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Highly efficient synthesis of [^{11}C]S12968 and [^{11}C]S12967, for the *in vivo* imaging of the cardiac calcium channels using PET

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Summary

The dihydrophyridines S12968 ((−)-S11568, absolute configuration S) and S12967 ((+)-S11568, absolute configuration R), 3-ethyl 5-methyl (−/+)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate, have both an *in vitro* profile of high potency and of high selectivity for the low-voltage-dependent L-type calcium channel. In this paper, the radiosynthesis of both enantiomers, S12968 and S12967, with carbon-11, a positron-emitting isotope (half-life : 20.4 min) was investigated and oriented towards the preparation of multi milliCuries of radiotracer. Typically, 130–250 mCi (4.81–9.25 GBq) of [^{11}C]S12968 and [^{11}C]S12967 were obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities ranging from 500 to 1000 mCi/μmol (18.5–37.0 GBq/μmol) using no-carrier-added [^{11}C]methyl triflate as the alkylating agent and the appropriate, enantiomerically pure carboxylic acid precursor at 100°C for 1 min. Based on preliminary PET experiments, only the *levo* enantiomer S12968 ((−)-[^{11}C]-1) appears to be suitable for myocardial PET imaging as demonstrated *in vivo* in beagle dogs: with S12968, 85% of the uptake of [^{11}C]S12968 could be inhibited in pretreatment experiments and up to 70% of [^{11}C]S12968 could be displaced. Further investigations are currently underway in order to provide an absolute quantification of ventricular calcium channels with PET. Copyright © 2001 John Wiley & Sons, Ltd.

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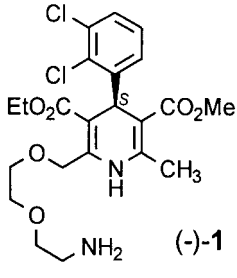
Introduction

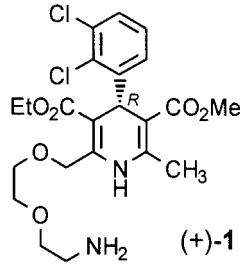
1,4-Dihydropyridines (DHP) are of interest for investigating the pharmacological properties of the L-type calcium channel. DHPs have already been intensively used to elucidate the molecular and conformational requirements for their interaction at the channel level.[1] Positron-emssion tomography (PET) is a high-resolution, sensitive and non-invasive imaging technique that could be used for *in vivo* determination of the myocardial density of DHP binding sites which is associated with heart disease.

S11568 (**1**, 3-ethyl 5-methyl (+/−)-2-[(2-(2-aminoethoxy)ethoxy)-methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate)[2,3] has an *in vitro* profile of high potency and of high selectivity for the slow voltage-dependent L-type calcium channel. In *in vitro* binding studies, it displaced specifically bound (−)-[^{3}H]PN 200-110 (isradipine, the reference molecule *in vitro* studies) from cardiac and vascular smooth muscle preparations with potencies of 5.6 and 25.3 nM, respectively.[4] It also appears as a pure pharmacological antagonist acting at a single channel L-type[5] and free of any interaction at the benzothiazepine binding site such as amlodipine.

As for most chiral DHPs, one enantiomer is more active than the other,[6] and in our case, the *levo* enantiomer S12968 ((−)-**1**, (−)-S11568, absolute configuration S) is described to be the more potent one (6–18 fold) compared to the *dextro* enantiomer S12967 ((+)-**1**, (+)-S11568, absolute configuration R).[7] Racemic S11568 (**1**), as well as enantiomerically pure S12968 ((−)-**1**) and S12967 ((+)-**1**) have been labelled with carbon-11, a positron-emitting isotope (half-life: 20.4 min) and preliminarily characterized (biodistribution in rodents and first PET experiments) as radiotracers.[7–9]



S12968 (S)-(-)-S11568



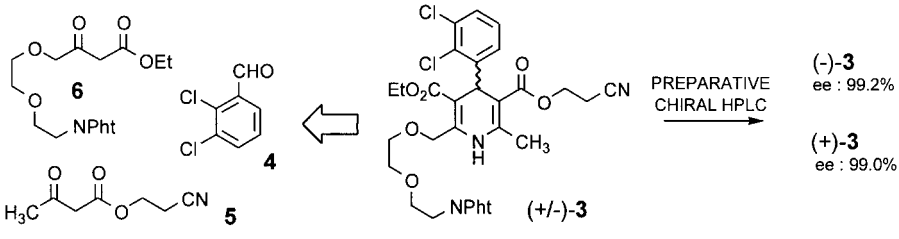
S12967 (R)-(+)-S11568

In this paper, the radiosynthesis of carbon-11-labelled S12968 ((−)-**1**) and –S12967 ((+)-**1**) has been investigated and oriented towards the preparation of hundreds of milliCuries of radiotracer, in order to satisfy the current demand for our PET investigations, based on mathematical compartmental ligand-channel models and on a multi-injection protocol.[10–13] Typical PET decay-corrected time-activity curves (left ventricle) obtained in *beagle* dogs following a single injection of a tracer dose of [^{11}C]S12968 ((S)-[^{11}C]-**1**) or [^{11}C]S12967 ((R)-[^{11}C]-**1**) are presented as well as preliminary estimation for both radiotracers of the ratio of specific-to-non-specific binding, determined during displacement and pretreatment experiments.

