Simple Synthesis of F-18-Labeled 2-Fluoro-2-Deoxy-D-Glucose: Concise Communication

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Fluorine-18 acetyl hypofluorite, produced by a gas/solid-phase reaction, was reacted with D-glucal in water, followed by treatment with HCl and serial passage through columns of charcoal, ion-retardation resin, and alumina. The product was an injectable solution of 2-[^{18}F]fluoro-2-deoxy-D-glucose. The radiochemical purity was greater than 95% and the radiochemical yield was about 40%. The synthesis was completed in about 15 min. Acetyl hypofluorite was shown to be a stable, highly stereoselective fluorinating agent in aqueous systems over a wide pH range.

J Nucl Med 25: 333–337, 1984

New methods are continuously being sought for the synthesis of 2-fluoro-2-deoxy-D-glucose (FDG), the most widely useful radiopharmaceutical for metabolic PET imaging. In addition to the methods involving electrophilic fluorination now in widespread use, nucleophilic reactions of $^{18}F^-$ are also finding increasing application (*1*). Both approaches involve the reaction of protected precursors in organic solvents, with subsequent deblocking, chromatographic purification, and removal of organic solvents—operations that are difficult to perform in remote, automated systems.

Acetyl hypofluorite [AcOF (*2*)] has recently been shown to react with protected 1,2-unsaturated sugars with high stereoselectivity to give the corresponding 2-fluoro-2-deoxy sugars in good yield (*3,4*). This reaction has been applied to the radiosynthesis of [F-18]FDG (*5–8*), resulting in higher yields and simpler procedures than for methods involving direct fluorination with [^{18}F]F_2. Recently we developed, for the synthesis of AcOF (*9*), a simple gas/solid-phase method that is readily applicable to the synthesis of AcO^{18}F. This method allowed considerable flexibility in the choice of solvent for the subsequent reaction of AcOF with an unsaturated compound. We therefore considered the possibility of reacting AcOF directly with an unprotected sugar in water.

When AcO^{18}F in neon was bubbled into a dilute solution of D-glucal in water, there resulted a mixture of F-18-labeled FDG and a radiolabeled acid-labile precursor of FDG. Addition of HCl followed by brief heating and serial passage through columns of charcoal, ion-retardation resin, and alumina converted the precursor to FDG and, at the same time, removed the excess starting materials, HCl and HF. The resulting solution of [F-18]FDG with high radiochemical purity was ready for injection after passage through a 0.2-μ filter.

Because the procedure requires no organic solvents, evaporations, or chromatographic separations, a very simple automatic system is adequate for remote synthesis. Since dry reagents or glassware are unnecessary, the system is ready for reuse after automatic washing with sterile, nonpyrogenic water and replacement of the columns.

MATERIALS AND METHODS

Potassium acetate – acetic acid complex for generating acetyl hypofluorite. Anhydrous KOAc (1.0 mole) was mixed with glacial acetic acid (1.5 mole) and stored in

Received July 21, 1983; revision accepted Oct. 24, 1983.

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a sealed container for 48 hr. The resulting solid cake was pulverized with a spoon and placed in a desiccator under vacuum over P_2O_5 for 24 hr. The product was stored in tightly sealed bottles to exclude moisture.

D-Glucal (*10*). Amberlyte CG 400*, OH^- form, was washed several times with anhydrous methanol. The resin was added to a solution of 27.2 g 3,4,6-tri-*O*-acetyl-D-glucal in 200 ml anhydrous MeOH. After stirring at 60° for 30 min, the solvent was removed on a rotary evaporator. A second 200 ml MeOH was added, and the suspension was stirred and heated for 30 min more. The resin was then removed by suction filtration and the solvent was removed on a rotary evaporator. The resulting syrup was maintained under a high vacuum for 24 hr. The resulting solid, mp 54–57° (58–60° Lit.) gave a single spot by TLC [MeCH/H_2O (95:5); silica gel; detected with I_2].

