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Design, synthesis, and biological evaluation of cytotoxic 11-aminoalkenylindenoisoquinoline and 11-diaminoalkenylindenoisoquinoline topoisomerase I inhibitors

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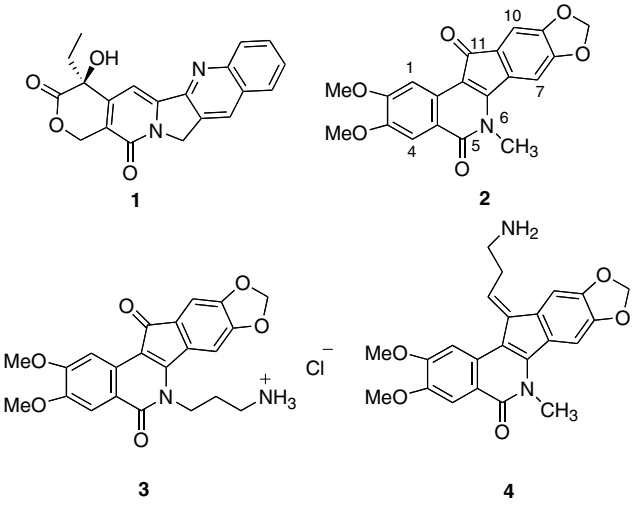
Abstract—The cytotoxic indenoisoquinolines are a novel class of noncamptothecin topoisomerase I inhibitors having certain features that compare favorably with the camptothecins. A new strategy was adopted to attach aminoalkenyl substituents at C-11 of the indenoisoquinoline ring system, which, according to molecular modeling, would orient the side chains toward the DNA minor groove. All of the newly synthesized compounds were more cytotoxic than the parent indenoisoquinoline NSC 314622. Despite an imperfect correlation between cytotoxicities and topoisomerase I inhibition results, the hypothetical structural model of the cleavage complex presented here provides a conceptual framework to explain the structure–activity relationships.

1. Introduction

Topoisomerases are essential nuclear enzymes responsible for resolving topological problems associated with DNA replication, transcription, recombination, repair, chromatin assembly, and chromosome segregation.[1] In particular, topoisomerase I mediates these processes in cells by triggering transient single-stranded breaks in DNA, followed either by strand passage through the nick or 'controlled rotation' of the free end of the broken strand around the unbroken strand, before resealing the break to relax supercoils in DNA.[1,2] Therefore, topoisomerase I is an important biological target for cancer chemotherapy. The prototypical topoisomerase I inhibitors are camptothecin (**1**), an alkaloid isolated from the Chinese tree *Camptotheca acuminata*, and its derivatives such as topotecan and irinotecan, which are currently used clinically as anticancer drugs.[3] As determined by X-ray crystallography, the pentacyclic ring system of the camptothecins mimics a DNA base pair and is therefore able to intercalate into DNA and thus stabilize the 'cleavage complex' formed between DNA and topoisomerase I.[4] The clinical application of the camptothecins is limited by several drawbacks resulting from instability due to the lactone ring opening and rapid reversibility of the 'cleavage complex' after drug removal.[5] As a



Keywords: Anticancer; Indenoisoquinoline; Topoisomerase I; McMurry coupling.
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consequence, there is a need for camptothecin analogues with superior chemical and pharmacological profiles.

Indenoisoquinoline **2** (referred to as NCS 314622) was found to be moderately cytotoxic against the National Cancer Institute's panel of 55 cancer cell lines. It acts by poisoning topoisomerase I via a mechanism similar to the camptothecins.[6] In spite of its moderate cytotoxicity, the chemical stability and persistency of the 'cleavage complex' stabilized by indenoisoquinoline **2** rendered it a good lead for further development of novel topoisomerase I inhibitors.[6] Also encouraging further work with the indenoisoquinolines was the finding that they produce a unique pattern of DNA cleavage sites relative to the camptothecins and therefore may target genes differently, which could result in a different spectrum of anticancer activity.[6–9] Some of the indenoisoquinolines have also been shown to be effective in trapping camptothecin-resistant, mutant top1 enzymes in DNA cleavage assays and to be active in camptothecin-resistant top1-mutant cell lines, suggesting the use of these compounds in the treatment of camptothecin-resistant tumors.[6,9]

In order to address the issue of the moderate cytotoxicity displayed by the original indenoisoquinolines, a number of analogues featuring different substituents on the lactam nitrogen were synthesized and biologically evaluated for both cytotoxicity and topoisomerase I inhibition.[5,7,8,10,11] Of these, compound **3** proved to be one of the most cytotoxic topoisomerase I inhibitors.[8] According to the hypothetical model of indenoisoquinoline binding in the 'cleavage complex', the substituents on the lactam nitrogen protrude toward the DNA major groove (Fig. 1).[11] This raises the question of whether attaching substituents at the 11-position of the indenoisoquinoline system **2**, which will project them into the DNA minor groove, will increase the biological activity. Preliminary studies showed that compound **4**, having a 3′-amino propylidene group at the C-11 position did show increased cytotoxicity and topoisomerase I inhibition.[5] The reasons for the increased bioactivities of compounds **3** and **4** are probably multifaceted. First, the aminoalkyl groups could have groove interactions with DNA, which include hydrogen bonding interactions with specific bases, hydrophobic interactions and electrostatic attraction between the negatively charged groove surfaces and the positively charged protonated amino groups.[12] Second, the increased water solubility of these analogues could also contribute to the enhanced activity.[5] Third, these analogues could be actively transported into cells by polyamine transporters (PAT) on the cancer cell membrane.[11,13] Therefore, a new series of indenoisoquinoline analogues with different amino- and diamino alkylidene substituents on C-11 were designed and synthesized to further explore the structure–activity relationships at this position, and the results of these studies are the subject of the present communication.

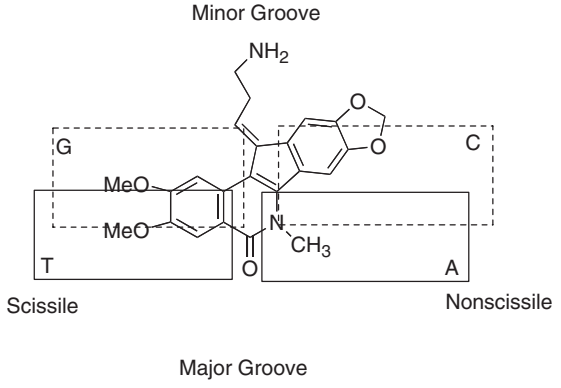
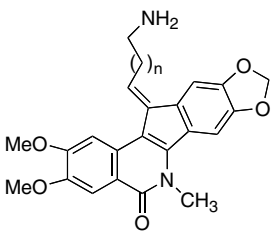


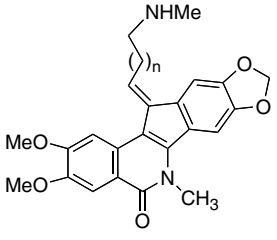
Figure 1. Hypothetical model of the orientation of indenoisoquinoline **4** relative to DNA in the ternary complex containing topoisomerase I, DNA, and the inhibitor **4**.

2. Chemistry

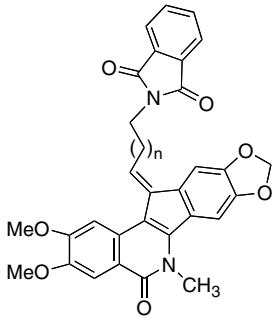
McMurry coupling between various aldehydes and indenoisoquinoline **2**[14] was employed to prepare analogues **5**–**16**.[5] The appropriately protected aldehydes were either commercially available or synthesized by the methods described below.



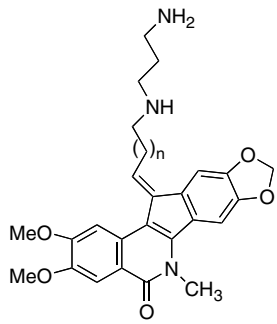
5 n = 0, **6** n = 2
7 n = 3, **8** n = 4



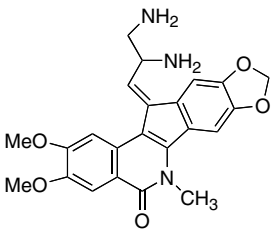
11 n = 1, **12** n = 2



9 n = 2, **10** n = 3



13 n = 0, **14** n = 1



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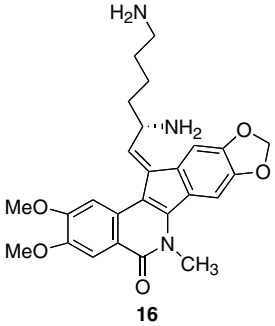

