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

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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

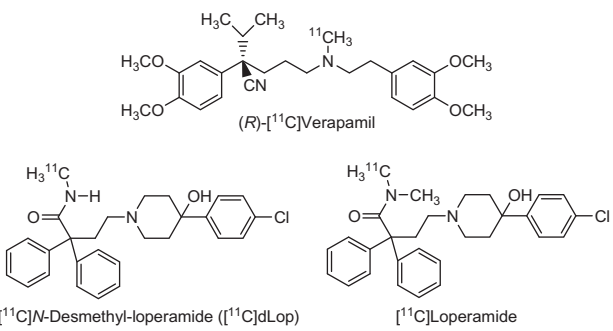

Figure 1. Chemical structure of (*R*)-[^{11}C]verapamil, [^{11}C]*N*-desmethyl-loperamide ([^{11}C]dLop) and [^{11}C]loperamide.

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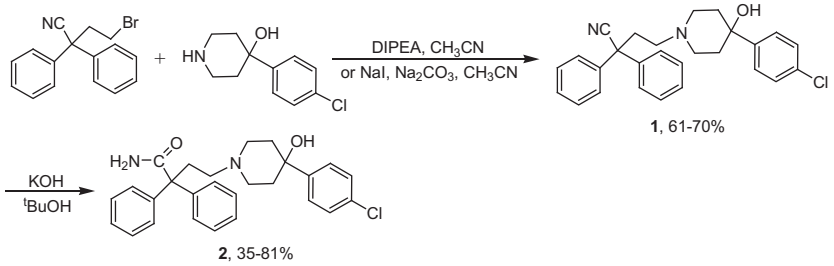
synthesis of [^{11}C]dLop has gaps in synthetic detail, and the key step methylation with CH_3I for synthesis of authentic standard *N*-desmethyl-loperamide (dLop) gave very poor yield (3%) and was difficult to reproduce in our hands. The overall chemical yield for the synthesis of dLop with 3 steps 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.

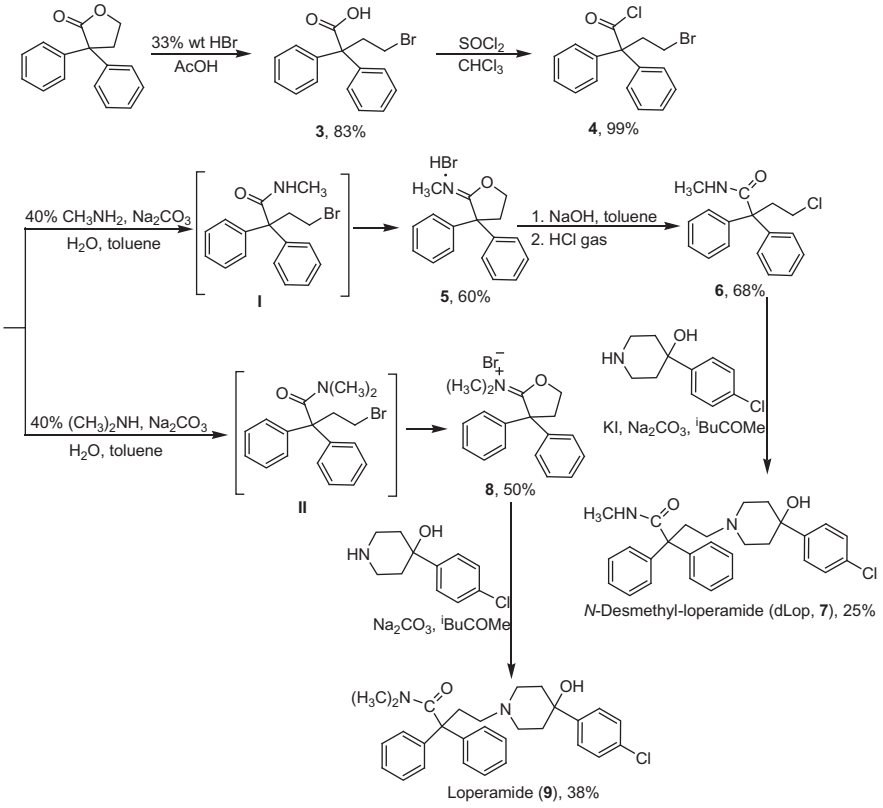
As indicated in Scheme 1, the amide precursor **2** was synthesized according to the literature method with modifications. When we applied the reaction condition reported in the literature[8] to synthesize compound **1** from commercially available 4-bromo-2,2-diphenylbutyronitrile and 4-(4-chlorophenyl)-4-hydroxypiperidine 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.

