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Synthesis of Two Optically Active Calcium Channel Antagonists Labelled with Carbon-11 for In Vivo Cardiac PET Imaging

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Abstract—(±)-S11568 (**1**, 3-ethyl-5-methyl-(±)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate), has an in vitro profile of high potency and of high selectivity for the low-voltage dependent L-type calcium channel. In in vitro binding studies, it displaced specifically bound (−)-[^{3}H]PN 200-110 (isradipine (**2**), the reference molecule for in vitro studies) from cardiac and vascular smooth muscle preparations with potencies of 5.6 and 51 nM, respectively. It also appears as a pure pharmacological antagonist acting at a single channel L-type and free of any interaction at the benzothiazepine binding site such as amlodipine (**3**). Both enantiomers of S11568 have in vitro activities, the dextro isomer S12967 ((+)-**1**) being 6 to 18-fold less potent than the *levo* one S12968 ((−)-**1**). Two couples of optically active labelling precursors of S11568, ((−)-**10**/(+)-**10** and (−)-**14**/(+)-**14**) have been synthesized using a modified Hantzsch's dihydropyridine synthesis. In both cases, the enantiomers were separated by preparative chiral HPLC. They both have been independently labelled with carbon-11, using [^{11}C]diazomethane or [^{11}C]iodomethane to give multimilliCurie quantities of (−)-**1** (S12968) and (+)-**1** (S12967) with high specific activities (500–1000 mCi/μmol, 18.5–37.0 GBq/μmol). Both enantiomers appear suitable for PET experiments: their myocardial concentration increases after a bolus injection to reach a maximum in 2 min and then remains on a plateau with a slight downslope while the blood concentration falls rapidly. Myocardial uptake was threefold higher than lung uptake, leading to a good contrast on PET images. The present preliminary biological results obtained in Beagle dogs showed that both enantiomers have similar myocardial kinetics and in vivo affinity for the left ventricular myocardium. © 1997 Elsevier Science Ltd.

Introduction

Among the calcium channel antagonists, 1,4-dihydropyridines (DHP) are of interest for investigating the pharmacological, electrophysiological, and structural 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] The in vivo determination of the myocardial density of DHP binding sites should be of interest for the assessment of pathophysiological changes associated with heart disease.

Recently, progress has been made in the search for new DHPs with wider clinical applicability, mainly characterized by a longer duration of action. S11568[2,3] (**1**, 3-ethyl-5-methyl-(±)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate), has an in vitro profile of high potency and of high selectivity for the low-voltage dependent L-type calcium channel. In in vitro binding studies, it displaced specifically bound ( )-[^{3}H]PN 200–110 (isradipine (**2**), the reference molecule for in vitro studies) from cardiac and vascular smooth muscle preparations with potencies of 5.6 and 51 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 (**3**) (Fig. 1).

PN 200-110 (isradipine (**2**)) and S11568 (**1**) have been labelled with carbon-11 for in vivo cardiac PET (positron emission tomography) imaging.[6] Labelling was performed by reaction of [^{11}C]diazomethane[7] with the corresponding carboxylic acid derivative. In spite of a K_d (in vitro binding studies) in the low nanomolar range, isradipine (PN 200-110, **2**) was not a suitable tracer for in vivo PET imaging.[8] The time course of the radioactivity showed a rapid wash-out of the tracer (myocardial and blood concentration were close together 10 min after injection) as well as a too low myocardium-to-lung ratio (1.5–1) 5 min after injection.

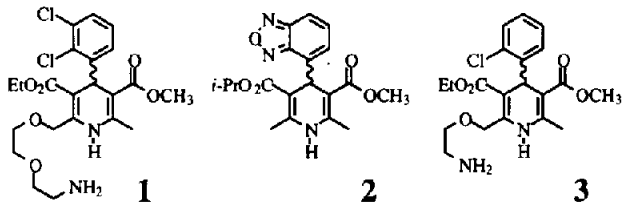


Figure 1. Structure of S11568 (**1**), isradipine (**2**), and amlodipine (**3**).

Currently: ChiralSep, Parc d'activités de la Boissière, 11 rue de la Boissière, F-76170 La Frenaye, France.

Other first-generation DHPs such as nifedipine,[9] nicardipine,[9] nisoldipine,[10] nitrendipine,[10] and nimodipine[11] have also been labelled with carbon-11, but none of them found use as a tracer in PET imaging.

In contrast with all these first-generation DHPs, S11568 (**1**) appears suitable for PET experiments.[8] Its myocardial concentration increases after a bolus injection to reach a maximum in 2 min and then remains on a plateau with a slight downslope while the blood concentration falls rapidly. Myocardial uptake was 2 to 4-fold higher than lung uptake, leading to a good contrast on PET images. Saturability of binding sites and proven displacement also validate the use of this ligand. Pretreatment with unlabelled S11568 (2 μmol/kg or 6 μmol/kg over 15 min) reduced myocardial uptake by 60 and 80%, respectively. Specific binding was estimated at 80% during displacement experiments (bolus of unlabelled S11568: 1 μmol/kg followed by a continuous infusion of 3 μmol/kg over 2 h).

Like for most chiral DHPs, one enantiomer is more active than the other,[12] and in our case, the *levo* isomer is described to be the more potent one (6 to 18-fold). The use of the racemate S11568 was now a limitation for the ongoing studies, demanding enantiomeric pure [^{11}C]labelled DHP. However, the resolution of both enantiomers of S11568 could not be processed post-labelling for the following reasons, mainly associated with the nature of the radioisotope used but to some extent with the target DHP: The drastic conditions used in carbon-11 syntheses, (e.g. 10- to 50-fold excess of precursors, no work up, and purification of the crude by direct injection on HPLC) are not compatible with the well-known fragility and sensitivity of chiral HPLC columns. Also, the 20.4 min half-life of carbon-11 excluded two successive HPLC purifications, the first one on a classical column for chemical purification followed by a second one on a chiral one for enantiomeric separation. Furthermore, the only chiral HPLC conditions known at this time for the resolution of our S11568 DHP involves, prior to the separation, an *N*-derivatization (see Experimental), which is not compatible with the non-analytical purpose.

The absolute configuration of the more active *levo* enantiomer ((−)-**1**, S12968), has recently been determined by X-ray diffraction studies and appears to be *S*[13] (Fig. 2), leading to 3-D structures comparable to the one already established for the chemically closely related amlodipine,[14,15] for example, as well as many other chiral DHPs.[12] Since at the onset of this work the *S* attribution of the absolute configuration was missing, discouraging enantioselective synthesis, a racemic synthesis of the appropriate precursors for carbon-11 labelling followed by enantiomeric resolution was planned. This approach would lead to the in vivo exploration of the two enantiomers, studies of particular interest since both have in vitro activities but show different affinity for vascular and myocardial tissues. The methods most frequently used for the separation of DHP enantiomers comprise resolution of the racemic via separation of diastereomeric salts or covalent derivatives followed by selective removal of the chiral auxiliary. In this work, a preparative chiral HPLC methodology was developed for the enantiomeric resolution at one stage of the total synthesis of the DHP precursors.

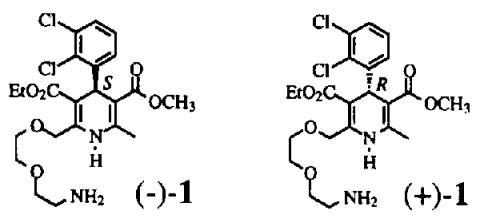


Figure 2. Structure of S12968 (−)-**1** and S12967 (+)-**1**.

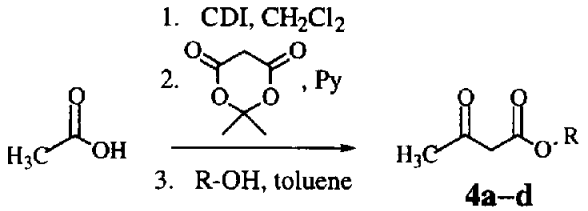
This paper describes (1) the synthesis and the enantiomeric separation of two different couples of enantiomericaly pure DHPs ((−)-**10**/(+)-**10** and (−)-**14**/(+)-**14**); (2) the labelling procedure, at the same site, with carbon-11 using [^{11}C]diazomethane or [^{11}C]iodomethane to afford multimilliCurie quantities of (−)-**1** (S12968) and (+)-**1** (S12967) with high specific activities; and (3) the preliminary PET experiments.

Results and Discussion

Chemistry

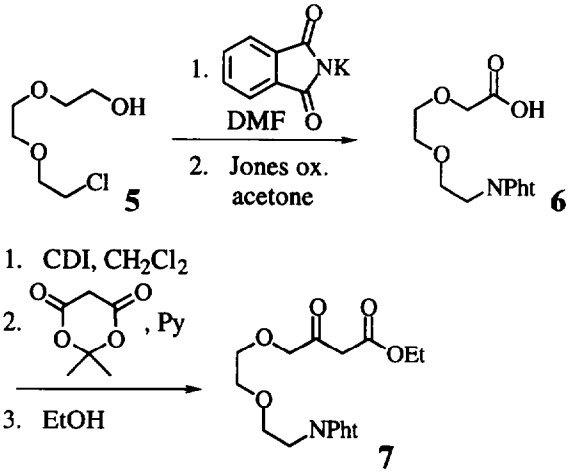
Synthesis of (−)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid (−)-(10) and its enantiomer (+)-(10), precursors for the [^{11}C]diazomethane labelling procedure.

The synthesis[16] of the first required β-keto ester **4a** started with the activation of the carboxylic acid function of acetic acid with CDI, followed by condensation with Meldrum's acid and finally opening with 2-cyanoethanol to give the desired building block in good yields (50–70%) (Scheme 1). The synthesis of the second required β-keto ester **7** started with 2-[2-(2-chloroethoxy)ethoxy]ethanol (**5**). Introduction of the phthalimido group followed by Jones oxidation afforded **6** in good yield (80%). Then, the use of the three-step procedure described above gave **7** as the other β-keto ester (40–60% yields) (Scheme 2).



R, **a**: -CH_2CH_2CN; **b**: -CH_2CCl_3; **c**: -$CH_2CH_2SiMe_3$; **d**: -CH_2Ph

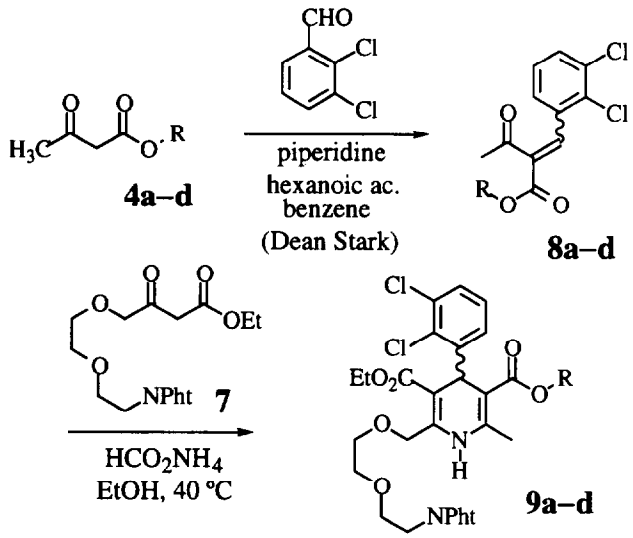
Scheme 1. Synthesis of β-ketoesters **4a–d**.



Scheme 2. Synthesis of β-ketoesters 7.

The modified Hantzsch[17] synthesis of the DHP **9a** started with the β-keto ester **4a** and commercial 2,3-dichlorobenzaldehyde. The corresponding benzylidene **8a** was obtained in moderate yields (50%) as a *Z/E* mixture (*Z/E*: 3:7, ratio determined by ^{1}H NMR). Condensation with the second β-keto ester 7 and a source of ammonia gave the expected DHP **9a** in good yields (50–70%) (Scheme 3).

