Synthesis of Tritiated (S)-10-Bromoacetamidomethylcamptothecin

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Summary

Tritiated (S)-10-bromoacetamidomethylcamptothecin was prepared starting from (S)-10-hydroxycamptothecin. The key step consisted of palladium catalyzed direct tritium exchange on the intermediate (S)-10-aminomethylcamptothecin. ^3H NMR spectroscopy indicated the tritium label was located exclusively at the C-5 position.

Key Words: 10-Bromoacetamidomethylcamptothecin, Camptothecin, Tritium exchange, ^3H NMR.

Introduction

Camptothecin (**1**), a naturally occurring antineoplastic agent,[1] binds reversibly to DNA-topoisomerase I covalent complex and stablizes the enzyme-mediated DNA cleavage, inhibiting religation.[2] In order to investigate whether the presence of a peripheral electrophilic substituent, for instance the bromoacetyl appendage in derivative **2** (SK&F S-106470), would render such binding irreversible, a radio-labeled analog of **2** was required.[2] Investigation of the nature of any covalent binding of [^3H]**2** could provide information relative to the position of camptothecin binding on the DNA-topoisomerase I complex. Based upon the precedent that a

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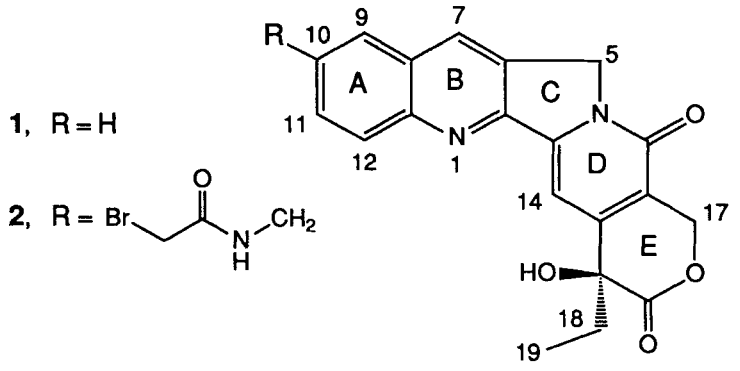
deuterated analog of camptothecin was prepared by deuterium exchange on the parent compound in the presence of Pd/C,[3] our synthetic strategy employed a similar approach in introducing the tritium label into the intermediate (S)-10-aminomethylcamptothecin (**6**). In this paper we report the synthesis of [^{3}H]SK&F S-106470 (**8**) and the use of ^{3}H NMR to elucidate the position of the tritium label.