Preparation of the columns. Powdered activated charcoal, USP grade, was mixed with diatomaceous earth 1:4 w/w. This material, 0.5 g, was packed loosely in a 7-mm column over a 10 μm sintered polyethylene frit. A small plug of cotton was placed above the charcoal to trap polymer particles that would otherwise clog the column bed. Before use, the column was washed with 20 ml sterile, pyrogen-free water. These columns were used once and discarded.

The ion-retardation resin and alumina were packed in a single column, which was regenerated after use. Neutral alumina, 100–200 mesh, was washed with water to remove fines, and slurry-packed to a depth of 70 mm in a glass column (7 mm i.d. × 300 mm) fitted with 10-μm sintered polyethylene frits at both ends. The remainder of the column was slurry-packed with AG 11A8 resin†. The column was regenerated by washing with 100 ml *N* NaOH followed by 100 ml sterile, pyrogen-free water. The upper frits become clogged with charcoal particles after several uses and are periodically replaced.

Synthesis of [F-18]FDG. $[^{18}F]F_2$, nominally 0.1% in neon, was produced by the $^{20}Ne(d,\alpha)^{18}F$ reaction. A 15.0-MeV deuteron beam from a CS-30 cyclotron was used (13.3 MeV D^+ on the target gas at 10 μA for 0.5 hr), with a nickel target (70 cc volume) containing 0.1% F_2 in neon at 80 psig. The total activity recovered from the target was 20 mCi after 15 min irradiation, or 40 mCi after 30 min.

After irradiation, the target gas was passed at 100 ml/min through a stainless steel tube, 4 mm × 100 mm, packed with $KOAc(HOAc)_{1.5}$. The effluent was passed into a reaction vessel similar to that described by Ido et al. (*11*) containing 30 mg D-glucal dissolved in 2.5 ml H_2O. The effluent gas from this vessel was passed through a small column of soda lime followed by a charcoal trap maintained at −70°.

After 5 min, 0.6 ml 37% HCl was added. The solution was transferred to a tube maintained at 100°. Heating was continued for 3 min, during which the excess D-glucal was converted to a green polymeric precipitate. The resulting suspension was drawn by a vacuum through the charcoal, resin, and alumina columns connected in series. The two reaction vessels and columns were washed with two 3.5-ml portions of sterile, pyrogen-free water. The combined fractions were passed through a 0.2 μm Millipore filter into a multidose vial.

Analysis of the product. The radiochemical purity of the product was determined by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC), and the chemical purity by gas-liquid chromatography (GLC) and TLC. For HPLC the radioactivity was measured directly with a detector consisting of a cell containing a solid glass scintillator coupled to two photomultiplier tubes. For TLC the chromatograms were cut into strips for gamma counting.

For HPLC we used a HPX-87C Ca^{++}-exchanged polystyrene sulfonate resin† with water as the mobile phase at 85° and at a pressure of 900 psig (*12*). Retention time for [F-18]FDG was 6.4 min.

For GLC‡ (*13*), a 100-μl aliquot was evaporated to dryness, the residue was dissolved in pyridine, and 25 μl hexamethyldisilazane and 15 μl of a 1.5 *M* solution of trimethylsilylimidazole in pyridine were added. The solution was kept at 60° for 1.5 hr. The resulting trimethylsilyl derivative, 1.5 μl, was injected on a 2-m SE30 column, temperature-programmed from 130° to 240° at the rate of 10°/min. Retention times for the alpha and beta anomers of FDG were 12.5 and 13.4 min, respectively (*6*).

For TLC, silica-gel coated strips were used with two solvent systems: MeCN/H_2O (95:5), R_f of FDG = 0.46 (*11*); and butanol/AcOH/H_2O (5:1:1), R_f of FDG = 0.52 (*14*). The strips were sprayed with alkaline $AgNO_3$ for visualization. Unreacted starting material was specifically detected by I_2.

