JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 401–405.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.562

Research Article

Synthesis and characterization of [vinylidene-^{3}H] (−)-α-kainic acid at high specific activity

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Summary

[Vinylidene-^{3}H] (−)-α-Kainic acid was prepared by means of a Wittig reaction with a protected ketone precursor and has proved useful as a tool to study neuroexcitatory amino acid receptors. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: tritium; kainic acid; Wittig reaction

Discussion

(−)-α-Kainic acid **1** is a pyrrolidine amino acid with three contiguous chiral centers, first isolated from the marine algae *Digenea simplex*[1] and the pronounced neuroexcitatory activity of **1** has prompted extreme interest in it and related substances.[2] Our laboratories had previously labelled compound **1** with tritium *via* an exchange procedure with tritiated water, providing low specific activity material for Kuhar and co-workers in one of the earliest binding studies in rat brain.[3] To enhance the utility of **1** for the study of the neuroexcitatory amino acid receptors, it became necessary for us to tritiate it at significantly higher specific activity, ideally employing a more specific labelling method. Because of competing functionality, the usual catalytic methods of

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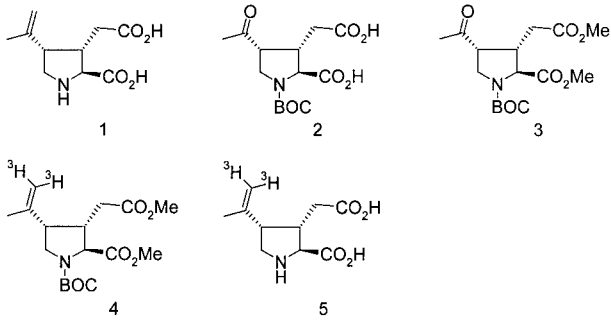
Received 5 November 2001
Accepted 21 November 2001

tritium labelling would be problematic. It seemed to us that **1** might be labelled with tritium on the vinylidene group *via* a Wittig reaction employing a suitable tritiated reagent with an appropriate ketone derived from **1**. Indeed, Wittig reactions on tritiated substrates, although not an especially common labelling strategy, have been accomplished in the past and we cite several examples.[4–6] Also, the literature has described the preparation of nitrogen protected ketone **2** from **1** and this appeared to be an attractive entry for a useful Wittig reaction precursor.[7]

Therefore, intermediate **2** could be prepared as described by the literature and methyl esterified to provide ketone precursor **3** or the latter could be obtained in a three step synthesis from **1** as recently described by Baldwin.[8] Ketone **3** was reacted with freshly prepared [methyl-^{3}H] methyltriphenylphosphonium iodide to obtain the olefin intermediate **4** shown below. Penultimate compound **4** could then be deprotected in straightforward fashion to obtain [vinylidene-^{3}H] (−)-α-kainic acid **5** in a good yield. Product **5** was radiochemically homogeneous on both TLC and HPLC and co-chromatographed with authentic **1**. A proton decoupled ^{3}H NMR (D_2O) was obtained for **1** and is seen in Figure 1, documenting exclusive and equivalent tritium labelling at the two vinyl positions (4.76 and 5.05 ppm), in excellent agreement with chemical shift values reported in the literature.[9] This spectrum, showing equivalent integrals for the two vinyl tritons, corroborated the retention of both remaining tritons from the originally fully tritiated Wittig reagent, indicating a theoretical specific activity for compound **1** as 58 Ci/mmol.

[Vinylidene-^{3}H] (−)-α-Kainic acid **5** has proved to be an immeasurably useful tool to elucidate the neurochemistry of the excitatory amino acid receptors.[10,11]



