

Translating the concept of peptide labeling with 5-deoxy-5-[¹⁸F]fluororibose into preclinical practice: ¹⁸F-labeling of Siglec-9 peptide for PET imaging of inflammation†

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Peptide glycosylation with 5-deoxy-5-[¹⁸F]fluororibose was translated into preclinical settings. The novel ¹⁸F-labeled Siglec-9 peptide was produced using an automated synthesis procedure. The ¹⁸F-labeled Siglec-9 peptide showed favorable binding in the animal model of inflammation *in vivo*.

Positron emission tomography (PET) is a sensitive and non-invasive *in vivo* molecular imaging modality. Fluorine-18 (¹⁸F) has favorable physical properties to generate high-resolution PET images and consequently ¹⁸F-labeled compounds are the most used tracers in clinical PET. Among the short-lived radio-nuclides, the physical half-life of ¹⁸F ($T_{1/2}$ = 109.7 minutes) allows multi-step synthesis of tracers for the same-day use in hospitals.¹ Versatile fluorinating agents have been developed for the synthesis of fluorine containing pharmaceuticals.¹ However, only a small proportion of the existing fluorination methods have been (or can be) translated into radiosynthesis of ¹⁸F-labeled compounds for preclinical and clinical applications.^{1a} This is especially true in the case of ¹⁸F-labeled biomolecules, *e.g.* peptides, proteins and antibodies. Although many strategies for ¹⁸F-labeling of peptides and proteins have shown some promise during the last two decades, only a few have proved amenable for routine large scale production for clinical use. Oxime formation between an aldehyde (or a ketone) and an aminoxy-functionalized compound is a classical and favorable reaction in bioconjugation due to the high chemoselectivity. Indeed, oxime formation has been successfully applied for the

production of [¹⁸F]fluciclatide (GE Healthcare) starting from 4-[¹⁸F]fluorobenzaldehyde ([¹⁸F]FBA). [¹⁸F]Fluciclatide is one of the few ¹⁸F-labeled peptide tracers currently in clinical use.^{1b} Among the ¹⁸F-labeled aldehydes, [¹⁸F]FBA is probably the most frequently used prosthetic group for peptide labeling. However, [¹⁸F]FBA is volatile requiring additional measures to ensure radiosafety and to reduce radioactive emission during the production. The attachment of [¹⁸F]FBA may cause increased lipophilicity, resulting in altered pharmacokinetics *in vivo*.^{1b} In addition to [¹⁸F]FBA, aldehydes 1–5 have recently been developed for peptide labeling (Fig. 1, [a]).^{1b,2}

It has been well documented that glycosylation may confer favorable characteristics to peptide tracers for *in vivo* imaging. The incorporation of a galactose fragment into the RGD peptide has generated a very valuable peptide tracer, [¹⁸F]galacto-RGD. So far, most of the published clinical PET imaging data concerning ¹⁸F-labeled peptides has actually been generated using this tracer. However, the synthesis procedure of [¹⁸F]galacto-RGD is rather complex and is a major challenge for its widespread use.³ As 2-deoxy-2-[¹⁸F]fluoroglucose ([¹⁸F]FDG, Fig. 1, [b]) is available in virtually every PET radiochemistry lab, it is an excellent concept to use [¹⁸F]FDG for peptide and protein labeling.⁴ [¹⁸F]FDG is an aldose and has an open chain aldehyde

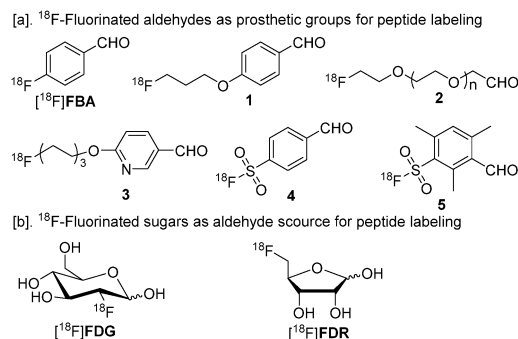


Fig. 1 ¹⁸F-Labeled prosthetic groups for oxime formation. ¹⁸F-Fluorinated sugars are presented in their cyclic forms.^{1b,2}

