
Efficient Stereospecific Synthesis of No-Carrier-Added 2-[¹⁸F]-Fluoro-2-Deoxy-D-Glucose Using Aminopolyether Supported Nucleophilic Substitution

K. Hamacher, H. H. Coenen, and G. Stöcklin

Institut für Chemie 1 (Nuklearchemie), Kernforschungsanlage Jülich GmbH, Jülich, FRG

An aminopolyether mediated synthesis of fluorine-18 (¹⁸F) 2-fluoro-2-deoxy-D-glucose (FDG) has been developed. The nucleophilic fluorination with accelerator-produced [¹⁸F]fluoride works at the no-carrier-added level and gives epimerically pure 2-¹⁸FDG with an uncorrected radiochemical yield of a maximum 50% in a synthesis time of ~ 50 min from EOB.

J Nucl Med 27:235-238, 1986

In conjunction with positron emission tomography (PET), 2-[¹⁸F]fluoro-2-deoxy-D-glucose (2-FDG) is presently the most important radiopharmaceutical and is used to measure regional cerebral glucose metabolism (1). The broad application of this radiolabeled carbohydrate leads to a variety of alternative syntheses with the aim of providing higher radiochemical yields and increasing the stereoselectivity of the fluorination reaction.

The synthesis routes of 2-FDG include electrophilic fluorinations with ¹⁸F-F₂ (2-4), Xe¹⁸F₂ (5-7), or acetylhypofluorite (4,8-10) as fluorinating agents and nucleophilic reactions with anhydrous [¹⁸F]fluoride (10,11). Although the electrophilic reaction of acetylhypofluorite with tri-*O*-acetyl-D-glucal (9) is the most commonly used method to produce 2-FDG for medical research, the synthesis exhibits only limited stereospecificity (12) similar to the reaction of F₂ with D-glucal. Any electrophilic process starting from ¹⁸F-F₂ leads to a loss of 50% in addition to losses by lacking stereospecificity of the available fluorine-18 (¹⁸F). Furthermore, the product has only low specific activity in the order of a few Ci/mmol.

The synthetic procedure starting from [¹⁸F]fluoride has several advantages. In contrast to the production of carrier-added ¹⁸F by the ²⁰Ne(d,α)¹⁸F reaction in the

presence of ¹⁹F₂, the [¹⁸F]fluoride can be obtained with very high specific activity (no-carrier-added), e.g., from the nuclear reaction of ¹⁸O(p,n)¹⁸F using an oxygen-18 (¹⁸O) enriched water target (13). This reaction can be carried out with a small 10 MeV proton accelerator.

Three successful nucleophilic syntheses of 2-¹⁸FDG are published. One based upon the replacement of the triflate group of methyl 4,6-*O*-benzylidene-3-*O*-methyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranoside by ¹⁸F⁻ (10) and the other on the reaction of [¹⁸F]fluoride with methyl 4,6-*O*-benzylidene-2,3-*O*-sulfuryl-β-D-mannopyranoside (11). The substitution of the triflate group proceeds with a yield of about 30%, but the difficulty in removing the methyl group from the 3-*O*-position reduced the overall yield significantly (~10%). The method developed by Tewson (11) leads to an excellent incorporation of ¹⁸F⁻ into the cyclic sulfuryl compound (>90%), but the hydrolysis of the glycoside resulted in a considerable reduction of the radiochemical yield to about 40% or less. A paper has recently been published describing the reaction of anhydrous no-carrier-added KH[¹⁸F]F₂ with 1,2-anhydro-3,4:5,6-di-*O*-isopropylidene-1-C-nitro-D-mannitol (14). Hydrolysis of the ¹⁸F-labeled derivative with trifluoroacetic acid afforded 2-¹⁸FDG in a radiochemical yield of 10%.

The goal of this study was to use (a) the tetraacetylated D-mannose, i.e., 1,3,4,6-tetra-*O*-acetyl-2-trifluoromethanesulfonyl-β-D-mannopyranose (1, Fig. 1) as a precursor, and (b) the aminopolyether potassium complex [K/2.2.2]⁺¹⁸F⁻ as a phase-transfer catalyst. This

Received May 29, 1985; revision accepted Sept. 18, 1985.

