Novel 7-Oxyiminomethyl Derivatives of Camptothecin with Potent in Vitro and in Vivo Antitumor Activity

Sabrina Dallavalle,[†] Anna Ferrari,[†] Barbara Biasotti,[†] Lucio Merlini,*[,†] Sergio Penco,[⊥] Grazia Gallo,[⊥] Mauro Marzi,[⊥] Maria Ornella Tinti,[⊥] Roberta Martinelli,[⊥] Claudio Pisano,[⊥] Paolo Carminati,[⊥] Nives Carenini,[§] Giovanni Beretta,[§] Paola Perego,[§] Michelandrea De Cesare,[§] Graziella Pratesi,[§] and Franco Zunino*[,§]

Dipartimento di Scienze Molecolari Agroalimentari, Sezione di Chimica, Università di Milano, Via Celoria 2, 20133 Milano, Italy, R & D, Sigma-tau, Pomezia, Italy, and Dipartimento Oncologia Sperimentale, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milano, Italy

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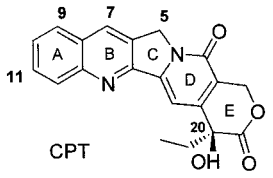
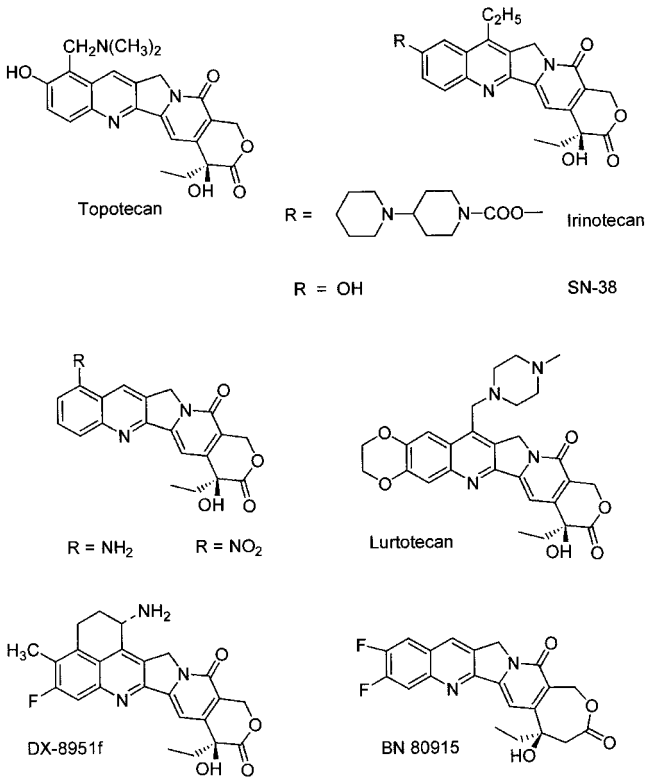
In an attempt to synthesize potential anticancer agents acting by inhibition of topoisomerase I (Topo I) a new series of oxyiminomethyl derivatives in position 7 of camptothecin (CPT) was prepared. The synthesis relied on the condensation of 20*S*-CPT-7-aldehyde or 20*S*-CPT-7-ketones with alkyl, aryl, heteroaryl, arylalkyl, and heteroarylalkyl O-substituted hydroxylamines. The compounds were tested for their cytotoxic activity in vitro against H460 non-small lung carcinoma cell line, the activity being for 24 out of 37 compounds in the 0.01−0.3 μM range. A QSAR analysis indicated that lipophilicity is the main parameter correlated with cytotoxicity. Investigation of the DNA−Topo I−drug cleavable complex showed a rough parallelism between cytotoxicity and inhibition of Topo I. Persistence of the DNA cleavage after NaCl-mediated disruption of the ternary complex suggests that for the most potent compounds, e.g., **15**, the cytotoxicity was at least in part related to stabilization of the complex, as also supported by the persistence of the DNA−enzyme complex in drug-treated cells. The in vivo antitumor efficacy of the most potent analogue (**15**) was evaluated in direct comparison with topotecan using human lung tumor xenograft models. In the range of optimal doses (2−3 mg/kg), the improved efficacy of **15** was documented in terms of inhibition of tumor growth and rate of complete response.

Introduction

Derivatives of camptothecin (CPT, **1**)[1] are an emerging class of antitumor drugs. Lack of interest for CPT itself for 20 years due to toxicity in humans was followed by a resurgence of studies when its mechanism of action, i.e., inhibition of the ubiquitous enzyme topoisomerase I, was discovered.[2] Topoisomerase I is an essential enzyme for topological DNA modifications during a number of critical cellular processes, including replication, transcription, and repair.[3] Stabilization of the covalent topoisomerase I−DNA complex by camptothecins (so-called "cleavable complex") results in topoisomerase I-mediated DNA breaks by preventing DNA religation.



Elucidation of the structural requisites for activity and extensive exploration of the possible modifications have led to the synthesis of very potent derivatives, two of which, topotecan (Hycamtin) (**2**) and irinotecan (Camptosar) (**3**), are already in clinical practice. Irinotecan is the prodrug of the active SN-38. Others, among them 9-aminocamptothecin, 9-nitrocamptothecin (Rubitecan), G7147211 (Lurtotecan), DX-8951f (Exatecan mesylate), and BN 80915 are in various stages of clinical development.[6]



* Authors to whom correspondence should be addressed. E-mail: lucio.merlini@unimi.it, zunino@istitutotumori.mi.it.
[†] Università di Milano.
[⊥] Sigma-tau.
[§] Istituto Nazionale Tumori.

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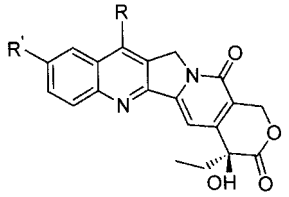
Table 1. Data and Cytotoxic Activity (IC_{50}, μM) on H460 Human NSCLC Cell Line of the Oximes



cpd	R′	R	method	yield[a]	mp (°C)	δH[b] syn (ppm)	δH[b] anti (ppm)	IC_{50} (μM)
CPT								0.33 ± 0.05
topotecan								1.38 ± 0.95
SN-38								0.08 ± 0.05
5	H	CH=NOH	A	89	257	9.27	8.35	0.032 ± 0.09
8	H	CH=NOCOC$_6$H$_5$			200 dec	10.05	-	1.77 ± 0.1
9	H	C(C$_6$H$_5$)=NOH	A	45	>200 dec	-	-	5.13 ± 0.8
10	H	CH=NOCH$_3$	A	67	230 dec	9.30	8.40	0.04 ± 0.015
11	H	CH=NOCH$_3$ 1-*N*-oxide			>200 dec	9.21	-	2.40 ± 0.15
12	OH	CH=NOCH$_3$			268 dec	9.04	8.30	0.20 ± 0.06
13	H	CH=NOCH$_2$CH=CH$_2$	A	62	235 dec	9.38	8.46	0.06 ± 0.015
14	H	CH=NOCH$_2$CH(CH$_2$)O			154 dec	9.45	8.50	0.02 ± 0.002
15	H	CH=NOC(CH$_3$)$_3$	B	82	250 dec	9.31	8.40	0.015 ± 0.006
16	H	CH=NOC(CH$_3$)$_3$ 1-*N*-oxide			190 dec	9.20	-	0.070 ± 0.018
17	OH	CH=NOC(CH$_3$)$_3$			195 dec	9.00	8.25	0.13 ± 0.03
18	OMe	CH=NOC(CH$_3$)$_3$	B	34	250 dec	9.33	8.39	0.070 ± 0.04
19	H	CH=NOC(CH$_3$)$_2$CH$_2$OH	B	88	245 dec	9.30	8.40	0.34 ± 0.1
20	H	CH=NOC(CH$_3$)$_2$COOtBu	A	62	180 dec	9.39	8.51	0.14 ± 0.06
21	H	CH=NOCH$_2$CH$_2$NH$_2$	A	50	220 dec	9.27	8.43	0.49 ± 0.014
22	H	CH=NOCH$_2$CH$_2$N(CH$_3$)$_2$	A	76	232 dec	9.34	8.62	0.30 ± 0.13
23	H	CH=NOCH$_2$COOH	B	90	208 dec	9.27	8.37	2.33 ± 0.2
24	H	CH=NOC(CH$_3$)$_2$COOH	A	79	193 dec	9.10	-	48 ± 9.6
25	H	CH=NOCH$_2$CONH(CH$_2$)$_3$NH$_3^+$·Cl$^-$			222 dec	9.44	-	5.2 ± 2.1
26	H	CH=NOCH$_2$CONH(CH$_2$)$_3$NHBoc			216 dec	9.30	8.21	1.7 ± 0.2
27	H	CH=NOCH$_2$CONH(CH$_2$)$_3$NHNos			160 dec	9.25	-	3.0 ± 0.09
28	H	CH=NOCH$_2$CH$_2$-morpholinyl	A	30	160 dec	9.36	8.44	0.08 ± 0.008
29	H	CH=NOCH$_2$CH$_2$-3-(N-Me)piperidinyl	A	35	185 dec	9.35	8.40	0.10 ± 0.02
30	H	CH=NOCH$_2$CH$_2$-1-uracylyl	A	42	200 dec	9.35	8.48	0.5 ± 0.35
31	H	CH=NO-6-galattosyl			210 dec	9.35	8.50	32 ± 12.2
32	H	CH=NO-6-(bis-isopropylidenegalattosyl)	C	14	155 dec	9.40	8.50	0.36 ± 0.02
33	H	CH=NOC$_6$H$_5$	B	80	210 dec	9.84	8.92	0.16 ± 0.04
34	H	CH=NOCH$_2$C$_6$H$_5$	A	65	200 dec	9.38	8.45	0.03 ± 0.002
35	H	C(CH$_3$)=NOCH$_2$C$_6$H$_5$	A	25	>200 dec	-	-	0.13 ± 0.006
36	H	CH=NOCH$_2$C$_6$H$_4$-p-CH$_3$	A	42	203 dec	9.35	8.42	0.02 ± 0.005
37	H	CH=NOCH$_2$C$_6$H$_4$-p-NO$_2$	C	20	212 dec	9.50	8.52	0.017 ± 0.002
38	H	CH=NOCH$_2$C$_6$F$_5$	A	76	200 dec	9.35	8.50	0.028 ±0.002
39	H	CH=NOCH$_2$C$_6$H$_4$-p-NH$_2$	B	20	146 dec	9.30	8.40	0.025 ± 0.003
40	H	CH=NOCH$_2$C$_6$H$_4$-p−C$_6$H$_5$	A	54	202 dec	9.42	8.46	0.28 ± 0.14
41	H	CH=NOC(C$_6$H$_5$)$_3$	A	20	140 dec	9.63	8.50	1.45 ± 0.3
42	H	CH=NO−CH$_2$-9-anthracenyl	A	40	202 dec	9.30	8.38	0.19 ± 0.08
43	H	CH=NOCH$_2$-4-pyridyl	A	50	190 dec	9.47	8.62	0.03 ± 0.02
44	H	CH=NOCH$_2$-2-imidazolyl	A	50	168−172	9.35	8.45	0.21 ± 0.04

[a] Yield of the condensation with hydroxylamines. [b] In DMSO-d_6.

