JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 67–71.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.644

Research Article

Synthesis and characterization of the potent cannabinoid agonist [naphthyl-^{3}H] WIN 55212-2 at high specific activity

Richard J. Seguin and Crist N. Filer*
PerkinElmer Life Sciences, Inc. 549 Albany Street, Boston, MA 02118, USA

Summary

[Naphthyl-^{3}H] WIN 55212-2 was prepared at high specific activity by the catalytic tritiation of dibromo precursor **3**. Product **4** was characterized by chromatography as well as tritium NMR and proven to be a useful radioligand. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: tritium; WIN 55212-2; cannabinoid; tritium NMR

Discussion

As a compound class the cannabinoids have demonstrated beneficial medicinal properties including analgesia, anti-emesis and the reduction of intraocular pressure.[1] However, the many untoward side effects of these substances has prompted the search for newer and safer alternative chemotherapeutic agents. During the last decade, the discovery and characterization of two distinct cannabinoid receptors, termed CB1 and CB2, has heightened interest in this area[2,3] and several diverse structures have been identified as potent agonists at these receptors.

One such lead structure is the indole pravadoline **1**, an analgesic and cyclooxygenase inhibitor.[4] To learn how the geometry of the morpho-

*Correspondence to: C. N. Filer, PerkinElmer Life Sciences, Inc., 549 Albany Street, Boston, MA 02118, USA. E-mail: Crist.Filer@perkinelmer.com

Received 8 July 2002
Accepted 29 July 2002

line ring influenced pravadoline's activity, conformationally restrained analogues of it were investigated.[5] Emerging from these studies was the result that the preferred geometry of the amine side chain was that shown in compound **2** (WIN 55212-2).

The options for tritium labelling of **2** at high specific activity appeared to be rather limited. There were no obvious olefin precursors to reduce and the structure of **2** did not appear to be an especially useful substrate for homogeneous transition metal catalyzed tritiation. It seemed, however, that a catalytic tritium dehalogenation with a polyhalo analogue of **2** might be an effective strategy. Toward this end, dibromo precursor **3**[5] was smoothly, catalytically dehalogenated with tritium to afford [naphthyl-^{3}H] WIN 55212-2 (**4**) after purification at 60 Ci/mmol. Although this specific activity value is essentially theoretical and very high for a catalytic dibromo tritium dehalogenation, it is not without precedent. In preparing the compound [phenyl-^{3}H] zolpidem, Allen and co-workers using a similar strategy reported a specific activity of 60.5 Ci/mmol for the radioligand.[6] A proton decoupled tritium NMR ($CDCl_3$) of product **4** displayed two peaks (Figure 1), establishing the specific tritium labelling at the aromatic positions.

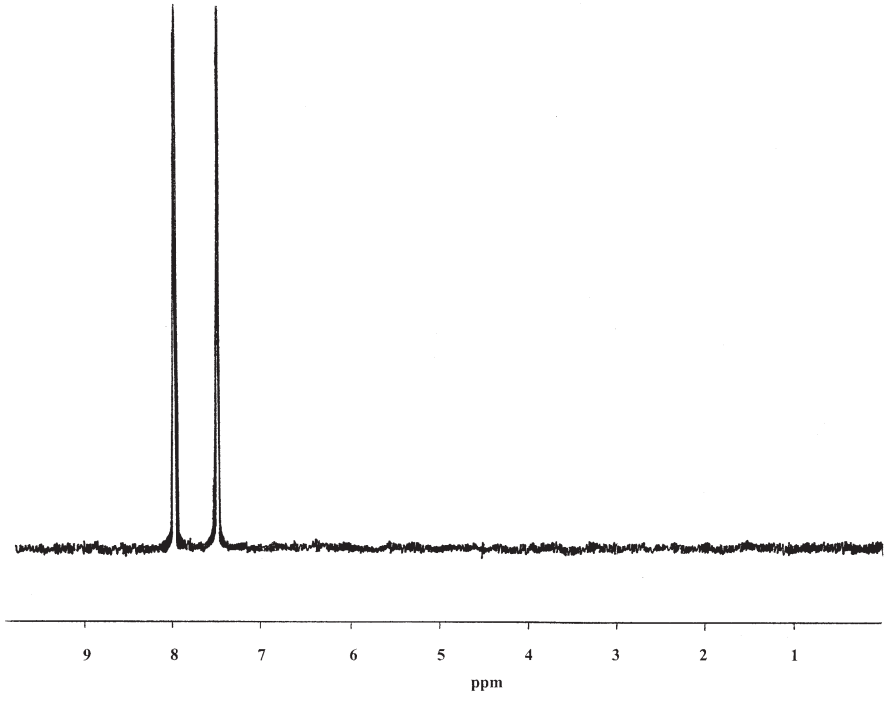
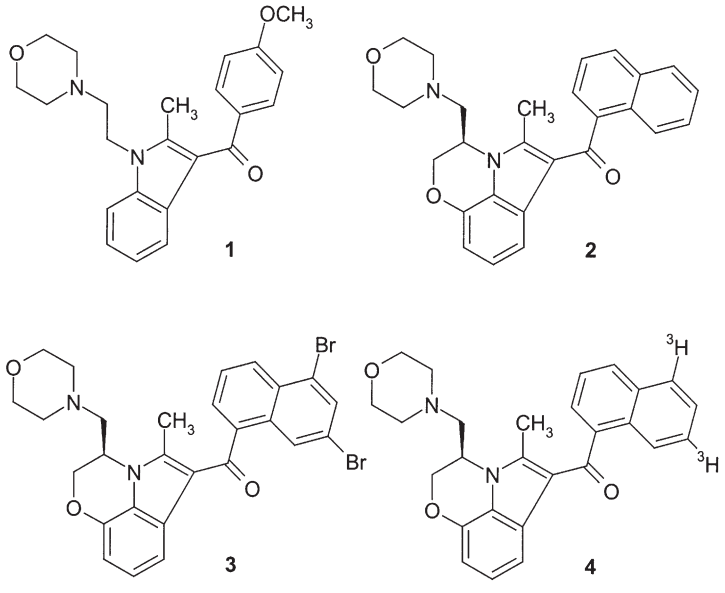


