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Research Article

Chemoenzymatic n.c.a synthesis of the coenzyme UDP-2-deoxy-2-(^{18}F)fluoro-α-D-glucopyranose as substrate of glycosyltransferases

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Abstract: The development of ^{18}F-labelling methods adopted to proteins and bioactive peptides is of great interest in radiopharmaceutical sciences. In order to provide ^{18}F-labelled sugars as a polar prosthetic group for an enzymatic ^{18}F-labelling procedure, an appropriate nucleotide activated sugar is needed. Here, we present the radiosynthesis of n.c.a. UDP-2-deoxy-2-[^{18}F]fluoro-α-D-glucopyranose (UDP-[^{18}F]FDG) as a substrate for glycosyltransferases. The MacDonald synthesis of [^{18}F]FDG-1-phosphate was successfully combined with an enzymatic activation to obtain UDP-[^{18}F]FDG directly in an aqueous medium located in the void volume of a solid phase cartridge. The radiochemical yield of UDP-[^{18}F]FDG was 20% (based on [^{18}F]fluoride) after a total synthesis time of 110 min. Thus, an intermediate was provided for the enzymatic transfer of [^{18}F]FDG using UDP-[^{18}F]FDG as glycosyl donor making use of a suitable glycosyltransferase. This would represent a highly selective and mild ^{18}F-labelling method for glycosylated biomolecules. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: ^{18}F; [^{18}F]FDG; UDP-2-deoxy-2-[^{18}F]fluoro-α-D-glucopyranose; glycosyl donor; ^{18}F-glycosylation

Introduction

The development of ^{18}F-labelling methods adopted to proteins and bioactive peptides for diagnostic PET-imaging has gained enormous interest in the qfield of nuclear medicine.[1] Particularly small peptides were optimized for their pharmacological properties and show remarkable advantages in comparison to large proteins or monoclonal antibodies due to the higher uptake of molecules with low molar mass in target tissue and improved blood clearance. The commonly used strategy to label peptides regioselectively via ^{18}F-labelled prosthetic groups such as ^{18}F-fluoroacylation requires multiple-step syntheses. So far, N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and 4-nitrophenyl 2-[^{18}F]fluoropropionate are some of the most effective and common ^{18}F-labelling agents as indicated by their prevalent application in a large number of radiosyntheses for ^{18}F-labelled peptide-based imaging agents (e.g. [^{18}F](D-Phe1)octreotide,[2] [^{18}F]SAA-RGD,[3] [^{18}F]NT(8-13)[4] or [^{18}F](Nle^4, D-Phe^7)-α-MSH;[5] for a review see.[1] However, this laborious strategy often suffers from the necessity of protecting groups to prevent side-reactions and/or complicated and time consuming tracer purification steps.

Thus, additional and improved radiofluorination techniques for important biomolecules such as proteins, oligosaccharides, oligonucleotides or antibodies are urgently needed especially in the case of selectively labelled compounds. In this paper, we focussed on a stereo- and regioselective method for the labelling of glycoproteins or saccharide acceptor molecules with fluorine-18. In the last decade, an enormous insight into the functional and biological properties of glycoproteins and their oligosaccharide moiety has been gained.[6,7] The ubiquity of glycoconjugates in nature reflects their broad function as markers in cell-cell adhesion,[8] inflammation[9] or host immune responses.[10] In order to further characterize the properties of glycoconjugates, the synthesis of defined

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substructures and the manipulation of their carbohydrate units is a predominant goal in glycoscience and glycoprotein remodelling (GPR).[11] The availability of a number of enzymes, such as glycosyltransferases, as synthetic tools in preparative organic chemistry of oligosaccharides has essentially supported this upcoming field of science.

Among the glycosyltransferases, β-(1,4)-galactosyltransferase is known as one of the most intensively studied transferases with regard to its acceptor substrate flexibility and donor substrate specificity.[12–16] In the biosynthesis of N-Asn glycosides the natural donor substrate of β-(1,4)-galactosyltransferase is UDP-galactopyranose, a nucleotide activated sugar donor, which is transferred to the terminal acceptor substrate N-acetyl-glucosamine (GlcNAc) in oligosaccharide units giving a single product with well defined regio- and stereochemistry.[17] Moreover, the use of UDP-glucose-4-epimerase (EC 5.1.3.2) could allow epimerization of structural analogues of UDP-galactopyranose, such as UDP-2-deoxy-2-fluoro-α-D-glucopyranose (UDP-FDG), to the corresponding galactosyl donor, so that β-(1,4)-galactosyltransferase represents an interesting transferase even for UDP-FDG.

