Synthesis of the Monosodium Salt of Carbon-14 Labeled Paclitaxel (Taxol®) 2'-Ethyl Carbonate 7-Phosphonooxymethyl Ether, a Potential Prodrug of Paclitaxel

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Summary

The monosodium salt of Carbon-14 labeled paclitaxel (Taxol®) [N3'-^{14}COPh] 2'-ethyl carbonate 7-phosphonooxymethyl ether, was prepared from C-14 labeled paclitaxel [N3'-^{14}COPh] in 5 steps. The radiochemical purity of the final product was greater than 99% and the specific activity was 25 μCi/mg.

Keywords: Carbon-14, Paclitaxel, Taxol®, Prodrug

Introduction

Paclitaxel (Taxol®), a diterpene isolated from the bark of the Pacific yew tree (Taxus brevifolia) in the early 1970's, is now a well established anticancer drug for the treatment of ovarian and breast carcinomas.[1] Recently it was reported that the use of paclitaxel in combination with Platinol® was highly active and well-tolerated in the treatment of advanced ovarian cancer patients.[2] Large numbers of clinical studies are ongoing to evaluate the use of paclitaxel in combination with other anticancer agents in the treatment of several types of cancer including ovarian, breast, lung and head and neck cancer. In spite of paclitaxel's promising antitumor profile in the clinic, the drug is not ideally suited for systemic delivery via intravenous infusion due to its poor water solubility (0.25 μg/mL).[3,4]

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To overcome this solubility problem paclitaxel is formulated in a mixture consisting of 50% Cremophore EL® (polyethoxylated castor oil) and 50% absolute ethanol which is then diluted with saline prior to patient administration. The Cremaphore EL® is thought to contribute at least in part to some of the adverse effects experienced during treatments with paclitaxel.[5] Significant effort has been spent during the past few years trying to enhance the water solubility of paclitaxel, and thereby eliminate the need for forumulation in Cremaphore EL®, by synthesizing a hydrophilic prodrug which would liberate the desired agent in vivo.[6] This approach has been the subject of many recent publications from our laboratories.[6,7,8,9]

This paper describes the synthesis of the monosodium salt of Carbon-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate 7-phosphonooxymethyl ether, **1**, a potential prodrug of paclitaxel, which was needed for biodistribution studies in tumor bearing mice. The monosodium salt of carbon-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate 7-phosphonooxymethyl ether, **1**, was prepared from carbon-14 labeled paclitaxel [N3'-^{14}COPh],[10] **2**, in a five step synthesis (Scheme 1).

Results and Discussion

Conversion of carbon-14 labeled paclitaxel, **2**, to the corresponding carbon-14 labeled 2'-ethyl carbonate, **3**, was essentially quantitative. The conversion of **3** to the carbon-14 labeled C-7 methylthiomethyl ether, **4**, in DMSO/acetic anhydride provided the desired product in a 65% yield with the remainder of the mass was being found in two unidentified impurities. The crude methylthiomethyl ether, **4**, was used directly in the next step since previous studies with nonradioactive material had shown that complete removal of both impurities from the product was not possible via silica gel chromatography.

