A general synthesis of dirhodium metallopeptides as MDM2 ligands

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Supporting information

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General considerations

Peptide synthesis. All peptides were synthesized with an Advanced ChemTech APEX 396 Automated Multipeptide Synthesizer using standard solid-phase Fmoc protocols. The purification was accomplished by reverse-phase HPLC with gradients of water-acetonitrile containing 0.1% trifluoroacetic acid, and peptides were isolated by lyophilization. Analysis and purity assessment was attained by mass spectrometry and analytical HPLC. Peptides were prepared using Rink amide MBHA resin (AAPPTEC) to afford the C-terminal amide and were acetylated at the N-terminus prior to cleavage from the resin.

HPLC. HPLC was performed on a Shimadzu CBM-20A instrument with Phenomenex Jupiter 4μ Proteo 90A (250 × 15 mm preparative) and Phenomenex Jupiter 4μ Proteo 90A (250 × 4.6 mm analytical) columns. Flow rates of 8 mL/min and 1 mL/min were used for preparative and analytical columns, respectively. Analytical and preparative HPLC were performed with gradient of acetonitrile in water. Both solvents contained 0.1% trifluoroacetic acid (TFA) unless otherwise noted. Data was collected using UV-vis absorption at 220 nm and 300 nm.

Mass Spectrometry. MALDI-MS and MS/MS analyses were performed on a Bruker Daltonics Autoflex MALDI- TOF/TOF mass spectrometer with CHCA matrix (10 mg/mL, Thermo Scientific Pierce). ESI–MS was performed on Bruker Daltonics micrOTOF instrument.

Experimental

General procedure for metallopeptide synthesis. Dirhodium metalation with *cis*-$Rh_2(tfa)_2(OAc)_2$ has been previously described.[1]

General procedure for palladium deprotection. Allyl deprotection was performed directly on the crude metalation reaction (1 mM peptide in MES buffer, pH 4.5). A solution of $Pd(PPh_3)_4$ (0.5 equiv) and morpholine (10 equiv) in tetrahydrofuran (volume equal to that of the buffer soln in the preceding rxn) were added to the crude metalation reaction and the pH was adjusted to 7.0 with KOH (0.1 M aq soln).[2] The reaction was monitored by HPLC and reached completion in 0.5–1.5 h. Metallopeptides were purified by RP-HPLC, isolated by lyophilization, and characterized by ESI–MS. Using this general procedure, P4 peptide (1.80 mg) was converted to the unlabeled P4-Rh metallopeptide (1.06 mg, 53%).

General procedure for labeling with fluorescein. A solution of fluorescein isothiocyanate (FITC) (1.5 equiv) in DMSO (0.1 mM concn of metallopeptide) was added to a lyophilized metallopeptide, followed by addition of diisopropylethylamine (5 equiv). The reaction mixture was quenched with methanol (5× total rxn volume), diluted with water (5× total rxn volume), purified by RP-HPLC (without TFA in the eluent), and characterized by ESI–MS. To prepare a stock soln for fluorescence polarization assays, a lyophilized, labeled metallopeptide was dissolved in DMSO (50 μL) and the concn of the solution was determined by absorbance at 492 nm (ε = 83000 $M^{-1}cm^{-1}$) after dilution in buffer (20 mM Tris pH 8, 200 mM NaCl) to achieve 0.2-1.0 absorbance values.[3] Using this general procedure P3-Rh unlabeled metallopeptide (0.64 mg) was converted to the P3-Rh metallopeptide (0.49 mg, 68%).

Protein expression. The protein MDM2 [5–109] was expressed in BL21 *E. coli* (Rosetta) cells as a fusion with its interaction partner, the p53 transactivation peptide, which has been shown to afford dramatically higher yields.[4] The MDM2 plasmid was purchased from Genscript in pET15b vector. After expression, cells were lysed by freezing at −80 °C. Due to instability of the protein, subsequent steps should be performed in minimal time. The lysate was purified on Ni-NTA affinity column with stepwise increase in concentration of imidazole in the eluent buffer (50 mM/ 100 mM/ 300 mM). After analysis by SDS-PAGE gel, most concentrated and clean fractions were combined and dialyzed. His-tag and the fusion peptide were cleaved with 100 U/mg TEV protease at rt overnight or 4 °C for 48 h. MDM2 protein was purified by a second Ni-column and used immediately in binding measurements. Concentration of the protein was determined by absorbance at 280 nm (ε= 8960 $M^{-1}cm^{-1}$)

Fluorescence polarization. Fluorescence polarization data were acquired on Nanolog (Horiba Jobin Yvon) Spectrometer with 16.5F-Q-10 quartz cells (1 cm path length). A 200-nM stock soln of the labeled metallopeptide in DMSO was prepared. Samples were made by adding 4 μL of metallopeptide solution (10 nM final concentration) to varying amounts of MDM2 (76 μL, 20 mM aq Tris pH 8.0 buffer with 200 mM NaCl and 2 μM mercaptoethanol) and were incubated for 30 min at rt. Measurements were obtained by excitation at 485 nm with 6-nm slit width and emission detection at 512 nm with 6-nm slit width. Ten data points each integrated over 3 seconds were collected. Binding curves and K_d values were generated in Excel using a non-linear least-squares fit to the equation[5]:

$$FP = FP_{min} \times PL \times \frac{(FP_{max} - FP_{min})}{FP_{max}}$$

$$PL = L_0 + P_0 + K_d - \frac{\sqrt{(L_0 + K_d + P_0)^2 - 4 \times L_0 \times P_0}}{2}$$

where L_0 is the concentration of the peptide, P_0 is the concentration of the protein, K_d is the dissociation constant, FP_{min} is the low limit of the curve and FP_{max} is the high limit of the curve.[6] K_d, FP_{min} and FP_{max} were all floating parameters during the non-linear least-squares fitting.

