

## Synthesis of [ $^{14}\text{C}_2$ ]Taxol: Application of (+)/(-)-[ $^{13,14}\text{C}_n$ ]BABS, part 3

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### Summary

A multiple step stereoselective procedure was successfully applied to doubly C-14 labelled Taxol **1** in its pharmacologically essential (2R,3S)-N-benzoyl-3-phenylisoserine side chain. A highly stereocontrolled aldol reaction of internally developed (+)-[ $^{14}\text{C}_2$ ]BABS **4a** with benzaldehyde followed by some functional group conversions provided (3R,4S)-3-(tert-butyl-dimethyl-silyloxy)-4-phenyl-[2,3- $^{14}\text{C}_2$ ]azetidin-2-one **9a** in an overall radiochemical yield of 34%. Attachment of the activated benzoyl  $\beta$ -lactam **9b** to the baccatine skeleton **10** and subsequent deprotection provided the expected doubly labelled drug substance.

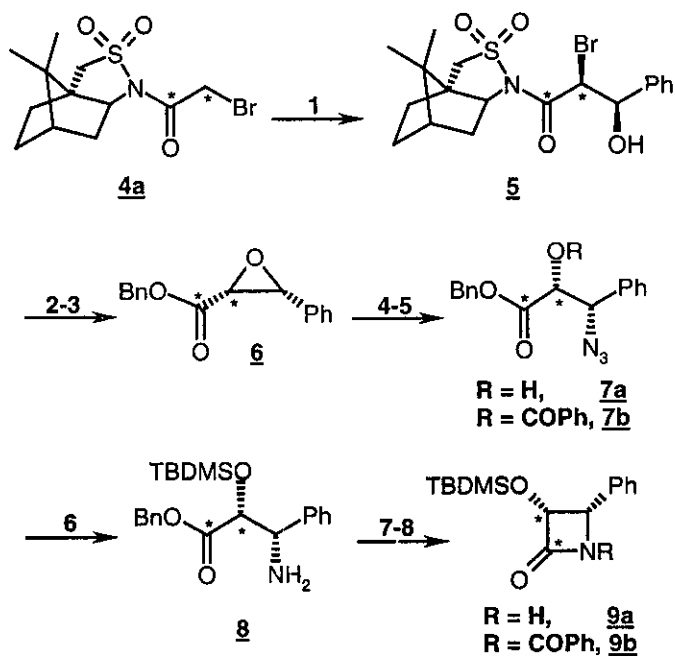
**Keywords:** (+)/(-)-[ $^{14}\text{C}_2$ ]BABS, C-14 labelled chiral synthons, simply/multiply C-14 labelled 1,2-functionalized carboxylic acids, e.p. C-14 labelled  $\beta$ -amino- $\alpha$ -hydroxycarboxylic acids, stereoselective aldol reaction, Taxol

### Introduction

The diterpenoid Taxol **1** still continues to provide significant benefits in the clinical treatment of breast and ovarian cancers in spite of several major drawbacks including toxic side effects, multidrug resistance and poor solubility in aqueous solution [1]. Taxol was originally isolated from the bark of Pacific Yew tree, *Taxus brevifolia*. Since this process was unsuitable because of low isolation yields and ecological problems [2], alternative sources as total synthetic and semisynthetic approaches were investigated and are now available [3]. Currently the drug is mainly produced from baccatine III **2** which is efficiently extracted from



**Scheme 1: (3R,4S)-3-(*tert*-Butyldimethylsilyloxy)-4-phenyl-[2,3- $^{14}\text{C}_2$ ]azetidin-2-one **9a****



**1a)**  $\text{TiCl}_4$ , 1-ethylpiperidine, dichloromethane,  $-78^\circ\text{C}$ , 5 min.; **1b)** PhCHO,  $-78^\circ\text{C}$ , 10 min.; **2)**  $\text{K}_2\text{CO}_3$ , DMF,  $\text{H}_2\text{O}$  cat., r.t.; **3)** BnOLi, THF,  $-10^\circ\text{C}$ ; **4)**  $\text{NaN}_3$ ,  $\text{HCO}_2\text{Me}$ , MeOH- $\text{H}_2\text{O}$ , 8:1,  $50^\circ\text{C}$ ; **5)** *tert*-butyldimethylsilyl triflate, 2,6-lutidine,  $\text{CH}_2\text{Cl}_2$ , r.t.; **6)**  $\text{PPh}_3$ , THF- $\text{H}_2\text{O}$  8:1,  $50^\circ\text{C}$ , 2h; **7)** *tert*-butylmagnesium chloride, ether, 2h,  $-10^\circ\text{C}$ ; **8)** PhCOCl,  $\text{Et}_3\text{N}$ , DMAP cat.,  $\text{CH}_2\text{Cl}_2$ ,  $-10^\circ\text{C}$ , 1h30 min.

