

RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Synthesis of No-Carrier-Added Fluorine-18 2-Fluoro-2-Deoxy-D-Glucose

Timothy J. Tewson

University of Texas Medical School at Houston, Houston, Texas

A new synthetic procedure for the preparation of fluorine-18 2-fluoro-2-deoxy-glucose has been developed. This procedure offers the advantages of flexibility in the source of the fluorine-18, high yields, and short synthesis times. The procedure works at the no-carrier-added level and gives a product of very high specific activity.

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Since the introduction in 1977 of 2-[¹⁸F]fluoro-2-deoxy-D-glucose by Ido et al. (1,2), the compound has provided a valuable tool for the study of glucose metabolism in both normal and diseased tissue (3). However, the published synthesis, based upon the addition of molecular fluorine-18 to triacetal glucal, suffers from some limitations that restrict the more widespread use of the radiopharmaceutical.

First the chemical nature of F₂ limits the nuclear reaction for the production of fluorine-18 to the ²⁰Ne(*d,α*)¹⁸F reaction, thus demanding an accelerator capable of accelerating deuterons. Some efforts have been made to overcome this problem by postbombardment exchange of F-18 species produced by the ¹⁸O(*p,n*)¹⁸F reaction with ¹⁹F₂, but the overall success of this procedure has not been established (4,5). Second, the target used for ¹⁸F-F₂ requires a deuteron beam, on the window, of an energy greater than 10 MeV, and this is higher than is available on some hospital cyclotrons (6). Third, the yield of ¹⁸F-F₂ is not a linear function of beam current. Published data on usable yield compared with current are limited to ≤15 μA but the extraction yield as a percentage of the theoretical production yield starts to fall below 15 μA (6). Other workers (7) run the target at 25 μA but with different energy, target pressure, and fluorine concentration, making it difficult to compare their data with the published extraction data. These currents are considerably lower than that available

on modern cyclotrons designed for isotope production, typically >50 μA. Fourth, the addition of F₂ to the triacetal glucal gives a mixture of 2-deoxy-2-fluoro glucal and manno-pyranosyl fluorides, which must be separated, and then the pyranosyl fluorides hydrolyzed, with loss of half of the fluorine-18 activity (2). The formation of the mannose derivative can be circumvented by use of acetyl hypofluorite (8), prepared from the ¹⁸F-F₂, but this still results in the loss of 50% of the available fluorine. These effects combine such that clinically useful quantities of 2-[¹⁸F]fluoro-2-deoxyglucose can be produced only with comparatively large cyclotrons, and even then only in sufficient quantities for one or two studies per synthetic preparation.

A synthetic procedure based upon ¹⁸F-fluoride ion would have three advantages. First the ¹⁸F-fluoride is available from all the known nuclear reactions that produce fluorine-18, and this would add considerable flexibility to the means of production of the radionuclide. Second, with fluoride all the radionuclide made can, in principle, be utilized in the required product. This will depend upon chemical yield and synthesis time, but there is no mandatory requirement to lose any isotope in obtaining the final product. Third, ¹⁸F-F⁻ can be obtained at very high specific activity, and nucleophilic displacement reactions have been successfully performed with no-carrier-added ¹⁸F-F⁻ (9). There is no known advantage in 2-deoxy-2-fluoroglucose of very high specific activity, but it has not been available in the past. One of the virtues of starting with no-carrier-added material is that the specific activity can be changed, by dilution, over several orders of magnitude, and thus the effects of specific activity can be determined.

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For reprints contact: Timothy J. Tewson, University of Texas Medical School, PO Box 20708, MSMB 1.246, Houston, TX 77025.

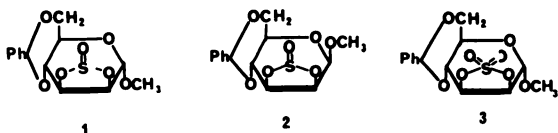


FIG. 1. 2,3-Cyclic sulfur esters of 4,6-benzylidene mannoside, which did not give fluorinated products on reaction with fluoride ion.

