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Radiolabeling of Paclitaxel with Electrophilic ^{123}I

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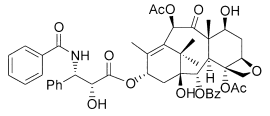
Abstract—^{123}I-Labeled paclitaxel, [^{123}I]-**1** was prepared by electrophilic aromatic radioiodination of 3′-*N*-(*p*-trimethylstannylbenzoyl)-3′-debenzoylpaclitaxel **2** with $^{123}I^-$ in the presence of peracetic acid.

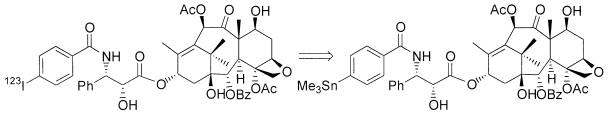
Introduction

Paclitaxel (Taxol®), a highly functionalized diterpene natural product isolated from the bark of Pacific yew (*Taxus brevifolia*) demonstrated anticancer activity in human malignancies such as ovarian, breast, lung, head and neck and gastrointestinal tract cancers.[1] A unique mechanism of paclitaxel as an antimitotic agent is based on its interaction with microtubule polymers which results in the formation of stable bundles of cellular microtubules that are resistant to depolymerization back to tubulin.[2–6]



Paclitaxel

Many of the paclitaxel derivatives have been evaluated in microtubule assembly assays and cytotoxicity assays against cancer cell lines.[7] Among them, analogues bearing a halogen substituent at the *para*-position of 3′-*N*-benzoyl group exhibited the ability to induce microtubule formation similar to that of paclitaxel.[8] From the strong microtubule binding affinity and antitumor activity against lung and ovarian cancers of 3′-*N*-(*p*-halo-benzoyl)-3′-debenzoylpaclitaxel, the 3′-*N*-(*p*-[^{123}I]-iodobenzoyl)-3′-debenzoylpaclitaxel [^{123}I]-**1** was designed as a cancer diagnostic radiopharmaceutical for SPECT (single photon emission computed tomography).



[^{123}I]-**1** **2**

In this paper, we report the synthesis of 3′-*N*-(*p*-[^{123}I]iodobenzoyl)-3′-debenzoylpaclitaxel [^{123}I]-**1** from the trimethyltin precursor **2**.

Results and Discussion

Synthesis of trimethyltin precursor 2

Trimethyltin precursor **2** and authentic iodopaclitaxel **1** were prepared as shown in Scheme 1.

Mixed anhydride **5** was prepared by the reaction of pivaloyl chloride and *p*-trimethylstannylbenzoic acid in the presence of NEt_3 in CH_2Cl_2. *p*-Trimethylstannylbenzoic acid **4** was prepared by the reaction of ethyl *p*-bromobenzoate and hexamethylditin in the presence of catalytic amounts of tetrakis(triphenylphosphine)palladium(0) in xylene, followed by hydrolysis with LiOH according to the known procedure.[9] The coupling of mixed anhydride **5** with 3′-amino-7-trichloroacetylpaclitaxel derivative **7** which was prepared

Keywords: 3′-*N*-(*p*-trimethylstannylbenzoyl)-3′-debenzoylpaclitaxel; electrophilic aromatic radioiodination; 3′-*N*-(*p*-[^{123}I]iodobenzoyl)-3′-debenzoylpaclitaxel; SPECT (single photon emission computed tomography).

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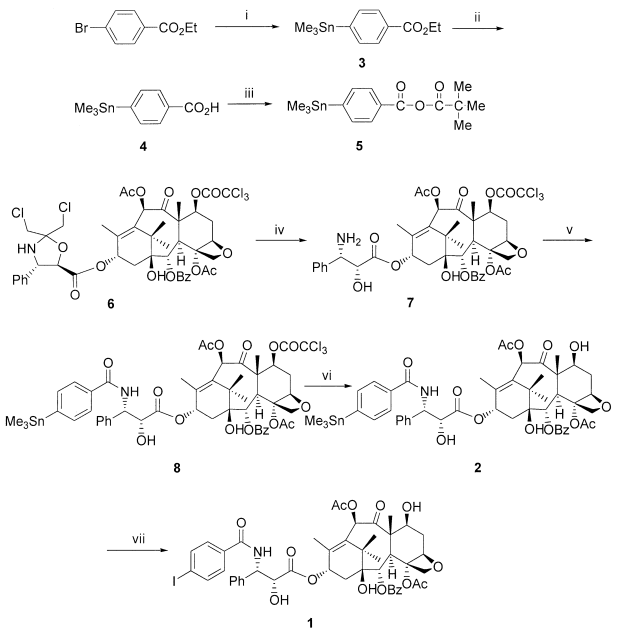
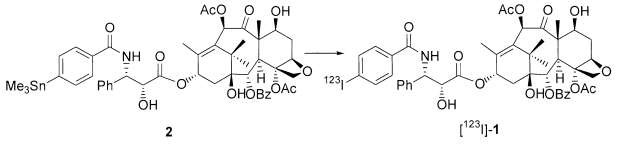


