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Synthesis and Radiolabelling of Ipratropium and Tiotropium for Use as PET Ligands in the Study of Inhaled Drug Deposition

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Ipratropium bromide [(1*R*,3*r*,5*S*,8*r*,2′*RS*)-3-(3′-hydroxy-2′-phenylpropionyloxy)-8-isopropyl-8-methyl-8-azabicyclo[3.2.1]octan-8-ium bromide] and tiotropium bromide [(1*R*,2*R*,4*S*,5*S*,7*s*)-7-[2′-hydroxy-2′,2′-di(thiophen-2″-yl)acetoxy]-9,9-dimethyl-9-aza-3-oxatricyclo[3.3.1.$0^{2,4}$]nonan-9-ium bromide] are inhaled drugs used in the treatment of chronic obstructive pulmonary disease (COPD) and asthma. Tertiary amine precursors have been synthesized and radiolabelled with carbon-11 by *N*-alkylation with [^{11}C]CH_3I. The [^{11}C]ipratropium and [^{11}C]tiotropium positron emission tomography (PET) ligands are obtained with high radiochemical purity, in 0.3 and 0.5% non-decay corrected yields based on [^{11}C]CO_2 at end-of-synthesis and specific activities of 11 and 18 GBq μmol^{-1}, respectively, calculated at end-of-synthesis. These PET radioligands can be used in the study of inhaled drug deposition.

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Introduction

Ipratropium **1** bromide and tiotropium **2** bromide (Fig. 1) are orally inhaled muscarinic receptor antagonists (anticholinergics, bronchodilators) used in the treatment of chronic obstructive pulmonary disease (COPD) and asthma.[1,2] They are structural analogues of atropine that share a quaternary ammonium functionality that gives rise to their low lipophilicity and, hence, minimal systemic anticholinergic side effects. While tiotropium and ipratropium show similar affinity for the human M_1, M_2, and M_3 muscarinic receptor subtypes expressed in Chinese hamster ovary-K1 (CHO) cells,[3] tiotropium has a 10-fold greater affinity than ipratropium for human lung muscarinic receptors[4–6] and dissociates 100-fold more slowly from the M_1 and M_3 receptors, which facilitate cholinergic neurotransmission and mediate bronchoconstriction and mucus secretion, respectively.[3–6] In addition, tiotropium dissociates more rapidly from the M_2 receptors, which functions to inhibit acetylcholine release when activated, thereby leads to a 'kinetic receptor subtype selectivity' that gives tiotropium its long duration of action.[3–6] This translates to a daily dosing of tiotropium in clinical application compared to the six-hourly administration of ipratropium.[2]

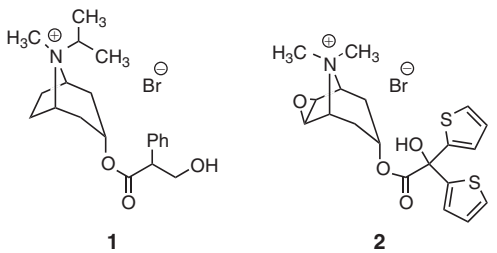

Fig. 1. Ipratropium **1** bromide and tiotropium **2** bromide.

In the USA and Australia, ipratropium **1** bromide is commercially sold as Apoven or Atrovert in a sodium chloride nebulizer solution or aerosol, and tiotropium **2** bromide is sold as Spiriva in a capsule with lactose to be inhaled as a dry powder via a HandiHaler device.[2] Pharmacokinetic studies show that for oral inhalation of ipratropium and tiotropium by pressurized-metered dose inhaler or dry powder inhaler, respectively, between 10 and 30% of the drug is delivered to the lungs, while most of the dose is swallowed and is unabsorbed in the gastrointestinal tract to be excreted in the faeces.[2,7–9]

Knowledge of the lung dose and distribution of inhaled drugs is critical for the assessment of the delivery technology.[10] Functional imaging is a non-invasive and direct technique for visualizing and quantifying airway deposition of inhaled radiolabelled drugs in vivo. The two-dimensional planar method of gamma-scintigraphy has been the most widely used to quantify deposition data because it is the simplest and most cost effective. However it is difficult to determine site-specific distribution patterns in

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the lung.[11] Three-dimensional imaging overcomes this problem by providing details of the anatomical location of the deposited drug. Positron emission tomography is a three-dimensional imaging modality that uses cyclotron-produced positron-emitters which can be incorporated into the structure of the drug molecule without altering the drug delivery characteristics of the product.[12] In this way the drug itself becomes the radioactive tracer and, unlike in single photon emission computed tomography (SPECT) where the formulation typically needs to be labelled with a surrogate marker for the drug, validation measurements demonstrating the drug/label association are not required.[11] The organic radioisotope carbon-11 (half-life 20 min, gamma-ray energy 511 keV) is usually the radiolabel of choice.

