of calmodulin-independent MLCK is stable for over 1 year at −70 °C.

Enzyme Assays. MLCK was assayed with the synthetic peptide substrate Kemptamide, a synthetic tridecapeptide with a sequence corresponding to residues 11–23 of gizzard myosin light chain except for a carboxy-terminal serine-NH_2.[5] The standard assay was performed in a total volume of 100 μL containing 20 mM Tris-HCl, pH 7.2, 10 μM $MgCl_2$, 100 μM [γ-^{32}P]ATP with a specific activity of 300–1000 cpm/pmol, 50 μM Kemptamide, 0.02 μg of enzyme. When calcium-dependent activity was measured, calmodulin was added at 200 nM, and assays contained 1 mM EGTA or 1 mM EGTA plus approximately 200 μM excess of free calcium. Calcium-independent activity was negligible in the calcium-dependent gizzard enzyme. Assays were initiated by addition of ATP and stopped by addition of 200 μM HCl. Phosphate incorporated into the basic substrate was measured by spotting an aliquot on phosphocellulose filter paper, washing, and counting the papers.

For determination of IC_{50} values, inhibitors were tested in duplicate at four concentrations, including concentrations above and below the IC_{50}, and values were determined graphically. Values are reported as the mean of measured IC_{50}'s or a percent inhibition at a concentration when the IC_{50} was not determined. K_i values were determined for key inhibitors by secondary plots of apparent K_m vs inhibitor concentration.

In order to determine whether the inhibitory peptides were phosphorylated by MLCK, a column anion-exchange method procedure was used.[32] Calmodulin-dependent chicken gizzard MLCK was used for this assay, and assay mixtures were as described above, except that 1 mM peptide was used in place of Kemptamide.

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Registry No. 1, 89315-28-6; 7, 73393-25-6; 8, 124318-95-2; 9, 124318-96-3; 10, 124318-97-4; 11, 124340-25-6; 12, 124318-98-5; 13, 124318-99-6; 14, 124319-00-2; 15, 124319-01-3; 16, 124319-02-4; 17, 124319-03-5; 18, 124319-04-6; 19, 124319-05-7; 20, 124319-06-8; 21, 124319-07-9; 22, 124319-08-0; 23, 124319-09-1; 24, 124319-10-4; 25, 124319-11-5; 26, 124340-26-7; 27, 124319-12-6; 28, 124319-13-7; 29, 124319-14-8; 30, 124319-15-9; 31, 124319-16-0; 32, 124319-17-1; 33, 124319-18-2; 34, 124319-19-3; 35, 124319-20-6; 36, 124319-21-7; 37, 124319-22-8; 38, 124319-23-9; 39, 124377-86-2; 40, 124377-87-3; 41, 124378-71-8; 42, 124377-88-4; 43, 124377-89-5; 44, 124319-24-0; 45, 124319-25-1; 46, 124319-26-2; 47, 124340-27-8; 48, 124340-28-9; 49, 124340-29-0; 50, 124319-27-3; 51, 124319-28-4; 52, 124319-29-5; 53, 124319-30-8; 54, 124319-31-9; 55, 124340-24-5; 56, 124319-32-0; 57, 124319-33-1; 58, 124377-90-8; 59, 124377-91-9; 60, 124319-34-2; 61, 124319-35-3; 62, 124319-36-4; 63, 124319-37-5; 64, 124319-38-6; 65, 124319-39-7; 66, 124319-40-0; 67, 124319-41-1; 68, 124340-30-3; 69, 124319-42-2; 70, 124377-92-0; 71, 124319-43-3; 72, 124319-44-4; 73, 124340-31-4; MLCK, 51845-53-5; (*R*)-$HOCH_2CHMeCOOMe$, 72657-23-9; (THP)OCH_2-(*R*)-CHMeCOOMe, 88557-53-3; (THP)OCH_2-(*S*)-$CHMeCH_2OH$, 88588-59-4; $PhCH_2Br$, 100-39-0; (THP)OCH_2-(*S*)-$CHMeCH_2OBzl$, 104265-23-8; (*S*)-$BrCH_2CHMeCH_2OBzl$, 63930-50-7; (*S*)-Ph_3P^+-$CH_2CHMeCH_2OBzl\cdot Br^-$, 124340-32-5; BOC-Phe-H, 72155-45-4; (BOC)NH-(*S*)-$CH(CH_2Ph)$-(*E*)-CH=CH-(*R*)-$CHMeCH_2OBzl$, 124319-45-5; (BOC)NH-(*S*)-$CH(CH_2Ph)$-(*Z*)-CH=CH-(*R*)-$CHMeCH_2OBzl$, 124319-46-6; BOC-Ser-Asn-Val-OH, 124319-47-7; (*S*)-(BOC)$NHCH(CH_2Ph)CH_2SO_2Ph$, 108385-55-3; (*E*)-PhCH=CHCHO, 14371-10-9; (BOC)$NHCH(CH_2Ph)CH(SO_2Ph)CH$-(OH)CH=CHPh, 124319-48-8; (2*S*,3*E*,5*E*)-(BOC)NHCH-(CH_2Ph)CH=CHCH=CHPh, 124319-49-9; (*R*)-(BOC)NHCH-$(CH_2Ph)(CH_2)_4Ph$, 124340-33-6; $Ph(CH_2)_3CO(CH_2)_3Ph$, 63434-46-8; $OHCCH[(CH_2)_3Ph]_2$, 124319-51-3; (2*S*,3*E*)-(BOC)NHCH-$(CH_2Ph)CH$=$CHCH[(CH_2)_3Ph]_2$, 124319-52-4; 3,4-dihydro-2*H*-pyran, 110-87-2; 2,2-bis(3-phenylpropyl)oxirane, 124319-50-2.

Plant Antitumor Agents. 29.[1] Synthesis and Biological Activity of Ring D and Ring E Modified Analogues of Camptothecin

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The total synthesis of the pentacyclic camptothecin analogues **3** and **4** in 11 steps from *p*-tolualdehyde is described. The overall shape of compound **3** is the same as that of potent, naturally occurring camptothecin (**1a**). Despite the near spatial identity of **3** and 1b (racemic, (20*RS*)-camptothecin) from a three-dimensional standpoint, the 9KB and 9PS cytotoxicity assays indicate at least a 40–60-fold decrease in activity of **3** compared to that of 1b, and the isomer **4** was inactive. Similarly, studies of the inhibition of topoisomerase I activity indicated only slight activity for **3** and no activity for **4**. It is evident that the pyridone ring D is essential for antitumor activity. Three E ring modified analogues of camptothecin, **2d–f**, are described in which the net change is replacement of O by N in ring E. Compared to (20*S*)-camptothecin (**1a**) or (20*RS*)-camptothecin (1b), the ring E modified analogues **2d–f** display little or no cytotoxic activity, greatly reduced effect on the inhibition of topoisomerase I, and total loss of life prolongation in the in vivo L-1210 mouse leukemia assay, indicative of the highly restricted structural and electronic requirements of ring E for biological activity in camptothecin.

The discovery of the natural product (20*S*)-camptothecin (**1a**) and its potent antitumor activity more than 20 years ago[2] has led to considerable research efforts to provide a derivative or analogue suitable for use as a clinically valuable anticancer agent. In the process, considerable insight into structural requirements for antitumor activity has been gained.[3] Recently the findings that

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[‡] National Cancer Institute.

