Synthesis of [3"-^{3}H]Taxol and [13-^{3}H]Taxol[1]

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SUMMARY

7-Triethylsilylbaccatin III (**11**) was coupled with cis-1-benzoyl-3-triethylsiloxy-4-(3'-bromophenyl)azetidin-2-one. Hydrolysis of the silyl groups gave 3"-bromotaxol which was reduced with tritium gas to give[3"-^{3}H]taxol with specific activity of 19.3 Ci mmol. Reduction of 7-triethylsilyl-13-oxobaccatin III with [^{3}H]borane-tetrahydrofuran complex gave 7-triethylsilyl[13-^{3}H]baccatin III (**17**). Coupling of **17** with cis-3-triethylsiloxy-4-phenyl-(3R,4S)-azetidin-2-one and hydrolysis gave [13-^{3}H]taxol with specific activity of 1.66 Ci/mmol.

Key words: taxol, tritium

INTRODUCTION

Since the discovery in 1971 of the diterpenoid taxol[2], with its anticancer activity and unusual ability to stabilize the assembly of microtubules[3,4], there has been a need for radiolabeled taxol to facilitate pharmacological studies. Although taxol has been labeled by tritium exchange[4] and by introducing a 7-[^{3}H]acetyl group[5], until recently taxol's complex structure has precluded site-specific labeling. Recent developments in taxol chemistry have provided methods for specifically labeling taxol both in the side chain and in the baccatin III ring system. Several research groups have reported the semi-synthesis of taxol based on coupling a side-chain synthon with suitably protected baccatin III.[6-10] We found that an adaptation of the methods of Holton[8,9] and Ojima[10] was the most effective for the semimicro scale required for synthesis of radiolabeled taxol at high specific activity.

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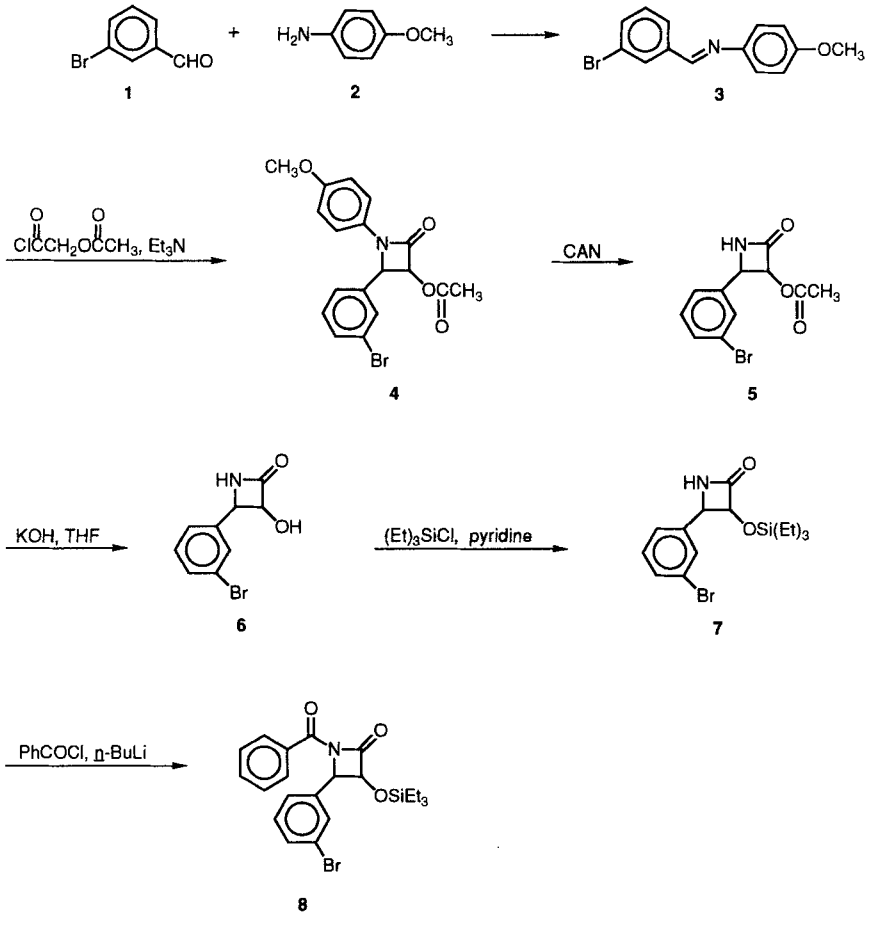
RESULTS AND DISCUSSION

The key intermediate for the preparation of side-chain labeled taxol, bromolactam **8**, was prepared by adapting the procedure of Holton.[8] The synthetic scheme is shown in Chart 1. Benzenimine **3** was prepared in 73% yield from the addition of p-anisidine to 3-bromobenzaldehyde. Imine **3** was treated with acetoxyacetyl chloride and triethylamine to give azetidinone **4** in 45% yield. The methoxyphenyl group was cleaved from **4** with ceric ammonium nitrate (CAN) to give ester **5** in 70% yield, and the ester group was hydrolyzed with potassium hydroxide to provide hydroxyazetidinone **6** in 57% yield. Because the coupling reaction with baccatin III favors the desired stereochemistry depending on reaction conditions and protecting group[9], **6** was not resolved. Several

