# Introduction of a SiFA Moiety into the D-Glutamate Chain of DOTA-PP-F11N Results in Radiohybrid-Based CCK-2R-Targeted Compounds with Improved Pharmacokinetics *in vivo*

# - Supplementary Materials -

**Nadine Holzleitner[1,#,*] Thomas Günther[1,#], Roswitha Beck[1], Constantin Lapa[2] and Hans-Jürgen Wester[1]**

[1]Chair of Pharmaceutical Radiochemistry, Department of Chemistry, Technical University of Munich, Garching, Germany

[2]Clinic for Nuclear Medicine, University Hospital Augsburg, Augsburg, Germany

**Corresponding co-authors:**

Nadine Holzleitner and Thomas Günther

Phone: +49.89.289.12203

Technical University of Munich,

Chair of Pharmaceutical Radiochemistry,

Walther-Meissner-Str. 3

85748 Garching

GERMANY

Fax: +49.89.289.12204

E-Mail: nadine.holzleitner@tum.de and thomas.guenther@tum.de

ORCID: https://orcid.org/0000-0001-8258-3526 (NH) and https://orcid.org/0000-0002-7412-0297 (TG)

**Analytical data of $^{nat/177}$Lu-labeled minigastrin analogues**

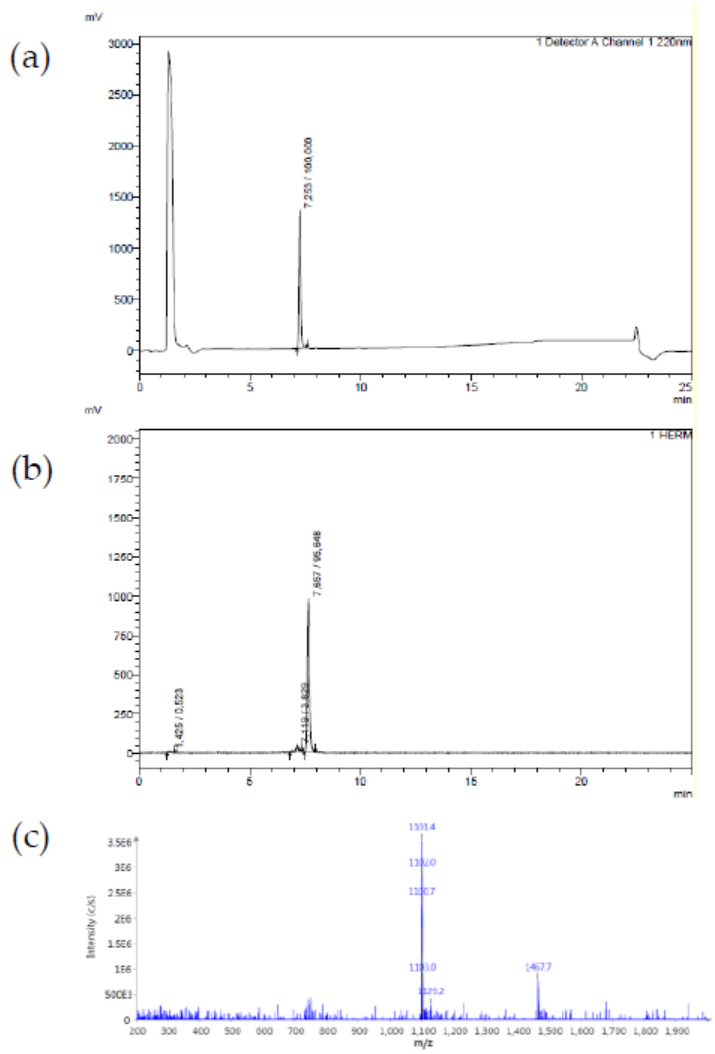
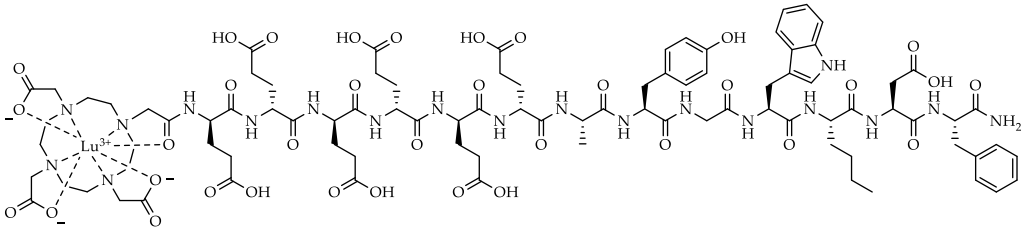
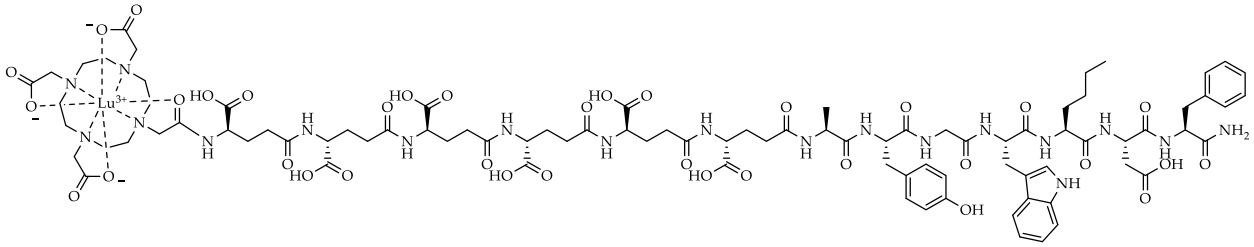


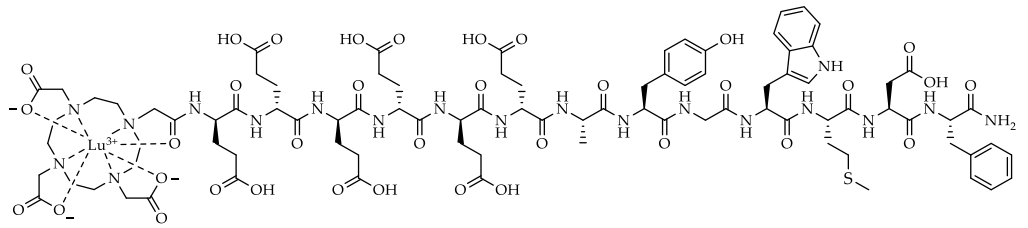

**Figure S1.** Confirmation of peptide identity and integrity for (**a**) [$^{nat}$Lu]Lu-DOTA-PP-F11N and (**b**) [$^{177}$Lu]Lu-DOTA-PP-F11N as analysed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, CS Chromatographie GmbH, Langerwehe, Germany; 10→90% MeCN in $H_2O$ + 0.1% TFA in 15 min). (**c**) Mass spectrum of [$^{nat}$Lu]Lu-DOTA-PP-F11N.



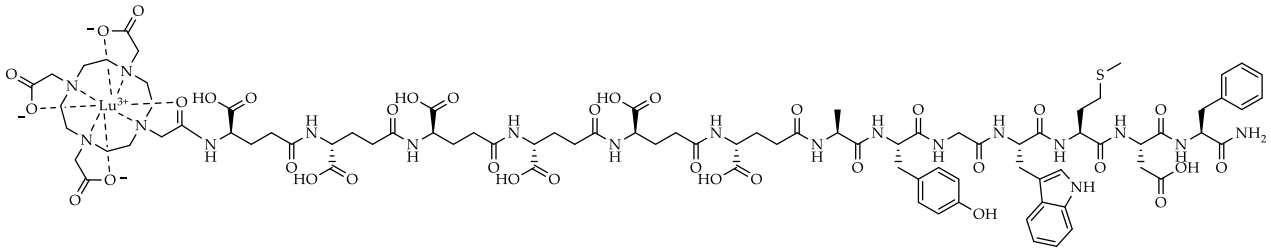
***[$^{nat}$Lu]Lu-DOTA-PP-F11N***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 7.55 min, K′ = 3.48; MS (ESI, positive): m/z calculated for $C_{90}H_{120}LuN_{19}O_{35}$: 2203.0, found: m/z = 1101.4 $[M+2H]^{2+}$.



