# THE SYNTHESIS OF [$^3$H]L-734,217, AN ORALLY ACTIVE FIBRINOGEN RECEPTOR ANTAGONIST.

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## Summary

The synthesis of [$^3$H]L-734,217, **1b**, an orally active fibrinogen receptor antagonist, is described. The conversion of 3-amino crotonate **6** to [$^3$H]L-734,217 was carried out via a two step sequence of catalytic tritiation followed by basic hydrolysis. Deuterium model reactions showed that the reduction of **6** with $PtO_2$ occurred via hydrogen transfer from the solvent (ethanol or acetic acid) leading to poor isotope incorporation. When this reduction was conducted in methanol with 10% Pd/C, good isotope incorporation resulted. Ultimately, [$^3$H]L-734,217 was formed with a specific activity of 42 Ci/mmol.

**Keywords:** Fibrinogen receptor antagonist, L-734,217, catalytic tritiation, tritium NMR.

## Introduction

Thrombus formation has been implicated in acute myocardial infarction, transient ischemic attacks, stroke, reocclusion following angioplasty and a variety of other occlusive vascular diseases (1). One of the key steps in thrombus formation is platelet aggregation triggered by the binding of the plasma protein fibrinogen to the activated glycoprotein (GP) receptor GPIIb/IIIa (2). By preventing binding of fibrinogen to its platelet receptor, fibrinogen receptor antagonists can inhibit platelet aggregation and thus prevent thrombus formation. For this reason, fibrinogen receptor antagonists are potentially useful therapeutic agents for cardiovascular diseases (3).

While it is known that antibodies (4,5) and synthetic peptides (6,7) containing the arginine-glycine-aspartic acid (RGD) sequence inhibit the binding of fibrinogen to GPIIb/IIIa, these types of agents are not suitable for chronic use since they are not well absorbed after oral administration. One of the goals of the fibrinogen receptor antagonist program at Merck is to develop nonpeptide, orally active fibrinogen receptor antagonists.

A variety of novel, non-peptide, low molecular weight fibrinogen receptor antagonists have been identified that mimic the RGD sequence of active peptides (8,9). One of the most potent analogs is L-734,217 (9), **1a** (Figure 1), a compound that shows good efficacy in dogs after oral administration as determined by *ex vivo* platelet aggregation. Radiolabeled L-734,217 was needed for use in preliminary absorption, distribution, metabolism and elimination (ADME) studies. The synthesis of [$^3$H]L-734,217 is described herein.

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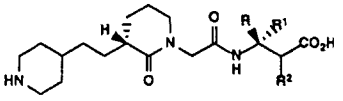
**Figure 1**

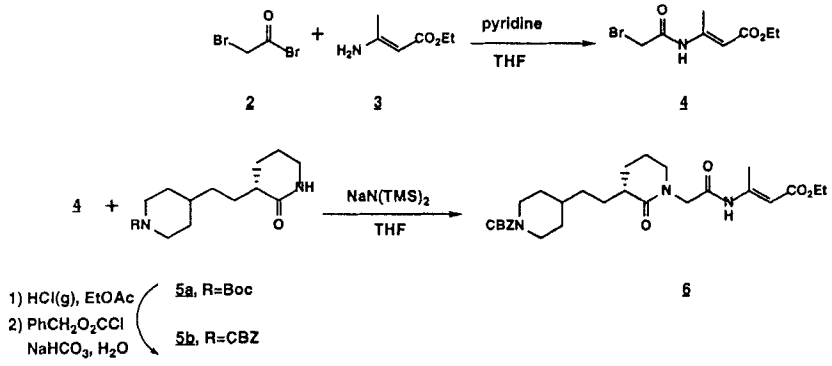


| | R | $R^1$ | $R^2$ | |
|---|---|---|---|---|
| 1a | H | Me | H | L-734,217 |
| 1b | T | $CH_2T$ | T | [$^3$H]L-734,217 |
| 1c | Me | H | H | L-734,350 |
| 1d | $CH_2T$ | T | T | [$^3$H]L-734,350 |

## Results and Discussion

Catalytic hydrogenation of olefins with tritium gas represents a convenient route for obtaining tritiated compounds with high specific activity (10). The 3-aminocrotonate **6** (Scheme 1) was selected as a suitable precursor for [$^3$H]L-734,217 since tritium could be introduced late in the synthesis by tritium reduction of the double bond. A final ester hydrolysis would produce [$^3$H]L-734,217.

