

## Supporting Information

### Multifunctional $\alpha_v\beta_6$ Integrin-Specific Peptide-Pt(IV) Conjugates for Cancer Cell Targeting

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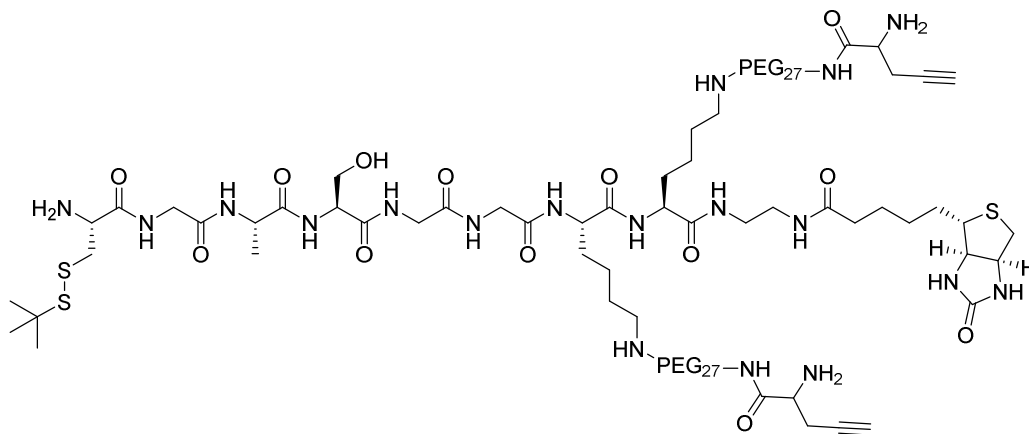
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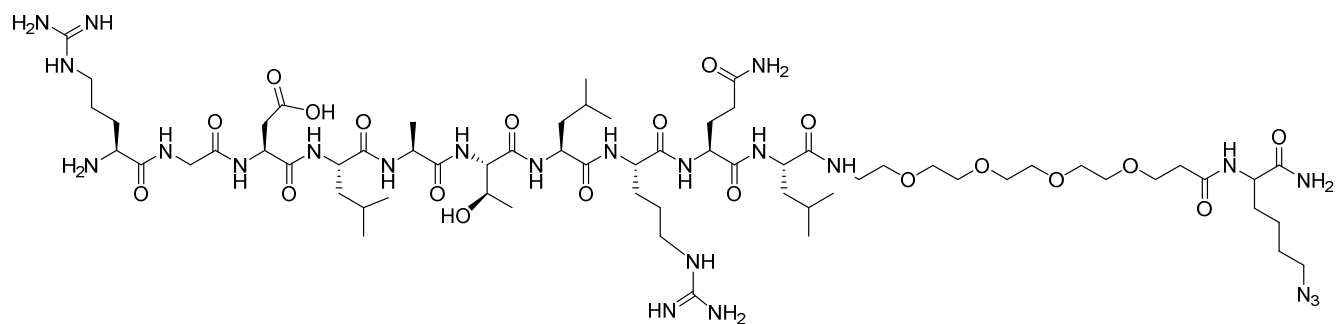
## Supporting Information