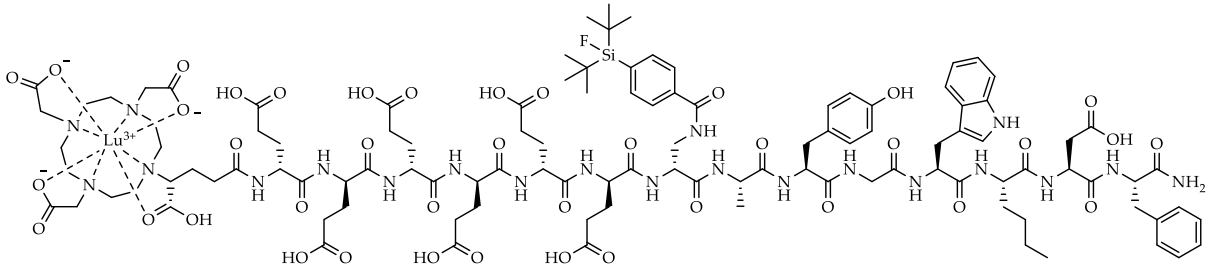
***[$^{nat}$Lu]Lu-DOTA-PP-γ-F11N***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 7.41 min, K′ = 3.39; MS (ESI, positive): m/z calculated for $C_{90}H_{120}LuN_{19}O_{35}$: 2203.0, found: m/z = 1101.9 $[M+2H]^{2+}$.



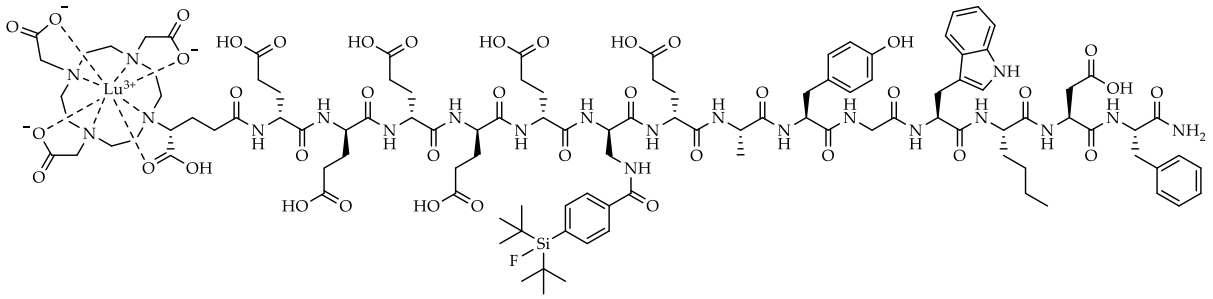
***[$^{nat}$Lu]Lu-CP04***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 7.60 min, K′ = 3.51; MS (ESI, positive): m/z calculated for $C_{89}H_{118}LuN_{19}O_{35}S$: 2219.7, found: m/z = 1110.3 $[M+2H]^{2+}$.



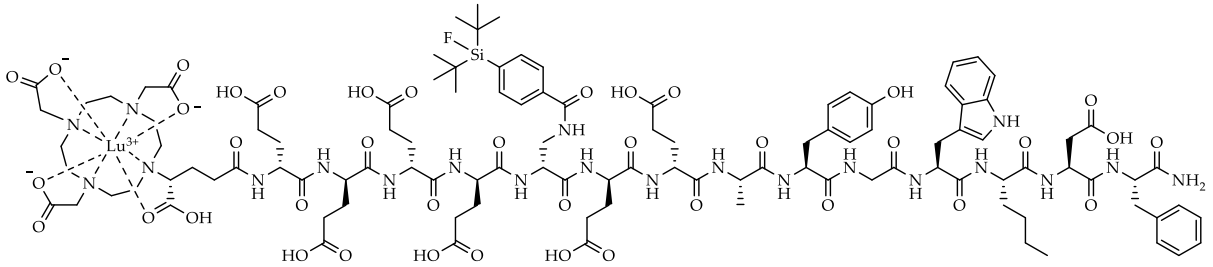
***[$^{nat}$Lu]Lu-γ-CP04***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 7.40 min, K′ = 3.39; MS (ESI, positive): m/z calculated for $C_{89}H_{118}LuN_{19}O_{35}S$: 2219.7, found: m/z = 1110.8 $[M+2H]^{2+}$.



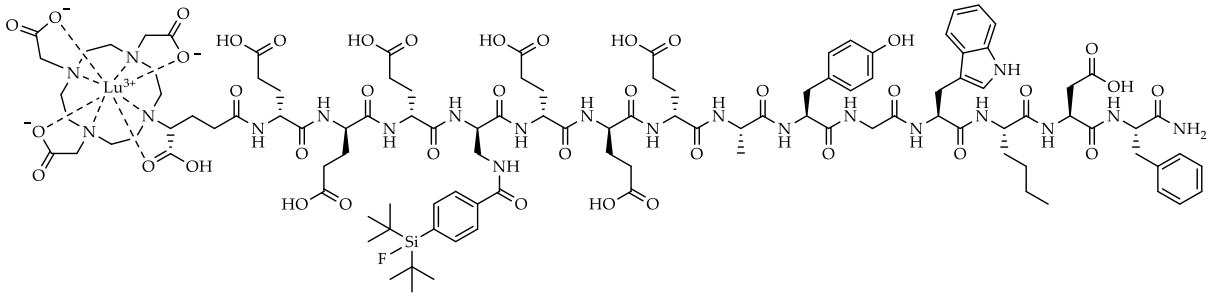
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-1***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 10.1 min, K′ = 4.99; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1312.8 $[M+2H]^{2+}$.



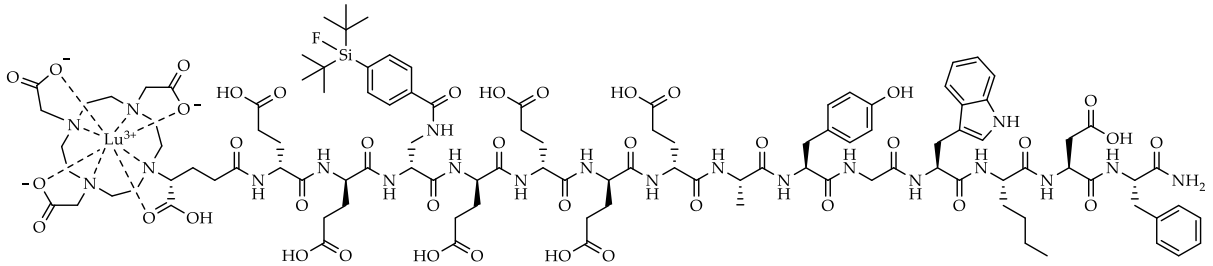
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-2***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.84 min, K′ = 4.83; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1312.7 $[M+2H]^{2+}$.



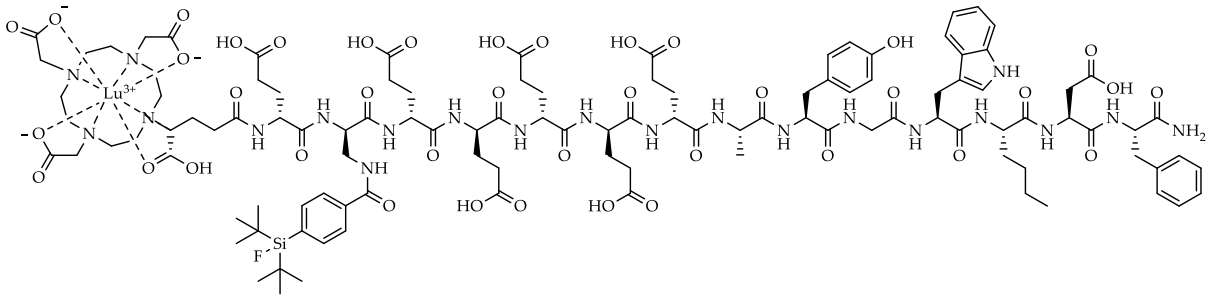
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-3***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.85 min, K′ = 4.84; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1312.3 $[M+2H]^{2+}$.