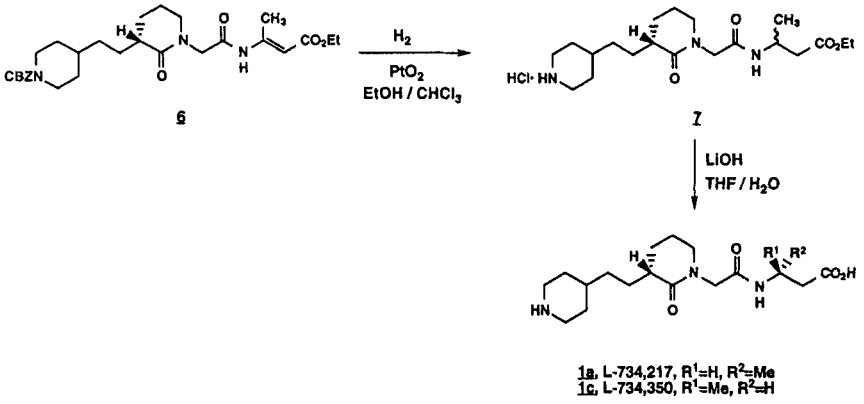
**Scheme 1: Synthetic pathway for 3-amino crotonate 6.**




The synthesis of the 3-aminocrotonate **6** is shown in Scheme 1. Acylation of ethyl 3-aminocrotonate **3** with bromoacetyl bromide **2** in the presence of pyridine gave the acylated aminocrotonate **4**. Treatment of the lactam **5b**, readily available from lactam **5a** (9), with sodium bis(trimethylsilyl)amide, followed by addition of the bromide **4**, furnished **6** in good yield. With precursor **6** in hand, the conditions necessary to convert it to L-734,217 were explored.

As shown in Scheme 2, the reaction conditions needed for the conversion of **6** to L-734,217, **1a**, and diastereomer L-734,350, **1c**, were developed using an hydrogenation, hydrolysis sequence. The initial model reduction and concomitant deprotection was found to work well using hydrogen gas and platinum oxide in an ethanol/chloroform mixture. The resulting diastereomeric mixture was hydrolyzed using lithium hydroxide in THF/$H_2O$ (11). L-734,217, **1a**, and its diastereomer **1c** could then be separated using preparative HPLC.

**Scheme 2: Conversion of 6 to L-734,217, 1a.**




When deuterium gas was used in place of the hydrogen gas to verify that isotopic incorporation would result from the catalytic reduction, the $^1$H NMR of the crude product showed very little deuterium incorporation. The use of deuterium or hydrogen gas under identical conditions led to compounds having $^1$H NMR spectra with signals displaying identical multiplicities and intensities.

This lack of deuterium incorporation was initially believed to be due to catalytic transfer hydrogenation (12). However, when this reaction was carried out in the absence of hydrogen or deuterium gas with catalyst present, the substrate was not reduced. The lack of deuterium incorporation could be due either to the presence of hydrogen in the deuterium gas or the catalyst, or a catalyst-mediated transfer of a solvent hydrogen to the substrate.