RESULTS AND DISCUSSION

Using the above method, [F-18]FDG was produced

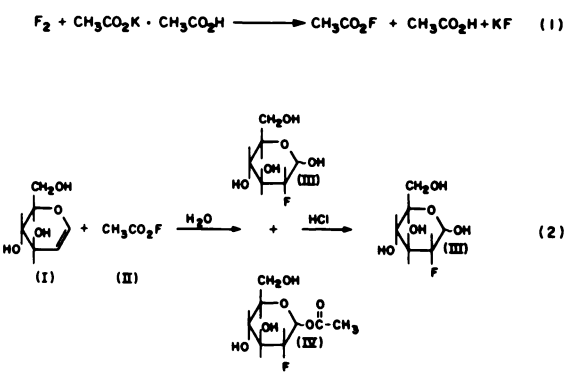

FIG. 1. Synthesis of 2-fluoro-2-deoxy-D-glucose from acetyl hypofluorite and D-glucal.

TABLE 1. PERCENT OF TOTAL RADIOACTIVITY ACCUMULATED IN VARIOUS FRACTIONS DURING SYNTHESIS OF [2-^{18}F]FDG

Experiment No.	Pure [2-^{18}F]FDG	Water solution after saturation with $AcO^{18}F$	$KOAc(HOAc)_{1.5}$	Soda-lime trap	Charcoal gas trap −70°
1	33.2	34.9	52.0	2.6	10.5
2	33.4	34.0	54.0	2.8	9.2
3	33.0	34.8	52.0	1.8	11.4
4	34.0	37.8	50.0	2.7	9.5
5	33.1	35.2	48.2	2.5	14.1
6	39.7	46.7	50.0	1.7	1.6
7	38.0	45.1	49.0	2.8	3.1
8	43.9	46.7	51.0	1.2	1.1
9	35.0	47.8	51.0	0.6	0.6
10	41.0	46.1	51.1	1.2	1.6
Average	36.4 ± 3.2	40.9 ± 5.6	50.8 ± 1.5	2.0 ± 0.7	6.3 ± 4.7

with a radiochemical yield of 34–43%, in greater than 95% radiochemical purity, in approximately 15 min after the end of irradiation. Specific activity was 4–6 mCi/mg.

Figure 1 summarizes the reaction sequence. In the gas-phase reaction between $[^{18}F]F_2$ and CH_3COOK, 1 mole of KF is created. This is in good agreement with the results in Table 1, which show 50.8% of the radioactivity in the column with the acetate complex at the end of the synthesis. The radioactivity in the aqueous D-glucal solution after addition of the $AcO^{18}F$ was variable, and less than the expected 50%. The radiochemical yield of $AcO^{18}F$ appeared to depend strongly on the dryness of the KOAc/HOAc complex. In Table 1, the $(KOAc)(HOAc)_{1.5}$ was dried in a vacuum over P_2O_5 for experiments 6–10, but not for experiments 1–5. We have observed that the presence of water in acetate salts leads to the formation of $CH_3{}^{18}F$, apparently by the reaction: $CH_3COO^{18}F \rightarrow CH_3{}^{18}F + CO_2$ (*15*). The $CH_3{}^{18}F$ is trapped on charcoal at −70° along with $[^{18}F]CF_4$ and $[^{18}F]NF_3$, which are produced during irradiation of the target (*16*).

$AcO^{18}F$ that failed to be transferred from the gas stream to the D-glucal solution would appear in the soda lime trap along with traces of $H^{18}F$ removed by the gas stream from the solution. The low radioactivity found in the soda-lime trap indicates that AcOF is readily transferred from the gas phase to the aqueous phase, even at the high rate of gas flow used (100 ml/min). This is in contrast to the situation for reactions of AcOF in Freon-11, where the gas flow rate could not exceed 20 ml/min without a severe reduction in radiochemical yield (*9*). Thus, the use of water as a reaction medium allows the target to be emptied very rapidly, an important factor in reducing the total time for the synthesis.

The aqueous solution was analyzed by TLC after introduction of the $AcO^{18}F$ but before the addition of HCl (Table 2). Radioactivity was distributed between two products, FDG and a precursor tentatively identified as 1-*O*-acetyl-2-fluoro-2-deoxy-D-glucose. When the solution was buffered at a low pH, more of the latter was detected. At high pH, the higher activity at the origin ($^{18}F^-$) indicates competition by base-catalyzed breakdown of the $AcO^{18}F$. Nevertheless, the good radiochemical yields over a broad pH range indicate AcOF to be a highly versatile electrophilic fluorinating reagent for use in aqueous systems.

