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Synthesis of deuterium-labelled paclitaxel and its hydroxyl metabolite

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This paper describes the synthesis of deuterium-labelled paclitaxel and its hydroxyl metabolite. Paclitaxel labelled with ^{2}H was obtained in four steps using the commercially available [$^{2}H_5$]benzoic chloride as the stable labelled reagent with a 40% overall yield. The hydroxyl metabolite labelled with ^{2}H was prepared starting from deuterium-labelled paclitaxel in six steps with a 42% overall yield based on unrecovered starting material.

Keywords: deuterium labelled; paclitaxel; metabolite; 6α-hydroxy-paclitaxel

Introduction

Paclitaxel, a substance originally isolated from the Pacific yew tree, has been approved for the clinical treatment of cancer patients and now became one of the most significant drugs in cancer therapy.[1] This molecule exerts its special anticancer activity by inhibiting mitosis through enhancement of the polymerization of tubulin and consequent stabilization of microtubules.[2] The determination of paclitaxel and its metabolites in biological fluids has been reported in the literature.[3–7] 6α-Hydroxy-paclitaxel was detected to be the principal metabolite of paclitaxel in human hepatic microsomes, human liver slices, and patient biliary excretions.[4,5] Metabolism studies revealed that paclitaxel is mainly metabolized by hydroxylation through CYP2C8 to the still active metabolite 6α-hydroxyl metabolite, as shown in Figure 1.[7]

Identification and quantification of drugs and metabolites by LC/MS relies very much on stable isotope-labelled analogues.[8,9] A renewed interest has been recently raised to develop a robust and validated LC/MS method to determine paclitaxel and its metabolite in biological fluids. The preparation of the stable labelled versions of the title compounds with M+5 was requested. Although ^{3}H-paclitaxel and ^{14}C-paclitaxel have been prepared for pharmacological studies,[10,11] the synthesis of its stable labelled internal standards has not been disclosed in detail.[12] In this paper, the synthetic routes to [$^{2}H_5$]paclitaxel and its 6α-hydroxyl metabolite are described in detail.

Experimental

General

All reagents were obtained from Sigma-Aldrich and CDN Isotope. Mass spectra were recorded using a Quattro micro API mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument. Chemical purities were determined by an Agilent 1200 HPLC with a XDB-C18 column, 5 μm, 4.6 × 150 mm.

7-Triethylsilyl baccatin III (2)

A solution of Baccatin III (10 g, 17 mmol) in dry pyridine (250 mL) was treated with chlorotriethylsilane (69.3 mL, 341 mmol) and stirred at room temperature for 8 h. The solvent and excess chlorotriethylsilane were removed under reduced pressure at 30°C. The remaining residue was dissolved in dichloromethane (200 mL) and washed with H_2O (5 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a white solid, which was recrystallized from CH_2Cl_2/hexanes to afford (2) as a white solid (10.01 g, 83.7%).

^{1}H-NMR($CDCl_3$) δ ppm: 8.12(dd, 2H, o-Ph), 7.62(*t*, 1H, p-Ph), 7.48(*m*, 2H, m-Ph), 6.46 (s, 1H, 10-H), 5.65(d, 1H, *J* = 6.0 Hz, 2-H), 4.95(d, 1H, *J* = 9.0 Hz, 5-H), 4.83 (*t*, 1H, 13-H), 4.50(dd, 1H, *J* = 12.0, 6.0 Hz, 7-H), 4.31(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.13 (d, 1H, *J* = 9.0 Hz, 20-H_β), 3.90(d, 1H, *J* = 6.0 Hz, 3-H), 2.51(*m*, 1H, 6-H_α), 2.29(s, 3H, 4-$COCH_3$), 2.27(*m*, 2H, 14-H), 2.19(s, 3H, 10-$COCH_3$), 2.17(s, 3H, 18-CH_3), 1.88(*m*, 1H, 6-H_β), 1.63(s, 3H, 19-CH_3), 1.20(s, 3H, 17-CH_3), 1.04(s, 3H, 16-CH_3), 0.93 (*t*, 3H, $SiCH_2$ CH_3), 0.55(*m*, 2H, $SiCH_2$ CH_3).

13-((4*S*, 5*R*)-3-(*tert*-butoxycarbonyl)-2-(4-methoxyl-benzyl)-4-phenyl-5-oxazolidinecarboxylic formyl)-7-trithylsilyl baccatin III (4)

To a stirred solution of (2) (5.00 g, 7.1 mmol) and (4*S*, 5*R*)-3-(*tert*-butoxycarbonyl)-2-(4-methoxyl-benzyl)-4-phenyl-5-oxazolidinecarboxylic acid (3) (8.55 g, 21.4 mmol) in dry toluene (214 mL) was added DCC (4.30 g, 20.8 mmol) and DMAP (0.44 g, 3.6 mmol). The reaction was allowed to proceed at 80°C for 1 h. The reaction mixture was cooled to room temperature and stirred for 1 h, followed by filtering the resulting dicyclohexylurea. The filter cake was rinsed thoroughly with toluene (50 mL) and the combined organic layer was concentrated under

