Synthesis of Racemic (*S*)-(+)- or (*R*)-(−)-[*Methyl*-^{11}C]amphetamine

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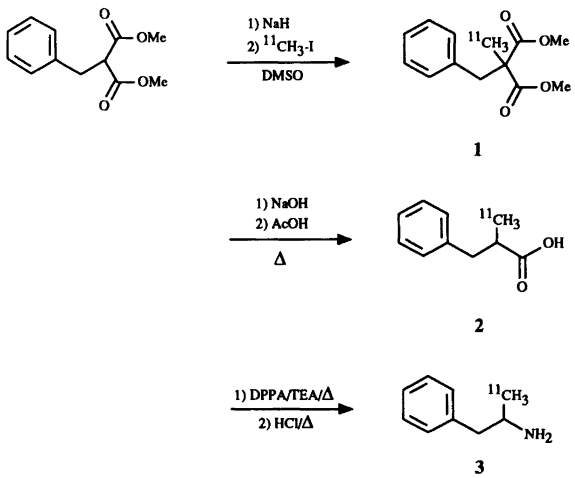
The synthesis of racemic (*S*)-(+)- or (*R*)-(−)-[*methyl*-^{11}C]amphetamine (**3**) is reported. The alkylation of dimethyl 2-benzylmalonate with [^{11}C]methyl iodide yielded dimethyl 2-benzyl-2-([^{11}C]methyl)malonate (**1**) which was used as an intermediate in the synthesis of **3**. Hydrolysis of **1** with NaOH and subsequent decarboxylation using glacial acetic acid and heating produced 2-benzyl-[3-^{11}C]propionic acid (**2**). Conversion of **2** into **3** was achieved via the intermediate isocyanate using diphenylphosphoryl azide (DPPA), and subsequently into the amine using conc. HCl. After purification by solid-phase extraction and preparative LC, (±)-**3** was obtained with a radiochemical purity greater than 98 %. Starting with 3 GBq (81 mCi) [^{11}C]carbon dioxide, 145 MBq (3.9 mCi) (±)-[*methyl*-^{11}C]amphetamine was obtained in 45 min with a 22 % decay-corrected radiochemical yield. Enantiomerically pure **3** was obtained by the preparative LC separation of the (+)- or (−)-10-camphorsulfonamide derivatives of the racemate with a total decay-corrected radiochemical yield of 7 % (counted from the start of methyl iodide synthesis). In a typical synthesis, 27 MBq (0.7 mCi) enantiomerically pure (*S*)-(+)- or (*R*)-(−)-[*methyl*-^{11}C]amphetamine were obtained starting from 3 GBq (81 mCi) [^{11}C]carbon dioxide. The position of labelling was confirmed by a ^{13}C synthesis using the same reaction pathway, and analysis by ^{13}C NMR spectroscopy.

With the advance of positron emission tomography (PET)[1,2] technology in recent years, there has arisen a corresponding demand for new synthetic methods which are suitable, considering the limitations imposed, for use with short-lived radionuclides. The short half-lives (^{11}C, ^{18}F, ^{13}N; $t_{1/2}$ = 20.3, 110 and 10.0 min, respectively) dictate that the production of the labelled precursor, synthesis of the target molecule, and subsequent purification, should be carried out as rapidly as possible. The development of ^{11}C and ^{18}F labelled precursors is therefore of prime importance for the advancement of PET. At present, the majority of ^{11}C-labelled tracers have been synthesised by the alkylation of nitrogen, oxygen and sulfur nucleophiles with [^{11}C]methyl iodide,[3] or by nucleophilic substitutions using [^{11}C]cyanide. However, due to the synthetic limitations of this approach, more advanced chemical manipulations are required in order to increase the flexibility of ^{11}C-labelling procedures. One strategy has been to use labelled multifunctional precursors to build up the target molecule. A notable example using this approach is the multi-enzymatic synthesis of [β-^{11}C]-L-dopa from [^{11}C]pyruvic acid.[4] With the aim of developing multi-functional precursors labelled with short-lived radionuclides, the possibility of using ^{11}C-labelled malonic ester derivatives in radiolabelling syntheses is being investigated. In combination with an array of chemical transformations developed for use in rapid labelling syntheses, a number of biologically interesting molecules have now been synthesised from labelled malonic ester substrates.[5] Presented in this paper is the synthesis of racemic, (*S*)-(+)- or (*R*)-(−)-[*methyl*-^{11}C]amphetamine (**3**) from dimethyl 2-benzyl-2-([^{11}C]methyl)malonate (**1**) according to Scheme 1.