To our knowledge, there is no evidence in the literature for the application of an enzyme-mediated glycosylation reaction making use of an ^{18}F-labelled nucleotide activated sugar donor and thus leading to a reliable, highly selective and mild ^{18}F-labelling method for glycosylated biomolecules. The often beneficial effect of glycosylated molecules on their biodistribution is amply demonstrated[18] and used to improve the biokinetics of ^{18}F-acylated peptides.[3,19]

Thus, the aim of this study is the development of a suitable radiosynthesis for UDP-2-deoxy-2-[^{18}F]fluoro-α-D-glucopyranose (UDP-[^{18}F]FDG) as a potential sugar donor substrate for glycosyltransferases, in order to combine the introduction of the ^{18}F-label with that of the polar sugar moiety. Our approach started from 1,3,4,6-tetra-O-acetyl-2-deoxy-2-[^{18}F]fluoroglucopyranose as an easily available intermediate in the radiosynthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG).[20] In this study we describe a rapid chemoenzymatic method for the synthesis of a n.c.a. ^{18}F-fluorinated coenzyme that allows final enzymatic activation of [^{18}F]FDG directly in aqueous medium making use of the small void volume of an anion exchange resin. Thereby the accessibility of a ^{18}F-labelled glycosyl substrate as a prosthetic group is a prerequisite for enzymatic transfer of [^{18}F]FDG in a regio- and stereoselective ^{18}F-glycosylation of target biopolymers without the use of protecting groups. After a previous short communication of this concept[21] an approach of using ^{18}F-labelled sugars was recently also achieved by a non-enzymatic method,[22] however, with less regioselectivity, due to glycosyl ester formation with interfering carboxyl groups.

Results and discussion

Enzymatic synthesis of uridine-5'-diphospho-2-deoxy-2-fluoro-α-D-glucopyranose (UDP-FDG)

For the cold synthesis of the nucleoside phosphate sugar UDP-FDG we emphasized an enzymatic strategy, because it can in principle be coupled in situ to the reaction of the glycosyltransferase (Scheme 1). This strategy closely followed the preparation of UDP-glucose, which has been scaled up to 40 mmol by Hirschbein *et al.*[28] The enzymatic synthesis of UDP-FDG starting from 2-deoxy-2-fluoro-α-D-glucopyranose required four enzymes which were commercially available: hexokinase (HK, EC 2.7.1.1), phosphoglucomutase (PGM, EC 5.4.2.2), UDP-glucose-pyrophosphorylase (UDP-Glc PP, EC 2.7.7.9), inorganic pyrophosphatase (PPase, EC 3.6.1.1). Furthermore, carbohydrates fluorinated in 2-position are known as substrates for some of these enzymes (HK,[29] PGM[30]). In addition, either the natural substrate ATP or UTP can be used as phosphate donor for the hexokinase-catalysed first step of the synthesis (Scheme 1). With regard to a one-pot-synthesis, we used UTP, since it acts as a participant in the pyrophosphorylase-catalysed last step of the synthesis. PGM was not applied in its commercially available form; rather the $(NH_4)_2SO_4$-suspension was removed by ultracentrifugation (Microcon-3, Amicon GmbH) and the membrane washed twice with Tris buffer in order to remove sulfate ions and thus to deminish inhibitory effects on UDP-Glc PP.[31]

Unfortunately, preliminary results indicated that the yield of UDP-FDG in a one step incubation starting from FDG was low (10% (HPLC), t=24 h). However, adequate yields were obtained in a two-step incubation precedure. This was accomplished by phosphorylation of FDG in 6-position using hexokinase involving ATP at aquimolar amounts in the first step. Inactivation of the enzyme by heating and centrifugation were sufficient to allow for increasing yields in the second incubation step. Thus, starting from FDG-6-phosphate the corresponding FDG-1-phosphate was produced by PGM-mediated catalysis. FDG-1-phosphate was condensed with UTP to give UDP-FDG. As a byproduct pyrophosphate (PP_i) was formed which was hydrolysed *in situ* by inorganic pyrophosphatase in order to make this reaction irreversible. This two-step procedure turned out to be fully adequat for the synthesis of UDP-FDG. Analysis by ion-pair HPLC before isolation of the product indicated 50% conversion of FDG to

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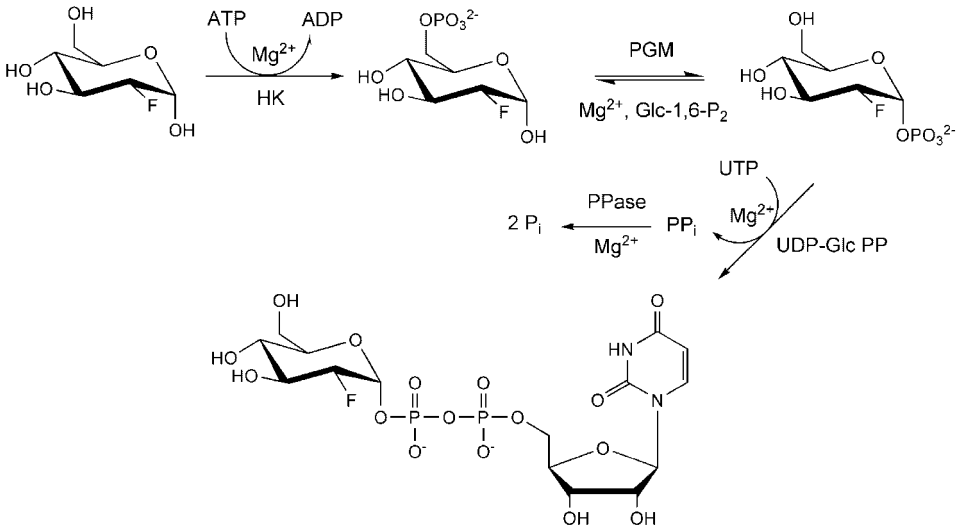

UDP-FDG

Scheme 1 Synthesis of uridine 5′-diphospho-2-deoxy-2-fluoro-α-D-glucopyranose (UDP-FDG).