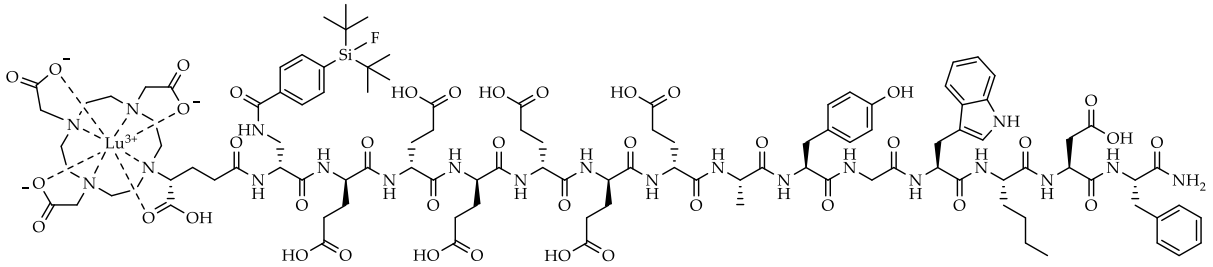
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-4***: RP-HPLC (10→70% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 11.9 min, K′ = 6.05; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.0 $[M+2H]^{2+}$.



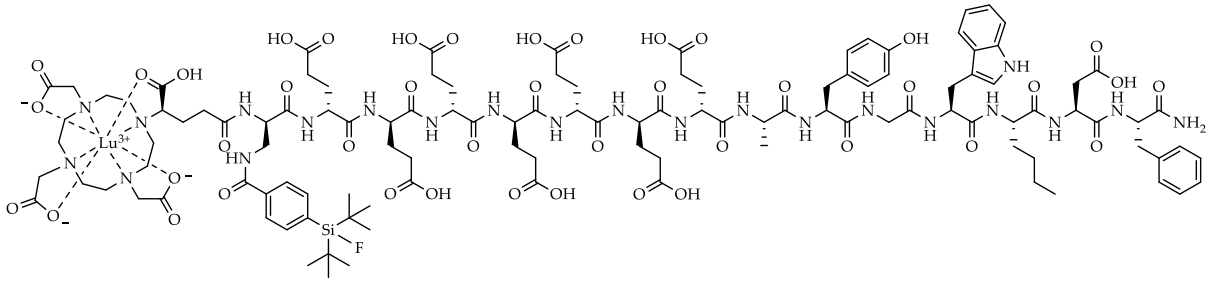
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-5***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.67 min, K′ = 4.73; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.3 $[M+2H]^{2+}$.



***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-6***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.71 min, K′ = 4.76; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1312.9 $[M+2H]^{2+}$.



***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-7***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.62 min, K′ = 4.70; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.0 $[M+2H]^{2+}$.



***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-8***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.77 min, K′ = 4.79; MS (ESI, positive): m/z calculated for $C_{116}H_{158}FLuN_{22}O_{42}Si$: 2754.7, found: m/z = 1378.2 $[M+2H]^{2+}$.

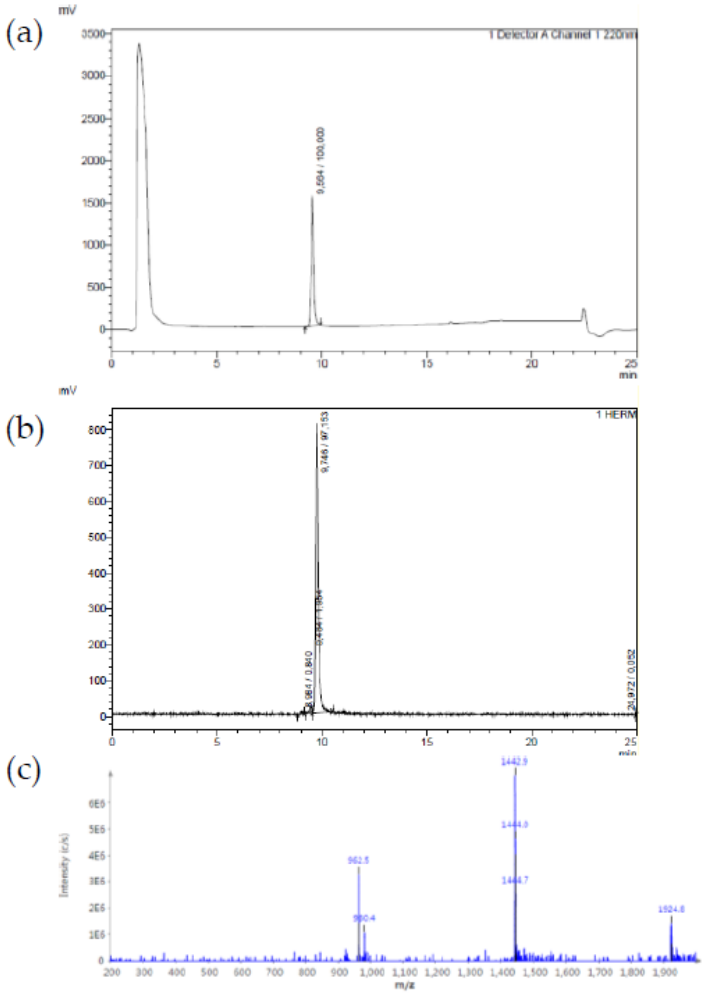
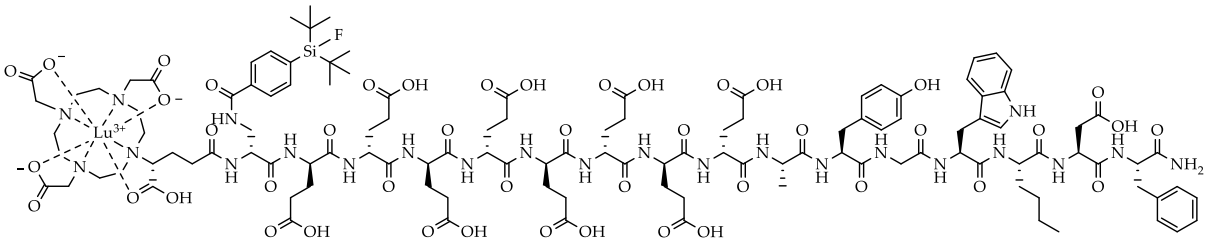
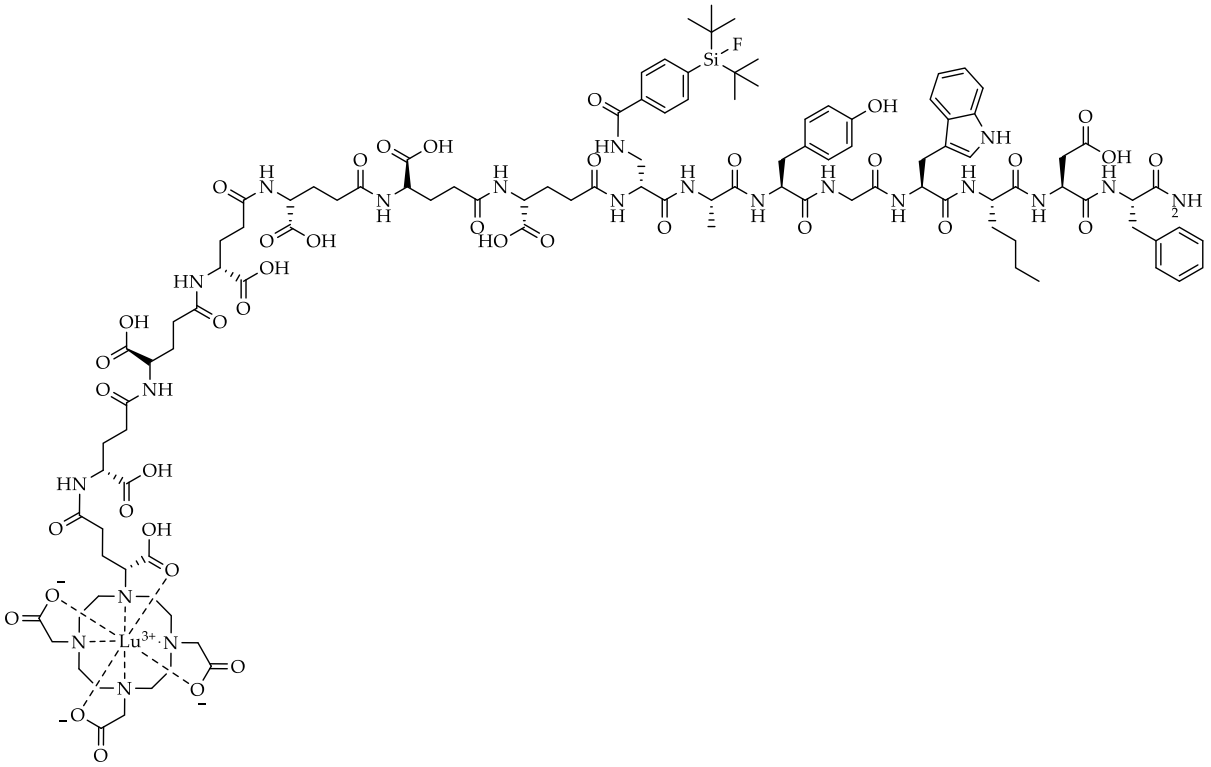


