Camptothecin and Minor-Groove Binder Hybrid Molecules: Synthesis, Inhibition of Topoisomerase I, and Anticancer Cytotoxicity *in Vitro*

Rulin Zhao,[†] Naim H. Al-Said,[†] Daniel L. Sternbach,[‡] and J. William Lown*,[†]

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2, and Glaxo-Wellcome (US), P.O. Box 13358, Research Triangle Park, North Carolina 27709

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The synthesis, characterization, inhibitory activity against topoisomerase I, and biological evaluation of a series of 14 camptothecin derivatives of polypyrrolecarboxamide (lexitropsin) conjugates of two structural classes: (A) camptothecin−NHCO−lexitropsin **44**−**51** and (B) camptothecin−CONH−lexitropsin **38**−**43** are described. All 16 compounds tested, 14 conjugates plus two functionalized camptothecin controls, inhibit topoisomerase I in the concentration range 1.12−16.6 μM that divide into three distinct categories based on activity. The most active enzyme inhibitors belong to structure class A with either cationic dimethylaminium or neutral amide end groups. Generally class B conjugates are less effective in inhibiting topoisomerase I. Cytotoxic potencies of the drugs was tested against four representative human tumor cell lines: SKOV3, SKLVB, HT29, and KB. All 16 drugs gave measurable IC_{50} values against the KB cell line and fell into two categories with IC_{50} values of 0.049−0.66 μM (largely structure class B) and 1.0−48 μM (largely class A). Thus the class B conjugates, while less potent against the enzyme, contain two of the most potent drugs, **38** and **39**, against KB cell lines. In contrast, in the case of the cell lines SKOV3 and HT29 there was a general correlation between the better topoisomerase inhibitors and their cell cytotoxicities.

Introduction

Camptothecin (**I**), isolated from *Camptotheca accuminata* (Nyssacease) by Wall and co-workers in 1966,[1] exhibits strong antitumor activity in several experimental tumor models including human colon, lung, and mammary tumor lines. However, severe side effects including myelosuppression, vomiting, and severe hemorrhagic cystitis led to the discontinuation of phase II trials.[2] Interest in camptothecin was revived in 1985, when camptothecin was tested against purified mammalian DNA topoisomerase and found to be a specific topoisomerase I inhibitor without affecting topoisomerase II, unlike many other antitumor agents that inhibit topoisomerase II.[3] Topoisomerase I is an enzyme that relaxes supercoiled DNA by a nicking resealing mechanism involving one strand only.[4]

It was found that camptothecin does not bind to topoisomerase I or to DNA alone but forms a three-component complex by binding to a covalent DNA−topoisomerase I complex, and thus inhibits DNA relaxation.[3,5] Studies of camptothecin derivatives showed a good correlation between topoisomerase I inhibition and antitumor activity.[6] Previous studies of ours have established the enhanced anticancer potencies resulting from the introduction of certain heterocyclic 10 substituents in camptothecin.[6c] An opportunity therefore exists for development of novel types of potent cytotoxic agents based on enhanced topoisomerase I inhibition.

The DNA minor groove binder, distamycin, also inhibits the enzyme catalytic activity at the high-affinity topoisomerase I-preferred binding sequence.[7] In contrast to camptothecin, this inhibition is not associated with cleavable complex formation, and is probably due to distamycin binding directly at the enzyme cleavage sites.

A promising initial strategy is to couple camptothecin to lexitropsin carriers for the following reasons. A number of minor-groove binding drugs (distamycin, Hoechst 33258 and DAPI) inhibit the catalytic activity of isolated topoisomerases (both I and II), while at low concentrations distamycin and netropsin were also able to stimulate enzymatic activity.[8] These data suggest that topological enzymes read DNA structure at least in part through the minor groove. Unlike many of the drugs known to interact with topological enzymes, none of the minor-groove binders to date induced cleavable complex formation. Altering the normal association of the topological enzyme with its DNA substrate could influence induction of the cleavable complex by topoisomerase poisons. Minor-groove binding effects on isolated enzymes parallel the influence of such agents on induction of cleavable complex formation in nuclei by topoisomerase poisons including camptothecin.[8,9] Pommier has published evidence demonstrating the ability of distamycin to prevent cleavable complex formation at minor-groove drug binding sites.[10] It has been proposed that modification of enzymatic activity occurs when distamycin acts to displace the enzyme from its binding sites.[10] All of these observations serve to support the rationale for enhancement of camptothecin potency by linking it to minor groove binding agents.

Earlier we have reported that bis-linked lexitropsins designed to address the phasing problem have proven to be potent inhibitors of topoisomerases.[11] We describe here the synthesis, inhibition of topoisomerase I, and cytotoxicity *in vitro* of certain hybrid molecules containing camptothecin and minor-groove binders. In addition, pharmacological factors concerned with drug solu-

* Address correspondence to: J. W. Lown, Department of Chemistry, University of Alberta, Edmonton, AB, Canada T6G 2G2. Tel: 403-492-3646. Fax: 403-492-8231.
[†] University of Alberta.
[‡] Glaxo-Wellcome (US).
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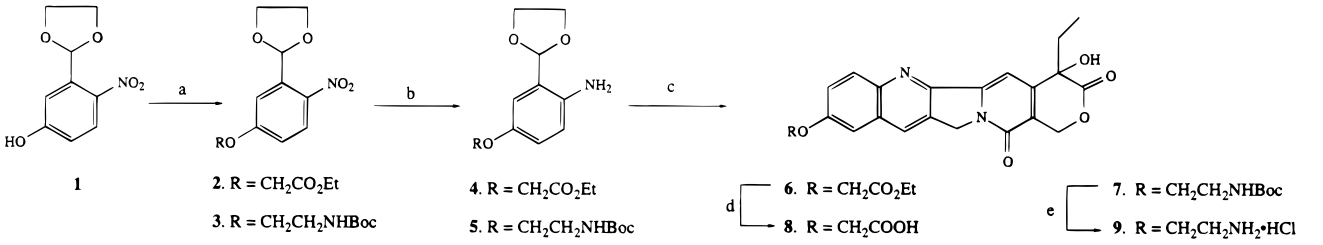
Scheme 1. Synthesis of Functionalized Camptothecin[a]



[a] Reaction conditions: (a) RBr, K_2CO_3, CH_3CN; (b) H_2, Raney Ni, EtOH; (c) II, TsOH, toluene; (d) K_2CO_3, EtOH; (e) dry HCl, MeOH.

bility and cellular uptake required incorporation of the methoxymethyl group into the heterocycles of lextropsin carriers. This required the development of the appropriate heterocyclic chemistry as shown in the following reaction schemes.

Synthesis

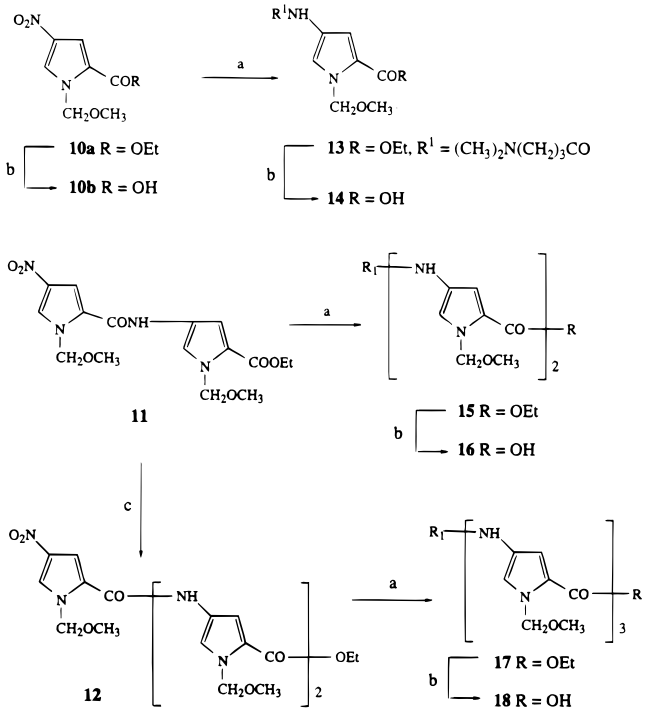
The synthesis of the hybrid compounds required the preparation of camptothecin derivative bearing an amino or carboxylic acid moiety to provide points of attachment of the carrier. One of the most adaptable and useful synthetic strategies for the present purposes is that of Wall and co-workers.[12] The overall strategy is that of Friedlander condensation of tricyclic ketone (**II**)[13] with amino acetals (**4**) to give the camptothecin pentacyclic structure (**6**) (Scheme 1). Amino acetals (**4**, **5**) were prepared from **1**[14] in two steps. Thus, **1** was condensed with ethyl bromoacetate (and *tert*-butyl *N*-(2-bromoethyl)carbamate) in the presence of potassium carbonate in dimethylformamide, followed by reduction of the nitro group in the presence of Raney Ni under hydrogen at atmospheric pressure to give **4**. Compound **6** was hydrolyzed to the acid **8** following the procedure described by Wani et al.[12a] The masked amine **7** was prepared in a similar fashion. Compound **7** was deprotected to amine **9** in dry HCl/MeOH solution. The two functionalized camptothecin derivatives (**8** and **9**) now afforded the opportunity to be coupled with lexitropsin carriers to form the desired hybrid molecules.

The syntheses of the polypyrrolecarboxamide−lextropsin carriers are essentially based on our method of syntheses of netropsin and distamycin.[15,16] The syntheses of **12**−**18** are shown in Scheme 2. Hydrogenation of **11** and condensation of 1-(methoxymethyl)nitropyrrole−acyl chloride in the presence of Et_3N afforded **12** in 67% yield.[15b] Compounds **10**,[15b] **11**, and **12** were hydrogenated to give the corresponding unstable amines which were treated immediately with 4-(dimethylamino)butanoic acid to give **13**, **15**, and **17**, respectively. The latter three compounds were then hydrolyzed under basic conditions to afford acids **14**, **16**, and **18**, respectively.

Scheme 3 shows the synthesis of **20**−**25**. Acylation of the 4-amino group in **19**[15b] with propionyl chloride afforded **20** which was hydrolyzed under basic conditions to gave the acid **21**. Compound **21** was allowed to condense with **19** in the presence of HOBt and DCC to give **22**, which was converted to the acid **23** following basic hydrolysis and neutralization. In a similar manner compound **25** was prepared from **19** and **23**.