### Structures of scaffolds and targeting peptide

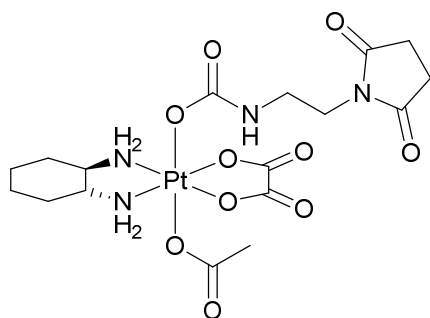
#### Peptide-PEG scaffold Y



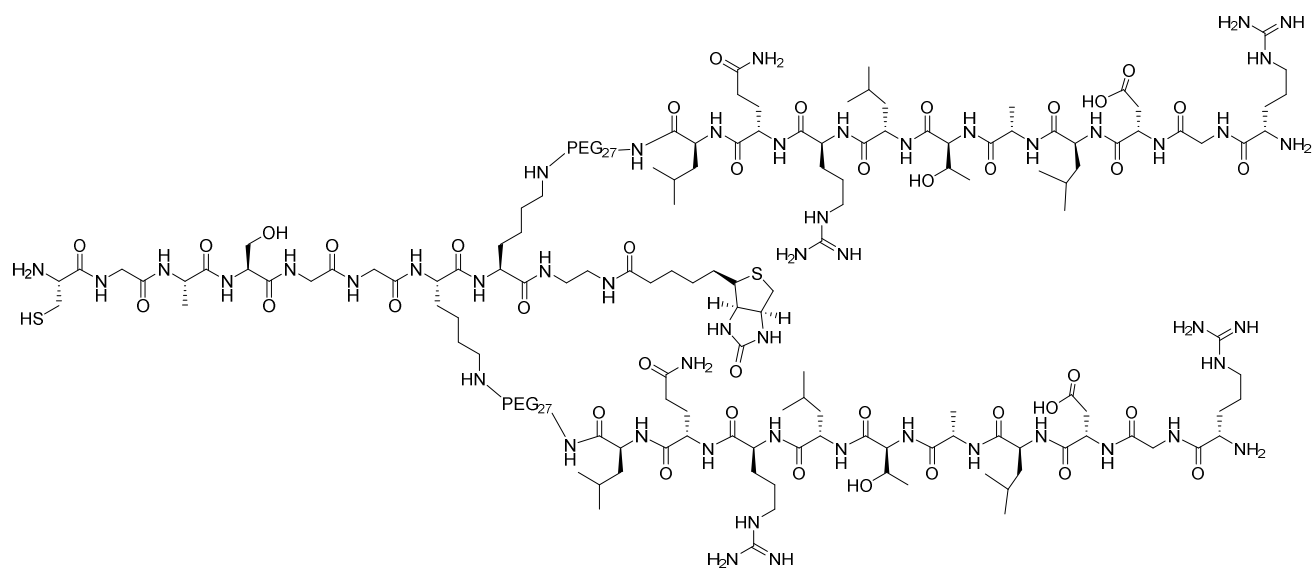
#### Integrin-targeting peptide P1



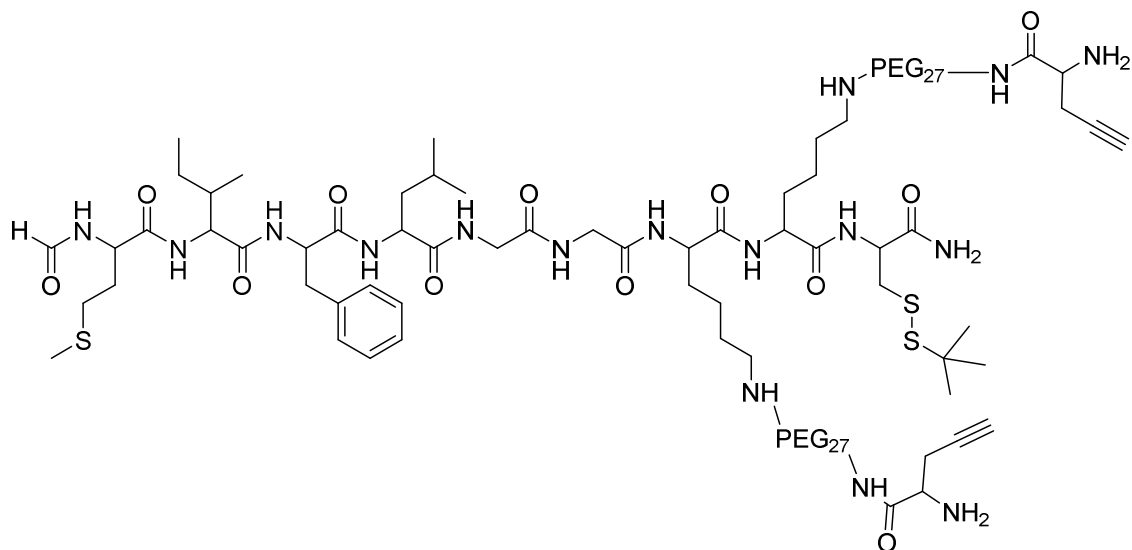
#### oxali-Pt-succ



## Combined Y-1 construct



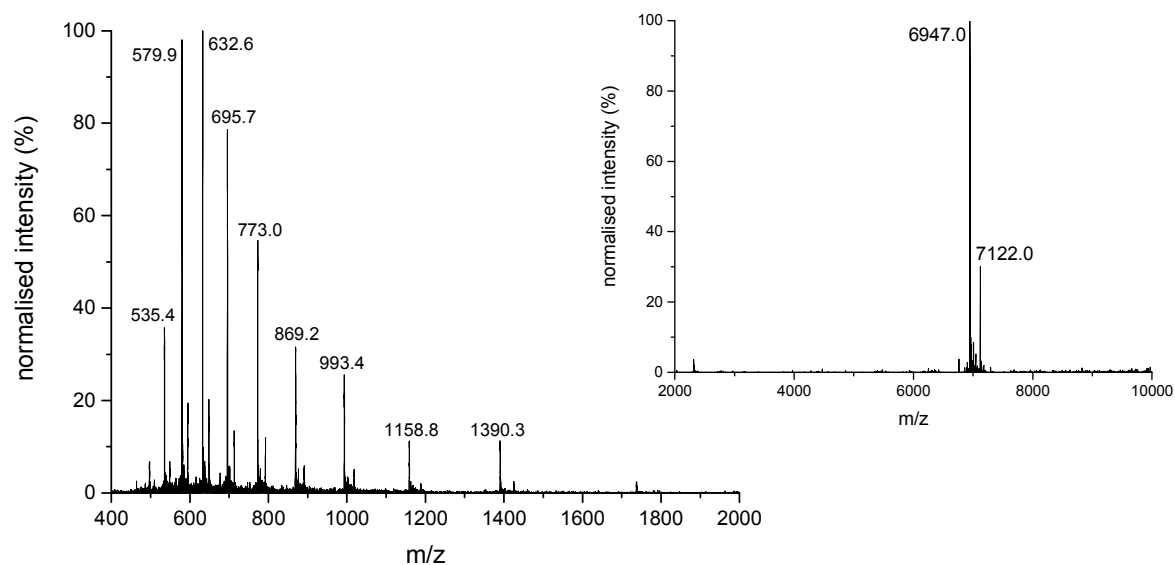
