

# <sup>111</sup>In-Tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid-HHHHHH-EAYGWMDf-NH<sub>2</sub> peptide

<sup>111</sup>In-DOTA-H6-Met

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<b>Chemical name:</b>	<sup>111</sup> In-Tetraazacyclododecane- <i>N,N',N'',N'''</i> -tetraacetic acid-HHHHHH-EAYGWMDf-NH <sub>2</sub> peptide	
<b>Abbreviated name:</b>	<sup>111</sup> In-DOTA-H6-Met	
<b>Synonym:</b>	Radiolabeled minigastrin	
<b>Agent Category:</b>	Peptide	
<b>Target:</b>	Gastrin/cholecystokinin-2 (CCK-2, CCK-B) receptor	
<b>Target Category:</b>	Receptor binding	
<b>Method of detection:</b>	Single-photon emission computed tomography (SPECT), planar gamma imaging	
<b>Source of signal/contrast:</b>	<sup>111</sup> In	
<b>Activation:</b>	No	
<b>Studies:</b>	<ul style="list-style-type: none"><li><i>In vitro</i></li><li>Rodents</li></ul>	Click on <a href="#">protein</a> , <a href="#">nucleotide</a> (RefSeq), and <a href="#">gene</a> for more information about the CCK-2 receptor.

## Background

[PubMed]

<sup>111</sup>In-Tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid-HHHHHH-EAYGWMDf-NH<sub>2</sub> peptide (<sup>111</sup>In-DOTA-H6-Met) is a radiolabeled gastrin analog that can be used for

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single-photon emission computed tomography (SPECT) imaging of tumors that express the gastrin/cholecystokinin-2 (CCK-2) receptor (1).  $^{111}\text{In}$  is a gamma emitter with a physical half-life ( $t_{1/2}$ ) of 2.8 days.

The gastrointestinal peptides gastrin and CCK have various regulatory functions in the brain and gastrointestinal tract (2). Gastrin and CCK have the same COOH-terminal pentapeptide amide sequence, which is the biologically active site (3). Human gastrin is a peptide composed of 34 amino acids and also exists in several C-terminal truncated forms (1). These C-terminal truncated forms include minigastrin, which is a 13-residue peptide with the sequence of LEEEEAYGWMDF-NH<sub>2</sub>. CCKs exist in a variety of biologically active molecular forms that are derived from a precursor molecule comprising 115 amino acids (4). These forms range from 4 to 58 amino acids in length and include sulphated and unsulphated CCK-8, which has the structure DYMGMDF-NH<sub>2</sub>. They bind to and act through transmembrane G-protein-coupled receptors (5). Two different CCK receptor subtypes have been identified in normal tissues. CCK-1 (CCK-A, alimentary) receptors have low affinity for gastrin, and CCK-2 (CCK-B, brain) receptors have high affinity for gastrin (4). They also differ in terms of molecular structure, distribution, and affinity for CCK. These receptors have also been found to be expressed or overexpressed on a multitude of tumor types (5). CCK-2 receptors have been found most frequently in medullary thyroid carcinoma, small-cell lung cancers, astrocytomas, and stromal ovarian cancers (2). CCK-1 receptors have been identified in gastroenteropancreatic tumors, meningiomas, and neuroblastoma.

Reubi et al. (6) designed a series of radiolabeled CCK-8 peptides that showed high specificity for potential *in vivo* imaging of tumors expressing CCK-2 receptors. de Jong et al. (7) developed an  $^{111}\text{In}$ -labeled non-sulfated CCK8 analog that used 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) as a bifunctional chelating agent. The radioligand showed high specific internalization rates in receptor-positive AR42J rat pancreatic tumor cells. von Guggenberg et al. (8) reported the synthesis of  $^{99\text{m}}\text{Tc}$ -hydrazinonicotinic acid (HYNIC)-minigastrin complexes and showed high tumor uptake in nude mice bearing AR42J tumors. Nock et al. (9) prepared  $^{99\text{m}}\text{Tc}$ -labeled minigastrin analogs and found that they displayed high specific localization in nude mice bearing AR42J tumors. Mather et al. (1) synthesized a library of different peptide sequences based on the C-terminal sequences of CCK-8 or minigastrin. These peptides were labeled with  $^{111}\text{In}$  by DOTA or diethylenetriamine pentaacetic acid (DTPA) conjugation. Although their ultimate goal was to identify an analog that could be used for radiotherapeutic applications,  $^{111}\text{In}$ -DOTA-H6-Met with a hexahistidine tag was evaluated as a potential molecular probe for CCK-2 receptors.

## Synthesis

[PubMed]

The peptide sequence HHHHHH-EAYGWMDF-amide was obtained by solid-phase peptide synthesis under standard conditions from commercial sources (1). The N-

terminus was capped with a DOTA chelating group to produce DOTA-H6-Met. The identity and purity were confirmed by matrix-assisted laser desorption/ionization mass spectrometry and reverse-phase high-performance liquid chromatography (HPLC). Radiolabeling was performed by mixing  $^{111}\text{In}$ -chloride in ammonium acetate and 0.04-M monothioglycerol (MTG), an antioxidant, with DOTA-H6-Met in 0.01-M phosphate-buffered saline (pH 7.2). The mixture was heated at 98°C for 15 min, and 0.1-M ethylenediamine tetraacetic acid (EDTA) was then added to quench the reaction. The labeling yield was >90%. The radiochemical purity and specific activity were not reported.

## In Vitro Studies: Testing in Cells and Tissues

[PubMed]

An *in vitro* receptor affinity assay of  $^{111}\text{In}$ -DOTA-H6-Met was performed by using AGS human gastric tumor cells transfected with the CCK-2 receptor (AGS-CCK2R) and  $^{125}\text{I}$ -G17 as the radioligand. The inhibition constant ( $K_i$ ) and half of the maximum binding fraction ( $\text{EC}_{50}$ ) were 2.1 nM and 3.1 nM, respectively.

## Animal Studies

### Rodents

[PubMed]

Biodistribution studies of  $^{111}\text{In}$ -DOTA-H6-Met were performed in nude mice bearing AR42J rat pancreatic tumors (1), HT29 human colorectal tumors, or CA20948 rat pancreatic tumors. Both AR42J and CA20948 tumors expressed different levels of gastrin receptors, and HT29 was a cell line transfected with a relatively low level of gastrin receptors. Each mouse received 0.2  $\mu\text{g}$  of  $^{111}\text{In}$ -DOTA-H6-Met by i.v. injection. The tumor radioactivity levels at 4 h ( $n = 3-4$ ), represented as percent injected dose per g (% ID/g), were  $0.59 \pm 0.14$ ,  $0.55 \pm 0.14$ , and  $0.26 \pm 0.04$  for AR42J, CA20948, and HT29 tumors, respectively. The blood radioactivity levels at 4 h were  $0.20 \pm 0.03$ ,  $0.21 \pm 0.01$ , and  $0.26 \pm 0.03$  for AR42J, CA20948, and HT29 tumors, respectively. The kidney radioactivity levels at 4 h were  $2.95 \pm 0.60$ ,  $3.08 \pm 0.77$ , and  $3.47 \pm 0.52$  for AR42J, CA20948, and HT29 tumors, respectively. No blocking study was performed.

### 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.

## References

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