Synthesis of Water-Soluble (Aminoalkyl)camptothecin Analogues: Inhibition of Topoisomerase I and Antitumor Activity†

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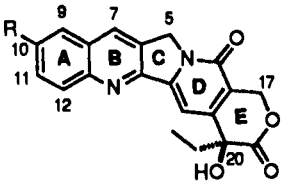
Water-soluble analogues of the antitumor alkaloid camptothecin (1) were prepared in which aminoalkyl groups were introduced into ring A or B. Most of the analogues were prepared by oxidation of camptothecin to 10-hydroxycamptothecin (2) followed by a Mannich reaction to give N-substituted 9-(aminomethyl)-10-hydroxycamptothecins (4–12) or by subsequent modification of Mannich product 4 (13, 15, 17, 19, 21). Others were obtained by modification of the hydroxyl group of 2 (25, 26) or by total synthesis (35, 42, 43). These analogues, as well as some of their synthetic precursors, were evaluated for inhibition of topoisomerase I, cytotoxicity, and antitumor activity. Although there was not a quantitative correlation between these assays, compounds that inhibited topoisomerase I were also cytotoxic and demonstrated antitumor activity in vivo. Further evaluation of the most active water-soluble analogue led to the selection of 9-[(dimethylamino)methyl]-10-hydroxycamptothecin (4, SK&F 104864) for development as an antitumor agent. In addition to its water solubility, ease of synthesis from natural camptothecin, and high potency, 4 demonstrated broad-spectrum activity in preclinical tumor models and is currently undergoing Phase I clinical trials in cancer patients.

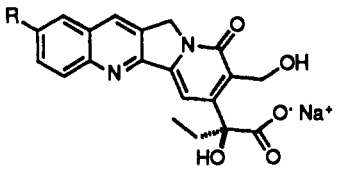
Camptothecin (1), an alkaloid with efficacy in animal tumor models, was isolated from the Chinese tree *Camptotheca acuminata* by Wall and co-workers in 1966[1] and more recently from the Indian tree *Nothapodytes foetida* (formerly *Mappia foetida*) by Govindachari and Viswanathan.[2] The molecule contains a pentacyclic ring system that includes a pyrrolo[3,4-*b*]quinoline moiety (rings A, B, and C) and one chiral center within the α-hydroxy lactone (ring E). Camptothecin has been a target for synthesis by numerous research groups due to its impressive biological activity and the paucity of naturally derived material (present in ~0.01% in the stem bark). The results of these efforts have been comprehensively reviewed.[3-6] Many camptothecin analogues have been prepared, and these examples constitute the basis for our present understanding of the relationship between structure and antitumor activity for this class of antitumor agents. In particular, an intact hydroxy lactone is essential for antitumor activity.[7,8] A-ring substitution at positions 9 and/or 10 is tolerated, while substitution at either position 11 or 12 results in diminished activity.[9-12] Substituents having limited steric bulk appear to be acceptable at position 11, since 11-hydroxycamptothecin and 10,11-(methylenedioxy)camptothecin are active against L1210 mouse leukemia.[12]

Camptothecin was evaluated clinically in the United States as the sodium salt 3, but was found to be ineffective in patients with advanced disseminated melanoma[13] or gastrointestinal cancer.[14] The unpredictable and severe toxicities that were observed—including myelosuppression, vomiting, diarrhea, and severe hemorrhagic cystitis—resulted in the discontinuation of Phase II trials. In China, however, sodium camptothecin (3) has been reported to be effective in the treatment of gastric cancer, head and neck tumors, and bladder carcinoma.[15] 10-Hydroxycamptothecin (2), formulated as the sodium salt, was reported to show improved antitumor activity and less bladder toxicity than camptothecin.[16]

The poor aqueous solubility of camptothecin (1) precluded its development as a clinical agent and necessitated



1, R = H, camptothecin
2, R = OH, 10-hydroxycamptothecin



3, sodium camptothecin

the use of the water-soluble sodium camptothecin (3). Although sodium camptothecin demonstrated antitumor

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(3) Schultz, A. G. *Chem. Rev.* **1973**, *73*, 385.
(4) Hutchinson, C. R. *Tetrahedron* **1981**, *37*, 1047.
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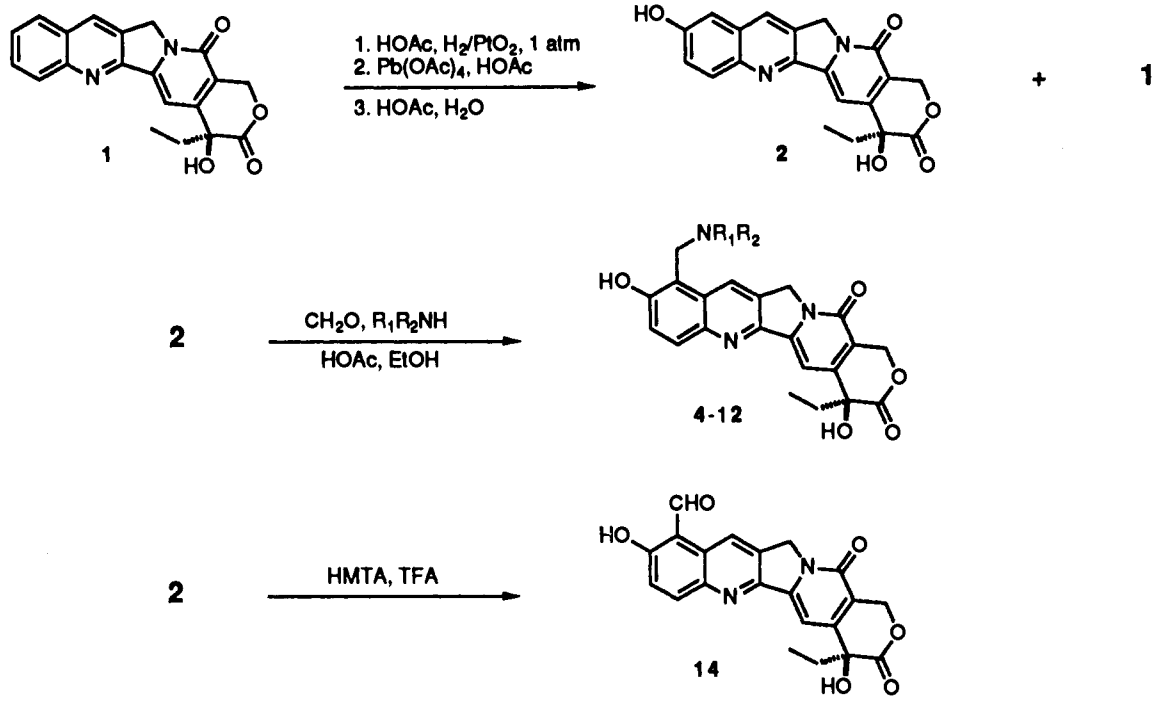
Scheme I



activity in animal models comparable to that of camptothecin, it was about 10-fold less potent with respect to therapeutic dosage range.[9] The antitumor activity of sodium camptothecin in vivo was probably a consequence of its conversion to camptothecin in plasma, since sodium camptothecin does not function as an inhibitor at the intracellular target, DNA topoisomerase I.[8,17] The problems encountered in the clinical trials of sodium camptothecin may have resulted in part from the formation of insoluble camptothecin in vivo.

Camptothecin has a unique mechanism of action; it produces DNA damage in the presence of topoisomerase I by binding to and stabilizing a covalent DNA–topoisomerase I complex in which one strand of the DNA is broken.[18-25] Recent structure–activity studies of camptothecin analogues have demonstrated a correlation between antitumor activity of a given analogue and its ability to stabilize the DNA topoisomerase I complex.[8,26,27] This supports the thesis that production of topoisomerase I mediated DNA damage by camptothecin leads to the expression of antitumor activity. Furthermore, camptothecin-resistant cell lines have been shown to contain an altered form of topoisomerase I that is resistant to inhibition by the drug,[20,21] and yeast cells deficient in topoisomerase I are resistant to camptothecin.[28,29] These results argue strongly that topoisomerase I is the cellular locus through which camptothecin produces its antitumor activity.

The elucidation of the mode of action of camptothecin provided a means of evaluating camptothecin analogues in vitro. On the assumption that the undesirable toxicities of camptothecin had derived, in part, from its lack of aqueous solubility, we attempted to synthesize water-soluble camptothecin derivatives that retain potent activity as an inhibitor of mammalian topoisomerase I and broad spectrum antitumor activity. Our approach focused on substitution of the A ring. Specifically, chemical synthesis was directed toward compounds in which an aminoalkyl group was attached to the A ring at either the 9- or the 10-position. This report describes the synthesis of these derivatives and the correlation between their ability to inhibit purified mammalian topoisomerase I, exhibit cytotoxicity toward mammalian cells, and produce antitumor activity in animal tumor models.

Chemistry

1. 9-Substituted 10-Hydroxycamptothecins. The preparation of these compounds is outlined in Scheme I. (20*S*)-Camptothecin (1) was converted to 10-hydroxycamptothecin (2) by a reduction–oxidation sequence.[30]

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(27) Hsiang, Y. H.; Liu, L. F.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Kirschenbaum, S.; Silber, R.; Potmesil, M. *Cancer Res.* **1989**, *49*, 4385.
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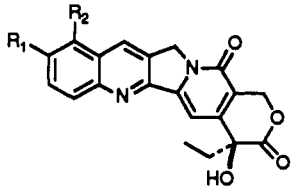
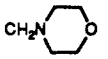
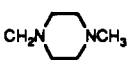
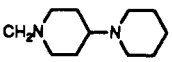
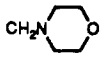
Table I. Topoisomerase I Inhibition, Cytotoxicity, and Antitumor Activity of 9- and 10-Substituted Camptothecin Derivatives[a]



no.	R_1	R_2	CC_{50}[a,b] μM	IC_{50},[c] nM	MTD[d]	% ILS[e]
1	H	H	0.82 ± 0.07[f]	23 ± 4[g]	22	118 ± 6[h]
2	HO	H	1.0	18	21	78
4	HO	$CH_2N(CH_3)_2$	3.2 ± 0.33[i]	56 ± 8[j]	30	173 ± 16[h]
5	HO		0.3	370	190	83
6	HO		0.5	72	74[k]	>200
7	HO	$CH_2N(CH_3)C_6H_5$	0.6	69	83	138
8	HO		1.0	270	77[k]	≥44
9	HO	$CH_2NHC_3H_5$	0.8	130	48	131
10	HO	$CH_2NHC_6H_{11}$	2.2	56	47	>200
11	HO	$CH_2NHCH_2CH_2OH$	0.8	250	82	62
12	HO	$CH_2NHCH_2CH_2N(CH_3)_2$	0.1	49	78[k]	≥94
13	HO	$CH_2N(CH_3)_3^+$	0.3	150	68	>200
14	HO	CHO	0.3	260	102[k]	≥75
15	HO	$CH_2OCH_2CH_2N(CH_3)_2$	0.9	180	50	188
16	HO	$CH_2OCH_2CH_3$	0.1	48	56	125
17	HO	$CH_2SCH_2CH_2N(CH_3)_2$	1.0	12	76[k]	≥112
18	HO	CH_2CN	0.4	400	28	>200
19	HO	$CH_2CH_2NH_2$	3.4	360	210	75
21	H	$CH_2N(CH_3)_2$	1.7	65	29	88
22	H		2.0	690	29	144
24	CN	H	0.5	80	7.8	100
25	CH_2NH_2	H	10	90	160	112
26	PO_3^{2-}	H	>100	26000	155[k]	neg
27	H	NH_2	0.9	12	8.9	94
28	H	CH_3	0.9	70	15	>200