Results and discussion

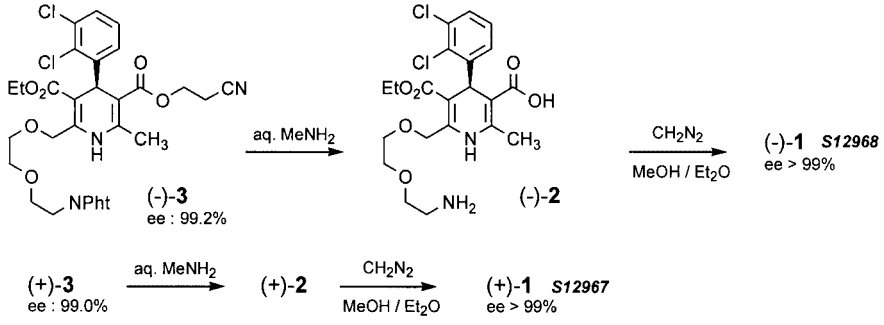
Chemistry

The total synthesis of both optically active precursors ((−)-**2**/(+)-**2**) and references ((−)-**1**/(+)-**1**) for carbon-11 labelling of the S12968/S12967 tandem have already been published.[7] Briefly, the dihydropyridine backbone (**3**) was synthesized using a modified Hantzsch procedure[14] and 2,3-dichlorobenzaldehyde (**4**), ammonium formate, 2-cyanoethyl acetylacetate (**5**) and ethyl 4-[2-(2-phthalimidoethoxy)ethoxy]-3-oxobutanoate (**6**).



Enantiomeric separation of both enantiomers of the *N*-phthalimidocyanoethyl ester **3** was performed by preparative chiral HPLC on a DAICEL Chiralcel® OF type column. Chemical purities of (−)-**3** and (+)-**3** were found to be greater than 95% as determined on a standard C18 reverse-phase column and enantiomeric purities were very high: ee: 99.2% for the *levo* isomer (−)-**2**($[\alpha]_{25}^{D}$: −44, $CHCl_3$) and ee: 99.0% for the dextro isomer (−)-**2**($[\alpha]_{25}^{D}$: +43, $CHCl_3$) as determined by chiral HPLC on an analytical DAICEL Chiralpak® AD column.

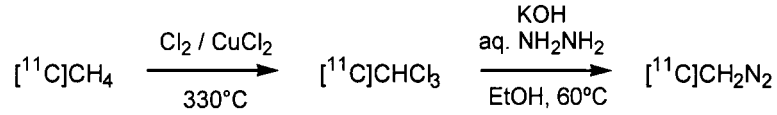
Treatment of the *levo* (−)-**3** or *dextro* (+)-**3** enantiomer with aqueous methylamine led in both cases to the simultaneous deprotection of the amino- and the carboxylic-acid function to afford the desired (−)-**2** and (+)-**2** precursors for carbon-11 labelling without racemization (ee unchanged as determined by chiral HPLC on an analytical DAICEL Chiralpak® AD column).



Treatment of the *levo* (−)-**2** or *dextro* (+)-**2** carboxylic acid on a micromolar scale with an etheral solution of diazomethane in a mixture of ether and methanol gave, as expected, S12968 (−)-**1** and S12967 (+)-**1**, respectively (Co-elution with authentic samples of S12968 and S12967 from Servier); In both cases, the enantiomeric purities were greater than 99%, as determined on an analytical Pirkle® column (the conditions used needed an *N*-derivatization with (S)-(+)-MPTA-Cl prior to separation).

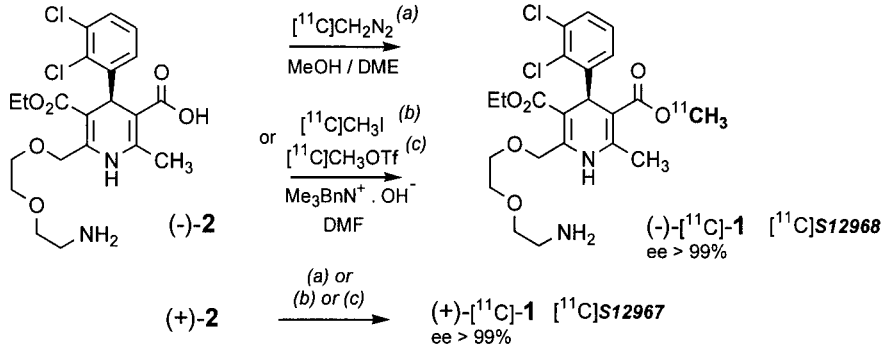
Radiochemistry

S11568 (**1**), the racemic mixture of S12968 ((−)-**1** and S12968 ((+)-**1**) has originally been labelled with carbon-11 diazomethane at its methyl ester function from the corresponding racemic carboxylic acid precursor 2.[8] For the first time, S12968 ((−)-**1**) and S12968 ((+)-**1**) were also labelled with [^{11}C]diazomethane from the corresponding carboxylic acid precursors (−)-**2** and (+)-**2**, respectively. [^{11}C]Diazomethane was prepared from [^{11}C]methane, according to a literature procedure, [15] using the two step protocol, consisting of the partial chlorination of [^{11}C]CH_4 giving [^{11}C]$CHCl_3$ (Cl_2, $CuCl_2$, 330°C) and conversion into [^{11}C]CH_2N_2 (KOH, Hydrazine, EtOH, 60°C).



