Bioorganic & Medicinal Chemistry Letters 23 (2013) 5259–5263

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A high-yield route to synthesize the P-glycoprotein radioligand [^{11}C]*N*-desmethyl-loperamide and its parent radioligand [^{11}C]loperamide

Min Wang, Mingzhang Gao, Qi-Huang Zheng *

Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16th Street, Room 202, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received 8 July 2013
Revised 26 July 2013
Accepted 5 August 2013
Available online 13 August 2013

Keywords:
[^{11}C]*N*-Desmethyl-loperamide ([^{11}C]dLop)
[^{11}C]Loperamide
P-glycoprotein (P-gp)
Automation
Positron emission tomography (PET)
Brain imaging

ABSTRACT

N-Desmethyl-loperamide and loperamide were synthesized from α,α-diphenyl-γ-butyrolactone and 4-(4-chlorophenyl)-4-hydroxypiperidine in five and four steps with 8% and 16% overall yield, respectively. The amide precursor was synthesized from 4-bromo-2,2-diphenylbutyronitrile and 4-(4-chlorophenyl)-4-hydroxypiperidine in 2 steps with 21–57% overall yield. [^{11}C]*N*-Desmethyl-loperamide and [^{11}C]loperamide were prepared from their corresponding amide precursor and *N*-desmethyl-loperamide with [^{11}C]CH_3OTf through *N*-[^{11}C]methylation and isolated by HPLC combined with solid-phase extraction (SPE) in 20–30% and 10–15% radiochemical yields, respectively, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB), with 370–740 GBq/μmol specific activity at EOB.

P-Glycoprotein (P-gp) is a cell membrane-associated protein that transports a variety of drug substrates, and P-gp plays a role in preventing exogenous or endogenous toxins from entering the cell.[1] P-gp is also known as adenosine triphosphate (ATP)-binding cassette (ABC) sub-family B member 1 (ABCB1), or multidrug resistance-associated protein 1 (MRP1), or cluster of differentiation 243 (CD243), encoded by the ABCB1 gene.[2] P-gp is highly expressed at various physiological barriers including blood–brain barrier (BBB), blood–testis barrier and blood–tumor barrier.[3] P-gp overexpression has been observed in several cancer types and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury.[4] P-gp has become an attractive target for molecular imaging of cancer and brain diseases using biomedical imaging technique positron emission tomography (PET). Several PET radioligands have been investigated for their feasibilities to visualize P-gp at the BBB.[5] Latest promising candidates progressing to human PET studies are (*R*)-[^{11}C]verapamil and [^{11}C]*N*-desmethyl-loperamide ([^{11}C]dLop), as indicated in Figure 1.[6–14] [^{11}C]dLop is derived from the parent radiotracer [^{11}C]loperamide (Fig. 1). The parent compound loperamide is a μ-opioid receptor agonist drug originally developed at Janssen Pharmaceutica and used against diarrhea resulting from gastroenteritis or inflammatory bowel disease.[15–17]

[^{11}C]dLop is originally developed and characterized at the National Institute of Mental Health.[8–14] The importance of this compound as a PET brain imaging agent is well recognized, and broader research investigation to fully explore and validate the utility of [^{11}C]dLop-PET is important. However, the limited commercial availability, complicated synthetic procedure, and high costs of starting materials and precursor can present an obstacle to more widespread evaluation of this intriguing agent. Wishing to study this compound in our PET center, we decided to make our own material by following the literature methods.[8] The published

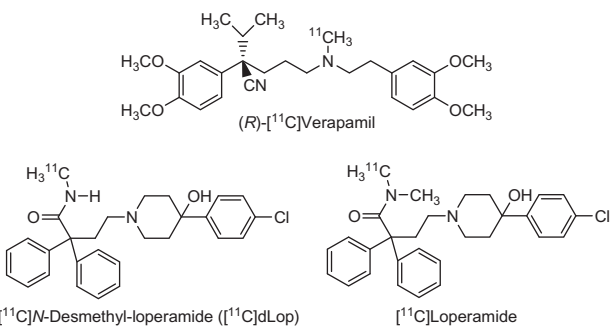


Figure 1. Chemical structure of (*R*)-[^{11}C]verapamil, [^{11}C]*N*-desmethyl-loperamide ([^{11}C]dLop) and [^{11}C]loperamide.