The synthesis of [*methyl*-^{11}C]-α-methylphenylalanine from **1** has recently been reported.[6] The synthetic route involved a selective enzymatic ester hydrolysis using pig liver esterase followed by a modified Curtius rearrangement and subsequent hydrolysis. In common with this synthesis, [*methyl*-^{11}C]amphetamine has now been made from the same precursor (**1**), as shown in Scheme 1.



Scheme 1. The synthetic pathway to [*methyl*-^{11}C]amphetamine starting from [^{11}C]methyl iodide.

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The enantiomers of amphetamine differ somewhat in biological activity, as is the case with most biomolecules (e.g. amino acids). Therefore, for the purpose of biological studies, the use of enantiomerically pure compounds is essential. In the synthesis of ^{11}C-amino acids this problem has been approached by a number of methods: labelling a substrate in which the stereochemistry is already established,[7] asymmetric synthesis (enzymatic[4,8–11] or chemical[12–14]), and resolution (enzymatic[15] or chromatographic[16–18]). In this paper, (+)- or (−)-10-camphorsulfonyl chloride was used in the diastereomeric resolution of (±)-**3** whereby both enantiomers could be obtained with ee values of >98 %.

Amphetamine† is an indirectly acting sympathomimetic amine which releases the catecholamines, noradrenaline and dopamine from the presynaptic dopamine neurons. Other actions include the block of catacholamine re-uptake and a certain degree of agonist activity at receptors for dopamine and 5-hydroxytryptophan. (+)-Amphetamine is slightly more potent centrally than that of the (−)-form, which is more potent peripherally.

A method enabling amphetamine to be labelled with a short-lived radionuclide in combination with PET, offers the possibility of increasing our understanding of this drug's mode of action *in vivo*, and to gain useful information about normal and diseased states in disorders such as schizophrenia and depression.

Results and discussion

(±)-[*Methyl*-^{11}C]amphetamine was synthesised according to Scheme 1 with a 22 % decay-corrected radiochemical yield in 45 min (counted from start of [^{11}C]methyl iodide synthesis) and a radiochemical purity of greater than 98 %. In a typical run starting from 3 GBq (81 mCi) [^{11}C]carbon dioxide, 145 MBq (3.9 mCi) of (±)-**3** was produced. Enantiomerically pure (*S*)-(+)- or (*R*)-(−)-**3** was synthesised within 60 min with a radiochemical yield (decay-corrected) of 7 % and a radiochemical purity grater than 98 %. The ^{11}C-methylation of dimethyl 2-benzylmalonate in DMSO using NaH as the base was achieved in ca. 95 % radiochemical yield (decay-corrected) at the end of [^{11}C]methyl iodide trapping. Decarboxylation of **1** to **2** was carried out using NaOH followed by the addition of glacial acetic acid and heating, in ca. 50 % decay-corrected radiochemical yield, the solid-phase extraction procedure and subsequent evaporation of THF being the main cause of yield reduction. The modified Curtius rearrangement of **2** using diphenylphosphoryl azide (DPPA)[20] and subsequent hydrolysis to **3** with conc. HCl was achieved in an 83 % decay-corrected radiochemical yield. Solid-phase extraction and preparative LC purification were carried out in 70 and 75 % decay-corrected radiochemical yields, respectively, leading to the pure product. It was found that the use of normal C-18 columns resulted in substantial peak broadening due to interaction of the amine with exposed silanol groups in the stationary phase. This problem was overcome by the use of a Suplex pKb-100 column,[21] with which sharp amphetamine peaks were obtained. A typical analytical LC chromatogram of the purified product is shown in Fig. 1. Enantiomeric resolution of the (+)- and

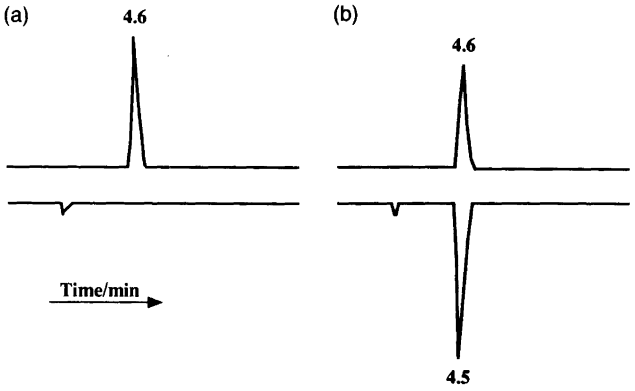

Fig. 1. Analytical LC chromatogram of (a) purified (±)-[*methyl*-^{11}C]amphetamine and (b) with added reference. Upper trace, radio detector; lower trace, UV.