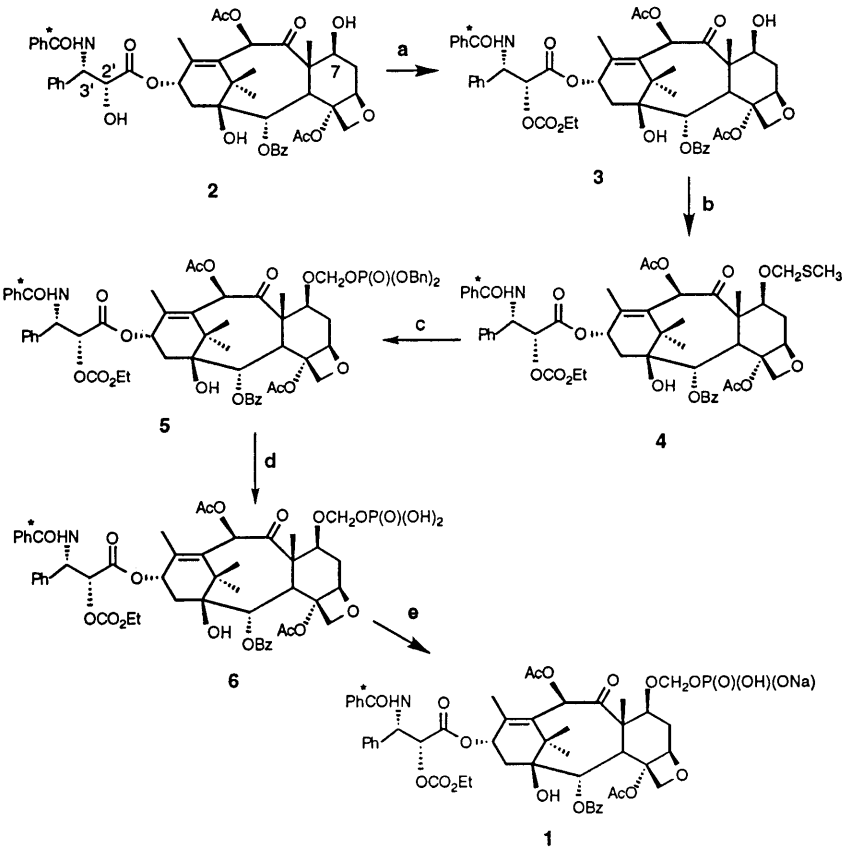
Synthesis of the carbon-14 labeled C-7 dibenzylphosphonooxymethyl ether, **5**, from the corresponding methylthiomethyl ether, **4**, was obtained in a 80% yield. Purification of **5** via flash chromatography was necessary since previous hydrogenation studies with crude nonradioactive dibenzylphosphonoxymethyl ether, **5**, resulted in a low yield of desired phosphonooxymethyl ether, **6**, with the major side product resulting from loss of the 2'-ethyl carbonate functionality. Conversion of the carbon-14 labeled C-7 phosphonooxymethyl ether of paclitaxel to the corresponding monosodium salt, **1**, followed by purification via C-18 column chromatography yield the desired product.

Experimental

Materials

Paclitaxel was obtained from Hauser Chemical Research, Inc. of Boulder, Colorado. Carbon-14 labeled paclitaxel [N3'-^{14}COPh] was

Scheme 1 Synthesis of the monosodium salt of Carbon-14 labeled Paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate 7-phosphonooxymethyl ether, **1**.



Reagents: (a) EtOCOCl, i-Pr$_2$EtN, CH_2Cl_2, Ar ; (b) Ac_2O, DMSO, Ar;
(c) N-iodosuccinimide, dibenzyl phosphate, 4A molecular sieves, CH_2Cl_2/THF;
(d) H_2, Pd/C, EtOAc; (e) $NaHCO_3$, H_2O, CH_3CN followed by C-18 chromatography.

prepared by Dr. D. Walker with a specific activity of 26.5 mCi/mmol and a radiochemical purity of 95%.[10] Preparative C18 125 A resin was obtained from Millipore Corporation of Milford, MA. Q12 ion pair cocktail (0.5 M solution of dodecyltriethylammonium phosphate) was obtained from Regis Chemical Company of Morton Grove, IL. All experimental conditions were optimized using non-labeled materials. All other reagents were obtained from Aldrich Chemical Company of Milwaukee, WI and were either ACS grade or the highest quality material commercially available. The identity of the final product was established by coelution of the radiolabeled material with authentic unlabeled compound on two

different HPLC systems. Radioactivity was measured on a Packard Tri-Carb liquid scintillation analyzer. Radiochemical purity was determined by HPLC. The HPLC system consisted of Rainin CPX pumps, a Rainin UV-1 detector for UV analysis and a *IN/US B*-RAM radioactive flowthrough detector for radioactivity measurements.

Analytical Methods

HPLC Method 1

In this method samples were loaded on a Jones C18 column (4.6 mm X 250 mm) equilibrated with 60% CH_3CN and 40% Buffer A (10 mmol NH_4HPO_4, pH 6.0, 0.005M Q12 ion-pairing reagent). Buffer A was prepared by adding a 10 ml aliquot of Q12 ion pair cocktail to a 1L solution of 10 mmol $NH_4H_2PO_4$, pH 6.0. The flowrate of the column was 1.5 ml/min.