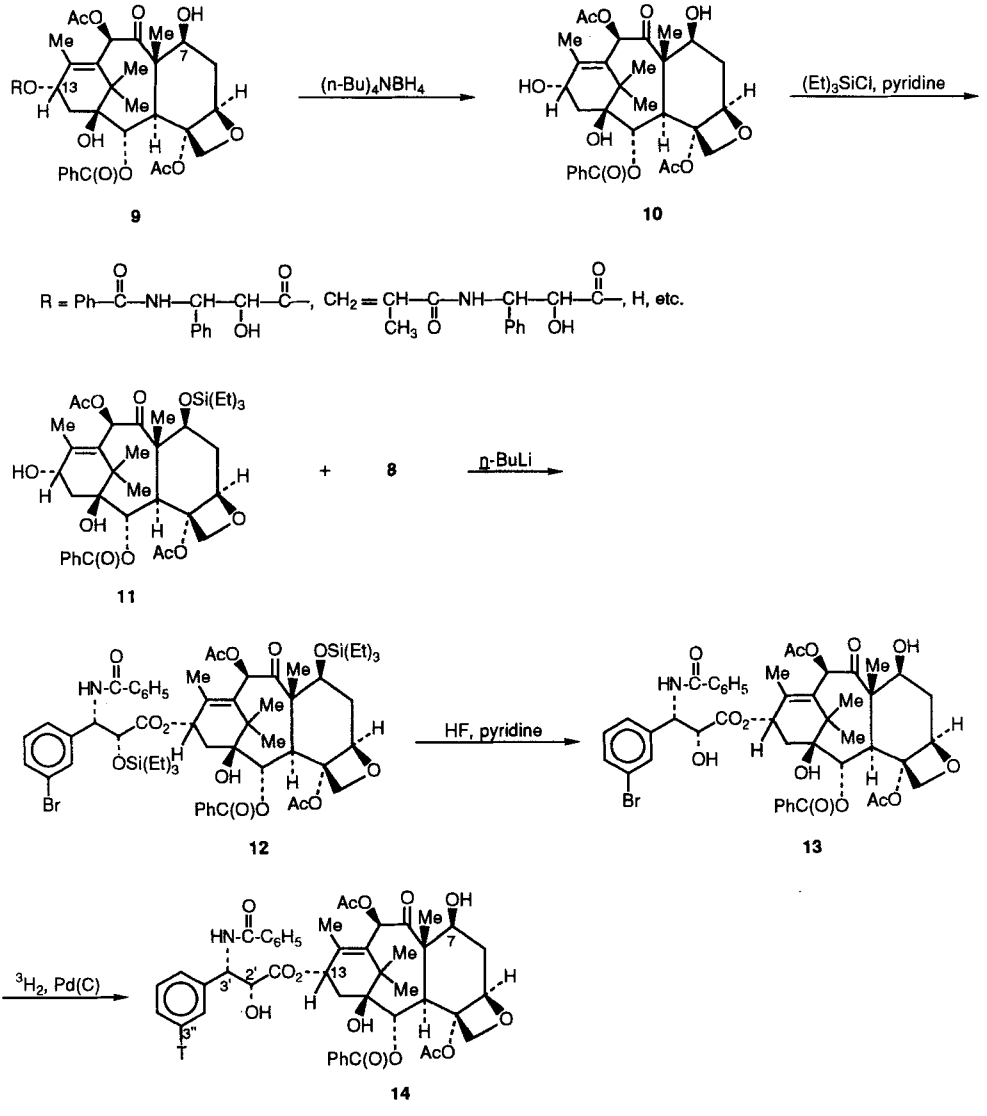
Chart 1



protecting groups have been proposed for **6**, among them ethoxyethyl[6] and 2,2,2-trichloroethoxymethyl.[8] We found that the triethylsilyl[9] group offered the best combination of stability and ease of removal for our purpose. **6** was treated with excess chlorotriethylsilane in pyridine to give **7**. Treatment of **7** with n-butyllithium and benzoyl chloride gave the desired lactam **8** in 86% yield.

[3"-^{3}H]Taxol (**14**) was prepared by condensing lactam **8** with 7-triethylsilylbaccatin III (**11**) as shown in Chart 2. We obtained baccatin III (**10**) by treating the partially purified residue of compounds left from the isolation of taxol with tetrabutylammonium