From structure−activity studies[7] it appears that the ring-E lactone and the natural 20*S*-configuration are essential for antitumor activity. The stability of the lactone ring in vivo appears to be an important factor for activity, because the hydrolysis in physiological conditions generates a substantial amount of the inactive carboxylate.[8] Whereas activity of compounds with substitutions in rings C and D is critically dependent on the size and type of substituents, most structural modifications have concerned rings A and B, where wide possibilities of variation exist, especially in positions 7, 9, 10, and 11. Recently the structure of the topoisomerase I covalent and noncovalent complexes with 22-base pair DNA duplexes has been solved by X-ray analysis. On this basis and on structure−activity relationships, a binding mode for camptothecin has been proposed.[9] In this and in an analogous model[10] there is wide space for substitutions in position 7 of camptothecin without steric clash.

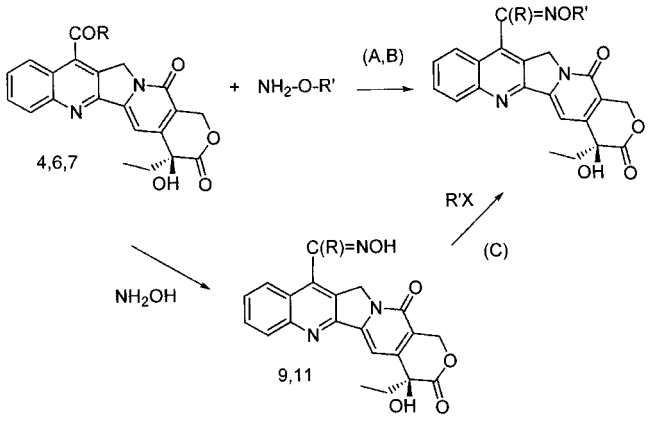
In two previous papers,[11,12] we have indicated the importance of a lipophilic group in position 7 of camptothecin for potent cytotoxic activity. This contention, already clearly stated some years ago by Burke and coworkers,[13] is supported by recent results of other groups, who have prepared and exploited lipophilic 7-silylcamptothecins.[14] The scarce solubility in water of these compounds does not represent a disadvantage, due to the possibility of successful administration per os of camptothecin derivatives.[15]

We report here on a novel series of 7-substituted camptothecins, the oxyiminomethyl derivatives, some of them exhibiting very potent in vitro and in vivo activity against a panel of human tumors.

Chemistry

The compounds prepared and tested are reported in Table 1. As a main starting material we used 20*S*-camptothecin-7-aldehyde (**4**), available from natural

Scheme 1

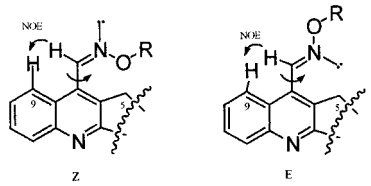


20*S*-camptothecin via Minisci free-radical hydroxymethylation and oxidation.[11,16,17] The condensation with O-substituted hydroxylamines to furnish the oximes (Scheme 1) was performed with free hydroxylamines (method B) or with the hydrochlorides in the presence of a base, usually pyridine (method A). In few cases the compounds were obtained in low yield by alkylation (method C) of the oxime of camptothecin-7-aldehyde (**5**).[16] The O-substituted hydroxylamines, when not commercially available, were prepared by alkylation of *N*-hydroxyphthalimide followed by hydrazinolysis or acid hydrolysis, or by similar alkylation of *N*-hydroxyurethane.[18] The derivatives of 10-hydroxycamptothecin-7-aldehyde (**12**, **17)** were synthesized by photochemical rearrangement[19] of the *N*-oxides of the corresponding oximes of 7-CPT. The synthesis of 10-methoxycamptothecin-7-aldehyde was reported in a preceding paper.[11]

The ketones 7-acetylcamptothecin (**6**) and 7-benzoylcamptothecin (**7**) were obtained by Minisci free radical acylation in sulfuric acid of CPT with the appropriate aldehydes in the presence of *t*-BuOOH and ferrous sulfate.[21] *N*-Oxides were prepared by oxidation with H_2O_2 in acetic acid.[19]

Some compounds (**8**, **11**, **12**, **14**, **16**, **17**, **24**−**27**, **31**) were prepared by further elaboration of an oxime. The synthesis of the oximes afforded in almost all cases a mixture of the *E* and *Z* diastereoisomers, with a high *E*/*Z* ratio. They showed separate signals in the ^{1}H NMR spectra, in particular the CH═N proton of the *E* isomer appeared constantly ca. 1 ppm at lower field (in DMSO) than that of the *Z* isomer. This is consistent with literature data on oximes[22] and *O*-alkyloximes.[23] The *Z* and *E* diastereoisomers are interconvertible at room temperature, the equilibrium ratio and kinetics depending on the solvent, light, and pH. Their relative amount could be measured by HPLC. UV spectra measured on HPLC peaks in CH_3CN/water showed a constant difference in pattern around 280−330 nm, which could be also used for distinction of the isomers. More interestingly, irradiation of the methyne hydrogen of both diastereoisomers in the ^{1}H NMR spectrum of, for example, compound **38** induced a remarkable NOE effect (ca. 7%) on H-9 of both isomer. No effect of the irradiation was observed on the signal of H-5 of both isomers. This indicates a strong preference for the conformation shown in Scheme 2. Molecular mechanics calculations for the same compound were consistent with these data, showing that in a conformation as that

Scheme 2



of Scheme 2 for the *E* isomer the distance between the two protons is ca. 2.09 Å.

The compounds synthesized and tested are reported in Table 1.

Results and Discussion

Cytotoxicity Studies. Cytotoxicities of the novel camptothecins were evaluated against a human non-small-cell lung carcinoma cell line, H460, using topotecan and SN-38 as reference compounds. This cell model was chosen for its sensitivity to topoisomerase I inhibitors, likely related to overexpression of the target enzyme.[24] The H460 cell line is also a useful model for in vivo studies of antitumor efficacy for its reproducible growth in athymic mice. The results of the cytotoxicity studies are summarized in Table 1.

All the 37 new oxyiminomethyl derivatives prepared showed potent cytotoxic activity, 27 of them being more active than topotecan, and 12 more active than SN-38. Among the alkyl derivatives, increase of the lipophilicity/bulkiness from Me to *t*-Bu was paralleled by an increase of activity, the *t*-Bu derivative **15** being the most active compound of the series. Introduction of an H-bonding or ionizable group (OH, COOH, primary amine) in the chain was detrimental (compare **13** vs **21** and **23**), the worst being the COOH (**15** vs **19** and **24**), whereas esterification of the carboxyl and alkylation or acylation of the primary amine or protection of the sugar OHs restored good activity (**20** vs **24**; **22**, **28** and **29** vs **21**, **26** and **27** vs **25**, **32** vs **31**). All these data pointed to the strong importance of a bulky lipophilic group linked to the oxime spacer. Substitution with a phenyl group (**33**) had a beneficial effect, but a benzyl (**34**, **36**−**40**) was undoubtedly better, possibly due to larger conformational mobility. According to the preceding observation, substitution on the benzyl group with groups with diverse electronic effects (including pyridine, **43**, and imidazole, **44**) had no relevance. There must be, however, some limit to the bulkiness and mobility of the groups (cf. **34** with **41**, and **42**). The introduction of steric hindrance near C-7 (derivatives of ketones instead of the aldehyde, i.e., **9** and **35**) was again detrimental, as well as N-oxidation (**11** vs **10**, and **16** vs **15**).

QSAR

A QSAR analysis was performed in order to predict cytotoxicity pIC_{50} values, employing descriptors related to the lipophilicity. A multiple regression linear analysis afforded the equation

$$Y = -0.039(X1) - 0.020(X2) + 0.332(X3) + 6.939$$

$$\text{with } N = 21,\ s = 0.441,\ F = 26.48,\ r^2 = 0.82$$

where X1 is the molecular volume (Å^3) of R1, X2 is the

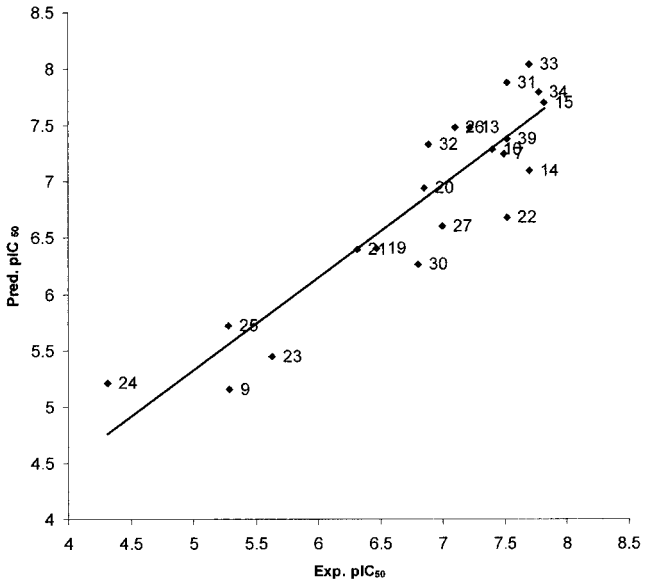
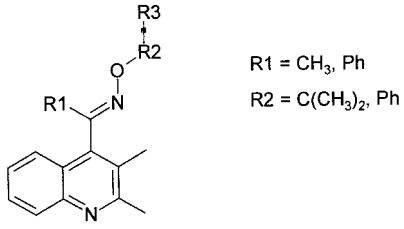


Figure 1. Plot of cytotoxic activity predicted on the basis of QSAR against experimental values on H460 cell line (IC_{50}, μM).

molecular volume (Å^3) of R2, X3 is ClogP or LogD (in case of ionizable groups). Compounds whose groups



R1 = CH_3, Ph

R2 = $C(CH_3)_2$, Ph

were not parametrized in ClogP (**8**, **31**, **32**) or with a logP larger than 4.0 (**40**−**43)** were excluded. The plot of the predicted pIC_{50} vs the experimental (Table 1) pIC_{50} values is reported in Figure 1. It can be seen that for most of the compounds (those with R1 = H) lipophilicity is the main factor correlated with the in vitro cytotoxicity.

Topoisomerase I-Mediated DNA Cleavage

The ability of selected camptothecin analogues of our series to inhibit topoisomerase I was investigated in the cleavable complex assay using purified human enzyme. The results presented in Figure 2 indicated that the most potent compound **15** as a cytotoxic agent was also very potent as a topoisomerase I poison. Conversely compound **9**, characterized by a low potency in the antiproliferative assay, was markedly less potent in the DNA cleavage assay.

