Synthesis, Topoisomerase I Inhibitory Activity, and *in Vivo* Evaluation of 11-Azacamptothecin Analogs

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A series of analogs based on a novel template, 11-aza-(20*S*)-camptothecin, were obtained from total synthesis and tested as potential anticancer drugs in the topoisomerase I enzyme cleavable complex assay. The parent compound 11-aza-(20*S*)-camptothecin (**8**) was derived from a Friedlander condensation between the known aminopyridine derivative 3-(3-amino-4-picolylidene)-*p*-toluidine and optically active tricyclic ketone **7**. Compound **8** had activity approximately twice that of (20*S*)-camptothecin in the calf thymus topoisomerase I cleavable complex assay. Compounds were prepared wherein the 11-aza nitrogen atom was quaternized as either the corresponding *N*-oxide or methyl iodide. Compounds with quaternized N-11 showed improved water solubility and were equipotent to the clinically investigated camptothecin analog topotecan in the cleavable complex assay. These compounds were evaluated *in vivo* in nude mice bearing HT-29 human colon carcinoma xenografts. The analog 11-aza-(20*S*)-camptothecin 11-*N*-oxide was found to significantly retard tumor growth when compared to untreated controls. Finally, 7,10-disubstituted 11-azacamptothecin analogs were synthesized using Pd(0) coupling reactions of 10-bromo-7-alkyl-11-aza-(20*S*)-camptothecins **19** and **20**, which in turn were available from a Friedlander condensation of the novel bromopyridine derivatives **17a** and **17b** with **7**. Among the 10-substituted series, a number of analogs displayed extremely high *in vitro* potency against topoisomerase I and improved aqueous solubility. A significant number of the compounds were found to be active in whole cell cytotoxicity assays and several were evaluated in nude mice bearing the HT-29 tumor xenografts. The most effective of these proved to be (*S*)-11-aza-7-ethyl-10-(aminohydroximinomethyl)camptothecin trifluoracetic acid salt (**27**), a potent topoisomerase I inhibitor which demonstrated excellent efficacy in both short term and in extended *in vivo* assays. A comparison between *in vitro* enzyme data and *in vivo* data from nude mouse studies in other compounds in this series revealed a poor overall correlation between topoisomerase inhibition *in vitro* and antitumor efficacy *in vivo*.

Introduction

The antitumor agent (20*S*)-(−)-camptothecin (**1**), discovered in 1966 by Wall and co-workers,[1] has attracted much attention as a lead structure in the search for effective agents for the treatment of solid tumors. While (20*S*)-(−)-camptothecin has long been known to exhibit antitumor properties both *in vitro* and in leukemic mouse models, it was not until the mid 1980s that these antitumor effects were correlated to its activity against the nuclear enzyme topoisomerase I.[2,3] Although the mechanism by which modulation of topoisomerase I leads to cytotoxicity is not well-understood, (20*S*)-camptothecin has been shown to stabilize the complex formed when topoisomerase I cleaves and covalently binds to a single strand of isolated double stranded DNA. This action of the drug results in fragmentation of DNA upon denaturation of the so-called "cleavable complex" and may be analogous to a lethal cellular DNA fragmentation process caused by the formation of the ternary complex of DNA with topoisomerase I and camptothecin.[4,5] Unfortunately, (20*S*)-(−)-camptothecin has fallen short of its initial promise as a therapeutic agent due to its poor solubility and toxicity in human clinical trials.[6] Attention has shifted toward the discovery of potent analogs of **1** with improved therapeutic potential, and during the past two decades numerous reports describing the synthesis and structure–activity relationships of camptothecin analogs have appeared.[7,8]

Much of the synthetic work in the camptothecin area has contributed to a general understanding of the SAR around the camptothecin structure, which in turn has led to the discovery of highly potent analogs as measured both *in vitro* and *in vivo* in leukemic and mouse models.[7] Poor water solubility, which may lead to difficulties in formulation and unpredictable plasma levels of the drug during administration in a clinical setting, has precluded further development of many of these analogs.[9] While the lactone ring-opened sodium salt of camptothecin (**2**) does possess solubility properties amenable to administration in the clinic, the unacceptable toxicity of the carboxylate salt, including severe hemorrhagic cystitis and unpredictable myelosuppression, led to the discontinuation of further clinical trials.[10] An alternative strategy in the search for water soluble analogs of camptothecin has centered around using known SAR information to attach water solubilizing substituents to positions of camptothecin tolerant to substitution, while at the same time retaining the

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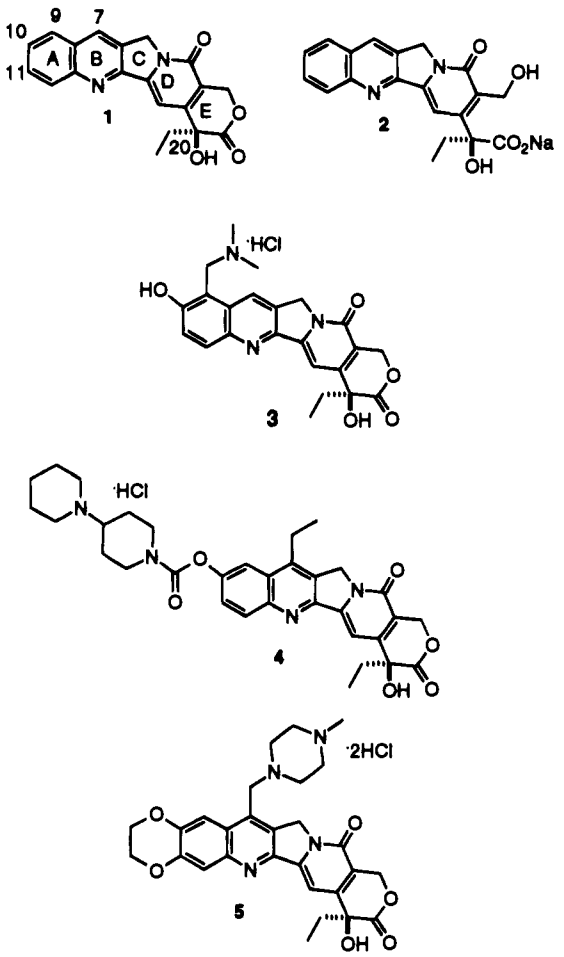
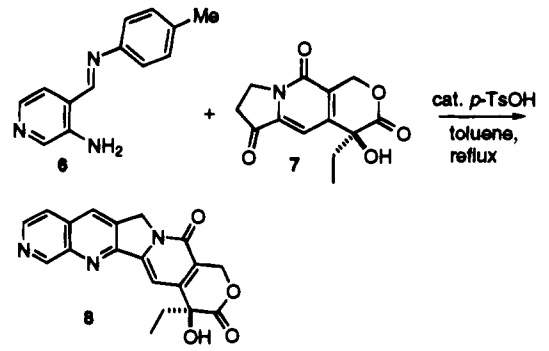
Chart 1



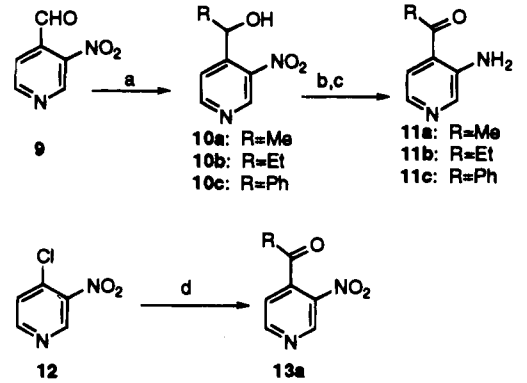
unmodified lactone E ring, an important pharmacaphore for enzyme activity.[11] Efforts in this area have resulted in the identification of the water soluble camptothecin analogs topotecan (**3**),[12] developed by workers at Smith-Kline Beecham, and CPT-11 (**4**), a water soluble prodrug of 7-ethyl-10-hydroxycamptothecin developed at Yakult-Honshu.[13] These promising antitumor agents are now undergoing extensive clinical evaluation in the United States.[14] Recently, we have reported the synthesis and pharmacological evaluation of GI 147211C (**5**),[15,16] which has demonstrated highly encouraging preclinical activity and is now in human clinical trials as well.

Most known potent analogs of camptothecin retain the quinoline A and B rings of camptothecin and are substituted at the 7, 9, 10, or 11 positions.[7] A few active camptothecin congeners that contain a modified template are known and include 10-azacamptothecin, 12-azacamptothecin, and 9-northiocamptothecin.[17] To further investigate this relatively unexplored area, we became interested in the 11-azacamptothecin template, which we considered making in order to mimic the electronic properties of the very potent analog 11-fluorocamptothecin.[18] We now describe the synthesis and *in vitro* and *in vivo* evaluation of a series of derivatives based upon the novel 11-aza-(20*S*)-camptothecin template. The good activity of the parent compound 11-aza-(20*S*)-(−)-camptothecin in the topoisomerase cleavable complex assay[2,19] encouraged us to synthesize analogs based around this structure. The 11-aza atom was modified as the quaternary *N*-oxide and *N*-methyl iodide salt in order to enhance water

Scheme 1



Scheme 2[a]



[a] (a) MeLi or Et_2Zn, −45 °C; (b) Dess−Martin periodinane, CH_2Cl_2; (c) $Na_2S_2O_4$, EtOH; (d) (1-ethoxyvinyl)tributyltin; HCl.

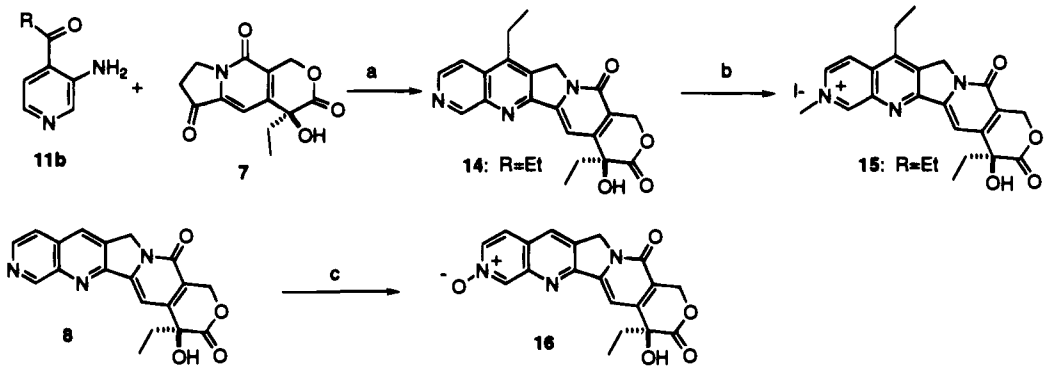
solubility. In addition, a series of 7-alkyl-11-azacamptothecin analogs substituted at the 10 position with groups that afforded further improvement in both *in vitro* potency and water solubility were made *via* a novel regioselective pyridine bromination. Many of these analogs were found to be active against a panel of human tumor cell lines in cell cytotoxicity assays. Several compounds that showed desirable *in vitro* and water solubility profiles were evaluated *in vivo* in nude mouse models bearing HT-29 xenografts.

