

Supporting Information

Integrin-targeted delivery into cancer cells of a Pt(IV) pro-drug through conjugation to RGD-containing peptides

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1. Experimental procedures

1.1. Materials and Methods.

Unless otherwise stated, common chemicals and solvents (HPLC grade or reagent grade quality) were purchased from commercial sources and used without further purification. Peptide grade DMF was purchased from Scharlau. Fmoc-protected amino acids, resins and coupling reagents for solid phase synthesis were obtained from Novabiochem, Bachem or Iris Biotech. Copper(I) catalyst (Cu nanopowder, CAS 7440-50-8) was purchased from Alfa Aesar. Milli-Q water was directly obtained from a Milli-Q system equipped with a 5000-Da ultrafiltration cartridge. Fluorescein-5(6)-carboxamidocaproic acid *N*-succinimidyl ester was purchased from Aldrich. All the assayed compounds displayed a purity $\geq 95\%$, determined by HPLC analysis.

Analytical reversed-phase HPLC analyses were carried out on a Jupiter Proteo column (250x4.6 mm, 4 μm , flow rate: 1 mL/min), using linear gradients of 0.045% TFA in H₂O (solvent A) and 0.036% TFA in ACN (solvent B). In some cases, small-scale purification was carried out using the same column. Large-scale purification was carried out on a Jupiter Proteo semipreparative column (250 x 10 mm, 10 μm , flow rate: 3 mL/min), using linear gradients of 0.1% TFA in H₂O (solvent A) and 0.1% TFA in ACN (solvent B). After several runs, pure fractions were combined and lyophilized.

NMR spectra were recorded at 25 °C on Varian Mercury 400 MHz spectrometers using deuterated solvents. Tetramethylsilane (TMS) was used as an internal reference (δ 0 ppm) for ¹H spectra recorded in CDCl₃ and the residual signal of the solvent (δ 77.16 ppm) for ¹³C spectra. For CD₃OD, acetone-*d*₆, DMSO-*d*₆ or D₂O, the residual signal of the solvent was used as a reference.

High resolution MALDI-TOF mass spectra were recorded on a 4800 Plus MALDI-TOF/TOF spectrometer (Applied Biosystems) in the positive-ion mode using 2,4-dihydroxybenzoic acid as a matrix. ESI mass spectra (ESI-MS) were recorded on a Micromass ZQ instrument with

single quadrupole detector coupled to an HPLC. High resolution electrospray mass spectra (HR ESI MS) were obtained on an Agilent 1100 LC/MS-TOF instrument.

1.2. Cell lines.

The SK-MEL-28 human melanoma and CAPAN-1 pancreas adenocarcinoma cell lines were from the American Tissue Culture Collection (ATCC, Rockville, MD, USA). The 1BR3G human skin fibroblasts were from the European Collection of Cell Cultures (ECACC, Porton, UK). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (Gibco-BRL, Grand Island, NY, USA) and 100 U mL⁻¹ penicillin-streptomycin (Gibco-BRL) at 37°C in a humidified atmosphere containing 5% CO₂. Cell growth and morphology were assessed using an inverted microscope.

1.3. Synthesis of *cis,cis,trans*-[PtCl₂(2-methylpyridine)(NH₃)(OH)₂] (3).

To a solution of picoplatin (100 mg, 0.26 mmol) in a 1:10 mixture of water and heptane (20 mL), hydrogen peroxide (15 mol equiv.) was added dropwise and the mixture was stirred at 80°C for 2 h. Once at room temperature, the reaction mixture was cooled with an ice bath for 1 h. The precipitate was filtered and washed with methanol. Overall yield (synthesis + purification): 54 mg of a yellow solid, 51%. Characterization: ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 9.02 (1H, m, H6 2-pic), 7.97 (1H, t, *J*= 7.6 Hz, H5 2-pic), 7.35-7.47 (2H, m, H3+H4 2-pic), 5.90 (m, 3H, NH₃), 3.08 (s, 3H); ¹⁹⁵Pt NMR (85.9 MHz, D₂O): δ (ppm): 1100.

1.4. Synthesis of *cis,cis,trans*-[PtCl₂(2-methylpyridine)(NH₃)(OH)(succinate)] (4).

To a cold suspension of *cis,cis,trans*-[PtCl₂(2-methylpyridine)(NH₃)(OH)₂] (3) (46 mg, 0.11 mmol) in anhydrous DMF (1 mL), a solution of succinic anhydride (1.2 mol equiv.) in DMF (1 mL) was added. The reaction mixture was stirred for 2 days at 40°C protected from light. After evaporation *in vacuo*, the solid residue was dissolved in acetone and filtered. The yellow solution was evaporated *in vacuo*, and diethyl ether was added to precipitate the compound. The solid was washed several times with a 1:1 mixture of acetone and diethyl ether to remove the excess of succinic anhydride. Overall yield (synthesis + purification): 15

mg of a yellow solid, 26%. Characterization: $R_t = 8.4$ min (Gracesmart C_{18} column (150 x 2.1 mm, 5 μ m, flow rate: 0.25 mL/min), analytical gradient: 0 to 25 % B in 30 min, A: 0.1% formic acid in H_2O , B: 0.1% formic acid in ACN); HR ESI MS, positive mode: m/z 510.0147 (calcd mass for $C_{10}H_{17}Cl_2N_2O_5Pt [M+H]^+$: 510.0156), m/z 531.9978 (calcd mass for $C_{10}H_{16}Cl_2N_2O_5PtNa [M+Na]^+$: 531.9976); 1H NMR (400 MHz, $DMSO-d_6$) δ (ppm): 12.0 (1H, br s, COOH), 8.84 (1H, br s, H6 2-pic), 8.03 (1H, t, $J = 7.6$ Hz, H5 2-pic), 7.49 (2H, m, H3+H4 2-pic), 6.63 (br, 3H, NH_3), 2.49 (3H, s, CH_3 2-pic), 2.43 (2H, m, succinate), 2.36 (2H, m, succinate).

1.5. Synthesis of RGD-containing peptides (8 and 11) and their fluorescein-labelled derivatives (12 and 13).

Solid-phase peptide syntheses were performed manually in a polypropylene syringe fitted with a polyethylene disc. Standard Fmoc/tBu chemistry was used with 2-chlorotriylchloride resin ($f = 1.5$ mmol/g, 100-200 mesh). The following protecting groups were used for the protection of trifunctional amino acids: Alloc (N^{ϵ} -allyloxycarbonyl, Lys), Boc (N^{ϵ} -tert-butoxycarbonyl, Lys), Pbf (N^G -2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, Arg) and t Bu (O -tert-butyl, Asp). Fmoc-Lys[N -4-pentynoic acid]-OH was synthesized following a previously reported procedure. First, the resin was washed with neutral DCM (2 x 5 min and 1 x 30 min) and the loading was reduced to *ca* 1 mmol/g by incorporation of Fmoc-Gly-OH (0.7 mol equiv.) in the presence of DIPEA (5 mol equiv.) in anhydrous DCM for 40 min. After capping with MeOH (1 x 10 min), the following Fmoc-protected amino acids (4 mol equiv.) were incorporated with DIPC (4 mol equiv.) and HOAt (4 mol equiv.) in anhydrous DMF for 2 h. The coupling efficiency was assessed by the ninhydrin test. Fmoc protecting groups were removed with 20% piperidine in DMF (2 x 10 min) in each synthesis cycle. After removal of the final N -terminal Fmoc group, linear peptides were released from the support by treatment with AcOH/TFE/DCM 1:1:8 (v/v/v) (2 x 30 min). The collected filtrates were evaporated *in vacuo*, and several co-evaporations with dioxane (4 x 25 mL) and DCM (4 x 25

mL) were carried out to remove completely acetic acid. The resulting residue was dissolved in the minimum amount of DCM and poured onto cold diethyl ether to precipitate the fully protected linear peptide. The crude was triturated and washed three times with ether. Cyclization was carried out in DMF (*ca* 1 mL/ mg crude peptide) at pH 8-9 (adjusted with DIPEA) by using PyBOP (1 mol equiv.). After it was stirred for 18 h at room temperature, the reaction mixture was evaporated *in vacuo*. Diethyl ether was used to precipitate the peptides **7** and **9**, which were used in the next step without further purification.