MDM2 model (Fig. 1). Model structure was prepared based on PDB coordinates 1YCR of p53-wild type peptide and N-terminal binding domain MDM2 [17-125]. The coordinates for p53 peptide were manually extracted into a separate file (pdb) and the structure was altered in Spartan to represent P1-[Rh_2]. The complete assembly was "frozen" and only the side chains of glutamates that are bound to the dirhodium core were released for molecular mechanic (MMFF) optimization. The optimized P1-[Rh_2] structure was exported in PDB format and overlaid with MDM2 in PyMOL.
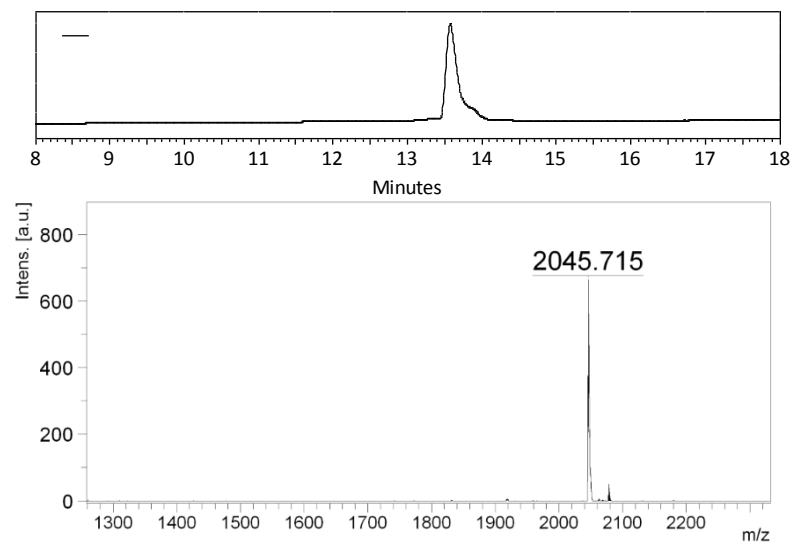
Analytical data

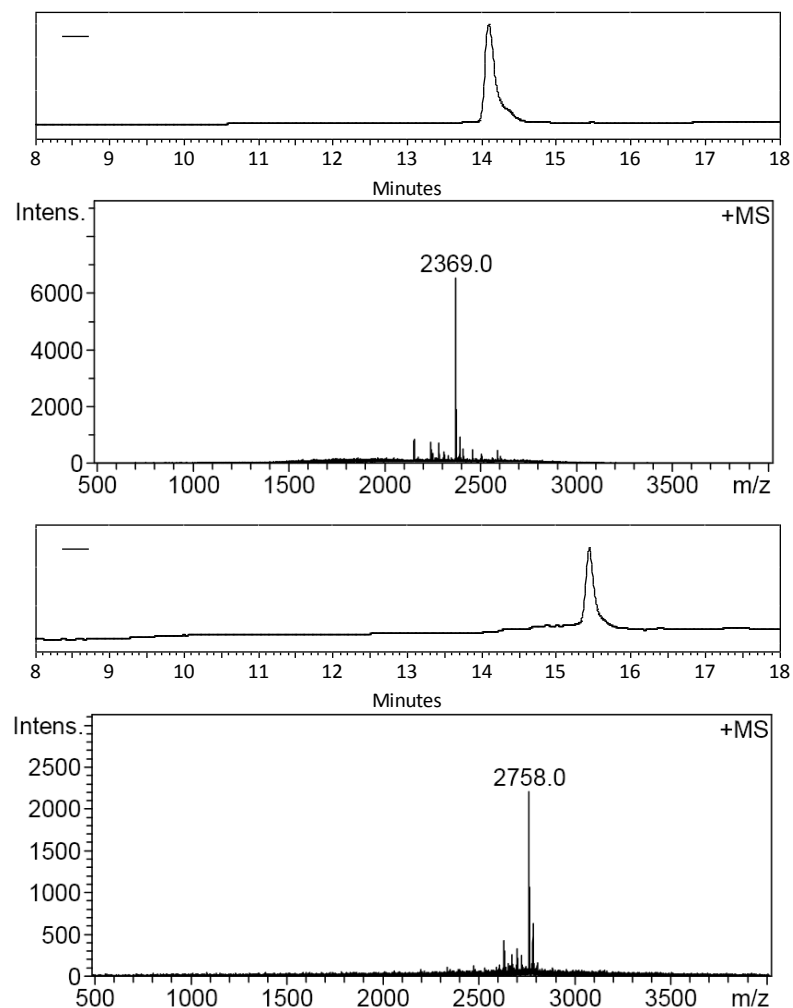**Figure S1.** HPLC trace and MS data for isolated P1 peptide.

