Title

Radiosynthesis of O-(1-[18 F]fluoropropan-2-yl)-O-(4-nitrophenyl)methylphosphonate: A novel PET tracer surrogate of sarin.

Journal

Journal of Labelled Compounds and Radiopharmaceuticals, 61(14)

Authors

Hayes, Thomas
Thompson, Charles
Blecha, Joseph
et al.

Publication Date

2018-12-01

DOI

10.1002/jlcr.3688

Peer reviewed

HHS Public Access

Author manuscript

J Labelled Comp Radiopharm. Author manuscript; available in PMC 2019 July 28.

Published in final edited form as:

J Labelled Comp Radiopharm. 2018 December ; 61(14): 1089–1094. doi:10.1002/jlcr.3688.

Radiosynthesis of O-(1-[^{18}F]fluoropropan-2-yl)-O-(4-nitrophenyl)methylphosphonate: A novel PET tracer surrogate of sarin

Thomas R. Hayes[1], **Charles M. Thompson**[2], **Joseph E. Blecha**[1], **John M. Gerdes**[2], **Henry F. VanBrocklin**[1]

[1]Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, CA, USA

[2]Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT, USA

Abstract

O-(1-Fluoropropan-2-yl)-O-(4-nitrophenyl) methylphosphonate is a reactive organophosphate ester (OP) developed as a surrogate of the chemical warfare agent sarin that forms a similar covalent adduct at the active site serine of acetylcholinesterase. The radiolabeled O-(1-[^{18}F]fluoropropan-2-yl)-O-(4-nitrophenyl) methylphosphonate ([^{18}F] fluorosarin surrogate) has not been previously prepared. In this paper, we report the first radiosynthesis of this tracer from the reaction of bis-(4-nitrophenyl) methylphosphonate with 1-[^{18}F]fluoro-2-propanol in the presence of DBU. The 1-[^{18}F]fluoro-2-propanol was prepared by reaction of propylene sulfite with Kryptofix 2.2.2 and [^{18}F] fluoride ion. The desired tracer O-(1-[^{18}F]fluoropropan-2-yl)-O-(4-nitrophenyl) methylphosphonate was obtained in a >98% radiochemical purity with a 2.4% ± 0.6% yield (n = 5, 65 minutes from start of synthesis) based on starting [^{18}F] fluoride ion and a molar activity of 49.9 GBq/μmol (1.349 ± 0.329 Ci/μmol, n = 3). This new facile radiosynthesis routinely affords sufficient quantities of [^{18}F] fluorosarin surrogate in high radiochemical purity, which will further enable the tracer development as a novel radiolabeled OP acetylcholinesterase inhibitor for assessment of OP modes of action with PET imaging in vivo.

Keywords

[^{18}F] fluorosarin surrogate; acetylcholinesterase; fluorine-18; positron emission tomography (PET)

1 | INTRODUCTION

Organophosphate esters (OP) are a class of compounds that include insecticides and chemical warfare agents (CWAs). CWAs such as the V-agents (eg, VX) and G-agents (eg,

Correspondence: Henry F. VanBrocklin, Department of Radiology and Biomedical Imaging, University of California, San Francisco, 185 Berry St. Suite 350, San Francisco, CA 94107. henry.vanbrocklin@ucsf.edu.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

sarin, soman) are nondiscriminating poisons (Scheme 1) that pose a threat to both military and civilian populations.[1,2] Human toxicity resulting from OP exposures occurs via the initial inactivation of acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh). At high OP exposure levels, inhibition of available AChE pools in the central nervous system causes a rapid increase in synaptic ACh and triggers neurotoxic sequelae,[3–6] which may be ameliorated using antidote combinations such as 2-pyridine aldoxime methiodide (2-PAM) and atropine, if given in temporal proximity to the exposure.[7–10]

The mechanism of inhibition of AChE by OP CWAs occurs through the displacement of a leaving group X (Scheme 1) by the active site serine hydroxyl of AChE.[4,11] The resultant OP-AChE adduct is relatively stable, but if the exposure is challenged early enough by certain antidotes such as 2-PAM, the OP can be nucleophilically displaced from AChE and the enzyme reactivated. Without antidote, the OP-AChE can remain intact or undergo "aging," a process in which the second phosphonoester group is lost forming a methyl phosphonate anion attached to the serine that is fully refractory to reactivation. Some OP CWAs such as soman undergo aging within minutes, whereas sarin ages within hours although both afford the same OP adduct following loss of the ester moiety.