During the final stages of this work, a paper was published describing the synthesis of 2-deoxy-2- ^{18}F -fluoroglucose based upon the nucleophilic displacement reaction by fluoride of the trifluoromethane sulfonate group of 4,6-benzylidene-1- β , 3-O-dimethylmannopyranoside-2-trifluoromethane sulfonate (10).* The fluorination proceeds in reasonable ($\sim 30\%$) yield but considerable difficulty was encountered in removing the methyl group from the 3-oxygen, and this reduced the overall yield. Before this work it was generally accepted that simple nucleophilic displacement reactions at C-2 would fail because of competing elimination and rearrangement reactions. To overcome these presumed difficulties, a synthetic strategy based upon a cyclic sulfate as a leaving group was developed. The concept behind this was that the cyclic nature of the leaving group would prevent the competing reactions from occurring, and that the stereochemistry of the mannoside derivative at C-2 and C-3 would direct the fluorine to C-2. To this end the 2,3-cyclic sulfites and sulfates of both α and β -O-methyl 4,6-benzylidene mannopyranoside (1-4) were prepared and reacted with fluoride ion (11).

Both sulfites and the α -O-methyl sulfate (1-3), (Fig. 1) gave nonfluorinated materials as the major products, but the β -O-methylcyclic sulfate gave the required fluoro sulfate (5) in excellent yield. This was attributed, at the time (12), to the ingenuity of the cyclic sulfate approach. With the success of Levy et al., this must be modified, and it is probable that a successful nucleophilic displacement reaction will be obtainable with any carbohydrate having a good leaving group axial at C-2, a

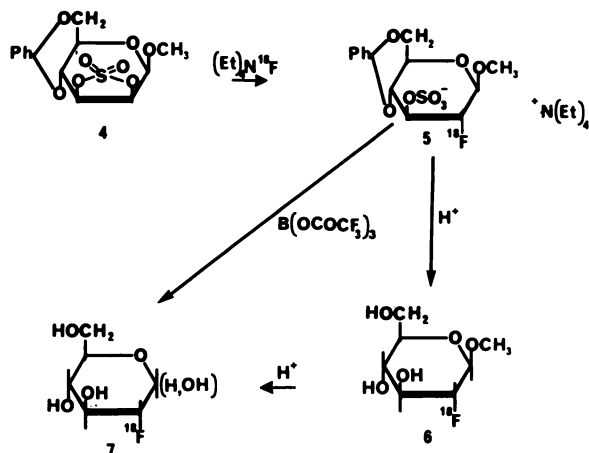


Fig. 2. Fluorination and deprotection of 4,6-benzylidene-1- β -methyl-mannopyranoside-2,3-cyclic sulfate.

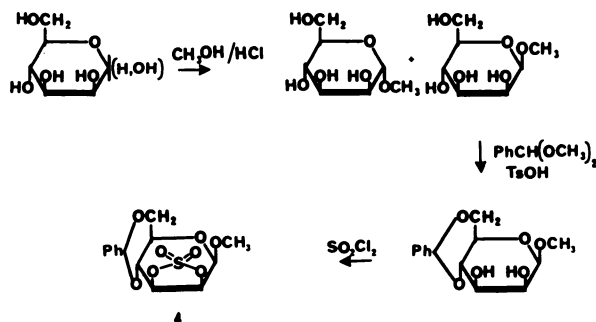


FIG. 3. Reaction sequence for synthesis of 4,6-benzylidene-1- β -methyl-mannopyranoside-2,3-cyclic sulfate.

protecting group β at C-1, and a nonparticipating protecting group at C-3. The cyclic sulfate serves as a convenient and easily removed protecting group for the 3-hydroxyl as well as a good leaving group at C-2, but any intrinsic properties in preventing rearrangements and elimination must be considered as yet unproven.