However, no precise correlation between cytotoxicity and effects at target level could be expected, since the cytotoxicity could be influenced not only by the ability to poison topoisomerase I but also by the intracellular drug accumulation which is dependent on the liphophilic nature of the compounds. For example, the hydrophilic compound **21** was appreciably less cytotoxic than compounds **15** or **34** despite an effective inhibition of topoisomerase I and a persistent stabilization of the cleavable complex. Indeed, an interesting observation of our study was the persistence of DNA cleavage by the most cytotoxic analogues (e.g., compounds **15**, **34** and **37**) after addition of high salt concentration (0.6 M NaCl), which favors the dissociation of the ternary drug−enzyme−DNA complex (Figure 3). Thus, it is likely that the impressive increase of cytotoxic potency in compound **15** is the result not only of potent inhibition of enzyme function but also of the long-lasting stabilization of the ternary complex.

Stability of Topoisomerase I−DNA Complex in Drug-Treated Cells

Since the cytotoxic potency is expected to be related to the drug-induced stabilization of the topoisomerase I−DNA complex, the extent of the enzyme−DNA complex was determined in PC3 prostate carcinoma cells. Cells were treated with drugs for 1 h during exponential growth and incubated for 6 h in drug-free medium. Under these conditions, compound **15** induced a persistent stabilization of enzyme−DNA−complex. (Figure 4). The stabilization of the complex by this novel camptothecin is consistent with the results obtained in the DNA cleavage assay (Figure 2).

Antitumor Activity

The in vivo antitumor efficacy of the most potent analogue (**15**) was evaluated using oral administration using two human lung tumor xenograft models (Table 2). Topotecan, a clinically relevant camptothecin, was chosen as a reference drug, since, in contrast to irinotecan which is a prodrug, it allowed a direct comparison of activity under optimal conditions (i.e., same route and schedule for each drug). Indeed, in contrast to irinotecan, the activity of topotecan by oral route is well-known.[25] The cytotoxic potency of **15** was also reflected in a marked potency as antitumor agent in vivo, since the maximum tolerated dose (3 mg/kg), using a therapeutic schedule of treatment (q4dx4), was 5 times lower

Table 2. Comparison of Antitumor Activity of Topotecan and Compound **15** (per os, q4dx4) against Human Non-Small-Cell Lung Tumor Xenografts

		NCI−H460					LX-1				
	dose	TVI[a]	LCK[b]	CR[c]	BWL[d] (%)	lethal toxicity[e]	TVI[a]	LCK[b]	CR[c]	BWL[d] (%)	lethal toxicity[e]
topotecan	5	80	1.3	0/8	0	0/4					
	9	91	1.7	0/8	0	0/4					
	15	98	2.1	0/8	8	0/4	99	3.7	4/8	6	0/4
compd **15**	1	90	1.4	0/8	0	0/4					
	2	99	2.4	5/8	1	0/4	99	2.4	4/8	5	0/4
	3						100	5.3	8/8	11	0/4
	4	100	2.5	8/8	23	2/5					

[a] Tumor volume inhibition percentage in treated versus control mice. [b] Log10 cell kill induced by the treatment. [c] Complete responses: no evidence of tumor for at least 10 days after the end of treatment. [d] Percentage of body weight loss after drug treatment. [e] Number of dead mice/total number of mice.

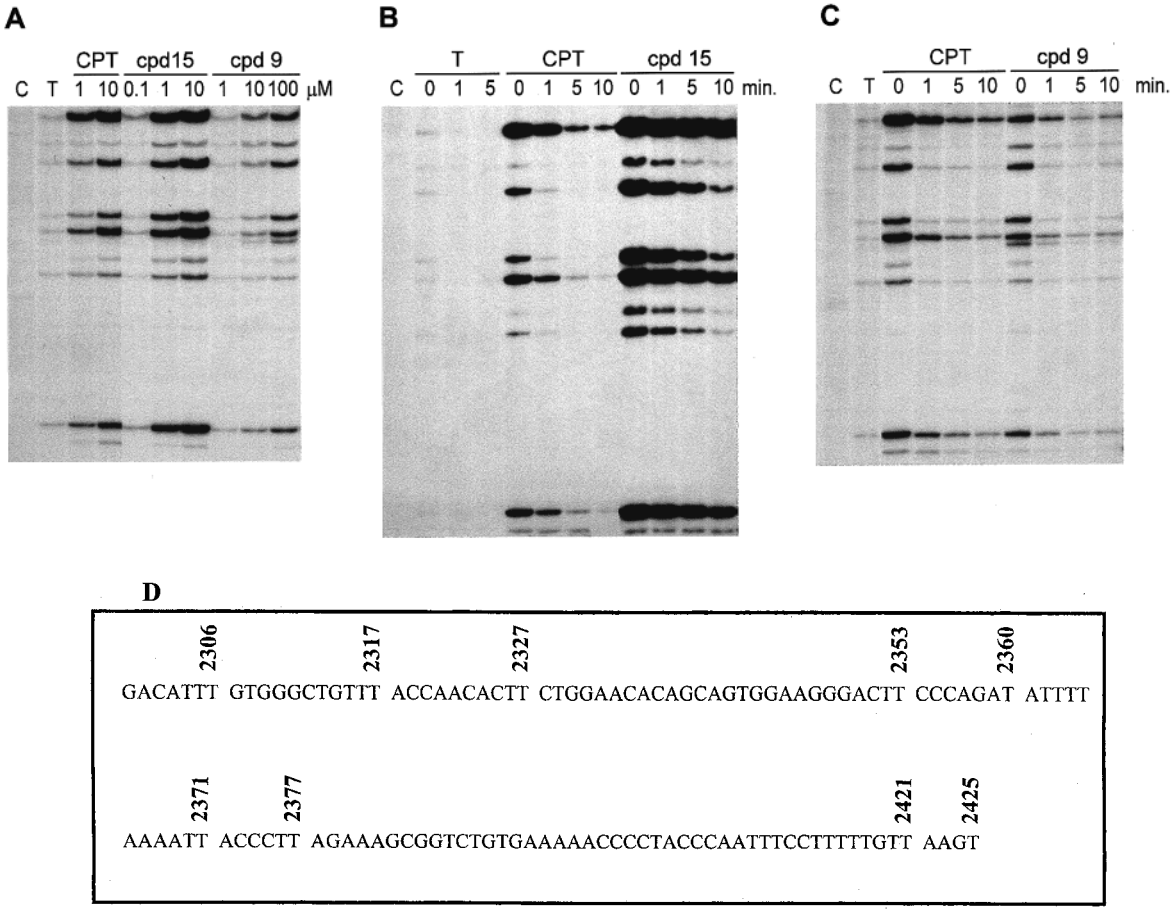


Figure 2. Stimulation of topoisomerase I-mediated DNA cleavage by camptothecin analogues (A) and persistence of DNA cleavage following addition of NaCl (B, C). C = DNA control, T = DNA and topoisomerase in the absence of drug. In experiments B and C aimed to examine the persistence of DNA cleavage, the concentrations of CPT, **15**, and **9** were 10, 10, and 100 μM, respectively. After 20 min of incubation of DNA with purified enzyme, DNA cleavage was reversed by adding 0.6 M NaCl to favor dissociation of DNA−enzyme complex and keeping the samples at 25 °C for the indicated times. A range of representative bands is shown. Details concerning the nucleotide positions of the cleavage sites are shown in D.

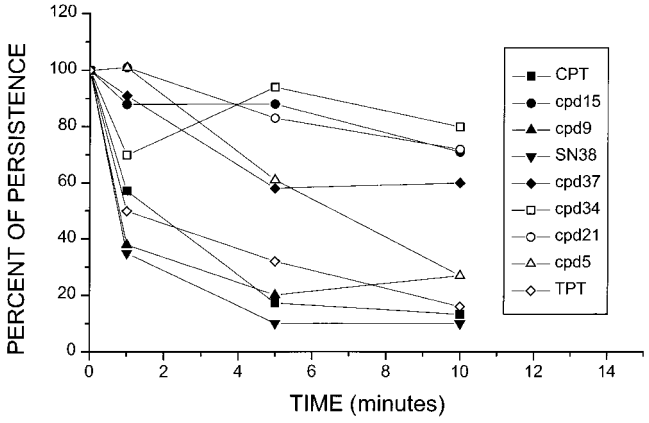


Figure 3. Persistence of topoisomerase I-mediated DNA cleavage in the presence of camptothecins. All the bands shown in Figure 2 were used to quantify the persistence of the cleavage. The samples were reacted for 20 min with 10 μM drug. DNA cleavage was then reversed by adding 0.6 M NaCl. The 100% value is referred to the extent of DNA cleavage at 20 min of incubation. See Experimental Section for details.

than that of topotecan (15 mg/kg). Using this intermittent treatment, topotecan was effective in reducing tumor growth achieving a TVI >90% in the tested lung tumors. Compound **15** exhibited a clearly superior activity in both tumors in terms of tumor growth inhibition and log-cell kill. (Figure 5). In addition, in the range of optimal doses (2−3 mg/kg), a high rate of complete response to **15** was found in both tumors. At the maximum tolerated dose (3 mg/kg), all treated mice bearing the lung carcinoma LX-1 achieved complete regression.

The present study extends previous work of our group on the design of 7-substituted analogues of camptothecin.[11,12] The results provide further support to the hypothesis that modification at the 7 position is a promising approach in the development of effective camptothecins. It is evident that the nature of the substituent is critical to achieve optimal activity. Indeed, lipophilicity could play an important role, because it promotes a rapid intracellular drug accumulation and tissue distribution, thus favoring lactone stabilization and enhancing interaction of the active lactone form with the intracellular target. This interpretation is consistent with an increased antiproliferative activity of highly lipophilic 7-silylcamptothecins (silatecans).[14] However, on the basis of a large difference in the cytotoxic activity of the 7-modified analogues presented in this study (Table 1), it appears that the lipophilic nature is not sufficient to account for the striking activity of some derivatives, including compounds **14** and **15**. The most active analogues of our series exhib-

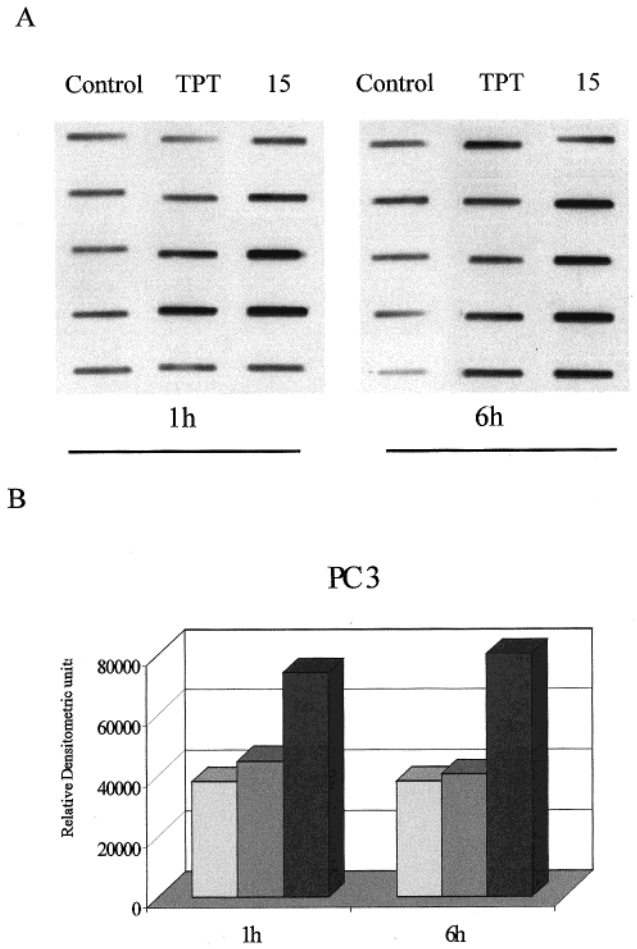


Figure 4. Stabilization of the topoisomerase I−DNA complex induced by camptothecins in prostate carcinoma cells. Human prostate carcinoma (PC3) cells were exposed for 1 h to 10 μM compound **15** (ST1481**)** or topotecan **(**TPT**)** in serum-free medium 1% DMSO. Then, cells were washed with serum-free medium and maintained in culture for 6 h. Different gradient fractions corresponding to bound and free topo I were collected and analyzed by spectrophotometric and immunoblotting methods. The amounts of topo I coupled covalently to DNA was determined by densitometric analysis of the immunoblot using a specific antibody anti human topo I. (A) Slot immunoblot of fractions containing DNA-bound topoisomerase I; (B) mean values from densitometric analysis of fractions shown in A for each sample.

