Supporting information

Design of Bimodal Ligands of Neurotensin Receptor 1 for PET Imaging and Fluorescence-Guided Surgery of Pancreatic Cancer

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Abbreviations

AEEAc : 8-amino-3,6-dioxaoctanoic acid
DCM: Dichloromethane
DIC: *N,N′*-diisopropylcarbodiimide
DIPEA: *N,N*-diisopropylethylamine
DMF: *N,N*-Dimethylformamide
ESI: Electrospray ionization
Fmoc: Fluorenylmethyloxycarbonyl
HPLC: High performance liquid chromatography
HRMS: High resolution mass spectrometry
LRMS: Low resolution mass spectrometry
MALDI-TOF: Matrix-assisted laser desorption ionization - Time of flight
PBS: Phosphate buffer saline
PEG: Polyethylene glycol
TEAB: Triethylammonium bicarbonate
Tle: *tert*-Leucine
TEA: Triethylammonium
TFA: Trifluoroacetic acid
TSTU: *N,N,N′,N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate
UV: Ultraviolet

Materials and general procedures

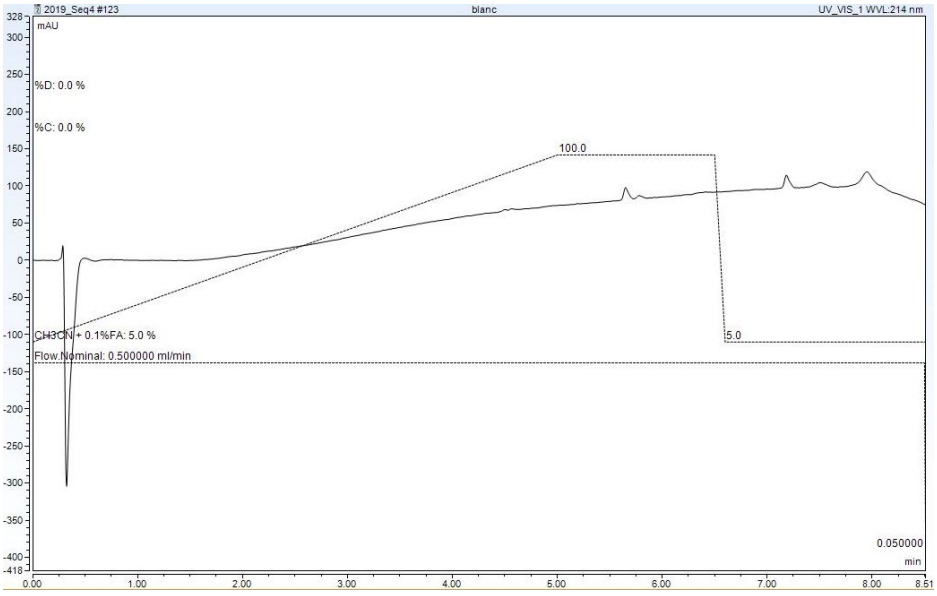
HPLC-gradient grade MeCN was obtained from Biosolve or Carlo Erba. LC-HRMS grade MeCN was obtained from Fisher Scientific. All aqueous buffers used in this work and aqueous mobile-phases for HPLC were prepared using water purified with a PURELAB Ultra system from ELGA (purified to 18.2 MΩ cm).

Purifications by semi-preparative HPLC were performed on an UltiMate 3000 system Dionex (Thermo Scientific) equipped with an UV-visible detector, on one of the following columns: BetaBasic-18 column (Thermo Scientific; 5 µm, 150 Å, 150 × 30 mm) at 20 mL/min or SiliaChrom® dt C_{18} column (Silicycle; 10 µm, 100 Å, 250 × 20 mm) at 20 mL/min, with HPLC grade eluents. The fractions of interest were analyzed by HPLC-MS, pooled, concentrated under reduced pressure to remove organic solvents and freeze-dried.

The peptides and related conjugates were characterized by high performance liquid chromatography (HPLC) analyses performed on an UltiMate 3000 system Dionex (Thermo Scientific) equipped with a DAD detector and coupled to a low-resolution mass spectrometry detector MSQ Plus (Thermo Scientific) equipped with an ESI source. Separation was achieved using an RP Kinetex™ column (Phenomenex) (2.6 µm, 100 Å, 50 × 2.1 mm) with ultrapure water and HPLC-grade MeCN (A: H_2O 0.1% FA and B: MeCN 0.1% FA). Analyses were performed with the following gradient program: 5% to 100% of B in 5 min, 100% B for 1.5 min, 100% to 5% B in 0.1 min and 5% B for 1.9 min, at a flow rate of 0.5 mL/min.

The purity of peptide derivatives was determined from the integration of HPLC-MS chromatograms at 214 nm.

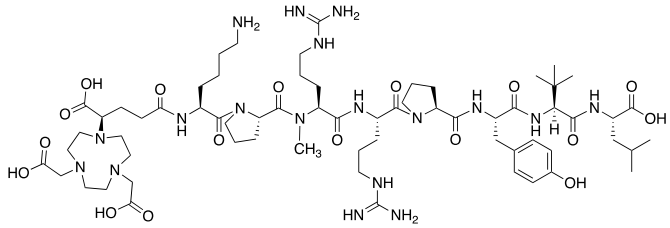
HPLC-MS chromatogram of H_2O injection at 214 nm (blank):



HRMS spectra were recorded on a mass spectrometer LTQ Orbitrap XL (Thermo Scientific) using an ESI source.

UV-visible spectra were obtained on an Agilent Cary 50 UV-Vis-Spectrophotometers by using a rectangular quartz cell (Hellma, 100-QS, 45 × 12.5 × 12.5 mm, pathlength: 10 mm, chamber volume: 3.5 mL), at 23 °C. Fluorescence spectroscopic studies were recorded on a JASCO FP8500 spectrofluorometer in a 10 mm path-length semimicro quartz cuvette (Starna), at 25 °C.

Analytical characterization of conjugates 1-3



Conjugate 1 ((*R*)-NODAGA-Lys-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH.3TFA): 103 mg, 59%, purity 98%. RP-HPLC-MS: t_r = 2.67 min. *m/z* calculated for $C_{65}H_{108}N_{18}O_{17}$ $[M+3H]^{3+}$ 471.94, found 472.2.

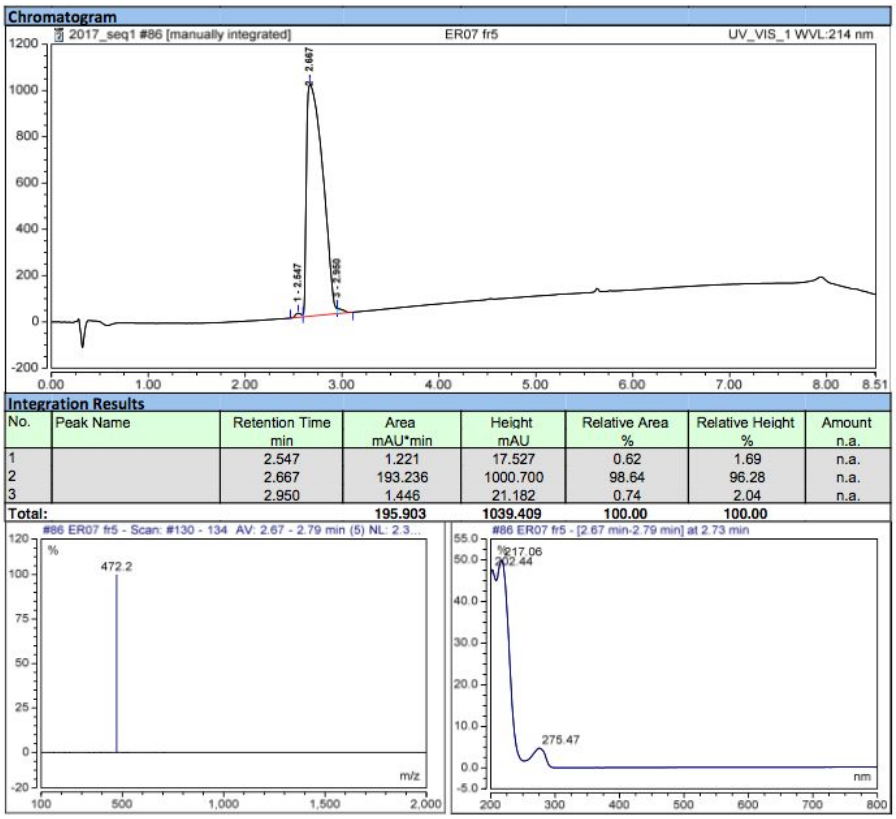
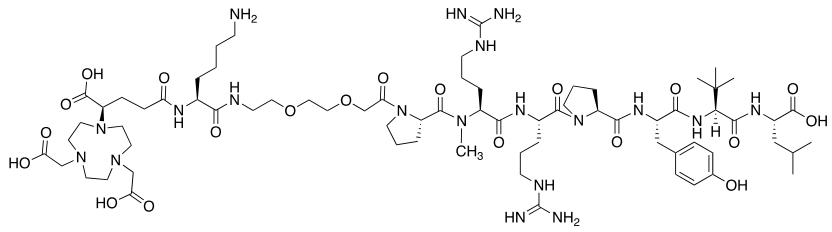


Figure S1. RP-HPLC-MS analysis of conjugate **1**. (Top) RP-HPLC chromatogram. (Bottom) ESI(+)-LRMS and UV-Vis absorbance spectrum.



Conjugate 2 ((*R*)-NODAGA-Lys-AEEAc-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH.3TFA): 81 mg, 43%, purity 98%. RP-HPLC-MS: t_r = 2.73 min. *m/z* calculated for $C_{71}H_{119}N_{19}O_{20}$ $[M+2H]^{2+}$ 779.95, found 780.2.

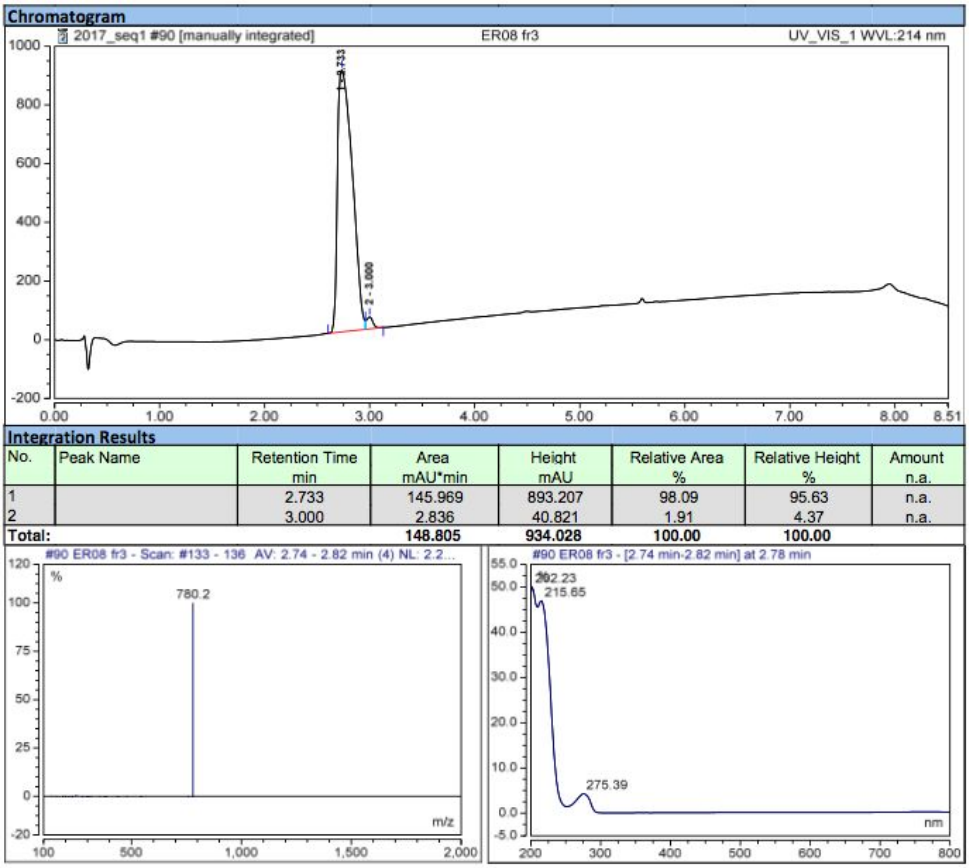
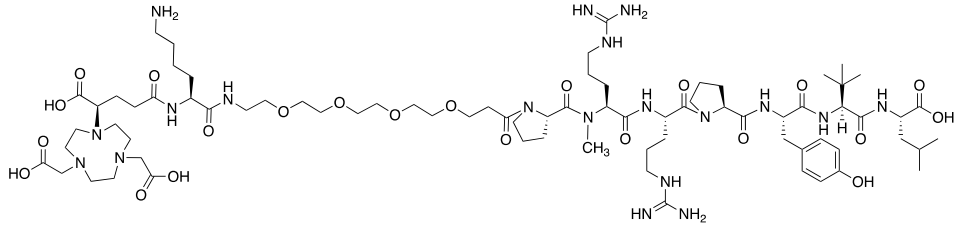


Figure S2. RP-HPLC-MS analysis of conjugate **2**. (Top) RP-HPLC chromatogram. (Bottom) ESI(+)-LRMS and UV-Vis absorbance spectrum.