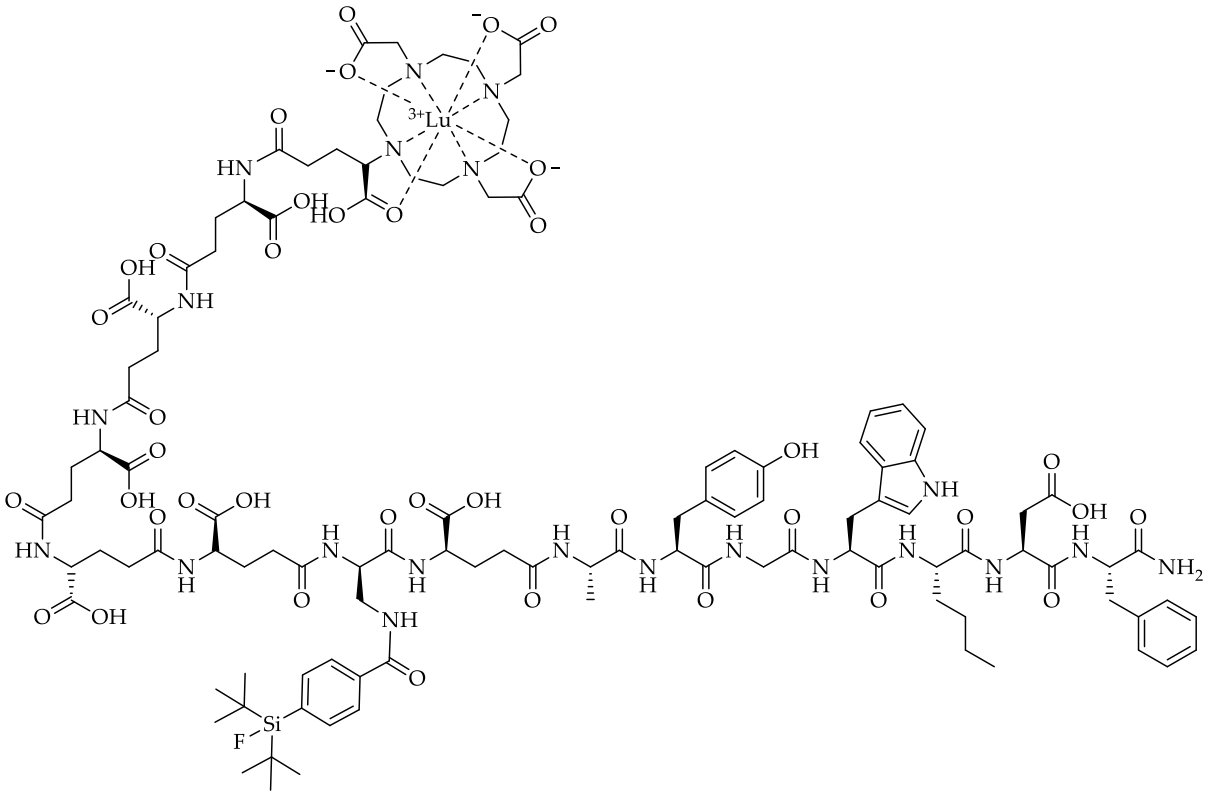

**Figure S2.** Confirmation of peptide identity and integrity for (**a**) [$^{nat}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 and (**b**) [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 as analysed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, CS Chromatographie GmbH, Langerwehe, Germany; 10→90% MeCN in $H_2O$ + 0.1% TFA in 15 min). (**c**) Mass spectrum of [$^{nat}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9.



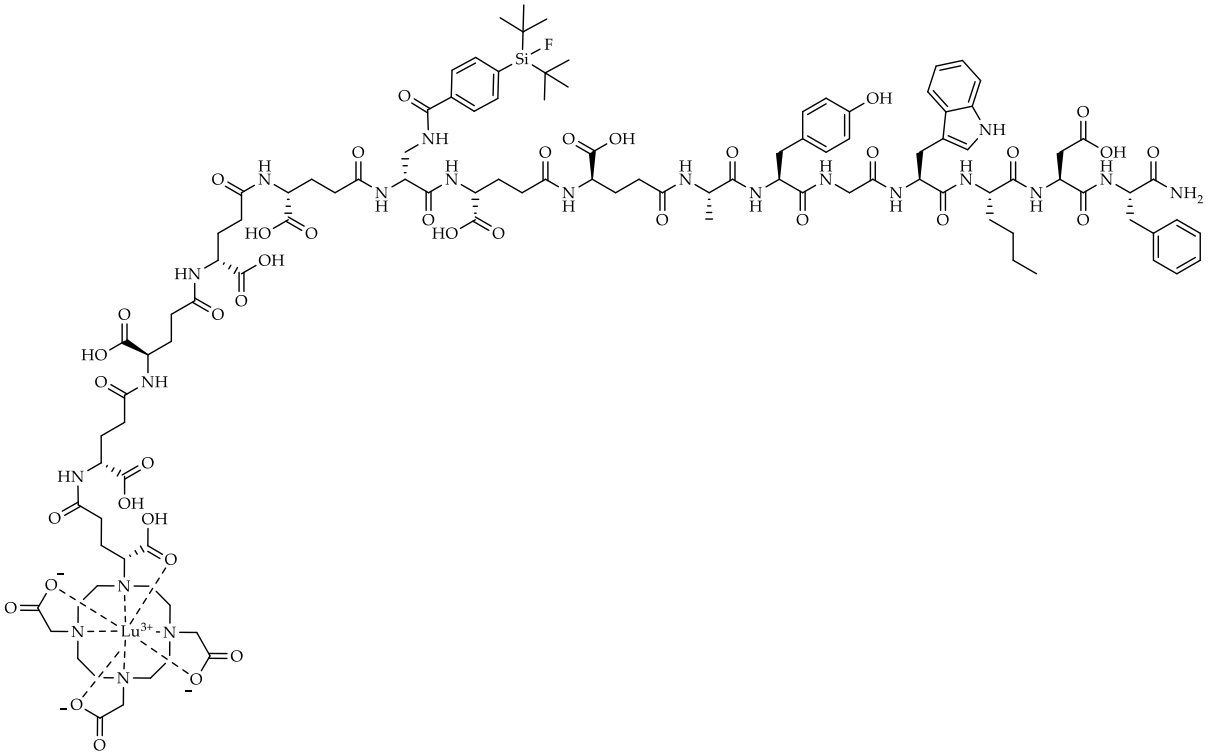
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-9***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.56 min, K′ = 4.67; MS (ESI, positive): m/z calculated for $C_{121}H_{165}FLuN_{23}O_{45}Si$: 2883.8, found: m/z = 1441.5 $[M+2H]^{2+}$.