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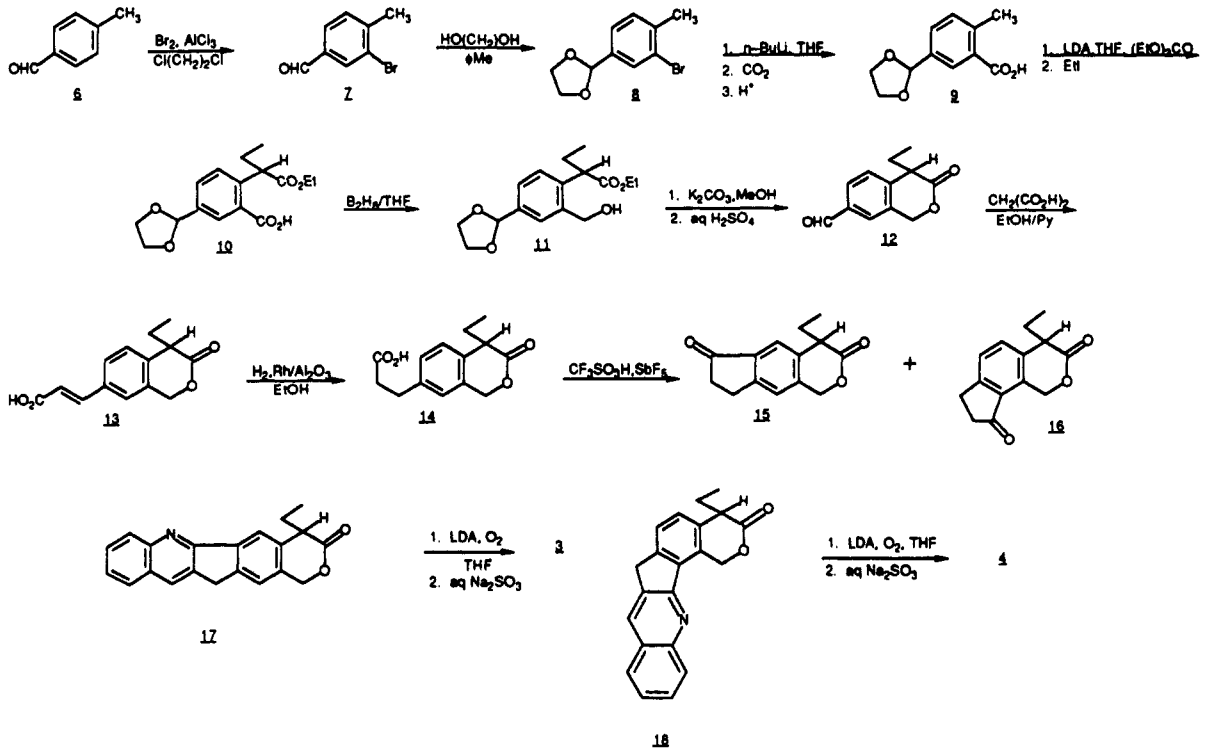
0022-2623/90/1833-0972$02.50/0

Scheme I



camptothecin and some of its analogues are potent inhibitors of the enzyme topoisomerase I and that there is excellent correlation with in vivo activity of the various active analogues provide a molecular basis for the activity.[4]

Studies in our laboratory have defined several structural requirements for antitumor activity in the camptothecin series. These may be summarized as follows: (1) the α-hydroxy lactone system of ring E is required for activity as evidenced by the poor activity of compounds **2a–c**;[2,5] (2) substitution in the A ring at position 9 or 10 is compatible with activity, and a wide range of activity and potency has been observed depending on the substituent;[3e,f,6] (3) substitution at position 11 in ring A leads to compounds of relatively low activity (notable exceptions are the extremely active and potent 10,11-methylenedioxy analogue **2g** and the highly active but less potent 11-hydroxy derivative **2h**[3f]); (4) substitution at the 12-position results in inactivation;[3c] and (5) the 20*S* enantiomeric configuration as found in the natural compound **1a** is a prerequisite for antitumor activity.[3g] The (20*R*)-camptothecin isomer **1c** has less than $^1/_{10}$ the cytotoxic activity of **1a** and is only marginally active in vivo (the slight activity found probably being due to the presence of a trace of the 20*S* isomer). Thus, advances have been made in defining some key structural features of camptothecin relating to its antitumor activity.

The subject of this paper is the description of the preparation and in vitro cytotoxic activity and inhibition of topoisomerase I activity of the unique ring D benzo compounds **3** and isomer **4** and the synthesis and in vitro and in vivo activities of the ring E modified camptothecin analogues **2d–f**. By design, compound **3** is practically indistinguishable from (20*RS*)-camptothecin (**1b**) in terms of three-dimensional structures obtained by molecular modeling. The unusual pentacyclic compound **4** which is isomeric with **3**, derives from a step late in the synthetic scheme. Compounds **2d–f** involve the substitution of nitrogen for oxygen in various positions of ring E of camptothecin. Compound **2e** to some extent would be expected to mimic the hydrogen-bonding characteristics

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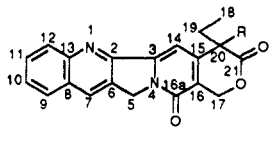
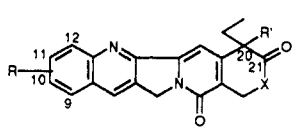
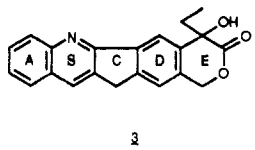


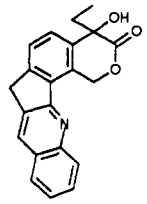
1a: 20(S); R = --- OH
b: 20(RS); R = —OH
c: 20(R); R = ▬OH



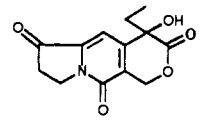
2a: R = R' = H, X = O
b: R = H, R' = OAc, X = O
c: R = H, R' = Br, X = O
d: R = H, R' = N_3, X = O
e: R = H, R' = NH_2·HCl, X = O
f: R = H, R' = --- OH, X = NH
g: R = 10,11-OCH_2O, R' = OH, X = O
h: R = 11-OH, R' = OH, X = O



3



4



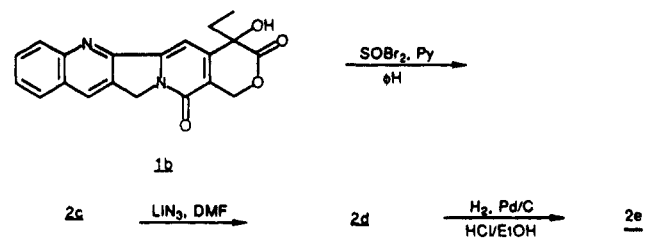
5

of the hydroxyl moiety of **1a** or **1b** of this ring.

