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Review

Preparation of fluorine-18 labelled sugars and derivatives and their application as tracer for positron-emission-tomography

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Abstract

The usefulness of ^{18}F-labelled carbohydrates, especially 2-deoxy-2-[^{18}F]fluoro-D-glucose, to study pathophysiological processes in man non-invasively using positron-emission-tomography (PET) led to a widespread investigation of different ^{18}F-labelled sugars and sugar derivatives. In consideration of the short half-life of fluorine-18 ($T_{1/2} = 110$ min) synthetic strategies concerning precursor design, labelling conditions and deprotection of the intermediate compounds were developed to guarantee an efficient high radiochemical yield synthesis for diagnostic purposes. Besides some aspects of medical application of 2-deoxy-2-[^{18}F]fluoro-D-glucose, a few synthetic strategies are described reflecting development work on promising ^{18}F-labelled sugars for diagnostic purposes during the last two decades.

Keywords: Positron-emission-tomography; ^{18}F-Labelled carbohydrates; Medical application

Contents

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1. Introduction

A second decade of studies with fluorine-18 labelled sugars has come to an end. During this time, various labelled sugars were developed for use as probes for investigating metabolic processes in vivo by positron-emission-tomography (PET). Meanwhile, PET is a routine tool in modern nuclear medicine diagnostics, as well as in research of physiological processes. In contrast to other imaging techniques, PET is capable of providing functional images based on quantitative data of metabolic rate constants and tissue distribution of a certain radiotracer in vivo. The method is based on the application of compounds that are labelled with positron emitting radionucleides such as fluorine-18 (half life 110 min). These positron emitters are the prerequisite for a quantitave measurement of radioactivity with high spatial resolution within living subjects.

Besides the need for the radionucleide fluorine-18 for PET application, the modification of substituents in carbohydrate ring positions, especially by fluorine or by functional groups bearing one or more fluorine atoms, was clearly shown to produce important carbohydrate analogues with dedicated pharmacological action. Simple fluorine substitution of a hydroxy-function results in almost all cases in molecules that are generally metabolized until the C–F bond will be attacked in the biochemical pathway. Fluorocarbohydrates therefore are most useful key tools for the isolation of a biochemical reaction sequence from a more general metabolic pathway, especially in functional imaging sciences. Accordingly, fluorine bearing carbohydrate molecules may exhibit certain therapeutic potential in the future.

Table 1
Outstanding importance of [^{18}F]FDG for nuclear medicine demonstrated by evaluation of scientific reports

Classification of radiotracer	Share in literature (%)	Share of patients (%)
[^{18}F]FDG	20	13.5
Other PET	9	1.5
Non PET	71	85

The first decade of PET research using labelled sugars was characterized by the exploration of a variety of ^{18}F-fluorinated carbohydrates with the aim of non-invasive investigation of selected biochemical processes. During this time, a couple of compounds have been described and basic work on labelling of sugars with fluorine-18 was performed. Overviews were given by Gatley et al. [1] and Tsuchiya [2].

During the last decade, further fluorine-18 labelled sugars for highly specialized applications have been developed. Determined by the demands of nuclear medicine application of the standard procedures for synthesis and purification for standard compounds were refined. However, only few of these compounds became radiopharmaceuticals of diagnostic importance.

2. 2-Deoxy-2-[^{18}F]fluoro-D-glucose [^{18}F-FDG] — the most frequently applied radiopharmaceutical for positron-emission-tomography

2.1 Aspects of medical application of [^{18}F]FDG.

2-Deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) is the most frequently applied radiotracer in PET — it is the workhorse of PET. It is produced routinely by some 130 PET centres worldwide [3]. It came into the focus of nuclear medicine mainly within the last 10 years. This is primarily due to the fact that [^{18}F]FDG has been shown to be a multi-purpose radiopharmaceutical with applications in a variety of diagnostic questions in neurology, oncology and cardiology. The growth of the diagnostic field was made possible by the development of whole body PET cameras in the late 1980s/early 1990s. The importance of the last mentioned development is the unique chance for detecting broad varieties of tumour tissue and its metastases at an early stage of the disease in the whole body.

The scientific interest of the nuclear medicine community in [^{18}F]FDG is demonstrated by analysing the scientific contributions in the 'Journal of Nuclear Medicine' and the 'European Journal of Nuclear Medicine' in 1998 (Table 1). The results are comparable for both journals.

Why is [^{18}F]FDG a tracer of such an outstanding importance?

The main advantage of the tracer is that it reflects the glucose metabolism, the main energy source in the human body. The general biochemical behaviour and the first steps of the metabolism of glucose and [^{18}F]FDG are very similar. After intravenous injection of [^{18}F]FDG, it is distributed within the body with the bloodstream and efficiently extracted by the cells. Transport into the cells is very quick and similar to that of glucose. There is a short and clear metabolic pathway, the first and important step of glucose metabolism being the phosphorylation by hexokinase to 2-deoxy-2-[^{18}F]fluoro-D-glucose-6-phosphate. In contrast to native glucose, the further metabolism can be neglected within about 1 h since 2-deoxy-2-[^{18}F]fluoro-D-glucose-6-phosphate is not a substrate for glucose-6-phosphate-isomerase. The enzyme for possible dephosphorylation, namely the glucose-6-phosphatase, is in most of the tissues under investigation (i.e., brain, tumor tissue) either non-existent or it exists only in tiny amounts. Also, other pathways usually starting from D-glucose-6-phosphate are blocked for 2-deoxy-2-[^{18}F]fluoro-D-glucose-6-phosphate [4]. This 'metabolic trapping' is the prerequisite for the isolated evaluation of the key step, the glucose utilization. This is due to the blocked 2-position of the molecule.