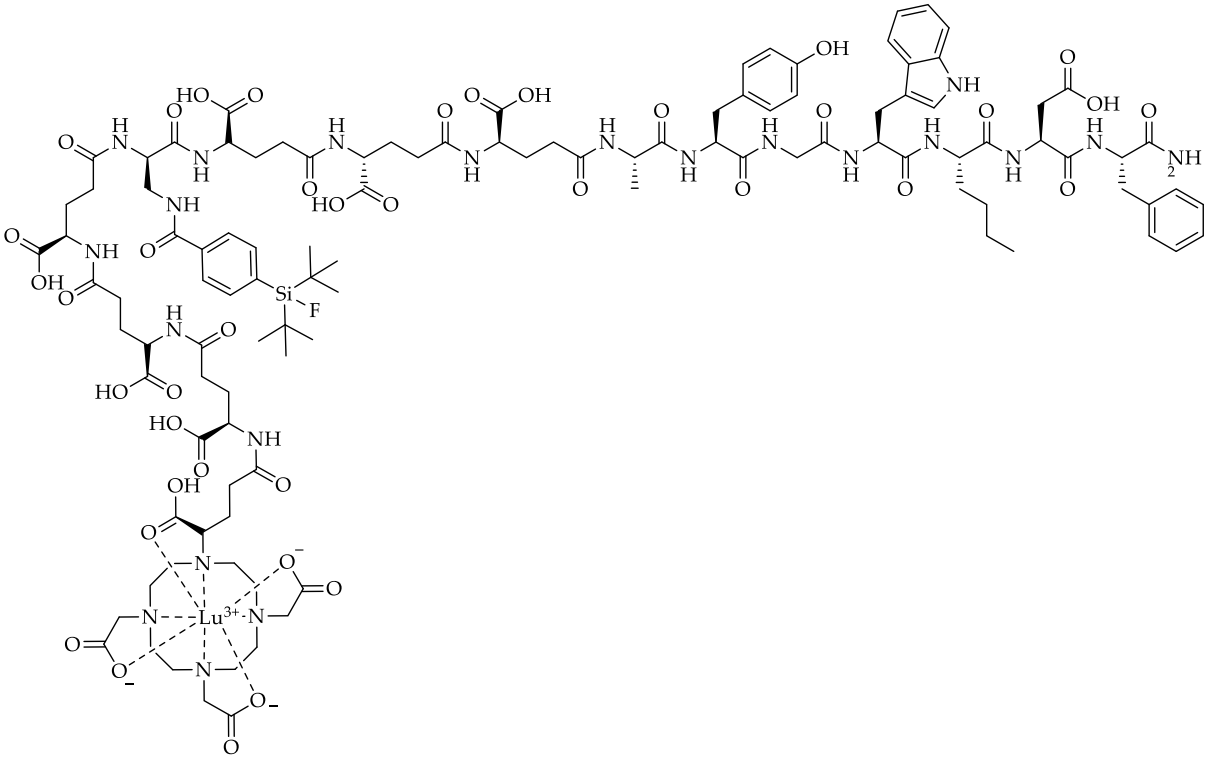
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-10***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 10.2 min, K′ = 5.05; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.6 $[M+2H]^{2+}$.



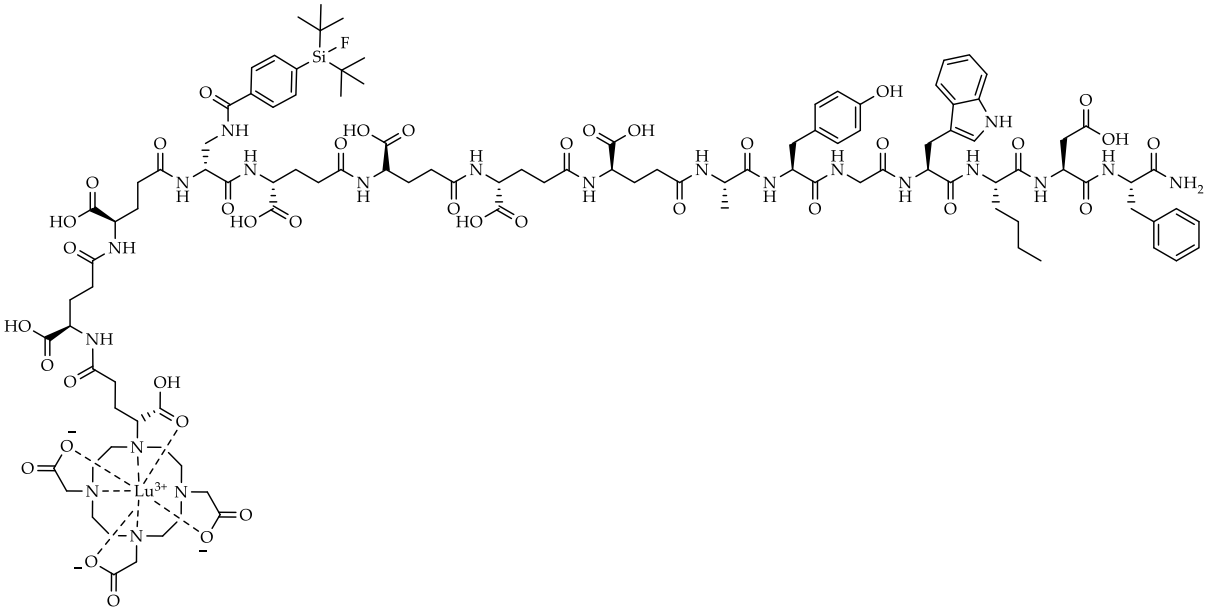
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-11***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.72 min, K′ = 4.76; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.0 $[M+2H]^{2+}$.



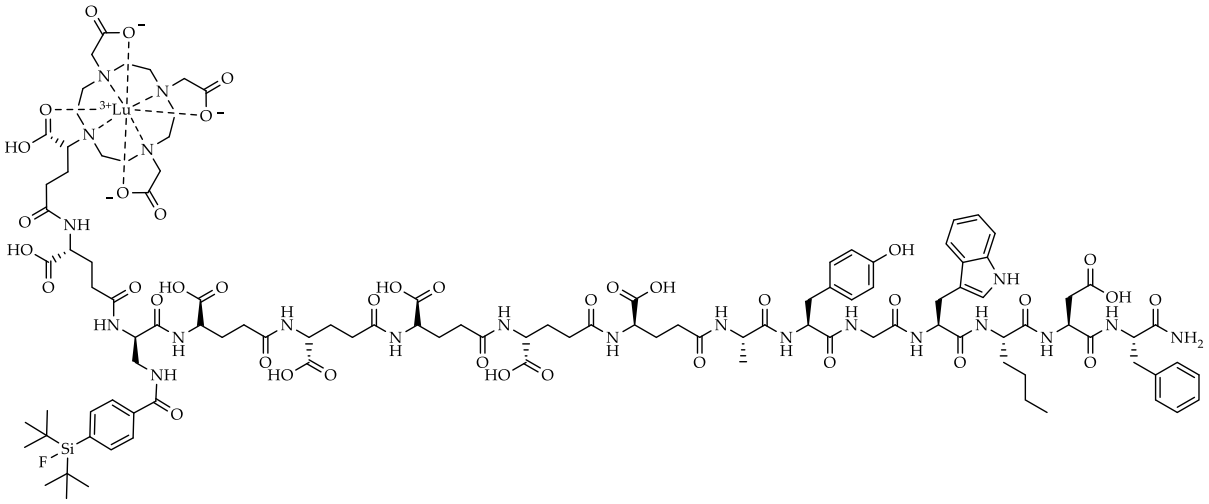
***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-12***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.87 min, K′ = 4.85; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1312.9 $[M+2H]^{2+}$.



***[natLu]Lu-(R)-DOTAGA-rhCCK-13***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.61 min, K′ = 4.70; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.2 $[M+2H]^{2+}$.



***[natLu]Lu-(R)-DOTAGA-rhCCK-14***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.60 min, K′ = 4.69; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.9 $[M+2H]^{2+}$.



***[natLu]Lu-(R)-DOTAGA-rhCCK-15***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.62 min, K′ = 4.70; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1313.3 $[M+2H]^{2+}$.