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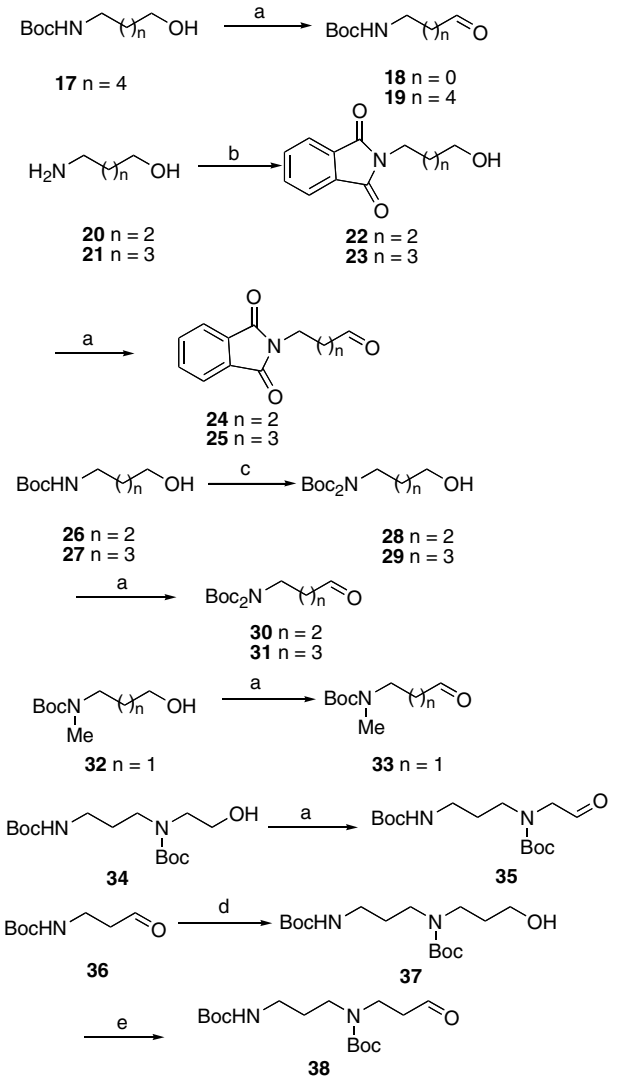
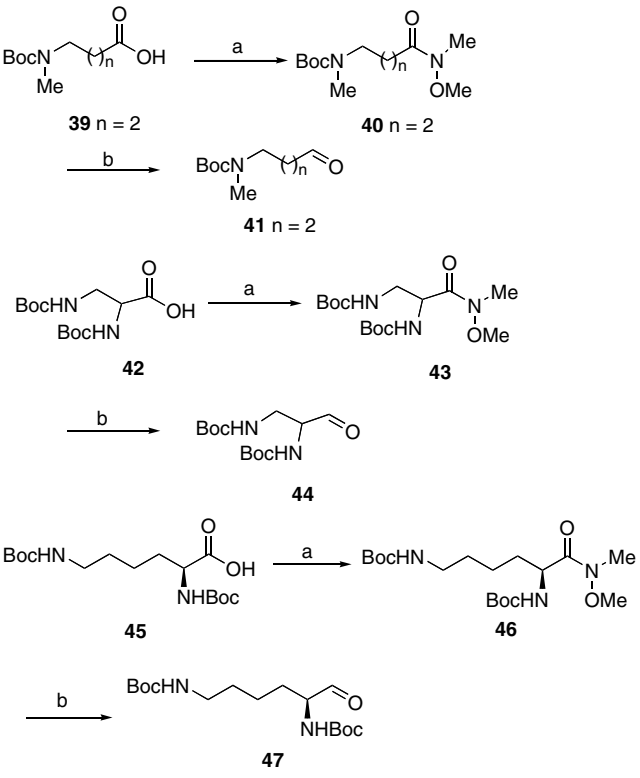


Scheme 1. Reagents and conditions: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C; (b) phthalic anhydride, benzene, reflux; (c) (i) TBSCl, imidazole, CH_2Cl_2, 0 °C; (ii) *n*-BuLi, Boc_2O, THF; (iii) TBAF, THF, rt, 4 h; (d) (i) 3-amino-1-propanol, acetic acid, $NaBH_3CN$, MeOH; (ii) Boc_2O, Et_3N, $CHCl_3$; (e) PDC, CH_2Cl_2, rt, 24 h.

Scheme 1 outlines the synthesis of various aldehydes prepared by Swern oxidation[15] or PDC oxidation of the corresponding alcohols. Although Boc-protected 6-aminohexanal (**19**) was successfully obtained by Swern oxidation of **17**, the related four- and five-carbon Boc-protected amino aldehydes were not isolable since they readily underwent cyclization to the cyclized lactamols,[16] which were unreactive toward McMurry coupling. Therefore, both of the two protons on the amino group were removed by utilization of a phthalic protecting group to give **22** and **23**, which were then subjected to Swern oxidation to afford the corresponding aldehydes **24** and **25**. However, the phthalic groups in the McMurry coupling products **9** and **10** were found to be resistant to either hydrazinolysis or hydrolysis to produce **6** and **7**. At this stage, another Boc protection of the remaining N–H groups in **26** and **27** was employed,[17] which entailed TBS protection of the free hydroxy groups, and a second Boc protection and TBS deprotection, to furnish the protected alcohols **28** and **29** that were easily oxidized to the aldehydes **30** and **31**. Likewise, Boc-protected alcohols **32** and **34** were converted to aldehydes **33** and **35**. Aldehyde **38** was elaborated from Borch reduction[18] of aldehyde **36** in the presence of 3-amino-1-propanol, followed by Boc protection and Swern oxidation.



Scheme 2. Reagents and conditions: (a) BOP reagent, Et_3N, *N*,*O*-dimethyl hydroxylamine hydrochloride, CH_2Cl_2; (b) $LiAlH_4$, THF, rt.

The other strategy used to prepare aldehydes was the lithium aluminum hydride reduction of Weinreb amides (Scheme 2).[19] Thus acids **39**, **42**, **45** were converted to Weinreb amides **40**, **43**, **46**, followed by reduction to aldehydes **41**, **44**, and **47**, respectively.

When the Boc-protected aldehydes from Schemes 1 and 2 were coupled with indenoisoquinoline **2** under standard McMurry coupling conditions ($TiCl_4$·2THF, Zn, THF), the Boc groups were hydrolyzed in each case to yield **5**–**16** directly.

3. Results and discussion

The new alkenylindenoisoquinolines 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

Table 1. Cytotoxicities and topoisomerase I inhibitory activities of indenoisoquinoline analogues

Compound	Cytotoxicity (GI_{50} in μM)[a]									MGM[b]	Top1cleavage[c]
	Lung HOP-62	Colon HCT-116	CNS SF-539	Melanoma UACC-62	Ovarian OVCAR-3	Renal SN12C	Prostate LDU-145	BreastMDA-MB-435			
2	1.3	35	41	4.2	73	68	37	96		20	++
3	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21		0.16	+++
4	0.071	0.028	0.42	0.20	0.56	0.58	0.37	1.8		0.34	+++
5	0.27	0.086	0.32	0.096	2.17	0.57	0.38	0.84		0.56	+++
6	3.64	4.64	1.65	2.13	13.8	NT[d]	5.57	8.60		5.25	+++
7	NT[d]	NT[d]	2.83	12.5	16.9	9.39	10.5	5.55		7.76	+
8	0.87	0.23	1.18	2.58	3.08	0.75	1.20	4.30		1.35	++
9	19.2	26.2	8.99	NT[d]	>40	NT[d]	6.73	>40		18.6	+
10	17.4	22.8	NT[d]	6.01	38.1	NT[d]	NT[d]	>100		11.0	+
11	1.01	0.13	0.41	0.39	1.06	0.36	0.43	0.86		0.58	++
12	0.60	0.40	0.14	0.25	1.48	0.86	1.40	1.96		0.78	0
13	0.74	0.12	0.96	0.24	4.22	0.15	1.69	1.95		0.61	+
14	3.85	1.49	1.65	NT[d]	NT[d]	1.18	NT[d]	2.48		2.34	+
15	1.37	1.16	1.50	0.96	1.92	1.91	2.06	2.58		1.73	++
16	3.82	0.36	0.34	0.50	3.84	0.46	0.86	2.82		2.03	+++

[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 semi-quantitatively as follows: 0: no activity; +: weak activity; ++: similar activity as the parent compound **2**; +++: greater activity than the parent compound **2**.
[d] NT = not tested.

tested (approximately 55) in which GI50 values below and above the test range (10^{-8}–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, the activities of the previously reported lead compound **2**[6] and its more potent *N*-3′-aminopropyl derivative **3**[8] and 3′-aminopropylidene analogue **4**[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 kept constant except for the substituent on the C-11 position. This was done to focus on the biological effects of side chain variation through the incorporation of amino groups.

All of the newly synthesized indenoisoquinoline analogues displayed increased cytotoxicity compared to the lead compound **2** as indicated by the MGM values. In the series of monoamines, a decrease in the carbon chain length from three (**4**, MGM 0.34 μM) to two (**5**, MGM 0.56 μM) only resulted in a slight decrease in cytotoxicity, while an increase in the chain length to four carbons (**6**, MGM 5.25 μM) caused a 15-fold decrease in the cytotoxicity. Interestingly, the topoisomerase I inhibitory potency was unchanged during this variation of the chain length. However, further homologation to five (**7**) or six carbons (**8**) resulted in both a decrease in cytotoxicity and topoisomerase I inhibition. Compounds **9** and **10**, without free amines at the end of the side chain, showed less topoisomerase I inhibitory potency than the lead compound **2**, but were still slightly more cytotoxic than **2**.