Chiral-HPLC separation potentials were evaluated at this stage of the synthesis on a selection of analytic DAICEL Chiralpak® AD/AS and DAICEL Chiralcel® OC, OD, OJ, OF columns. Preparative separation of hundreds of milligrams of the two enantiomers was finally performed on an OF-type column. Chemical purities were found to be greater than 95% as determined on a standard C18 reverse-phase column. Enantiomeric purities were determined also by chiral HPLC on an analytical DAICEL Chiralpak® AD column and were both very high: ee: 99.2% for the *levo* isomer ( )-**9a** ($[\alpha]_D^{25}$ 44, $CHCl_3$) and ee: 95.0% for the *dextro* isomer (+)-**9a** ($[\alpha]_D^{25}$ +43, $CHCl_3$).

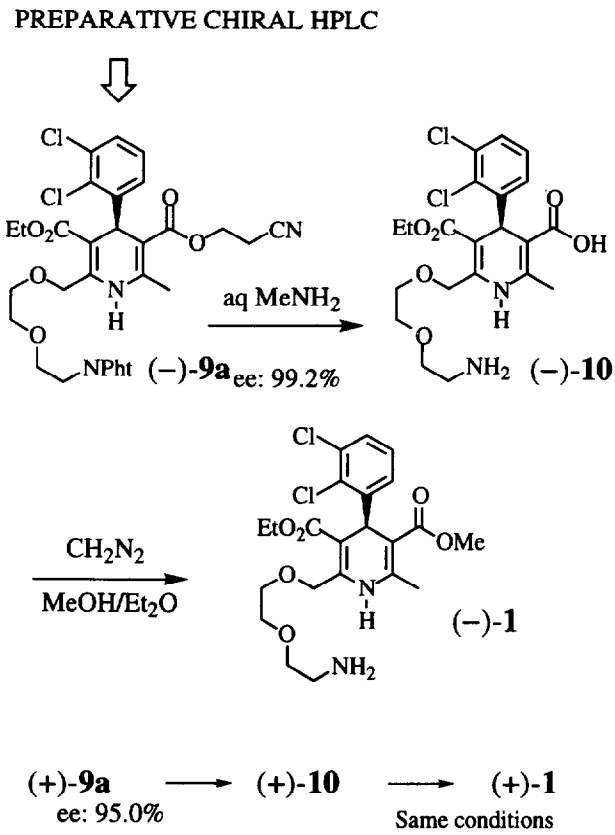


R, **a**: $-CH_2CH_2CN$; **b**: $-CH_2CCl_3$; **c**: $-CH_2CH_2SiMe_3$; **d**: $-CH_2Ph$

Scheme 3. Synthesis of DHPs **9a–d**.

Treatment of the *levo* ( )-**9a** or *dextro* (+)-**9a** DHP enantiomers with aqueous methylamine lead in both cases to the simultaneous deprotection of the amino and the carboxylic acid function to afford the desired ( ) and (+)-**10** precursors in excellent yields, 92 and 94%, respectively, without racemization (Scheme 4). Enantiomeric purities were determined by chiral HPLC on an analytical DAICEL Chiralpak® AD column with unapplicable conditions (unfortunately) to a preparative separation at this stage. The ee were unchanged. Treatment of the *levo* ( )-**10** or *dextro* (+)-**10** DHPs 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 (coelution with authentic samples of S12968 and S12967 from Servier). The enantiomeric attribution as well the enantiomeric purities were determined on an analytical Pirkle column. The conditions used needed an *N*-derivatization with (S)-(+)-MPTA-Cl prior to separation.

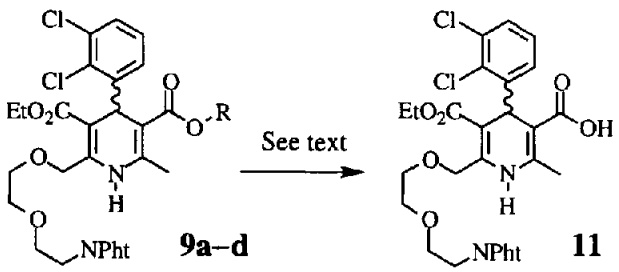
Synthesis of (±)-2-[(2-(2-phthalimidoethoxy)ethoxy)-methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid (11), putative precursor for the [^{11}C]iodomethane labelling procedure.



Scheme 4. Preparation of ( ) and (+)-**10**, precursors for the [^{11}C]CH_2N_2 labelling procedure.

Since the free amino group present at the extremity of the alkyloxy chain could react with iodomethane during the carbon-11 methylation process using standard, conventional labelling conditions, several approaches have been tried to synthesize derivative **11**, an *N*-phthalimido protected precursor, presenting the free carboxylic acid function. In a first set of experiments, trials including the selective removal of the cyanoethoxy group from DHP **9a** (various basic conditions tried) and the reprotection of the DHP **10** with phthaloyl dichloride or anhydride were unsuccessful.

Consequently, DHPs bearing a selective non-basic removable ester protective group such as trichloroethyl **9b**, trimethylsilylethyl **9c**, or benzyl **9d** were synthesized (Scheme 3). Briefly, DHPs **9b–d** were obtained using similar conditions to those described above. The required β-ketoesters **4b–d** were synthesized using the general three-step procedure described above for **4a** with yields varying from 53 to 63%. The corresponding benzylidenes **8b–d** were obtained in moderate yields by condensation with commercial 2,3-dichlorobenzaldehyde (26–75% yield) as a *Z*/*E* mixture (*Z*/*E*: 3:7–4:6, ratio determined by ^{1}H NMR). Condensation with the second β-keto ester **7** and a source of ammonia gave the expected DHPs **9b–d** in moderate yields (13–42%). The possible enantiomeric separation by chiral HPLC was evaluated on the same selection of analytic DAICEL Chiralpak® AD/AS and DAICEL Chiralcel® OC, OD, OJ, OF columns as previously used for DHP **9a**, without leading to better resolution. Furthermore, none of the DHPs **9b–d** were suitable for the preparation of appreciable quantities of **11** (Scheme 5). Selective removal of the trichloroethyl moiety (**9b**) using standard conditions such as Zn in acetic acid or selective removal of the TMSethyl moiety (**9c**) using fluoride anion were unsuccessful (degradation was observed in the first case and removal of the phthalimido group in the other). Only debenzylation of DHP **9d** using a heterogenous catalyst such as Pd on charcoal gave the desired derivative **11** with desperately poor recovered yield (<10%). The use of different Pd contents (10–20% Pd) or Pt on various supports (charcoal or $CaCO_3$) did not improve the yields. No reaction was observed with the homogeneous Wilkinson catalyst. Even though these hydrogenolysis reactions are described in the literature,[12] no further work in this direction was done since, in a similar synthesis, chlorine atoms were removed together with the benzyl group of a felodipine precursor.[18] Finally, stability of the phthalimido group in the different synthesized DHPs **9a–d** in the conditions used for the standard carbon-11 methylation procedure using [^{11}C]iodomethane was evaluated and appears to be variable and not reproducible.



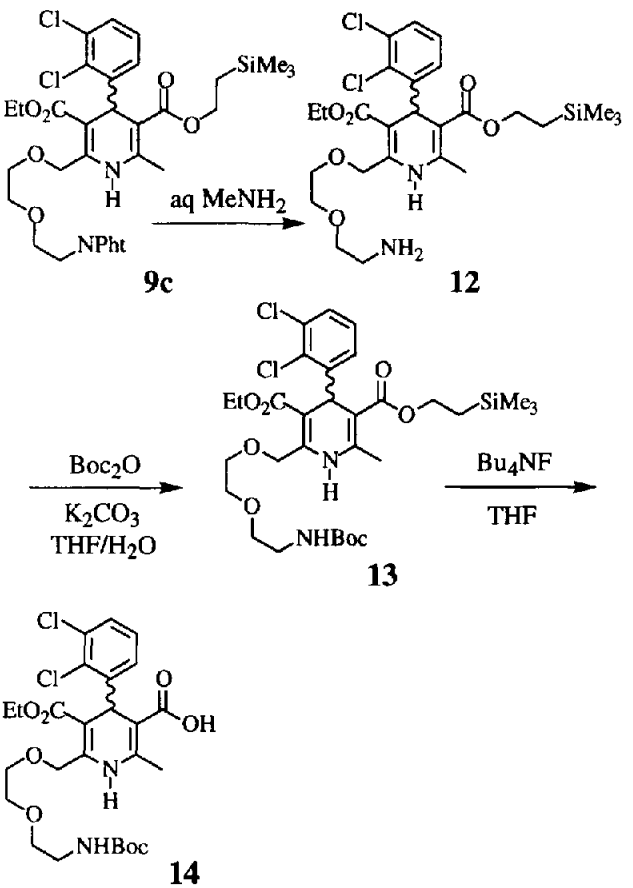
R, **a**: -CH_2CH_2CN; **b**: -CH_2CCl_3; **c**: -$CH_2CH_2SiMe_3$; **d**: -CH_2Ph

Scheme 5. Synthesis of racemic DHP **11**.

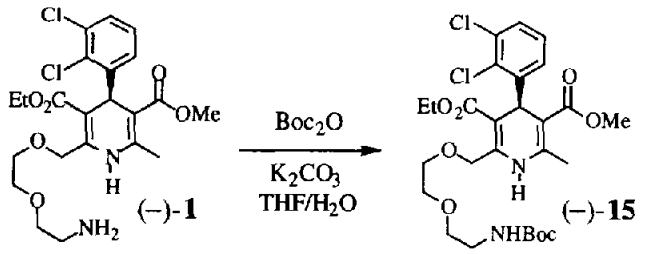
Synthesis of (−)-2-[(2-(2-*tert*-butoxycarbonylaminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid (−)-14 and its enantiomer (+)-14, precursors for the [^{11}C]iodomethane labelling procedure.

N-Boc DHP **14** was synthesized in three steps from **9c** using common procedures with good yields (Scheme 6). Regeneration of the amino function using aqueous methylamine gave compound **12** in 85–90% yields. Protection with di-*tert*-butyl dicarbonate in aqueous THF containing K_2CO_3 afforded **13** quantitatively (99% yield). Selective removal of the TMSethyl with tetrabutylammonium fluoride in THF gave the expected DHP **14** in 65–75% yield. It is interesting to note that direct protection of the DHP **10** with di-*tert*-butyl dicarbonate in aqueous (K_2CO_3, THF/H_2O) or non-aqueous (CH_2Cl_2, DMAP) standard procedures gave no reaction or poor yields (5–10%).

For analytical purpose, *N*-Boc-S12968 ((−)-**15**) was synthesized in 97% yield, as an HPLC standard, by



Scheme 6. Synthesis of DHP **14**.

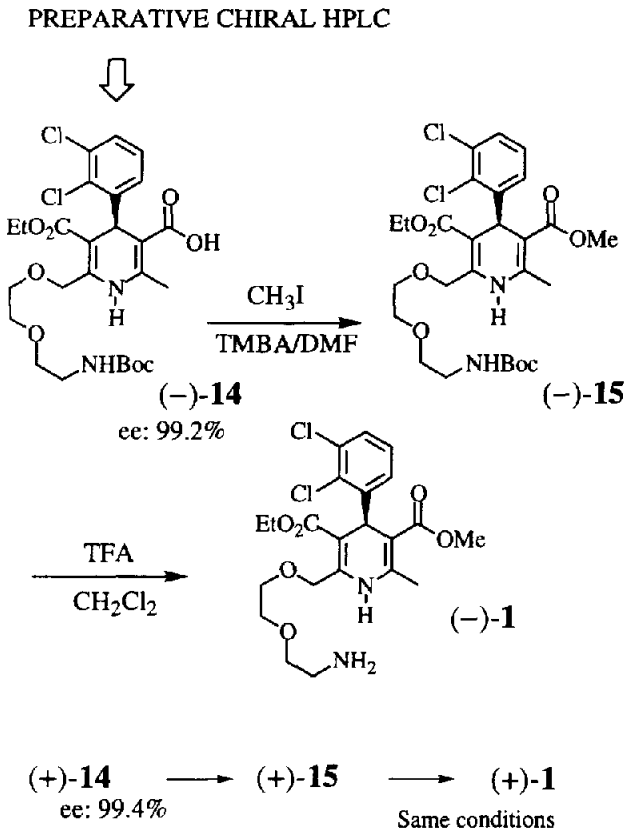


Scheme 7. Synthesis of *N*-Boc-S12968 ((−)-15).

treatment of the corresponding free amine (S12968 ((−)-1)) with di-*tert*-butyl dicarbonate in aqueous THF containing K_2CO_3 (Scheme 7).