c(RGDfK(N^ε-PEG-NH₂)) (**8**). To a solution of the cyclic peptide c[-Arg(Pbf)-Gly-Asp(tBu)-D-Phe-Lys(Alloc)-] (**7**) (750 mg, 0.75 mmol) in anhydrous DCM/DMF 3:1 (40 mL) under Ar, phenylsilane (9.8 mL, 105 mol equiv.) was added and the resulting yellow mixture was stirred for 3 min. Then, Pd(PPh₃)₄ (23.4 mg, 0.027 mol equiv.) was added and the resulting orange mixture was stirred for 30 min at room temperature. After evaporation *in vacuo*, the oily residue was dissolved in the minimum amount of DCM/MeOH 1:1 and added over cold diethyl ether. The crude peptide was triturated and washed three times with ether. For coupling the polyethyleneglycol spacer, 8-(9-fluorenylmethyloxycarbonyl-amino)-3,6-dioxaoctanoic acid (1 mol equiv.) was first pre-activated with HATU (0.95 mol equiv.) and DIPEA (4 mol equiv.) for 2 min in anhydrous DMF. The resulting yellow solution was added to the crude peptide c[-Arg(Pbf)-Gly-Asp(tBu)-D-Phe-Lys-] (75 mg, 82 μmol) previously dissolved in anhydrous DMF and DIPEA (10 mol equiv.). After stirring for 2 h at rt, the reaction mixture was evaporated *in vacuo*, and the Fmoc group removed by treatment with 5% piperidine in DMF for 30 min. The solvent was evaporated *in vacuo* and side-chain deprotection was performed with TFA/TIS/H₂O 95:2.5:2.5 for 4 h at room temperature. After evaporation under reduced pressure, the crude peptide was triturated and washed three times with cold diethyl ether. After purification by semipreparative HPLC (gradient from 0 to 50% B in 30 min, flow rate: 3 mL/min, R_t = 19.5 min), the trifluoroacetate salt of **8** was obtained as a white solid. Overall yield (synthesis + purification) from c[-Arg(Pbf)-Gly-Asp(tBu)-D-Phe-

Lys-]: 22 mg, 27%. Characterization: $R_t = 14.1$ min (analytical gradient: 0 to 100% B in 30 min); HR ESI MS, positive mode: m/z 749.3938 (calcd mass for $C_{33}H_{53}N_{10}O_{10}$ $[M+H]^+$: 749.3941), m/z 375.2002 (calcd mass for $C_{33}H_{54}N_{10}O_{10}$ $[M+2H]^{2+}$: 375.2007).

Fluorescein-labeled c(RGDfK) (12). Peptide **8** (3 mg, 3.1 μ mol) was allowed to react with 6-[Fluorescein-5(6)-carboxamido]hexanoic acid *N*-hydroxysuccinimide ester (1.2 mol equiv) in a 1:1 mixture of ACN and aqueous phosphate buffer (100 mM, pH 7) for 48 h protected from light. Purification was carried out by semipreparative HPLC (gradient from 0 to 100% B in 30 min, flow rate: 3 mL/min, $R_t = 17.5$ min). Overall yield (synthesis + purification): 1.9 mg of a yellow solid, 46%. Characterization: $R_t = 18.3$ min (analytical gradient: 0 to 100% in 30 min); HR ESI MS, positive mode: m/z 1220.5251 (calcd mass for $C_{60}H_{74}N_{11}O_{17}$ $[M+H]^+$: 1220.5259), m/z 610.7664 (calcd mass for $C_{60}H_{75}N_{11}O_{17}$ $[M+2H]^{2+}$: 610.7666).

RAFT-{c(RGDfK)}₄ (11). The Boc-protected RAFT cyclodecapeptide scaffold **9**, c[-Lys(alkyne)-Lys(Boc)-Lys(alkyne)-Pro-Gly-Lys(alkyne)-Ala-Lys(alkyne)-Pro-Gly-] (15.5 mg, 11 μ mol), the azide-containing cyclopentapeptide **10**, c[-Arg(Pbf)-Gly-Asp(tBu)-*D*-Phe-Lys(COCH₂N₃)] (38 mg, 4.4 mol equiv.), and the copper catalyst (Cu nanopowder, Cu(core)/CuO(shell)) (17 mg) were dissolved in a 6:4 mixture of tBuOH/PBS buffer (10 mL). The reaction mixture was stirred overnight at room temperature and then filtered to remove the catalyst. The aqueous phase was diluted with acidic Milli-Q water (0.1 % TFA) and lyophilized. Finally, deprotection was performed by treatment with TFA/H₂O 9:1 for 2 h at room temperature. After evaporation *in vacuo*, purification was accomplished by MPLC with a gradient from 0 to 60% of B as eluent (A: 0.1% TFA in H₂O; B: 0.1% TFA in ACN; 600 mL each solvent). Pure fractions by analytical HPLC were combined and lyophilized, providing the desired product (**11**) as a white solid. Overall yield (click reaction + Boc deprotection + purification): 8.2 mg of a white solid, 18%. Characterization: $R_t = 13.7$ min (analytical gradient: 0 to 100% B in 30 min); HR ESI MS, positive mode: m/z 1022.2739 (calcd mass for $C_{183}H_{272}N_{63}O_{46}$ $[M+3H]^{3+}$: 1022.2734).

-Fluorescein-labeled RAFT-*c*(RGDfK)₄ 13. RAFT peptide **11** (1.5 mg, 0.32 μmol) was allowed to react with 6-[Fluorescein-5(6)-carboxamido]hexanoic acid *N*-hydroxysuccinimide ester (1.2 mol equiv) in a 1:1 mixture of ACN and aqueous phosphate buffer (100 mM, pH 7) for 48 h protected from light. Overall yield (synthesis + purification by analytical HPLC): 1.0 mg of a yellow solid, 62%. Characterization: $R_t = 16.9$ min (analytical gradient: 0 to 100 % in 30 min); HR ESI MS, positive mode: m/z 912.2466 (calcd mass for C₂₁₀H₂₉₅N₆₄O₅₃ [M+5H]⁵⁺: 912.2465), m/z 1140.0552 (calcd mass for C₂₁₀H₂₉₄N₆₄O₅₃ [M+4H]⁴⁺: 1140.0563), m/z 1519.7375 (calcd mass for C₂₁₀H₂₉₃N₆₄O₅₃ [M+3H]³⁺: 1519.7394).

1.6. Synthesis of Pt-peptide conjugates (5 and 6).

-Picoplatin(IV)-*c*(RGDfK) conjugate (5). To a solution of **4** (4.3 mg, 1.5 mol equiv.) and HATU (3.0 mg, 1.4 mol equiv.) in anhydrous DMF (0.5 mL), DIPEA (5.8 μL, 4.5 mol equiv.) was added. After stirring for 2 min at room temperature, the reaction mixture was added to peptide **8** (5.5 mg, 5.6 μmol) previously dissolved in anhydrous DMF (0.3 mL) and DIPEA (9.8 μL, 10 mol equiv.). After stirring for 2 h at room temperature, the solvent was evaporated *in vacuo* and the conjugate was purified by semipreparative HPLC (gradient from 0 to 80% B in 30 min, flow rate: 3 mL/min, $R_t = 16.0$ min). Overall yield (synthesis + purification): 4 mg of a white solid, 52%. Characterization: $R_t = 13.6$ min (analytical gradient: 0 to 100 % in 30 min); HR ESI MS, positive mode: m/z 1240.3927 (calcd mass for C₄₃H₆₇Cl₂N₁₂O₁₄Pt [M+H]⁺: 1240.3924).