Chart 2

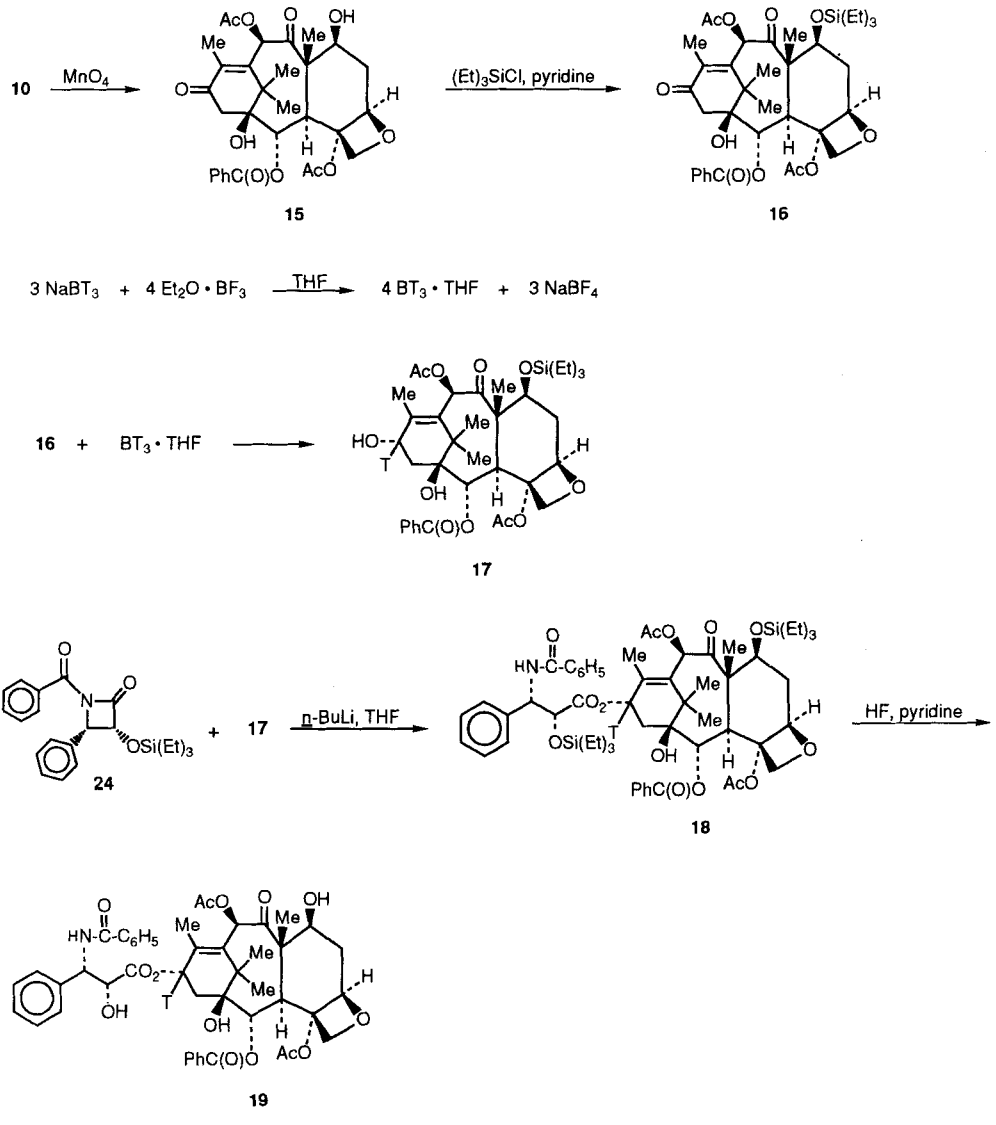


borohydride.[11] This residue contained a mixture of taxol, cephalomanine, and baccatin III, and after cleavage and purification, yielded baccatin III identical with a sample prepared from pure taxol.

The C-7 hydroxyl group of baccatin III was protected by treatment with chlorotriethyl silane in pyridine.[6] A solution of 7-triethylsilylbaccatin III (**11**) in tetrahydrofuran was treated with a slight excess of n-butyllithium and then with a five-fold excess of bromolactam **8**. The protected 3"-bromotaxol **12** was obtained as a mixture of isomers in 93% yield after purification. The 2',3'-isomers of **12** were separated by preparative-HPLC to give the desired 2'R,3'S-isomer in 64% yield from **11**. The 2'- and 7-triethylsilyl groups were removed with a mixture of hydrogen fluoride and pyridine[9] in tetrahydrofuran to give 3"-bromotaxol (**13**) in 60% yield. The 7-triethylsilyl group was readily cleaved, but the 2'-triethylsilyl group was resistant to cleavage and required treatment over several days with gradually increasing amounts of hydrogen fluoride while monitoring the reaction by TLC. Attempts to accelerate this reaction by adding the acid over a shorter time reduced the yield. Treatment of 3"-bromotaxol (**13**) with carrier-free tritium gas and palladium on carbon catalyst gave [3"-^{3}H]taxol (**14**) in 75% chemical yield with a specific activity of 19.3 Ci/mmol. The chromatographic and spectral properties of **14** were identical with an authentic sample of taxol.[12] A solution of **14** in pH 7 phosphate buffer showed 0.2% exchangeable tritium after 24 hours at room temperature. The ^{3}H NMR spectrum of **14** showed the expected triplet at δ 7.42 which collapsed to a singlet in the proton-decoupled spectrum. The ^{3}H NMR spectrum showed that at least 98% of the tritium was in the 3"-position.

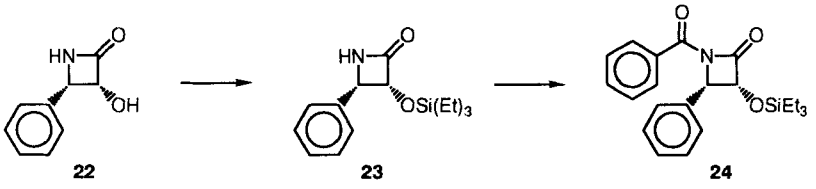
[13-^{3}H]Taxol was prepared using the scheme shown in Chart 3. Baccatin III was oxidized to its 13-ketoanalogue[2,13], **15**, in 74% yield using neutral, activated manganese dioxide.[14] Ketone **15** was treated with chlorotriethylsilane to give protected ketone **16**. 13-Oxo-10-deacetylbaccatin III is reported[15] to be regioselectively reduced by sodium borohydride to 10-deacetylbaccatin III. Treatment of **16** with sodium borohydride in ethanol gave a 35% yield of silylbaccatin **11** with considerable loss of yield due to cleavage of ester groups. Borane-tetrahydrofuran complex is reported[16] to selectively reduce the α,β-unsaturated ketone of progesterone. Based on this report, **16** was treated with borane-tetrahydrofuran and afforded a 74% yield of 7-triethylsilylbaccatin III (**11**). [^{3}H]Borane-tetrahydrofuran compelx was prepared by treating sodium