Reaction of (+)-[ $^{14}\text{C}_2$ ]BABS **4a** with equimolar amounts of titanium tetrachloride and 1-ethyl piperidine ( $-78^\circ\text{C}$ , dichloromethane) followed by trapping of the resulting titanium enolate with benzaldehyde afforded the syn- $\alpha$ -bromo aldol **5** with excellent diastereomeric purity after recrystallization (78%, de > 99%).

The aldol **5** was stereospecifically and cleanly converted to the respective 2,3-cis-epoxy sultam derivative with potassium carbonate in DMF [9]. Subsequent cleavage of the auxiliary with lithium benzoxide efficiently provided the (2R,3R)-epoxy benzyl ester **6** in 74% yield over the two steps. Simultaneous cleavage of the auxiliary and epoxide formation upon treatment with 2 equiv. of lithium benzoxide proceeded with lower yield

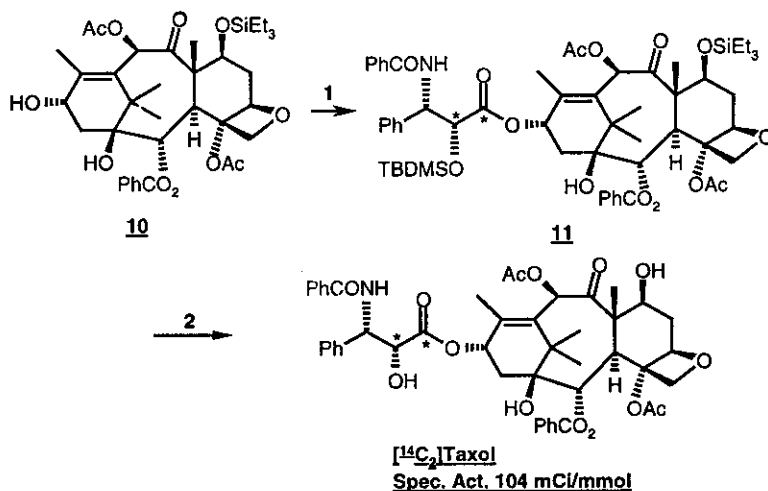
due to formation of side compounds [10]. Regioselective opening of the epoxide according to the procedures developed by Sharpless [11] provided the azide **7a** in 85% yield.

O-silylation with *tert*-butyldimethylsilyl triflate and 2,6-lutidine (95%) followed by reduction of the azide **7b** with triphenylphosphine in THF-water provided (2R,3S)-O-*tert*-butyldimethylsilyl-phenylisoserinate **8** in 95% yield.

Exposure of **8** to 2.1 equiv. of *tert*-butylmagnesium chloride in ether at  $-10^{\circ}\text{C}$  for 2h cleanly afforded the  $\beta$ -lactam **9a** in 86% yield. The synthesis of **9a** from (+)-[ $^{14}\text{C}_2$ ]BABS **4a** was achieved in 38% overall chemical yield and 34% radiochemical yield.

Attachment of **9a** to the baccatine skeleton was performed according to literature procedures [6,12]. Activation of the  $\beta$ -lactam **9a** with benzoylchloride (74%), followed by reaction of the resulting N-benzoyl  $\beta$ -lactam **9b** with the deprotonated allylic hydroxy group of 7-TES-baccatine III **10** (nBuLi,  $-40^{\circ}\text{C}$ , THF) afforded the protected taxol **11** in 67% yield. Conclusive desilylation with HF-pyridine provided doubly labelled Taxol in 79% yield and with a specific activity of 104 mCi/mmol. The identity of the labelled Taxol was confirmed by  $^1\text{H-NMR}$  spectroscopy and HPLC-comparison with commercially available unlabelled material.

