Semisynthesis, Biological Activity, and Molecular Modeling Studies of C-Ring-Modified Camptothecins†

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The synthesis, biological activity, and molecular modeling studies of C-ring-modified camptothecins are reported. A general synthetic protocol, based on "C-5 camptothecin (C-5-CPT) enolate chemistry", allows one to obtain various C5-substituted analogues. All new compounds, obtained as 1:1 epimeric mixtures, were tested for their antiproliferative activity. Experimental data showed that all novel derivatives are less active than the reference compounds and that one of the two epimers is more active than the other. Molecular docking simulations were performed to achieve more insight into the interactions between the new C5-modified CPTs and Topo I. A good correlation was observed when the data of cytotoxicity and the values calculated for the free binding energy were combined.

Introduction

Camptothecin (CPT,[a] **1**, Figure 1) is a naturally occurring quinoline alkaloid from *Camptotheca acuminata*, having remarkable antitumoral and antileukemic activity.[1] The FDA has approved two water soluble semisynthetic camptothecin analogues, namely, topotecan **2**[2] and irinotecan **3**[3] for the clinical treatment of the ovarian, small-cell lung, and refractory colorectal cancers. The molecular target of the CPT family is the DNA topoisomerase I (Topo I), an enzyme that catalyzes the relaxation of supercoiled DNA during DNA replication.[4]

The drug acts by forming a reversible and stable enzyme−DNA−drug complex, thus preventing the religation step of the enzyme action. SAR studies on CPT derivatives led to the synthesis of several analogues with improved pharmacological properties, mostly regarding synthetic modifications of the quinoline system[5] (A/B rings, Figure 1) and especially positions C7, C9, and C10. Additional studies have been made on the E-ring modification; in particular the 20-OH esterification has been intensively studied to overcome poor solubility and improve delivery of CPT analogues.[6] Moreover, quite recently, a new family of very active seven-member β-hydroxy lactone derivatives were synthesized by Pommier and co-workers.[7]

So far, very few studies have been made on C-ring synthetic modifications, probably because of the low antineoplastic activity observed for the analogues so far reported, such as the hydroxy, alkoxy, acyloxy,[8] and hydroxymethyl[9] 5-substituted derivatives. Similarly, the anticancer activity of 5-amino substituted camptothecins was generally lower with respect to CPT except for the methylamine and hydroxylamine substituents, which showed good biological activity and water solubility.[10]

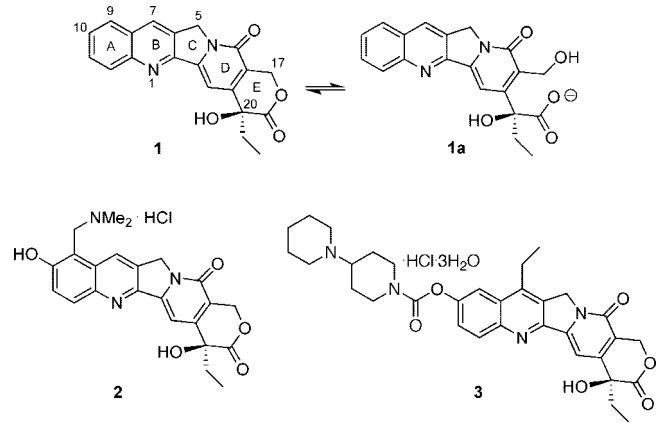

Figure 1. Camptothecin **1** and its carboxylate **1a** and their analogues topotecan **2** and irinotecan **3**.

Accordingly, it appears quite clear that ABCD system planarity is required for an efficient Topo I inhibition. Since the 5-position is adjacent to the pyridone moiety, the docking to the receptor active site might be inhibited when substituents are present at this position or when hydrogen bonding, favored by the presence of a hydroxy or amino group, occurs between the 5-position and the pyridone ring.[9] These findings seem to be confirmed by the behavior of 5-ethylene CPT analogues, in fact, where the presence of the ethylene moiety, assumed to lie in the molecular plane, does not reduce its antitumor activity.[11] However, further studies on 5-substituted CPT derivatives gave contradictory results. For instance, C5-propionyl moieties[12] and different 5-ethoxy derivatives bearing hydroxy, methoxy, or fluoro groups at the 2-position[13] showed good antitumoral activity. One of these analogues, DRF-1042, is currently in phase II clinical trials.[14] Finally, it is worth noting that 5-amino-substituted CPT inhibitory activity against hypoxia inducible

† Dedicated to the memory of Dr. Arturo Battaglia. He committed his life to the organic synthesis of biologically active molecules and to teaching his alumni the great importance of chemistry for life.

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[a] Abbreviations: CPT, camptothecin; Topo I, topoisomerase I; TESCl, triethylsilyl chloride; DMF, dimethylformamide; DMAP, dimethylaminopyridine; LHMDS, lithium bis(trimethylsilyl)amide; TBDMS, *tert*-butyldimethylsilyl group; QPLD, quantum mechanics polarized ligand docking.

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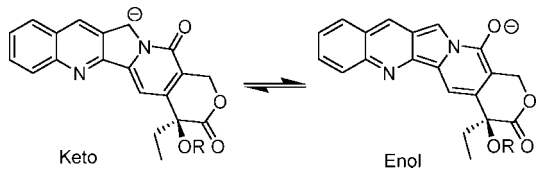
Scheme 1. CPT Resonance System after Base-Induced Removal of the 5-CH



factors HIF-1α[15,16] was better than that displayed by **1** and topotecan. For these reasons, more studies were needed to better understand the role of modification at the C-5 position.

The facile removal of a proton from C-5 of CPT has been well established by H^1/H^2 and H^1/H^3 hydrogen exchange experiments,[17] as well as by the synthesis of a number of 5-alkylated CPT analogues performed under basic conditions.[9,18] These derivatizations are successfully achieved because of the formation of a stable tautomeric enolate through an extended four-ring resonance system, which stabilizes the C ring as a heteroaromatic pyrrole system (Scheme 1). However, such reactivity has only been exploited for a few selected alkylation reactions, while other derivatizations such as hydroxylation[13] and amination[19] were achieved under oxidative conditions, whereas alkylations, such as C5-propionyl substitution, were achieved through a Wittig reaction on the 5-OH camptothecin.[12] Thus, C-5 CPT enolate chemistry deserves further investigation in order to develop a general synthetic protocol able to provide more diversified C-5 CPT analogues. In particular, besides alkylation, electrophilic amination, oxygenation, and fluorination were also considered.

Chemistry

The C-5 CPT functionalization initially required suitable protection of the 20-OH group, which was achieved by treatment of **1** with TESCl in DMF in the presence of an excess of imidazole and DMAP according to a modified Corey protocol.[20] As shown in Scheme 2, the 20-OTES camptothecin **4** was obtained in quantitative yield. The silyl protective group highly enhanced the solubility of **4** with respect to **1** in ethereal solvents, allowing the heterofunctionalization of the 5-position under mild conditions and low temperatures. Accordingly, treatment of **4** with LHMDS at −78 °C provided the corresponding lithium enolate. After reaction with suitable electrophiles, a number of 5-substituted derivatives were obtained as a mixture of α/β epimers, which were separated, where possible, by silica gel flash column chromatography. Removal of the silicon group with the complex $Et_3N \cdot 3HF$ afforded the 5-substituted CPT analogues (Scheme 2).

(a) Alkylation. For our purpose methyl iodide (MeI) and *tert*-butyl(3-iodopropoxy)dimethylsilane were used as alkylating agents. The addition was carried out under more demanding reaction conditions with respect to those reported above. The reaction was performed according to a slightly modified literature procedure,[18] using potassium *tert*-butoxide (^{t}BuOK) in DMF at 0 °C. Compounds **5** and **6** were obtained as inseparable 1:1 α/β-epimeric mixtures in moderate overall yields (40% and 41%, respectively) (Scheme 3). Partial deprotection of the 20-OH group was observed for compound **6** under these conditions. Quantitative removal of the TES and the TBDMS protecting groups from **5** and **6**, respectively, afforded the target 5-Me and 5-$(CH_2)_3$OH CPT analogues **7** and **8**, as an inseparable 1:1 α/β-epimeric mixture. Interestingly, the 5(*RS*)-(3-hydroxypropionyl)-20(*S*)-CPT analogue **8** represents the carbon isostere of DRF-1042 [5(*RS*)-(2-hydroxyethoxy)-20(*S*)-CPT].[14] Compound **8** could be more resistant toward enzymatic degradation than DRF-1042.

(b) Oxygenation. As an alternative to this acidic oxidation and in line with the enolate chemistry, 5-OH-CPT was synthesized through a direct oxidation of the enolate with 2-benzenesulfonyl-3-phenyloxaziridine.[21,22] The reaction afforded, after silyl group removal, the desired compound **10** as a 1:1 inseparable α/β-epimeric mixture (Scheme 4).

(c) Fluorination. Electrophilic fluorination with *N*-fluorobenzenesulfonimide (NFSI) of **4a** followed by 20-OTES deprotection gave the 5-fluorocamptothecin **12** as a 1:1 α/β-epimeric mixture, which was separated by silica gel flash chromatography. To the best of our knowledge, the synthesis of CPT analogues bearing a halo substituent at C-5 has not been previously reported (Scheme 4).

(d) Amination. So far, the 5-nitrogen functionalization has been obtained from the corresponding 5-OH derivatives via a two-step procedure, comprising the nucleophilic substitution of the OH with an amino function.[10,15] Instead, we chose the dibenzyl- and di-*tert*-butyl-azodicarboxylate[23] as suitable reagents for the **4a** enolate amination. The reaction provided the corresponding 5-hydrazino derivatives **13** and **14**, respectively; these compounds were obtained as a 1:1 α/β epimeric mixture, which was separated by silica gel column chromatography, affording the pure epimers **13a** and **13b** and pure epimers **14a** and **14b**. Although the hydrazino derivatives are potential precursors of the 5-aminocamptothecin, their conversion was not attempted.

The hydrolysis of the 20-OTES substituent of the pure epimers **13a** and **13b** yielded the corresponding CPT analogues **15a** and **15b**, whereas the hydrolysis of **14a** and **14b** gave compounds **16a** and **16b** (Scheme 5).