Figure S2. HPLC trace and MS data of purified product for P1-Rh metallopeptide synthesis before (top) and after (bottom) fluorescein labelling with FITC.
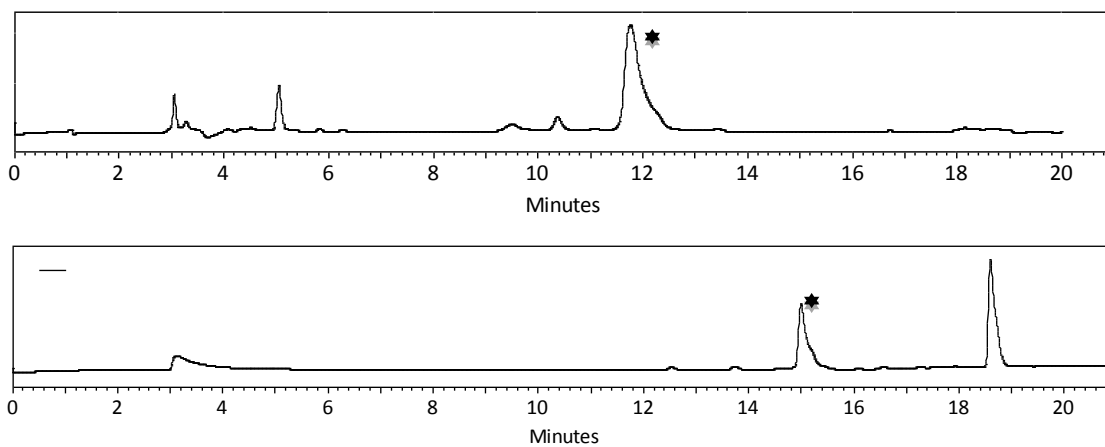
Figure S3. HPLC trace of crudes of metallation reaction (top) and fluorescein labelling with FITC (bottom) for P1-Rh metallopeptide synthesis.
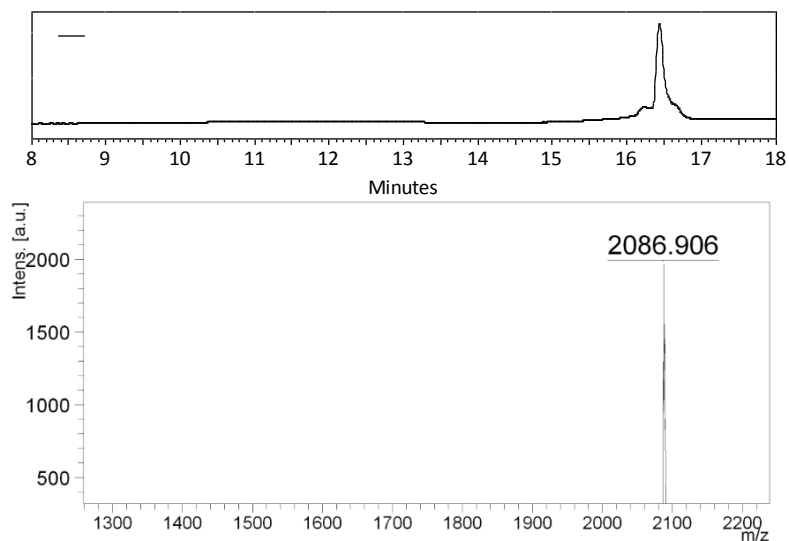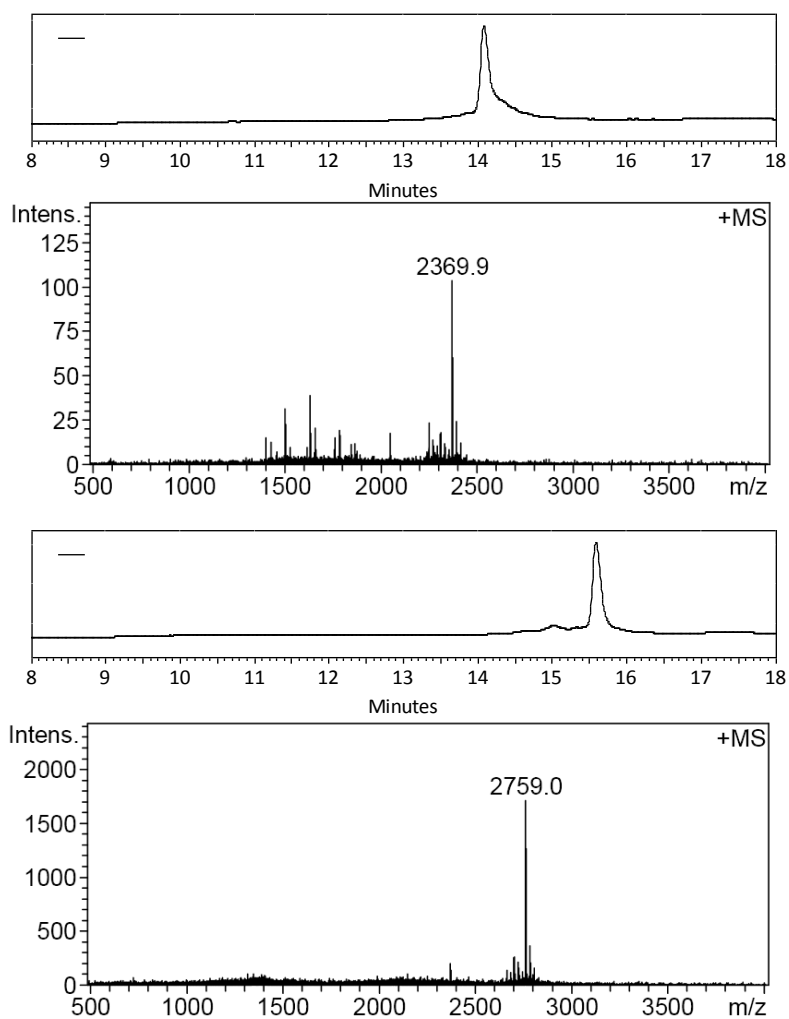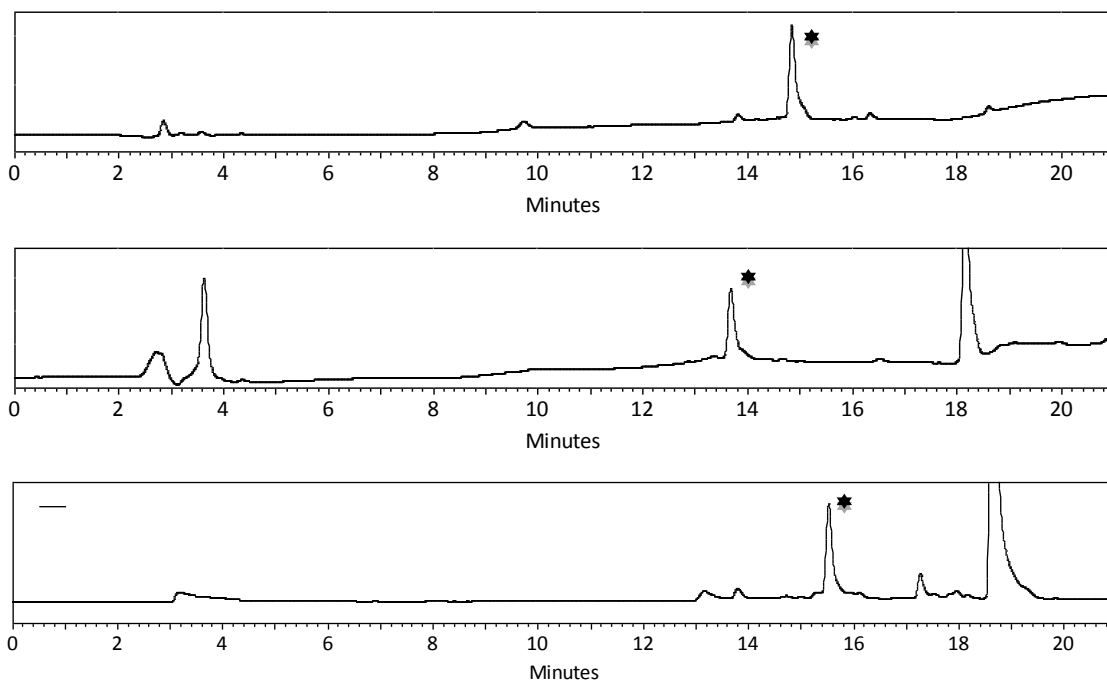
Figure S4. HPLC trace and MS data for isolated P2 peptide.

