Synthetic Methods

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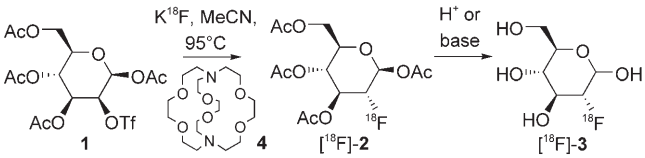
A Solid-Phase Route to ^{18}F-Labeled Tracers, Exemplified by the Synthesis of [^{18}F]2-Fluoro-2-deoxy-D-glucose**

*Lynda J. Brown, Denis R. Bouvet, Sue Champion, Alex M. Gibson, Yulai Hu, Alex Jackson, Imtiaz Khan, Nianchun Ma, Nicholas Millot, Harry Wadsworth, and Richard C. D. Brown**

Positron emission tomography (PET) is an important diagnostic tool in modern medicine,[1] where it is routinely used to locate and assess abnormalities in oncology,[2] neurology,[3] and cardiology.[4] In addition, PET and microPET are also finding increased applications in the areas of drug discovery and development.[5] [^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG, [^{18}F]-**3**) is the most widely used radiochemical tracer in PET applications that possesses the positron-emitting radionuclide ^{18}F (half-life = 110 min).[6] [^{18}F]FDG is used to measure glucose uptake by tissue, and produces real-time images for diagnosis, management, and study of diseases such as cancer.

The radiosynthesis of [^{18}F]FDG was first achieved by electrophilic fluorination of D-glucal with [^{18}F]F_2, but relatively low yields and limited stereoselectivity were obtained.[7] Since that time, various synthetic routes have been reported,[6,8–10] including the one used in PET centers throughout the world: this method involves the reaction of excess (10^5 equiv) tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl D-mannose (**1**) with the [^{18}F]fluoride ion in the presence of a phase-transfer agent **4** (Scheme 1). Deprotection of the FDG precursor **2** thus obtained, under basic or acidic conditions, produces [^{18}F]FDG. Although the chemical yields for the nucleophilic fluoridation reaction are rather poor (based on **1** in the stoichiometric reaction), the use of a massive excess of triflate **1** and a limiting amount of the [^{18}F]fluoride ion in the radiolabeling step ensures high yields of the radiolabeled product (> 60 %).



Scheme 1. Conventional radiosynthesis of [^{18}F]FDG ([^{18}F]-**3**). Tf = triflate.

Current methods of [^{18}F]FDG preparation have the disadvantage that the FDG is produced as a mixture with a large stoichiometric excess of D-glucose and other degradation products that arise from side reactions of the starting material **1** during the fluoridation and deprotection steps.[11] In the case of FDG, the presence of the by-products is not considered to be harmful to the patient or to affect the efficacy of the injected product. However, the above may not hold true for other ^{18}F-containing radiotracers under development for research and diagnostic applications. In addition, the short half-life of ^{18}F requires the radiotracer to be synthesized and purified as rapidly as possible, ideally within one hour for clinical use. This makes reverse-phase HPLC purification undesirable for commercial applications.

In recognition of the issues discussed above we set out to develop a platform technology based on the nucleophilic cleavage of solid-supported precursors using the [^{18}F]fluoride ion which would allow the production of ^{18}F-labeled compounds, as exemplified by the synthesis of [^{18}F]FDG.[12,13] Preparation of radiopharmaceuticals by this method would be advantageous in terms of the purity of the cleaved fluorinated products, whilst facilitating automation of the process and thus offering increased reproducibility and further protection from radiation exposure.[8,14] The new technology would produce ^{18}F-labeled tracers quickly and with high specific activity, as well as minimize time-consuming purification steps.

Our chosen strategy was to attach the FDG precursor to the solid-support through a sulfonate linker that would allow specific cleavage of the radiotracer into solution by using the [^{18}F]fluoride ion.[15] Any precursor present at the end of the reaction would remain attached to the resin, thus permitting its separation from the product by simple filtration, and avoiding the presence of the excess reactive triflate in the deprotection step. Following removal of the protecting groups, the ^{18}F tracer could be purified by ion-exchange chromatography to leave pure product ready for administration.