Chart 3



boro[^{3}H]hydride in tetrahydrofuran with an equivalent amount of boron trifluoride etherate.[17] Silyl-ketone **16** was treated with this mixture to give 7-triethylsilyl[13-^{3}H]-baccatin III (**17**) in 32% chemical, 4.8% radiochemical yield. In order to avoid loss of radiolabel to the undesired stereoisomer, enantiometrically pure lactam **24** was prepared by adapting the method reported by Ojima et.al.[10,18] Lactam **22**, obtained from this procedure, was protected with chlorotriethylsilane and benzoylated as for the

bromo analogues **7** and **8** to give the desired **24**. HPLC analysis with a Chiralcel OD column[10] showed **24** to be 98.8% the desired 3R,4S-isomer. The coupling reaction



22 **23** **24**

of **17** and **24** was performed as for the bromoanalogue, **12**, to give 2',7-bis(triethylsilyl)-[13-^{3}H]taxol (**18**) in 68% radiochemical yield. The silyl groups were removed as for bromoanalogue **13** to give 13.3 mCi of [13-^{3}H]taxol (52% yield) with specific activity of 1.66 Ci/mmol (1.94 mCi/mg). The ^{3}H NMR spectrum of the product showed the expected triplet at δ 6.19 which collapsed to a singlet in the proton-decoupled spectrum. The ^{3}H NMR spectrum showed that at least 98% of the tritium was in the 13-position.

EXPERIMENTAL

Ultraviolet spectra were recorded on a Varian Model 2290 spectrometer. ^{3}H NMR spectra were recorded on a Bruker/AMX-500 multi-nuclear Fourier transform spectrometer, equipped wtih a tritium/proton dual probe, a tritium E coupler, and a selective pre-amplifier for tritium. Radioactive samples were counted with a Packard Tri-carb 400 liquid scintillation spectrometer in Packard Optima Gold cocktail using the internal standard method of quench correction. E. Merck silica gel 60F-254 plates were used for analytical TLC. Analtech silica gel GF and E. Merck RP-18F plates were used for preparative-TLC. Radioactive TLC plates were scanned on a Berthold Model LB 283 Linear Analyzer system. HPLC was done using a Waters Associates Model 6000A dual pump system with a model U6K septumless injector and with a Schoeffel Instrument Co. Spectroflow UV monitor and IN/US β-RAM radioactivity flow monitor as detectors. Mass spectra were done on a Fisons ZAB-E high resolution mass spectrometer using FAB with a thioglycerol/glycerol 30:70 matrix. Carrier-free tritium gas was purchased from DuPont/New England Nuclear Corporation. Taxol samples and taxol isolation residues were provided by the National Cancer Institute.

N-(3'-Bromophenylmethylene)-4-methoxybenzenamine (3)

p-Anisidine (10.56 g, 0.086 mol) was dissolved in 80 mL of benzene, and 10.0 mL (0.086 mol) of 3-bromobenzaldehyde was added to the solution. The reaction was

heated at 83°C for 1 h in a flask equipped with a Dean-Stark trap. The reaction should not be heated above 90°C or extensive decomposition of the imine results. The crude solid obtained after removal of the benzene was crystallized from warm absolute ethanol-acetone (95:5). The resulting orange crystals were recrystallized once from acetone-hexane (4:1) to provide a tan solid, 18.23 g (73%): m.p. 76-77°C; TLC, silica gel 60F, hexane-EtOAc 3:1, R_f 0.60; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.43 (s, 1, HC=N), 8.08 (t, 1, ArH), 7.5 - 7.8 (dd, 2, ArH), 7.35 - 7.20 (m, ArH), 7.27 - 6.90 (dd, 4, ArH), 3.84 (s, 3, OCH_3). Anal. calcd. for $C_{14}H_{12}BrNO$: C, 57.95; H, 4.17; N, 4.83; Br, 27.54. Found: C, 57.93; H, 4.14; N, 4.75; Br, 27.48.

cis-1-(4-Methoxyphenyl)-3-acetoxy-4-(3'-bromophenyl)azetidine-2-one (4)

