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Radiosynthesis of [^{11}C]ximelagatran via palladium catalyzed [^{11}C]cyanation

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***N*-hydroxyamidines (amidoximes) may be used in prodrug technology in improving oral bioavailability of drugs containing amidino functional groups. In the body, amidoximes are reduced quickly to amidines by enzymes that are present in several organs. Ximelagatran is a benzamidoxime and ethyl ester prodrug of melagatran, which is a thrombin inhibitor. Our aim was to develop a fast and efficient labeling route for the synthesis of [^{11}C]ximelagatran ([^{11}C]3) with a label in a metabolically stable position. [^{11}C]3 was synthesized via a two-step synthesis sequence, starting from palladium catalyzed [^{11}C]cyanation of its corresponding bromide precursor (2-[2-(4-bromo-benzylcarbamoyl)-azetidin-1-yl]-1-cyclohexyl-2-oxo-ethyl amino-acetic acid ethyl ester) (1), followed by a reaction with hydroxylamine. [^{11}C]3 was synthesized with 27 ± 17% total overall decay corrected yield (specific radioactivity of 2360 ± 165 Ci/mmol at EOS), with a total synthesis time of 45 min. A fast and efficient labeling route for the synthesis of [^{11}C]3 was developed.**

Keywords: *N*-hydroxyamidine; amidoxime; serine protease inhibitor; radiosynthesis; PET; ximelagatran

Introduction

N-hydroxyamidine functionalities (amidoximes) may be used in prodrug technology in improving oral bioavailability of drugs containing amidino functional groups.[1–4] Amidino groups are very strong bases and they are protonated under physiological conditions, which causes poor absorption of amidino-based drugs from the gastrointestinal tract. In *N*-hydroxyamidines, an oxygen atom is introduced on one of the nitrogen atoms of the amidino group, which leads to lower pK_a and thus higher lipophilicity, increasing permeability across epithelial cells. In the body, the hydroxyamidine group is reduced quickly to its active amidino moiety by enzymes that are present in several organs.[1]

Amidino groups are a common feature of many serine protease inhibitors.[5] The functional group binds to the enzymatic pocket of the protease, inhibiting binding of the endogenous substrate.[6,7] Many serine proteases, such as thrombin and factors VIIa, IXa, Xa, XIa and XIIa, are involved in the blood coagulation cascade.[8] Serine protease inhibitors targeting especially the inhibition of the function of thrombin have been popular targets in the recent development of novel drugs in the treatment of thromboembolic diseases.[9]

Ximelagatran is a prodrug of an oral direct thrombin inhibitor melagatran.[10,11] Melagatran belongs to the class of serine protease inhibitors with a benzamidine functional group for binding to the arginine binding S1 pocket of thrombin (Figure 1). Melagatran is a peptidomimetic, mimicking the D-Phe-Pro-Arg sequence. The pK_a's of the benzamidine (pK_a = 11.5) and the carboxylic acid (pK_a = 2.0) groups of melagatran cause its zwitterionic character at physiological pH, leading to poor oral bioavailability of the drug.[12] The prodrug ximelagatran (Exanta, AstraZeneca) was designed to overcome this problem by using ester and amidoxime (pK_a 5.2) functionalities in order to avoid ionization of the drug at physiological pH. In the body, after absorption from the gastrointestinal tract, ximelagatran is metabolized quickly in two steps, via hydrolysis of the ethyl ester group and reduction of the hydroxyamidine moiety, to the active drug melagatran. Melagatran itself is eliminated through urine and faeces without further metabolism.[13]

Positron emission tomography is a useful, but still rarely used tool in evaluating the bioavailability of prodrugs *in vivo*.[14] The technique enables simultaneous observation of the biodistribution of the investigated prodrug, metabolized intermediates and the active drug, which provides important information about possible accumulation of the prodrug or the intermediates in non-target organs. The technique also enables analysis of kinetics of the metabolic processes liberating the drug from its prodrug moiety. All this may provide crucial information on *in vivo* behavior of a novel prodrug in early clinical stages.

[^{11}C]Amidination of [^{11}C]nitriles was reported by Siméon *et al.* in the radiosynthesis of [^{11}C](Z,Z)-BABCH.[15] According to our knowledge, [^{11}C](Z,Z)-BABCH is the only ^{11}C-labeled benzamidine reported so far. However, synthesis of [^{11}C]benzamidoximes has not been reported before and our aim was to develop a fast and efficient labeling route for the synthesis of ^{11}C-labeled benzamidoximes with a label in a metabolically stable position. We used ximelagatran as a model compound.