Initial efforts were focussed on adapting conventional FDG solution-phase chemistry to produce a solid-supported analogue of triflate **1**. However, major limitations in this approach became apparent: fluoridation of triflate **1** using a stoichiometric amount of the [^{19}F]fluoride ion afforded [^{19}F]FDG tetraacetate ([^{19}F]-**2**) only as a minor product (< 5 %; Scheme 2).[16]

[*] Dr. L. J. Brown, Dr. Y. Hu, Dr. N. Ma, Dr. R. C. D. Brown
School of Chemistry
University of Southampton
Highfield, Southampton, SO171BJ (UK)
Fax: (+44) 238-059-6805
E-mail: rcb1@soton.ac.uk

Dr. D. R. Bouvet, Dr. S. Champion, Dr. A. M. Gibson, Dr. A. Jackson, Dr. I. Khan, Dr. N. Millot, Dr. H. Wadsworth
GE Healthcare
The Grove Centre
White Lion Road, Amersham, Bucks, HP79LL (UK)

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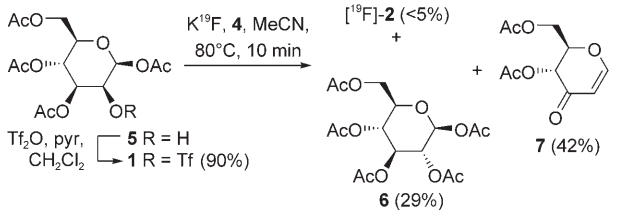
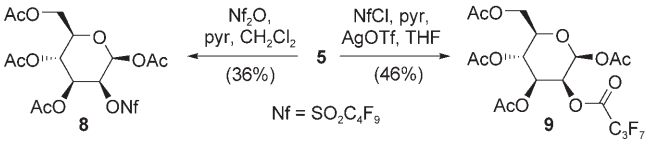


Scheme 2. Synthesis of [^{19}F]FDG by using stoichiometric amounts of [^{19}F]fluoride ions.

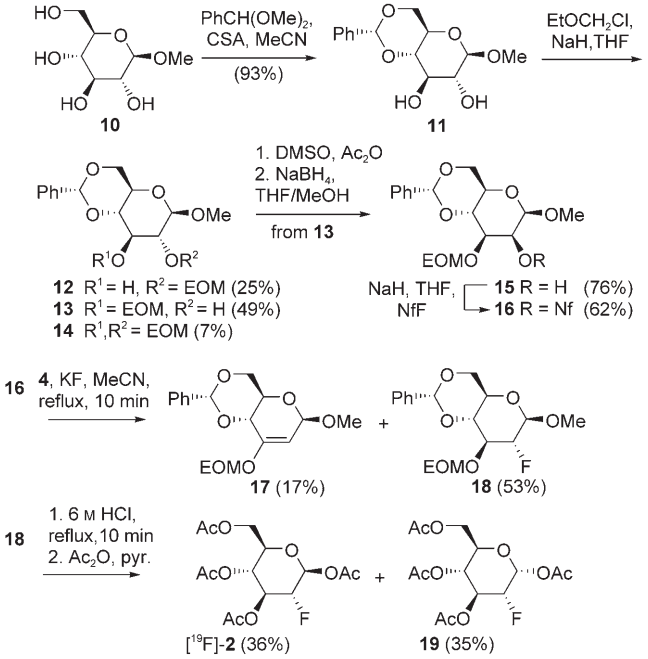
Furthermore, synthesis of the nonaflate **8** as a model for a solid-supported sulfonate ester could only be effected in reasonable yield using nonaflic anhydride (Nf_2O; Scheme 3);[17] the corresponding sulfonyl fluorides and chlorides failed to react effectively with the hindered 2-hydroxy group of **5** under similar conditions. One curious observation was the formation of perfluorobutanoate **9** when perfluoroalkylsulfonation was attempted in the presence of pyridine and AgOTf in THF (Scheme 3).



Scheme 3. Reactions of **5** with $F_9C_4SO_2Cl$ and $(F_9C_4SO_2)_2O$. pyr = pyridine.

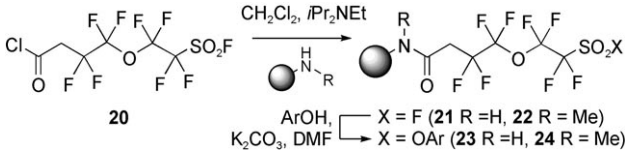
At this point the high reactivity of the perfluoroalkylsulfonic anhydrides prevented us from preparing a suitably functionalized linker, while the base-sensitive acetyl protecting groups would not allow the formation of a more reactive alkoxide from **5** to facilitate its coupling with a sulfonyl halide linker. Fortunately, by changing to base-stable acetal protecting groups,[18] it was possible to couple a β-D-mannose derivative **15** with a perfluoroalkylsulfonyl halide to give the sugar-linker model compound **16** in good yield (Scheme 4).[17,19] The use of the β anomer was crucial to obtain the desired stereoselectivity in the hydride reduction (d.r. = ca. 9:1),[18] and to ultimately open up C2 for nucleophilic attack by the fluoride ion. Our change in protecting-group strategy was further vindicated when the reaction of nonaflate **16** with [^{19}F]KF afforded the desired protected FDG **18** in a greatly improved 53 % yield along with a smaller quantity of an eliminated by-product **17**. Global deprotection of **18** was achieved by refluxing it in 6 N HCl for 10 minutes to afford unlabeled FDG [^{19}F]-**3**, which was characterized following conversion into the α- and β-tetraacetates **19** and [^{19}F]-**2**.[16a,20]