About 175 mCi (6.47 GBq) of $[^{11}C]CH_2N_2$ is routinely obtained in our laboratory in 10 min after EOB in 20% decay-corrected yield (240 mCi or 8.88 GBq, EOB), based on starting $[^{11}C]CH_4$ (1.2 Ci or 44.40 GBq, EOB).

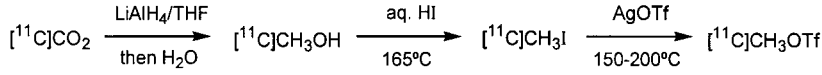
Reaction of the carboxylic acid precursors (−)-**2** and (+)-**2** with $[^{11}C]$diazomethane was highly regioselective, as expected with diazomethane (*procedure a*).



The conditions used were the following: (1) trapping at –10°C of the $[^{11}C]CH_2N_2$ in a mixture of dimethoxyethane (300 µl) and methanol (50 µl) containing 0.5–1.0 mg of precursor (−)-**2** or (+)-**2**; (2) leaving the reaction mixture at room temperature for 5–10 min; (3) concentration to dryness of the reaction mixture (at 120°C, using a nitrogen stream); (4) taking up the crude with 0.5 ml of the HPLC mobile phase and (5) HPLC purification. However, only 20–50 mCi (0.74–1.85 GBq) of $[^{11}C]$S12968 ((−)-**1**) or $[^{11}C]$S12967 ((+)-**1**) were obtained in 35 to 40 min (HPLC purification included) with specific radioactivities as high as 1200 mCi/µmol (44.4 GBq/µmol, typical values 400–900 mCi/µmol (14.8–33.3 GBq/µmol)). The radiochemical yield, decay-corrected and based on $[^{11}C]CH_2N_2$, was estimated at 20–50%.

Surprisingly, S12968 ((−)-**1**)) and S12967 ((+)-**1**)) were also labelled with carbon-11 by reaction of labelled methyl iodide or methyl triflate using the same precursors that are described above ((−)-**2** and (+)-**2**). $[^{11}C]$Methyl iodide was prepared from $[^{11}C]$carbon dioxide using the well-known two-step, one-pot protocol, consisting of the trapping of $[^{11}C]CO_2$ and conversion into $[^{11}C]$methanol ($LiAlH_4$) followed by

iodination using aqueous HI giving [^{11}C]methyl iodide.[16] [^{11}C]Methyl triflate was prepared according to a literature procedure from [^{11}C]methyl iodide using silver triflate.[17]



Reaction of the carboxylic acid precursors (−)-**2** or (+)-**2** with [^{11}C]methyl iodide yielded, in a moderate yield, [^{11}C]S12968 ((−)-[^{11}C]-**1**) and [^{11}C]S12967 ((+)-[^{11}C]-**1**), respectively (*procedure b*). The conditions used were the following: (1) trapping at room temperature of the [^{11}C]CH_3I in dimethylformamide (200 µl) containing 5–10 µl of a 0.25 M solution of trimethylbenzylammonium hydroxyde (TMBAH, 1.15 equivalents with respect to starting DHP) in ethanol; (2) heating the reaction mixture at 40–60°C for 5 min; (3) dilution of the reaction mixture with 0.5 ml of the HPLC mobile phase and (4) HPLC purification. Typically, 100–120 mCi (3.70–4.40 GBq) of [^{11}C]S12968 ((−)-[^{11}C]-**1**) and [^{11}C]S12967 ((+)-[^{11}C]-**1**) were obtained in 25–30 min (HPLC purification included) with specific radioactivities as high as 1500 mCi/µmol (55.5 GBq/µmol, typical values 500–1000 mCi/µmol (18.5–37.0 GBq/µmol)). The radiochemical yield, decay-corrected and based on [^{11}C]CH_3I, was estimated at 30–35%. No difference could be observed when the originally defined reaction mixture (0.5–1.0 mg of precursor (−)-**2** or (+)-**2**) was heated at 100°C (instead of 40–60°C).

Using [^{11}C]methyl triflate as the alkylating agent and the carboxylic acid precursor (−)-**2** or (+)-**2** (0.5–1.0 mg) at only 100°C for 1 min immediately led to a large increase of the radiochemical yield. The conditions used were similar to those described above: (1) trapping at room temperature of the [^{11}C]CH_3OTf; (2) heating the reaction mixture at 100°C for 1 min; (3) dilution of the reaction mixture with 0.5 ml of the HPLC mobile phase and (4) HPLC purification. Typically, 130–250 mCi (4.81–9.25 GBq) of [^{11}C]S12968 ((−)-[^{11}C]-**1**) and [^{11}C]S12967 ((+)-[^{11}C]-**1**) were obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities similar to those described above (up to 1500 mCi/µmol (55.5 GBq/µmol); typical values 500–1000 mCi/µmol (18.5–37.0 GBq/µmol)). The radiochemical yield, decay-corrected and based on [^{11}C]CH_3OTf, was estimated at 40–70%.

As demonstrated by HPLC analysis, the radiotracers were found to be >95% chemically and >97% radiochemically pure. The collected fraction containing [^{11}C]S12968 ((−)-[^{11}C]-**1**) or [^{11}C]S12967 ((+)-[^{11}C]-**1**) from the preparative HPLC was shown to be free on non-radioactive

precursor. The enantiomeric attribution as well as the enantiomeric purities were determined on an analytical Pirkle column using the same protocole as described above for the unlabelled S12968 and S12967 (*N*-derivatization with (S)-(+)-MPTA-Cl prior to HPLC separation). In both cases, the enantiomeric purities were greater than 99%.

