SYNTHESIS OF CARBON-14 LABELED TAXOL® (PACLITAXEL)

Donald G. Walker,[1] John E. Swigor,§,* Joydeep Kant¶ and Daniel R. Schroeder¶

§Bristol-Myers Squibb Co., Pharmaceutical Research Institute, P.O. Box 4755, Syracuse, NY 13221

¶Bristol-Myers Squibb Co., Pharmaceutical Research Institute, P.O. Box 5100, Wallingford, CT 06492

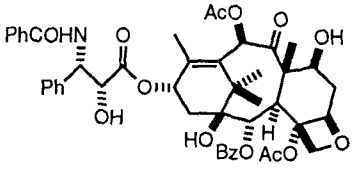
SUMMARY

Reductive cleavage of the C13 side chain of Taxol® (**1**, paclitaxel) followed by regioselective silylation gave 7-triethylsilylbaccatin III (**4**). *3-O-Triethylsilylation of* **5** *and subsequent reaction with benzoyl* chloride-*C7-^{14}C* gave azetidinone **7**. Coupling of **4** and **7** followed by deprotection gave 1.26 g of Taxol®-*N3'-^{14}C* (**11**) having a specific activity of 26.5 mCi/mmol and a radiochemical purity of 95%.

Key words: Taxol®, paclitaxel, carbon-14

INTRODUCTION

The diterpenoid Taxol® (**1**, paclitaxel)[2] continues to show promise as an effective anticancer agent.[3-8] A number of researchers have reported preparations of various tritium-labeled taxols[9-11] which were used for a variety of pharmacological studies. Other reports have described the *de novo* synthesis of sub-milligram amounts of radiolabeled Taxol® (*^{3}H*, *^{14}C* or dual labeled) from cell[12,13] or fungus[14] cultures using appropriately labeled precursors. However, the preparation of a specifically labeled carbon-14 Taxol® in synthetically useful quantities remains unreported. We required such a labeled Taxol® to support ongoing pharmacokinetic investigations and for use as a starting material for carbon-14-labeled prodrugs of Taxol®.[15] In this paper we report the synthesis of gram quantities of Taxol®-*N3'-^{14}C* (**11**).



1

*: author to whom correspondence should be addressed

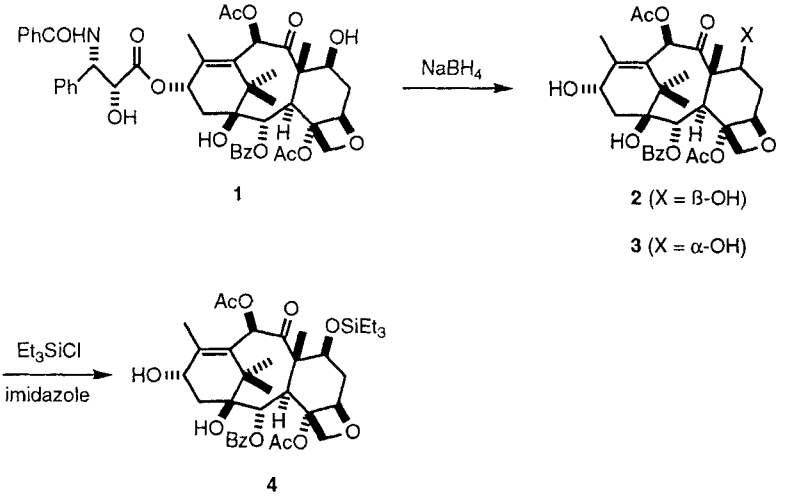
CCC 0362-4803/94/100973-08

Received 6 May, 1994
Revised 10 May, 1994

RESULTS AND DISCUSSION

The requisite taxane component **4** was derived from Taxol® (**1**)[2] by modified literature procedures (Scheme 1). Reductive cleavage of the C13 side chain[16] was accomplished on reacting **1** with sodium borohydride in tetrahydrofuran (THF) / pH 7 phosphate buffer. Yields of baccatin III (**2**)[16] were generally 75-80% after chromatographic purification, which removed minor amounts of 7-*epi*-baccatin III (**3**) produced under these conditions. Regioselective 7-*O*-triethylsilylation of **2**[17] by reaction with excess triethylchlorosilane and imidazole in dichloromethane afforded gram quantities of 7-triethylsilylbaccatin III (**4**)[17] after chromatographic purification.