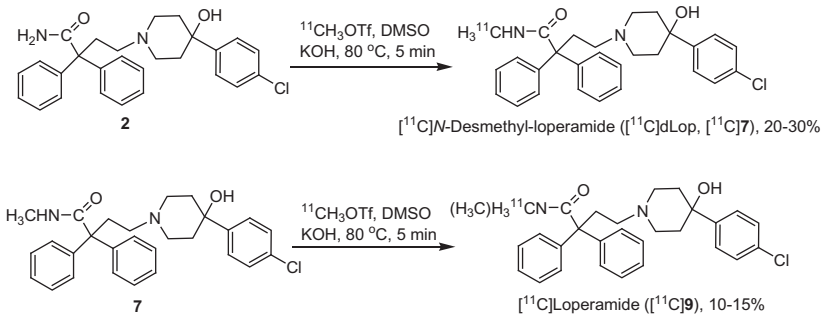
Synthesis of the target tracer [^{11}C]dLop ([^{11}C]**7**) and its parent radioligand [^{11}C]loperamide ([^{11}C]**9**) is indicated in Scheme 3. The amide precursor **2** or dLop **7** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[18,19] through *N*-[^{11}C]methylation[20,21] at 80 °C under 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**).



Scheme 2. Synthesis of *N*-desmethyl-loperamide (dLop, **7**) and loperamide (**9**).



Scheme 3. Synthesis of [^{11}C]*N*-desmethyl-loperamide ([^{11}C]dLop, [^{11}C]**7**) and [^{11}C]loperamide ([^{11}C]**9**).

in hours of the radionuclide. For carbon-11, carrier-free ^{11}C, maximum (theoretical) ^{11}C SA = 340,918 GBq/μmol.[31] Actual SAs of the ^{11}C-tracers in the PET chemistry facility are depended on two parts: (1) carrier from the ^{11}C-target, and (2) carrier from the ^{11}C-radiosynthesis unit.[30] Furthermore, actual SA for the ^{11}C-tracers synthesized by ^{11}C-methylation with [^{11}C]CH_3OTf in our PET chemistry facility is depended on two parts: (1) carrier from the cyclotron consisted of the ^{11}C gas irradiation target system, and (2) carrier from the [^{11}C]CH_3OTf system, ^{11}C radiolabeled precursor or called ^{11}C radiolabeled methylating reagent. The ^{11}C gas target we used is the Siemens RDS-111 Eclipse cyclotron ^{11}C gas target. The [^{11}C]CH_3OTf production system we used is an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module, convenient gas phase bromination of [^{11}C]methane and production of [^{11}C]CH_3OTf, a 'dry' method using Br_2 different with other 'dry' method using I_2 and 'wet' method using $LiAlH_4$ and HI. Our system will have much less ^{12}C carrier-added in comparison with other 'dry' method and 'wet' method.[19] The SA for our ^{11}C-tracers usually ranges from 370 to 925 GBq/μmol at EOB according to our previous works. Our study has proved that the maximum in-target ^{11}C SA is ~925 GBq/μmol (25 Ci/μmol) at EOB in our cyclotron and [^{11}C]CH_3-OTf system. The SA of the title tracer was 370–740 GBq/μmol at EOB, which was similar to the values previously reported by our lab. A historical perspective on the SA of radiopharmaceticals indicated it is difficult to achieve high SA for the ^{11}C-tracers.[31] However, a recent paper reported the maximum in-target ^{11}C SA 2775 GBq/μmol (75Ci/μmol) at EOB has been obtained, using GE PETtrace cyclotron and TRACERLab FXC automated synthesizer for the synthesis of a ^{11}C-tracer, [^{11}C]GR103545.[32] Chemical purity and radiochemical purity were determined by analytical HPLC.[33] The chemical purity of the precursors and reference standards **2**, **7**, and **9** was >96%. The radiochemical purity of the target tracers [^{11}C]**7** and [^{11}C]**9** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of [^{11}C]**7** and [^{11}C]**9** was >90% determined by reverse-phase HPLC through UV flow detector. A C-18 Plus Sep-Pak cartridge instead of rotatory

