General on the synthetic procedures

All chemicals were obtained from commercial sources and used without further purification. For the solid phase peptide synthesis the Tentagel S RAM-Cys(Trt)-Fmoc was purchased from RAPP Polymere (Tuebingen, Germany). HPLC was performed on a Waters HPLC system using a 1525EF pump and a 2489 UV detector. For preparative HPLC a Dr. Maisch GmbH Reprosil-Pur 120 C18-AQ 10 µm (250 × 20 mm) column was used (12 mL/min). For analytical HPLC a Dr. Maisch GmbH Reprosil-Pur C18-AQ 5 µm (250 × 4.6 mm) column was used and a gradient of 0.1% TFA in H_2O/CH_3CN 95:5 to 0.1% TFA in H_2O/CH_3CN 5:95 in 20 minutes (1 mL/min) was employed. Mass spectrometry was performed on a Bruker microflex MALDI-TOF.

Synthesis

*cyclo[D-Phe-Cys-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys-Thr(tBu)]-resin (**1**).* Resin-bound D-Phe-Cys(Acm)-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Acm)-Thr(tBu) was synthesized by SPPS using Fmoc-L-Thr(tBu)-PEG-PS resin (loading 0.16 mmol/g). Standard Fmoc/tBu strategy was used with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as coupling reagent. The linear side chain-protected peptide was cyclized on resin using $Tl(TFA)_3$ (*1*): the resin-bound peptide (0.1 mmol) was swollen in 3 mL CH_2Cl_2. $Tl(TFA)_3$ (109 mg, 0.2 mmol) in 3 mL DMF was added to the resin and the mixture was stirred for 1 hour at room temperature. The resin was washed with DMF (3×) and CH_2Cl_2 (3×) and dried under vacuum yielding peptidyl resin **1**. The cyclization was confirmed with analytical HPLC by cleavage and deprotection of a small amount of peptide.

DTPA-Tyr^3-octreotate. Peptidyl resin **1** (25 µmol) was swollen in CH_2Cl_2. DTPA-tetra (t-Bu ester) (46 mg, 75 µmol), PyBOP (39 mg, 75 µmol) and N,N-Diisopropylethylamine (DiPEA) (26 µL, 150 µmol) in DMF (3 mL) was added and the mixture was stirred overnight at room temperature. The resin was washed with DMF (3×) and CH_2Cl_2 (3×) and dried under vacuum. The peptide was cleaved from the resin and the side chains were de-protected with a solution of TFA/H_2O/TIS 90:5:1.5 (5 mL) for 3 h. The resin was removed from the solution by filtration. The peptide was precipitated with MTBE/hexane 1:1 v/v at -20 °C and lyophilized from CH_3CN/H_2O 1:1 v/v yielding 21.7 mg of crude peptide. The peptide was purified by preparative HPLC using a gradient of 0.1% TFA in H_2O/CH_3CN 9:1 to 0.1% TFA in H_2O/CH_3CN 1:9 in 60 minutes. After pooling of the appropriate fractions and lyophilisation, 4.4 mg of pure DTPA-Tyr^3-octreotate peptide was obtained. MS: $[M+H]^+$ calculated 1425.6, found 1425.6; $[M+Na]^+$ calculated 1447.5, found 1447.9; $[M+K]^+$ calculated 1463.7, found 1463.5.

Cy5-Tyr^3-octreotate. Peptidyl resin **1** (25 µmol) was swollen in CH_2Cl_2. Cy5 (38 mg, 50 µmol), PyBOP (26 mg, 50 µmol) and DiPEA (17 µL, 100 µmol) in DMF (3 mL) was added and the mixture was stirred overnight at room temperature. The resin was washed with DMF (3×) and CH_2Cl_2 (3×) and dried under vacuum. The peptide was cleaved from the resin and the side chains were deprotected with a solution of TFA/H_2O/TIS 90:5:1.5 (5 mL) for 3 h. The resin was removed from the solution by filtration. The peptide was precipitated with MTBE/hexane 1:1 v/v at -20 °C and lyophilized from CH_3CN/H_2O 1:1 v/v yielding 25.7 mg of crude peptide. The peptide was purified by preparative HPLC using a gradient of 0.1% TFA in H_2O/CH_3CN 9:1 to 0.1% TFA in H_2O/CH_3CN 1:9 in 60 minutes. After pooling of the appropriate fractions and lyophilisation, 6.8 mg of pure Cy5-Tyr^3-octreotate peptide was obtained. MS: $[M+H]^+$ calculated 1797.1, found 1798.4.

