Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

for

Design of RGD-ATWLPPR peptide conjugates for dual targeting of $\alpha_v\beta_3$ integrin and neuropilin-1

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TABLE OF CONTENTS

1) Reagents and materials

All Fmoc amino acid derivatives and resins were purchased from Advanced ChemTech Europe Brussels, Belgium), Bachem Biochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). PyBOP was purchased from France Biochem.

Tetrasulfate-Cyanine 5.5 was obtained from Interchim (Montluçon, France), and other reagents were obtained from either Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France).

RP-HPLC analyses were performed on Waters equipment consisting of a Waters 600 controller, a Waters 2487 Dual Absorbance Detector and a Waters In-Line Degasser. The analytical column used was a Nucleosil 120 Å 3µm C18 particles, 125x4 mm operated at 1 mL/min with linear gradient programs in 20 min run time (routine program: 5% to 100 % B in 20 min).

UV monitoring was performed most of the time at 214 nm and 250 nm. Solvent A consisted of H_2O containing 0.1% TFA and solvent B consisted of CH_3CN containing 9.9% H_2O and 0.1% TFA. Water was of Milli-Q quality. CH_3CN and TFA were of HPLC use quality.

RP-UHPLC analyses were performed on Waters equipment consisting of a Waters Acquity H-Class Bio UPLC combined to a Waters SQ Detector 2 mass spectrometer. The analytical column used was a ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm x 50 mm operated at 0.6 mL/min with linear gradient programs in 2.20 min run time (routine program: 5% to 100 % B in 2.20min).

UV monitoring was performed at 214 nm or 678 nm. Solvent A consisted of H_2O containing 0.1% formic acid (FA) and solvent B consisted of CH_3CN containing 0.1% FA.

Water was of Milli-Q quality. CH_3CN and FA were LC-MS grade.

RP-HPLC purifications were either performed on Gilson GX-281 (high quantities, hundreds of mg, starting material) or GX-271 equipment (low quantities, few mg, molecular assemblies).

For GX-281, the preparative column, Macherey-Nagel 100 Å 7 µm C18 particles, 250 x 21 mm was operated at 20.84 mL/min. For GX-271, the preparative column, Macherey-Nagel 100 Å 7µm C18 particles, 250 x 10 mm was operated at 4.65 mL/min. For very polar and complex compounds, a C5 column was used to improve the resolution: Discovery BIO 300 Å 10µm C5 particles, D.250 × 10 mm. Linear gradient programs in 20 min run time were used and solvents A and B were the same as the ones used in RP-HPLC analysis.

Electron spray ionization (ESI-MS) mass spectra were obtained on an Esquire 3000 (Bruker).

The multiply charged data produced by the mass spectrometer on the m/z scale were converted to the molecular weight.

NMR spectra were recorded on BRUKER Avance 400 spectrometers. Chemical shifts are expressed in ppm and calculated taking the solvent peak as an internal reference.

2) General procedures for peptide synthesis

a) General Procedure for Solid-Phase Peptide Synthesis

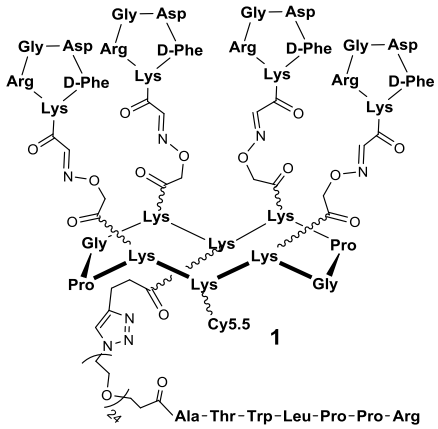
Assembly of all protected peptides was carried out using the Fmoc/*t*-Bu strategy manually in a glass reaction vessel fitted with a sintered glass frit or automatically on a peptide synthesizer (Biotage – Syro II) using 2-chlorotritylchloride® resin. Coupling reactions were performed manually by using 2 eq. of N-Fmoc-protected amino acid (relative to the resin loading) activated in situ with 2 eq. of PyBOP and 3-5 eq. of diisopropylethylamine (DIPEA) in DMF (10 mL/g resin) for 30 min. The coupling efficiency in manual synthesis was assessed by TNBS tests. N-Fmoc protecting groups were removed by treatment with a piperidine/DMF solution (1:4) for 10 min (10 mL/g resin). The process was repeated three times and the deprotection was verified by reading the absorbance of the piperidine washings at 299 nm. The linear peptides were then released from the resin by treatments with a solution of trifluoroacetic acid/dichloromethane (1:99, 10 mL/mg resin, 2x30 min). After evaporation of the cleavage solution, the crude peptide was solubilized in a minimum of DCM and added dropwise to ether for precipitation.

Then they were triturated and washed three times with diethyl ether to obtain crude materials.

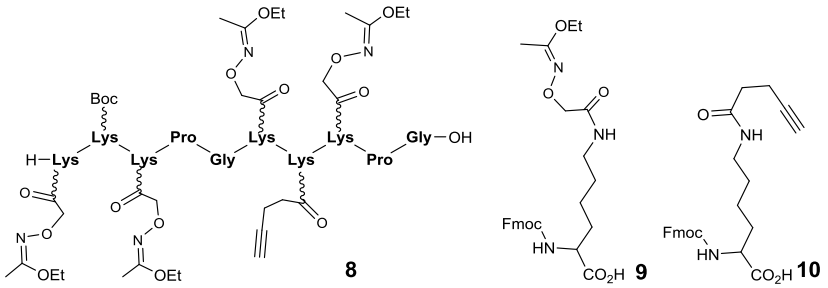
b) General Procedure for Cyclization Reactions

All linear peptides were dissolved in DMF (0.5 mM) and the pH values were adjusted to 8-9 by addition of DIPEA. PyBOP (1.3 eq.) was added and the solution stirred at room temperature for 1 h. Solvent was removed under reduced pressure and the residue dissolved in a minimum of dichloromethane. Diethyl ether was added to precipitate peptides. They were then triturated and washed three times with diethyl ether to obtain crude materials.

3) Synthesis of peptide conjugate 1



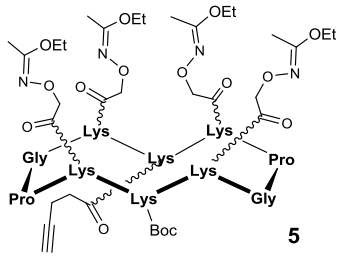
a) Synthesis of linear decapeptide intermediate 8



Linear decapeptide **8** was assembled on 2-chlorotritylchloride® resin (500 mg, loading of 0.54 mmol/g) using the general procedure and modified amino acid **9** and **10** that were produced as described.[1] The anchoring of the first amino acid (Fmoc-Gly-OH) was performed following the standard procedure yielding a convenient resin loading of 0.6 mmol/g. The peptide was released from the resin using a TFE/AcOH/CH_2Cl_2 (2/1/7) cleavage solution. The linear protected peptide was obtained as a light brown powder after precipitation, triturating and washing with diethyl ether (554 mg, 0.3 mmol).

[1] M. Galibert, P. Dumy, D. Boturyn. *Angew. Chem. Int. Ed.* **2009**, *48*, 2576-2579.

b) Synthesis of cyclodecapeptide 5



The cyclization reaction was carried out as described in the general procedure using crude linear peptide **8** (554 mg, 0.3 mmol). Precipitation from ether afforded cyclic peptide **5** as a light brown powder (550 mg, 0.3 µmol). This crude material was used without further purification.

For further analysis, compound **5** was deprotected under mild TFA conditions.

RP-UHPLC: RT = 0.97 min (C18, 214 nm, 5-100% B in 2.20 min)

MS (ESI-MS, positive mode): C63H109N20O19 Calcd : MW = 1449.8 g.mol-1 ; Found MW = 1450.2 g.mol-1

Figure S1: RP-UHPLC profile of deprotected compound **5**

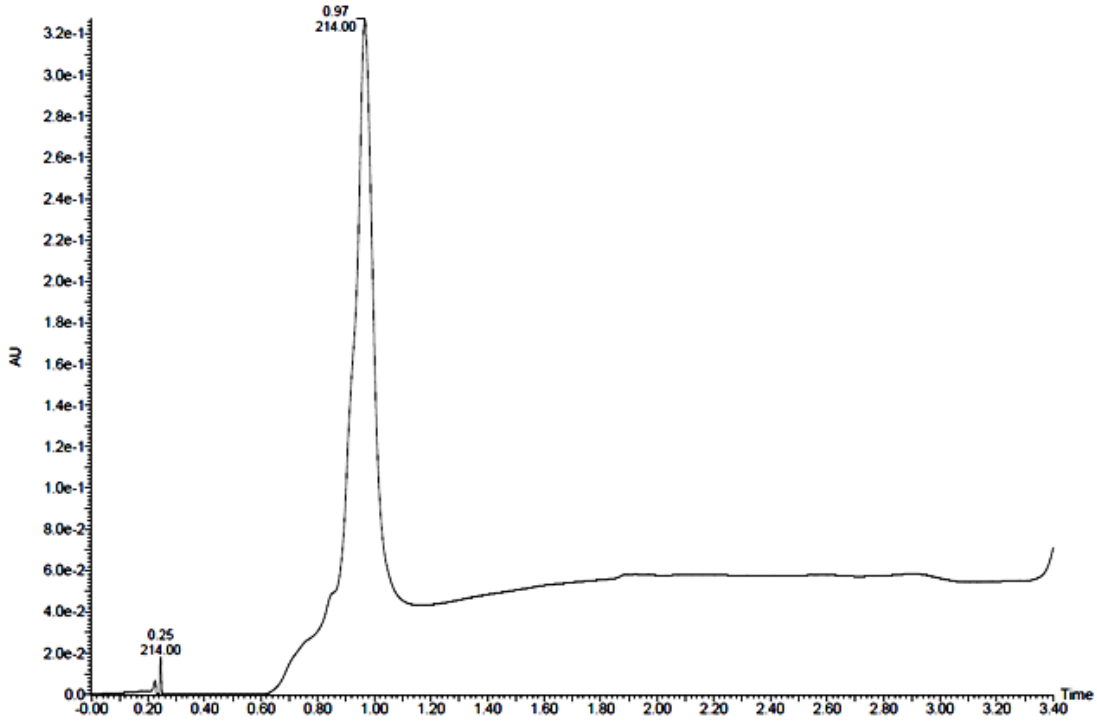
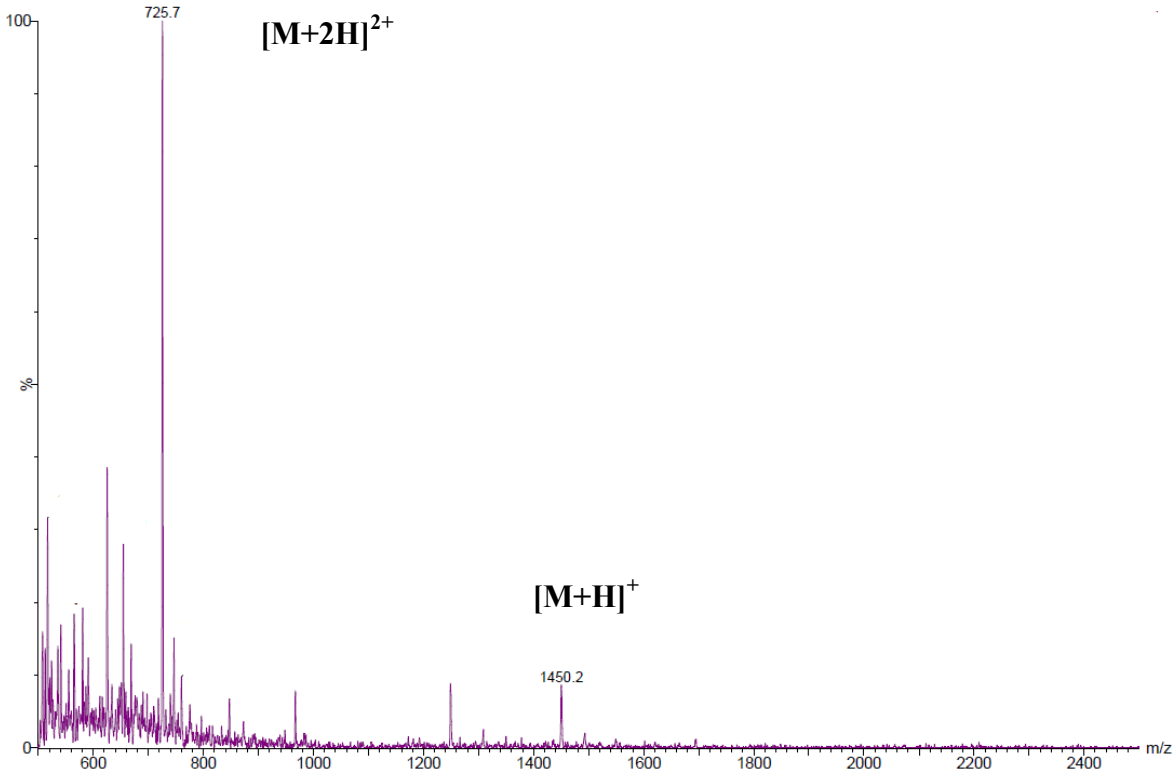
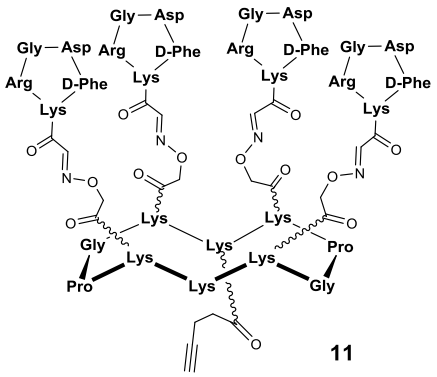


