Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 933-941 (2000)

Synthesis of the Taxane Analogue PNU-105298 Labelled with Deuterium and with Carbon-14

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SUMMARY: In this paper the preparations of the taxane analogue PNU-105298 (**1**), labelled with deuterium and with carbon-14 are described. A three-step sequence starting from the commercially available (*tert*-[$^2H_9$]butyl)amine afforded the deuterium specifically labelled final compound [$^2H_9$]PNU-105298 (**1a**). The preparation of the carbon-14 labelled final compound [^{14}C]PNU-105298 (**1b**) was carried out in a similar fashion yielding the radiochemically pure product with a specific activity of 2.15 GBq/mmol.

KEYWORDS: PNU-105298, Taxane Analogue, Paclitaxel, Deuterium, Carbon-14, Antitumor Agent.

INTRODUCTION

PNU-105298 (**1**, see Figure 1) belongs to a new class of taxane analogues discovered at Pharmacia & Upjohn in carrying out a program whose goal was to find a product more potent and less toxic than paclitaxel (Taxol®).

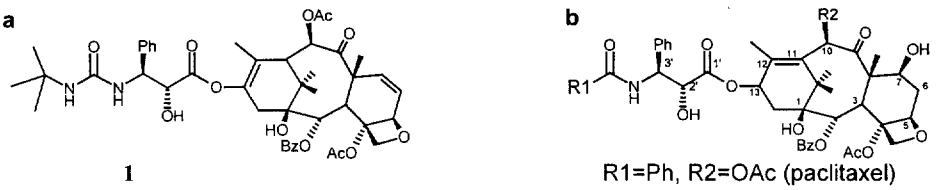

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Figure 1. a) The taxane analogue PNU-105298; b) Structure of taxanes

Received 30 March 2000
Revised 10 April 2000
Accepted 8 May 2000

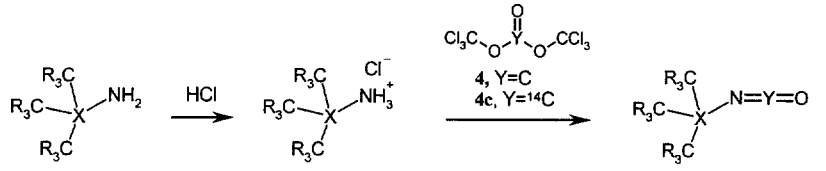
As with paclitaxel, the anticancer activity of these compounds involved an unusual ability to interfere with the microtubuletubulin system, which stops the cellular division [ref.1]. In comparison with paclitaxel, **1** has three structural modifications: the 3' *tert*-butylurea (TBU) terminus in the C13 side-chain [ref.2], the double bond at the 12-13 position instead of 11-12 [ref.3]and the 6-7 double bond that substitutes the C7 hydroxy group [ref.4]. As the development of the drug candidate has progressed, a specifically labelled carbon-14 form of PNU-105298 was required for metabolism and disposition studies. Moreover, as it is generally agreed that the stable isotopically labelled analogues with the same molecular structure of a compound are the best internal standards (IS) for a liquid chromatography-mass spectrometry (LC-MS) assay, a non-radioactive labelled form of the compound under investigation was required for use as IS. This IS should have a molecular weight at least three mass unit higher than that of the non-labelled material. The radiolabelling of the acetyl groups was avoided, as in the literature of paclitaxel it is reported that a small amount ofdeacetylation could occur [ref.5]. Preliminary *in vivo* metabolism studies carried out in mice with the unlabelled PNU-105298 as well as its MS fragmentation, indicated that the C13 side chain could allow a convenient carbon-14 or stable labelled isotopes introduction. In this paper the syntheses of deuterium and carbon-14 specifically labelled forms of the title compound are described.

DISCUSSION AND RESULTS

The chemistry of taxanes is not trivial due to the unexpected and unusual transformations of the highly functionalized diterpenoid nucleus. Several research groups reported the preparation of paclitaxel or its analogues specifically labelled in the C13 side chain with carbon-14 [ref.6a-d] or tritium[ref.7]. All these preparations are based on coupling a radiolabelled side-chain synthon, obtained through multi-step syntheses, with a suitably protected baccatin ring systems. In case of **1**, the TBU moiety seemed at first glance to be the most accessible site to label. Among the several possible methods to prepare the *tert*-butylisocyanate from which the TBU

moiety can be obtained [ref.8], the pathway outlined in Scheme 1 was chosen. As shown in this scheme, by means of the same synthetic steps, the introduction of the label in different TBU positions could be achieved starting from commercially available labelled materials (i.e. **2a**, **2b** or **4c**).