Experiments with $[\text{F-19}]$ fluoride established that the reactions of the β -O-methyl sulfate (4) (Fig. 2) with fluoride met the requirements for a successful reaction with fluorine-18. The reaction was rapid (< 5 min in refluxing acetonitrile), high yielding ($> 90\%$ based upon either substrate or fluoride), and stereochemically pure. Analysis of the reaction mixtures established that any of the positional or stereochemical alternatives could not be detected, and make up less than 2% of the total fluorinated product (11). With the reactions with stable fluoride reasonably well understood, the reaction was applied to $[\text{F-18}]$ fluoride and the results are reported here.

MATERIALS AND METHODS

Synthesis of (4). The synthesis and characterization of the sulfites and sulfates of mannopyranoside (1-4) are reported in detail elsewhere (11). Experimental details for the synthesis of (4) (Fig. 3) are as follows:

1- β -Methylmannopyranoside (13) (9 g, crystallized from isopropanol) was dissolved in 25 ml of dry DMF and evaporated to dryness. This removes the isopropanol of crystallization. The residue was dissolved in 50 ml of dry DMF and 9 g of benzaldehyde dimethyl ketal and 200 mg of paratoluene sulfonic acid were added. The solution was placed on a rotary evaporator with the water bath at 70°C and evacuated with a water aspirator. The flask was rotated for 90 min in the water bath. The solution was neutralized with triethylamine, the water aspirator replaced with a mechanical pump, and the DMF removed. The residue was dissolved in methylene chloride, washed with water, dried with sodium sulfate, and evaporated to dryness. The product was redissolved in methylene chloride and chromatographed on silica (150 g). Elution with methylene chloride washes off the dibenzylidene product. The column was then eluted with

2% methanol/methylene chloride to give the required product. Crystallization from ethyl acetate gives 5.2 g of 4,6-benzylidene-1- β -methylmannopyranoside: m.p. 173–4° (14), $R_f = 0.4$ on silica gel TLC plates run in 2% methanol/methylene chloride.

This product (2.82 g) was dissolved in dry ethyl acetate (200 ml) containing triethylamine (5.0 g). Sulfuryl chloride (2.5 g) in ethyl acetate (25 ml) was run in slowly (1 hr). The solution was stirred for a further hour, filtered and evaporated to dryness. Chromatography on Florisil (110 g) and elution with methylene chloride gives, in the first 250 ml of solvent, 4,6-benzylidene-1- β -methylmannopyranoside-2,3-cyclic sulfate (2.3 g) (4) on crystallization from heptane, m.p. 149–153°C (decomp).

Fluorine-18 tetra-ethyl ammonium fluoride. 1. *Reactor production.* LiOH was irradiated in quartz ampoules in a 1-MW research reactor to produce fluorine-18 by the ${}^6\text{Li}(n,\alpha){}^3\text{H}$, ${}^{16}\text{O}({}^3\text{H},n){}^{18}\text{F}$ reaction. Details of the conversion of the fluorine-18 activity to a reproducible and reactive solution of tetraethylammonium [${}^{18}\text{F}$]fluoride have been reported (15).

2. *Accelerator production.* Oxygen-18 was bombarded in a glass-lined target with 11-MeV protons at 2 μA (4). After bombardment the ${}^{18}\text{O}_2$ was removed and retained by cryopumping, and the glass liner removed. The fluorine-18 activity produced is absorbed on the liner. Boiling this liner with water gave an aqueous solution that contains ~80% of the fluorine-18 (4). The solution was passed through a cation-exchange resin and then treated in the same fashion as the reactor-produced material.

Fluorination Reactions. To a dry acetonitrile solution (5 ml), containing the no-carrier-added tetraethylammonium fluoride (F-18) and tetraethylammonium hydroxide (20 μmole), was added the cyclic sulfate (4) (8 mg, 20 μmole). The solution was heated to 55°C for 15 min and the extent of reaction was routinely monitored by passage of a small aliquot of the reaction mixture through an alumina column and elution with water. Fluoride ion is totally retained by alumina and the sulfate salt (5) washes through with water. The column and the eluant were then counted in a dose calibrator. Blank runs—that is, solutions containing everything but the sulfate (4)—established that fluoride ions are totally retained on the alumina. These results were occasionally checked by chromatography on a C_{18} reverse-phase column eluted with a 1:1 mixture of 0.01 M ammonium acetate and acetonitrile at 4 ml/min. Under these conditions fluoride ion elutes at the void volume and the sulfate (5) is eluted 2 min later. The retention time of the fluorine-18-labeled sulfate, as detected by a flowthrough scintillation counter, was compared with a sample of authentic material detected by uv absorption at 254 nm. The yield was determined by collecting the radioactive peak for (5) and comparing its activity with that of a

