities of the other compounds as well. However, the previously published COMPARE analysis of the cytotoxicity profile of the lead compound **1** provides convincing evidence that inhibition of topoisomerase I is likely to be responsible for the cytotoxicity of this compound and many of the other indenoisoquinolines as well.[1]

Within the series of triamines **19**, the two compounds having central ethylenediamine linkages [**19a** (MGM 2.4 μM) and **19b** (MGM 3.0 μM), $m = 1$] were significantly more cytotoxic than the two corresponding propylenediamines **19c** (MGM 41 μM) and **19d** (MGM 59 μM) ($m = 2$). Also, the two compounds having terminal ethylenediamine moieties ($n = 1$, **19a** and **19c**) were more potent topoisomerase I inhibitors than the two having terminal propylenediamine moieties ($n = 2$, **19b** and **19d**).

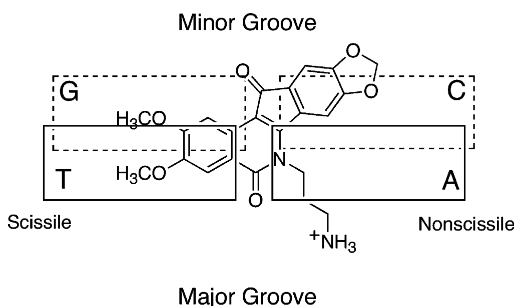


Figure 1. Hypothetical model of the orientation of indenoisoquinoline **2** relative to DNA in the ternary complex containing topoisomerase I, DNA, and the inhibitor **2**.

Dimethylation of the terminal amino group of **2** (MGM 0.16 μM) did not have a large effect on cytotoxicity (see **6a**, MGM 0.35 μM). Shortening the side chain by one methylene unit decreased cytotoxicity (**7b**, MGM 3.1 μM), but increased topoisomerase I inhibitory activity. Overall, these results, in conjunction with those obtained with **19a**–**d** and **23**, reveal that there is not an exact correlation of topoisomerase I inhibition potency and cytotoxicity in the present series of polyamine derivatives. In this regard, it is interesting to note that in a related series of tetraamine–(DNA–intercalator) conjugates, the less potent topoisomerase II inhibitors proved to be more cytotoxic to L1210 cells than the more potent topoisomerase II inhibitors.[13,14] In this previously reported case, as well as in the present case with the tetraamine **23**, differences in cellular uptake and the possibility of additional targets may play a role. Prior investigations with tetraamine–(DNA–intercalator) conjugates have indicated that strong binding of tetraamines in the cell membrane may disrupt its structure and function.[29]

Compound **26**, having two 2-hydroxyethyl substituents on the side chain nitrogen atom, is the most cytotoxic compound in the present series (MGM 0.07 μM). Its potency approaches that of camptothecin (MGM 0.04), and it is also as potent as camptothecin as a topoisomerase I poison. The previously reported compound **30**, having a single 2-hydroxyethyl substituent, was also very cytotoxic (MGM 0.11 μM).[5]

Assuming the lactam ring of the 3-aminopropyl analogue **2** is oriented in the ternary cleavage complex in the same way that it is in the published crystal structure of the camptothecin derivative topotecan (**31**), the aminopropyl side chain of **2** points out from the major groove of DNA (Figure 1).[2,34] This would also suggest that the polyamine side chains of the indenoisoquinolines in the present series are also directed out of the duplex through the major groove, which would explain the bulk tolerance for the biological effect of replacing the *N*-methyl group in the lead compound **1** with a variety of relatively large polyamine chains. A hypothetical model (Figure 2) was constructed by overlapping the structure of the 3-aminopropyl analogue **2** with the structure of topotecan (**31**) in the published crystal structure of the ternary complex.[34] During this operation, the inhibitor was oriented relative to DNA according to the schematic representation of the structure displayed in Figure 1. The structure of topotecan (**31**) was then removed and the energy of the new complex was minimized with Sybyl, using the MMFF94s force field and MMFF94 charges. During energy minimization, the structures of the protein, nucleic acid, and the surrounding water molecules were frozen while the inhibitor was allowed to move. According to this model, the C-11 ketone is hydrogen bonded directly to the Arg364 side chain. In addition, the lactam oxygen and the amino group of the side chain are hydrogen bonded to a network of water molecules that hydrogen bond with the protein and the nucleic acid residues. The stabilization of the side chain through hydrogen bonding would provide an explanation for the more potent topoisomerase I inhibitory activity of **2** and related amines and amino alcohols relative to the lead compound **1**. The model indicates that it is possible for the indenoisoquinoline ring system to intercalate into the DNA structure of the cleavage complex, with the ring system occupying the space that would normally be filled with a base pair. We assume that, like the camptothecins, the indenoisoquinolines effectively replace a base pair in the duplex at the cleavage site in the ternary cleavage complex, thereby sterically hindering the religation reaction.[34]

With respect to the molecular modeling, it is worth noting that the indenoisoquinoline **32** (MGM 0.34 μM), which has a C-11 aminoalkenyl substituent on the opposite side of the molecule, also has enhanced activity relative to the lead compound **1** (MGM 20 μM).[2] We assume that in the case of **32**, the aminoalkyl group projects outward from the minor groove. Taken together, these results indicate that the C-11 region and the lactam region of the indenoisoquinolines will be fruitful locations for the placement of large substituents, since they will not clash with the surrounding DNA structure in the ternary complex.

In addition to being an unexpected product formed during the attempted synthesis of the aminopyrazine derivative **27**, the trifluoroacetate **28** displayed unexpectedly potent cytotoxicity and topoisomerase I inhibitory activity. Its cytotoxicity MGM was 0.15 μM, reflecting a potency 133 times that of the lead compound **1**. The reason for the high potency of **28** remains to be established, but it is obvious that the trifluoroacetate is a good leaving group that provides a potential for alkylation of nucleophiles in DNA and the enzyme in the ternary complex. In this regard, it is worth noting that the cytotoxic potency of **28** is 91 times greater than the previously reported 3-bromopropyl compound **10** (MGM 13.7 μM), both of which bear a good leaving group.[5] The gain in both cytotoxicity and topoisomerase I inhibitory activity of compound **28** in comparison to **10** will undoubtedly stimulate additional work on structurally related topoisomerase I inhibitors.

In summary, a new series of indenoisoquinolines has been synthesized with aminoalkyl and polyamine side chains on the lactam nitrogen. These compounds are more potent as topoisomerase I poisons and as cytotoxic agents in human cancer cell cultures than the lead compound **1**. The structure–activity relationships and the molecular models displayed in Figures 1 and 2 provide a possible explanation for the enhanced activity of the amines, and they also provide a framework for the design of future indenoisoquinoline topoisomerase I inhibitors.

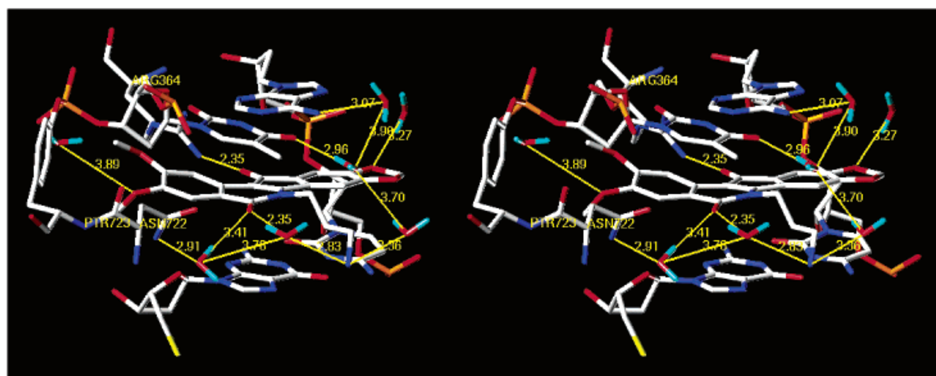


Figure 2. Model of the binding of the indenoisoquinoline **2** in the ternary complex consisting of DNA, topoisomerase I, and the inhibitor. The diagram is programmed for wall-eyed viewing.

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. Except where noted, ^{1}H NMR spectra were obtained using $CDCl_3$ as solvent and TMS as internal standard. ^{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 Analtech silica gel GF 1000-micron glass plates. Compounds were visualized with short wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

***cis*-2-[3-(*N,N*-Dimethylamino)-1-propyl]-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (5a).** Piperonal (4.00 g, 26.6 mmol) was added to a stirred solution of commercially available 3-(dimethylamino)propylamine (3.2 g, 32.0 mmol) and anhydrous magnesium sulfate (10.0 g) in chloroform (100 mL) and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered through a Celite pad and the residue was washed with chloroform (40 mL). The combined organic layer was washed with water (100 mL), brine, and dried (Na_2SO_4). The organic solution was concentrated on a rotary evaporator and the resulting Schiff base was used in the next reaction without purification. 4,5-Dimethoxyhomophthalic anhydride **3** (4.10 g, 18.4 mmol) was added to a chloroform (100 mL) solution of the crude imine **4a** (4.30 g, 18.4 mmol) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated to give an oily residue. The residue was dissolved in a minimum amount of methanol (20 mL) and ether (70 mL) was added slowly with stirring to obtain a white precipitate. The resultant solid was filtered off and washed with CH_3OH-ether (2:1, 30 mL) to yield pure indenoisoquinolone **5a** (4.8 g, 57%) as a pale white solid: mp 230−232 °C. ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 1 H), 6.64 (s, 1 H), 6.63 (bs, 2 H), 6.57 (s, 1 H), 5.86 (s, 1 H), 5.84 (s, 1 H), 5.28 (s, 1 H), 4.30 (t, J = 9.5 Hz, 1 H), 3.82 (bs, 7 H), 3.61 (s, 1 H), 3.15−3.13 (m, 1 H), 3.04−2.96 (m, 1 H), 2.63 (s, 6 H), 2.11 (bs, 1 H), 1.89 (bs, 1 H); ESIMS m/z (relative intensity) 458 (24), 457 (MH^+, 100), 413 (7), 412 (28); HRESIMS calcd for $C_{24}H_{28}N_2O_7$: 457.1975. Found: 457.1979.

