# SYNTHESIS OF PACLITAXEL-*C3'-14C*

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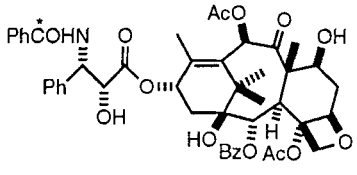
## SUMMARY

Reductive cleavage of the C13 side chain of paclitaxel (**3**) followed by regioselective silylation gave 7-triethylsilylbaccatin III (**6**). Successive reaction of L-threonine methyl ester hydrochloride (**7**) with *tert*-butoxydiphenylchlorosilane, benzaldehyde-*C7-14C* and acetoxyacetyl chloride / triethylamine gave a 92:8 ratio (NMR) of azetidinones **10**:**11** in 57% yield from **7**. Removal of the chiral auxiliary led to **16**, which after 3-*O*-triethylsilylation and *N*-benzoylation provided (3R,4S)-*cis*-1-benzoyl-3-*O*-(triethylsilyl)-4-phenylazetidin-2-one-*C4-14C* (**18**). Coupling of **18** and **6** followed by deprotection gave 1.12 g of paclitaxel-*C3'-14C* (**2**) having a specific activity of 16.4 mCi/mmol and a radiochemical purity of 96%.

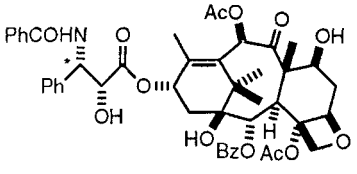
Key words: Taxol®, paclitaxel, carbon-14

## INTRODUCTION

We recently reported the synthesis of paclitaxel-*N3'-14C* (**1**)[2] which was used primarily for the preparation of a carbon-14-labeled prodrug of paclitaxel.[3] We also had need of a second carbon-14-labeled paclitaxel, with the label placed in a more internal position, for a number of other pharmacological studies. In considering alternative labeling sites and synthetic methodology available for their incorporation, we chose paclitaxel-*C3'-14C* (**2**) as our candidate. In this paper we report the synthesis of gram quantities of **2**.




**1**




**2**

* indicates position of carbon-14 label

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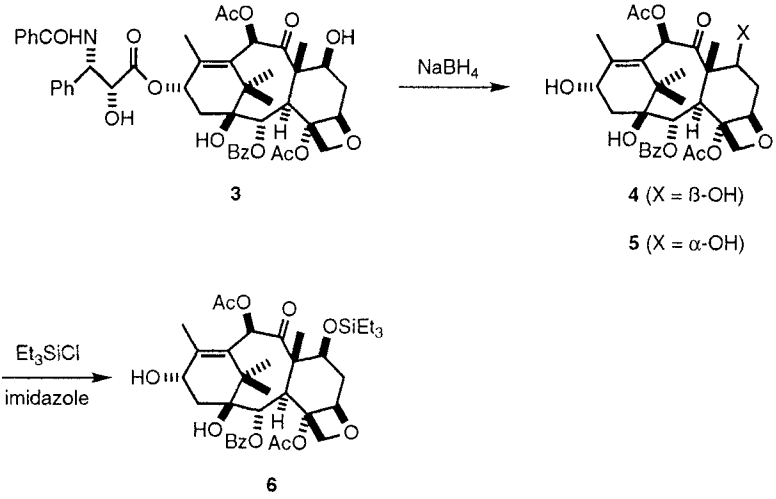
## RESULTS AND DISCUSSION

The requisite taxane component **6** was derived from paclitaxel (**3**)[4] exactly as described previously (Scheme 1).[2] Reductive cleavage of the C13 side chain[5] was accomplished on reacting **3** with sodium borohydride in tetrahydrofuran (THF) / pH 7 phosphate buffer.[2] Yields of baccatin III (**4**)[2,5] were generally 75-80% after chromatographic purification, which removed minor amounts of 7-*epi*-baccatin III (**5**) produced under these conditions. Regioselective 7-*O*-triethylsilylation of **4** by reaction with excess triethylchlorosilane and imidazole in dichloromethane[2] afforded gram quantities of 7-triethylsilylbaccatin III (**6**)[2,6] after chromatographic purification.

Scheme 1




**3**

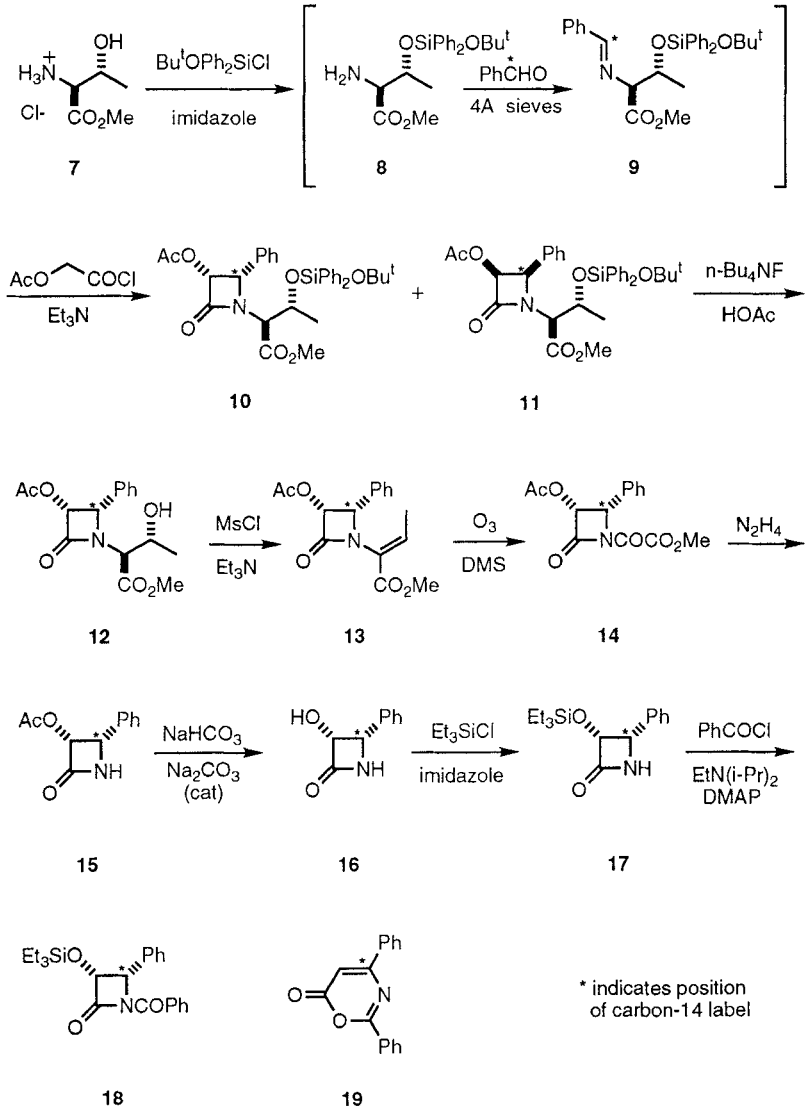
**4** (X = β-OH)

