Synthesis of Position-Specific Tritium-Labeled 20(S)-Camptothecin, 9-Amino-20(S)-Camptothecin, and 10,11-Methylenedioxy-20(S)-Camptothecin

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SUMMARY

The synthesis is given for three ring A tritiated camptothecin (CPT) analogs as biological probes in the study of the parent compounds which are of current widespread interest as potent anticancer agents. The strategy of catalytic tritolysis of aryl halide bonds was employed, and thus the preparations of the requisite precursors 9-chloro-20(S)-CPT (9), 9-amino-10,12-dibromo-20(S)-CPT (14), and 9-chloro-10,11-methylenedioxy-20(S)-CPT (18) are given; catalytic tritiation of these respective precursors under polar, alkaline solvent conditions using palladium/carbon provides smooth conversion to [9-^{3}H]-20(S)-CPT (10), 9-amino-[10,12-^{3}H]-20(S)-CPT (15), and [9-^{3}H]-10,11-methylenedioxy-20(S)-CPT (19).

Keywords: Camptothecin, tritium, anticancer agents.

INTRODUCTION

The natural plant alkaloid 20(S)-camptothecin (1) (CPT) was first isolated from a crude plant extract in this laboratory more than 25 years ago, guided by a simple but tedious process of tracing cytotoxicity during extract refinement.[1] Subsequent in vivo testing of 1 in mouse L-1210 leukemia identified a considerable degree of antitumor activity.[2] Early enthusiasm waned, however, when clinical trials employing the water-soluble open-lactone salt 2 revealed significant toxic side effects.[3] Various studies continued nonetheless[4], and after 20 years, the revelation that 1 was unique as an inhibitor of the DNA topoisomerase I enzyme[5] led to substantial renewed interest in this

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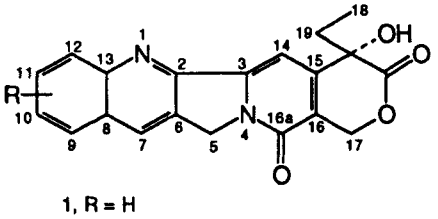
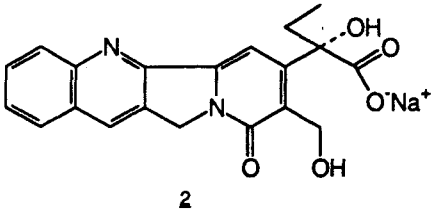
class of compounds. The discovery of a novel cellular target may provide ways for overcoming the problem of resistance to conventional drug treatment such as in the treatment of chronic lymphatic leukemia with chlorambucil. Furthermore, the finding that topoisomerase I enzyme levels in several human tumor systems are significantly elevated over those in normal tissue[6] offers hope of greater selectivity in the cytotoxic action of CPT analogs. Extensive synthetic efforts by our group[7] and others[8,9] have recently helped to define the structural parameters providing for the best interaction with the topoisomerase I enzyme. As a result, several CPT analogs are now at various stages of preclinical and clinical evaluation as anticancer agents, notably compounds 1, 3, 4, 5[8] and 6[9].

The development of any of these compounds as anticancer agents requires tissue distribution, metabolism, excretion and absorption studies. The high sensitivity of detection methods for radioisotope labels has led to the establishment of radiolabeling as a valuable tool in such work. At the molecular level, it is anticipated that the radiolabel will assist in the probe to determine more precisely the nature of the CPT drug-enzyme-DNA interaction. Hence we undertook studies to incorporate a radiolabel into CPT and its analogs 3 and 4 which are of current interest. To this end, we have employed the extremely useful and general reaction of tritium-halogen exchange (tritolysis). This paper provides the details of these studies.