Chemistry

The parent A-ring aza analog **8** was obtained by a Friedlander condensation between the optically active tricyclic ketone **7**[20] and the known aminopyridine derivative **6**[21] (Scheme 1). The synthesis of analogs additionally substituted at C-7 with methyl or ethyl groups by analogous Friedlander condensations required the novel ketoaminopyridine derivatives **11a** and **11b**, which were made through one of two routes shown in Scheme 2. In the first route, an organometallic agent was added to 3-nitro-4-pyridinecarboxaldehyde (**9**)[21] to afford, after Dess−Martin oxidation of the resulting secondary alcohol[22] and reduction of the nitro group, the desired ketoaminopyridine derivatives. In the second route, which was applied to the synthesis of **11a**, 3-chloro-2-nitropyridine[23] and (1-ethoxyvinyl)tributyltin were coupled using catalytic palladium(0) to afford intermediate **13a** after acid hydrolysis of the unisolated pyridine vinyl ether intermediate.

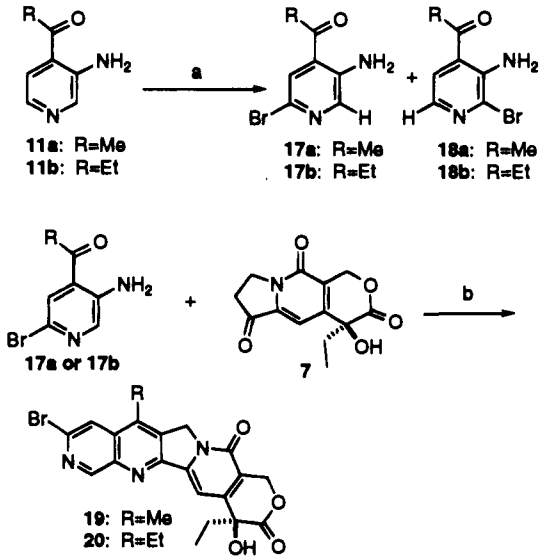
Two 11-azacamptothecin analogs quaternized at the 11-nitrogen atom were synthesized as shown in Scheme 3. Friedlander condensation of ketoaminopyridine derivative **11b** afforded 7-ethyl-11-aza-(20*S*)-camptothecin

Scheme 3[a]



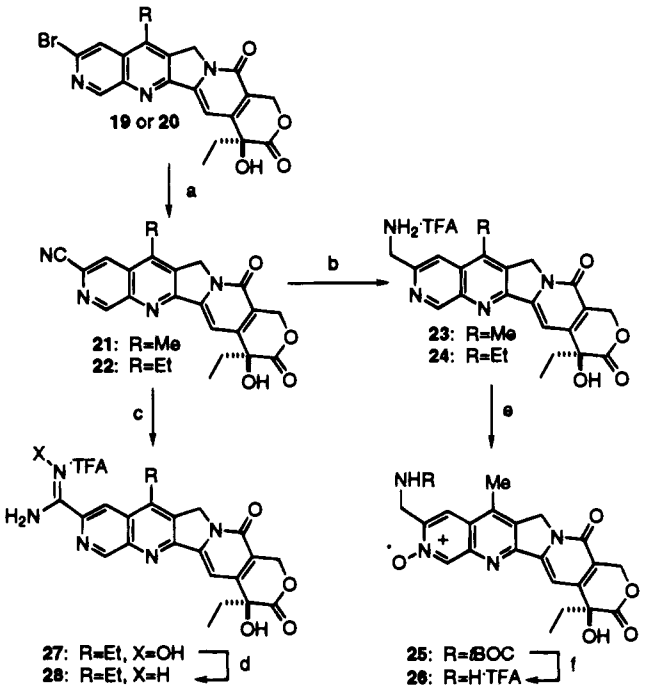
[a] (a) Catalytic *p*-toluenesulfonic acid, toluene, reflux; (b) methyl iodide, 5:1 $CHCl_3$/MeOH; (c) *m*-CPBA, CH_2Cl_2.

Scheme 4[a]



[a] (a) Pyrrolidine hydrotribromide, 1 equiv THF; (b) catalytic *p*-toluenesulfonic acid, toluene, reflux.

Scheme 5[a]



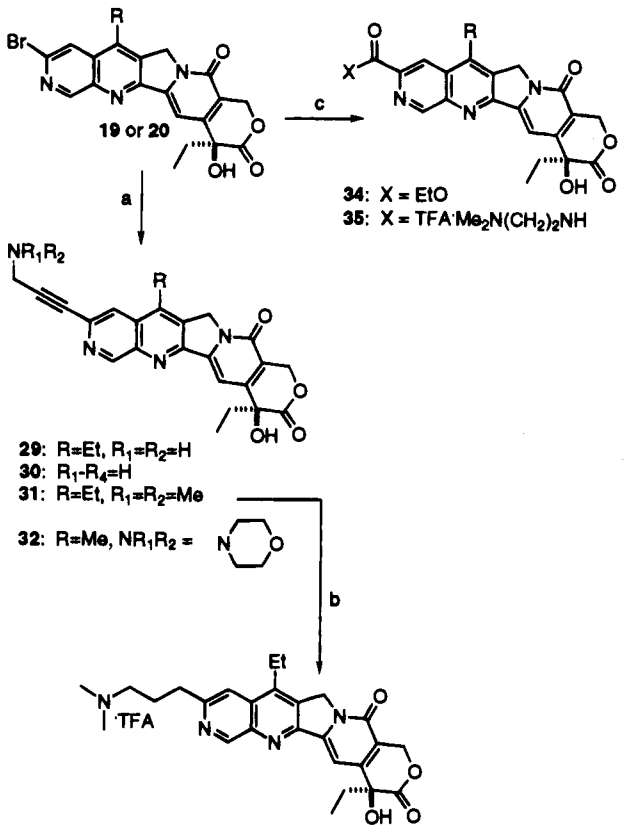
[a] (a) Bu_3SnCN, $Pd(PPh_3)_4$, 1,2-dichloroethane; (b) H_2, Pd/C; (c) H_2NOH·HCl, Na_2CO_3; (d) H_2, Raney Ni; (e) $(Boc)_2O$, Et_3N; *m*-CPBA; (f) TFA (neat).

(**14**), which in turn was quaternized with iodomethane to give the *N*-methyl iodide salt **15**. The 11-*N*-oxide **16** was prepared by treatment of 11-aza-(20*S*)-camptothecin (**8**) with *m*-CPBA. Both the methyl quaternization and oxidation reactions were found to be completely regioselective with respect to reaction at N-11 *vs* N-1, even though more vigorous oxidation conditions have been reported to produce camptothecin N-1 oxides.[18]

For the preparation of various 10-substituted 11-azacamptothecin analogs, we employed the key intermediate 11-aza-10-bromocamptothecin analogs **19** and **20**, which were synthesized as shown in Scheme 4. Treatment of **11a** or **11b** with a small excess of pyrrolidone hydrotribromide afforded a 5−6:1 mixture of the regioisomeric derivatives **17** and **18**. Subjecting the mixture of **17** and **18** to the usual Friedlander reaction conditions with (*S*)-tricyclic ketone **7** resulted in the rapid formation of either **19** or **20** at the exclusion of products arising from the participation of **18** in the condensation reaction.

The (*S*)-11-aza-10-bromo-7-alkylcamptothecin analogs **19** and **20** underwent facile palladium(0)-catalyzed insertion reactions to afford a diverse set of derivatives substituted at C-10 with water solubilizing groups.[24] Palladium-catalyzed exchange to afford the 10-cyano compounds **21** and **22** occurred smoothly under standard conditions (Scheme 5). Hydrogenation of the cyano group of **21** or **22** using palladium on carbon[25] afforded the 10-aminomethyl derivatives **23** and **24**, the former of which was protected, oxidized with mCPBA to afford *t*-Boc *N*-oxide **25**, which was deprotected to give the corresponding A-ring pyridine *N*-oxide derivative **26**. The cyano compound **22** was converted into the corresponding carboxamide oxime which was reduced using Raney nickel to supply the amidino derivative **28**. Palladium-catalyzed substitution of analogs **19** and **20** with propargylamines afforded gave the acetylenic analogs **29**−**32**. Reduction of acetylene **31** afforded the 10-*n*-propylamino derivative **33** (Scheme 6). Finally, Pd-catalyzed CO insertion[26] in the presence of ethanol or ethanol plus *N*,*N*-dimethylethylenediamine afforded the 10-ethyl carboxylate **34** and the aminoethyl amide derivative **35**, respectively. In general, derivatives containing water-solubilizing amine, amidoxime, and amidine substituents were purified by reverse-phase HPLC and tested as the corresponding trifluoroacetic acid salt.

Scheme 6[a]



[a] (a) Propargyl or *N,N*-dialkylpropargylamine, $Pd(PPh_3)_4$; (b) H_2, Pd/C; (c) CO, EtOH, $Pd(PPh_3)_4$; or CO, $Me_2N(CH_2)_2NH_2$, $Pd(PPh_3)_4$

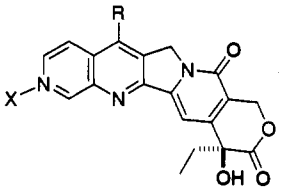
Results and Discussion

All of the azacamptothecin analogs described above were tested in the topoisomerase I cleavable complex assay, in which (20*S*)-camptothecin (**1**) was used as a positive control. The results are given as IC_{50} values, which is the concentration of the analog required to produce approximately 50% fragmentation of P-32 end-labeled DNA in the presence of calf thymus topoisomerase I. ((*S*)-Camptothecin and topotecan were measured in this assay to have values of 700 and 1080 nM, respectively.) Selected 11-azacamptothecin analogs were also tested against the human colon cell line HT-29, the ovarian cell line SKOV3, and also against SKVLB (an ovarian cell line with upregulated MDR-1 P-glycoprotein) in *in vitro* cytotoxicity assays. Compounds that displayed good enzyme and cell activity (<1 μM) and contained structural features thought to enhance water solubility were then analyzed to determine their aqueous solubility either in pH 5 acetate buffer using an HPLC method or in D_2O using ^1H NMR and 1,4-dioxane as an internal standard. Several analogs demonstrating both potent *in vitro* activity and adequate water solubility (>300 μg/mL) were evaluated *in vivo* for efficacy in nude mice bearing human colon carcinoma xenografts, using either a protocol that measures tumor growth over approximately a 2 week period or one in which tumor volume over a 5 week period is monitored. In the 2 week assay, the maximum tolerated dose of the compound was first determined, and this dose was administered in three portions to tumor bearing animals in 1 day. The subsequent delay in tumor growth (T−C, expressed in days) of treated *vs* tumor-bearing controls was then measured as the difference in time that the mean tumor volume required to reach 500% of initial mean volume in the two groups of animals ($N = 6$). In the longer assay, the compound was dosed twice weekly over a period of 5 weeks, and the relative mean tumor volume in drug-treated animals ($N = 8$) was compared between the beginning and end of the 5 week period (expressed as %T/B).