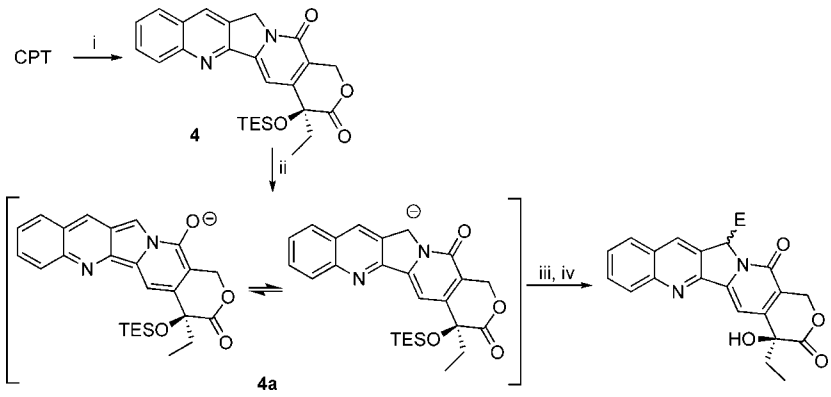
Among the electrophilic azides usually employed for reaction with enolates (phenylsulfonyl-, tosyl-, and 2,4,6-triisopropylbenzenesulfonyl azide),[24] we selected tosyl azide as the less sterically demanding. Moreover, reaction parameters should be carefully examined, since this azide could serve for diazo or azide transfer.[25] Nevertheless, in our case, the azide transfer occurred almost quantitatively and the 5-azido derivative **17** was obtained with only trace amounts of the diazide derivative **19** (Table 1). Chromatographic workup of the mixture gave the pure epimers **17a** and **17b**, which were deprotected giving the CPT derivatives **18a** and **18b**, respectively (Scheme 5). Treatment of the azido epimer **18a** with hydrogen over 10% palladium on activated carbon gave the 5-amino-camptothecin **22** as a 1:1 unseparable epimeric mixture (Scheme 5).

With the di-Boc-5-hydrazino compound **14** in our hands, we envisioned the possibility of synthesizing a new six-membered ring CPT derivative in which the amido substituents of the pyridone moiety and the nitrogen atom at the 5-position are joined into an additional heteroaromatic triazole ring. To this end, sequential TFA-induced removal of the BOC protecting groups, followed by cyclization of the corresponding trifluoro acetate salt, yielded a 1:1 epimeric mixture of 5,17-dihydro, 4,17,18-triazole derivatives **20** whose oxidation with DDQ provided the corresponding CPT analogue **21** in almost quantitative yield (Scheme 6).

Cytotoxic Activity

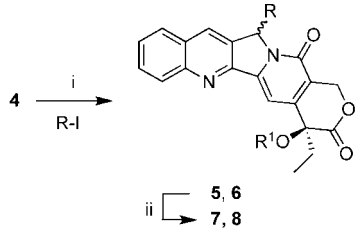
Antiproliferative Activity. The new 5-modified CPT analogues were tested for their antiproliferative activity against the human non-small-cell lung cancer carcinoma NCI-H460 cell line with a short-term exposure (1 h).[26] Topotecan, CPT (**1**), and SN38, the active metabolite of irinotecan, were used as

Scheme 2. Synthetic Protocol of 5-Substituted CPT Derivatives via Enolate Route[a]



[a] (i) TESCl, imidazole, DMAP, DMF; (ii) LHMDS, THF, −78 °C; (iii) electrophile, 4−5 h; (iv) $Et_3N \cdot 3HF$, THF, 20 °C, 35−40 h.

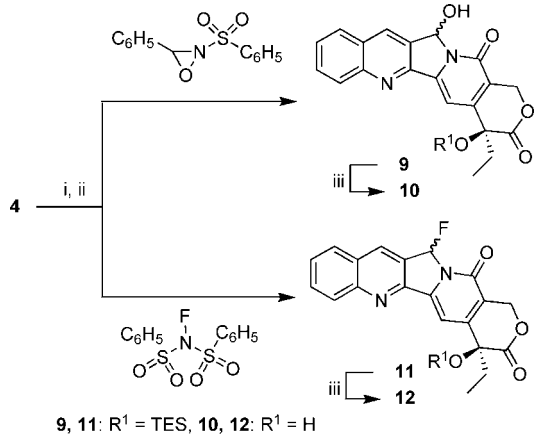
Scheme 3. Synthesis of 5-Alkylcamptothecins[a]



5: R^1 = TES, R = Me; **6**: R^1 = H, R = -$(CH_2)_3$OSi $Me_2{}^tBu$;
7: R^1 = H, R = Me; **8**: R^1 = H, R = -$(CH_2)_3$OH

[a] (i) ^{t}BuOK, DMF, 0 °C, then R−I; (ii) $Et_3N \cdot 3HF$, THF, 20 °C, 35−40 h.

Scheme 4. Synthesis of 5-Hydroxycamptothecin **10** and 5-Fluorocamptothecin **12**[a]



9, 11: R^1 = TES, **10, 12**: R^1 = H

[a] (i) LHMDS, THF, −78 °C; (ii) THF, −78 °C; (iii) $Et_3N \cdot 3HF$, 20 °C.

reference drugs. The collected data (Table 2) show that all new compounds are less active than the reference CPTs. Moreover, it appears that, except for compounds **12a** and **12b**, one of the two epimers is more active than the other, the 5-N_3 (**18a** and **18b**) derivatives (entries 12−13) being the most active of the series.

The presence of two N_3 in position 5 did not produce a substantial change in cytotoxic activity (**19** vs **18a** and **18b**). Methyl substituent (**7**) partially increased the potency, while the introduction of hydrophilic groups (**22**, **8**) was detrimental to the antiproliferative activity. Although the capability to cross the plasma membrane is strongly influenced by the lipophilic properties of the compounds, a reduced potency was observed for the most hydrophobic derivatives (**15a**, **15b**, **16a**, and **16b**). The bulky dimension of the substituents could produce a steric hindrance at the target level and thus might explain this feature.

The presence of an additional ring (CPTs **20** and **21**) influenced the antiproliferative activity of CPTs, with compound **21** about 5-fold more active than the corresponding compound **20**.

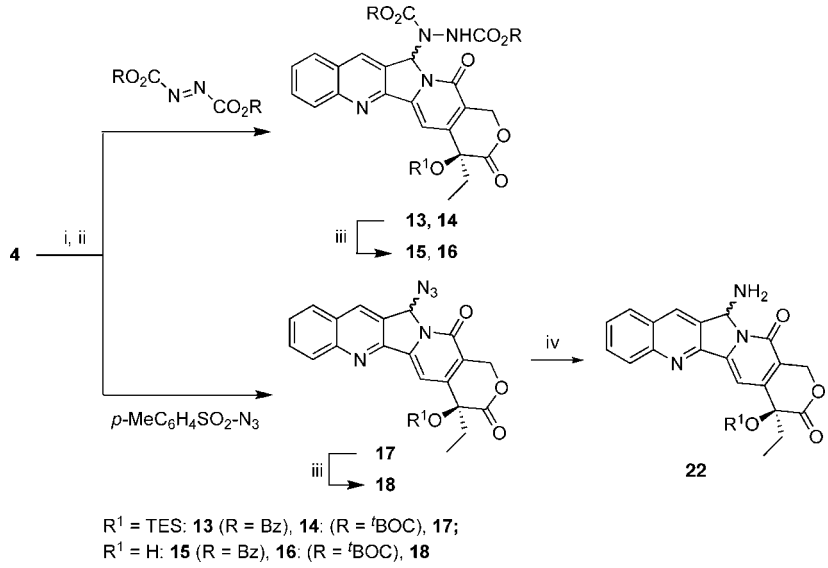
DNA Cleavage Assays. Topoisomerase I-mediated DNA cleavage assays with purified human topoisomerase I were performed to investigate the capability of the C5-modified CPTs to stimulate DNA damage.[27] SN38 was used as reference compound (Figure 3). The cleavage pattern was found to be similar to that of SN38 for all the tested CPT derivatives. Compound **18b** exhibited an intensity of DNA damage similar to that of SN38. A slightly reduced capability to damage DNA was observed for **12a**, **12b**, **21**, and **7**, whereas **15b**, **20**, and **22** were substantially less efficient.

Theoretical Calculations. To achieve more insight into the interactions of the new C-5 CPT analogues with Topo I, a molecular modeling study of the structures of the potential ternary cleavage complexes of Topo I, DNA, and both α/β-epimeric C-5 CPT analogues was carried out using molecular docking simulations. In particular, it is now well recognized that electrostatic interactions play a critical role in noncovalent interactions between DNA/ligand, protein/ligand, and DNA/protein complexes. Because of the highly charged nature of DNA, we assumed that the electrostatic interactions between DNA and its ligand might play a critical role in the accurate estimation of the binding free energy. In order to estimate the binding free energy with improved accuracy, we carried out molecular docking simulations based on the quantum mechanics (QM) polarized ligand docking protocol using QPLD-derived partial charges (see Experimental Section for details).

In general, all ternary complexes with Topo I and DNA are characterized by a negative free energy of binding ΔG_{bind}, as shown in Table 3. Thus, local energy minima were observed for structures in which all the new C-5 CPT analogues are accommodated into the DNA intercalation site (Figure 4) adopting a binding motif which is similar to the crystallographic pose of topotecan for most derivatives, although slightly different in some cases, as will be discussed later in detail.

In particular, smaller C-5 substituents such as 5-methyl (**7**), 5-hydroxy (**8**), 5-fluorine (**12**), 5-azido (**18**), and 5-amino (**22**) are apparently accepted in both epimeric configurations, with a slight preference for the β-epimer (up configuration) in the case of the 5-azido (**18**) derivative. Consistently, the 5-diazido derivative (**19**) is also accepted. In each case, the large overlap of the planar ABCD ring system with the nucleic bases

Scheme 5. Amination of Compound **4**[a]



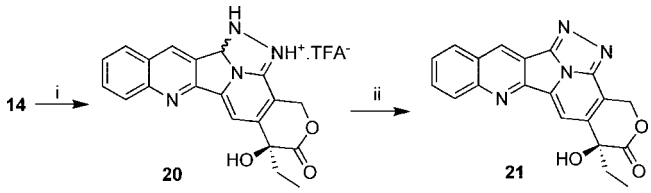
[a] (i) LHMDS, THF, −78 °C; (ii) THF, −78 °C; (iii) $Et_3N \cdot 3HF$, 20 °C; (iv) Pd/C.