The synthesis of **27** and **28** is summarized in Scheme 4. Catalytic reduction of **26**[15b] and then treatment with 1-(methoxymethyl)-4-nitropyrrole−acyl chloride afforded

Scheme 2. Synthesis of Lexitropsin Carriers[a]



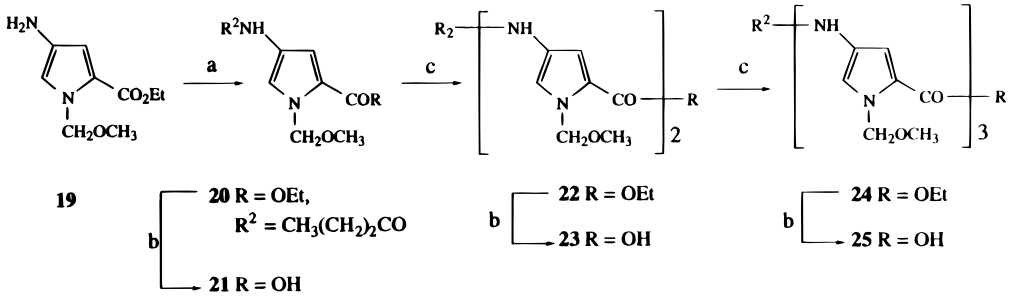
[a] Reaction conditions: (a) H_2, Pd/C, THF, then $(CH_3)_2N(CH_2)_3COOH$, Im_2CO, THF; (b) 2 N NaOH, $MeOH-H_2O$ (1:1), then 1 N HCl; (c) H_2, Pd/C, THF; **10b**, $SOCl_2$, THF then THF, Et_3N.

compound **27**. In a similar manner, **28**[15b] was also prepared from **27**.

Scheme 5 shows the synthesis of lextropsin carriers containing imidazole moieties. Intermediate **33** was prepared from *N*-(methoxymethyl)imidazole (**30**) which was synthesized by alkylation of imidazole with chloromethyl methyl ether in EtOH in the presence of EtONa. Reaction of **30** with trichloroacetyl chloride in CH_2Cl_2 afforded **31**. Nitration of **31** afforded **32**, which was allowed to condense with EtOH to give the desired **33**. Compound **33** was hydrogenated to give the corresponding unstable amine, which was acylated immediately with butyl chloride to generate **34** and then hydrolyzed to give acid **35**. Acid **35** was coupled with the reduction product of **32** to afford **36** and then hydrolyzed to give acid **37**.

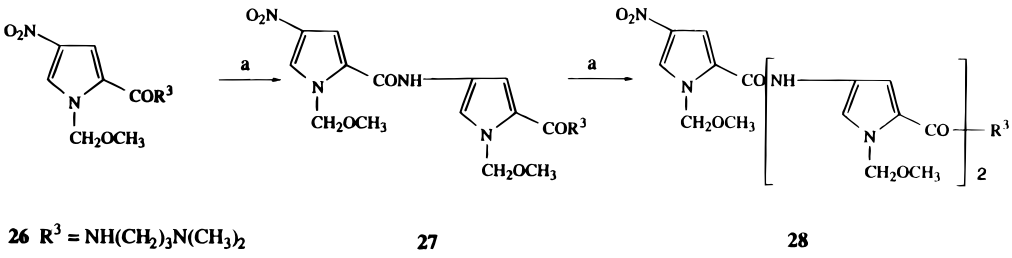
Synthesis of the final hybrid molecules (**38**−**51**) was accomplished by using dicyclohexylcarbodiimide (DCC) and HOBt or EDCI as the coupling agent (Scheme 6). Thus, the acid groups in camptothecin or the lexitropsin were first condensed with HOBt to give the corresponding active ester which were allowed to react with the amine group in the lexitropsin or camptothecin to afford hybrid molecules. The polar hybrid compounds contain-

Scheme 3. Elaboration of Polypyrrolecarboxamide Moieties[a]



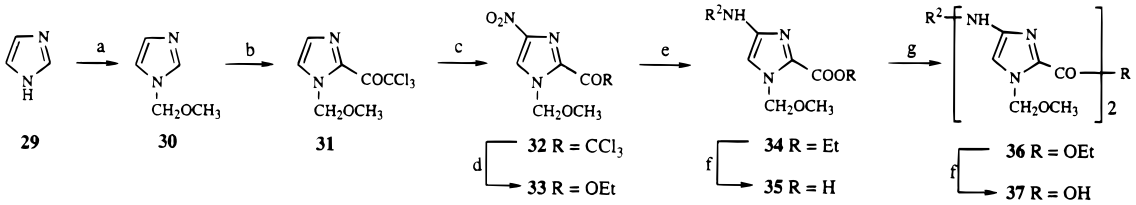
[a] Reaction conditions: (a) $CH_3(CH_2)_3COCl$, Et_3N; (b) NaOH, $MeOH/H_2O$; (c) **19**, EDCl, THF/DMF.

Scheme 4. Synthesis of Intermediate Nitrolexitropsin[a]



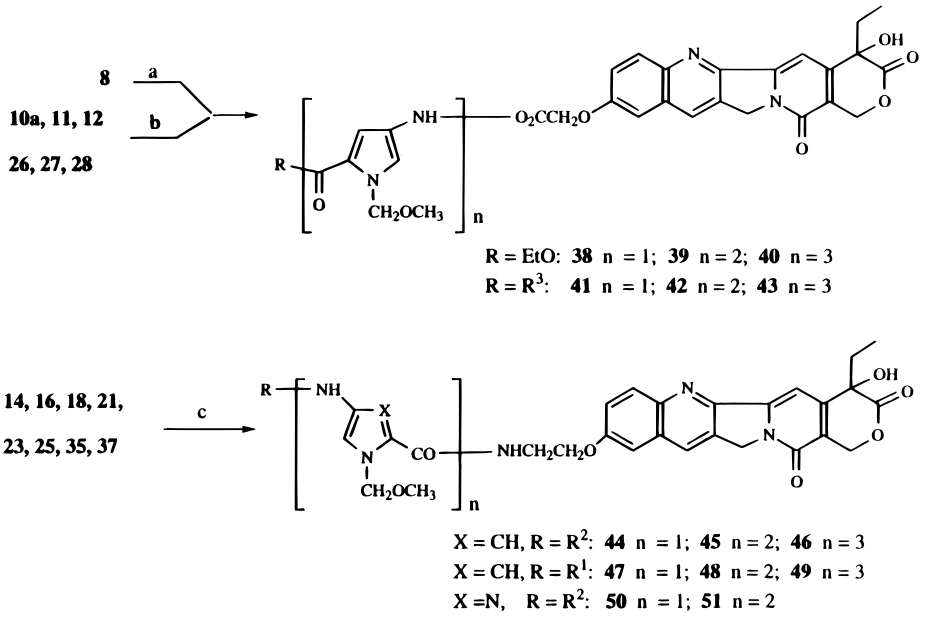
[a] Reaction conditions: (a) H_2, Pd/C, MeOH; **10b**, $SOCl_2$, THF then Et_3N, THF.

Scheme 5. Synthesis of Lexitropsincarboxylic Acid[a]



[a] Reaction conditions: (a) EtONa, EtOH then $ClCH_2OCH_3$, THF; (b) Cl_3CCOCl, Et_3N, CH_2Cl_2; (c) HNO_3, CH_2Cl_2; (d) NaH, EtOH; (e) H_2, Pd/C, THF, then $CH_3CH_2CH_2COCl$, Et_3N; (f) NaOH, $MeOH/H_2O$, then 1 N HCl; (g) $SOCl_2$, THF, then reduction product of **33**, Et_3N.

Scheme 6. Synthesis of Final Camptothecin−Lexitropsin Conjugates[a]



[a] Reaction conditions: (a) DCC, HOBt, DMF/THF; (b) H_2, Pd/C, MeOH or THF; (c) DCC, HOBt, DMF/THF then **9**, Et_3N.

ing a dimethylamino group were purified by TLC on silica gel with MeOH as eluent and Et_3N as a coeluent.

Results and Discussion

Topoisomerase I Inhibition. The inhibitory activity of these novel conjugates toward the topoisomerase I relaxation reaction was evaluated by agarose gel electrophoresis[16] (Table 1).

The ability of these compounds to inhibit topoisomerase I was quantified by measuring the action on supercoiled pBR322 DNA substrate as a function of increasing concentration of the ligands. Supercoiled

Table 1. Inhibition of Topoisomerase I and Cytotoxicity in Vitro

		in vitro cytotoxicity: IC_{50} (μM)[b]			
compound	topoisomerase I[a] IC_{50} (μM)	SKOV3	SKVLB	HT29	KB cells
8	1.118	1.2	2.1	2.8	0.250
9					0.175
38	4.842	3.4	9.7	>10	0.049
39	4.290	1.8	>10	>10	0.093
40	3.882	3.0	5.8	6.1	0.571
41	4.597	>10	>10	>10	48.08
42		>10	>10	>10	0.663
43	11.918	>10	>10	>10	5.50
44		5.1	>10	8.1	15.29
45	16.618	>10	>10	>10	3.25
46		3.6	>10	7.1	5.09
47	2.602	1.5	3.5	3.4	5.68
48	4.818	4.3	>10	6.2	6.29
49	1.973	9.7	>10	>10	1.00
50	2.382	1.3	>10	6.9	1.00
51	2.417	3.2	>10	6.0	3.08
distamycin	2.7[c]		>10	54.7	8.4
netropsin	62[d]		>10	44.7	47.1
camptothecin	0.70	0.051	0.053	0.0878	0.008
doxorubicin		0.0251	6.54	0.28	0.015

[a] IC_{50} (μM) measures concentration of drug required to inhibit supercoil relaxation property of topoisomerase I by 50%. [b] IC_{50}: drug concentration that reduced the viability of the cell population by 50%. Values given are an average of three or more determinations. [c] Reference 19. [d] Reference 20.

