LABELED 2-DEOXY-D-GLUCOSE ANALOGS. ^{18}F-LABELED 2-DEOXY-
2-FLUORO-D-GLUCOSE, 2-DEOXY-2-FLUORO-D-MANNOSE AND
^{14}C-2-DEOXY-2-FLUORO-D-GLUCOSE

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SUMMARY

A convenient method for the synthesis of ^{18}F-2-deoxy-2-fluoro-
D-glucose (4) and ^{18}F-2-deoxy-2-fluoro-D-mannose (8) by the direct
fluorination of 3,4,6-tri-0-acetyl-D-glucal with ^{18}F-F$_2$ is described.
^{14}C-2-deoxy-2-fluoro-D-glucose has been synthesized from ^{14}C-3,4,6-
tri-0-acetyl-D-glucal (from D-[^{14}C(U)]-glucose) by fluorination with
F$_2$ (Method 1) and CF$_3$OF (Method 2). These labeled analogs of
2-deoxy-D-glucose were required for the study of local cerebral
glucose metabolism.

INTRODUCTION

It has recently been shown that ^{14}C-deoxyglucose can be used to map the

regions in brain with altered glucose utilization in response to alterations in

local functional activity.[1] An extension of this technique to the in vivo

estimation of local glucose metabolism in man especially as it is altered by

disease induced changes in functional state required a metabolic analog to

2-deoxyglucose labeled with a radionuclide which is both short-lived and decays

by the emission of radiation which can be detected externally using emission

tomography. The 2-deoxyglucose analog, ^{18}F-2-deoxy-2-fluoro-D-glucose, appeared

to satisfy these requirements since (1) the non-radioactive compound has been

shown to be metabolically similar to 2-deoxyglucose in that it is a good

substrate for yeast hexokinase and (2) fluorine-18, $t_{1/2}$ = 110 min decays by positron emission resulting in two 511 KeV photons which can readily be detected external to the organism. Preliminary studies in which the suitability of 2-deoxy-2-fluoro-D-glucose as an analog to 2-deoxyglucose was established also required the synthesis of ^{14}C-2-deoxy-2-fluoro-D-glucose.

Two synthetic routes to 2-deoxy-2-fluoro-D-glucose have been reported previously by Pacák and coworkers[3] and by Adamson et al.[4] These procedures involved fluoride displacement with potassium bifluoride (KHF_2)[3] and electro-philic fluorination with trifluoromethyl hypofluorite (CF_3OF)[4] to introduce fluorine. Although electrophilic fluorination with CF_3OF was suitable for preparation of ^{14}C-2-deoxy-2-fluoro-D-glucose, the convenience of this method has decreased since there is presently no commercial source of CF_3OF.

Since labeling with ^{18}F is limited to rapid synthetic reactions with readily available chemical forms of ^{18}F which can be produced in high yield and in high specific activity, neither of the reported procedures were adequate. We have developed an alternative synthesis of 2-deoxy-2-fluoro-D-glucose by direct fluorination of 3,4,6-tri-0-acetyl-D-glucal with elemental fluorine[5] and have used this synthesis to produce both ^{18}F- and ^{14}C-labeled 2-deoxy-2-fluoro-D-glucose (Fig. 1). The development of a new method for synthesizing the required anhydrous ^{18}F-F_2 reagent in high yield and in high specific activity[6] allowed the use of this method for preparing 15-17 mCi of ^{18}F-2-deoxy-2-fluoro-D-glucose at end of synthesis (EOS). Synthesis time was one half-life, 110 min.

RESULTS AND DISCUSSION

The ^{20}Ne(d,α)^{18}F nuclear reaction is used to reliably produce large quantities (> 1 Ci if desired) of anhydrous ^{18}F-F_2 with high specific activity.[6] Purging the ^{18}F-F_2 through a solution of 3,4,6-tri-0-acetyl-D-glucal (1) yields a mixture of 2 and 3 which are separated by preparative glpc. Hydrolysis of 2 or 3 in HCl followed by passage over an ion retardation column (neutralization) followed by an alumina column (fluoride removal) and another ion retardation column yielded ^{18}F-2-deoxy-2-fluoro-D-glucose (^{18}F-4) or ^{18}F-8 in 8% and 3% radiochemical yield respectively. The radiochemical purity of ^{18}F-4 was > 98% with very high specific activity (as high as 8750 mCi/mmol).