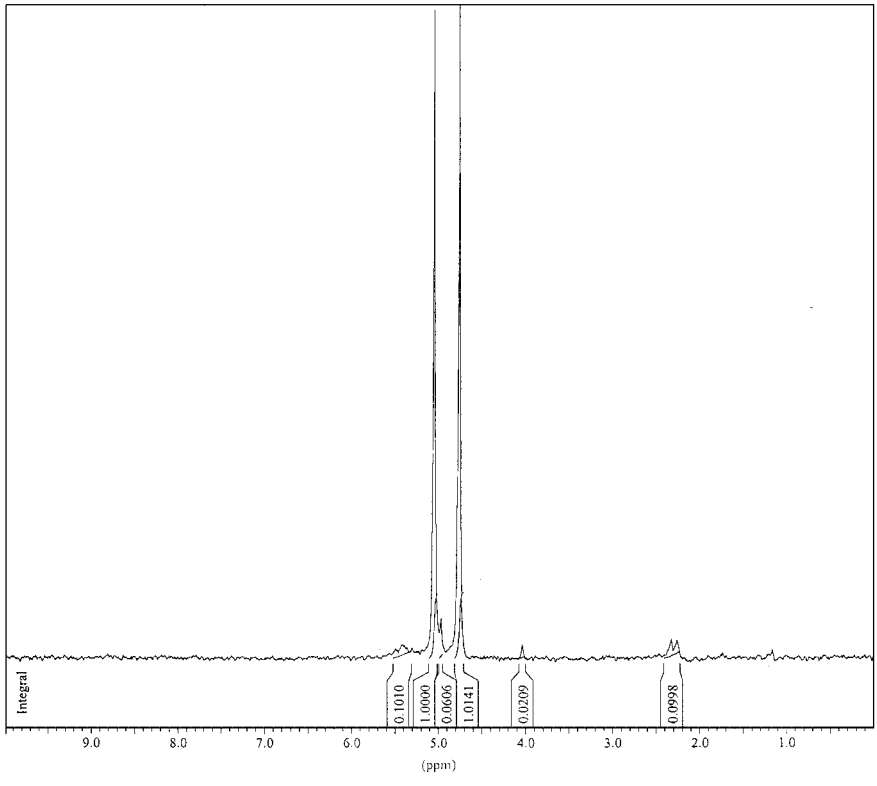


Figure 1. Proton decoupled ^{3}H NMR (D_2O) of 5

Experimental

Evaporations were carried out on a Buchi rotary evaporator *in vacuo* at bath temperatures $<40^{\circ}C$. 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 ($\sim$3 min) for radioactivity ($\sim$10 μCi). Preparative and 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.

[Vinylidene-^{3}H] N-BOC-(−)-α-kainic acid, dimethyl ester (4)

To 25 mg (0.062 mmol) of [methyl-^{3}H] methyltriphenylphosphonium iodide (prepared *in situ* from the reaction of triphenylphosphine and

[^{3}H] methyl iodide at 87 Ci/mmol in dry THF for 16 h at ambient temperature) in 0.3 ml of dry THF was added *via* syringe 40 µl (0.079 mmol) of 1.98 M *n*-butyllithium with rapid stirring at ambient temperature under argon. To the resulting yellow–orange solution was added a solution of 20.6 mg (0.060 mmol) of ketone **3** in 100 µl of dry THF *via* syringe at ambient temperature and the milky white solution was stirred at ambient temperature for 2 h. After this time labile tritium was removed by several methanol evaporations and the resulting crude product (1.9 Ci) was dissolved in 2 ml of THF. The crude product was purified by preparative TLC on two 500 µm silica gel plates developed with toluene:dioxane:acetic acid (90:25:4). After development, the main radioactive band (Rf = 0.7) was visualized by UV, scraped and eluted with ethanol to afford 590 mCi of product **4** which was homogeneous in this TLC system (where ketone precursor **3** had an Rf of 0.6).

*[Vinylidene-^{3}H] (−)-α-kainic acid (**5**)*

A solution of 236 mCi of **4** was stirred in 1 ml of TFA for 16 h at ambient temperature. After this time excess TFA was removed by rotary evaporation and the residue was saponified in 2 ml of 1 N sodium hydroxide for 16 h at ambient temperature. The solution was then acidified to pH 4–5 with 6N aqueous acetic acid and passed through a Biorad 50 ion exchange column (hydrogen ion form). The crude product was eluted from the column with 75 ml of 2 N ammonium hydroxide affording 198 mCi of reasonably pure product. Final purification was accomplished by preparative HPLC on a reverse phase column eluted with 1% aqueous triethylammonium acetate (pH = 4). Pure fractions were combined and dissolved in 2% ethanol in water to afford 79 mCi of product **1** (a 5% radiochemical yield based on precursor **3**) which was homogeneous on both TLC (silica gel – toluene:dioxane:acetic acid (90:25:4)) and HPLC (reverse phase – 0.01 M potassium phosphate (pH = 3):methanol (90:10)). Product **5** also co-chromatographed with authentic standard **1** and afforded the proton decoupled ^{3}H NMR as seen in Figure 1, demonstrating a specific activity of 58 Ci/mmol.

Acknowledgements

We would like to thank Tom Brewitt of PerkinElmer Life Sciences Inc. for obtaining the ^{3}H NMR of **5** as seen in Figure 1. Also we want to

acknowledge Dr James F. Collins of the Department of Chemistry, Sir John Cass School of Science and Technology (London, England) for an initial generous gift of precursor ketone **3**.

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