Scheme 1.



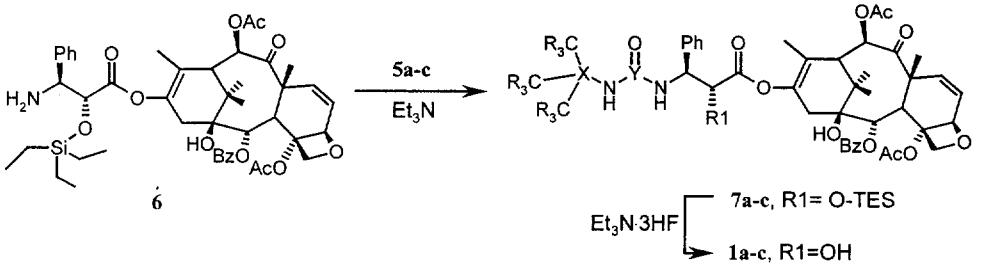
2a, R=2H; X=C
2b, R=H; X=14C

3a, R=2H; X=C
3b, R=H; X=14C

5a, R=2H; X=Y=C
5b, R=H; X=14C; Y=C
5c, R=H; X=C; Y=14C

Two different approaches to prepare the stable- and radio-labelled analogues with the same molecular structure of **1** were then considered using the labelled isocyanates **5a-c** as precursors. Both routes were based on modifications of known methods carried out with good results during the preparation of other unlabelled taxane analogues [ref.2].

Scheme 2.



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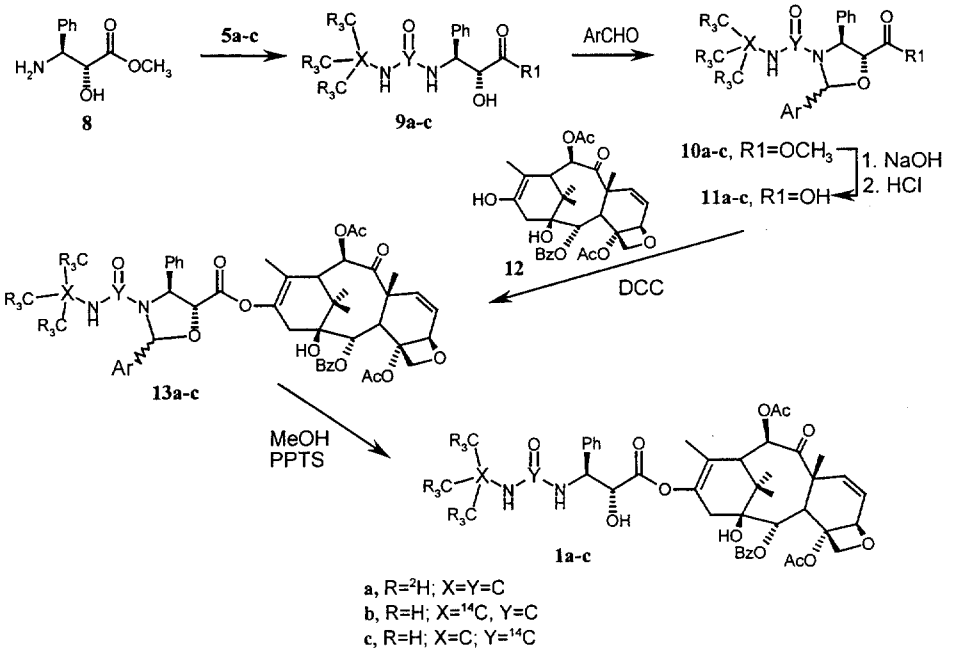
a, R=2H; X=Y=C
b, R=H; X=14C, Y=C
c, R=H; X=C; Y=14C

