Supporting Information

Laccase-Catalysed Tyrosine Click Reaction

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1. Supporting Data

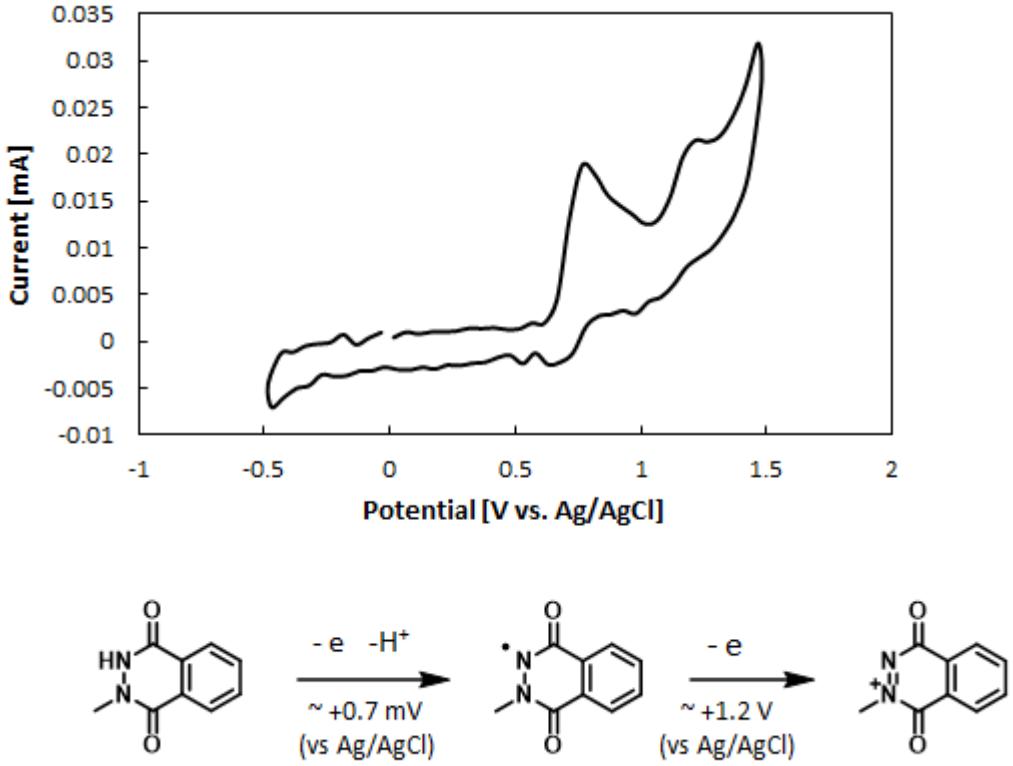


Figure S1. Redox property of *N*-methylluminol derivative.

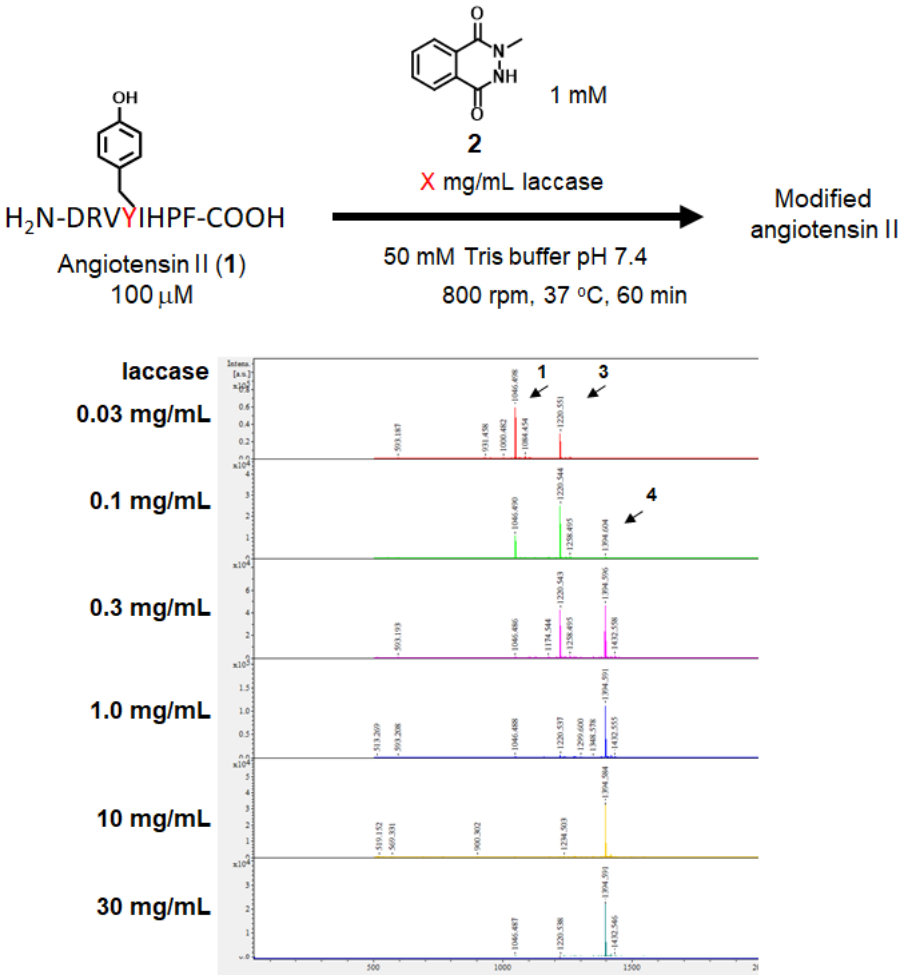


Figure S2. Investigation of suitable laccase concentration.

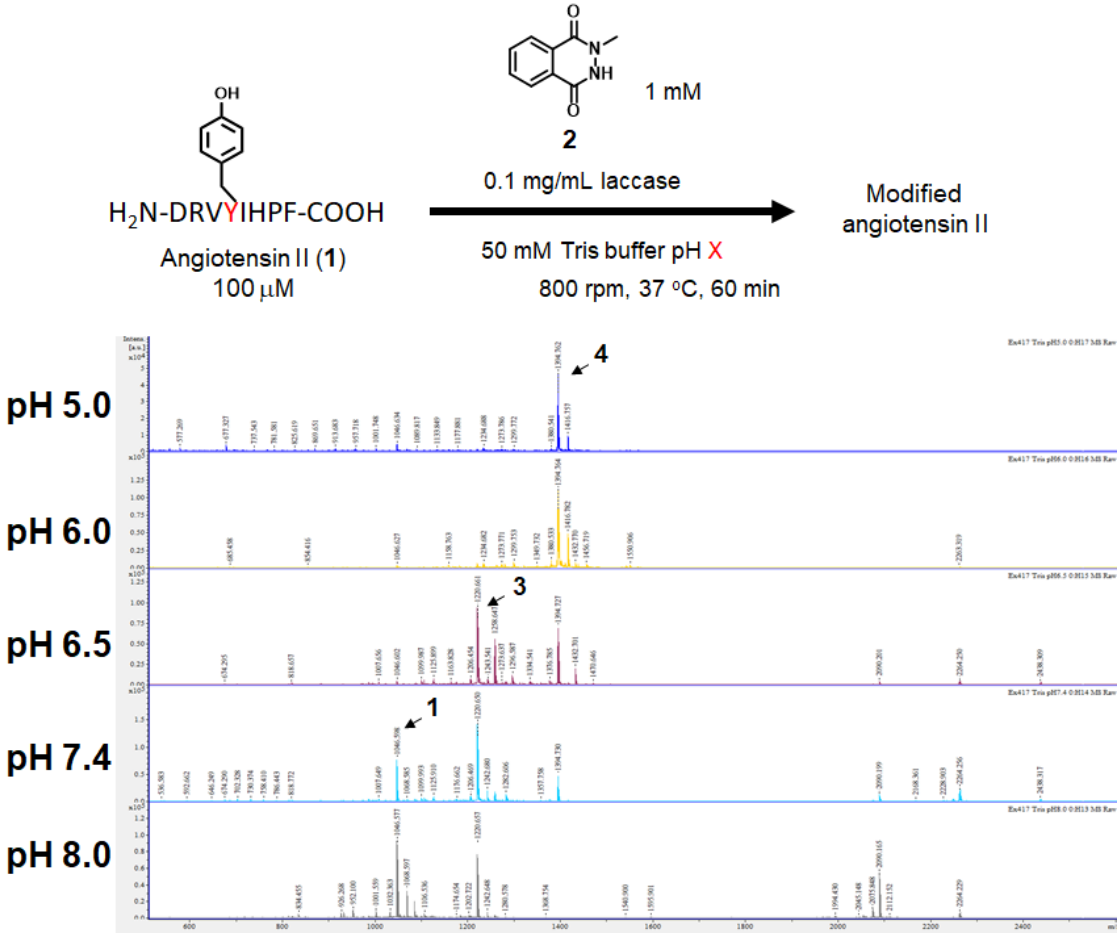


Figure S3. Investigation of suitable buffer pH.

