# $^{177}$Lu-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys))

$^{177}$Lu-AM3

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| **Chemical name:** | $^{177}$Lu-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)) | |
| **Abbreviated name:** | $^{177}$Lu-AM3 | |
| **Synonym:** | | |
| **Agent Category:** | Peptides | |
| **Target:** | Somatostatin receptors (SSTRs) | |
| **Target Category:** | Receptors | |
| **Method of detection:** | Single-photon emission computed tomography (SPECT) and planar imaging | |
| **Source of signal / contrast:** | $^{177}$Lu | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Rodents | For structures of octreotide analogues, click on PubChem. |

## Background

[PubMed]

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The $^{177}$Lu-labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-Tyr-cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)), abbreviated as $^{177}$Lu-AM3, is a somatostatin (SST)-based, bicyclic peptide conjugate developed by Fani et al. for SST receptor (SSTR)–targeted imaging of neuroendocrine tumors (1).

The human SSTR family is a group of G-protein–coupled receptors with five members (SSTR1–SSTR5). All receptor members have seven α-helical transmembrane domains and possess a highly conserved sequence motif (YANSCANPI/VLY) in the seventh topology, which serves as a signature sequence for this family (2-4). Overall, there is 39%–57% sequence identity among the members, with the highest homology between SSTR1 and SSTR4, and among SSTR2, SSTR3, and SSTR5, respectively. The two groups of receptors also differ in their interactions with SST and its analogs (3-6). SSTR2, SSTR3, and SSTR5 have a high affinity for octreotide and seglitide, whereas SSTR1 and SSTR4 exhibit a very low affinity for them. With the exception of SSTR2, the precise contributions of other members remain to be elucidated. This is largely due to the lack of highly selective ligands and the co-expression of different members in single cells. SSTRs are distributed widely in cells both in the nervous system and periphery, and they have been shown to be overexpressed in a large number of malignancies, with particularly high density in neuroendocrine tumors (2, 7, 8).

As the targets of SST radiopharmaceuticals, SSTRs are of considerable clinical relevance for tumor imaging and radionuclide therapy (2, 9, 10). Because the native SST has a very short biological half-life (<2 min), various analogs have been synthesized, including a group of bicyclic peptides. Bicyclic peptides were first synthesized to understand the bioactive conformation and pharmacophoric amino acid sequence, and to increase the metabolic stability of the natural peptide SST-14 (also known as somatotropin release-inhibiting factor (SRIF)-14) by increasing the rigidity of bicyclic peptides (11, 12). Thereafter, various modified bicyclic peptides have been synthesized and have exhibited higher potency and longer duration of biological activity than SRIF-14 (1, 11, 12). As seen in the structure of the lead peptide cyclo(Aha,cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)), conformational constraints are introduced by head-to-tail coupling of a 16-atom ring with 7-aminoheptanoic acid (Aha) to the N- and C-terminally amino acid–deleted octreotide. To identify metabolically stable pansomatostatin analogs, a group of investigators from Switzerland have generated a set of bicyclic peptides by forming a second 16-atom ring with Arg and γ-aminobutyric acid (GABA) while keeping the octreotide 20-atom sequence as an inner circle (13-15). These modifications led to development of another lead peptide, cyclo(Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys)-GABA), which is named HR3005 (1). Arg has been shown to be important for broad binding to SSTR subtypes, and GABA can be exchanged with diaminobutyric acid (DAB) for chelator coupling. Based on the HR3005 sequence, four bicyclic analogs have been further synthesized by Fani et al. from the same group (1). The influence of the conformational constraints on receptor binding profiles and the pharmacokinetics of the respective radiopeptides have been investigated. Studies by Fani et al. have shown that the high rigidity of these bicyclic radiopeptides leads to agonistic ligands with good affinity for all five SSTRs (1). The pharmacokinetic data of the DOTA-conjugated bicyclic peptide AM3 (DOTA-Tyr-

cyclo(DAB-Arg-cyclo(Cys-Phe-D-Trp-Lys-Thr-Cys))), make it an excellent candidate as an imaging radiotracer (1).

### Related Resource Links:

- MICAD chapters on SSTR imaging
- Gene information on SSTRs
- Articles on SSTRs in Online Mendelian Inheritance in Man (OMIM)
- Somatostatin analogs in PubChem
- Bioassays of SSTRs in PubChem
- SSTR-related clinical trials in ClinicalTrial.gov

## Synthesis

[PubMed]

Fani et al. synthesized AM3 on the basis of the lead peptide HR3005 (1). The GABA in HR3005 was replaced with DAB, followed by coupling of the peptide with DOTA through Tyr as a spacer. The $^{177}$Lu-labeled AM3 ($^{177}$Lu-AM3) was prepared by incubation of AM3 with $^{177}LuCl_3$ at 95°C for 30 min in ammonium acetate buffer (pH 5.0). The radiotracer solution was prepared by dilution with 0.9% NaCl containing 0.1% bovine serum albumin. The overall yield was ~30%. The $^{177}$Lu-labeling yield was >97% with a specific activity of 17 GBq/μmol (0.46 Ci/μmol). The radiochemical purity of $^{177}$Lu-AM3 was >95%.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Fani et al. characterized AM3 and $^{177}$Lu-AM3 for their binding affinity, cell internalization, stimulating effect on intracellular $Ca^{2+}$ release, and stability (1).