**5** (X = α-OH)

**6**

The C13 side chain synthon **18**[2,7,8] was assembled by the combined methodologies of Farina[9] and Holton[7,8] (Scheme 2).[10] Reaction of **7** with a slight excess of *tert*-butoxydiphenylchlorosilane and imidazole in dichloromethane gave **8**, which was sufficiently pure (NMR) for further manipulation.[9] Thus, formation of **9** by reaction of **8** with benzaldehyde-*C7-$^{14}$C* (limiting reagent)[14] and 4A sieves in dichloromethane was monitored by NMR ($CD_2Cl_2$). As the condensation proceeded, the doublet for the proton α to the ester shifted downfield from 3.29 ppm in **8** to 4.00 ppm in **9** and the benzaldehyde proton signal at 10.00 ppm was replaced by the aldimine proton signal at 8.27 ppm. It is of interest to note that the reaction employing carbon-14-labeled benzaldehyde was complete after 21 h while 72 h was necessary when unlabeled benzaldehyde was used. Following removal of the 4A sieves and cooling to low temperature (-40°C), excess triethylamine and acetoxyacetyl chloride were added and reaction progress was monitored by NMR. In this instance, loss of the aldimine proton signal at 8.27ppm was

Scheme 2




accompanied by the appearance of two baseline resolved doublets representing **10** (5.84 ppm, J = 5.2 Hz, C4 ß-lactam H) and **11** (4.96 ppm, J = 4.9 Hz, C3 ß-lactam H) in a ratio of 92:8, respectively. The mixture of **10/11** was isolated in an overall yield of 57% from **7** after chromatographic purification.[9]

Having directed introduction of the chirality at C3 and C4, removal of the chiral auxiliary was now necessary. Treatment of **10/11** with glacial acetic acid and *n*-$Bu_4NF$ in tetrahydrofuran (THF) was

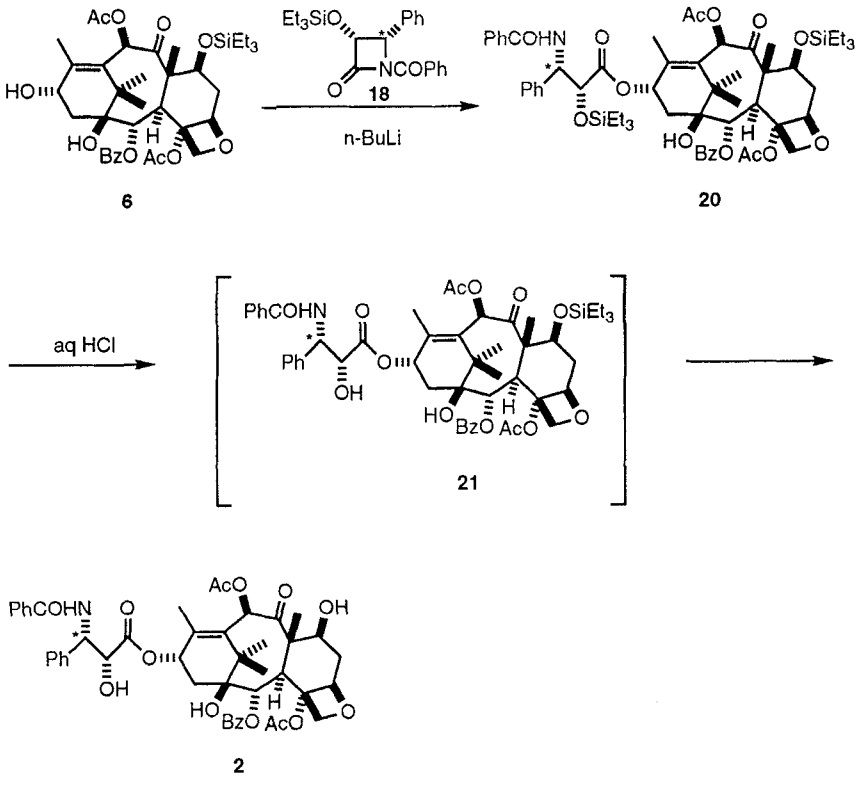
complete after 2 h (TLC).[9] The highly crystalline alcohol **12** was isolated as a single diastereomer in 75% yield following an immediate chromatographic purification of the crude reaction mixture. Formal elimination of water on exposure of **12** to excess methanesulfonyl chloride and triethylamine afforded olefin **13** (100%), which was then converted to oxalimide **14** by ozonolysis and reductive workup with dimethyl sulfide (100%).[9] Selective removal of the oxalimide resulted on reacting **14** with hydrazine hydrate (10 equiv) at low temperature (-78°C) in THF (68%). Hydrolysis of the 3-*O*-acetyl group of **15** proceeded smoothly in a two phase medium composed of methanol - saturated aqueous $NaHCO_3$ to afford alcohol **16**[9,15] in 83% yield (56% overall yield from **12**). It was subsequently found that **14** could be directly converted to **16**, via the intermediacy of **15** (TLC), in quantitative yield on exposure to methanol - saturated aqueous $NaHCO_3$.[9]