-Picoplatin(IV)-RAFT-*c*(RGDfK)₄ conjugate (6). To a solution of **4** (0.68 mg, 1.6 mol equiv.) and HATU (0.48 mg, 1.5 mol equiv.) in anhydrous DMF (0.3 mL), DIPEA (0.7 μL, 4.5 mol equiv.) was added. After stirring for 2 min at room temperature, the reaction mixture was added to RAFT peptide **11** (3.8 mg, 0.82 μmol) previously dissolved in anhydrous DMF (0.1 mL) and DIPEA (1.4 μL, 10 mol equiv.). After stirring for 2 h at room temperature, the solvent was evaporated *in vacuo* and the conjugate was purified by semipreparative HPLC (gradient from 0 to 70% B in 30 min, flow rate: 3 mL/min, $R_t = 20.0$ min). Overall yield

(synthesis + purification): 1.1 mg of a white solid, 27%. Characterization: $R_t = 14.6$ min (analytical gradient: 0 to 100 % in 30 min); HR ESI MS, positive mode: m/z 1145.0359 (calcd mass for $C_{193}H_{286}Cl_2N_{65}O_{50}Pt$ $[M+4H]^{4+}$: 1145.0234), 1526.3668 (calcd mass for $C_{193}H_{286}Cl_2N_{65}O_{50}Pt$ $[M+3H]^{3+}$: 1526.3620).

1.7. Integrin expression analysis.

The expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins on the cell surface was analyzed by double immuno-fluorescence. The cells were incubated for 30 min at 4°C with monoclonal antibodies against human $\alpha_v\beta_3$ (clone LM609) (Millipore, Temecula, CA) and $\alpha_v\beta_5$ integrin (sc-81632) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or medium alone as negative control. After washing with phosphate-buffered saline (PBS) (Gibco-BRL), cells were incubated for additional 30 min at 4°C with the Alexa-Fluor 488-conjugated goat anti-mouse IgG antibody (Invitrogen, Carlsbad, CA, USA). Next, the cell fluorescence was analyzed using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) equipped with CellQuest™ software (Becton Dickinson). 10.000 cells were analysed in each experiment. Fluorescence intensity was represented on a four orders of magnitude log scale (1-10.000).

1.8. Internalization experiments with 12 and 13.

The uptake efficiency of **12** and **13** by the cells was quantified by flow cytometry. 60.000 cells were seeded onto 24-well plates and allowed to attach for 24 h. Then, the cells were treated with either **12** or **13** at 25 μ M, or medium alone as a control, for 1 h at 37°C. After washing the cells three times with cold PBS (Gibco), the cells were harvested by trypsinization and the fluorescence of the cells, corresponding to the internalization of **12** or **13**, was analysed using a FACSCalibur (Becton Dickinson Immunocytometry Systems). The geometric mean fluorescence of 10.000 cells was determined with the CellQuest™ software (Becton Dickinson).

1.9. Cytotoxicity studies.

The cytotoxicity of the compounds in CAPAN-1 and SK-MEL-28 tumor cells and in 1BR3G non-malignant cells was determined by the MTT assay. Peptides and conjugates were dissolved in Milli-Q water to obtain 1 mM stock solutions. Picoplatin (Abcam Biochemicals) and cisplatin (Sigma-Aldrich, St. Louis, MO) were dissolved in DMSO to provide a 10 mM solution, which was then diluted in Milli-Q to obtain a 1 mM stock solution. Appropriate aliquots of these solutions were diluted in the cell culture medium to obtain the final working solutions. Aliquots of 5000 CAPAN-1, 4000 SK-MEL-28 and 5000 1BR3G cells were seeded on 96-well plates 24 h prior to the treatments. Then, cells were treated for 72 h with the corresponding compound at concentrations ranging from 0 to 50 μ M. After removal of the treatment, cells were washed with PBS and incubated for additional 2 h in the darkness with fresh culture medium (100 μ L) with MTT (10 μ L). The medium was discarded and DMSO (100 μ L) was added to each well to dissolve the purple formazan crystals. Plates were agitated at room temperature for 2 min and the absorbance of each well was determined with an absorbance microplate reader (ELx800, BioTek, Winooski, USA) at a wavelength of 570 nm. Three replicates for each compound were used, and all treatments were tested at least in three independent experiments. For each treatment, the cell viability was determined as a percentage of the control untreated cells, by dividing the mean absorbance of each treatment by the mean absorbance of the untreated cells. The concentration that reduces by 50% the cell viability (IC_{50}) was established for each compound using a four-parameter curve fit (Gen5 Data Analysis Software, BioTeck).

1.10. Platinum accumulation in cancer cells.

For platinum cellular uptake studies, 1.5×10^6 SK-MEL-28 and CAPAN-1 cells were plated in 100 mm Petri dishes and allowed to attach for 24 h. Next, the plates were exposed to picoplatin or to conjugates **5** or **6** at a concentration (0.5 μ M) below their corresponding IC_{50} values. Additional plates were incubated with medium alone as negative control. After 24 h of

incubation, the cells were rinsed three times with cold PBS and harvested by trypsinization. The number of cells in each sample was counted manually in a haemocytometer using the trypan blue dye exclusion test. Then the cells were centrifuged to obtain the whole cell pellet for ICP-MS analysis. All experiments were conducted in triplicate.

1.11. ICP-MS analysis.

The whole cell pellets were dissolved in 400 μL of concentrated 60 % v/v nitric acid, and the samples were then transferred into wheaton v-vials (Sigma-Aldrich) and heated in an oven at 373 K for 18 h. The vials were then allowed to cool, and each cellular sample solution was transferred into a volumetric tube and combined with washings with Milli-Q water (1.6 mL). Digested samples were diluted 10 times with Milli-Q to obtain a final HNO_3 concentration of approximately 1.2% v/v. Platinum content was analyzed on an ICP-MS Perkin Elmer Elan 6000 series machine at the Centres Científics i Tecnològics of the Universitat de Barcelona. The solvent used for all ICP-MS experiments was Milli-Q water with 1% HNO_3 . The platinum standard (High-Purity Standards, 1000 $\mu\text{g}/\text{mL} \pm 5 \mu\text{g}/\text{mL}$ in 5% HNO_3) was diluted with 1% HNO_3 to 20 ppb. Platinum standards were freshly prepared in Milli-Q water with 1% HNO_3 before each experiment. The concentrations used for the calibration curve were in all cases 0, 0.2, 0.4, 1, and 2 ppb. The isotope detected was ^{196}Pt and readings were made in triplicate. Rhodium was added as an internal standard at a concentration of 10 ppb in all samples.

1.12. Statistical analysis.

The statistical analysis was performed with the SPSS statistical software for Windows (version 15.0; SPSS Inc., Chicago, IL, USA). Quantitative variables were expressed as mean and standard deviation (SD).

2. Characterization of RGD-containing peptides (8 and 11) and fluorescein-labelled derivatives (12 and 13).

c(RGDfK(N^ε-PEG-NH₂)) (8).