Concerning DHP **14**, chiral-HPLC separation potentials were evaluated, at this stage of the synthesis, on a similar selection as the one previously used of analytical DAICEL Chiralpak® AD/AS and DAICEL Chiralcel® OC, OD, OJ, OF columns. Preparative separation of hundreds of milligrams of the two enantiomers of DHP **14** was finally performed on an OF-type column. Chemical purities were found to be greater than 95% as determined on a standard C18 reverse-phase column. Enantiomeric purities were determined also by chiral HPLC on an analytical DAICEL Chiralpak® AD column and were both very high: ee: 99.2% for the *levo* isomer (−)-**14** ($[\alpha]_D^{25}$ −102, CH_2Cl_2) and ee: 99.4% for the *dextro* isomer (+)-**14** ($[\alpha]_D^{25}$ +102, CH_2Cl_2).

Treatment of the *levo* (−)-**14** or *dextro* (+)-**14** DHP enantiomers on a micromolar scale with a DMF solution of iodomethane in the presence of trimethylbenzylammonium hydroxide at 45 °C gave *N*-Boc-S12968 (−)-**15** and *N*-Boc-S12967 (+)-**15**, respectively (Scheme 8). Deprotection with trifluoroacetic acid in CH_2Cl_2 regenerated the amino function to afford (−) and (+)-**1**, respectively, without trace of racemization (coelution with authentical samples of S12968 and S12967 from Servier). The enantiomeric attribution as well the enantiomeric purities were determined on an analytical Pirkle column. The conditions used needed an *N*-derivatization with (S)-(+)-MPTA-Cl prior to separation.



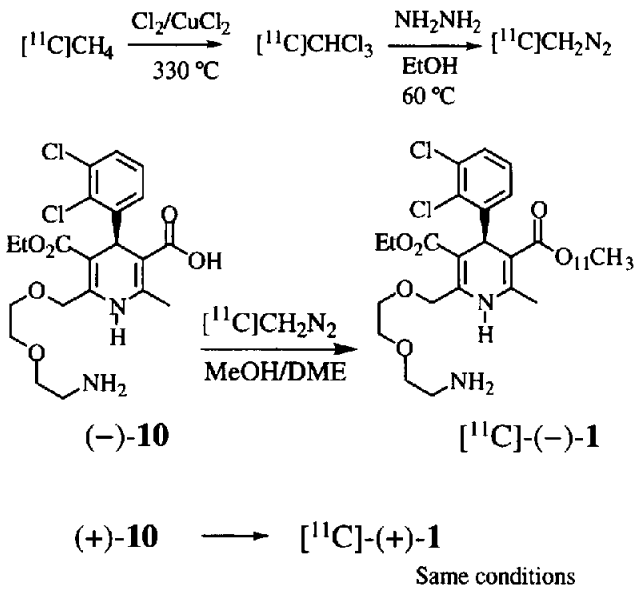
Scheme 8. Preparation of (−) and (+)-**15**, precursors for the $[^{11}C]CH_3I$ labelling procedure.

Radiochemistry

Radiosynthesis of carbon-11 labelled S12968 ((−)-1) and S12967 ((+)-1)

Labelling process using $[^{11}C]CH_2N_2$. S12968 (−)-**1** and S12967 (+)-**1** were labelled with carbon-11 by reaction of $[^{11}C]$diazomethane[7] with the corresponding carboxylic acid derivative (−)-**10** and (+)-**10**, respectively, using a slightly modified process of a previously described method[6] (Scheme 9). The reaction occurred cleanly at room temperature within 5 min in a mixture of dimethoxyethane and methanol. Twenty to 50 mCi (0.74–1.85 GBq) of pure $[^{11}C]$DHP (−)-**1** or (+)-**1** were obtained at 35–40 min after end of bombardment (HPLC purification included) with specific radioactivities as high as 1200 mCi/μmol (44.4 GBq/μmol, typical values 400–900 mCi/μmol (14.8–33.34 GBq/μmol)); yield 20–50% decay-corrected based on $[^{11}C]CH_2N_2$.

Labelling process using $[^{11}C]CH_3I$. *N*-Boc-S12968 (−)-**15** and *N*-Boc-S12967 (+)-**15** were labelled with carbon-11 by reaction of $[^{11}C]$iodomethane[9] (Scheme 10) with the corresponding carboxylic acid derivative (−)-**14** and (+)-**14**, respectively. The reaction occurred cleanly at 40 °C within 5 min in DMF containing 0.7–1.4 equiv of trimethylbenzylammonium hydroxide with respect to starting DHP. Between 100 and 200 mCi (3.70–7.40 GBq) of pure $[^{11}C]$DHP (−)-**15** or (+)-**15** were obtained at 25–30 min after end of bombardment (HPLC purification included) with specific radioactivities as high as 1700 mCi/μmol (62.9 GBq/μmol, typical values 600–1000 mCi/μmol (22.2–37.0 GBq/μmol)). Deprotection with trifluoroacetic acid in CH_2Cl_2 regenerated the amino function affording 40–80 mCi (1.48–2.96 GBq) of pure $[^{11}C]$DHP (−)-**1** or (+)-**1** at 45–50 min after end of bombardment (second HPLC purification included) with specific radioactivities of 300–500 mCi/μmol (11.1–18.5 GBq/μmol) and without trace of racemization; yield 25–50% decay-corrected based on $[^{11}C]CH_3I$. The enantiomeric attribution as well the enantiomeric purities were determined on an analytical Pirkle column. The conditions used needed an *N*-derivatization with (S)-(+)-MPTA-Cl prior to separation.

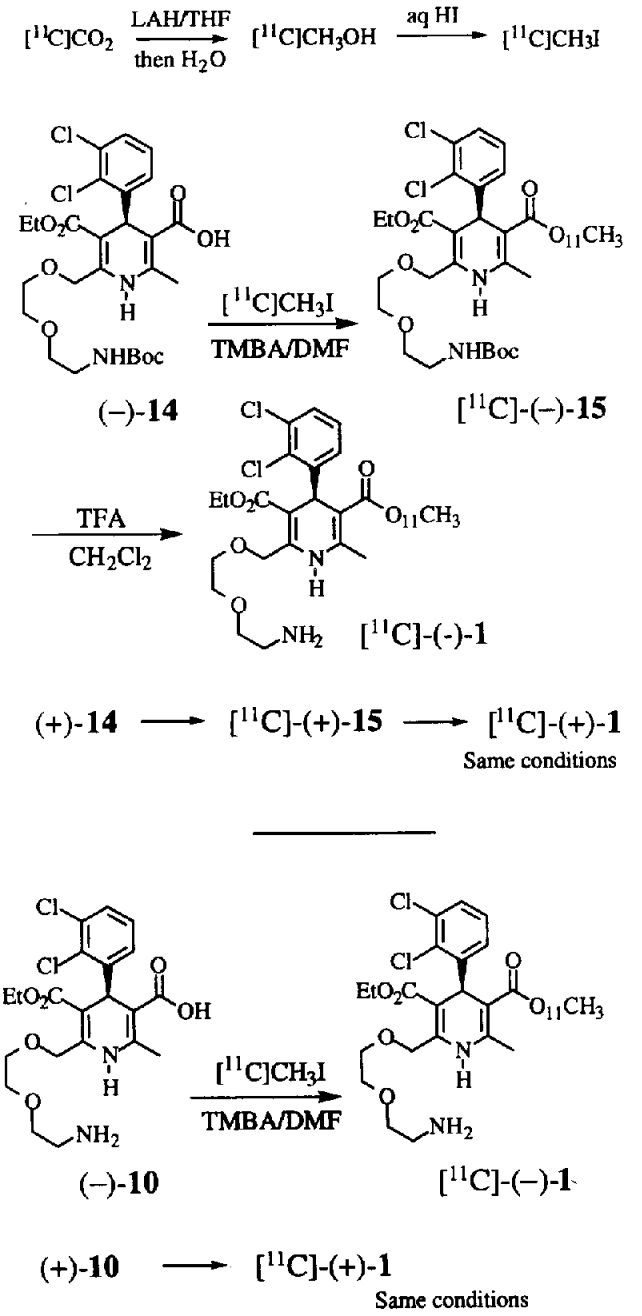


Scheme 9. Radiosynthesis of [^{11}C]S12968 ((−)-**1**) and [^{11}C]S12967 ((+)-**1**) using [^{11}C]diazomethane.

Surprisingly, S12968 (−)-**1** and S12967 (+)-**1** were also labelled with carbon-11 by reaction of [^{11}C]iodomethane (Scheme 10) with the corresponding carboxylic acid derivative (−)-**10** and (+)-**10**, respectively. The reaction occurred very cleanly at 40 °C within 5 min under the same conditions as the one described above (in DMF, containing 0.8–1.6 equiv of trimethylbenzylammonium hydroxide with respect to starting DHP). Between 100 and 120 mCi (3.70–4.40 GBq) of pure [^{11}C]DHP (−)-**1** or (+)-**1** were obtained at 25–30 min after end of bombardment (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)); yield 25–30% decay-corrected based on [^{11}C]CH_3I. Again, the enantiomeric attribution as well the enantiomeric purities were determined on an analytical Pirkle column. The conditions used needed an *N*-derivatization with (S)-(+)-MPTA-Cl prior to separation.

Competitive *N*-methylation, if existent at all, seems relatively unimportant as the radiochemical yield is only slightly reduced compared to the one obtained with the *N*-Boc-protected precursor. Any other radioactivity eluted in the HPLC dead volume (probably unreacted [^{11}C]iodomethane), well before the unlabelled precursor, and thus is unlikely to correspond with any *N*-methylated side product, even though none of them were available as standards.

Although all methylation methods described here are satisfactory, providing multimilliCuries of product with similar specific radioactivities, we have found for routine preparation the latter method with [^{11}C]iodomethane (direct pathway) more convenient for reasons of shorter synthesis time and technical simplicity.



Scheme 10. Radiosynthesis of [^{11}C]S12968 ((−)-**1**) and [^{11}C]S12967 ((+)-**1**) using [^{11}C]iodomethane (direct and indirect pathway).

PET imaging

Positron emission tomography (PET) imaging can provide a sensitive and powerful means for visualizing myocardial calcium channels. Imaging of these binding sites in vivo may be useful to the exploration of several cardiac diseases (idiopathic dilated cardiomyopathy, hypertrophic cardiomyopathy). The feasibility of synthetizing the two enantiomers of S11568 combined with the results obtained previously[8] in vivo with [^{11}C]S11568 were encouraging enough to initiate the evaluation of [^{11}C]S12968 and [^{11}C]S12967 as PET imaging ligands in Beagle dogs.