Results and Discussion

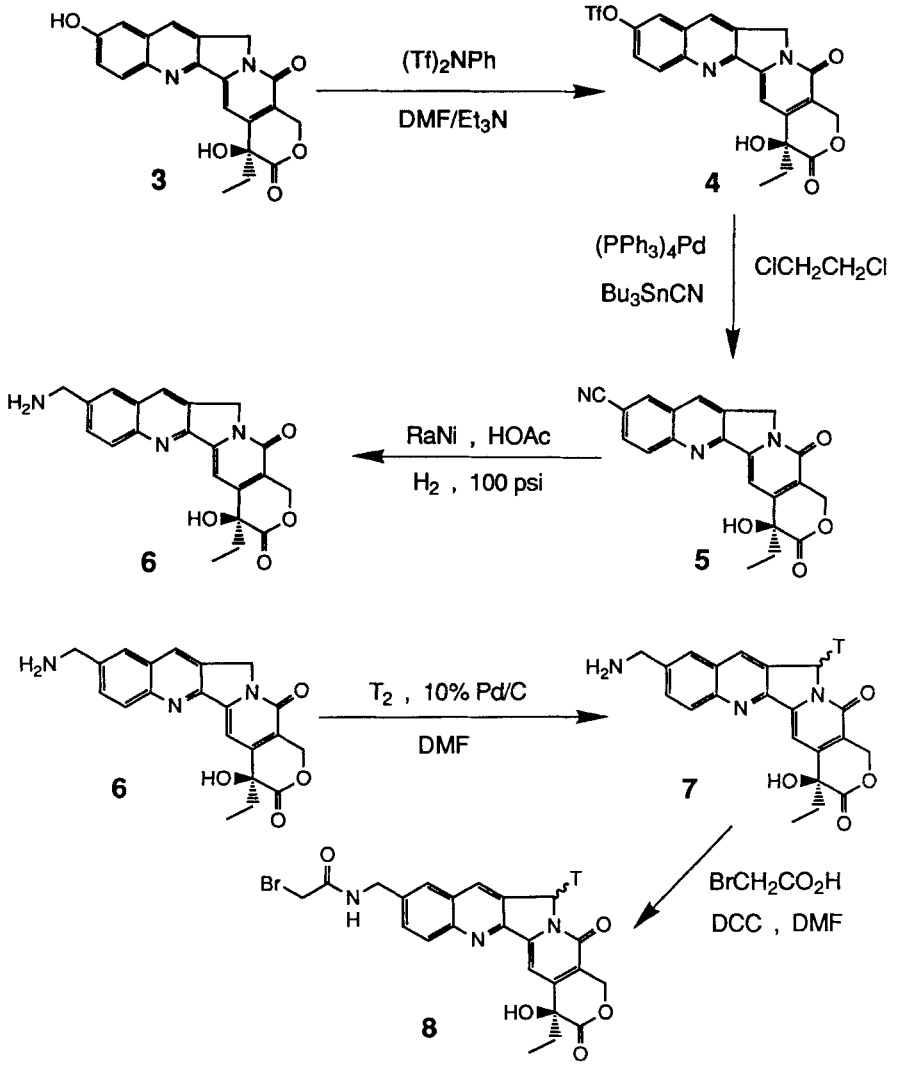
Synthesis of **8**. [^{3}H]SK&F S-106470 (**8**) was prepared by coupling bromoacetic acid with the tritiated product derived from direct tritium exchange on (S)-10-aminomethylcamptothecin (**6**, Scheme I). Substrate **6** was synthesized by a three step sequence from (S)-10-hydroxycamptothecin (**3**). Treatment of **3** with N-phenyltrifluoromethanesulfonimide led to triflate **4**.[4] Cyanation *via* palladium mediated coupling[5] of **4** gave the 10-cyano derivative **5**, which was reduced with Raney nickel under 100 psi of hydrogen to afford the desired substrate **6** in an overall yield of 61%. Tritium exchange of **6** was carried out in the presence of 10% Pd/C and 10 Ci of carrier-free tritium gas in DMF for 24 h at room temperature, furnishing the tritiated analog **7** with a radiochemical purity of 50% and a radioactivity recovery of 443 mCi. The crude **7** was directly treated with excess bromoacetic acid and 1,3-dicyclohexylcarbodiimide to give, after HPLC purification, the desired [^{3}H]SK&F S-106470 (**8**) in 25% radiochemical yield. Further HPLC purification raised the radiochemical purity of **8** from 93% to 98%.

^{3}H NMR of **8**. ^{1}H NMR and ^{3}H NMR of [^{3}H]SK&F S-106470 (**8**) indicate the tritium label is located exclusively at C-5, and the presence of t_0:t_1:t_2 species in the ratio of 44:44:12.

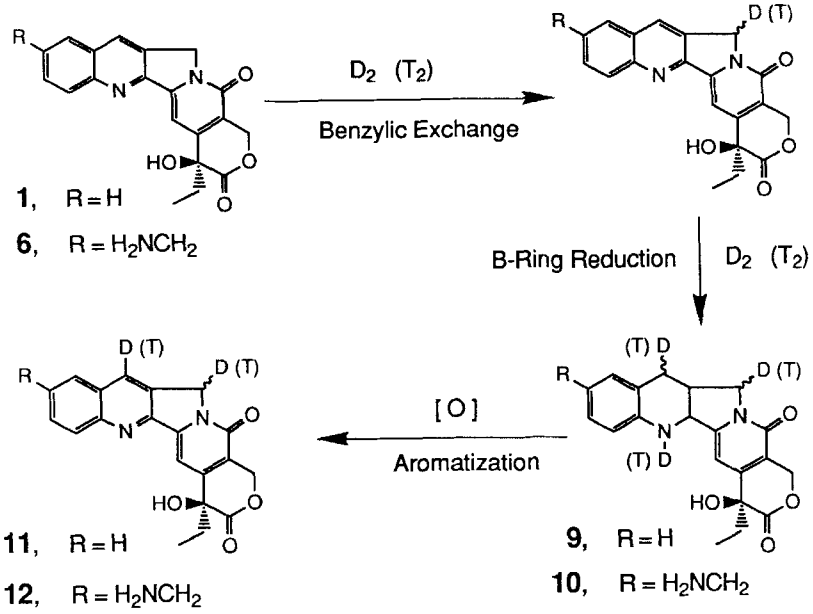
Comparison of the ^{1}H NMR of unlabeled **2** with the ^{3}H NMR of **8**, depicted in Figure 1, reveals that the only tritium signals observed are