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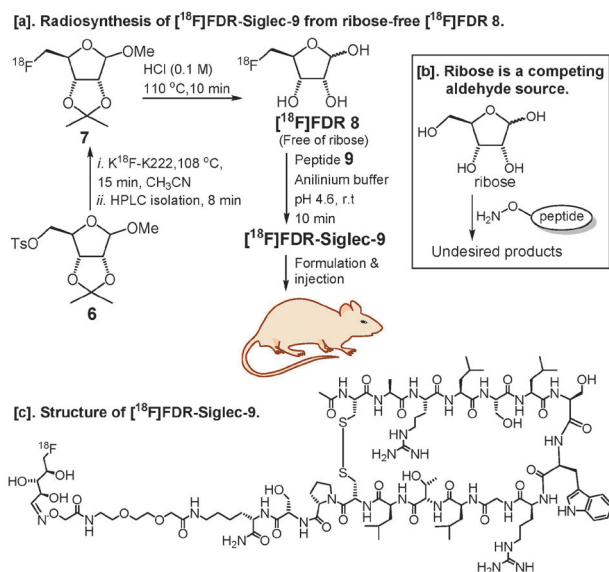
† Electronic supplementary information (ESI) available: Radiosynthesis of ribose-free [¹⁸F]FDR 8 and [¹⁸F]FDR-Siglec-9 and PET imaging in rats. See DOI: 10.1039/c3cc40738a

form in solution.^{1b} However, the conjugation between [¹⁸F]FDG and aminoxy-functionalized peptides generally needs elevated temperature and low pH and forms a complex product profile.^{1b,4b} Very recently, it has been discovered that 5-deoxy-5-[¹⁸F]fluoro-ribose ([¹⁸F]FDR, Fig. 1, [b]) conjugates to peptides much more efficiently than [¹⁸F]FDG does.⁵ Although we were able to produce [¹⁸F]FDR in large scale in an automated procedure, we were not able to perform the conjugation step efficiently enough for preclinical PET studies. Furthermore, the quality of radiolabeled peptides was not acceptable (*e.g.* low chemical purity) for *in vivo* animal studies. Thus, peptide glycosylation with [¹⁸F]FDR remained at a conceptual stage with respect to preclinical and clinical utilities. In this communication, we report the translation of [¹⁸F]FDR-based bench chemistry into practical pre-clinical use, and exemplify this utility on PET imaging of vascular adhesion protein 1 (VAP-1), which is a promising new target protein for PET imaging of inflammation.

In the previous work, [¹⁸F]FDR was prepared in high radiochemical purity (>98%) from precursor **6** (Scheme 1, [a]), and the synthesis was scaled up to 4 GBq of radioactivity. However, chemical purity of the synthesized [¹⁸F]FDR was not optimal and the final product contained ribose in a mM-range, originating from precursor **6**. Subsequently, only a small proportion of the obtained [¹⁸F]FDR (8–20 MBq) was conjugated to model peptides. In the presence of an appropriate amount of peptides (*e.g.* at 23 mM of concentration), the conjugation reached completion in 10 minutes in sodium acetate buffer (pH 4.6) at rt.⁵ Scaling up the conjugation reaction was hampered due to the high ribose content of the produced [¹⁸F]FDR. When starting with 100 MBq–4 GBq of [¹⁸F]FDR, only 5% conversion yields were achieved in an hour, even at high concentrations of peptide (*e.g.* 40 mM). Since only nano- or picomoles of non-carrier added and high specific radioactivity [¹⁸F]FDR was used, the ribose was present in the conjugation mixture in a large excess. Its presence as a competing aldehyde source resulted in the inefficient conjugation of [¹⁸F]FDR (Scheme 1, [b]).