Figure S2: ESI analysis of deprotected compound **5**



c) Synthesis of RGD derivative 11



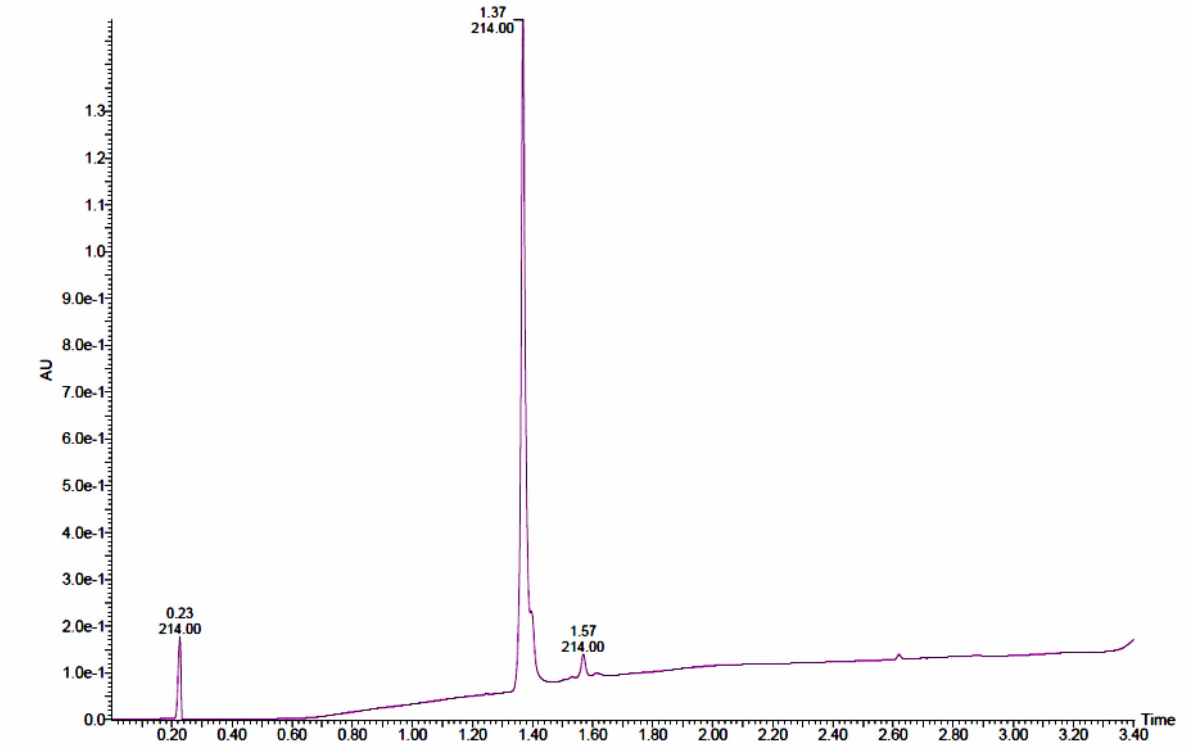
Cyclopetapeptide c[-Arg-Gly-Asp-D Phe-Lys(COCHO)-] **6** was obtained as described.[2]

Cyclodecapeptide **5** (20 mg, 11 µmol) and 6 equiv of **6** (44 mg, 66 µmol) were dissolved in 1.2 mL of a TFA/H_2O (7/3) solution. The mixture was stirred for 30min and the product was purified by RP-HPLC affording pure conjugate **11** as a white powder in 41% yield (18 mg, 4.48 µmol).

RP-UHPLC: RT = 1.37 min (C18, 214 nm, 5-100% B in 2.30 min)

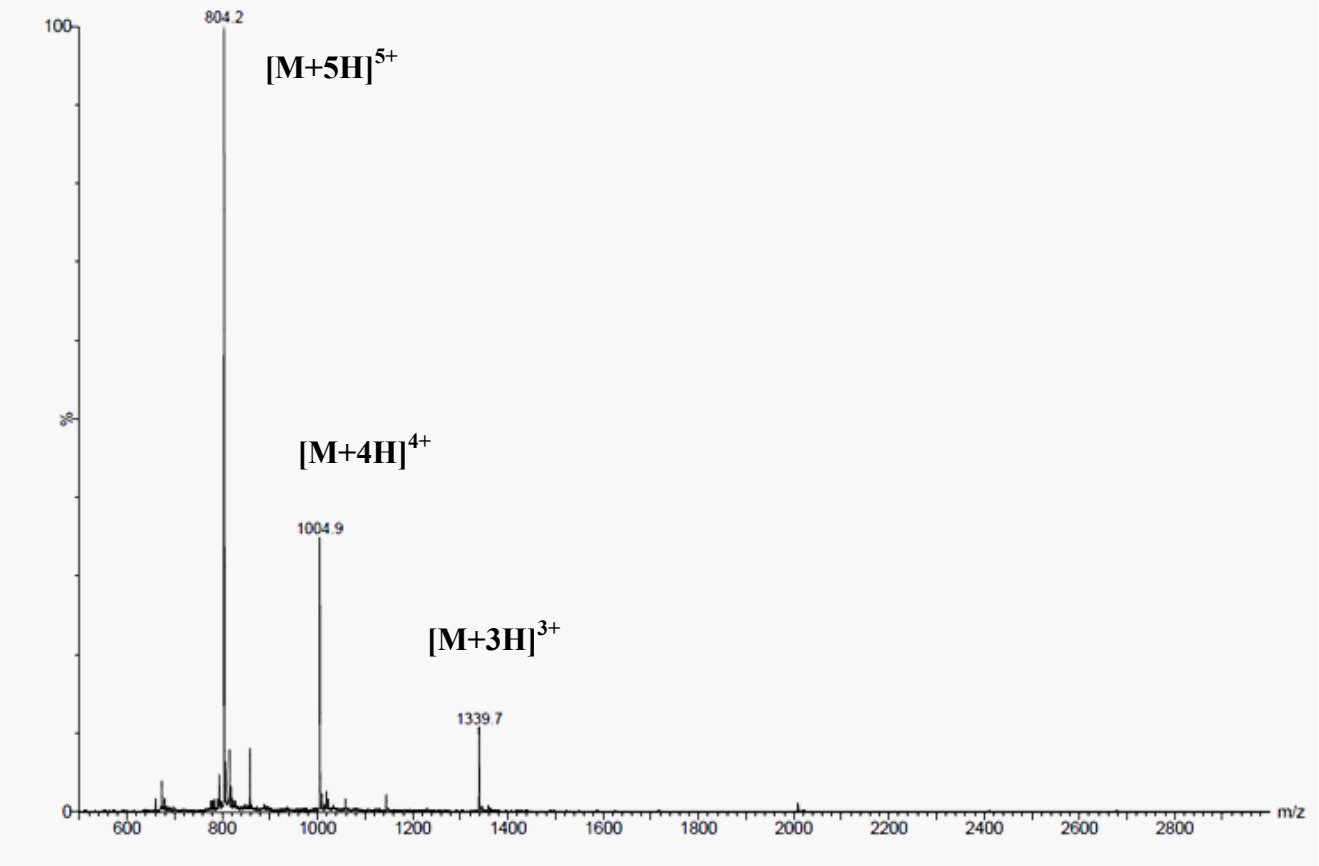
MS (ESI-MS, positive mode): $C_{179}H_{264}N_{56}O_{51}$ Calcd MW = 4016.4 g.mol^{-1} ; Found MW = 4016.0 g.mol^{-1}

Figure S3: RP-UHPLC profile of **11**

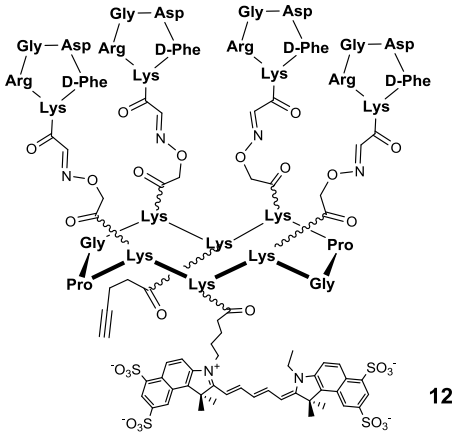


[2] D. Boturyn, J.-L. Coll, E. Garanger, M.-C. Favrot, P. Dumy. *J. Am. Chem. Soc.* **2004**, *126*, 5730-5739.

Figure S4: ESI analysis of **11**



d) Synthesis of Cy5.5 RGD derivative 12



12

RGD dericative **11** (4.1 mg, 1 µmol) and 2 equiv of tetrasulfo-Cy5.5-mono-NHS-ester (3.4 mg, 2 µmol) were dissolved in 2 mL of a DMF/DIPEA (pH = 9) solution. The mixture was stirred for 30min and the product was purified by RP-HPLC affording pure conjugate **12** as a blue powder in 80% yield (3.2 mg, 0.8 µmol).

RP-UHPLC: RT = 1.28 min (C18, 214 nm, 5-100% B in 2.30 min)

MS (ESI-MS, positive mode): $C_{220}H_{307}N_{58}O_{64}S_4$ Calcd MW = 4916.5 g.mol^{-1} ; Found MW = 4915.2 g.mol^{-1}

Figure S5: RP-UHPLC profile of **12**

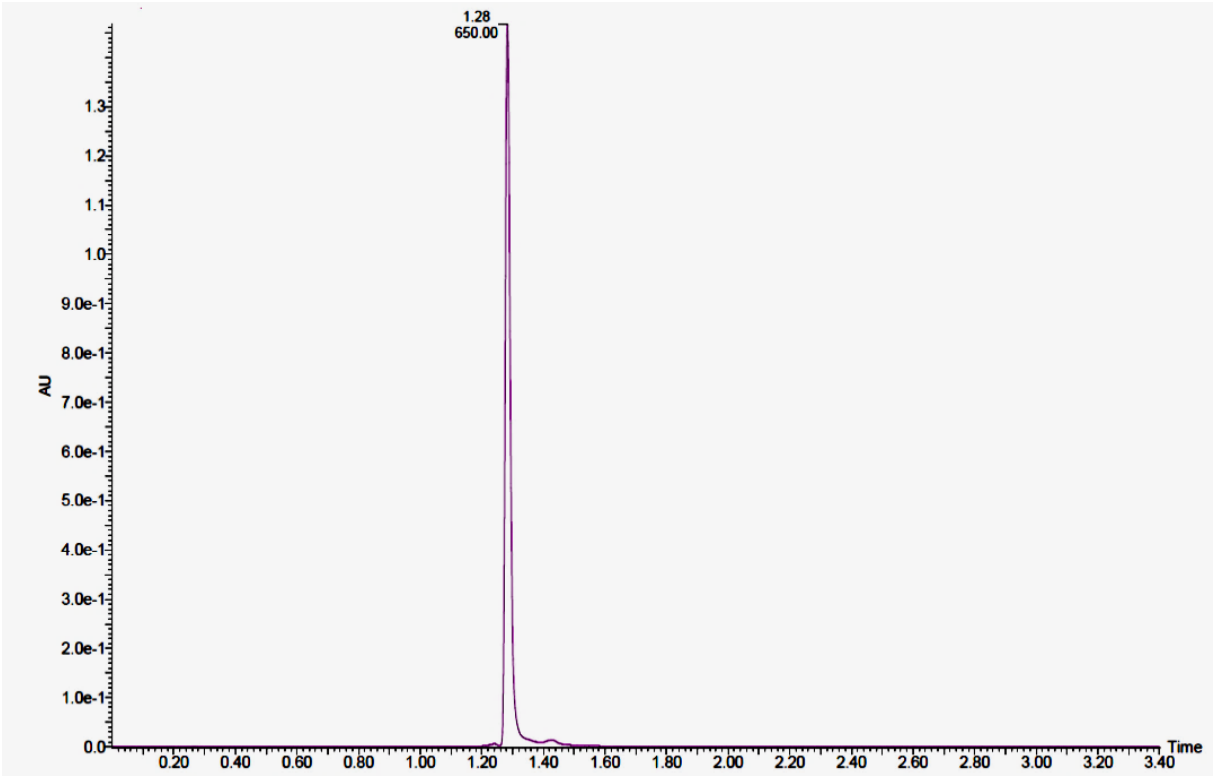
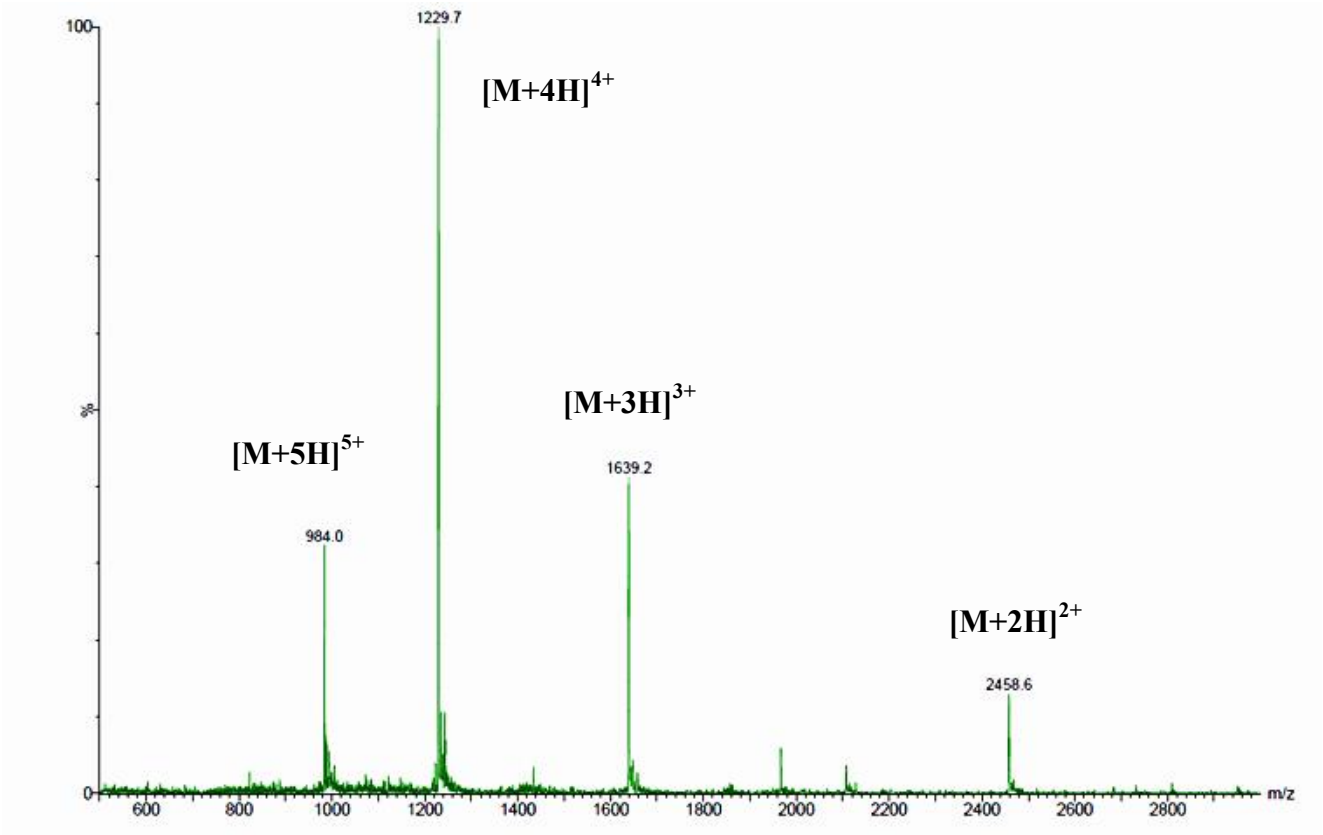


Figure S6: ESI analysis of **12**



e) Synthesis of peptide A7R 7

7

Protected peptide is assembled on 2-chlorotrytilchloride® resin (20 mg, loading of 0,54 mmol/g) using the general procedure and N_3-PEG24-OH. The anchoring of the first amino acid (Fmoc-Arg(Pbf)-OH) was performed following the standard procedure yielding a resin loading of 0.35 mmol/g. The protected peptide was released from the resin using a TFA/CH_2Cl_2 (1/99) cleavage solution, and stirred 4h in a TFA/TIS/H_2O (95/2.5/2.5) deprotection solution. The linear peptide **7** was obtained in 20% yield as a white powder after RP-HPLC and lyophilisation (3.3 mg, 1.4 µmol).