A solution of imine **3** (5.65 g, 0.0195 mol) and triethylamine (4.2 mL, 0.03 mol) in methylene chloride (70 mL) was cooled to -10°C with an ice-salt bath. Acetoxyacetyl chloride (2.15 mL, 0.02 mol) in 5 mL of CH_2Cl_2 was added dropwise over 15 min. The reaction was allowed to warm to ambient temperature and was stirred overnight. The reaction mixture was partitioned between 50 mL of CH_2Cl_2 and 75 mL of 1 N HCl. The acidic layer was back extracted with CH_2Cl_2 (50 mL) and the combined organic layers were washed with 1 N HCl, H_2O, saturated $NaHCO_3$ solution, and brine. After drying (Na_2SO_4) the CH_2Cl_2 was removed on the rotary evaporator. The crude solid was dissolved in a minimum quantity of ethyl acetate and triturated with hexane. The solid was collected by filtration and was washed with cold absolute ethanol. A tan solid (3.5 g, 45%) was obtained after air drying: m.p. 154 - 155 °C; IR ($CHCl_3$) 1753 cm^{-1} (C=O, lactam), 1510 cm^{-1}, 1224 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.50 - 7.45 (m, 2, ArH) 7.29 - 7.19 (m, 4, ArH), 6.82 (d, 2, ArH), 5.94 (d, 1, CH) 5.28 (d, 1, CH), 3.76 (s, 3, OCH_3) 1.75 (s, 3, CH_3). Anal. calcd. for $C_{18}H_{16}BrNO_4$: C, 55.40; H, 4.13; N, 3.59; Br, 20.48. Found: C, 55.48; H, 4.25; N, 3.57; Br, 20.55.

cis-3-Acetoxy-4-(3'-bromophenyl)azetidin-2-one (5)

Cis-1-(4-methoxyphenyl)-3-acetoxy-4-(3'-bromophenyl)azetidin-2-one (4.5 g, 0.012 mol) in 180 mL of acetonitrile was cooled to -10 °C in an ice/salt bath. A solution of ceric ammonium nitrate (19.0 g, 0.035 mol) in 100 mL of distilled H_2O was added dropwise over 45 min., and the reaction was stirred at -10 °C for an additional 30 min. The reaction mixture was added to 150 mL of ethyl acetate and 100 mL of distilled H_2O and partitioned. The aqueous layer was extracted with ethyl acetate (2 x 75 mL). The

combined organic layers were washed with saturated $NaHCO_3$ solution, saturated sodium bisulfite solution, saturated $NaHCO_3$ solution, and brine. After drying (Na_2SO_4) and evaporation of solvent, the crude solid was dissolved in methylene chloride and treated with silica gel 60 (230-400 mesh) to form a thick slurry. The silica slurry was stirred for 45 min, then filtered over a pad of Celite. The bright yellow filtrate was evaporated, and the solid residue was crystallized from ethyl acetate-hexane (1:1) to yield a white solid (2.29 g, 70%): m.p. 133 - 135 °C; IR ($CHCl_3$), 1780 cm^{-1}, 1750 cm^{-1} (C=O), 1330 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.50 - 7.47 (m, 2, ArH), 7.26 - 7.23 (m, 2, ArH), 6.3 (s, 1, NH), 5.91 - 5.88 (dd, 1, CH), 5.0 (d, 1, CH), 1.75 (s, 3, CH_3). Anal. calcd. for $C_{16}H_{10}BrNO_3$: C, 46.50; H, 3.55; N, 4.93; Br, 28.12. Found: C, 46.61; H, 3.62; N, 4.92; Br, 28.19.

cis-3-Hydroxy-4-(3'-bromophenyl)azetidin-2-one (6)

Ester **5** (1.63 g, 0.0057 mol) was dissolved in 60 mL of THF, and the solution was added dropwise to a solution of 1 N KOH (60 mL, 10 equivalents) in 45 mL of THF at -5 °C over a 40 min period. The solution was stirred an additional 45 min at -5 ° to 0 °C then added to 75 mL of saturated $NaHCO_3$ solution and 75 mL of distilled H_2O. This mixture was extracted with ethyl acetate (3 x 75 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to give a crude orange solid (1.75 g) which gave a white solid after crystallization from CH_2Cl_2 (0.785 g, 57%): m.p. 201 - 203 °C; IR ($CHCl_3$) 3220 cm^{-1} (OH), 1785 cm^{-1} (C=O). ^1H NMR (250 MHz, d_6-acetone) δ 7.53 - 7.33 (m, 4, ArH), 5.13 (d, 1, CH), 4.90 (d, 1, CH). This material was sufficiently pure for further reaction.

Cis-3-triethylsiloxy-4-(3'-bromophenyl)azetidin-2-one (7)

Cis-3-hydroxy-4-(3'-bromophenyl)azetidin-2-one (0.784 g, 0.0032 mol) was dissolved in 8 mL of anhydrous pyridine and stirred with chlorotriethylsilane (1.0 mL, 0.005 mol) at ambient temperature. The reaction was complete within 30 min (TLC silica gel 60F, hexane-EtOAc 3:1). Heptane (10 mL) was added, and the azeotrope removed with the rotary evaporator. Heptane was added again followed by evaporation, and the crude residue was dissolved in CH_2Cl_2 and washed with 1 N HCl. The organic layer was dried (Na_2SO_4), and the solvent was removed on the rotary evaporator. Chromatography of the residue on silica gel 60 (230-400 mesh) using hexane (60 mL) and then hexane-ethyl acetate 3:1 (300 mL) as eluants yielded 0.94 g (82%) of a white

paste: ^{1}H NMR (250 MHz $CDCl_3$) δ 7.48 - 7.41 (m, 2, ArH), 7.24 - 7.19 (m, 2, ArH), 6.10 (s, 1, NH), 5.10 - 5.07 (m, 1, CH), 4.76 (d, 1, CH), 0.828 - 0.761 (m, 9, CH_3), 0.515 - 0.404 (m, 6, CH_2). Anal. calcd. for $C_{15}H_{22}BrNO_2Si$: C, 50.56; H, 6.22; N, 3.93; Br, 22.42. Found: C, 50.61; H, 6.20; N, 3.89; Br, 22.50.

