General Information

The Fmoc-(9-fluorenylmethoxycarbonyl-) and all other protected amino acid analogues were purchased from *Bachem Inc.* (Bubendorf, Switzerland), *Merck KGaA* (Darmstadt, Germany) or *Iris Biotech GmbH* (Marktredwitz, Germany). The *H*-Rink amide ChemMatrix® resin (35-100 mesh particle size, 0.4-0.6 mmol/g loading) was purchased from *Merck KGaA* (Darmstadt, Germany). *CheMatech* (Dijon, France) delivered the chelators DOTA(^{t}Bu)$_3$ as well as DOTAGA(^{t}Bu)$_4$. Peptide syringes were obtained from *VWR International GmbH* (Bruchsal, Germany).

All necessary solvents and other organic reagents were purchased from either, *Alfa Aesar*™ (Karlsruhe, Germany), *Merck KGaA* (Darmstadt, Germany) or *VWR International GmbH* (Bruchsal, Germany). Solid-phase synthesis of the peptides was carried out by manual operation using a Scilogex MX-RL-E Analog Rotisserie Tube Rotator (*Scilogex®*, Rocky Hill, CT, USA). H_2O was used after purification by a Barnstead MicroPure system (*Thermo Fisher Scientific Inc.*, Waltham, MA, USA).

Analytical and preparative reversed-phase high performance liquid chromatography (RP-HPLC) were performed using Shimadzu gradient systems (*Shimadzu Deutschland GmbH*, Neufahrn, Germany), each equipped with a SPD-20A UV/Vis detector (220 nm, 254 nm). Different gradients of MeCN (0.1% TFA) in H_2O (0.1% TFA) were used as eluents for all RP-HPLC operations.

For analytical measurements, a Nucleosil 100 C18 (125 × 4.6 mm, 5 µm particle size) column (*CS Chromatographie Service GmbH*, Langerwehe, Germany) was used at a flow rate of 1 mL/min. Both, specific gradients and the corresponding retention times t_R as well as the capacity factor K' are cited in the text.

Preparative RP-HPLC purification was done with a Multospher 100 RP 18 (250 × 10 mm, 5 µm particle size) column (*CS Chromatographie GmbH*, Langerwehe, Germany) at a constant flow rate of 5 mL/min.

Analytical and preparative radio RP-HPLC was performed using a MultoKrom 100-5 C18 (5 µm, 125 × 4.6 mm) column (*CS Chromatographie GmbH*, Langerwehe, Germany).

Electrospray ionization-mass spectra for characterization of the substances were acquired on an expression^{L} CMS mass spectrometer (*Advion Ltd.*, Harlow, UK).

For radiolabeling, $^{177}LuCl_3$ (Molar Activity (A_M) >3000 GBq/mg, 740 MBq/mL, 0.04 M HCl, *ITG GmbH*, Garching, Germany) was used. Radioactivity was detected through connection of the outlet of the UV-photometer to an AceMate 925-Scint NaI(Tl) well-type scintillation counter from *EG&G Ortec®* (Oak Ridge, TN, USA). Radioactive samples were measured by a WIZARD$^{2®}$ 2480 Automatic γ-Counter (*Perkin Elmer Inc.*, Waltham, MA, USA) and determination of IC_{50} values was carried out using GraphPad Prism 6 (*GraphPad Software Inc.*, San Diego, CA, USA). For radio TLC, a Scan-RAM™ Scanner with Laura™ software (*LabLogic Systems Ltd.*, Broomhill, Sheffield, United Kingdom) was used.

Lyophilization was accomplished using an Alpha 1-2 LDplus lyophilizer (*Martin Christ Gefriertrocknungsanlagen GmbH*, Osterode am Harz, Deutschland) combined with a RZ-2 vacuum pump (*Vacuubrand GmbH & Co KG*, Olching, Germany).

For *in vitro* and *in vivo* studies, the used nutrition mixture Dulbecco’s modified eagle's medium/Ham’s F-12 (DMEM/F-12, *v*/*v* = 1/1, with stable glutamine), fetal bovine serum (FBS Superior), phosphate buffered saline (PBS Dulbecco, without Ca^{2+}/Mg^{2+}), trypsin/EDTA (0.05%/0.02% in PBS without Ca^{2+}/Mg^{2+}) solution as well as Hank’s balanced salt solution (HBSS, with 0.35 g/L $NaHCO_3$ and Ca^{2+}/Mg^{2+}) were obtained from *Biochrom GmbH* (Berlin, Germany). Solution of purified products was applied using Tracepur® H_2O (*Merck KGaA*, Darmstadt, Germany). Bovine serum albumin (BSA) was purchased from *Merck KGaA* (Darmstadt, Germany).

Cells were cultured in CELLSTAR® cell culture flasks and seeded in 24-well plates (*Greiner Bio-One GmbH*, Kremsmünster, Austria) after being counted with a Neubauer hemocytometer (*Paul Marienfeld*, Lauda-Königshofen, Germany) using Trypan Blue (0.4% in 0.81% NaCl and 0.06% potassium phosphate) solution (*Sigma-Aldrich GmbH*, Munich, Germany). Cells were handled inside a MSC Advantage laminar flow cabinet and maintained

in a Heracell 150i incubator (*Thermo Fisher Scientific Inc.*, Waltham, MA, USA) at 37 °C in a humidified 5% CO_2 atmosphere.

General Procedures (GP) and Execution Protocols

On-resin Peptide Formation (GP1). The respective side-chain protected Fmoc-AA-OH (1.5 eq.) is dissolved in NMP and pre-activated by adding TBTU (1.5 eq.), HOAt (1.5 eq.) and DIPEA (4.5 eq.). After activation for 10 min, the solution is added to resin-bound free amine peptide and shaken for 1.5 h at rt. Subsequently, the resin is washed with NMP (6 × 20 mL/g resin) and after Fmoc deprotection (GP2), the next amino acid is coupled analogously.

On-resin Fmoc Deprotection (GP2). The resin-bound Fmoc-peptide is treated with 20% piperidine in NMP (*v*/*v*) for 5 min and subsequently for 15 min. Afterwards, the resin is washed with NMP (6 × 20 mL/g resin).

Conjugation of Chelator (GP3). The protected chelator DOTA(^{t}Bu)$_3$ or DOTAGA(^{t}Bu)$_4$ (1.5 eq.) is dissolved in NMP and pre-activated by adding TBTU (1.5 eq.), HOAt (1.5 eq.) and DIPEA (4.5 eq.). After activation for 10 min, the solution is added to resin-bound *N*-terminal deprotected peptide (1.0 eq.) and shaken for 3 h at rt. Subsequently, the resin is washed with NMP (3 × 20 mL/g resin) and DCM (3 × 20 mL/g resin).

Peptide Cleavage from the Resin with additional Deprotection of acid labile Protecting Groups (GP4). The fully protected resin-bound peptide is washed with DCM, afterwards dissolved in a mixture of TFA/TIPS/DCM (*v*/*v*/*v*; 95/2.5/2.5) and shaken for 45 min. The solution is filtered off and the resin is treated in the same way for another 45 min. Both filtrates are combined and concentrated under a stream of nitrogen. After dissolving the residue in MeOH and precipitation in diethyl ether, the liquid is decanted and the remaining solid is dried. As the deprotection of the ^{t}Bu groups is usually not complete using this procedure (see Supplemental Fig. 11 for AMTG as an example), a further deprotection method is used (GP5).

Complete Deprotection of ^{t}Bu (GP5). Removal of remaining ^{t}Bu protecting groups after peptide cleavage from the resin (GP4) is carried out by dissolving the crude DOTA or DOTAGA

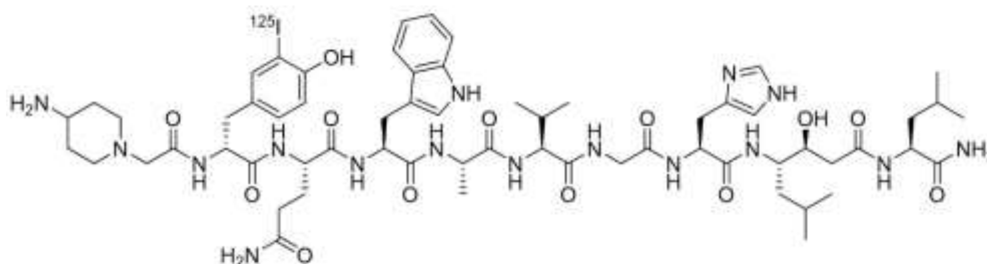
coupled product in TFA and stirring for 6 h and 16 h at rt, respectively. After removing TFA under a stream of nitrogen, the crude unprotected product is obtained.

Cold Complexation

The purified chelator-containing ligand (10^{-3} M in Tracepur® H_2O, *Merck KGaA*, Darmstadt, Germany, 1.0 eq.) and $^{nat}LuCl_3$ (20 mM in Tracepur® H_2O, 2.5 eq.) were diluted with Tracepur® H_2O to a final concentration of 10^{-4} M and heated to 95 °C for 30 min. After cooling to room temperature, the crude product was obtained and used without further purification for IC_{50} studies. In order to confirm that the remaining excess of 1.5 eq. $^{nat}LuCl_3$ did not affect the cell-based assay, a validation experiment was carried out (see *In Vitro Experiments* below).

Radiolabeling

^{125}I-Labeling. Briefly, 0.2 mg of D-Tyr^6-MJ9 were dissolved in 20 µL Tracepur® H_2O and 280 µL TRIS buffer (25 mM TRIS · HCl, 0.4 M NaCl, pH = 7.9). After solution was transferred to a vial containing 150 µg surface-bound Iodo-Gen® (1,3,4,6-Tetrachloro-3*α*,6*α*-diphenylglycouril, *Merck KGaA*, Darmstadt, Germany), 5.0 µL (16 MBq) ^{125}I-NaI (74 TBq/mmol, 3.1 GBq/mL, 40 mM NaOH, *Hartmann Analytic*, Braunschweig, Germany) were added. The reaction solution was incubated for 15 min at room temperature and purified by RP-HPLC. Immediately after purification, sodium ascorbate (0.1 M in Tracepur® H_2O, 10 vol-%) was added to prevent radiolysis.

