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Total and semisynthesis and in vitro studies of both enantiomers of 20-fluorocamptothecin

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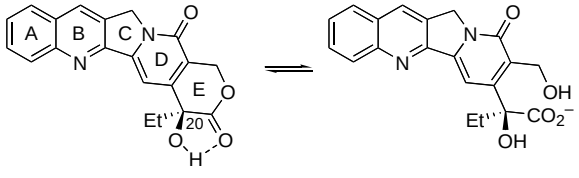
Dedicated to Professor Bernd Giese on the occasion of his 65th birthday.

Abstract—Both enantiomers of 20-fluorocamptothecin and the racemate have been prepared by total synthesis. The (*R*)-enantiomer is essentially inactive in a topoisomerase-I/DNA assay, while the (*S*)-enantiomer is much less active than (20*S*)-camptothecin. The lactone ring of 20-fluorocamptothecin hydrolyzes more rapidly than that of camptothecin in PBS. The results provide insight into the role of the 20-hydroxy group in the binding of camptothecin to topoisomerase-I and DNA.

Members of the camptothecin family are among the most important current and prospective drugs for clinical treatment of solid tumors.[1] Structure/activity studies on (*S*)-camptothecin **1** (Fig. 1) and congeners have identified the hydroxy lactone E-ring as important for function,[2] and several suggestions have been made about how the crucial 20-hydroxy group interacts in the topoisomerase/DNA cleavable complex that is responsible for the anticancer activity.

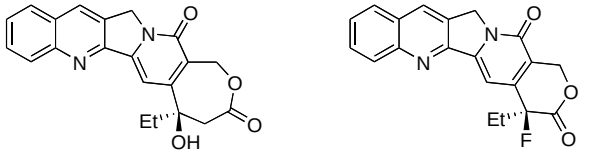
A crystal structure of a ternary complex of topoisomerase I/DNA and the drug topotecan (present in both closed lactone and open hydroxy acid forms) shows a lone hydrogen bond between the 20-OH and Asp533.[3] A similar interaction was found for camptothecin lactone.[4] Other models propose different H-bonding interactions,[1a,5] but again the 20-OH is a key group that interacts with topo-I. This hydroxy group is also proposed to be an important factor in the pharmacodynamics of camptothecin, which suffers rapid lactone opening to an inactive hydroxy acid form **2** under physiological conditions.[6] The 20-hydroxy group can activate the lactone carbonyl group towards attack by water inductively and by intramolecular hydrogen bonding.



1, (*S*)-camptothecin closed lactone form

2, lactone-opened (hydroxy acid) form

the 20-hydroxy group is suggested to:
• bind to asp 533 in the topo I/DNA complex
• facilitate lactone opened in vivo by
(a) H-bonding and (b) induction



3, (*R*)-homocamptothecin

4, (*S*)-20-fluorocamptothecin (20-deoxy-20-fluorocamptothecin)

Figure 1. Structures of (20*S*)-camptothecin and analogs.

Keywords: Camptothecin; Topoisomerase I; Isonitrile radical annulation.
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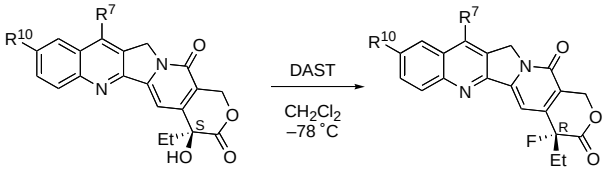
Hecht and others have prepared an assortment of E-ring analogs of camptothecin, including 20-amino-, thio-, chloro-, bromo-, and 20-deoxycamptothecin.[7] Most of these analogs are not very active either against isolated topoisomerase I/DNA complex or in cells, and for some time the α-hydroxy lactone functionality was thought to be indispensable. More recently, homocamptothecin **3** has been shown to be highly active,[8] and other non-lactone analogs have also shown some activity.[9,10] Both homocamptothecins and the non-lactone analogs bear a 20-hydroxy group.