UDP-FDG after 6 days of incubation. A shorter incubation time would probably be sufficient. The strategy for isolation and purification of UDP-FDG was simplified by the application of alkaline phosphatase from calf intestine (CIAP, EC 3.1.3.1). This enzyme selectively catalyses the hydrolysis of terminal phosphoric esters, such as UTP, to nucleosides and has also been used as a tool to avoid inhibitory effects due to high nucleotide concentrations in the field of sialyltransferase mediated synthesis.[32] After selective hydrolysis of nucleotides and sugar phosphates over night at room temperature and separation of enzymes, a weak anion exchange resin can be used to separate resulting ortho-phosphate from the UDP-FDG containing fractions. Here, we used DEAE-Sephadex and the volatile buffer ammonium hydrogencarbonate as eluent allowing isolation of UDP-FDG by repeated lyophilization.

UDP-FDG could be obtained in a chemical purity of 90% (HPLC) and a yield of about 35% (referring to FDG). This study discovered that FDG-1-phosphate serves as a substrate of bovine liver UDP-glucose-pyrophosphorylase (UDP-Glc PP, EC 2.7.7.9). In order to determine the effects of the fluorinated substrate on enzyme activity we performed an HPLC analyses in more detail.

Assay of UDP-glucose-pyrophosphorylase (UDP-Glc PP)

The HPLC assay of UDP-glucose-pyrophosphorylase was performed as described under Experimental. The enzyme activity was shown to be related to the Mg^{2+}/UTP ratio. For two different concentrations of Mg^{2+} (2, 10 mM) the UTP-concentration was varied from 0.5 to 10 mM. We found the highest enzyme activity of UDP-Glc PP with 10 mM Mg^{2+} and 1.4–3.5 mM UTP. 'Free' UTP which is not masked by Mg^{2+} lead to substrate inhibition of UDP-Glc PP indicated by a decreased enzyme activity. In comparison with the reference substrate α-D-glucopyranose 1-phosphate, we found the enzyme activity to be decreased by a factor of 30 when using 2-deoxy-2-fluoro-α-D-glucopyranose 1-phosphate under the same incubation conditions (Figure 1).

Chemoenzymatic radiosynthesis of uridine-5'-diphospho-2-deoxy-2-(^{18}F)fluoro-α-D-glucopyranose (UDP-(^{18}F)FDG)

With regard to the adaptability of the multiple enzyme system for the radiosynthesis of n.c.a. UDP-[^{18}F]FDG a rapid hexokinase-mediated phosphorylation of [^{18}F]FDG utilizing ATP or UTP as phosphate donor was successful. However, further isomerization of n.c.a. [^{18}F]FDG-6-phosphate to n.c.a. [^{18}F]FDG-1-phosphate was hindered due to the formation of n.c.a. [^{18}F]FDG-1,6-diphosphate as main product. Applying carrier added conditions (5 mM FDG-6-phosphate), [^{18}F]FDG-1,6-diphosphate was not detected, but the radiochemical yield of UDP-[^{18}F]FDG did not exceed 4% within 2 h. Noteworthy, decreased enzyme activity could be excluded by addition of non-isotopic carrier (α-D-glucose-6-phosphate) which was transformed to UDP-Glc properly as shown by HPLC analysis. This 'monitor-reaction' to assay enzyme

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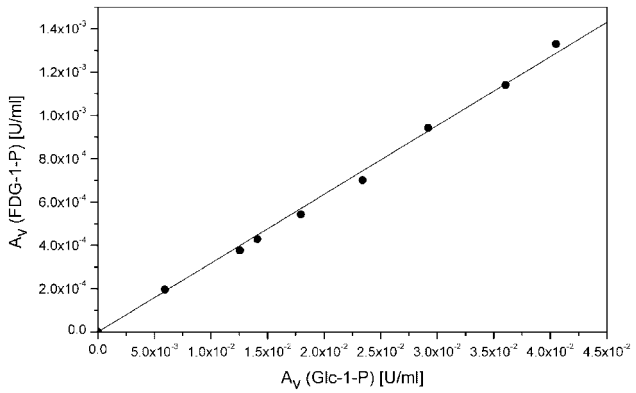


Figure 1 Determination of enzymatic activity of UDP-glucose-pyrophosphorylase (UDP-Glc PP) for the substrates 2-deoxy-2-fluoro-α-D-glucose 1-phosphate (FDG-1-P) and α-D-glucose 1-phosphate (Glc-1-P) as a reference. The activities were measured in terms of the activity unit (U) which is defined as the amount which will catalyse the transformation of 1 μmol of α-D-glucose 1-phosphate per minute (see Experimental). Values are expressed as volume activity (A_V [U/ml]). A_V(FDG-1-P) = 0.0318 · A_V(Glc-1-P); R=0.9985.