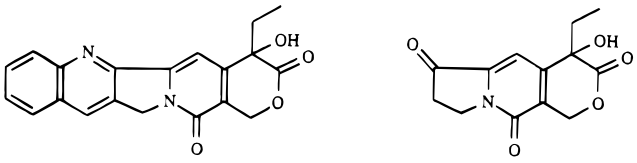
DNA was converted to relaxed DNA in the presence of topoisomerase I. For comparison, camptothecin, a recognized inhibitor of topoisomerase I, was included. As expected, the supercoiled DNA remaining in the assay system increased with the increasing concentration of camptothecin. The percentage of supercoiled DNA remaining was determined by densitometry from which IC_{50} values (concentration of drug required to inhibit the relaxation of supercoiled DNA in the presence of topoisomerase I) were determined (Table 1). All 11 camptothecin conjugates together with the chromophores **8** and **9** tested in the assay inhibit the relaxation of supercoiled DNA by the topoisomerase I in the IC_{50} range 1.12−16.6 μM compared with camptothecin itself under these conditions of 0.70 μM. Doxorubicin as a control was, as expected, ineffective in this assay. The order of the inhibitory activities against topoisomerase I is **8** > **49** > **50** > **51** > **47** > **40** > **39** > **41** > **48** > **38** > **43** > **45**. These divide into three distinct categories in terms of the ranges of inhibitory activity, viz. **8**, **49**, **50**, **51**, **47** and then **40**, **39**, **41**, **48**, **38** and finally the least active pair of **43** and **45**. Compounds **8**, **49**, **50**, **51**, and **47** are more potent than or comparable with distamycin but much more potent inhibitors than netropsin. Structurally the camptothecin−lexitropsin conjugates are of two types: (A) camptothecin−NHCO−lexitropsin (**44**−**51**) and (B) camptothecin−CONH−lexitropsin (**38**−**43**). The most active enzyme inhibitors **49**, **50**, **51**, and **47** all belong to structural type A with either a cationic dimethylaminium end group (**49** and **47**) or an amide end group (**50** and **51**) on the lexitropsin carrier. The next group of somewhat less active topoisomerase I enzymatic inhibitors (**40**, **39**, **41**, **48**, and **38**) with the exception of **48** all belong to structure class B. Within this group the two most active inhibitors **40** and **39** have a neutral ester end group on the lexitropsin moiety. The significant effect of the two structural types of conjugates may be seen with **49** (IC_{50} = 1.97, structure class A) and **43** (IC_{50} = 11.4, structure class B). It is also noteworthy that the only examples of conjugates in which the pyrrole moiety in the carrier is replaced by imidazoles **50** and **51**, both in structure class A, are among the most potent enzyme inhibitors.

Cytotoxicities. The camptothecin−lexitropsin conjugates were also evaluated for cytotoxic activity against four tumor cell lines: SKOV3, SKVLB, HT29, and KB (Table 1). SKOV3 is a human adenocarcinoma of the ovary and HT29 is a human adenocarcinoma of the colon, while KB are human naseopharengeal tumor cells. These cell lines were selected by Glaxo-Wellcome (US) as being representative of and of known predictive value for camptothecin compounds. The most detailed and informative structure−activity correlations could be attempted with the KB cell data since all 16 agents gave measurable IC_{50} values in contrast to, *e.g.*, the SKVLB data where only four drugs exhibited measurable IC_{50} values significantly below 10 μM. In the case of the KB data the drugs fell into two classes with IC_{50} values of 0.049−0.66 μM (**38**, **39**, **4**, **8**, **40**, and **42**) and of 1.0−48 μM (**49**, **50**, **51**, **45**, **46**, **43**, **47**, **48**, **44**, and **41**). With the exception of **9**, all of the former and more potent drugs belong to conjugate structure class B, whereas with the exception of **41**, those in the less potent category all belong to structure class A. The two most potent drugs against KB cells, **38** and **39** (IC_{50} values of 0.049 and 0.093 μM, respectively), bear neutral terminal groups on the lexitropsin moiety, whereas the least potent drug, **41** (IC_{50} = 48 μM) bears a cationic terminus, suggesting the effects of cationic charge on decreasing cellular uptake.[18] With SKOV3 cells cytotoxic potencies range from 1.2−9.7 μM while for HT29 cells the range is 2.8−8.1 μM. In both cases and, with the exception of only **38**, **39**, and **40**, in a total of 18 sets of data all the active compounds belong to conjugate structure class A. Thus it would appear that, given an admittedly small number of cell types tested, there is a camptothecin−lexitropsin conjugate structural type preference A or B, depending on the type of tumor cells. The main conclusions that can be drawn are (a) structural class B, while less potent against the enzyme, are more potent against KB cell lines and (b) for *both* classes A and B there is a reasonable correlation of enzyme activity with potency against SKOV3 and HT29 cell lines.

Experimental Section

Chemistry. Melting points were determined using an Electrohome apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker WH-200 spectrometer. High-resolution mass spectra (FAB-HRMS) were recorded on a modified MS50 mass spectrometer equipped with a VG 11-250J data system. Accurate masses were calculated interactively with the data system using a reference (such as CsI in glycerol) peak. Analytical thin layer chromatography was performed on silica-coated plastic plates (silica gel 60 F-254, Merck) and visualized under UV light. Preparative separations were performed by flash chromatography on silica gel (Merck, 70−230 or 230−400 mesh). Tetrahydrofuran was dried by distillation from sodium benzophenone ketyl. Dimethylformamide and triethylamine were dried over molecular sieves (4A) before use. The above solvents were stored over molecular sieves (4A). All other solvents were used as received and were reagent grade where available.

5-[(Carbethoxymethyl)oxy]-2-nitrobenzaldehyde Ethylene Acetal (2). To a solution of **1** (2.11 g, 10 mmol) in 100 mL of CH_3CN was added ethyl bromoacetate (1.83 g, 11 mmol) and K_2CO_3 (2.07 g, 15 mmol). The reaction mixture was



I II

Figure 1. Structure of camptothecin (**I**) and tricyclic ketone (**II**).

refluxed for 1 h. The solution was extracted with $CHCl_3$, and the extract was washed successively with aqueous $NaHCO_3$ solution and brine. After evaporation of the solvent in vacuo and purification on a silica gel column eluting with EtOAc:Hex (1:1) afforded **2** (2.90 g, 97% yield) as a yellow solid: mp 60 °C; ^{1}H-NMR ($CDCl_3$) δ 7.95 (d, J = 7.5 Hz, 1H), 7.25 (d, J = 3 Hz, 1H), 6.87 (dd, J = 7.5, 3 Hz, 1H), 6.48 (s, 1H), 4.65 (s, 2H), 4.25 (q, J = 7 Hz, 2H), 3.98 (m, 4H), 1.25 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{13}H_{15}NO_7$ 297.0848, found 297.0827 (M^+, 32). Anal. ($C_{13}H_{15}NO_7$) C, H, N.

5-[2-[(*tert*-Butoxycarbonyl)amino]ethoxy]-2-nitrobenzaldehyde Ethylene Acetal (3). Compound **3** was synthesized in 85% yield from **1** and *tert*-butyl *N*-(2-bromoethyl)carbamate using a procedure similar to that described for above **2**: mp 56 °C; ^{1}H-NMR ($CDCl_3$) δ 8.02 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 3 Hz, 1H), 6.90 (dd, J = 7.5, 3 Hz, 1H), 6.52 (s, 1H), 4.95 (bs, 1H), 4.08 (m, 6H), 3.55 (m, 1H), 1.42 (s, 9H); EIMS calcd for $C_{16}H_{22}N_2O_7$ 354.1427, found 354.10 (M^+, 4). Anal. ($C_{16}H_{22}N_2O_7$) C, H, N.

5-[(Carbethoxymethyl)oxy]-2-aminobenzaldehyde Ethylene Acetal (4). A solution of **2** (2.97 g, 10 mmol) in 100 mL of EtOH was hydrogenated (55 psi) in the presence of Raney nickel (5 g) for 12 h. The catalyst was removed by filtration, and the solvent was removed in vacuo to yield **4** as an oil (2.54 g, 95% yield): ^{1}H-NMR ($CDCl_3$) δ 6.90 (d, J = 3 Hz, 1H), 6.72 (dd, J = 7, 3 Hz, 1H), 6.52 (d, J = 7 Hz, 1H), 5.73 (s, 1H), 4.48 (s, 2H), 4.18 (q, J = 7 Hz, 2H), 3.98 (m, 4H), 3.82 (bs, 2H, NH_2), 1.20 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{13}H_{17}NO_5$ 267.1106, found 267.1101 (M^+, 78).

5-[2-[(*tert*-Butoxycarbonyl)amino]ethoxy]-2-aminobenzaldehyde Ethylene Acetal (5). Compound **5** was prepared in 91% yield from **3** in a manner similar to that described for **4** above: ^{1}H-NMR (CH_3OH-d_4) δ 6.90 (d, J = 3 Hz, 1H), 6.71 (d, J = 7.5 Hz, 1H), 6.78 (dd, J = 7.5, 3 Hz, 1H), 5.75 (s, 1H), 4.02 (m, 4H), 3.90 (t, J = 7 Hz), 3.37 (t, J = 7 Hz, 2H), 1.42 (s, 9H); EIHRMS calcd for $C_{16}H_{24}N_2O_5$ 324.1685, found 324.1682 (M^+, 27).

10-[(Carbethoxymethyl)oxy]-20(*R,S*)-camptothecin (6). A mixture of **4** (400 mg, 1.5 mmol) and the tricyclic ketone **II** (Figure 1) (263 mg, 1 mmol) in toluene (30 mL) was refluxed under N_2 for 5 min in a flask equipped with a Dean−Stark trap. *p*-Toluenesulfonic acid (5 mg) was then added, and refluxing was continued for an additional 1 h. The solvent was removed in vacuo, and the residue was chromatographed eluting with EtOAc:hex (4:1) to yield **6 (**288 mg, 64% yield) as a yellow solid: mp 248−250 °C; ^{1}H-NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.07 (d, J = 8 Hz, 1H), 7.52 (dd, J = 8, 2 Hz, 1H), 7.49 (d, J = 2 Hz, 1H), 7.28 (s, 1H), 6.50 (s, 1H, OH), 5.38 (s, 2H), 5.21 (s, 2H), 4.98 (s, 2H), 4.20 (q, J = 7 Hz, 2H), 1.86 (m, 2H), 1.22 (t, J = 7 Hz, 3H), 0.94 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{24}H_{22}N_2O_7$, 450.1427, found 450.1423 (M^+, 88). Anal. ($C_{24}H_{22}N_2O_7 \cdot 0.5H_2O$) C, H, N.

10-[2-[(*tert*-Butoxycarbonyl)amino]ethoxy]-20(*R,S*)-camptothecin (7). Compound **7** was prepared in a manner similar to that described for **6** above from compound **5** (534 mg, 1.5 mmol) and tricyclic ketone **II** (263 mg, 1 mmol) in 61% yield: mp 238 °C; ^{1}H-NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.02 (d, J = 9 Hz, 1H), 7.44 (m, 2H), 7.26 (s, 1H), 7.12 (t, J = 6 Hz, 1H), 6.50 (s, 1H, OH), 5.40 (s, 2H), 5.21 (s, 2H), 4.16 (t, J = 6 Hz, 2H), 3.42 (m, 2H), 1.95 (m, 2H), 1.42 (s, 9H), 0.94 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{27}H_{29}N_3O_7$ 507.2005, found 507.2003 (M^+, 3.94). Anal. ($C_{27}H_{29}N_3O_7$) H, N; C: calcd, 63.90; found 63.03.

