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CONF-730309-1

BIOLOGICALLY ACTIVE F-18-FLUOROORGANIC COMPOUNDS:
RAPID, HIGH SPECIFIC ACTIVITY SYNTHESIS OF
F-18-FLUOROCARBOXYLATES AND DERIVATIVES*

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This work was supported by AEC Contract AT(04-1) GEN-12 between the U.S. Atomic Energy Commission and the University of California at Los Angeles.

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BIOLOGICALLY ACTIVE F-18-FLUOROORGANIC COMPOUNDS:
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The participation of small ions or molecules in specific metabolic processes is potentially a powerful mechanism for improving the localization of radiopharmaceuticals in target organs. Interest in the preparation of F-18-fluoroorganic agents is due to the strong evidence that the F for H substitution in some biologically active compounds leads to analogs having almost unchanged biological behavior.

Potassium fluoride is commonly used to prepare fluoroorganic compounds from the corresponding bromo-, chloro-, or iodoorganics. This interhalogen exchange reaction has been adapted for the synthesis of a series of high specific activity F-18-fluoroorganic compounds. F-18-fluoride in aqueous solution is prepared by the $O-16(He-3,p)F-18$ reaction on water in our Biomedical Cyclotron facility. The F-18-fluoride is absorbed on anion exchange resin ("Dowex 1-4X", fluoride) and dried under vacuum at 40°C. This dry F-18 "reagent" is then sealed in a glass ampule with an appropriate ethyl halocarboxylate and reacted at elevated temperature (<180°C) for up to 90 minutes. After cooling, the ampule is opened and the labeled product (ethyl F-18-fluorocarboxylate) is isolated by gas chromatography. Hydrolysis of the product gives labeled carboxylic acid, while reduction gives labeled alcohol. Reaction of the

free carboxylic acid with SOCl_2 gives the highly reactive acid chloride. Radiochemical yields vary from 10 to 40 percent with "available" yields ranging from 3 to 20 percent. Specific activities range from 5 to 20 mCi/mg (available).

The F-18 labeled analog molecules are currently being tested in animals as potential radiodiagnostic agents, primarily as tracers for rapid, dynamic metabolic functions. The lipid soluble compounds (2-fluorobutyrate, 2-fluorotetradecanoate, 2-fluoroethanol, etc.) are expected to show promise as agents for the evaluation of regional brain perfusion due to the apparent absence of a blood-brain barrier for many such substances. The medium and long chain fatty acids (2-fluorohexanoate, 2-fluorotetradecanoate, etc.) are being evaluated as potential agents for myocardial imaging and liver function studies. As a result of their relatively non-specific, high tissue extractability, fluoroacetate and 2-fluoroethanol show promise as agents for the measurement of fractional cardiac output. In addition, F-18-fluoroacetyl chloride has considerable potential for use in the rapid preparation of a wide variety of high specific activity compounds, such as fluoroacetylsalicylic acid (an aspirin analog), cholesteryl fluoroacetate, etc.

1. INTRODUCTION

Gamma-emitting analog molecules are potentially useful tools for monitoring specific metabolic processes and for developing improved radiopharmaceuticals. Continued interest in the preparation of F-18 labeled compounds of biological importance is due to the fact that F for H substitution in some biologically active compounds leads to analogs having almost unchanged biological behaviors [1,2].

The Schiemann reaction, which involves thermal decomposition of a diazonium F-18-tetrafluoroborate salt, has been used for the preparation of aromatic F-18-fluoroamino acids [3,4]. Although, this synthetic route is relatively rapid and the decomposition is generally under mild conditions, the use of considerable carrier is required, and low overall yields lead to compounds of low specific activity ($\mu\text{Ci}/\text{mg}$).

Hot atom (recoil) techniques have been investigated for the direct production of F-18-fluoroanalog compounds with little success [5]. This is largely due to the high energies associated with hot atom processes and the resultant radiolytic decomposition of the target molecules. High specific activity F-18 labeled degradation products are the usual result. Wolf, et al. have recently reported direct hot atom production of reactive F-18 intermediates which can be subsequently used to synthesize high specific activity (mCi/mg) compounds [6].

Metal fluorides have been used for the synthesis of non-radioactive fluoroorganic compounds by interhalogen exchange with the corresponding bromo-, chloro-, or iodoorganics under anhydrous conditions [7-10]. This approach, however, is limited by the low solubility of metal fluorides in organic solvents. We have modified this approach by utilizing F-18-fluoride on anion exchange resin as a fluoride source for the interhalogen exchange reaction, and have begun to prepare high specific activity ($10 \text{ mCi}/\text{mg}$) F-18-fluorocarboxylates and derivatives. These compounds are currently being evaluated as potential agents for use in clinical nuclear medicine, and as models for the development of new radiopharmaceuticals.

