Plant Antitumor Agents. 30.[1a,b] Synthesis and Structure Activity of Novel Camptothecin Analogs

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Received February 8, 1993•

A large number of camptothecin (CPT) analogs have been prepared in the 20*S*, 20*RS*, and 20*R* configurations with a number of ring A substituents. Topoisomerase I (T-I) inhibition data (IC_{50}) have been obtained by standard procedures. In general, substitution at the 9 or 10 positions with amino, halogeno, or hydroxyl groups in compounds with 20*S* configuration results in compounds with enhanced T-I inhibition. Compounds in the 20*RS* configuration were less active in vitro and in vivo and those in the 20*R* configuration were inactive. Compounds with 10,11-methylenedioxy substitution on ring A displayed a marked increase in potency in the T-I inhibition assay. The activities of some of the analogs as determined in a variety of in vivo assays including the L-1210 mouse leukemia assay were, in general, in accord with T-I inhibition. A number of water-soluble analogs such as 20-glycinate esters, 9-glycinamides, or hydrolyzed lactone salts were prepared and tested in in vitro and in vivo assays. In general, these compounds were less active than CPT both in terms of T-I inhibition and life prolongation in the L-1210 assay. However, certain 20-glycinate esters showed good in vivo activity after iv administration.

Introduction

Camptothecin (1, CPT; Chart I), an optically active (20*S*) alkaloid from a tree, *Camptotheca acuminata*, which is native to China, was isolated by Wall and co-workers 27 years ago.[2] It aroused immediate interest as a potential cancer chemotherapeutic agent because of the high activity found for the compound in the L1210 mouse leukemia assay.[3] Because 1 was water insoluble it was tested clinically in the form of the water-soluble sodium salt **2a**. The clinical results with **2a** were disappointing,[4–6] and interest in 1 decreased. It was not realized at the time that the salt **2a** with the open lactone ring was actually only $^1/_{10}$ as active as 1.[7] Moreover, structure–activity studies initiated as early as 1969 showed conclusively that the intact α-hydroxy lactone ring was essential for antitumor activity.[8] The discovery in 1985 that 1 uniquely inhibited the activity of the enzyme topoisomerase I (T-I)[9] resulted in a rapid resurgence of interest, not only in 1 but also in more potent synthetic and/or semisynthetic analogs.[1a,10–16]

It was found that in the case of 1 and analogs that there was an excellent, but not absolute, correlation between T-I inhibition and in vivo activity in such assays as life prolongation in the L-1210 mouse leukemia assay[13,17,18] and inhibition of the growth of human colon cancer xenografts in nude mice.[19]

Access to a number of CPT analogs with 20*RS* stereochemistry substituted principally in the A ring was obtained previously in our laboratory as a consequence of the development of a facile synthesis involving Friedlander reaction of appropriately substituted *o*-aminobenzaldehyde derivatives with the tricyclic (*RS*)-hydroxy ketone **3a**.[1a,7,10–12] Subsequently, we developed a procedure for resolving **3a** into the corresponding *S* and *R* isomers **3b** and **3c**, respectively,[20] thus permitting the synthesis of the (20*S*)- and (20*R*)-CPT analogs previously available only in the racemic 20*RS* configuration. In this paper we present the preparation and T-I inhibition activities of a number of new 20*S* and 20*R* analogs of 1 and, where available, *in vivo* data on some of these compounds. Since most of these analogs of 1 are water insoluble, we have prepared a number of water-soluble sodium salts or hydrochlorides for which both the preparation, T-I inhibition, and, where available, *in vivo* activity are presented.

Chemistry

Friedlander Reaction Conditions. The various ring A substituted analogs were prepared by the Friedlander condensation of the key oxytricyclic synthon **3** with the appropriately substituted *o*-aminobenzaldehyde **4**. Generally, this reaction was conducted in refluxing toluene solution in the presence of *p*-toluenesulfonic acid (*p*-TsOH). However, when nitro groups were present as substituents in **4**, such as 2-amino-6-nitrobenzaldehyde (**4a**) and 2-amino-5-nitrobenzaldehyde (**4b**), this condition was insufficiently vigorous, and it was necessary to conduct the reaction in acetic acid–hydrochloric acid solution utilizing **3** or the corresponding ketal and **4** or the corresponding protected acetal. This method proved superior for the preparation of 10-nitro compounds **5d** and **5e** as compared to the neat fusion process we reported earlier for the synthesis of **5d**.[11]

Nitro and Amino Analogs. Reaction of **3a**, **3b**, or **3c** (as ketals) with **4a** (as acetal) in glacial acetic acid–concentrated hydrochloric acid yielded the corresponding 9-nitro-CPT analogs **5a**, **5b**, and **5c**, respectively.[21] Reaction of **3a** and **3b** with **4b** gave the corresponding 10-nitro-CPT analogs **5d** and **5e**, respectively. The corresponding 9-amino analogs **5f–h** were prepared by reduction of the nitro group.[22] Reduction of **5d** and **5e** gave 10-amino-(20*RS*)-CPT (**5i**) and 10-amino-(20*S*)-CPT (**5j**), respectively.

Reduction of the nitro groups of the various analogs **5a–e** has at times presented difficulties. We have utilized

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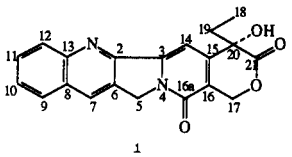
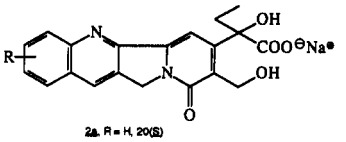
• Abstract published in *Advance ACS Abstracts*, August 15, 1993.

0022-2623/93/1836-2689$04.00/0

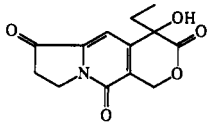
Chart I



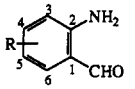
1



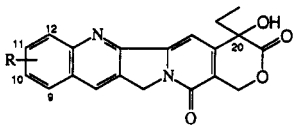
2a, R = H, 20(S)
2b, R = 10-ONa, 20(S)
2c, R = 9-NH_2, 20(RS)
2d, R = 10,11-OCH_2O-, 20(RS)
2e, R = 10,11-OCH_2O-, 20(S)
2f, R = 9-NH_2-10,11-OCH_2O-, 20(S)
2g, R = 10,11-OCH_2O-, 20(R)
2h, R = H, 20(RS)
2i, R = 9-NH_2-10,11-OCH_2O-, 20(RS)



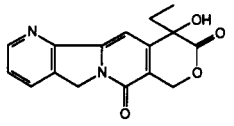
3a, RS
3b, S
3c, R



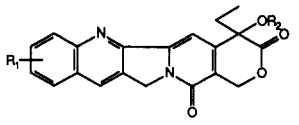
4a, R = 6-NO2
4b, R = 6-NO2
4c, R = 6-Cl
4d, R = 5-Cl
4e, R = 6-F
4f, R = 5-F
4g, R = 6-Br
4h, R = 5-Br
4i, R = 5,6-Cl_2
4j, R = 6-OMe
4k, R = 6-Me
4l, R = 5-Me
4m, R = 4,5-OCH_2O-
4n, R = 4,5-$O(CH_2)_2O$-
4o, R = 5,6-OCH_2O-



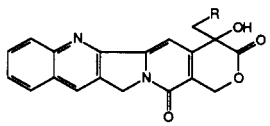
5a, R = 9-NO_2, 20(RS)
5b, R = 9-NO_2, 20(S)
5c, R = 9-NO_2, 20(R)
5d, R = 10-NO_2, 20(RS)
5e, R = 10-NO_2, 20(S)
5f, R = 9-NH_2, 20(RS)
5g, R = 9-NH_2, 20(S)
5h, R = 9-NH_2, 20(R)
5i, R = 10-NH_2, 20(RS)
5j, R = 10-NH_2, 20(S)
5k, R = 9-Cl, 20(S)
5l, R = 10-Cl, 20(S)
5m, R = 9-F, 20(S)
5n, R = 10-F, 20(S)
5o, R = 9-Br, 20(S)
5p, R = 10-Br, 20(S)
5q, R = 9,10-Cl_2, 20(S)
5r, R = 9-OCH_3, 20(RS)
5s, R = 9-OH, 20(RS)
5t, R = 9-OH, 20(S)
5u, R = 9-CH_3, 20(S)
5v, R = 10-CH_3, 20(S)
5w, R = 10-CN, 20(S)
5x, R = 10-COOH, 20(S)
5y, R = 10,11-OCH_2O-, 20(RS)
5z, R = 10,11-OCH_2O-, 20(S)
5aa, R = 10,11-OCH_2O-, 20(R)
5ab, R = 9-NO_2-10,11-OCH_2O-, 20(S)
5ac, R = 9-NO_2-10,11-OCH_2O-, 20(R)
5ad, R = 9-NO_2-10,11-OCH_2O-, 20(RS)
5ae, 9-NH_2-10,11-OCH_2O-, 20(RS)
5af, 9-NH_2-10,11-OCH_2O-, 20(S)
5ag, 9-NH_2-10,11-OCH_2O-, 20(R)
5ah, 9-Cl-10,11-OCH_2O-, 20(S)
5ai, 10,11-$O(CH_2)_2O$-, 20(RS)
5aj, 9-10-OCH_2O-, 20(RS)
5ak 10-OH, 20(S)
5al, 9-$(CH_3)_2NCH_2$-10-OH, 20(S)
5am, 10-(4-methylpiperizino)-carbonyloxy-, 20(S)
5 an, 10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-, 20(S)
5ao, R = H, 20(R)
5ap, R = 9-Cl, 20(RS)
5aq, R = H, 20(RS)
5ar, R = 9-NH2-10,12-Br_2-, 20(RS)
5as, R = 10-CH_3, 20(RS)
5at, R = 10-Cl, 20(RS)
5au, R = 9-$(CH_3)_2NCH_2$-10-OH·HCl



6, 20(RS)



7a, R_1 = 9-NHCOCH$_2$NH-t-BOC, R_2 = H, 20(RS)
7b, R_1 = 9-NHCOCH$_2$NH$_2$·HCl, R_2 = H, 20(RS)
7c, R_1 = 9=NHCOCH$_2$NH-t-BOC-10,11-OCH_2O-, R_2 = H, 20(RS)
7d, R_1 = 9-NHCOCH$_2$NH$_2$·HCl-10,11-OCH_2O-, R_2 = H, 20(RS)
7e, R_1 = 9-NO_2, R_2 = COCH$_2$NH-t-BOC, 20(RS)
7f, R_1 = 9-NH_2, R_2 = COCH$_2$NH-t-BOC, 20(RS)
7g, R_1 = 9-NH_2, R_2 = COCH$_2$NH$_2$·HCl, 20(RS)
7h, R_1 = 10-NO_2, R_2 = COCH$_2$NH-t-BOC, 20(RS)
7i, R_1 = 10-NH_2, R_2 = COCH$_2$NH-t-BOC, 20(RS)
7j, R_1 = 10-NH_2, R_2 = COCH$_2$NH$_2$·HCl, 20(RS)
7k, R_1 = 10,11-OCH_2O-, R_2 = COCH$_2$NH-t-BOC, 20(RS)
7l, R_1 = 10,11-OCH_2O-, R_2 = COCH$_2$NH$_2$·HCl, 20(RS)



8a, R = CH_2Br, 20(RS)
8b, R = H, 20(RS)
8c, R = CH_2OH, 20(RS)

various conditions for this purpose, including palladium on charcoal, ferrous sulfate, nickel boride, and most recently, stannous chloride. Stannous chloride has given particularly good results in the reduction of **5b** to **5g**,[23] and **5e** to **5j**, and **5ab** to **5af**.

9- or 10-Halogenated Analogs. 9-Chloro-(20*S*)-CPT (**5k**) was prepared by reaction of **3b** with 2-amino-6-chlorobenzaldehyde (**4c**)[24] in toluene–acetic acid in the presence of *p*-TsOH. By similar reaction with **3b**, 10-chloro-(20*S*)-CPT (**5l**) was prepared from 2-amino-5-chlorobenzaldehyde (**4d**),[25] 9-fluoro-(20*S*)-CPT (**5m**) was prepared from 2-amino-6-fluorobenzaldehyde (**4e**) (derived from the nitroaldehyde precursor),[26] and 10-fluoro-(20*S*)-CPT (**5n**) resulted from 2-amino-5-fluorobenzaldehyde (**4f**).[25] 9-Bromo-(20*S*)-CPT (**5o**) and 10-bromo-(20*S*)-CPT (**5p**) were prepared in a similar manner from reaction of **3b** with 2-amino-6-bromobenzaldehyde (**4g**) (derived from a nitro precursor in analogy to **4c**)[24] and 2-amino-5-bromobenzaldehyde (**4h**) (from the nitro precursor as for **4f**),[25] respectively, and 9,10-dichloro-(20*S*)-CPT (**5q**) was prepared from **3b** and 2-amino-5,6-dichlorobenzaldehyde (**4i**) (derived from the reported nitroaldehyde precursor).[27]

Other Camptothecin Analogs. 9-Hydroxycamptothecin. 9-Methoxy-(20*RS*)-CPT (**5r**)[28] was prepared in the usual manner from **3a** and 2-amino-6-methoxybenzaldehyde (**4j**).[29] 9-Hydroxy-(20*RS*)-CPT (**5s**) was prepared by demethylation of **5r** with aluminum chloride. The activity of **5s** was surprisingly low in vitro and in vivo (cf. **5s**, Table II and Table V). In order to confirm this unexpected observation, the corresponding 20*S* analog **5t** was prepared by an alternate route involving diazotization of the 9-amino analog **5g** followed by hydrolysis to **5t** in aqueous sulfuric acid. However, the T-1 inhibition activity of **5t** was also much lower than expected; compare **5ak** and **5t** (Table I).

