Synthesis and Cytotoxic Activity of Polyamine Analogues of Camptothecin

Sabrina Dallavalle,*[,†] Giuseppe Giannini,*[,‡] Domenico Alloatti,[‡] Andrea Casati,[†] Elena Marastoni,[‡] Loana Musso,[†] Lucio Merlini,[†] Gabriella Morini,[†] Sergio Penco,[†] Claudio Pisano,[‡] Stella Tinelli,[§] Michelandrea De Cesare,[§] Giovanni Luca Beretta,[§] and Franco Zunino[§]

Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Via Celoria 2, 20133 Milano, Italy, Sigma-tau, R&D, Via Pontina km 30,400, 00040 Pomezia, Italy, and Unità Operativa "Chemioterapia e Farmacologia Antitumorale Preclinica", Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milano, Italy

Received March 14, 2006

A number of derivatives of camptothecin with a polyamine chain linked to position 7 of camptothecin via an amino, imino, or oxyiminomethyl group were synthesized and tested for their biological activity. All compounds showed marked growth inhibitory activity against the H460 human lung carcinoma cell line. In particular, the iminomethyl derivatives where the amino groups of the chain were protected with Boc groups exhibited a high potency, with IC_{50} values of $\sim 10^{-8}$ M. The pattern of DNA cleavage in vitro and the persistence of the cleavable ternary complex drug−DNA−topoisomerase I observed with polyamine conjugates containing free amino groups support a contribution of specific drug interaction with DNA as a determinant of activity. Modeling of compound **7c** in the complex with topoisomerase 1 and DNA is consistent with this hypothesis. The lack of a specific correlation between stabilization of the cleavable complex and growth inhibition likely reflects multiple factors including the cellular pharmacokinetic behavior related to the variable lipophilicity of the conjugate, and the nature and linkage of the polyamine moiety.

Introduction

DNA is recognized as the primary target for several clinically effective antitumor agents. Drug-induced DNA damage or alterations of critical DNA functions (e.g., DNA transcription or replication) inhibit cell proliferation and induce cell death. Despite their relative nonselectivity, DNA-damaging agents remain the cornerstone of antitumor chemotherapy. Chemical efforts to improve the selectivity and the efficacy of DNA-interactive drugs have focused on approaches to increase DNA binding affinity or tumor cell selectivity by modification with a targeting moiety.[1]

Among the many ways so far devised for these aims, conjugation of a cytotoxic drug to polyamines appears to be an attractive possibility. The naturally occurring polyamines, such as putrescine, spermidine, and spermine, are ubiquitous cellular cations that play multifunctional roles in cell growth and differentiation.[2,3]

These polyamines bind to polyanions and to proteins with anionic binding sites. Their balanced hydrophilicity/hydrophobicity ratio allows them to perform physiological functions without impairing the functionality of other structures.[4] In eukaryotic cells, there are specific active transport systems that allow uptake and release of polyamines.[5] These systems seem to be more active in rapidly proliferating cells than in resting cells,[6] and the uptake of polyamines by tumor tissues is larger than in other tissues.[7] Polyammonium salts have a high DNA-binding ability through electrostatic interactions in an essentially non-sequence-specific manner.[8] Recently, Lindemose and co-workers have provided the first evidence for the sequence-specific binding of polyamines to DNA, demonstrating that polyamines at submillimolar concentrations bind preferentially to bent adenine tracts in double-stranded DNA.[9] Importantly, it has been shown that, while constrained to remain close to DNA, the polyammonium cations retain a high degree of freedom of motion along the polyanion backbone.[10] Thus, conjugation of a drug to a polyammonium cation will confer a high DNA affinity but the mobility will allow drugs with high specificity to locate appropriate sites of DNA.

The literature reports successful examples of polyamine conjugates with cytotoxic drugs, such as chlorambucil,[11−13] nitroimidazole,[14] aziridines,[15−17] acridine,[18] and enediynes,[19] that gave enhanced cytotoxicity and/or increased drug selectivity for tumor cells.

Camptothecin (CPT) and its analogues are strong inhibitors of topoisomerase I (top1), a nuclear enzyme that plays a role in the uncoiling, relaxation, and repair of DNA.[20] Two analogues of CPT, topotecan and irinotecan, are already used in clinical practice as antitumor drugs, whereas a number of other analogues are at an advanced stage of development.[21]

It is well established that CPTs target top1 by binding to a covalent top1−DNA complex, giving a ternary complex called "cleavable complex".[22] Because CPTs bind more efficiently to the enzyme−DNA complex than to DNA alone, the introduction of a chain that confers affinity to the DNA could improve the stabilization of the ternary complex and/or modify the pattern of cleavage. The aim of the present study was to explore the possibility of modulating the biological activity of CPTs by tethering the drug to a polyamine chain. An increased affinity and/or specificity is expected by drug location at appropriate sites on DNA[23,24] and may therefore have influence on the pharmacological activity.

We have recently reported on the synthesis and the preclinical activity of a series of imino[25] and oxyimino[26] derivatives of CPT-7-aldehyde. In this paper we report on the synthesis, biological activity, and the evaluation of the inhibition of top1 of a series of CPT derivatives substituted with a polyamine chain linked to position 7 of CPT via an amino, imino, or oxyiminomethyl group. To the best of our knowledge, the conjugation of a polyamine to CPT has not been attempted before.

* To whom correspondence should be addressed. For S.D.: phone, +39 0250316816; fax, +39 0250316801; e-mail, sabrina.dallavalle@unimi.it. For G.G.: phone, +39 0691393640; fax, +39 0691393638; e-mail, giuseppe.giannini@sigma-tau.it.

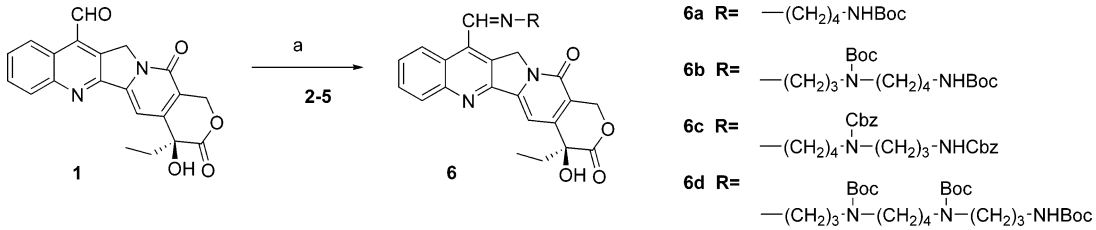
† Università di Milano.

‡ Sigma-tau, R&D.

§ Istituto Nazionale dei Tumori.

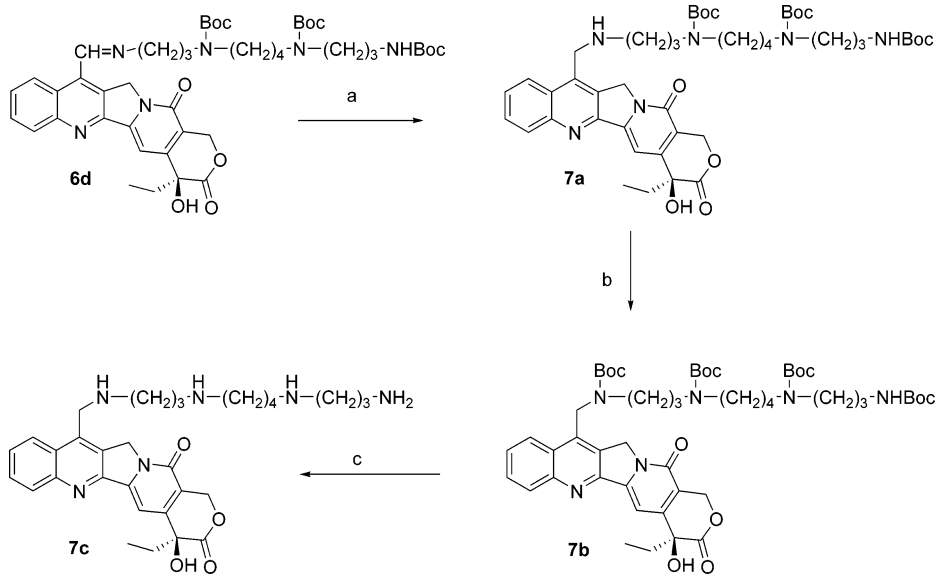
10.1021/jm060285b CCC: $33.50
Published on Web 07/29/2006

Scheme 1[a]



[a] Reagents: (a) $Yb(OTf)_3$, CH_2Cl_2, room temp.

Scheme 2[a]



[a] Reagents: (a) Pd/C, dry methanol, H_2 (20 psi), room temp, 1 h, 50%; (b) $(Boc)_2O$, TEA, CH_2Cl_2, room temp, 16 h, 80%; (c) HCl (gas), CH_2Cl_2, 82%.

Chemistry

The starting material for all the synthesized compounds was camptothecin-7-aldehyde (**1**),[27] a versatile precursor of the imino and oxyimino derivatives of camptothecin.

We first prepared a series of imines (**6**) by condensation of suitably protected polyamines such as putrescine, spermidine, and spermine with **1** in the presence of ytterbium triflate as previously described[25] (Scheme 1). N^1,N^2,N^3-tri-Boc-spermine (**2**) was prepared by monotrifluoroacetylation of spermine at the primary NH_2 group followed by reaction with *tert*-butyl dicarbonate as described.[28] Application of the same method to the unsymmetrical spermidine produced N^2,N^3-di-Boc-spermidine (**3**),[29] whereas the same reaction with dibenzyl dicarbonate unexpectedly gave N^1,N^2-di-Z-spermidine (**4**).[30] *N*-Boc-putrescine (**5**) was commercially available.

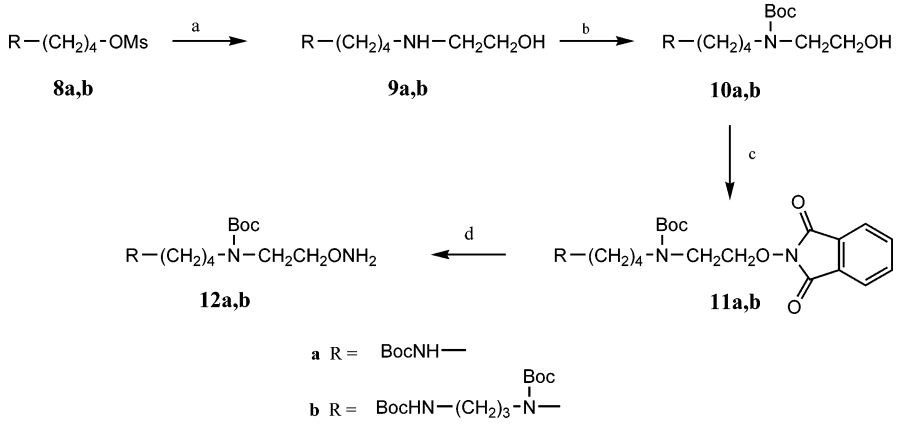
To obtain the corresponding free polyamine derivatives, we needed to deprotect **6**, but unfortunately treatment with trifluoroacetic acid (TFA) gave unstable products that readily decomposed so that the free imines could not be obtained. Hydrogenation of the imino group of the tri-Boc derivative **6d** with Pd/C produced the corresponding amine **7a**, which was converted into the tetra-Boc derivative **7b**. This was, in turn, deprotected with TFA to give the amine **7c** (Scheme 2).