ited a potent topoisomerase I inhibition and persistent stabilization of the topoisomerase I-mediated DNA cleavage. Although the structural basis of the improved activity of compound **15** over other analogues (with particular reference to SN38 or topotecan) remains unclear, a plausible explanation for the enhanced interaction with the enzyme−DNA complex is a favorable fitting of the drug in the ternary complex, rather than a different mechanism of topoisomerase I poisoning. Indeed compound **15** shares a common binding site with camptothecin in the DNA cleavable complex, as documented by a similar pattern of DNA cleavage. The concomitant substitutions in the rings A and B (in particular, the presence of a hydroxy group at the position 10) reduced the cytotoxic activity as indicated by comparison of compounds **15**, **17**, and **18**. This effect could be counterbalanced by a lesser interaction with human serum albumin due to the 10-OH group.[26] However, on the basis of its potency as a topoisomerase

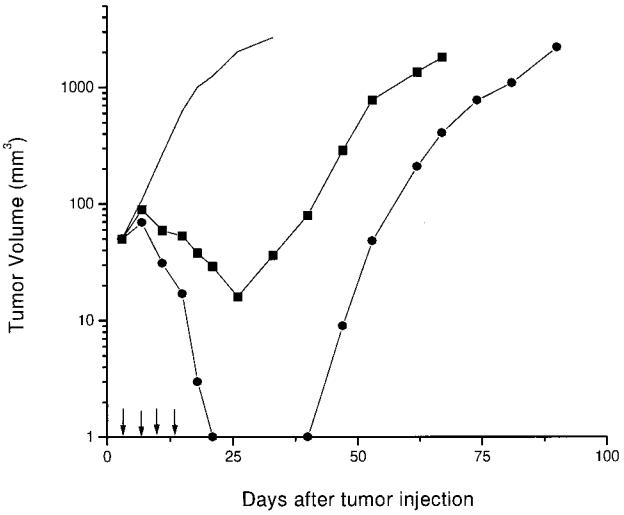


Figure 5. Antitumor activity of compound **15** and topotecan against LX-1 lung carcinoma xenografts. Drugs were delivered per os, with a q4dx4 schedule. Arrows indicate the days of treatment. Compound **15**, 3 mg/kg (●); topotecan, 15 mg/kg (■); untreated control (▲). Each point represents the mean value of the tumor volumes of a group of mice treated with the same drug and dose.

I poison and a cytotoxic agent, compound **15** (ST 1481) was selected for preclinical development despite expected affinity for human serum albumin.

In conclusion, it is likely that the efficacy of the potent analogues of our series is the result of a combination of multiple factors, including lipophilicity, a potent topoisomerase I inhibition, and stability of the DNA−enzyme cleavable complex.

Experimental Section

General Methods. 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. HPLC analysis of the mixture of diastereoisomers was performed on an HP 1050 quaternary pump fitted with a Rheodyne injector (20 μl loop) and a HP-1050 diode-array detector. Chromatograms were recorded at 360 and 400 nm. The column was a Rainin C18, 25 × 0.4 cm Varian, flow 1 mL/min, with a gradient from CH_3CN:H_2O 30:70 to 100:0, in 20 min.

Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were obtained by distillation from sodium-benzophenone ketyl; 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.

7-Benzoylcamptothecin (7). To a suspension of CPT (200 mg, 0.57 mmol) in 50% aqueous acetic acid (1.6 mL) were added dropwise 0.17 mL of concentrated H_2SO_4 and benzaldehyde (304 mg, 2.87 mmol). After cooling at 0 °C, 80% *t*-BuOOH (128 mg, 1.14 mmol) and a solution of 317 mg of $FeSO_4$ in 0.56 mL of water were added, and the mixture was stirred overnight at room temperature. Dilution with water, filtration of the precipitate, extraction with dichloromethane,

and chomatography of the extract (CH_2Cl_2/MeOH 98/2) gave 90 mg (35%) of **7**: mp > 200 °C; ^{1}H NMR δ 0.90 (t, J = 7 Hz, H_3-18), 1.75−1.95 (m, H_2-19), 2.82 (s, CO-CH_3) 5.42 (s, H_2-5), 5.48 (s, H_2-17), 6.58 (s, OH), 7.39 (s, H-14), 7.55−7.85 (5H, H-10, H-11 and 3H Ar), 7.9−8.0 (3H, H-12 and 2H Ar), 8.31 (dd, J = 8.46 Hz, J = 1.47 Hz, H-9).

7-Acetylcamptothecin (6) was analogously prepared starting from CPT (250 mg, 0.72 mmol) and acetaldehyde (154 mg, 3.5 mmol) in 2 mL of 50% aqueous acetic acid. Purification by chromatography (CH_2Cl_2/MeOH 99.2/0.8 gave the expected product in 36% yield: mp >200 °C; ^{1}H NMR δ 0.88 (t, H_3-18, J = 7 Hz), 1.85 (m, H_2-19), 5.0 (s, H_2-5), 5.40 (s, H_2-17), 6.6 (s, OH), 7.40 (s, H-14), 7.80 (ddd, H-11, J = 1.47 Hz, J = 8.46 Hz, J = 8.46 Hz) 7.95 (ddd, H-10, J = 1,47 Hz, J = 8.46 Hz, J = 8.46 Hz) 8.2 (dd, H-12, J = 8.46 Hz, J = 1.47 Hz,), 8.3 (d, H-9, J = 8.46 Hz, J = 1.47 Hz); MS *m/z* 390 (10, M^+), 346 (10), 331 (15), 290 (28) 218 (10), 140(20), 57 (20), 43 (100).

7-Benzoyloxyiminomethylcamptothecin (8). To a solution of PhCOCl (0.16 mL, 1.4 mmol) in 5 mL of pyridine was added 500 mg (1.3 mmol) of 7-hydroxyimino-CPT (**5**),[16] and the mixture was stirred overnight at room temperature. Evaporation, taking up with aqueous $NaHCO_3$, extraction with CH_2Cl_2, and chromatography (CH_2Cl_2/MeOH 98/2) gave 200 mg (32%) of **8**: mp 200 °C (dec); ^{1}H NMR δ 0.80 (t, J = 7 Hz,H_3-18), 1.82 (m, H_2-19), 5.45 (s, H_2-5), 5.55 (s, H_2-17), 6.6 (s, OH), 7.30 (s, H-14), 7.75−8.00 (5H, H-10, H-11 and 3H Ar), 8.25 (m, 2H Ar), 8.31 (dd, H-12, J = 8.46 Hz, J = 1.47 Hz), 8.75 (dd, H-9, J = 8.46 Hz, J = 1.47 Hz), 10.05 (s, CH═N). MS *m/z*: 373 (100), 329 (75), 314 (90), 300 (80) 272 (60), 243 (70).

General Procedure for the Synthesis of Oximes (Method A). A solution of 500 mg (1.33 mmol) of CPT-7-aldehyde in 100 mL of EtOH was added with 15 mL of pyridine and 4 mmol of the appropriate O-substituted hydroxylamine·HCl and refluxed 5 h. The product was obtained after evaporation and chromatography with an hexane/AcOEt or CH_2Cl_2/MeOH mixture.

7-Hydroxyimino(phenyl)methylcamptothecin (9). The solution was refluxed 2 days. The crude product was purified by chromatography with CH_2Cl_2/MeOH 98:2: yield 45%; mp >200 °C (dec); ^{1}H NMR δ 0.9 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.85 (m, H_2-19*E* +H_2-19*Z*), 4.80 (m, H_2-5*E* + H_2-5*Z*), 5.85 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z*), 6.60 (s, -OH*E*), 7.35−7.55 (m, Ar*E* +Ar*Z* + H-10*E* + H-10*Z* + H-11*E* +H-11*Z* + H-14*E* + H-14*Z*), 7.60−7.70 (m, H-12*E* + H-12*Z*), 8.22−8.27 (m, H-9*E* + H-9*Z*), 12.07 (s, N-OH*E*), 12.40 (s, N-OH*Z*).

7-Methoxyiminomethylcamptothecin (10). The solution was refluxed 5 h. The crude product was purified by chromatography with hexane/AcOEt 2:8: yield 67%; mp 230 °C (dec); ^{1}H NMR δ 0.87 (t, H_3-18), 1.75−1.95 (m, H_2-19), 4.13 (s, -OCH_3), 5.32 (s, H_2-5), 5.42 (s, H_2-17), 6.50 (s, -OH), 7.26 (s, H-14), 7.76 (d, H-9, J = 2.6 Hz), 8.08 (d, H-12, J = 9.2 Hz,), 8.30 (s, CH═N*Z*), 9.04 (s, CH═N*E*).

7-Allyloxyiminomethylcamptothecin (13). The solution was refluxed 4 h. Purification of the product was obtained by chromatography with hexane/AcOEt 2:8: yield 62%; mp 235 °C (dec); ^{1}H NMR δ 0.90 (t, H_3-18*E* + H_3-18*Z*), 1.75−2.0 (m, H_2-19*E* +H_2-19*Z*), 4.7 (d, -CH_2 All.*Z*, J = 7 Hz), 4.85 (d, -CH_2 All.*E*, J = 7 Hz), 5.20 (s, H_2-5*Z*), 5.30 (s, H_2-5*E*), 5.35−5.55 (m, H_2-17*E* + H_2-17*Z* + -CH_2 = All.*Z* + -CH_2 = All.*E*), 5.90−6.05 (m, CH = All.*Z*), 6.10−6.25 (m, CH= All.*E*), 6.60 (s, -OH*Z* + -OH*E*), 7.37 (s, H-14*E* + H-14*Z*), 7.75 (m, H-11*E* +H-11*Z*), 7.90 (m, H-10*E* + H-10*Z* + H-12*Z*), 8.05 (dd, H-9*Z* J = 8.46 Hz, J = 1.47 Hz), 8.22 (dd, H-12*E*, J = 8.46 Hz, J = 1.47 Hz), 8.46 (s, CH═N*Z*), 8.60 (dd, H-9*E*, J = 8.46 Hz, J = 1.47 Hz), 9.38 (s, CH═N*E*); MS *m/z* 431 (100, M^+), 373 (50), 330 (20).