sample of the same size that was not chromatographed. In all cases the two methods agreed within experimental error.

Deprotection of the sulfate (5). The acetonitrile solution from the previous step was evaporated to dryness, and boron tris(trifluoroacetate) (2 ml of a 1 M solution in trifluoroacetic acid) was added and stirred until the residue dissolved. The solution was kept at room temperature for 10 min and the trifluoroacetic acid evaporated on a rotary evaporator. Water was added and the pH of the solution adjusted to 10 with sodium hydroxide solution. This solution was then passed through an ion-retardation resin column† (5 g) to remove the borate salts, and a short alumina column to remove any fluoride ion produced during the hydrolysis.

The resulting solution was then analyzed for 2-deoxy-2-fluoroglucose by chromatography on an amino-column† eluted with 70% acetonitrile/water at 2 ml/min. The retention times of the fluoro sugar (7) (5 min) and the methyl glucoside (6) (3.5 min) were measured by detection of radioactivity with a flowthrough scintillation detector and compared with authentic compounds detected with a differential refractometer. Yields were measured by collecting and counting the peaks and comparing the results with an equivalent sample of the reaction solution.

RESULTS AND DISCUSSION

Reactions using tetramethylammonium[${}^{19}\text{F}$]fluoride on a macroscopic scale had shown that in refluxing acetonitrile, reaction with the cyclic sulfate was complete in less than 5 min. However, with tetraethylammonium fluoride the reflux temperature of acetonitrile (88°C) is high enough to give some decomposition, so reaction temperatures were maintained at 55°C. Under these conditions, using the reactor-produced fluorine-18, reaction to give the sulfate salt (5) went to >90% completion based upon fluoride, in 15 min. Results with the accelerator-produced fluorine-18 were broadly comparable but with more variability of the yields from run to run. This variation is apparently due to differences in the glass liner.

With the sulfate salt (5) in hand, the final necessary step was removal of the protecting groups to give 2-deoxy-2-fluoroglucose. The benzylidene and sulfate groups were labile and easily removed by mild acid hydrolysis to give (6). However, the β -O-methyl group was considerably more resistant and rapid hydrolysis presented some difficulties. A variety of acidic reagents were tried, all of which removed the glycosidic methyl group but also resulted in partial defluorination of the product to give the 1,6-anhydrocompound, apparently through the 2,6-anhydro compound (11,16).

Of the reagents tried for the hydrolysis, boron tris(trifluoroacetate) gave the best combination of speed and

selectivity, although even with this reagent some defluorination did occur with longer reaction times. Thus, reaction for 5 min at room temperature gave the fluoro sugar (7) in an overall yield of 70%, but analysis showed that this solution contained approximately 15% of β -methyl-2-deoxy-2-fluoro-glucopyranoside (6). Reaction for 10 min gave an overall yield of 40% with impurities amounting to less than 2%.

Thus under standard conditions, with initial solutions containing 400 μ Ci of tetraethylammonium fluoride (F-18), 280 μ Ci of the fluoro sugar at 85% purity or 160 μ Ci at >98% purity were obtained with synthesis times of 35–40 min. The specific activity was not measured, but no special precautions were taken to exclude extraneous fluoride, so it was probably two or three orders of magnitude off the theoretical value (9).

CONCLUSION

The synthetic method described for 2-[¹⁸F]fluoro-2-deoxy glucose provides greater flexibility in the source of fluorine-18 than the previous procedure. On a small scale there is also a considerable improvement in yield. Although constraints on the availability of accelerator time have limited our experiments with larger quantities of fluorine-18, there are reasonable grounds for optimism that the procedure can be transferred to the multimilliecurie level required for clinical utility.