The activity of the parent compound 11-aza-(20*S*)-(−)-camptothecin and the quaternary *N*-methyl iodide and *N*-oxide analogs in the topoisomerase I cleavable complex assay, and water solubility data for some of these analogs is shown in Table 1. As can be seen, the enzyme activities of both 11-aza-(20*S*)-camptothecin (**8**) and 7-ethyl-11-aza-(20*S*)-camptothecin (**14**) are approximately twice that of camptothecin itself, while the *N*-oxide derivative (**16**) and the methyl iodide quaternary salt (**15**) are somewhat less active. Replacement of the 11 carbon atom of camptothecin with nitrogen in itself failed to significantly improve water solubility, as is indicated by the low value of **14** (10 μg/mL) in pH 5 acetate buffer, while quaternization of N-11 as the *N*-oxide or *N*-methyl iodide salt improved aqueous solubility. Although less active than the parent 11-aza compound, *N*-methyl iodide **15** and *N*-oxide **16** had topoisomerase I activities comparable to topotecan. These compounds were further investigated *in vivo*. The *N*-oxide was evaluated in the 5 week assay in nude mice, and its effect on tumor growth was measured and compared to controls (Figure 1). As can be seen (Figure 1A), compound **16** exhibited a dose dependent reduction in the rate of tumor growth, with low toxicity as indicated by lack of appreciable body weight loss (Figure 1B). By comparison, the clinical standard chemotherapeutic agent 5-fluorouracil[27] was less effective than **16** when administered in this protocol at maximum tolerated dose (60 mg/kg, data not shown). The *N*-methyl iodide **15** was investigated using the 2 week *in vivo* protocol (see below).

Encouraged by these results, we prepared and evaluated a series of 11-azacamptothecin analogs with substitution at the 10-position. Because of synthetic accessibility and the fact that active topoisomerase inhibitors such as CPT-11 contain a lower alkyl group at C-7, we incorporated a methyl or ethyl group at C-7 in the 10-substituted series. The cleavable complex data for the 10-substituted 11-azacamptothecin analogs (Table 2) revealed that several of the analogs were substantially more potent *in vitro* than (20*S*)-camptothecin. Compounds with small substituents at C-10 (Br, CN) or which contained a substituent capable of hydrogen bond donation two atoms from the A ring (e.g., C(=NH)-NH_2, CH_2NH_2, and CH_2NH*t*-BOC) were especially active in the enzyme assay, while propargylamine derivatives **29**−**32**, which were made to probe a region spanning a three-atom link from the A ring, were generally less potent than camptothecin. On the basis of this data, we believe that the compounds containing a hydrogen bond-donating substituent adopt an intramolecular H-bond with the A ring nitrogen atom, affording a roughly planar 5- or 6-membered ring which augments activity.[28] In support of this argument, it is noteworthy that while compound **24** is roughly twice as potent as **14**, which lacks the 10-aminomethyl substituent, 10-(aminomethyl)-(20*S*)-camptothecin (which does not have a heteroatom in which to form an intramo-

Table 1. 11-Aza-(20*S*)-camptothecin *N*-Oxides or *N*-(Methyl iodide) Salts



compound	R	X	mp (°C)	aqueous solubility (μg/mL)[a]	average IC_{50} (nM)[b]
11-aza-(20*S*)-camptothecin (**8**)	H		200 dec	ND	383 (104)
14	Et		150 dec	10	336 (46)
15	Et	MeI	213–215	>2500	1461
16	H	O	215 dec	300	1184 (484)
(20*S*0-(−)-camptothecan (1)				<5[32]	700 (105)
topotecan (3)				3100	1080 (220)

[a] Solubility determinations were performed in pH 5 acetate buffer (see Experimental Section). [b] IC_{50} = the minimum drug concentration that produced 50% fragmentation of DNA in the presence of excess calf thymus topoisomerase. Standard deviations given in parentheses.

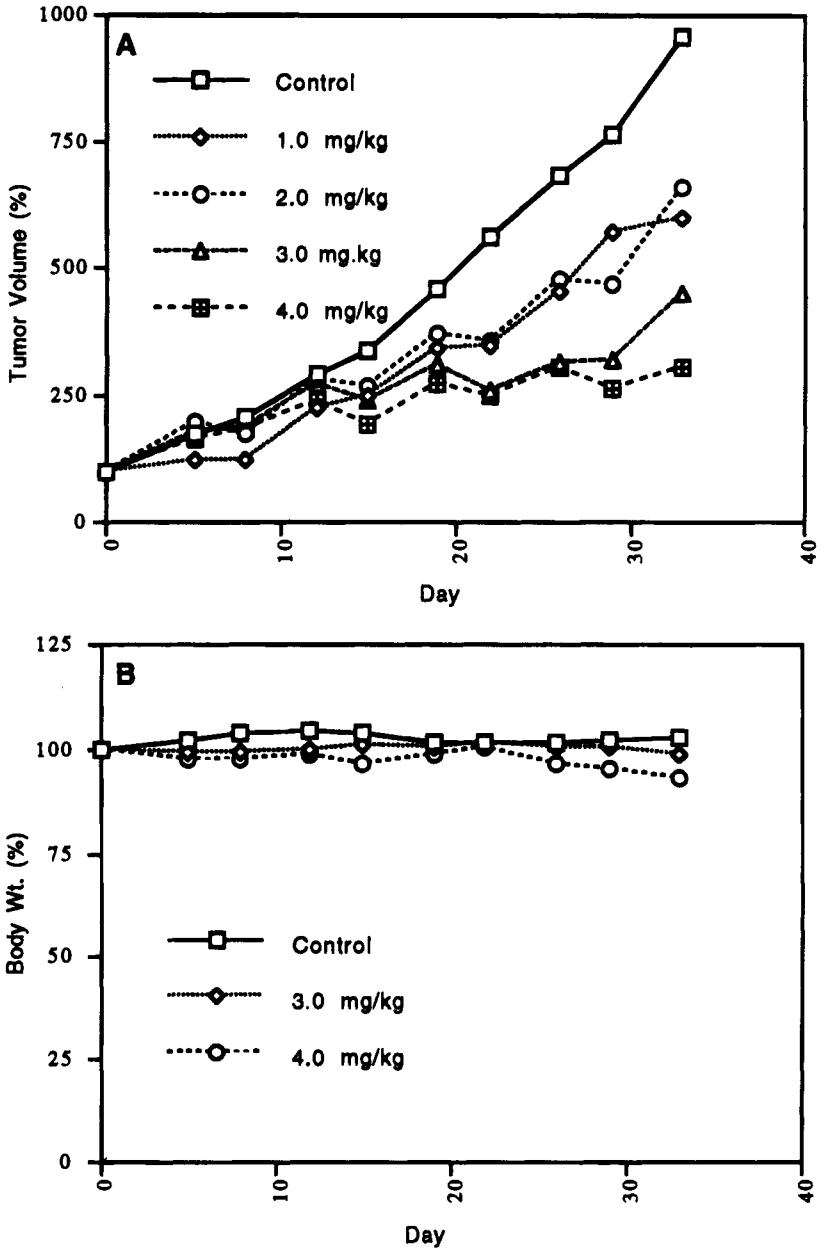
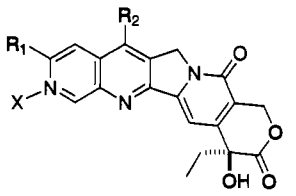
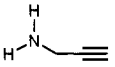
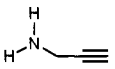
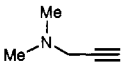
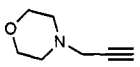
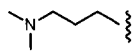
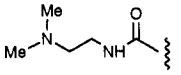


Figure 1. (A) Tumor volume of HT-29 nude mice (N = 8) treated subcutaneously twice/week with **16** at 1.0, 2.0, 3.0, and 4.0 mg/kg compared to controls. (B) Body weight of animals treated at 3.0 and 4.0 mg/kg compared to controls.

lecular hydrogen bond) has been reported to be only $^1/_{12}$ as potent as (20*S*)-(−)-camptothecin itself.[12] A compound that does not follow the observed trend is the (*N*,*N*-dimethylamino)ethyl carboxamide derivative (**35**),

Table 2. 10-Substituted 11-Azacamptothecin Analogs



compd	R_1	R_2	X	mp (°C)	SKOV IC_{50} (nM)[a]	SKVLB IC_{50} (nM)[b]	HT29 IC_{50} (nM)[c]	IC_{50} (nM)[d] (SE)
19	Br	Me		274 dec	1.0	0.5	1.0	28 (11)
20	Br	Et		273–275			7	122 (39)
21	NC	Me		293 dec				143 (97)
22	NC	Et		160 dec			17	268 (36)
23[e]	H2NCH2	Me		230 dec	17	42	123	232 (30)
24[e]	H2NCH2	Et		160 dec	32	444		167 (37)
25	t-BocNHCH$_2$	Me	O	250 dec				217 (18)
26[e]	H_2NCH_2	Me	O	230 dec	66	527	731	190 (18)
27[e]	$H_2NC{=}NOH$	Et		260 dec	0.8	15	7	71 (7)
28[e]	$H_2NC{=}NH$	Et		250 dec	71	1030	311	33 (7)
29[e]		Me		132 dec	245	258	355	505 (105)
30[e]		Et		175–177	243	384	351	1075 (315)
31[e]		Et		130 dec				1331 (314)
32[e]		Me		178–180				1113 (792)
33[e]		Et		115–117				1494 (824)
34	EtO_2C	Et		175 dec			26	949 (157)
35[e]		Et		153 dec				9328 (3806)

[a] Cytotoxicity concentration against a human ovarian cancer cell line. [b] Cytotoxicity concentration against a vinblastine resistant ovarian cancer cell line. [c] Cytotoxicity concentration against a human colon carcinoma cell line. [d] See Table 1. [e] Data is for TFA salt.

which contains a substituent potentially capable of intramolecular bonding but has poor activity in the cleavable complex assay. The other compounds we prepared that contained an amine linked three or more atoms from the A ring, which include the propargylamine derivatives **29**–**32** and the propylamine derivative **33**, were also less active than the parent compound **8**. We believe that the generally weak (>1 μM) topoisomerase I activity of compounds **29**–**33** and **35** is a result of a lack of tolerance for the charged 10-alkylamino group in the active site of the enzyme–DNA–drug ternary complex.[29]

A number of the 11-azacamptothecin derivatives were tested in whole cell assays against the human ovarian carcinoma cell line SKOV3, a vinblastine resistant variant (SKVLB), and the human colon carcinoma cell line used in the *in vivo* xenografts (HT-29). In general, compounds active in the cleavable complex assay had IC_{50}'s in the submicromolar range against all three cell lines (Table 2). Interestingly, the derivatives that did not contain an amino salt (**19**, **20**, **22**, **34**) generally showed greater cytoxicity against HT-29 cells than those containing a charged amino group, suggesting that a charged group in these analogs may reduce cell permeability and therefore cytotoxicity. With the exception of **27**, compounds bearing a positive charge were also somewhat less potent in the vinblastine resistant cell line than in the nonvinblastine ovarian cell line.

