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ABSTRACT

There is currently no ideal radiotracer for imaging protein synthesis rate (PSR) by positron emission tomography (PET). Existing fluorine-18 labelled amino acid-based radiotracers predominantly visualize amino acid transporter processes, and in many cases they are not incorporated into nascent proteins at all. Others are radiolabelled with the short half-life positron emitter carbon-11 which is rather impractical for many PET centers. Based on the puromycin (6) structural manifold, a series of 10 novel derivatives of 6 was prepared via Williamson ether synthesis from a common intermediate. A bioluminescence assay was employed to study their inhibitory action on protein synthesis which identified fluoroethyl analogue (7b) as a lead compound. The fluorine-18 analogue was prepared via nucleophilic substitution of the corresponding tosylate precursor in modest radiochemical yield 2±0.6% and excellent radiochemical purity (>99%) and showed complete stability over 3 h at ambient temperature.

INTRODUCTION

Protein translation is a fundamental process for function and survival of all organisms. Enzymes, membrane receptors, structural proteins, growth factors amongst many more are constantly produced within the cell under tight control. Disruption of cellular protein synthesis indicates disease, characterized for instance by an increase of the rate of protein synthesis in malignant growth, or by a reduction in rate in certain neurodegenerative disorders. The ability to visualize protein synthesis rate (PSR) is therefore an important goal in diagnosis, treatment and monitoring of these conditions. Positron emission tomography (PET) is an ideal technique
with which to do this. Since PET requires sub-nanomolar concentrations of radiolabelled tracer molecules, cellular processes such as PSR can be probed without causing a pharmacological effect.\textsuperscript{4} Several amino acids labelled with positron emitters have been investigated to this end, although their application in PSR imaging has several critical limitations. Carbon-11 labelled methionine and leucine ([\textsuperscript{11}C]1, [\textsuperscript{11}C]MET and [\textsuperscript{11}C]2, [\textsuperscript{11}C]LEU, Figure 1) are used for imaging PSR, but the short physical half-life of carbon-11 (20 min) makes their use practically impossible at PET scanning centers without a cyclotron, severely limiting their accessibility.\textsuperscript{5,6} Furthermore, [\textsuperscript{11}C]1 produces brain-penetrating radiolabelled metabolites.\textsuperscript{6} Fluorine-18 (t\textsubscript{1/2} = 110 min) labelled analogues such as S-(3-[^18]F]fluoropropyl)homocysteine ([\textsuperscript{18}F]\textsubscript{3}, [\textsuperscript{18}F]FPHCYS) and O-(2-[\textsuperscript{18}F]fluoroethyl)tyrosine ([\textsuperscript{18}F]\textsubscript{4}, [\textsuperscript{18}F]FET), are not recognized by the cellular protein synthesis mechanism, and therefore report amino acid active uptake, rather than PSR.\textsuperscript{7,8} The tyrosine analogue 2-[^18]F]fluorotyrosine ([\textsuperscript{18}F]\textsubscript{5}, [\textsuperscript{18}F]FTYR), is incorporated into nascent proteins, but in addition to its challenging electrophilic radiosynthesis via [\textsuperscript{18}F]F\textsubscript{2} gas rather than [\textsuperscript{18}F]F\textsubscript{−}, the resulting PET images are also dominated by the amino acid transporter processes.\textsuperscript{8} (Figure 1). A new fluorine-18 labelled radiotracer capable of imaging PSR is therefore required.
Figure 1. Selected carbon-11 and fluorine-18 amino acid radiotracers.

The aim of this project was to develop a novel radiotracer which: (1) enables measurement of PSR in vivo; (2) is not an amino acid and thus does not participate in the amino acid active transport mechanism; and (3) is radiolabelled with fluorine-18, via $^{18}$F fluoride.

Puromycin (6, PURO, Figure 2) is an aminonucleoside antibiotic that inhibits protein synthesis in both bacteria and eukaryotes by mimicking aminoacyl-tRNA (8) at the ribosome.$^{9,10}$

Compound 6 and some structural analogues, enter the ribosomal site A and form an amide bond with the C-terminus of a nascent protein, thus anchoring at sites of active protein growth.$^9$

Studies have demonstrated that 6 can be used directly to assess ribosome mediated peptide bond formation since it does not require soluble translation factors for function, it does not induce EF-Tu-GTPase activity and it can enter the ribosome independently.$^{11-14}$ Furthermore, incorporation of 6 into newly synthesized protein has been established as a direct readout of translation rate, by comparison with classical $^{35}$S MET studies.$^{15}$ Fluorescent analogues of 6 have been successfully used to monitor protein synthesis (PS) in tissue samples (ex vivo)$^{16}$ and scandium-44, gallium-68 and carbon-11 radiolabelled 6 derivatives have been described for PET.$^{11,17,18}$ However, the scandium-44 and gallium-68 complexes are structurally dominated by
the metal chelate, and carbon-11 radiolabelled 6 is limited by the short physical half-life of the radionuclide. We hypothesized that a fluorinated analogue of 6 would be a preferable radiotracers for PET imaging, as well as incorporating a more widely available radioisotope. The literature precedent demonstrates that derivatives of 6 modified at the phenyl ring retain the inhibitory activity of the parent,\textsuperscript{13,16,19} which prompted us to investigate the effect of introduction of a fluorine atom into the scaffold of 6 by varying functional groups at the phenol as point of modification (7b-k, Figure 2). We also considered potential routes for facile introduction of fluorine-18 in this position using a nucleophilic approach. Herein, we report the synthesis of several derivatives of 6, their ability to inhibit PS, as well as the radiolabelling of the most prominent derivative as a potential PET radiotracer.