Figure S5. HPLC trace and MS data of purified product for P2-Rh metallopeptide synthesis before (top) and after (bottom) fluorescein labelling with FITC.

Figure S6. HPLC trace of crudes of metalation reaction (top), in-situ palladium deprotection (center) and fluorescein labeling with FITC (bottom) for P2-Rh metallopeptide synthesis.
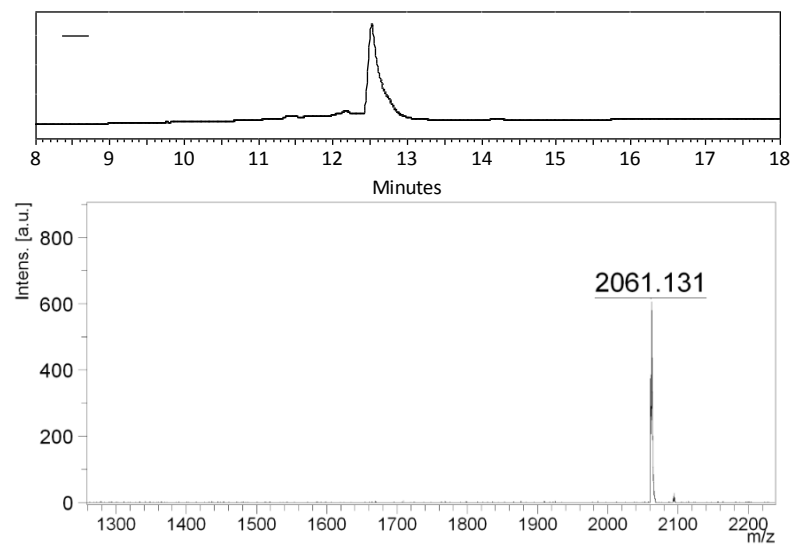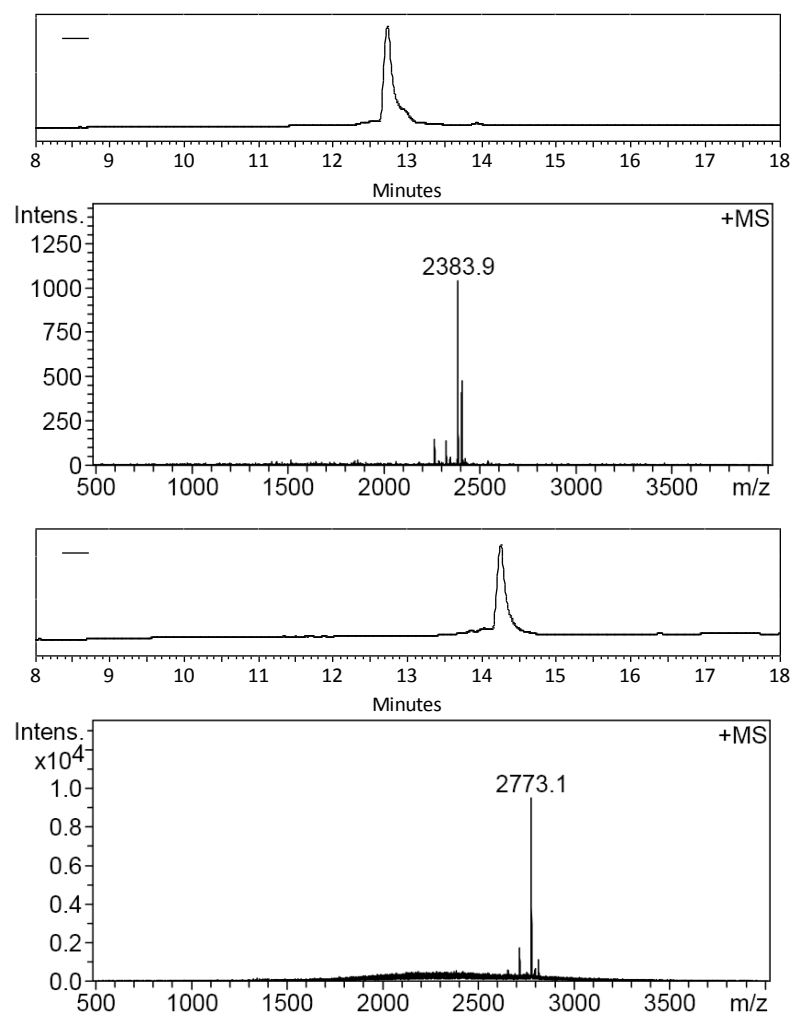
Figure S7. HPLC trace and MS data for isolated P3 peptide.