Scheme 1



1

2 (X = ß-OH)

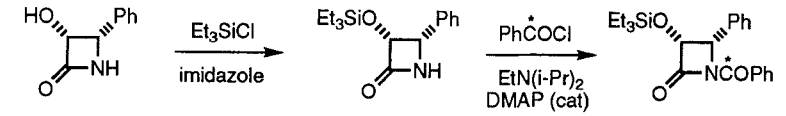
3 (X = α-OH)

4

The C13 side chain synthon **7**[18-21] was assembled as shown in Scheme 2. 3-*O*-Triethylsilylation of **5**[22] *proceeded* smoothly in near quantitative yield to afford **6**, which was sufficiently pure for conversion to azetidinone **7**. Thus, *N*-benzoylation of **6** was observed on reaction with benzoyl chloride-*C7-*^{14}*C*,[23] *N,N*-diisopropylethylamine and a catalytic amount of 4-dimethylaminopyridine in dichloromethane. Care *must* be taken not to store crude **7** *in vacuo* at ambient temperature, since rapid conversion to a mixture of **8** and unidentifiable decomposition product(s) occurs. Yields consistently in the range of 80-85% (**5** -> **7**) were observed when the process stream containing **7** was concentrated to near dryness, redissolved in minimal dichloromethane and *immediately* purified by flash chromatography over silica gel. Under these conditions, formation of **8** and decomposition were suppressed.

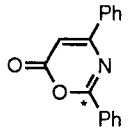
Coupling[18,19] of the lithium alkoxide of **4** with **7** (1.35 equiv) in THF gave 2',7-bis(triethylsilyl)taxol-*N3'-*^{14}*C* (**9**) in good yield after chromatographic purification (Scheme 3). Hydrolysis of the triethylsilyl protecting

Scheme 2



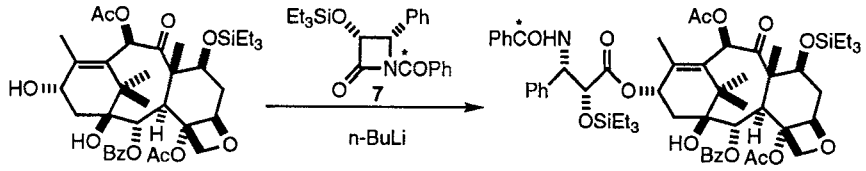
5 6 7

*: denotes position of carbon-14 label

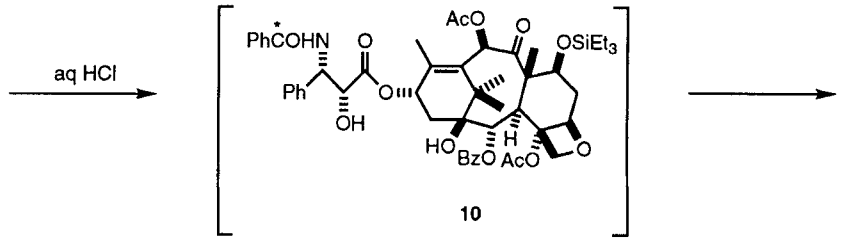


8

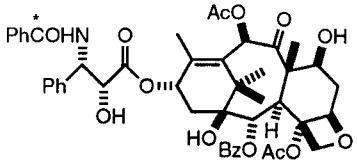
Scheme 3



4 9



10



11

*: denotes position of carbon-14 label

groups resulted on exposure of **9** to 6*M* aqueous HCl (4.5 equiv) in acetonitrile (MeCN) at -5°C for 3-4h. This conversion proceeded through the intermediacy of 7-triethylsilyltaxol-*N3'-14C* (**10**), to which **9** was completely converted within 5 min (TLC, NMR). Slow conversion of **10** to **11** was monitored by TLC. After chromatographic purification, 1.26 g of Taxol®-*N3'-14C* (**11**) was isolated. The NMR spectrum, TLC R_f and chromatographic behavior of **11** were consistent with a sample of natural Taxol® (**1**).[2] This material had a specific activity of 26.5 mCi/mmol and a radiochemical purity of 95%, which were sufficient for our studies.[15]