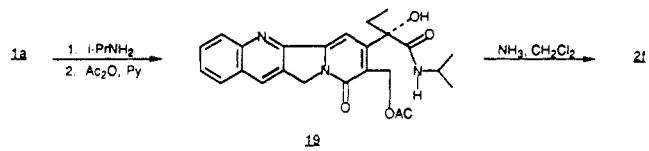
Chemistry

The sequence of reactions which was ultimately successful in the synthesis of compounds **3** and **4** is shown in Scheme I.[7] However, it is noteworthy to point out here that attempts to parallel some of the key synthetic steps which we earlier developed for the versatile tricyclic camptothecin synthon **5**[3d,6] met with unexpected difficulties.[8]

Scheme II



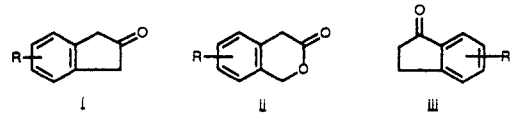
Scheme III



Bromination of *p*-tolualdehyde (**6**) in refluxing 1,2-dichlorethane containing anhydrous $AlCl_3$ gave **7** in 74% yield.[9] Standard reaction conditions employing ethylene glycol and tosyl acid in refluxing toluene gave a 94% yield of acetal **8**. Metalation of **8** using *n*-butyllithium in THF at −78 °C followed by quenching with powdered dry ice and acidification provided a 62% yield of the benzoic acid derivative **9**. By the application of the general procedure of Hauser and Rhee[10] for acylation of the methyl group of *o*-toluic acid, we were able to further elaborate compound **9**. Thus, treatment of **9** in THF solution with 3 equiv of lithium diisopropylamide (LDA) in the presence of diethyl carbonate at low temperature followed by further reaction with ethyl iodide in a one-pot process gave acid ester derivative **10** in overall 49% yield. Standard diborane reduction of **10** in THF afforded alcohol ester compound **11** in 84% yield, and treatment of **11** with potassium carbonate in MeOH followed by aqueous H_2SO_4 provided a 38% yield of bicyclic lactone **12**. Condensation of the aldehyde group in **12** with malonic acid under conditions of refluxing ethanol/pyridine[11] resulted in concomitant dehydration/decarboxylation of the intermediate hydroxy malonic acid derivative to give a 67% yield of cinnamic acid derivative **13**. Hydrogenation of **13** using rhodium on alumina at ambient conditions provided the propionic acid analogue **14** in 86% yield. When this reaction was attempted in the presence of palladium on carbon or platinum oxide, there was substantial hydrogenolysis of the lactone function as a competing reaction. Cyclodehydration of **14** in a triflic acid/antimony pentafluoride mixture at 90 °C afforded the tricyclic isomers **15** (12%) and **16** (19%). Friedlander condensation of each tricyclic ketone with o-aminobenzaldehyde hydrochloride by neat fusion at 130–140 °C provided pentacyclic systems **17** (54%) and **18** (35%), respectively. The anions of **17** and **18** were generated by reaction with LDA in THF, and treatment with bubbling oxygen followed by reduction of the intermediate hydroperoxides with sodium sulfite resulted in compound **3** (20%) and the isomeric structure **4** (14%), respectively.

The synthesis of two ring E modified camptothecin analogues, 20-deoxy-20-azide **2d** and 20-amine hydrochloride **2e**, is shown in Scheme II. Following the procedure previously reported for the reaction of camptothecin with thionyl chloride,[2] compound **1b** was reacted with

(7) Other approaches to the synthesis of **3** which received limited attention and were ultimately abandoned include the following: (1) synthesis of substituted 2-indanones i and elaboration



i ii iii

into the isochromanone system ii via Baeyer–Villiger reaction; (2) Friedel–Crafts reaction on the unsubstituted isochromanone system ii (R = H); (3) various syntheses of substituted 1-indanones iii. All of these attempts were designed to take advantage of the intact bicyclic systems of these molecules. However, the incorporation of appropriate R groups would not have been feasible.

(8) There are additional features of Scheme I which warrant further comment, and these serve to emphasize chemical dissimilarity between tricyclic ketones **5** and **15**. The methyl group on the carbocyclic ring of compound **8** is considerably less acidic than that of the methylpyridones leading to ketone **5**. Our attempts to acylate the methyl group in the cyano and nitro analogues of **9** (i.e., CN or NO_2 instead of CO_2H) failed even when LDA was used. In the corresponding cyanopyridone case, sodium ethoxide was sufficiently basic for acylation to take place. During the cyclization of **11** to **12** it was possible to also introduce the OH group at the tertiary postion in analogy to the pyridone series. However, when hydroxylated **12** was warmed with triflic acid (conditions for cyclodehydration of **14** to tricyclics **15** and **16**), there was NMR evidence of dehydration to the two exocyclic double bond isomers. In the pyridone series, there is a remarkable resistance to such dehydration. In the absence of the activating influence of the *p*-CHO group in compounds following lactone **12**, oxygenation at the tertiary position was surprisingly difficult and required the use of a strong amide base in the final low-yield conversions of **17** to **3** and **18** to **4**. In these oxygenations it was necessary to maintain strict control of base quantity because of additional oxidation in ring C. Finally, standard acid-catalyzed Friedlander condensation conditions which worked nicely with tricyclic ketone **5**[3d-f] failed to give useful products with **15** or **16**.

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Table I. In Vivo and in Vitro Activities of Camptothecin and Analogues

compd	DNA relaxation[a] by topoisomerase I: dose, μm	DNA relaxation[a] by topoisomerase I: % inhibition	cytotoxic activity:[b] ED_{50}, μg/mL: 9KB	cytotoxic activity:[b] ED_{50}, μg/mL: 9PS	life prolongation:[b] T/C × 100 (mg/kg): L1210	life prolongation:[b] T/C × 100 (mg/kg): P388
1a	10	76	0.02	0.08[c]	200 (4)	199 (4)
	30	85				
1b	10	39	0.03	0.04	NT[d]	220 (8)
	30	48				
	100	70				
2d	inactive at 10–30 μm		>10.0	>10.0	inactive, nontoxic at 30 mg/kg	
2e	inactive at 10–30 μm		1.6	3.4	inactive, nontoxic at 40 mg/kg	
2f	inactive at 10–30 μm		>10.0	3.0	inactive, nontoxic at 80 mg/kg	
3	30	6	1.3	2.5	NT	NT
	100	38				
4	30	0	>10.0	>10.0	NT	NT

[a] Determined by the method of Jaxel et al.[4c] [b] Determined by the method of Geran et al.[15] [c] Evaluated in another assay. [d] NT = not tested.

refluxing thionyl bromide and pyridine in benzene to provide the intermediate 20-deoxy-20-bromo-(20*RS*)-camptothecin (**2c**, R′ = Br) in 57% yield. The nucleophilic potency of the azide ion is well-known and displacements at sterically hindered tertiary positions have been documented.[12] When bromo compound **2c** was treated at room temperature with lithium azide in DMF, the displacement of bromide ion was remarkably facile and was complete in a matter of minutes with 20-deoxy-20-azido-(20*RS*)-camptothecin (**2d**) being isolated in 87% yield. Hydrogenation of **2d** using palladium/carbon in EtOH containing HCl gave a near quantitative conversion to amine hydrochloride **2e**. Somewhat surprisingly, salt **2e** was poorly water soluble.

The third ring E modified camptothecin analogue **2f** was synthesized as shown in Scheme III. We have reported some biological activity data for **2f** earlier,[4d] and very recently an alternate method for the synthesis of **2f** has been suggested.[13] Camptothecin (**1a**) was reacted with isopropylamine at reflux overnight to give an intermediate hydroxy amide;[14] a controlled acetylation over a couple of hours provided 17-acetoxy-(20*S*)-camptothecin-21-isopropylamide (**19**).[14] Upon treatment of **19** with anhydrous ammonia in methylene chloride in a sealed vessel at room temperature, the lactam **2f** resulted in 75% yield.