For reprints contact: Kurt Hamacher, PhD, Institut für Chemie 1 (Nuklearchemie), Kernforschungsanlage Jülich GmbH, Postfach 1913, D-5170 Jülich, FRG.

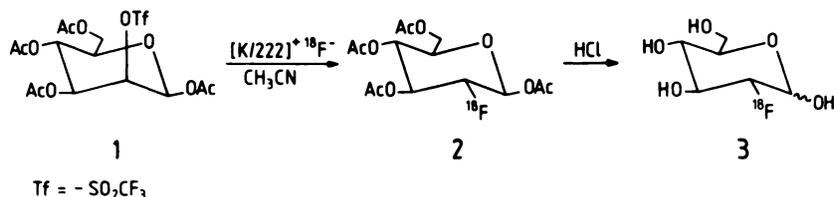


FIGURE 1
Reaction scheme—syntheses of 2-
fluoro-2-deoxy-D-glucose

complex has recently been shown to allow a mild and efficient nucleophilic fluorination at a no-carrier-added level (15–17). The resulting increase of nucleophilicity greatly facilitates the fluorination procedure. By using the tetraacetylated precursor which can be selectively prepared (19), the removal of the protecting groups can be carried out rapidly under mild conditions and hence higher yields of 2-FDG can be obtained.

MATERIALS AND METHODS

Accelerator Production of [^{18}F]Fluoride

Fluoride-18 was produced* by the $^{20}\text{Ne}(d,\alpha)^{18}\text{F}$ reaction using a Ne (15% H_2) target (18) to produce no-carrier-added ^{18}F -HF, which was removed from the target wall after bombardment by rinsing with triply distilled water. The conversion of no-carrier-added ^{18}F activity to a reproducible and reactive fluoride labeling system using the bicyclic aminopolyether Kryptofix 222† was carried out similar to the method reported previously (15–17).

Cold Syntheses

The synthesis and characterization of the precursor 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl- β -D-mannopyranose have been published elsewhere (19).

For the purpose of confirming the structure of the synthetic product by ^{19}F nuclear magnetic resonance (NMR) spectroscopy, it was necessary to carry out an inactive synthesis to obtain macroscopic amounts of unlabeled 2-fluoro-2-deoxy-D-glucose. The reaction scheme is shown in Fig. 1. The experimental details for the synthesis are as follows: 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl- β -D-mannopyranose (0.48 g; 1 mmol) and 0.38 g (1 mmol) of the cryptand Kryptofix 222 were dissolved in 10 ml of dry acetonitrile and heated under reflux for 15 min in the presence of 41 mg (0.7 mmol) KF and 25 mg (0.15 mmol) K_2CO_3 . The residue was filtered off and the solution concentrated on a rotary evaporator to dryness (bath-temperature $<50^\circ\text{C}$). To remove the phase-transfer catalyst and inorganic components the viscous residue was extracted three times with 5 ml of water and then heated under reflux in the presence of 10 ml of 1M hydrochloric acid for 20 min. The acid solution was deionized using ion retardation resin AG11A8 (50–100 mesh)‡, concen-

trated to dryness and dissolved in D_2O to measure the ^{19}F -NMR spectrum.

Sugar Analysis

The crude reaction mixture of the 2-FDG synthesis was analyzed by anion exchange chromatography (AEC) of the sugar borate complexes. The AEC was performed with an automated sugar analyzer Biotronik ZA 5100§ as described previously (20).

Thin layer chromatography (TLC) was performed on silica gel 60† with the solvent system acetonitrile/water (95:5). The spray reagent orcinol-sulfuric acid was used for detection. The radiochemical purity was ascertained by TLC on monosodium phosphate impregnated silica plates (12). This modified TLC method allows the separation of FDG and FDM by developing the plates several times with $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, (95:5) as eluent. Radio high performance liquid chromatography (HPLC) on Lichrosorb- NH_2 (10) (column 250×4 †, eluent $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 95:5, flow: 1 ml/min) of the no-carrier-added product was compared with authentic samples of 2-FDG/2-FDM.