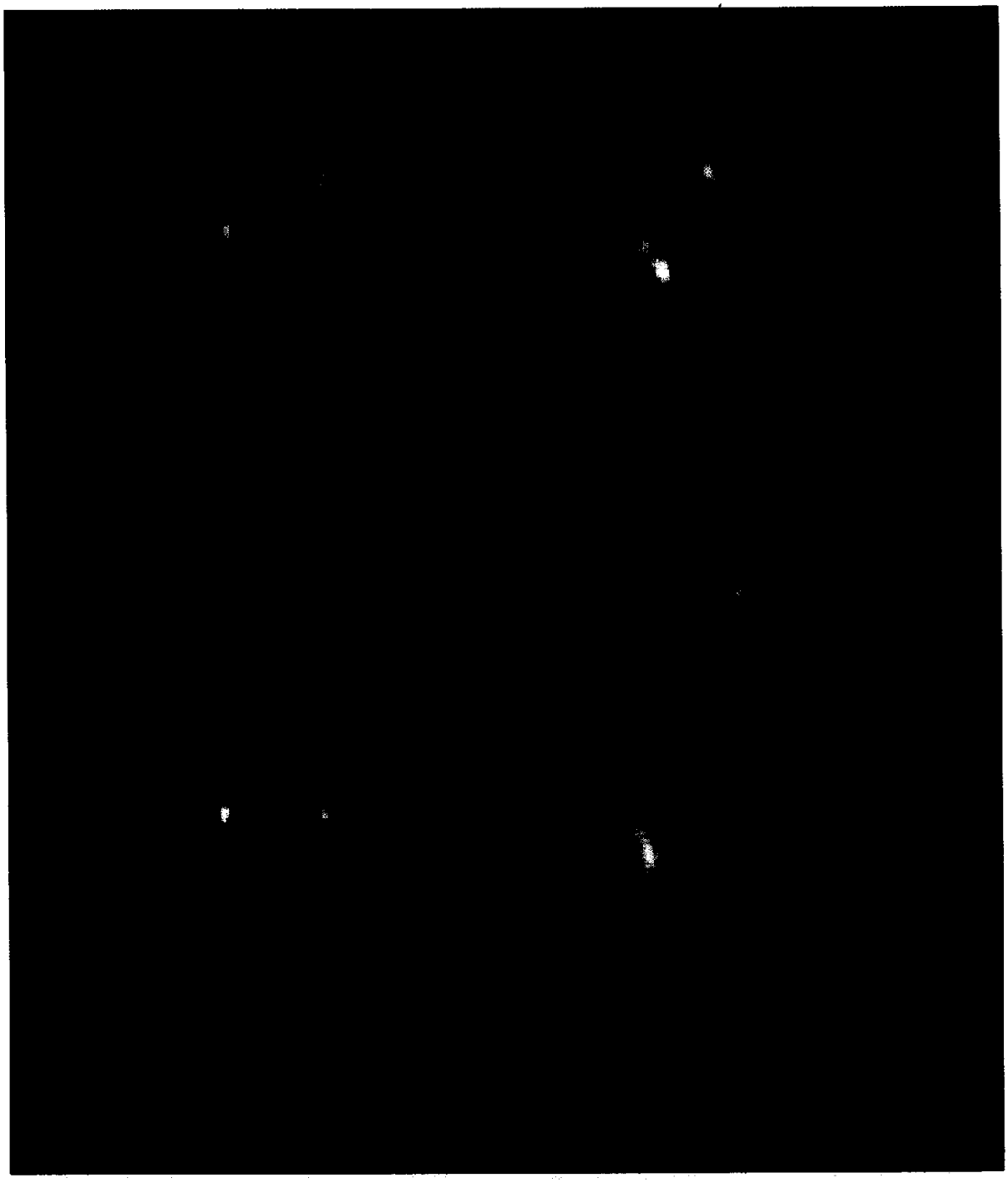


Figure 3. Left ventricular uptake in Beagle dogs at 5 and 15 min after iv injection of the corresponding carbon-11 labelled tracers S12967 (+)-(**1**) (left column) and S12968 (−)-(**1**) (right column); apparent disparities in orientation are only due to minor differences in lateral tilt of the animals in the scanner; differences in brightness have no significance as the used colour-scales are not normalized.

In Figure 3, myocardial images of two dogs injected with [^{11}C]S12968 and [^{11}C]S12967, respectively (4.46 mCi or 5.42 nmole and 5.1 mCi or 7.08 nmole, respectively), show that the accumulation was evident within 5 min for both enantiomers with a good contrast between heart and lung (heart-to-lung ratio: 3.1 and 3.3, respectively). At 5 min, per cent of injected dose/100 mL myocardial tissue was 3.4 and 3.9 for S12968 and S12967, respectively. At that time heart-to-lung ratios were 3 for S12968 and 3.2 for S12967. At 15 min, dynamic images showed a persistent high myocardial uptake (% injected dose/100 mL myocardial tissue was 3.1 and 3.5 for S12968 and S12967, respectively).

In Figure 4, myocardial and blood–time radioactivity curves obtained in a Beagle dog injected with [^{11}C]S12968 show that the maximal myocardial accumulation of the tracer was observed 2 min after iv injection while the clearance from the blood was rapid. At 30 min after injection, clearance from the myocardium was 21% for S12968. In Figure 5, data concerning [^{11}C]S12967 is presented (clearance from the myocar-

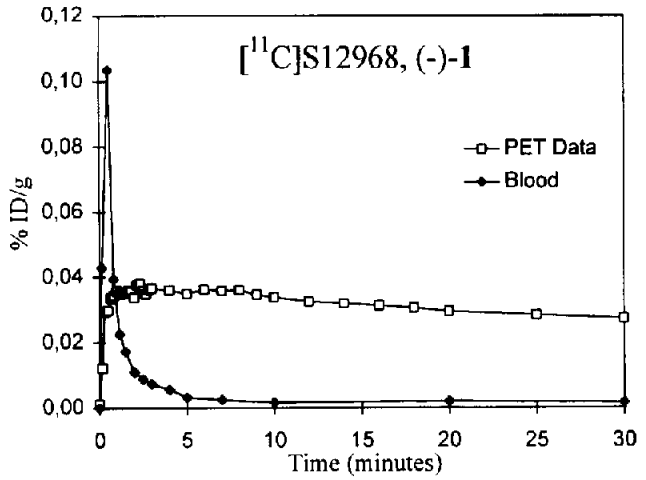


Figure 4. Myocardial and blood kinetics of [^{11}C]S12968 (−)-(**1**).

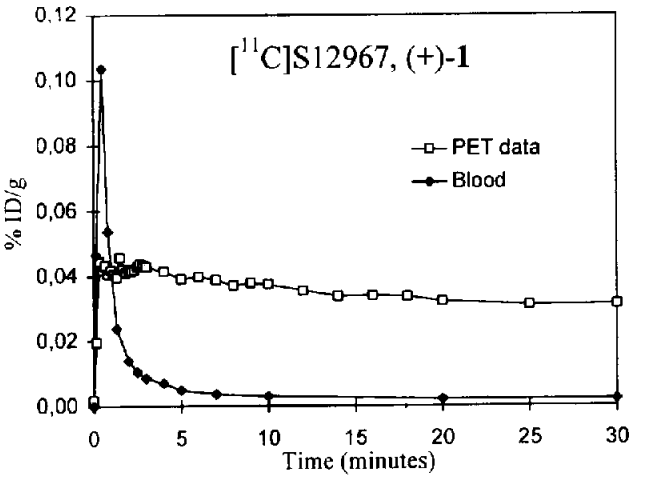


Figure 5. Myocardial and blood kinetics of [^{11}C]S12967 (+)-(**1**).

dium at 30 min: 21%). Similar myocardial and blood–time radioactivity curves were observed for both enantiomers. This result was likely since the racemic mixture and the (+)-enantiomer have similar affinity for rat myocytes in vitro studies (K_i = 5.7 and 5.6 nM, respectively). Apparent disparities in the initial uptake slope shown in the peaks of the Figures 4 and 5 are most likely not significant, but probably due to differences in syringe handling.

The ratio of specific to non-specific binding was determined during displacement experiments (bolus injection of 0.5 mg/kg over 1 min of the corresponding cold enantiomer followed by an infusion of 0.5 mg/kg over 1 h). From these experiments, specific binding could be estimated to be at least 55% for both enantiomers. This value is probably underestimated because of the rather low doses of cold calcium channel antagonist that could be used and by the inability of PET using short half-life carbon-11 to perform long-lasting displacement experiments. Higher doses of each enantiomer could not be used because they provoked severe bradycardia in halothane anaesthetized dogs. The bradycardia observed during displacement experiment is the essential criterion for distinguishing a displaceable binding with no signal transmission and a receptor binding site that is related to physiological response.[20] Pretreatment with the cold enantiomer (1 mg/kg over 1 h) inhibited myocardial uptake by 60% for both enantiomers.

The shape of the curves in Figures 4 and 5 suggest that the obtained images are flow dependent and may not represent the true channel distribution, which may be an explanation for the observed similar kinetics in vivo of both enantiomers. An initial flow image is not necessarily incompatible with quantitative receptor-PET imaging. Models using partial saturation experiments exist to overcome this problem.[21] Further experiments will be needed to be able to determine the calcium channel number.

Conclusion

(±)-S11568 (**1**, 3-ethyl-5-methyl-(±)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate), has an in vitro profile of high potency and of high selectivity for the low-voltage dependent L-type calcium channel. In in vitro binding studies, it displaced specifically bound (−)-[^{3}H]PN 200-110 (isradipine (**2**), the reference molecule for in vitro studies) from cardiac and vascular smooth muscle preparations with potencies of 5.6 and 51 nM, respectively. It also appears as a pure pharmacological antagonist acting at a single channel L-type and free of any interaction at the benzothiazepine binding site such as amlodipine (**3**). Both enantiomers of S11568 have in vitro activities, the *dextro* isomer S12967 ((+)-**1**) being 6 to 18-fold less potent than the *levo* one S12968 (( )-**1**). Two couples of optically active labelling precursors of S11568, ((−)-**10**/(+)-**10** and (−)-**14**/(+)-**14**) have been synthesized using a modified Hantzsch's dihydropyridine synthesis. In both cases, the enantiomers were separated by preparative chiral HPLC. They both have been independently labelled with carbon-11, using [^{11}C]diazomethane or [^{11}C]iodomethane to give multimilliCurie quantities of (−)-**1** (S12968) and (+)-**1** (S12967) with high specific activities (500–1000 mCi/μmol, 18.5–37.0 GBq/μmol). Both enantiomers appear suitable for PET experiments: their myocardial concentration increases after a bolus injection to reach a maximum in 2 min and then remains on a plateau with a slight downslope while the blood concentration falls rapidly. Myocardial uptake was 3-fold higher than lung uptake, leading to a good contrast on PET images. The present preliminary biological results obtained in Beagle dogs showed that both enantiomers have similar myocardial kinetics and in vivo affinity for the left ventricular myocardium.

Experimental

General

Chemicals were purchased from Aldrich, Fluka or Sigma and were used without further purification. TLC were run on precoated 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 iodine staining and/or (3) by dipping the TLC plates in a 1% ninhydrin solution in ethanol (or 1% aqueous $KMnO_4$) and heating on a hot plate. Flash chromatography was conducted on silica gel 63–200 μm (Merck) at 0.3 bars. HPLC were run on a Waters system equipped with a 510 pump and a 490E UV-multiwavelength detector. The eluent was also monitored for radioactivity with a Geiger–Müller counter. NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuterated solvents ($CDCl_3$, δ 7.26 ppm, CD_2Cl_2, δ 5.32 ppm, or DMSO-d_6, δ 2.50 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvents ($CDCl_3$, δ 77.0 ppm, CD_2Cl_2, δ 53.8 ppm or DMSO-d_6, δ 39.5 ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t and b for singlet, doublet, triplet and broad, respectively). Mass spectra (MS, DCI/NH_4^+) were measured on a Nermag R10-10 apparatus; an ionization potential of 70 eV was used. Optical rotations ($[\alpha]_D$) were measured on a Perkin-Elmer 141 polarimeter and an Atago Polax-D. Air or moisture-sensitive reactions were conducted in heat gun-dried glassware, under an inert atmosphere and with freshly distilled solvents.

$[^{11}C]CH_4$ was produced by irradiation of an Air Liquide 95/5 mixture of nitrogen and hydrogen with a 20 MeV proton beam via the $^{14}N[p,\alpha]^{11}C$ nuclear reaction on a CGR-MeV 520 cyclotron. Similar irradiation of an ultrapure N60 Air Liquide N_2 target produced $[^{11}C]CO_2$. Synthesis of [^{11}C]S12967 and [^{11}C]S12968 were carried out remote-controlled in radiation-shielded cells.