Formulation and quality control

Formulation of [^{11}C]S12968 ((−)-[^{11}C]-**1**) or [^{11}C]S12967 ((+)-[^{11}C]-**1**) for i.v. injection was effected as follows: The HPLC-collected fraction containing the desired carbon-11-labelled tracer was diluted with water (50–100 ml). The resulting solution was passed through a C18 Sep-pak cartridge, which was then washed twice with water and partially dried with nitrogen. [^{11}C]S12968 ((−)-[^{11}C]-**1**) or [^{11}C]S12967 ((+)-[^{11}C]-**1**) was finally eluted with ethanol, the solution being then sterile-filtered and diluted with physiological saline.

As demonstrated by HPLC analysis, the radiopharmaceutical preparation was found to be >95% chemically and >97% radiochemically pure and was radiochemically stable for at least 120 min (the first injection in PET experiments was done within 15 min after the end of synthesis).

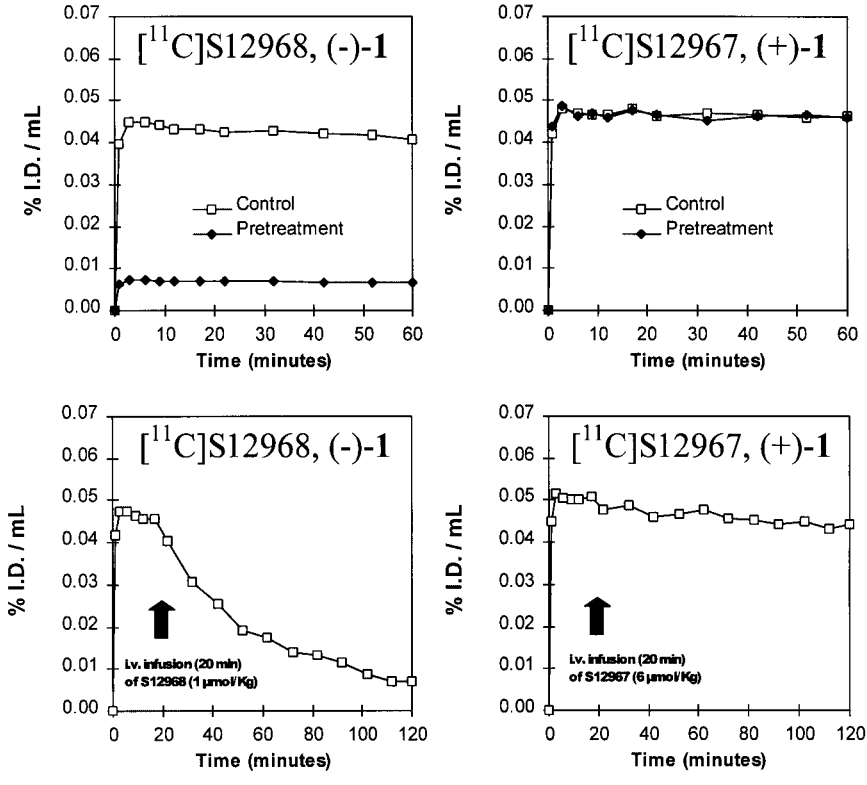
A previously used procedure for the formulation of [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**) led to unsatisfactory chemical and radiochemical purity (less than 70%). The formulation process was the following: (1) HPLC solvent removal by evaporation; (2) taking up the residue, while heating gently (45°C), in 5 ml of physiological saline and (3) filtration on a 0.22 μm Millipore filter. The addition of 50 μl of 1,3-propanediol just before concentration led to 85–90% radiochemical purity, without ever reaching the QC-required 95% level.

Myocardial PET studies in Beagle dogs

Myocardial images of dogs injected with [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**) respectively, show that the accumulation was evident within 5 min for both enantiomers with a good contrast between heart and lung. Typical decay-corrected time-activity curves obtained in the left ventricle after i.v. administration of a tracer dose of [^{11}C]S12968 ((S)-[^{11}C]-**1**), 4.5 mCi or 6.0 nmol) and [^{11}C]S12967 ((R)-[^{11}C]-**1**, 4.01 mCi or 5.7 nmol) are represented (open symbols). Values are expressed in % of injected dose per ml of myocardial tissue (% ID/ml). At 5 min,

%ID/ml was 0.045 and 0.048 for S12968 and S12967, respectively. At that time heart to lung ratios were 3.0 for S12968 and 3.2 for S12967 (data not shown). At 15 min, dynamic images showed a persistent high myocardial uptake (% ID/ml was 0.043 and 0.047 for S12968 and S12967, respectively).

For both tracers, the ratio of specific-to-non-specific binding was determined during displacement and pretreatment experiments. In a



first set of experiments, dogs were pretreated with the corresponding cold enantiomer (1 µmol/kg over 20 min for S12968 and 6 µmol/kg over 20 min for S12967), 20 min before the radiotracer injection ([^{11}C]S12968 and [^{11}C]S12967, respectively). No significant change in the myocardial uptake curve could be observed with S12967, whereas 85% of this uptake was inhibited with S12968.

In a second set of experiments, dogs were infused with the corresponding cold enantiomer (1 µmol/kg over 20 min for S12968 and 6 µmol/kg over 20 min for S12967), 20 min after the radiotracer injection ([^{11}C]S12968 and [^{11}C]S12967, respectively). With S12967, no

displacement of the myocardial uptake of the radiotracer ([^{11}C]S12967) could be observed. On the contrary, the myocardial uptake [^{11}C]S12968 was displaced up to 70% in 40 min, the final value of % ID/ml being almost superimposable to the curve obtained in the pretreatment experiment described above.