![Figure 2](image-url)  
*Figure 2. The structures of 6, envisioned derivatives (7) and aminoacyl-tRNA (8).*

RESULTS AND DISCUSSION
Syntheses of investigated derivatives 7 (Figure 2 and Table 2) were envisioned following the method previously reported for phenol 12 (Scheme 1) by the Aigbirhio group. Starting with the commercially available Boc-protected tyrosine 9, EDC/HOBt amide coupling with TBS-protected puromycin aminonucleoside 11 afforded phenol 12 which served as a common intermediate to access TBS and Boc-protected derivatives of 6 (13a-f). These derivatives were prepared from 12 by employing the classical Williamson ether synthesis with several bromides, or electrophiles containing tosylate, mesylate or iodide as leaving groups (direct method). Alternatively, acid 9 was esterified to afford methyl ester 10 which was successfully converted to ethers 15g-h under analogous Williamson conditions in excellent yields (>95%). Removal of the methyl ester group was performed using trimethyltin hydroxide in order to avoid possible racemization at the chiral center and acids 16g-h were coupled with 11 to afford amides 13g-h (Scheme 1, indirect method). Using the indirect method ether 15g was prepared in excellent yield of 95% but the additional reaction step in the synthesis of 13g negligibly lowered overall yield to 57% from 62% for the indirect and direct methods respectively.
Scheme 1. Syntheses of TBS and Boc protected derivatives 7 and 14, using direct and indirect Williamson ether methods (please refer to Supporting Information for respective yields).

Conversion to products with some electrophiles using the Williamson method was sluggish, likely because of the structural complexity of phenol 12. In support of this theory, the reactivity of 10 and 12 with fluoroethyl electrophiles was compared (Table 1). Using mesylate as a leaving group in reaction with 12, conversion of 16% was observed by NMR after 25 h (entry 1) due to the significant amount of by-products formed. Reaction of 12 with 2-fluoroethyl tosylate gave only trace amount of product (entry 2) whereas heating the reaction mixture to 70 °C increased the yield of 13b to a modest 11% after 2 h and further to 44% when mixture was heated over 17 h (entries 3 and 4). On the other hand, reaction of 10 with 2-fluoroethyl tosylate proceeded with 37% NMR conversion (entry 6) at ambient temperature. Further improvement
to 42% of 15b (entry 7) was achieved by longer reaction time at ambient temperature as well as the addition of 18-crown-6, thus avoiding the possibility of decomposition or racemization upon extended heating. The yield could be improved further to 64% by heating to reflux in MeCN (entry 8), however racemization was observed (by chiral HPLC) and therefore these reaction conditions were not pursued. To our surprise, reaction of 10 with 2-fluoroethyl mesylate showed only slightly improved conversion (vs reaction with 12) of 20% by NMR (entry 5), for which reason we deterred from employing mesylate as the leaving group.

Table 1. Williamson ether synthesis with 10 and 12 and fluoroethyl electrophiles.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Phenol</th>
<th>LG</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
<th>(S):(R)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>OMs</td>
<td>23</td>
<td>25</td>
<td>16a</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>OTs</td>
<td>23</td>
<td>19</td>
<td>trace</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>OTs</td>
<td>70</td>
<td>2</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>OTs</td>
<td>70</td>
<td>17</td>
<td>44</td>
<td>—</td>
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<td>5</td>
<td>10</td>
<td>OMs</td>
<td>23</td>
<td>25</td>
<td>20a</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>OTs</td>
<td>23</td>
<td>16</td>
<td>37a</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>OTs</td>
<td>23</td>
<td>48</td>
<td>42b</td>
<td>99:1</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>OTs</td>
<td>reflux</td>
<td>21</td>
<td>64bc</td>
<td>89:11</td>
</tr>
</tbody>
</table>

* NMR conversion; b 18-c-6 used as additive; c MeCN was used as solvent

Alkyne 13f was further reacted with aryl azides under Cu(I)-mediated [2 + 3] cycloaddition conditions to give “click” chemistry analogues 14a-c (Scheme 1).16,21
Structural characterization of derivative 7f has been previously reported\textsuperscript{16} and we selected 7f as a reference to establish conditions for deprotection of both the TBS and Boc groups without epimerization (Scheme 2). Alkyne 13f was therefore treated with TBAF·THF resulting in deprotection of both primary and secondary TBS groups in 17f. TFA was employed for the removal of the Boc protecting group and 7f was afforded in 34% yield. NMR analysis of 7f was in complete agreement with that previously reported (see Supporting Information).\textsuperscript{16} An alternative two step deprotection was explored in which the Boc group was removed by TFA quantitatively first, and 18f was subsequently treated with TBAF·THF to yield 7f in 57% yield. Interestingly, the primary TBS group in 13f was cleaved during the Boc removal with TFA; however it was the secondary TBS which required application of TBAF.

Scheme 2. Two step deprotection of 13f via two routes, yielding desired 7f in structural agreement with the literature report.\textsuperscript{16}
With the deprotection route established, the syntheses of other derivatives of 6 were completed in a one pot two step method. Intermediates 17 and 18 were not isolated; instead the second step was performed with the crude material to afford 7b-k (Scheme 3a). Treatment of 13a and 13k with TBAF in a single step yielded Boc-protected analogues 17a and 17k (Scheme 3b). Using the synthetic method depicted in Schemes 1 and 2, derivative 7f was obtained in 20% yield over 4 steps from commercially available starting materials, which was an improvement to the previously reported preparation of 7f in a total of six steps, with 9% overall yield.16 Yields obtained for the syntheses of various derivatives of 6 are shown in Table 2.