Table 1

entry	electrophyle	E	product[a]	yield(%)
1	CbzN═NCbz	(Cbz)N−NH(Cbz)	**13**	>95
2	BOCN═NBOC	(BOC)N−NH(BOC)	**14**	>95
3	$Me(C_6H_4)SO_2N_3$	N_3[b]	**17**	>95

[a] a and b diastereoisomer formation. [b] Diazidation occurs.

Scheme 6. Synthesis of the Six-Membered Ring CPT Derivative **21**[a]



[a] (i) TFA, 20 °C, 20 h, 80%; (ii) DDQ, THF, 60 °C, 3 h, 95%.

Table 2. Cytotoxic Activity for C5-Substituted Camptothecins on a Human H460 Cell Line[a]

entry	compd	E	IC_{50} (μM), 1 h
1	CPT (**1**)	H	0.33
2	topotecan	H	0.61
3	SN38	H	0.13
4	**7**	CH_3	5.9
5	**8**	$(CH_2)_3OH$	16
6	**12a**	F	56
7	**12b**	F	56
8	**15a**	NBoc−NHBoc	>173
9	**15b**	NBoc−NHBoc	77
10	**16a**	NCbz−NHCbz	85
11	**16b**	NCbz−NHCbz	60
12	**18a**	N_3	11.5
13	**18b**	N_3	6.7
14	**19**[b]	$(N_3)_2$	9.3
15	**20**[b]		46.8
16	**21**[b]		10.6
17	**22**	NH_2	30

[a] Sensitivity was assessed by growth inhibition assay after 1 h of drug exposure. Cells were counted 72 h after drug-free medium incubation. [b] Formulas are reported in Figure 2.

guarantees the significant influence of the $\pi-\pi$ stacking interactions on the orientation of the ligand within the intercalation site and on the overall binding energy.

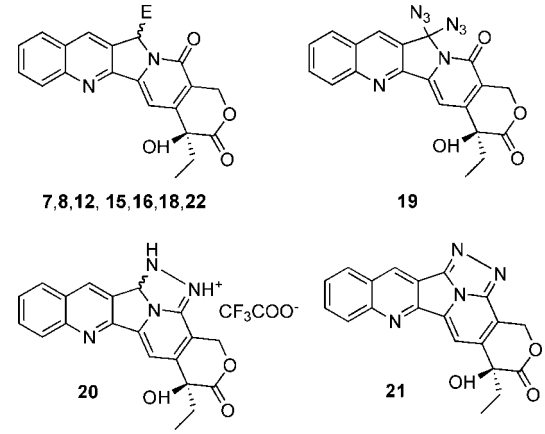


Figure 2. Chemical structures for all tested compounds.

Also, the stabilizing interactions with the Arg364 and the Asp533 are maintained, as shown in Figure 5 (panel A). In contrast, bulkier C-5 substituents such as 5-NBoc-NHBoc (**15**) and 5-NCbz-NHCbz (**16**) significantly modify the occupancy of these derivatives into the intercalation site. In fact, to permit a sterically favorable allocation of these bulky substituents inside the major groove of the DNA, the planar ABCD ring system shifts back its position with respect to that observed in the crystal structure of topotecan (Figure 5, panel B). This movement reduces the intensity of the $\pi-\pi$ stacking interactions and abolishes the formation of both stabilizing interactions with the Arg364 and Asp533. Moreover, the β-epimer at C-5 position of both derivatives **15** and **16** seems to better stabilize the ternary complex compared to the α-epimer (down stereochemistry) (ΔG_{bind}(**15**α) = −5.79 kcal/mol; ΔG_{bind}(**15**β) = −6.95 kcal/mol). Our results indicate that the introduction of substituents in position 5 of CPT resulted in derivatives with reduced cytotoxic potency compared to the reference drugs. In general, the presence of a small lipophilic substituent is well tolerated while the modification with hydrophilic groups resulted in reduced potency (e.g.,**7** vs **22**). In contrast, the presence of a bulky group is detrimental for cytotoxic potency (**15**, **16**, and **20**). This observation is not a general feature, since the expansion of the CPT structure with an additional aromatic ring (compound **21**) produced a derivative with good activity. The

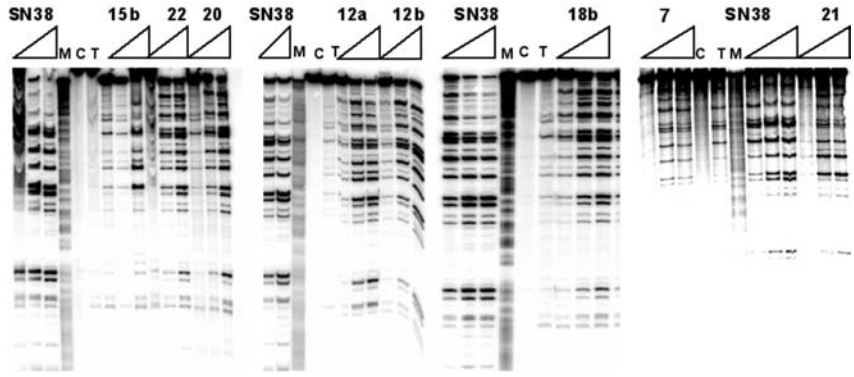
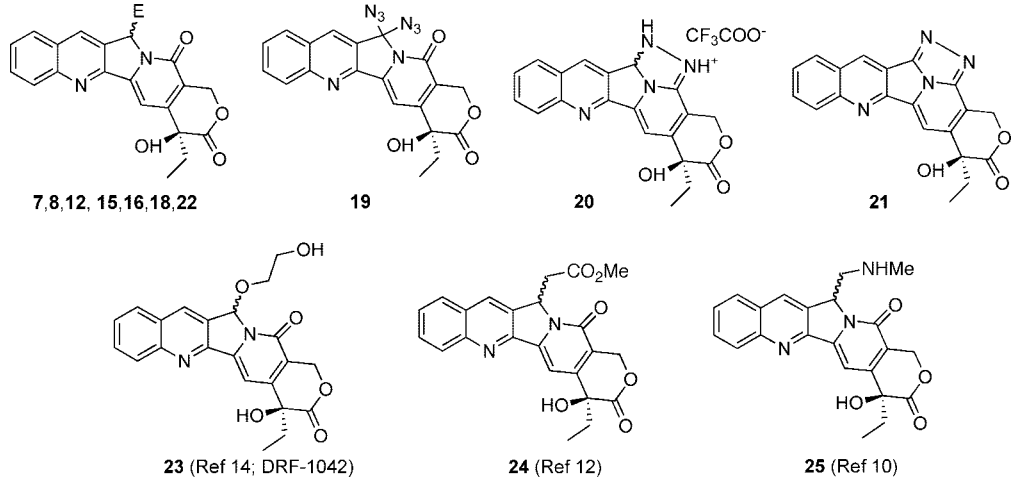


Figure 3. Topoisomerase I-mediated DNA cleavage by SN38 and different camptothecin derivatives. Samples were reacted with 1, 10, and 50 μM drug at 37 °C for 30 min. The reaction was then terminated by adding 0.5% SDS and 0.3 mg/mL proteinase K and incubating for 45 min at 42 °C before loading on a denaturing 8% polyacrylamide gel: C, control DNA; T, reaction without drug; M, purine markers. The experiment was repeated three times, and the results of a representative value are reported.

Table 3. Free Energy of Binding ΔG_{bind} Calculated for All α/β-Epimeric C-5 CPT Analogues with the QM-Polarized Ligand Docking Protocol



entry	compd	5-CPT substituent	*G* score (kcal/mol)
1	CPT (**1**)	H	−11.82
2	**7**α[a]	CH_3	−10.63
3	**7**β[b]	CH_3	−10.98
4	**8**α	$(CH_2)_3OH$	−9.90
5	**8**β	$(CH_2)_3OH$	−10.02
6	**12**α	F	−8.93
7	**12**β	F	−9.08
8	**15**α	NBoc−NHBoc	−5.79
9	**15**β	NBoc−NHBoc	−6.95
10	**16**α	NCbz−NHCbz	−8.17
11	**16**β	NCbz−NHCbz	−8.40
12	**18**α	N_3	−10.82
13	**18**β	N_3	−11.12
14	**19**	$(N_3)_2$	−10.76
15	**20**α		−7.94
16	**20**β		−7.35
17	**21**[c]		−10.50
18	**22**α		−9.12
19	**22**β		−9.74
20	**23**α	$O(CH_2)_2OH$	−8.04
21	**23**β	$O(CH_2)_2OH$	−10.95
22	**24**α	CH_2COOMe	−9.86
23	**24**β	CH_2COOMe	−10.25
24	**25**α	$NHCH_3$	−7.98
25	**25**β	$NHCH_3$	−9.92

[a] α-Epimer: down stereochemistry. [b] β-Epimer: up stereochemistry. [c] Formulas are reported in Figure 2.

finding that the β-epimer is more active than the corresponding α-epimer suggests a possible steric hindrance between the Topo I−DNA structure and the α-oriented group.