Experimental

General

TLCs were run on pre-coated plates of silicagel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by iodine staining and/or (3) by dipping the TLC-plates in a 1% ethanolic ninhydrin solution (or in a 1% aqueous $KMnO_4$) and heating on a hot plate. Radioactive spots were detected using Berthold TraceMaster 20 automatic TLC linear analyzer.

HPLCs

HPLC A: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; column: analytical Kromasil 5C18 (250×4.6 mm); porosity: 5 μm; conditions: gradient elution: to: CH_3CN/1% aqueous $HClO_4$ 40/60 (v : v)—t10 to t50: linear gradient CH_3CN/1% aqueous $HClO_4$ 40/60 (v : v) to 90/10 (v : v); flow rate : 1.0 ml/min; temperature: 30°C; UV detection at λ: 210 nm; *HPLC B*: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; column: analytical Chiralpak AD (250×4.6 mm); porosity: 5 μm; conditions: isocratic elution with heptane/EtOH/TFA; 90/10/0.1 (v : v : v); flow rate: 1.0 ml/min; temperature: 20°C; UV etection at λ: 210 nm; *HPLC C*: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger–Muller counter; column: analytical Pyrkle® (250×4.6 mm); conditions: isocratic elution with: *n*-hexane/*i*-PrOH/THF: 95/5/2 (v : v : v); flow rate: 1.5 ml/min; temperature: 45°C; UV detection at λ: 360 nm; *HPLC D*: Equipment: Waters or Shimadzu systems. For example, Waters systems

equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger–Müller counter; column: semi preparative C-18 μBondapak, Waters (300 × 7.8 mm); porosity: 10 μm; conditions: isocratic elution with: CH_3CN/H_2O/TFA: 35/65/0.1 (v : v : v); flow rate: 4.0 ml/min; temperature: RT; UV detection at λ: 254 nm; *HPLC E*: Equipment: Waters or Shimadzu systems. For example, waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger–Müller counter; column: semipreparative C-18 Zorbax® SB, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; conditions: isocratic elution with: CH_3CN/H_2O/TFA: 45/55/0.1 (v : v : v); flow rate: 4.0 ml/min; temperature: RT; UV detection at λ: 254 nm; *HPLC F*: Equipment: HPLC system consisted of two Shimadzu (Kyoto, Japan) LC-10AS pumps, a 2.6 ml mixing chamber, a Valco injector (model C6W; Vici Valco Instruments, TX, USA) with a 1 ml loop, a UV detector (Shimadzu SPD-10A) operated at 254 nm and a radioisotope detector (Berthold, Wildbad, Germany; model LB 506, 500 μl cell). A Berthold LB 5035 pump was used to add liquid scintillator (Quickszint Flow 302; Zinsser Analytic, Frankfurt, Germany) to the eluent at a flow rate of 8.0 ml/min, just before the radioactivity detector. The data acquisition and handling were done on a PC using the software Winflow (version 1.21, JMBS Developments, Grenoble, France); column: semi-preparative C-18, μBondapak®, waters (300 × 7.8 mm); porosity: 10 μm; conditions: gradient elution from 30% acetonitrile in 0.01 M aqueous phosphoric acid up to 90% in 6 min; flow rate before addition of the liquid scintillator: 6.0 ml/min; temperature: RT; UV detection at λ: 254 nm; *HPLC G*: Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, waters (3.9 × 150 mm); porosity: 5 μm; conditions: isocratic elution with solvA/solvB: 10/90 (v : v) [solvA: H_2O containing Low-UV PIC® B7 reagent (Waters), 20 ml for 1000 ml; solvB: H_2O/CH_3CN: 50/50 (v : v) containing Low-UV PIC® B7 reagent (Waters, 20 ml for 1000 ml]; flow rate: 1.5 ml/min; temperature: 30°C; UV detection at λ: 214 nm.

NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated sovlents ($CDCl_3$, δ: 7.26 ppm; CD_2Cl_2, δ: 5.32 ppm) and/or TMS as internal standards for ^1H NMR as well as the deuteriated solvents (CD_2Cl_2, δ: 53.8 ppm) and/

or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield either from TMS (s, d, t, dd, q, b for singlet, doublet, triplet, doublet of doublet, quadruplet and broad respectively). The mass spectra (MS), were measured on a Nermag R10-10—or a Quadripolair Finnigan 4600 instrument (DCI/NH_4^+).

Radiosyntheses were performed in a 5 cm lead shielded confinement. Specific radioactivity was determined as follows: The area of the UV absorbance peak corresponding to the radiolabelled product was measured on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance. Animal subject: All animal-use procedures were in strict accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Chemistry

*Preparation of S12967 ((−)-**1**) and S12967 ((+)-**1**) as HPLC standards*. 3-ethyl 5-methyl (−)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate ((−)-**1** and 3-ethyl 5-methyl (+)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate ((+)-**1**) were synthesized according to the literature.[2,3,7] All analytical properties and HPLC chromatographic profiles were consistent with the assigned structures. The synthesized compounds also co-elute with authentic samples of S12968 and S12967 from Servier.

