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Divergent Synthesis of Organophosphate [^{11}C]VX- and [^{11}C]Sarin-Surrogates from a Common Set of Starting Materials

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Abstract

Radiolabeled 1-[^{11}C]ethyl, 4-nitrophenyl methylphosphonate (VX-surrogate) and 2-[^{11}C]-propanyl, 4-nitrophenyl methylphosphonate (sarin surrogate) were developed as organophosphate (OP) tracers. The [^{11}C]ethyl- and [^{11}C]isopropyl-iodide radiolabeled synthons were obtained by temperature controlled, in loop reactions of [^{11} C]CO_2 with MeMgBr followed by reduction with $LiAlH_4$, then reaction with HI. Distillation of the [^{11}C]alkyl iodides into a solution of hydrogen (4-nitrophenyl)methylphosphonate and cesium carbonate afforded the desired tracers in > 95% radiochemical purity, yields from [^{11}C]CO_2 of 1-3% and 1.7-15.1 GBq/mmol molar activities.

Keywords

Organophosphate; [^{11}C]sarin surrogate; [^{11}C]VX surrogate; acetylcholinesterase; [^{11}C]ethyl iodide; [^{11}C]isopropyl iodide

1. Introduction

Organophosphate (OP) compounds are a class of chemicals which were initially developed as insecticides. Their novel mechanism of toxicity and rapid effect, however, led to their further development into chemical warfare agents (CWAs). CWAs such as the V- (VX) and G-agents (sarin, soman) are non-discriminating neuropoisons that pose a threat to both military and civilian populations (Fig. 1). Toxicity in humans following OP exposure occurs mainly through the inactivation of acetylcholinesterase (AChE), the enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh), which is responsible for proper function of muscle stimulation. At high exposure levels, inhibition of available AChE pools in the CNS causes a

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rapid increase in ACh and triggers neurotoxic sequelae (Taylor, 2018). If administered soon after exposure, OP toxicity can be ameliorated using antidote combinations such as 2-pyridine aldoxime methyl iodide (2-PAM) and atropine (Fukuto, 1990; Sidell and Borak, 1992; Taylor, 2018; Voicu et al., 2010).

The mechanism of inhibition of AChE by an OP occurs through interaction with a serine residue in the active site concomitant with loss of a leaving group (Fig. 2). The resultant OP-AChE adduct is relatively stable leading to prolonged stimulation of post-synaptic receptors by ACh. The OP-AChE adduct can undergo reactivation by oximes (e.g., 2-PAM) that restores the enzyme activity or participate in a process called 'aging,' which results from phosphoester dealkylation and forms a methyl phosphonate anion permanently inactivating AChE. The stability (or lack thereof) of the OP-AChE adduct is dictated by the phosphoester group with the sarin-AChE adduct bearing an isopropyl ester undergoing aging in minutes, whereas the VX-AChE adduct bearing an ethyl ester ages over hours yet both result in the same OP-AChE adduct after the aging process. Due to this unique stepwise loss of the initial leaving group and possible aging of an esteratic moiety, placement of the isotopic label is critical to measure intact OP-AChE adduct or aged moiety.

Native ^{11}C-sarin was previously prepared (Prenant and Crouzel, 1990) but biological data was not reported, in part, due to the extreme toxicity and handling concerns with OP CWA agents. Recenetly, researchers have turned to the OP 'surrogates' (Chao et al., 2016; Chao et al., 2018; Fukuto and Metcalf, 1959; Meek et al., 2012) in which a *p*-nitrophenoxy (PNP) replaces the reactive fluorine or thiocholine ester leaving group of a CWA. PNP is an excellent leaving group (pKa ~ 7.2) and is present on the potent anti-AChE molecule paraoxon. It confers safer handling due to low volatility and adds a chromophore that is important for analysis. The mechanism of inhibition of PNP-substituted surrogates was recently validated for the VX-surrogate in nonradioactive (cold) (Chao et al., 2016; Chao et al., 2018) and [^{18}F]-tracer (James et al., 2014) forms.