Cis-1-benzoyl-3-triethylsiloxy-4-(3'-bromophenyl)azetidine-2-one (8)

Triethylsilyl β-lactam **7** (0.94 g, 0.00264 mol) was dissolved in 15 mL of anhydrous THF and cooled to -78 °C. n-Butylithium in hexane (1.7 mL of a 1.6 M solution, 0.0027 mol) was slowly added, and the mixture was stirred for 10 min. Benzoyl chloride (0.313 mL, 0.0027 mol) in 3 mL of THF was added dropwise, and the mixture was stirred at -75°C for 1 h. The mixture was diluted with saturated $NaHCO_3$ solution (50 mL) and extracted with CH_2Cl_2 (2 x 50 mL). The combined CH_2Cl_2 layers were dried (Na_2SO_4), and the solvent removed on the rotary evaporator to leave an oil. The oil was chromatographed on silica gel 60 (230-400 mesh) with hexane (130 mL) and then hexane-ethyl acetate 3:1 (150 mL) as eluants to yield 0.98 g (0.0023 mol, 86%) of an oil that solidified under vacuum to a white paste: IR ($CHCl_3$) 2955 (aromatic), 1796 (C=O), 1677 cm^{-1} (amide C=O); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.06 - 8.02 (m, 2, ArH), 7.61 - 7.44 (m, 7, ArH), 5.36 (d, 1, CH), 5.14 (d, 1, CH), 0.82 (m, 9, CH_3), 0.48 (m, 6, CH_2).

Baccatin III (10)[11]

Two grams of the crude plant residue (**9**) left from the isolation of taxol was dissolved in 40 mL of methylene chloride, and 1.0 g of tetrabutylammonium borohydride was added in portions to avoid foaming. The mixture was stirred at room temperature (~ 25 °C) for 2 h. Acetic acid was added dropwise until there was no more gas evolution. The mixture was evaporated to a yellow syrup and chromatographed on a column of 400 g of silica gel 60 (230-400 mesh) packed in $CHCl_3$ and eluted with $CHCl_3$-MeOH 98:2. The baccatin III fractions were combined and crystallized from $CHCl_3$-hexane to give 582 mg of **10** that was 98% pure by HPLC [μ-bondapak phenyl; linear gradient in 30 min from CH_3CN-MeOH-H_2O (15:20:65) to CH_3CN-MeOH-H_2O (55:20:25), UV-227, t_R 16.5 min]. The retention time and ^{1}H NMR (500 MHz, $CDCl_3$) spectrum were identical to baccatin III prepared by the hydrolysis of taxol.

7-Triethylsilylbaccatin III (11)[6]

A solution of 100 mg (0.171 mmol) of baccatin III in 8 mL of pyridine was treated

with 0.7 mL of chlorotriethylsilane and stirred under N_2 for 24 h at room temperature (~26 °C). The volatiles were removed under reduced pressure; heptane was used to azeotrope the pyridine. The residue was chromatographed on two 20 x 20 cm x 2 mm silica gel GF plates eluted with ethyl acetate-cyclohexane 1:1. The major band shown by UV-quench was washed from the silica with $CHCl_3$-EtOH 1:1. The solvent was removed to give white crystals that were dried under vacuum (0.1 torr) at 100 °C: mp 221-223 °C [lit.[6] mp 253-254 °C]. Anal. calcd. for $C_{37}H_{52}O_{11}Si$:C, 63.40; H, 7.48. Found: C, 63.39; H, 7.48. The yield was 87 mg (73%). The ^1H NMR (250 MHz, $CDCl_3$) spectrum was consistent with the structure.

2',7-Bis(triethylsilyl)-3"-bromotaxol (12)

Compound **12** was prepared by a method analogous to that described by Holton et.al[9] for the preparation 2',7-bis(triethysilyl)taxol. A solution of 50 mg (0.0714 mmol) of 7-triethylsilylbaccatin III (**11**) in 0.4 mL of THF was cooled to -45 °C under dry N_2. A solution of n-butyllithium in hexane, 58 μL (0.0857 mmol), was added dropwise over ~4 min, and the mixture was stirred at -45 °C for 10 min. A solution of 164 mg (0.357 mmol) of bromolactam **8** in 0.5 mL of THF was added to the cold solution of the lithium salt of **11**. The -45 °C bath was replaced with an ice bath, and the mixture was stirred at 0 °C for 2.5 h. The reaction mixture was added to 20 mL of saturated aqueous $NaHCO_3$ solution, and the resulting mixture was extracted with $CHCl_3$ (3 x 20 mL). The $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated, and the residue was chromatographed on two 20 x 20 cm x 2 mm silica gel GF plates eluted with ethyl acetate-hexane 1:1. The product band was washed from the silica with $CHCl_3$-EtOH 1:1 to give 91 mg of crude **12** as 3.5:1 mixture of isomers.