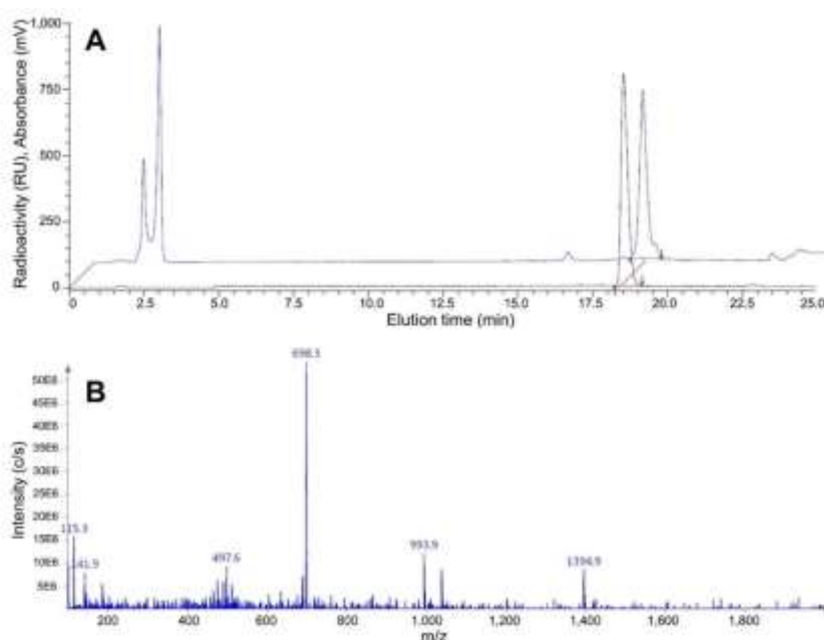


SUPPLEMENTAL FIGURE 1. Structural formula of the radiolabeled reference 3-^{125}I-D-Tyr^6-MJ9.

3-^{125}I-D-Tyr^6-MJ9. RP-HPLC (20→35% MeCN in 20 min). t_R = 18.5 min, *K'* = 12.21.

3-I-D-Tyr^6-MJ9. RP-HPLC (20→35% MeCN in 20 min). t_R = 18.4 min, *K'* = 12.14.

Calculated monoisotopic mass ($C_{78}H_{118}N_{20}O_{19}$): 1394.6, found: m/z = 698.3 $[M+2H]^{2+}$, 1394.9 $[M+H]^{+}$.



SUPPLEMENTAL FIGURE 2. (A) Confirmation of peptide integrity for 3-^{125}I-D-Tyr^{6}-MJ9 (black), as analyzed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min) *via* co-injection of the cold ligand (blue). (B) Mass spectrum of 3-I-D-Tyr^{6}-MJ9.

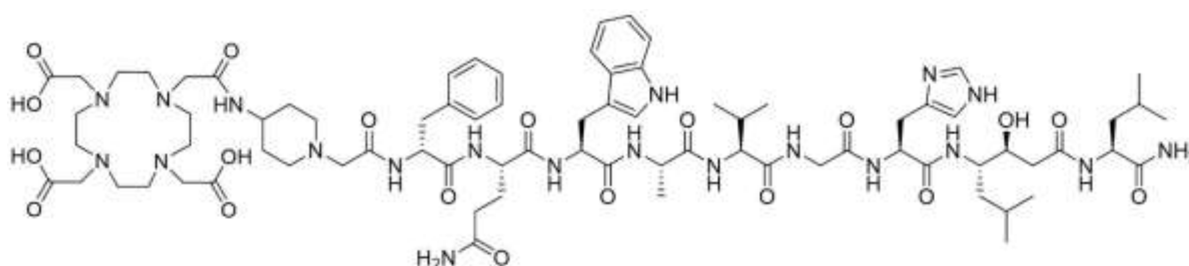
^{177}Lu-Labeling. A solution of the purified chelator-containing ligand (10^{-3} M in Tracepur® H_2O, 1 µL), NaOAc buffer (1.0 M, pH = 5.50, 10 µL) and approximately 10-30 MBq $^{177}LuCl_3$ (0.04 M in HCl) were diluted with HCl (0.04 M) to a total volume of 90 µL and heated to 95 °C for 10 min. Immediately after labeling, sodium ascorbate (0.1 M, 10 µL) was added to prevent radiolysis. Incorporation of ^{177}Lu was determined by radio TLC (ITLC-SG chromatography paper, mobile phase: 0.1 M trisodium citrate). Radiochemical purity of the labeled compound was determined by radio RP-HPLC.

Characterization of RM2 Derivatives and NeoBOMB1

All mentioned compounds based on the core structure of RM2 were synthesized by standard Fmoc-based SPPS (**GP1-5**) using a *H*-Rink amide ChemMatrix® resin (35-100 mesh

particle size, 0.4-0.6 mmol/g loading, *Merck KGaA*, Darmstadt, Germany). After finishing the peptide sequence with slightly modifications within the RM2 sequence, a chelator was coupled at the resin (**GP3**). Thereafter, the peptide was cleaved (**GP4**) and furthermore, remaining acid labile protection groups were deprotected by TFA (**GP5**) and purified by RP-HPLC.

RM2 (DOTA-Pip-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$)



SUPPLEMENTAL FIGURE 3. Structural formula of the parent compound RM2.

RM2. RP-HPLC (10→90% MeCN in 15 min): t_R = 6.8 min, *K'* = 3.25.

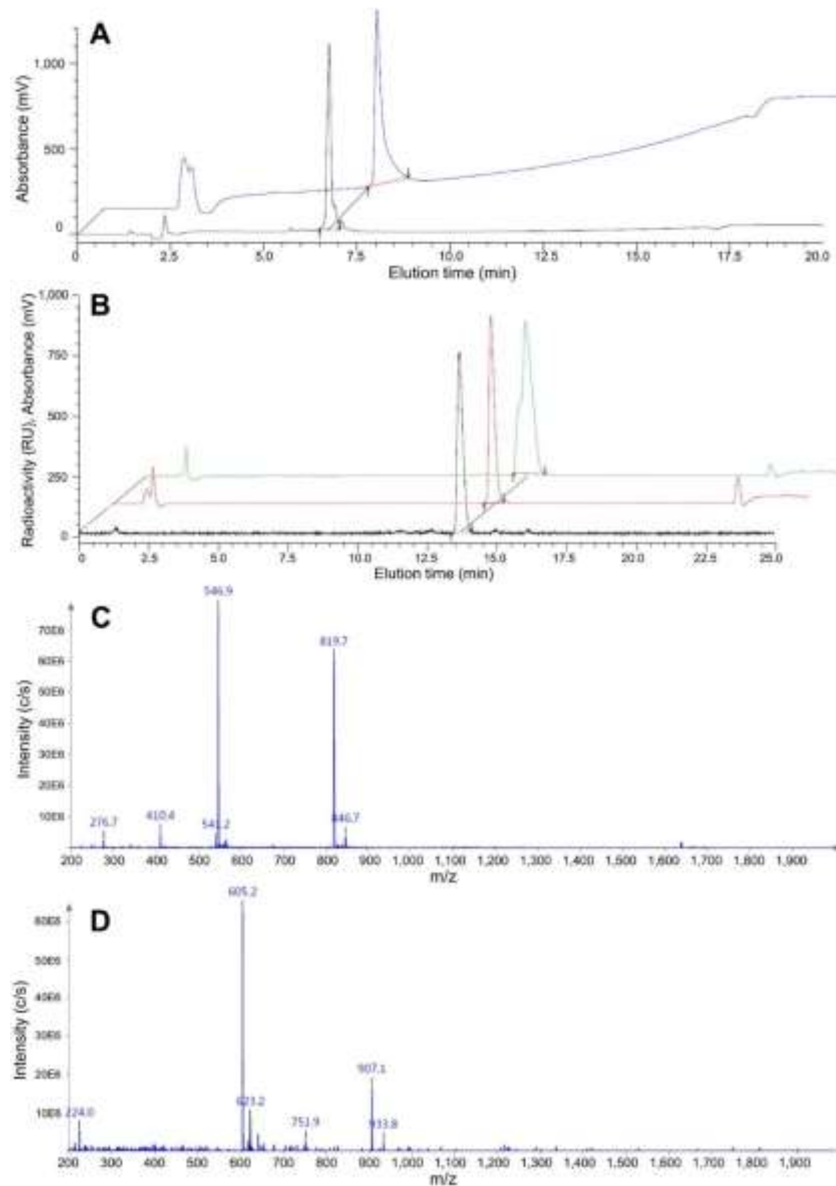
RP-HPLC (20→35% MeCN in 15 min): t_R = 13.6 min, *K'* = 9.46.

Calculated monoisotopic mass ($C_{78}H_{118}N_{20}O_{19}$): 1638.9, found: m/z = 546.9 $[M+3H]^{3+}$, 819.7 $[M+2H]^{2+}$.

^{nat}Lu-RM2. RP-HPLC (10→90% MeCN in 15 min): t_R = 7.3 min, *K'* = 2.65.

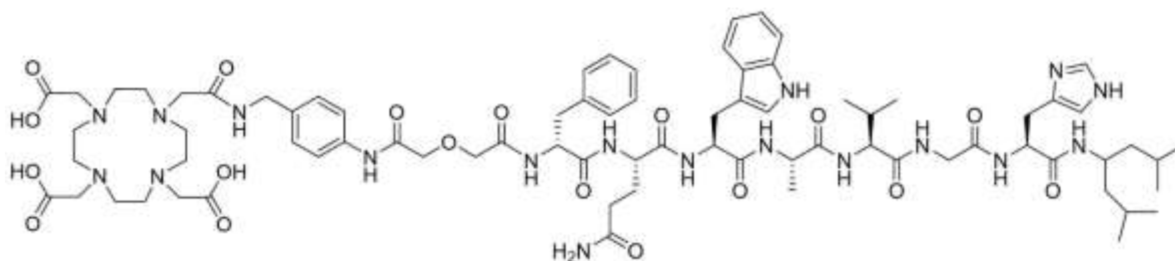
RP-HPLC (20→35% MeCN in 15 min): t_R = 13.6 min, *K'* = 9.46.