Oximes were obtained by condensation of **1** with the appropriate alkoxyamines using a previously described procedure.[26] We reasoned that the presence of the alkoxyimino oxygen atom required shortening of the carbon chain in order to maintain the same overall length of the polyamine moiety. The synthesis of the analogues of **6b** and **6d** (containing spermine and spermidine moieties) required stepwise synthesis of the intermediate *O*-alkylhydroxylamines **12a**,**b** (Scheme 3). Substitution of the mesylates **8a**[31] and **8b**[32] with ethanolamine produced the amino alcohols **9**, which were first protected with a Boc group (**10**), then subjected to a Mitsunobu reaction with *N*-hydroxyphthalimide to give the phthalimides **11**. Hydrazinolysis of **11** produced the alkoxyamines **12**. The oximes **13a** and **13b** were prepared by treatment with $(Boc)_2O$ of the corresponding free amino derivatives **14a** and **14b**, which were synthesized as previously reported.[26] The oximes **14c** and **14d** were obtained from **13c** and **13d** with TFA (Scheme 4).

Results

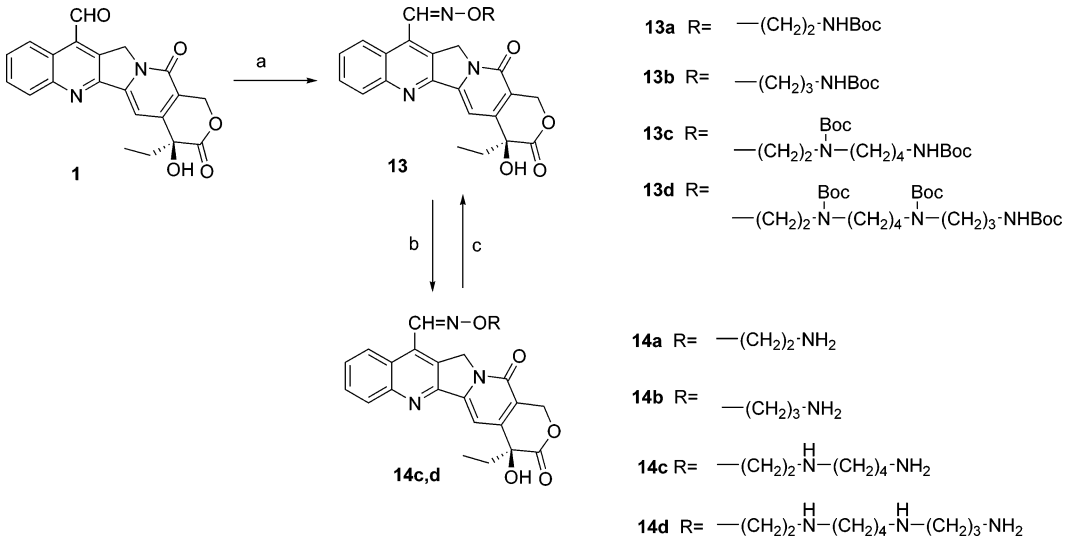
Cellular Pharmacology Studies. The prepared compounds were tested for their growth inhibitory activity against the human non-small-cell lung carcinoma cell line H460 (Table 1), a cell model selected for its sensitivity to top1 inhibitors.[33] Topotecan, one of the two CPT drugs currently in clinical practice, and SN38, the active metabolite of irinotecan, were used as reference compounds. The cellular pharmacology study revealed that, except for **7b**, all compounds exhibited potent growth inhibitory activity similar or superior to that of topotecan. IC_{50} values were strongly related to the nature of the substituent in position 7 of the CPT. The most potent compounds were the imino derivatives (**6a**−**d**). Compound **6a**, carrying *N*-Boc-putrescine linked via the imino group, was less potent than imino derivatives with N-protected spermidine (**6b** and **6c)** and spermine (**6d**). When compared to imino compounds (**6**), amino (**7**) and oxyimino derivatives (**13**, **14**) showed a general reduction in growth inhibitory potency. Compound **7a**, with a tri-Boc-substituted chain, displayed the same inhibitory effect on cell growth as the free polyamine **7c**. The introduction of another bulky

Scheme 3[a]



[a] Reagents: (a) 2-aminoethanol, 80 °C, 4 h; (b) Boc_2O, TEA, CH_2Cl_2, room temp, 4 h; or Boc_2O, dioxane/water, Na_2CO_3, room temp, 2 h; (c) *N*-hydroxyphthalimide, PPh_3, DIAD, THF, room temp, 5 h; (d) hydrazine, 70 °C, 5 h.

Scheme 4[a]



[a] Reagents: (a) **12a**,**b**, EtOH, reflux; (b) TFA, CH_2Cl_2; (c) $(Boc)_2O$, TEA, THF, room temp, 30 h.

Table 1. In Vitro Growth Inhibitory Activity (IC_{50})[a] of Polyamino Derivatives on H460 Cell Line

compd	H460 IC_{50} (μM)	compd	H460 IC_{50} (μM)
topotecan	1.38 ± 0.95	**13a**	0.084 ± 0.014
SN 38	0.08 ± 0.05	**13b**	0.3 ± 0.1
6a	0.68 ± 0.358	**13c**	1.15 ± 0.38
6b	0.015 ± 0.002	**13d**	1.06 ± 0.79
6c	0.039 ± 0.003	**14a**	0.49 ± 0.014[24]
6d	0.029 ± 0.008	**14b**	1.77 ± 0.3
7a	1.4 ± 0.1	**14c**	1.16 ± 0.29
7b	3.27 ± 1.83	**14d**	1.07 ± 0.84
7c	1.43 ± 0.673		

[a] Concentration required for 50% reduction of cell growth compared with untreated controls after 1 h of exposure to the drug. Mean ± SD values are reported from at least three experiments.

substituent on the nitrogen of the remaining free amino group (**7b**) resulted in reduction of the inhibitory activity. Like for the amino compounds, the activity of oxyiminopolyamine derivatives did not substantially change in relation to the presence or absence of substituents on the amino groups of the polyamine chain (**13c** vs **14c**; **13d** vs **14d**). Interestingly, among the oxyimino compounds, the most active were found to be the short-chain compounds **13a**, **13b**, and **14a**.

Topoisomerase I Mediated DNA Cleavage. Top1-mediated DNA cleavage experiments were used to investigate the ability of some selected compounds to induce DNA damage. For this purpose, purified human top1 was used. SN38 was used as the reference compound. Except for **7a** and **7b**, the tested compounds showed an intensity of DNA damage similar to that of SN38 (Figure 1). As indicated by the cleavage pattern, compounds **7c**, **14c**, and **14d** displayed a higher intensity at some cleavage sites. The densitometric analysis revealed that compound **7c** exhibited a 2-fold increased intensity for one site as indicated in Figure 1. Interestingly, we observed a general reduction for the other sites. No change was observed for compound **6d**. The site selectivity observed for compound **7c** was also evident for compound **14d** (not shown). Sequence analysis of these sites revealed a guanine in position +1. The stabilization of the ternary cleavable complex was evaluated after the addition of a high salt concentration (0.6 M NaCl), which favors the dissociation of the ternary drug−enzyme−DNA complex (Figure 2). The densitometric analysis of the sites indicated in Figure 2A revealed that the complexes with N-unsubstituted polyamines, **7c**, **14c**, and **14d**, were more stable and gave a persistence of DNA damage of around 60−80% after 10 min.

The stabilization of the ternary complex can be related to a stronger interaction of DNA with compounds with a positively charged polyamino chain (**7c**, **14a**−**d**) with respect to the

