Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

1-Methyl-4-aryl-urazole (MAUra) Labels Tyrosine in Proximity to Ruthenium Photocatalyst

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1. Supporting Figures

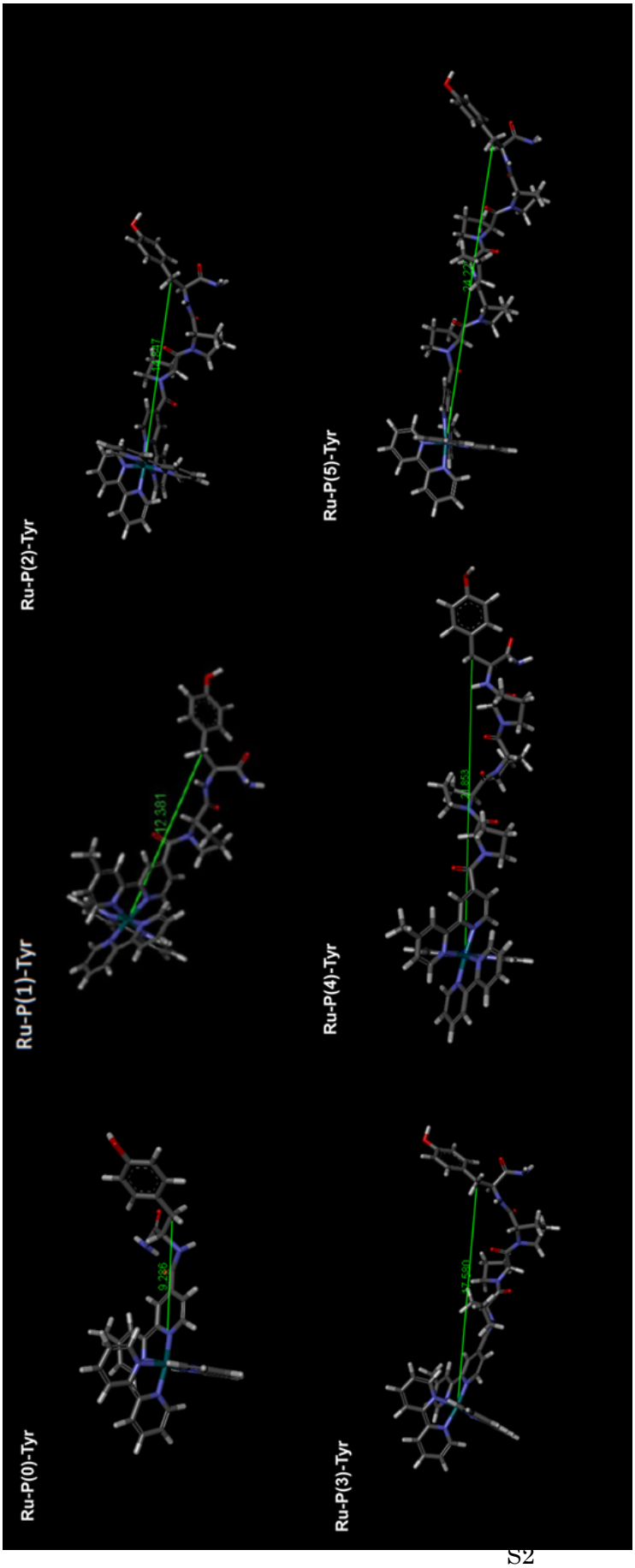


Figure S1. The distances between ruthenium atom and methylene carbon of tyrosine residues in Ru-(Pro)n-Tyr peptides

n = 0: 0.93 nm
n = 1: 1.24 nm
n = 2: 1.48 nm
n = 3: 1.76 nm
n = 4: 2.18 nm
n = 5: 2.42 nm

The PPII backbone dihedral angles applied to as setting: phi-75 psi 145 degrees (*Omega*=180 degrees)[1]

Drawing the molecules and distance calculation were performed using Discovery Studio 4.5 software.

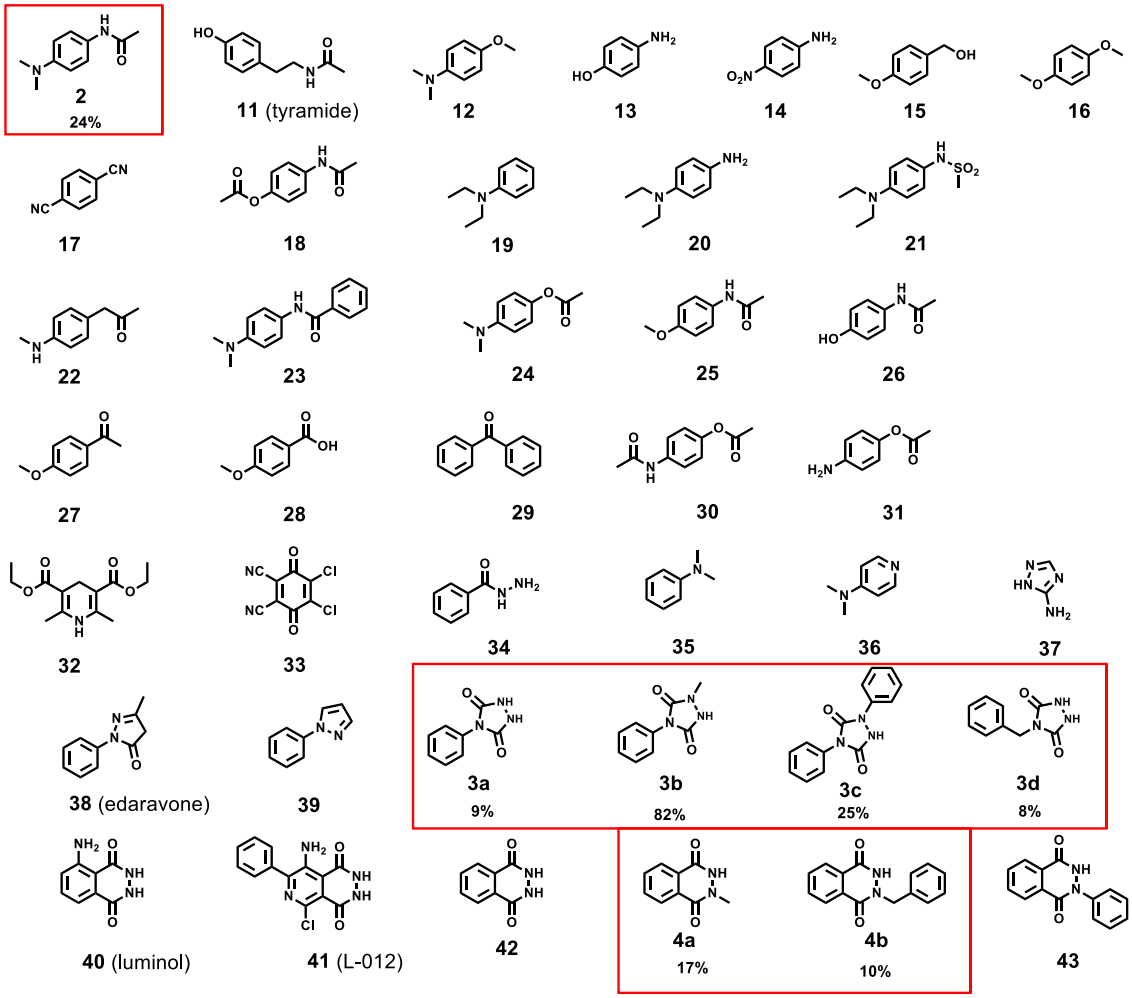


Figure S2. Compounds tested by the screening system using peptide **1a.**

The labelling reaction of peptide **1a** by compounds except **2**, **3a-d** and **4a-c** were not detected by MALDI-MS analysis. Reaction conditions: peptide **1a** (10 μM), labelling reagent candidates (300 μM) in 10 mM MES buffer (pH 6.0), blue light (455 nm LED) irradiation 5 min on ice. The ratios (%) of labelling peptides in the total peptide MS peaks are listed.

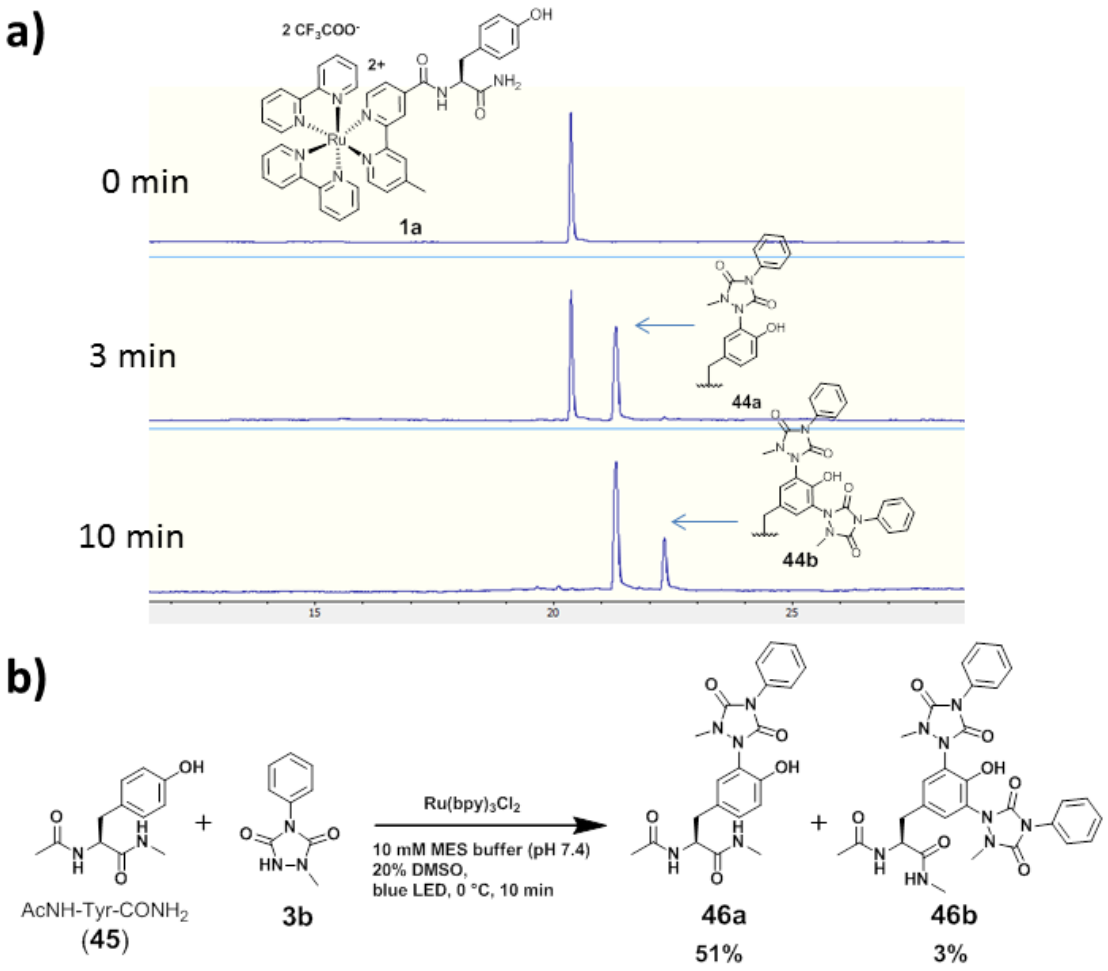


Figure S3. Efficient labelling of tyrosine residue by labelling reagent **3b**. (a) LC-MS analysis by monitoring the reaction between **1a** and **3b** up to 10 minutes blue LED irradiation. (b) Tyrosine labelling with **3b** and structures of tyrosine adducts clarified by NMR analysis.

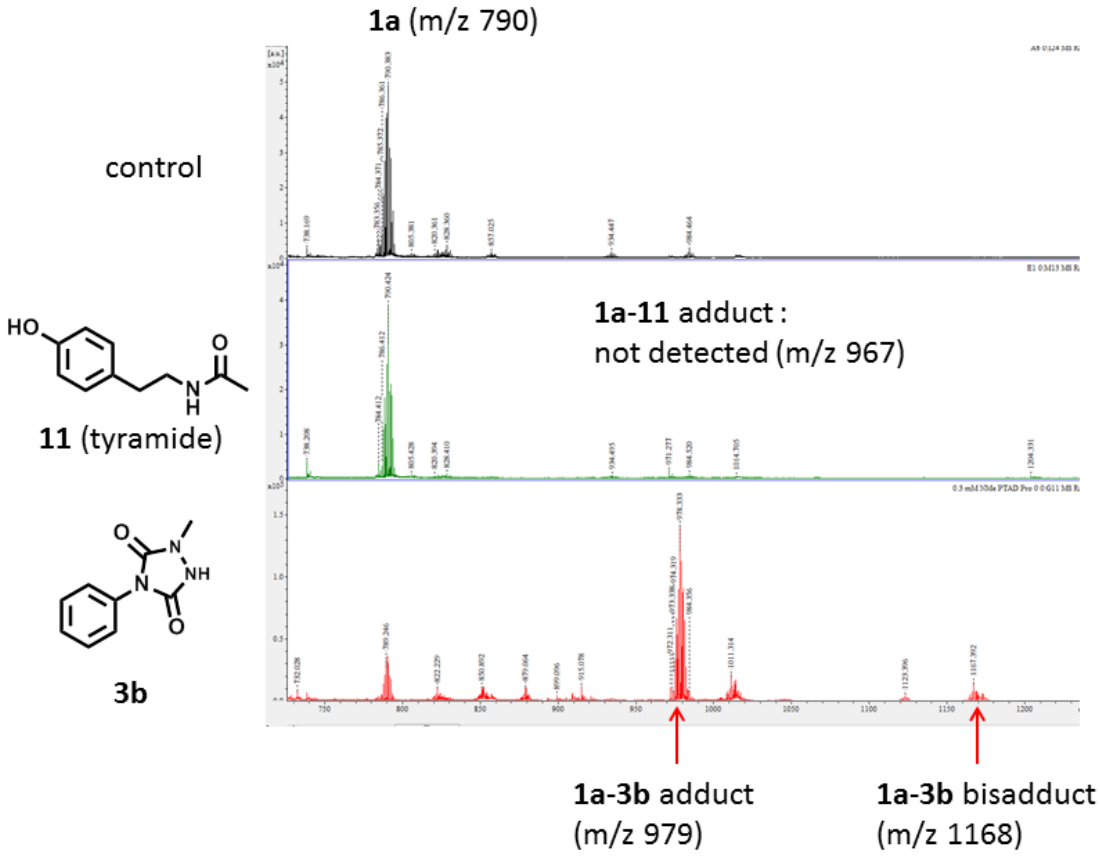


Figure S4. The reactivity of tyramide under the condition using ruthenium photocatalyst.
The labelling reaction was not observed under the condition using **1a** and tyramide **11**.
Reacrtion condition: **1a** (10 μM), labelling reagent (300 μM) in MES buffer (10 mM, pH 6.0), light irradiation (455 nm) 0.5 cm from the light source(230 mW/cm^2) on ice for 5 min.

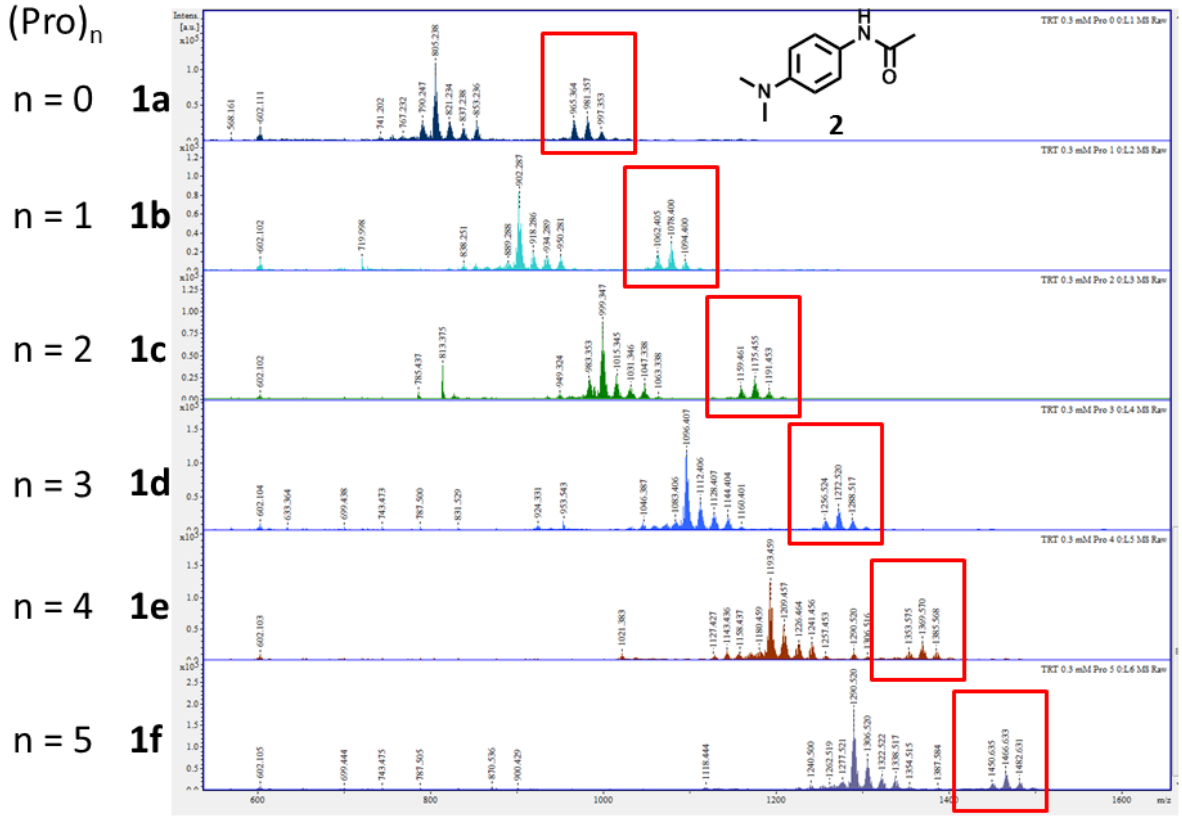


Figure S5. Labelling of peptides **1a-1f** with **2**. Peaks surrounded by red squares: labelled peptides.

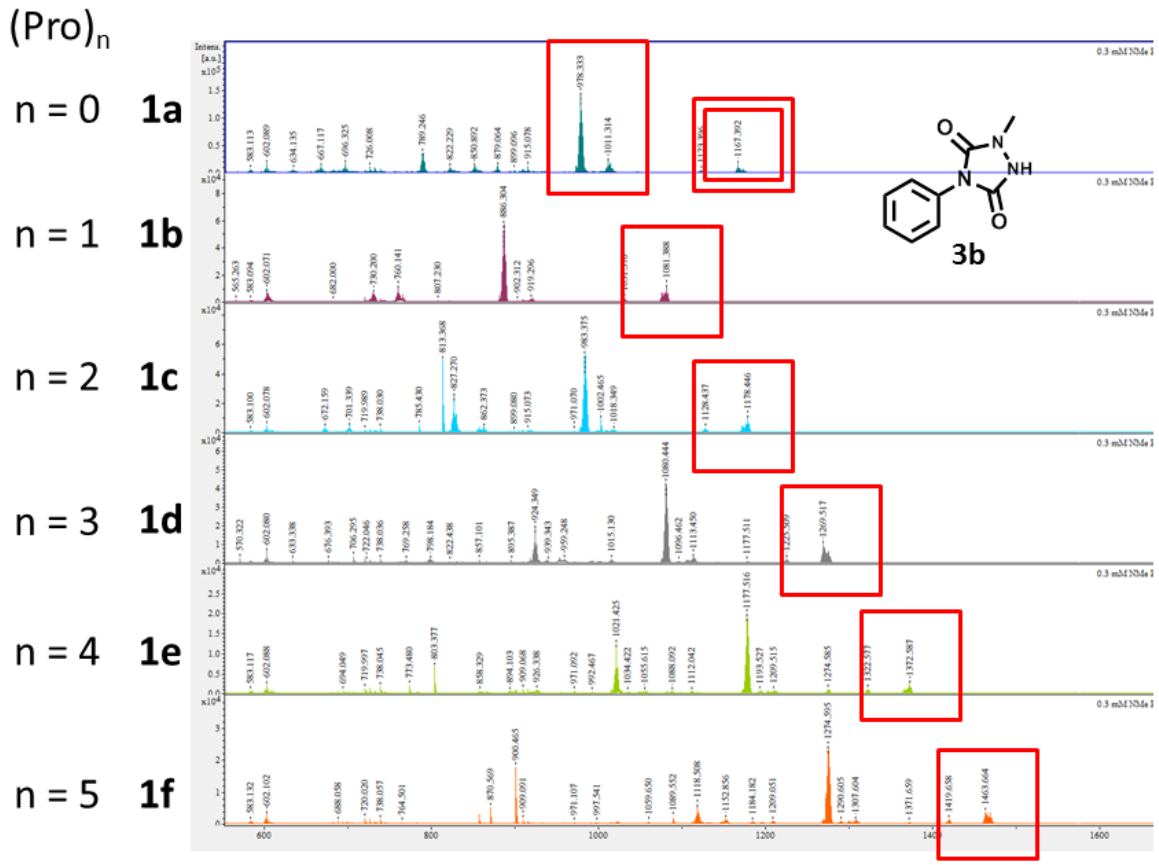


Figure S6. Labelling of peptides **1a-1f** with **3b**. Peaks surrounded by red squares: labelled peptides. Peak surrounded by double red squares: double labelled peptide.