Calculated monoisotopic mass ($C_{78}H_{115}LuN_{20}O_{19}$): 1810.8, found: m/z = 605.2 $[M+3H]^{3+}$, 907.1 $[M+2H]^{2+}$.



SUPPLEMENTAL FIGURE 4. (A) Confirmation of peptide identity and integrity for RM2 (black) and ^{nat}Lu-RM2 (blue), as analyzed by analytical RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 10→90% MeCN in H_2O + 0.1% TFA in 15 min). (B) Confirmation of peptide identity and integrity for ^{177}Lu-RM2 (black), ^{nat}Lu-RM2 (red) and RM2 (green), as analyzed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). Mass spectra of (C) RM2 and (D) ^{nat}Lu-RM2.

NeoBOMB1 (DOTA-pABzA-DIG-D-Phe-Gln-Trp-Ala-Val-Gly-His-NH-CH[CH_2-$CH(CH_3)_2$]$_2$)



SUPPLEMENTAL FIGURE 5. Structural formula of the second reference compound NeoBOMB1.

NeoBOMB1. RP-HPLC (10→90% MeCN in 15 min): t_R = 9.5 min, K' = 3.75.

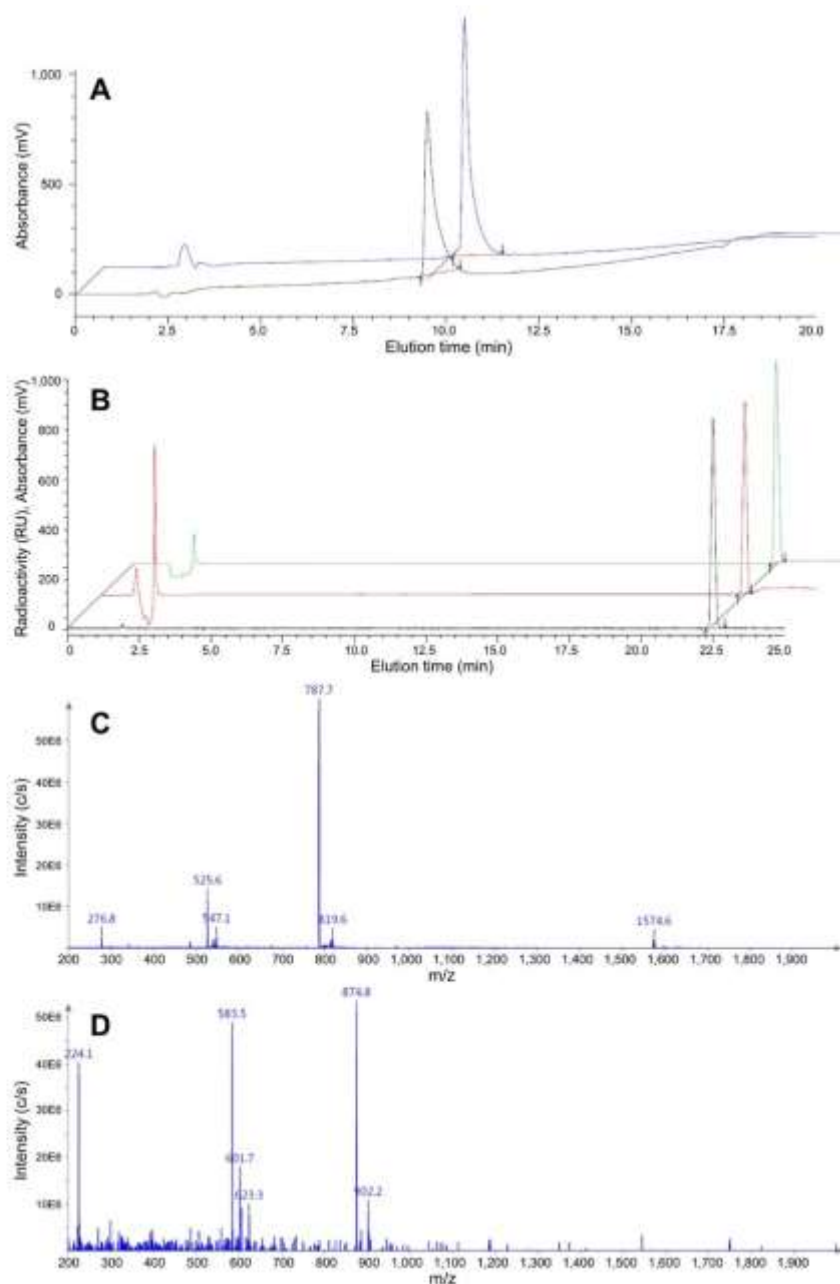
RP-HPLC (20→35% MeCN in 15 min): t_R = 22.4 min, K' = 16.23.

Calculated monoisotopic mass ($C_{77}H_{110}N_{18}O_{18}$): 1574.8, found: m/z = 787.7 $[M+2H]^{2+}$, 1574.6 $[M+H]^{+}$.

^{nat}Lu-NeoBOMB1. RP-HPLC (10→90% MeCN in 15 min): t_R = 9.7 min, K' = 3.85.

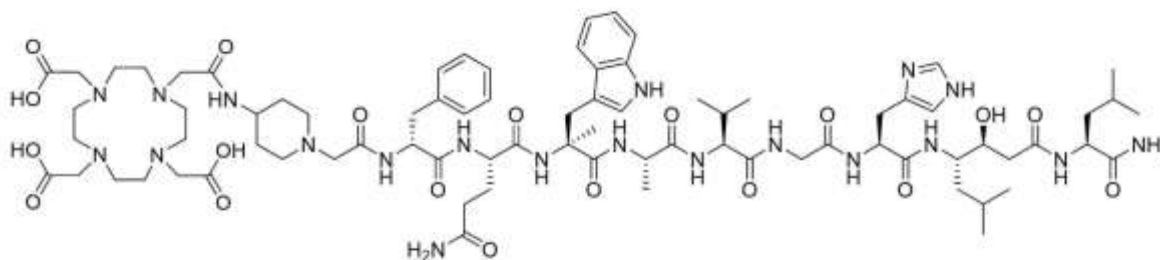
RP-HPLC (20→35% MeCN in 15 min): t_R = 22.4 min, K' = 16.23.

Calculated monoisotopic mass ($C_{77}H_{107}LuN_{18}O_{18}$): 1746.7, found: m/z = 583.5 $[M+3H]^{3+}$, 874.8 $[M+2H]^{2+}$.



SUPPLEMENTAL FIGURE 6. (A) Confirmation of peptide identity and integrity for NeoBOMB1 (black) and natLu-NeoBOMB1 (blue), as analyzed by analytical RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 10→90% MeCN in H_2O + 0.1% TFA in 15 min). (B) Confirmation of peptide identity and integrity for ^{177}Lu-NeoBOMB1 (black), natLu-NeoBOMB1 (red) and NeoBOMB1 (green), as analyzed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). Mass spectra of (C) NeoBOMB1 and (D) natLu-NeoBOMB1.

AMTG (DOTA-Pip-D-Phe-Gln-α-Me-Trp-Ala-Val-Gly-His-Sta-Leu-NH_2)



SUPPLEMENTAL FIGURE 7. Structural formula of AMTG.

AMTG. RP-HPLC (10→90% MeCN in 15 min): t_R = 7.5 min, K' = 2.75.

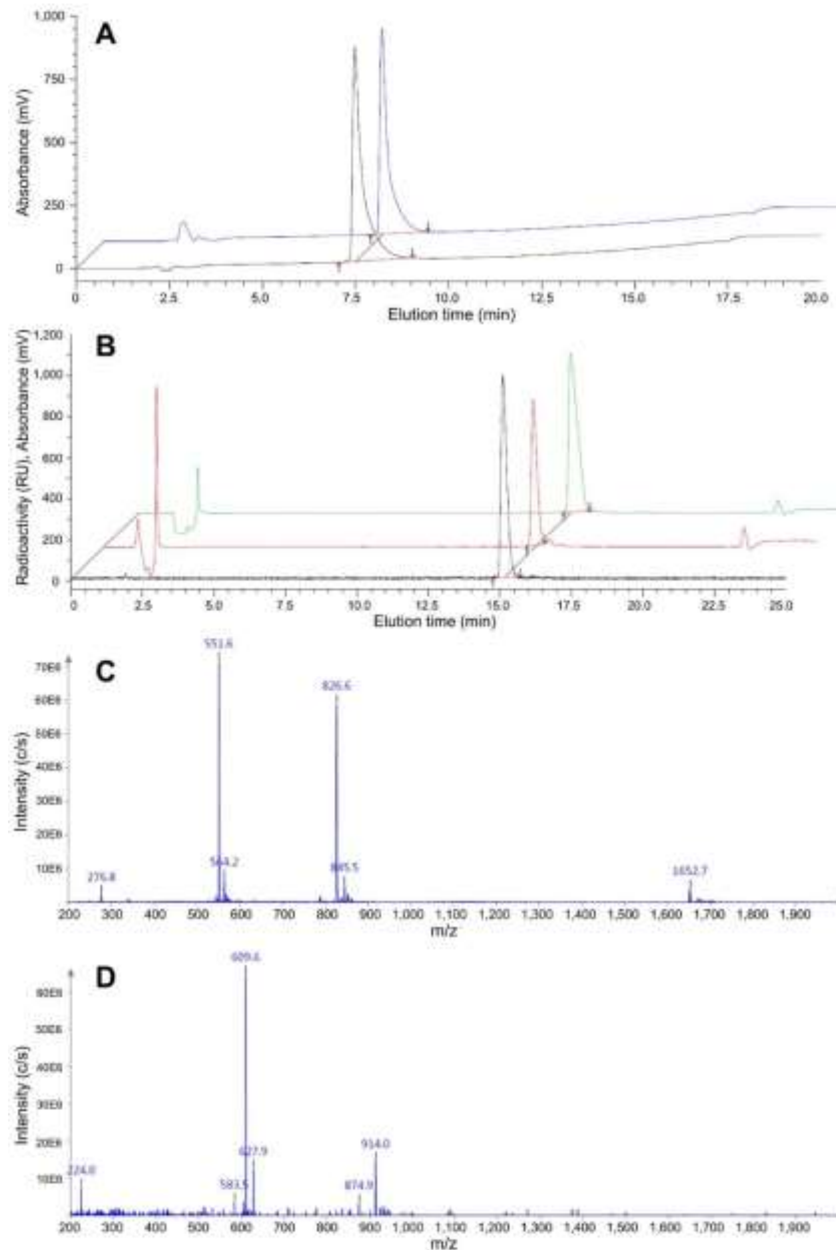
RP-HPLC (20→35% MeCN in 15 min): t_R = 15.2 min, K' = 10.69.