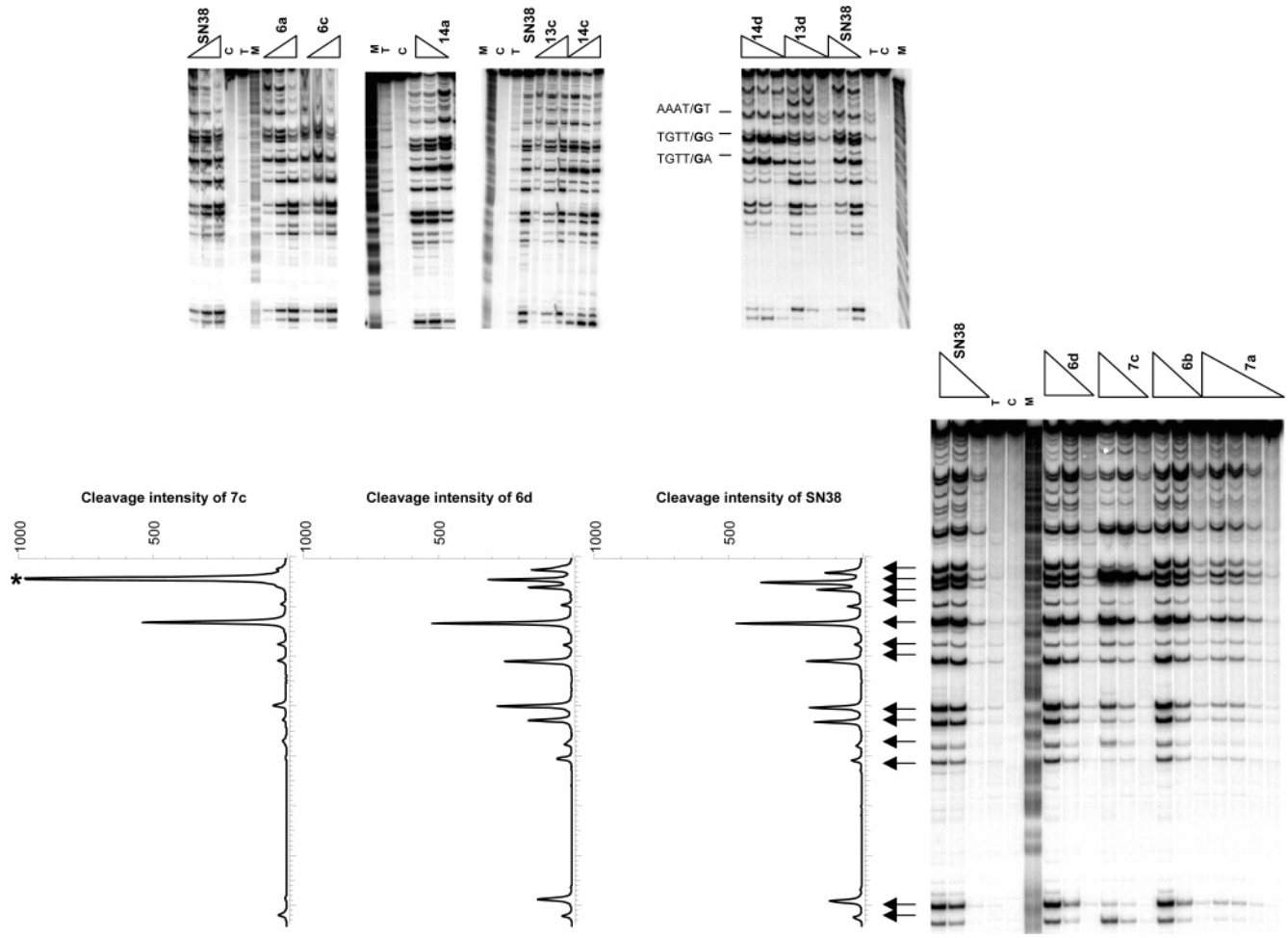


Figure 1. Topoisomerase I mediated DNA cleavage by SN38 and camptothecin analogues. Samples were reacted with 1, 10, and 50 μM drug (also 100 μM for compound **7a**) at 37 °C for 30 min. Reaction was stopped by adding 1% SDS and 0.3 mg/mL of proteinase K and incubating for 45 min at 42 °C before loading on a denaturating 8% polyacrylamide gel: C, control DNA; T, reaction mixture with enzyme without drug; M, purine markers. A graphical comparison of DNA cleavage intensity for compounds **7c**, **6d**, and SN38 at 50 μM is reported. Arrows indicate the sites analyzed.

standard SN38 and the compounds with protected amino groups (Figure 2). To support this hypothesis, a model of compound **7c** in the top1−DNA complex was constructed by overlapping the minimized structure of this compound with the structure of topotecan in the published crystal structure[34] of the ternary complex. The structure of topotecan was then removed, and the energy of the new complex was minimized. According to this model, the side chain is directed out of the duplex into a zone where there is no steric hindrance. The amino groups of the side chain are within interaction distance with the nucleic acid residues T-9, T-10, and C-12 (Figure 3).

This interpretation was also supported by comparison of migration in agarose gel of SV40 DNA plasmid in the presence of some CPTs. As reported in Figure 4, compound **7c** revealed a dose-dependent reduced migration in comparison to compound **7a** and SN38, which displayed a gel migration identical to that of the control.

Discussion

As already observed in our previous work,[25,26] the results reported in this study provide further evidence that modification at the 7 position of the CPT is a promising approach to the development of effective derivatives. It is clear that the nature of the substituent is critical in achieving an optimal cytotoxic effect and that lipophilicity may play an important role in promoting rapid intracellular drug accumulation.[24] However, from the present results it appears that the lipophilic nature of the conjugate is not the only factor accounting for the cell growth inhibition produced by the tested derivatives. Indeed, the structure of the linker joining the polyamine with the CPT is an important determinant of the growth inhibitory effect. The marked activity observed for imino derivatives compared to amino and oxyimino polyamines confirms this hypothesis. It is well-known that pharmacokinetic behavior at the cellular level is a critical aspect of the antitumor efficacy of CPTs because, as a consequence of the potential reversibility of the cleavable complex, adequate intracellular concentrations and exposure are required to produce a lethal extent of DNA damage. Moreover, the subcellular distribution could play an important role because it may affect the drug−target interaction. Finally, the persistence of the cleavable complex is expected to be a critical event resulting in lethal lesions during DNA replication. The increase in drug binding affinity could therefore provide favorable stabilization of the ternary drug−enzyme−DNA complex.

The data of Table 1 show that the insertion of a free polyamine chain at position 7 of CPT did not result in increased growth inhibitory potency (e.g., **7c** and **14c**,**d**) with respect to the reference compounds, notwithstanding that these compounds were effective inhibitors of top1 in isolated systems and resulted in increased stabilization of the cleavable complex (Figures 1 and 2). This could indicate limited penetration into the intracellular compartments. The increase in lipophilicity due to the

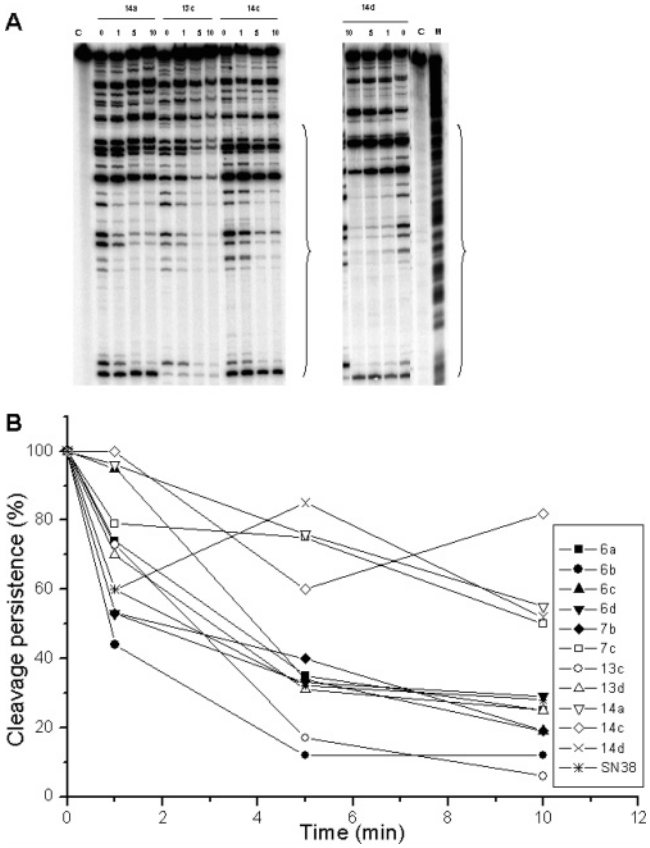


Figure 2. Persistence of topoisomerase I mediated DNA cleavage in the presence of SN38 and camptothecin analogues: (A) DNA damage reversion of compounds **14a**, **14c**, **14d**, and **13c**. Samples were reacted for 30 min with 10 μM drug. DNA cleavage was then reversed by adding 0.6 M NaCl. The 100% value is referenced to the extent of DNA cleavage at 30 min of incubation: C, control DNA; M, purine markers. (B) Time course analysis of cleavage persistence in the presence of SN38 and camptothecin analogues. Each value was obtained by densitometric analysis of the sites indicated by braces in part A. Experiments were repeated two to three times, and a representative value is reported.

presence of the protecting groups (see compounds **7a**, **7b**, **13c**, **13d**) should favor penetration into the cells, but this possible favorable effect could be counterbalanced by the increase in steric hindrance at the target level due to the length and the bulkiness of the chain. Similar effects have been observed in the series of oxyimino compounds reported previously[26] and again appear here, where the short-chain compounds **13a** and **14a** are more active than similar compounds with longer chains. On this basis, the decrease in activity from **7a** to **7b** is not surprising. A different behavior is shown by the imino derivatives, which are much more active than the corresponding amines and oximes. The structural differences are so small that it is difficult to assume that they account for the strong difference in activity, considering also the comparable inhibitory effect on top1. A possible explanation for this behavior could be the lower stability in hydrolytic conditions of imino compared to oxyimino linkage. The lipophilic moiety of compounds **6a**−**d** could promote a rapid uptake across cellular membranes. Once accumulated in the intracellular compartment, these compounds could behave as prodrugs, releasing CPT-7-aldehyde, a compound endowed with good activity,[35] after hydrolysis because of the lower pH in some compartments of the tumor cell, such as lysosomes. To confirm this hypothesis, we carried out tests on the stability of imino derivatives **6a** and **6d** in buffered solutions at different pH values. After 3 h at pH 5.0 compounds

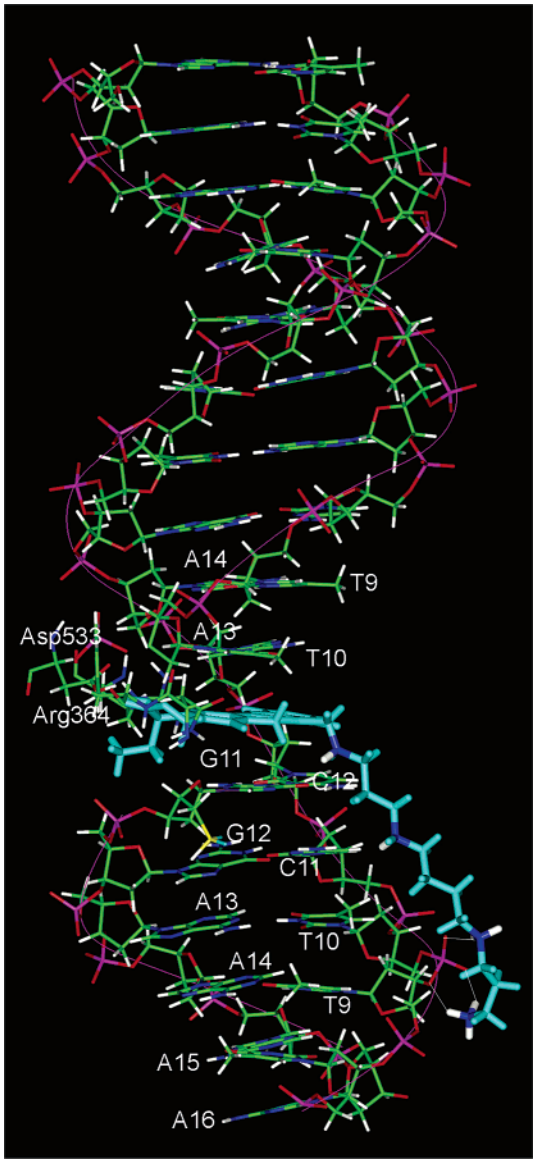


Figure 3. Structure of compound **7c** in the ternary complex. For the sake of clarity all the base pairs and only a few amino acids are shown.

6a and **6d** were almost completely converted into CPT-7-aldehyde (conversion was monitored by HPLC analysis; for details see Experimental Section). At pH 6.0 the amount of aldehyde with respect to the initial imino derivatives was about 20% after 24 h, whereas at pH 6.8 the imino derivatives appeared remarkably stable. This hypothesis is consistent with the data for the other derivatives, in particular with the lower activity of the oxyimino compounds, which are more stable in acid conditions.