Biology

The effect of compounds **3** and **4** on the inhibition of DNA relaxation by topoisomerase I is shown in Table I. For comparison, similar data for (20*S*)-camptothecin (**1a**) and (20*RS*)-camptothecin (**1b**) are presented. It is evident that **3** is much less active than **1b** and that **4** is essentially inactive. Also shown in Table I are the ED_{50} values of **3** and **4** for 9KB and 9PS compared with those of **1a** and **1b** assayed at the same time. It is evident that **3** is about 40–60 times less active than **1b**, and **4** is essentially inactive. Because of the limited quantity of **3** and **4** available and because of the excellent correlation of in vivo and in vitro activities including inhibition of topoisomerase I in the camptothecin series,[3e,f,4b,c] no in vivo assays were conducted with **3** and **4**. Although the inactivity of **4** is not surprising, the relative inactivity of **3** was unexpected. Molecular modeling studies show that the shape of **3** and **1b** are virtually identical. Therefore it appears that the pyridone ring D is a key feature in the binding of camptothecins to the cleavable enzyme–DNA complex. These studies clearly show that the pyridone ring D can be added to the requirements for activity in the camptothecin series.

Table I gives in vitro and in vivo data for the nitrogen analogues **2d–f**. Lactam **2f** was inactive in 9KB cytotoxicity and 100 times less active than **1b** in 9PS cytoxicity. 20-Azido analogue **2d** was inactive in both 9KB and 9PS assays, and 20-amino analogue **2e**, allowing for the probable difference between 20*S* and 20*RS* forms, was about 40 times less active than **1a**.[15] In the in vivo L-1210 mouse leukemia assay, lactam **2f** was inactive and nontoxic at doses as high as 80 mg/kg, as was 20-azido analogue **2d** at doses of 30 mg/kg, and somewhat surprisingly, 20-amino analogue **2e** was inactive at doses of 40 mg/kg. In contrast, **1a** and **1b** are highly active at doses of 4–8 mg/kg. The inactivity of **2d** was not surprising given the requirement that the C-20 hydroxyl moiety be present for activity in the camptothecin series.[3c,5] The inactivity of lactam **2f** was also not unexpected as it has become increasingly evident that the requirements for topoisomerase I activity necessitate the reactive α-hydroxy lactone function in ring E which can easily open and then relactonize (cf. Hertzberg et al.);[13] the lactam moiety is obviously much less reactive. The inactivity of 20-amino compound **2e** may be rationalized as follows: It is believed that **1a** interferes with the DNA breakage–reunion reaction by reversibly trapping the enzyme DNA intermediate termed the "cleavable complex".[4a–d,13] We have known for many years that the lactone carbonyl of **1a** was unusually reactive, due probably to intramolecular hydrogen bonding by the C-20 hydroxyl.[2,5] However, in **2e** such activation of the lactone carbonyl is not possible in the protonated form, and even in the free base such activation would be greatly reduced.

Experimental Section

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.,

(12) Edwards, O. E.; Grieco, C. *Can. J. Chem.* **1974**, *52*, 3561.
(13) (a) Hertzberg, R. P.; Caranfa, M. J.; Holden, K. G.; Jakas, D. R.; Gallagher, G.; Mattern, M. R.; Mong, S-M.; Bartus, J. O.; Johnson, R. K.; Kingsbury, W. D. *J. Med. Chem.* **1989**, *32*, 715. (b) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629.
(14) Adamovics, J. A.; Hutchinson, C. R. *J. Med. Chem.* **1979**, *22*, 310.
(15) Although we have shown that the (20*RS*)-camptothecin analogues have exactly $^1/_2$ the potency of the 20*S* forms (cf. P388 activity of compounds **1a** and **1b** in Table I) and that the 20(*R*) form is inactive,[3g] we have not made the resolved forms of **2e**. However the lack of activity and toxicity of **2e** suggests that neither the *R* nor *S* form alone would be active.

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.

Topoisomerase I inhibition assays were conducted by the procedure of Jaxel et al.[4c] In vivo L-1210 mouse leukemia life-prolongation assays and in vitro 9KB and 9PS cytotoxicity assays were conducted under protocols described by Geran et al.[16]

3-Bromo-4-methylbenzaldehyde, Ethylene Acetal (8). 3-Bromotolualdehyde (7,[9] 5.00 g, 25.13 mmol), ethylene glycol (5 mL, excess), and *p*-TsOH·H_2O (100 mg) were refluxed in toluene (125 mL), and the H_2O azeotrope was collected (Dean–Stark trap). After 2 h, the hazy pale yellow solution was cooled and diluted with H_2O (50 mL) and EtOAc (20 mL). After mixing, the H_2O was discarded and the organic phase was washed with additional H_2O (25 mL). After drying (Na_2SO_4) and evaporation, ethylene acetal 8 was obtained as a colorless oil (5.73 g, 94%). Compound 8 of >90% purity resulted and could be purified further by silica gel chromatography (2% EtOAc in hexanes) to provide a clear colorless oil: IR ($CHCl_3$) 2950, 2880, 1415, 1340, 1080 (acetal), 1035, 962, 938, 880, and 818 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 2.36 (s, 3, CH_3), 3.98 (m, 4, $-O(CH_2)_2O-$), 5.70 (s, 1, acetal *H*), 7.22 (m, 2, aromatic *H*), 7.63 (s, 1, aromatic *H*). Anal. ($C_{10}H_{11}BrO_2$) C, H, N.

3-Carboxy-4-methylbenzaldehyde, Ethylene Acetal (9). A solution of bromo compound 8 (45.58 g, 0.188 mol) in dry THF (390 mL) was added over 10 min to a stirred solution of *n*-BuLi in hexanes (1.6 M, 120 mL, 0.192 mol) at −78 °C. The pale yellow turbid mixture was stirred for 35 min at −78 °C and then poured over 1 kg of powdered dry ice. After the mixture had warmed to >0 °C, H_2O (1 L) and Et_2O (400 mL) were added. The aqueous phase was separated and acidified with HOAc to pH 5. The turbid white mixture was extracted with EtOAc (2 × 250 mL); the extract was dried (Na_2SO_4) and evaporated to afford the acid 9 as a white solid (24.34 g, 62%). Recrystallization of the product from EtOAc provided colorless needles: mp 155–157 °C; IR (KBr) 2300–3300 (acid OH), 1686 (carboxyl CO), 1618, 1573, 1450, 1432, 1407, 1381, 1370, 1308, 1276, 1220, 1078 (acetal), 990, 954, 907, 842, 836, 781, and 676 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 2.66 (s, 3, CH_3), 4.07 (m, 4, $-O(CH_2)_2O-$), 5.80 (s, 1, acetal *H*), 7.27 (d, 1, J = 7 Hz, H-5), 7.56 (dd, 1, J = 2, 7 Hz, H-6), 8.17 (d, 1, J = 2 Hz, H-2), 10.5 (br s, 1, CO_2H). Anal. ($C_{11}H_{12}O_4$) C, H.

Ethyl 2-[2-Carboxy-4-[1-(2,5-dioxolyl)]phenyl]butanoate (10). A solution of benzoic acid derivative 9 (13.59 g, 65.34 mmol) and diethyl carbonate (11.56 g, 98.00 mmol, 1.5 equiv) in dry THF (130 mL) was added over 15 min to a cold, stirred solution (−78 °C) of LDA in dry THF/hexanes [196.01 mmol, prepared by the addition of 124 mL of a 1.6 M solution of *n*-BuLi in hexanes (198.4 mmol) to a stirred solution of 19.80 g (196.01 mmol) of diisopropylamine in 130 mL of dry THF at 0 °C]. The resulting red-brown solution was allowed to warm to room temperature over 1.5 h whereupon a light orange slurry was formed.

