Supplementary information

In vivo metal-catalyzed SeCT therapy by a proapoptotic peptide

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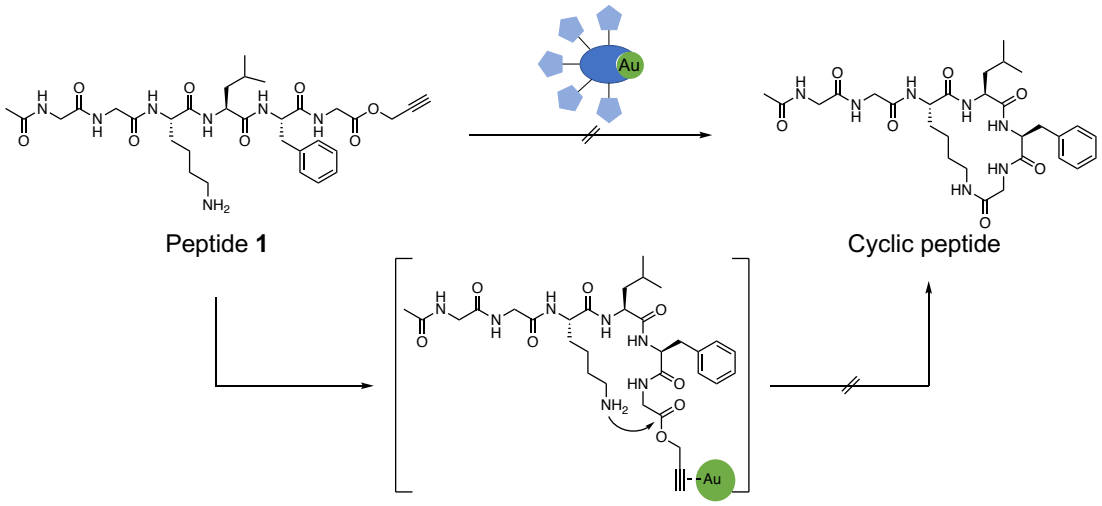
Serendipity from peptide cyclization strategy

During our different project, we aimed to synthesize bioactive cyclic peptide like a cyclin inhibitory peptide[1] from linear peptide with C-terminal propargyl ester (PE) using previously developed gold catalyzed amide bond formation[2]. However, the reaction did not produce the cyclic peptide. On the other hand, we evaluated the cytotoxicity of peptide derivatives in the presence or absence of gold complex, HSA(*c*RGD)-Au (20 μM). Surprisingly, peptide **1** shows cytotoxicity in the presence of catalyst, while the other peptides did not show such cytotoxic feature.

a) Cyclization strategy based on gold-catalyzed amide bond formation



b) Peptide derivatives for cyclization

Entry	Sequence	Cell viability against SW620 cell (%)	
		Without catalyst	With catalyst (20 μM)
1	Ac-GGKLFG-PE (peptide **1**)	88	23
2	Ac-GYKLFG-PE (peptide **2**)	86	84
3	Ac-GSKLFG-PE	68	72
4	Ac-FYKLFG-PE	75	77

Figure S1. a) The failure strategy of gold catalyzed cyclization of peptide. b) the typical peptide derivatives for cyclization and its cytotoxicity against SW620 at concentration of 600 μM in the presence or absence of HSA(*c*RGD)-Au (20 μM).

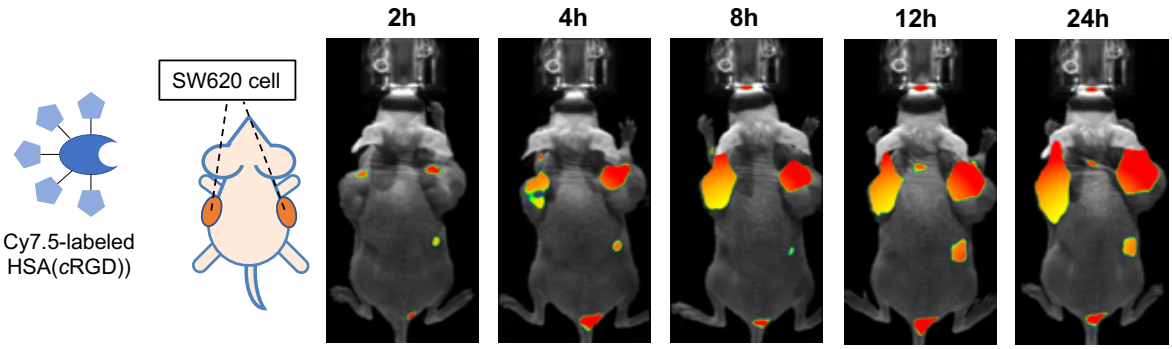


Figure S2. Targeting ability of catalyst carrier, HSA(*c*RGD). Time-dependent imaging after intravenous injection of Cy7.5-labeled HSA(*c*RGD) to the SW620 bearing mice.

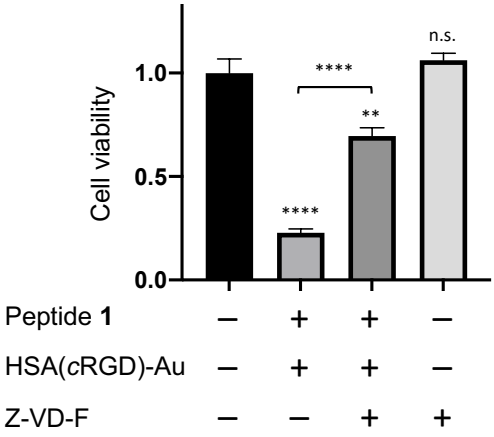


Figure S3. Cytotoxicity of peptide **1** and HSA(*c*RGD)-Au using a caspase inhibitor. n.s.: not significant, *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001. Data (n = 3) are shown as means ± SD.

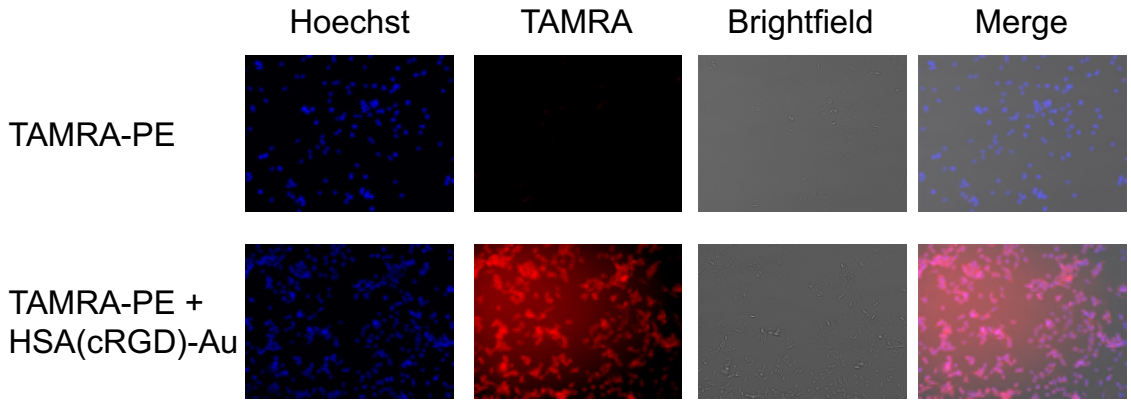


Figure S4. Fluorescence labeling of SW620 cell by reaction with TAMRA-PE and HSA(*c*RGD)-Au (1 h, 37 ºC). The images are obtained by fluorescence microscopy via Hoechst33258, TAMRA fluorescence (3 s exposure time) and brightfield.

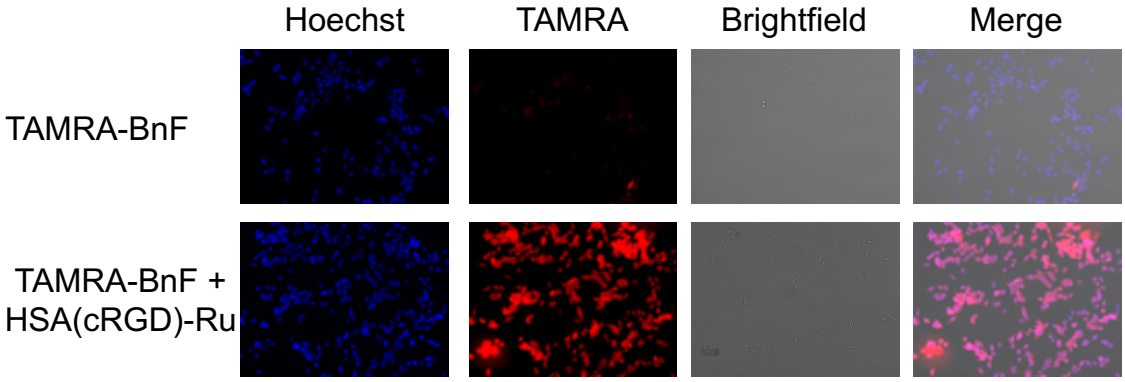


Figure S5. Fluorescence labeling of SW620 cell by reaction with TAMRA-BnF and HSA(*c*RGD)-Ru (1 h, 37 ºC). The images are obtained by fluorescence microscopy via Hoechst33258, TAMRA fluorescence (1/6 s exposure time) and brightfield.

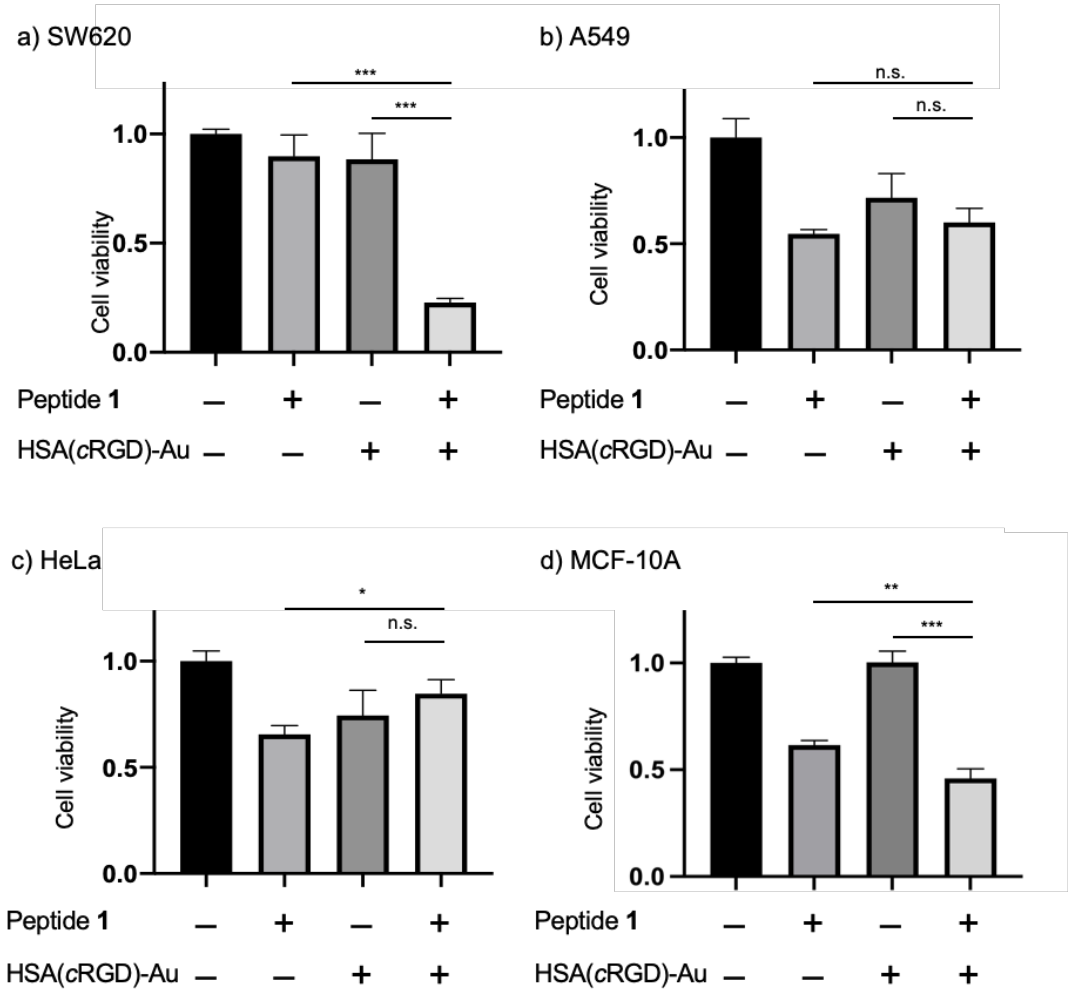


Figure S6. Cytotoxicity assay of peptide **1** (600 μM) in the presence or absence of HSA(*c*RGD)-Au (20 μM) against (a) SW620, (b) A549, (c) HeLa S3 and (d) MCF-10A cell lines. The cytotoxicity was determined by ATP assay after 48h incubation with samples. n.s.: not significant, *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001. The Data (n = 3) are shown as means ± SD.

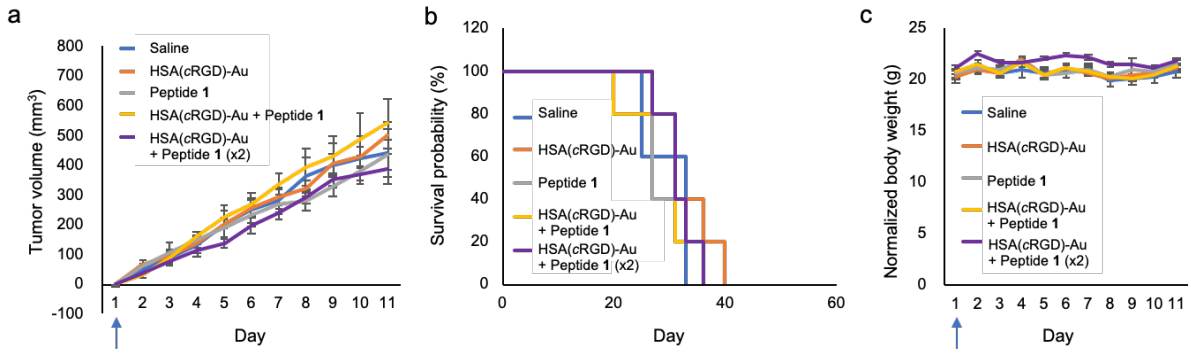


Figure S7. In vivo antitumor activity of peptide **1** and HSA(cRGD)-Au against SW620 xenografted tumor. (a) Tumor volume (n = 5), (b) survival rate (n = 5), and (c) body weight (n = 5) change of various treatments group mice; vehicle (blue), HSA(cRGD)-Au (70.0 mg/kg, orange), peptide **1** (8.0 mg/kg, gray), and peptide **1** + HSA(cRGD)-Au (8.0 or 16.0 mg/kg and 70.0 or 140.0 mg/kg, yellow or purple, respectively). Blue arrows under the horizontal axis indicate the day of treatment with the compounds. Data are shown as means ± SE. n.s.: not significant, *p < 0.05, **p < 0.01, ***p < 0.005. ****p < 0.001.

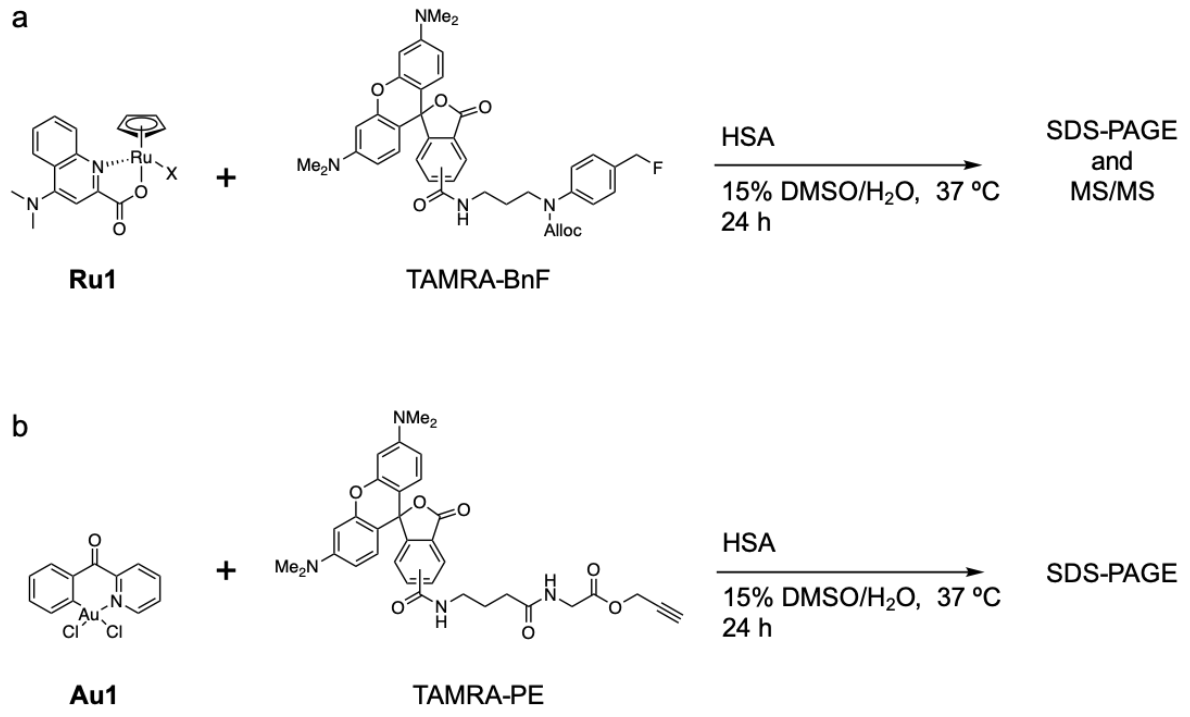


Figure S8. HSA modification reaction with (a) **Ru1**/TAMRA-BnF for SDS-PAGE and MSMS analysis (for **Figure 4b** and **4c**) or (b) **Au1**/TAMRA-PE for SDS-PAGE analysis.

Figure S9. MSMS analysis.

Sequence: LC(TAMRA)T(TAMRA)VATLR [HSA(74-81)]

Annotated Sequence	Modifications	Theo. MH+ [Da]	Found in Sample: [S3] F3: Control, AspN control	Found in Sample: [S7] F7: Control, AspN control	Found in Sample: [S4] F4: Sample, AspN sample	Found in Sample: [S8] F8: Sample, AspN sample	Found in Sample: [S9] F9: Control, Chymotrypsin control	Found in Sample: [S11] F11: Sample, Chymotrypsin control	Found in Sample: [S10] F10: Sample, Chymotrypsin sample	Found in Sample: [S12] F12: Sample, Chymotrypsin sample	Found in Sample: [S1] F1: Control, Trypsin control	Found in Sample: [S5] F5: Control, Trypsin control	Found in Sample: [S2] F2: Sample, Trypsin sample	Found in Sample: [S6] F6: Sample, Trypsin sample	Confidence (by Search Engine): Mascot	Ions Score (by Search Engine): Mascot	Top Apex RT [min]
[K].LCTVATLR.[E]	2xTAMRA [C2; T3]	2025.01316	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Peak Found	Medium	Medium	14	50.64
[K].LCTVATLR.[E]	1xPropionamide [C2]	947.53426	Peak Found	Peak Found	Not Found	Not Found	Peak Found	Peak Found	Not Found	Not Found	High	High	High	High	High	40	38.66
[K].LCTVATLR.[E]		876.49715	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	High	Peak Found	Not Found	Not Found	High	37	34.37

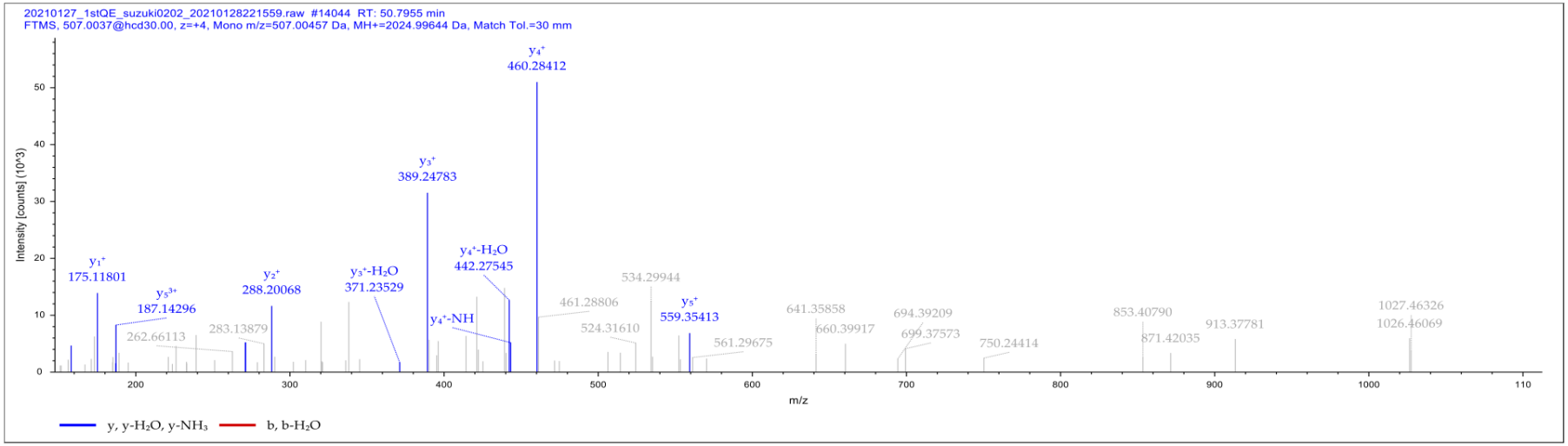


Figure S10. MSMS analysis.

Sequence: S(TAMRA)LHTLFGDK [HSA(65-73)]

Annotated Sequence	Modifications	Theo. MH+ [Da]	Found in Sample: [S3] F3: Control, AspN control	Found in Sample: [S7] F7: Control, AspN control	Found in Sample: [S4] F4: Sample, AspN sample	Found in Sample: [S8] F8: Sample, AspN sample	Found in Sample: [S9] F9: Control, Chymotrypsin control	Found in Sample: [S11] F11: Sample, Chymotrypsin control	Found in Sample: [S10] F10: Sample, Chymotrypsin sample	Found in Sample: [S12] F12: Sample, Chymotrypsin sample	Found in Sample: [S1] F1: Control, Trypsin control	Found in Sample: [S5] F5: Control, Trypsin control	Found in Sample: [S2] F2: Sample, Trypsin sample	Found in Sample: [S6] F6: Sample, Trypsin sample	Confidence (by Search Engine): Mascot	Ions Score (by Search Engine): Mascot	Top Apex RT [min]
[K].SLHTLFGDK.[L]	1xTAMRA [S/K]	1591.79438	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	High	High	High	46	48.55
[K].SLHTLFGDK.[L]		1017.53637	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Peak Found	Not Found	Not Found	High	High	High	High	High	43	39.83

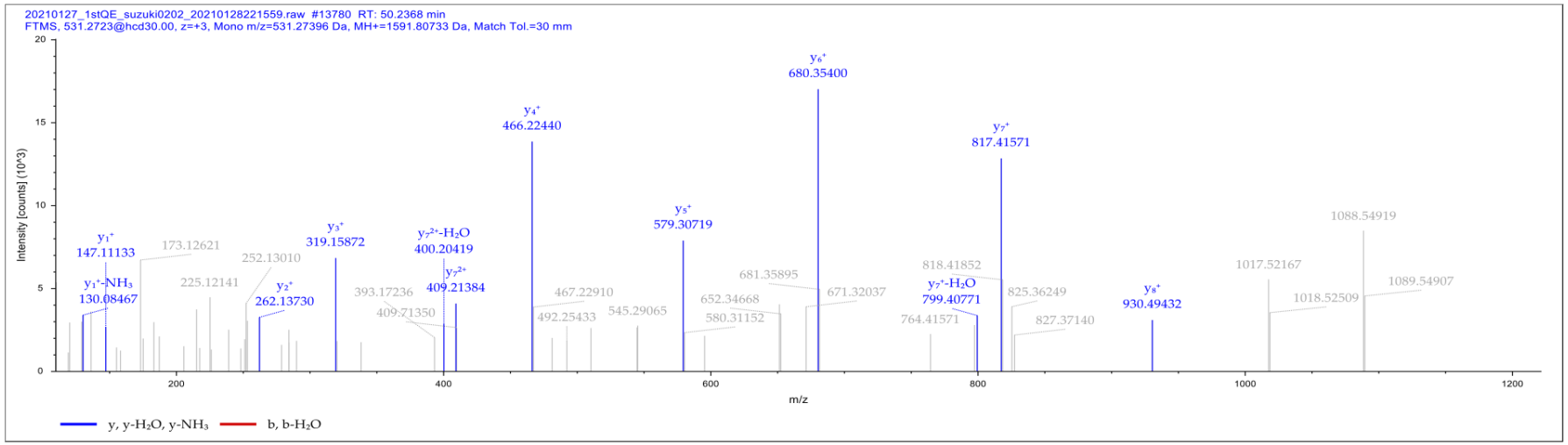
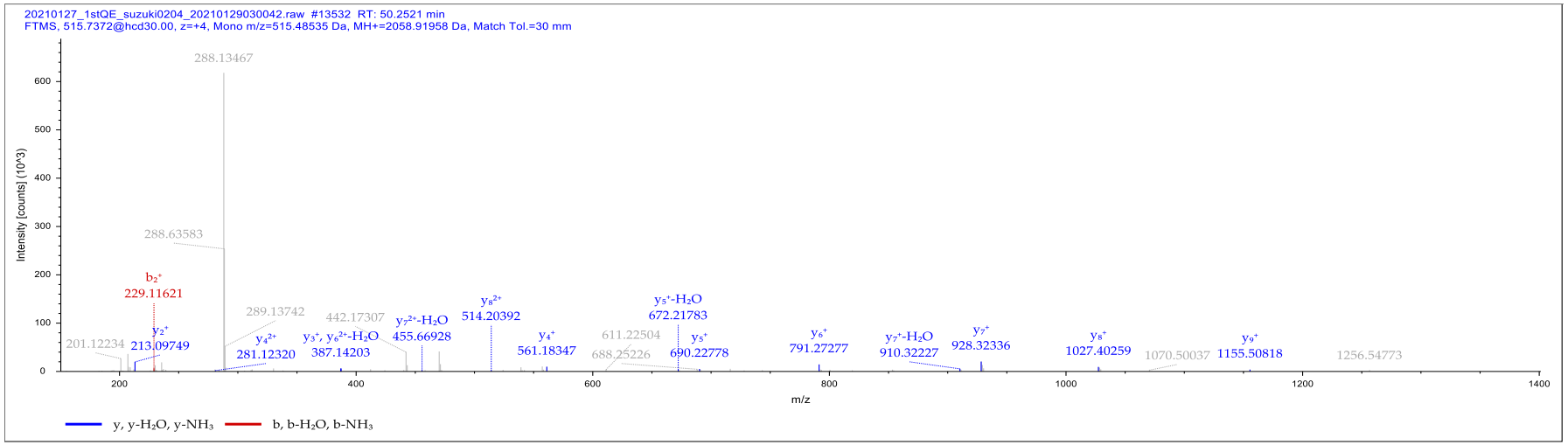


Figure S11. MSMS analysis.