Chemistry

General synthesis procedure for the β-ketoesters (4a–d). To 9.2 mL of glacial acetic acid (160 mmol, MW 60.05, d 1.049) in 100 mL of methylene chloride (freshly distilled) under a N_2 atmosphere at room temperature, was added at once 27.7 g of CDI (carbonyldiimidazole, 165 mmol, 1.03 equiv, MW 162.15, dried over P_2O_5 under vacuum 24 h prior to use). The mixture was stirred at room temperature for 6 h (strong evolution of CO_2).

To this solution was then rapidly added 24.2 g of Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione, 168 mmol, 1.05 equiv, MW 144.13) dissolved in 100 mL of methylene chloride and 20 mL of pyridine. The resulting mixture was stirred at room temperature for 48 h, then washed with 1 N aq H_2SO_4, water, brine, and dried over Na_2SO_4. Concentration gave 23–29 g of a brown oily residue, which was used in the next synthetic step without further purification (crude yield 75–95%). Chromatography on silica gel of an aliquot gave 5-acetyl-2,2-dimethyl-1,3-dioxane-4,6-dione as a pale-yellow oil (eluent: CH_2Cl_2:MeOH, 90:10). R_f (CH_2Cl_2) 0.2; ^{1}H NMR (DMSO-d_6, 298.0 K) δ 2.30 (s, 3H), 2.09 (s, 1H), 1.55 (s, 6H).

The above brown oily residue was redissolved in toluene (1 g/mL) and 1.1 equiv of the appropriate alcohol was added. The mixture was refluxed for 3–10 h, concentrated to dryness and chromatographed on silica gel.

2-Cyanoethyl acetylacetate (4a). The procedure described above was used with 5.46 mL (80 mmol, MW 71.08, d 1.041) of 3-hydroxypropionitrile as the alcohol. Chromatography (eluent: CH_2Cl_2:EtOAc, 95:5) gave 10.6 g of **4a** as a yellow oil (68% from acetic acid). R_f (CH_2Cl_2:MeOH, 95:5) 0.5; ^{1}H NMR ($CDCl_3$, 298.0 K) δ 4.36 (t, J = 6.0 Hz, 2H), 3.55 (s, 2H), 2.76 (t, J = 6.0 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 300.0 K) δ 200.9 [C], 166.9 [C], 117.7 [C], 59.6 [CH_2], 49.6 [CH_2], 30.2 [CH_3], 17.8 [CH_2].

2,2,2-Trichloroethyl acetylacetate (4b). The procedure described above was used with 2.59 mL (27 mmol, MW 149.40, d 1.557) of 2,2,2-trichloroethanol as the alcohol. Chromatography (eluent: CH_2Cl_2:heptane, 80:20) gave 5.2 g of **4b** as a pale-brown oil (63% from acetic acid). R_f (CH_2Cl_2:heptane, 80:20) 0.5; ^{1}H NMR (CD_2Cl_2, 298.1 K) δ 4.81 (s, 2H); 3.61 (s, 2H), 2.28 (s, 3H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 200.1 [C], 166.3 [C], 95.4 [C], 75.1 [CH_2], 50.2 [CH_2], 30.9 [CH_3].

2-Trimethylsilylethyl acetylacetate (4c). The procedure described above was used with 7.74 mL (54 mmol, MW 118.25, d 0.825) of 2-trimethylsilylethanol as the alcohol. Chromatography (eluent: CH_2Cl_2:heptane, 90:10) gave 7.5 g of **4c** as a pale-brown oil (53% from acetic acid). R_f (CH_2Cl_2:heptane, 80:20) 0.4; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 4.20 (t, J = 8.7 Hz, 2H), 3.41 (s, 2H), 2.21 (s, 3H), 1.09 (t, J = 8.7 Hz, 2H), 0.05 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 201.1 [C], 167.9 [C], 64.0 [CH_2], 50.8 [CH_2], 30.6 [CH_3], 17.9 [CH_2], −1.0 [CH_3].

Benzyl acetylacetate (4d). The procedure described above was used with 5.59 mL (54 mmol, MW 108.14, d 1.045) of benzylalcohol as the alcohol. Chromatography (eluent: CH_2Cl_2) gave 7.3 g of **4d** as a yellow-brown oil (57% from acetic acid). R_f (CH_2Cl_2) 0.4; ^{1}H NMR ($CDCl_3$, 298.0 K) δ 7.28 (b, 5H), 5.08 (s, 2H), 3.39 (s, 2H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 298.0 K) δ 199.8 [C], 166.3 [C], 135.0 [C], 127.9 [CH], 127.6 [CH], 127.5 [CH], 66.0 [CH_2], 49.0 [CH_2], 29.2 [CH_3].

[2-(2-Phthalimidoethoxy)ethoxy]acetic acid (6). A DMF solution (70 mL) containing 18.8 g of 2-[2-(2-chloroethoxy)ethoxy]ethanol (**5**, 0.11 mol, MW 168.62) and 14.6 g of potassium phthalimide (0.08 mol, MW 185.23) was refluxed for 17 h. The mixture was then

diluted with methylene chloride, washed twice with brine, dried over Na_2SO_4, and concentrated. Distillation (Kugelrohr) at 0.05 mm Hg (180–185 °C) gave 19.8 g of pure [2-(2-phthalimidoethoxy)ethoxy]ethanol (89% yield). ^{1}H NMR ($CDCl_3$, 298.0 K) δ 7.90–7.60 (m, 4H), 4.00–3.40 (b, 12H), 2.70 (b, 1H, D_2O exch).

To a solution of 3.00 g of [2-(2-phthalimidoethoxy)-ethoxy]ethanol (0.011 mol, MW 279.29) in 150 mL of acetone Jones reagent was slowly added (temperature range of 20–25 °C). The mixture was stirred for 1 h, concentrated, diluted with methylene chloride, washed with water, and dried over Na_2SO_4. Concentration gave 2.93 g of **6** (90% yield). R_f (CH_2Cl_2:MeOH, 80:20) 0.3; ^{1}H NMR ($CDCl_3$, 345.0 K) δ 9.99 (b, $w_{1/2}$ = 15 Hz, 1H, D_2O exch.), 7.90–7.60 (m, 4H), 4.09 (s, 2H), 3.89 (t, J = 5.4 Hz, 2H), 3.77 (t, J = 5.2 Hz, 2H), 3.72–3.65 (b, 4H); ^{13}C NMR ($CDCl_3$, 345.0 K) δ 172.3 [C], 169.9 [C], 133.7 [CH], 131.9 [C], 122.9 [CH], 70.8 [CH_2], 69.7 [CH_2], 68.1 [CH_2], 67.7 [CH_2], 37.1 [CH_2].

Ethyl 4-[2-(2-phthalimidoethoxy)ethoxy]-3-oxo-butanoate (7). (2-(2-Phthalimidoethoxy)ethoxy)acetic acid (**6**, 7.5 g, 26 mmol; MW 293.27) was dissolved in 50 mL of methylene chloride under an N_2 atmosphere at room temperature. CDI (carbonyldiimidazole, 4.4 g, 27 mmol, 1.05 equiv, MW 162.15, dried over P_2O_5 under vacuum 24 h prior to use) was added at once (strong evolution of CO_2) and the mixture was stirred at room temperature for 3 h. To this solution was then rapidly added 4.0 g of Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione, 28 mmol, 1.1 equiv, MW 144.13) dissolved in 10 mL of methylene chloride and 2 mL of pyridine. The resulting mixture was stirred at room temperature for 48 h, then washed with 1 N aq H_2SO_4, water, brine, dried over Na_2SO_4, and concentrated. The resulting brown oily residue was redissolved in ethanol and was refluxed for 4 h. The mixture was concentrated to dryness and chromatographed on silica gel. Elution with CH_2Cl_2:MeOH (97:3) gave 5.19 g of **7** as an oil (55% yield). R_f (CH_2Cl_2:MeOH, 80:20) 0.5; ^{1}H NMR ($CDCl_3$, 298.0 K) δ 7.80–7.60 (m, 4H), 4.19 (s, 2H), 4.19–4.13 (b, 2H), 3.89 (t, J = 5.3 Hz, 2H), 3.75 (t, J = 5.3 Hz, 2H), 3.72–3.65 (b, 4H), 3.53 (s, 2H), 1.26 (t, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 298.0 K) δ 201.2 [C], 167.3 [C], 166.3 [C], 133.2 [CH], 131.2 [C], 122.4 [CH], 75.3 [CH_2], 70.2 [CH_2], 69.4 [CH_2], 67.2 [CH_2], 60.4 [CH_2], 44.9 [CH_2], 36.5 [CH_2], 13.3 [CH_3].

General synthesis procedure for the benzylidenes (8a–d). To a solution of 3.5 g of 2,3-dichlorobenzaldehyde (20 mmol, 1 equiv, MW 173.01), 0.395 mL of piperidine (4 mmol, 0.2 equiv, MW 85.15, d 0.861), 0.500 mL of hexanoic acid (4 mmol, 0.2 equiv, MW 116.16, d 0.927) in 50 mL of benzene was added 20 mmol of the appropriate β-ketoester. The mixture was refluxed for 3–10 h with continuous removal of the water formed (Dean–Stark apparatus). The reaction mixture was then concentrated to dryness, dissolved in methylene chloride, washed with 10% aq $NaHCO_3$, water, 10% aq HCl, water again, and brine. The methylene chloride solution was dried over Na_2SO_4, concentrated and chromatographed on silica gel.

2-Cyanoethyl-(*Z*/*E*)-2-(2,3-dichlorobenzylidene)-3-oxo-butanoate (8a). The procedure described above was used with 8.0 g (52 mmol, MW 155.15) of 2-cyanoethyl acetylacetate (**4a**) as the β-ketoester. Chromatography (eluent: CH_2Cl_2) gave 8.4 g of **8a** as a yellow oil (52%, *Z*/*E*: 3/7). R_f (CH_2Cl_2:EtOAc, 95:5) 0.2 and 0.25; ^{1}H NMR ($CDCl_3$, 298.0 K) δ 7.96 (s, Z-1H), 7.86 (s, E-1H), 7.60–7.45 (b, Z-1H & E-1H), 7.45–7.15 (b, Z-2H and E-2H), 4.47 (t, J = 6.0 Hz, Z-2H), 4.36 (t, J = 6.0 Hz, E-2H), 2.83 (t, J = 6.0 Hz, Z-2H), 2.65 (t, J = 6.0 Hz, E-2H), 2.49 (s, E-3H), 2.26 (s, Z-3H); ^{13}C NMR ($CDCl_3$, 298.0 K) δ 200.9 [Z-C], 194.3 [E-C], 165.7 [E-C], 163.2 [Z-C], 139.4 [E-C], 139.0 [Z-C], 136.7 [E-C], 135.8 [Z-C], 134.1 [E-C], 134.0 [Z-C], 134.0 [E-C], 133.7 [Z-C], 132.5 [Z-C], 132.5 [E-C], 132.1 [E-CH], 132.0 [Z-CH], 128.4 [Z-CH], 127.7 [E-CH], 127.6 [Z-CH], 127.3 [E-CH], 116.3 [Z-C], 116.3 [E-C], 59.8 [Z-CH_2], 59.4 [E-CH_2], 31.3 [Z-CH_3], 26.6 [E-CH_3], 17.7 [Z-CH_2], 17.5 [E-CH_2].