Although the interactions of ^{18}F-VX surrogate in wild-type rats showed rapid equilibration in body tissues (James et al., 2014) and retained the desired mechanism of AChE inactivation, in some instances [^{11}C]tracers can be an inherently better alternative for PET imaging, as these tracers would not introduce a second non-native chemical moiety to the OP surrogates. Integration of the carbon-11 into the phosphoester moiety of the OP would result in a viable PET OP imaging agent as this group is retained upon phosphylation of AChE.

With the goal of preparing new ^{11}C-labeled PET tracers for evaluating the exposure mechanism of OP agents and reactivation therapies, we sought a radiosynthetic route to produce [^{11}C]VX and [^{11}C]sarin surrogates. As indicated, OP surrogates differ from the parent compounds by a PNP leaving group. The resultant [^{11}C]OP-surrogates, namely, 1-[^{11}C]ethyl, 4-nitrophenyl methylphosphonate ([^{11}C]2) and 2-[^{11}C]propan-yl, 4-nitrophenyl methylphosphonate ([^{11}C]3) have been reported to possess similar properties of inhibition, reactivation and adduct formulation as the parent VX and sarin compounds (Meek et al., 2012). We present the efficient radiosyntheses and purification protocols of [^{11}C]2 and [^{11}C]3 PET tracers for utilizing a common set of starting reagents.

2. Experimental

Materials.

All reagents and solvents (purchased and used as anhydrous) were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification unless otherwise noted. MeMgBr in diethyl ehter (3M), diethyl ether, and $LiAlH_4$ in THF (1M) were obtained in Sure/Seal™ containers from Sigma-Aldrich. Containers were stored in a dessicator between uses and were used up to 10 times before loss of molar activity was noted. 4-Nitrophenyl hydrogen methylphosphonate **1**, VX surrogate **2** and sarin surrogate **3** were synthesized by literature procedures (Chao et al., 2016; Chao et al., 2018; Tawfik et al., 1993). The loop and valve used in this experiment were cleaned after each run by sequential washing with 10% citric acid (10 mL), water (10 mL) and acetone (10 mL), the system was then dried under a continuous flow of nitrogen for 20 minutes. Nitrogen flow was initiated 20 minutes prior to the start of synthesis to eliminate endogenous CO_2.

Analytical HPLC was performed using a Waters 590 LC pump (Midland, MA), connected in series to a Shimadzu SPD-UV-Visible detector (Columbia, MD) (λ = 254 nm) and a gamma counting in-line radiation flow detector (Model 105a, CRA; Berkeley, CA) with a Phenomenex Luna® 5μm C-18(2) 100 Å 250 × 4.6 mm LC column using a isocratic system of 40% acetonitrile in 60% 5 mM pH 6.8 phosphate buffer. Semi-preparative purification of tracer was performed using a Waters 600 LC pump (Milford, MA) connected in series to a Shimadzu SPD-UV-Visible detector (Columbia, MD) (λ = 254 nm) and a gamma counting in-line radiation flow detector (Model 105a, CRA; Berkeley, CA) with a Phenomenex Luna® 10μm C-18(2) 100 Å 250 × 10 semi-preparative HPLC column using an isocratic solvent system of 50% acetonitrile in 5 mM pH 6.8 phosphate buffer. HPLC chromatograms were acquired using SRI PeakSimple software (version 304 – Torrence, CA).

Radiosynthesis of 1-[^{11}C]ethyl, 4-nitrophenyl methylphosphonate ([^{11}C]2).