In preparation for coupling, 3-*O*-triethylsilylation[2,7,8] of crude **16** (from **14**) furnished silyl ether **17** (73% overall yield from **12**), which upon further reaction with benzoyl chloride, *N,N*-diisopropylethylamine and a catalytic amount of 4-dimethylaminopyridine in dichloromethane afforded **18**.[2,7,8] As was previously observed,[2] care *must* be taken not to store crude **18** in vacuo at ambient temperature, since rapid conversion to a mixture of **19** and unidentifiable decomposition product(s) occurs. A yield of 82% was observed when the process stream containing **18** was concentrated to near dryness, redissolved in minimal dichloromethane and *immediately* purified by flash chromatography over silica gel. Under these conditions, formation of **19** and decomposition were suppressed.[2]

Coupling[2,7,8] of the lithium alkoxide of **6** with **18** (1.35 equiv) in THF gave 2',7-bis(triethylsilyl)taxol-*C3'-$^{14}$C* (**20**) in excellent yield after chromatographic purification (Scheme 3). Hydrolysis of the triethylsilyl protecting groups resulted on exposure of **20** to 6*M* aqueous HCl (4.5 equiv) in -5°C acetonitrile (MeCN) for 3-4h. This conversion proceeded through the intermediacy of 7-triethylsilyltaxol-*C3'-$^{14}$C* (**21**), to which **20** was completely converted within 5 min (TLC, NMR).[2] Slow conversion of **21** to **2** was monitored by TLC. After chromatographic purification, 1.12 g of paclitaxel-*C3'-$^{14}$C* (**2**) was isolated. The NMR spectrum, TLC $R_f$ and chromatographic behavior of **2** were consistent with a sample of natural paclitaxel (**3**).[4] This material had a specific activity of 16.4 mCi/mmol and a radiochemical purity of 96%, which were sufficient for our studies.

The overall chemical yield for the conversion of **7** to **2** was 17% while the radiochemical yield of the process was 11%.

Scheme 3




* denotes position of carbon-14 label

## EXPERIMENTAL

$^1$H NMR spectra were recorded on a Bruker AM360 spectrometer. Chemical shifts are expressed on the $\partial$ scale downfield of tetramethylsilane internal standard. Thin layer chromatography plates (silica gel GF, catalog no. 21521) were purchased from Analtech, Inc. (Newark, DE). Developed TLC plates were viewed under short wavelength UV light (254 nm) unless otherwise noted. Flash chromatography silica gel (32-63 μm, 60Å) was purchased from ICN (Costa Mesa, CA). Radiochemical measurements were taken on a Beckman LS9000 liquid scintillation counter. The specific activity was determined using an IN/US Systems Model 2 β-RAM system. Benzaldehyde-*C7-$^{14}$C* was purchased as a solution in dichloromethane from Moravek Biochemicals, Inc. (Brea, CA) and was used as received. Acetoxyacetyl chloride, benzaldehyde, benzoyl chloride, tetra-*n*-butylammonium fluoride / THF solution, *n*-BuLi / hexanes solution, *N,N*-diisopropylethylamine, 4-dimethylaminopyridine, dimethyl sulfide, hydrazine hydrate, imidazole, methanesulfonyl chloride, 4A sieves (catalog no. 33430-8), sodium borohydride, triethylamine, triethylchlorosilane and anhydrous acetonitrile (MeCN), dichloromethane ($CH_2Cl_2$) and tetrahydrofuran

(THF) were purchased from Aldrich Chemical Co. (Milwaukee, WI) in the highest purity available and were used as received. The *tert*-butoxydiphenylchlorosilane was purchased from Fluka (Ronkonkoma, NY) and was used as received. The L-threonine methyl ester hydrochloride (**7**) was purchased from Sigma Chemical Co. (St. Louis, MO) and was used as received. Glacial acetic acid, pH 7 phosphate buffer, anhydrous magnesium sulfate ($MgSO_4$) and sodium carbonate ($Na_2CO_3$) and HPLC grade acetone, chloroform ($CHCl_3$), ethyl acetate, hexanes and methanol were purchased from Fisher Scientific Co. (Rochester, NY) and were used as received. All other reagents were purchased from Fisher Scientific Co. in the highest purity grade available.

**Baccatin III (4)**[2,5]

A 250 mL 3-neck flask equipped with a thermometer and overhead stirrer was charged with 5 g (5.9 mmol) paclitaxel (**3**) and THF (58 mL) at ambient temperature. Next, pH 7 phosphate buffer (29 mL) was added, resulting in an opaque solution. A total of 875 mg (23.1 mmol, 3.95 mol equiv) sodium borohydride was added in one portion (vigorous gas evolution noted). The progress of the reaction was monitored by TLC (65:35 $CHCl_3$-MeCN, v/v). After 70 min, 5.8 mL acetone was added dropwise and stirring continued 5 min. A second 5.8 mL portion of acetone was added dropwise, stirring continued an additional 5 min then EtOAc (58 mL) and deionized water (58 mL) were added. After stirring vigorously for 10 min, the layers were separated, the aqueous phase extracted with EtOAc (4 X 75 mL) and discarded. The combined organic phases were dried 15 min over anhydrous $MgSO_4$, filtered and concentrated in vacuo to constant weight to yield crude **4**. Flash chromatographic purification over silica gel using 65:35 $CH_2Cl_2$-MeCN (v/v) as eluant returned 2.70 g (78.8%) baccatin III (**4**) as a colorless powder: $R_f$ = 0.50 (65:35 $CH_2Cl_2$-MeCN, v/v). The $^1H$ NMR spectrum ($CD_2Cl_2$) was consistent for **4**.