10-[(Carboxymethyl)oxy]-20(*R,S*)-camptothecin (8). Compound **8** was prepared from **6** following the reported procedure:[12a] mp 250−265 °C; ^{1}H-NMR (DMSO-d_6) δ 8.45 (s, 1H), 8.00 (d, J = 8 Hz, 1H), 7.45 (m, 2H), 7.22 (s, 1H), 7.10−7.60 (bs, 2H), 5.40 (s, 2H), 5.18 (s, 2H), 4.90 (s, 2H), 1.91 (m, 2H), 0.94 (t, J = 7 Hz, 3H); FAB-HRMS calcd for $C_{22}H_{18}N_2O_7H$ 423.1192, found 423.1203 (MH^+, 17). Anal. ($C_{22}H_{18}N_2O_7 \cdot H_2O$) C, H, N.

10-(2-Aminoethoxy)-20(*R,S*)-camptothecin (9). Compound **7** (25.3 mg, 0.05 mmol) was dissolved in a solution of dry HCl in methanol (5%, 20 mL). After 30 min, absolute ether (50 mL) was added, and the precipitated hydrochloride **9** was collected and recrystallized from MeOH−ether to give **9** (21.8 mg, 98% yield): mp 245−255 °C; ^{1}H-NMR (DMSO-d_6) δ 8.60 (s, 1H), 8.19 (bs, 3H), 8.10 (d, J = 8 Hz, 1H), 7.57 (m, 2H), 7.19 (s, 1H), 6.80−6.40 (bs, 1H, OH), 5.42 (s, 2H), 5.28 (s, 2H), 4.36 (t, J = 6 Hz, 2H), 3.34 (m, 2H), 1.89 (m, 2H), 0.85 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{22}H_{21}N_3O_5$ 407.1481, found 407.1472 (M^+, 42). Anal. ($C_{22}H_{21}N_3O_5 \cdot 2.25HCl$) C, H, N.

Ethyl 1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[1-(methoxymethyl)-4-nitropyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxylate (12). A stirred solution of **11** (3.8 g, 10 mmol) and Pd/C (10%, 0.3 g) in ethanol (100 mL) was purged with nitrogen. After 10 min the solution was stirred under a hydrogen atmosphere for 12 h. The solution was filtered through Celite, the filtrate was concentrated, and the residue was lyophilized with THF. The residue was dissolved in THF (100 mL) and cooled to −15 °C, and diisopropylethylamine (1.3 mL) was added. Separately, a solution of 1-(methoxymethyl)-4-nitropyrrole-2-carboxylic acid (**10b**) (2.2 g, 11 mmol) in THF (10 mL) was heated under reflux with $SOCl_2$ (5 mL) for 30 min. The solvent and the excess of $SOCl_2$ were removed in vacuo, and the evaporation was repeated with some THF. The residual acid chloride was dissolved in THF and added dropwise to the solution of amine prepared above. The reaction mixture was stirred for 1 h at room temperature, the solvent was removed, and the residue was purified on silica gel (flash chromatography, 50% ethyl acetate in hexane) to afford **12** (4.94 g, 93% yield): mp 206 °C; IR (KBr) 3400, 3360, 1698, 1671, 1657, 1563, 1545, 1287, 1306 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 10.45 and 10.20 (2s, 1H each), 8.38, 7.47, 7.13 and 7.02 (4d, J = 2 Hz each, 1H each), 7.64 (d, J = 2 Hz, 2H), 5.75, 5.66 and 5.58 (3s, 2H each), 4.41 (q, J = 7 Hz), 3.24, 3.18, and 3.16 (3s, 3H each), 1.27 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{23}H_{28}N_6O_9$ 532.1917, found 532.192 6 (M^+, 100). Anal. ($C_{23}H_{28}N_6O_9$) C, H, N.

Ethyl 1-(Methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxylate (13). A solution of **10** (0.32 g, 1.4 mmol) and 5% Pd/C (20 mg) in a mixture of EtOH and ethyl acetate (1:1, 30 mL) was stirred under a hydrogen atmosphere. After the completion of the hydrogenation (4 h), the mixture was filtered through Celite and the filtrate was evaporated and dissolved in THF (15 mL). Separately, *N,N′*-carbonyldiimidazole (0.275 g, 1.7 mmoL) was added to a solution of 4-(dimethylamino)butyric acid hydrochloride (0.267 g, 1.6 mmol) in DMF (5 mL), and the solution was stirred mechanically for 2 h. This activated ester was added to the resulting amine, and the mixture was stirred for 4 h. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ and washed with NaOH (5%). The organic layer was removed, dried, and concentrated to give the ester **13** (0.40 g, 92% yield): IR 2977, 2941, 1704, 1679, and 1399 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 10.10 (s, 1H each), 7.57 and 6.72 (2d, J = 2 Hz, 1H each), 5.56 (s, 2H), 4.26 (q, J = 7 Hz), 3.28 (s, 3H), 2.45 (m, 4H), 2.35 (s, 6H), 1.84 (m, 2H), 1.33 (t, J = 7 Hz); EIHRMS calcd for $C_{15}H_{25}N_3O_4$ 311.1845, found 311.1846 (M^+, 18). Anal. ($C_{15}H_{25}N_3O_4$) C, H, N.

1-(Methoxymethyl)-4-[4-(dimethylamino)butyramido]-pyrrole-2-carboxylic Acid (14). A solution of ester **13** (0.25 g, 0.8 mmol) was heated under reflux in 3 N NaOH (2 mL) and methanol (10 mL) for 2 h. Ethyl acetate (10 mL) was added, and the mixture was heated for an additional 30 min. The solution was cooled to 0 °C, and the pH was adjusted to 8 with dilute HCl. The mixture was concentrated, and the residue was purified on silica gel (flash chromatography, ethyl acetate in MeOH, 1:1, then MeOH) to afford the acid **14** (180 mg, 79% yield): IR ($CHCl_3$ cast) 3200−2000, 1652, 1575, 1472, 1386 cm^{-1}; ^{1}H-NMR (MeOH-d_4) δ 7.32 and 6.62 (2d, J = 2 Hz,

1H each), 5.68 (s, 2H), 3.25 (s, 3H), 2.35 (m, 4H), 2.25 (s, 6H), 1.85 (m, 2H); FABMS calcd for $C_{13}H_{21}N_3O_4$ 283.15, found 283.80 (M^+, 58). Anal. ($C_{13}H_{21}N_3O_4$) C, H, N.

Ethyl 1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]pyrrole-2-carboxylate (15). *N,N′*-Carbonyldiimadizole (0.97 g, 6 mmoL) was added to a solution of 4-(dimethylamino)butyric acid hydrochloride (0.92 g, 5.5 mmol) in DMF (5 mL), and the solution was stirred mechanically for 2 h. Separately, a solution of **11** (0.19 g, 0.5 mmol) and 5% Pd/C (20 mg) in DMF (5 mL) was stirred under a hydrogen atmosphere. After the completion of the hydrogenation (24 h), the activated ester was added to the resulting amine and the mixture was stirred for 12 h. The solvent was removed under reduced pressure, and the residue was purified on silica gel (flash chromatography, 10% MeOH in $CHCl_3$) to give the ester **15** (0.20 g, 86% yield): mp 207 °C; IR 3258, 2671, 1695, 1664, 1652, and 1248 cm^{-1}; ^{1}H-NMR (MeOH-d_4) δ 7.58, 7.42, 7.02 and 6.91 (4d, J = 2 Hz, 1H each), 5.63 and 5.61 (2s, 2H each), 4.26 (q, J = 7 Hz), 3.26 and 3.24 (2s, 3H each), 3.18 (m, 2H), 2.88 (s, 6H), 2.53 (t, J = 7 Hz, 2H), 2.05 (m, 2H), 1.32 (t, J = 7 Hz); HRMS calcd for $C_{22}H_{33}N_5O_6$ 463.2431, found 463.2436 (M^+, 8). Anal. ($C_{22}H_{35}N_5O_6$) C, H, N.

1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]pyrrole-2-carboxylic Acid (16). A solution of 1 N NaOH (2 mL) was added to a solution of **15** (710 mg, 1.53 mmol) in a mixture of methanol (10 mL), and the mixture was kept in a water bath (45 °C). After 2 h the reaction mixture was cooled, and the pH was adjusted to 8 with 2 N HCl. The solvent was evaporated in vacuo, and the product was purified on silica gel (flash chromatography, MeOH) to give **16** (580 mg, 87% yield): IR (KBr) 3700−2600, 1700, 1692, 1652, 1646, 1569, and 1382 cm^{-1}; ^{1}H-NMR (MeOH-d_4) δ 7.40 (m, 4H), 6.82 and 6.72 (2 d, J = 2 Hz, 1H each), 5.72 and 5.62 (2s, 2H each), 3.25 and 3.27 (2s, 3H each), 2.70 (m, 2H), 2.52 (s, 6H), 1.95 (m, 2H); FABMS calcd for $C_{20}H_{29}N_5O_6$ 436.21, found 436.29 (MH^+, 18). Anal. ($C_{20}H_{29}N_5O_6$) C, H, N.

Ethyl 1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[1-(methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxylate (17). *N,N′*-Carbonyldiimidazole (0.18 g, 1.1 mmoL) was added to a solution of 4-(dimethylamino)butyric acid hydrochloride (0.184 g, 1.1 mmol) in DMF (5 mL), and the solution was stirred mechanically for 1 h. Separately, a solution of **12** (0.532 g, 1 mmol) and 5% Pd/C (50 mg) in DMF (20 mL) was stirred under a hydrogen atmosphere. After completion of the hydrogenation (15 h), the activated ester was added to the resulting amine and the mixture was stirred for 6 h. The solvent was removed under reduced pressure, and the residue was purified on silica gel (flash chromatography, EtOAc in MeOH, 1:1, then methanol) to give the ester **17** (0.60 g, 98% yield): mp 72 °C; IR 3292, 3286, 2823, 1703, 16554, and 1084 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 9.55, 9.50, and 9.45 (3s, 1H each), 7.68, 7.47, 7.42, 7.08, 7.03, and 6.90 (6d, J = 2 Hz each, 1H each), 5.74, 5.71, and 5.66 (3s, 2H each), 4.24 (q, J = 7 Hz), 3.25 (s, 6H), 3.22 (s, 3H), 2.40 (m, 4H), 2.25 (s, 6H), 1.82 (m, 2H), 1.32 (t, J = 7 Hz); FABMS calcd for $C_{29}H_{41}N_7O_8$ 616.31, found 616.22 (MH^+, 53). Anal. ($C_{29}H_{41}N_7O_8$) C, H, N.