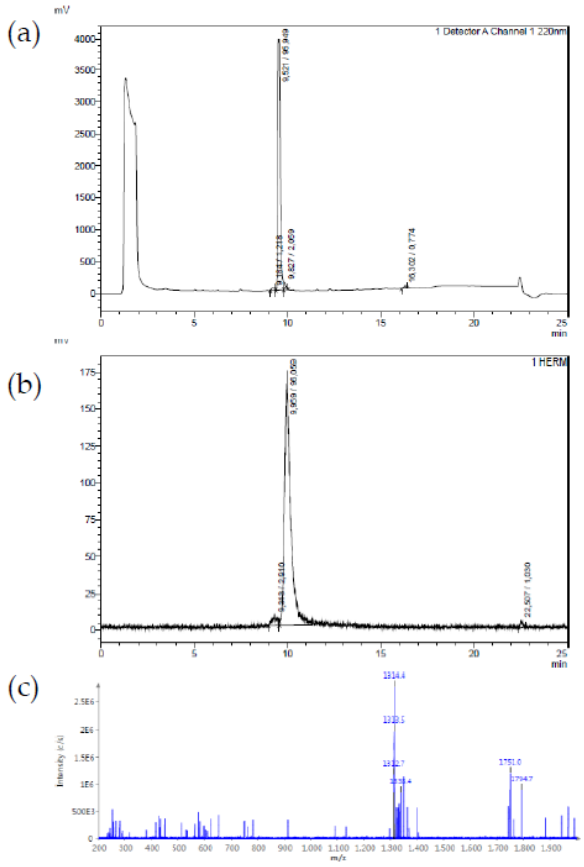
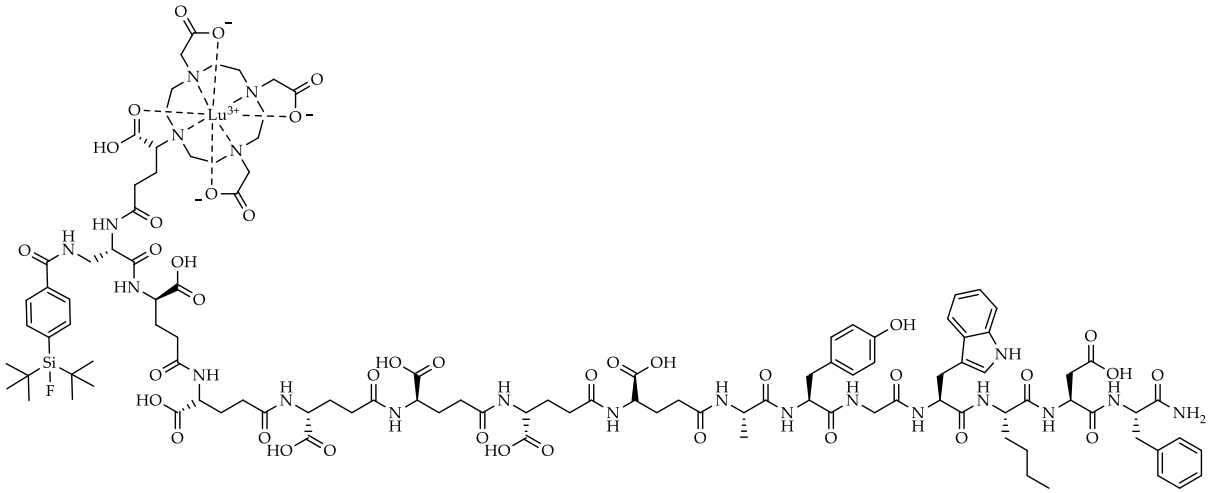
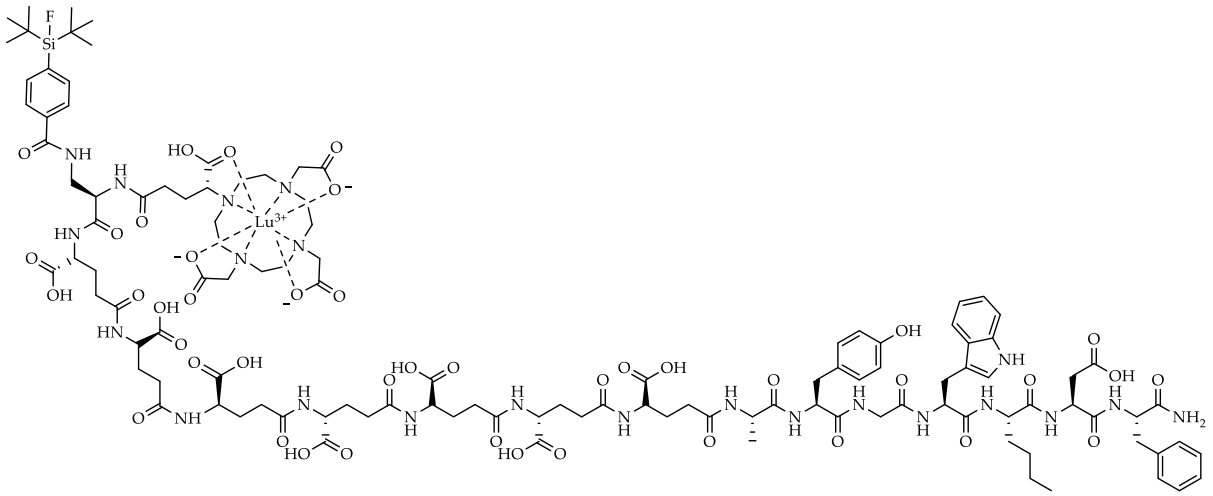


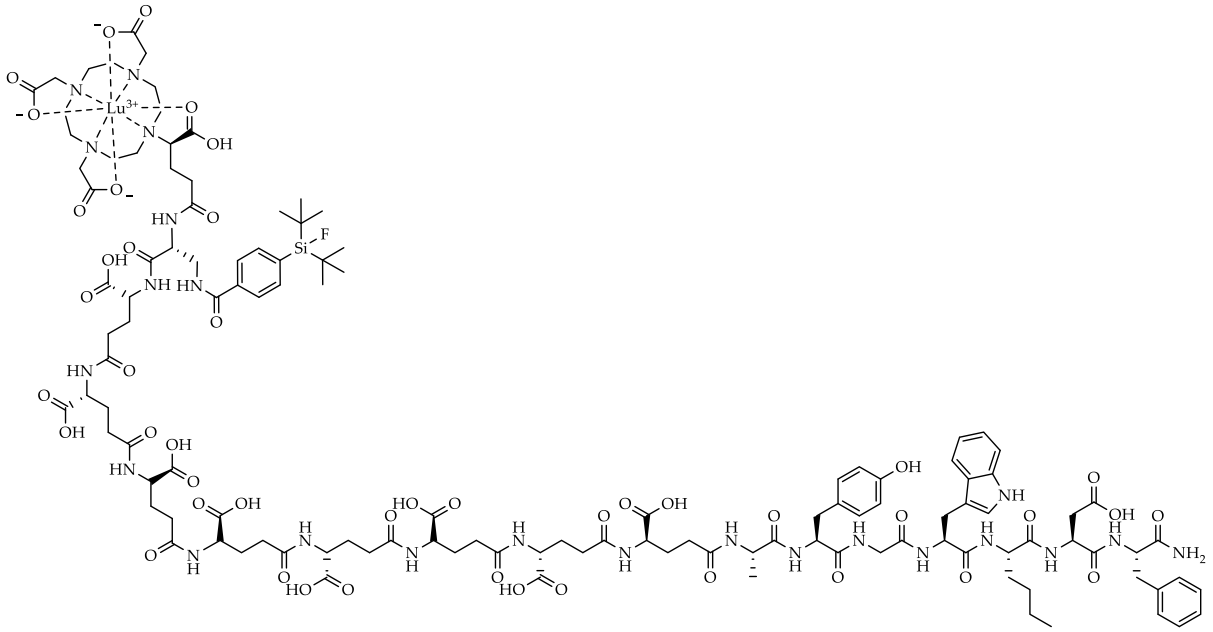

**Figure S3.** Confirmation of peptide identity and integrity for (**a**) [natLu]Lu-(*R*)-DOTAGA-rhCCK-16 and (**b**) [177Lu]Lu-(*R*)-DOTAGA-rhCCK-16 as analysed by analytical (radio-)RP-HPLC (MultoKrom 100-5 C18, 5 µm, 125 × 4.6 mm, CS Chromatographie GmbH, Langerwehe, Germany; 10→90% MeCN in $H_2O$ + 0.1% TFA in 15 min). (**c**) Mass spectrum of [natLu]Lu-(*R*)-DOTAGA-rhCCK-16.