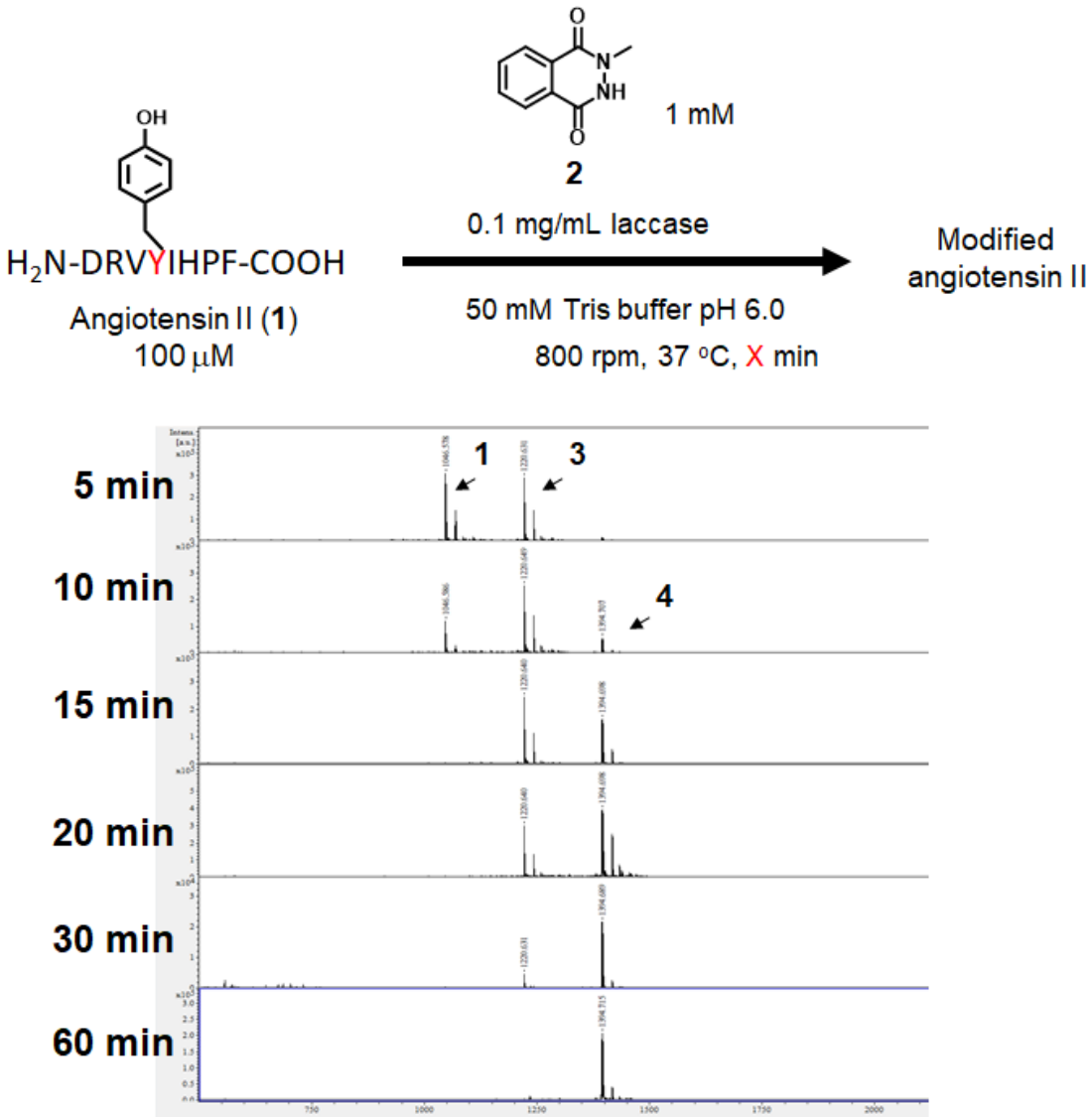


Figure S4. Reaction time-dependent peptide modification catalysed by laccase.

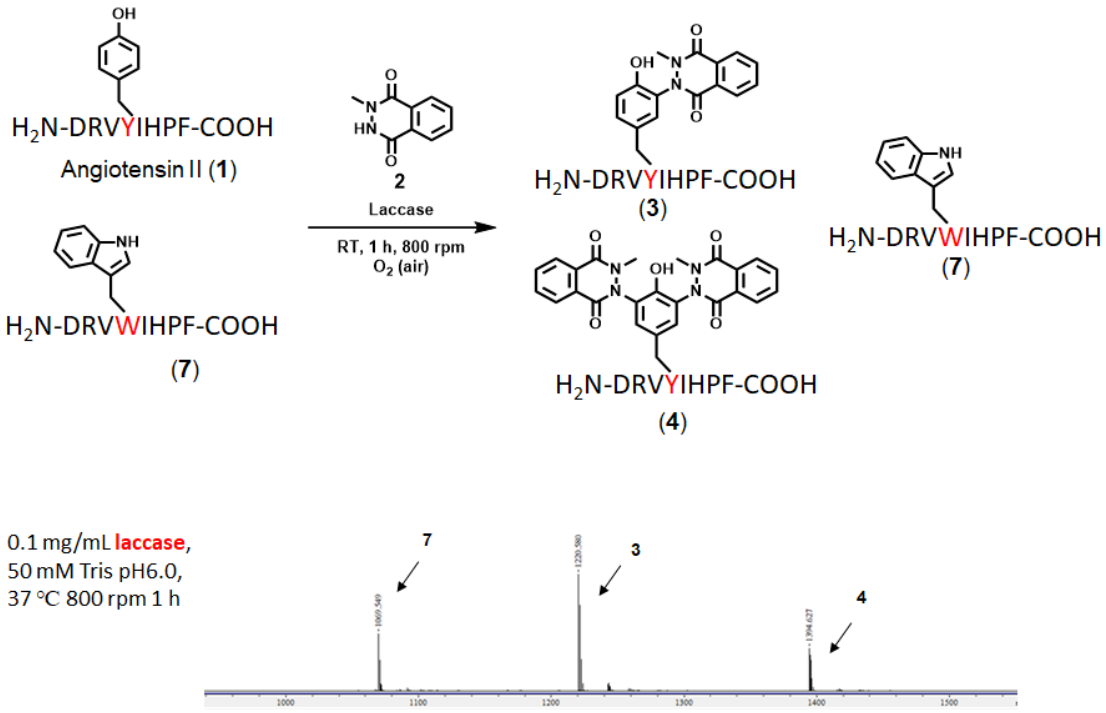


Figure S5. Tyrosine residue selectivity of laccase-catalysed peptide modification.

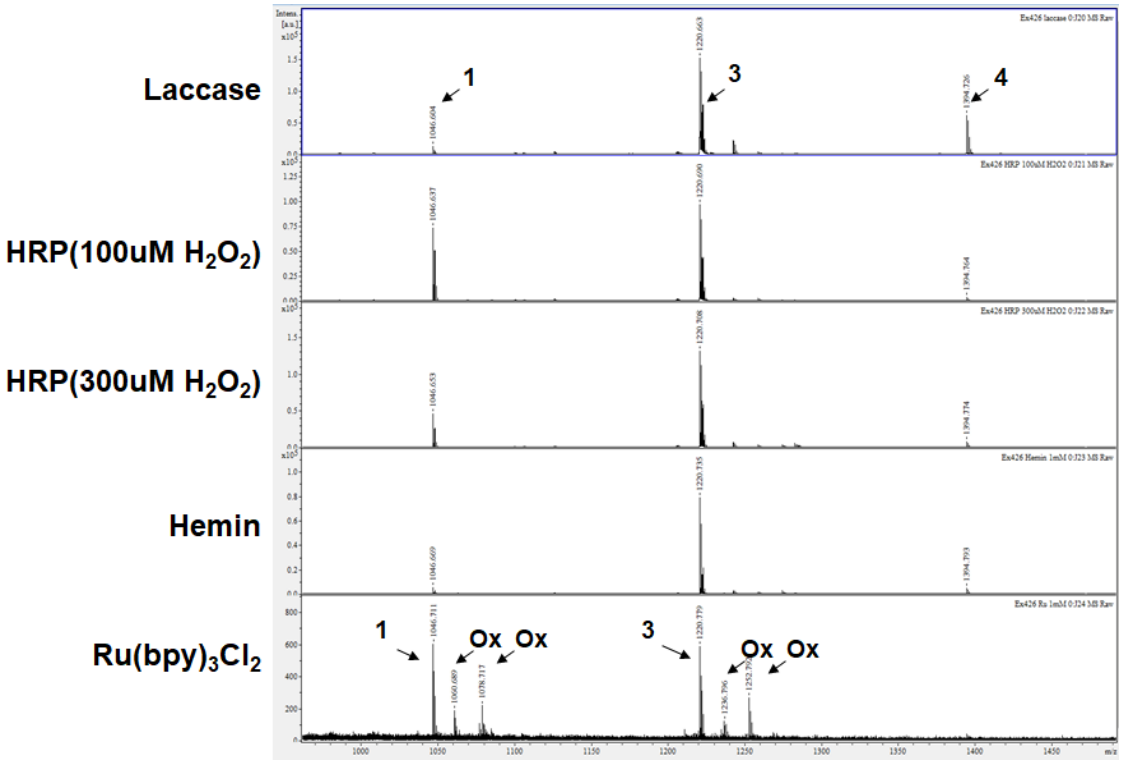


Figure S6. Comparison of peptide modification efficiency by various Y-click reactions. Peptide 1 (100 μM) was modified by Y-click reactions using 1 mM of **2**.

Laccase method: 0.1 mg/mL laccase, 50 mM Tris pH 6.0, 37 °C, 800 rpm.1 hr.

HRP method: 45 nM HRP, 100 or 300 μM H_2O_2, 50 mM Tris pH 7.4, r.t.,1 hr.

Hemin method: 10 μM hemin, 1 mM H_2O_2, 50 mM Tris pH 7.4, r.t.,1 hr.

Ruthenium photocatalyst method: 1 mM Ru(bpy)$_3$Cl$_2$, 10 mM MES pH 6.0, 455 nm LED, on ice, 5 min.

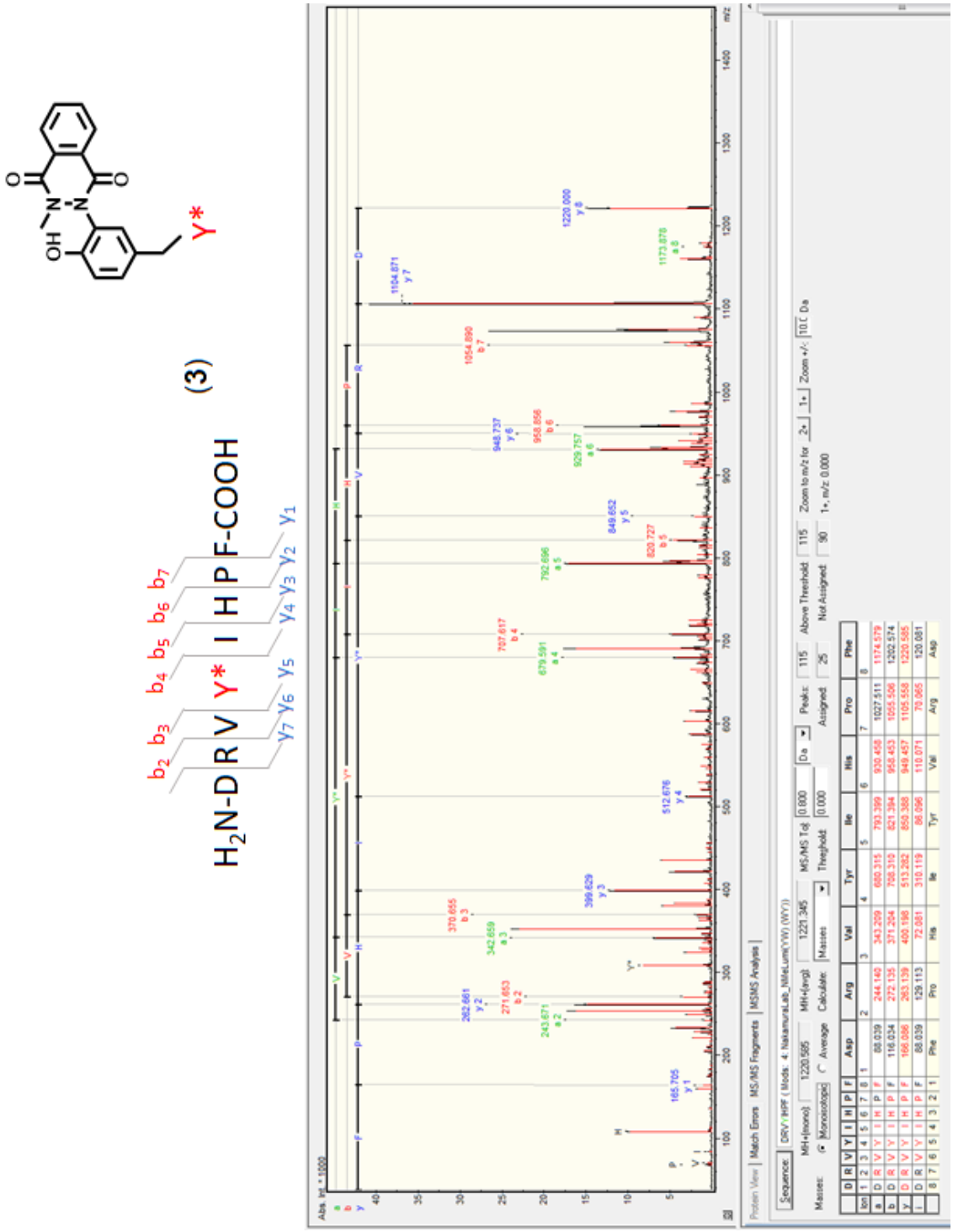


Figure S7. MS/MS analysis of angiotensin II modification by laccase-catalysed Y-click.

