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Review Article

Radiohalogenated carbohydrates for use in PET and SPECT

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Summary

Radiohalogenated carbohydrates have been of significant interest to the Nuclear Medicine community since the success of 2-Deoxy-2-fluoro-glucose (FDG)/positron emission tomography (PET) to measure glucose metabolic rates in humans. This review of this important class of compounds will be written in two parts, and will concentrate on the chemistry. The first part will cover the development of FDG, the many attempts to synthesize other radiohalogenated PET and SPECT analogs, and future prospects of a SPECT analog. The second will cover the synthesis of other radiohalogenated carbohydrate compounds such as nucleosides, where the halogen is located on the sugar rings, as potential imaging or therapeutic agents.

2-deoxy-2-fluoro-D-glucose (FDG)

FDG was first synthesized[1] via electrophilic fluorination with trifluoromethyl hypofluorite (CF_3OF) for the structure activity study of Hexokinase. The first practical synthesis of ^{18}F-FDG was carried out[2] by the addition of ^{18}F-F_2 to triacetyl glucal (Scheme 1).

Because of the highly reactive nature of F_2, it adds unselectively to both faces of the sugar ring. This yields a significant amount of glucose and mannose products (35% gluco, 26% manno of total yield), thus requiring the need for silica gel separation. Subsequent hydrolysis of the

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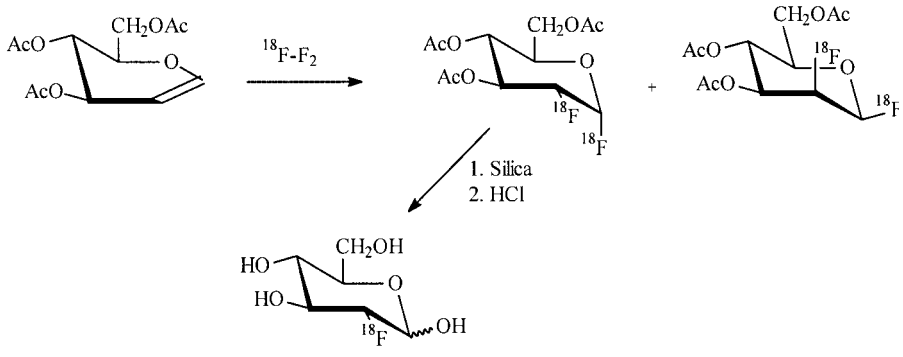


Scheme 1.

acetylated glucosyl fluoride gave the FDG product. Since this development FDG has grown to be the single most important radiopharmaceutical used in PET, and has become a significant radiopharmaceutical in the broader field of Nuclear Medicine.

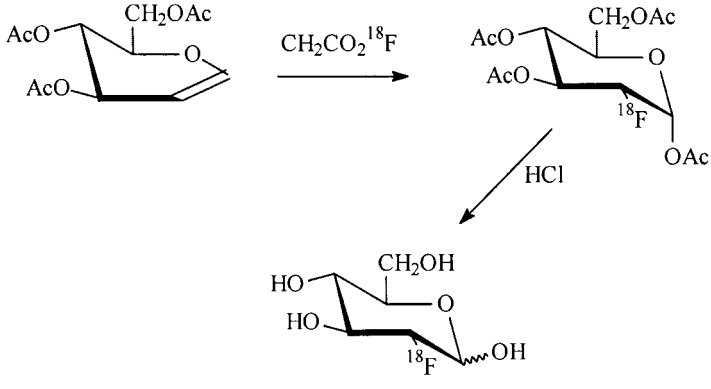
The Chemistry for the synthesis of FDG has undergone several advances over the years.[3,4] There are literally dozens of papers reported by several groups, including our own, on improvements and changes to the synthesis since the original $^{18}F_2$ procedure. This review will not chronicle all of these incremental developments, but instead will focus on the milestones.