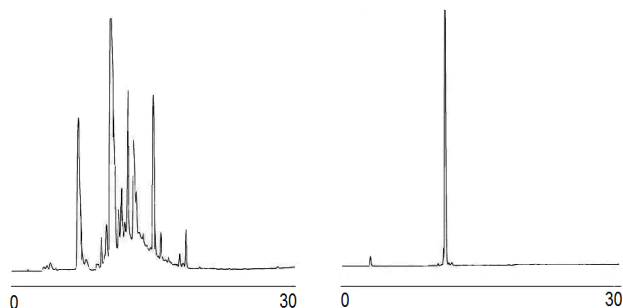


Figure S-1 Reversed-phase HPLC traces for peptide **8**: reaction crude (left) and purified (right).

Fluorescein-labeled c(RGDfK) (12).

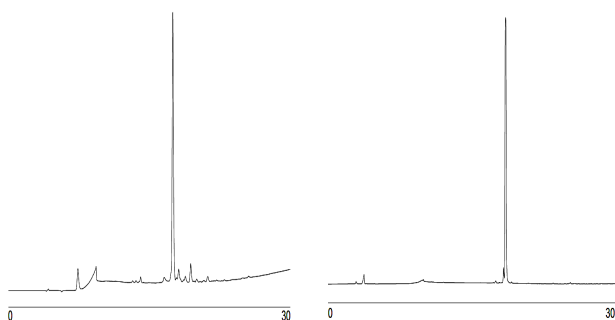


Figure S-2 Reversed-phase HPLC traces for peptide **12**: reaction crude (left) and purified (right).

RAFT-{*c*(*RGDfK*)₄ (**11**).

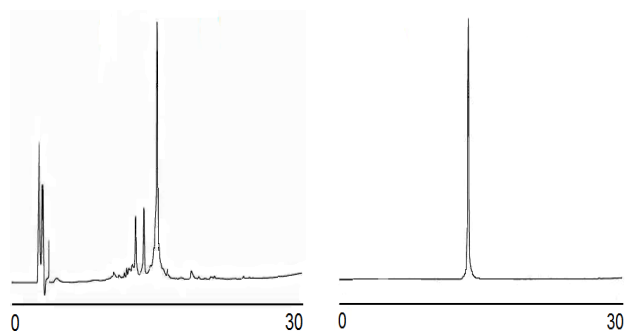


Figure S-3 Reversed-phase HPLC traces for the reaction crude of the Boc-protected peptide intermediate (left), and for the purified peptide **11** (right).

Fluorescein-labeled RAFT-{*c*(*RGDfK*)₄ **13**.

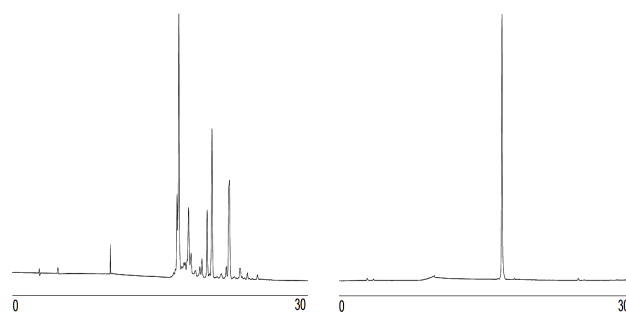


Figure S-4 Reversed-phase HPLC traces for peptide **13**: reaction crude (left) and purified (right).

3. Characterization of Pt(IV)-peptide conjugates (5 and 6).

Picoplatin(IV)-c(RGDfK) conjugate (5).

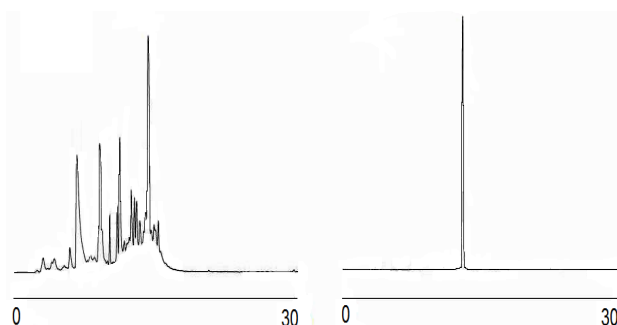


Figure S-5 Reversed-phase HPLC traces for conjugate **5**: reaction crude (left) and purified (right).

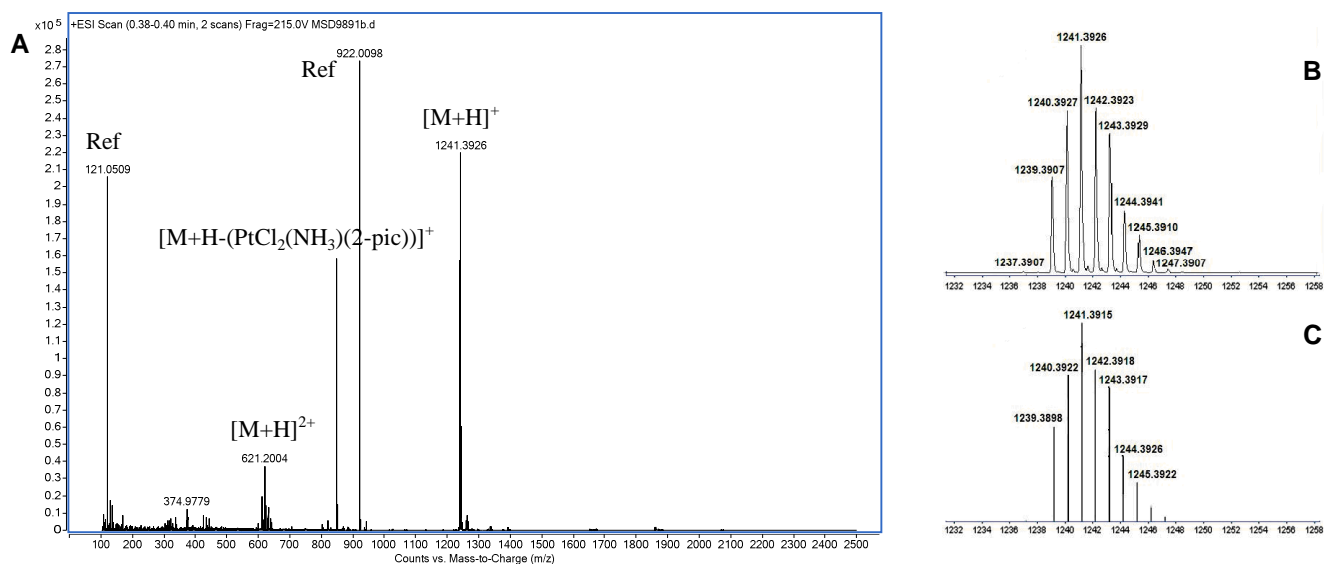


Figure S-6. High resolution ESI MS of conjugate **5**: experimental (A, B) and calculated (C).

Picoplantin(IV)-RAFT- $\{c(\text{RGDfK})\}_4$ conjugate (6).

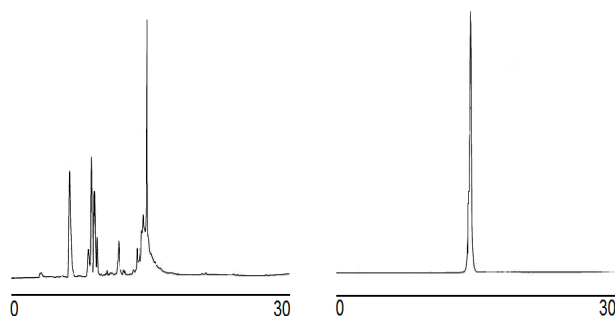


Figure S-7 Reversed-phase HPLC traces for conjugate **6**: reaction crude (left) and purified (right).