Because very pure deuterium gas was used, the amount of hydrogen gas present was low; this could not explain the lack of deuterium incorporation. Since the deuterium gas and catalyst had also been used successfully in other deuteration reactions, the role of the solvent in this reduction was investigated. Various conditions were investigated where the sole deuterium source was either the gas ($D_2$), the chloroform ($CDCl_3$) or the ethanol ($d_6$-EtOH). This series of experiments clearly showed that using $PtO_2$ as the catalyst, the source for incorporated hydrogen or deuterium was ethanol. When deuterated ethanol was used, deuterium was incorporated, even in the presence of hydrogen gas. When deuterated ethanol was not used, deuterium was not incorporated, even in the presence of deuterium gas. When methanol or THF was used in place of ethanol, little or none of the desired reduction product was obtained. Alternate hydrogenation conditions that were successful for the conversion of **6** to **7** (10% HOAc/EtOAc/$PtO_2$) also showed that deuterium was incorporated only if the solvent contained $d_4$-acetic acid.

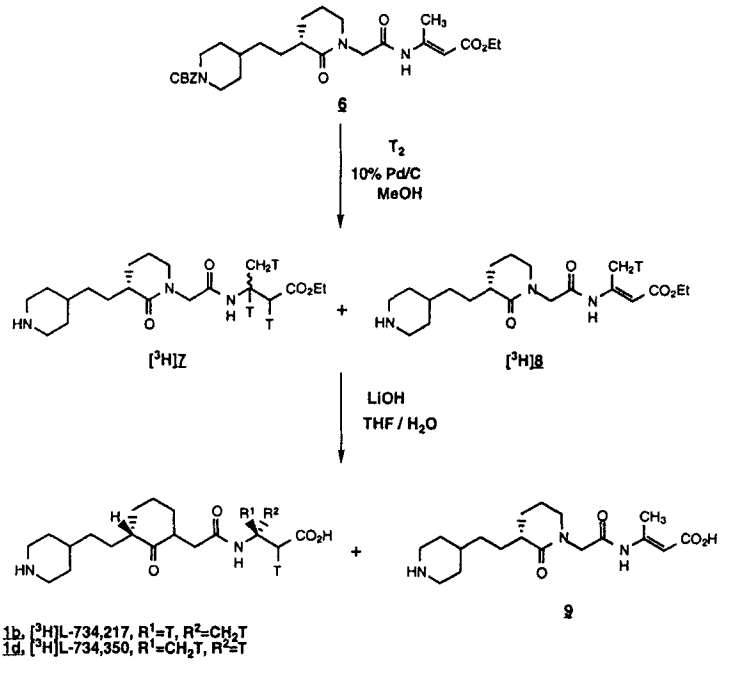
By changing the catalyst from $PtO_2$ to 10%Pd/C, and using methanol in place of ethanol, the desired product was formed with deuterium incorporation. The $^1$H NMR showed that deuterium was incorporated into **7** at the α- and β-carbons (relative to the ester). Deuterium was also incorporated at the β-methyl group via allylic exchange before the olefin was reduced. Hydrolysis of the resulting [$^2$H]**7** gave [$^2$H]L-734,217, which coeluted with an authentic sample of L-734,217 on HPLC. Comparison of the $^1$H NMR spectrum of the deuterated hydrolysis product with the $^1$H NMR spectrum of L-734,217 showed that deuterium had been incorporated into L-734,217.

When the tritiation procedure was carried out following the optimized conditions for deuterium incorporation, a mixture of labelled compounds resulted, as indicated in Scheme 3. To better characterize this crude mixture and to determine the impurities, the $^1H$ and $^3H$ NMR spectra of this mixture were taken. The $^1H$ NMR spectrum of the reduced mixture showed the presence of both [$^3H$]**7** and [$^3H$]**8** (13), indicating incomplete reduction of **6**. In addition to the $^1H$ signals due to [$^3H$]**7**, there was a vinyl proton signal (δ4.9) and a vinyl methyl signal (δ2.4) while the signals for the CBZ group were absent, consistent with **8**. Additionally, the $^1H$ coupled $^3H$ NMR of this same mixture showed four signals. Comparison of the δ values of these $^3H$ NMR signals with the $^1H$ NMR signals of unlabelled **7** and **8** showed that tritium had been incorporated into **7** α (δ 2.5) and β (δ 4.4) to the ester, as well as at the β-methyl group (δ 1.2). The $^1H$ coupled $^3H$ NMR also showed a signal at δ 2.4 indicating that unreduced, deprotected **8** had tritium incorporated into the vinyl methyl group. This incorporation was also seen in the deuterium model reductions.