1, R = H
3, R = 9-NH_2
4, R = 10,11-OCH_2O
5, R = 9-CH_2NMe_2•HCl, 10-OH
6, R = 7-CH_2CH_3, 10-OCON



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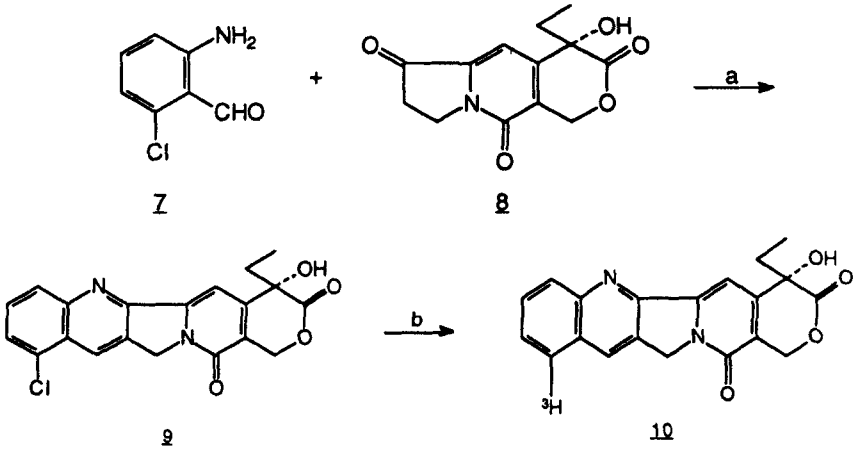
RESULTS AND DISCUSSION

The synthetic methods employed to obtain the halogen-substituted camptothecin derivatives 9-chloro-20(S)-CPT (9), 9-amino-10,12-dibromo-20(S)-CPT (14), and 9-chloro-10,11-methylenedioxy-20(S)-CPT (18) are outlined in Schemes I, II,

and III, respectively. These schemes use similar palladium catalyzed tritolysis steps to afford target compounds [9-^{3}H]-20(S)-CPT (10), 9-amino-[10,12-^{3}H]-20(S)-CPT (15), and [9-^{3}H]-10,11-methylenedioxy-20(S)-CPT (19), respectively.

In the synthesis of [9-^{3}H]-20(S)-CPT (10) (Scheme I), the synthesis of 9-chloro-20(S)-CPT (9)[7] was carried out by acid catalyzed Friedlander condensation of aminoaldehyde 7[7] and the key oxytricyclic ketone 8[10] in a 75% yield. Synthon 8 has been developed in our laboratory through a multistep synthesis specifically as a versatile precursor to dozens of CPT analogs. Tritiation of 9 was carried out after hydrogenolysis of chlorine was confirmed under the conditions to be used for the tritolysis. Thus a mixture of chloro compound 9 in dioxane, triethylamine, and 10% palladium on carbon was exposed for four hours to carrier-free tritium gas at ambient conditions.

Scheme I



a. toluene, HOAc, p-TsOH, reflux, 18 h
b. $^3H_2$, 10% Pd/C, dioxane, Et_3N, RT, 4 h

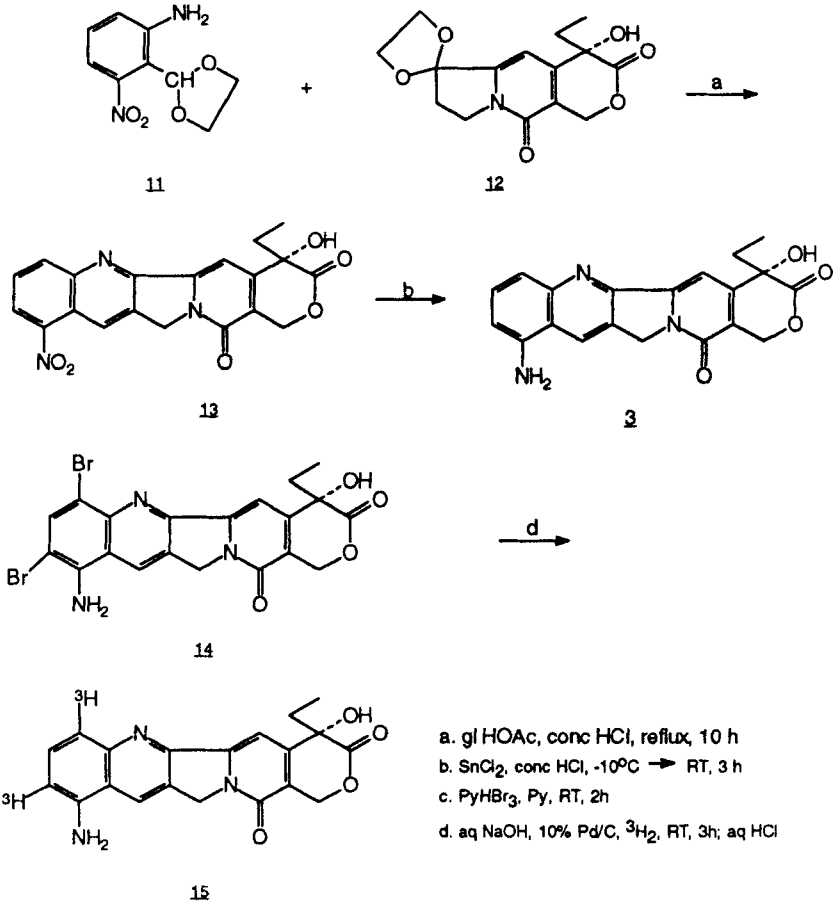
Standard scintillation counting measurements and quantitative UV determinations were performed on the purified [9-^{3}H]-20(S)-CPT (10) obtained by a flash column chromatography. Thus a yield of 168 mCi of 10 (from 0.47 mmol of 9) with specific activity of 17.3 Ci/mmol (49.8 mCi/mg) was obtained (21% chemical yield). Radiochemical homogeneity of 10 was assessed at 95% by thin layer radioscan. Solutions of 10 showed no appreciable hydrogen tritium exchange at pH 7 or 9 after 24 hours. However, 3% exchange was observed after 24 hours at pH 3.