Conspicuously missing from the list of camptothecin analogs until very recently was 20-fluorocamptothecin **4**.[11] From the activity standpoint, this is a crucial compound to test since fluorine is generally considered to be the closest substituent to hydroxy with respect to size and electronic effects, but has very different hydrogen bonding properties.[12]

Sporadic recent reports of 20-fluorocamptothecin provide conflicting information. In their 2002 review, Ulukan and Swaan state that 20-fluorocamptothecin is inactive, but they do not provide a supporting reference.[13] In another 2002 review, Zunino and coworkers state that the compound is active and cite unpublished results.[14] As this work was being completed, Shibata, Toru, and co-workers reported a synthesis of enantioenriched (*S*)-20-fluorocamptothecin by asymmetric fluorination of (*rac*)-20-deoxycamptothecin.[15] They resorted to fluorination of the deoxy compound after attempts to directly deoxyfluorinate camptothecin itself failed. They also speculated that the 20-fluoro substituent would deactivate the E-ring toward opening relative to camptothecin, and stated in a footnote that the compound was less active than camptothecin.

We report here the synthesis of both enantiomers and the racemate of 20-fluorocamptothecin. In contrast to Shibata and Toru's observations, we find that it is possible to directly fluorinate camptothecin and analogs, but these reactions occur primarily with inversion to give the 20*R* enantiomer. The racemate and both enantiomers are accessed by total synthesis. The 20*S* enantiomer is only weakly active in a standard topo I/DNA assay, but the fluorine atom is found to activate the lactone toward opening relative to camptothecin. These results help to clarify further the key role of the 20-hydroxy group in the camptothecin family of natural products.

Semisynthesis of 20-fluorocamptothecin. The preparation of 20-fluorocamptothecin by direct deoxyfluorination of camptothecin is shown in Eq. 1. Diethylaminosulfur trifluoride (DAST)[16,17] was added dropwise to a suspension of (*S*)-camptothecin S-**1** in dichloromethane at −78 °C. After 3 h, the reaction mixture was warmed to room temperature, quenched, and worked up, and the crude product was purified by flash chromatography to give (*R*)-20-fluorocamptothecin R-**4** as a yellow solid in 61% yield. The structure was assigned by a detailed spectroscopic analysis. Especially diagnostic were the one- and two-bond couplings to ^{19}F in the broadband decoupled ^{13}C NMR spectrum[18] (see Supplementary data for details). The ee of the sample was 89%, as measured by chiral HPLC, and 92%, as measured by optical rotation; the absolute configuration was assigned as *R* by the assays described below. This assignment is consistent with both HPLC and optical rotation data of Shibata, Toru, and co-workers[15] who tentatively assigned absolute configuration by the CD lactone sector rule. Thus, the absolute configuration of 20-fluorocamptothecin is confirmed, and the fluorination occurs with predominant inversion.



S-**1** $R^7 = R^{10} = H$
S-**5** $R^7 = Si(^tBu)Me_2$, $R^{10} = OH$

R-**4**, 61% yield, 89% ee
R-**6**, 61% yield, ee not known

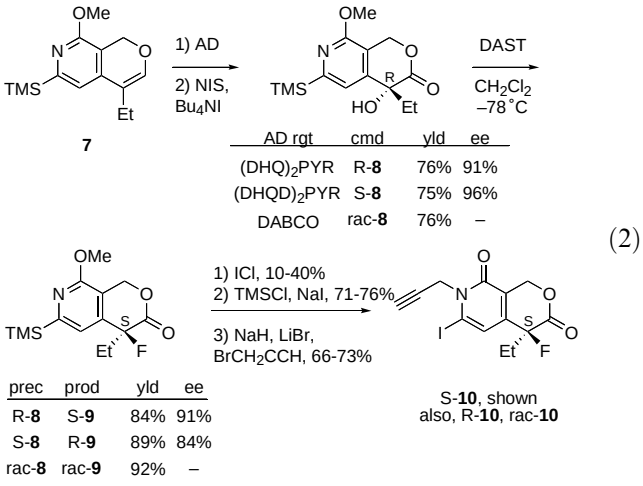
(1)

The important camptothecin analog DB-67[19] S-**5** was also fluorinated under the same conditions to provide 20-fluoroDB-67 R-**6** in 61% yield. The enantiomeric excess of this sample was not determined, but we presume that it is predominantly the *R*-enantiomer.

