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Radiosynthesis of (*S*)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-yl] oxazolidin-2-[^{11}C]one ([^{11}C]SL25.1188), a novel radioligand for imaging monoamine oxidase-B with PET

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Within a novel series of 2-oxazolidinones developed in the past by Sanofi-Synthélabo, SL25.1188 ((*S*)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-yl]oxazolidin-2-one), a compound that inhibits selectively and competitively MAO-B in human and rat brain (Ki values of 2.9 and 8.5 nM for MAO-B, respectively, and $ED_{50\ (rat)}$: 0.6 mg/kg p.o.), was considered an appropriate candidate for imaging this enzyme with positron emission tomography. SL25.1188 was labelled with carbon-11 ($T_{1/2}$: 20.38 min) in one chemical step using the following process: (i) reaction of [^{11}C]phosgene with the corresponding ring-opened precursor (1.2–2.5 mg) at 100°C for 2 min in dichloromethane (0.5 mL) followed by (ii) concentration to dryness of the reaction mixture and finally (iii) semi-preparative HPLC purification on a Waters Symmetry® C18. A total of 300–500 MBq of [^{11}C]SL25.1188 (> 95% chemically and radiochemically pure) could be obtained within 30–32 min (Sep-pak-based formulation included) with specific radioactivities ranging from 50 to 70 GBq/μmol (3.5–7% decay-corrected radiochemical yield, based on starting [^{11}C]CH_4).

Keywords: SL25.1188; carbon-11; phosgene; MAO-B

Introduction

Monoamine oxidase (MAO) is an integral protein of outer mitochondrial membranes and occurs in neuronal and non-neuronal cells in the brain and in peripheral organs.[1,2] Two isoforms, termed MAO-A and MAO-B, have been described, each consisting of two subunits[3,4] coded by different genes and with molecular weights of about 59 700 and 58 800 D,[5] respectively. MAOs catalyse the oxidative deamination of both endogenous and exogenous amines[6] with its two isoforms differing in substrate specificity and inhibitor sensitivity. MAO-A preferentially deaminates 5-hydroxytryptamine (serotonin), norepinephrine and epinephrine and is selectively inactivated by low concentrations of clorgyline.[7] MAO-B preferentially deaminates phenethylamine and benzylamine and is selectively inhibited by low concentrations of deprenyl.[8] Dopamine and tyramine are metabolized by both forms.[9–11] Nevertheless, this specificity is relative and the deamination of a given substrate by MAO-A or MAO-B depends not only on the substrate itself but also on the relative concentration of each form of MAO. Histochemical, immunohistochemical and autoradiographic studies have revealed that the two isoforms of MAO also have a distinct regional brain distribution, with MAO-A found primarily in catecholaminergic neurones and MAO-B localized in serotoninergic neurones and glial cells.

Fluctuations in functional MAO activity, directly impacting the concentration of neurotransmitters as well as many xenobiotics, have been associated with human diseases such as Parkinson's disease, depression and certain psychiatric disorders,[12–16] reinforcing the potential role of positron emission tomography (PET) to the *in vivo* and non-invasive study of these enzymes. A few compounds have already been proposed as MAO-radiotracers, most of them having been labelled with the positron-emitter carbon-11 ($T_{1/2}$: 20.38 min). The irreversible inhibitors [^{11}C]deprenyl and deuterium-substituted [^{11}C]deprenyl are currently the radioligands of choice for PET-imaging of MAO-B activity.[17–31] On the other hand, MAO-A selective PET-ligands include not only [^{11}C]harmine,[32–37] [^{11}C]brofaromine[38] and [^{11}C]clorgyline[19,29,39–43] but also [^{11}C]befloxatone,[44–47] the lead-compound of a novel series of 2-oxazolidinones, developed in the past by Sanofi-Synthélabo,[48–53] as highly potent reversible inhibitors. SL25.1188 is another chemically closely related

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2-oxazolidinone derivative, which inhibits not only selectively but also competitively MAO-B in both human and rat brain (Ki values of 2.9 and 8.5 nM for MAO-B, respectively.[54] SL25.1188 is also a pharmacologically active compound at a reasonably low dose ($ED_{50\ (rat)}$: 0.6 mg/kg p.o.) and was considered another appropriate candidate for imaging the MAO-B form with PET. It was, therefore, selected for carbon-11 labelling with [^{11}C]phosgene at its oxazolidinone function, similar to [^{11}C]befloxatone (Figure 1).