7-(*tert*-Butoxycarbonyl(dimethyl)methoxy)iminomethylcamptothecin (20). The solution was refluxed 8 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 98:2: yield 62%; mp 180 °C (dec), ^{1}H NMR δ: 0.88 (t, H_3-18, J = 7 Hz), 1.44 (s, 3 -CH_3), 1.60 (s, 2 -CH_3), 1.80−1.92 (m, H_2-19), 5.27 (s, H_2-5), 5.43 (s, H_2-17), 6.53 (s, -OH), 7.35 (s, H-14), 7.76 (ddd, H-11, J = 8.46 Hz, J = 8.46 Hz, J = 1.47 Hz), 7.92 (ddd, H-10, J = 8.46 Hz, J = 8.46 Hz, J = 1.47 Hz), 8.23 (dd, H-12, J = 8.46 Hz, J = 1.47 Hz), 8.51 (s, CH═N*Z*), 8.65 (dd, H-9, J = 8.46 Hz, J = 1.47 Hz), 9.39 (s, -CH═N); Mass *m/z* 534 (13, M^+), 477 (29), 374 (55), 273 (10), 57 (100), 41(57).

7-(Carboxy(dimethyl)methoxyiminomethylcamptothecin (24). A total of 40 mg (0.08 mmol) of **20** was dissolved in 5 mL of dichloromethane. Trifluoroacetic acid (1 mL) was then added, and the solution was stirred at room temperature overnight. After evaporation of the solvent, the precipitate was redissolved in dichloromethane and treated with a saturated solution of Na_2CO_3. The two phases were separated. The aqueous layer was acidified with HCl and extracted with CH_2Cl_2. Drying, evaporation, and chromatography gave the desired product as a yellow solid: yield 79%; mp 193 °C (dec); ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, H_3-18, J = 7.35 Hz), 1.70 (s, -CH_3) 1.81−1.95 (m, H_2-19), 3.60 (s, -OH), 5.24 (d, H-17A, J = 16.55 Hz), 5.32 (s, H_2-5), 5.65 (d, H-17B, J = 16.55 Hz), 7.64 (s, H-14), 7.67 (ddd, H-11, J = 6.99 Hz, J = 8.47 Hz, J = 1.47 Hz), 7.80 (ddd, H-10, J = 6.99 Hz, J = 8.47 Hz, J = 1.47 Hz), 8.10−8.16 (m, H-9 + H-12) 9.10 (s, -CH═N).

7-(2-Aminoethoxy)iminomethylcamptothecin (21). The solution was refluxed 3 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 90:10: yield 50%; mp 220 °C (dec); ^{1}H NMR δ 0.82 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.85 (m, H_2-19*E* +H_2-19*Z*), 3.20−3.30 (m, -CH_2-N.*Z* + -CH_2-N.*E*), 4.35−4.5 (m, -CH_2-O.*Z* + -CH_2-O.*E*), 5.30 (s, H_2-5*Z* + H_2-5*E*), 5.38 (s, H_2-17*E* + H_2-17*Z*), 6.50 (s, -OH*Z* + -OH*E*), 7.30 (s, H-14*E* + H-14*Z*), 7.70 (m, H-11*E* +H-11*Z*), 7.85 (m, H-10*E* + H-10*Z* + H-12*Z*), 8.15 (m, H-9*Z* + H-12*E*), 8.43 (s, CH═N*Z*), 8.45 (dd, H-9*E*, J = 8.46 Hz, J = 1.47 Hz), 9.27 (s, CH═N*E*).

7-(*N*,*N*-Dimethylaminoethoxy)iminomethylcamptothecin (22). The solution was refluxed 4 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 90:10: yield 76%; mp 232 °C (dec); ^{1}H NMR δ 0.87 (t, H_3-18*E* + H_3-18*Z*), 1.80−1.95 (m, H_2-19*E* +H_2-19*Z*), 2.65 (s, 2CH_3-N*E* + 2CH_3-N*Z*), 3.15−3.25 (m, -CH_2-N.*Z* + -CH_2-N.*E*), 4.52−4.60 (m, -CH_2-O.*Z* + -CH_2-O.*E*), 5.30 (s, H_2-5*Z* + H_2-5*E*), 5.45 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z* + -OH*E*), 7.35 (s, H-14*E*), 7.52 (s, H-14*Z*), 7.80 (m, H-11*E* +H-11*Z*), 7.95 (m, H-10*E* + H-10*Z* + H-12*Z*), 8.25 (m, H-9*Z* + H-12*E*), 8.62 (m, H-9*E* + CH═N*Z*), 9.34 (s, CH═N*E*).

7-[2-(4-Morpholinyl)ethoxy]iminomethylcamptothecin (28). The solution was refluxed 2 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 95:5: yield 30%; mp 158−160 °C (dec), ^{1}H NMR ($CDCl_3$) δ: 1.06 (t, H_3-18, J = 7.35 Hz), 1.84−2.00 (m, H_2-19), 2.62 (t, -CH_2-N morf, J = 4.78 Hz), 2.87 (t, -CH_2-N, J = 5.52 Hz), 3.60 (s, -OH), 3.79 (t, -CH_2-O morf., J = 4.78 Hz), 4.59 (t, -CH_2-O, J = 5.52 Hz), 5.33 (d, H-17A, J = 16.18 Hz), 5.45 (s, H_2-5), 5.77 (d, H-17B, J = 16.18 Hz), 7.69 (s, H-14), 7.73 (ddd, H-11, J = 1.47 Hz, J = 8.46 Hz, J = 8.46 Hz), 7.87 (ddd, H-10, J = 1.47 Hz, J = 8.46 Hz, J = 8.46 Hz), 8.19−8.31 (m, H-9 + H-12), 9.12 (s, -CH═N). Mass *m/z*: 504 (4, M^+), 373 (23), 329 (26), 272 (18), 244 (20), 216 (13), 100 (100).

7-[2-(3-Methylpiperidin-1-yl)ethoxy]iminomethylcamptothecin (29). The solution was refluxed 4 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 98:2: yield 35%; mp 185 °C (dec); ^{1}H NMR δ 0.9 (t, H_3-18*E* + H_3-18*Z*), 1.75 (s, N-CH_3*E* + N-CH_3*Z*)1.5−1.95 (m, H_2-19*E* +H_2-19*Z* + -CH_2 pip.*E* + -CH_2 pip.*Z*), 2.55−2.70 (m, CH_2-N pip.*E* + CH_2-N pip.*Z*), 3.75−3.85 (m, -CH pip.*Z* + -CH pip.*E*), 4.20−4.40 (m, -CH_2-O.*Z* + -CH_2-O.*E*), 5.20 (s, H_2-5*Z* + H_2-5*E*), 5.35 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z* + -OH*E*), 7.37 (s, H-14*E* + H-14*Z*), 7.80 (m, H-11*E* +H-11*Z*), 7.95 (m, H-10*E* + H-10*Z*) 8.10 (dd, H-12*Z*, J = 8.46 Hz, J = 1.47 Hz), 8.25 (m, H-9*Z* + H-12*E*), 8.40 (s, CH═N*Z*), 8.60 (dd, H-9*E*, J = 8.46 Hz, J = 1.47 Hz), 9.35 (s, CH═N*E*). MS *m/z*: 502 (80, M^+) 373 (60) 329 (100) 314 (80) 300 (60) 272 (40) 243 (60) 128 (80) 97 (50).

7-[2-(1-Uracylyl)ethoxy]iminomethylcamptothecin (30). The solution was refluxed 4 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 95:5: yield 42%; mp 197−200 °C (dec); ^{1}H NMR δ: 0.88 (t, H_3-18*E* + H_3-18*Z*, J = 7.35 Hz) 1.80−1.95 (m, H_2-19*E* +H_2-19*Z*) 3.90 (t, -CH_2N*Z*, J = 6 Hz), 4.15 (t, -CH_2N*E*, J = 6 Hz), 4.35 (t, -CH_2O*Z*, J = 6 Hz), 4.58 (t, -CH_2O*E*, J = 6 Hz), 5.00 (d, H-5 U *Z*, J = 8 Hz),

5.35−5.50 (m, H_2-5*Z* + H_2-5*E* + H_2-17*E* + H_2-17*Z*), 5.55 (d, H-5 U *E*, *J* = 8 Hz) 6.55 (s, -OH*Z* + -OH*E*), 7.15 (d, H-6 U *Z*, *J* = 8 Hz), 7.40 (s, H-14*E* + H-14*Z*), 7.64 (d, H-6 U *E*, *J* = 8 Hz), 7.70−7.82 (m, H-11*E* +H-11*Z*), 7.85−8.00 (m, H-10*E* + H-10*Z* + H-12*Z*), 8.23 (m, H-12*E* + H-9*Z*), 8.48 (s, CH=N*Z*), 8.60 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.35 (s, -CH=N*E*) 11.3 (br s, NH U).

7-Benzyloxyiminomethylcamptopthecin (34). It was obtained in 65% yield after refluxing 2 h. Purification by chromatography with hexane/AcOEt 3:7 gave the pure product as a yellow solid, mp 200 °C (dec); ^{1}H NMR δ 0.88 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* + H_2-19*Z*), 5.18 (s, H_2-5*Z*), 5.21 (s, H_2-Ph*Z*) 5.30 (s, H_2-Ph*E*) 5.40(s, H_2-5*E*), 5.45 (s, H_2-17*E* + H_2-17*Z*), 6.53 (s, OH), 7.35 (s, H-14*E*), 7.3−7.6 (m, Ar*E* + Ar*Z* + H-14*Z*), 7.70−7.80 (m, H-11*E* + H-11*Z*), 7.85−7.95 (m, H-10*E* + H-10*Z*), 7.98 (dd, H-12*Z*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.18−8.27 (m, H-12*E* + H-9*Z*), 8.45 (s, CH=N*Z*), 8.60 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.38 (s, CH=N*E*); MS *m*/*z*: 481 (100, M^+), 374 (30), 330 (70), 273 (20), 243 (20), 91 (34). HPLC retention times: *E*: 14.5 min, *Z*: 12.5 min

7-(1-Benzyloxyiminoethyl)camptothecin (35). The solution was refluxed 4 h. The crude product was purified by chromatography with hexane/AcOEt 3:7: yield 25%; mp >200 °C; ^{1}H NMR δ 0.88 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* + H_2-19*Z*), 2.3 (s CH_3-C= *Z*), 2.4 (s, CH_3-C= *E*), 5.0 (s, -CH_2O*Z*) 5.2 (s, -CH_2O*E*), 5.30(s, H_2-5*E* + H_2-5*Z*), 5.45 (s, H_2-17*E* + H_2-17*Z*), 6.53 (s, OH), 7.10−7.75 (m, H-14*E* + Ar*E* + Ar*Z* + H-14*Z* + H-11*E* + H-11*Z* + H-10*E* + H-10*Z*), 7.85−8.0 (m, H-12*Z* + H-12*E*), 8.20−8.30 (m, H-9*Z* + H-9*E*).