2,2,2-Trichloroethyl-(*Z*/*E*)-2-(2,3-dichlorobenzylidene)-3-oxo-butanoate (8b). The procedure described above was used with 5.0 g (21 mmol, MW 233.47) of 2,2,2-trichloroethyl acetylacetate (**4b**) as the β-ketoester. Chromatography (eluent: CH_2Cl_2:heptane, 30:70) gave 5.4 g of **8b** as a pale-yellow oil (65%, *Z*/*E*: 3/7). R_f (CH_2Cl_2:heptane, 50:50) 0.4 and 0.45; ^{1}H NMR (CD_2Cl_2, 295.2 K) δ 8.05 (s, Z-1H), 7.90 (s, E-1H), 7.60–7.45 (b, Z-1H and E-1H), 7.45–7.15 (b, Z-2H and E-2H), 4.93 (s, Z-2H), 4.76 (s, E-2H), 2.50 (s, E-3H), 2.31 (s, Z-3H); ^{13}C NMR (CD_2Cl_2, 296.9 K) δ 200.8 [Z-C], 194.5 [E-C], 165.2 [E-C], 163.0 [Z-C], 140.9 [E-CH], 140.2 [Z-CH], 137.1 [E-C], 136.4 [Z-C], 135.1 [E-C], 134.4 [E-C], 134.5 [Z-C], 133.1 [Z-C], 132.8 [E-C], 132.7 [Z-CH], 132.6 [Z-C], 132.5 [E-CH], 129.2 [Z-CH], 128.6 [E-CH], 128.5 [Z-CH], 128.4 [E-CH], 95.3 [Z-C], 94.8 [E-C], 75.6 [E-CH_2], 75.3 [Z-CH_2], 31.8 [Z-CH_3], 27.5 [E-CH_3].

2-Trimethylsilylethyl-(*Z*/*E*)-2-(2,3-dichlorobenzylidene)-3-oxo-butanoate (8c). The procedure described above was used with 7.5 g (37 mmol, MW 202.32) of 2-trimethylsilylethyl acetylacetate (**4c**) as the β-ketoester. Chromatography (eluent: CH_2Cl_2:heptane, 60:40) gave 10.0 g of **8c** as a yellow oil (75%, *Z*/*E*: 3/7). R_f (CH_2Cl_2:heptane, 80:20) 0.2 and 0.3; ^{1}H NMR (CD_2Cl_2, 295.2 K) δ 7.88 (s, Z-1H), 7.82 (s, E-1H), 7.65–7.50 (b, Z-1H and E-1H), 7.45–7.20 (b, Z-2H and E-2H), 4.39 (t, J = 8.4 Hz, Z-2H), 4.26 (t, J = 8.7 Hz, E-2H), 2.48 (s, E-3H), 2.26 (s, Z-3H), 1.31 (t, J = 8.4 Hz, Z-2H), 0.91 (t, J = 8.7 Hz, E-2H), 0.12 (s, Z-9H); 0.02 (s, E-9H); ^{13}C NMR (CD_2Cl_2, 296.1 K) δ 201.9 [Z-C], 195.2 [E-C], 167.1 [E-C], 164.7 [Z-C], 138.6 [E-C], 138.5 [Z-C], 138.3 [E-CH], 137.6 [Z-CH], 135.4 [E-C], 135.1 [Z-C], 134.4 [Z-C], 134.3 [E-C], 133.1 [E-C], 132.9 [Z-C], 132.3 [E-CH], 132.2 [Z-CH], 129.2 [Z-CH], 128.4 [Z-CH], 128.4 [E-CH], 128.3 [E-CH], 65.0 [Z-CH_2], 64.9 [E-CH_2], 31.8 [Z-CH_3], 27.5 [E-CH_3], 18.0 [E-CH_2], 18.0 [Z-CH_2], -1.1 [Z-CH_3], −1.2 [E-CH_3].

Benzyl-(*Z*/*E*)-2-(2,3-dichlorobenzylidene)-3-oxo-butanoate (8d). The procedure described above was used with 6.9 g (36 mmol, MW 192.21) of benzyl acetylacetate (**4d**) as the β-ketoester. Chromatography (eluent: CH_2Cl_2:heptane, 70:30) gave 3.3 g of **8d** as a yellow-orange oil (26%, *Z*/*E*: 4/6). R_f (CH_2Cl_2:heptane, 80:20) 0.45 and 0.5; ^{1}H NMR (CD_2Cl_2, 293.5 K) δ 7.91 (s, Z-1H), 7.79 (s, E-1H), 7.60–7.10 (b, Z-8H and E-7H), 6.97 (t, J = 7.8 Hz, E-1H), 5.31 (s, Z-2H), 5.16 (s, E-2H), 2.43 (s, E-3H), 2.21 (s, Z-3H); ^{13}C NMR (DMSO-d_6, 305.0 K) δ 200.8 [Z-C], 194.3 [E-C], 166.0 [E-C], 163.6 [Z-C], 145.0-125.0 (over 20 peaks), 67.3 [Z/E-CH_2], 31.0 [Z-CH_3], 26.6 [E-CH_3].

General synthesis procedure for the 1,4-dihydropyridines (9a–d). To a solution of 3.63 g (10 mmol, MW 363.37) of **7** in 10 mL of ethanol, was added 631 mg (10 mmol, MW 63.06) of ammonium formate and 10 mmol of the appropriate benzylidene. The resulting mixture was stirred at 40 °C under a N_2 atmosphere for 72 h, concentrated to dryness and chromatographed on silica gel.

5-(2-Cyanoethyl)-3-ethyl-(±)-2-[(2-(2-phthalimidoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (9a). The procedure described above was used with 1.1 g (3.5 mmol, MW 312.15) of **8a** as the benzylidene. Chromatography (eluent: heptane:EtOAc, 70:30) gave 1.15 g of **9a** as a yellow flocky powder (50%). R_f (heptane:EtOAc, 50:50) 0.2; ^{1}H NMR ($CDCl_3$, 298.0 K) δ 7.90–7.65 (b, 4H), 7.57 (s, 1H), 7.33 (d, J = 7.5 Hz, 1H), 7.25 (d, J = 7.5 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 5.43 (s, 1H), 4.65 (bs, 2H), 4.15–3.55 (b, 12H), 2.68 (t, J = 6.3 Hz, 2H), 2.34 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 298.0 K) δ 167.8 [C], 166.3 [C], 166.2 [C], 147.6 [C], 146.1 [C], 145.2 [C], 133.8 [CH], 132.4 [C], 131.6 [CH], 130.4 [C], 129.6 [CH], 128.0 [CH], 126.9 [CH], 122.9 [CH], 116.8 [C], 101.8 [C], 100.9 [C], 70.3 [CH_2], 69.1 [CH_2], 67.7 [CH_2], 67.5 [CH_2], 59.5 [CH_2], 57.9 [CH_2], 38.1 [CH], 36.6 [CH_2], 19.3 [CH_3], 17.6 [CH_2], 13.9 [CH_3]; MS 677 [M+NH_4^+], 675 [M+NH_4^+], 673 [M+NH_4^+], 660 [M+H^+], 658 [M+H^+], 656 [M+H^+].

3-Ethyl-5-(2,2,2-trichloroethyl)-(±)-2-[(2-(2-phthalimidoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (9b). The procedure described above was used with 3.0 g (7.7 mmol, MW 390.48) of **8b** as the benzylidene. Chromatography (eluent: heptane:EtOAc, 70:30) gave 2.4 g of **9b** as a yellow flocky powder (42%). R_f (heptane:EtOAc, 60:40) 0.2; ^{1}H NMR (CD_2Cl_2, 297.6 K) δ 7.90-7.65 (b, 4H), 7.65 (s, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 5.48 (s, 1H), 4.85–4.55 (b, 4H), 4.15–3.55 (b, 10H), 2.34 (s, 3H), 1.14 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 168.4 [C], 166.8 [C], 165.8 [C], 147.6 [C], 147.4 [C], 145.8 [C], 134.3 [CH], 133.1 [C], 131.4 [C], 129.9 [CH], 128.6 [CH], 127.0 [CH], 123.4 [CH], 101.4 [C], 101.2 [C], 96.0 [C], 73.8 [CH_2], 71.0 [CH_2], 69.8 [CH_2], 68.2 [CH_2], 68.0 [CH_2], 60.0 [CH_2], 39.6 [CH], 37.2 [CH_2], 20.0 [CH_3], 14.1 [CH_3]; MS 758 [M+NH_4^+], 756 [M+NH_4^+], 754 [M+NH_4^+], 752 [M+NH_4^+], 750 [M+NH_4^+], 741 [M+H^+], 739 [M+H^+], 737 [M+H^+], 735 [M+H^+], 733 [M+H^+].

3-Ethyl-5-(2-trimethylsilylethyl)-(±)-2-[(2-(2-phthalimidoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (9c). The procedure described above was used with 5.0 g (13.9 mmol, MW 359.32) **8c** as the benzylidene. Chromatography (eluent: heptane:EtOAc, 80:20) gave 4.0 g of **8c** as a yellow flocky powder (41%). R_f (heptane:EtOAc, 65:35) 0.3; ^{1}H NMR (CD_2Cl_2, 297.6 K) δ 7.85-7.65 (b, 4H), 7.40 (s, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 5.41 (s, 1H), 4.61 (bs, 2H), 4.15–3.55 (b, 12H), 2.26 (s, 3H), 1.20 (t, J = 8.5 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H), 0.01 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ : 168.4 [C], 167.8 [C], 167.0 [C], 148.6 [C], 146.3 [C], 144.3 [C], 134.7 [CH], 132.8 [C], 132.4 [C], 131.2 [C], 130.5 [CH], 128.4 [CH], 127.3 [CH], 123.5 [CH], 104.1 [C], 100.7 [C], 71.0 [CH_2], 69.8 [CH_2], 68.3 [CH_2], 68.2 [CH_2], 62.0 [CH_2], 59.8 [CH_2], 39.2 [CH], 37.4 [CH_2], 19.3 [CH_3], 17.7 [CH_2], 14.4 [CH_3], -1.1 [CH_3]; MS 724 [M+NH_4^+], 722 [M+NH_4^+], 720 [M+NH_4^+], 707 [M+H^+], 705 [M+H^+], 703 [M+H^+].

5-Benzyl-3-ethyl-(±)-2-[(2-(2-phthalimidoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (9d). The procedure described above was used with 3.2 g (9.2 mmol, MW 349.21) of **8d** as the benzylidene. Chromatography (eluent: heptane:EtOAc, 60:40) gave 840 mg of **9d** as a yellow oil (13%). R_f (heptane:EtOAc, 50:50) 0.4; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 7.85–7.60 (b, 4H), 7.45 (s, 1H), 7.35–7.10 (b, 7H), 7.05 (t, J = 7.8 Hz, 1H), 5.43 (s, 1H), 5.06 (bs, 2H), 4.65 (bs, 2H), 4.15–3.55 (b, 12H), 2.26 (s, 3H), 1.12 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 168.3 [C], 167.1 [C], 166.8 [C], 148.4 [C], 145.9 [C], 145.3 [C], 137.7 [C], 134.2 [CH], 132.7 [C], 132.6 [C], 131.1 [C], 130.3 [C], 128.4 [CH], 128.2 [C], 128.1 [CH], 127.8 [C], 127.2 [C], 123.2 [CH], 103.1 [C], 101.0 [C], 70.8 [CH_2], 69.7 [CH_2], 68.1 [CH_2], 68.0 [CH_2], 65.3 [CH_2], 59.8 [CH_2], 38.9 [CH_3], 37.2 [CH_2], 19.3 [CH_3], 14.3 [CH_3]; MS 714 [M+NH_4^+], 712 [M+NH_4^+], 710 [M+NH_4^+], 697 [M+H^+], 695 [M+H^+], 693 [M+H^+].

Preparation of the precursors for the [^{11}C]labelling with CH_2N_2

Preparative chiral HPLC: Separation of both enantiomers of dihydropyridines 9a. 400 mg of (+)-**9a** ($[\alpha]_D^{25}$ +43, $CHCl_3$) and 380 mg of (−)-**9a** ($[\alpha]_D^{25}$ −44, $CHCl_3$) were purified in 10 successive injections by Chiral preparative HPLC. Chemical purities were higher than 95% as were the enantiomeric purities: (+)-**9a** (ee: 95.0) and (−)-**9a** (ee: 99.2). Column: Prochrom LC50 (500 × 50 mm); Phase: OF type[22] (300 g); Granulometry: 5 μm; porosity: 100 Å; eluent: heptane:isopropanol, 50:50; flowrate: 80.0 mL/min; temperature: rt; UV detection at λ: 210 nm; retention times: (+)-**9a**: 50–70 min and (−)-**9a**: 73–100 min.

