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Synthesis of [$^{14}C_2$]Taxol: Application of (+)/(-)-[$^{13,14}C_n$]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 (+)-[$^{14}C_2$]BABS __4a__ with benzaldehyde followed by some functional group conversions provided (3R,4S)-3-(tert-butyl-dimethyl-silanyloxy)-4-phenyl-[2,3-$^{14}C_2$]azetidin-2-one __9a__ in an overall radiochemical yield of 34%. Attachement of the activated benzoyl β-lactam __9b__ to the baccatine skeleton __10__ and subsequent deprotection provided the expected doubly labelled drug substance.*

Keywords: (+)/(-)-[$^{14}C_2$]BABS, C-14 labelled chiral synthons, simply/multiply C-14 labelled 1,2-functionalized carboxylic acids, e.p. C-14 labelled β-amino-α-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

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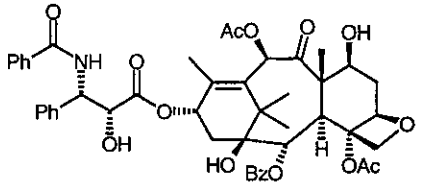
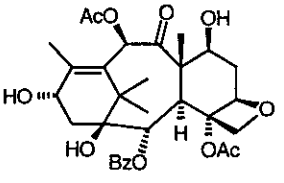
renewable yew leaves of *Taxus Baccata.* The semisynthetic process involves the coupling of baccatine III derivatives to a suitable precursor of (2R,3S)-N-benzoyl-3-phenylisoserine. Therefore, much effort has been made in the preparation of enantiomerically pure phenylisoserine derivatives and consequently many elegant approaches have been developed and reported [4].

We were asked to supply doubly C-14 labelled **Taxol** to support pharmaceutical and pharmacokinetic investigations. Availability of baccatine III **2** made the semisynthetic approaches more practical, and therefore, the label was placed in the pharmacologically essential (2R,3S)-phenylisoserine side chain. Among several efficient methods which have so far been reported for the synthesis of the (2R,3S)-N-benzoyl-3-phenylisoserine chain, β-lactam **3** proved to be the most convenient precursor for the subsequent attachment to the skeleton baccatine III [5,6]. The total diastereoselective synthesis of the doubly labelled lactam **9a** was efficiently completed from (+)-[$^{14}C_2$]**BABS 4a** as shown in scheme 1.



1: Taxol (Paclitaxel)



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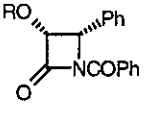

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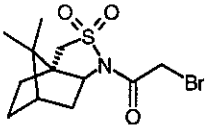
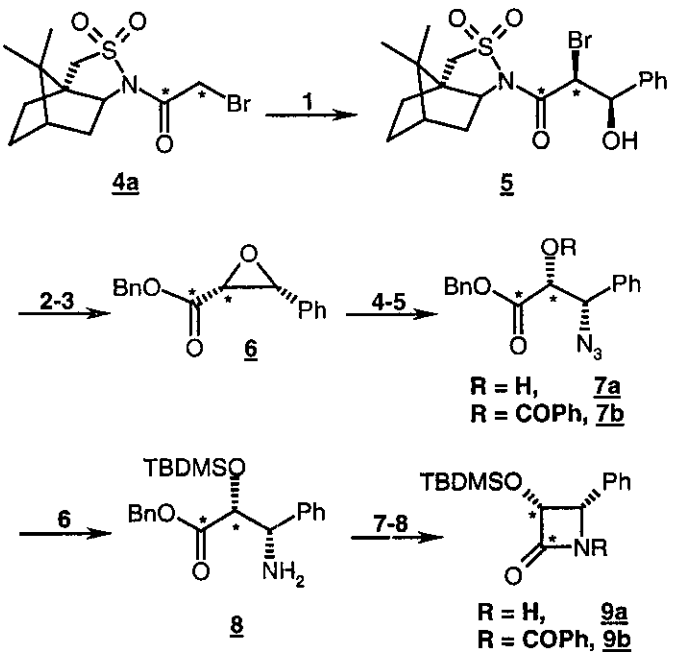


4: (+)-[$^{14}C_n$]BABS

Discussion and Results

Oppolzer [7] has extensively demonstrated that acylbornanesultams are very useful intermediates since they provide exceptionally high fase discrimination in a wide variety of reactions of their enolate derivatives with several electrophiles. Their utility has been extended by the conception of using (+)/(-)-[$^{14}C_n$]**BABS 4** [8] as a highly valuable chiral building block to prepare enantiomerically pure singly/multiply labelled intermediates.