Calculated monoisotopic mass ($C_{79}H_{120}N_{20}O_{19}$): 1652.9, found: m/z = 551.6 $[M+3H]^{3+}$, 826.6 $[M+2H]^{2+}$, 1652.7 $[M+H]^{+}$.

^{nat}Lu-AMTG. RP-HPLC (10→90% MeCN in 15 min): t_R = 7.5 min, K' = 2.75.

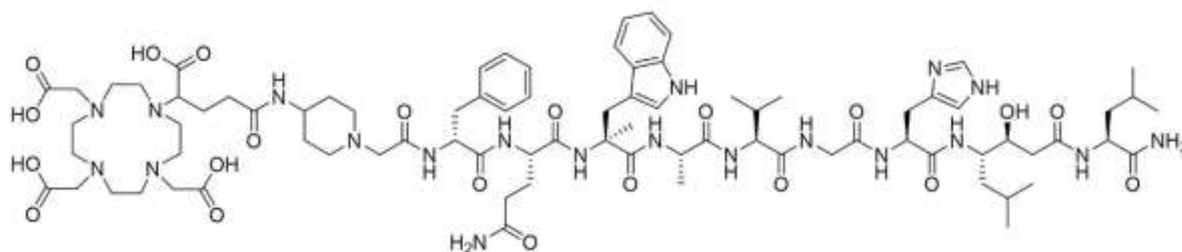
RP-HPLC (20→35% MeCN in 15 min): t_R = 15.0 min, K' = 10.54.

Calculated monoisotopic mass ($C_{79}H_{117}LuN_{20}O_{19}$): 1824.8, found: m/z = 609.6 $[M+3H]^{3+}$, 914.0 $[M+2H]^{2+}$.



SUPPLEMENTAL FIGURE 8. (A) Confirmation of peptide identity and integrity for AMTG (black) and natLu-AMTG (blue), as analyzed by analytical RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 10→90% MeCN in H_2O + 0.1% TFA in 15 min). (B) Confirmation of peptide identity and integrity for ^{177}Lu-AMTG (black), natLu-AMTG (red) and AMTG (green), as analyzed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). Mass spectra of (C) AMTG and (D) natLu-AMTG.

AMTG2 (DOTAGA-Pip-D-Phe-Gln-*α*-Me-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$)



SUPPLEMENTAL FIGURE 9. Structural formula of AMTG2.

AMTG2. RP-HPLC (10→90% MeCN in 15 min): t_R = 7.5 min, K' = 2.75.

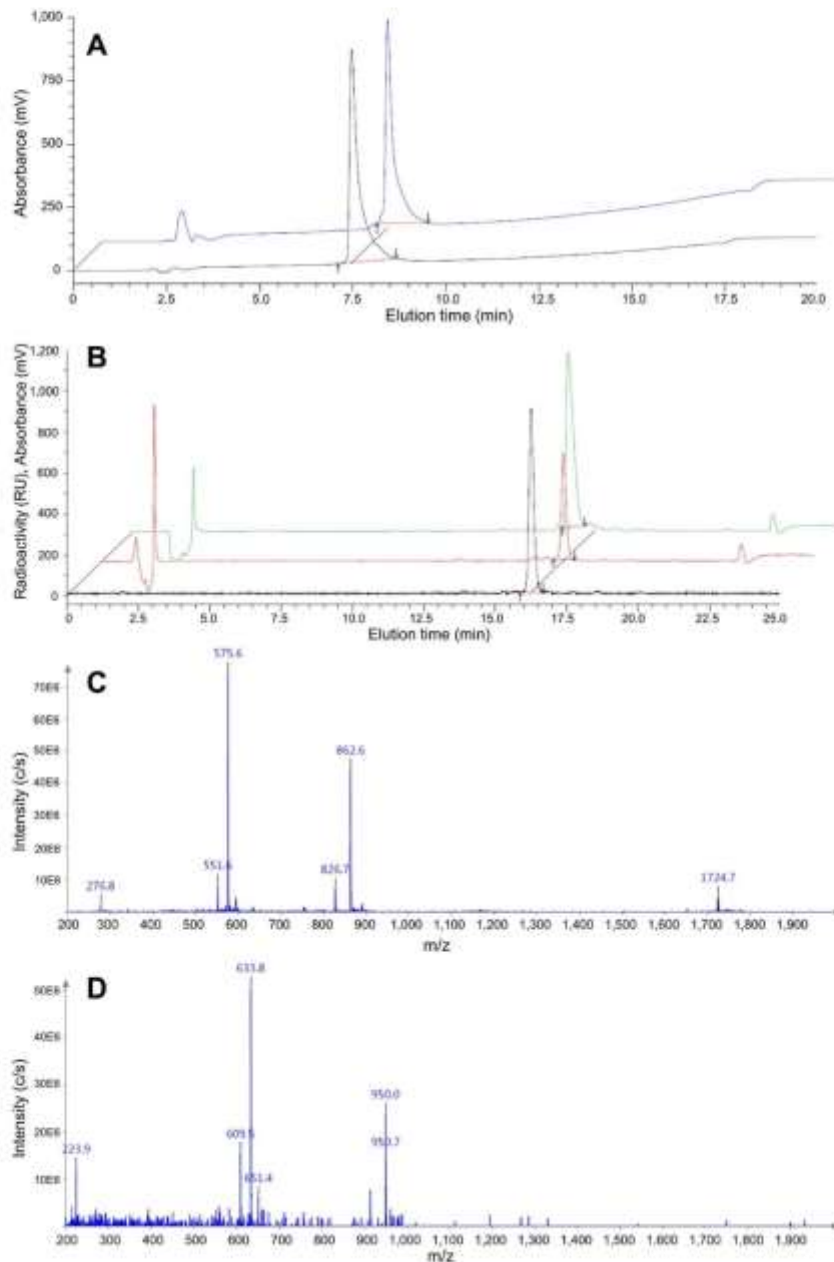
RP-HPLC (20→35% MeCN in 15 min): t_R = 15.2 min, K' = 10.69.

Calculated monoisotopic mass ($C_{82}H_{124}N_{20}O_{21}$): 1724.9, found: m/z = 575.6 $[M+3H]^{3+}$, 862.6 $[M+2H]^{2+}$, 1724.7 $[M+H]^{+}$.

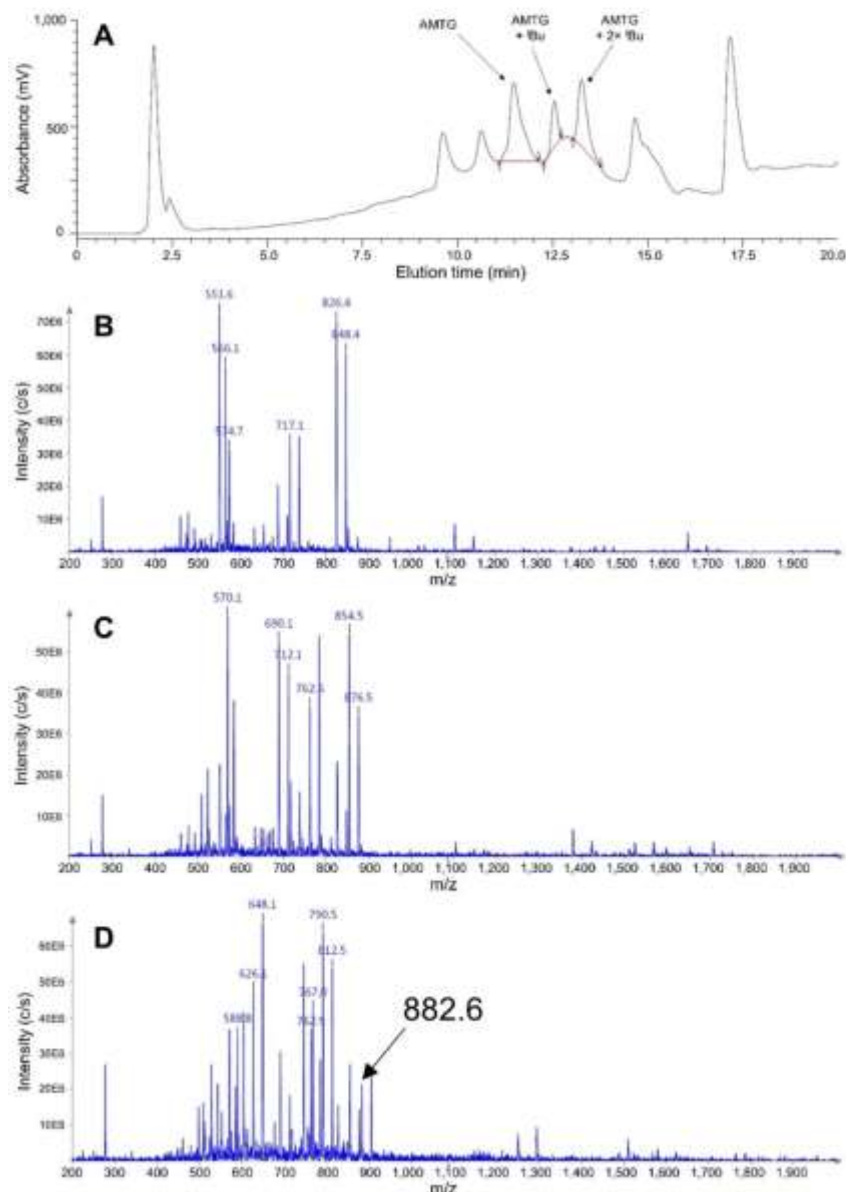
^{nat}Lu-AMTG2. RP-HPLC (10→90% MeCN in 15 min): t_R = 7.7 min, K' = 2.85.