Scheme 1. Reagents and conditions: (i) $Me_3SnSnMe_3$, $Pd(PPh_3)_4$, xylene, 115 °C, 15 h; (ii) $LiOH{\cdot}H_2O$, THF-MeOH; (iii) pivaloyl chloride, NEt_3, CH_2Cl_2; (iv) c-HCl, then aqueous $NaHCO_3$, EtOAc; (v) **5**, pyridine; (vi) NH_4OAc, MeOH-THF; (vii) NaI, peracetic acid, EtOH.

by oxazolidine cleavage of the compound **6** with concd HCl in ethyl acetate succeeded smoothly in pyridine to give **8** in 58% yield. Trimethylstannylpaclitaxel **2** was then obtained in 85% yield by deprotection of trichloroacetyl group with excess of ammonium acetate. The ^1H NMR (300 MHz, $CDCl_3$) and HRMS (FAB) of **2** were consistent with the assigned structure. Electrophilic aromatic iodination of **2** with NaI in the presence of peracetic acid was then carried out to give the authentic compound **1**, and the reaction conditions for electrophilic aromatic radioiodination of **7** with $^{123}I^-$ were optimized. Iodopaclitaxel **1** was obtained in good yield and its structure was confirmed by ^1H NMR and HRMS (FAB) analyses.

^{123}I-Labeling of paclitaxel

Electrophilic aromatic radioiodination on **2** was carried out using $^{123}I^-$ and peracetic acid in ethanol (Scheme 2).[10] Progress of the reaction was monitored by radio-TLC, and the substitution reaction gave essentially one major radioactive product which corresponded to ^{123}I-labeled paclitaxel, [^{123}I]-**1**. After completion of the reaction (ca. 30 min) and work up, the crude product was purified by high performance liquid chromatography (HPLC) to give the pure radioactive product [^{123}I]-**1** in 63–65% radiochemical yield with effective specific activity greater than 37 GBq/μmol (1000 mCi/μmol).



Scheme 2. Reagents: $Na^{123}I$, CH_3CO_3H, EtOH.

Conclusion

Electrophilic aromatic radioiodination of **2** afforded the ^{123}I-labeled paclitaxel [^{123}I]-**1** in 63–65% radiochemical yield with effective specific activity greater than 37 GBq/μmol. To the best of our knowledge, this is the first reported example of the radioiododestannylation on a paclitaxel analogue. Biological studies with [^{123}I]iodopaclitaxel are currently in progress and electrophilic aromatic radiofluorinations of **2** with both [^{18}F]CH_3COOF and [^{18}F]F_2 are also being currently pursued.

Experimental

Materials and methods

High radionuclidic purity $Na^{123}I$ was obtained from Korea Cancer Center Hospital (Seoul, South Korea). Peracetic acid (32 wt% in acetic acid) was purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and HPLC solvents from J. T. Baker, Inc. (Phillipsburg, NJ, USA). HPLC was carried out on a Thermo Separation Products System (Fremont, CA, USA) with a semipreparative column (Alltech Econosil silica gel, 10 μ, 10×250 mm) and an analytical column (Alltech Econosil C18, 5 μ, 4.6×250 mm). The eluent was simultaneously monitored by a UV detector (254 nm) and a NaI(T1) radioactivity detector. Thin layer chromatography (TLC) was performed on Merck F_{254} silica plates and visualized by UV illumination. For radiolabeled compounds, TLC was analyzed on a Bioscan TLC scanner (Washington, DC, USA). Radioactivity was measured in a dose calibrator, and all radiochemical yields are not decay-corrected unless noted. ^1H NMR (300 MHz) spectra were recorded on a Varian Gemini 300 spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a MIDAC 101025 FT–IR spectrometer and main absorption frequencies were given in cm^{-1}. HRMS (FAB) analysis was carried out by Mass Spectrometry Analysis Group at Korea Basic Science Institute.