evaporation was used to significantly improve the chemical purity of the tracer solution.[22–24,33] In this study, the Sep-Pak purification further increased the chemical purity by 10–20%.[22–24]

The experimental details and characterization data for compounds **1**–**9** and for the tracers [^{11}C]**7** and [^{11}C]**9** are given.[34]

In summary, a high-yield synthetic route to PET P-gp radioligand [^{11}C]dLop and its parent radioligand [^{11}C]loperamide has been developed. This new synthetic approach provided amide precursor, dLop and loperamide in high overall chemical yields. An automated self-designed multi-purpose [^{11}C]-radiosynthesis module for the synthesis of [^{11}C]dLop and [^{11}C]loperamide has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed *N*-[^{11}C]methylation radiolabeling on nitrogen position of the amide precursor. Radiolabeling procedures incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3OTf, produced by gas-phase production of [^{11}C]methyl bromide ([^{11}C]CH_3Br) from our laboratory. The target tracers were isolated and purified by a semi-preparative HPLC combined with SPE procedure in relatively high radiochemical yields, short overall synthesis time, and high specific activity. These results facilitate the potential preclinical and clinical PET studies of [^{11}C]dLop and [^{11}C]loperamide as brain P-gp at BBB imaging agents in animals and humans.

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34. (a). *General.* All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[19] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker Avance II 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). Liquid chromatography-mass spectra (LC–MS) analysis was performed on an Agilent system, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. Semi-preparative reverse-phase (RP) HPLC purification for compounds **7** and **9** employed a Shimadzu LC-20AT pump, and a SPD-M20A diode array detector set at 254 nm. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; mobile phase 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 phosphate (buffer solution); flow rate 1.0 mL/min; and UV (225 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10 × 250 mm; mobile phase 60% CH_3CN/40% H_2O for [^{11}C]**7** and 70% CH_3CN/30% H_2O for [^{11}C]**9**; 5.0 mL/min flow rate; UV (225 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).
(b). *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanenitrile (**1**).* Method A. To a stirred suspension of 4-(4-chlorophenyl)-4-hydroxypiperidine (705 mg, 3.3 mmol) in CH_3CN (5 mL) was added DIPEA (1.16 mL, 6.7 mmol), followed by 4-bromo-2,2-diphenylbutyronitrile (1.0 g, 3.3 mmol). The reaction mixture was heated and refluxed overnight. After concentration in vacuo, the residue was dissolved in CH_2Cl_2 and purified by column chromatography with ammonium hydroxide solution (2 M) in MeOH/CH_2Cl_2 (from 0 to 6:250) to afford compound **1** (0.88 g, 61%) as a white solid. Method B. A mixture of 4-bromo-2,2-diphenylbutyronitrile (8.0 g, 26.7 mmol), 4-(4-chlorophenyl)-4-hydroxypiperidine (5.92 g, 28.0 mmol), NaI (4.40 g, 29.4 mmol) and Na_2CO_3 (5.65 g, 53.3 mmol) in CH_3CN (50 mL) was heated and refluxed overnight. The solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 and washed with water, brine and dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with ammonium hydroxide solution (2 M) in MeOH/CH_2Cl_2 (from 0 to 3:125) to afford compound **1** (8.05 g, 70%) as a white solid, mp 106–108 °C (lit.