This prompted us to derive a strategy to produce [¹⁸F]FDR free of ribose ([¹⁸F]FDR **8**, Scheme 1, [a]). After the fluorination of precursor **6** with K¹⁸F-K222, intermediate **7** was isolated using a preparative HPLC column. The method gave an excellent separation between the starting material **6** (retention time = 10.6 min) and intermediate **7** (retention time = 4.9 min) in a short HPLC protocol. Thus, the synthesis procedure was not significantly prolonged. The HPLC analysis of the isolated **7** proved the success of the intermediate purification step (ESI[†]). After the acid-catalyzed hydrolysis of **7**, [¹⁸F]FDR **8** was obtained in high radiochemical purity (>98%). Subsequently, the pH of [¹⁸F]FDR **8** solution was adjusted to 4.6 at which the efficiency of peptide conjugation was optimal. The total preparation time of the high purity [¹⁸F]FDR **8** was typically 85 min starting from end-of-bombardment (EOB), including a concentration step of the [¹⁸F]FDR solution before the conjugation reaction. In addition to **6**, 1,2,3-tri-*O*-acetyl-5-*O*-tosyl ribose (AcRib, ESI[†]) was tested as a precursor to produce [¹⁸F]FDR **8**. Unfortunately, only residual amount of [¹⁸F]FDR **8** was obtained from AcRib. In this respect, compound **6** was a very applicable precursor in radio-synthesis (*n* = 40). The next step was to prepare VAP-1 targeting [¹⁸F]FDR-Siglec-9 peptide for *in vivo* imaging of inflammation in rats (Scheme 1, [a] and [c]). VAP-1 is a 90 kDa glycoprotein and is a unique target in inflammation.⁶ Inflammation is highly relevant in major diseases including cancer, diabetes, obesity and Alzheimer's disease.^{6b} In endothelial cells, VAP-1 stays in the intracellular storage granules under normal physiological conditions. Upon inflammation, VAP-1 relocates rapidly to the endothelial cell surface. This makes VAP-1 an ideal target for diagnostic imaging of inflammation. Recently, it has been discovered that sialic acid-binding Ig-like lectin 9 (Siglec-9) is a leukocyte ligand of VAP-1 and ⁶⁸Ga-labeled Siglec-9 motif peptide can be used as a PET tracer for *in vivo* imaging of inflammation and cancer.⁷ Accordingly, peptide **9** (ESI[†]) was designed in order to enable conjugation to [¹⁸F]FDR **8** in this work.

In general, oxime formation is not the most efficient chemical reaction and the substrates need to be used at relatively high concentrations in order to reduce the reaction time. Specific radioactivity of the cyclotron produced ¹⁸F is high and the amount of the synthesized [¹⁸F]FDR **8** is in a nano- to picomolar level. Following the reported conjugation procedure,⁵ the initial tests for the synthesis of [¹⁸F]FDR-Siglec-9 peptide were carried out in sodium acetate buffer (pH 4.6, 90 mM) at rt. However, the concentration of peptide **9** used (2.1 kDa) had to be at over 15 mM (32.2 mg ml⁻¹), which was absolutely not applicable. In a previous study, aniline was used to catalyze the oxime formation between [¹⁸F]FBA and leptin.⁸ Accordingly, we tested aniline-catalysis in the conjugation reactions between 5-deoxy-5-fluororibose ([¹⁸F]FDR) and **9** (Table 1). Being the “cold” counterpart of [¹⁸F]FDR **8**, [¹⁹F]FDR was used to prepare [¹⁹F]FDR-Siglec-9, which was needed as a reference in radio-synthesis of [¹⁸F]FDR-Siglec-9. In anilinium buffer (0.3 M, pH 4.6), the conjugation efficiency was dramatically enhanced (Table 1, entries 1 and 2). Aniline has been proposed to catalyze oxime formation with a rapid transimination mechanism.^{1b} The input concentration of peptide **9** could be as low as 0.3 mM

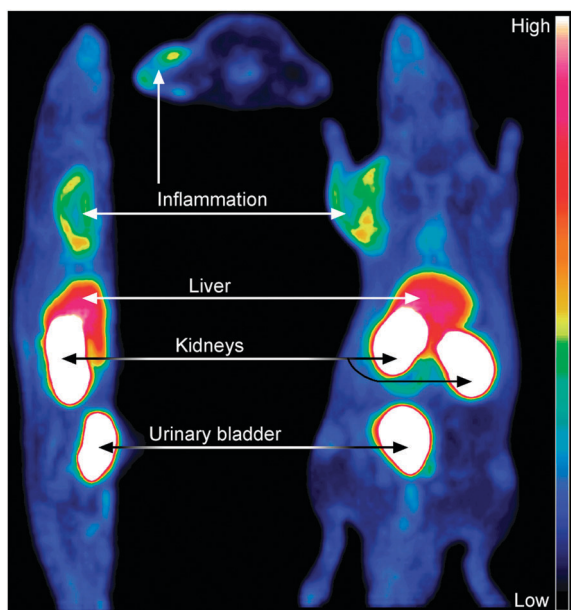


Scheme 1 Synthesis and preclinical utilization of [¹⁸F]FDR-Siglec-9.