Results and discussion

Chemistry

The ring-opened precursor for carbon-11 labelling, compound **2** ((*S*)-1-methoxy-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-ylamino]propan-2-ol), was prepared in non-optimized 93% yield from SL25.1188 (**1**) using 5 equivalents of NaOH in a mixture of water and ethanol (Scheme 1) according to the published procedures.[55,56] The batch obtained was >99% chemically pure (as determined by HPLC) and shown to be free of the starting material SL25.1188 (**1**), which would give rise to unwanted carrier in the carbon-11 preparation. Analytical data were all in accordance with the structure.

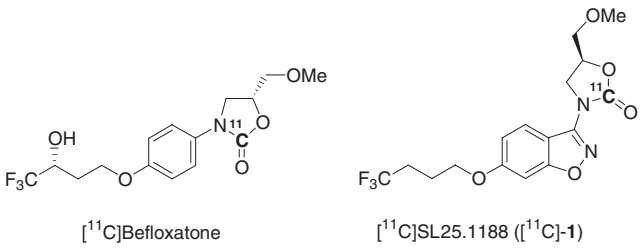


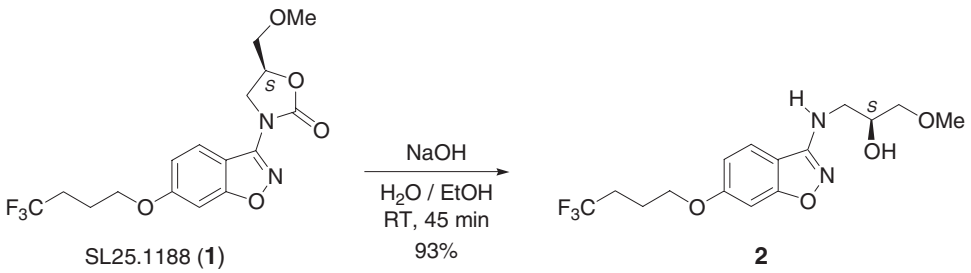
Figure 1. Chemical structures of the two carbon-11-labelled 2-oxazolidinones: [^{11}C]befloxatone and [^{11}C]SL25.1188.

Radiochemistry

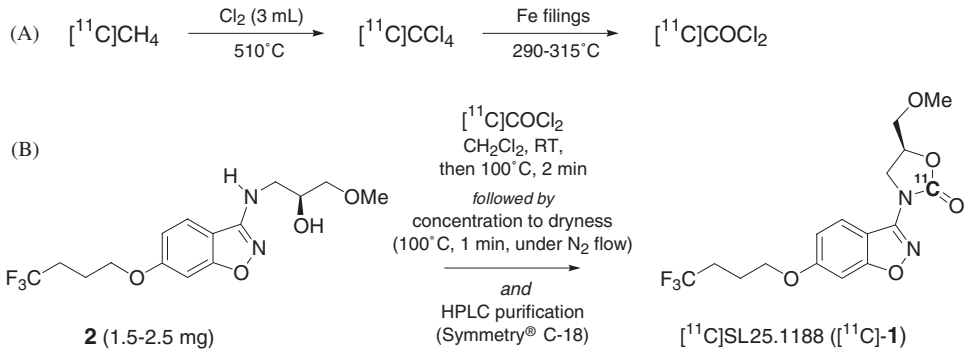
SL25.1188 (**1**) presents in its chemical structure one methoxy group, which could have been the site for carbon-11 labelling using either [^{11}C]methyl iodide or -triflate as reagent.[57–60] Metabolism studies have shown that desmethyl-SL25.1188 and its glucuronic acid conjugate were the main metabolites, therefore prohibiting the labelling approach suggested above. SL25.1188 (**1**) was eventually labelled with carbon-11 using no-carrier-added [^{11}C]phosgene and the corresponding ring-opened precursor **2**.