The overall chemical yield for the conversion of **5** to **11** was 57% while the radiochemical yield of the process was 36%.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker AM360 spectrometer. Chemical shifts are expressed on the ∂ 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 ß-RAM system. Benzoyl chloride-*C7-14C* was purchased as a solution in dichloromethane from Moravek Biochemicals, Inc. (Brea, CA) and was used as received. Anhydrous tetrahydrofuran (THF), dichloromethane (CH_2Cl_2) and acetonitrile (MeCN), imidazole, triethylchlorosilane, sodium borohydride, *N,N*-diispropylethylamine, 4-dimethylaminopyridine, n-BuLi/hexanes solution and benzoyl chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI) in the highest purity available and were used as received. Anhydrous $MgSO_4$, HPLC grade ethyl acetate (EtOAc), hexanes, acetone and chloroform ($CHCl_3$) and pH 7 phosphate buffer 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 (2)[16]

A 250 mL 3-neck flask equipped with a thermometer and overhead stirrer was charged with 5 g (5.9 mmol) Taxol® (**1**) 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, acetone (5.8 mL) was added dropwise and stirring continued 5

min. A second portion of acetone (5.8 mL) 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 **2**. 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 (**2**) 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 **2**.

7-Triethylsilylbaccatin III (4)[17]

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) **2** 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 **4**. Flash chromatographic purification over silica gel using 40:60 EtOAc-hexanes (v/v) returned 2.73 g (85.2%) **4** as a colorless powder: R_f = 0.41 (40:60 EtOAc-hexanes, v/v). The ^1H NMR spectrum (CD_2Cl_2) was consistent for **4**.

(3R,4S)-*cis*-3-*O*-(Triethylsilyl)-4-phenylazetidin-2-one (6)[18-21]

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 571 mg (3.5 mmol) **5**[22] and dry THF (18 mL) at ambient temperature. Stirring for 10 min produced a clear, colorless solution. After cooling in an ice water bath for 15 min, 238 mg (3.5 mmol, 1.0 equiv) imidazole was added in one portion, followed by dropwise addition (neat) of 0.59 mL (3.5 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, an additional 24 mg (0.35 mmol, 0.1 equiv) imidazole and 59 µL (0.35 mmol, 0.1 equiv) triethylchlorosilane were added. After 2.3 h, solids were removed by suction filtration, washed with EtOAc (3 X 24 mL) and discarded. The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (2 X 10 mL) and deionized water (2 X 10 mL), dried 15 min over anhydrous

$MgSO_4$, filtered and concentrated *in vacuo* to constant weight to give 965 mg (99.4%) **6** as a colorless solid: R_f = 0.71 (50:50 EtOAc-hexanes, v/v); ^1H 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-H), 5.06 (dd, 1H, J = 2.7, 4.8 Hz, C4-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-*N1*-^{14}*C* (7)[18,19]

Crude **6** (965 mg, 3.5 mmol) was dissolved in CH_2Cl_2 (8 mL) under an inert atmosphere of nitrogen. The resulting clear, colorless solution was cooled 15 min in an ice water bath with good stirring. Next, 0.67 mL (3.85 mmol, 1.1 equiv) *N,N*-diisopropylethylamine was added dropwise by microsyringe, followed by addition of a solution composed of benzoyl chloride-*C7*-^{14}*C* (2.12 mmol, 0.61 equiv)[23] and 0.20 mL (1.74 mmol, 0.50 equiv) benzoyl chloride in CH_2Cl_2 (10 mL). The ampule which contained the benzoyl chloride-*7*-^{14}*C* was rinsed forward with CH_2Cl_2 (3 X 2 mL). Next, 85 mg (0.7 mmol, 0.2 equiv) 4-dimethylaminopyridine was added, and the solution was warmed to ambient temperature (t-zero). The progress of the reaction was monitored by TLC (30:70 EtOAc-hexanes, v/v). After 2.3 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 **7** 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 (84.8%) **7** 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-H), 5.39 (d, 1H, J = 6.1 Hz, C4-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-*N3'*-^{14}*C* (9)[18,19]

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 1.53 g (2.18 mmol) **4** 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, 1.44 mL (2.29 mmol, 1.05 equiv) 1.59*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 (2.95 mmol, 1.35 equiv) **7** in THF (2 mL) was added dropwise. The flask which contained **7** 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 75 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 2.57 g (>100%) crude **9**. Flash chromatographic purification over silica gel using 25:75 EtOAc-hexanes (v/v) as eluant returned 1.91 g (80.8%) **9** as a colorless foam: R_f = 0.65 (30:70 EtOAc-hexanes, v/v). The ^1H NMR spectrum (CD_2Cl_2) was consistent for **9**.

