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Synthesis of 1-[^{18}F]fluorodeoxyglucose: an unexpected rearrangement in the reaction of 2-*O*-methanesulfonyl-ß-D-mannopyranose with [^{18}F]fluoride

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SUMMARY

2-*O*-Methanesulfonyl-ß-D-mannose was reacted with kryptofix/K_2CO_3/[^{18}F]fluoride in CH_3CN/THF (9:1) at 60°C. Unexpectedly, a mixture of 1α- and 1ß-glucopyranosyl [^{18}F]fluoride (**4** and **5**, respectively) was obtained in 50% radiochemical yield (EOB); 2-[^{18}F]FDG was not detected. Modification of temperature, solvent or pH did not result in the formation of 2-[^{18}F]FDG. Uptake of radioactivity in heart and brain of mice was significantly lower for **4** and **5** than for 2-[^{18}F]FDG, although **5** seems to pass the blood-brain barrier. Uptake in bone was more pronounced for **4** than for **5** and negligible for 2-[^{18}F]FDG.

Key-words : fluorine-18, 1-FDG, 2-FDG, rearrangement, PET.

INTRODUCTION

2-[^{18}F]Fluoro-2-deoxy-D-glucose (2-[^{18}F]FDG) is a widely applied radiopharmaceutical in positron emission tomography (PET). Today, it is not only used to address questions of scientific interest, but it is also applied in routine clinical studies. Therefore, the demand for 2-[^{18}F]FDG still increases. At present, the method of choice for the preparation of 2-[^{18}F]FDG is based on a two-step synthesis starting from 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-D-mannose as described by Hamacher *et al.* (1). First, fluorine-18 is introduced in the 2-position by a nucleophilic

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substitution of 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-D-mannose with [^{18}F]fluoride, followed by acidic hydrolysis of the four protecting acetate esters. An important modification suggested recently, is alkaline hydrolysis of the protecting acetate groups instead of acidic hydrolysis (2). The alkaline hydrolysis proceeds faster than the acidic procedure and the possibility of formation of 2-chloro-2-deoxy-D-glucose is reduced. A disadvantage of the hydrolysis in alkaline conditions is the risk of epimerisation and formation of 2-[^{18}F]fluoro-2-deoxy-D-mannose (3). However, at room temperature this risk is minimal.

We sought to simplify and shorten the procedure for the preparation of 2-[^{18}F]FDG. The possibility to synthesise 2-[^{18}F]FDG by a one step procedure was investigated, by performing the nucleophilic substitution with [^{18}F]fluoride on a non-protected mannose precursor, i.e. 2-*O*-methanesulfonyl-ß-D-mannose (**2**). The mesylate was chosen as leaving group because it combines both reactivity and stability. The reaction sequence would eliminate the need for hydrolysis of the fluorine-18 labelled tetraacetylglucose intermediate and lead to a very simple synthesis set-up. This would greatly enhance the flexibility and reliability of the 2-[^{18}F]FDG production.

MATERIALS AND METHODS

1α-Fluoro-1-deoxyglucose (**4**) was obtained from Sigma (St. Louis, Mo.). ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Gemini 200 MHz spectrometer (Varian, Palo Alto, CA). Chemical shifts are reported in ppm relative to TMS ($\delta = 0$). ^{13}C-NMR spectra were assigned on the basis of chemical shifts, or by using APT and selectively decoupled spectra. Melting points were determined in open capillaries immersed in an oil bath (Electrothermal, Southend-on-Sea, U.K.) and are not corrected. Preparative column chromatography was performed on silica gel 60 (Merck, Darmstadt, Germany). Batches of [^{18}F]fluoride were prepared by a ^{18}O(p,n)^{18}F reaction

by bombarding 500-µl ^{18}O-enriched water (Isotec, Miamisburg, OH) in a silver target using an IBA cyclone 10/5 cyclotron (IBA, Louvain-la-Neuve, Belgium). 2-[^{18}F]FDG was prepared according to literature procedures (1) using an IBA synthesis module. HPLC analysis was performed on a carbohydrate-NH_2 column (Alltech, Deerfield, IL) eluted with CH_3CN/H_2O (95:5, v/v), at a flow rate of 1.5 ml/min. The column effluent was monitored with a refractive index detector and a 2-in. NaI(Tl) scintillation detector coupled to a single channel analyser. Output of both detectors was analysed by a Rachel analysis program (Lablogic, Sheffield, UK).