Previously, a novel radiolabeled OP surrogate based upon the CWA compound VX was prepared by replacing the β-aminothiol leaving group with a *p*-nitrophenyl (X = PNP) and the addition of a fluorine-18 atom to the ethyl ester side chain (R = CH_2CH_2F; Scheme 2) providing an OP-based PET imaging tracer.[12–15] The PNP group effectively mimics the leaving group in the CWA preserving the mechanism of inhibition forming the O-(2-fluoro-ethyl) methylphosphonate-AChE adduct thereby leaving the [^{18}F]-OP attached to AChE. The rate of AChE inhibition by the VX and sarin PNP surrogates was slightly less than VX and sarin, but the presence of a fluorine on the alkoxy ester group reduced the rate of aging while favoring reactivation.[12,16] Thus, these surrogate compounds labeled with fluorine-18 furnish stable OP-AChE adducts that can aptly report on pools of active AChE in small animal models.[14]

Given our goal of developing tools for evaluation of exposure mechanism and reactivation therapies, we sought to prepare the [^{18}F] fluorosarin surrogate PET tracer. The fluorine leaving group in the sarin molecule was replaced with a PNP group. An additional PNP leaving group (compound 1, Scheme 3) facilitates the incorporation of the fluorine-18-labeled isopropyl group. The fluorine-18 atom is located on the terminal methyl group of the isopropyl moiety to minimize interference with AChE reactivity. The resultant fluorosarin surrogate structure, O-(1-fluoropropan-2-yl)-O-(4-nitrophenyl) methylphosphonate (compound 2, Scheme 3) has been reported to possess similar inhibition, reactivation, and AChE adduct formation to sarin (without handling concerns)[16] while demonstrating minimal aging. The bis PNP starting material allows for the incorporation of a fluorine-18 isopropyl group and formation of the radiolabeled AChE adduct with fluorine-18 in place of the methyl hydrogen on the sarin-AChE adduct (Scheme 2). In this study, we report the synthesis and purification of the PET tracer sarin surrogate O-(1-[^{18}F] fluoropropan-2-yl)-O-(4-nitrophenyl)methylphosphonate.

2 | EXPERIMENTAL

2.1 | 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. Flash chromatography was conducted using silica gel (200–300 mesh), and thin-layer chromatography (TLC) was visualized by shortwave UV and/or staining by 2,5-dinitrobromoquinone-4-chlorimide (DBQ) and/or iodine. The nonradioactive molecular characterizations by ^{1}H-NMR, ^{19}F-NMR, and ^{31}P-NMR were recorded in $CDCl_3$ on a Varian Avance 400-MHz spectrometer, and chemical shifts (δ) are reported in ppm (relative to7.26 ppm) and coupling constants (J) in hertz (Hz). For radiochemical transformations, reversed-phase analytical HPLC was performed using a Waters 590 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 5 μm C-18 (2) 100 Å 250 × 4.6 mm LC column. An isocratic solvent system of 40% acetonitrile/60% 5 mM pH 6.8 phosphate buffer at a flow rate of 1 mL/minute was used for analytical reversed-phase HPLC. Semipreparative reversed-phase HPLC 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 10 μm C-18 (2) 100 Å 250 × 10 semipreparative HPLC column. HPLC chromatograms were acquired using SRI PeakSimple software (version 304—Torrance, CA). QMA light cartridges for concentrating [^{18}F] fluoride ion and C-18 light Sep-Paks were purchased from Waters Scientific (Milford, MA).