***cis*-2-[2-(*N,N*-Dimethylamino)-1-ethyl]-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (5b).** Piperonal (1.3 g, 8.5 mmol) was added to a stirred solution of commercially available 2-(dimethylamino)ethylamine (3.0 g, 9.4 mmol) and anhydrous magnesium sulfate (5.0 g) in chloroform (100 mL) and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered through a Celite pad and the residue was washed with chloroform (40 mL). The combined organic layer was washed with water (100 mL), brine, and dried (Na_2SO_4). The organic solution was concentrated on a rotary evaporator and the resulting Schiff base was used in the next reaction without purification. 4,5-Dimethoxyhomophthalic anhydride **3** (2.0 g, 9.0 mmol) was added to a chloroform (100 mL) solution of the imine **4b** (2.0 g, 9.1 mmol) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated to give an oily residue. The residue was dissolved in a minimum amount of methanol (20 mL) and ether (70 mL) was added slowly with stirring to obtain a white precipitate. The resultant solid was filtered off and washed with CH_3OH-ether (2:1, 30 mL) to afford pure indenoisoquinolone **5b** (2.8 g, 70%) as a pale white solid: mp 188−190 °C. ^{1}H NMR (DMSO-d_6) δ 7.49 (s, 1 H), 7.23 (s, 1 H), 6.76 (d, J = 8.0 Hz, 1 H), 6.58 (d, J = 7.8 Hz, 1 H), 6.50 (s, 1 H), 5.93 (s, 2 H), 5.10 (d, J = 5.7 Hz, 1 H), 4.47 (d, J = 5.3 Hz, 1 H), 3.94−3.88 (m, 2 H), 3.81 (s, 3 H), 3.74 (s, 3 H), 2.91−2.88 (m, 1 H), 2.49 (m, 1 H, merged with DMSO-d_6 peak), 2.22 (s, 6 H); ESIMS m/z (relative intensity) 444 (23), 443 (MH^+, 100), 398 (38). Anal. ($C_{23}H_{26}N_2O_7{\cdot}0.6H_2O$) C, H, N.

***cis*-2-(2-FMOCaminoethyl)-4-carboxy-3,4-dihydro-6,7-dimethoxy-3-(3,4-methylenedioxyphenyl)-1(2*H*)-isoquinolone (5c).** Piperonal (1.3 g, 8.5 mmol) was added to a stirred solution of FMOC-protected aminoethylamine hydrochloride **9**[23,24] (3.0 g, 9.4 mmol), Et_3N (2.00 mL, 14.2 mmol) and anhydrous magnesium sulfate (5.0 g) in chloroform (100 mL) and the mixture was stirred further at room temperature for 24 h. The reaction mixture was filtered through a Celite pad and the residue was washed with chloroform (40 mL). The combined organic layer was washed with water (100 mL), brine, and dried (Na_2SO_4). The organic solution was concentrated on a rotary evaporator and used in the next reaction without purification. 4,5-Dimethoxyhomophthalic anhydride **3** (1.6 g, 7.2 mmol) was added slowly in portions to a chloroform (100 mL) solution of the imine **4c** (3.0 g, 7.2 mmol) at 0 °C and the reaction mixture was further stirred at room temperature for 12 h. The precipitated solid was filtered off through a sintered glass funnel, washed with chloroform (50 mL), and dried to give pure indenoisoquinolone **5c** (2.6 g, 48%) as a white solid: mp 210−212 °C. ^{1}H NMR (DMSO-d_6) δ 7.86 (d, J = 7.2 Hz, 2 H), 7.65 (bs, 2 H), 7.50 (s, 1 H), 7.39 (bs, 2 H), 7.30 (t, J = 7.0 Hz, 2 H), 7.12 (s, 1 H), 6.76 (d, J = 7.8 Hz, 1 H), 6.54 (d, J = 7.6 Hz, 1 H), 6.45 (s, 1 H), 5.94 (s, 2 H), 5.04 (d, J = 5.7 Hz, 1 H), 4.69 (d, J = 5.2 Hz, 1 H), 4.25 (bs, 2 H), 4.17 (d, J = 6.0 Hz, 2 H), 3.99 (t, J = 6.0 Hz, 1 H), 3.78 (s, 3 H), 3.74 (s, 3 H), 3.24 (bs, 2 H); ESIMS m/z 637 (MH^+, 24), 434 (11), 399 (12), 398 (45), 369 (22), 368 (100), 355 (10), 354 (42). Anal. ($C_{36}H_{32}N_2O_9{\cdot}1.5H_2O$) C, H, N.

6-[3-(*N,N*-Dimethylamino)-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (6a). Thionyl chloride (100 mL) was added to the indenoisoquinolone **5a** (3.2 g, 7.02 mmol), and the reaction mixture was stirred at room temperature for 6 h. Benzene (50 mL) was added to the red solution and the mixture was concentrated under reduced pressure. Et_3N (20 mL) was added to a mixture of the resultant purple solid in chloroform (50 mL) and the mixture was stirred at room temperature for 2 h. The mixture was concentrated on a rotary evaporator and methanol (50 mL) was added to the resultant solid, which was filtered off through a sintered glass funnel.

The solid was further washed with $CH_3OH-CHCl_3$ (9:1, 30 mL) to obtain pure indenoisoquinoline **6a** (2.0 g, 65%) as a purple solid: mp 235−240 °C. ^1H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 7.62 (s, 1 H), 7.47 (s, 1 H), 7.03 (s, 1 H), 6.06 (s, 2 H), 4.47 (t, J = 8.2 Hz, 2 H), 4.03 (s, 3 H), 3.96 (s, 3 H), 2.48 (t, J = 6.2 Hz, 2 H), 2.29 (s, 6 H), 2.02−1.92 (m, 2 H); ESIMS m/z (relative intensity) 437 (MH^+, 100), 392 (5). Anal. ($C_{24}H_{24}N_2O_6$·0·3H_2O) C, H, N.

6-[2-(*N,N*-Dimethylamino)-1-ethyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (6b). Thionyl chloride (80 mL) was added to the indenoisoquinolone **5b** (2.5 g, 5.7 mmol) and the mixture was stirred at room temperature for 6 h. Benzene (50 mL) was added to the red solution and the mixture was concentrated under reduced pressure. Et_3N (20 mL) was added to a mixture of the resultant purple solid in chloroform (50 mL) and the mixture was stirred at room temperature for 2 h. The mixture was concentrated on a rotary evaporator and methanol (50 mL) was added to the resultant solid, which was filtered off through a sintered glass funnel. The solid was further washed with $CH_3OH-CHCl_3$ (9:1, 30 mL) to obtain pure compound **6b** (1.53 g, 64%) as a purple solid: mp 300−302 °C. ^1H NMR ($CDCl_3$) δ 8.00 (bs, 1 H), 7.63 (bs, 1 H), 7.24 (bs, 1 H), 7.06 (bs, 1 H), 6.07 (bs, 2 H), 4.54 (bs, 2 H), 4.03 (s, 3 H), 3.96 (s, 3 H), 2.72 (bs, 2 H), 2.39 (bs, 6 H); ESIMS m/z (relative intensity) 424 (23), 423 (MH^+, 100), 378 (32), 306 (39), 118 (12); HRESIMS calcd for $C_{23}H_{22}N_2O_6$: 423.1556. Found: 423.1558.

6-[2-(*N*-FMOCaminoethyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (6c). Thionyl chloride (50 mL) was added to the indenoisoquinolone **5c** (2.0 g, 3.15 mmol) and the mixture was stirred at room temperature for 6 h. Benzene (50 mL) was added to the red solution and the reaction mixture was concentrated under reduced pressure. The resultant solid was directly loaded on a silica gel column and eluted with a 0−1% gradient of methanol in $CHCl_3$ to give FMOC-protected aminoindenoisoquinoline **6c** (1.3 g, 67%) as a purple solid: mp 242−244 °C (dec). ^1H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 7.74 (d, J = 7.6 Hz, 2 H), 7.59 (s, 1 H), 7.54 (d, J = 3.3 Hz, 2 H), 7.51 (s, 1 H), 7.37 (t, J = 7.4 Hz, 2 H), 7.25 (m, 2 H), 6.99 (s, 1 H), 5.91 (s, 2 H), 5.45 (bs, 1 H), 4.55 (t, J = 6.4 Hz, 2 H), 4.40 (d, J = 7.2 Hz, 2 H), 4.17 (t, J = 7.2 Hz, 1 H), 4.02 (s, 3 H), 3.95 (s, 3 H), 3.68 (m, 2 H); ESIMS m/z (relative intensity) 617 (MH^+, 3), 611 (3), 444 (24), 443 (MH^+ − FMOC, 100), 426 (78), 395 (26), 378 (21); HRESIMS calcd for $C_{36}H_{28}N_2O_8$: 617.1924. Found: 617.1921. Anal. ($C_{36}H_{28}N_2O_8$·1.7H_2O) C, H, N.