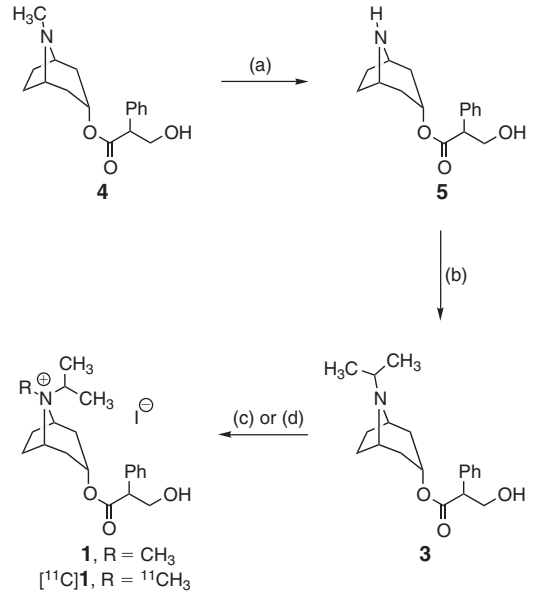
The direct labelling of ipratropium **1** bromide with bromine-77 (half-life 56 h, gamma-emitter, 245 keV) and incorporation into a metered dose inhaler by Short and co-workers[9] is currently the only study of its kind undertaken to quantify inhaled drug deposition patterns by gamma scintigraphy. While novel in its approach, the study was limited by the assumption that the [^{77}Br]bromide label remains in association with the drug during the first few minutes after inhalation, making it unsuitable for drug clearance studies. Furthermore, bromine-77 emits significant gamma radiation peaks at 520 and 580 keV, which are a source of error in quantitative measurements of ^{77}Br deposition.[13]

To date, few inhaled drug deposition studies have involved the direct labelling of the drug molecule,[12] and the deposition of inhaled ipratropium **1** and tiotropium **2** formulations by newer delivery system designs containing positron-emitting radionuclides has not been investigated. Given the growing importance of these drugs for the treatment of COPD and asthma a greater understanding of drug delivery methods is needed. In this paper we report the development of ipratropium **1** and tiotropium **2** PET radioligands containing carbon-11 suitable for imaging studies on inhaled drug deposition.

Results and Discussion

*Synthesis of Ipratropium **1** Iodide and Tiotropium **2** Iodide*

To access ipratropium **1** iodide and the tertiary amine precursor **3**, a short route based on the reductive alkylation of noratropine was developed (Scheme 1). The photochemical *N*-demethylation[14] of free base atropine **4** using oxygen and tetraphenyl porphine (TPP) as the photosensitizing agent under irradiation with visible light afforded noratropine **5** in 24% yield.[15] Reductive amination of **5** with acetone and sodium cyanoborohydride proceeded smoothly to give *N*-isopropyl tropane **3** in 62% yield.[16] Finally, *N*-methylation of *N*-isopropyl tropane **3** with methyl iodide in diethyl ether and *N*,*N*-dimethylformamide (DMF) as co-solvent proceeded to give a single isomer of ipratropium **1** iodide in low yield (2%).[16] The *N*-methylation reaction conditions were subsequently optimized for the tiotropium **2** iodide target compound before labelling studies (see below). The synthetic sample correlated closely with a commercially derived sample of ipratropium bromide by high-performance



Scheme 1. (a) TPP, O_2, CH_2Cl_2, $h\nu$ (160 W), 5.75 h. (b) CH_3COCH_3, $NaBH_3CN$, CH_3OH, pH 7.0, room temperature (RT), 5 h. (c) CH_3I, Et_2O, DMF, RT, 16.5 h; Δ, 2.5 h. (d) [^{11}C]CH_3I, DMF, −40°C, 2 min; 88°C; 5 min.

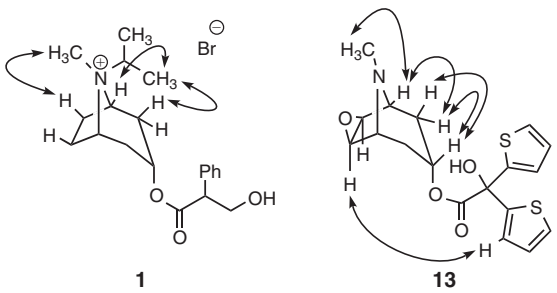
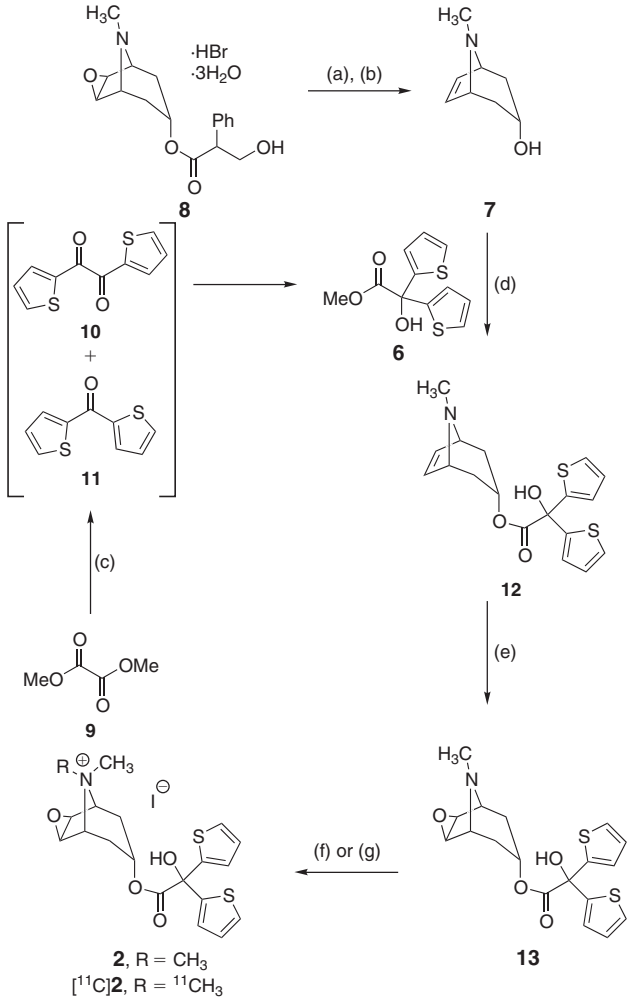