1, R = H
2, R = Br
R
10
9
7
5
A
B
C
D
E
11
12
1
N
O
14
17
HO
18
19

Scheme I


HO
3
(Tf)2NPh
DMF/Et3N
TfO
4
(PPh3)4Pd
Bu3SnCN
ClCH2CH2Cl
NC
5
RaNi , HOAc
H2 , 100 psi
H2N
6
T2 , 10% Pd/C
DMF
7
BrCH2CO2H
DCC , DMF
Br
8

Scheme II



those corresponding to the C-5 position of the camptothecin nucleus. This is in contrast to the previously reported deuteration of camptothecin, in which the label was found at both the C-5 and C-7 sites.[3] This outcome could be attributed either to a difference in reactivity between these two compounds or to the methods utilized to prepare them, despite the fact that both were prepared by a similar direct exchange process. In the case of deuterated camptothecin **11**, the previous workers observed apparent B-ring reduction to **9**, which they oxidized with air or oxygen to regenerate camptothecin. This resulted in deuterium incorporation at C-7 in addition to the initial benzylic exchange at C-5 (Scheme II). On the other hand, we obtained evidence that the B ring of (S)-10-aminomethylcamptothecin (**6**) is more resistant to catalytic reduction. In a control experiment, none of the saturated B-ring product **10** was observable in the crude hydrogenation mixture by ^{1}H NMR. Moreover, in the present work, [^{3}H]SK&F S-106470 (**8**) was derived from an exchanged mixture that had not been deliberately subjected to oxidation. Consequently, if the saturated B-ring product **10** had been formed to a minute extent, it probably would not have undergone