[^{11}C]CO_2 was cryotrapped in an aluminum tube charged with glass wool, submerged in liquid nitrogen. While the [^{11}C]CO_2 is being trapped, a Teflon tube (40 cm, 0.030” ID) at 0 °C was coated with 1 mL of 1M MeMgBr in ether (diluted with anhydrous ether from commercially available 3.0 M MeMgBr in ether). Nitrogen gas was used to lightly blow dry the ether (15-30 sec) and then the [^{11}C]CO_2 was delivered through the MeMgBr coated loop at a flow rate of 5 mL/min with nitrogen as the carrier gas while the loop was maintained at 0 °C. Once the activity had been transferred, the nitrogen flow was stopped and the loop was left at 0 °C for 90 sec. The activity was eluted off the loop with 0.2 M $LiAlH_4$ (1 mL) in THF. The solvent was then removed under a flow of nitrogen at 100 °C, and concentrated HI (300 μL) was added at 0 °C. The reaction was then heated to 180 °C and distilled through P_2O_5 and ascarite traps in series into a vial containing **1** (5 mg, 23 μmol) with Cs_2CO_3 (9-11 mg, 27-34 μmol) in DMF (300 μL). After approximately 4 min the distillation was ended and the reaction vial was heated to 115 °C for 5 min. The mixture was then cooled to room temperature, diluted with 20% acetonitrile in 0.1% ascorbic acid in water, and injected onto a semi-prep HPLC column (C18(2) 250 × 10 mm, 50:50 acetonitrile/0.1% ascorbic acid, 6 mL/min, 254 nm) for purification. The desired peak was collected (t_R = 5.2 min), diluted to 30 mL with 10 mM pH 6.8 phosphate buffer and loaded onto a C-18 light Sep-Pak. The Sep-

Pak was washed with an addition 5 mL of phosphate buffer and eluted with 500 μL acetonitrile. The acetonitrile was concentrated to 100 μL and reconstituted to give a solution of 10% acetonitrile in 10 mM pH 6.8 phosphate buffered saline. Molar activity was accessed using analytical HPLC on a Luna C18(2) column (250 × 2.6 mm, 40% acetonitrile/60% 5 mM pH 6.8 phosphate buffer, 1 mL/min, 254 nm). This process yielded **[^{11}C]2** in 2.8 % ± 1.5% decay corrected yield (EOB; 45 min) from [^{11}C]CO_2 with a molar activity of 1.7 ±0.6 GBq/μmol (42 ± 16 Ci/mmol, n = 6, 55 min).

Radiosynthesis of 2-[^{11}C]propyl (4-nitrophenyl)methylphosphonate ([^{11}C]3).

The exact same procedure as **[^{11}C]2** was used with the following exceptions: once the [^{11}C]CO_2 had been transferred into the MeMgBr coated loop, the nitrogen flow was stopped and the loop was warmed to 25 °C for 5 min. The reaction was loaded on the HPLC as described above. The desired peak was collected (t_R = 6.1 min), diluted to 30 mL with 10 mM pH 6.8 phosphate buffer and loaded onto a C-18 light Sep-Pak. The Sep-Pak was washed with an addition 5 mL of phosphate buffer and eluted with 500 μL acetonitrile. The acetonitrile was concentrated to 100 μL and reconstituted to give a solution of 10% acetonitrile in 10 mM pH 6.8 phosphate buffered saline. Molar activity was accessed using analytical HPLC on a Luna C18(2) column (250 × 2.6 mm, 40% acetonitrile/60% 5 mM pH 6.8 phosphate buffer, 1 mL/min, 254 nm). This process yielded **[^{n}C]3** in 1.04 % ± 0.96% decay corrected yield (EOB; 50 min) from [^{11}C]CO_2 with a molar activity of 15.1 ± 6.9 GBq/μmol (409 ± 187 Ci/mmol, n = 6, 60 min).

3. Results and Discussion

Initially, a transesterification reaction between [^{11}C]-labeled alcohols and *bis-(p*-nitrophenyl) methylphosphonate (Tawfik et al., 1993) was attempted to prepare the labeled surrogates of VX and sarin. However, production of the corresponding alcohols required a strong reducing agent and workup with water that caused hydrolysis of the product due to surplus Cs_2CO_3 present. Due to this issue, a different approach was needed to yield the desired [^{11}C]OP surrogates. One alternate approach was to react alkyl iodides with a phosphate oxyanion to introduce a labeled ester group rather than couple to an alcohol. The labeled surrogates could then be accessed via the readily available methyl phosphonic acid precursor **1** (Fig. 3). In general, while [^{11}C]methyl iodide is a common precursor for carbon-11 syntheses, there are very few reliable methods for the production of [^{11}C]ethyl iodide and [^{11}C]isopropyl iodide.(Långström et al., 1986; Zhang et al., 2006) Therefore, an investigation was undertaken to achieve these important labeled synthons.