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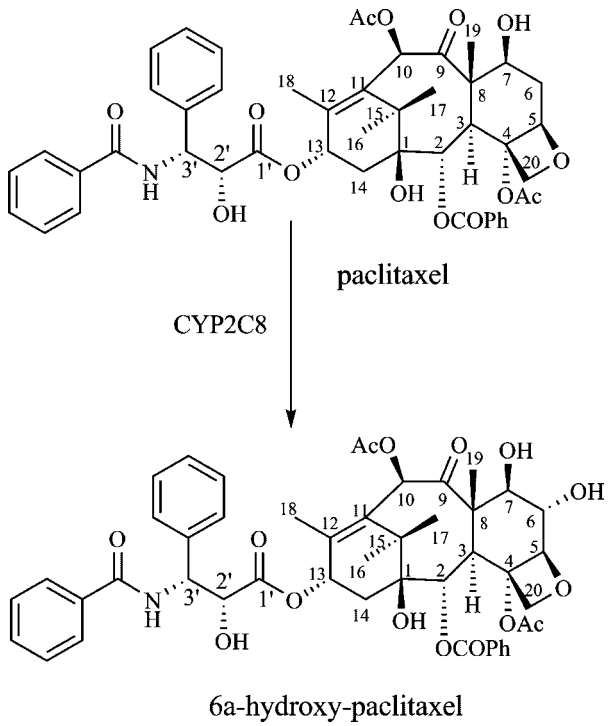


Figure 1. Paclitaxel is mainly metabolized by CYP2C8 through hydroxylation at α-C-6 to provide 6α-hydroxy-paclitaxel, which is still active.

reduced pressure to give a yellow solid. The crude product was purified on a silica gel column, eluted with hexanes/ethyl acetate (7:3) to afford (4) as a light yellow solid (6.42 g, 82.9%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.03(dd, 2H, o-Ph), 7.61(*t*, 1H, p-Ph), 7.50(*m*, 2H, m-Ph), 7.47(*m*, 2H, Ph), 7.42(*m*, 7H, Ph), 6.35(s, 1H, 10-H), 6.08(*t*, 1H, *J* = 9.0 Hz, 13-H), 5.65(d, 1H, *J* = 6.0 Hz, 2-H), 5.04(*m*, 1H, 4′-H), 4.87(dd, 1H, *J* = 9.0, 3.0 Hz, 5-H), 4.46(d, 1H, *J* = 6.0 Hz, 5′-H), 4.45(dd, 1H, *J* = 9.0, 6.0 Hz, 7-H), 4.25(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.10(d, 1H, *J* = 9.0 Hz, 20-H_β), 3.81(s, 3H, OCH_3), 3.80(d, 1H, *J* = 6.0 Hz, 3-H), 2.51(*m*, 1H, 6-H_α), 2.18(s, 3H, 4-$COCH_3$), 2.17(s, 3H, 10-$COCH_3$), 2.16(*m*, 2H, 14-H), 2.05(s, 1H, 18-CH_3), 1.82(*m*, 1H, 6-H_β), 1.66(s, 1H, 19-CH_3), 1.22(s, 1H, 17-CH_3), 1.21(s, 1H, 16-CH_3), 1.10(brs, 9H, Boc), 0.92(*t*, 3H, $SiCH_2CH_3$), 0.56(*m*, 2H, Si CH_2 CH_3).

13-(*O*-2*R*-Hydroxy-3*S*-amine-phenylpropionyl)-baccatin III (5)

A solution of (4) (8.47 g, 7.83 mmol) in formic acid (176.1 mL) was stirred at room temperature for 2 h. The mixture was concentrated to about 20 mL, diluted with CH_2Cl_2 (50 mL), neutralized with saturated $NaHCO_3$ solution and solid Na_2CO_3 to pH 8. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2/methanol 95:5 (6 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a yellow solid. The crude product was purified by flash chromatography on a silica gel column, eluted with CH_2Cl_2/MeOH (95:5) to afford (5) as a brown solid (3.3 g, 56.2%).

^{1}H-NMR($CDCl_3$)δ ppm: 7.80(dd, 2H, o-Ph), 7.66(*m*, 1H, p-Ph), 7.62(*m*, 2H, o-Ph), 7.53(*m*, 2H, m-Ph), 7.36(*m*, 3H, Ph), 6.32(s, 1H, 10-H), 5.98(*t*, 1H, *J* = 9.0 Hz, 13-H), 5.53(d, 1H, *J* = 6.0 Hz, 2-H), 4.93(d, 1H, *J* = 9.0 Hz, 3′-H), 4.86(d, 1H, *J* = 9.0 Hz, 5-H), 4.78(d, 1H, *J* = 9.0 Hz, 2′-H), 4.27(brt, 1H, *J* = 6.0 Hz, 7-H), 4.18(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.06(d, 1H, *J* = 9.0 Hz, 20-H_β), 3.60(d, 1H, *J* = 6.0 Hz, 3-H), 2.40(*m*, 1H, 6-H_α), 2.18(s, 3H, 4-$COCH_3$), 2.16(*m*, 2H, 14-H), 2.14(s, 3H, 10-$COCH_3$), 1.78(s, 3H, 18-CH_3), 1.78(s, 3H, 19-CH_3), 1.68(s, 1H, 6-H_β), 1.59(s, 3H, 17-CH_3), 1.11(s, 3H, 16-CH_3).