***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-16***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.52 min, K′ = 4.64; MS (ESI, positive): m/z calculated for $C_{111}H_{151}FLuN_{21}O_{39}Si$: 2625.6, found: m/z = 1314.4 $[M+2H]^{2+}$.



***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-17***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.48 min, K′ = 4.62; MS (ESI, positive): m/z calculated for $C_{116}H_{158}FLuN_{22}O_{42}Si$: 2754.7, found: m/z = 1377.7 $[M+2H]^{2+}$.



***[$^{nat}$Lu]Lu-(R)-DOTAGA-rhCCK-18***: RP-HPLC (10→90% MeCN in $H_2O$ with 0.1% TFA, 15 min, $\lambda$ = 220 nm): $t_R$ = 9.50 min, K' = 4.63; MS (ESI, positive): m/z calculated for $C_{121}H_{165}FLuN_{23}O_{45}Si$: 2883.8, found: m/z = 1443.1 $[M+2H]^{2+}$.

**$^{177}$Lu-labelling**

For $^{177}$Lu-labelling experiments, [$^{177}$Lu]$LuCl_3$ dissolved in hydrochloric acid (0.04 M, 40 GBq/mL) was acquired from *ITM Isotope Technologies Munich SE* (Garching, Germany). Radiolabelling of the peptide precursor (1 nmol) was performed at 90 °C for 15 min in a NaOAc-buffered (1 M, pH = 5.5) hydrochloric acid (0.04 M) solution. After radiolabelling, a radiolysis quencher (sodium ascorbate, 1 M in $H_2O$) was added and radiochemical purity was determined via radio-RP-HPLC and radio-TLC (instant thin layer chromatography paper impregnated with silica gel (iTLC-SG, *Agilent Technologies Inc.*, Folsom, United States); sodium citrate*1.5 $H_2O$ (0.1 M)).

***In Vitro* Experiments**

*Cell Culture.* CCK-2R expressing rat pancreatic cancer cells AR42J (*CLS GmbH,* Eppelheim, Germany) were cultivated in monolayers in CELLSTAR® cell culture flasks acquired from *Greiner Bio-One GmbH* (Frickenhausen, Germany) at 37 °C in a humidified atmosphere (5% $CO_2$) using a HERAcell 150i-Incubator (*Thermo Fisher Scientific Inc.*, Waltham, United States). As nutrient medium RPMI 1640 medium, supplemented with 5 mM L-Gln 5 mL non-essential amino acids (100×) and 10% FCS, was used. Furthermore, a Dulbecco's PBS solution with 0.1% EDTA (*v*/*v*) was applied to detach the cells for cell passaging. The detached cells were counted using a Neubauer hemocytometer (*Paul Marienfeld*, Lauda-Königshofen, Germany). In addition, all operations under sterile conditions were accomplished using a MSC-Advantage safety workbench (*Thermo Fisher Scientific Inc*., Waltham, United States).

*Determination of* $IC_{50}$. AR42J cells ($2.0 \times 10^5$ cells/well) were seeded into 24-well plates 24 ± 2 h prior to testing, using 1 mL of nutrient medium (RPMI 1640, 5 mM L-Gln, 5 mL non-essential amino acids (100×), 10% FCS). Cells were incubated at 37 °C in a humidified atmosphere (5% $CO_2$).
After removal of the medium, each well was washed with 500 µL PBS. For the cell-based assay, 200 µL of nutrient medium (+5% BSA), [$^{177}$Lu]Lu-DOTA-PP-F11N (25 µL, 0.3 pmol) as a radiolabeled reference and 25 µL of the peptide of interest in increasing concentrations ($10^{-10}$ to $10^{-4}$ M) in triplicate were added to the cells. Thereafter, the assay was incubated for 3 h at 37 °C and thereafter, the supernatant was collected. The cells were washed with 300 µL PBS and the collected supernatant fractions were unified. After lysis of the cells with NaOH (300 µL, 1 N) for 15 min, the respective wells were washed with NaOH (300 µL, 1 N) and both fractions were unified. The radioactivity of both, the supernatant and the lysed fractions was quantified using a $\gamma$-counter (*PerkinElmer Inc.,* Waltham, United States). The obtained data were evaluated *via* the GraphPad PRISM software (*GraphPad Software Inc*., La Jolla, United States), which calculates the halfmaximal inhibitory concentration ($IC_{50}$) of the peptides.

*Internalisation Studies*. For the determination of the internalisation kinetics of the various peptides, AR42J cells ($3.0 \times 10^5$ cells/well) were seeded into polylysine coated 24-well plates adding 1 mL of nutrient medium. Afterwards, the cells were incubated for 24 ± 2 h at 37 °C in a humidified atmosphere (5% $CO_2$).
On the day of the experiment, the medium was removed, and each well was washed with nutrient medium (300 µL). Afterwards 200 µL of nutrient medium, 25 µL of the $^{177}$Lu-labeled peptide (0.3 pmol, $n = 6$) and either 25 µL of nutrient medium for internalisation studies ($n = 3$) or 25 µL of [$^{nat}$Lu]Lu-DOTA-PP-F11N (10 µmol) for competition studies ($n = 3$) were added. Thereafter, the assay was incubated for various time points (1, 2, 4and 6 h) at 37 °C in a humidified atmosphere (5% $CO_2$). After incubation, the cells were put on ice for at least 1 min to stop internalisation kinetics and the supernatant was collected. Then, the cells were washed with an ice-cold nutrient medium (300 µL) and both fractions were unified. For the acid wash, 300 µL of an ice-cold glycine buffer (1 M, p1 M, pAfter lysis of the cells with NaOH (300 µL, 1 N) for 15 min, the respective wells were washed with NaOH (300 µL, 1 N) and both fractions were unified. The radioactivity of the supernatant, the acid wash and the lysed fractions were quantified using a $\gamma$-counter.

**Table S1.** Affinity and lipophilicity data of the compounds evaluated. Affinity data were determined on AR42J cells ($2.0 \times 10^5$ cells/well) and [$^{177}$Lu]Lu-DOTA-PP-F11N (0.3 pmol/well) as radiolabeled reference (3 h, 37 °C, RPMI 1640, 5 mM L-Gln, 5 mL non-essential amino acids (100×), 10% FCS + 5% BSA (*v*/*v*)).

| peptide | $IC_{50}$ [nM] | log$D_{7.4}$ |
|---|---|---|
| [$^{nat/177}$Lu]Lu-DOTA-PP-F11N | 12.8 ± 2.8 | −4.75 ± 0.07 |
| [$^{nat/177}$Lu]Lu-DOTA-PP-$\gamma$-F11N | 7.55 ± 0.48 | −4.38 ± 0.05 |
| [$^{nat/177}$Lu]Lu-CP04 | 11.2 ± 0.2 | −3.80 ± 0.10 |
| [$^{nat/177}$Lu]Lu-$\gamma$-CP04 | 8.88 ± 0.67 | −4.30 ± 0.08 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-1 | 629 ± 48 | −1.71 ± 0.10 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-2 | 156 ± 18 | −1.95 ± 0.09 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-3 | 156 ± 4 | −2.03 ± 0.10 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-4 | 185 ± 14 | −2.19 ± 0.08 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-5 | 90.0 ± 2.3 | −2.67 ± 0.05 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-6 | 56.8 ± 6.4 | −2.22 ± 0.08 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-7 | 75.7 ± 10.7 | −2.63 ± 0.08 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-8 | 58.0 ± 11.0 | −2.19 ± 0.08 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 | 55.3 ± 7.8 | −2.84 ± 0.04 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-10 | 863 ± 148 | −2.15 ± 0.08 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-11 | 111 ± 13 | −2.23 ± 0.07 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-12 | 89.7 ± 14.5 | −2.34 ± 0.05 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-13 | 68.5 ± 11.4 | −2.57 ± 0.06 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-14 | 51.3 ± 9.3 | −2.26 ± 0.05 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-15 | 33.0 ± 11.1 | −2.50 ± 0.02 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-16 | 20.4 ± 2.7 | −2.54 ± 0.05 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-17 | 29.5 ± 1.9 | −2.10 ± 0.06 |
| [$^{nat/177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-18 | 20.4 ± 2.0 | −2.16 ± 0.09 |