[^{11}C]Phosgene ([^{11}C]$COCl_2$) was synthesized from cyclotron-produced [^{11}C]methane ([^{11}C]CH_4) via [^{11}C]carbon tetrachloride ([^{11}C]CCl_4) using slight modifications of the already published processes.[61–66] Briefly, [^{11}C]CH_4 was separated from the target contents, trapped and concentrated on Porapak-Q. [^{11}C]CH_4 was then swept by a flow of helium gas (Scheme 2(A)), mixed with 3 mL of chlorine and this mixture was carried through an empty linear horizontal glass tube at a temperature of 510°C using the same vector gas. The online synthesized [^{11}C]CCl_4 was continuously swept away by the same helium vector gas and passed through a glass U-tube containing iron filings at a temperature of 290–310°C, giving [^{11}C]$COCl_2$ in 9–10 min radiosynthesis time and in 35–45% decay-corrected radiochemical yield, based on starting [^{11}C]CH_4.

Reaction of derivative **2** with [^{11}C]$COCl_2$, employing our recently optimized conditions used in our laboratory for the synthesis of [^{11}C]befloxatone, another 2-[*carbonyl*-^{11}C]oxazolidi-



Scheme 1



Scheme 2

none, from its corresponding ring-opened precursor ((*R*)-1-methoxy-3-[[4-[(3*R*)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]amino]-2-propanol), gave unexpected extremely low yields for the preparation of [^{11}C]SL25.1188 ([^{11}C]-**1**) (Scheme 2(B)). The conditions used were as follows: (**1**) trapping at room temperature (RT) of [^{11}C]$COCl_2$ in 300 μL of acetonitrile containing only 0.5–1.5 mg of precursor **2** (1.44–4.31 μmol); (**2**) dilution of the crude reaction mixture with 0.5 mL of water containing 2% of Et_2NH [v:v] and (3) semi-preparative HPLC purification on a Waters Symmetry® C18 column. Although 3.0–3.3 GBq of [^{11}C]befloxatone were routinely obtained within 20–25 min of radiosynthesis using these conditions and starting from a 22–24 GBq [^{11}C]CH_4 batch (25–35% decay-corrected radiochemical yield), less than 50 MBq of [^{11}C]SL25.1188 ([^{11}C]-**1**) could be isolated (<1% decay-corrected radiochemical yield). Noteworthy, [^{11}C]SL25.1188 ([^{11}C]-**1**) was the only detectable product on the radiochromatogram besides the characteristic peaks at the front emanating from unreacted [^{11}C]phosgene. Heating the reaction mixture at 90–100°C (sealed vessel) did not significantly improve the yields or the use of a larger amount of the labelling precursor **2** (for example, 2.5 mg or 7.2 μmol). The use of toluene, the reaction solvent proposed in the past for the in-house synthesis of [^{11}C]CGP-12177,[67–69] a benz[*carbonyl*-^{11}C]imidazolone, as well as for the synthesis of [^{11}C]MD-230254,[70] a 1,3,4-[*carbonyl*-^{11}C]oxadiazolone was completely unsuccessful since no traces of [^{11}C]SL25.1188 ([^{11}C]-**1**) could be isolated, with or without heating (90–100°C) the reaction mixture. The best and also the most reproducible yields were observed using dichloromethane as the solvent and conditions including the use of 1.5–2.5 mg of the labelling precursor **2** (4.3–7.2 μmol), a short heating of the mixture (2 min at 100°C) followed by its concentration to dryness before final HPLC purification (4–10% decay-corrected radiochemical yield, based on starting [^{11}C]CH_4).

Formulation of [^{11}C]SL25.1188 ([^{11}C]-**1**) as an i.v. injectable solution was performed using a home-made Sep-Pak® Plus C18 device.[71] The HPLC-collected fraction containing the radiotracer was diluted with water and the resulting solution was passed through a C18 Sep-Pak® cartridge. The cartridge was then washed with water, partially dried with helium and finally eluted with ethanol followed by physiological saline. The solution was then diluted with physiological saline to an ethanol concentration below 10%.