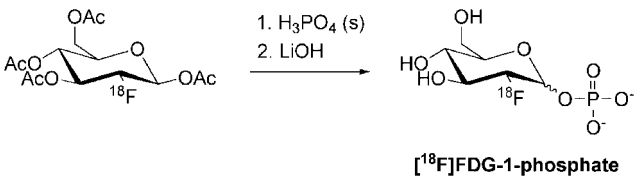
activity demonstrated almost 70% yield of UDP-Glc after 2 h. Due to the necessity of carrier added conditions and limited radiochemical yield, we focussed on the development for a chemoenzymatic synthesis of n.c.a. UDP-[^{18}F]FDG including chemical phosphorylation and avoiding the critical isomerization step under no-carrier-added conditions.

MacDonald phosphorylation of tetraacetylated (^{18}F)FDG

According to MacDonald,[33] glycosyl phosphates could be prepared by fusion of fully acetylated carbohydrates with anhydrous crystalline phosphoric acid at moderate temperatures as depicted in Scheme 2. The reaction was highly sensitive to moisture and performed under high-vacuum. In order to adopt this procedure to the phosphorylation of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranose some features had to be optimized. Using a glassy carbon vial and limiting the amount of phosphoric acid to 20–30 mg improved the radiochemical yield significantly. Furthermore, the deprotection of the intermediate could be performed by neutralizing the melt with aqueous LiOH, so that organic solvents were avoided. The chemical phosphorylation via MacDonald reaction and subsequent deprotection led to a radiochemical yield of 55% of [^{18}F]FDG-1-phosphate (decay-corrected and related to teraacetylated [^{18}F]FDG). The product was separated from [^{18}F]FDG, which occurs as a by-product, using an anion exchange cartridge (Accell QMA, Waters).

Enzymatic synthesis of UDP-(^{18}F)FDG

The MacDonald procedure provided excellent conditions to perform the following enzymatic coupling reaction directly in the void volume of the anion exchange resin (Scheme 3). Particularly with regard to an automated chemoenzymatic synthesis of UDP-[^{18}F]FDG we examined the influence of UTP-concentration. As shown in Figure 2 a maximum radiochemical yield of UDP-[^{18}F]FDG (60%) was determined for 0.5 M < c(UTP) < 0.8 M. Although the concentration of Mg^{2+} was chosen to be in excess when compared to UTP (c(Mg^{2+})=4c(UTP)), a significant loss of UDP-Glc-PP activity was observed for UTP-concentrations above 0.8 M. It seems likely that this effect is due to substrate enzyme inhibition these reaction conditions. Because of the fact that the pyrophosphorylase activity is decreased by a factor of 30 (Figure 1), we intended to increase the enzyme concentration in order to accelerate conversion of [^{18}F]FDG-1-phosphate to UDP-[^{18}F]FDG. This was realized by minimizing the amount of anion exchange resin (50 mg) used to isolate [^{18}F]FDG-1-phosphate and concurrently using high enzyme concentrations. Increasing the enzyme concentration alone led to a shorter reaction time of about 15 min compared to a total reaction time of approximately 60 min as graphically depicted in Figure 3. As referred to [^{18}F]FDG-1-phosphate the n.c.a. enzymatic activation interstitially in the anion exchange resin provided a decay-corrected radiochemical yield of UDP-[^{18}F]FDG of about 60% after an incubation time of 60 min. Thus, the overall radiochemical yield of UDP-[^{18}F]FDG was 20% after a total radiosynthesis time of 110 min and provided the nucleotide activated sugar within an aqueous medium.



[^{18}F]FDG-1-phosphate

Scheme 2 Phosphorylation of tetraacetylated [^{18}F]FDG following the procedure of MacDonald (1962).

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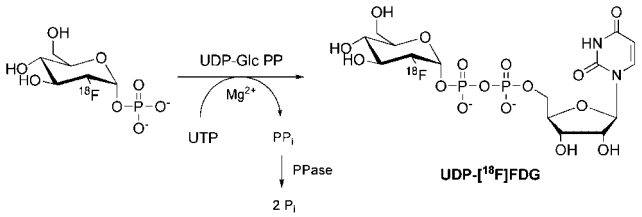
Experimental

Reagents and equipment

1,3,4,6-Tetra-O-acetyl-β-D-mannopyranose and 1,3,4,6-tetra-*O*-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose were synthesized as described elsewhere.[20,23,24] The ammonium molybdate reagent (Mo(V)-Mo(VI) reagent) was prepared as described by Baba *et al.*,[25] 2-Deoxy-2-fluoro-D-glucopyranose 6-phosphate (FDG-6-P) was obtained from Sigma (Deisenhofen, GER). The following enzymes were purchased from Sigma (Deisenhofen, GER): inorganic pyrophosphatase (EC 3.6.1.1, yeast), hexokinase (EC 2.7.1.1, yeast), phosphoglucomutase (EC 5.4.2.2, chicken muscle) and UDP-glucose-pyrophosphorylase



Scheme 3 Radiosynthesis of the ^{18}F-labelled nucleotide activated sugar donor UDP-[^{18}F]FDG by enzymatic coupling of UTP to n.c.a. [^{18}F]FDG-1-phosphate in the void volume of the anion exchange resin.