Scheme 1: (3R,4S)- 3-(*tert*-Butyldimethylsilanyloxy)-4-phenyl-[2,3-$^{14}C_2$]azetidin-2-one 9a



1a) $TiCl_4$, 1-ethylpiperidine, dichloromethane, -78°C, 5 min.; **1b)** PhCHO, -78°C, 10 min.; **2)** K_2CO_3, DMF, H_2O cat., r.t.; **3)** BnOLi, THF, -10°C; **4)** NaN_3, HCO_2Me, MeOH-H_2O, 8:1, 50°C; **5)** *tert*-butyldimethylsilyl triflate, 2,6-lutidine, CH_2Cl_2, r.t.; **6)** PPh_3, THF-H_2O 8:1, 50°C, 2h; **7)** *tert*-butylmagnesium chloride, ether, 2h, -10°C; **8)** PhCOCl, Et_3N, DMAP cat., CH_2Cl_2, -10°C, 1h30 min.

Reaction of (+)-[$^{14}C_2$]BABS **4a** with equimolar amounts of titanium tetrachloride and 1-ethyl piperidine (-78°C, dichloromethane) followed by trapping of the resulting titanium enolate with benzaldehyde afforded the syn-α-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

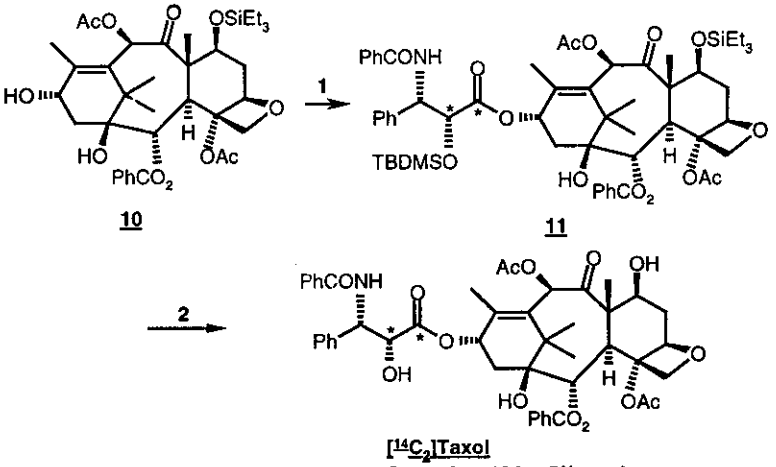
due to formation of side compounds [10]. Regioselective opening of the epoxide according to the procedures developed by *Sharpless* [11] provided the azide **7a** in 85% yield.

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°C for 2h cleanly afforded the β-lactam **9a** in 86% yield. The synthesis of **9a** from (+)-[$^{14}C_2$]**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 β-lactam **9a** with benzoylchloride (74%), followed by reaction of the resulting N-benzoyl β-lactam **9b** with the deprotonated allylic hydroxy group of 7-TES-baccatine III **10** (nBuLi, -40°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}H-NMR spectroscopy and HPLC-comparison with commercially available unlabelled material.

Scheme 2: Conclusive Synthesis of [1',2'-$^{14}C_2$]Taxol



1a) nBuLi, THF, -40°C; **1b)** **9b**; 2) HF-Pyridine, 0°C

Experimental Section

General:
Chemical reagents were purchased from *Aldrich Chemical Co.* or *Fluka* and used without further purification. **(+)-[$^{14}C_2$]BABS** was purchased from *NEN*. The identity of intermediates was confirmed by MS and ^{1}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'-$^{14}C_2$] propanoyl] bornan-10,2-sultam 5

A solution of (+)-[$^{14}C_2$]BABS **4a** (250 mCi, 830 mg, 2.47mmol) 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°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 (Na_2SO_4) and evaporated. Purification by recrystallization from dichloromethane-hexane gave the syn α-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'-$^{14}C_2$]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 (Na_2SO_4) and evaporated to give 705 mg of crude epoxide which was used without further purification.

(2R,3R)- 3-phenyl-[1,2-$^{14}C_2$]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°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°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-$^{14}C_2$]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°C for 24 h. The methanol was evaporated, the residue taken up in ethyl acetate, washed with water, dried (Na_2SO_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*-butyldimethylsilanyloxy)-3-phenyl-[1,2-$^{14}C_2$] 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°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-silanyloxy)-3-phenyl-[1,2-$^{14}C_2$] 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°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*-Butyldimethylsilanyloxy)-4-phenyl-[2,3-$^{14}C_2$]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°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 (Na_2SO_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 β-lactam **7a** with a specific activity of 103 mCi/mmol.

(3R,4S)-1-Benzoyl-3-(*tert*-butyldimethylsilanyloxy)-4-phenyl-[2,3-$^{14}C_2$] azetidin-2-one 9b

A solution of the azetidin-2-one **9a** (120 mg, 0.43 mmol) in dichloromethane kept at -10°C was consecutively treated with triethylamine (73 µl, 0.52 mmol), benzoyl chloride (61 µl, 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'-$^{14}C_2$]Taxol 11

Under argon, a solution of n-butyllitium (1.6M hexane, 150 µl) 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°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°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 → 3:1, hexane-ethyl acetate) gave 175 mg (67%) of **11**.

[1',2'-$^{14}C_2$]Taxol

The silyl-protected taxol **11** (175 mg, 0.16 mmol) was dissolved in pyridine (2.5 ml) and reacted with an exces 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_2SO_4 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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