CtPyPyIm-(R)$^{H}_{2}N\gamma$-PyImPyPy-C_3-^{18}F

[^{18}F]PIPAM8

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Chemical name:	CtPyPyIm-(R)$^{H}_{2}N\gamma$-PyImPyPy-C_3-^{18}F	
Abbreviated name:	[^{18}F]PIPAM8	
Synonym:		
Agent category:	macromolecule	
Target:	DNA	
Target category:	Nucleic acid binding molecule	
Method of detection:	Positron emission tomography (PET)	
Source of signal/contrast:	^{18}F	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is current available in PubChem.

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

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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 motifs 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}F], with a half-life of 109.7 min and low β^+-energy (0.64 MeV), represents the ideal radionuclide for position emission tomography (PET). The ^{18}F-produced positron is annihilated with an electron, leading to the emission of two 511-keV photons ~180° apart, which is detected coincidentally with PET. Various peptides have been successively fluorinated with multistep ^{18}F-acylation, using ^{18}F-labeled prosthetic groups such as amino-reactive ^{18}F-labeling agent N-succinimidyl 4-[^{18}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 aminooxy group in the peptide and an ^{18}F-labeled aldehyde such as 4-[^{18}F]fluorobenzaldehyde (6). CtPyPyIm-(R)$^{H}_{2}{}^{N}$γ-PyImPyPy-C_3-^{18}F ([^{18}F]PIPAM8) is an ^{18}F-labeled PAM used for PET that is obtained with the oxime ligation approach (5). [^{18}F]PIPAM8 contains eight aromatic amino acids connected with a (*R*)-2,4-diaminobutyric acid, which is denoted as (*R*)$^{H}_{2}{}^{N}$ and is also known as an eight-ring hairpin motif. The use of chiral diaminobutyric acid as the γ-turn increases the overall binding affinity by 10-fold without reduction of sequence specificity (1). PIPAM8 is designed to bind specifically to the sequence 5'-ATACGT-3' found in the vascular endothelial growth factor (VEGF) hypoxia response element (HRE) and can downregulate the hypoxia-induced VEGF expression in cell culture (7).

Synthesis

[PubMed]

Harki et al. reported the synthesis of [^{18}F]PIPAM8 (5). Initially, 4-[^{18}F]-fluorobenzaldehyde was obtained by nucleophilic fluorination of a trimethylammonium benzaldehyde derivative with cyclotron-produced [^{18}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 hairpin PAM CtPyPyIm-(R)$^{H}_{2}{}^{N}$γ-PyImPyPy was synthesized on a Kaiser oxime resin according to standard protocols. Briefly, after a single Py amino acid and a trimer PyImPy were sequentially loaded on the resin, a chiral turn was introduced *via* coupling of α-Boc-N-γ-Fmoc-D-diamionbutyric acid, followed by a coupling with a tetramer CtPyPyIm. Then the PAM was hydroxylamine-functionalized in DMF by reaction with tert-butyl-3-aminopropoxycarbamate in the presence of benzotriazolyloxy-tris-(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) and *N*,*N*,-diisopropylethylamine (DIEA). Finally, the obtained CtPyPyIm-(R)$^{H}_{2}{}^{N}$γ-PyImPyPy-

hydroxylamine was ligated with the 4-[^{18}F]-fluorobenzaldehyde with aniline as a catalyst to produce [^{18}F]PIPAM8 at radiochemical yield of 7%. 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}F]PIPAM8 to evaluate its affinity to DNA *in vitro* (5). Quantitative DNaseI footprint titrations were performed on the 5'-^{32}P-polymerase chain reaction fragment from plasmid pGL2-VEGF-Luc. In this method, equilibrium mixtures of ^{32}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, $3.3 \pm 0.3 \times 10^9$ M^{-1} for [^{19}F]PIPAM8.

Animal Studies

Rodents

[PubMed]

Harki et al. examined the biodistribution of [^{18}F]PIPAM8 *in vivo* with PET and computed tomography (5). C57 mice were injected intravenously with [^{18}F]PIPAM8 at doses of 543, 191, and 84 μCi (20.1, 7.06 and 3.108 MBq), respectively, and PET images were collected for 2 to 3 h. At 4 min after injection, ~36% of injected [^{18}F]PIPAM8 was found in the liver and maintained a constant level throughout the duration of PET scan. The excretion from liver to gallbladder was slow; ~5% of injected [^{18}F]PIPAM8 was observed in the gastrointestinal tract 20 min after injection. No significant radioactivity was found in the brain, heart, or bone. Thus, the clearance of [^{18}F]PIPAM8 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

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