The next important development in the synthesis of FDG was the use of acetyl hypofluorite as the fluorinating agent instead of elemental fluorine.[5,6]

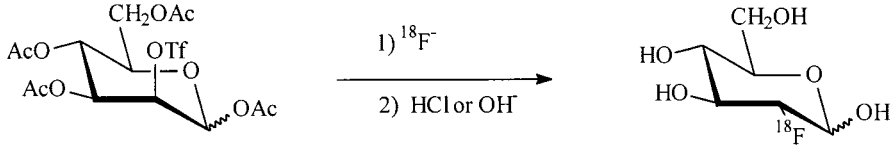
This fluorinating agent[7] has the advantage of being a milder fluorinating agent than F_2, and of adding more selectively across the double bond in triacetyl glucal. This method gives approximately 95% of the desired glucose configuration if carried out under the correct conditions (in Freon-11 at low temperature).[5] Approximately 5% of the product was in the mannose configuration. However, if the reaction is carried out in other solvents,[8] such as water,[9] the mannose configuration can be the dominant product. The hypofluorite method eliminated the need for the cumbersome chromatographic purification step of the crude product, as was needed for the F_2 reaction. The crude reaction mixture was simply evaporated and treated with HCl to give the final product after ion exchange and C-18 Sep Pak purification. Conveniently, the preparation of ^{18}F-acetyl hypofluorite is carried out by passing $^{18}F_2$ gas through a small column packed with a powdered

mixture of acetic acid and sodium acetate.[10] This procedure gave a maximum radiochemical yield of approximately 20% (decay corrected). The main drawbacks to the hypofluorite method is that 50% of the ^{18}F is lost, as is the case with all F_2 reactions, and the product is carrier added resulting in low specific activity (Scheme 2).

The synthesis of 3-fluoro-glucose via a nucleophilic displacement, several years ago, paved the way for the next major milestone in FDG synthesis.[11] The final chapter in the synthesis of FDG came with the development of the ^{18}F-fluoride synthesis (Scheme 3) using a mannose triflate precursor.[12] This method uses a specific anomeric form (β) of the triflate precursor, and gives FDG after deprotection and purification in 50–70% radiochemical yield (decay corrected). The advantages of this synthesis are the much higher yields (because all of the fluorine is available to the reaction), higher specific activity (10^3 Ci/mmol), and higher overall amounts of final product (currie quantities). This method also relied on the successful development of the ^{18}F-fluoride target system based on ^{18}O-water. The water target system can result in the production of currie amounts of ^{18}F, thus, the production of large amounts (up to and exceeding 1 Ci) of FDG is also possible. This



Scheme 2.



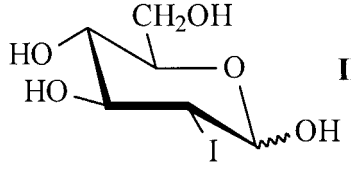
Scheme 3.

combination of developments has made it feasible for commercial producers to make FDG and distribute it from a central location. The fluoride FDG synthesis is also the preferred method adopted by most research labs that carry out PET imaging studies. The success of this new chemistry coupled with the emergence of clinical FDG/PET, has also prompted commercial manufactures to market several models of automated FDG synthesis devices. All of these chemistry modules are based on the same fluoride chemistry, with only minor variations.

Halogenated PET and SPECT analogs of FDG

The success of FDG as a clinical and research radiopharmaceutical has prompted chemists over the years to attempt to synthesize other halogenated sugar derivatives that might have similar properties to FDG. The development of a SPECT analog would, of course, be significant, since this would open up the FDG/PET technique to the wider medical community.

^{123}I is the halogen isotope of choice due to its excellent physical properties that make it ideal for imaging. The most logical analog of FDG for use in SPECT is the iodinated version 2-deoxy-2-iodo-glucose



IDG

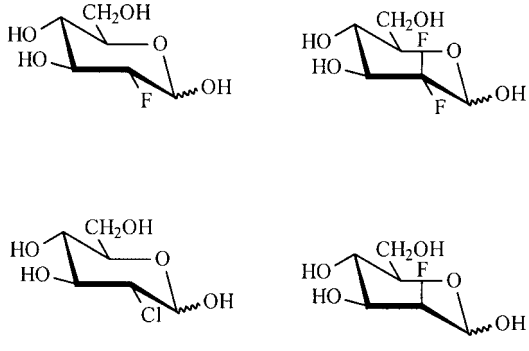
(IDG). However, several years ago[13] it was determined that this compound was very unstable since the iodine in the 2 position of the glucose molecule is α to an aldehyde, when in the open chain form, making elimination of HI favorable. Since that time many attempts to make other iodinated analogs have been published. Before summarizing some of these attempts, a brief overview of the structural requirements of Hexokinase and glucose carriers will be given. Being a substrate for hexokinase is a prerequisite for a compound to be used as an agent for determing glucose metabolic rates. An excellent review/editorial of the prospects of developing an ^{123}I analog of FDG has previously been published.[14]

There are two main structural requirements for phosphorylation of a sugar derivative by hexokinase. One is that only Glucose and Mannose derivatives are potential substrates, and the other is that only positions C2, C3 and C4 are suitable for substitution. Previous work on 3 and 4 fluoro-glucose[15] has determined that these compounds are poor substrates for hexokinase. Since fluorine is the smallest halogen, it is unlikely that any other halogenated 3 or 4 halo-glucose analog would be a better substrate. Also, the enzyme cannot tolerate bulky groups on position C2. Clearly, there are very tight constraints to the extent of structural change that can be accommodated.