Basic hydrolysis of this reduced mixture yielded [$^3H$]L-734,217, **1b**, along with diastereomer [$^3H$]L-734,350, **1d**, and unlabelled **9** (13), as indicated by HPLC and NMR analysis. The $^1H$ coupled $^3H$ NMR of the crude hydrolysis mixture had three tritium signals (δ 4.2, 2.3 and 1.2) which were assigned to the α and β tritiums as well as the β-methyl group of [$^3H$]L-734,217. The signal due to the allylic tritium label in **8** was absent due to exchange during the hydrolysis step.

Analytical HPLC analysis (using a radiodetector) of the tritiated mixture from the hydrolysis reaction showed only two peaks for the diastereomers **1b** and **1d**, while preparative HPLC purification (using a UV detector) of this same mixture showed three peaks identified as **1b**, **1d** and **9**. This preparative HPLC purification gave [$^3H$]L-734,217 with chemical and radiochemical purities of >98% and a specific activity of 42 Ci/mmol.

**Scheme 3: Conversion of 6 to [$^3H$]L-734,217, 1b.**



## Experimental

$^1$H NMR were recorded using a Varian Infinity-300 spectrometer operating at 300 MHz. The samples were dissolved in $CDCl_3$ with tetramethylsilane as the internal reference or $D_2O$ with 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS) as the internal reference. $^3$H NMR were recorded using a Varian VXR 400S spectrometer operating at 426.6 MHz with a tritium probe tuned to tritium. Samples were placed in a 5mm teflon sleeve and placed in a 10mm glass NMR tube. Tritium chemical shifts were measured by ghost-referencing from internal non-tritiated TMS. Melting points were taken using a Thomas Hoover capillary melting point apparatus. Analytical and preparative HPLC was carried out using a Waters 600E Powerline Multi Solvent Delivery System with 100 µL heads with a Rheodyne 7125 injector and a Waters 990 Photodiode Array Detector with a Gilson FC203 Microfraction collector. The acetonitrile used for the HPLC analyses was Fisher Optima grade. The HPLC radiodetector used was a Beckman 171 Radioisotope detector with a Beckman 110B solvent delivery system and Beckman Ready Flow III liquid scintillation cocktail was used. A Waters C-18 µ Bondapak column, 3.9 X 150 mm was used for analytical HPLC and a Waters C-18 µ Bondapak column, 3.9 X 300 mm was used for preparative HPLC of the tritiated product. Solutions of radioactivity were concentrated using a Jouan vacuum centrifuge. Calibration curves and chemical concentrations were determined using a Hewlett Packard Model 8452A UV/Vis Diode Array Spectrophotometer. Sample radioactivities were determined in an LKB Wallac 1410 liquid scintillation counter. The identity of labelled compounds were determined by HPLC coelution with authentic compounds. Reagents were purchased from Aldrich Chemical Co. unless otherwise noted.

**Ethyl 3-(bromoacetyl)aminocrotonate (4):**

A solution of ethyl 3-aminocrotonate (1.29 g, 10 mmol) and pyridine (0.808 mL, 10 mmol) in THF (20 mL) was heated to reflux. A solution of bromoacetyl bromide (0.915 mL, 10.5 mmol) in THF (4mL) was added over 1.5 hours. After the addition was complete, the reaction was continued to be heated at reflux overnight. The THF was removed *in vacuo* and the residue was partitioned between ethyl acetate and $H_2O$. The layers were separated and the ethyl acetate layer was washed with 1 M $NaHSO_4$ (10 mL), brine (3 x 10 mL) and dried ($MgSO_4$). Filtration and concentration gave a yellow semi-solid. Flash chromatography (15% ethyl acetate/hexane) gave crude **4** (1.02 g) as a colorless solid. This material was resubmitted to flash chromatography (methylene chloride then 5% acetone/methylene chloride) to give **4** (0.73 g, 29%) as a colorless solid: $^1$H NMR (δ, $CDCl_3$): 11.1 (1H, bs), 5.0 (1H, s), 4.2 (2H, q, J=7 Hz), 3.9 (2H, s), 2.4 (3H, s), 1.3 (3H, t, J= 7 Hz).