### Scheme 2: Conclusive Synthesis of [ $1',2'-^{14}\text{C}_2$ ]Taxol



1a) nBuLi, THF,  $-40^{\circ}\text{C}$ ; 1b) **9b**; 2) HF-Pyridine,  $0^{\circ}\text{C}$

## Experimental Section

### General:

Chemical reagents were purchased from *Aldrich Chemical Co.* or *Fluka* and used without further purification. (+)-[ $^{14}\text{C}_2$ ]BABS was purchased from *NEN*. The identity of intermediates was confirmed by MS and  $^1\text{H-NMR}$ . The specific activity was determined by scintillation counting and confirmed by MS.

### Procedures:

#### (2R,2'S,3'R)-N-[2'-Bromo-3'-hydroxy-3'-phenyl-[1',2'- $^{14}\text{C}_2$ ]propanoyl] bornan-10,2-sultam **5**

A solution of (+)-[ $^{14}\text{C}_2$ ]BABS **4a** (250 mCi, 830 mg, 2.47 mmol) and 1-ethyl piperidine (0.48 ml, 3.45 mmol) in dichloromethane was slowly treated with titanium tetrachloride (0.32 ml, 2.96 mmol) at  $-78^\circ\text{C}$ . The resulting dark-red solution was stirred for 5 min. and then reacted with benzaldehyde (0.37 ml, 3.66 mmol). After 10 min. the reaction mixture was quenched with buffer phosphate pH7, the layers were separated, the aqueous phase extracted with ethyl acetate, and the combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. Purification by recrystallization from dichloromethane-hexane gave the syn  $\alpha$ -bromo aldol **5** as a single diastereomer (850 mg, 78%, de > 99%).

#### (2R,2'R,3'R)-N-[3'-Phenyl-2',3'-epoxy-[1',2'- $^{14}\text{C}_2$ ]propanoyl]bornan-10,2-sultam

A solution of aldol **5** (840 mg, 1.9 mmol) in dimethylformamide (7 ml) was reacted with potassium carbonate (340 mg, 2.45 mmol) and a catalytic amount of water. The resulting suspension was kept at room temperature overnight, then partitioned between ethyl acetate and water. The layers were separated, the aqueous phase once washed with ethyl acetate, the combined organic layers washed with water, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give 705 mg of crude epoxide which was used without further purification.

#### (2R,3R)-3-phenyl-[1,2- $^{14}\text{C}_2$ ]oxirane-2-carboxylic acid benzyl ester **6**

To a solution of benzyl alcohol (0.23 ml, 2.21 mmol) in THF (2 ml), kept under argon and cooled to  $-30^\circ\text{C}$ , was added n-butyllithium (1.6M, 1.35

ml, 2.16 mmol). After 15 min., the crude epoxide in THF solution (10 ml) was added and the reaction was kept below  $-10^{\circ}\text{C}$  for 2 h 30 min. Addition of acetic acid, evaporation of solvent, and purification by flash chromatography on silica gel (6:1, hexane-ethyl acetate) gave the pure epoxide **6** (350 mg, 74% over two steps).

**(2R,3S)-3-Azido-2-hydroxy-3-phenyl-[1,2- $^{14}\text{C}_2$ ]propionic acid benzyl ester **7a****

Following the general procedure of Sharpless [11] a solution of epoxide **6** (350 mg, 1.82 mmol) in methanol-water (8:1, 13.5 ml) was treated with methyl formate (1.7 ml, 27.4 mmol) and sodium azide (0.72 g, 11.04 mmol) and then stirred at  $50^{\circ}\text{C}$  for 24 h. The methanol was evaporated, the residue taken up in ethyl acetate, washed with water, dried ( $\text{Na}_2\text{SO}_4$ ), and the organic solvent evaporated. Purification of the residue by flash chromatography on silica gel (4:1, hexane-ethyl acetate) gave 350 mg (85%) of the desired hydroxy azide **7a**.

**(2R,3S)-3-Azido-2-(tert-butyldimethylsilyloxy)-3-phenyl-[1,2- $^{14}\text{C}_2$ ]propionic acid benzyl ester **7b****

tert-Butyldimethylsilyl trifluoromethanesulfonate (0.35 ml, 1.52 mmol) was added dropwise to a solution of the compound **7a** (350 mg, 1.18 mmol) and 2,6-lutidine (0.2 ml, 1.7 mmol) in dichloromethane (6 ml) at  $0^{\circ}\text{C}$ . The reaction was warmed up to r.t. and stirred for 1 h. After addition of saturated aqueous sodium bicarbonate and dilution with ethyl acetate the organic layer was separated, washed with brine, dried and evaporated. Purification of the residue by flash chromatography on silica gel (9:1, hexane-ethyl acetate) gave 460 mg (95%) of the pure compound **7b**.