To date the only halogenated glucose/mannose analogs that have been found to have significant hexokinase activity are shown in Scheme 4.

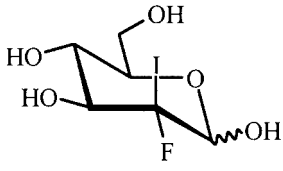
Two of these are FDG and 2-deoxy-2-fluoro-mannose (FDM), another is 2-deoxy-2,2-difluoro-glucose (DFDG),[16] and the only nonfluorinated glucose derivative is the 2-chloro compound.[17] However, the 2-chloro derivative is not a very good substrate when compared to FDG. The only other non 2 or 3-substituted ^{18}F-fluorinated deoxy glucose with slight hexokinase[15] activity is ^{18}F-4FDG.[18] There have also been many hopeful iodinated glucose analogs of FDG prepared over the years, and a few of these are shown in Scheme 5.[19–25]

Prompted by the successful result that the 2,2-difluoro sugar ^{18}F-DFDG was a substrate of hexokinase and was similar to ^{18}F-FDG in biodistribution and imaging studies, the mixed 2,2-dihalo sugars 2-deoxy-2-fluoro-2-Iodo-mannose (FIM) and -glucose (FIG) were synthesized as possible SPECT analogs. They are

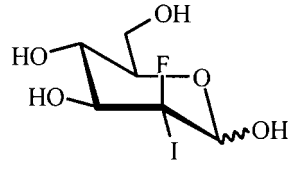


Scheme 4.

structurally very similar to FDG, and are stable *in vitro* as compared to IDG.[26]



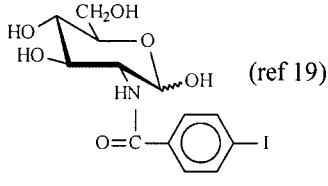
2-FIG



2-FIM

Unfortunately, as with all the other iodinated analogs shown in Scheme 5, neither of these two was found to be substrates for hexokinase. Also, the radioiodine on FIM was found to be unstable *in vivo*.[27]

The many failed attempts to synthesize an ^{123}I analog of FDG, combined with the published structure-activity studies for hexoki-



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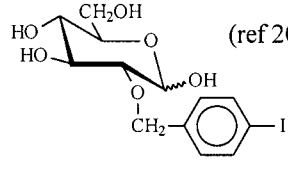

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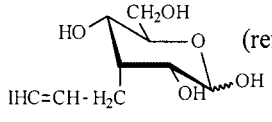

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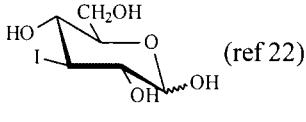

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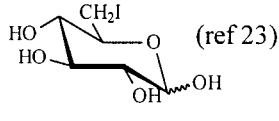

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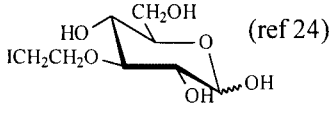

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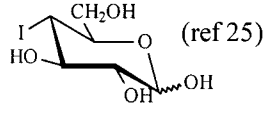
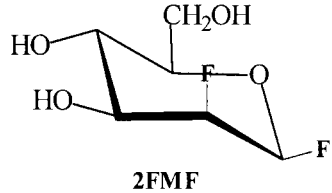


(ref 25)

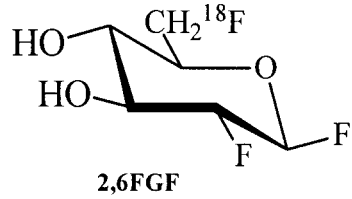
Scheme 5.

nase,[17,28] make the prospects of developing a fully functional SPECT halogenated analog of FDG remote. However, the door is still open in two areas for a potential SPECT analog, as was pointed out in a previous review.[14] A well transported SPECT analog might still be useful in studying diseases such as Alzheimer's and Huntington's Disease, or a glucose transport (GLUT 1) selective SPECT analog might prove useful as a tumor marker. Therefore, further work in this area may still be warranted.