**3(R)-[N-CBZ-2-(Piperidin-4-yl)ethyl]-2-piperidone (5b):**

The lactam **5a** (9) (1.0 g, 3.22 mmol) was dissolved in ethyl acetate (20 mL), cooled to 0°C and saturated with HCl (g). After 45 minutes, TLC (20% isopropanol/hexane) indicated the absence of starting material. Argon was bubbled through the solution to remove some of the HCl. Concentration resulted in 800 mg of a colorless foam which was dissolved in $H_2O$ (20 mL). This solution was treated with $NaHCO_3$ (1.08 g, 12.88 mmol) and cooled to 0°C. Benzylchloroformate (0.58 mL, 3.54 mmol) was added via syringe over 5 minutes. After the addition was complete, the reaction was stirred at ambient temperature for 12 hours. The reaction mixture was acidified with solid $NaHSO_4$ and extracted with methylene chloride (3 x 50 mL). The organic extracts were combined and washed with brine (10 mL), saturated $NaHCO_3$ (20 mL), brine (20

mL), dried ($MgSO_4$), filtered and concentrated to give 900 mg (81%) of **5b** as a solid: $^1H$ NMR (δ, $CDCl_3$): 7.36-7.30 (5H,m), 5.98 (1H, bs), 5.12 (2H, s), 4.15 (2H, m), 3.3 (2H, m), 2.75 (2H, m), 2.28-1.20 (12H, m), 1.10 (2H, m).

**Ethyl [3(R)-[2-[N-[(benzyloxy)carbonyl]piperidin-4yl]ethyl-2-piperidone-1]acetyl-3-aminocrotonate (6):**

A solution of lactam **5b** (0.344 g, 1.0 mmol) in dry THF (10 mL) was cooled to -78°C and treated with sodium bis(trimethylsilyl)amide (2.2 mL, 2.2 mmol, 1M in THF). After stirring for 15 minutes, a solution of **4** (0.275 g, 1.1 mmol) in THF (5 mL) was added via cannula. After the addition was complete, the reaction was stirred for 15 minutes and then the cold bath was replaced with an ice bath. After stirring for 2 hours at 0°C, the reaction was quenched by adding saturated $NH_4Cl$ solution (10 mL) and then poured into ether (100mL). The aqueous layer was acidified by the addition of 1M $NaHSO_4$ and the ether layer was separated. The ether layer was then washed with brine (10 mL), saturated $NaHCO_3$ (20 mL), brine (2 x 10 mL), dried ($MgSO_4$), filtered and concentrated to give 550 mg of a yellow viscous oil. Flash chromatography (20% isopropanol/hexane) gave **6** (0.425 g, 83%) as a viscous oil: $^1H$ NMR (δ, $CDCl_3$): 7.36-7.31 (5H, m), 5.12 (2H, s), 4.93 (1H, s), 4.12 (2H, q, J=7Hz), 4.2-4.0 (4H, m), 3.36 (2H, m), 2.74 (2H, m), 2.41 (1H, m), 2.38 (3H, s), 2.1-1.5 (11H, m), 1.25 (3H, t, J=7Hz), 1.20 (2H, m).