Conjugate 3 ((*R*)-NODAGA-Lys-PEG_4-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH.3TFA): 138 mg, 69%, purity 90%. RP-HPLC-MS: t_r = 2.9 min. *m/z* calculated for $C_{76}H_{129}N_{19}O_{22}$ $[M+2H]^{2+}$ 830.98, found 830.9; *m/z* calculated for $[M+3H]^{3+}$ 554.32, found 554.6.

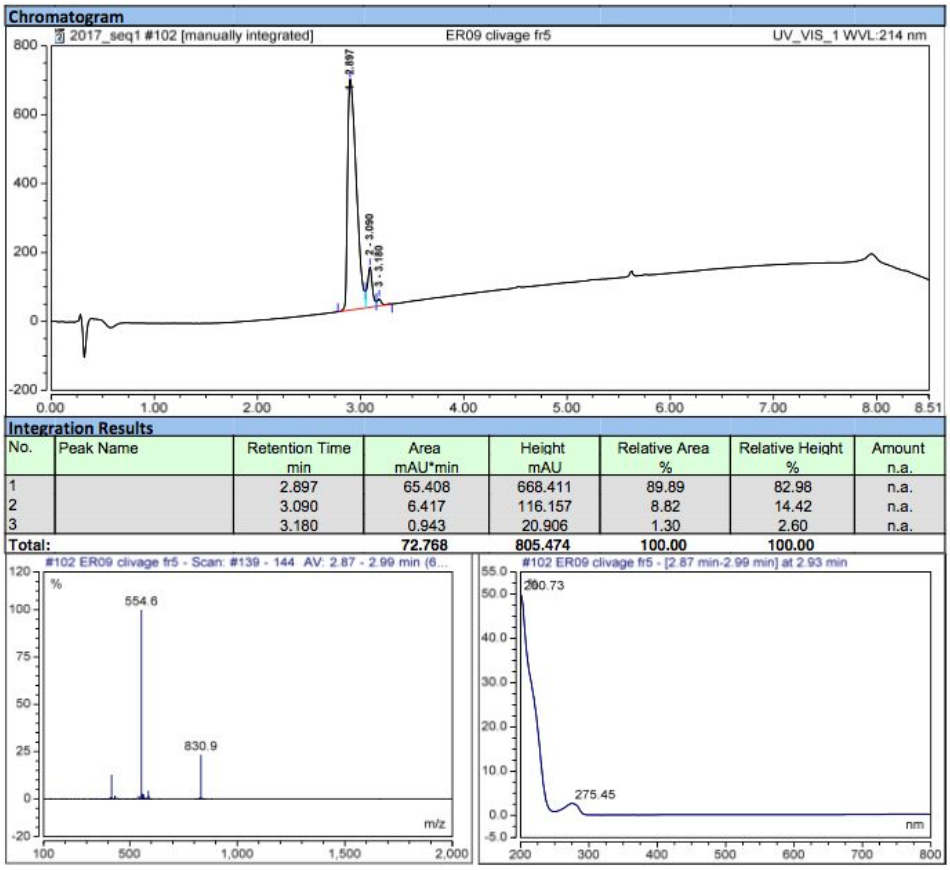
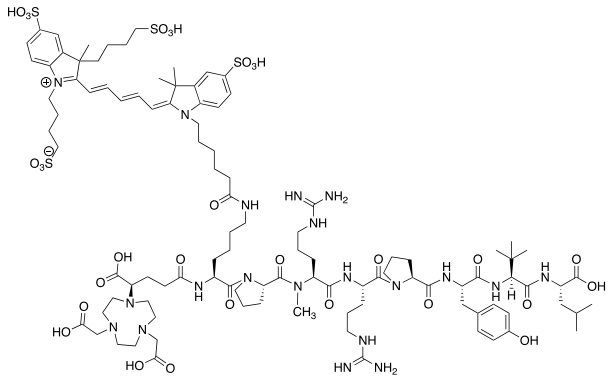


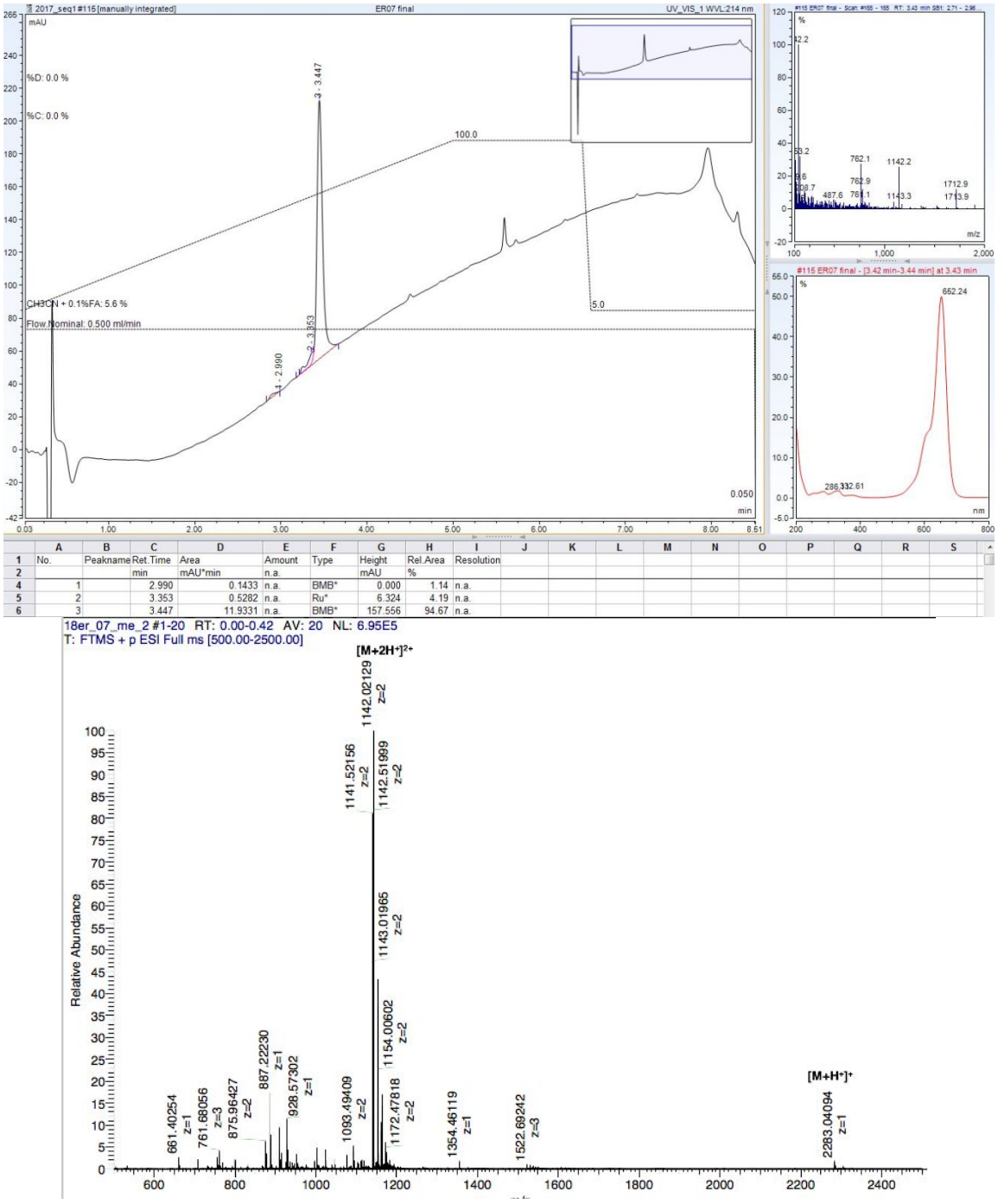
Figure S3. RP-HPLC-MS analysis of conjugate **3**. (Top) RP-HPLC chromatogram. (Bottom) ESI(+)-LRMS and UV-Vis absorbance spectrum.

Synthesis and analytical characterization of conjugates 1a-3a

To a solution of tetrasulfonated cyanine 5.0 Cy5** (1.3 equiv.) in DMF was added DIPEA (7.8 equiv.) and TSTU (1.3 equiv.). The reaction mixture was stirred at room temperature for 1 h. This solution was added to a solution of peptide (1 equiv.) and DIPEA (10 equiv.) in DMF. The reaction mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the solid was purified by semi-preparative HPLC on a SiliaChrom® dt C_{18} column (Silicycle) (A: TEAB 50 mM, B: MeCN with the following gradient program: 5% of B for 10 min, 5% to 60% of B in 60 min, at a flow rate of 20 mL/min) to afford the target peptide.

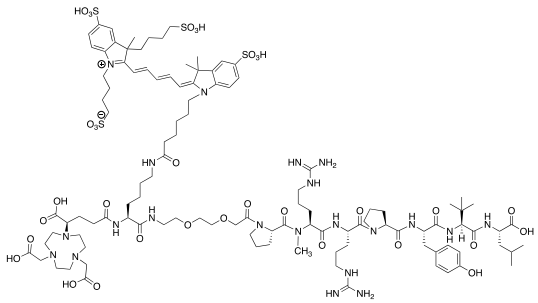


Conjugate 1a (*R*)-NODAGA-(Cy5**)Lys-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH.8TEA: a blue powder was obtained (36.4 mg, 20%, purity: 95%). RP-HPLC-MS: t_r = 3.45 min., HRMS: *m/z* calculated for $C_{103}H_{156}N_{20}O_{30}S_4$ $[M+H]^+$ 2282.02518, found 2282.03806.

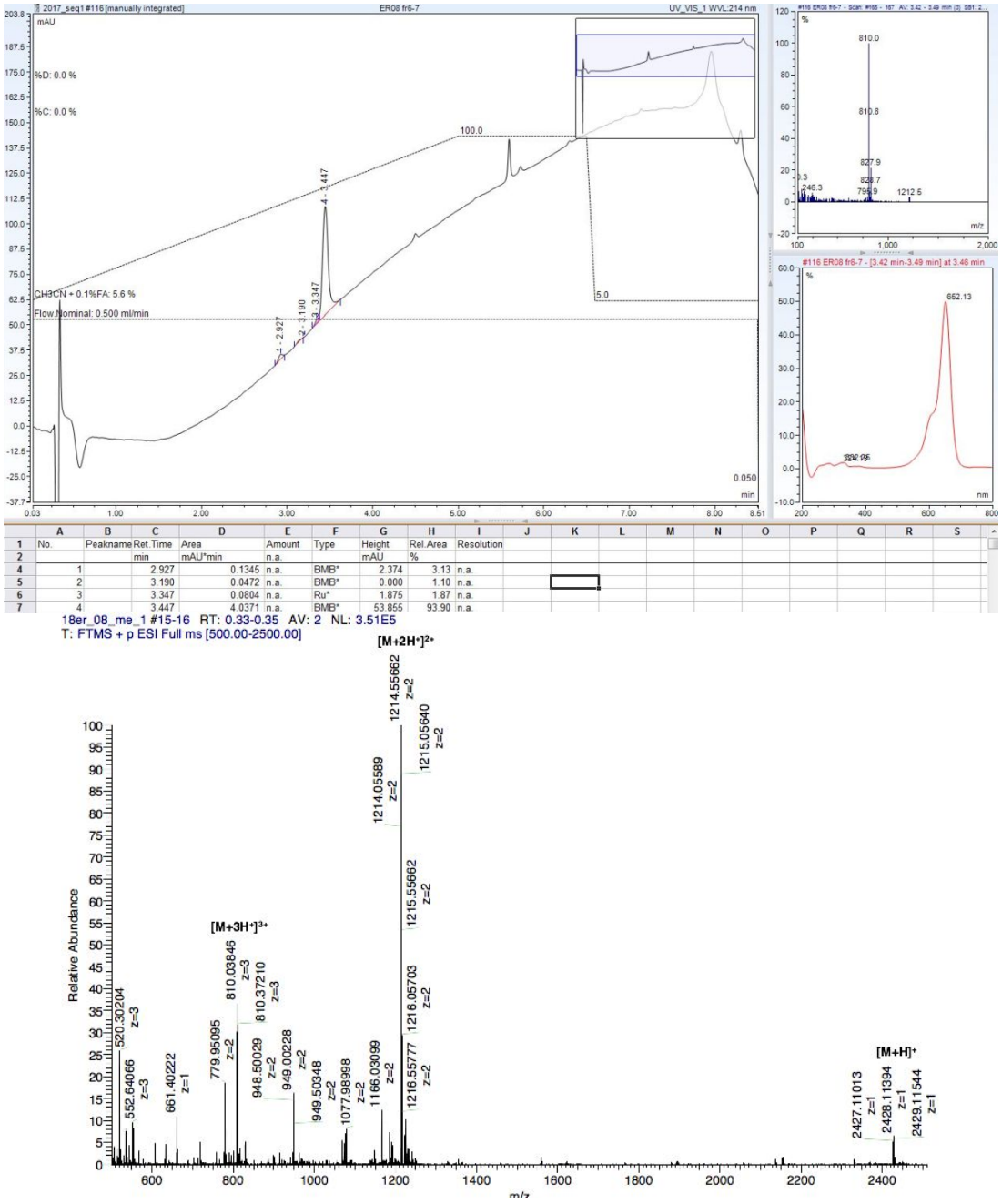


	A	B	C	D	E	F	G	H	I
1	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
2			min	mAU*min	n.a.		mAU	%	
4	1		2.990	0.1433	n.a.	BMB*	0.000	1.14	n.a.
5	2		3.353	0.5282	n.a.	Ru*	6.324	4.19	n.a.
6	3		3.447	11.9331	n.a.	BMB*	157.556	94.67	n.a.