2.2 | Synthesis of O-(1-fluoropropan-2-yl)-O-(4-nitrophenyl) methylphosphonate, 2

Solid bis-(4-nitrophenyl) methylphosphonate,[17] 1 (0.890 g; 2.63 mmol), was added to a conical flask (10 mL) containing 2.50 mL of anhydrous CH_2Cl_2 and stirred at 25°C. To this solution, 1,8-diazobicycloundec-7-ene (DBU; 430 μL, 2.9 mmol) was added giving a light yellow-gold solution that was stirred for 15 to 30 minutes whereupon 1-fluoro-2-propanol (220 μL, 2.9 mmol) was added via syringe, and the reaction was monitored by TLC. After 5 minutes, the solution was diluted with 50 mL $CHCl_3$ and extracted with 0.1 M HCl (2 × 25 mL), 1% NaOH (1 × 25 mL), water (1 × 25 mL), and brine (1 × 25 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated to give 662 mg (91%) of 2. As an alternative to aqueous workup, the product can be isolated in 45% yield by silica gel flash chromatography (EtOAc/hexanes 1:1) of the crude oil following removal of solvent and reagents by rotary evaporation and high vacuum (<0.5 mmHg). ^{1}H-NMR (400.18 MHz, $CDCl_3$) δ 8.25 (dd, J = 17.8 Hz, 2H),7.42 (dd, J = 9.1 Hz, 2H), 4.95 to 4.82 (m, 1H), 1.76,1.74 (dd, J = 17.8 Hz), 1.38 (d, J = 6.5 Hz, 1.5H), 1.24 (d, J = 7.1 Hz, 1.5H). ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 155.2 (d, J_{C-P} = 20.1 Hz), 144.6, 125.6, 121.1 (d, J_{C-P} = 11.0 Hz), 86.0 (d, J_{C-P} = 24.1 Hz), 84.3 (d, J_{C-P} = 25.0 Hz), 73.0 and 72.9 (d, J_{C-P} = 19.0, 26.2 Hz),17.0 (d, J_{C-P} = 29.0 Hz), 16.9 (d, J_{C-P} = 34.0 Hz), 13.0 (d, J_{C-P} = 35.0 Hz), 11.5 (d, J_{C-P}= 33.40 Hz). ^{31}P-NMR (162 MHz, $CDCl_3$) δ 27.7, 29.2. ^{19}F-NMR (376.3 MHz, $CDCl_3$) δ −223.9, −225.1. HRMS (ESI): Expected (M + H): 278.058815, Found: 278.059061.

2.3 | Radiosynthesis of O-(1-[^{18}F] fluoropropan-2-yl)-O-(4-nitrophenyl) methylphosphonate ([^{18}F] fluorosarin surrogate), [^{18}F]-2