RP-UHPLC: RT = 1.78 min (C18, 214 nm, 5-100% B in 2.20 min)

MS (ESI-MS, positive mode): $C_{91}H_{160}N_{14}O_{34}$ Calcd MW = 1994.4 g.mol^{-1} ; Found MW = 1994.8 g.mol^{-1}

Figure S7: RP-UHPLC profile of **7**

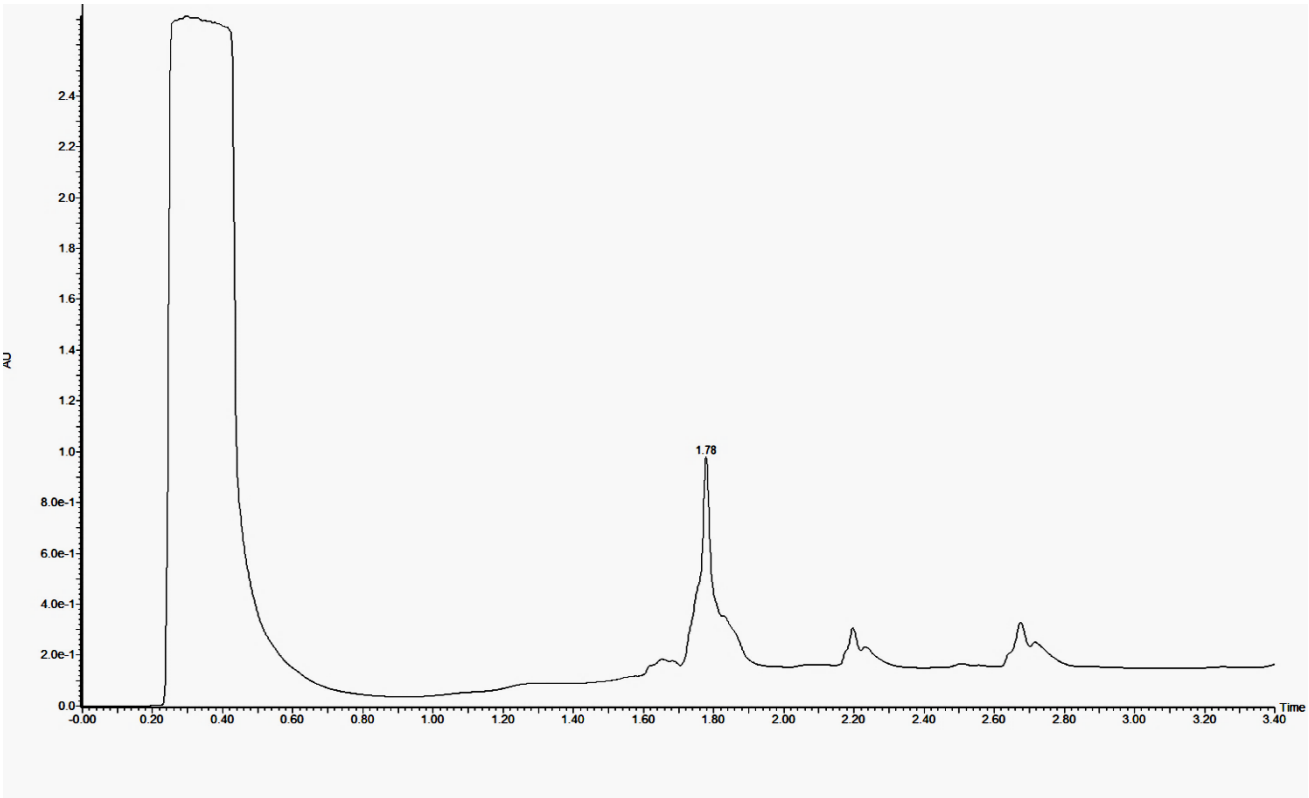
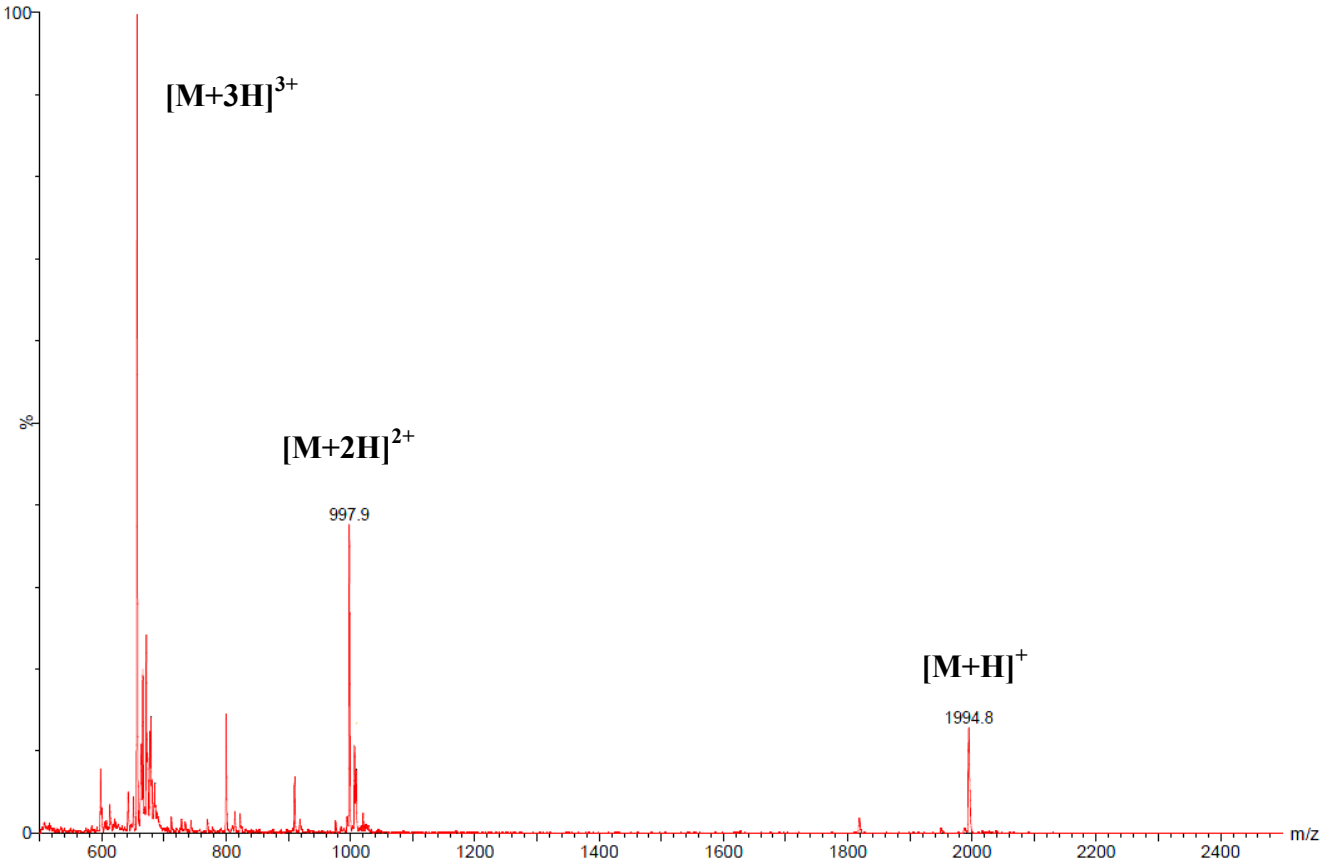


Figure S8: ESI analysis of **7**



f) Synthesis of conjugate 1

To a stirred solution of Cy5.5 RGD derivative **12** (1.2 mg, 245 nmol) and peptide **7** (1.2 eq., 640 µg, 318 nmol) in 200 µL DMF/PBS (pH = 7.4, 1 mM) (5/5) was added a solution of $CuSO_4$ (66 µg, 265 nmol, 1 eq.) and THPTA (345 µg, 795 nmol, 3 eq) in 30 µL of PBS (7.4, 1 mM). All solutions were degased under argon. To this blue stirred solution, was added a solution of ascorbate (233 µg, 1.325 µmol, 5 eq.) in 30 µL PBS (7.4, 1 mM). Both solutions were degased under argon. The uncolored resulting solution is stirred 4h at 40°C. The final compound **1** was obtained pure as a blue powder in 25% yield after RP-HPLC purification and lyophilisation (420 µg, 61 nmol).

RP-UHPLC: RT = 1.54 min (C18, 678 nm, 5-100% B in 2.3 min)

MS (ESI-MS, positive mode): $C_{311}H_{468}N_{72}O_{38}S_4$ Calcd MW = 6910.9 g.mol^{-1}; Found MW = 6910.5 g.mol^{-1}

Figure S9: RP-UHPLC profile of **1**

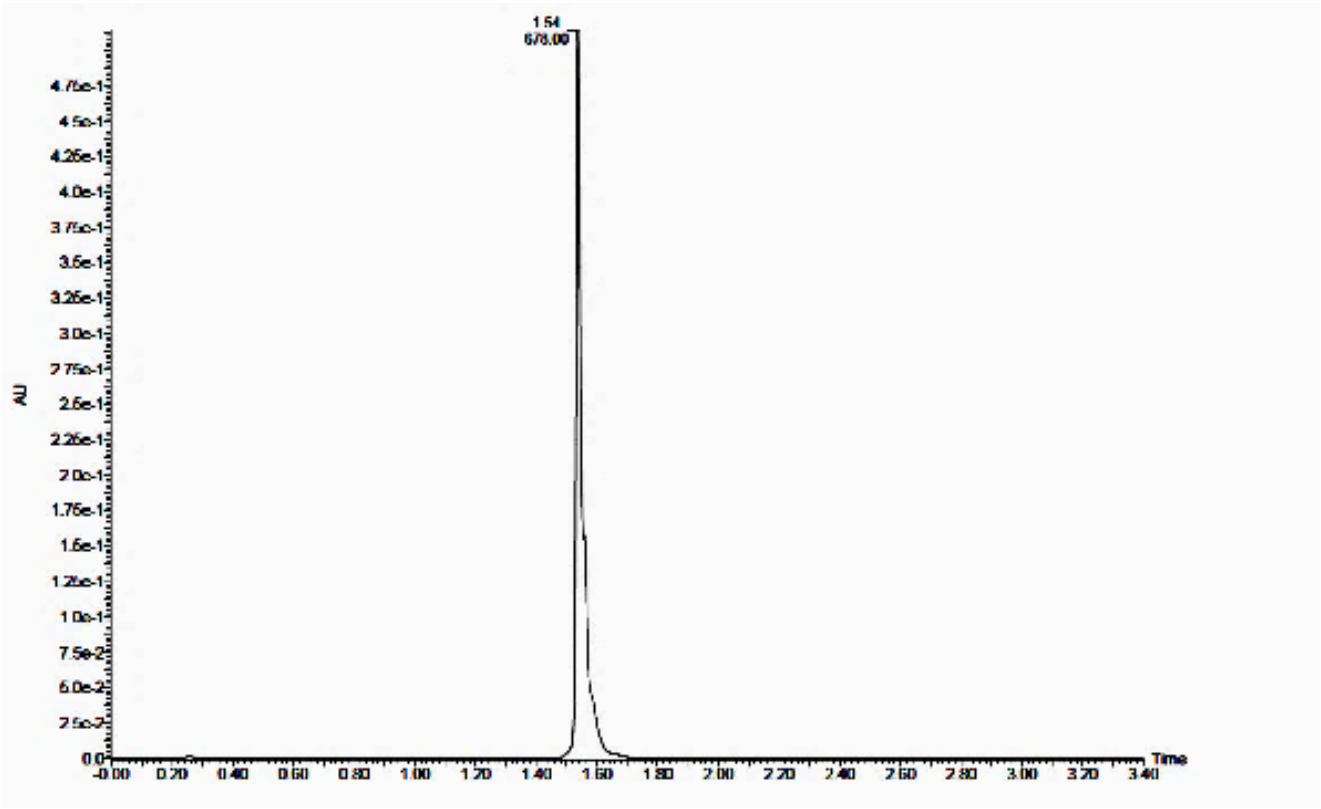
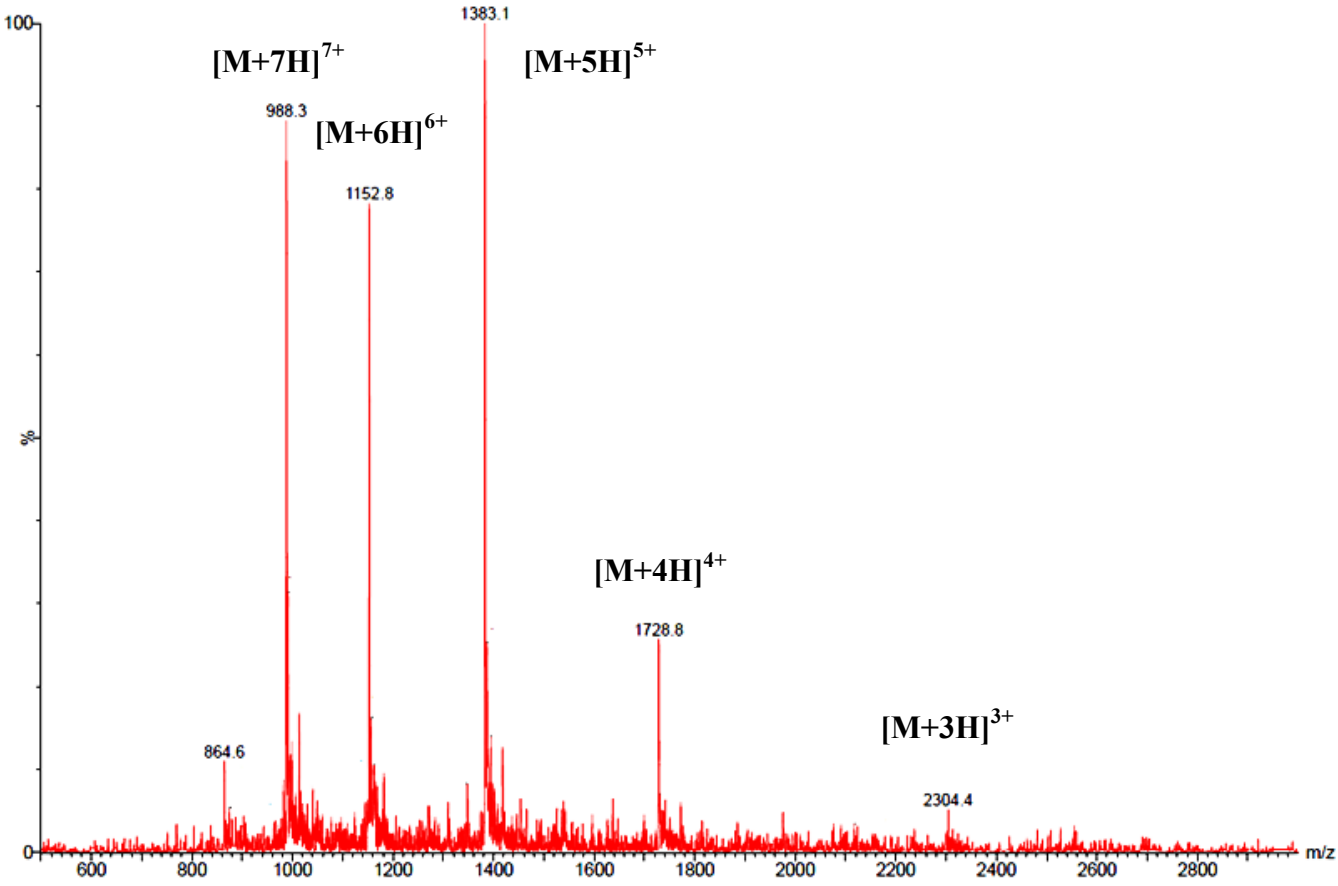
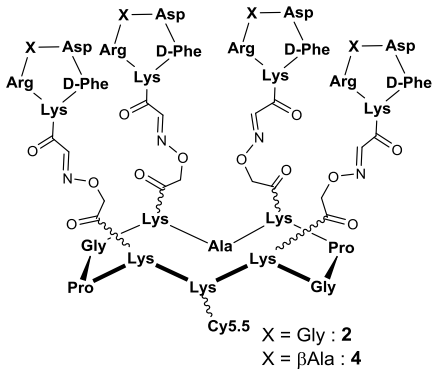