Figure S4. (Top) RP-HPLC chromatogram. (Middle) ESI(+)-LRMS and UV-Vis absorbance spectrum. (Bottom) HRMS analysis of conjugate **1a**.

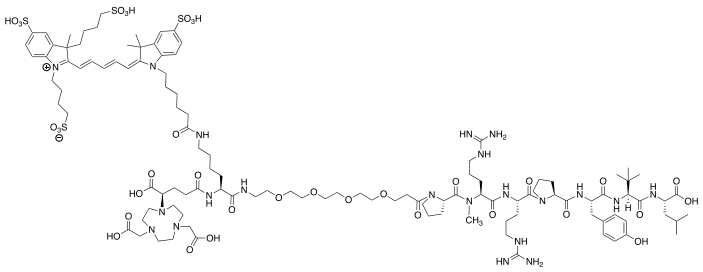


Conjugate 2a (*R*)-NODAGA-(Cy5**)Lys-AEEAc-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH.8TEA: a blue powder was obtained (15.8 mg, 23%, purity 94%). RP-HPLC-MS: t_r = 3.45 min., HRMS: *m/z* calculated for $C_{109}H_{167}N_{21}O_{33}S_4$ $[M+H]^+$ 2427.09908, found 2427.11013.

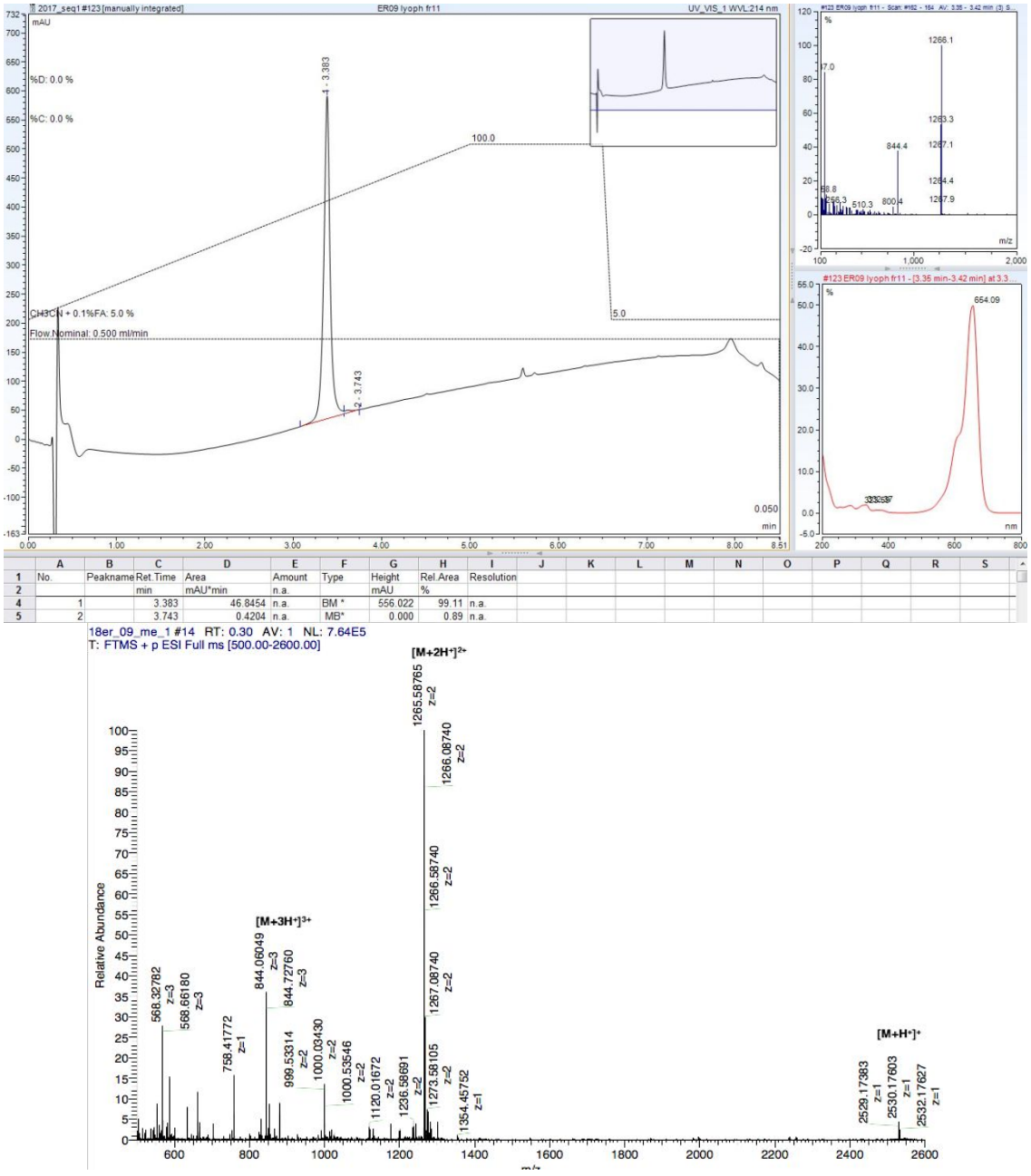


	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
			min	mAU*min	n.a.		mAU	%	
4	1		2.927	0.1345	n.a.	BMB*	2.374	3.13	n.a.
5	2		3.190	0.0472	n.a.	BMB*	0.000	1.10	n.a.
6	3		3.347	0.0804	n.a.	Ru*	1.875	1.87	n.a.
7	4		3.447	4.0371	n.a.	BMB*	53.855	93.90	n.a.

Figure S5. (Top) RP-HPLC chromatogram. (Middle) ESI(+)-LRMS and UV-Vis absorbance spectrum. (Bottom) HRMS analysis of conjugate **2a**.



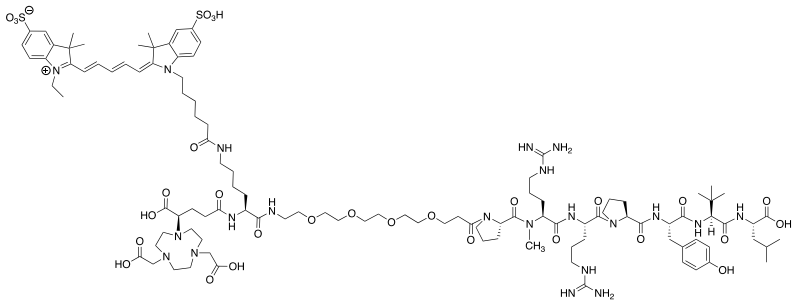
Conjugate 3a (*R*)-NODAGA-(Cy5**)Lys-PEG$_4$-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH.8TEA: a blue powder was obtained (15.4 mg, 18%, purity 99%). RP-HPLC-MS: t_r = 3.38 min., HRMS: *m/z* calculated for $C_{114}H_{177}N_{21}O_{35}S_4$ $[M+H]^+$ 2529.16715, found 2529.17383.



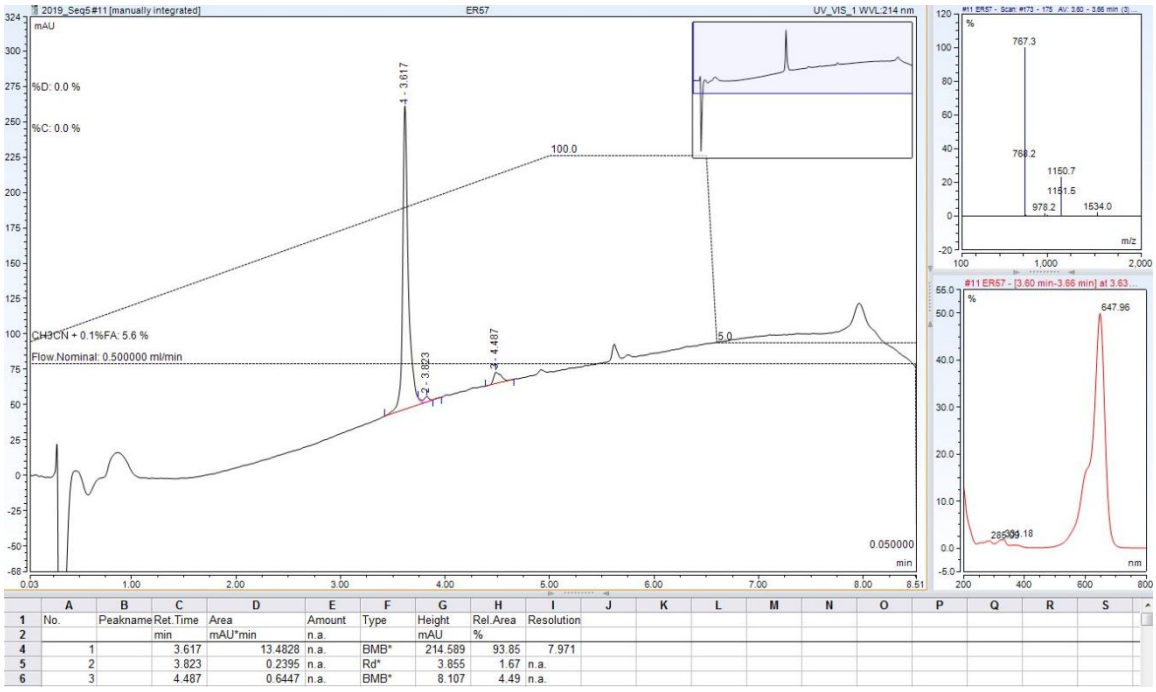
	A	B	C	D	E	F	G	H	I
1	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
2			min	mAU*min	n.a.		mAU	%	
4	1		3.383	46.8454	n.a.	BM *	556.022	99.11	n.a.
5	2		3.743	0.4204	n.a.	MB*	0.000	0.89	n.a.

Figure S6. (Top) RP-HPLC chromatogram. (Middle) ESI(+)-LRMS and UV-Vis absorbance spectrum. (Bottom) HRMS analysis of conjugate **3a**.

Synthesis and analytical characterization of conjugate 3b



To a solution of disulfonated cyanine 5.0 (8.7 mg, 0.013 mmol, 1.1 equiv.) in DMF (1 mL) was added DIPEA (8.4 µL, 0.048 mmol, 4 equiv.) and TSTU (4.3 mg, 0.014 mmol, 1.2 equiv.). The reaction mixture was stirred at room temperature for 1 h. This solution was added to a solution of (*R*)-NODAGA-Lys-PEG_4-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH (20 mg, 0.012 mmol, 1 equiv.) and DIPEA (21 µL, 0.12 mmol, 10 equiv.) in DMF (1 mL). The reaction mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the solid was purified by semi-preparative HPLC on a SiliaChrom® dt C_{18} column (Silicycle) (A: H_2O 0.1% TFA, B: MeCN 0.1% TFA with the following gradient program: 15% of B for 10 min, 15% to 60% of B in 60 min, at a flow rate of 20 mL/min) to give a blue powder (TFA salt, 14 mg, 48%, purity: 94%). RP-HPLC-MS: t_r = 3.62 min., *m/z* calculated for $C_{109}H_{167}N_{21}O_{29}S_2$ $[M+3H]^{3+}$ 767.39, found 767.3. HRMS: *m/z* calculated for $C_{109}H_{167}N_{21}O_{29}S_2$ $[M+2H]^{2+}$ 1150.09128, found 1150.09588.



