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Current Radiopharmaceuticals, **2017**, *10*, **1-9**

RESEARCH ARTICLE

Automated One-pot Radiosynthesis of [^{11}C]*S*-adenosyl Methionine

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ARTICLE HISTORY

Received: May 23, 2017
Revised: July 05, 2017
Accepted: July 07, 201?

DOI:
10.2174/1874471010666170718171441

Abstract: ***Background*:** Glycine *N*-methyltransferase is an enzyme overexpressed in some neoplastic tissues. It catalyses the methylation of glycine using *S*-adenosyl methionine (SAM or AdoMet) as substrate. SAM is involved in a great variety of biochemical processes, including transmethylation reactions. Thus, [^{11}C]SAM could be used to evaluate transmethylation activity in tumours. The only method reported for [^{11}C]SAM synthesis is an enzymatic process with several limitations. We propose a new chemical method to obtain [^{11}C]SAM, through a one-pot synthesis.

***Method*:** The optimization of [^{11}C]SAM synthesis was carried out in the automated TRACERlab® FX C Pro module. Different labelling conditions were performed varying methylating agent, precursor amount, temperature and reaction time. The compound was purified using a semi-preparative HPLC. Radiochemical stability, lipophilicity and plasma protein binding were evaluated.

***Results*:** The optimum labelling conditions were [^{11}C]CH_3OTf as the methylating agent, 5 mg of precursor dissolved in formic acid at 60 ºC for 1 minute. [^{11}C]SAM was obtained as a diastereomeric mixture. Three batches were produced and quality control was performed according to specifications. [^{11}C]SAM was stable in final formulation and in plasma. Log P_{OCT} obtained for [^{11}C]SAM was (-2,01 ± 0,07) (n=4), and its value for plasma protein binding was low.

***Conclusion*:** A new chemical method to produce [^{11}C]SAM was optimized. The radiotracer was obtained as a diastereomeric mixture with a 53:47 [(*R*,*S*)-isomer: (*S*,*S*)-isomer] ratio. The compound was within the quality control specifications. In vitro stability was verified. This compound is suitable to perform preclinical and clinical evaluations.

Keywords: *S*-adenosyl methionine; Glycine *N*-methyltransferase; ^{11}C[SAM], PET radiotracers, radiochemistry, ^{11}C-labelling, automated synthesis.

1. INTRODUCTION

Molecular imaging by positron emission tomography (PET) is a minimally invasive technique that provides valuable biological information about molecular function and metabolic activity. It constitutes a useful quantitative tool that allows the study of physiological, biochemical and pharmacological processes in living subjects [1-4].

PET/CT plays an important role in the field of oncology. This technique can be useful in the diagnosis and therapy of tumour processes, such as initial localization and staging of tumour tissue, monitoring of the therapeutic response and diagnosis of recurrence [2, 5-7]. In neoplastic tissues, many metabolic pathways are altered [8], including: glucose metabolism, DNA and protein synthesis, amino acid transport, receptor and enzyme expression. Several PET radiotracers have been developed with the purpose of evaluating the functional or molecular changes associated with tumour biology [9, 10]. To achieve this, it is necessary to find a specific target on tumour cells, as well as a radiotracer with high affinity and specificity for this target [11].

It has been reported that glycine N-methyltransferase (GNMT EC 2.1.1.20) levels are increased in prostate cancer progression [12]. This enzyme is implicated in other neoplastic processes [13]. Identifying GNMT ligands that can be labelled with PET radionuclides provides a way to develop new radiotracers for the diagnosis of different tumours.

GNMT catalyses the methylation of glycine to produce sarcosine using *S*-adenosyl methionine (SAM or AdoMet) as substrate. SAM is a natural cofactor involved in a great variety of biochemical process, including transmethylation reactions [14, 15]. It is the main biological donor of methyl groups [16]. In the organism, these methyl groups can be transferred to proteins, lipids, DNA, RNA and low molecular weight metabolites [17, 18]. Tumour accumulation of SAM labelled with ^{11}C in two animal models has been reported: in mice bearing mammary carcinomas and rats bearing ascitic hepatomas. This report showed that [^{11}C]SAM could be a

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useful radiopharmaceutical to evaluate the transmethylation activity in tumours [13].

Currently, the only method reported for [^{11}C]SAM synthesis is an enzymatic process. Ishiwata and colleagues describe the preparation of [^{11}C]SAM using [^{11}C]*L*-methionine, ATP and rat-liver extract as the enzyme source. This is a two-step process, the first one being the synthesis of [^{11}C]*L*-methionine using [^{11}C]CH_3I and *L*-homocysteine thiolactone as precursor. The second one is the enzymatic step, which involves the incubation of [^{11}C]*L*-methionine with ATP and the liver extract, followed by the product purification [19].

The method above described has several limitations: a) the preparation of the enzyme source from rat liver; b) the two-step synthesis ([^{11}C]*L*-methionine preparation prior to the enzymatic reaction); c) the tedious product isolation involving various techniques to denature and precipitate the extract proteins as well as purification and formulation of the final product [20, 21]. In subsequent work, referred to an enzymatic synthesis of non-radiolabelled SAM, the production of the enzyme *L*-methionine-*S*-adenosyl transferase has been improved [22].

To date, no direct method to obtain [^{11}C]SAM has been described. Here, we present a new chemical method for the labelling of SAM with ^{11}C, through a one-pot synthesis. This process is based on the direct [^{11}C]S-methylation of *S*-adenosyl homocysteine (SAH) to obtain the desired product, using the automated platform GE TRACERlab® FX C Pro.

In vitro stability studies were also conducted to characterize [^{11}C]SAM as a prerequisite for future preclinical evaluations. In that sense, the stability of [^{11}C]SAM in the final formulation and in plasma, as well as its plasma protein binding (PPB) and lipophilicity were evaluated.