[a] Compounds 1, 2, 14, 16, 18, 24, 27, and 28 are insoluble in water and were dissolved in DMSO prior to dilution into the biological assays. Compound 26 was tested as an aqueous solution of the hydrated diacid. All other compounds were water soluble as their acid salts and were dissolved in water for testing. Compound 4 was tested as both the acetate and hydrochloride salts; both forms were equivalent in all assays. [b] Concentration that produced 50% DNA cleavage in the presence of topoisomerase I. [c] Concentration that inhibited the proliferation of L1210 cells by 50% upon continuous exposure. [d] Maximally tolerated dose (μmol/kg) in mice bearing L1210 leukemia. [e] Increase in life span of mice bearing L1210 leukemia, relative to untreated controls, when treated at the MTD. [f] Values reported as the mean ± SEM, $n = 12$. [g] $n = 16$. [h] $n = 17$. [i] $n = 4$. [j] $n = 8$. [k] Highest dose tested; no toxicity was observed.

Controlled catalytic reduction of camptothecin produced 1,2,6,7-tetrahydrocamptothecin, which, because of air sensitivity, was treated with lead tetraacetate immediately to afford a mixture of 10-hydroxycamptothecin, 10-acetoxycamptothecin, and camptothecin in an approximate ratio of 2:1:1. Treatment of this mixture with acetic acid–water resulted in the hydrolysis of 10-acetoxycamptothecin and provided a mixture of 10-hydroxycamptothecin and camptothecin. Due to the extremely poor solubility of 10-hydroxycamptothecin, only partial purification was effected before conversion to the desired 9-[(dialkylamino)methyl]-10-hydroxycamptothecin (**4–12**, Table I) by treatment with an appropriately substituted amine, aqueous formaldehyde, and acetic acid. The use of an ethanolic solution of dimethylamine hydrochloride in this reaction produced 9-(ethoxymethyl)-10-hydroxycamptothecin (**16**). Also, treatment of 10-hydroxycamptothecin with hexamethylenetetraamine and trifluoroacetic acid afforded 9-formyl-10-hydroxycamptothecin (**14**). In addition, other 9-substituted camptothecin derivatives could be prepared from the 9-(dimethylamino)methyl derivative **4**. For example, treatment of **4** with (dimethylamino)ethanol and trace aqueous hydrochloric acid gave **15**; treatment of **4** with (dimethylamino)ethanethiol hydrochloride in DMF afforded **17**. Treatment of **4** with methyl methanesulfonate afforded the trimethyl quaternary salt **13**. Treatment of **13** with sodium cyanide yielded **18**, which, upon Raney nickel catalyzed reduction, gave **19**.

2. 9-[(Dimethylamino)methyl]camptothecin (21). This compound was prepared via the palladium-catalyzed reduction of an aryl triflate (Scheme II).[31] Thus, treatment of **4** with *N*-phenyltrifluoromethanesulfonimide provided triflate **20**, which was converted to **21** in situ. This reaction did not proceed to completion; the use of more forcing conditions (e.g., increased reaction times or elevated reaction temperatures) led to increased amounts of a side product identified as 9-methylcamptothecin (**28**, Table I). Compound **22** was prepared from the corresponding triflate in a similar manner.

3. 10-(Aminomethyl)camptothecin (25). 10-Hydroxycamptothecin (**2**) was treated with *N*-phenyltrifluoromethanesulfonimide to give a quantitative yield of crude triflate **23**, which was converted to 10-cyano-

(30) Miyasaka, T.; Sawada, S.; Nogata, K.; Mutai, M. *Japanese* Patent J59 05, 188; *Chem. Abstr.* **1984**, *100*, 192141.

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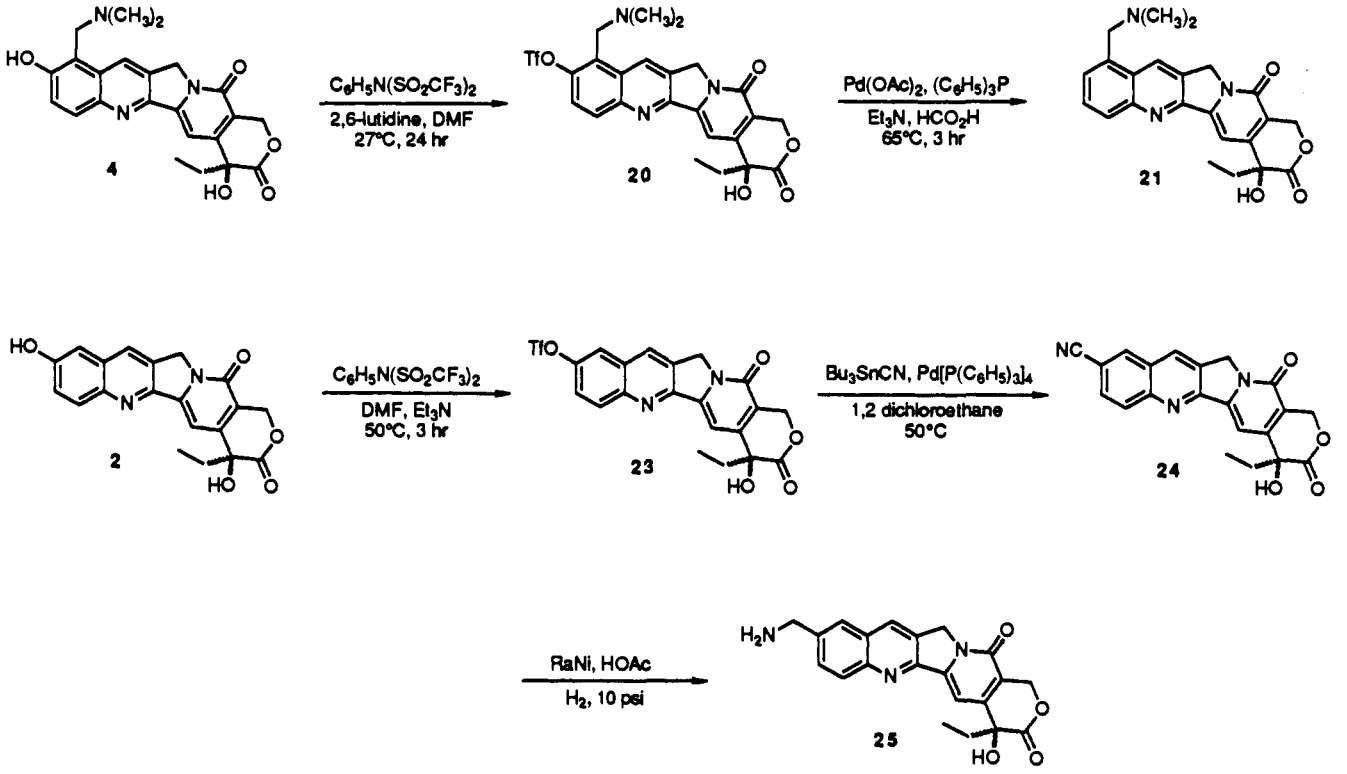
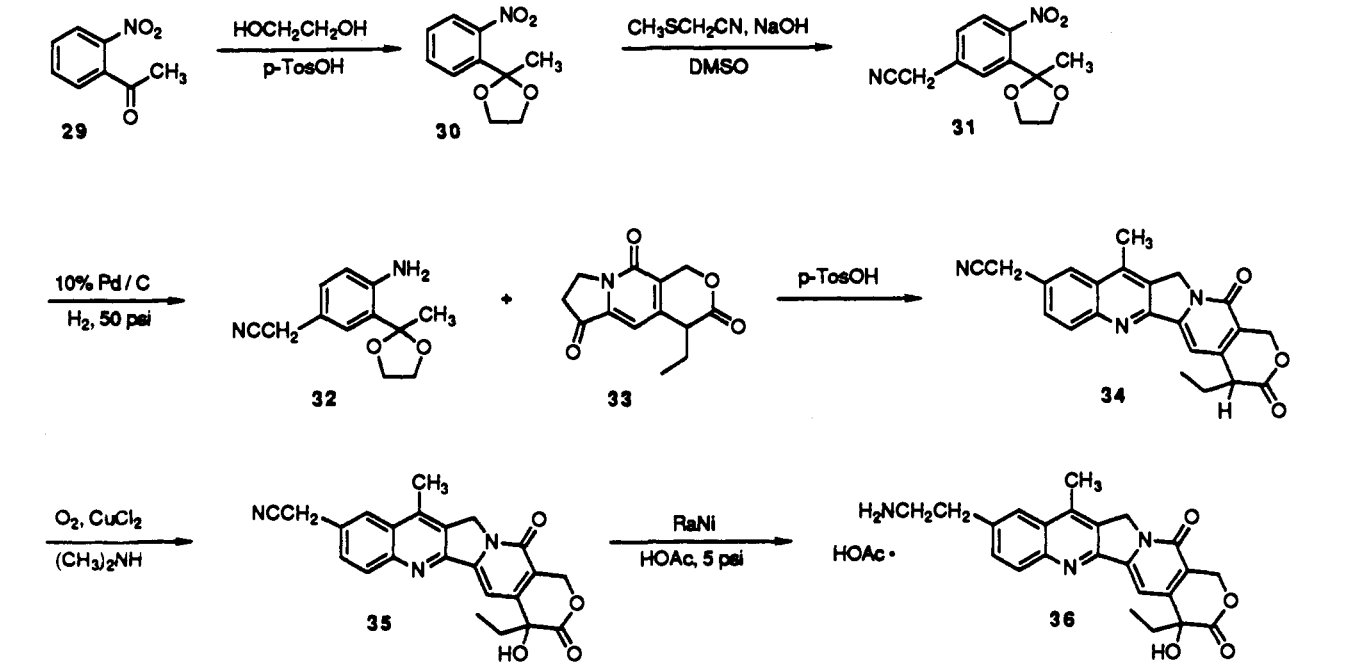
Scheme II



Scheme III



camptothecin (**24**) by the palladium-catalyzed cyanation method of Kosugi.[32] Catalytic reduction of **24** then gave the desired 10-(aminomethyl)camptothecin (**25**) (Scheme II). The palladium-catalyzed reaction of triflate **23** with diethyl phosphite produced the corresponding diethyl phosphonate, which was hydrolyzed to afford phosphonate salt **26**.[33]

4. **7-Substituted camptothecins (35, 36, 41–42)** were prepared by total synthesis in analogy with published methods (Schemes III and IV).[9] Tricyclic ketone **33** was condensed with the appropriate aromatic amino ketone (e.g., **32** and **39**), and the resulting deoxycamptothecin derivative was hydroxylated with oxygen in the presence of cupric chloride.