2. EXPERIMENTAL METHOD

2.1. Production of F-18-Fluoride

F-18-fluoride in aqueous solution is produced by the $^{16}\text{O}(\text{He-3},\text{p})\text{F-18}$ reaction on a water target in the UCLA Biomedical Cyclotron facility. With an incident He-3 beam energy of 29 MeV and a beam current of 20 μAmp , approximately 100 mCi of F-18 are produced during a 45-min bombardment. Short lived nuclides (principally ^{15}O , $t_{1/2} = 2.1 \text{ min}$) are allowed to decay for 30 min before recovery of the target. The small quantities of longer lived isotopes which are produced have no effect upon subsequent chemical manipulations.

The chemical identity of the F-18 activity as fluoride ion was confirmed by anion exchange column chromatography.

2.2. Preparation of Anion Exchange Resin (Fluoride Form)

Dowex 1-X4 (50-100 mesh, chloride form) is converted to the hydroxide form by passing 1 liter of 1 N NaOH through a 50 cm X 1 cm dia. column at a flow rate of 12 ml/hr. No chloride is detectable in the eluate after this treatment. Following a thorough rinsing with several volumes of distilled water, the hydroxide resin is converted to fluoride form by passing 100 ml of 1 N HF through the column at 120 ml/hr. The column is rinsed with distilled water until the pH of the eluate is above 3. A single batch of anion exchange resin prepared in this manner served as the fluoride source for exchange reactions in all subsequent experiments.

2.3. Sample Preparation and Exchange

Ion exchange column-reaction ampules are fabricated as shown in Figure 1. A length of 2 mm i.d. X 3 mm o.d. Pyrex tubing is constricted at one end and a small glass wool plug is positioned inside at the constriction. The column-ampule is then formed by a second constriction 2 cm above the first. A slight vacuum is created on the downstream side and the column-ampule is filled to capacity with the fluoride form anion exchange resin. Finally, the excess glass tubing, beyond the constrictions, is broken off.

Immediately prior to use, the column-ampule is connected to the output from a Cheminert CMP-2 metering pump and washed with 30 ml of 1% aqueous KF solution and rinsed with 30 ml of distilled water (both at a flow rate of 60 ml/hr). F-18-fluoride is exchanged onto the resin by recycling the F-18 solution through the column-ampule, as shown in Figure 2, for at least 15 min at 120 ml/hr. Recirculation of the F-18-fluoride is stopped by removing the inlet to the pump from the solution and allowing the system to pump to dryness. Finally, the column-ampule is rinsed with methanol and then diethyl ether (15 ml each at 120 ml/hr) and dried under vacuum at 40°C.

The ampule containing dry, F-18-fluoride labeled resin is sealed at the bottom by flame and 10-20 µl of bromocarboxylate ester is added by microliter syringe. The top of the ampule is sealed immediately and the sealed ampule is enclosed in a steel Parr bomb half filled with water for exchange at 180°C for up to 90 min. The water in the bomb tends to balance the pressure inside the exchange ampule as the temperature is raised.

2.4. Isolation of F-18-Fluorocarboxylate Esters

F-18-fluorocarboxylate esters are the products of interhalogen exchange between bromocarboxylate esters and F-18-fluoride on the anion exchange resin. After heating for exchange, the ampule is removed from the Parr bomb and opened. The organic exchange reaction products are removed by syringe and the resin is washed with 50 µl of an appropriate organic solvent (one which will not interfere with the subsequent gas

chromatographic separation). The organic products and wash solution are combined and then analyzed by gas chromatography with thermal conductivity detection. The separation is carried out on a 6 ft X 1/4 in. stainless steel column packed with 5% diethylene glycol succinate (DEGS) on Anaprep A (50-60 mesh) and a helium carrier gas flow rate of 80 ml/min.

F-18-fluorocarboxylates are trapped at the gas chromatograph exit port by condensation in a 2 ml multidose vial which is cooled by liquid nitrogen.

2.5. Preparation of F-18-Fluorocarboxylates and 2-F-18-Fluoroethanol

Free F-18-fluoroacetate is prepared by alkaline hydrolysis of the labeled ethyl ester. The trap containing ethyl F-18-fluoroacetate is rinsed several times with distilled water and the combined rinse solutions (≈ 5 ml) are placed in a 10 ml pear shaped flask. 0.5 ml of 1 N NaOH are added and the solution is brought to boiling at such a rate that half of the volume is evaporated in 30 min. The remaining solution is then cooled and neutralized with 0.5 ml of 1 N HCl.