The binding profile of AM3 was determined with receptor autoradiography. The cell membrane pellets were prepared from human embryonic kidney (HEK) cells expressing different subtypes of SSTRs (16). The binding affinity was expressed as 50% inhibitory concentration ($IC_{50}$; $n > 3$). The lead peptide HR3005 showed high affinity (comparable to SRIF-28) for SSTR2 (2.4 ± 0.6 nM) and SSTR3 (3.4 ± 0.8 nM), and moderate affinity for SSTR5 (14.5 ± 4.5 nM), SSTR1 (34 ± 10 nM), and SSTR4 (68 ± 22 nM), conferring almost pansomatostatin potency to this peptide. Replacement of GABA with DAB in the HR3005 did not change the affinity profile, whereas DOTA-coupling led to marked loss of affinity for all SSTR subtypes. By introducing Tyr as a spacer between DOTA and the peptide, the affinity of AM3 recovered to levels comparable to those of HR3005. The $IC_{50}$ values of AM3 were 119 ± 6 nM for SSTR1, 2.3 ± 0.2 nM for SSTR2, 4.0 ± 0.03 nM for SSTR3, 97 ± 21 nM for SSTR4, and 27 ± 1 nM for SSTR5. The binding profile of $^{177}$Lu-AM3 was not examined.

The immunofluorescence microscopy–based internalization assay for SSTR2 and SSTR3 with HEK-SSTR2 and HEK-SSTR3 cells showed that unlabeled AM3 elicited a pronounced relocation of SSTR2 and SSTR3 from the plasma membrane to the cytoplasm at 30 min after it was applied to the cells, an effect similar to that of the control, SRIF-28.

The stimulating effect of AM3 on the intracellular $Ca^{2+}$ release was tested with HEK cells expressing SSTR2, SSTR3, and SSTR5. Intracellular $Ca^{2+}$ release is a part of the signal pathway regulated by native SST. On SSTR3 and SSTR5, no signal was found. On SSTR2, a large, concentration-dependent increase of the signal was observed. AM3 has been considered to be an agonist because of its stimulating effect on $Ca^{2+}$ release and high potencies similar to those of SRIF-14. The agonist-elicited response in terms of the half maximal effective concentration value was 21.5 ± 3.5 nM.

The internalization rate and efflux of $^{177}$Lu-AM3 were studied with HEK-SSTR2 and HEK-SSTR3 cells. Internalization was expressed as percentage of the total applied radioactivity. In HEK-SSTR2 cells, 10.5 ± 1.2% of the $^{177}$Lu-AM3 was internalized at 4 h, and in HEK-SSTR3 cells, 15.3 ± 2.3% of the $^{177}$Lu-AM3 was internalized at 4 h. $^{177}$Lu-AM3 internalization reached a plateau in both HEK-SSTR2 and HEK-SSTR3 cells after 4 h. $^{177}$Lu-AM3 had a lower internalization rate than the reference molecule $^{177}$Lu-DOTATOC (23.8 ± 1.6% in HEK-SSTR2 cells). Nonspecific internalization was <1% (data not shown), indicating receptor-mediated internalization. The efflux of $^{177}$Lu-AM3 was found to be faster in HEK-SSTR2 cells than in HEK-SSTR3 cells. Within 4 h, 45%–50% of the $^{177}$Lu-AM3 was released from HEK-SSTR2 cells, whereas only ~35% was released from HEK-SSTR3 cells.

$^{177}$Lu-AM3 was extremely stable in human blood and serum at 37°C. No metabolites were observed in the plasma and serum after 24 h and 7 days, respectively.

# Animal Studies

## Rodents

[PubMed]

Five-week-old athymic female Swiss nude mice with HEK-SSTR2 tumors in the right front leg and HEK-SSTR3 tumors in the other leg ($n$ = 4–7 mice/group) were used for biodistribution studies after injection of $^{177}$Lu-AM3 (100 µl/10 pmol per 0.15 MBq (0.0041 mCi)) into the tail vein. Nonspecific uptake was determined with co-injection of 2,000-fold excess DOTANOC (17, 18). One group of mice was pre-injected with lysine 10 min before the injection of $^{177}$Lu-AM3 to study kidney blocking. The results were expressed as percentage of injected dose per gram of tissue (% ID/g).

$^{177}$Lu-AM3 demonstrated fast blood clearance (only 0.01% ID/g was left at 4 h after injection), fast nontarget clearance, and high receptor-mediated uptake in the tumors. The kidneys were the only other tissue to accumulate radioactivity. At 1 h after injection, both tumors accumulated the greatest amount of $^{177}$Lu-AM3, with the SSTR3 tumors (12.74

± 0.75% ID/g) exhibiting slightly higher uptake than the SSTR2 tumors (10.37 ± 0.76% ID/g). Four hours after injection, ~60% of the initially accumulated radioactivity remained in both tumors, with 7.56 ± 2.16% ID/g in the SSTR3 tumors and 6.05 ± 1.13% ID/g in the SSTR2 tumors. At 24 h after injection, <30% was tumor-bound. Blocking experiments with DOTANOC (high affinities for SSTR2, SSTR3, and SSTR5) confirmed the specific and receptor-mediated uptake, showing only 0.37 ± 0.07% ID/g in the SSTR3 tumors and 1.82 ± 0.66% ID/g in the SSTR2 tumors at 4 h after injection. The radioactivity in the kidneys was 7.81 ± 0.24% ID/g and 5.09 ± 1.10% ID/g at 1 h and 4 h, respectively. The kidney uptake was not affected by the blocking agent DOTANOC, indicating that this uptake is not receptor-mediated. The tumor/kidney ratio was >1 at 1 h after injection, but the slower washout from the kidneys resulted in decreasing tumor/kidney ratios with time. The SSTR2/kidney and SSTR3/kidney ratios were 1.3 and 1.6 at 1 h, respectively, and 0.8 and 1.2 at 24 h after injection, respectively. Pre-injection of lysine (20 mg/100 µl) resulted in ~50% reduction of the kidney uptake with no significant impact on the tumor uptake.

### 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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