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Research Article

Tritiation of CEP-1347 at high specific activity using several methods

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Summary

[Thiomethylenes-^{3}H] CEP-1347 (**5**) was synthesized by the tritiation of diformyl precursor **3** with NaB^3H_4 followed by treatment with ethanethiol. [Methyl ester-^{3}H] CEP-1347 (**7**) was prepared at even higher specific activity by the alkylation of precursor **6** with C^3H_3I. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: CEP-1347; C^3H_3I; NaB^3H_4; neurotrophic; tritium

Introduction

CEP-1347 (**2**), a semi-synthetic derivative of the indolocarbazole natural product (+) K-252a (**1**) (Figure 1), promotes neuronal survival by blocking the JNK signalling cascade via inhibition of the mixed lineage kinases (MLKs) and is currently in late phase III clinical trials for Parkinson's disease.[1–6] To support studying this novel mechanism, [^{3}H] CEP-1347 at high specific activity was desired for use in radioligand binding assays. This paper describes two synthetic methods to label [^{3}H] CEP-1347 in different regions of the molecule. The first approach was to incorporate tritium at the 3- and 9-thiomethylene positions on the heteroaryl rings via a reductive tritiation of the diformyl precursor using NaB^3H_4 and the second strategy was to incorporate tritium on the methyl ester of **2** at even higher specific activity.

Discussion

The first approach employed for the tritiation of **2** is seen in Scheme 1 and closely paralleled the unlabelled synthesis strategy.[2,3,6] Diformyl precursor **3**

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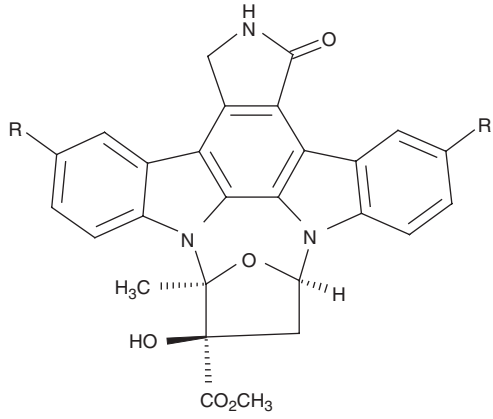
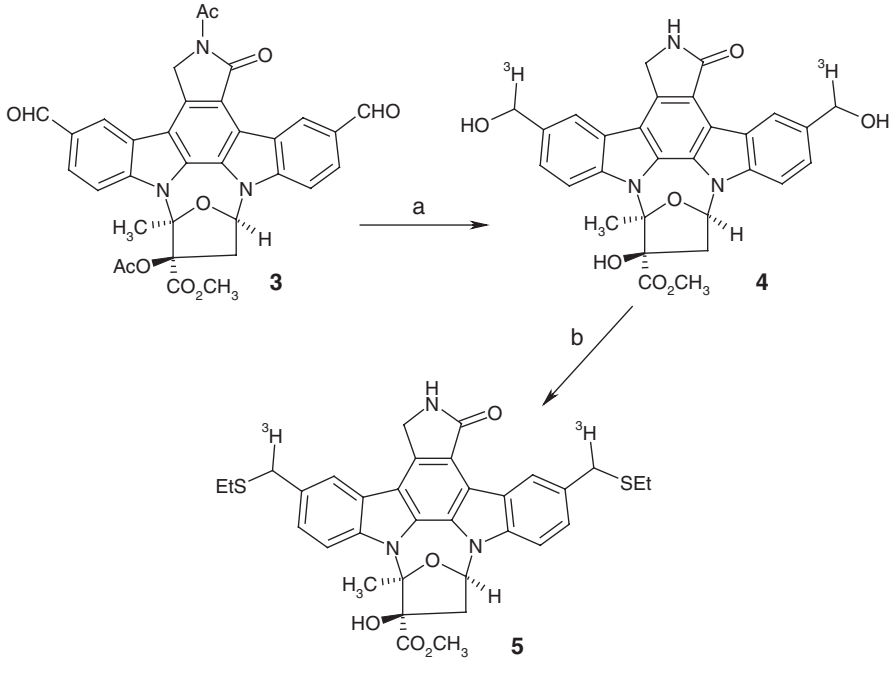


1 (+)-K-252a R = H

2 CEP-1347 R = CH_2SEt

Figure 1.