Determination of chemical purity. Column: analytical Kromasil 5C18 (250 × 4.6 mm); eluent: t_0: acetonitrile: water, 50:50, t_{10} to t_{50}: linear gradient acetonitrile:water, 50:50 to 90:10; flowrate: 1.0 mL/min; temperature: 30 °C; UV detection at λ: 210 nm; retention time: (±)-**9a**: 31.5 min.

Determination of enantiomeric purity. Column: analytical Chiralpak AD (250 × 4.6 mm); eluent: heptane:isopropanol, 60:40; flowrate: 1.0 mL/min; temperature: rt; UV detection at λ: 210 nm; retention times: (−)-**9a**: 14.0 min and (+)-**9a**: 17.0 min.

General procedure for the simultaneous deprotection of the phthalimido and cyanoethoxy groups of 9a

(±)-2-[(2-(2-Aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid (10). 100 mg (0.15 mmol, MW 656.52) of (**9a**) were dissolved in 4 mL of 40% aqueous methylamine. The solution was stirred at room temperature for 3 h, concentrated to dryness and the residue chromatographed on silica gel (elution with EtOAc first, then MeOH) to give 81 mg of **10** (80%). R_f (MeOH) 0.2; ^{1}H 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], 130.1 [CH], 129.9 [C], 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$^+$].

Determination of chemical purity. Column: analytical Kromasil 5C18 (250 × 4.6 mm); eluent: t_0: acetonitrile/1% aqueous $HClO_4$ 40/60, t_{10} to t_{50}: linear gradient acetonitrile: 1% aqueous $HClO_4$ 40:60 to 90:10; flowrate: 1.0 mL/min; temperature: 30 °C; UV detection at λ: 210 nm; retention time: (±)-**10**: 22.8 min.

Determination of enantiomeric purity. Column: analytical Chiralpak AD (250 × 4.6 mm); eluent: heptane: ethanol:TFA, 90:10:0.1; flowrate: 1.0 mL/min; temperature: 20 °C; UV detection at λ: 210 nm; retention times: (−)-**10**: 13.2 min and (+)-**10**: 18.9 min.

(−)-2-[(2-(2-Aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((−)-10). The procedure described above was used with 150 mg of (−)-**9a** to give 85 mg of (−)-**10** (79%). $[\alpha]_D^{25}$ −21 (CH_2Cl_2) and other analytical data identical to (±)-**10**; chemical purity: >95%; enantiomeric purity: ee: 99.2.

(+)-2-[(2-(2-Aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid ((+)-10). The procedure described above was used with 150 mg of (+)-**9a** to give 100 mg of (+)-**10** (92%). $[\alpha]_D^{25}$ +20 (CH_2Cl_2) and other analytical data identical to (±)-**10**; chemical purity: >95%; enantiomeric purity: ee : 95.0.

Micromolar-scale synthesis of S12968 ((−)-1) and S12967 ((+)-1) from (−)- and (+)-10

3-Ethyl-5-methyl-(−)- and (+)-2-[(2-(2-aminoethoxy)-ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate ((−)- and (+)-1). To 2.0 mg (4.2 µmol, MW 473.35) of (−)-**10** (respectively, (+)-**10**) in 1 mL of 8:2 mixture of Et_2O:MeOH was added 20 drops of a 1.6 mM solution of diazomethane in Et_2O (a typical procedure using Diazald as diazomethane generator is described in the Aldrich Technical Bulletin number AL-180). The solution was stirred 10 min at 0 °C, then concentrated to dryness. HPLC purification (see the labelling section for the fully described HPLC conditions) gave pure S12968 ((−)-**1**) (respectively S12967 ((+)-**1**)). The synthesized compounds as well as their *N*-MTPA-derivatives coelute with samples of original S12968 and S12967 from Servier and *N*-MTPA derivatives of S12968 and S12967, respectively.

General procedure for the *N*-derivatization of S12968 (respectively S12967) with (*S*)-(+)-MTPA-Cl and chiral HPLC analysis of the derivatives. 0.1–5 mg of S12968 ((−)-**1**) (respectively S12967, ((+)-**1**)) was dissolved in 2 mL of CH_2Cl_2, 10 µL of (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and 50 µL of triethylamine were added and the solution was stirred for 1–5 min. The mixture was then washed once with 1 mL of 1.0 N aq NaOH, 1 mL of water, 1 mL of 1.0 N aq 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. The residue was taken up in 1 mL of CH_2Cl_2 and analysed by HPLC. Column: analytical Pyrkle (250 × 4.6 mm); eluent: hexane:isopropanol:THF, 95:5:2; flowrate: 1.5 mL/min; temperature: 45 °C; UV detection at λ: 360 nm; retention times: S12967 ((+)-**1**): 24.0 min and S12968 ((−)-**1**): 25.6 min.

Preparation of the precursors for the [^{11}C]labelling with CH_3I

3-Ethyl-5-(2-trimethylsilylethyl)-(±)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (12). A solution of 4.0 g (57 mmol, MW : 703.69) of **9c** in 40 mL of 40% aq methylamine was stirred at room temperature for 5 h and then concentrated to dryness. The residue was redissolved in EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated. Chromatography on silica gel (eluent: EtOAc:MeOH, 85:25) gave 2.3 g of **12** as an oily residue (71%). R_f (EtOAc:MeOH, 65:35) 0.2; ^{1}H NMR (CD_2Cl_2, 296.2 K) δ 7.58 (s, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 5.47 (s, 1H), 4.72 (AB system, looks like a q, 2H), 4.15–3.55 (b, 12H), 2.33 (s, 3H), 1.14 (t, J = 8.5

Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H), 0.01 (s, 9H); ^{13}C NMR (CD_2Cl_2, 296.2 K) δ 168.1 [C], 167.5 [C], 149.1 [C], 146.8 [C], 144.7 [C], 133.3 [C], 131.6 [C], 130.9 [CH], 128.8 [CH], 127.7 [CH], 104.5 [C], 101.4 [C], 74.3 [CH_2], 71.6 [CH_2], 70.6 [CH_2], 68.7 [CH_2], 62.5 [CH_2], 60.4 [CH_2], 42.3 [CH_2], 39.7 [CH], 19.8 [CH_3], 18.1 [CH_2], 14.9 [CH_3], −1.1 [CH_3]; MS 577 [M+H$^+$], 575 [M+H$^+$], 573 [M+H$^+$].

3-Ethyl-5-(2-trimethylsilylethyl)-(±)-2-[(2-(2-*tert*-butoxycarbonylaminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (13). 2.3 g (4.0 mmol, MW 573.59) of **12** were dissolved in 40 mL of THF and 4 mL of H_2O. 1.5 g of K_2CO_3 were added and the solution was cooled to 0 °C. 1.0 mL of di-*tert*-butyl dicarbonate (4.4 mmol, 1.1 equiv, MW 218.25, d 0.950) was added and the mixture was stirred at 0 °C for 2 h. After dilution with EtOAc, the solution was washed with water, brine, dried over $MgSO_4$, and concentrated to dryness. The residue was chromatographed on silica gel (eluent: heptane:EtOAc, 50:50) to give 2.3 g of **13** as an oily residue (85%). R_f (heptane:EtOAc, 50:50) 0.3; ^{1}H NMR (CD_2Cl_2, 295.8 K) δ 7.42 (s, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.26 (d, J = 7.6 Hz, 1H), 7.10 (t, J = 7.6 Hz, 1H), 5.45 (s, 1H), 4.72 (AB system, looks like a q, 2H), 4.15–3.95 (b, 4H), 3.75–3.50 (b, 6H), 3.40–3.20 (b, 2H), 2.31 (s, 3H), 1.15 (t, J = 6.9 Hz, 3H), 0.92 (t, J = 6.5 Hz, 2H), 0.00 (s, 9H); ^{13}C NMR (CD_2Cl_2, 296.2 K) δ 168.1 [C], 167.5 [C], 156.5 [C], 149.1 [C], 146.8 [C], 144.7 [C], 133.3 [C], 131.6 [C], 130.9 [CH], 128.8 [CH], 127.7 [CH], 104.5 [C], 101.6 [C], 79.9 [C], 71.5 [CH_2], 71.0 [CH_2], 70.6 [CH_2], 68.6 [CH_2], 62.5 [CH_2], 60.4 [CH_2], 40.8 [CH_2], 39.3 [CH], 28.8 [CH_3], 19.7 [CH_3], 18.1 [CH_2], 14.9 [CH_3], 1.1 [CH_3]; MS 677 [M+H$^+$], 675 [M+H$^+$], 673 [M+H$^+$].

(±)-2-[(2-(2-*tert*-Butoxycarbonylaminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-6-methyl-1,4-dihydropyridine-5-carboxylic acid (14). To a solution of 2.0 g (3.0 mmol, MW 673.7045) of **13** in 40 mL of THF were added over a period of 5 h 7.83 g (30 mmol, 10 equiv, MW 261.46) of tetrabutylammonium fluoride at room temperature. The mixture was stirred for 24 h at room temperature, then 20 mL of H_2O was added and the solution stirred for another 2 h. The mixture was then diluted with EtOAc, washed with water, brine, dried over $MgSO_4$, and concentrated to dryness. The residue was chromatographed on silica gel (eluent EtOAc first, then EtOAc:MeOH, 95:5) to give 1.3 g of **14** as a yellow powder (76%). R_f (heptane:EtOAc, 50:50) 0.1; ^{1}H NMR (CD_2Cl_2, 294.2 K) δ 7.56 (s, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 5.43 (s, 1H), 4.71 (AB system, looks like a q, 2H), 4.01 (b, 2H), 3.75–3.50 (b, 6H), 3.30 (b, 2H), 2.33 (s, 3H), 1.15 (t, J = 6.9 Hz, 3H); ^{13}C NMR (CD_2Cl_2, 296.2 K) δ 173.3 [C], 167.4 [C], 156.6 [C], 148.4 [C], 147.5 [C], 146.4 [C], 133.4 [C], 131.9 [C], 131.1 [CH], 129.0 [CH], 127.6 [CH], 102.9 [C], 102.0 [C], 79.8 [C], 71.5 [CH_2], 71.0 [CH_2], 70.6 [CH_2], 68.5 [CH_2], 60.6 [CH_2], 40.9 [CH_2], 39.8 [CH], 28.8 [CH_3], 20.3 [CH_3], 14.8 [CH_3]; MS 594 [M+NH$_4^+$], 592 [M+NH$_4^+$], 590 [M+NH$_4^+$], 577 [M+H$^+$], 575 [M+H$^+$], 573 [M+H$^+$].

Preparative chiral HPLC: Separation of both enantiomers of dihydropyridines 14. 425 mg of (+)-**14** ($[\alpha]_D^{25}$ +102, $CHCl_3$) and 380 mg of (−)-**14** ($[\alpha]_D^{25}$ −102, $CHCl_3$) were purified in 11 successive injections by chiral preparative HPLC. Chemical purities were higher than 95% were the enantiomeric purities: (+)-**14** (ee: 99.4) and (−)-**14** (ee: 99.2). Column: Prochrom LC50 (500 × 50 mm), Phase: OF type[22] (300 g); granulometry: 5 μm; porosity: 100 Å; eluent: heptane:isopropanol: 85:15; flowrate: 80.0 mL/min; temperature: rt; UV detection at λ: 210 nm; retention times: (+)-**14**: 34–48 min and (−)-**14**: 57–80 min.

Determination of chemical purity. Column: analytical Hypersil BDS 5C18 (250 × 4.6 mm); eluent: t_0 to t_{50}: linear gradient acetonitrile:water, 50:50 to 80:20; flowrate: 1.0 mL/min; temperature: 40 °C; UV detection at λ: 210 nm; retention time: (±)-**14**: 11.5 min.

Determination of enantiomeric purity. Column: analytical Chiralpak AD (250 × 4.6 mm); eluent: heptane:isopropanol:TFA, 85:15:0.1; flowrate: 1.0 mL/min; temperature: 35 °C; UV detection at λ: 210 nm; retention times: (−)-**14**: 11.5 min and (+)-**14**: 19.0 min.