2. MATERIALS AND METHOD

2.1. Organic Synthesis

2.1.1. General

All chemicals, solvents and reagents were purchased from commercial sources (Merck, Sigma-Aldrich, Carlo Erba and Dorwil). They were analytical grade and were used without further purification. The SAH precursor and the SAM standard were purchased from Sigma-Aldrich.

2.1.2. Synthetic Procedures

2.1.2.1. S-methylation of SAH with CH_3I

The SAM synthesis was performed according to the method reported by Matos *et al.* [17]. SAH (26 μmol) was dissolved in 99% formic acid (283 μL) with magnetic stirring. Methyl iodide (5.4 mmol) was added and the solution was kept in dark at room temperature for 5 days. Reaction samples were drawn and analysed by HPLC using the following conditions: 0.1 M sodium acetate buffer pH 4.5 (mobile phase A); acetonitrile (mobile phase B), elution gradient: isocratically at 4.2% B from 0 to 7 minutes, then a linear gradient from 4.2 to 50% B from 7 to 13 minutes and isocratically at 50% B from 13 to 20 minutes, flow rate was 1.5 mL/min on a C_{18} column [23]. Chromatograms were registered using an UV detector (260 nm). The retention time of the precursor (SAH) was 9.6 minutes, and the product (SAM) eluted with a retention time of 5.6 minutes.

2.1.2.2. S-methylation of SAH with CH_3OTf

The procedure was performed according to the synthesis method for SAM analogues described by Stecher *et al.* [24]. SAH (6.5 μmol) was dissolved in 99% formic acid (500 μL). Silver triflate (AgOTf) (20 μmol) and methyl iodide (0.4 mmol) were added with magnetic stirring. Different reaction mixtures were kept at two temperatures: room temperature for 4 hours and 40 ºC for 1 hour. The reactions were monitored by HPLC using the conditions above described.

2.2. Radiosynthesis

2.2.1. Materials

The Strata XC-SPE cartridges were purchased from Phenomenex. The 0.22 mm sterilizing filters were purchased from Waters. The semipreparative HPLC column was a 250/10 mm Luna 5 μm C_{18} (Phenomenex). The analytical HPLC column was an EC 250/4.6 mm Nucleodur 100-5 C_{18}ec (Macherey-Nagel). The analytical GC column was a DB-WAX 30 m long, 0.53 mm in diameter and with 1.00 mm film thickness (Agilent). The molecular exclusion columns were a MicrospinTM G-50, GE Healthcare. Pooled human plasma, donated by a registered clinical laboratory, was used for physicochemical studies.

2.2.2. Instruments

[^{11}C]CO_2 was produced in a PET Trace® 16.5 MeV cyclotron (GE Healthcare). A high-performance target was used for [^{11}C]CO_2 production. Target content was a mixture of N_2 and 1.0% O_2 (Praxair). Radiosynthesis was carried out using the TRACERlab® FX C Pro module (GE Healthcare). The HPLC analyses were performed with a Shimadzu UFLC equipped with diode array and gamma detectors. The GC analyses of ethanol and residual solvents were done with a Shimadzu GC-2010 Plus equipped with a FID detector. Activity measurements were performed with a dose calibrator (Capintec CRC 25R, CRC 25 PET) or a 3" x 3" well type NaI(Tl) solid scintillation detector coupled to a multichannel analyser from ORTEC. Stability studies were conducted using a Thermo Scientific Sorvall ST 16R centrifuge.

2.2.3. Production of Secondary Precursors [^{11}C]CH_3I and [^{11}C]CH_3OTf

The secondary precursors [^{11}C]CH_3I and [^{11}C]CH_3OTf were produced in the automated TRACERlab® FX C Pro module, according to the method described by Buccino *et al.* [25]. Briefly, the synthetic process began with the production of [^{11}C]CO_2 in the cyclotron via the ^{14}N(p, α)^{11}C nuclear reaction. [^{11}C]CO_2 was delivered from the target to the module, where it was trapped for purification and further reduction to [^{11}C]CH_4. Then, [^{11}C]CH_4 was reacted with elemental iodine to produce [^{11}C]CH_3I in a recirculation loop. [^{11}C]CH_3I could be optionally converted into [^{11}C]CH_3OTf by reaction with silver triflate. [^{11}C]CH_3I or [^{11}C]CH_3OTf were finally bubbled into the reactor loaded with the solvent and the precursor. Trapping of ^{11}C-methylating agents in precursor solution were carried out at different temperatures depending on the solvent melting points, in these cases from -10 to 15 °C.

2.2.4. Radiosynthesis of [^{11}C]SAM

All the fully automated syntheses were carried out in the TRACERlab® FX C Pro module. A variety of labelling conditions of precursor (SAH) were performed in the reactor. During test runs, different amounts of precursor were used (1, 3 or 5 mg), dissolved in formic acid (400 µL). The precursor solution was vigorously mixed using a vortex agitator and the mixture was loaded into the sealed reactor. Then the ^{11}C-methylating agent was bubbled into the precursor solution. In these assays [^{11}C]CH_3I with AgOTf (2.5 mg) or [^{11}C]CH_3OTf have been tested as ^{11}C-methylating agents. Then, the reaction mixture was heated to a range of temperatures (40, 60, 80, 100 or 120 °C) for different reaction times (1, 5, 10 or 30 min). Subsequently, the crude reaction mixture was cooled to 40 °C. To evaluate the presence of the expected ^{11}C-labelled compound and its radiochemical purity, the crude reaction mixture was diluted with analytical mobile phase A (600 µL), and a sample was injected into an analytical HPLC. Chromatographic system conditions for HPLC were: 0.1 M sodium acetate buffer pH 4.5 (mobile phase A); acetonitrile (mobile phase B). The elution was carried out using the following gradient: isocratically at 2% B from 0 to 7 minutes, then a linear gradient from 2 to 50% B from 7 to 13 minutes and isocratically at 50% B from 13 to 15 minutes. The flow rate was 1 mL/min on a C_{18} column. Chromatograms were registered using UV (260 nm) and gamma detectors. The retention time of the precursor (SAH) was 11.8 minutes, and the product [^{11}C]SAM was 5.4 minutes.