9- and 10-Methyl-(20*S*)-camptothecin. Reaction of **3b** with 2-amino-6-methylbenzaldehyde (**4k**), prepared in four steps from commercially available 6-methyl-2-nitrobenzoic acid[30] (cf. Experimental Section) gave 9-methyl-(20*S*)-CPT (**5u**).[31] In a similar manner, as reported for the *RS* compound,[12] 10-methyl-(20*S*)-CPT (**5v**) was prepared by reaction of **3b** with 2-amino-5-methylbenzaldehyde (**4l**).

10-Carboxy-(20*S*)-camptothecin (5x). Reaction of the 10-bromo analog (**5p**) with cuprous cyanide[32] gave the corresponding 10-cyano derivative **5w** which was then hydrolyzed to give acid **5x**.

(Methylenedioxy) Analogs. The methylenedioxy moiety, when introduced into ring A of **1** at the C-10/C-11 position greatly potentiates the biological activity of **1** (cf., Results and Discussion). Reaction of **3a**, **3b**, and **3c** with the amino aldehyde **4m**[11] in toluene solution in the presence of *p*-TsOH gave, respectively, the known 10,11-(methylenedioxy)-(20*RS*)-CPT **5y**[11] and the new 20*S* and 20*R* analogs, **5z** and **5aa**. Nitration of **5y**, **5z**, and **5aa** in cold sulfuric–nitric acid gave exclusively the corresponding 9-nitro-10,11-(methylenedioxy) analogs **5ad**, **5ab**, and **5ac** and after catalytic hydrogenation yielded for the first time the 9-amino 20*RS*, 20*S*, and 20*R* analogs **5ae**, **5af**, and **5ag**, respectively. The *in vitro* and *in vivo* activities of these compounds will be discussed in detail in the Results and Discussion. It can be stated at this point that both **5ae** and **5af** are highly active, potent compounds which exhibit much less toxicity *in vivo* than **5y** and **5z**. Diazotization of **5af** with sodium nitrite–HCl followed by reaction with cuprous chloride gave 9-chloro-10,11-(methylenedioxy)-CPT (**5ah**). 10,11-(Ethylenedioxy)-(20*RS*)-

CPT (**5ai**) was prepared by reaction of **3a** with 2-amino-4,5-(ethylenedioxy)benzaldehyde (**4n**) (derived from the nitroaldehyde precursor)[33] in toluene–acetic acid in the presence of *p*-TsOH. In a similar manner, **3a** was treated with **4o** (from nitro precursor)[34] to give 9,10-(methylenedioxy)-(20*RS*)-CPT (**5aj**).

Tetracyclic De-A-Ring Analog of CPT. A prediction was made some years ago based on theoretical principles that a tetracyclic (A-ring removed) analog **6** might be active.[35] Reaction of **3a** with β-amino acrolein[36] in the presence of ammonium acetate yielded the inactive 20*RS* de-A-ring analog **6** (cf. Table II) in very low yield.

Water-Soluble Analogs. A number of water-soluble analogs of **1** have been prepared. Since the methodology is quite similar for a variety of structural analogs, only a few examples of each type will be presented.

Sodium Salts. A number of water-soluble sodium carboxylate salts of **1** and analogs were readily prepared by reaction of the parent compound with equivalent quantities (molar basis) of sodium hydroxide in aqueous methanol. Thus the sodium salts **2a–d** were prepared from the corresponding lactones.

Hydrochloride Salts. 9-Glycinamide Hydrochloride Salts.[37] 9-Amino-(20*RS*)-CPT (**5f**) on treatment with *t*-BOC-glycine in the presence of dicyclohexylcarbodiimide (DCC) was converted to the corresponding amide derivative **7a**, and on treatment with hydrogen chloride–saturated dioxane, the water-soluble 9-glycinamido-(20*RS*)-CPT hydrochloride (**7b**) was formed. In a similar manner, 9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT (**5ae**) was converted to **7c** and then to 9-glycinamido-10,11-(methylenedioxy)-(20*RS*)-CPT hydrochloride (**7d**).

20-Glycinate Ester Hydrochloride Salts. The introduction of the same *t*-BOC-glycine moiety as an ester at C-20 was accomplished by the same process with 4-(*N*,*N*-dimethylamino)pyridine (DMAP) added as a catalyst. Thus 9-nitro-(20*RS*)-CPT (**5c**) afforded glycinate ester **7e**, which after catalytic hydrogenation over Pd/C gave **7f** and after treatment with hydrogen chloride provided **7g**. In a similar fashion, 10-nitro-(20*RS*)-CPT (**5d**) gave ester **7h** and then the glycinate ester hydrochloride **7j** after hydrogenation to **7i** followed by hydrolysis. 10,11-(Methylenedioxy)-(20*RS*)-CPT (**5y**) was converted to the 20-glycinate ester **7k** by esterification with *t*-BOC-glycine in the presence of DCC and DMAP, and subsequent treatment with hydrogen chloride in dioxane afforded the water-soluble hydrochloride salt **7l**.

Results and Discussion

As a consequence of the successful resolution of the tricyclic synthon **3a**, the chiral synthons **3b** and **3c** are now readily available.[20] Friedlander reactions of **3b** and **3c** with appropriately substituted *o*-aminobenzaldehydes have yielded for the first time chiral (20*S*)- and (20*R*)-CPT analogs, respectively. Table I presents T-I inhibition IC_{50} values for 20*S* and 20*R* analogs substituted on the 9, 10, or 10,11-positions and Table II gives similar data for various 20*RS* analogs.

The compounds are listed in order of decreasing potency against purified T-I (as assessed by formation of the cleavable complex), and the discussion will relate in this case to the numerical listings of the IC_{50} potency data. Four distinct areas of potency can be more or less arbitrarily assigned: class 1, compounds with IC_{50} values between 0.01 and 0.10 μM; class 2, compounds with values between 0.1 and 1.0 μM; class 3, compounds with values between 1.0 and 10.0 μM; and class 4, compounds with values >10.0 μM.

Table I. Topoisomerase I Inhibition by Cleavable Complex Formation by CPT Analogs

compound	IC_{50} (μM)[a]	SE
10,11-(methylenedioxy)-(20*S*)-CPT (**5z**)	0.027	0.0050
9-methyl-(20*S*)-CPT (**5u**)	0.038	0.013
9-amino-10,11-(methylenedioxy)-(20*S*)-CPT (**5af**)	0.048	0.016
9-chloro-10,11-(methylenedioxy)-(20*S*)-CPT (**5ah**)	0.061	0.019
9-chloro-(20*S*)-CPT (**5k**)	0.086	0.054
10-hydroxy-(20*S*)-CPT (**5ak**)[b]	0.11	0.031
9,10-dichloro-(20*S*)-CPT (**5q**)	0.11	0.029
9-amino-(20*S*)-CPT (**5g**)	0.11	0.024
10-bromo-(20*S*)-CPT (**5p**)	0.13	0.040
10-amino-(20*S*)-CPT (**5j**)	0.14	0.022
10-chloro-(20*S*)-CPT (**5l**)	0.14	0.027
9-nitro-10,11-(methylenedioxy)-(20*S*)-CPT (**5ab**)	0.15	0.043
9-fluoro-(20*S*)-CPT (**5m**)	0.16	0.074
10-methyl-(20*S*)-CPT (**5v**)	0.30	0.080
10-fluoro-(20*S*)-CPT (**5n**)	0.37	0.19
10-nitro-(20*S*)-CPT (**5e**)	0.64	0.12
(20*S*)-CPT (**1**)	0.68	0.22
10,11-(methylenedioxy)-(20*S*)-CPT Na salt (**2e**)	0.84	0.68
9-hydroxy-(20*S*)-CPT (**5t**)	0.87	0.30
10-carboxy-(20*S*)-CPT (**5x**)	1.0	0.32
9-[(dimethylamino)methyl]-10-hydroxy-(20*S*)-CPT (**5al**)[c]	1.0	0.44
9-[(dimethylamino)methyl]-10-hydroxy-(20*S*)-CPT·HCl (**5au**)[c]	1.1	0.12
10-cyano-(20*S*)-CPT (**5w**)	1.9	0.36
10-hydroxy-(20*S*)-CPT $2Na^+$ salt (**2b**)	3.2	0.60
9-amino-10,11-(methylenedioxy)-(20*S*)-CPT Na^+ salt (**2f**)	4.3	2.5
10-[[(4-methylpiperazino)carbonyl]oxy]-(20*S*)-CPT (**5am**)[d]	4.4	6.5
(20*S*)-CPT Na^+ salt (**2a**)[e]	12	5.9
10-[[(4-piperidinopiperidino)carbonyl]oxy]-(20*S*)-CPT (**5an**)[d]	21	19
10,11-(methylenedioxy)-(20*R*)-CPT Na^+ salt (**2g**)	>30	
9-amino-10,11-(methylenedioxy)-(20*R*)-CPT (**5ag**)	>30	
10,11-(methylenedioxy)-(20*R*)-CPT (**5aa**)	>30	
(20*R*)-CPT (**5ao**)[f]	>30	

[a] IC_{50} = the minimum drug concentration (μM) that inhibited the cleavable complex formation by 50%. [b] Reference 29. [c] Reference 15. [d] Reference 16. [e] Reference 2. [f] Reference 20.

Table III, which contains data from Tables I and II, shows that the 20*S* form of a particular CPT analog is always much more potent than the 20*R* form and is approximately twice as potent as the 20*RS* form (range 1.5–4.0 fold). The same rank order of potency observed against purified T-I was also observed when an assay for the formation of the cleavable complex was conducted in intact HL-60 cells (Table IV).

The compounds with maximal IC_{50} values, for example classes 1 and 2, in general are water insoluble 20*S* and 20*RS* analogs substituted at the 9 or 10 position. CPT analogs with amino, hydroxyl, halogeno, or methyl substituents show in general high T-I inhibition activity. The relatively low activity of 9-hydroxy-CPT is an exception. The presence of a 10,11-(methylenedioxy) substituent greatly enhances the basic CPT activity in T-I inhibition either when present singly or when 9-amino or 9-chloro substituents are also present (cf. **5z**, **5af**, and **5ah**, Table I). Planarity of the substituents in ring A appears crucial for enhancing biological activity. For example, the 10,11-(ethylenedioxy) analog **5ai** (Table II) is only about one-

Table II Topoisomerase I Inhibition by Cleavable Complex Formation by Racemic CPT Analogs

compound	IC_{50} (mM)[a]	SE
10,11-(methylenedioxy)-(20*RS*)-CPT (**5y**)	0.067	0.038
9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT (**5ae**)	0.076	0.030
9-chloro-(20*RS*)-CPT (**5ap**)[b]	0.19	0.088
9-nitro-10,11-(methylenedioxy)-(20*RS*)-CPT (**5ad**)	0.22	0.11
9-glycinamido-10,11-(methylenedioxy)-(20*RS*)-CPT (**7d**)	0.26	0.057
10,11-(methylenedioxy)-20-glycinyl-(20*RS*)-CPT·HCl (**7l**)	0.43	0.22
9-amino-(20*RS*)-CPT (**5f**)	0.44	0.12
10,11-(ethylenedioxy)-(20*RS*)-CPT (**5ai**)	0.47	0.14
10,11-(methylenedioxy)-(20*RS*)-CPT Na^+ salt (**2d**)	0.55	0.18
9-glycinamido-(20*RS*)-CPT·HCl (**7b**)	0.57	0.083
9-hydroxy-(20*RS*)-CPT (**5s**)	0.90	0.61
(20*RS*)-CPT (**5aq**)[c]	1.4	0.26
9-methoxy-(20*RS*)-CPT (**5r**)	1.6	0.17
(20*RS*)-CPT Na^+ salt (**2h**)	2.0	0.42
18-bromo-(20*RS*)-CPT (**8a**)[d]	3.1	0.89
9,10-(methylenedioxy)-(20*RS*)-CPT (**5aj**)	3.8	0.63
20-desethyl-20-methyl-(20*RS*)-CPT (**8b**)[e]	4.0	1.4
9-amino-20-glycinyl-(20*RS*)-CPT·HCl (**7g**)	5.5	5.4
9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT Na^+ salt (**2i**)	5.5	4.2
18-hydroxy-(20*RS*)-CPT (**8c**)[d]	9.6	3.1
9-amino-(20*RS*)-CPT Na^+ salt (**2c**)	13	5.7
9-amino-10,12-dibromo-(20*RS*)-CPT (**5ar**)[f]	>30	
de-A-ring-(20*RS*)-CPT (**6**)	>30	

[a] See footnote *a* of Table I. [b] Reference 12. [c] Reference 7. [d] Obtained from a mixture of **8a** and **8c** resulting from the corresponding 18-methoxy analog[7] upon treatment with refluxing aqueous HBr; unpublished results. [e] Prepared by total synthesis in a manner similar to that of (20*RS*)-CPT **95a**);[7] unpublished results. [f] Prepared from 9-amino-(20*RS*)-CPT (**5f**) by treatment with Br_2 in HOAc; unpublished results.