Scheme 3. (a) Two step, one pot deprotection of TBS and Boc groups in 13/14 to afford derivatives 7. (b) TBAF deprotection of TBS groups to obtain derivatives 17.
Table 2. Structures of derivatives 7 and 17, deprotection yields and inhibitory affinity data.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Derivative</th>
<th>OR₁</th>
<th>Yield (%)</th>
<th>GLuc (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>OCH₃</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>7b</td>
<td>O—F</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>7c</td>
<td>O—F</td>
<td>52</td>
<td>195</td>
</tr>
<tr>
<td>4</td>
<td>7d</td>
<td>O—O</td>
<td>14</td>
<td>184</td>
</tr>
<tr>
<td>5</td>
<td>7e</td>
<td>O—O</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>7f</td>
<td>O—O</td>
<td>27</td>
<td>87</td>
</tr>
<tr>
<td>7</td>
<td>7g</td>
<td>O—F</td>
<td>48</td>
<td>99</td>
</tr>
<tr>
<td>8</td>
<td>7h</td>
<td>O—F</td>
<td>33</td>
<td>136</td>
</tr>
<tr>
<td>9</td>
<td>7i</td>
<td>O—O</td>
<td>24</td>
<td>165</td>
</tr>
<tr>
<td>10</td>
<td>7j</td>
<td>O—O</td>
<td>15</td>
<td>187</td>
</tr>
<tr>
<td>11</td>
<td>7k</td>
<td>O—O</td>
<td>37</td>
<td>150</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>OR₁ = OH, R₂ = TBS</td>
<td>—</td>
<td>112</td>
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<tr>
<td>13</td>
<td>13a</td>
<td>OR₁ = OCH₃, R₂ = TBS</td>
<td>—</td>
<td>192</td>
</tr>
<tr>
<td>14</td>
<td>17a</td>
<td>OR₁ = OCH₃, R₂ = H</td>
<td>99</td>
<td>156</td>
</tr>
<tr>
<td>15</td>
<td>17f</td>
<td>OR₁ = O—O, R₂ = H</td>
<td>96</td>
<td>184</td>
</tr>
<tr>
<td>16</td>
<td>17k</td>
<td>OR₁ = O—O, R₂ = H</td>
<td>78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>177</td>
</tr>
</tbody>
</table>

<sup>a</sup> % GLuc production (vs. 6 inhibition) at 50 µM

<sup>b</sup> Material was accessed via two different methods;

<sup>c</sup> Material was prepared via [2 + 3] cycloaddition of azide directly with 17f.

Please refer to Supporting information for details.
With the target molecules (6, 7b-k, 12, 13a, 17a, 17f, 17k) in hand, their PS inhibitory activity was assessed using a bioluminescent protein reporter system in bacteria. This technique has been previously established for the assessment of compounds that inhibit PS. In this assay, Gram positive bacteria *Staphylococcus aureus* 8325.4 were engineered to produce Gaussia Luciferase (GLuc), a 19 kD photoprotein. On addition of the GLuc substrate coelenterazine, a bioluminescent signal at 475 nm is produced proportional to the concentration of the GLuc protein, thus providing a sensitive method with which to detect differences in PS in bacterial culture. The test compound or 6 dihydrochloride was independently applied to *S. aureus* culture, and the quantity of GLuc protein synthesized was measured by bioluminescence after 2.5 h. Cell number was estimated by optical density (OD) to normalize the response. The efficiency of the novel derivatives for PS inhibition compared with 6·2HCl (set at 100%) is displayed in Table 2.

Comparable PS inhibitory activity of 7f to that of 6 was observed, as expected based on the published data (Table 2, entries 6 and 1). Only three other analogues showed comparable or better PS inhibitory effects at 50 µM concentration: 7b, 7e and 7g (entries 2, 5 and 7). At this concentration, non-fluorinated 7e proved the most potent PS inhibitor. Functionalization of 6 with either aromatic substituents (entries 3, 4 and 8) or [2 + 3] cycloaddition products, the “click” analogues (entries 9-11), resulted in reduction in inhibitory potency compared with 6. Similarly, masking of the hydroxyl functional groups (entries 12 and 13), in addition to amine group (entries 14-16) confirmed importance of free hydroxyl and amine functionalities to maintain inhibitory activity. Figure 3 shows the concentration dependence of PS inhibitory activity of the novel derivatives. At all concentrations tested, 7b was the most potent of the
fluorinated derivatives. At the lowest concentration tested (5 μM), 7b reduced protein production by 36% compared with 6, and proved to be a superior PS inhibitor to 7e (25% reduction compared with 6).

Figure 3. Effect of derivatives of 6 on protein synthesis, as indicated by inhibition of luciferase synthesis. RLU/OD = relative light units/optical density.

With evidence to support the use of 7b (FEPURO, Table 2) as the lead fluorinated compound in the series, we next sought to develop the respective radiolabelling precursor (Scheme 4). An obvious selection of phenol 12 (Scheme 1) as a precursor for radiosynthesis with 2-[18F]fluoroethyl tosylate was quickly discarded for following reasons: the presence of TBS protecting groups in 12 would facilitate side reactions due to the affinity of fluoride for silicon; and reaction of 12 with 2-fluoroethyl tosylate (Scheme 1 and Supporting Information) proceeded with modest yield therefore predicting similarly poor conversion with 2-[18F]fluoroethyl tosylate. Silicon-free protecting groups on the hydroxyl groups were necessary for successful radiolabelling, and we chose to investigate a direct fluorination approach. The
radiolabelling precursor 24 (Scheme 4) was therefore envisioned as most appropriate. Alongside synthesis of the radiolabelling precursor, we revisited the route to access 7b. Methyl ester 10 was reacted with 2-fluoroethyl tosylate to afford 15b in 42% (Table 1), and in analogy, reaction of 10 with 1,2-dibromoethane gave 19, in 89% yield, using crown ether 18-c-6 as additive. Heating this reaction to 80 °C did not induce racemization of the bromo-analogue (see the Supporting Information). Hydrolysis of the methyl esters was accomplished with LiOH, and using DCC the N-succinimidyl ester was successfully installed to yield 20 and 21. Amide coupling of activated esters 20 and 21 with commercially available puromycin aminonucleoside 22 furnished 77% of 17b and 96% of 23. For 17b, the Boc group was removed under acidic conditions leading to 7b in total of five steps and 7% overall yield. The hydroxyl groups in bromide 23 were acetylated catalysed by DMAP, and subsequently the bromide functionality was converted to a tosylate leaving group using silver tosylate, providing 24 in 46% yield from 23 (Scheme 4).

