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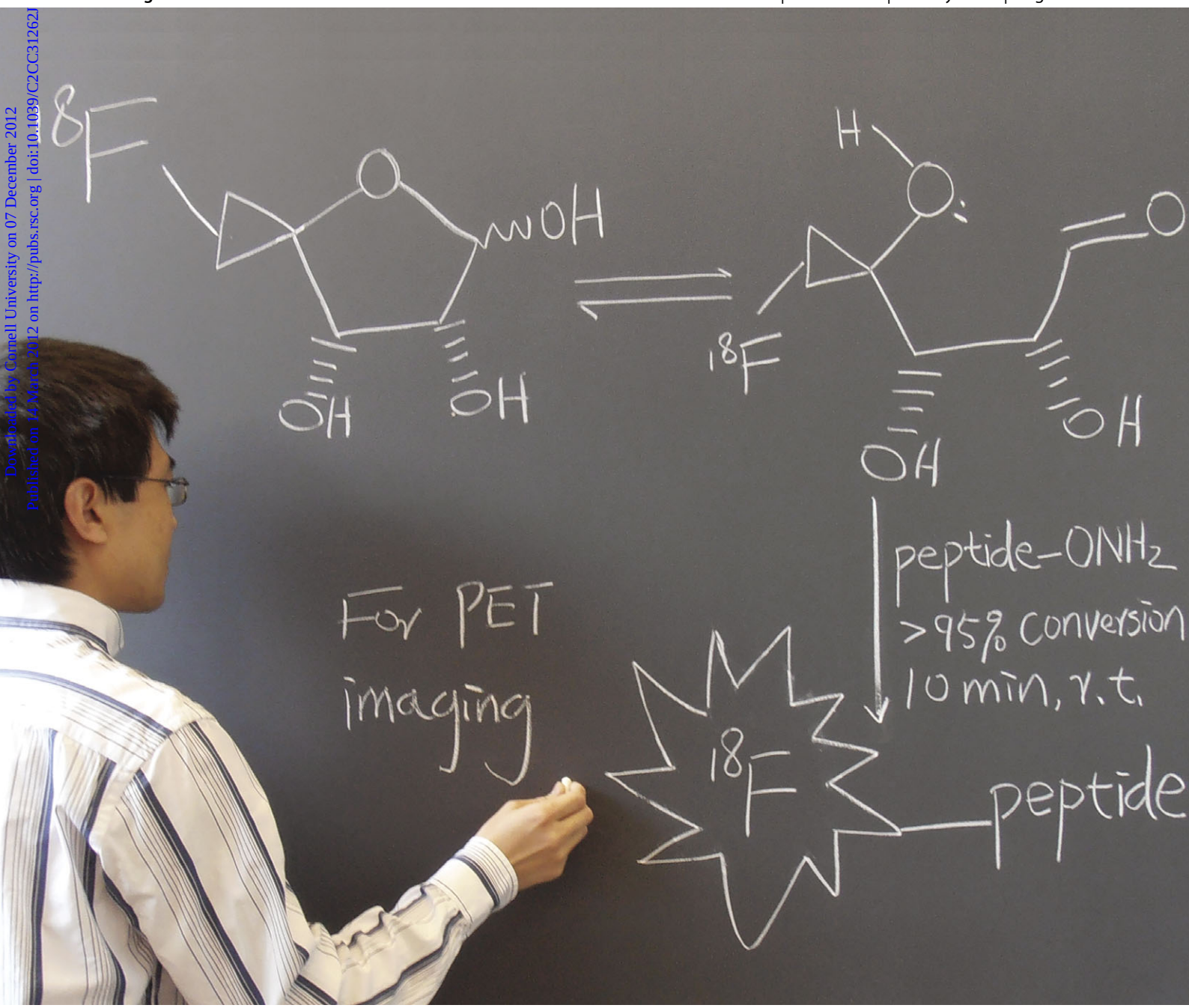

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[^{18}F]-5-Fluoro-5-deoxyribose, an efficient peptide bioconjugation ligand for positron emission tomography (PET) imaging†