Sequence: DLT(TAMRA)KVHTEC(propionamide)C(propionamide)HG [HSA(237-248)]

Annotated Sequence	Modifications	Theo. MH+ [Da]	Found in Sample: [S3] F3: Control, AspN control	Found in Sample: [S7] F7: Control, AspN control	Found in Sample: [S4] F4: Sample, AspN sample	Found in Sample: [S8] F8: Sample, AspN sample	Found in Sample: [S9] F9: Control, Chymotrypsin control	Found in Sample: [S11] F11: Sample, Chymotrypsin control	Found in Sample: [S10] F10: Sample, Chymotrypsin sample	Found in Sample: [S12] F12: Sample, Chymotrypsin sample	Found in Sample: [S1] F1: Control, Trypsin control	Found in Sample: [S5] F5: Control, Trypsin control	Found in Sample: [S2] F2: Sample, Trypsin sample	Found in Sample: [S6] F6: Sample, Trypsin sample	Confidence (by Search Engine): Mascot	Ions Score (by Search Engine): Mascot	Top Apex RT [min]
[T].DLTKVHTECCHG.[D]	2xPropionamide [C9; C10]; 1xTAMRA [T3]	2058.92007	Not Found	Not Found	Peak Found	Medium	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Medium	18	50.29
[T].DLTKVHTECCHG.[D]	2xPropionamide [C9; C10]	1484.66206	High	High	High	High	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	High	78	29.71
[T].DLTKVHTECCHG.[D]	1xPropionamide [C]	1413.62495	Peak Found	Peak Found	High	High	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	Not Found	High	45	29.72



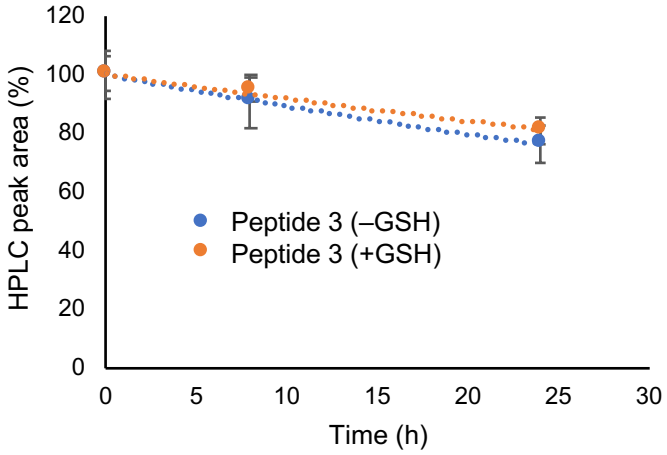


Figure S12. Stability of peptide **3** with the condition of 50 μM of substrate in 1% DMSO/H_2O with or without glutathione (200 μM) at 25 ºC. The solution was analyzed by reverse-phase HPLC with a linear gradient of 0.1 % TFA/CH_3CN (25-45%, 20 min) in 0.1 % TFA/H_2O at a flow rate of 1.0 mL/min, detected by UV absorption at 215 nm (COSMOSIL, $5C_{18}$ AR-300, 4.6 I.D. x 200 mm). The data (n = 3) are shown as means ± SD.

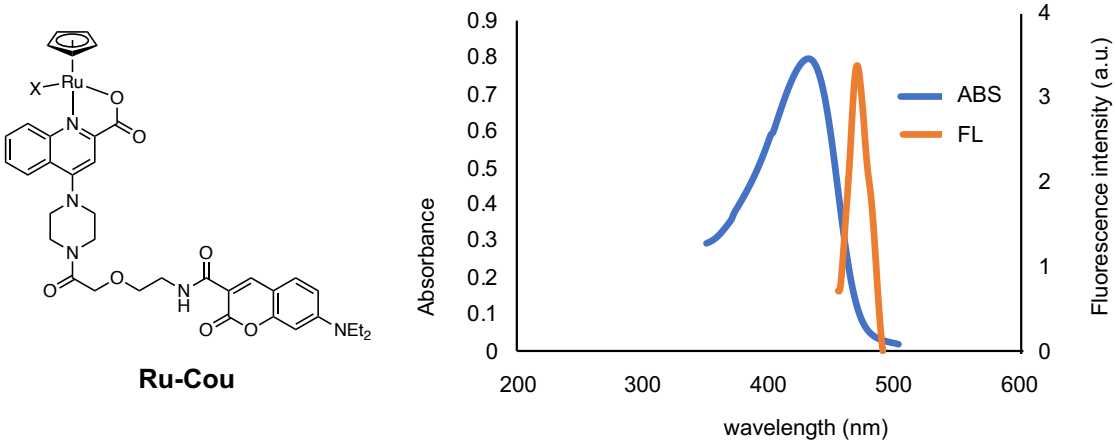


Figure S13. Absorbance (blue) and fluorescence (orange, ex: 425 nm) properties of **Ru-Cou** in 10% DMSO/H_2O measured by V-630 spectrophotometer and FP-6500 fluorometer, respectively.

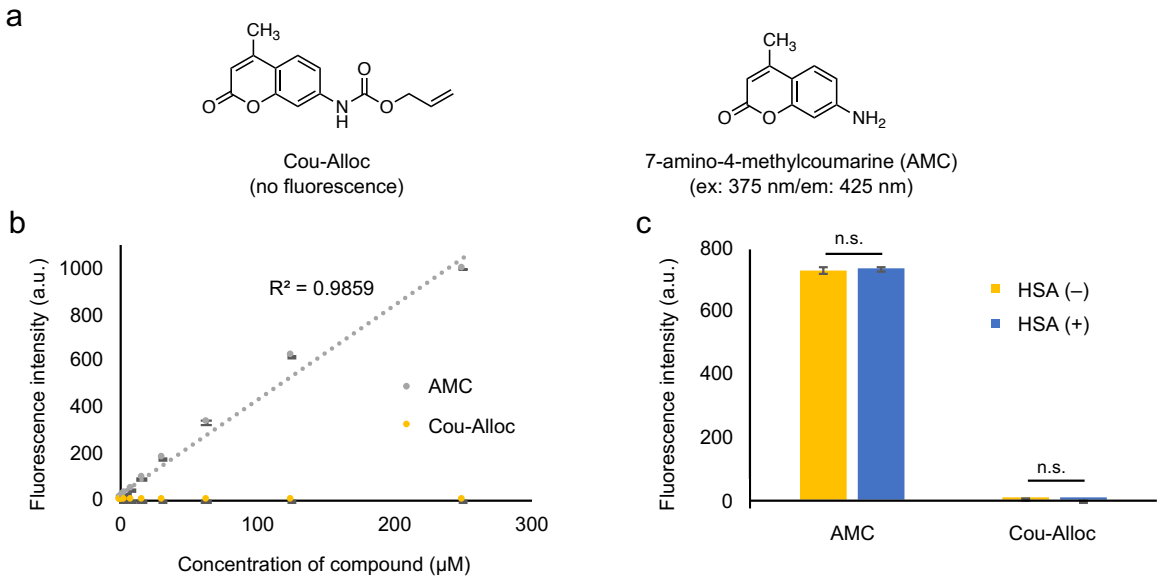


Figure S14. Fluorescence properties of Alloc protected Coumarin (Cou-Alloc) and 7-amino-3-methyl coumarin (AMC). a) Structures of Cou-Alloc and AMC. b) Concentration dependent fluorescence intensity of Cou-Alloc and AMC (ex: 375 nm, em: 450 nm). c) The effect of HSA to the fluorescence of coumarin compounds (150 μM). HSA did not affect the fluorescence properties of AMC as DEAC, since AMC is not environment-sensitive dye as reported[3]. n.s. not significant. Data (n = 3) are shown as means ± SD.

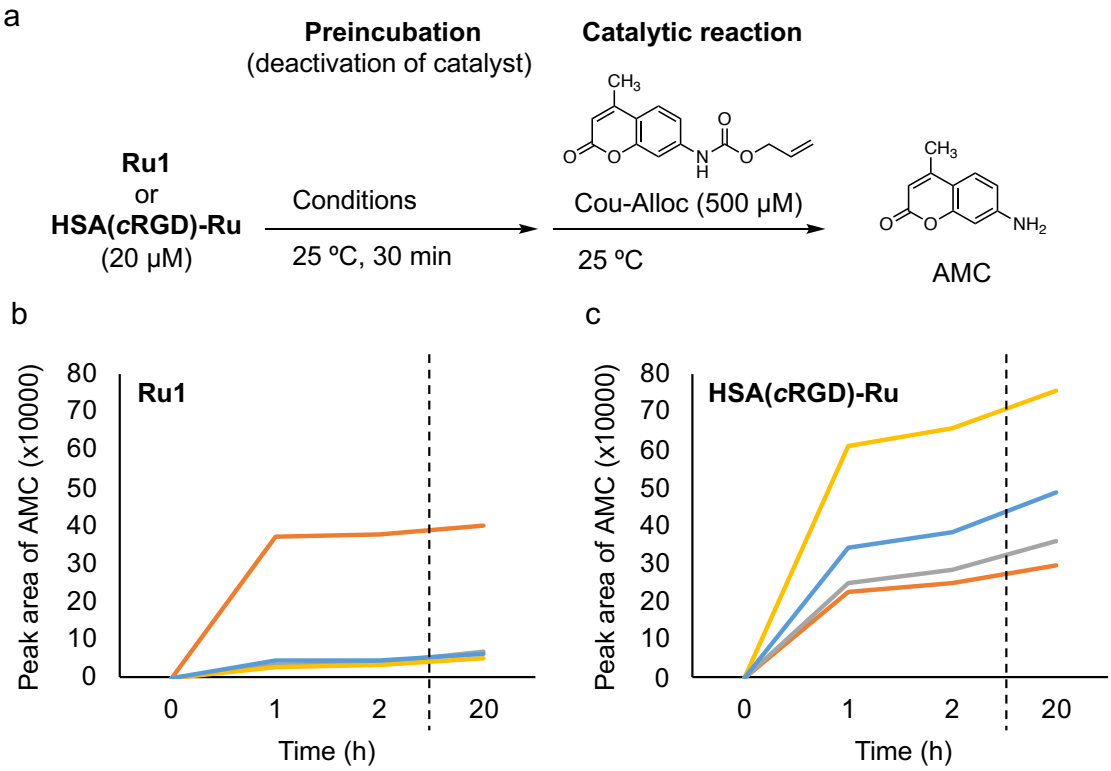


Figure S15. a) Catalytic activity evaluation with/without preincubation under following conditions for 30 min; Orange: no preincubation (in 5% acetone/H_2O), gray: preincubation with 5% acetone/H_2O, blue: preincubation with 5% acetone/PBS, Yellow: preincubation with glutathione (200 μM) in 5% acetone/H_2O. Time course of product (AMC) generation determined by HPLC using b) **Ru1** and c) **HSA(*c*RGD)-Ru**. The activity of HSA(cRGD)-Ru was increased in the presence of GSH (yellow line), while the series of Ru catalysts originally have the both of activation[4] and deactivation[5] effects by thiol compounds. The results suggested that HSA not only can protect the catalytic center, but also the catalytic process is activated by thiol, presumably by scavenging ally moiety.

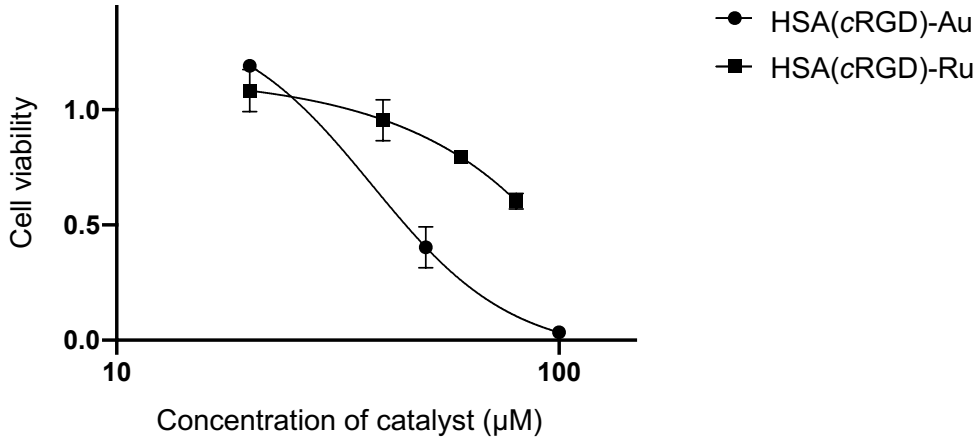


Figure S16. The dose-cytotoxicity curve of HSA(*c*RGD)-Au and HSA(*c*RGD)-Ru against SW620 cells. The data (n = 3) are shown as means ± SD.

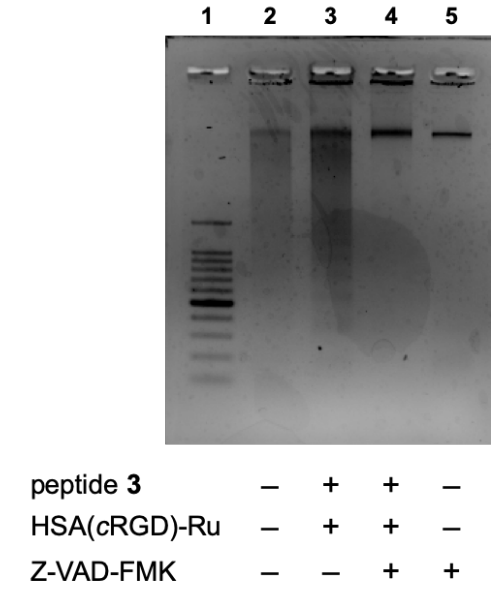


Figure S17. DNA fragmentation analysis of HSA(*c*RGD)-Ru/peptide **3** by DNA ladder assay. DNA fragmentation of the co-treated cell with peptide **3** and HSA(*c*RGD)-Ru (lane 3) exhibited an excellent DNA laddering by showing fragmentations up to 150 bp which indicated a characteristic of "DNA ladders" where it showed a good marker of apoptotic cell death. The DNAs remained unfragmented in the presence of Z-VAD-FMK (lane 4).

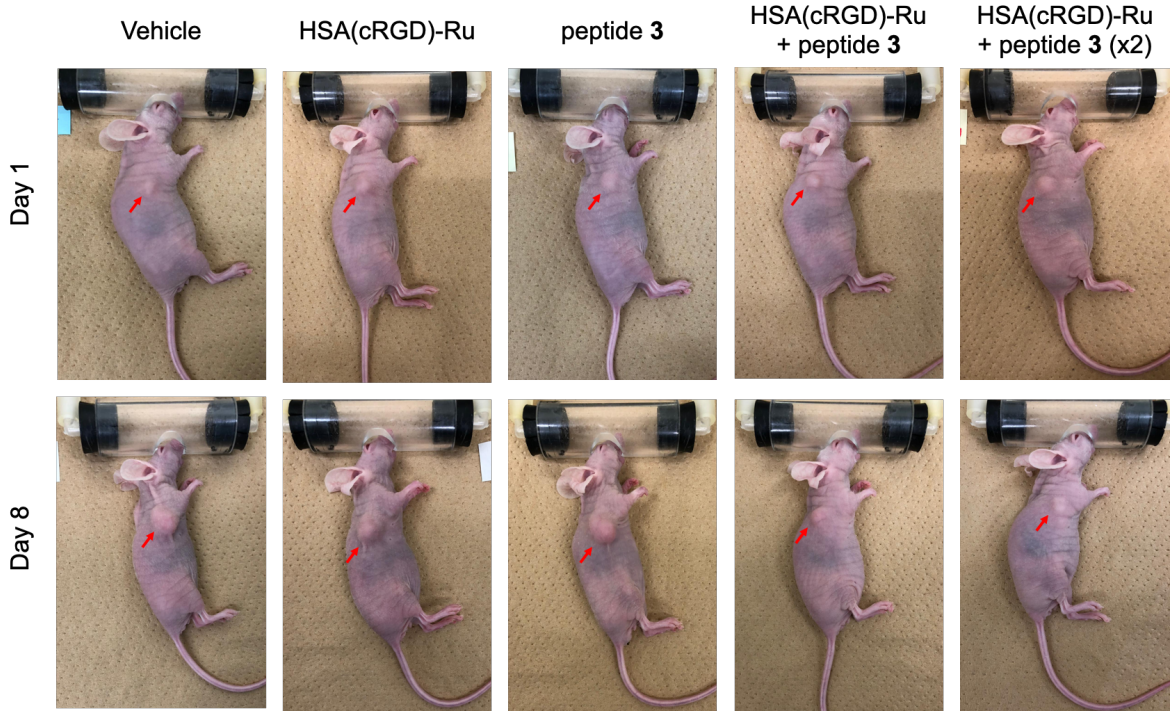


Figure S18. Photographs of SW620-xenografted mice treated with vehicle, HSA(cRGD)-Ru (69.0 mg/kg), peptide **3** (11.8 mg/kg), or peptide **3** + HSA(cRGD)-Ru (11.8 or 23.6 mg/kg and 69.0 or 138.0 mg/kg, respectively) on day 1 and day 8. Red allow indicates tumor region.

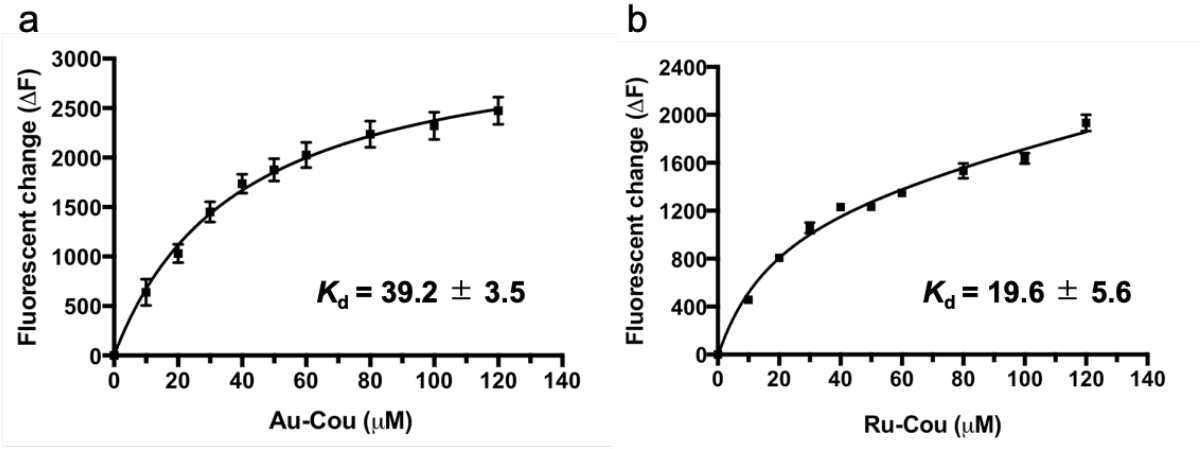


Figure S19. Saturation binding curves based on the fluorescent quenching of albumin when bound to A) **Ru-Cou** in 10% (DMSO/dixoane(v/v=1/4))/PBS or B) **Au-Cou** in 10% dioxane/PBS. Experiments were conducted at albumin concentrations of 10 μM. Incubations were performed at 37 ºC for 1 h and monitored at λ_{EX} =280 nm/λ_{EM}=320 nm. The measured equilibrium dissociation constants (K_D) were determined by non-linear regression. Error bars represent the standard deviation of three replicate measurements.

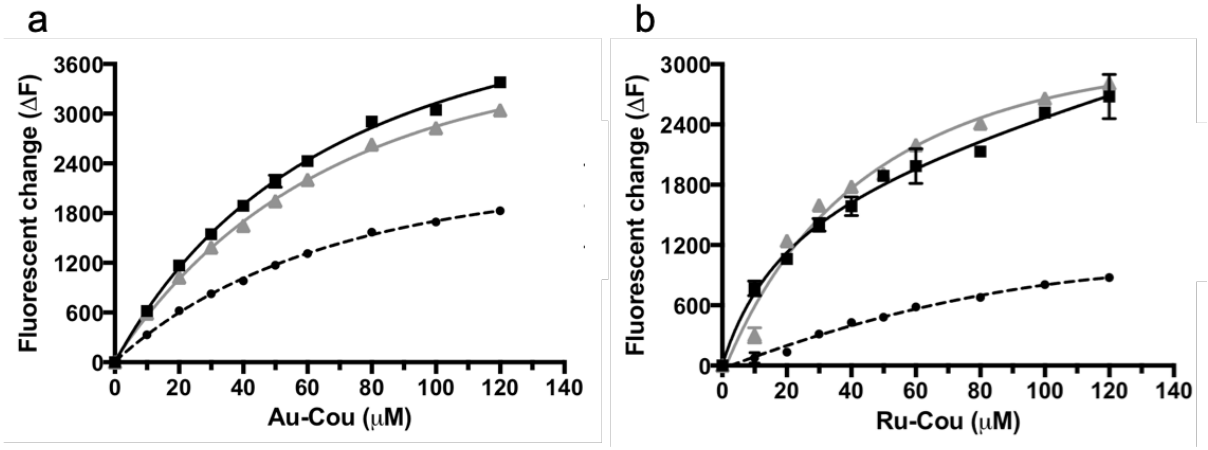


Figure S20. Saturation binding curves based on the fluorescent quenching of albumin (black line), albumin preincubated with warfarin (dotted line), and albumin preincubated with ibuprofen (gray line). Preincubation was carried out with 20 μM of albumin and 40 μM of either buffer (control), warfarin, or ibuprofen for 1 hr at 37 ºC. Incubation at 37 ºC for 1 h in 10% dioxane/PBS buffer pH 7.4 was done for a) **Au-Cou** or b) **Ru-Cou**. Fluorescence quenching was monitored at λ_{EX}=280 nm/λ_{EM}=320 nm. Error bars represent the standard deviation of three replicate measurements.

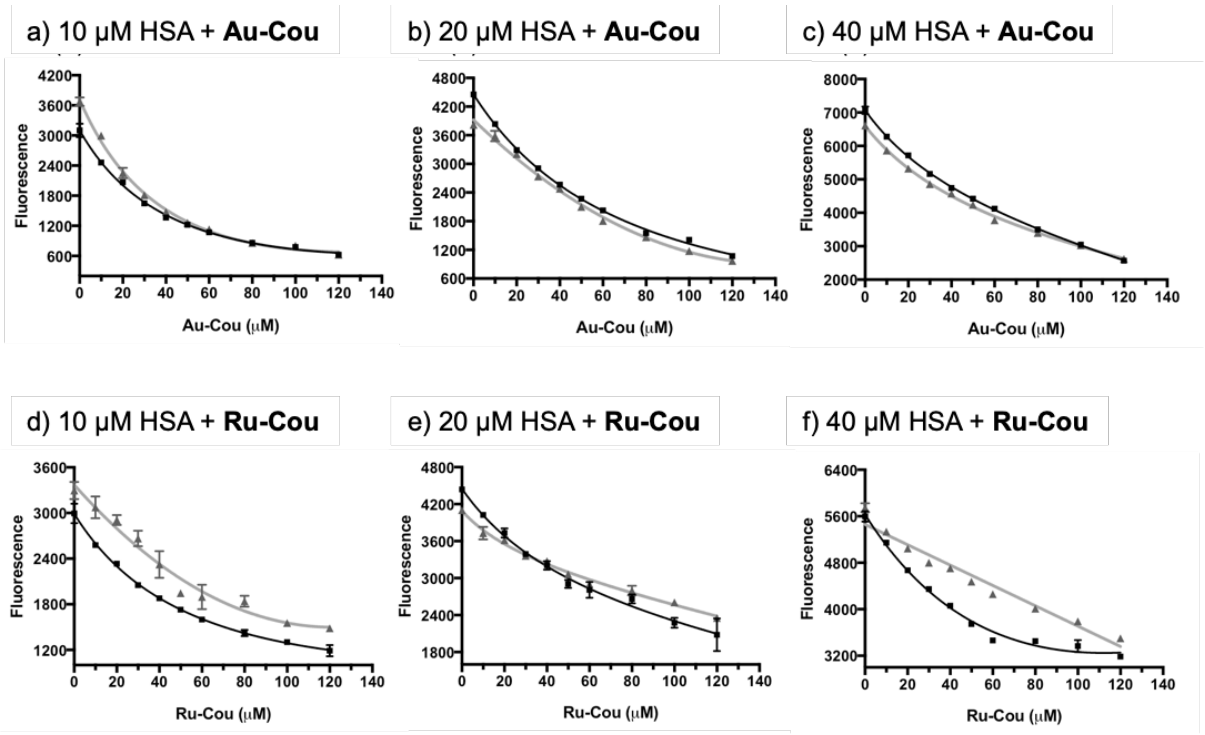


Figure S21. Fluorescence quenching measurements from **Au-Cou** (a-c) and **Ru-Cou** (d-f) binding to a,d) 10 µM of albumin, b,e) 20 µM of albumin, c,f) 40 µM of HSA. Shown are the values obtained before (grey line) and after (black line) spin-column purification. Incubations were done in 10% dioxane/PBS buffer pH 7.4 for 1 h at 37 ºC and monitored at λ_{EX} = 280 nm/λ_{EM} = 320 nm. Error bars represent the standard deviation of three replicate measurements.