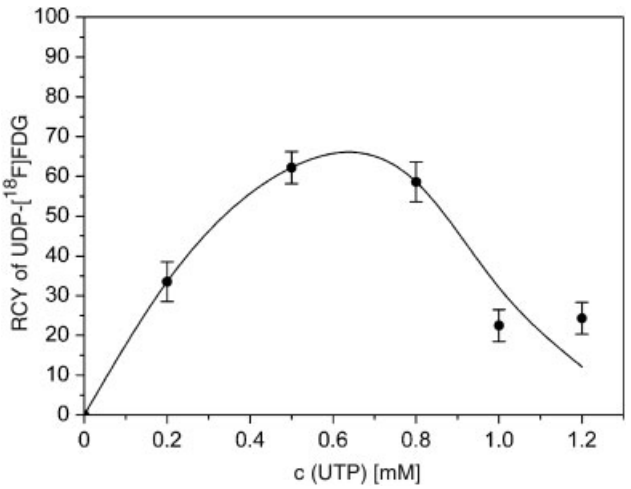


Figure 2 Dependence of the radiochemical yield (RCY) of UDP-[^{18}F]FDG on the concentration of UTP.

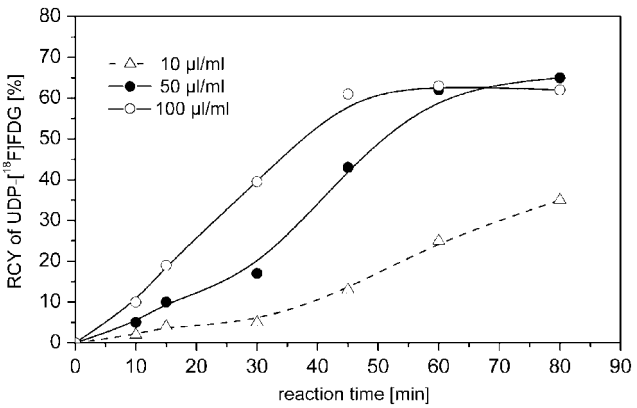


Figure 3 Dependence of the radiochemical yield (RCY) of UDP-[^{18}F]FDG on reaction time and UDP-glucose-pyrophosphorylase concentration. The specification 50 µl/ml refers to the addition of 50 µl UDP-Glc-PP (corresponding to 10 units) to a reaction volume of 1 ml.

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(EC 2.7.7.9, bovine liver). Prior to use, the $(NH_4)_2SO_4$-suspension of phosphoglucomutase was removed by ultracentrifugation (Microcon-3, Amicon GmbH) and the membrane washed twice with Tris buffer. Alkaline phosphatase (EC 3.1.3.1, calf intestine) was purchased from Roche (Mannheim, GER). ortho-H_3PO_4 (s) was obtained from Sigma (Deisenhofen, GER) and dried over P_4O_{10} before use. All enzyme-mediated reactions were performed in degassed twice distilled H_2O. Other chemicals and biochemicals were obtained from standard commercial sources. [^{18}F]Fluoride and [^{18}F]FDG[20] were produced by standard procedures at the JSW-BC1710 cyclotron and by the service group for radiopharmaceuticals, respectively. Sep-Pak™ C-18 plus-cartridges were purchased from Waters. Flash silica gel column chromatography was carried out on silica gel (230–400 mesh). Thin layer chromatography (TLC) was carried out on silica gel-coated aluminium plates (60F254, Merck). Compounds were visualized by UV light (254 nm). Radio-TLC was detected and analysed by electronic autoradiography (Instant Imager™, Canberra Packard). Analytical HPLC was performed on the following system: HPLC-pump (Merck, Hitachi L-6000), UV/VIS-detector (Merck, Hitachi L-4000) with variable wavelength and a radioactivity detector (EG&G Ortec, model 276). Computer analysis of HPLC data was performed with Nina-software (Vers. 1.4, Nuclear Interface). Melting points (m.p.) were determined on a Mettler FP-61 apparatus (uncorrected). Proton nuclear magnetic resonance (^{1}H-NMR) spectra and ^{13}C-NMR, ^{19}F-NMR and ^{31}P-NMR spectra were recorded on a Bruker Avance 200 instrument at 200 MHz (at the Institute of Nuclear Chemistry) or on a Bruker AM 300 spectrometer at 300 MHz (at the Institute of Organic Chemistry at the University of Cologne). Chemical shifts are reported in ppm relative to TMS as an internal standard. Mass spectra were measured on a Finnigan mass spectrometer Automass III (electrospray-ionization, ESI).

