A NEW SYNTHESIS OF 2-DEOXY-2-[^{18}F]FLUORO-D-GALACTOSE USING [^{18}F]FLUORIDE ION

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A nucleophilic radiofluorination of methyl 3,4-O-isopropylidene-2-O-(trifluoromethanesulfonyl)-6-O-trityl-β-D-talopyranoside (1) with aminopolyether (Kryptofix 2.2.2.) supported potassium [^{18}F]fluoride in acetonitrile gave methyl 2-deoxy-2-[^{18}F]fluoro-3,4-O-isopropylidene-6-O-trityl-β-D-galactopyranoside (2). Hydrolysis of 2 with 6N HCl followed by passage through an ion retardation resin column and a neutral alumina column gave 2-deoxy-2-[^{18}F]fluoro-D-galactose (2-^{18}FDGal) in 36-39% overall radiochemical yield without correction for decay. The total preparation time was about 90 min from start of the radiofluorination.

Keyword: radiofluorination, no-carrier-added, methyl 3,4-O-isopropylidene-2-O-(trifluoromethanesulfonyl)-6-O-trityl-β-D-talopyranoside, 2-deoxy-2-[^{18}F]fluoro-D-galactose

INTRODUCTION

^{18}F-Labeled 2-deoxy-2-fluoro-D-galactose (2-^{18}FDGal) has recently been proposed as an useful tracer for the measurement of galactose metabolism in the liver by positron emission tomography (1). The current synthesis of this radiopharmaceutical is based on the electrophilic addition of either [^{18}F]F_2 (2) or [^{18}F]CH_3COOF (3) to 3,4,6-tri-O-acetyl-D-galactal followed by acid hydrolysis, giving 2-^{18}FDGal in low radiochemical yields of 10-13%. A higher yield route to 2-^{18}FDGal is still desirable for its clinical application. [^{18}F]Fluoride ion has become a vital precursor to the preparation of a wide range of ^{18}F-labeled compounds, since reactive nucleophilic [^{18}F]fluorinating agents can easily be prepared from [^{18}F]fluoride ion generated in an ^{18}O-enriched water target (4), as exemplified by recent developments in ^{18}F-neuroleptic syntheses (5). A nucleophilic approach to the synthesis of 2-^{18}FDGal based on the displacement of a highly reactive leaving group by [^{18}F]fluoride ion, similar to that developed

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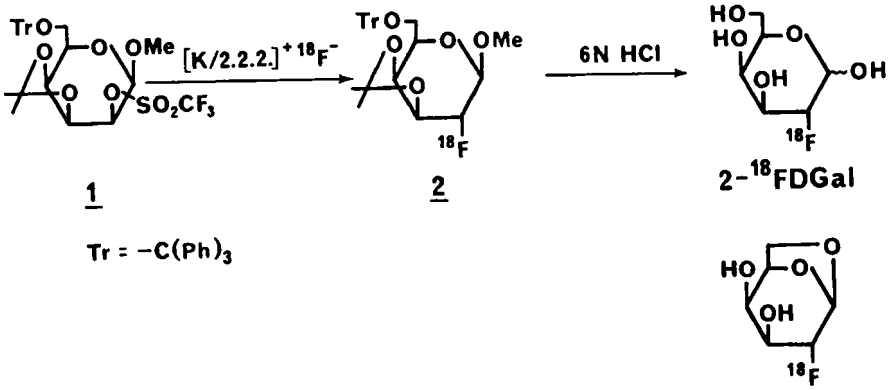
recently to prepare no-carrier-added (NCA) 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-^{18}FDG) (6-8), appeared to be an attractive alternative for the purpose of improving the yield. This paper describes a new improved synthesis of 2-^{18}FDGal using the nucleophilic displacement of the C_2-triflate function of a protected talopyranoside with [^{18}F]fluoride ion.



