



## ImPyβImPyβImβ-C<sub>3</sub>-<sup>18</sup>F [<sup>18</sup>F]PIPAM5

Huiming Zhang, PhD<sup>1</sup>

Created: November 4, 2008; Updated: December 1, 2008.

<b>Chemical name:</b>	ImPyβImPyβImβ-C <sub>3</sub> - <sup>18</sup> F	No structure is current available in <a href="#">PubChem</a> .
<b>Abbreviated name:</b>	[ <sup>18</sup> F]PIPAM5	
<b>Synonym:</b>		
<b>Agent category:</b>	Macromolecule	
<b>Target:</b>	DNA	
<b>Target category:</b>	Nucleic acid binding molecule	
<b>Method of detection:</b>	Positron emission tomography (PET)	
<b>Source of signal/contrast:</b>	<sup>18</sup> F	
<b>Activation:</b>	No	
<b>Studies:</b>	<ul style="list-style-type: none"> <li><i>In vitro</i></li> <li>Rodents</li> </ul>	

## Background

[PubMed]

Polyamides (PAM) constructed from N-methylpyrrole (Py), N-methylimidazole (Im), 3-chlorothiophene (Ct), and N-methylhydroxypyrrole (Hp) amino acids comprise a class of synthetic oligomeric ligands that bind to the minor groove of DNA (1, 2). The aromatic heterocycles in the PAM orientate antiparallel with respect to the Watson-Crick base pair (bp), which leads to a specific recognition of DNA sequences (3). The recognition process follows a series of pairing rules; i.e., an ImPy specifies for G·C, a PyPy binds both A·T and T·A, an HpPy discriminates T·A over A·T, and a CtPy prefers T·A over A·T at the N-terminus. These aromatic amino acids can be programmed to a strand with more than two residues to recognize longer DNA sequences; for example, an ImPyPy motif specifies for the five-bp sequence 5'-WGWCW-3' (W=A, T) instead of 5'-WGWWW-3' (4). More complicated PAM motifs can be designed by adding small molecules such as β-alanine or γ-aminobutyric acid to covalently link between two antiparallel PAM strands, yielding substantial increases in affinities and specificities. For instance, an eight-ring hairpin motif, which has a γ-aminobutyric acid (γ-turn) linker to connect the carboxylic terminus of one polyamide to the amino terminus of another, exhibits ~100-fold higher affinity for binding a six-bp DNA sequence compared to the unlinked homodimers (4). PAM are molecules that can

permeate cell membranes and have been used in targeting a variety of DNA sequences in cell culture (5). The binding of PAM replaces the DNA-binding proteins and thus regulates the transcription of selected genes. The use of radiolabeled PAM aims at imaging gene regulations *in vivo*.

Fluorine-18 [ $^{18}\text{F}$ ], with a half-life of 109.7 min and low  $\beta^+$ -energy (0.64 MeV), represents the ideal radionuclide for position emission tomography (PET). The  $^{18}\text{F}$ -produced positron is annihilated with an electron, leading to the emission of two 511-keV photons  $\sim 180^\circ$  apart, which is detected coincidentally with PET. Various peptides have been successively fluorinated with multistep  $^{18}\text{F}$ -acylation, using  $^{18}\text{F}$ -labeled prosthetic groups such as amino-reactive  $^{18}\text{F}$ -labeling agent N-succinimidyl 4- $^{18}\text{F}$ fluorobenzoate (6). To increase labeling efficiency, the fluorination also can be conducted *via* a two-step synthetic approach in which an oxime is formed between an aminoxy group in the peptide and an  $^{18}\text{F}$ -labeled aldehyde such as 4- $^{18}\text{F}$ fluorobenzaldehyde (6). ImPy $\beta$ ImPy $\beta$ Im $\beta$ -C<sub>3</sub>- $^{18}\text{F}$  ( $^{18}\text{F}$ PIPAM5) is an  $^{18}\text{F}$ -labeled PAM used for PET that is obtained with the oxime ligation approach (5).  $^{18}\text{F}$ PIPAM5 contains five aromatic amino acids connected with  $\beta$ -alanine, which is denoted as  $\beta$  and is also known as a five-ring  $\beta$ -linked motif. Its unlabeled form with an *N,N*-dimethylaminopropyl tail has been exhibit ability to upregulate the repressed gene frataxin in a cell culture mode of Friedreich's ataxia (7).

## Synthesis

[PubMed]

Harki et al. reported the synthesis of  $^{18}\text{F}$ PIPAM5 (5). Initially, 4- $^{18}\text{F}$ -fluorobenzaldehyde was obtained by nucleophilic fluorination of a trimethylammonium benzaldehyde derivative with cyclotron-produced  $^{18}\text{F}$ fluoride in the presence of 5,6-benzo-4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacos-5-ene (Kryptofix[2.2.2]). The  $\beta$ -linked PAM ImPy $\beta$ ImPy $\beta$ Im $\beta$  was synthesized on a Boc- $\beta$ -alanine phenylacetamidomethyl resin according to standard protocols. Then the PAM was hydroxylamine-functionalized in DMF by reaction with tert-butyl-3-aminopropoxycarbamate in the presence of benzotriazolyl-oxy-tris-(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) and *N,N*-diisopropylethylamine. Finally, the obtained ImPy $\beta$ ImPy $\beta$ Im $\beta$ -hydroxylamine was ligated with the 4- $^{18}\text{F}$ -fluorobenzaldehyde with aniline as a catalyst to produce  $^{18}\text{F}$ PIPAM5 at radiochemical yield of 12%. The whole synthetic procedure was completed in 100 min after the end of bombardment.

## In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Harki et al. used the cold PAM analog  $^{19}\text{F}$ PIPAM5 to evaluate its affinity to DNA *in vitro* (5). Quantitative DNaseI footprint titrations were performed on the 5'- $^{32}\text{P}$ -polymerase chain reaction fragment from plasmid pJWP-16. In this method, equilibrium mixtures of  $^{32}\text{P}$  end-labeled DNA and a range of PAM concentrations were partially digested by DNase I followed by gel electrophoresis and autoradiography. The PAM bound DNA was protected from cleavage, which produced a band gap on the gel. Quantification of the binding fraction as a function of PAM concentration was used to the apparent association constant,  $3.5 \pm 2.1 \times 10^9 \text{ M}^{-1}$  for  $^{19}\text{F}$ PIPAM5.

## Animal Studies

### Rodents

[PubMed]

Harki et al. examined the biodistribution of [<sup>18</sup>F]PIPAM5 *in vivo* by PET and computed tomography (CT) (5). C57 mice were injected intravenously with [<sup>18</sup>F]PIPAM5 at doses of 472, 218, and 193 μCi (17.5, 8.06 and 7.141 MBq), respectively, and PET images were collected for 2 to 3 h. At 4 min after injection, ~46% of injected [<sup>18</sup>F]PIPAM5 was found in the liver. At 20 min after injection, 35% to 40% of injected [<sup>18</sup>F]PIPAM5 was observed in the gastrointestinal tract and maintained a constant for the entire PET scan. No significant radioactivity was found in the brain, heart, or bone. Thus, the clearance of [<sup>18</sup>F]PIPAM5 was primarily *via* the liver by excretion through the gallbladder and entry into small intestine; the renal clearance was <1.5%.

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available.

## Human Studies

[PubMed]

No publication is currently available.

## NIH Support

GM 27681, EB 01943, CA 92865

## References

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