[^{18}F] Fluoride ion was produced in the UCSF GE PETtrace cyclotron by the $^{18}O(p,n)^{18}F$ reaction on enriched ^{18}O-water. [^{18}F] Fluoride ion was trapped on a QMA Light Sep-Pak preconditioned with 1 M Na_2CO_3 (10 mL) and H_2O (10 mL). A solution of Kryptofix[2.2.2] (18 mg) and 0.1 M K_2CO_3 (0.2 mL) dissolved in0.8 mL of CH_3CN was used to elute the [^{18}F] fluoride ion from the QMA Sep-Pak. The solution was dried at 115°C under N_2 and repetitive azeotropic drying with CH_3CN (2 × 1 mL). A solution of 1,2-propyleneglycol sulfite (15 μL) in 300 μL CH_2Cl_2 was added, and the vial was sealed with a screw cap with a teflon septa and heated to 70°C for 20 minutes. The reaction was removed from heat and allowed to cool for 3 minutes, and a solution of 1 (8 mg, 23.6 μmol) and DBU (2 μL) in 300 μL CH_2Cl_2 was added. After 10 minutes, the reaction was dried at 80°C with a stream of N_2 under reduced pressure, dissolved in CH_3CN (300 μL), and diluted with 1.7 mL of0.1 M NaH_2PO_4. The solution was purified by semipreparative HPLC using 50% CH_3CN/50% aqueous0.1% (w/v) ascorbic acid. The fraction containing [^{18}F]-2was diluted to 30 mL with pH 6.8 10 mM phosphate buffer, passed through a C-18 Light Sep-Pak preconditioned with EtOH (10 mL) and H_2O (10 mL). The Sep-Pak was washed with 5 mL of pH 6.8 10 mM phosphate buffer. The activity was then eluted with CH_3CN and formulated by dilution with pH 6.8 phosphate-buffered saline to 10% CH_3CN/PBS final concentration. The desired tracer identity, [^{18}F]-2, was confirmed using the identical HPLC conditions and coelution with cold standard 2. Radiochemical purity of the final product was >98% with a yield of 2.4% ± 0.6% (n = 5, 65 minutes from start of synthesis (SOS)) based on starting [^{18}F] fluoride ion and a molar activity of49.9 GBq/μmol (1.349 ± 0.329 Ci/μmol) at the end of synthesis (n = 3). Starting from 11.1 to 18.5 GBq (300–500 mCi) of [^{18}F] fluoride ion, the range of final isolated yields of [^{18}F]-2 was 0.11 to 0.37 GBq (3–10 mCi). Identity of [^{18}F]-2 was confirmed by HPLC coinjection with 2 (data not shown).

3 | RESULTS AND DISCUSSION

The inhibition of AChE occurs through the displacement of a leaving group on the OP by nucleophilic reaction with an active-site AChE serine hydroxyl (Scheme 1).[4,5,11]

In order to follow the fate of a radiolabeled sarin, the radioisotope must be on either the methyl or the isopropoxy moieties. As previously described for the [^{18}F]-VX surrogate,[12–15] the fluorine-18 label was placed on the alkoxy group, where the electronegativity would have minimal effect at phosphorus both in terms of the enzyme reaction and also subsequent hydrolysis of the alkoxy substituent.

Two approaches were applied to prepare the [^{18}F]-VX surrogate. The first approach involved alkylation of a cesium phosphonate salt with 2-[^{18}F] fluoroethyltosylate ([^{18}F]FCH_2CH_2OTs) using microwave acceleration.[14] Alternatively, transesterification of bis-(4-nitrophenyl) methylphosphonate 1 with 2-[^{18}F] fluoroethanol in the presence of DBU provided more efficient and reproducible preparation of the [^{18}F]-VX surrogate.[15] While both of these radiosyntheses were successful, improved yields, overall synthesis time, and the efficiency of the transesterification process prompted application of this approach to prepare the sarin surrogate (Scheme 3).

Direct application of the reaction conditions used to produce the [^{18}F]-VX surrogate[12,18] including the acetonitrile solvent resulted in low yields of [^{18}F]-2. The ring opening of 1,2-propyleneglycol sulfite with [^{18}F] fluoride ion in CH_3CN gave the desired 1-[^{18}F]fluoro-2-propanol in high yield although absolute quantification of the conversion measured by radio-TLC may be underestimated due to product volatility. In spite of the suitable yield of 1-[^{18}F]fluoro-2-propanol, the transesterification radio-chemical yields were less than 20% by analytical reversed-phase HPLC. The lower transesterification yields may be due to the greater steric demands of the 1-fluoro-2-propyloxy group as compared with the 1-fluoroethoxy group. Additionally, losses of the final product during HPLC isolation contributed to the low yield of [^{18}F]-2 with CH_3CN. Overall, the CH_3CN route to form the [^{18}F]-2 gave inadequate product yield for further evaluation.