2.2.5. Purification and Formulation

The desired ^{11}C-labelled compound from the reactor was purified using a semi-preparative HPLC. After cooling down to 40 ºC, the reaction mixture was diluted by the addition of semi-preparative HPLC eluent (1 mL), and subsequently transferred to the 5 mL injection loop. The injection of the crude reaction mixture to the semi-preparative HPLC system was controlled by an automated fluid detector. The semi-preparative HPLC conditions were: a C_{18} semi-preparative column, 0.1 M sodium acetate buffer pH 4,5: acetonitrile (98:2) as mobile phase with an isocratic flow rate of 6.0 mL/min. The chromatograms were registered using UV (254 nm) and gamma detectors. The fraction containing [^{11}C]SAM was collected and diluted with 40 mL of water for injection. The resulting solution was purified through a Strata XC-SPE cartridge (pre-activated with 1 mL of absolute ethanol followed by 1 mL of 0.1 M sodium acetate buffer pH 4.5). The cartridge was washed with 3 mL of water for injection. The trapped product was eluted with 4 mL of Na_2HPO_4 0.1 M pH 8.5: EtOH (9:1) and formulated in 6 mL of 0.9% NaCl solution. The final solution was transferred to a sterile vial through a 0.22 µm sterilizing filter. The total radiotracer solution volume was 10 mL.

2.2.6. Quality Control

The chemical and radiochemical purity of [^{11}C]SAM was determined by analytical radio-HPLC. The mobile phases consisted of 50 mM ammonium acetate buffer pH 5.4 with 1% TFA (mobile phase A) and acetonitrile (mobile phase B) [26]. The elution was carried out using the following gradient: isocratically at 0% B from 0 to 15 minutes, then a linear gradient from 0 to 100% B from 15 to 20 minutes. The flow rate was 2 mL/min on a C_{18} analytical column. Chromatograms were registered using UV (260 nm) and gamma detectors. The retention time of the precursor (SAH) was 17.6 minutes. The product [^{11}C]SAM was eluted with a retention time of 10.8 minutes for the (*S*,*S*)-isomer and 11.8 minutes for the (*R*,*S*)-isomer. Chemical identity of [^{11}C]SAM was determined by comparison with the unlabelled reference compound. The radiochemical purity was calculated from the portion of the two isomers of [^{11}C]SAM in relation to the total radioactivity. The specific activity was determined by calculating the [^{11}C](*S*,*S*)-SAM radioactivity at the end of synthesis and the amount of this unlabelled compound. The residual solvents (such as acetone, ethanol and acetonitrile) were analysed by gas chromatography (GC) in accordance with the USP standard [27]. The appearance of the solution was checked by visual inspection, and pH was determined using a calibrated pH-meter. Radionuclidic purity was assessed by recording the corresponding gamma spectrum and the radionuclidic identity by measuring the half-life.

2.2.7. Radiochemical Stability of [^{11}C]SAM in the Final Formulation

The radiochemical stability of [^{11}C]SAM was checked in the final formulation at room temperature for 100 minutes. Three samples of [^{11}C]SAM were taken at different incubation times (30, 70 and 100 minutes). The samples were analysed by analytical radio-HPLC using the conditions described for quality control.

2.2.8. Radiochemical Stability of [^{11}C]SAM in Plasma

The radiochemical stability of [^{11}C]SAM was checked in plasma at 37 °C for 60 minutes. Human plasma (1000 µL) was incubated with [^{11}C]SAM (100 µL) at 37 ºC for 60 minutes. Three plasma samples (100 µL) were taken at different incubation times (15, 30 and 60 minutes). The plasma samples were mixed with TFA 25% (100 µL) denature the proteins and the precipitated proteins were removed by centrifugation (2 minutes, 4696 x g, at 4 °C). The supernatants were injected in a radio-HPLC and the radiochemical purity of [^{11}C]SAM was determined according to the method described for [^{11}C]SAM radiosynthesis.

2.2.9. Lipophilicity

The experimental determination of partition coefficient of [^{11}C]SAM was performed in 1-octanol and 0.1 M phosphate buffer at a pH of 7.0. The two phases were presaturated with each other, 1-Octanol (2.0 mL) and phosphate buffer (2.0 mL) were each pipetted into two test tubes. 100 µL of [^{11}C]SAM was added in each tube, the mixture was shaken by vortex for 2 minutes and centrifuged (5 minutes, 4696 x g, at 4°C). After phase separation, three samples (50 - 100 µL) of each phase were taken and measured for radioactivity on a gamma counter. The partition coefficient was calculated using:

$$\log P_{OCT} = \log (\text{counts in octanol} / \text{counts in buffer})$$

2.2.10. Plasma Protein Binding Determination

Molecular exclusion columns were prepared by centrifugation (2 minutes, 2046 x g, at 4 ºC). Human plasma (1000 µL) was incubated with [^{11}C]SAM (100 µL) at 37 ºC for 120 minutes. Plasma samples (50 µL) were applied to the columns at different incubation times (30, 60 and 120 minutes)

and incubated for 2 minutes. The columns were centrifuged (2 minutes, 2046 x g, at 4 ºC) and the eluent collected. Radioactivity of both, columns and eluates, was measured by gamma counter. A blank was carried out at 120 minutes of incubation, replacing plasma for 0.1 M phosphate buffer.