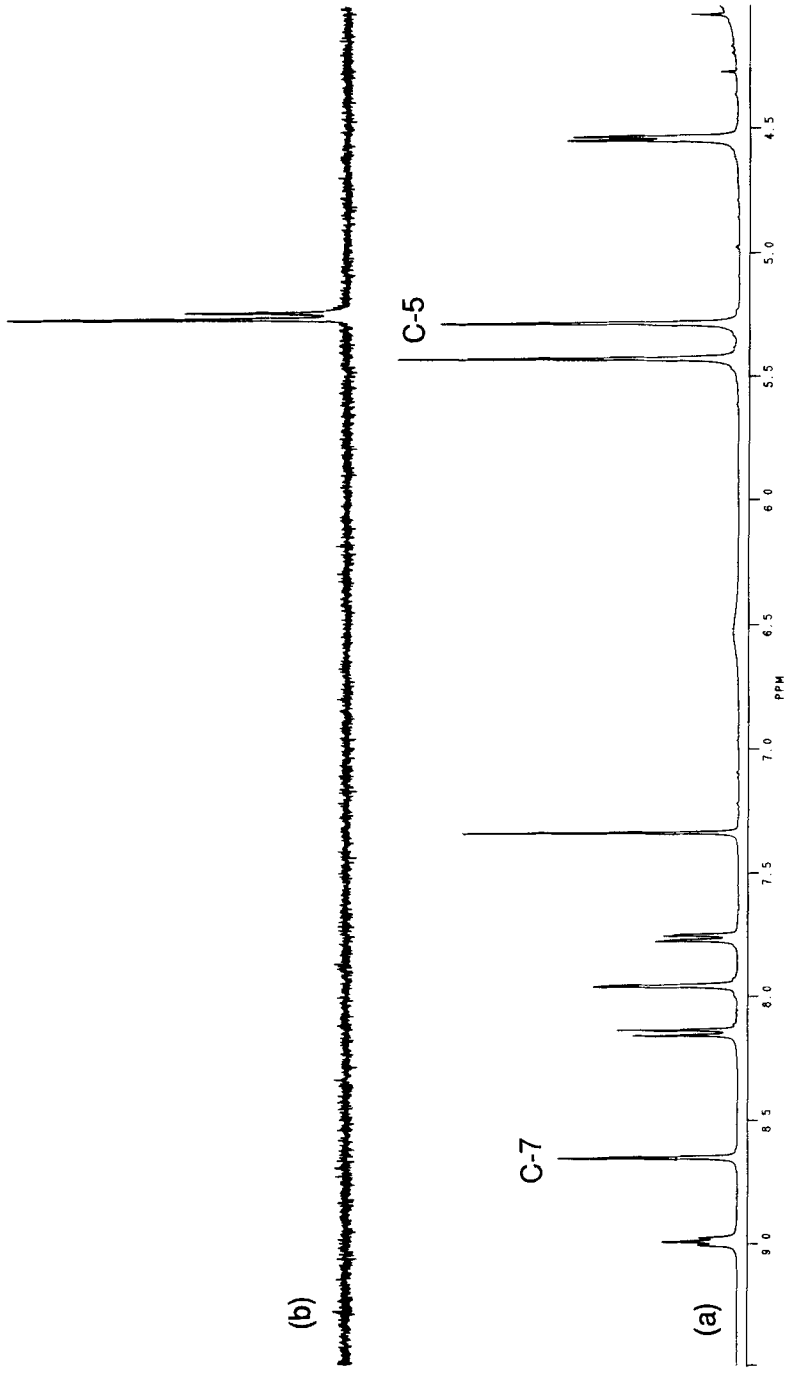


Figure 1. (a) ^{1}H NMR of unlabeled **2**. (b) ^{3}H NMR (proton-decoupled mode) of **8**.

reoxidation, and its bromoacetylated derivative would have been separated from **8** during purification of the latter by HPLC. We did not observe any product arising from double-site (C-5 and C-7) labeled **12**.