Quality controls of [^{11}C]SL25.1188 ([^{11}C]-**1**) were performed on an aliquot of the preparation ready for i.v. injection. The radiotracer preparation was a clear and colourless solution with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis, the radiotracer preparation was found to be >95% chemically and radiochemically pure (**1**, t_R: 4.25 min). The preparation was also shown to be chemically and radiochemically stable for at least 60 min.

Experimental set up

General

Chemicals

Chemicals were purchased from Aldrich-, Fluka- or Sigma France and were used without further purification. SL25.1188 (**1**, (*S*)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-yl]oxazolidin-2-one) was synthesized by Sanofi-Synthélabo (31 Avenue Paul Vaillant Couturier, F-92200 Bagneux, France).

TLC and HPLC analyses

TLCs were run on pre-coated plates of silica gel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV lamp and/or (2) by dipping the TLC-plates in a 1% ethanolic ninhydrin solution and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser. HPLCs: Equipment, column and conditions; [HPLC A]: equipment: Waters Alliance 2695 and a Waters 996 UV-multi-wavelength detector; column: analytical SunFire® C18, Waters (50 × 2.1 mm); porosity: 3.5 μm; conditions: linear gradient elution with solvA/solvB (95:5–50:50 (v:v) in 15 min) [solvent A: aq. 5 mM NH_4OAc (pH 6.8)/CH_3CN (97:3 (v:v)); solvent B: CH_3CN]; flow rate: 1.0 mL/min; temperature: 30°C; absorbance detection at $\lambda = 254$ nm. [HPLC B]: Equipment: Waters 510 pump (or a Waters 600 pump and Waters 600 Controller), a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semipreparative Symmetry® C18, Waters (300 × 7.8 mm); porosity: 7 μm; conditions: isocratic elution with H_2O/CH_3CN/TFA (48:52:0.1 (v/v/v)); flow rate: 8 mL/min; temperature: RT; absorbance detection at $\lambda = 240$ nm. [HPLC C]: Equipment: Waters Alliance 2690 (or a Waters binary HPLC pump 1525) equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C18, Waters (50 × 4.6 mm); porosity: 5.0 μm; conditions: isocratic elution with solvA/solvB (7:93 (v:v)) [solvent A: H_2O containing low-UV PIC® B7 reagent (20 mL for 1000 mL, composition (% by weight): methanol (18–22%), heptane sulphonic acid–sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters); solvent B: H_2O/CH_3CN: 50:50 (v/v) containing low-UV PIC® B7 reagent (20 mL for 1000 mL)]; flow rate: 2.0 mL/min; temperature: 30°C; absorbance detection at $\lambda = 254$ nm.

Physicochemical analyses including spectroscopies

Melting points were measured on a Buchi B-540 and are uncorrected. Optical rotations ($[\alpha]_D$) were measured on a Perkin-Elmer 341 polarimeter in CH_2Cl_2. NMR spectra were recorded on a Bruker DPX200 (200 MHz) apparatus or on a Bruker Advance (400 MHz) apparatus using the hydrogenated residue of the deuteriated solvents ($CDCl_3$, $\delta = 7.26$ ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuteriated solvents ($CDCl_3$, $\delta = 77.0$ ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q, q^5, dd, td, m, b for singlet, doublet, triplet, quadruplet, quintuplet, doublet of doublet, triplet of doublet, multiplet (or multi sharp-peak system) and broad, respectively). The mass spectra (MS) were measured on an MS Platform II™ (Waters) spectrometer. Elemental analyses were performed on a Thermo Finnigan EA flash 1112 and results were within ± 0.4% of theoretical values.

Miscellaneous

Radiosyntheses using carbon-11, including the HPLC purifications, were performed in a 5-cm lead-shielded cell using a homemade automated system.