The first one is depicted in Scheme 2 and involved the reaction of the labelled isocyanates **5a-c** with the taxoid amine **6** to give the labelled intermediates **7a-c**. The removal of the 2'-OH triethylsilyl-protecting group (TES) afforded the labelled final compounds **1a-c**. Another possibility (see Scheme 3) foresaw the preparation of the TBU-labelled oxazolidines **11a-c** through the N-acylation of the phenylisoserine methyl ester (**8**) with the labelled isocyanates **5a-c**, subsequent reaction with a

suitable aromatic aldehyde and final methyl ester cleavage. The key side-chain precursors **11a-c** coupled with the modified baccatin enol **12** afforded the intermediates **13a-c**, from which the labelled final compounds **1a-c** were obtained after removal of the oxazolidine protecting group.

Scheme 3.



Due to the availability of the synthon **6** as well as the stability of the intermediates involved, the method shown in Scheme 2 seemed to be more attractive to prepare the two labelled compounds (out of the three possible) [$^2H_9$]PNU-105298 (**1a**, R=^{2}H; X=Y=C) and [^{14}C]PNU-105298 (**1b**, R=H; X=^{14}C; Y=C) that were selected for use.

The perdeuterated isocyanate **5a** was prepared by treatment of (*tert*-[$^2H_9$]butyl)amine (**2a**) with HCl followed by reaction of the resulting hydrochloride **3a** with *bis*(trichloromethyl)carbonate (**4**) in dioxane at reflux. An excess of the crude **5a** in dioxane/*n*-hexane solution in the presence of triethylamine was used to acylate the taxoid amine **6**. The obtained crude compound **7a** was purified by flash chromatography and preparative high performance liquid chromatography (HPLC).

After the 2'-O-TES cleavage with triethylamine trihydrofluoride ($Et_3N{\cdot}3HF$) in acetonitrile, the final compound **1a** was obtained as a white solid >97% chemically pure. The chemical yield was about 50% from **6**. The isotopic enrichment was >98% of ^2H atom in the *tert*-butyl moiety and MS and ^1HNMR data were in agreement with those of the non-labelled material. The carbon-14 labelled form of PNU-105298 was prepared in a similar manner yielding **1b** with a radiochemical purity >97% and a specific activity of 2.15 GBq/mmol. MS data were in agreement with that from the non-labelled material. Although the above product was stored as a dry powder under argon atmosphere at -20 °C in the dark, a radiochemical purity loss of about 10% during three months after its preparation was observed. As a radiochemical purity of about 90% was not suitable for most of the scheduled studies, a preparative-HPLC method was developed to purify **1b**.

EXPERIMENTAL

General Methods: (*tert*-[$^2H_9$]butyl)amine (**2a**, >98% ^2H in the *tert*-butyl moiety) was purchased from Isotec. The N-acylation of **6** with **5b** to afford the TBU-labelled side chain of **7b** was performed by Amersham Int. (Specific activity: 2.15 GBq/mmol) according to a provided method. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Activity measurementsby liquid scintillation counting were performed on a Tri-Carb 2100 TR liquid scintillation analyzer (Packard) using Rialuma (Lumac System) as liquid scintillation cocktail. Counting efficiencies were calculated by transformed spectral index of the external standard (tSIE). Chemical purities were determined by HPLC performed at 25°C using a series-200 pump (Perkin-Elmer) equipped with a LC-295 UV/VIS detector (Perkin-Elmer) and PE-Nelson Turbochrom 4.0 software. Radiochemical purities were determined by HPLC using an A-515TR radio-HPLC analyser (Packard) equipped with a 0.5 ml homogeneous cell (liquid scintillation cocktail:Ultima Flo-M (Packard); ratio to HPLC effluent: 2/1). Preparative-HPLC was performed at 25°C using a series 410 pump (Perkin-Elmer) equipped a SP-100 UV/VIS detector

(Spectra-Physics) on line with a model 561 recorder (Perkin-Elmer) and a B684 fraction collector (Büchi).

