Protocols and Methods

Journal of Labelled Compounds and Radiopharmaceuticals

Received 12 January 2014, Revised 23 February 2014, Accepted 15 March 2014 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3198

Synthesis and characterization of [*N*-methyl-^{3}H]loperamide

Crist N. Filer,* Judith A. Egan, and Richard P. Nugent

Loperamide is a piperidine butyramide mu-opiate receptor agonist and currently employed to treat diarrhea. Because a single past report of tritiating loperamide was limited to only a very low specific activity product without technical details or extensive analysis, the synthesis of [*N*-methyl-^{3}H]loperamide at high specific activity is now described in detail. An imine precursor was alkylated with [^{3}H]methyl iodide to obtain a quaternary intermediate, which was then reacted with 4-(4-chlorophenyl)-4-hydroxypiperidine to afford the desired product [*N*-methyl-^{3}H]loperamide, characterized by thin layer chromatography (TLC), HPLC, MS, UV, and proton-decoupled tritium NMR.

Keywords: loperamide; mu-opiate receptor; tritium; tritium NMR

Introduction

Any discussion of the mu-opiate receptor system is understandably dominated by the topic of analgesia. However, there are other profound and useful biological effects mediated by this crucial binding site besides pain relief. In 1973, Janssen medicinal chemists described[1] the remarkable antidiarrheal properties of the mu-opiate receptor agonist loperamide (**1**), based on its selective activity at the myenteric plexus of the large intestine. Additionally, the structure of loperamide had been cleverly crafted (with *p*-chlorine substitution on the 4-phenylpiperidine) to deprive it of any appreciable central nervous system (CNS) activity. Over the years, the life-saving benefit of this valuable substance, especially in severely dehydrated children, has been well-documented.[2]

However, beyond its role as an effective medicine, there has been recent heightened interest in loperamide for an entirely different reason. The permeability-glycoprotein (P-gp) efflux pump, also known as the multidrug resistance protein 1 is widely expressed in various tissues. As its name implies, P-gp readily transports many drugs across cell membranes, often interfering with their biological target destination. It has been suggested that some disorders may be associated with abnormalities of P-gp. For instance, P-gp may play a role in limiting the access of chemotherapeutic agents to tumor cytoplasm.[3] Furthermore, it has also been proposed that reduced function of P-gp at the blood–brain barrier may hinder the clearance of beta-amyloid peptide from the brain, thereby increasing the chance of amyloid plaque formation and resulting Alzheimer's disease.[4] Recently, loperamide has been demonstrated to be a superior substrate for P-gp and may be one of the best probes to measure its function.[5]

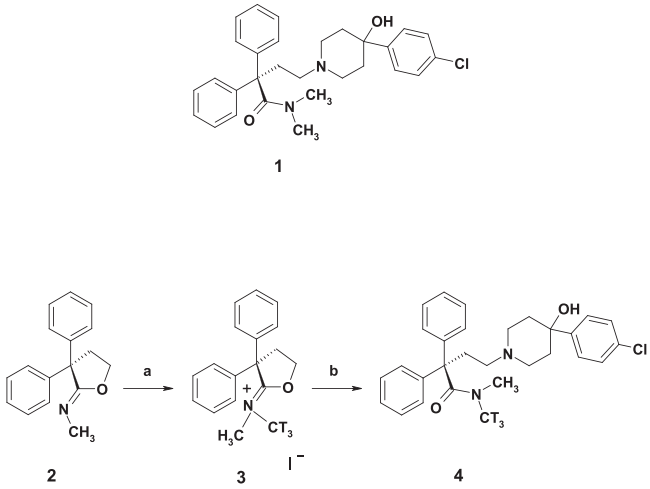
The tritiation of loperamide has been reported but just briefly referenced by only a structural reaction scheme lacking any experimental details.[6] Also, the product obtained was of extremely low (45 mCi/mmol) specific activity, analyzed only by TLC and without proof of tritium location. Because of the recent renewed interest in loperamide and our continued fascination with the opiate receptor family,[7] we now describe the synthesis and complete characterization of high specific activity [*N*-methyl-^{3}H]loperamide.