The basis for this application was furnished by the development of a method for measurement of the local cerebral glucose utilization using [^{14}C]deoxy-D-glucose [5]. It was demonstrated to be very similar to [^{18}F]FDG [6], the prerequisite for the application in PET. Furthermore, the half-life of ^{18}F (110 min) is very suitable for the time needed for medical investigations.

The [^{18}F]FDG method originally introduced for brain studies in order to understand the pathophysiology was extended to heart investigations [4] and to oncology.

In the following the main clinical applications are mentioned.

2.1.1 Oncology.

The value of [^{18}F]FDG for tumour diagnosis is based on an increased glucose metabolism [7] of tumour cells. The transport into the tumour cells is increased and accordingly the uptake in the tumour tissue is significantly higher in comparison with the 'normal' tissue. The accumulated activity is measured by the PET camera and visualized.

What are the main fields in tumour diagnosis?

- Localization of local or distant metastatic spread. Even if the primary tumour was identified by other imaging modalities, a knowledge of locoregional or distant metastases is crucial for treatment planning [8]. In relation to contrast and resolution of the scanner, PET can identify metastases of 3–5 mm in diameter.
- The grading of tumours according to their [^{18}F]FDG uptake provides valuable information on their tumorpathophysiology, and is of importance for further treatment planning and for the assessment of prognostic factors [9].
- Evaluation of tumour response to different kinds of therapy like chemotherapy or radiation therapy based on repeated measurements at different timepoints [10,11].
- Detection of tumour recurrence. After therapy regimes like radiation therapy differentiation between tumor recurrences and scar tissue may be impossible by conventional imaging techniques [12,13]. A tumour recurrence is delineated by a higher [^{18}F]FDG uptake in comparison with necrosis or scar tissue, which has normally a low energy consumption.

2.1.2 Cardiology.

The main task for [^{18}F]FDG-PET in cardiology is to identify viable myocardial tissue, which would benefit from revascularisation procedures [14]. In patients with coronary heart disease perfusion imaging, i.e., with ^{201}Tl-perfusion-SPECT, may identify areas of myocardial perfusion defects, the result of heavily narrowed coronary arteries, identified by angiography. The question is whether these areas of compromised myocardium are still viable, with the possibility to regain normal function, or if these areas of malperfusion are due to myocardial infarction and represent scar tissue, which would not benefit from

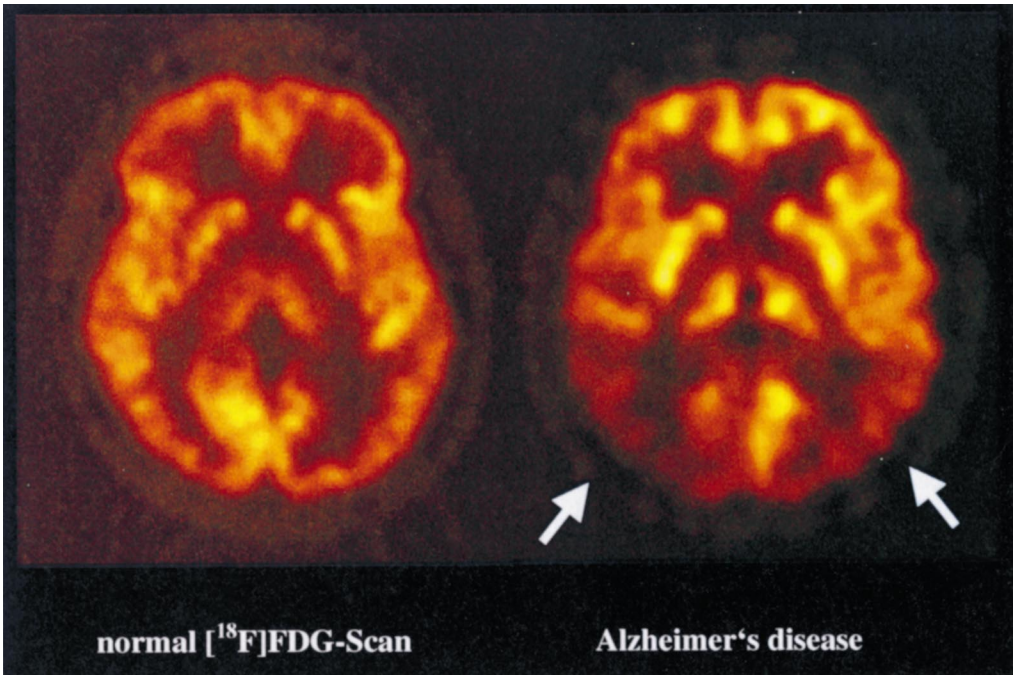


Fig. 1. Typical reduction of glucose metabolism of the parietal-temporal lobes in Alzheimer's disease.

revascularisation. In the state of chronic malperfusion and low energy supply, myocardium reaches the state of so called 'hibernating myocardium' [15], which is characterized by decreased energy consumption and lack of contractility. The minimum energy level for the survival of these cells is derived from glycolysis, whereas the normal myocytes take their energy supply mainly from free fatty acids. Accordingly, hibernating myocardium shows a significantly higher [^{18}F]FDG uptake than the surrounding myocardial tissue. Scar tissue has no glucose metabolism any more.