Reactive [^{18}F]Fluoride Labeling System

In a cylindrical reaction vessel of pyrolytic carbon (Sigradur-G, 18×70 mm)** the aqueous solution of no-carrier-added ^{18}F (0.5–1 mCi for the test runs and 20–50 mCi for a production run) was added to a solution of 4.6 mg (0.03 mmol) potassium carbonate and 26 mg (0.06 mmol) Kryptofix 222 in acetonitrile- H_2O (86:14) (v/v). At an oil bath temperature of about 105°C the solution was purged with helium (~ 50 ml/min) and concentrated to dryness. After the solvent was removed (~ 3 min) the drying process was extended for about 3 min to remove traces of remaining water.

Synthesis of 2- ^{18}F FDG

A solution of 20 mg (0.04 mmol) 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl- β -D-mannopyranose in 1 ml anhydrous acetonitrile was added to the dry residue of [^{18}F] fluoride containing aminopolyether (APE)-complexed potassium carbonate. This mixture was heated under reflux for about 5 min. The solution was concentrated to ~ 0.4 ml, transferred into a syringe with about 5 ml distilled water, and passed through a C18 SEP-PAK cartridge†† which had been previously washed with 2 ml of THF and 5 ml of water. Residual

aminopolyether was desorbed completely by washing the C18 SEP-PAK cartridge with 5 ml of hydrochloric acid (0.1 mol/l). The acetylated carbohydrates were subsequently eluted from the cartridge with 2 ml THF and the solution evaporated to dryness in the Sigradur reaction vessel. Two milliliters of 1M hydrochloric acid was added and heated under reflux for 15 min (bath temperature: 130°C). The hydrolysate was decolorized by passing through the same C18 SEP-PAK cartridge as used before. The carbon vessel was rinsed with 1 ml of water and used to elute residual ¹⁸FDG from the cartridge. For deionizing the hydrolysate, the acid solution was transferred to a column packed with AG11A8 retardation resin and neutral aluminium oxide 90† (4). The resulting neutral eluent was adjusted to an isotonic solution and finally sterilized by passage through a Millipore filter (0.22 μm).

RESULTS AND DISCUSSION

Fluorination of 1.3.4.6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose using APE-complexed potassium fluoride on a macroscopic scale yielded peracetylated 2-fluoro-2-deoxy-D-glucose (2, Fig. 1), in ~ 50%. Based on the ¹⁹F-NMR data of the crude synthetic product after acid hydrolysis, it is obvious that the nucleophilic substitution gave pure 2-¹⁹FDG without any formation of the epimer 2-fluoro-2-deoxy-D-mannose.

The chemical shifts observed were in agreement with data available from the literature for 2-FDG (4,21). The δ-values found for 2-FDG were 32.44 ppm (α-anomer) and 32.26 ppm (β-anomer) with a ¹H-¹⁹F coupling constant of J_{H-2,F} = 50 Hz and J_{H-3,F} = 15 Hz, respectively. TLC of the hydrolyzed crude reaction product showed two major products, with R_f 0.37 identical to 2-FDG and a spot with R_f 0.05 which was due to aldohexoses. Ion exchange chromatography of the sugar borate complexes demonstrated that besides 2-FDG only glucose could be detected in the acid hydrolysate of the crude reaction mixture.

The displacement of the triflate group using the aminopolyether-complexed potassium salt of no-carrier-added [¹⁸F]fluoride in the presence of potassium carbonate occurred within less than 5 min (80° C). Although some decomposition was observed during refluxing of acetonitrile, the no-carrier-added [¹⁸F]fluoride was incorporated with formation of acetylated 2-¹⁸FDG to about 95%. Labeling results of no-carrier-added substitution reaction are reproducible since the amount of [¹⁸F]fluoride remaining on the wall of the glassy carbon reaction vessel is <3%. The acetylated 2-¹⁸FDG and the excess of triflate were separated from the water-soluble components using a C18 SEP-PAK cartridge that adsorbed the lipophilic substances while the hydrophilic aminopolyether (Kryptofix 222) and

inorganic salts were eluted completely by water-acetonitrile (90:10) and subsequently washed with 0.1M hydrochloric acid. After desorption of the labeled acetylated carbohydrate from the cartridge, the final step was to remove the acetyl groups to give 2-¹⁸FDG.

