3-Cyano-4-[^{18}F]fluoro-benzoyl-Arg-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH_2

[^{18}F]6b

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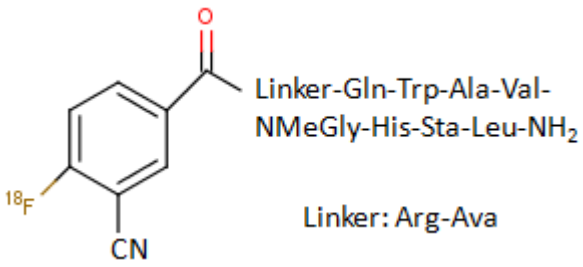
Chemical name:	3-Cyano-4-[^{18}F]fluoro-benzoyl-Arg-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH_2
Abbreviated name:	[^{18}F]6b
Synonym:	
Agent Category:	Peptides
Target:	Gastrin-releasing peptide receptor (GRPR)
Target Category:	Receptors
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



Structure of [^{18}F]6b

Background

[PubMed]

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3-Cyano-4-[^{18}F]fluoro-benzoyl-Arg-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH_2, abbreviated as [^{18}F]6b, is a bombesin (BN)-based ^{18}F-labeled peptide synthesized by Mu et al. for positron emission tomography (PET) of tumors expressing gastrin-releasing peptide receptor (GRPR) (1).

BN is an amphibian neuropeptide consisting of 14 amino acids (pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2) (2, 3). The C-terminal region of BN is responsible for its receptor binding and signal transduction. BN and its mammalian counterpart, gastrin-releasing peptide (GRP), produce a wide range of biological responses in diverse tissues (3). They also act as growth factors for cancer cells. Of the BN receptors, GRPR (also known as BB_2 or BRS2) is best characterized (2). GRPR is a glycosylated G-protein–coupled receptor and is normally expressed in non-neuroendocrine tissues of the breast and pancreas and in neuroendocrine cells of the brain, gastrointestinal tract, lung, and prostate (3, 4). Because GRPR is overexpressed in various tumors, a large number of BN analogs have been tested for GRPR-targeted imaging and therapy (5, 6). These analogs have been synthesized on the basis of either truncated BN (6–14 or 7–14) or full-length BN (1–14), and most analogs exhibit a high affinity to GRPR (7, 8). The truncated BN analogs appear more stable *in vivo* than the full-length tetradecapeptides, but the full-length peptides offer more labeling options by attachment of functional groups to the amino acids on positions 1 to 6 (6, 9). For most analogs, the amino acids on positions 13 (Leu) and 14 (Met) have been replaced with non-natural amino acids for increasing stability, and Lys has been placed on position 3 for attaching radiolabels (1, 10). Spacers, chelators, or radiometals have also been widely used for conjugation and for favorable kinetics (1, 11, 12). Functionally, most BN analogs act as agonists, and only a few to date are antagonists (9). Agonists are internalized into and accumulate within cells, and they have been assumed to exhibit higher uptake by cancer cells than antagonists. However, some studies have shown that tumor uptake of antagonists is higher than that of agonists because antagonists may have stronger binding for GRPR than agonists (9).

Mu et al. synthesized a series of BN-based peptides by using different linkers, peptide sequences, and non-natural amino acids (1). These peptides have been labeled with ^{18}F with a one-step approach *via* ^{18}F-for-$^{+}N(CH_3)_3$ substitution using a less lipophilic benzonitrile labeling moiety. Amino acids such as His, Trp, Arg, and non-natural amino acids such as statine (Sta) and cysteine sulfonic acid (Ala(SO_3H)) in the peptide sequence did not require any protection group during radiosynthesis. Two analogs, one named as [^{18}F]6b and another [^{18}F]7b, exhibited specific uptake in GRPR-expressing PC-3 tumors and the pancreas in nude mice (1). [^{18}F]6b is positively charged, while [^{18}F]7b is negatively charged. This chapter describes the data obtained with [^{18}F]6b. The data obtained with [^{18}F]7b are described in the MICAD chapter on [^{18}F]7b.

Related Resource Links:

- Protein, nucleotide (RefSeq), and gene information for GRPR
- Structure information of BN and analogs in PubChem

- GRPR-targeted imaging agents in MICAD

Synthesis

[PubMed]

Mu et al. synthesized [^{18}F]6b on the basis of the amino acid sequence 7–14 of the natural BN (1). Met^{14} was replaced with Leu for stabilization against aminopeptidases, and Leu^{13} was replaced with Sta for prevention of neutral endopeptidase cleavage. Gly^{11} was changed to its methylated version (NMeGly). A polar spacer, Arg-Ava, was inserted between the labeling moiety and the binding sequence to improve the peptide pharmacokinetics and to avoid the interference of radiolabels with the binding region. The ^{18}F labeling was carried out according to the method described by Becaud et al. (12). The reaction time was 15 min at 70°C. The ^{18}F-incorporation was 51%. The decay-corrected radiochemical yield of [^{18}F]6b was ~20%. The specific activity and radiochemical purity were not defined in this report (refer to Becaud et al. (12)). The lipophilicity, expressed as distribution coefficient *D* (log $D_{7.4}$), was −1.1. The fluorinated peptide had calculated and found molecular weights of 1,381.7 and 692.4, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The receptor binding affinity of [^{18}F]6b was determined with the use of cell membranes transfected with human GRPR (1). Nonspecific binding was determined with an excess of 10 μM Tyr^4-BN. The binding affinity of the non-labeled peptide (Arg-Ava-Gln-Trp-Ala-Val-NMeGly-His-Sta-Leu-NH_2) was 0.7 nM, and its half maximal inhibitory concentration was 2.7 nM.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{18}F]6b was studied in nude mice bearing subcutaneous PC-3 human prostate tumors (n = 3 mice/group) (1). The tumor uptake was 2.36 ± 0.47% injected dose per gram of tissue (ID/g) at 30 min after injection, which decreased slightly to 1.8 ± 1.56, 1.28 ± 0.27, and 1.61 ± 0.23% ID/g at 1, 2, and 4 h after injection, respectively. Similarly, uptake in the GRPR-rich pancreas reached 2.57 ± 0.03% ID/g at 30 min after injection and then decreased slightly over time. The binding of [^{18}F]6b toward GRPR could be blocked by ~40% in the tumor and by ~60% in the pancreas at 60 min after injection, indicating that a large part of the tracer uptake in the tumor and pancreas can be attributed to specific GRPR binding. After the tumor and pancreas, the highest accumulation of [^{18}F]6b was found in excretory organs, specifically the gallbladder, intestine, kidneys, and liver. Activity uptake in the gallbladder and intestine was much

higher than uptake in the kidneys, suggesting a preponderance of hepatobiliary elimination *versus* renal excretion. The % ID/g values in bone were 4.36 ± 0.34, 4.64 ± 0.42, 7.80 ± 0.89, and 7.98 ± 1.58 at 0.5, 1, 2, and 4 h after injection, respectively. The increasing uptake in bone over time indicates that ^{18}F-fluoride represents a major radiometabolite.

The comparative *in vivo* analysis of [^{18}F]6b and [^{18}F]7b showed that [^{18}F]7b possesses superior characteristics with respect to the total tracer accumulation in tumors and the GRPR-rich pancreas and the specificity of uptake in target tissues (1). At physiological pH, Arg and Ala(SO_3H) become positively and negatively charged, respectively. Although [^{18}F]6b and [^{18}F]7b bear the same binding peptide sequence and have the same lipophilicity, the charge of the linker obviously plays a major role in *in vivo* pharmacokinetics.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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