The mixture containing the enolate of the intermediate acetate was again chilled to −78 °C and treated over 10 min with a solution of EtI (12.74 g, 81.67 mmol) in dry THF (30 mL). The turbid orange slurry was warmed to room temperature over 1.5 h, during which time the slurry became light yellow. The reaction was poured into ice/H_2O (700 mL) containing HOAc (50 mL,) the resulting mixture was extracted with EtOAc (3 × 250 mL), and the extract was washed with aqueous $NaHCO_3$ (3 × 100 mL). The aqueous phase was acidified with HOAc until pH 5 was reached to give a turbid white solution which was extracted with EtOAc (3 × 150 mL). The extract was dried (Na_2SO_4) and evaporated to afford substituted ethyl butanoate 10 as a heavy yellow syrup (9.92 g, 49%). Further purification by silica gel chromatography using 5% MeOH in $CHCl_3$ containing 1% HOAc gave 10 as a thick, colorless oil: IR ($CHCl_3$) 3300–2400, 2960, 2935, 2875, 1720, 1690, 1370, 1260, 1175, 1085, 1018, and 940 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 0.95 (t, 3, J = 7 Hz, $RR'CHCH_2CH_3$), 1.21 (t, 3, J = 7 Hz, OCH_2CH_3), 1.6–2.2 (m, 2, $RR'CHCH_2CH_3$), 4.1 (m, 6, OCH_2CH_3 and $-O(CH_2)_2O-$), 4.65 (t, 1, J = 7 Hz, $RR'CHCH_2CH_3$), 5.83 (s, 1, acetal *H*), 7.46 (d, 1, J = 7 Hz, 6-*H*), 7.67 (d, 1, J = 7 Hz, 5-*H*), 8.15 (s, 1, 3-*H*), 9.2 (br s, 1, CO_2H). Anal. ($C_{16}H_{20}O_6$) C, H.

The intermediate substituted phenyl ethyl acetate ester can be isolated by quenching the reaction after 2 h of the addition of diethyl carbonate and then carrying out a workup as described above: 90-MHz ^1H NMR ($CDCl_3$) δ 1.29 (t, 3, J = 7 Hz, OCH_2CH_3), 3.9–4.3 (m, 8, OCH_2CH_3, $ArCH_2CO_2Et$, and $-O(CH_2)_2O-$), 5.85 (s, 1, acetal *H*), 7.29 (d, 1, J = 7 Hz, 6-*H*), 7.64 (dd, 1, J = 2, 7 Hz, 5-*H*), 8.22 (d, 1, J = 2 Hz, 3-*H*), 10.55 (br s, 1, CO_2H).

Ethyl 2-[4-[1-(2,5-Dioxolyl)]-2-(hydroxymethyl)phenyl]butanoate (11). A stirred solution of acid ester 10 (9.92 g, 32.21 mmol) in dry THF (200 mL) was cooled by H_2O bath during treatment with BH_3/THF (1 M, 64.4 mL, 64.4 mmol) over 15 min. The pale yellow solution slowly (1.5 h) became clear and colorless. The reaction mixture was poured into chilled 5% aqueous HOAc and the resulting mixture was extracted with EtOAc (2 × 400 mL). The extract was washed with portions of saturated aqueous $NaHCO_3$ solution until the washings were alkaline. Drying (Na_2SO_4) and evaporation afforded the product alcohol ester 11 as a faint yellow syrup (7.93 g, 84%). This product eluded attempts at purification by chromatography in that there was always evidence by TLC of some formation of the cyclized lactone product 12 as an impurity. Compound 11 was analyzed by TLC and NMR prior to further reaction: 90-MHz ^1H NMR ($CDCl_3$) δ 0.86 (t, 3, J = 7 Hz, $RR'CHCH_2CH_3$), 1.18 (t, 3, J = 7 Hz, OCH_2CH_3), 1.65–2.25 (m, 2, $RR'CHCH_2CH_3$), 3.6–4.2 (m, 6, OCH_2CH_3, $-O(CH_2)_2O-$), 4.5–4.8 (m, 3, $RR'CHEt$, $ArCH_2OH$), 5.85 (s, 1, acetal *H*), 7.0–7.5 (m, 3, 3 arom *H*).

4-Ethyl-7-formylisochroman-3-one (12). A stirred solution of crude hydroxy ester 11 from the preceding step (11.62 g, 39.52 mmol) in MeOH (310 mL) at 20 °C was treated over several minutes with powdered anhydrous K_2CO_3 (5.5 g, 39.86 mmol) in portions. The resulting bright yellow-orange suspension was stirred for 1.5 h at room temperature and acidified to pH 1–2 with 2 N aqueous H_2SO_4. Most of the MeOH was removed from the white turbid mixture under reduced pressure, and H_2O (250 mL) and $CHCl_3$ (300 mL) were added. The $CHCl_3$ phase was reserved, and the aqueous phase was reextracted with $CHCl_3$. The combined $CHCl_3$ was washed with H_2O, dried (Na_2SO_4), and evaporated to provide crude lactone 12 (9.69 g) as a yellow syrup. The sample was purified on SiO_2 (300 g, $CHCl_3$) to give the pure lactone 12 as a thick pale yellow syrup (3.05 g, 38%): IR ($CHCl_3$) 2963, 2937, 2879, 2837, 1739, 1698, 1613, 1588, 1466, 1460, 1440, 1371, 1342, 1308, 1290, 1233, 1180, 1146, 1120, 1060, 1043, and 821 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 1.02 (t, 3, J = 7 Hz, $RR'CHCH_2CH_3$), 1.75–2.2 (m, 2, $RR'CHCH_2CH_3$), 3.56 (t, 1, J = 7 Hz, $RR'CHEt$), 5.35 (AB q, 2, J = 15 Hz, $\Delta\nu$ = 21 Hz, $ArCH_2O$), 7.32 (d, 1, J = 7 Hz, 6-*H*), 7.68 (d, 1, J = 2 Hz, 3-*H*), 7.80 (dd, 1, J = 2, 7 Hz, 5-*H*), 9.94 (s, 1, *CHO*). Anal. ($C_{12}H_{12}O_3 \cdot 0.2H_2O$) C, H.

4-Ethyl-7-(2-carboxyvinyl)isochroman-3-one (13). Aldo lactone 12 (1.6 g, 7.8 mmol) was refluxed with malonic acid (2.75 g, 23.3 mmol) in a solution of 1:1 EtOH/pyr (20 mL). After 12 h, the solution was evaporated under reduced pressure and the residue was taken up in $CHCl_3$/saturated, aqueous $NaHCO_3$ (50 mL of each). The $CHCl_3$ phase was further extracted with bicarbonate solution, and the combined extracts were acidified with concentrated HCl and extracted with $CHCl_3$ (3 × 75 mL). Drying (Na_2SO_4) and evaporation under reduced pressure provided cinnamic acid derivative 13 as a beige, crystalline solid (1.2 g, 62%). The neutral material from this reaction was recycled to provide an additional quantity (110 mg) of 13 for a total yield of 67%. Recrystallization from EtOAc/hexanes gave 13 as a white, crystalline solid: mp 173 °C; IR ($CHCl_3$) 3330–2450 (CO_2H), 2970 (CH), 2940 (CH), 2880 (CH), 1742 (lactone), 1692 (acid), 1640 (arom), 1589, 1502, 1470, 1440, 1412, 1382, 1321, 1280–1290, 1182, 1160, 1046, 981, 868, and 828 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 1.03 (t, 3, J = 7 Hz, CH_2CH_3), 1.9 (m, 2, CH_2CH_3), 3.5 (t, 1, $EtCHRR'$), 5.3 (AB q, 2, J = 14 Hz, $\Delta\nu$ = 25 Hz, $ArCH_2O$), 6.4 (d, 1, J = 15 Hz, $RCH{=}CHCO_2H$), 7.15–8.07 (m, 3, aromatic *H*), 7.7 (d, 1, J = 15 Hz, $RCH{=}CHCO_2H$). Anal. ($C_{14}H_{14}O_4 \cdot 0.1H_2O$) C, H.