[8] 108–109 °C). ^{1}H NMR ($CDCl_3$): δ 7.43–7.28 (m, 14H), 2.76 (d, J = 11.5 Hz, 2H), 2.66–2.63 (m, 2H), 2.54–2.51 (m, 2H), 2.44 (dt, J = 2.0, 12.0 Hz, 2H), 2.08 (dt, J = 4.0, 13.0 Hz, 2H), 1.69 (dd, J = 2.0, 14.0 Hz, 2H), 1.61 (br s, 1H).
(c). *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylbutanamide (**2**).* To a stirred suspension of compound **1** (8.0 g, 18.6 mmol) in ^{t}BuOH (100 mL) was added newly ground KOH powder. The reaction mixture was heated and refluxed for 2 days. The solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2, washed with water, brine and dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography with ammonium hydroxide solution (2 M) in MeOH/CH_2Cl_2 (from 1:20 to 3:50) to afford compound **2** (6.76 g, 81%) as a white solid, mp 208–210 °C (lit.[8] 208–210 °C). ^{1}H NMR ($CDCl_3$): δ 7.41 (d, J = 8.5 °Hz, 2H), 7.32–7.27 (m, 14H), 6.54 (s, 1H), 5.72 (s, 1H), 2.80 (d, J = 10.5 Hz, 2H), 2.66 (t, J = 7.0 Hz, 2H), 2.44–2.35 (m, 4H), 2.08 (t, J = 11.0 Hz, 2H), 1.69 (d, J = 12.5 Hz, 2H), 1.27 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 176.8, 146.8, 143.3, 132.9, 128.8, 128.5, 127.2, 126.2, 70.8, 60.0, 55.0, 49.6, 38.2, 35.8. LC-MS (ESI, m/z): Calcd for $C_{27}H_{30}ClN_2O_2$ ([M+H]$^+$) 449.2, found: 449.2.
(d). *4-Bromo-2,2-diphenylbutyric acid (**3**).* A mixture of α,α-diphenyl-γ-butyrolactone (20.0 g, 83.9 mmol) in 33% wt HBr in AcOH (60 mL) was stirred

at room temperature for 2 days. The precipitate was collected by filtration, washed with water and toluene. The crude product was crystallized from $^{i}Pr_2O$ to afford compound **3** (22.2 g, 83%) as a white solid, mp 137–138 °C (lit.[15] 135–137 °C). ^{1}H NMR ($CDCl_3$): δ 7.34–7.27 (m, 10H), 3.10–3.07 (m, 2H), 2.97–2.93 (m, 2H).

(e). *4-Bromo-2,2-diphenylbutanoyl chloride (**4**).* To a stirred suspension of compound **3** (10.0 g, 31.4 mmol) in $CHCl_3$ (70 mL) was added $SOCl_2$ dropwise. The reaction mixture was heated and refluxed for 4 h. The solvent was removed in vacuo to afford the crude acid chloride **4** (10.4 g, 99%) as a yellow oil, which was used for next step without further purification.

(f). *N-(Tetrahydro-3,3-diphenyl-2-furylidene)methylamine hydrobromide (**5**).* To a stirred mixture of 40% aqueous methylamine (1.03 mg, 13.2 mmol) and Na_2CO_3 (1.24 g, 11.7 mmol) in water (10 mL) and toluene (8 mL) was added compound **4** (3.95 g, 11.7 mmol) in toluene (5 mL) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and the precipitate was collected by filtration. The solid was dissolved in $CHCl_3$ and dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was crystallized from ^{i}BuCOMe to afford compound **5** (2.3 g, 60%) as a white solid, mp 158–159 °C (lit.[15] 159–161 °C). ^{1}H NMR ($CDCl_3$): δ 12.7 (s, 1H), 7.46–7.20 (m, 10H), 4.88 (t, J = 6.5 Hz, 2H), 3.28 (d, J = 4.0 Hz, 3H), 3.23 (t, J = 6.5 Hz, 2H).