Taxol®-*N3'-^{14}C* (11)

To the flask containing 1.91 g (1.76 mmol) **9** 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, 1.32 mL (7.92 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-*N3'-^{14}C* (**10**, R_f = 0.36) was noted. After 3.25 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 1.79 g (>100%) crude **11**. Flash chromatographic purification over silica gel using 40:60 acetone-hexanes (v/v) as eluant returned 1.26 g (83.6%) Taxol®-*N3'-^{14}C* (**11**) as a colorless powder: R_f = 0.15 (50:50 EtOAc-hexanes, v/v). The specific activity was found to be 26.5 mCi/mmol while the radiochemical purity was measured at 95%. The ^1H NMR spectrum (CD_2Cl_2), TLC R_f and chromatographic behavior were consistent with that of natural Taxol® (**1**).[2]

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Bill Winter for supplying a sample of chiral, non-racemic azetidinone **5**. We are also indebted to Dr. George Crull, Bud Floor, Betty Noga and Priscilla Richberg for supporting analytical work. Finally, we thank Dr. Robert Holton of Florida State University for supplying details of his coupling procedure prior to publication.

REFERENCES AND NOTES

1. Present address: The R.W. Johnson Pharmaceutical Research Institute, Welsh & McKean Roads, Spring House, PA 19477.
2. Wani M.C., Taylor H.L., Wall M.E., Coggon P. and McPhail A.T. - J. Am. Chem. Soc. 93: 2325 (1971).
3. Rowinsky E.K., Cazenave L.A. and Donehower R.C. - J. Natl. Cancer Inst. 82: 1247 (1990).
4. Slichenmeyer W.J. and VanHoff D.D. - Anti-Cancer Drugs 2: 519 (1991).
5. Rose W.C. - Anti-Cancer Drugs 3: 311 (1992).
6. Rowinsky E.K., Onetto N., Canetta R.M. and Arbuck S.G. - Semin. Oncol. 19: 646 (1992).
7. Vanhaelen M.H. - J. Pharm. Belg. 47: 417 (1992).
8. Lenaz L. and DeFuria M.D. - Fitoterapia 64(1, Suppl.): 27 (1993).
9. Parnes J. and Horwitz S.B. - J. Biol. Chem. 91: 479 (1981).
10. Chenu J., Takondju M., Wright M., Senilh V. and Guénard D. - J. Labelled Cpds. and Radiopharm. 24: 1245 (1987).
11. Taylor G.F., Thornton S.S., Tallent C.R. and Kepler J.A. - J. Labelled Cpds. and Radiopharm. 33: 501 (1993).
12. Zamir L.O., Nedea M.E. and Gameau F.X. - Tetrahedron Lett. 33: 5235 (1992) and references cited therein.
13. Stroebel G.A., Stierle A. and VanKuijk F.J.G.M. - Plant Sci. 84: 65 (1992).
14. Stierle A., Stroble G. and Stierle D. - Science 260: 214 (1993).
15. Chen S.H., Dischino D.D., Farina V., Hauck S.I., Walker D.G. and Wong H. - J. Labelled Cmpds. and Radiopharm., to be submitted.
16. Magri N.F., Kingston D.G.I., Jitrangsri C. and Picariello T. - J. Org. Chem. 51: 3239 (1986).
17. Denis J.-N., Greene A.E., Guénard D., Guéritte-Voegelein F., Mangatal L. and Potier P. - J. Am. Chem. Soc. 110: 5917 (1988).
18. Holton R.A. - PCT Int. Appl. WO9306079, April 1, 1993 (Chem. Abst. 119: 139574j (1993)).
19. Holton R.A. - US 5229526, July 20, 1993.
20. Ojima I., Habus, I., Zhao M., Georg G.I. and Jayasinghe L.R. - J. Org. Chem. 56: 1681 (1991).
21. Ojima I., Habus I., Zhao M., Zucco M., Park Y.H., Sun C.M. and Brigaud T. - Tetrahedron 48: 6985 (1992).
22. A chiral, non-racemic sample of **5** was kindly provided by Dr. Bill Winter of Bristol-Myers Squibb.
23. Moravek Biochemicals Inc. (Brea, CA), 52 mCi/mmol; supplied as a 200 mCi solution in 10 mL dichloromethane.