ImPyβImPyβImβ-C_3-^{18}F

[^{18}F]PIPAM5

Huiming Zhang, PhD[1]

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

Chemical name:	ImPyβImPyβImβ-C_3-^{18}F	
Abbreviated name:	[^{18}F]PIPAM5	
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

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Zhang H. ImPyβImPyβImβ-C_3-^{18}F. 2008 Nov 4 [Updated 2008 Dec 1]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

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}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). ImPyβImPyβImβ-C_3-^{18}F ([^{18}F]PIPAM5) is an ^{18}F-labeled PAM used for PET that is obtained with the oxime ligation approach (5). [^{18}F]PIPAM5 contains five aromatic amino acids connected with β-alanine, which is denoted as β and is also known as a five-ring β-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}F]PIPAM5 (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 β-linked PAM ImPyβImPyβImβ was synthesized on a Boc-β-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 benzotriazolyloxy-tris-(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) and *N*,*N*,-diisopropylethylamine. Finally, the obtained ImPyβImPyβImβ-hydroxylamine was ligated with the 4-[^{18}F]-fluorobenzaldehyde with aniline as a catalyst to produce [^{18}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}F]PIPAM5 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 pJWP-16. 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, $3.5 \pm 2.1 \times 10^9$ M^{-1} for [^{19}F]PIPAM5.

Animal Studies

Rodents

[PubMed]

Harki et al. examined the biodistribution of [^{18}F]PIPAM5 *in vivo* by PET and computed tomography (CT) (5). C57 mice were injected intravenously with [^{18}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 [^{18}F]PIPAM5 was found in the liver. At 20 min after injection, 35% to 40% of injected [^{18}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 [^{18}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

1. Hsu C.F., Phillips J.W., Trauger J.W., Farkas M.E., Belitsky J.M., Heckel A., Olenyuk B.Z., Puckett J.W., Wang C.C., Dervan P.B. Completion of a Programmable DNA-Binding Small Molecule Library. Tetrahedron. 2007;**63**(27):6146–6151. PubMed PMID: 18596841.
2. Nickols N.G., Jacobs C.S., Farkas M.E., Dervan P.B. Improved nuclear localization of DNA-binding polyamides. Nucleic Acids Res. 2007;**35**(2):363–70. PubMed PMID: 17175539.
3. Belitsky J.M., Nguyen D.H., Wurtz N.R., Dervan P.B. Solid-phase synthesis of DNA binding polyamides on oxime resin. Bioorg Med Chem. 2002;**10**(8):2767–74. PubMed PMID: 12057666.
4. Dervan P.B., Edelson B.S. Recognition of the DNA minor groove by pyrrole-imidazole polyamides. Curr Opin Struct Biol. 2003;**13**(3):284–99. PubMed PMID: 12831879.
5. Harki D.A., Satyamurthy N., Stout D.B., Phelps M.E., Dervan P.B. In vivo imaging of pyrrole-imidazole polyamides with positron emission tomography. Proc Natl Acad Sci U S A. 2008;**105**(35):13039–44. PubMed PMID: 18753620.
6. Poethko T., Schottelius M., Thumshirn G., Hersel U., Herz M., Henriksen G., Kessler H., Schwaiger M., Wester H.J. Two-step methodology for high-yield routine radiohalogenation of peptides: (18)F-labeled RGD and octreotide analogs. J Nucl Med. 2004;**45**(5):892–902. PubMed PMID: 15136641.
7. Burnett R., Melander C., Puckett J.W., Son L.S., Wells R.D., Dervan P.B., Gottesfeld J.M. DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA.TTC repeats in Friedreich's ataxia. Proc Natl Acad Sci U S A. 2006;**103**(31):11497–502. PubMed PMID: 16857735.