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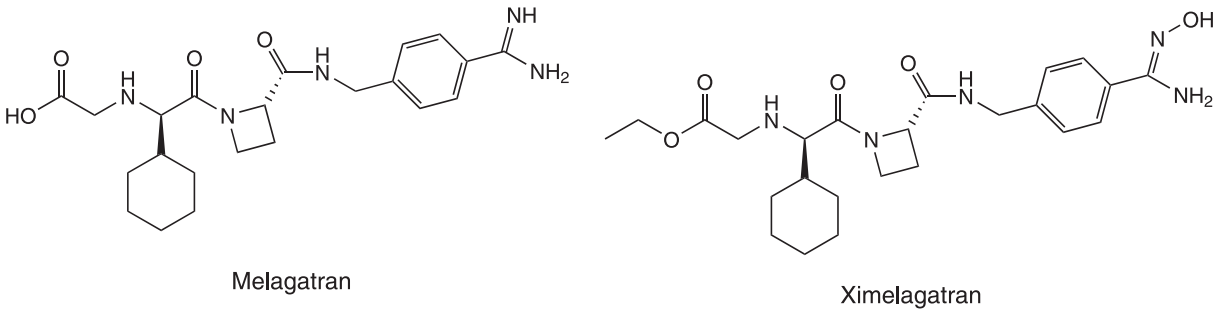


Figure 1. Structures of melagatran and its prodrug ximelagatran.

Results and discussion

Production of [^{11}C]HCN

[^{11}C]Hydrocyanic acid was produced according to previously published methods with minor modifications.[16] In short, [^{11}C]CH_4 was converted online in a platinum oven ($T = 990^{\circ}C$) to [^{11}C]ammonium cyanide ([^{11}C]NH_4CN), which was immediately bubbled through H_2SO_4 to generate [^{11}C]HCN.[17] The [^{11}C]HCN gas was passed through a P_2O_5 column to a solution containing KOH and Kryptofix 2.2.2 in dimethyl sulfoxide (DMSO) to generate K^{11}CN (Figure 2).

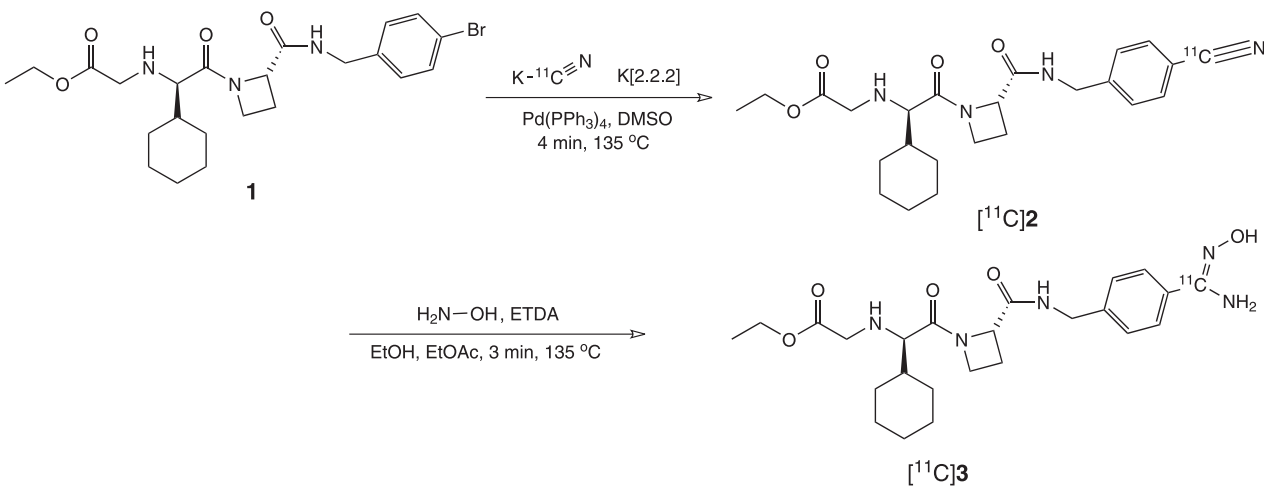
NH_3 concentration is very important for the [^{11}C]HCN formation, and in our system 10–15 vol% was found to be sufficient. A furnace temperature of 990°C gave significantly better conversion (42 ± 17%, $n = 3$) than a temperature of 950°C (11 ± 2%, $n = 2$), which is not in accordance with previously published data.[18] The over-all conversion yields were lower than what has been previously reported. Explanations for the low yields could be the contact time for the gas with platinum or possibly the platinum amount. It has been reported that platinum amount below 3 g reduces the conversion yield linearly.[18] The amount, 1.3 g, which was used in our system, may not be sufficient. Another possible reason is that oxygen or H_2O is interfering with the catalytic reaction of [^{11}C]CH_4 with NH_3 on Pt. Iwata *et al.* reported the effect of absorbers to improve the yield, achieving close to quantitative conversion when metallic sodium coated on quartz wool was used.