Scheme 1. Preparation of 5. Reagents and conditions. (a) NaB $^3H_4$, CH_3OH/$CHCl_3$, 0°C, (b) EtSH, CH_2Cl_2, TFAA, 24°C

was treated with NaB^3H_4, producing intermediate **4**. Interestingly, not only were both aldehyde groups reduced but apparently the reaction conditions were sufficiently basic enough to also promote the concomitant hydrolysis of both the acetamide and acetate protecting groups. This was not observed in

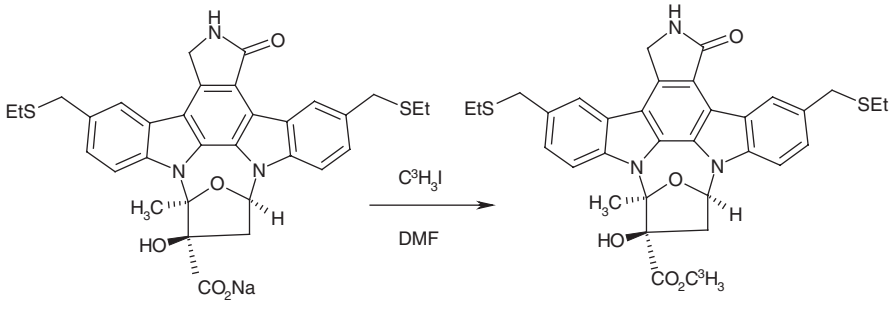
the unlabelled synthesis where a separate hydrolysis step using sodium methoxide was needed. Treatment of **4** with ethanethiol afforded **5** of sufficient purity for biological testing. Product **5** was characterized by TLC and HPLC co-chromatography with **2** as well as UV analysis and its specific activity of 27.4 Ci/mmol was in complete agreement with our experience for such NaB^3H_4 aldehyde reductions.

[^{3}H] **5** was used to develop a recombinant human MLK3 binding assay. Competition studies at 4°C with a 2-h incubation showed ATP displaced [^{3}H] **5** with an IC_{50} value of 65 μM. The IC_{50} value for CEP-1347 to displace [^{3}H] **5** was 0.39 nM, compared to an IC_{50} value of 23 nM in an MLK3 ELISA-based enzyme assay.[3]

To tritiate **2** at even higher specific activity an entirely different approach was required and is illustrated in Scheme 2. This alternate strategy involved tritium labelling of the methyl ester and although this method has not been commonly employed, it is not without encouraging precedent. For instance, the successful tritiation and valuable neurochemical applications of [methyl ester-^{3}H] rauwolscine and [methyl ester-^{3}H] yohimbine have generated hundreds of published studies in the alpha-2 adrenergic area.[7] Our initial campaign to prepare **7** was an attempt to form the acid chloride of **6** with oxalyl chloride followed by reaction with C^3H_3OH, but no product was obtained. However, the method of Scheme 2 worked very well and afforded product **7** which was characterized in essentially the same way as that of **5**. The UV derived specific activity of 67.8 Ci/mmol for **7** was again entirely reasonable for the method employed.

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



6 **7**

Scheme 2. Preparation of 7

(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 of reagent grade.