7-(4-Methylbenzyl)oxyiminomethylcamptothecin (36). The solution was stirred overnight at room temperature. The crude product was purified by chromatography with hexane/AcOEt 1:1: yield 42%; mp 203 °C (dec); ^{1}H NMR δ 0.9 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* + H_2-19*Z*), 2.30 (s, pCH_3*E* + pCH_3*Z*), 4.98 (s, -OCH_2-*Z*), 5.25 (s, -OCH_2-*E*), 5.38 (s, H_2-5*Z* + H_2-5*E*), 5.48 (s, H_2-17*E* + H_2-17*Z*), 6.53 (s, OH), 7.25 (d, 2Ar*E* + 2Ar *Z*, *J* = 8.46 Hz), 7.35 (s, H-14*E* + H-14*Z*), 7.45 (d, 2Ar*E* + 2Ar *Z*, *J* = 8.46 Hz), 7.75 (ddd, H-11*E* + H-11*Z*, *J* = 8.46 Hz, *J* = 8.46 Hz, *J* = 1.47 Hz), 7.95 (ddd, H-10*E* + H-10*Z*, *J* = 8.46 Hz, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.25 (dd, H-12*E* + H-12*Z*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.42 (s, CH=N*Z*), 8.60 (dd, H-9*Z* + H-9*E*), 9.35 (s, CH=N*E*).

7-Pentafluorobenzyloxyiminomethylcamptothecin (38). The solution was refluxed 2 h. The crude product was purified by chromatography with hexane/AcOEt 3:7: yield 76%; mp 200 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*), 1.80−1.95 (m, H_2-19*E* +H_2-19*Z*), 5.10−5.5 (m, -CH_2-O.*Z* + -CH_2-O.*E* + H_2-5*Z* + H_2-5*E* + H_2-17*E* + H_2-17*Z*), 6.50 (s, -OH*Z* + -OH*E*), 7.30 (s, H-14*E*),7.33 (s, H-14*Z*), 7.65−7.75 (m, H-10*E* + H-10*Z*), 7.81−7.84 (m, H-11*E* + H-11*Z* + H-9*Z*), 8.12−8.24 (H-12*E* + H-12*Z*), 8.50 (m, CH=N*Z* + H-9*E*), 9.35 (s, CH=N*E*).

7-(4-Phenylbenzyl)oxyiminomethylcamptothecin (40). The solution was refluxed 4 h. The crude product was purified by chromatography with hexane/AcOEt 4:6: yield 54%; mp 202 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*), 1.80−1.95 (m, H_2-19*E* +H_2-19*Z*), 5.3−5.5 (m, -CH_2-O.*Z* + -CH_2-O.*E* + H_2-5*Z* + H_2-5*E* + H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z* + -OH*E*), 7.35−7.5 (m, H-14*E* + H-14*Z* + 4Ar*E* + 4Ar*Z*), 7.55−7.75 (m, 5Ar*E* + 5Ar*Z* + H-11*Z* + H-10*Z*), 7.85−8.0 (H-11*E*+ H-10*E* + H-12*Z*), 8.20−8.30 (m, H-9*Z* + H-12*E*), 8.46 (s, CH=N*Z*), 8.60 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.42 (s, CH=N*E*).

7-Triphenylmethoxyiminomethylcamptothecin (41). The solution was refluxed 16 h. The crude product was purified by chromatography with hexane/AcOEt 1:1: yield 20%; mp 140 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* +H_2-19*Z*), 4.8 (s, H_2-5*E*), 5.15 (s, H_2-5*Z*), 5.45 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z* + -OH*E*), 7.0−7.5 (m, H-14*E* + H-14*Z* + Ar*E* + Ar*Z*), 7.55−8.0 (m, H-11*Z* + H-10*Z* + H-11*E*+ H-10*E* + H-12*Z*), 8.20 (dd, H-12*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.33 (dd, H-9*Z*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.50 (s, CH=N*Z*), 8.55 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.63 (s, CH=N*E*).

7-(9-Anthracenyl)methoxyiminomethylcamptothecin (42). The solution was refluxed 4 h. The crude product was purified by chromatography with hexane/AcOEt 3:7, then with CH_2Cl_2/MeOH 99:1: yield 40%; mp 202 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* +H_2-19*Z*), 4.8 (s, H_2-5*E* + H_2-5*Z*), 5.45 (s, H_2-17*E* + H_2-17*Z*), 6.25 (s, -CH_2-O.*Z* + -CH_2-O.*E*), 6.55 (s, -OH*Z* + -OH*E*), 7.3 (s, H-14*Z*), 7.35−8.7(m, H-14*E* + 9Ar*E* + 9Ar*Z* + H-11*Z* + H-10*Z* + H-11*E*+ H-10*E* + H-12*Z* + H-12*E* + H-9*Z* + H-9*E*), 8.38 (s, CH=N*Z*), 9.30 (s, CH=N*E*).

7-(4-Pyridyl)methoxyiminomethylcamptothecin (43). The solution was refluxed 2.5 h. The crude product was purified by chromatography from CH_2Cl_2/MeOH 98:2 to CH_2Cl_2/MeOH 95:5: yield 50%; mp 190 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* +H_2-19*Z*), 5.25−5.35 (s, -CH_2-O.*Z* + -CH_2-O.*E*), 5.45 (s, H_2-5*E* + H_2-5*Z*), 5.50 (s, H_2-17*E* + H_2-17*Z*), 6.50 (s, -OH*Z* + -OH*E*), 7.27 (m, 2H pyr*Z*), 7.34 (s, H-14*E*), 7.38 (s, H-14*Z*), 7.53 (m, 2H pyr*E*), 7.74−7.82 (m, H-11*Z* + H-11*E*), 7.87−7.98 (m, H-10*Z* +H-10*E*), 8.02 (dd, H-12*Z*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.20−8.28 (m, H-12*E* + H-9*Z*), 8.5 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.58−8.65 (m, CH=N*Z* + 2H pyr*E* + 2H pyr*Z*), 9.47 (s, CH=N*E*).

7-(2-Imidazolyl)methoxyiminomethylcamptothecin (44). The solution was refluxed 8 h. The crude product was purified by chromatography with CH_2Cl_2/MeOH 95:5: yield 50%; mp 168−172 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*), 1.75−1.95 (m, H_2-19*E* +H_2-19*Z*), 5.30−5.50 (m, -CH_2-O.*Z* + -CH_2-O.*E* + H_2-5*E* + H_2-5*Z* + H_2-17*E* + H_2-17*Z*), 6.53 (s, -OH*Z* + -OH*E*), 6.90−7.20 (m, 2H, Im), 7.36 (s, H-14), 7.76 (m, H-11), 7.91 (m, H-10), 8.25 (dd, H-12, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.45 (s, CH=N*Z*), 8.60 (dd, H-9, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.35 (s, CH=N*E*), 12.33 (NH Im).

General Procedure for the Synthesis of Oximes (Method B). The appropriate O-substituted hydroxylamine·HCl (4 mmol) was dissolved in EtOH (15 mL), treated with 3.6 mmol of NaOH, and stirred until all NaOH had reacted. Then CPT-aldehyde (1.32 mmol) was added, and the mixture stirred until TLC indicated the end of the reaction. Evaporation of the solvent and chromatography as above gave the product.

7-*tert*-Butoxyiminomethylcamptothecin (15). The crude product was purified by chromatography with hexane/AcOEt 3:7. It was obtained in 82% yield: mp 250 °C (dec); $[\alpha]_D$ +58° ($CHCl_3$ *c* 0.1 for isomer *E*); IR (isomer *E*, KBr) 1751, 1662, 1605 cm $^{-1}$; UV (isomer *E*, DMSO:EtOH 3:997) λ_{max} 259, 304, 327, 371, 384 nm, (ϵ 25800, 15100, 16000, 18400, 18800); ^{1}H NMR δ 0.88 (t, H_3-18*E* + H_3-18*Z*), 1.30 (s, *t*-Bu*Z*), 1.30 (s, *t*-Bu*E*),1.87−2.0 (m, H_2-19*E* + H_2-19*Z*), 5.18 (s, H_2-5*Z*), 5.37 (s, H_2-5*E*), 5.42 (s, H_2-17*E* + H_2-17*Z*), 6.53 (s, -OH*Z* + -OH*E*), 7.35 (s, H-14*E*), 7.36 (s, H-14*Z*), 7.69−7.83 (m, H-11*E* + H-11*Z*), 7.85−7.98 (m, H-10*E* + H-10*Z*), 8.07 (dd, *J* = 9.06 Hz, *J* = 1.46 Hz, H-9*Z*), 8.16−8.27 (m, H-12*Z* + H-9*E*), 8.40 (s, CH=N*Z*), 8.62 (dd, *J* = 9.06 Hz, *J* = 1.46 Hz, H-12*E*), 9.31-(s, CH=N*E*); MS *m*/*z* 448 (28, M^+), 391 (40), 374 (100), 362 (40), 330 (70). HPLC retention times: *E*: 14.6 min, *Z*: 12.9 min.

7-*tert*-Butoxyiminomethyl-10-methoxycamptothecin (18). The compound was obtained starting from 10-methoxy-camptothecin-7-aldehyde according to method C. The solution was stirred 24 h at room temperature, then 10 h at 65 °C. The crude product was purified by chromatography with hexane/AcOEt 4:6: yield 34%; mp 250 °C (dec); ^{1}H NMR δ: 0.88 (t, H_3-18*E* + H_3-18*Z*), 1.47 (s, *t*-Bu*Z* + *t*-Bu*E*), 1.80−1.93 (m, H_2-19*E* + H_2-19*Z*), 3.95 (s, -OCH_3 *Z*), 3.98 (s, -OCH_3 *E*), 5.17 (s, H_2-5 *Z*), 5.30−5.45 (m, H_2-5*E* + H_2-17*E* + H_2-17*Z*), 6.50 (s, -OH*Z* + -OH*E*), 7.29 (s, H-14*Z* + H-14*E*), 7.56 (dd, H-11*E* + H-11*Z*, *J* = 9.19 Hz; *J* = 2.57 Hz), 7.90 (d, H-9*E* + H-9*Z*, *J* = 2.57 Hz), 8.12 (d, H-12*E* + H-12*Z*, *J* = 9.19 Hz), 8.39 (s, -CH=N*Z*), 9.33 (s, -CH=N*E*). MS *m*/*z*: 477 (56, M^+), 421 (75), 404 (100), 392 (66), 360 (18), 303 (6), 274 (8).