**7-Triethylsilylbaccatin III (6)**[2,6]

An oven-dried 50 mL 2-neck flask and stir bar were cooled to ambient temperature under a stream of dry nitrogen. The flask was charged with 2.68 g (4.6 mmol) **4** and $CH_2Cl_2$ (29 mL). The resulting clear, colorless solution was stirred 15 min in an ice water bath. Next, 1.25 g (18.3 mmol, 4.0 equiv) imidazole was added in one portion, followed by dropwise addition (neat) of 3.07 mL (18.3 mmol, 4.0 equiv) triethylchlorosilane (t-zero). The heterogeneous mixture was allowed to warm to ambient temperature. After a reaction time of 55 min, the mixture was diluted with $CH_2Cl_2$ (400 mL) , washed with deionized water (40 mL), saturated aqueous $NaHCO_3$ solution (40 mL), deionized water (40 mL) and saturated aqueous NaCl solution (40 mL). Drying 15 min over anhydrous $MgSO_4$, filtration and concentration in vacuo to

constant weight gave 3.26 g (>100%) crude **6**. Flash chromatographic purification over silica gel using 40:60 EtOAc-hexanes (v/v) returned 2.73 g (85.2%) **6** as a colorless powder: $R_f$ = 0.41 (40:60 EtOAc-hexanes, v/v). The $^1$H NMR spectrum ($CD_2Cl_2$) was consistent for **6**.

**(2S,3R)-2-Amino-3-(*tert*-Butoxydiphenylsiloxy)butyric acid methyl ester (8)[9]**

An oven-dried 2-neck flask and stir bar were cooled to ambient temperature under a stream of dry nitrogen. The flask was charged with 1.98 g (11.7 mmol) **7** and dry $CH_2Cl_2$ (50 mL). After adding 1.59 g (23.4 mmol, 2.0 equiv) imidazole in one portion and cooling 15 min in an ice water bath, 3.32 mL (12.3 mmol, 1.05 equiv) *tert*-butoxydiphenylchlorosilane was added dropwise (neat) by syringe (t-zero). The resulting mixture was stirred under nitrogen while warming to ambient temperature. After 17 h, insoluble material was removed by suction filtration, washed with fresh $CH_2Cl_2$ (2 X 19 mL) and discarded. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (2 X 19 mL), distilled water (19 mL) and saturated NaCl solution (19 mL) , dried 15 min over anhydrous $MgSO_4$, filtered and concentrated in vacuo to constant weight to yield 4.63 g (>100%) crude **8** as a viscous syrup: NMR ($CD_2Cl_2$,) $\partial$ 1.25 (d, 3H, J = 6.3 Hz, -CH(Osilyl)C$\underline{H}_3$), 1.26 (s, 9H, SiOBu$^t$), 3.29 (d, 1H, J = 2.8 Hz, -C$\underline{H}$CO$_2$CH$_3$), 3.60 (s, 3H, -CO$_2$C$\underline{H}_3$), 4.39 (dq, 1H, J = 2.8, 6.3 Hz, -CH(CO$_2$CH$_3$)C$\underline{H}$ (Osilyl)CH$_3$), 7.25-7.70 (m, 10H, aromatic).

**(2S,3R)-2-*anti*-Benzylideneamino-3-(*tert*-butoxydiphenylsiloxy)butyric acid methyl ester-*N2-$^{14}$C* (9)[9]**

Crude **8** (4.63 g, 11.7 mmol, 1.25 equiv) was dissolved in $CH_2Cl_2$ (20 mL) under an inert atmosphere of nitrogen. A solution consisting of benzaldehyde (0.559 mL, 5.5 mmol, 0.59 equiv) and benzaldehyde-*C7-$^{14}$C* (3.85 mmol, 0.41 equiv)[14] in $CH_2Cl_2$ (10 mL) was added. The ampule which contained the benzaldehyde-*C7-$^{14}$C* was rinsed forward with $CH_2Cl_2$ (4 X 5 mL). The solution was stirred 5 min and an aliquot (0.2 mL) removed for NMR analysis (0.5 mL $CD_2Cl_2$). Next, 6.8 g (150 weight % of the theoretical yield for **8**) 4A sieves (oven-dried at 105°C and cooled to ambient temperature under nitrogen just prior to use) were added. Progress of the reaction was monitored by NMR. After 21 h, the 4A sieves were removed by filtration through a 5µm nylon filter (47 mm) into a 250 mL 3-neck flask, washed with fresh $CH_2Cl_2$ (3 X 15 mL) and discarded. The resulting clear, light yellow filtrate containing **9** was used immediately. NMR ($CH_2Cl_2$ / $CD_2Cl_2$, partial spectrum) $\partial$ 3.70 (s, 3H, -CO$_2$C$\underline{H}_3$), 4.00 (d, 1H, J = 6.8 Hz,-C$\underline{H}$(CO$_2$CH$_3$)), 4.46 (m, 1H, -C$\underline{H}$(Osilyl)CH$_3$), 8.27 (s, 1H, -N=C$\underline{H}$Ph).