## Scaffold for Cy5-labelled Y-1



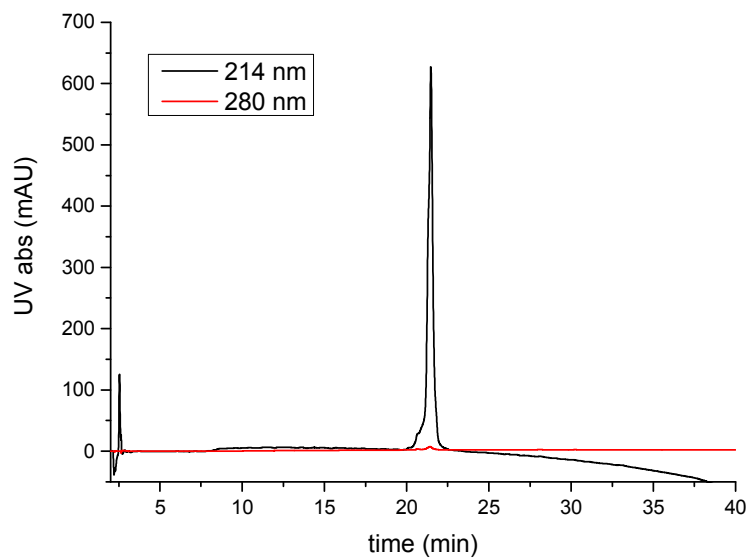
## Characterization of ligation products

Y-1

ESI MS:  $MW_{\text{calc}}$ : 6948.2,  $MW_{\text{obs}}$ : 6947.0

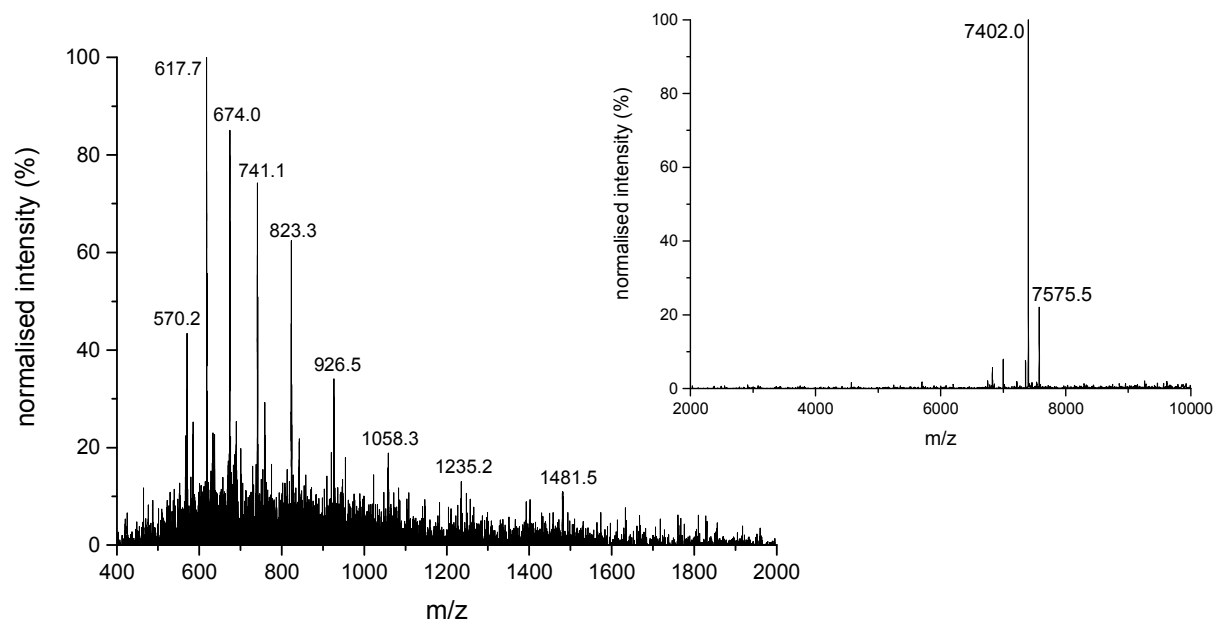


Analytical HPLC, 5-65% ACN in H<sub>2</sub>O (0.1% TFA) over 30 min, C4 column.