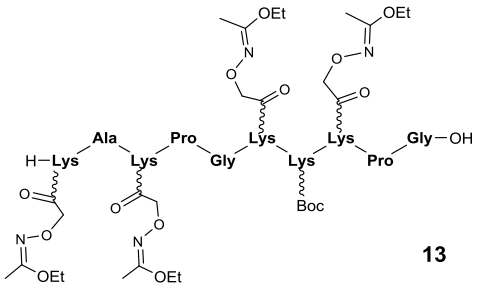
Figure S10: ESI analysis of **1**



4) Synthesis of fluorescent peptide conjugates 2 and 4

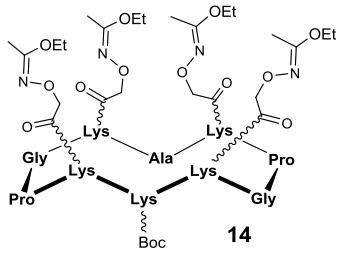


a) Synthesis of linear decapeptide intermediate 13



The linear decapeptide **13** was assembled on 2-chlorotritylchloride® resin (1 g, loading of 0.7 mmol/g) using the general procedure and modified amino acid **9**. The anchoring of the first amino acid (Fmoc-Gly-OH) was performed following the standard procedure yielding a convenient resin loading of 0.68 mmol/g. The peptide was released from the resin using cleavage solution of TFE/AcOH/CH_2Cl_2 (2:1:7). Linear protected peptide was obtained as a white solid powder after precipitation, triturating and washing with diethyl ether (1.04 g, 605 µmol).

b) Synthesis of cyclodecapeptide 14



14

The cyclization reaction was carried out as described in general procedure using the crude linear peptide **13** (1.04 g, 605 µmol). Solubilisation in a small amount of CH_2Cl_2 and dropwise addition in Et_2O afforded precipitation of cyclic decapeptide **14** as a white solid powder (922 mg, 544 µmol). This crude material was used without further purification.

RP-HPLC: RT = 2.12 min (C18, 214 nm, 5-100% B in 2.20 min)

MS (ESI-MS, positive mode): $C_{76}H_{129}N_{19}O_{24}$ Calcd MW = 1693.0 g.mol^{-1} ; Found MW = 1693.1 g.mol^{-1}

Figure S11: RP-UHPLC profile of **14**

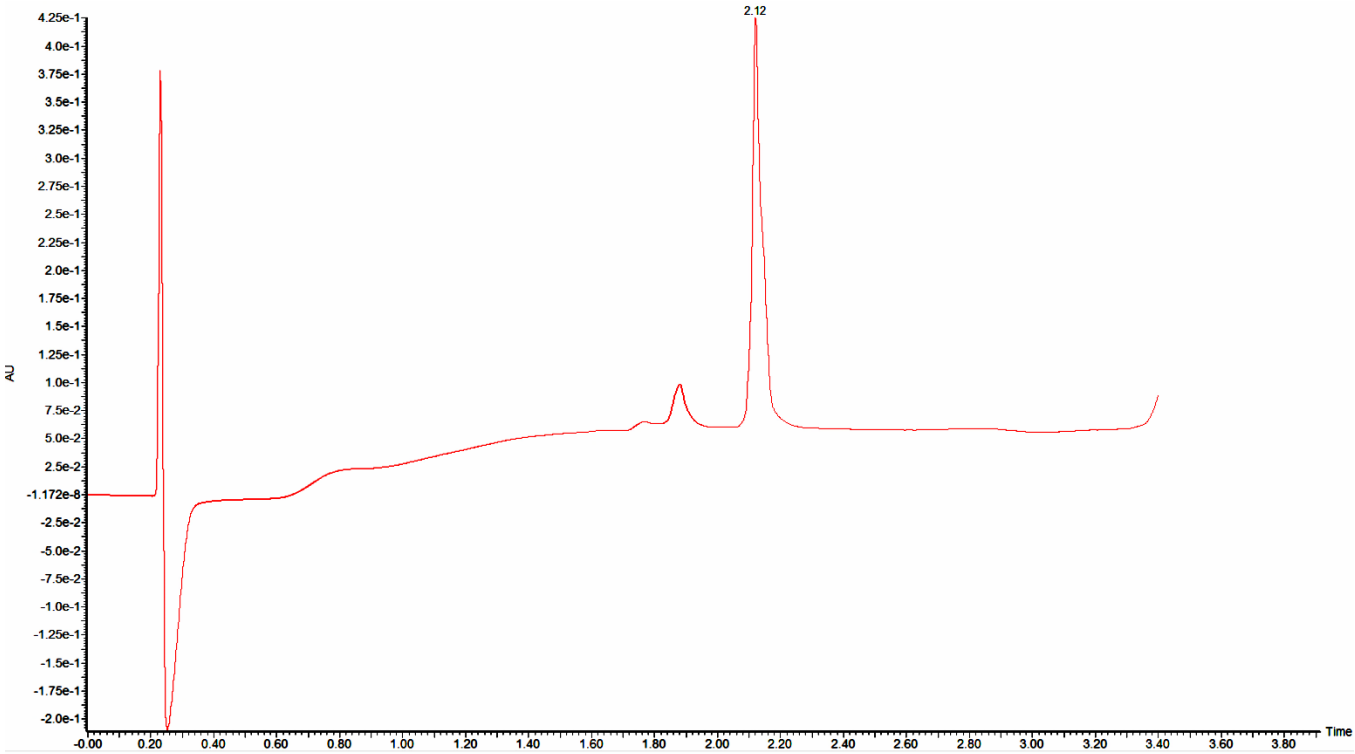
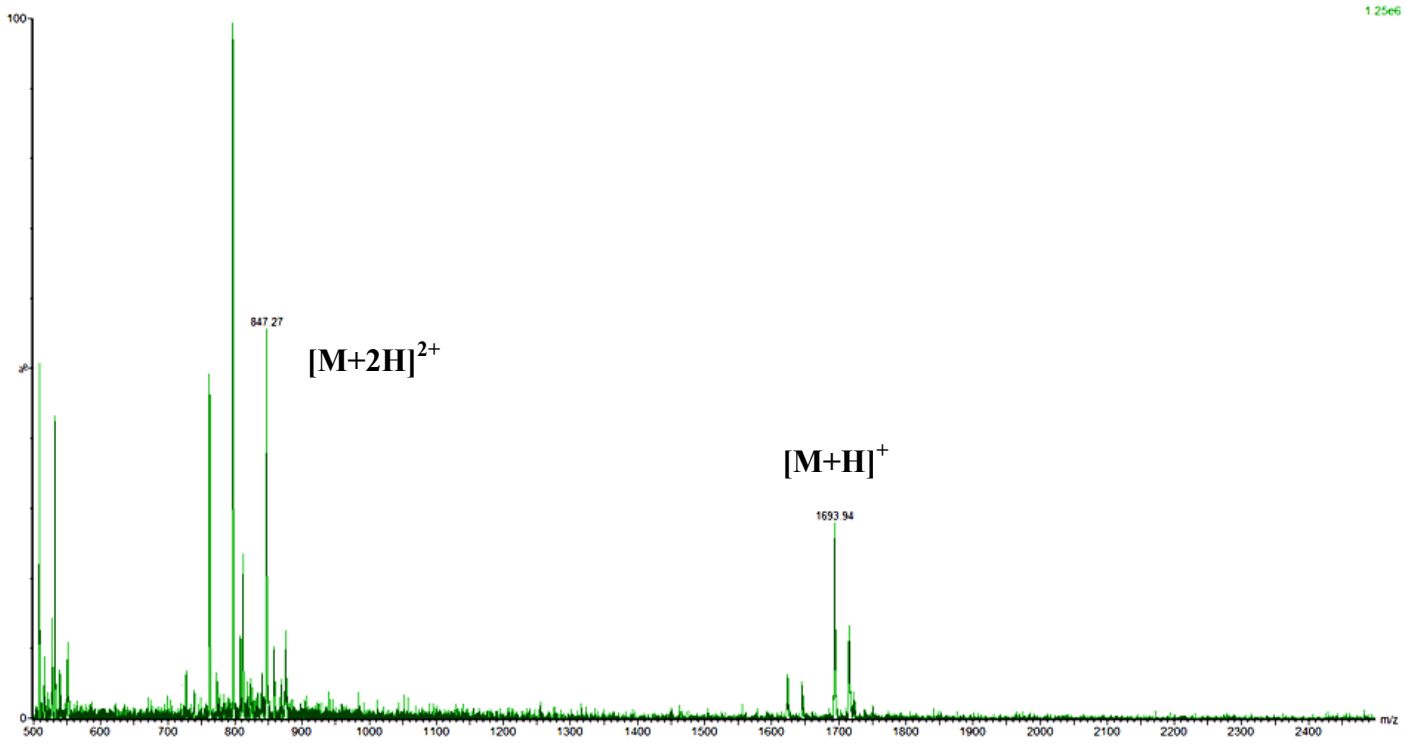
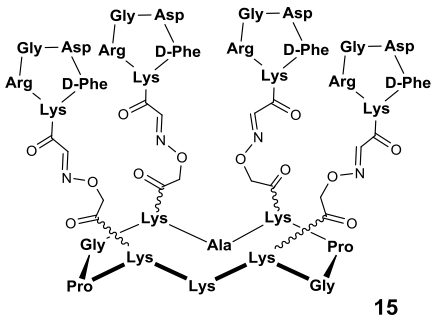


Figure S12: ESI analysis of **14**



c) Synthesis of RGD derivative 15



The cyclodecapeptide **14** (10 mg, 6.0 µmol) and 6 equiv. of peptide **6** (23.5 mg, 36 µmol) were dissolved in 1 mL of TFA/H_2O (7/3) solution. The mixture was stirred for 2h and the product was purified by RP-HPLC affording pure conjugate **15** as a white powder in 42% yield (9.78 mg, 2.52 µmol).

RP-UHPLC: RT = 1.17 min (C18, 214 nm, 5-100% B in 2.20 min)

MS (ESI-MS, positive mode): $C_{171}H_{253}N_{55}O_{50}$ Calcd MW = 3879.3 g.mol^{-1} ; Found MW = 3878.6g.mol^{-1}