4-Ethyl-7-(2-carboxyethyl)isochroman-3-one (14). A solution of cinnamic acid derivative 13 (1.2 g, 4.9 mmol) in ethanol (20 mL) containing rhodium on alumina catalyst (600 mg) was

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hydrogenated at 1 atm for 18 h. The catalyst was collected on Celite and the filtrate was evaporated to afford product **14** as a pale yellow syrup (1.05 g, 86%). Further purification by column chromatography (SiO_2, 2% MeOH/$CHCl_3$) gave the title compound **14** as a colorless syrup: IR ($CHCl_3$) 3250–2400 (acid), 2930 (CH), 1740 (lactone) with 1715 shoulder (acid), 1600 (arom), 1460, 1378, 1280, 1146, 1038, and 1010 cm^{-1}; 90-MHz ^1H NMR ($CDCl_3$) δ 0.95 (t, 3, J = 7 Hz, CH_2CH_3), 1.85 (m, 2, CH_2CH_3), 2.62 (m, 2, RCH_2CHCO_2H), 2.81 (m, 2, $RCH_2CH_2CO_2H$), 3.4 (t, 1, J = 7 Hz, EtCHRR'), 5.2 (AB q, 2, J = 15 Hz, $\Delta\nu$ = 21 Hz, $ArCH_2O$), 6.9–7.2 (m, 3, aromatic H), 8–9 (br s, 1, CO_2H). Anal. ($C_{14}H_{16}O_4 \cdot 0.3H_2O$) C, H.

(4*RS*)-4-Ethyl-1,3,4,6,7,8-hexahydro-3,6-dioxocyclopenta[*g*]-2-benzopyran (15) and (4*RS*)-4-Ethyl-1,3,4,7,8,9-hexahydro-3,9-dioxocyclopenta[*h*]-2-benzopyran (16). A solution of propionic acid derivative **14** (1.9 g, 7.7 mmol) and SbF_5 (2 mL) in triflic acid (6 mL) was heated at 85–90 °C under N_2 for 1 h. The sample was poured into H_2O (50 mL) and the resulting mixture was extracted with $CHCl_3$ (2 × 30 mL). The extract was dried (Na_2SO_4) and evaporated to provide a mixture of crude **15** and **16**. This sample was combined with that generated by a repetition of the above experiment to afford 3.1 g of crude **15** and **16**. Chromatography (SiO_2 column, EtOAc/hexanes, 2:3) gave pure **15** (420 mg, 12%) as the more polar isomer and **16** (680 mg, 19%) both as beige solids. **15**: IR ($CHCl_3$) 2938 (CH), 1750 (lactone), 1712 (ketone), 1623, 1462, 1450, 1384, 1337, 1270, 1245, 1156, 1045, and 876 cm^{-1}; 250-MHz ^1H NMR ($CDCl_3$) δ 1.09 (t, 3, J = 7 Hz, CH_2CH_3), 1.89–2.15 (m, 2, CH_2CH_3), 2.75 (m, 2, $COCH_2CH_2Ar$), 3.16 (m, 2, $COCH_2CH_2Ar$), 3.60 (t, 1, J = 7 Hz, EtCHRR'), 5.41 (AB q, 2, J = 15 Hz, $\Delta\gamma$ = 38 Hz, $ArCH_2O$), 7.36 (s, 1, H-5), 7.62 (s, 1, H-8). Anal. ($C_{14}H_{14}O_3$) C, H. Recrystallization of the less polar isomer **16** from EtOAc gave a cream colored solid: mp 130 °C; IR ($CHCl_3$) 2970 (CH), 2940 (CH), 1740 (lactone), 1704 (ketone), 1615, 1597, 1487, 1458, 1407, 1380, 1296, 1270, 1257, 1186, 1075, 1050, 977, and 837 cm^{-1}; 250-MHz ^1H NMR ($CDCl_3$) δ 1.02 (t, 3, J = 7 Hz, CH_2CH_3), 1.84–2.04 (m, 2, CH_2CH_3), 2.73 (m, 2, $COCH_2CH_2Ar$), 3.17 (m, 2, $COCH_2CH_2Ar$), 3.63 (t, 1, J = 7 Hz, EtCHRR'), 5.89 (AB q, 2, J = 16 Hz, $\Delta\nu$ = 104 Hz, $ArCH_2O$), 7.36 (d, 1, J = 8 Hz, H-5), 7.46 (d, 1, J = 8 Hz, H-4). Anal. ($C_{14}H_{14}O_3$) C, H.

Deoxy Ring D Modified Analogue 17. A mixture of tricyclic ketone **15** (400 mg, 1.739 mmol) and *o*-aminobenzaldehyde hydrochloride (480 mg, 3.491 mmol) was ground with NH_4OAc (100 mg), and the powder was heated at 130–140 °C under nitrogen for 2 h. After cooling, MeOH (5 mL) and then aqueous $NaHCO_3$ (15 mL) were added, and the mixture was extracted with $CHCl_3$ (2 × 30 mL). The $CHCl_3$ phase was dried (Na_2SO_4) and evaporated to afford crude **17**; chromatography (SiO_2, $CHCl_3$) gave the product as a yellow solid (295 mg, 54%), and recrystallization from EtOAc provided **17** of analytical purity as a beige solid: mp 224 °C; IR ($CHCl_3$) 2964 (CH), 2940 (CH), 2860 (CH), 1742 (lactone), 1630 (aromatic), 1575, 1503, 1461 with 1470 shoulder, 1417, 1400, 1384, 1360, 1337, 1312, 1270, 1200–1240, 1188, 1043, 958, 868, and 860 cm^{-1}; 250-MHz ^1H NMR ($CDCl_3$) δ 1.16 (t, 3, J = 7 Hz, CH_2CH_3), 1.99–2.21 (m, 2, CH_2CH_3), 3.72 (t, 1, J = 7 Hz, EtCHRR'), 4.06 (s, 2, $ArCH_2Ar'$), 5.44 (AB q, 2, J = 14 Hz, $\Delta\nu$ = 48 Hz, $ArCH_2O$), 7.45–8.25 (m, 7, arom H). Anal. ($C_{21}H_{17}NO_2$) C, H, N.

Deoxy Angular Ring D Modified Analogue 18. A mixture of tricyclic ketone **16** (280 mg, 1.217 mmol), *o*-aminobenzaldehyde hydrochloride (255 mg, 1.855 mmol), and NH_4OAc (50 mg) was fused at 130–140 °C for 2 h under N_2. The cooled mixture was extracted with $CHCl_3$ (2 × 50 mL) and the extract was washed with H_2O, dried (Na_2SO_4), and evaporated to give crude angular analogue **18**. Column chromatographic purification (SiO_2, EtOAc/hexanes, 1:3) provided pure analogue **18** as a beige solid (133 mg, 35%), and recrystallization from EtOAc gave the analytical sample: mp 221 °C; IR ($CHCl_3$) 2932 (CH), 1737 (lactone), 1629 (aromatic), 1573, 1503, 1487, 1462, 1412, 1381, 1317, 1190, 1132, 1050, 978, 960, 891, 860, and 818 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) δ 1.09 (t, 3, J = 7 Hz, CH_2CH_3), 1.98–2.11 (m, 2, CH_2CH_3), 3.71 (t, 1, J = 7 Hz, ArCHRR'), 4.08 (s, 2, $ArCH_2Ar'$), 6.48 (AB q, 2, J = 16 Hz, $\Delta\nu$ = 164 Hz, $ArCH_2O$), 7.29–8.22 (m, 7, aromatic H). Anal. ($C_{21}H_{17}NO_2$) C, H, N.