Synthesis of *N*-Boc-S12968 ((−)-15) as an HPLC standard

3-Ethyl-5-methyl-(−)-2-[(2-(2-*tert*-butoxycarbonylaminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate ((−)-15). 4.9 g (10.0 mmol, MW 487.50) of (−)-**1** were dissolved in 40 mL of THF and 5 mL of H_2O. 4.1 g of K_2CO_3 were added and the solution was cooled to 0 °C. 2.5 mL of di-*tert*-butyl dicarbonate (11.0 mmol, 1.1 equiv, MW 218.25, d 0.950) were added and the mixture was stirred at 0 °C for 2 h. After dilution with EtOAc, the solution was washed with water, brine, dried over $MgSO_4$, and concentrated to dryness. The residue was chromatographed on silica gel (eluent: heptane:EtOAc, 50:50) to give 5.7 g (−)-**15** as yellow powder (97%). R_f (heptane:EtOAc, 50:50) 0.25; ^{1}H NMR (CD_2Cl_2, 293.0 K) δ 7.55 (s, 1H), 7.35 (d, J = 7.6 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 5.47 (s, 1H), 4.73 (AB system, looks like a q, 2H), 4.02 (q, J = 6.9 Hz, 2H), 3.75–3.50 (b, 6H), 3.56 (s, 3H), 3.32 (b, 2H), 2.34 (s, 3H), 1.15 (t, J = 6.9 Hz, 3H); ^{13}C NMR (CD_2Cl_2, 293.0 K) δ 168.3 [C], 167.4 [C], 156.5 [C], 149.2 [C], 146.7 [C], 145.2 [C], 133.3 [C], 131.4 [C], 130.6 [CH], 128.8 [CH], 127.8 [CH], 104.1 [C], 101.6 [C], 79.8 [C], 71.5 [CH_2], 71.0 [CH_2], 70.6 [CH_2], 68.6 [CH_2], 60.4 [CH_2], 51.3 [CH_3], 40.8 [CH_2], 39.3 [CH], 28.8 [CH_3], 19.7 [CH_3], 14.8 [CH_3]; MS 608 [M+NH$_4^+$], 606 [M+NH$_4^+$], 604 [M+NH$_4^+$], 591 [M+H$^+$], 589 [M+H$^+$], 587 [M+H$^+$].

Micromolar-scale synthesis of S12968 ((−)-1) and S12967 ((+)-1) from (−)- and (+)-14

3-Ethyl-5-methyl-(−)- and (+)-2-[(2-(2-aminoethoxy)-ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate ((−)- and (+)-1). 2.0 mg (3.5 mmol, MW 573.47) of (−)-**14** (respectively, (+)-**14**) were dissolved in 0.4 mL of freshly distilled DMF containing 10 µL of 0.25 M trimethylbenzylammonium hydroxide in EtOH. After addition of 19 µL of a freshly prepared 0.01 M solution of iodomethane in DMF (3.0 mmol), the reaction mixture was heated for 10 min at 40 °C and then cooled. The crude was then diluted with 0.5 mL of the HPLC mobile phase and was injected onto the column. HPLC purification (see the labelling section for the fully described conditions) gave pure DHP (−)-**15** (respectively, (+)-**15**). The solvents were evaporated and the residue was taken up in 2 mL of a 4:1 mixture of CH_2Cl_2:TFA (v/v). The solution was stirred 15 min at rt, then concentrated to dryness. HPLC purification (see the labelling section for the fully described HPLC conditions) gave pure S12968 ((−)-**1**) (respectively S12967 ((+)-**1**)). The synthesized compounds as well as their *N*-MTPA-derivatives coelute with samples of original S12968 and S12967 from Servier and *N*-MTPA derivatives of S12968 and S12967, respectively.

Radiochemistry

Labelling process using $[^{11}C]CH_2N_2$

Preparation of $[^{11}C]CH_2N_2$ synthon. $[^{11}C]CH_4$ was produced by irradiation of a 95:5 mixture of nitrogen and hydrogen with a 20 MeV proton beam from a 520-CGR-MeV cyclotron (25 µA, 45 min). After being separated from the target gas by trapping on Porapak-Q at −186 °C (liquid argon), the $[^{11}C]CH_4$ was released from the traps by simply raising them to room temperature (with nitrogen as vector gas). Then after having been mixed with a few millilitres of chlorine gas (20 mL), it was transformed into $[^{11}C]CHCl_3$ by being passed through a catalyst of pumice stone impregnated with $CuCl_2$ (1:1, w/w) at a temperature of 330 °C. The $[^{11}C]CHCl_3$ was converted into $[^{11}C]CH_2N_2$ by reaction with a solution of hydrated hydrazine (300 µL in ethanol (300 µL)) in the presence of potassium hydroxide (140 mg) at 60 °C. The $[^{11}C]CH_2N_2$ is carried away by a flow of N_2/O_2 into the reaction flask. On average, about 250 mCi (9.25 GBq) of $[^{11}C]CH_2N_2$ is routinely obtained in our laboratory[7] in 10 min after end of bombardment in 30% decay-corrected yield, based on starting $[^{11}C]CH_4$ (1.2 Ci or 44.40 GBq, EOB).

Formation of the [^{11}C]methyl ester. 0.5–1.0 mg of (−)- or (+)-**10** were dissolved in a mixture of freshly distilled dimethoxyethane (300 µL) and methanol (50 µL). The $[^{11}C]CH_2N_2$ was trapped in this solution at 0 °C for 3 min. Bubbling was continued for another 5 min at room temperature. The solvents were then evaporated off and the residue was dissolved in 1–2 mL of the HPLC mobile phase used for purification and was injected onto the column.

Between 20 and 50 mCi (0.74–1.85 GBq) of radiochromatographically pure (−)-**1** or (+)-**1** were obtained at 35–40 min after end of bombardment (HPLC purification included) with specific radioactivities as high as 1200 mCi/µmol (44.4 GBq/µmol, typical values 400–900 mCi/µmol (14.8–33.34 GBq/µmol)); yield 20–50% decay-corrected based on $[^{11}C]CH_2N_2$. Column: semipreparative C-18 µBondapak Waters (300 × 7.8 mm); porosity: 10 µm; eluent: acetonitrile:water:TFA, 35:65:0.1; flowrate: 4.0 mL/min; temperature: rt; UV detection at λ: 254 nm; retention times: precursors (+)-**10** and (−)-**10**, 5.5–6.0 min; labelled S12967 ((+)-**1**) and S12968 ((−)-**1**): 8.5–9.0 min.

Labelling process using $[^{11}C]CH_3I$

Preparation of $[^{11}C]CH_3I$ synthon. $[^{11}C]CO_2$ was produced by irradiation of ultrapure nitrogen N60 Air Liquide with a 20 MeV proton beam from a 520-CGR-MeV cyclotron (30 µA, 30 min). After being separated from the target gas by trapping at −186 °C (liquid argon), the $[^{11}C]CO_2$ was released from the trap by simply raising the latter to room temperature with nitrogen as vector gas. The $[^{11}C]CO_2$ was converted into $[^{11}C]CH_3OH$ by reduction at room temperature with a 1.0 M THF solution of lithium aluminium hydride (5 µL) in THF (50 µL), followed by concentration and hydrolysis (50 µL of deionized water). The [^{11}C]MeOH was distilled into 1 mL of an aqueous 57% HI solution, the $[^{11}C]CH_3I$ thus synthesized was continously distilled away by a flow of nitrogen gas and trapped at 0 °C into the reaction flask. On average, about 750 mCi (27.75 GBq) of $[^{11}C]CH_3I$ is routinely obtained in our laboratory[9] in 7–8 min after end of bombardment in 80% decay-corrected yield, based on starting $[^{11}C]CO_2$ (1.2 Ci or 44.40 GBq, EOB).

Formation of the [^{11}C]methyl ester: indirect pathway, via [^{11}C]-(−)- or [^{11}C]-(+)-15. Between 0.5 and 1.0 mg of (−)- or (+)-**14** were dissolved in 0.2 mL of freshly distilled DMF containing 5 µL of 0.25 M trimethylbenzylammonium hydroxide (0.7–1.4 equiv with respect to starting DHP) in EtOH. The $[^{11}C]CH_3I$ was trapped in this solution at 0 °C for 5 min. The reaction vessel was then isolated, heated for 5 min at 40 °C and cooled. The crude was then diluted with 0.5 mL of the HPLC mobile phase and was injected onto the column.

Between 100 and 200 mCi (3.70–7.40 GBq) of radiochromatographically pure (−)-**15** or (+)-**15** were obtained at 25–30 min after end of bombardment (HPLC purification included) with specific radioactivities as high as 1700 mCi/µmol (62.9 GBq/µmol, typical values 600–1000 mCi/µmol (22.2–37.0 GBq/µmol)); yield 25–50% decay-corrected based on $[^{11}C]CH_3I$. Column: semipreparative C-18 µBondapak Waters (300 × 78 mm); porosity: 10 µm; eluent: acetonitrile: water:TFA, 70:30:0.1; flowrate: 4.0 mL/min; tempera-

ture: rt; UV detection at λ: 254 nm; retention times: precursors (+)-**14** and (−)-**14**: 3.5–4.0 min; labelled (+)-**15** and (−)-**15**: 6.0–6.5 min.

After concentration, the residue was taken up in 2 mL of a 4:1 mixture of CH_2Cl_2:TFA (v/v). The solution was allowed to react at room temperature for 2 min, then concentrated to dryness. The residue was dissolved with 1.0 mL of the HPLC solvent and was injected onto the column.

Between 40 and 80 mCi (1.48–2.96 GBq) of radiochromatographically pure (−)-**1** or (+)-**1** were obtained at 45–50 min after end of bombardment (second HPLC purification included) with specific radioactivities of 300–500 mCi/μmol (11.1–18.5 GBq/μmol); total yield: 20–40% decay-corrected based on $[^{11}C]CH_3I$. Column: semipreparative C-18 μBondapak Waters (300 × 7.8 mm); porosity: 10 μm; eluent: acetonitrile:water:TFA, 35:65:0.1; flowrate: 4.0 mL/min; temperature: rt; UV detection at λ: 254 nm; retention times: labelled S12967 ((+)-**1**) and S12968 ((−)-1): 8.5–9.0 min.

Poor and not reproducible results were obtained when the intermediate labelled (+)-**15** or (−)-**15** were directly submitted to the TFA deprotection without HPLC purification.

Formation of the [^{11}C]methyl ester: direct pathway. Between 0.5 and 1.0 mg of (−)- or (+)-**10** were dissolved in 0.2 mL of freshly distilled DMF containing 5 μL of 0.25 M trimethylbenzylammonium hydroxide (0.8–1.6 equiv with respect to starting DHP) in EtOH. The $[^{11}C]CH_3I$ was trapped in this solution at 0 °C for 5 min. The reaction vessel was then isolated, heated for 5 min at 40 °C and cooled. The crude was then diluted with 0.5 mL of the HPLC mobile phase and was injected onto the column.

Between 100 and 120 mCi (3.70–4.40 GBq) of radiochromatographically pure (−)-**1** or (+)-**1** were obtained at 25–30 min after end of bombardment (HPLC purification included) with specific radioactivities as high as 1500 mCi/μmol (55.5GBq/μmol, typical values 500–1000 mCi/μmol (18.5–37.0 GBq/μmol)); yield 25–30% decay-corrected based on $[^{11}C]CH_3I$. Column: semipreparative C-18 μBondapak Waters (300 × 7.8 mm); porosity: 10 μm; eluent: acetonitrile:water:TFA: 35:65:0.1; flowrate: 4.0 mL/min; temperature: rt; UV detection at λ: 254 nm; retention times: precursors (+)-**10** and (−)-**10**: 5.5–6.0 min; labelled S12967 ((+)-**1**) and S12968 ((−)-**1**): 8.5–9.0 min.

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