**(2R,3S)-3-Amino-2-(tert-butyldimethyl-silyloxy)-3-phenyl-[1,2- $^{14}\text{C}_2$ ]propionic acid benzyl ester **8****

A solution of azide **7b** (450mg, 1.09 mmol) was reacted with triphenylphosphine (0.43 g, 1.64 mmol) and water (0.5 ml) in THF (4 ml) at  $60^{\circ}\text{C}$  for 3h. The reaction was diluted with ethyl acetate washed with brine and evaporated. Purification of the residue by flash chromatography on silica gel (3:1, hexane-ethyl acetate) gave 370 mg (95%) of pure compound **8**.

**(3R,4S)-3-(*tert*-Butyldimethylsilyloxy)-4-phenyl-[2,3- $^{14}\text{C}_2$ ]azetidin-2-one 9a**

A solution of *tert*-butylmagnesium chloride (1M THF, 2.1 ml) was dropwise added to a solution of the compound 8 (370 mg, 0.96 mmol) in ether (10 ml) kept at  $-10^\circ\text{C}$ . After 2 h at this temperature the reaction was quenched with saturated aqueous ammonium chloride and diluted with ethyl acetate. The organic layer was separated, washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. Purification of the residue by flash chromatography on silica gel (4:1, hexane-ethyl acetate) gave 230 mg (85.9 mCi, 86%) of the pure  $\beta$ -lactam 7a with a specific activity of 103 mCi/mmol.

**(3R,4S)-1-Benzoyl-3-(*tert*-butyldimethylsilyloxy)-4-phenyl-[2,3- $^{14}\text{C}_2$ ]azetidin-2-one 9b**

A solution of the azetidin-2-one 9a (120 mg, 0.43 mmol) in dichloromethane kept at  $-10^\circ\text{C}$  was consecutively treated with triethylamine (73  $\mu\text{l}$ , 0.52 mmol), benzoyl chloride (61  $\mu\text{l}$ , 0.52 mmol) and a catalytic amount of DMAP. The solution was stirred for 1 h 30 min. and concentrated in vacuo to a yellow oil which was immediately purified by flash chromatography on silica gel (6:1, hexane-ethyl acetate) and directly used for the following coupling step.

**2'-*tert*-Butyl-dimethylsilyl-7-triethylsilyl-[1',2'- $^{14}\text{C}_2$ ]Taxol 11**

Under argon, a solution of *n*-butyllithium (1.6M hexane, 150  $\mu\text{l}$ ) was added to a solution of the protected baccatine III 10 [12] (168 mg, 0.24 mmol) in THF (1.0 ml) kept at  $-50^\circ\text{C}$ . After 30 min at this temperature a solution of the azetidine-2-one 9b (120 mg, 0.31 mmol) in THF (1.5 ml) was dropwise added and the reaction mixture stirred at  $0^\circ\text{C}$  for an additional 1 h 30 min. The reaction was quenched with saturated aqueous ammonium chloride and diluted with ethyl acetate. The layers were separated, the aqueous phase extracted with ethyl acetate, and the combined organic phases were dried and evaporated. Purification of the residue by flash chromatography on silica gel (6:1  $\rightarrow$  3:1, hexane-ethyl acetate) gave 175 mg (67%) of 11.

**[1',2'- $^{14}\text{C}_2$ ]Taxol**

The silyl-protected taxol 11 (175 mg, 0.16 mmol) was dissolved in pyridine (2.5 ml) and reacted with an excess of HF-pyridine (70%, 1.3 ml)

at 0°C for 5 h. The reaction was quenched with water (5 ml) and diluted with ethyl acetate. The organic layer was separated and washed consecutively with water, 1N HCl, water, saturated aqueous sodium bicarbonate, brine and finally dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. Purification by flash chromatography on silica gel (1:1 → 2:3, hexane-ethyl acetate) gave 108 mg (79%), 13.2 mCi of chemically and radiochemically pure Taxol with a specific activity of 104.3 mCi per mmol.

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