3. RESULTS AND DISCUSSIONS

3.1. Organic Synthesis of SAM

In order to study the chemical reactivity of the SAH-SAM pair, different methylation reactions were performed (Fig. **1**) varying the methylating agent, solvent, precursor amount, temperature and reaction time as shown in Table **1**. In all cases, the reaction was carried out under acidic conditions, in order to protonate the amino and hydroxyl groups and suppress their undesired methylation [20].

Initially, the reactions were carried out employing CH_3I as methylating agent. These conditions are described in Table **1**. The first methylation was performed with 10 mg of precursor in formic acid at room temperature for 5 days (Table **1**, entry 1). In this occasion, SAM was obtained with a % conversion of 39.9%. Conditions similar to those commonly used in the ^{11}C-methylating reactions were also attempted. In these cases, it is usual to employ lower amount of precursor, as well as, polar aprotic organic solvents (such as DMSO, DMF, acetonitrile) to trap the gaseous ^{11}C-methylating agent [1, 28]. Therefore, the precursor amount was decreased to 1 mg and DMF was incorporated to the solvent mixture (Table **1**, entry 2). With this reaction, performed at 40 ºC, it was not possible to obtain the desired product.

Finally, it was decided to test CH_3OTf as methylating agent. The use of this methylating agent has several advantages compared to CH_3I due to its higher reactivity. Generally, methylation reactions with CH_3OTf result in higher yields using shorter reaction times or lower reaction temperatures [1, 28]. For the assays, CH_3OTf was generated *in situ* by the combined addition of AgOTf and CH_3I in the reaction vessel. The methylation was carried out in formic acid at room temperature and 40 ºC (Table **1**, entries 3-4). In both cases, it was possible to obtain SAM with high % conversion (91.4% and 84.9% respectively).

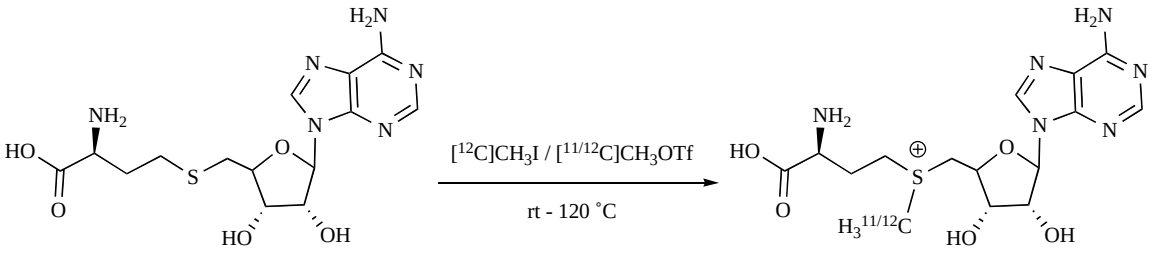
As it is known, "cold" organic methylation conditions are different from those used in the ^{11}C-methylating reactions. However, the above mentioned tests provided valuable information about SAH-SAM reactivity, which was useful in the subsequent radiosynthesis stage.

3.2. Radiosynthesis

3.2.1. Radiosynthesis of [^{11}C]SAM

In order to optimize [^{11}C]SAM synthesis, different labelling conditions were tested. Based on the results obtained above, [^{11}C]CH_3OTf from the module or generated from a mixture of [^{11}C]CH_3I with AgOTf was used as methylating agent, with formic acid employed as the solvent. The parameters evaluated during the optimization process (^{11}C-methylating agent, precursor amount, labelling temperature and reaction time), are summarized in Table **2**.

At first, a mixture of [^{11}C]CH_3I and AgOTf was used to generate [^{11}C]CH_3OTf *in situ*, which was previously tested in the "cold" experiments. However, in this instance the assay was performed with lower amount of precursor because in ^{11}C-methylating reactions the desmethyl precursor is present in a molar excess compared to the methylating agent, the stoichiometrical relation can reach a factor of 10^4 : 1 [28]. So, the reaction was conducted at 40 ºC for 5 minutes employing 1 mg of precursor (Table **2**, entry 1). With this condition it was not possible to obtain the desired product.



(A) (B)

Fig. (1). Organic synthesis and radiosynthesis of SAM. Precursor and product: (**A**) SAH and (**B**) [$^{11/12}$C]SAM).

Table 1. Table of reaction conditions and SAH-SAM conversion for organic synthesis.

Entry	Methylating Agent	Solvent	Precursor Amount (mg)	Reaction Temperature (ºC)	Reaction Time (h)	SAH-SAM Conversion (%) [a]
1	CH_3I [b]	Formic acid	10	rt	120	39.9
2	CH_3I [b]	Formic acid/DMF [c]	1	40	1	0
3	CH_3I/AgOTf [d]	Formic acid	2.5	rt	4	91.4
4	CH_3I/AgOTf [d]	Formic acid	2.5	40	1	84.9

[a] SAH-SAM conversion was determined by HPLC using the conditions described in synthetic procedures section.
[b] Molar excess.
[c] Solvent ratio (1:1).
[d] Methyl iodide used in molar excess and silver triflate in 3 mol. eq. relative to precursor SAH.

Table 2. Table of reaction conditions and [^{11}C]SAM radiochemical purity by radio-HPLC.