In a previous report microfluidics was used to produce [^{11}C]labeled ethyl and isopropyl iodide (Zhang et al., 2006). This was achieved through temperature control of a loop coated with MeMgBr. Low temperatures (−11 °C) favored mono-addition of Grignard reagent to yield the ethyl derivative, while warming the loop (25 °C) afforded a second addition of Grignard to form the isopropyl product. This temperature-dependent approach was adapted for use with a loop setup by employing a six-way Rheodyne 7720 valve (Fig. 4). The loop was attached to allow flow either from a syringe port or from the [^{11}C]CO_2 feed line from the cyclotron. A positive pressure of nitrogen was initially applied through both of these

lines to minimize contamination by cold CO_2 gas. Temperature of the loop was controlled using either an ice or warm water bath, depending on the desired reaction temperature. Temperature control, as in the previous work, was essential for production of one or the other desired alkyl iodides.

Selection of the mono- and bis-addition of MeMgBr was controlled through changes in temperature and reaction time. Mono-addition of the Grignard reagent was performed by first coating the loop with MeMgBr at 0 °C. Flow of [^{11}C]CO_2 in nitrogen carrier was used to load the loop. The reaction was held at 0 °C for 90 s. Subsequent rinsing of the loop with a THF solution of $LiAlH_4$ gave [^{11}C]ethanol that was converted into the major product [^{11}C]ethyl iodide by heating with HI. Initial Grignard reactions were conducted at −11 °C. However, when the temperature was raised from −11 °C to 0 °C, to simplify the reaction setup, double Grignard addition occurred, albeit [^{11}C]ethyl iodide still predominated as the primary product at this temperature. [^{11}C]Isopropyl iodide was selectively produced via the same general method as [^{11}C]ethyl iodide with two minor process changes. Following the transfer of the [^{11}C]CO_2 to the loop, the loop was moved into a 25 °C water bath and reacted for 5 min rather than 90 s. The higher temperature and longer time enabled more [^{11}C]isopropanol to be produced versus [^{11}C]ethanol. HPLC analysis (Fig. 5), of the iodides, collected by distillation, confirmed that the increase in reaction temperature favored the formation of the isopropyl product, however, significant amounts of methyl, ethyl and isopropyl iodides were seen in each reaction mixture.

Reaction of the [^{11}C]ethyl- or [^{11}C]isopropyl iodide preparation with **1** and Cs_2CO_3 under basic conditions gave a mixture of **[^{11}C]2** and **[^{11}C]3** along with labeled byproducts that may include the [^{11}C]alkyl alcohols and labeled hydrolyzed OP. Neutralization of the reactions with NaH_2PO_4 and injection onto the HPLC was carried out rapidly to reduce hydrolysis of the product in the basic reaction solution. Semi-preparative HPLC (Fig. 5) afforded the purified [^{11}C]VX or [^{11}C]sarin surrogates, **[^{11}C]2** and **[^{11}C]3**, respectively. The purified products were concentrated by solid phase extraction on a C-18 light Sep-Pak. Elution with acetonitrile, concentration and dilution with phosphate buffered saline gave the 10% acetonitrile/ PBS formulation that was used in preclinical studies. Final decay corrected yields for **[^{11}C]2** and **[^{11}C]3** were 2.8 % ± 1.5% and 1.04 ± 0.96% with molar activities of 1.7 ±0.6 GBq/μmol and 15.1 ± 6.9 GBq/μmol, respectively. Despite suitable quantities of the [^{11}C]ethyl- or [^{11}C]isopropyl iodide the low yields of the final products were believed to be a function of the reaction of the iodides with the OP intermediate and solution stability of the OP product once formed. It is interesting to note that inspite of the increase in isopropyl iodide formation in the warm loop reaction, **[^{11}C]2** appears to dominate in the OP coupling reaction. This may be due to the lower stability of the isopropyl OP surrogates, as has been shown previously.(Hayes et al., 2018) While iodide optimization was not undertaken in this study, there may be efficiencies and yield gained by reducing the time necessary to conduct the synthetic manipulations, adjusting the loop temperature to 0 °C or 25 °C at the start of the desired iodide synthesis, adjusting precursor and reagent quantities, or considering alternate loop materials and sizes. (Matarrese et al., 2002; Wadsak et al., 2007)