Synthesis of trimethyltin precursor 2

p-Trimethylstannylbenzoic acid **4** was prepared by the reaction of ethyl *p*-bromobenzoate and hexamethylditin in the presence of catalytic amounts of tetrakis(triphenylphosphine)palladium(0) in xylene, followed by hydrolysis with LiOH according to the known procedure.[9] The oxazolidine-protected 7-trichloroacetylpaclitaxel **6** was prepared according to the reported procedure.[11]

Pivaloyl *p*-trimethylstannylbenzoate 5. To a stirred mixture of *p*-trimethylstannylbenzoic acid (0.1 g, 0.35 mmol), triethylamine (0.07 mL, 0.53 mmol) in dichloromethane (10 mL) was added dropwise pivaloyl chloride (0.05 mL, 0.38 mmol) at 0 °C and stirring was continued for 3 h at room temperature. The mixed anhydride **5** generated in situ as above was used without further purification for the coupling reaction with 7-trichloroacetyl-3′-debenzoylpaclitaxel **7**.

7-Trichloroacetyl-3′-debenzoylpaclitaxel 7. To a solution of oxazolidine-protected 7-trichloroacetylpaclitaxel **6** (0.2 g, 0.19 mmol) in ethyl acetate (5 mL) was added concd HCl (0.05 mL) at 0 °C. After stirring for 30 min, the reaction mixture was neutralized by addition of aqueous $NaHCO_3$ and the organic phase was washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to afford the crude product of **7** which was used without further purification for the coupling reaction with pivaloyl *p*-trimethylstannyl benzoate **5**.

7-Trichloroacetyl-3′-(*p*-trimethylstannylbenzoyl)-3′-debenzoylpaclitaxel 8. To a stirred solution of the crude product of **7** (50 mg, 0.05 mmol) in pyridine (5 mL) was added dropwise *p*-trimethylstannylbenzoyl pivaloyl anhydride (0.17 mL) at 0 °C. After stirring for 2 h at the same temperature, the reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:2) to give **8** (38 mg, 58% yield) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 7.1 Hz, 2H), 7.30–7.72 (m, 11H), 6.95 (d, J = 8.3 Hz, 1H), 6.23 (m, 2H), 5.74 (dd, J = 8.9, 2.2 Hz, 1H), 5.62 (d, J = 7.2 Hz, 1H), 4.82 (d, J = 2.8 Hz, 1H), 4.76 (m, 1H), 4.23 (d, J = 8.4 Hz, 1H), 4.12 (d, J = 8.5 Hz, 1H), 3.82 (d, J = 6.9 Hz, 1H), 2.35 (s, 3H), 2.22 (s, 3H), 1.73 (s, 3H), 1.63 (s, 3H), 1.22 (s, 3H), 0.21 (s, 9H).

3′-(*p*-Trimethylstannylbenzoyl)-3-debenzoylpaclitaxel 2. To a stirred solution of **8** (38 mg, 0.03 mmol) in MeOH (5 mL) and THF (5 mL) was added ammonium acetate (13 mg, 0.16 mmol) at room temperature and the reaction was monitored by TLC. After completion of the reaction (ca. 2 h), the reaction mixture was diluted with ethyl acetate and washed with brine. After concentration in vacuo, the crude product was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:1) to give **2** (28 mg, 85% yield) as a white solid: IR (KBr) 3400–3500, 2900, 1700, 1650, 1516, 1431, 1381, 1227 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J = 7.0 Hz, 2H), 7.25–7.70 (m, 11H), 6.92 (d, J = 8.3 Hz, 1H), 6.23 (m, 2H), 5.72 (dd, J = 2.8, 2.9 Hz, 1H), 5.61 (d, J = 7.2 Hz, 1H), 4.93 (d, J = 2.8 Hz, 1H), 4.72 (m, 1H), 4.35 (m, 1H), 4.23 (d, J = 8.4 Hz, 1H), 4.12 (d, J = 8.5 Hz, 1H), 3.78 (d, J = 6.9 Hz, 1H), 2.53 (m, 1H), 2.35 (s, 3H), 2.23 (s, 3H), 1.72 (s, 3H), 1.61 (s, 3H), 1.21 (s, 3H), 0.22 (s, 9H); HRMS (FAB) m/z calcd for $C_{50}H_{60}NO_{14}Sn$: 1018.3048. Found: 1018.3036.