As originally reported, the DBU-mediated transesterification used CH_2Cl_2 as solvent.[17] In order to avoid a solvent change, CH_3CN was replaced by CH_2Cl_2 as the solvent for both labeling and transesterification. The initial [^{18}F] fluorination reaction to the intermediate 1-[^{18}F]fluoro-2-propanol gave radiochemical conversion comparable with the CH_3CN reaction by radio-TLC. The subsequent CH_2Cl_2 transesterification reaction gave improved yields of [^{18}F]-2. Analytical HPLC of the crude reaction showed sufficient production of [^{18}F]-2 (Figure 1B). In order to purify [^{18}F]-2 by reversed-phase HPLC, it was necessary to remove the CH_2Cl_2 by evaporation and reconstitute the residue in CH_3CN and phosphate buffer. This solvent exchange process takes time and with the extra handling usually leads to loss of radioactive product. In this case, the increased transesterification yield in CH_2Cl_2 allowed isolation of [^{18}F]-2 post HPLC in spite of the extra time and handling involved. Additionally, the necessary pre-HPLC solvent exchange enabled facile removal of unreacted 1-[^{18}F] fluoro-2-propanol by evaporation. The semipreparative HPLC was carried out in 50/50 CH_3CN/0.1% ascorbic acid. The ascorbic acid was added to the HPLC eluent, as previously noted in Neumann et al,[15] to buffer the pH <7 in order to reduce tracer decomposition during the purification step. The final yield of [^{18}F]-2 was 2.4% ± 0.6% (SOS, 65 min) with a molar activity of49.9 GBq/μmol (1349 ± 329 Ci/mmol) and >98% radiochemical purity (Figure 1A). Thereafter, tracer was formulated in 10% CH_3CN/pH 6.8 PBS as developed for the [^{18}F]-VX (James et al[14]) post HPLC to enable meaningful in vivo assessments.

Tracer product stability was an issue. The analytical HPLC of the CH_2Cl_2 transesterification reaction (Figure 1B) shows unreacted starting material and the fluorine-18-labeled phosphonic acid analog, resulting from the loss of PNP, indicating that [^{18}F]-2 may be hydrolyzing even in the mild room temperature transesterification reaction. It was noted that upon the addition of DBU that the reaction solution turns yellow presumably from the liberation of PNP. Additionally, exchanging the solvent from CH_2Cl_2 to CH_3CN followed by the addition 0.1 M aqueous NaH_2PO_4, to facilitate the reversed-phase semipreparative HPLC purification, may have contributed to further loss of [^{18}F]-2. Fortu nately, the higher yields afforded by the CH_2Cl_2 transesterification reaction provided an opportunity to isolate adequate quantities of the pure [^{18}F]-2 for subsequent evaluation.

4 | CONCLUSIONS

A new fluorine-18-labeled surrogate of the organophosphate nerve agent sarin was prepared. Transesterification of *bis*-(4-nitrophenyl) methylphosphonate with DBU and 1-[^{18}F]fluoro-2-propanol in CH_2Cl_2 gave the desired product. This approach uses mild conditions with modest radiochemical yields and high specific activity. Facile production of [^{18}F] sarin surrogate in sufficient quantities will enable its use as a tracer for assessing OP modes of action with PET imaging in vivo.

ACKNOWLEDGEMENTS

This work was supported by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Grant Award Number U01NS092495. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Funding information

National Institute of Neurological Disorders and Stroke (NINDS), Grant/Award Number: U01NS092495; National Institutes of Health Office of the Director (NIH OD); CounterACT Program