Next, conditions for radiolabelling of 24 were explored (Scheme 5) with small (288-480 MBq) amounts of $[^{18}\text{F}]\text{F}^-$ in manually conducted experiments. Aqueous $[^{18}\text{F}]\text{F}^-$ was azeotropically dried with acetonitrile in the presence of base. Variables investigated included time (5-30 min), reaction temperature (80-130 °C), reaction solvent (DMSO, MeCN, DMF), base (KHCO$_3$, K$_2$CO$_3$), phase transfer agent (18-c-6, K$_{222}$, TBAHCO$_3$) and precursor quantity (5-10 mg). Full details can be found in Table 3. The optimal conversion of 22% was achieved using DMSO at 120 °C over 15 min as analysed by HPLC (entry 8). Heating the reaction to 130 °C resulted in only a slight increase in conversion to 24%, however five additional, radiolabelled species were observed by HPLC that eluted closely with $[^{18}\text{F}]25$ (entry 9). Use of
the mild base KHCO$_3$ with K$_{222}$ also favoured [$^{18}$F]25 formation. Reducing the amount of 24 used in the reaction resulted in a significant reduction in conversion to 12% (entry 11).

Table 3. Optimisation of radiolabelling for preparation of [$^{18}$F]25 from 10 mg of 24 (0.3 mL reaction volume).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Additive</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>HPLC conversion (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>K$_2$CO$_3$</td>
<td>K$_{222}$</td>
<td>DMF</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>K$_2$CO$_3$</td>
<td>K$_{222}$</td>
<td>DMSO</td>
<td>80</td>
<td>13</td>
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<tr>
<td>3</td>
<td>K$_2$CO$_3$</td>
<td>K$_{222}$</td>
<td>MeCN</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>TBAHCO$_3$</td>
<td>—</td>
<td>DMSO</td>
<td>80</td>
<td>12$^a$</td>
</tr>
<tr>
<td>5</td>
<td>KHCOC$_3$</td>
<td>K$_{222}$</td>
<td>DMSO</td>
<td>80</td>
<td>18</td>
</tr>
<tr>
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<td>KHCOC$_3$</td>
<td>K$_{222}$</td>
<td>DMF</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>KHCOC$_3$</td>
<td>K$_{222}$</td>
<td>DMSO</td>
<td>120</td>
<td>20$^b$</td>
</tr>
<tr>
<td>8</td>
<td>KHCOC$_3$</td>
<td>K$_{222}$</td>
<td>DMSO</td>
<td>120</td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td>KHCOC$_3$</td>
<td>K$_{222}$</td>
<td>DMSO</td>
<td>130</td>
<td>24$^a$</td>
</tr>
<tr>
<td>10</td>
<td>KHCOC$_3$</td>
<td>18-c-6</td>
<td>DMSO</td>
<td>120</td>
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<td>KHCOC$_3$</td>
<td>K$_{222}$</td>
<td>DMSO</td>
<td>120</td>
<td>12$^{a,c}$</td>
</tr>
</tbody>
</table>

$^a$ Formation of by-products observed; $^b$ 10 min reaction time; $^c$ 7.5 mg of 24 used
Scheme 5. Radiosynthesis of $[^{18}\text{F}]7\text{b}$ via nucleophilic substitution and deprotection. Overlaid radio (red) and UV (blue) HPLC traces of formulated $[^{18}\text{F}]7\text{b}$, and 7b. Axes have been slightly offset for clarity.

After formation of $[^{18}\text{F}]25$, the removal of protecting groups was investigated. Attempted total deprotection to form $[^{18}\text{F}]7\text{b}$ directly from $[^{18}\text{F}]25$ by acidification of the DMSO reaction mixture with HCl was unsuccessful, resulting in no reaction at ambient temperature, or decomposition upon heating. For this reason a two-step method was established. In the first step, quantitative removal of both acetyl groups was accomplished in a short reaction time of 5 min using NaOH (aq.). An SPE purification was next performed to remove the DMSO from the mixture. Application of the aqueous base in the first step facilitated transfer to the SPE cartridge by solubilizing unreacted precursor 24. The Boc-group was then removed in the second step. Successfully employed reaction conditions to form 7b from 17b with TFA (Scheme 4) were investigated, and although $[^{18}\text{F}]17\text{b}$ was completely consumed, an unidentified by-product was
additionally formed resulting in only 47% of $[^{18}\text{F}]7\text{b}$ by HPLC. Use of TFA was not pursued for this reason, as well as its incompatibility with the polymer components of automated radiochemistry modules. Instead, hydrochloric acid (3M) was successfully employed for Boc-deprotection to form $[^{18}\text{F}]7\text{b}$ without by-products at ambient temperature over 5 min. $[^{18}\text{F}]7\text{b}$ was purified by semi-preparative HPLC and formulated in phosphate buffered saline (PBS) containing 10% ethanol. Structural identity of $[^{18}\text{F}]7\text{b}$ was confirmed by HPLC co-injection with reference sample 7b (Scheme 5).