Table III Comparison of Topoisomerase I Inhibition of (20*S*), (20*RS*), and (20*R*)-CPT Compounds

compound	IC_{50} (μM)[a]
(20*S*)-CPT (**1**)	0.52
(20*RS*)-CPT (**5aq**)	1.4
(20*R*)-CPT (**5ao**)	>60
9-amino-(20*S*)-CPT (**5g**)	0.12
9-amino-(20*RS*)-CPT (**5f**)	0.50
10,11-(methylenedioxy)-(20*S*)-CPT (**5z**)	0.03
10,11-(methylenedioxy)-(20*RS*)-CPT (**5y**)	0.08
10,11-(methylenedioxy)-(20*R*)-CPT (**5aa**)	>60
9-amino-10,11-(methylenedioxy)-(20*S*)-CPT (**5af**)	0.05
9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT (**5ae**)	0.07
9-amino-10,11-(methylenedioxy)-(20*R*)-CPT (**5ag**)	>60

[a] See footnote *a* for Table I.

Table IV Cleavable Complex Formation by Racemic CPT Analogs on Intact HL-60 Cells

compound	IC_{50} (μM)[a]	SE[a]
10,11-(methylenedioxy)-(20*RS*)-CPT (**5y**)	0.038	0.02
9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT (**5ae**)	0.069	0.005
9-amino-(20*RS*)-CPT (**5f**)	0.84	0.03
(20*RS*)-CPT (**5aq**)	1.6	0.2
10,11-(methylenedioxy)-20-*O*-glycinyl-(20*RS*)-CPT·HCl (**7l**)	2.5	0.2
9-amino 20-*O*-glycinyl(20*RS*)-CPT·HCl (**7g**)	6.8	2
9-glycinamido-10,11-(methylenedioxy)-(20*RS*)-CPT·HCl (**7d**)	>30	
9-glycinamido-(20*RS*)-CPT·HCl (**7b**)	>30	

[a] See footnote *a* for Table I.

seventh as potent in T-I inhibition as the corresponding 10,11-(methylenedioxy) analog **5y**. Moreover the location of the planar substituent in ring A also appears to be crucial for activity. Thus the planar analog, 9,10-(methylenedioxy)-(20*RS*)-CPT (**5aj**) has only $^1/_5$ the activity of **5y**. Water-soluble analogs which cannot undergo bioactive transformation in the T-I assay, or which interact poorly with the DNA–T-I cleavable complex,[13,14] show poor to modest activity in T-I inhibition. Compare in Table I, for example, **5z** with the corresponding sodium salt **2e** or 10-hydroxy-(20*S*)-CPT (**5ak**) with the water-soluble hydroxy analogs of CPT-11,[16] **5am**, and **5an**, which are inactive until hydrolyzed to the free 10-hydroxy compound; in Table II, in the 20*RS* series of the 9-amino analogs, compare **5f** with **7g** or **5ae** with **2i**.

As shown in Table I, all the 20*R* analogs are essentially inactive in T-I inhibition. (20*R*)-CPT (**5ao**) has been shown to be inactive also in 9KB cytotoxicity and in the *in vivo* L1210 mouse leukemia assay.[13,20]

Steric factors which either enhance or prevent interaction of CPT analogs with the DNA–T-I cleavable complex are of great importance. In previous reports,[11-13] we have commented on the adverse effects on T-I inhibition and/or *in vivo* antitumor activity of substituents at positions 11 or 12 or substituents at both positions 10 and 11, as with 10,11-dimethoxy groups or disubstitution at positions 9 and 10.

Marked enhancements of both T-I inhibition and in vivo activity are seen with various 10,11-(methylenedioxy) analogs. Molecular modeling studies have shown that such compounds have much higher maximum values (kcal mol^{-1}) than 1 and many other CPT analogs.[38] It is attractive to speculate that the higher energy of the 10,-11-(methylenedioxy) analogs contributes to the greater activity of such compounds in T-I inhibition.

In many, but not all, cases, the relationship between the T-I inhibition potency of a CPT analog (Tables I–IV) and *in vivo* antitumor activity (Tables V–VII) is in good agreement, but this relationship is subject to many other factors which must be considered. If a compound is a prodrug which must be converted to the active species *in vivo*, then inactivity in the T-I assay cannot be related to the compound's *in vivo* potential. Thus, with the exception of prodrugs **5am** and **5an**, the other compounds in categories 3 and 4 with weak or no T-I inhibition are marginally active or inactive *in vivo*.

The analogs of (20*S*)-CPT (**1**) which have shown *both* strong T-1 inhibition and excellent L1210 *in vivo* life prolongation activity are 9-amino (**5g**), 10-amino (**5j**), 9-amino-10,11-(methylenedioxy) (**5af**), and 9-methyl (**5u**) analogs. Both the 10,11-(methylenedioxy) 20*S* (**5z**) and the corresponding 20*RS* analogs are potent T-I inhibitors (cf. Tables I and II) but are too toxic for potential therapeutic use. When 9-amino- or 9-chloro substituents are in conjunction with the 10,11-(methylenedioxy) moiety, toxicity is considerably reduced. Thus when the 9-amino-10,11-(methylenedioxy) analog **5af** (Table I) was tested in the L1210 mouse leukemia assay, the majority of the mice survived for 60 days with T/C values of 536 and were tumor free (cf. Table V).

The majority of the water-soluble analogs prepared by us were less potent *in vitro* and *in vivo* (cf. Tables I, II, and VI). We have prepared three types of water-soluble analogs (cf. Table VI). Typical examples are (1) sodium salts of the carboxylic acid obtained by hydrolytic cleavage of the ring E lactone,[1] (2) 20-glycinate ester hydrochloride salts,[39] and (3) 9-glycinamido hydrochloride salts reported for the first time in this paper. The objective was to

Table V. L1210 Mouse Leukemia Life Prolongation by Water-Insoluble CPT Analogs[a,b]

compound	highest active dose, mg/Kg (% T/C)[c]	active dose range, mg/kg	KE[d]	cures	toxic dose, mg/kg
(20*S*)-CPT (**1**)	12 (250)	5.3–12	4.8	1/6	10
9-amino-(20*S*)-CPT (**5g**)	5 (361)	0.6–5	5.97	3/6	10
10-amino-(20*S*)-CPT (**5j**)[e]	4.5 (565)	2–4.5	>5.99	6/6	
9-nitro-(20*S*)-CPT (**5b**)	10 (348)	1.25–20	5.97	5/6	40
9-amino-10,11-(methylenedioxy)-(20*S*)-CPT (**5af**)[f]	1.5 (536)	0.3–1.5	5.8	4/6	
9-chloro-(20*S*)-CPT (**5k**)	12 (150)	2.37–12.00	0	0/6	
9-methyl-(20*S*)-CPT (**5u**)	8 (274)	2.37–8.00	5.6	4/6	12
9-hydroxy-(20*RS*)-CPT (**5s**)	20 (134)	10–40		0/6	
10-nitro-(20*RS*)-CPT (**5d**)	7.25 (233)	3.63–15.5	5.97	0/6	31
10-methyl-(20*RS*)-CPT (**5as**)[g]	10 (207)	10–80	4.66	0/6	
10-amino-(20*RS*)-CPT (**5i**)	3.75 (365)	1.35–3.74	5.97	3/6	6.25
10-chloro-(20*RS*)-CPT (**5at**)[g]	10 (280)	5–20	5.97	2/6	40
10,11-(methylenedioxy)-(20*RS*)-CPT (**5y**)[g]	2 (325)	2–4	5.97	2/6	8.0

[a] Intraperitoneal implants. [b] Drug dosing ip on days 1 and 5. [c] % TC = (median survival time of treated/control animals) × 100. [d] log of initial tumor cell population minus log of tumor cell population at the end of treatment. [e] 100% long term (day 45) tumor-free survivors. [f] 4/6 long term (day 60) tumor-free survivors. [g] For synthesis, cf. ref 12.

Table VI. L1210 Life Prolongation[a] by Water-Soluble CPT Analogs

compound	dose regimen[b]	route	highest active dose, mg/kg (% T/C)[c]	active dose range, mg/kg	KE[d]	cures	toxic dose, mg/kg
9-amino-20-glycinyl-(20*RS*)-CPT·HCl (**7g**)	Q04DX02[b]	ip	10 (132)	10	−1.00	0	NT[e] at 10
9-amino-20-glycinyl-(20*RS*)-CPT·HCl (**7g**)	Q04HX02	iv	5 (168)	2.5–5.0	1.67	1/6	NT at 5
10-amino-20-glycinyl-(20*RS*)-CPT·HCl (**7j**)	Q04HX02	iv	20 (225)	1.25–20	>5.97	0	NT at 20
10,11-(methylenedioxy)-20-glycinyl-(20*RS*)-CPT·HCl (**7l**)	Q04HX02	iv	10 (236)	1.25–20	5.97	3/6	NT at 20
9-glycinamido-(20*RS*)-CPT·HCl (**7b**)	Q04HX02	iv	20 (180)	1.25–20	2.95	0	NT at 20
10,11-(methylenedioxy)-(20*RS*)-CPT Na^+ salt (**2d**)	Q04HX02	iv	10 (157)	2.85–10	4.79	0	NT at 20
10-hydroxy-(20*S*)-CPT $2Na^+$ salt (**2b**)	Q04HX02	iv	20 (184)	2.5–20	3.24	0	NT at 20

[a,c,d] For footnotes *a*, *c*, and *d* see Table V. [b] Q04DX02 = ip injection on days 1 and 5. Q04HX02 = iv drug dosing on hours 1 and 5. [e] NT = not toxic.

Table VII. *In Vivo* Antitumor Activity of (20*S*) and (20*RS*)-CPT Analogs

compound	dose, mg/kg	L1210[a]	RAW117-H10[a]	K1735-M2[b]	HT-29[c]
9-amino-(20*S*)-CPT (**5g**)	2.5	2.44	1.15	73	25
10,11-(methylenedioxy)-(20*S*)-CPT Na^+ salt (**2e**)	4	2.13	1.36	15	40
10,11-(methylenedioxy)-(20*S*)-CPT (**5z**)	3	1.49	1.33	9	18
9-amino-10,11-(methylenedioxy)-(20*S*)-CPT Na^+ salt (**2f**)	3.5	2.56	1.75	51	38
9-amino-10,11-(methylenedioxy)-(20*S*)-CPT (**5af**)	1.5	1.98	1.25	17	17
10,11-(methylenedioxy)-(20*RS*)-CPT Na^+ Salt (**2d**)	6	2.13	1.32	4	
10,11-(methylenedioxy)-(20*RS*)-CPT (**5y**)	3	1.22	1.04	65	
9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT Na^+ Salt (**2i**)	6	1.78	1.40	98	
9-amino-10,11-(methylenedioxy)-(20*RS*)-CPT (**5ae**)	3	3.00	1.60	25	

[a] Relative survival (T/C); compounds were injected sc on days 1, 4, 6, 8; L-1210 cells were implanted ip and RAW 177 cells were implanted iv. [b] Number of metastases (% of control); compounds were injected sc on days 1, 4, 6, 8. [c] Tumor volume (% of control); compounds were injected sc on days 16, 20, 23, 27, 30, 34, 37, 41, 44, 48.

determine if the glycinate ester and 9-glycinamido hydrochloride salts would be hydrolyzed by esterases and amidases known to be present in human plasma and tissues or whether facile ring closure of sodium salts would occur in vivo. The results shown in Table VI are typical of a much larger body of data. Although much more testing would be required for a definitive conclusion, it would appear that certain water-soluble 20-glycinate esters, when administered at 10–20 mg/kg, afforded substantial life prolongation when assayed in L1210 mouse leukemia (cf. **7j** and **7l**, Table VI). It should be noted that compounds **7j** and **7l**, reported in Table VI, were 20*RS* analogs. More active compounds might be anticipated from the corresponding 20*S* analogs. Results obtained with water-soluble sodium salts or with 9-glycinamides were less favorable (cf. Table VI).

At present CPT (**1**) and three CPT analogs, 9-amino-(20*S*)-CPT (**5g**), 9-[(dimethylamino)methyl]-10-hydroxy-CPT·HCl (topotecan, **5au**), and CPT-11, a 7-ethyl analog of **5an**, are in clinical trial. The water-insoluble **1** is in phase I clinical trial administered orally. The water-insoluble **5g** and the water-soluble analogs topotecan and CPT-11, all administered by IV infusion, are in phase I or phase II trial.

