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Note

High specific activity tritium labeling of anti-tumor agent CEP-2563

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Summary

[Ester Methylene-^{3}H] CEP-2563 (**9**) was synthesized by the tritiation of precursor **6** with NaB^3H_4 followed by esterification with BOC-Lys(BOC)-β-Ala-OH and deprotection. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: anti-tumor; CEP-751; CEP-2563; NaB^3H_4; tritium

Introduction

The indolocarbazole alkaloid (+)-K-252a (**1**) is an inhibitor of several serine/threonine kinases as well as the tyrosine kinase domain of the high affinity nerve growth factor (NGF) receptor *trk A*. Because NGF also appears to play a key role in cancer growth, a program was initiated using **1** as a template to identify other candidate lead compounds as anti-tumor agents. Emerging from that effort was a promising compound CEP-751 (**2**).[1] However, one drawback to CEP-751 as well as other members of this compound class was its poor water solubility. To enhance the water solubility a series of soluble esters were prepared as a pro-drug approach.[2] The optimum compound identified based on aqueous solubility and stability to serve as an intravenous pro-drug form was the dipeptide, Lys-β-Ala ester, CEP-2563 (**3**). For further biological evaluation tritium labeled CEP-2563 was needed and this paper describes the synthetic method used to accomplish that.

Discussion

To tritium label **3** we employed a strategy somewhat similar to that earlier utilized for the tritiation of CEP-1347 (**4**).[3] At that time *bis*-aldehyde precursor

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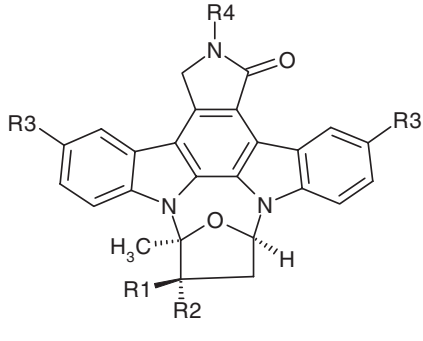
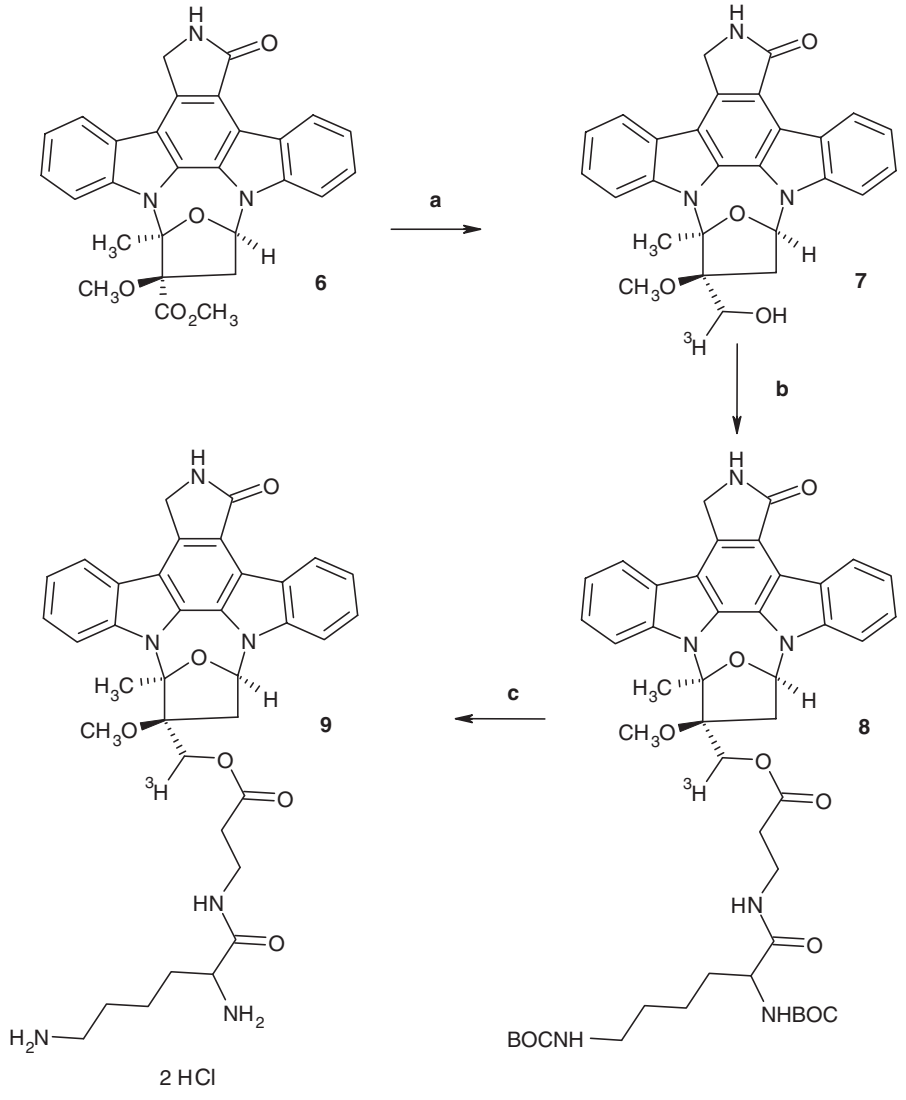
5 was reduced with high specific activity NaB^3H_4 to yield a [hydroxymethylene-^{3}H] intermediate which was further elaborated to afford [hydroxymethylene-^{3}H] CEP-1347. In this earlier work the use of stoichiometrically limited NaB^3H_4 for a short reaction time (30 min) at low temperature (0°C) prevented concomitant reduction of the methyl ester. In the current campaign we reasoned that reduction of **6**[4] with excess NaB^3H_4 at elevated temperature for a longer time period should give [hydroxymethylene-^{3}H] CEP-751 (**7**). Although not common, such a reduction of esters by sodium borohydride does have precedent.[5] In our hands the transformation of **6** to **7** went very smoothly and in good yield (Scheme 1).