6-[2-(*N*-*tert*-BOCamino)-1-ethyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (6d). Compound **6c** (1.5 g, 2.4 mmol) was dissolved in chloroform−piperidine (1:1, 100 mL) and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated on a rotary evaporator to dryness to give purple solid. BOC_2O (0.76 g, 3.5 mmol) in chloroform (20 mL) was added dropwise to a stirred solution of crude product (0.91 g, 2.3 mmol) and Et_3N (0.65 mL, 4.6 mmol) in chloroform (100 mL) at 0 °C and the reaction mixture was further stirred at room temperature for 8 h. The reaction mixture was diluted with water (100 mL), washed with 1% HCl (100 mL), brine and dried (Na_2SO_4). The reaction mixture was concentrated under reduced pressure and purified by silica gel column chromatography, eluting with a 0−1% gradient of methanol in chloroform to afford compound **6d** (0.84 g, 70%) as a purple solid: mp 252−254 °C. ^1H NMR ($CDCl_3$) δ 7.92 (s, 1 H), 7.56 (s, 1 H), 7.50 (s, 1 H), 7.00 (s, 1 H), 6.06 (s, 2 H), 5.25 (bs, 1 H, −NH), 4.52 (t, J = 6.7 Hz, 2 H), 4. 01 (s, 3 H), 3.96 (s, 3 H), 3.61 (q, J = 6.4 Hz, 2 H), 1.41 (s, 9 H); ESIMS m/z (relative intensity) 517 (MNa^+, 20), 495 (MH^+, 9), 455 (10), 395 (MH^+ − BOC, 31), 379 (21), 378 (100), 352 (18). Anal. ($C_{26}H_{26}N_2O_8$·0.3H_2O) C, H, N.

6-[2-(*N,N*-Dimethylamino)-1-ethyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline Hydroformate (7b). The indenoisoquinoline **6b** (0.3 g, 0.7 mmol) was dissolved in 98% formic acid (10 mL) and the solution was stirred at room temperature for 30 min. The reaction mixture was then concentrated on a rotary evaporator and the resultant solid was mixed with chloroform (30 mL) and filtered off through a sintered glass funnel to obtain pure compound **7b** (270 mg, 82%) as a pale purple solid: mp 296−298 °C. ^1H NMR (DMSO-d_6) δ 8.32 (bs, 1 H, COOH), 6.82 (bs, 1 H), 6.58 (bs, 2 H), 6.26 (bs, 1 H), 5.95 (bs, 2 H), 4.31 (bs, 2 H), 3.61 (s, 3 H), 3.53 (s, 3 H), 3.37 (bs, 2 H), 2.98 (bs, 6 H); ESIMS m/z (relative intensity) 424 (22), 423 (MH^+−HCOOH, 100), 379 (15), 378 (70). Anal. ($C_{24}H_{24}N_2O_6$·HCOOH·0.6H_2O) C, H, N.

6-(2-Aminoethyl)-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (7e). BOC-protected indenoisoquinoline **6d** (0.5 g, 1.01 mmol) was dissolved in chloroform (50 mL) and an anhydrous solution of HCl in ether (2 M, 10.1 mL, 20.2 mmol) was added at 0 °C. The reaction mixture was then stirred at room temperature for 6 h. The precipitated product was filtered off and washed with chloroform (50 mL) and dried over P_2O_5 for 24 h to afford pure indenoisoquinoline hydrochloride **7e** (0.33 g, 76%) as a purple solid: mp 294−296 °C. ^1H NMR (DMSO-d_6) δ 8.16 (bs, 2 H, −NH_2), 7.79 (s, 1 H), 7.47 (s, 1 H), 7.42 (s, 1 H), 7.02 (s, 1 H), 6.19 (s, 2 H), 4.60 (bs, 2 H), 3.89 (s, 3 H), 3.84 (s, 3 H), 3.19 (bs, 2 H); ESIMS m/z (relative intensity) 397 (7), 396 (24), 395 (MH^+, 100), 394 (7), 380 (7), 379 (20), 378 (79), 355 (9), 277 (14), 255 (11), 215 (24). Anal. ($C_{21}H_{19}N_2O_6Cl$·0.4H_2O) C, H, N.

General Procedure for the Synthesis of Amino Indenoisoquinolines 11a, 11b, 18a−18d, and 22. A mixture of indenoisoquinoline bromide **10** (0.3 g, 0.63 mmol), BOC-protected amines (1.89 mmol), and anhydrous K_2CO_3 (0.44 g, 3.2 mmol) in anhydrous 1,4-dioxane (25 mL) was heated to 100 °C and kept at that temperature for 4 h. The reaction mixture was cooled and concentrated on a rotary evaporator. The reaction mixture was diluted with chloroform (100 mL) and washed with a 1% HCl solution (50 mL), water, and brine and dried (Na_2SO_4). The reaction mixture was concentrated, loaded on a silica gel column, and eluted with a 0−1% gradient of methanol in chloroform to provide indenoisoquinolines **11a**, **11b**, **18a**−**18d**, and **22** in 25−68% yields.

6-[3-(2-*tert*-BOCaminoethyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (11a). The indenoisoquinoline **11a** (87 mg, 25%) was isolated as a purple solid: mp 199−201 °C. IR (thin film) 3450 (br), 2970, 2965, 1712, 1699, 1693, 1650, 1481, 1280 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.96 (s, 1 H), 7.60 (s, 1 H), 7.40 (s, 1 H), 7.00 (s, 1 H), 6.06 (s, 2 H), 5.06 (bs, 1 H), 4.49 (t, J = 7.3 Hz, 2 H), 4.02 (s, 3 H), 3.96 (s, 3 H), 3.25 (q, J = 5.6 Hz, 2 H), 2.77 (q, J = 6.3 Hz, 4 H), 2.01 (m, 2 H), 1.42 (s, 9 H); ESIMS m/z (relative intensity) 554 (5), 553 (28), 552 (MH^+, 100), 496 (12), 453 (14), 452 (MH^+ − BOC, 57), 392 (21). Anal. ($C_{29}H_{33}N_3O_8$·1.2H_2O) C, H, N.

6-[3-(3-*tert*-BOCaminopropyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (11b). The indenoisoquinoline **11b** (108 mg, 30%) was isolated as a purple solid: mp 197−199 °C. ^1H NMR ($CDCl_3$) δ 8.02 (s, 1 H), 7.63 (s, 1 H), 7.40 (s, 1 H), 7.06 (s, 1 H), 6.07 (s, 2 H), 4.98 (bs, 1 H), 4.52 (t, J = 7.8 Hz, 2 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 3.22 (m, 2 H), 2.78 (t, J = 6.3 Hz, 2 H), 2.69 (t, J = 6.7 Hz, 2 H), 2.00 (m, 2 H), 1.70 (m, 2 H), 1.40 (s, 9 H); ESIMS m/z (relative intensity) 568 (6), 567 (29), 566 (MH^+, 100), 510 (8), 467 (10), 466 (MH^+ − BOC, 39). Anal. ($C_{30}H_{35}N_3O_8$·0.5H_2O) C, H, N.

6-{3-[2-(2-*tert*-BOCamino)-ethyl-(*tert*-BOCamino)-ethylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18a). The indenoisoquinoline **18a** (760 mg, 52%) was isolated as a purple solid: mp 123−125 °C. ^1H NMR ($CDCl_3$) δ 8.07 (s, 1 H), 7.61 (s, 1 H), 7.32 (bs, 1 H), 7.04 (s, 1 H), 6.07 (s, 2 H), 5.49 (bs, 1 H), 5.31 (bs, 1 H), 4.50 (t, J = 7.4 Hz, 2 H), 4.02 (s, 3 H), 3.96 (s, 3 H), 3.34−3.29 (bs, 6 H), 2.80 (t, J = 5.3 Hz, 4 H), 2.01 (m, 2 H), 1.44 (s, 9 H), 1.38 (s, 9 H); ESIMS m/z (relative intensity) 696 (34), 695 (MH^+, 100), 639 (24), 595 (MH^+ − BOC), 495 (MH^+ − 2 × BOC). Anal. ($C_{36}H_{46}N_4O_{10}$·0.2H_2O) C, H, N.

6-{3-[2-(3-*tert*-BOCamino)-propyl-(*tert*-BOCamino)-ethylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18b). The indenoisoquinoline **18b** (510 mg, 68%) was isolated as a purple solid: mp 155−158 °C. ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1 H), 7.62 (s, 1 H), 738 (s, 1 H), 7.05 (s, 1 H), 6.07 (s, 2 H), 4.50 (t, J = 6.8 Hz, 2 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 3.29 (bs, 4 H), 3.10 (apparent t, J = 6.4 Hz, 2 H), 2.80−2.77 (m, 4 H), 2.01−1.98 (m, 2 H), 1.66 (m, 2 H), 1.44 (s, 9 H), 1.41 (s, 9 H); ESIMS m/z (relative intensity) 709 (MH^+, 100), 652 (10), 610 (6), 609 (MH^+ − BOC, 18), 509 (MH^+ − 2 × BOC, 10). Anal. ($C_{37}H_{48}N_4O_{10}$·0.3H_2O) C, H, N.

6-{3-[3-(2-*tert*-BOCamino)-ethyl-(*tert*-BOCamino)-propylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18c). The indenoisoquinoline **18c** (750 mg, 50%) was isolated as a purple solid: mp 115−118 °C. ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1 H), 7.63 (s, 1 H), 7.44 (s, 1 H), 7.05 (s, 1 H), 6.07 (s, 2 H), 5.04 (bs, 1 H), 4.85 (bs, 1 H), 4.51 (t, J = 7.4 Hz, 2 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 3.27 (bs, 6 H), 2.78 (t, J = 6.1 Hz, 2 H), 2.62 (t, J = 6.8 Hz, 2 H), 1.99 (m, 2 H), 1.75 (m, 2 H), 1.44 (s, 9 H), 1.40 (s, 9 H); ESIMS m/z (relative intensity) 710 (35), 709 (MH^+, 100), 653 (6), 609 (MH^+ − BOC, 16), 594 (8), 509 (MH^+ − 2 × BOC, 67), 494 (6). Anal. ($C_{37}H_{48}N_4O_{10}$) C, H, N.