Figure 1. Proton decoupled tritium NMR ($CDCl_3$) of 4

A receptor binding assay for product **4** was reported[7] and since that time it has proven extremely useful to clarify issues regarding the cannabinoid receptor family.[8–12]



Experimental

Evaporations were carried out on a Buchi rotary evaporator *in vacuo* at bath temperatures less than 40°C. TLC was performed on Analtech plates coated with silica gel (250 μm for analytical and 500 μm 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). Analytical HPLC was performed on a Waters instrument and peak detection was done simultaneously by UV (280 nm–Waters 440 UV detector) and a liquid scintillation flow monitor. The tritium NMR spectrum was obtained on a Bruker 300 MHz instrument and chemical shifts are reported in parts per million (ppm) downfield from TMS.

[Naphthyl-^{3}H] WIN 55212-2 (4)

A solution of 25 mg (0.044 mmol) of precursor **3** in 2 ml of ethanol with 25 mg of 10% Pd/C and 0.025 ml of triethylamine was vigorously stirred

with 80 Ci of tritium gas at ambient temperature and atmospheric pressure for 4 h. After this time the catalyst was filtered, labile tritium was removed by several evaporations of methanol and the crude product (1960 mCi) was dissolved in 20 ml of ethanol. Purification was accomplished on two 500 μm silica gel plates eluted with hexane: ethyl acetate (1:1) to afford 988 mCi (a 37% radiochemical yield based on precursor **3**) of product **4** that was found to be >98% radiochemically pure and coelute with authentic **2** both on silica gel TLC (hexane : ethyl acetate (1:1)) and reverse phase HPLC (water : acetonitrile (35:65)). The specific activity of product **4** was measured to be 60 Ci/mmol by UV assay (where $E_{246} = 20{,}958$) and the UV of product **4** was superimposable on that of unlabelled **2**. A proton decoupled tritium NMR ($CDCl_3$) of product **4** showed two peaks at 7.49 and 7.98 ppm (Figure 1).

Acknowledgements

We would like to thank Dr Thomas D'Ambra (formerly of the Sterling Research Group) for a generous gift of precursor **3** and Dr Puliyer Srinivasan of PerkinElmer Life Sciences Inc. for obtaining the tritium NMR of **4** as seen in Figure 1.

References

1. Kumar RN, Chambers WA, Pertwee RG. *Anaesthesia* 2001; **56**: 1059–1068.
2. Porter AC, Felder CC. *Pharmacol Ther* 2001; **90**: 45–60.
3. Pertwee RG. *Progr Neurobiol* 2001; **63**: 569–611.
4. Bell MR, D'Ambra TE, Kumar V, *et al*. *J Med Chem* 1991; **34**: 1099–1110.
5. D'Ambra TE, Estep KG, Bell MR, *et al*. *J Med Chem* 1992; **35**: 124–135.
6. Allen J, Parent G, Tizot A. *J Label Compd Radiopharm* 1986; **23**: 807–813.
7. Kuster JE, Stevenson JI, Ward SJ, D'Ambra TE, Haycock DA. *J Pharmacol Exp Ther* 1993; **264**: 1352–1363.
8. Das SK, Paria BC, Chakraborty I, Dey SK. *Proc Nat Acad Sci* 1995; **92**: 4332–4336.
9. Stark S, Pacheco MA, Childers SR. *Cell Mol Neurobiol* 1997; **17**: 483–493.
10. Shim J-Y, Collantes ER, Welsh WJ, Subramaniam B, Howlett AC, Eissenstat MA, Ward SJ. *J Med Chem* 1998; **41**: 4521–4532.

11. Hernandez M, Berrendero F, Suarez I, Garcia-Gil L, Cebeira M, Mackie K, Ramos JA, Fernandez-Ruiz *J. Brain Res* 2000; **857**: 56–65.
12. Breivogel CS, Griffin G, Di Marzo V, Martin BR. *Mol Pharmacol* 2001; **60**: 155–163.