(g). *4-Chloro-N-methyl-2,2-diphenylbutanamide (**6**).* A cooled solution of compound **5** (1.8 g, 5.4 mmol) in water (2 mL) was adjusted pH to 12 with 2 N NaOH. The mixture was extracted with toluene. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the free base. The base was dissolved in $CHCl_3$. The mixture was heated and refluxed while dry HCl gas was bubbled through for 1 h. The solvent was removed in vacuo. The residue was crystallized from $^{i}Pr_2O$ to afford compound **6** (1.06 g, 68%) as a white solid, 150–151 °C (lit.[17] 150–152 °C). ^{1}H NMR ($CDCl_3$): δ 7.31–7.21 (m, 10H), 5.42 (s, 1H), 3.52 (t, J = 8.0 Hz, 2H), 2.88 (t, J = 8.0 Hz, 2H), 2.76 (d, J = 5.0 Hz, 3H).

(h). *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-N-methyl-2,2-diphenylbutanamide (N-desmethyl-loperamide, **7**).* A mixture of 4-(4-chlorophenyl)-4-hydroxypiperidine (350 mg, 1.67 mmol), KI (27.6 mg, 0.17 mmol), Na_2CO_3 (264 mg, 2.49 mmol) in ^{i}BuCOMe (20 mL) was distilled azeotropically to dry with the aid of a Dean-Stark trap. After cooling to 80 °C, Compound**6** (500 mg, 1.66 mmol) was added. The reaction mixture was heated and refluxed overnight. The solvent was removed in vacuo. The residue was diluted with water, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was first purified by column chromatography with ammonium hydroxide solution (2 M) in MeOH/CH_2Cl_2 (1:10). Then the product purified by silica gel column was injected onto a Luna C18 column (5 μm, 100 A, 10 × 250 mm), and eluted at 5.0 mL/min with 50% solvent A (water/0.1% TFA) and 50% solvent B (acetonitrile/0.1% TFA) to afford compound **7** (238 mg, 25%, TFA salt) as a pale yellow solid, mp 225–226 °C. ^{1}H NMR ($CDCl_3$): δ 11.30 (s, 1H), 7.39–7.28 (m, 10H), 7.16–7.15 (m, 4H), 5.76 (s, 1H), 5.53 (d, J = 5.0 Hz, 1H), 3.37 (d, J = 10.5 Hz, 2H), 3.22 (q, J = 10.5 Hz, 2H), 3.03–3.02 (m, 2H), 2.88–2.86 (m, 2H), 2.75 (d, J = 4.5 Hz, 3H), 2.46–2.41 (m, 2H), 1.89 (d, J = 14.0 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 175.3, 144.7, 141.9, 133.7, 129.3, 128.9, 128.4, 128.2, 126.1, 69.1, 59.6, 55.6, 49.4, 35.7, 33.6, 27.1. LC–MS (ESI, m/z): Calcd for $C_{28}H_{32}ClN_2O_2$ ([M+H]$^+$) 463.1, found: 463.1. The free base used as precursor was prepared by dissolving the TFA salt in water and basified with concd ammonium solution, followed by extraction with $CHCl_3$, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo.

(i). *Dimethyl(tetrahydro-3,3-diphenyl-2-furylidene)ammonium bromide (**8**).* To a stirred mixture of 40% aqueous dimethylamine (1.35 mg, 12.0 mmol) and Na_2CO_3 (2.54 g, 24.0 mmol) in water (8 mL) was added compound **4** (3.38 g, 10.0 mmol) in toluene (5 mL) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature. The mixture was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was crystallized from ^{i}BuCOMe to afford compound **8** (1.72 g, 50%) as a white solid, mp 186–187 °C (lit.[15] 181–182 °C). ^{1}H NMR ($CDCl_3$): δ 7.55–7.45 (m, 10H), 4.86 (t, J = 6.5 Hz, 2H), 3.83 (s, 3H), 3.47 (t, J = 6.5 Hz, 2H), 2.98 (s, 3H).