Figure S8. HPLC trace and MS data of purified product for P3-Rh metallopeptide synthesis before (top) and after (bottom) fluorescein labeling with FITC.
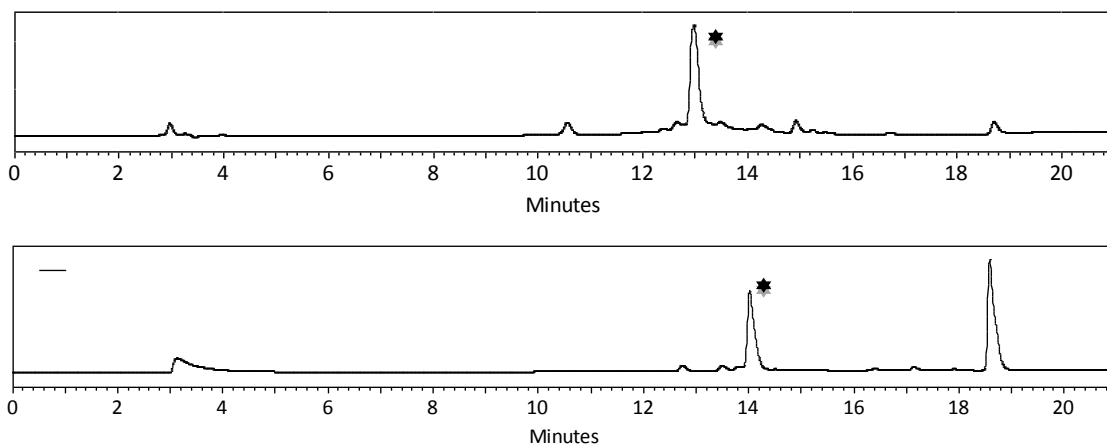
Figure S9. HPLC trace of crudes of metalation reaction (top) and fluorescein labeling with FITC (bottom) for P3-Rh metallopeptide synthesis.
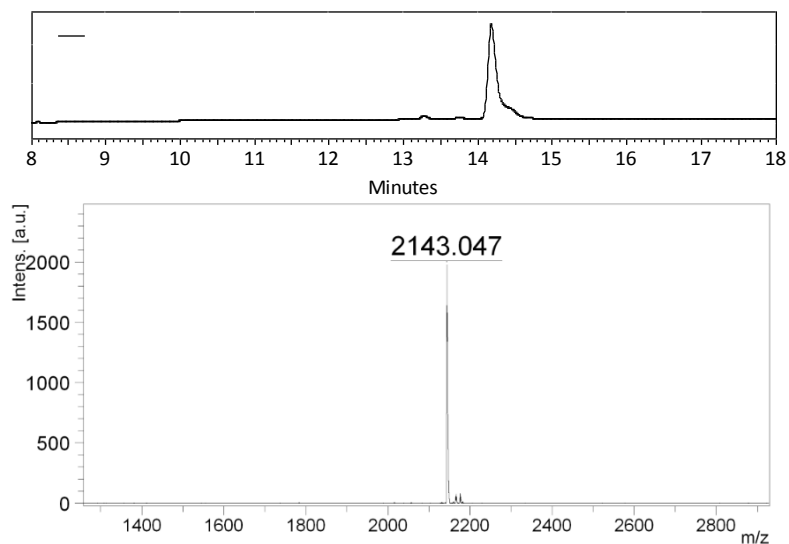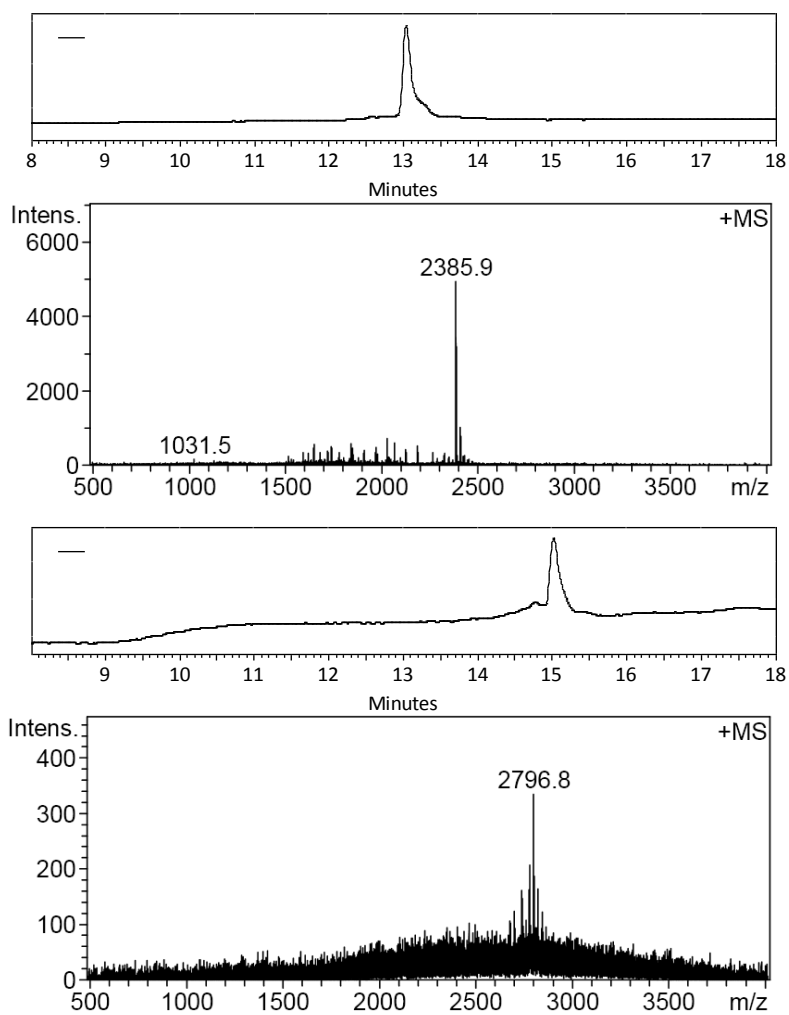
Figure S10. HPLC trace and MS data for isolated P4 peptide.