The synthesis was completed in a manner similar to that reported for the unlabeled compound.[2] Our initial concern was the effect that the very small scale needed for the esterification and deprotection would have on the required chemistry, but this was also achieved in satisfactory yield to provide mCi quantities of [ester methylene-^{3}H] CEP-2563 (**9**). One small modification was noted in the deprotection step. Initially we had planned to remove the BOC groups with HCl saturated ethyl acetate, but an incomplete reaction was observed. We found it far more efficient to use trifluoroacetic acid for 1 h at ambient temperature. Product **9** was found to be essentially radiochemically homogeneous by HPLC and co-chromatographed with authentic standard **3**. The high specific activity of 35.3 Ci/mmol measured for **9** is also consonant with the NaB^3H_4 reduction of a methyl ester.



1 K-252a	R1 = OH	R2 = CO_2CH_3	R3 = H	R4 = H
2 CEP-751	R1 = CH_3O	R2 = CH_2OH	R3 = H	R4 = H
3 CEP-2563	R1 = CH_3O	R2 = CH_2O-β-Ala-Lys	R3 = H	R4 = H
4 CEP-1347	R1 = OH	R2 = CO_2CH_3	R3 = CH_2SEt	R4 = H
5	R1 = OAc	R2 = CO_2CH_3	R3 = CHO	R4 = Ac
6	R1 = CH_3O	R2 = CO_2CH_3	R3 = H	R4 = H



Scheme 1. Preparation of 9.
Reagents and conditions: (a) NaB^3H_4, CH_3OH/THF, 24°C, (b) BOC-Lys(BOC)-β-Ala-OH DCC/DMAP 24°C and (c) TFA, HCI, 24°C

Experimental

Evaporations were carried out on a Buchi rotary evaporator (Model RE 111) *in vacuo* at bath temperatures less than 40°C. TLC was performed on Analtech plates coated with silica gel (250 μm for analytical (5 × 15 cm) and 500 μm (20 × 20 cm) for preparative). Autoradiography was performed at 0°C after spraying with PPO and exposing the plates to X-ray film. TLC plates were also scanned (~3 min) for radioactivity (~10 μCi) using a Vangard Autoscanner.