Biological Results and Discussion

All of the camptothecin derivatives were evaluated for inhibition of mammalian topoisomerase I, cytotoxicity toward mammalian cells, and antitumor activity in mice

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(33) Petrakis, K.; Nagabhushan, T. L. *J. Am. Chem. Soc.* 1987, *109*, 2831.

Scheme IV

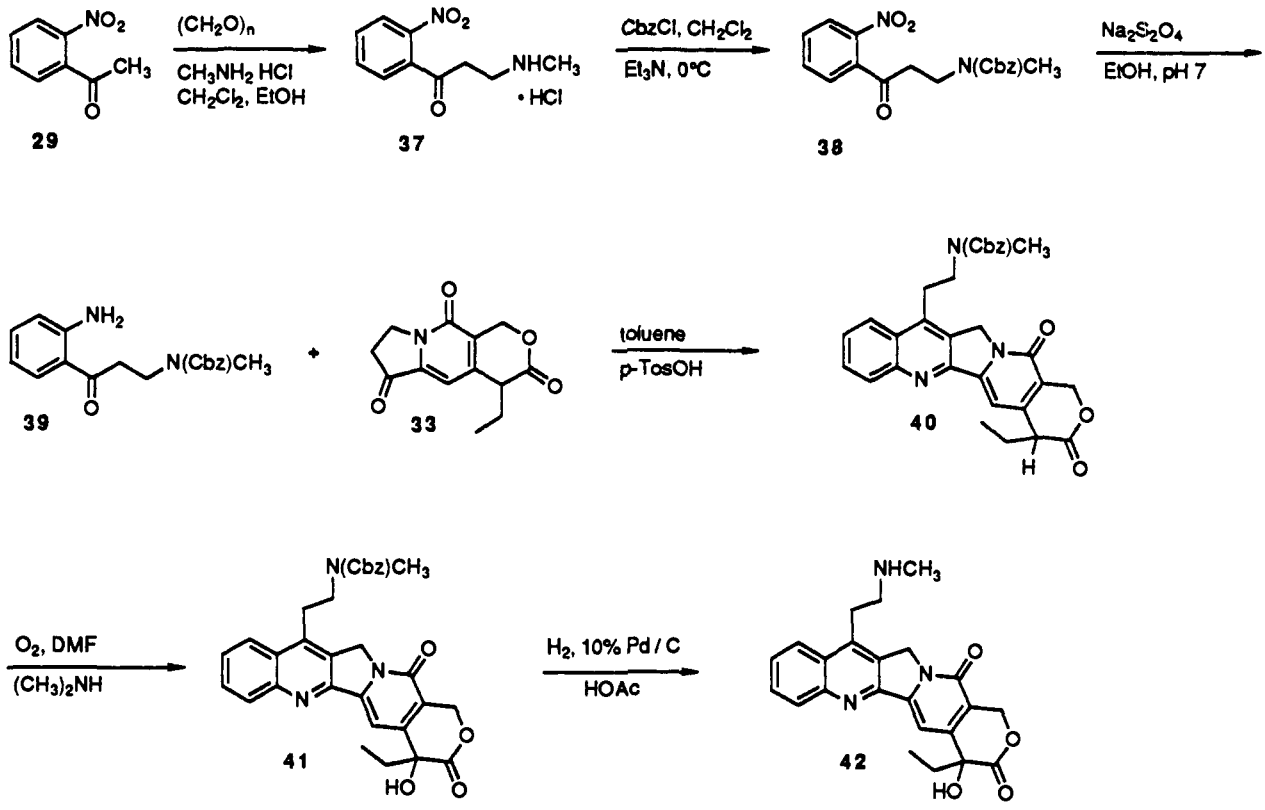
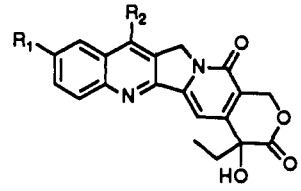
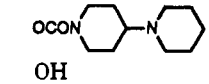


Table II. Topoisomerase I Inhibition, Cytotoxicity, and Antitumor Activity of 7- and 10-Substituted Camptothecin Derivatives[a]



no.	R_1	R_2	CC_{50}, μM	IC_{50}, nM	MTD	% ILS
35	CH_2CN	CH_3	0.5	7.5	6.4	106
36	$CH_2CH_2NH_2$	CH_3	3.0	29	68[b]	≥33
41	H	$CH_2CH_2N(Cbz)CH_3$	2.0	62	128	62
42	H	$CH_2CH_2NHCH_3$	0.4	44	122	43
43		CH_2CH_3	>100	1200	245	138
44	OH	CH_2CH_3	0.8	8.9	86	144

[a] Definitions of table headings are as in Table I. Compounds 35, 41, and 44 are insoluble in water and dissolved in DMSO for testing. Compounds 36, 42, and 43 are water soluble as their acid salts and were dissolved in water for testing. [b] Highest dose tested; no toxicity was observed.

bearing L1210 leukemia. Camptothecin inhibits topoisomerase I by binding to a covalent enzyme–DNA complex, resulting in the production of protein-associated DNA single strand breaks.[18,23-25] We determined the concentration dependence with which individual camptothecin derivatives stabilized the DNA–topoisomerase I covalent complex by the use of agarose gel electrophoresis (Figure 1).[23] Supercoiled DNA (form I) was converted to relaxed DNA (form I') in the presence of topoisomerase I (lane 17). The addition of camptothecin derivatives resulted in a stabilization of the nicked DNA intermediate (form II) in a concentration-dependent manner (lanes 1–16). The proportion of form II DNA was determined by densitometry (Figure 1b), and the concentration of each camptothecin derivative that resulted in 50% DNA cleavage (CC_{50}) is shown in Tables I and II. Some of the compounds were also examined with topoisomerase I isolated from human colon carcinoma cells (COLO 201) or mouse leukemia cells (P388). No differences in drug sensitivity were noted when comparing enzymes from these different mammalian sources (data not shown).

With the exceptions of compounds **26** and **43**, all of the 9- and 10-substituted camptothecin derivatives produce DNA strand breaks in the presence of topoisomerase I. This suggests that the enzyme–DNA complex has a large degree of bulk tolerance for substituents at position 9 of camptothecin, and is supported by the potent activity of compounds such as **8** and **12**. There was little difference in the potencies of the various 9-substituted derivatives, consistent with the interpretation that this region of the molecule undergoes little or no interaction with the enzyme–DNA complex. Small substituents at the 10-position were tolerated, but the low potency of **25** and the inactivity of **26** and **43** suggest that larger groups at position 10 were

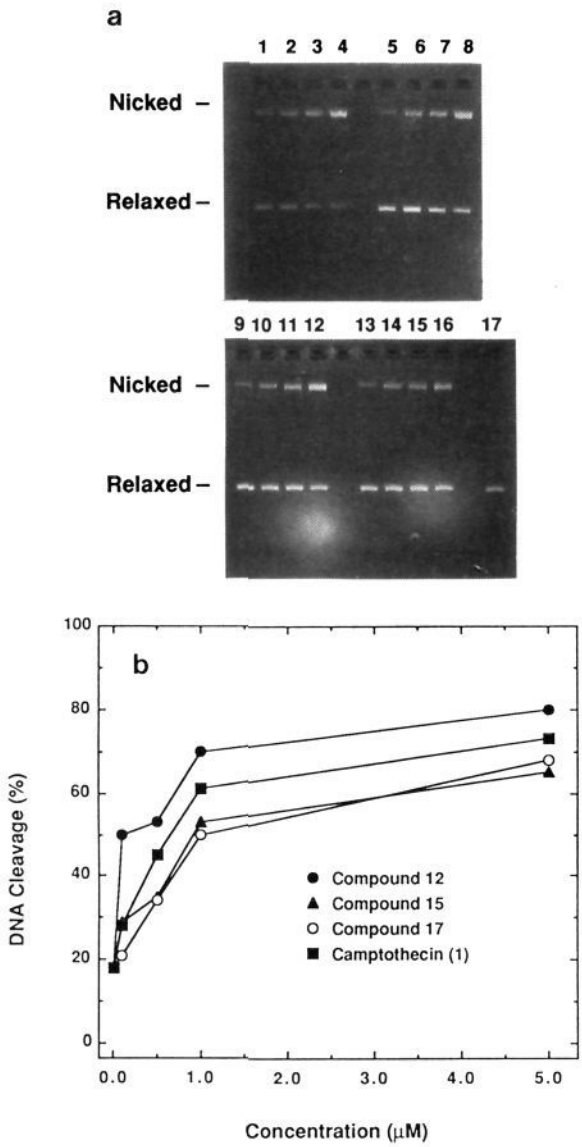


Figure 1. Cleavage of supercoiled pDPT2789 DNA by topoisomerase I in the presence of camptothecin analogues. In panel a, supercoiled DNA was treated with 10 nM topoisomerase I and a camptothecin analogue for 30 min; the reaction was terminated with SDS/proteinase K and then analyzed on a 1% agarose gel. Lanes 1–4, compound **12**; lanes 5–8, compound **15**; lanes 9–12, compound **17**; lanes 13–16, camptothecin (**1**); lane 17, topoisomerase I with no drug. Each group of four lanes contained 0.1, 0.5, 1.0, and 5.0 μM camptothecin analogue, respectively. Panel b illustrates the formation of nicked DNA (form II) as a function of camptothecin analogue concentration, based on densitometric analysis of the gel in panel a. The CC_{50} values in Table I and II are determined from this analysis.

not well tolerated by the enzyme–DNA complex. In contrast, the enzyme–DNA complex was tolerant of aminoalkyl substitution at position 7 (e.g., **41** and **42**).