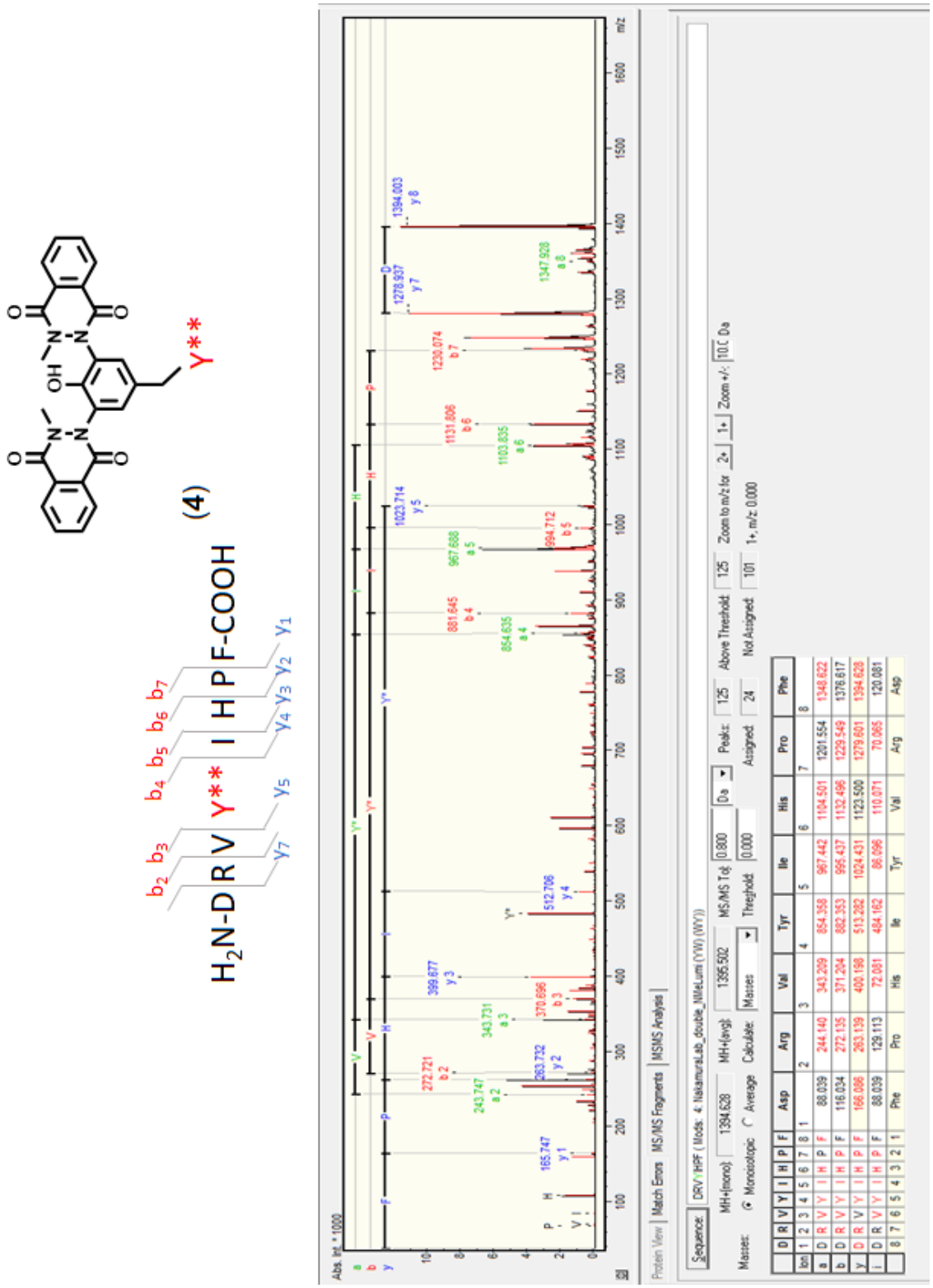


Figure S8. MS/MS analysis of double-modified angiotensin II.

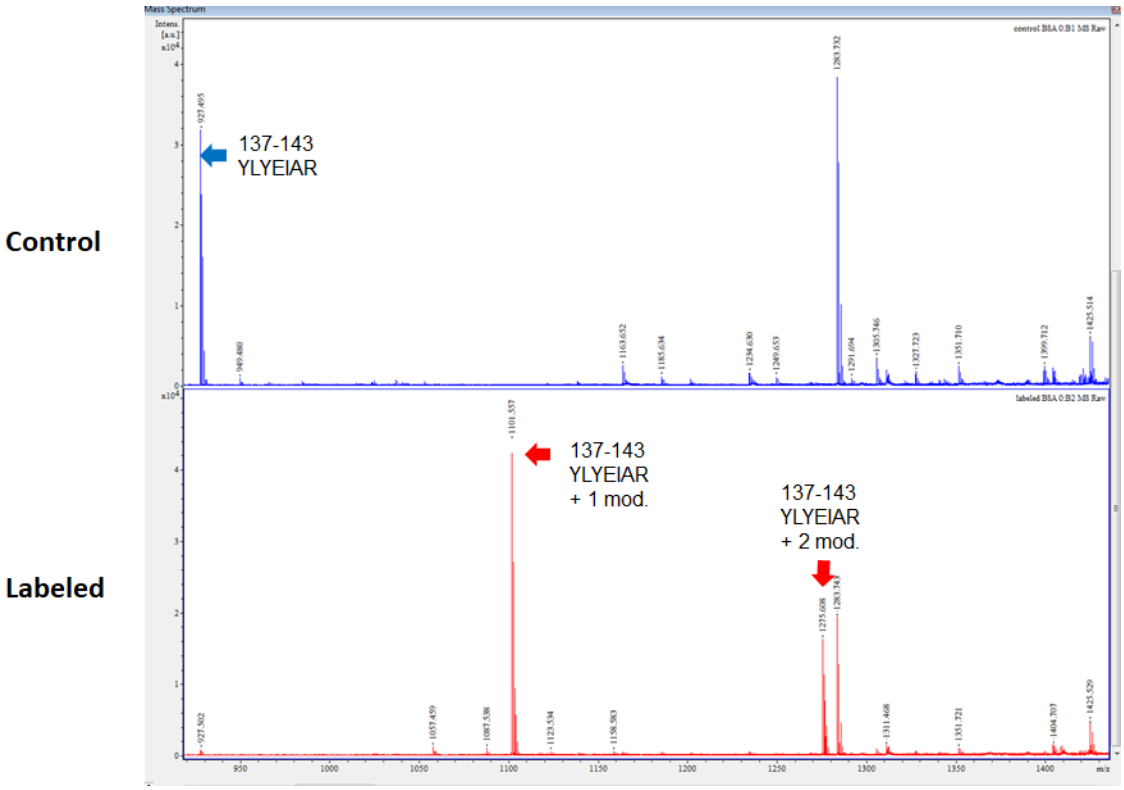


Figure S9. MALDI-MS analysis of trypsin-digested BSA (MW 900–1450): (upper) control BSA; (lower) BSA modified by laccase-catalysed Y-click (condition: 10 µM BSA, 300 µM **2**, 0.1 mg/mL laccase, 50 mM Tris pH 6.0, 37 °C, 800 rpm, 1 h).

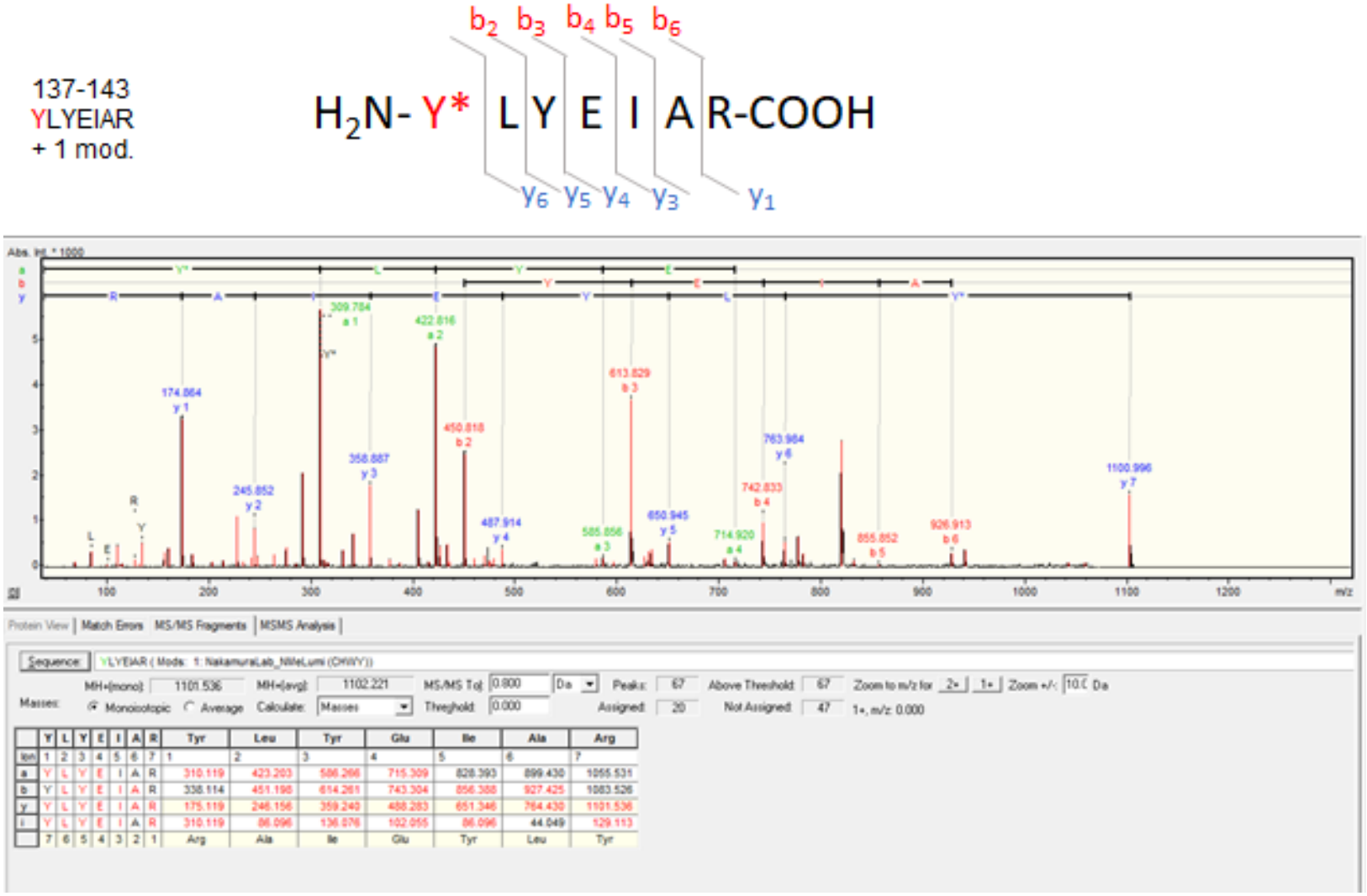


Figure S10. MS/MS analysis of modified peptide corresponding to Tyr137–Arg143.