**(2S, 3R, 3'R, 4'S)-2-(*cis*-3'-Acetoxy-2'-oxo-4'-phenylazetidin-1'-yl)-3-*tert*-butoxydiphenylsiloxy)butyric acid methyl ester-*C4'-$^{14}$C* (10) and (2S,3R,3'S,4'R)-2-(*cis*-3'-acetoxy-2'-oxo-4'-phenylazetidin-1'-yl)-3-(*tert*-butoxydiphenylsiloxy)butyric acid methyl ester-*C4'-$^{14}$C* (11)[9]**

The $CH_2Cl_2$ solution containing **9** was cooled to -40 to -45°C (reaction temperature) under an inert atmosphere of nitrogen. With good stirring, 1.64 mL (11.8 mmol, 1.26 equiv based on total benzaldehyde charge) triethylamine was added dropwise, followed by dropwise addition of 1.51 mL (14.0 mmol, 1.5 equiv based on total benzaldehyde charge) acetoxyacetyl chloride. An exotherm from -43 to -37°C during the acid chloride addition was noted. The solution was allowed to slowly warm to ambient temperature. Progress of the reaction was monitored by NMR (0.2 mL reaction aliquot / 0.5 mL $CD_2Cl_2$). After 4 h, no starting **9** was detected (NMR). After 5 h, the solution was diluted with fresh $CH_2Cl_2$ (62 mL), washed with saturated aqueous $NaHCO_3$ solution (19 mL), distilled water (19 mL) and saturated NaCl solution (19 mL), dried 15 min over anhydrous $MgSO_4$, filtered and concentrated in vacuo to constant weight to afford 6.21 g (>100%) crude **10/11**. Gradient flash chromatographic purification over silica gel ($CH_2Cl_2$, then 20:80 EtOAc-hexanes, v/v) returned 3.80 g (57%) **10/11** in a 92:8 ratio of diastereomers, respectively, as a colorless, sticky foam: $R_f$ 0.23 (silica, 20:80 EtOAc-hexanes, v/v); NMR ($CD_2Cl_2$) **1 0** (major diastereomer, partial spectrum) ∂ 1.08 (d, 3H, J = 6.4 Hz, -CH(Osilyl)C$\underline{H}_3$), 1.15 (s, 9H, -SiOBu$^t$), 3.67 (s, 3H, -$CO_2C\underline{H}_3$), 4.35 (d, 1H, J = 3.7 Hz, -C$\underline{H}$($CO_2CH_3$)), 4.57 (m, 1H, -C$\underline{H}$(Osilyl)$CH_3$), 5.34 (d, 1H, J = 5.2 Hz, C3 ß-lactam H), 5.84 (d, 1H, J = 5.2 Hz, C4 ß-lactam H); **11** (minor diastereomer, partial spectrum) ∂ 4.96 (d, 1H, J = 4.9 Hz, C3 ß-lactam H), 5.65 (d, 1H, J = 4.9 Hz, C4 ß-lactam H).

**(2S,3R,3'R,4'S)-2-(*cis*-3'-Acetoxy-2'-oxo-4'-phenylazetidin-1'-yl)-3-hydroxybutyric acid methyl ester-*C4'-$^{14}$C* (12)[9]**

To a solution of 3.80 g (6.6 mmol, 1.0 equiv) **10/11** in dry THF (34 mL) at ambient temperature under an inert atmosphere of nitrogen was added 2.37 mL (41.4 mmol, 6.3 equiv) glacial acetic acid followed by 19.7 mL (19.7 mmol, 3.0 equiv) 1 *M* tetra-*n*-butylammonium fluoride / THF solution with good stirring. After 2 h the reaction was complete (TLC). The solution was diluted with EtOAc (125 mL), washed with saturated aqueous $NaHCO_3$ solution (31 mL), dried 15 min over anhydrous $MgSO_4$, filtered and concentrated in vacuo for 1 h to yield 5.44 g (>100%) crude **12**. An immediate gradient flash chromatographic purification over silica gel (10:90 EtOAc-hexanes, then 20:80 EtOAc-hexanes, then 40:60 EtOAc-hexanes, v/v) returned 1.60 g (75%) **12** as a colorless, highly crystalline solid: $R_f$ 0.29 (silica, 40:60 EtOAc-hexanes, v/v); NMR ($CD_2Cl_2$) ∂1.18 (d, 3H, J = 6.6 Hz, -CH(Osilyl)C$\underline{H}_3$), 1.73 (s, 3H, -OCOC$\underline{H}_3$),

2.96 (d, 1H, J = 8.7 Hz, -OH), 3.69 (s, 3H, -$CO_2C\underline{H}_3$), 3.96 (d, 1H, J = 4.7 Hz, -$C\underline{H}(CO_2CH_3)$), 4.24 (m, 1H, -$C\underline{H}(OH)CH_3$), 5.06 (d, 1H, J = 4.8 Hz, C3 ß-lactam H), 5.87 (d, 1H, J = 4.8 Hz, C4 ß-lactam H), 7.30-7.45 (m, 5H, aromatic).