HPLC Method 2

This method is identical to Method 1 except that the percentage of acetonitrile is increased to 70% CH_3CN.

HPLC Method 3

In this method samples were loaded on a Zorbax Cyano column (4.6 mm X 25 cm) equilibrated with 35% CH_3CN and 65% Buffer B (50 mmol NaH_2PO_4, pH 3). The flowrate of the column was 1 ml/min. In this analysis, a linear gradient starting at 35% CH_3CN and ending at 60% CH_3CN was run over 20 minutes. The linear gradient was started immediately after injection. After 20 minutes post-injection, the mobile phase was maintained for 5 minutes after which time a linear gradient starting at 65% CH_3CN and ending at 35% CH_3CN was completed during the next 15 minutes.

Synthesis

C-14 labeled Paclitaxel [N3'-^{14}COPh] 2'-Ethyl carbonate (3)

To a solution of Carbon-14 labeled paclitaxel [N3'-^{14}COPh][10], **2**, (500 mg, 0.585 mmol) dissolved in 6.25 ml of anhydrous CH_2Cl_2, was added diisopropylethylamine (227 mg, 1.750 mmol, 3 eq). The solution allowed to stir at RT for 5 minutes and then placed in an ice-bath for 10 minutes. After 10 minutes, ethyl chloroformate (190 mg, 1.75 mmol, 3 eq) was added and the solution allowed to stir under Ar for 3 h. After 3 h, CH_2Cl_2 (15 mL) was added to the reaction mixture and then the solution was extracted with cold 0.1N HCl (25 ml), followed by of 0.1N $NaHCO_3$ (25 ml), H_2O (25 ml), and brine (25 ml). All solutions were backextracted to maximize recovery. The CH_2Cl_2 layer was dried ($MgSO_4$), filtered and concentrated to a solid. The solid was then dried under vacuum at RT for 1 h to yield 490 mg of **3** as a white solid. The radiochemical purity of **3** was

91% (HPLC Method 1). In this system paclitaxel, **2**, has a R_t of approximately 2.4 min and paclitaxel 2'-ethyl carbonate, **3**, has a R_t of approximately 4.1 min. This material was used as is in the next step without further purification.

C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-methylthiomethyl ether, **4**).

To **3** (490 mg, 0.530 mmol) was added 4.0 ml of anhydrous DMSO and 4.0 ml of acetic anhydride. The solution allowed to stir at RT under Ar for 30 h. After 30 h, the reaction mixture was diluted with EtOAc (100 mL) and washed with 0.1N $NaHCO_3$ (3 X 50 ml), H_2O (2 X 50 mL) and brine (50 mL). The organic layer dried ($MgSO_4$), and concentrated on a rotary evaporator. The crude material was dissolved in a minimum amount of CH_2Cl_2 (1-2 mL) and applied to a silica gel column (2.5 X 15 cm). The material was eluted from the column with 60% EtOAc/hexane and the solution was concentrated in vacuo and then transferred to a 50 mL RB flask. The material was then dried under vacuum at RT to yield 400 mg of crude **4** as a white solid. The radiochemical purity of **4** was 83% (HPLC Method 1), with two unidentified radiolabeled impurities eluting at approximately 6.5 and 7.4 minutes. In this system, C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-methylthiomethyl ether, **4**, has a R_t of approxi- mately 8.5 minutes and C-14 labeled paclitaxel-N'3-^{14}C 2'-ethyl carbonate, **3**, has a R_t of approximately 4.1 minutes.

C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-dibenzylphosphonoxymethyl ether, **5**.