6-{3-[3-(3-*tert*-BOCamino)-propyl-(*tert*-BOCamino)-propylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (18d). The indenoisoquinoline **18d** (360 mg, 47%) was isolated as a purple solid: mp 96−99 °C. ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1 H), 7.61 (s, 1 H), 7.31 (s, 1 H), 7.04 (s, 1 H), 6.06 (s, 2 H), 5.25 (bs, 1 H), 4.51 (t, J = 7.4 Hz, 2 H), 4.02 (s, 3 H), 3.96 (s, 3 H), 3.24 (bs, 4 H), 3.08 (apparent t, J = 5.7 Hz, 2 H), 2.79 (t, J = 5.9 Hz, 2 H), 2.64 (t, J = 6.1 Hz, 2 H), 2.04 (bs, 2 H), 1.76 (bs, 2 H), 1.65 (bs, 2 H), 1.43 (s, 9 H), 1.41 (s, 9 H); ESIMS m/z (relative intensity) 724 (35), 723 (MH^+, 100), 624 (14), 623 (MH^+ − BOC, 40), 524 (17), 523 (MH^+ − 2× BOC, 67), 332 (10). Anal. ($C_{38}H_{50}N_4O_{10}$·0.2H_2O) C, H, N.

6-{3-[3−3-(3-*tert*-BOCamino)-propyl-(*tert*-BOCamino)-propyl-(*tert*-BOCamino)-propylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (22). The indenoisoquinoline **22** (467 mg, 50%) was isolated as a dark purple solid: mp 102−104 °C. ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 7.60 (s, 1 H), 7.32 (s, 1 H), 7.04 (s, 1 H), 6.06 (s, 2 H), 4.52 (bs, 2 H), 4.03 (s, 3 H), 3.96 (s, 3 H), 3.22 (bs, 4 H), 3.13 (bs, 6 H), 2.84 (bs, 2 H), 2.68 (bs, 2 H), 1.73 (bs, 4 H), 1.63 (bs, 4 H), 1.43 (s, 18 H), 1.41 (s, 9 H); ESIMS m/z (relative intensity) 881 (45), 880 (MH^+, 100), 803 (40), 780 (MH^+ − BOC, 20), 747 (14), 691 (10), 680 (MH^+ − 2 × BOC, 18), 580 (MH^+ − 3 × BOC, 9). Anal. ($C_{46}H_{65}N_5O_{12}$·0.8H_2O) C, H, N.

General Procedure for the Synthesis of Amino Indenoisoquinoline Hydrochlorides 12a, 12b, 19a−19d, and 23. Indenoisoquinolines **11a**, **11b**, **18a**−**18d**, and **22** (1.0 mmol) were dissolved separately in chloroform (50 mL) and an anhydrous solution of HCl in ether (2 M, 30.0 mmol) was added at 0 °C. The reaction mixture was then stirred at room temperature for 8 h. The precipitated product was filtered off and washed with chloroform (50 mL), methanol (20 mL), and dried over P_2O_5 for 24 h to afford pure amino indenoisoquinoline hydrochlorides **12a**, **12b**, **19a**−**19d**, and **23** in 51−83% yield as dark purple solids.

6-[3-(2-Aminoethylamino)-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (12a). The indenoisoquinoline **11a** was converted to the hydrochloride **12a** (304 mg, 80%), which was obtained as a purple solid: mp 280−283 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.29 (bs, 1 H), 8.26 (bs, 2 H), 7.86 (s, 1 H), 7.48 (s, 1 H), 7.40 (s, 1 H), 7.09 (s, 1 H), 6.20 (s, 2 H), 4.50 (bs, 2 H), 3.89 (s, 3 H), 3.85 (s, 3 H), 3.14 (bs, 6 H), 2.16 (bs, 2 H); ESIMS m/z (relative intensity) 453 (24), 452 (MH^+ − 2 × HCl, 95), 393 (22), 392 (100). Anal. ($C_{24}H_{27}Cl_2N_3O_6$·0.5H_2O) C, H, N.

6-[3-(3-(Aminopropyl)amino)-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (12b). The indenoisoquinoline **11b** was converted to the hydrochloride **12b** (251 mg, 75%), which was obtained as a purple solid: mp 273−276 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.15 (bs, 1 H), 8.79 (bs, 2 H), 7.68 (s, 1 H), 7.36 (s, 1 H), 7.29 (s, 1 H), 6.96 (s, 1 H), 6.18 (s, 2 H), 4.42 (bs, 2 H), 3.85 (s, 3 H), 3.82 (s, 3 H), 2.98 (bs, 4 H), 2.85 (bs, 2 H), 2.12 (bs, 2 H), 1.69 (bs, 2 H); ^{1}H NMR (D_2O) δ 6.63 (bs, 1 H), 6.47 (bs, 1 H), 6.36 (bs, 1 H), 6.08 (bs, 1 H), 5.89 (bs, 2 H), 3.94 (bs, 2 H), 3.42 (s, 3 H), 3.29 (s, 3 H), 3.04−2.95 (bs, 6 H), 1.98 (bs, 2 H), 1.74 (bs, 2 H); ESIMS m/z (relative intensity) 467 (18), 466 (MH^+ − 2 × HCl, 72), 393 (25), 392 (100). Anal. ($C_{24}H_{25}N_3O_6$·2.2H_2O) C, H, N.

6-{3-[2-(2-Aminoethylamino)-ethylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19a). The indenoisoquinoline **18a** was converted to the hydrochloride **19a** (305 mg, 70%), which was obtained as a dark purple solid: mp 257−260 °C (dec). ^{1}H NMR (D_2O) δ 6.66 (bs, 1 H), 6.52 (bs, 2 H), 6.18 (bs, 1 H), 5.90 (bs, 2 H), 3.97 (bs, 2 H), 3.52 (s, 3 H), 3.49 (s, 3 H), 3.45−3.37 (m, 6 H), 3.32−3.27 (m, 2 H), 3.19 (bs, 2 H), 2.06 (bs, 2 H); ESIMS m/z (relative intensity) 496 (27), 495 (MH^+ − 3 × HCl, 100), 478 (7), 435 (19), 409 (6), 393 (15), 392 (68). Anal. ($C_{26}H_{33}N_4O_6Cl_3$·2.7H_2O) C, H, N.

6-{3-[2-(3-(Aminopropyl)amino)-ethylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19b). The indenoisoquinoline **18b** was converted to the hydrochloride **19b** (340 mg, 83%), which was obtained as a purple solid: mp 270−273 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.52 (bs, 1 H), 9.39 (bs, 1 H), 8.02 (bs, 2 H), 7.86 (s, 1 H), 7.49 (s, 1 H), 7.40 (s, 1 H), 7.09 (s, 1 H), 6.20 (s, 2 H), 4.50 (bs, 2 H), 3.90 (s, 3 H), 3.86 (s, 3 H), 3.15 (bs, 4 H), 3.11 (bs, 2 H), 3.03 (bs, 2 H), 2.90 (bs, 2 H), 2.16 (bs, 2 H), 1.96 (bs, 2 H); ESIMS m/z (relative intensity) 510 (28), 509 (MH^+ − 3 × HCl, 100), 435 (6), 392 (14). Anal. Calcd ($C_{27}H_{35}Cl_3N_4O_6$·1.8H_2O) C, H, N.

6-{3-[3-(2-Aminoethylamino)-propylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19c). The indenoisoquinoline **18c** was converted to the hydrochloride **19c** (270 mg, 65%), which was obtained as a dark purple solid: mp 263−265 °C (dec). ^{1}H NMR (D_2O) δ 6.59 (bs, 1 H), 6.48 (bs, 1 H), 6.40 (bs, 1 H), 6.13 (bs, 1 H), 5.89 (bs, 2 H), 3.90 (bs, 2 H), 3.48 (s, 3 H), 3.44 (s, 3 H), 3.36−3.24 (m, 4 H), 3.16−3.07 (m, 6 H), 2.13−1.97 (m, 4 H); ESIMS m/z (relative intensity) 510 (21), 509 (MH^+ − 3 × HCl, 88), 495 (28), 494 (100), 393 (11), 392 (48). Anal. ($C_{27}H_{35}Cl_3N_4O_6$·1.3H_2O) C, H, N.

6-{3-[3-(3-(Aminopropyl)amino)-propylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (19d). The indenoisoquinoline **18d** was converted to the hydrochloride **19d** (205 mg, 66%), which was obtained as a dark purple solid: mp 288−291 °C (dec). ^{1}H NMR (D_2O) δ 6.57 (bs, 1 H), 6.46 (bs, 1 H), 6.38 (bs, 1 H), 6.11 (bs, 1 H), 5.88 (bs, 2 H), 3.89 (bs, 2 H), 3.47 (s, 3 H), 3.43 (s, 3 H), 3.11−3.04 (m, 8 H), 2.98 (t, J = 7.8 Hz, 2 H), 2.11−1.93 (m, 6 H); ESIMS m/z (relative intensity) 525 (22), 524 (63), 523 (MH^+- 3 × HCl, 100), 392 (37). Anal. ($C_{28}H_{37}Cl_3N_4O_6$·1.2H_2O) C, H, N.

6-{3-[3−3-(3-(Aminopropyl)amino)-(propylamino)-propylamino]-1-propyl}-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (23). The indenoisoquinoline **22** was converted to the hydrochloride **23** (232 mg, 51%), which was obtained as a dark brown solid: mp 280−282 °C. ^{1}H NMR (D_2O) δ 6.79 (bs, 1 H), 6.63 (bs, 2 H), 6.29 (bs, 1 H), 5.96 (bs, 2 H), 4.06 (bs, 2 H), 3.61 (s, 3 H), 3.58 (s, 3 H), 3.10−2.99 (bs, 14 H), 2.06 (bs, 8 H); ESIMS m/z (relative intensity) 581 (32), 580 (MH^+, 100), 523 (9), 466 (7), 392 (24), 290 (12). Anal. ($C_{27}H_{35}Cl_3N_4O_6$·1.8H_2O) C, H, N.