Figure S11. MS/MS analysis of double-modified peptide corresponding to Tyr137–Arg143.

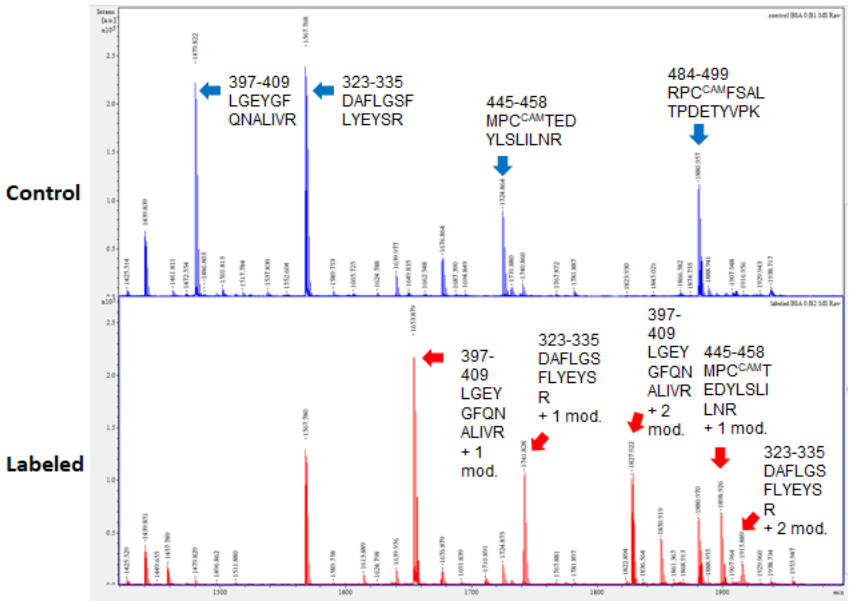


Figure S12. MALDI-MS peaks of trypsin-digested BSA (MW 1400–2000): (upper) control BSA. (lower) BSA modified by laccase-catalysed Y-click (condition: 10 μM BSA, 300 μM **2**, 0.1 mg/mL laccase, 50 mM Tris pH 6.0, 37 °C, 800 rpm, 1 h).

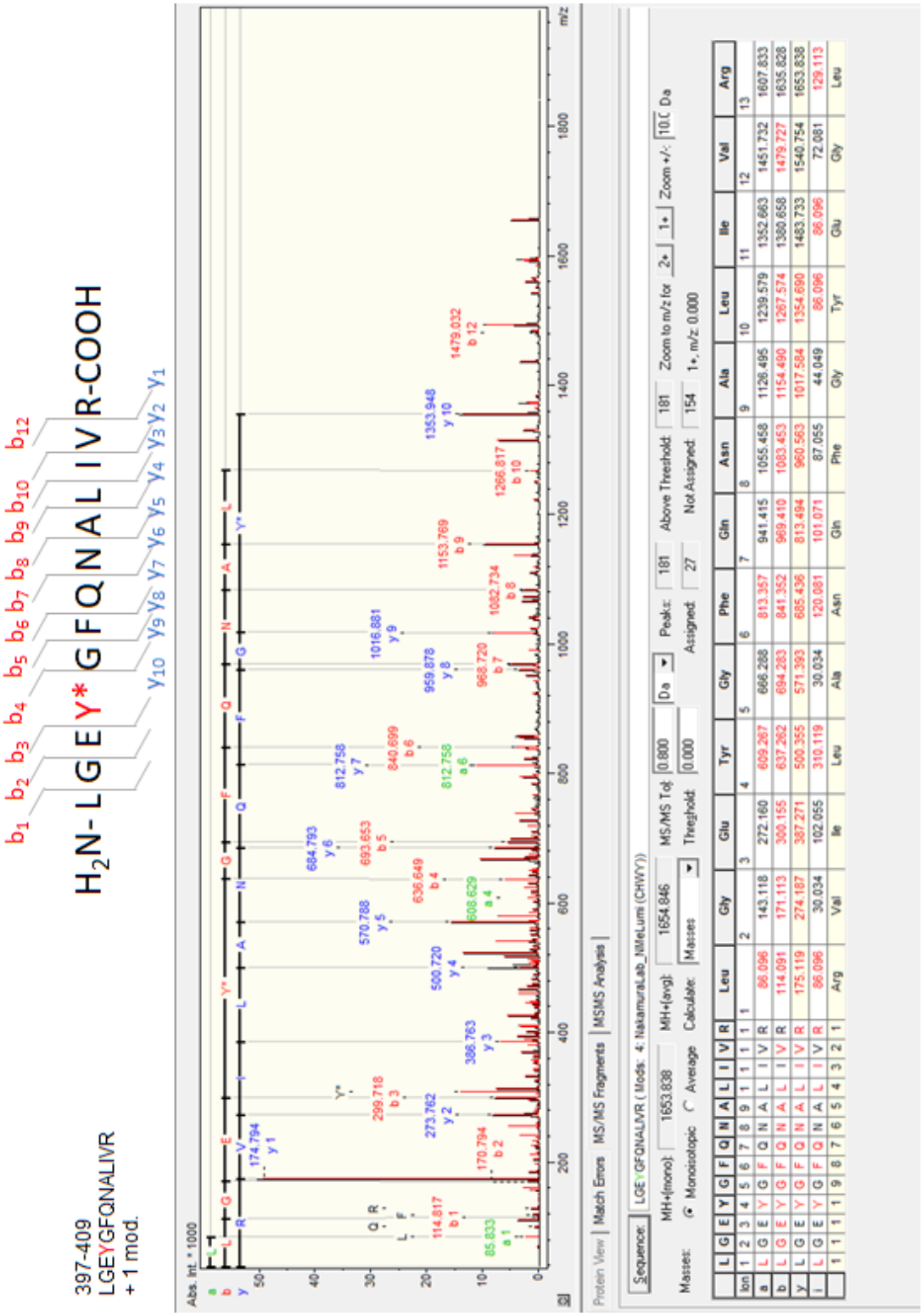


Figure S13. MS/MS analysis of modified peptide corresponding to Leu397–Arg409.

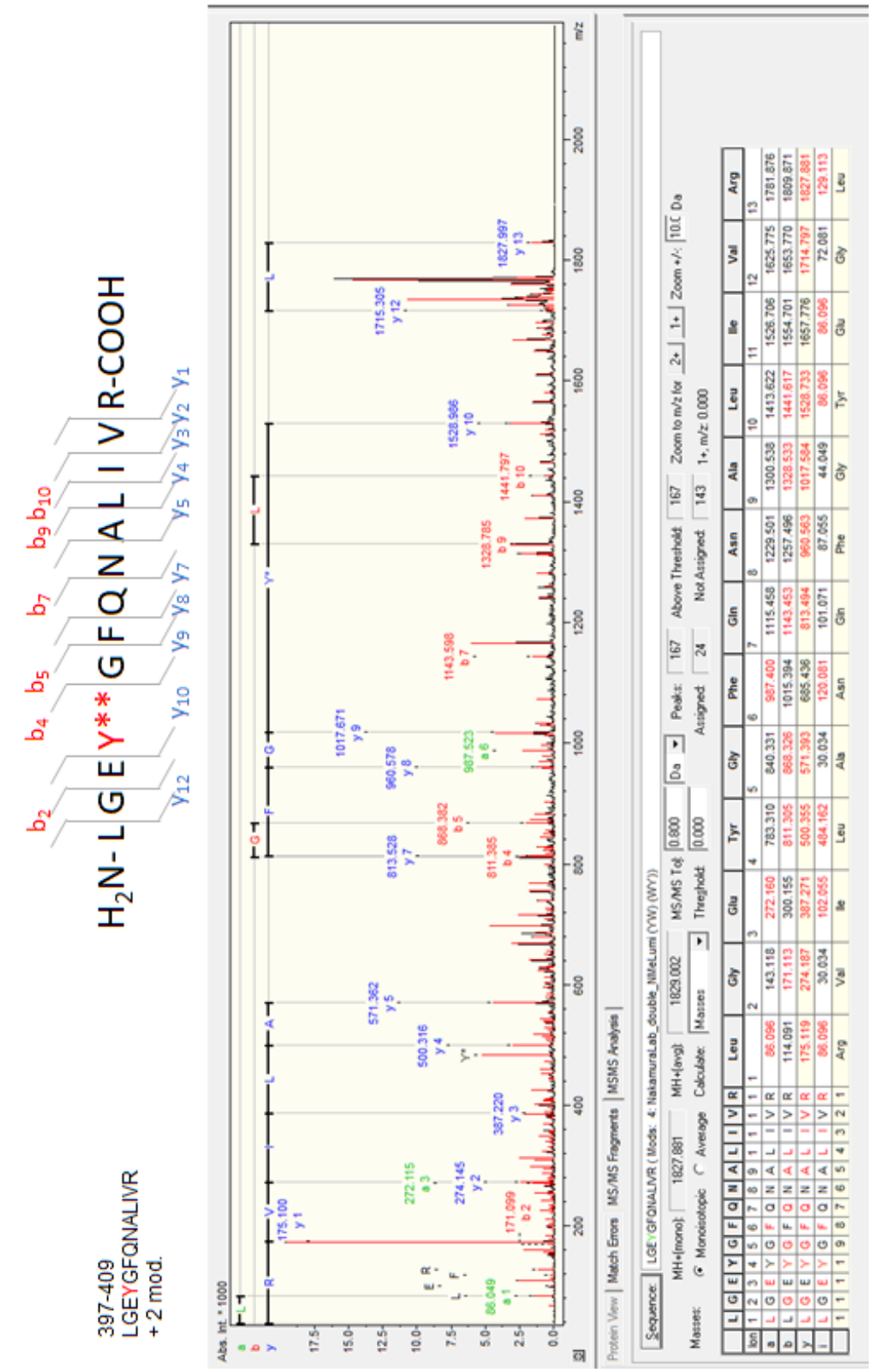


Figure S14. MS/MS analysis of double-modified peptide corresponding to Leu397–Arg409.