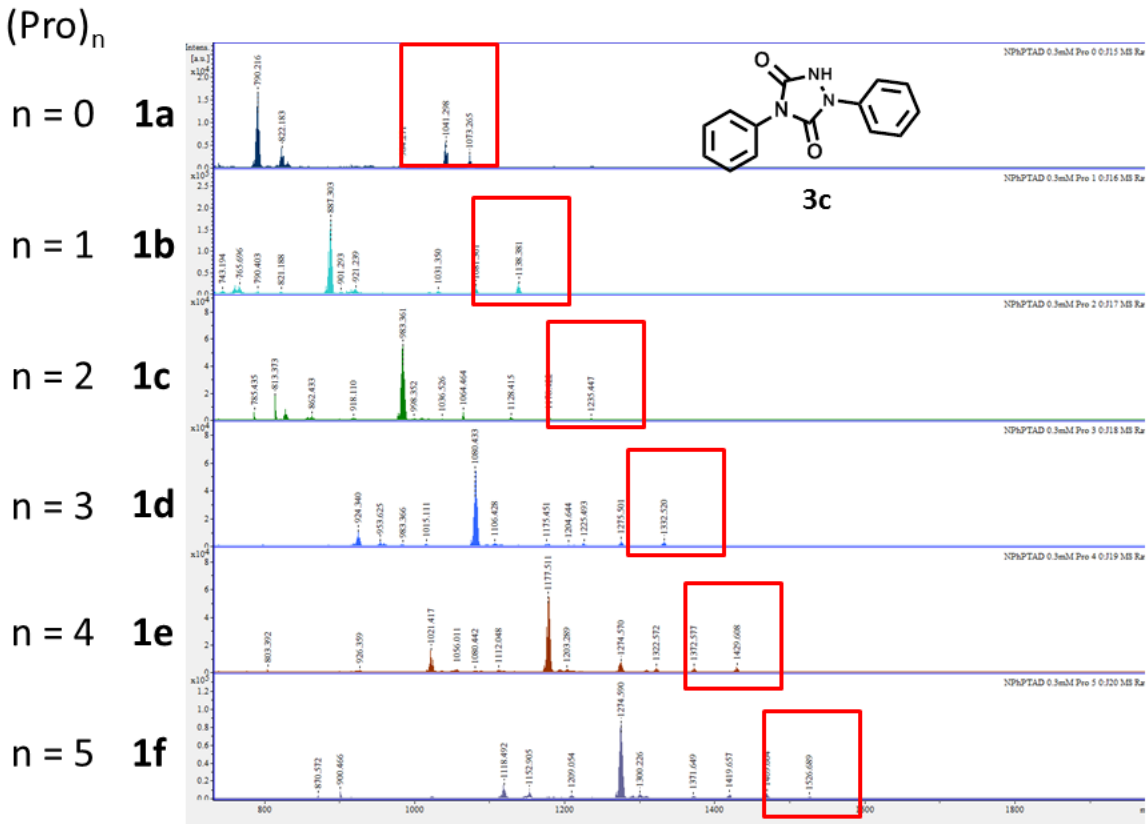


Figure S7. Labelling of peptides **1a-1f** with **3c**. Peaks surrounded by red squares: labelled peptides.

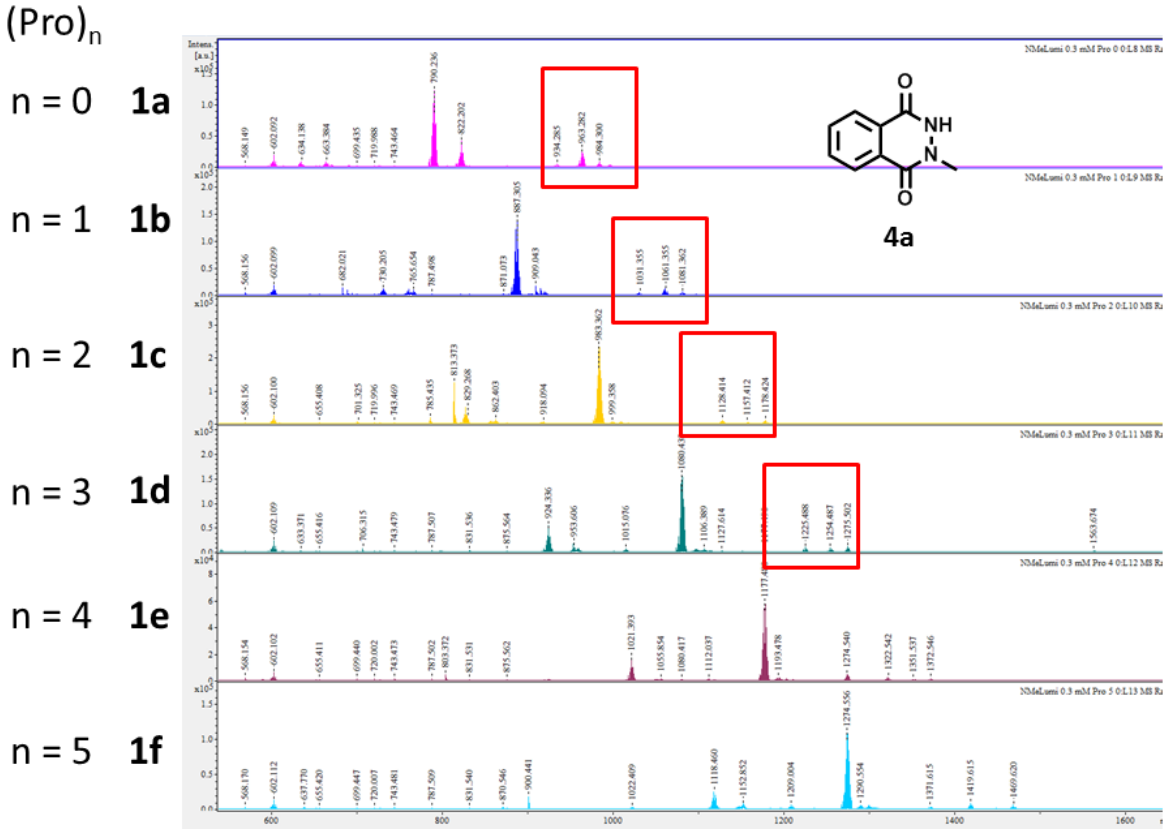
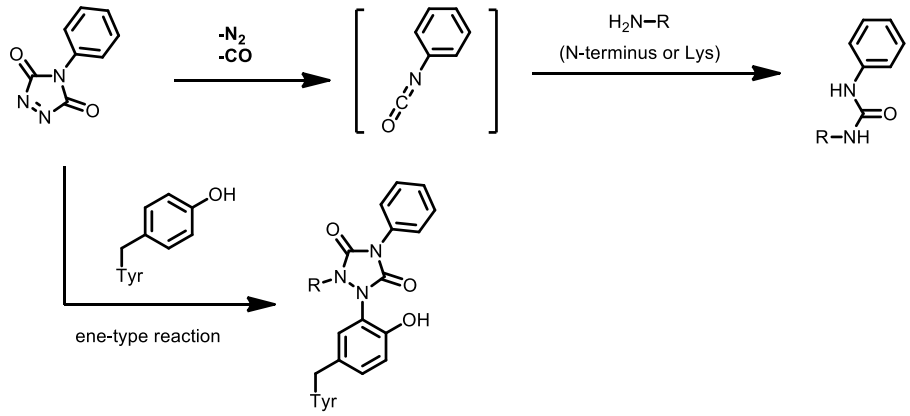


Figure S8. Labelling of peptides **1a-1f** with **4a**. Peaks surrounded by red squares: labelled peptides.



Scheme S1. Isocyanate generation by PTAD degradation and their reactivity.

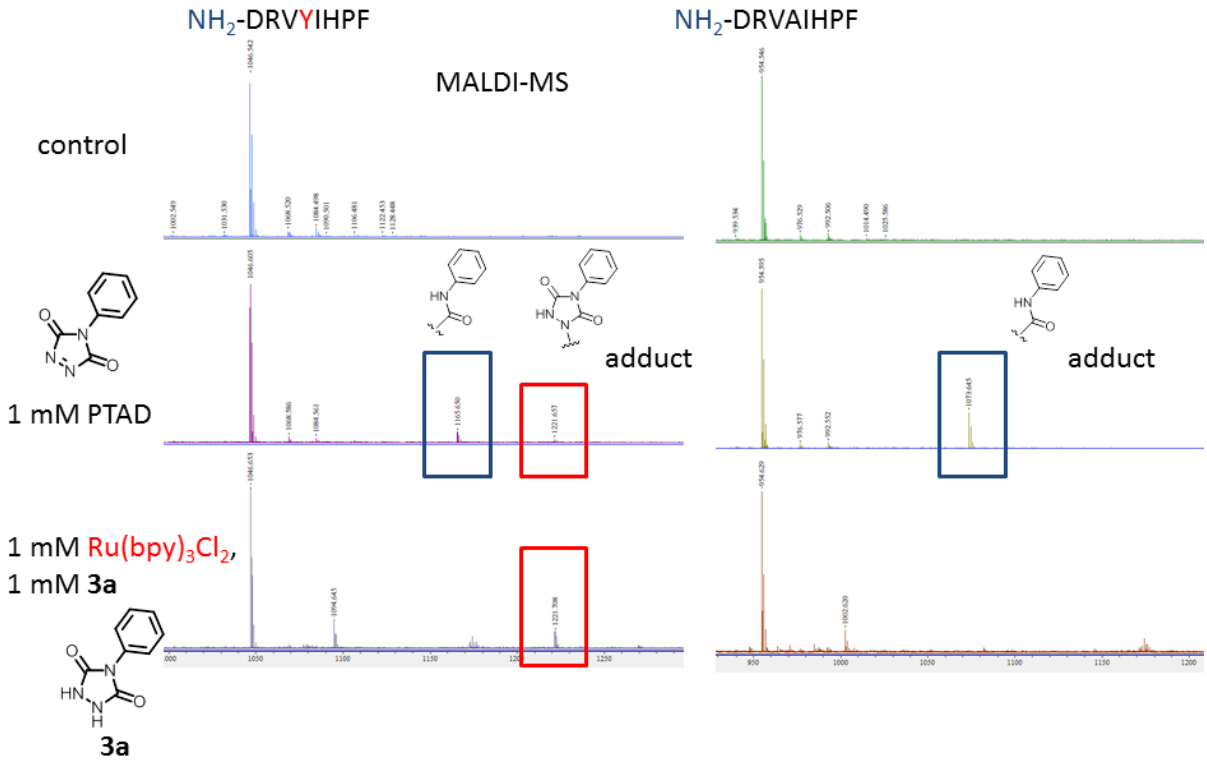


Figure S9. Comparison of products in labelling with PTAD and **3a**.

Reaction condition for PTAD labelling: peptides (DRVYIHPF and DRVAIHPF) (100 μM) in 10 mM Phosphate (pH 7.4) at room temperature for 60 min.

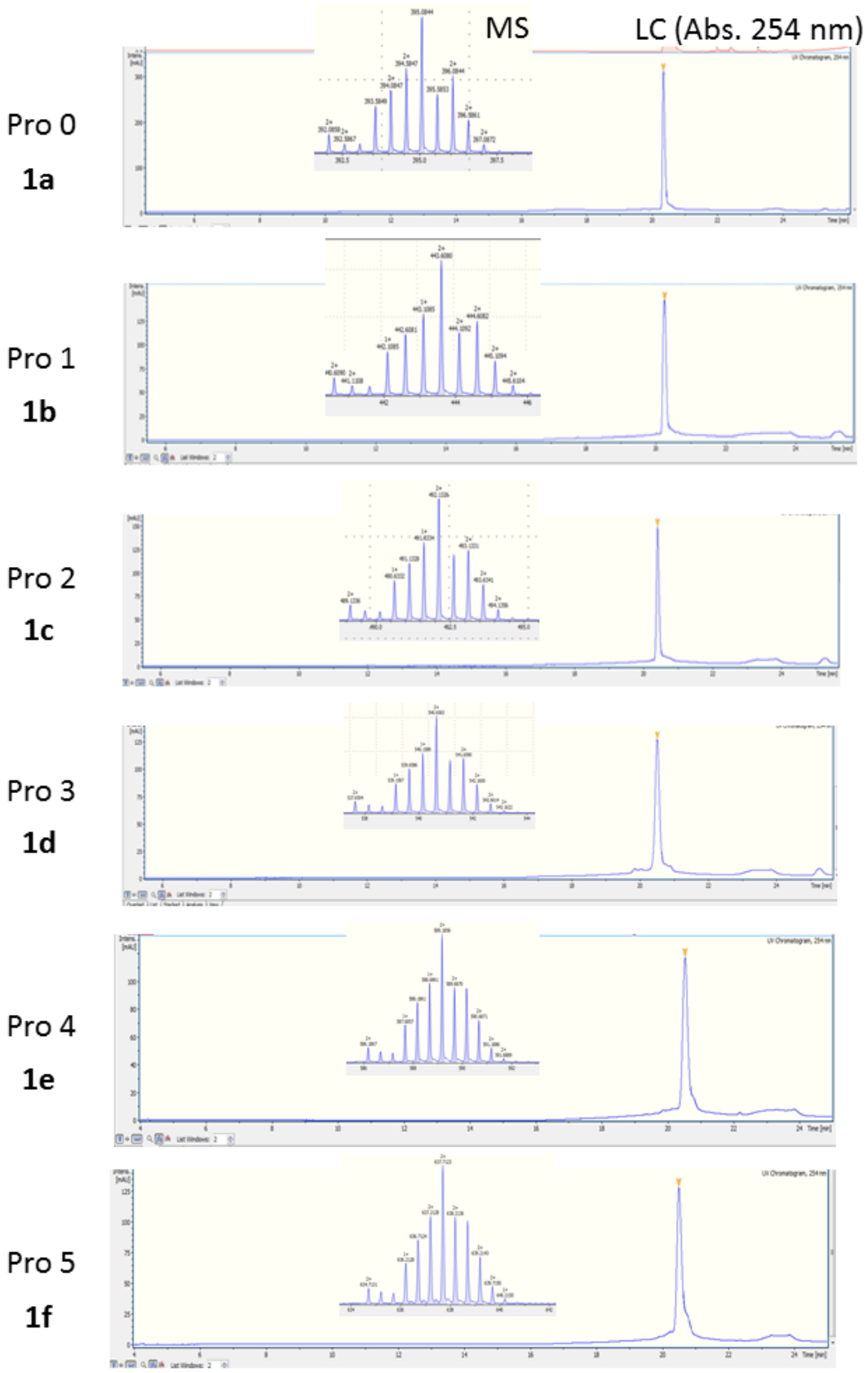


Figure S10. Confirmation of purity of **1a-f** by LC-MS analysis.

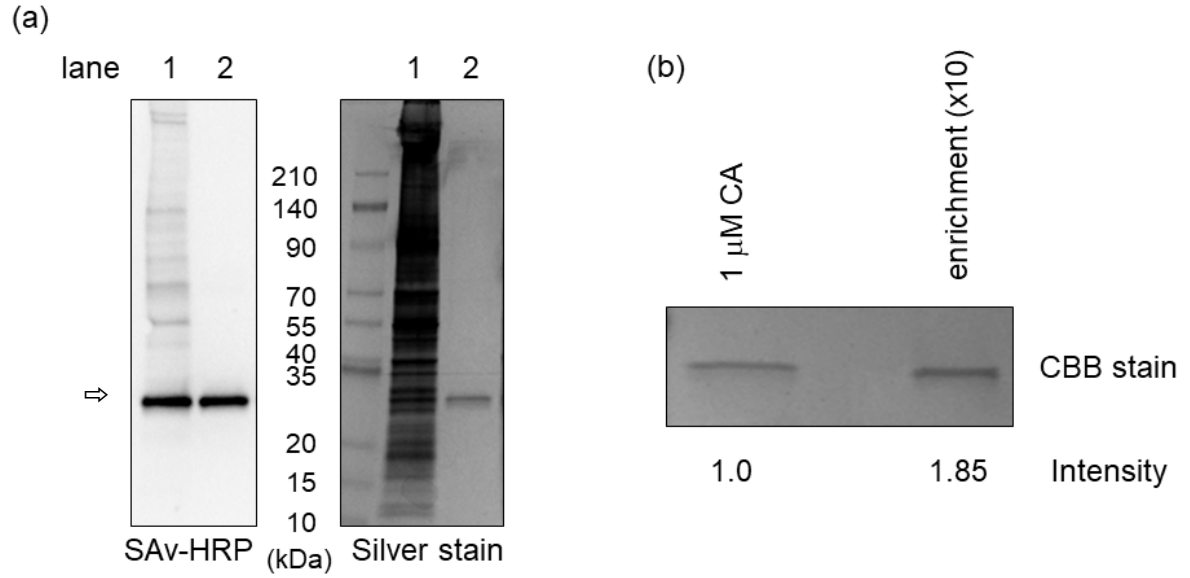


Figure S11. The efficiency of labelling and enrichment on CA. (a) CA-labelling with labelling reagent **10** and pohotocatalyst **5**. Lane 1: Labelling was operated in 1.0 mg/mL HeLa cell lysate containing 1 μM CA. Lane 2: Labelling was operated in 1 μM CA solution. The result shows that the efficiency of CA-selective labelling is similar in either cell lysate or isolated system. (b) Enrichment of desthiobiotin-labelled CA using streptavidin beads. The desthiobiotin-labelled CA was observed in 18.5% yield using SAv beads at a 1 μM concentration of CA after labelled with **10** and **5**.

Figure S12. Enzymatic digestion of non-labelled and labelled CA.

(a) MS pattern of digested non-labelled CA with trypsin and Glu-C. (a) MS pattern of peptide fragments of CA which was labelled, purified (Figure S11b labelled and enriched CA) and digested with trypsin and Glu-C. Major peak with mass number 2637 corresponds to the acetylated N-terminal sequence (Ser1-Lys17) labelled with compound **10**. This peak was the only peptide fragment that was attributed to labelled peptide with compound **10** and was detected in Figure S12b.