At this stage, we were interested in employing the SYBYL 6.9 molecular modeling package to dock the most potent analogue in this series into the topoisomerase I-DNA complex by assuming the structural similarities[5] between the indenoisoquinolines and camptothecin. Thus, the structure of the 3′-aminopropylidene compound **4** was overlaid with that of topotecan in the recently published ternary complex[4] and then the topotecan molecule was deleted from the complex. During this process, it was assumed that the lactam rings of the two systems would be oriented similarly relative to DNA. The substituent at C-11 was therefore oriented toward the DNA minor groove (Fig. 1). The energy of the newly generated complex was minimized using the Powell method and the MMFF94s force field with MMFF94 charges and a distance-dependent dielectric function ($\varepsilon \propto r$) to calculate the electrostatic interactions. During energy minimization, the coordinates of the protein, nucleic acid, and the surrounding water molecules were kept frozen while the inhibitor was allowed to move. The final structure, shown in Figure 2, was converged on the criterion of an energy gradient of 0.05 kcal/(mol Å). In this hypothetical model, the lactam oxygen was hydrogen bonded with topoisomerase I through a series of mediating water molecules as ob-

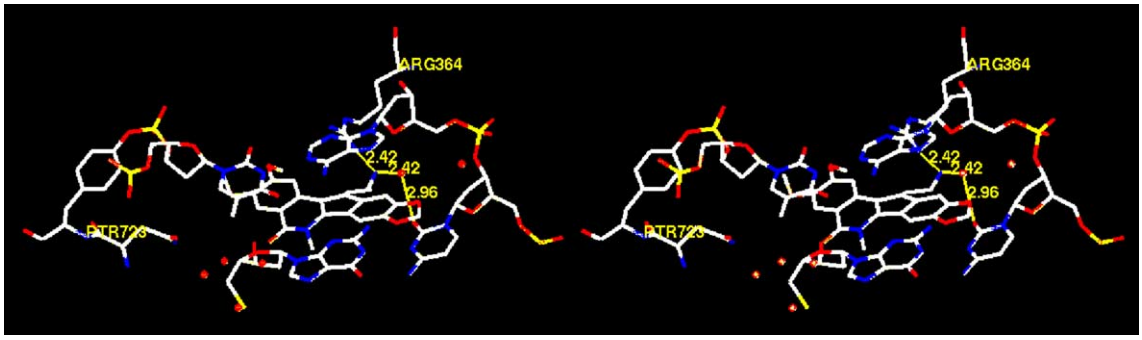


Figure 2. Model of the binding of the indenoisoquinoline **4** in the ternary complex consisting of DNA, top1, and the inhibitor. For simplicity, the distances between lactam carbonyl oxygen and the mediating water molecules are not shown. The diagram is programmed for wall-eyed viewing.

served in the previously published hypothetical model of compound **3**.[11] Moreover, the amino group on the side chain is hydrogen bonded directly with Arg364 and indirectly with one of the flanking bases via a mediating water molecule. This overall network of hydrogen bonding interactions and the π–π stacking interaction between the aromatic ring system of the indenoisoquinoline and the flanking base pairs effectively stabilize the 'ternary complex', which in turn inhibits the religation step by increasing the distance between the 5′-hydroxyl group and the phosphodiester group that would be involved in the reaction.[4,9] The snug fit of compound **4** in the cleavage site was further visualized by examining the electrostatic potential surfaces of **4** and the cleavage site (Fig. 3), which indicated good electrostatic complementarity, both in the aromatic ring system and the side chain. With an increase of the chain length between the amino group and indenoisoquinoline nucleus, or through protection of the free amine, the amino group would not be able to hydrogen bond with Arg364, which explains the decreased potency in inhibiting topoisomerase I by **7**, **8** and **9**, **10**.

Since only one of the two hydrogens on the amino groups of the indenoisoquinolines is proposed to be involved in hydrogen bond formation with a water molecule (Fig. 2), methylation of the amino group would be beneficial for binding since it should decrease the desolvation penalty.[20] To test this hypothesis, two methylamino analogues **11** and **12** were synthesized. Although **12** showed significantly increased cytotoxicity (MGM 0.78 μM) in comparison to **6** (MGM 5.25 μM), the topoisomerase I inhibitory activity was completely abolished, while both cytotoxicity and topoisomerase I inhibition were decreased for compound **11** (MGM 0.58 μM) as opposed to **4** (MGM 0.34 μM). This trend persists even for diamines **13** (MGM 0.61 μM) and **14** (MGM 2.34 μM), in which one of the amino hydrogens was substituted by a 3-aminopropyl group. Taken together, these results suggest that a primary amine separated from the indenoisoquinoline nucleus by an appropriate chain length is necessary for high biological potency of these analogues both as cytotoxic agents and topoisomerase I inhibitors. Steric factors may play a role in the decreased enzyme inhibitory activities of the methylamine analogues. The apparent discrepancy between the cytotoxicity and topoisomerase I inhibition results also

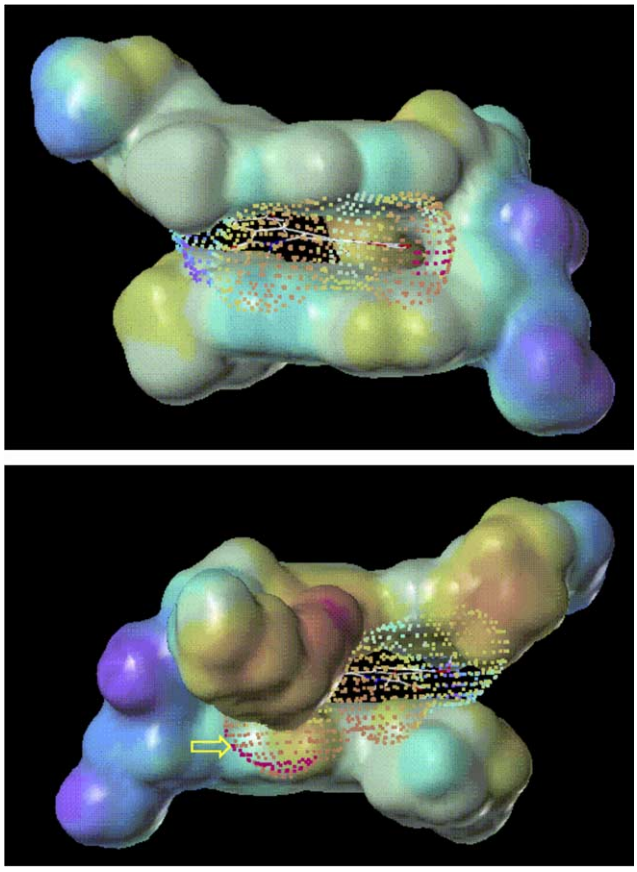


Figure 3. Electrostatic potentials on the molecular surfaces of the indenoisoquinoline **4** (dotted surface) in the cleavage site showing the flanking base pairs and ARG364 only. Electronegative potentials are colored blue and electropositive potentials are colored red. Top, view from the DNA major groove. Bottom, view from the DNA minor groove. The arrow highlights the position of the amino side chain of compound **4**.

indicates that topoisomerase I may not be the sole biological target for the cytotoxic indenoisoquinolines as observed in some other analogues.[11] Alternatively, differences in the cell membrane penetration, distribution within the cell, and metabolism could also play a role.

The fact that compound **5** is both very cytotoxic and a potent topoisomerase I inhibitor implies that attachment of an additional amino group at the 2′-position