Our attention then turned to the synthesis of a sugar-linker construct. Recent publications described a perfluoroalkylsulfonyl fluoride linker, which was used to prepare aryl triflate equivalents (Scheme 5).[15a,b] However, poor yields were obtained for the coupling of D-mannose derivative **15** to this linker in solution or after attachment to the resin. In addition, elimination of the [^{19}F]fluoride ion from the linker



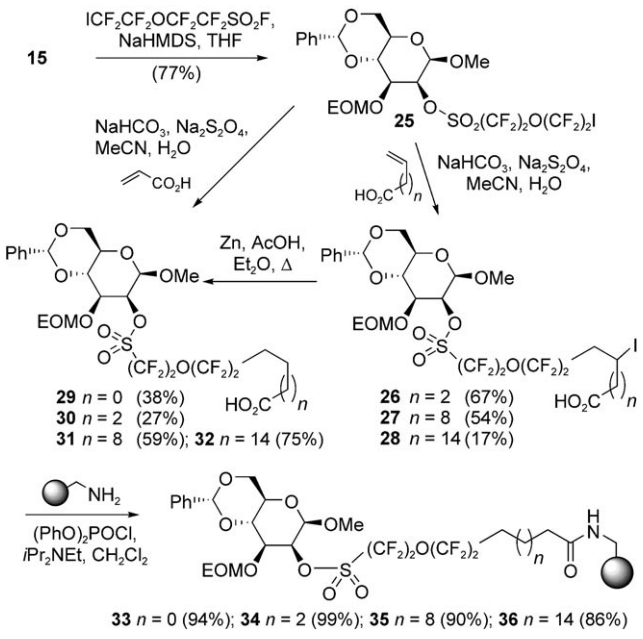
Scheme 4. Synthesis of [^{19}F]FDG by using the sugar-linker model **16**. CSA = camphorsulfonic acid, EOM = ethoxymethyl.

occurred under basic conditions. It is important to recognize that any $^{19}F^-$ ions released from the resin, through elimination or $^{18}F^-$ exchange with residual resin-bound sulfonyl fluoride (for example, **21**), would be detrimental to the yield and specific activity of the labeled product. A different linker was therefore sought, which would be less prone to elimination and that would be suitable for the formation of a sugar-linker conjugate in solution ready for coupling with an aminomethyl resin (Scheme 6).



Scheme 5. Previous syntheses of perfluoroalkylsulfonate linkers.[15] DMF = *N*,*N*-dimethylformamide.

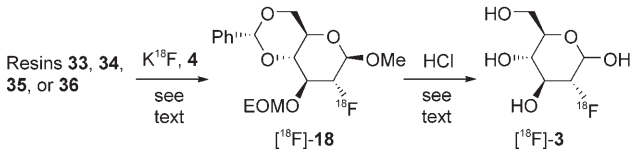
Linker-sugar conjugates **29**–**32** were prepared by coupling D-mannose derivative **15** to 5-iodooctafluoro-3-oxapentanesulfonyl fluoride, followed by radical-mediated coupling reactions of the resulting iodide **25** with a series of enoic acids of various chain lengths.[21] The yield of this reaction using l6-heptadecenoic acid was lower because of the poor solubility of this olefin. Reaction of acrylic acid with iodide **25** provided the reduced product **29** directly, whilst radical coupling products **26**–**28** required de-iodination using zinc powder in refluxing Et_2O/AcOH.[22] The resulting acids were coupled to aminomethyl-functionalized polystyrene to give resins **33**–**36** in high yields (estimated from the masses of dry resins). Successful formation of the supported sulfonate esters



Scheme 6. Synthesis of solid-supported FDG precursors **33**–**36**. HMDS = 1,1,1,3,3,3-hexamethyldisilazane.

was confirmed by on-bead IR spectroscopy, elemental analysis, and ^{19}F NMR as well as MAS ^{1}H and ^{13}C NMR spectroscopy.