Entry	^{11}C-Methylating Agent	Solvent	Precursor Amount (mg)	Reaction Temperature (ºC)	Reaction Time (min)	Radiochemical Purity (%) of [^{11}C]SAM in Reaction Crude
1	[^{11}C]CH_3I/AgOTf	Formic acid	1	40	5	0 [a]
2	[^{11}C]CH_3OTf	Formic acid	1	40	30	9.8 [a]
3	[^{11}C]CH_3OTf	Formic acid	1	60	10	12.5 [b]
4	[^{11}C]CH_3OTf	Formic acid	1	80	10	4.2 [a]
5	[^{11}C]CH_3OTf	Formic acid	1	100	10	2.9 [a]
6	[^{11}C]CH_3OTf	Formic acid	1	120	10	0.5 [a]
7	[^{11}C]CH_3OTf	Formic acid	3	60	10	23.0 [a]
8	[^{11}C]CH_3OTf	Formic acid	5	60	10	34.3 [a]
9	[^{11}C]CH_3OTf	Formic acid	5	60	5	30.7 [a]
10	[^{11}C]CH_3OTf	Formic acid	5	60	1	31.7 [b]

[a] (n=1)
[b] (n=2)

Comparative experiments were then performed using gaseous [^{11}C]CH_3OTf that was supplied by the automated module. The methylations were carried out with different amounts of precursor, temperatures and time lengths (Table **2**, entries 2-10). When 1 mg of precursor was employed, the highest radiochemical purity was obtained at 60 ºC with a 10 min reaction time (Table **2**, entry 3). Above this temperature, a decrease in the radiochemical purity was observed. This fact could be related to the decomposition of the precursor at high temperatures. Using this optimum temperature and timespan, different precursor amounts were tested (1, 3 or 5 mg) and showed that 5 mg gave the best result (Table **2**, entry 8). Subsequently, varying the reaction times tested between 1, 5 and 10 minutes (Table **2**, entries 8-10) gave similar results in terms of radiochemical purity.

In summary, the optimum conditions for the labelling reaction were: [^{11}C]CH_3OTf as the methylating agent, 5 mg of precursor, a labelling temperature of 60 ºC for 1 minute (Table **2**, entry 10). The purity of [^{11}C]SAM in the crude reaction mixture was 31.7%. Analytical radio-HPLC chromatogram of this mixture disclosed a split peak corresponding to the [^{11}C]SAM, (Fig. **2A**). This can be explained by the chiral trivalent sulphur atom obtained in the methylation reaction, which results in the formation of a diastereomeric mixture of [^{11}C]SAM, (Fig. **3**).

Analytical radio-HPLC chromatogram showed, besides the ^{11}C-methylated product, two radiochemical impurities. One of this impurities was the unreacted [^{11}C]CH_3OTf and the other the [^{11}C]CH_3OH (product of [^{11}C]CH_3OTf hydrolysis).

3.2.2. Purification and Formulation

It is reported that the (*S,S*) configuration of SAM is the only enzymatically produced form. This isomer is the only biologically active form for almost all SAM dependent methyltransferases, including GNMT. Nevertheless, there are a few methyltransferases that are capable of recognizing the (*R,S*) form [29-31]. Thus initially the purification step was focused on trying to isolate the (*S,S*) isomer by semi-preparative HPLC. Here, investigations were performed by injecting a standard sample of SAM containing the two isomers while varying the chromatographic column, components of mobile phase and flow rate (Table **3**).

Several chromatographic conditions were tested to isolate the active isomer, however with most of them it was not possible to achieve it. With some conditions, both isomers eluted at the same retention time, showing a single peak in the semi-preparative HPLC chromatogram (Table **3**, entries 1; 4-7; 13-14). Using the chromatographic conditions 2-3; 12, described in Table **3**, it was obtained two overlapping peaks, so that isomers could not be completely isolated.

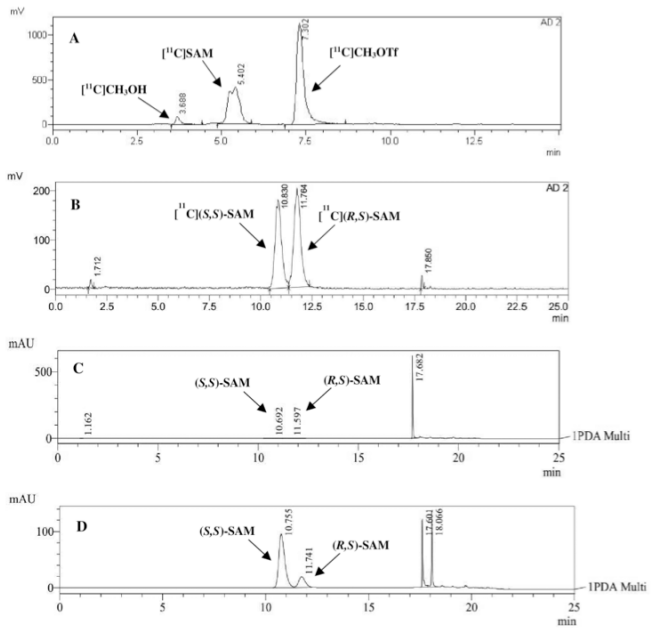
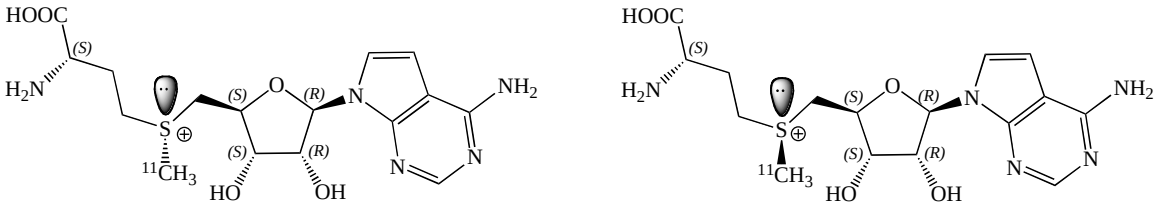


Fig. (2). A. Analytical radio-HPLC chromatogram of [^{11}C]SAM in the reaction crude: **B**. Analytical radio-HPLC chromatogram of [^{11}C]SAM post-purification: **C**. Analytical UV-HPLC chromatogram of [^{11}C]SAM post-purification: **D**. Analytical UV-HPLC chromatogram of SAM standard.



(A) (B)

Fig. (3). [^{11}C]SAM isomers: (**A**) isomer [^{11}C](*R,S*)-SAM and (**B**) isomer [^{11}C](*S,S*)-SAM.