Figure S13: RP-UHPLC profile of **15**

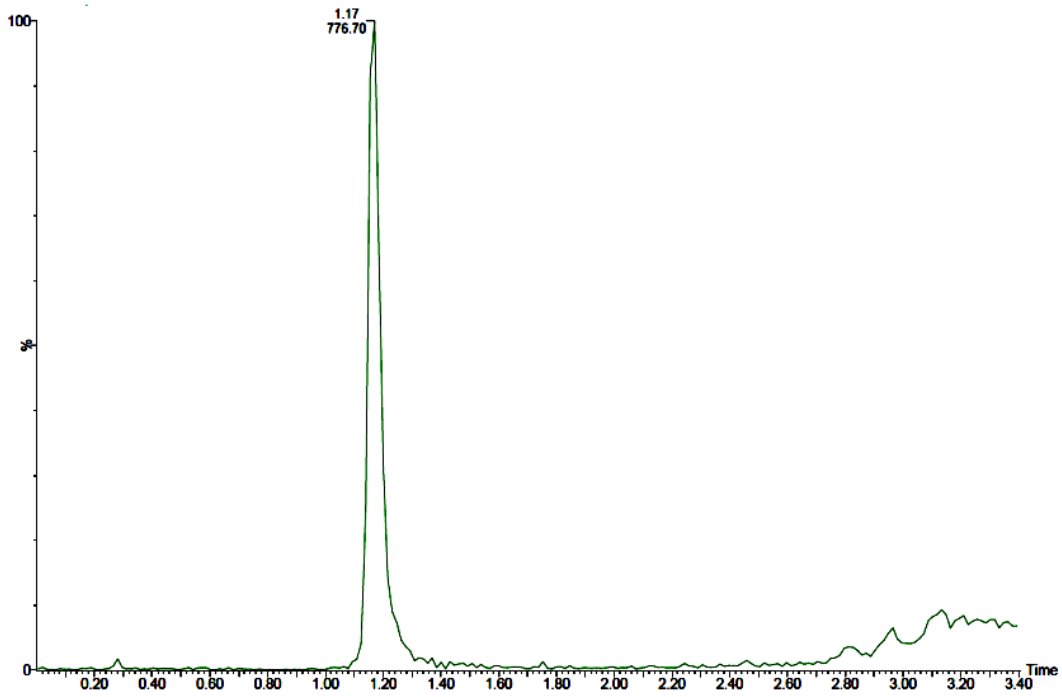
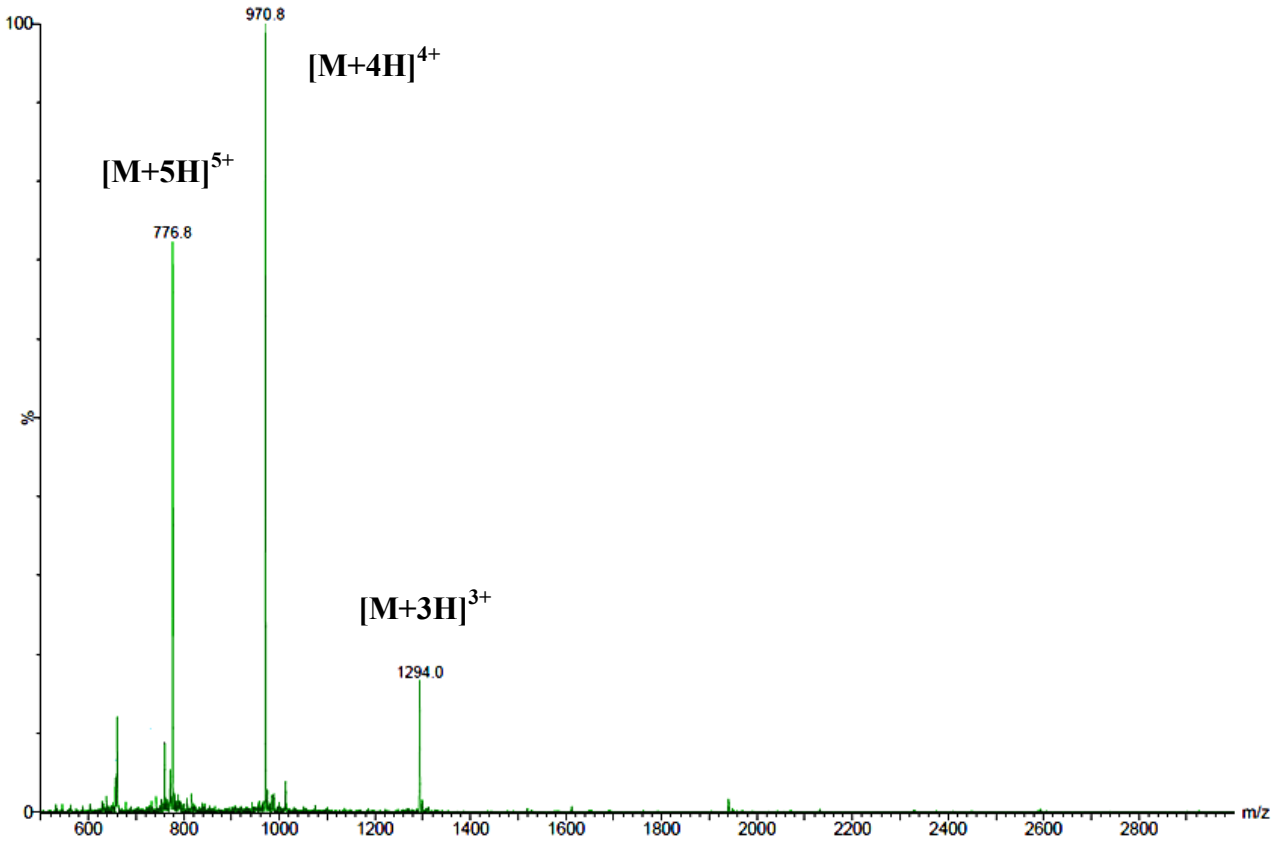
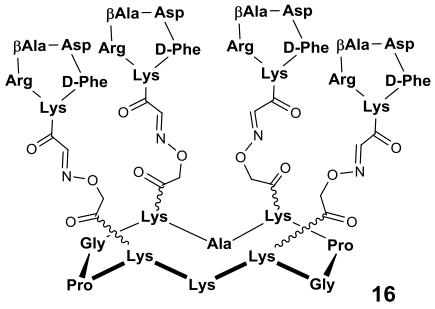


Figure S14: ESI analysis of **15**



d) Synthesis of RβAD derivative 16



Cyclopentapeptide c[-Arg-βAla-Asp-D Phe-Lys(COCHO)-] **17** was obtained as described.[2]

The cyclodecapeptide **14** (10 mg, 6.0 µmol) and 6 equiv. of peptide **17** (23.5 mg, 36 µmol) were dissolved in 1 mL of TFA/H2O (7/3) solution. The mixture was stirred for 2h and the product was purified by RP-HPLC affording pure conjugate **16** as a white powder in 34% yield (8 mg, 2.52 µmol).

RP-UHPLC: RT = 1.06 min (C18, 214 nm, 5-100% B in 2.20 min)

MS (ESI-MS, positive mode): $C_{175}H_{261}N_{55}O_{50}$ Calcd MW = 3935.4 g.mol^{-1} ; Found MW = 3935.1 g.mol^{-1}

Figure S15: RP-UHPLC profile of **16**

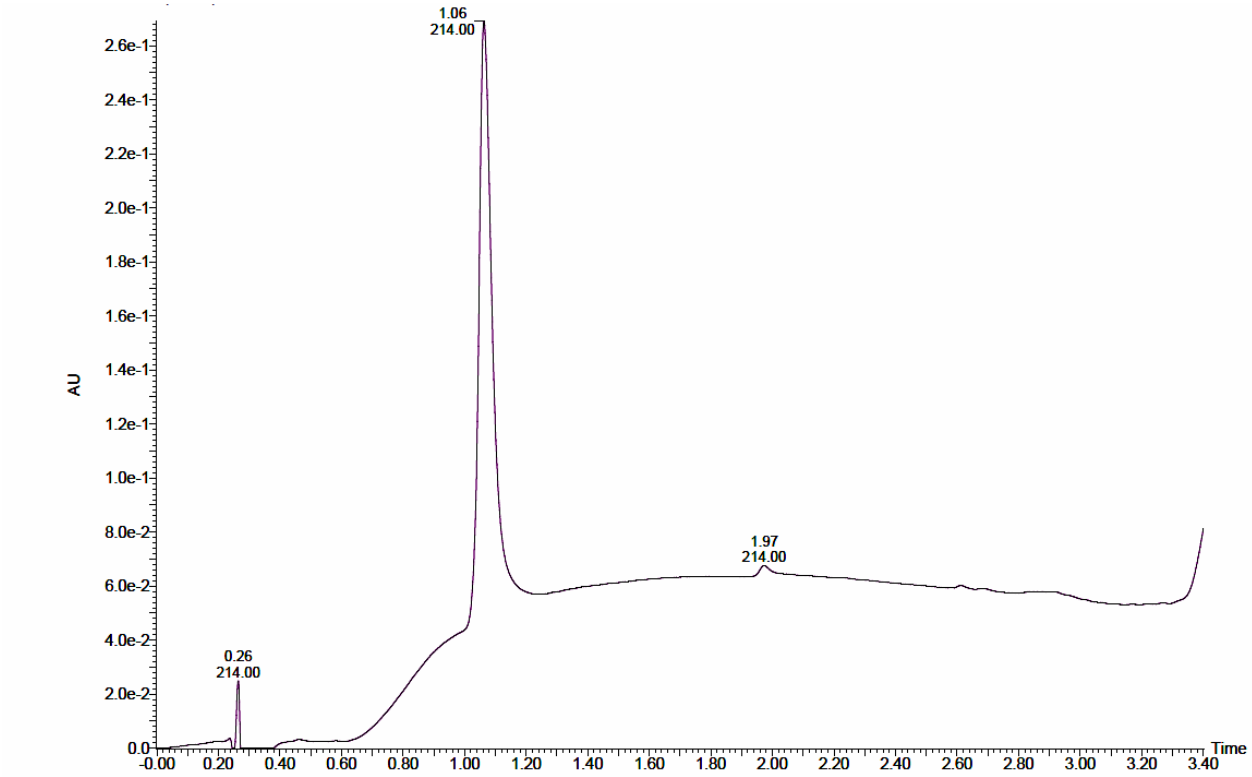
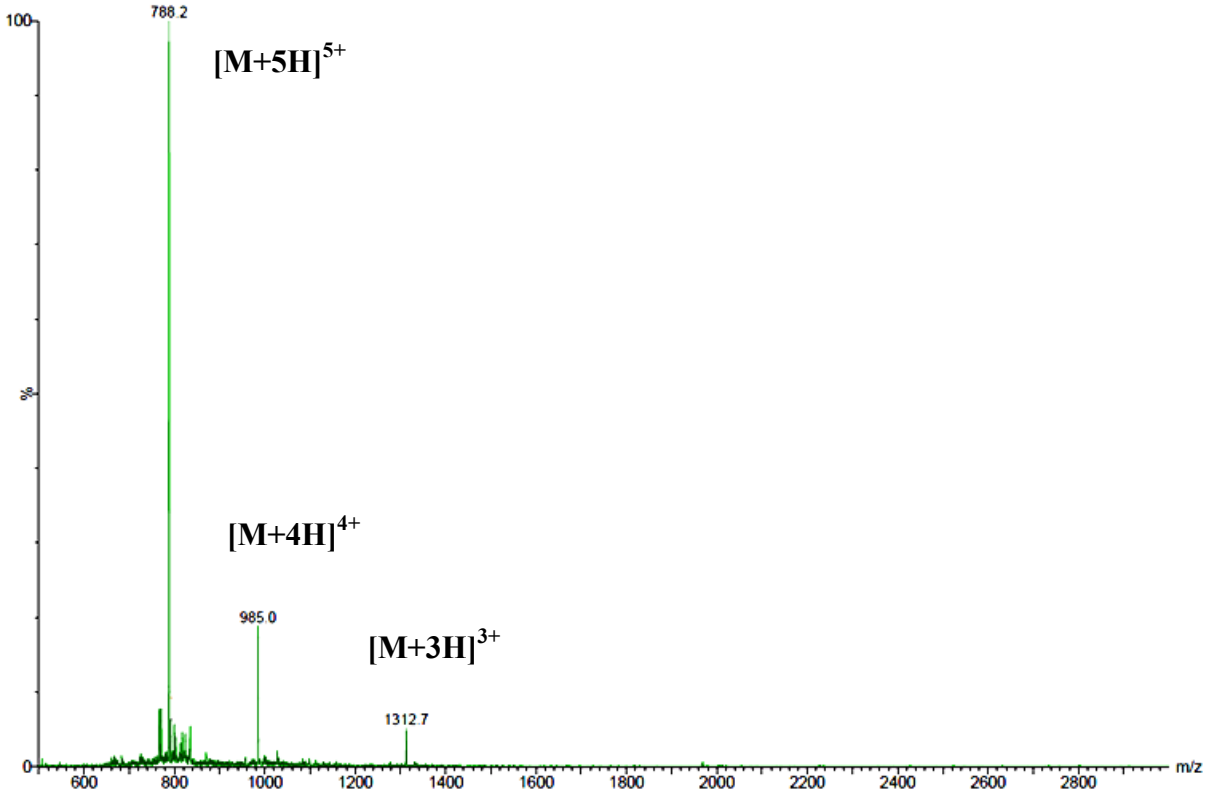


Figure S16: ESI analysis of **16**



e) Synthesis of fluorescent RGD peptide conjugate 2

The RGD derivative **15** (2 mg, 515 nmol) and 2 equiv. of Cy5.5-NHS (1.05 mg, 1.03 µmol) were dissolved in 1 mL of DMF/DIPEA solution (pH=9). The mixture was stirred for 2h and the product was purified by RP-HPLC affording pure conjugate **2** as a blue powder in 90% yield after lyophilisation (2.21 mg, 464 nmol).

RP-UHPLC: RT = 1.33 min (C18, 214 nm, 5-100% B in 2.30min)

MS (ESI-MS, positive mode): $C_{212}H_{297}N_{57}O_{63}S_4$ Calcd MW = 4778.3 g.mol^{-1} ; Found MW = 4778.0 g.mol^{-1}

Figure S17: RP-UHPLC profile of **2**

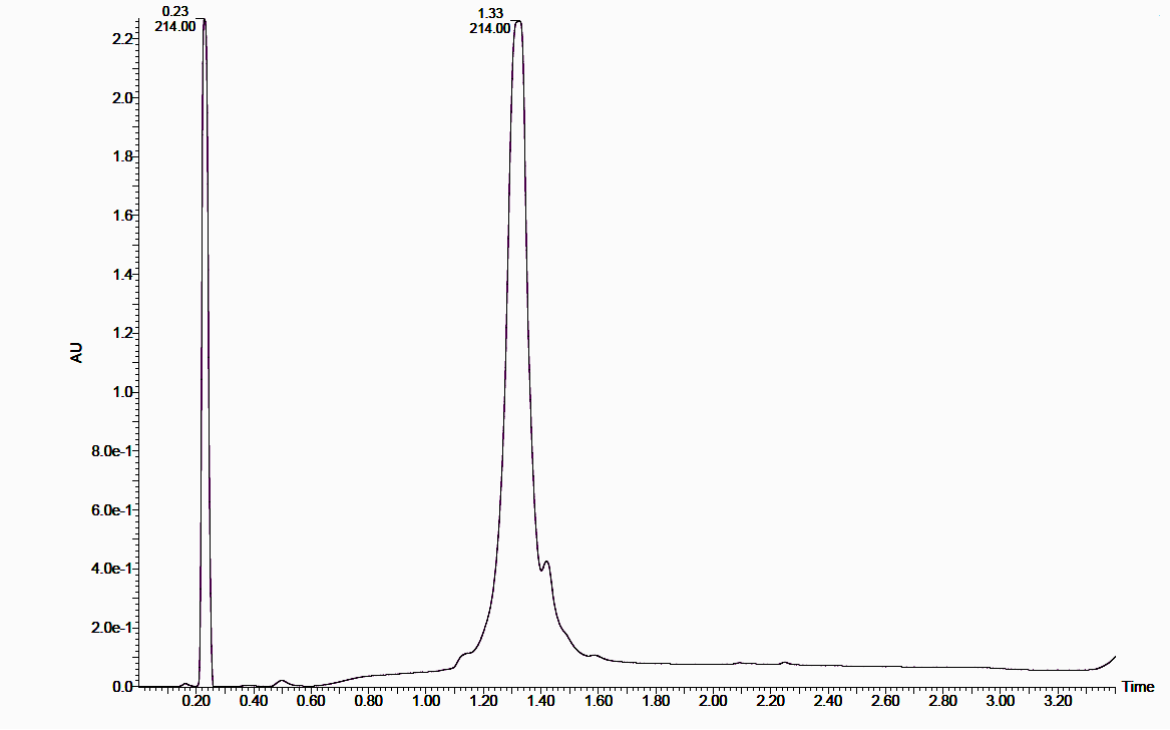
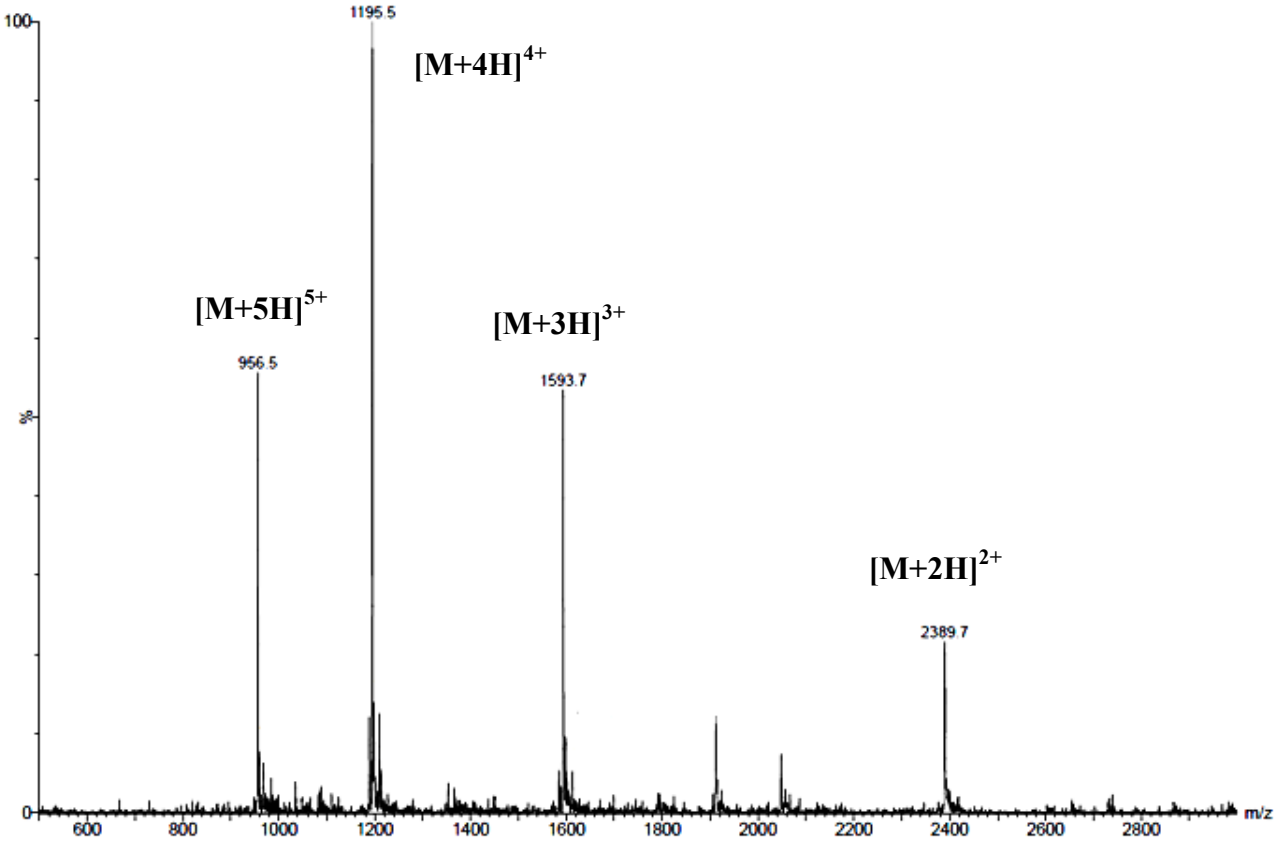