Based on cardiac [^{18}F]FDG-PET, risk stratification can be done to identify candidates for revascularisation and patients for whom the risk of procedures like bypass operation outweighs their benefit. Cardiac PET is a valuable tool to direct heart operations only to those patients who will benefit and to reduce the high costs of unnecessary procedures [16].

2.1.3 Neurology.

The exclusive energy source of the human brain is glucose. The energy consumption is closely connected to the function of the brain and the neuronal activity. Investigations of the cerebral glucose metabolism gives insight into regional or global impairment of cerebral function in certain brain disorders [17].

One main indication of cerebral [^{18}F]FDG-PET concerns the identification of epileptic foci in patients with intractable epilepsy before surgical intervention [18]. During epileptic activity the focus is identified by high glucose consumption. In times without epileptic activity the focus normally exhibits glucose metabolism below the surrounding brain tissue. In routine application, cerebral [^{18}F]FDG-PET is normally done in times without epileptic activity.

Growing interest is related to early diagnosis and differentiation of different forms of dementia [19]. Dementia may be due to different neurologic diseases, like Alzheimer's disease or multi infarct dementia. Many of these syndromes display different patterns in cerebral [^{18}F]FDG-PET like the typical reduction of glucose metabolism of the parietal-temporal lobes in Alzheimer's disease (Fig. 1).

Some diseases even show typical changes in cerebral [^{18}F]FDG-PET before neurological symptoms can be distinguished. Huntington's disease, inherited in a autosomal-dominat fashion, shows a reduction of glucose metabolism of the basal ganglia before psychiatric symptoms, chorea or dementia may be present [19].

As already mentioned in the oncology section, especially in brain tumours the assessment of glucose metabolism in relation to surrounding brain tissue, the potential of identification of viable tumour tissue for tumour biopsy and identification of tumor recurrence within necrotic areas are of importance for patient management and treatment planning. Growing interest is directed towards the evaluation of changes of glucose metabolism in psychiatric disorders like depression and schizophrenia in order to gain insight into underlying pathophysiological mechanisms [20–22].

2.2 Methods for synthesizing [^{18}F]FDG.

The development of the chemistry of [^{18}F]FDG was driven all this time by the needs of radiopharmacy/nuclear medicine for PET. The main objectives were high yield, short preparation times, low costs, and high purity of the final product to meet the pharmaceutical criteria. The necessity to produce large amounts of the radiotracer has dictated the need to develop a convenient and reliable synthesis method.

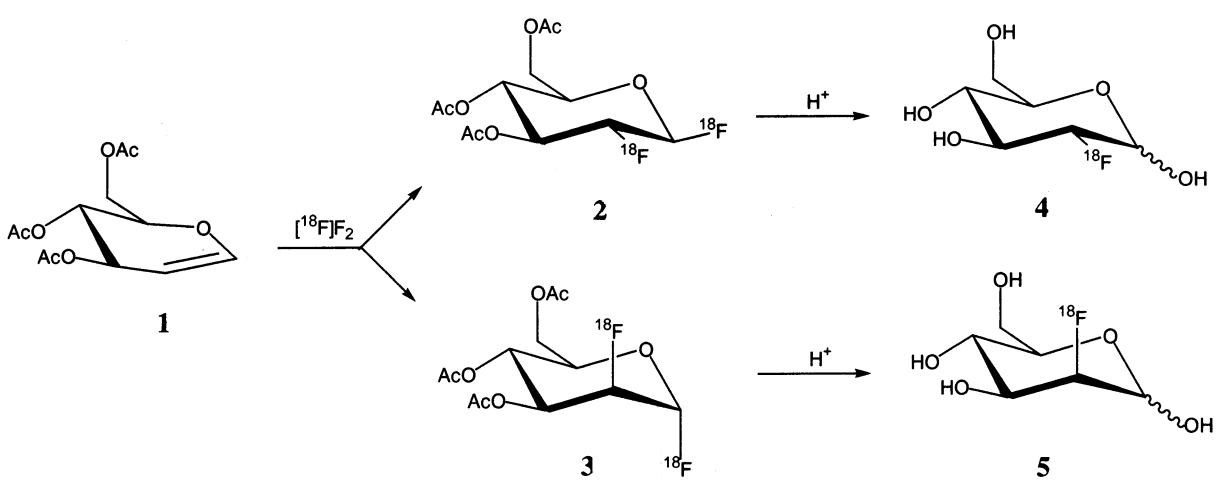
2.2.1 The historical view.

A short overview of the most important methods for synthesizing [^{18}F]FDG (**4**) is repeated even though it was reported similarly in a previous article [1]. This seems to be justified due to the dominance of [^{18}F]FDG in medical application. It should be mentioned that a non-radioactive FDG synthesis was already developed in 1969 [23,24], about 10 years before radiofluorine labelled [^{18}F]FDG was synthesized.