Figure S11. HPLC trace and MS data of purified product for P4-Rh metallopeptide synthesis before (top) and after (bottom) fluorescein labeling with FITC.
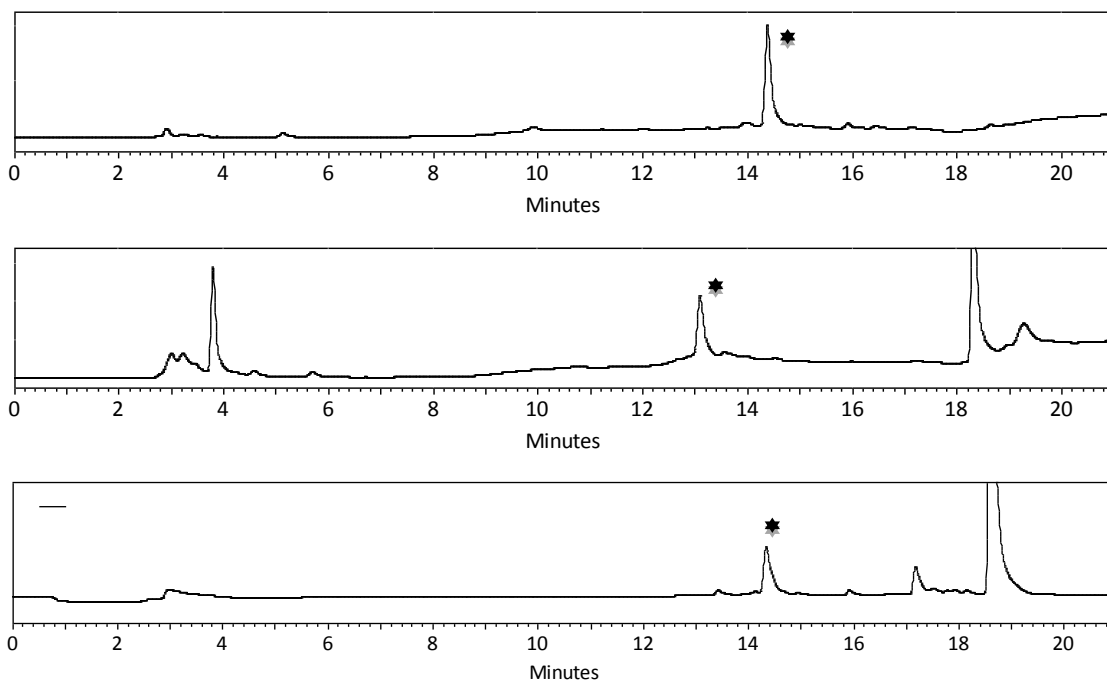
Figure S12. HPLC trace of crudes of metalation reaction (top), in-situ palladium deprotection (center) and fluorescein labeling with FITC (bottom) for P4-Rh metallopeptide synthesis.
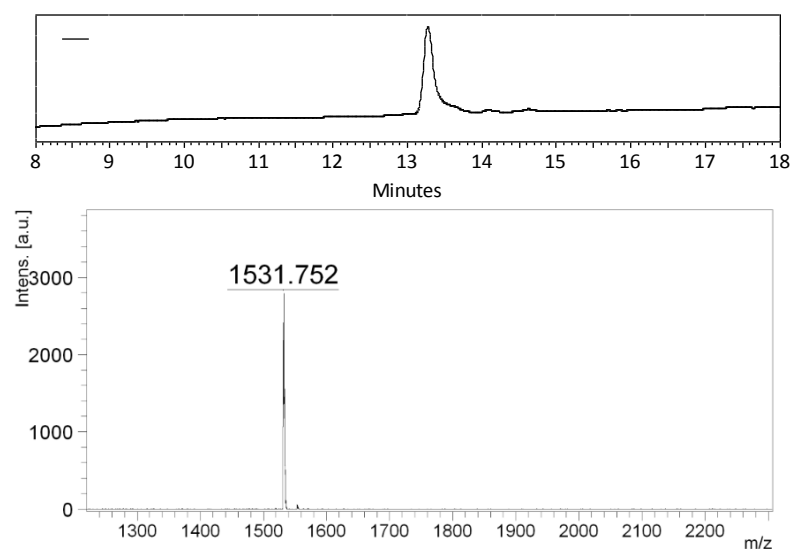
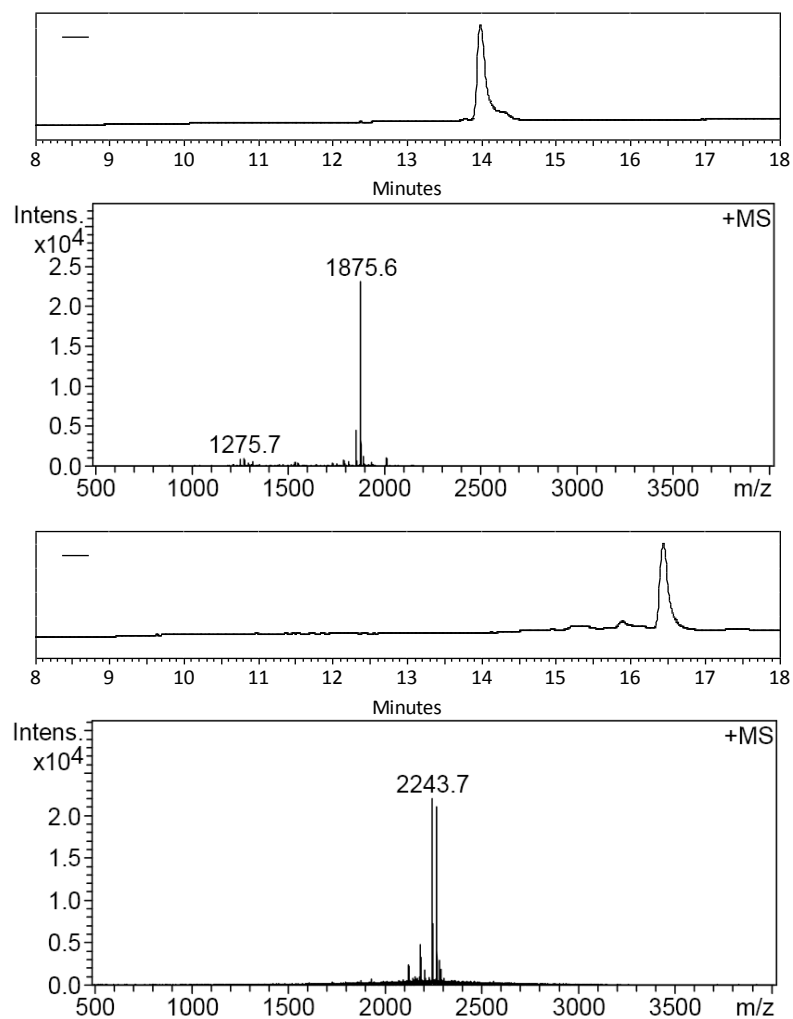**Figure S13.** HPLC trace and MS data for isolated P6 peptide.