[$^2H_5$]Paclitaxel (6)

The solution of [$^2H_5$]benzoyl chloride (0.47 g, 3.23 mmol) in CH_2Cl_2 (5 mL) was added slowly to a solution of (5) (2.19 g, 2.92 mmol) in CH_2Cl_2 (29.2 mL) containing Et_3N (0.55 mL, 3.96 mmol) while cooling in an ice-bath. The reaction mixture was stirred at room temperature for half an hour and the solvent was removed under reduced pressure to give a white solid. The crude product was purified by flash chromatography on a silica gel column, eluted with CH_2Cl_2/MeOH (99:1) to afford (6) as a white solid (2.01 g, 80.4%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.14(dd, 2H, o-Ph), 7.61(dd, 2H, o-Ph), 7.53(*t*, 1H, p-Ph), 7.48(*m*, 2H, m- Ph), 7.36–7.43(*m*, 3H, Ph), 6.98(d, 1H, *J* = 9.0 Hz, NH), 6.28(s, 1H, 10-H), 6.23 (*t*, 1H, *J* = 9.0 Hz, 13-H), 5.80(dd, *J* = 9.0, 3.0 Hz, 1H), 5.68(d, 1H, *J* = 7.0 Hz, 2-H), 4.97(d, 1H, *J* = 9.0 Hz, 5-H), 4.80(d, 1H, *J* = 3.0 Hz, 2′-H), 4.40(dd, 1H, *J* = 12.0, 6.0 Hz, 7-H), 4.29(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.20(d, 1H, *J* = 9.0 Hz, 20- H_β), 3.80(d, 1H, *J* = 6.0 Hz, 3-H), 2.54(*m*, 1H, 6-H_α), 2.38(s, 3H, 4-$COCH_3$), 2.30(*m*, 1H, 14-H), 2.23(s, 3H, 10-$COCH_3$), 1.88(*m*, 2H, 6-H_β,14-H), 1.80(s, 3H, 18-CH_3), 1.69(s, 3H, 19-CH_3), 1.24(s, 3H, 17-CH_3), 1.15(s, 3H, 16-CH_3). HPLC (XDB-C18, CH_3CN/H_2O = 53/47, 1.0 mL/min): t_R 5.96 min (> 99.3%). MS-EI (*m/z*): 860.1(20), 881.3(MNa^+, 30), 932.4(100), 933.4(55).

[$^2H_5$]2′-*O*-(*t*-Butyldimethylsilyl)-paclitaxel (7)

To a solution of [$^2H_5$]paclitaxel (2.5 g, 2.91 mmol) in dry DMF(25 mL) was added imdazole (0.9908 g, 14.55 mmol) and *t*-butyldimethylchlorosilane (1.0982 g, 7.28 mmol). The mixture was stirred at 70°C for 8 h. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with H_2O (50 mL), 2% HCl solution (50 mL), $NaHCO_3$ solution (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a white solid. The crude product was purified by column chromatography on a silica gel column, eluted with hexanes/ethyl acetate (6:4) to afford (7) as a white solid (2.2 g, 77.7%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.14(dd, 2H, o-Ph), 7.60(dd, 2H, o-Ph),7.55(*t*, 1H, p-Ph), 7.32–7.43(*m*, 5H, Ph), 7.05(d, 1H, *J* = 9.0 Hz, NH), 6.30(s, 1H, 10-H), 6.28(*t*, 1H, *J* = 9.0 Hz, 13-H), 5.73(dd, 1H, *J* = 9.0, 6.0 Hz, 3′-H), 5.68(d, 1H, *J* = 6.0 Hz, 2-H), 4.98(d, 1H, *J* = 9.0 Hz, 5-H), 4.66(d, 1H, *J* = 3.0 Hz, 2′-H), 4.42(dd, 1H, *J* = 12.0, 6.0 Hz, 7-H), 4.29(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.20(d, 1H, *J* = 9.0 Hz, 20-H_β), 4.12(*m*, H, OH), 3.82(d, 1H, *J* = 6.0 Hz, 3-H), 2.57(s, 3H, 4-$COCH_3$), 2.45(*m*, 1H, 6-H_α), 2.36(*m*, 1H, 14-H), 2.23(s, 3H, 10-$COCH_3$), 2.12(*m*, 2H, 6-H_β, 14-H), 1.90(s, 3H, 18-CH_3), 1.80(s, 3H, 19-CH_3), 1.24(s, 3H, 17-CH_3), 1.14(s, 3H, 16-CH_3), 0.80 (s, 9H, $C(CH_3)_3$), −0.04(*t*, 3H, $SiCH_3$), 0.29(s, 3H, $SiCH_3$).