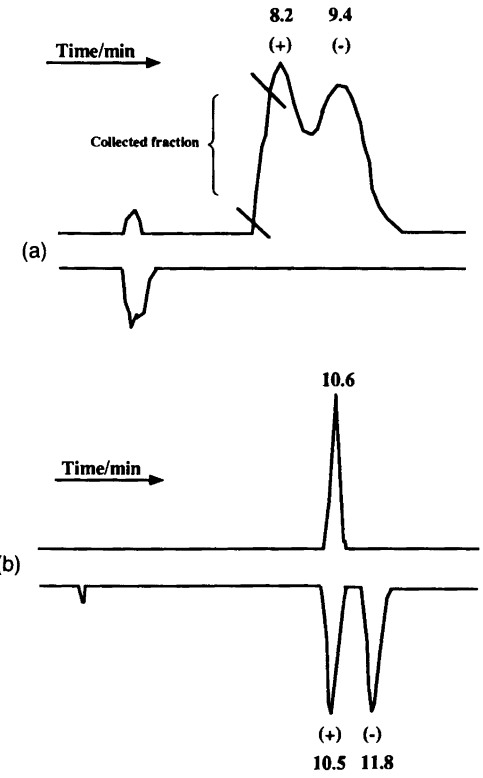

Fig. 2. (a) Preparative LC chromatogram showing the resolution of the (−)-10-camphorsulfonamide derivatives of (+)- and (−)-[*methyl*-^{11}C]amphetamine and (b) and analytical LC chromatogram of the fraction taken from the above resolution showing enantiomerically pure (+)-[*methyl*-^{11}C]amphetamine [reference (±)-amphetamine added]. Upper trace, radio detector; lower traces, UV.

† For review of pharmacological effects of amphetamine, see Ref. 19 and references therein.

(−)-form was achieved with a 32% decay-corrected radiochemical yield by the separation of the (+)- or (−)-10-camphorsulfonamide diastereomers using preparative LC. It has previously been reported that amphetamine can be resolved analytically using the (+)-10-camphorsulfonamide derivative, where the (−)-amphetamine diastereomer is eluted first.[22] In this paper the use of both the (+)- and (−)-camphor derivatives to achieve a preparative separation of the desired enantiomer is reported. In this way, the order of elution of the diastereomers could be changed so that the required diastereomer was eluted first. Preparative LC fractionation was carried out as indicated in Fig. 2. The collection of larger fractions resulted in contamination by the unwanted antipode. Preparative and analytical LC chromatograms from a typical resolution procedure are presented in Fig. 2. The position of labelling was confirmed by a ^{13}C synthesis using the same reaction pathway as indicated in Scheme 1. Analysis by ^{13}C NMR spectroscopy showed a large peak at 23 ppm corresponding to the methyl carbon from an authentic sample of amphetamine.

In this paper, the decarboxylation of a malonic ester to the monocarboxylic acid is shown to be a useful reaction for use in rapid labelling synthesis. Work is now in progress to synthesise [3-^{11}C]carboxylic acids by this route using various ^{11}C-labelled alkyl halides. The modified Curtius rearrangement using DPPA has been shown to be a fast and efficient method for the transformation of ^{11}C-labelled carboxylic acids into the corresponding amines. This transformation has recently been used in the synthesis of labelled α-methyl amino acids from ^{11}C-substituted dimethylmalonic esters.[6] Here, a selective enzymatic hydrolysis of **1** using pig liver esterase to the corresponding monoester and subsequent conversion into the amine using DPPA was used. Further development of other functional group transformations is in progress. It has now been shown that labelled malonic esters in combination with an array of such reactions are versatile and efficient precursors for use in rapid labelling synthesis.