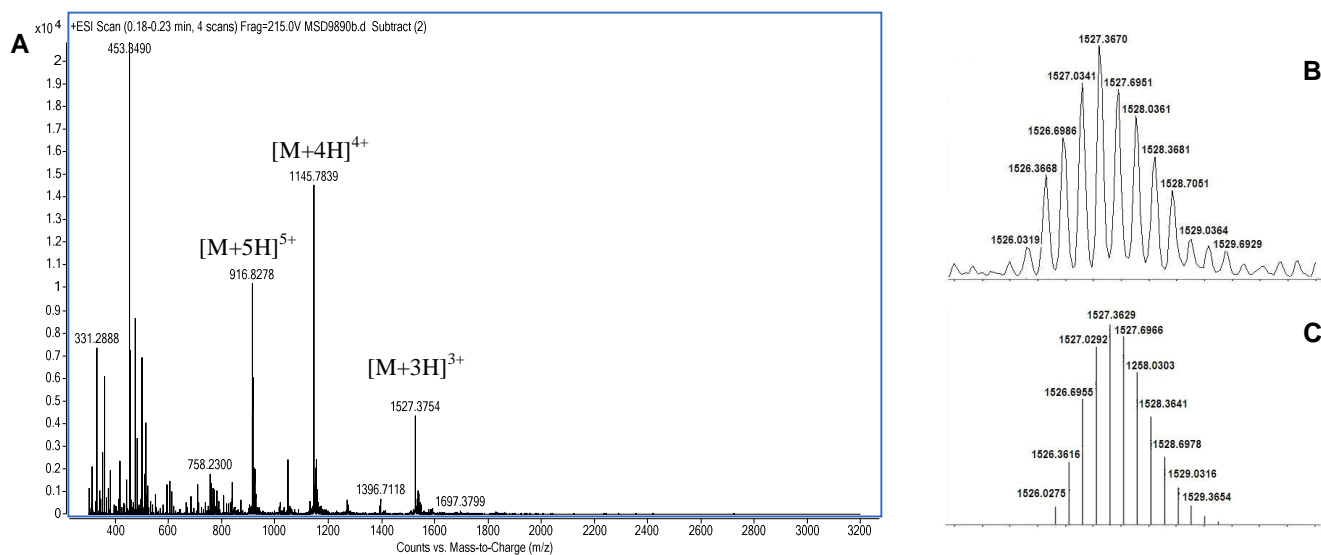


Figure S-8. High resolution ESI MS of conjugate **6**: experimental (A, B) and calculated (C).

4. ^1H NMR spectra of complexes 3 and 4, and HPLC trace of complex 4.

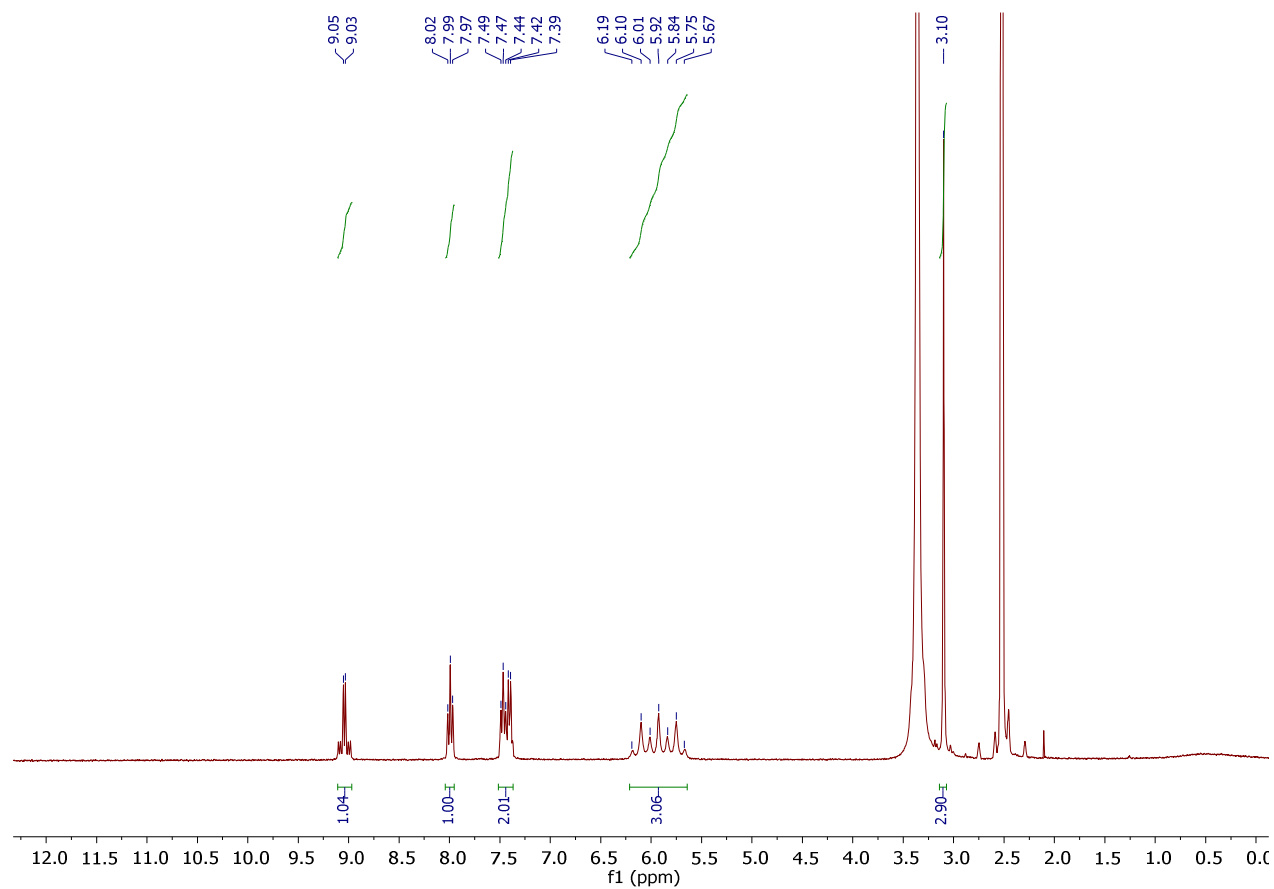


Figure S-9. ^1H NMR spectra of complex 3 in $\text{DMSO-}d_6$ (400 MHz).