The longer chain F-18-fluorocarboxylate esters are taken up in diethyl ether which is then layered over 5 ml of distilled water in a 10 ml pear shaped flask. Upon heating, the ether is rapidly evaporated leaving the higher boiling F-18 labeled ester behind. Hydrolysis then proceeds with the addition of NaOH as described above.

2-F-18-fluoroethanol is prepared from the F-18-fluoroacetate ester by reduction in benzene solution. 0.5 ml of benzene is added to the vented trap containing ethyl F-18-fluoroacetate. 0.25 ml of a 70% solution of sodium dihydro-bis(2-methoxyethoxy)aluminate (SDMA) in benzene is added dropwise with stirring. Reduction is complete within 30 min at which time excess SDMA is hydrolyzed by the dropwise addition, with cooling, of 1.0 ml of distilled water. The aqueous phase, which contains the 2-F-18-fluoroethanol, is separated and the labeled alcohol is isolated by gas chromatography on the column described above at 35°C. The compound is then taken up in normal saline.

2-F-18-fluoroethanol is also prepared directly from 2-bromoethanol in the manner described above for the F-18-fluorocarboxylate series. In this case, simple preparative gas chromatography isolates the product which is taken up in isotonic saline.

3. RESULTS AND DISCUSSION

The use of metal fluorides as fluorinating agents for several classes of haloorganic compounds has been extensively investigated. Due to the stability of the C-F bond, the exchange is essentially irreversible and ease of exchange of halogen increases in the order $\text{Cl} < \text{Br} < \text{I}$ [11]. The bromocarboxylates were chosen as parent molecules for our studies because of ready commercial availability, relative ease of exchange, the multitude of information on the F for Br exchange that exists in the chemical literature, and the biological activities of the fluorocarboxylates pro-

duced. Anion exchange resins were chosen as a source of F-18-fluoride to avoid the poor solubilities of metal fluorides in organic solvents and, in addition, because of the simplicity of preparing the required anhydrous F-18-fluoride from an aqueous solution.

The relationship between time of heating, temperature of exchange and F-18 radiochemical yield for ethyl F-18-fluoroacetate and ethyl F-18-2-fluorotetradecanoate are shown in Figure 3. Qualitatively, the curves are similar, showing an initial rise in yield which eventually reaches a limiting value. Quantitatively, the early rates of exchange and, indeed, the magnitudes of the ultimate plateaus are strongly influenced by both temperature and the "complexity" (e.g., aliphatic chain length) of the F-18-fluorocarboxylate being produced. In general, the simpler compounds give higher yields, at lower temperatures, in less time than the more complex, longer chain molecules. This is also reflected in lower quantities of non-radioactive organic by-products being produced during synthesis of the simpler F-18-compounds, as shown in Figure 4.

Yields of the F-18-fluorocarboxylates under "optimal" conditions are shown in Table I. These conditions were chosen with respect to achieving maximum available radioactive yields (mCi) of the products. Highest specific activities are, of course, obtained early, when the F-18 specific activity is highest; however, radiochemical yields are highest, until the limiting value is reached, at longer heating times. Thus, the "optimal" yield represents a compromise between these two factors. A more detailed description of the preparation of F-18-fluoroacetate under "optimal" conditions is given in Table II.

The limit to F-18 radiochemical yields, as illustrated in Figure 3, is characteristic of all of the compounds studied. This plateau phenomenon has yet to be adequately interpreted, since all of the F-18-fluoride on the resin is expected to be irreversibly exchangeable and, therefore, no limiting value should be observed if excess bromocarboxylate remains (although the reaction is expected to slow somewhat as reactants are consumed). Even when significantly larger quantities of bromocarboxylate are used (200-500 μ l), only slight increases in ultimate radiochemical yields were obtained.

4. APPLICATIONS IN NUCLEAR MEDICINE

Aliphatic fluorine compounds which are catabolized in vivo to fluoroacetate show uniformly high toxicities [12]. It has been shown that fluoroacetate is converted to fluorocitrate, which in turn blocks the tricarboxylic acid cycle by enzyme inhibition. The ability of fluorocarboxylates and their derivatives to function, to a limited extent, as biological analogs of the natural compounds has suggested their use for a variety of applications in clinical nuclear medicine. Unfortunately, the high levels of toxicity of many of the compounds ($LD_{50} = 5$ mg/kg body weight in rats) require that the syntheses be carried out at high specific activities or carrier free. While the latter has not yet been accomplished, the specific activities listed in Table I are in the range required for preliminary investigations in animals.