The top1−DNA cleavage experiments showed that although the DNA cleavage pattern for the CPTs carrying N-unsubstituted polyamines in position 7 (**7c**, **14c** and **14d**) demonstrated a sequence specificity similar to that of other CPTs, specific cleavage sites were preferred. This feature was observed for amino and oxyimino derivatives. Among the sites observed for N-unsubstituted derivatives, the highest intensity was found for the site carrying guanine in positions +1 and +2. This feature could reflect a role of the polyamine chain as a DNA sequence selector.

The N-unsubstituted polyamines also revealed long-lasting stabilization of the ternary complex. Indeed, the persistence of DNA damage was found to be around 60−80% under conditions

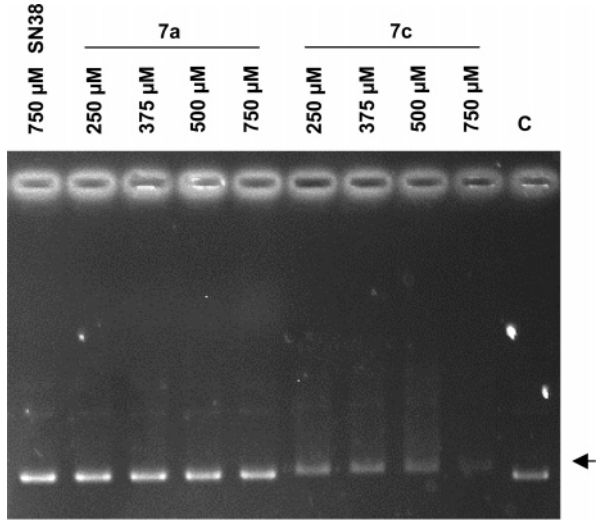


Figure 4. DNA binding of compounds **7c**, **7a**, and SN38. DNA and CPT analogues were incubated in 20 μL of phosphate buffer saline for 1 h at 37 °C in the presence of 200 ng of plasmid and the indicated concentrations of compounds. Samples were analyzed on 0.7% agarose gel, followed by ethidium bromide staining. The arrow indicates the reduced migration of compound **7c**.

favoring dissociation of the ternary complex. The stability of the ternary complex is likely the result of an increase of drug affinity for DNA by electrostatic attraction of the positively charged polyamine to the DNA in the cleavable complex, as supported by modeling studies and comparison of patterns of plasmid DNA migration (see above). This interaction could also be maintained after introduction of DNA strand breaks and partial local modification of DNA conformation, as shown by the crystal structure of top1 in its covalent complex with DNA, indicating that no marked structural changes in the DNA had occurred.[34]

The lack of a correlation between the cell growth inhibition and top1 inhibition of the derivatives tested suggests that differences in cellular uptake, subcellular distribution, and additional biological targets may all play important roles in determining antitumor activity. A similar finding was reported previously with anthracene and acridine polyamine conjugates and topoisomerase II inhibition.[36] These results support the concept that optimal drug efficacy can be achieved as a balance between cellular pharmacokinetics and intrinsic ability to induce DNA cleavage.

One of the most potent derivatives in cell growth inhibition assay, **6d**, was evaluated in vivo against a human gastric carcinoma cell line, MKN-28, grown as tumor xenograft. The compound was found to be very potent; low dose levels (1−4 mg/kg) were effective in tumor growth inhibition, with maximum inhibition of around 70% (Table 2). Although topotecan at optimal doses had superior efficacy, compound **6d** was also well tolerated at the tested doses. On the basis of this tolerability, optimization of the antitumor effects is conceivable with the use of prolonged treatment schedules.

In conclusion, the results reported in this study indicate that polyamine analogues of CPTs have an intrinsic ability to induce DNA cleavage in the presence of isolated top1. The appreciable change in the pattern of DNA cleavage supports a contribution of drug−DNA interaction in modulating the recognition of specific sequences. The lack of a specific correlation between top1 inhibition and cell growth inhibition likely reflects multiple events including limited intracellular accumulation of polyamine derivatives with free amino groups.

Table 2. Antitumor Effects of **6d** and Topotecan against MKN-28 Human Gastric Carcinoma Xenograft in Nude Athymic Mice[a]

drug		dose (mg/kg)	TVI[b] (%)	LCK[c]	BWL[d] (%)	toxicity[e]
topotecan	po	5	80	1.3	0	0/4
		9	91	1.7	0	0/4
		15	98	2.1	8	0/4
6d	iv	1	34	0.2	1	0/4
		2	36	0.2	6	0/4
		4	66	0.8	0	0/4
6d	po	1	28	0.1	5	0/4
		2	62	0.5	1	0/4
		4	72	1	7	0/4

[a] Tumor fragments were inoculated on both flanks at day 0. Treatment started when tumors were just palpable (day 3−4). [b] Tumor volume inhibition (%) in treated over control animals 20 days after last treatment. [c] The $\log_{10}$ cell kill to reach 1000 mm^3 of tumor volume. [d] Body weight loss (%) induced by drug treatment. [e] Dead/treated mice.

Experimental Section

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries on a Büchi melting point apparatus and are uncorrected. Column chromatography was carried out on flash silica gel (Merck 230−400 mesh). TLC analysis was conducted on silica gel plates (Merck 60F_{254}). NMR spectra were recorded in DMSO-d_6 (when not otherwise stated) at 300 MHz with a Bruker instrument. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. Mass spectra were recorded at an ionizing voltage of 70 eV on a Finnigan TQ70 spectrometer. The relative intensities of mass spectrum peaks are listed in parentheses. Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were obtained by distillation from sodium benzophenone ketyl, and dry methylene chloride was obtained by distillation from phosphorus pentoxide. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and all glassware was oven-dried and/or flame-dried. Analyses indicated by the symbols of the elements or functions were within ±4% of the theoretical values.

4-(Camptothecinyl-7-methylidene)amino)butylcarbamic Acid *tert*-Butyl Ester (6a). 6a was prepared using the synthesis procedure described below for **6d**. Yield: 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.0−1.1 (t, 3H, CH_3), 1.45 (s, 9H, *t*Bu), 1.65−2.0 (m, 4H, 2-CH_2), 3.2−3.35 (m, 2H, CH_2), 3.9−4.0 (t, 2H, CH_2), 5.3−5.4 (d, 1H, CH), 5.55 (s, 2H, CH_2), 5.75−5.85 (d, 1H, CH), 7.7−7.9 (m, 3H, 2Ar + CH═), 8.25−8.35 (d, 1H, 1Ar), 8.45−8.55 (d, 1H, 1Ar), 9.4 (s, 1H, CH═). MS (IS), *m*/*z*: 547.7 $[MH]^+$. Anal. ($C_{30}H_{34}N_4O_6$) C, H, N.

(4-{[3-(Camptothecinyl-7-methylidene)amino)propyl]-*tert*-butoxycarbonylamino}butyl)carbamic Acid *tert*-Butyl (6b). 6b was prepared using the same procedure described for **6d**. Yield: 22%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.0−1.1 (t, 3H, CH_3), 1.4−2.1 (m, 26H, 2*t*Bu + 4 × CH_2), 3.0−3.4 (m, 4H, 2 −CH_2), 3.75−3.95 (m, 4H, 2 −CH_2), 5.25−5.35 (d, 1H, −CH), 5.55 (s, 2H, −CH_2), 5.75−5.85 (d, 1H, −CH), 7.7−7.9 (m, 3H, 3Ar + CH═), 8.25−8.35 (d, 1H, −CH), 8.45−8.55 (d, 1H, −CH), 9.4 (s, 1H, −CH═). ^{13}CNMR (75.4 MHz,$CDCl_3$) δ: 8.0, 28.6, 28.7, 32.1, 47.4, 51.7, 52.9, 53.6, 66.7, 69.7, 72.9, 79.7, 98.0, 98.4, 119.0, 122.5, 123.1, 126.4, 127.7, 128.6, 130.2, 130.4, 131.0, 131.3, 146.4, 150.1, 153.1, 156.0, 156.4, 157.9, 174.2. MS (IS), *m*/*z*: $[MH]^+$ = 704.6, $[M + Na]^+$ = 726.6. Anal. ($C_{38}H_{49}N_5O_8$), C,H, N.

(4-{[3-(Camptothecinyl-7-methylidene)amino)propyl]-benzyloxycarbonylamino}butyl)carbamic Acid Benzyl Ester (6c). 6c was prepared using the synthesis procedure described for **6d.** Yield: 35%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.0−1.1 (t, 3H, −CH_3), 1.4−2.1 (m, 8H, 4 −CH_2), 3.2−3.6 (m, 6H, 3 −CH_2), 3.95 (m, 2H, CH_2), 5.1−5.2 (m, 4H, 2 −CH_2), 5.4−5.9 (m, 4H, 2-CH_2), 7.2−7.45 (m, 10H, 10Ar), 7.7−7.9 (m, 3H, 2Ar + CH═), 8.25−8.5 (m, 2H, 2Ar), 9.4 (s, 1H, CH═). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 8.0, 27.2, 31.7, 41.0, 52.8, 66.5, 66.6, 67.2, 72.9, 98.0, 118.9, 122.8, 126.2, 127.4, 127.8, 128.0, 128.2, 128.5, 130.3, 130.9,

136.7, 146.0, 150.0, 152.9, 156.3, 156.7, 157.7, 174.0. MS (IS), *m/z*: $[MH]^+ = 772.9$, $[M + Na]^+ = 794.9$. Anal. ($C_{44}H_{45}N_5O_8$) C, H, N.

{3-[(4-{[3-(Camptothecinyl-7-methylidene)amino)propyl]-*tert*-butoxycarbonylamino}butyl)-*tert*-butoxycarbonylamino]propyl}-carbamic Acid *tert*-Butyl Ester (6d). An amount of 272 mg (0.72 mmol) of **1** was dissolved in 20 mL of anhydrous CH_2Cl_2 in a 100 mL flamed flask. $Yb(OTf)_3$ (44 mg, 0.07 mmol) was added to the solution, and then 700 mg (1.4 mmol) of tri-Boc-spermine was dissolved in 12 mL of anhydrous CH_2Cl_2 in the presence of 0.4 nm molecular sieves, keeping the reaction flask sheltered from the light. After 16 h at room temperature, an amount of 1.9 g (4.2 mmol) of a resin functionalized with isocyanate groups (loading, 2.2 mmol/g) was added as a scavenger of the amine in excess. The reaction mixture was left for 16 h at room temperature and filtered on Celite. The solvent was removed in vacuo, and the crude product was purified by preparative HPLC chromatography (CH_3CN/MeOH = 90:10; 8 mL/min; RP-18, 250 mm × 25 mm, 7 μm) to give 500 mg (81%) of **6d** as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ: 1.0−1.1 (t, 3H, CH_3), 1.4−2.0 (m, 35H, 3 *t*Bu + $4CH_2$), 2.0−2.1 (m, 2H, CH_2), 3.0−3.3 (m, 10H, $5CH_2$), 3.85−3.95 (m, 2H, CH_2), 5.3−5.4 (d, 1H, CH), 5.55 (s, 2H, CH_2), 5.75−5.85 (d, 1H, CH), 7.7−7.9 (m, 3H, 2Ar + CH═), 8.25−8.35 (d, 1H, 1Ar), 8.45−8.55 (d, 1H, 1Ar), 9.4 (s, 1H, CH═). ^{13}C NMR (75.4 MHz,$CDCl_3$) δ: 8.0, 28.6, 28.7, 31.8, 47.0, 52.7, 66.6, 72.9, 79.5, 97.8, 118.9, 126.2, 127.6, 128.5, 139.3, 130.8, 146.2, 149.9, 150.0, 152.9, 155.7, 157.6, 174.0. MS (IS), *m/z*: 860.8 $[MH]^+$, 858.7 $[M - 1]^-$. Anal. ($C_{46}H_{64}N_6O_{10}$) C, H, N.