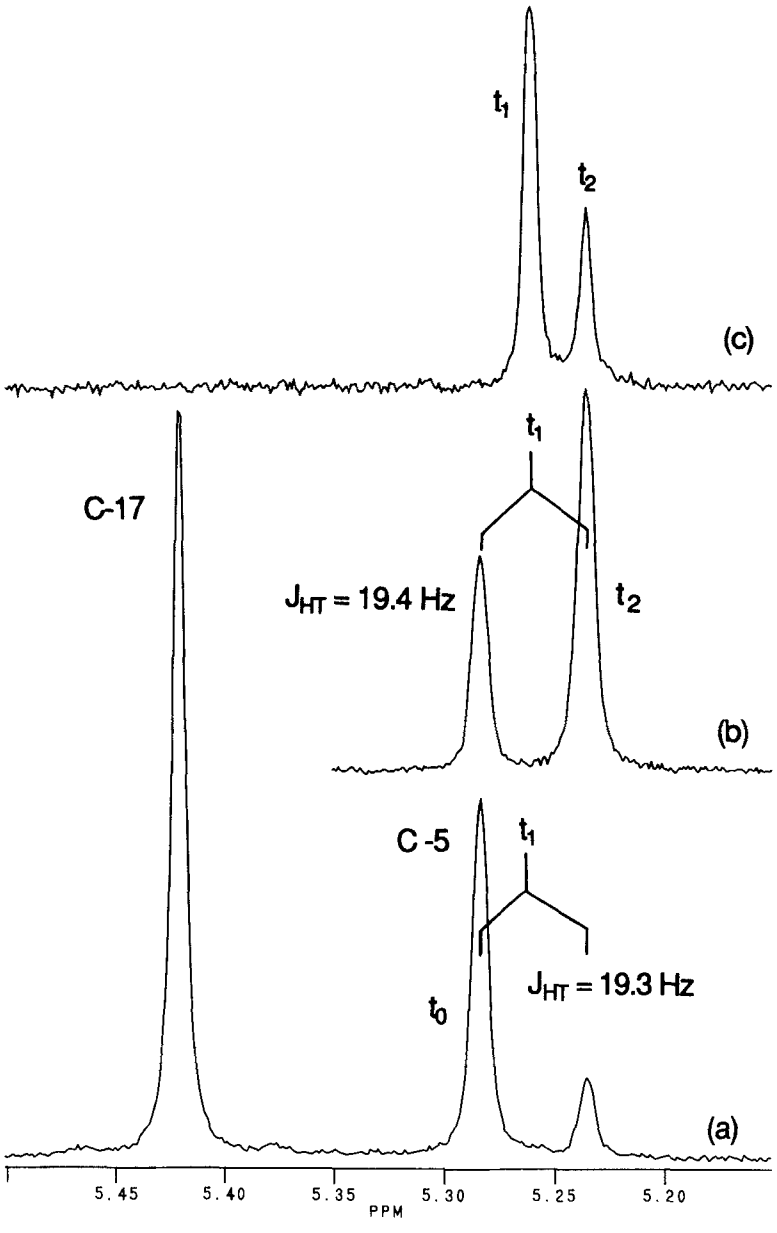


Figure 2. NMR spectra of **8**. (a) ^{1}H NMR. (b) ^{3}H NMR in proton-undecoupled mode. (c) ^{3}H NMR in proton decoupled mode.

The expanded regions at C-5 of the ^{1}H NMR and ^{3}H NMR of **8**, depicted in Figure 2, disclose the presence of three isotopic species. In the ^{1}H NMR (Figure 2a), the C-5 protons of the unlabeled (t_0) species appear as a

singlet at 5.281 ppm; whereas that of the monotritiated (t_1) species, coupled to a geminal triton, shows up as a doublet (J = 19.3 Hz) at 5.257 ppm. The upfield shift (0.024 ppm) observed in the latter is due to the primary isotope effect exerted by the geminal triton.[6] Similarly, in the ^3H NMR, the C-5 triton of the t_1 species is split by a geminal proton into a doublet (J = 19.4 Hz) when measured in the proton-undecoupled mode (Figure 2b), but collapses into a singlet in the proton-decoupled mode (Figure 2c). Finally, in both the proton- undecoupled and decoupled modes, the C-5 tritons of the ditritiated (t_2) species remain as a singlet at 5.235 ppm. Again, the primary isotope effect shifts the signal of the t_2 species upfield (0.022 ppm) relative to that of the t_1 species. From peak area integration, the ratio of t_0:t_1:t_2 is determined to be 44:44:12, which is in good agreement with the numerical value (43:45:12) obtained from FAB-MS measurement.

In summary, [^3H]SK&F S-106470 was synthesized *via* a direct tritium exchange method with the resulting label located exclusively at the C-5 position as determined by ^3H NMR.

Experimental

All chemicals used were purchased from Aldrich. Carrier-free tritium gas was obtained from New England Nuclear. Reaction solvents were distilled prior to use. ^1H NMR (400 MHz) and ^3H NMR (426 MHz) spectra were recorded on a Bruker AM-400 spectrometer in dimethylsulfoxide-d_6 (reference at 2.500 ppm). Referencing of chemical shifts in ^3H NMR, measured on a 25 mCi sample, was achieved initially by the reported ghost referencing method,[7] but were later adjusted to match the appropriate signals (especially the t_1 species) in ^1H NMR. Radiochemical purities were measured on a Ramona-D radioactivity detector. Mass spectra of unlabeled

intermediates were recorded in the chemical ionization mode using methane as reagent gas.

(S)-10-Trifluoromethylsulfonyloxycamptothecin (**4**)