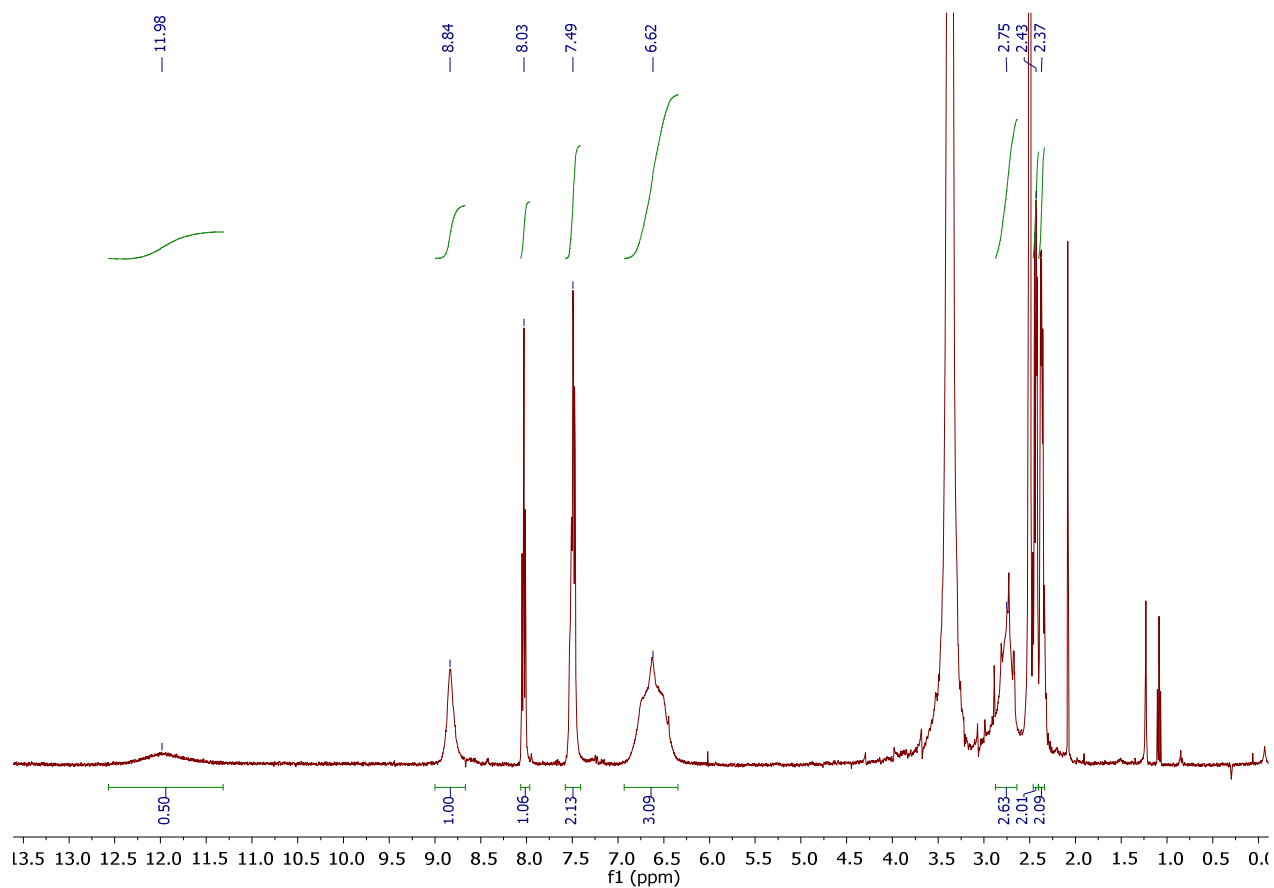


Figure S-10. ^1H NMR spectra of complex **4** in $\text{DMSO-}d_6$ (400 MHz).

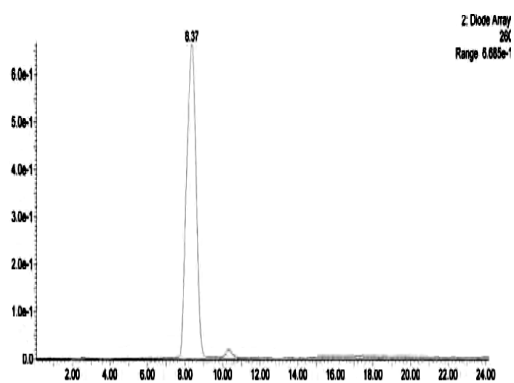


Figure S-11 Reversed-phase HPLC traces for complex **4**.

5. ^1H NMR spectra of conjugate **5**.

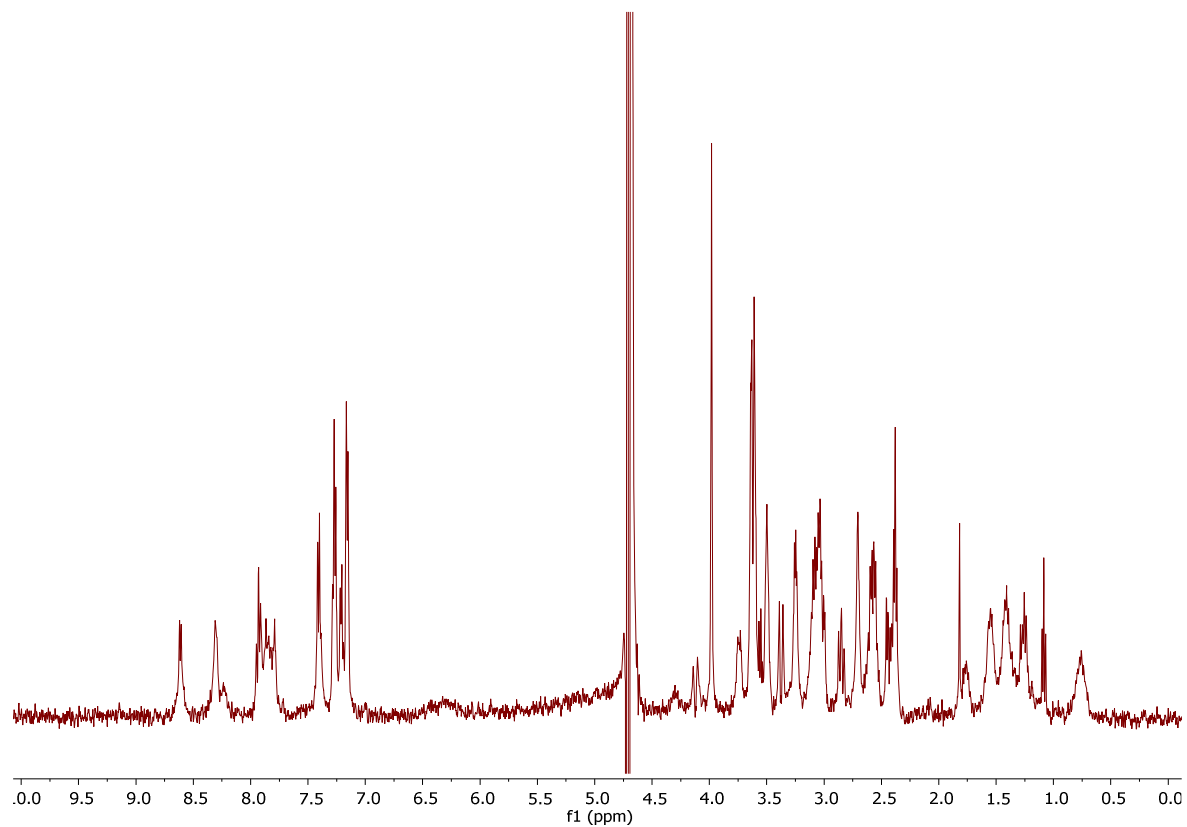


Figure S-12. ^1H NMR spectra of conjugate **5** in $\text{H}_2\text{O}/\text{D}_2\text{O}$ 9:1 (500 MHz).