Scheme 1

RESULTS AND DISCUSSION

The present methodology is based on our previous report on the synthesis of unlabeled 2-FDGal, involving the fluorination of methyl 3,4-O-isopropylidene-2-O-(trifluoromethanesulfonyl)-6-O-trityl-β-D-talopyranoside (1) and subsequent acid hydrolysis (9). The NCA [^{18}F]fluoride activity obtained from the ^{18}O(p, n)^{18}F reaction on a 8% ^{18}O-enriched water target was converted to the aminopolyether (Kryptofix 2.2.2.) potassium complex ([K/2.2.2.]$^{+18}$F$^-$) by a method similar to that described in the literature (10, 11). The preparation of this [^{18}F]fluorinating agent and the subsequent substitution step were carried out using a Polymethylpentene (TPX) vessel, in which resolubilization of the fluoride has previously been shown to give a high yield (8, 12). The reaction of 1 with NCA [K/2.2.2.]$^{+18}$F$^-$ in acetonitrile at 75°C for 20 min gave a single radioactive product with identical chromatographic characteristics to authentic methyl 2-deoxy-2-fluoro-3,4-O-isopropylidene-6-O-trityl- β-D-galactopyranoside when analyzed by normal-phase HPLC and TLC. After purification by HPLC, the ^{18}F-intermediate (2) with >99% radiochemical purity was isolated in 62-63% yield

with a processing time of about 40 min.

The deprotection by acid hydrolysis of the ^{18}F-intermediate (2) proceeded by heating with 6N HCl at 115°C, requiring a 30 min period to remove all the protecting groups. Thus 2-^{18}FDGal, after passage through an ion retardation resin column and a neutral alumina column, was obtained in 36-39% overall radiochemical yield (without correction for decay). The total preparation time was about 90 min.

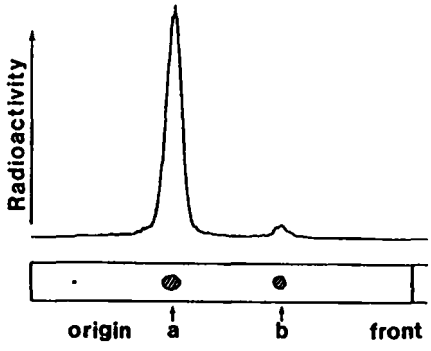


Fig. 1

A TLC chromatogram of the 2-^{18}FDGal fraction obtained by adding authentic carriers as standard.
Solvent system: CH_3CN - H_2O (95:5)
a: 2-FDGal; b: 1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose

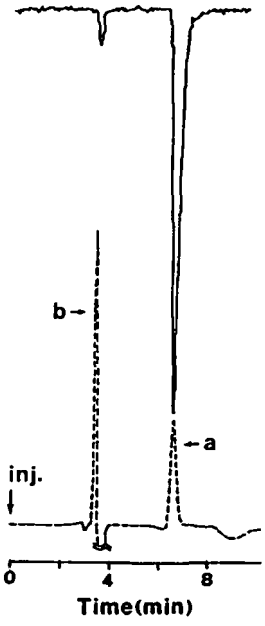


Fig. 2

A HPLC chromatogram of the 2-^{18}FDGal fraction obtained by adding authentic carriers as standard.
Mobile phase: CH_3CN - H_2O (85:15);
Flow rate: 1 ml/min; a: 2-FDGal;
b: 1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose; Radioactivity (——);
Refractive index (---)

When the final product was analyzed by both TLC (silica gel; CH_3CN : H_2O = 95 : 5) and HPLC (carbohydrate analysis column, Waters; CH_3CN : H_2O = 85 : 15), the radiochemical purity of 2-^{18}FDGal was about 95%. The only detectable contaminant was a small amount (-5%) of 1,6-anhydro-2-deoxy-2-[^{18}F]fluoro-β-D-galactopyranose (3) as determined by the TLC and HPLC comparison with authentic sample (Fig. 1 and Fig. 2). A similar type of anhydride formation has been