Several of the most potent analogs in the 10-substituted series contained substituents thought to enhance water solubility and were considered for further evaluation of their antitumor properties. Among these, six 11-azacamptothecin analogs with structurally diverse substituents at the 10 position (including the 10-*H*-methyl iodide salt **15**) were found to possess water solubilities in excess of 2 mg/mL and were chosen for *in vivo* evaluation in nude mice. These compounds were investigated using the 2 week HT-29 xenograft assay in which drug efficacy was measured as the delay in tumor growth, T–C, of drug treated animals relative to controls. As can be seen in Table 3, many of these derivatives showed less effect on tumor growth in this assay than topotecan, a surprising observation in view of the uniformly high potency of these analogs in the

Table 3. Solubility and In Vivo Results for Selected 10-Substituted 11-Azacamptothecin Analogs

compound	IC_{50} (nM)	H_2O solubility (μg/mL)	dose (mg/kg)	tumor growth delay (T–C, days)[a]
topotecan	1080	3500[b]	54	10.3
15	1462	>2500[b]	100	3.5
23	232	7200[b]	200	3.4
26	190	>3000[c]	120	5.3
27	71	>3000[c]	25	**15**
28	33	>3000[c]	90	6
30	1075	5700[b]	146	1.5

[a] Delay in tumor growth to 500% volume of day 1 relative to control (see Experimental Section). [b] Solubility determined in pH 5 acetate buffer using HPLC method (see Experimental Section). [c] Solubility determined in D_2O using ^1H NMR method (see Experimental Section).

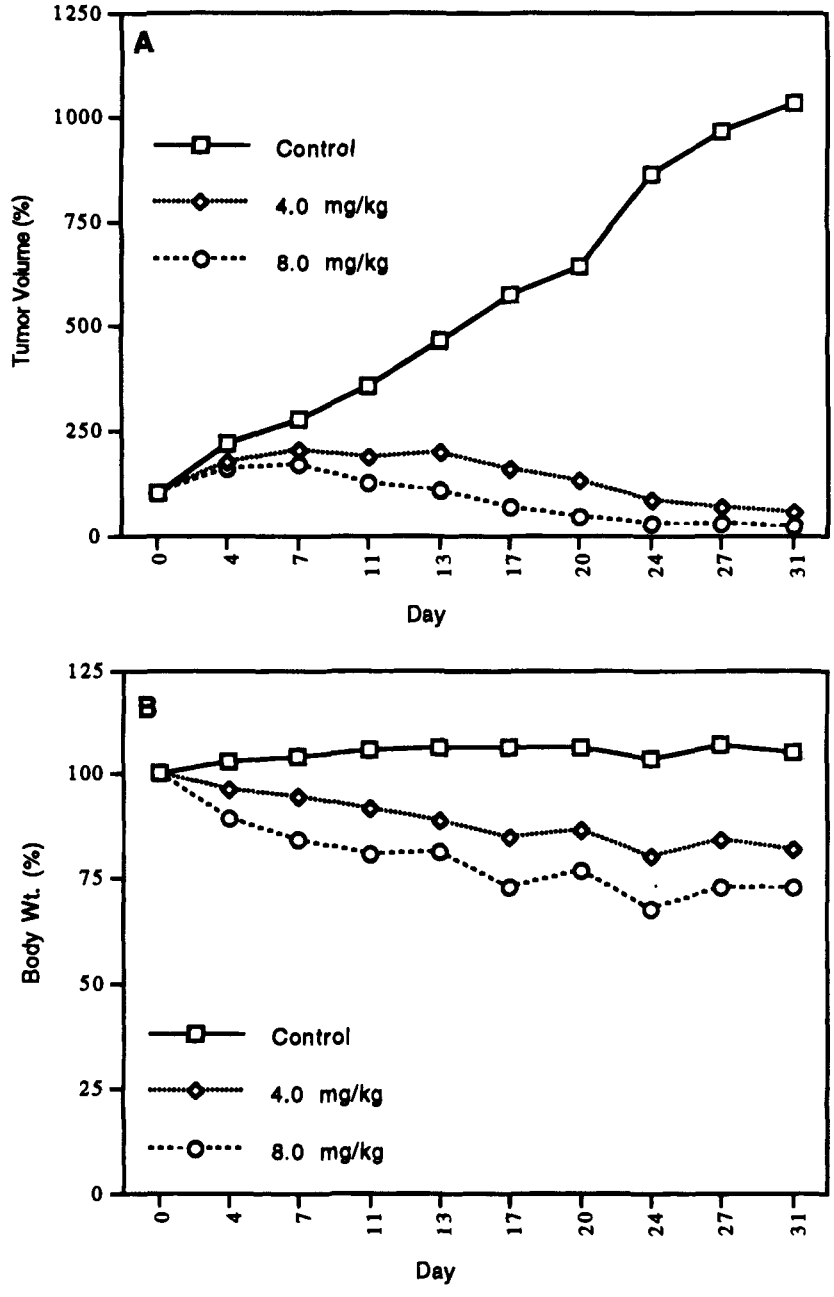


Figure 2. (A) Mean tumor volume of HT-29 nude mice ($N = 8$) treated subcutaneously twice/week with **27** at 4.0 and 8.0 mg/kg compared to controls. (B) Body weight of animals treated at 4.0 and 8.0 mg/kg. (Note: After day 17, $N = 7$; after day 24, $N = 5$; after day 27, $N = 2$ for dose of 8.0 mg/kg.)

cleavable complex assay. For example, compounds **23** and **30** were both at least as active as topotecan in the cleavable complex assay but showed significantly less effect on delay of tumor growth. Amidine **28**, *the most potent camptothecin analog we have identified to date that has significant water solubility* (with an IC_{50} value in the cleavable complex assay that is >30× that of **3**), also showed less efficacy in the two week assay than did topotecan. Compound **28** is active against HT-29 cells in the cytotoxicity assay, so the unexpectedly low efficacy of this compound *in vivo* may be related to rapid clearance and short half-life. In contrast, the carboxamide oxime trifluoroacetic acid salt **27** showed excellent activity *in vivo* in the 2 week protocol relative to controls (15 *vs* 10.3 days for topotecan) which was nearly comparable to our clinical candidate GI 147211C (T−C = 16.8 days). The maximum antitumor effect of **27** was achieved at a much lower dose than other 11-azacamptothecin analogs tested *in vivo* as well as with **3**. Compound **27** was further examined in the extended 5 week HT-29 xenograft assay (Figure 2A). At 4 mg/kg, **27** caused significant regression in the size of the tumors (T/B = 0.58) with less toxicity as judged by body weight loss during the experiment than was observed in this protocol with topotecan at its maximum tolerated dose. (In a separate experiment, we determined the %T/B for topotecan as 2.9 at a dose which caused a 22% body weight loss *vs* 18% for **27** at 4.0 mg/kg).[30] At a higher dose (8 mg/kg), **27** showed marked toxicity, causing death in most of the animals before the conclusion of the study. Since, in contrast to the other 11-azacamptothecin derivatives, carboxamide oxime **27** shows potent antitumor activity *in vivo*, we have concluded that the disappointing activity observed with several of 11-azacamptothecin derivatives in the 2 week assay is probably a consequence of poor pharmocokinetics re-

lated specifically to the water-solubilizing substituent at the 10 position, rather than to the 11-aza template itself.

Conclusion

We have synthesized a novel series of 11-azacamptothecin analogs which are potent inhibitors of topoisomerase I and have improved water solubility relative to camptothecin. Although substitution of 11-*C*-*H* with nitrogen failed to afford satisfactory water solubility in itself, the activity of parent compound **8** was approximately twice that of (20*S*)-(−)-camptothecin in the cleavable complex assay, suggesting that 11-azacamptothecin represented a promising template for further substitution. One modification designed to enhance water solubility involved quaternization at the N-11 atom so as to render it permanently charged. When N-11 was quaternized as the *N*-oxide or *N*-methyl iodide, water solubility was improved, but with some loss of *in vitro* potency relative to the parent compound. Nevertheless, 11-aza-(20*S*)-(−)-camptothecin *N*-oxide showed significant activity *in vivo* in the 5 week nude mouse xenograft model with minimal toxicity. On the basis of this encouraging result, additional 11-azacamptothecin analogs substituted with water solubilizing groups at C-10 were made and tested. In addition to showing good water solubility, many of these compounds were potent *in vitro*, and a number were progressed *in vivo*. In general, the *in vivo* results we have gathered fail to correlate with activity in the *in vitro* cleavable complex assay. This was most striking for amidine **28** which is highly active *in vitro* but has modest antitumor properties *in vivo*. In contrast, the 10-carboxamide oxime trifluoroacetic acid salt **27** was potent *in vitro* and efficacious both in the 2 and 5 week HT-29 xenograft assay protocols. In spite of the lack of correlation between *in vitro* and *in vivo* activity, the results we have obtained here indicate that 11-azacamptothecin represents an important new class of potential antitumor agents and that further studies are required to understand the *in vivo* factors that determine antitumor efficacy in this novel series of compounds.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Unless stated otherwise, reagents were obtained from commercial sources and were used directly. Reactions involving air- or moisture-sensitive reagents were carried out under a nitrogen atmosphere. Anhydrous solvents were obtained from Aldrich (Sure Seal) except CH_2Cl_2 which was distilled from CaH prior to use. ^1H NMR spectra were recorded on a Varian 300 MHz spectrometer; chemical shifts are reported in parts per million (ppm) relative to TMS, and coupling constants are reported in hertz. The following abbreviations are used to describe peak patterns when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High-performance liquid chromatography (HPLC) was performed on a Beckman 126 with a Beckman 166 UV Detector or Waters 3000 Delta Prep with a Rainin Dynamax-60A column and Waters Lamda-Max 481 UV detector (monitoring at 254 nm). Elemental analyses, performed by Atlantic Microlab, Inc., Norcross, GA, were within 0.4% of the theoretical values calculated for C, H, and N.

11-Aza-(20*S*)-camptothecin (8). A round-bottomed flask was charged with (*S*)-(−)-tricyclic ketone **7** (382 mg, 1.45 mmol) and 3-(3-amino-4-picolylidene)-*p*-toluidine (**6**)[21] (257 mg, 1.21 mmol) and anhydrous toluene (25 mL). A condensor and Dean−Stark trap with molecular sieves was attached, and the mixture was heated at reflux for 30 min. The reaction mixture was allowed to cool to just below reflux, and *p*-toluenesulfonic acid (10 mg, 0.053 mmol) was added. The mixture was heated at reflux for 18 h. The dark mixture was allowed to cool to room temperature, and the volatiles were removed with a rotary evaporator. The residue was purified by silica gel chromatography (EtOAc followed by 6:5:1 EtOAc/$CHCl_3$/MeOH elution) to afford 115.4 mg of the product (27% yield) as a tan-colored solid: mp 200 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7.3), 1.85 (m, 2H), 5.33 (s, 2H), 5.43 (s, 2H), 6.57 (s, 1H), 7.39 (s, 1H), 8.06 (d, 1H), J = 5.6), 8.68 (d, 1H, J = 5.6), 8.74 (s, 1H), 9.54 (s, 1H); high-resolution MS (MH^+) m/e 350.1140 ($C_{19}H_{16}N_3O_4$ requires 350.1141). Anal. ($C_{19}H_{15}N_3O_4 \cdot 1.25H_2O$) C, H, N.