[$^2H_5$]2′-*O*-(*t*-Butyldimethylsilyl)-7β-*O*-trifluoromethanesulfonylpaclitaxel (8)

To a stirred solution of silyl ether (7) (2.66 g, 2.73 mmol) in dry trichloromethane (26.6 mL) was added DMAP (0.5009 g, 4.1 mmol) and trifluoromethanesulfonyl chloride (0.5527 g, 3.28 mmol). The mixture was kept stirring at room temperature for 2.5 h. The reaction mixture was diluted with trichloromethane (150 mL) and washed with H_2O (2 × 100 mL), 1% HCl solution (100 mL), H_2O (100 mL), saturated $NaHCO_3$ solution (100 mL), dried over

anhydrous Na_2SO_4, and concentrated under reduced pressure to give a white solid. The crude product was purified by column chromatography on a silica gel column, eluted with hexanes/ethyl acetate (3:1) to afford (8) as a white solid (2.75 g, 91%).

^{1}H-NMR(300 MHz, $CDCl_3$) δ ppm: 8.13(dd, 2H, o-Ph), 7.61(d, 2H, o-Ph), 7.53(*t*, 1H, p-Ph), 7.33–7.42 (*m*, 5H, Ph), 7.06(d, 1H, *J* = 9.0 Hz, NH), 6.62(s, 1H, 10-H), 6.25(*t*, 1H, *J* = 9.0 Hz, 13-H), 5.75(dd,2H, *J* = 9.0, 3.0 Hz, 2-H), 5.48(dd, 1H, *J* = 12.0, 6.0 Hz, 7-H), 4.96(d, 1H, *J* = 9.0 Hz, 5-H), 4.64(d, 1H, *J* = 3.0 Hz, 2′-H), 4.36(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.22(d, 1H, *J* = 9.0 Hz, 20-H_β), 4.14(*m*, 1H, OH), 4.12(*m*, 1H, OH), 3.96(d, 1H, *J* = 6.0 Hz, 3-H), 2.86(*m*, 1H, 6-H_α), 2.60(s, 3H, 4-$COCH_3$), 2.39(*m*, 1H,14-H), 2.20(s, 3H, 10-$COCH_3$), 2.17(*m*, 1H, 6-H_β), 2.17(*m*, 1H, 14-H), 2.06(s, 3H, 18-CH_3), 1.90(s, 3H, 19-CH_3), 1.22(s, 3H, 17-CH_3), 1.19(s, 3H, 16-CH_3), 0.80(s, 9H, $C(CH_3)_3$), −0.02(s, 3H, $SiCH_3$), −0.29(s, 3H, $SiCH_3$).

[$^2H_5$]2′-*O*-(*t*-Butyldimethylsilyl)-6,7-dehydropaclitaxel (9)

To a stirred solution of (8) (1.75 g, 1.58 mmol) in CH_2Cl_2 (17.5 mL) was added 1, 8- diazabicyclo [5, 4, 0] undec-7-ene (2.4104 g, 15.8 mmol). The mixture was kept stirring at 40°C for 8 h. The solvent was removed under reduced pressure to afford a white solid. The crude product was purified by flash chromatography on a silica gel column, eluted with hexanes/ethyl acetate (7:3) to afford (9) as a white solid (1.54 g, 98.7%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.10(dd, 2H, o-Ph), 7.50(d, 2H, o-Ph), 7.38(*t*, 1H, p-Ph), 7.33–7.42(*m*, 5H, Ph), 7.06(d, 1H, *J* = 9.0 Hz, NH), 6.25(*t*, 1H, *J* = 9.0 Hz, 13-H), 6.24(s, 1H, 10-H), 6.10(dd, 1H, *J* = 9.0, 6.0 Hz, 6-H), 5.88(d, 1H, *J* = 9.0 Hz, 7-H), 5.86(d, 1H, *J* = 6.0 Hz, 2-H), 5.55(d, 1H, *J* = 9.0 Hz, 3′-H), 4.67(d, 1H, *J* = 3.0 Hz, 2′-H), 4.20(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.12(d, 1H, *J* = 9.0 Hz, 20-H_β), 4.03(d, 1H, *J* = 6.0 Hz, 3-H), 2.58(s, 3H, 4-$COCH_3$), 2.40(*m*, 1H, 14-H_α), 2.22(*m*, 2H, 6-H, 14-H_β), 2.11(s, 3H, 10-$COCH_3$), 1.88(s, 3H, 18-CH_3), 1.82(s, 3H, 19-CH_3), 1.26(s, 3H, 17-CH_3), 1.15(s, 3H, 16-CH_3), 0.79(s, 9H, $C(CH_3)_3$), −0.05(s, 3H, $SiCH_3$), −0.31(s, 3H, $SiCH_3$).