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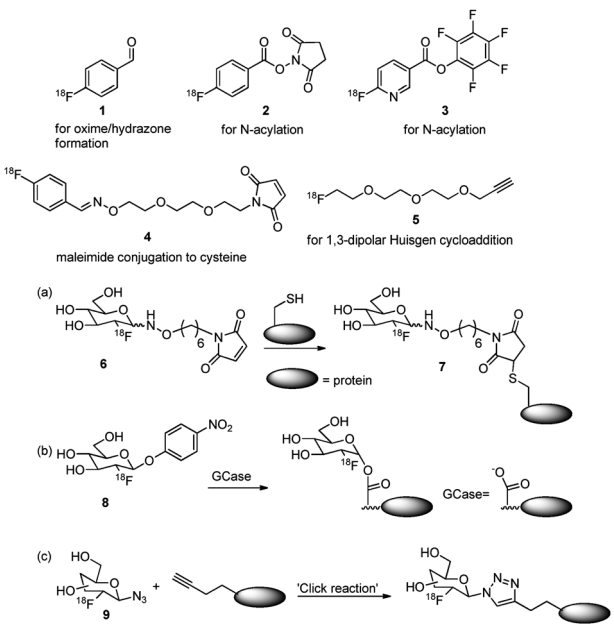
[^{18}F]-5-Fluoro-5-deoxyribose ([^{18}F]-FDR) conjugates much more rapidly than [^{18}F]-FDG under mild reaction conditions to peptides and offers new prospects for mild and rapid bioconjugation for fluorine-18 labelling in PET imaging.

Positron emission tomography (PET) is an important imaging modality used widely in clinical diagnosis.[1] Fluorine-18 is an ideal isotope for PET applications in the clinic and in research and pharmaceuticals development programmes. It has a relatively long half-life ($t_{1/2}$ = 109.8 min) and it is readily generated at very high specific activity as [^{18}F]F$^-$ fluoride ion in hospital and research centre localized cyclotrons. One of the current challenges in PET is to efficiently attach a readily available fluorine-18 containing small molecule to the peptide by bioconjugation through a linker. This is generally achieved by reaction of, *e.g.*, a benzaldehyde *e.g.* **1**, or an activated benzoic acid *e.g.* **2**, or a pyridine carboxylic acid *e.g.* **3**, or a maleimide linker *e.g.* **4** or a water soluble acetylene **5** carrying a fluorine-18 label (for 'click' conjugation) with an appropriate linker attached to the peptide/protein, or by conjugation directly.[2] A drawback of aromatic moieties is that the ligated peptide has an increased hydrophobicity due to the attachment of an aromatic moiety. Furthermore, the preparation of [^{18}F]-labelled prosthetic groups such as **1–5** can be difficult to adapt to automated PET radiochemistry synthesis platforms and this limits widespread application of the technology. Thus [^{18}F]-FDG is an attractive candidate for direct peptide conjugation as it is a hydrophilic sugar and it is readily available at PET centres worldwide. However, [^{18}F]-FDG does not conjugate well with peptide linkers under mild conditions. Instead, successful reactions with hydrazine/alkoxyamine bioconjugate linkers require low pH (~pH 1–3)[3a] and/or high temperature[3b] (~80–130 °C), and even then extended conjugations times (~30 min to several hours), a combination often incompatible with peptides, and the desire to carry out rapid protocols.[3a–d] Thus to circumvent these issues [^{18}F]-FDG has been pre-modified prior to bioconjugation. Recent developments have therefore involved the pre-preparation of [^{18}F]-FDG-maleimidehexyloxime **6** which is then conjugated to an appropriate cysteine containing/modified peptide to give **7**.[4] Other recent approaches to [^{18}F]-FDG derivatisation have involved nitrophenol acetal **8**[5] and α-azide **9**[6] followed by substitution and 'click' reactions respectively as illustrated in Scheme 1. In these cases the prepared [^{18}F]-FDG requires modification to generate a suitable linker prior to bioconjugation.

In this *Communication* we present a conjugation method which utilizes [^{18}F]-5-fluoro-5-deoxyribose ([^{18}F]-FDR **10**) as a novel [^{18}F]-prosthetic group for the efficient and direct labelling of biopolymers. Our interest in [^{18}F]-FDR **10** was stimulated by its availability by biotransformation involving the fluorinase enzyme.[7] However we also reveal that [^{18}F]-FDR can be efficiently prepared by adaptation of the standard automated



Scheme 1 Compounds **1–4** are representative of fluoroaryl acylation or alkylation precursors used for fluorine-18 bioconjugation to peptides/proteins. Click conjugations are represented by **5**. Methods *e.g.* (a)–(c) involving [^{18}F]-FDG all require pre-derivatisation of [^{18}F]-FDG prior to bioconjugation.