3-Nitro-4-pyridinecarboxaldehyde (9). The procedure used is a modification of that of Baumgarten and Krieger.[21] A mixture of selenium(IV) oxide (4.64 g, 36.95 mmol), *tert*-butyl hydroperoxide (90%, 14.78 mL, 147.8 mmol), and dry CH_2Cl_2 was stirred for 1 h at 35−40 °C, and 4-methyl-3-nitropyridine (10.2 g, 73.9 mmol) was added. The mixture was heated at just below reflux (35 °C) for 3 days. The reaction mixture was concentrated and dissolved in 1:1 acetic acid/water (50 mL), and dimethyl sulfide (10 mL) was added. The solution was stirred at 35−40 °C for 3.5 h, then neutralized by addition of solid K_2CO_3, and extracted with ether until TLC showed no UV activity of the ethereal extract. The combined ether layers were dried over Na_2SO_4, filtered, and concentrated to afford a brown semisolid. Recrystallization from water afforded 7.58 g (60% yield) of 3-nitro-4-pyridinecarboxaldehyde dihydrate as tan needles: mp 79−82 °C (lit. mp 91−93 °C); ^1H NMR (300 MHz, acetone-d_6) δ 6.07 (bs, 2H), 6.51 (bs, 1H), 7.91 (d, 1H, J = 5.0), 8.84 (d, 1H, J = 5.0), 8.98 (s, 1H). The free 3-nitro-4-pyridinecarboxaldehyde was obtained as clear needles, mp 49−51 °C, in 70−80% yield by sublimation of the dihydrate over P_2O_5 at 45−55 °C (lit.[21] mp 53−54 °C).

(*R*,*S*)-3-Nitro-4-(1-hydroxyethyl)pyridine (10a). A dry nitrogen-flushed round-bottomed flask was charged with 3-nitro-4-pyridinecarboxaldehyde (**9**) (520 mg, 3.41 mmol) and anhydrous THF (12.0 mL). The solution was cooled to −78 °C, and methyllithium (2.44 mL of a 1.4 M solution in diethyl ether, 3.42 mmol) was added dropwise over 10 min. The reaction mixture was allowed to stir at −78 °C for 25 min, and the reaction was quenched by addition of saturated aqueous $NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (3 × 30 mL), and the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to afford an orange semisolid. Purification by radial silica gel chromatography (2:1 hexanes/EtOAc followed by 1:1 hexane/EtOAc) provided 274 mg (50% yield) of the product as a yellow semisolid: ^1H NMR (300 MHz, acetone-d_6) δ 1.55 (d, 3H, J = 6.5), 3.04 (bs, 1H), 5.54 (q, 1H, J = 6.5), 7.84 (d, 1H, J = 5.1), 8.78 (d, 1H, J = 5.1), 9.10 (s, 1H). Anal. ($C_7H_8N_2O_3$) C, H, N.

(*R*,*S*)-4-(1-Hydroxypropyl)-3-nitropyridine (10b). A solution of **9** (1000 mg, 6.58 mmol) in anhydrous toluene (100 mL) was cooled to −78 °C, and TMEDA (248 μL, 1.65 mmol) was added, followed by dropwise addition of diethylzinc (19.7 mL of a 1.0 M solution in toluene, 19.7 mmol). After 5 min, the reaction mixture was allowed to warm to room temperature over 30 min and poured into saturated aqueous sodium potassium tartrate (100 mL). The mixture was stirred vigorously for 30 min and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford the crude product as red semisolid. Purification by radial chromatography (1:1 hexane/EtOAc) afforded 607 mg (51% yield) of the product as a yellow semisolid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.01 (t, 3H, J = 7.2), 1.66 (m, 1H), 1.77 (m, 1H), 3.73 (bs, 1H), 5.29 (d, 1H, J = 7.5), 7.78 (d, 1H, J = 5.1), 8.71 (d, 1H, J = 5.1), 9.03 (s, 1H); low-resolution MS shows parent ion (MH^+) at 183.

(*R*,*S*)-4-(1-Hydroxypropyl)-3-nitropyridine (Alternative Procedure). To a −50 °C solution of **9** (0.63 g, 4.14 mmol) in anhydrous *N*,*N*-DMF (8.0 mL) was added dropwise over 1 min 5.34 mL of diethylzinc in hexanes (1.0 M, 5.34 mmol). The rapidly stirring mixture was allowed to warm to 0 °C and stirred for 15 min. The mixture was quenched by addition of 10 mL of saturated sodium potassium tartrate, and

the resulting mixture was extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were washed with water (2 × 10 mL), dried over Na_2SO_4, filtered, and concentrated to afford the crude product. Purification by flash chromatography (2:1 hexanes/ethyl acetate then 1:1 hexanes/ethyl acetate) afforded 475 mg of the product (63% yield).

3-Amino-4-(1-oxoethyl)pyridine (11a). A round-bottomed flask was charged with **10a** (268 mg, 1.59 mmol), Dess–Martin periodinane[22] (1.35 g, 3.19 mmol), and dry CH_2Cl_2 (25 mL), and the yellow slurry was stirred at ambient temperature for 14 h. The mixture was passed through a pad of Celite, washing with *ca.* 50 mL of ether, and then through a pad of silica gel. The resulting solution was washed with saturated aqueous $NaHCO_3$ followed by brine and dried over Na_2SO_4. Filtering and concentrating the solution afforded the product (234 mg, 88% crude yield) as a yellow semisolid which ^1H NMR analysis showed to be the desired methyl ketone **13a** contaminated by a small amount of periodinane-related material. This material was used in the next step without further purification: ^1H NMR (300 MHz, acetone-d_6) δ 2.60 (s, 3H), 7.37 (d, 1H, J = 5), 8.96 (d, 1H, J = 5), 9.38 (s, 1H).

In a small round-bottomed flask were combined the 3-nitro-4-(1-oxoethyl)pyridine (550 mg, 3.31 mmol) and $Na_2S_2O_4$ (3.45 g, 19.87 mmol). The mixture was taken up in 95% ethanol (35 mL), and the mixture was heated at reflux for 14 h. The mixture was allowed to cool to room temperature and concentrated with a rotary evaporator. The residue was partitioned between EtOAc (30 mL) and water (30 mL). The organic layer was separated, and the aqueous layer was reextracted with EtOAc (2 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated with a rotary evaporator and the residual solvent removed with high vacuum to afford 320 mg (70% yield) of the product (**11a**) as a yellow semisolid: ^1H NMR (300 MHz, acetone-d_6) δ 2.59 (s, 3H), 7.43 (d, 1H, J = 5.1), 7.93 (d, 1H, J = 5.1), 8.21 (s, 1H). Anal. ($C_7H_8N_2O$) C, H, N.

3-Amino-4-(1-oxopropyl)pyridine (11b). The procedure was the same as above using **10b** (34 mg, 0.187 mmol) in dry CH_2Cl_2 (4.0 mL) and 160 mL (0.374 mmol) of Dess–Martin periodinane. The reaction afforded 34 mg of a yellow solid which ^1H NMR showed to be the ketone contaminated by a trace of periodinane-related impurities (100% crude yield): ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H, J = 7), 2.80 (q, 2H, J = 7), 7.29 (d, 1H, J = 5), 7.93 (d, 1H, J = 5), 8.39 (s, 1H). A mixture of this crude ketone (34 mg, 0.189 mmol) and $Na_2S_2O_4$ (197 mg, 1.13 mmol) in 95% ethanol (3.0 mL) was heated at reflux for 14 h. The mixture was allowed to cool to ambient temperature, and the solvent was removed with a rotary evaporator. Purification by radial chromatography afforded 13 mg (48% yield) of the product as a bright yellow semisolid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.21 (t, 3H, J = 7.5), 2.99 (q, 2H, J = 7.5), 6.20 (bs, 1H), 7.50 (d, 1H, J = 5.4), 7.94 (d, 1H, J = 5.4), 8.25 (s, 1H); low-resolution MS shows parent ion (MH^+) at 151.

3-Nitro-4-(1-oxoethyl)pyridine (13a) from 4-chloro-3-nitropyridine. A N_2-flushed flask was charged with 4-chloro-3-nitropyridine (**12**) (900 mg, 5.69 mmol), (1-ethoxyvinyl)-tributyltin (4.05 mL, 11.4 mmol), $(PPh_3)_4Pd$ (960 mg, 0.83 mmol), LiCl (1.5 g, 35.9 mmol), and anhydrous THF (50 mL). The mixture was heated at reflux for 22 h and was allowed to cool to ambient temperature and diluted with ether. The mixture was washed with water and 5% aqueous NH_4OH and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford the crude vinylpyridine as a dark brown oil. This material was dissolved in 10 mL of THF, and 5 mL of 2 N aqueous HCl was added. The mixture was stirred for 18 h and concentrated. The concentrated residue was made basic by the addition of Na_2CO_3 and extracted with ether to give crude **13a**. Purification by radial chromatography eluting with hexanes/EtOAc gave 350 mg (37% yield) of **13a**, whose ^1H NMR spectrum corresponded to that obtained from the procedure of **11a**.

7-Ethyl-11-aza-(20*S*)-campotothecin (14). The procedure was the same as that of **8** except using **11b** (13 mg, 0.087 mmol) and **7** (29.6 mg, 0.113 mmol). The crude product was purified by radial chromatography to afford 14 mg (43% yield) of **14** as a tan solid: mp 150 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H, J = 7), 1.27 (t, 3H, J = 7), 1.80–1.95 (m, 2H), 3.30 (q, 2H, J = 7), 5.38 (s, 2H), 5.42 (s, 2H), 6.57 (s, 1H), 7.39 (s, 1H), 8.18 (d, 1H, J = 5), 8.69 (d, 1H, J = 5), 9.55 (s, 1H). High-resolution MS (MH^+) *m/e* 378.1450 ($C_{21}H_{20}O_4N_3$ requires 378.1454). Anal. ($C_{21}H_{19}N_3O_4$·$CHCl_3$) C, H, N.

11-Aza-(20*S*)-camptothecin 11-*N*-Oxide (16). A round-bottomed flask was charged with **14** (115 mg, 0.33 mmol) and *m*-chloroperbenzoic acid (50–60%, 143 mg, 0.41–50 mmol). The mixture was dissolved in CH_2Cl_2 (50 mL), and the solution was allowed to stir at ambient temperature for 15 h. Additional *m*-CPBA (17 mg) was added, and the reaction mixture was allowed to stir for an additional 2 h. The mixture was partitioned between 5:1 $CHCl_3$/*i*-PrOH (50 mL) and saturated aqueous Na_2SO_3 (10 mL). The organic layer was separated, and the aqueous layer was reextracted with 5:1 $CHCl_3$/*i*-PrOH (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the crude product. Purification by silica gel chromatography (6:5:1 EtOAc/$CHCl_3$/MeOH followed by 5:1 $CHCl_3$/MeOH) afforded 119 mg of the product (99% yield) as a bright yellow solid: mp 215 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 7.0), 1.87 (m, 2H), 5.28 (s, 2H), 5.43 (s, 2H), 6.57 (s, 1H), 7.33 (s, 1H), 8.14 (d, 1H, J = 7.3), 8.34 (d, 1H, J = 7.3), 8.69 (s, 1H), 9.06 (s, 1H); HRMS (FAB) *m/z* 366.1095 ($C_{19}H_{15}N_3O_5^+$ requires 366.1090). Anal. ($C_{19}H_{15}N_3O_5$·$2H_2O$) C, H, N.