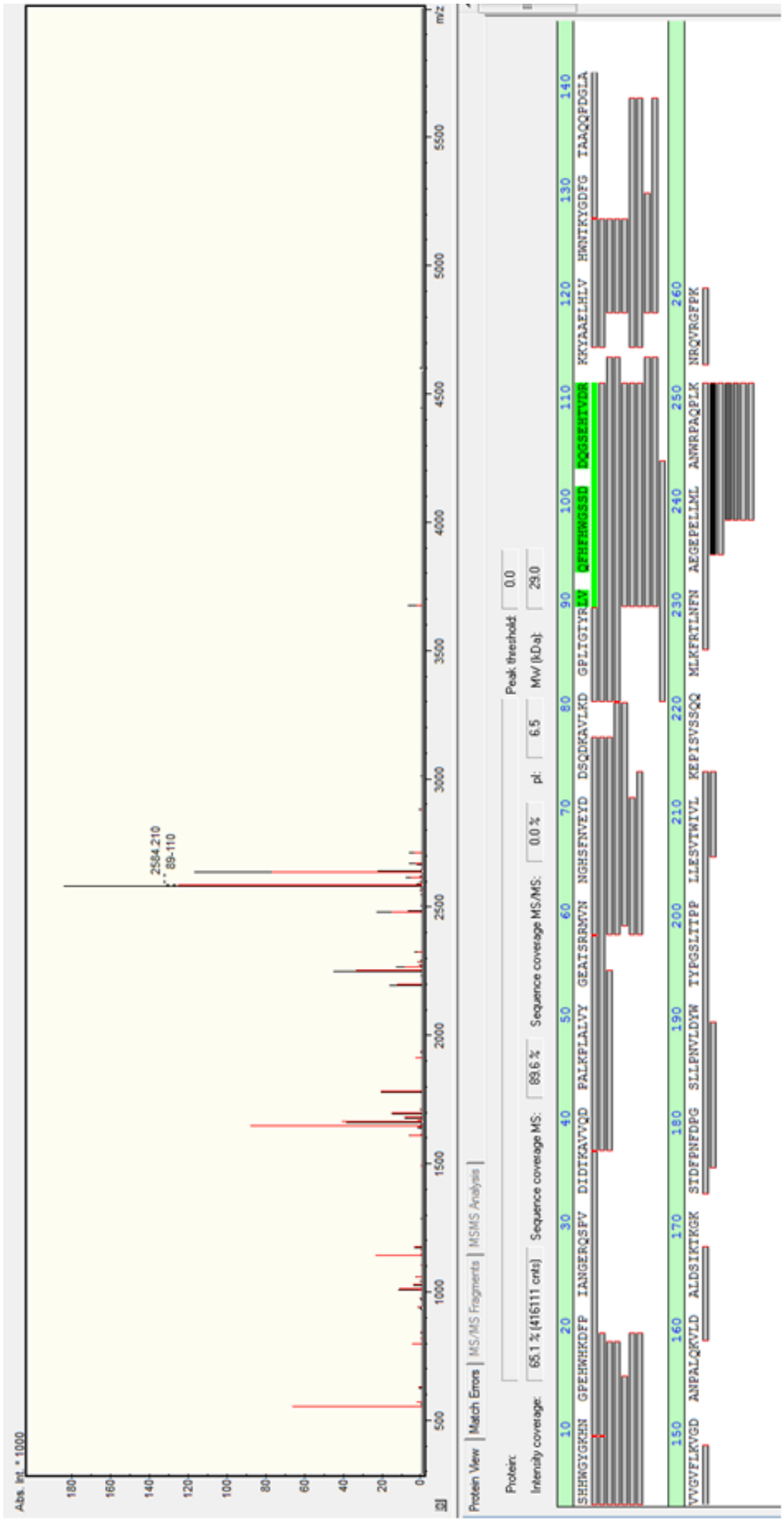


Figure S13. Assignment of detected peptide fragments of Figure S12b. N-terminal Ser1 was acetylated in this bovine carbonic anhydrase II. (sequence coverage :89.6%)

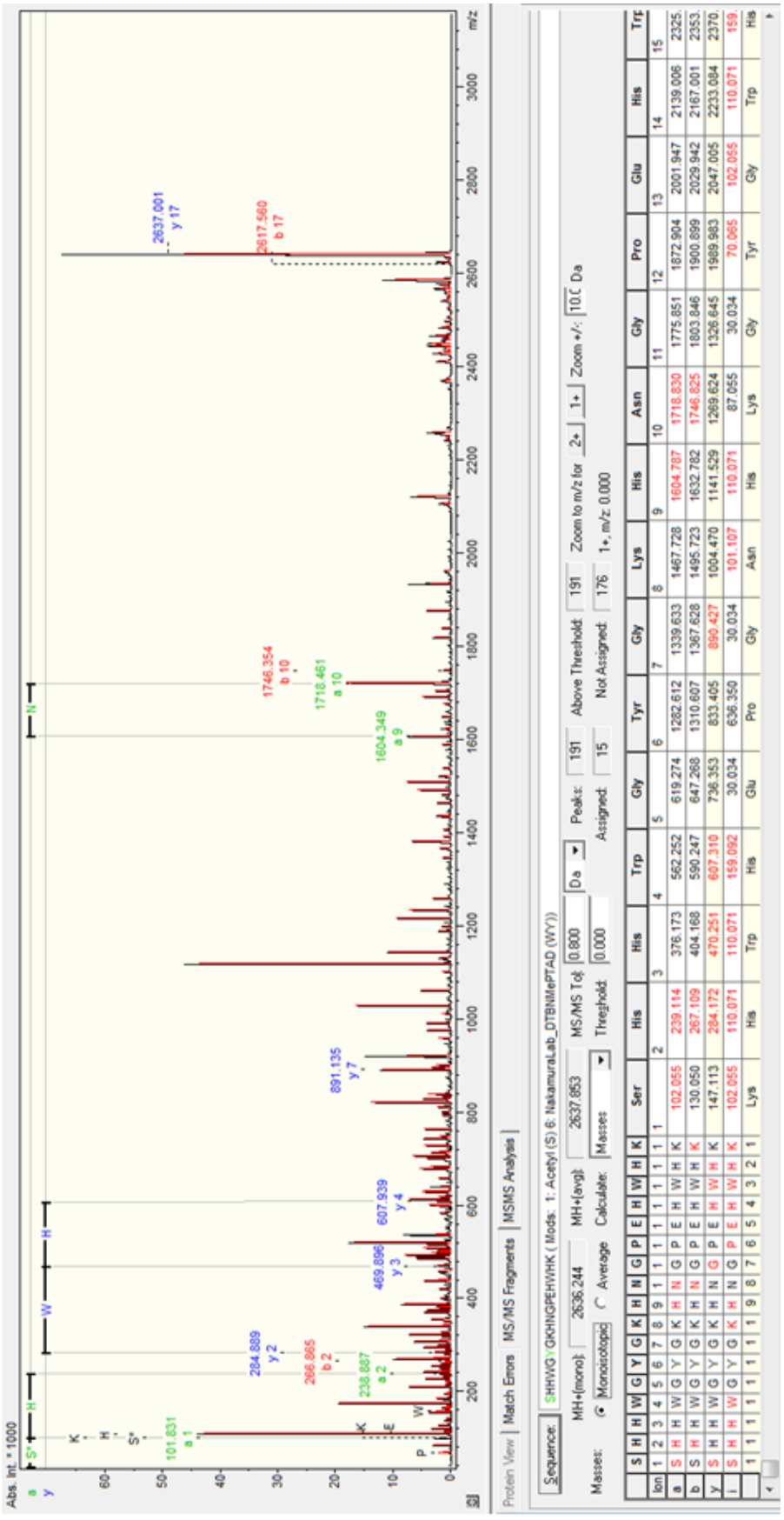


Figure S14. MS/MS analysis of the peptide fragment with mass number 2637. Tyr6 belonged as **10**-labelled Tyr. This result suggested that Tyr6 was labelled with compound **10**.

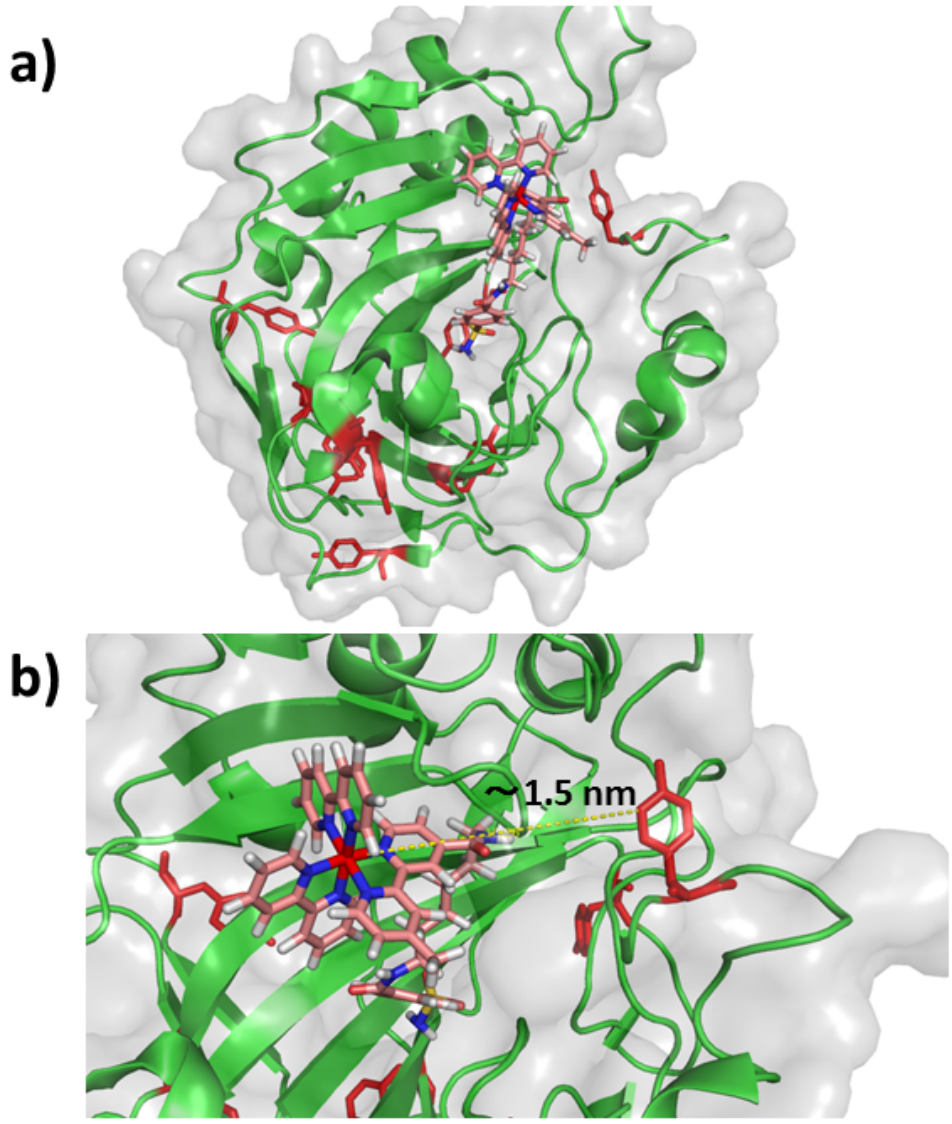


Figure S15. Three dimensional (3D) image of labelled tyrosine (Tyr6) and binding of compound **5** to CA. (a) Binding mode of CA with **5** based on the X-ray crystal structure of CA inhibitor having benzensulfonamide moiety (PDB: 4ILX). The 3D structure of 5 was calculated on Chem3D Pro using MM2 method, and overlapped with reported structure (PDB: 4ILX). Although, The PDB file (4ILX) was obtained using human carbonic anhydrase II, and bovine carbonic anhydrase II was used in this paper, this binding site and Tyr6 is preserved. Therefore, this 3D model is suitable for consideration of these results. (b) Zoom up image around the ruthenium complex. The distance between carbon atom of Tyr6 and Ru atom is about 1.5 nm. Considering the flexibility of the liker of **5**, the ruthenium complex moiety can be brought into proximity to the range where single electron can move from tyrosine residue.

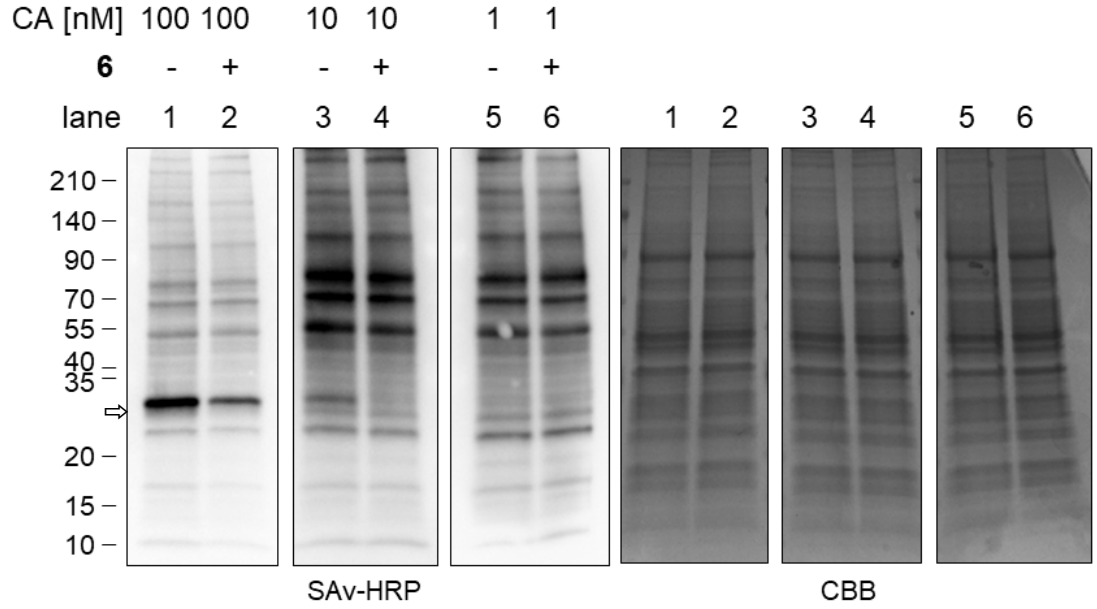


Figure S16. The CA-selective labelling at various concentration of CA (100, 10, and 1 nM) in HeLa cell lysate. The labelling was operated with labelling reagent **10** (500 μM) and photocatalyst **5** (1 equiv. for CA) with or without ligand **6** (1000 equiv. for CA). The labelling of CA was detected at 10 nM or higher concentrations of it.

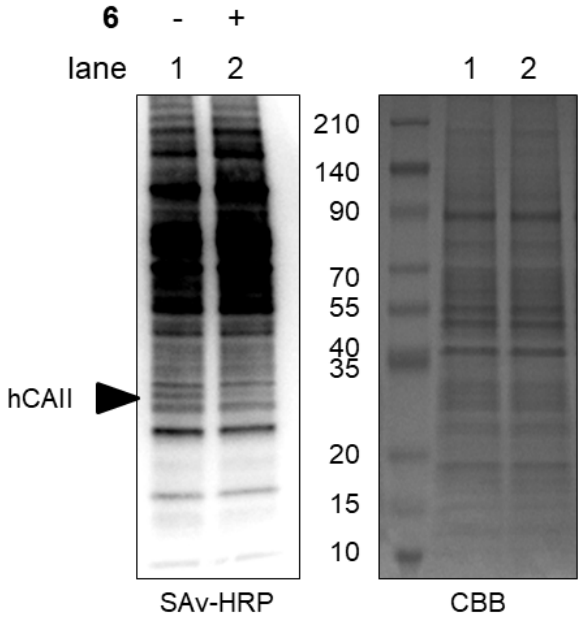


Figure S17. CA-selective labelling in 1.0 mg/mL MCF7 cell lysate. Labelling was operated with labelling reagent **10** (500 μM) and **5** (30 nM). Endogenous hCAII was detected (ca. 29 kDa).

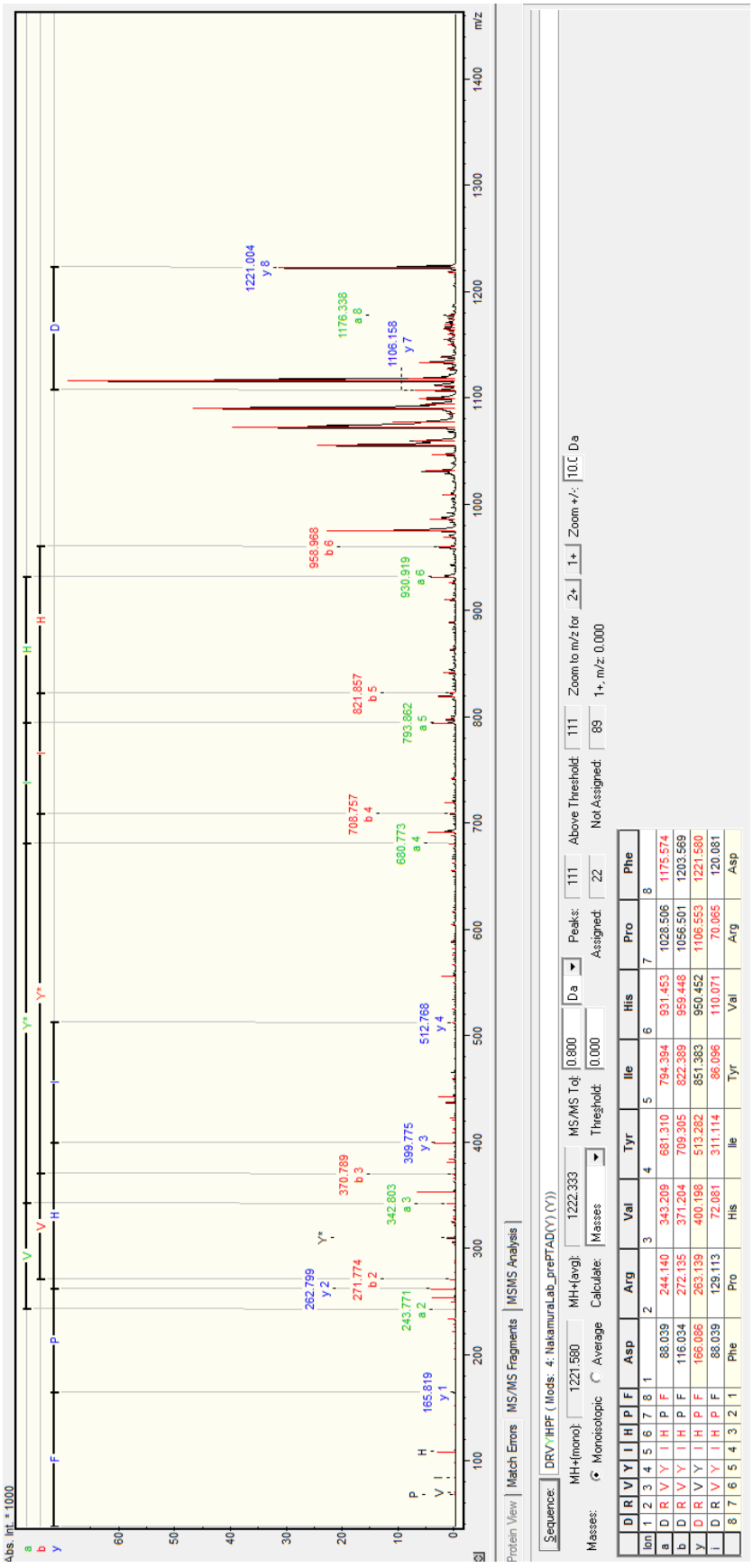


Figure S18. MS/MS analysis of PTAD-modified angiotensin II (MS: 1221, Figure S9 middle left). Tyr belonged as PTAD-labelled Tyr.