**[2-(piperidin-4-yl)ethyl]-2-piperidone-1]-acetyl-3-methyl-β-alanine, ethyl ester hydrochloride (7):**

A mixture of ethyl [3(R)-[2-[N-[(benzyloxy)carbonyl]piperidin-4-yl]ethyl]-2-piperidone-1]acetyl-3-aminocrotonate (**6**) (10 mg, 19 μmol), absolute ethanol (1.8 mL), chloroform (0.2 mL), and platinum oxide (5 mg) was stirred under 1 atmosphere (balloon) of hydrogen gas at ambient temperature overnight. After twenty hours, the reaction mixture was purged with argon then filtered through a celite pad and the catalyst was washed with ethanol (2 x 2 mL). The filtrate was then concentrated to dryness to give **7** (8.5 mg, 100%) as an amorphous solid which was used directly in the next step. $^1H$ NMR (δ, $CDCl_3$): 6.83 (1H, br s), 4.30 (1H, m), 4.12 (2H, q, J=7 Hz), 3.94 (2H, m), 3.45 (2H, m), 3.35 (2H, m), 2.82 (2H, m), 2.46 (2H, d, J=5 Hz), 2.32 (1H, m), 2.00-1.20 (13H, m), 1.26 (3H, t, J=7 Hz), 1.20 (3H, d, J=7 Hz). For the deuterated compound, a mixture of ethyl [3(R)-[2-[N-[(phenylmethoxy)carbonyl]piperidin-4-yl]ethyl]-2-piperidone-1]acetyl-3-aminocrotonate **6** (4.98 mg, 9.7 μmol) in methanol (680 μL) was treated with 10% palladium on activated carbon (4.5 mg), degassed and stirred under 1 atmosphere (balloon) of deuterium gas for 2 hours at room temperature. The reaction mixture was filtered through a celite pad, the celite was rinsed with ethanol and the solution was concentrated *in vacuo* to give 4.15 mg of [$^2H$]**7** as on off white solid which was used directly in the next step: $^1H$ NMR (δ, $CDCl_3$): 6.83 (1H, br s), 4.12 (2H, q, J=7 Hz), 3.94 (2H, m), 3.45 (2H, m), 3.35 (2H, m), 2.82 (2H, m), 2.46 (1H, br s), 2.32 (1H, m), 2.00-1.20 (13H, m), 1.26 (3H, t, J=7 Hz), 1.20 (2.5H, s). HPLC analysis showed the product (9.8 minute retention time) with no starting material (14.5 minute retention time). HPLC conditions: Waters C-18 μ Bondapak column, 3.9 x 150 mm, 1 mL/min, 5% acetonitrile:$H_2O$ (0.1% trifluoroacetic acid) to 95% acetonitrile:$H_2O$ (0.1% trifluoroacetic acid) over 10 minutes, linear gradient, hold at 95% acetonitrile:$H_2O$ (0.1% trifluoroacetic acid) for 10 minutes)

**[3(R)-[2-(piperidin-4-yl)ethyl]-2-piperidone-1]-acetyl-3-methyl-β-alanine (1a + 1c):**

Ester **7** (8.5 mg, 0.02 mmol) was dissolved in 1:1 THF:$H_2O$ (1 mL) and was treated with LiOH monohydrate (4.1 mg, 0.098 mmol). After stirring for 1 hour at ambient temperature, TLC (5:3:1