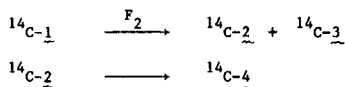<u>^{18}F-2-deoxy-2-fluoro-D-glucose and ^{18}F-2-deoxy-2-fluoro-D-mannose</u>

^{20}Ne$(d,\alpha)^{18}$F

^{18}F + F$_2$ → ^{18}F - F$_2$
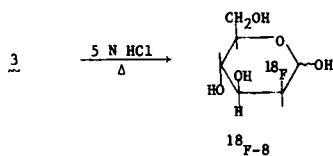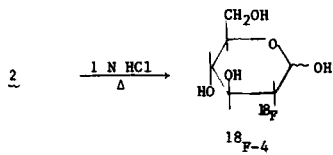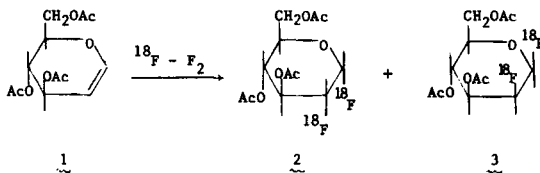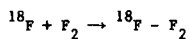
^{14}C-2-deoxy-2-fluoro-D-glucose

Method I (fluorination with F$_2$)
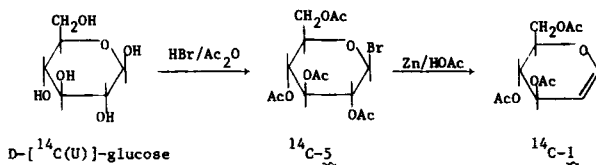
Method II (fluorination with CF$_3$OF)

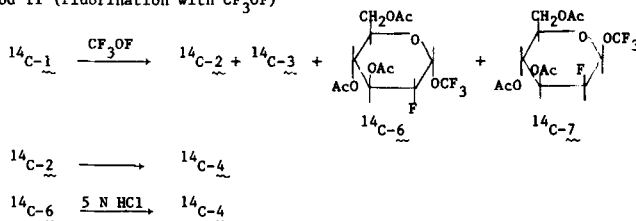**Figure 1**. Synthesis of ^{18}F-2-deoxy-2-fluoro-D-glucose.
^{18}F-2-deoxy-2-fluoro-D-mannose and ^{14}C-Labeled 2-deoxy-2-fluoro-D-glucose.