*[Thiomethylenes-^{3}H] CEP-1347 (**5**)*

To a solution of 30 mg (0.05 mmol) of diformyl precursor **3** in 2 ml of chloroform:methanol (7:1) was added 10 mg (0.26 mmol, 21 Ci at 80 Ci/mmol) of NaB^3H_4 for 30 min at 0°C. 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 (1698 mCi) was dissolved in 120 ml of chloroform:methanol (1:1). A 300 mCi portion of this crude reaction was purified by TLC on one plate developed with chloroform:methanol:ammonium hydroxide (8:2:0.1) with authentic unlabelled standard allowed to migrate at each side of the plate to facilitate product location by UV. After plate development and UV visualization, the appropriate band was scraped and eluted with a 50 ml solution of chloroform:methanol (1:1) to afford 164 mCi of intermediate **4** (a 33% extrapolated radiochemical yield based on precursor **3**). A 100 mCi portion of intermediate **4** was concentrated by rotary evaporation to near dryness and dissolved in 0.2 ml of methylene chloride under argon. To this stirred solution was added 10 μl (0.12 mmol) of ethanethiol along with 3 μl of trifluoroacetic anhydride and the reaction was stirred at ambient temperature overnight. It was then diluted with 0.5 ml of methylene chloride and purified by TLC on one plate developed with chloroform:methanol:ammonium hydroxide (95:5:1) while the development tank was flushed with argon. Authentic standard **2** was allowed to migrate at each side of the plate to facilitate product location by UV. After plate development and UV visualization, the appropriate band was scraped and eluted with two 10 ml portions of argon degassed ethanol to afford 23 mCi of product **5** (a 23% radiochemical yield based on intermediate **4**) which was found to be 98.9% radiochemically pure by TLC (chloroform:methanol:ammonium hydroxide (95:5:1)) and 94.8% radiochemically pure by reverse phase HPLC (10 mmol aqueous triethylammonium acetate (pH 4):acetonitrile (35:65)) and co-eluted with authentic standard **2** in these chromatographic systems. Also, its specific activity was measured to be 27.4 Ci/mmol by a parallel radioassay and mass assay by UV where $E_{295} = 76\,869$ for **2**. The UV spectrum of **5** was completely superimposable on that of **2** as well.

*[Methyl ester-^{3}H] CEP-1347 (**7**)*

Precursor **6** (30 mg, 0.06 mmol) was dissolved in 1 ml of DMF. Then, by vacuum transfer, 4.25 Ci of [^{3}H] methyl iodide (0.05 mmol) was added and the solution was stirred at ambient temperature for 4 h. After this time volatile tritium was removed by several evaporations of methanol and the solution was filtered through a small plug of silica gel to afford 3 Ci of crude product **7** dissolved in 200 ml of acetonitrile. Half of this crude material was evaporated to near dryness and purified by preparative TLC on one plate developed with chloroform:methanol:acetic acid (100:5:1). Authentic standard **2** was allowed to migrate at each side of the plate to facilitate product location by UV. After plate development and UV visualization, the appropriate band was scraped and eluted with three 10 ml portions of ethyl acetate to afford 208 mCi (an extrapolated 12% radiochemical yield based on precursor **6**) which was found to be 98.8% radiochemically pure by TLC (same system as above) and 96.7% radiochemically pure by reverse-phase HPLC (0.2% aq TFA:acetonitrile (4:6)) and co-eluted with authentic standard **2** in these chromatographic systems. Also, its specific activity was measured to be 67.8 Ci/mmol by UV where $E_{295} = 76\,869$ for **2**. The UV spectrum of **7** was completely superimposable on that of **2** as well.

References

1. Bozyczko-Coyne D, Saporito MS, Hudkins RL. *Curr Drug Targets*: *CNS Neurol Disorders* 2002; **1**: 31–49.
2. Saporito MS, Hudkins RL, Maroney AC. *Progress Med Chem* 2002; **40**: 23–62.
3. Murakata C, Kaneko M, Gessner G, Angeles TS, Ator MA, O'Kane TM, McKenna BAW, Thomas BA, Mathiasen JR, Saporito MS, Bozyczko-Coyne D, Hudkins RL. *Bioorg Med Chem Lett* 2002; **12**: 147–150.
4. Maroney AC, Saporito MS, Hudkins RL. *Curr Med Chem*: *CNS Agents* 2002; **2**: 143–155.
5. Maroney AC, Finn JP, Connors TJ, Durkin JT, Angeles T, Gessner G, Xu Z, Meyer SL, Savage MJ, Greene LA, Scott RW, Vaught JL. *J Biol Chem* 2001; **276**: 25 302–25 308.
6. Kaneko M, Saito Y, Saito H, Matsumoto T, Matsuda Y, Vaught JL, Dionne CA, Angeles TS, Glicksman MA, Neff NT, Rotella DP, Kauer JC, Mallamo JP, Hudkins RL, Murakata C. *J Med Chem* 1997; **40**: 1863–1869.
7. Cheung YD, Barnett DB, Nahorski SR, Stefan R. *Eur J Pharmacol* 1982; **84**: 79–85.