REFERENCES

1. Barthold CL, Schier JG. Organic phosphorus compounds— nerve agents. Crit Care Clin. 2005;21(4):673–689, v-vi. [PubMed: 16168308]
2. Sidell FR, Borak J. Chemical warfare agents: II. Nerve agents. Ann Emerg Med. 1992;21(7):865–871. [PubMed: 1610046]
3. Bajgar J Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. Adv Clin Chem. 2004;38:151–216. [PubMed: 15521192]
4. Eto M Organophosphorus Pesticides; Organic and Biological Chemistry. Cleveland: CRC Press; 1974.
5. Fest C, Schmidt KJ. The Chemistry of Organophosphorus Pesticides; Reactivity, Synthesis, Mode of Action, Toxicology. New York: Springer Verlag, Berlin; 1973.
6. Taylor P In: Hardman JG, Limbird LE, Molinoff PB, Richards AN, Ruddon RW, eds. Goodman & Gilman's the Pharmacological Basis of Therapeutics. New York: McGraw-Hill; 1996: 161–176.
7. Bajgar J, Fusek J, Kuca K, Bartosova L, Jun D. Treatment of organophosphate intoxication using cholinesterase reactivators: facts and fiction. Mini Rev Med Chem. 2007;7(5):461–466. [PubMed: 17504181]
8. Jokanovic M Structure-activity relationship and efficacy of pyridinium oximes in the treatment of poisoning with organo-phosphorus compounds: a review of recent data. Curr Top Med Chem. 2012;12(16):1775–1789. [PubMed: 23030612]
9. Kassa J Review of oximes in the antidotal treatment of poisoning by organophosphorus nerve agents. J Toxicol Clin Toxicol. 2002;40:803–816. [PubMed: 12475193]
10. Worek F, Thiermann H. The value of novel oximes for treatment of poisoning by organophosphorus compounds. Pharmacol Ther. 2013;139(2):249–259. [PubMed: 23603539]
11. Kovacic P Mechanism of organophosphates (nerve gases and pesticides) and antidotes: Electron transfer and oxidative stress. Curr Med Chem. 2003;10(24):2705–2709. [PubMed: 14529460]
12. Chao CK, Ahmed SK, Gerdes JM, Thompson CM. Novel organophosphate ligand O-(2-fluoroethyl)-O-(p-nitrophenyl) methylphosphonate: synthesis, hydrolytic stability and analysis of the inhibition and reactivation of cholinesterases. Chem Res Toxicol. 2016;29(11):1810–1817. [PubMed: 27551891]
13. Gerdes JM, James S, Ahmed SA, et al. A novel high affinity F-18 organophosphonate tracer for CNS acetylcholinesterase. J Nucl Med. 2013;54:323.

14. James SL, Ahmed SK, Murphy S, et al. A novel fluorine-18 beta-fluoroethoxy organophosphate positron emission tomography imaging tracer targeted to central nervous system acetylcholinesterase. ACS Chem Nerosci. 2014;5(7):519–524.

15. Neumann KD, Thompson CM, Blecha JE, Gerdes JM, VanBrocklin HF. An improved radiosynthesis of O-(2-[(18) F] fluoroethyl)-O-(p-nitrophenyl)methylphosphonate: a first-in-class cholinesterase PET tracer. J Labelled Comp Radiopharm. 2017;60(7):337–342. [PubMed: 28406525]

16. Chao CK, Balasubramanian N, Gerdes JM, Thompson CM. The inhibition, reactivation and mechanism of VX-, sarin-, fluoro-VX and fluoro-sarin surrogates following their interaction with HuAChE and HuBuChE. Chem Biol Interact. 2018;291:220–227. [PubMed: 29920286]

17. Tawfik DS, Eshhar Z, Bentolila A, Green BS. 1,8-Diazabicyclo[5.4.0] undecene mediated transesterification of p-nitrophenyl phosphonates: a novel route to Phosphono esters. Synthesis. 1993;1993(10):968–972.

18. Ahmed SK, Belabassi Y, Sankaranarayanan L, Chao C-K, Gerdes JM, Thompson CM. Synthesis and anti-acetylcholinesterase properties of novel β- and γ-substituted alkoxy organophosphonates. Bioorg Med Chem Lett. 2013;23(7):2048–2051. [PubMed: 23453838]