Preparation of the precursors for carbon-11 labelling. (−)-2-[(2-(2-Aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-eth-oxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((−)-**2**) was synthesized according to Reference 7. R_f (MeOH): 0.2; R_t (HPLC A): 22.8 min; R_t (HPLC B): 13.2 min; $[\alpha]_{25}^{D}$: -21 (CH_2Cl_2); ^1H NMR ($CDCl_3$, 323.0 K): δ: 7.37 (s, 1H); 7.31 (d, $J = 7.5$ Hz, 1H); 7.26 (d, $J = 7.5$ Hz, 1H); 7.05 (t, $J = 7.5$ Hz, 1H); 5.48 (s, 1H); 4.74 (bq, 2H); 4.04 (b, 2H), 3.80-3.40 (b, 6H); 2.90 (b, 2H); 2.50 (b, 1H, D_2O exch.); 2.30 (s, 3H); 1.16 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (CD_2Cl_2, 293.0 K): δ: 173.8 [C]; 166.9 [C]; 148.8 [C]; 146.2 [C]; 144.9 [C]; 133.6 [C]; 129.9 [C]; 130.1 [CH]; 128.3 [CH]; 127.4 [CH]; 103.4 [C]; 101.3 [C]; 73.6 [CH_2]; 71.1 [CH_2]; 70.0 [CH_2]; 68.0 [CH_2]; 59.8 [CH_2]; 42.0 [CH_2]; 38.8 [CH]; 19.1 [CH_3]; 14.3 [CH_3]; MS: 477 [$M + H^+$]; 475 [$M + H^+$]; 473 [$M + H^+$].

(+)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((+)-**2**) was synthesized according to Reference 7. R_t (HPLC B): 18.9 min; $[\alpha]_D^{25}$: +20 (CH_2Cl_2) and other analytical data identical to (−)-**2**.

General procedure for the N-derivatization of S12968 and S12967 with (S)-(+)-MTPA-Cl

0.1–5 mg of S12968 ((−)-**1**) (respectively S12967, ((+)-**1**) was dissolved in 2 ml of CH_2Cl_2. (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 µl) and triethylamine (50 µl) were added and the solution was stirred for 1–5 min. The mixture was then successively washed once with 1 ml of 1 N aqueous NaOH, 1 ml of water, 1 ml of 1 N aqueous HCl, 1 ml of water and 1 ml of brine. The organic layer was then dried with Na_2SO_4, filtered and concentrated to dryness.

Chiral HPLC analysis of the N-MTPA-derivatives of S12968 and S12967

The above residue was taken up in 1 ml of CH_2Cl_2 and analysed by HPLC (HPLC C; R_t: *N*-MTPA-S12967 (*N*-MTPA-(+)-**1**): 24.0 min and N-MTPA-S12968 (N-MTPA-(−)-1): 25.6 min).

Radiochemistry

Preparation of [^{11}C]CH_4. [^{11}C]CH_4 was produced by irradiation of an ultrapure Air Liquid 95/5 mixture of N_2/H_2 target with a 20 MeV proton beam (30 µA) *via* the ^{14}N[p,α]^{11}C nuclear reaction on a CGR-MeV 520 cyclotron (54 000 µC in 30 min). At the end of the bombardment, the target contents were transferred to the 5 cm lead shielded hot cell dedicated to the radiosynthesis of the tracer and passed first 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 ammonia (produced during the irradiation) and then through a glass P_2O_5-guard (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).

Preparation of [^{11}C]CO_2. [^{11}C]CO_2 was produced by irradiation of an ultrapure N60 Air Liquid N_2 target with a 20 MeV proton bream (30 μA) *via* the ^{14}N[p,α]^{11}C nuclear reaction on a CGR-MeV 520 cyclotron (54 000 μC in 30 min). At the end of the bombardment, the target contents were transferred to the 5 cm lead-shielded hot cell dedicated to the radiosynthesis of the tracer and passed first through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. [^{11}C]CO_2 was then separated from the target gas by trapping in an empty stainless-steel coil (1500 mm length, 0.51 mm internal diameter), cooled at –186°C using liquid argon.

On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CO_2 or [^{11}C]CH_4 is routinely obtained in our laboratory for a 30 μA, 30 min (54 000 μC) irradiation.

Preparation of [^{11}C]CH_2N_2. [^{11}C]CH_4 was released from the trap by simply warming the Porapak-Q-tube to room temperature (water bath) and using helium as vector gas (40 ml/min). [^{11}C]CH_4 was then passed through a P_2O_5 glass-guard (70 mm length, 10 mm internal diameter) and concentrated in a second 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 room temperature and sweeping (15 ml/min) a volume of 1–2 ml of helium into a gas mixing chamber containing 10 ml of chlorine (99.99%, Air Liquid). Using nitrogen/oxygen (98/2) as vector gas (15 ml/min), the [^{11}C]CH_4–chlorine mixture was passed through a glass U-tube (200 mm length, 6 mm internal diameter) containing 3.0 g of pumice stone impregnated with $CuCl_2$ (see footnote for the preparation this catalyst)[†] at a temperature of 330°C. The on-line synthesized [^{11}C]$CHCl_3$ was swept, using the same vector gas as described above, into a reaction flask heated at 60°C and containing a solution of hydrated hydrazine (300 μl in ethanol (300 μl)) and potassium hydroxide (140 mg). The [^{11}C]CH_2N_2 thus synthesized was continuously swept away and passed through a glass antimony trap (70 mm length, 3 mm internal diameter, containing a 2/1 ration (v/v) of antimony (400 mg) and glass beads (1 mm external diameter) in order to remove the excess of chlorine. About 175 mCi (6.47 GBq) of

[†] Pumice stone impregnated with $CuCl_2$: 65 g of $CuCl_2$ (Aldrich) were dissolved in 56 ml of milli-Q water. To this solution, pumice stone (40 g, Merck) was added and the mixture was stirred for 20 h. After filtration, the mass was dried for 2 h at 110°C. Usually, a 1/1 ratio (v/v) of this catalyst on pumice stone and non-impregnated pumice stone was used in the chlorination process.