[3-({4-[(3-(Camptothecinyl-7-methyl)amino)propyl]-*tert*-butoxycarbonylamino}butyl)-*tert*-butoxycarbonylamino]propyl}-carbamic Acid *tert*-Butyl Ester (7a). A suspension of 100 mg (0.116 mmol) of **6d** in dry CH_3OH and 20 mg of Pd/C was hydrogenated at 20 psi at room temperature. After 1 h, the mixture was passed through a short pad of Celite to remove the catalyst, and the crude product was purified by chromatography on silica gel with CH_2Cl_2/MeOH, 98:2, to give 50 mg (50%) of a yellow oil. $R_f = 0.51$ in CH_2Cl_2/MeOH, 98:2. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.0−1.1 (t, 3H, CH_3), 1.4−2.0 (m, 35H, 3-*t*Bu + 4-CH_2), 2.0−2.1 (m, 2H, CH_2), 2.7−2.8 (t, 2H, CH_2), 3.0−3.3 (m, 10H, 5-CH_2), 3.85−3.95 (m, 2H, CH_2), 5.4 (s, 2H, CH_2), 5.3−5.4 (d, 1H, CH), 5.75−5.85 (d, 1H, CH), 7.7−7.9 (m, 3H, 3 × CH), 8.25−8.35 (d, 1H, CH), 8.45−8.55 (d, 1H, CH), 9.4 (s, 1H, CH). MS (IS), *m/z*: $[MH]^+ = 863.2$. Anal. ($C_{46}H_{66}N_6O_{10}$) C, H, N.

{3-[(4-{[3-(Camptothecinyl-7-methyl)-*tert*-butoxycarbonylamino)propyl]-*tert*-butoxycarbonylamino}butyl)-*tert*-butoxycarbonylamino]propyl}carbamic Acid *tert*-Butyl Ester (7b). To a solution of 7 mg (0.008 mmol) of **7a** in 3 mL of dry CH_2Cl_2 cooled in an ice bath, 4 mg (0.016 mmol) of $(Boc)_2O$ and 3 μL (0.0016 mmol) of Et_3N were added, and the mixture was left at room temperature for 16 h. Dilution with CH_2Cl_2 (10 mL) and washing with 0.5 N HCl (5 mL) and with brine gave a crude product that was purified by preparative HPLC on RP-18 silica gel with CH_3CN/H_2O, 85:15, to give 6 mg (80%) of a yellow oil. $R_f = 0.5$ in CH_2Cl_2/MeOH, 92:8. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.0−1.1 (t, 3H, CH_3), 1.4−1.9 (m, 44H, 4 *t*Bu + 4 $-CH_2$), 1.9−2.0 (m, 2H, CH_2), 2.7−2.8 (t, 2H, CH_2), 3.0−3.3 (m, 10H, 5-CH_2), 3.85−3.95 (m, 2H, CH_2), 5.4 (s, 2H, CH_2), 5.3−5.4 (d, 1H, CH), 5.75−5.85 (d, 1H, CH), 7.7−7.9 (m, 3H, 2H + CH═), 8.25−8.35 (d, 1H, 1Ar), 8.45−8.55 (d, 1H, 1Ar), 9.4 (s, 1H, CH). MS (IS), *m/z*: $[MH]^+ = 963.2$. Anal. ($C_{51}H_{74}N_6O_{12}$) C, H, N.

***N*-(3-Aminopropyl)-*N*′-(3-(camptothecinyl-7-methyl)aminopropyl)butane-1,4-diamine (7c).** Gaseous HCl was bubbled for 15 min into a solution of 80 mg (0.09 mmol) of **7b** in 4 mL of CH_2Cl_2. The reaction mixture was stirred for one more hour to allow the disappearance of the starting material. The solvent was removed under vacuum, and the residue was dissolved in water and washed with CH_2Cl_2. The aqueous phase was lyophilized, and the residue was triturated with EtOH to give a yellow solid (50 mg, 82%). ^{1}H NMR (300 MHz, D_2O) δ: 0.8−0.9 (t, 3H, CH_3), 1.6−1.7 (m, 4H, 2-CH_2), 1.8−2.2 (m, 6H, 3-CH_2), 2.9−3.1 (m, 10H, 5-CH_2), 3.3−3.4 (t, 2H, $-CH_2$), 4.8−4.9 (d, 2H, $-CH_2$), 5.2−5.3 (d, 1H, −CH), 5.3 (s, 2H, $-CH_2$), 5.4−5.5 (d, 1H, −CH), 7.3 (s, 1H, CH═), 7.6−7.7 (m, 2H, 2Ar), 7.7−7.8 (t, 1H, 1Ar), 7.9−8.0 (d, 1H, 1Ar), 8.0−8.1 (d, 1H, CH═). MS (IS), *m/z*: $[MH]^+ = 563.2$. Anal. ($C_{31}H_{42}N_6O_4$) C, H, N.

[4-(2-Hydroxyethylamino)butyl]carbamic Acid *tert*-Butyl Ester (9a). A solution of **8a** (780 mg, 2.9 mmol) in 2-aminoethanol (1.75 mL, 29 mmol) was stirred and heated at 80 °C for 4 h. The reaction mixture was then poured into 15 mL of water and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford 650 mg (95%) of crude product. ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s, $3CH_3$), 1.49−1.58 (4H, m, 2-CH_2), 2.67 (2H, t, $-CH_2$, $J = 6.70$ Hz), 2.79 (2H, t, $-CH_2$, $J = 4.84$ Hz), 2.97−3.05 (2H, m, $-CH_2$), 3.10 (2H, brs, NH−OH), 3.67 (2H, t, $-CH_2$, $J = 4.48$ Hz), 4.89 (1H, bs, NH).

(3-*tert*-Butoxycarbonylaminopropyl)-[4-(2-hydroxyethylamino)butyl]carbamic Acid *tert*-Butyl Ester (9b). A solution of **8b** (2.1 g, 5 mmol) in 2-aminoethanol (3 mL, 50 mmol) was stirred and heated at 80 °C for 4 h. The reaction mixture was then poured into 30 mL of water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford 1.8 g (92%) of crude product. ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s, 3-CH_3), 1.44 (9H, s, 3-CH_3), 1.55−1.84 (6H, m, 3-CH_2), 2.83−3.34 (10H, m, 5-CH_2), 3.69 (1H, t, OH, $J = 4.47$ Hz), 3.77−3.96 (2H, m, $-CH_2$).

(4-*tert*-Butoxycarbonylaminobutyl)-(2-hydroxyethyl)carbamic Acid *tert*-Butyl Ester (10a). To a solution of **9a** (650 mg, 2.8 mmol) and triethylamine (0.8 mL, 5.6 mmol) in CH_2Cl_2 (5 mL) a solution of Boc_2O (670 mg, 3.1 mmol) in CH_2Cl_2 (2 mL) was added. The reaction mixture was stirred at room temperature for 4 h and then washed with water and brine. The organic extracts were dried over Na_2SO_4 and evaporated to give 1.150 g of crude product. Purification by flash chromatography on silica gel using 1−3% methanol in CH_2Cl_2 gave 500 mg of pure product (55%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s, 3-CH_3), 1.44 (9H, s, 3 $-CH_3$), 1.40−1.62 (4H, m, 2-CH_2), 2.52 (1H, brs, OH), 3.11 (2H, t, $-CH_2$, $J = 6.70$ Hz), 3.23 (2H, t, $-CH_2$, $J = 6.70$ Hz), 3.35 (2H, t, $-CH_2$, $J = 5.21$ Hz), 3.72 (2H, t, $-CH_2$, $J = 5.21$ Hz), 4.56 (1H, bs, NH).

(3-*tert*-Butoxycarbonylaminopropyl)-{4-[*tert*-butoxycarbonyl-(2-hydroxyethyl)amino]butyl}carbamic Acid *tert*-Butyl Ester (10b). To a solution of **9b** (1.8 g, 4.6 mmol) in dioxane/water (20 mL:10 mL), Na_2CO_3 (250 mg, 2.3 mmol) and a solution of Boc_2O (1.1 g, 5 mmol) in dioxane/water (6 mL:3 mL) were added. The mixture was stirred for 2 h at room temperature. The organic phase was evaporated, and the aqueous phase was acidified to pH 4 and then extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH, 98:2, to give 1.2 g of the title compound as an oil (56%). ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s, 3-CH_3), 1.43 (9H, s, 3-CH_3), 1.44 (9H, s, 3-CH_3), 1.47 (4H, m, 2-CH_2), 1.63 (2H, m, $-CH_2$), 2.62 (1H, brs, OH), 3.07 (2H, t, $-CH_2$, $J = 6.63$ Hz), 3.22 (4H, m, 2-CH_2), 3.13 (2H, m, $-CH_2$), 3.34 (2H, t, $-CH_2$, $J = 5.58$ Hz), 3.71 (2H, t, $-CH_2$, $J = 5.21$ Hz).

(4-*tert*-Butoxycarbonylaminobutyl)-[2-(1,3-dioxo-1,3-dihydroisoindol-2-yloxy)ethyl]carbamic Acid *tert*-Butyl Ester (11a). To a solution of **10a** (250 mg, 0.75 mmol), triphenylphosphine (300 mg, 1 mmol), *N*-hydroxyphthalimide (170 mg, 1 mmol) in 10 mL of THF, and 210 mg (1 mmol) of diisopropyl azodicarboxylate were dropped at 0 °C. The reaction mixture was stirred for 5 h under nitrogen at room temperature. After removal of the solvent in vacuo, the crude product was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH, 98:2, and then ethyl acetate/hexane, 40:60, to give 250 mg of an oil (63%). ^{1}H NMR ($CDCl_3$) δ: 1.41 (9H, s, 3-CH_3), 1.43 (9H, s, 3-CH_3), 1.55−1.70 (4H, m, 2-CH_2), 3.07−3.18 (2H, m, $-CH_2$), 3.36 (2H, t, $-CH_2$, $J = 7.07$ Hz), 3.48−3.68 (2H, m, $-CH_2$), 4.22−4.40 (2H, m, $-CH_2$), 4.61 (1H, brs, NH), 7.68−7.90 (4H, m, Ar).