Lower molar activities of **[^{11}C]2** and **[^{11}C]3** were likely due to presence of cold CO_2 introduced that was present in the reactor system, or while diluting the MeMgBr to the

desired concentration. Thus, care should be taken during the setup of the reaction to minimize the introduction of exogenous CO_2. Dilution of solutions under inert atmosphere would be desirable and reduce atmospheric CO_2 exposure. In the current process the syringe port is opened several times to atmosphere. Intoducing multiple port valve attached to the syringe port could allow introduction of the precursors and reagents without introducing CO_2. These improvements may increase the molar activity. The [^{11}C]VX and [^{11}C]Sarin surrogates interact with the high capacity acetylcholinesterase enzyme system so the reported molar activities are adequate for initial the imaging studies.

Importantly, the radiosynthetic method is able to provide both [^{11}C]ethyl- and [^{11}C]isopropyl-iodides using the same reagents and by the same reaction, whereby the loop reaction using cooler temperature and shorter reaction time favors the formation of [^{11}C]ethanol, and higher temperature and longer reaction time favors [^{11}C]isopropanol preparation that are subsequently transformed to their respective iodide forms.

4. Conclusions

New organophosphate carbon-11 labeled surrogates of VX and sarin were prepared. To achieve this, a loop synthesis method was developed for the production of both [^{11}C]ethyl and [^{11}C]isopropyl iodides using the same reagents. The selective formation of one radiolabeled alkyl iodide fragment over the other was effectively achieved through control of reaction temperature and time. Sufficient amounts of the radiolabeled VX and sarin surrogates tracers **[^{11}C]2** and **[^{11}C]3**, respectively tracers from production are obtained to enable testing of biological properties. The divergent radiolabeling method described in this paper for the production of [^{11}C]ethyl and [^{11}C]isopropyl iodide is considered generally useful for application to the formation of a broad spectrum of carbon-11 PET tracers.

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Highlights

- Loop preparation of [^{11}C]Et-I and [^{11}C]iPr-I from [^{11}C]CO_2 and common reagents was developed.
- Increase in Grignard temperature/time shifted the intermediate from [^{11}C]EtI to [^{11}C]iPrI.
- [^{11}C]alkyl iodide reaction with (4-NO_2PhO)(Me)P(O)O^-Cs^+ affords VX and sarin OP surrogates.

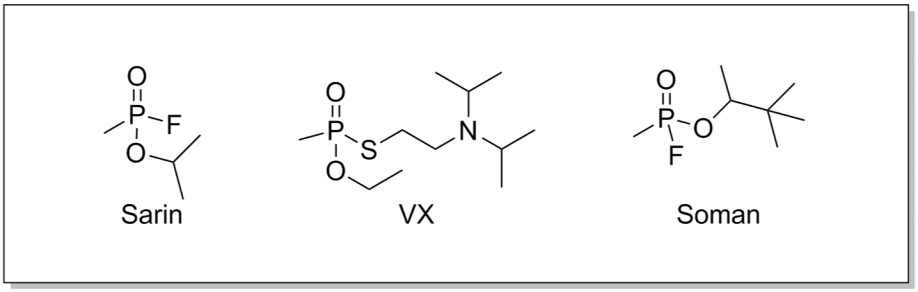

Fig. 1.
Structures of common chemical warfare agents.