Although not surprising, the inversion result is not favorable since the 20*S* enantiomer of fluorocamptothecin is the more interesting one. Thus, we decided to prepare both the *S* and *R* enantiomers and the racemate by total synthesis.

Total synthesis of 20-fluorocamptothecin. The total synthesis approach employs the cascade radical annulation of isonitriles and *N*-propargyl iodopyridones that has been so useful in preparing diverse analogs of camptothecin and homocamptothecin.[20] The synthesis of the key radical precursor, iodopyridone **10**, is shown in Eq. 2. Enol ether **7** was subjected to dihydroxylation followed by oxidation with NIS to provide the pivotal hydroxy lactone **8**. The usual use of $(DHQD)_2PYR$ in the Sharpless asymmetric dihydroxylation[21] reaction provided S-**8** in 75% yield and 96% ee, while the use of $(DHQ)_2PYR$ provided R-**8** in 76% yield and 91% ee. The ee analysis was conducted by chiral hplc. The racemate of **8** was prepared in 76% yield by dihydroxylation with DABCO.[22]

Fluorinations of each of the subsequent intermediates in the synthesis were attempted with DAST,[16,17] and the best results in terms of yields, ees, and ease of analysis were obtained in the fluorination of **8**.[18] Fluorination of **8** with deoxofluor[23] was also possible, but DAST was superior. Fluorination of R-**8** with DAST at −78 °C, as above, provided a clean crude product S-**9** in 84% yield and 91% ee, while fluorination of S-**8** provided R-**9** in 89% yield and 84% ee. As before, predominant but not complete inversion of configuration was observed. The racemate of **8** was also fluorinated to give rac-**9**.



(2)

Iododesilylation of **9** with ICl was problematic, and the best results were obtained in runs of 1 h with 5 equiv ICl where about 40% conversion was observed along with recovered starting material (which was reused). In other runs with different times and amounts of ICl, as low as 10% conversion to the iodide was observed. The purified iodides from the various runs were pooled and taken forward through demethylation and N-propargylation to give **10** as both enantiomers and the racemate.

The results of the cascade radical annulation reactions with **10** and several isonitriles are shown in Figure 2. The reaction of phenyl isonitrile with S-**10** provided (*S*)-20-fluorocamptothecin S-**4** and the reaction with rac-**10** provided the racemate of **4**. Likewise, the reaction of R-**10** with phenyl isonitrile, 4-fluorophenylisonitrile, and 3,4-methylene-dioxyphenylisonitrile provided (*R*)-20-fluorocamptothecin R-**4** and the corresponding analogs R-**11a**,**b** and R-**12** in the indicated yields. The crude product of the reaction with methylene-dioxyphenylisonitrile was a 1/2.5 mixture of regioisomers **11a** and **11b**, which were isolated in pure form by chromatography.

Analysis of the ees of pure samples of (*R*)- and (*S*)-20-fluorocamptothecin by chiral HPLC and optical rotation provided somewhat differing results, with rotation providing a higher value. The (*S*)-enantiomer was 69% ee by HPLC but 84% ee by rotation, while the (*R*)-enantiomer was 79% ee by chiral HPLC and 98% ee by rotation. We presume that the HPLC numbers are more accurate, and in that case there has been a slight decrease in ee over the course of synthesis from **8**. The source of that decrease is not clear. The ees of the derivatives **11a,b** and **12** could not be determined because they did not resolve under the chiral HPLC conditions and the rotations of enantiopure samples are not known. However, based on the results above, we presume that they are (*R*)-enantiomers with at least 70% ee, possibly higher.

Topoisomerase I assays. The detailed procedures for the topoisomerase I assays are contained in the supplementary data. Briefly, labeled DNA was incubated with recombinant topo I with and without drug. After 20 min, the reaction was stopped and the samples were denatured and analyzed for cleavage on a polyacrylamide gel.