(3-*tert*-Butoxycarbonylaminopropyl)-(4-{*tert*-butoxycarbonyl-[2-(1,3-dioxo-1,3-dihydroisoindol-2-yloxy)ethyl]amino}butyl)-carbamic Acid *tert*-Butyl Ester (11b). To a solution of **10b** (1.2 g, 2.6 mmol), triphenylphosphine (820 mg, 3.1 mmol), *N*-hydroxy-

phthalimide (510 mg, 3.1 mmol) in 35 mL of THF, and 630 mg (3.1 mmol) of diisopropyl azodicarboxylate were dropped at 0 °C. The reaction mixture was stirred for 5 h under nitrogen at room temperature. After removal of the solvent in vacuo, the crude product was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH, 98:2, and then ethyl acetate/hexane, 40:60, to give 950 mg of pure product as an oil (60%). ^{1}H NMR ($CDCl_3$) δ: 1.42 (27H, s, 9-CH_3), 1.51 (4H, m, 2-CH_2), 1.63 (2H, m, $-CH_2$), 3.07 (2H, m, $-CH_2$), 3.13 (4H, m, $-CH_2$), 3.22 (2H, m, $-CH_2$), 3.44 (2H, m, $-CH_2$), 3.56 (2H, m, $-CH_2$), 4.29 (2H, m, $-CH_2$), 7.68−7.90 (4H, m, Ar).

(2-Aminooxyethyl)-(4-*tert*-butoxycarbonylaminobutyl)carbamic Acid *tert*-Butyl Ester (12a). To a solution of **11a** (220 mg, 0.46 mmol) in 3 mL of ethanol, hydrazine (30 mg, 0.55 mmol) was dropped at room temperature. The reaction mixture was heated at 70 °C for 3 h and then was filtered. After solvent removal in vacuo, the crude product was purified by flash chromatography on silica gel using 50−35% of hexane in ethyl acetate to obtain 100 mg of pure product as an oil (65%). ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s, 3 $-CH_3$), 1.44 (9H, s, 3 $-CH_3$), 1.33−1.65 (4H, m, 2-CH_2), 3.00−3.60 (6H, m, 3-CH_2), 4.00−4.20 (2H, m, $-CH_2$), 4.59 (1H, bs, NH).

{4-[(2-Aminooxyethyl)-*tert*-butoxycarbonylamino]butyl}-(3-*tert*-butoxycarbonylaminopropyl)carbamic Acid *tert*-Butyl Ester (12b). To a solution of **11b** (950 mg, 1.56 mmol) in 15 mL of ethanol, hydrazine (100 mg, 1.87 mmol) was dropped at room temperature. The reaction mixture was heated at 70 °C for 5 h and then filtered. After solvent removal in vacuo, the crude product was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH, 95:5, to obtain 535 mg of the title compound as an oil (65%). ^{1}H NMR ($CDCl_3$) δ: 1.42−1.50 (27H, brs, 9-CH_3), 1.50−1.65 (6H, m, 3-CH_2), 3.05−3.30 (8H, m, 4-CH_2), 3.45−3.55 (2H, m, $-CH_2$), 4.30−4.35 (2H, m, $-CH_2$), 4.75 (1H, brs, NH).

(2-(7-(Camptothecinyl-7-methylidene)aminooxyethyl)carbamic Acid *tert*-Butyl Ester (13a). To a suspension of 7-[(2-aminoethoxyiminomethyl)]-20*S*-camptothecin hydrochloride[26] (21 mg, 0.044 mmol) in 2 mL of anhydrous CH_2Cl_2 at 0 °C were added 19 mg of $(Boc)_2O$, 18 μL of Et_3N, and a catalytic amount of DMAP. The mixture was stirred at this temperature for 45 min and then at room temperature for 1 h. The solvent was evaporated, and the crude product was diluted with AcOEt, washed once with 0.5 N HCl, and twice with brine. The organic phase was dried with Na_2SO_4, filtered, and brought to dryness. After purification by preparative HPLC (eluent CH_3CN/H_2O = 55/45, flow 10 mL/min, column Merck Lichrosorb C18 250 mm × 25 mm, 7 μm, UV detector 375 nm), the product (17 mg, 70%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (t, $-CH_3$), 1.44 (s, *tert*-butyl), 1.88 (m, $-CH_2-$), 3.60 (t, $-CH_2-N-$), 3.80 (broad s, OH), 4.49 (t, $-CH_2-O-$), 4.90 (broad s, NH), 5.32 (d, $-CH-$), 5.44 (s, $-CH_2-$), 5.76 (s, $-CH-$), 7.71 (t, 1Ar), 7.73 (s, $-CH=$), 7.88 (t, 1Ar), 8.20 (d, 1Ar), 8.30 (d, 1Ar), 9.32 (s, $-CH=$N). Anal. ($C_{28}H_{30}N_4O_7$) C, H, N.

(2-(7-(Camptothecinyl-7-methylidene)aminooxypropyl)carbamic Acid *tert*-Butyl Ester (13b). To a suspension of 7-[(3-amino)propoxyiminomethyl)-20*S*-camptothecin (**14b**, 20 mg, 0.045 mmol) in 5 mL of anhydrous THF were added 10 mg of $(Boc)_2O$ and 7 μL of Et_3N. The mixture was left at room temperature for 30 h. The solvent was evaporated, and the solid was extracted with CH_2Cl_2. The organic phase was washed twice with water and once with brine, then dried with Na_2SO_4, filtered, and brought to dryness. The product (16 mg, 64%) was obtained as a mixture of *E* and *Z* isomers, mp 141−142 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.87 (t, $-CH_3$ *E* + CH_3 *Z*), 1.37 (s, *tert*-butyl *E*), 1.30 (s, *tert*-butyl *Z*), 1.67 (m, 2-CH_2 *Z*), 1.87 (m, 2 $-CH_2$ *E*), 2.83 (t, $-CH_2-N-$ *Z*), 3.07 (t, $-CH_2-N-$ *E*), 4.12 (t, $-CH_2-O-$ *Z*), 4.35 (t, $-CH_2-O-$ *E*), 5.17 (s, $-CH$ *Z*), 5.32 (s, $-CH$ *E*), 5.40 (s, $-CH$ *E*), 6.50 (s, $-OH$ *E* + $-OH$ *Z*), 6.75 (t, NH *Z*), 6.90 (t, NH *E*), 7.25 (s, $-CH$ *Z*), 7.32 (s, $-CH$ *E*), 7.75 (m, 1Ar *E* + 1Ar *Z*), 7.90 (m, 1Ar *E* + 1Ar *Z*), 8.02 (d, 1Ar *Z*), 8.20 (d, 1Ar *E* + 1Ar *Z*), 8.40 (s, $-CH=$N *Z*), 8.6 (d, 1Ar *E*), 9.32 (s, $-CH=$N *E*). *E*/*Z* ratio = 88:22 (by NMR). Anal. ($C_{29}H_{32}N_4O_7$) C, H, N.

{4-[(2-(7-(Camptothecinyl-7-methylidene)aminooxyethyl)-*tert*-butoxycarbonylamino]butyl}carbamic Acid *tert*-Butyl Ester (13c). To a solution of **1** (55 mg, 0.145 mmol) in 2 mL of ethanol was added a solution of **12a** (100 mg, 0.29 mmol) in 1 mL of ethanol. The mixture was heated for 8 h and then evaporated. Purification by flash chromatography on silica gel using as eluent 3% of methanol in CH_2Cl_2 afforded 52 mg of the title compound (50%). Yellow solid, mp 153 °C. ^{1}H NMR ($CDCl_3$) δ: 0.86 (3H, t, *J* = 6.70 Hz, $-CH_3$), 1.02 (3H, t, *J* = 7.07 Hz, $-CH_3$), 1.17−1.70 (22H, m, 6-CH_3 + 2 $-CH_2$), 1.77−2.01 (2H, m, $-CH_2$), 2.86−3.02 (2H, m, $-CH_2$), 3.07−3.20 (2H, m, $-CH_2$), 3.25−3.49 (4H, m, 2-CH_2), 3.54−3.68 (2H, m, $-CH_2$), 4.31 (2H, t, *J* = 5.95 Hz, $-OCH_2$), 4.43−4.53 (2H, m, $-OCH_2$), 4.62 (1H, brs, NH), 5.10 (1H, brs, NH), 5.19 (2H, s, $-CH_2$), 5.25−5.31 (2H, dd, *J* = 2.98 Hz, $-CH_2$), 5.41 (2H, s, $-CH_2$), 5.72 (2H, dd, *J* = 4.09 Hz, $-CH_2$), 7.65−7.74 (m, 1Ar), 7.78−7.87 (m, 1Ar), 7.92 (1H, d, *J* = 8.19 Hz, 1Ar), 8.04 (1H, s, CH=N *Z*), 8.16−8.31 (m, 1Ar), 9.05 (1H, s, CH=N *E*). MS *m*/*z*: 705 (M^+,100), 661 (32), 615 (32), 587 (66), 418 (78), 374 (58). Anal. ($C_{37}H_{47}N_5O_9$) C, H, N.

[3-({4-[(2-(7-(Camptothecinyl-7-methylidene)aminooxyethyl)-*tert*-butoxycarbonylamino]butyl}-*tert*-butoxycarbonylamino)-propyl]carbamic Acid *tert*-Butyl Ester (13d). To a solution of **1** (200 mg, 0.53 mmol) in 8 mL of ethanol was added a solution of **12b** (535 mg, 1.06 mmol) in 8 mL of ethanol. The mixture was heated for 8 h and then evaporated. Purification by flash chromatography on silica gel using as eluent 1% of methanol in CH_2Cl_2 afforded 300 mg of pure product (70%). Yellow solid, mp 85 °C. ^{1}H NMR ($CDCl_3$) δ: 1.03 (3H, t, $-CH_3$, *J* = 7.44 Hz), 1.40−1.63 (31H, m, 9-CH_3 + $-CH_2$ + $-CH_2$), 1.80−1.99 (2H, m, $-CH_2$), 2.86−3.49 (8H, m, 4-CH_2), 3.59 (2H, brs, $-CH_2$), 4.49 (2H, brs, $-CH_2$), 5.41 (2H, s, $-CH_2$), 5.27−5.32 (1H, d, *J* = 15.88 Hz), 5.71−5.76 (1H, d, *J* = 15.88 Hz), 7.66 (1H, s, Ar), 7.70 (1H, t, Ar, *J* = 7.82 Hz), 7.84 (1H, t, Ar, *J* = 7.82 Hz), 8.24 (2H, m, Ar), 9.05 (1H, s, CH=N). MS *m*/*z*: 862 (10) M^-, 567 (64), 541 (100), 512 (79). Anal. ($C_{45}H_{62}N_6O_{11}$) C, H, N.