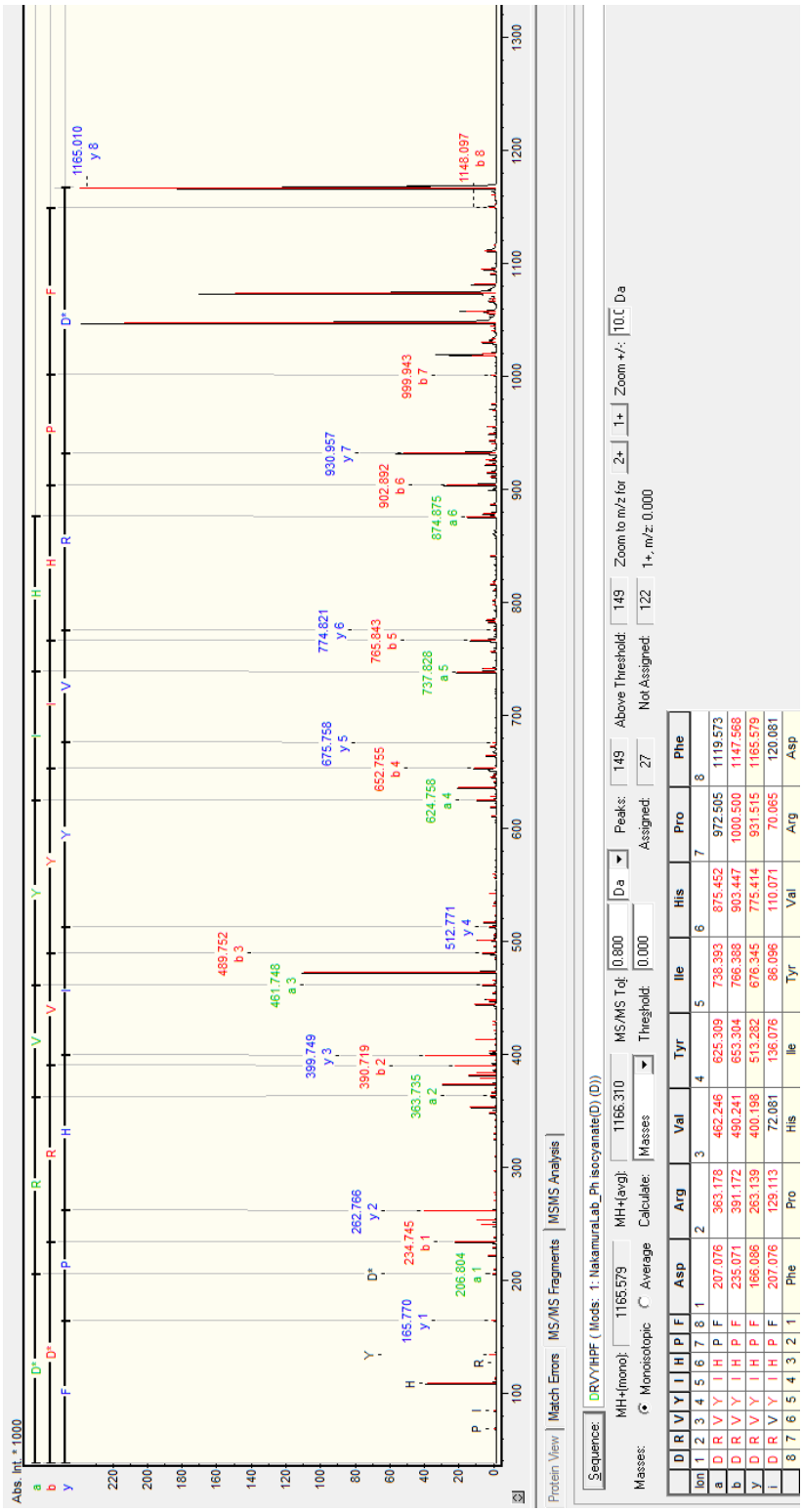
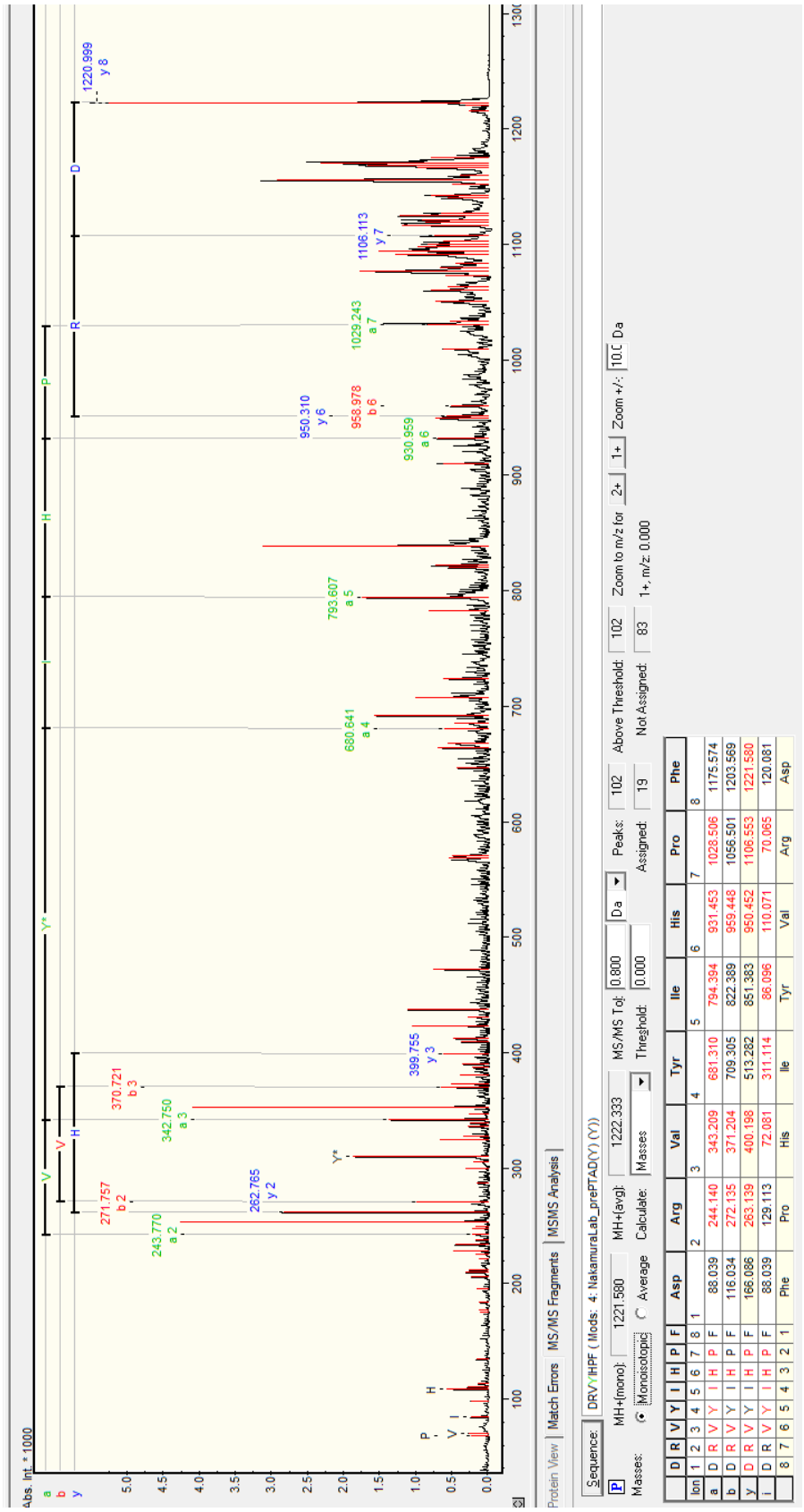


Figure S19. MS/MS analysis of phenyl isocyanate-modified angiotensin II (MS: 1165, Figure S9 middle left). N-terminal Asp belonged as phenyl isocyanate-labelled Asp.



	D	R	V	Y	I	H	P	F	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe
Ion	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
a	D	R	V	Y	I	H	P	F	88.039	244.140	343.209	681.310	794.394	931.453	1028.506	1175.574
b	D	R	V	Y	I	H	P	F	116.034	272.135	371.204	709.305	822.389	959.448	1056.501	1203.569
y	D	R	V	Y	I	H	P	F	166.086	263.139	400.198	513.282	851.383	950.452	1106.553	1221.580
i	D	R	V	Y	I	H	P	F	88.039	129.113	72.081	311.114	86.096	110.071	70.065	120.081
	8	7	6	5	4	3	2	1	Phe	Pro	His	Ile	Tyr	Val	Arg	Asp

Figure S20. MS/MS analysis of modified angiotensin II under the condition using $Ru(bpy)_3Cl_2$ and **3a** (MS: 1221, Figure S9 bottom left).
Tyr belonged as **3a**-labelled Tyr.

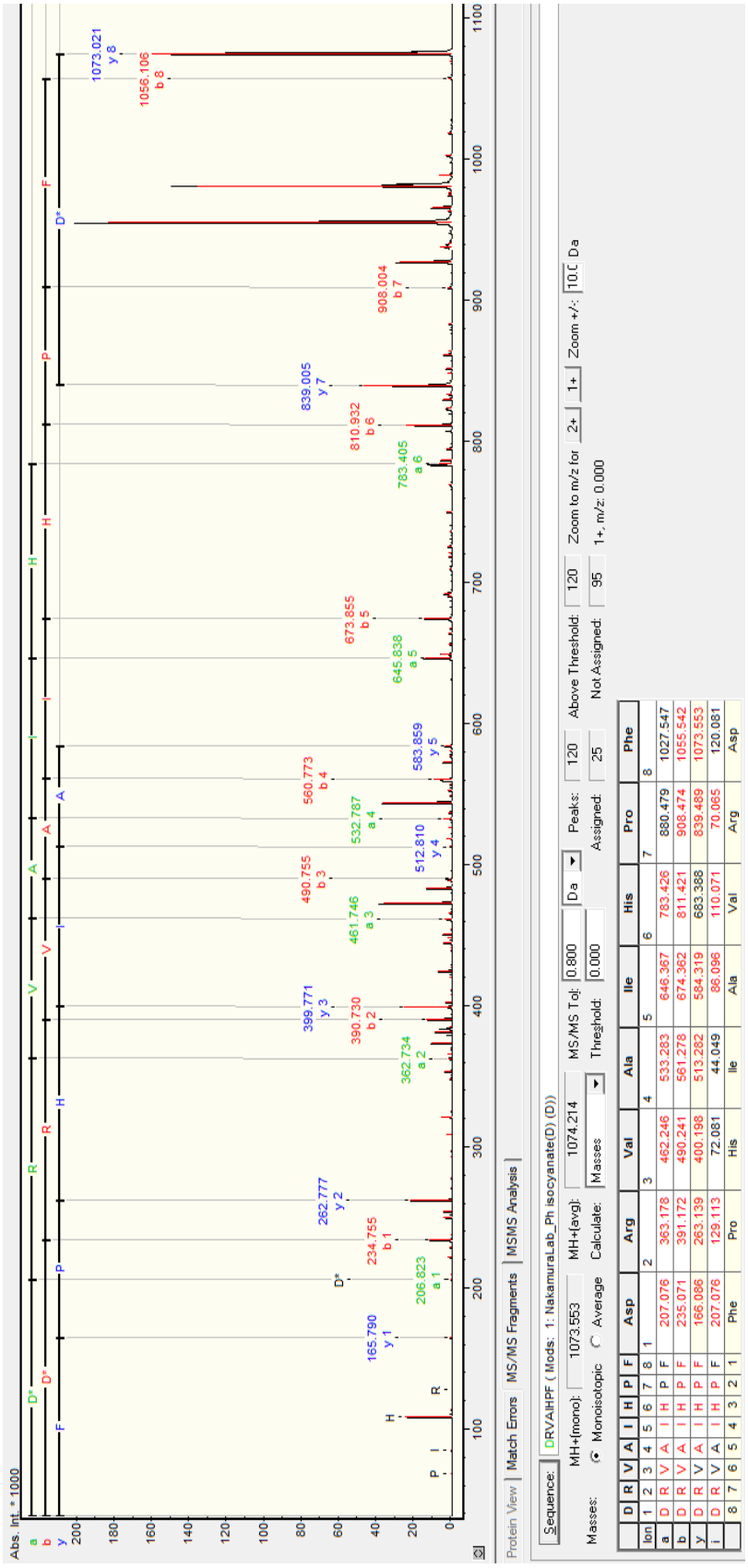


Figure S21. MS/MS analysis of phenyl isocyanate-modified peptide (DRVAIHPF) (MS: 1073, Figure S9 bottom right). N-terminal Asp belonged as phenyl isocyanate-labelled Asp.

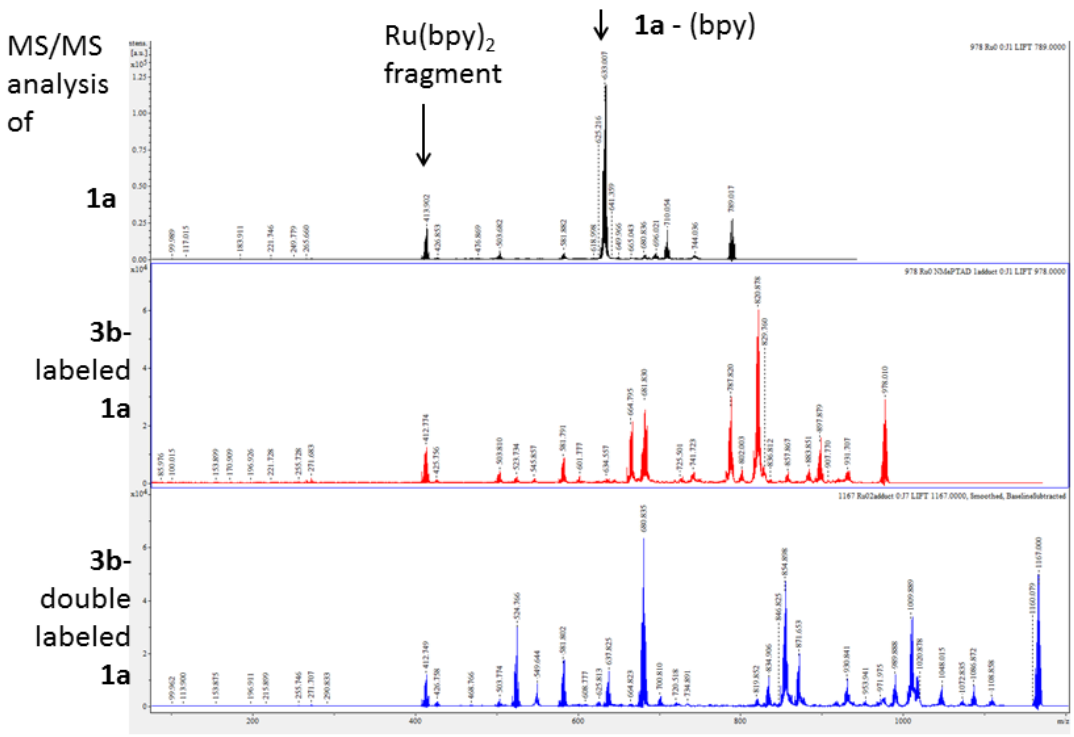


Figure S22. MS/MS analysis of **3b**-labled **1a**.

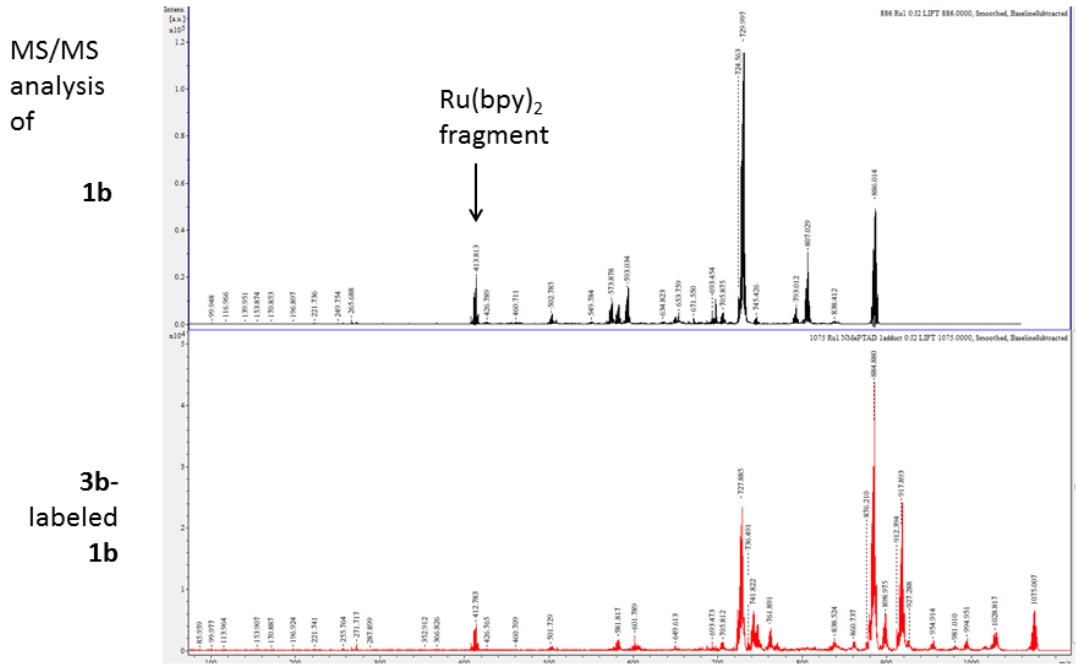


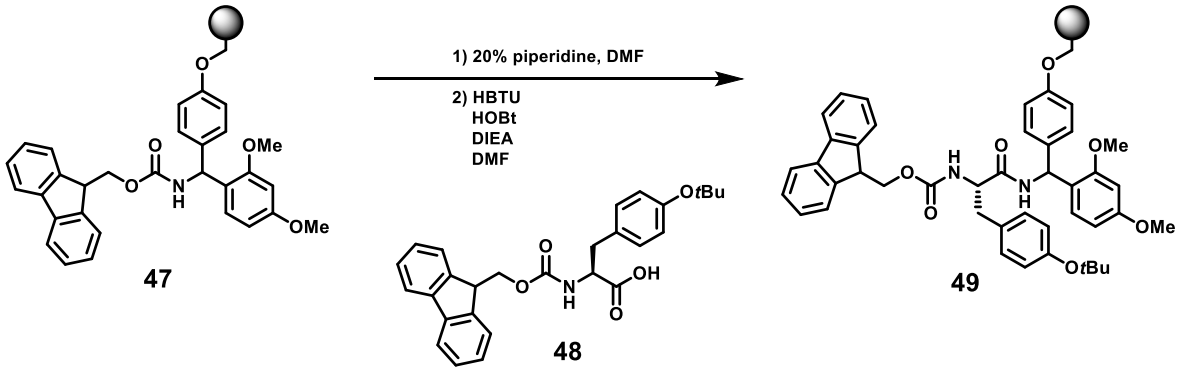
Figure S23. MS/MS analysis of **3b**-labled **1b**.

Due to the cationic structure of the ruthenium complex, only the fragments containing ruthenium complex were detected. These data indicated that bipyridine (bpy) structures were not labelled in these reactions.

2. Experimental Section

General. NMR spectra were recorded on a Bruker biospin AVANCE III (500 MHz for ^{1}H, 125 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in units parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C) or CD_3CN (1.94 ppm for ^{1}H, 118.26 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Only the strongest and/or structurally important peaks are reported as IR data given in cm^{-1}. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-TOF-MS (micrOTOF II). Analytical thin layer chromatography (TLC) was performed on a glass plate of silica gel 60 GF254 (Merck). Silica gel (Fuji Silysia, CHROMATOREX PSQ 60B, 50-200 μm) was used for column chromatography. Reverse phased column chromatography was performed with GL Science InterSep C18 or semi-preparative HPLC (YMC, Multiple Preparative HPLC LC-forte/R) using a C18 reverse phase column (Kanto, Mightysil RP-18 250 × 20 mm, 5 μm) with a 20% to 100% gradient of acetonitrile in water containing 0.1% TFA over 120 minutes. Most commercially supplied chemicals were used without further purification.