Results and discussion

In selecting a strategy to tritium label **1,** we initially explored the method of halogenation–catalytic tritium dehalogenation. However, for reasons that are not clear, we had no success in either the bromination or iodination of **1** and it appears that the literature also contains no examples of this derivatization. In view of these results, there appeared to be no better tritiation option than the original Janssen synthesis[1] itself, but some experimental modifications were made to accommodate the small scale and radiochemistry. Higher boiling solvents were chosen because the two-step synthesis was conducted in a sealed glass apparatus. Imine **2** was prepared as reported[1] with nothing unusual noted beyond what was experimentally described. It was then reacted with high specific activity [^{3}H] methyl iodide to give the iminium salt **3** as seen in Scheme 1. Because of instability concerns, this intermediate was not purified or characterized but instead immediately reacted with commercially available 4-(4-chlorophenyl)-4-hydroxypiperidine. After the final reaction, TLC analysis of the crude reaction mixture revealed significant amounts of desired product [*N*-methyl-^{3}H]loperamide (**4**), which was conveniently purified by reverse phase HPLC. The observed HPLC purification recovery was somewhat lower than expected and this may be due in part to a degree of crude product decomposition during concentration prior to HPLC injection. The product specific activity was easily

PerkinElmer Life Sciences & Technology Inc., 940 Winter Street, Waltham, MA 02451, USA

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



Scheme 1. Synthesis of [*N*-methyl-3H]loperamide (**4**). Reagents and conditions: (a) [3H]methyl iodide, methyl ethyl ketone, 65 0C, 8 h. (b) 4-(4-chlorophenyl)-4-hydroxypiperidine, sodium carbonate, benzene, 65 °C, 1 h.

determined by mass spectrometry with the appearance of a large parent ion for **4** at *m/z* 483. Alternatively, the specific activity could also be measured by a companion radioassay and UV (ethanol) mass determination of **4** for which $\epsilon_{260} = 644$ for **1**.

Perhaps the most intriguing spectral property of product **4** was its proton-decoupled tritium NMR as shown in Figure 1. The two large singlets observed are attributed to fully tritiated *N*-methyl groups, but each of them is also accompanied by a downfield singlet corresponding to smaller amounts of their C^3H_2H methyl isotopologues. Interestingly, the tritium resonances of the two main radiolabelled methyl peaks are widely separated by virtue of what apparently are their very dissimilar chemical and magnetic environments. Recently, the crystal structure of loperamide monohydrate was measured with high precision by single crystal X-ray analysis[8] and is the same three-dimensional structure as portrayed in **1**. However, this may not necessarily be the actual conformation of **1** in solution. The literature appears to contain only a single study[9] of the proton NMR of **1** in solution. Examining the host–guest inclusion complexation of **1** with beta-cyclodextrin, these authors also assigned the very same methyl chemical shift values (δ 2.33 and 2.95 ppm), which we observed for **4** but made no attempt to explain the dramatic differences for them. A possible explanation is that because of restricted rotation about the crowded amide bond, the two amide methyl resonances are a consequence of their different exposures to the shielding and deshielding regions of the amide carbonyl and/or phenyl rings. Finally, with striking evidence that both methyls of **4** are almost equally radiolabelled, the tritium NMR also demonstrates that mechanistically, there is apparently no stereochemical preference for either the methylation of precursor **2** or the ring opening rearrangement of intermediate **3** to product **4**.

We have performed the robust reaction sequence reported here a number of times, and under the described experimental conditions, it affords tens of mCi of pure product **4** at high specific activity.

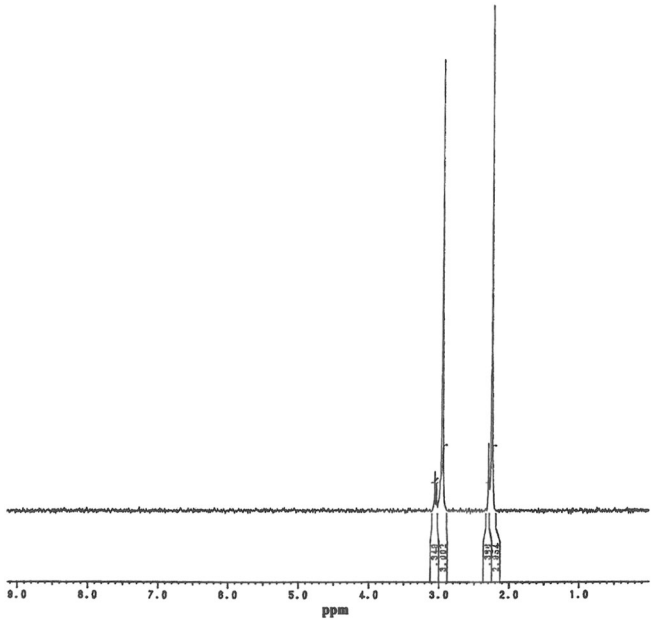


Figure 1. Proton decoupled tritium NMR (CD_3OD) of [N-methyl-^{3}H]loperamide.