$CH_2Cl_2$:MeOH:HOAc) showed no starting material left. The reaction was acidified with acetic acid (58 μL) and the solution was concentrated to dryness. The residue was treated with 3 x 5 mL of toluene for azeotropic removal of $H_2O$ to give **1a** + **1c** (7 mg, 100%) as a colorless semi-solid: $^1$H NMR (δ, $D_2O$): 4.02 (1H, m), 3.83 (2H, m), 3.28 (4H, m), 2.81 (2H, m), 2.25 (2H, m), 2.18 (1H, m), 1.8-1.1 (13 H, m), 1.1 (3H, d, J=7 Hz). For the deuterated compound, a solution of [$^2$H][3(R)-[2-(piperidin-4-yl)ethyl]-2-piperidone-1]-acetyl-3-methyl-β-alanine, ethyl ester (2.26 mg, 5.93 μmol) in 1:1 THF:$H_2O$ (312 μL) at room temperature was treated with LiOH monohydrate (1.4 mg, 3.3 μmol). The pH of the solution was basic as determined by wet litmus paper. After stirring for 1 hour at room temperature the reaction mixture was quenched by adding acetic acid (20 μL). The acidity of the mixture was verified using wet litmus paper. The reaction mixture was concentrated *in vacuo*, treated with several portions of toluene and concentrated for azeotropic removal of water to give a semi-solid. $^1$H NMR (δ, $D_2O$): 3.83 (2H, m), 3.28 (4H, m), 2.81 (2H, m), 2.25 (1H, m), 2.18 (1H, m), 1.8-1.1 (13 H, m), 1.1 (2.5H, s). This material coeluted with an authentic standard of L-734,217 (9 minute retention time). HPLC conditions: C-18 μBondapak, 3.9 x 150 mm, 215nm, 1 mL/min, linear gradient of 5% acetonitrile:$H_2O$ (0.1% TFA) to 95% acetonitrile over 10 minutes and held there for 10 minutes.

**[$^3$H][3(R)-[2-(piperidin-4-yl)ethyl]-2-piperidone-1]-acetyl-3(R)-methyl-β-alanine (1b):**

The tritiation reaction was carried out at NEN/Dupont. A mixture of 20 mg (39 mmol) of **6** in 3.5 mL of methanol containing 18 mg of 10% Pd/C was stirred for 2 hours at room temperature under 50 Ci of tritium gas at atmospheric pressure. The reaction mixture was filtered, rinsed with 10 mL of ethanol and concentrated. Volatiles were chased using 2 x 2 mL of ethanol leaving 520 mCi of activity in 10 mL of ethanol. A 100 mCi aliquot was diluted to 5 mCi/mL in ethanol. For conversion to the final product, a 4 mL aliquot (20 mCi) of this material was concentrated to dryness and treated with 1:1 THF:$H_2O$ (104 μL) and LiOH monohydrate (16.6 μL of a solution of 1.69 mg LiOH monohydrate in 84 μL of 1:1 THF:$H_2O$) giving a basic solution when checked by pH paper. The reaction was stirred for two hours at room temperature and quenched with 5 μL of acetic acid (acidic to pH paper), lyophilized and diluted with 200 μL of $H_2O$ for preparative HPLC purification [Beckman C-18 Ultrasphere ODS, 4.6 x 250 mm, 210 nm, linear gradient of 5% AcCN:$H_2O$ (1 mL $Et_3N$/liter + 1 mL $HClO_4$/liter) to 15% AcCN:$H_2O$ (1 mL $Et_3N$/liter + 1 mL $HClO_4$/liter) over 45 minutes] at 1 mL/min. Beginning at 30 minutes, 0.2 mL fractions were collected. UV peaks were present at 31 (**9**), 33 (**1a**) and 35 (**1d**) minutes. The pure fractions containing most of the first radioactive peak (33 minutes, **1a**) were combined, lyophilized and diluted with 3 mL of $H_2O$ to give 3.35 mCi of [$^3$H]L-734,217. The chemical concentration of [$^3$H]L-734,217 was determined by reading the absorption against a calibration curve for L-734,217 and an aliquot was counted giving a specific activity of 41.9 Ci/mmol. This material coeluted (analytical HPLC conditions were the same as the preparative HPLC conditions listed above) with an authentic sample of L-734,217 and was found to have radiochemical and chemical purities of >98%.

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(13) To verify that [$^3$H]**8** and ultimately **9** were present, standards of **8** and **9** were synthesized. If the reduction of **6** was carried out for only 45 minutes instead of 2 hours, **8** (along with **7**) was produced. The crude $^1$H NMR spectrum had a $\delta$4.93 (vinyl proton) and a $\delta$2.38 (vinyl methyl) signal in addition to the peaks due to **7**. This sample was hydrolyzed to produce **9** (along with unlabelled **1**).