	A	B	C	D	E	F	G	H	I
1	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
2			min	mAU*min	n.a.		mAU	%	
4	1		3.617	13.4828	n.a.	BMB*	214.589	93.85	7.971
5	2		3.823	0.2395	n.a.	Rd*	3.855	1.67	n.a.
6	3		4.487	0.6447	n.a.	BMB*	8.107	4.49	n.a.

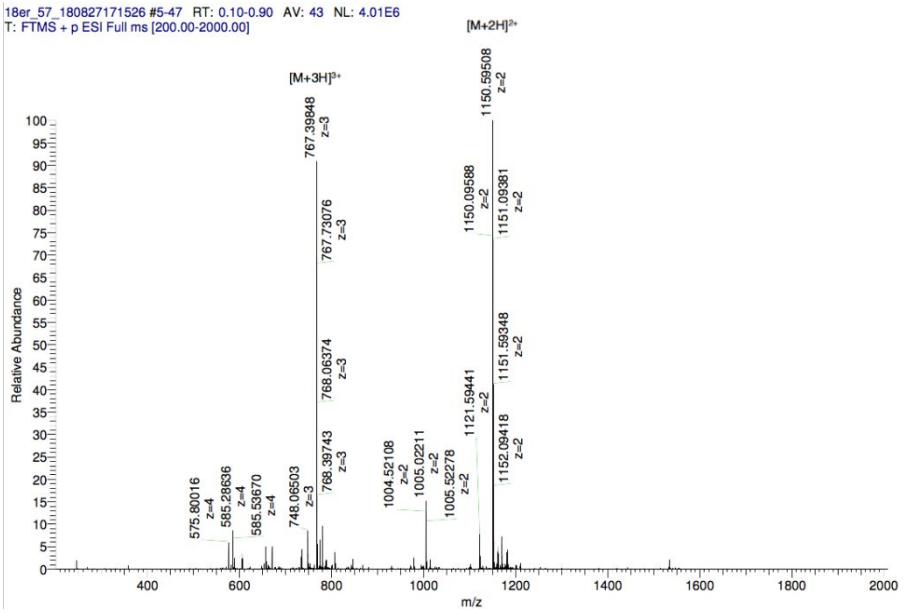
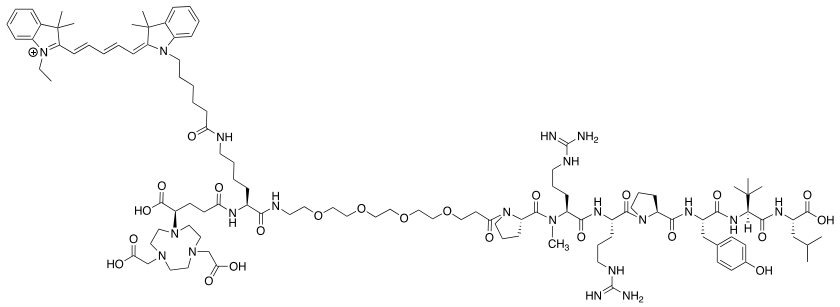
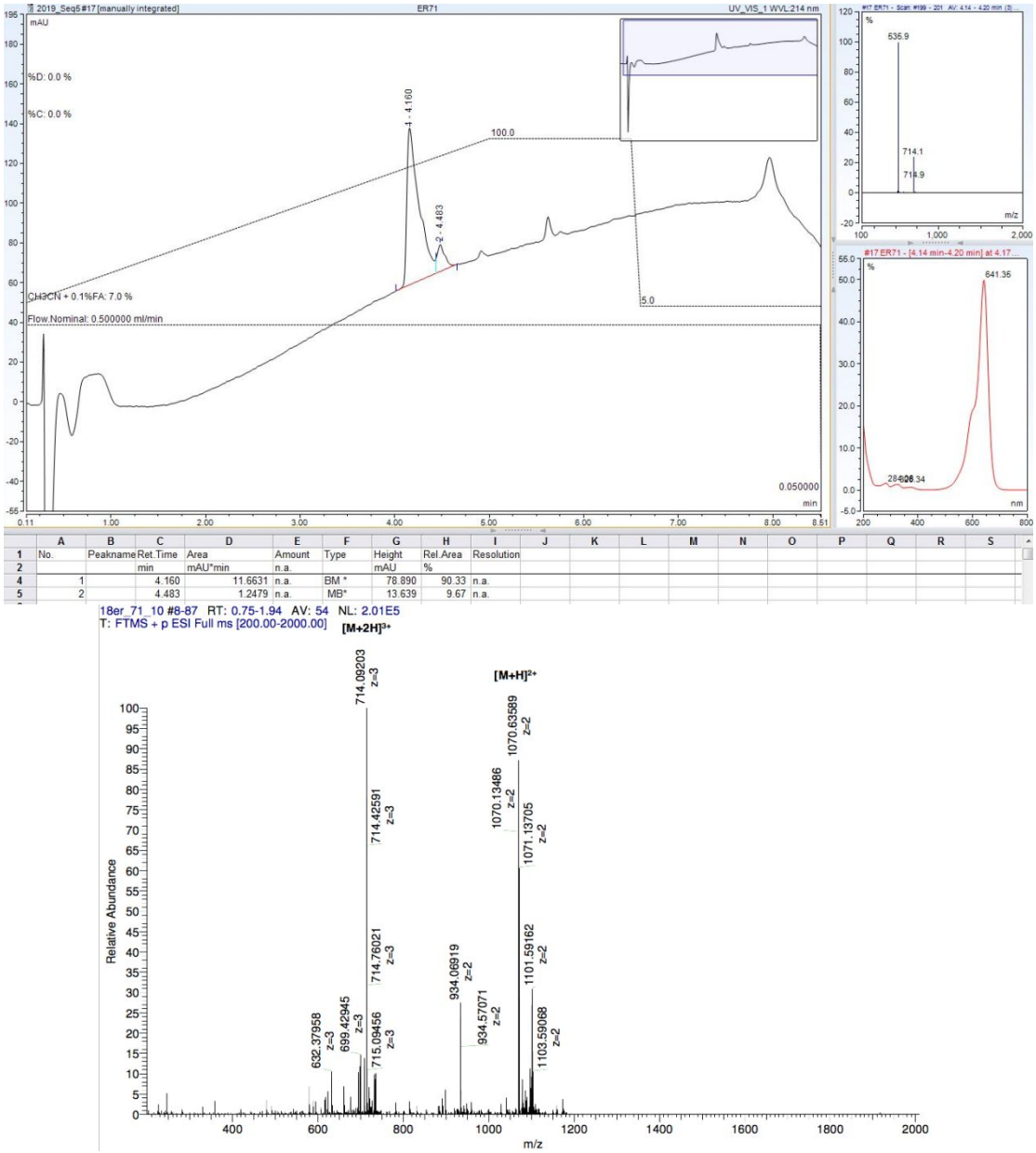


Figure S7. (Top) RP-HPLC chromatogram. (Middle) ESI(+)-LRMS and UV-Vis absorbance spectrum. (Bottom) HRMS analysis of conjugate **3b**.

Synthesis and analytical characterization of conjugate 3c



To a solution of cyanine Cy5 (3.55 mg, 0.0072 mmol, 1.2 equiv.) in DMF (0.5 mL) was added DIPEA (2.1 µL, 0.012 mmol, 2 equiv.) and TSTU (2.35 mg, 0.0078 mmol, 1.3 equiv.). The reaction mixture was stirred at room temperature for 1 h. This solution was added to a solution of (*R*)-NODAGA-Lys-PEG$_4$-Pro-N-Me-Arg-Arg-Pro-Tyr-Tle-Leu-OH (10 mg, 0.006 mmol, 1 equiv.) and DIPEA (11 µL, 0.06 mmol, 10 equiv.) in DMF (0.5 mL). The reaction mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the solid was purified on semi-preparative HPLC on a SiliaChrom® dt C_{18} column (Silicycle) (A: H_2O 0.1% TFA, B: MeCN 0.1% TFA with the following gradient program: 20% of B for 10 min, 20% to 70% of B in 70 min, at a flow rate of 20 mL/min) afforded to a blue powder (TFA salt, 5.4 mg, 38%, purity: 90%). RP-HPLC-MS: t_r = 4.16 min., *m/z* calculated for $C_{109}H_{168}N_{21}O_{23}$ $[M+3H]^{3+}$ 714.42, found 714.2. HRMS: *m/z* calculated for $C_{109}H_{168}N_{21}O_{23}$ $[M+2H]^{2+}$ 1070.13446, found 1070.13486.



	A	B	C	D	E	F	G	H	I
1	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
2			min	mAU*min	n.a.		mAU	%	
4	1		4.160	11.6631	n.a.	BM *	78.890	90.33	n.a.
5	2		4.483	1.2479	n.a.	MB*	13.639	9.67	n.a.

Figure S8. (Top) RP-HPLC chromatogram. (Middle) ESI(+)-LRMS and UV-Vis absorbance spectrum. (Bottom) HRMS analysis of conjugate **3c**.

Spectral properties of compounds 2a, 3b and 3c

UV-Vis absorbance and fluorescence spectra of compounds were recorded in the range 200-800 nm in PBS (pH 7.4, 0.01 M) with concentrations in the micromolar range. Emission spectra were recorded in the range 630-800 nm after excitation at 620 nm (shutter: Auto Open, Ex. Slit = 5 nm and Em. slit = 5 nm). Excitation spectra were recorded in the range 200-800 nm with emission measurement at 690 nm (shutter: Auto Open, Ex. slit = 5 nm and Em. slit = 5 nm).

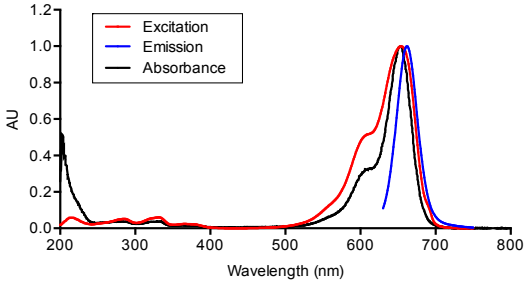


Figure S9. Normalized photophysical spectra (absorbance, excitation and emission) of compound **2a**. $\lambda_{Ex,\ max}$ = 652-653 nm, $\lambda_{Em,\ max}$ = 665-666 nm.

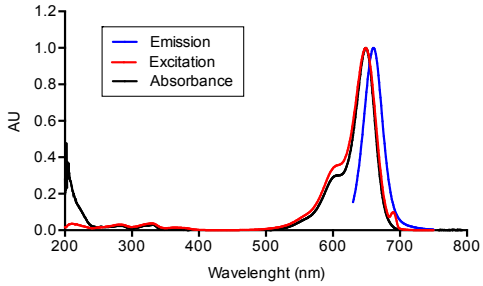


Figure S10. Normalized photophysical spectra (absorbance, excitation and emission) of the compound **3b**. $\lambda_{Ex,\ max}$ = 649 nm, $\lambda_{Em,\ max}$ = 661 nm.