1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[1-(methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxylic Acid (18). A solution of 1 N NaOH (0.5 mL) was added to a solution of **17** (130 mg, 1.53 mmol) in a mixture of EtOH and THF (10 mL), and the mixture was kept in a constant temperature water bath (50 °C). After 2 h the reaction mixture was cooled, and the pH was adjusted to 5 with 2 N HCl. The solvent was evaporated in vacuo, and the product was purified on silica gel (flash chromatography, MeOH−EtOAc, 7:3, containing a few drops of Et_3N) to give **18** (100 mg, 77% yield): mp 168 °C; IR 3283, 3278, 3125, 2823, 1652, 1575, 1201, and 1081 cm^{-1}; ^{1}H NMR (MeOH-d_4) δ 7.48, 6.96, 6.90, and 6.78 (4d, J = 2 Hz each, 1H each), 7.41 (d, J = 2 Hz, 1H), 5.75 (s, 2H), 5.62 (s, 4H), 3.28, 3.27, 3.25 (3s, 3H each), 2.40 (m, 4H), 2.30 (s, 6H), 1.88 (m, 2H); FABMS calcd for $C_{27}H_{37}N_7O_8$ 588.28, found 588.24 (MH^+, 8). Anal. ($C_{22}H_{37}N_7O_8$) C, H, N.

Ethyl 1-(Methoxymethyl)-4-butyramidopyrrole-2-carboxylate (20). Compound **19** (1.74 g, 8.76 mmol) was treated with butyl chloride (0.91 mL, 8.76 mmol) and Et_3N (1.34 mL, 9.6 mmol) in 100 mL of THF. The reaction mixture was stirred at ambient temperature for 2 h and extracted with ethyl acetate. The extract was washed successively with 1 N HCl, aqueous $NaHCO_3$, and brine. The solution was dried over Na_2SO_4 and then concentrated in vacuo to give a crude oil which was purified by column chromatography (50% hexane in ethyl acetate) to afford **20** as an oil (1.9 g, 81% yield): ^{1}H NMR ($CDCl_3$) δ 7.52 (d, br, 2H), 6.77 (d, J = 2 Hz, 1H), 5.57 (s, 2H), 4.42 (q, J = 7 Hz, 2H), 3.25 (s, 3H), 2.28 (t, J = 7 Hz, 2H), 1.70 (m, J = 7 Hz, 2H), 1.29 (t, J = 7 Hz, 3H), 0.96 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{13}H_{20}N_2O_4$ 268.1423, found 268.1423 (M^+, 100). Anal. ($C_{13}H_{20}N_2O_4$) C, H, N.

1-(Methoxymethyl)pyrrole-4-butyramido-2-carboxylic Acid (21). A suspension of **20** (1.9 g, 7.08 mmol) in 30 mL of 1 N NaOH (50% methanol and water) was stirred at room temperature overnight. Methanol was evaporated, and the remaining aqueous solution was cooled to 5 °C and adjusted to pH 2 using cold 1 N HCl. The precipitate was collected and washed with water to afford **21** (1.62 g, 95% yield): mp 174 °C; ^{1}H-NMR (DMSO-d_6) δ 12.30 (br, 1H), 9.80 (s, 1H), 7.48 (d, J = 2 Hz, 1H), 6.72 (d, J = 2 Hz, 1H), 5.52 (s, 2H), 3.16 (s, 3H), 2.20 (t, J = 7 Hz, 2H), 1.58 (m, 2H), 0.90 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{11}H_{16}N_2O_4$ 240.1110, found 240.1108 (M^+, 70). Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

Ethyl 1-(Methoxymethyl)-4-[4-butyramido-2-formamido-*N*-(methoxymethyl)pyrrole]pyrrole-2-carboxylate (22). To a solution of **19** (1.65 g, 8.32 mmol) in 50 mL of CH_2Cl_2 and 10 mL of DMF were added **21** (2 g, 8.32 mmol) and EDCI (1.75 g, 9.15 mmol). The reaction mixture was stirred overnight at room temperature and extracted with ethyl acetate. The organic phase was washed with 1 N HCl, aqueous $NaHCO_3$, and brine, successively. The solution was dried over Na_2SO_4, and then the solvent was removed in vacuo to give a crude oil which was purified by column chromatography (5% MeOH in $CHCl_3$) to give the solid **22** (2.66 g, 76% yield): mp 112 °C; ^{1}H-NMR ($CDCl_3$) δ 8.77 (s, 1H), 8.18 (s, 1H), 7.52 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 6.82 (d, J = 2 Hz, 1H), 6.73 (d, J = 2 Hz, 1H), 5.52 (s, 2H), 5.47 (s, 2H), 4.21 (q, J = 7 Hz, 2H), 3.30 (s, 3H), 3.21 (s, 3H), 2.22 (t, J = 7 Hz, 2H), 1.65 (m, J = 7 Hz), 1.28 (t, J = 7 Hz, 2H), 0.89 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{20}H_{28}N_4O_6$ 420.2016, found 420.2017 (M^+, 100). Anal. ($C_{20}H_{28}N_4O_6$) C, H, N.

4-[4-Butyramido-2-formamido-*N*-(methoxymethyl)pyrrolyl]-*N*-(methoxymethyl)pyrrole-2-carboxylic Acid (23). Compound **23** was prepared from **22** (2.66 g, 6.32 mmol), using a similar procedure as that described for **21**, in 93% yield: mp 97 °C; ^{1}H-NMR (DMSO-d_6) δ 12.38 (br, 1H), 10.70 (br s, 1H), 9.88 (br s, 1H), 7.60 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 6.92 (s, 2H), 5.64 (s, 2H), 5.60 (s, 2H), 3.18 (s, 3H), 3.15 (s, 3H), 2.22 (t, J = 7 Hz, 2H,), 1.60 (m, J = 7 Hz, 2H), 0.90 (t, J = 7 Hz, 3 H); EIHRMS calcd for $C_{17}H_{24}N_4O_4$ (M − CO_2) 348.1801, found 348.1801 ($M^+ - CO_2$, 70). Anal. ($C_{18}H_{24}N_4O_6$) C, H, N.

Ethyl 4-[4-[4-Butyramido-*N*-(methoxymethyl)pyrrole-2-carboxamido]-*N*-(methoxymethyl)pyrrole-2-carboxamido]-*N*-(methoxymethyl)pyrrole-2-carboxylate (24). A solution of acid **23** (1.1 g, 3.16 mmol), DCC (716 mg, 3.47 mmol), and HOBt (470 mg, 3.47 mmol) in DMF (10 mL) and THF (20 mL) was stirred for 4 h. Then amine **19** (627 mg, 3.16 mmol) was added to the above solution. The mixture was stirred overnight. Workup was similar to that used for **22**, and purification was achieved by flash chromatograph (3% MeOH in HCl_3) to give solid **24** (1.54 g, 85% yield): mp 105 °C; ^{1}H-NMR ($CDCl_3$) δ 8.90 (s, 1H), 8.49 (bs, 1H), 8.42 (bs, 1H), 7.55 (d, J = 2 Hz, 1H), 7.40 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 6.90 (d, J = 2 Hz, 1H), 6.68 (d, J = 2 Hz, 1H), 6.62 (d, J = 2 Hz, 1H), 5.50 (s, 2H), 5.47 (s, 2H), 5.41 (s, 2H), 4.18 (q, J = 7 Hz, 2H), 3.19 (s, 3H), 3.18 (s, 3H), 3.17 (s, 3H), 2.20 (t, J = 7 Hz, 2H), 1.60 (m, 2H), 1.21 (t, J = 7 Hz, 3H), 0.85 (t, J = 7 Hz, 3H); FABMS calcd for $C_{27}H_{36}N_6O_8$ 572.26, found 572.28 (M^+, 16). Anal. ($C_{27}H_{36}N_6O_8$) C, H, N.

4-[4-[4-Butyramido-*N*-(methoxymethyl)pyrrole-2-carboxamido]-*N*-(methoxymethyl)pyrrole-2-carboxamido]-

***N*-(methoxymethyl)pyrrole-2-carboxylic Acid (25).** Compound **25** was prepared from **24** (1.5 g, 2.62 mmol) in 93% yield using a similar procedure as that described for **23**: mp 125 °C; ^{1}H-NMR (DMSO-d_6) δ 12.38 (bs, 1H), 10.10 (bs, 2H), 9.84 (s, 1H), 7.62 (d, J = 2 Hz, 1H), 7.45 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 7.10 (d, J = 2 Hz, 1H), 6.90 (d, J = 2 Hz, 2H), 5.70 (s, 2H), 5.68 (s, 2H), 5.58 (s, 2H), 3.18 (s, 3H), 3.17 (s, 3H), 3.16 (s, 3H), 2.21 (t, 2H, J = 7 Hz), 1.60 (m, 2H), 0.84 (t, J = 7 Hz, 3H); FABMS calcd for $C_{25}H_{32}N_6O_8$ 544.23, found 544.24 (M^+, 30). Anal. ($C_{25}H_{32}N_6O_8$) C, H, N.

3-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-nitropyrrole-2-carboxamido]pyrrole-2-carboxamido]-1-(dimethylamino)propane (27). Compound **27** was prepared from **26** (284 mg, 1 mmol) and 1-(methoxymethyl)-4-nitropyrrole-2-carboxylic acid (**10b**) (201 mg, 1 mmol) in 75% yield in similar manner previously described for **12**: mp 124 °C; ^{1}H-NMR ($CDCl_3$) δ 9.05 (br, 1H), 7.90 (t, 1H, J = 7 Hz), 7.78 (d, J = 2 Hz, 1H,), 7.48 (d, J = 2 Hz, 1H),7.42 (d, J = 2 Hz, 1H), 6.95 (bs, 1H), 6.73 (d, J = 2 Hz, 1H), 5.72 (s, 2H), 5.58 (s, 2H), 3.42 (m, 2H), 3.39 (s, 3H), 3.30 (s, 3H), 2.42 (t, 2H, J = 7 Hz), 2.28 (s, 6H), 1.70 (m, 2H); EIHRMS calcd for $C_{19}H_{28}N_6O_6$ 436.2070, found 436.2080 (M^+, 14). Anal. ($C_{19}H_{28}N_6O_6$) C, H, N.