FOOTNOTES

* After submission of this manuscript another synthesis of 2-deoxy-2-fluoroglucose was published, based upon the reaction of KHF₂ with an acyclic nitroepoxide. Szarek WA, et al: *JCS Chem Commun* 1253, 1982.

† Sigma.

‡ Altech, 10 μ m.

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REFERENCES

1. IDO T, WAN C-N, FOWLER JS, et al: Fluorination with F₂. A convenient synthesis of 2-deoxy-2-fluoro-D-glucose. *J Org Chem* 42:2341–2342, 1977
2. IDO T, WAN C-N, CASELLA V, et al: Labeled 2-deoxy-D-glucose analogs. ¹⁸F-labeled 2-deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose, and ¹⁴C-2-deoxy-2-fluoro-D-glucose. *J Label Comp Radiopharm* 14:175–183, 1978
3. PHELPS ME, MAZZIOTTA JC, HUANG SC: Study of cerebral function with positron computed tomography. *Cerebral Blood Flow Metab* 2:113–162, 1982
4. NICKLES RJ, DAUBE ME, HUTCHINS GD: Smaller, Colder Targets. 4th International Symposium on Radiopharmaceutical Chemistry. Jülich, West Germany, August, 1982. *J Label Comp Radiopharm* (in press)
5. STRAATMANN MG, SCHLYER DJ, CHASKO J: Synthesis of F-18-Fluorine gas from no carrier added F-18-HF. 4th International Symposium on Radiopharmaceutical Chemistry. Jülich, West Germany, August, 1982. *J Label Comp Radiopharm* (in press)
6. CASELLA V, IDO T, WOLF AP, et al: Anhydrous F-18 labeled elemental fluorine for radiopharmaceutical preparation. *J Nucl Med* 21:750–757, 1980
7. BARRIO JR, MACDONALD NS, ROBINSON GD, et al: Remote semiautomated production of F-18-labeled 2-deoxy-2-fluoro-D-glucose. *J Nucl Med* 22:372–375, 1981
8. SHIUE C-Y, SALVADORI PA, WOLF AP, et al: A new improved synthesis of 2-deoxy-2-[¹⁸F]Fluoro-D-glucose from ¹⁸F-labeled acetyl hypofluorite. *J Nucl Med* 23:899–903, 1982
9. TEWSON TJ, WELCH MJ: Preparation and preliminary biodistribution of “no carrier added” fluorine-18 fluoroethanol. *J Nucl Med* 21:559–564, 1980
10. LEVY S, ELMALEH DR, LIVNI E: A new method using anhydrous [¹⁸F]fluoride to radiolabel 2-[¹⁸F]fluoro-2-deoxy-D-glucose. *J Nucl Med* 23:918–922, 1982
11. TEWSON TJ: Cyclic sulfur esters as substrates for nucleophilic substitution: A new synthesis of 2-deoxy-2-fluoro-glucose. *J Org Chem* (in press)
12. TEWSON TJ, GOULD KL: Synthesis of 2-deoxy-2-fluoro-glucose using fluoride ion as a source of fluorine. *J Nucl Med* 23:P109, 1982
13. BOTT HG, HAWORTH WN, HIRST EL: The structure of carbohydrates and their optical rotatory power. Part IV. Derivatives of α and β -methylmannopyranoside. *J Chem Soc* 2653–2659, 1931
14. GAREGG PJ: Benzylidene derivatives of mannose. *Ark Kimi* 23:255–268, 1965
15. GATLEY SJ, HICHIWA RD, SHAUGHNESSY WJ, et al: ¹⁸F-labeled lower fluoroalkanes: Reactor-produced gaseous physiological tracers. *Int J Appl Radiat Isot* 32:211–214, 1981
16. MILLER JN: Acid hydrolysis of glycosides. *Adv Carbohydr Chem* 22:25–108, 1967