**(3'R,4'S)-2-(*cis*-3'-Acetoxy-2'-oxo-4'-phenylazetidin-1'-yl)but-2-enoic acid methyl ester-*C4'-$^{14}$C* (13)[9]**

An oven-dried 100 mL 2-neck flask and stir bar were cooled to ambient temperature under a stream of dry nitrogen. The flask was charged with 1.60 g (4.95 mmol, 1.0 equiv) **12** and dry $CH_2Cl_2$ (62 mL). After stirring 15 min under nitrogen in an isopropanol-dry ice cooled bath, 0.39 mL (5.0 mmol, 1.01 equiv) methanesulfonyl chloride was added dropwise (neat), followed by dropwise addition of 1.38 mL (9.9 mmol, 2.0 equiv) of triethylamine (t-zero). The cooling bath was removed and the solution allowed to slowly warm to ambient temperature. Progress of the reaction was monitored by TLC. At 30 min (reaction at 18°C), the flask was plunged into an ice water bath while awaiting the TLC result. At 45 min, an additional 0.39 mL (5.0 mmol, 1.01 equiv) methanesulfonyl chloride and 1.38 mL (9.9 mmol, 2.0 equiv) triethylamine were added, and the reaction was allowed to warm to ambient temperature. After 2 h, the solution was diluted with EtOAc (61 mL), washed with distilled water (11 mL), saturated aqueous $NaHCO_3$ solution (11 mL), 0.1 *N* aqueous HCl (11 mL), saturated aqueous $NaHCO_3$ solution (11 mL), distilled water (11 mL) and saturated aqueous NaCl solution (11 mL). Drying 15 min over anhydrous $MgSO_4$, filtration and concentration in vacuo to constant weight gave 1.62 g (>100%) crude **13** as a clear orange oil that was used immediately without further purification: $R_f$ 0.61 (silica, 40:60 EtOAc-hexanes, v/v); NMR ($CD_2Cl_2$) ∂ 1.72 (s, 3H, -OCOC$\underline{H}_3$), 2.02 (d, 3H, J = 7.4 Hz, =CHC$\underline{H}_3$), 3.72 (s, 3H, -$CO_2C\underline{H}_3$), 5.64 (d, 1H, J = 5.0 Hz, C3 ß-lactam H), 5.91 (d, 1H, J = 5.0 Hz, C4 ß-lactam H), 6.89 (q, 1H, J = 7.4 Hz, =C$\underline{H}CH_3$), 7.26-7.37 (m, 5H, aromatic).

**(3R,4S)-(*cis*-3-Acetoxy-2-oxo-4-phenylazetidin-1-yl)oxoacetic acid methyl ester-*C4-$^{14}$C* (14)[9]**

A solution of 1.62 g (1.51 g ,4.95 mmol (theory), 1.0 equiv) crude **13** in $CH_2Cl_2$ (170 mL) was ozonized at low temperature (-78°C) to a persistent pale blue solution. Excess ozone was removed by degassing the solution with nitrogen for 15 min. To the clear, colorless solution was added 9.1 mL (124 mmol, 25 equiv) dimethyl sulfide followed by warming to ambient temperature. Concentration in vacuo to constant weight provided 1.49 g (>100%) **14** as a light beige colored, amorphous solid: NMR ($CD_2Cl_2$) ∂ 1.70 (s, 3H,-OCOC$\underline{H}_3$), 3.89 (s, 3H, -$CO_2C\underline{H}_3$), 5.55 (d, 1H, J = 6.1 Hz, C3 ß-lactam H), 6.03 (d, 1H, J = 6.1 Hz, C4 ß-lactam H), 7.25-7.28 (m, 2H, aromatic), 7.38-7.41 (m, 3H, aromatic).

**(3R,4S)-Acetic acid *cis*-2-oxo-4-phenylazetidin-1-yl ester-*C4-$^{14}$C* (15)**

After cooling a solution of 407 mg (1.40 mmol, 1.0 equiv) **14** in dry THF (8.5 mL) to -78°C under an inert atmosphere of nitrogen, 0.68 mL (14.0 mmol, 10 equiv) hydrazine hydrate was added dropwise by microsyringe with good stirring. After 30 min, additional THF (2 mL) was added to facilitate stirring of the gummy, heterogeneous mixture. After 1 h, the mixture was partitioned between EtOAc and water. The organic phase was washed with water (2X) and saturated aqueous NaCl solution, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to constant weight to afford 196 mg (68%) **15** as a colorless powder: $R_f$ 0.84 (silica, EtOAc); NMR ($d_6$-DMSO) ∂1.62 (s, 3H, -OCOC$\underline{H}_3$), 4.98 (d, 1H, J = 4.9 Hz, C3 ß-lactam H), 5.80 (d, 1H, J = 4.9 Hz, C4 ß-lactam H), 7.18 - 7.39 (m, 5H, aromatic), 8.94 (s, 1H, NH).

**(3R,4S)-*cis*-3-Hydroxy-4-phenylazetidin-2-one-*C4-$^{14}$C* (16) from 15**

To a solution of 194 mg (0.95 mmol, 1.0 equiv) **15** in methanol (10 mL) was added saturated aqueous $NaHCO_3$ solution (10 mL) followed by 50 mg (0.47 mmol, 0.5 equiv) anhydrous $Na_2CO_3$ with good stirring. The progress of the reaction was monitored by TLC (EtOAc, $I_2$ chamber development). After 5 h, insoluble material was removed by suction filtration, washed with methanol (4X) and discarded. The clear, colorless filtrate was concentrated *in vacuo* to constant weight. The resulting solid was stirred in MeCN at ambient temperature. Insoluble material was removed by suction filtration, washed with MeCN (2X) and discarded. The clear, colorless filtrate was concentrated *in vacuo* to constant weight to yield 128 mg (83%) **16**[15] as a colorless solid: $R_f$ 0.60 (silica, EtOAc, $I_2$ chamber development); NMR ($d_6$-DMSO) ∂4.70 (d, 1H, J = 4.8 Hz, C3 ß-lactam H), 4.94 (dd, 1H, J = 1.9, 4.8 Hz, C4 ß-lactam H), 5.95 (br s, 1H, NH), 7.18-7.73 (m, 5H, aromatic), 8.49 (br s, 1H, OH).