3-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[1-(methoxymethyl)-4-nitropyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-1-(dimethylamino)-propane (28). Compound **28** was prepared from **27** in 87% yield, in a similar procedure as that described for **27**. The ^{1}H-NMR and MS spectra were in agreement with the data previously reported.[15b]

1-(Methoxymethyl)imidazole (30). To a solution of sodium ethoxide (6.8 g, 0.1 mol) in absolute ethoxide (200 mL) at 0 °C was added imidazole (6.8 g, 0.1 mol), and the resulting mixture was stirred for 1 h before concentration in vacuo. The residue was dissoved in dry THF (200 mL), and chloromethyl methyl ether (8.9 g, 0.11 mol) was added over a period of 10 min. The mixture was stirred for 2 h at room temperature and then was filtered. After evaporation of the solvent the residue was distilled through a 20 cm Vigreux column to yield **30** (7.5 g, 67% yield): bp 65 °C/1.5 mmHg; ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1H), 6.85 (m, 2H), 5.10 (s, 2H), 3.15 (s, 3H); EIHRMS calcd for $C_5H_8N_2O$ 112.0636, found 112.0632 (M^+, 100). Anal. ($C_5H_8N_2O$) C, H, N.

1-(Methoxymethyl)-2-(trichloroacetyl)imidazole (31). A solution of **30** (5.6 g, 0.05 mol) in dry CH_2Cl_2 (100 mL) was added to a solution of trichloroacetyl chloride (10 g, 0.055 mol) in dry CH_2Cl_2 (100 mL) over 2 h. The reaction mixture was stirred for 3 h at room temperature, E_3N (1 mmol) was added over 30 min, and then the reaction mixture was concentrated. The residue was purified on a silica gel column eluting with EtOAc:Hex (1:1) to give **31** as a yellow oil (9 g, 70% yield): ^{1}H-NMR ($CDCl_3$) δ 7.38 (s, 2H), 5.7 (s, 2H), 3.36 (s, 3H); EIHRMS calcd for $C_7H_7N_2O_2Cl_3$ 255.9579, found 255.9577 (M^+, 4).

1-(Methoxymethyl)-4-nitro-2-(trichloroacetyl)imidazole (32). To a solution of **31** (5.15 g, 0.02 mol) in acetic anhydride (30 mL) at −40 °C was added fuming HNO_3 (8 mL) over 30 min, and then the reaction mixture was allowed to warm up slowly to room temperature. After being stirred for 2 h, the mixture was poured into ice water and extracted with $CHCl_3$. The organic phase was removed, washed with brine, and then dried (Na_2SO_4). After evaporation of the solvent and acetic anhydride, the residue was purified by column chromatograph eluting with EtOAc:Hex (1:1) to give **32** as an oil (2.78 g, 46% yield): ^{1}H-NMR δ 8.20 (s, 1H), 5.81 (s, 2H), 3.57 (s, 3H); EIHRMS calcd for $C_7H_6N_3O_4Cl_3$ 300.9424, found 300.9425 (M^+, 2).

Ethyl 1-(Methoxymethyl)-4-nitroimidazole-2-carboxylate (33). To a solution of **32** (3.01 g, 0.01 mol) in absolute EtOH (50 mL) was added 80% NaH (10 mg). The mixture was stirred for 1 h. The ethanol was removed in vacuo, and the residue was diluted with water and then extracted with EtOAc (3 × 30 mL). The organic phase was washed with water, dried (Na_2SO_4), and evaporated to yield an oil, which was recrystallized (EtOAc:Hex, 2:1) to give **33** (2.20 g, 96% yield): ^{1}H-NMR δ 8.08 (s, 1H), 5.78 (s, 2H), 4.38 (q, J = 7 Hz, 2H), 3.40 (s, 3H), 1.36 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_8H_{11}N_3O_5$ 229.0699, found 229.0699 (M^+, 20). Anal. ($C_8H_{11}N_3O_5$) C, H, N.

Ethyl 4-Butyramido-*N*-(methoxymethyl)imidazole-2-carboxylate (34). Compound **34** was prepared from **33** (4.5 g, 1.96 mmol) in 81% yield, using a similar procedure as described for **20**: mp 58 °C; ^{1}H-NMR ($CDCl_3$) δ 8.95 (s, 1H), 7.61 (s, 1H), 5.58 (s, 2H), 4.27 (q, J = 7 Hz, 2H), 3.22 (s, 3H), 2.19 (t, J = 7 Hz, 2H), 1.58 (m, J = 7 Hz, 2H), 1.24 (t, J = 7 Hz, 3 H), 0.81 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{12}H_{19}N_3O_4$ 269.1375, found 269.1374 (M^+, 31). Anal. ($C_{12}H_{19}N_3O_4$) C, H, N.

4-Butyramido-*N*-(methoxymethyl)imidazole-2-carboxylic Acid (35). Compound **35** was made from **34** (3.86 g, 1.43 mmol) in 94% yield, using a procedure similar to that described for **21**: mp 148 °C; ^{1}H-NMR (DMSO-d_6) δ 10.58 (s, 1H), 7.50 (d, J = 2 Hz, 1H), 5.70 (s, 2H), 3.20 (s, 3H), 2.25 (t, J = 7 Hz, 2H), 1.57 (m, J = 7 Hz, 2H), 0.88 (t, J = 7 Hz, 3 H); HRFABMS calcd for $C_{10}H_{15}N_3O_4H$ 242.1141, found 242.1140 (MH^+). Anal. ($C_{10}H_{15}N_3O_4$) C, H, N.

Ethyl 4-[4-Butyramido-2-formamido-*N*-(methoxymethyl)imidazolyl]-*N*-(methoxymethyl)imidazole-2-carboxylate (36). A solution of **35** (1.0 g, 4.14 mmol) and an excess of $SOCl_2$ in anhydrous THF was heated to reflux for 2 h. The solvent and excess $SOCl_2$ were removed in vacuo to give a crude acid chloride **35**, which was treated with the reduction product of **32** (from 0.95 g, 1 mmol) and Et_3N (0.63 mL, 4.56 mmol) in 40 mL of THF at 0 °C. The reaction mixture was stirred at ambient temperature for 2 h and then extracted with ethyl acetate. The extract was washed with aqueous $NaHCO_3$ and concentrated in vacuo to give a crude oil which was purified by chromatography (50% hexane and ethyl acetate) to give solid **36** (1.08 g, 62% yield): mp 138 °C; ^{1}H-NMR ($CDCl_3$) δ 9.65 (br s, 1H), 8.57 (br s, 1H), 7.73 (s, 1H), 7.62 (s, 1H), 5.72 (s, 2H), 5.68 (s, 2H), 4.38 (q, J = 7 Hz, 2H), 3.32 (s, 3H), 3.28 (s, 3H), 2.32 (t, J = 7 Hz, 2H), 1.68 (m, J = 7 Hz, 2H), 1.33 (t, J = 7 Hz, 2H), 0.92 (t, J = 7 Hz, 3 H); HRFABMS calcd for $C_{18}H_{26}N_6O_6$ 422.1919, found 422.1914 (M^+, 70). Anal. ($C_{18}H_{26}N_6O_6$) C, H, N.

4-[4-Butyramido-2-formamido-*N*-(methoxymethyl)imidazolyl]-*N*-(methoxymethyl)imidazole-2-carboxylic Acid (37). Compound **37** was made from **36** in 81% yield (1.0 g, 2.36 mmol), using a similar procedure as that described for **35**: mp 117 °C; ^{1}H-NMR (DMSO-d_6) δ 10.44 (br s, 1H), 9.96 (br s, 1H), 7.79 (s, 1H), 7.63 (s, 1H), 5.71 (s, 2H), 5.69 (s, 2H), 3.24 (s, 6H), 2.30 (t, J = 7 Hz, 2H), 1.58 (m, J = 7 Hz, 2H), 0.90 (t, J = 7 Hz, 3H); EIHRMS calcd for $C_{16}H_{22}N_6O_6H$ 395.1679, found 395.1672 (M^+, 18). Anal. ($C_{16}H_{22}N_6O_6$) C, H, N.

10-[[[1-(Methoxymethyl)-2-carbethoxypyrrol-4-yl]carbamoyl]methoxy]-2(*R*,*S*)-camptothecin (38). DCC (11.31 mg, 0.055 mmol) and HOBt (7.4 mg, 0.055 mmol) were added to a solution of **8** (21.2 mg, 0.05 mmol) in 20 mL of DMF−THF (1:4), and the mixture was stirred for 4 h. A solution of **19** (15.8 mg, 0.08 mmol) in THF was added to the above solution, which was stirred overnight and then filtered. The filtrate was evaparated to dryness in vacuo, and the residue was purified by column chromatography (silica gel, THF:$CHCl_3$ (1:1)) to give **38** as a solid (20 mg, 66% yield): ^{1}H-NMR (DMSO-d_6) δ 10.21 (s, 1H), 8.52 (s, 1H), 8.15 (d, J = 8 Hz, 1H), 7.65 (dd, J = 8, 2 Hz, 1H), 7.60 (d, J = 2 Hz, 1H), 7.53 (d, J = 2 Hz, 1H), 7.26 (s, 1H), 7.00 (d, J = 2 Hz, 1H), 6.50 (s, 1H, OH), 5.59 (s, 2H), 5.40 (s, 2H), 5.22 (s, 2H), 4.83 (s, 2H), 4.19 (q, J = 7 Hz, 2H), 3.18 (s, 3H), 1.83 (m, 2H), 1.23 (t, J = 7 Hz, 3H), 0.83 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{31}H_{30}N_4O_9H$ 603.2091, found 603.2092 (MH^+). Anal. ($C_{31}H_{30}N_4O_9$) C, H, N.