(j). *4-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-N,N-dimethyl-2,2-diphenylbutanamide (loperamide, **9**).* A mixture of 4-(4-chlorophenyl)-4-hydroxypiperidine (123 mg, 0.58 mmol), Na_2CO_3 (231 mg, 2.18 mmol) in ^{i}BuCOMe (18 mL) was distilled azeotropically to dry with the aid of a Dean–Stark trap. After cooling to 80 °C, compound **8** (200 mg, 0.58 mmol) was added. The reaction mixture was heated and refluxed overnight. The solvent was removed in vacuo. The residue was diluted with water, and extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was first purified by column chromatography with ammonium hydroxide solution (2 M) in MeOH/CH_2Cl_2 (1:10). Then the product purified by silica gel column was injected onto onto a Luna C18 column (5 μm, 100 A, 10 × 250 mm), and eluted at 5.0 mL/min 40% solvent A (water/0.1% TFA) and 60% solvent B (acetonitrile/0.1% TFA) to afford compound **9** (126 mg, 38%, TFA salt) as a white solid, mp 103–104 °C. ^{1}H NMR ($CDCl_3$): δ 11.10 (s, 1H), 7.43–7.40 (m, 4H), 7.37–7.26 (m, 10H), 5.44 (s, 1H), 3.34 (d, J = 10.0 Hz, 2H), 3.20 (q, J = 10.5 Hz, 2H), 2.94 (s, 3H), 2.76–2.75 (m, 2H), 2.64–2.61 (m, 2H), 2.44–2.40 (m, 2H), 2.29 (s, 3H), 1.83 (d, J = 14.5 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 144.7, 138.9 133.6, 129.2, 128.8, 128.0, 127.9, 126.1, 69.1, 60.2, 55.6, 49.2, 39.6, 39.4, 37.4, 35.6. LC–MS (ESI, m/z): Calcd for $C_{29}H_{34}ClN_2O_2$ ([M+H]$^+$) 477.2, found: 477.2.

(k). *General procedure for preparation of [^{11}C]N-desmethyl-loperamide ([^{11}C]dLop, [^{11}C]**7**) and [^{11}C]loperamide ([^{11}C]**9**).* [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas from Praxair, Indianapolis, IN. Typical irradiations used for the development were 55 μA beam current and 30 min on target. The production run produced approximately 45.5 GBq of [^{11}C]CO_2 at EOB. In a small reaction vial (5 mL), the precursor **2** or **7** (0.3–0.5 mg) was dissolved in DMSO (400 μL). To this solution was added the newly ground KOH powder (1–2 mg). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[19] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial at room temperature, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 5 min. The contents of the reaction vial were diluted with $NaHCO_3$ (0.1 M, 1 mL), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2), followed by 10 mL saline, to release [^{11}C]**7** or [^{11}C]**9**. The eluted product was then sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected into a sterile vial. Total radioactivity (2.3–4.9 GBq for [^{11}C]**7** and 1.2–2.5 GBq for [^{11}C]**9**) was assayed and total volume (10–11 mL) was noted for tracer dose dispensing. The overall synthesis, purification and formulation time was 30–40 min from EOB. Retention times in the analytical HPLC system were: t_R **2** = 3.58 min, t_R **7** = 5.11 min, t_R **9** = 6.24 min, t_R [^{11}C]**7** = 5.11 min, and t_R [^{11}C]**9** = 6.24 min. Retention times in the semi-preparative HPLC system were: t_R **2** = 5.62 min, t_R **7** = 8.78 min, t_R [^{11}C]**7** = 8.78 min; and t_R **7** = 5.35 min, t_R **9** = 7.65 min, t_R [^{11}C]**9** = 7.65 min. The radiochemical yields were 20–30% for [^{11}C]**7** and 10–15% for [^{11}C]**9**, respectively, decay corrected to EOB, based on [^{11}C]CO_2.