Reactions performed using 0.9-1.8 GBq of aqueous $[^{18}\text{F}]$fluoride yielded formulated $[^{18}\text{F}]7\text{b}$ in non-decay corrected yield of 2±0.6% (n = 3) in 140 min, with specific activity of 5 GBq/µmol and radiochemical purity of >99% after purification (Scheme 5). Conducting the radiosynthesis manually limited any further increase in the amount of starting activity and therefore specific activity.

Radiochemical stability of a formulated solution of $[^{18}\text{F}]7\text{b}$ was tested by analytical HPLC over a three hour period, and showed no reduction in radiochemical purity.

CONCLUSIONS

We have developed a series of novel derivatives of 6, with potential for imaging PSR by PET. The synthesis of a common intermediate was accomplished via EDC/HOBt mediated amide coupling from commercially available materials, and derivatisation of the phenol functionality was achieved employing the classical Williamson synthesis in good yields. Using a luciferase PS assay 7b was identified as the lead compound having higher PSR inhibitory potency than
The radiolabelling precursor \( \text{24} \) was prepared in seven steps with 14\% overall yield and successfully employed in the radiosynthesis of \( [\text{\textsuperscript{18}}\text{F}]\text{7b} \) \textit{via} nucleophilic substitution. Radiolabelled \( [\text{\textsuperscript{18}}\text{F}]\text{7b} \) was prepared in excellent radiochemical purity. Implementation of a modular radiosynthesis of \( [\text{\textsuperscript{18}}\text{F}]\text{7b} \) as well as \textit{in vitro} and \textit{in vivo} evaluation of \( [\text{\textsuperscript{18}}\text{F}]\text{7b} \) are currently underway in our laboratories and will be reported in a due course.

**EXPERIMENTAL SECTION**

**General.** All reactions requiring anhydrous conditions were conducted in oven-dried glass apparatus under an atmosphere of inert gas. All reagents were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification, unless otherwise stated. Preparative chromatographic separations were performed on Aldrich Science silica gel 60 (35-75 \( \mu \text{m} \)) and reactions followed by TLC analysis using Sigma-Aldrich silica gel 60 plates (2-25 \( \mu \text{m} \)) with fluorescent indicator (254 nm) and visualized with UV or potassium permanganate. \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR spectra were recorded in Fourier transform mode at the field strength specified on a Bruker Avance 400 MHz or a Bruker Avance 300 MHz spectrometer. Spectra were obtained in the specified deuterated solvents in 5 mm diameter tubes. Chemical shift in ppm is quoted relative to residual solvent signals calibrated as follows: \( \text{CDCl}_3 \delta_{\text{H}} (\text{CHCl}_3) = 7.26 \text{ ppm}, \delta_{\text{C}} = 77.2 \text{ ppm}; (\text{CD}_3)_2\text{SO} \delta_{\text{H}} (\text{CD}_3\text{SOCH}_2\text{D}) = 2.50 \text{ ppm}, \delta_{\text{C}} = 39.5 \text{ ppm}; \text{MeOD-d}_4 \delta_{\text{H}} (\text{CD}_2\text{HOD}) = 3.31 \text{ ppm}, \delta_{\text{C}} = 49.0 \text{ ppm}; (\text{CD}_3)_2\text{NC(O)}\text{D} \delta_{\text{H}} ((\text{CD}_3)_2\text{NC(O)}H) = 7.92 \text{ ppm}, \delta_{\text{C}} = 165.5 \text{ ppm}.

Multiplicities in the \( ^1\text{H} \) NMR spectra are described as: \( s = \) singlet, \( d = \) doublet, \( t = \) triplet, \( q = \) quartet, \( \text{quint.} = \) quintet, \( m = \) multiplet, \( b = \) broad; coupling constants are reported in Hz.

Numbers in parentheses following carbon atom chemical shifts refer to the number of attached
hydrogen atoms as revealed by the DEPT/HSQC spectral editing technique. Mass spectra were recorded on a Waters Micromass LCT instrument, a Bruker MicroTOF mass spectrometer using electrospray Ionisation (ESI), or by the EPSRC Mass Spectrometry Service at the University of Swansea. Purity of compounds was \( \geq 95\% \) as determined by analytical LC on a Waters Acquity-H UPLC or Agilent Series 1200 system with UV detection \((\lambda = 254 \text{ nm})\). The general UPLC/HPLC methods were as follows. Waters general method: 0-4 min, 5-95\% aq. MeCN at 0.6 mL/min (column: BEH C18, 1.7 \( \mu \)m, 2.1x50 mm. Agilent general method: 0- 1 min 5\% B; 1-15 min 5-95\% B; 15-18 min 95\% B; 18-20 min 95-5\% B; 20-25 min 5\% B. A flow rate of 1 mL/min was used. Unless otherwise stated, solvent A = H\(_2\)O and B = MeCN. A Phenomenex Luna C18(2) column (150 mm x 4.6 mm) was used. All radioactive manipulations were performed in designated lead shielded hot cells to reduce operator dose. \[^{18}\text{F}]\)Fluoride was purchased from PETNET Nottingham, and was supplied as \[^{18}\text{F} \text{fluoride in H}_2\text{(^{18}\text{O})O}\). Seppak tC18 light cartridges were purchased from Waters. Radiochemical incorporation was determined by the percentage of the desired product from integration of the analytical HPLC radiotracer. Where radiochemical yields are given, they are calculated from HPLC purified material as a percentage of starting aqueous \[^{18}\text{F} \text{fluoride, and are not corrected for decay. Semi-}

preparative HPLC was performed using a Knauer Smartline pump, equipped with Knauer Azura UV detector (254 nm) and diode radiodetector (Carroll Ramsey, USA), and a custom built apparatus for HPLC loop load and product collection. Data were collected using SingleStream software (Dr. R. Fortt). A Phenomenex Synergi Hydro-RP column (250 x 10 mm) 4\( \mu \) 80A with guard was used, with an isocratic method. The eluant was composed of 30\% MeCN in 70\% ammonium acetate (aq. 50 mM), with a flow rate of 2 mL/min.
(S)-2-Amino-N-{(2S,3S,4R,5R)-5-[6-(dimethylamino)-9H-purin-9-yl]-4-hydroxy-2-(hydroxymethyl)tetrahydrofuran-3-yl]-3-[4-(2-fluoroethoxy)phenyl]propanamide (7b).