[$^2H_5$]2′-*O*-(*t*-Butyldimethylsilyl)-6α-hydroxy-7-epi-paclitaxel (10)

To a solution of (9) (1.42 g, 1.49 mmol) in THF (14.2 mL) was added *N*-Methylmoprpholine N-oxide (NMO, 0.26 g, 2.2 mmol) in H_2O (4.3 mL) and OsO_4 solution (4 wt. 4% solution in H_2O, 0.0945 mL, 0.015 mmol) under nitrogen atmosphere. The mixture was stirred at room temperature for 4 h. Additional OsO_4 solution (0.2835 mL, 0.045 mmol) and NMO (0.086 g, 0.73 mmol) was added and the mixture was stirred for 5 h. TLC analysis showed that about 20% product was formed. This process was repeated several times till more than 90% product was formed. The reaction mixture was diluted with CH_2Cl_2 (12 mL) and sodium metabisulfite (0.572 g) was added while cooled in ice-bath and stirred for 1 h. Anhydrous Na_2SO_4 (4 g) was added and the mixture was stirred at room temperature for another 1 h. The suspension was filtered and the solid residue was rinsed thoroughly with THF (3 × 8 mL). The filtrate was concentrated to give a brown paste, which was re-dissolved in EtOAc (60 mL) and washed with water (2 × 40 mL), 0.25 M H_2SO_4 (2 × 40 mL), brine (2 × 40 mL). The organic layer was dried with Na_2SO_4 and concentrated under reduced pressure to give a light green semi-solid. The crude product was purified by flash-chromatography on a silica gel column, eluted with hexanes/ethyl acetate (7:3) to afford (10) as a white solid (1.16 g, 78.9%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.16(dd, 2H, o-Ph), 7.59(d, 2H, o-Ph), 7.52(*t*, 1H, p-Ph), 7.32–7.43(*m*, 5H, Ph), 7.06(d, 1H, *J* = 9.0 Hz, NH), 6.83(s, 1H, 10-H), 6.29(*t*, 1H, *J* = 9.0 Hz, H-13), 5.77(dd, 1H, *J* = 9.0, 3.0 Hz, 3′-H), 5.74(d, 1H, *J* = 6.0 Hz, 2-H), 4.67(d, 1H, *J* = 3.0 Hz, 5-H), 4.66(brs, 2H, 20-H), 4.37(s, 1H, 2′-H), 4.12(*m*, 1H, 6-H), 3.87(d, 1H, *J* = 7.0 Hz, H-3), 3.71(dd, 1H, *J* = 12.0, 6.0 Hz, 7-H), 2.90(d, 1H, *J* = 9.0 Hz, OH), 2.68(s, 3H, 4-$COCH_3$), 2.33(*m*, 1H, 14-$H_á$), 2.20(s, 3H, 10-$COCH_3$), 2.13(*m*, 1H, 14-H_β), 1.91(s, 3H, 18-CH_3), 1.60(s, 3H, 19-CH_3), 1.24(s, 3H, 17-CH_3), 1.14(s, 3H, 16-CH_3), 0.78(s, 9H, $C(CH_3)_3$), −0.04(*t*, 3H, $SiCH_3$), −0.30(s, 3H).

[$^2H_5$]2′-*O*-(*t*-Butyldimethylsilyl)-6α-hydroxy-paclitaxel (11)

To a solution of (10) (1.95 g, 1.971 mmol) in dry toluene(19.5 mL) was added 1, 8-diazobicyclo [5, 4, 0] udec-7-ene (DBU, 0.6 g, 3.94 mmol) and the mixture was stirred at 80°C for 1 h. The reaction mixture was concentrated under reduced pressure to give a brown solid. The crude product was purified by flash-chromatograph on a silica gel, eluted with hexanes/ethyl acetate (7:3) to afford (11) as a white solid (0.15 g, 7.67%) and (10) as a white solid too (1.6 g, 82%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.14(dd, 2H, o-Ph), 7.60(d, 2H, o-Ph), 7.53(*t*, 1H, p-Ph), 7.32–7.43 (*m*, 5H, Ph), 7.06(d, 1H, *J* = 9.0 Hz, NH), 6.29(s, 1H, 10-H), 6.29(*m*, 1H, *J* = 9.0 Hz, H-13), 5.77(d, 1H, *J* = 9.0, 3.0 Hz, 3′-H), 5.74(d, 1H, *J* = 9.0 Hz, 2-H), 4.86 (d, 1H, 5-H), 4.66(d, 1H, *J* = 3.0 Hz, 2′-H), 4.34(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.24(*m*, 1H, 7-H), 4.23(d, 1H, *J* = 9.0 Hz, 20-H_β) 3.96(*m*, 2H, 6-H and 3-H), 2.59(s, 3H, 4-$COCH_3$), 2.42(*m*, 1H, 14-H_α), 2.26(s, 3H, 10-$COCH_3$), 2.14(*m*, 1H, 14-H_β), 1.91(s, 3H, 18-CH_3), 1.64(s, 3H, 19-CH_3), 1.20(s, 3H, 17-CH_3), 1.14(s, 3H, 16-CH_3), 0.78(s, 9H, $C(CH_3)_3$), −0.05(*t*, 3H, $SiCH_3$), −0.30(s, 3H, $SiCH_3$).

