JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 497–500.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.946

Research Article

New radiolabelling chemistry: synthesis of phosphorus–[^{18}F]fluorine compounds

Andrei R. Studenov[1,*], Michael J. Adam[1], John S. Wilson[2] and Thomas J. Ruth[1]
[1] *TRIUMF, 4004 Wesbrook Mall, Vancouver, BC, Canada V6T 2A3*
[2] *Nuclear Medicine, Cross Cancer Institute, Edmonton, AB, Canada T6G 1Z2*

Summary

The feasibility of synthesizing compounds containing the P–^{18}F bond has been demonstrated by labelling the pesticide, cholinesterase inhibitor Dimefox (*N*,*N*,*N*′*N*′-tetramethylphosphorodiamidic fluoride) with F-18. Radiolabelling was achieved in high radiochemical yield (96%) by nucleophilic substitution of the chloro group attached to phosphorus, in the oxidation state P(V), by $^{18}F^-$ (activated with tetrabutylammonium carbonate in acetonitrile). Given the large number of important biological molecules possessing phosphorus such as oligonucleotides, phospholipids as well as phosphorylated proteins, sugars and steroids, this new labelling chemistry may provide an additional route to radiolabelling these biologically important compounds for use in PET. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: positron emission tomography; fluorine-18; phosphorus; radiolabelling

Introduction

Compounds containing the phosphorus–fluorine bond have been used in biological studies[1] and as poisons.[2,3] An extensive literature exists on the chemistry of the P–F bond, with special attention being paid to fluorinated oligonucleotide analogs.[4] None of these compounds, however, has yet to be labelled with the no-carrier-added (n.c.a.) positron emitter fluorine-18 (half-life, 110 min) or used as a PET radiotracer. Commonly, most of the fluorine-18 PET radiotracers contain a C–^{18}F bond.[5,6] As an exception, radiotracers containing an Si–^{18}F bond have been proposed.[7] However, until now these

*Correspondence to: Andrei R. Studenov, TRIUMF, PET Group, 4004 Wesbrook Mall, Vancouver, BC, Canada V6T 2A3. E-mail: studenov@triumf.ca

Contract/grant sponsor: Canadian Institute for Health Research
Contract/grant sponsor: TRIUMF Life Sciences Program

Received 18 October 2004
Revised 23 February 2005
Accepted 24 February 2005

compounds have found limited application. A synthesis of sodium fluorophosphate double labelled with ^{18}F and ^{32}P, accomplished via the high temperature (800°C) solid-phase reaction of the carrier-added [^{18}F]NaF with carrier-added [^{32}P]$NaPO_3$, has been described back in the earlier 1960s.[8]

Before the development of any new radiotracer labelled with fluorine-18 the question of stability must be answered. Although some oligonucleotide fluorophosphodiesters are hydrolytically unstable for *in vivo* use,[9] a negative charge on the oxygen adjacent to the P–F bond renders fluorophosphomonoesters stable. For example, an AZT analog containing a P–F bond was sufficiently stable to be considered as a potential therapeutic drug.[10,11] (Storage of this compound in aqueous solution for 3 days did not lead to any decomposition. The most 'aggressive' bacterial media studied decomposed it with a 2 h half-life.)[11]

A simple model compound, Dimefox, (tetramethylphosphorodiamidic fluoride; pesticide/cholinesterase inhibitor) was chosen to demonstrate the feasibility of n.c.a. radiofluorination of [^{18}F] P–F compounds.

Experimental

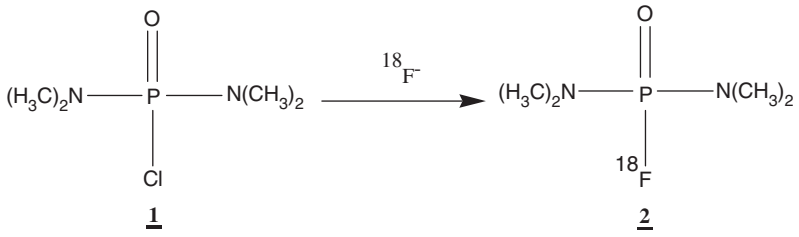
All chemicals were acquired from Sigma-Aldrich, TCI America, Lancaster or Acros, unless noted otherwise. Dimefox was purchased from Riedel de Haen. The chemical and radiochemical purity were determined by radio TLC (Macherey-Nagel, G/UV_{254} plastic-back TLC plates, 4×8 cm, Bioscan system 200). Ninhydrin spray was applied to visualize the compounds on the TLC plate.

Preparation of reactive dried [^{18}F]F^- (fluoride activated with tetrabutylammonium carbonate)

Tetrabutylammonium carbonate (10 µl of 1.5 M solution in acetonitrile prepared as per literature)[12] was added to the aqueous [^{18}F]F^-, and the solution was evaporated under argon (oil bath temperature 80°C). Acetonitrile (3×0.3 ml) was added and evaporated to ensure complete removal of water.