TLC of the reaction solution after heating with HCl (Table 2, Solvent 1) shows that less than 1% of the radioactivity occurs at the position of 2-fluoro-2-deoxy-D-mannose, the other expected product of the addition of AcOF to D-glucal. Thus, the high stereospecificity already demonstrated (*3–5*) for the addition of AcOF to protected unsaturated sugars in nonpolar solvents can be generally expected for the unprotected sugars in water as well.

The precursor of FDG obtained by the reaction of AcOF with aqueous D-glucal, and tentatively identified as 1-*O*-acetyl-2-fluoro-2-deoxy-D-glucose, decomposed rapidly to a compound identical by TLC to FDG after chromatographic isolation. Thus, we failed to obtain a pure, stable sample that would allow unequivocal identification. However, several kinds of evidence indicate the identification to be correct. The product of the reaction of the analogous tri-*O*-acetyl-D-glucal with AcOF has been shown to be 1,3,4,6-tetra-*O*-acetyl-2-fluoro-2-deoxy-D-glucose (*3,4*). A compound identical to the precursor by TLC was the main product when D-glucal was reacted with AcOF in acetic acid instead of water. By TLC [$MeCN/H_2O$ (95:5), silica gel] the precursor

TABLE 2. SUMMARY OF PERCENT DISTRIBUTION OF RADIOACTIVITY BY TWO DIFFERENT TLC SYSTEMS DURING SYNTHESIS OF [2-^{18}F]FDG

pH of aqueous solution of D-Glucal[a]		TLC System 1 (see text)			TLC System 2 (see text)	
	R_f	0.0[c]	0.46[b]	0.7[d]	0.0[c]	0.53[b]
4.5						
e		7.2	73.8	24.1	3.2	96.0
f		3.7	94.2	0.3	3.6	95.2
5.7						
e		3.9	74.2	19.2	3.6	95.5
f		4.2	93.1	0.6	3.8	94.8
7.1						
e		4.8	75.4	19.3	3.9	94.4
f		4.7	93.6	0.8	4.2	93.8
9.1						
e		5.3	72.1	21.3	4.3	92.9
f		5.5	92.1	0.8	5.1	91.6
12.1						
e		7.2	90.4	1.1	7.7	88.6
f		6.9	91.1	0.4	7.4	88.4

[a] Solutions were made by mixing 0.5 *N* NaOH and 0.5 *N* HOAc to the specified pH value.
[b] R_f of [2-^{18}F]FDG.
[c] R_f of fluoride ion.
[d] R_f of putative 1-*O*-acetyl-FDG.
[e] After addition of AcO^{18}F, but before hydrolysis.
[f] After heating with HCl.

had an R_f of 0.67 compared with 0.46 for FDG, indicating a lower polarity. After treatment with aqueous acid, it yielded a material identical to FDG by TLC. It was rapidly converted by treatment with trimethylsilylimidazole in pyridine to a product with the same gas chromatographic retention time as tetra-(trimethylsilyl)-FDG. The ^{1}H-NMR spectrum of the material, immediately after chromatographic purification, showed a methyl singlet 2.10 ppm downfield from Me_4Si, indicating —O—C(O)—CH_3. The presence of an acetyl ester was also confirmed by the IR spectrum (1750 cm^{-1}, C=O).

FOOTNOTES

* Rohm and Haas Co., Philadelphia, PA.

† Bio-Rad, Richmond, CA 94804.

‡ See Pierce Chemical Company "Handbook and General Catalogue" for an excellent up-to-date discussion and description of silylation methods and references.

ACKNOWLEDGMENTS

This research was supported by Grant 5T32 CA09015 awarded by the National Cancer Institute, DHEW, by Grant P01 NS 15655 awarded by NINCDS, DHEW, and by the Nuclear Medicine Research Fund.

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