Our group had previously prepared isomeric [12-^{3}H]-20(S)-CPT in a similar fashion from the corresponding 12-bromo compound.[11] This in turn was obtained in two steps from 12-nitro-20(S)-CPT, the major isomer from nitration of 20(S)-CPT

(1).[11,12] By analogy, an alternate route to the 9-tritiated compound 10 by way of the minor 9-nitro isomer (compound 13, Scheme II) is suggested. Preliminary hydrogenolysis experiments have shown that both 10-bromo and 10-chloro-CPT are suitable substrates for similar tritiations. However, there is no clear advantage of one substrate over the others. This serves to illustrate the versatility of the method with the selection of substrate made primarily on the basis of substrate availability.

The preparation of 9-amino-[10,12-$^3H_2$]-20(S)-CPT (15) is mentioned in Scheme II. 9-Nitro-20(S)-CPT (13) was prepared and reduced to 9-amino-20(S)-CPT (3) as described in the literature.[7] The overall yield of 3 from 11 and 12 was 60%. It may be noted that the stringent acidic conditions required for reaction of electron deficient aminober.zaldehydes (e.g., unprotected 11) in the Friedlander reaction obviates the need to deprotect either synthon 11 or 12 which carry these groups during their synthesis.

Scheme II



Treatment of 3 with pyridinium hydrobromide perbromide in pyridine at ambient temperature gave dibromo derivative 14 in 57% yield. Interestingly, earlier procedures which were worked out for the racemic 20(RS) series included a simple bromine/acetic acid reaction at this stage, but proved to be unsatisfactory for the 20(S) enantiomer because of its lower solubility. Tritiation of 14 was conducted on the opened lactone sodium salt (generated in situ) in aqueous sodium hydroxide by exposure to pure tritium gas in the presence of 10% palladium on carbon at ambient conditions.