*[^{18}F]Dimefox {[^{18}F]N,N,N′,N′-tetramethylphosphorodiamidic fluoride, **2**, Scheme 1}*

The solution of *N*,*N*,*N*′,*N*′-tetramethylphosphorodiamidic chloride (**1**, 10 µl, 67 µmol) in acetonitrile (0.4 ml) was added to the dried [^{18}F]F^- activated with tetrabutylammonium carbonate, and the mixture was left at room temperature for 5 min. After a small sample was taken for analysis, the solution was heated at 75°C for 10 min. Analysis of the reaction and proof of the product identity was accomplished by three different radioTLC conditions: Silica, 1:1 acetone: hexane, R_f (**2**) 0.47, (chloride precursor) 0.55. Alumina-N, 1:1 acetone: hexane, R_f (**2**) 0.60. C18, 30 mM ammonium acetate–40% acetonitrile, R_f (**2**) 0.60. The spots of unlabelled Dimefox and its chloride precursor were visualized by



Scheme 1.

spraying TLC plates with a freshly prepared 1:2 concentrated aqueous HCl: 2% ninhydrin in acetone followed by heating.[13] This treatment produced red–brown spots on the light-red background on Silica; the brown spots were observed on the white and the gray backgrounds in the case of Alumina-N and C18 plates accordingly. Conversion of the solubilized [^{18}F]F$^-$ into **2** was 96%. *Assessment of hydrolytic stability:* An aliquot (50 µl) of the reaction mixture was mixed with water (50 µl). TLC analysis was done at 10 and 30 min time points.

Results and discussion

Because of the lack of publications on the synthesis of phosphorus–fluorine-18 containing compounds (except for carrier-added [^{18}F]sodium fluorophosphate),[8] we limited the studies to a simple, readily obtainable precursor and reference standard. Acetonitrile was used as the reaction solvent, while [^{18}F]F$^-$ was activated with tetrabutylammonium carbonate (for mild ^{18}F-fluorination).[12] The synthesis of phosphorofluoridates can be achieved starting with either P(III) or P(V) precursors. Although P(III) compounds are more reactive, it is advantageous to use P(V)-containing precursors for ^{18}F-labelling since the later compounds, in general, have better stability. Moreover, the use of the P(III) precursors necessitates an additional step (typically oxidation[14] or sulfurization[15]) to bring phosphorus to a stable oxidation state P(V).

To investigate the possibility of radiolabelling P(V) compounds, the P(V)–Cl bond containing precursor of Dimefox was chosen for the initial tests. Radiolabelling of Dimefox (**2**, Scheme 1), resulted in conversion of 96% of soluble [^{18}F]F$^-$ into the product at room temperature.

Assessment of the hydrolytic stability of ^{18}F-labelled Dimefox showed that it did not undergo an immediate defluorination in aqueous solution. More than 75% of n.c.a. [^{18}F]dimefox remained unchanged after 30 min of storage in aqueous media. It is anticipated that future P–^{18}F bond-based PET radiotracers, possibly containing phosphorofluoridatemonoester groups, would possess higher hydrolytic stability. (A negative charge on the oxygen

attached to phosphorus disfavors nucleophilic attack on the phosphorus atom.)

Conclusions

The no-carrier-added radiotracer Dimefox containing a P–^{18}F bond was synthesized in high radiochemical yield (96%), demonstrating that P(V) compounds can be labelled with n.c.a. F-18. Although molecules of biological interest have not yet been labelled via this P–^{18}F chemistry it suggests that this new labelling method may provide an alternate method for the radiolabelling of phosphorus-containing biomolecules for use in PET.

Acknowledgements

We are thankful to Paul Piccioni for the preparation of aqueous [^{18}F]F^{-}. This research was supported by the Canadian Institute for Health Research and the TRIUMF Life Sciences Program.

References

1. Anderson C, Freeman J, Lucas LH, Farley M, Dalhoumi H, Widlanski TS. *Biochemistry* 1997; **36**: 2586–2594.
2. Green AL, Sainsbury GL, Saville B, Stansfield M. *J Chem Soc Abstr* 1958; 1583–1587.
3. Ashani Y, Leader H, Rothschild N, Dosoretz C. *Biochem Pharmacol* 1998; **55**: 159–168.
4. Bollmark M, Stawinski J. *Nucleos Nucleot* 1998; **17**: 663–680.
5. Iwata R. Division of Radiopharmaceutical Chemistry, CYRIC TOHOKU University Website. 2003. http://kakuyaku.cyric.tohoku.ac.jp [31 October 2003].
6. Fowler JS, Ding YS, Volkow ND. *Semin Nucl Med* 2003; **33**: 14–27.
7. Walsh JC, Akhoon KM, Satyamurthy N, Barrio JR, Phelps ME, Gambhir SS, Toyokuni T. *J Label Compd Radiopharm* 1999; **42**: S1–S3.
8. Ericson Y. *Int J Appl Radiat Isot* 1961; **10**: 177–180.
9. Misiura K, Szymanowicz D, Kusnierczyk H. *Bioorg Med Chem* 2001; **9**: 1525–1532.
10. Dyatkina N, Arzumanov A, Krayevsky A, Ohara B, Gluzman Y, Baron P, Maclow C, Polsky B. *Nucleos Nucleot* 1994; **13**: 325–337.
11. Egron D, Arzumanov AA, Dyatkina NB, Aubertin AM, Imbach JL, Gosselin G, Krayevsky A, Perigaud C. *Bioorg Chem* 2001; **29**: 333–344.
12. Culbert PA, Adam MJ, Hurtado ET, Husser JM, Jivan S, Lu J, Ruth TJ, Zeisler SK. *Appl Radiat Isot* 1995; **46**: 887–891.
13. Coha F. *J Chromatogr* 1968; **34**: 558–559.
14. Hayakawa Y, Uchiyama M, Noyori R. *Tetrahedron Lett* 1986; **27**: 4191–4194.
15. Iyer RP, Egan W, Regan JB, Beaucage SL. *J Am Chem Soc* 1990; **112**: 1253–1254.