All of the compounds that inhibited topoisomerase I were found to be cytotoxic to mammalian cells and to have antitumor activity (Tables I, II). Jaxel and co-workers examined a set of camptothecin analogues and suggested that there is a quantitative correlation between topoisomerase I activity and in vivo antitumor efficacy.[26] While we found that compounds that fail to inhibit topoisomerase I have no antitumor activity[8] (with one exception, **43**; see below), we were unable to establish a quantitative relationship between potency of enzyme inhibition and in vivo potency (i.e., maximally tolerated dose) or efficacy (i.e., increase in life span at the maximally tolerated dose). Similarly, no quantitative relationship between cytotoxic potency and enzyme inhibition could be established. For example, while compounds such as **5**, **11**, and **42** were at least as potent as camptothecin as inhibitors of topoisomerase I, they were less potent than camptothecin with respect to in vitro cytotoxicity and in vivo maximally tolerated dose. These compounds also demonstrated lower efficacy than camptothecin in vivo. Furthermore, compounds such as **4**, **10**, and **25**, which were 3–12-fold less potent than camptothecin as inhibitors of purified topoisomerase I, were at least as active as camptothecin in prolonging the life span of mice bearing L1210 leukemia. It is likely that cellular uptake and pharmacokinetics play an important role in determining, respectively, cytotoxic potency in vitro and antitumor activity in vivo.

Of all of the camptothecin derivatives described to date, only one exhibits antitumor activity without inhibiting topoisomerase I. This compound, CPT-11 (**43**), is in clinical trials in Japan. CPT-11 does not inhibit topoisomerase I at concentrations 120 times greater than the CC_{50} of camptothecin. It is 50 times less potent than camptothecin as a cytotoxic agent and about 10 times less potent as an antitumor agent (Table II). When tested at a high enough dose, however, CPT-11 is as efficacious as camptothecin in L1210 in vivo and has been reported to be effective in a spectrum of animal tumor models.[34] These results suggest that CPT-11 may be a prodrug; it has little or no inherent activity as an inhibitor of the target enzyme but may be converted to 7-ethyl-10-hydroxycamptothecin (**44**) upon hydrolysis in vivo. The lability anticipated for the carbamate linkage in CPT-11 is consistent with this hypothesis. In support of this hypothesis, we have demonstrated that **44** is a potent inhibitor of topoisomerase I, has potent cytotoxic activity, and has efficacy against L1210 leukemia in vivo similar to that observed for CPT-11 (Table II).

In searching for a water-soluble camptothecin analogue to develop as a clinically useful agent, we set a number of criteria. We felt that the compound should (1) inhibit topoisomerase I by the same mechanism as camptothecin and with similar potency, (2) be active as the parent compound rather than as a water-soluble prodrug, (3) be at least as active as camptothecin as an antitumor agent in a panel of preclinical tumor models, (4) be accessible semisynthetically in good yield from camptothecin because of the complexity and low yields of the total syntheses reported to date,[3-6,9,12] (5) demonstrate potency in vivo (i.e., a maximally tolerated dose) at similar or lower dose levels than camptothecin in order to minimize the requirements for camptothecin as a starting material. We particularly wished to avoid the use of a sodium salt analogous to **3** to achieve water solubility.

Compound **4** met all of the above criteria. The hydrochloride salt is soluble in water at concentrations up to 1 mg/mL; the comparable value for camptothecin itself is 2.5×10^{-3} mg/mL. Analogue **4** stabilizes the DNA–topoisomerase I covalent complex, albeit at concentrations 4-fold higher than camptothecin (Table I). Compound **4** is about 2-fold less potent than camptothecin as a cytotoxic agent against L1210 cells in tissue culture (Table I); however, the therapeutic dosage range for **4** is virtually identical with that of camptothecin. Most importantly, **4** is reproducibly and significantly superior to camptothecin

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as an antitumor agent in mice bearing L1210 leukemia at the respective maximally tolerated doses (173 ± 16% ILS for 4 as opposed to 118 ± 6% ILS for camptothecin). Several other analogues shown in Tables I and II have in vivo antitumor activity superior to that of camptothecin, but these compounds either lack adequate water solubility (e.g., 18, 28) or demonstrate superior antitumor activity only at a higher dose level (e.g., 6, 10, 13, 15).

9-Aminocamptothecin (27) recently has been reported to have a high degree of activity against human colon cancer xenografts in mice.[35] We found 27 to be active in L1210 leukemia at relatively low doses, but it is not superior to camptothecin and is not water-soluble. We have subsequently carried out extensive comparative studies of 4, 27, and camptothecin in a panel of animal and xenografted tumor models.[36] Compound 4 demonstrates superior activity in certain tumor models such as Lewis lung carcinoma, B16 melanoma, and L1210 leukemia; compound 4 is no more effective than 27 and/or camptothecin in murine colon tumors or the HT29 human colon tumor xenograft. Each of these compounds demonstrates a high degree of antitumor efficacy. Because of its broad-spectrum activity in preclinical tumor models, its water solubility and accessibility via semisynthesis, the hydrochloride salt of compound 4 (SK&F 104864) has been selected for development and is currently undergoing Phase I clinical trials in cancer patients.[37]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were obtained on a Perkin-Elmer 137 spectrometer; proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a JEOL FX-90 Q instrument; all values are reported in parts per million (δ) from $(CH_3)_4Si$ unless otherwise stated. Elemental analyses were performed in the Analytical and Physical Chemistry Department of Smith Kline & French Laboratories. Mass spectra were obtained by the Physical and Structural Chemistry Department of Smith Kline & French Laboratories.

Analytical thin-layer chromatography (TLC) was carried out with Merck silica gel 60 F-254 glass backed plates or with Whatman KC 18 F reversed-phase RP-18 glass backed plates. High-performance liquid chromatography (HPLC) was performed on an Altex Model 110 gradient liquid chromatograph with a UV wavelength detector set at 254 nM. Preparative chromatography was carried out on a medium-pressure liquid chromatography (MPLC) system using an Altex column packed with either Merck silica gel (40–60 μm), Whatman Partisil 40 ODS3, or Merck Licroprep RP-18 (25–40 μm). Camptothecin was obtained from Tainjain-SK&F Ltd., Tainjain, China. Tricyclic ketone 33[9] and 9-aminocamptothecin (27)[38] were prepared according to literature procedures; compounds 43 and 44 were prepared as reported with minor modification.[39]

Topoisomerase I was purified from calf thymus and enzyme-mediated DNA plasmid cleavage was performed as described.[8,23] Growth inhibition assays in L1210 murine leukemia cells[8] and experiments in mice bearing L1210 leukemia[40] were performed as described.

10-Hydroxy-(20*S*)-camptothecin (2). A mixture of camptothecin (32.0 g, 0.092 mol), Pt^0 (prepared by prereduction of 8.0 g of amorphous PtO_2 in 800 mL of HOAc for 1.5 h under 1 atm H_2), and HOAc (1.6 L), was subjected to 1 atm of H_2 for 8.5 h, after which the theoretical amount of H_2 had been absorbed (slightly more than 4.1 L) and the uptake of H_2 had slowed considerably. The reaction mixture was degassed under a stream of argon, filtered through Celite, and washed with HOAc (200 mL). The resulting solution of 1,2,6,7-tetrahydrocamptothecin was treated immediately with $Pb(OAc)_4$ (64 g, 0.144 mol) in one portion and the reaction mixture was stirred vigorously under argon for 30 min. Evaporation of the solvent afforded a gummy residue that was triturated with cold H_2O (1 L) to produce a light brown solid. The solid was collected, washed with additional cold H_2O (200 mL), and air-dried overnight to afford 10-hydroxycamptothecin (44.3%), 10-acetoxycamptothecin (26.9%), and camptothecin (32.1%) on the basis of HPLC analysis (Whatman Partisil 5 ODS3 Rac II, 60% CH_3OH/H_2O). This crude mixture was combined with 1.7 L of 50% aqueous HOAc and heated at reflux overnight. The reaction mixture was then cooled, concentrated to 50–100 mL, and treated with cold H_2O (1 L), to produce a precipitate that was filtered, washed with additional cold H_2O (200 mL), and dried to afford 21.2 g of a solid containing 10-hydroxycamptothecin (70.9%), 10-acetoxycamptothecin (1.2%), and camptothecin (21.3%) on the basis of HPLC analysis.

9-[(Dimethylamino)methyl]-10-hydroxy-(20*S*)-camptothecin (4). 10-Hydroxycamptothecin (20 g, 62% by HPLC), prepared as described above, was combined with HOAc (620 mL), 37% aqueous CH_2O (12.4 mL), and 40% aqueous dimethylamine (12.4 mL), and the reaction mixture was stirred for 18 h. Analysis by TLC (silica gel, 9:1 CH_2Cl_2/CH_3OH) indicated that some starting material remained. Therefore, the reaction mixture was treated with an additional 6 mL of 37% aqueous CH_2O and 6 mL of 40% aqueous dimethylamine, and stirring was continued for 24 h. The reaction mixture was then concentrated to dryness and the residue was triturated with 0.5% aqueous HOAc (1 L) to dissolve the water-soluble camptothecin adduct. The insoluble camptothecin was removed by filtration to afford 6.3 g of a solid identified as camptothecin by HPLC and ^{1}H NMR. The combined aqueous filtrate was extracted with EtOAc (3 × 600 mL) and petroleum ether (600 mL) and lyophilized. Purification of the crude residue was achieved by RP-18 HPLC as follows: the solid was dissolved in solvent A (99% H_2O, 1% HOAc) and injected onto a 50 mm × 600 mm steel column packed with 680 g of Whatman Partisil 40 ODS 3 and eluted at the rate of 350 mL/min with a 34-min linear gradient from 100% solvent A to 40% solvent B (99% CH_3OH, 1% HOAc). The progress of the chromatography was monitored at 410 nm, and 1-L fractions were collected. Those which assayed at ≥99% purity by analytical HPLC (Whatman Partisil 10 ODS 3, 50% CH_3OH/H_2O, retention time 9 min) were pooled, concentrated, redissolved in a minimum of 0.5% aqueous HOAc, and lyophilized to afford desired product 4 (acetate salt) as an off-white hygroscopic solid: yield 10.6 g (62%); IR (KBr) 3400, 2960, 1740, 1650, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3/CD_3OD$) δ 1.04 (t, 3, J = 7 Hz, C18), 1.96 (q, 2, J = 7 Hz, C19), 2.01 (s, 3, CH_3CO_2), 2.50 (s, 6, $(CH_3)_2NH$), 4.20 (s, 2, $ArCH_2N$), 5.28 (d, 1, J = 19 Hz, C17), 5.29 (s, 2, C5), 5.50 (d, 1, J = 19 Hz, C17), 7.42 (d, J = 9 Hz, C11), 7.67 (s, 1, C14), 8.05 (d, J = 9 Hz, C12), 8.51 (s, C7). Anal. ($C_{23}H_{23}N_3O_5 \cdot HOAc \cdot H_2O$) C, H, N. The hydrochloride salt of 4 was readily prepared by the addition of dilute aqueous hydrochloric acid to a solution of the acetate salt of 4, followed by lyophilization.