KEL-F VALVE

$^{18}F-F_2$

TEFLON 1/8" OD

SODA LIME TRAP
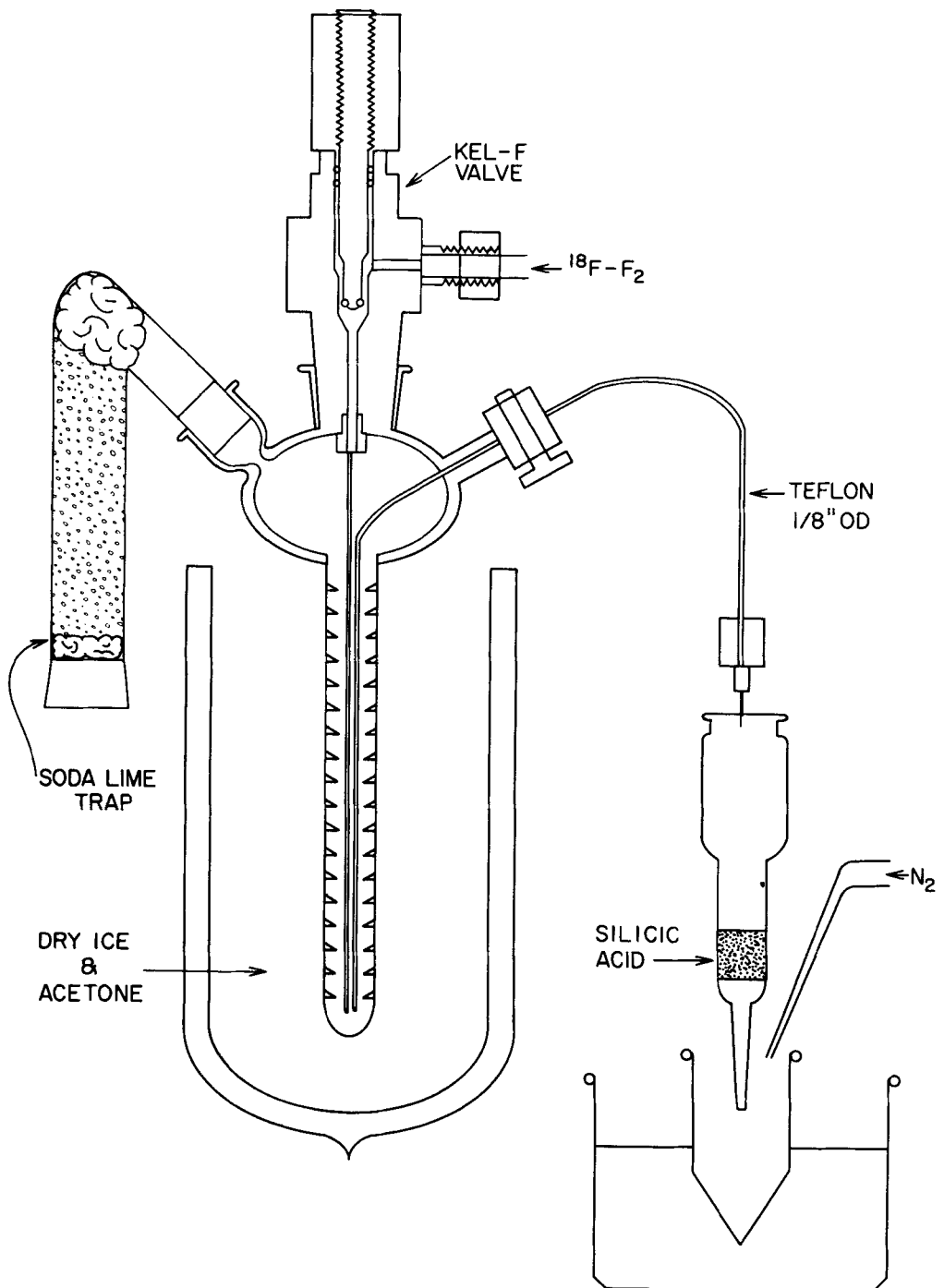
DRY ICE & ACETONE

SILICIC ACID

N_2

Figure 2. Reaction vessel for the fluorination of 1.

Efforts are currently underway to optimize the yield of ^{18}F-2-deoxy-2-fluoro-D-glucose by varying reaction conditions. Furthermore, other γ-emitting analogs to 2-deoxyglucose are being synthesized and evaluated for (1) their ability to serve as substrates for hexokinase, (2) in vivo stability of the radioactive lable, and (3) their ability to indicate local glucose metabolism.

EXPERIMENTAL

^{18}F-2-Deoxy-2-fluoro-D-glucose. The target,[6] consisting of neon (Matheson Research Grade) containing 0.1% (60 μmol) of fluorine carrier, was irradiated with deuterons at the Brookhaven National Laboratory 60-in cyclotron. The ^{18}F-labeled fluorine was produced from the ^{20}Ne(d,α)^{18}F nuclear reaction. The beam was degraded from 13.8 to 0 MeV in the target. Approximately 60-80% of the ^{18}F-F$_2$ produced was slowly purged from the target chamber (flow rate ∿ 45 ml/min, total time 25 min) through an automatic flow control system[6] into the solution of 20 mg (73.5 μmol of 3,4,6-tri-O-acetyl-D-glucal (1) (Aldrich) in 6 ml of fluorotrichloromethane (Freon-11) at -78°C. The specially designed reaction vessel is shown in Fig. 2. After all the gas had bubbled through, the whole system was purged with helium for 2 min in order to force the remaining ^{18}F-F$_2$ into the reaction mixture. The cold bath was then removed and the Freon-11 was blown down by a stream of helium. The residue was transferred to a small test tube through a 0.5 x 1.0 cm column of silicic acid[7] using 6 ml x 2 of methylene chloride. This was blown to dryness with nitrogen, the residue was diluted with 50 μl of acetone and the mixture separated by preparative gas liquid phase chromatography.[8a] The chromatogram showed three major peaks besides acetone at 1.1, 4.5, and 8.1 min corresponding to 1, ^{18}F-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl fluoride (2), and ^{18}F-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-mannopyranosyl fluoride (3).[9] The peak area ratio of 2 and 3 was 4:1. 2 and 3 were obtained in 17.6% and 4.8% radiochemical yield respectively.