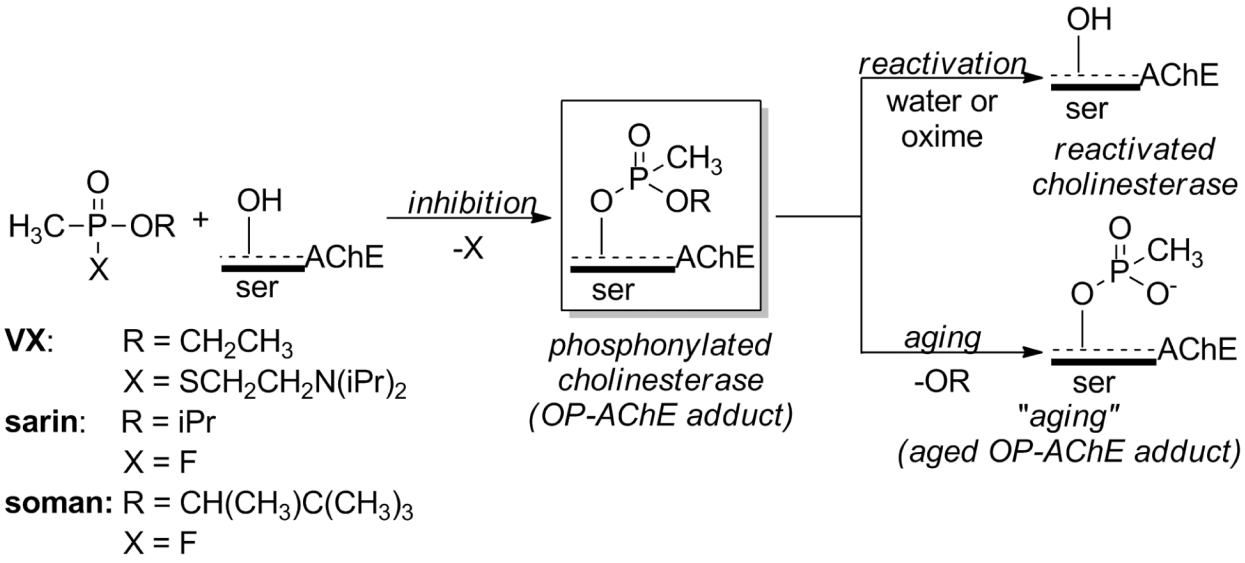

Fig. 2.
Inhibition of acetylcholinesterase by OP CWAs and the aging or reactivation processes.

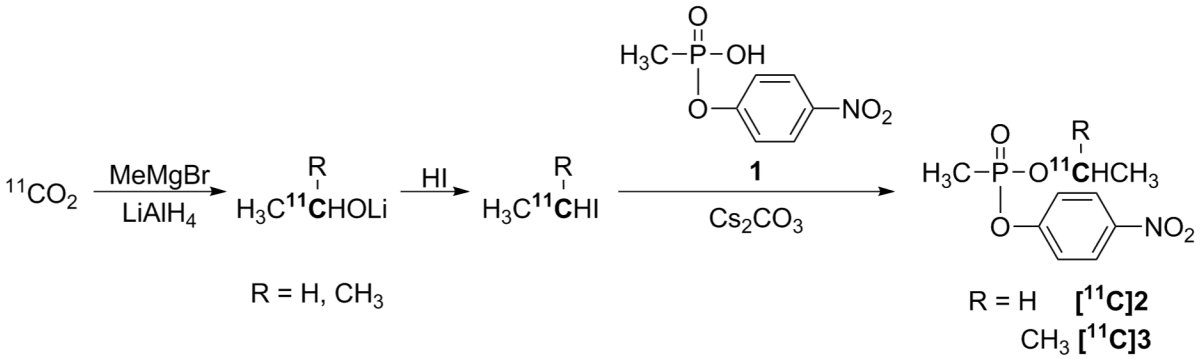

Fig. 3.
Synthesis of PET tracers **[^{11}C]2** and **[^{11}C]3**.