Fig. 2. Two-dimensional NOESY correlations for ipratropium **1** bromide and epoxide **13**.

liquid chromatography (HPLC) retention time, and ^{1}H and ^{13}C NMR spectroscopic analysis. Furthermore, the expected (8*r*) configuration resulting from equatorial quaternization of tertiary amine **3** was consistent with the two-dimensional nuclear overhauser and exchange spectroscopy (NOESY) correlations observed for commercially derived ipratropium bromide (Fig. 2).[17]

Tiotropium **2** iodide was synthesized through the transesterification of ester **6** with alcohol **7**, an adaption of the reported patent literature (Scheme 2). The two-step synthesis of tropenol **7**, the first coupling partner for the transesterification reaction, was initiated by deoxygenation of scopolamine hydrobromide trihydrate **8**, with an excess of a zinc–copper couple in acetonitrile (73%).[18,19] The use of a non-protic solvent and the hydrogen bromide salt instead of free base minimized the formation of side products. Hydrolysis of the ester sidechain in aqueous sodium hydroxide solution and extraction then gave alcohol **7** as a volatile liquid (58%).[20] Due to this volatility, tropenol **7** was engaged in the proceeding transesterification reaction without further purification.

Methyl 2-hydroxy-2,2-di(thiophen-2′-yl)acetate **6** (Scheme 2) was synthesized in two steps from dimethyl



Scheme 2. (a) Zn–Cu, CH_3CN, pressure tube, Ar, 156°C, 3 days. (b) NaOH, H_2O, Δ, 2.5 h. (c) Thiophen-2-yllithium, tetrahydrofuran, −78°C, 1.5 h; KOH, H_2O, Δ, 1 h; trimethylsilyldiazomethane, Et_2O, RT, 1 h. (d) NaH, **6**, heptane, Δ, 2.5 h. (e) V_2O_5, 30% H_2O_2, DMF, 54°C, 4.5 h; $NaHSO_3$, $NaHCO_3$. (f) CH_3I, DMF, 57°C, 4.5 h. (g) [^{11}C]CH_3I, DMF, −40°C, 2 min; 88°C, 5 min.

oxalate **9**.[21,22] Addition of thiophen-2-yllithium to dimethyl oxalate **9** afforded a mixture containing dione **10**,[21] which was subjected to base promoted benzylic acid-type rearrangement and immediate methylation with trimethylsilyldiazomethane to give methyl ester **6**.[22] The formation of di(thiophen-2-yl)methanone **11**[23] as a minor by-product of the organometallic addition step was also observed but this could readily be separated from **6** by chromatography.

Transesterification of ester **6** with tropenol **7** using sodium hydride in heptane at reflux afforded ester **12** in 58% yield.[24] Chemoselective oxidation of compound **12** by treatment with hydrogen peroxide in DMF at 54°C and catalytic vanadium(v) oxide afforded 80% of the labelling precursor **13**[24] after a basic workup and purification by flash chromatography. The stereochemistry at the epoxide carbon centres of **13** was confirmed by a two-dimensional NOESY experiment (Fig. 2), with a cross-peak observed between the epoxide protons and the thiophene ring consistent with the *exo*-orientation of the epoxide oxygen. Methylation of tertiary amine **13** was conducted in order to isolate the tiotropium **2** iodide target for comparison with the carbon-11 labelled analogue by HPLC. Heating a DMF solution of epoxide **13** with methyl iodide afforded tiotropium **2** iodide, which was purified by reverse phase HPLC to afford the trifluoroacetate salt in 97% yield. The synthetic sample of the tiotropium correlated with a commercially derived Spiriva (tiotropium bromide and lactose) by co-injection on reverse-phase HPLC.