To a solution of crude **4** (400 mg, 0.406 mmol) in 6.5 mL of CH_2Cl_2 under Ar was added 2 g of activated 4A molecular sieves. To this was then added a solution of N-iodosuccinimide (0.100 g, 0.00047 mol, 1.7 eq) and 0.130 g (0.00046 mol, 1.7 eq) of dibenzylphosphate dissolved in 5 mL of anhydrous THF. The solution was allowed to stir at RT and within 15 minutes the solution had turned a reddish brown color. The reaction was allowed to stir for 3 h. After 3 h, the reaction mixture was filtered through Celite bed and the bed rinsed with CH_2Cl_2 (10 mL). The reddish brown solution was then concentrated on a rotary evaporator to a viscous syrup, and then diluted with EtOAc (50 mL). The organic layer was rinsed with 1% $Na_2S_2O_3$ (30 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated on a rotary evaporator to yield **5**, as a crude product. The crude product was dissolved in CH_2Cl_2 (1.5 mL) and applied to a silica gel column (5 x 2.5 cm). (An additional 4 mL of EtOAc and 4 mL of 50% EtOAc/hexane was added to the column to assist the crude material entering the column bed). The column was then eluted with 30% EtOAc (500 mL) followed by 40% EtOAc (1L). The effluent from the column was collected in 100 mL aliquots. Fractions containing the desired compound were then combined and concentrated on a rotary evaporator to yield 400 mg of **5**. (*Care must be taken to minimize the length of time that **5** stays on the column during this purification since we have observed various degrees of product decomposition during this*

procedure). The radiochemical purity of **5** was 92% (HPLC Method 2). In this system, C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-dibenzylphosphonoxy methyl ether, **5**, has a R_t of approximately 6.8 minutes and C-14 labeled paclitaxel-N'3-^{14}C 2'-ethyl carbonate-7-methylthiomethyl ether, **4**, has a R_t of approximately 3.7 minutes.

C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-phosphonooxymethyl ether (**6**).

To a 250 mL Parr bottle containing 670 mg of 10% Pd/C was added **5** (400 mg, 0.330 mmol) dissolved in EtOAc (40 mL). The solution was hydrogenated at 50-60 psi of H_2 for 16 h. After 16 h, the solution was filtered through a Celite bed and then passed through a 0.45 micron filter (to remove residual catalyst), and concentrated on a rotary evaporator to yield 270 mg of **6**. The radiochemical purity of **6** was 88% (HPLC Method 2). In this system, C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-phosphonooxymethyl ether, **6**, has a R_t of approximately 1.1 minutes and C-14 labeled paclitaxel-[N3'-^{14}COPh] 2'-ethyl carbonate-7-dibenzyl- phosphonoxy methyl ether, **5**, has a R_t of approximately 6.8 min. The crude product, **6**, was used in the next step without further purification.

C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-phosphonooxymethyl ether, monosodium salt, **1**.

Into a 1L RB flask containing **6** (270 mg, 0.260 mmol) was added CH_3CN (18 mL) and the mixture was sonicated at RT for 10 minutes. To this solution was then added $NaHCO_3$ (21.6 mg, 0.257 mmol) dissolved in H_2O (3 mL). Within minutes a white flocculent solid appeared in the flask. To this mixture was added CH_3CN (45 mL) and H_2O (90 mL). The suspension was then sonicated for 1 h during which time the suspension slowly went into solution. After 1 h, the solution was diluted with H_2O (800 mL) and applied to a C-18 resin column (2.5 x 50 cm). The flowrate of the column was approximately 7-8 mL/min. The eluent was monitored by HPLC (Method 1). The column was rinsed with H_2O (400 mL), and then the eluent was changed to 50% CH_3CN. Fractions (20 ml) containing the desired product were combined and the acetonitrile removed on a rotary evaporator (bath temp<30°C.). The aqueous solution was then lyophilized to yield 230 mg of **1**. The radiochemical purity of **1** as determined by HPLC Method 1 was only 95%. Therefore a second purification was necessary and a 90 mg sample of **1** was repurified via the above method (scaled down to reflect the smaller amount of material) to yield 66 mg of **1**. The radiochemical purity of the repurified sample as determined by HPLC Methods 1 and 3 was over 99%. The R_t of C-14 labeled paclitaxel [N3'-^{14}COPh] 2'-ethyl carbonate-7-phosphonooxymethyl ether, monosodium salt, **1**, in HPLC Methods 1 and 3 is approximately 1.8 minutes and 13.7 minutes respectively. The specific activity of **1** was found to be 25 μCi/mg.

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Footnotes

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