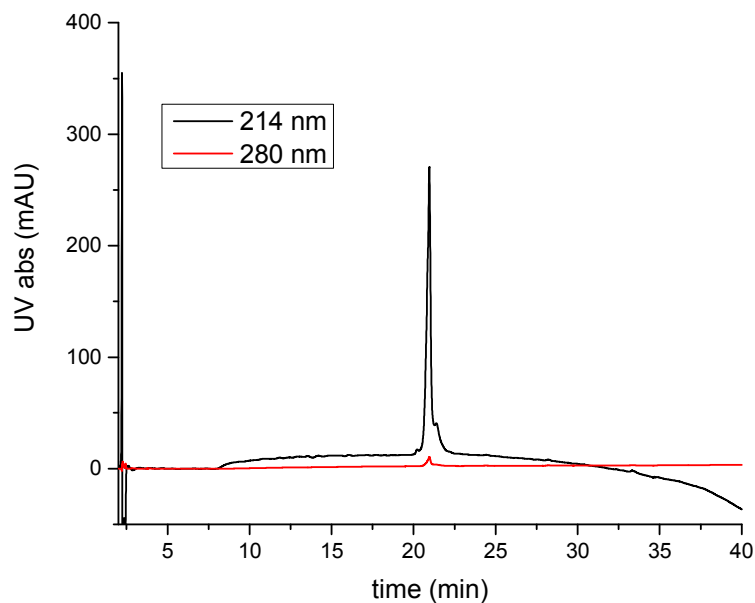


**cis-Pt-Y-1 (modular synthesis)**

ESI MS:  $MW_{calc}$ : 7402.3,  $MW_{obs}$ : 7402.0

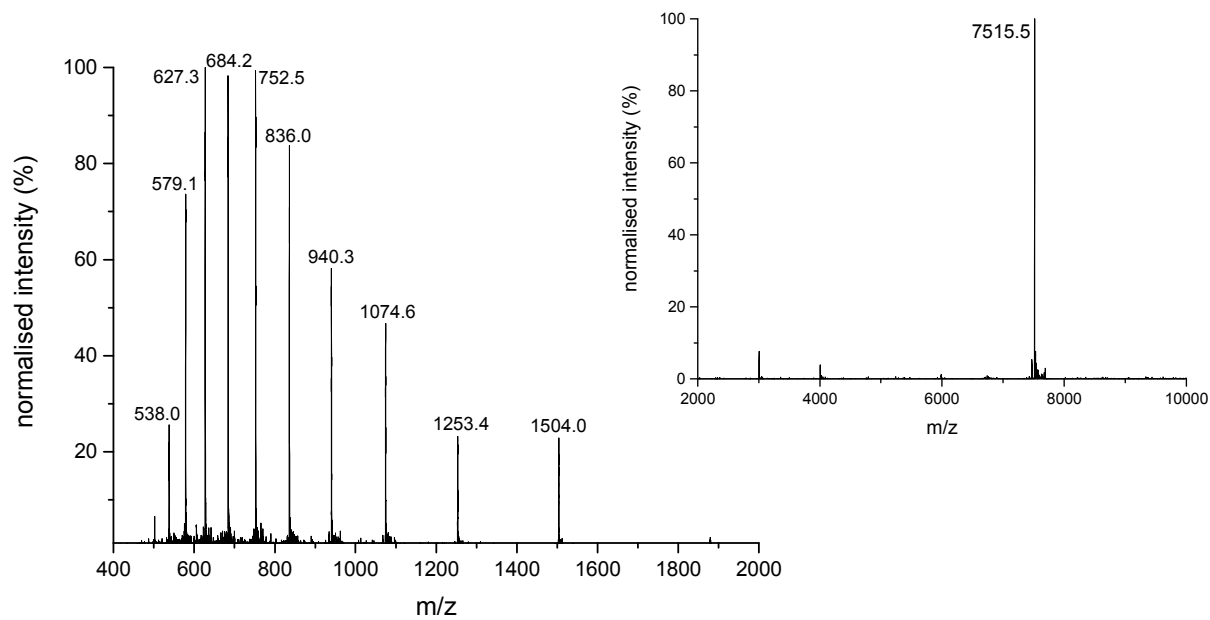


Analytical HPLC, 5-65% ACN in H<sub>2</sub>O (0.1% TFA) over 30 min, C4 column.