Figure S14. HPLC trace and MS data of purified product for P6-Rh metallopeptide synthesis before (top) and after (bottom) fluorescein labeling with FITC.
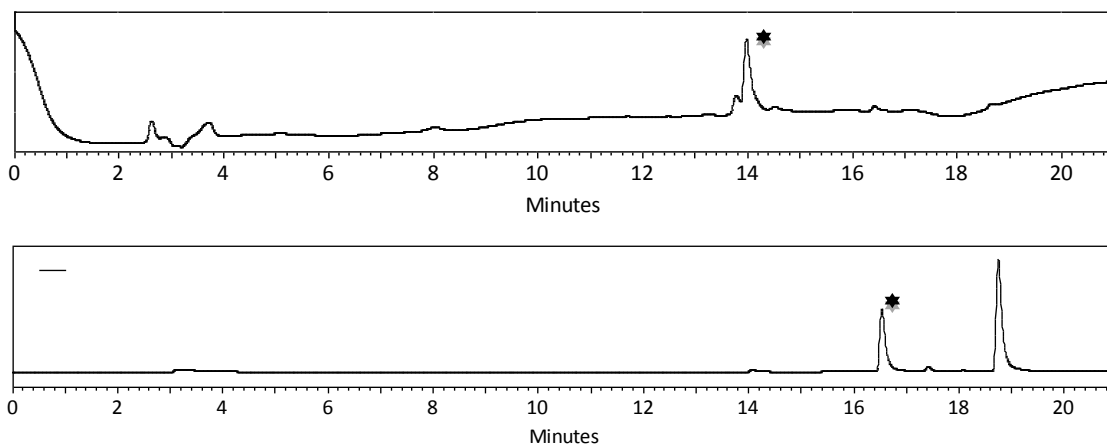
Figure S15. HPLC trace of crudes of metalation reaction (top) and fluorescein labeling with FITC (bottom) for P6-Rh metallopeptide synthesis.
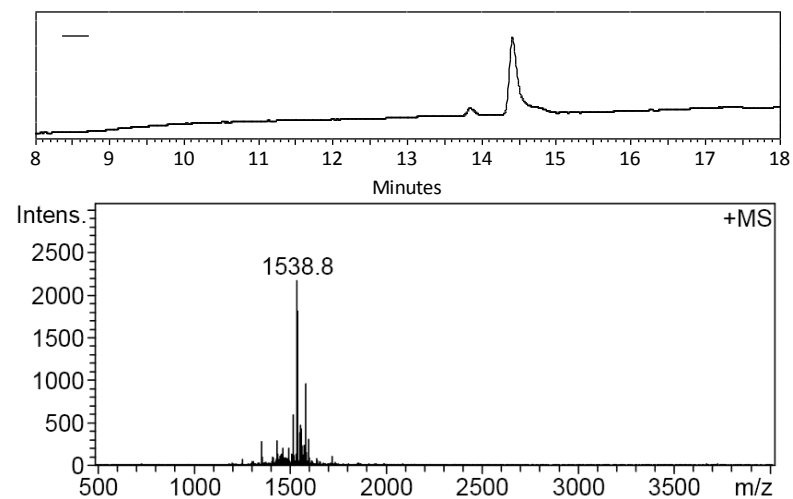
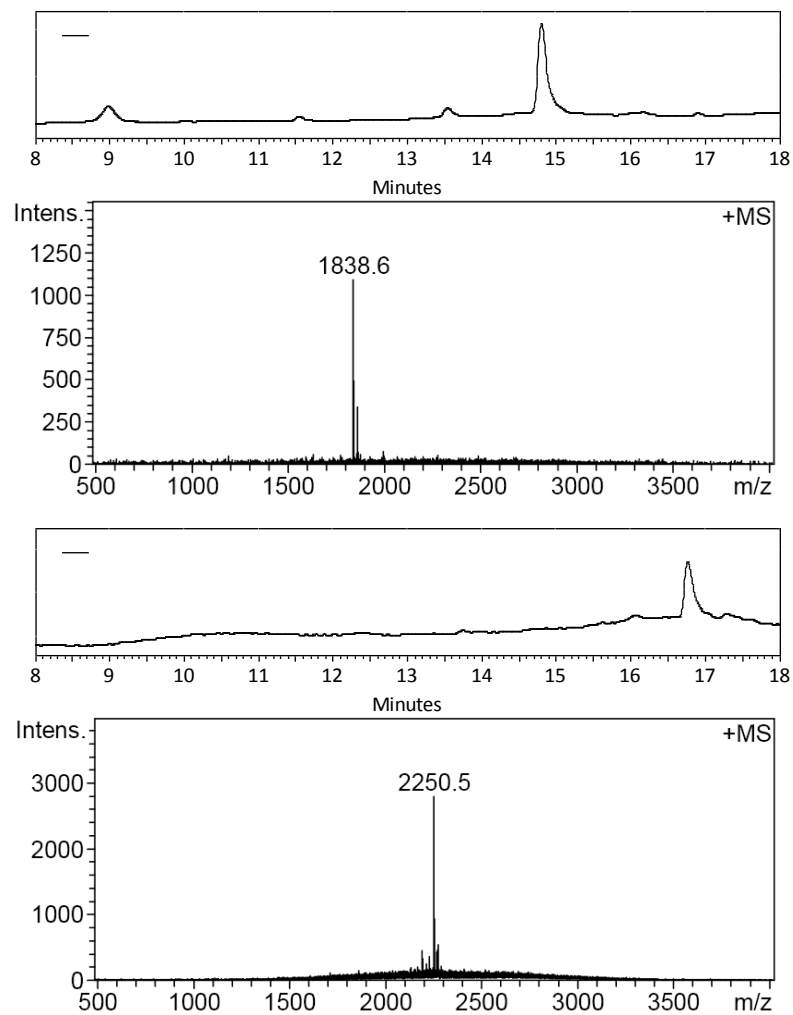**Figure S16.** HPLC trace and MS data for isolated P7 peptide.