In the present docking study, we have also included three known 5-substitued CPT derivatives (compounds **23**−**25**, entries 20−24, Table 3) that have shown, experimentally, appreciable antitumoral activity.[10,12,14] These molecules fit into the Topo I−DNA complex in a binding mode, which is comparable to that of the new 5-substituted CPTs synthesized herein, and their predicted ΔG_{bind} values fall within the range of ΔG_{bind} calculated

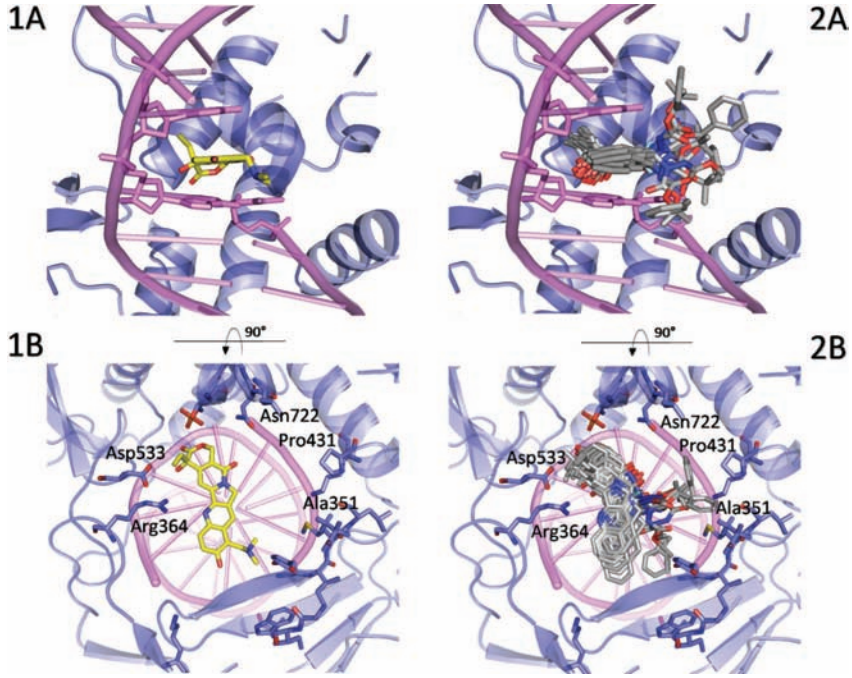


Figure 4. Docking results for the C-5 modified CPTs. In panels 1A (side view) and 1B (top view) the binding conformation of topotecan (yellow) from the crystal structure of the Topo I−DNA−topotecan ternary complex (PDB code 1k4t) is shown as a reference. Panels 2A (side view) and 2B (top view) show the superimposition of the best conformations for all C-5 modified CPTs, from docking simulations into the Topo I−DNA binding site. Topo I and DNA are represented by cartoon structure, colored in blue and pink, respectively.

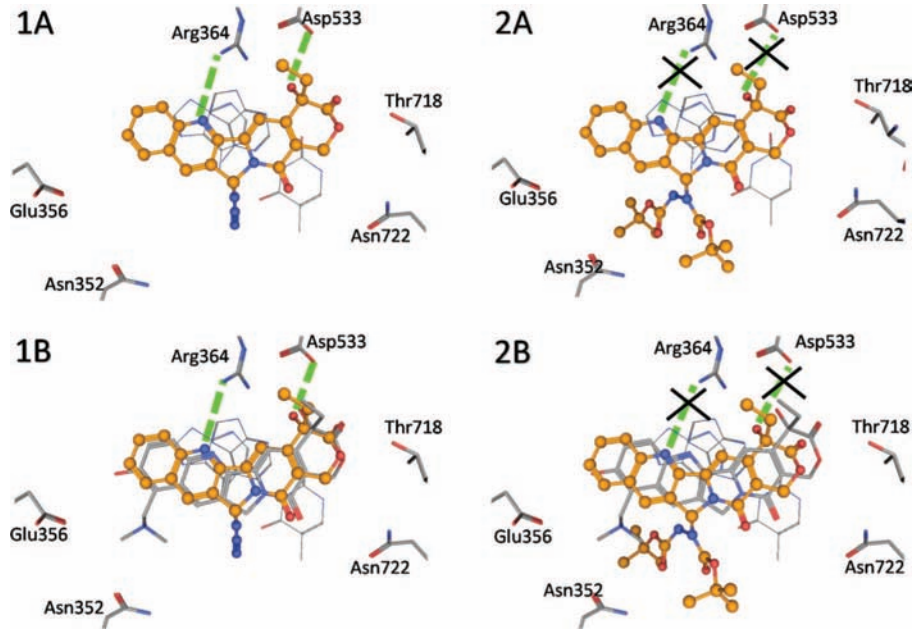


Figure 5. Binding conformation of 5-N_3 and 5-NBocNHBoc derivatives from docking simulations. Panels 1A and 2A: binding conformations of 5-N_3 and 5-NBocNHBoc, respectively (colored in orange). Panels 2A and 2B: conformations of 5-N_3 and 5-NBocNHBoc, respectively, superimposed on the topotecan binding conformation (in gray) for comparison. Polar interactions are represented by green dashed lines.

for our compounds. Moreover, also for these derivatives we observed that the β-epimer is better accepted than the α-epimer.

Finally, the 4,17,18-triazole derivative **21** increasing the extension of the aromaticity of the planar ring system is also properly accommodated within the intercalation site as demonstrated by the calculated ΔG_{bind} value (ΔG_{bind}(**21**) = −10.50 kcal/mol).

Interestingly, we have also found that an acceptable correlation was observed when the data of cytotoxicity and the values calculated for the free energy of binding were combined (Figure 6).

To make such a correlation, we assumed that the antiproliferative activity data are mostly pharmacodynamically driven, since the IC_{50} values and the cleavage potency show a similar trend for compounds that were tested in the cleavage assay. The cleavage potency was defined by comparing the intensity of cleavage bands of the tested compounds with that of the reference drug (SN38). As shown in Figure 6, a linear correlation between antiproliferative potency and ΔG_{bind} was observed ($r^2 = 0.76$).

Conclusions

A general synthetic protocol, based on the "C-5-CPT enolate chemistry", allowed the synthesis of various C5-substituted

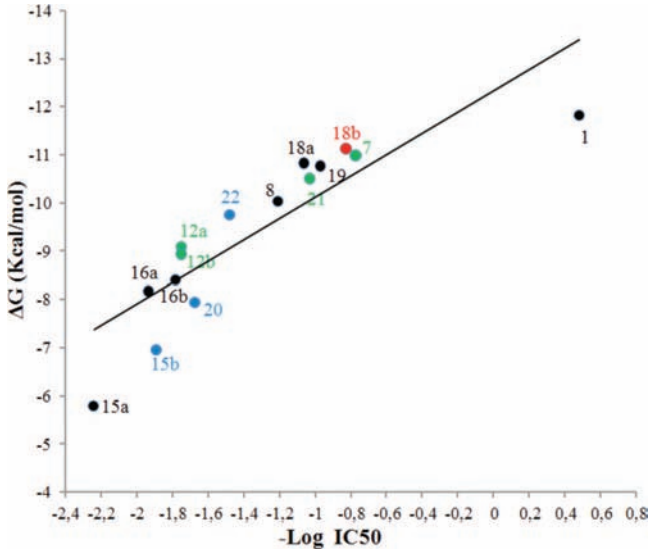


Figure 6. Correlation between cytotoxicity and ΔG_{bind}: red, the most active compound of the series; green, compounds with slightly reduced DNA damage activity; blue, less active compounds; black, compounds not tested in the cleavage assay.

analogues. In particular, the reported methodology provided, besides C5-alkylated derivatives, new C5-fluorinated and aminated analogues, along with the first six-membered ring CPT derivative **21**. All compounds were obtained as 1:1 epimeric mixtures, which were separated by silica gel flash chromatography where possible. The new C5-modified CPT analogues were tested for their antiproliferative activity against the human non-small-cell lung cancer carcinoma NCI-H460. The collected data showed that all new compounds are less active than the reference compounds and that, except for compounds **12a** and **12b**, one of the two epimers is more active than the other, the C5-N_3 **18** derivatives being the most active of the series. Molecular docking simulations were performed to achieve more insight into the interactions between the new C5-modified CPTs and Topo I. The experiments were carried out on the structures of the potential ternary cleavage complexes of Topo I, DNA, and both α/β-epimeric C5-CPTs in order to estimate the binding free energy values. Interestingly, in this study a good correlation was observed when the data of cytotoxicity and the values calculated for the free energy of binding were combined. In particular, a good correlation was identified for compound **18b**, the most active in the cleavage assay, which was effective in reducing cell proliferation and in producing a stable, cleavable complex.

Experimental Section

Chemistry. General Remarks. All reactions were performed under an atmosphere of dry nitrogen using oven-dried glassware. Tetrahydrofuran, toluene, and ethyl ether were distilled from sodium benzophenone ketal. Dichloromethane and DMF were distilled from calcium hydride. All other solvents were HPLC grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with E. Merck silica gel 60-F254 plates. Flash column chromatography was performed with Merck silica gel (0.04−0.63 μm, 240−400 mesh) under high pressure. NMR spectra were recorded on 400 MHz spectrometer. Unless otherwise stated, all NMR spectra were measured in $CDCl_3$ solutions and referenced to the $CHCl_3$ signal. All ^{1}H and ^{13}C shifts are given in ppm (s = singlet; d = doublet; t = triplet; dd = quadruplet; dt = doublet of triplets, m = multiplet; br s = broad signal). Coupling constants J are given in Hz. Assignments of proton resonances were confirmed, when possible, by selective homonuclear decoupling experiments or by correlated spectroscopy. IR spectra were recorded on a FT-IR ESP spectrometer as thin films on NaCl plates. Mass spectra were recorded on an ion trap spectrometer with an ionization potential of 70 eV. High resolution mass spectra (HRMS) were performed with a resolution of 10 000 against a suitable mass standard. The commercially available reagents were used as received without further purification.