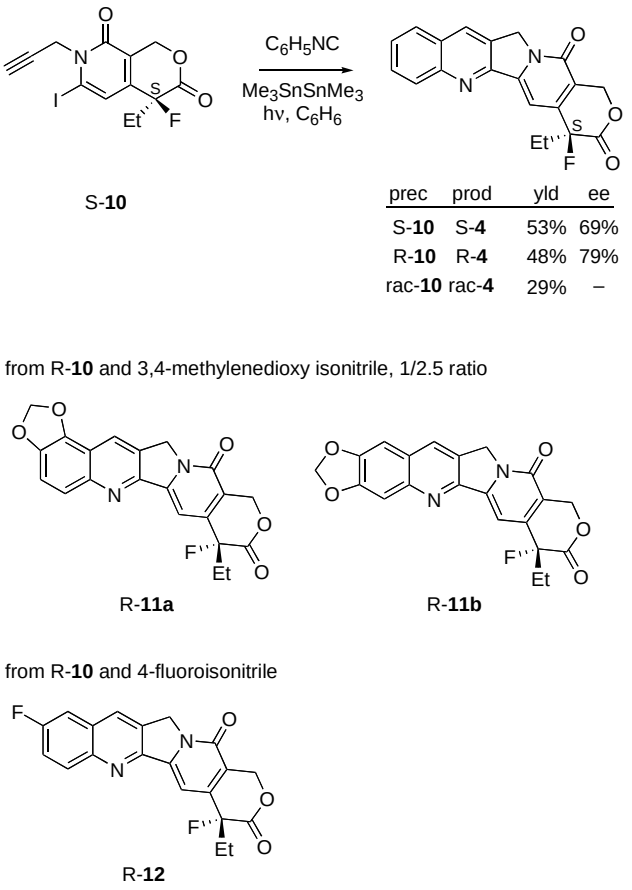


Figure 2. Radical annulations to make 20-fluorocamptothecin and analogs.

The results of the topoisomerase assay of the 20-fluorocamptothecins are shown in Figure 3. Lanes 1 and 2 are controls with no drug, while lanes 3–5 are the camptothecin standard at 1, 10, and 100 μm. Lanes 6–11 are the three samples at 10 and 100 μM. None of the 20-fluorocamptothecin samples shows much activity at 10 μM, but there is a clear trend at 100 μM with S-**4** showing significant activity, rac-**4** showing moderate activity, and R-**4** showing a trace of activity. These results form the basis of the enantiomer assignment.

The analogs **6**, **11a**,**b**, and **12** of camptothecin are all (*R*)-enantiomers and are therefore not expected to be very active. This was confirmed by topoisomerase assays (not shown); none of the samples showed activity at 10 μM, while a few showed slight activity at 100 μM. Because the samples are not enantiopure, the low activity might be due to the residual (*S*)-enantiomer.

From these results, we conclude that the (*R*)-enantiomer of 20-fluorocamptothecin is essentially inactive, while the (*S*)-enantiomer is at least 100–1000 times less potent than the natural product. Clearly, the seemingly small substitution of hydroxy by fluoro has a significant detrimental effect on interactions of the drug with the topo1/DNA complex.

Kinetics of lactone ring opening. The stability and hydrolysis reaction of (*S*)-20-fluorocamptothecin S-**4** were

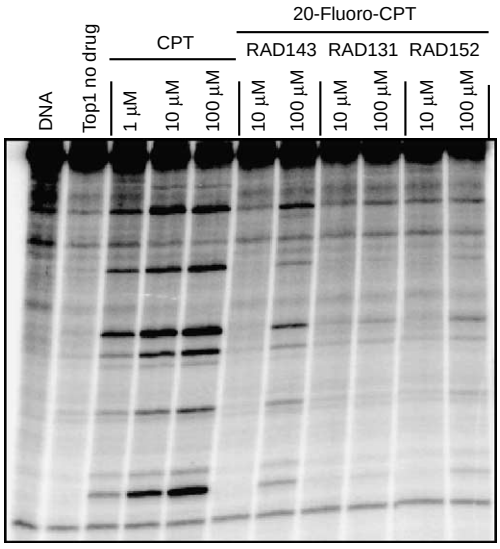


Figure 3. Topoisomerase-I/DNA cleavage assays of *S*, *R*, and *rac*-20-fluorocamptothecin. Lanes 1 and 2, controls; lanes 3–5, camptothecin at 1, 10, and 100 μm; lanes 6–11 are the three samples at 10 and 100 μM. RAD143 is (20S)-fluorocamptothecin, S-**4**; RAD131 is R-**4**; RAD-152 is rac-**4**.