Table 3. Table summarizing the semi-preparative HPLC conditions tested.

Entry	Chromatographic Column	Mobile Phase	Flow (mL/min)	SAM Retention Time, t_R (min)
1	250/10 mm Luna 5 µm C_{18} (Phenomenex)	0.1 M sodium acetate buffer pH 4,5: acetonitrile (98:2)	6.0	(3.4-4.7) single peak
2	250/10 mm Luna 5 µm C_{18} (Phenomenex)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	6.0	(11.0-12.0) split peak
3	250/10 mm Luna 5 µm C_{18} (Phenomenex)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	4.0	(18.0-19.0) split peak
4	100/10 mm SemiPrep RP_{18}e (Chromolith)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	2.0	(8.0-9.0) single peak
5	100/10 mm SemiPrep RP_{18}e (Chromolith)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	4.0	(5.0-6.0) single peak
6	VP 125/10 mm Nucleodur 5 µm C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	4.0	(8.0-9.0) single peak
7	VP 125/10 mm Nucleodur 5 µm C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	2.0	(17.0) single peak
8	VP 250/16 mm Nucleosil 100-7 C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	8.0	(35.0 and 37.5) two peaks
9	VP 250/16 mm Nucleosil 100-7 C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	10.0	(26.0 and 28.0) two peaks
10	VP 250/16 mm Nucleosil 100-7 C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	15.0	(15.0-16.0 and 17.0) two peaks
11	VP 250/16 mm Nucleosil 100-7 C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	17.0	(14.0 and 15.2) two peaks
12	VP 250/10 mm Nucleosil 100-5 C_{18} (Macherey-Nagel)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	6.0	(13.5-14.0) split peak
13	50/3 mm Synergi 4 µm (Phenomenex)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	1.0	(1.2) single peak
14	50/3 mm Synergi 4 µm (Phenomenex)	50 mM ammonium acetate buffer pH 5.4 1% TFA [a]	1.9	(1.9) single peak

[a] [25].

The chromatographic conditions shown in entries 8-11 (Table **3**) resulted in the separation of the isomers, giving two peaks in the semi-preparative HPLC chromatogram. Nevertheless, to achieve this separation extremely high flow rates had to be used. The retention times obtained were incompatible with the short half-life of a ^{11}C-labelled molecule (entries 8 and 9) or not suitable resolution was accomplished (entries 10 and 11). Likewise, conditions 10 and 11 were used to evaluate the isolation of [^{11}C]SAM isomers in the module. In this occasion, it was not possible to achieve a suitable resolution.

As it was not possible to separate the radioactive isomers, subsequent studies were conducted with the product as a mixture of the two diastereomers. So, in order to purify [^{11}C]SAM from the other radiochemical impurities ([^{11}C]CH_3OTf and [^{11}C]CH_3OH), the conditions stated in entry 1 of Table **3** were chosen. These conditions were selected because they allow the product to elute with a suitable retention time using an optimal flow.

Employing the results of all above investigations, a complete synthesis was performed with the best labelling (Table **2**, entry 10) and purification (Table **3**, entry 1) conditions selected. As it is required for any radiopharmaceutical to be injected a formulation step was performed in order to adjust isotonicity and pH [32]. Using the optimum conditions described, [^{11}C]SAM was obtained as a diastereomeric mixture

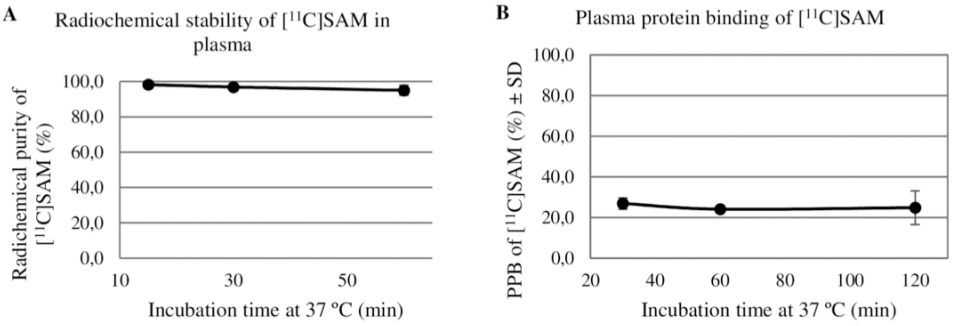


Fig. (4). A. Graph of radiochemical stability of [^{11}C]SAM in plasma against time (n=3) : **B**. Graph of plasma protein binding of [^{11}C]SAM against time (n=3).

Table 4. Specifications and results of quality control assessment for [^{11}C]SAM.

Test	Specifications	Batch 1	Batch 2	Batch 3
Appearance	Clear, colourless and particle free	OK	OK	OK
pH	4.5-8.5	7.5	7.5	7.5
Radiochemical Purity	> 90%	97.4%	98.0%	97.7%
Radiochemical Identity	RT difference of radioactivity and reference peaks ≤ 10%	100	101	98
Residual solvents:				
Acetonitrile	< 0.04%	0.0011%	0.0009%	0.0009%
Acetone	< 0.5%	0.0001%	0.0002%	0.0002%
Ethanol	< 10%	3.8%	4.1%	3.8%
Radionuclidic Identity ($t_{1/2}$)	19.9 – 20.9 min	19.9 min	20.3 min	20.3 min
Specific Activity	> 30 GBq/µmol (EOS)	83 GBq/µmol	75 GBq/µmol	129 GBq/µmol

with a global radiochemical purity of (97,7 ± 0,3)% and an end of synthesis (EOS) radiochemical yield from [^{11}C]CH_3I of (10 ± 2)%, non-decay corrected ((17 ± 4)%, decay corrected), (n=3) (Fig. **4B-D**). (The yield of [^{11}C]CH_3I from [^{11}C]CO_2 in the module was (22 ± 2)%, non-decay corrected, n=3). The process was completed in (28 ± 1) min (from delivery of the [^{11}C]CO_2 to the formulated final product). In the ^{11}C-methylation process the inactive isomer was obtained as the major product in a 53:47 [(*R*,*S*)-isomer: (*S*,*S*)-isomer] ratio. The isomer ratio could be determined through an analytical HPLC method (as described in 2.2.6), which allowed the isomer separation (this separation was possible in the analytical column but not in the semi-preparative column because of the higher resolution of the former).