Figure S18: ESI analysis of **2**



f) Synthesis of fluorescent RβAD peptide conjugate 4

The RAD derivative **16** (4 mg, 1 µmol) and 2 equiv. of Cy5.5-NHS (2.03 mg, 2 µmol) were dissolved in 2 mL of DMF/DIPEA solution (pH=9). The mixture was stirred for 2h and the product was purified by RP-HPLC affording pure conjugate **4** as a blue powder in 92% yield after lyophilisation (4.45 mg, 920 nmol).

RP-UHPLC: RT = 1.31 min (C18, 214 nm, 5-100% B in 2.30min)

MS (ESI-MS, positive mode): $C_{216}H_{303}N_{57}O_{63}S_4$ Calcd MW = 4834.4 g.mol^{-1} ; Found MW = 4834.8 g.mol^{-1}

Figure S19: RP-UHPLC profile of **4**

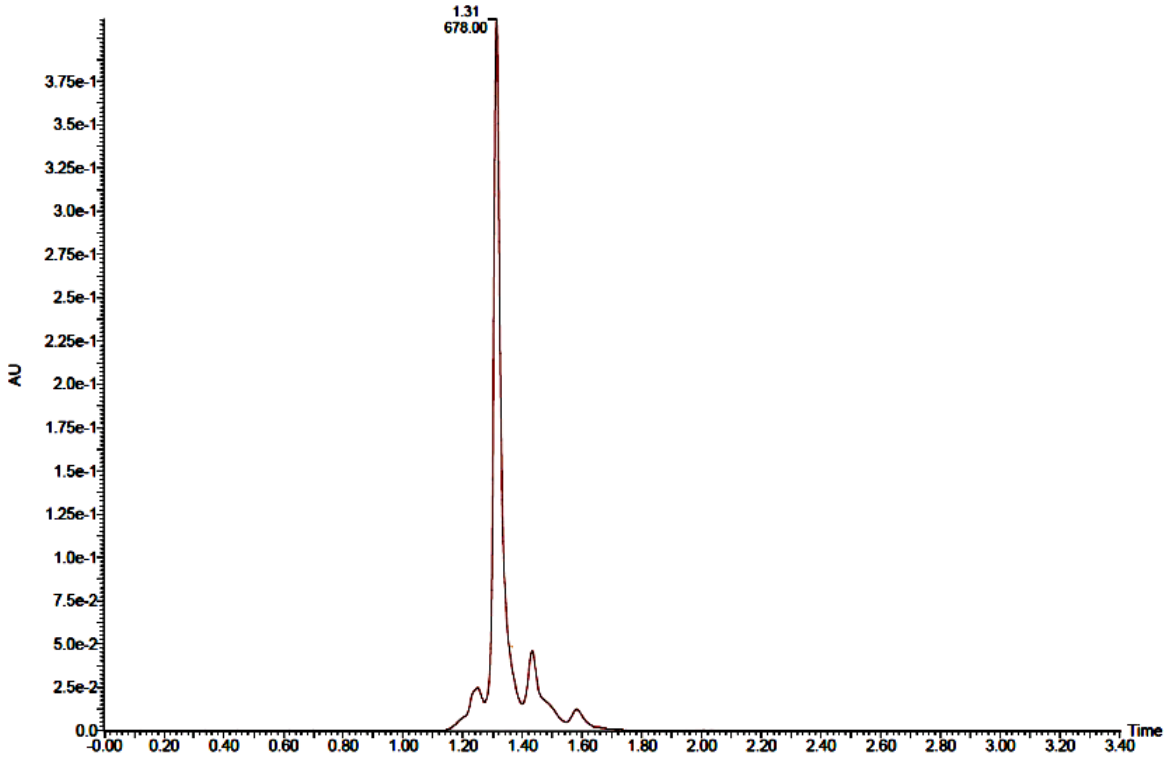
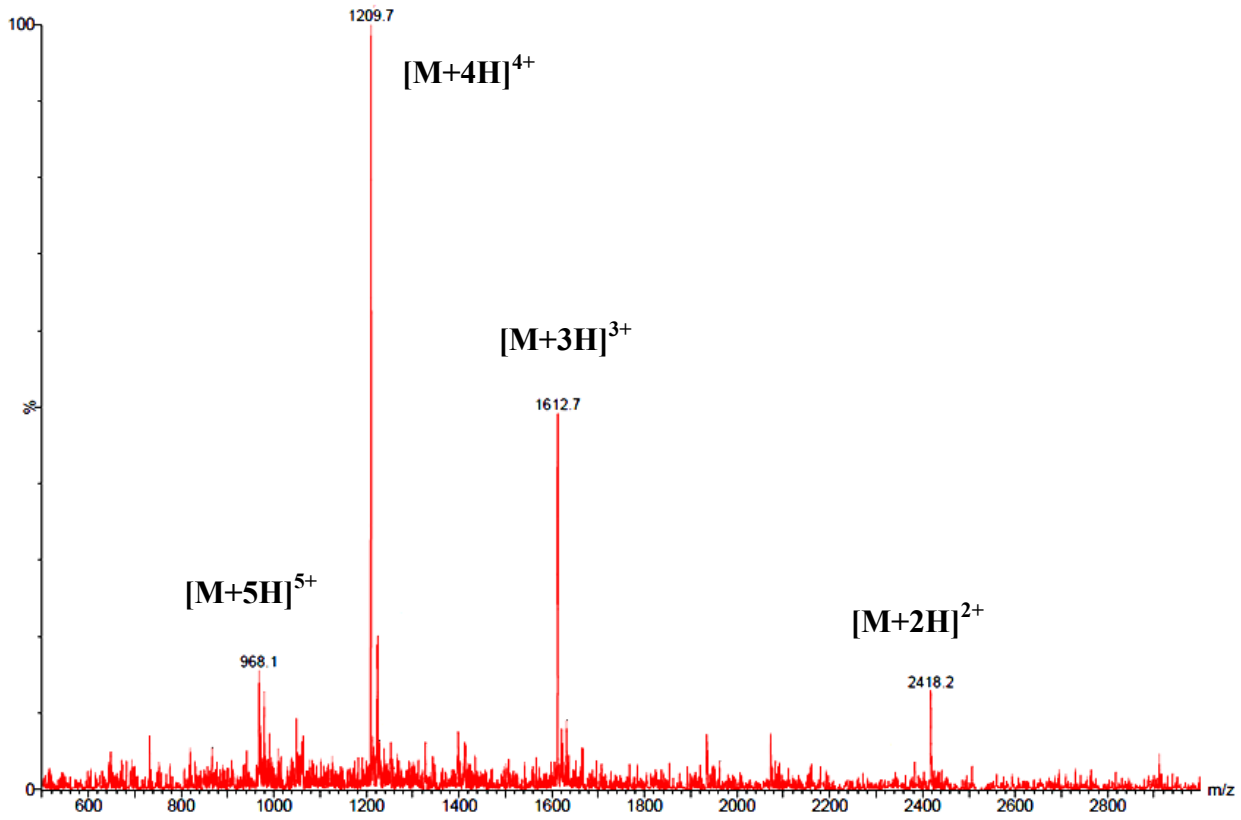


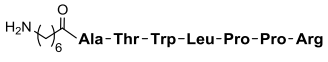
Figure S20: ESI analysis of **4**



5) Synthesis of fluorescent A7R peptide 3

3

a) Synthesis of peptide intermediate 18



18

Linear peptide **18** was assembled on 2-chlorotritylchloride® resin (2 g, loading of 0.7 mmol/g) using the general procedure and automatised synthesis. The anchoring of the first amino acid (Fmoc-Arg(Pbf)-OH) was performed manually following the standard procedure yielding a resin loading of 0.37 mmol/g. The peptide was released from the resin using a TFA/CH2Cl2 (99/1) cleavage solution and stirred 4h in a TFA/TIS/H2O (95/2.5/2.5) deprotection solution. The linear peptide was obtained in 85% yield after RP-HPLC and lyophilisation (600 mg, 630 µmol).

RP-UHPLC: RT = 0.88 min (C18, 214 nm, 5-100% B in 2.20 min)

MS (ESI-MS, positive mode): $C_{46}H_{72}N_{12}O_{10}$ Calcd MW = 953.2 g.mol^{-1} ; Found MW = 953.6 g.mol^{-1}

Figure S21: RP-UHPLC profile of **18**

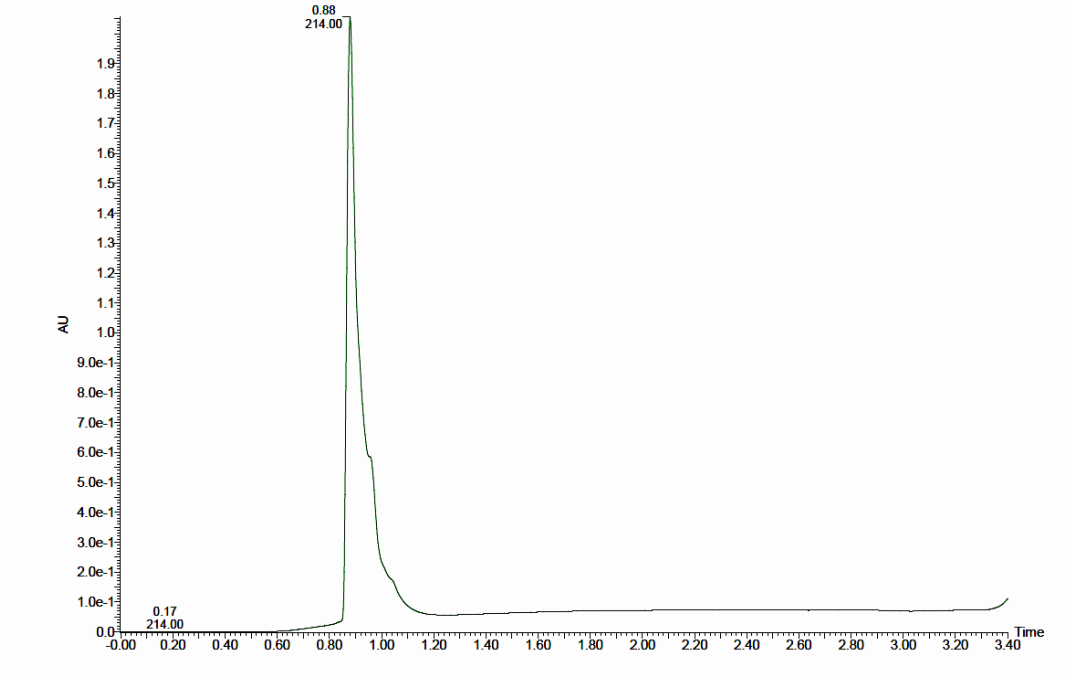
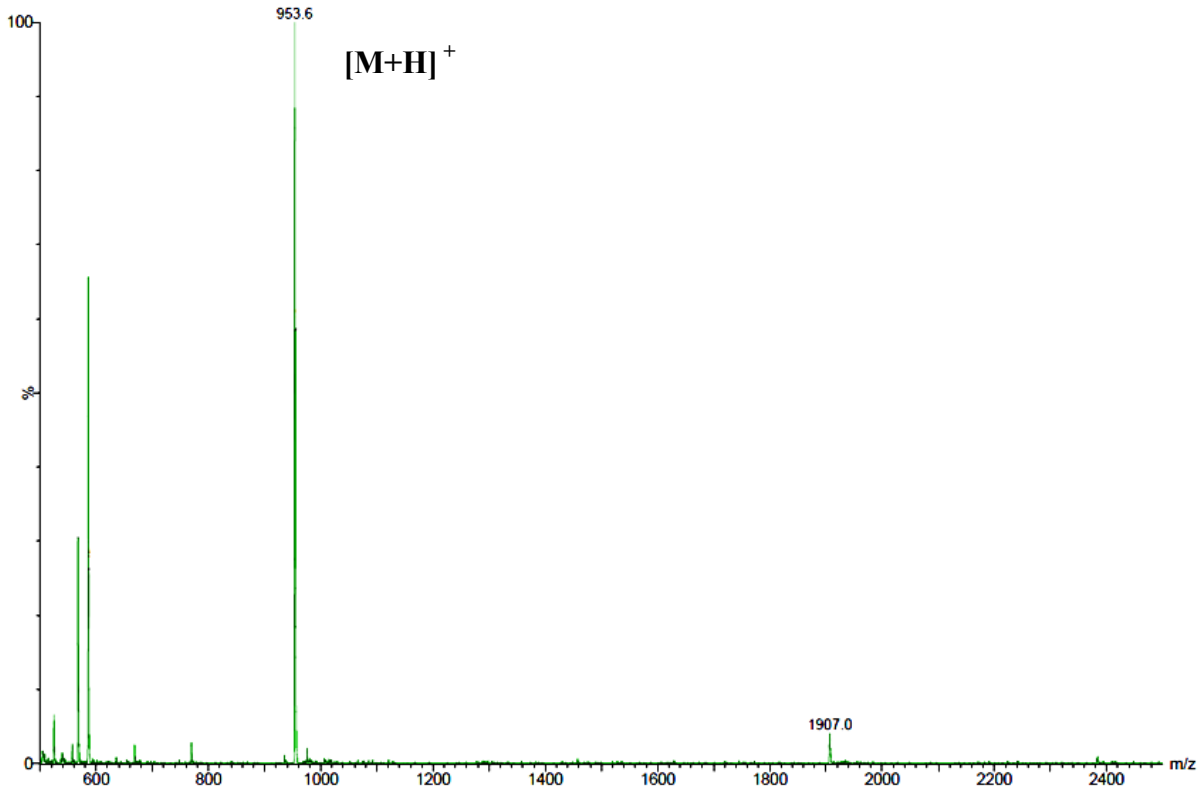


Figure S22: ESI analysis of **18**



b) Synthesis of fluorescent A7R peptide 3

To a stirred mixture of peptide **18** (1.32 mg, 1.38 µmol) in DMF and DIPEA (pH = 9) was added 2 eq. of tetrasulfo-Cy5.5-mono-NHS-ester (2.8 mg, 2.76 µmol). The dark blue solution was stirred 1h at room temperature. After solvents evaporation under reduced pressure, the crude was purified by RP-HPLC, affording the product **3** as a dark blue powder in 70% yield after lyophilisation (1.78 mg, 966 nmol).