**(3R,4S)-*cis*-3-Hydroxy-4-phenylazetidin-2-one-*C4-$^{14}$C* (16) from 14[9]**

To a solution of 1.49 g (1.44 g, 4.95 mmol (theory)) crude **14** in methanol (8 mL) was added saturated aqueous $NaHCO_3$ solution (8 mL) and 53 mg (0.5 mmol, 0.1 equiv) anhydrous $Na_2CO_3$. The resulting heterogeneous mixture was stirred at ambient temperature while monitoring reaction progress by TLC (EtOAc, $I_2$ chamber development). After 3.5 h, insoluble material was removed by suction filtration, washed with methanol (5 X 25 mL) and discarded. The filtrate was further clarified by passing through a 5µm nylon filter (47 mm) under suction. Concentration of the filtrate in vacuo to constant weight gave 1.71 g (>100%) **14** as a colorless solid which was used immediately without further purification: $R_f$ and NMR data as for the previous procedure.

**(3R,4S)-*cis*-3-*O*-(Triethylsilyl)-4-phenylazetidin-2-one-*C4-$^{14}$C* (17)**[2,7,8,11-13]

To the flask containing crude **16** (1.71 g, 10.4 mmol based on 100% purity) was added dry THF (55 mL) under an inert atmosphere of nitrogen. After stirring the heterogeneous mixture 15 min in an ice water bath, 705 mg (10.4 mmol, 1.0 equiv) imidazole was added in one portion, followed by dropwise addition (neat) of 1.74 mL (10.4 mmol, 1.0 equiv) triethylchlorosilane (t-zero). The reaction was allowed to warm to ambient temperature. Reaction progress was monitored by TLC (50:50 EtOAc-hexanes, v/v; iodine chamber development). After 45 min, solids were removed by suction filtration, washed with EtOAc (3 X 90 mL) and discarded. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (2 X 35 mL), deionized water (2 X 35 mL) and saturated aqueous NaCl solution (35 mL). Drying 15 min over anhydrous $MgSO_4$, filtration and concentration in vacuo to constant weight gave 1.77 g (>100%) **17**. Since the NMR spectrum showed contamination of the crude product with ca. 20 mol% starting **16**, the triethylsilylation reaction was repeated. Flash chromatographic purification over silica gel using 25:75 EtOAc-hexanes (v/v) as eluant returned 1.01 g (73% overall yield from **12**) **17** as a colorless, crystalline solid: $R_f$ = 0.71 (50:50 EtOAc-hexanes, v/v); $^1$H NMR ($CD_2Cl_2$) ∂ 0.43 (m, 6H, -SiC$\underline{H}_2$CH$_3$), 0.76 (t, 9H, J = 7.9 Hz, -SiCH$_2$C$\underline{H}_3$), 4.79 (d, 1H, J = 4.8 Hz, C3 ß-lactam H), 5.06 (dd, 1H, J = 2.7, 4.8 Hz, C4 ß-lactam H), 6.26 (br s, 1H, NH), 7.28-7.38 (m, 5H, aromatic).

**(3R,4S)-*cis*-1-Benzoyl-3-*O*-(triethylsilyl)-4-phenylazetidin-2-one-*C4-$^{14}$C* (18)**[2,7,8]

An oven-dried 3-neck flask and stir bar were cooled to ambient temperature under a stream of dry nitrogen. The flask was charged with 1.00 g (3.59 mmol, 1.0 equiv) **17** and $CH_2Cl_2$ (20 mL). The resulting clear, colorless solution was cooled 15 min in an ice water bath with good stirring. Next, 0.69 mL (3.95 mmol, 1.1 equiv) *N,N*-diisopropylethylamine was added dropwise by microsyringe, followed by addition of 0.46 mL (3.95 mmol, 1.1 equiv) benzoyl chloride and 88 mg (0.72 mmol, 0.2 equiv) 4-dimethylaminopyridine (t-zero). The solution was warmed to ambient temperature and reaction progress was monitored by TLC (30:70 EtOAc-hexanes, v/v). After 2.25 h, the clear, light yellow solution was diluted with $CH_2Cl_2$ (95 mL), washed with saturated aqueous $NaHCO_3$ solution (2 X 10 mL), deionized water (2 X 10 mL) and saturated aqueous NaCl solution (10 mL). The organic phase was dried 15 min over anhydrous $MgSO_4$ and filtered to afford a clear, light yellow solution of **18** in $CH_2Cl_2$ (volume ca. 225 mL). This solution was concentrated in vacuo at 30°C (bath temperature) to a residue which was *immediately* redissolved in $CH_2Cl_2$ (15 mL) and applied to a flash chromatography column (Aldrich, 50 mm i.d.; 6" silica). Elution with $CH_2Cl_2$ was monitored by TLC ($CH_2Cl_2$). The appropriate fractions were pooled, concentrated and dried in vacuo for 1 h to yield 1.13 g (82.2%) **18** as a light yellow oil: $R_f$ = 0.74 (30:70

EtOAc-hexanes, v/v); NMR ($CD_2Cl_2$) ∂ 0.48 (m, 6H, -SiC$\underline{H}_2$CH$_3$), 0.79 (t, 9H, J = 7.9 Hz, -SiCH$_2$C$\underline{H}_3$), 5.16 (d, 1H, J = 6.1 Hz, C3 ß-lactam H), 5.39 (d, 1H, J = 6.1 Hz, C4 ß-lactam H), 7.32-7.39 (m, 5H, aromatic), 7.50 (m, 2H, aromatic), 7.62 (m, 1H, aromatic), 7.97 (m, 2H, aromatic).