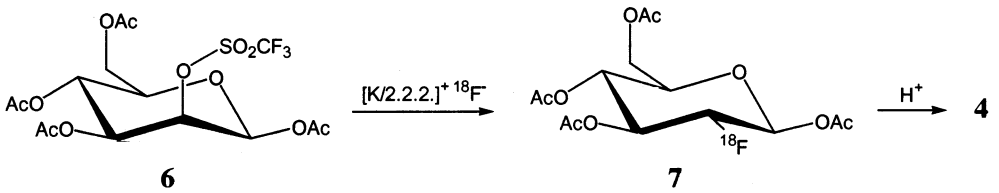
Fluorination methods starting with elemental ^{18}F-fluorine ([^{18}F]F_2): these methods are only of historical importance because of the relatively low radiochemical yields obtained. [^{18}F]F_2 is either directly applied as labelling reagent or after conversion into other agents, e.g., [^{18}F]acetylhypofluorite. [^{18}F]F_2 is usually produced by the ^{20}Ne(d,α)^{18}F reaction in a gas target at a cyclotron. For this purpose, a considerable amount of non-radioactive fluorine has to be added to stabilize the chemical form. Unfortunately, 50% of the fluorine is lost by the electrophilic pathway.

[^{18}F]FDG was first synthesized in 1977 by reaction of elemental fluorine with 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-arabino-hex-1-enitol (tri-*O*-acetyl-D-glucal (**1**) [25–27] (Scheme 1). A stream of diluted ^{18}F-labelled fluorine (0.1–2% in neon) is passed through the solution of 3,4,6-tri-*O*-acetyl-D-glucal in $CFCl_3$. By adding the fluorine to the double bond, two isomers are formed: the 1,2-difluoro-glucose isomer **2** and the 1,2-difluoro-mannose isomer **3** in a ratio of 3:1. These substances are separated on a silica gel column and the 1,2-difluoro-glucose isomer is hydrolyzed by hydrochloric acid. The [^{18}F]FDG yield was in the range of 10% (maximum 1 GBq) after purification.

Even by reaction of [^{18}F]F_2 with unprotected glucal in aqueous solution [^{18}F]FDG can be



Scheme 1. Reaction course of the first [^{18}F]FDG (**4**) synthesis via electrophilic ^{18}F-fluorination [26].



Scheme 2. Reaction scheme of the commonly used phase-transfer-mediated [^{18}F]FDG-synthesis [33].

synthesized. In that case the ratio of the glucose isomer ([^{18}F]FDG) to the mannose-isomer (2-deoxy-2-[^{18}F]fluoro-D-mannose, [^{18}F]-FDM) is about 2:1 [28].

A considerable improvement of achievable yields was reached by fluorination of triacetyl-glucal with [^{18}F]acetylhypofluorite [29] in $CFCl_3$. The yields increased to about 20% and the selectivity of the reaction was also improved. The ratio of [^{18}F]FDG to [^{18}F]FDM was 7:1 [30].

Besides these procedures, [^{18}F]XeF_2 was also used as a fluorinating agent [31,32].

All these electrophilic production methods are characterized by low to medium yields and limited stereoselectivity of the addition reactions, thus leading only to an epimeric mixture of 2-deoxy-2-[^{18}F]fluoro-D-glucose (**4**) and 2-deoxy-2-[^{18}F]fluoro-D-mannose (**5**).

2.2.2 Synthesis of [^{18}F]FDG via nucleophilic fluorination.

The above named disadvantages could be overcome by using nucleophilic S_N2 substitution leading to pure isomers. Firstly, high yield nuclear reactions producing no-carrier-added [^{18}F]fluoride can be utilized. The most common is the ^{18}O(p,n)^{18}F reaction, which is capable of giving a tenfold absolute yield of ^{18}F. Therefore, highly ^{18}O-enriched water as target material is irradiated by protons in an appropriate target holder and the ^{18}F formed is primarily as [^{18}F]HF in the solution. It has to be fixed by a base. Usually this is performed by adding a potassium carbonate solution.

Various syntheses have been reported. They differ in their precursors, the methods of introduction of fluoride, the cleavage of the protecting groups and the technical realization.

The most important method, which is routinely applied nowadays in the vast majority of the worlds PET facilities, utilizes 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-manno-pyranose as a precursor [33] (Scheme 2). This precursor combines the efforts towards an ideal substance; the powerful leaving group triflate leads to a high fluorination yield and the cleavage of the protecting groups in it can be performed under hydrochloric acid conditions. This was combined with an efficient method for introduction of [^{18}F]fluoride via aminopolyether support (Kryptofix® 2.2.2), generating a highly reactive fluoride ion by masking the potassium ions. The potassium cations are the counter part of the no-carrier-added [^{18}F]fluoride isolated from the ^{18}O–water target. The fluorination yield is in the range of 95%, whereas the overall yield of the whole procedure including purification is about 60%.

Numerous variants were worked out utilizing the same precursor. Of importance is the use of the tetrabutylammonium fluoride as the reactive fluoride source [34,35] or the synthesis with resin supported [^{18}F]fluoride [36].

The first important attempts for [^{18}F]FDG synthesis starting from [^{18}F]fluoride are nowadays of historical interest. They utilized the methyl 4,6-*O*-benzylidene-3-*O*-methyl-2-*O*-triflyl-β-D-mannopyranoside [37] and methyl 4,6-*O*-benzylidene-β-D-mannopyranoside-2,3-sulfate [38], respectively as the precursors. Both methods suffered from low fluorination yields (30% [37]), but mainly from problems to remove the methoxy groups. Therefore, the overall yields obtained were not sufficient for widespread medical application.