17b (40.1 mg, 0.07 mmol) was dissolved in TFA (0.5 mL). The reaction stirred at room temperature for 10 min, after which the volatiles were removed under a stream of N₂. The resulting solid was rinsed with diethyl ether (2x1 mL) and dried under a stream of N₂. The solid was redissolved in EtOAc (15 mL), and extracted into H₂O (15 mL). The aqueous layer was retained, and saturated aq. NaHCO₃ (10 mL) was added. The product was extracted into EtOAc (2x10 mL), and washed with H₂O (10 mL). The organic layer was dried (MgSO₄), and evaporated under reduced pressure. The product was purified by column chromatography on silica (eluting with 5% MeOH/CH₂Cl₂ containing 1% NEt₃) yielding a white solid (11.4 mg, 0.02 mmol, 34%):¹H NMR (400 MHz, DMSO-d₆) δ 8.44 (s, 1H), 8.23 (s, 1H), 8.08 (b s, 1H), 7.16 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.13 (b d, J = 2.9 Hz, 1H), 5.98 (d, J = 2.7 Hz, 1H), 5.15 (bt, J = 5.2 Hz, 1H), 4.80-4.64 (dm, J₁,F₁-C₂=H = 47.8 Hz, 2H), 4.51-4.42 (m, 2H), 4.25-4.13 (dm, J₁,F₁-C₂=H = 30.2 Hz, 2H), 3.96-3.90 (bm, 1H), 3.72-3.65 (b m, 1H) 3.54-3.26 (b m, 8H), 2.92 (dd, J = 13.5, 5.0 Hz, 1H), 2.57 (dd, J = 13.5, 8.5 Hz, 1H) ppm; ¹³C NMR (100 MHz, DMSO-d₆) δ 174.3 (0), 156.6 (0), 154.2 (0), 151.7 (1), 149.5 (0), 137.8 (1), 130.7 (0), 130.2 (1, 2C), 119.5 (0), 114.1 (1, 2C), 89.3 (1), 83.5 (1), 82.1 (2, d, J = 166.6 Hz), 73.1 (1), 66.9 (2, d, J = 19.0 Hz), 60.9 (2), 56.0 (1), 50.0 (1), 39.7 (2, obscured by solvent, visible by DEPT-135), 37.7 (b, 3, 2C) ppm; ¹⁹F NMR (376 MHz, DMSO-d₆) δ -224.7 (tt, J₁,F₁-C₂=H = 47.8, J₁,F₁-C₂=H = 30.2 Hz) ppm; HRMS m/z [M + H]⁺ 504.2372 (calcd. 504.2365 for C₂₃H₁₅FN₇O₅⁺); HPLC (Solvent A = ammonium acetate, 50 mM, solvent B = MeOH) Rₚ = 12.2 min.

(S)-2-amino-N-{(2S,3S,4R,5R)-5-[6-(dimethylamino)-9H-purin-9-yl]-4-hydroxy-2-(hydroxymethyl)tetrahydrofuran-3-yl]-3-[4-[2-[(¹⁸F)fluoro]ethoxy]phenyl]propanamide
The optimized procedure was as follows: aqueous $[^{18}\text{F}]}$fluoride (0.9-1.8 GBq) was added to a mixture of $\text{K}_2\text{CO}_3$ (5.0 mg) and $\text{KHCO}_3$ (50 $\mu$L, 0.1 M) in MeCN (0.5 mL) in a v-vial equipped with stirring bar. The mixture was dried azeotropically at 110 °C under a stream of $\text{N}_2$ (without stirring). Two further portions of MeCN (2x0.5 mL) were added to ensure complete drying (total drying time ca. 30 min). Precursor 24 (10.0 mg), was dissolved in DMSO (0.3 mL, anhydrous) and added to the dried $[^{18}\text{F}]}$fluoride. The reaction was heated to 120 °C for 15 min with stirring. After cooling to room temperature, NaOH (1 M, 3 mL) was added and stirred for 5 min, followed by DMSO (0.7 mL). The reaction mixture was loaded to a pre-conditioned (MeCN 2 mL; $\text{H}_2\text{O}$ 5 mL) 'C18 Seppak light cartridge, and the cartridge was washed with $\text{H}_2\text{O}$ (4 mL). The cartridge was briefly dried for 2 min after washing. The cartridge was eluted with MeCN (0.5 mL), concentrated under a stream of $\text{N}_2$ at 80 °C for 5 min, cooled, and HCl (3 M, 300 $\mu$L) added. The reaction stirred for 5 min at room temperature. After neutralization with NaOH (1M) and dilution with mobile phase (1 mL), the mixture was loaded to a semi-preparative HPLC system for purification. The radioactive peak corresponding to the product ($R_t$ = 12 min) was collected. The HPLC cut was diluted with $\text{H}_2\text{O}$ (15 mL), loaded to a primed 'C18 light cartridge (EtOH 2mL; $\text{H}_2\text{O}$ 5 mL), and washed with $\text{H}_2\text{O}$ (2 mL). The desired product $[^{18}\text{F}]}$7b was eluted with EtOH (300 $\mu$L) and diluted in phosphate buffered saline (2.7 mL).

Product $[^{18}\text{F}]}$7b was isolated in non-decay corrected yield of 2±0.6% (n = 3), with specific activity of 5 GBq/µmol and radiochemical purity of >99%.