Compound 2 was transferred into a hydrolysis tube with anhydrous ether (10 ml) and the solvent was removed with nitrogen. This residue was heated at 130°C with 0.5 ml 1 N HCl for 30 min. The cooled mixture was passed through

three 0.5 x 5.0 cm columns in series, one aluminum oxide (Woelm, neutral, Grade I) in between two ion-retardation resin columns (Bio-Rad, AG11A8, 50-100 mesh) and eluted with 3 ml of deionized water. This gave an 8% chemical yield (8% radiochemical yield) of ^{18}F-2-deoxy-2-fluoro-D-glucose (4) in > 98% purity. Thin-layer chromatography [cellulose (Eastman Kodak 13254) with isobutyric acid:ammonia:water (66:1:33) as developing solvent] showed an R_f 0.67 and the radioactivity was congruent with a spot corresponding to an authentic sample of 4.[9] The chemical yield of 4 in this small scale reaction can be conveniently determined by high pressure liquid chromatography using Waters µBondapak carbohydrate column (#84038; 4 mm x 30 cm) eluting with $CH_3CN:H_2O$ (85:15) at 1.5 ml/min using the refractive index detector. The retention time of 4 in this system is 6 min.

Assay of ^{18}F-2-deoxy-2-fluoro-D-glucose for ^{18}F-fluoride. To an aliquot of ^{18}F-4 was added a 0.04 M solution of a mixture of $PbCl_2$ and $Pb(NO_3)_2$ and 0.1 M solution of a mixture of $PbCl_2$ and $Pb(NO_3)_2$ and 0.1 M NaF solution was added as a fluoride ion carrier to facilitate separation of the PbF_2. The concentration of ^{18}F-fluoride was insignificant (0.04-0.08%).

2-Deoxy-2-fluoro-D-mannose. Compound 3 was hydrolyzed using 5 N HCl at 130° for 15 min and a similar workup was used to give ^{18}F-2-deoxy-2-fluoro-D-mannose (8). T.l.c. (same system as that described for 4) showed that 8 had R_f 0.6.

^{14}C-2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl bromide (^{14}C-5). A mixture of ^{14}C-D-glucose [5 mCi, 1.7 mmol (calculated value based on radioactivity) Amersham] and 1 ml of acetic anhydride saturated with HBr was stirred at room temperature for 1 hr. An additional 1 ml of acetic anhydride/HBr reagent was added and the mixture stirred for 1 hour. Most of the excess HBr was removed with a stream of nitrogen. Chloroform (2 ml) was added to the mixture, and this solution was washed with water (2 ml x 2), saturated aqueous $NaHCO_3$ (2 ml x 2), and water (2 ml x 2). After drying (Na_2SO_4), chloroform was evaporated, leaving 727 mg of 5 which had 4.74 mCi of total activity (94.7% radiochemical

yield). Thin-layer chromatography on silica gel plate (Kodak 13181) with ether

as developing solvent showed that $\underset{\sim}{1}$ had R_f value 0.48 and in 97% purity.

^{14}C-3,4,6-tri-O-acetyl-D-glucal ($\underset{\sim}{1}$). A mixture of $\underset{\sim}{1}$ (10.9 mCi, 4.05 mmol),

1.5 g zinc dust, and 12 ml of 50% aqueous acetic acid was stirred at room

temperature for 13 hr. The zinc dust was removed by filtration, the filtrate

was concentrated under vacuum, water (10 ml) was added, and the mixture extracted

with chloroform. The chloroform solution was washed carefully with saturated

aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated to give 908 mg (82.5%)

of oil (8.81 mCi, 80.8% radiochemical yield).

Fluorination of ^{14}C-3,4,6-tri-O-acetyl-D-glucal (^{14}C-$\underset{\sim}{1}$) with F_2.[5]

A solution of $\underset{\sim}{1}$ (8.81 mCi, 3.3 mmol) in fluorotrichloromethane (Freon-11, 20 ml,

in a specially designed reaction vessel was cooled at -78°C. Fluorine ($\sim$ 2.1

equiv.) diluted with argon was passed into the solution (flow rate 6 ml/min)

during a period of 5 hr. The reaction mixture was allowed to warm to room

temperature, and the excess fluorine and Freon-11 were removed with a stream

of nitrogen. The residue was dissolved in 20 ml of chloroform, and was washed

with saturated aqueous $NaHCO_3$ (25 ml). The separated chloroform layer was dried

(Na_2SO_4), and concentrated yielding 909 mg (87.8%) of viscous oil (8.14 mCi,

92.4% radiochemical yield).