RP-HPLC (20→35% MeCN in 15 min): t_R = 16.2 min, K' = 11.46.

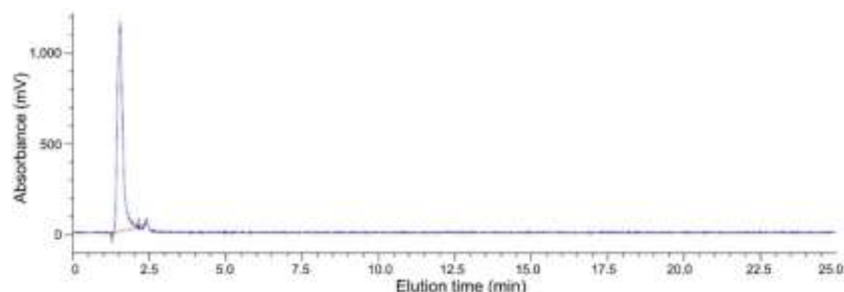
Calculated monoisotopic mass ($C_{82}H_{121}LuN_{20}O_{21}$): 1896.8, found: m/z = 633.8 $[M+3H]^{3+}$, 950.0 $[M+2H]^{2+}$.



SUPPLEMENTAL FIGURE 10. (A) Confirmation of peptide identity and integrity for AMTG2 (black) and natLu-AMTG2 (blue), as analyzed by analytical RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 10→90% MeCN in H_2O + 0.1% TFA in 15 min). (B) Confirmation of peptide identity and integrity for 177Lu-AMTG2 (black), natLu-AMTG2 (red) and AMTG2 (green), as analyzed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). Mass spectra of (C) AMTG2 and (D) natLu-AMTG2.



SUPPLEMENTAL FIGURE 11. Incomplete deprotection of ^{t}Bu groups of the DOTA moiety in AMTG (calculated monoisotopic mass: 1652.9), as analyzed by (A) analytical RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 15 min) and mass spectrometry (B-D). (B) Mass spectrum of the fully deprotected compound (AMTG: K' = 6.2, t_R = 11.5 min, mass found: m/z = 826.4 $[M+2H]^{2+}$). (C) Mass spectrum of the compound carrying one ^{t}Bu group (AMTG + ^{t}Bu: K' = 6.8, t_R = 12.5 min, mass found: m/z = 854.5 $[M+2H]^{2+}$). (D) Mass spectrum of the compound carrying two ^{t}Bu groups (AMTG + 2× ^{t}Bu: K' = 7.3, t_R = 13.2 min, mass found: m/z = 882.6 $[M+2H]^{2+}$).



SUPPLEMENTAL FIGURE 12. Chromatogram of ^{177}Lu-LuCl$_3$, (elution time t_R = 1.54 min) as analyzed by analytical radio-RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min).

In Vitro **Experiments**

Cell Culture. GRPR$^+$ PC-3 cells (*Merck KGaA*, Darmstadt, Germany) were cultivated in Dulbecco's modified eagle's medium/Ham's F-12 (DMEM/F-12, *v*/*v* = 1/1, with stable glutamine, *Biochrom GmbH*, Berlin, Germany) supplemented with fetal bovine serum (10%, FBS Superior, *Biochrom GmbH*, Berlin, Germany) at 37 °C in a humidified 5% CO_2 atmosphere. GRPR$^+$ T-47D cells (*American Type Culture Collection*, Manassas, VA, USA) were cultivated in Gibco™ RPMI 1640 Medium (*Fisher Scientific GmbH*, Schwerte, Germany) supplemented with fetal bovine serum (10%, FBS Superior, *Biochrom GmbH*, Berlin, Germany) at 37 °C in a humidified 5% CO_2 atmosphere. A mixture of trypsin and ethylenediaminetetraacetic acid (0.05%, 0.02%) in PBS (*Biochrom GmbH*, Berlin, Germany) was used in order to harvest cells. Cells were counted with a Neubauer hemocytometer (*Paul Marienfeld*, Lauda-Königshofen, Germany).

Determination of IC_{50}. For determination of the GRPR affinity on both PC-3 and T-47D cells (IC_{50}), cells were harvested 24 ± 2 h before the experiment and seeded in 24-well plates (1.5 × 10^5 cells in 1 mL/well).

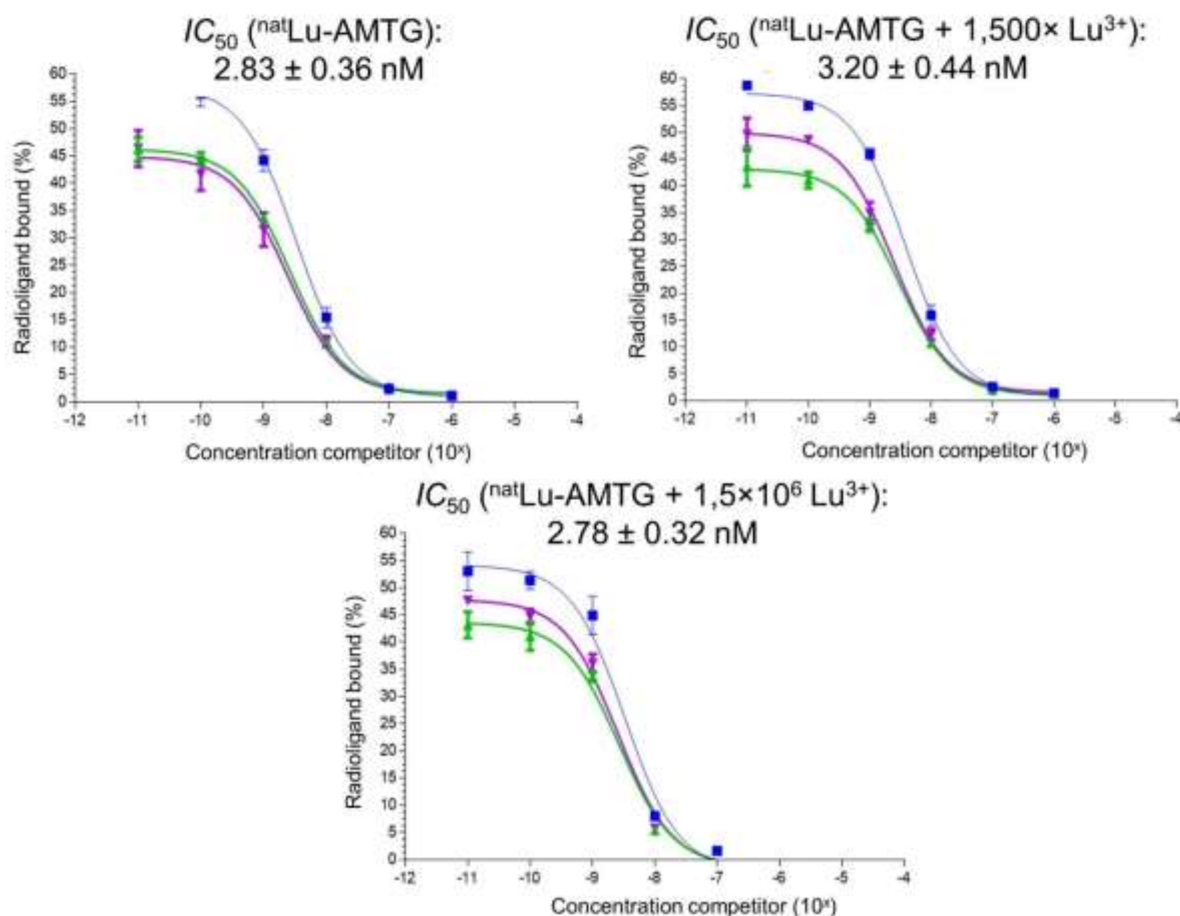
After removal of the culture medium, the cells were washed once with 500 µL of HBSS (Hank's balanced salt solution, *Biochrom GmbH*, Berlin, Germany, with addition of 1% bovine

serum albumin (BSA, *v*/*v*)) and left in 200 µL HBSS (1% BSA, *v*/*v*) for 9 min at room temperature for equilibration. Next, 25 µL per well of solutions, containing either HBSS (1% BSA, *v*/*v*) as control or the respective compound in increasing concentration (10^{-10}–10^{-4} M in HBSS (1% BSA, *v*/*v*)), were added with subsequent addition of 25 µL of 3-^{125}I-D-Tyr^{6}-MJ9 (2.0 nM) in HBSS (1% BSA, *v*/*v*).

All experiments were performed in triplicate for each concentration. After 2 h incubation at rt, the experiment was terminated by removal of the medium and consecutive rinsing with 300 µL of HBSS (1% BSA, *v*/*v*). The media of both steps were combined in one fraction and represent the amount of free 3-^{125}I-D-Tyr^{6}-MJ9. Afterwards, the cells were lysed with 300 µL of 1 M NaOH for at least 15 min and united with the 300 µL NaOH of the following washing step. Quantification of bound and free 3-^{125}I-D-Tyr^{6}-MJ9 was accomplished in a *γ*-counter. IC_{50} determination for each conjugate was repeated twice.

Brief Competition Study to validate Assay Conditions. In order to exclude an impact of free $^{nat}LuCl_3$ (from labeling procedure, see *Cold Complexation* above) in the above-described IC_{50} assay, the study was repeated for ^{nat}Lu-AMTG applying the same conditions. However, in addition to the used dilution series (10^{-10}–10^{-4} M in HBSS (1% BSA, *v*/*v*)) of ^{nat}Lu-AMTG (1.5-fold excess of free $^{nat}Lu^{3+}$ present), two further dilution series were prepared. On the one hand, a 1,500-fold and on the other hand, a 1.5×10^{6}-fold excess of free $^{nat}Lu^{3+}$ was added to each dilution of ^{nat}Lu-AMTG.

The study confirmed that no noticeable effect of free $^{nat}Lu^{3+}$ on the cellular uptake or displacement of 3-^{125}I-D-Tyr^{6}-MJ9 and thus IC_{50} values in the above-described assay could be determined (Supplemental Fig. 13).