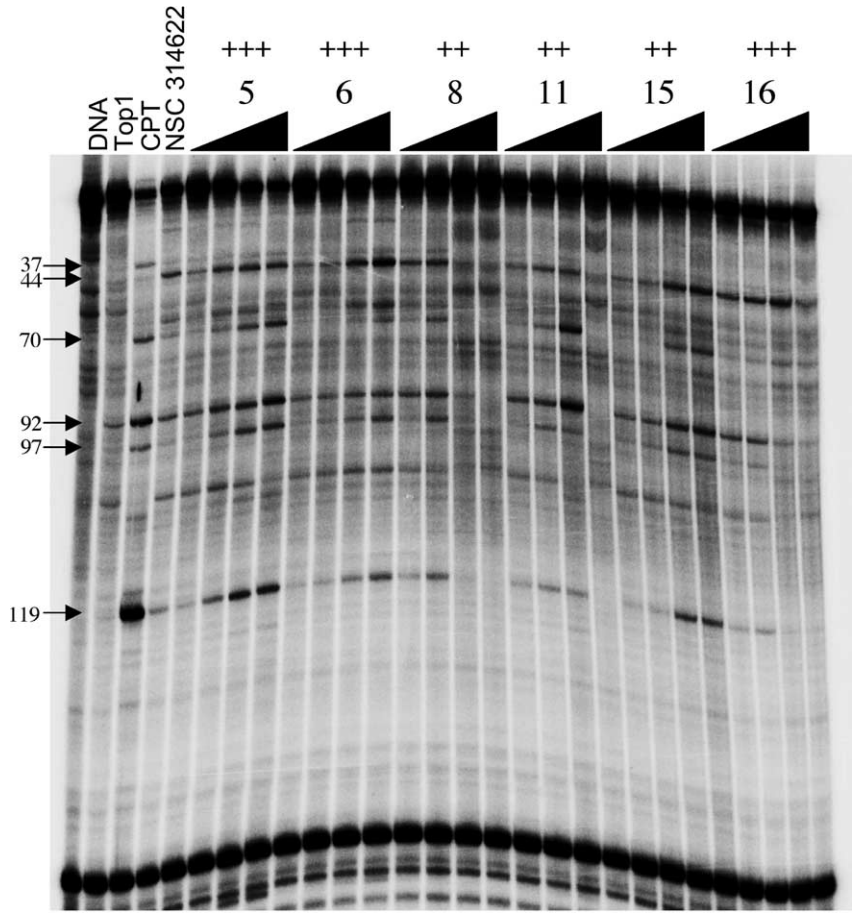


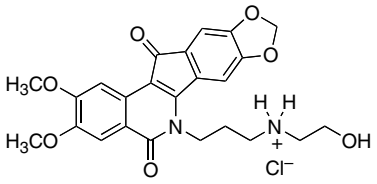
Figure 4. Comparison of the top1-mediated DNA cleavages at different drug concentrations. The DNA used corresponds to the 3′-end-labeled *Pvu*II/*Hind*III fragment of pBluescript SK(−) phagemid DNA. The four concentrations of the inhibitors used were 0.1, 1.0, 10, and 100 μM. Reactions were performed 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 in the control lane. Control: DNA with neither top1 nor any drug.

of such potent analogues as **4** and **8** might further increase the biological potency. To this end, compounds **15** and **16** were synthesized and evaluated. A gain in topoisomerase I inhibition was indeed obtained with compound **16** versus **8**; however, cytotoxicity was slightly decreased, which might be due to the poorer capability of compound **16**, having two positive charges on the side chain, to penetrate into the cancer cell membrane or nuclear membrane.[21] Less promising results came from compound **15**, which showed both less cytotoxicity and topoisomerase I inhibition than either **4** or **5**. The possible explanation of this unexpected result could be related to van der Waal's repulsion between the side chains of the ligand and Arg364 of the enzyme, or to the stereochemistry at C-2′.

All of the compounds were examined for induction of DNA cleavage in the 3′-end-labeled *Pvu*II/*Hind*III fragment of pBluescript SK(−) phagemid DNA in the presence of top1.[6] The resulting cleavage patterns of some of the more potent indenoisoquinolines are displayed in Figure 4. The results were compared with camptothecin (**1**) and the lead compound **2** (NSC 314622). Some, but not all, of the DNA cleavage sites observed with the indenoisoquinolines were different from those observed with camptothecin. For example, the camptothecin band at site 37 is very low in intensity with the indenoisoquinolines in Figure 4, and the band at site 44 was observed with the indenoisoquinolines but not with camptothecin. Also, the bands observed for cleavage at identical sites varied in intensity among the indenoisoquinolines, as well as in comparison with camptothecin. These differences are important because they indicate that different cancer cell genes could be targeted more selectively with the indenoisoquinolines versus the camptothecins. Also, similar to the situation with other anticancer drugs that share a single target (e.g., top2 and tubulin inhibitors), it can be expected that different top1 inhibitors will have different spectra of antitumor activities.[22] Similar conclusions have been reached in prior top1–DNA cleavage studies involving the other indenoisoquinoline top1 inhibitors.[7,8,10,23]

In general, the intensities of the DNA cleavage bands produced by the amines in this study varied in intensity as a function of drug concentration (Fig. 4). Two types of cleavage patterns emerged. In one type, the intensities of the DNA cleavage bands detected by gel electrophoresis increased as the concentration of the top1 inhibitor increased from 0.1 to 100 μM. This pattern was observed in most of the cleavage sites produced by compounds **5**, **6**, and **15**. On the other hand, the intensities of the cleav-

age bands produced by the top1 inhibitors **8**, **11**, and **16** increased as the concentration of the inhibitor was increased in the lower concentration range, but then decreased as the concentration was increased further. The latter dose-response pattern has been observed previously with a variety of different indenoisoquinolines, including the amine **3**.[8] With compound **8**, maximum cleavage was observed at 1 μM, while with the top1 poison **11**, it was usually observed at 10 μM. With inhibitor **16**, the maximum DNA cleavage seemed to occur at 1 or at 10 μM, depending on the cleavage site. These results indicate that these inhibitors suppress top1-mediated DNA cleavage at high drug concentration, which is similar to the situation observed with DNA unwinding or intercalating inhibitors.[24–26] Prior results with the aminoalcohol **48** demonstrated a low affinity DNA intercalation that could be responsible for suppression of DNA cleavage at higher drug concentration.[8] Alternatively, higher concentrations of the amines may suppress top1-mediated DNA cleavage through a direct effect on the enzyme resulting in a conformational change, as has been proposed with saintopin E.[25] It is possible that compounds **5**, **6**, and **15** could also suppress DNA cleavage at concentrations higher than the 100 μM maximum concentration tested.



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In conclusion, a number of new indenoisoquinolines were synthesized with enhanced cytotoxicity relative to the lead compound **2**. Although the cytotoxicity results did not correlate perfectly with topoisomerase I inhibitory activities, the hypothetical model which orients the substituents at C-11 position toward the DNA minor groove provided a conceptual framework to explain the structure–activity relationships.

4. Experimental

All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents, unless otherwise stated. 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, ^1H NMR spectra were obtained using $CDCl_3$ or DMSO-d_6 as solvent and the chemical shifts of the residual $CHCl_3$ (δ 7.24) or DMSO (δ 2.49) were taken as reference. ^1H NMR spectra were recorded at 300 MHz except where stated. Microanalyses were performed at the Purdue University Microanalysis Laboratory. Analytical thin-layer chromatography was carried out on Analtech silica gel GF 1000 μm glass plates. Compounds were visualized with short wavelength UV light. Silica gel flash chromatography was performed using 230–400 mesh silica gel.

4.1. 11-(2′-Aminoethylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (5)

$TiCl_4$–THF (1:2) complex (962 mg, 2.88 mmol) and zinc dust (374 mg, 5.76 mmol) were put in a two-necked round-bottomed flask. THF (30 mL) was added. The resulting suspension was heated under reflux for 4 h. At this point, a mixture of commercially available *tert*-butyl *N*-(2-oxoethyl)carbamate (**18**) (184 mg, 1.16 mmol) and indenoisoquinoline **2**[14] (352 mg, 0.96 mmol) in THF (30 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 4 h. Then 4 N HCl (10 mL) was added after cooling to room temperature and then to 0 °C. The resulting mixture was stirred at room temperature overnight. The mixture was recooled to 0 °C and solid $NaHCO_3$ was added to neutralize HCl. The solvent was removed in vacuo and the resulting residue was subjected to flash chromatography, eluting with $CHCl_3$ followed by $CHCl_3$–MeOH (4:1) providing a yellow powder (112 mg, 30%): mp > 170 °C (dec.). ^1H NMR (300 MHz, DMSO-d_6) δ 7.70 (s, 1H), 7.65 (s, 1H), 7.51 (s, 1H), 7.32 (s, 1H), 6.87 (t, J = 5.4 Hz, 1H), 6.16 (s, 2H), 4.31 (d, J = 5.4 Hz, 2H), 4.00 (s, 3H), 3.97 (s, 3H), 3.88 (s, 3H). ESIMS *m*/*z* (rel. intensity) 393 (91, MH^+), 376 (100, MH^+-NH_3). Anal. Calcd for $C_{22}H_{20}N_2O_5{\cdot}0.6CHCl_3$: C, 58.50; H, 4.48; N, 6.04. Found: C, 58.18; H, 4.98; N, 5.56.

4.2. 11-(4′-Aminobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (6)

Prepared from **2** (100 mg, 0.27 mmol) and **30** (95 mg, 0.33 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH–$NH_3{\cdot}$ H_2O (100:10:1.0), yielding a yellow powder (40 mg, 35%): mp > 230 °C (dec). ^1H NMR (300 MHz, DMSO-d_6) δ 7.64 (s, 1H), 7.59 (s, 1H), 7.57 (s, 1H), 7.22 (s, 1H), 7.04 (t, J = 6.1 Hz, 1H), 6.10 (s, 2H), 4.16 (br s, 2H), 3.96 (s, 6H), 3.89 (s, 3H), 2.74–2.80 (m, 4H), 1.33–1.40 (m, 2H); ESIMS *m*/*z* (rel. intensity) 421 (100, MH^+). Anal. Calcd for $C_{24}H_{24}N_2O_5{\cdot}0.2CHCl_3$: C, 65.42; H, 5.49; N, 6.30. Found: C, 65.54; H, 5.28; N, 5.87.