3′-(*p*-Iodobenzoyl)-3′-debenzoylpaclitaxel 1. To a solution of **2** (2 mg, 0.002 mmol) in EtOH were added NaI (0.32 mg, 0.002 mmol) and peracetic acid (32% in acetic acid, 0.62 μL, 0.003 mmol) at 0 °C and the reaction was monitored by TLC. After completion of the reaction (ca. 1 h), the reaction mixture was diluted with ethyl acetate, washed with brine and dried over anhydrous $MgSO_4$. After evaporation of organic volatiles, the crude product was purified by flash column chromatography on silica gel (EtOAc:*n*-hexane = 1:1) to give **1** (1.5 mg, 80% yield) as a white solid: IR (KBr) 3400–3500, 1740, 1710, 1621, 1561, 1222 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 7.4 Hz, 2H), 7.67 (d, J = 8.3 Hz, 2H), 7.60–7.25 (m, 10H), 6.96 (d, J = 8.9 Hz, 1H), 6.19 (s, 1H), 6.14 (t, J = 8.8 Hz, 1H), 5.69 (dd, J = 8.8, 2.1 Hz, 1H), 5.59 (d, J = 7.0 Hz, 1H), 4.87 (d, J = 8.2 Hz, 1H), 4.71 (d, J = 2.4 Hz, 1H), 4.32 (dd, J = 6.7, 10.7 Hz, 1H), 4.23 (d of ABq, J = 8.4 Hz, 1H), 4.11 (d of ABq, J = 8.4 Hz, 1H), 3.71 (d, J = 7.0 Hz, 1H), 2.5 (sym.m, 1H), 2.31 (s, 3H), 2.17 (s, 3H), 1.71 (s, 3H), 1.61 (s, 3H), 1.16 (s, 3H), 1.07 (s, 3H); HRMS (FAB) m/z calcd for $C_{47}H_{51}INO_{14}$: 980.2354. Found: 980.2407.

Radiolabeling

3′-(*p*-[^{123}I]Iodobenzoyl)-3′-debenzoylpaclitaxel [^{123}I]-1. $Na^{123}I$ in 0.01 N NaOH (220 μL) was treated with 0.05 N H_3PO_4 (40 μL). To this solution were added successively the solution of **2** (2 mg, 0.002 mmol) in EtOH (300 μL) and peracetic acid (10 μL of 10 μL 0.32 wt%/2.4 mL H_2O, 0.2 μmol) at room temperature. After 30 min, the reaction was quenched by addition of aqueous $NaHSO_3$ solution (10 μL of 1 mg/mL H_2O, 0.096 mmol) and the reaction mixture was diluted with CH_2Cl_2. After washing with water, the organic layer was passed through a short plug filled with 7 cm of anhydrous Na_2SO_4. The solvents were evaporated under a gentle stream of N_2, and the residue was redissolved in 1 mL of CH_2Cl_2 and injected onto a semi-preparative HPLC column, which was eluted with a 35:65 mixture of CH_2Cl_2 (5% *i*-PrOH) and *n*-hexane at a flow rate of 4 mL/min. The desired product [^{123}I]-**1** eluted at 26–28 min was collected (Fig. 1) and the solvents were removed. The residue was resolubilized in ethanol and passed through a sterile membrane filter (0.22 μm), and then diluted with sterile saline to give a final solution of 10% ethanol in saline. Radiochemical purity of [^{123}I]-**1** was determined to be greater than 99% by a radio-TLC developed in a 1:1 mixture of ethyl acetate and *n*-hexane (Fig. 2). Effective specific activity of [^{123}I]-**1** was determined by HPLC method (Fig. 3). An aliquot of the final