Other halogenated carbohydrates that have been synthesized for purposes other than as FDG analogs include the synthesis of 2-deoxy-2-[^{18}F]-fluoro-galactose for imaging galactose metabolism in tumors such as liver tumors.[29,30] Rat studies indicated that this agent could be used with PET for the biochemical characterization of hepatomas. More examples of non-FDG analogs include potential imaging agents for glycosidase activity. The synthesis of ^{18}F-labelled 2-deoxy-2-fluoro-β-D-glucosyl fluorides via electrophilic ^{18}F-fluorination have been carried out to produce derivatives which might be useful in studying lysosomal



2FMF



2,6FGF

storage diseases such as Gaucher's Disease.[31,32] Two compounds, 2-deoxy-2-fluoro-β-mannosyl fluoride (2FMF, label is on 1 or 2 but not both)[31] and 2,6-dideoxy-2-fluoro-6-[^{18}F]fluoro-β-D-glucopyroanosyl fluoride (2,6FGF)[32] have been synthesized for this purpose. It was shown that 2FMF covalently derivatized *Agrobacterium* sp.*β-glucosidase* and that the trapped species was catalytically competent since it was capable of 'turnover' with release of 2-deoxy-2-mannose and the native enzyme. Unfortunately, tests on this derivative *in vivo* revealed that hydrolysis occurred with formation of 2-deoxy-2-fluoro-mannose. A possible solution to this problem was the synthesis of 2,6FGF, since the 6-fluoro substituent prohibits possible phosphorylation and offers a simpler means of radiolabelling using higher specific activity ^{18}F-fluoride. We await enzyme binding and animal studies for these compounds before further developing them as imaging agents.

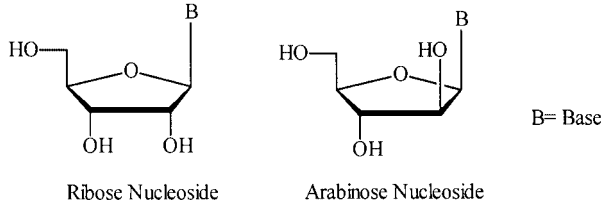
Radiohalogenated nucleosides

This section of the review will cover radiohalogenated nucleosides, where the radiohalogen is attached to the sugar ring and not on the base moiety. A review by L. Wiebe, elsewhere in this series, will include this other class of halogenated nucleosides.

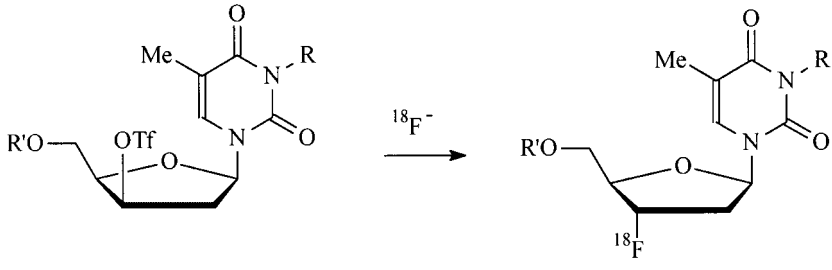
Radiohalogenated nucleosides are an important class of radiopharmaceuticals that have been used to study and treat a wide variety of disorders such as hypoxia in tumors,[33] tumor proliferation[34], gene therapy, Herpes[35] and more.

The basic structure of some common nucleosides is shown in Scheme 6.

There are three positions on the sugar ring[2,3,5] of most nucleosides for incorporation of radiohalogens. In 2-deoxy ribose derivatives, such as thymidine, there are only 2 available positions for halogenation. One of the standard methods for incorporating a radiohalogen into the sugar ring is by the nucleophilic displacement of a good leaving group, such as a triflate, as shown in Scheme 7 for the synthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT). FLT has been shown to be useful for imaging cellular proliferation *in vivo*.[36] In general the halogenation, via



Scheme 6.