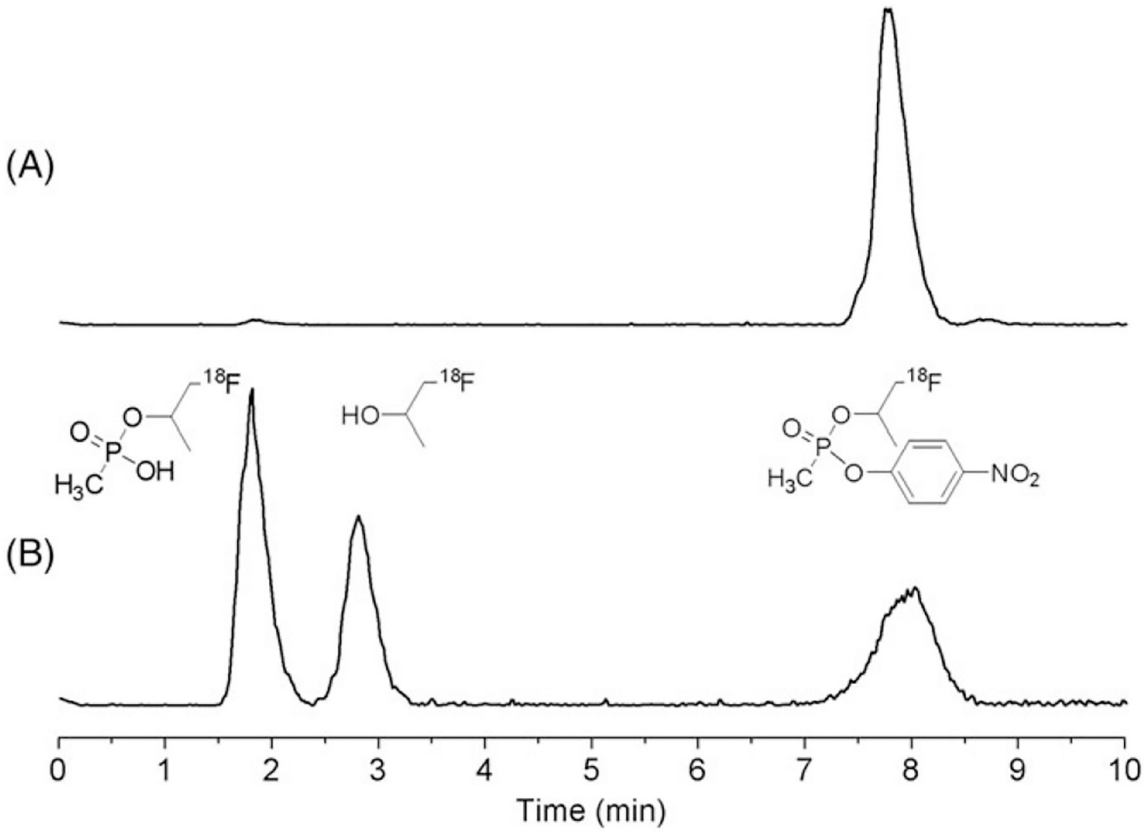
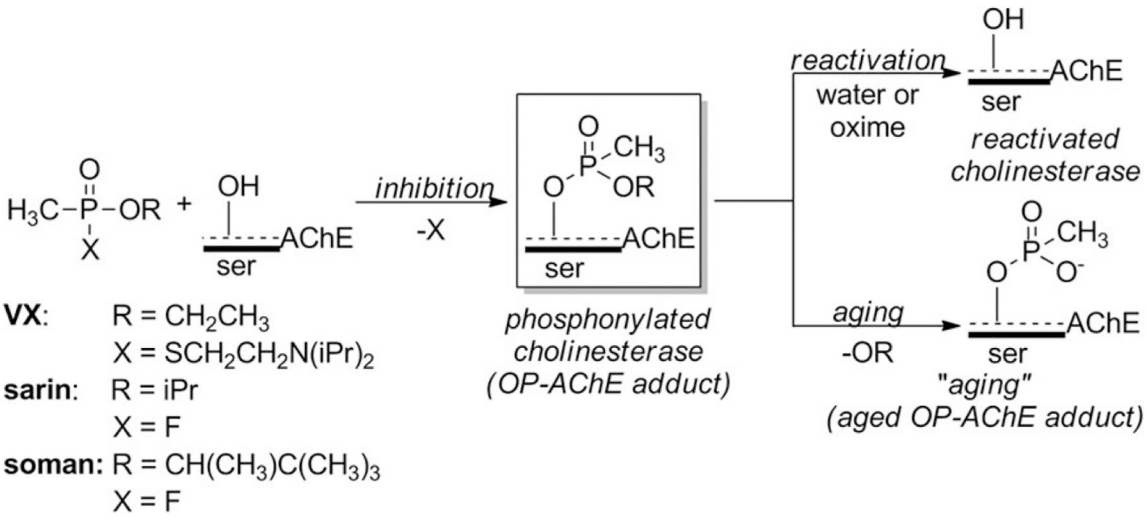
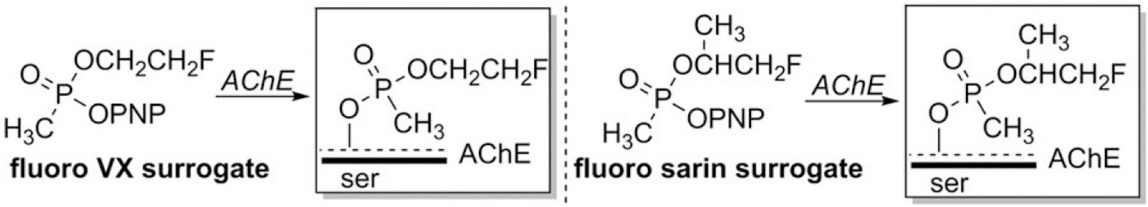