Automatic procedures were developed for all these methods in order to minimize the radiation risk and for improving the reliability and reproducibility [39,40]. An overview with recommendations for a practical production of [^{18}F]FDG summarizes the previous work [41].

2.2.3 Aspects concerning deprotection and purification.

During the 1990s, no real new synthesis route to [^{18}F]FDG was introduced. All efforts were directed to improve the requirements of a routine application of this radio-tracer. Whereas in the beginning of [^{18}F]FDG application, single charges for individual patients were produced, nowadays large scale batches are produced and distributed. Accordingly, the most common synthesis according to Scheme 2 [33] was optimized with respect to radionucleide yield, rapidity and cost. Simultaneously, the pharmaceutical criteria of the production became dominant. Besides the pharmaceutical quality, the chemical purity of the final product became the focus of interest, i.e., traces of by-products. Additionally, various methods of automation and application of polymer supports were tested.

The second reaction step, the cleavage of the acetyl protecting groups of the intermediate tetraacetyl-[^{18}F]FDG, became of interest.

The first possibility of deblocking without hydrochloric acid was demonstrated using a solid phase acid to apply milder conditions and to avoid the neutralization step [42]. Reacting the protected compound with Dowex 50 sulfonic acid resin (H^+-form) at 100 °C gave within 10 min the final product. Because of the high cost of the equipment, this method was less convenient.

Another reason for the search for an alternative deprotecting method was the generation of two major by-products, the D-glucose and another unknown product. This was identified to be the 2-chloro-2-deoxy-D-glucose [43,44]. The appearance of this substance in the [^{18}F]FDG solution in traces of < 100 μg could be proven by GC analysis. The presence of 2-chloro-2-deoxy-D-glucose is undesirable from a pharmaceutical point of view, even if it can be considered to be toxicologically irrelevant [45]. It is formed during the deprotection procedure of tetraacetyl-[^{18}F]FDG by nucleophilic chlorination of the still remaining precursor, 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose in the presence of hydrochloric acid. This problem could be overcome by deprotection of the acetyl ester functions under basic conditions using sodium hydroxide [46]. Additionally, the deprotection step is much faster in comparison with the acid catalyzed hydrolysis and takes place within only 1 min. Accordingly, the radiochemical yield could be increased as a consequence of a shorter reaction time of 25 min in comparison with 35 min.

The acidic hydrolysis had been favoured over a long time in order to avoid epimerization at the C-2 position according to the 'Lobry de Bruyn–van Eckstein' rearrangement of aldoses under alkaline conditions. In order to ensure safe production conditions without significant epimerisation, an appropriate study was performed, which demonstrated the time and temperature dependence of this process. Using 0.33 M sodium hydroxide solution below 40 °C, within 5 min the epimerization can be limited to 0.5% [47]. This reaction could be applied for epimerization of [^{18}F]FDG to produce [^{18}F]FDM [48]; however, the selective synthesis of 2-deoxy-2-[^{18}F]fluoro-D-mannose can be performed under phase transfer conditions and acid hydrolysis using methyl 4,6-*O*-benzylidene-3-*O*-benzyl-2-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside [49].

Another topic reflected the purity of the radiopharmaceutical [^{18}F]FDG concerns the content of the cryptand Kryptofix® 2.2.2. as a contaminant. The need for elimination is given by its toxicity [50] and therefore it is indispensable to minimize the level of this compound in the [^{18}F]FDG solution to be injected. The toxicity of tetraalkylammonium salts seems to be even higher than that of Kryptofix® 2.2.2.

The solid phase supported [^{18}F]fluorination using special quaternary ammonium salts [36,51] aims to simplify the labelling procedure. Although this method avoids product contamination with the phase transfer catalysts like Kryptofix® 2.2.2. or tetrabutylammonium salts, the insufficient fluorination yield, however, is a hindrance in its application. Because the [^{18}F]fluoride is fixed in a small amount of special anion exchange resins, the resulting very high activity concentration causes radiation effects. Therefore, the yield decreases significantly with increasing activity, making the method unsuitable at high activity levels.

Another interesting approach is the use of microwave heating for the whole process. The high overall yield of about 72% (corrected for decay) is of considerable interest even if the required microwave equipment is extraordinarily expensive [52].

[^{18}F]FDG will keep its importance in PET even if new and more specific tracers are introduced. Therefore, every improvement with respect to yield and pharmaceutical quality is desirable.