7-Hydroxymethyl(dimethyl)methoxyiminomethyl-camptothecin (19). The solution was heated 6 h at 50 °C. The crude product was purified by chromatography with hexane/AcOEt 2:8: yield 88%; mp 245 °C (dec); ^{1}H NMR δ 0.9 (t, H_3-18*E* + H_3-18*Z*), 1.40 (s, -$C(CH_3)_2$*E* + -$C(CH_3)_2$*Z*), 1.75−1.95 (m, H_2-19*E* +H_2-19*Z*), 3.6 (s, -CH_2-O.*Z* + -CH_2-O.*E*), 5.37

(s, H_2-5*E* + H_2-5*Z*), 5.45 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z* + -OH*E*), 7.35 (s, H-14*E* + H-14*Z*), 7.75 (m, H-11*Z* + H-11*E*), 7.90 (m, H-10*Z* +H-10*E* + H-12*Z*), 8.1 (dd, H-9*Z*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.20 (dd, H-12*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 8.40 (s, CH=N*Z*), 8.63 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.30 (s, CH=N*E*). MS *m*/*z*: 463 (80, M^+), 419 (90), 374 (90), 330 (100), 273 (20).

7-Carboxymethoxyiminomethylcamptothecin (23). Prepared according to the general procedure stirring 9 h at room temperature. The crude product was purified by chromatography with CH_2Cl_2/MeOH (from 95:5 to 80:20): yield 90%; mp 208 °C (dec); ^{1}H NMR δ 0.9 (t, H_3-18*E* + H_3-18*Z*), 1.80−1.95 (m, H_2-19*E* +H_2-19*Z*), 4.35 (s, -CH_2-COOH.*Z*), 4.55 (s,-CH_2-COOH.*E*), 5.20 (s, H_2-5*E* + H_2-5*Z*), 5.40 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, -OH*Z* + -OH*E*), 7.35 (s, H-14*E* + H-14*Z*), 7.75 (m, H-11*Z* + H-11*E*), 7.85 (m, H-10*Z* +H-10*E* + H-12*Z*), 8.25 (m, H-9*Z* + H-12*E*), 8.37 (s, CH=N*Z*), 8.63 (dd, H-9*E*, *J* = 8.46 Hz, *J* = 1.47 Hz), 9.27 (s, CH=N*E*).

7-*tert*-Butoxycarbonylaminotrimethylenaminocarbonylmethoxyiminomethylcamptothecin (26). A mixture of 32 mg (0.07 mmol) of **23,** 14 mg (0.10 mmol) of 1-hydroxybenzotriazole (HOBt), and 20 mg (0.10 mmol) of *N*′-[3-(dimethylamino)propyl]-*N*-ethylcarbodiimide hydrochloride (WSC) in CH_3CN (8 mL) and anhydrous THF (6 mL) was heated at 45 °C under nitrogen for 3 h. N-Boc-diaminopropane (14 mg, 0.07 mmol) was then added, and the mixture was refluxed for 2 h. After cooling, the solvent was removed under reduced pressure; the residue was dissolved in water and extracted with CH_2Cl_2, dried, and concentrated in vacuo. Purification by chromatography (CH_2Cl_2/MeOH 97:3) afforded the pure product as a yellow solid: yield 63%; mp 216 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.06 (t, H_3-18*E* + H_3-18*Z*, *J* = 7.35 Hz), 1.40 (s, tBu*E* + tBu*Z*),1.70−1.85 (m, -CH_2-*E* + -CH_2-*Z*), 1.80−1.95 (m, H_2-19*E* +H_2-19*Z*), 3.03−3.23 (m, -CH_2-N *E* + -CH_2-N *Z*), 3.30−3.42 (m, -CH_2-NHBoc *E* + -CH_2-NHBoc *Z*), 3.73 (s, -OH*Z* + -OH*E*), 4.72 (s, -O-CH_2-CO.*Z*), 4.91 (s,-O-CH_2-CO *E*), 5.28−5.37 (s, H_2-17A *E* + H_2-17*Z* + H_2-5*Z*), 5.43 (H_2-5*E*), 5.77 (d, H_2-17B *E*, *J* = 16.55 Hz), 7.65−7.78 (m, H-14*E* + H-14*Z* + H-11*Z* + H-11*E*), 7.82−7.93 (m, H-10*Z* +H-10*E* + H-12*Z*), 8.09 (m, H-9*Z*), 8.21 (s, CH=N*Z*), 8.30 (m, H-9*E* + + H-12*E*), 9.30 (s, CH=N*E*).

7-Aminotrimethylenaminocarbonylmethoxyiminomethylcamptothecin Hydrochloride (25). A total of 14 mg of **26** were dissolved in 1 mL of ethyl acetate, and HCl was bubbled into the solution. The precipitate was collected by filtration, washed with diethyl ether, and dried: yield 73%; mp 222 °C (dec); ^{1}H NMR δ: 0.88 (t, H_3-18, *J* = 7.35 Hz), 1.67−1.96 (m, H_2-19 + -CH_2), 2.68−2.90 (m, -*CH_2*N; -*CH_2*NHCO), 4.79 (s, -OCH_2), 5.30 (s, H_2-5), 5.44 (s, H_2-17), 6.50 (s, -OH), 7.38 (s, H-14), 7.66−7.84 (m, H-11+ NH_3^+), 7.94 (m, H-10), 8.21−8.34 (m, H-12 + NH) 8.61 (dd, H-9, *J* = 8.47 Hz, *J* = 1.46 Hz), 9.44 (s, -CH=N).

7-(2-Nitrobenzenesulfonylamino)trimethylenaminocarbonylmethoxyiminomethylcamptothecin (27). Prepared according to the same procedure used to obtain **26.** Yield 60%; mp 155−160 °C (dec); ^{1}H NMR ($CDCl_3$) δ: 1.05 (t, H_3-18), 1.85−1.98 (m, H_2-19 + -CH_2), 3.22 (m, -C*H_2*-NHCO), 3.54-(m, -C*H_2*-NH−SO_2), 4.85 (s, -*CH_2*O), 5.35 (d, H-17A, *J* = 16.55 Hz), 5.39 (s, H_2-5), 5.76 (d, H-17B, *J* = 16.55 Hz), 6.51 (NH), 6.82 (NH), 7.6−8.30 (m, 4Ar + H-9 + H-10 + H-11 + H-12 + H-14), 9.25 (s, CH=N).

7-Phenoxyiminomethylcamptothecin (33). Prepared according to the general procedure B, stirring the mixture 3.5 h at room temperature. The crude product was purified by chromatography with hexane/AcOEt 1:1: yield 80%; mp 210 °C (dec), ^{1}H NMR δ: 0.89 (t, H_3-18*E* + H_3-18*Z*, *J* = 7.35 Hz), 1.81−1.95 (m, H_2-19*E* +H_2-19*Z*), 5.25 (s, H_2-5*Z*), 5.42 (s, H_2-17*Z*), 5.45 (s, H_2-5*E*), 5.52 (s, H_2-17*E*), 6.56 (s, -OH*Z* + -OH*E*), 7.15−7.55 (m, 5Ar*E* + 5Ar*Z* + H-14*E* + H-14*Z*), 7.83−7.96 (m, H-11*Z* + H-11*E* + H-10*Z* +H-10*E*), 8.28 (dd, H-12*E* + H-12*Z*, *J* = 8.09 Hz; *J* = 1.10 Hz), 8.73 (dd, H-9*E* + H-9*Z* *J* = 8.09 Hz; *J* = 1.10 Hz), 8.92 (s, -CH=N*Z*), 9.84 (s, -CH=N*E*). MS *m*/*z*: 467 (33, M^+), 373 (100), 329 (60), 314 (70), 273 (60), 244 (50), 135 (40), 57 (25), 43 (40).

7-(4-Aminobenzyl)oxyiminomethylcamptothecin (39). Prepared according to the general procedure B, stirring the mixture 2 days at room temperature. The crude product was purified by chromatography (CH_2Cl_2/MeOH 98:2): yield 20%; mp 146 °C (dec), ^{1}H NMR δ: 0.87 (t, H_3-18*E* + H_3-18*Z*, *J* = 7.35 Hz), 1.80−2.00 (m, H_2-19*E* +H_2-19*Z*), 5.00 (s, H_2-5*Z*), 5.10−5.55 (m, H_2-17*Z* +H_2-5*E* + H_2-17*E* + -CH_2O*Z* + -CH_2O*E*), 6.40−6.65 (m, 2Ar*Z* + 2Ar*E* +-OH*Z* + -OH*E*), 6.90−7.00 (m, 2Ar*Z*), 7.15−7.30 (m, 2Ar*E*) 7.35 (s, H-14*E* + H-14*Z*), 7.70−8.00 (m, H-11*Z* + H-11*E* + H-10*Z* +H-10*E* + H9*Z* + H-12*Z*), 8.20 (dd, H-12*E*, *J* = 8.47 Hz; *J* = 1.47 Hz), 8.40 (s, -CH=N*Z*), 8.60 (dd, H-9*E*, *J* = 8.47 Hz; *J* = 1.47 Hz), 9.30 (s, -CH=N*E*). MS *m*/*z*: 496 (13, M^+), 373 (58), 329 (42), 273 (42), 244 (51), 106 (100).

General Procedure for the Synthesis of Oximes (Method C). To a suspension of 7-hydroxyiminocamptothecin (**5**) (40 mg, 0.1 mmol) and Na_2CO_3 (11 mg, 0.1 mmol) in 4 mL of EtOH was added the appropriate halide or tosylate (0.1 mmol), and the mixture refluxed 3 h. Evaporation of the solvent and chromatography with hexane/AcOEt gave the expected product.

7-(4-Nitrobenzyl)oxyiminomethylcamptothecin (37). It was obtained from 4-nitrobenzylbromide in 20% yield, mp. 212 °C; ^{1}H NMR δ 0.88 (t, H_3-18*E* + H_3-18*Z*, *J* = 7.35 Hz), 1.81−1.95 (m, H_2-19*E* +H_2-19*Z*), 5.23 (s, CH_2O*Z* + CH_2O*E*), 5.30 (s, H_2-5*Z*), 5.40 (s, H_2-5*E*), 5.57 (s, H_2-17*E* + H_2-17*Z*), 6.55 (s, OH), 7.35 (s, H-14*E* + H-14*Z*), 7.75−7.95 (m, 2 Ar*E* + 2Ar*Z* + H-10*E* + H-10*Z* + H-11*E* + H-11*Z*), 8.2−8.4 (m, 2 Ar*E* + 2Ar*Z* + H-12*E* + H-12*Z* + H-9*Z*), 8.52 (CH=N*Z*) 8.65 (dd, H-9E, *J* = 8.5 Hz, *J* = 1.5 Hz), 9.50 (s, CH=N*E*).