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† Electronic supplementary information (ESI) available: [^{18}F]-FDR production and peptide conjugations are described in detail. See DOI: 10.1039/c2cc31262j
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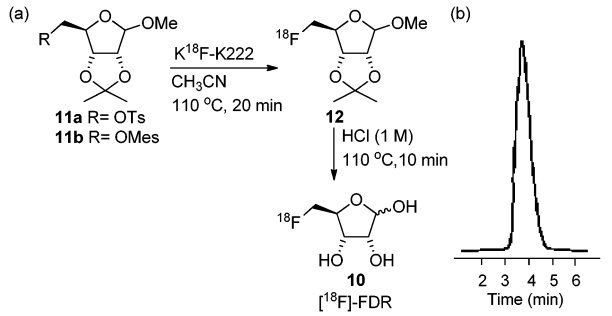
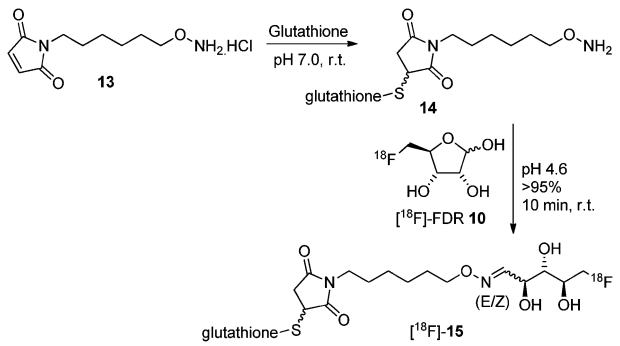


Scheme 2 Automated production of [^{18}F]-FDR **10** in a GE TRACERLab® module. (a) Reactions involved in the synthesis of [^{18}F]-FDR. (b) Radio-HPLC analysis demonstrates an [^{18}F]-FDR product of over 98% radiochemical purity.

protocol used for the synthesis of [^{18}F]-FDG in PET laboratories worldwide (Scheme 2).[8] Accordingly, activated sulfonate ester precursors **11a** and **11b** were readily prepared in two steps from D-ribose (ESI†). Precursor **11** was converted to [^{18}F]-FDR **10** in a reprogrammed GE TRACERLab® module which was intended for routine production of [^{18}F]-FDG. Intermediate [^{18}F]-**12** was formed by reaction of **11** with K^{18}F-Kryptofix 2,2,2 (K222) in dry acetonitrile, and the product was then hydrolyzed with aqueous HCl (1 M) to give [^{18}F]-FDR **10**. Finally the reaction mixture is purified through a Chromabond cartridge. This protocol took 50 min in our hands. The decay corrected radiochemical yield (RCY) considering the activity transferred in the reaction vessel was (35.0 ± 5)% (n = 6) for **11a** and (33.0 ± 8)% (n = 3) for **11b**. Thus both precursors **11a** and **11b** proved very practical. Production was scaled to achieve 4 GBq of radioactivity without any difficulty. Notably, the radiochemical purity was over 98% after discharge from the automated system, without any need for further purification (Scheme 2). Upon reverse phase HPLC, the retention time of [^{18}F]-FDR **10** (RT = 4 min) was found to be clearly different from that of intermediate [^{18}F]-**12** (RT = 18 min, ESI†).

Aldose sugars (*e.g.* FDG and FDR) exist in several forms, including α- and β-anomers and the open chain aldehyde. During bioconjugation the aldehyde reacts with an alkoxyamine (*e.g.* **13** and **14**, Scheme 3) to form an oxime (*e.g.* **15**). Accordingly, the tendency for ring opening of the sugar dictates its conjugation efficiency. Non-fluorinated sugars have been treated with hydroxylamine to form oximes, with D-ribose emerging as the most efficient conjugation ligand under mild reaction conditions (pH 4.6, 25 °C).[9] In the case of [^{18}F]-FDR **10** the 5-membered ring and the fluorine positioned at C-5 are features which promote ring opening to the aldehydic form. We anticipated



Scheme 3 Oxime bioconjugation strategy of GSH with [^{18}F]-FDR **10**.