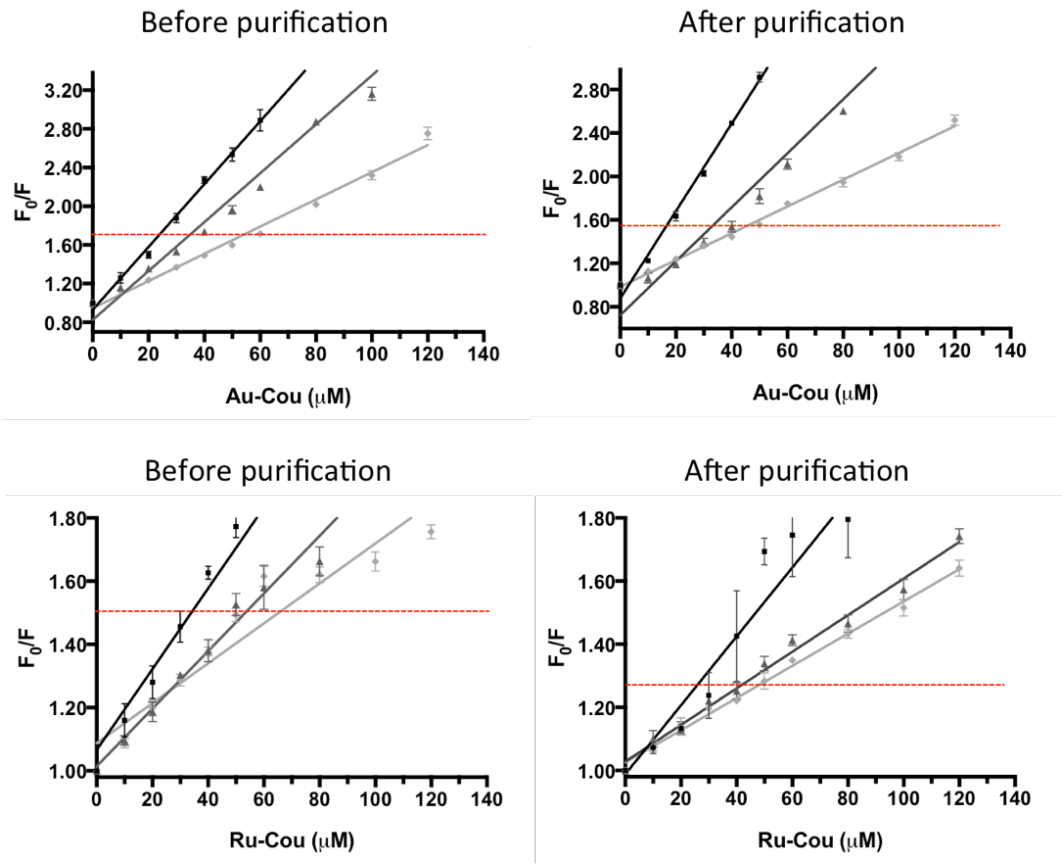


Figure S22. Stern-Volmer plots for the quenching of albumin intrinsic fluorescence by ligand **Au-Cou** and **Ru-Cou** under varying concentrations of HSA, as depicted: 10 μM (■); 20 μM (△); 40 μM (◇). Graphs are arranged to show values obtained before and after spin-column purification. The horizontal lines depict the F_0/F values used for **Au-Cou** (1.7075 and 1.5911) and **Ru-Cou** (1.508 and 1.2604) before and after purification, respectively. Error bars represent the standard deviation of three replicate measurements.

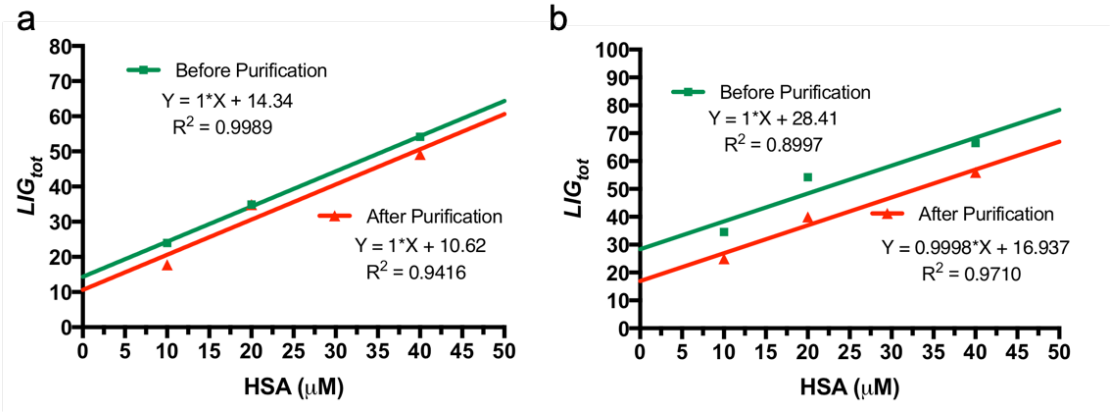
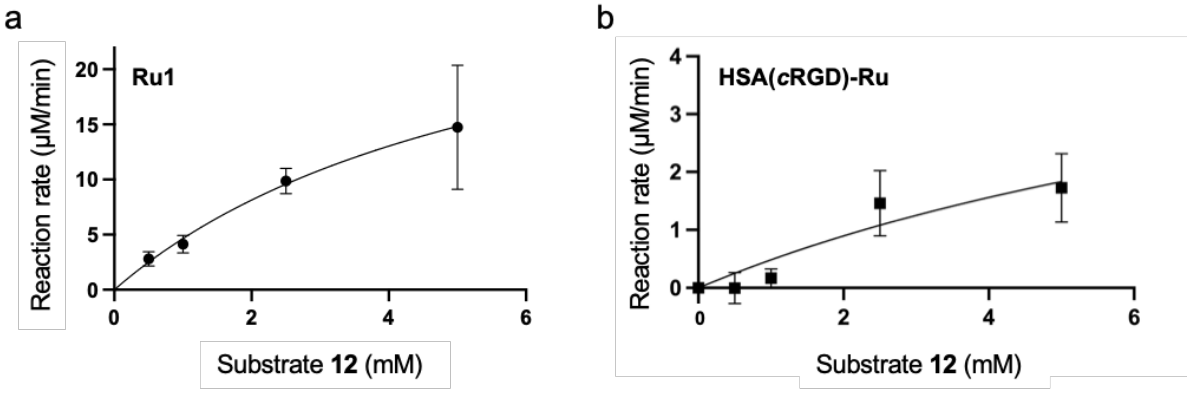


Figure S23. Total ligand concentrations needed to produce a given F_0/F value as a function of HSA concentration. Shown are the plots obtained for the ligand A) **Ru-Cou** and B) **Au-Cou** under study.



c

	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} · min^{-1})	TON
Ru1	6.05	3.27	0.545	28.6
HSA(*c*RGD)-Ru	11.5	0.605	0.0522	7.4

Figure S24. Michaelis-Menten plots of a) **Ru1** and b) HSA(*c*RGD)-Ru for the substrate **12**. The concentration of catalyst used was 10 μM. HSA(*c*RGD)-Ru was prepared by the condition determined in Figure S23 for 1 : 1 stoichiometry (20 μM of HSA was incubated for 1 h at 37 ºC with 48 μM of **Ru-Cou)**. The data are shown as means ± SD (n = 3). c) Summarized kinetic parameters; substrate affinity (K_M), turnover frequency (k_{cat}), catalytic efficiency (k_{cat}/K_M), and turnover number (TON, determined by 20 h incubation).

Experimental section

General information

All commercially available reagents were used without further purification. General reagents and solvents were purchased from FUJIFILM Wako Pure Chemical Industries, Sigma-Aldrich, Watanabe Chemical Industries, Tokyo Chemical Industries, Fisher Scientific, Cosmo Bio Co. Ltd. Funakoshi (Tokyo, Japan). All were used as received. Human Serum Albumin was purchased from Sigma-Aldrich (Tokyo, Japan). Cyanine7.5 NHS ester was purchased from Lumiprobe Life Science Solutions (Maryland, USA). Resin and amino acid derivatives for peptide synthesis were purchased from Watanabe Chemical Industry (Tokyo, Japan). Ultrapure water used for all synthetic experiments described in this paper was obtained from a Milli-Q Advantage® A10 Water Purification System sold by Merck Millipore (Burlington, USA). In addition, Amicon® Ultra Centrifugal Filters (10 and 30 kDa) and Durapore PVDF 0.45 µm® filters were also purchased from by Merck Millipore (Burlington, USA). HPLC purification was conducted on Nacalai Tesque reversed phase column ($5C_{18}$-AR300, I10 x 250 mm, 4.6 x 250 mm) (Kyoto, Japan). Fluorescence data were recorded on a plate reader SpectraMax iD3 (Kyoto, Japan). In vivo kinetics and biodistribution analysis data were recorded on an In Vivo Optical Imaging System (Clairvivo OPT plus, Shimadzu). TLC analyses (F-254) were performed with 60 Å silica gel from Merck. ^{1}H, ^{13}C and ^{19}F NMR spectra were measured on either a JEOL JNM-AL300 (300 MHz) or JNM-ECZ400R/S1 (400 MHz) instrument with the solvent peaks as internal standards: δH 0.00, 7.26 or 1.94 (tetramethylsilane, $CDCl_3$ or CD_3CN), δC 77.0, 39.5, or 49.0 ($CDCl_3$, DMSO-d_6, or CD_3OD, respectively) and δF -76.6 (trifluoroacetic acid). When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. For chemical synthesis, high-resolution mass spectra (HRMS) were obtained on a Bruker MicroTOF-QIII spectrometer® by electron spray ionization time-of-flight (ESI-TOF-MS). High molecular weight molecules were measured on a Bruker Daltonics Maldi-TOF UltrafleXtreme.

Cell lines and reagents

SW620 cells were obtained from our stock kept in liquid nitrogen. They were cultured in Leibovitz's L-15 medium (Wako, Japan) supplemented with 10% fetal bovine serum (FBS) (Biowest, France), 1% penicillin streptomycin (Gibco, Saint Aubin, France) at 37°C in 5% CO_2-humidified atmosphere.

Binding affinity measurement

Binding affinity parameters obtained in this study were done via spectrofluorometric analysis. For sample preparation, 10× stock solutions of compound **Au-Cou** were made in dioxane, and the other 10× stock solutions of compound **Ru-Cou** were made in DMSO/dioxane (v/v=1/4). 10× stock

solutions of albumin were alternatively prepared in PBS buffer pH 7.4. For binding affinity experiments, reagents were diluted from their stock solutions to 1× final concentrations of 10% Dioxane/PBS buffer for **Au-Cou** and 1× final concentrations of 10% [DMSO/dioxane (v/v=1/4)]/PBS buffer for **Ru-Cou**. Adjustments to experimental conditions were made to albumin concentration (10 μM), ligand concentrations (0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80, 100, 120 μM), as described. During incubations, mixtures in separate Eppendorf tubes were placed in a temperature-controlled oven for 1h at 37 ºC. The mixtures were first cooled to room temperature and then aliquots (100 μl) were pipetted into 96-well microtiter plates for analysis. Saturation curves were collected based on the fluorescent quenching of albumin measured at λ_{EX} = 280 nm/λ_{EM} = 320 nm (Figure S19). The measured equilibrium dissociation constants (K_d) were determined by non-linear regression.

Binding site confirmation

By utilizing known site marker ligands of albumin, the potential binding site for **Ru-Cou** and **Au-Cou** can be indirectly determined. In literature, there are two well-known binding regions: Sudlow's site I (located in subdomain IIA) and Sudlow's site II (located in subdomain IIIA). Bulky, heterocyclic molecules (ex/ warfarin) are known to bind to site I while aromatic carboxylates (ex/ ibuprofen) have a preference for site II.[6] Both warfarin and ibuprofen are known to bind albumin with low micromolar K_d. Experimentally, a 20 μM solution of albumin was preincubated with 2 equivalents of either site marker ligand (warfarin, ibuprofen) for 1 hr at 37 ºC. The albumin/site marker ligand mixture was then used to construct saturation binding curves, which are shown in Figure S20. In Supplementary Figure S20, it can be clearly seen that binding of **Au-Cou** and **Ru-Cou** remains unaffected in the presence of ibuprofen, but decreases significantly with warfarin. This data suggests that the main binding site of both **Au-Cou** and **Ru-Cou** is Sudlow's site I.

Stoichiometry of binding

To determine the stoichiometry of binding between albumin and ligand **Ru-Cou** and **Au-Cou**, the Encinas-Lissi method was employed. The main premise behind this approach is to carry out Stern-Volmer plots, as defined in Eq. (1), with several protein concentrations to better characterize the stoichiometry of the binding step (n).

$$F_0/F = 1 + K_{SV}(LIG_{tot}) \quad (1)$$

where K_{SV} is the Stern-Volmer quenching constant, LIG_{tot} is the total ligand (quencher) concentration, F_0 is the fluorescent intensity without a quencher, and F is the fluorescent intensity with a quencher.

To begin, albumin under various concentrations was incubated with varying concentrations of ligand (**Ru-Cou** and **Au-Cou**) in 10% dioxane/PBS buffer for 1 hr at 37 ºC. The resultant

fluorescence measurements are shown in Figure S21, where greater quenching is correlated to higher ligand concentrations. Following Eq. (1), this data can be further transformed to fit Stern-Volmer plots, as shown in Figure S22. As a note, all data sets were obtained separately under *Before Purification* and *After Purification* conditions (as outlined in the general protocol) in order to quantify the loss of ligand-binding due to spin-column filtration.

From the Stern-Volmer plots of Figure S22, a set of LIG_{total} values can be obtained at different protein concentrations for any given (F^0/F) ratio, which is represented by the horizontal bars. Another means to define LIG_{total} is given by Eq. (2), which states it as the sum of unbound ligand (LIG_{free}) and bound ligand (LIG_{bound}) concentrations.

$$LIG_{tot} = LIG_{free} + LIG_{bound} \quad (2)$$

Eq. (2) can be further rewritten as Eq. (3), which redefines LIG_{bound} to incorporate the concentration of albumin protein (HSA) with the value of n, which is the ratio ligand moles bound per moles of HSA protein.

$$LIG_{tot} = LIG_{free} + n[\text{HSA}] \quad (3)$$

Using Eq. (3), a plot LIG_{total} vs. [HSA] should theoretically give the value of n from the slope and the value of LIG_{free} from the y-intercept. Given that for 1:1 ligand-to-protein complexes, the value of n should be equal to 1, the plots shown in Figure S23 were constructed for ligand **Ru-Cou** and **Au-Cou** at F^0/F ratios calculated to produce n values of 1. These plots theoretically give the necessary total ligand concentrations to ensure 1:1 ligand-to-protein complexes within a certain protein range. Using this data as a guide, reagent ratios used in the preparation of the protein-metal catalyst complexes were adjusted accordingly. (e.g. In case of **Ru-Cou**, 20 μM of protein was incubated for 1 h at 37 ºC with 48 μM of **Ru-Cou**. In case of **Au-Cou**, 20 μM of protein was incubated for 1 h at 37 ºC with 34 μM of **Au-Cou**).

Kinetics evaluation

To the solution of substrate **12** in DMSO/H_2O was added **Ru1** or HSA(cRGD)-Ru (final concentrations: 0.5, 1, 2.5 or 5 mM of substrate **12**, and 10 μM of catalyst in 5% DMSO/H_2O). After incubation at 37 ºC for 20 min, the reaction was stopped by adding 80 μL of potassium 2-isocyanoacetate in MeOH (200 μM). The consume of substrate was analyzed by reverse-phase HPLC with a linear gradient of 0.1 % TFA/CH_3CN (30-60%, 15 min) in 0.1 % TFA/H_2O at a flow rate of 1.0 mL/min, detected by UV absorption at 254 nm. The kinetic parameters are determined by using GraphPad Prism 9 software by curve fitting with Michaelis-Menten plot. TON of catalysts was

determined by the condition with 0.5 mM of substrate **12**, 10 μM of catalyst and incubation at 37 ºC for 20 h.

Protein modification

TAMRA-BnF or TAMRA-PE was mixed with HSA in 15% DMSO/H_2O as final concentrations of 150 μM and 1.5 mM, respectively. Then, the **Ru1** or **Au1**[7] was added to the mixture with the concentration of 150 μM, and incubate at 37 ºC for 24 h. To remove the small molecules, the reaction mixture was washed with 10% DMSO aq. twice and H_2O twice by using Amicon Ultra 30K. The solution was applied to SDS-PAGE or MSMS analysis.

SDS-PAGE

The protein solution was mixed with a sample buffer (2 % SDS, 10% glycerol and 0.02% bromophenol blue in 0.5 M Tris/HCl), boiling at 95 ºC for 5 min and vortexed. The sample solution was applied to 10% acrylamide gel. After 1 h of electrophoresis with 20 mA, fluorescence band was detected by Typhoon 9400, and Coomassie brilliant blue stain was performed by using quick CBB plus (FUJIFILM Wako Pure Chemical Industries). Precision plus protein dual color standard (Bio-Rad Laboratories) was used as a standard protein ladder.

MSMS analysis

The TAMRA-modified and non-modified human serum albumin samples were subjected to SDS-PAGE and were stained by Coomassie blue. The gel bands reduced with dithiothreitol, alkylated with acrylamide and digested with a trypsin (TPCK-treated; Worthington Biochemical, Worthington, OH, USA). The digestion mixture was separated on a nanoflow LC (Easy-nLC 1000; Thermo Fisher Scientific, Inc.) using a nano-electrospray ionization spray column (NTCC analytical column; C18, φ75 μm x 100 mm, 3 μm; Nikkyo Technology Co., Ltd., Tokyo, Japan) with a linear gradient of 0-80% buffer B (0.1% formic acid in acetonitrile) in buffer A (0.1% formic acid in water) and a flow rate of 300 nl/min over 60 min, coupled on-line to a Q Exactive mass spectrometer (Thermo Fisher Scientific, Inc.) that was equipped with a nanospray ion source. The mass spectrometer was operated in positive-ion mode, and MS and MS/MS spectra were acquired in a data dependent TOP 10 method. Proteins were identified and quantified using Proteome Discoverer 2.4 (Thermo Fisher Scientific, Inc.) with MASCOT program ver 2.7 (Matrix science) using in-house database. The modified amino acid was visualized on the crystal structure of HSA (PDB: 1AO6)[8] by using PyMOL software.

Catalytic activity measurement

(For Figure 5c) To the solution of Cou-Alloc in acetone-d_6/H_2O was added **Ru1** or HSA(*c*RGD)-Ru (final concentrations: 150 μM of Cou-Alloc and 5 μM of catalyst in 2% acetone-

d_6/H_2O), and the reaction solution was plated in black bottom 96 well-plate containing 50 μL in each well. After incubation at 25 ºC, the reaction was stopped at 0, 30 60, 90 and 120 min by adding 200 μL of potassium 2-isocyanoacetate in MeOH (200 μM). The fluorescence intensity was measured by FP-6500 fluorometer with the band width 3 nm and 5 nm for excitation and emission, respectively, and 0.5 s of response time.

(For Figure S15) The solution of **Ru1** or HSA(*c*RGD)-Ru was incubated at 25 ºC for 30 min with following conditions; 1. no incubation [in 5% acetone/H_2O (control)], 2. preincubation with 5% acetone/H_2O, 3. preincubation with 5% acetone/PBS and 4. preincubation with glutathione (200μM) in 5% acetone/H_2O. To start the catalytic reaction, Cou-Alloc was added, and the reaction solution was divided to 0.5 mL tube containing 40 μL in each (final concentrations: 500 μM Cou-Alloc and 20 μM of catalyst). After incubation at 25 ºC, the reaction was stopped at 0, 1, 2 and 20 h by adding 160 μL of potassium 2-isocyanoacetate in MeOH (200 μM). The produced AMC was analyzed by reverse-phase HPLC with a linear gradient of 0.1 % TFA/CH_3CN (30-60%, 15 min) in 0.1 % TFA/H_2O at a flow rate of 1.0 mL/min, detected by UV absorption at 330 nm.

Cell Surface Labelling

The SW620 cells (2.5 x 10^4 cells/100 ml/well) were seeded into a 96 well black-colored plate and incubated at 37 ºC in the 5% CO_2 atmosphere for overnight. Before the treatment, the medium was removed, changed with 90 μL medium containing either HSA(*c*RGD)-Au or HSA(*c*RGD)-Ru, and incubated at culture condition. After incubating cells for 15 min, the cells were added 10 μL of **TAMRA-PE** or **TAMRA-BnF** to give total volume culture of 100 μL with a final concentration of 20 μM of HSA(*c*RGD)-Au or HSA(*c*RGD)-Ru and 200 μM of **TAMRA-PE** or **TAMRA-BnF**. After incubation at 37 ºC in the 5% CO_2 atmosphere for 1h, the medium was removed, changed with 1% DMSO-contained medium, and incubated for another 1 h to wash non-specific binding of the substrates. Then, the cells were washed with PBS (2x 100 μL), fixed with 4% PFA by incubating the cells at room temperature for 10 min. After fixation, the cells were treated with 100 μL of Hoechst33258 (1 mg/mL) solution in PBS, incubated at 37 ºC for 10 min, washed with PBS (2x 100 mL), and added 100 μL of PBS to each well. The cells were then observed by using a fluorescence microscope, BZ-X700 Keyence.

Cell proliferation assay

The SW620 cells (2.0 x 10^4 cells/well) were seeded into 96 well white-colored plate and incubated for overnight at 37 ºC in the presence of 5% CO_2-humidified atmosphere. The cells were added with 1 μl of peptide solution in DMSO and/or 1 μl of catalyst solution in H_2O to give various final concentrations. The cells were incubated for 48 h at 37 ºC with 5% CO_2 atmosphere. For ATP assay, added 100 μl ATPlite 1step kit solution (PerkinElmer, Netherlands). After 10 minutes

incubation at room temperature, the luminescence intensity of the treated cells was measured by using SpectraMax iD3 at all wavelength. The luminescence intensity was divided by control to give relative luminescence intensity where the final value of control is 1. For MTT assay, the cultured media were removed and added 100 µl of medium in the presence of 10% MTT and incubated for 3 h. Media were then removed and added 100 ml of DMSO and measured the absorbance at 570 nm.

Caspase assay

The SW620 cells (2.0 x 10^4 cells/well) were seeded into 96 well black plate and incubated for overnight at 37°C in the presence of 5% CO_2-humidified atmosphere. The cells were added 1 µl of samples to give various final concentrations and incubated for additional 3 or 6 or 12 h. The Apo 3/7 HTS kit solution was prepared according to the kit instruction (CTI, USA) and added 100 µl to each well (procedure refer to protocol kit) and incubated for another 1 h. The fluorescence intensity of the treated cells was recorded by using microplatereader SpectraMax iD3 with excitation and emission wavelength at 488 and 525 nm, respectively. The fluorescence intensities were simplified to give relative fluorescence intensity values.

DNA ladder assay

The SW620 cells were seeded in 60 mm dish with the number of cells about 5.8 x 10^6 and incubated for 24 h at 37°C in the presence of 5% CO_2-humidified atmosphere. The medium was removed and then added sample solution in 2 mL medium containing corresponding peptides and/or catalysts. The cells were incubated for additional 24h at the culture temperature and condition. The treated cells were lysed and the DNAs were extracted according to the kit instruction (BioVision, catalog #K170-50). The DNA extracts were run on 1.7% agarose gel and imaged using ImageQuant LAS 4000 imager.

Animal Experiments

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of RIKEN and approved by the Animal Ethics Committee of RIKEN (W2019-2-049). For all imaging experiments and tumor measurements, mice were anesthetized with 2.5% isoflurane in oxygen at a flowrate 2.5-3.0 L/min.

SW620 bearing mice xenograft models

The five weeks-old female nude mice BALB/cAJcl-nu/nu were subcutaneously injected with 2.5 x 10^6 cells of SW620 in 100 µL cold unnourished Leibovitz's L-15 medium into the shoulder. The mice were kept in controlled temperature, salinity and aeration room with sufficient food and water for 12 h day and 12 h night. When tumor reach 200-400 mm^3, the SW620 tumor bearing mice were

ready to be used for in vivo therapy, while for in vivo imaging, the bigger size (approximately 1000 – 1500 mm^3) of tumors were used.

In vivo imaging

The SW620 tumor bearing mice were intravenously injected with 100 μL of 100 μM of cy7.5 labeled RGD-modified HSA. The mice were imaged by using Clair Vivo Shimadzu after injection at 0, 0.5, 1, 2, 4, 6, 8, and 24 h. The mice were exposed at the same shooting time and intensity. To image the mice, excitation and emission wavelength of fluorescence was adjusted at 785 and 849 nm, respectively and used 5 s exposure time.

In vivo therapy

Peptide 1 and HSA(*c*RGD)-Au (For Figure 3e-g): The 200–400 mm^3 sized SW620-bearing mice were randomized divided into 4 groups and assigned with I.V. injection of saline (n = 4), 35 mg/kg of HSA(*c*RGD)-Au (n = 7), 4.0 mg/kg of peptide **1** (n = 7), mixture of 35 mg/kg of HSA(*c*RGD)-Au and 4.0 mg/kg of peptide **1** (n = 7). The therapy was repeated for 10 times IV administration within 12 days. The tumor volume and bodyweight of the treated mice were recorded in every single day until the tumor size reached 2000 mm^3. The tumor size was calculated by the equation of $V = W^2xL/2$, where W and L represented minor and major length of the tumor, respectively. When the tumor size reached 2000 mm^3, the mice were painlessly sacrificed and the survival rates were calculated by using Kaplan Meier method.