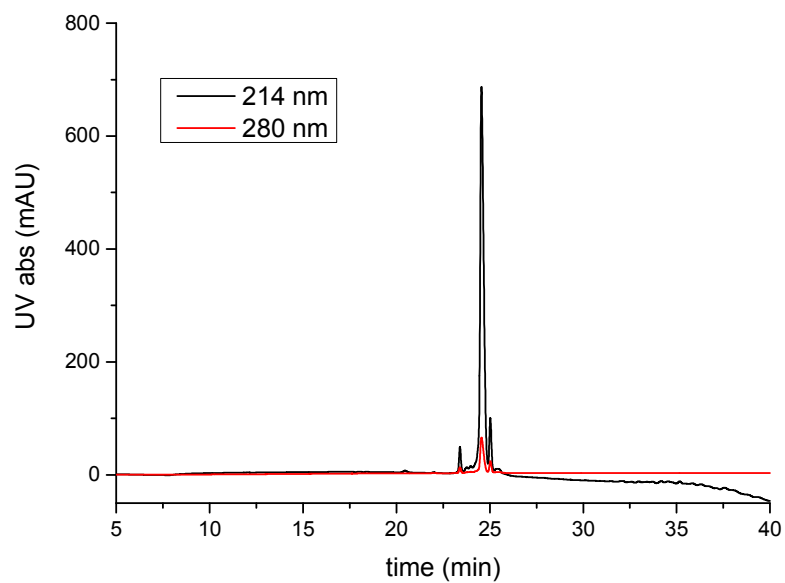


# Cy5-Y-1

ESI MS:  $MW_{\text{calc}}$ : 7513.5,  $MW_{\text{obs}}$ : 7515.5

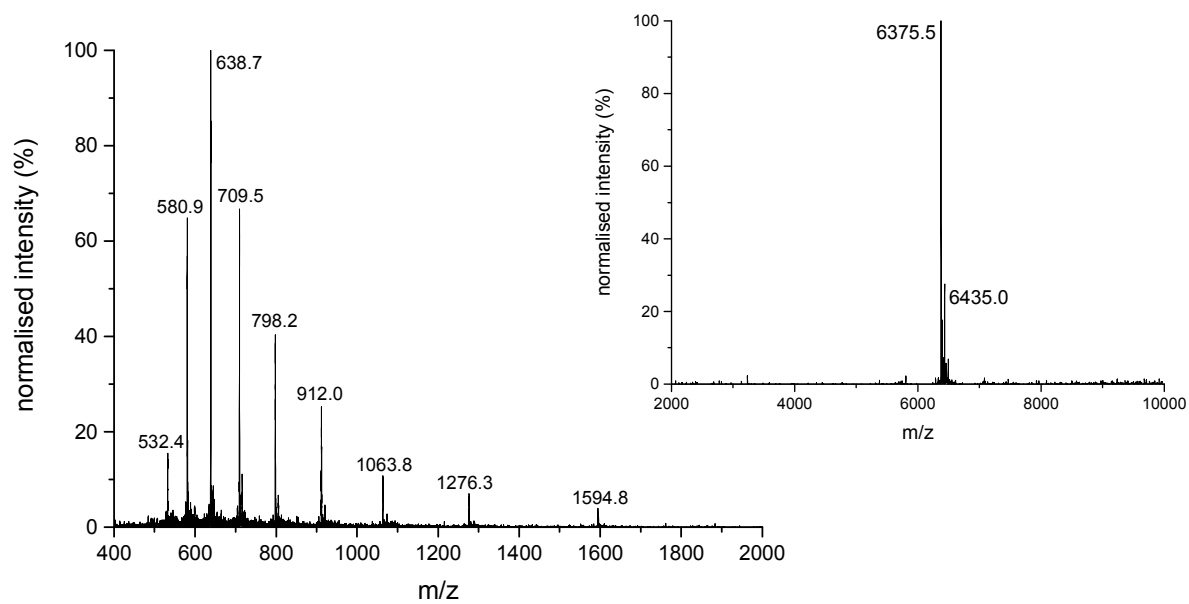


Analytical HPLC, 5-65% ACN in H<sub>2</sub>O (0.1% TFA) over 30 min, C4 column.