[$^2H_5$]6α-Hydroxy-paclitaxel (12)

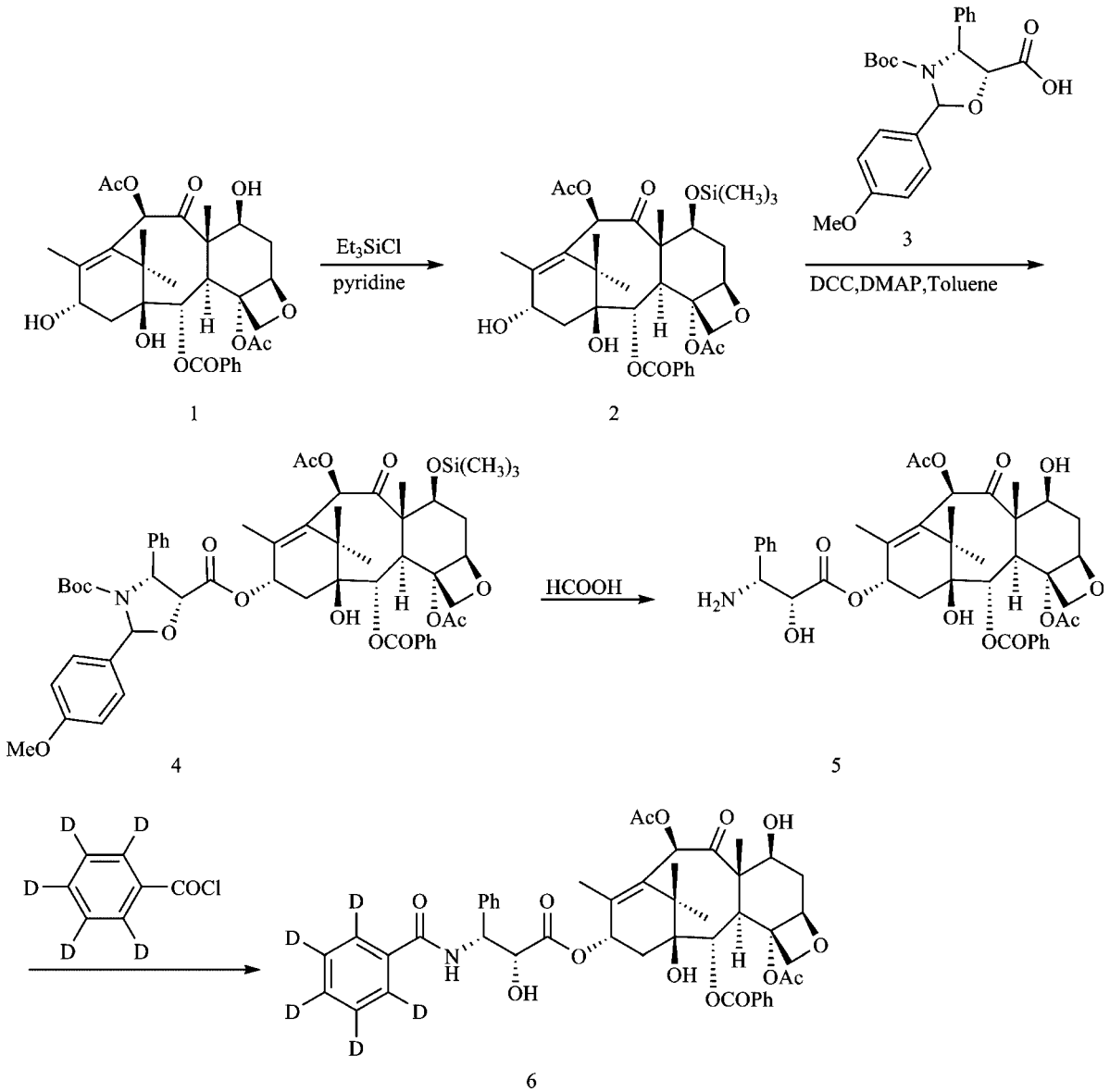
The reaction mixture of (11) (0.23 g, 0.233 mmol), acetic acid (0.112 g, 0.106 mL, 1.86 mmol), and tetrabutyl ammonium fluoride hydrate (0.1822 g, 0.69 mmol) was stirred in THF(3.0 mL) at room temperature for 4 h. It was diluted with EtOAc (10 mL). The excess TBAF was quenched with 2% HCl solution (10 mL) and the mixture was extracted with ethyl acetate (5 × 10 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution (2 × 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to afford the desired product (12) as a white solid (0.18 g, 79.1%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 8.14(dd, 2H, o-Ph), 7.62(*t*, 1H, p-Ph), 7.48(*m*, 2H, o-Ph), 7.35–7.42(*m*, 5H), 6.96(d, 1H, *J* = 9.0 Hz, NH), 6.26(s, 1H, 10-H), 6.25(*t*, *J* = 9.0 Hz, 13-H), 5.78(dd, 1H, *J* = 9.0, 3.0 Hz, 3′-H), 5.66(d, 1H, *J* = 6.0 Hz, 2-H), 4.82 (s, 1H, 5-H), 4.79(dd, 1H, *J* = 9.0, 3.0 Hz, 2′-H), 4.30(d, 1H, *J* = 9.0 Hz, 20-H_α), 4.21(d, 1H, *J* = 9.0 Hz, 20-H_β), 4.20(d, 1H, *J* = 9.0 Hz, 7-H), 3.94 (*m*, 2H, 6-H and 3-H), 2.39(s, 3H, 4-$COCH_3$), 2.33(*m*, 2H, 14-H), 2.24(s, 3H, 10-$COCH_3$), 1.90(brs, 1H, OH), 1.82(s, 3H, 18-CH_3), 1.65(s, 3H, 19-CH_3), 1.25(s, 3H, 17-CH_3), 1.12(s, 3H, 16-CH_3). MS-EI (*m/z*): 242.2(100), 243.2(18), 897.2(MNa^+, 25), 898.2(17), HPLC (XDB-C18, CH_3CN/H_2O = 47/53, 1.0 mL/min): t_R 6.05 min (>98.9%). MS-EI analysis of the compound(12) have the same deuterium enrichment as compound(6).