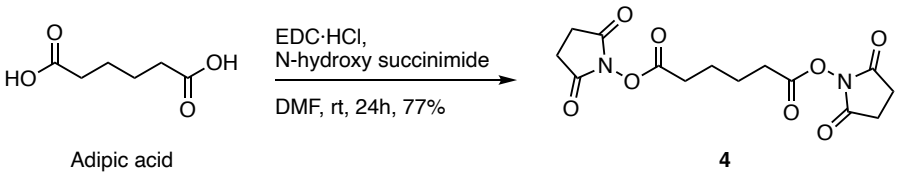
Peptide 1 and HSA(*c*RGD)-Au (For Figure S7): The 200–400 mm^3 sized SW620-bearing mice were randomized divided into 5 groups and assigned with I.V. injection of saline (n = 5), 70.0 mg/kg of HSA(*c*RGD)-Au (n = 5), 8.0 mg/kg of peptide **1** (n = 5), mixture of 70.0 mg/kg of HSA(*c*RGD)-Au and 8.0 mg/kg of peptide **1** (n = 5) and mixture of 140.0 mg/kg of HSA(*c*RGD)-Au and 16.0 mg/kg of peptide **1** (n = 5). A single IV injection was performed and tumor growths, bodyweights, and survival rates were daily controlled. The tumor size was calculated by the equation of $V= W^2xL/2$, where W and L represented the minor and major lengths of the tumor, respectively. When the tumor size reached 2000 mm^3, the mice were painlessly sacrificed and the survival rates were calculated by using the Kaplan Meier method.

Peptide 3 and HSA(*c*RGD)-Ru (For Figure 5e-g): The 200–400 mm^3 sized SW620-bearing mice were randomized divided into 5 groups and assigned with I.V. injection of saline (n = 8), 69.0 mg/kg of HSA(*c*RGD)-Ru (n = 8), 11.8 mg/kg of peptide **3** (n = 8), mixture of 69.0 mg/kg of HSA(*c*RGD)-Ru and 11.8 mg/kg of peptide **3** (n = 8) and mixture of 138.0 mg/kg of HSA(*c*RGD)-Ru and 23.6 mg/kg of peptide **3** (n = 8). A single IV injection was performed in this cancer therapy while tumor

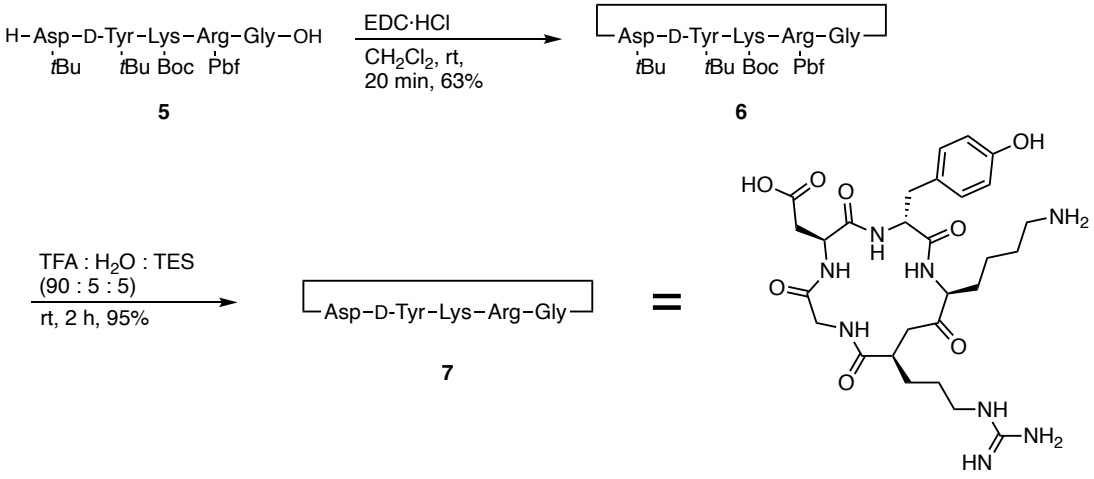
growth, bodyweight and survival rate were daily controlled. The tumor size was calculated by the equation of $V= W^2xL/2$, where W and L represented minor and major length of the tumor, respectively. When the tumor size reached 2000 mm^3, the mice were painlessly sacrificed and the survival rates were calculated by using Kaplan Meier method.

Statistics

Statistical analysis was performed by using the GraphPad Prism 9 software. Student's t-test with two-tailed, two-way ANOVA and log-rank (Mantel-Cox) test were adopted for the comparison of two groups, evaluation of synergistic effect and comparison of survival curves, respectively.



Synthesis of compound 4: To a stirred solution of adipic acid (1.0 g, 6.84 mmol) in DMF 20 mL was added *N*-hydroxysuccinimide (3.0 g, 27.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl) (5.14 g, 27.4 mmol). After the solution was stirred at room temperature for 24 hours, the reaction mixture was evaporated *in vacuo*, and the crude was dissolved into acetone 200 mL and poured into 1M HCl aq. 250 mL. After 2 h, white precipitate was filtered and washed with water and acetone to give Bis-NHS compound (1.75 g, 77%). The characterization matched with previous report of this known compound[9]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.81 (s, 8H), 2.78 – 2.70 (m, 4H), 1.77–1.65 (m, 4H). HRMS (ESI) m/z calcd for $C_{14}H_{17}N_2O_8$ $[M+H]^+$ 341.0979, found 341.0973.

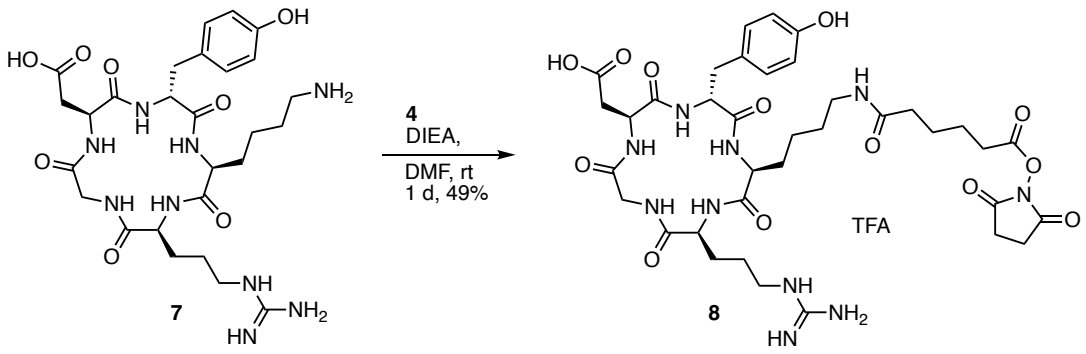


Synthesis of peptide 5: A 218 mg of H-Gly-Trt(2-Cl) resin (0.2 mmol) was swollen by dipping in 3 mL DMF for 30 min in 6 mL reservoir. To the swollen-resin was added Fmoc-Arg(Pbf)-OH • 0.1 acetone (532 mg, 0.8 mmol), *O*-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) (331 mg, 0.8 mmol) HOBt(6-Cl) (136 mg, 0.8 mmol) in 2 mL DMF and *N*,*N*-diisopropylethylamine (DIEA) (204 μL, 1.2 mmol). The suspension was shaken for 30 min at room temperature. The coupling reaction was monitored by KISER test reagent for detection of amino group. After reaction was completed, the resin was washed with DMF (1 min, thrice). Fmoc deprotection was performed by the use of 20% piperidine in DMF (2 min, thrice) and followed by washing step using DMF (1 min, thrice). Peptide elongation and Fmoc deprotection reactions were carried out manually by using Fmoc-amino acid/HCTU/HOBt(6-Cl)/DIEA (4/4/4/6 eq) in DMF for

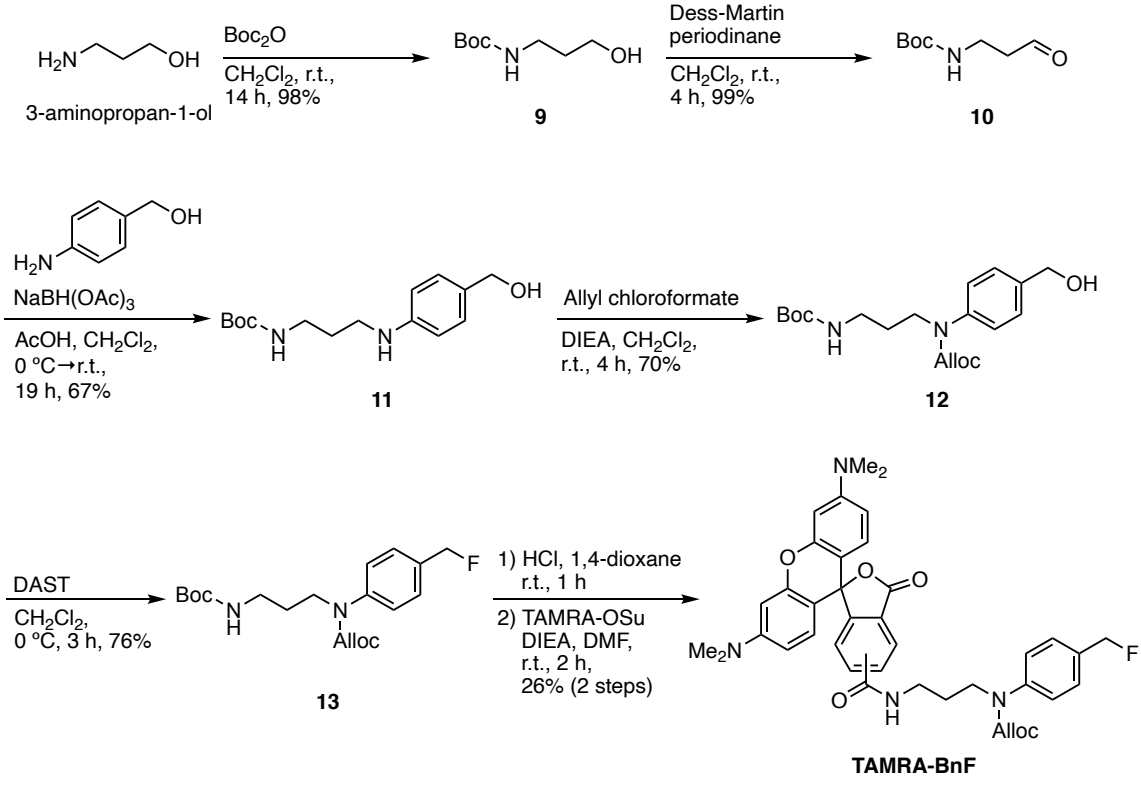
30 min and 20% piperidine in DMF (2 min, thrice) treatments, respectively. After peptide elongation of 3 amino acids, the protected peptide was treated with 20% hexafluoro-2-propanol (HFIP)/$CHCl_3$ for 1 hour. The crude peptide was precipitated with cold diethyl ether, and the precipitate was dried *in vacuo* to obtain H-Asp(^{t}Bu)-D-Tyr(^{t}Bu)-Lys(Boc)-Arg(Pbf)-Gly-OH **5** as white solid (111.9 mg, 51%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.12 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 4.51 (t, J = 7.6 Hz, 1H), 4.46-4.38 (m, 1H), 4.21 (dd, J = 8.4, 5.2 Hz, 1H), 4.06-3.96 (m, 1H), 3.94 (d, J = 17.2 Hz, 1H), 3.82 (d, J = 17.6 Hz, 1H), 3.22-3.11 (m, 1H), 3.01-2.92 (m, 6H), 2.82-2.69 (m, 2H), 2.54 (s, 3H), 2.48 (s, 3H), 1.95-1.82 (m, 1H), 1.81-1.66 (m, 2H), 1.66-1.52 (m, 2H), 1.45 (s, 9H), 1.43 (s, 6H), 1.40 (s, 9H), 1.37-1.31 (m, 2H), 1.30 (s, 9H), 1.29-1.25 (m, 2H), 1.02-0.91 (m, 2H); HRMS (ESI) m/z calcd for $C_{53}H_{84}N_9O_{14}S$ $[M+H]^+$ 1102.5853, found 1102.5880.

Synthesis of peptide 6: To the stirred solution of H-Asp(^{t}Bu)-D-Tyr(^{t}Bu)-Lys(Boc)-Arg(Pbf)-Gly-OH **5** (111.9 mg, 0.102 mmol) in 2 mL CH_2Cl_2 was added EDC•HCl (29.3 mg, 0.153 mmol) and HOBt (25.9 mg, 0.153 mmol). The solution was stirred for 20 min at room temperature and extracted with 20 mL CH_2Cl_2. The organic layer was concentrated *in vacuo* to obtain c(Asp(^{t}Bu)-D-Tyr(^{t}Bu)-Lys(Boc)-Arg(Pbf)-Gly) **6** (69.3 mg, 63%) as white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.11 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.4 Hz, 2H), 4.72 (t, J = 7.6 Hz, 1H), 4.48 (t, J = 8.4 Hz, 1H), 4.22-4.10 (m, 2H), 4.92-3.82 (m, 1H), 3.39 (d, J = 5.2 Hz, 1H), 3.20-3.08 (m, 2H), 3.02-2.88 (m, 6H), 2.78-2.66 (m, 1H), 2.54 (d, J = 4.8 Hz, 3H), 2.48 (d, J = 5.2 Hz, 3H), 2.05 (d, J = 4.8 Hz, 3H), 1.85-1.72 (m, 1H), 1.69-1.52 (m, 2H), 1.49-1.31 (m, 6H), 1.44 (s, 9H), 1.42 (s, 6H), 1.29 (s, 9H), 0.99 (m, 2H); HRMS (ESI) m/z calcd for $C_{53}H_{82}N_9O_{13}S$ $[M+H]^+$ 1084.5747, found 1084.5788.

Synthesis of peptide 7: A 100 mg of c(Asp(^{t}Bu)-D-Tyr(^{t}Bu)-Lys(Boc)-Arg(Pbf)-Gly) **6** (0.092 mmol) in a 10 mL round bottom flask was added TFA-TES-H_2O (90:5:5) 2 mL and stirred for 2 hours at room temperature. The solution was concentrated *in vacuo* and purified with RP-HPLC (Column: $5C_{18}$-AR-300, 10 ID x 250 mm, with gradient solvent MeCN in H_2O (10–80% over 40 min) to obtain c(RGDyK) **7** (54.3 mg, 95%) as white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.01 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 4.74 (t, J = 6.8 Hz, 1H), 4.41 (q, J = 7.2 Hz, 1H), 4.28 (t, J = 6.8 Hz, 1H), 4.22 (d, J = 14.8 Hz, 1H), 3.91 (dd, J = 11.6, 3.6 Hz, 1H), 3.31 (d, J = 14.4 Hz, 1H), 3.25-3.05 (m, 2H), 2.94-2.72 (m, 5H), 2.56 (dd, J = 16.0 and 6.8 Hz, 1H), 1.90-1,78 (m, 1H), 1.74-1.35 (m, 7H), 0.95 (t, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.4, 172.8, 172.4, 172.1, 171.4, 170.9, 157.3, 156.0, 130.2, 127.3, 116.9, 115.1, 56.0, 54.6, 52.4, 49.6, 43.6, 40.6, 39.1, 36.2, 34.9, 30.4, 28.1, 26.6, 25.0, 22.8; HRMS (ESI) m/z calcd for $C_{27}H_{42}N_9O_8$ $[M+H]^+$ 620.3151, found 620.3146.



Synthesis of peptide 8: To the stirred solution of **7** (28.8 mg, 0.034 mmol) and Bis-NHS compound **4** (23.2 mg, 0.068 mmol) in DMF 680 μL was added DIEA (16.8 μL, 0.10 mmol). After the solution was stirred at room temperature for 24 hours, the resulting residue was purified by RP-HPLC using a preparative column (COSMOSIL 5C_{18}-AR-300, 10ID x 250 mm) at a flow rate of 4.5 mL/min with a linear gradient of MeCN in H_2O (10–80% over 40 min) to obtain *c*(RGDyK)-OSu (**8**, 14.0 mg, 49%). ^{1}H NMR (400 MHz, CD_3OD, 25 °C) δ 7.00 (d, J = 6.5 Hz, 2H), 6.68 (d, J = 6.5 Hz, 2H), 4.74 (t, J = 7.0 Hz, 1H), 4.41 (q, J = 7.0 Hz, 1H), 4.26-4.30 (m, 2H), 3.91-3.86 (m, 1H), 3.22-3.18 (m, 1H), 3.14-3.10 (m, 1H), 3.09 (t, J = 6.5 Hz, 2H), 2.91-2.85 (m, 2H), 2.84-2.78 (m, 5H), 2.59 (t, J = 9.5 Hz, 2H), 2.64-2.60 (m, 1H), 2.58 (dd, J = 16.5 and 9.0 Hz, 1H), 2.19 (t, J = 7.0 Hz, 2H), 2.22 (t, J = 7.0 Hz, 2H), 1.92-1.82 (m, 1H), 1.74-1.60 (m, 6H), 1.57-1.44 (m, 3H), 1.44-1.32 (m, 2H), 1.09-0.90 (m, 2H); HRMS (ESI) m/z calcd for $C_{37}H_{53}N_{10}O_{13}$ $[M+H]^+$ 845.3788, found 845.3769.



Synthesis of compound 9: To the solution of 3-aminopropan-1-ol (2.0 g, 26.6 mmol) in CH_2Cl_2 (20 mL) was added Boc_2O (6.97 g, 31.9 mmol) at 0 ºC. After stirring for 2 h at room temperature, the reaction solution was diluted with Sat. $NaHCO_3$ aq., extracted with $CHCl_3$, dried over Na_2SO_4, filtered and concentrated. The crude was purified by silica gel column chromatography (Hex : AcOEt = 2 : 1) to give compound **9** as a clear oil. (4.6g, 98%) The characterization matched with previous report of this known compound[10]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (s, 1H), 3.65 (q, *J* = 5.6 Hz, 2H), 3.28 (q, *J* = 6.3 Hz, 2H), 3.04 (s, 1H), 1.65 (quint., *J* = 5.9 Hz, 2H), 1.44 (s, 9H). HRMS (ESI) m/z calcd for $C_8H_{18}NO_3$ $[M+H]^+$ 176.1287, found 176.1295.

Synthesis of compound 10: To a solution of compound **9** (177 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) was added Dess-Martin periodinane (545 mg, 1.2 mmol) at 0 ºC. After the solution was stirred at room temperature for 3 h, the reaction mixture was diluted with Et_2O, 20% $Na_2S_2O_3$ aq. and Sat. $NaHCO_3$ aq. and extracted with Et_2O twice. The combined organic layer was washed with 20% $Na_2S_2O_3$ aq., Sat. $NaHCO_3$ aq., Brine, dried over Na_2SO_4, filtered and concentrated. The obtained clear oil **10** was used in next reaction without further purification (173.4 mg, 99%). The characterization matched with previous report of this known compound[10]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (s, 1H), 4.98 (s, 1H), 3.42 (q, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 5.8 Hz, 2H), 1.43 (s, 9H).

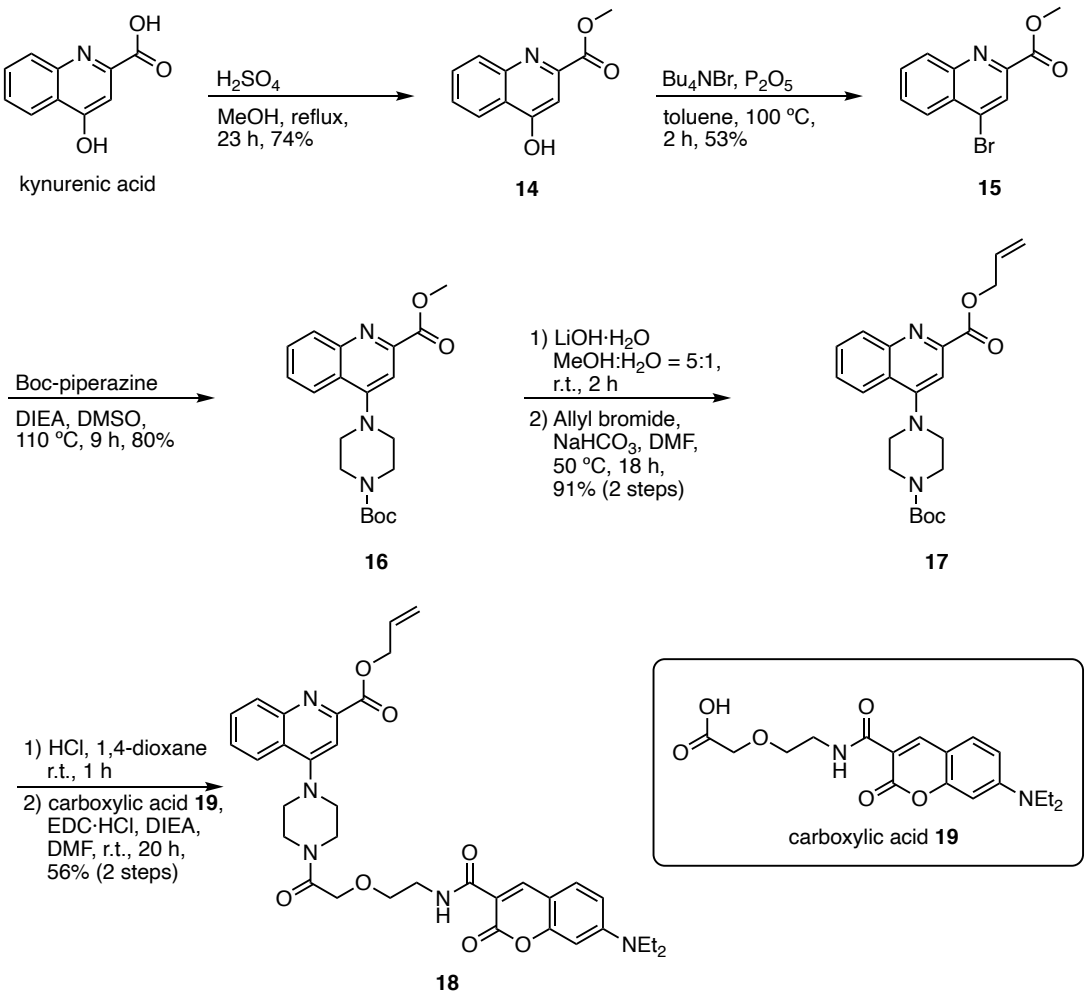
Synthesis of compound 11: To a solution of compound **10** (173.4 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) was added *p*-aminobenzyl alcohol (225.2 mg, 1.3 mmol) and 1 drop of AcOH at 0 ºC. After stirring for 30 min, sodium triacetoxybrohydride (275.5 mg, 1.3 mmol) was added and the reaction mixture was stirred for 19 h. the solution was diluted with AcOEt and washed with Sat. $NaHCO_3$ aq., H_2O twice and Brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by silica gel column chromatography using Smart flash to give compound **11** as brown oil. (187.5 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17–7.11 (m, 2H), 6.62–6.53 (m, 2H), 4.75 (s, 1H), 4.50 (s, 2H), 3.19 (q, *J* = 6.5 Hz, 2H), 3.13 (t, *J* = 6.6 Hz, 2H), 1.72 (quint., *J* = 6.7 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 147.8, 129.7, 128.8, 112.8, 79.3, 65.2, 40.9, 37.9, 29.5, 28.3. HRMS (ESI) m/z calcd for $C_{15}H_{25}N_2O_3$ $[M+H]^+$ 281.1865, found 281.1879.

Synthesis of compound 12: To a solution of compound **11** (294.4 mg, 1.05 mmol) in CH_2Cl_2 (6 mL) was added DIEA (951 μL, 0.21 mmol) and allyl chloroformate (144.3 μL, 1.37 mmol) at 0 ºC. After stirring for 4 h at room temperature, the reaction mixture was poured into H_2O and acidified to around pH3 by adding 1M HCl aq., and then extracted with $CHCl_3$, washed with Brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by silica gel column chromatography (Hex : AcOEt = 2 : 1) to give compound **12** as a clear oil. (267.8 mg, 70%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J*

= 8.2 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.00–5.76 (m, 1H), 5.14 (d, *J* = 9.7 Hz, 2H), 4.71 (d, *J* = 5.9 Hz, 2H), 4.57 (d, *J* = 5.3 Hz, 2H), 3.74 (t, *J* = 6.7 Hz, 2H), 3.17 (q, *J* = 6.4 Hz, 2H), 1.74 (t, *J* = 5.9 Hz, 1H), 1.67 (quint., *J* = 6.7 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.98, 155.63, 140.56, 139.66, 132.54, 127.66, 127.40, 79.08, 66.24, 64.65, 47.57, 37.27, 28.39. HRMS (ESI) m/z calcd for $C_{19}H_{29}N_2O_5$ $[M+H]^+$ 365.2076, found 365.2071.

Synthesis of compound 13: To a solution of compound **12** (50 mg, 0.14 mmol) in CH_2Cl_2 (2.7 mL) was dropwise added *N*,*N*-diethylaminosulfur trifluoride (27.2 µL, 0.21 mmol) at 0 ºC. After stirring for 3 h at 0 ºC, the reaction mixture was poured into Sat $NaHCO_3$ aq. and extracted with $CHCl_3$ twice. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by silica gel column chromatography using Smart flash to give compound **13** as a clear oil. (38.3 mg, 76%) HRMS (ESI) m/z calcd for $C_{19}H_{28}FN_2O_4$ $[M+Na]^+$ 389.1853, found 389.1863. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.35 (m, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 5.92–5.80 (m, 1H), 5.38 (d, *J* = 47.6 Hz, 2H), 5.22–5.12 (m, 2H), 4.58 (d, *J* = 5.3 Hz, 2H), 3.76 (t, *J* = 6.7 Hz, 2H), 3.17 (q, *J* = 6.5 Hz, 2H), 1.67 (quint., *J* = 6.5 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 155.6, 141.8, 134.9 (d, *J* = 68 Hz), 132.6, 128.4 (d, *J* = 24 Hz), 127.6, 117.5, 84.1 (d, J = 664 Hz), 79.2, 66.4, 47.7, 37.4, 28.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ -208.0.