1,3,4,6-tetra-O-acetyl-2-O-methanesulfonyl-ß-D-mannose (1)

An amount of 5.15 g (14.8 mmol) of 1,3,4,6-tetra-*O*-acetyl-ß-D-mannose (4) was dissolved in 50 ml of dry pyridine. The solution was cooled to -20 °C and 1.25 ml (16.3 mmol) of methanesulfonyl chloride was added dropwise with a syringe. The mixture was allowed to warm to 0 °C, and stirring was continued for 2 h. Next, the solution was poured into a mixture of 80 ml of 6N HCl and 80 g of crushed ice and the resulting yellow solution was extracted twice with CH_2Cl_2. The organic layer was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the crude product was recrystallised from absolute ethanol, yielding 3 g (50%) of white crystals (mp. 119-121 °C). ^{1}H-NMR δ 1.99, 2.01, 2.04, 2.09 (4x s, CH_3COO), 3.29 (s, CH_3SO_2), 3.98 (d, $J_{5,6}$=10 Hz, 6-*H*), 4.11 (m, 5-*H*), 4.14 (dd, 6-*H'*), 5.03 (t, $J_{3,4}$=$J_{4,5}$=10.1 Hz, 4-*H*), 5.06 (dd, $J_{1,2}$=0.8 Hz, $J_{2,3}$=3.3 Hz, 2-*H*), 5.39 (dd, $J_{2,3}$=3.3 Hz, $J_{3,4}$=10.1 Hz, 3-*H*), 6.13 (d, $J_{1,2}$=0.8 Hz, 1-*H*). ^{13}C-NMR δ 20.58 (4xCH_3COO), 38.21 (CH_3SO_2), 61.71 (6-*C*), 64.68 (4-*C*), 69.37 (3-*C*), 71.67 (5-*C*), 75.89 (2-*C*), 89.50 (1-*C*), 168.18, 169.56, 170.09 (4xCH_3COO).

2-O-methanesulfonyl-ß-D-mannose (2)

A solution of 1.6 g (3.7 mmol) of **1** in 100 ml of 1N HCl was heated to reflux for 30 min and subsequently concentrated *in vacuo*. The oily residue was purified by

chromatography on silica using acetonitrile as eluent. As a final purification, the product was crystallised from CH_3CN/THF, yielding 500 mg (50%) of a white solid (mp. 125-130 °C). ^{1}H-NMR δ 3.17 (s, C*H*$_3$SO$_2$), 3.3-3.8 (m, 5H, 4-*H*,5-*H*,6-*H*,3-*H*), 4.50 (m, $J_{1,2}$=1.7 Hz, 2-*H*), 4.55 (t, *J*=5.9 Hz, 6-O*H*), 5.0 (d, *J*=5.9 Hz, 4-O*H*), 5.05 (dd, $J_{1,2}$=1.7 Hz, *J*=4.8 Hz, 1-*H*), 5.33 (d, *J*=5.7 Hz, 3-O*H*), 6.92 (d, *J*=4.8 Hz, 1-O*H*). ^{13}C-NMR δ 37.84 (*C*H$_3$SO$_2$), 61.01 (6-*C*), 67.04 (4-*C*), 68.38 (3-*C*), 73.28 (5-*C*), 81.66 (2-C), 91.82 (1-*C*).