Separation of ^{14}C-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl

fluoride (^{14}C-$\underset{\sim}{2}$) and ^{14}C-3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-β-D-mannopyranosyl

fluoride (^{14}C-$\underset{\sim}{3}$). The mixture obtained from the fluorination reaction (909 mg,

8.14 mCi) was separated by column chromatography on 2.5 x 30 cm silicic acid

(100 mesh) column, eluting with n-hexane, ether and methylene chloride. ^{14}C-$\underset{\sim}{2}$

and $\underset{\sim}{3}$ were obtained in yields of 950 µCi and 2450 µCi respectively.

^{14}C-2-deoxy-2-fluoro-D-glucose (^{14}C-$\underset{\sim}{4}$). Compound ^{14}C-$\underset{\sim}{2}$ (770 µCi) was

hydrolyzed by heating with 3 ml 1 N HCl at 130° for 45 min. The cooled reaction

mixture was extracted with ether. The aqueous layer was then neutralized with

Dowex 1 x 8 anion exchange resin and eluted with 75 ml of water. After evapora-

tion, the residue was purified by column chromatography on a 1.3 x 15 cm silica

gel column, eluting with ethyl acetate:ethanol (1:1), yielding ^{14}C-4 (604.8 μCi, 78.6% radiochemical yield). Thin-layer chromatography on cellulose plate (Kodak 13254) with isobutyric acid:NH_3:H_2O (66:1:33) as developing solvent had R_f 0.67[9] and 98% purity.

Fluorination of ^{14}C-3,4,6-tri-O-acetyl-D-glucal (^{14}C-1) with CF_3OF.

^{14}C-Triacetylglucal (^{14}C-1) (250 μCi, 0.191 mmol) was fluorinated with CF_3OF according to the method of Adamson et al.[4] The desired adducts 2 and 6 were isolated by preparative GLPC[8b] and had retention times of 12.2 min and 7.4 min respectively. Adduct 2 (15.2 μCi) was hydrolyzed in 0.3 ml of 1 M HCl for 35 min at 110°, neutralized (AG 1X-8) and evaporated to yield 13 μCi of ^{14}C-2-fluoro-2-deoxy-D-glucose (^{14}C-4). Adduct 6 (28.3 μCi) was hydrolyzed with 0.25 ml of 5 M HCl at 115° for 4 hrs and the solution was evaporated to dryness. The residue was dissolved in ethyl acetate:ethanol (1:1) and passed over 0.5 g of silica gel eluting with 20 ml of the solvent. The solvent was removed to give 24.5 μCi of ^{14}C-labeled 4. The radiochemical purity of 4 was determined to be > 98% by tlc (Silica gel G, ethyl acetate:ethanol, 1:1, I_2 detection) with an R_f = 0.67 which was identical to an authentic sample of 2-fluoro-2-deoxy-D-glucose.[9] The radiochemical yield was 15% (based on starting activity of (^{14}C-1) and the specific activity was 1.31 mCi/mmol.

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   (b) Pacák J., Podešva J., Točík Z. and Černý M. - Collection Czechoslov. Chem. Commun 37: 2589 (1972)

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6. Casella V., Ido T. and Wolf A.P. – manuscript submitted

7. Most of compound 3 decomposes on GLPC under the described conditions. We
   found that the decomposition of 3 can be avoided if the reaction mixture was
   passed through a short silicic acid column before Feron-11 was removed.

8. GLPC conditions were as follows (a) XE-60 nitrile (20%) on Chromosorb P,
   6' x ¼" columns, column temperature 248°, injector temperature 250° detector
   temperature 260°, carrier gas (He) flow rate 86 ml/min; (b) XE-60 nitrile
   (12.33%) on Chromosorb P, 6' x ¼" column, column temperature 250°, flow rate
   40 ml/min.

9. Authentic samples of 2 and 3, 6, 7 and 4 were prepared according to the
   procedure reported by J. Adamson et al.[4] The authors are grateful to
   Dr. R. H. Hesse for providing an authentic sample of 2-fluoro-2-deoxy-D-
   glucose for which served as seed crystals as well as a spectral and
   chromatographic standard.