10-Hydroxy-9-(morpholinomethyl)-(20*S*)-camptothecin (5). 10-Hydroxycamptothecin (100 mg, 0.27 mmol), prepared as described above, 37% aqueous CH_2O (0.5 mL), morpholine (0.1 mL), and 2:1 HOAc/EtOH (10 mL) were combined and stirred overnight, after which 10-hydroxycamptothecin reacted completely as judged by silica gel TLC (9:1 CH_2Cl_2/CH_3OH). The reaction mixture was concentrated to dryness, dissolved in 5 mL of H_2O

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containing several drops of HOAc, and filtered to remove insoluble camptothecins, and the filtrate was lyophilized. The lyophilizate was purified by chromatography (MPLC; 15 mm × 250 mm silica gel column, 0–2% CH_3OH/CH_2Cl_2); the fractions containing product were combined and evaporated to produce a residue. This residue was dissolved in dilute aqueous HOAc and lyophilized to give the title compound **5** (acetate salt) as a hygroscopic white solid: yield 53 mg (38%); IR (KBr) 3400, 3100, 2960, 2920, 2840, 1740, 1650, 1590 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 1.12 (t, 3, J = 7 Hz), 1.94 (q, 2, J = 7 Hz), 2.02 (s, 3), 2.73 (m, 4), 3.83 (m, 4), 4.19 (s, 2), 5.26 (s, 2), 5.25 (d, 1, J = 16 Hz), 5.76 (d, 1, J = 16 Hz), 7.27 (s, 1), 7.40 (d, 1, J = 8 Hz), 8.07 (d, 1, J = 8 Hz), 8.36 (s, 1). Anal. ($C_{25}H_{25}N_3O_6$·HOAc) C, H, N.

10-Hydroxy-9-[(*N*-methylpiperazinyl)methyl]-(20*S*)-camptothecin (6). 10-Hydroxycamptothecin (100 mg, 0.27 mmol), HOAc/EtOH (2:1, 10 mL), 37% aqueous CH_2O (0.5 mL), and *N*-methylpiperazine (0.1 mL) were combined and stirred at room temperature for 20 h, after which the reaction mixture was concentrated to produce a residue that was dissolved in H_2O (50 mL) and washed with EtOAc (5 × 20 mL) and petroleum ether (20 mL). The aqueous phase was lyophilized, and the resulting residue was redissolved in dilute aqueous HOAc and purified by MPLC (Whatman Partisil 40 ODS3, 9 mm × 250 column); elution was with 0–20% CH_3OH/H_2O containing 0.02% HOAc. The desired product was pooled, concentrated, and lyophilized to afford **6** (acetate salt) as a hygroscopic white solid: yield 67 mg (46%); IR (KBr) 3400, 2960, 2910, 1740, 1650, 1590 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 1.03 (t, 3, J = 7 Hz), 1.89 (q, 2, J = 7 Hz), 2.02 (s, 3), 2.37 (s, 3), 2.65 (m, 8), 4.21 (s, 2), 5.26 (s, 2), 5.30 (d, 1, J = 16 Hz), 5.71 (d, 1, J = 16 Hz), 7.45 (d, 1, J = 7 Hz), 8.05 (d, 1, J = 7 Hz), 8.47 (s, 1). Anal. ($C_{26}H_{28}N_4O_5$·1.5HOAc·0.3H_2O) C, H, N.

10-Hydroxy-9-[(*N*-methylanilino)methyl]-(20*S*)-camptothecin (7). A mixture of 10-hydroxycamptothecin (254 mg, 0.7 mmol), 37% aqueous formaldehyde (1.0 mL), *N*-methylaniline (0.75 mL, 0.7 mmol), HOAc (16 mL), and EtOH (8 mL) was combined and stirred at ambient temperature for 40 h. Concentration of the reaction mixture provided a crude oil that was partially purified by flash chromatography (silica gel; 1–3% CH_3OH/CH_2Cl_2). Final purification was obtained by MPLC (silica gel; 2% CH_3OH/CH_2Cl_2) to afford the desired product **7** as a yellow solid: yield 77 mg (24%); ^1H NMR ($CDCl_3$) δ 1.0 (t, 3, J = 7 Hz), 1.2 (t, 3), 1.9 (q, 2, J = 7 Hz), 2.8 (s, 3), 5.0 (s, 2), 5.2 (s, 2), 5.3 (d, 1, J = 16 Hz), 5.7 (d, 1, J = 16 Hz), 6.9 (m, 5), 7.5 (s, 1), 7.9 (q, 2), 8.4 (s, 1). Anal. ($C_{28}H_{25}N_3O_5$·1.2H_2O) C, H, N.

10-Hydroxy-9-[(4-piperidinopiperidinyl)methyl]-(20*S*)-camptothecin (8). A reaction mixture containing 10-hydroxycamptothecin (100 mg, 0.27 mmol), 4-piperidinopiperidine (100 mg, 0.60 mmol), 37% aqueous CH_2O (0.5 mL), and HOAc/EtOH (2:1, 10 mL) was stirred for 20 h at room temperature; analysis by TLC (silica gel; 9:1 CH_2Cl_2/CH_3OH) indicated complete disappearance of 10-hydroxycamptothecin. The reaction mixture was concentrated, and the residue was dissolved in 1% aqueous HOAc, filtered, and lyophilized. MPLC [Whatman Partisil 40 ODS3, 9 × 250 mm column eluting with H_2O (100 mL) and 0–80% CH_3OH in 0.02% aqueous HOAc] afforded the desired product **8** as an amorphous white solid after lyophilization: yield 44 mg (30%); IR (KBr) 3400, 2940, 1745, 1660, 1590 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 0.99 (t, 3, J = 7 Hz), 1.3–3.3 (m, 19), 4.11 (s, 2), 5.21 (s, 2), 5.25 (d, 1, J = 16 Hz), 5.70 (d, 1, J = 16 Hz), 7.85 (d, 1, J = 7 Hz), 7.59 (s, 1), 8.00 (d, 1, J = 7 Hz), 8.35 (s, 1). Anal. ($C_{31}H_{36}N_4O_5$·1.5H_2O) C, H, N.

9-[(Cyclopropylamino)methyl]-10-hydroxy-(20*S*)-camptothecin (9). A mixture of 10-hydroxycamptothecin (254 mg, 0.7 mmol), 37% aqueous formaldehyde (1.0 mL), cyclopropylamine (400 mg, 0.7 mmol), HOAc (16 mL), and EtOH (8 mL) was stirred overnight at ambient temperature and concentrated to dryness. The resulting residue was triturated with H_2O, filtered, and dried to afford 260 mg (75% yield) of the desired product **9** (acetate salt) as a white hygroscopic solid: yield 260 mg (75%). The acetate salt was converted to the hydrochloride salt by triturating with 0.1 N HCl: ^1H NMR (D_2O) δ 0.96 (m, 7), 2.1 (m, 2), 2.8 (m, 1), 4.6 (s, 2), 4.8 (s, 2), 5.2 (s, 2), 7.2 (s, 1), 7.4 (d, 1, J = 9 Hz), 7.7 (d, 1, J = 9 Hz), 8.6 (s, 1). Anal. ($C_{24}H_{23}N_3O_5$·HCl·3H_2O) C, H, N.

9-[(Cyclohexylamino)methyl]-10-hydroxy-(20*S*)-camptothecin (10). A mixture of 10-hydroxycamptothecin (364 mg, 1.0 mmol), 37% aqueous formaldehyde (1.5 mL), cyclohexylamine (1.3 mL, 10 mmol), HOAc (25 mL), and EtOH (12 mL) was stirred overnight at ambient temperature and concentrated to dryness. The resulting residue was purified by MPLC (RP-18; 15% aqueous MeOH containing 0.02% glacial HOAc), and the combined product fractions were concentrated to a small volume and lyophilized to afford desired product **10** (acetate salt) as a white hygroscopic solid: yield 250 mg (47%); the acetate salt was converted to the hydrochloride salt by addition of 0.1 N HCl; ^1H NMR (DMSO-d_6) δ 0.9 (t, 3), 1.0–2.0 (m, 10), 3.1 (s, 1), 4.5 (s, 2), 5.2 (s, 2), 5.4 (s, 2), 7.2 (s, 1), 7.7 (d, 1, J = 9 Hz), 8.1 (d, 1, J = 9 Hz), 8.9 (s, 1). Anal. ($C_{27}H_{29}N_3O_5$·HCl·1.1H_2O) C, H, N.

10-Hydroxy-9-[[(2-hydroxyethyl)amino]methyl]-(20*S*)-camptothecin (11). A mixture containing 10-hydroxycamptothecin (200 mg, 0.55 mmol), paraformaldehyde (61 mg, 0.55 mmol), ethanolamine (61 mg, 1.1 mmol), and HOAc (6 mL) was stirred for 48 h, after which most of the 10-hydroxycamptothecin had reacted as judged by TLC. The reaction mixture was concentrated, redissolved in dilute HOAc (200 mL), washed successively with EtOAc (4 × 30 mL), and petroleum ether (30 mL), and the resulting aqueous solution was lyophilized. The crude lyophilizate was dissolved in H_2O (50 mL) and purified by MPLC (Whatman Partisil 40 ODS3, 15 mm × 250 mm column); elution was with H_2O (100 mL) followed by 0–10% CH_3OH in 0.02% aqueous HOAc. After lyophilization, the desired product **11** (acetate salt) was isolated as a hygroscopic solid: yield 88 mg (33%); IR (KBr) 3400, 2980, 2940, 1750, 1570 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 1.03 (t, 3, J = 7 Hz), 1.89 (q, 2, J = 7 Hz), 2.00 (s, 3, HOAc), 3.03 (m, 2), 3.75 (m, 2), 4.49 (s, 2), 5.24 (s, 2), 5.30 (d, 1, J = 16 Hz), 5.70 (d, 1, J = 16 Hz), 7.41 (d, 1, J = 8 Hz), 7.61 (s, 1), 8.00 (d, 1, J = 8 Hz), 8.48 (s, 1). Anal. ($C_{23}H_{23}N_3O_6$·HOAc·1.8H_2O) C, H, N.