1β-fluoro-1-deoxyglucose (5)

A solution of 2 g (8.1 mmol) of **2** and 3 g (16.2 mmol) of tetraethylammonium fluoride dihydrate in 150 ml of CH_3CN/THF (9:1, v/v) was stirred overnight at room temperature. The solvent was removed *in vacuo* and the crude product was purified on a silica column eluted with CH_3CN, yielding 500 mg of a white crystalline product (mp. 70 °C (dec)). ^{1}H-NMR (^{1}H- and COSY spectrum) δ 3.08 (m, $J_{2,F}$=14 Hz, $J_{2,3}$=10 Hz, J_{1-2}=6.9 Hz, 2-H), 3.0-3.3 (m, 3H, 3-*H*,4-*H*,5-*H*), 3.5 (dd, 6-*H*), 3.7 (dd, 6-*H'*), 4.7 (t, 6-O*H*), 5.06 (dd, $J_{1,F}$=53.7 Hz, $J_{1,2}$=6.9 Hz, 1-*H*), 5.1 (d, O*H*), 5.15 (d, O*H*), 5.5 (d, O*H*). ^{13}C-NMR δ 60.73 (6-*C*), 69.42 (d, 4-*C*, ^4J=4.6 Hz), 73.64 (d, 2-*C*, ^2J=20.5 Hz), 75.72 (d, 3-*C*, ^3J=11.8 Hz), 77.12 (5-*C*), 110.05 (d, 1-*C*, ^1J= 208 Hz).

1-[^{18}F]fluoro-1-deoxyglucose (1-[^{18}F]FDG)

After irradiation of 500 µl of [^{18}O]water with 10-MeV protons, the contents of the target were passed over a 4-mm diameter ion exchange membrane (AG1-X8, OH^- form, Bio-Rad, Hercules, CA) to trap [^{18}F]fluoride. The membrane was eluted with a solution of 18 mg of kryptofix 2.2.2. and 1.6 mg of K_2CO_3 in 0.5 ml of CH_3CN/H_2O (95:5 v/v). The eluate containing the kryptofix-[^{18}F]fluoride complex was added to a solution of 10 mg of **2** in 1.2 ml of CH_3CN/THF (9:1, v/v) and the mixture was heated in a closed vial at 85 °C during 5 min. Next, the reaction mixture was purified over a

short alumina column (Alumina N Cartridge, Waters, Milford, MA.). The column was eluted with 3 ml of water, yielding 1-[^{18}F]FDG as a mixture of anomers with a radiochemical yield of 25-50% (corrected for decay). HPLC purification was performed as described above. Retention time of 1ß-[^{18}F]FDG was 21.6 min and of 1α-[^{18}F]FDG 26.3 min; both compounds coeluted with the respective authentic materials. The radiochemical purity was determined using ITLC plates (ITLC™ SG, Gelman, Ann Arbor, MI), eluted with CH_3CN and was found to be >90%.

Biodistribution in mice

Animal studies were performed according to the Belgian code of practice for the care and use of animals. The preparations of fluorine-18 labelled sugars were diluted with normal saline to a concentration of 3.7 MBq/ml. Male NMRI mice (body mass 25-30 g) were sedated by i.m. injection of 0.05 ml of a 1:4 diluted solution of Hypnorm® (Duphar, Oss, The Netherlands). Then, 0.1 ml of the tracer solution was injected in each of three mice via a tail vein. The mice were sacrificed by decapitation at 10 or 30 min post injection (p.i.). Blood was collected in a tared tube and weighed. All organs and other body parts were dissected and their fluorine-18 radioactivity was determined in a 3-in. NaI(Tl) well crystal coupled to a dual-channel analyser and scaler. Corrections were made for background radiation and physical decay during counting.