4.3. 11-(5′-Aminopentylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (7)

Prepared from **2** (200 mg, 0.55 mmol) and **31** (198 mg, 0.66 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH (6:1) yielding a yellow powder (46.5 mg, 20%): mp > 220 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.844 (s, 1H), 7.837 (s, 1H), 7.81 (s, 1H), 7.45 (s, 1H), 7.08 (t, J = 6.0 Hz, 1H), 6.03 (s, 2H), 4.05 (s, 3H), 4.02 (s, 3H), 4.00 (s, 3H), 2.54–2.83 (m, 4H), 1.20–1.40 (m, 4H); ESIMS *m*/*z* (rel. intensity) 435 (100, MH^+). Anal. Calcd for $C_{25}H_{26}N_2O_5{\cdot}0.5CHCl_3$: C, 61.98; H, 5.41; N, 5.67. Found: C, 61.89; H, 5.88; N, 5.82.

4.4. 11-(6′-Aminohexylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (8)

Prepared from **2** (100 mg, 0.27 mmol) and **19** (71 mg, 0.33 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH (5:1), yielding a yellow powder (54 mg, 45%): mp > 245 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (br s, 2H), 7.68 (s, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 6.98 (t, J = 6.1 Hz, 1H), 6.13 (s, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 2.78–2.86 (m, 4H), 1.58–1.73 (m, 4H), 1.45–1.57 (m, 2H); ESIMS *m*/*z* (rel. intensity) 449 (100, MH^+). Anal. Calcd for $C_{26}H_{28}N_2O_5 \cdot 0.85CHCl_3$: C, 58.64; H, 5.29; N, 5.09. Found: C, 58.32; H, 5.73; N, 5.09.

4.5. 11-(4′-Phthalylaminobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (9)

Prepared from **2** (300 mg, 0.82 mmol) and **24** (214 mg, 0.99 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH (20:1), yielding a yellow powder (202 mg, 44%): mp 262–264 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.63–7.66 (m, 2H), 7.55–7.57 (m, 2H), 7.42 (s, 1H), 7.37 (s, 1H), 7.28 (s, 1H), 6.87 (t, J = 6.3 Hz, 1H), 6.04 (s, 2H), 4.08 (s, 3H), 4.01 (s, 3H), 4.00 (s, 3H), 3.85 (t, J = 6.6 Hz, 2H), 2.91 (q, J = 6.6 Hz, 2H), 2.12 (quin, J = 6.6 Hz, 2H); IR (film) 3460, 2938, 1769, 1709, 1634, 1611, 1517, 1484, 1396, 1253, 1030, 722 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 551 (100, MH^+). Anal. Calcd for $C_{32}H_{26}N_2O_7 \cdot 0.4H_2O$: C, 68.91; H, 4.86; N, 5.02. Found: C, 68.91; H, 4.88; N, 5.13.

4.6. 11-(5′-Phthalylaminopentylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (10)

Prepared from **2** (300 mg, 0.82 mmol) and **25** (229 mg, 0.99 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH (20:1) yielding a yellow powder (210 mg, 45%): mp 205–207 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.79–7.82 (m, 2H), 7.66–7.71(m, 2H), 7.45 (s, 1H), 7.40 (s, 1H), 7.33 (s, 1H), 6.86 (t, J = 7.2 Hz, 1H), 6.04 (s, 2H), 4.04 (s, 3H), 4.02 (s, 3H), 4.00 (s, 3H), 3.77 (t, J = 7.2 Hz, 2H), 2.88 (t, J = 7.2 Hz, 2H), 1.91 (quin, J = 7.2 Hz, 2H), 1.77 (quin, J = 7.2 Hz, 2H); ESIMS *m*/*z* (rel. intensity) 565 (100, MH^+). Anal. Calcd for $C_{33}H_{28}N_2O_7$: C, 70.20; H, 4.80; N, 4.96. Found: C, 69.88; H, 4.98; N, 4.90.

4.7. 11-(3′-Methylaminopropylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (11)

Prepared from **2** (100 mg, 0.27 mmol) and **33** (61.7 mg, 0.33 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH (5:1) yielding a yellow powder (55 mg, 48%): mp 216–218 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.69 (s, 1H), 7.64 (s, 1H), 7.54 (s, 1H), 7.51 (s, 1H), 6.95 (t, J = 6.1 Hz, 1H), 6.15 (s, 2H), 3.99 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 3.21 (t, J = 7.0 Hz, 2H), 3.13 (q, J = 6.6 Hz, 2H), 2.60 (s, 3H). ESIMS *m*/*z* (rel. intensity) 421 (100, MH^+). Anal. Calcd for $C_{24}H_{24}N_2O_5 \cdot 0.55CHCl_3$: C, 60.66; H, 5.09; N, 5.76. Found: C, 60.99; H, 5.56; N, 5.15.

4.8. 11-(4′-Methylaminobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (12)

Prepared from **2** (100 mg, 0.27 mmol) and **41** (66 mg, 0.33 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$ and $CHCl_3$–MeOH (6:1), yielding a yellow powder (57 mg, 48%): mp > 215 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (s, 1H), 7.64 (s, 1H), 7.58 (s, 1H), 7.52 (s, 1H), 6.91 (t, J = 6.1 Hz, 1H), 6.15 (s, 2H), 4.00 (s, 3H), 3.97 (s, 3H), 3.87 (s, 3H), 3.07 (t, J = 6.0 Hz, 2H), 2.89–2.94 (m, 2H), 2.58 (s, 3H), 1.95–2.05 (m, 2H); ESIMS *m*/*z* (rel. intensity) 435 (100, MH^+). Anal. Calcd for $C_{25}H_{26}N_2O_5 \cdot 1.4H_2O$: C, 65.32; H, 6.32; N, 6.09. Found: C, 65.01; H, 6.21; N, 5.81.

4.9. 11-[2′-*N*-(3′-Aminopropyl)-aminoethylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (13)

Prepared from **2** (100 mg, 0.27 mmol) and **35** (104 mg, 0.33 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH–$NH_3 \cdot H_2O$ (100:20:2.0), yielding a yellow powder (30 mg, 24%): mp 183–185 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.69 (s, 1H), 7.62 (s, 1H), 7.53 (s, 1H), 7.03 (s, 1H), 7.03 (t, J = 6.1 Hz, 1H), 6.13 (s, 2H), 3.96 (s, 6H), 3.89 (s, 3H), 2.83–2.89 (m, 2H), 2.65–2.75 (m, 4H), 1.52–1.62 (m, 2H); ESIMS *m*/*z* (rel. intensity) 450 (100, MH^+). Anal. Calcd for $C_{25}H_{27}N_3O_5 \cdot 0.5CHCl_3$: C, 60.15; H, 5.44; N, 8.25. Found: C, 60.08; H, 5.37; N, 8.23.

4.10. 11-[3′-*N*-(3′-Aminopropyl)-aminopropylidene]-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (14)

Prepared from **2** (100 mg, 0.27 mmol) and **38** (132 mg, 0.4 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH–$NH_3 \cdot H_2O$ (100:20:2), affording a yellow solid powder (45 mg, 36%): mp > 210 °C (dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71 (s, 1H), 7.65 (s, 1H), 7.57 (s, 1H), 7.52 (s, 1H), 7.04 (t, J = 6.0 Hz, 1H), 6.15 (s, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 3.88 (s, 3H), 3.08–3.12 (m, 4H), 2.85–2.91 (m, 4H), 1.87–1.90 (m, 2H). ESIMS *m*/*z* (rel. intensity) 464 (100, MH^+); HRESIMS *m*/*z* $C_{26}H_{30}N_3O_5$: Calcd 464.2185, Found 464.2202. Anal. Calcd for $C_{26}H_{29}N_3O_5 \cdot 0.8CHCl_3$: C, 57.58; H, 5.37; N, 7.52. Found: C, 57.23; H, 5.11; N, 7.34.

4.11. 11-(2′,3′-Diaminopropylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (15)

Prepared from **2** (150 mg, 0.41 mmol) and **44** (137 mg, 0.49 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$ and $CHCl_3$–MeOH–$NH_3{\cdot}H_2O$ (100:20:2.0), yielding a yellow powder (78 mg, 45%): mp 162–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 (s, 1H), 7.60 (s, 1H), 7.57 (s, 1H), 7.54 (s, 1H), 6.87 (d, J = 6.1 Hz, 1H), 6.13 (s, 2H), 4.14–4.25 (m, 1H), 3.96 (s, 6H), 3.87 (s, 3H), 2.77–2.85 (m, 1H), 2.61–2.70 (m, 1H); ESIMS *m*/*z* (rel. intensity) 422 (44, MH^+), 405 (100, $M-NH_3^+$). Anal. Calcd for $C_{23}H_{23}N_3O_5{\cdot}0.4CHCl_3$: C, 59.90; H, 5.03; N, 8.96. Found: C, 60.19; H, 5.11; N, 8.46.