* Corresponding author. Tel.: +1 317 278 4671.
E-mail address: qzheng@iupui.edu (Q.-H. Zheng).

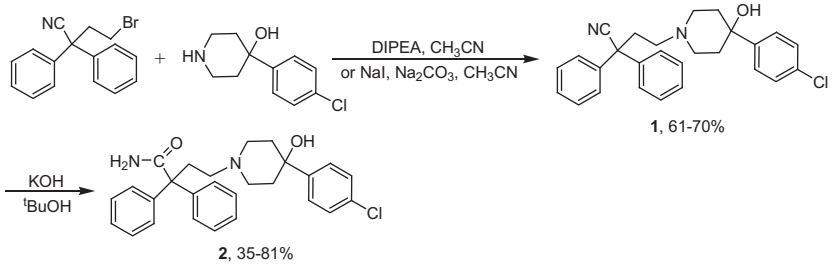
0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.08.024

synthesis of [^{11}C]dLop has gaps in synthetic detail, and the key step methylation with CH_3I for synthesis of authentic standard *N*-desmethyl-loperamide (dLop) gave very poor yield (3%) and was difficult to reproduce in our hands. The overall chemical yield for the synthesis of dLop with 3 steps was <1%. Consequently, we revisited the reported literature methods[15–17] and investigated alternate approaches and modifications that eventually resulted in a high-yield synthesis of [^{11}C]dLop and its parent radioligand [^{11}C]loperamide starting from very beginning materials 4-bromo-2,2-diphenylbutyronitrile, 4-(4-chlorophenyl)-4-hydroxypiperidine and α,α-diphenyl-γ-butyrolactone that was superior to previous works or addressed more synthetic details to reveal and explain technical tricks. In this letter we provide complete experiment procedures, yields, analytical details and new findings for this high-yield synthetic route, as well as dLop, loperamide and amide precursor, and present a fully automated radiosynthesis of [^{11}C]dLop and [^{11}C]loperamide with [^{11}C]methyl triflate ([^{11}C]CH_3OTf) in relatively high radiochemical yields.

As indicated in Scheme 1, the amide precursor **2** was synthesized according to the literature method with modifications. When we applied the reaction condition reported in the literature[8] to synthesize compound **1** from commercially available 4-bromo-2,2-diphenylbutyronitrile and 4-(4-chlorophenyl)-4-hydroxypiperidine in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in CH_3CN, the yield was 61%. The reaction condition was optimized in the presence of NaI and Na_2CO_3 in CH_3CN to afford the desired product **1** in 70% yield. Hydrolysis of nitrile **1** with pellet KOH in ^{t}BuOH gave the precursor **2** in 35% yield. With the newly ground KOH powder, the yield was increased to 81% in a shortened reaction time (from 3 days to 2 days).