RESULTS

The precursor for the labelling experiments, 2-*O*-methanesulfonyl-ß-D-mannose (**2**), was prepared by a three-step sequence starting from D-mannose. First, 1,3,4,6-tetra-*O*-acetyl-ß-D-mannopyranose was prepared according to the method described by Deferrari *et al.* (4). The subsequent reaction with methanesulfonyl chloride in pyridine yielded 1,3,4,6-tetra-*O*-acetyl-2-*O*-methanesulfonyl-ß-D-mannose (**1**, Figure 1) with a

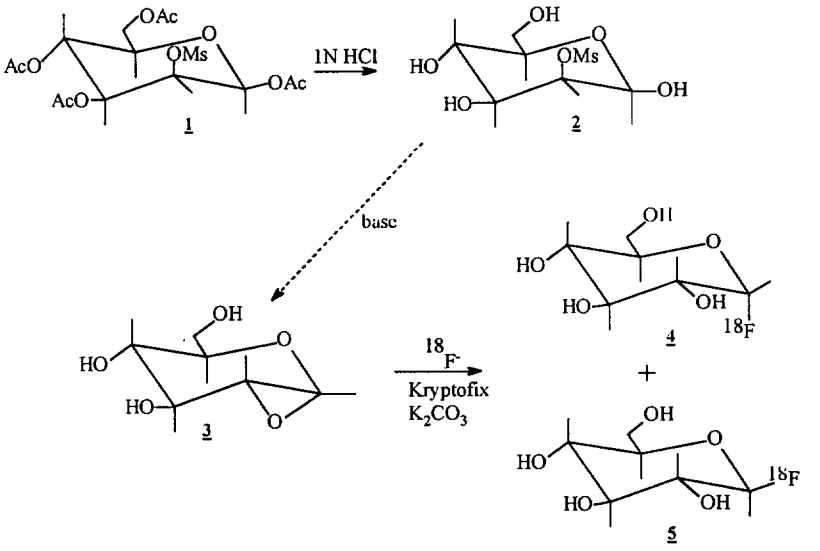

Figure 1. Synthesis of 1α-[^{18}F]FDG (4) and 1β-[^{18}F]FDG (5).

yield of 50%. Selective hydrolysis of the four protective acetate groups was accomplished by refluxing in 1N HCl, yielding 2 as a white, crystalline product in 50% yield.

The identity of 2 was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy. Assignments in the ^{1}H-spectra were based on selective homo-decoupling experiments. The presence of an O*H*-signal at δ 6.9 and the appearance of 1-*H* at δ 5.05 as a double doublet in the ^{1}H-NMR spectrum indicated that the anomeric hydroxyl group was free. The small coupling (^{3}J=1.7 Hz) of 1-*H* with the signal at δ 4.50 (C*H*OMs) indicated that the mesylate was retained at C-2 in the expected axial position and therefore no rearrangement had occurred.

The reaction of 2 with millimolar amounts of tetraethylammonium fluoride yielded predominantly a single fluorinated species. ^{1}H-NMR spectroscopy revealed that the characteristic signal of the anomeric O*H* at δ 6.9 had disappeared. Moreover, a far downfield double doublet for the C*H*F signal (δ 5.09, $^{2}J_{H1,F}$=53.7 Hz) indicated that the fluorine substituent was at position 1. The stereochemistry at carbon-1 and carbon-2

was confirmed by determining the other coupling constants. The coupling constant $^{3}J_{H2,H3}$=10 Hz indicated an axial-axial relationship between 2-*H* and 3-*H* and the coupling between 1-*H* and 2-*H* ($^{3}J_{H1,H2}$=6.9 Hz) also suggested an axial-axial relationship. Due to the anomeric character this coupling is somewhat smaller than normally would be expected. Finally, the ^{19}F coupling with 2-*H*, $^{3}J_{H2,F}$=14 Hz, is in agreement with an axial-equatorial relationship of 2-*H* and *F*. The compound can therefore be identified as 1ß-glucopyranosyl fluoride (5). These results are in agreement with an earlier study by Hall *et al.* (5), who investigated the NMR spectra of fully *O*-acetylated hexopyranosyl fluorides.

Several solvents were used in the nucleophilic substitution reaction with [^{18}F]fluoride. (Table 1). Surprisingly, the presence of water during the nucleophilic substitution had only a small negative effect on the radiochemical yield. Best results were found in CH_3CN/THF 9:1, where THF was added to improve the solubility of 2.