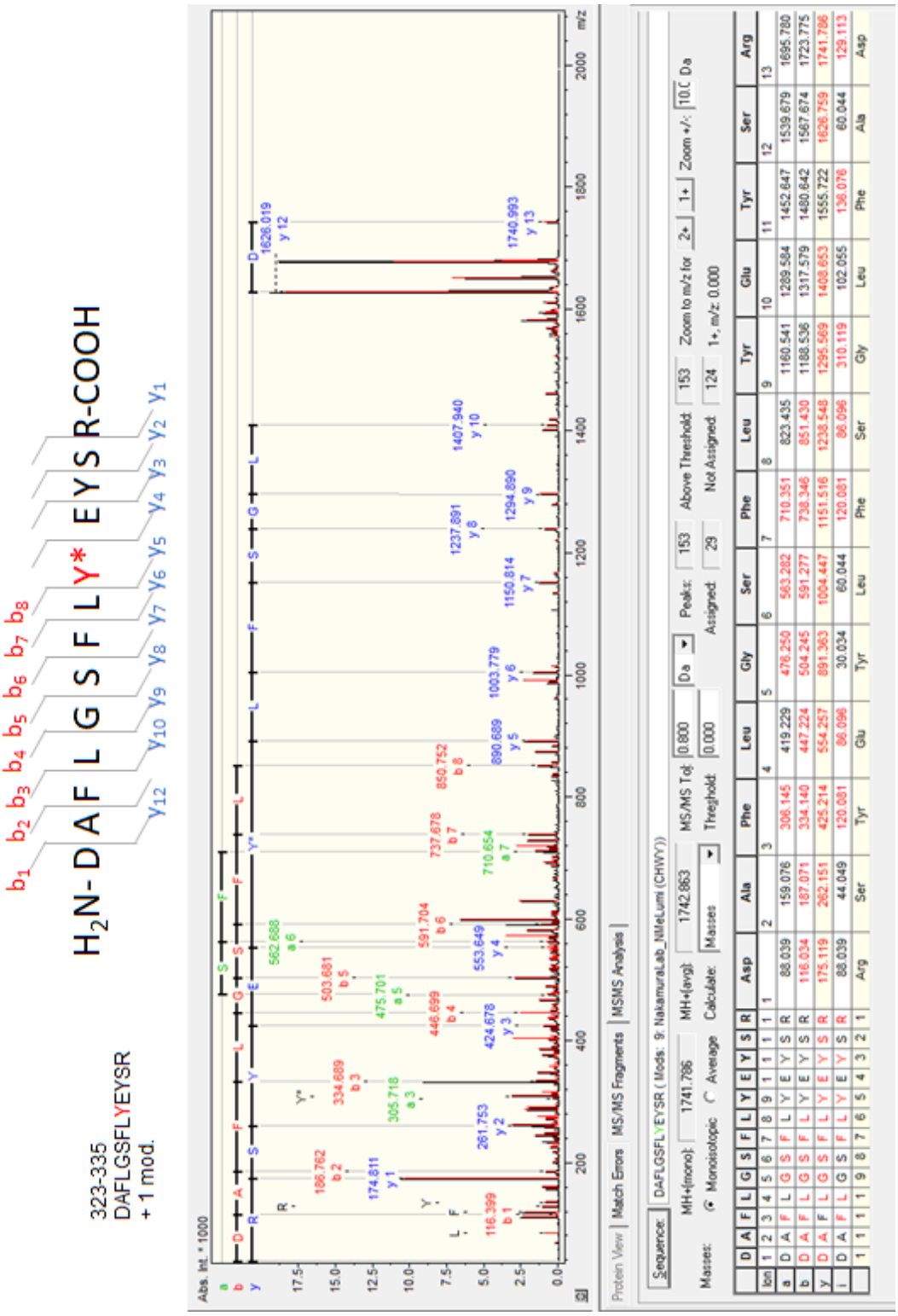


Figure S15. MS/MS analysis of modified peptide corresponding to Asp323–Arg335 (modification site assigned to Tyr331). This modified peptide peak corresponds to both Tyr331- and Tyr333-modified peptide.

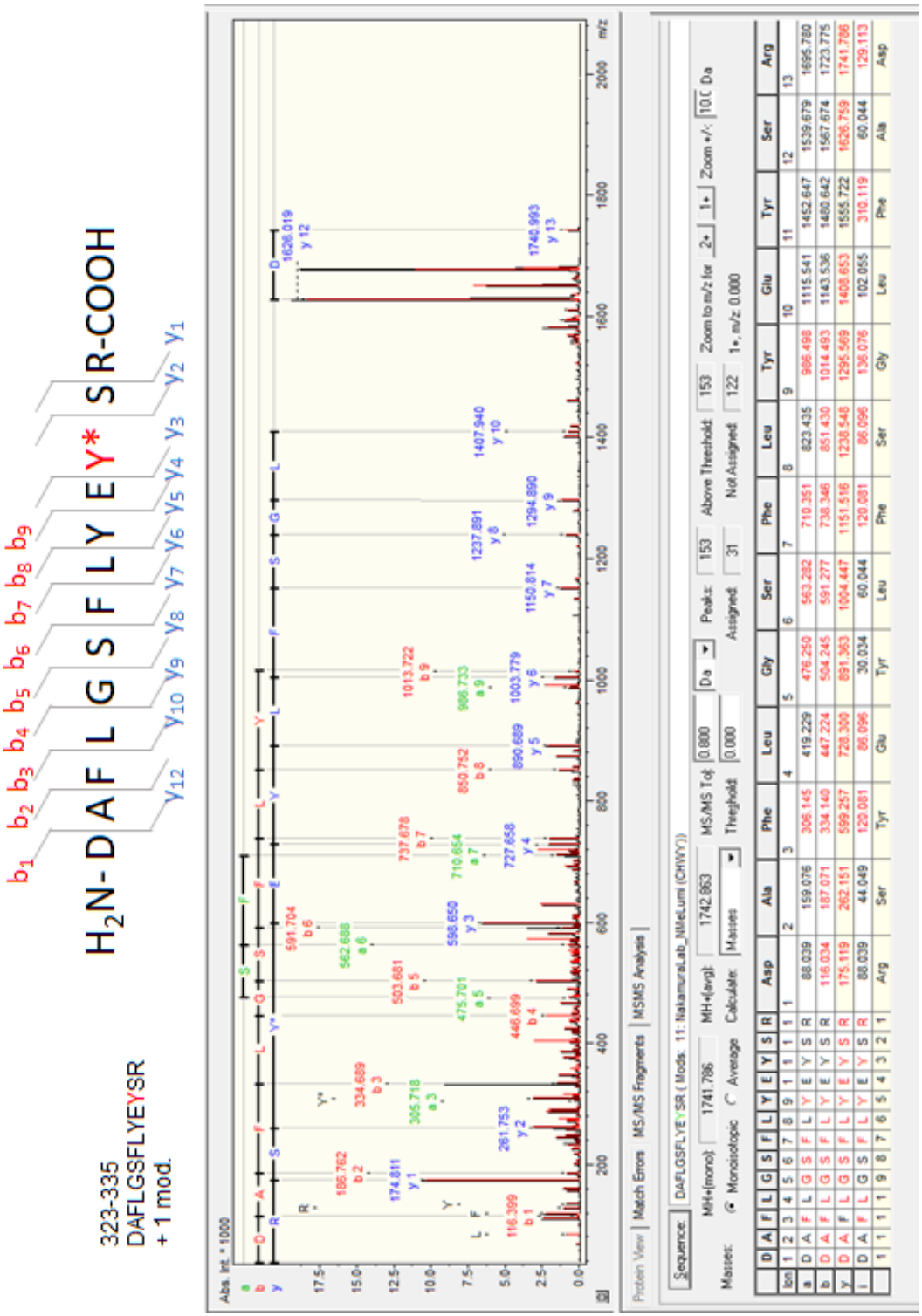


Figure S16. MS/MS analysis of modified peptide corresponding to Asp323–Arg335 (modification site assigned to Tyr333). This modified peptide peak corresponds to both Tyr331- and Tyr333-modified peptide.

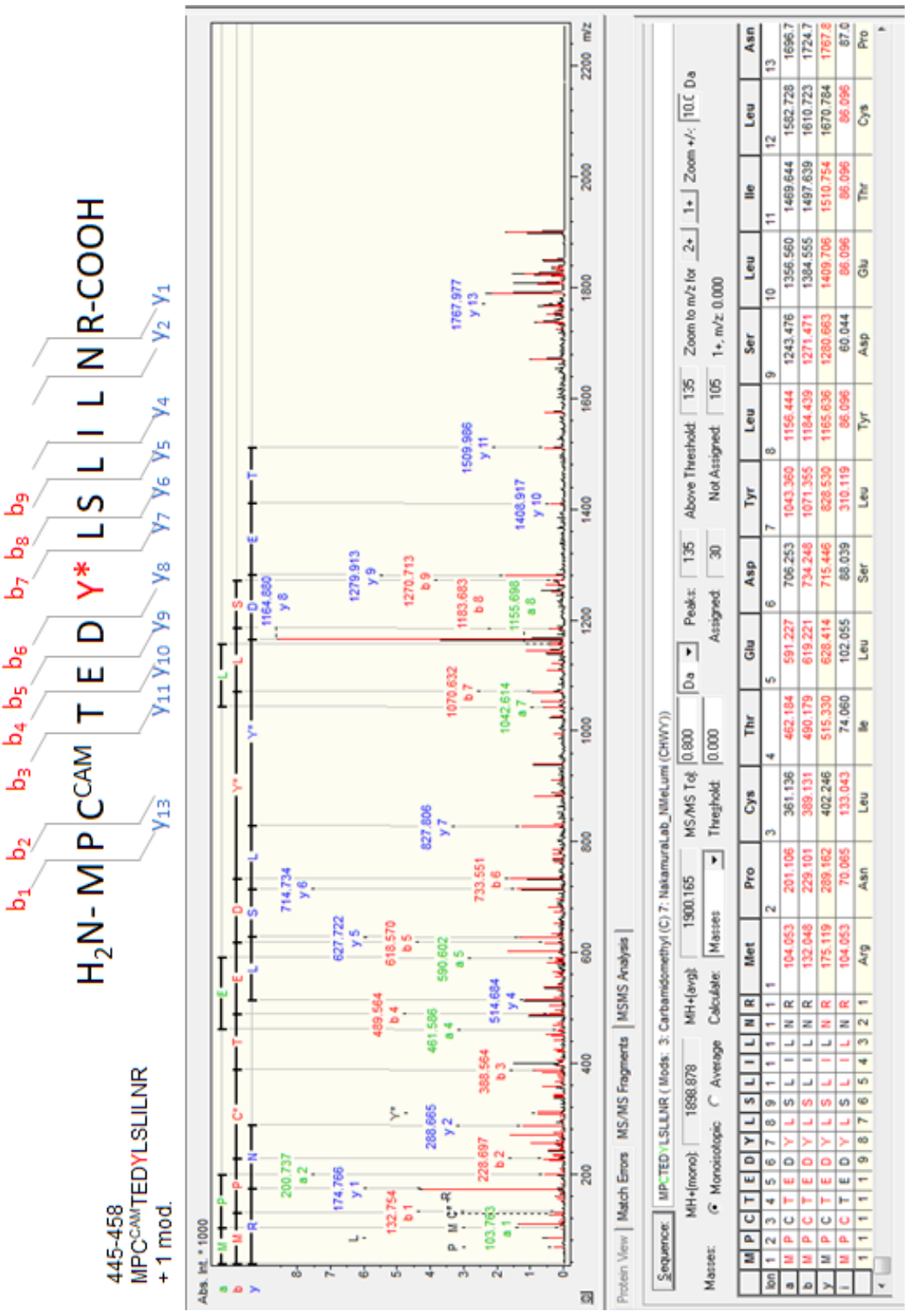


Figure S17. MS/MS analysis of modified peptide corresponding to Met445–Arg458.