The isomers were separated by HPLC using two Waters Associates 100 x 25 μ-bondapak RCM columns eluted with acetonitrile, 15 mL/min, monitored by UV at 227 nm. The yield was 53 mg (64%) of the desired 2'R,3'S-isomer (t_R 11.6 min) as a colorless glass and 15 mg of the 2'S,3'R-isomer (t_R 10.4 min). The ^1H NMR (250 MHz, $CDCl_3$) spectrum of the desired isomer was the same as 2',7-bis(triethylsilyl)taxol prepared from authentic taxol except for the aromatic region δ 7.2 - 7.8 and a small upfield shift (0.07 ppm) of the 3'-hydrogen.

3"-Bromotaxol (13)

The triethylsilyl protecting groups were removed from **12** by a method analogous to

that described by Holton et al[9] for preparing taxol. A solution of 50 mg (0.043 mmol) of **12** in 3 mL of THF was cooled in an ice bath and 15 μL of pyridine and 45 μL of 48% HF were added. The ice bath was removed, and the mixture was stirred at room temperature (~26 °C). Additional HF (a total of 240 μL) and pyridine (a total of 145 μL) were added in portions with monitoring by TLC (silica gel, ethyl acetate-hexane 6:4) over a period of 3 days until both of the silyl groups were removed. The reaction mixture was added to 20 mL of saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with $CHCl_3$ (3 x 20 mL). The $CHCl_3$ extracts were dried (Na_2SO_4), evaporated, and chromatographed on a 20 x 20 cm x 1 mm silica gel GF plate eluted with ethyl acetate-hexane 1:1. The product band was washed from the silica with $CHCl_3$-EtOH 1:1 to give 24 mg (60%) of **13**. The product crystallized from MeOH-H_2O, mp 173-174 °C. HPLC showed the product to be 99% pure [Dupont Zorbax phenyl, MeOH-CH_3CN-H_2O 20:43:37, 1 mL/min, UV-227, t_R 22 min.] The ^{1}H NMR (250 MHz, $CDCl_3$) spectrum of **13** was the same as authentic taxol except for the aromatic region δ 7.2-7.8 and very small shifts for the protons at 2' and 3' [taxol 2', δ 4.79, 3', δ 5.78; 3"-bromotaxol 2', δ 4.75, 3', δ 5.75]. High resolution mass spectrum calcd. for $C_{47}H_{51}NO_{14}Br$: m/z 932.2493. Found m/z 932.2485.

[3"-^{3}H]taxol (14)

A solution of 20 mg (0.0214 mmol) of bromotaxol **13** in 0.5 mL of THF and 20 μL of triethylamine with 10 mg of 10% palladium on carbon catalyst was exposed to 5 Ci of carrier-free tritium gas for 4 h at room temperature (26 °C). The catalyst was removed by filtration through Celite, and the solvent was removed by vacuum transfer. The residue was exchanged three times with methanol, the methanol being removed by vacuum transfer. The residue was chromatographed on four 20 x 20 cm analytical C_{18} TLC plates eluted with CH_3CN-MeOH-H_2O (1:1:1). The taxol band was washed from the C_{18}-silica with methanol to give 311 mCi of **14** that was 98% pure by TLC-RAM [silica gel; $CHCl_3$-MeOH 95:5, R_f 0.36 and ethyl acetate-hexane 6:4, R_f 0.23] with R_f's the same as authentic taxol. HPLC showed the material to be 98% pure by both radioactivity and UV (227 nm) monitors [Dupont Zorbax phenyl, CH_3CN-MeOH-H_2O 37:20:43, 1 mL/min, t_R 23 min]. The specific activity was determined to be 19.3 Ci/mmol (22.6 mCi/mg) using UV (methanol) λ_{max} 227 ε 29,800. Exchange for 24 h in pH 7 phosphate buffer at room temperature (~26 °C) showed 0.2% exchange.

The ^3H NMR (533 MHz, $CDCl_3$) spectrum showed the expected triplet at δ 7.42 which collapsed to a singlet in the proton-decoupled spectrum. The ^3H NMR spectrum indicated that at least 98% of the tritium was in the 3"-position.

13-Oxo-baccatin III (15)[2,13]

Baccatin III (**10**), 100 mg (0.17 mmol) was dissolved in 11 mL of dry (3A molecular sieve) chloroform and stirred with 500 mg of manganese dioxide for 20 h at room temperature (~ 25 °C). The mixture was centrifuged, and the supernatant was filtered through a 0.5 μ PTFE filter and evaporated. The residue was chromatographed on a 20 x 20 cm x 1 mm silica gel plate eluted with $CHCl_3$-MeOH 95:5. The major band was washed from the silica with $CHCl_3$-EtOH 1:1 to give 55 mg of **15** that was 97% pure by HPLC [system as for baccatin III above]: ^1H NMR (250 MHz, $CDCl_3$) was essentially identical to that reported[13] for **15**; IR ($CHCl_3$) 1676 cm^{-1} (conjugated ketone). Crystals from ether-pentane mp 210-213 °C [lit.[2] 210-212 °C].