7-Ethyl-11-Aza-(20*S*)-camptothecin 11-*N*-(Methyl iodide) (15). A sample of **14** (12 mg, 0.032 mmol) was taken up in 1:1 $CHCl_3$/MeOH (2 mL), and MeI (1.0 mL) was added. The mixture was heated at a gentle reflux for 2 days and concentrated with a rotary evaporator to leave a reddish residue, which was taken up in *ca.* 2 mL of water and passed through a fine glass frit. The filtrate was lyophylized to afford the product (16 mg, 96% yield) as a deep yellow fluffy powder: ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7.5), 1.80–1.95 (m, 2H), 2.89 (s, 3H), 4.58 (s, 3H), 5.45 (s, 4H), 7.40 (s, 1H), 8.82 (d, 1H, J = 5.5), 8.95 (d, 1H, J = 5.5), 10.20 (s, 1H); low-resolution MS shows parent ion ($M-I^+$) at 392; HRMS (FAB) *m/z* 392.1606 ($C_{22}H_{22}N_3O_4 - I^+$ requires 392.1610). Anal. ($C_{22}H_{22}IN_3O_4$·$2H_2O$·$0.5CHCl_3$) C, H, N.

5-Amino-2-bromo-4-(1-oxoethyl)pyridine (17a). In a small flask was dissolved 3-amino-4-(1-oxoethyl)pyridine (214 mg, 1.57 mmol) in anhydrous THF (43 mL), and $NaHCO_3$ (264 mg, 3.14 mmol) was added. The stirred slurry was treated by the dropwise addition of pyrrolidone hydrotribromide in THF (701 mg in 10 mL), which was added via pipette. Monitoring by thin layer chromatography showed the formation of two higher R_f spots relative to starting material, the highest gradually disappearing over 10 min. Additional pyrrolidone hydrotribromide (77 mg in 3 mL THF) was added, and the yellow slurry was allowed to stir at ambient temperature for 14 h. Without concentration, the reaction mixture was passed directly through a plug of silica, eluting with 100% EtOAc followed by 10:1 EtOAc/Et_3N. The resulting clear yellow solution was concentrated with a rotary evaporator, and the semisolid residue was purified by radial chromatography (3:1 hexane/EtOAc eluent) to afford 214 mg (63% yield) of the product obtained as a 6:1 mixture of regioisomers, as judged by ^1H NMR analysis: ^1H NMR (300 MHz, $CDCl_3$) (minor regioisomer in parentheses) δ 2.60 (s, 3H), (2.62) (7.45) 6.10 (bs, 2H), (7.75) 7.60 (s, 1H), 7.98 (s, 1H); low-resolution MS shows parent ion (MH^+) at 215.

5-Amino-2-bromo-4-(1-oxopropyl)pyridine (17b). The foregoing procedure was used substituting 3-amino-4-(1-oxopropyl)pyridine (180 mg, 1.20 mmol) and using $NaHCO_3$ (202 mg, 2.4 mmol) and 595 mg (1.20 mmol) pyrrolidone hydrotribromide, which was added in one portion. The mixture was stirred at ambient temperature for 4 h and without concentration was passed through a short silica gel column, washing with EtOAc followed by 5% Et_3N in EtOAc. The crude product, which eluted with the 100% EtOAc fraction, was obtained by concentration and silica gel chromatography (2:1 hexanes/EtOAc eluent) to afford 183 mg (67% yield) of a yellow semisolid which analysis by ^1H NMR spectroscopy showed was a 6:1 mixture of **17b:18b**: ^1H NMR (300 MHz, $CDCl_3$) (major

isomer) δ 1.20 (t, 3H, J = 6.9), 2.96 (q, 2H, J = 6.9), 6.10 (bs, 2H), 7.65 (s, 1H), 7.98 (s, 1H); (minor isomer) δ 1.21 (t, 3H, J = 7.2), 3.00 (q, 2H, J = 7.2), 6.75 (bs, 2H), 7.49 (d, 1H, J = 5.4), 7.71 (d, 1H, J = 5.4); low-resolution MS shows parent ion (MH^+) at 229.

10-Bromo-7-methyl-11-aza-(20*S*)-camptothecin (19). A flask was charged with **17a** (82.2 mg, 0.382 mmol, contaminated with 20% of **18a**) and (*S*)-(−)-tricyclic ketone **7** (105 mg, 0.40 mmol). Anhydrous toluene (4.0 mL) was added, and the mixture was brought to gentle reflux for 10 min. The mixture was allowed to cool to just below reflux, and *p*-toluenesulfonic acid (4.5 mg, 0.024 mmol) was added. The mixture was heated at reflux for 20 h and allowed to cool to ambient temperature. The dark mixture was concentrated with a rotary evaporator, and the residue was triturated with 5:1 $CHCl_3$/MeOH to afford 73.9 mg of the product as a pale tan solid, mp 274 °C dec. The mother liquor obtained from the trituration was concentrated to leave a solid residue which was further triturated as described above to afford 53.9 mg of additional product. (78% total yield): ^1H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, 3H, J = 7), 1.85 (q, 2H, J = 7), 2.79 (s, 3H), 5.38 (s, 2H), 5.43 (s, 2H), 6.59 (s, 1H), 7.38 (s, 1H), 8.45 (s, 1H), 9.37 (s, 1H); low-resolution MS shows parent ion (MH^+) at 442.0. Anal. ($C_{20}H_{16}N_3O_4Br^{7}/_{8}H_2O$) C, H, N.

10-Bromo-7-ethyl-11-aza-(20*S*)-camptothecin (20). A mixture of **17b** (220 mg, 0.96 mmol), **7** (259 mg, 0.98 mmol), and anhydrous toluene (7.0 mL) was heated at reflux for a 10 min. The solution was allowed to cool to just below reflux, and catalytic *p*-toluenesulfonic acid (10 mg) was added. The mixture was heated at reflux for 15 h. The dark brown reaction mixture was allowed to cool to ambient temperature, and the solid precipitate was collected by suction filtration and washed with EtOAc several times to afford 327 mg (75% yield) of the product as a yellow-brown solid: mp 273–275 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H, J = 7.2), 1.26 (t, 3H, J = 7.2), 1.78–1.90 (m, 2H), 3.18–3.25 (m, 2H), 5.37 (s, 2H), 5.43 (s, 2H), 6.55 (s, 1H), 7.36 (s, 1H), 8.45 (s, 1H), 9.34 (s, 1H); low-resolution MS shows parent ion (MH^+) at 456.1. Anal. ($C_{21}H_{18}N_3O_4Br^{3}/_{4}H_2O$) C, H, N.

10-Cyano-7-methyl-11-aza-(20*S*)-camptothecin (21). A dry N_2-flushed flask equipped with a stirring bar was charged with Bu_3SnCN (488 mg, 1.54 mmol), $(PPh_3)_4Pd$ (48 mg, 0.042 mmol), and anhydrous 1,2-dichloroethane (32 mL). The mixture was heated at a gentle reflux in an oil bath for 45 min. The mixture was allowed to cool to just below reflux, and bromide **19** (340 mg, 0.769 mmol) was added. The mixture heated at a gentle reflux for 15 h. Additional catalyst (10 mg) was added, and the mixture was heated at reflux for another 8 h. The dark brown mixture was allowed to cool to ambient temperature, concentrated to ca. 50% volume, and allowed to stand at 5 °C for 12 h. The product (215.4 mg) was collected as a greenish yellow precipitate by suction filtration and drying in a vacuum oven. The filtrate was concentrated, and the residue was purified by silica gel chromatography (eluting with 100% EtOAc followed by 6:5:1 EtOAc/$CHCl_3$/MeOH) to afford 64.3 mg of additional product: mp 293 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7.3), 1.90 (m, 2H), 2.85 (s, 3H), 5.38 (s, 2H), 5.43 (s, 2H), 6.60 (s, 1H), 7.40 (s, 1H), 9.00 (s, 1H), 9.60 (s, 1H); low-resolution MS shows parent ion (MH^+) at 389.2. Anal. ($C_{21}H_{16}N_4O_4 \cdot 0.4CHCl_3 \cdot 0.25H_2O$) C, H, N.

10-Cyano-7-ethyl-11-aza-(20*S*)-camptothecin (22). The procedure used was the same as the foregoing procedure, substituting **20** (383 mg, 0.839 mmol) for the corresponding 7-methyl derivative, using 539 mg (1.71 mmol) of Bu_3SnCN, 58 mg (0.05 mmol) of $(PPh_3)_4Pd$, and 35 mL of anhydrous 1,2-dichloroethane. The reaction was allowed to proceed for 26 h. Following partial concentration of the reaction mixture and cooling at 10 °C overnight, 134 mg of product was obtained as a precipitate by suction filtration, mp 160 °C dec. An additional 254 mg (388 mg total, 100% yield) was obtained by radial chromatography of the concentrated filtrate: ^1H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H, J = 7), 1.28 (t, 3H, J = 7), 1.85 (m, 2H), 5.42 (s, 2H), 5.45 (s, 2H), 6.59 (s, 1H), 7.40 (s, 1H), 9.02 (s, 1H), 9.59 (s, 1H). Anal. ($C_{22}H_{18}N_4O_6$) C, H, N.

10-(Aminomethyl)-7-methyl-11-aza-(20*S*)-camptothecin Trifluoroacetic Acid Salt (23). A mixture of 10-cyano-7-methyl-11-aza-(20*S*)-camptothecin (**21**) (100.7 mg, 0.259 mmol) and 10% Pd/C (77 mg) in glacial acetic acid (160 mL) was stirred rapidly under 1 atm of H_2 for 4 h. The mixture was filtered through a pad of Celite and concentrated with a rotary evaporator. The residue was purified by reverse-phase HPLC (Rainin Dynamax 60A column, eluting with 98:20:5:2 H_2O/CH_3CN/THF/TFA) to afford, after lyophylization of the most intense UV active fraction, 107 mg of the product as a bright yellow lyophylate (81% yield): mp 230 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7), 1.87 (m, 2H), 2.80 (s, 3H), 4.42 (s, 2H), 5.37 (s, 2H), 5.45 (s, 2H), 6.56 (s, 1H), 7.37 (s, 1H), 8.30 (s, 1H), 9.59 (s, 1H); low-resolution MS shows parent ion (MH^+) at 393.2; HRMS (FAB) *m/z* 393.1562 ($C_{22}H_{22}N_4O_4H^+$ requires 393.1563). Anal. ($C_{21}H_{20}N_4O_4 \cdot 2TFA \cdot 1.5H_2O$) C, H, N.

10-(Aminomethyl)-7-ethyl-11-aza-(20*S*)-camptothecin Trifluoroacetic Acid Salt (24). The foregoing procedure was carried out using **22** (100 mg, 0.259 mmol) to afford 120 mg of the crude acetic acid salt. A sample of this material (12 mg) was purified by reverse-phase HPLC as described above to afford 4.5 mg of the title compound after lyophylization: mp 160 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7.5), 1.35 (t, 3H, J = 7.5), 1.87 (m, 2H), 3.21 (q, 2H, J = 7.5), 4.43 (d, 2H, J = 6), 5.41 (s, 2H), 5.45 (s, 2H), 6.56 (bs, 1H), 7.37 (s, 1H), 8.32 (s, 1H), 8.41 (s, 1H), 9.60 (s, 1H); low-resolution MS shows parent ion (MH^+) at 407.1. Anal. ($C_{22}H_{22}N_4O_4 \cdot 1.5TFA \cdot H_2O$). C, H, N.