Synthesis of TAMRA-BnF: Compound **13** (16.7 mg, 45.5 µmol) was treated with 4M HCl/1,4-dioxane for 1 h at room temperature. The solution was concentrated under vacuo. Then, the residue was dissolved in DMF (400 µL), and 5(6)-TAMRA succinimidyl ester (20 mg, 38 µmol) and DIEA (, 76 µmol) was added. After stirring 2h at room temperature, the reaction solution was purified by RP-HPLC (5C_{18} AR-300, 20 I.D. x 250 mm) to obtain the title compound **13**. The single isomer with less polar was separately purified as a purple powder (8.0 mg, 26%) ^{1}H NMR (400 MHz, CD_3CN) δ 8.66 (s, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 7.66 (s, 1H), 7.47 – 7.34 (m, 5H), 7.11 (d, *J* = 9.4 Hz, 2H), 6.93 (d, *J* = 9.6 Hz, 2H), 6.84 (s, 2H), 5.88 (s, 1H), 5.40 (d, *J* = 48.0 Hz, 2H), 5.16 (dd, *J* = 22.3, 14.0 Hz, 2H), 4.56 (d, *J* = 5.1 Hz, 2H), 3.82 (t, *J* = 7.2 Hz, 2H), 3.49– 3.41 (m, 2H), 3.24 (s, 12H), 1.87–1.80 (m, 2H), ^{13}C NMR (100 MHz, CD_3CN) δ 175.2, 166.3, 158.1, 156.1, 134.1, 132.0, 131.7, 130.8, 130.0, 129.5 (d, *J* = 23 Hz), 128.7, 117.4, 114.9, 113.8, 97.3, 85.9 (d, *J* = 648 Hz), 66.8, 48.6, 41.2, 37.9, 28.7. ^{19}F NMR (376 MHz, CD_3CN) δ -206.4. HRMS (ESI) m/z calcd for $C_{39}H_{40}FN_4O_6$ $[M+H]^+$ 679.2932, found 679.2930.



Synthesis of compound 14: To the solution of kynurenic acid (2 g, 10.6 mmol) in dry MeOH (20 mL) under N_2 atmosphere was added H_2SO_4 (1.2 mL) at room temperature. After stirring for 23 h under reflux condition, the reaction mixture was concentrated and neutralized with Sat. $NaHCO_3$ aq. at 0 ºC. The residue was purified by silica-gel column chromatography ($CHCl_3$: MeOH = 10 : 1) to obtain the desired compound as a white solid (1.6g, 74%). The characterization matched with previous report of this known compound[11]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.10 (s, 1H), 8.12–8.00 (m, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.71 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.38 (t, J = 7.6 Hz, 1H), 6.63 (s, 1H), 3.96 (s, 3H), HRMS (ESI) m/z calcd for $C_{11}H_{10}NO_3$ $[M+H]^+$, 204.0661 found 204.0644.

Synthesis of compound 15: To the solution of compound **14** (1.6 g, 7.87 mmol) in anhydrous toluene (40 mL), P_2O_5 (6.7 g, 47.2 mmol) and Bu_4NBr (6.08 g, 18.9 mmol) was added under N_2 atmosphere. After stirring for 2 h at 100 ºC, the reaction solution was cooled to room temperature and toluene layer was separated from brown precipitate. Then, toluene and H_2O were added to the residual precipitate, vigorously stirred and extracted with toluene. The combined organic layer was concentrated and purified by silica-gel column chromatography (Hex : AcOEt = 2 : 1) to obtain the

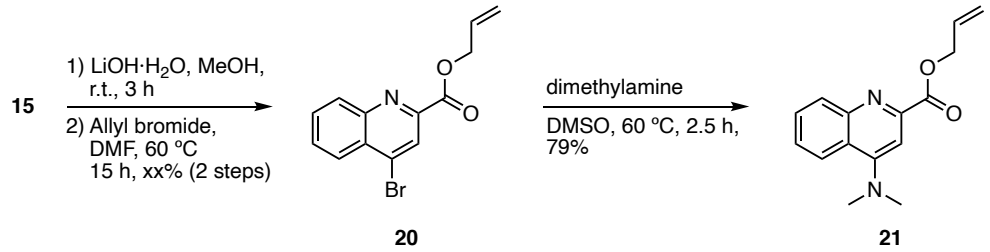
desired compound **15** as a white solid (1.1 g, 53%). The characterization matched with previous report of this known compound[11]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H), 8.25-8.18 (m, 2H), 8.02-7.95 (m, 1H), 7.95-7.85 (m, 1H), 3.97 (s, 3H). HRMS (ESI) m/z calcd for $C_{11}H_9BrNO_2$ $[M+H]^+$, 265.9817, found 265.9814.

Synthesis of compound 16: To the solution of compound **15** (500 mg, 1.88 mmol) in DMSO (4 mL) was added Boc-piperazine (700 mg, 3.76 mmol) and DIEA (655 μL, 3.76 mmol) at room temperature. After stirring 9 h at 110 ºC, the reaction was cooled to room temperature. The reaction mixture was diluted with H_2O, extracted with AcOEt thrice, washed with H_2O and Brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica-gel column chromatography (Hex : AcOEt = 5 :1 to 3 : 1) to obtain the desired compound **16** as a yellow oil. (555 mg, 80%). The characterization matched with previous report of this known compound[11]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 8.5, 1.1 Hz, 1H), 8.04 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.74 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.68 (s, 1H), 7.60 (ddd, *J* = 8.2, 6.6, 1.3 Hz, 1H), 4.07 (s, 3H), 3.77–3.70 (m, 4H), 3.29–3.22 (m, 4H), 1.51 (s, 9H), HRMS (ESI) m/z calcd for $C_{20}H_{26}N_3O_{34}$ $[M+H]^+$, 372.1923 found 372.1922.

Synthesis of compound 17: To the solution of compound **16** (530 mg, 1.43 mmol) in MeOH (5 mL) was added $LiOH·H_2O$ (240 mg, 5.71 mmol) in H_2O (1 mL) at 0 ºC to hydrolyze the methyl ester. After stirring 2 h at room temperature, the solvent was removed under reduced pressure. The residue was dissolved in DMF (15 mL), then allyl bromide (363 μL, 4.29 mmol) and $NaHCO_3$ (264 mg, 3.15 mmol) was added to the reaction mixture at room temperature. After stirring 18 h at 50 ºC, the reaction mixture was diluted with H_2O, extracted with AcOEt, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica-gel column chromatography to obtain the desired compound **17** as a yellow solid (516 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.3 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.73– 7.65 (m, 1H), 7.63 (s, 1H), 7.61–7.51 (m, 1H), 6.16–6.02 (m, 1H), 5.48–5.39 (m, 1H), 5.30 (d, *J* = 10.4 Hz, 1H), 4.94 (d, *J* = 6.0 Hz, 2H), 3.74–3.67 (m, 4H), 3.25–3.18 (m, 4H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.38, 157.70, 154.60, 148.93, 148.42, 131.78, 131.43, 129.72, 127.35, 124.19, 123.20, 119.14, 108.90, 80.08, 66.70, 51.96, 28.31. HRMS (ESI) m/z calcd for $C_{22}H_{28}N_3O_4$ $[M+H]^+$ 398.2080, found 398.2069.

Synthesis of compound 18: To the compound **17** (45 mg, 0.12 mmol) was added 4M HCl/1,4-dioxane (1 mL) at 0 ºC. After stirring at room temperature for 1 h, solvent was removed under reduced pressure. The residue was dissolved in DMF (1 mL) and condensate with carboxylic acid **19**[12] (50 mg, 0.14 mmol) with EDC·HCl (26.5 mg, 0.14 mmol) and DIEA (60.1 μL, 0.34 mmol). After stirring for 20 h, the reaction solution was diluted with H_2O and extracted with AcOEt, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica-gel column chromatography to

obtain the compound **18** as a yellow solid (43.0 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (t, *J* = 5.1 Hz, 1H), 8.69 (s, 1H), 8.25 (dd, *J* = 8.7, 1.3 Hz, 1H), 8.04 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.74 – 7.67 (m, 1H), 7.66 (s, 1H), 7.63 – 7.54 (m, 1H), 7.39 (d, *J* = 8.9 Hz, 1H), 6.62 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.42 (d, *J* = 2.6 Hz, 1H), 6.19 – 6.04 (m, 1H), 5.51 – 5.41 (m, 1H), 5.38 – 5.29 (m, 1H), 5.03 – 4.93 (m, 2H), 4.30 (s, 2H), 3.92 (t, J = 4.8 Hz, 4H), 3.78 – 3.68 (m, 4H), 3.45 (q, *J* = 7.2 Hz, 4H), 3.35 – 3.25 (m, 4H), 1.23 (t, J = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 165.4, 163.3, 162.6, 157.6, 157.4, 152.6, 148.97, 148.50, 148.2, 131.8, 131.5, 131.1, 129.7, 127.5, 124.2, 123.3, 119.3, 109.9, 109.1, 108.3, 96.5, 71.2, 70.2, 66.8, 52.4, 51.9, 45.4, 45.1, 41.8, 39.3, 12.4. HRMS (ESI) m/z calcd for $C_{35}H_{40}N_5O_7$ $[M+H]^+$ 642.2928, found 642.2924. .



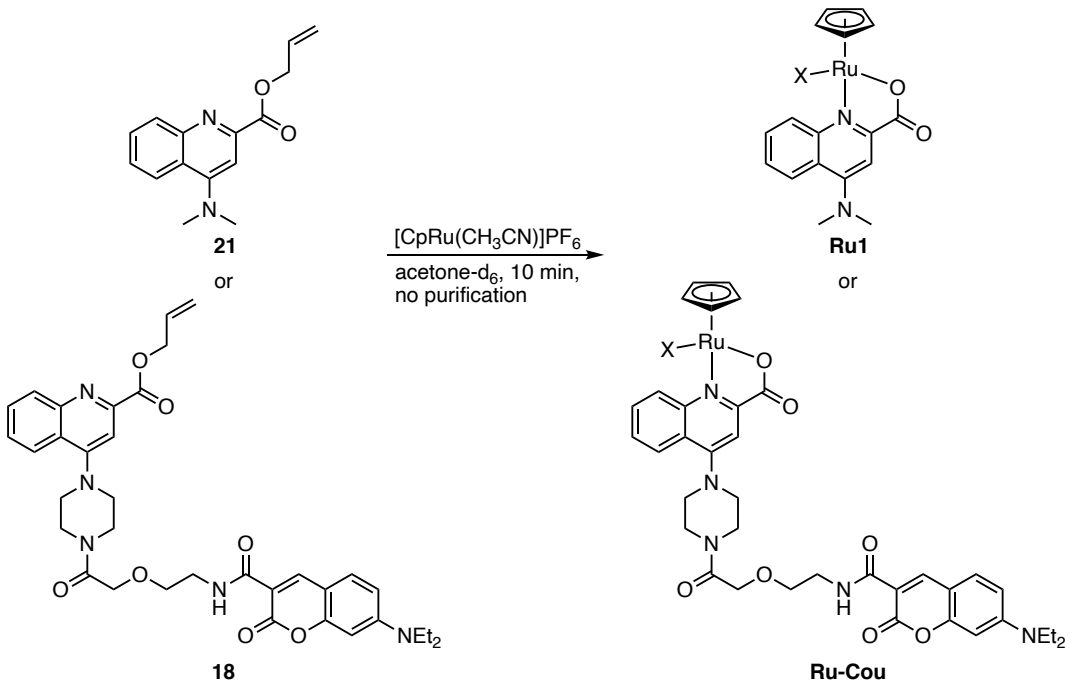
20 **21**

Synthesis of compound 20: To the solution of compound **15** (200 mg, 0.75 mmol) in MeOH (4 mL) was added $LiOH·H_2O$ (63.1 mg, 1.50 mmol) at 0 ºC. After stirring 3 h at room temperature, the solvent was removed under reduced pressure. Then, the residue was dissolved in DMF (4 mL), and allyl bromide (191 µL, 2.3 mmol) was added at room temperature. After stirring for 15 h at 60 ºC, the reaction solution was diluted with H_2O and AcOEt, and then extracted with AcOEt, washed with Sat. $NaHCO_3$ aq., washed with Brine, dried over Na_2SO_4, filtered and concentrated. The residual white solid was used in next reaction without further purification. (163 mg, 74%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.31 (dd, *J* = 8.4, 1.3 Hz, 1H), 8.21 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.83 (ddd, *J* = 8.5, 6.8, 1.5 Hz, 1H), 7.73 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 6.13 (ddt, *J* = 16.5, 10.3, 5.9 Hz, 1H), 5.61 – 5.28 (m, 2H), 5.00 (dt, *J* = 5.9, 1.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) 164.0, 148.1, 147.6, 135.2, 131.6, 131.3, 131.1, 130.0, 128.9, 126.7, 125.1, 119.6, 67.1, HRMS (ESI) m/z calcd for $C_{13}H_{11}BrNO_2$ $[M+H]^+$ 291.9973, found 291.9982.

Synthesis of compound 21: To the solution of compound **20** (100 mg, 3.42 mmol) in DMSO (1.5 mL) was added dimethyl amine in H_2O (360 µL, 3.42 mmol) at room temperature. After stirring for 2.5 h at 60 ºC, the reaction solution was diluted with AcOEt. The organic layer was washed with H_2O and Brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica-gel column chromatography to obtain the compound **21** as a white solid. (68.9 mg, 79%). The characterization matched with previous report of this known compound[4]. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.19 (dd, *J* = 8.3, 1.6 Hz, 1H), 8.06 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.75 (ddd, *J* = 8.6, 6.9, 1.6 Hz, 1H), 7.61 (ddd, *J* = 8.2, 6.7, 1.4 Hz, 1H), 7.52 (s, 1H), 6.20–6.07 (m, 1H), 5.54–5.45 (m, 1H),

5.34–5.26 (m, 1H), 4.93–4.87 (m, 2H), 3.13 (s, 6H). HRMS (ESI) m/z calcd for $C_{15}H_{17}N_2O_2$ $[M+H]^+$ 257.1290, found 257.1285.

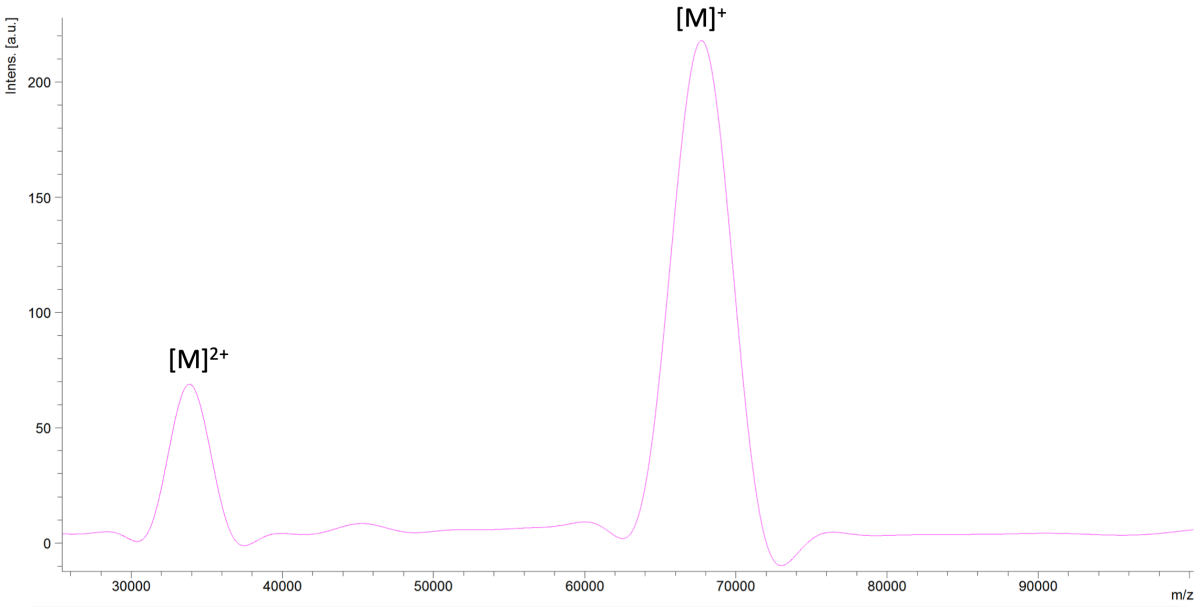
Preparation of Ru catalysts: The solution of compound **18** or **21** (1.8 μmol) in acetone-d_6 (600 μL) was degassed by freeze-pump-thaw cycling. Then, $[CpRu(CH_3CN)_3]PF_6$ (2.2 μmol) was added at room temperature. After 10 min, the catalyst was concentrated with argon flow.



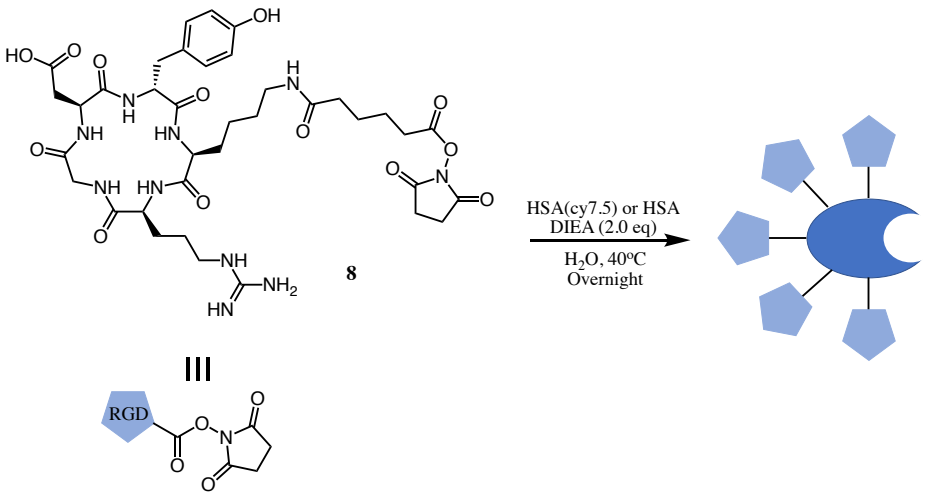
Ru1[4]: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.36–8.30 (m, 1H), 7.96–7.88 (m, 2H), 7.69 (ddd, J = 8.4, 6.2, 1.9 Hz, 1H), 7.29 (s, 1H), 6.43 (s, 5H), 4.80 (d, J = 10.6 Hz, 1H), 4.70 (tt, J = 10.7, 6.2 Hz, 1H), 4.58 (d, J = 10.9 Hz, 1H), 4.29 (dd, J = 6.2, 2.9 Hz, 1H), 4.20 (dd, J = 6.1, 2.8 Hz, 1H), 3.38 (s, 6H). HRMS (ESI) m/z calcd for $C_{20}H_{21}N_2O_2Ru$ [M (X = C_3H_5)]$^+$ 423.0647, found 423.0659.

Ru-Cou: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.90 (s, 1H), 8.67 (s, 1H), 8.35 – 8.28 (m, 1H), 8.10 – 8.05 (m, 1H), 8.04 – 7.98 (m, 1H), 7.83 – 7.78 (m, 1H), 7.64 (d, J = 9.0 Hz, 1H), 7.53 (s, 1H), 6.88 – 6.84 (m, 1H), 6.56 (d, J = 9.0 Hz, 1H), 6.50 – 6.47 (m, 5H), 4.93 – 4.61 (m, 3H), 4.39 – 4.32 (m, 1H), 4.30 (s, 1H), 4.29 – 4.25 (m, 1H), 4.04 – 3.81 (m, 4H), 3.79 – 3.67 (m, 4H), 3.67 – 3.52 (m, 8H) 1.28 – 1.20 (m, 6H). ^{13}C NMR (100 MHz, DMSO) δ 172.4, 167.5, 162.3, 161.8, 157.4, 157.2, 153.2, 152.5, 149.2, 147.8, 139.0, 131.6, 130.5, 127.4, 124.3, 123.7, 113.3, 110.1, 109.5, 109.2, 107.7, 95.9, 95.7, 86.0, 85.6, 84.0, 80.3, 72.4, 69.5, 69.3, 61.8, 51.5, 44.3, 41.0, 12.3. HRMS (ESI) m/z calcd for $C_{37}H_{40}N_5O_7Ru$ $[M–X+H]^+$ 768.1971, found 768.1977.

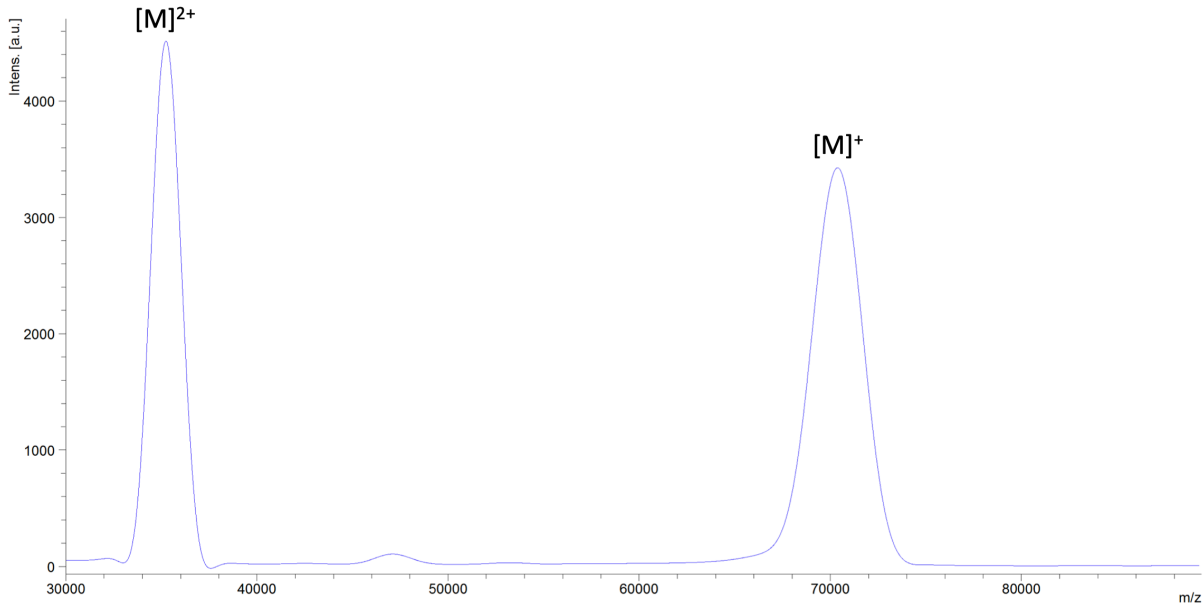
Preparation of Cy7.5-labelled HSA (HSA(cy7.5)): A 150 µL solution of 100 µM HSA in PBS was diluted to 474.9 µl with PBS and added 25.1 ml solution of 17.9 mM of cy7.5-NHS solution in DMSO to give final solution 5% DMSO. The solution mixture was incubated at 37°C for overnight and then purified by centrifugation using Amicon 10K at 14,000 rpm for 10 min. After 3 times washed with ultrapure water by the same centrifugation manner, the solution was diluted to 450 µl in ultrapure water to give final concentration 100 µM of HSA(cy7.5). MALDI-TOF-MS detected the molecular weight of HSA(cy7.5) at 68 kDa indicating 2 molecules cy7.5 was attached on the surface of HSA.



MALDI-TOF mass spectrum of HSA(cy7.5).



Preparation of HSA(*c*RGD): To a 400 µl of 1.5 mM (1.0 eq) HSA solution in PBS was added 200 µl of 40 mM of *c*RGD peptide **8** (13.3 eq.) in DMSO, 2.4 µL of 5.7 mM DIEA (3.4 eq.) and PBS to give 5% DMSO in 4 mL final solution. The mixture solution was incubated at 40 ºC for overnight. The HSA(*c*RGD) was purified by using Amicon 10K by centrifugation at 14,000 rpm for 10 min. The residue was washed with ultrapure water and centrifuged for 3 times. The resulting solution was diluted in ultrapure water up to 400 µl to afford 1.5 mM solution of HSA(*c*RGD). MALDI-TOF-MS (positive mode) detected the molecular weight of HSA(*c*RGD) at 70.0 kDa, which indicated that 5 molecules of *c*RGD peptide was attached to albumin on an average.

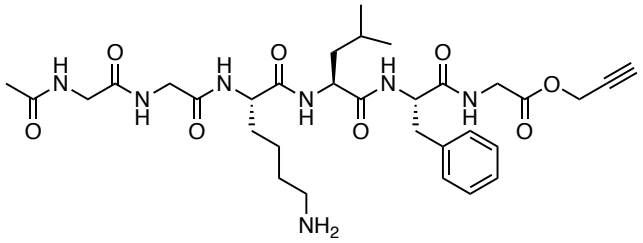


MALDI-TOF mass spectrum of HSA(*c*RGD).

Preparation of HSA(*c*RGD)-Au: a 20 µL solution of 37.5 mM of known compound **Au-Cou**, which is synthesized by same procedure with previous report[2], was added to the solution mixture of 300 µl of 500 µM of HSA(*c*RGD) and 80 µl of ultrapure water. The solution mixture was incubated at 37 ºC for overnight and then purified by centrifugation using Amicon 10K at 14,000 g for 10 min and washed three times by ultrapure water. The resulting solution was diluted to 200 µl in dH_2O to give stock solution with final concentration 750 µM of HSA(*c*RGD)-Au.