FIGURE 1.

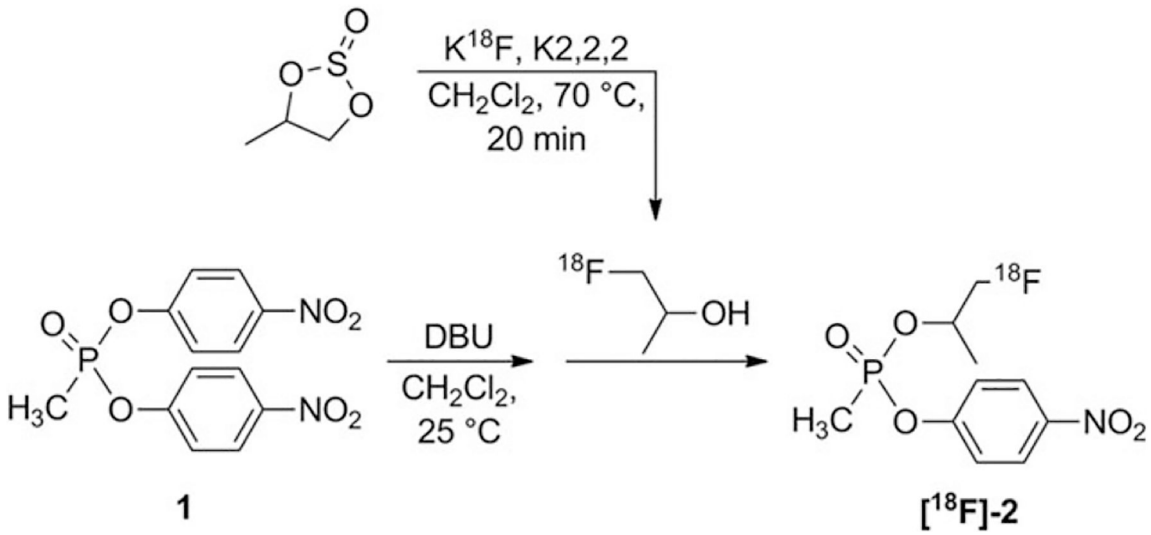
A, Analytical HPLC radiochromatogram of [^{18}F]-2 post purification. B, Analytical HPLC radiochromatogram of crude transesterification reaction mixture



SCHEME 1.
Inactivation of AChE by OP CWAs and postinhibitory pathways



SCHEME 2.
CWA fluoro-surrogates and their AChE adducts



SCHEME 3.
Synthesis of [^{18}F]-2