(S)-10-Hydroxycamptothecin (**3**, shown by ^{1}H NMR to contain 20% camptothecin, 820 mg, 1.8 mmol after correction) and N-phenyltrifluoromethanesulfonimide (1.07 g, 3.0 mmol) in DMF (25 mL) and Et_3N (1 g) were heated at 50 °C under argon for 65 min. The mixture was concentrated *in vacuo*. Addition of H_2O (30 mL) induced a precipitate which was collected by filtration and dried under vacuum. Chromatography was carried out on a silica gel column, packed in CH_2Cl_2 and eluted with mixtures of CH_2Cl_2/CH_3OH in a step gradient from 0% to 6% CH_3OH increasing in 0.5% increments. The 3-4% CH_3OH fractions were combined and concentrated *in vacuo* to give triflate **4** (868 mg, shown by ^{1}H NMR to contain 20% camptothecin, 78% corrected yield). ^{1}H NMR: 0.880 (3H, t, J = 7.3 Hz, C18-**H**), 1.872 (2H, m, C19-**H**), 5.330 (2H, s, C5-**H**), 5.441 (2H, s, C17-**H**), 6.572 (1H, s, O-**H**), 7.376 (1H, s, C14-**H**), 7.984 (1H, dd, J = 9.3 Hz, J = 2.8 Hz, C11-**H**), 8.356 (1H, d, J = 9.3 Hz, C12-**H**), 8.436 (1H, d, J = 2.8 Hz, C9-**H**), 8.822 (1H, s, C7-**H**).

(S)-10-Cyanocamptothecin (**5**)

Tetrakis(triphenylphosphine)palladium (3.76 g, 3.2 mmol) and tributyltin cyanide (2.83 g, 8.9 mmol) in 80 mL of dichloroethane were heated under argon at 80 °C for 2 h. To this refluxing solution was added in one portion triflate **4** (520 mg, 0.8 mmol after correction) in 70 mL of dichloroethane. The reaction was heated at 80 °C under argon for 19 h. The mixture was cooled to room temperature and then applied to a silica gel column packed in CH_2Cl_2. Elution was carried out in a step gradient manner using initially mixtures of CH_2Cl_2/EtOAc and then mixtures of CH_2Cl_2/EtOAc/CH_3OH in increasing polarity. Obtained from chromatography was

(S)-10-cyanocamptothecin (**5**, 380 mg, shown by ^{1}H NMR to contain 20% camptothecin and traces of triphenylphosphine type impurities, 102% corrected yield). ^{1}H NMR: 0.875 (3H, t, J = 7.4 Hz, C18-**H**), 1.870 (2H, m, C19-**H**), 5.330 (2H, s, C5-**H**), 5.445 (2H, s, C17-**H**), 6.583 (1H, s, O-**H**), 7.394 (1H, s, C14-**H**), 8.154 (1H, dd, J = 8.8 Hz, J = 1.8 Hz, C11-**H**), 8.315 (1H, d, J = 8.8 Hz, C12-**H**), 8.795 (1H, s, C7-**H**), 8.832 (1H, d, J = 1.6 Hz, C9-**H**); CI-MS, m/z (%): 374 (100, $(M+H)^+$), 349 (43, $(M+H-CN)^+$), 328 (23), 303 (9).

(S)-10-Aminomethylcamptothecin (**6**)

Raney nickel (290 mg) was washed sequentially with H_2O (100 mL), C_2H_5OH (100 mL), and glacial HOAc (100 mL). To a solution of (S)-10-cyanocamptothecin (**5**, 200 mg) in glacial HOAc (50 mL) was added the prewashed Raney nickel in one portion. The mixture was shaken in a parr shaker under 100 psi of hydrogen gas at room temperature for 6.5 h. The mixture was filtered. The filtrate was concentrated *in vacuo* to give crude **6**, which was purified by preparative reverse-phase HPLC (Dynamax C_{18} preparative column (8 μm, 2.54 cm I.D. x 25 cm), 80/20/6 (v/v/v) H_2O/CH_3OH/HOAc, 9.0 mL/min, VIS at 366 nm, retention time at 18-26 min). Removal of HPLC solvents and drying under vacuum led to pure **6** (125 mg, 78% corrected yield). ^{1}H NMR (20 μL of TFA added): 0.878 (3H, t, J = 7.3 Hz, C18-**H**), 1.888 (2H, m, C19-**H**), 4.299 (2H, d, J = 5.6 Hz, Ar**CH**$_2$N), 5.303 (2H, s, C5-**H**), 5.433 (2H, s, C17-**H**), 7.358 (1H, s, C14-**H**), 7.929 (1H, dd, J = 8.8 Hz, J = 1.8 Hz, C11-**H**), 8.162 (1H, s, C9-**H**), 8.235 (1H, d, J = 8.8 Hz, C12-**H**), 8.701 (1H, s, C7-**H**); CI-MS, m/z (%): 378 (62, $(M+H)^+$), 362 (7, $(M+C_2H_5-CO_2)^+$ and/or $(M-CH_3)^+$), 350 (3, $(M+H-C_2H_4)^+$ and/or $(M+H-CO)^+$), 344 (100, $(M+H-CO_2)^+$), 115 (37).