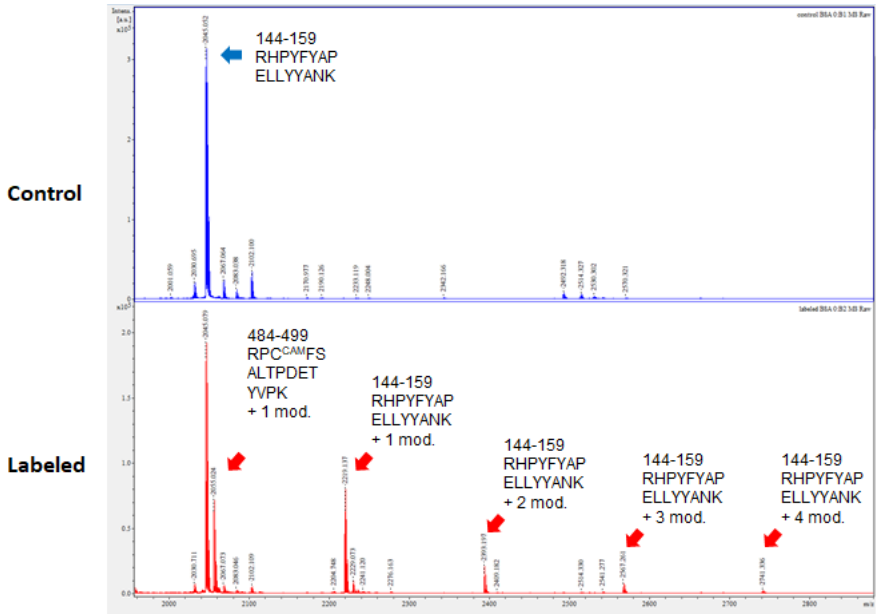


Figure S18. MALDI-MS peaks of trypsin-digested BSA (MW 1950–2900). (upper) control BSA. (lower) BSA modified by laccase-catalysed Y-click. (Condition: 10 μM BSA, 300 μM **2**, 0.1 mg/mL laccase, 50 mM Tris pH 6.0, 37 °C, 800 rpm, 1 hr.)

The modification site of single-, double-, triple-, and quadruple-modified peptide corresponding to Arg144–Lys159 could not be identified due to the complex mixture of peptide modified in different sites.

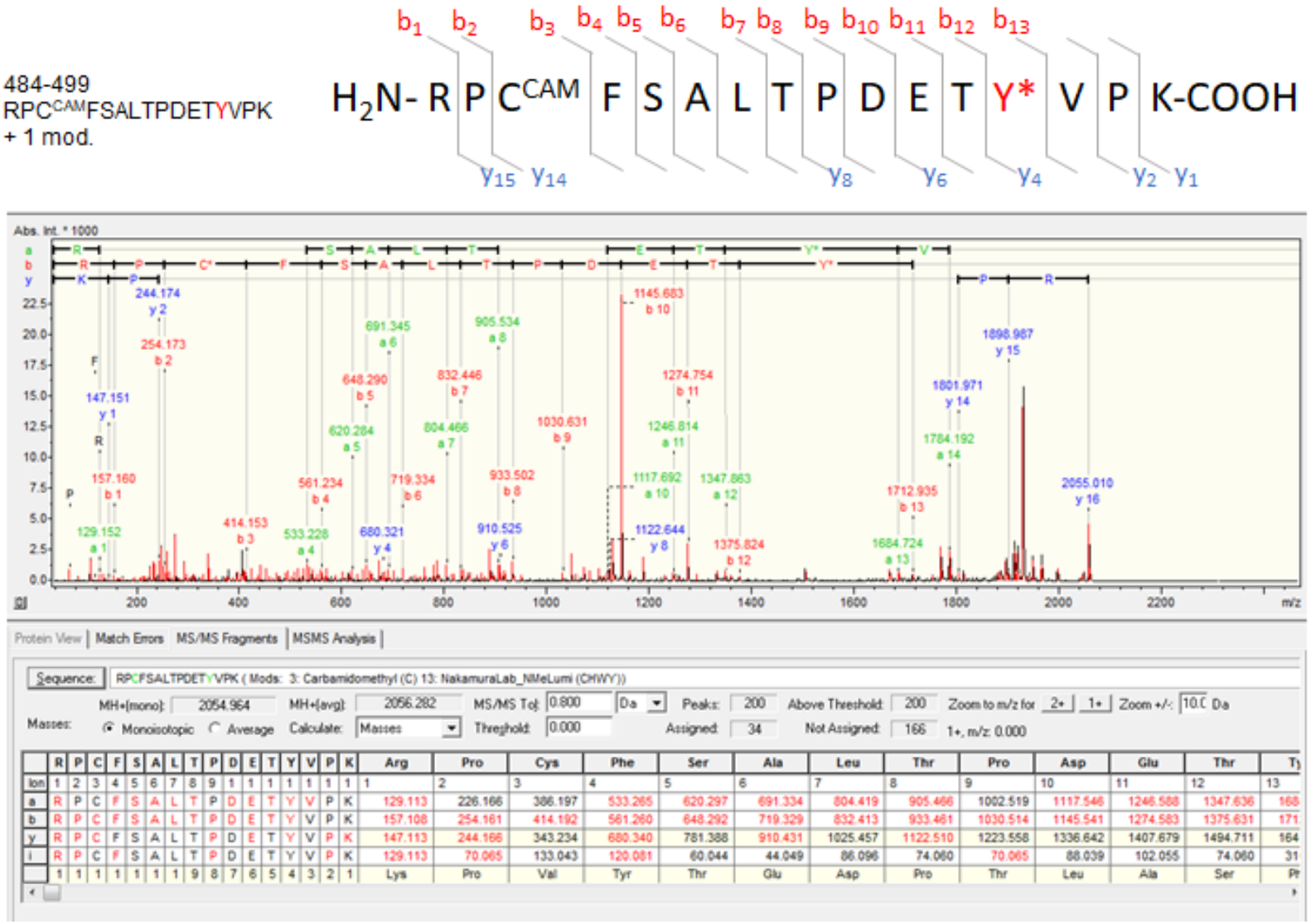


Figure S19. MS/MS analysis of modified peptide corresponding to Arg484–Lys499.

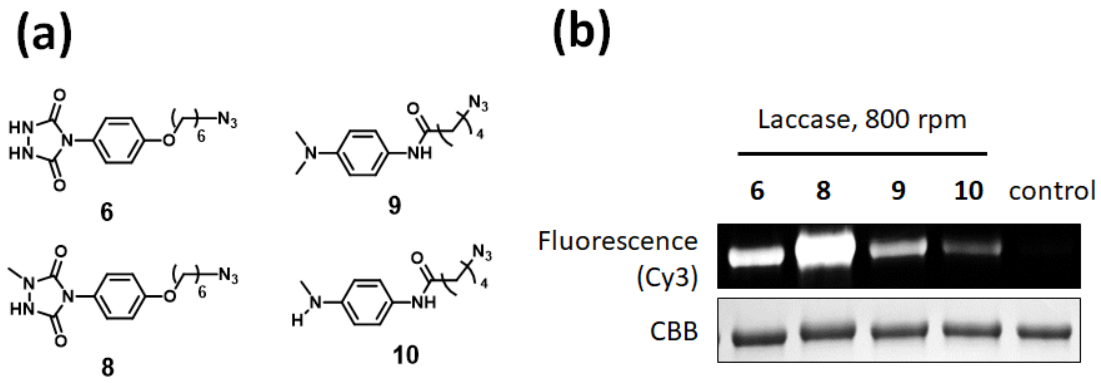


Figure S20. BSA modification with tyrosine modification reagents **6**, **8**–**10** using laccase. (a) Chemical structure of modification regents **6**, **8**–**10**. (b) Detection of fluorescent BSA. Laccase method: 10 μM protein, 300 μM **6**, **8**–**10**, 0.1 mg/mL laccase, 50 mM Tris pH 6.0, 37 °C, 800 rpm, 1 h. Fluorescent visualization: 20 μM DBCO-Cy3, 37 °C, 30 min.