4.12. 11-[2′(*S*),6′-Diaminohexylidene]-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (16)

Prepared from **2** (100 mg, 0.27 mmol) and **47** (109 mg, 0.33 mmol) following the procedure for **5**. The chromatography column was eluted with $CHCl_3$–MeOH–$NH_3{\cdot}$ H_2O (100:25:2.5) yielding a yellow powder (40 mg, 32%): mp > 168 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (s, 1H), 7.63 (s, 1H), 7.54 (s, 1H), 7.49 (s, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.14 (s, 2H), 4.21 (q, J = 8.4 Hz, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 2.70 (t, J = 7.0 Hz, 2H), 1.59–1.66 (m, 2H), 1.42–1.55 (m, 4H); ESIMS *m*/*z* (rel. intensity) 464 (5, MH^+), 447 (100, MH^+-NH_3). Anal. Calcd for $C_{26}H_{29}N_3O_5{\cdot}0.55CHCl_3$: C, 60.26; H, 5.63; N, 7.94. Found: C, 60.74; H, 5.96; N, 7.27.

4.13. *tert*-Butyl-6-oxo-hexyl carbamate (19).[27]

A solution of DMSO (568 μL, 8.0 mmol) in CH_2Cl_2 (4 mL) was added over 30 min to a stirred solution of oxalyl chloride (350 μL, 4.0 mmol) in CH_2Cl_2 (15 mL) at −78 °C. Upon completion of the addition, the mixture was stirred at −78 °C for 5 min, followed by addition of a solution of commercially available alcohol **17** (434 mg, 2.0 mmol) in CH_2Cl_2 (4 mL) over 30 min at −78 °C. The resulting mixture was stirred at −78 °C for 40 min. Then Et_3N (1.67 mL, 12.0 mmol) was added dropwise over 10 min. The resulting mixture was allowed to warm to 0 °C and stirred at 0 °C for 1 h. Water (10 mL) was added to quench the reaction. Et_2O (120 mL) was added to dilute the reaction mixture. The organic layer was then separated from the water layer, which was further washed with water (2 × 15 mL) and brine (2 × 15 mL). The thus obtained organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was subjected to flash chromatography, eluting with *n*-hexane–ethyl acetate (5:1), yielding a colorless oil (391 mg, 91%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (t, J = 1.5 Hz, 1H), 4.45 (br s, 1H), 3.09 (q, J = 6.6 Hz, 2H), 2.42 (td, J = 7.2, 1.5 Hz, 2H), 1.63 (quin, J = 7.2 Hz, 2H), 1.45 (quin, J = 7.2 Hz, 2H), 1.42 (s, 9H), 1.29–1.37 (m, 2H); ESIMS *m*/*z* (rel. intensity) 238 (100, MNa^+).

4.14. 4-Phthalylamino-1-butanol (22).[28]

A mixture of 4-amino-1-butanol (**20**) (890 mg, 10 mmol) and phthalic anhydride (1.48 g, 10 mmol) in toluene (30 mL) was heated to reflux under Dean–Stark conditions for 3 h. After cooling, removal of solvent in vacuo produced the crude product which was purified by flash column chromatography eluting with $CHCl_3$ providing a colorless oil, which upon standing became a colorless crystalline solid (2.14 g, 98%): mp 48–49 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.71 (m, 2H), 7.57–7.62 (m, 2H), 3.59 (t, J = 6.9 Hz, 2H), 3.56 (t, J = 6.9 Hz, 2H), 2.94 (s, 1H), 1.66 (quin, J = 6.9 Hz, 2H), 1.50 (t, J = 6.9 Hz, 2H); IR (film) 3462, 2942, 1771, 1709, 1467, 1399, 1046, 720 cm^{-1}; CIMS *m*/*z* (rel. intensity) 220 (100, MH^+).

4.15. 4-Phthalylamino-1-pentanol (23).[28]

Prepared from 5-amino-1-pentanol (**21**) (1.03 g, 10 mmol) and phthalic anhydride (1.48 g, 10 mmol) following the procedure for **22**. The column was eluted with $CHCl_3$, providing a colorless oil (2.10 g, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.62 (m, 2H), 7.48–7.54 (m, 2H), 3.48 (t, J = 7.2 Hz, 2H), 3.44 (t, J = 6.6 Hz, 2H), 3.05 (s, 1H), 1.52 (quin, J = 7.2 Hz, 2H), 1.43 (quin, J = 6.6 Hz, 2H), 1.18–1.28 (m, 2H); ^{13}C NMR (75 M Hz, $CDCl_3$) δ 168.7 (2C), 134.2 (2C), 132.3 (2C), 123.4 (2C), 62.5, 38.2, 32.4, 28.7, 23.4; IR (film) 3460, 2939, 2863, 1771, 1715, 1683, 1615, 1456, 1338, 1188, 1052, 963, 720, 693 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 234 (100, MH^+), 256 (20, MNa^+).

4.16. 4-Phthalylamino-1-butanal (24).[28]

Prepared from alcohol **22** (700 mg, 3.2 mmol) following the procedure for **19**. The column was eluted with *n*-hexane–ethyl acetate (4:1) yielding a colorless solid (625 mg, 90%): mp 61–62 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 7.76–7.79 (m, 2H), 7.65–7.68 (m, 2H), 3.67 (t, J = 6.9 Hz, 2H), 2.48 (t, J = 6.9 Hz, 2H), 1.95 (quin, J = 6.9 Hz, 2H); IR (film) 3457, 2951, 1745, 1705, 1468, 1335, 1130, 1061, 864, 716 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 218 (100, MH^+).

4.17. 4-Phthalylamino-1-pentanal (25).[28]

Prepared from alcohol **23** (700 mg, 3.0 mmol) following the procedure for **19**. The column was eluted with *n*-hexane–ethyl acetate (4:1), yielding a colorless oil (580 mg, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (t, J = 1.2 Hz, 1H), 7.73–7.78 (m, 2H), 7.63–7.68 (m, 2H), 3.63 (t, J = 6.9 Hz, 2H), 2.44 (td, J = 6.9, 1.2 Hz, 2H), 1.55–1.70 (m, 4H); ^{13}C NMR (75 M Hz, $CDCl_3$) δ 202.2, 168.7 (2C), 134.3 (2C), 132.4 (2C), 123.6 (2C), 43.6, 37.8, 28.3, 19.6; IR (film) 3464, 2942, 1772, 1709, 1398, 1374, 1051, 721 cm^{-1}.

4.18. Di-*tert*-butyl-(4-hydroxyl-butyl) dicarbamate (28)

Imidazole (414.8 mg, 6.1 mmol) and TBS-Cl (442 mg, 2.93 mmol) were sequentially added to a stirred solution

of commercially available alcohol **26** (462 mg, 2.44 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. Then Et_2O (100 mL) was added to dilute the reaction mixture, which was further washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over Na_2SO_4, filtered, concentrated yielding a colorless oil, which was used for next operation without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72 (br s, 1H), 3.57 (t, J = 5.7 Hz, 2H), 3.07 (q, J = 5.1 Hz, 2H), 1.46–1.50 (m, 4H), 1.38 (s, 9H), 0.83 (s, 9H), 0.01 (s, 6H); IR (film) ν 3355, 2931, 2859, 1694, 1520, 1366, 1253, 1175, 837 cm^{-1}; ESIMS m/z (rel. intensity) 326 (100, MNa^+). n-BuLi (2.0 M in cyclohexane, 1.1 mL, 2.2 mmol) was added to a stirred solution of TBS ether (554 mg, 1.83 mmol) obtained as above in THF (8 mL) at 0 °C. The reaction mixture was stirred for 15 min at 0 °C before the addition of a solution of Boc_2O (478 mg, 2.2 mmol) in THF (3 mL). The resulting reaction mixture was warmed to room temperature and stirred for 1 h. Then Et_2O (100 mL) was added to dilute the reaction mixture, which was then washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over Na_2SO_4, filtered, and concentrated giving the crude product as a colorless oil (737 mg, 100%), which was used for the next operation without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49–3.58 (m, 4H), 1.40–1.48 (m, 22H), 0.83 (s, 9H), −0.02 (s, 6H); IR (film) ν 2933, 2859, 1794, 1748, 1473, 1368, 1256, 1124, 8374, 776 cm^{-1}; ESIMS m/z (rel. intensity) 326 (426, MNa^+). TBAF (1.0 M in THF, 2.75 mL, 2.75 mmol) was added slowly to a stirred solution of the crude TBS ether (737 mg, 1.83 mmol) obtained as above in THF (5 mL) at room temperature. The resulting solution was stirred at room temperature for 4 h. Then Et_2O (100 mL) was added to dilute the reaction mixture, which was further washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over Na_2SO_4, filtered, concentrated and the residue was subjected to flash column chromatography, eluting with n-hexane–EtOAc (4:1) yielding a colorless oil (415 mg, 79% in three steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41–3.49 (m, 4H), 2.91 (br s, 1H), 1.33–1.51 (m, 22H); IR (film) ν 3479, 2979, 2871, 1780, 1731, 1479, 1368, 1122, 856, 783 cm^{-1}; ESIMS m/z (rel. intensity) 312 (100, MNa^+), 212 (20, MH^+−Boc). Anal. Calcd for $C_{14}H_{27}NO_5$: C, 58.11; H, 9.40; N, 4.84. Found: C, 58.43; H, 9.72; N, 4.90.