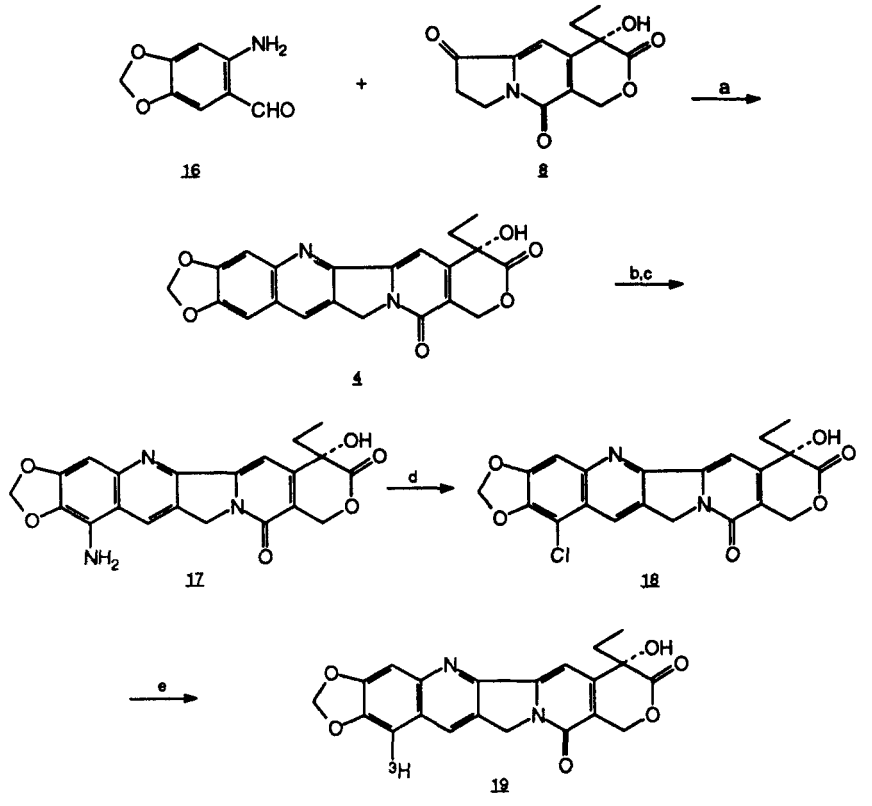
9-Amino-[10,12-$^3H_2$]-20(S)-CPT (15) was obtained by preparative reverse phase chromatography in 98% purity as shown by both radioactivity and UV-254 detection. Scintillation counting and quantitative UV analysis indicated a yield of 302 mCi of 15 (from 0.021 mmol of 8) of specific activity 21.3 Ci/mmol (61.1 mCi/mg) and a 68% chemical yield. The ^3H NMR spectrum (proton decoupled) indicated the relative tritium distribution at C-10, C-11, and C-12 to be 53%, 9%, and 38%, respectively. Back exchange of tritium in aqueous solution over 24 hours at ambient temperature was extremely slow at pH 7 and 9 where less than 1% was measured for both. However, at pH 3, with other conditions the same, a sample of 15 showed an 8% loss of tritium. Enhanced exchange in 15 compared to 10 under acidic conditions is not surprising, since the relatively electron-rich A ring of 15 provides for increased rate of interaction with positively charged species such as the proton.

The preparation of [9-^3H]-10,11-methylenedioxy-20(S)-CPT (12) was carried out as outlined in Chart III. 9-Chloro-10,11-methylenedioxy-20(S)-CPT (18) was prepared according to the literature procedure.[7] Thus, Friedlander condensation of ketone 8 and 2-aminopiperonal (16) provided 4 in 80% yield. Nitration gave an 80% yield of the intermediate 9-nitro derivative which was reduced with stannous chloride to afford 9-amino-10,11-methylenedioxy-20(S)-CPT (17) in 60% yield. Diazotization of 17 followed by treatment with cuprous chloride gave 18 in 50% yield. We also prepared the 9-bromo analog by a similar reaction with cuprous bromide, although the yields were poor possibly due to steric crowding by the large bromine atom. Hydrolysis to the 9-hydroxy side product appeared to be a competing process. Earlier efforts to prepare the 9-bromo analog by direct bromination of 4 were unsuccessful, and thus the alternate more circuitous route of Scheme III was employed. Tritolysis of 4 as its sodium salt in dioxane/aqueous sodium hydroxide using tritium gas and 10% palladium on carbon gave the target compound [9-^3H]-10,11-methylenedioxy-20(S)-CPT (19) after acidification.

Purification of 19 by flash column chromatography removed unreacted 9 and yielded 76.5 mCi (from 0.042 mmol) of pure 19 with specific activity of 4.59 Ci/mmol. Tritium exchange was <1% upon treatment of 19 with pH 7 and 9 aqueous buffers. At pH 5, 5% exchange was found.

In conclusion, tritolysis of nuclear halogen in CPT analogs has been successful in providing tritium labeled compounds for pharmacological evaluations. We would expect this general method to be applicable to other CPT analogs as well.

Scheme III



a) Toluenne, HOAc, p-TsOH, reflux, 15 h

b) conc. HNO_3, conc. H_2SO_4, -10°D, 2 h

c) $SnCl_2$, conc. HCl, 0-10°C → RT, 5 h

d) $NaNO_2$, conc. HCl, 0-10°C, 20 min; CuCl, 60°C

e) 10% Pd/C, $^3H_2$, dioxane, aq.NaOH, RT, 4 h; aq. HCl

EXPERIMENTAL SECTION

Tritiations were carried out using carrier-free tritium gas purchased from DuPont (New England Nuclear Corp.). Radioactivity was measured by a Packard Tri-Carb 4000 liquid scintillation counter, using Ultima Gold (Packard) as the scintillation fluor, and zones of radioactivity on thin layer chromatograms were determined by scanning with a Berthold Tracemaster 20 Automatic TLC-Linear Analyzer system. The molar quantities of tritiated products obtained were determined from quantitative ultraviolet (UV) spectra as recorded on a Varian Model 2290 spectrophotometer. ^1H NMR spectra were recorded by a Bruker WM-250 spectrometer at 250 MHz with tetramethylsilane as internal standard and a ^3H NMR spectrum of 15 was obtained on a Bruker spectrometer at 533 MHz. Analytical TLC was carried out using E. Merck silica gel