9-[[[2-(*N*,*N*-Dimethylamino)ethyl]amino]methyl]-10-hydroxy-(20*S*)-camptothecin (12). 10-Hydroxycamptothecin (364 mg, 1.0 mmol), *N*,*N*-dimethylethylenediamine (100 mg, 1.1 mmol), 37% aqueous formaldehyde (1.5 mL), HOAc (25 mL), and EtOH (10 mL) were stirred at room temperature for 64 h. The solvents were evaporated, and the solid residue was treated with water (10 mL) and 2-propanol (10 mL) containing 3 N HCl (3 mL). The resulting finely precipitated solid was collected, washed with 2-propanol, and dried to give **12** as the hydrochloride salt: yield 218 mg (40%); ^1H NMR (CD_3OD) δ 1.0 (t, 3, J = 7 Hz), 1.9 (q, 2, J = 7 Hz), 2.9 (s, 6), 4.5 (s, 2), 5.1 (m, 4), 5.4 (q, 2), 7.3 (d, 1, J = 9 Hz), 7.5 (s, 1), 7.8 (d, 1, J = 9 Hz), 8.4 (s, 1). Anal. ($C_{25}H_{28}N_4O_5$·2HCl) C, H, N.

10-Hydroxy-9-[(trimethylammonio)methyl]-(20*S*)-camptothecin (13). Compound **4** (65 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (70 mL) and filtered. The filtrate was combined with methyl methanesulfonate (1 mL), cooled to 0 °C, and partially concentrated under a stream of argon. After 4 h the solvent was concentrated to one-half volume and cooled. The precipitate was filtered, dissolved in H_2O (10 mL), washed successively with EtOAc (3 × 10 mL) and petroleum ether (10 mL), and the aqeuous solution was lyophilized to afford the desired methanesulfonate salt **13** as an amophorous solid: yield 50 mg (60%); IR (KBr) 3400, 2950, 2900, 1760, 1660, 1600 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 1.03 (d, 3, J = 7 Hz), 2.01 (q, 2, J = 7 Hz), 2.78 (s, 3), 2.94 (s, 9), 4.72 (s, 2), 5.20 (s, 2), 5.22 (d, 1, J = 16 Hz), 5.67 (d, 1, J = 16 Hz), 7.62 (d, J = 7 Hz), 7.71 (s, 1), 8.16 (d, 1, J = 7 Hz), 8.89 (s, 1). Anal. ($C_{24}H_{25}N_3O_5$·1.5CH_3SO_3H·2H_2O) C, H, N.

9-Formyl-10-hydroxy-(20*S*)-camptothecin (14). A mixture of 10-hydroxycamptothecin (100 mg, 0.27 mmol), HMTA (0.80 g, 5.5 mmol), and TFA (15 mL) was heated at reflux under argon for 20 h. The reaction mixture was concentrated, combined with H_2O (15 mL), and stirred for 1 h; additional H_2O (75 mL) was added and the pH was adjusted to 8.4 ($NaHCO_3$). The aqueous phase was washed with EtOAc (3 × 75 mL), acidified to pH 1.5 (3 N HCl), and extracted with EtOAc (5 × 75 mL). The combined organic extract was washed successively with 1 N HCl (5 × 75 mL), H_2O (75 mL), and saturated aqueous NaCl (25 mL) and then concentrated. The resulting residue was purified by flash chromatography (silica gel, 1 cm × 15 cm column with the crude material preadsorbed on a 1 cm × 1 cm plug of Na_2SO_4); elution was with 1% CH_3OH/CH_2Cl_2 to give the desired product **14** as

an off-white solid: yield 50 mg (47%). An analytical sample was obtained by fractional precipitation of 14 from 25% CH_3OH in CH_2Cl_2, through gradually cooling and concentrating the solution under a stream of nitrogen: IR (KBr) 3400, 3100, 2950, 1755, 1660, 1600 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 1.04 (t, 3, J = 7 Hz), 1.96 (d, 2, J = 7 Hz), 5.32 (d, 1, J = 14 Hz), 5.33 (s, 2), 5.68 (d, 1, J = 14 Hz), 7.50 (d, 1, J = 9 Hz), 7.67 (s, 1), 8.33 (d, 1, J = 9 Hz), 9.34 (s, 1), 10.85 (s, 1). Anal. ($C_{21}H_{16}N_2O_6 \cdot 1H_2O$) C, H, N.

9-[[2-(*N*,*N*-Dimethylamino)ethoxy]methyl]-10-hydroxy-(20*S*)-camptothecin (15). Compound 4 (100 mg, 0.2 mmol) was treated with 2-(dimethylamino)ethanol (4 mL) containing 3 drops of 3 N HCl and heated under argon at 80 °C for 24 h. The semisolid reaction mixture was treated with H_2O (5 mL) and 2-propanol (10 mL), stirred, and filtered to give the desired product 15 (hydrochloride salt) an an amorphous solid: yield 60 mg (59%); ^1H NMR (DMSO-d_6) δ 0.9 (t, 3, J = 7 Hz), 1.85 (q, 2, J = 7 Hz), 2.3 (s, 6), 3.3 (s, 2), 4.1 (s, 2), 5.2 (s, 2), 5.4 (s, 2), 7.3 (s, 1), 7.4 (d, 1, J = 9 Hz), 8.0 (d, 1, J = 9 Hz), 8.7 (s, 1). Anal. ($C_{25}H_{27}N_3O_6 \cdot HCl \cdot 0.5H_2O$) C, H, N.

9-(Ethoxymethyl)-10-hydroxy-(20*S*)-camptothecin (16). A mixture of 10-hydroxycamptothecin (364 mg, 1.0 mmol), diethylamine hydrochloride (90 mg, 1.1 mmol), and 37% aqueous formaldehyde (1.5 mL) was heated at reflux in 95% EtOH (25 mL) for 6 H and then concentrated to a small volume. The resulting precipitate was collected, dried, and purified by MPLC (silica gel; 3% CH_3OH/CH_2Cl_2) to afford 16 as an off-white solid: yield 85 mg (20%); ^1H NMR (DMSO-d_6) δ 0.85 (t, 3, J = 7 Hz), 1.1 (t, 3), 1.9 (q, 2, J = 7 Hz), 3.5 (s, 2), 4.8 (s, 2), 5.2 (s, 2), 5.4 (s, 2), 7.2 (s, 1), 7.5 (d, 1, J = 9 Hz), 7.9 (d, 1, J = 9 Hz), 8.6 (s, 1). Anal. ($C_{23}H_{22}N_2O_6$) C, H, N.

9-[[[2-(*N*,*N*-Dimethylamino)ethyl]thio]methyl]-10-hydroxy-(20*S*)-camptothecin (17). Compound 4 (100 mg, 0.2 mmol), 2-(dimethylamino)ethanethiol hydrochloride (560 mg, 4 mmol), and DMF (13 mL) were heated at 80 °C under argon for 5 h. The insoluble solid (excess thiol) was removed by filtration, and the filtrate was concentrated to an afford oily residue, which was purified by MPLC(RP-18; 5–10% CH_3OH/H_2O). The desired hydrochloride salt 17 was obtained as a yellow solid: yield 45 mg (41%); ^1H NMR (D_2O) δ 1.0 (t, 3, J = 7 Hz), 1.9 (q, 2, J = 7 Hz), 2.8 (s, 6), 4.4 (s, 2), 5.3 (s, 2), 7.1 (d, 1, J = 9 Hz), 7.2 (s, 1, J = 9 Hz), 7.6 (d, 1), 8.2 (s, 1). Anal. ($C_{25}H_{28}N_3O_5S \cdot HCl \cdot 3H_2O$) C, H, N.

9-(Cyanomethyl)-10-hydroxy-(20*S*)-camptothecin (18). A solution of compound 13 (0.42 g, 0.8 mmol) in 95% EtOH (35 mL) was treated with sodium cyanide (1.26 g, 25 mmol) and the resulting reaction mixture was refluxed under argon for 3 h. The solvent was evaporated, H_2O added (20 mL), and the pH adjusted to 1.5 with 3 N HCl. The resulting precipitated solid was collected, dried, and purified by flash chromatography (silica gel; 5% CH_3OH/CH_2Cl_2) to afford 110 mg (33%) of desired product 18: ^1H NMR (Cd_3Cl_3/CD_3OD_4) δ 0.9 (t, 3, J = 7 Hz), 1.8 (q, 2, J = 7 Hz), 4.2 (s, 2), 5.2 (s, 2), 5.3 (d, 1, J = 16 Hz), 5.6 (d, 1, J = 16 Hz), 7.5 (d, 1, J = 9 Hz), 7.6 (s, 1), 7.9 (d, 1, J = 9 Hz), 8.4 (s, 1). Anal. ($C_{22}H_{17}N_3O_5 \cdot 1.8H_2O$) C, H, N.

9-(Aminoethyl)-10-hydroxy-(20*S*)-camptothecin (19). Compound 18 (60 mg, 0.15 mmol) in HOAc (30 mL) was treated with 1 g (wet weight) of activated Raney nickel and hydrogenated at 10 psi for 6 h. The catalyst was removed and the solvent was evaporated. The residue was dissolved in H_2O and purified by MPLC (RP-18; 10% CH_3OH/H_2O containing 0.02% glacial acetic acid) to afford the desired product 19 (acetate salt) as an amorphous solid: yield 23 mg (33%); ^1H NMR (DMSO-d_6) δ 0.9 (t, 3, J = 7 Hz), 1.9 (q, 2, J = 7 Hz), 3.2 (s, 2), 5.0 (s, 2), 5.1 (s, 2), 5.4 (s, 2), 7.2 (s, 1), 7.3 (d, 1, J = 16 Hz), 7.6 (d, 1, J = 16 Hz), 8.4 (s, 1). Anal. ($C_{22}H_{21}N_3O_5 \cdot HOAc \cdot 10H_2O$) C, H, N.