*Synthesis of [^{11}C]Ipratropium ([^{11}C]**1**) and [^{11}C]Tiotropium ([^{11}C]**2**) PET Radioligands*

PET radioligands [^{11}C]**1** (Scheme 1) and [^{11}C]**2** (Scheme 2) were prepared by *N*-alkylation of the tertiary amine precursors **3** and **13**, respectively, with [^{11}C]CH_3I. The [^{11}C]CH_3I was trapped at low temperature in a solution of the precursor **3** or **13** in DMF for 2 min and then the reaction was heated at 88°C for 5 min to promote methylation. Purification was carried out by HPLC using acetonitrile/phosphate buffer solvent mixes followed by evaporation and reconstitution. Unfortunately, attempts to use an ethanol/0.45% saline mobile phase, which would have eliminated the evaporation and formulation requirements in the preparation of the radioligands for administration, failed to give adequate separation. The radiochemical yield of ligand [^{11}C]**1** (>98% chemical and radiochemical purity) was 61 ± 3 MBq ($n = 3$), at end-of-synthesis (0.3%, calculated from [^{11}C]CO_2, non-decay corrected) and specific activity 11 GBq μmol^{-1}. Radioligand [^{11}C]**2** (>98% chemical and radiochemical purity) was obtained in a radiochemical yield of 129 ± 8 MBq ($n = 3$) at end-of-synthesis (0.5%, calculated from [^{11}C]CO_2, non-decay corrected) and 18 GBq μmol^{-1} specific activity. The total synthesis time for both radioligands, including formulation and quality control, was 35–37 min.

Initially, radiolabelling was performed at room temperature, but yielded no radiolabelled product formation, with the major radiochemical peak observed by HPLC corresponding to unreacted [^{11}C]CH_3I. Increasing the reaction temperature was necessary for radiolabelling while increasing the time of reaction from 5 to 10 min did not change the radiochemical yield. No radiolabelled or unlabelled by-products were evident by HPLC in any of the radiolabelling experiments.

Based on our previous experience of lung deposition studies,[25] administration of 20 MBq of each radioligand would be sufficient for PET studies, and this dose can be achieved with high-efficiency delivery systems.[26] Imaging of the initial deposition of aerosols takes a few minutes from inhalation, therefore, radioactive decay poses no problem. Similar studies using carbon-11 labelled radioligands in aqueous nasal sprays and metered dose inhalers have been reported in the literature.[12] Although the radiochemical yields obtained were low, no further attempts were made to the increase yields, by either increasing the production of [^{11}C]CO_2 or optimizing the radiolabelling conditions. Sufficient quantities of each radioligand were produced for preliminary imaging experiments.

Conclusions

[^{11}C]Ipratropium [^{11}C]**1** and [^{11}C]tiotropium [^{11}C]**2** PET radioligands have been synthesized in three and five steps,

respectively. Tertiary amine precursors for both ligands were radiolabelled by *N*-alkylation with $[^{11}C]CH_3I$ in the final step of the synthesis. The $[^{11}C]$ipratropium and $[^{11}C]$tiotropium were obtained with high radiochemical purity, in 0.3 and 0.5% non-decay corrected yields based on $[^{11}C]CO_2$ and specific activities of 11 and 18 GBq μmol^{-1}, respectively, calculated at end-of-synthesis. These radioligands can be used for the study of inhaled drug deposition in the human lung.

Experimental

General

Reagents were purchased from Aldrich or Lancaster Synthesis. Solvents and reagents were dried and purified according to literature methods. Thin layer chromatography was performed using 0.2 mm thick precoated aluminium-backed silica gel plates (Merck kieselgel 60 F254). Compounds were visualized by ultraviolet absorption or by staining with vanillin in ethanolic sulfuric acid or potassium permanganate. Flash chromatography was performed using Merck kieselgel 60 (230–400 mesh) with the specified solvents. Preparative HPLC was performed with a Waters 515 or Waters 600 Controller pump, a Waters 2487 dual or Waters 486 tuneable absorbance detector, and a single sodium iodide crystal flow radioactivity detector. HPLC chromatograms were recorded using *RadioStar ver. 3.0* and Waters *Millennium ver. 3.05.01* software.

Infrared absorption spectra were recorded using a Shimadzu 8400S Fourier-transform infrared spectrophotometer. Compounds were prepared as either thin films between sodium chloride plates or as potassium bromide discs. Absorption maxima are expressed in wavenumbers (cm^{-1}). NMR spectra were recorded on a Bruker Avance DPX200, DPX300, or DPX400 spectrometer at 300 K. Spectra were recorded in deuterochloroform or deuteromethanol. Data are expressed in parts per million downfield shift from tetramethylsilane and are reported as chemical shift, relative integral, multiplicity, coupling constant (*J*, Hz), and assignment. Low-resolution mass spectra were recorded using electron impact (EI+) on a Finnigan Polaris Q ion trap at 70 eV with PFK as standard or positive electrospray ionization (ESI+) on a Finnigan LCQ ion trap. High-resolution mass spectra were recorded using EI+ on a Kratos MS25 RFA at 70 eV in magnetic scan with PFK as standard or ESI+ on a 4.7 T Bruker FT-ICR/MS.

$[^{11}C]CO_2$ was produced using a PET trace cyclotron by a 40 μA, 5 min irradiation of nitrogen gas using a proton beam by the $^{14}N[p,\alpha]^{11}C$ nuclear reaction. The $[^{11}C]CO_2$ was converted into $[^{11}C]CH_3I$ via $[^{11}C]CH_4$ in a General Electric methyl iodide module and transported through tubing by a stream of helium gas into the reaction vial contained in a lead shielded cell.