Table 1 Comparative conjugation times and conversions between **14** (20 mM) and the aldose sugars (20 mM) at pH 4.6 (sodium acetate buffer, 0.25M) at 25 °C

Entry	Sugar	Time/min	Conversion[a] (%)
1	5-FDR	7	>98
2	D-Ribose	7	17
		60	60
3	D-Glucose	7	2
		60	14
4	6-FDG	60	25
5	FDG	1080	48

[a] Conversions were determined by HPLC.

that [^{18}F]-FDR **10** and its "cold" counterpart [^{19}F]-FDR (named 5-FDR) could be an efficient ligand for rapid bioconjugations. To explore this, the endogenous tripeptide, glutathione (GSH), was chosen as a model peptide to study its conjugation with 5-FDR. The maleimide linker **13** was prepared according to a literature protocol and was attached to GSH through the sulfur of the cysteine residue,[10] affording **14** in 3 min and in quantitative yield (Scheme 3 and ESI†). The reaction between 5-FDR (20 mM) and **14** (20 mM) was then evaluated and was shown to be complete within 7 min (pH 4.6 at 25 °C) (Table 1, entry 1). Under the same conditions, conjugation of D-ribose to **14** was significantly slower (60% conversion in 1 h, entry 2). Conjugation with D-glucose was slower again (entry 3), indicative of the relative rates of five-membered and six-membered ring opening under the reaction conditions. 6-Fluoro-substitution of D-glucose (6-FDG) did not accelerate the conjugation to a significant extent (entry 4). Among the sugars studied FDG (2-fluoro-2-deoxyglucose) was the most sluggish conjugation partner (48% conversion in 45 h, entry 5) which is reasonably due to the electronegative fluorine which, at the 2-position of the sugar, disfavours oxocarbenium ion formation required for ring opening to the aldehydic form necessary for oxime formation.

pH is an important factor in the reaction between D-ribose and hydroxylamine.[9] Conjugation between either **13** or **14** and 5-FDR was explored at different pHs (between pH 2.6–6.0 at 25 °C) and pH 4.6 emerged as an optimum with reactions taking only a few minutes. However even at pH 7.0, the conjugation with **14** went to completion in 2 h. For analysis purposes, conjugated "cold" **15** ([^{19}F]-**15**) was isolated from a preparative-scale reaction between **14** and 5-FDR, which proceeded to 99% completion in 7 minutes. The isolation process was carried out by passing the product mixture through a C18 cartridge. It was observed that [^{19}F]-**15** was stable to hydrolysis at pH 7.0 in phosphate-buffered saline (PBS) for at least 8 h at 37 °C, suitable for *in vivo* PET imaging applications.

An oxime, formed between an alkoxyamine and an aldose, can exist in ring-opened and/or ring-closed forms.[11] When [^{19}F]-**15** was subjected to ^{1}H-NMR analysis in D_2O, it became clear that the conjugate exists exclusively in ring-opened E/Z forms in a ratio of 4 : 1 (Fig. 1). The presence of the imine bonded protons of the E and Z isomers was diagnostic (E-isomer, 7.40 ppm, N = C*H*, d, J = 6.8 Hz; Z-isomer, 6.78 ppm, d, J = 6.0 Hz). There was no evidence for cyclic forms by ^{1}H-NMR. This product profile contrasts with conjugates with [^{18}F]-FDG, which existed in both ring-opened and closed forms.[3b] With this background a radiolabelled conjugation of [^{18}F]-FDR **10** with **14**

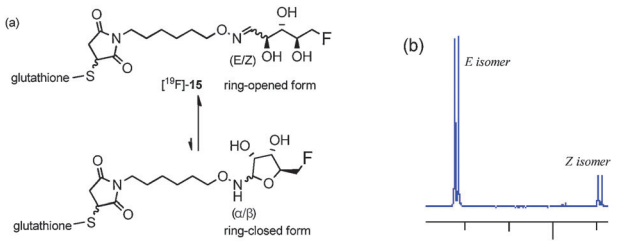
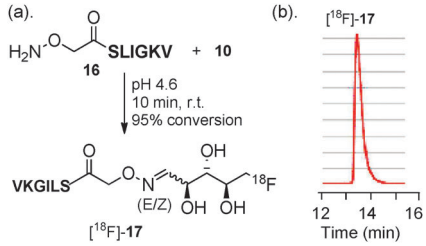


Fig. 1 (a) Proposed forms of [^{19}F]-**15** in aqueous solution; (b) ^{1}H-NMR signals representing the proton at the imine bond carbon of *E*- and *Z*-[^{19}F]-**15**, respectively.