Experimental

General. The ^{11}C was prepared by means of the ^{14}N(p,α)^{11}C reaction using a nitrogen gas target and 10 MeV protons produced by the tandem Van der Graff accelerator at the Svedberg Laboratory, Uppsala University. The [^{11}C]carbon dioxide obtained was trapped in 4 Å molecular sieves and after transport to the chemistry laboratory, [^{11}C]methyl iodide was prepared by a routine procedure described previously.[23]

Analytical LC was carried out on a Hewlett Packard 1090 liquid chromatograph equipped with a diode array detector (λ = 254 nm) in series with a β$^+$-flow detector.[24] The following columns and mobile phases were used: A, Nucleosil C-18, 250×4.6 mm, 5 μm and B, Suplex pKb-100,[21] 250×4.6 mm, 5 μm; eluents: C, methanol, D, ammonium formate, 0.05 M, pH 3.5, E, acetonitrile/water (50/7 : v/v) and F, potassium diphosphate, 0.1 M, pH 7.02. Preparative HPLC was performed on a Waters M-600 pump with a Waters M-45 fixed wavelength detector (254 nm) in series with G, a 250×10 mm C-18 column (Nucleosil 30 μm) and a β$^+$-flow detector. Analytical GC was carried out on a Hewlett Packard 5880 A gas chromatograph equipped with a FI detector in series with a β$^+$-gas flow detector[25] and a 1 m 1/8″ glass column packed with 20% SE-52 on 110/120 Chromosorb W, with a flow rate of 30 ml min^{-1} (N_2) and a temperature gradient of 100–250 °C, 10 °C min^{-1}. ^{13}C NMR spectra were recorded on a Varian XL-300 NMR spectrometer.

The (+)- and (−)-10-camphorsulfonyl chloride was obtained from Aldrich.

[^{11}C]Methyl iodide. [^{11}C]Carbon dioxide was released from lead-shielded molecular sieves upon heating and transferred in a stream of nitrogen gas to a specially designed one-pot reaction vessel[23] containing a solution of lithium aluminium hydride in THF (0.8 M, 0.5 ml). After evaporation of the THF, hydriodic acid (57%, 2 ml) was added and the reaction mixture refluxed, during which the [^{11}C]methyl iodide formed was distilled over in a stream of nitrogen gas and trapped in the reaction vial containing the substrate.

Dimethyl 2-benzyl-2-([^{11}C]methyl)malonate (**1**). Approximately 15 min before the start of the [^{11}C]methyl iodide synthesis, sodium hydride (0.4 mg, 8 μmol, 50% oil dispersion) was added to a 0.5 ml pear-shaped reaction vessel followed by 100 μl DMSO and 2 mg (8 μmol) dimethyl 2-benzylmalonate and heated at 80 °C. The freshly prepared [^{11}C]methyl iodide (< 0.2 μmol) was then passed into the solution at ambient temperature in a stream of nitrogen gas, **1** being produced almost instantaneously. Analytical LC conditions: column A, mobile phase C/D, 70/30 isocratic, flow 2 ml min^{-1}, retention time 3.2 min.

2-Benzyl-[3-^{11}C]propionic acid (**2**). Sodium hydroxide (100 μl, 10 M) was added to **1** at the end of [^{11}C]methyl iodide trapping, and heated with shaking at 150 °C for 3 min. After addition of 500 μl glacial acetic acid, the vessel was heated and shaken at 150 °C for a further 5 min and the resulting solution was purified by solid-phase extraction. A C-18 Sep-Pak® was pre-conditioned with 2 ml THF followed by 10 ml water. The decarboxylation product was diluted to 10 ml with water, passed through the column, and washed with a further 2×4 ml water. Purified **2** was eluted into a 7 ml screw-cap vessel with 4×300 μl THF (the first fraction was discarded), and the solvent removed by heating and shaking at 150 °C. Analytical LC conditions: column A, mobile phase C/D, 70/30 isocratic, flow 2 ml min^{-1}, retention time 1.6 min.