Attempts to methylate the amide **2** with CH_3I provided dLop **7** in very low yield (<2%). Alternatively, we turned to another synthetic route as designed and outlined in Scheme 2. This synthetic route is based on the reported literature methods[15–17] with significant modification and optimization. Ring opening was accomplished by treatment of α,α-diphenyl-γ-butyrolactone with HBr in AcOH to afford 4-bromo-2,2-diphenylbutyric acid **3** in 83% yield. Transformation of acid **3** to its corresponding acid chloride **4** was achieved with $SOCl_2$. Condensation of acid chloride **4** with aqueous primary amine provided intermediate **I**, which was rearranged spontaneously under the same reaction condition to form hydrobromide salt **5** in 60% yield. Neutralization of the hydrobromide salt with 1 N NaOH yielded the free base, and gaseous HCl passed through the refluxed free base in $CHCl_3$ to afford the ring opening compound **6** in 68% yield. Treatment of ω-chloro-2,2-diarybutyramide **6** with 4-(4-chlorophenyl)-4-hydroxypiperidine in the presence of KI and Na_2CO_3 in ^{i}BuCOMe obtained dLop **7** in 25% yield. As shown in Scheme 2, condensation of acid chloride **4** with aqueous secondary amine provided intermediate **II**. The intermediate was rearranged spontaneously under the same reaction condition to form ammonium salt **8** in 50% yield, which was treated with 4-(4-chlorophenyl)-4-hydroxypiperidine in the presence of Na_2CO_3 in ^{i}BuCOMe to obtain loperamide (**9**) in 38% yield.

Synthesis of the target tracer [^{11}C]dLop ([^{11}C]**7**) and its parent radioligand [^{11}C]loperamide ([^{11}C]**9**) is indicated in Scheme 3. The amide precursor **2** or dLop **7** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[18,19] through *N*-[^{11}C]methylation[20,21] at 80 °C under basic condition (newly ground KOH powder) and isolated by a semi-preparative high performance liquid chromatography (HPLC) with a C-18 column and a solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge (a second purification or isolation process)[22–24] to produce the corresponding pure radiolabeled compound [^{11}C]**7** or [^{11}C]**9** in 20–30% or 10–15% radiochemical yield, respectively, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. In comparison with the results reported in the literature,[8,10] several significant improvements in the radiosynthesis have been made. [^{11}C]CH_3OTf was used as a radiolabeled precursor, which is a proven methylation reagent with greater reactivity than commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I),[25] and thus, the radiochemical yield of [^{11}C]**7** was relatively higher. However, there was no significant difference for the radiochemical yield of [^{11}C]**9** between the use of [^{11}C]CH_3OTf and [^{11}C]CH_3I, because it is much more difficult to ^{11}C-methylate the secondary amide **7** than primary amide **2** using either [^{11}C]CH_3OTf or [^{11}C]CH_3I. Fortunately, metabolism study indicated that [^{11}C]dLop is superior to [^{11}C]loperamide in measuring P-gp function at the BBB.[10] It is important to note that newly ground KOH powder would help the *N*-[^{11}C]methylation of amide precursor and significantly increase the radiochemical yield of both [^{11}C]**7** and [^{11}C]**9**. Meanwhile, small amount of the precursor (0.3–0.5 mg) was used for radiolabeling instead of large amount of the precursor (1.0–1.5 mg), which improved the chemical purity of the final tracer solution. In addition, in order to make more product radioactivity, we also modified the reported semi-preparative HPLC conditions including column, mobile phase and flow rate to shorten the retention time of [^{11}C]**7** and [^{11}C]**9**. Addition of $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of [^{11}C]**7** from its amide precursor **2** or [^{11}C]**9** from its precursor **7**.[22–24,26] Therefore, the radiochemical yields for [^{11}C]**7** and [^{11}C]**9** in our method (20–30% and 10–15%) are relatively higher than that reported previously (18% and 11.3% ± 1.4%).[8,10] The radiosynthesis was performed in a home-built automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific radioactivity during synthesis.[27–29] This ^{11}C-radiosynthesis module includes the overall design of the reaction, purification and reformulation capabilities of the prototype system. In addition, ^{11}C-tracer specific activity (SA, GBq/μmol at EOB) can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system.[29,30] The SA was in a range of 370–740 GBq/μmol at EOB. SA is defined as the radioactivity per unit mass of a radionuclide or a labeled compound. SA (MBq/mg) = $3.13 \times 10^9/A \times t_{1/2}$, where A is the mass number of the radionuclide, and $t_{1/2}$ is the half-life



Scheme 1. Synthesis of amide precursor (**2**).