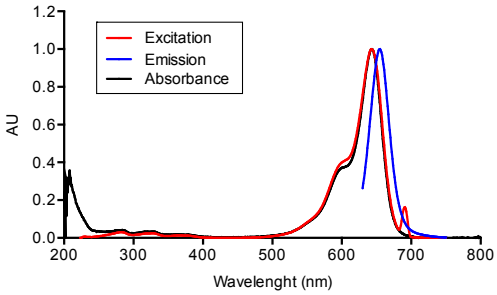
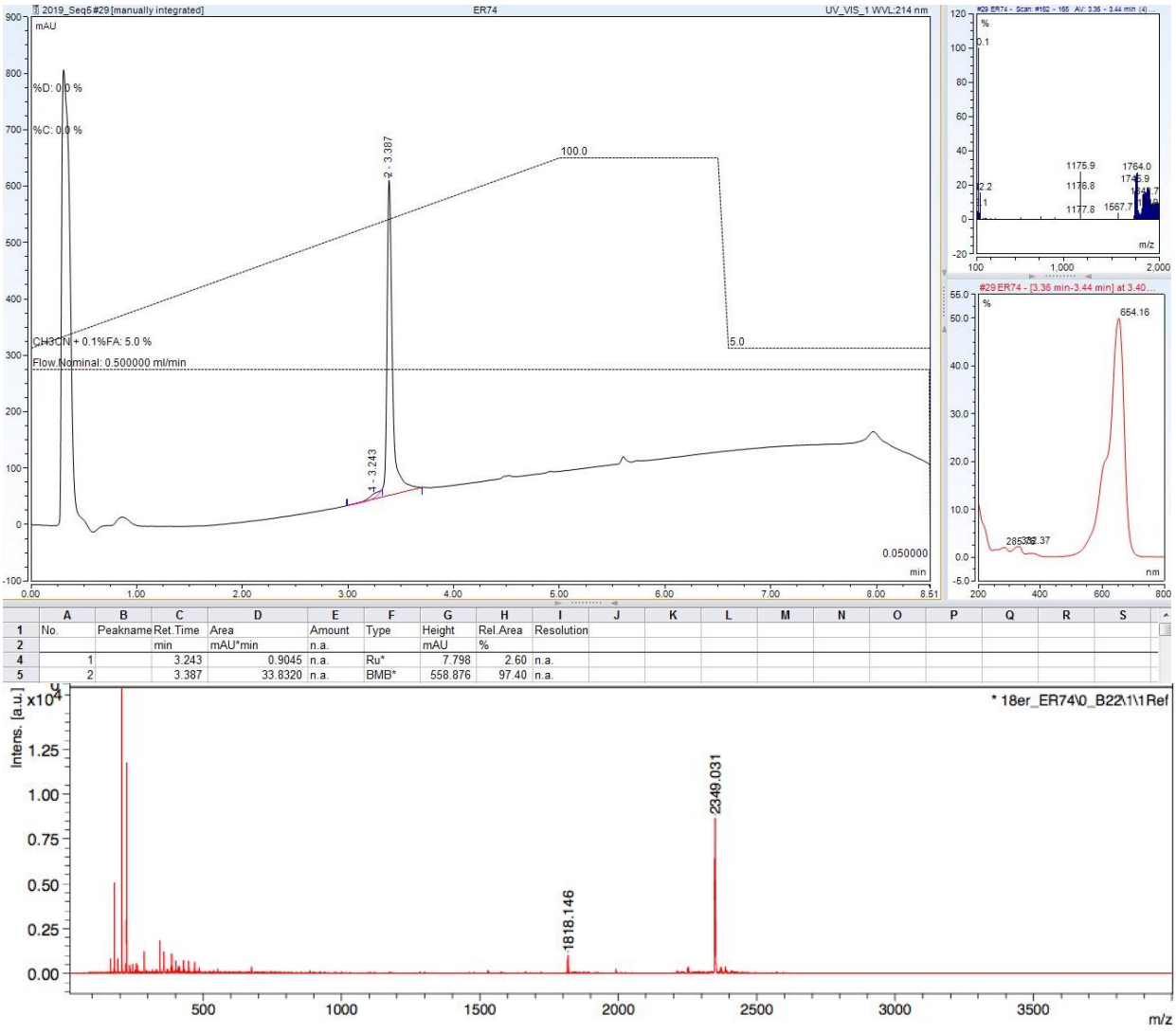


Figure S11. Normalized photophysical spectra (absorbance, excitation and emission) of the compound **3c**. $\lambda_{Ex,\ max}$ = 643 nm, $\lambda_{Em,\ max}$ = 655 nm.

Metalation of peptides with ^{nat}Ga

The compound was dissolved in acetate buffer (0.1 M, pH 3.48). A solution of $Ga(NO_3)_3$ (1.5 equiv.) in acetate buffer was then added, eventually with a small amount of acetonitrile to improve the solubility. The reaction mixture was stirred for 5 h at 40 °C. The excess of free gallium was removed by semi-preparative RP-HPLC.

^{nat}Ga-**1a**: on a SiliaChrom® dt C_{18} column (Silicycle) (A: H_2O 0.1% TFA, B: MeCN 0.1% TFA with the following gradient program: 10% to 60% of B in 50 min, at a flow rate of 20 mL/min). The TFA salt was obtained as a blue powder (6 mg, 59%, purity: 97%). RP-HPLC-MS: t_r = 3.37 min. MALDI-TOF: *m/z* calculated for $C_{103}H_{153}GaN_{20}O_{30}S_4$ $[M+H]^+$ 2348.92, found 2349.03.



	A	B	C	D	E	F	G	H	I
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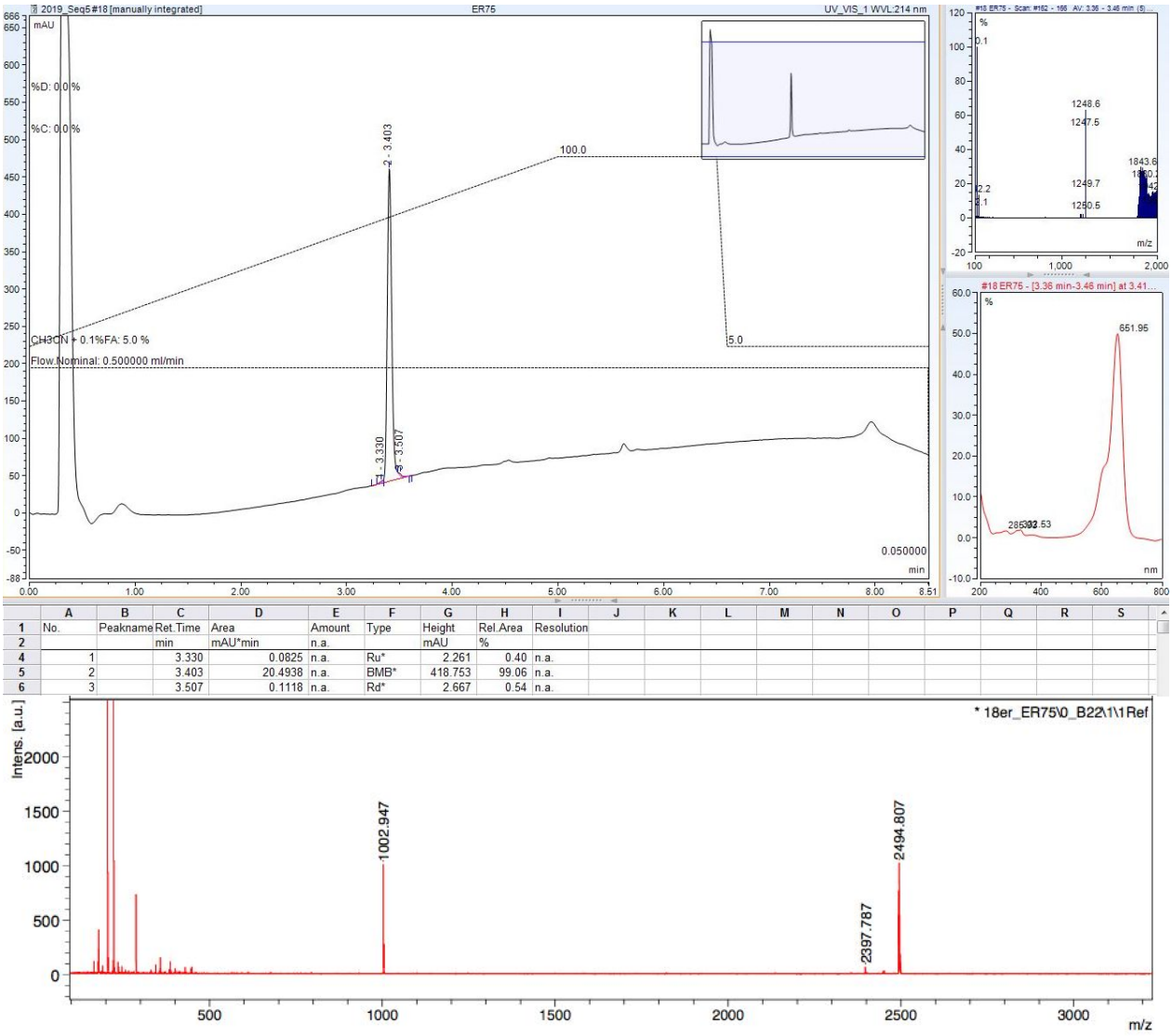
2			min	mAU*min	n.a.		mAU	%	
4	1		3.243	0.9045	n.a.	Ru*	7.798	2.60	n.a.
5	2		3.387	33.8320	n.a.	BMB*	558.876	97.40	n.a.

Figure S12. (Top) RP-HPLC chromatogram. (Bottom) MALDI-TOF analysis of conjugate ^{nat}Ga-**1a**.

^{nat}Ga-**2a**: on a SiliaChrom® dt C_{18} column (Silicycle) (A: TEAB 50 mM, B: MeCN with the following gradient program: 10% to 60% of B in 50 min, at a flow rate of 20 mL/min). The tetratriethylammonium salt was obtained as a blue powder (8 mg, 84%, purity 99%). RP-HPLC-MS: t_r = 3.4 min. MALDI-TOF: *m/z* calculated for $C_{109}H_{164}GaN_{21}O_{33}S_4$ $[M+H]^+$ 2494.0, found 2494.81.



	A	B	C	D	E	F	G	H	I
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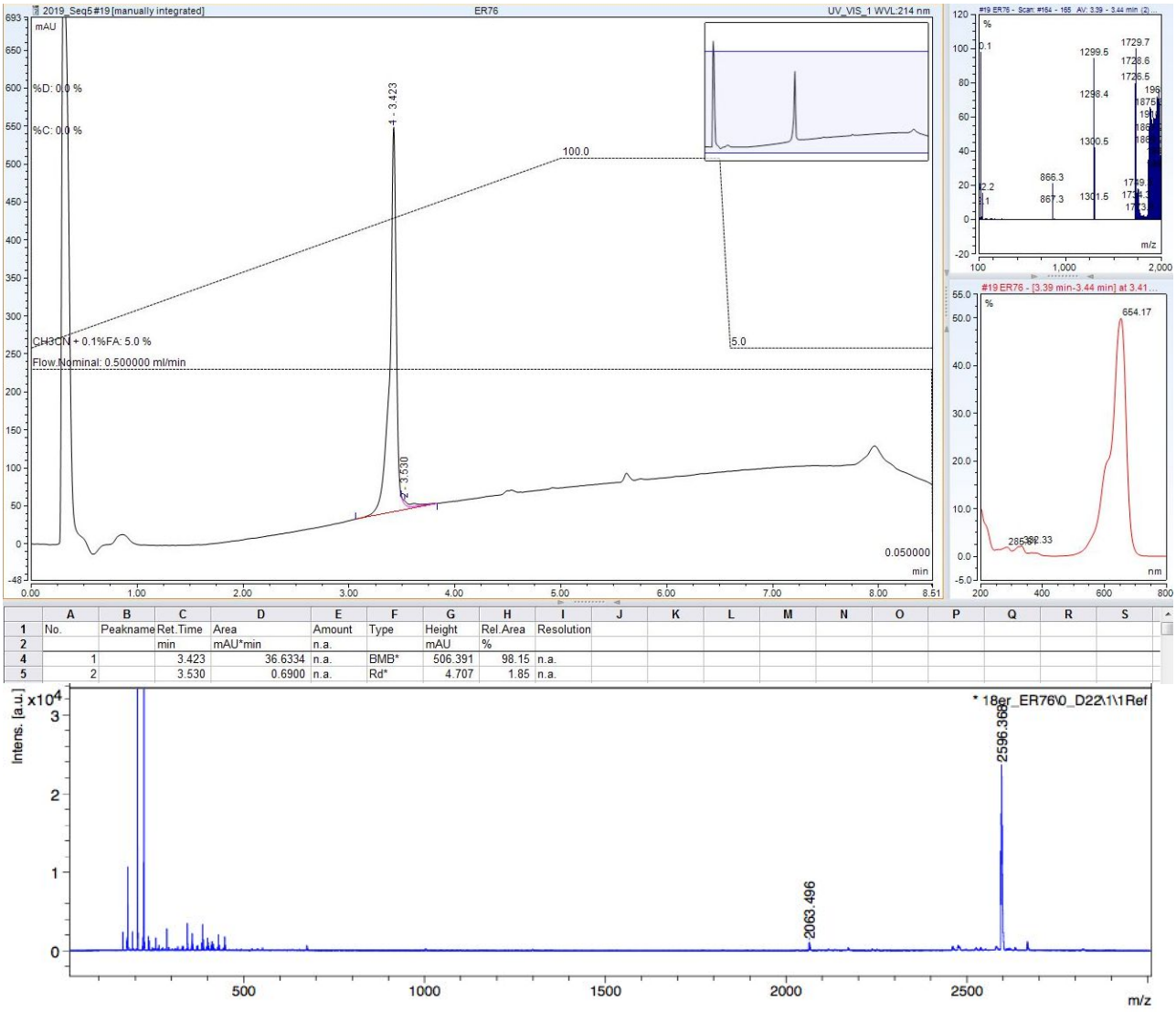
2			min	mAU*min	n.a.		mAU	%	
4	1		3.330	0.0825	n.a.	Ru*	2.261	0.40	n.a.
5	2		3.403	20.4938	n.a.	BMB*	418.753	99.06	n.a.
6	3		3.507	0.1118	n.a.	Rd*	2.667	0.54	n.a.