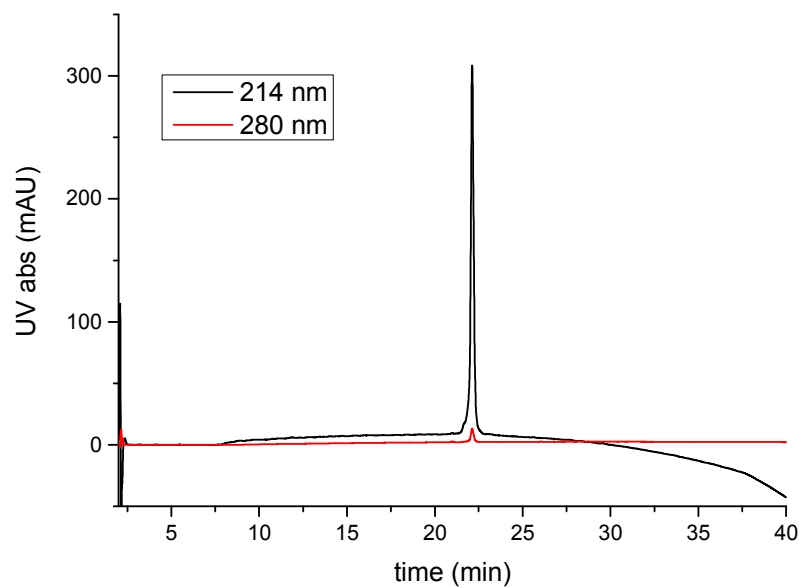


**cis-Pt-Y-1 (from combined Y-1 scaffold)**

ESI MS:  $MW_{\text{calc}}$ : 6375.2,  $MW_{\text{obs}}$ : 6375.5

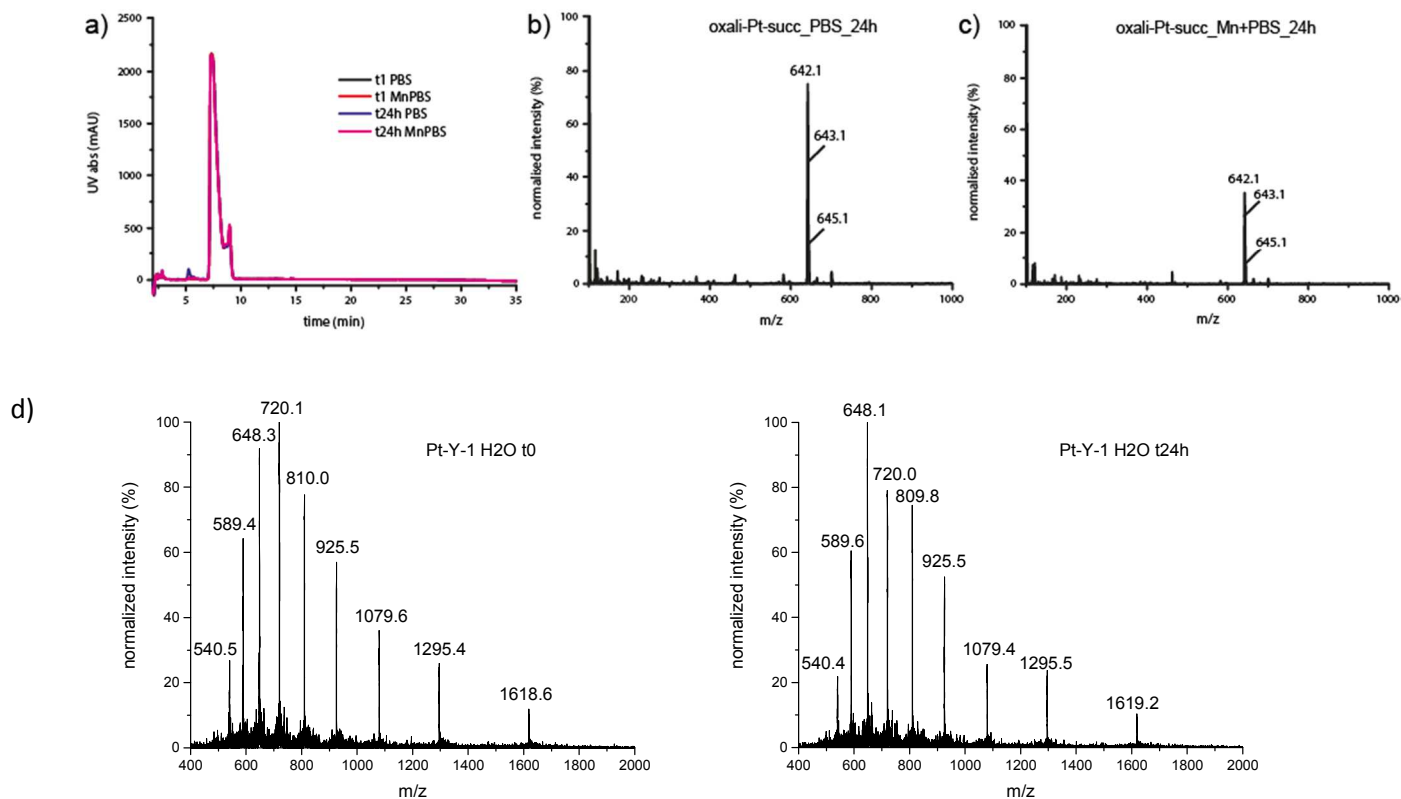


Analytical HPLC, 5-65% ACN in H<sub>2</sub>O (0.1% TFA) over 30 min, C4 column.



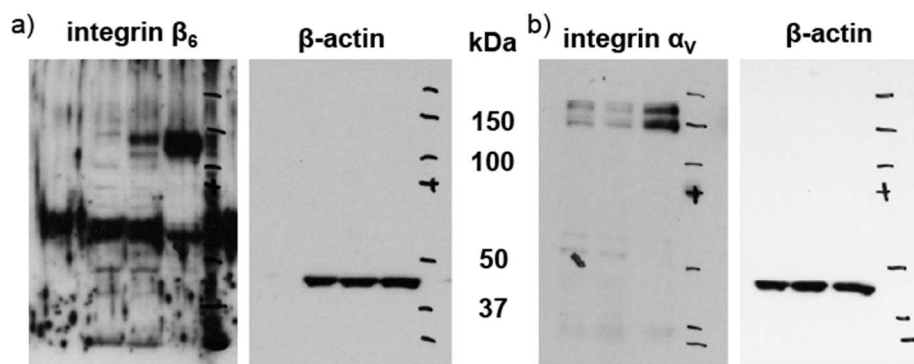
## Stability of maleimide-functionalised platinum(IV) prodrugs

The oxaliplatin-based succinimide **oxali-Pt-succ** was incubated at 1 mM in either PBS or PBS + 4 mM MnCl<sub>2</sub> for 24 h and the stability was monitored by LC-MS. As shown in Figure S1, the complex showed no degradation over this time period.

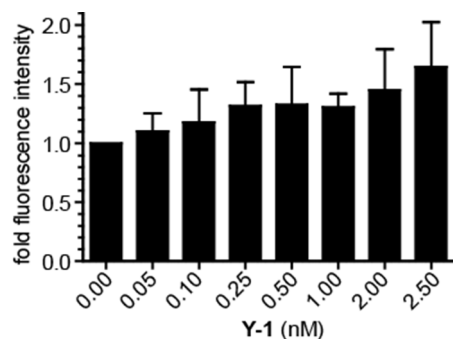


**Figure S1.** Stability of **oxali-Pt** prodrugs. a) Analytical HPLC trace (C18) of **oxali-Pt-succ** incubated in PBS or PBS + 4 mM MnCl<sub>2</sub> (MnPBS) for 1 h (t1) and 24 h (t24). b) and c) Mass spectra of **oxali-Pt-succ** incubate in PBS or PBS + 4 mM MnCl<sub>2</sub> for 24 h. MW<sub>calc</sub>: 641.5, MW<sub>obs</sub>: 642.1. d) Mass spectra of **oxali-Pt-Y-1** in H<sub>2</sub>O at t0 and after 24 h. MW<sub>calc</sub>: 6472.4, MW<sub>obs</sub>: 6471.6 (t0) and 6470.7 (t24).