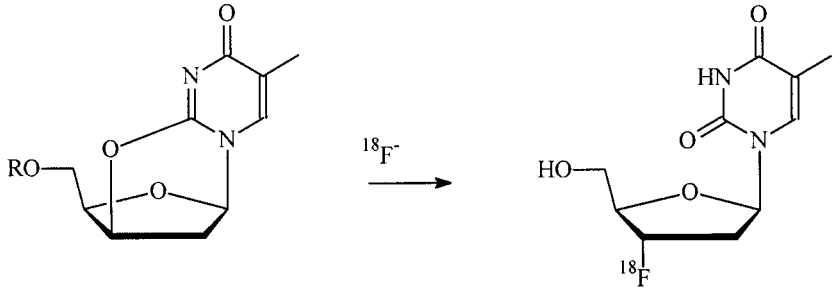
Scheme 7.

nuleophilic displacement, of the sugar ring in nucleosides is no more or less difficult than that of a simple sugar ring. The stereochemistry of the sugar is the most important issue, and the displacement of the leaving group, needs to be set up well. Elimination to the alkene by abstraction of the C-1 or C-3 hydrogen is the most significant problem with these types of reactions and plagues both nucleoside and non-nucleoside sugars alike.

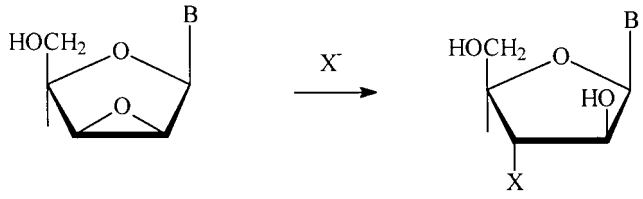
Another route to FLT and other similar nucleosides is the fluoride displacement of the anhydro derivative, as shown in Scheme 8.[37] This method provides reasonable yields of the radiolabelled product, and the anhydro precursor is easier to prepare than the triflate. In fact, the 2,2′ and 2,3′ anhydro intermediates are a well known, excellent route to a variety of 2′ and 3′ halogentated nucleosides.[38] Another favorite method of nucleoside halogenation involves the use of 2′,3′ epoxy compounds,[39] as shown in Scheme 9a. For the incorporation of halogens such as ^{123}I into the 5' position, exchange reactions, as shown in Scheme 9b for the synthesis of IAZA,[40] have been used.

These types of exchange reactions are usually carried out on a primary halide, such as the terminal 5-position of the arabinose sugar, as illustrated since the iodine carbon bond is more labile. In cases where the direct halogenation of the sugar ring on the intact nucleoside is not possible, an alternative 2 step approach has been developed.[41] These researchers incorporated ^{18}F at the C-2 position of the arabino sugar, followed by coupling with the pyrimidine to form the arabinonucleoside as illustrated in Scheme 10.

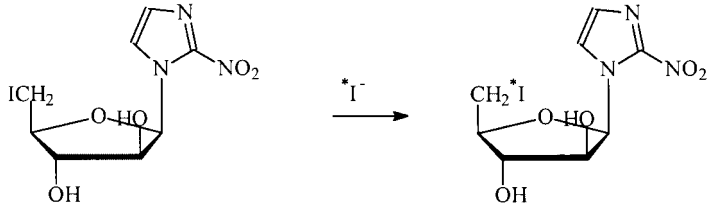
Although this route is not the preferred method for radiolabelling with short lived isotopes, such as ^{18}F-fluorine, it does provide an alternative when the direct method fails. It is also a more general route that could be applied to other arabinonucleosides.



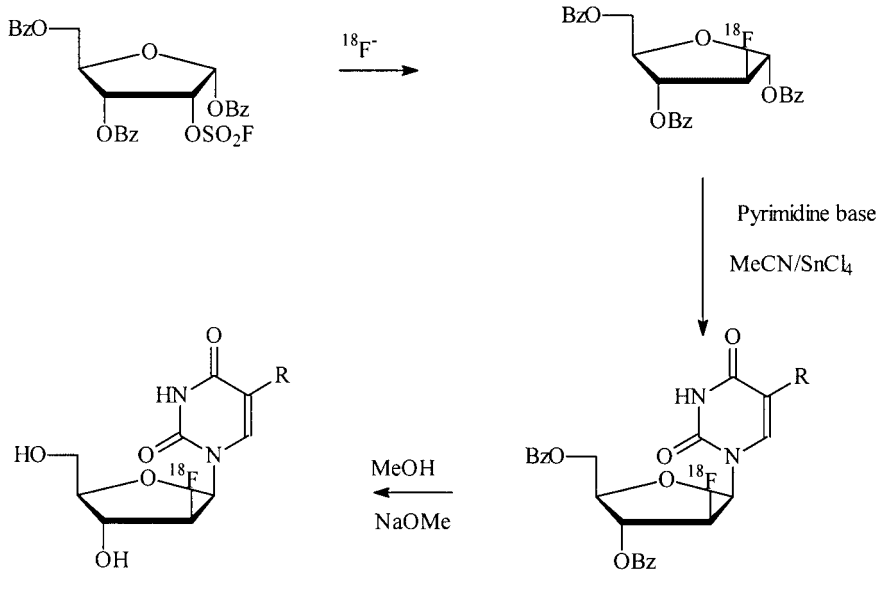
Scheme 8.