Data from initial clinical trials of **1** and **5g** are as yet not available. Initial phase I and phase II trials with CPT-11 have shown objective responses to lung, colorectal, ovarian, and cervical cancers.[40a,b] Topotecan has also given responses in lung, ovarian, and colorectal cancer.[41a,b]

Several other CPT analogs may eventually receive consideration for clinical trial. These include the 10-amino analog **5j** and 9-amino-10,11-(methylenedioxy) analog **5af**, both highly active in L1210 mouse leukemia assay (cf. Table V). Compound **5af** has recently been shown to have considerable cytotoxicity toward β-lymphocytes obtained from patients with β-cell chronic lymphocytic leukemia (CLL) with activity much greater than chlorambucil currently in clinical use for CLL patients or other CPT analogs.[42]

Experimental Section

Chemistry. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were recorded on either a Perkin-Elmer 267 spectrophotometer or a

Shimadzu Model IR-460 spectrophotometer. Proton NMR spectra were obtained at 90 MHz on a Varian EM-390 spectrometer or at 250 MHz on a Bruker WM-250 Supercon. High-resolution mass spectra were determined by an Associated Electrical Industries MS-902, and elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA; analyses were correct within ±0.4% of the formulas shown. Where anhydrous conditions were required, a nitrogen atmosphere was employed, and solvents were freshly distilled from CaH_2.

2-Amino-6-nitrobenzaldehyde (4a). A stirred mixture of 2,6-dinitrobenzaldehyde (5.00 g, 25.51 mmol), ethylene glycol (20 mL), *p*-TsOH·H_2O (150 mg), and toluene (250 mL) was refluxed for 4.5 h during which time the H_2O azeotrope was collected (Dean–Stark trap). The yellow solution was cooled and diluted with H_2O (100 mL) and EtOAc (200 mL). After mixing, the EtOAc was collected and the aqueous phase was reextracted with EtOAc (100 mL). The combined extract was dried (Na_2SO_4) and evaporated to afford 2,6-dinitrobenzaldehyde ethylene acetal as a pale yellow solid (6.02 g, 98%). Recrystallization from MeOH gave this compound as colorless rods: mp 107–110 °C; IR ($CHCl_3$) 2910, 1612, 1550, 1366, 1190, 1151, 1110, 1081, 1034, 981, 946, 840 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 3.9 (m, 4, OCH_2CH_2O), 6.4 (s, 1, acetal C-*H*), 7.5–7.9 (m, 3, arom). Anal. ($C_9H_8H_2O_6$) C, H, N.

A stirred mixture of 2,6-dinitrobenzaldehyde ethylene acetal (6.02 g, 25.08 mmol), $Na_2S·9H_2O$ (12.26 g, 51.11 mmol), EtOH (265 mL), and H_2O (80 mL) was refluxed for 30 min. After cooling slightly, the ethanol was distilled from the clear red solution under reduced pressure and the aqueous residue was extracted with $CHCl_3$ (7 × 50 mL). The extract was dried (Na_2SO_4) and evaporated to give 2-amino-6-nitrobenzaldehyde ethylene acetal as an orange-yellow solid (4.73 g, 90%). Recrystallization from MeOH provided the pure product as fine yellow needles: mp 103–105 °C; IR ($CHCl_3$) 3500, 3405, 2904, 1625, 1530, 1480, 1370, 1330, 1315, 1080, 1053, 990, 960, 940 cm^{-1}; 90 MHz ^1H NMR ($CDCl_3$) δ 4.0 (m, 4, OCH_2CH_2O), 4.7 (br s, 2, NH_2), 6.0 (s, 1, acetal *H*), 6.8–7.3 (m, 3, arom). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

A stirred solution of the amino nitro acetal (4.72 g, 22.48 mmol) in THF (150 mL) was treated over 1 min with 2 N aqueous H_2SO_4 (6 mL) to give, after transient turbidity, a clear red-orange solution. After 1 h the THF was removed under reduced pressure, and the residue was mixed with H_2O (30 mL). The mixture was extracted with $CHCl_3$ (2 × 125 mL) and the extract dried (Na_2SO_4) and evaporated to give the desired aldehyde **4a** as an orange-yellow solid (3.32 g, 89%). Recrystallization from heptane gave pure **4a** as a yellow microcrystalline solid: mp 105–107 °C; IR ($CHCl_3$) 3510, 3360, 1670, 1629, 1592, 1550, 1532, 1465, 1356, 1190, 1175, 845 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 6.2–6.9 (br s, 2, NH_2), 6.8–7.4 (m, 3, arom), 10.0 (s, 1, C*H*O). Anal. ($C_7H_6N_2O_3$) C, H, N.

9-Nitro-CPT Enantiomers 5b and 5c and Racemate 5a. 9-Nitro-(20*RS*)-CPT (5a). A stirred suspension of aldehyde **4a** (2.00 g, 12.05 mmol) and tricyclic ketone **3a** (2.13 g, 8.08 mmol) in glacial HOAc (100 mL) was heated to reflux during which time a clear orange solution resulted. Concentrated HCl (15 mL) was added over 3 min, and refluxing was continued for 4 h to give a turbid orange-brown mixture. The solvents were evaporated, and the residue was recrystallized from MeOH/$CHCl_3$ to provide **5a** as a yellow solid (1.62 g). The mother liquor was adsorbed on Celite (10 g), and the resulting powder was chromatographed (SiO_2, 150 g, 0.5% MeOH in $CHCl_3$) to provide additional nitro compound **5a** (1.11 g) for a total of 2.73 g (86%). Recrystallization from MeOH/$CHCl_3$ gave fine pale yellow needles: mp 295–300 °C (for 20*S* enantiomer, lit.[11] mp 190 °C); the IR (KBr) and 250-MHz ^1H NMR (DMSO-d_6) were identical to those reported for the *S* enantiomer **5b**.[11]

Similarly, enantiomer **5b** was prepared in 85% yield from tricyclic ketal **3b** (1.20 g, 3.96 mmol) and the acetal of **4a** (1.10 g, 5.34 mmol). Recrystallization from MeOH gave pale yellow microneedles: mp 192–193 °C (lit.[11] mp 190 °C); $[\alpha]^{21}_D$ −19° (*c* = 0.282, DMF).

Enantiomer **5c** resulted in 90% yield from reaction of ketal **3c** (220 mg, 0.726 mmol) and aminoaldehyde **4a** (220 mg, 1.06 mmol). Data for this compound was identical to that for the racemate **5a** described earlier;[12] $[\alpha]^{22}_D$ +18° (*c* = 0.261, DMF).

10-Nitro-(20*S*)-CPT (5e). A stirred mixture of tricyclic ketal of **3b** (220 mg, 0.726 mmol), aminoaldehyde **4b**[12] (220 mg, 1.067 mmol), concentrated hydrochloric acid (4 mL), and glacial HOAc (20 mL) was refluxed for 10 h. The solvent was removed under reduced pressure to afford crude **5e**, which was chromatographed as a dispersion on Celite (SiO_2, 40 g, 0.5% MeOH in $CHCl_3$) to provide **5e** as a yellow solid (295 mg, 86%). Recrystallization from MeOH provided pure 10-nitro-(20*S*)-CPT (**5e**): mp (Kofler) >300 °C dec (lit.[12] dec); the 250-MHz ^1H NMR (DMSO-d_6) for **5e** matched that for the authentic racemate **5d** prepared earlier;[12] $[\alpha]^{21}_D$ −24° (*c* = 0.274, DMF).

9-Amino-CPT Racemate 5f and Enantiomers 5g and 5h. 9-Amino-(20*RS*)-CPT (5f). A stirred suspension of nitro compound **5a** (1.62 g) and Pd/C (10%, 475 mg) in absolute EtOH (700 mL) was subjected to 1 atm of H_2 for 20 h. The mixture was filtered through Celite, which was then washed gradually with hot MeOH/$CHCl_3$ and hot DMF (450 mL). High-vacuum distillation of the solvents provided crude 9-amino-20(*RS*)-camptothecin (**5f**, 1.52 g) as a brown solid. Recrystallization from MeOH/$CHCl_3$ gave **5f** as a tan-orange solid (1.11 g, 74%). The sample was dispersed on Celite and chromatographed (SiO_2, 150 g, 5% MeOH in $CHCl_3$) to afford the pure racemic amino compound **5b** (0.89 g, 65%): mp darkening at 250 °C with gradual decomposition. The spectral properties [250-MHz ^1H NMR and IR (KBr)] for **5f** were identical to those of the (*S*) enantiomer.[11]

9-Amino-(20*S*)-CPT (**5g**) was prepared by $SnCl_2$ reduction as follows: Nitro analog **5b** (0.97 g, 2.47 mmol) was added to a cold (−12 °C) stirred solution of anhydrous $SnCl_2$ (1.70 g, 8.94 mmol) in concentrated hydrochloric acid (15 mL). The bright yellow mixture was stirred at ambient temperature for 1.5 h, during which time a homogenous solution resulted followed by another bright yellow suspension. The mixture was cooled to −12 °C, and the solid was collected by filtration and washed with cold concentrated HCl (3 mL). The product was suspended in H_2O (100 mL) and solid $NaHCO_3$ was added in portions until pH 7 was achieved. The orange solid was collected by filtration and washed with H_2O (10 mL), and the resulting wet material was stirred in absolute EtOH (110 mL) for 1 h. The yellow solid was removed by filtration, washed with EtOH (12 mL) and Et_2O (35 mL), and then extracted with DMF (125 mL and 2 × 60 mL). The yellow extract was reduced to a 10-mL volume under reduced pressure and diluted with Et_2O (20 mL). The orange solid was collected, washed with Et_2O (8 mL), and dried at 110 °C at high vacuum to afford **5g** as a bright orange-yellow solid (660 mg, 74%). Material of very high purity resulted by further recrystallization from DMF followed by drying at 110 °C under high vacuum: $[\alpha]^{21}_D$ −13° (*c* = 0.285, DMF), +4° (*c* = 0.210, DMSO).

20(*R*)-9-Nitro analog **5c** (200 mg, 0.509 mmol) was reacted with anhydrous $SnCl_2$ (350 mg, 1.842 mmol) in concentrated hydrochloric acid (3 mL) at −10 °C in a manner identical to that described for *S* isomer **5b**. *R* enantiomer **5h** resulted as a yellow solid (110 mg, 57%): $[\alpha]^{22}_D$ +12° (*c* = 0.262, DMF).

10-Amino-(20*S*)-CPT (5j). Nitro analog **5e** (200 mg, 0.509 mmol) was added to a cold (−10 °C) solution of anhydrous $SnCl_2$ (350 mg, 1.842 mmol) in concentrated hydrochloric acid (3 mL). The mixture was stirred for 2 h at ambient temperature, and after chilling to −10 °C, the yellow solid was collected (Büchner) and washed with cold concentrated hydrochloric acid (1 mL). Crude **5j** was suspended in H_2O (20 mL) and neutralized with solid $NaHCO_3$. The resulting solid was collected (Büchner), washed with H_2O (2 mL), and then stirred for 1 h in absolute EtOH (20 mL). The solid was again collected, washed with EtOH (3 mL) and Et_2O (10 mL), and dried. This material was extracted with DMF (5 × 35 mL), and the resulting yellow-orange solution was concentrated *in vacuo* to a 1–2-mL volume. During this time, 10-amino-(20*S*)-CPT (**5j**) crystallized as a yellow-orange solid (95 mg, 50%): mp (Kofler) >250 °C dec; the 250-MHz NMR (DMSO-d_6) spectrum of **5j** was in close agreement to that obtained in earlier studies for the racemic compound **5i**; $[\alpha]_D$ −57° (*c* = 0.274, DMF).

9-Chloro-(20*S*)-CPT (5k).[43] A solution of 2-amino-6-chlorobenzaldehyde[24] (**4c**, 100 mg, 0.64 mmol) and oxytricyclic ketone **3b** (100 mg, 0.38 mmol) in toluene (20 mL) was refluxed for 5 min. *p*-Toluenesulfonic acid (10 mg) and acetic acid (0.5 mL) were then added, and refluxing was continued for an additional 18 h. The solvent was removed in vacuo, and the product obtained

was crystallized ($CHCl_3$/MeOH) to give pure **5k** (130 mg, 90%): mp 262–265 °C; IR (KBr) 3400, 1740 (lactone), 1655 (pyridone), 1595, 1460, 1240, 1160 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 7.5 Hz, H-18), 1.86 (m, 2, H-19), 5.32 (s, 2, H-17), 5.44 (s, 2, H-5), 7.36 (s, 1, H-14), 7.52 (d, 1, J = 6.9 Hz, H-12), 7.9 (t, 1, J = 12.5 Hz, H-11), 8.18 (d, 1, J = 7.5 Hz, H-10), 8.93 (s, 1, H-7); $[\alpha]^{24}_D$ +21° (c = 0.75, MeOH/$CHCl_3$, 1:4). Anal. ($C_{20}H_{15}N_2ClO_4$) C, H, N, Cl.

10-Chloro-(20*S*)-CPT (5l).[43] A solution of the 2-amino-5-chlorobenzaldehyde[25] (**4d**, 102 mg, 0.66 mmol) and oxytricyclic ketone **3b** (100 mg, 0.38 mmol) in toluene (60 mL) was refluxed for 15 min. p-Toluenesulfonic acid (10 mg) and acetic acid (0.5 mL) were then added, and refluxing was continued for an additional 18 h. The solvent was removed *in vacuo*, and the product obtained was crystallized ($CHCl_3$/MeOH) to give **5l** (138 mg, 95%): mp 259–261 °C; IR (KBr) 3425, 1740 (lactone), 1655 (pyridone), 1600, 1495, 1230, 1160 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.86 (t, 3, J = 7 Hz, H-18), 1.84 (m, 2, H-19), 5.25 (s, 2, H-5), 5.41 (s, 2, H-17), 7.31 (s, 1, H-14), 7.45 (d, 1, J = 8 Hz), H-11), 7.82 (d, 1, J = 8 Hz, H-12), 8.24 (s, 1, H-9), 8.61 (s, 1, H-7); $[\alpha]^{24}_D$ +32° (c = 0.075, MeOH/$CHCl_3$, 1:4). Anal. Calcd. ($C_{20}H_{15}N_2ClO_4$) C, H, N, Cl.