F-254 (0.25 mm) plates and column chromatography employed E. Merck silica gel 60 (230-400 mesh). HPLC was conducted in a Waters Assoc. Model 6000A dual pump system with a Model U6K septum-less injector with detection by radioactivity monitor (Berthold Model LB503-HDS radioactivity monitor) and/or Waters Model 450 Variable UV Detector at 254/nm. For preparative HPLC, a Waters μ-bondapak C18 25 cm x 10 mm cartridge with precolumn and 60% MeOH in H_2O as solvent at 6.0 mL/min. was used; for analytical purposes, a DuPont Zorbax RxC8 25 cm x 4.6 mm column with precolumn and H_2O/MeOH containing ≤0.1% trifluoracetic acid (pH > 2, Et_3N) at 1.0 mL/min. The pH-dependent isotope exchange was determined by measuring radioactivity in the water from the lyophilized buffer solutions. A portion of 9-amino-20(S)-CPT (3) used in this study was provided by the National Cancer Institute. The remaining camptothecin analogs and intermediates were synthesized as previously reported, or are described more fully here.

[9-^{3}H]-20(S)-Camptothecin (10)

A stirred mixture of chloro compound 9[7] (18 mg, 0.047 mmol), 10% palladium on carbon (18 mg), dioxane (0.9 mL, dried by passing through Al_2O_3), and triethylamine (0.2 mL) was exposed to 5 curies of carrier-free tritium gas at ambient temperature for 4 h. The mixture was frozen [N_2(l)] and the excess tritium gas removed by evacuation. The mixture was thawed and filtered through Celite. The filter cake was rinsed with methanol/chloroform and the combined filtrate was concentrated by closed-system vacuum distillation. The labile tritium in crude 10 was exchanged by three successive treatments with 5 mL portions of methanol followed by distillation as before. The residue was dissolved in a minimum of chloroform, and the resulting brown solution was rapidly passed (slight N_2 pressure) through a 1.5 g bed of silica gel in a Pasteur pipet using 0.25% methanol in chloroform and collecting fractions of 2-3 mL volume. Fractions 7-10 (>85% purity by TLC in chloroform:acetone:methanol, 80:15:5; radioscan, R_f 0.65; and UV detection) were evaporated at ambient temperature under reduced pressure to afford a yellow solid which was immediately triturated with a few drops of methanol. The supernatant was removed by pipet, and the pale tan [9-^{3}H]-20(S)-camptothecin (10) of 95% purity as determined by radioscan of the TLC plate as before was quickly diluted with 2 mL of 1:1 methanol/chloroform, sonicated, and diluted to 400.0 mL with methanol for storage under nitrogen at <-15°C. The chromatographic properties (TLC R_f 0.65, $CHCl_3$:Me_2CO:MeOH, 80:15:5) and UV spectrum of 10 matched those of nonlabeled authentic 20(S)-camptothecin (1). The yield was 168 mCi of material with specific activity of 17.3 Ci/mmol (mass determination based on UV_{max} 253 nm, ξ = 32,900 for authentic CPT, chemical yield 21%). Back exchange in aqueous buffers for 24 hours at pH 7 and 9 showed less than 1% exchange, while the exchange at pH 3 was 3% over the same period.

9-Amino-[10,12-$^3H_2$]-20(S)-Camptothecin (15)

A suspension of amino compound 3[7] (50 mg, 0.137 mmol) in pyridine (25 mL) was sonicated and warmed until solution was complete. After cooling to room temperature the stirred solution was treated with pyridinium hydrobromide perbromide