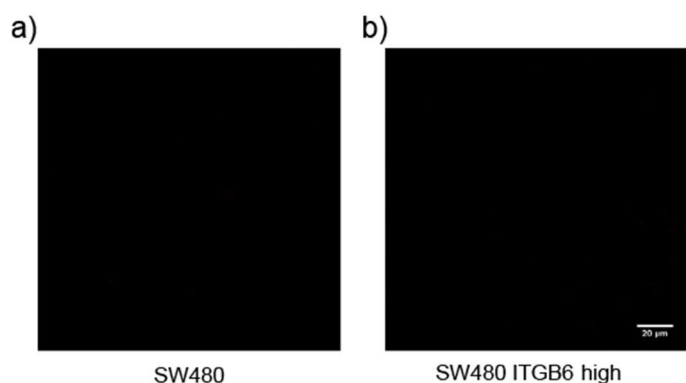




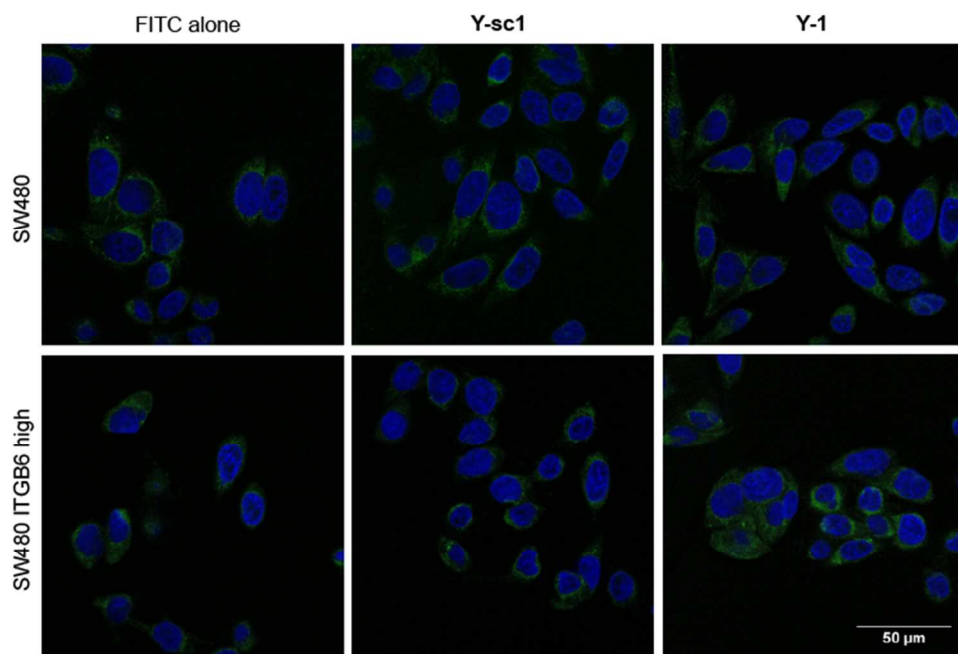
**Figure S2.** Total films of western blot experiment shown in Figure 3b. Integrin  $\beta_6$  and  $\alpha_V$  expression of a) SW480, SW480 ITGB6 high, and b) endogenous ITGB6-expressing A431 cells measured of total protein lysates by western blot.  $\beta$ -actin levels were used as a loading control.



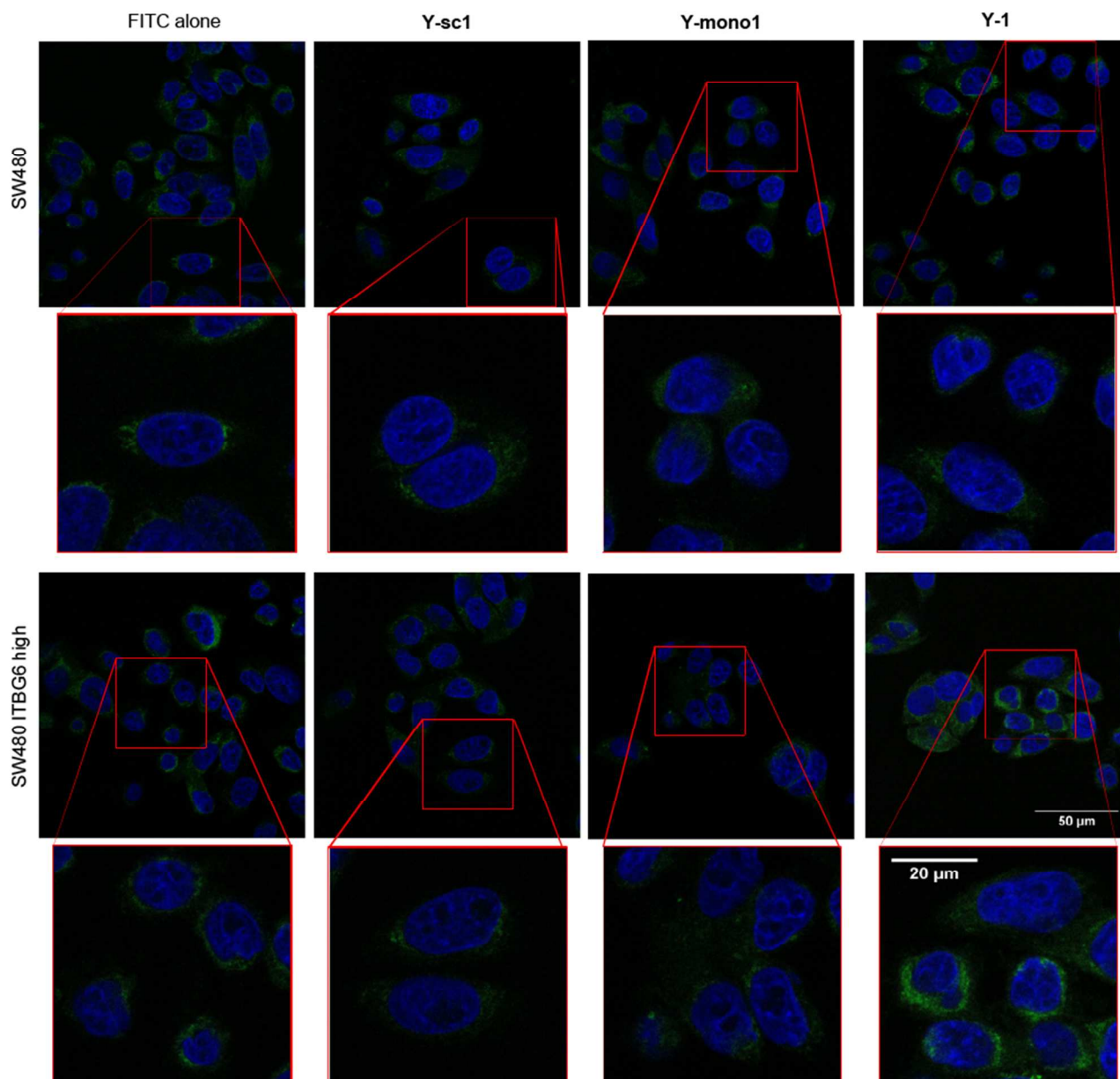
**Figure S3.** Binding of biotinylated Y-1 to A431 cells. Fluorescence of peptide-binding avidin-FITC was measured by flow cytometry in two independent experiments and normalized to control without peptide. Values given are the mean  $\pm$  standard deviation.