Radiosynthesis of [^{11}C]ximelagatran ([^{11}C]3)

For [^{11}C]cyanation of the bromide-precursor (**1**), we considered two synthetic strategies, cyanation via traditional copper catalyzed Rosenmund reaction or via palladium catalyzed reaction.[19,20] We decided to utilize the palladium-catalyzed approach in the radiosynthesis of [^{11}C]**3** (Scheme 1), because in the synthesis of [^{11}C](Z,Z)-BABCH Siméon *et al.* reported a comparison of copper-catalyzed and palladium-catalyzed [^{11}C]cyanations and in their labeling conditions, they found the palladium-catalyzed reaction more efficient.

The K^{11}CN solution, which was produced as described above, was transferred into a vial containing the bromide precursor (**1**, 2-[2-(4-bromo-benzylcarbamoyl)-azetidin-1-yl]-1-cyclohexyl-2-oxo-ethyl amino-acetic acid ethyl ester) and $Pd(PPh_3)_4$ dissolved in DMSO and heated for 4 min at 135°C. The reaction provided the ^{11}CN-intermediate ([^{11}C]**2**) with good incorporation yield, with only small variations between synthesis repetitions (between 70 and 80%) (Figure 3). The high-performance liquid chromatography (HPLC) analysis of the reaction mixture revealed one unidentified radiolabeled side product eluting before the intermediate ([^{11}C]**2**). The intermediate ([^{11}C]**2**) was identified by comparing with the retention time of the corresponding non-labeled analogue (**2**).

In classical organic chemistry, hydroxyamidines are synthesized from nitriles by treatment with hydroxylamine at room temperature. To complete the reaction, reaction times from several hours to days are often needed.[3,21] However, in tracer