SUPPLEMENTAL FIGURE 13. Sigmoidal binding curves and calculated mean IC_{50}´s as obtained by competitive binding studies of ^{nat}Lu-AMTG, ^{nat}Lu-AMTG with a 1,500-fold excess of free Lu^{3+} and ^{nat}Lu-AMTG with a 1.5×10^6-fold excess of free Lu^{3+}. Binding studies have been carried out in triplicate, using 1.5×10^5 PC-3 cells/ml/well, rt, 2 h; using 3-^{125}I-D-Tyr^6-MJ9 (0.2 nM/well) as radiolabeled reference (n = 3).

Receptor-mediated Internalization. For internalization studies, PC-3 cells were harvested 24 ± 2 h before the experiment and seeded in poly-L-lysine coated 24-well plates (1.5×10^5 cells/well, 1 mL, *Greiner Bio-One*, Kremsmünster, Austria). Subsequent to the removal of the culture medium, the cells were washed once with 500 µL DMEM/F-12 (5% BSA, *v*/*v*) and left to equilibrate at 37 °C for at least 15 min in 200 µL DMEM/F-12 (5% BSA, *v*/*v*). Each well was treated with either 25 µL of DMEM/F-12 (5% BSA, *v*/*v*) or 25 µL ^{nat}Lu-RM2 (10^{-3} M) for blockade. Next, 25 µL of the ^{177}Lu-labeled GRPR analogue (10 nM) was added and the cells were incubated at 37 °C for 60 min.

The experiment was terminated by placing the 24-well plate on ice for 1 min and consecutive removal of the medium. Each well was rinsed with 300 µL ice-cold PBS and the fractions from these first two steps were combined, representing the amount of free 3-^{125}I-D-Tyr^{6}-MJ9. Removal of surface bound activity was accomplished by incubation of the cells with 300 µL of ice-cold Acid Wash solution (0.02 M NaOAc, pH = 5.0) for 10 min at room temperature and rinsed again with 300 µL of ice-cold PBS. The internalized activity was determined by incubation of the cells in 300 µL NaOH (1 M) and the combination with the fraction of a subsequent washing step with 300 µL NaOH (1 M).

Each experiment (control and blockade) was performed sixfold. Free, surface bound and internalized activity was quantified in a *γ*-counter. Data was corrected for non-specific internalization.

In Vivo **Experiments**

Establishment of Tumor Xenografts. PC-3 cells (5.0×10^6 cells per 200 µL) were suspended in a mixture (*v*/*v* = 1/1) of Dulbecco's modified eagle's medium/Ham's F-12 (DMEM/F-12) with Glutamax-I (1/1) and Cultrex® Basement Membrane Matrix Type 3 (*Trevigen Inc.*, Gaithersburg, MD, USA) and inoculated subcutaneously onto the right shoulder of 6–10 weeks old female CB17-SCID mice (*Charles River Laboratories International Inc.*, Sulzfeld, Germany). Mice were used for experiments when tumor volume was 125-500 mm^3 (2–3 weeks after inoculation).

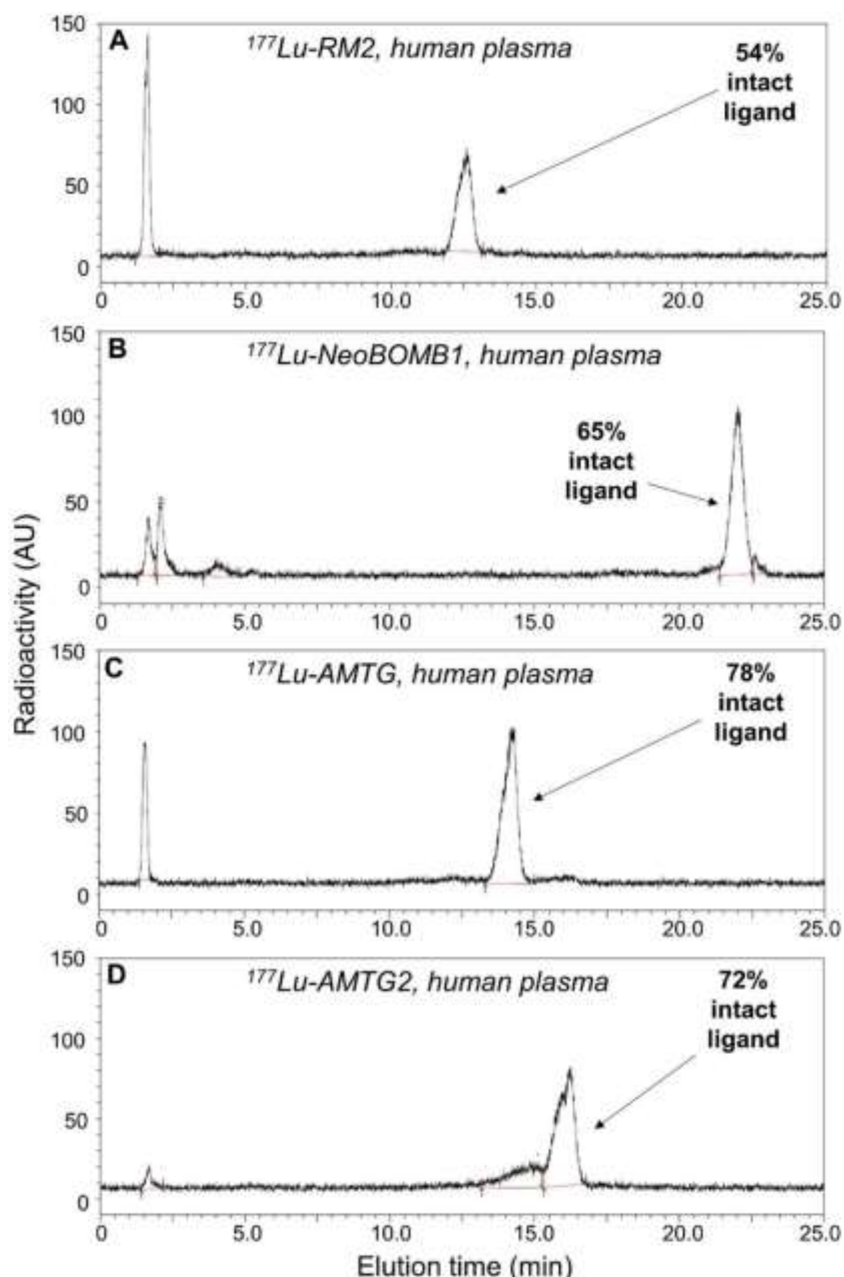
Supplemental Data

SUPPLEMENTAL TABLE 1. Preclinical data of ^{nat}Lu-RM2, ^{nat}Lu-NeoBOMBM1, ^{nat}Lu-AMTG and ^{nat}Lu-AMTG2. Affinity data were determined on PC-3 and T-47D cells (1.5×10^5 cells/well) and 3-^{125}I-D-Tyr^6-MJ9 (c = 0.2 nM) as radiolabeled reference (2 h, rt, HBSS + 1% BSA, *v*/*v*). Receptor-mediated internalization (0.25 pmol/well) was determined on PC-3 cells as percent (%) of the applied activity after incubation for 1 h (37 °C, DMEM/F-12 + 5% BSA (*v*/*v*), 1.5×10^5 cells/well). Data are corrected for non-specific binding (10^{-3} M ^{nat}Lu-RM2). Metabolic stability *in vitro* was determined in human plasma by incubation at 37 °C for 72 ± 2 h (n = 4). Metabolic stability *in vivo* was determined on CB17-SCID mice at 30 min p.i. (n = 3). Data are expressed as mean ± SD. Metabolic stability of the ^{177}Lu-RM2 derivatives as determined *in vitro* and *in vivo*. * ^{nat}Lu-labeled, ** ^{177}Lu-labeled.

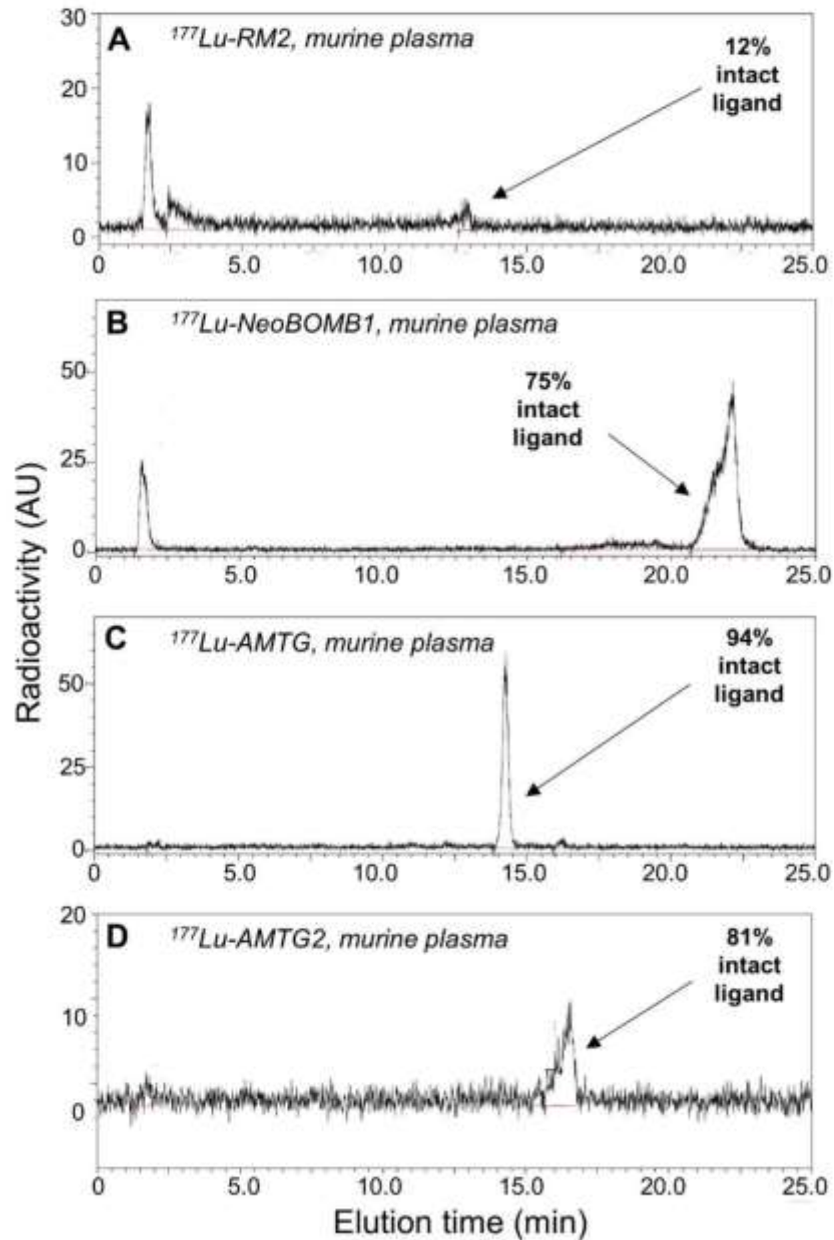
	IC_{50} (nM)		GRPR-mediated internalization (%)	log$D_{7.4}$	Fraction intact tracer (%)		
GRPR-targeted compound	PC-3 cells (n = 3)*	T-47D-cells (n = 3)*	(n = 5)**	(n = 6)**	human plasma, *in vitro* at 72 ± 2 h (n = 4)**	murine blood, *in vivo* at 30 min p.i. (n = 3)**	murine urine, *in vivo* at 30 min p.i. (n = 3)**
RM2	3.5 ± 0.2	1.2 ± 0.2	2.92 ± 0.20	−2.51 ± 0.02	38.7 ± 9.3	11.4 ± 3.7	0.5 ± 0.1
NeoBOMB1	4.2 ± 0.1	1.1 ± 0.2	13.91 ± 0.64	−0.57 ± 0.03	61.9 ± 2.1	75.9 ± 0.6	3.9 ± 1.3
AMTG	3.0 ± 0.1	1.0 ± 0.1	3.03 ± 0.18	−2.28 ± 0.06	77.7 ± 8.7	92.9 ± 0.7	68.2 ± 3.1
AMTG2	4.7 ± 0.2	4.6 ± 0.2	5.88 ± 0.33	−2.51 ± 0.11	66.2 ± 5.1	77.6 ± 3.1	61.6 ± 1.6