**2',7-Bis(triethylsilyl)taxol-*C3'-$^{14}$C* (20)[2,7,8]**

An oven-dried 50 mL 2-neck flask and stir bar were cooled to ambient temperature under a stream of dry nitrogen. The flask was charged with 1.37 g (1.96 mmol) **6** and dry THF (12 mL). Stirring for 10 min produced a clear, colorless solution which was then cooled 15 min in a dry ice - isopropanol bath maintained at -40 to -50°C. Next, 1.25 mL (2.06 mmol, 1.05 equiv) 1.64*M* *n*-BuLi/hexanes (freshly titrated) was added dropwise over ca. 2.5 min. The resulting clear, light yellow solution was stirred at -40 to -45°C (bath temperature) for an additional 30 min. A solution of 1.13 g (2.64 mmol (based on 90 mol% purity (NMR)), 1.35 equiv) **18** in THF (2 mL) was added dropwise. The flask which contained **18** was rinsed forward with THF (2 X 2 mL). Following the addition, the flask was plunged into an ice water bath (t-zero). Reaction progress was monitored by TLC (30:70 EtOAc-hexanes, v/v). After 90 min, the clear yellow solution was quenched into saturated aqueous $NH_4Cl$ solution (35 mL). The reaction flask was rinsed forward with EtOAc (4 X 50 mL). The phases were separated, the aqueous phase extracted with EtOAc (2 X 100 mL) and discarded. The combined organic phases were dried 15 min over anhydrous $MgSO_4$, filtered and concentrated in vacuo to constant weight to afford 2.40 g (>100%) crude **20**. Flash chromatographic purification over silica gel using 25:75 EtOAc-hexanes (v/v) as eluant returned 1.97 g (92.6% ) **20** as a colorless foam: $R_f$ = 0.65 (30:70 EtOAc-hexanes, v/v). The $^1$H NMR spectrum ($CD_2Cl_2$) was consistent for **20**.

**Paclitaxel-*C3'-$^{14}$C* (2)[2]**

To the flask containing 1.97 g (1.81 mmol) **20** was added MeCN (100 mL). The resulting solution was stirred 15 min in a dry ice - isopropanol bath maintained at -5 to -10°C. Next, 1.36 mL (8.16 mmol, 4.5 equiv) 6*M* aqueous HCl was added dropwise. The progress of the reaction was monitored by TLC (40:60 EtOAc-hexanes, v/v). Within 5 min, complete conversion to 7-triethylsilyltaxol-*C3'-$^{14}$C* **21** ($R_f$ of 0.36) was noted.[2] After 4 h, the mixture was diluted with EtOAc (975 mL) and washed with deionized water (95 mL), saturated aqueous $NaHCO_3$ solution (95 mL), deionized water (95 mL) and saturated aqueous NaCl solution (95 mL). Drying 15 min over anhydrous $MgSO_4$, filtration and drying to constant weight in vacuo gave 1.88 g (>100%) crude **2**. Flash chromatographic purification over silica gel using 40:60 acetone-hexanes (v/v) as eluant returned 1.12 g (72.3%) paclitaxel-*C3'-$^{14}$C* (**2**) as a colorless powder: $R_f$ = 0.15 (50:50 EtOAc-hexanes, v/v). The specific activity was found to be 16.4 mCi/mmol while the radiochemical

purity was measured at 96%. The $^1$H NMR spectrum ($CD_2Cl_2$), TLC $R_f$ and chromatographic behavior were consistent with that of natural paclitaxel (**3**).[2,4]

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REFERENCES AND NOTES

1. Present address: The R.W. Johnson Pharmaceutical Research Institute, Welsh & McKean Roads, Spring House, PA 19477-0776.
2. Walker D.G., Swigor J.E., Kant J. and Schroeder D.R. - J. Labelled Cpds. and Radiopharm. 34: 973 (1994).
3. Chen S.H., Dischino D.D., Farina V., Hauck S.I., Walker D.G. and Wong H. - J. Labelled Cpds. and Radiopharm., to be submitted.
4. Wani M.C., Taylor H.L., Wall M.E., Coggon P. and McPhail A.T. - J. Am. Chem. Soc. 93: 2325 (1971).
5. Magri N.F., Kingston D.G.I., Jitrangsri C. and Picariello T. - J. Org. Chem. 51: 3239 (1986).
6. Denis J.-N., Greene A.E., Guénard D., Guéritte-Voegelein F., Mangatal L. and Potier P. - J. Am. Chem. Soc. 110: 5917 (1988).
7. Holton R.A. - PCT Int. Appl. WO9306079 (1993) (Chem. Abst. 119: 139574j).
8. Holton R.A. - US 5229526 (1993).
9. Farina V., Hauck S.I. and Walker D.G. - Synlett, 761 (1992).
10. Following its successful preparation from **7**, a much simpler method for synthesis of **17** was reported (see reference 11). It is suggested that future preparations of carbon-14-labeled **17** be approached either in this manner, or by adapting chemistry used to prepare the corresponding 3-*O*-triisopropyl analog of **17** (see references 12, 13).
11. Georg G.I., Cheruvallath Z.S., Harriman G.C.B., Hepperle M. and Park H. - Bioorg. Med. Chem. Lett. 3: 2467 (1993).
12. Ojima I., Habus I., Zhao M., Georg G.I. and Jayasinghe L.R. - J. Org. Chem. 56: 1681 (1991).

13. Ojima I., Habus I., Zhao M., Zucco M., Park Y.H., Sun C.M. and Brigaud T. - Tetrahedron 48: 6985 (1992).

14. Moravek Biochemicals Inc. (Brea, CA), 52 mCi/mmol; supplied as a 200 mCi solution in 10 mL dichloromethane.

15. Patel R.N., Banerjee A., Ko R.Y., Howell J.M., Li W-S., Comezoglu F.T., Partyka R.A. and Szarka L. - Biotechnol. Appl. Biochem. 20: 23 (1994).