RP-UHPLC: RT = 1.60 min (C18, 214 nm, 5-100% B in 2.30min)

MS (ESI-MS, positive mode): $C_{86}H_{114}N_{14}O_{23}S_4$ Calcd MW = 1837.2 g.mol^{-1} ; Found MW = 1837.6 g.mol^{-1}

Figure S23: RP-UHPLC profile of **3**

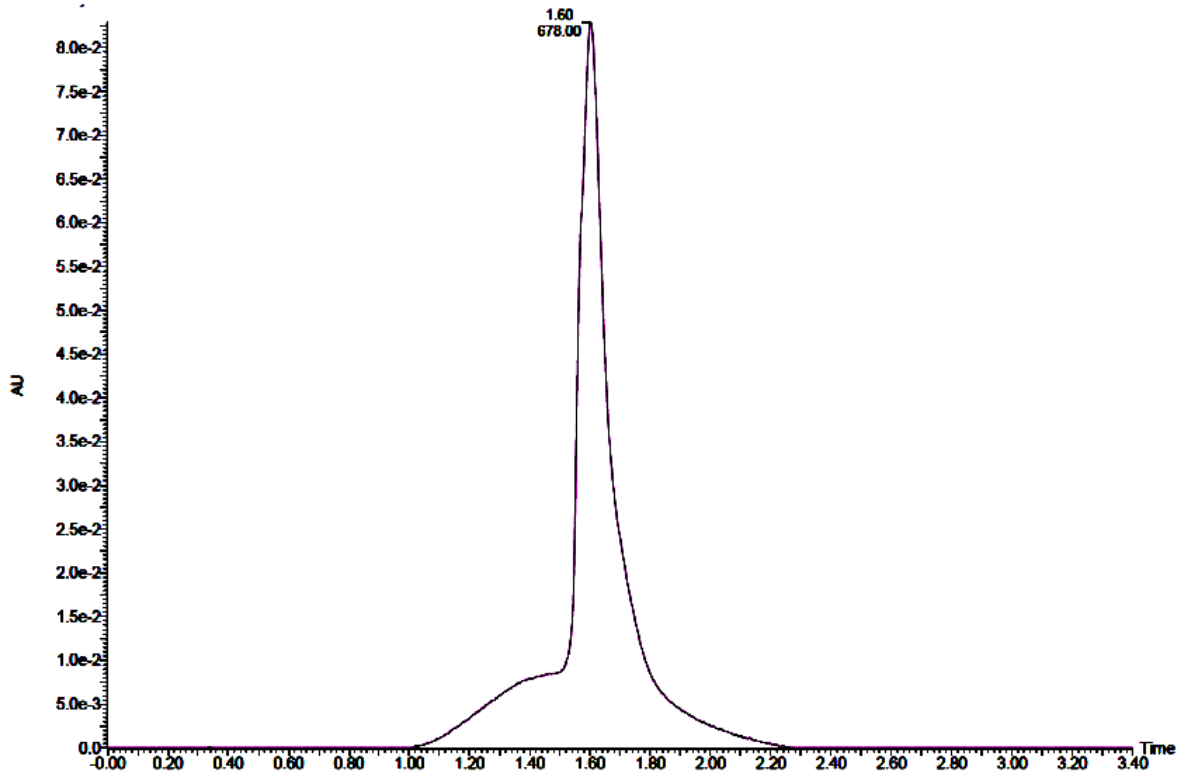
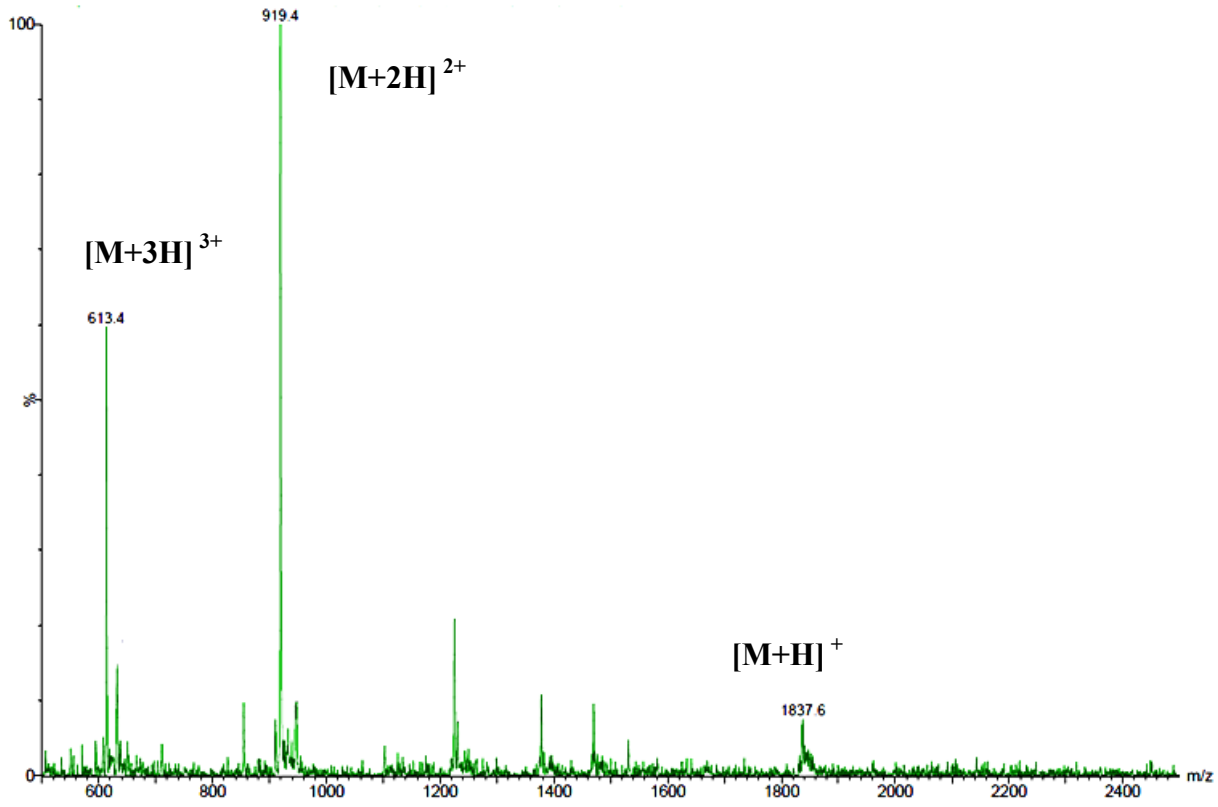


Figure S24: ESI analysis of **3**



6) U87MG Cell line culture

Cells were maintained in DMEM + 10% SVF (from ATCC®), which are listed in Table A. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

7) Animals

Female Naval Medical Research Institute (NMRI) nude mice (5 weeks old) weighting 24.0 ± 0.5 g were purchased from Janvier (Le Genest). Before the beginning of the experiment, the animals were acclimatized in a temperature-controlled environment for 1 wk. Facility rooms were maintained at constant temperature (25°C), humidity (30–50% relative humidity), and 12-h light: dark illumination cycle. Access to food and tap water was available ad libitum. Experiments were carried out following Institut National de la Santé et de la Recherche Médicale guidelines regarding the fair treatment of animals with approval of the Comité d'Éthique en Expérimentation Animale de Grenoble.

8) Fluorescence of organs (*ex vivo* imaging)

- Fluorescence of organs at t=5h post-injection

RGD peptide **2** was injected to mice S4 to S6, RGD-A7R peptide **1** was injected to mice S10 to S12. 5h after injection, mice S4, S5, S6, S10, S11 and S12 were sacrificed. Immediately after sacrifice, dissection and *ex vivo* imaging of organs (on Hamamtsu®) were performed.

Organs are placed on a paper as disclosed in the following table:

Heart	Lung	Brain	Skin
Muscle	Kidney	Adrenal	Bladder
Intestine	Spleen	Pancreas	Fat
Stomach	Uterus-Ovary	Liver	Tumour

Table S1 Organ repartition for *ex vivo* fluorescence imaging

Ex vivo fluorescence imaging realised for each mice are presented above:

Figure S25: **S4** (organs 5h after injection of RGD compound **2**):

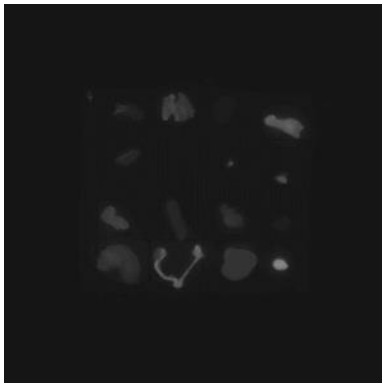


Figure S26: **S5** (organs 5h after injection of RGD compound **2**):

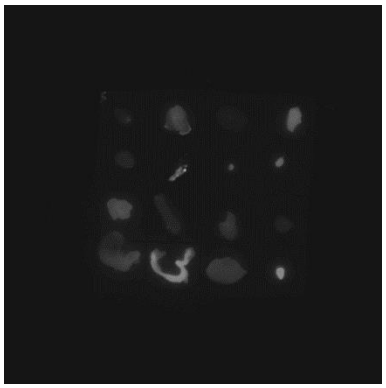


Figure S27: **S6** (organs 5h after injection of RGD compound **2**):

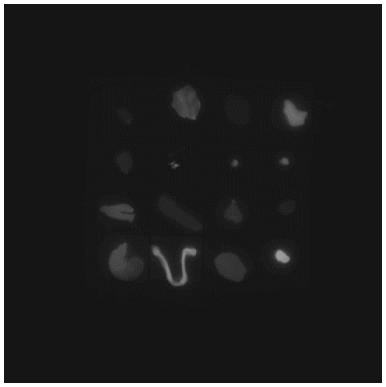


Figure S28: **S10** (organs 5h after injection of RGD-A7R compound **1**):

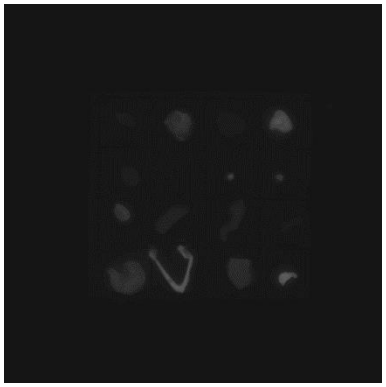


Figure S29: **S11** (organs 5h after injection of RGD-A7R compound **1**):

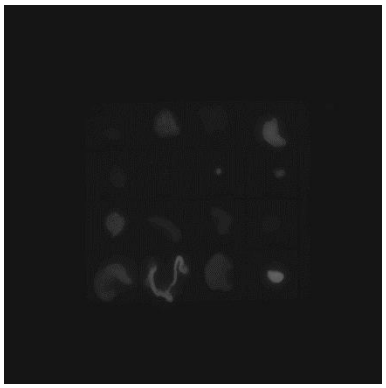
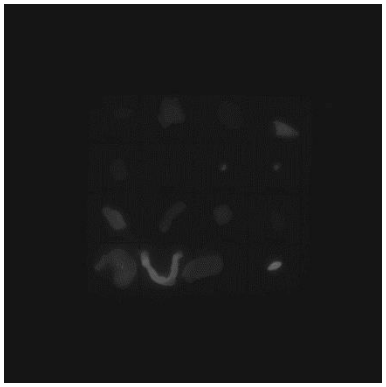
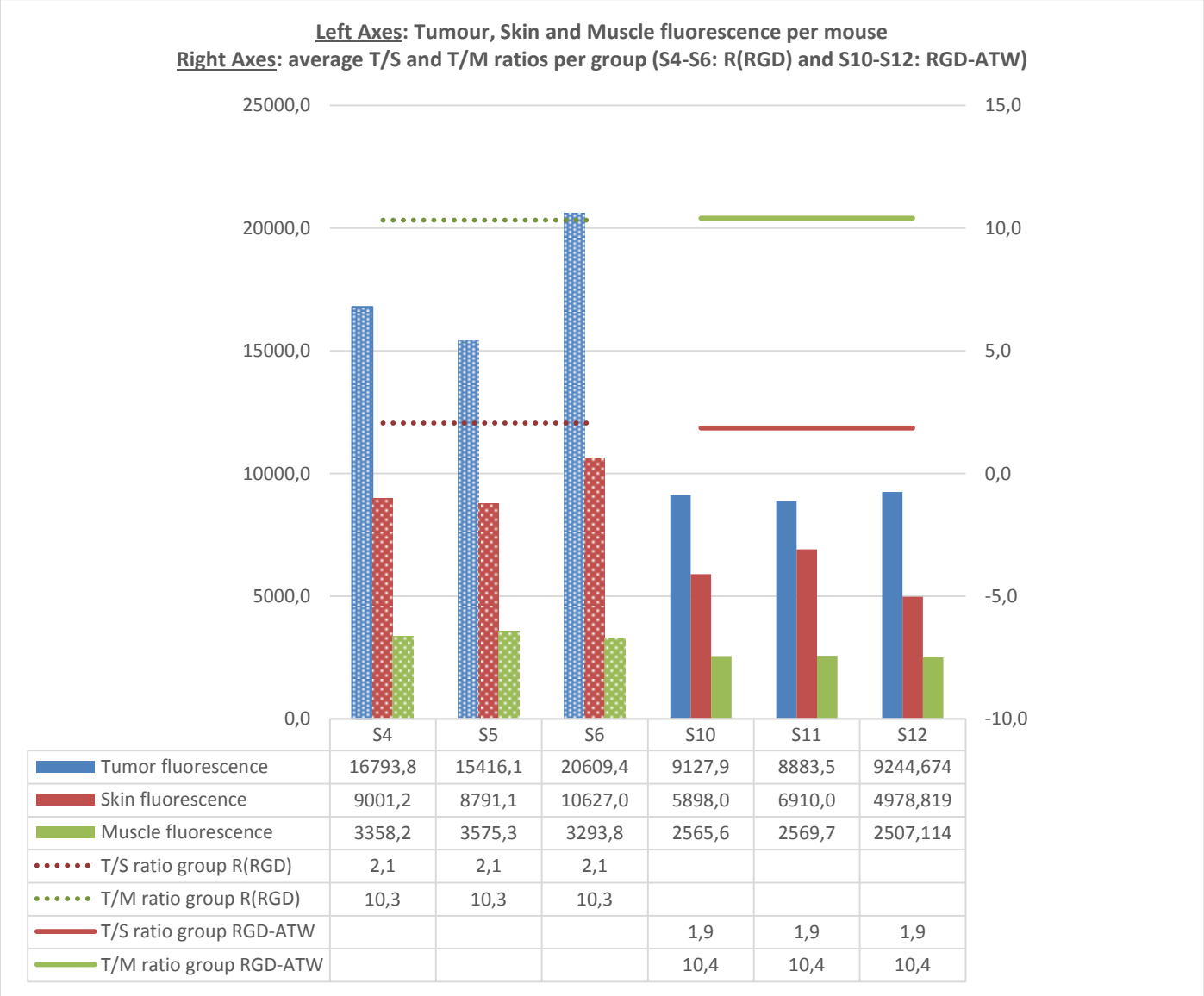