Determination of UDP-glucose-pyrophosphorylase activity

Ion-pair high-performance liquid chromatography (IP-HPLC) was used for the assay of UDP-glucose-pyrophosphorylase (EC 2.7.7.9, bovine liver). The mobile phases were phosphate buffered solutions and tetrabutylammonium hydrogensulphate (TBHS) was used as pairing agent as described by Ryll and Wagner.[26] Buffer A: 100 mM potassium dihydrogenphosphate-dipotassium hydrogenphosphate buffer, 8 mM TBHS (pH 6); buffer B: 70% buffer A, 30% methanol (pH 6). A LiChrosorb RP-18 column (250 mm × 4 mm, 7 μm particle size, CS-Chromatographie, Langerwehe, Germany) was used. The gradient elution was: 100% buffer A for 2.5 min, 0–50% buffer B for 14 min, 50–100% buffer B for 1 min, 100% buffer B for 8 min, 100–0% buffer B for 1 min, followed by an equilibration period. The flow rate was 1.0 ml/min. FDG, FDG-phosphates, UDP-2-deoxy-2-fluoro-α-D-glucopyranose and nucleoside phosphates (UTP, UDP and UMP) were separated within 23 min using these chromatographic conditions. For determination of UDP-glucose-pyrophosphorylase activity the enzyme concentration was varied and the conversion of substrate was limited to max. 5%. Incubations were performed in 50 mM Tris buffer containing 1 mM α-D-glucopyranose 1-phosphate (Glc-1-P) or FDG-1-phosphate (FDG-1-P), 1 mM UTP, 5 mM $MgCl_2$ at pH 8 and T=25°C. Incubations were stopped by heating at 100°C for 5 min and centrifugation. The enzyme activity was calculated using the integral of the UV absorbance signal (HPLC) of the respective product (UDP-Glc in case of the reference substrate Glc-1-P or UDP-FDG in case of FDG-1-P) and expressed as the volume activity A_v [$\mu mol \cdot min^{-1} \cdot ml^{-1}$].

Chemistry

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-fluoro-β-d-glucopyranose. The Synthesis of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranose was based on the method of Card *et al.*[34] and performed as recently described,[35] but with slight modifications concerning the work-up procedure. 3.45 g (10.35 mmol) 1,3,4,6-Tetra-O-acetyl-β-D-mannopyranose were dissolved in 50 ml abs. CH_2Cl_2 under nitrogen and the solution was cooled to −40°C. 5 ml (40 mmol) diethylamino sulfurtrifluoride (DAST) was added slowly and the reaction mixture was stirred for further 30 min. After removing cooling the solution was stirred over night. The excess reagent was removed by addition of methanol in the cold. After removing the solvent under reduced pressure the residue was taken up in toluene/acetone (9:1, v/v) and the crude product was purified by flash-chromatography on silica gel (EtOAc/hexane 7:3, v/v) yielding 2.2 g (6.3 mmol, 61%) of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranose. M.p. 92°C (from CH_3OH) (Lit.: 91–92°C).[27] ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.81 (1H, dd, $J_{1,2}$=8.1 Hz, $J_{1,F}$=3.1 Hz, H-1), 5.40 (1H, dt, $J_{3,F}$=14.3 Hz, $J_{2,3}$=9.1 Hz, $J_{3,4}$=9.2 Hz, H-3), 5.10 (1H, t (dd), $J_{4,3} \approx J_{4,5}$=9.8 Hz, H-4), 4.46 (1H, ddd, $J_{2,3}$=9.1 Hz, $J_{2,F}$=51 Hz, $J_{2,1}$=8.2 Hz, H-2), 4.33 (1H, dd, $J_{6a,6b}$=12.3 Hz, H-6a), 4.13 (1H, dd, $J_{6b,5}$=2.3 Hz, H-6b), 3.89 (1H, ddd, $J_{5,6a}$=4.5 Hz, $J_{5,6b}$=2.2 Hz, H-5), 2.22 (3H, s, OAc), 2.13 (3H, s, OAc), 2.11 (3H, s, OAc), 2.07 (3H, s, OAc). ^{19}F-NMR

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(188 MHz, $CDCl_3$): δ −201.3 (ddd, $J_{F,2}$=51 Hz, $J_{F,1}$=3.0 Hz, $J_{F,3}$=15 Hz).