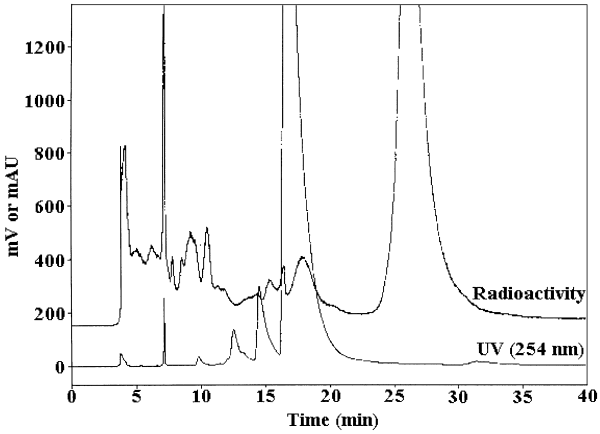


Figure 1. HPLC trace of purification of [^{123}I]iodopaclitaxel, [^{123}I]-**1**. Purification was carried out on a silica gel column (10 μ, 10×250 mm) eluted with a 35:65 mixture of CH_2Cl_2 (5% *i*-PrOH) and *n*-hexane at 4 mL/min. The eluent was simultaneously monitored by a UV detector (254 nm) and a NaI(T1) radioactivity detector. The peak eluted between 26 and 28 min is [^{123}I]-**1** and the major UV peak at 17–18 min is the trimethyltin precursor.

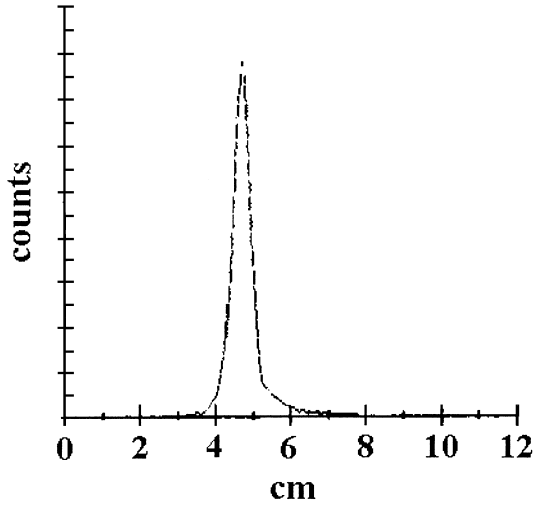


Figure 2. Radio-thin layer chromatogram of the purified [^{123}I]iodopaclitaxel.

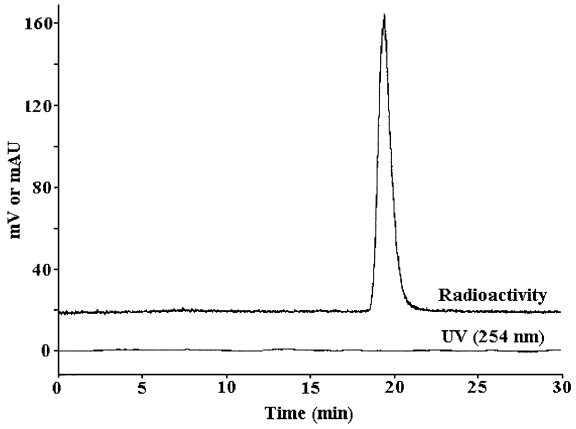


Figure 3. HPLC analysis of the purified [^{123}I]iodopaclitaxel in 10% ethanol-saline. Analysis was performed on an analytical C18 column (5 μ, 4.6×250 mm) eluted with a 30:70 mixture of 0.1 M NH_4HCO_3: methanol at 1 mL/min. The peak at 19–20 min is [^{123}I]-**1**.

solution was analyzed on an analytical C18 column eluted with a 30:70 mixture of 0.1 M NH_4HCO_3: methanol with a flow rate of 1 mL/min. The area of UV peak coeluted with [^{123}I]-**1** was compared to the standard curve obtained from different concentrations of the unlabeled standard **1** and the corresponding UV peak area. Radiochemical yield was 63–65% and effective specific activity was greater than 37 GBq/μmol. Total synthesis time including HPLC purification was 90 min. Authenticity of [^{123}I]-**1** was confirmed by coinjection with unlabeled standard **1** on HPLC.

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