9-Fluoro-(20*S*)-CPT (5m). 6-Fluoro-2-nitrobenzaldehyde was prepared in three steps from 2-fluoro-6-nitrotoluene according to literature procedure[26] and was in turn converted to 2-amino-6-fluorobenzaldehyde (**4e**) by ferrous sulfate reduction. A solution of crude 2-amino-6-fluorobenzaldehyde (**4e**, 120 mg, 0.8 mmol) and oxytricyclic ketone **3b** (120 mg, 0.46 mmol) in toluene (5 mL) was refluxed for 5 min. p-Toluenesulfonic acid (2 mg) and acetic acid (0.5 mL) were added, and refluxing was continued for an additional 18 h. After cooling, the precipitate was filtered and washed with ether to give **5m** (11 mg, 6%): mp 268–269 °C; IR (KBr) 3390, 1740 (lactone), 1660 (pyridone), 1595, 1440, 1225, 1160 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.89 (t, 3, J = 7.2 Hz, H-18), 1.86 (m, 2, H-19), 5.26 (s, 2, H-5), 5.41 (s, 2, H-17), 7.34 (s, 1, H-14), 7.53 (t, 1, H-12), 7.82 (t, 1, H-11), 8.01 (d, 1, H-10), 8.80 (s, 1, H-7); $[\alpha]^{24}_D$ +34° (c = 0.0625, MeOH); MS m/z 366 (M^+). Anal. ($C_{20}H_{15}FN_2O_4$) C, H, N, F.

10-Fluoro-(20*S*)-CPT (5n). 5-Fluoro-2-nitrobenzaldehyde and 2-amino-5-fluorobenzaldehyde (**4f**) were prepared, respectively, by nitration of 3-fluorobenzaldehyde followed by ferrous sulfate reduction. A solution of crude 2-amino-5-fluorobenzaldehyde (**4f**, 120 mg, 0.86 mmol) and oxytricyclic ketone **3b** (110 mg, 0.42 mmol) in toluene (20 mL) was refluxed for 5 min, p-toluenesulfonic acid (10 mg) and acetic acid (0.5 mL) were added, and refluxing was continued for an additional 18 h. The solvent was removed *in vacuo*, and the crude product obtained was recrystallized ($CHCl_3$/MeOH) to give **5n** (72 mg, 47%); mp 265–267 °C; IR (KBr) 3400, 1745 (lactone), 1655 (pyridone), 1595, 1460, 1240 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.86 (t, 3, J = 7.2 Hz, H-18), 1.84 (m, 2, H-19), 5.25 (s, 2, H-5), 5.41 (s, 2, H-17), 6.51 (s, 1, OH), 7.30 (s, 1, H-14), 7.76 (t, 1, J = 7.2 Hz, H-11), 7.91 (d, 1, J = 8 Hz, H-12), 8.24 (m, 1, H-9), 8.63 (s, 1, H-7); $[\alpha]^{24}_D$ +28° (c = 0.1, MeOH/$CHCl_3$, 1:4). Anal. ($C_{20}H_{15}FN_2O_4$) C, H, N, F.

9-Bromo-(20*S*)-CPT (5o).[43] A solution of crude aminoaldehyde **4g**[24] (300 mg) and tricyclic ketone **3b** (180 mg, 0.68 mmol) in toluene (120 mL) and acetic acid (1 mL) was refluxed for 8 h. The toluene was evaporated, and the brown residue was chromatographed (SiO_2, 2% MeOH/$CHCl_3$) to give **5o** as a light brown solid (235 mg, 81%). It was recrystallized from $CHCl_3$/ether: mp 262–264 °C; IR (KBr) 3400, 1740, 1660, 1600 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.87 (t, 3, J = 7 Hz, H-18), 1.86 (m, 2, H-19), 5.30 (s, 2, H-17), 5.42 (s, 2, H-5), 6.54 (s, 1, 20-OH), 7.34 (s, 1, H-14), 7.76 (t, 1, J = 7 Hz, H-11), 8.04 (d, 1, J = 7 Hz, H-12), 8.2 (d, 1, J = 7 Hz, H-10), 8.85 (s, 1, H-7). Anal. ($C_{20}H_{15}BrN_2O_4$) C, H, N, Br.

10-Bromo-(20*S*)-CPT (5p).[43] In analogy to fluoro synthon **4f**,[25] nitration of m-bromobenzaldehyde gave 5-bromo-2-nitrobenzaldehyde, which was converted to the unstable 2-amino-5-bromobenzaldehyde (**4h**) by a literature procedure.[24] A solution of the crude 2-amino-5-bromobenzaldehyde (**4h**, 180 mg, 0.9 mmol) and oxytricyclic ketone **3b** (130 mg, 0.5 mmol) in toluene (12 mL) was refluxed for 5 min. p-Toluenesulfonic acid (5 mg) and acetic acid (0.2 mL) were added, and refluxing was continued for an additional 17 h. The solvent was removed in vacuo, and the product obtained was crystallized from MeOH/$CHCl_3$ (1:4) to give **5p** (90 mg, 42%); mp 269–270 °C; IR (KBr) 3400, 1740 (lactone), 1650 (pyridone), 1595, 1450, 1220, 1150 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 7.5 Hz, H-18), 1.86 (m, 2, H-19), 5.28 (s, 2, H-5), 5.42 (s, 2, H-17), 6.45 (s, 1, OH), 7.95 (d, 1, H-12), 8.09 (d, 1, H-11), 8.43 (s, 1, H-9), 8.64 (s, 1, H-7); $[\alpha]^{24}_D$ +33° (c = 0.012, 10% MeOH in $CHCl_3$). Anal. ($C_{20}H_{15}BrN_2O_4$) C, H, N, Br.

9,10-Dichloro-(20*S*)-CPT (5q). An excess of the crude amino aldehyde **4j**[27] and oxytricyclic ketone **3b** (150 mg, 0.57 mmol) in toluene (150 mL) and acetic acid (1 mL) were refluxed for 12 h. After evaporation, the crude product was purified by column chromatography (SiO_2; 2% MeOH/$CHCl_3$) to yield **5q** as a yellow solid (25 mg, 11%): mp 264–266 °C; IR (KBr) 3450, 1745, 1660, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.87 (t, 3, J = 7 Hz, H-18), 1.85 (m, 2, H-19), 5.29 (s, 2, H-15), 5.42 (s, 2, H-17), 6.54 (s, 1, 20-OH), 7.33 (s, 1, H-14), 7.95 (d, 1, J = 9 Hz, H-12), 8.15 (d, 1, J = 9 Hz, H-11), 8.93 (s, 1, H-7); $[\alpha]^{24}_D$ +26° ($CHCl_3$/MeOH 3:1). Anal. Calcd for $C_{20}H_{14}N_2O_4Cl_2$ 416.0334, found 416.0333.

9-Methoxy-(20*RS*)-CPT (5r).[43] Aminoaldehyde **4j**[29] (3.34 g, 22.0 mmol) and tricyclic ketone **3a** (0.989, 3.7 mmol) were combined and refluxed in toluene (500 mL) for 5 min. p-Toluenesulfonic acid (300 mg) and acetic acid (8 mL) were added and refluxing continued for 10 h. The solvent was evaporated, and the residue was chromatographed (SiO_2, 230–400 mesh, 2% MeOH/$CHCl_3$) to give a pale yellow powder (0.862 g, 61%): mp 251–253 °C (MeOH/$CHCl_3$/ether); IR (KBr) 1740, 1655, 1620, 1600, 1465, 1370 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.80–1.95 (m, 2, H-19), 4.04 (s, 3, 9-OCH_3), 5.25 (s, 2, H-5), 5.43 (s, 2, H-17), 6.52 (s, 1, OH), 7.16 (J = 1.4; 7.4 Hz, H-12), 7.32 (s, 1, H-14), 7.70–7.81 (m, 2, H-10 and H-11), 8.84 (s, 1, H-7). Anal. Calcd for $C_{21}H_{18}N_2O_5$ 378.1215, found 378.1219; $C_{21}N_{18}N_2O_5$·0.5H_2O) C, H, N.

9-Hydroxy-CPT Racemate 5s and Enantiomer 5. 9-Hydroxy-(20*RS*)-CPT 5s. A mixture of 9-methoxy-(20*RS*)-CPT (860 mg, 2.28 mmol), aluminum chloride (2.5 g), and toluene (300 mL) was refluxed for 15 h. After cooling, the solution was poured over 300 g of crushed ice, and the light orange precipitate was collected. The filtrate was concentrated and extracted with $CHCl_3$/MeOH. The total crude product was chromatographed (SiO_2, 230–400 mesh, 5% MeOH/$CHCl_3$) to provide pure 9-hydroxy-(20*RS*)-CPT (**5s**, 140 mg, 17%): mp 266 °C; IR (KBr) 3400 (br), 3180 (br), 1745, 1644, 1620, 1595, 1365, 1280, 1230, 1185, 1160, 815 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.9 (m, 2, H-19), 5.26 (s, 2, H-5), 5.4 (s, 2, H-17), 6.51 (s, 1, 20-OH), 7.0–7.04 (m, 1, H-12), 7.32 (s, 1, H-14), 7.58–7.67 (m, 2, H-10 and H-11), 8.81 (s, 1, H-7), 10.72 (s, 1, 9-OH). Anal. Calcd for $C_{20}H_{16}N_2O_5$ 364.1059, found 364.1053. ($C_{20}H_{16}N_2O_6$·H_2O) C, H, N.

S enantiomer 9-hydroxy-(20*S*)-CPT (**5t**)[43] was prepared as follows: A cold (0–5 °C) stirred solution of 9-amino-(20*S*)-CPT (**5g**, 36.3 mg, 0.100 mmol) in 50% aqueous H_2SO_4 (1 mL) was treated dropwise over 1 min with aqueous $NaNO_2$ (8.3 mg, 0.120 mmol, 0.2 mL H_2O). The mixture was refluxed for 1 h, cooled, and poured over ice/H_2O (7 mL). The resulting suspension was extracted repeatedly with $CHCl_3$ (6 × 25 mL) to remove (20-*S*)-CPT (1) obtained as a side product. The remaining suspension was centrifuged, and the solid was collected, mixed with H_2O (2 mL), and again centrifuged. The solid residue was dissolved/suspended in MeOH (0.5 mL) and slowly diluted with Et_2O (3 mL). 9-Hydroxy-(20*S*)-CPT (**5t**) resulted as a pale tan-yellow solid (12 mg, 60%). The 250-MHz ^1H NMR (DMSO-d_6) of this material was identical to that of the racemate **5s**; $[\alpha]^{21}_D$ −20° (c = 0.175, DMF).

9-Methyl-(20*S*)-CPT (5u).[31] A mixture of 2-amino-6-methylbenzaldehyde[30] (**4k**, 300 mg, 2.272 mmol) and tricyclic ketone **3b** (150 mg, 0.57 mmol), toluene (100 mL), and acetic acid (1 mL) was refluxed for 8 h. The solvent was removed and the residue was triturated with ether and filtered. The product **5u**[31] was recrystallized from $CHCl_3$ ether (169 mg, 82%): mp 278–280 °C; IR (KBr) 3400 br, 1750, 1660, 1595 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.87 (t, 3, J = 7 Hz, H-18), 1.85 (a, 2, J = 7 Hz, H-19), 2.72 (s, 3, 9-CH_3), 5.26 (s, 2, H-5), 5.41 (s, 2, H-17), 6.51 (s, 1, 20-OH), 7.32 (s, 1, H-14), 7.53 (d, 1, J = 7 Hz, H-10), 7.72 (t, 1, J = 7 Hz, H-11), 7.99 (d, 1, J = 7 Hz, H-10), 8.79 (s, 1, H-7). Anal. ($C_{21}H_{18}N_2O_4$·0.75H_2O) C, H, N.

10-Cyano-(20*S*)-CPT (5w).[31] A mixture of 10-bromo-(20*S*)-CPT (120 mg, 0.28 mmol) and an excess of cuprous cyanide (500 mg) in dry DMF (20 mL) was refluxed for 5 h. The solution was passed through a silica pad and washed with DMF. The combined solution was evaporated to dryness, and the crude product was chromatographed (SiO_2, 2% MeOH/$CHCl_3$) to yield pure 10-cyano-(20*S*)-CPT (39 mg, 37%) with spectral properties identical to those reported.[31]

10-Carboxy-(20*S*)-CPT (5x). An ethanolic solution (100 mL) containing 2 N HCl (10 mL) and 10-cyano-(20*S*)-CPT (80 mg, 0.2 mmol) was refluxed for 30 h. The solution was evaporated to give the product **5x**, which was further purified by crystallization from ethanol/ether (73 mg, 87%): mp 254–256 °C; IR (KBr) 3400, 1740, 1660, 1620, 1525 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.87 (t, 3, J = 7 Hz, H-18), 1.86 (m, 2, H-19), 5.25 (s, 2, H-5), 5.41 (s, 2, H-17), 6.54 (s, 1, 20-OH), 7.33 (s, 1, H-14), 8.06 (d, 1, J = 8.5 Hz, H-12), 8.32 (d, 1, J = 8.5 Hz, H-11), 8.58 (s, 1, H-9), 8.71 (s, 1, H-7). Anal. ($C_{21}H_{16}N_2O_6 \cdot 1.5H_2O$) C, H, N.