6-[3-(3-Aminopropyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (13). A 2 M NaOH solution was added to a stirred solution of compound **12b** (160 mg, 0.30 mmol) in a 1:1 mixture of chloroform−water (40 mL) at 0 °C, until a basic pH of 7−8 obtained. The organic portion was then

separated from the aqueous portion and the organic layer was concentrated, loaded on a silica gel column, eluted with a 5−10% gradient of methanol in chloroform, along with 1 mL of aqueous NH_4OH to provide pure diamino indenoisoquinoline **13** (105 mg, 76%) as a purple solid: mp 204−206 °C. ^1H NMR ($CDCl_3$) δ 7.99 (s, 1 H), 7.61 (s, 1 H), 7.37 (s, 1 H), 7.03 (s, 1 H), 6.06 (s, 2 H), 4.99 (bs, 1 H), 4.51 (t, $J = 7.1$ Hz, 2 H), 4.02 (s, 3 H), 3.97 (s, 3 H), 3.23 (apparent t, $J = 5.8$ Hz, 2 H), 2.78 (t, $J = 5.9$ Hz, 2 H), 2.70 (t, $J = 6.4$ Hz, 2 H), 2.01 (m, 2 H), 1.70 (m, 2 H); ESIMS *m/z* (relative intensity) 467 (27), 466 (MH^+, 100), 393 (17), 392 (74). Anal. ($C_{25}H_{27}N_3O_6 \cdot 0.6H_2O$) C, H, N.

General Procedure for the Preparation of Compounds 15a−15d. The BOC-protected amino alcohols **14a** and **14b**[35] (1.0 mmol) and Et_3N (1.5 mmol) were separately dissolved in $CHCl_3$ (100 mL) and cooled to 0 °C. Methanesulfonyl chloride (1.1 mmol) was added dropwise to this ice-cold solution and the reaction mixture was further stirred at that temperature for 4 h. The excess methanesulfonyl chloride was quenched with 1 N NaOH solution (5 mL). The organic portion was then washed with water (2 × 200 mL), brine, dried over Na_2SO_4, concentrated and used in the next step without purification. The above crude product (1.0 mmol) and ethanolamine (10.0 mmol) and/or propanolamine (10.0 mmol) were heated at 80 °C for 8 h. The reaction mixture was cooled to room temperature, diluted with water (50 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The organic portion was washed with brine, dried over Na_2SO_4, concentrated, and used in the next reaction without purification. BOC_2O (1.1 mmol) was added to the above crude product (1.0 mmol) and Et_3N (2.0 mmol) in $CHCl_3$ (100 mL) and the reaction mixture was stirred further at 23 °C for 8 h. The reaction mixture was washed with water, brine, dried over Na_2SO_4, and concentrated. The product was further purified by silica gel column and eluted with a 1−3% gradient of methanol in chloroform to afford compounds **15a−15d** as colorless, viscous oils in 70−75% overall yield in three steps.

(2-*tert*-BOCamino-ethyl)-(2-hydroxy-ethyl)-carbamic Acid *tert*-Butyl Ester (15a).[36] The amino alcohol **15a** (16.0 g, 73%, three steps) was isolated as a viscous, colorless oil: IR (neat) 3356 (br), 2976, 2932, 1670, 1249 and 1146 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.72 (bs, 2 H), 3.34 (bs, 6 H), 1.46 (s, 9 H), 1.42 (s, 9 H); ESIMS *m/z* (relative intensity) 328 (12), 327 (MNa^+, 100), 279 (17), 271 (35), 227 (42).

(3-*tert*-BOCamino-propyl)-(2-hydroxy-ethyl)-carbamic Acid *tert*-Butyl Ester (15b). The amino alcohol **15b** (20.5 g, 75%, three steps) was isolated as a viscous, colorless oil: IR (neat) 3358 (br), 2975, 2931, 1681, and 1169 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.73 (bs, 2 H), 3.33 (bs, 4 H), 3.10 (bs, 2 H), 1.63 (quin, $J = 6.6$ Hz, 2 H), 1.44 (s, 9 H), 1.40 (s, 9 H); ESIMS *m/z* 342 (16), 341 (MNa^+ − 100), 285 (10), 266 (10), 241 (10). Anal. ($C_{15}H_{30}N_2O_5$) C, H, N.

(2-*tert*-BOCamino-ethyl)-(3-hydroxy-propyl)-carbamic Acid *tert*-Butyl Ester (15c). The amino alcohol **15c** (19.4 g, 70%, three steps) was isolated as a viscous oil: IR (neat) 3363 (br), 2976, 2933, 1695 (br), 1523, 1480, 1366, 1250, 1170, 1061 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.52 (bs, 2 H), 3.30 (bs, 2 H), 3.23 (bs, 4 H), 1.64 (bs, 2 H), 1.44 (s, 9 H), 1.40 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 156.3, 155.8, 80.1, 78.9, 58.2, 46.4, 43.2, 39.1, 30.4, 28.1; ESIMS *m/z* (relative intensity) 320 (13), 319 (MH^+, 100), 219 (MH^+ − BOC, 48), 119 (MH^+ − 2 × BOC, 3); HRESIMS calcd for $C_{15}H_{30}N_2O_5$: 319.2233. Found: 319.2234.

(3-*tert*-BOCamino-propyl)-(3-hydroxy-propyl)-carbamic Acid *tert*-Butyl Ester (15d). The amino alcohol **15d** (10.43 g, 73%, three steps) was isolated as a viscous oil: IR (neat) 3361 (br), 2976, 2933, 1685, 1681, 1251, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.75 (bs, 1 H), 3.50 (bs, 2 H), 3.31−3.16 (m, 4 H), 3.06 (apparent t, $J = 5.8$ Hz, 2 H), 1.64 (m, 4 H), 1.42 (s, 9 H), 1.39 (s, 9 H); ESIMS *m/z* 356 (18), 355 (MNa^+, 100), 303 (10), 289 (8), 281 (6), 255 (MNa^+ − BOC, 11). Anal. ($C_{16}H_{32}N_2O_5$) C, H, N.

General Procedure for the Preparation of Compounds 16a−16d. Diethyl azodicarboxylate (5.7 g, 36.0 mmol) was added separately, over 10 min, to an ice-cold mixture of compounds **15a−15d** (33.0 mmol), PPh_3 (9.6 g, 36.0 mmol) and phthalimide (5.4 g, 36.0 mmol) in dry THF (100 mL). The reaction mixture was slowly brought to room temperature and stirred at that temperature for 5 h. THF was evaporated under vacuum and the residue was directly loaded on a silica gel column and purified by flash chromatography (230−400 mesh, 8:2 benzene-EtOAc as an eluant) to afford compounds **16a−16d** in 69−85% yield.

(2-*tert*-BOCamino-ethyl)-[2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl]-carbamic Acid *tert*-Butyl Ester (16a). The protected triamine **16a** (11.76 g, 82%) was isolated as a white solid: mp 41−43 °C. IR (neat) 3375 (br), 2977, 2935, 1773, 1714, 1515, 1250, 1156 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.81 (m, 2 H), 7.69 (m, 2 H), 5.13 (bs, 1 H), 3.81 (bs, 2 H), 3.48 (bs, 2 H), 3.30 (m, 4 H), 1.40 (s, 9 H), 1.20 (s, 9 H); ESIMS *m/z* 457 (24), 456 (MNa^+, 100), 356 (MNa^+ − BOC, 26), 400 (17). Anal. ($C_{22}H_{31}N_3O_6$) C, H, N.

(3-*tert*-BOCamino-propyl)-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propyl]-carbamic Acid *tert*-Butyl Ester (16b). The protected triamine **16b** (12.02 g, 85%) was isolated as a semisolid: IR (neat) 3376 (br), 2977, 2936, 1800, 2770, 1714, 1505, 1393, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.82 (m, 2 H), 7.68 (m, 2 H), 5.26 (bs, 1 H), 3.81 (bs, 2 H), 3.44 (m, 2 H), 3.26 (m, 2 H), 3.10 (bs, 2 H), 1.63 (m, 2 H), 1.40 (s, 9 H), 1.25 (s, 9 H); ESIMS *m/z* 471 (23), 470 (MNa^+, 100), 370 (MNa^+ − BOC, 5). Anal. ($C_{23}H_{33}N_3O_6 \cdot 0.4H_2O$) C, H, N.

(2-*tert*-BOCamino-ethyl)-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propyl]-carbamic Acid *tert*-Butyl Ester (16c). The protected triamine **16c** (12.6 g, 69%) was isolated as a viscous oil: IR (neat) 3358 (br), 2976, 2933, 2874, 1692 (br), 1688, 1524, 1420, 1366, 1170, 1059 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83−7.77 (m, 2 H), 7.71−7.66 (m, 2 H), 5.01 (bs, 1 H), 3.65 (t, $J = 7.2$ Hz, 2 H), 3.30−3.28 (m, 4 H), 3.23 (t, $J = 4.7$ Hz, 2 H), 1.93−1.78 (m, 2 H), 1.39 (s, 9 H), 1.36 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 168.3, 156.2, 133.9, 131.9, 123.2, 80.1, 79.2, 50.3, 44.2, 35.6, 28.2; ESIMS *m/z* (relative intensity) 471 (22), 470 (MNa^+, 100), 414 (11), 370 (12), 292 (13); HRESIMS calcd for $C_{23}H_{33}N_3O_6$: 448.2448. Found: 448.2442.

(3-*tert*-BOCaminopropyl)-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propyl]-carbamic Acid *tert*-Butyl Ester (16d). The protected triamine **16d** (11.2 g, 77%) was isolated as a semisolid: IR (neat) 3364, 2976, 2933, 1770, 1714, 1694, 1504, 1249, 1172 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.82−7.73 (m, 2 H), 7.72−7.68 (m, 2 H), 5.24 (bs, 1 H), 3.66 (t, $J = 7.1$ Hz, 2 H), 3.24 (m, 4 H), 3.06 (m, 2 H), 1.88 (m, 2 H), 1.60 (m, 2 H), 1.40 (s, 18 H); ESIMS *m/z* 485 (23), 484 (MNa^+, 100), 428 (11), 384 (MNa^+ − BOC, 17). Anal. ($C_{24}H_{35}N_3O_6$) C, H, N.