Figure S30: **S12** (organs 5h after injection of RGD-A7R compound **1**):



Mean fluorescence for Tumour, Skin and Muscle for each mouse (S4 to S6 and S10 to S12) at t=5h after injection are reported in the following figure S31. Average Tumour/Skin and Tumour/Muscle ratios for R(RGD) group (S4 to S6) and RGD-ATW group (S10 to S12) are also indicated.



	S4	S5	S6	S10	S11	S12
Tumor fluorescence	16793,8	15416,1	20609,4	9127,9	8883,5	9244,674
Skin fluorescence	9001,2	8791,1	10627,0	5898,0	6910,0	4978,819
Muscle fluorescence	3358,2	3575,3	3293,8	2565,6	2569,7	2507,114
T/S ratio group R(RGD)	2,1	2,1	2,1			
T/M ratio group R(RGD)	10,3	10,3	10,3			
T/S ratio group RGD-ATW				1,9	1,9	1,9
T/M ratio group RGD-ATW				10,4	10,4	10,4

Figure S31. *Ex vivo* mean fluorescence for tumour, skin and muscle ratios along with T/S and T/M ratios at t=5h for R(RGD) peptide group (compound **2**) and RGD-ATW peptide group (compound **1**).

- *Ex vivo* fluorescence of organs at t=24h post-injection

RGD peptide **2** was injected to mice S1 to S3, RGD-A7R peptide **1** was injected to mice S7 to S9, RβAD peptide **4** was injected to mice S16 to S18. 24h after injection, mice S1, S2, S3, S7, S8, S9, S16, S17 and S18 were sacrificed. Immediately after sacrifice, dissection and *ex vivo* imaging of organs (on Hamamtsu®) were performed.
Organs are placed on a paper as disclosed in the following table :

Heart	Lung	Brain	Skin
Muscle	Kidney	Adrenal	Bladder
Intestine	Spleen	Pancreas	Fat
Stomach	Uterus-Ovary	Liver	Tumour

Table S2 Organ repartition for *ex vivo* fluorescence imaging

Ex vivo fluorescence imaging realised for each mice are presented above:

Figure S32: **S1** (organs 24h after injection of RGD compound **2**):

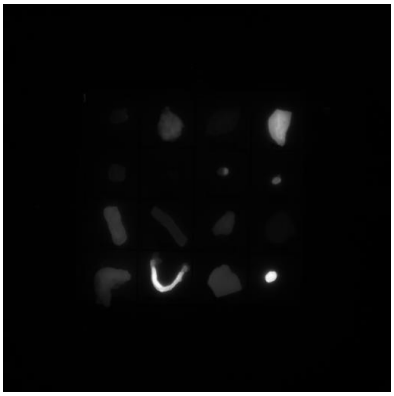


Figure S33: **S2** (organs 24h after injection of RGD compound **2**):

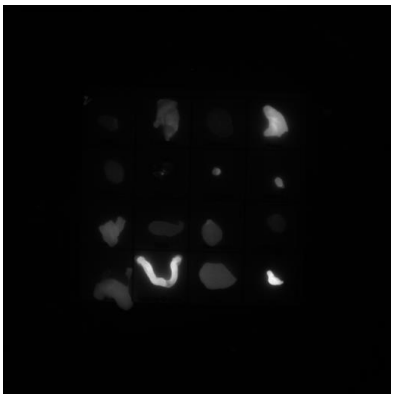


Figure S34: **S3** (organs 24h after injection of RGD compound **2**):

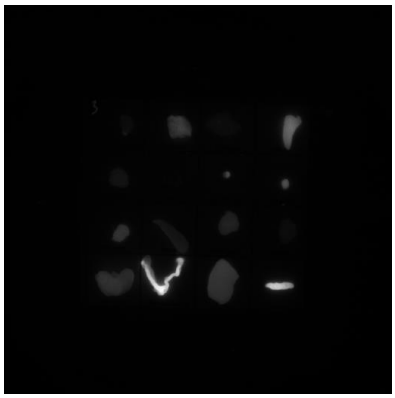


Figure S35: **S7** (organs 24h after injection of RGD-A7R compound **1**):

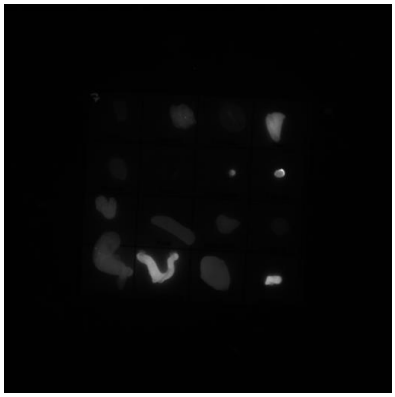


Figure S36: **S8** (organs 24h after injection of RGD-A7R compound **1**):

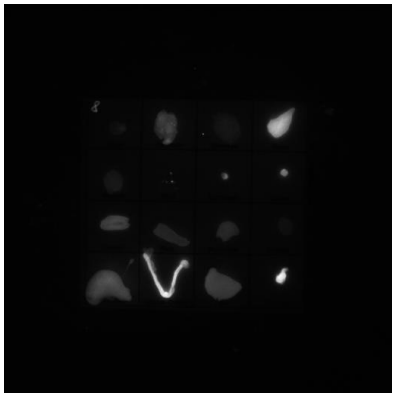


Figure S37: **S9** (organs 24h after injection of RGD-A7R compound **1**):

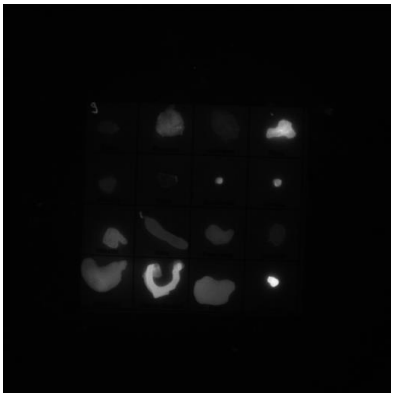


Figure S38: **S16** (organs 24h after injection of RβAD compound **4**):

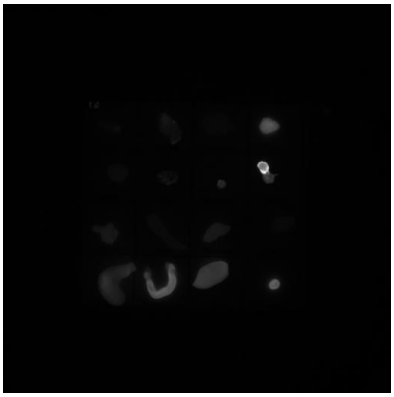


Figure S39: **S17** (organs 24h after injection of RβAD compound **4**):

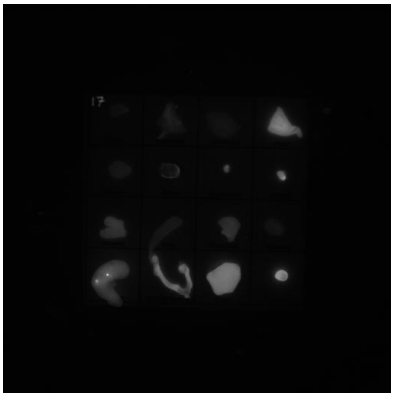
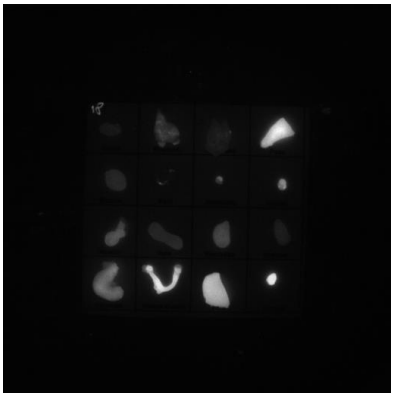
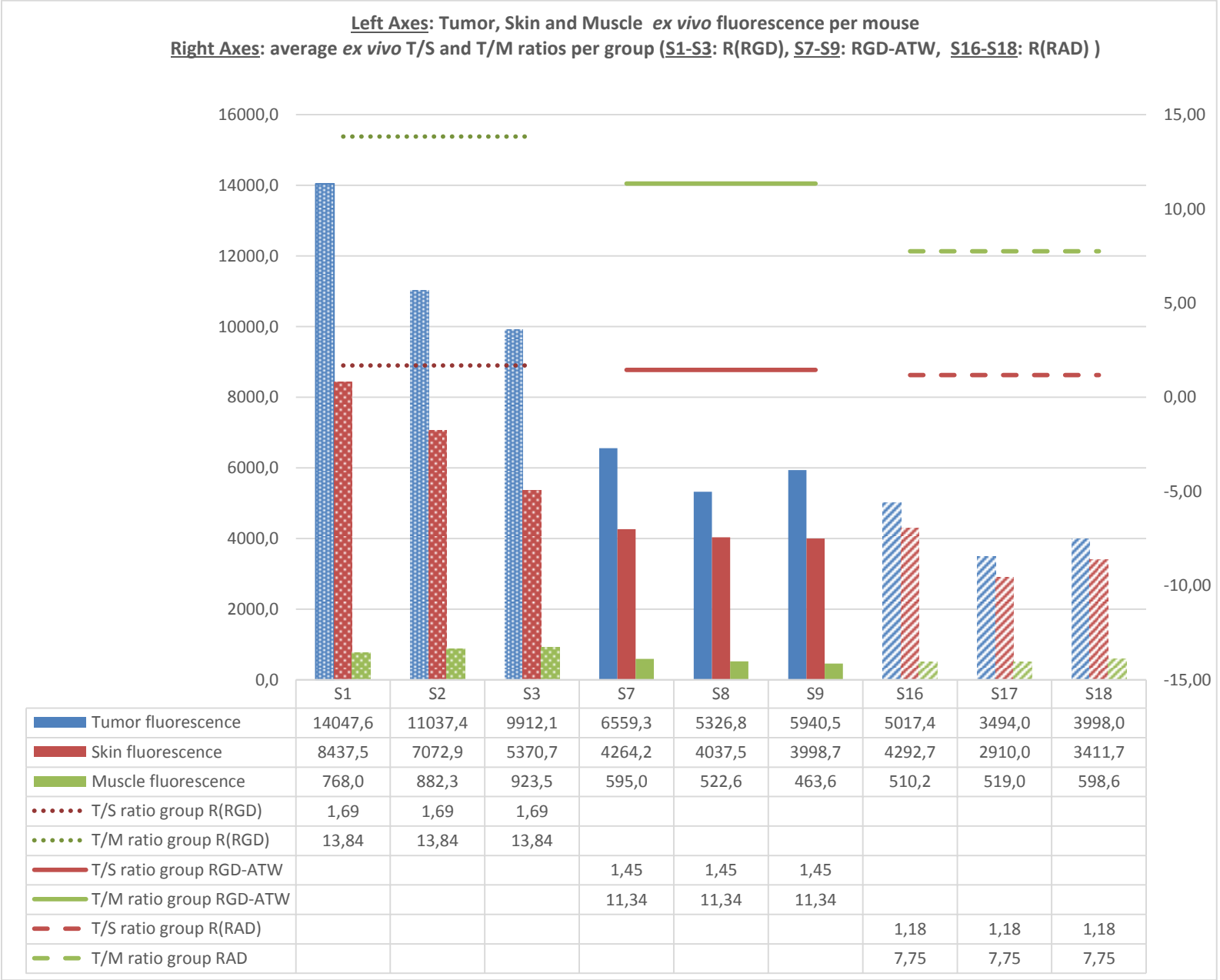


Figure S40: **S18** (organs 24h after injection of RβAD compound **4**):



Mean fluorescence for tumour, skin and muscle for each mouse (S1 to S3, S7 to S9 and S16 to S18) at 24h after injection are reported in figure S41. Average Tumour/Skin and Tumour/Muscle ratios for R(RGD) group (S1 to S3), RGD-ATW group (S7 to S9 to S12) and R(RAD) group (S16 to S18) are also indicated.



	S1	S2	S3	S7	S8	S9	S16	S17	S18
Tumor fluorescence	14047,6	11037,4	9912,1	6559,3	5326,8	5940,5	5017,4	3494,0	3998,0
Skin fluorescence	8437,5	7072,9	5370,7	4264,2	4037,5	3998,7	4292,7	2910,0	3411,7
Muscle fluorescence	768,0	882,3	923,5	595,0	522,6	463,6	510,2	519,0	598,6
T/S ratio group R(RGD)	1,69	1,69	1,69						
T/M ratio group R(RGD)	13,84	13,84	13,84						
T/S ratio group RGD-ATW				1,45	1,45	1,45			
T/M ratio group RGD-ATW				11,34	11,34	11,34			
T/S ratio group R(RAD)							1,18	1,18	1,18
T/M ratio group RAD							7,75	7,75	7,75

Figure S41. *Ex vivo* Mean Fluorescence for Tumour, Skin and Muscle ratios along with T/S and T/M ratios at t=24h for R(RGD) group (compound **2**), RGD-ATW group (compound **1**), and R(RAD) group (compound **4**).