20-OTES-camptothecin (4). To a suspension of camptothecin (**1**) (100 mg, 0.29 mmol) in dry DMF (3 mL), imidazole (98 mg, 1.44 mmol) was added. After 10 min triethylsilyl chloride (0.19 mL, 1.15 mmol) was introduced dropwise, followed by DMAP (40 mg, 0.29 mmol). The mixture was stirred at room temperature until TLC analysis (CH_2Cl_2−MeOH, 30:1) showed complete disappearance of the starting material (60 h). Excess of solvents was removed under diminished pressure, and water was added to the residue. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3), and the organic phases were dried over Na_2SO_4, filtered, and concentrated under vacuum. The resulting solid residue was purified by silica gel flash chromatography (CH_2Cl_2−MeOH, 30:1) to afford **4** (130 mg, 98%) as a pale-yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.37 (s, 1 H, Ar, H-7), 8.25 (d, 1 H, J = 8.4 Hz, Ar), 7.92 (d, 1 H, J = 8.0 Hz, Ar), 7.82 (t, 1 H, J = 8.0 Hz, Ar), 7.65 (t, 1 H, J = 8.4 Hz, Ar), 7.57 (s, 1 H, H-14), 5.67 (d, 1 H, J = 16.4 Hz, H-17), 5.29 (s, 2 H, H-5), 5.25 (d, 1 H, J = 16.4 Hz, H-17), 2.00−1.84 (m, 2 H, H-19), 1.03−0.93 (m, 12 H), 0.80−0.71 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.7, 157.6, 152.5, 151.5, 149.0, 145.9, 130.9, 130.4, 130.0, 128.4, 128.1, 128.0, 127.9, 118.9, 94.4, 75.3, 66.0, 50.0, 33.2, 7.9, 7.2, 6.4. MS (70 eV): m/z (rel intensity), 433 [M^+ − 29] (89), 390 [M^+ − 72] (100). HRMS m/z calcd for $C_{26}H_{30}N_2O_4Si$ 462.197, m/z found 462.193.

General Procedure Electrophilic Addition to 20-OTES-CPT (4). Method A. A solution of 20-OTES-Camptothecin (**4**) (1 equiv) in dry THF (6 mL) was cooled to −78 °C, and 1.0 M LHMDS in THF (1.2 equiv) was added dropwise. The solution was stirred at this temperature for 30 min, and then a solution of the electrophile (1.3 equiv) in THF (2 mL) was introduced dropwise. The solution was stirred at −78 °C for 2 h and then warmed to 20 °C and stirred for additional 2 h. The reaction mixture was treated with saturated NH_4Cl and extracted with CH_2Cl_2, dried over Na_2SO_4, and filtered, and the solvent was removed under vacuum. The crude mixture was purified by silica gel flash chromatography employing the above-reported eluents.

General Procedure for He Electrophilic Addition to 20-OTES-CPT (4). Method B. A solution of 20-OTES-camptothecin (**4**) (1.0 equiv) in dry DMF (4 mL) was cooled to 0 °C, and ^{t}BuOK (1.3 equiv) was added. The solution was stirred at 0 °C for 5 min, and then a solution of the electrophile (5.0 equiv) in dry DMF (0.2 mL) was introduced dropwise. The solution was stirred for 15 min at this temperature and allowed to warm to 20 °C. After an additional 30 min, the reaction mixture was diluted with water (10 mL) and extracted with EtAcO. The organic layers were washed with brine, dried over Na_2SO_4, and filtered, and the solvents were removed under vacuo. The mixture of diastereoisomers was purified by flash chromatography employing the above-reported eluents.

20-OTES-5-fluorocamptothecin (11). Method A was employed. The crude mixture was purified by silica gel flash chromatography. Step gradient elution with n-hexane−AcOEt, 3:1 followed by 2:1 and 1:1, allowed us to separate the 1:1 mixture of diastereoisomers **11a** and **11b** (overall yield 97%). **11a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (s, 1 H, Ar, H-7), 8.25 (d, 1 H, J = 8.4 Hz, Ar), 7.96 (d, 1 H, J = 8.4 Hz, Ar), 7.87 (t, 1 H, J = 8.4 Hz, Ar), 7.69 (t, 1 H, J = 8.4 Hz, Ar), 7.47 (d, 1 H, $^1J_{HF}$ = 61.2 Hz, H-5), 7.45 (s, 1 H, H-14), 5.62 (d, 1 H, J = 16.8 Hz, H-17), 5.22 (d, 1 H, J = 16.8 Hz, H-17), 2.02−1.84 (m, 2 H, H-19), 1.03−0.93 (m, 12 H), 0.80−0.71 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4, 157.5, 152.3, 151.2, 150.3, 143.6, 133.7, 131.7, 130.2, 128.9, 128.4, 127.9, 126.3, 121.8, 98.9, 93.8 (d, $^1J_{CF}$ = 213.2 Hz, C-5), 75.1, 65.7, 33.1, 7.8, 7.2, 6.4. HRMS m/z calcd for $C_{26}H_{29}FN_2O_4Si$ 480.188, m/z found 480.195. **11b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.51 (s, 1 H, Ar, H-7), 8.25 (d, 1 H, J = 8.4 Hz, Ar), 7.96 (d, 1 H, J = 8.4 Hz, Ar), 7.87 (t, 1 H, J = 8.4 Hz, Ar), 7.68 (t, 1 H, J = 8.4 Hz, Ar),

7.51 (d, 1 H, $^1J_{HF}$ = 60.8 Hz, H-5), 7.42 (s, 1 H, H-14), 5.62 (d, 1 H, *J* = 17.2 Hz, H-17), 5.20 (d, 1 H, *J* = 17.2 Hz, H-17), 2.02−1.82 (m, 2 H, H-19), 1.04−0.93 (m, 12 H), 0.80−0.71 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.2, 157.8, 152.5, 151.2, 150.3, 143.7, 133.7, 131.7, 130.2, 128.9, 128.3, 127.9, 126.3, 121.8, 99.0, 93.8 (d, $^1J_{CF}$ = 214.8 Hz, C-5), 75.0, 65.8, 33.3, 7.9, 7.1, 6.4. HRMS *m*/*z* calcd for $C_{26}H_{29}FN_2O_4Si$ 480.188, *m*/*z* found: 480.192.

20-OTES-5-azidocamptothecin (17). Method A was employed. The crude mixture was purified by silica gel flash chromatography. Step gradient elution with *n*-hexane−AcOEt, 3:1 followed by 2:1 and 1:1, allowed us to separate the 1:1 mixture of diastereoisomers **17a** and **17b** (overall yield 97%). **17a**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.45 (s, 1 H, Ar, H-7), 8.25 (d, 1 H, *J* = 8.4 Hz, Ar), 7.95 (d, 1 H, *J* = 8.4 Hz, Ar), 7.86 (t, 1 H, *J* = 8.4 Hz, Ar), 7.68 (t, 1 H, *J* = 8.4 Hz, Ar), 7.49 (s, 1 H, H-14), 6.97 (s, 1 H, H-5), 5.65 (d, 1 H, *J* = 16.8 Hz, H-17), 5.26 (d, 1 H, *J* = 16.8 Hz, H-17), 2.01−1.84 (m, 2 H, H-19), 1.03−0.94 (m, 12 H), 0.80−0.71 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.6, 158.3, 152.2, 150.8, 150.0, 144.0, 132.9, 131.4, 130.1, 128.6, 128.3, 128.2, 128.1, 120.8, 98.7, 75.4, 75.2, 65.7, 33.1, 7.9, 7.2, 6.4. HRMS *m*/*z* calcd for $C_{26}H_{29}N_5O_4Si$ 503.199, *m*/*z* found: 503.191. **17b**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.45 (s, 1 H, Ar, H-7), 8.24 (d, 1 H, *J* = 8.4 Hz, Ar), 7.95 (d, 1 H, *J* = 8.4 Hz, Ar), 7.86 (t, 1 H, *J* = 8.4 Hz, Ar), 7.68 (t, 1 H, *J* = 8.4 Hz, Ar), 7.46 (s, 1 H, H-14), 6.99 (s, 1 H, H-5), 5.66 (d, 1 H, *J* = 16.8 Hz, H-17), 5.22 (d, 1 H, *J* = 16.8 Hz, H-17), 2.02−1.84 (m, 2 H, H-19), 1.03−0.94 (m, 12 H), 0.80−0.71 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.4, 158.4, 152.3, 150.9, 150.0, 144.0, 132.9, 131.4, 130.1, 128.6, 128.3, 128.2, 128.1, 120.8, 98.7, 75.3, 75.1, 65.8, 33.3, 7.9, 7.2, 6.4. HRMS *m*/*z* calcd for $C_{26}H_{29}N_5O_4Si$ 503.199, *m*/*z* found 503.192.

20-OTES-5-DTBAC-camptothecin (14). Method A was employed. The crude mixture was purified by silica gel flash chromatography. Elution with *n*-hexane−AcOEt, 3:1, allowed the separation of the 1:1 mixture of diastereoisomers **14a** and **14b** as pale-yellow solids (overall yield 97%). **14a**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.80 (br s, 1 H, Ar), 8.23 (d, 1 H, *J* = 8.4 Hz, Ar), 8.01 (br d, 1 H, Ar), 7.90−7.71 (m, 2 H, Ar), 7.70−7.45 (m, 2 H, Ar + H-14), 6.52 (br s, 1 H, H-5), 5.61 (d, 1 H, *J* = 16.8 Hz, H-17), 5.23 (d, 1 H, *J* = 16.8 Hz, H-17), 2.03−1.81 (m, 2 H, H-19), 1.79−1.08 (br s, 18 H), 1.06−0.92 (m, 12 H), 0.80−0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.7, 157.8, 155.5, 155.5, 152.0, 152.0, 151.2, 149.4, 145.0, 132.1, 130.6, 130.0, 128.7, 128.4, 127.9, 119.9, 98.2, 82.7, 81.5, 79.7, 75.2, 65.7, 33.2, 28.3, 27.6, 7.7, 7.2, 6.4. HRMS *m*/*z* calcd for $C_{36}H_{48}N_4O_8Si$ 692.324, *m*/*z* found 692.319. **14b**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.79 (br s, 1 H, Ar), 8.23 (d, 1 H, *J* = 8.4 Hz, Ar), 8.01 (br d, 1 H, Ar), 7.85−7.76 (m, 2 H, Ar), 7.65 (br t, 1 H, *J* = 8.4 Hz, Ar), 7.52 (s, 1 H, H-14), 6.54 (br s, 1 H, H-5), 5.61 (d, 1 H, *J* = 16.8 Hz, H-17), 5.22 (d, 1 H, *J* = 16.8 Hz, H-17), 2.03−1.82 (m, 2 H, H-19), 1.76−1.08 (br s, 18 H), 1.04−0.92 (m, 12 H), 0.80−0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.5, 157.9, 155.5, 155.5, 152.3, 152.0, 151.2, 149.4, 145.1, 132.1, 130.6, 130.0, 128.7, 128.4, 127.9, 119.9, 98.2, 82.9, 81.5, 79.6, 75.2, 65.8, 33.3, 28.3, 27.4, 7.8, 7.2, 6.4. HRMS *m*/*z* calcd for $C_{36}H_{48}N_4O_8Si$ 692.324, *m*/*z* found 692.317.