7-(3-Aminopropoxy)iminomethylcamptothecin (14b). To a suspension of **1** (235 mg, 0.625 mmol) in 20 mL of absolute ethanol were added 203 mg (1.25 mmol) of *O*-(3-aminopropyl)hydroxylamine hydrochloride and 0.7 mL of anhydrous pyridine, and the mixture was refluxed for 1 h. The mixture was cooled to room temperature, and the precipitate was filtered and washed with cold ethanol and ethyl ether to give 138 mg of a yellow solid (49%), mp 187 °C (dec). R_f = 0.11, CH_3OH/H_2O, 9:1 (RP 18). ^{1}H NMR δ: 0.86 (3H, t, $-CH_3$, *J* = 7.44 Hz), 1.86 (2H, m, $-CH_2$), 2.07 (2H, m, CH_2), 2.96 (2H, bs, CH_2NH_2), 4.43 (2H, t, $-OCH_2$, *J* = 5.95 Hz), 5.34 (2H, s, $-CH_2$ *E*), 5.41 (2H, s, $-CH_2$ *E*), 6.52 (1H, bs, OH), 7.35 (1H, s, $-CH$ *E*), 7.70−8.04 (2H, bs, NH_2), 7.76 (1H, dd, 1Ar, *J* = 7.44, 8.19 Hz), 7.90 (1H, dd, 1Ar, *J* = 7.44, 7.82 Hz), 8.22 (1H, d, 1Ar, *J* = 8.19 Hz), 8.60 (1H, d, 1Ar, *J* = 7.82 Hz), 9.34 (1H, s, $-CH=N-$ *E*). MS *m*/*z*: $[M]^+$ 449 (100), 374 (20), 154 (45), 97 (20). Anal. ($C_{24}H_{24}N_4O_5$) C, H, N.

7-[2-(4-Aminobutyl)aminoethoxy]iminomethylcamptothecin (14c). To a solution of **13c** (15 mg, 0.02 mmol) in 0.5 mL of CH_2Cl_2 was added 0.5 mL of TFA. The reaction mixture was stirred for 1 h. After solvent removal in vacuo, the pure product was obtained by crystallization with hexane (15 mg, 97%), mp 172 °C. ^{1}H NMR δ: 0.86 (3H, t, CH_3, *J* = 7.44 Hz), 1.48−1.74 (4H, m, 2 $-CH_2$), 1.77−1.94 (2H, m, $-CH_2$), 2.74−2.87 (2H, bs, $-CH_2$), 2.97−3.09 (2H, bs, $-CH_2$), 3.25−3.35 (2H, bs, $-CH_2$), 4.60 (2H, t, $-CH_2O$, *J* = 5.21 Hz), 5.38 (2H, s, $-CH_2$), 5.42 (2H, s, $-CH_2$), 6.53 (1H, s, OH), 7.36 (1H, s, Ar), 7.75 (3H, brs, NH_3), 7.78 (1H, ddd, 1Ar, *J* = 1.49, 8.56, 9.68 Hz), 7.92 (1H, ddd, 1Ar, *J* = 1.12, 8.19, 9.68 Hz), 8.24 (1H, dd, 1Ar, *J* = 1.12, 8.56 Hz), 8.56 (1H, d, 1Ar, *J* = 8.19 Hz), 8.85 (2H, brs, NH_2), 9.35 (1H, s, CH=N). MS *m*/*z*: 506 M^+ , 489 (100). Anal. ($C_{27}H_{31}N_5O_5$) C, H, N.

7-{2-[4-(3-Aminopropyl)aminobutyl]aminoethoxy}iminomethylcamptothecin (14d). To a solution of **13d** (50 mg, 0.06 mmol) in 2 mL of CH_2Cl_2 was added 2 mL of TFA. The reaction mixture was stirred for 5 h. After solvent removal in vacuo, the pure product was obtained by taking up with hexane (50 mg, 97%).

^{1}H NMR (D_2O) δ: 0.88 (3H, t, $-CH_3$, J = 7.28 Hz), 1.60−2.03 (8H, m, 4-CH_2), 2.90−3.17 (10H, m, 5-CH_2), 3.49 (2H, bs, $-CH_2O$), 5.35 (2H, dd, $-CH_2$, J = 7.28 Hz), 7.21 (1H, s, 1Ar), 7.51 (1H, t, 1Ar, J = 7.28, 8.44 Hz), 7.72 (1H, t, 1Ar, J = 7.28, 8.55 Hz), 7.82 (1H, d, 1Ar, J = 8.44 Hz), 7.89 (1H, d, 1Ar, J = 8.55 Hz), 8.91 (1H, s, CH═N). MS *m*/*z*: 563 M^+. Anal. ($C_{30}H_{38}N_6O_5$) C, H, N.

Hydrolysis of 6a,d. Compounds **6a**,**d** (50 μL of a 0.003 M solution in DMSO) were added with 450 μL of a standard acetate buffer solution at pH 5.0 and standard phosphate buffer solutions at pH 6.0 and pH 6.8. The conversion was monitored by TLC analysis using CH_2Cl_2/MeOH, 90:10, (R_f: **1**, 0.30; **6a**, 0.46; **6b**, 0.62) and by HPLC. HPLC analyses were performed on a HP-1050 quaternary pump fitted with a Rheodyne injector (20 mL loop) and a HP-1050 diode array detector. The column was a Rainin C-18, 25 cm × 0.4 cm, flow 1 mL/min, with a gradient of CH_3CN/H_2O from 40:60 to 100:0 in 10 min, then 100% CH_3CN for 10 min. **6d**: t_R = 11.00 min. **1**: t_R = 2.88 min.

Molecular Modeling. The three-dimensional molecular model of fully protonated compound **7c** was built on a Silicon Graphics O2, using the programs Insight II and Discover (Accelrys Inc., San Diego, CA). Minimizations were performed with the AMBER all-atom force field[37] and the conjugate gradients algorithm. For atomic partial charges of the ligand atoms, Mulliken charges calculated on the minimized structure using the MOPAC program[38] with the MNDO Hamiltonian were used.

The structure of the ternary complex containing topoisomerase I, DNA, and topotecan was downloaded from the Protein Data Bank (PDB entry 1K4T). All the atoms were fixed according to AMBER atom type, and hydrogens were added. The minimized structure of compound **7c** was appropriately overlapped with the structure of topotecan in the ternary complex, and the structure of topotecan was then deleted. The new complex was energy-refined using the AMBER force field. During energy minimization the structure of compound **7c** was allowed to relax, while the structure of the protein−DNA complex was frozen.

DNA Binding Assay. SV40 DNA plasmid was used to evaluate the DNA binding of polyamine derivatives. Reactions were performed in 20 μL of phosphate buffer saline (10 mM sodium phosphate, 150 mM NaCl, pH 7.4) for 1 h at 37 °C in the presence of 200 ng of plasmid and different amounts of compounds. Samples were analyzed on 0.7% agarose gel followed by ethidium bromide staining.

Assessment of Biological Activity. Cells were cultured in RPMI-1640 containing 10% fetal calf serum. Growth inhibition assay was used to evaluate the activity of the compounds after 1 h of drug exposure. Cells in the logarithmic phase of growth were harvested and seeded in duplicates into six-well plates. Twenty-four hours after seeding, cells were exposed to the drug and harvested 72 h after exposure and counted with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth over that of untreated control. All compounds were dissolved in DMSO prior to dilution into the biological assay. In vivo antitumor activity was determined as previously described.[26]

Plasmid Construction and Purification of Topoisomerase I. The plasmid pEMBLyex4 containing a hybrid GAL-CYC galactose-inducible promoter was used.[39] The cDNAs coding for the *Schistosoma japonicum* GST protein and for the 3′-untranslated region of human top1 (3340 base pairs) were inserted into the polylinker of the vector, thus resulting in the pEZ2ThTop1 plasmid. JEL1 yeast cells were transformed with the obtained vector, which allows the expression full length top1 with a GST domain linked to the NH_2 terminus of the enzyme. Yeast cells were galactose-induced, as described,[40] collected by centrifugation, and resuspended in PBS buffer containing 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.3. Protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 μg/mL pepstatin A, 10 μg/mL leupeptin, and 10 μg/mL aprotinin) were included in all buffers during purification. Cells were then lysed with glass beads by vortexing vigorously, and proteins were fractionated (50% and 70%) with ammonium sulfate precipitation. Protein extract was resuspended in PBS, loaded onto a GSH-Sepharose 4B (Pharmacia Biotech) chromatography column, and allowed to bind to the resin. The column was washed with PBS several times, and then the product was eluted with 10 mM glutathione in 50 mM Tris-HCl (pH 8.0). The top1 was finally concentrated by a phosphocellulose column and stored in 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.1 mM DTT, 1 M KCl, 200 μg/mL BSA, and 30% glycerol at −80 °C. The Bradford method was used to determine the protein concentration, 320 ng/μL.

Topoisomerase I Dependent DNA Cleavage Assay. A gel-purified 751-base-pair BamHI-EcoRI fragment of SV40 DNA was used for the cleavage assay. DNA fragments were uniquely 3′-end labeled. Top1 DNA cleavage reactions (20 000 cpm/sample) were performed in 20 μL of 10 mM Tris-HCl (pH 7.6), 150 mM KCl, 5 mM $MgCl_2$, 15 μg/mL BSA, 0.1 mM dithiotreitol, and the human recombinant enzyme (full length topoisomerase I)[41] for 30 min at 37 °C. Reactions were stopped by 0.5% SDS and 0.3 mg/mL of proteinase K for 45 min at 42 °C. Persistence of DNA cleavage at different time points was examined by adding 0.6 M NaCl after 30 min of incubation. After precipitation DNA was resuspended in denaturing buffer (80% formamide, 10 mM NaOH, 0.01 M EDTA, and 1 mg/mL dyes) before loading on a denaturing 7% polyacrylamide gel in TBE buffer. Overall, DNA cleavage levels were measured with a PhosphoImager 425 model (Molecular Dynamics).

An aliquot of 640 ng of protein was used in each cleavage assay. This amount completely relaxed 250 ng of wind pBR322 plasmid. The DNA damage was expressed as the ratio of the radioactivity present in selected cleavage bands to that of uncleaved DNA for all the time points (0, 1, 5, and 10 min). The potency of the CPT derivatives was assessed as a percentage of cleavage persistence defining the level of DNA damage for each time point with respect to time 0, as previously reported.[26]

Acknowledgment. We are indebted to A.I.R.C. and to the Italian Ministry of Health for partial financial support.

Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hurley, L. H. DNA and its associated processes as targets for cancer therapy. *Nat. Rev. Cancer* **2002**, *2*, 188−200.
(2) Cohen, S. S. *A Guide to the Polyamines*; Oxford University Press: New York, 1998.
(3) Thomas, T.; Thomas, T. J. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications *Cell. Mol. Life Sci.* **2001**, *58*, 244−258.
(4) Seiler, N. Pharmacological aspects of cytotoxic polyamine analogs and derivatives for cancer therapy. *Pharmacol. Ther.* **2005**, *107*, 99−119.
(5) (a) Seiler, N.; Dezeure, F. Polyamines transport in mammalian cells. *Int. J. Biochem.* **1990**, *22*, 211−218. (b) Seiler, N.; Dezeure, F.; Moulinoux, J. P. Polyamines transport in mammalian cells. An update. *Int. J. Biochem. Cell Biol.* **1996**, *28*, 843−861.
(6) Chen, K. Y.; Rinehart, C. A. Difference in putrescine transport in undifferentiated versus differentiated mouse NB-15 neuroblastoma cells. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 243−249.
(7) Volkow, N.; Goldman, S. S.; Flamm, E. S.; Cravioto, H.; Wolf, A. P.; Brodie, J. D. Labeled putrescine as a probe in brain tumors. *Science* **1983**, *221*, 673−675.
(8) (a) Braunlin, W. H.; Strick, T. J.; Record, M. T., Jr. Equilibrium dialysis studies of polyamine binding to DNA. *Biopolymers* **1982**, *21*, 1301−1314. (b) Plum, G. E.; Bloomfield, V. A. Effects of spermidine and hexaamminecobalt(III) on thymine imino proton exchange. *Biochemistry* **1990**, *29*, 5934−5940. (c) Burton, D. R.; Forsen, S.; Reimarsson, P. The interaction of polyamines with DNA: a ^{23}Na NMR study. *Nucleic Acids Res.* **1981**, *9*, 1219−1228. (d) Edwards, M. L.; Snyder, R. D.; Stemerick, D. M. Synthesis and DNA-binding properties of polyamine analogues. *J. Med. Chem.* **1991**, *34*, 2414−2420.
(9) Lindemose, S.; Nielsen, P. E.; Mollegaard, N. E. Polyamines preferentially interact with bent adenine tracts in double-stranded DNA. *Nucleic Acids Res.* **2005**, *33*, 1790−1803.

(10) Manning, G. S.; Ray, J. Counterion condensation revisited. *J. Biomol. Struct. Dyn*. **1998**, *16*, 461−476.
(11) Holley, J. L.; Mater, A.; Wheelhose, R. T.; Cullis, P. M.; Hartley, J. A.; Bingham, J. P.; Cohen, G. M. Targeting of tumor cells and DNA by a chlorambucil spermidine conjugate. *Cancer Res*. **1992**, *52*, 4190−4195.
(12) Cullis, P. M.; Merson-Davis, L.; Weaver, R. Mechanism and reactivity of chlorambucil and chlorambucil−spermidine conjugates. *J. Am. Chem. Soc*. **1995**, *117*, 8033−8034.
(13) Stark, P. A.; Thrail, B. D.; Meadows, G. G.; Abdel-Monem, M. M. Synthesis and evaluation of novel spermidine derivatives as targeted cancer chemotherapeutic agents. *J. Med. Chem*. **1992**, *35*, 4264−4269.
(14) (a) Holley, J. L.; Mater, A.; Cullis, P. M.; Symons, M. R.; Wardman, P.; Watt, R. A.; Cohen, G. M. Uptake and cytotoxicity of novel nitroimidazole−polyamine conjugates in Ehrlich ascite tumour cells. *Biochem. Pharmacol*. **1992**, *43*, 763−769. (b) Papadopoulou, M. V.; Rosenzweig, H. S.; Bloomer, W. D. Synthesis of a novel nitroimidazole-spermidine derivative as a tumor-targeted hypoxia-selective cytotoxin. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 1519−1522.
(15) Heston, W. D.; Uy, L.; Fair, W. R.; Covey, D. F. Cytotoxic activity of aziridinyl putrescine enhanced by polyamine depletion with alpha-difluoromethylornithine. *Biochem. Pharmacol*. **1985**, *34*, 2409−2410.
(16) Yuan, Z. M.; Egorin, M. J.; Rosen, D. M.; Simon, M. A.; Callery, P. S. Cellular pharmacology of N1- and N-8-aziridinyl analogues of spermidine. *Cancer Res*. **1994**, *54*, 742−748.
(17) Eiseman, J. L.; Rogers, F. A.; Guo, Y.; Kaufmann, J.; Sentz, D. L.; Klinger, M. F.; Callery, P. S.; Kyprianou, N. Tumor-targeted apoptosis by a novel spermine analogue, 1,12-diaziridinyl-4,9-diazadodecane, results in therapeutic efficacy and enhanced radiosensitivity of human prostate cancer. *Cancer Res*. **1998**, *58*, 4864−4870.
(18) Delcros, J.-G.; Tomasi, S.; Carrington, S.; Martin, B.; Renault, J.; Blagbrough, I. S.; Uriac, P. Effect of spermine conjugation on the cytotoxicity and cellular transport of acridine. *J. Med. Chem*. **2002**, *45*, 5098−5111.
(19) Suzuki, I.; Shigenaga, A.; Nemoto, H.; Shibuya, M. Synthesis and DNA damaging ability of enediyne−polyamine conjugates. *Tetrahedron Lett.* **2004**, *45*, 1955−1959.
(20) Bailly, C. Topoisomerase I poisons and suppressors as anticancer drugs. *Curr. Med. Chem*. **2000**, *7*, 39−58.
(21) Zunino, F.; Dallavalle, S.; Laccabue, D.; Beretta, G.; Merlini, L.; Pratesi, G. Current status and perspectives in the development of camptothecins. *Curr. Pharm. Des.* **2002**, *8*, 2505−2520.
(22) Pommier, Y. Eucaryotic DNA topoisomerase I: genome gate keeper and its intruders, camptothecins. *Semin. Oncol*. **1996**, *23*, 1−10.
(23) 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.
(24) Nagarajan, M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Design, synthesis and biological evaluation of indenoisoquinoline topoisomerase I inhibitors featuring polyamine side chains on the lactam nitrogen. *J. Med. Chem*. **2003**, *46*, 5712−5724.
(25) Dallavalle, S.; Ferrari, A.; Merlini, L.; Penco, S.; Carenini, N.; Perego, P.; De Cesare, M.; Pratesi, G.; Zunino, F. Novel cytotoxic 7-iminomethyl and 7-aminomethyl derivatives of camptothecin. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 291−294.
(26) Dallavalle, S.; Ferrari, A.; Biasotti, B.; Merlini, L.; Penco, S.; Gallo, G.; Marzi, M.; Tinti, M. O.; Martinelli, R.; Pisano, C.; Carminati, P.; Carenini, N.; Beretta, G.; Perego, P.; De Cesare, M.; Pratesi, G.; Zunino, F. Novel 7-oxyiminomethyl derivatives of camptothecin with potent in vitro and in vivo antitumor activity. *J. Med. Chem.* **2001**, *44*, 3264−3274.
(27) Sawada, S.; Nokata, K.; Furuta, T.; Yokokura, T.; Miyasaka, T. Chemical modification of an antitumor alkaloid camptothecin: synthesis and antitumor activity of 7-C-substituted camptothecins. *Chem. Pharm. Bull.* **1991**, *39*, 2574−2580.
(28) Blagbrough, I. S.; Geall, G. J. Practical synthesis of unsymmetrical polyamine amides. *Tetrahedron Lett.* **1998**, *39*, 439−442.
(29) Gardner, R. A.; Kinkade, R.; Wang, C.; Phanstiel, O. Total synthesis of petrobactin and its homologues as potential growth stimuli for *Marinobacter hydrocarbonoclasticus*, an oil-degrading bacteria. *J. Org. Chem.* **2004**, *69*, 3530−3537 and references therein.
(30) Almeida, M.; Lurdes, S.; Grehn, L.; Ragnarsson, U. Facile synthesis of monoacetylated spermidines, illustrating selective deacetylation and application of a common precursor. *Acta Chem. Scand.* **1989**, *43*, 990−994.
(31) Lemaire-Audoire, S.; Savignac, M.; Genet, J.-P. A new strategy for the synthesis of selectively protected spermidine and norspermidine derivatives**.** *Synlett* **1996**, 75−78.
(32) Veznik, F.; Guggisberg, A.; Hesse, M. Synthesis of *N*1,4-di(*p*-coumaroyl)spermine, a possible biogenetic precursor of aphelandrine**.** *Helv. Chim. Acta* **1991**, *74*, 654−661.
(33) (a) Giaccone, G.; Gazdar, A. F.; Beck, H.; Zunino, F.; Capranico, G. Multidrug sensitivity phenotype of human lung cancer cells associated with topoisomerase II expression. *Cancer Res*. **1992**, *52*, 1666−1674. (b) Marchesini, R.; Colombo, A.; Camerini, C.; Perego, P.; Supino, R.; Capranico, G.; Tronconi, M.; Zunino, F. *Int. J. Cancer* **1996**, *66*, 342−346.
(34) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B.; Stewart, L. The mechanism of topoisomerase I poisoning by a camptothecin analog. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387−15392.
(35) Dallavalle, S.; Delsoldato, T.; Ferrari, A.; Merlini, L.; Penco; S.; Carenini, N.; Perego, P.; De Cesare, M.; Pratesi, G.; Zunino, F. Novel 7-substituted camptothecins with potent antitumor activity. *J. Med. Chem.* **2000**, *43*, 3963−3969.
(36) Wang, L.; Prince, H. L.; Juusola, J.; Kline, M.; Phanstiel, O., IV. Influence of the polyamine architecture on the transport and topoisomerase II inhibitory properties of polyamine DNA−intercalator conjugates. *J. Med. Chem.* **2001**, *44*, 3682−3691.
(37) Weiner, P. *J. Comput. Chem*. **1981**, *2*, 287−303. Weiner, S. J. K.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc*. **1984**, *10* (6), 765−784.
(38) Stewart, J. J. P. *J. Comput.-Aided Mol. Des*. **1990**, *4*, 1−105.
(39) Baldari, C.; Murray, J. A. H.; Ghiara, P.; Cesareni, G.; Galeotti, C. L. A novel leader peptide which allows efficient secretion of a fragment of human interleukin 1 in *Saccharomyces cerevisiae*. *EMBO J*. **1987**, *6*, 229−234.40.
(40) Nitiss, J.; Wang, J. C. DNA topoisomerase-targeting antitumor drugs can be studied in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7501−7505.
(41) Beretta, G. L.; Binaschi, M.; Zagni, E.; Capuani, L.; Capranico, G. Tethering a type IB topoisomerase to a DNA site by enzyme fusion to a heterologous site-selective DNA-binding protein domain. *Cancer Res*. **1999**, *59*, 3689−3697.

JM060285B