observed in the case of the preparation of NCA 2-^{18}FDG involving a deblocking step by strong acid (8). It was previously shown by ^{19}F-NMR spectroscopy that interconversion of 2-FDG and 2-deoxy-2-fluoro-D-mannose (2-FDM) is caused by treatment with strong acids, the extent of which depends on the concentration of acid (13). Separate experiments using ^{19}F-NMR spectroscopy revealed that 2-FDGal, on heating in 6N HCl solution for 30 min, underwent intramolecular dehydration to give appreciable extent (2-4%) of 1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose, but epimerization to 2-deoxy-2-fluoro-D-talose was only less than 1%, indicating that 2-FDGal is considerably more resistant to acid-catalyzed epimerization at C_2 when compared with 2-FDG or 2-FDM. Therefore, the contamination of 2-deoxy-2-[^{18}F]fluoro-D-talose due to epimerization of 2-^{18}FDGal during the deblocking process in the present study is so little as to be neglected, although this remains to be determined conclusively.

In conclusion, the present method provides the first successful synthesis of NCA 2-^{18}FDGal using [^{18}F]fluoride ion and produces a greater radiochemical yield than the previously published methods using electrophilic [^{18}F]fluorinating agents (2,3,14). Although the 2-^{18}FDGal obtained by this procedure contained a small amount (~5%) of the dehydration product (3) as an impurity, the radiochemical purity of 95% may be acceptable for medical research.

EXPERIMENTAL

2-FDGal was prepared according to the method published previously (9). Unless stated otherwise, all chemicals and reagents were obtained commercially and used without further purification. Acetonitrile was distilled from CaH_2. All melting points (mp) are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL FX-100 spectrometer with tetramethylsilane as an internal standard. High performance liquid chromatography (HPLC) was performed using a Waters Liquid Chromatograph System equipped with either a 254 nm U.V. absorbance detector (Model 441) or a differential refractometer (R 401). The effluent from the column was continuously monitored for radioactivity using NaI(Tl) scintillation detector system. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck). Products were co-spotted with authentic samples and, after develop-

ment, spots were visualized by spraying with aqueous sulfuric acid followed by heating, and activity distribution on the plates were measured by a TLC radiochromatogram scanner (Aloka). Conditions for all chromatographic separations are given in the following experimental sections. The radioactivity was also quantified with a Capintec Radioisotope Calibrator (CRC-30). The radiochemical yields are expressed at the end of synthesis (not corrected for decay) relative to the amount of the $[K/2.2.2.]^{+18}F^-$ measured as total radioactivity present in the reaction vessel.

Preparation of 1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose

Conventional sulfonylation of 1,6-anhydro-3,4-O-isopropylidene-β-D-talopyranose (300 mg, 1.5 mmol) (15) with trifluoromethanesulfonic anhydride (0.4 ml, 2.4 mmol) in a mixture of CH_2Cl_2 (15 ml) and pyridine (0.74 ml) followed by column chromatography on silica gel with $CHCl_3$ afforded 1,6-anhydro-3,4-O-isopropylidene-2-O-(trifluoromethanesulfonyl)-β-D-talopyranose (470 mg, 95%) as a white powder, after recrystallization from ether, mp 104-106°C (decomp.). ^{1}H-NMR ($CDCl_3$) ppm: 1.38, 1.62 (3H, s, CH_3), 3.72-3.84 (1H, m, H-6exo), 4.33 (1H, d, $J_{6exo,6endo}$=8.1Hz, H-6endo), 4.43-4.80 (3H, m, H-3,4,5), 4.88 (1H, dd, $J_{1,2}$=2.2Hz, $J_{2,3}$=6.2Hz, H-2), 5.48 (1H, d, H-1). Anal. Calcd for $C_{10}H_{13}F_3O_7S$: C, 35.93; H, 3.92. Found: C, 36.01; H, 3.95. A solution of the triflate (530 mg, 1.6 mmol) in freshly distilled acetonitrile (5 ml) was heated under reflux with anhydrous tetramethylammonium fluoride (294 mg) for 50 min. After the solvent was evaporated under vacuum, the residue was chromatographed on silica gel with n-hexane - ethyl acetate (10:1) to give 1,6-anhydro-2-deoxy-2-fluoro-3,4-O-isopropylidene-β-D-galactopyranose (146 mg, 45%) as colorless needles, after recrystallization from n-hexane - ether, mp 86°C. ^{1}H-NMR ($CDCl_3$) ppm: 1.36, 1.53 (3H, s, CH_3), 3.53-3.66 (1H, m, H-6exo), 4.09 (1H, d, $J_{6exo,6endo}$=7.6Hz, H-6endo), 4.16-4.61 (3H, m, H-3,4,5), 4.51 (1H, dd, $J_{2,3}$=1.0Hz, $J_{2,F}$=45.0Hz, H-2), 5.49 (1H, d, $J_{1,F}$=4.2Hz, H-1). Anal. Calcd for $C_9H_{13}FO_4$: C, 52.94; H, 6.42. Found: C, 53.01; H, 6.37.