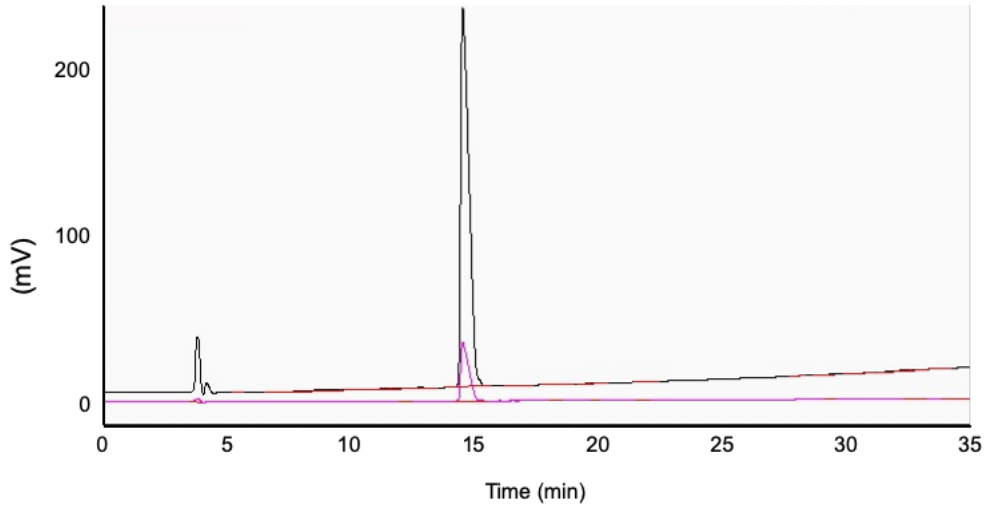
Preparation of HSA(*c*RGD)-Ru: a 7.5 µL solution of 60.0 mM of **Ru-Cou** was added to the 150 µL aqueous solution of 1.5 mM of HSA(*c*RGD). The solution mixture was incubated at 37 ºC for 1 h and then purified by centrifugation using Amicon 10K at 14,000 g for 10 min and washed three times by

ultrapure water. The resulting solution was diluted to 112.5 μL in dH_2O to give stock solution with a final concentration 2 mM of complex HSA(*c*RGD)-Ru.



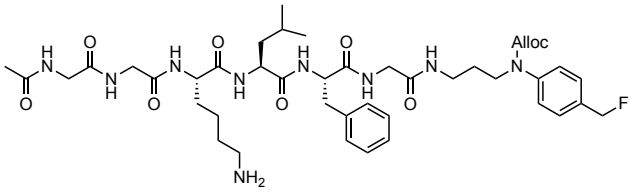
Synthesis of peptide 22: A 218 mg H-Gly-Trt(2-Cl) resin (0.2 mmol) was swollen by dipping it in 3 mL DMF for 30 min in 6 mL reservoir. To the swollen-resin was added Fmoc-Phe-OH (310 mg, 0.8 mmol), HCTU (331 mg, 0.8 mmol), HOBt(6-Cl) (136 mg, 0.8 mmol) in 2 mL DMF and DIEA (204 μL, 1.2 mmol). The suspension mixture was shaken for 30 min at room temperature. The coupling reaction was monitored by KISER test reagent for detection of amino group. The suspension mixture was then washed with DMF (1 min x 3) and treat with 20% piperidine in 2 mL DMF in 2 min for three times to deprotect Fmoc moiety. The peptide elongation and Fmoc deprotection reaction were carried out manually by treating the swollen-resin with Fmoc amino acid/HCTU/HOBt(6-Cl)/DIEA (4/4/4/6 eq) in 2 mL DMF for 30 min and 20% piperidine in DMF (2 min x 3), respectively. After peptide elongation of 3 amino acids, *N*-Acetyl amino acid was used as *N*-terminus residue of peptide. The treated resin was treated with 20% HFIP/$CHCl_3$ for 2 hours at room temperature to give peptide-OH. The resulted-peptide was precipitated with cold diethyl ether and dried *in vacuo* to obtain crude of peptide **22** (Ac-GGK(Boc)LFG-OH)**.**

Synthesis of Ac-GGKLFG-PE (peptide 1): To a stirred solution of the crude peptide **22** (5 mg, 0.0076 mmol) in 0.5 mL DMF was added K_2CO_3 and propargyl bromide at room temperature. After stirring for 1 d, DMF was removed *in vacuo* and the crude was extracted with $CHCl_3$ and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give propargyl ester. To a solution of propargyl ester (5 mg, 0.0076 mmol) in 0.2 mL TFA was stirred for 1 h at room temperature to deprotect the peptide. The mixture was then dried *in vacuo* and purified by RP-HPLC column ($5C_{18}$-AR-300, 4.6 I.D. x 250 mm) at a flow rate of 1 mL/min with a linear gradient of MeCN in H_2O containing 0.1%TFA (0-80% for 40 min, 80-100% for 5 min) to obtain Ac-Gly-Gly-Lys-Leu-Phe-Gly-OPropargyl. ESI-HRMS *m/z* calcd for $C_{32}H_{48}N_7O_8$ ($[M+H]^+$) 658.3559, found 658.3570.

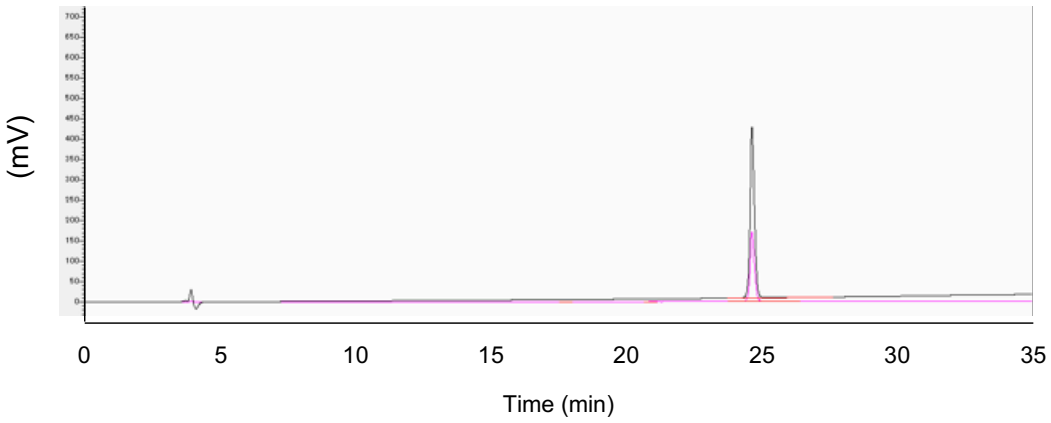


Reverse-phase HPLC chromatogram of purified peptide **1** with a linear gradient of 0.1 % TFA/CH_3CN (20-50%, 30 min) in 0.1 % TFA/H_2O at a flow rate of 1.0 mL/min, detected by UV absorption at 230 (black) and 254 nm (pink).

Synthesis of Ac-GGKLFG-BnF (peptide 3)



To a stirred solution of the crude peptide **22** (Ac-Gly-Gly-Lys(Boc)-Leu-Phe-Gly-OH, 0.2 mmol) in 2 mL DMF was added HOBt(6-Cl) (44.1 mg, 0.26 mmol), EDC (56.5 uL, 0.32 mmol) and DIEA (34.8 μL, 0.2 mmol) at room temperature. After stirring for 5 min, amine compound (95 mg, 0.26 umol), which is obtained from compound **13** by treating with 4 M HCl/1,4-dioxane for 1 h, in DMF was added to the mixture. After stirring overnight, the reaction solution was diluted with $CHCl_3$ and washed with 0.5 M HCl aq., dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The residue was dissolved in 4 M HCl/1,4-dioxane at 0 ºC and stirred for 30 min at room temperature to deprotect the Boc group. The reaction mixture was then concentrated *in vacuo* and purified by RP-HPLC ($5C_{18}$-AR-300, 20 I.D. x 250 mm) at a flow rate of 9 mL/min with a linear gradient of MeCN in H_2O containing 0.1%TFA (0-80% for 40 min, 80-100% for 5 min) to obtain peptide **3** as a white powder (45.7 mg, 23% from resin). HRMS (ESI) *m/z* calcd for $C_{43}H_{63}FN_9O_9$ $[M+H]^+$ 868.4733, found 868.4751.



Reverse-phase HPLC chromatogram of purified peptide **3** with a linear gradient of 0.1 % TFA/CH_3CN (20-50%, 30 min) in 0.1 % TFA/H_2O at a flow rate of 1.0 mL/min, detected by UV absorption at 230 (black) and 254 nm (pink).

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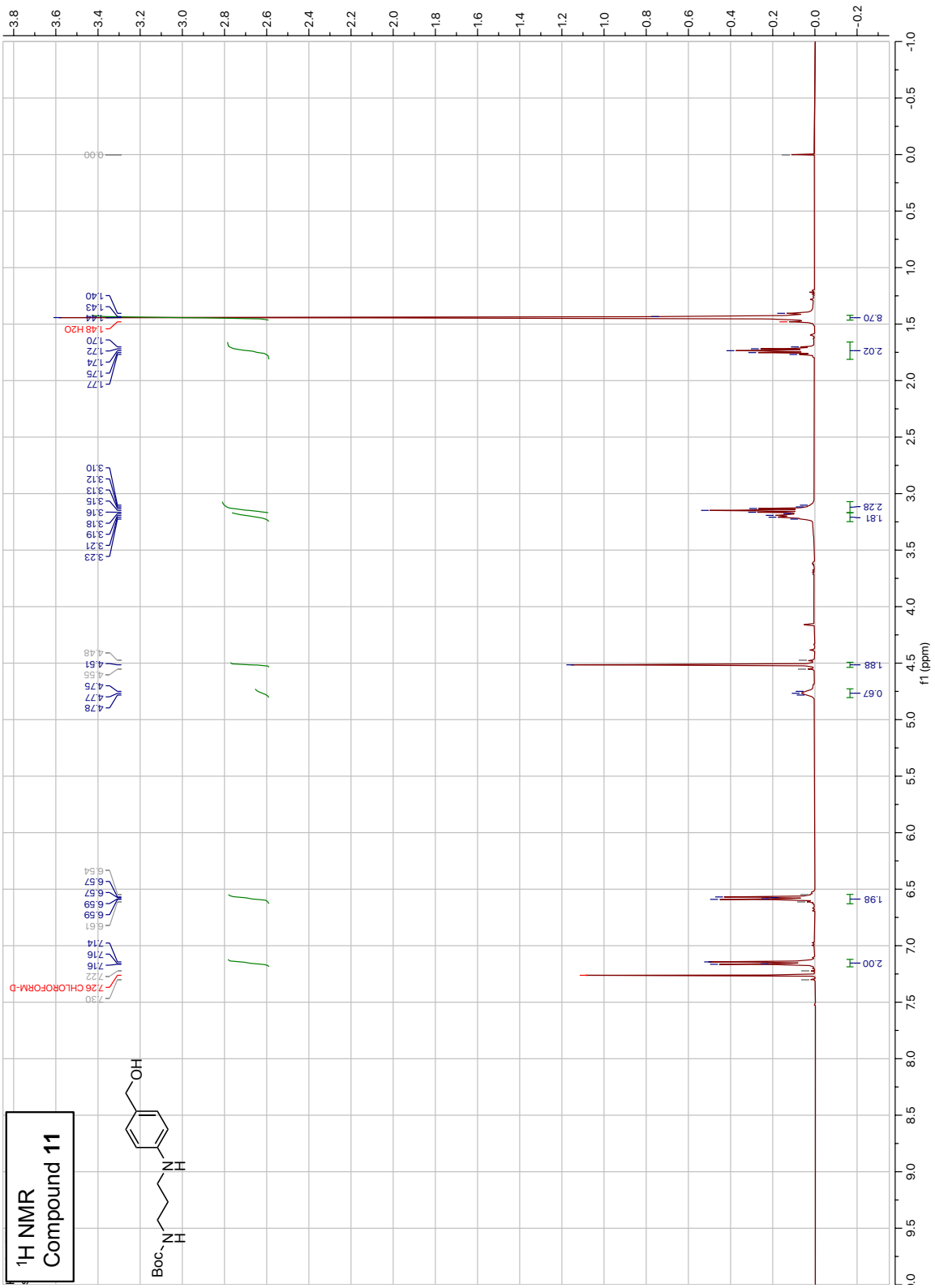
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Compound **11**
Boc
HO
f1 (ppm)
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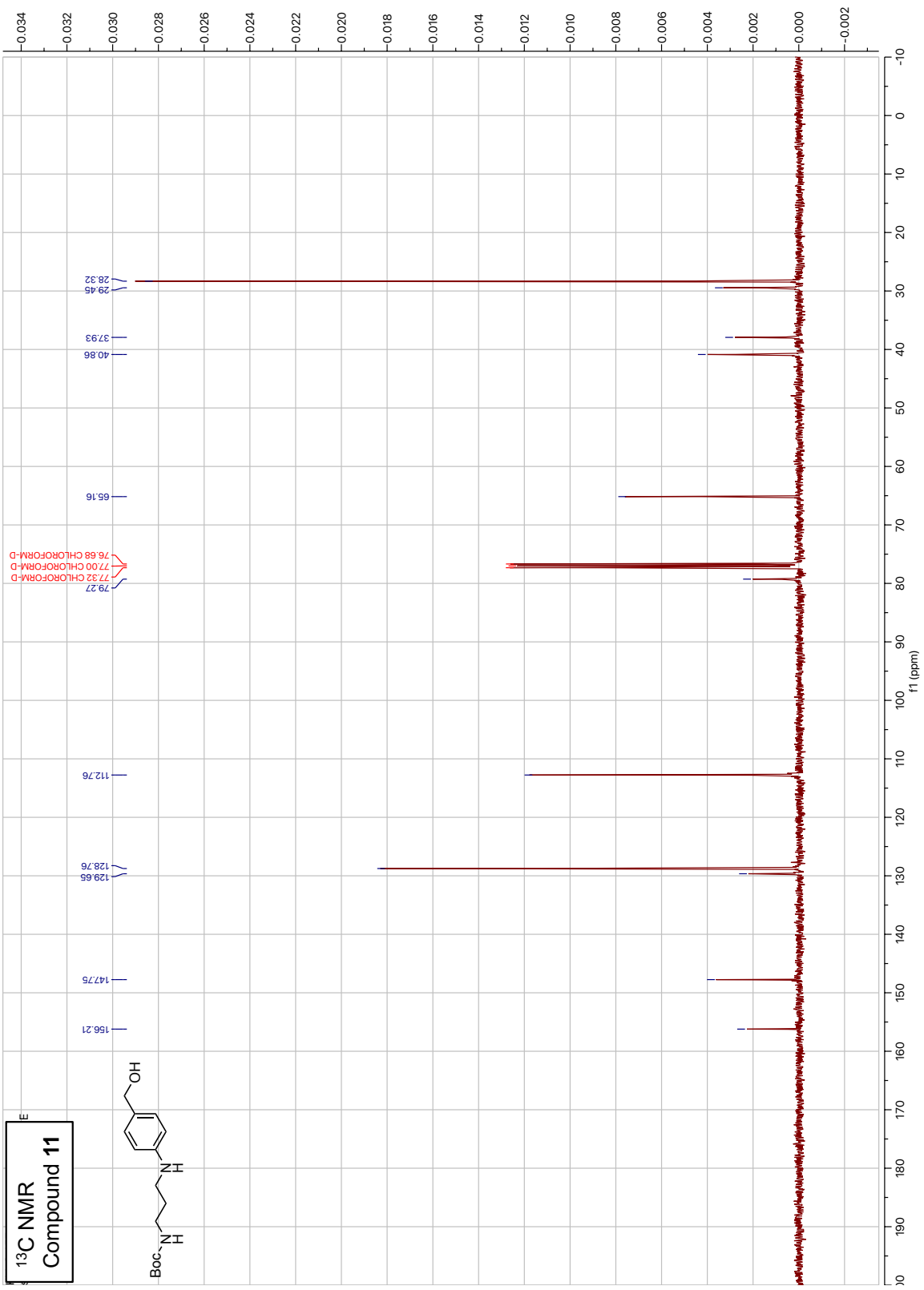
8.70
2.02
1.81
2.28
1.88
0.67
1.98
2.00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Compound 11
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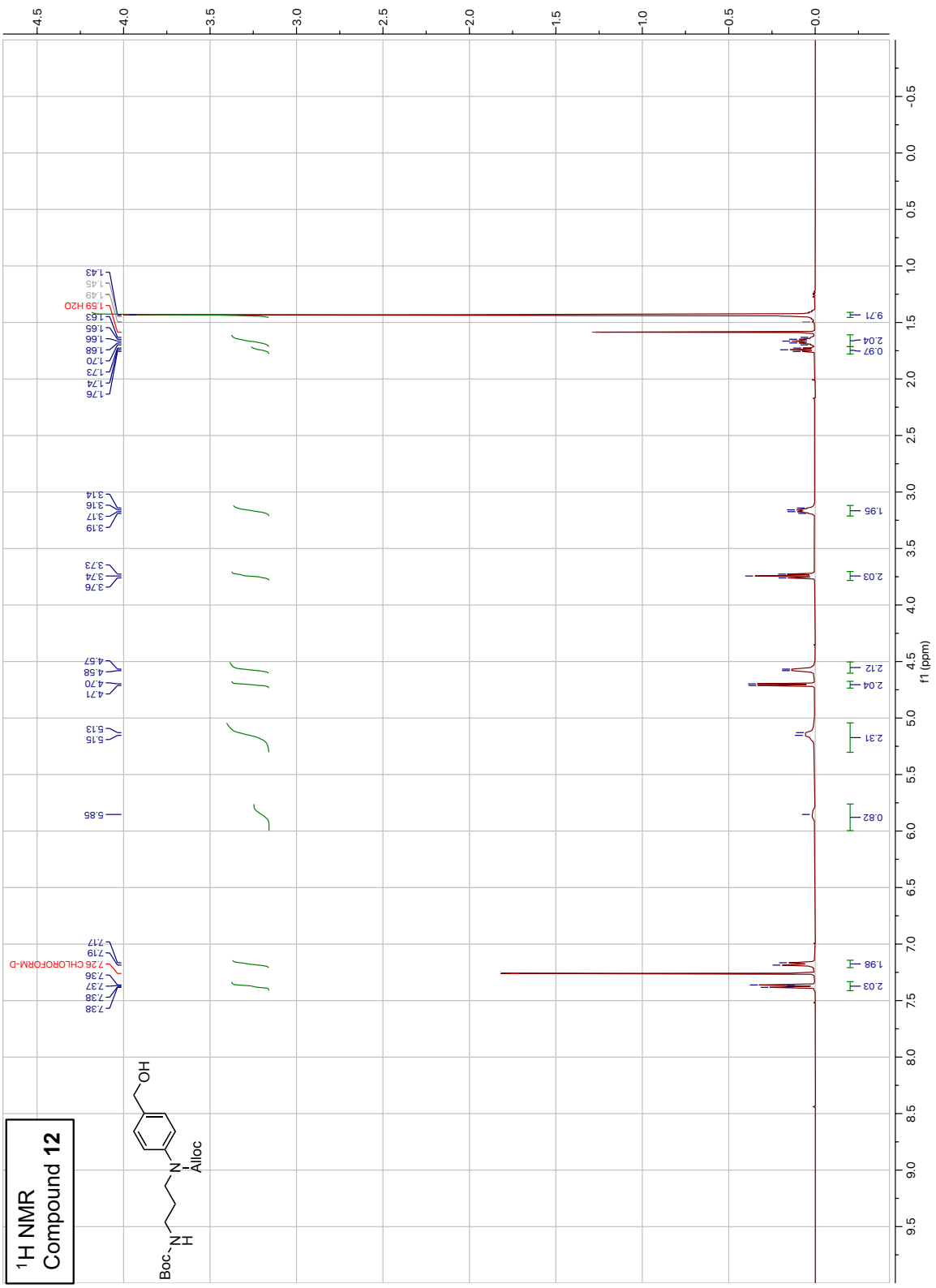
29.45
37.93
40.86
65.16
76.68 CHLOROFORM-D
77.00 CHLOROFORM-D
77.32 CHLOROFORM-D
79.27
112.76
128.76
129.65
147.75
156.21
Boc
HO
f1 (ppm)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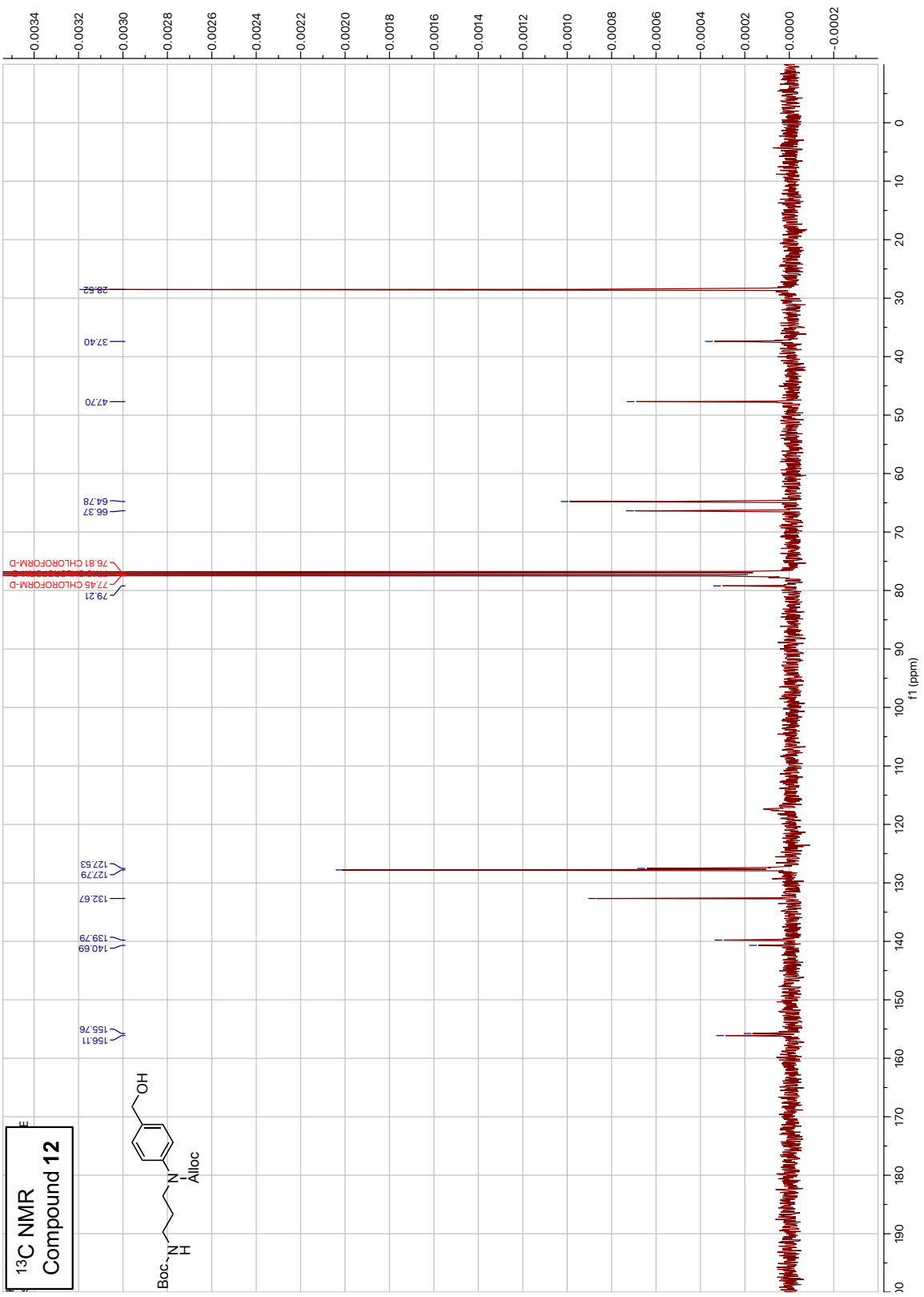
Compound 12
Boc
N
H
N
Alloc
OH
f1 (ppm)
CHLOROFORM-D
H2O