Table 1.
Effect of solvent on overall radiochemical yield of 1-[^{18}F]FDG

Solvent	Yield[a]
CH_3CN/THF 9:1	47
THF	34
CH_3CN/H_2O 8:2	20
pH=8.5	26
pH=7	3
DMF	10
DMSO	8
Dioxane	17
Ethylacetate	14
Isopropanol	38
Methanol	4

[a] Radiochemical yield corrected for decay (end of bombardment, EOB).

The separation of the two anomers 4 and 5 was accomplished with HPLC (Figure 2), the first eluting compound being 1ß-[^{18}F]FDG (5). The identity of both anomers was verified by coinjection of the corresponding non-radioactive materials. Coinjection of a sample of 2-[^{18}F]FDG revealed a third peak at t_R=24.3 min, indicating that neither of the reaction products coeluted with 2-[^{18}F]FDG.

The tissue distribution of the two anomers 4 and 5 was studied in mice and compared to that of 2-[^{18}F]FDG. (Table 2). The results show some striking differences between the distribution of 4 or 5 and that of 2-[^{18}F]FDG. Uptake of both 4 and 5 in the heart was much lower than that of 2-[^{18}F]FDG. The same holds true for the uptake in the brain, although 1ß-[^{18}F]FDG (5) seemed to cross the blood-brain barrier to a higher extent than 1α-[^{18}F]FDG (4). A high uptake in bone was found for 4. For 5 and especially for 2-[^{18}F]FDG the amount of radioactivity found in bone was much lower.

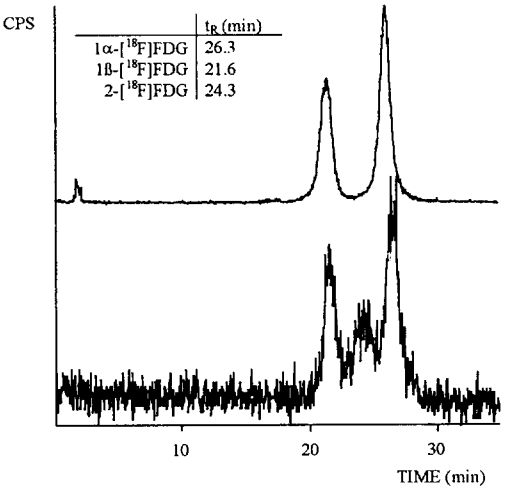

Figure 2. HPLC chromatogram of the separation of 1α-[^{18}F]FDG (4) and 1β-[^{18}F]FDG (5) (top line). The bottom line features the result after coinjection with 2-[^{18}F]FDG.

Table 2.
Tissue distribution of 1α-[^{18}F]FDG (4), 1β-[^{18}F]FDG (5) and 2-[^{18}F]FDG after IV injection in mice. Depicted is % injected dose/gram (mean ± SD; n=3).

	1α-[^{18}F]FDG (4)		1β-[^{18}F]FDG (5)		2-[^{18}F]FDG	
	10'	30'	10'	30'	10'	30'
Kidneys	9.0±0.8	4.8±0.5	6.7±0.5	5.1±1.2	8.4±1.4	7.5±0.6
Liver	9.4±1.4	3.1±0.1	18.0±4.4	4.8±1.8	6.2±0.6	4.5±0.4
Spleen	1.9±0.3	1.0±0.2	1.7±0.1	1.1±0.3	2.5±0.2	3.7±0.1
Lungs	3.6±0.3	1.7±0.1	4.3±0.3	1.9±0.5	5.1±0.6	4.3±0.3
Heart	3.3±0.1	2.4±0.5	4.0±0.8	2.8±0.7	5.9±0.6	31.9+8.9
Cerebrum	1.2±0.2	1.3±0.1	4.2±0.4	3.5±0.9	6.8±0.7	10.4±0.9
Cerebellum	1.4±0.1	1.3±0.3	4.4±0.6	3.7±0.8	6.8±0.6	9.4±0.7
Blood	3.2±1.7	2.3±1.1	2.0±1.2	1.7±0.8	6.3±2.5	1.8±0.2
Bone	12.0±1.1	29.0±1.0	7.0±1.5	18.1±1.4	2.5±0.3	3.6±0.1

DISCUSSION

The reaction of 2-*O*-methanesulfonyl-ß-D-mannose (2) with tetraethylammonium fluoride gave 1ß-FDG (5) as the predominant product. No evidence was found for the formation of 2-FDG or 1α-FDG (4).