**Methyl-(S)-2-[(tert-butoxycarbonyl)amino]-3-[4-(2-fluoroethoxy)phenyl]propanoate**

(BocTyr(EtF)OMe, 15b). Boc(t1)TyrOMe (1.01 g, 3.4 mmol, 1eq) was dissolved in DMF (6 mL) and $\text{K}_2\text{CO}_3$ (3.23 g, 23.4 mmol, 6.9 eq) was added. 18-Crown-6 (0.10 g, 0.4 mmol, 0.1 eq) and 2-fluoroethyl tosylate (0.82 g, 3.8 mmol, 1.1 eq) were added and the reaction stirred at
room temperature for 48 h. H$_2$O (25 mL) was added to the reaction, and the product extracted into EtOAc (2x30 mL). The combined organics were washed with brine (2x30 mL), dried (MgSO$_4$), and evaporated under reduced pressure. The product was purified by silica chromatography (eluting with 20% EtOAc/hex) yielding a colorless oil, which solidified on standing (0.49 g, 1.4 mmol, 42%): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.07 (d, $J$ = 8.6 Hz, 2H), 6.88 (d, $J$ = 7.9 Hz, 1H), 4.77 (dt, $J$ = 7.9, 4.2 Hz, 2H), 4.57 (m, 1H), 4.22 (dt, $J$ = 27.7, 4.2 Hz, 2H), 3.73 (s, 3H), 3.09-2.96 (m, 2H), 1.44 (s, 9H) ppm; HRMS $m/z$ [M + Na]$^+$ 364.1542 (calcd. 364.1531 for C$_{17}$H$_{24}$FNNaO$_5$)$^+$; HPLC $R_t$ = 13.3 min. The spectral data were in complete agreement with the literature reported values.$^{25}$

**Tert-butyl [(S)-1-[(2S,3S,4R,5R)-5-[6-(dimethylamino)-9H-purin-9-yl]-4-hydroxy-2-(hydroxymethyl)tetrahydrofuran-3-yl]amino]-3-[4-(2-fluoroethoxy)phenyl]-1-oxopropan-2-yl]carbamate (17b).** Puromycin aminonucleoside (0.14 g, 0.5 mmol, 1 eq) was dissolved in THF (8 mL) and BocTyr(EtF)OSu 20 (0.20 g, 0.5 mmol, 1 eq) was added. The reaction stirred at room temperature for 20 h. The reaction was diluted with H$_2$O and product extracted with EtOAc (3x20 mL). The organic extracts were washed with H$_2$O (20 mL) and brine (20 mL), dried (MgSO$_4$), and evaporated to dryness. The product was purified by column chromatography on silica (eluting with 5% MeOH/CH$_2$Cl$_2$) yielding a white solid (0.22 g, 3.6 mmol, 77%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.44 (s, 1H), 8.24 (s, 1H), 8.00 (d, $J$ = 7.6 Hz, 1H), 7.20 (d, $J$ = 8.5 Hz, 2H), 6.90-6.84 (m, 3H), 6.06 (d, $J$ = 4.7 Hz, 1H), 5.99 (d, $J$ = 2.8 Hz, 1H), 5.16 (bm, 1H), 4.72 (m, $J_{H\cdotsC\cdotsF} = 47.8$ Hz, 2H), 4.53-4.43 (m, 2H), 4.24-4.13 (m, $J_{H\cdotsC\cdotsC\cdotsF} = 30.2$ Hz, 3H), 3.96-3.91 (bm, 1H), 3.68 (bd, $J$ = 11.6 Hz, 1H), 3.61-3.34 (bm, 7H), 2.91 (dd, $J$ = 13.6, 4.6 Hz, 1H), 2.70 (dd, $J$ = 13.3, 9.9 Hz, 1H), 1.30 (s, 9H) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 172.0 (0), 156.6 (0), 155.1 (0), 154.2 (0), 151.8 (1), 149.6 (0), 137.8 (1), 130.2 (1,
2C), 119.5 (0), 114.0 (1, 2C), 89.2 (1), 83.3 (1), 82.1 (2, d, \( J = 166.5 \) Hz), 78.0 (0), 73.0 (1), 66.9 (2, d, \( J = 19.0 \) Hz), 60.8 (2), 55.8 (1), 50.2 (1), 37.8 (3, 2C), 36.8 (2), 28.0 (3, 3C) ppm.

One carbon resonance was missing (quaternary aromatic carbon); \(^1^9\)F NMR (376 MHz, DMSO-\( d_6 \)) \( \delta = -224.7 \) (tt, \( J_{H-C-F} = 47.8, J_{H-C-C-F} = 30.2 \) Hz) ppm; HRMS [M + H]^+ \( m/z \) 604.2887 (calcd. 604.2890 for \( C_{28}H_{39}FN_7O_7 \)); HPLC \( R_t = 10.9 \) min.

(S)-2-\([(Tert-butoxycarbonyl)amino]-3-[4-(2-fluoroethoxy)phenyl]propanoic acid

(BocTyr(EtF)OH, \textit{en route to 20}). BocTyr(EtF)OMe 15b (0.46 g, 1.4 mmol) was dissolved in THF (8 mL) and cooled to 0 °C in an ice bath. LiOH (aq. 2M, 2 mL) was added and the reaction stirred for 2 h. The THF was evaporated and the mixture acidified with KHSO\(_4\) (5%, 30 mL).