13C NMR
Compound 12
Boc
N
H
N
Alloc
OH
156.11
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139.79
132.67
127.79
127.53
79.21
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76.81 CHLOROFORM-D
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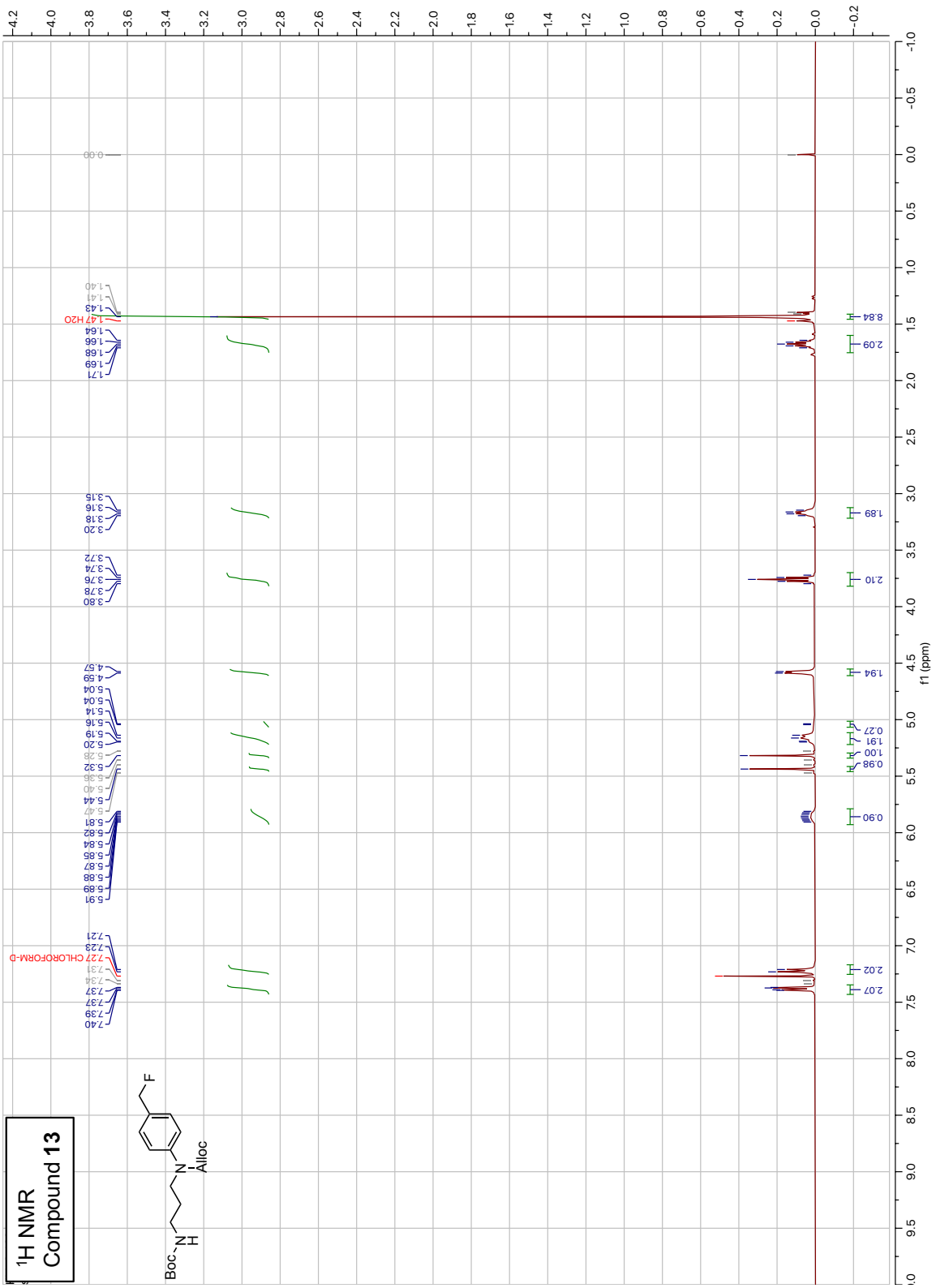
47.70
37.40
28.62
f1 (ppm)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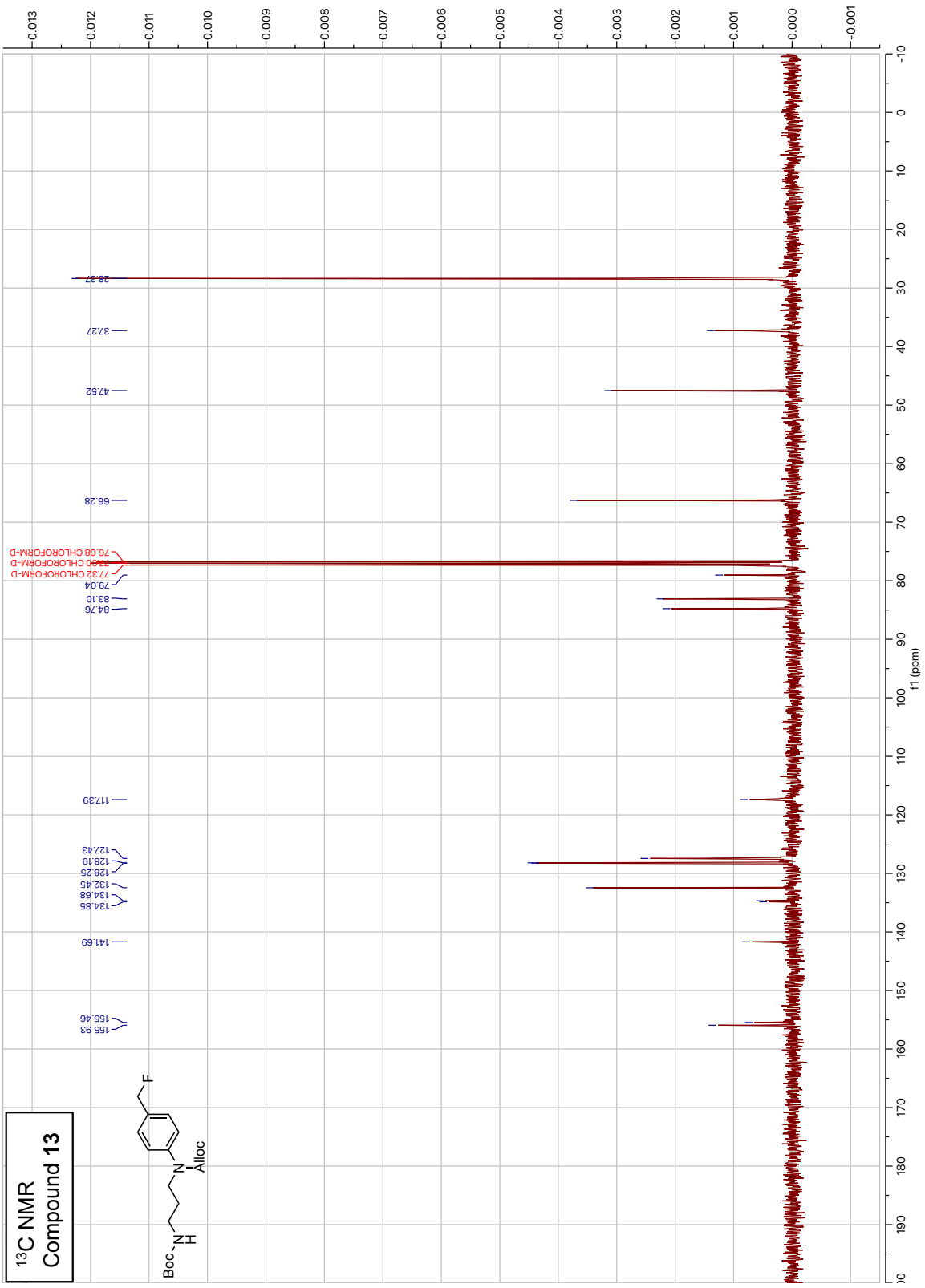
Compound **13**
Boc
N
H
N
Alloc
F
CHLOROFORM-D
f1 (ppm)


13C NMR
Compound 13
Boc
N
H
N
Alloc
F
28.37
37.27
47.52
66.28
76.68 CHLOROFORM-D
77.00 CHLOROFORM-D
77.32 CHLOROFORM-D
79.04
83.10
84.76
117.39
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128.19
128.25
132.45
134.68
134.85
141.69
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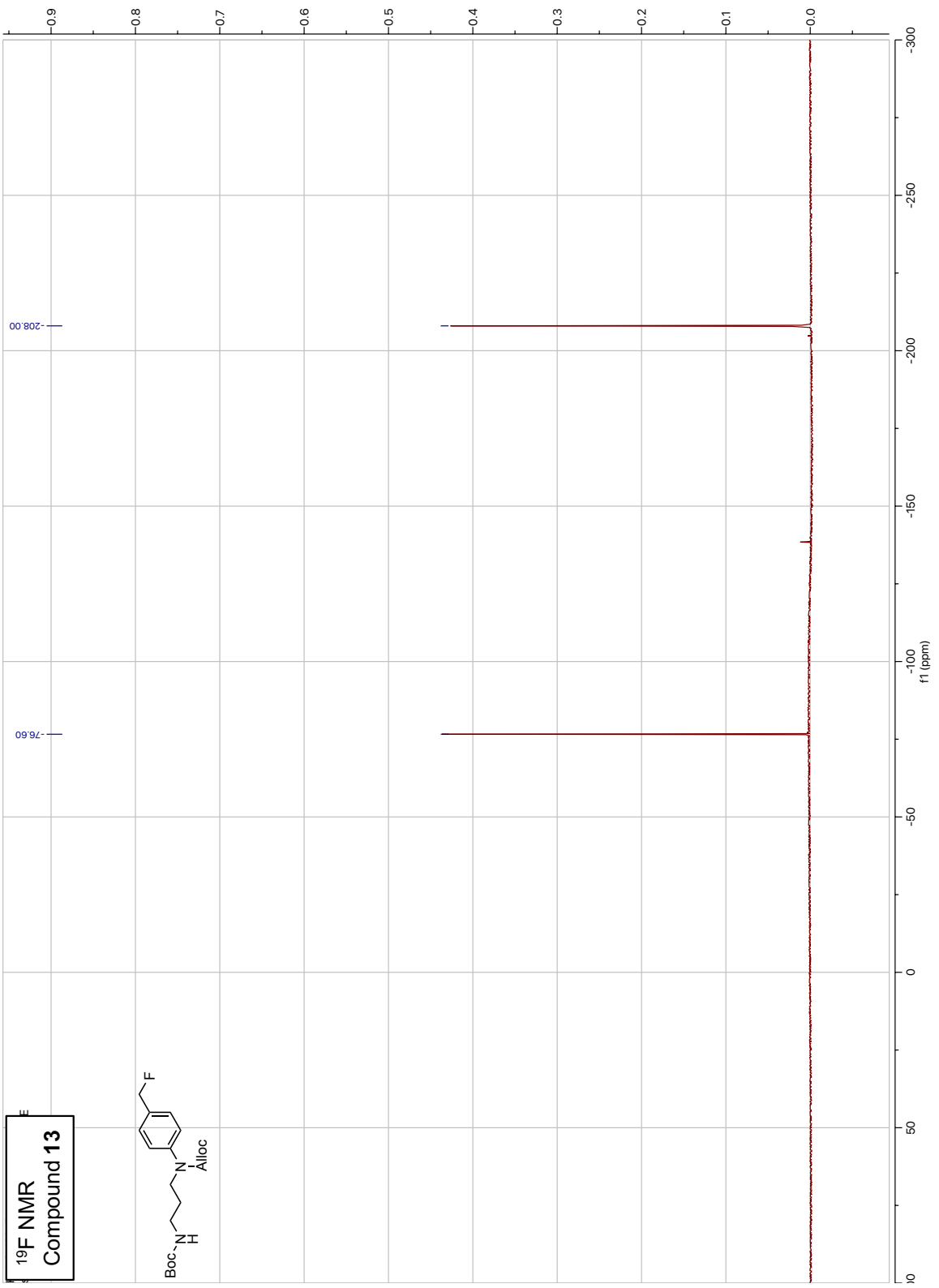
155.46
f1 (ppm)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F
N
Alloc
Boc
N
H
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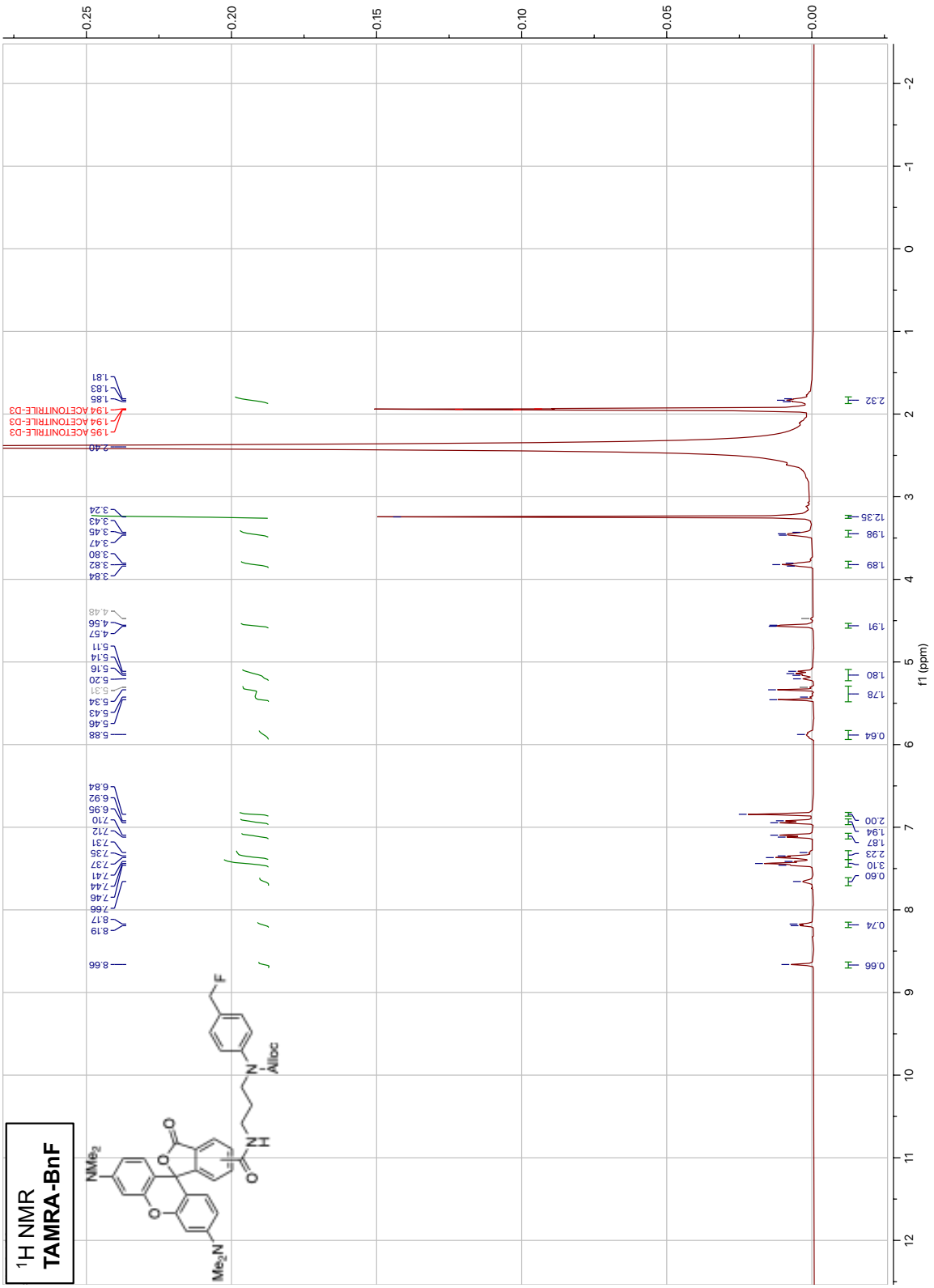
-76.60
f1 (ppm)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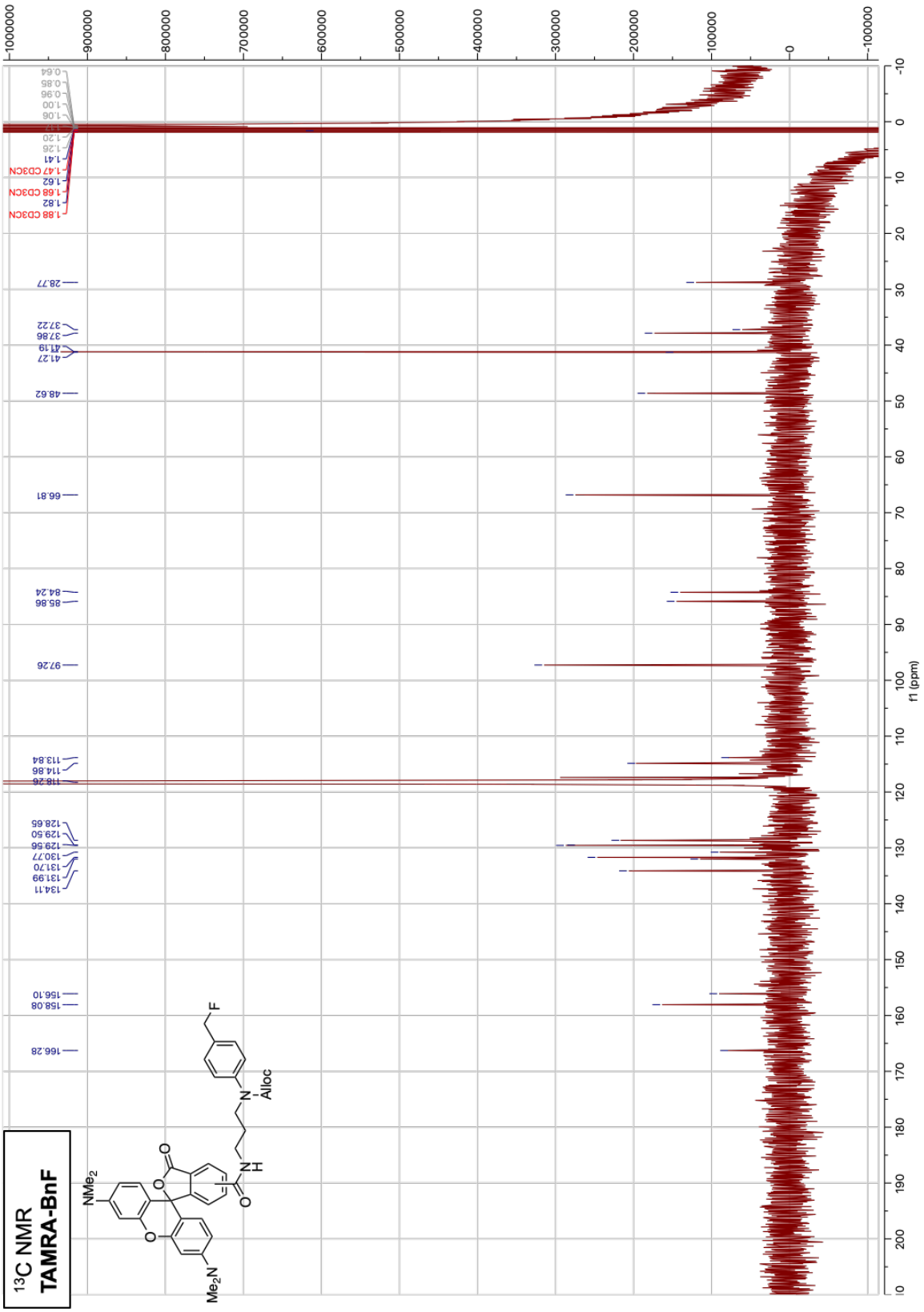
TAMRA-BnF
NMe2
Me2N
Alloc
F
f1 (ppm)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TAMRA-BnF
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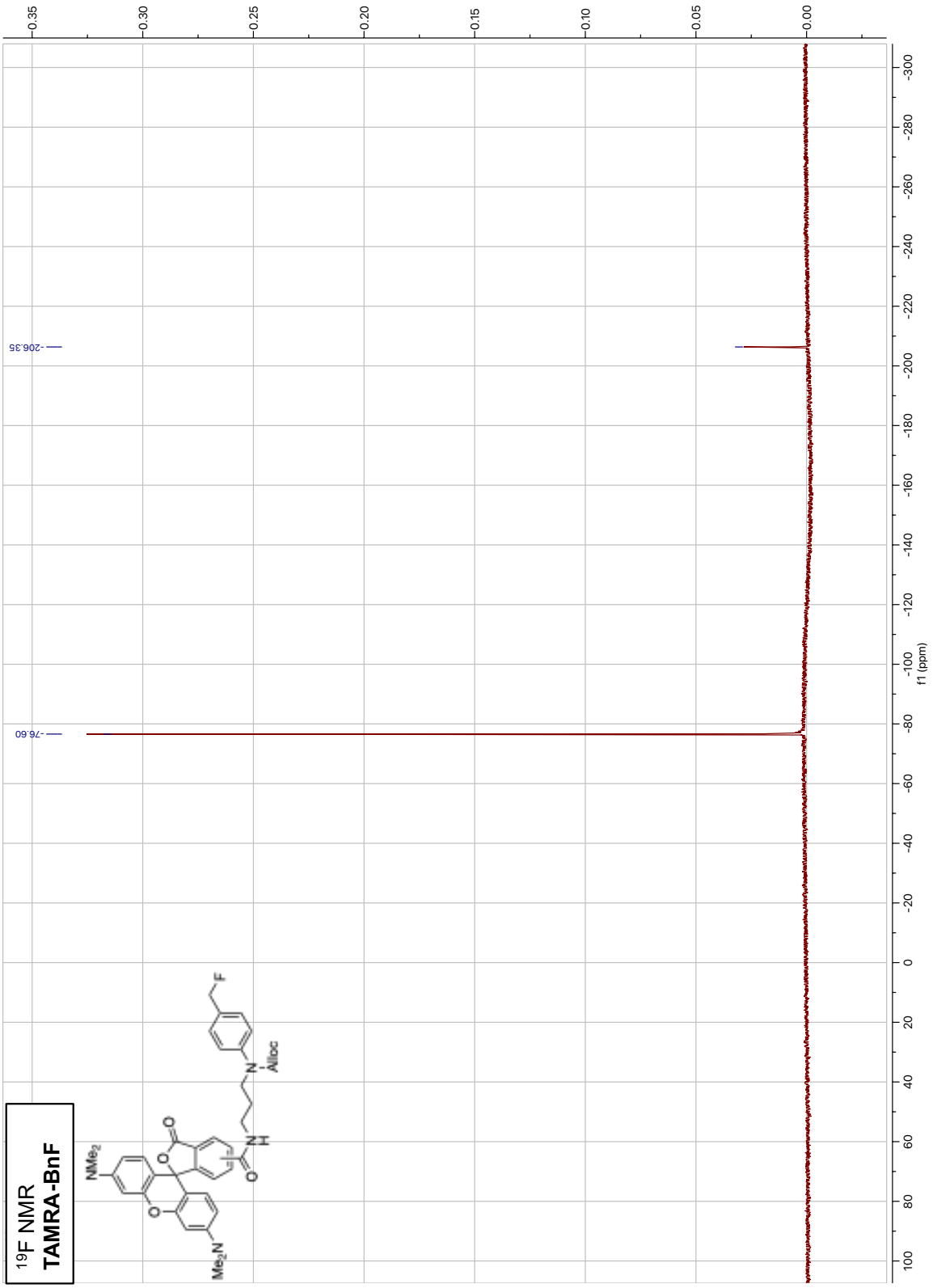
Me2N
F
N-Alloc
NH
O
f1 (ppm)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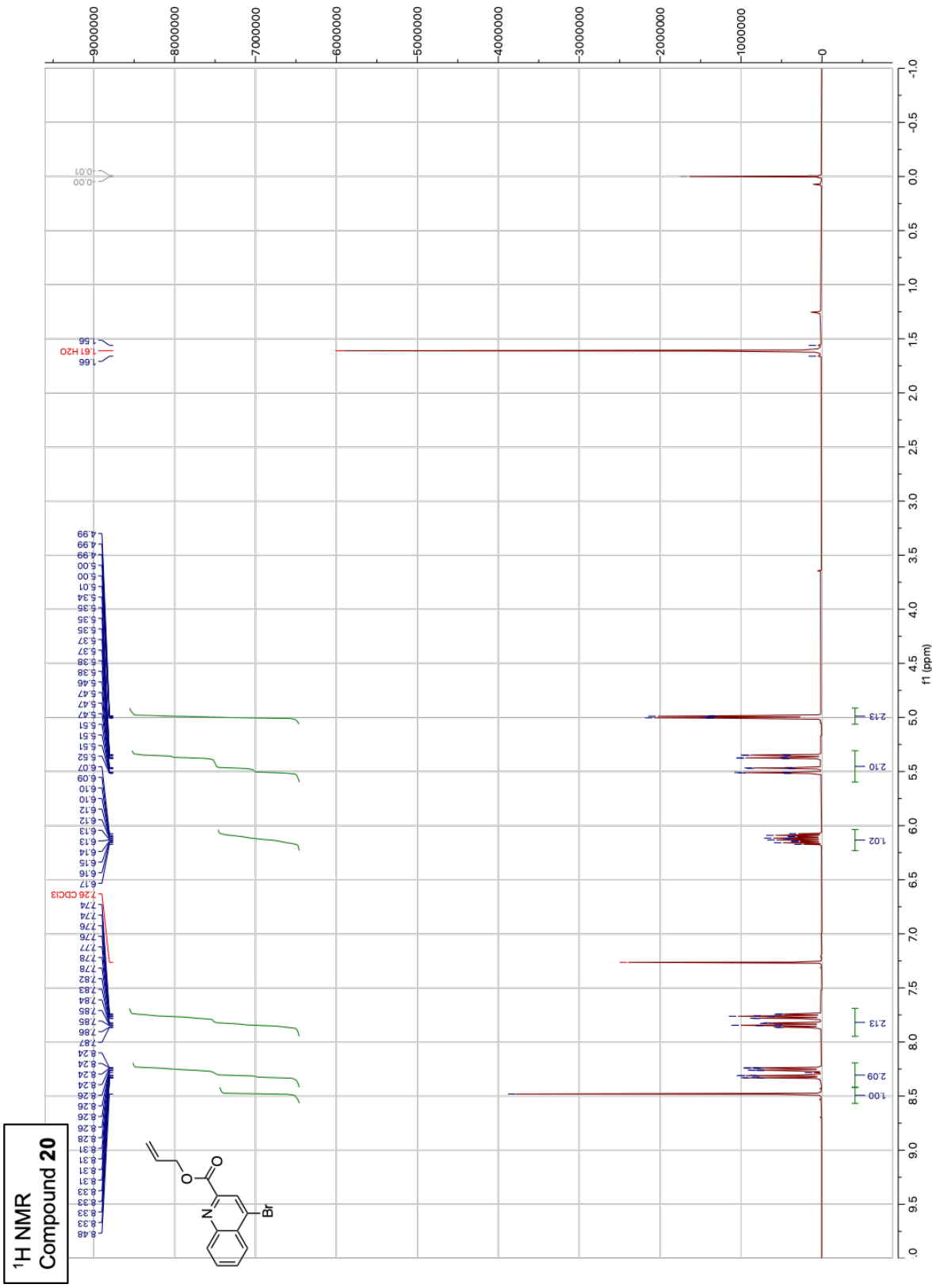
TAMRA-BnF
-76.60
-206.35
f1 (ppm)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Compound **20**
f1 (ppm)
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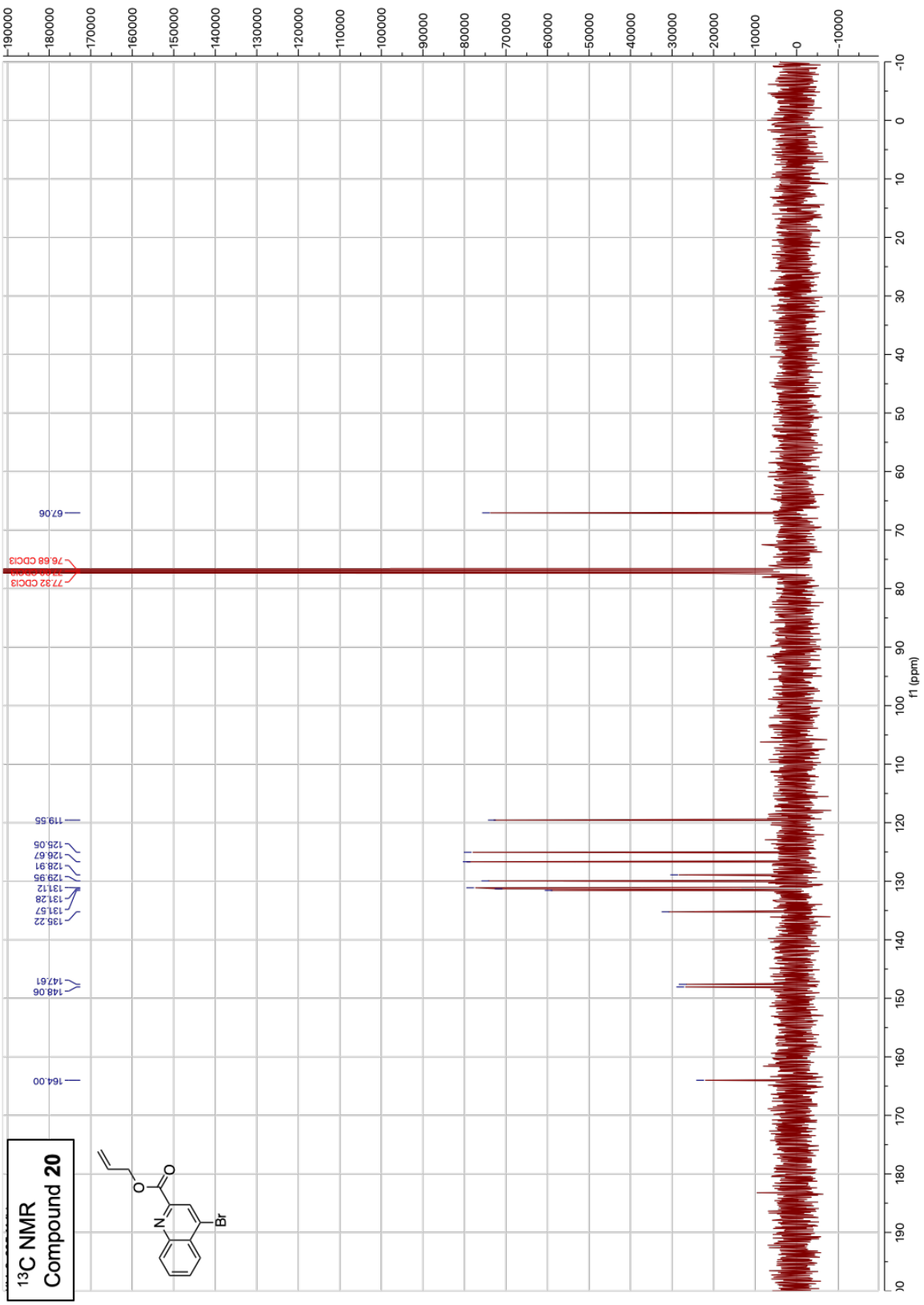
1.61 H2O
Br
N
O
O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Compound **20**
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148.06
147.61
135.22
131.57
131.28
131.12
129.95
128.91
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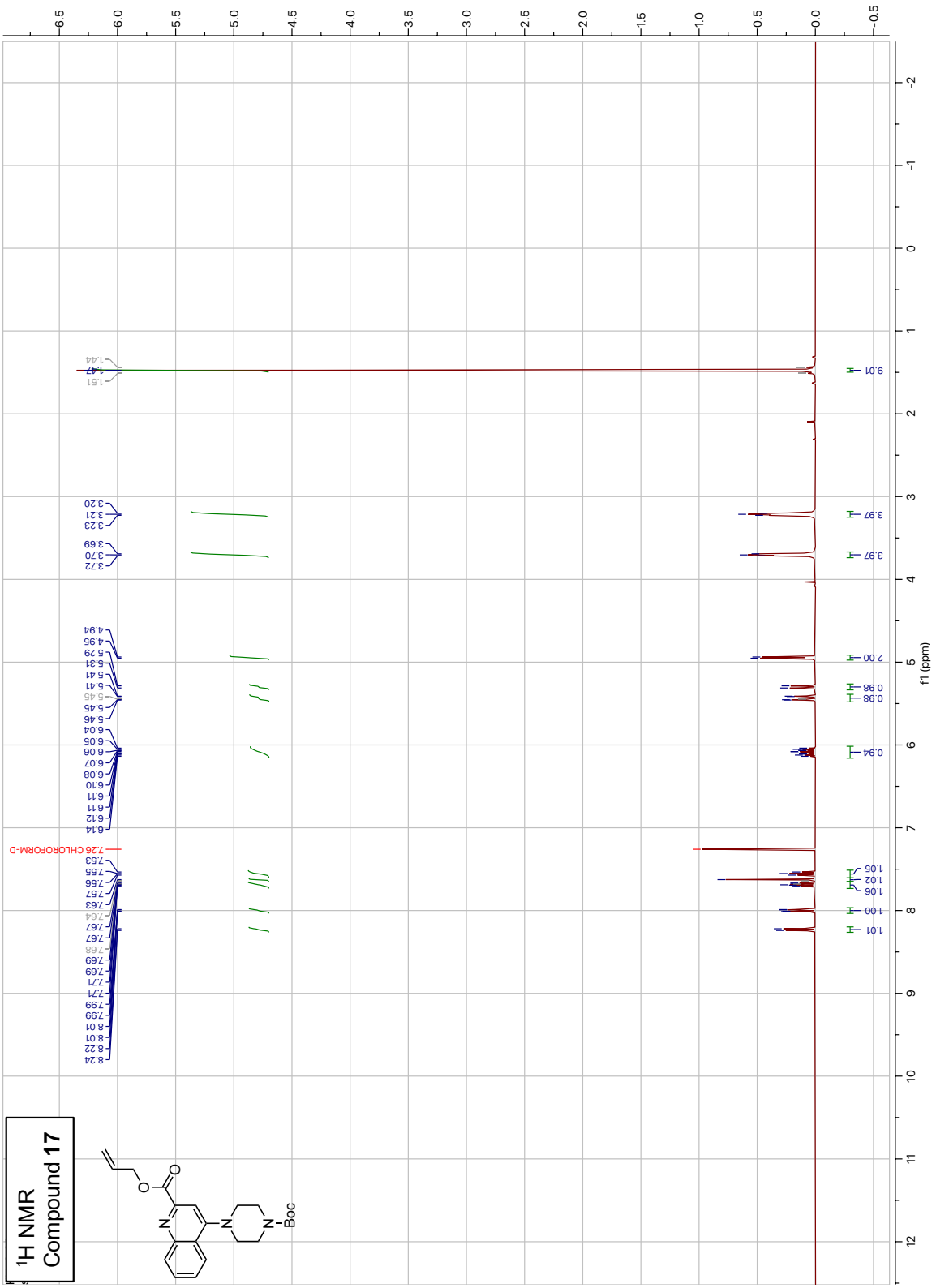
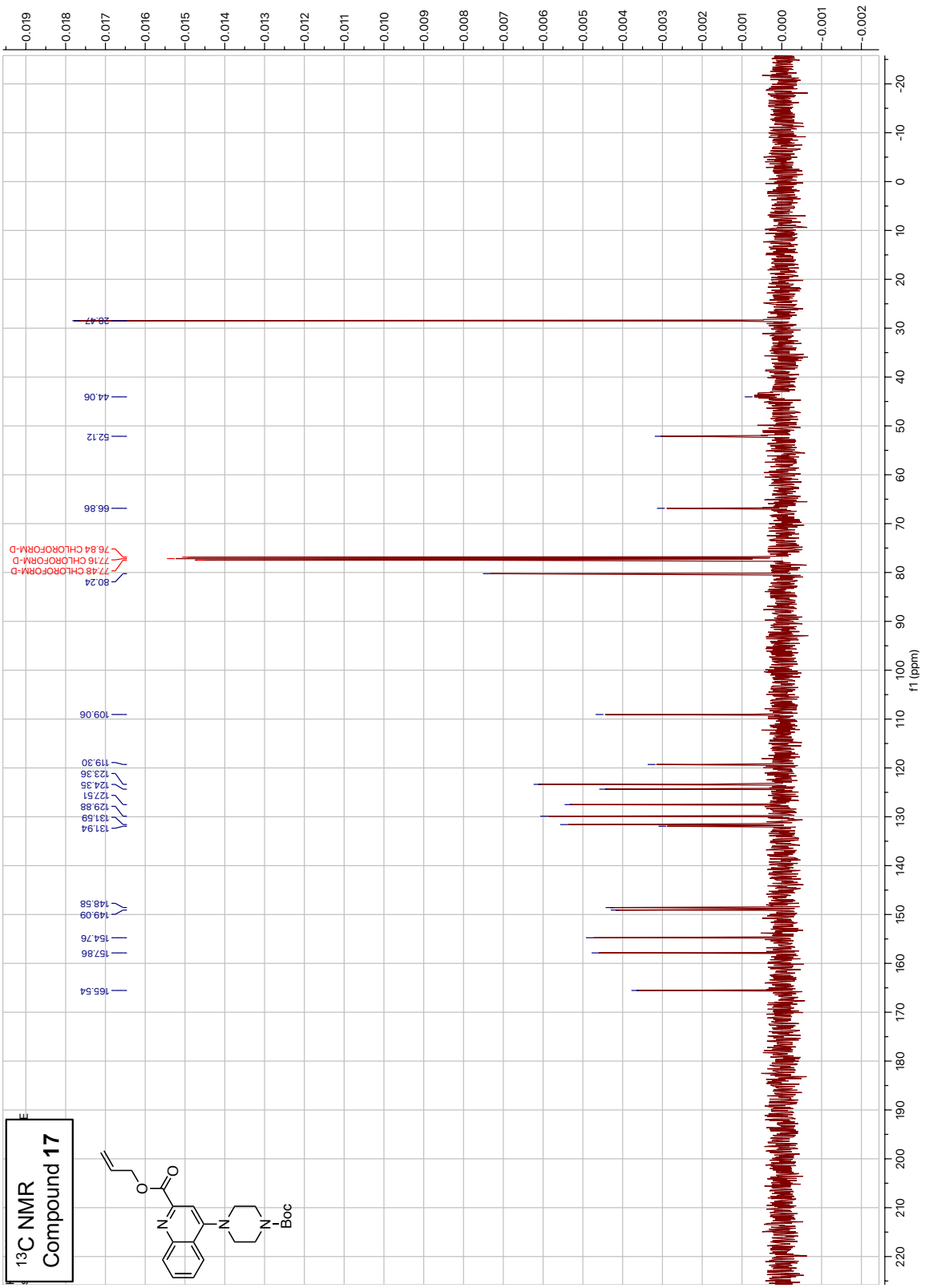
76.88 CDCl3
67.06
Br
f1 (ppm)