Chemistry

*(S)-1-Methoxy-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-ylamino]propan-2-ol (**2**)*

To a suspension of SL25.1188 (**1**, (*S*)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-yl]oxazolidin-2-one,

0.87 g, 2.32 mmol) in EtOH (10 mL) was added 9 mL of aq. 1.3 N NaOH (11.7 mmol, 5 equivalent). The reaction mixture was stirred at RT for 45 min. The resulting solution was diluted with water (100 mL) and CH_2Cl_2 (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The solid obtained was triturated with petroleum ether to give 0.75 g of **2** as a white powder (93%). HPLC A; t_R: (**2**): 7.33 min (>99%). M_p: 118.5–119.5°C. $[\alpha]_D^{20}$: −9.7 (CH_2Cl_2, 1.006). ^{1}H NMR ($CDCl_3$, 200 MHz): δ: 2.05–2.20 (m, 2H); 2.20–2.40 (m, 2H); 2.83 (bd, NH, 1H); 3.39 (s, 3H); 3.41–3.70 (m, 4H); 4.03 (t, J = 5.9 Hz, 2H); 4.14 (m, J = 4.0 Hz, 1H); 4.65 (bt, OH, 1H); 6.79 (d, J = 7.1, 1H); 6.82 (s, 1H); 7.37 (d, J = 9.7 Hz, 1H).^{1}H NMR ($CDCl_3$, 400 MHz): δ: 2.05–2.10 (m, 2H); 2.23–2.36 (m, 2H); 3.36 (s, 3H); 3.40 (q, J = 7.4 Hz, 1H); 3.44 (d, J = 6.4 Hz, 1H); 3.50 (dd, J = 9.6 and 4.0 Hz, 1H); 3.57 (dd, J = 14.1 and 3.7 Hz, 1H); 4.01 (t, J = 6.7 Hz, 2H); 4.13 (m, J = 3.6 Hz, 1H); 6.73 (dd, J = 6.7 and 2.1 Hz, 1H); 6.75 (s, 1H); 7.39 (d, J = 9.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ: 21.9 (q, $^3J_{C-F}$ = 3.2 Hz); 30.5 (q, $^2J_{C-F}$ = 29.5 Hz); 46.4; 59.1; 66.4; 68.9; 74.6; 93.5; 109.4; 112.6; 126.6; 127.0 (q, $^1J_{C-F}$ = 274.4 Hz); 158.6; 161.2; 164.3. MS: 349 $[M+H]^+$. Anal. Calcd $C_{15}H_{19}F_3N_2O_4$: C, 51.72; H, 5.50; N, 8.04; Found: C, 51.75; H, 5.60; N, 7.95.

Radiochemistry

Preparation of [^{11}C]methane

[^{11}C]Methane ([^{11}C]CH_4) was produced by irradiation of a pressurized target consisting of an ultrapure 95/5 mixture of N_2/H_2 (Air Liquide) with an 18 MeV proton beam via the ^{14}N[p,α]^{11}C nuclear reaction on an IBA Cyclone-18/9 cyclotron. At the end of the bombardment, the target contents were transferred by expansion to the 5-cm lead-shielded hot cell dedicated to the radiosynthesis of the tracer and passed firstly through an empty tube (stainless steel coil, 500 mm length, 4 mm internal diameter) cooled at −186°C using liquid argon in order to remove traces of [$^{13/14}$N]ammonia (produced during the irradiation) and secondly through a guard of P_2O_5 (glass tube, 70 mm length, 3 mm internal diameter) in order to remove residual moisture. [^{11}C]CH_4 was then separated from the target gas by trapping in a copper-U-tube (150 mm length, 4 mm internal diameter) filled with Porapak-Q (80-100 mesh, Waters) and cooled at −186°C (liquid argon). Typical average production of [^{11}C]CH_4 at the end of the bombardment for a 15 μA, 30 min irradiation: 22–24 GBq (595–650 mCi). Target to hot cell transfer and concentration time: 4–5 min.