The lipid soluble, long chain F-18 fatty acids may be useful agents for the evaluation of regional brain perfusion. Due to the apparent absence of a blood-brain barrier for such substances, the uptake and release of these molecules is a rapid, dynamic process [13,14]. Altered regional blood flow may be reflected in abnormal rates and/or amounts of uptake and clearance of tracer from affected areas.

Radioiodinated fatty acids, primarily I-131-oleic acid, have been used in the past as agents for heart imaging [15,16]. The F-18 compounds are currently under investigation as potential agents for improved myocardial imaging. Although the gamma-ray energy of F-18 ($E_\gamma = 150$ KeV) is poorly suited for most current imaging systems, its short half-life ($t_{1/2} = 110$ min) could allow the use of substantially higher doses of activity than are now possible with other isotopes.

As a result of their relatively non-specific, high tissue extractability, the more polar, short chain F-18 compounds (i.e., fluoroacetate, 2-fluoroethanol, etc.) show promise as agents for the measurement of fractional cardiac output. In organs where extraction of these molecules is near 100% on a single pass, the percent of dose in the tissue is a measure of the fraction of the cardiac output flowing to the organs. This potential application is also currently being investigated.

There are, of course, a multiplicity of organic reactions which can be used to convert the F-18-fluorocarboxylates to other, more complex, derivative forms. The conversion of fluorocarboxylic acids to fluoroacetyl chlorides by treatment with SOCl_2 is of great potential significance in this sense. The F-18 labeled acid chlorides can rapidly and efficiently convert alcohols to F-18-fluorocarboxylic esters (i.e., cholesterol to cholesteryl F-18-fluoroacetate), amino acids to N-F-18-fluoroacyl amino acids (i.e., phenylalanine to N-F-18-fluoroacetyl phenylalanine), etc. We are currently studying the preparation of O-F-18-fluoroacetyl salicylic acid by the action of F-18-fluoroacetyl chloride on salicylic acid. It is anticipated that this molecule will serve as a useful analog for investigations of the biological dynamics of O-acetyl salicylic acid or, more commonly, aspirin.

F-18-fluoride as a dry "reagent" on anion exchange resins is useful for the rapid synthesis of high specific activity F-18-fluorocarboxylates and derivatives under mild conditions. Potentially, this same synthetic method offers an attractive route to the synthesis of more complex agents. Knowledge of the biological behaviors of these simple compounds is most important in forming a logical basis for the design of those more complex, F-18 labeled, biologically active radiopharmaceuticals.

5. REFERENCES

- [1] HEIDELBERGER, C., *Nucleic Acid Res. Mol. Biol.* 4 (1965) 1.
- [2] WEBB, J.L., *Enzyme and Metabolic Inhibitors*, Vol. I, Academic Press, New York and London (1963) 436.
- [3] HOYTE, R.M., LIN, S.S., CHRISTMAN, D.R., ATKINS, H.L., HAUSER, W., WOLF, A.P., *J. Nucl. Med.* 12 (1971) 280.
- [4] GOULDING, R.W., PALMER, A.J., *Int. J. Appl. Radiat. Isotopes* 23 (1972) 133.
- [5] WOLF, A.P., in *Preparation and Bio-Medical Application of Labeled Molecules* (Sirchis, J., Ed.), EURATOM EUR 2200.e (1964) 423.
- [6] FOWLER, J.S., FINN, R.D., LAMBRECHT, R.M., WOLF, A.P., *J. Nucl. Med.* 14 (1973) 63.
- [7] HENNE, A.L., MIDGLEY, T., Jr., *J. Am. Chem. Soc.* 58 (1936) 882.
- [8] *Methoden der Organischen Chemie (HOUBEN-WEYL)*, Vol. 5, Part 3, Georg Thieme Verlag, Stuttgart (1962) 206.
- [9] HERZ, J.E., FRIED, J., GRABOWICH, P., SABO, E.F., *J. Am. Chem. Soc.* 78 (1956) 4812.
- [10] PATTISON, F.L.M., BUCHANAN, R.L., DEAN, F.H., *Can. J. Chem.* 43 (1965) 1700.
- [11] SHEPPARD, W.A., SHARTS, C.M., *Organic Fluorine Chemistry*, Benjamin, New York (1969) 83.
- [12] PATTISON, F.L.M., *Toxic Aliphatic Fluorine Compounds*, Elsevier, Amsterdam and New York (1959).
- [13] DHOPESHWARKAR, G.A., MEAD, J.F., *Biochim. Biophys. Acta* 210 (1970) 250.
- [14] DHOPESHWARKAR, G.A., SUBRIMANIAN, C., MEAD, J.F., *Biochim. Biophys. Acta* 231 (1971) 8.
- [15] EVANS, J.R., GUNTON, R.W., BEANLANDS, D.S., *Circulation* 261 (1962) 714.
- [16] EVANS, J.R., GUNTON, R.W., BAKER, R.G., BEANLANDS, D.S., SPEARS, J.C., *Circulation Research* 16 (1965) 1.