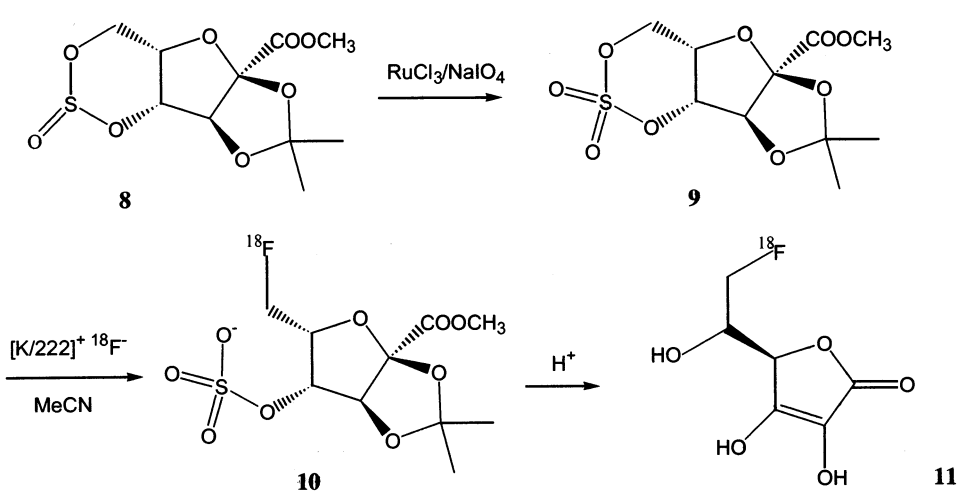
3. Further ^{18}F-fluorinated sugars as radiotracers of potential diagnostic interest

Besides [^{18}F]FDG, the workhorse of PET, a couple of other ^{18}F-fluorinated sugar derivatives have been synthesized in order to prove the usefulness of these compounds as radiotracers for diagnostic purposes.

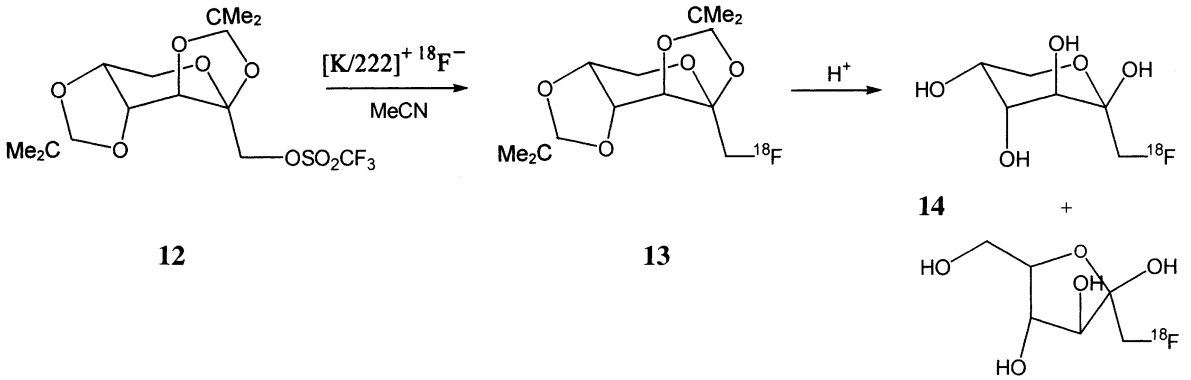
The aim to develop positron emitting radiopharmaceuticals with efficient antioxidant properties leads to the synthesis of 6-deoxy 6-[^{18}F]fluoro-L-ascorbic acid (**11**) for imaging of in vivo biochemistry of antioxidants in humans [53]. Biokinetic studies with such a labelled compound by PET imaging may provide new insights into the pathophysiology of diseased states resulting from oxidative damages as well as better understanding of biochemical functions of antioxidants [54]. The necessity to prevent a significant change in antioxidant activity requires the ^{18}F-fluorination be done at the C-6 hydroxymethyl group because the enediol moiety of the γ-lactone ring is attributed to the reductive potential.

The synthesis of 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid is based on the phase transfer mediated nucleophilic displacement of a cyclic sulfate with no-carrier-added [^{18}F]fluoride Scheme 3. The 4,6-cyclic sulfite (**8**) was prepared from the corresponding diol using thionyl chloride, which was oxidized with $NaIO_4$–$RuCl_3$ to give the cyclic sulfate (**2**). Nucleophilic ^{18}F-fluorination with opening of the 4,6-cyclic sulfate in **10** was performed in the presence of the carbonate containing cryptate $[K \subset 2.2.2.]_2CO_3$ in a dipolar aprotic solvent like acetonitrile. After acid hydrolysis the desired 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid (**11**) was obtained within about 90 min and with a radiochemical yield of 15%.

Tissue distribution studies with 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid in rats showed high uptakes of activity in those organs that have high concentrations of L-ascorbic acid, such as adrenals, kidneys, liver and small intestine. The in vivo uptake and distribution of 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid in rat brain following postischemic reperfusion indicates that this radiotracer increasingly accumulates in vulnerable regions of the brain at the late period of recirculation [55]. This distribution profile of 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid might possibly serve as a tool for understanding the pathophysiology of cerebral reperfusion injury by PET.



Scheme 3. Synthetic route to 6-deoxy-6-[^{18}F]fluoro-L-ascorbic acid (**11**).



Scheme 4. Synthesis of 1-deoxy-1-[^{18}F]fluoro-D-fructose (**14**).

Analogous to 2-deoxy-2-[^{18}F]fluoro-D-glucose, a representative metabolic tracer to investigate the differences in energy production in humans with PET, D-fructose, a physiological ketohexose, has been labelled with fluorine-18. Taking into account the metabolic interrelationships between D-glucose and D-fructose [56], the development of the positron emitter labelled tracers, which undergo metabolic trappings in the process of D-fructose metabolism, would offer a new approach to study human energy metabolism by PET.