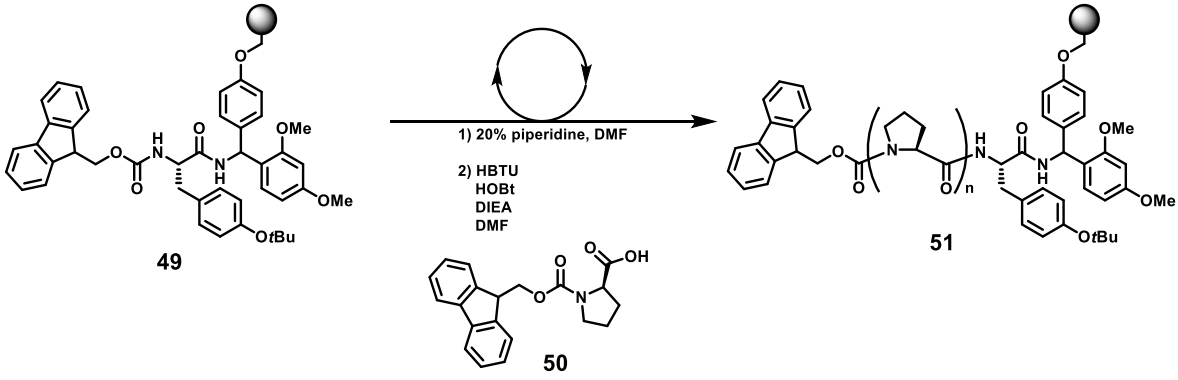
Loading of Fmoc-Tyr(*t*Bu)-OH to Fmoc-NH-SAL Resin (49).



Solid-phase peptide synthesis (SPPS) was performed in PD-10 Empty Column (GE). Fmoc-NH-SAL Resin **1** (0.1 mmol) were treated with DMF at room temperature for 12 h. After the treated was completed, the resin was washed with CH_2Cl_2 (2 mL× 3), DMF (2 mL× 3). To the Fmoc-NH-SAL Resin was added to 20% piperidine in DMF and the mixture was stirred. After being left for 1min, the solvent was removed, the Fmoc-NH-SAL Resin was added to 20% piperidine in DMF and the mixture was stirred using a vortex mixer at room temperature for 10 min. After mixing, the Fmoc-NH-SAL Resin were washed with DMF (2 mL× 5), then treated with a Fmoc-Tyr(*t*Bu)-OH (0.3 mmol), condensing agent cocktail (HBTU 3.05 g, HOBt 1.25g, DMF 16 mL) 700 μL and DIEA

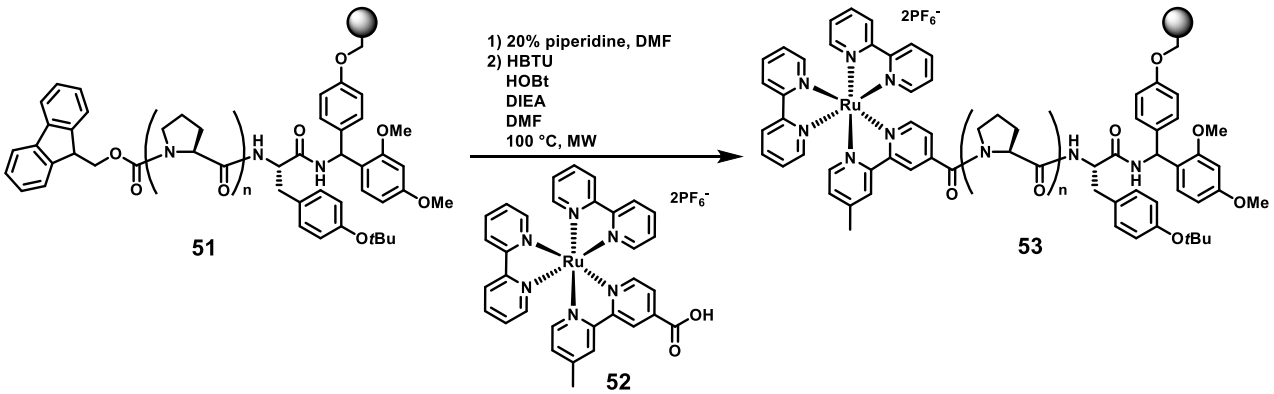
cocktail (DIEA 2.75 mL, NMP 14.25 mL) 700 μL. The mixture was stirred using a vortex mixer at room temperature for 1 h. The resulting resin was washed with DMF (2 mL×5), CH_2Cl_2 (2 mL×5), DMF (2 mL× 5) after removal of the solvent to give Fmoc-Tyr(*t*Bu)-Resin **49**. The condensation reactions were checked by Kaiser test reagent (nynhydrin/EtOH 20 μL phenol/EtOH 20 μL KCN/pyridine 20 μL).

Fmoc-(Pro)$_n$-Tyr(*t*Bu)-Resin (51).



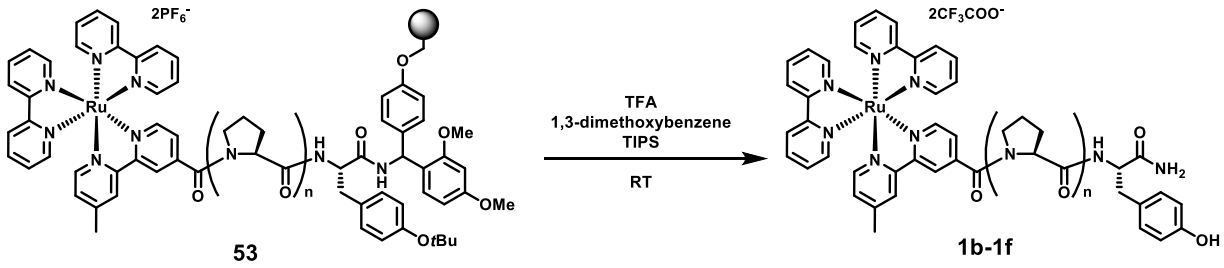
The Fmoc-Tyr(*t*Bu)-Resin **49** (0.3 mmol) was added to 20% piperidine in DMF and the mixture was stirred. After being left for 1min, the solvent was removed and the Fmoc-Tyr(*t*Bu)-Resin was added to 20% piperidine in DMF and the mixture was stirred using a vortex mixer at room temperature for 10 min. After mixing, the Fmoc-Tyr(*t*Bu)-Resin were washed with DMF (2 mL× 5), then treated with a Fmoc-Pro-OH **50** (0.3 mmol), condensing agent cocktail 700μL and DIEA cocktail 700 μL. The mixture was stirred using a vortex mixer for 1 h. The resulting resin was washed with DMF (2 mL×5), CH_2Cl_2 (2 mL×5), DMF (2 mL×5) after removal of the solvent. And the coupling reaction was repeated. After the each times reaction was completed, to give Fmoc-(Pro)$_n$-Tyr(*t*Bu)-Resin **51**. The condensation reactions were checked by Kaiser test reagent.

Ru complex-(Pro)$_n$-Tyr-Resin (53)



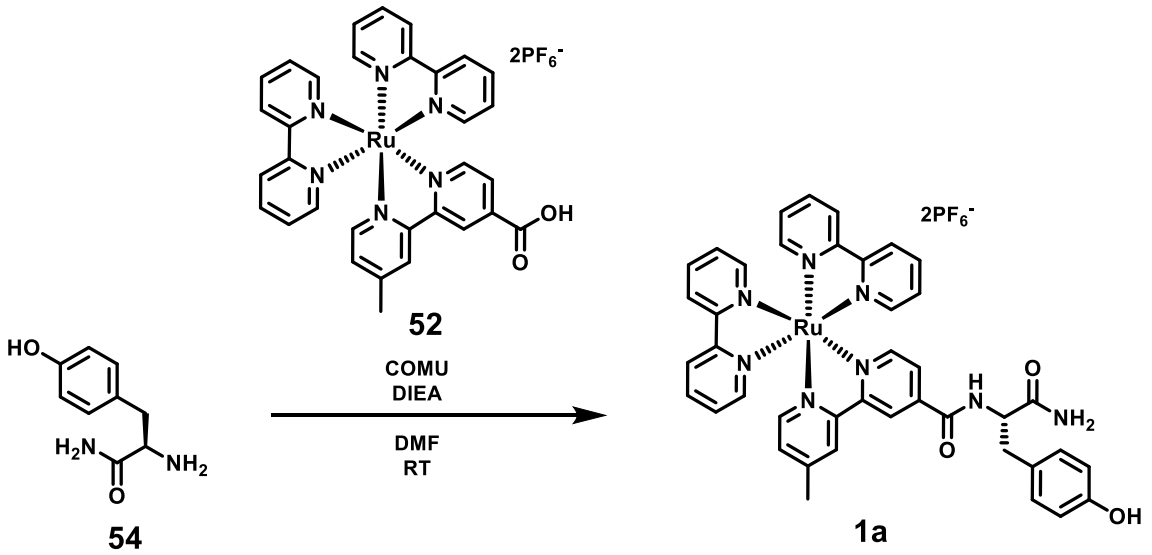
The Fmoc-$(Pro)_n$-Tyr(*t*Bu)-Resin. **8** (0.3 mmol) was added to 20% piperidine in DMF and the mixture was stirred. After being left for 1min, the solvent was removed and the Fmoc-$(Pro)_n$-Tyr(*t*Bu)-Resin. was added to 20% piperidine in DMF and the mixture was stirred using a vortex mixer for 10 min. After the reaction was, the Fmoc-$(Pro)_n$-Tyr(*t*Bu)-Resin. were washed with DMF (2 mL×5), then treated with a Ru complex (54 μmol), HBTU (540 μmol), HOBt (270 μmol), DIEA (540 μmol), DMF (2 mL) and CH_3CN (2 mL) at 100 °C for 10 min using a micro wave reactor (Biotage Initiator+). The resulting resin was washed with CH_2Cl_2 (2 mL×3), DMF (2 mL×3), CH_3OH (2 mL×3) after removal of the solvent and dried *in vacuo* to give Ru complex-$(Pro)_n$-Tyr-Resin (**53**).

Cleavage from resin and deprotection of side chain by strong acid



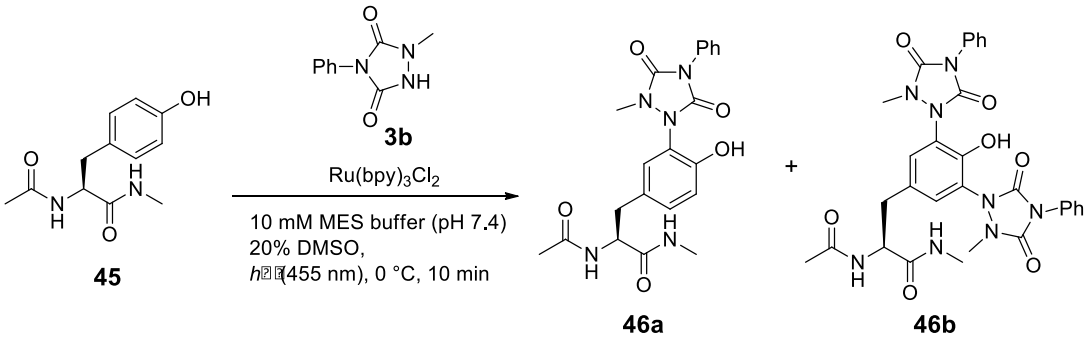
To the Ru complex-$(Pro)_n$-Tyr-Resin (**53**) was added TFA (2.38 mL), 1,3-dimethoxybenzene (128 μL), TIPS(64 μL) and the mixture was stirred at room temperature for 90 min. The mixture was filtered by using TFA (1.5 mL) and the filtrate was concentrated *in vacuo*. After the dried was, the crude compound added to Et_2O (40 mL),then the mixture was centrifuge at 3500 rpm, 4 °C for 15 min and dried *in vacuo*. The Centrifugation was repeated was three times. The Ru-conjugated peptides was purified by reverse phase column chromatography (InertSep C18 g/mL, 0.1% TFA , 5% MeCN in H_2O to 25% MeCN in H_2O), reverse phase GPC (0.1% TFA in MeOH) or semi-preparative HPLC (YMC, Multiple Preparative HPLC LC-forte/R) using a C18 reverse phase column (Kanto, Mightysil RP-18 250 × 20 mm, 5 μm) with a 20% to 100% gradient of acetonitrile in water containing 0.1% TFA over 120 minutes. Ru-conjugated peptides were obtained as red solids (up to 15.6 μmol). The purities of synthesized peptides were checked by LC-MS detecting 254 nm absorbance (see Figure S10). The micropump gradient method was used, as follows. Mobile phase A: 0.1% FA, mobile phase B: 100% acetonitrile. 0−10 min: 5% B, 10−20 min: 5−60% B, 20−22 min: 60−100% B, 22−24 min: 100% B, 24−25 min: 100−5% B, 25−35 min: 5% B.

Bis-(2,2'-bipyridine)-(4'-methyl-N-[(s)-Tyrosilamide)-(2,2'-bipyridine)-4-carboxamide]rutheniu m (II) bis-(hexafluorophosphate) (1a).



Compound **54** (11.9 mg, 0.013 mmol) was dissolved in DMF (2 mL) and **52** (5.59 mg, 0.031 mmol), COMU (6.85 mg, 0.016 mmol), DIEA (4.9 mg, 0.026 mmol) were added. The reaction mixture stirred at room temperature for 24 h. And the solvent was removed under reduced pressure. Purification by reverse phase chromatography (19:1 to 4:1 H_2O : MeOH), reverse phase GPC (MeOH 0.1% TFA) and reverse phase HPLC (80:20 to 0:10 H_2O:MeOH) to afford a solid product (6.1 mg, 44%).

^{1}H NMR (500 MHz, methanol-d_4) δ 8.87 (d, *J* = 7.8 Hz, 1H)8.66 (d, *J* = 8.1 Hz, 4H), 8.55 (d, *J* = 4.9 Hz, 1H), 8.09 (t, *J* = 7.3 Hz, 4H), 7.88 (d, *J* = 5.8 Hz, 1H), 7.76 (d, *J* = 5.8 Hz, 4H), 7.64 (ddd, *J* = 1.5, 4.3, 11.7 Hz, 1H), 7.59 (d, *J* = 5.8 Hz, 1H)7.46-7.43 (m, 4H), 7.33 (d, *J* = 5.7 Hz, 1H), 7.08 (q, *J* = 4.0 Hz, 2H), 6.64 (q, *J* = 4.0 Hz, 2H), 3.57 (q, *J* = 7.0 Hz, 1H), 2.95-2.89 (m, 1H), 2.57 (s, 3H); ^{13}C NMR (125 MHz, methanol-d_4) δ 176.0, 165.8, 159.4 (d, *J* = 3.8 Hz), 158.5-158.4 (m), 157.5, 157.4, 153.2, 152.6 (m), 152.5, 152.4, 151.8, 143.7, 139.4-139.3 (m), 131.3, 130.2, 129.0-128.9 (m), 126.8, 126.2 (d, *J* = 6.6 Hz), 125.7-125.6 (m), 123.0 (d, *J* = 8.8 Hz), 116.2, 56.9, 38.2, 21.3



Compound 46a and 46b. *N*-acyl tyrosine methylamide **45** (23.6 mg, 0.100 mmol) $Ru(bpy)_3Cl_2$ (64.1 mg, 0.100 mmol) and *N*-methyl-4-phenyl-1,2,4-triazolidine-3,5-dione **3b** (38.3 mg, 0.200 mmol) were dissolved in 10.0 mL of 10 mM MES buffer (pH 7.4) contained 20% DMSO. 1.0 mL of the mixture was stirred open to air at 0 °C for 10 min under irradiation with a blue light (RELYON, Twin LED light, 455 nm) in 50 mL round bottom flask. This reaction was repeated 10 times to react all of the mixture. After all of reaction, the reaction mixture was concentrated *in vacuo*, the residue was dissolved in MeOH, the solution was passed through a pad of silica gel and concentrated *in vacuo*. The residue was purified by PTLC with CH_2Cl_2 : MeOH = 10 : 1 and Recycle Gel Permeation Chromatography (JAIGEL-GS310,$CHCl_3$) to give **46a** (21.7 mg, 51%) and **46b** (2.0 mg, 3%) as white solid.

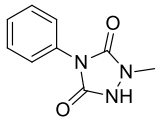
Compound 46a

Mp 146-148 °C ; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.0 Hz, 2H), 7.44 (t, *J* = 7.0 Hz, 2H), 7.36 (t, *J* = 7.0 Hz, 1H), 7.04-7.02 (m, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 6.83-6.80 (m, 2H), 4.52 (d, *J* = 7.0 Hz, 1H), 3.07 (s, 1H), 2.92-2.88 (m, 1H), 2.81-2.77 (m, 1H), 2.55 (d, *J* = 4.0 Hz, 3H), 1.83-1.81 (m, 2H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 171.0, 153.2, 152.7, 152.2, 131.9, 131.3, 129.4, 129.1, 128.8, 128.7, 125.9, 121.9, 118.5, 54.8, 37.6, 32.6, 26.2, 22.9; FT-IR (neat) 3309, 3102, 3079, 3013, 2941, 1765, 1704, 1651, 1541, 1515, 1505, 1433, 1407, 1304, 1159 cm^{-1}; HRMS (ESI, Positive): *m/z* calced. for $C_{21}H_{23}N_5O_5Na$ $[M+Na]^+$: 448.1591, found 448.1587.

Compound 46b

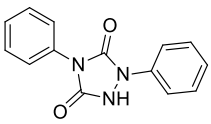
Mp 168-170 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.0 Hz, 4H), 7.49 (t, *J* = 7.5 Hz, 4H), 7.41 (t, *J* = 7.0 Hz, 2H), 7.26-7.25 (m, 2H), 6.38-6.36 (m, 1H), 6.24-6.21 (m, 1H), 4.60 (quint, *J* = 7.5 Hz, 1H), 3.21 (s, 6H), 3.06 (d, *J* = 6.5 Hz, 2H), 2.70 (d, *J* = 4.5 Hz, 3H), 1.99 (s, 3H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 153.8, 153.0, 131.0, 129.5, 128.8, 128.1, 126.4, 125.8, 54.5, 37.6, 33.7, 29.8, 26.4, 23.3; FT-IR (neat) 3338, 3066, 3014, 2949, 2925, 1769, 1711, 1652, 1541, 1504, 1430, 1405, 1291, 1164 cm^{-1}; HRMS (ESI, Positive): *m/z* calced. for $C_{21}H_{23}N_5O_5Na$ $[M+Na]^+$: 637.2130, found 637.2134.

General Procedure for preparation of 1-alkyl-4-phenylurazole 3b, 3c and 1-alkyl-4-benzylurazole 3d. To a solution of phenyl isocyanate or benzyl isocyanate (1.0 eq.) in toluene (15 mL) was added 2-alkylcarbazic acid ethyl aster (1.0 eq.) at room temperature. After stirring at 90 °C for 3 h, to the reaction mixture was added EtOAc and H_2O. The organic layer was washed by aqueous HCl solution (1 M) and saturated aqueous NaCl solution, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography with Hexane : AcOEt = 1 : 1 to give the desired 2-alkyl-1-(phenylcarbamoyl) carbazic acid ethyl ester or 2-(benzylcarbamoyl) carbazic acid ethyl ester as a precursor of **3b**, **3c** or **3d**. The precursor was dissolved in 5.0 mL of aqueous KOH solution (4 M) and stirred at 80 °C for 3 h. Then, to the reaction mixture was added aqueous HCl solution (6 M) to make the solution acidic (pH = 1), extracted by CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **3b**, **3c** or **3d**.



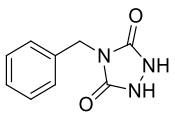
1-methyl-4-phenylurazole (3b).

White solid (Mp 186-188 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51-7.47 (m, 4H), 7.41-7.38 (m, 1H), 3.26 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.0, 153.3, 131.3, 129.4, 128.6, 125.8, 33.7; FT-IR (neat) 3183, 3061, 2977, 1766, 1685, 1596, 1506, 1441, 1407, 1314, 1152 cm^{-1}; HRMS (ESI, Negative): *m/z* calced. for $C_9H_8N_3O_2$ $[M-H]^-$: 190.0611, found 190.0613.



1,4-diphenylurazole (3c).