$[^{11}C]CH_2N_2$ is routinely obtained in our laboratory in 10 min after EOB in 20% decay-corrected yield (240 mCi or 8.88 GBq, EOB), based on starting $[^{11}C]CH_4$ (1.2 Ci or 44.4 GBq, EOB).

Preparation of [^{11}C]CH_3I and [^{11}C]CH_3OTf. $[^{11}C]CO_2$ was released from the trap by simply raising the stainless-steel coil to room temperature, swept away by a flow of nitrogen gas (40 ml/min) and trapped at −10°C (EtOH-ice bath) into 55 µl of THF containing 5 µl of 1.0 M THF solution of lithium aluminum hydride. Concentration to dryness (evaporation of solvent at 165°C using a stream of nitrogen) followed by hydrolysis (100 µl of deionized water) of the formed aluminum complex afforded $[^{11}C]CH_3OH$, which was distilled using a flow of nitrogen gas into 1 ml of an aqueous 57% HI solution (heating block at 165°C). The $[^{11}C]CH_3I$ thus synthesized was continuously swept away by a flow of nitrogen gas, passed through a combined 1/1 (v : v) soda lime/P_2O_5-guard (35 mm length each, 3 mm internal diameter) and converted into $[^{11}C]CH_3OTf$ by passing through a glass column (33 cm length, 5 mm internal diameter), heated at 200°C and containing silver triflate-impregnated graphitized carbon (200 mg). About 750 mCi (27.75 GBq) of $[^{11}C]CH_3I$ (or $[^{11}C]CH_3TOf$) is routinely obtained in our laboratory in 7–8 min after EOB in 80% decay-corrected yield (960 mCi or 35.52 GBq, EOB), based on starting $[^{11}C]CO_2$ (1.20 Ci or 44.40 GBq, EOB).

Preparation of [^{11}C]S12968 ((S)-[^{11}C]-1)

Typical procedure using [^{11}C]CH_2N_2. $[^{11}C]CH_2N_2$, carried by a flow of nitrogen/oxygen (98/2) gas, was trapped (bubbling through) at –10°C (EtOH–ice bath) in a reaction vessel containing 0.5–1.0 mg of (−)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((−)-**2** 1.1–2.1 µmol) dissolved in a mixture of freshly distilled dimethoxyethane (300 µl) and methanol (50 µl). Trapping of $[^{11}C]CH_2N_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (3 min usually), bubbling was continued for another 5 min at room temperature and the reaction mixture was then gently concentrated to dryness at 120°C under a nitrogen/oxygen stream (2–3 min). The crude was taken up with 0.5 ml of the HPLC mobile phase and was injected onto the column (HPLC D; R_t: (1): 8.5–9.0 min; (2) 5.5–6.0 min). 20–50 mCi (0.74–1.85 GBq, 23–57% decay-corrected

yield, based on [^{11}C]CH_2N_2) of [^{11}C]S12968 ((S)- [^{11}C]-**1**)) with a radiochemical-and chemical purity of more than 95% were obtained within 40 min of radiosynthesis (including HPLC purification) with specific radioactivities of 400–900 mCi/μmol (14.8–33.3 GBq/μmol).

Typical procedure using [^{11}C]CH_3I. [^{11}C]CH_3I, carried by a flow of nitrogen gas, was trapped (bubbling through) at 0°C (EtOH-ice bath) in a reaction vessel containing 0.5–1.0 mg of (−)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((−)-**2** 1.1–2.1 μmol) dissolved in freshly distilled DMF (200 μl) containing 5–10 μl of a 0.25 M solution of trimethylbenzylammonium hydroxyde in EtOH (1.25–2.5 μmol of base). Trapping of [^{11}C]CH_3I was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction vessel was then isolated, heated 40°C using a heating block for 5 min and then cooled (EtOH–ice bath). Finally, the reaction mixture was diluted with 0.5 ml of HPLC mobile phase was injected onto the column (HPLC D; R_t: (1) 8.5–9.0 min; (2) 5.5–6.0 min). 100–120 mCi (3.70–4.40 GBq, 29–35% decay-corrected yield based on [^{11}C]CH_3I of [^{11}C]S12968 ((S)-[^{11}C]-1)) with a radiochemical and chemical purity of more than 95% were obtained within 30 min of radiosynthesis (including HPLC purification) with specific radioactivities of 500–1000 mCi/μmol (18.5–37.0 GBq/μmol).