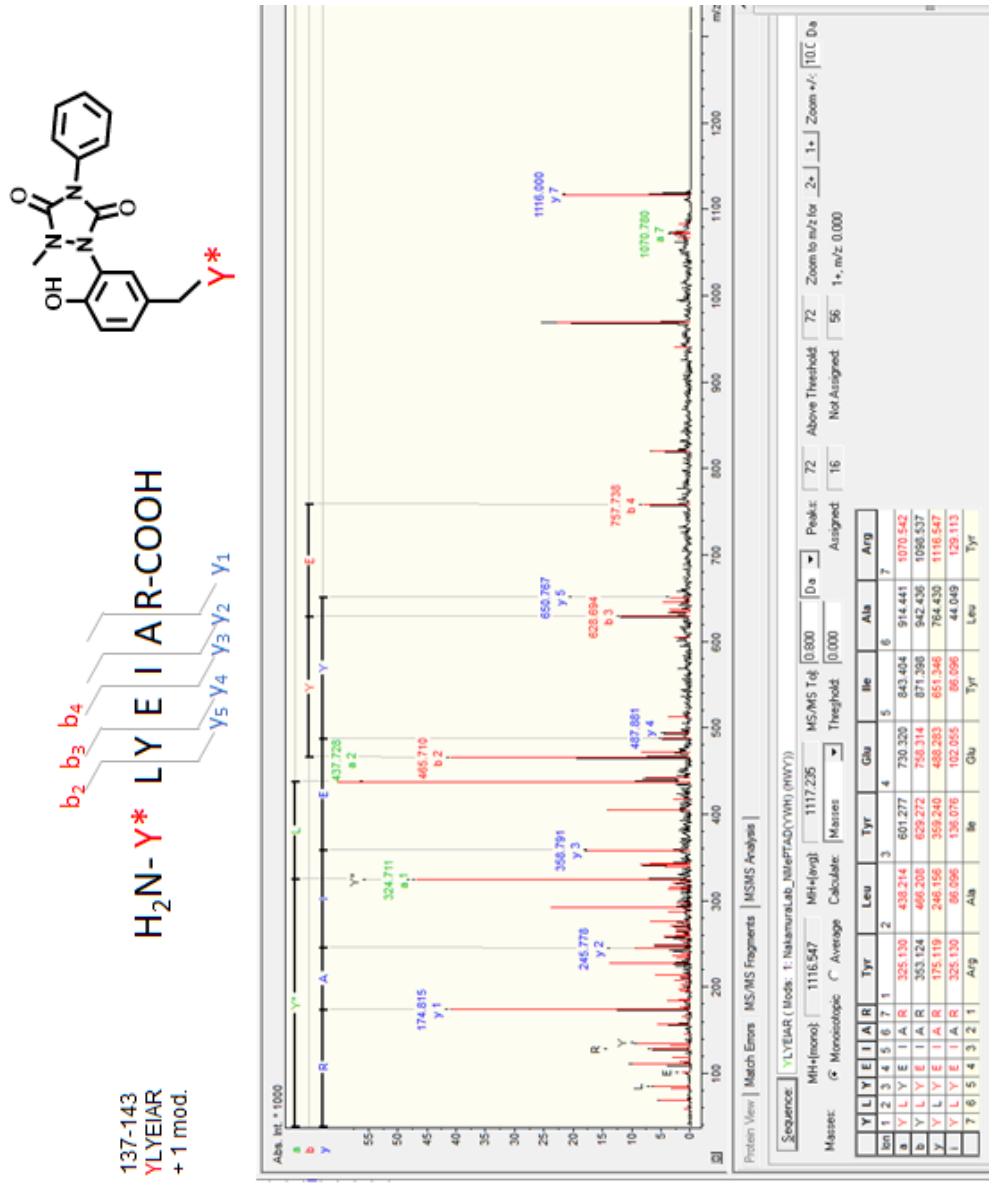


Figure S21. MS/MS analysis of peptide corresponding to Tyr137–Arg143 modified with 1-methyl-4-pheylurazole.

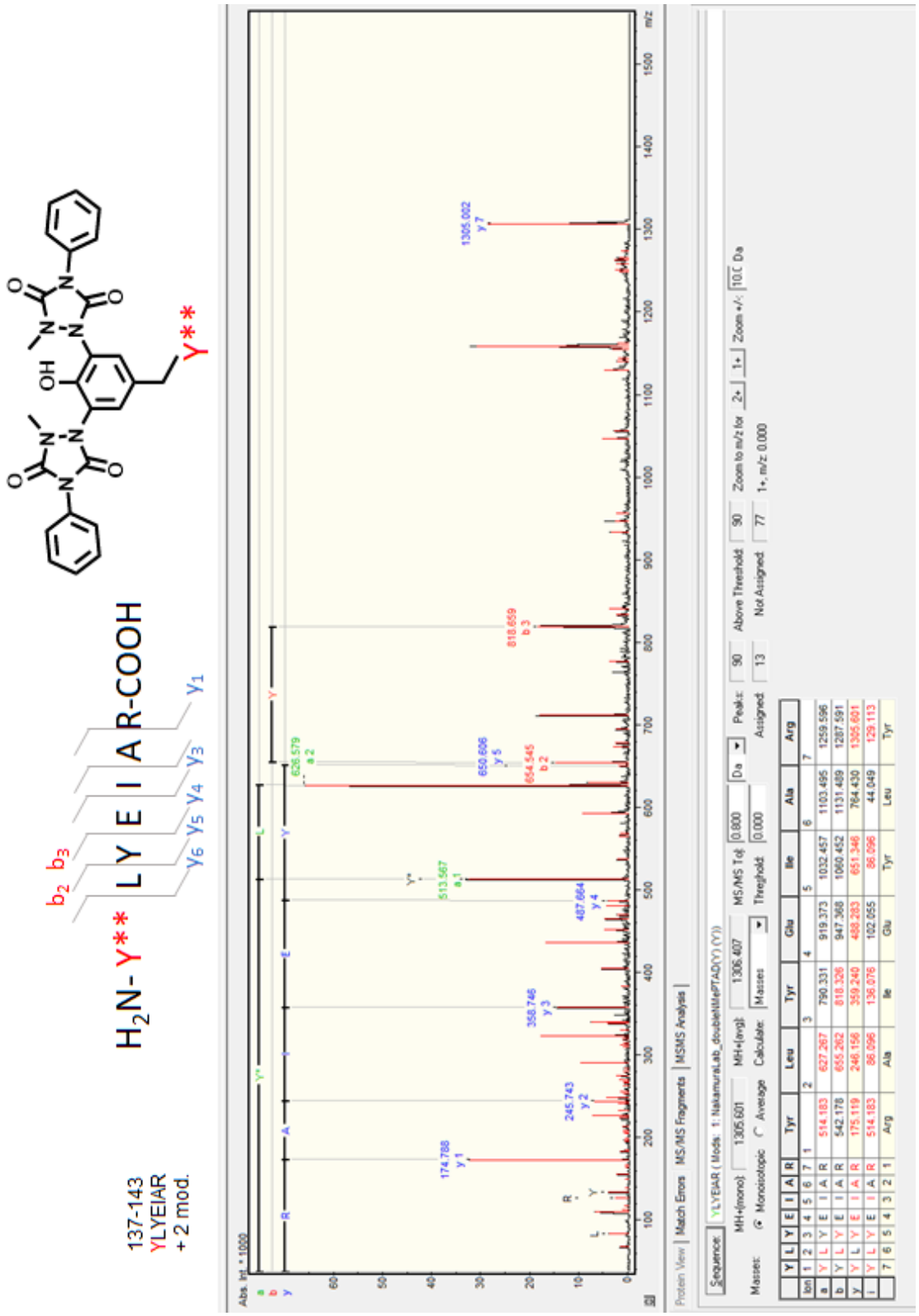


Figure S22. MS/MS analysis of peptide corresponding to Tyr137–Arg143 double-modified with 1-methyl-4-pheylurazole.

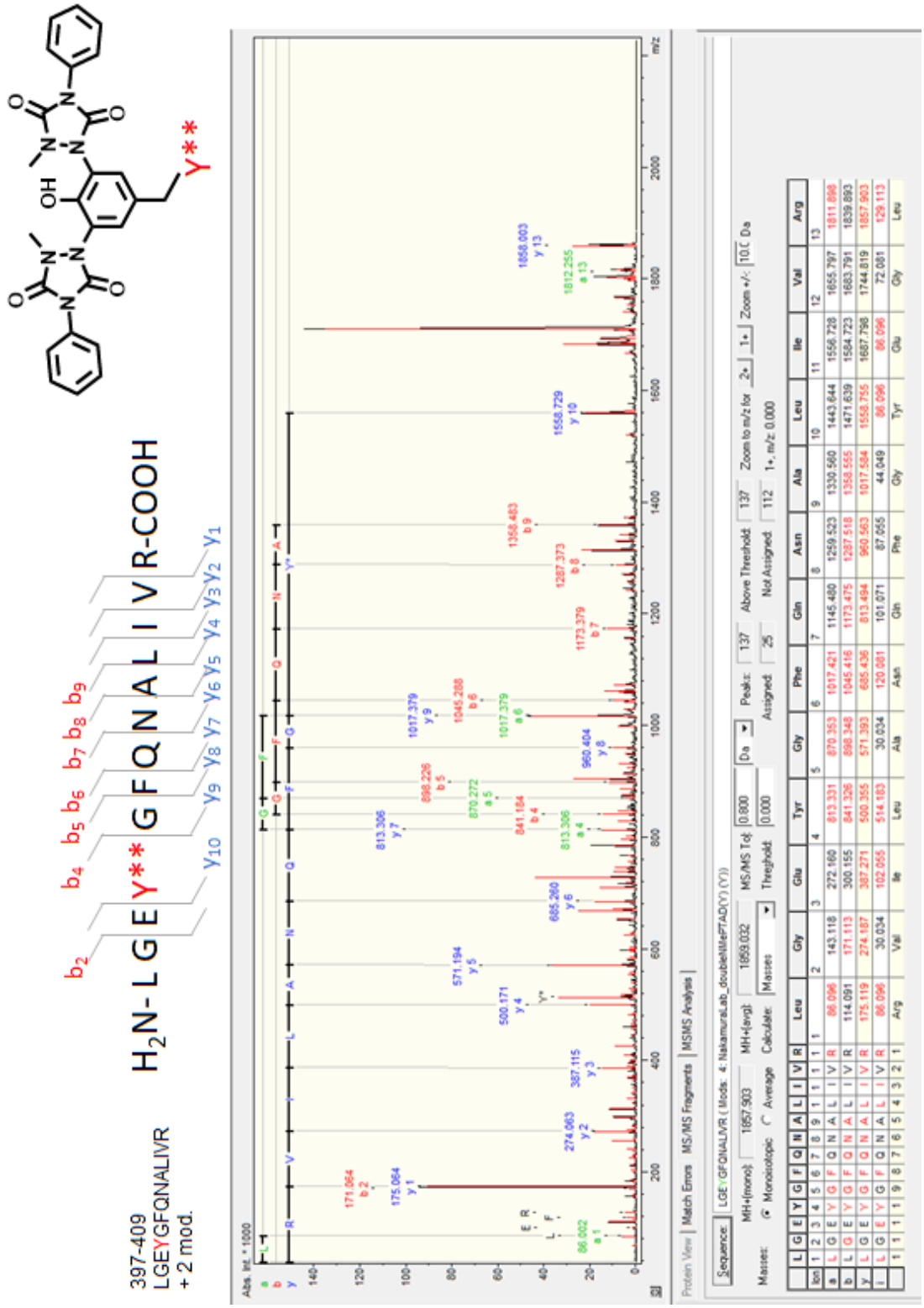


Figure S23. MS/MS analysis of peptide corresponding to Leu397–Arg409 double-modified with 1-methyl-4-pheylurazole.