Scheme 1

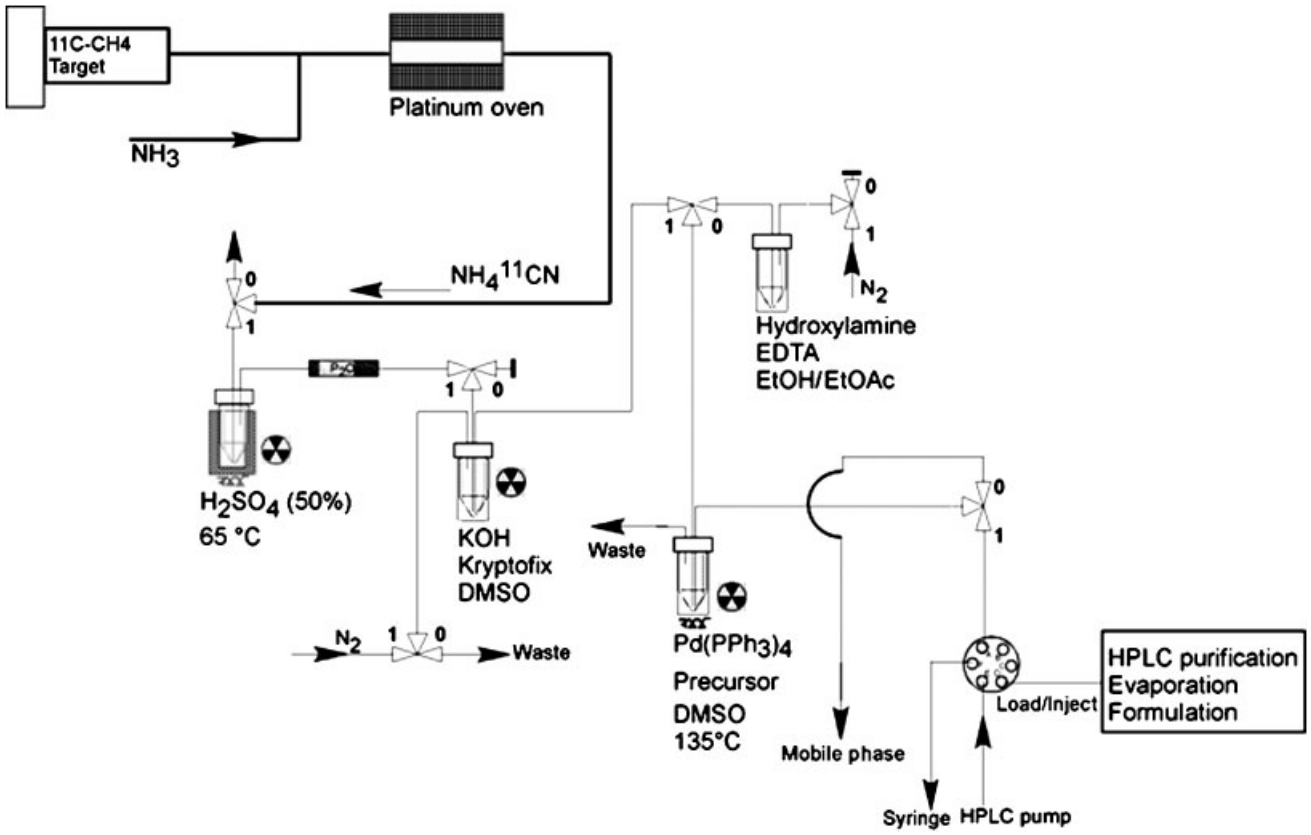


Figure 2. Schematic diagram of the synthesis apparatus for H^{11}CN production and for the synthesis of [^{11}C]**3**.

conditions, the reaction between the ^{11}CN-intermediate (**2**) and hydroxylamine was complete in 3 min at 135°C in a mixture of EtOH and EtOAc in the presence of a small amount of EDTA. Two unidentified radiolabeled side products were observed in the reaction mixture corresponding to 12 and 10% of the total radioactivity (Figure 3). These side products were most probably originating from the previous reaction step, followed by further reaction. No ^{11}CN-intermediate (**2**) was left in the reaction mixture.

The synthesized [^{11}C]**3** was purified with semi-preparative HPLC and evaporated to dryness under reduced pressure (Figure 4). The evaporation residue, containing the product, was dissolved in isotonic phosphate buffer solution (pH 7.4). The total overall yield starting from [^{11}C]CH_4 was 27 ± 16% (decay corrected yield (DCY) at EOS) with specific radioactivity 87 ± 6 GBq/μmol (2360 ± 165 Ci/mmol) in a total synthesis time of 45 min. The relatively high specific radioactivity was achieved due to the in-target produced [^{11}C]CH_4, which was used as a starting material.[22] The difficulties in the H^{11}CN production caused the large variation in the overall yields. Radiochemical purity of the final product was 98 ± 0.7% (Figure 4). The product was identified by co-injection with the corresponding non-labeled ximelagatran.

Experimental

General

All the chemicals were purchased from commercial suppliers and used without further purification. The starting material 2-[2-(4-bromo-benzylcarbamoyl)-azetidin-1-yl]-1-cyclohexyl-2-oxo-ethyl amino-acetic acid ethyl ester (**1**) and the references of 2-[2-(4-cyano-benzylcarbamoyl)-azetidin-1-yl]-1-cyclohexyl-2-oxo-ethyl amino-acetic acid ethyl ester (**2**) and ximelagatran (**3**) were provided by Astrazeneca R&D, Mölndal, Sweden. Radiosynthesis was performed in a homemade semi-automated synthesis module. A carbolite furnace, MTF 10/15, and platinum wire (1.3 g, 0.127 mm, 99.99%) from Sigma-Aldrich were used for [^{11}C]cyanide production.

Semi-preparative HPLC system consisted of Merck-Hitachi L-7100 Pump, L-7400 UV-detector and GM-tube for radioactivity detection. Waters μBondapak C18 column (300 × 7.8 mm, 10 μm) was used, eluted with 30% acetonitrile in HCO_2NH_4 (0.1 M) with a flow of 6 mL/min. A wavelength of 235 nm was used. The analytical HPLC system included Merck-Hitachi L-7100 Pump, L-7400 UV-detector, D-7000 interface and Beckman radiodetector (Model 170). The system was controlled by Merck-Hitachi Chromatography Data Station Software D-7000 (version 4.1). Waters μBondapak C18 column (300 × 3.9 mm, 10 μm) was used, eluted with 35% acetonitrile in HCO_2NH_4 (0.1 M) with a flow of 2 mL/min and the chromatogram was monitored with a wavelength of 235 nm.