10,11-(Methylenedioxy)-CPT Enantiomers 5z and 5aa. As reported for racemate **5y**,[12] *S* isomer **5z** was prepared from (*S*) ketone **3b** (1.769 g, 6.726 mmol), 2-aminopiperonal[11] (**4m** 1.800 g, 11.613 mmol), glacial HOAc (2.5 mL), and *p*-TsOH·H_2O (30 mg) in refluxing toluene (100 mL) in 86% yield: mp 270 °C; $[\alpha]^{21}_D$ −27° (c = 0.122, DMSO); the 250-MHz ^1H NMR (DMSO-d_6) for **5z** was identical to that reported earlier for the racemate **5y**.

In the same fashion as described for the synthesis of *S* isomer **5z**, (*R*)-ketone **3c** (107 mg, 0.407 mmol) was condensed with 2-aminopiperonal (**4m**, 110 mg, 0.710 mmol) in refluxing toluene containing HOAc and *p*-TsOH. Chromatography on SiO_2 (10 g, 0.25% MeOH in $CHCl_3$) afforded **5aa** as a beige solid (88 mg, 55%). The 250-MHz ^1H NMR (DMSO-d_6) matched that of racemate **5y** prepared in an earlier study;[12] $[\alpha]^{21}_D$ +25° (c = 0.20, DMF).

9-Nitro-10,11-(Methylenedioxy)-CPT Enantiomers 5ab and 5ac and Racemate 5ad. 9-Nitro-10,11-(methylenedioxy)-(20*S*)-CPT (5ab). To stirred concentrated H_2SO_4 (6 mL) at −10 °C was added finely powdered 10,11-(methylenedioxy)-(20*S*)-CPT (**5z**, 440 mg, 1.122 mmol) over 10–15 min in small portions to prevent clumping of the solid. The resulting iridescent brown-green solution was stirred vigorously at −10 °C while a precooled mixture of concentrated HNO_3 and concentrated H_2SO_4 (16 drops each) was added dropwise over 8 min. The clear deep red-brown solution was left stirring at ambient temperature for 1 h and then solution was carefully quenched onto a bed of crushed ice (~30 g) at a dropwise rate sufficiently slow to prevent localized heating. The bright yellow precipitate was collected by vacuum filtration, washed with H_2O, cold EtOH, and finally Et_2O. The nitro compound **5ac** resulted after vacuum drying as a yellow powder (371 mg). The aqueous phase was extracted thoroughly with $CHCl_3$ (5 × 40 mL). The extract was back-washed with H_2O, dried (Na_2SO_4), and evaporated to give additional yellow **5ac** (88 mg, total 459 mg, 94%). Chromatography (SiO_2, 230–400 mesh, 0.25% MeOH in $CHCl_3$) removed some dark pigment, and recrystallization from MeOH/$CHCl_3$ EtOAc provided pure **5ac**: darkening without melting above 255 °C; IR (KBr) 3430, 2920, 1741, 1654, 1596, 1525, 1450, 1343, 1242, 1191, 1043, 928, 785, 565 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.87 (t, 3, J = 7 Hz, H-18), 1.85 (m, 2, H-19), 5.21 (s, 2, H-5), 5.41 (s, 2, H-17), 6.52 (s, 2, OCH_2O), 7.24 (s, 1, H-14), 7.78 (s, 1, H-12), 8.96 (s, 1, H-7); $[\alpha]^{21}_D$ −30° (c = 0.279, DMF). Anal. ($C_{21}H_{15}N_3O_8 \cdot 2H_2O$) C, H, N.

The *R* isomer of 10,11-(methylenedioxy)-CPT **5aa** (73 mg, 0.186 mmol) was nitrated in cold (−10 °C) concentrated H_2SO_4 (1 mL) using a concentrated HNO_3 and concentrated H_2SO_4 mixture (5 drops each) as described for the *S* isomer **5z**. Workup afforded (*R*)-nitro compound **5ac** as a yellow solid (66 mg, 89%): $[\alpha]^{22}_D$ +28° (c = 0.212, DMF); the 250-MHz ^1H NMR (DMSO-d_6) spectrum was identical to those recorded for racemate **5ad** and *S* isomer **5ab**.

Also, the *RS* isomer **5ad** was obtained in an analogous manner from **5y** in 85% yield.

9-Amino-10,11-(methylenedioxy)-CPT Racemate 5ae and Enantiomers 5af and 5ag. A mixture of nitro compound **5ab** (220 mg) in absolute EtOH (50 mL) containing CF_3CO_2H (1 mL) was warmed and sonicated to dissolve as much of the sample as possible. The stirred yellow suspension was treated with 10% Pd/C (80 mg) and subjected to 1 atm of H_2 for 18 h. The brown-green suspension was filtered through Celite, and the pad was slowly rinsed successively with several 15-mL portions each of hot MeOH/$CHCl_3$ (1:1), 1 N aqueous HCl, and DMSO. The washing process was terminated when the filtrate changed from orange-brown to faint yellow. The combined filtrate was again filtered and concentrated at <40 °C under vacuum to afford a DMSO solution of amine **5af**. Most of the DMSO was removed over 48 h by directing a stream of N_2 across the surface of the stirred solution. The sample was further dried under high vacuum to afford **5af** as an orange-brown solid (160 mg, 78%). The product dissolved in boiling MeOH/$CHCl_3$, and the resulting solution was concentrated to faint turbidity and diluted ≈10% with EtOAc. Purified amine **5af** resulted as an orange-tan powder (112 mg, 55%): darkening above 250 °C with no discreet melting point below 350 °C; IR (KBr) 3450, 3370, 1745, 1655, 1590, 1445, 1247, 1160, 1040, 935 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.87 (m, 2, H-19), 5.22 (s, 2, H-5), 5.41 (s, 2, H-17), 5.74 (s, 2, NH_2), 6.18 (s, 2, OCH_2O), 6.47 (s, 1, OH), 6.91 (s, 1, H-12), 7.23 (s, 1, H-14), 8.74 (s, 1, H-7); $[\alpha]^{21}_D$ −26° (c = 0.324, DMF), −20° (c = 0.274, DMSO), +22° (c = 0.04, $CHCl_3$/MeOH, 4:1). Anal. ($C_{21}H_{17}N_3O_6$) C, H, N.

Alternatively, chemical reduction of **5ab** employing $SnCl_2$ (as in the conversion of 9-nitro-(20*S*)-CPT (**5b**) to amine **5g** and in the reduction of 10-nitro-(20*S*)-CPT (**5e**) to the corresponding 10-amino analog **5j**) afforded **5af** of superior purity as a bright yellow solid. As for **5b** and **5e**, **5ab** (174 mg, 0.537 mmol) was treated at −10 °C with $SnCl_2$ (370 mg, 1.948 mmol) in concentrated hydrochloric acid (3.2 mL) and then was stirred at room temperature for 4 h (development of the yellow product precipitate was notably slower with this substrate). Workup provided **5af** as a bright yellow solid (87 mg, 55%) with spectral properties identical to those of the earlier sample.

Compound **5ac** (72 mg, 0.165 mmol) was dissolved/suspended in stirred MeOH/H_2O, 2:1 (6 mL). Aqueous NaOH (0.1 N, 2.0 mL, 0.20 mmol) was added and the mixture heated to 50 °C. After 30 min, the hazy tan solution was brought to reflux while a hot solution of $FeSO_4 \cdot 7H_2O$ (500 mg, excess) in H_2O (2 mL) was added. The dark green-brown mixture was refluxed gently for 5 min and filtered hot through a Celite pad. The pad residue was washed with hot MeOH (3 × 2 mL) and 1 N aqueous NaOH (2 × 1 mL). The clear orange-brown filtrate was acidified to pH 2 using concentrated hydrochloric acid. The amino compound **5ag** resulted as a fine orange-yellow suspension which was collected by centrifugation and recrystallized from MeOH/$CHCl_3$ to give **5ag** as a tan-orange solid (41 mg, 84%): $[\alpha]^{21}_D$ −20° (c = 0.0650, MeOH/$CHCl_3$, 1:4); the 250-MHz ^1H NMR (DMSO-d_6) of **5ag** was identical with those recorded for *RS* analog **5ae** and *S* analog **5af**.

Racemate **5ae** resulted in 50% yield by hydrogenation as described for **5af**.

9-Chloro-10,11-(methylenedioxy)-(20*S*)-CPT (5ah). A stirred orange-brown suspension of amine **5af** (104 mg, 0.255 mmol) in concentrated HCl (5 mL) at 0 °C was treated dropwise over 1 min with a solution of $NaNO_2$ (24 mg, 0.348 mmol) in H_2O (12 drops). After 20 min the tan-orange suspension was treated with CuCl (48 mg, 0.484 mmol) and heated at 50 °C. After 30 min the reaction mixture was poured over ice (40 g). The crude chloro analog **5ah** was collected by filtration as a yellow-gray solid (82 mg, 75%). Further purification was effected by column chromatography (dispersion on Celite, 0.5 g, 230–400 mesh SiO_2, 6 g, 0.2% MeOH in $CHCl_3$) and recrystallization from MeOH to afford pure **5ah** as a pale yellow solid (55 mg, 51%): mp 294–297 °C; IR (KBr) 3300, 2960, 2910, 1738 (s), 1654 (s), 1581 (s), 1557, 1483, 1438 (s), 1243 (s), 1151, 1110, 1038, 941, 861, 851 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.85 (m, 2, H-19), 5.22 (s, 2, H-5), 5.42 (s, 2, H-17), 6.39 (s, 2, OCH_2O), 7.25 (s, 1, H-14), 7.52 (s, 1, H-12), 8.66 (s, 1, H-7); $[\alpha]^{21}_D$ −25° (c = 0.191, DMF). Anal. ($C_{21}H_{15}ClN_2O_6$) C, H, Cl, N.

10,11-(Ethylenedioxy)-(20*RS*)-CPT (5ai). 6-Formyl-2,3-dihydro-7-nitro-1,4-benzodioxane was prepared by nitration of the corresponding aldehyde as reported.[33] The nitro aldehyde (1.0 g, 4 mol) was dissolved in 50% absolute EtOH (100 mL) and warmed to 70 °C. This hot solution was added to a boiling solution of $FeSO_4 \cdot 7H_2O$ (10 g, 36 mol) in water (100 mL). The solution

was boiled for 1 min and then concentrated. Concentrated NH_4OH (15 mL) was added with rapid stirring over 2–3 min, when the yellow mixture immediately became dark green-brown. The mixture was kept at the boiling point for 5 min after the addition was complete and filtered hot (sintered glass), and the pad was washed with hot water (10 mL). The aqueous filtrate, after cooling, gave a pale yellow solid (0.475 g, 53%). Aminoaldehyde **4n** was further purified by crystallization from water: mp 101–102 °C; IR (KBr) 3505, 3360 (NH_2), 1660 (CHO), 1600, 1550, 1440, 1330, 1282, 1180, 1150, 1080 cm^{-1}. Anal. ($C_9H_9NO_3$) C, H, N.

Aminoaldehyde **4n** (102 mg, 0.5 mmol) and oxytricyclic ketone **3a** (102 mg, 0.38 mmol) were reacted in the same fashion as reported for racemic 10,11-(methylenedioxy)-CPT **5y**[12] to provide 10,11-(ethylenedioxy)-(20*RS*)-CPT (**5a**) in 93% yield. Recrystallization from 13% MeOH in $CHCl_3$ afforded an orange-yellow powder: mp 296–300 °C; IR (KBr) 1750 (lactone), 1665 (pyridone), 1585 (aromatic) cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 12 Hz, H-18), 1.88 (m, 2, H-19), 4.42 (s, 4, OCH_2CH_2O), 5.18 (s, 2, H-5), 5.40 (s, 2, H-17), 7.2 (s, 1, H-9), 7.52 (s, 1, H-12), 8.43 (s, 1, H-7). Anal. ($C_{22}H_{18}H_2O_6$) C, H, N.

9,10-(Methylenedioxy)-(20*RS*)-CPT (5aj). A stirred mixture of 2,3-(methylenedioxy)-6-nitrobenzaldehyde (200 mg, 1.02 mmol)[34] in 50% aqueous EtOH (20 mL) was brought to reflux, and the resulting solution was added to a gently refluxing solution of $FeSO_4 \cdot 7H_2O$ (2.0 g, 7.10 mmol) in H_2O (10 mL). Concentrated NH_4OH (2.6 mL) was added over 20 min and, after 2 min at reflux, the brown-green suspension was chilled rapidly (ice/H_2O bath), diluted with $CHCl_3$ (30 mL), and filtered (Celite). The bright yellow $CHCl_3$ layer was separated and the aqueous phase again extracted with $CHCl_3$ (30 mL). Combined $CHCl_3$ extract was washed with H_2O (20 mL), dried (Na_2SO_4), and evaporated to afford compound **4o** as a gummy yellow-orange solid (161 mg). Recrystallization from MeOH/Et_2O gave pure **4o** as a pale green-yellow solid (81 mg, 49%): mp 206–209 °C; IR ($CHCl_3$) 3350, 1675 (CHO), 1625, 1440, 1272, 1180, 1080 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 6.2 (s, 2, OCH_2O), 6.8 (d, 1, J = 9 Hz, H-3), 7.1 (d, 1, J = 9 Hz, H-4), 8.9 (s, 1, CHO). Anal. ($C_8H_7NO_3$) C, H, N.