Chemistry

*(1*R*,3*r*,5*S*,2′*RS*)-3′-Hydroxy-2′-phenylpropionic Acid 8-Azabicyclo[3.2.1]oct-3-yl Ester* ***5****(Noratropine* ***5****)*[15]

Atropine **4** (1.34 g, 4.63 mmol, 1.0 equiv.) was converted into noratropine **5**[15] (308 mg, 24%) according to the method of Ripper et al.,[14] using a Rayonet RPR-100 photoreactor (160 W). The ^{1}H NMR spectrum was consistent with the data reported in the literature.[15]

*(1*R*,3*r*,5*S*,2′*RS*)-3′-Hydroxy-2′-phenylpropionic Acid 8-Isopropyl-8-azabicyclo[3.2.1]oct-3-yl Ester* ***3***[16]

To a solution of noratropine **5** (220.4 mg, 0.801 mmol, 1.0 equiv.) in anhydrous methanol (6.1 mL) was added dry acetone (1.2 mL, 16.0 mmol, 20 equiv.) followed by sodium cyanoborohydride (174 mg, 2.77 mmol, 3.5 equiv.) in one portion at room temperature. After stirring under an atmosphere of nitrogen for 20 min, the basic reaction mixture was adjusted to pH 7.0 with glacial acetic acid (Universal indicator paper). The dark brown mixture was stirred at room temperature for a further 4.5 h and then diluted with dichloromethane (10 mL) and washed with saturated sodium hydrogencarbonate solution (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated. Purification of the residue by flash chromatography on a short column (EtOAc/MeOH/NH_3 90/10/0.5) afforded the *N*-isopropyl tropane **3**[16] as a white solid (157 mg, 62%). R_f (CH_2Cl_2/MeOH/NH_3 90/10/1) 0.30. R_t (Spherisorb S10 SCX 10 μm, 10 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 60/40, 5 mL min^{-1}) 6.06 min. ν (thin film)/cm^{-1} 3385 (OH), 2966, 2949 (C–H), 1718 (C=O ester), 732, 698 (C–H). δ_H (400 MHz, $CDCl_3$) 7.37–7.24 (5H, m, Ar-H), 5.09 (1H, t, *J* 5.3, C_3-H), 4.19 (1H, dd, *J* 10.6 & 8.3, $C_{2'}$-H), 3.86–3.78 (2H, m, $C_{3'}$-H), 3.46 (1H, m, C_1-H or C_5-H), 3.34 (1H, m, C_1-H or C_5-H), 2.63 (1H, septet, *J* 6.2, Pr^i-CH), 2.28–1.88 (3H, m, C_2-H^a, C_4-H^a, OH), 1.85–1.77, 1.66–1.57 and 1.26–1.17 (5H, m, C_2-H^b or C_4-H^b, C_6-H, C_7-H), 1.40 (1H, d, *J* 15.5, C_2-H^b or C_4-H^b), 1.09 (6H, d, *J* 6.1, Pr^i-CH_3). δ_C (75 MHz, $CDCl_3$) 172.3, 135.6, 128.9, 128.2, 127.8, 68.4, 64.2, 54.3, 54.3, 54.1, 46.7, 33.2, 33.0, 26.5, 26.0, 21.3 (2C). *m/z* (ESI+) 318 (M + H, 100%). (HRMS (ESI+) calc. 318.2069. $C_{19}H_{28}NO_3$ (M + H) found 318.2062.)

*(1*R*,3*r*,5*S*,8*r*,2′*RS*)-3-(3′-Hydroxy-2′-phenylpropionyloxy)-8-isopropyl-8-methyl-8-azabicyclo[3.2.1]octan-8-ium* ***1*** *Iodide (Ipratropium* ***1*** *Iodide)*[16]