9-[(Dimethylamino)methyl]-(20*S*)-camptothecin (21). The trifluoromethanesulfonate of 4 was prepared in situ as follows: under a stream of argon, a mixture of 4 (482 mg, 1 mmol) in DMF (40 mL) was treated with 2.6-lutidine (268 mg, 2.5 mmol) and *N*-phenyltrifluoromethanesulfonimide (0.54 g, 1.5 mmol), and the reaction mixture was stirred overnight at room temperature. To the resulting trifluoromethanesulfonate was added Et_3N (0.4 mL, 3.0 mmol), $Pd(OAc)_2$ (8 mg, 0.04 mmol), $(C_6H_5)_3P$ (20 mg, 0.08 mmol), and concentrated formic acid (0.08 mL, 2 mmol), and the reaction mixture was heated at 60 °C for 8 h. The solvent was evaporated and the residue was triturated with a small amount of H_2O, filtered, and dried ($MgSO_4$) to give 550 mg of crude 21 that was purified by flash chromatography (silica gel; 4% CH_3OH/CH_2Cl_2). In addition to a trace amount of the starting triflate of 4 and 25 mg (7%) of 9-methyl-(20*S*)-camptothecin (28), chromatographic separation provided the desired compound 21 as an amorphous solid: yield 88 mg (20%). A portion of 21 was converted to the acetate salt by treatment with dilute HOAc: ^1H NMR ($CDCl_3$) δ 0.9 (t, 3, J = 7 Hz), 1.8 (q, 2, J = 7 Hz), 2.2 (s, 6), 3.7 (s, 2), 5.2 (s, 2), 5.3 (d, 1, J = 16 Hz), 5.6 (d, 1, J = 16 Hz), 7.3 (d, 1), 7.5 (d, 1), 7.6 (2, 1), 8.0 (d, 1), 3.3 (s, 1). Anal. ($C_{23}H_{23}N_3O_4 \cdot HOAc \cdot 2.5H_2O$) C, H, N.

9-(Morpholinomethyl)-(20*S*)-camptothecin (22). A 1-mmol solution of the trifluoromethanesulfonate of 10-hydroxy-9-(morpholinomethyl)camptothecin, prepared from 5 in analogy with the conversion of 4 to 21, was dissolved in DMF (25 mL) and treated with Et_3N (0.4 mL), $Pd(OAc)_2$ (8 mg, 0.04 mmol), $(C_6H_5)_3P$ (20 mg, 0.08 mmol), and 99% formic acid (0.08 mL, 2 mmol). The reaction mixture was heated under argon at 60 °C for 6 h, concentrated, and treated with H_2O. Both the desired compound 22 and the major byproduct, 9-methyl-(20*S*)-camptothecin (28), were precipitated (300 mg) and collected by filtration. Purification by flash chromatography (silica gel; 1–2% CH_3OH/CH_2Cl_2) afforded 28 as a colorless solid (45 mg, 13%) and the desired compound 22 as an off-white amorphous solid (93 mg, 20%): ^1H NMR ($CDCl_3/CD_3OD_4$) δ 1.0 (t, 3, J = 7 Hz), 2.0 (q, 2, J = 7 Hz), 2.5 (m, 4), 3.7 (m, 4), 4.0 (s, 2), 5.2 (t, 2), 5.3 (d, 1, J = 16 Hz), 5.7 (d, 1, J = 16 Hz), 7.5 (d, 1), 7.6 (s, 1), 7.7 (d, 1), 8.2 (d, 1), 9.0 (s, 1). Anal. ($C_{25}H_{25}N_3O_5 \cdot 0.5H_2O$) C, H, N.

Trifluoromethanesulfonate of 10-Hydroxy-(20*S*)-camptothecin (23). To a mixture of 10-hydroxycamptothecin (1.44 g, 4.0 mmol) in DMF (40 mL) was added Et_3N (1.2 g, 12 mmol) followed by *N*-phenyltrifluoromethanesulfonimide (2.0 g, 6 mmol). The reaction mixture was heated at 50 °C for 3 h, the solvent was evaporated, and the residue was triturated with water, filtered, and dried. The crude product was obtained as a white solid in theoretical yield; analysis by TLC (silica gel; 5% CH_3OH/CH_2Cl_2) indicated a single spot at R_f 0.4. A small sample was purified by flash chromatography (silica gel; 2% CH_3OH/CH_2Cl_2): mp 145–147 °C; ^1H NMR ($CDCl_3$) δ 1.0 (t, 3, J = 7 Hz), 1.9 (q, 2, J = 7 Hz), 5.3 (s, 2), 5.4 (d, 1, J = 16 Hz), 5.7 (d, 1, 16 Hz), 7.7 (m, 2), 7.8 (d, 1, J = 9 Hz), 8.2 (d, 1, 9 Hz), 8.5 (s, 1); MS m/e (FAB) 497 (M + H), 495 (M - H). Anal. ($C_{21}H_{15}F_3N_2O_7S$) C, H, N.

10-Cyano-(20*S*)-camptothecin (24). A solution of (n-Bu)$_3$SnCN (444 mg, 1.4 mmol) and $Pd[P(C_6H_5)_3]_4$ (276 mg, 0.6 mmol) in 1,2-dichloroethane (20 mL) was heated to reflux under argon for 2 h. Trifluoromethanesulfonate 23 (266 mg, 0.6 mmol) was added and the reaction mixture was heated at reflux for an additional 3.5 h. The reaction mixture was concentrated to one-third its original volume and triturated with an equal volume of Et_2O to afford 183 mg of a yellow solid. Flash chromatography (silica gel; 2% CH_3OH/CH_2Cl_2) yielded the desired cyanide 24 as a light yellow solid: yield 160 mg (67%); ^1H NMR ($CDCl_3/CD_3OD$) δ 1.0 (t, 3, J = 7 Hz), 1.9 (q, 2, J = 7 Hz), 5.3 (d, 1, J = 16 Hz), 5.4 (s, 2), 5.7 (d, 1, J = 16 Hz), 7.7 (s, 1), 7.7–8.4 (m, 3), 8.6 (s, 1). Anal. ($C_{21}H_{15}N_3O_4 \cdot 0.5H_2O$) C, H, N.

10-(Aminomethyl)-(20*S*)-camptothecin (25). 10-Cyanocamptothecin (24) (160 mg, 0.42 mmol) was dissolved in HOAc (45 mL) and treated with activated Raney nickel and the resulting mixture hydrogenated at 10 psi for 7 h. The catalyst was removed by filtration and the solvent was evaporated. Purification of the resulting residue by MPLC (RP-18; 10% CH_3OH/H_2O containing 0.02% HOAc) afforded after lyophilization the desired product 25 (acetate salt) as a light yellow amorphous solid: yield 26 mg (14%); ^1H NMR (D_2O/CD_3OD) δ 1.0 (t, 3, J = 7 Hz), 2.0 (q, 2, J = 7 Hz), 4.3 (s, 2), 5.2 (s, 2), 5.5 (s, 2), 7.5 (s, 1), 8.0 (m, 3), 8.6 (s, 1). Anal. ($C_{21}H_{21}N_3O_4 \cdot HOAc \cdot 6H_2O$) C, H, N.

(20*S*)-Camptothecin-10-phosphonic Acid (26). To solution of 23 (132 mg, 0.3 mmol) in dry DMF (10 mL) under argon was added diethyl phosphite (49.8 mg, 0.36 mmol), diisopropylethylamine (50.7 mg, 0.39 mmol), and $Pd[P(C_6H_5)_3]_4$ (12 mg, 0.009 mmol). The reaction mixture was heated at 75 °C for 5.5 h, and the solvent was evaporated. The residue was purified by flash chromatography (silica gel; 2% CH_3OH/CH_2Cl_2) to afford 10-(diethylphosphono)-(20*S*)-camptothecin as an oil (110 mg, 70%): ^1H NMR ($CDCl_3$) δ 0.9 (t, 3), 1.2 (m, 6), 1.8 (m, 2), 4.1 (m, 4), 5.2 (s, 2), 5.4 (q, 2), 7.6 (s, 1), 7.9–8.6 (m, 4). A solution of this

compound (0.2 g, 0.4 mmol) in 48% HBr (40 mL) was heated at reflux for 7 h, and the solvent was evaporated. The residue was treated with H_2O (10 mL) and filtered, and the filtrate was purified by MPLC (RP-18; 15% CH_3OH in H_2O) to afford **26** as a white solid (125 mg, 73%): ^1H NMR (DMSO-d_6) δ 0.9 (t, 3, J = 7 Hz), 1.9 (q, 2, J = 7 Hz), 5.3 (s, 2), 5.5 (s, 2), 8.1–8.7 (m, 4), 8.9 (s, 1). Anal. ($C_{20}H_{17}N_2O_7P\cdot1.3H_2O$) C, H, N.

4-(Cyanomethyl)-2-nitroacetophenone Ethylene Acetal (31). A solution of o-nitroacetophenone (7.5 g, 0.045 mol), ethylene glycol (6.2 g, 0.1 mol), and *p*-TsOH (300 mg) in dry toluene (200 mL) was refluxed with a Dean–Stark trap for 20 h. The solvent was evaporated, the residue was dissolved in EtOAc, and the EtOAc solution was washed with H_2O. The separated organic layer was dried ($MgSO_4$) and concentrated to afford **30** as an amorphous solid: yield 6.9 g (73%). A small sample was recrystallized from Et_2O/petroleum ether, mp 65–66 °C.

DMSO (40 mL) was deoxygenated (N_2, 0 °C, 2 h) and powdered NaOH was added. The contents were stirred vigorously as a solution of **30** (2.6 g, 0.125 mol) and (methylthio)acetonitrile (1.2 g, 0.13 mol) in deoxygenated DMSO (17 mL) was added dropwise. The reaction mixture was stirred for 18 h under a nitrogen atmosphere and then poured into cold 2 N HCl (125 mL). The resulting solution was extracted with EtOAc (4 × 75 mL) and the organic extract was washed successively with concentrated NaCl (3 × 75 mL) and water (5 × 75 mL) and dried ($MgSO_4$). Concentration of the solvent produced an oil that was purified by flash chromatography (silica gel, CH_2Cl_2) to afford **31** as an oil: yield 70 mg (22%); 38% of the starting ketal was also recovered; ^1H NMR ($CDCl_3$) δ 1.6 (s, 3), 3.3 (s, 2), 3.4–3.8 (m, 4), 7.2–7.6 (m, 3); MS m/e MH^+ 249. Anal. ($C_{12}H_{12}N_2O_4$) C, H, N.