20-OTES-5DBAC-camptothecin (13). Method A was employed. The crude mixture was purified by silica gel flash chromatography. Step gradient elution with *n*-hexane−AcOEt, 4:1 followed by 7:2, allowed the separation of the 1:1 mixture of diastereoisomers **13a** and **13b** (overall yield 98%). **13a**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.70 (br s, 1 H, Ar), 8.39 (br s 1 H, Ar), 8.22 (br d, 1 H, *J* = 7.6 Hz, Ar), 7.95 (br d, 1 H, *J* = 7.6 Hz, Ar), 7.83 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.65 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.64−7.00 (m, 11 H, Ar + H-14), 6.49 (br s, 1 H, H-5), 5.57 (d, 1 H, *J* = 16.4 Hz, H-17), 5.47−4.44 (m, 5 H), 1.98−1.82 (m, 2 H, H-19), 1.02−0.89 (m, 12 H), 0.80−0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.6, 158.0, 156.3, 156.3, 153.0, 152.2, 151.0, 149.6, 144.8, 135.3, 132.1, 130.6, 130.0, 128.6−127.8 (11 C), 119.9, 98.4, 79.5, 75.2, 68.4, 67.9, 65.6, 33.0, 7.9, 7.2, 6.4. HRMS *m*/*z* calcd for $C_{42}H_{44}N_4O_8Si$ 760.293, *m*/*z* found 760.289. **13b**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.85 (br s, 1 H, Ar), 8.58 (br s 1 H, Ar), 8.20 (br s, 1 H, Ar), 7.93 (br s, Ar), 7.81 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.63 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.56−6.90 (m, 11 H, Ar + H-14), 6.52 (br s, 1 H, H-5), 5.55 (d, 1 H, *J* = 16.8 Hz, H-17), 5.44−4.71 (m, 5 H), 1.98−1.80 (m, 2 H, H-19), 1.05−0.90 (m, 12 H), 0.81−0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.5, 157.9, 156.4, 156.4, 152.9, 152.4, 150.9, 149.4, 144.8, 135.3, 132.1, 130.6, 129.9, 128.6−127.8 (11 C), 119.9, 98.5, 79.3, 75.2, 68.4, 67.8, 65.6, 32.9, 7.8, 7.2, 6.4. HRMS *m*/*z* calcd for $C_{42}H_{44}N_4O_8Si$ 760.293, *m*/*z* found 760.297.

20-OTES-5-OH-camptothecin (9). Method A was employed. The crude mixture was purified by silica gel flash chromatography. Elution with *n*-hexane−AcOEt, 3:1 followed by 7:2, afforded **9** as pale-yellow solids in 95% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.48 (s, 0.5 H, Ar, H-7), 8.47 (s, 0.5 H, Ar, H-7), 8.25 (d, 1 H, *J* = 8.8 Hz, Ar), 7.96 (d, 1 H, *J* = 8.8 Hz, Ar), 7.85 (t, 1 H, *J* = 8.8 Hz, Ar), 7.67 (t, 1 H, *J* = 8.8 Hz, Ar), 7.49 (s, 0.5 H, H-14), 7.48 (s, 0.5 H, H-14), 7.09 (d, 0.5 H, *J* = 2.8 Hz, H-5), 7.05 (d, 0.5, *J* = 2.8 Hz, H-5), 5.63 (d, 0.5 H, *J* = 16.8 Hz, H-17), 5.62 (d, 0.5 H, *J* = 16.8 Hz, H-17), 5.55 (bs, 0.5 H, OH), 5.51 (bs, 0.5 H, OH), 5.23 (d, 0.5 H, *J* = 16.8 Hz, H-17), 5.21 (d, 0.5 H, *J* = 16.8 Hz, H-17), 1.94−1.86 (m, 2 H, H-19), 0.98−0.92 (m, 12 H), 0.78−0.72 (m, 6 H). HRMS *m*/*z* calcd for: $C_{26}H_{30}N_2O_5Si$ 478.192, *m*/*z* found: 478.187.

20-OTES-5-methylcamptothecin (5). Method B was employed. For the electrophile, the crude mixture was purified by silica gel flash chromatography. Elution with *n*-hexane−EtOAc, 1:3, afforded **5** as an inseparable 1:1 mixture of epimers (pale-yellow solid, 40% yield). ^{1}H NMR ($CDCl_3$) *δ* 8.25 (d, 1 H, *J* = 8.4 Hz, Ar), 8.25 (s, 1 H, Ar), 7.92 (d, 1 H, *J* = 8.4 Hz, Ar), 7.81 (t, 1 H, *J* = 8.4 Hz, Ar), 7.64 (t, 1 H, *J* = 8.4 Hz, Ar), 7.54 (s, 0.5 H, Ar), 7.52 (s, 0.5 H, Ar), 5.74 (m, 1 H, H-5), 5.66 (d, 0.5 H, *J* = 16.8 Hz), 5.63 (d, 0.5 H, *J* = 16.8 Hz), 5.24 (d, 0.5 H, *J* = 16.8 Hz), 5.22 (d, 0.5 H, *J* = 16.8 Hz), 1.98−1.84 (m, 5 H, H-19 + Me), 1.04−0.96 (m, 12 H), 0.82−0.74 (m, 6 H). ^{13}C NMR ($CDCl_3$) *δ* 171.9, 158.1, 151.7, 151.2, 149.3, 145.4, 134.7, 130.3, 130.1, (130.0−130.0), 128.3, 128.1, 127.8, 119.9, (98.1−98.0), 75.3, (66.2−66.0), (59.2−59.1), (33.2−33.2), (18.9−18.5), (7.9−7.9), 7.2, 6.5. HRMS *m*/*z* calcd for $C_{27}H_{32}N_2O_4Si$ 476.213, *m*/*z* found 476.209.

20-OTES-5-(3-hydroxypropyl)camptothecin (6). Method B was employed. For the electrophile, the crude mixture was purified by silica gel flash chromatography. Elution with *n*-hexane−EtOAc, 1:3 followed by 1:1, afforded **6** as an inseparable 1:1 mixture of epimers (pale-yellow solid, 41% yield). ^{1}H NMR ($CDCl_3$) *δ* 8.29 (s, 1 H, Ar), 8.22 (d, 1 H, *J* = 8.4 Hz, Ar), 7.91 (d, 1 H, *J* = 8.4 Hz, Ar), 7.82 (t, 1 H, *J* = 8.4 Hz, Ar), 7.65 (t, 1 H, *J* = 8.4 Hz, Ar), 7.63 (s, 0.5 H, Ar), 7.61 (s, 0.5 H, Ar), 5.86 (dd, 0.5 H, J_1 = 6.0 Hz, J_2 = 2.8 Hz, H-5), 5.82 (dd, 0.5 H, J_1 = 6.0 Hz, J_2 = 2.8 Hz, H-5), 5.72 (d, 0.5 H, *J* = 16.4 Hz, H-17), 5.70 (d, 0.5 H, *J* = 16.4 Hz, H-17), 5.29 (d, 0.5 H, *J* = 16.4 Hz, H-17), 5.27 (d, 0.5 H, *J* = 16.4 Hz, H-17), 3.75 (s, 1 H, O*H*), 3.55−3.49 (m, 2 H), 2.68−2.58 (m, 1 H), 2.52−2.44 (m, 1 H), 1.98−1.74 (m, 2 H, H-19), 1.25−1.10 (m, 2 H), 1.05 (t, 1.5 H, *J* = 7.6 Hz, Me), 1.04 (t, 1.5 H, *J* = 7.6 Hz, Me), 0.82 (s, 9 H), −0.03 (s, 3 H), −0.07 (s, 3 H). ^{13}C NMR ($CDCl_3$) *δ* (174.0−173.9), 157.9, 152.1, (149.8−149.8), 149.2, (146.5−146.5), (133.0−133.0), 130.5 (2C), 129.8, 128.2, 127.9, 119.4, (97.6−97.6), (72.7−72.7), (66.5−66.4), 62.8, (62.4−62.3), 31.6, (27.6−27.1), (26.3−26.1), 25.9, 18.2, (7.9−7.8), (−5.4 to −5.4). HRMS *m*/*z* calcd for $C_{29}H_{36}N_2O_5Si$ 520.239, *m*/*z* found 520.231.

General Procedure for Silyl Group Removal. To a solution of 20-OTES-CPT 5-substituted derivatives (1 equiv) in dry THF (5 mL), $Et_3N\cdot 3HF$ (7.5 equiv) was added dropwise. The mixture was reacted at room temperature until TLC analysis (*n*-hexane-AcOEt, 1:1) showed the complete conversion of the starting material (35 h). The solvent was removed under diminished pressure, and the residue was purified by silica gel flash chromatography.