Results and discussion

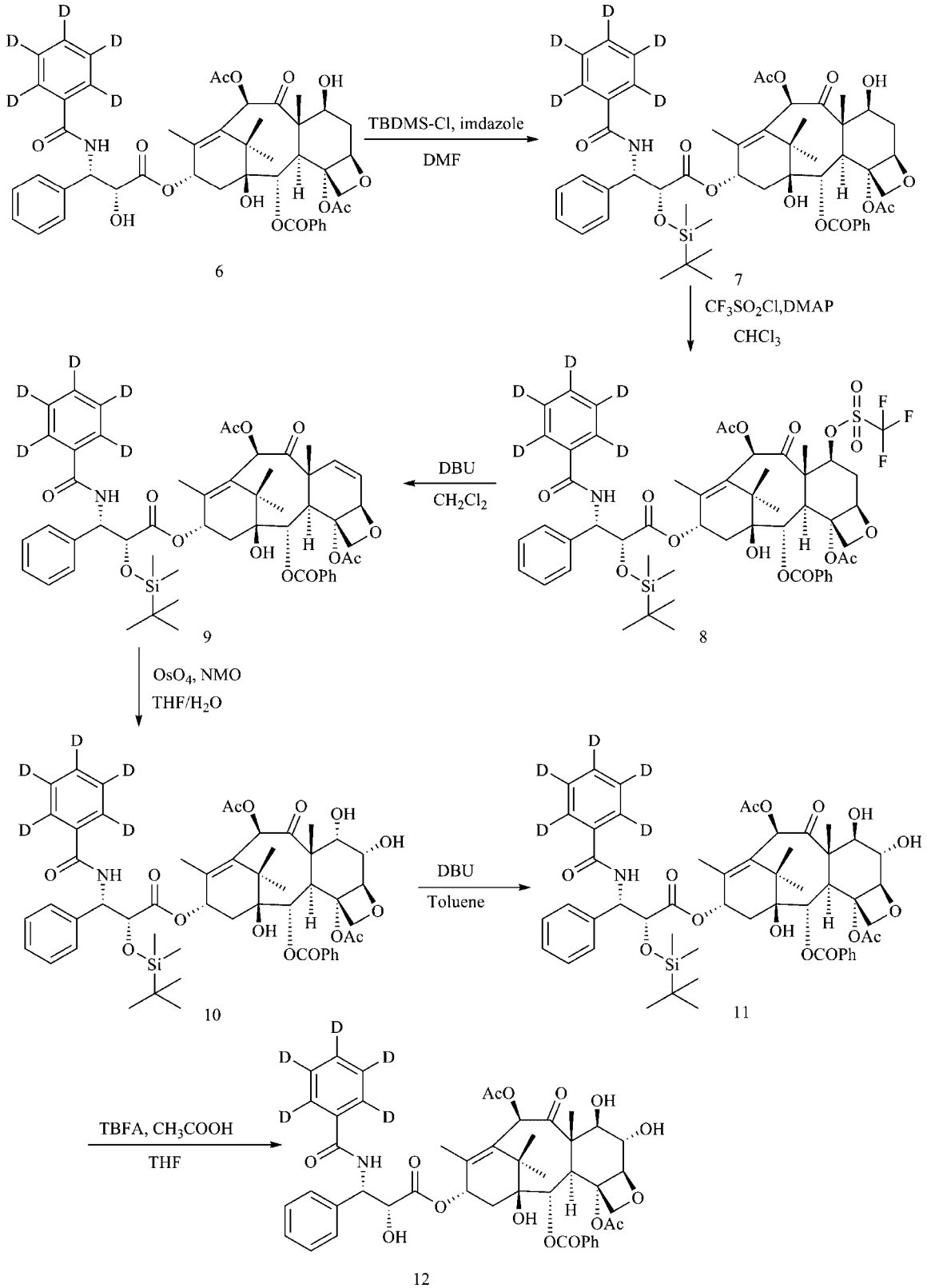
The synthesis of unlabelled and radioactive isotope (^{14}C, ^3H) labelled paclitaxel has been reported in detail.[10–16] The chiral side chain of taxol contains phenylisoserine ester and β-lactam. For our synthesis, the [$^2H_5$]benzoyl group was introduced