The product was extracted into EtOAc (2x30 mL), and the combined organic extracts were dried (MgSO\(_4\)), filtered and evaporated to dryness. The product was isolated as a white solid (0.42 g, 1.3 mmol, 93%). No further purification was necessary. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \)

- 7.11 (d, \( J = 8.6 \) Hz, 2H), 6.87 (d, \( J = 8.6 \) Hz, 2H), 4.92 (d, \( J = 7.3 \) Hz, 1H), 4.74 (dt, \( J = 47.4, 4.2 \) Hz, 2H), 4.57 (m, 1H), 4.19 (dt, \( J = 27.8, 4.2 \) Hz, 1H), 3.17-3.00 (m, 2H), 1.42 (s, 9H) ppm;

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta \)

- 176.5 (0), 157.8 (0), 155.6 (0), 130.7 (1, 2C), 128.6 (0), 115.0 (1, 2C), 82.1 (2, d, \( J = 170.6 \) Hz), 80.5 (0), 67.3 (2, d, \( J = 20.5 \) Hz), 54.6 (1), 37.1 (2), 28.5 (3, 3C) ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \( \delta = -224.9 \) (tt, \( J_{H-C-F} = 47.4, J_{H-C-C-F} = 27.8 \) Hz) ppm;

HRMS \( m/z \) [M - H]^- 326.1408 (calcd. 326.1409 for \( C_{16}H_{21}FNO_5^- \)); HPLC (Solvent A = H\(_2\)O + 0.1% TFA; B = MeCN + 0.1% TFA) \( R_t = 11.7 \) min.

2,5-Dioxopyrrolidin-1-yl (S)-2-\([(Tert-butoxycarbonyl)amino]-3-[4-(2-fluoroethoxy)phenyl]propanoate (BocTyr(EtF)OSu, 20). BocTyr(EtF)OH (0.41 g, 1.3 mmol, 1 eq), was dissolved in EtOAc (10 mL) and N-hydroxysuccinimide (0.15 g, 1.3 mmol, 1 eq) was
added. The mixture was cooled to 0 °C in an ice bath. Dicyclohexylcarbodiimide (0.26 g, 1.3 mmol, 1 eq) was added dropwise as a solution in EtOAc (10 mL). The reaction stirred at room temperature for 16 h, after which the white precipitate was filtered off. The filtrate was evaporated to dryness, then recrystallized from hot 2-propanol. The resulting solid was filtered and dried in vacuo (0.36 g, 0.8 mmol, 68%): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.21 (d, $J$ = 8.6 Hz, 2H), 6.88 (d, $J$ = 8.6 Hz, 2H), 4.95-4.83 (m, 1H), 4.74 (dt, $J$ = 47.4, 4.2 Hz, 2H), 4.20 (dt, $J$ = 27.7, 4.2 Hz, 2H), 3.28-3.07 (m, 2H), 2.86 (s, 4H), 1.42 (s, 9H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.8 (0), 167.9 (0), 157.9 (0), 154.8 (0), 151.0 (1, 2C), 127.4 (0), 115.0 (1, 2C), 82.1 (2, d, $J$ = 170.6 Hz), 80.7 (0), 67.3 (2, d, $J$ = 20.6 Hz) 52.9 (1), 37.5 (2), 28.4 (3, 3C), 25.8 (2, 2C) ppm; $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -224.9 (tt, $J_{H-C-F}$ = 47.4, $J_{H-C-C-F}$ = 27.7 Hz) ppm; HRMS [M + Na]$^+$ m/z 447.1530 (calcd. 447.1538 for C$_{20}$H$_{25}$FN$_2$NaO$_7$$^+$); HPLC: BocTyr(EtF)OSu appeared to decompose on HPLC analysis under all conditions tested, using both normal and reverse phase columns.

**In vitro assays.** *S. aureus* 8325.4 (pUNKP$_{xyl/tet}$::GLuc) was grown overnight in 5 mL tryptone soya broth (TSB), with erythromycin (5 µg/mL) and lincomycin (25 µg/mL) at 37 °C with 250 rpm shaking. The overnight culture was diluted to an optical density (OD$_{600}$) of 0.05 with fresh TSB, and anhydrotetracycline (ATc, 40 ng/mL) was added to induce GLuc expression. Compounds for assessment were prepared as stock solutions of 50 mM in DMSO, and diluted to final concentrations of 5 µM, 50 µM and 500 µM, containing 1% DMSO. To a 96-well plate (Corning, black, clear bottom) was added bacterial culture (180 µL) and test compound (20 µL), control wells contained 1% DMSO; all experiments were performed in triplicate. **Bacterial cell number:** Plates were incubated at 37 °C in a 96 well plate reader (Tecan), and optical density (OD$_{600}$) was recorded every 15 min for 2.5 h. **Bioluminescence:** After 2.5 h, and whilst in the
Tecan plate reader, coelenterazine (20 µM, 50 µL) was added to each well and bioluminescence immediately recorded. The bioluminescent signal was normalized with optical density to correct for cell number and reported in relative light units per OD (RLU/OD). Error bars indicate standard deviation from the mean.

ASSOCIATED CONTENT

Supporting Information.

Full experimental details for the synthesis of analogues of 6, NMR spectral data, radio-HPLC traces and stability study data are available in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS

DSC disuccinimidyl; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; \[^{\text{18}}\text{F}\]FET, O-(2-\[^{\text{18}}\text{F}\]fluoroethyl) tyrosine; \[^{\text{18}}\text{F}\]FPHCYS, S-(3-\[^{\text{18}}\text{F}\]fluoropropyl)homocysteine; \[^{\text{18}}\text{F}\]FTYR, 2-\[^{\text{18}}\text{F}\]fluorotyrosine; HOBt, hydroxybenzotriazole; \[^{\text{1}}\text{C}\]LEU, carbon-11 leucine; \[^{\text{11}}\text{C}\]MET, carbon-11 methionine; PET, positron emission tomography; PURO, puromycin; SPE, solid phase extraction; TBS, \textit{tert}-butyldimethylsilyl.

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