General Procedure for the Preparation of Compounds 17a−17d. Hydrazine hydrate (40.0 mmol) was added separately to a solution of compounds **16a−16d** (20.0 mmol) in methanol (100 mL) at room temperature and the mixture was stirred further under reflux for 12 h, after which the TLC showed the complete disappearance of the starting phthalimide derivative. The reaction mixture was cooled and the byproduct phthalhydrazide separated out as a white solid. The reaction mixture was then filtered off through a sintered glass funnel and the solid washed with cold methanol and the filtrate was concentrated. The reaction product was purified by silica gel column chromatography, eluting with a 2−10% gradient of methanol in chloroform, to provide pure BOC-protected triamines **17a−17d** in 65−72% yield.

(2-Amino-ethyl)-(2-*tert*-BOCamino-ethyl)-carbamic Acid *tert*-Butyl Ester (17a).[37] The BOC-protected triamine **17a** (3.52 g, 72%) was isolated as a white solid: mp 74−76 °C. IR (neat) 3363 (br), 2976, 2932, 1693 (br), 1517, 1479, 1414, 1366, 1250, 1165, 1067 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.27 (bs, 1 H), 3.29−3.24 (m, 8 H), 2.81 (bs, 2 H), 1.43 (s, 9 H), 1.40 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 155.9, 79.6, 78.7, 50.7, 47.5, 40.6, 39.3, 28.2; ESIMS *m/z* (relative intensity) 326 (MNa^+, 37), 305 (12), 304 (MH^+, 100), 247 (27), 204 (MH^+ − BOC, 26), 104 (MH^+ − 2 × BOC, 10). Anal. ($C_{14}H_{29}N_3O_4 \cdot 0.22H_2O$) C, H, N.

3-[(2-Amino-ethyl)-*tert*-BOCamino]-propyl-carbamic Acid *tert*-Butyl Ester (17b). The BOC-protected triamine **17b** (6.9 g, 65%) was isolated as a viscous yellow oil: IR (neat) 3360 (br), 2976, 2932, 2871, 1693 (br), 1516, 1480, 1417, 1366,

1250, 1167, 1068 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.26 (bs, 1 H), 4.80 (bs, 2 H), 3.27 (bs, 2 H), 3.20 (bs, 2 H), 3.09 (apparent t, J = 6.0 Hz, 2 H), 2.81 (t, J = 6.2 Hz, 2 H), 1.66 (m, 2 H), 1.45 (s, 9 H), 1.42 (s, 9 H); ^{13}C NMR (CDCl$_3$) δ 155.8, 79.4, 78.6, 49.7, 44.3, 40.4, 37.2, 28.1; ESIMS *m/z* (relative intensity) 318 (MH$^+$, 100), 302 (21), 258 (17), 218 (MH$^+$ − BOC, 31), 118 (MH$^+$ − 2 × BOC, 15). Anal. ($C_{15}H_{31}N_3O_4 \cdot 0.1H_2O$) C, H, N.

(3-Amino-propyl)-(2-*tert*-BOCamino-ethyl)-carbamic Acid *tert*-Butyl Ester (17c).[38] The BOC-protected triamine **17c** (7.3 g, 68%) was isolated as a viscous yellow oil: IR (neat) 3361 (br), 2976, 2933, 2871, 1694 (br), 1519, 1417, 1366, 1251, 1168, 1068 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.13−4.97 (bs, 1 H), 3.18 (bs, 6 H), 2.61 (bs, 2 H), 2.01 (bs, 2 H, NH$_2$), 1.62−1.53 (m, 2 H), 1.37 (s, 9 H), 1.30 (s, 9 H); ^{13}C NMR (CDCl$_3$) δ 155.9, 79.8, 79.1, 46.2, 44.4, 39.3, 32.2, 28.3; ESIMS *m/z* (relative intensity) 319 (16), 318 (MH$^+$, 100), 218 (MH$^+$ − BOC). HRESIMS Calcd for $C_{15}H_{31}N_3O_4$: 318.2393. Found: 318.2393. Anal. ($C_{15}H_{31}N_3O_4 \cdot 0.6CH_2Cl_2$) C, H, N.

(3-Amino-propyl)-(3-*tert*-BOCamino-propyl)-carbamic Acid *tert*-Butyl Ester (17d).[39,40] The BOC-protected triamine **17d** (7.3 g, 68%) was isolated as a viscous yellow oil: IR (neat) 3360 (br), 2976, 2933, 2870, 1694, 1683, 1519, 1480, 1419, 1366, 1275, 1251, 1170, 1083 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.25 (bs, 1 H), 4.71 (bs, 2 H), 3.22 (bs, 4 H), 3.07 (apparent t, J = 6.0 Hz, 2 H), 2.67 (t, J = 5.8 Hz, 2 H), 1.63 (m, 4 H), 1.43 (s, 9 H), 1.41 (s, 9 H); ^{13}C NMR (CDCl$_3$) δ 155.9, 79.5, 78.5, 43.5, 39.2, 37.3, 32.3, 28.3; ESIMS *m/z* (relative intensity) 333 (16), 332 (MH$^+$, 100), 276 (16), 232 (MH$^+$ − BOC, 62), 132 (MH$^+$ − 2 × BOC, 27). Anal. ($C_{16}H_{33}N_3O_4$) C, H, N.

(3-*tert*-BOCamino-propyl)-{3-[*tert*-BOC-(2-cyano-ethyl)-amino]-propyl}-carbamic Acid *tert*-Butyl Ester (20). Acrylonitrile (1.5 mL, 23.0 mmol) was added to a solution of BOC-protected amine **17d** (5.8 g, 18.0 mmol) in methanol (30 mL) at 0 °C and the reaction mixture was stirred for 16 h, protected from light. The solvent was evaporated in vacuo, and the residue was used in the next reaction without purification. BOC$_2$O (3.3 g, 15.0 mmol) was added slowly to the above crude product (4.9 g, 13.0 mmol) and Et$_3$N (3.5 mL, 25.0 mmol) in CHCl$_3$ (40 mL) at 0 °C and the reaction mixture was stirred further at RT for 12 h. The reaction mixture was washed with water, brine, dried over Na$_2$SO$_4$, and concentrated. The reaction mixture was purified by silica gel column using CHCl$_3$−CH$_3$OH (95:5) as an eluant to afford compound **20** (4.1 g, 67%) as a viscous oil: IR (neat) 3364 (br), 3004, 2976, 2933, 2249, 1694 (br), 1513, 1479, 1417, 1303, 1250 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.44 (t, J = 6.6 Hz, 2 H), 3.27−3.22 (m, 4 H), 3.16−3.07 (m, 4 H), 2.60 (bs, 2 H), 1.79−1.70 (m, 2 H), 1.65−1.58 (m, 2 H), 1.44 (s, 18 H), 1.41 (s, 9 H); ESIMS *m/z* (relative intensity) 508 (25), 507 (MNa$^+$, 100), 485 (MH$^+$, 23), 385 (MH$^+$ − BOC, 7), 329 (5), 285 (MH$^+$ − 2 × BOC, 3), 185 (MH$^+$ − 3 × BOC, 3). Anal. ($C_{24}H_{44}N_4O_6$) C, H, N.

[3-({3-[(3-Aminopropyl)-*tert*-BOCamino]-propyl}-*tert*-BOCamino)-propyl]-carbamic Acid *tert*-Butyl Ester (21).[41,42] Raney nickel slurry in water (pore size ~50 μ, 80−100 m^2/g) (~2.0 g) and 2 M NaOH solution (30 mL) were added to a solution of compound **20** (3.2 g, 6.6 mmol) in ethanol (70 mL) at room temperature. The reaction mixture was stirred at room temperature for 16 h under atmospheric hydrogen pressure (~20 psi). The reaction mixture was carefully filtered through a Celite pad and the solvent was evaporated. The product was passed through a short pad of silica gel column using CHCl$_3$−CH$_3$OH−NH$_4$OH (6:3:1) as an eluant to give amine **21** (1.7 g, 53%) as a viscous oil: IR (neat) 3361 (br), 2975, 2932, 1686 (br), 1478, 1419, 1365, 1249, 1163 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.22 (bs, 4 H), 3.11 (bs, 6 H), 2.70 (bs, 2 H), 2.30 (bs, 2 H), 1.74−1.63 (m, 6 H), 1.42 (bs, 18 H), 1.40 (s, 9 H); ESIMS *m/z* (relative intensity) 490 (25), 489 (MH$^+$, 100), 389 (MH$^+$ − BOC, 19), 289 (MH$^+$ − 2 × BOC, 18), 189 (MH$^+$ − 3 × BOC, 8). Anal. ($C_{24}H_{48}N_4O_6$) C, H, N.

6-[3-(1,1′-Dimethyl-2-hydroxyethyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (24). A mixture of indenoisoquinoline bromide **10** (0.3 g, 0.63 mmol), 2-amino-2-methyl-1-propanol (0.17 g, 1.9 mmol) and anhydrous K$_2$CO$_3$ (0.44 g, 3.2 mmol) in anhydrous DMF (30 mL) was heated to 100 °C and kept at that temperature for 4 h. The hot reaction mixture was filtered, diluted with ethanol (5 mL), and cooled in an ice bath. The precipitated product was filtered off through a sintered glass funnel, washed with ethanol (15 mL), and dried. The product was then crystallized in ethyl acetate−chloroform (3:1) to give a purple solid, which was further treated with 2 M HCl in ether (6.3 mL, 12.7 mmol) in chloroform at room temperature for 8 h to provide indenoisoquinoline aminol hydrochloride **24** (140 mg, 43%) as a dark purple solid: mp 274−276 °C. ^{1}H NMR (DMSO-d_6) δ 8.50 (bs, 1 H), 7.80 (s, 1 H), 7.44 (s, 1 H), 7.36 (s, 1 H), 7.05 (s, 1 H), 6.20 (s, 2 H), 5.59 (bs, 1 H), 4.46 (bs, 2 H), 3. 88 (s, 3 H), 3.84 (s, 3 H), 3.41 (bs, 2 H), 3.05 (bs, 2 H), 2.16 (bs, 2 H), 1.20 (s, 6 H); ESIMS *m/z* (relative intensity) 482 (27), 481 (MH$^+$ − HCl, 100), 478 (9), 393 (11), 392 (47); HRESIMS calcd for (MH$^+$) $C_{26}H_{29}N_2O_7Cl$: 481.1975; Found: 481.1978. Anal. ($C_{26}H_{29}ClN_2O_7 \cdot 1.3CHCl_3$) C, H, N.