SUPPLEMENTAL TABLE 2. Tumor/background ratios of ^{177}Lu-RM2 and its analogues as well as ^{177}Lu-NeoBOMB1 for the selected organs of PC-3 tumor-bearing CB17-SCID mice at 24 h p.i. (n = 4).

Organ	^{177}Lu-RM2	^{177}Lu-AMTG	^{177}Lu-AMTG2	^{177}Lu-NeoBOMB1
Blood	688.7 ± 79.4	2702.0 ± 321.0	723.1 ± 109.2	175.5 ± 100.2
Heart	152.8 ± 7.4	575.3 ± 98.3	646.9 ± 597.4	87.5 ± 37.0
Lung	91.4 ± 16.4	357.2 ± 107.8	181.2 ± 29.6	16.7 ± 2.0
Liver	18.8 ± 1.3	85.3 ± 19.5	27.4 ± 6.4	5.6 ± 2.7
Spleen	42.8 ± 5.2	114.3 ± 24.9	59.4 ± 14.2	5.3 ± 3.1
Pancreas	19.9 ± 2.5	26.1 ± 12.0	8.4 ± 0.8	0.9 ± 0.2
Stomach	50.8 ± 19.3	128.3 ± 47.7	64.9 ± 5.0	5.6 ± 0.2
Intestine	39.7 ± 7.0	80.0 ± 49.7	27.5 ± 5.7	8.5 ± 0.6
Kidney	4.7 ± 0.1	10.5 ± 2.5	4.3 ± 0.4	4.7 ± 2.3
Adrenal	10.9 ± 1.7	34.3 ± 21.8	22.7 ± 2.9	2.1 ± 0.1
Muscle	1680.2 ± 978.9	2247.8 ± 687.3	4133.8 ± 2593.0	1017.0 ± 598.5
Bone	7.9 ± 3.6	234.3 ± 69.4	37.9 ± 11.3	42.2 ± 17.4

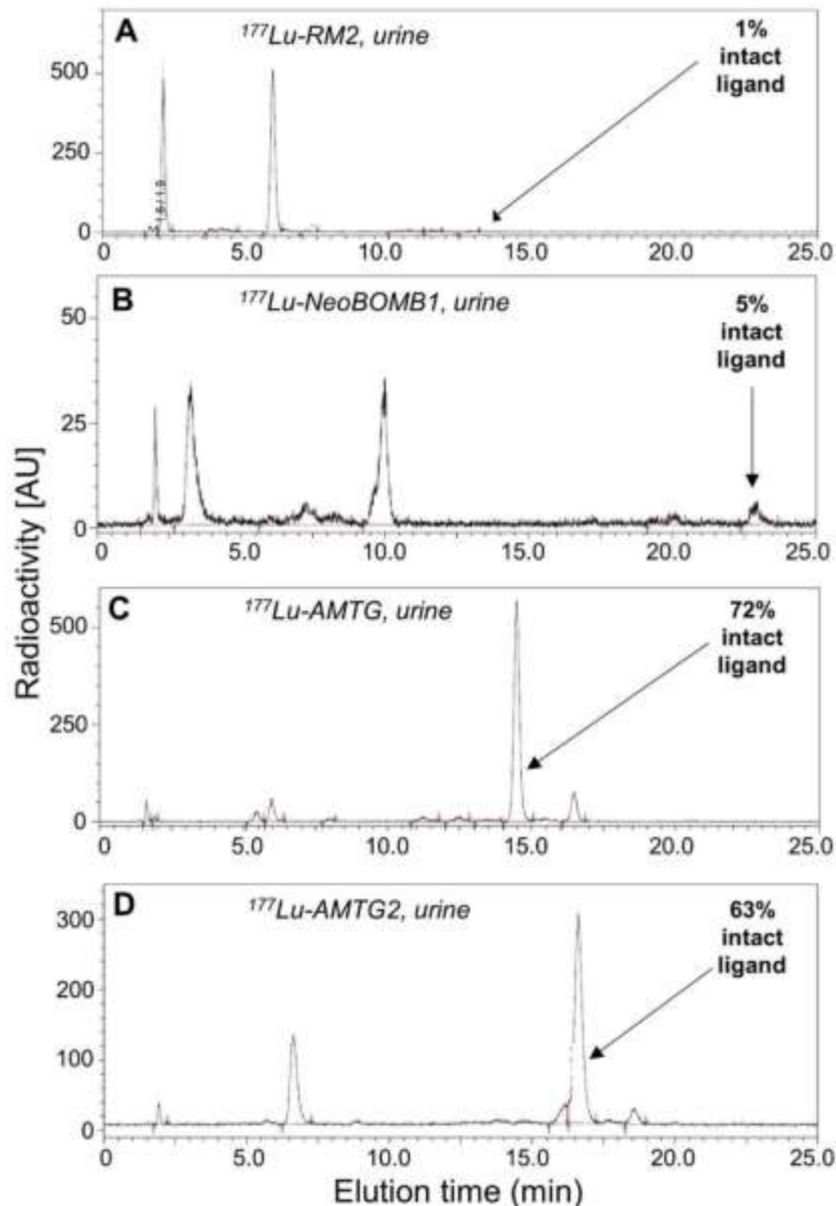


SUPPLEMENTAL FIGURE 14. *In vitro* stability of ^{177}Lu-labeled GRPR ligands incubated in human plasma at 37 °C for 72 ± 2 h, as analyzed by analytical radio-RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, *CS Chromatographie GmbH*, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). (A) ^{177}Lu-RM2, (B) ^{177}Lu-NeoBOMB1, (C) ^{177}Lu-AMTG and (D) ^{177}Lu-AMTG2. Fractions representing intact compounds are indicated by black arrows (^{177}Lu-RM2: K' = 8.7, t_R = 12.6 min; ^{177}Lu-NeoBOMB1: K' = 15.9, t_R = 22.0 min; ^{177}Lu-AMTG: K' = 9.9, t_R = 14.2 min; ^{177}Lu-AMTG2: K' = 11.5, t_R = 16.3 min).



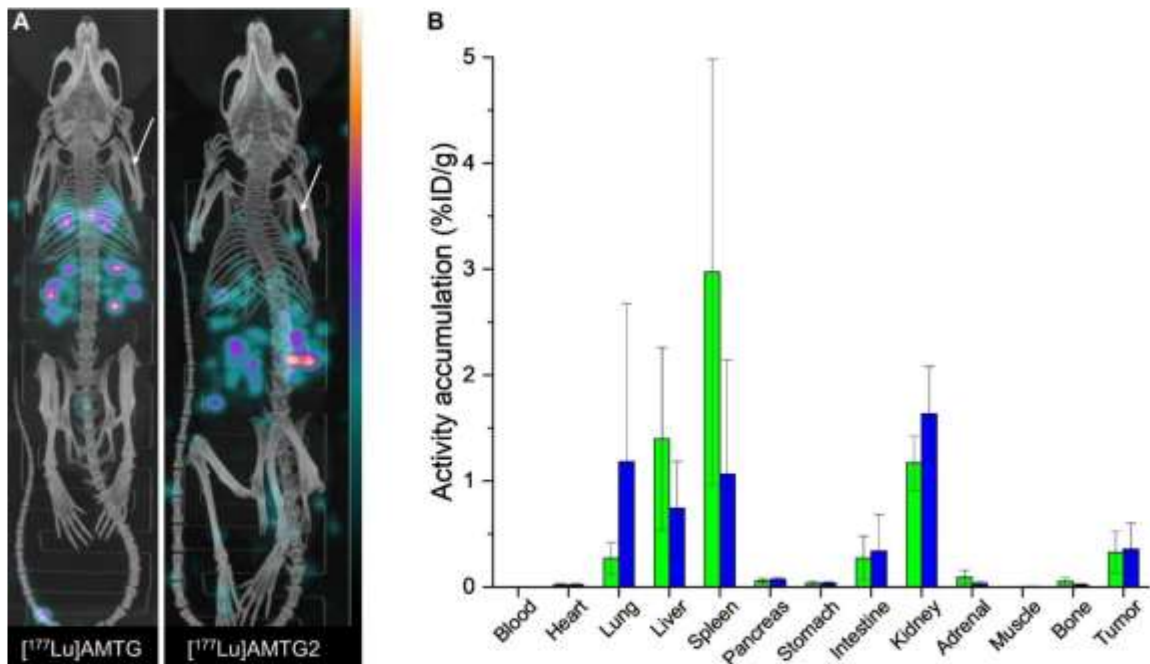
SUPPLEMENTAL FIGURE 15. *In vivo* stability of ^{177}Lu-labeled GRPR ligands in murine plasma (A-D) at 30 min p.i., as analyzed by analytical radio-RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, CS Chromatographie GmbH, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). (A) ^{177}Lu-RM2, (B) ^{177}Lu-NeoBOMB1, (C) ^{177}Lu-AMTG and (D) ^{177}Lu-AMTG2. Fractions representing intact compounds indicated by black arrows (^{177}Lu-RM2: K' = 8.7,

t_R = 12.8 min; ^{177}Lu-NeoBOMB1: *K'* = 15.9, t_R = 22.9 min; ^{177}Lu-AMTG: *K'* = 9.9, t_R = 14.5 min, ^{177}Lu-AMTG2: *K'* = 11.5, t_R = 16.6 min).