A stirred suspension of amino aldehyde **4o** (113 mg, 0.685 mmol) and tricyclic ketone **3a** (75 mg, 0.285 mmol) in toluene (30 mL) was heated to gentle reflux at which point p-TsOH·H_2O (10 mg) was added to the clear yellow solution. Heating at reflux was continued for 4 h, during which time the reaction became a rusty-colored suspension. The solvent was removed under vacuum and the residue subjected to column chromatography (SiO_2, 10 g, $CHCl_3$) as a dispersion on Celite (1 g). The product **5aj** resulted as a lime-yellow solid (62 mg, 55%) from evaporation of those fractions which displayed yellow fluorescence under long-wavelength UV irradiation. Recrystallization of a sample from MeOH/$CHCl_3$ gave 9,10-(methylenedioxy)-(20*RS*)-CPT (**5aj**) as a pale lime powder: mp 271–274 °C dec; IR (KBr) 3200–3700, 3120, 2935, 1745 (lactone), 1660 (pyridone), 1604 (aromatic), 1480, 1272, 1160, 1050 cm^{-1}; 250 MHz ^1H NMR (DMSO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.87 (m, 2, H-19), 5.22 (s, 2, H-5), 5.42 (s, 2, H-17), 6.34 (s, 2, OCH_2O), 6.50 (s, 1, 20-OH), 7.28 (s, 1, H-14), 7.66 (d, 1, J = 9 Hz, H-11), 7.79 (d, 1, J = 9 Hz, H-12), 8.48 (s, 1, H-7). Anal. Calcd for $C_2H_{16}N_2O_6$ 392.1008, found 392.1005 ($C_{12}H_{16}N_2O_6 \cdot 0.5H_2O$) C, H, N.

De-A-Ring Analog of CPT (6). A mixture of oxytricyclic ketone **3a** (185 mg, 0.7 mmol) and aminoacrolein (185 mg, 2.6 mmol) was dissolved in 5% methanol/$CHCl_3$. The solution was evaporated to dryness and ammonium acetate (35 mg) was added followed by acetic acid (1 mL). The mixture was heated under vacuum in an oil bath (80–90 °C) for 1 h. The residue was chromatographed (SiO_2, 230–400 mesh, 1% MeOH/$CHCl_3$) to afford tetracyclic analog **6**: mp 267 °C; IR (KBr) 3400, 3250 (br), 1745, 1660, 1610, 1160 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.86 (t, 3, H-14), 1.85 (m, 2, H-15), 5.16 (s, 2, H-5), 5.39 (s, 2, H-13), 6.45 (s, 1, 16-OH), 7.16 (s, 1, H-10), 7.59 (m, 1, H-9), 8.18 (m, 1, H-8), 8.75 (m, 1, H-7). Anal. Calcd for $C_{16}H_{14}N_2O_4$ 298.0953, found 298.0966 ($C_{16}H_{14}N_2O_4 \cdot 0.25H_2O$) C, H, N.

Sodium Salts 2d–i. 10,11-(Methylenedioxy)-(20*S*)-CPT Sodium Salt (2e). A suspension of 10,11-(methylenedioxy)-(20*S*)-CPT (**5z**, 131 mg, 0.33 mmol) in 90% aqueous MeOH and 0.1 NaOH (3.3 mL, 0.33 mmol) was stirred at 65 °C for 3 h. The solvent was removed under reduced pressure, and the brown solid was dissolved in distilled water (10 mL), filtered (0.45-μm membrane), and freeze-dried to give 10,11-(methylenedioxy)-(20*S*)-CPT sodium salt **2e** (140 mg, 97%): IR (KBr) 3400, 1640 (pyridone), 1595, 1460, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3, J = 7.5 Hz), 2.09 (m, 2, H-19), 4.62 (d, 1, H-17), 4.88 (d, 1, H-17), 5.12 (s, 2, H-5), 6.12 (s, 1, OH), 6.25 (s, 2, OCH_2O), 7.46 (s, 1, H-14), 7.52 (s, 1, H-9), 7.61 (s, 1, H-12), 8.40 (s, 1, H-7). Anal. ($C_{21}H_{17}N_2O_7Na$) C, H, N, Na.

The sodium salts **2c**, **2d**, and **2f–i** were similarly prepared from the corresponding parent lactones.

9-Glycinamido-(20*RS*)-CPT Hydrochloride (7b). To a stirred solution of racemic 9-amino-CPT **5f** (88 mg, 0.242 mmol) and t-BOC-glycine (110 mg, 0.629 mmol) in dry DMF (10 mL) under N_2 was added DCC (125 mg, 0.607 mmol) at room temperature. During 18 h, the reaction became turbid white from precipitated DCU. This was removed by filtration and the solvent removed by high vacuum distillation to provide crude **7a** as a tan yellow solid. Column chromatography of this material (SiO_2, 25 g, 250 mL each $CHCl_3$, 1% MeOH/$CHCl_3$, 2% MeOH/$CHCl_3$) provided **7a** as a yellow solid (55 mg, 44%). Recrystallization from MeOH gave the t-BOC-glycinamide **7a** as a beige solid: mp 208–210 °C; IR (KBr) 3360, 1750 (lactone), 1710 (carbamate), 1692 (amide), 1660 (lactone), 1622, 1598, 1493, 1235, 1165, 1110, 1058 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.89 (5, 3, J = 7 Hz, H-18), 1.44 (s, 9 $C(CH_3)_3$), 1.88 (m, 2, H-19), 3.92 (d, 2, J = 6 Hz, $COCH_2N$), 5.29 (s, 2, H-5), 5.44 (s, 2, H-17), 6.53 (s, 1, OH), 7.19 (t, 1, J = 6 Hz, NHCO), 7.37 (s, 1, H-14), 7.79 (d, 1, J = 7 Hz, H-10), 7.85 (t, 1, J = 7 Hz, H-11), 8.03 (d, 1, J = 7 Hz, H-12), 8.79 (s, 1, H-7), 10.20 (s, 1, amide). Anal. ($C_{27}H_{28}N_4O_7 \cdot H_2O$) C, H, N.

The t-BOC derivative **7a** (21 mg, 0.040 mmol) was suspended in CH_2Cl_2 (10 mL) followed by the addition of MeOH (0.7 mL), thereby giving a clear solution. After chilling to 0 °C, the stirred solution was treated dropwise over 5 min with a solution of HCl-saturated anhydrous dioxane (4.5 min). The turbid yellow mixture was left to warm to ambient temperature, and after 2 h the solvents were evaporated under reduced pressure to provide **7b** as an orange yellow solid (18 mg). This material was dissolved in deionized H_2O (5 mL), filtered (0.45 μm membrane), and lyophilized to provide pure hydrochloride **7b** as a fluffy yellow solid (14 mg, 77%): darkening above 245 °C without melting to 310 °C; IR (KBr) 2400–3650, 1742 (lactone), 1700 (amide), 1658 (pyridone), 1590, 1550, 1495, 1234, 1164, 1110, 1050, 900, 820, 720 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.89 (m, 2, H-19), 4.03 (d, 2, J = 5 Hz, $COCH_2N$), 5.30 (s, 2, H-5), 5.44 (s, 2, H-17), 7.37 (s, 1, H-14), 7.86 (d, 1, J = 7 Hz, H-12), 7.92 (t, 1, J = 7 Hz, H-11), 8.07 (d, 1, J = 7 Hz, H-10), 8.35 (br s, 3, NH_3^+), 8.95 (s, 1, H-7), 10.88 (s, 1, amide H). Anal. ($C_{22}H_{21}ClN_4O_5 \cdot 3H_2O$) C, H, Cl, N.

9-Glycinamido-10,11-(methylenedioxy)-(20*RS*)-CPT Hydrochloride (7d). A stirred mixture of racemic amino derivative **5ae** (186 mg, 0.457 mmol) and t-BOC-glycine (150 mg, 0.85 mmol) in DMF (15 mL) containing pyridine (1 mL) was treated at 0 °C with DCC (200 mg, 0.971 mmol). The reaction was stirred for 65 h at room temperature, the solvents were evaporated, and the residue was redissolved in MeOH/$CHCl_3$. The sample was evaporated in the presence of Celite (3 g), and the resulting powder was subjected to chromatography (SiO_2, 20 g, 200 mL of $CHCl_3$, 500 mL of 5% MeOH in $CHCl_3$, 500 mL of 12% MeOH in $CHCl_3$). Isolation and evaporation of the appropriate fractions gave intermediate **7c** (98 mg, 38%). This material was dissolved in CH_2Cl_2 (30 mL) containing MeOH (0.5 mL) and the resulting stirred solution was cooled to 5 °C. A cold solution of HCl-saturated dioxane (5 mL) was added over 5 min to give a bright yellow suspension. After 5 h at room temperature, the mixture was concentrated under reduced pressure, the residue was dissolved in H_2O (50 mL), and the yellow solution was filtered (0.45-μm membrane). Lyophilization provided an amber gummy solid which on trituration with absolute EtOH gave the hydrochloride salt **7d** as a yellow microcrystalline solid (57 mg, 73%): darkening above 230 °C with no melting below 340 °C; IR (KBr) 3680–2300, 3220, 2990, 2920, 1740, 1700, 1655, 1585, 1492, 1447, 1390, 1249, 1160, 1108, 1075, 1041, 933, 845 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.89 (t, 3, J = 7 Hz, H-18), 1.87 (m, 2, H-19), 4.02 (d, 2, J = 5.4 Hz, $COCH_2N$), 5.17 (s, 2, H-5), 5.42 (s, 2, H-17), 6.32 (s, 2, OCH_2O), 7.26 (s, 1, H-14), 7.47 (s, 1, H-12), 8.38 (br s,

3, NH_3), 8.59 (s, 1, H-7), 10.75 (s, 1, amide H). Anal. ($C_{23}H_{21}ClN_4O_7 \cdot 2.5H_2O$) C, H, N.

9-Amino-20*O*-glycinyl-(20*RS*)-CPT Hydrochloride (7g). A stirred clear yellow solution of 9-nitro-(20*RS*)-CPT (**5a**, 78.6 mg, 0.200 mmol), *t*-BOC-glycine (75 mg, 0.400 mmol), and DMAP (12 mg) in DMF (2 mL) was treated with DCC (84 mg, 0.400 mmol) at ambient temperature under N_2. Over the course of 1.5 h the reaction turned brown-green and hazy. The DMF was removed under high vacuum, and the residue was chromatographed (SiO_2 column, 20 g) as a dispersion on Celite (1.3 g) with $CHCl_3$ as eluting solvent. The crude product was isolated as a yellow solid which was recrystallized from MeOH/$CHCl_3$ to provide **7e** as a pale yellow solid (62 mg, 56%): mp 169–172 °C dec; IR (CH_2Cl_2) 3445, 2940, 2862, 1752, 1711, 1667, 1620, 1530, 1502, 1370, 1234, 1162, 1060, 832 cm^{-1}; 250-MHz ^1H NMR ($CDCl_3$) δ 1.01 (t, 3, J = 7 Hz, H-18), 1.41 (s, 9, *t*-Bu), 2.20 (m, 2, H-19), 4.13 (d AB q, 2, J = ~7, 18 Hz, $\Delta\gamma$ = 49 Hz, $COCH_2NH$), 5.36 (s, 2, H-5), 5.24 (AB q, 2, J = 18 Hz, $\Delta\gamma$ = 76 Hz, H-17), 7.35 (s, 1, H-14), 7.93 (t, 1, J = 8 Hz, H-11), 8.48 (d, 1, J = 8 Hz, H-12), 8.56 (d, 1, J = 8 Hz, H-10), 9.27 (s, 1, H-7). Anal. ($C_{27}H_{28}N_4O_9 \cdot 0.5H_2O$) C, H, N.

The nitro compound **7e** (20 mg, 32.7 μmol) was stirred with 10% Pd/C (12 mg) in absolute EtOH (12 mL) under 1 atm of H_2 for 1 h. The mixture was filtered (Celite) and the pad was rinsed with MeOH/$CHCl_3$ (1:1, 3 × 5 mL). The filtrate was evaporated under reduced pressure to give amine **7g** as a bright orange-yellow solid (18 mg). Recrystallization from MeOH gave the pure compound (13 mg, 76%) as a pale orange gold solid: mp 182–185 °C dec; IR (CH_2Cl_2) 1743, 1702, 1652, 1640, 1350; 250-MHz ^1H NMR (DMSO-d_6) δ 0.92 (t, 3, J = 7 Hz, H-18), 1.39 (s, 9, *t*-Bu), 2.13 (m, 2, H-19), 3.87 (d AB q, 2, J = 6, 18 Hz, $\Delta\gamma$ = 33 Hz), 5.36 (s, 2, H-5), 5.50 (s, 2, H-17), 6.11 (s, 2, NH_2), 6.81 (d, 1, J = 7 Hz, H-10), 7.26 (s, 1, H-14), 7.36 (d, 1, J = 7 Hz, H-12), 7.41 (t, 1, J = 6 Hz, N*H*CO), 7.57 (t, 1, J = 7 Hz, H-11), 8.81 (s, 1, H-7). Anal. ($C_{27}H_{28}N_4O_7 \cdot 1.1H_2O$) C, H, N.