***dl*-10-(Cyanomethyl)-7-methyl-20-deoxycamptothecin (34).** A solution of **31** (70 mg, 0.28 mmol) in dry CH_3OH (15 mL) was treated with 10% Pd/C (50 mg) was subjected to 50 psi of H_2 for 1.5 h. The catalyst was filtered and the filtrate was concentrated to afford **32** as an oil: yield 40 mg. A solution of **32** (0.5 g, 2.3 mmol) and tricyclic ketone **33** (0.4 g, 1.6 mmol) in dry toluene (75 mL) was refluxed for 30 min, pTsOH (0.1 g) was added, and refluxing was continued for 5 h with a Dean–Stark trap. Upon cooling of the reaction mixture, **34** precipitated as a brick-red solid: yield 315 mg (52%). The crude solid was recrystallized from CH_2Cl_2/EtOAc to give an analytical sample of **34** as a pale yellow solid: ^1H NMR ($CDCl_3$) δ 1.1 (t, 3), 2.1 (m, 2), 2.8 (s, 3), 3.6 (t, 1), 4.1 (s, 2), 5.2 (s, 2), 5.5 (q, 2), 7.6–8.4 (m, 4); MS m/e MH^+ 386. Anal. ($C_{23}H_{19}N_3O_3\cdot0.75H_2O$) C, H, N.

***dl*-10-(Cyanomethyl)-7-methylcamptothecin (35).** A mixture of **34** (65 mg, 0.17 mmol), $CuCl_2$ (100 mg), and 40% aqueous dimethylamine in dry DMF (14 mL) was heated at 40 °C as oxygen gas was bubbled into the reaction mixture over a 4-h period. The reaction mixture was concentrated to one-third volume and treated with EtOH to precipitate **35** as a crude solid that was purified by MPLC (silica gel, 2% CH_3OH/CH_2Cl_2): yield 35 mg; ^1H NMR (DMSO-d_6) δ 0.9 (t, 3), 1.8 (m, 2), 2.7 (s, 3), 4.3 (s, 2), 5.3 (s, 2), 5.8 (s, 2), 7.3 (s, 1), 7.6–8.2 (m, 3); MS m/e MH^+ 402. Anal. ($C_{23}H_{19}N_3O_4\cdot2H_2O$) C, H, N.

***dl*-10-(Aminoethyl)-7-methylcamptothecin (36).** A partial solution of **35** (400 mg, 1 mmol) in HOAc (200 mL) was treated with activated Raney Ni (13 mg) and subjected to 5 psi of H_2 for 4.5 h. The catalyst was removed by filtration and the filtrate was concentrated to afford **36** as a crude solid that was purified by MPLC (RP-18 silica gel, 15% CH_3OH/H_2O). The combined product fractions were lyophilized to afford **36** (acetate salt) as a white solid: yield 44 mg; ^1H NMR (D_2O) δ 1.1 (t, 1), 2.0 (m, 2), 2.5 (s, 3), 2.7 (s, 3), 3.2 (m, 2), 3.6 (m, 2), 4.6 (s, 2), 5.3 (s, 2), 7.1 (s, 1), 7.4–8.1 (m, 3). Anal. ($C_{23}H_{23}N_3O_4\cdot HOAc\cdot5H_2O$) C, H, N.

***dl*-7-[2-(*N*-Carbobenzyloxy-*N*-methylamino)ethyl]-20-deoxycamptothecin (40).** A solution containing o-nitroacetophenone (33.0 g, 0.2 mol), methylamine hydrochloride (21.2 g, 0.26 mol), and paraformaldehyde (8 g, 0.88 mol) in 95% EtOH (65 mL) containing concentrated HCl (0.5 mL) was heated at reflux for 30 h after which time the solution was cooled and poured into 500 mL of acetone. The excess methylamine hydrochloride was filtered and the filtrate was concentrated to afford **37** as a crude oil: yield 50 mg.

In a flame-dried flask, **37** (50 g, 0.2 mol) was dissolved in CH_2Cl_2 (400 mL), the solution was cooled to 0 °C, and Et_3N (42 g, 0.41 mol) was added followed by a dropwise addition of 40 mL of benzyl chloroformate. The dark reaction mixture was stirred at 0 °C for 1 h, the reaction mixture was allowed to warm to room temperature, and stirring was continued for an additional 5 h. The solvent was evaporated, the residue was dissolved in EtOAc, and the resulting solution was washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated to afford crude **38**, which was purified by flash chromatography (silica gel, 20–30% hexane/CH_2Cl_2 to afford **38** as a light yellow oily residue: yield 2.5 g; ^1H NMR ($CDCl_3$) δ 2.3 (m, 2), 2.9 (s, 3), 3.6 (t, 3), 5.1 (s, 2), 7.3 (s, 8), 7.6 (m, 1).

A solution of **38** (2.2 g, 6 mmol) in 95% EtOH (100 mL) was treated with sodium dithionite (5.2 g, 0.03 mol) and the resulting solution heated at reflux for 3 h while the pH was kept near 7.0 by dropwise addition of 5% $NaHCO_3$ when appropriate. After cooling, the solvent was removed and the residue was triturated with EtOAc and H_2O. The layers were separated, and the organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (silica gel, CH_2Cl_2). The product fractions were combined and evaporated to give **39** as an off-white solid: yield 0.7 g; ^1H NMR ($CDCl_3$) δ 2.3 (m, 2), 2.9 (s, 3), 3.5 (m, 3), 5.1 (s, 2), 6.6 (d, 2), 7.3 (s, 9).

A solution of **39** (0.35 g, 1.1 mmol) and the tricyclic ketone **33** (0.18 g, 1.0 mmol) in 40 mL of toluene was heated at reflux for 30 min, *p*-TsOH (25 mg) was added, and the refluxing was continued for 9 h with a Dean–Stark trap. The reaction mixture was concentrated to a dark oil and treated with EtOAc, the dark residue was removed by filtration, and the filtrate was concentrated to give **40** as a dark solid: yield 300 mg. A sample was purified by flash chromatography (silica gel, 1% CH_3OH/CH_2Cl_2): ^1H NMR ($CDCl_3$) δ 1.0 (t, 3), 2.1 (m, 2), 2.9 (s, 3), 3.5 (m, 4), 5.0–5.3 (m, 4), 5.5 (q, 2), 7.1–8.3 (m, 10).

***dl*-7-[2-(*N*-Carbobenzyloxy-*N*-methylamino)ethyl]camptothecin (41).** The crude solid **40** (300 mg, 0.57 mmol) was dissolved in 30 mL of DMF and treated with 40% aqueous dimethylamine. Oxygen gas was bubbled through the dark solution for 6.5 h, the solvent was evaporated, and the resulting dark oil residue was triturated with CH_3OH and filtered. After drying, **41** was obtained as a yellow solid: yield 270 mg. A small sample was purified by MPLC (silica gel, 1% CH_3OH/CH_2Cl_2): ^1H NMR ($CDCl_3$) δ 1.0 (t, 3), 1.9 (m, 2), 2.9 (s, 3), 3.5 (m, 4), 5.1 (s, 2), 5.3 (s, 2), 5.5 (q, 2), 7.2–8.3 (m, 9); MS m/e MH^+ 540.

***dl*-7-[2-(*N*-Methylamino)ethyl]camptothecin (42).** A solution of **41** (200 mg, 0.37 mmol) dissolved in 55 mL of glacial HOAc (55 mL) was treated with 10% Pd/C (100 mg) and subjected to 10 psi of H_2 for 6 h. The catalyst was removed by filtration, the filtrate was concentrated, and the residue was triturated with H_2O. The insoluble starting material **41** (85 mg, 28%) was removed and the filtrate concentrated to a small volume. The desired product **42** was purified by MPLC (RP-18 silica gel, 10% CH_3OH/H_2O) to afford **42** as a white solid: yield 80 mg; ^1H NMR (CD_3OD) δ (t, 3), 1.9 (m, 2), 2.5 (s, br, 3), 3.0 (m, 2), 3.5 (m, 2), 5.2 (s, 2), 5.4 (q, 2), 7.6 (s, 1), 7.7–8.3 (m, 4). Anal. ($C_{23}H_{23}N_3O_4\cdot1.8HOAc\cdot3H_2O$) C, H, N.

Registry No. 1, 7689-03-4; **2**, 19635-09-7; **2** (R_1 = AcO), 19685-11-1; **4**, 123948-87-8; **4**·HOAc, 123948-88-9; **4**·HCl, 119413-54-6; **5**, 123948-89-0; **5**·HOAc, 123948-90-3; **6**, 123948-91-4; **6**·1.5HOAc, 130064-58-3; **7**, 123949-02-0; **8**, 123948-93-6; **9**, 123949-17-7; **9**·HOAc, 130064-59-4; **9**·HCl, 123949-00-8; **10**, 123949-18-8; **10**·HOAc, 130064-60-7; **10**·HCl, 123949-03-1; **11**, 123948-95-8; **11**·HOAc, 123948-96-9; **12**, 123949-19-9; **12**·2HCl, 123949-06-4; **13**, 123948-98-1; **14**, 123948-99-2; **15**, 123949-20-2; **15**·HCl, 123949-04-2; **16**, 123949-01-9; **17**, 123949-21-3; **17**·HCl, 123949-05-3; **18**, 123949-14-4; **19**, 123949-22-4; **19**·HOAc, 130064-61-8; **20**, 130064-45-8; **21**, 123969-94-8; **21**·HOAc, 130064-62-9; **22**, 123969-95-9; **23**, 123949-09-7; **24**, 123949-10-0; **25**, 123949-12-2; **25**·HOAc, 123949-13-3; **26**, 130064-46-9; **26** diethyl ester, 130064-65-2; **27**, 91421-43-1; **28**, 123949-23-5; **29**, 577-59-3; **30**, 37456-50-1; **31**, 130064-47-0; **32**, 130064-48-1; *dl*-+33, 73428-00-9; *dl*-+34, 130064-49-2; *dl*-+35, 130064-50-5; *dl*-+36, 130064-51-6; *dl*-+36·HOAc, 130064-63-0; **37**·HCl, 130064-52-7; **38**, 130064-53-8; **39**, 130064-54-9; *dl*-**40**, 130064-55-0; *dl*-**41**, 130064-56-1; *dl*-**42**, 130064-57-2; *dl*-**42**·1.8HOAc, 130064-64-1; *dl*-**43**, 130144-33-1; *dl*-**44**, 130144-34-2; topoisomerase I, 80449-01-0.