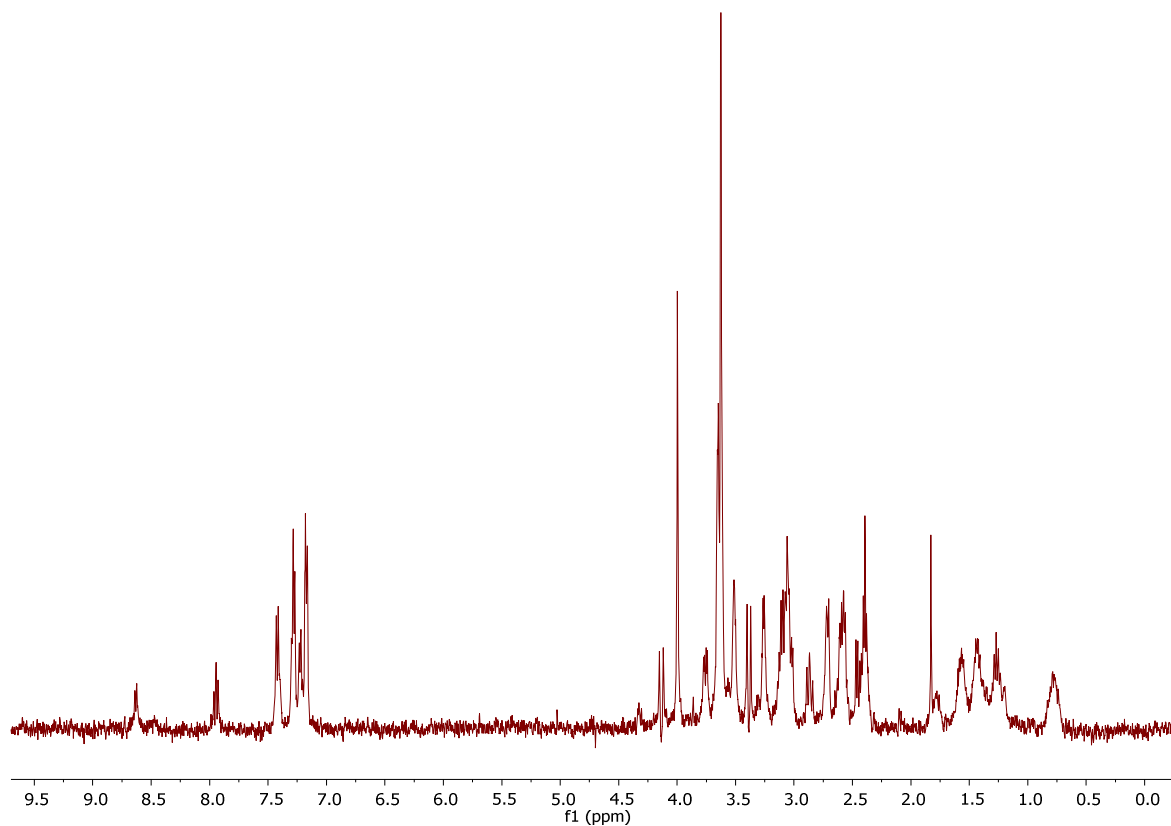


Figure S-13. ^1H NMR spectra of conjugate **5** in D_2O (500 MHz).

6. Integrin expression analysis.

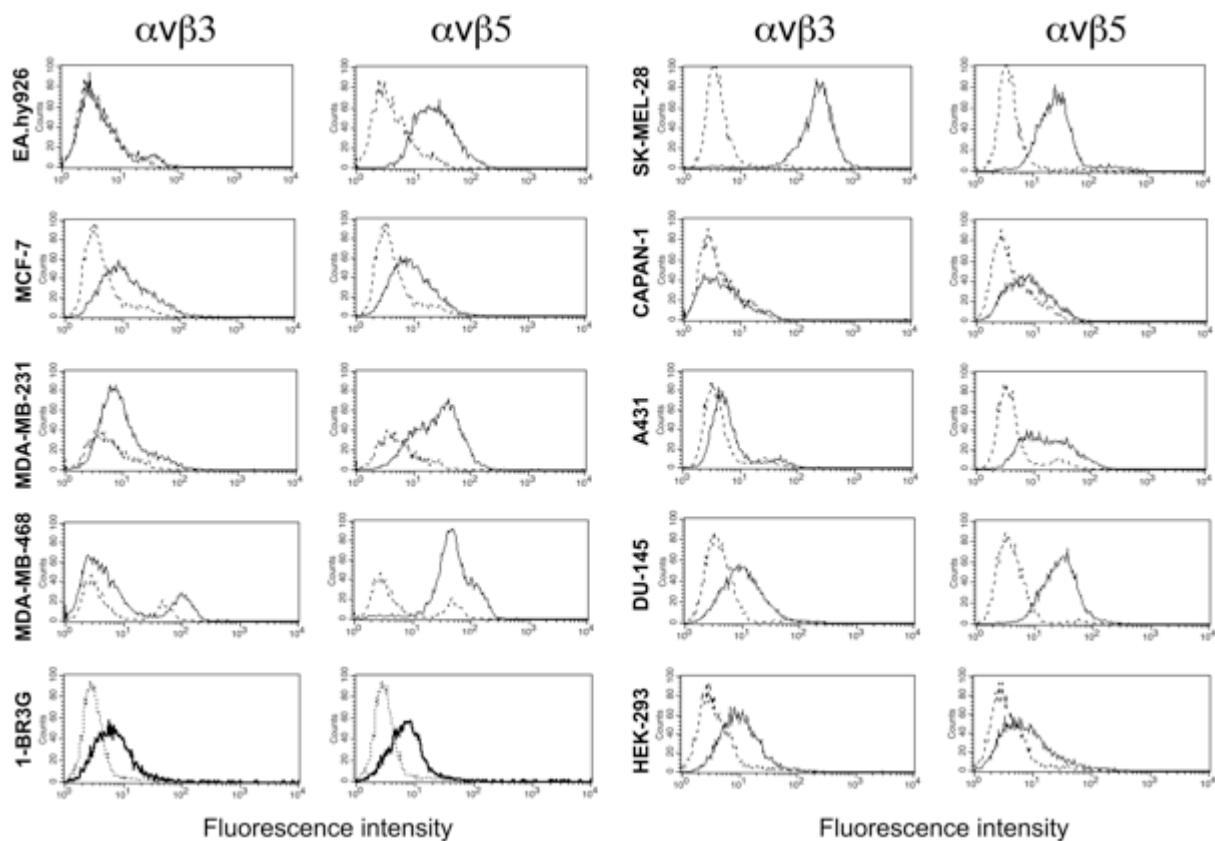


Figure S-14. Expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins on a broad panel of human cell lines. AE.hy926 hybrid umbilical vein cells, MCF-7, MDA-MB-231 and MDA-MB-468 breast cancer lines, 1BR3G fibroblasts, SK-MEL-28 melanoma cells, CAPAN-1 pancreas adenocarcinoma cells, A431 epidermoid carcinoma cells, DU-145 prostate adenocarcinoma cells and HEK-293 embryonic kidney cells were incubated with monoclonal antibodies against $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins followed by the incubation with secondary antibody conjugated to Alexa-Fluor 488. Representative flow cytometry histograms obtained after the immunofluorescence staining of the cells are showed. Solid lines represent the fluorescence intensity of the cells after the incubation with the corresponding antibodies. Dotted lines indicate the background staining with the secondary antibody alone. 10.000 cells were analysed in each experiment.