The exclusive formation of the 1-fluorodeoxyglucose isomer suggests a rapid conversion of 2, probably into the 1,2-epoxide 3 (Fig. 1). This type of rearrangement has been described in literature for the acetyl protected analogue of 3, 3,4,6-tri-*O*-acetyl-1,2-anhydroglucose (6). This compound is referred to as *Brigl's anhydride* and is formed by treatment of 3,4,6-tri-O-acetyl-ß-D-glucopyranosyl chloride with base. Unfortunately, in our study attempts to isolate the intermediate 1,2-anhydroglucose (3) in the reaction of 2 with tetraethylammonium fluoride failed.

From a mechanistic point of view it can be expected that the 1ß isomer (5) would be the predominant product due to S_N2 attack of the fluoride anion. For bulky substituents the equatorial (ß-) position is thermodynamically favourable, but in the case of electronegative substituents an axial position of the substituent at C-1 is preferred. In this way interaction of the electronegative (fluoro) substituent with the unshared electrons of the pyranose ring oxygen is prevented. This effect is referred to as the *anomer effect* (7).

The reaction of 2 with [^{18}F]fluoride proceeded very smoothly and the presence of water in the nucleophilic substitution was well tolerated. It proved not to be necessary to dry the kryptofix-[^{18}F]fluoride complex prior to the substitution reaction. The use of different solvents, temperatures and pH was investigated, but none of these conditions did result in the formation of 2-[^{18}F]FDG. In the reaction with [^{18}F]fluoride, 1α-[^{18}F]FDG (4) is also found as a product, probably due to racemisation of the 1ß-epimer (5). Apparently 5 is the kinetically controlled reaction product. The ratio of the 1α- and 1ß-epimer in the reaction product was variable but apparently did not correlate with the reaction conditions employed.

To our knowledge, this is the first report of a synthesis of no-carrier-added 1α,ß-[^{18}F]FDG. Earlier, in 1978 the synthesis of 5 was described by Lemire and Reed (8). They reacted 2,3,4,6-tetra-O-acetyl-α-D-glucosyl bromide with carrier added silver [^{18}F]fluoride, resulting in the formation of 5 with low specific activity.

As could be expected, uptake of 2-[^{18}F]FDG in heart and brain of the mice is high. Neither 4 nor 5 is taken up in the heart to the same extent as 2-[^{18}F]FDG. Probably, 4 and 5 have no affinity for the glucose transporter and/or cellular hexokinase. The metabolic instability of the two epimers is reflected in the results of the tissue distribution study. Uptake of radioactivity in bone was higher after injection of 4 or 5 than after injection of 2-[^{18}F]FDG. This increased uptake is most probably due to uptake of released [^{18}F]fluoride formed by metabolic degradation of 4 and 5. The increased metabolic instability can be attributed to the lability of the fluorine-18 substituent at the anomeric carbon atom.

CONCLUSION

The proposed method for a simple and faster preparation of 2-[^{18}F]FDG did not result in the formation of the desired 2-[^{18}F]FDG. Taking into account the predominant formation of the 1-[^{18}F]FDG isomers in this study, it is unlikely that reaction conditions will be found that enable the exclusive formation of 2-[^{18}F]FDG. A high radiochemical purity is a prerequisite for any radiochemical procedure to be used for routine preparation of fluorine-18 radiopharmaceuticals in PET. An HPLC purification of the final product would in any case not be of advantage with respect to the present production methods of 2-[^{18}F]FDG. *In vivo* evaluation of 1α-[^{18}F]FDG (4) and 1β-[^{18}F]FDG (5) suggest that both products are not suitable as *in vivo* tracers of glucose metabolism, due to their metabolic instability.

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