***tert*-[$^2H_9$]Butylisocianate (5a)**: to a cooled (10°C) solution of**2a** (700 mg, 8.5 mmol) in dry dioxane (7 ml), 4N HCl (anhydrous, dioxane solution, 2.15 ml; 8.6 mmol) was slowly added with stirring under nitrogen. After 10 min. a white precipitate of**3a** was obtained, then a solution of **4** (950 mg, 3.1 mmol) in dry dioxane (7 ml) was added to the above cooled suspension and a reflux condenser was connected to the reaction flask. The mixture was refluxed with stirring under nitrogen for 5 hours, then the suspension was cooled to room temperature (r.t.), diluted with*n*-hexane (about 13 ml) and stirred for further 10 min. After removal of the non-reacted**3a** by filtration under nitrogen, a portion (10 ml out of 26 ml) of the yellowish acidic solution of**5a** was rapidly washed in a separating funnel with cold water containing crushed ice (2 x 10 ml). This clear light yellowish neutral solution was immediately used in the next step.

2'-Triethylsilyl-N-debenzoyl-N-(*tert*-[$^2H_9$]butyl)aminocarbonyl-7-deoxy-$\Delta^{6,7}$-12,13-*iso*taxol (7a): the cooled solution of **5a** in dioxane/*n*-hexane (1:1 by vol., 10 ml) prepared in the previous step was added with a solution of**6** (71.5 mg, 84.5 μmol) in dry dioxane (about 2 ml), TEA (20 μl) and stirred at r.t. for 3.5 hours. The reaction mixture was stored at -20°C overnight then stirred again atr.t.. After 24 hours the solvents were removed under vacuum and a white glassy residue was recovered. The HPLC [ref.9] and MS analyses showed that the compound **7a** and three main unidentified by-products were present in the above residue with the following retention times (r_t) and m/z (FAB+) values: **7a**, r_t~26', m/z=954; unknown 1 (**UK1**), r_t~25', m/z=918; unknown 2 (**UK2**), r_t~25.5', m/z=996 and unknown 3 (**UK3**), r_t~28.5', m/z=979. The crude material was flash-chromatographed on a silica gel column eluting with mixtures of*n*-hexane/ethyl acetate from 8:2 to 7:3 by vol. After combination and evaporation of the collected fractions, two samples containing**7a** were obtained: sample A (24.6 mg), 96% chemically pure (**UK3** < 3%) and sample B (8.2 mg) 85% chemically pure (**UK3** about 10%). The sample B was dissolved in a mixture of acetonitrile/water and purified by preparative-HPLC [ref.10]. After solvent

evaporation, **7a** (7.4 mg) was recovered >98% chemically pure. This product was added to the sample A obtaining **7a** as a white glassy solid (30.7 mg) to be used without further purification in the next step.

N-Debenzoyl-N-(*tert*-[$^2H_9$]butyl)aminocarbonyl-7-deoxy-$\Delta^{6,7}$-12,13-*iso*taxol (1a): to a cooled (4°C) solution of **7a** (30.7 mg, 32 μmol) in dry acetonitrile (500 μl), $Et_3N.3HF$ (110 μl, 640 μmol) was added with stirring under argon over two minutes and the solution was allowed to warm to r.t.. After 30 minutes the reaction mixture was diluted with ethyl acetate (8 ml) and 8% $NaHCO_3$ (4 ml) was dripped into the reaction flask under vigorous stirring. The two phases wereseparated and the organic layer was washed with water, brine and dried (Na_2SO_4). After solvent evaporation to dryness, **1a** (27.8 mg) was recovered >97% chemically pure (by HPLC [ref.11]). The overall yield from **6** was about 50%. MS (FAB+): m/z 840 (13, $[MH]^+$); m/z 732 (5, $[M-(CD_3)_3CNCO+2H]^+$); m/z 272 (45, $[(CD_3)_3CNHCONHCH(C_6H_5)CH(OH)CO]^+$); m/z 244 (24, $[(CD_3)_3CNHCONHCH(C_6H_5)CH(OH)]^+$); m/z 136 (100, $[H_2NCH(C_6H_5)CHOH]^+$); m/z 106 (29, $[H_2NCH(C_6H_5)]^+$); m/z 105 (29, $[(C_6H_5)CO]^+$). NMR (^{1}HNMR; $CDCl_3$; 400 MHz): 1.05 and 1.30 δ (two singlets, 6H, 16+17); 1.50 δ (s, 3H, 18); 1.68 δ (s, 1H, OH-1); 1.74 δ (s, 3H, 19); 2.09 δ (d, J=18.7 Hz, 1H, 14β); 2.19 and 2.62 δ (two singlets 6H, two CH_3CO); 2.73 δ (s, 1H, 11); 2.95 δ (d, J=18.7 Hz, 1H, 14α); 3.37 δ (d, 4.1 Hz, 1H, OH-2'); 3.68 δ (d, J=5.6 Hz, 1H, 3); 4.26 δ (s, 1H, NH-6'); 4.34 and 4.54 δ (two doublets, J=8.2 Hz, 2H, 20); 4.71 δ (dd, J=4.1and 2.7 Hz, 1H, 2'); 4.95 δ (d, J=9.1 Hz, 1H, NH-4'); 5.13 δ (d, J=5.0 Hz, 1H, 5); 5.17 δ (s, 1H, 10); 5.52 δ (dd, J=9.1and 2.7 Hz, 1H, 3'); 5.72 δ (d, J=5.6 Hz, 1H, 2); 6.05 δ (m, 2H, 6+7); 7.2-8.2 δ (m, 10H, two Ph).