The synthetic pathway to the formation of 1-deoxy-1-[^{18}F]fluoro-D-fructose (**14**) is according to the commonly used phase transfer, mediated nucleophilic ^{18}F-fluorination with subsequent acid catalyzed deketalyzation [57] (Scheme 4). Using this procedure 1-deoxy-1-[^{18}F]fluoro-D-fructose was obtained in 30 % uncorrected yield and a synthesis time of 80 min.

It was anticipated that fluorine substitution at the C-1 position of D-fructose would be susceptible to metabolism, primarily by hexokinase and that the 1-deoxy-1-[^{18}F]fluoro-D-fructose-6-phosphate possibly formed would remain unmetabolized in tissue because of the blocking effect of the fluorine atom for further metabolism and low membrane permeability of the phosphate. It was expected that this ^{18}F-labelled ketohexose shows biochemical behaviors closely resembling those of [^{18}F]FDG.

From in vivo studies, however, it has become apparent that the replacement of a hydroxyl group at carbon-1 of D-fructose with a fluorine atom leads to a fluorinated analog, which does not enter the cellular metabolism in vivo. Thus, the ^{18}F-labelled analog, 1-deoxy-1-[^{18}F]fluoro-D-fructose, has no features of a metabolic trapping tracer showing no organ or tumour specific localization in tumours.

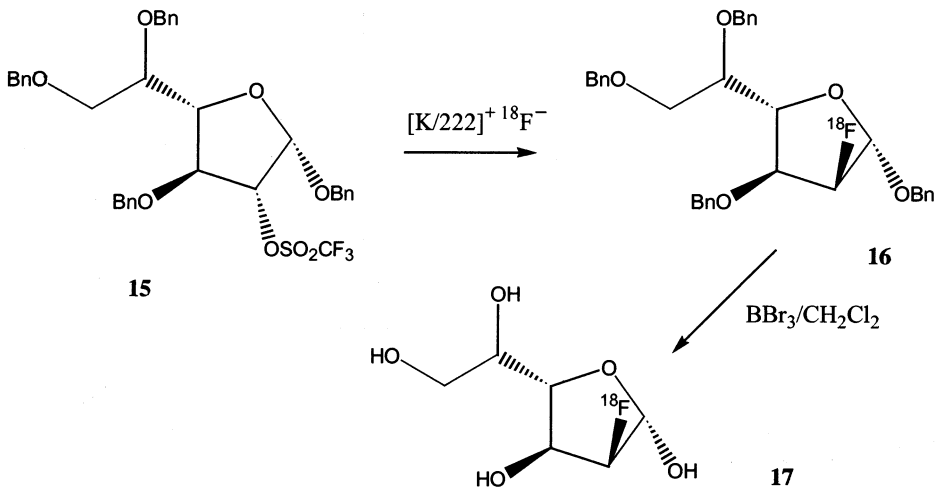
To compare the in vivo behavior of isomeric [^{18}F]fluoro-deoxyhexoses in tumor-bearing animals, 2-deoxy-2-[^{18}F]fluoro-D-talose (**17**) has been synthesized according to Scheme 5 [58]. 2-Deoxy-2-[^{18}F]fluoro-D-talose has an interesting structural feature in that it is isomeric not only with 2-deoxy-2-[^{18}F]fluoro-D-galactose ([^{18}F]FDGal) at C-2, but also with 2-deoxy-[^{18}F]fluoro-D-mannose at C-4. D-Talose, one of the eight D-aldohexoses, is not a physiological substrate in mammals but studies on galactokinase specificity have shown that D-talose can be accepted as a substrate for *Saccharomyces fragilis* galactokinase [59]. When the biodistribution pattern of fibrosarcoma bearing mice was compared with those reported for the three fluorinated D-hexoses, [^{18}F]FDG, [^{18}F]FDM and [^{18}F]FDGal, it was clearly evident that the pattern of 2-deoxy-2-[^{18}F]fluoro-D-talose, [^{18}F]FDTal, has a marked resemblance to that of [^{18}F]FDGal, with moderate capability to accumulate in tumours [60]. The results obtained suggest that 2-deoxy-2-[^{18}F]fluoro-D-talose might be metabolized through the galactose metabolic pathway, analogous to that observed with [^{18}F]FDGal.

Glycoproteins with carbohydrate components like 2-acetamido-2-deoxy-D-galactose have been positively correlated with the metastatic potential, e.g., of melanoma cells [61]. As 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine) is a component of hyaluronic acid, one of the mucopolysaccha-