Production of [^{11}C]HCN

[^{11}C]CH_4 was produced in-target with a GEMS PETtrace cyclotron using 16.4 MeV protons in the ^{14}N(p,α)^{11}C reaction on N_2(g) and 10% H_2(g). The target gas was irradiated for 20 min with a beam intensity of 35 μA. NH_3 gas (flow of 20–30 mL/min) was added to the produced [^{11}C]CH_4 (flow of 200 mL/min) and the mixture was passed through a carbolite furnace containing a quartz tube containing a heated platinum wire (990°C). The produced

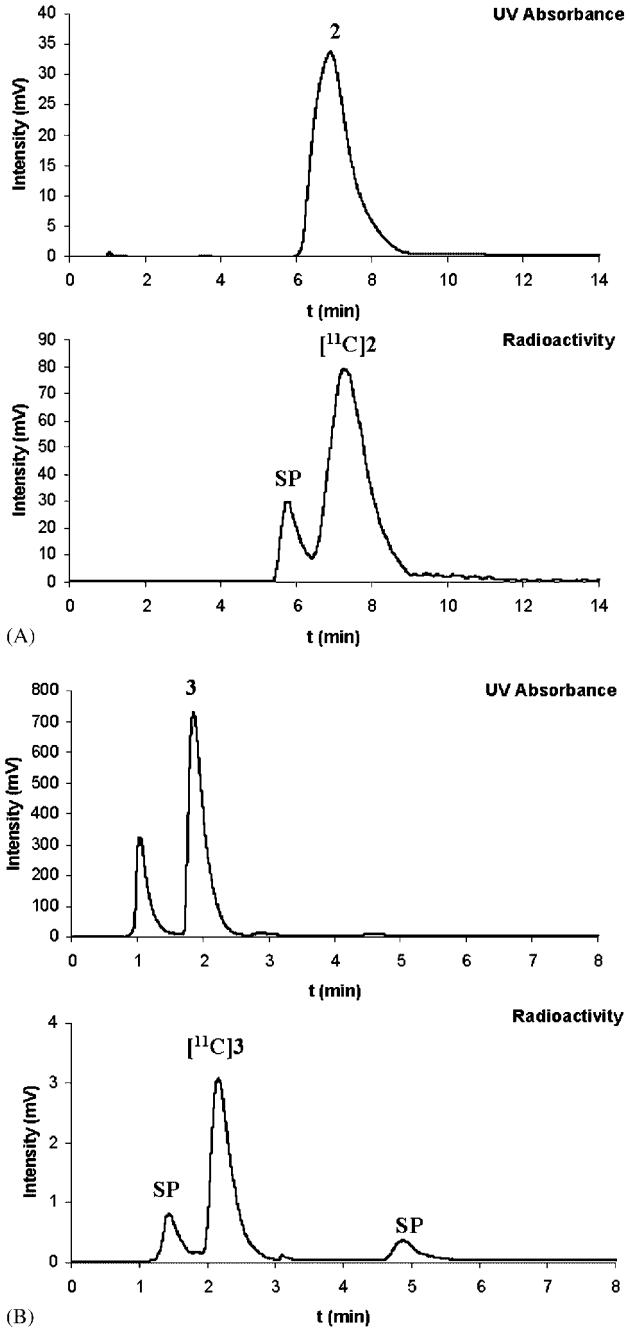


Figure 3. HPLC chromatograms of (A) the reaction mixture after cyanation co-injected with unlabeled reference **2**, R_t([^{11}C]**2**) = 7.2 min (analytical μBondapak C18, 20% acetonitrile, 80% H_3PO_4 (0.01 M), flow of 3 mL/min) and (B) reaction mixture after reaction with hydroxylamine co-injected with ximelagatran reference (**3**), R_t([^{11}C]**3**) = 2.2 min (analytical μBondapak C18, 5% acetonitrile, 95% H_3PO_4 (0.01 M), flow of 3 mL/min). SP = radiolabeled un-identified side product.