investigated by reversed-phase high-performance liquid chromatographic (HPLC) methods.[6,24] Figure 4 depicts the progress of the hydrolysis reaction of S-**4** in phosphate-buffered saline (PBS) solution at pH 7.4 and 37 °C. The HPLC method was optimized by using a mobile phase consisting of 50% acetonitrile and 50% triethylamine acetate buffer (pH 5.5, 1% in water), such that the retention times of the carboxylate and lactone forms were approximately 2 and 4 min, respectively. This allows enough data points for kinetic analysis of the hydrolysis reaction of 20-F-CPT.

(*S*)-20-Fluorocamptothecin S-**4** hydrolyzes with a half-life ($t_{1/2}$ value) of 7.8 min. In contrast, the $t_{1/2}$ of camptothecin under these conditions is 30 min.[25] Accordingly,

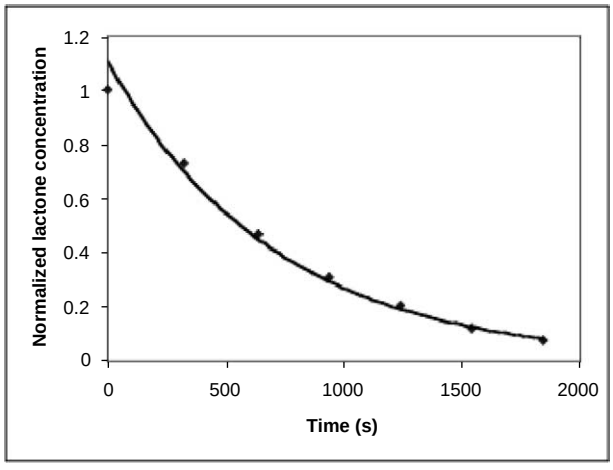


Figure 4. Kinetic evaluation of the rate of opening of the lactone ring for (*S*)-20-fluorocamptothecin in PBS (pH 7.4 ± 0.05) at 37 °C. Shown are averages of at least three independent experiments with the same time points. The standard deviation of each point is typically 5% or less. Pseudo-first-order hydrolysis rate constant is: $k_{obs} = 1.48 \cdot 10^{-3} \pm 0.013$ 1/s. So the $t_{1/2}$ is 7.80 ± 0.067 min.

the fluorine substituent accelerates the hydrolysis. Moreover, 20-F-CPT is almost completely hydrolyzed at pH 7.4 with an equilibrium lactone ratio of <0.5%, while the equilibrium lactone ratio of CPT at pH 7.4 is about 20%.

The important analog 20-fluorocamptothecin has been prepared in one step by semisynthesis from camptothecin, but the reaction occur with predominant inversion to give the (*R*)-enantiomer. Total synthesis by the cascade radical annulation approach provides both enantiomers of 20-fluorocamptothecin along with the racemate. The (*R*)-enantiomer is essentially inactive in a topoisomerase-I/DNA assay, while the (*S*)-enantiomer is much less active than (20*S*)-camptothecin. This suggests that the hydrogen bonding contribution of the 20-hydroxyl group is crucial in stabilizing the ternary complex of camptothecin, topoisomerase I, and DNA. The lactone ring of 20-fluorocamptothecin hydrolyzes more rapidly than that of camptothecin in PBS. This result suggests that the inductive effect of the 20-hydroxyl group of camptothecin is more important than its intramolecular hydrogen bonding effect to the lactone carbonyl group in hydrolytic lactone ring opening.

Acknowledgment

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Supplementary data

Supporting information contains experimental information and characterization data for all new compounds. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.07.074.

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