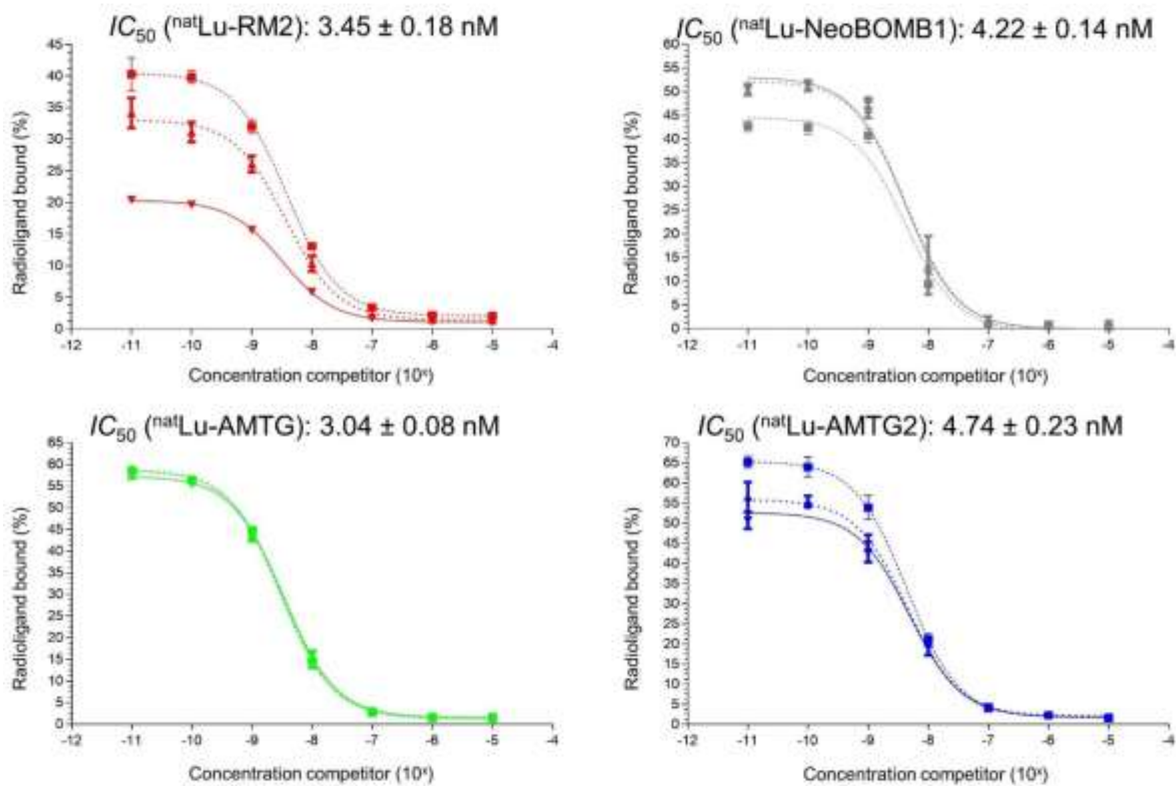


SUPPLEMENTAL FIGURE 16. *In vivo* stability of ^{177}Lu-labeled GRPR ligands in murine urine (A-D) at 30 min p.i., as analyzed by analytical radio-RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, CS Chromatographie GmbH, Langerwehe, Germany; 20→35% MeCN in H_2O + 0.1% TFA in 20 min). (A) ^{177}Lu-RM2, (B) ^{177}Lu-NeoBOMB1, (C) ^{177}Lu-AMTG and (D) ^{177}Lu-AMTG2. Fractions representing intact compounds indicated by black arrows (^{177}Lu-RM2: *K'* = 8.7,

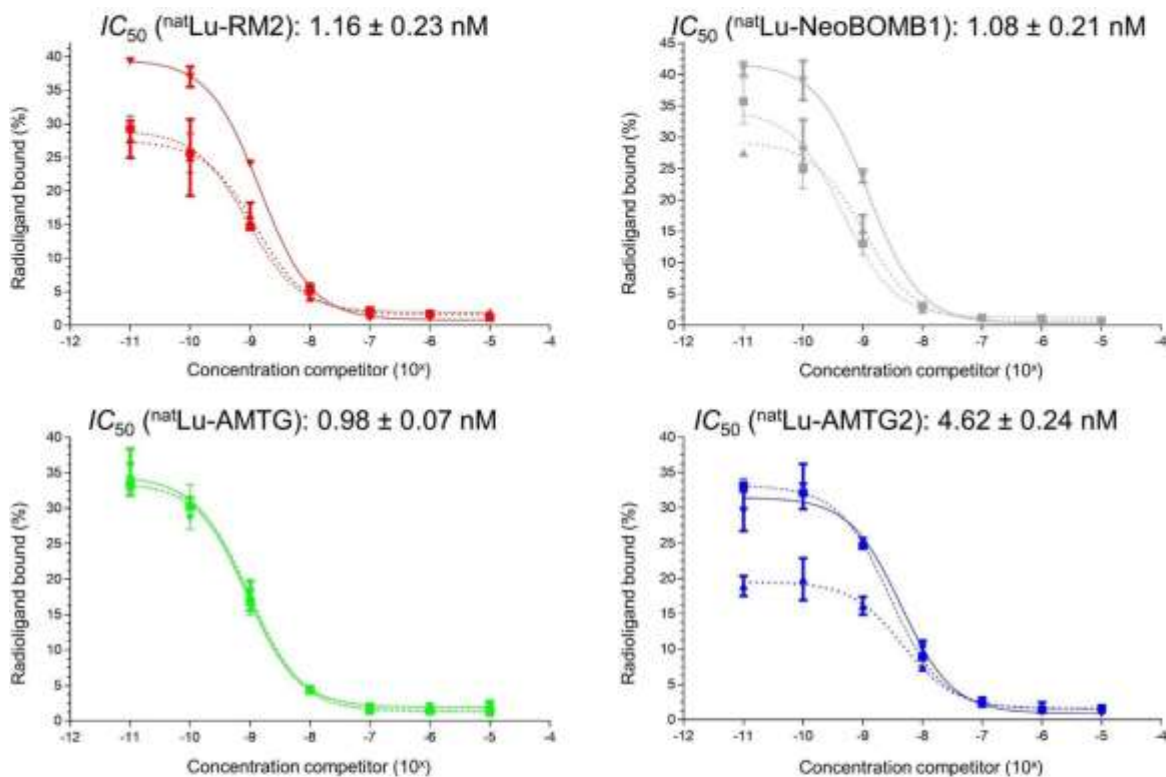
t_R = 12.8 min; ^{177}Lu-NeoBOMB1: K' = 15.9, t_R = 22.9 min; ^{177}Lu-AMTG: K' = 9.9, t_R = 14.5 min, ^{177}Lu-AMTG2: K' = 11.5, t_R = 16.6 min).



SUPPLEMENTAL FIGURE 17. (A) Maximum intensity projection of PC-3 tumor-bearing CB17-SCID mice injected with each 100 pmol of ^{177}Lu-AMTG (left) and ^{177}Lu-AMTG2 (right) and co-injected with an excess of ^{nat}Lu-RM2 (3.62 mg/kg). Images were acquired at 24 h p.i. PC-3 tumors are depicted by white arrows; (B) Biodistribution of ^{177}Lu-AMTG (green) and ^{177}Lu-AMTG2 (blue) co-injected with an excess of ^{nat}Lu-RM2 (3.62 mg/kg) in selected organs (in %ID/g) at 24 h p.i. in PC-3 tumor-bearing CB17-SCID mice (100 pmol each). Data is expressed as mean ± SD (n = 3).



SUPPLEMENTAL FIGURE 18. Sigmoidal binding curves and calculated mean IC_{50}´s as obtained by competitive binding studies of ^{nat}Lu-RM2, ^{nat}Lu-NeoBOMB1, ^{nat}Lu-AMTG and ^{nat}Lu-AMTG2. Binding studies have been carried out in triplicate, using 1.5 × 10^5 PC-3 cells/ml/well, rt, 2 h; using 3-^{125}I-D-Tyr^6-MJ9 (0.2 nM/well) as radiolabeled reference (n = 3).



SUPPLEMENTAL FIGURE 19. Sigmoidal binding curves and calculated mean IC_{50}´s as obtained by competitive binding studies of ^{nat}Lu-RM2, ^{nat}Lu-NeoBOMB1, ^{nat}Lu-AMTG and ^{nat}Lu-AMTG2. Binding studies have been carried out in triplicate, using 1.5 × 10^5 T-47D cells/ml/well, rt, 2 h; using 3-^{125}I-D-Tyr^6-MJ9 (0.2 nM/well) as radiolabeled reference (n = 3).