The procedure was adapted from that of Dei et al.[27] To a solution of *N*-isopropyl tropane **3** (135 mg, 0.425 mmol, 1.0 equiv.) in 87/13 diethyl ether/DMF (12.6 mL) was added methyl iodide (179 μL, 2.88 mmol, 6.8 equiv.) under an atmosphere of nitrogen. The mixture was stirred at room temperature for 16.5 h and then heated at reflux for 2.5 h. The resulting white precipitate was isolated by filtration to give ipratropium **1** iodide[16] (10 mg, 2%) as a white solid. R_t (Alltech Altima C18 5 μm, 4.6 × 250 mm^2, acetonitrile/water 1/9 to 1/1 gradient with 0.05% trifluoroacetic acid at 1 mL min^{-1}) 21.0 min; and (Spherisorb S10 SCX 10 μm, 10 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 60/40, 5 mL min^{-1}) 7.73 min. δ_H (300 MHz, MeOD) 7.43–7.25 (5H, m, Ar-H), 5.19 (1H, t, *J* 5.8, C_3-H), 4.25–4.09 (2H, m, $C_{2'}$-H, Pr^i-CH), 4.00 (1H, m, C_1-H or C_5-H), 3.93–3.79 (3H, m, C_1-H or C_5-H, $C_{3'}$-H), 2.87 (3H, s, N-CH_3), 2.75–2.56 (2H, m, C_2-H^a, C_4-H^a), 2.39–2.25 (2H, m, C_6-H^a, C_7-H^a), 2.17 (1H, m, C_6-H^b or C_7-H^b), 2.05 (1H, d, *J* 17.1, C_2-H^b or C_4-H^b), 1.85 (1H, m, C_6-H^b or C_7-H^b), 1.81 (1H, d, *J* 16.5, C_2-H^b or C_4-H^b), 1.39 (6H, t, *J* 6.0, Pr^i-CH_3). δ_C (75 MHz, MeOD) 172.9, 137.1, 130.0, 129.3, 128.9, 67.3, 67.1, 64.5, 64.5, 57.1, 55.9, 40.2, 32.4 (2C), 25.8, 25.6, 16.6 (2C). *m/z* (ESI+) 332 (M, 100%). (HRMS (ESI+) calc. 332.2226. $C_{20}H_{30}NO_3$ (M) found 332.2218.) Concentration of the filtrate afforded recovered nortropane **3**. The HPLC, ^{1}H and ^{13}C NMR data obtained for ipratropium **1** iodide strongly correlated with that of a commercially derived sample of ipratropium bromide.

*(1*R*,3*s*,5*S*,2′*RS*)-3′-Hydroxy-2′-phenylpropionic Acid 8-Methyl-8-azabicyclo[3.2.1]oct-6-en-3-yl Ester*[20]

The procedure was adapted from that of Malpass et al.[19] Scopolamine hydrobromide trihydrate **8** (800 mg, 1.83 mmol) in acetonitrile (22 mL) was treated with a zinc–copper couple (4.40 g) in a pressure tube. The tube was flushed with Ar, sealed, and heated at 156°C for 3 days. After cooling, the reaction mixture was filtered through a celite plug and the plug washed with dichloromethane. The filtrate was concentrated to dryness and the residue taken up in dichloromethane (30 mL). The organic phase was washed with saturated sodium hydrogencarbonate solution (2 × 20 mL), dried (Na_2SO_4), filtered, and the solvent removed by rotary evaporation. The oily residue was purified by flash chromatography (MeOH/CH_2Cl_2/NH_3 6/94/1) to afford the title compound[20] (383 mg, 73%) as a colourless oil. The ^{1}H NMR and IR spectra were consistent with the data reported in the literature.[20]

*(1*R*,3*s*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-6-en-3-ol* ***7***[20]

A solution of (1*R*,3*s*,5*S*,2′*RS*)-3′-hydroxy-2′-phenylpropionic acid 8-methyl-8-azabicyclo[3.2.1]oct-6-en-3-yl ester (687 mg, 2.39 mmol, 1.0 equiv.) in sodium hydroxide (3 M, 25.5 mL, 32.0 equiv.) was heated at reflux (oil bath 150°C) for 2.5 h. After cooling, the reaction mixture was diluted with brine and extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with saturated sodium hydrogencarbonate solution, dried (Na_2SO_4), filtered, and the solvent removed by rotary evaporation (water bath 25°C) to give tropenol **7**[20] (193 mg, 58%) as a volatile colourless oil. Due to the volatility of the product,

tropenol **7** was taken onto the next step without further purification. The ^{1}H NMR and IR spectra were consistent with the data reported in the literature.[20]

*2-Hydroxy-2,2-di(thiophen-2′-yl)acetic Acid Methyl Ester **6***[22]

Ester **6** was synthesized according to the literature with only minor modifications.[21,22] Purification of the crude reaction mixture by flash chromatography (EtOAc/hexane 1/9) gave methyl ester **6**[22] (75%) as white crystalline plates that darkened on standing. R_f (EtOAc/hexane 1/9) 0.21. ν (thin film)/cm^{-1} 3477 (OH), 3105, 2960, 2924, 2849 (C–H), 1722 (C=O ester) cm^{-1}. δ_H (200 MHz, $CDCl_3$) 7.29 (2H, dd, *J* 5.1 & 1.2, $C_{5'}$-H), 7.16 (2H, dd, *J* 3.6 & 1.3, $C_{3'}$-H), 6.98 (2H, dd, *J* 5.1 & 3.7, $C_{4'}$-H), 4.68 (1H, s, OH), 3.90 (3H, s, OCH_3). δ_C (75 MHz, $CDCl_3$) 172.8, 145.6, 126.8, 126.0, 125.9, 76.5, 54.1. *m/z* (EI+) 237 (M-OH, 27%), 195 (M-$C_2H_3O_2$, 48), 111 (C_5H_3OS, 100). A second fraction afforded di(thiophen-2-yl)methanone **11**,[23] for which the ^{1}H NMR and IR spectra were consistent with the data reported in the literature.[23]

*(1R,3s,5R)-2′-Hydroxy-2′,2′-di(thiophen-2″-yl)acetic Acid 8-Methyl-8-azabicyclo[3.2.1]oct-6-en-3-yl Ester **12***[24]