White solid (Mp 155-157 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, J = 8.5 Hz, 2H), 7.51-7.46 (m, 4H), 7.42-7.39 (m, 3H), 7.23 (t, J = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.6, 149.5, 136.3, 131.0, 129.5, 129.4, 128.7, 126.0, 125.9, 118.9; FT-IR (neat) 3141, 3067, 1775, 1710, 1597, 1498, 1427, 1314, 1149 cm^{-1}; HRMS (ESI, Negative): *m/z* calced. for $C_{14}H_{10}N_3O_2$ $[M-H]^-$: 252.0768, found 252.0764.

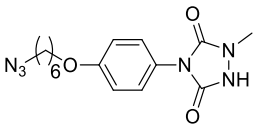


4-benzylurazole (3d).

White solid (Mp 177-179 °C); ^{1}H NMR (500 MHz, CD_3OH) δ 7.36-7.26 (m, 5H), 4.66 (s, 2H); ^{13}C NMR (125 MHz, CD_3OH) δ 156.9, 137.6, 129.7, 128.9, 128.8, 43.2; FT-IR (neat) 3156, 3027, 2928, 1767, 1682, 1657, 1595, 1495, 1432, 1315, 1218, 1146 cm^{-1}; HRMS (ESI, Negative): *m/z* calced. for $C_9H_8N_3O_2$ $[M-H]^-$: 190.0611, found 190.0613.

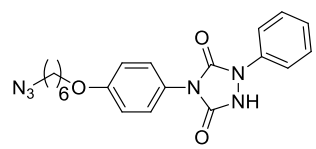
General procedure for preparation of 4-(4-((6-azidohexyl)oxy)phenyl)-1-methylurazol (8b) and 4-(4-((6-azidohexyl)oxy)phenyl)-1-phenylurazol (8c). 4-((6-azidohexyl)oxy)aniline was prepared according to previously reported procedure.[2] To a solution of triphosgene (1.0 eq.) in 3.0 mL of CH_2Cl_2 was dropwisely added the solution of 4-((6-azidohexyl)oxy)aniline (1.0 eq.) in 1.0 mL CH_2Cl_2 at 0 °C for 10 min and TEA (3.0 eq.) at 0 °C for 5 min under Ar. After stirring at room temperature for 1 h, the reaction mixture was washed by saturated aqueous NaCl solution, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in 5.0 mL of toluene and added to 2-alkyl-1-(phenylcarbamoyl) carbazic acid ethyl ester (0.9 eq.) at room temperature. After stirring at 90 °C for 3 h, the reaction mixture was quenched by MeOH and concentrated *in vacuo*. The residue was purified by silica gel chromatography with Hexane : AcOEt = 1 : 2 to give 2-((4-((6-azidohexyl)oxy)phenyl)carbamoyl)- 2-alkylcarbazic acid ethyl ester as precursor of **8b** and **8c**. Then, the precursor was dissolved in 3.0 mL of aqueous KOH solution (4 M) and stirred at 80 °C for 3 h. To the reaction mixture was added aqueous HCl solution (6 M) to make the solution acidic (pH = 1), extracted by CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **8b** or **8c**.

Compound **6**[3], **7**[3], **8a**[2] and **9**[2] were prepared according to the previously reported procedures.



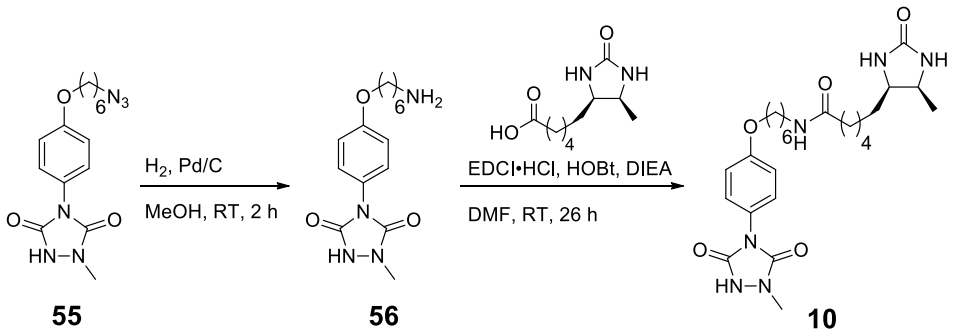
4-(4-((6-azidohexyl)oxy)phenyl)-1-methylurazol (8b).

White solid (Mp 98-99 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 9.2 Hz, 2H), 6.98 (d, *J* = 9.2 Hz, 2H), 3.99 (t, *J* = 6.6 Hz, 2H), 3.30 (t, *J* = 6.8 Hz, 2H), 3.21 (s, 3H), 1.83-1.77 (m, 2H), 1.67-1.60 (m, 2H), 1.54-1.44 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 154.2, 153.3, 127.4, 123.6, 115.2, 68.1, 51.5, 33.5, 29.1, 28.9, 26.6, 25.7; FT-IR (neat) 3186, 3066, 2939, 2865, 2097, 1761, 1692, 1611, 1545, 1471, 1437, 1324, 1177 cm^{-1}; HRMS (ESI, Negative): *m/z* calced. for $C_{15}H_{19}N_6O_3$ $[M-H]^-$: 331.1513, found 331.1512.



4-(4-((6-azidohexyl)oxy)phenyl)-1-phenylurazol (8c).

White solid (Mp 102-104 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.0 Hz, 2H), 7.43-7.37 (m, 4H), 7.26-7.22 (m, 1H), 6.96 (d, *J* = 4.5 Hz, 2H), 3.97 (t, *J* = 6.0 Hz, 2H), 3.28 (t, *J* = 7.0 Hz, 2H) 1.82-1.78 (m, 2H), 1.66-1.61 (m, 2H), 1.52-1.43 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.2, 153.8, 149.8, 136.4, 129.5, 127.5, 125.8, 123.3, 118.8, 115.2, 114.9, 68.1, 51.5, 29.1, 28.9, 26.6, 25.8; FT-IR (neat) 3184, 3069, 2938, 2860, 2095, 1772, 1702, 1597, 1435, 1303, 1249, 1173 cm^{-1}; HRMS (ESI, Negative): *m/z* calced. for $C_{20}H_{21}N_6O_3$ $[M-H]^-$: 393.1670, found 393.1669.



55 **56** **10**

Compound 10. To a solution of **55** (41.5 mg, 0.125 mmol) in 1.0 mL of MeOH was added 4.0 mg of 10% Pd/C (50% moisture content). After stirring for 2 h under H_2 at room temperature, Pd/C was removed by Celite filtration and the filtrate was concentrated under the reduced pressure to give **56**. Then, D-desthiobiotin (24.1 mg, 0.113 mmol), HOBt·H_2O (26.0 mg, 0.170 mmol) and EDCI·HCl (20.1 mg, 0.136 mmol) were added to a solution of **56** in 2.0 mL of DMF at room temperature. After stirring at room temperature for 26 h, the reaction mixture was concentrated *in vacuo*. The residue was purified PTLC with $CHCl_3$:MeOH = 6 : 1 and HPLC (50-100% MeOH/H_2O) to give **10** as a white solid (14.9 mg, 26%).

Mp 170-172 °C ; ^{1}H NMR (500 MHz, CD_3OD) δ 7.31 (d, *J* = 9.0 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 4.02 (t, *J* = 6.0 Hz, 2H), 3.82-3.79 (m, 1H), 3.71-3.67 (m, 2H), 3.22 (s, 3H), 3.18 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 7.3 Hz, 2H), 1.81-1.77 (m, 2H), 1.64-1.60 (m, 2H), 1.57-1.31 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 160.5, 154.9, 154.8, 128.9, 125.3, 115.9, 69.2, 57.4, 52.7, 40.2, 37.0, 33.0, 30.7, 30.3, 30.2, 30.1, 27.7, 27.1, 26.9, 26.8, 15.6; FT-IR (neat) 3293, 3079, 2932, 2860, 1698, 1515, 1432, 1248, 1222 cm^{-1}; HRMS (ESI, Negative): *m/z* calced. for $C_{25}H_{37}N_6O_5$ $[M-H]^-$: 501.2820, found 501.2816.

Labelling of ruthenium-conjugated peptide.

To a solution of Ru-$(Pro)_n$-Tyr **1a-1f** (final concentration 10 μM) in MES buffer (10 mM, pH 6.0), labelling reagent (a 30 mM stock solution in DMSO, final concentration 300 μM) were added, and the mixture was briefly vortexed and incubated at room temperature for 5 min. The solution was vortexed and the reaction was performed with the irradiation of the light (RELYON, Twin LED light, 455 nm) on ice 0.5 cm from the light source for 5 min irradiated with light on ice for 5 min. The reaction mixture (0.5 μL) with 0.1% TFA 0.5 μL was mixed with 0.5 μL of CHCA solution (0.5 mg/mL solution in acetonitrile : 0.1% TFA = 1 : 1) on MALDI-TOF plate and dried at room temperature. The modified protein peaks were detected by MALDI-TOF MS (Bruker, UltrafleXtreme).

LC-MS analysis of Labelled 1a with NMePTAD

The peptide labelled according to the above method was detected by LC-MS detecting 455 nm absorbance for ruthenium complex. The micropump gradient method was used, as follows. Mobile phase A: 0.1% FA, mobile phase B: 100% acetonitrile. 0−10 min: 5% B, 10−20 min: 5−60% B, 20−22 min: 60−100% B, 22−24 min: 100% B, 24−25 min: 100−5% B, 25−35 min: 5% B.

Preparation of crude cellular extract

HeLa cells (2.0×10^7 cell) or MCF7 cells (1.0×10^7 cell) was washed with phosphate buffered saline three times and added to 1 mL of EDTA. After incubation for 5 min at 37 °C, the suspension was added to 4 mL of phosphate buffered saline and supernatant were removed by centrifugation (1500 rpm, r.t., 3 min). The cells were washed with phosphate buffered saline three times and 1.0 mL of Lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 v/v% Triton X) was added. After incubation on the ice for 15 min, the sample was vortexed for 10 s and centrifuged (13200 rpm, 4 °C, 7 min) to give supernatant as HeLa cell lysate or MCF7 cell lysate. The concentration of HeLa cell lysate or MCF7 cell lysate was determined by protein BCA assay.

CA-selective labelling in HeLa cell lysate

HeLa cell lyate was diluted in MES buffer (1.0 mg/mL proteins, pH 6.0) adding bovine carbonic anhydrase II (Aldrich, 1 μM). Labelling reagent (a 50 mM stock solution in DMSO, final concentration 0.5 mM) and **5** (a 100 μM stock solution in DMSO, final concentration 1 μM) were added to the solution (50 μL), and the mixture was incubated at room temperature for 15 min. The light irradiation ((RELYON, Twin LED light, 455 nm, 230 mW/cm^2) was performed on ice 0.5 cm from the light source for 5 min. The reaction mixture was added to

2-iodoacetamide (a 1 M stock solution in H_2O, final concentration 1mM), incubated at room temperature for 90 min. Then, DBCO-Cy3 (Aldrich) (10 mM stock solution in DMF, final concentration 100 μM) was added, and the mixture was incubated at 37 °C for 30 min. The resulted samples were added 5 x SDS-PAGE sample buffer and heated 95 °C for 5 min. Proteins were separated by SDS-PAGE using 4-20% acrylamide gels (Biorad). Fluorescence of modified proteins was detected with a Molecular Imager Fusion Solo S (VILBER LOURMAT). After obtaining of fluorescent image, the same gel was visualized with Coomassie brilliant blue (CBB) stain, and the image was obtained with a Molecular Imager ChemiDoc XRS system (Bio-Rad).

Enrichment of desthiobiotin-labelled proteins

Labelling reagent **10** (a 50 mM stock solution in DMSO, final concentration 0.5 mM) and **5** (a 100 μM stock solution in DMSO, final concentration 1 μM) were added to CA containing HeLa cell lysate (50 μL x 4 vials, 1 μM CA, 1.0 mg/mL proteins, in MES buffer pH 6.0), and the mixture was incubated at room temperature for 15 min. The light irradiation (RELYON, Twin LED light, 455 nm, 230 mW/cm^2) was performed on ice 0.5 cm from the light source for 5 min. After removing excess amount of **10** by Sephadex G-25 column (GE), streptavidin FG beads (Tamagawa Seki) (0.25 mg) was added to the labelled protein solution 200 μL, and shaken at room temperature for 30 min. The beads was washed with lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 v/v% Triton X) three times. For the elution of destiobiotin labelled proteins, 2 mM biotin containing lysis buffer (10 μL) was incubated with the beads at room temperature for 60 min. The supernatant was collected by magnetic separation and analyzed by SDS-PAGE. The proteins in SDS-PAGE gel were visualized by Silver Stain KANTO III (Kanto). The bands at ~60 kDa in the fractions of enriched proteins are thought to be the contaminated keratin even by carefully manipulation in clean bench.

Determination of labelling efficiency

Labelling reagent **10** (a 50 mM stock solution in DMSO, final concentration 0.5 mM) and **5** (a 100 μM stock solution in DMSO, final concentration 1 μM) were added to 1 μM CA solution (50 μL x 10 vials, in MES buffer pH 7.4), and the mixture was incubated on ice for 60 min. Labelling reaction was operated above described procedure, and protein was precipitated by 2-D clean up kit (BioRad). The residue was rehydrated with lysis buffer, to the solution was added Dynabeads^{TM}MyOne^{TM}Streptavidin C1 (Invitrogen) (0.5 mg) and shaken at room temperature for 60 min. The beads were washed with lysis buffer three times. For the elution of destiobiotin labelled proteins, to the beads was added DTB elution buffer (2 mM biotin, 200 mM DTT, 1x NuPAGE buffer (Thermo)) (50 μL) and incubated at 95 °C for 5 min. Then, the supernatant was

collected by magnetic separation, analyzed by above-described method.

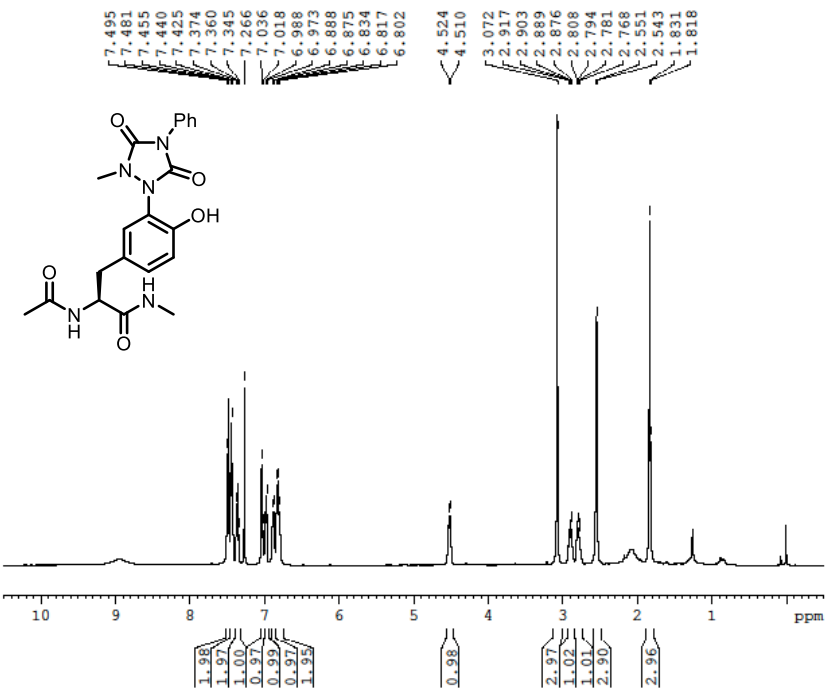
Electrochemical measurement

Electrochemical measurements were made with a Hokuto-Denko HSV-110 analyzer. A three electrode system which consists of glassy carbon working electrode, a platinum wire counter electrode and an Ag/AgCl (in saturated aqueous KCl solution) reference electrode was used. The CV measurement were performed in 200 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.4) which contained 10% DMSO (for dissolution of substrate) and 1 mM substrate at room temperature. A scan rate of 100 mV/s was applied.

Enzymatic digestion of CA

The SDS-PAGE gel bands of CA and labelled CA were cut and destained using 50% acetonitrile / 0.1% TFA. The cut gel was treated with trypsin (Promega) in Tris buffer (pH 8.0), and incubated overnight at 37 °C. After quenching with final 0.1% TFA, 100 mM Na_2HPO_4 was added to pH 7.4, added Glu-C endoproteinase (Thermo), and incubated overnight at 37 °C. The digested peptide mixtures were puridied and concentrated on Millipore Zip Tip® C18 Pipette. The peaks were detected by MALDI-TOF (Bruker, UltrafleXtreme), and were assigned by Biotools (Bruker).

3. ^{1}H and ^{13}C NMR Spectra of Compounds

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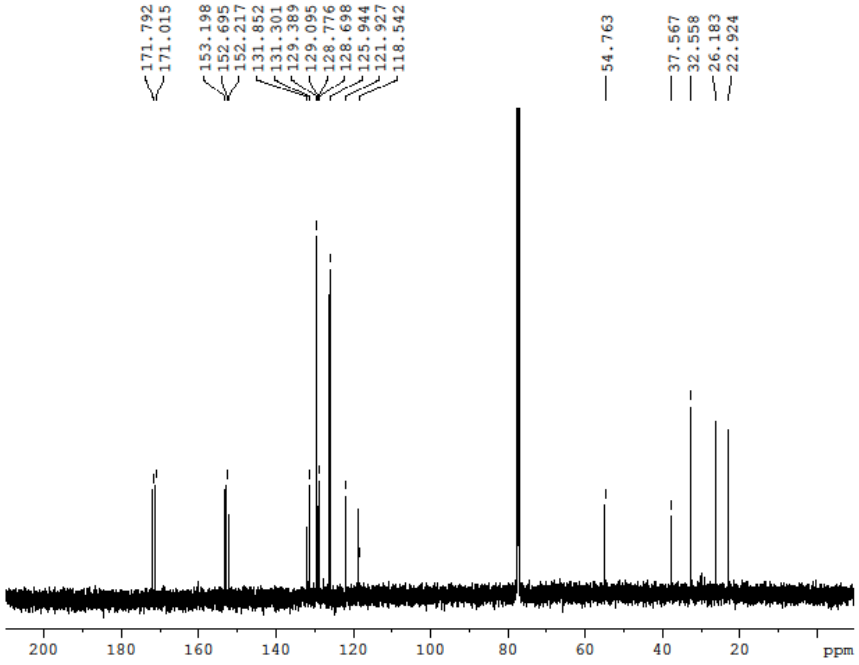


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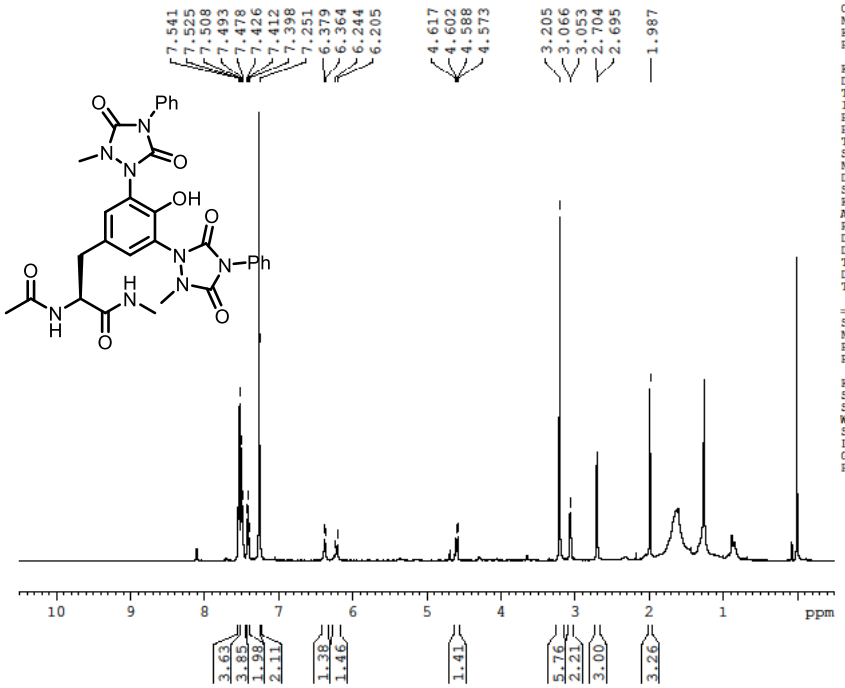
SFO2         500.1323506 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         14.50000000 W
PLW12         0.32624999 W
PLW13         0.20880000 W