6. LEGENDS

- Figure 1: Exchange column-reaction ampule fabrication: (1) Constrict 2 mm i.d. X 3 mm o.d. tubing and insert glass wool plug. (2) Form second constriction 2 cm above the first. (3) Fill column-ampule with resin and break away excess tubing.
- Figure 2: Labeling fluoride resin with F-18. Pumping speed of 60 ml/hr gives 2 complete cycles of F-18-fluoride solution (7 ml total volume) within 15 min.
- Figure 3: F-18 radiochemical yields as a function of time and temperature of exchange.
- Figure 4: Gas chromatographic analysis of exchange reaction organic products as described in Section 2.4. Identity of peaks is as follows: (1) Ethyl fluoroacetate, (2) ethyl chloroacetate, (3) ethyl bromoacetate, (4) bromine, (5) ethyl 2-fluorotetradecanoate, and (6) ethyl 2-bromotetradecanoate.

FIG. 1. Exchange column-reaction ampule fabrication:

- 1) Constrict 2 mm i.d. X 3 mm o.d. tubing and insert glass wool plug,
- 2) Form second constriction 2 cm above the first,
- 3) Fill column-ampule with resin and break away excess tubing.

FIG. 2. Labeling fluoride resin with F-18. Pumping speed of 60 ml/hr gives 2 complete cycles of F-18-fluoride solution (7 ml total volume) within 15 min.

FIG. 3. F-18 radiochemical yields as a function of time and temperature of exchange.

A. F-18-fluoroacetate preparation

B. F-18-2-fluorotetradecanoate preparation

FIG. 4. Gas chromatographic analysis of exchange reaction organic products as described in Section 2.4. Identity of peaks is as follows: 1) ethyl fluoroacetate, 2) ethyl chloroacetate, 3) ethyl bromoacetate, 4) bromine, 5) ethyl 2-fluorotetradecanoate, 6) ethyl 2-bromotetradecanoate.

TABLE I. OPTIMAL YIELDS OF F-18-FLUOROCARBOXYLATES AND DERIVATIVES

	<u>Radiochemical Yield (%)</u>	<u>Available^a Yield (mCi)</u>	<u>Specific Activity (mCi/mg)</u>	<u>Heating Time^b (min)</u>
F-18-Fluoroacetate	36	18	15	30
F-18-Fluoroacetamide	32	22	15	30
F-18-2-Fluoroethanol ^c	26	17	17	30
F-18-2-Fluoroethanol ^d	34	12	17	30
F-18-2-Fluorohexanoate	16	7	8.7	60
F-18-2-Fluorotetradecanoate	7	3	4.7	90

a = Available yields vary as much as 30% between individual runs. The values cited represent average yields assuming 100 mCi of F-18-fluoride.

b = In all cases, temperature of heating was 180°C.

c = Prepared by direct exchange with 2-bromoethanol.

d = Prepared by reduction of ethyl F-18-fluoroacetate.

TABLE II. PREPARATION OF F-18-FLUOROACETATE

<u>Procedure</u>	<u>Time</u> (min)	<u>Yield</u> (relative)
Obtain F-18-fluoride from Biomedical Cyclotron (in 7 ml).	--	1.00
Absorb F-18-fluoride on 10 mg "Dowex 1-4X", 50-100 mesh, fluoride form.	15	0.93
Wash F-18 labeled resin with equal volumes (15 ml) of methanol and diethyl ether and then dry under vacuum at 40°C.	15	--
Add 10 μ l of ethyl bromoacetate (= 10 mg) and seal glass ampule.	5	--
Heat ampule under pressure at 180°C.	20	--
Cool and open ampule; chromatograph organic reaction mixture (trapping ethyl F-18-fluoroacetate).	15	0.38
Hydrolyze ester by heating (boiling) with 0.1 N NaOH.	30	--
Neutralize and "Millipore" filter.	5	--
TOTAL (RADIOCHEMICAL) YIELD	105	0.35
AVAILABLE (END OF PROCESSING) YIELD	--	0.18

Gas chromatographic data indicates that the chemical yield of ethyl fluoroacetate is approximately 1 mg. Routine preparative runs begin with 100 mCi of F-18-fluoride resulting in the production of 15-20 mCi (available) of F-18-fluoroacetate at a specific activity of 15 mCi/mg.