*(±)-[*Methyl-^{11}*C]amphetamine* (**3**). Toluene (400 μl), diphenylphosphoryl azide[5] (DPPA, 200 μl) and triethylamine (TEA, 100 μl) were placed in a 7 ml flask which was sealed, shaken and heated at 150 °C for 5 min. Hydrolysis of the

isocyanate to **3** was achieved by heating and shaking at 150 °C for 5 min with 1 ml conc HCl in the sealed vessel. The crude product was diluted to 9 ml, made basic with 1 ml 10 M NaOH and passed through a C-18 Sep-Pak® previously conditioned with 2 ml acetonitrile followed by 10 ml water. After being washed with 2×4 ml H_2O the product was eluted with 5×300 µl acetonitrile and diluted to 5 ml with water. The crude product was purified by preparative LC using column G and 30/70 C/D as an isocratic eluent, flow 6 ml min^{-1}. Analytical LC conditions: column B, mobile phase E/F, 10/90 isocratic 0–2 min, 10/90–100/0 gradient 2–10 min, flow 2 ml min^{-1}, retention time 4.6 min. Analytical GC conditions: see general, retention time 2.7 min.

*(S)-(+)-[*Methyl-^{11}*C]amphetamine.* After the final solid-phase purification of racemic **3**, 200 µl 10 M NaOH was added to the eluted fraction in a 7 ml screw-cap vessel. (−)-10-Camphorsulfonyl chloride (60 mg, 240 µmol) and H_2O (500 µl) were added, and the vessel sealed and heated at 80 °C for 5 min. The resulting solution was diluted to 4 ml with the mobile phase and subjected to preparative LC purification using the following conditions: column G, mobile phase 55/45, C/D, flow 6 ml min^{-1}. The fractions were collected as shown in Fig. 2. Retention times for the (*S*)-(+)- and (*R*)-(−)-amphetamine derivatives were 7.7 and 9.0 min, respectively. The relevant fraction was diluted to 20 ml with H_2O and subjected to solid-phase extraction as described earlier. After elution with THF, the solvent was evaporated by heating and shaking at 150 °C in a 7 ml reaction vessel. Hydrolysis to the enantiomerically pure (*S*)-(+)-[*methyl*-^{11}C]amphetamine was carried out with HCl (2 ml, 9 M), with heating and shaking at 150 °C for 4 min. The enantiomeric purity was checked by derivatisation with (−)-10-camphorsulfonyl chloride as described above. Analytical LC conditions: column B, mobile phase C/F, 50/50 isocratic, flow 2 ml min^{-1}. Retention times for the diastereomers of (*S*)-(+)- and (*R*)-(−)-**3** were 10.6 and 11.9 min, respectively.

*(R)-(−)-[*Methyl-^{11}*C]amphetamine.* As above, except that (+)-10-camphorsulfonyl chloride was used instead of the (−)-form, and that the order of elution was reversed. Preparative LC as above; retention times for (*S*)-(+)- and (*R*)-(−)-**3** = 10.9 and 9.8 min, respectively. The enantiomeric purity of the resolved product was established by the LC analysis of the (−)-10-camphorsulfonyl chloride derivative as described above.

*(±)-(*Methyl-^{13}*C)amphetamine.* Dimethyl 2-benzylmalonate (10 mg, 45 µmol) was added to NaH (2 mg, 42 µmol, 50 % oil dispersion) in 100 µl DMSO. The synthesis was carried out with 7 mg (49 µmol) [^{13}C]methyl iodide as described above but with increased reaction times: alkylation (15 min, 80 °C), decarboxylation (NaOH 5 min, 150 °C; glacial acetic acid 10 min, 150 °C), Curtius rearrangement (DPPA/TEA/toluene 15 min, 150 °C) and hydrolysis (conc. HCl 10 min, 150 °C). After preparative LC purification, the relevant fraction was made basic and passed through a pre-conditioned Sep-Pak® cartridge (2 ml $CHCl_3$ followed by 10 ml H_2O). After being washed with 2×4 ml water followed by 500 µl D_2O, the product was eluted with 4×250 µl $CDCl_3$ (the first fraction was discarded). The product was analysed by ^{13}C NMR spectroscopy without further treatment and was compared with the spectrum of an authentic reference sample.

(*Methyl*-^{13}C)amphetamine: ^{13}C NMR (75.5 M Hz, $CDCl_3$): δ 23.3 (CH_3). Amphetamine (reference): ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 23.3 (CH_3), 46.4 (NCH), 48.2 (CH_2), 125.9 (ArCH), 128.2, 129.0 (2×ArCH), 139.4 (ArC).

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