10-[[[1-(Methoxymethyl)-2-[[1-(methoxymethyl)-2-carbethoxypyrrol-4-yl]carbamoyl]pyrrole-4-yl]carbamoyl]-methoxy]-2(*R*,*S*)-camptothecin (39). DCC (11.3 mg, 0.055 mmol) and HOBt (7.4 mg, 0.055 mol) were added to a solution of **8** (21.2 mg, 0.05 mmol) in 20 mL of DMF−THF (1:3), and the solution was stirred for 4 h. Separately, a solution of **11** (31 mg, 0.08 mol) and 5% Pd/C (5 mg) in 10 mL of THF was stirred under a hydrogen atmosphere. After the completion of the hydrogenation (8 h), a solution of the above activated

ester was added to the resulting amine and the mixture was stirred for 8 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (silica gel, THF:$CHCl_3$) to give **39** (21.8 mg, 57% yield): ^{1}H-NMR (DMSO-d_6) δ 10.26 (s, 1H), 10.05 (s, 1H), 8.52 (s, 1H), 8.13 (d, J = 8 Hz, 1H), 7.62 (dd, J = 8, 2 Hz, 1H), 7.60 (d, J = 2 Hz, 1H), 7.56 (d, J = 2 Hz, 1H), 7.42 (d, J = 2 Hz, 1H), 7.23 (s, 1H), 7.10 (d, J = 2 Hz, 1H), 6.99 (d, J = 2 Hz, 1H), 6.50 (s, 1H, OH), 5.63 (s, 2H), 5.58 (s, 2H), 5.40 (s, 2H), 5.23 (s, 2H), 4.84 (s, 2H), 4.09 (q, J = 7 Hz, 2H), 3.18 (s, 3H), 3.16 (s, 3H), 1.82 (m, 2H), 1.23 (t, J = 7 Hz, 3H), 0.83 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{38}H_{38}N_6O_{11}H$, 755.2677, found 755.2641 (MH^+). Anal. ($C_{38}H_{38}N_6O_{11}\cdot 0.5H_2O$) C, H, N.

10-[[[1-(Methoxymethyl)-2-[[1-(methoxymethyl)-2-[[1-(methoxymethyl)-2-carbethoxypyrrol-4-yl]carbamoyl]-pyrrol-4-yl]carbamoyl]pyrrol-4-yl]carbamoyl]methoxy]-2(*R,S*)-camptothecin (40). Compound **11** (42.56 mg, 0.08 mmol) and 10% Pd/C (10 mg) in 20 mL of THF−MeOH (1:1) was hydrogenated at atmospheric pressure for 4 h and then filtered. The filtrate was concentrated in vacuo, and then DMF (10 mL), CH_2Cl_2 (10 mL), **8** (21.2 mg, 0.05 mmol), and EDCI (0.055 mmol) were added successively. The solution was stirred under argon overnight and then evaporated to dryness. The residue was purified on a silica gel column eluting with THF:$CHCl_3$ (1:1) to yield **40** (23.6 mg, 52% yield): ^{1}H-NMR (DMSO-d_6) δ 10.30 (s, 1H), 10.18 (s, 1H), 10.12 (s, 1H), 8.55 (s, 1H), 8.14 (d, J = 8 Hz, 1H), 7.63 (dd, J = 8, 2 Hz, 1H), 7.62 (d, J = 2 Hz, 1H), 7.55 (d, J = 2 Hz, 1H), 7.42 (s, 2H), 7.28 (s, 1H), 7.13 (d, J = 2 Hz, 1H), 7.10 (d, J = 2 Hz, 1H), 7.01 (d, J = 2 Hz, 1H), 6.50 (s, 1H, OH), 5.68 (s, 4H), 5.58 (s, 2H), 5.40 (s, 2H), 5.24 (s, 2H), 4.88 (s, 2H), 4.20 (q, J = 7 Hz, 2H), 3.16 (s, 9H), 1.83 (m, 2H), 1.26 (t, J = 7 Hz, 3H), 0.85 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{45}H_{46}N_8O_{13}H$ 907.3262, found 907.3204 (MH^+). Anal. ($C_{45}H_{46}N_8O_{13}\cdot H_2O$) C, H, N.

10-[[[1-(Methoxymethyl)-2-[[3-(dimethylamino)propyl]-carbamoyl]pyrrol-4-yl]carbamoyl]methoxy]-20(*R,S*)-camptothecin (41). Acid **8** (21.2 mg, 0.05 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (20 mL) for 6 h. Separately, a solution of **26** (22.7 mg, 0.08 mmol) and 10% Pd/C in 20 mL of MeOH was stirred under a hydrogen atmosphere (4 h) and then filtered. The filtrate was concentrated and dried under vacuum. The residue was added to the above solution, and stirring was continued for 8 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (silica gel, $CHCl_3$:MeOH) to give **41** (19 mg, 55% yield): ^{1}H-NMR (DMSO-d_6) δ 10.22 (s, 1H), 8.56 (s, 1H), 8.19 (t, J = 6 Hz, 1H), 8.12 (d, J = 8 Hz, 1H), 7.63 (dd, J = 8, 2 Hz, 1H), 7.53 (d, J = 2 Hz, 1H), 7.35 (d, J = 2 Hz, 1H), 7.25 (s, 1H), 6.82 (d, J = 2 Hz, 1H), 6.50 (bs, 1H), 5.60 (s, 2H), 5.42 (s, 2H), 5.24 (s, 2H), 4.83 (s, 2H), 3.19 (m, 2H), 3.14 (s, 3H), 2.22 (t, 2H, J = 6 Hz), 2.05 (s, 6H), 1.82 (m, 2H), 1.58 (m, 2H), 0.86 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{34}H_{38}N_6O_8H$ 659.2829, found 659.2822 (MH^+). Anal. ($C_{34}H_{38}N_6O_8$) C, H, N.

10-[[[1-(Methoxymethyl)-2-[[1-(methoxymethyl)-2-[[3-(dimethylamino)propyl]carbamoyl]pyrrolyl-4-yl]carbamoyl]pyrrol-4-yl]carbamoyl]methoxy]-20(*R,S*)-camptothecin (42). Compound **42** was prepared in 65% yield from acid **8** (21.2 mg, 0.05 mmol) and **27** (26.16 mg, 0.06 mmol) in a manner similar to that described for **41**: ^{1}H-NMR (DMSO-d_6) δ 10.32 (s, 1H), 10.08 (s, 1H), 8.53 (s, 1H), 8.21 (t, J = 6 Hz, 1H), 8.12 (d, J = 8 Hz, 1H), 7.63 (dd, J = 8, 2 Hz, 1H), 7.53 (d, J = 2 Hz, 1H), 7.42 (d, J = 2 Hz, 1H), 7.35 (d, J = 2 Hz, 1H), 7.26 (s, 1H), 7.08 (d, J = 2 Hz, 1H), 6.88 (d, J = 2 Hz, 1H), 5.63 (s, 2H), 5.60 (s, 2H), 5.29 (s, 2H), 4.85 (s, 2H), 3.20 (m, 2H), 3.12 (s, 6H), 2.58 (t, 2H, J = 6 Hz), 2.35 (s, 6H), 1.85 (m, 2H), 1.66 (m, 2H), 0.85 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{41}H_{46}N_8O_{10}H$ 811.3415, found 811.3377 (MH^+). Anal. ($C_{41}H_{46}N_8O_{10}$) C, H, N.

10-[[[1-(Methoxymethyl)-2-[[1-(methoxymethyl)-2-[[1-(methoxymethyl)-2-[[3-(dimethylamino)propyl]carbamoyl]pyrrol-4-yl]carbamoyl]pyrrol-4-yl]carbamoyl]pyrrol-4-yl]carbamoyl]methoxy]-20(*R,S*)-camptothecin (43). Compound **43** was prepared in 58% yield from acid **8** (21.2 mg, 0.05 mmol) and **28** (47 mg, 0.08 mmol) in a manner similar to that described for **41**: ^{1}H-NMR (DMSO-d_6) δ 10.38 (s, 1H), 10.16 (s, 1H), 10.08 (s, 1H), 8.55 (s, 1H), 8.21 (t, J = 6 Hz, 1H), 8.18 (d, J = 8 Hz, 1H), 7.62 (dd, J = 8, 2 Hz, 1H), 7.54 (d, J = 2 Hz, 1H), 7.43 (s, 2H), 7.36 (d, J = 2 Hz, 1H), 7.26 (s, 1H), 7.06 (s, 2H), 6.90 (d, J = 2 Hz, 1H), 6.50 (bs, 1H, OH), 5.64 (s, 4H), 5.40 (s, 2H), 5.23 (s, 2H), 4.85 (s, 2H), 3.20 (m, 2H), 3.16 (s, 6H), 3.13 (s, 3H), 2.60 (t, J = 6 Hz, 2H), 2.40 (s, 6H), 1.85 (m, 2H), 1.72 (m, 2H), 0.96 (t, J = 7 Hz, 3H); FABMS calcd for $C_{48}H_{54}N_{10}O_{12}H$ 963.40, found 963.40 (MH^+). Anal. ($C_{48}H_{54}N_{10}O_{12}\cdot H_2O$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-butyramidopyrrole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (44). Acid **21** (13.2 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.05 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **38**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **44** (22.4 mg, 71% yield): ^{1}H-NMR (DMSO-d_6) δ 9.80 (s, 1H), 8.52 (s, 1H), 8.39 (t, J = 6 Hz, 1H), 8.07 (d, J = 8 Hz, 1H), 7.58 (d, J = 2 Hz, 1H), 7.50 (dd, J = 8, 2 Hz, 1H), 7.31 (d, J = 2 Hz, 1H), 7.28 (s, 1H), 6.80 (d, J = 2 Hz, 1H), 6.50 (bs, 1H, OH), 5.60 (s, 2H), 5.40 (s, 2H), 5.24 (t, 2H, J = 6 Hz), 5.23 (s, 2H), 3.64 (m, 2H), 3.15 (s, 3H), 2.20 (t, 2H, J = 7 Hz), 1.86 (m, 2H), 1.58 (m, 2H), 0.90 (t, J = 7 Hz, 3H); FABMS calcd for $C_{33}H_{35}N_5O_8H$ 630.25, found 630.36 (MH^+). Anal. ($C_{33}H_{35}N_5O_8\cdot H_2O$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-butyramidopyrrole-2-carboxamido)pyrrole-2-carboxyamido]ethoxy]-20(*R,S*)-camptothecin (45). Acid **23** (19.14 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.05 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **38**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **45** (26.6 mg, 68% yield): ^{1}H-NMR (DMSO-d_6) δ 10.04 (s, 1H), 9.82 (s, 1H), 8.52 (s, 1H), 8.42 (t, J = 6 Hz, 1H), 8.05 (d, J = 8 Hz, 1H), 7.58 (d, J = 2 Hz, 1H), 7.51 (dd, J = 8, 2 Hz, 1H), 7.39 (d, J = 2 Hz, 1H), 7.36 (d, J = 2 Hz, 1H), 7.26 (s, 1H), 6.98 (d, J = 2 Hz, 1H), 6.90 (d, J = 2 Hz, 1H), 6.50 (bs, 1H, OH), 5.62 (s, 2H), 5.60 (s, 2H), 5.40 (s, 2H), 5.22 (s, 2H), 4.28 (t, J = 6 Hz, 2H), 3.63 (m, 2H), 3.18 (s, 3H), 3.16 (s, 3H), 2.18 (t, J = 7 Hz, 2H), 1.84 (m, 2H), 1.58 (m, 2H), 0.90 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{40}H_{43}N_7O_{10}H$ 782.3149, found 782.3149 (MH^+). Anal. ($C_{40}H_{43}N_7O_{10}$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[1-(methoxymethyl)-4-butyramidopyrrole-2-carboxamido]-pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (46). Acid **25** (30 mg, 0.05 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.05 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **38**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **46** (27.1 mg, 58% yield): ^{1}H-NMR (DMSO-d_6) δ 10.10 (s, 2H), 9.83 (s, 1H), 8.52 (s, 1H), 8.42 (t, J = 6 Hz, 1H), 8.05 (d, J = 8 Hz, 1H), 7.58 (d, J = 2 Hz, 1H), 7.50 (dd, J = 8, 2 Hz, 1H), 7.43 (d, J = 2 Hz, 1H), 7.40 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 7.25 (s, 1H), 7.10 (d, J = 2 Hz, 1H), 7.00 (d, J = 2 Hz, 1H), 6.92 (d, J = 2 Hz, 1H), 6.50 (bs, 1H, OH), 5.62 (s, 6H), 5.40 (s, 2H), 5.23 (s, 2H), 4.25 (t, J = 6 Hz, 2H), 3.64 (m, 2H), 3.18 (s, 3H), 3.17 (s, 3H), 3.16 (s, 3H), 2.22 (t, 2H, J = 7 Hz), 1.83 (m, 2H), 1.58 (m, 2H), 0.90 (m, 6H); FABMS calcd for $C_{47}H_{51}N_9O_{12}H$ 934.37, found 934.42 (MH^+). Anal. ($C_{47}H_{51}N_9O_{12}$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (47). Acid **14** (15.6 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.05 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **41**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **47** (17.17 mg, 51% yield): ^{1}H-NMR (DMSO-d_6) δ 9.93 (s, 1H), 8.50 (s, 1H), 8.42 (t, J = 6 Hz, 1H), 8.02 (d, J = 8 Hz, 1H), 7.50 (m, 2H), 7.30 (d, J = 2 Hz, 1H),