4.19. Di-*tert*-butyl-(5-hydroxyl-pentyl) dicarbamate (29)

Imidazole (753 mg, 11.08 mmol) and TBS-Cl (802 mg, 5.32 mmol) were sequentially added to a stirred solution of commercially available alcohol **27** (900 mg, 4.43 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. Then Et_2O (200 mL) was added to dilute the reaction mixture, which was further washed with water (2 × 20 mL) and brine (2 × 20 mL). The organic solution was dried over Na_2SO_4, filtered, and concentrated to give a colorless oil (1.5 g, 100%), which was used for the next operation directly without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.51 (br s, 1H), 3.58 (t, J = 6.0 Hz, 2H), 3.08 (q, J = 6.0 Hz, 2H), 1.34–1.50 (m, 15H), 0.88 (s, 9H), 0.03 (s, 6H). n-BuLi (2.0 M in cyclohexane, 1.1 mL, 2.2 mmol) was added to a stirred solution of TBS ether (579 mg, 1.83 mmol) obtained as above in THF (8 mL) at 0 °C. The reaction mixture was stirred for 15 min at 0 °C before the addition of a solution of Boc_2O (478 mg, 2.2 mmol) in THF (3 mL). The resulting reaction mixture was warmed to room temperature and stirred for 1 h. Then Et_2O (100 mL) was added to dilute the reaction mixture, which was then washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over Na_2SO_4, filtered, and concentrated giving the crude product as a colorless oil (720 mg, 95%), which was used for next operation without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (t, J = 6.0 Hz, 2H), 3.53 (t, J = 6.0 Hz, 2H), 1.46–1.58 (m, 22H), 1.27–1.32 (m, 2H), 0.88 (s, 9H), 0.02 (s, 6H). TBAF (1.0 M in THF, 2.60 mL, 2.60 mmol) was added slowly to a stirred solution of the crude TBS ether (720 mg, 1.73 mmol) obtained as above in THF (5 mL) at room temperature. The resulting solution was stirred at room temperature for 4h. Then Et_2O (100 mL) was added to dilute the reaction mixture, which was further washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over Na_2SO_4, filtered, concentrated and the residue was subjected to flash column chromatography, eluting with n-hexane–EtOAc (4:1), yielding a colorless oil (397 mg, 72% in three steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (t, J = 6.6 Hz, 2H), 3.54 (t, J = 7.5 Hz, 2H), 1.55–1.60 (m, 4H), 1.48 (s, 18H), 1.32–1.37 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.8 (2C), 82.1 (2C), 62.8, 46.2, 32.3, 28.7, 28.1 (6C), 22.9; IR (film) 3470, 2979, 2935, 2866, 1780, 1732, 1696, 1457, 1368, 1140, 856 cm^{-1}; ESIMS m/z (rel. intensity) 629 (100, $2MNa^+$), 326 (44, MNa^+). Anal. Calcd for $C_{15}H_{29}NO_5$: C, 59.38; H, 9.63; N, 4.62. Found: C, 59.62; H, 9.47; N, 4.52.

4.20. Di-*tert*-butyl-(4-oxo-butyl) dicarbamate (30)

Prepared from alcohol **28** (350 mg, 1.21 mmol) following the procedure for **19**. The column was eluted with n-hexane–EtOAc (4:1), yielding a colorless oil (347 mg, 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (t, J = 1.5 Hz, 1H), 3.57 (t, J = 7.2 Hz, 2H), 2.42 (td, J = 7.2, 1.5 Hz, 2H), 1.85 (quin, J = 6.9 Hz, 2H), 1.46 (s, 18H); ESIMS m/z (rel. intensity) 310 (100, MNa^+). Anal. Calcd for $C_{14}H_{25}NO_5$: C, 58.52; H, 8.77; N, 4.87. Found: C, 58.52; H, 8.56; N, 4.88.

4.21. *N*,*N*-Di(*tert*-butoxycarbonyl)-5-amino-1-pentanal (31)

Prepared from alcohol **29** (318 mg, 1.05 mmol) following the procedure for **19**. The column was eluted with n-hexane–EtOAc (4:1), yielding a colorless oil (310 mg, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (br s, 1H), 3.56 (t, J = 6.9 Hz, 2H), 2.43 (t, J = 6.3 Hz, 2H), 1.41–1.69 (m, 22H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 152.7 (2C), 82.3 (2C), 45.8, 43.5, 28.4, 28.1 (6C), 19.2; IR (film) 2980, 2936, 1788, 1728, 1696, 1394, 1368, 1146, 1122, 857 cm^{-1}; ESIMS m/z (rel. intensity) 340 (100, MK^+), 324 (81, MNa^+). Anal. Calcd for $C_{15}H_{27}NO_5$: C,

59.78; H, 9.03; N, 4.65. Found: C, 59.90; H, 8.91; N, 4.44.

4.22. *tert*-Butyl Methyl-(3-oxo-propyl) carbamate (33)

Prepared from alcohol **32**[29] (270 mg, 1.4 mmol) following the procedure for **19**. The column was eluted with *n*-hexane–ethyl acetate (10:1), yielding a colorless oil (255 mg, 96%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (t, J = 1.5 Hz, 1H), 3.47 (t, J = 6.3 Hz, 2H), 2.79 (s, 3H), 2.60 (td, J = 6.6, 1.5 Hz, 2H), 1.37 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 155.5, 79.7, 42.7, 34.5, 31.5, 28.3 (3C); IR (film) 3429, 2977, 2933, 1724, 1694, 1482, 1393, 1367, 1176, 1062, 878, 774 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 210 (100, MNa^+).

4.23. *tert*-Butyl-(3-*tert*-butoxycarbonylamino-propyl)-(2-oxo-ethyl)carbamic acid (35)

Prepared from alcohol **34**[30] (636 mg, 2.0 mmol) following the procedure for **19**. The column was eluted with $CHCl_3$–MeOH (20:1), yielding a colorless oil (621 mg, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (s, 1H), 3.81 (s, 2H), 3.27 (t, J = 6.3 Hz, 2H), 3.06 (q, J = 6.3 Hz, 2H), 1.56 (quin, J = 6.6 Hz, 2H), 1.39 (s, 9H), 1.36 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) 199.0, 156.5, 155.9, 81.3, 79.4, 58.1, 53.0, 46.1, 37.6, 28.8 (3C), 28.5 (3C); ESIMS *m*/*z* (rel. intensity) 315 (100, $M-H^+$).

4.24. *tert*-Butyl (3-*tert*-butoxycarbonylamino-propyl)-(3-hydroxy-propyl)carbamic acid (37)

Aldehyde **36**[5] (162.2 mg, 0.94 mmol) and 3-amino-1-propanol (70.3 mg, 0.94 mmol) were dissolved in methanol (8 mL). To this stirred solution was added acetic acid (2.5 mL) and $NaBH_3CN$ (88.6 mg, 1.41 mmol). The resulting mixture was stirred at room temperature for 5 h. The reaction mixture was cooled to 0 °C and $NaHCO_3$ powder was added to neutralize the acetic acid. The precipitate was filtered out and the resulting filtrate was evaporated in vacuo yielding a crude product, which was used for next operation without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) 3.77 (t, J = 5.4 Hz, 2H), 3.15 (t, J = 6.6 Hz, 2H), 2.82 (t, J = 5.7 Hz, 2H), 2.62 (t, J = 6.9 Hz, 2H), 1.58–1.70 (m, 4H), 1.42 (s, 9H). Et_3N (245 μL, 1.76 mmol) and Boc_2O (349 mg, 1.6 mmol) were added sequentially to a stirred solution of amino alcohol obtained as above in $CHCl_3$ (10 mL) at room temperature. The resulting mixture was stirred at room temperature for 24 h. The solvent was evaporated in vacuo and the residue was subjected to flash column chromatography, eluting with $CHCl_3$–MeOH (20:1), giving a colorless oil (234 mg, 75% in two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (br s, 2H), 3.33 (br s, 2H), 3.18 (br s, 2H), 3.09 (br s, 2H), 1.60–1.72 (m, 4H), 1.44 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 155.9, 80.5, 79.2, 58.2, 44.2, 42.2, 37.7, 30.4, 28.8, 28.4 (6C); IR (film) ν 3356, 2976, 2932, 1674, 1520, 1480, 1420, 1367, 1251, 1165, 866 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 687 (100, $2MNa^+$), 355 (23, MNa^+), 333 (68, MH^+).