6-[(3-Dimethoxyethyl)amino-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (25). A mixture of indenoisoquinoline bromide **10** (0.4 g, 0.85 mmol), aminoacetaldehyde dimethyl acetal (0.2 g, 1.86 mmol) and anhydrous K$_2$CO$_3$ (0.47 g, 3.40 mmol) in anhydrous 1,4-dioxane (25 mL) was heated to 100 °C and kept at that temperature for 4 h. The reaction mixture was cooled and then concentrated. The reaction mixture was diluted with water (50 mL) and extracted with CHCl$_3$ (2 × 50 mL), and washed with brine and dried (Na$_2$SO$_4$). The reaction mixture was concentrated and loaded on the silica gel column (50 g), eluting with a 1−2% gradient of methanol in chloroform, to provide pure indenoisoquinoline **25** (0.23 g, 55%) as a purple solid: mp 206−208 °C. ^{1}H NMR (CDCl$_3$) δ 7.99 (s, 1 H), 7.61 (s, 1 H), 7.33 (s, 1 H), 7.03 (s, 1 H), 6.06 (s, 2 H), 4.57 (t, J = 5.4 Hz, 1 H), 4.51 (t, J = 6.9 Hz, 2 H), 4.02 (s, 3 H), 3.96 (s, 3 H), 3.39 (s, 6 H), 2.84 (t, J = 6.1 Hz, 2 H), 2.79 (d, J = 5.6 Hz, 2 H), 2.07 (m, 2 H); ^{13}C NMR (CDCl$_3$) δ 189.9, 162.7, 154.9, 153.3, 151.5, 149.1, 148.7, 132.5, 130.4, 128.3, 116.8, 107.9, 105.3, 103.2, 102.9, 102.6, 56.3, 56.0, 54.2, 51.0, 46.5, 42.5; ESIMS *m/z* 498 (26), 497 (MH$^+$, 100), 465 (26). Anal. ($C_{25}H_{26}N_2O_8$) C, H, N.

6-[3-(Bis-hydroxyethylamino)-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (26). A mixture of indenoisoquinoline bromide **10** (0.3 g, 0.63 mmol), bis-(2-*tert*-butyldimethylsilyloxyethyl)amine[43] (0.63 g, 1.9 mmol) and anhydrous K$_2$CO$_3$ (0.44 g, 3.2 mmol) in anhydrous 1,4-dioxane (25 mL) was heated to 100 °C and kept at that temperature for 4 h. The reaction mixture was then cooled and concentrated. The reaction mixture was diluted with chloroform (50 mL), washed with water, brine and dried (Na$_2$SO$_4$). The product was purified by silica gel column and eluted with a 1−5% gradient of methanol in chloroform to give silyl protected indenoisoquinoline aminol (0.24 g, 0.33 mmol, 52%), which was further treated with 2 M HCl in ether (7.0 mL, 13.2 mmol) in chloroform (30 mL) at room temperature for 8 h to afford pale purple solid. The resultant solid was filtered through a sintered glass funnel, and washed with chloroform (30 mL) to provide indenoisoquinoline aminol **26** (105 mg, 61%) as a pale purple solid: mp 270−272 °C (dec). ^{1}H NMR (DMSO-d_6) δ 7.72 (s, 1 H), 7.37 (s, 1 H), 7.27 (s, 1 H), 6.97 (s, 1 H), 6.17 (s, 2 H), 5.27 (bs, 2 H, −OH groups), 4.39 (bs, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H), 3.73 (bs, 4 H), 3.24 (bs, 6 H), 2.19 (bs, 2 H); ESIMS *m/z* 498 (27), 497 (MH$^+$ − HCl, 100), 392 (20), 335 (6), 334 (25). Anal. ($C_{26}H_{29}N_2O_8Cl \cdot 0.6CHCl_3$) C, H, N.

6-[(3-Trifluoroacetyloxy)-1-propyl]-5,6-dihydro-2,3-dimethoxy-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (28). A mixture of indenoisoquinoline bromide **10** (0.3 g, 0.63 mmol), aminopyrazine (0.12 g, 1.3 mmol), and anhydrous K$_2$CO$_3$ (0.35 g, 2.5 mmol) in anhydrous 1,4-dioxane (25 mL) was heated to 100 °C and kept at that temperature for 4 h. The reaction mixture was cooled and concentrated on a rotary evaporator. The reaction mixture was diluted with chloroform (100 mL) and washed with 1% HCl solution (50 mL), water, and brine and dried (Na$_2$SO$_4$). The

reaction mixture was concentrated, loaded on the silica gel column, and eluted with a 2−5% gradient of methanol in chloroform to provide indenoisoquinoline derivative, which was further treated with neat CF_3COOH (10 mL) at room temperature for 30 min to provide indenoisoquinoline trifluoroacetate ester **28** (46 mg, 15%) as a pale purple solid: mp 280−282 °C. ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1 H), 7.61 (s, 1 H), 7.06 (s, 1 H), 7.02 (s, 1 H), 6.08 (s, 2 H), 4.57 (t, J = 6.2 Hz, 4 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 2.35 (m, 2 H); ESIMS m/z (relative intensity) 507 (26), 506 (MH^+, 100), 505 (6), 481 (14), 465 (6), 433 (32), 410 (7), 393 (6), 392 (1). Anal. ($C_{24}H_{18}F_3NO_8$) C, H, N.

Topoisomerase I-Mediated DNA Cleavage Reactions. Human recombinant topoisomerase I was purified from Baculovirus as described previously.[44] 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 1× 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 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 topoisomerase I at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration).[1] 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).

Molecular Modeling. The structure of the ternary complex, containing topoisomerase I, DNA, and topotecan, was downloaded from the Protein Data Bank (PDB code 1K4T).[34] One molecule of PEG and the topotecan carboxylate form were deleted. All of the atoms were then fixed according to Sybyl atom types. Hydrogens were added and minimized using the MMFF94s force field and MMFF94 charges. The structure of the indenoisoquinoline **2**, constructed in Sybyl and energy minimized with the Tripos force field and Gasteiger-Hückel charges, was overlapped with the structure of topotecan according to the proposed structural similarity[2] in the ternary complex, and the structure of topotecan was then deleted. The new whole complex was subsequently subjected to energy minimization using the MMFF94s force field with MMFF94 charges. During energy minimization, the structure of the indenoisoquinoline was allowed to move, while the structures of the protein, nucleic acid, and water molecules were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/mol Å energy gradient convergence criterion and a distance-dependent dielectric constant.

Acknowledgment. This work was made possible by the National Institutes of Health (NIH) through support of this work with Research Grant UO1 CA89566 and Training Grant ST32CA09634. The in vitro and in vivo testing was conducted through the Developmental Therapeutics Program, DCTD, NCI under Contract NO1-CO-56000.

References

(1) Kohlhagen, G.; Paull, K.; Cushman, M.; Nagafuji, P.; Pommier, Y. Protein-Linked DNA Strand Breaks Induced by NSC 314622, a Novel Noncamptothecin Topoisomerase I Poison. *Mol. Pharmacol.* **1998**, *54*, 50−58.

(2) Fox, B. M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline-Camptothecin Hybrids. *J. Med. Chem.* **2003**, submitted.

(3) Pommier, Y. Eukaryotic DNA Topoisomerase I: Genome Gate Keeper and Its Intruders, Camptothecins. *Semin. Oncol.* **1996**, *23*, 1−10.

(4) Strumberg, D.; Pommier, Y.; Paull, K.; Jayaraman, M.; Nagafuji, P.; Cushman, M. Synthesis of Cytotoxic Indenoisoquinoline Topoisomerase I Poisons. *J. Med. Chem.* **1999**, *42*, 446−457.

(5) Cushman, M.; Jayaraman, M.; Vroman, J. A.; Fukunaga, A. K.; Fox, B. M.; Kohlhagen, G.; Strumberg, D.; Pommier, Y. Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors. *J. Med. Chem.* **2000**, *43*, 3688−3698.

(6) Jayaraman, M.; Fox, B. M.; Hollingshead, M.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of New Dihydroindeno-[1,2-*c*]isoquinoline and Indenoisoquinolinium Chloride Topoisomerase I Inhibitors Having High in Vivo Anticancer Activity in the Hollow Fiber Animal Model. *J. Med. Chem.* **2002**, *45*, 242−249.

(7) Edwards, M. L.; Snyder, R. D.; Stemerick, D. M. Synthesis and DNA-Binding Properties of Polyamine Analogues. *J. Med. Chem.* **1991**, *34*, 2414−2420.

(8) Cohen, G. M.; Cullis, P. M.; Hartley, J. A.; Mather, A.; Symons, M. C. R.; Wheelhouse, R. T. Targeting of Cytotoxic Agents by Polyamines: Synthesis of a Chlorambucil-Spermidine Conjugate. *J. Chem. Soc., Chem. Commun.* **1992**, 298−300.