10-[[(*tert*-Butyloxycarbonyl)amino]methyl]-7-methyl-11-aza-(20*S*)-camptothecin 11-Oxide (25). A slurry of **23** (186.5 mg, 0.42 mmol) in anhydrous CH_2Cl_2 (25 mL) was treated with di-*tert*-butyl dicarbonate (180.5 mg, 0.827 mmol) and Et_3N (0.5 mL, 3.59 mmol). The mixture was allowed to stir at ambient temperature for 1.5 h. Additional di-*tert*-butyl dicarbonate (56 mg, 0.26 mmol) was added, and the mixture was stirred for a further 1.25 h. The mixture was partitioned between H_2O (50 mL) and CH_2Cl_2 (50 mL). The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated to afford a greenish residue. Purification by silica gel chromatography (6:5:1 EtOAc/$CHCl_3$/MeOH, 1% Et_3N as eluent) afforded 128.7 mg of the *N-t*-Boc-aminomethyl derivative. This material was treated with *m*-CPBA (135.0 mg, 50–60%, *ca.* 0.4 mmol) and $NaHCO_3$ in CH_2Cl_2 (20 mL). The slurry was stirred at ambient temperature for 7 h and partitioned between CH_2Cl_2 (50 mL) and aqueous Na_2SO_3 (20 mL). The organic layer was separated and washed with H_2O (10 mL). The combined aqueous layers were extracted with CH_2Cl_2 (20 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford the crude product (164.7 mg) as a yellow solid. This material was purified by flash chromatography (eluting with 100% EtOAc followed by 6:5:1 EtOAc/CH_2Cl_2/MeOH containing 1% Et_3N) to afford 84.5 mg (40% overall yield) of **25** as a bright yellow solid: mp 250 °C dec; ^1H NMR (300 MHz, $CDCl_3$) δ 1.04 (t, 3H, J = 7.3), 1.41 (s, 9H), 2.78 (s, 3H), 4.66 (s, 2H), 5.25 (s, 2H), 5.30 (d, 1H, J = 16.6), 5.74 (d, 1H, J = 16.6), 7.63 (s, 1H), 8.01 (s, 1H), 9.10 (s, 1H); low-resolution MS shows parent ion (MH^+) at 509.0. Anal. ($C_{26}H_{23}N_4O_7 \cdot H_2O$) C, H, N.

10-(Aminomethyl)-7-methyl-11-aza-(20*S*)-camptothecin 11-*N*-Oxide (26). A solution of **25** (79.3 mg, 0.156 mmol) in CH_2Cl_2 (28 mL) was treated with trifluoroacetic acid (1.35 mL). The solution was stirred at ambient temperature for 4.5 h and concentrated with a rotary evaporator. The residue was purified by reverse-phase HPLC (Rainin Dynamax 60A column, eluting with 98:20:5:2 H_2O/CH_3CN/THF/TFA) and afforded 82.8 mg of the product as a bright yellow lyophylized powder: mp 230 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (t, 3H, J = 7.1), 1.86 (m, 2H), 2.76 (s, 3H), 4.39 (d, 2H, J = 4.9), 5.32 (s, 2H), 5.44 (s, 2H), 8.32 (s, 1H), 8.56 (s, 1H), 9.27 (s, 1H); low-resolution MS shows parent ion (MH^+) at 409 (calcd 409); HRMS (FAB) *m/z* 409.1518 ($C_{23}H_{20}N_4O_6H^+$ requires 409.1512). Anal. ($C_{23}H_{20}N_4O_6 \cdot 2TFA \cdot 2.5H_2O$) C, H, N.

10-(Aminohydroximinomethyl)-7-ethyl-11-aza-(20*S*)-camptothecin (27). A small round-bottomed flask was charged with **22** (375 mg, 0.93 mmol), 1.5 g (21.6 mmol) $HONH_2 \cdot HCl$, 0.92 g (8.71 mmol) Na_2CO_3, and 100 mL of 95% ethanol. The mixture was heated at reflux for 8 h, and AcOH

(15 mL) was added. The mixture was further heated at reflux for 45 min, allowed to cool to room temperature, and worked up by concentrating the mixture with a rotary evaporator. The solid residue was collected by suction filtration and washed several times with water to afford, after drying 299 mg (74% yield) of the free base amidoxime as a tan solid: mp 260 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H, J = 7.5), 1.30 (t, 3H, J = 7.5), 1.85 (m, 2H), 3.22 (q, 2H, J = 7.5), 5.39 (s, 2H), 5.44 (s, 2H), 6.07 (s, 2H), 6.59 (s, 1H), 7.40 (s, 1H), 8.52 (s, 1H), 9.57 (s, 1H), 10.03 (s, 1H). A portion (98.9 mg) of this material was purified by reverse-phase HPLC (80:20 A/B, A = 2% TFA in H_2O, B = 4:1 CH_3CN/THF to afford the trifluoroacetic acid salt (94.7 mg) after collecting and lyophylizing the major UV active fraction): mp 145–147 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, 3H, J = 7.5), 1.33 (t, 3H, J = 7.5), 1.85 (m, 2H), 3.27 (m, 2H), 5.40 (s, 2H), 5.43 (s, 2H), 6.60 (bs, 1H), 7.40 (s, 1H), 8.79 (bs, 1H), 9.60 (s, 1H); HRMS (FAB) m/z 436.1616 ($C_{22}H_{21}N_5O_5H^+$ requires 436.1621). Anal. ($C_{22}H_{21}N_5O_5$·TFA·1.5H_2O) C, H, N.

10-Amidino-7-ethyl-11-aza-(20*S*)-camptothecin Trifluoroacetic Acid Salt (28). To a solution of the free base of **27** (prepared as described in the above procedure) (31.0 mg, 0.071 mmol) in 1.0 N HCl (3.0 mL) was added Raney nickel (73 mg, 50% aqueous). The mixture was stirred vigorously under 1 atm of H_2 for 24 h. The mixture was filtered through a pad of Celite, and the solvent was removed with a rotary evaporator to afford a greenish residue. Purification by reverse-phase HPLC (Rainin Dynamax 60A column, eluting with 98:20:5:2 water/acetonitrile/THF/TFA) afforded, after lyophylization, 5.2 mg (14% yield) of the product as a yellow powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 7.3), 1.34 (t, 3H, J = 7.3), 3.32 (q, 2H, J = 7.3), 5.45 (s, 2H), 5.46 (s, 2H), 6.60 (s, 1H), 7.43 (s, 1H), 9.12 (s, 1H), 9.34 (s, 2H), 9.67 (s, 1H), 9.76 (s, 2H); low-resolution MS shows parent ion (MH^+) at 420.1; HRMS (FAB) m/z 420.1672 ($C_{22}H_{21}N_5O_4H^+$ requires 420.1672). Anal. ($C_{22}H_{21}N_5O_4$·2.5TFA·3H_2O) C, H, N.

10-(3-Amino-1-propynyl)-7-methyl-11-aza-(20*S*)-camptothecin (29). In a dry N_2-flushed Kontes tube a solution of **19** (50.3 mg, 0.114 mmol) in anhydrous DMF (5.0 mL) was cooled in an ice bath, evacuated, flushed three times with N_2, and allowed to warm to ambient temperature. Triethylamine (20 μL, 0.143 mmol) and $Pd(PPh_3)_4$ (9.8 mg, 8.5 × 10^{-3} mmol) were added, and the reaction mixture was allowed to stir for 30 min. *tert*-Butyl 1-propargylaminecarboxylate (84.5 mg, 0.544 mmol) and copper(I) iodide (17.5 mg, 0.092 mmol) were added, and the reaction flask was covered with foil. The reaction mixture was stirred at ambient temperature for 17 h and was concentrated with a rotary evaporator under high vacuum. The residue was purified by flash silica chromatography (eluting with 100% EtOAc followed by 6:5:1 EtOAc/$CHCl_3$/MeOH) to afford 39.9 mg of *N*-Boc-protected propargylamine. This material was dissolved in CH_2Cl_2 (5.0 mL) and treated with trifluoroacetic acid (0.75 mL). The mixture was stirred at ambient temperature for 2 h, and the volatiles were removed with a rotary evaporator. The residue was purified by reverse-phase HPLC (eluting with 128:20:5:2 H_2O/CH_3CN/THF/TFA) to afford, after lyophylization, 38.3 mg (53% yield) of the product as a yellow lyophylate: mp 132 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H, J = 7.3), 1.86 (m, 2H), 2.79 (s, 3H), 4.14 (s, 2H), 5.37 (s, 2H), 5.45 (s, 2H), 6.60 (bs, 1H), 7.37 (s, 1H), 8.29 (s, 1H), 8.40 (bs, 2H), 9.49 (s, 1H); low-resolution MS shows parent ion (MH^+) at 417.1. Anal. ($C_{23}H_{20}N_4O_4$·2TFA·1.5H_2O) C, H, N.

10-(3-Amino-1-propynyl)-7-ethyl-11-aza-(20*S*)-camptothecin (30). The procedure followed was the same as that used to give **29** except substituting the ethyl derivative **20** (100 mg), for **19**, and using 163 mg (1.05 mmol) *tert*-butyl 1-propargylaminecarboxylate, 27 mg (0.023 mmol) of $(PPh_3)_4Pd$, 46 μL (0.33 mmol) of Et_3N, 33.4 mg (0.18 mmol) of CuI, and 7.5 mL of anhydrous DMF. The reaction afforded, after silica gel chromatography, 56 mg of *N*-*t*-Boc-protected amine, which was deprotected by dissolving in a solution of 0.35 mL of TFA in 2.5 mL of CH_2Cl_2 and stirring for 5 h. The mixture was concentrated, and the residue was purified by reverse-phase chromatography (same conditions as used for **29**) to afford, after collecting and lyophylizing the eluent corresponding to the major UV active peak (retention time with flow rate of 10 mL/min = 19.4 min), 52 mg (35% yield) of the product: mp 175–177 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H, J = 7.3), 1.27 (t, 3H, J = 7.3), 1.85 (m, 2H), 3.21 (q, 2H, J = 7.3), 4.12 (bs, 2H), 5.39 (s, 2H), 5.44 (s, 2H), 6.55 (bs, 1H), 7.36 (s, 1H), 8.37 (s, 1H), 8.38 (bs, 2H), 9.49 (s, 1H); low-resolution MS shows parent ion (MH^+) of 431.2. Anal. ($C_{24}H_{22}O_4N_4$·TFA·H_2O) C, H, N.

10-(3-(Dimethylamino)-1-propynyl)-7-ethyl-11-aza-(20*S*)-camptothecin (31). The foregoing procedure was employed with **20** (15 mg, 0.032 mmol) and using 17.7 μL (0.164 mmol) of 1-(dimethylamino)-2-propyne, 4.0 mg of $(PPh_3)_4Pd$, 6.7 μL (0.048 mmol) of Et_3N, and 5 mg (0.046 mmol) of CuI in 2.0 mL of DMF. Purification of the crude product by radial chromatography (100% EtOAc eluent) afforded 12.0 mg (82% yield) of the product: mp 130 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, 3H, J = 7.2) 1.42 (t, 3H, J = 7.5), 1.89 (m, 2H), 2.50 (bs, 6H), 3.16 (q, 2H, J = 7.2), 3.67 (bs, 1H), 5.28 (d, 1H, J = 16.5), 5.28 (s, 2H), 5.72 (d, 1H, J = 16.5), 7.64 (s, 1H), 8.02 (s, 1H), 9.50 (s, 1H); low-resolution MS shows parent ion (MH^+) at 458.9 (calcd 459). Anal. ($C_{26}H_{26}N_4O_4$·1.5TFA·1.5H_2O) C, H, N.