Figure S17. HPLC trace and MS data of purified product for P7-Rh metallopeptide synthesis before (top) and after (bottom) fluorescein labeling with FITC.
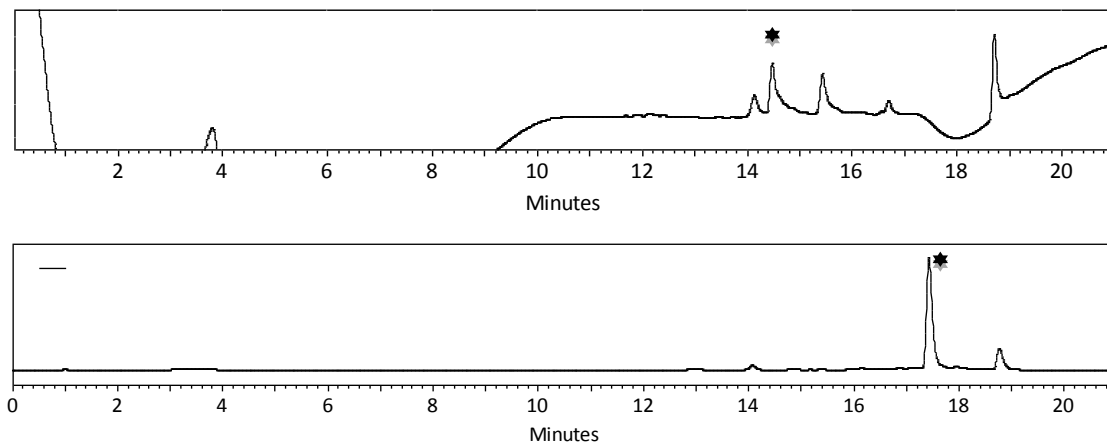
Figure S18. HPLC trace of crudes of metalation reaction (top) and fluorescein labeling with FITC (bottom) for P7-Rh metallopeptide synthesis.
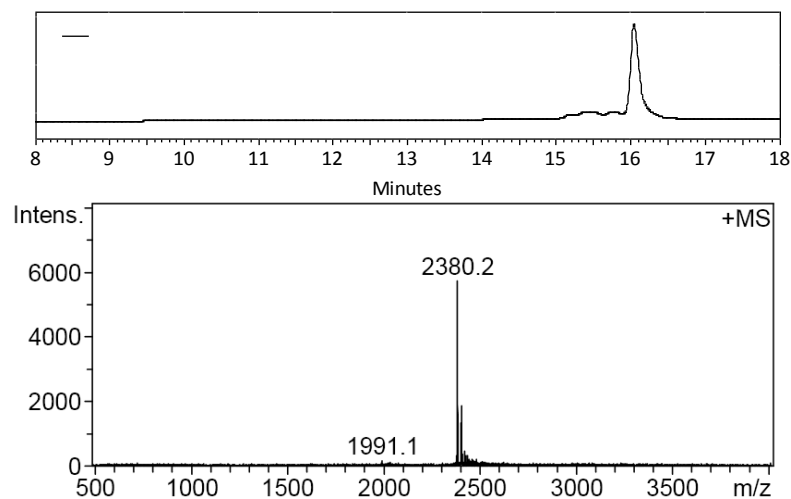

Figure S19. HPLC trace and MS data for isolated fluorescein-p53-wt peptide.
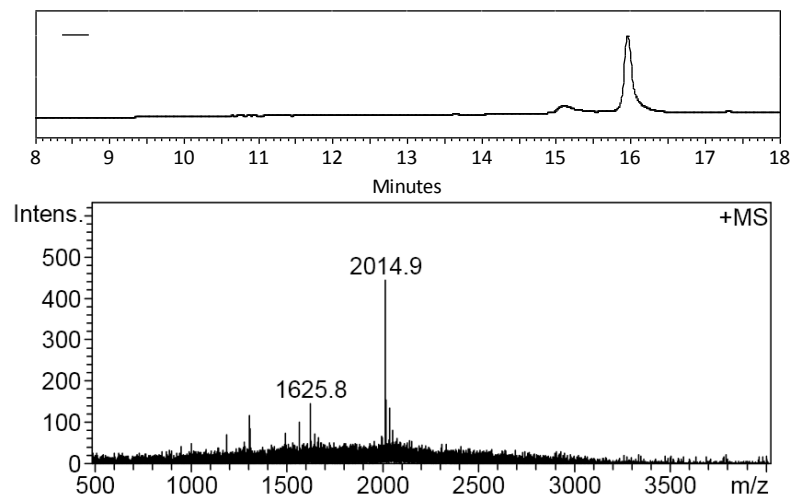

Figure S20. HPLC trace and MS data for isolated fluorescein-P5 peptide.

References.

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5. G. Kemmer and S. Keller, *Nat Protocols*, **5**, 267-281.
6. F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky and G. L. Verdine, *J Am Chem Soc*, 2007, **129**, 2456-2457.