Table 1 The influence of aniline on conjugation efficiency of [^{18}F]FDR to peptide **9** in 10 min of reaction (pH 4.6, rt)

Entry	Concentration of [^{18}F]FDR and 9 ^a (mM)	Buffer (M)	Conversion ^b (%)
1	0.3	Sodium acetate (0.3)	5
2	0.3	Anilinium acetate (0.3)	95
3	0.3	Anilinium acetate (0.2)	89
4	0.3	Anilinium acetate (0.05)	63
5	0.1	Anilinium acetate (0.3)	48

^a The two reagents were used in equal amounts. ^b Conversions were determined using HPLC.

**Fig. 2** Representative sagittal (left), transaxial (middle) and coronal (right) multiplane PET images of [^{18}F]FDR-Siglec-9 biodistribution in a rat. The images are summation from 10–60 min post-injection.

(0.6 mg ml⁻¹) and the conversion was 95% in 10 min at rt. The concentration of the anilinium buffer also had an influence on the conjugation efficiency (entries 2–4). Our results together with the previous work demonstrated that aniline-catalyzed oxime formation is chemoselective in the presence of any amino acid side chain from all the 20 standard amino acids under ^{18}F -labeling conditions.⁸

A general challenge in developing peptide PET tracers is to isolate the labeled products (e.g. [^{18}F]FDR-Siglec-9) from their unlabeled peptide precursors (e.g. **9**). The possible residual peptide precursors may compete with the tracer on binding to the target protein or even saturate the target to be imaged. Bearing this in mind, we developed a reproducible HPLC method for efficient separation of [^{18}F]FDR-Siglec-9 from **9** in a preparative scale (ESI[†]). Finally, [^{18}F]FDR-Siglec-9 was prepared from the conjugation of [^{18}F]FDR **8** to **9** (0.3 mM) in anilinium buffer (pH 4.6) at rt and the conversion was 50–60% in 10 min. The final product was isolated by HPLC with high radiochemical purity (>98%). The amount of the peptide precursor **9** was under the detection limit of the UV-detection. The total synthesis time was typically 120 minutes and the

decay-corrected radiochemical yield was 27% starting from ^{18}F -fluoride (EOB). The two HPLC-purifications were well integrated in the protocol and were performed with remote-control. Both radiation exposure and radioactive emission were minimal. The synthesis was scaled up to 1.1 GBq of the final product [^{18}F]FDR-Siglec-9. The specific radioactivity was 36–43 GBq μmol^{-1} at the end-of-synthesis (EOS) (ESI[†]). [^{18}F]FDR-Siglec-9 was formulated in phosphate-buffered saline (PBS) for intravenous injection. In PBS, [^{18}F]FDR-Siglec-9 was stable for >4 hours and thus suitable for *in vivo* imaging studies. The [^{18}F]FDR-Siglec-9 (18.3 \pm 5.1 MBq) was intravenously injected into rats having sterile, turpentine oil induced inflammation.⁷ Dynamic PET imaging lasting for 1 hour was performed by using a High Resolution Research Tomograph (Siemens). The inflammation focus on the right shoulder area of the rats ($n = 8$) was clearly visualized (Fig. 2). The target-to-muscle ratio was 9:1. The observed liver uptake and rapid excretion through kidneys to the urine were in line with our previous studies with [^{68}Ga]-DOTA-Siglec-9.⁷ Very importantly, bones were not visualized, which was an indication that there was no *in vivo* defluorination of the ^{18}F -label on the prosthetic group [^{18}F]FDR **8**.

In conclusion, we have successfully translated [^{18}F]FDR-based oxime formation into the production of [^{18}F]FDR-Siglec-9 peptide for preclinical use. [^{18}F]FDR-Siglec-9 has been produced in high radiochemical quality and sufficient specific radioactivity facilitating *in vivo* PET imaging of experimental inflammation. Further development of the tracer for clinical use is warranted. [^{18}F]FDR-conjugation is independent of peptide sequence and the whole synthesis procedure can be automated. Thus, [^{18}F]FDR-based glycosylation should be generally applicable in the development of peptide PET tracers.

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