^{1}H NMR
Compound **17**
Boc
CHLOROFORM-D
f1 (ppm)


^{13}C NMR
Compound **17**
28.47
44.06
52.12
66.86
76.84 CHLOROFORM-D
77.16 CHLOROFORM-D
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80.24
109.06
119.30
123.36
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154.76
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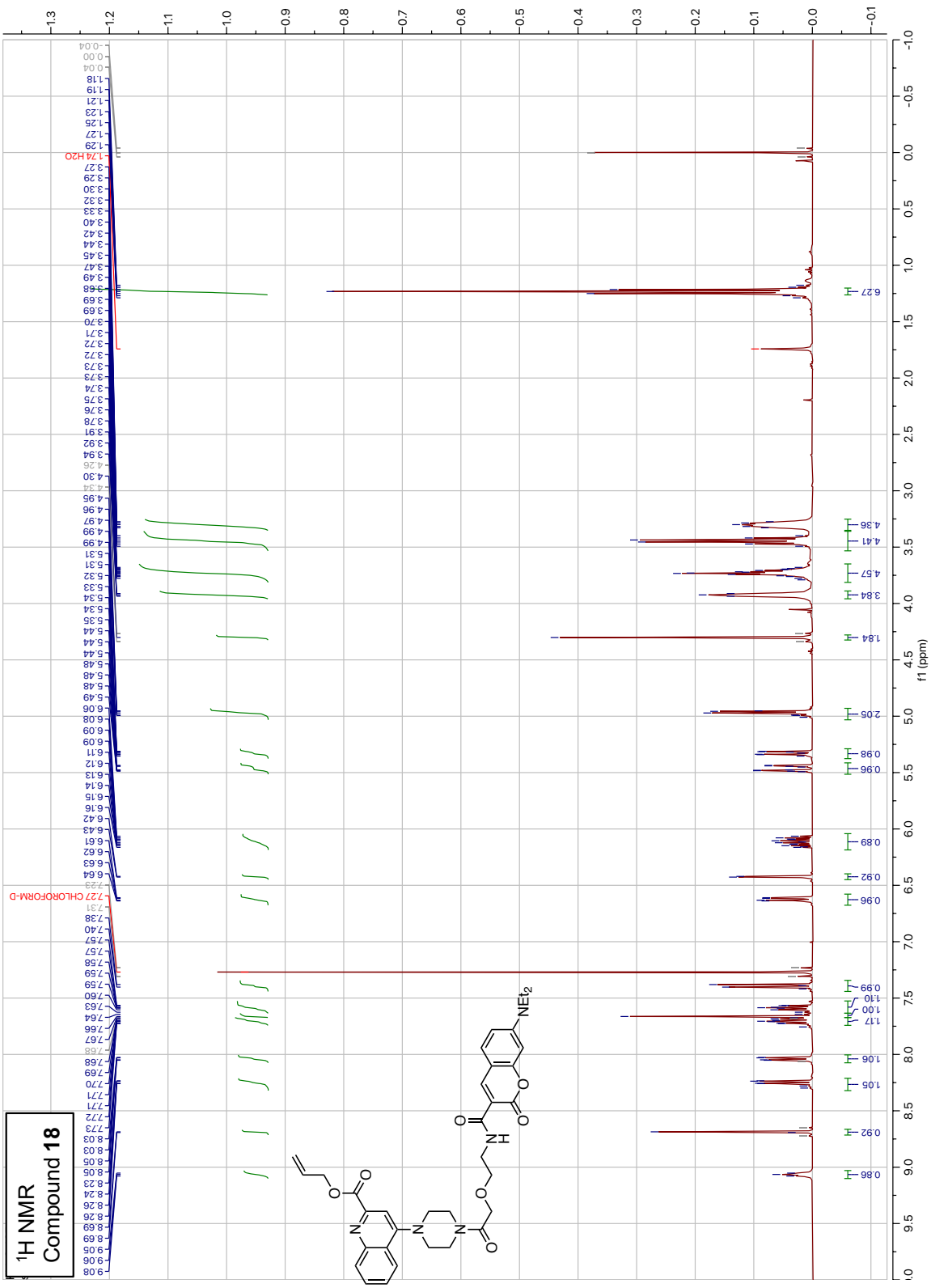
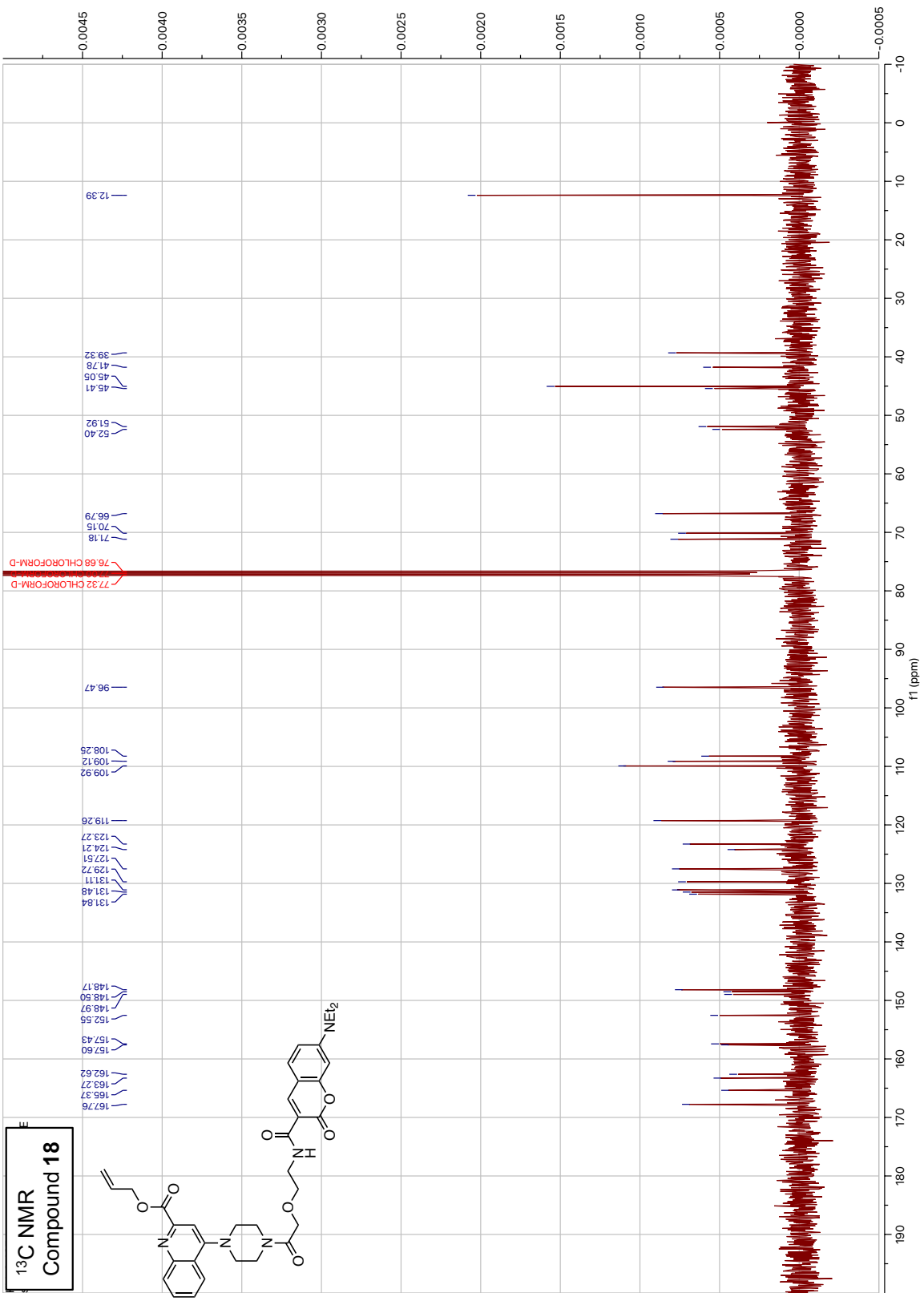
Boc
f1 (ppm)


^{1}H NMR
Compound **18**
f1 (ppm)


^{13}C NMR
Compound **18**
NEt$_2$
f1 (ppm)
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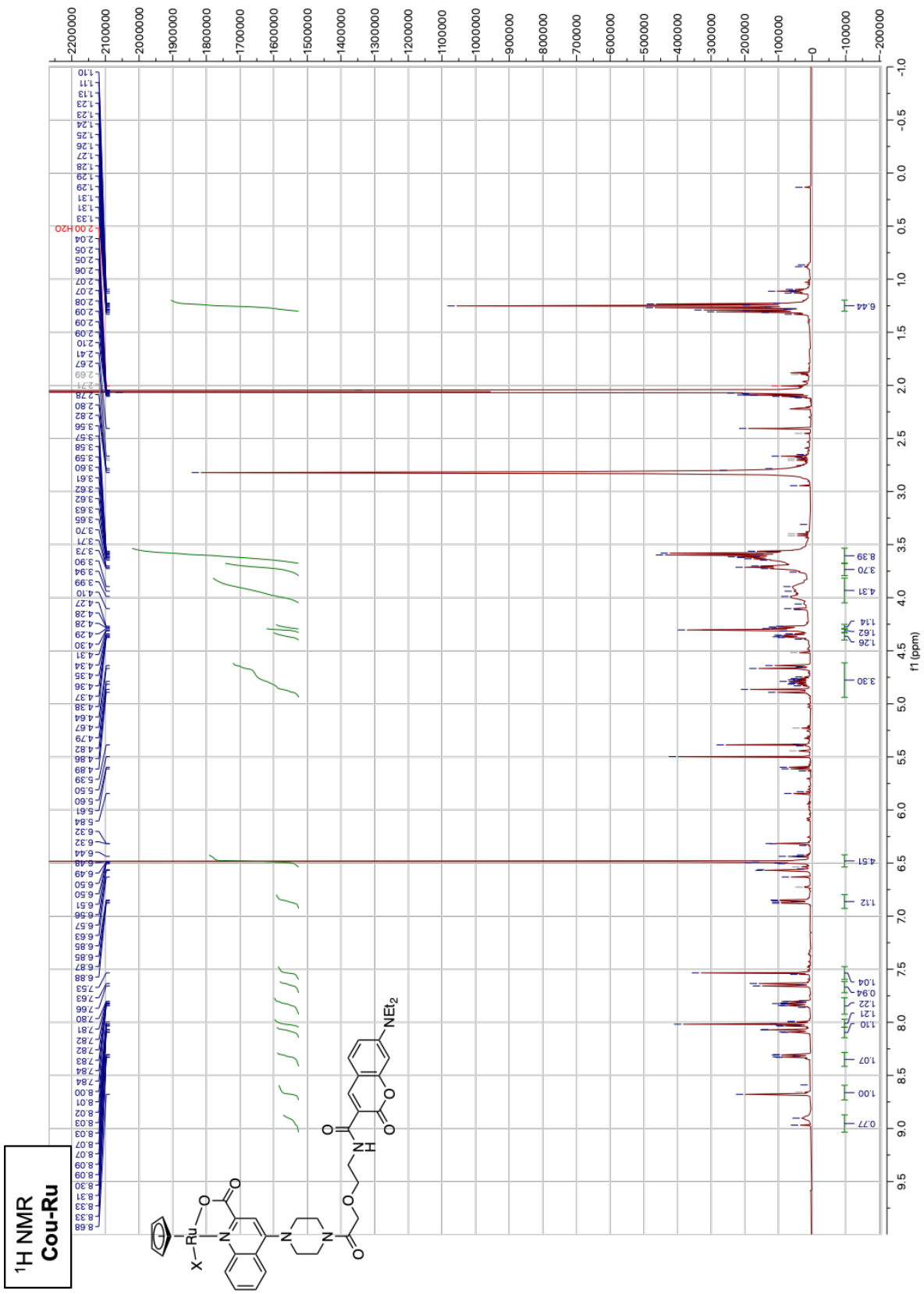
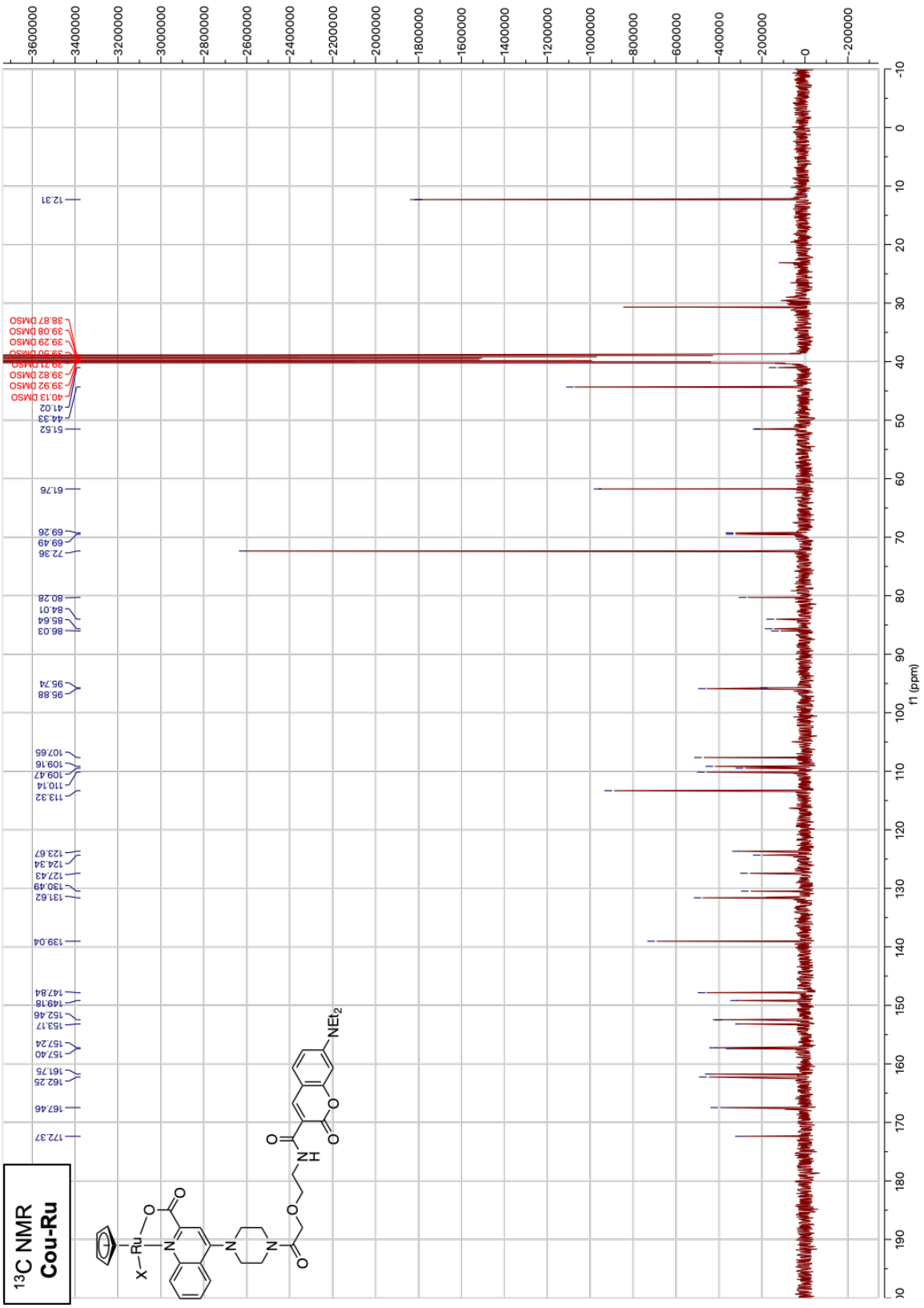
76.68 CHLOROFORM-D


1H NMR
Cou-Ru
f1 (ppm)


13C NMR
Cou-Ru
f1 (ppm)