The *t*-BOC-protected derivative **7f** (18 mg) was dissolved in CH_2Cl_2 (3 mL) and the resulting stirred bright yellow solution was treated dropwise with HCl-saturated dioxane (4 mL). Initial foaming subsided quickly, and after 1 h the solvents were distilled under reduced pressure to give **7g** as a gray-brown solid. This material was dissolved in H_2O (4 mL), and the resulting deep orange-yellow solution was filtered (0.45 μm membrane) and lyophilized to provide **7g** as a fluffy tan-brown solid (17 mg, 100%): mp >300 °C dec; IR (KBr) 2400–3520, 1760, 1665, 1610, 1362, 1262, 1225, 1060, 820 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.97 (t, 3, J = 7 Hz, H-18), 2.19 (m, 2, H-19), 4.20 (AB q, J = 18 Hz, $\Delta\gamma$ = 70 Hz, $COCH_2N$), 5.33 (s, 2, H-5), 5.55 (s, 2, H-17), 7.03 (d, 1, J = 8 Hz, H-10), 7.34 (s, 1, H-14), 7.50 (d, 1, J = 8 Hz, H-12), 7.63 (t, 1, J = 8 Hz, H-11), 8.50 (br s, 3, NH_3^+), 8.92 (s, 1, H-7). Anal. ($C_{22}H_{20}N_4O_5 \cdot 1.75HCl \cdot 4.0H_2O$) C, H, Cl, N.

10-Amino-20-*O*-glycinyl-(20*RS*)-CPT Hydrochloride (7j). A stirred solution of 10-nitro-(20*RS*)-CPT[11] (**5d**, 50 mg, 0.127 mmol), *t*-BOC-glycine (50 mg, 0.286 mmol), and DMAP (10 mg) in CH_2Cl_2 (1 mL) and DMF (10 mL) under N_2 was treated at room temperature with DCC (70 mg, 0.340 mmol). Over 2 h the reaction became turbid and brown-green. The mixture was concentrated under reduced pressure, redissolved in $CHCl_3$, and subjected to column chromatography (SiO_2, 10 g, $CHCl_3$). Compound **7h** was isolated from the appropriate fractions as a yellow solid (43 mg, 61%). Recrystallization from MeOH/$CHCl_3$ gave **7h** as a pale yellow microcrystalline solid: mp 253–255 °C; IR (CH_2Cl_2) 1753, 1714, 1667, 1625, 1540, 1497, 1348, 1150 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.94 (t, 3, J = 7 Hz, H-18), 1.41 (s, 9, *t*-Bu), 2.14 (m, 2, H-19), 3.89 (d AB q, 2, J = 6, 18 Hz, J = 30 Hz), 5.33 (s, 2, H-5), 5.51 (s, 2, H-17), 7.34 (s, 1, H-14), 7.46 (t, 1, J = 6 Hz, NHCO), 8.27 (d, 1, J = 9 Hz, H-12), 8.53 (dd, 1, J = 2.5, 9 Hz, H-11), 8.96 (s, 1, H-7), 9.18 (d, 1, J = 2.5 Hz, H-9). Anal. ($C_{17}H_{26}N_4O_9$) C, H, N.

The nitro compound **7h** (20 mg) was dissolved as completely as possible in absolute EtOH (15 mL) by sonication. The hazy yellow solution was treated with H_2 (1 atmosphere) in the presence of 10% Pd/C for 1 h. The resulting bright iridescent green mixture was filtered (Celite) and the filter pad was further washed with MeOH/$CHCl_3$. Evaporation of the solvent provided **7i** of good purity as an orange-yellow solid (18 mg, 95%). A sample recrystallized from MeOH/$CHCl_3$ gave amino compound **7i** as a fine orange powder: darkening above 170 °C, no discreet melting below 300 °C; IR (CH_2Cl_2) 3240, 1755, 1670, 1610, 1340, 1150 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.91 (t, 3, J = 7 Hz, H-18), 1.38 (s, 9, *t*-Bu), 2.11 (m, 2, H-19), 3.87 (d AB q, 2, J = 6, 18 Hz, $\Delta\gamma$ = 31 Hz, $COCH_2N$), 5.18 (s, 2, H-5), 5.46 (s, 2, H-17), 5.95 (s, 2, NH_2), 6.88 (d, J = 23 Hz, H-9), 7.03 (s, 1, H-14), 7.26 (dd, 1, J = 2.3, 9 Hz, H-11), 7.37 (t, 1, J = 6 Hz, NHCO), 7.81 (d, 1, J = 9 Hz, H-12), 8.20 (s, 1, H-7). Anal. ($C_{27}H_{28}N_4O_7 \cdot 1.4H_2O$) C, H, N.

The amino derivative **7i** (25 mg) was stirred in CH_2Cl_2 (8 mL) to give a turbid solution which became clear yellow-orange upon the addition of MeOH (1 mL). The solution was chilled to 0 °C and treated over 3 min with HCl-saturated dioxane (4 mL). The mixture was left to warm to room temperature, and after 2 h, the solvent was evaporated under reduced pressure. The resulting orange solid was dissolved in H_2O (3 mL) to give a bright orange solution. After filtration (0.45 μm membrane), the solution was frozen and lyophilized to afford **7j** as a fluffy orange solid (18 mg, 75%): darkening above 240 °C with no discreet melting below 325 °C; IR (KBr) 2400–3520, 1755, 1665, 1610, 1355, 1260, 1070, 820; 260-MHz ^1H NMR (DMSO-d_6) δ 0.95 (t, 3, J = 7 Hz, H-18), 2.19 (m, 2, H-19), 4.07 (d AB q, J = 5, 18 Hz, $\Delta\gamma$ = 30 Hz, $COCH_2N$), 5.23 (s, 2, H-5), 5.52 (s, 2, H-17), 7.10 (d, 1, J = 2.5 Hz, H-9), 7.18 (s, 1, H-14), 7.38 (dd, 1, J = 2.5, 9 Hz, H-11), 7.91 (d, 1, J = 9 Hz, H-12), 8.34 (s, 1, H-7), 8.41 (br s, 3, NH_3^+). Anal. ($C_{22}H_{20}N_4O_5 \cdot 1.9HCl \cdot 3.5H_2O$) C, H, Cl, N.

10,11-(Methylenedioxy)-20-*O*-glycinyl-(20*RS*)-CPT Hydrochloride (7l). To a stirred turbid mixture of 10,11-(methylenedioxy)-(20*RS*)-CPT (**5y**, 425 mg, 1.084 mmol) and dry CH_2Cl_2 (500 mL) was added *t*-BOC-glycine (475 mg, 2.714 mmol) and DMAP (125 mg). The mixture was chilled to 0 °C treated with DCC (600 mg, 2.913 mmol), and then left to warm to room temperature. After 20 h, the reaction mixture was concentrated to 50 mL and filtered to remove white DCU. The sample was concentrated further to 20 mL, filtered once more, and then applied to a SiO_2 column (40 g, $CHCl_3$). Evaporation of the appropriate fractions gave pure **7k** as an off-white solid (185 mg). The remainder of the material isolated from the chromatography consisted of a mixture of **5y** and **7k** (140 mg). This was combined with additional starting **5y** (97 mg) recovered by $CHCl_3$ extraction of the DCU obtained above by filtration. This was again reacted under the same conditions, and the product was chromatographed to afford **7k** as a pale white solid (176 mg, 361 mg total, 61%). Recrystallization of a sample from MeOH/CH_2Cl_2 gave pure **7n** as a white microcrystalline solid: mp 251–253 °C; IR (KBr) 3420, 3300, 2970, 2925, 1747, 1697, 1655, 1605, 1595, 1458, 1363, 1247, 1153, 1050, 1030, 940, 860 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.92 (t, 3, J = 7 Hz, H-18), 1.38 (s, 9, *t*-Bu), 2.13 (m, 2, H-19), 3.89 (d AB q, 2, J = 6 (18) Hz, $\Delta\gamma$ = 30 Hz), 5.16 (s, 2, H-5), 5.48 (s, 2, H-17), 6.28 (s, 2, OCH_2O), 7.11 (s, 1, H-14), 7.41 (s, 1, H-9), 7.47 (s, 1, H-12), 8.43 (s, 1, H-7). Anal. ($C_{28}H_{27}N_3O_9 \cdot H_2O$) C, H, N.

The ester **7k** (57 mg) was dissolved in stirred CH_2Cl_2 (15 mL) and the solution was cooled to 0 °C. A solution of HCl-saturated dioxane (8 mL) was added dropwise over 3 min resulting in turbid yellow solution. The mixture was warmed to room temperature, and after 1.5 h the solvent was evaporated to give crude salt **7l**. This material was triturated with CH_2Cl_2 to remove unreacted **7k**. The remaining solid was dissolved in H_2O (20 mL), the hazy blue-yellow solution was filtered (0.45 μm membrane), and the translucent yellow-blue filtrate was frozen and lyophilized to provide **7l** as a bright yellow fluffy solid (32 mg, 64%): mp 240 °C dec; IR (KBr) 2900–3600, 1760, 1656, 1610, 1595, 1495, 1464, 1383, 1255, 1220, 1156, 1025, 860 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95 (t, 3, J = 7 Hz, H-18), 2.18 (m, 2, H-19), 4.22 (d AB q, s, J = 6, 18 Hz, $COCH_2N$), 5.22 (s, 2, H-5), 5.53 (s, 2, H-17), 6.29 (s, 2, OCH_2O), 7.18 (s, 1, H-14), 7.43 (s, 1, H-12), 7.52 (s, 1, H-9), 8.48 (s, 1, H-7), 8.50 (s, 3, NH_3^+). Anal. ($C_{23}H_{19}N_3O_7 \cdot 3.5H_2O \cdot 1.4HCl$) C, H, Cl, N.

Biology. Camptothecin analogs were dissolved in DMSO as stock solutions ranging from 1.25 to 12.5 μg/mL and stored at 4 °C. Radioactive dATP and dTTP were purchased from NEN. The large fragment of *Escherichia coli* DNA polymerase I and all restriction endonucleases were obtained from Bethesda Research Labs. DNA topoisomerase I was isolated from calf thymus.[44]

Gel-Based Cleavable Complex Assay for Topoisomerase I Inhibition. The 3′ end-labeled pBR322 DNA was prepared using published procedures.[45] A modification of a published procedure was used for topoisomerase I mediated DNA cleavage. Reactions were performed in 10 μL-aliquots containing 50 mM Tris, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, and 30 μg/mL BSA. Each drug was tested over a wide range of concentrations in the presence of topoisomerase I and 3′ end-labeled pBR322 DNA. The reactions were incubated at room temperature for 30 min and then terminated by the addition of 1.2 μL of 1.5 mg/mL proteinase K and 1.0 μL of 10% SDS. The reactions were incubated at 50 °C for 30 min. In order to visualize single strand breaks, the DNA was denatured with NaOH and electrophoresed on agarose gels. The gels were blotted on nitrocellulose paper and exposed to X-ray film.

Intact Cell Assay for Topoisomerase I Inhibition. A modification of a published procedure[46] was used to quantitate the amount of topoisomerase I mediated DNA cleavage in intact cells. The DNA of HL-60 cells growing in culture was labeled by [^{3}H]thymidine incorporation. The cells were exposed to drugs and lysed, and protein was precipitated. Radioactive DNA in cleavable complex formation with topoisomerase I coprecipitates with the protein. The amount of cleavable complex formation was quantitated by counting the pellet with a liquid scintillation counter.

***In Vivo* Antitumor Assays.** *In vivo* anti-tumor assays were performed using the L1210 murine lymphoid leukemia,[47] RAW 117-H10 murine lymphosarcoma,[48,49] K1735-M2 murine melanoma,[50,51] and HT-29 human colon adenocarcinoma models.[19] L1210 leukemia cells (10^5) were implanted ip in female DBA/2 mice, RAW 117-H10 lymphosarcoma cells (5×10^5) were injected into the lateral tail vein of female BALB/C mice, K1735-M2 melanoma cells (10^5) were injected iv into female C3H mice, and HT-29 adenocarcinoma cells (10^6) were injected sc into female nude (Nu/Nu) mice. Drug treatment for the murine models was administered sc beginning 1 day after tumor implant. L1210 and RAW 117-H10 were evaluated by median survival time, with results being expressed as a percentage of control survival time. K1735-M2 was evaluated by mean number of metastases at day 14, expressed as a percentage of control metastases. Drug treatment for the HT-29 model was initiated when tumor weight reached 100–200 mg [based on the formula for an ellipsoid sphere, where weight (mg) = length (mm) × width^2 (mm)/2] and continued twice weekly for 10 administrations. HT-29 was evaluated by tumor weight expressed as percentage of control.

Acknowledgment. We thank John Bisi and Francis Sun at Glaxo for assistance in evaluating certain compounds in vivo. We are grateful to NIH-NCI for major support under CA-38996-01-06 and CA50529. We wish to thank Glaxo Research Institute for support.

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