It was possible to obtain a tracer which fulfilled the quality control specifications. These specifications, as well as the results of quality control assessment for three different [^{11}C]SAM production batches are summarized in Table **4**.

3.2.3. In Vitro Stability Studies

In order to characterize the radiopharmaceutical, the following studies were conducted: final formulation stability, plasma stability, plasma protein binding and lipophilicity. These studies allow to predict the *in vivo* behavior of the labelled compound, thus they constitute a prerequisite for future preclinical evaluations.

The stability studies were carried out by measurement of radiochemical purity over time. [^{11}C]SAM was stable in final formulation for 100 minutes, with a radiochemical purity > 90%. The studies in plasma showed that [^{11}C]SAM was stable at all incubation times. This assay was performed up to 60 minutes, the amount of time required to perform preclinical evaluations such as *in vivo* imaging or biodistribution studies. These results are represented in Figure 4A.

Plasma protein binding of [^{11}C]SAM was performed by gel filtration method. The product showed a low PPB percentage, which was constant over time (Fig. **4B**). This result is desirable since only the unbound molecules are available to cross membranes and penetrate into the cells.

The lipophilicity was determined by the partition coefficient against octanol. The value of log P_{OCT} obtained for [^{11}C]SAM was (-2,01 ± 0,07) (n=4). This value characterized the product as a hydrophilic compound, which is in concordance with its chemical structure.

CONCLUSION

A novel chemical method to produce [^{11}C]SAM, through a one-pot synthesis, was optimized using the commercially available GE TRACERlab® FX C Pro platform. The “cold” organic chemistry trials provided valuable information about chemical reactivity of the SAH-SAM pair, which guided the

selection of the radiosynthesis conditions to be tested. The radiotracer was obtained as a diastereomeric mixture with a 53:47 [(*R*,*S*)-isomer: (*S*,*S*)-isomer] ratio. It was possible to optimize an analytical HPLC method to separate the diastereomers. Nevertheless, this separation was not possible in the semi-preparative HPLC system. The ^{11}C-labelled compound was within the quality control specifications and its stability was verified by in vitro studies. Future preclinical evaluations should be carried out to conclude about the potential of this radiotracer.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

National Research and Innovation Agency (ANII) for a grant to Florencia Zoppolo as research fellowship; PEDECIBA; Andrea Boné for her support in quality control analysis; Pablo Buccino for his collaboration in drafting this paper.

REFERENCES

[1] Li, Z.; Conti, P.S. Radiopharmaceutical chemistry for positron emission tomography. *Adv. Drug Deliv. Rev*, **2010**, *62*, 1031-1051.

[2] Riola-Parada, C.; García-Cañamaque, L.; Pérez-Dueñas, V.; Garcerant-Tafur, M.; Carreras-Deñgado, J.L. Simultaneous PET/MRI *vs*. PET/CT in oncology. A systematic review. *Rev Esp Med Nucl Imagen Mol*, **2016**, *35*(5), 306-312.

[3] Mikla, V.I.; Mikla, V.V. Positron Emission Tomography In: *Medical Imaging Technology*; Elsevier Insights, **2014**; pp. 53-64.

[4] Strauss, L.G.; Dimitrakopoulou-Strauss, A. Positron Emission Tomography (PET) Applications. *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, **2017**, 718-722.

[5] Papathanassiou, D.; Bruna-Muraille, C.; Liehn, J.C.; Nguyen, T.D.; Curé, H. Positron Emission Tomography in oncology: Present and future of PET and PET/CT. *Crit Rev Oncol Hematol*, **2009**, *72*, 239–254.

[6] Wechalekar, K.; Sharma, B.; Cook, G. PET/CT in oncology—a major advance. *Clin Radiol*, **2005**, *60*, 1143-1155.

[7] Sheikhbahaei, S.; Mena, E.; Pattanayak, P.; Taghipour, M.; Solnes, L.B.; Subramaniam, R.M. Molecular Imaging and Precision Medicine PET/Computed Tomography and Therapy Response Assessment in Oncology. *PET Clin*, **2017**, *12*, 105-118.

[8] Naqa, I.E. Perspectives on making big data analytics work for oncology. *Methods*, **2016**, *111*, 32-44.

[9] Vallabhajosula, S. (18)F-Labeled positron emission tomographic radiopharmaceuticals in oncology: an overview of radiochemistry and mechanisms of tumor localization. *Semin Nucl Med*, **2007**, *37*(6), 400-19.

[10] Pantel, A.R.; Mankoff, D.A. Molecular imaging to guide systemic cancer therapy: llustrative examples of PET imaging cancer biomarkers. *Cancer Lett*, **2017**, *387*, 25-31.

[11] Sharma, P.; Kumar, R.; Alavi, A. PET/Computed Tomography Using New Radiopharmaceuticals in Targeted Therapy. *PET Clin*, **2015**, *10*, 495-505.

[12] Song, Y.H.; Shiota, M.; Kuroiwa, K.; Naito, S.; Oda, Y. The important role of glycine *N*-methyl-transferase in the carcinogenesis and progression or prostate cancer. *Mod Pathol*, **2011**, *24*, 1272-1280.