(88 mg, 0.28 mmol). After 2 h, the volatile components were removed under reduced pressure, and residual pyridine was removed by azeotropic distillation with heptane. The residue was sonicated in methanol (40 mL), and the resulting suspension was refluxed for 5 min. After storage at -10°C for 18 h, the sample was centrifuged, and the solid dried at room temperature under vacuum to afford the target 9-amino-10,12-dibromo-20(S)-camptothecin (14) as an orange solid (41 mg, 57%). Further purification by preparative HPLC gave the following analytical data: ^{1}H NMR (DMSO-d_6) δ 0.90 (3, t, J = 7 Hz, H-18), 1.87 (2, m, H-19), 5.32 (2, s, H-5), 5.44 (2, s, H-17), 6.46 (2, s, D_2O exchangeable, 9-NH_2), 6.57 (1, s, D_2O exchangeable, 20-OH), 7.34 (1, s, H-14), 8.13 (1, s, H-11), 9.05 (1, s, H-7); UV_{max} (MeOH) 211 nm (ξ 34,970), 225 (28,330), 267 (20,190), 342 (28,170), 376 (18,260).

9-Amino-10,12-dibromo-20(S)-camptothecin (14, 11 mg, 0.021 mmol) was warmed gently in 1 N aqueous sodium hydroxide (2.0 ml) until solution was complete. After cooling, 10% palladium on carbon (5 mg) was added, and the stirred mixture was exposed to carrier-free tritium gas (5 Ci, 0.086 mmol) at ambient temperature for 3 h. After removal of excess tritium, the mixture was filtered through a Celite plug into 1 N aqueous hydrochloric acid (2.1 mL). The resulting suspension of 15 was concentrated to dryness by closed-system vacuum distillation. The residue was then subjected to 3 successive labile isotope exchanges with methanol by the addition of a portion of methanol followed by distillation as above in each case. Immediate purification of 15 was effected by preparative HPLC and after removal of the solvent under vacuum at <30°C, the purified 9-amino-[10,12-$^3H_2$]-20(S)-camptothecin (15) was dissolved without delay in methanol and stored at -70°C. The yield of 15 was 302 mCi which was 98% pure by analytical HPLC (R_T 9.0 min, 50% MeOH in 0.1% TFA, pH 2.4 with Et_3N) by both radioactivity and UV detectors. The specific activity was 21.3 Ci/mmol (mass determination based on UVmax (MeOH) ξ_{336} = 20,850, ξ_{262} = 17,820, ξ_{224} = 30,000 for 9-amino-camptothecin, 68% chemical yield). Back exchange in aqueous buffers for 24h showed very little (<1%) exchange of tritium at pH 7 and 9, but at pH 3 the exchange was 8%. From the proton-decoupled ^{3}H NMR (MeOH) spectrum of 15 the relative tritium distribution was found to be 53% at C-10 (δ 6.99), 9% at C-11 (δ 7.63), and 38% at C-12 (δ 7.56).

[9-^{3}H]-10,11-Methylenedioxy-20(S)-Camptothecin (19)

A stirred suspension of chloro derivative 18[7] (17 mg, 0.042 mmol) in dioxane (0.3 mL) was treated dropwise with 1 N aqueous sodium hydroxide (0.7 mL) with slight warming. When solution was complete, 10% palladium on carbon (5 mg) was added and the stirred mixture was exposed to carrier-free tritium gas (5.0 Ci, 0.086 mmol) at ambient temperature. After 4 h, the degassed reaction was filtered through Celite, rinsing with methanol, into 1 N aqueous hydrochloric acid (0.8 mL). The filtrate was concentrated to a solid by closed system vacuum distillation, and by the same treatment the residue was subjected to 3 labile tritium exchanges using portions of methanol. The crude 19 was dissolved in methanol/chloroform, dispersed onto Celite, and immediately placed on a 2 g column of silica gel followed by elution with 0.25%

methanol in chloroform under slight nitrogen pressure. Fractions of 1-2 mL were collected, and those fractions containing a bright blue fluorescing spot matching the TLC R_f of 0.75 (SiO_2 $CHCl_3$:Me_2CO:MeOH, 8:1.5:0.5) for the nonlabeled analog 4 were combined, evaporated and diluted with methanol to 100 mL. Measured radioactivity for the sample of [9-^{3}H]-10,11-methylenedioxy-20(S)-camptothecin (19) was 76.5 mCi. The specific activity was 4.59 Ci/mmol (mass determination based on UV_{max} 255 nm, ξ = 11,230 for authentic 4). Purity assessment by radiochromatogram (SiO_2, $CHCl_3$:Me_2CO:MeOH, 8:1.5:0.5) was 90%. Less than 1% exchange of tritium was found over 24 h in pH 7 and 9 buffers, whereas exchange at pH 5 was 5%.

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