7-Triethylsilyl-13-oxo-baccatin III (16)

13-Oxobaccatin III (**15**), 55 mg, was dissolved in 8.5 mL of pyridine, and 0.8 mL of chlorotriethylsilane was added. The mixture was stirred at room temperature for 24 h. The volatiles were evaporated under reduced pressure, and heptane was used to azeotrope the last of the pyridine from the product. The crude mixture was chromatographed on a 20 x 20 cm x 1 mm silica gel 60F plate eluted with ethyl acetate-hexane 1:1. The product was washed from the silica with acetone. The acetone was removed under reduced pressure to give 37 mg (56%) of **16** after drying under vacuum at 80 °C: mp 163-166 °C. The ^1H NMR spectrum (250 mHz, $CDCl_3$) was consistent with the structure. HIgh resolution mass spectrum calcd. for $C_{37}H_{51}O_{11}Si$: m/z 699.3201. Found m/z 699.3196.

7-Triethylsilyl[13-^3H]baccatin III (17)

A slurry of 500 mCi (0.055 mmol) of sodium [^3H]borohydride in 0.25 mL of tetrahydrofuran was cooled in a dry ice-isopropanol bath, and 9 μL of boron trifluoride etherate (0.0733 mmol) was added. The bath was removed, and the reaction mixture was stirred for 1 h at ambient temperature. The cooling bath was replaced, and a solution of 32.7 mg (0.0438 mmol) of silyl ketone **16** in 0.25 mL of THF was added. The bath was removed, and the reaction mixture was stirred at ambient temperature for 1 h. The volatile components of the mixture were vacuum transferred into a solution of 0.5 g

of triphenylphosphine in 10 mL of ether to trap unreacted [^{3}H]borane-tetrahydrofuran. Methanol (1 ml) was added to the reaction mixture residue, and the solution was stirred for 30 min. The methanol was removed by vacuum transfer, and the residue was chromatographed on a 20 x 20 cm x 1 mm silica gel GF plate eluted with chloroform-methanol 95:5. The product was washed from the silica with $CHCl_3$-EtOH 1:1 to give 12 mg of partially pure **17**. The product was chromatographed again on a 20 x 20 analytical TLC C_{18} plate eluted with CH_3CN-MeOH 1:1. The product was washed from the plate with $CHCl_3$-EtOH 1:1 to give 10.5 mg (32% chemical yield) of **17** with specific activity of 1.62 Ci/mmol (2.32 mCi/mg) and that was 98% pure by TLC-RAM (systems above) and 95% pure by HPLC-RAM (baccatin system above), with R_f's and t_R the same as 7-triethylsilylbaccatin III prepared by the method of Denise et al.[6]

2',7-Bis(triethylsilyl)[13-^{3}H]taxol (18)

Compound **18** was prepared by a method analogous to that described by Holton et.al.[9] for the preparation 2',7-bis(triethysilyl)taxol. A solution of 10.5 mg (24.4 mCi) of 7-triethylsilyl[13-^{3}H]baccatin III (0.015 mmol) in 0.1 mL of THF was cooled to -45 °C, and 13 μL of a solution of n-butyllithium (1.49 molar) in hexane was added dropwise. The mixture was stirred at -45 °C for 10 min. A solution of 30 mg (0.0786 mmol) of silyllactam **24** in 0.15 mL of THF was added dropwise. The -45 °C bath was replaced with an ice bath, and the reaction mixture was stirred at 0 °C for 2 h. The mixture was partitioned between 30 mL of $CHCl_3$ and 20 mL of H_2O. The water was washed with 20 mL of $CHCl_3$, and the $CHCl_3$ extracts were dried with Na_2SO_4 and evaporated. The crude product was chromatographed on a 20 x 20 cm x 1 mm silica gel TLC plate eluted with ethyl acetate-hexane 1:1. The band corresponding to product was washed from the silica with $CHCl_3$-EtOH 1:1 to give 15.4 mg of **18** that was 98% pure by TLC-RAM (system above) but contained some non-radioactive impurities. The 16.7 mCi (68.4% radiochemical yield) of **18** was used in the following reaction without further purification.

[13-^{3}H]Taxol (19)

The triethylsilyl protecting groups were removed from **18** by a method analogous to that described by Holton et.al[9] for preparing taxol. The disilyl[13-^{3}H]taxol **18**, 15.4 mg, was dissolved in 1.5 mL of THF in a plastic vial. Pyridine (7 μL) and 48% hydrofluoric acid (20 μL) were added, and the mixture was stirred at ambient temperature. Additional portions of 7 μL pyridine and 20 μL of 48% HF were added after 3 h, 22 h, and

32 h. After a total of 48 h, the reaction mixture was added to 25 mL of saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$ (2 x 20 mL). The extracts were dried (Na_2SO_4) and evaporated, and the residue was chromatographed on a 20 x 20 cm x 0.5 mm silica gel GF plate eluted with ethyl acetate-hexane 1:1. The product was washed from the silica with $CHCl_3$-EtOH 1:1 to give 13.3 mCi (6.86 mg or 52%) of **19**. The specific activity was determined by UV absorbance at 227 nm, ε 29,800[2] to be 1.66 Ci/mmol (1.94 mCi/mg). The **19** was 98% pure by TLC-RAM and by HPLC-RAM [systems as for **14**, above] with t_R and Rf's the same as for authentic taxol. The ^1H NMR (500 MHz, $CDCl_3$) spectrum of the product was identical to that of authentic taxol, and the ^3H NMR (533 MHz, $CDCl_3$) showed a triplet at δ 6.19 (J = 9.1 Hz) as expected for a triton in the 13-position. Exchange for 24 h in pH 7 phosphate buffer showed 0.03% exchangeable tritium.

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