The procedure was adapted from that of Nilles and Schuetz.[22] To a round-bottomed flask charged with sodium hydride (67.1 mg, 2.80 mmol, 2.4 equiv.) was added ester **6** (293.7 mg, 1.16 mmol, 1.0 equiv.) followed by a pre-dried ($CaSO_4$) solution of tropenol **7** (193 mg, 1.39 mmol, 1.2 equiv.) in *n*-heptane (13.9 mL). The reaction mixture was heated at reflux under a nitrogen atmosphere for 2.5 h. After cooling, the mixture was quenched carefully with water (10 mL) and dichloromethane (10 mL). The organic phase (top layer) was separated and the aqueous phase extracted with dichloromethane (2 × 15 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness to give a tan coloured solid. Purification by flash chromatography ($MeOH/CH_2Cl_2/NH_3$ 10/90/1) afforded ester **12**[24] (241 mg, 58%) as a white solid. R_f ($MeOH/CH_2Cl_2/NH_3$ 10/90/1) 0.30. ν (thin film)/cm^{-1} 3472 (OH), 3101, 3069, 2955, 2934, 2870 (C–H), 1728 (C=O ester). δ_H (200 MHz, $CDCl_3$) 7.29 (2H, dd, *J* 5.1 & 1.2, $C_{5''}$-H), 7.16 (2H, dd, *J* 3.7 & 1.2, $C_{3''}$-H), 7.00 (2H, dd, *J* 5.0 & 3.6, $C_{4''}$-H), 5.63 (2H, s, C_6-H, C_7-H), 5.11 (1H, t, *J* 6.0, C_3-H), 3.33 (2H, m, C_1-H, C_5-H), 2.25 (3H, m, C_2-H^a, C_4-H^a, OH), 2.23 (3H, s, N-CH_3), 1.69 (2H, d, *J* 14.6, C_2-H^b, C_4-H^b). δ_C (50 MHz, $CDCl_3$) 171.3, 145.7, 131.5, 126.7, 126.0, 125.7, 71.0, 65.2, 41.3, 33.1 (one signal overlapping). *m/z* (ESI+) 362 (M + H, 100%).

*(1R,2R,4S,5S,7s)-2′-Hydroxy-2′,2′-di(thiophen-2″-yl)acetic Acid 9-Methyl-9-aza-3-oxatricyclo[3.3.1.0^{2,4}]non-7-yl Ester **13***[24]

The procedure was adapted from that of Rapp and Sobbota.[24] To a mixture of ester **12** (142 mg, 0.393 mmol, 1.0 equiv.) and vanadium(v) oxide (7.2 mg, 0.0393 mmol, 0.1 equiv.) was added DMF (2.3 mL) followed by 30% hydrogen peroxide (107 µL, 0.944 mmol, 2.4 equiv.) dropwise. The reaction mixture was stirred at 57°C for 4.5 h. After cooling to 40°C, the reaction was quenched with sodium bisulfite (a mixture of $NaHSO_3$ and $Na_2S_2O_5$) and then acidified with 2 M hydrochloric acid. After stirring at 40°C for 10 min, the mixture was cooled to room temperature and diluted with 5% sodium hydrogencarbonate solution. The product was extracted into ethyl acetate (4 × 10 mL) and the combined organic layers washed with brine, dried (Na_2SO_4), filtered, and concentrated under vacuum. Residual DMF was removed under high vacuum. Flash chromatographic purification ($MeOH/CH_2Cl_2/NH_3$ 6/94/1) afforded epoxide **13**[24] (119 mg, 80%) as a white solid. R_f ($MeOH/CH_2Cl_2/NH_3$ 10/90/1) 0.56. R_t (Spherisorb S10 SCX 10 µm, 10 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 40/60, 5 mL min^{-1}) 7.20 min. ν (thin film)/cm^{-1} 3481 (OH), 3103, 3039, 2959, 2945 (C–H), 1732 (C=O ester). δ_H (200 MHz, $CDCl_3$) 7.31 (2H, dd, *J* 5.1 & 1.2, $C_{5''}$-H), 7.12 (2H, dd, *J* 3.6 & 1.2, $C_{3''}$-H), 6.99 (2H, dd, *J* 5.1 & 3.6, $C_{4''}$-H), 5.13 (1H, t, *J* 5.4, C_7-H), 4.81 (1H, br s, OH), 3.09 (2H, m, C_1-H, C_5-H), 3.00 (2H, s, C_2-H, C_4-H), 2.47 (3H, s, N-CH_3), 2.16 (2H, dt, *J* 15.7 & 5.2, C_6-H^a, C_8-H^a), 1.60 (2H, d, *J* 15.4, C_6-H^b, C_8-H^b). δ_C (50 MHz, $CDCl_3$) 171.4, 145.2, 126.8, 126.2, 126.1, 70.5, 57.8, 56.1, 42.5, 31.0 (one signal overlapping). *m/z* (ESI+) 378 (M + H, 100%). (HRMS (ESI+) calc. 378.0834. $C_{18}H_{20}NO_4S_2$ (M + H) found 378.0824.)