Experimental

General

All chemicals used were reagent grade. Evaporations were carried out on a Buchi rotary evaporator (model RE 111) at bath temperatures less than 40 °C. Analytical TLC was performed on Analtech silica gel glass plates. Analytical HPLC was accomplished on a PerkinElmer instrument, and peak detection was performed simultaneously by UV and an IN/US Systems Beta RAM Model 3 radioactivity detector. Solution assays were performed with a PerkinElmer Tri-Carb 3100TR instrument. The mass spectrum was recorded on a Kratos Model MS25 RF instrument, and the UV spectrum was performed on a PerkinElmer Lambda 35 UV spectrophotometer. The proton-decoupled tritium NMR was obtained on a Bruker 300-MHz instrument with chemical shifts being reported as parts per million (ppm) downfield from internal tetramethylsilane.

[*N*-Methyl-^{3}H]loperamide (4)

To a solution of **2**[1] (12.6 mg, 0.05 mmol) in 0.25 mL of methyl ethyl ketone was added 21 Ci (0.25 mmol) of [^{3}H]methyl iodide, and the reaction was heated at 65 °C for 8 h. in a sealed glass vessel fitted with several break seal joints. After this time, the vessel was cooled, opened, and 16 mg (0.076 mmol) of 4-(4-chlorophenyl)-4-hydroxypiperidine (Aldrich C66056), 16 mg of sodium carbonate, and 0.25 ml of benzene were added. The reaction vessel was then resealed and heated at 65 °C for 1 h. It was then cooled and opened, and labile tritium was removed by the vacuum evaporation of several ethanol portions to give 1.1 Ci of crude product. TLC analysis of the reaction product mixture (silica gel (chloroform: methanol–ammonium hydroxide (19:1:0.1)) indicated that approximately 60% of it was desired product **4**. Half of the crude product was purified by preparative reverse phase HPLC (acetonitrile–1% aqueous triethylammonium acetate (pH 4) (60:40)) to afford 62 mCi (an extrapolated 3% yield based on precursor **2**) of product **4,** which was 98% radiochemically pure on TLC and HPLC (same systems as previously mentioned) and also co-chromatographed with authentic **1** (Aldrich L4762). The specific activity of **4** was measured to be 84.5 Ci/mmol by mass spectrometry with a prominent parent ion peak at 483 *m/z*. Its UV (ethanol) spectrum was superimposable on that of authentic **1** as well. A proton-decoupled tritium NMR (CD_3OD) of **4** is shown in Figure 1 and reveals that the tritium had been exclusively and almost equally installed on two methyl groups of **4**.

Acknowledgement

We would like to acknowledge the contribution of Dr. Puliyer Srinivasan of PerkinElmer Life Sciences & Technology in obtaining the tritium NMR spectrum.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] R. A. Stokbroekx, J. Vandenberk, A. H. M. T. Van Heertum, G. M. L. W. van Laar, M. J. M. C. Van der Aa, W. F. M. Van Bever, P. A. J. Janssen, *J. Med. Chem.* **1973**, *16*, 782–786.
[2] B. K. Sandhu, J. H. Tripp, P. J. Milla, J. T. Harries, *Arch. Dis. Child.* **1983**, *58*, 39–43.
[3] M. M. Gottesman, T. Fojo, S. E. Bates, *Nat. Rev. Cancer* **2002**, *2*, 48–58.
[4] S. Vogelgesang, I. Cascorbi, E. Schroeder, J. Pahnke, H. K. Kroemer, W. Siegmund, C. Kunert-Keil, L. C. Walker, R. W. Warzok, *Pharmacogenetics* **2002**, *12*, 535–541.
[5] J.-S. Taur, E. L. Schuck, N. Y. Wong, *Drug Metab. Lett.* **2012**, *6*, 285–291.
[6] J. Heykants, M. Michiels, A. Knaeps, J. Brugmans, *Arzneim.-Forsch.* **1974**, *24*, 1649–1653.
[7] C. N. Filer, *J. Labelled Compd. Radiopharm.* **2013**, *56*, 639–648.
[8] J. P. Jasinski, C. J. Guild, A. S. Dayananda, H. S. Yathirajan, A. R. Ramesha, *Acta Cryst.* **2012**, *E68*, o539–o540.
[9] S. K. Upadhyay, S. M. Ali, *J. Chem. Sci.* **2009**, *121*, 521–527.