(S)-10-Aminomethyl [5-^{3}H] camptothecin (**7**)

(S)-10-Aminomethylcamptothecin (**6**, 11 mg) and 10% Pd/C (9.5 mg) were mixed in DMF (4 mL) in an 8 mL round-bottomed flask. Carrier-free tritium

gas (10 Ci) was transferred into this round-bottomed flask by means of a Toepler pump. The mixture was stirred at room temperature for 24 h. Excess tritium gas was removed by the Toepler system. The tritiated mixture was filtered, and washed with 8 mL of 4:1 (v/v) CH_3OH/HOAc. Solvents of the filtrate were removed by static vacuum distillation. Tritium labiles were removed by repeated addition of CH_3OH and subsequent static vacuum distillation. The resulting tritiated **7** was taken up in 2 mL of DMF (total activity: 443 mCi, radiochemical purity: 50%).[8]

(S)-10-Bromoacetamidomethylcamptothecin-5-^{3}H (**8**)

To a stirred solution of **7** at room temperature was added in one portion a solution of bromoacetic acid (30 mg) and 1,3-dicyclohexylcarbodiimide (40 mg) in DMF (1 mL). The mixture was stirred at room temperature for 1 h. Then CH_3OH (2 mL) was added to quench the reaction, which was allowed to stir at room temperature for an additional 10 min. Solvents were then removed by static vacuum distillation, resulting in crude **8** (radiochemical purity: 41%).[9] Purification by preparative reverse-phase HPLC (Beckman C_{18} preparative column (10 μm, 2.54 cm I.D. x 15 cm), 55/45/1 (v/v/v) H_2O/CH_3OH/TFA, 3.0 mL/min, VIS at 366 nm, retention time at 45.2-51.2 min) led to pure **8**. ^{1}HNMR: 0.869 (3H, t = 7.6 Hz, C18-**H**), 1.858 (2H, m, C19-**H**), 3.968 (2H, s, Br**CH**$_2$CON), 4.533 (2H, d, J = 5.9 Hz, N**CH**$_2$Ar), 5.257 (0.5H, d, J = 19.3 Hz, C5-**H** of t_1 species), 5.281 (1H, s, C5-**H** of t_0 species), 5.420 (2H, s, C17-**H**), 7.326 (1H, s, C14-**H**), 7.755 (1H, dd, J = 8.8 Hz, J = 1.8 Hz, C11-**H**), 7.951 (1H, s, C9-**H**), 8.143 (1H, d, J = 8.7 Hz, C12-**H**), 8.644 (1H, s, C7-**H**), 8.996 (1H, t, J = 5.9 Hz, CON**H**); ^{3}H NMR: 5.257 (66.8T, d, J = 19.4 Hz, C5-**T** of t_1 species), 5.235 (32.6T, s, C5-**T** of t_2 species); total activity: 110 mCi; radiochemical purity: 93%;[9] specific activity: 19.9 Ci/mmol.

Repurification with careful fractionation using the same HPLC system mentioned above raised the radiochemical purity to 98%.[9]

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References and Notes

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8. This was assayed by the following method: Altex Ultrasphere ODS column (5 μm, 4.6 mm I.D. x 25 cm), 80/20/0.1 (v/v/v) H_2O/CH_3OH/TFA (A), 55/45/0.1 (v/v/v) H_2O/CH_3OH/TFA (B), 1.0

mL/min, step gradient: 0-15 min (100% A) and 15-40 min (100% B), retention time at 17.9 min.

9. This was assayed by the following method: Altex Ultrasphere ODS column (5 μm, 4.6 mm I.D. x 25 cm), 60/40/1 (v/v/v) H_2O/CH_3OH/TFA, 1.0 mL/min, retention time at 21.2 min.