2-Deoxy-2-fluoro-D-glucopyranose 1-phosphate. 0.3 g (0.82 mmol) of crystalline 1,3,4,6-tetra-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranose was added to 320 mg (3.26 mmol) of dry and crystalline phosphoric acid (H_3PO_4(s)) and slowly heated to 50°C under high vaccum (10^{-2} mbar). The reaction temperature was maintained at 50°C for 2 h. For the next 48 h the temperature was increased to 70–80°C. The reaction mixture was cooled with ice and 6.7 ml of cold 2 M LiOH (13.4 mmol LiOH) was added. The precipitate (Li_3PO_4) was filtered off using a Celite pad and the residue was washed with ice-cold 0.2 M LiOH. The pH of the combined solutions was readjusted to 7 by adding ion-exchange material (Dowex-X8, H^+). The neutral solution was filtered and lyophilized. The white residue was dissolved in 30 mM NH_4HCO_3 and applied to a DEAE-Sephadex column (elution by gradient: 30–200 mM). The product containing fractions were identified by IR-absorption (830 nm) after addition of Mo(V)-Mo(VI) reagent. The remaining NH_4HCO_3 was removed by repeated lyophilization to yield 60 mg (0.14 mmol, 17%) 2-deoxy-2-fluoro-D-glucopyranose 1-phosphate as a white solid (mixture of anomers; ammonium salt). M.p. 135°C. ^{1}H-NMR (200 MHz, D_2O): δ 5.43 (dd, $J_{1,P}$=8.2 Hz, $J_{1,2}$=3.6 Hz, $J_{1,F}$<0.5 Hz, H-1 (α)), 4.94 (ddd (q), $J_{1,P}$=$J_{1,2}$=7.8 Hz, $J_{1,F}$=2.7 Hz, H-1(β)), 4.23 (1H, dddd, $J_{2,F}$=51.8 Hz, $J_{2,3}$=9.9 Hz, $^4J_{2,P}$=1.7 Hz, H-2), 3.88 (1H, dd, $J_{6a,6b}$=9.4 Hz, $J_{6a,5}$=3.8 Hz, H-6a), 3.78 (1H, ddd, H-5), 3.72 (1H, dd, $J_{6b,5}$=2.3 Hz, H-6b). ^{13}C-NMR (50 MHz, D_2O): δ 91.3 (C-1, $J_{C\text{-}1,F}$=26 Hz), 90.3 (C-2, $J_{C\text{-}2,F}$=181 Hz, $J_{C\text{-}2,P}$=10 Hz), 72.1 (C-4), 71.5 (C-3, $J_{C\text{-}3,F}$=17.7 Hz), 69.7 (C-5), 60.9 (C-6). ^{19}F-NMR (188 MHz, D_2O): δ −199.72 (dd, $J_{F,2}$=53 Hz, $J_{F,3}$=14 Hz, α-anomer), −199.4 (ddd, $J_{F,2}$=51 Hz, $J_{F,3}$=15 Hz, $J_{F,1}$=2.8 Hz, β-anomer). ^{31}P-NMR (81 MHz, D_2O): δ 3.48 (d (br.), $J_{1,P}$=8.1 Hz). ^{31}P-NMR (81 MHz, D_2O, ^{1}H-decoupled) : δ 3.48 (s, $COPO_3^{2-}$).

Uridine-5'-diphospho-2-deoxy-2-fluoro-α-D-glucopyranose (UDP-FDG). The multienzymatic synthesis of UDP-FDG consisted of two incubation steps. The first incubation comprised 22 mg (121 μmol) FDG, 70 mg (126 μmol) ATP and 39 mg (189 μmol) $MgCl_2$ in a final volume of 4.8 ml (50 mM Tris buffer, pH 7.2). After degassing with helium, hexokinase (100–150 U in 200 μl Tris buffer, pH 7.2) was added in free form. The reaction was maintained at 25°C on a thermostate (Eppendorf Thermomixer 5436) over night. The reaction was followed by HPLC-analysis. A maximum of ADP indicated total conversion of educt. The enzyme was denaturated by heating at 100°C for 2 min. After centrifugation 12 mg (21 μmol) UTP in 20 μl Tris-buffer, 100 μl phosphoglucomutase (150 U in Tris buffer, pH 8), 0.02 μmol glucose 1,6-diphosphate (20 nmol in 100 μl Tris buffer, pH 8), 200 μl UDP-glucose-pyrophosphorylase (10 U in 20 μl Tris buffer, pH 8), 200 μl inorganic pyrophosphatase (10 U in 20 μl, Tris buffer, pH 8) were added to the cooled incubation mixture (25°C). The pH was readjusted to 7.8 using 0.5 M Tris buffer (pH 7.2) and the reaction was maintained at 25°C. After 6 d HPLC indicated conversion to UDP-FDG of about 60%. The enzymes were removed by heating at 100°C for 2 min followed by centrifugation. 5–10 U alkaline phosphatase (calf intestine, Roche) were added to hydrolize nucleotides. The turnover was followed by HPLC. After total conversion, the enzymes were removed as described above and anion exchange chromatography using a DEAE-Sephadex column (1.0 × 30 cm, previously equilibrated with 30 mM NH_4HCO_3) was performed. A linear gradient of NH_4HCO_3 was applied (30–200 mM). UDP-FDG was eluted at a concentration of approximately 60 mM NH_4HCO_3 as detected by UV-absorbance at 262 nm. Product containing fractions were combined and most of the NH_4HCO_3 was removed by repeated lyophilization. A final purification step was performed on Bio-Gel P2 (1.6 × 13 cm). The yield of UDP-FDG was determined to be 4.8 μmol (38%; diammonium salt). ^{1}H-NMR (200 MHz, D_2O): δ 7.85 (1H, d, $J_{5,6}$=8.1 Hz, U:H-6), 5.90–5.81 (2H, s,d, $J_{5,6}$=8.1 Hz, U:H-5, rib-H-1'), 5.67 (1H, dd, $J_{1,P}$=7.7 Hz, $J_{1,2}$=3.5 Hz, $J_{1,F}$<0.6 Hz, H-1), 4.30–4.0 (5H, m, H-3, H-4, H-5, rib-H-2', rib-H-3'), 3.97–3.65 (5H, m, rib-H-4', rib-H_2-5', H_2-6). ^{19}F-NMR (188 MHz, D_2O): δ −200.73 (dd, $J_{F,2}$=49 Hz, $J_{F,3}$=11 Hz). ^{31}P-NMR (81 MHz, D_2O): δ −12.2 (dm, $J_{P,P}$=19.4 Hz), −10.4 (dm, $J_{P,P}$=19.4 Hz). ESI-MS: m/z 566.8 $[M]^{2-}$.