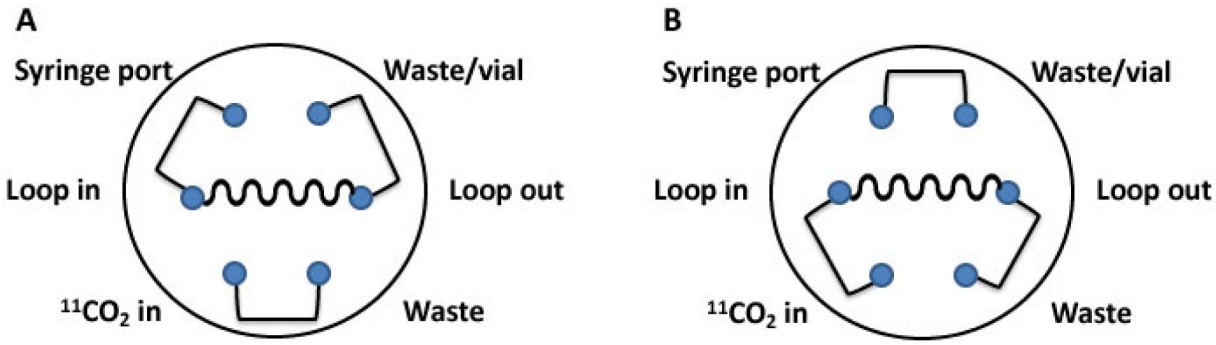

Fig. 4.

Schematic for the HPLC injection port used in the loop synthesis of [^{11}C]ethyl iodide and [^{11}C]isopropyl iodide. A. Configuration for the introduction of the MeMgBr reagent and release of the ^{11}C labeled compound. B. Configuration for the introduction of the $^{11}CO_2$ from the cyclotron.

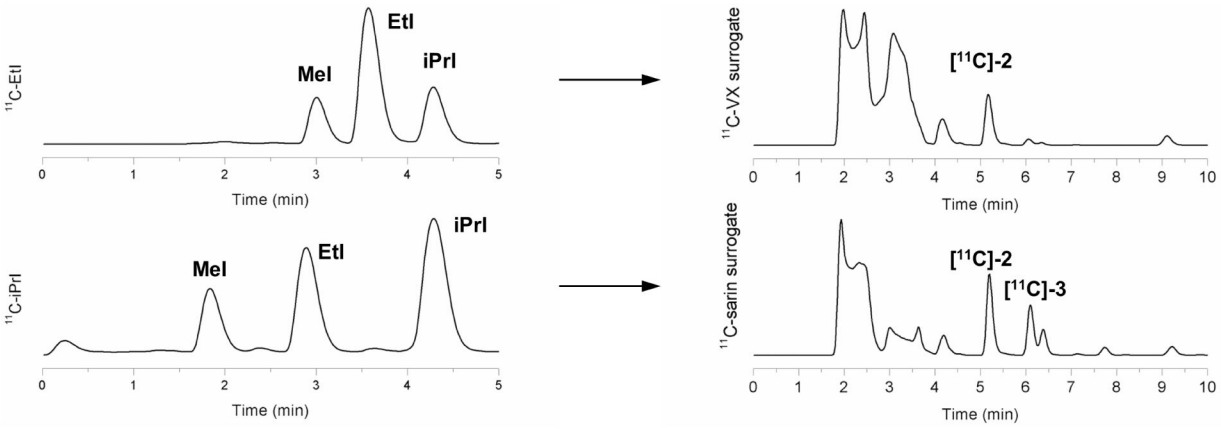

Fig. 5.

Reverse phase HPLC radio-chromatograms of the crude [^{11}C] intermediates and products. [^{11}C]ethyl iodide (Luna C18(2) analytical column, 75% acetonitrile/25% 10 mM NH_4OAc H2O, 1 mL/min) and [^{11}C]isopropyl iodide (Luna C18(2) analytical column, 70% acetonitrile/30% 10 mM NH_4OAc H2O, 2 mL/min).