Preparation of [^{11}C]phosgene

[^{11}C]CH_4 was released from the trap by warming the copper U-tube to RT (hot air) and swept away by a flow of helium gas (40 mL/min). [^{11}C]CH_4 was then passed through a guard of P_2O_5 (glass tube, 70 mm length, 10 mm internal diameter) and concentrated in a second smaller copper U-tube (150 mm length, 2 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186°C (liquid argon). [^{11}C]CH_4 was released from the trap by warming the latter to RT and swept (15 mL/min) in a volume of 1–2 mL of helium into a gas-mixing chamber containing 3 mL of chlorine (99.99%, Air Liquide). Using the same helium as vector gas (15 mL/min), the [^{11}C]CH_4–chlorine mixture was passed through an empty horizontal glass tube (215 mm length, 7 mm internal diameter) at a temperature of 510°C. The thus formed [^{11}C]CCl_4 then passed online through a glass U-tube (200 mm length, 4 mm internal diameter) containing 1.5 g of iron filings (Telar 57, Weber) at a temperature of 290–310°C. Finally, the gaseous reaction mixture now containing [^{11}C]$COCl_2$ passed online through an antimony-guard (glass tube, 70 mm length, 3 mm internal diameter), containing a 2/1 ratio [v/v] of antimony powder (400 mg) and glass beads (1 mm diameter) in order to remove the excess of chlorine.

Preparation of (S)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)-benzo[d]isoxazol-3-yl]-oxazolidin-2-[^{11}C]one ([^{11}C]SL25.1188, [^{11}C]-1)

The online synthesized [^{11}C]$COCl_2$ was trapped (bubbling through) at RT in a reaction vessel containing 1.5–2.5 mg of the labelling precursor (**2**, (*S*)-1-methoxy-3-[6-(4,4,4-trifluorobutoxy)benzo[*d*]isoxazol-3-ylamino]propan-2-ol, 4.3–7.2 μmol) dissolved in 500 μL of CH_2Cl_2. Trapping of [^{11}C]$COCl_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reactor was sealed and heated at 100°C for another 2 min. The reaction mixture was then concentrated to dryness at 65–75°C under a gentle helium stream for 1 min. The residue was redissolved in 0.5 mL of CH_3CN, and the solution was diluted with 0.5 mL of H_2O containing 4% of Et_2NH [v:v] and injected onto the column. (HPLC B; t_R: (**1**): 8.5–9.0 min; (**2**): <3 min).

Formulation of (S)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)-benzo[d]isoxazol-3-yl]-oxazolidin-2-[^{11}C]one ([^{11}C]SL25.1188, [^{11}C]-1)

The formulation of the labelled product for i.v. injection was effected as follows: The HPLC-collected fraction containing the radiotracer was diluted with water (50 mL). The resulting solution was passed through a Sep-Pak® Plus C18 cartridge (Waters, washed with 2 mL of EtOH and then rinsed with 10 mL of water prior to use). The cartridge was washed with water (10 mL) and partially dried by applying a helium stream for 10 s. The radiotracer was eluted with 2 mL of EtOH (less than 10% of the total radioactivity was left on the cartridge), followed by 8 mL of physiological saline and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to take the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated homemade device based on a literature procedure.[71]

Quality control of (S)-5-methoxymethyl-3-[6-(4,4,4-trifluorobutoxy)-benzo[d]isoxazol-3-yl]-oxazolidin-2-[^{11}C]one ([^{11}C]SL25.1188, [^{11}C]-1)

The radiotracer preparation was visually inspected for clarity, absence of colour and particulates. An aliquot of the preparation was removed for determination of pH using standard pH-paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC (HPLC C), with a sample of authentic **1** (t_R: 4.25 min). Chemical and radiochemical stabilities of the entire preparation were tested by HPLC (HPLC C) at regular 5-min intervals during 30 min. Specific radioactivity of the radiotracer was calculated from three consecutive HPLC (HPLC C) analyses (average) and determined as follows: The area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the HPLC chromatogram and compared with a standard curve relating mass to UV absorbance. The HPLC fraction containing the radiolabelled product was collected; its radioactivity was then measured; and the

corresponding value divided by the found mass gives the specific radioactivity.

Conclusion

SL25.1188, a selective and competitive MAO-B inhibitor, was labelled with [^{11}C]phosgene at its oxazolidinone function. A total of 300–500 MBq of [^{11}C]SL25.1188 ([^{11}C]-**1**, >95% chemically and radiochemically pure) could be obtained within 30–32 min (Sep-pak-based formulation included) with specific radioactivities ranging from 50 to 70 GBq/μmol. To date, no attempts were made to further optimize these reactions, as sufficient material was nevertheless obtained to allow for preliminary PET-radiopharmacological characterization in non-human primates.[72]

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