In contrast to the etherified and glycosylated sugar derivatives (10,11) the acetyl groups could be removed easily using acid hydrolysis conditions as described for the corresponding glucal derivative (9).

The light-yellow acid hydrolysate containing the unprotected 2-¹⁸FDG could be decolorized by filtration through the same C18-cartridge used before. The 2-¹⁸FDG solution was neutralized by an ion retardation resin and traces of fluoride were adsorbed on aluminium oxide. After this purification step the uncorrected yield of 2-[¹⁸F]fluoro-2-deoxy-D-glucose was 44±4% (n = 7) and the total time for the preparation was 45 to 50 min. Within experimental error no change in radiochemical yield was observed for runs ranging from 1 to 50 mCi of no-carrier-added [¹⁸F]fluoride. TLC (MeCN:H₂O/95:5) of the FDG solution has shown that ~ 99% of the [¹⁸F]fluorine was present as 2-¹⁸FDG (R_f 0.37) whereas only 0.5 to 1% of the ¹⁸F⁻ activity was located at the starting point. The TLC on monosodium phosphate impregnated silica plates as described by van Rijn et al. (12) makes it feasible to separate the epimeric sugars FDG and FDM. Using this modified TLC method, only one radioactive component with a R_f-value equivalent to that of FDG appeared to be present.

Additionally, the isocratic HPLC (Lichrosorb-NH₂ column) gave the same retention time for the radiochemical product and the authentic 2-FDG sample (HPLC retention time 3.9 min). As in the case of the TLC, radiochemical impurities were not detected.

The nucleophilic ¹⁸F fluorination was also performed satisfactorily in anhydrous THF but the reaction time of 25 to 30 min was significantly longer than in the dipolar solvent acetonitrile (5 min).

CONCLUSION

The advantage of the synthetic method presented here is the high yield (max. 55% uncorrected) of no-carrier-added 2-¹⁸FDG based on the phase-transfer mediated substitution of triflate by [¹⁸F]fluoride. The stereochemical specificity of the nucleophilic displacement combined with a rapid hydrolysis of the acetylated sugar derivative makes it possible to synthesize epimerically pure 2-¹⁸FDG with high specific activity. The synthesis of 2-¹⁸FDG was carried out successfully with larger quantities of [¹⁸F]fluoride suitable for clinical use. In addition, the precursor 1.3.4.6 tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose can be easily prepared in a two step reaction starting with D-mannose.

FOOTNOTES

- * Jülich (Compact cyclotron CV-28), Jülich, FRG.
- † E. Merck AG, Darmstadt, FRG.
- ‡ BIO-RAD, Richmond, CA.
- § Biotronik, Munich, FRG.
- ¶ Merck, Darmstadt, FRG.
- ** SIGRI, Meitingen, FRG.
- †† Waters Chromatography Div., Millipor, MA.

ACKNOWLEDGMENT

The authors thank Mr. G. Blessing for carrying out the ^{18}F production and Mr. P. Laufer for performing the NMR analyses.