The fluoro galactopyranose (36 mg, 0.18 mmol) thus obtained was added to a solution of methanol (10 ml) containing 0.5N HCl (3 ml). The mixture was heated

under reflux for 1 h and neutralized with 1N NaOH. After the solvent was evaporated under vacuum, the residue was chromatographed on silica gel with $CHCl_3$ - methanol (20:1) to give 1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose (26 mg, 90%) as colorless needles, after recrystallization from ether, mp 161-165°C. Anal. Calcd for $C_6H_9FO_4$: C, 43.91; H, 5.53. Found: C, 44.03; H, 5.59. This was further characterized by conversion into 3,4-di-O-acetyl-1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose using acetic anhydride in pyridine in the usual manner, mp 81°C, after recrystallization from n-hexane - ether. ^{1}H-NMR ($CDCl_3$) ppm: 2.04, 2.13 (3H, s, $OCOCH_3$), 3.74 (1H, dd, $J_{5,6exo}$=6.4Hz, $J_{6exo,6endo}$=7.3Hz, H-6exo), 4.30 (1H, d, H-6endo), 4.40 (1H, dt, $J_{1,2}$=$J_{2,3}$=1.7Hz, $J_{2,F}$=44.4Hz, H-2), 4.51 (1H, t, $J_{4,5}$=6.4Hz, H-5), 5.23-5.53 (3H, m, H-1,3,4). Anal. Calcd for $C_{10}H_{13}FO_6$: C, 48.39; H, 5.28. Found: C, 48.38; H, 5.27.

Radiofluorination of the triflate (1)

Fluorine-18 was produced from 8% enriched [^{18}O]H_2O by the $^{18}O(p, n)^{18}F$ reaction as described previously (8, 12). Aminopolyether (Kryptofix 2.2.2.) supported potassium [^{18}F]fluoride ([K/2.2.2.]$^{+18}F^-$) as [^{18}F]fluorinating agent was prepared by the addition of K_2CO_3 (1.5 mg, 10 μmol) and Kryptofix 2.2.2. (8 mg, 21 μmol, Merck) to the irradiated water and subsequent removal of the water by co-evaporation with acetonitrile as described in the literature (8,10,11).

A solution of the triflate (1) (11 mg, 18 μmol) in dry acetonitrile (250 μl) was added to a TPX vessel containing the [K/2.2.2.]$^{+18}F^-$ (0.52-1.4 mCi). The vessel was capped and heated at 75°C for 20 min. After the solvent was evaporated with a stream of argon, the residue was extracted with n-hexane - CH_2Cl_2 (1:1) (500 μl). The extract was injected onto a HPLC column (Partisil M9 10/50 PAC, 50 cm, Whatman; detectors: U.V. at 254 nm and radioactivity) attached a precolumn (silica gel, 30μ). Elution with a mixture of n-hexane and ethyl acetate (3:1) at a flow rate of 4 ml/min gave methyl 2-deoxy-2-[^{18}F]fluoro-3,4-O-isopropylidene-6-O-trityl-β-D-galactopyranoside (2) (R_t= 11 min) in 62-63% radiochemical yields. The identity of 2 was verified by HPLC and TLC comparison with authentic sample, and the radiochemical purity of 2 was

found to be >99% by TLC (n-hexane : ethyl acetate = 3 : 1, R_f = 0.42). The time required in this radiofluorination and subsequent HPLC separation was about 40 min.