through *N*-esterification of [$^2H_5$]benzoyl chloride with an amine. Initially, we choose the synthesis of [$^2H_5$]azetidinone. Based on protocols from the literature,[12] [$^2H_5$]azetidinone was synthesized in three steps starting from *β*-lactam. The deuterium-labelled reagent was introduced in the second step. The coupling of the lithium alkoxide of [$^2H_5$]azetidinone with 7-triethylsilyl baccatin III in THF gave [$^2H_5$]2′, 7-*bis*(triethylsilyl)paclitaxel. Removal of the carefully chosen protecting groups in [$^2H_5$]2′, 7-*bis*(triethylsilyl)paclitaxel could be cleanly accomplished with 6 M aqueous HCl in acetonitrile at −5°C to give [$^2H_5$]paclitaxel. However, the esterization of [$^2H_5$]benzoyl chloride with 13-(*O*-2*R*-hydroxy-3*S*-amine-phenylpropionyl)-baccatin III(5) to give paclitaxel(6) is much more economic. The total yield is much higher. In addition, the conditions of the whole process were milder. In order to obtain 13-(*O*-2*R*-hydroxy-3*S*-amine-phenyl-propionyl)-baccatin III(5), we selected baccatin III(1) and phenylisoserine ester derivative(chiral oxazolidine) (3)[17,18] as starting materials (Scheme 1). Selective protection of baccatin III (1) with triethylsilyl chloride in anhydrous pyridine produced 7-triethylsilyl baccatin III (2),[19] which was condensed with the excess chiral oxazolidine(3) in the presence of dicyclohexyl-carbodiimide (DCC) and 4-dimethylamino-pyridine (DMAP) in anhydrous toluene at 80°C to afford 13-*O*-oxazolidinyl derivative(4).[17,18,20] Hydrolysis of (4) with formic acid yielded 13-(*O*-2*R*-hydroxy-3*S*-amine-phenylpropionyl)-baccatin III(5). Yields for this reaction were found to be highly dependent on careful temperature control (if the reaction temperature was allowed to rise above 30°C, yields decreased significantly). The resulting amine (5), after treatment with [$^2H_5$]benzoyl chloride and Et3N in dry CH_2Cl_2, gave [$^2H_5$]paclitaxel (6) in 80.4% yield. After purification by flash-chromatography, the desired product (6) was obtained with over 99% chemical purity. The ^1H NMR spectrum, R_f, and chromatographic behaviour of compound (6) were consistent with that of natural paclitaxel.[21] Mass spectrometry analysis of (6) revealed that the compound has more than 99% deuterium enrichment.



Scheme 1.

The synthesis of unlabelled 6α-hydroxy-paclitaxel have been described in the literature, but lacks experimental details.[22–24] Those general procedures were adopted in the synthesis of [$^2H_5$]6α-hydroxyl paclitaxel (12). With [$^2H_5$]paclitaxel (6) as the starting material, Scheme 2 presents the synthesis of [$^2H_5$] 6α-hydroxyl paclitaxel. Selective protection of [$^2H_5$]paclitaxel (6) with *t*-butyldimethylchlorosilane in dry DMF produced [$^2H_5$]2′-*O*- (*t*-butyldimethylchlorosilyl) palictaxel (7). Reaction



Scheme 2.

of (7) with trifluoromethanesulfonyl chloride and DMAP in dry $CHCl_3$ furnished [$^2H_5$]2′-*O*-(*t*-butyldimethylchlorosilyl)-7β-*O*-trifluromethanesulfonylpaclitaxel (8). The boiling point of trifluoromethanesulfonyl chloride is 29°C, so the reaction temperature was kept below 30°C to ensure good yield. Treatment of compound (8) with 1, 8-diazabicyclo [5, 4, 0] undec-7-ene (DBU) at 40°C in dry CH_2Cl_2 gave the key intermediate [$^2H_5$]2′-*O*-(*t*-butyldimethylsilyl)-6, 7-dehydropaclitaxel (9). The R_f of the starting material and product was very close, therefore 10 equivalents of DBU were used to ensure that the reaction was complete. The oxidation of (9) with osmium tetroxide and *N*-Methylmoprpholine N-oxide (NMO) in H_2O/THF produced [$^2H_5$]2′-*O*-(*t*-butyldimethylsilyl)-6α-hydroxy-7-epi-paclitaxel (10).[25] The high yield was achieved by repeatedly adding osmium tetroxide and NMO. Treatment of (10) with 1,8-Diazobicyclo [5, 4, 0]udec-7-ene(DBU) in anhydrous toluene at 80°C afforded a single product which was isolated and fully characterized as [$^2H_5$]2′-*O*-(*t*-butyldimethylsilyl)-6α-hydroxyl paclitaxel (11) in 7% yield, along with 80% unreacted starting material. Since the epimerization was essentially an equilibrium reaction,[26] reaction time cannot be longer than 1.5 h. Otherwise it will lead to the formation of other impurities. Deprotection of (11) with tetrabutylammonium fluoride and acetic acid at ambient temperature gave the major metabolite [$^2H_5$]6α-hydroxy-paclitaxel (12) in 79.1% yield. It has over 98% chemical purity and isotopic enrichment. The ^{1}H NMR spectrum of (12) was identical to that reported in the literature.[5,6] Other spectra, including TOCSY, HMQC, and NOESY, were consistent with the assigned structure.[5,6]

In conclusion, five deuterium-labelled paclitaxel and its hydroxyl metabolite have been successfully synthesized and characterized. It provides important internal standards for the clinical studies of paclitaxel.

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