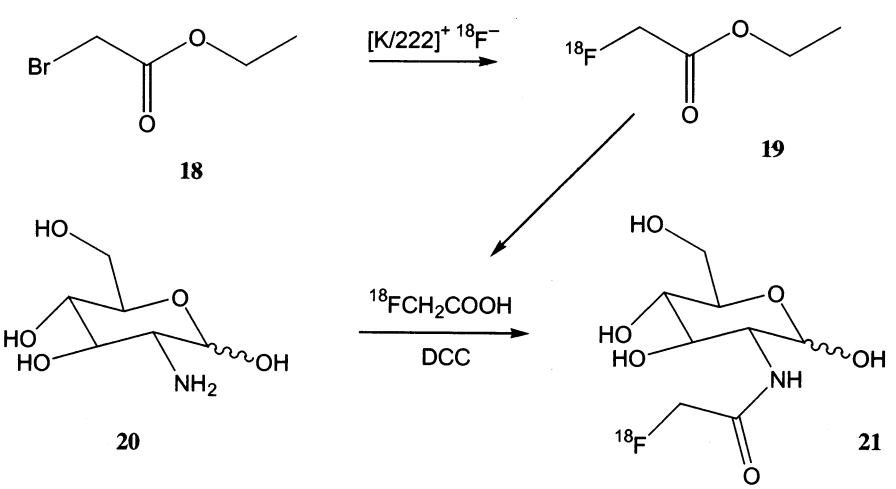
rides, the structural analog *N*-[^{18}F]fluoroacetyl-D-glucosamine **21** has been synthesized according to Scheme 6 [62]. The synthesis was accomplished by a combination of phase transfer mediated nucleophilic halogen exchange, alkaline hydrolysis of the labelled ester and condensation of the [^{18}F]fluoroacetic acid with glucosamine in the presence of dicyclohexylcarbodiimide. The one-pot synthesis combined with chromatographic purification on ion retardation resin yielded the desired sugar in about 9% yield with a radiochemical purity of $>98\%$ within about 90 min.

The fluoro-derivative of *N*-acetyl-D-glucosamine is incorporated into hyaluronic acid by mammalian cells and it was shown that the regions of increased hyaluronate concentrations were in the connective tissue interface between the tumour mass and the neighboring host tissue. PET measurements have demonstrated that the radiotracer can be used as a tumor tracer; however, metabolite analysis showed that the incorporation rate of analog *N*-[^{18}F]fluoroacetyl-D-glucosamine into glycoconjugate synthesis was slower than that of ^{14}C-labelled *N*-acetyl-D-glucosamine. These data suggested that *N*-acetylglucosamine labelled with ^{18}F seems to have a too short half-life to detect the differences of glycoconjugate synthesis in the tumour in vivo.

A well investigated sugar with respect to the isolation of a biochemical reaction sequence from a more general metabolic pathway is 2-deoxy-2-[^{18}F]fluoro-D-galactose ([^{18}F]FDGal) [63]. The compound is actively



Scheme 5. Synthesis of no-carrier-added 2-deoxy-2-[^{18}F]fluoro-D-talofuranose.



Scheme 6. Two step synthesis to *N*-[^{18}F]fluoroacetyl-D-glucosamine (**21**).

metabolized in the hepatic D-galactose pathway. Its administration led at first to an accumulation of 2-deoxy-2-fluoro-D-galactose-1-phosphate, followed by the almost irreversible formation of the corresponding 2-deoxy-2-fluoro-D-galactose-uridine-diphospate and its 4-epimerized analogue. This uridylate trapping action and accumulation of uridinediphosphate fluorocarbohydrate products can cause an uridinetriphosphate deficiency and in turn to a secondary stimulation of the de novo uridylate synthesis. The compound 2-deoxy-2-fluoro-D-galactose may therefore be employed in combination with an inhibitor of the de novo pyrimidine synthesis as a growth inhibition and chemotherapeutic agent against hepatocellular carcinoma. The drug combination has the distinct advantage that, prior and during chemotherapy, its metabolism and distribution can be followed by PET and 2-deoxy-2-[^{18}F]fluoro-D-galactose.

Additionally, 2-deoxy-2-fluoro-D-galactose was shown to be a potent inhibitor of N-glycosylation of secretory and membrane glycoproteins [64], and may be a selective tool for studying the final biological role of protein N-glycosylation and the function of glycoprotein glycans.

The compound 2-deoxy-2-fluoro-D-galactose is best prepared by a direct fluorination procedure of 3,4,6-tri-*O*-aceyl-1,5-anhydro-2-deoxy-D-lyxo-hex-1-enitol (3,4,6-tri-*O*-acetyl-D-galactal). Several methods were reported, such as addition of CF_3OF [65], CH_3COOF [66,67] or directly F_2 in a highly diluted gas mixture. Direct fluorination of the unsaturated carbohydrate derivative also seemed the method of choice for producing the labelled 2-deoxy-2-[^{18}F]fluoro-D-galactose using simply [^{18}F]F_2. Direct fluorination of the galactal delivered yet a stereochemically pure product with regard to the configuration at the C-2 position of the molecule. Only a minor amount of the C-3 epimer of 2-deoxy-2-fluoro-D-galactose was formed during the fluorine addition [68]. Easily available XeF_2 has also been used to produce 2-deoxy-2-fluoro-D-galactose in a good yield [69,70], but this procedure was not suited for labelling purposes. A nucleophilic substitution reaction using a protected 2-*O*-trifluoromethanesulfonyl-talopyranoside has been tried with the fluoride anion and the [^{18}F]fluoride anion [71,72]. It may be a method of choice when no [^{18}F]F_2 can be produced for the labelling reaction or if a high activity level is required.

Easily available 2,3-exo- and endo-epoxides of 1,6-anhydrohexopyranoses may be used in some cases to prepare monofluoro substituted hexoses through nucleophilic oxirane ring opening reactions [73–76]. Especially the hexose ring positions 1, 3, and 4, normally difficultly available for fluorination, may be substituted by this way. The most notable aspect of the bicyclooctane synthetic pathway is the evident simplicity by which the ring positions C-2 to C-4 may be accessible for selective mono fluorination [77]. However, this pathway still remains to be worked out using [^{18}F]fluoride as a reagent.

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