7. Cell morphology study in SK-MEL-28 cells.

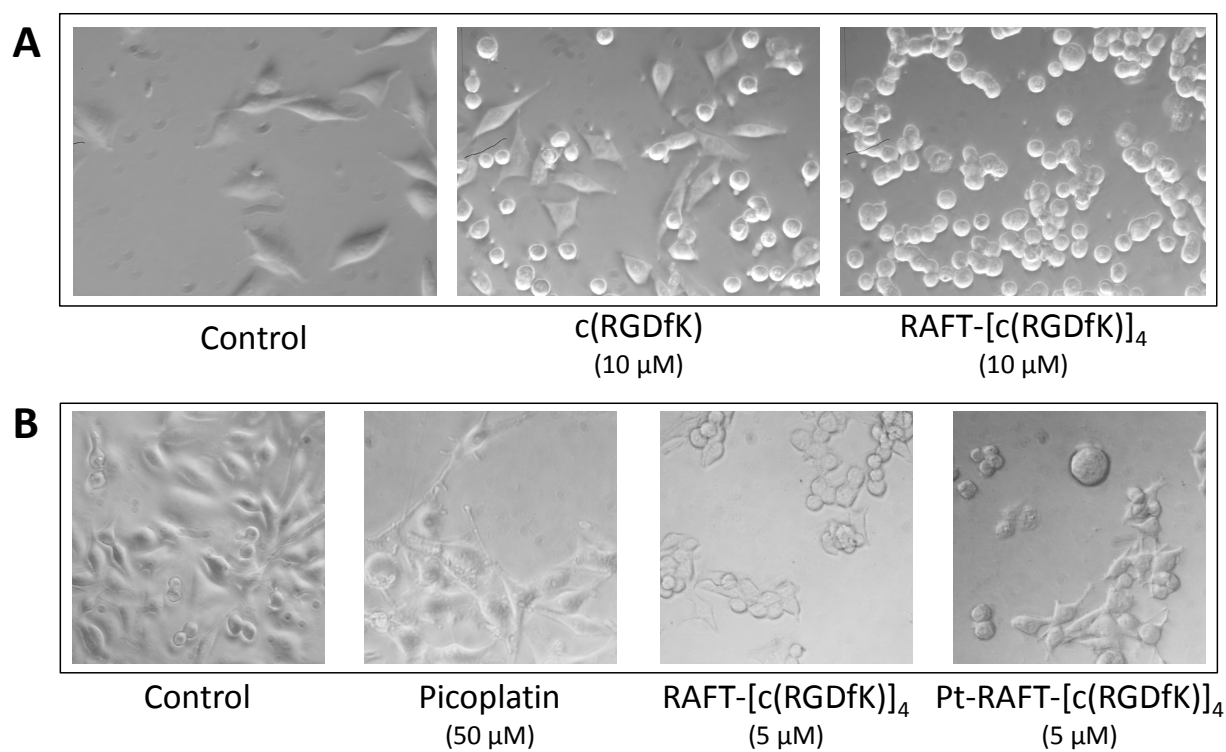


Figure S-15. Representative microscopy images of SK-MEL-28 cells. A) Changes in the cell morphology after exposure to c(RGDfK) and RAFT-{c(RGDfK)}₄ at 10 μM, medium alone was used as a control. B) Changes in the cell morphology after exposure to medium alone (control), picoplatin (50 μM), RAFT-{c(RGDfK)}₄ and Pt-RAFT-{c(RGDfK)}₄ (5 μM).

8. Activation by reduction of the Pt-peptide conjugates.

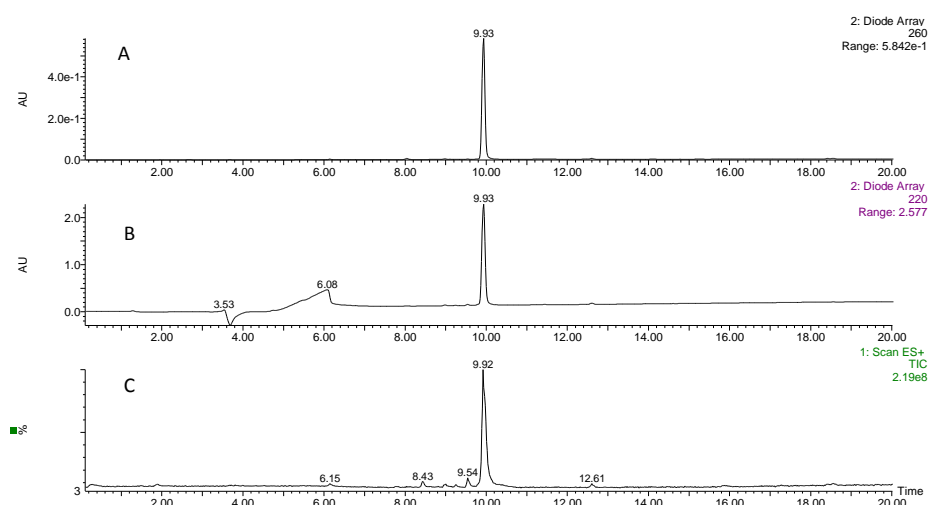


Figure S-16. MS-HPLC analysis of conjugate **5**: reversed-phase HPLC traces at 260 nm (A) and 220 nm (B), and total ion chromatogram (C).

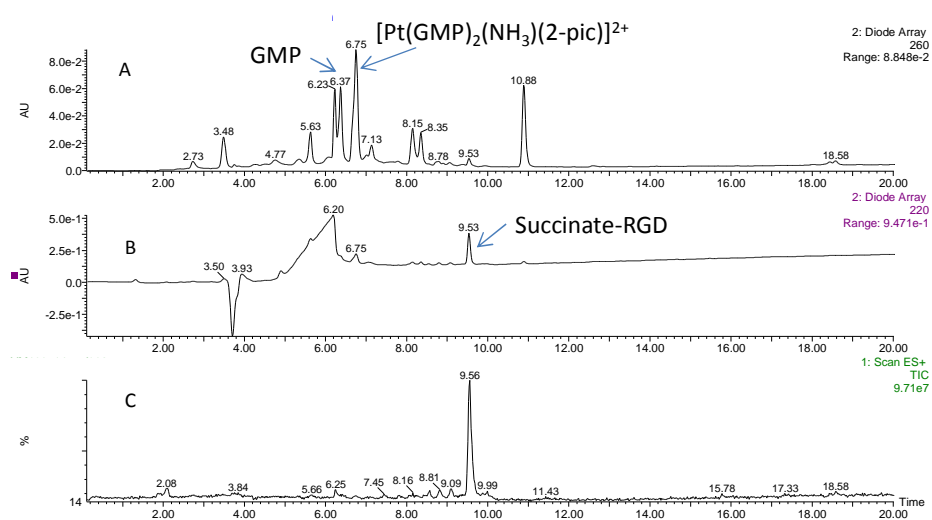


Figure S-17. MS-HPLC analysis of the reaction between conjugate **5** and ascorbate (5 mol equiv.) in the presence of 5'-GMP (5 mol equiv.) at 37°C for 24 h: reversed-phase HPLC traces at 260 nm (A) and 220 nm (B), and total ion chromatogram (C).

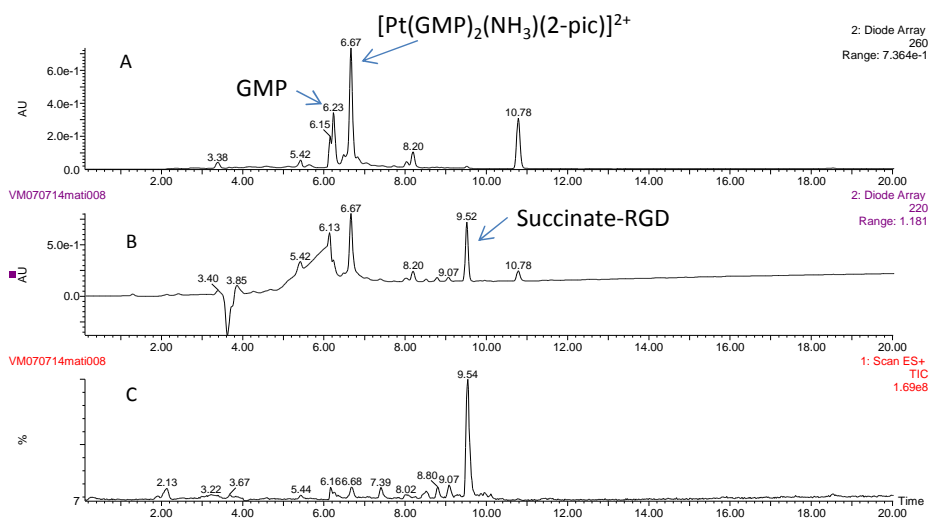


Figure S-18. MS-HPLC analysis of the reaction between conjugate 5 and ascorbate (5 mol equiv.) in the presence of 5'-GMP (5 mol equiv.) at 37°C for 72 h: reversed-phase HPLC traces at 260 nm (A) and 220 nm (B), and total ion chromatogram (C).