F2 - Processing parameters
SI                32768
SF           125.7577743 MHz
WDW                  EM
SSB       0
```

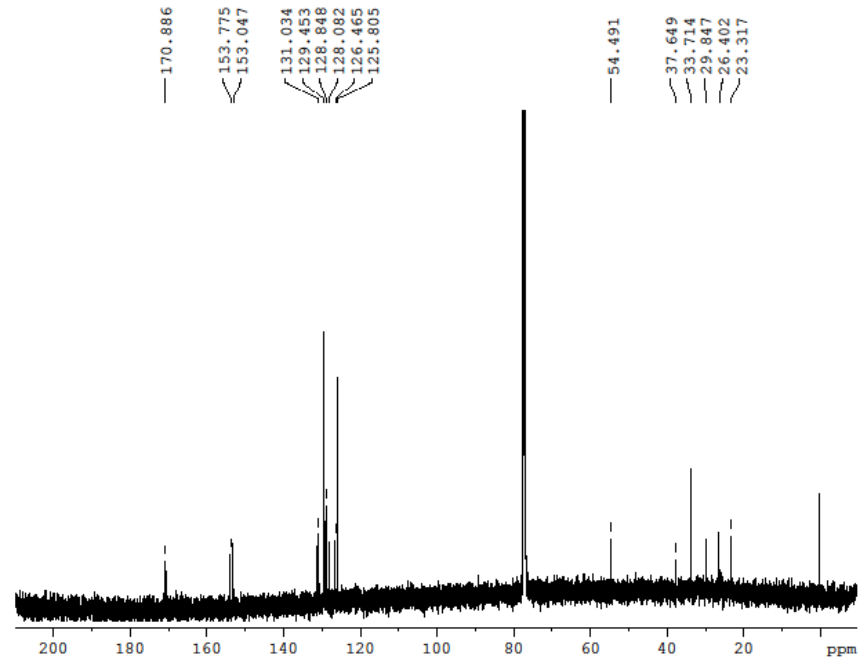



```
Current Data Parameters
NAME      tsushima-170802-CRL20-357-
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20170802
Time              10.53
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10000.000 Hz
FIDRES        0.152588 Hz
AQ            3.2767999 sec
RG                46.16
DW              50.000 usec
DE              10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1         500.1323506 MHz
NUC1                 1H
P1                12.00 usec
PLW1         14.50000000 W

F2 - Processing parameters
SI                65536
SF           500.1300121 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



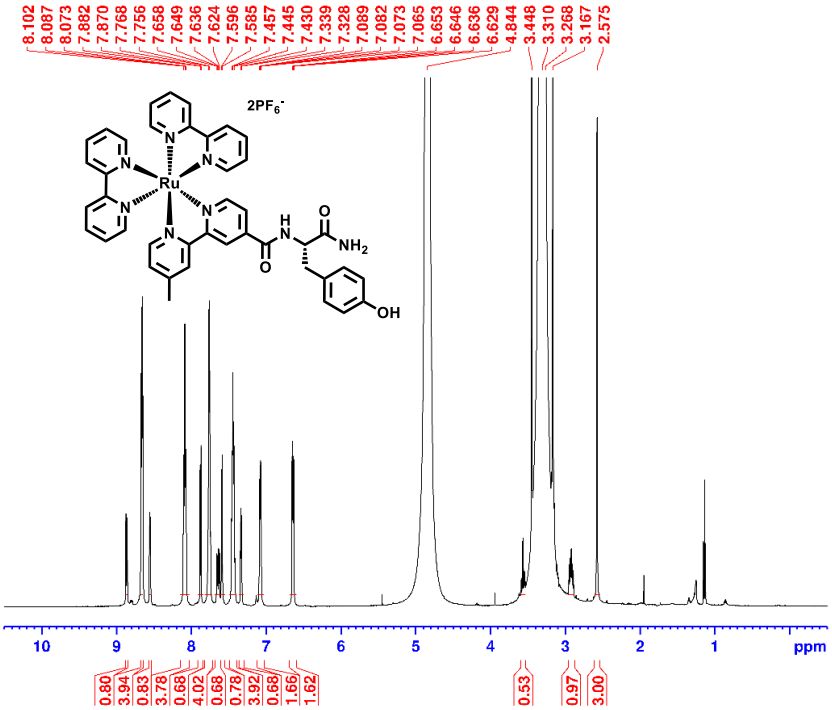

```
Current Data Parameters
NAME      tsushima-170802-CRL20-357-
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20170802
Time              20.42
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG         zgpg30
TD               65536
SOLVENT           CDCl3
NS                  780
DS                    4
SWH           27573.529 Hz
FIDRES        0.420739 Hz
AQ            1.1883861 sec
RG                191.56
DW              18.133 usec
DE                18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1         125.7703648 MHz
NUC1                 13C
P1                10.00 usec
PLW1         55.70000076 W

======== CHANNEL f2 =======
SFO2         500.1323506 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         14.50000000 W
PLW12         0.32624999 W
PLW13         0.20880000 W

F2 - Processing parameters
SI                32768
SF           125.7577718 MHz
WDW                  EM
SSB       0
```

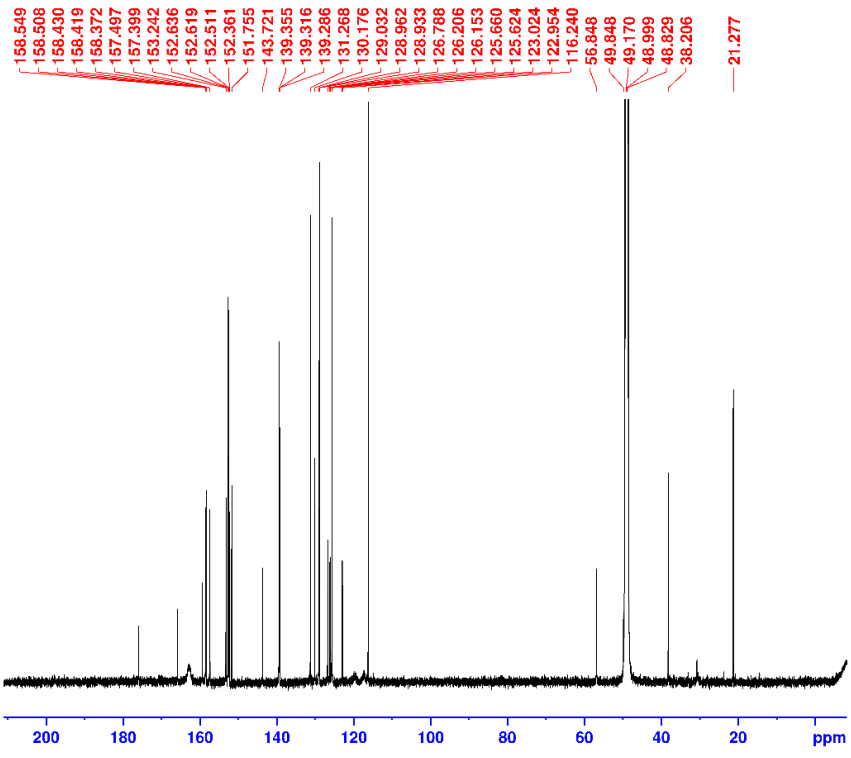


Current Data Parameters
NAME CRL16-462-HPLC-shigemi 01 1H
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20161129
Time 20.55
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 256
DS 4
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 11.85
DW 50.000 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 500.1323506 MHz
NUC1 1H
P1 12.00 usec
PLW1 14.50000000 W

F2 - Processing parameters
SI 65536
SF 500.1300291 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



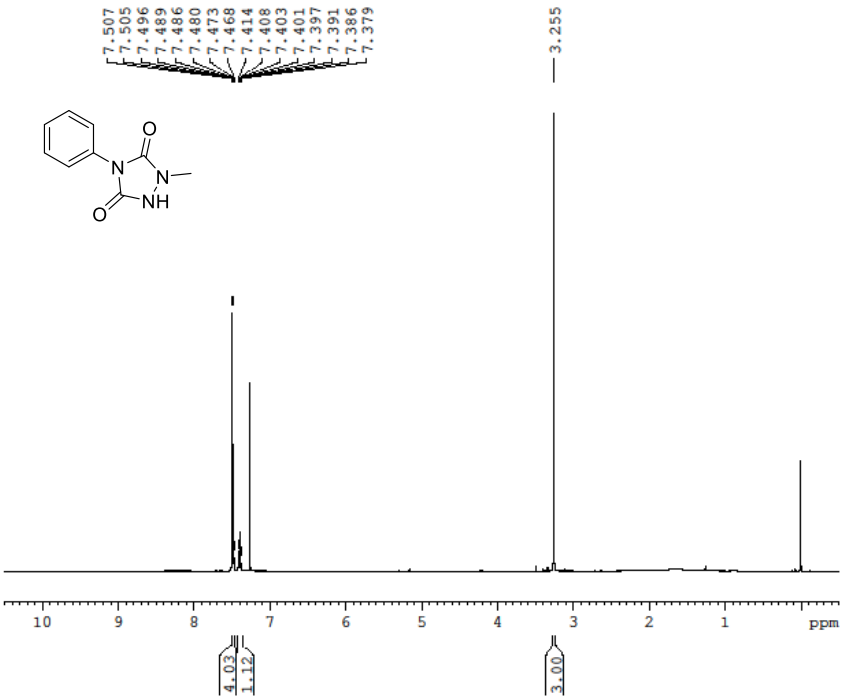
Current Data Parameters
NAME CRL16-462-HPLC-shigemi 01 13C
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20161201
Time 9.21
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 11838
DS 4
SWH 27573.529 Hz
FIDRES 0.420739 Hz
AQ 1.1883861 sec
RG 191.56
DW 18.133 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 125.7703648 MHz
NUC1 13C
P1 10.00 usec
PLW1 55.70000076 W

======== CHANNEL f2 ========
SFO2 500.1323506 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 14.50000000 W
PLW12 0.32624999 W
PLW13 0.20880000 W

F2 - Processing parameters
SI 32768
SF 125.7576131 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

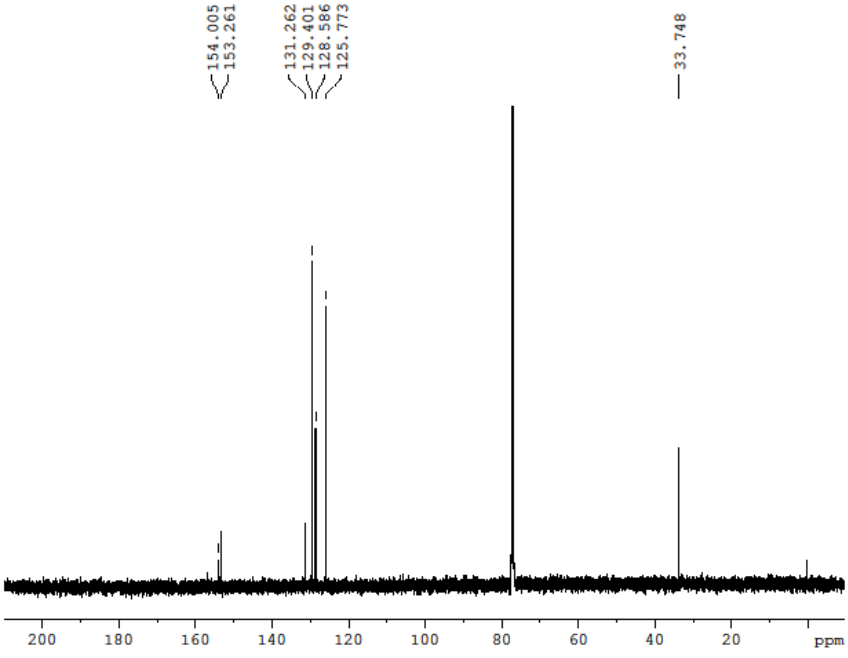



```
Current Data Parameters
NAME      tsushima-170617-CRL20-346-
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20170617
Time              14.53
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10000.000 Hz
FIDRES            0.152588 Hz
AQ            3.2767999 sec
RG                46.16
DW                50.000 usec
DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1          500.1323506 MHz
NUC1                  1H
P1                12.00 usec
PLW1          14.50000000 W

F2 - Processing parameters
SI                65536
SF            500.1300118 MHz
WDW                   EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



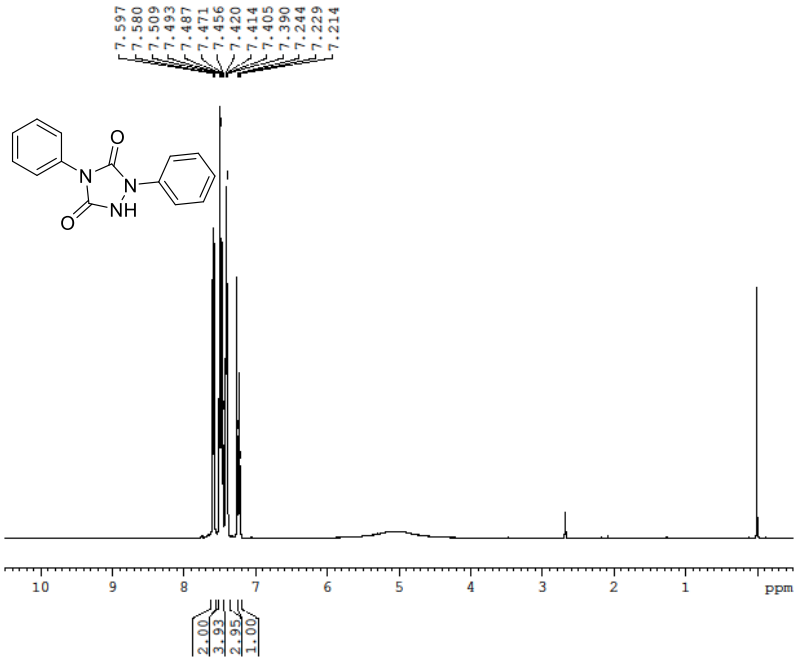

```
Current Data Parameters
NAME      tsushima-171108-NMePTAD-ca
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20171108
Time              11.50
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zgpg30
TD               65536
SOLVENT           CDCl3
NS                   42
DS                    4
SWH           27573.529 Hz
FIDRES            0.420739 Hz
AQ            1.1883861 sec
RG                191.56
DW                18.133 usec
DE                18.00 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1          125.7703648 MHz
NUC1                  13C
P1                10.00 usec
PLW1          55.70000076 W

======== CHANNEL f2 =======
SFO2          500.1323506 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          14.50000000 W
PLW12          0.32624999 W
PLW13          0.20880000 W

F2 - Processing parameters
SI                32768
SF            125.7577726 MHz
WDW                   EM
SSB       0
```

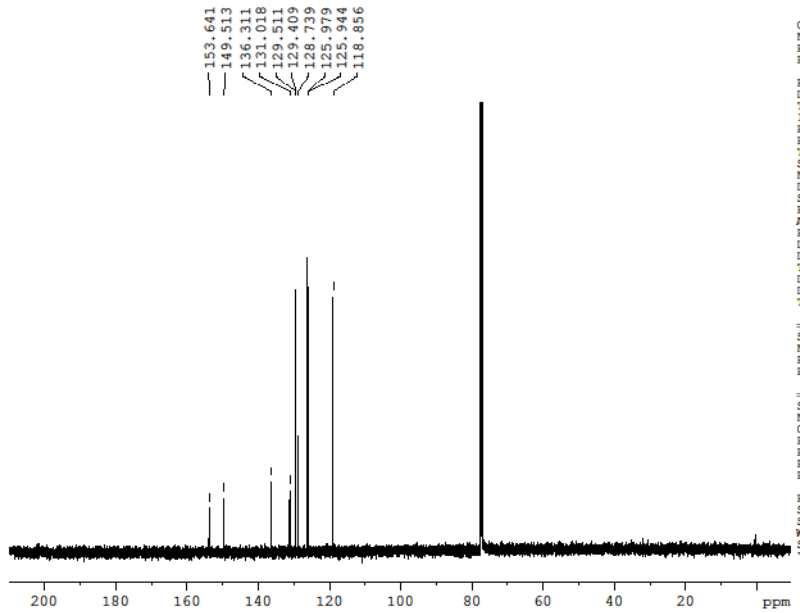



```
Current Data Parameters
NAME       tsushima-171108-NPhPTAD-pr
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20171108
Time              11.17
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   4
SWH          10000.000 Hz
FIDRES         0.152588 Hz
AQ             3.2767999 sec
RG                46.16
DW                50.000 usec
DE                10.00 usec
TE                298.2 K
D1            1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         500.1323506 MHz
NUC1                1H
P1                 12.00 usec
PLW1        14.50000000 W

F2 - Processing parameters
SI                 65536
SF           500.1300132 MHz
WDW                EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



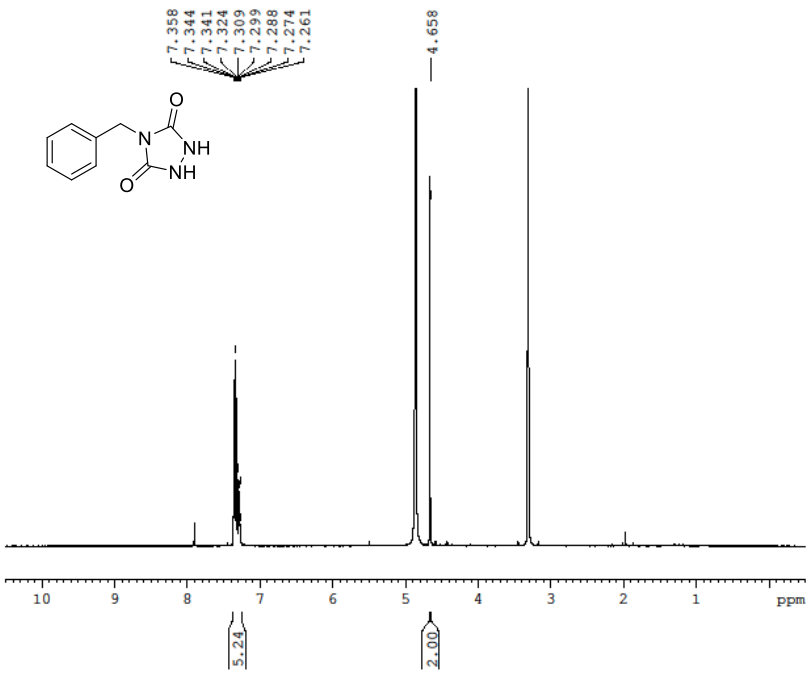

```
Current Data Parameters
NAME       tsushima-171108-NPhPTAD-ca
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20171108
Time              11.57
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG          zgpg30
TD               65536
SOLVENT          CDCl3
NS                  63
DS                   4
SWH          27573.529 Hz
FIDRES         0.420739 Hz
AQ             1.1883861 sec
RG                191.56
DW                18.133 usec
DE                18.00 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         125.7703648 MHz
NUC1                13C
P1                 10.00 usec
PLW1        55.70000076 W

======== CHANNEL f2 ========
SFO2         500.1323506 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2        14.50000000 W
PLW12        0.32624999 W
PLW13        0.20880000 W

F2 - Processing parameters
SI                 32768
SF           125.7577729 MHz
WDW                EM
SSB       0
```