10-(3-(4-Morpholino)-1-propynyl)-7-methyl-11-aza-(20*S*)-camptothecin (32). The foregoing procedure was used with 20 mg (0.045 mmol) of **19**, 1-(*N*-morpholino)-2-propyne (30 mg, 0.226 mmol), CuI (7 mg, 0.036 mmol), Et_3N (10 μL, 0.072 mmol), and $(PPh_3)_4Pd$ (8 mg). Purification by radial chromatography as above afforded 18.1 mg of the title compound: mp 178–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (t, 3H, J = 7.2), 1.88 (m, 2H), 2.72 (m, 4H), 2.77 (s, 3H), 3.63 (s, 2H), 3.78 (m, 4H), 3.93 (bs, 1H), 5.27 (s, 2H), 5.29 (d, 1H, J = 16.8), 5.73 (d, 1H, J = 16.8), 7.26 (s, 1H), 7.65 (s, 1H), 7.99 (s, 1H), 9.49 (s, 1H); low-resolution MS shows parent ion (MH^+) at 487.1 (calcd 487). Anal. ($C_{27}H_{16}N_4O_5$·1.20TFA·2H_2O) C, H, N.

10-(3-(Dimethylamino)propyl)-7-ethyl-11-aza-(20*S*)-camptothecin (33). A sample of propyne **31** (12 mg, 0.026 mmol) with 10% Pd/C (7 mg) in glacial acetic acid (1.5 mL) was stirred under 1 atm of H_2 for 5 h. The reaction mixture was filtered and subjected to reverse-phase HPLC using the conditions described above to afford 6 mg (40% yield) of the title compound as a yellow powder: mp 115–117 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H, J = 7.5), 1.40 (t, 3H, J = 7.5), 0.85 (m, 2H), 2.18 (quintet, 2H, J = 7), 2.79 (s, 3H), 2.81 (s, 3H), 3.04 (t, 2H, J = 7), 3.20 (m, 2H), 3.22 (q, 2H, J = 7.5), 5.38 (s, 2H), 5.43 (s, 2H), 6.65 (bs, 1H), 7.37 (s, 1H, 8.03 (s, 1H), 9.38 (bs, 1H), 9.49 (s, 1H); low-resolution MS shows parent ion (MH^+) at 463.1. Anal. ($C_{26}H_{30}N_4O_4$·2.5TFA·H_2O) C, H, N.

Ethyl 7-ethyl-11-aza-(20*S*)-10-camptothecincarboxylate (34). A Parr bomb was charged with a mixture of **20** (20 mg, 0.044 mmol), Et_3N (10 μL, 0.053 mmol), and $(PPh_3)_4Pd$ (15 mg) in degassed ethanol (8 mL). The mixture was heated at 100 °C under 80 psi of CO for 20 h, then allowed to cool to ambient temperature, and concentrated to afford the crude product. Purification by radial chromatography (eluting with 100% EtOAc) afforded 9 mg (46% yield) of the product as a yellow solid: mp 175 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 7.2), 1.32 (t, 3H, J = 7.5), 1.86 (m, 2H), 3.28 (q, 2H, J = 7.5), 5.42 (s, 2H), 5.45 (s, 2H), 6.58 (bs, 1H), 7.41 (s, 1H), 8.78 (s, 1H), 9.59 (s, 1H); low-resolution MS shows parent ion (MH^+) at 450.0. Anal. ($C_{24}H_{23}N_3O_6$·0.25H_2O) C, H, N.

***N*-(2-(Dimethylamino)ethyl)-7-ethyl-11-aza-(20*S*)-10-camptothecincarboxamide (35).** A high-pressure Parr reaction vessel (bomb) was charged with a degassed mixture of **20** (20 mg, 0.044 mmol), $(PPh_3)_4Pd$ (20 mg), and *N*,*N*-dimethylethylenediamine (12 μL, 0.113 mmol) in ethanol (3 mL). The apparatus was evacuated, placed under CO gas (100 psi), and heated at 100 °C for 20 h while stirring. The reaction vessel was allowed to cool to ambient temperature, and the contents were removed and concentrated with a rotary evaporator to afford a residue which was purified by radial chromatography (EtOAc followed by 5:1 CHCl3/MeOH eluent) to afford 7 mg (54% yield) of the title compound: mp 153 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 7.2), 1.32 (t, 3H, J = 7.2), 1.86 (m, 2H), 2.85 (s, 3H), 2.87 (s, 3H), 3.25–

3.55 (m, 4H), 3.70 (q, 2H, J = 5.7), 5.43 (s, 2H), 5.45 (s, 2H), 6.58 (bs, 1H), 7.40 (s, 1H), 8.74 (s, 1H), 9.30 (bs, 1H), 9.40 (t, 1H, J = 5.7), 9.60 (s, 1H); low-resolution MS shows parent ion (MH^+) at 492.0 (calcd 492). Anal. ($C_{26}H_{29}N_5O_5 \cdot 1.5H_2O \cdot 1.5TFA$) C, H, N.

Topoisomerase Cleavable Complex Assays. The ability of camptothecin analogs to inhibit topoisomerase I was quantified in the cleavable complex assay as previously described.[2,19] Topoisomerase I was isolated from calf thymus to a high degree of purity and was devoid of topoisomerase II.[30] All reactions were carried out in 10 μL volumes of reaction buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 30 μg/mL BSA) in microtiter plates. The camptothecin analogs were dissolved in DMSO at 10 mg/mL and serially diluted in 96-well microtiter plates to which the [^{32}P] end-labeled pBR322 DNA and topoisomerase enzyme were added. The reaction mixture was incubated at room temperature for 30 min and then stopped by adding 2 μL of a mixture of sodium dodecyl sulfate and proteinase K (Boehringer Mannheim, Indianapolis, IN) (1.6% and 0.14 mg/mL final concentrations, respectively). The plates were heated at 50 °C for 30 min, 10 μL of standard stop mixture containing 0.45 N NaOH was added in order to generate single stranded DNA, and the samples were electrophoresed in 1.5% agarose gels in TBE buffer. Gels were blotted on nitrocellulose paper (BioRad, Richmond, CA) dried and exposed to X-ray film. The units of cleavage were calculated from the autoradiographs and plotted against the log drug concentration using the Nonlin84 software package from SCI Software (Lexington, KY). The IC_{50}'s for each drug were determined from three separate topoisomerase mediated cleavable complex experiments, and values expressed as a mean ± the standard error.

Solubility Determinations (HPLC Method). Compounds were assayed for solubility using an HPLC method which separated the lactone and carboxylate forms. A BDS Hypersil C-18 column, 250 × 4.6 mm, 5 μm particle size, was used with a mobile phase of 40% organic/60% aqueous with 0.005 M tetrabutylammonium phosphate added as a competing base. The solubilities were determined at pH 5.0 in 0.1 M sodium acetate buffer by equilibrating an excess of solid compound in 0.25 mL of buffer at 25 °C for 24 h. The samples were filtered through a 0.22 μm Millipore (Millex GV4) filter unit and injected on the column. Calibration curves were obtained by plotting the peak areas of standards as a function of drug concentration.

^{1}H NMR Method. The drug was equilibrated in 0.5–1.0 mL of deuterium oxide containing a known amount of 1,4-dioxane as internal standard for at least 30 min, and the mixture was filtered and its 300 MHz spectrum was taken. The concentration of the solubilized drug was determined by comparing the 1,4-dioxane integral at 3.75 ppm and the camptothecin analog ethyl triplet at 0.8–0.9 ppm. In no case where this method was used was ring opening of the lactone observed by ^{1}H NMR.

Cell Culture Cytotoxicity Assays. The cytotoxicity of compounds was determined using a micro culture tetrazolium assay. The reported values were usually obtained from a single curve, so error margins are not included. (As an indication of assay reproducibility, doxorubicin was routinely used as an internal control and has an IC_{50} of 331 nM with a statistical error of ±36 nM against HT-29 cells. The potency values of doxorubicin against SKOV3 and SKVLB cell lines are 36(±7) and 5650(±940) nM, respectively.) All cell lines used for the cytotoxicity assay were grown under identical conditions in α-MEM medium containing 15% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 unit/mL insulin, nonessential amino acids, and 0.5 mg/mL gentamycin (GibcoBRL, Grand Island, NY) at 37 °C in an atmosphere of 5% CO_2 in air. Tumor cells were plated in 96-well microtiter plates and allowed to adhere overnight. Cells were incubated with compound for 48 h and then with fresh medium for 48 h. The drugs were tested over a 0.17 nM to 10 μM range, in quadruplicate at each concentration tested. Following a 4 h incubation of treated cells to MTT, the reduced dye product was extracted from the cells with DMSO and quantitated spectrophotometrically. Assay data for each compound were fitted using a four-parameter logistic equation to obtain IC_{50} values.

Xenograft Studies (Five Week Assay). Female nu/nu mice (18–24 g, 10–14 weeks old) were housed in micro isolator filtration racks and maintained with filtered acidified water and sterile lab chow *ad libitum*. Mice were allowed to acclimate for 1 week prior to testing. Before treating tumor-bearing animals with test compounds, a dose ranging study of compound was performed in naive mice to determine the highest dose for the 5 week schedule. For this purpose, mice were dosed twice a week for 5 weeks and their body weight was monitored twice weekly. The loss of 30% body weight or greater was considered lethal, and the highest dose was defined as that dose which caused sufficient morbidity as determined by body weight loss. Tumors were established by injecting harvested HT-29 tumor cells in a single subcutaneous site on the flank of the mice in the left axillary region. The mice were then sorted according to body weight, grouped four mice/cage, and tattooed on the tail for permanent identification. Within a treatment group, a narrow range in body weight ± 1 g and tumor size was established. Efficacy studies were performed over a dose range which included the highest dose. The tumor volume for each mouse was determined by measuring two dimensions with vernier calipers and calculated using the formula: tumor volume = [length × (width)^2]/2. The data was plotted as the percent change in mean values of tumor volume and body weight for each group. The overall growth of tumors was expressed as a ratio of T/B where the tumor volume at the end of treatment (T) was divided by the initial volume at the beginning of the experiment (B). Thus, any tumor group which did not respond to treatment and grew over the course of the experiment displayed a T/B ratio of >1, and treatment groups in which tumors regressed displayed T/B ratios of <1. For dosing of compound **22**, the vehicle used was DMSO, whereas for compound **39** the vehicle was water.

Two Week Assay. This protocol was carried out as above with a modification in the dosing schedule. The maximum tolerated dose for the compound was first determined by evaluation of body weight loss in nude mice (without xenografts) upon administration of a single bolus dose of drug subcutaneously. This dose was then divided into three portions, which were administered to xenograft-bearing mice (N = 6) over the course of a single day. The delay in tumor growth, the time in days required for the tumor to reach 500% of initial volume in drug treated animals (T) compared to controls (C), was determined and expressed as T–C.

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