Scheme 9a.



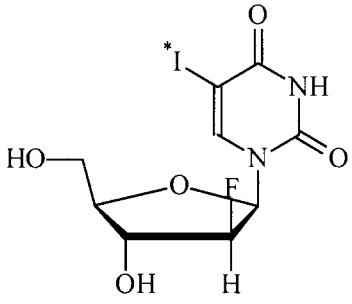
Scheme 9b.



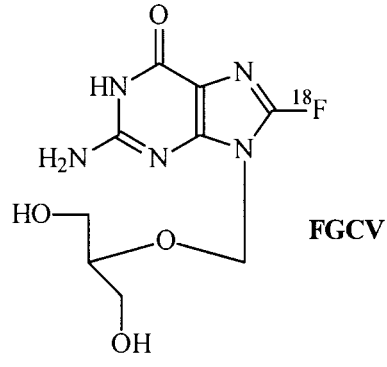
Scheme 10.

There are many other examples of radiohalogenated nucleosides. Some of the more recent exciting developments are in the area of imaging reporter gene expression as a tool for monitoring the expression of genes in animals and humans. Examples of these agents include uracil

nucleosides such as FIAU labelled with radioiodines on the base moiety,[42] and acyclo derivatives such as ^{18}F-labelled fluoroganciclovir.[43]



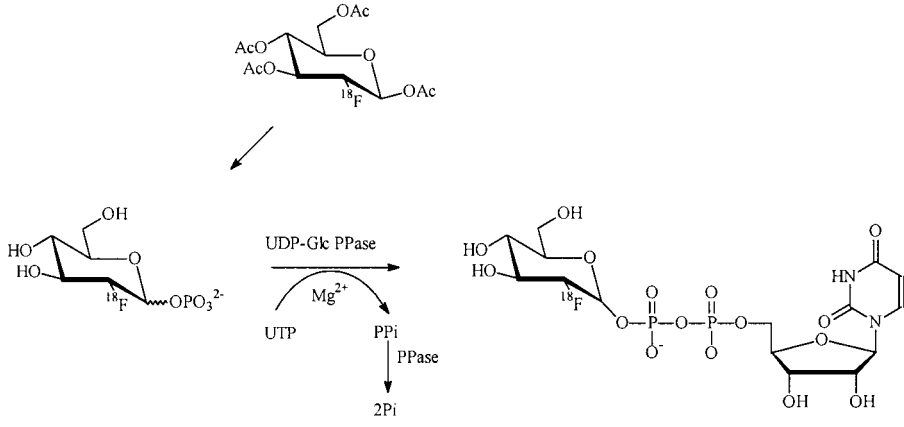
FIAU



FGCV

Radiolabelled nucleosides such as these will be discussed in a separate review in this journal. Another novel example of the application of radiohalogenated nucleosides is related to the field of glycoconjugates. In this paper, the label is on the sugar ring as shown in scheme 11.[44] The authors suggest that this may lead to a general labelling method for glycosylated biopolymers.

In conclusion, the area of radiohalogenated carbohydrates continues to be an active, important area of research with exciting new agents and applications. FDG continues to be the most significant radiohalogenated sugar in this class, and will undoubtedly remain so for some time. However, newly developed agents such as the radiohalogented nucleo-



Scheme 11.

side FLT are beginning to attract a great deal of attention. The future looks bright for radiohalogenated carbohydrates simply due to the important role carbohydrates play in biological systems. One area of application for radiohalogentated carbohydrates, not mentioned in this review, would be the synthesis of glycoconjugates labelled with radiohalogens designed to residualize the label in tumors for possible cancer therapy. This exciting area of research has been carried out with glycoconjugates such radioiodinated tyramine-cellobiose bound to specific antibodies.[45,46] This may well be an area of research that could benefit from a glycoconjugate that has the radiohalogen directly labelled on the sugar ring of the carbohydrate. These and other new developments in radiohalogenated carbohydrates will ensure that this area of radiochemistry research will be active for many years to come.

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