20-OH-5-fluorocamptothecin (12a, 12b). Reaction of **11a** (**11b**) with $Et_3N\cdot 3HF$ afforded, after silica gel flash chromatography purification (*n*-hexane/EtOAc, 1:1), **12a** (**12b**) in 97% yield as a pale-yellow solid. **12a**: ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.52 (s, 1 H, Ar, H-7), 8.25 (d, 1 H, *J* = 8.4 Hz, Ar), 7.96 (d, 1 H, *J* = 8.4 Hz, Ar), 7.87 (t, 1 H, *J* = 8.4 Hz, Ar), 7.69 (t, 1 H, *J* = 8.4 Hz,

Ar), 7.59 (s, 1 H, H-14), 7.46 (d, 1 H, $^1J_{HF}$ = 61.2 Hz, H-5), 5.69 (d, 1 H, *J* = 16.8 Hz, H-17), 5.26 (d, 1 H, *J* = 16.8 Hz, H-17), 3.87 (br s, 1 H, OH), 2.01−1.81 (m, 2 H, H-19), 1.05 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5, 157.6, 151.1, 151.0, 150.2, 144.1, 133.9, 131.9, 130.0, 129.0, 128.5, 127.8, 126.4, 121.7, 98.8, 93.8 (d, $^1J_{CF}$ = 214.0 Hz, C-5), 72.5, 66.0, 31.5, 7.8. HRMS *m*/*z* calcd for $C_{20}H_{15}FN_2O_4$ 366.102, *m*/*z* found 366.109. **12b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (s, 1 H, Ar, H-7), 8.24 (d, 1 H, *J* = 8.4 Hz, Ar), 7.96 (d, 1 H, *J* = 8.4 Hz, Ar), 7.88 (t, 1 H, *J* = 8.4 Hz, Ar), 7.69 (t, 1 H, *J* = 8.4 Hz, Ar), 7.56 (s, 1 H, H-14), 7.51 (d, 1 H, $^1J_{HF}$ = 60.4 Hz, H-5), 5.69 (d, 1 H, *J* = 16.4 Hz, H-17), 5.25 (d, 1 H, *J* = 16.4 Hz, H-17), 3.87 (br s, 1 H, OH), 1.98−1.78 (m, 2 H, H-19), 1.04 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 157.7, 151.2, 151.2, 150.2, 144.2, 133.8, 131.9, 130.0, 129.0, 128.5, 127.8, 126.4, 121.6, 98.9, 93.7 (d, $^1J_{CF}$ = 214.0 Hz, C-5), 72.5, 66.1, 31.6, 7.8. HRMS *m*/*z* calcd for $C_{20}H_{15}FN_2O_4$ 366.102, *m*/*z* found 366.110.

20-OH-5-azidocamptothecin (18a, 18b). Reaction of **17a** (**17b**) with $Et_3N{\cdot}3HF$ afforded, after silica gel flash chromatography purification (*n*-hexane/EtOAc, 1:1), **18a** (**18b**) in 97% yield as a pale-yellow solid. **18a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.44 (s, 1 H, Ar, H-7), 8.24 (d, 1 H, *J* = 8.4 Hz, Ar), 7.93 (d, 1 H, *J* = 8.4 Hz, Ar), 7.85 (t, 1 H, *J* = 8.4 Hz, Ar), 7.67 (t, 1 H, *J* = 8.4 Hz, Ar), 7.63 (s, 1 H, H-14), 6.97 (s, 1 H, H-5), 5.70 (d, 1 H, *J* = 16.8 Hz, H-17), 5.29 (d, 1 H, *J* = 16.8 Hz, H-17), 3.99 (br s, 1 H, OH), 2.00−1.84 (m, 2 H, H-19), 1.04 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6, 158.3, 150.8, 150.7, 149.8, 144.4, 133.1, 131.5, 129.9, 128.6, 128.3, 128.3, 128.1, 120.6, 98.6, 75.4, 72.7, 66.0, 31.5, 7.8. HRMS *m*/*z* calcd for $C_{20}H_{15}N_5O_4$ 389.112, *m*/*z* found: 389.108. **18b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.45 (s, 1 H, Ar, H-7), 8.23 (d, 1 H, *J* = 8.4 Hz, Ar), 7.95 (d, 1 H, *J* = 8.4 Hz, Ar), 7.85 (t, 1 H, *J* = 8.4 Hz, Ar), 7.68 (t, 1 H, *J* = 8.4 Hz, Ar), 7.60 (s, 1 H, H-14), 7.00 (s, 1 H, H-5), 5.74 (d, 1 H, *J* = 16.8 Hz, H-17), 5.28 (d, 1 H, *J* = 16.8 Hz, H-17), 3.86 (br s, 1 H, OH), 1.98−1.82 (m, 2 H, H-19), 1.04 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 158.4, 150.9, 150.7, 149.8, 144.5, 133.0, 131.5, 129.9, 128.6, 128.4, 128.3, 128.1, 120.6, 98.6, 75.3, 72.6, 66.1, 31.6, 7.8. HRMS *m*/*z* calcd for $C_{20}H_{15}N_5O_4$ 389.112, *m*/*z* found 389.109.

20-OH-5-DTBAC-camptothecin (16a, 16b). Reaction of **14a** (**14b**) with $Et_3N{\cdot}3HF$ afforded, after silica gel flash chromatography purification (*n*-hexane/EtOAc, 3:2), **16a** (**16b**) in 97% yield as a pale-yellow solid. **16a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.77 (br s, 1 H, Ar), 8.16 (br d, 1 H, *J* = 8.0 Hz, Ar), 7.97 (br s, 1 H, Ar), 7.86−7.50 (m, 4 H, Ar), 6.51 (br s, 1 H, H-5), 5.66 (d, 1 H, *J* = 16.4 Hz, H-17), 5.24 (d, 1 H, *J* = 16.4 Hz, H-17), 3.86 (br s, 1 H, OH), 2.00−1.80 (m, 2 H, H-19), 1.79−1.13 (br s, 18 H), 1.03 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 157.9, 155.5, 155.5, 152.1, 151.3, 150.7, 149.6, 145.7, 132.3, 130.7, 129.9, 128.7, 127.9, 127.6, 120.0, 97.9, 82.8, 81.6, 79.7, 72.7, 66.1, 31.8, 28.3, 27.7, 7.7. **16b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.79 (br s, 1 H, Ar), 8.22 (br d, 1 H, *J* = 8.4 Hz, Ar), 7.99 (br s, 1 H, Ar), 7.88−7.50 (m, 4 H, Ar), 6.53 (br s, 1 H, H-5), 5.65 (d, 1 H, *J* = 16.4 Hz, H-17), 5.26 (d, 1 H, *J* = 16.4 Hz, H-17), 3.80 (br s, 1 H, OH), 2.00−1.80 (m, 2 H, H-19), 1.79−1.13 (br s, 18 H), 1.03 (t, 3 H, *J* = 7.2 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6, 157.9, 155.4, 155.4, 152.1, 151.3, 150.8, 149.5, 145.6, 132.3, 130.8, 129.8, 128.7, 127.9, 127.8, 119.8, 98.0, 83.0, 81.5, 79.7, 72.7, 66.3, 31.8, 28.3, 27.7, 7.8.

20-OH-5-DBAC-camptothecin (15a, 15b). Reaction of **13a** (**13b**) with $Et_3N{\cdot}3HF$ afforded, after silica gel flash chromatography purification (*n*-hexane/EtOAc, 1:1 followed by 2:3), **15a** (**15b**) in 98% yield as a pale-yellow solid. **15a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.67 (br s, 1 H, Ar), 8.39 (br s 1 H, Ar), 8.12 (br d, 1 H, *J* = 7.6 Hz, Ar), 7.95 (br s, 1 H, Ar), 7.74 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.65−6.66 (m, 12 H, Ar + H-14), 6.48 (br s, 1 H, H-5), 5.55 (d, 1 H, *J* = 16.0 Hz, H-17), 5.42−4.44 (m, 5 H), 3.86 (br s, 1 H, OH), 1.92−1.72 (m, 2 H, H-19), 0.95 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5, 158.0, 156.2, 156.0, 153.0, 150.9, 150.9, 149.5, 145.3, 135.4, 132.2, 130.7, 129.8, 128.7−127.8 (11 C), 119.9, 98.2, 79.6, 72.7, 68.5, 68.0, 65.9, 31.6, 7.8. **15b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.71 (br s, 1 H, Ar), 8.34 (br s 1 H, Ar), 8.18 (br s, 1 H, Ar), 7.94 (br s, 1 H, Ar), 7.79 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.70−6.70 (m, 12 H, Ar + H-14), 6.52 (br s, 1 H, H-5), 5.53 (d, 1 H, *J* = 16.4 Hz, H-17), 5.44−4.48 (m, 5 H), 3.87 (br s, 1 H, OH), 1.90−1.70 (m, 2 H, H-19), 0.99 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 158.0, 156.3, 156.1, 153.0, 151.0, 150.9, 149.6, 145.3, 135.5, 132.3, 130.8, 129.8, 128.7−127.8 (11 C), 119.8, 98.4, 79.5, 72.7, 68.5, 67.8, 66.0, 31.6, 7.7.

20-OH-5-methylcamptothecin (7). Reaction of **5** with $Et_3N{\cdot}3HF$ afforded, after silica gel flash chromatography purification (*n*-hexane/EtOAc, 1:3), **7** in 98% yield as a pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1 H, Ar), 8.24 (d, 1 H, *J* = 8.4 Hz, Ar), 7.92 (d, 1 H, *J* = 8.4 Hz, Ar), 7.82 (t, 1 H, *J* = 8.4 Hz, Ar), 7.65 (t, 1 H, *J* = 8.4 Hz, Ar), 7.63 (s, 1 H, Ar), 5.80−5.70 (m, 2 H, H-5 + H-17), 5.30 (d, 0.5 H, *J* = 16.4 Hz, H-17), 5.28 (d, 0.5 H, *J* = 16.4 Hz, H-17), 1.98−1.84 (m, 5 H, H-19 + Me), 1.06 (t, 1.5 H, *J* = 7.6 Hz, Me), 1.05 (t, 1.5 H, *J* = 7.6 Hz, Me).

20-OH-5-(3-hydroxypropyl)camptothecin (8). Reaction of **6** with $Et_3N{\cdot}3HF$ afforded, after silica gel flash chromatography purification (CH_2Cl_2−MeOH, 30:1), **8** in 98% yield as a as pale-yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1 H, Ar), 8.22 (d, 1 H, *J* = 8.4 Hz, Ar), 7.92 (d, 1 H, *J* = 8.4 Hz, Ar), 7.82 (t, 1 H, *J* = 8.4 Hz, Ar), 7.66 (t, 1 H, *J* = 8.4 Hz, Ar), 7.65 (s, 0.5 H, Ar), 7.63 (s, 0.5 H, Ar), 5.88 (dd, 0.5 H, J_1 = 6.4 Hz, J_2 = 2.8 Hz, H-5), 5.82 (bt, 0.5 H, *J* = 4.8 Hz, H-5), 5.71 (d, 0.5 H, *J* = 16.0 Hz, H-17), 5.70 (d, 0.5 H, *J* = 16.0 Hz, H-17), 5.29 (d, 0.5 H, *J* = 16.0 Hz, H-17), 5.27 (d, 0.5 H, *J* = 16.0 Hz, H-17), 3.76 (s, 1 H, O*H*), 3.71−3.48 (m, 2 H), 2.68−2.48 (m, 2 H), 1.98−1.74 (m, 2 H, H-19), 1.48−1.30 (m, 2 H), 1.06 (t, 1.5 H, *J* = 7.6 Hz, Me), 1.04 (t, 1.5 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$) δ 173.9, 158.0, 150.1, 149.2, 148.8, 146.4, 133.2, 130.6, 130.3, 129.8, (128.3−128.2), 128.0, 119.4, 97.9, (72.7−72.7), (66.4−66.3), 62.4, 62.0, 31.6, (27.9−27.4), (26.5−26.3), (7.9−7.8).