7.14 (d, J = 2 Hz, 1H), 7.05 (bs, 1H), 6.80 (d, J = 2 Hz, 1H), 5.60 (s, 4H), 5.40 (s, 2H), 5.21 (s, 2H), 4.22 (t, J = 6 Hz, 2H), 3.65 (m, 2H), 3.16 (s, 3H), 2.23 (m, 4H), 2.10 (s, 6H), 1.84 (m, 2H), 1.68 (m, 2H), 0.88 (t, J = 7 Hz, 3H); FABMS calcd for $C_{35}H_{40}N_6O_8H$ 673.29, found 673.64 (MH^+). Anal. ($C_{35}H_{40}N_6O_8$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (48). Acid **16** (24 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.055 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **41**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **48** (19.8 mg, 49% yield): ^{1}H-NMR (DMSO-d_6) δ 10.12 (s, 1H), 10.04 (s, 1H), 8.46 (s, 1H), 8.43 (t, J = 6 Hz, 1H), 8.01 (d, J = 8 Hz, 1H), 7.50 (m, 2H), 7.40 (d, J = 2 Hz, 1H), 7.37 (d, J = 2 Hz, 1H), 7.23 (s, 1H), 6.98 (d, J = 2 Hz, 1H), 6.93 (d, J = 2 Hz, 1H), 6.50 (bs, 1H, OH), 5.60 (s, 4H), 5.39 (s, 2H), 5.18 (s, 2H), 4.26 (t, J = 6 Hz, 2H), 3.65 (m, 2H), 3.16 (s, 3H), 3.14 (s, 3H), 2.23 (m, 4H), 1.83 (m, 2H), 1.70 (m, 2H), 0.85 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{42}H_{48}N_8O_{10}H$ 825.3571, found 825.3543 (MH^+). Anal. ($C_{42}H_{48}N_8O_{10}\cdot 0.5H_2O$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-[1-(methoxymethyl)-4-[4-(dimethylamino)butyramido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (49). Acid **18** (32.35 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.055 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **38**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **49** (21.5 mg, 44% yield): ^{1}H-NMR (DMSO-d_6) δ 10.10 (s, 2H), 9.92 (s, 1H), 8.56 (s, 1H), 8.45 (t, J = 6 Hz, 1H), 8.08 (d, J = 8 Hz, 1H), 7.58 (d, J = 2 Hz, 1H), 7.52 (dd, J = 8, 2 Hz, 1H), 7.42 (d, J = 2 Hz, 1H), 7.38 (d, J = 2 Hz, 1H), 7.34 (d, J = 2 Hz, 1H), 7.24 (s, 1H), 7.08 (d, J = 2 Hz, 1H), 6.98 (d, J = 2 Hz, 1H), 6.92 (d, J = 2 Hz, 1H), 6.50 (bs, 1H, OH), 5.62 (s, 6H), 5.40 (s, 2H), 5.24 (s, 2H), 4.28 (t, J = 6 Hz, 2H), 3.64 (m, 2H), 3.12 (s, 9H), 2.23 (m, 4H), 1.85 (m, 2H), 1.72 (m, 2H), 0.85 (t, J = 7 Hz, 3H); HRFABMS calcd for $C_{49}H_{56}N_{10}O_{12}H$ 977.4157, found 977.4119 (MH^+). Anal. ($C_{49}H_{56}N_{10}O_{12}\cdot H_2O$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-butyramidoimidazole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (50). Acid **35** (12.10 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.055 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **38**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THF) to give **50** (20.2 mg, 64% yield): ^{1}H-NMR (DMSO-d_6) δ 10.32 (s, 1H), 8.51 (s, 1H), 8.22 (t, J = 6 Hz, 1H), 8.03 (d, J = 8 Hz, 1H), 7.59 (d, J = 2 Hz, 1H), 7.57 (s, 1H), 7.48 (dd, J = 8, 2 Hz, 1H), 7.23 (s, 1H), 6.50 (bs, 1H, OH), 5.78 (s, 2H), 5.67 (s, 2H), 5.40 (s, 2H), 5.23 (s, 2H), 4.30 (t, 2H, J = 6 Hz), 3.76 (m, 2H), 3.21 (s, 3H), 2.25 (t, J = 7 Hz, 2H), 1.84 (m, 2H), 1.58 (m, 2H), 0.85 (m, 6H); FABMS calcd for $C_{32}H_{34}N_6O_8H$ 631.25, found 631.42 (MH^+). Anal. ($C_{32}H_{34}N_6O_8\cdot 0.25H_2O$) C, H, N.

10-[2-[1-(Methoxymethyl)-4-[1-(methoxymethyl)-4-butyramidoimidazole-2-carboxamido]imidazole-2-carboxamido]ethoxy]-20(*R,S*)-camptothecin (51). Acid **37** (19.78 mg, 0.055 mmol), DCC (11.3 mg, 0.055 mmol), and HOBt (7.4 mg, 0.055 mmol) were stirred in DMF (30 mL) for 8 h. Amine **9** (22.2 mg, 0.05 mmol) and triethylamine (10 mg, 0.1 mmol) were added, and stirring was continued for 8 h. Workup was similar to that used for **38**, and purification was achieved by flash chromatography (silica gel, $CHCl_3$:THE) to give **51** (26.2 mg, 67% yield): ^{1}H-NMR (DMSO-d_6) δ 10.38 (s, 1H), 9.58 (s, 1H), 8.56 (t, J = 6 Hz, 1H), 8.48 (s, 1H), 8.05 (d, J = 8 Hz, 1H), 7.56 (s, 1H), 7.64 (s, 1H), 7.52 (d, J = 2 Hz, 1H), 7.45 (dd, J = 8, 2 Hz, 1H), 7.23 (s, 1H), 6.50 (bs, 1H, OH), 5.78 (s, 2H), 5.73 (s, 2H), 5.40 (s, 2H), 5.22 (s, 2H), 4.30 (t, 2H, J = 6 Hz), 3.75 (m, 2H), 3.26 (s, 6H), 2.30 (t, 2H, J = 7 Hz), 1.85 (m, 2H), 1.58 (m, 2H), 0.90 (m, 6H); HRFABMS calcd for $C_{38}H_{41}N_9O_{10}H$ 784.3054, found 784.3018 (MH^+). Anal. ($C_{38}H_{41}N_9O_{10}\cdot 1.5H_2O$) C, H, N.

Topoisomerase I Relaxation Assay. The assays were done as described previously.[16,17] In brief, 0.25 μg of pBR322 DNA and 0.5 unit of topoisomerase I were incubated for 30 min at 37 °C in the presence of the ligands in a final volume of 10 μL. Following the incubation, an equal volume of agarose gel loading buffer (2 × TBAA, 0.1% bromophenol blue, 0.2% SDS, and 20% glycerol) was added, and the mixture was incubated for another 30 min at 37 °C prior to loading. The gel was run overnight and stained in 0.5 μg/mL ethidium bromide solution, and the DNA was visualized using a 300 nm wavelength transilluminator and photographed with Polaroid film. The negative was scanned on an LKB ultroscan XL laser densitometer. The IC_{50} values, *i.e.* concentration required to inhibit 50% relaxation of the supercoiled DNA in the presence of topoisomerase, were determined.

Cell Culture Cytotoxicity Assay. In vitro cytotoxicity assay of compounds was performed using KB cancer cell line (ATCCCCL 17) and the other cell lines.[18] Cells were cultivated in Eagle's minimum essential medium supplemented with 10% calf serum and incubated in a humidified 5% CO_2 atmosphere at 37 °C. Cells were counted on a Neubauer hemocytometer and seeded at 100 μL of 3 × 10^3 cells per mL per well and allowed to culture for 24 h. Test compounds were added in triplicate at different concentrations. Control wells were identical except that the test compound was absent. After 3 days, the cells were fixed in 25% glutaraldehyde, washed with water, dried, and then stained with 100 μL of 0.05% crystal violet. The wells were eluted with 0.05 M NaH_2PO_4/ethanol (1:1 v/v) and read at OD_{450} on a multiscan spectrophotometer. TD_{50} values were determined as the concentrations required to reduce KB cell count by 50%.

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