N-Debenzoyl-N-(*tert*-[1-^{14}C]butyl)aminocarbonyl-7-deoxy-$\Delta^{6,7}$-12,13-*iso*taxol (1b): to a cooled (4°C) solution of **7b** (20.8 MBq; 9.2 mg; 9.67 μmol) in dry acetonitrile (150 μl), $Et_3N{\cdot}3HF$ (34 μl, 206 μmol) was slowly added with stirring under argon and the solution was allowed to warm to r.t.. After 30 minutes the reaction mixture was diluted with ethyl acetate (1.5 ml), then 8% $NaHCO_3$ (2 ml) was dripped under

vigorous stirring. The two phases were separated and the organic layer washed with brine and dried (Na_2SO_4). After solvent evaporation to dryness, **1b** (17.8 MBq) was recovered >97% radiochemically pure. The radiochemical yield of this step was about 86% from **7b**. A ten-fold higher scale gave radiochemically pure **1b** with a 95% yield. *HPLC-Purification*: a batch of **1b** 91% radiochemically pure (55.5 MBq) was dissolved in a mixture of acetonitrile/water and purified by preparative-HPLC [ref12]. The fractions containing the compound were combined and after evaporation to dryness, **1b** (51.8 MBq) was recovered >97% radiochemically pure (by radio-HPLC [ref.11]). The radiochemical yield of the purification step was about 90%.

ACKNOWLEDGMENTS

The authors would like to thank Robert C. Kelly, P&U Medicinal Chemistry, Kalamazoo (Michigan) for helpful discussions during the course of this work and his group for preparing the taxoid amine **6**. We are also grateful to Vittorio Pinciroli and Emanuele Arlandini, P&U Structural & Predevelopment Analysis, Nerviano (Milan) for NMR and MS spectra.

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9. Zorbax TMS column (mm 250 x 4.6 ID, 5μm, supplied by RTI) at 25°C eluting with acetonitrile (A) and 50mM phosphatebuffer at pH=3 (B) mixtures: isocratic at 60% A for 10 min, linear gradient from 60% to 78% A over 15 min., isocratic at 78% A for 15 min. Flow rate: 1 ml/min. Analytical wavelength: 210nm.

10. Zorbax TMS column (mm 250 x 9.4 ID, 5μm, supplied by RTI) at 25°C eluting with acetonitrile (A) and water (B) mixtures: isocratic at 70% A for 20 min, linear gradient from 70% to 90% A over 15 min., isocratic at 90% A for 10 min. Flow rate: 4.5 ml/min. Analytical wavelength: 210nm.

11. Symmetry C8 column (mm 250 x 4.6 ID, 5μm, supplied by Waters) at 25°C eluting with methanol (A) and water (B) mixture: isocratic at 80% A for 30 min. Flow rate: 1 ml/min. Analytical wavelength: 210nm.

12. Zorbax TMS column (mm 250 x 9.4 ID, 5μm, supplied by RTI) at 25°C eluting with acetonitrile (A) and water (B) mixtures: isocratic at 50% A for 20 min, linear gradient from 50% to 85% A over 15 min., isocratic at 85% A for 10 min. Flow rate: 4.5 ml/min. Analytical wavelength: 210nm.