*(1R,2R,4S,5S,7s)-7-[2′-Hydroxy-2′,2′-di(thiophen-2″-yl) acetoxy]-9,9-dimethyl-9-aza-3-oxatricyclo[3.3.1.0^{2,4}] nonan-9-ium **2** Trifluoroacetate (Tiotropium **2** Trifluoroacetate)*

A solution of epoxide **13** (27 mg, 0.0716 mmol, 1.0 equiv.) in anhydrous DMF (0.9 mL) was treated with methyl iodide (44.6 µL, 0.716 mmol, 10 equiv.) dropwise at room temperature. The reaction mixture was heated at 57°C for 4.5 h. After cooling to room temperature, milliQ water (5 mL) was added. The aqueous phase was washed with ethyl acetate and then lyophilized to dryness. Purification by reverse-phase HPLC (Alltech Altima C18 10 µm, 22 × 300 mm^2, acetonitrile/water 40/60 with 0.05% trifluoroacetic acid, 7 mL min^{-1}) afforded tiotropium **2** trifluoroacetate (27.2 mg, 97%) as a white solid. R_t (Spherisorb S10 SCX 10 µm, 10 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 40/60, 5 mL min^{-1}) 9.10 min. ν (KBr plate)/cm^{-1} 3427 (OH), 1742 (C=O ester), 1686 (COO^-). δ_H (200 MHz, MeOD) 7.43 (2H, dd, *J* 5.1 & 1.2, $C_{5''}$-H), 7.17 (2H, dd, *J* 3.6 & 1.2, $C_{3''}$-H), 7.02 (2H, dd, *J* 5.1 & 3.7, $C_{4''}$-H), 5.22 (1H, t, *J* 6.0, C_7-H), 4.04 (2H, m, C_1-H, C_5-H), 3.40 (2H, s, C_2-H, C_4-H), 3.34 (3H, s, N-CH_3), 3.10 (3H, s, N-CH_3), 2.79 (2H, ddd, *J* 18.0 & 6.0 & 4.2, C_6-H^a, C_8-H^a), 2.02 (2H, d, *J* 17.6, C_6-H^b, C_8-H^b). δ_C (75 MHz, MeOD) 171.5, 147.4, 127.8, 127.5, 127.2, 78.1, 66.8, 65.1, 57.2, 55.0, 48.2, 29.8. *m/z* (ESI+) 392 (M, 100%). (HRMS (ESI+) calc. 392.0990. $C_{19}H_{22}NO_4S_2$ (M) found 392.0996.) The HPLC retention time for tiotropium **2** trifluoroacetate correlated with that of a commercially derived sample of tiotropium bromide.

Radiochemistry

General Procedures

A small septum-sealed vial containing a solution of the labelling precursor (1.5 mg) dissolved in anhydrous DMF (200 µL) was cooled to −40°C and [^{11}C]methyl iodide was transferred into the vial by a stream of helium carrier gas for 2 min. The solution was heated at 88°C in a water bath for 5 min and then diluted with 200 µL of HPLC solvent. The mixture was injected into a HPLC and the effluent from the column was monitored with both UV and radioactivity detectors. The peak corresponding to the carbon-11 labelled product was collected in a rotary evaporator, the solvent was removed under reduced pressure, and then the residue was diluted with water. The radiochemical purity and specific radioactivity was determined by HPLC (Waters Spherisorb S10 SCX, 5 µm, 4.6 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 50/50, 2 mL min^{-1}). The area of the absorbance peak corresponding to the radiolabelled product of known radioactivity was measured at 210 nm (for **1**) or 238 nm (for **2**) by an automated integrating recorder and compared to a standard curve relating mass to UV absorbance.

*Synthesis of [^{11}C]Ipratropium **1** Iodide [^{11}C]**1***

The labelling was conducted according to the general procedure to afford [^{11}C]ipratropium **1** iodide [^{11}C]**1** (>98% chemical and radiochemical purity). R_t (Spherisorb S10 SCX 10 µm, 10 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 60/40, 6 mL min^{-1}) 6.67 min. The radiochemical yield was 61 ± 3 MBq (n = 3) at end-of-synthesis (0.3%, calculated from [^{11}C]CO_2, non-decay corrected) and the specific radioactivity at end-of-synthesis including formulation and quality control (35 min) was measured at 11 GBq µmol^{-1}.

*Synthesis of [^{11}C]Tiotropium **2** Iodide [^{11}C]**2***

The labelling was conducted according to the general procedure to afford [^{11}C]tiotropium **2** iodide [^{11}C]**2** (>98% chemical and radiochemical purity). R_t (Spherisorb S10 SCX 10 µm, 10 × 250 mm^2, acetonitrile/0.1 M NaH_2PO_4 40/60, 6 mL min^{-1}) 6.68 min. The radiochemical yield calculated was 129 ± 8 MBq (n = 3) at end-of-synthesis (0.5%, calculated from [^{11}C]CO_2, non-decay corrected) and the specific

radioactivity at end-of-synthesis including formulation and quality control (37 min) was measured at 18 GBq μmol^{-1}.

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