[13] Ishiwata, K.; Ido, T.; Abe, Y.; Matsuzawa, T.; Iwata, R. Tumor Uptake Studies of *S*-Adenosyl-*L*-[methyl-11C]Methionine and *L*-[methyl-11C]Methionine. *Nucl Med Biol*, **1988**, *15*, 123-126.

[14] Krijt, J.; Dutá, A.; Kozich, V. Determination of *S*-Adenosylmethionine and *S*-Adenosylhomocysteineby LC–MS/MSandevaluationof theirstabilityin mice tissues. *J. Chromatogr. B*, **2009**, *877*, 2061-2066.

[15] Clarke, S.; Banfield, K. *S*-Adenosylmethionine-dependent Methyltransferases In: *Homocysteine in Health and Disease*; Carmel, R.; Jacobsen, D.W. Eds.; Cambridge University Press: Cambridge, **2001**; pp. 63-78.

[16] Stabler, S.P.; Allen, R.H. Quantification of Serum and Urinary *S*-Adenosylmethionine and *S*-Adenosylhomocysteine by Stable-Isotope Dilution Liquid Chromatography–Mass Spectrometry. *Clin Chem*, **2004**, *50*(2), 365-372.

[17] Matos, J.R.; Raushel, F.M.; Wong, C.H. *S*-Adenosylmethionine: Studies on Chemical and Enzymatic Synthesis. *Biotechnol Appl Biochem*, **1987**, *9*, 39-52.

[18] Iwig, D.F.; Booker, S.J. Insight into the Polar Reactivity of the Onium Chalcogen Analogues of *S*-Adenosyl-*L*-methionine. *Biochemistry*, **2004**, *43*, 13496-13509.

[19] Ishiwata, K.; Ido, T.; Sato, H.; Iwata, R.; Kawashima, K.; Yanai, K.; Watanuki, S.; Ohtomo, H.; Kogure, K. Simplified enzymatic synthesis and biodistribution of 11C-*S*-adenosyl-*L*-methionine. *Eur J Nucl Med*, **1986**, *11*, 449-452.

[20] Deshpande, P.B.; Senthilkumar, U.P.; Padmanabhan, R. Chemical synthesis of *S*-adenosyl-*L*-methionine with enrichment of (*S*,*S*)-isomer. U.S. Patent 6,881,837 B2, April 19, **2005**.

[21] Senthilkumar, U.P.; Padmanabhan, R.; Sivasankaran, V.; Mohan, S. Improved process for the chemical synthesis of *S*-adenosyl-*L*-methionine with enrichment of (*S*,*S*)-isomer. W.O. Patent 2006/000883 A2, January 5, **2006**.

[22] Iwig, D.F.; Booker, S.J. Insight into the Polar Reactivity of the Onium Chalcogen Analogues of *S*-Adenosyl-*L*-methionine. *Biochemistry*, **2004**, *43*, 13496-13509.

[23] Castro, R.; Struys, E.A.; Jansen, E.E.W.; Blom, H.J.; Tavares de Almeida, I.; Jakobs, C. Quantification of plasma *S*-adenosylmethionine and *S*-adenosylhomocysteine as their fluorescent 1,N6-etheno derivatives: an adaptation of previously described methodology. *J Pharm Biomed Anal*, **2002**, *29*, 963-968.

[24] Stecher, H.; Tengg, M.; Ueberbacher, B.J.; Remler, P.; Schwab, H.; Griengl, H.; Gruber-Khadjawi, M. Biocatalytic Friedel-Crafts Alkylation using Non-natural Cofactors. *Angew. Chem. Int. Ed.*, **2009**, *48*, 9546-9548.

[25] Buccino, P.; Kreimerman, I.; Zirbesegger, K.; Porcal, W.; Savio, E.; Engler, H. Automated radiosynthesis of [11C]*L*-deprenyl-D2 and [11C]D-deprenyl using a commercial platform.. *Appl Radiat Isot*, **2016**, *110*, 47-52.

[26] Zhang, J.; Klinman, J.P. HPLC Separation of the (*S*,*S*)- and (*R*,*S*)-forms of *S*-Adenosyl-*L*-methionine. *Anal Biochem*, **2015**, *476*, 81-83.

[27] U.S. Pharmacopeia-National Formulary [USP 40 NF 35], Rockville, Md: United States Pharmacopeial Convention, Inc; 2017. <467> *General chapter Residual Solvents*; **2017**, Volume 1; pp. 369-384.

[28] Wuest, F.; Berndt, M.; Kniess, T. Carbon-11 Labeling Chemistry Based upon [11C]Methyl Iodide. In: *PET Chemistry*; Schubiger, P.A.; Lehmann, L.; Friebe, M., Ed.; Ernst Schering Research Foundation Workshop: Springer, Berlin, Heidelberg, **2007**; Vol. 64, pp. 183-213.

[29] Vinci, C.R.; Clarke, S.G. Recognition of age-damaged (*R*,*S*)-adenosyl-*L*-methionine by two methyltransferases in the yeast Saccharomyces cerevisiae. *J Biol Chem*, **2007**, *282*, 8604-8612.

[30] Vinci, C.R.; Clarke, S.G. Yeast, plants, worms, and flies use a methyltransferase to metabolize age-damaged (*R,S*)-AdoMet, but what do mammals do? *Rejuvenation Res*, **2010**, *13*, 362-364.

[31] Vinci, C.R.; Clarke, S.G. Homocysteine methyltransferases Mht1 and Sam4 prevent the accumulation of age-damaged (*R,S*)-AdoMet in the yeast *Saccharomyces cerevisiae*. *J Biol Chem*, **2010**, *285*, 20526-20531.

[32] U.S. Pharmacopeia-National Formulary [USP 40 NF 35], Rockville, Md: United States Pharmacopeial Convention, Inc; 2017. <1823> *Positron Emission Tomography Drugs-Information*; **2017**, Volume 1; pp. 2190-2201.

PMID: 28721805