Ring D Modified Analogue 3. D ring modified derivative **17** (500 mg, 1.587 mmol) was oxygenated in 10 50-mg batches as follows: under anhydrous conditions, a stirred solution of THF (0.5 mL) and $(i\text{-}Pr)_2NH$ (30 μL, 0.21 mmol) was cooled to −78 °C and treated with *n*-BuLi/hexanes (125 μL, 0.20 mmol). The solution was permitted to warm to 0 °C and after stirring for 10 min was cooled to −25 °C. A suspension of derivative **17** (50 mg, 0.159 mmol) in THF (2 mL) was slowly introduced, the temperature was raised to 0 °C, and the stirring was continued at 0 °C for 30 min. Dry O_2 was bubbled through for 10 min, and then the mixture was poured into saturated, aqueous Na_2SO_3 (2 mL). The sample was stirred for 10 min and the THF was evaporated. The residue was treated with a few drops of 5 N aqueous HCl followed by careful adjustment of pH to 7 using dilute aqueous NaOH. Crude product **3** was collected by filtration (~40 mg). The combined products (10 batches) from the above process were purified by column chromatography as a Celite (2 g) dispersion (SiO_2, $CHCl_3$, 1% MeOH/$CHCl_3$). The appropriate fractions were concentrated whereupon pure **3** separated as an off-white solid (109 mg, 20%). Recrystallization from MeOH/$CHCl_3$ provided the analytical sample: mp 288 °C; IR (KBr) 3460 (OH), 3050, 2950, 2910, 1742 (lactone), 1612 (aromatic), 1563, 1500, 1467, 1460, 1455, 1408, 1398, 1378, 1356, 1328, 1273, 1225, 1179, 1115, 1105, 1040, 1000, 950, 855, 790, 762, and 742 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.86 (t, 3, J = 7 Hz, CH_2CH_3), 1.86 (m, 2, CH_2CH_3), 4.16 (s, 2, $ArCH_2Ar'$), 5.60 (AB q, 2, J = 15 Hz, $\Delta\nu$ = 69 Hz, $ArCH_2O$), 6.29 (s, 1, ArCOCHRR'), 7.59 (t, 1, J = 8 Hz, ring A arom), 7.65 (s, 1, ring D arom), 7.76 (t, 1, J = 8 Hz, ring A arom), 8.01, (d, 1, J = 8 Hz, ring A arom), 8.09 (d, 1, J = 8 Hz, ring A arom), 8.30 (s, 1, ring D arom), 8.48 (s, 1, ring B arom). Anal. Calcd for $C_{21}H_{17}NO_3$: 331.1208. Found: 331.1199. Anal. ($C_{21}H_{17}NO_3$) C, H, N.

Angular Ring D Modified Analogue 4. Title compound **4** was prepared in a fashion analogous to that for the preceding compound **3** with the exception that warming to ambient temperature was required for the formation of the anion of the angular deoxy compound **18**. Oxygenation was then carried out as usual upon cooling again to 0 °C. Thus, eight 50-mg batches of **18** provided pure angular oxy isomer **4** as a beige solid (56 mg, 14%) after chromatography and recrystallization from MeOH/$CHCl_3$: mp 236 °C; IR (KBr) 3405 (OH), 3050, 2955, 2930, 2870, 1735 (lactone), 1622 (aromatic), 1560, 1495, 1480, 1465, 1450, 1370, 1310, 1290, 1232, 1186, 1170, 1148, 1127, 1057, 1034, 998, 920, 812, 763, 755, and 747 cm^{-1}; 250-MHz ^1H NMR (DMSO-d_6) δ 0.83 (t, 3, J = 7 Hz, CH_2CH_3), 1.87 (m, 2, CH_2CH_3), 4.16 (s, 2, $ArCH_2Ar'$), 6.24 (s, 1, ArCOHRR'), 6.45 (AB q, 2, J = 16 Hz, $\Delta\nu$ = 256 Hz, $ArCH_2O$), 7.59–7.84 (m, 4, aromatic), 8.02 (d, 1, J = 9 Hz, aromatic), 8.12 (d, 1, J = 9 Hz, aromatic), 8.48 (s, 1, H-7). Anal. Calcd for $C_{21}H_{17}NO_3$: 331.1208. Found: 331.1214. Anal. ($C_{21}H_{17}NO_3 \cdot 0.5H_2O$) C, H, N.

20-Bromo-20-deoxy-(20*RS*)-camptothecin (2c). (20*RS*)-Camptothecin (1b, 500 mg, 1.437 mmol) was suspended in a mixture of thionyl bromide (4.56 g, 21.90 mmol), pyridine (1.7 mL), and benzene (65 mL), and the sample was refluxed for 1.25 h to give a clear red solution. The solvents were removed in a nitrogen stream, and the residue was chromatographed as a dispersion on Celite through silica gel (30 g, $CHCl_3$). Evaporation of the appropriate fractions provided 20-bromo compound **2c** as a yellow solid (339 mg, 57%), and recrystallization from MeOH/$CHCl_3$ gave **2c** as a yellow microcrystalline solid: mp 290 °C dec; IR (KBr) 1727 (lactone), 1655 (pyridone), 1618 (aromatic), 1522, 1483, 1376, 1285, 1262, 1240, 1166, 1142, 1050, 748, and 672 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.01 (t, 3, J = 7 Hz, H-18), 2.83 (m, 1, H-19), 3.04 (m, 1, H-19), 5.37 (s, 2, H-5), 5.49 (AB q, 2, J = 19 Hz, $\Delta\nu$ = 80 Hz, H-17), 7.47 (s, 1, H-14), 7.69 (t, 1, J = 8 Hz, H-11), 7.86 (t, 1, J = 8 Hz, H-10), 7.93 (d, 1, J = 8 Hz, H-9), 8.26 (d, 1, J = 8 Hz, H-12), 8.42 (s, 1, H-7). Anal. ($C_{20}H_{15}N_2BrO_3 \cdot 0.25H_2O$) C, H, N, Br.

20-Azido-20-deoxy-(20*RS*)-camptothecin (2d). Racemic 20-bromo compound **2c** (212 mg, 0.516 mmol) was suspended in dimethylformamide (12 mL) at room temperature and treated with lithium azide (76 mg, 1.551 mmol). The reaction mixture rapidly turned clear orange and then over 10 min changed to turbid tan-grey. The solvent was removed by high-vacuum distillation at 40 °C, and the residue was dispersed on Celite and subjected to column chromatography (12 g of SiO_2, $CHCl_3$). Combination and evaporation of the suitable fractions gave azide **2d** as an off-white solid (167 mg, 87%) which gave a white solid

upon recrystallization from MeOH/$CHCl_3$: mp 255–258 °C dec; IR (KBr) 2114 (N_3), 1752 (lactone), 1662 (pyridone), 1614 (aromatic), 1440, 1392, 1995, 1260, 1250, 1230, 1100, 1048, 785, 760, 722, and 170 cm^{-1}; ^1H NMR ($CHCl_3$) δ 1.03 (t, 3, J = 7 Hz, H-18), 2.20 (m, 2, H-19), 5.30 (s, 2, H-5), 5.49 (AB q, 2, J = 17 Hz, $\Delta\nu$ = 90 Hz, H-17), 7.52 (s, 1, H-14), 7.68 (t, 1, H = 8 Hz, H-11), 7.84 (t, 1, 8 Hz, H-10), 7.94 (d, 1, 8 Hz, H-12), 8.24 (d, 1, 8 Hz, H-9), 8.41 (s, 1, H-7). Anal. ($C_{20}H_{15}N_5O_3 \cdot 0.25H_2O$) C, H, N.