7-[6-(1,2:3,4-Di-*O*-isopropylidene-D-galactopyranosyloxy)]iminomethylcamptothecin (32). A solution of 40 mg (0.1 mmol) of **5,** 2.5 mg (0.1 mmol) of NaH, 43 mg (0.1 mmol) of 1,2:3,4-diisopropylidene-α-D-galactopyranosyltrifluoromethanesulfonate in 4 mL of EtOH was stirred at room temperature for 24 h. After evaporation of the solvent, the product was purified by chromatography (hexane/AcOEt 4:6, then CH_2Cl_2/MeOH 95:5): yield 14%; mp 155 °C (dec); ^{1}H NMR δ: 0.87 (t, H_3-18*E* + H_3-18*Z*, *J* = 7.35 Hz), 1.30−1.45 (m, 4 -CH_3), 1.81−1.95 (m, H_2-19*E* +H_2-19*Z*), 3.90−4.70 (m, H_2-6′; H-5′; H-4′; H-3′; H-2′), 5.35 (s, H_2-5*Z* + H_2-5*E*), 5.45 (s, H_2-17*Z* + H_2-17*E*), 5.60 (d, H-1′, *J* = 5.52 Hz,) 6.52 (s, -OH*Z* + -OH*E*), 7.35 (s, H-14*E* + H-14*Z*), 7.75 (m, H-10*E*; H-10*Z*), 7.90 (m, H-11*E* + H-11*Z*) 8.05 (dd, H-12*Z*, *J* = 8.47 Hz; *J* = 1.47 Hz), 8.20 (m, H-12*E* + H-9*Z*), 8.50 (s, -CH=N*Z*), 8.65 (dd, H-9*E*, *J* = 8.47 Hz, *J* = 1.47 Hz), 9.40 (s, -CH=N*E*). MS *m*/*z*: 634 (13, M+1), 576 (10), 486 (20), 347 (35), 329 (45), 314 (50), 302 (30), 246 (100), 242 (55), 187 (26).

7-(6-D-Galactopyranosyloxy)iminomethylcamptothecin (31). A total of 6 mg (0.01 mmol) of **32** was stirred 3 h at room temperature in 0.5 mL of TFA 80%. Evaporation of the solvent and chromatography (CH_2Cl_2/MeOH 97:3) gave the pure product in 60% yield: mp 210 °C (dec); ^{1}H NMR δ 0.85 (t, H_3-18*E* + H_3-18*Z*, *J* = 7.35 Hz), 1.75−1.95 (m, H_2-19*E* +H_2-19*Z*), 3.50−5.0 (m, 10H galact.), 5.35 (s, H_2-5*Z* + H_2-5*E*) 5.45 (s, H_2-17*Z* + H_2-17*E*), 6.25 (d, -OH galact.), 6.55 (s, -OH*Z* + -OH*E*), 6.65 (d, -OH galact.), 7.35 (s, H-14*E* + H-14*Z*), 7.80 (m, H-10*E* + H-10*Z*), 7.98 (m, H-11*E* + H-11*Z*), 8.25 (dd, H-12*E* + H-12*Z*, *J* = 8.47 Hz, *J* = 1.46 Hz), 8.50 (s, CH=N*Z*); 8.60 (dd, H-9*E* + H-9*Z*, *J* = 8.47 Hz, *J* = 1.46 Hz) 9.35 (s, CH=N*E*).

7-*tert*-Butoxyiminomethylcamptothecin *N*-oxide (16). Compound **15** (0.067 mmol) was dissolved in AcOH (5 mL), and 1 mL of 30% H_2O_2 was added. The mixture was heated at 70−80 °C for 10 h, then concentrated to one-third and poured into iced water. The precipitate was collected by suction and chromatographed with hexane/AcOEt 1:1 to give the expected product: yield 50%; mp 185−190 °C; ^{1}H NMR δ 0.87 (t, H_3-18), 1.48 (s, *t*-Bu), 1.75−1.95 (m, H_2-19), 5.37 (s, H_2-5), 5.42 (s, H_2-17), 6.60 (s, OH), 7.85−8.00 (m, H-10 + H-11), 8.15 (s, H-14), 8.65−8.75 (m, H-9 + H-12), 9.20 (s, CH=N).

7-Methoxyiminomethylcamptothecin *N*-Oxide (11). It was prepared according to the procedure described for **16.** Chromatography with CH_2Cl_2/MeOH 98:2 gave **11** as a yellow

solid: Yield 53%; mp >200 °C (dec); ^{1}H NMR δ 0.87 (t, H_3-18, J = 7.35 Hz), 1.78−1.93 (m, H_2-19), 4.12 (s, -OCH_3), 5.35 (s, H_2-5), 5.43 (s, H_2-17), 6.54 (s, -OH), 7.84−8.00 (m, H-10 + H-11), 8.11 (s, H-14) 8.68−8.73 (m, H-9 + H-12), 9.21 (s, -CH=N).

7-Methoxyiminomethyl-10-hydroxycamptothecin (12). A suspension of **11** (8.3 mg, 0.02 mmol) in 5 mL of acetonitrile was added with 40 μL of 0.5 N H_2SO_4, degassed with N_2, and stirred while irradiated with UV light at 365 nm for 10 h. Evaporation and chromatography (CH_2Cl_2/MeOH 97:3) gave 6.9 mg of **(12)**, yield 83%; mp 268 °C (dec).

7-*tert*-Butoxyiminomethyl-10-hydroxycamptothecin (17). The compound was prepared according to the procedure followed to obtain **12.** Purification by chromatography (CH_2Cl_2/MeOH 98:2) gave the desired product in 22% yield, mp 195°(dec); ^{1}H NMR δ 0.88 (t, H_3-18*E* + H_3-18*Z*, J = 7.35 Hz), 1.35 (s, *t*-Bu *Z*) 1.45 (s, *t*-Bu *E*) 1.80−1.90 (m, H_2-19*E* + H_2-19*Z*), 5.10−5.40 (m, H_2-5*E* + H_2-5*Z* + H_2-17*E* + H_2-17*Z*), 6.53 (s, OH), 7.25−7.50 (m, H-14*E* + H-14*Z* + H-11*E* + H-11*Z* + H-9*Z*), 7.70 (d,. H-9*E*, J = 2.57 Hz) 8.05 (d, H-12*E* + H-12*Z*, J = 9.19 Hz), 8.25 (s, CH=N *Z*), 9.0 (s, CH=N *E*) 10.35 (s, 10-OH).

7-Oxiranylmethoxyiminomethylcamptothecin (14). To a suspension of compound **13** (15 mg, 0.035 mmol) in 18 μL of CF_3CH_2OH and 4 μL of pyridine were added 0.01 mg of $MeReO_3$ and 4 μL of 35% H_2O_2, and the mixture was stirred for 5 h at room temperature. Chromatography of the mixture with hexane/AcOEt 3:7 afforded 4 mg (26%) of (**14**), mp 154 °C (dec); ^{1}H NMR ($CDCl_3$) δ: 0.87 (t, H_3-18, J = 7 Hz), 0.80−2.00 (m, H_2-19), 2.80 (1H, m, CH_2O), 3.05 (1H, m, CH_2O), 3.40 (m, CHO-), 3.75 (s, -OH), 4.30 (1H, m, CH_2ON), 4.73 (1H, m, CH_2ON), 5.33 (d, H-17A, J = 16 Hz), 5.45 (s, H_2-5), 5.75 (d, H-17B, J = 16 Hz), 7.70 (s, H-14), 7.75 (m, H-11), 7.85 (m, H-10), 8.15−8.35 (m, H-9 + H-12), 9.12 (s, -CH=N).

QSAR Methods. LogP values were generated using CLOGP (BioByte); volume indices were calculated by TSAR (OMG). Multiple regression analysis was performed using TSAR (OMG).

In Vitro Studies. The human tumor cell lines used in this study included H460, a human lung large cell carcinoma cell line (ATCC HTB 177), and a topotecan-resistant subline (H460/TPT); IGROV-1, an ovarian carcinoma cell line,[27] and its cisplatin-resistant variant IGROV-1/Pt1. The resistant cell lines were selected in our laboratory after exposure to increasing drug concentrations; their growth characteristics were similar to those of the correspondent parental cell lines. All the cell lines were cultured in RPMI-1640 containing 10% fetal calf serum. Cytotoxicity was assessed by growth inhibition assay after 1 h drug exposure. Briefly, 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 are insoluble in water and were dissolved in DMSO prior to dilution into the biological assay.

In Vivo Studies. Nude athymic CD1 mice (Charles River Lab.), 8−10 weeks old, were used for the studies. Animals were maintained in laminar flow rooms and experimental protocols were approved by the Ethical Committee for Animal Experimentation of Istituto Nazionale dei Tumori Human lung tumor lines were maintained s.c. by serial passages. For chemotherapy studies, mice were xenografted s.c. in both flanks with tumor fragments as already described.[27] Tumor growth was monitored by diameters measurement and tumor volume (TV) was calculated as: TV = $d2 \times D/2$, where d and D represent the shortest and the longest diameter, respectively. Drugs were delivered in a volume of 10 mL/kg body weight, starting treatment when tumors were visible but not measurable. The effects of drug treatment were assessed as folows: TV inhibition percent in treated versus control tumors, 7−10 days after the last treatment, and log10 cell kill (LCK) induced by the treatment and calculated as $T - C/3.32 \times$ tumor doubling time, where T and C represent days in treated and in control tumors to reach a mean TV of 1000 mm^3. Student's t test was used to compared tumor volumes of treated mice. P values < 0.05 were considered significant.

Topoisomerase I-Dependent DNA Cleavage Assay. A gel purified 751 bp BamHI-EcoRI fragment of SV40 DNA was used for the cleavage assay. DNA fragments were uniquely 3′-end labeled. Topoisomerase I−DNA cleavage reactions (20000 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)[28]for 20 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 20 min of incubation. Three volumes of denaturing buffer (80% formamide, 10 mM NaOH, 0.01 M EDTA, and 1 mg/mL dyes) were added before loading on a denaturing 7% polyacrylamide gel in TBE buffer.

Overall DNA cleavage levels were measured with a PhosphoImager 425 model (Molecular Dynamics) and expressed as the ratio of the radioactivity present in selected cleavage bands with respect to uncleaved DNA. The drug-stimulative effects were calculated as the ratio of DNA cleavage levels with drugs with respect to topoisomerase I alone. To normalize among different experiments the stimulation factor of camptothecin at 1 μM, included in all of the experiments, was used as internal standard.

Determination of Topoisomerase−DNA Complexes. Cells were plated in 100 mm dish plates at different number to reach a subconfluent density (2.5×10^6 cells/dish) the day of treatment (after 24 h). Then, cells were washed with serum-free medium and incubated at 37 °C for 1 h with camptothecin analogue (10 μM). Drugs were dissolved in serum-free medium containing 1% DMSO. After treatment, cells were washed with serum-free medium to remove drugs and then maintained in culture for 6 h in complete medium. Different samples were collected at 1 h, corresponding to the end of treatment, and after 6 h from drug removal using TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) with 1% Sarkosil. The complex was purified according to Muller and Metha[29] and Trask and Muller.[30] Briefly, lysates were overlaid on a CsCl gradient (g/cm^3: 1.8, 1.7, 1.5, 1.3) and centrifuged for 18 h at 31000 rpm at 25 °C. Fractions (400 μL each) of the gradients were collected, and the DNA peak was localized by absorbance at 260 nm. Fractions were transferred under vacuum to a nitrocellulose membrane using a Slot Blot apparatus (Bio-Rad), and filters were incubated with a monoclonal antibody against human topoisomerase I (Topogen) followed by treatment with horseradish peroxidase-linked anti mouse IgG (SIGMA). Chemiluminescence was detected by exposure to ECL-plus reagent (Amersham). The intensity of bands was quantified by a densitometer.

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