Scheme 4 ^{18}F-labelling of peptide **16**: (a) efficient reaction under mild conditions; (b) radioactive detection of labelled product [^{18}F]-**17**.

was explored. Accordingly, compound **14** (2 mg) was incubated with [^{18}F]-FDR **10** (8–12 MBq) in sodium acetate buffer (110 μL, 90 mM, pH 4.6) in an Eppendorf tube at room temperature. Now the molar ratio of **14** relative to **10** is in large excess, and the reaction proceeded extremely rapidly (~10 min) and to completion (>95%, n = 3) as determined by radiochemical conversion (HPLC). The identity of product [^{18}F]-**15** was confirmed by comparison with a reference sample of [^{19}F]-**15**. During the production of [^{18}F]-FDR **10**, D-ribose was generated as a side product, just as glucose is generated during FDG production. Based on the results in Table 1 (entries 1 and 2), the oxime formation with D-ribose is anticipated to be much slower in the presence of [^{18}F]-FDR **10** as a competing conjugation ligand. Also there was distinct separation of conjugate [^{18}F]-**15** from unreacted peptide **14** on HPLC such that it is not a contaminant in the radiolabelled product (ESI†).

A peptide of clinical relevance was then explored for bioconjugation with [^{18}F]-FDR **10**. The hexapeptide H-SLIGKV-OH is a well-characterized agonist of the human protease activated receptor 2 (PAR-2).[12] PAR-2 is involved in a number of pathological processes, including its role in tumor progression.[13] Recent studies have shown that PAR-2 is involved in the cell invasion of hepatocellular carcinoma (HCC). HCC is a metastatic malignancy and its early diagnosis remains a challenge.[14] Peptide **16**, which is commercially available, has an aminoxyacetyl (Aoa) group attached to the N-terminus of the H-SLIGKV-OH peptide (Scheme 4). Conjugation between **16** (2 mg, 22.6 mM) and [^{18}F]-FDR **10** in sodium acetate buffer was again very rapid (~95% conversion in 10 min) at room temperature. In the hot labeling experiments, peptide **16** was used in excess of [^{18}F]-FDR **10**. Analytical radio-HPLC purification was performed to demonstrate a clear separation of labeled product [^{18}F]-**17** from unreacted peptide **16**. Thus [^{18}F]-**17** was obtained in high radiopurity (>99%) and was free of precursor **16**. The analytical method did not allow an accurate determination of the specific activity. Additionally, it is satisfactory to use a reduced amount of peptide **16** (0.5–1.0 mg) for hot labeled runs. For example, when the amount of **16** was reduced to 0.5 mg (5.6 mM), the conjugation took 1 h at room temperature (ESI†). The experiments were reproducible and in each case the resultant conjugate [^{18}F]-**17** was the only radioactive product. The identity of [^{18}F]-**17** was confirmed by comparison with a reference sample obtained independently from the conjugation between **16** and 5-FDR. Thus bioconjugation to this PAR-2 agonist proved very straightforward.

In summary it is demonstrated that [^{18}F]-FDR **10** is an efficient bioconjugation ligand for [^{18}F]-fluoro-labelling of suitably modified peptides. An attractive aspect of [^{18}F]-FDR **10** over [^{18}F]-FDG utilising protocols, there is no requirement for harsh conditions or pre-derivatisation of the [^{18}F]-sugar, and labelling can occur at pH 4.6–7.0 in one step. The ligation is extremely rapid relative to FDG due to the location of the fluorine at C-5 and the increased reactivity of 5-membered over 6-membered aldol sugars with alkoxyamines. So there are special electronic features of FDR that render it particularly suitable in this context. Additionally an automated production method for [^{18}F]-FDR **10** is developed by adapting a standard GE TRACERLab® module used for routine [^{18}F]-FDG synthesis. With its ease of synthesis and its conjugation efficiency [^{18}F]-FDR **10** emerges as a very attractive bioconjugation tool for clinical and preclinical peptide PET imaging studies.

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