Synthesis of 20. To a solution of 20-OTES-5-DTBAC-CPT (**14**) (225 mg, 0.32 mmol) in dry dichloroethane (8 mL), TFA (0.9 mL, 11.7 mmol) was added. The mixture was stirred at room temperature for 20 h and then refluxed for 4 h. The excess of solvents was removed under reduced pressure, and the residue was purified by silica gel flash chromatography (CH_2Cl_2−MeOH, 30:1). The product was further purified by a second chromatography (toluene−AcOEt, 1:1) affording **20** as a yellow solid (84 mg, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.61 (br s, 0.5 H, N^5−N*H*=C^{16a}), 10.39 (br s, 0.5 H, N^5−N*H*=C^{16a}), 8.67 (s, 1 H, Ar, H-7), 8.22−8.15 (m, 1 H, Ar), 7.96−7.92 (m, 1 H, Ar), 7.88−7.78 (m, 1 H, Ar), 7.69−7.60 (m, 2 H, Ar), 6.38−6.36 (m, 1 H, Ar, H-5), 5.72−5.62 (m, 1 H, Ar, H-17), 5.32−5.20 (m, 2 H, Ar, H-17 + N^5*H*), 4.08−3.86 (br s, 1 H, OH), 1.96−1.74 (m, 2 H, H-19), 1.05−0.98 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4 (0.5 C), 173.8 (0.5 C), 159.1, 159.0, 156.7 (q CF_3COOH), 156.5 (q CF_3COOH) 151.5, 151.3, 150.7, 150.5, 150.1, 149.9, 144.8, 144.7, 134.0, 133.8, 131.6, 131.5, 129.9, 129.8, 128.7, 128.7, 128.4, 128.4, 128.2, 128.2, 127.1, 126.9, 120.5, 120.3, 99.1(2 C), 78.9, 78.6, 72.7, 72.7, 66.0 (2 C), 31.7 (2 C), 7.7, 7.7. HRMS *m*/*z* calcd for $C_{22}H_{17}F_3N_4O_5$ 474.115, *m*/*z* found 474.109.

Synthesis of 21. DDQ (25 mg, 0.110 mmol) was added to a solution of **20** (20 mg, 0042 mmol) in dry THF (4 mL). The reaction mixture was warmed to 60 °C and reacted at this temperature for 3 h. The solvent was removed under reduced pressure, and water was added. The aqueous layers were extracted with CH_2Cl_2 (15 mL × 3), and the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (*n*-hexane−OEtAc, 1:1) to afford **21** as a yellow solid (14 mg, 94%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.89 (s, 1 H, Ar, H-7), 8.20 (d, 1 H, *J* = 8.4 Hz, Ar), 8.00 (d, 1 H, *J* = 8.4 Hz, Ar), 7.88 (t, 1 H, *J* = 8.4 Hz, Ar), 7.79 (s, 1 H, Ar H-14), 7.69 (t, 1 H, *J* = 8.4 Hz, Ar), 5.70 (d, 1 H, *J* = 17.2 Hz, H-17), 5.28 (d, 1 H, *J* = 17.2 Hz, H-17), 3.83 (br s, 1 H, OH), 2.00−1.74 (m, 2 H, H-19), 1.08 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6, 157.4, 152.5, 150.8, 148.9, 143.7, 134.9, 132.5, 132.4, 130.0, 129.5, 128.7,

127.5, 122.6, 121.4, 101.2, 72.4, 66.0, 31.6, 7.7. HRMS *m/z* calcd for $C_{20}H_{14}N_4O_3$ 358.107, *m/z* found 358.101.

20-OH-5-NH_2-CPT (22). To a solution of 20-OH-5-azido-camptothecin (**18**) (50 mg, 0.129 mmol) in a mixture of dry THF (1.5 mL) and dry MeOH (6 mL) under nitrogen, 10% palladium on activated carbon (14 mg) was added. The oxygen dissolved in the reaction mixture was removed under vacuum. Then a hydrogen balloon was mounted and the mixture was stirred for 3 h at room temperature. The catalyst was removed by filtration through a Celite pad, and the filtrate was concentrated under reduced pressure to remove solvents. The crude product was purified by silica gel flash chromatography CH_2Cl_2−MeOH (step gradient elution 35:1 and 25:1) to afford **22** as a pale-yellow solid (46 mg, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.48 (s, 1 H, Ar, H-7), 8.22−8.17 (m, 1 H, Ar), 7.95−7.90 (m, 1 H, Ar), 7.85−7.78 (m, 1 H, Ar), 7.68−7.60 (m, 1 H, Ar), 7.58 (s, 0.5 H, H-14), 7.54 (s, 0.5 H, H-14) 6.50 (s, 0.5 H, H-5), 6.47 (s, 0.5 H, H-5), 5.74−5.64 (m, 1 H, H-17), 5.28−5.22 (m, 1 H, H-17), 4.00−2.40 (br s, 3 H, OH + NH_2), 1.98−1.82 (m, 2 H, H-19), 1.07−1.01 (m, 3 H, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8 (2 C), 158.5 (2 C), 151.2 (2 C), 150.4 (2 C), 149.7 (2 C), 144.5 (2 C), 132.7 (2 C), 131.0 (2 C), 129.8 (2 C), 128.5 (2 C), 128.3 (2 C), 128.0 (2 C), 127.8 (2 C), 120.2 (2 C), 113.8 (2 C), 97.7 (2 C), 72.7 (2 C), 66.3, 66.0 31.5 (2 C), 7.8, 7.8.

Growth Inhibition Assay. Human non-small-cell lung cancer NCI-H460 cells[26] were cultured in RPMI 1640 containing 10% FCS. Cell sensitivity was assessed by growth inhibition assay. Briefly, cells in the logarithmic phase of growth were seeded in duplicate into six-well plates. Twenty-four hours later, cells were exposed for 1 h to the drug and counted with a Coulter counter 72 h later. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth over that of untreated control.

Topoisomerase I Dependent DNA Cleavage Assay. A gel-purified 751-bp BamHI-EcoRI fragment of SV40 DNA was used for the cleavage assay.[27] DNA fragments were uniquely 3′-end labeled. Topoisomerase I-DNA cleavage reactions (20 000 cpm per sample) were done in 20 μL of 10 mM Tris-HCl (pH 7.6), 150 mM KCl, 5 mM $MgCl_2$, 15 μg/mL bovine serum albumin, 0.1 mM DTT, and the human recombinant enzyme (full-length topoisomerase I) for 30 min at 37 °C. Reactions were stopped using 0.5% SDS and 0.3 mg/mL of proteinase K for 45 min at 42 °C. DNA was resuspended in denaturing buffer (80% formamide, 10 mM NaOH, 0.01 mM EDTA, and 1 mg/mL dyes) before loading on a denaturing 8% polyacrylamide gel in Tris-borate EDTA buffer. Overall, DNA cleavage levels were measured with a PhosphoImager 425 model (Molecular Dynamics, Sunnyvale, CA).[28]

Computational Calculations. Topo I−DNA Complex Preparation. The structure of the ternary complex containing topoisomerase I (Topo I), DNA, and topotecan was downloaded from the Protein Data Bank (PDB code 1K4T).[29] One molecule of PEG and both topotecan forms (closed and open lacton ring) were removed. All of the atoms were fixed according to the MOE atom types.[30] Hydrogens were added and minimized using the AMBER99[3] force field and AMBER99 charges[31] until the rms value of the truncated Newton method (TN) was <0.01 kcal mol^{-1} Å^{-1}.

The structures of the 5-subsituted CPT analogues were constructed in MOE, and energy was minimized using the MMFF94 force field.[32]

Molecular Docking (QM-Polarized Ligand Docking). In order to predict the binding mode of the new 5-modified CPT analogues into the Topo I−DNA binary complex, we used the quantum mechanics (QM) polarized ligand docking (QPLD) protocol,[33,34] which is implemented in the Schrödinger software suite. The QPLD protocol consists of three steps: in the first one protein−ligand complexes are generated with GLIDE. The docking algorithm in GLIDE utilizes a hierarchical search protocol, in which the final step is minimization of a flexible ligand in the field of the Coulomb and van der Waals potential of the protein, as represented by the OPLS-AA[35] molecular mechanics potential energy function. The scoring function for computing binding affinity, called GLIDE score, is an extension of an empirically based Chem-Score function.[36] Briefly, the value of the calculated *G*-score is a sum of van der Waals and Coulomb energy contributions to ligand−protein interaction; hydrogen-bonding, lipophilic contact, and metal binding (if present) terms are also taken into account. In the second step, a mixed quantum mechanical/molecular mechanics (QM/MM) method is used to compute the ligand charge distribution. For QM/MM calculations the QSITE program is used. The protein is defined as the MM region (force field, OPLS-AA), and the ligand is defined as the QM region. To represent the QM region, ab initio density functional theory (DFT) methods were used (6-31G* basis set and hybrid DFT functional B3LYP). At each configuration, a single point energy calculation was performed and the output was used for further computation. The optimization of the QM wave function was performed incorporating coupling of the surrounding MM point charges. It is the effect of these point charges that leads to polarization of the ligand charge distribution. After performance of the DFT calculation for the ligand, atomic charges are assigned employing electrostatic potential (ESP) fitting. In the third step, the ligands are submitted to another GLIDE docking run where the ligand charges are substituted with the new charge sets calculated in the second step. Finally, GLIDE returns the most energetically favorable poses.

Supporting Information Available: Elemental analysis data for all new and enantiomeric pure compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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