Radiochemistry

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(^{18}F)fluoro-glucopyranose. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranose was synthesized following the radiosynthesis of [^{18}F]FDG[20] and isolated by HPLC as described recently.[22] Briefly, the crude reaction mixture of [^{18}F]fluoride, Kryptofix® 2.2.2, K_2CO_3 and 1,3,4,6-tetra-*O*-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose was passed through a SiO_2-cartridge (200 mg) and eluted with 1.0 ml CH_3CN. After evaporation of the solvent the residue was dissolved in 500 μl CH_3CN/H_2O (30:70) and submitted to semipreparative HPLC (Kromasil C8, 125 × 8, 3.5 ml/min, acetonitrile/ water 30:70). The product was isolated by solid phase extraction (C18-cartridge, 100 mg) for further experimental use. The radiochemical yield of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranose was 93% as determined by analytical HPLC and

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radio-TLC (silica gel, R_f=0.84, ethyl acetate/hexane 7:3 (v/v)) from a sample withdrawn from the reaction mixture.

2-Deoxy-2-(^{18}F)fluoro-D-glucopyranose 1-phosphate. In a glassy-carbon reaction vessel a solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranose in dry acetonitrile was evaporated to dryness under low pressure. After the reaction vessel was maintained under high vacuum (10^{-3} mbar) at 50°C for further 10 min the heating source was removed and the reactor was filled with argon. 20 mg H_3PO_4 (s) was added in the presence of a shielding gas. The reaction mixture was slowly heated to 70°C under high-vacuum (10^{-3} mbar). After 30 min the melt was taken up by addition of 0.4 ml THF. After cooling to room temperature 200 µl of 1 M LiOH was added and the precipitate was removed by centrifugation. The solution was taken up with 4 ml of water and passed through an anion exchange cartridge (Accell plus, Waters). The solid phase was washed with additional 6 ml of water to remove free [^{18}F]FDG. 2-Deoxy-2-[^{18}F]fluoro-D-glucopyranose 1-phosphate was obtained by elution with 0.1 M Tris buffer (pH 8; 2 ml) in a radiochemical yield of 55%.

Chemoenzymatic synthesis of uridine-5'-diphospho-2-deoxy-2-(^{18}F)fluoro-α-D-glucopyranose (UDP-(^{18}F)FDG)

The MacDonald phosphorylation of tetraacetylated [^{18}F]FDG was performed as described above. After addition of 200 µl of an 1 M LiOH solution, isolation of 2-deoxy-2-[^{18}F]fluoro-D-glucopyranose 1-phosphate and removing of the Li_3PO_4 precipitate was realized by connecting a glass filter panel to the entrance side of the anion exchange cartridge (Accell plus-cartridge, 50–100 mg). After washing with 4 ml water, the cartridge was dried in a stream of argon. With a 1 ml syringe 120 µl of a 0.1 M Tris buffer solution (pH 8) containing UTP (0.5 mM), $MgCl_2$ (4 mM), 10 U UDP-glucose-pyrophosphorylase (UDP-Glc PP) and 10 U inorganic pyrophosphatase was added to the solid support. The cartridge was heated at 40 C for 60 min. For analytical purposes the resin can be eluted with 1 ml 0.1 M Tris buffer and the product can be isolated by solid phase extraction on a Sep-Pak plus-cartridge (RP-18, 500 mg, Waters). The Tris buffer eluant was diluted with water (1:10) and passed through the Sep-Pak cartridge. After washing (5 ml water) and drying, the cartridge was eluted with 3 ml 0.1 M triethylammonium hydrogencarbonate followed by 7 ml methanol/0.1 M triethylammonium hydrogencarbonate (1:1), in order to provide separation of UDP-[^{18}F]FDG from residual [^{18}F]FDG-1-phosphate. The radiochemical yield of uridine-5'-diphospho-2-deoxy-2-[^{18}F]fluoro-α-D-glucopyranose was 20% (decay-corrected and related to [^{18}F]fluoride) after a total synthesis time of 110 min.

Conclusion

Our approach for the development of a reliable radiosynthesis of the ^{18}F-labelled nucleotide activated sugar donor UDP-[^{18}F]FDG is based on the commonly available intermediate 1,3,4,6-tetra-O-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranose, which occurs as an intermediate in the [^{18}F]FDG synthesis. The chemoenzymatic synthesis of UDP-[^{18}F]FDG was successfully performed in the void volume of an anion exchange cartridge and provides a satisfying radiochemical yield. This should enable the enzymatic transfer of [^{18}F]FDG using UDP-[^{18}F]FDG as glycosyl donor in combination with a suitable glycosyltransferase and model acceptor substrates in order to develop a mild ^{18}F-labelling method of highly selective glycosylated biomolecules.

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