(9) Cullis, P. M.; Green, R. E.; Merson-Davies, L.; Travis, N. Probing the Mechanism of Transport and Compartmentalization of Polyamines in Mammalian Cells. *Chem. Biol.* **1999**, *6*, 717−729.

(10) Delcros, J.-G.; Tomasi, S.; Carrington, S.; Martin, B.; Renault, J. Effect of Spermine Conjugation on the Cytotoxicity and Cellular Transport of Acridine. *J. Med. Chem.* **2002**, *45*, 5098−5111.

(11) Bergeron, R. J.; McManis, J. S.; Weimar, W. R.; Schreier, K. M.; Gao, F.; Wu, Q.; Ortiz-Ocasio, J.; Luchetta, G. R.; Porter, C.; Vinson, J. R. T. The Role of Charge in Polyamine Analogue Recognition. *J. Med. Chem.* **1995**, *38*, 2278−2285.

(12) Seiler, N.; Delcros, J. G.; Moulinoux, J. P. Polyamine Transport in Mammalian Cells. An Update. *Int. J. Biochem. Cell. Biol.* **1996**, *28*, 843−861.

(13) Phanstiel, O., IV; Price, H. L.; Wang, L.; Juusola, J.; Kline, M.; Shah, S. M. The Effect of Polyamine Homologation on the Transport and Cytotoxicity Properties of Polyamine-(DNA-Intercalator) Conjugates. *J. Org. Chem.* **2000**, *65*, 5590−5599.

(14) Wang, L.; Price, H. L.; Juusola, J.; Kline, M.; Phanstiel, O., IV. Influence of Polyamine Architecture on the Transport and Topoisomerase II Inhibitory Properties of Polyamine DNA-Intercalator Conjugates. *J. Med. Chem.* **2001**, *44*, 3682−3691.

(15) Siddiqui, A. Q.; Merson-Davies, L.; Cullis, P. M. The Synthesis of Novel Polyamine-Nitroimidazole Conjugates to Probe the Structural Specificities of the Polyamine Untake System in A549 Lung Carcinoma Cells. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3243−3252.

(16) Porter, C. W.; Miller, J.; Bergeron, R. J. Aliphatic Chain Length Specificity of the Polyamine Transport System in Acites L1210 Leukemia Cells. *Cancer Res.* **1984**, *44*, 126−128.

(17) Bergeron, R. J.; Feng, Y.; Weimar, W. R.; McManis, J. S.; Dimova, H.; Porter, C.; Raisler, B.; Phanstiel, O. A Comparison of Structure−Activity Relationships between Spermidine and Spermine Analogue Antineoplastics. *J. Med. Chem.* **1997**, *40*, 1475−1494.

(18) Cullis, P. M.; Symons, C. R.; Cohen, G. M.; Wardman, P. A General Method for Efficient Drug Delivery to DNA. *Med. Sci. Res.* **1990**, *18*, 87−88.

(19) Cushman, M.; Gentry, J.; Dekow, F. W. Condensation of Imines with Homophthalic Anhydrides. A Convergent Synthesis of *cis*- and *trans*-13-Methyltetrahydroprotoberberines. *J. Org. Chem.* **1977**, *42*, 1111−1116.

(20) Rastogi, S. N.; Bindra, J. S.; Anand, N. α-Methyldopa in a Rigid Framework: 3-Amino-3-methyl-6,7-dihydroxy-3,4-dihydroxycoumarins and 2-Amino-6,7-dihydroxytetralin-2-carboxylic Acids. *Indian J. Chem.* **1971**, *9*, 1175−1182.

(21) Cushman, M.; Cheng, L. Total Synthesis of Nitidine Chloride. *J. Org. Chem.* **1978**, *43*, 286−288.

(22) Cushman, M.; Cheng, L. Stereoselective Oxidation by Thionyl Chloride Leading to the Indeno[1,2-*c*]isoquinoline System. *J. Org. Chem.* **1978**, *43*, 3781−3783.

(23) Boeijin, A.; Ameijde, J. V.; Liskamp, R. M. Solid-Phase Synthesis of Oligourea Peptidomimetics Employing the Fmoc Protection Strategy. *J. Org. Chem.* **2001**, *66*, 8454−8462.

(24) Adamcyzk, M.; Grote, J. A. A Practical Method for the Synthesis of *N*-Fluorenylmethoxycarbonyl Diamines. *Org. Prep. Proc. Int.* **1995**, *27*, 239−242.

(25) Blagbrough, I. S.; Geall, A. J. Practical Synthesis of Unsymmetrical Polyamine Amides. *Tetrahedron Lett.* **1998**, *39*, 439−442.

(26) Geall, A. J.; Blagbrough, I. S. Homologation of Polyamines in the Synthesis of Lipo-Spermine Conjugates and Related Lipoplexes. *Tetrahedron Lett.* **1998**, *39*, 443–446.
(27) Kuksa, V.; Buchan, R.; Lin, P. K. T. Synthesis of Polyamines, Their Derivatives, Analogues and Conjugates. *Synthesis* **2000**, 1189–1207.
(28) Wang, C.; Delcros, J.-G.; Biggerstaff, J.; Phanstiel, O., IV. Synthesis and Biological Evaluation of N^1-(Anthracen-9-ylmethyl)triamines as Molecular Recognition Elements for the Polyamine Transporter. *J. Med. Chem.* **2003**, *46*, 2663–2671.
(29) Wang, C.; Delcros, J. G.; Biggerstaff, J.; Phanstiel, O., IV. Molecular Requirements for Targeting the Polyamine Transport System. Synthesis and Biological Evaluation of Polyamine-Anthracene Conjugates. *J. Med. Chem.* **2003**, *46*, 2672–2682.
(30) Chen, A. Y.; Yu, C.; Gatto, B.; Liu, L. F. DNA Minor Groove-binding Ligands: A Different Class of Mammalian DNA Topoisomerase I Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8131–8135.
(31) Fujii, N.; Yamashita, Y.; Mizukami, T.; Nakano, H. Correlation Between the Formation of Cleavable Complex with Topoisomerase I and Growth Inhibitory Activity for Saintopin-Type Antibiotics. *Mol. Pharmacol.* **1997**, *51*, 269–276.
(32) Fujii, N.; Yamashita, Y.; Saitoh, Y.; Nakana, H. Induction of Mammalian DNA Topoisomerase I-Mediated DNA Cleavage and DNA Winding by Bulgarein. *J. Biol. Chem.* **1993**, *268*, 13160–13165.
(33) Kim, D.-K.; Kim, Y.-W.; Gam, J.; Kim, G.; Lim, J.; Lee, N.; Kim, H.-T.; Kim, K. H. Synthesis and Anti-HIV-1 Activity of a Series of 1-(Alkoxymethyl)-5-alkyl-6-(arylselenenyl)uracils and -2-thiouracils). *J. Heterocyclic Chem.* **1996**, *33*, 1275–1283.
(34) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin Jr., A. B.; Stewart, L. The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analogue. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387–15392.
(35) Mattingly, P. G. Monoprotected Diamines. $N\alpha$-(*tert*-Butoxycarbonyl)-α,ω-Alkanediamine Hydrochlorides from Amino Alcohols. *Synthesis* **1990**, *4*, 366–368.
(36) Schutte, M. T.; Schumacher, P.; Unger, C.; Mulhaupt, R.; Kratz, F. Synthesis of Two Maleimide Derivatives of Cis-Configurated Platinum(II) Complexes for the Preparation of Chemoimmunoconjugates. *Inorg. Chem.* **1998**, *267*, 133–136.
(37) Verheijen, J. C.; Deiman, B. A. L. M.; Yeheskiely, E.; van der Marcel, G. A.; Van Boom, J. H. Efficient Hydrolysis of RNA by a PNA-Diethylenetriamine Adduct. *Angew. Chem., Int. Ed.* **2000**, *39*, 369–372.
(38) Lochner, M.; Geneste, H.; Hesse, M. General Access to Polyamines Containing Ethane-1,2-diamine Units. Synthesis of Unnatural Homologs and Isomeric N1,4-Di(4-coumaroyl)spermines. *Helv. Chim. Acta* **1998**, *81*, 2270–2281.
(39) Garrett, S. W.; Davies, O. R.; Milroy, D. A.; Wood, P. J.; Pouton, C. W.; Threadgill, M. D. Synthesis and Characterisation of Polyamine-Poly(ethylene glycol) Constructs for DNA Binding and Gene Delivery. *Bioorg. Med. Chem.* **2000**, *8*, 1779–1797.
(40) Nakamura, Y. Actinocynyl Bis(Hemin): DNA-intercalating Cleavage Agent. *Heterocycles* **1988**, *27*, 1873–1879.
(41) Geall, A. J.; Al-Hadithi, D.; Blagbrough, I. S. Efficient Calf Thymus DNA Condensation upon Binding with Novel Bile Acid Polyamine Amides. *Bioconj. Chem.* **2002**, *13*, 481–490.
(42) Geall, A. J.; Taylor, R. J.; Earll, M. E.; Eaton, M. A. W.; Balgbrough, I. S. Synthesis of Cholesteryl Polyamine Carbamates: pK_a Studies and Condensation of Calf Thymus DNA. *Bioconj. Chem.* **2000**, *11*, 314–326.
(43) Grote, G.; Marcel, H. B.; Steemers, F. J.; Verboom, W.; Reinhoudt, D. N. Synthesis of Neutral, Water-Soluble Calix[4]arenes. *Synthesis* **1997**, *6*, 643–648.
(44) Pourquier, P.; Ueng, L.-M.; Fertala, J.; Wang, D.; Park, H.-J.; Essigmann, J. M.; Bjornsti, M.-A.; Pommier, Y. Induction of Reversible Complexes between Eukaryotic DNA Topoisomerase I and DNA-containing Oxidative Base Damages. 7,8-Dihydro-8-Oxoguanine and 5-Hydroxycytosine. *J. Biol. Chem.* **1999**, *274*, 8516–8523.

JM030313F