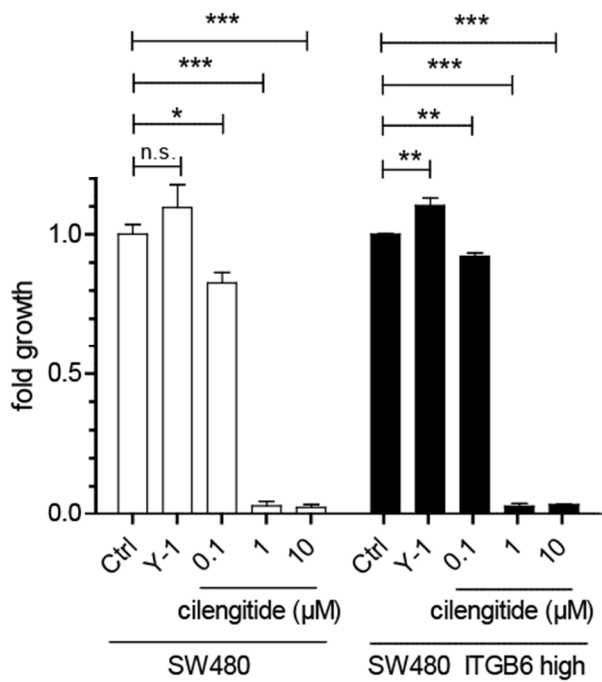
**Figure S4.** Control microscopy images that show the absence of any autofluorescence of SW480 (a) and SW480 ITGB6 (b) high cells treated and prepared in the same way as cells in Figure 3e and 3f but without the Cy5-labeled peptide (**Cy5-Y-1**). Scalebar: 20  $\mu$ m.



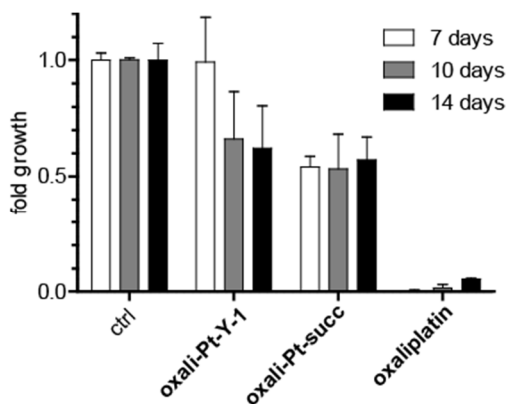
**Figure S5.** Representative confocal microscopy images used for fluorescence quantification in Figure 4. SW480 and SW480 ITGB6 high cells were incubated with no peptide (FITC alone), biotinylated **Y-sc1** or biotinylated **Y-1** (1  $\mu$ M) for 10 min before fixation, permeabilization and staining with avidin-FITC (Scalebar: 50  $\mu$ m).



**Figure S6.** Representative confocal microscopy images used for fluorescence quantification in Figure 3d. SW480 and SW480 ITGB6 high cells were incubated with no peptide (FITC alone), biotinylated **Y-sc1**, biotinylated **Y-mono1** or biotinylated **Y-1** (1  $\mu$ M) for 10 min before fixation, permeabilization and staining with avidin-FITC (Scalebar: 50  $\mu$ m or 20  $\mu$ m in enlarged sections).



**Figure S7.** Cytotoxicity of 10 μM Y-1 and indicated concentrations of cilengitide after long term exposure (14 days) to SW480 and SW480 ITGB6 high cells. Cell viability was measured by crystal violet staining from duplicates of one experiment. Values given are the mean ± standard deviation and significances were established using one-way ANOVA with Dunnett's multiple comparison test (\*\*\*)  $p \leq 0.001$ ; \*\*  $p \leq 0.01$ ; \*  $p \leq 0.05$ ; n.s. not significant).



**Figure S8.** Cytotoxicity of 10 μM oxali-Pt-Y-1, oxali-Pt-succ and oxaliplatin alone after long term exposure (7, 10 and 14 days) to A431 cells. Cell viability was measured by crystal violet staining and experiment was performed in duplicates. Values given are the mean ± standard deviation.

**Table S1.** Parameters for ICP-MS Agilent 7500ce

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<b>RF power (W)</b>	1560
<b>Cone material</b>	Nickel
<b>Carrier gas (l/min)</b>	0.8 – 1.0
<b>Make up gas (l/min)</b>	0.1 – 0.3
<b>Plasma gas (l/min)</b>	15
<b>Monitored isotopes</b>	$^{185}\text{Re}$ , $^{194}\text{Pt}$ , $^{195}\text{Pt}$
<b>Dwell time (s)</b>	0.3
<b>Number of replicates</b>	10

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