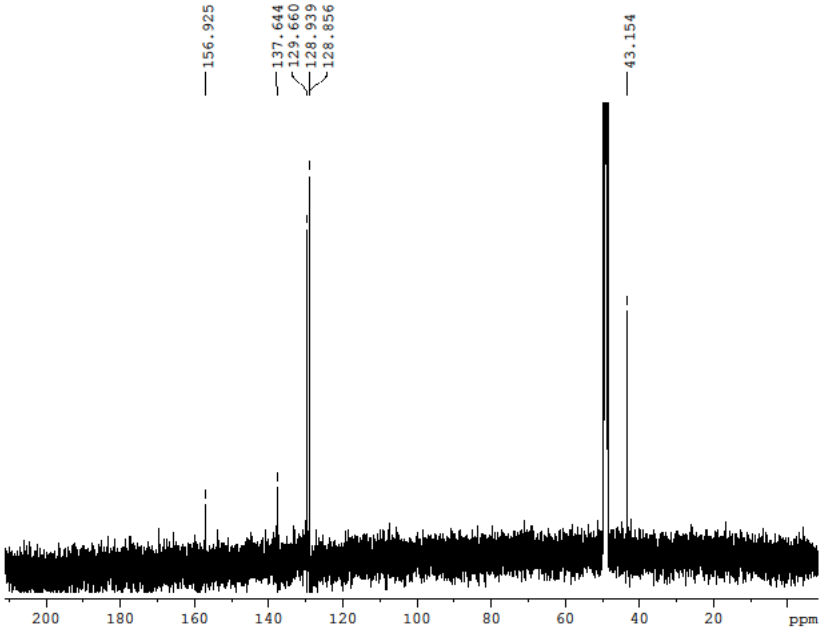



```
Current Data Parameters
NAME      tsushima-171109-BnTAD-prot
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20171109
Time              1.22
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    4
SWH           10000.000 Hz
FIDRES            0.152588 Hz
AQ             3.2767999 sec
RG                 46.16
DW                50.000 usec
DE                10.00 usec
TE                298.2 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1          500.1323506 MHz
NUC1                  1H
P1                 12.00 usec
PLW1          14.50000000 W

F2 - Processing parameters
SI                65536
SF          500.1300096 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



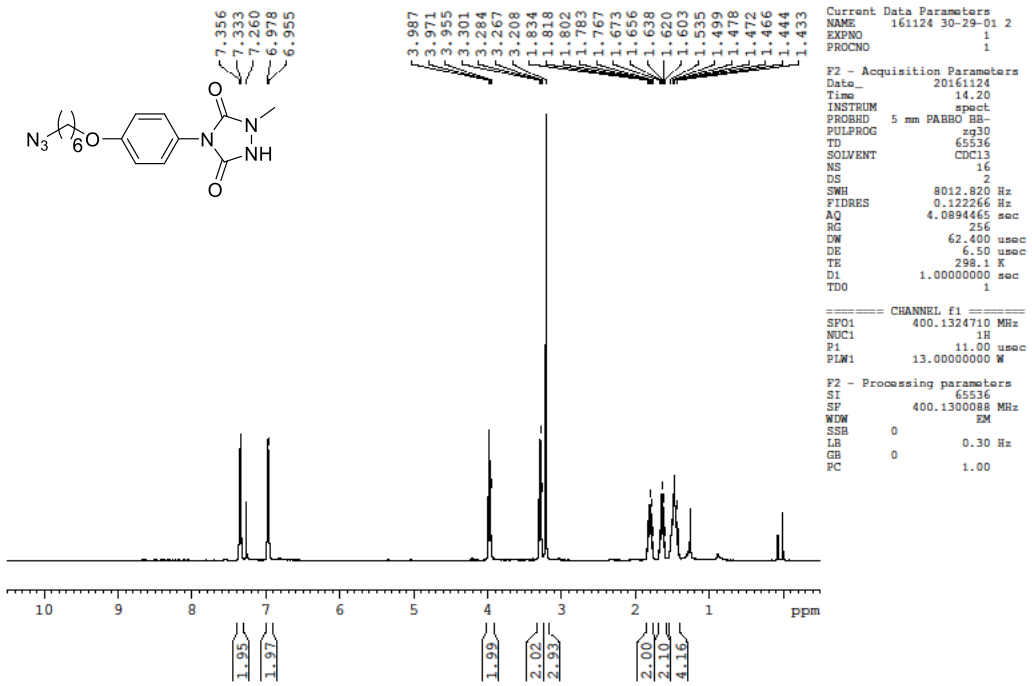

```
Current Data Parameters
NAME      tsushima-171109-BnTAD-carb
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20171109
Time              1.49
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                  433
DS                    4
SWH           27573.529 Hz
FIDRES            0.420739 Hz
AQ             1.1883861 sec
RG                191.56
DW                18.133 usec
DE                18.00 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 =======
SFO1          125.7703648 MHz
NUC1                  13C
P1                10.00 usec
PLW1          55.70000076 W

======== CHANNEL f2 =======
SFO2          500.1323506 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          14.50000000 W
PLW12          0.32624999 W
PLW13          0.20880000 W

F2 - Processing parameters
SI                32768
SF          125.7576114 MHz
WDW                  EM
SSB       0
```

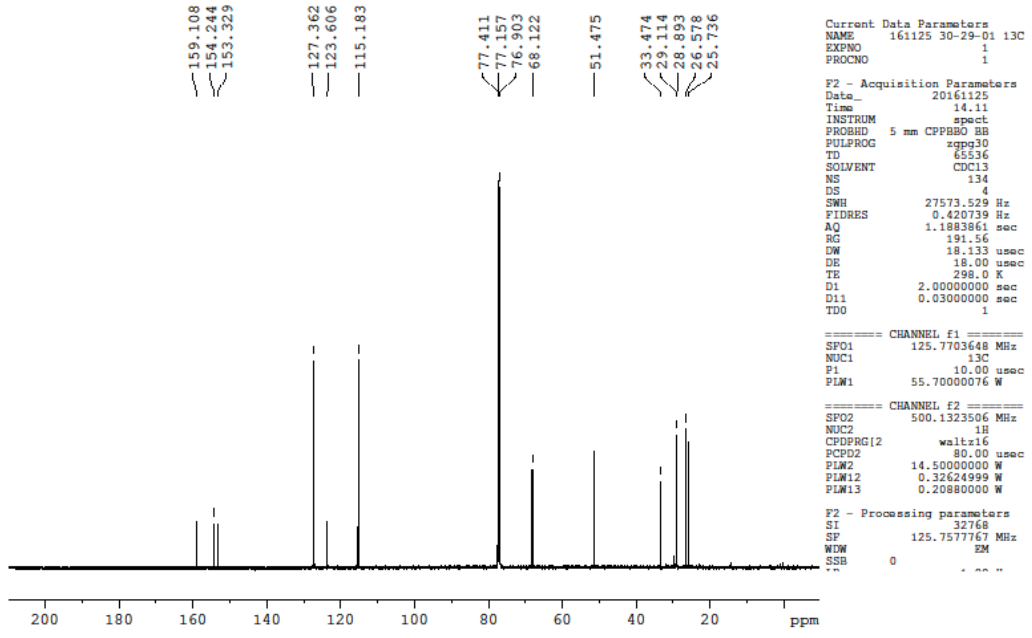



```
Current Data Parameters
NAME      161124 30-29-01 2
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20161124
Time                  14.20
INSTRUM               spect
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       16
DS                        2
SWH                8012.820 Hz
FIDRES             0.122266 Hz
AQ                 4.0894465 sec
RG                      256
DW                   62.400 usec
DE                    6.50 usec
TE                   298.1 K
D1                1.00000000 sec
TD0                      1

======== CHANNEL f1 =======
SFO1             400.1324710 MHz
NUC1                     1H
P1                    11.00 usec
PLW1             13.00000000 W

F2 - Processing parameters
SI                    65536
SF               400.1300088 MHz
WDW                      EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.00
```



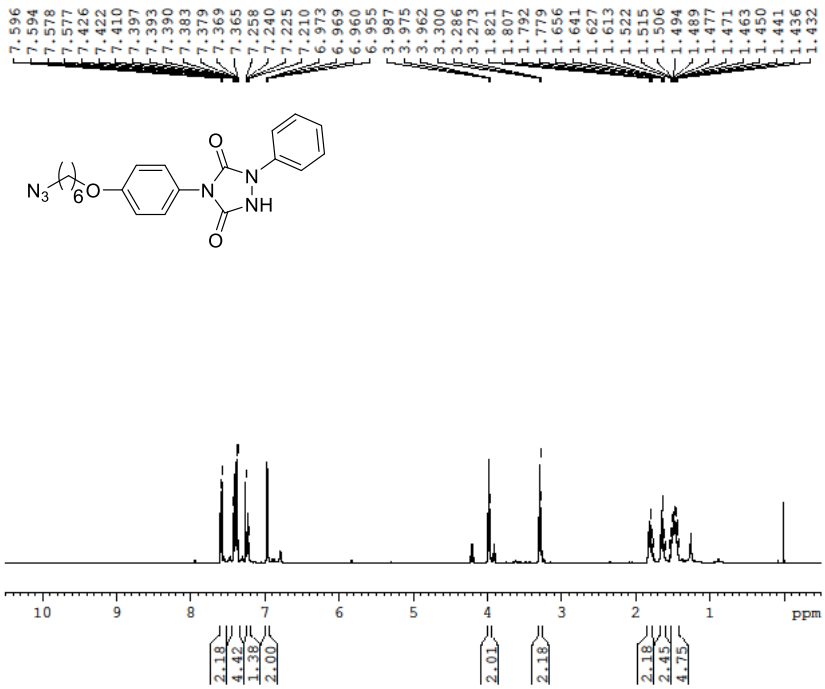

```
Current Data Parameters
NAME      161125 30-29-01 13C
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20161125
Time                  14.11
INSTRUM               spect
PROBHD    5 mm CPPBBO BB
PULPROG             zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      134
DS                        4
SWH               27573.529 Hz
FIDRES             0.420739 Hz
AQ                 1.1883861 sec
RG                    191.56
DW                   18.133 usec
DE                    18.00 usec
TE                   298.0 K
D1                2.00000000 sec
D11               0.03000000 sec
TD0                      1

======== CHANNEL f1 =======
SFO1             125.7703648 MHz
NUC1                    13C
P1                    10.00 usec
PLW1             55.70000076 W

======== CHANNEL f2 =======
SFO2             500.1323506 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                 80.00 usec
PLW2             14.50000000 W
PLW12             0.32624999 W
PLW13             0.20880000 W

F2 - Processing parameters
SI                    32768
SF               125.7577767 MHz
WDW                      EM
SSB          0
```

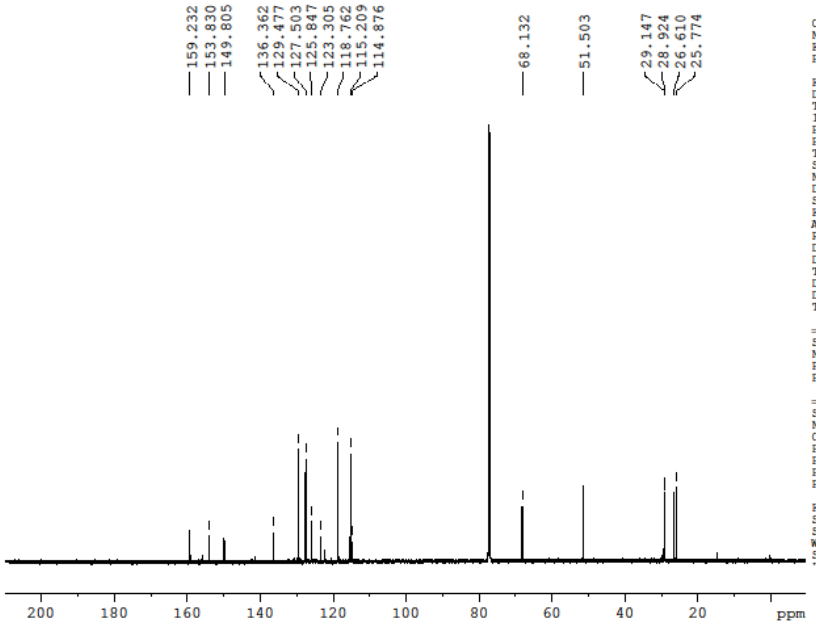



```
Current Data Parameters
NAME      tsushima-170314-CRL20-309-
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20170314
Time              20.36
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zg30
TD               65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10000.000 Hz
FIDRES        0.152588 Hz
AQ            3.2767999 sec
RG                42.17
DW               50.000 usec
DE               10.00 usec
TE               298.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         500.1323506 MHz
NUC1                 1H
P1                12.00 usec
PLW1         14.50000000 W

F2 - Processing parameters
SI                65536
SF           500.1300128 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



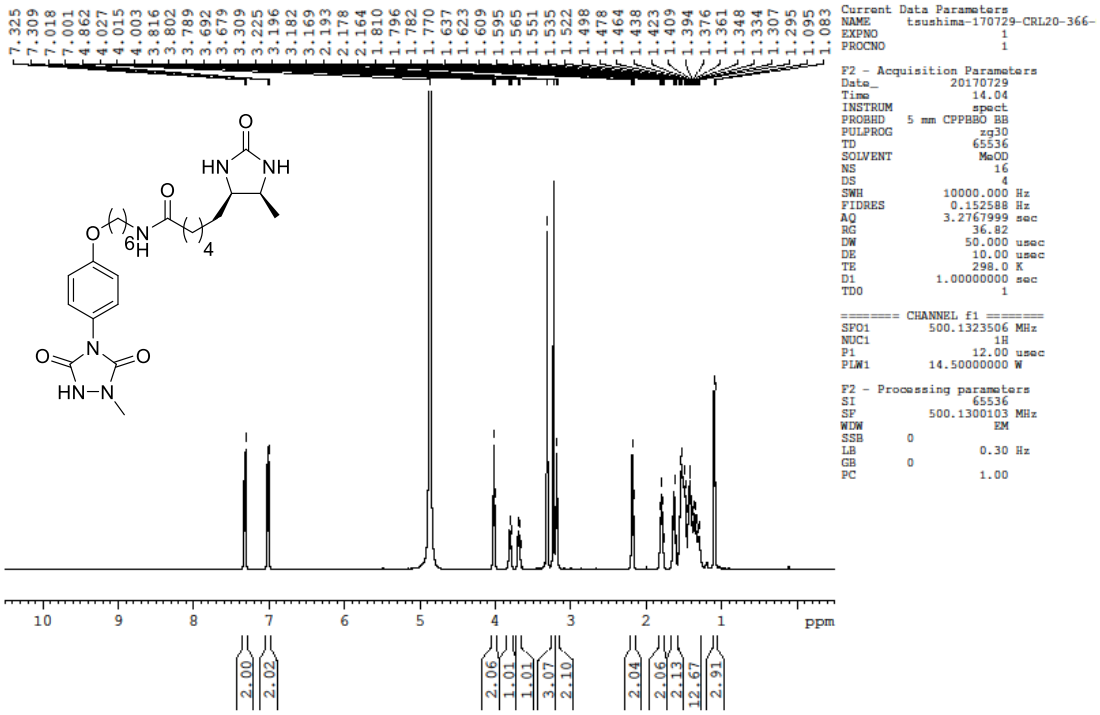

```
Current Data Parameters
NAME      tsushima-170314-CRL20-309-
EXPNO                 1
PROCNO                1

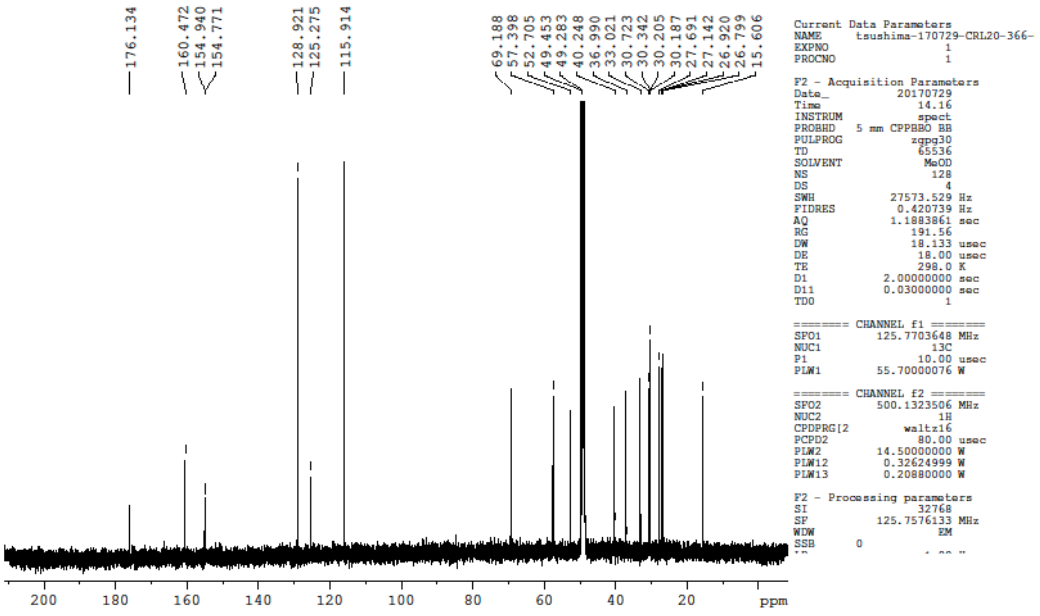
F2 - Acquisition Parameters
Date_           20170314
Time              20.48
INSTRUM           spect
PROBHD   5 mm CPPBBO BB
PULPROG           zgpg30
TD               65536
SOLVENT           CDCl3
NS                  128
DS                    4
SWH           27573.529 Hz
FIDRES        0.420739 Hz
AQ            1.1883861 sec
RG               191.56
DW               18.133 usec
DE               18.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.7703648 MHz
NUC1                 13C
P1                10.00 usec
PLW1         55.70000076 W

======== CHANNEL f2 ========
SFO2         500.1323506 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2         14.50000000 W
PLW12         0.32624999 W
PLW13         0.20880000 W

F2 - Processing parameters
SI                32768
SF           125.7577742 MHz
WDW                  EM
SSB       0
```


7.325 7.309 7.018 7.001 4.862 4.027 4.015 4.003 3.816 3.802 3.789 3.692 3.679 3.309 3.225 3.196 3.182 3.169 2.193 2.178 2.164 1.810 1.796 1.782 1.770 1.637 1.623 1.609 1.595 1.565 1.551 1.535 1.522 1.498 1.478 1.464 1.438 1.423 1.409 1.394 1.376 1.361 1.348 1.334 1.307 1.295 1.095 1.083
HN NH O O O N H 6 4 O N O HN N
10 9 8 7 6 5 4 3 2 1 ppm
2.00 2.02 2.06 1.01 1.01 3.07 2.10 2.04 2.06 2.13 12.67 2.91
Current Data Parameters
NAME tsushima-170729-CRL20-366-
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170729
Time 14.04
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 4
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 36.82
DW 50.000 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.1323506 MHz
NUC1 1H
P1 12.00 usec
PLW1 14.50000000 W
F2 - Processing parameters
SI 65536
SF 500.1300103 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


176.134 160.472 154.940 154.771 128.921 125.275 115.914 69.188 57.398 52.705 49.453 49.283 40.248 36.990 33.021 30.723 30.342 30.205 30.187 27.691 27.142 26.920 26.799 15.606
200 180 160 140 120 100 80 60 40 20 ppm
Current Data Parameters
NAME tsushima-170729-CRL20-366-
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170729
Time 14.16
INSTRUM spect
PROBHD 5 mm CPPBBO BB
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 128
DS 4
SWH 27573.529 Hz
FIDRES 0.420739 Hz
AQ 1.1883861 sec
RG 191.56
DW 18.133 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.7703648 MHz
NUC1 13C
P1 10.00 usec
PLW1 55.70000076 W
======== CHANNEL f2 ========
SFO2 500.1323506 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 14.50000000 W
PLW12 0.32624999 W
PLW13 0.20880000 W
F2 - Processing parameters
SI 32768
SF 125.7576133 MHz
WDW EM
SSB 0

4. References

1. B. J. Stapley, T. P. Creamer, *Protein Sci.* 1999, **8**, 587-95.

2. S. Sato, K. Nakamura, H. Nakamura, *ACS Chem. Biol.* 2015, **10**, 2633−2640.

3. S. Sato, H. Nakamura, *Angew. Chem. Int. Ed.* 2013, **52**, 8681−8684.

4. G. C. Neil, E. G. William, *Chem. Rev.* 1996, **96**, 877-910.