20-Amino-20-deoxy-(20*RS*)-camptothecin Hydrochloride (2e). A solution of azide **2d** (100 mg, 0268 mmol) in absolute EtOH (15 mL) containing concentrated HCl (0.6 mL) and 10% Pd/C (75 mg) was subjected to 1 atm of H_2 at room temperature for 20 h. The catalyst was removed by Celite filtration and the solvent was evaporated to afford **2e** as a pale orange-yellow solid (96 mg, 93%), and recrystallization from MeOH/$CHCl_3$ gave the pure material as a beige solid: mp 285–287 °C dec; IR (KBr) 3650–3120, 3120–2300 (C-H, amine HCl), 1750 (lactone), 1660 (pyridone), 1595 (aromatic), 1498, 1470, 1403, 1245, 1172, 1132, 1045, 785, 765, and 718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, 3, J = 7.5 Hz, H-18), 1.91 (m, 2, H-19), 1.96 (b s, 3, NH_3), 5.29 (s, 2, H-5), 5.49 (AB q, 2, J = 17 Hz, $\Delta\nu$ = 92 Hz, H-17), 7.65 (t, 1, J = 7 Hz, H-11), 7.78 (s, 1, H-14), 7.83 (t, 1, J = 7 Hz, H-10), 7.92 (d, 1, J = 7 Hz, H-12), 8.22 (d, 1, J = 7 Hz, H-9), 8.38 (s, 1, H-7). Anal. ($C_{20}H_{17}N_3O_3 \cdot 1.4HCl \cdot 2.2H_2O$) C, H, Cl, N.

(20*S*)-Camptothecin-21-lactam (2f). 17-Acetoxy-21-isopropyl amide derivative **19**[14] of 20(*S*)-camptothecin (267 mg, 0.595 mmol) was suspended in CH_2Cl_2 (2 mL) in a 23-mL capacity Teflon-lined bomb (Parr 4745) containing a magnetic stirbar and the mixture was chilled to -78 °C. Ammonia (8 mL) was condensed in the bomb, the bomb was sealed, and the stirred mixture was left at ambient temperature for 18 h. After recooling, the bomb was opened, and the contents were evaporated to provide a yellow solid consisting primarily of lactam **2f**. The sample was chromatographed as a dispersion on Celite through SiO_2 (18 g, 50 mL of $CHCl_3$, 300 mL each of 2% MeOH/$CHCl_3$ and 3% MeOH/$CHCl_3$) to provide pure **2f** as a pale yellow solid (155 mg, 75%) which was recrystallized from MeOH/$CHCl_3$: mp 310–315 °C with prior darkening above 250 °C; IR (KBr) 3400, 3240 (NH, OH), 2975, 2937, 2880, 1672 (lactam), 1655 (pyridone), 1600, 1583, 1496, 1460, 1440, 1400, 1354, 1228, 1176, 1132, 1083, 1000, 918, 838, 789, 773, 764, and 726; ^1H NMR (DMSO-d_6) δ 0.81 (t, 3, J = 7 Hz, H-18), 1.76 (m, 2, H-19), 4.26 (AB q, 2, J = 17.5 Hz, $\Delta\nu$ = 50 Hz, with one proton further coupled, J = 4 Hz, with lactam proton), 5.28 (s, 2, H-5), 5.66 (s, 1, 20-OH), 7.35 (s, 1, H-14), 7.70 (t, 1, J = 7 Hz, H-11), 7.86 (t, 1, J = 7 Hz, H-10), 8.15 (m, 2, H-9 and H-12), 8.34 (d, 1, J = 4 Hz, NH), 8.68 (s, 1, H-7). Anal. ($C_{20}H_{17}N_3O_3 \cdot 0.5H_2O$) C, H, N.

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Registry No. **1a**, 7689-03-4; **1b**, 31456-25-4; **2c**, 124717-74-4; **2d**, 124717-75-5; **2e**, 124717-76-6; **2f**, 118514-60-6; **3**, 124717-72-2; **4**, 124717-73-3; **7**, 36276-24-1; **8**, 124717-60-8; **9**, 124717-61-9; **10**, 124717-62-0; **11**, 124717-64-2; **12**, 124717-65-3; **13**, 124717-66-4; **14**, 124717-67-5; **15**, 124717-68-6; **16**, 124717-69-7; **17**, 124717-70-0; **18**, 124717-71-1; **19**, 69181-16-4; $HO_2CCH_2CO_2H$, 141-82-2; *o*-$NH_2C_6H_4CHO \cdot HCl$, 51723-15-0; ethyl 2-[2-carboxy-4-[1-(2,5-dioxolyl)]phenyl]ethanoate, 124717-63-1; topoisomerase I, 80449-01-0.

Acid-Stable 2′-Fluoro Purine Dideoxynucleosides as Active Agents against HIV

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2′,3′-Dideoxy purine nucleosides have anti-HIV activity in vitro and the inosine analogue is being clinically evaluated. The instability of these compounds toward acidic conditions complicates oral administration. The effect of the addition of a fluorine atom to the 2′-position was investigated by preparing the fluorine-containing 2′-*erythro* and 2′-*threo* isomers of ddA and the *threo* isomer of ddI. All fluorine-containing compounds were indefinitely stable to acidic conditions which completely decomposed ddI (1) and ddA (2) in minutes. While the fluorine-containing *erythro* isomer, **5**, was inactive, the *threo* isomers, 2′-F-dd-ara-A (**3**) and 2′-F-dd-ara-I (**4**), were just as potent and active in protecting CD4+ ATH8 cells from the cytopathogenic effects of HIV-1 as the parent drugs. Exposure to pH 1 at 37 °C prior to testing destroyed the activity of ddA and ddI but left the anti-HIV properties of **3** and **4** unchanged. The fluorinated analogues also protected cells exposed to HIV-2 and inhibited *gag* gene product expression but not as effectively as the parent compounds. The fluorine-containing analogues appear to be somewhat more toxic in vitro to the antigen- and mitogen-driven proliferation of immunocompetent cells than their corresponding parent compounds.

Fluorine substitution has been extensively investigated in drug research and biochemistry as a means of enhancing biological activity and increasing chemical or metabolic stability.[1] Important factors in the substitution of fluorine for hydrogen are (1) the comparable size of the two atoms, (2) the powerful electron withdrawing properties of fluorine relative to hydrogen, and (3) the increased stability of the carbon–fluorine bond relative to the carbon–hydrogen bond. In terms of size, fluorine has a small van der Waals radius (1.35 Å) which closely resembles that of hydrogen (1.20 Å).[2] Therefore, replacement of a hydrogen by fluorine in a bioactive molecule is expected to cause minimal steric perturbations with respect to the molecule's mode of binding to receptors or enzymes. In contrast, since fluorine is the most electronegative of the elements,[2] its powerful electron withdrawing properties can profoundly

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