Figure S13. (Top) RP-HPLC chromatogram. (Bottom) MALDI-TOF analysis of conjugate ^{nat}Ga-**2a**.

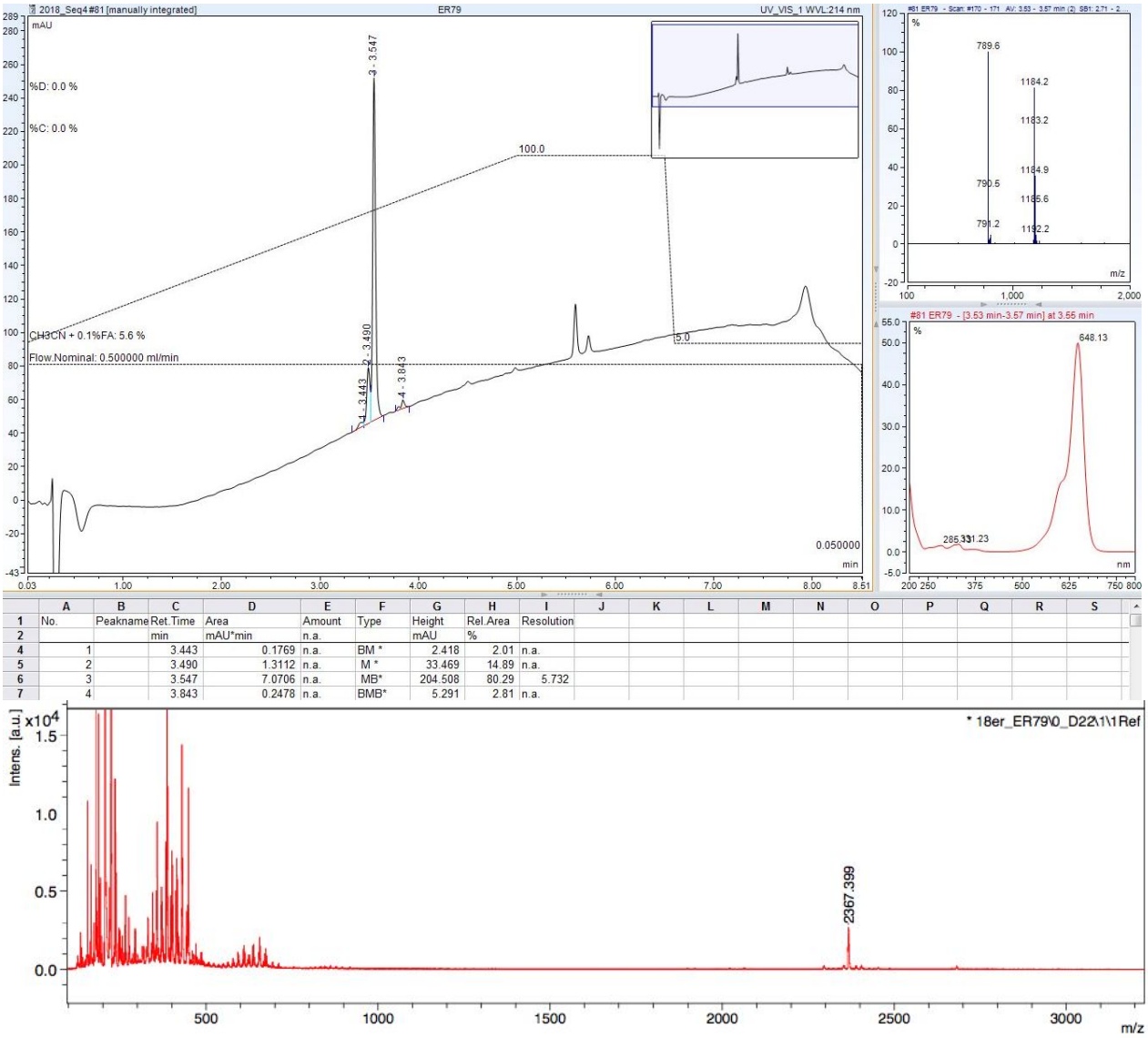
^{nat}Ga-**3a**: on a SiliaChrom® dt C_{18} column (Silicycle) (A: TEAB 50 mM, B: MeCN with the following gradient program: 10% to 60% of B in 50 min, at a flow rate of 20 mL/min). The tetratriethylammonium salt was obtained as a blue powder (7 mg, 74%, purity 98%). RP-HPLC-MS: t_r = 3.42 min. MALDI-TOF: *m/z* calculated for $C_{114}H_{174}GaN_{21}O_{35}S_4$ $[M+H]^+$ 2596.07, found 2596.37.



	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
			min	mAU*min	n.a.		mAU	%	
4	1		3.423	36.6334	n.a.	BMB*	506.391	98.15	n.a.
5	2		3.530	0.6900	n.a.	Rd*	4.707	1.85	n.a.

Figure S14. (Top) RP-HPLC chromatogram. (Bottom) MALDI-TOF analysis of conjugate ^{nat}Ga-**3a**.

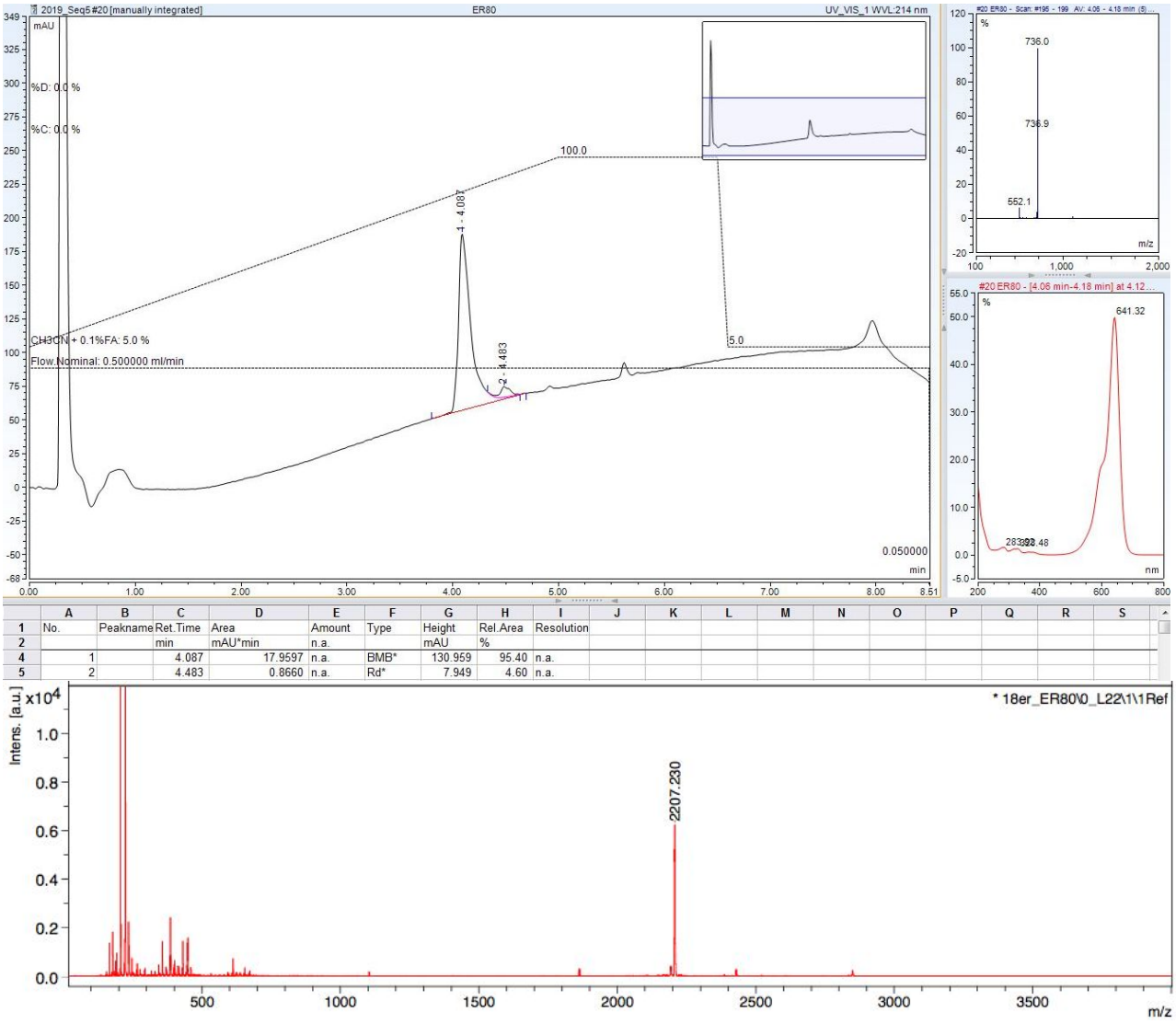
^{nat}Ga-**3b**: on a SiliaChrom® dt C_{18} column (Silicycle) (A: H_2O 0.1% TFA, B: MeCN 0.1% TFA with the following gradient program: 10% to 60% of B in 50 min, at a flow rate of 20 mL/min). The TFA salt was obtained as a blue powder (4 mg, 76%, purity: 80%). RP-HPLC-MS: t_r = 3.55 min. MALDI-TOF : *m/z* calculated for $C_{109}H_{164}GaN_{21}O_{29}S_2$ $[M+H]^+$ 2366.07, found 2367.40.



No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min	n.a.		mAU	%	
1		3.443	0.1769	n.a.	BM *	2.418	2.01	n.a.
2		3.490	1.3112	n.a.	M *	33.469	14.89	n.a.
3		3.547	7.0706	n.a.	MB*	204.508	80.29	5.732
4		3.843	0.2478	n.a.	BMB*	5.291	2.81	n.a.

Figure S15. (Top) RP-HPLC chromatogram. (Bottom) MALDI-TOF analysis of conjugate ^{nat}Ga-**3b**.

^{nat}Ga-**3c**: on a SiliaChrom® dt C_{18} column (Silicycle) (A: H_2O 0.1% TFA, B: MeCN 0.1% TFA with the following gradient program: 30% to 70% of B in 40 min, at a flow rate of 20 mL/min). The TFA salt was obtained as a blue powder (3.3 mg, 68%, purity: 95%). RP-HPLC-MS: t_r = 4.09 min. MALDI-TOF: *m/z* calculated for $C_{109}H_{165}GaN_{21}O_{23}$ $[M+H]^+$ 2207.17, found 2207.23.



	A	B	C	D	E	F	G	H	I
1	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
2			min	mAU*min	n.a.		mAU	%	
4	1		4.087	17.9597	n.a.	BMB*	130.959	95.40	n.a.
5	2		4.483	0.8660	n.a.	Rd*	7.949	4.60	n.a.

Figure S16. (Top) RP-HPLC chromatogram. (Bottom) MALDI-TOF analysis of conjugate ^{nat}Ga-**3c**.

In vitro stability

Samples were analyzed on a RP Kinetex™ column (Phenomenex) (2.6 µm, 100 Å, 150 × 2.1 mm) with ultrapure water and HPLC-grade MeCN (A: H_2O 0.1% FA and B: MeCN 0.1% FA). Analyses were performed with the following gradient program: 5% to 100% of B in 23 min, 100% B for 4 min at a flow rate of 0.3 mL/min.