**Table S2.** Receptor-mediated internalisation values (37 °C, RPMI 1640, 5 mM L-Gln, 5 mL non-essential amino acids (100×), 10% FCS, 0.25 pmol/well) determined as percent (%) of the applied activity of [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 and -16 as well as the references [$^{177}$Lu]Lu-DOTA-PP-F11N and [$^{177}$Lu]Lu-CP04 using AR42J cells ($3.0 \times 10^5$ cells/well) at different time points (1, 2, 4 and 6 h). Data are corrected for non-specific binding (10 µmol/well, [$^{nat}$Lu]Lu-DOTA-PP-F11N).

| Peptide | Internalisation Values (%) | | | | Internalisation Values* (% of reference) |
|---|---|---|---|---|---|
| | 1 h | 2 h | 4 h | 6 h | 6 h |
| [$^{177}$Lu]Lu-DOTA-PP-F11N | 6.44 ± 0.32 | 10.1 ± 0.4 | 17.5 ± 1.0 | 22.4 ± 0.6 | - |
| [$^{177}$Lu]Lu-CP04 | 5.16 ± 0.44 | 9.33 ± 0.36 | 15.4 ± 0.7 | 20.5 ± 0.5 | 91.6 ± 2.4 |
| [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 | 2.08 ± 0.12 | 3.44 ± 0.55 | 8.01 ± 0.53 | 13.2 ± 0.5 | 65.7 ± 2.5 |
| [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-16 | 7.63 ± 0.13 | 14.0 ± 1.3 | 23.2 ± 2.7 | 32.2 ± 2.1 | 135 ± 9 |

* Internalisation values are depicted as % of the reference [$^{177}$Lu]Lu-DOTA-PP-F11N

**Table S3.** Total cell uptake (37 °C, RPMI 1640, 5 mM L-Gln, 5 mL non-essential amino acids (100×), 10% FCS, 0.25 pmol/well) determined as percent (%) of the applied activity of [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 and -16 as well as the references [$^{177}$Lu]Lu-DOTA-PP-F11N and [$^{177}$Lu]Lu-CP04 using AR42J cells ($3.0 \times 10^5$ cells/well) at different time points (1, 2, 4 and 6 h). Data are corrected for non-specific binding (10 μmol/well, [$^{nat}$Lu]Lu-DOTA-PP-F11N).

| **Peptide** | **Total cell uptake** (%) | | | |
|---|---|---|---|---|
| | **1 h** | **2 h** | **4 h** | **6 h** |
| [$^{177}$Lu]Lu-DOTA-PP-F11N | 7.03 ± 1.45 | 10.6 ± 1.5 | 18.5 ± 2.5 | 23.5 ± 1.4 |
| [$^{177}$Lu]Lu-CP04 | 6.00 ± 0.42 | 10.0 ± 0.4 | 16.5 ± 0.9 | 20.4 ± 1.7 |
| [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 | 2.25 ± 0.23 | 3.46 ± 0.56 | 8.31 ± 0.59 | 13.4 ± 0.7 |
| [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-16 | 8.37 ± 0.07 | 14.4 ± 1.3 | 23.6 ± 2.8 | 32.8 ± 2.3 |

**Table S4.** Biodistribution data of [$^{177}$Lu]Lu-DOTA-PP-F11N, [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9 and [$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-16 in selected organs at 24 h p.i. in AR42J tumour-bearing CB17-SCID mice (100 pmol each). Data are expressed as %ID/g, mean ± SD.

| **Organ** | **[$^{177}$Lu]Lu-DOTA-PP-F11N** *(n = 4)* | **[$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-9** *(n = 4)* | **[$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-16** *(n = 4)* | **[$^{177}$Lu]Lu-(*R*)-DOTAGA-rhCCK-16 competition studies** *(n = 2)* |
|---|---|---|---|---|
| Blood | 0.00 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.05 ± 0.00 |
| Heart | 0.02 ± 0.01 | 0.09 ± 0.03 | 0.11 ± 0.01 | 0.13 ± 0.01 |
| Lung | 0.03 ± 0.02 | 0.10 ± 0.05 | 0.09 ± 0.02 | 2.59 ± 1.27 |
| Liver | 0.67 ± 0.64 | 1.07 ± 0.54 | 1.18 ± 0.27 | 2.31 ± 0.66 |
| Spleen | 0.07 ± 0.03 | 0.70 ± 0.69 | 0.62 ± 0.03 | 1.73 ± 0.01 |
| Pancreas | 0.05 ± 0.01 | 0.31 ± 0.20 | 0.39 ± 0.12 | 0.20 ± 0.01 |
| Stomach | 0.36 ± 0.05 | 1.80 ± 0.37 | 3.51 ± 0.50 | 0.15 ± 0.04 |
| Intestine | 0.04 ± 0.02 | 0.26 ± 0.04 | 0.20 ± 0.04 | 0.16 ± 0.03 |
| Kidney | 3.08 ± 0.51 | 84.4 ± 22.7 | 85.5 ± 11.3 | 105 ± 21 |
| Adrenal | 0.03 ± 0.03 | 0.45 ± 0.30 | 1.49 ± 1.50 | 0.40 ± 0.11 |
| Muscle | 0.00 ± 0.00 | 0.03 ± 0.01 | 0.05 ± 0.01 | 0.06 ± 0.04 |
| Bone | 0.03 ± 0.01 | 0.82 ± 0.42 | 0.17 ± 0.02 | 0.24 ± 0.04 |
| Tumour | 1.88 ± 0.82 | 6.40 ± 1.48 | 15.70 ± 3.27 | 0.91 ± 0.04 |

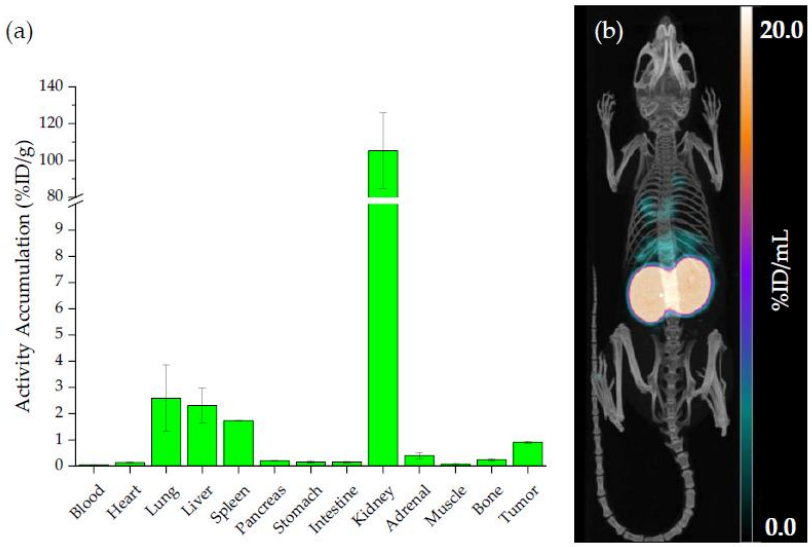



**Figure S4.** (**a**) Biodistribution of [$^{177}$Lu]Lu-DOTA-rhCCK-16 (100 pmol) co-injected with [$^{nat}$Lu]Lu-DOTA-MGS5 (40 nmol) in selected organs (%ID/g) at 24 h p.i. in AR42J tumour-bearing CB17-SCID mice. Data is expressed as mean ± SD ($n = 2$). (**b**) Representative $\mu$SPECT/CT images of [$^{177}$Lu]Lu-DOTA-rhCCK-16 co-injected with [$^{nat}$Lu]Lu-DOTA-MGS5 (40 nmol) at 24 h p.i. in AR42J tumour-bearing CB17- SCID mice.