4.25. *tert*-Butyl (3-*tert*-butoxycarbonylamino-propyl)-(3-oxo-propyl)carbamic acid (38)[31]

PDC (473 mg, 1.26 mmol) was added to a stirred solution of alcohol **37** (208 mg, 0.63 mmol) in CH_2Cl_2 (4 mL) at room temperature. The resulting mixture was stirred at room temperature for 24 h. Evaporation of the solvent in vacuo resulted in a residue, which was subjected to flash column chromatography, eluting with $CHCl_3$–MeOH (50:1) yielding a colorless oil (132 mg, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (br s, 1H), 3.45 (t, J = 6.6 Hz, 2H), 3.24 (br s, 2H), 3.06 (br s, 2H), 2.67 (t, J = 6.3 Hz, 2H), 1.63–1.67 (m, 2H), 1.42 (s, 9H), 1.40 (s, 9H); IR (film) ν 3360, 2976, 2933, 1693, 1516, 1479, 1366, 1250, 1171, 864 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 683 (100, $2MNa^+$).

4.26. *tert*-Butyl-[3-(methoxy-methyl-carbomoyl)-propyl]-methyl-carbamic acid (40)

Et_3N (369 μL, 2.65 mmol) was added to a stirred solution of acid **39**[32] (575 mg, 2.65 mmol) in CH_2Cl_2 (12 mL) at room temperature. Then BOP reagent (1.17 g, 2.65 mmol) was added. After 5 min, *N*,*O*-dimethylhydroxylamine hydrochloride (284 mg, 2.91 mmol) and an additional portion of Et_3N (406 μL, 2.91 mmol) were added sequentially at room temperature. The reaction mixture was stirred at room temperature for 1 h. Then CH_2Cl_2 (100 mL) was added to dilute the reaction mixture, which was then washed with 1 N HCl (2 × 10 mL), saturated solution of $NaHCO_3$ (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over Na_2SO_4, filtered, concentrated, and the residue was subjected to flash column chromatography, eluting with *n*-hexane–EtOAc (2:1) providing a colorless oil (563 mg, 82%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (s, 3H), 3.20 (t, J = 6.9 Hz, 2H), 3.10 (s, 3H), 2.78 (s, 3H), 2.35 (t, J = 7.5 Hz, 2H), 1.77 (quin, J = 7.2 Hz, 2H), 1.38 (s, 9H); ESIMS *m*/*z* (rel. intensity) 283 (100, MNa^+). Anal. Calcd for $C_{12}H_{24}N_2O_4$: C, 55.36; H, 9.29; N, 10.76. Found: C, 55.34; H, 9.57; N, 10.64.

4.27. *tert*-Butyl-methyl-(4-oxo-butyl)-carbamic acid (41)

$LiAlH_4$ (47 mg, 1.25 mmol) was added at room temperature to a stirred solution of Weinreb amide **40** (264 mg, 1.0 mmol) in THF (10 mL). The resulting mixture was stirred at room temperature for an additional 1 h. The reaction mixture was then cooled to 0 °C and HCl (1 N, 10 mL) was added to hydrolyze the intermediate complex. Et_2O (100 mL) was added and the organic layer was separated, which was further washed with 1 N HCl (2 × 10 mL), saturated aqueous solution of $NaHCO_3$ (2 × 10 mL), water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered, concentrated, and the residue was subjected to flash column chromatography, eluting with *n*-hexane–EtOAc (4:1), yielding a colorless oil (125 mg, 61%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (s, 1H), 3.21 (t, J = 7.2 Hz, 2H), 2.80 (s, 3H), 2.42 (t, J = 7.2 Hz, 2H), 1.80 (quin, J = 7.2 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6, 155.7, 79.4, 47.7, 40.9, 34.0, 28.4 (3C), 20.2; IR (film) ν 2976, 2933,

1725, 1693, 1482, 1394, 1172, 879, 773 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 224 (100, MNa^+).

4.28. *N*-Methoxy-*N*′-methyl-2,3-di(*tert*-butoxycarbonylamino)propionic amide (43)

Prepared from acid **42**[33] (600 mg, 1.97 mmol) following the procedure for **40**. The column was eluted with *n*-hexane–EtOAc (2:1), providing a colorless oil, which became a white solid upon standing (685 mg, 100%): mp 78–79 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 5.49 (br s, 1H), 4.85 (br s, 1H), 4.70 (br s, 1H), 3.75 (s, 3H), 3.37–3.52 (m, 2H), 3.18 (s, 3H), 1.42 (s, 9H), 1.39 (s, 9H); ESIMS *m*/*z* (rel. intensity) 370 (100, MNa^+), 348 (43, MH^+). Anal. Calcd for $C_{15}H_{29}N_3O_6$: C, 51.96; H, 8.41; N, 12.10. Found: C, 51.53; H, 8.56; N, 11.74.

4.29. 2,3-Di(*tert*-butoxycarbonylamino)propanal (44)

Prepared from Weinreb amide **43** (690 mg, 1.99 mmol) following the procedure for **41**. The column was eluted with *n*-hexane–EtOAc (3:1), yielding a colorless oil, which gave a white solid (463 mg, 81%) upon standing: mp 51–52 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 9.52 (s, 1H), 5.74 (br s, 1H), 5.22 (br s, 1H), 4.14 (br s, 1H), 3.37–3.57 (m, 2H), 1.34 (s, 9H), 1.31 (s, 9H); IR (film) 3356, 2979, 2934, 1706, 1517, 1368, 1252, 1167, 1064, 783 cm^{-1}; ESIMS *m*/*z* (rel. intensity) 599 (100, $2MNa^+$), 311 (38, MNa^+). Anal. Calcd for $C_{13}H_{24}N_2O_5$: C, 54.15; H, 8.39; N, 9.72. Found: C, 54.08; H, 8.47; N, 9.53.

4.30. *N*-Methyl-*N*′-methoxy-2(*S*),6-bis-(*tert*-butoxycarbonylamino)hexamide (46)

Prepared from acid **45** (1.0 g, 2.89 mmol) following the procedure for **40**. The column was eluted with $CHCl_3$–MeOH (5:1) to afford a colorless oil (1.05 g, 94%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.33 (q, $J = 5.7$ Hz, 1H), 4.81 (br s, 1H), 4.57 (br s, 1H), 3.68 (s, 3H), 3.11 (s, 3H), 2.99 (q, $J = 6.0$ Hz, 2H), 1.25–1.42 (m, 24H); ESIMS *m*/*z* (rel. intensity) 412 (100, MNa^+). Anal. Calcd for $C_{18}H_{35}N_3O_6 \cdot 0.25CHCl_3$: C, 52.27; H, 8.47; N, 10.02. Found: C, 52.27; H, 8.45; N, 10.04.

4.31. 2(*S*),6-Bis-(*tert*-butoxycarbonylamino)hexanal (47)

Prepared from Weinreb amide **46** (1.05 g, 2.7 mmol) following the procedure for **41**. The column was eluted with $CHCl_3$–MeOH (10:1), yielding a colorless oil (490 mg, 55%): ^1H NMR (300 MHz, $CDCl_3$) δ 9.49 (s, 1H), 5.30 (q, $J = 5.7$ Hz, 1H), 4.69 (br s, 1H), 4.08 (br s, 1H), 3.04 (q, $J = 6.0$ Hz, 2H), 1.32–1.58 (m, 24H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.8, 156.6, 156.2, 80.4, 79.5, 60.0, 40.1, 30.1, 28.8 (3C), 28.7 (3C), 22.6, 18.7; ESIMS *m*/*z* (rel. intensity) 353 (100, MNa^+). Anal. Calcd for $C_{16}H_{30}N_2O_5 \cdot 0.25CHCl_3$: C, 54.18; H, 8.46; N, 7.78. Found: C, 54.48; H, 8.20; N, 7.85.

4.32. Topoisomerase I-mediated DNA cleavage reactions

Human recombinant top1 was purified from Baculovirus as described previously.[34] 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 [α-^{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 unit 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).[6] 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% polyacrylamine, 7 M urea). Gels were dried and visualized by using a Phosphoimager and IMAGEQUANT software (Molecular Dynamics, Sunnyvale, CA). The strongest cleavage band produced by 1 μM CPT was semiquantified as ++++.

4.33. Molecular modeling

The structure of the ternary complex, containing topoisomerase I, DNA, and topotecan, was downloaded from the Protein Data Bank (PDB code 1K4T).[4] One molecule of PEG and the topotecan carboxylate form were deleted. All of the atoms were then fixed according to the SYBYL atom types. Hydrogens were added and minimized using MMFF94s force field and MMFF94 charges. The structure of the indenoisoquinoline **4**, 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[5] in the ternary complex, and the structure of topotecan was then deleted. The new whole complex was subsequently subjected to energy minimization using MMFF94s force field with MMFF94 charges. During the energy minimization, the structure of the indenoisoquinoline was allowed to move, while the structures of the protein, nucleic acids, and the surrounding water molecules were frozen. The energy minimization was performed using the Powell method with a 0.05 kcal/mol Å energy gradient convergence criterion and a distance-dependent dielectric function.

Molecular surfaces were created using the MOLCAD module implemented in SYBYL 6.9 with MMFF94 charges.

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