Hydrolysis of the ^{18}F-intermediate (2) to 2-^{18}FDGal

The fraction of 2 from HPLC was collected in a 5 ml Pyrex vessel and evaporated with a flow of argon at 100°C. To the residue was added 6N HCl (500 μl), and the vessel was capped and heated at 115°C for 30 min. The reaction mixture was cooled and water (1 ml) was added. The acid solution was passed through an AG11A8 ion retardation resin column (6 x 1.3 cm) and a neutral alumina column (0.5 x 1.3 cm) to remove any fluoride ion. By this procedure, an aqueous solution of 2-^{18}FDGal was obtained in a volume of 4 ml of water with overall radiochemical yields of 36-39% at the end of synthesis. The total preparation time was about 90 min from start of the radiofluorination.

HPLC and TLC analyses of 2-^{18}FDGal

The aqueous solution of 2-^{18}FDGal was evaporated to dryness with a flow of argon at 100°C, and the residue was redissolved in a small amount of CH_3CN - H_2O (85 : 15). This solution was analyzed by both TLC (CH_3CN : H_2O = 95 : 5) and HPLC (column: Waters, carbohydrate analysis; mobile phase: CH_3CN : H_2O = 85 : 15; flow rate: 1 ml/min; detectors: radioactivity and refractive index). The radiochemical purity of 2-^{18}FDGal (R_f = 0.29, R_t = 6.5 min) was about 95%. The only detectable impurity (R_f = 0.60, R_t = 3.5 min), accounting for about 5% of the total radioactivity, was identified as 1,6-anhydro-2-deoxy-2-[^{18}F]fluoro-β-D-galactopyranose (3) by the comparison with authentic sample.

Treatment of 2-FDGal with 6N HCl and product analysis by ^{19}F-NMR

2-FDGal (40 mg) was heated with 6N HCl (1 ml) at 110±2°C for 30 min. After exact neutralization with 2N NaOH, the solution was evaporated to dryness and the residue was chromatographed on dry silica gel with ethyl acetate - ethanol (4:1). The sugar fractions were collected and then evaporated to dryness under reduced pressure. The sample (39.5 mg) thus obtained was dissolved in a mixture of H_2O (1 ml) and D_2O (0.5 ml). ^{19}F-NMR spectra of the sample were taken with a

JEOL FX-100 standard high resolution spectrometer operated at 93.7 MHz at ambient temperature (22-23°C). The ^{19}F chemical shifts were referenced to an external sample of hexafluorobenzene and negative signs were assigned for the chemical shifts upfield of the standard. The relative ratios of the products observed were calculated from the integrated peak areas obtained by the Fourier-transform technique.

^{19}F-NMR spectral analysis of the sample showed, in addition to resonances with strong signal intensities corresponding to the two anomers [-40.16 (β-anomer) and -40.68 ppm (α-anomer)] of the unchanged 2-FDGal, the presence of four resonances with different chemical shifts. One of the four resonances had the same chemical shift (-22.88 ppm) and ^{1}H-^{19}F coupling constants ($J_{1,F}$<1Hz, $J_{2,F}$=53.3Hz, $J_{3,F}$=13.2Hz) as those of authentic 1,6-anhydro-2-deoxy-2-fluoro-β-D-galactopyranose, which constituted 2-4% of the total intensity. Fluorinated substances with chemical shifts of -35.05 ppm ($J_{1,F}$=9.2Hz, $J_{2,F}$=48.8Hz, $J_{3,F}$=34.2Hz) and -55.07 ppm ($J_{1,F}$=20.8Hz, $J_{2,F}$=51.3Hz, $J_{3,F}$=34.2Hz) were identified as the α- and β-anomers of 2-deoxy-2-fluoro-D-talose; these assignments are in good agreement with those in the literature (14). The amount of 2-deoxy-2-fluoro-D-talose in the sample was only less than 1% of the total intensity in several experiments. The chemical identity of an additional resonance at -41.91 ppm (about 1%) remained to be elucidated.

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