Low-activity labeling experiments were carried out in a carbon glass vessel using [^{18}F]KF (185–350 MBq) and kryptofix[2.2.2] (**4**) in CH_3CN. Radiochemical yields were calculated from the reversed-phase HPLC chromatograms of the protected product [^{18}F]-**18** recorded with γ-ray detection (Scheme 7). Typically, solution-phase ^{18}F-labeling results in



Scheme 7. Synthesis of [^{18}F]FDG from resin-bound precursors.

80–90% incorporation after 2 minutes at 86 °C in a reaction volume of 1–2 mL.[8] For heterogeneous reactions involving the solid-supported precursors **33**–**36** it was advantageous to have the fluoride ion as concentrated as possible (reaction volume: 0.2 mL). Using resin **35**, labeling times of 3–4 minutes at 86 °C resulted in 70–91% incorporation of ^{18}F into [^{18}F]-**18**. Furthermore, high-activity labeling studies (up to 6.16 Ci) resulted in similar incorporation yields (68–77%).

The chemical purity of the crude protected FDG [^{18}F]-**18** from the resin precursor **35** was compared against the crude protected FDG product [^{18}F]-**2** obtained by conventional solution-phase synthesis. HPLC analyses, with γ-ray detection, indicated that the desired protected fluorinated sugars and $^{18}F^-$ were the only significant ^{18}F-containing components in both reactions. By contrast, the corresponding UV activity traces (205 and 210 nm) showed that the protected ^{18}F-labeled sugar obtained from the resin precursor **35** contained significantly reduced levels of chemical impurities. The absence of a significant UV-active peak corresponding to the protected FDG from the solid-phase synthesis indicated very little leaching of $^{19}F^-$ ions from the linker during labeling, and a high specific radioactivity of [^{18}F]-**18**. Five successive labeling experiments re-using the same sample of resin **35** all led to the formation of the protected [^{18}F]FDG with consistently high radiochemical yields. These experiments clearly demonstrated that the majority of the protected D-mannose derivative remained attached to the resin through the fluoroalkylsulfonyl linker during the ^{18}F-labeling experiments.

The effect of altering the chain length of the linker upon the radiochemical yield was also investigated for resins **33**–**36** (Figure 1). There was a steady rise in the radiochemical yield with increasing length of the alkyl chain up to four methylene groups, after which it leveled off and then began to fall. Sulfonate ester **35** was ultimately considered to be the linker of choice based on radiochemical yield, economy, and efficiency of synthesis.

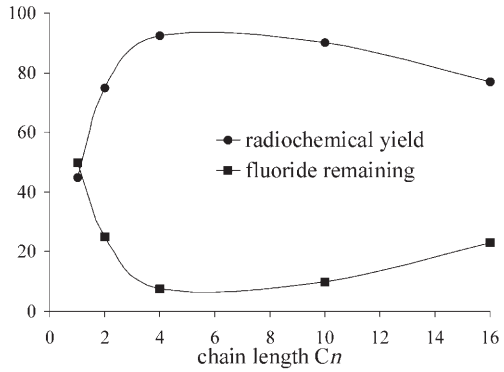


Figure 1. Effect of the linker chain length on the radiochemical yield. (C_1 resin synthesized by coupling **15** to resin **21**.)

To identify the optimal conditions required for removal of the protecting groups a sample of the protected [^{18}F]fluorosugar [^{18}F]-**18** was subjected to a range of acidic conditions, with Dionex anion-exchange HPLC fitted with γ-ray detection used to quantify the [^{18}F]FDG produced. The preferred conditions for the generation of [^{18}F]FDG required deprotection of [^{18}F]-**18** with 6 M HCl for five minutes at 125 °C, which gave a radioactive ion-exchange chromatogram with one main peak ([^{18}F]FDG) and one small peak (< 3%) corresponding to partially deprotected [^{18}F]FDG. This result produced an average radiochemical yield of 73% (decay corrected) and activity losses on the resin between 3–8%, with 91–97% of the activity collected.

In conclusion, we have presented a reliable route for the synthesis of a solid support that liberated protected [^{18}F]FDG in high radiochemical yield on treatment with $^{18}F^-$ ions. The product [^{18}F]-**18** was deprotected to give [^{18}F]FDG with excellent chemical purity. The method lends itself to automation, as the solid-supported precursor could be provided as

part of a kit to a radiopharmacy. The production of a wide range of other ^{18}F-labeled radiopharmaceuticals will be facilitated by this new platform technology, thus making such compounds more widely available for future applications.

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