REFERENCES

1. Walker MD: Research issues in positron emission tomography. *Ann Neurol* (Suppl)15: 1984
2. Ido T, Wan C-N, Fowler JS, et al: Fluorination with molecular fluorine. A convenient synthesis of 2-deoxy-2-fluoro-D-glucose. *J Org Chem* 43:2341-2342, 1977
3. Fowler JS, MacGregor RR, Wolf AP, et al: A shielded synthesis system for production of 2-deoxy-2- ^{18}F fluoro-D-glucose. *J Nucl Med* 22:376-380, 1981
4. Bida TG, Satyamurthy N, Barrio JR: The synthesis of 2- ^{18}F fluoro-2-deoxy-D-glucose using glycals: A reexamination. *J Nucl Med* 25:1327-1334, 1984
5. Korytnyk W, Valentekovic-Horvat S: Reactions of glycals with xenon fluoride: An improved synthesis of 2-deoxy-2-fluoro-saccharides. *Tetrahedron Lett* 21:1493-1496, 1980
6. Shiue C-Y, To K-C, Wolf AP: A rapid synthesis of 2-deoxy-2-fluoro-D-glucose from xenon difluoride suitable for labelling with ^{18}F . *J Label Comp Radiopharm* 20:157-162, 1983
7. Sood S, Firnau G, Garnett ES: Radiofluorination with xenon difluoride: A new high yield synthesis of ^{18}F 2-fluoro-2-deoxy-D-glucose. *J Nucl Med* 24:718-721, 1983
8. Shiue C-Y, Salvadori PA, Wolf AP, et al: A new improved synthesis of 2-deoxy-2- ^{18}F fluoro-D-glucose from ^{18}F -labeled acetyl hypofluorite. *J Nucl Med* 23:899-903, 1982
9. Ehrenkauf RE, Potocki JF, Jewett DM: Simple synthesis of F-18-labeled 2-fluoro-2-deoxy-D-glucose: Concise communication. *J Nucl Med* 25:333-337, 1984
10. Levy S, David RE, Livni E: A new method using anhydrous ^{18}F fluoride to radiolabel 2- ^{18}F fluoro-2-deoxy-D-glucose. *J Nucl Med* 23:918-922, 1982
11. Tewson TJ: Synthesis of no-carrier-added fluorine-18 2-Fluoro-2-deoxy-D-glucose. *J Nucl Med* 24:718-721, 1983
12. Van Rijn CJS, Herscheid JDM, Visser GW, et al: On the stereoselectivity of the reaction of ^{18}F acetyl hypofluorite with glycals. *Int J Appl Radiat Isot* 36, 2:111-115, 1985
13. Kilbourn MR, Hood JT, Welch MJ: A simple ^{18}O water target for ^{18}F production. *Int J Appl Radiat Isot* 35(7):599-602, 1984
14. Beeley PA, Szarek WA, Hay GW, et al: A synthesis of 2-deoxy-2- ^{18}F fluoro-D-glucose using accelerator-produced ^{18}F -fluoride ion generated in a water target. *Can J Chem* 62: 2709-2711, 1984
15. Klatte B: Ph.D. Thesis, University of Hamburg, FRG, 1984
16. Coenen HH, Colosimo M, Schüller M, et al: Mild and effective aliphatic and aromatic n.c.a. ^{18}F -fluorination using crown ether. *J Nucl Med* 26:P37, 1985 (abstr)
17. Coenen HH, Schüller M, Stöcklin G, et al: Verfahren zur Herstellung von ^{18}F -Alkyl- und Arylverbindungen durch nukleophilen Austausch. German patent application P. 3424525.1
18. Lambrecht RM, Neirinckx R, Wolf AP: Cyclotron isotopes and radiopharmaceuticals XXIII. Novel anhydrous ^{18}F -fluorinating intermediates. *J Appl Radiat Isot* 29:175, 1978
19. Hamacher K: Phase-transfer catalysed synthesis of 4-S- β -D-glucopyranose-4-thio-D-glucopyranose (thiocellobiose) and 2-S- β -D-glucopyranosyl-2-thio-D-glucopyranose (thiosophorose). *Carbohydrate Res* 128:291-295, 1984
20. Hamacher K, Schmid G, Sahn H, et al: Structural heterogeneity of celooligomers homogeneous according to high-resolution size-exclusion chromatography. *J Chrom* 319: 311-318, 1985
21. Phillips L, Wray V: Stereospecific electronegative effects. Part 1. The ^{19}F nuclear magnetic resonance spectra of deoxyfluoro-D-glucopyranoses. *J Chem Soc (B)*: 1618-1624, 1971