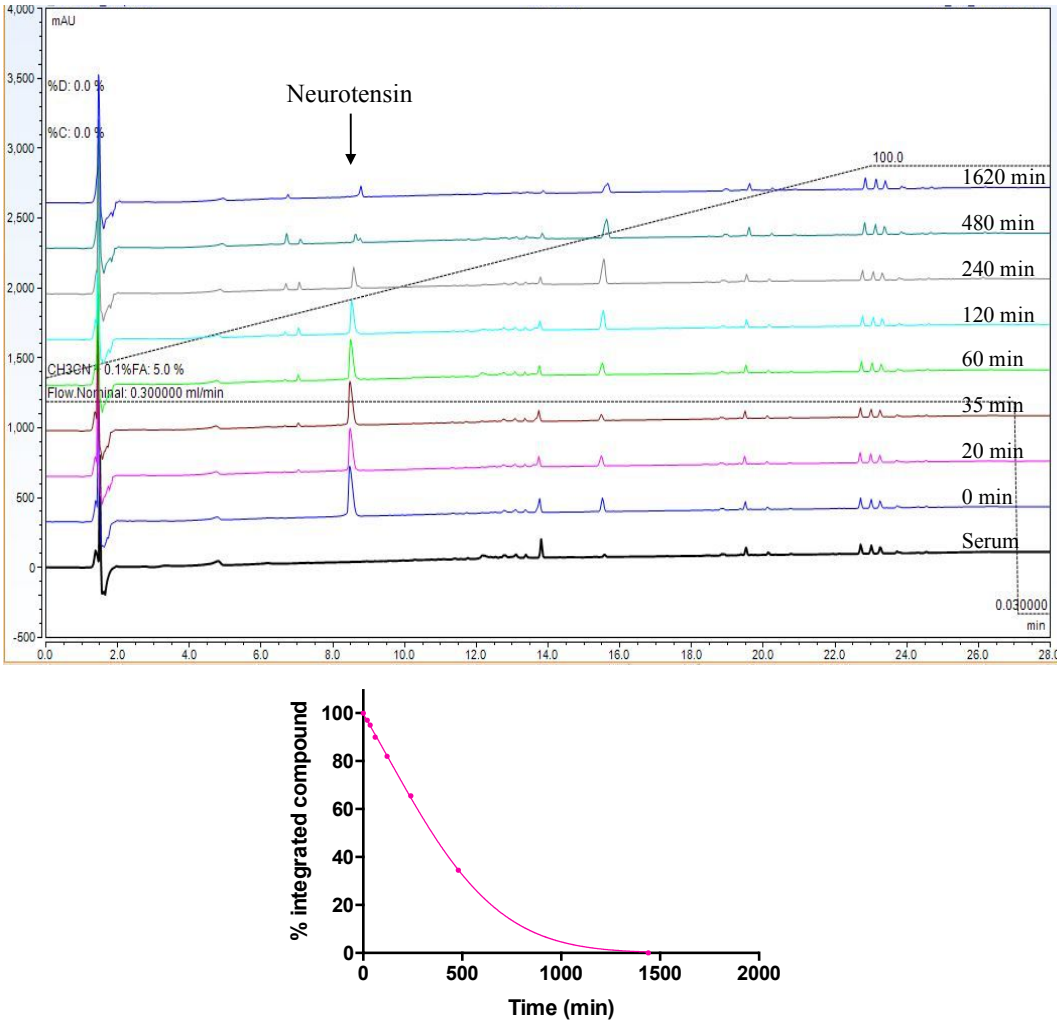


Figure S17. (Top) HPLC chromatogram of neurotensin after 0 min, 20 min, 35 min, 1 h, 2 h, 4 h, 8 h and 27 h of incubation. (Bottom) Time-dependent changes in the amount of intact neurotensin upon incubation in mouse serum at 37 °C.

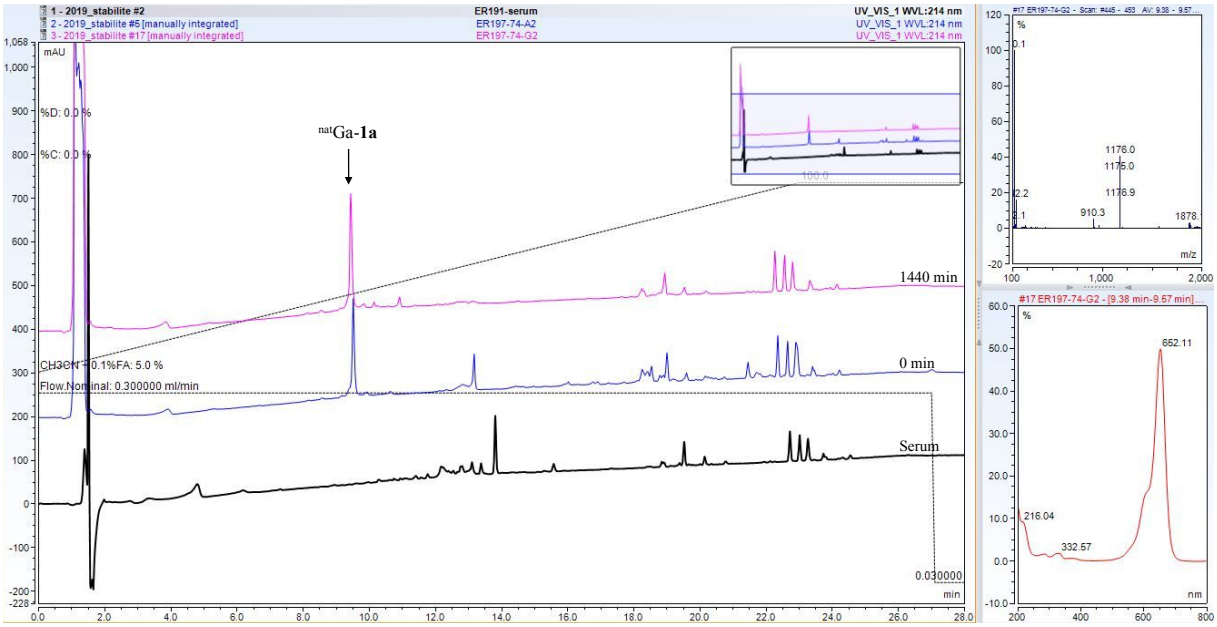


Figure S18. HPLC chromatogram of ^{nat}Ga-**1a** after 0 min and 24 h of incubation in mouse serum at 37 °C.

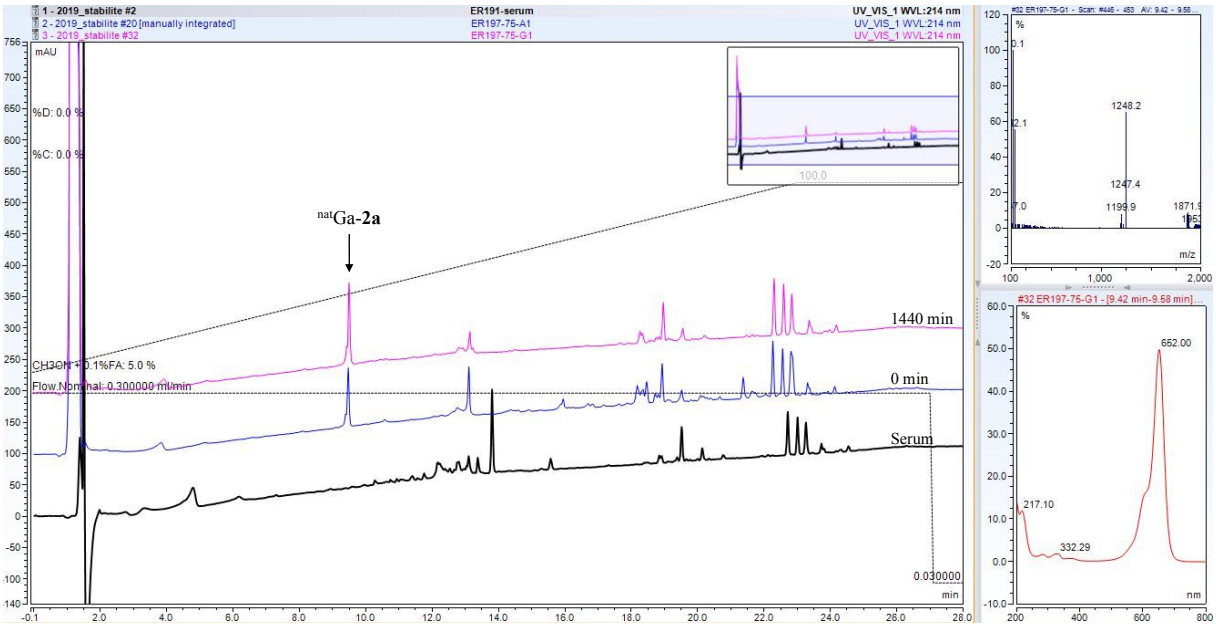


Figure S19. HPLC chromatogram of ^{nat}Ga-**2a** after 0 min and 24 h of incubation in mouse serum at 37 °C.

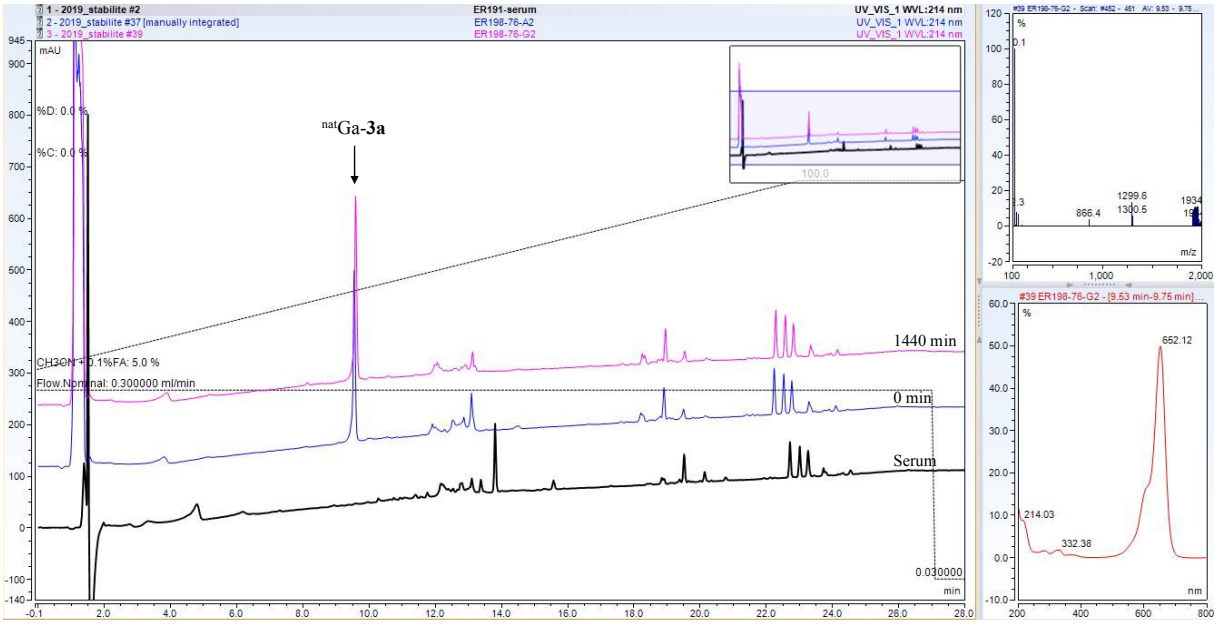


Figure S20. HPLC chromatogram of ^{nat}Ga-**3a** after 0 min and 24 h of incubation in mouse serum at 37 °C.

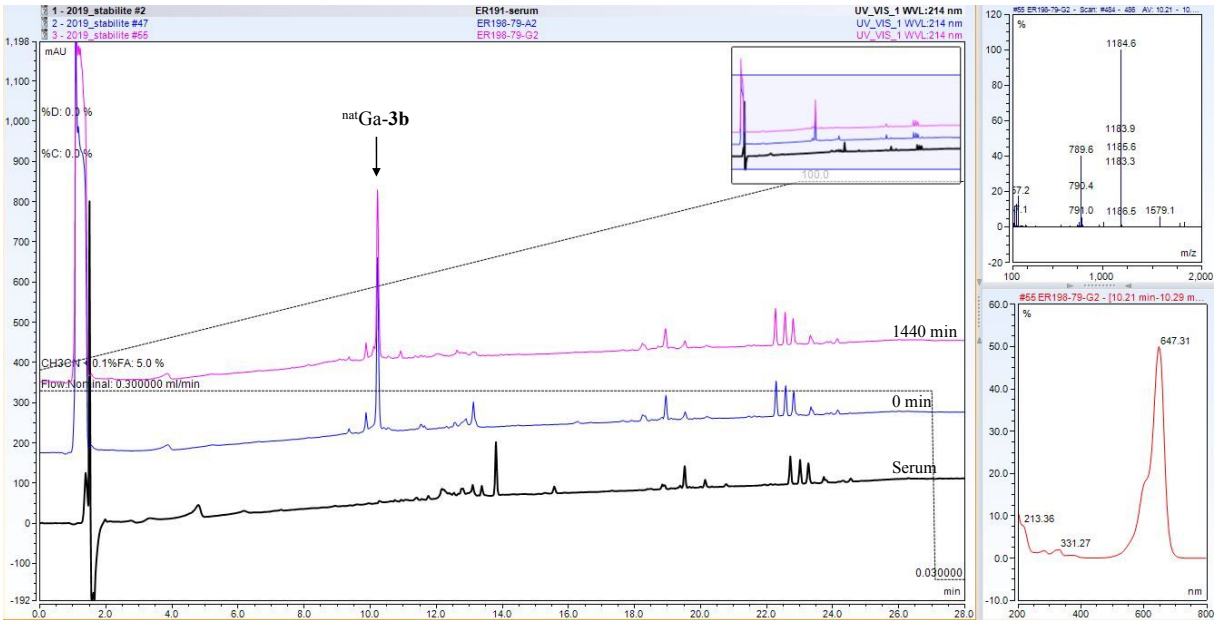


Figure S21. HPLC chromatogram of ^{nat}Ga-**3b** after 0 min and 24 h of incubation in mouse serum at 37 °C.