Typical procedure using [^{11}C]CH_3OTf. [^{11}C]CH_3OTf, carried by a flow of nitrogen gas, was trapped (bubbling through) at 0°C (EtOH–ice bath) in a reaction vessel containing 0.5–1.0 mg of (−)-2-[(2-(2-aminoethoxy) ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((−)-**2** 1.1–2.1 μmol) dissolved in freshly distilled DMF (200 μl) containing 5–10 μl of 0.25 M solution of trimethylbenzylammonium hydroxide in EtOH (1.25–2.5 μmol of base). Trapping of [^{11}C]CH_3OTf was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min usually), the reaction vessel was the isolated, heated at 100°C using a heating block for 1 min and then cooled (EtOH–ice bath). Finally, the reaction mixture was diluted with 0.5 ml of the HPLC mobile phase and was injected onto the column (HPLC E; R_t: (1) 9.5–1.0 min; (2) 4/5–5.0 min). 130–250 mCi (4.81–9.25 GBq, 37–72% decay-corrected yield based on [^{11}C]CH_3OTf) of [^{11}C]S12968 ((S)- [^{11}C]-1)) with a radiochemical and chemical purity of more than 95% were obtained

within 30 min of radiosynthesis (including HPLC purification) with specific radioactivities of 500–1000 mCi/μmol (18.5–37.0 GBq/μmol)).

*Preparation of [^{11}C]S12967 ((R)-[^{11}C]-**1**).* The typical [^{11}C]CH_2N_2-, [^{11}C]CH_3I- and [^{11}C]CH_3TOf procedures described above for the preparation of [^{11}C]S12968 ((S)-[^{11}C]-**1**) were used with the corresponding (+)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((+)-**2** as precursor for labelling.

*General procedure for the N-derivatization of [^{11}C]S12968 ((S)-[^{11}C]-**1**) and S12967 ((R)-**1**) with (S)-(+)-MTPA-Cl*

The procedure described above for the *N*-derivatization of S12968 ((S)-**1**) and S12967 ((R)-**1**) with (S)-(+)-MTPA-Cl was used with [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**)), respectively.

*Chiral HPLC analysis of the N-MTPA-derivatives of [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**)*

The procedure described above for the chiral HPLC analysis of the *N*-MTPA-derivatives of S12968 ((S)-**1**) and S12967 ((R)-**1**) was used with the *N*-MTPA-derivatives [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**), respectively (HPLC C; R_t: *N*-MTPA-[^{11}C]S12967 (*N*-MTPA-(+)-[^{11}C]-**1**): 24.0 min and *N*-MTPA-[^{11}C]S12968 (*N*-MTPA-(−)-[^{11}C]-**1**): 25.6 min).

*Formulation of [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**)*

Formulation of labelled product of i.v. injection was effected as follows: The HPLC collected fraction containing the desired carbon-11 labelled tracer was diluted with water (50–100 ml). The resulting solution was passed through a C18 Sep-pak cartridge (Waters). The cartridge was washed twice with 5 ml of water and partially dried for 10 s by applying a nitrogen stream. The carbon-11 labelled tracer was eluted with 2 ml of EtOH (less than 5% of the total radioactivity was left on the cartridge) and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to lower the EtOH concentration below 10%. This whole process was performed by the chemist using a remote-

controlled dedicated home-made device based on a literature procedure.[18] The first injection in PET experiments was done within 15 min after the end of synthesis.

*Quality control of [^{11}C]S12968 ((S)-[^{11}C]-**1**) and [^{11}C]S12967 ((R)-[^{11}C]-**1**)*

As demonstrated by HPLC analysis (HPLC F and HPLC G), the radiolabelled products were found to be >95% chemically and radiochemically pure and also co-elute with a sample of authentic S12968 ((S)-**1**) or S12967 ((R)-**1**) (HPLC F; retention time: 4.7 min; HPLC G; retention time: 3.0 min). The preparations were shown to be free of non-radioactive precursors and radiochemically stable for at least 180 min.

As also demonstrated by HPLC analysis (HPLC C), the radiolabelled products were found to be >95% enantiomerically pure (ee >95% for both tracers). The HPLC conditions used needed an N-derivatization with (S)-(+)-MPTA-Cl prior to separation. The *N*-MTPA derivative of [^{11}C]S12968 ((S)-[^{11}C]-**1**) co-elutes with a sample of authentic *N*-MTPA-S12968 (HPLC C; retention time: 25.6 min). In the same way, the *N*-MTPA derivative of [^{11}C]S12967 ((R)- [^{11}C]-**1**) co-elutes with a sample of authentic *N*-MTPA-S12967 (HPLC C; retention time: 24.0 min).

Conclusion

In this paper, the radiosynthesis of carbon-11 labelled S12968 ((−)-[^{11}C]-**1**) and S12967 ((+)-[^{11}C]-**1**), two *in vitro* highly potent and selective antagonists of the slow voltage-dependent L-type calcium channel was investigated and oriented towards the preparation of multi milliCuries of radiotracer. Typically, using no-carrier-added [^{11}C]methyl triflate as the alkylating agent and the dedicated, enantiomerically pure carboxylic acid precursor (0.5–1.0 mg) at 100°C only for 1 min led to a 40–70% radiochemical yield (decay-corrected and based on [^{11}C]CH_3OTf). 130 to 250 mCi (4.81–9.25 GBq) of [^{11}C]S12968 ((−)-[^{11}C]-**1**) and [^{11}C]S12967 ((+)-[^{11}C]-**1**) were obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities ranging from 500 to 1000 mCi/μmol (18.5–37.0 GBq/μmole). Only the *levo* enantiomer S12968 ((−)-[^{11}C]-**1**) appears to be suitable for PET as demonstrated *in vivo* in *beagle* dogs in a set of preliminary experiments.

Further investigations are currently underway in order to provide an absolute quantification of ventricular calcium channels with PET.

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