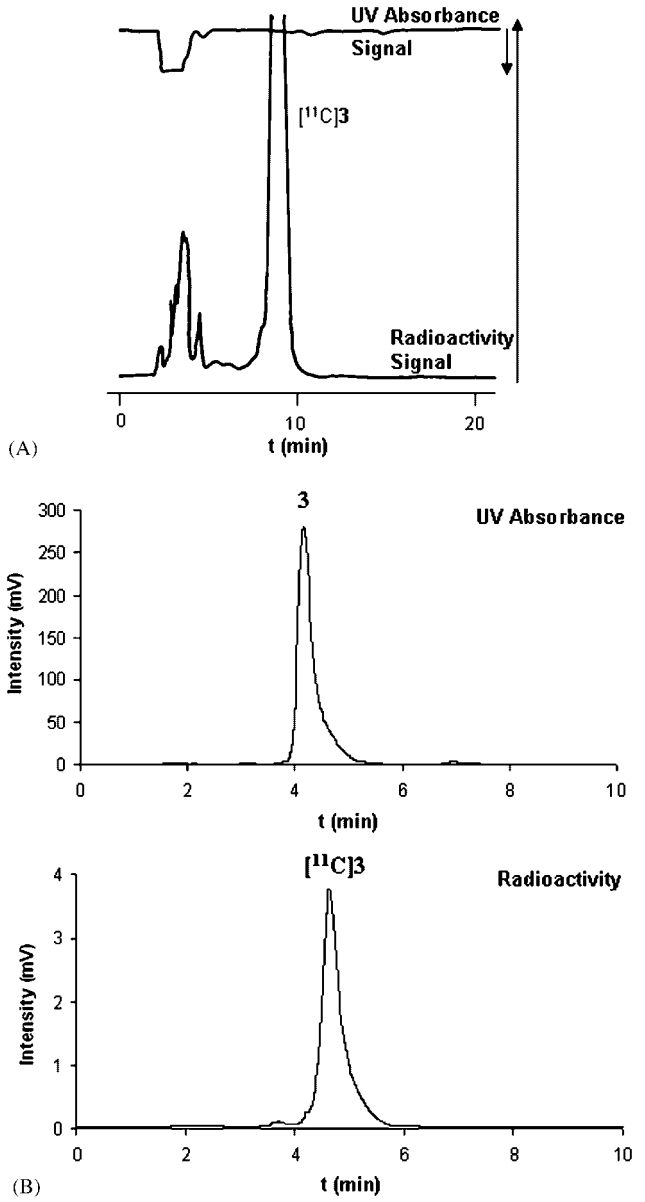


Figure 4. HPLC chromatograms of (A) the semi-preparative purification, R_t([^{11}C]**3**) = 9.5 min (semi-prep HPLC conditions) and (B) the purified [^{11}C]**3**, co-injected with ximelagatran reference (**3**) , R_t([^{11}C]**3**) = 4.7 min (analytical HPLC conditions).

[^{11}C]NH_4CN was bubbled through H_2SO_4 (50%, 2 mL) at 65°C to generate [^{11}C]HCN.

Synthesis of [^{11}C]3

[^{11}C]HCN gas was directed through a P_2O_5 column to a solution of powdered KOH (1.0 mg, 17.8 μmol) and Kryptofix 2.2.2 (5.0 mg, 13.3 μmol) in DMSO (400 μL). The K^{11}CN/Kryptofix solution was transferred into a vial containing the bromide precursor (**1**, 2-[2-(4-bromo-benzylcarbamoyl)-azetidin-1-yl]-1-cyclohexyl-2-oxo-ethyl amino-acetic acid ethyl ester, 4.0 mg, 8.1 μmol) and $Pd(PPh_3)_4$ (5.5 mg, 4.8 μmol) dissolved in DMSO (400 μL). The reaction mixture was heated for 4 min at 135°C. The reaction vial was cooled to room temperature and a mixture of EDTA (1.0 mg, 3.4 μmol), 50% wt hydroxylamine in H_2O (100 μL, 1.63 mmol), EtOH (180 μL) and ethylacetate (100 μL) was added. The reaction mixture was heated at 135°C for 3 min, cooled to 40°C after which 800 μL of the mobile phase was added and the diluted reaction mixture was injected into the semi-preparative HPLC. The fraction, which contained the product (R_t = 9.5 min), was evaporated to dryness under

reduced pressure and dissolved into phosphate buffer solution, pH 7.4.

Conclusion

A fast and efficient method for synthesizing [^{11}C]**3** was developed. [^{11}C]**3** was synthesized with good yield (27 ± 17%, total overall DCY), giving 1.75 ± 1.0 GBq (47 ± 27 mCi) of radiochemically pure compound (> 97%) with a total synthesis time of 45 min and relatively high specific radioactivity. The developed labeling procedure has a general utility to be also used in ^{11}C-labeling of other benzamidoximes.

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