Cy5-DTPA-Tyr^3-octreotate. Peptidyl resin **1** (25 µmol) was swollen in CH_2Cl_2. Cy5-DTPA hybrid label (30 mg, 18 µmol) and DiPEA (44 µL, 255 µmol) in DMF (2 mL) was added and the mixture was stirred overnight at room temperature. The resin was washed with DMF (3×) and CH_2Cl_2 (3×) and dried under vacuum. The peptide was cleaved from the resin and the side chains were de-protected with a solution of TFA/H_2O/TIS 90:5:1.5 (5 mL) for 3 h. The resin was removed from the solution by filtration. The peptide was precipitated with MTBE/hexane 1:1 v/v at -20 °C and lyophilized from CH_3CN/H_2O 1:1 v/v yielding 25.5 mg of crude peptide. The peptide was purified by preparative HPLC using a gradient of 0.1% TFA in H_2O/CH_3CN 9:1 to 0.1% TFA in H_2O/CH_3CN 1:9 in 120 minutes. After pooling of the appropriate fractions and lyophilisation 1 mg of pure Cy5-DTPA-Tyr3-octreoatate peptide was obtained. MS: $[M+H]^+$ calculated 2586.0, found 2586.1.

Labeling with ^{111}In

For the labeling with ^{111}In peptides were incubated for 20 minutes following the following the procedure previously described (*2,3*). After that, the peptide solutions were diluted with 0.1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) to obtain the appropriate concentrations for the different evaluations.

In vitro experiments

Nuclear based imaging. Nuclear SPECT/CT images for in vivo analyses were acquired using were acquired with a four-headed helical NanoSPECT system (NanoSPECT/CT, Bioscan, Inc, Washington D.C., USA). A multi-pinhole mouse collimator with 9 pinholes (1.4 mm diameter) per head, with a matrix of 256 x 256 was used. The machinery was equipped with a heated bed system to prevent hypothermia

in the animals. All animals received a SPECT scan of 24 projections (90 sec per projection) and a CT scan (240 projections, 500 ms exposure time at 55 kV) for anatomical reference. For those mice that received both SPECT/CT and FMT scan the acquisition time of nuclear imaging was reduced to 20 minutes (20 projections, 60 second per projection).

Optical and multimodal imaging. Optical imaging for in vivo analyses was performed using fluorescence tomography device (FMT, 2500XL, PerkinElmer Inc., Boston, MA) and IVIS (Caliper Life Science, Hopkinton, MA, USA). For the tomographic analyses with FMT the mice were placed in a supine position in the imaging cassette, which was inserted into the docking system of the FMT imaging chamber that was saturated with a mixture of isoflurane and oxygen (1.5% and 0.8% respectively). The light source consisted of a laser diode (80mW). The region of interest was drawn around the tumor, to highlight the uptake. The signals were collected at the opposite side of the animal body by a low noise TE-cooled back illuminated high quantum efficiency CCD camera with air assist. Using the preconfigured channel 1 (λ_{ex}=635 nm, λ_{em}=650-670 nm) both fluorescent and excitation data sets were collected and multiple source-detector fluorescence projections were normalized to laser excitation data.

In our protocol, for acquisition of optical images with the IVIS system, the mice were euthanized and placed supine in the chamber. IVIS system is equipped with a tungsten halogen lamp and filters for selection of excitation wavelength. In our evaluation optical images were acquired using an excitation length of 640 nm. The animals were top illuminated by the excitation light and the emission light subsequently filtered at different wavelength from 680 nm to 780 nm (band pass = 20 nm). Acquisition time was approximately 60 second.

Data analysis and statistical methods. The competition studies were fitted using the normalized geometric means were fitted with equations in the GraphPad Prism 5 software. The K_D and IC_{50} values

of Cy5-Tyr^3-octreotate, Cy5-DTPA-Tyr^3-octreotate and Cy5-In-DTPA-Tyr^3-octreotate were calculated using the 'Binding – Saturation, One site – Total' nonlinear regression equation (Eq. 1).

$$y = \frac{B_{\max} * x}{K_D + x} + NS * x + Background \quad \text{(Eq. 1)}$$

Where, y is the normalized fluorescence; B_{max} is the maximum specific binding in the units of the y axis; x is the concentration of Cy5-Tyr^3-octreotate, Cy5-DTPA-Tyr^3-octreotate or Cy5-In-DTPA-Tyr^3-octreotate in nM; K_D = equilibrium dissociation constant of Cy5-Tyr^3-octreotate, Cy5-DTPA-Tyr^3-octreotate or Cy5-In-DTPA-Tyr^3-octreotate in nM; NS is the slope of nonspecific binding; Background is the amount of normalized fluorescence with no added compound.

The K_D values of Tyr^3-octreotate, DTPA-Tyr^3-octreotate and In-DTPA-Tyr^3-octreotate were calculated using the 'Binding – Competitive, One site – Fit K_i' nonlinear regression equation (Eq. 2 and 3).

$$\log IC_{50} = \log(10^{\log K_D} * (1 + \frac{[a]}{K_{D,a}})) \quad \text{(Eq. 2)}$$

$$y = Bottom + \frac{Top - Bottom}{1 + 10^{(x - \log IC_{50})}} \quad \text{(Eq. 3)}$$

Where IC_{50} is the concentration of competitor that results in binding half-way between Bottom and Top; K_D is the equilibrium dissociation constant of the competitor in nM; [**a**] is the concentration of Cy5-DTPA-Tyr^3-octreotate (100 nM); $K_{D,a}$ is the dissociation constant of Cy5-DTPA-Tyr^3-octreotate (387.7 nM); y is the normalized fluorescence; Bottom and Top are the plateaus in the units of the y axis.

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