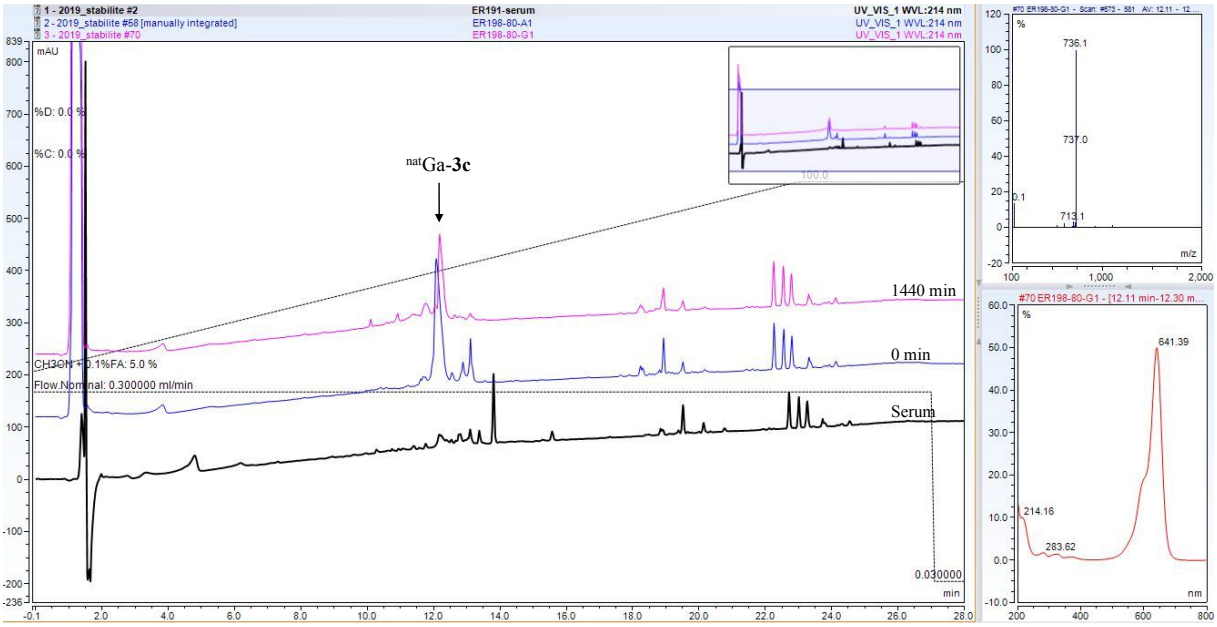


Figure S22. HPLC chromatogram of ^{nat}Ga-**3c** after 0 min and 24 h of incubation in mouse serum at 37 °C.

Radiolabeling of peptides with ^{68}Ga

Compound	Radiochemical yield	Molar activity (MBq/nmol)	Radiochemical purity
1a (n=3)	69 % ± 30	7.2 ± 2.2	> 99%
2a (n=4)	90 % ± 3	7.4 ± 0.2	> 99%
3a (n=4)	84 % ± 16	10.7 ± 4.3	> 99%
3b (n=2)	51 % ± 46	5.1 ± 5.1	> 99%
3c (n=2)	69 % ± 4	3.5 ± 1.3	> 99%

Table S1. Summary of radiochemical yields, molar activities and radiochemical purities.

Small animal PET imaging and biodistribution studies

	[^{68}Ga]Ga**1a**	[^{68}Ga]Ga**2a**	[^{68}Ga]Ga**3a**	[^{68}Ga]Ga-DOTA-NT-20.3[a]
Uptake (%ID/g)	n=3	n=3	n=3	n=4
Blood	0.317 ± 0.091	0.283 ± 0.097	0.450 ± 0.361	0.99 ± 0.44
Heart	0.198 ± 0.025	0.210 ± 0.061	0.313 ± 0.176	0.52 ± 0.10
Lung	0.307 ± 0.141	0.217 ± 0.126	0.397 ± 0.083	0.68 ± 0.16
Spleen	0.518 ± 0.525	0.203 ± 0.047	0.337 ± 0.035	0.34 ± 0.07
Liver	0.142 ± 0.054	0.303 ± 0.031	0.240 ± 0.036	0.31 ± 0.09
Kidneys	17.409 ± 5.068	16.690 ± 3.528	17.490 ± 1.129	5.38 ± 0.54
Pancreas	0.137 ± 0.036	0.090 ± 0.017	0.187 ± 0.025	0.22 ± 0.03
Stomach	0.275 ± 0.215	0.263 ± 0.081	0.840 ± 1.109	0.14 ± 0.03
Intestine	0.570 ± 0.199	0.377 ± 0.146	0.450 ± 0.108	0.53 ± 0.08
Colon	0.229 ± 0.273	0.110 ± 0.056	0.240 ± 0.141	0.41 ± 0.23
Muscle	0.198 ± 0.095	0.107 ± 0.058	0.210 ± 0.173	0.29 ± 0.02
Bone	0.512 ± 0.426	0.130 ± 0.035	0.283 ± 0.157	0.49 ± 0.11
Tumor	2.976 ± 0.497	2.563 ± 0.965	0.947 ± 0.257	5.28 ± 0.93

	[^{68}Ga]Ga**1a**	[^{68}Ga]Ga**2a**	[^{68}Ga]Ga**3a**	[^{68}Ga]Ga-DOTA-NT-20.3[a]
Uptake	n=3	n=3	n=3	n=4
Tumor/Blood	9.881 ± 2.865	9.030 ± 2.525	3.140 ± 2.539	5.93 ± 1.82
Tumor/Muscle	18.293 ± 10.386	27.963 ± 9.683	6.130 ± 3.543	18.21 ± 4.01
Tumor/Kidneys	0.18 ± 0.06	0.17 ± 0.08	0.05 ± 0.02	1 ± 0.26
Tumor/Pancreas	23.805 ± 11.700	28.340 ± 10.394	5.103 ± 1.621	24.56 ± 6.18

Table S2. (Top) Ex vivo biodistribution of [^{68}Ga]Ga**1a,** [^{68}Ga]Ga**2a**, [^{68}Ga]Ga**3a** and [^{68}Ga]Ga-DOTA-NT-20.3. Values are expressed as the percentage of injected dose per gram of tissue (%ID/g ± SD). (Bottom) Tumor-to-organs ratios from the ex vivo biodistribution. [a]Values are derived from Prignon et al., *Mol. Pharmaceutics* **2019,** *16*, 2776-2784.

Near-Infrared Fluorescence Imaging

	Injected Dose	1 h	3 h	24 h
Tumor	50 pmol	$1.62^{E}7$	-	-
	500 pmol	$3.90^{E}7 \pm 6.58^{E}6$	$3.77^{E}7$	$1.41^{E}7$
	5 nmol	$2.44^{E}8 \pm 6.86^{E}6$	$1.5^{E}8$	$9.98^{E}6$

	Injected Dose	1 h	3 h	24 h
Muscle	50 pmol	$6.97^{E}6$	-	-
	500 pmol	$8.09^{E}6 \pm 2.04^{E}6$	$5.83^{E}6$	$7.44^{E}6$
	5 nmol	$1.68^{E}7 \pm 1.61^{E}6$	$9.98^{E}6$	$1.32^{E}7$

	Injected Dose	1 h	3 h	24 h
Pancreas	50 pmol	$7.68^{E}6$	-	-
	500 pmol	$8.40^{E}6 \pm 2.46^{E}5$	$7.72^{E}6$	$6.62^{E}6$
	5 nmol	$1.60^{E}7 \pm 2.83^{E}5$	$1.45^{E}7$	$1.15^{E}7$

	Injected Dose	1 h	3 h	24 h
Kidneys	50 pmol	$2.44^{E}7$	-	-
	500 pmol	$8.44^{E}7 \pm 1.70^{E}7$	$1.16^{E}8$	$6.99^{E}7$
	5 nmol	$4.90^{E}8 \pm 6.72^{E}7$	$5.53^{E}8$	$7.47^{E}8$

	Injected Dose	1 h	3 h	24 h
Ratio Tumor/Muscle	50 pmol	2.33	-	-
	500 pmol	4.87 ± 0.42	6.46	1.90
	5 nmol	12.64 ± 0.96	15.02	2.96

	Injected Dose	1 h	3 h	24 h
Ratio Tumor/Pancreas	50 pmol	2.11	-	-
	500 pmol	4.63 ± 0.65	4.88	2.14
	5 nmol	15.27 ± 0.16	10.30	3.40

Table S3. Summary table of fluorescence signals measured from the images of ex vivo organs, 1 h, 3 h, 24 h post injection of 50 pmol, 500 pmol or 5 nmol of **2a**. Values are expressed as the average radiant efficiency for each organ ([p/s/cm²/sr] / [µW/cm²]).

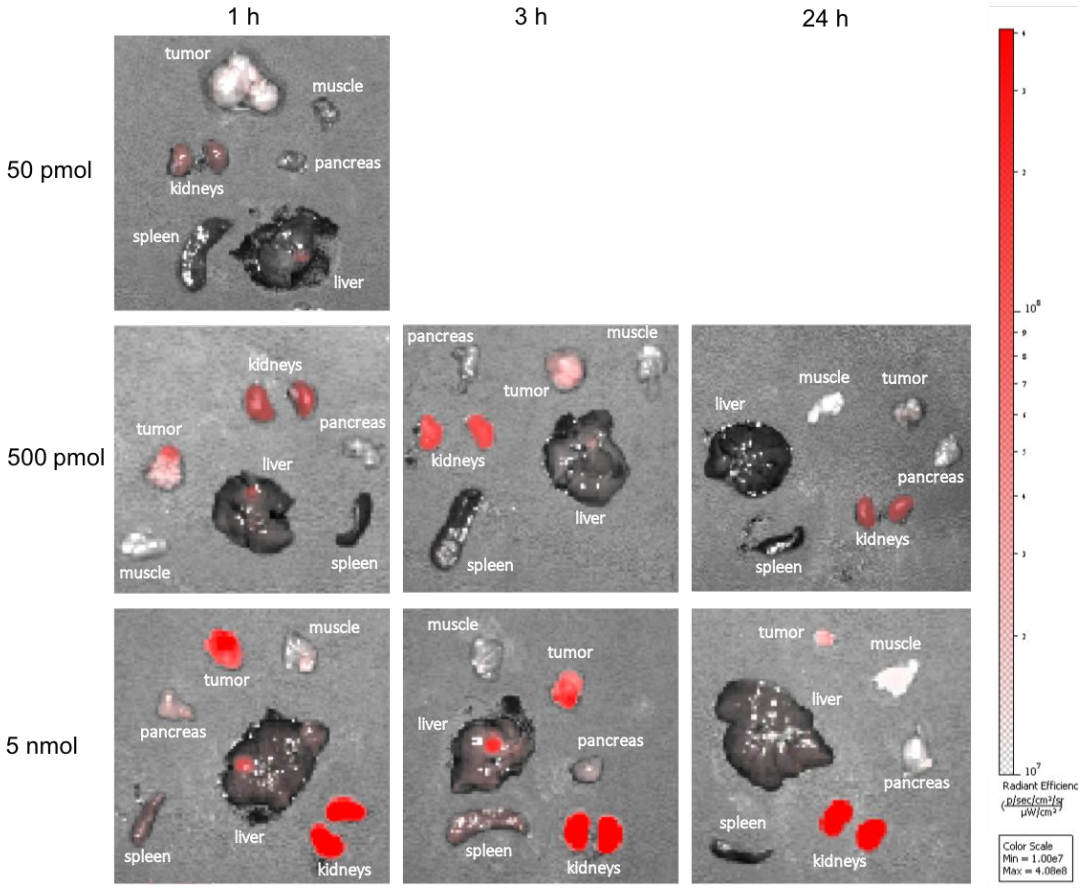


Figure S23. Overlays of optical and fluorescence images of isolated organs after injection of 50 pmol, 500 pmol and 5 nmol of **2a** at 1 h, 3 h and 24 h post injection. White light images and fluorescence signal were captured using an IVIS Lumina Imaging System.