Analytical and preparative HPLC were performed on a Waters instrument (Model 510 pump) with peak detection done simultaneously by UV (280 nm - Waters 440 UV detector) and a Beta RAM Model 3 radioactivity detector. Solution radioassays were conducted with a Beckman Model LS 3801 instrument. All chemicals used were reagent grade.

[Hydroxymethylene-^{3}H] CEP-751 (7)

To a solution of 25 mg (0.052 mmol) of precursor **6** in 2 ml of THF:methanol (4:1) was added 20 mg (0.52 mmol, 42 Ci at 80 Ci/mmol) of NaB^3H_4 and the reaction was stirred at ambient temperature overnight. After this time the reaction was quenched with 2 ml of water and volatile tritium was removed by several evaporations of ethanol. The crude product (2437 mCi) was dissolved in 50 ml of methanol and by analytical TLC (chloroform:methanol:ammonium hydroxide (9:1:0.1)) it showed ~85% of a main radioactive peak that co-chromatographed with authentic **2**. A 975 mCi portion of this crude product was purified by TLC on two plates (same system as above) with authentic unlabeled standard **2** allowed to migrate at the side of each plate to facilitate product location by UV. After plate development and UV visualization, the appropriate yellow band was scraped and eluted with a 50 ml of chloroform to afford 342 mCi of intermediate **7** (an extrapolated 47% radiochemical yield based on precursor **6**). Purified product **7** was observed to be >95% radiochemically pure by analytical TLC (same system as above) and was used directly for the next step.

[EsterMethylene-^{3}H] CEP-2563 (9)

Precursor **7** (342 mCi, 0.01 mmol) was rotary evaporated to near dryness and reconstituted in 0.2 ml of anhydrous methylene chloride. To this was added 7 mg (0.017 mmol) of BOC-Lys(BOC)-β-Ala-OH along with 1.8 mg (0.015 mmol) of 4-dimethylaminopyridine in 0.011 ml of methylene chloride and the solution was stirred for 5 min at ambient temperature. After this time 3 mg of dicyclohexylcarbodiimide (0.015 mmol) in 0.012 ml of methylene chloride was added and the reaction was stirred for 4 h at ambient temperature. The reaction was then rotary evaporated to near dryness, reconstituted in 0.5 ml of ethyl acetate and filtered free of a white solid to afford a filtrate with 313 mCi of activity. An analytical TLC (same system as above) showed ~80% of a main radioactive peak that co-chromatographed with authentic standard unlabeled **8** and this intermediate was used directly for the next deprotection step. All of intermediate **8** was rotary evaporated to near dryness, reconstituted in 10 ml of trifluoroacetic acid (TFA) and stirred at ambient temperature for 1 h. After this time the reaction was rotary evaporated to near dryness and slurried with 15 ml of ethyl acetate, revealing

a solid. The solid was isolated by filtration and itself dissolved in 20 ml of ethanol to afford 88 mCi of crude product **9**. An analytical TLC showed ~80% of a main radioactive peak that co-chromatographed with authentic standard **3**. Final purification was accomplished by preparative HPLC. All of crude product **9** was rotary evaporated to near dryness, dissolved in 2 ml of the mobile phase acetonitrile:0.2% aq TFA (35:65) and purified by reverse phase HPLC. Appropriate fractions were combined to afford 52 mCi (an extrapolated 15% radiochemical yield based on precursor **7**) of product **9** which was found to be 97.4% radiochemically pure by reverse phase HPLC (same system as above) and co-eluted with authentic standard **3**. Also, its specific activity was measured to be 35.3 Ci/mmol by UV where $E_{290} = 50\,452$ for **3**. The UV spectrum of **9** was completely superimposable on that of **3** as well .

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