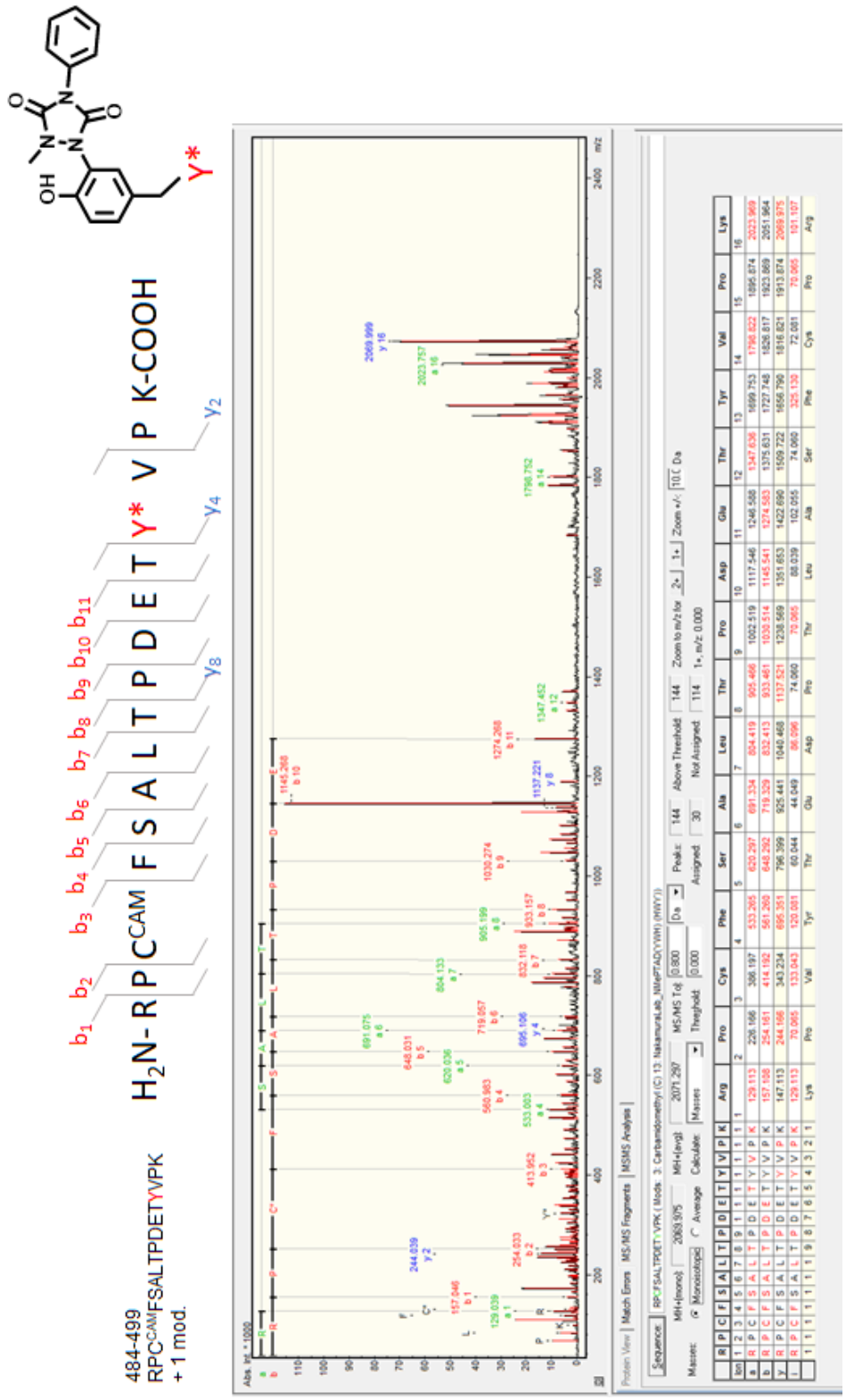


Figure S24. MS/MS analysis of peptide corresponding to Arg484–Lys499 modified with 1-methyl-4-pheylurazole.

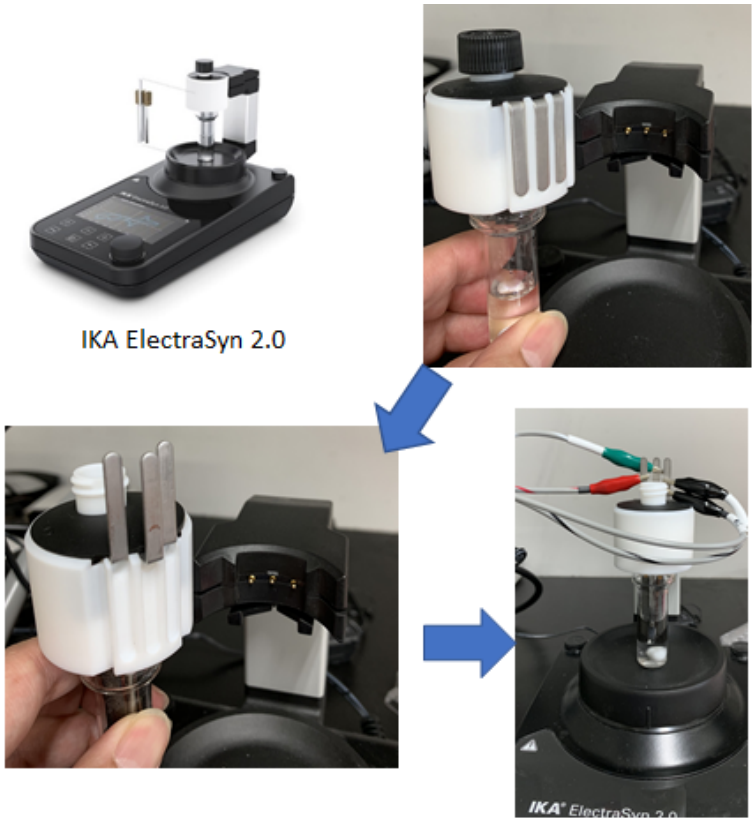


Figure S25. The reaction device for e-Y-click.

2. Experimental section

Preparation of modification reagents

Luminol derivatives (**2** and **5**)[1], urazole derivative (**6**[1] and **8**[2]) and phenylene diamine derivative (**9**[3] and **10**[4]) were synthesized according to previously reported procedure. ^{1}H-NMR data was attached in this supporting information section 3.

Peptide labeling using laccase

A solution of **2** (final conc. 1 mM) was added to a solution of peptide (final conc. 100 μM in 50 mM Tris or phosphate buffer, pH5.0-8.0). Laccase (Amano Enzyme inc., final conc. 0.03-3.0 mg/mL) was added to the mixture and mixed, and the mixture was stirred at 0 or 800 rpm (using Thermo shaker incubater), 37 °C for 5-90 min. Then, the reaction was quenched by adding TFA aq. (final conc. 0.1%). Peaks of the modified peptides were detected by MALDI-TOF analysis (Bruker, UltrafleXtreme).

Protein labeling using laccase

A solution of **2** or **5** (final conc. 300 μM) was added to a solution of protein (final conc. 10 μM in 50 mM Tris buffer (pH6.0)). Laccase (Amano Enzyme inc., final conc. 0.1 mg/mL) was added to the mixture and mixed, and the mixture was stirred at 800 rpm (using Thermo shaker incubater), 37 °C for 1 hr. After 1 hr, excess modification reagents were removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000×g, 4 min). A solution of DBCO-Cy3 (final conc. 20 μM) was added to the filtrate, and the mixture was incubated at 37 °C for 30 min. After 30 min, excess DBCO reagent was removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000×g, 4 min). Sephadex G-25 gel filtration removes antibodies aggregated by oxidative damage. SDS-PAGE was performed using the same amount of protein in each well.

In-gel digestion of proteins

The BSA band separated by SDS-PAGE were cut out and excised into approximately 1 mm pieces. The gel pieces were transferred into a tube and 1 mL of water was added. The tube was incubated at 37 °C for 10 min and the solution was removed (repeat three times). A solution of 50% MeCN in 100 mM NH_4HCO_3 aq. was added to the tube for gel de-staining. The tube was incubated at 37 °C for 10 min and the solution in the tube was removed. Next, MeCN was added to the tube for dehydration. The tube was incubated at 37 °C for 10 min and the solution was removed. After that, dithiothreitol (100 mM) in 100 mM NH_4HCO_3 aq. was added to the tube for Cys reduction. The tube was incubated at 37 °C for 30 min and the solution was removed. Subsequently, iodoacetamide (250 mM) in 100 mM NH_4HCO_3 aq. was added to the tube for Cys alkylation. The tube was incubated at room temperature

for 30 min in the dark and the solution was removed. The gels were washed with 100 mM NH_4HCO_3 aq. and 50% MeCN in 100 mM NH_4HCO_3 aq. Thereafter, MeCN was added to the tube for dehydration. The tube was incubated at 37 °C for 10 min and the solution was removed. Finally, trypsin solution was added, the tube was incubated at 37 °C overnight, and the obtained solution was quenched by adding TFA aq. (final conc. 0.1%).

MALDI-TOF MS analysis of digested samples

The digested solutions were desalted using C18 pipette tips (Agilent). Each sample was mixed with 1 μL of α-cyano-4-hydroxycinnamic acid (CHCA) solution on a MALDI-TOF plate. Peaks of the modified peptides were detected by MALDI-TOF analysis (Bruker, UltrafleXtreme).

E-Y-click of BSA

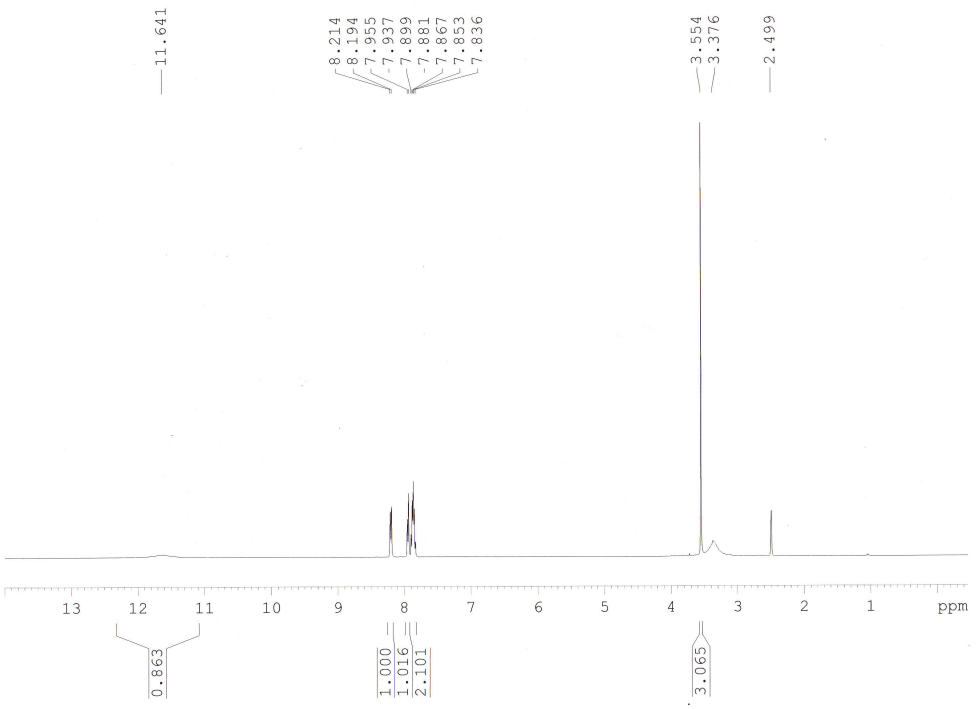
Voltage was applied to a mixture of BSA (10 μM) and **6** (300 μM) in 50 mM Tris buffer (pH 7.4) using the experimental device shown in Figure S20 (400 mV vs Ag/AgCl, room temperature, 5–30 min, stirring speed: 400 rpm). Excess modification reagent was removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000×g, 4 min). A solution of DBCO-Cy3 (final conc. 10 μM) was added to the filtrate and the mixture was incubated at 37 °C for 30 min. After 30 min, excess DBCO reagent was removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000×g, 4 min).

Estimation of free cysteine oxidation of BSA

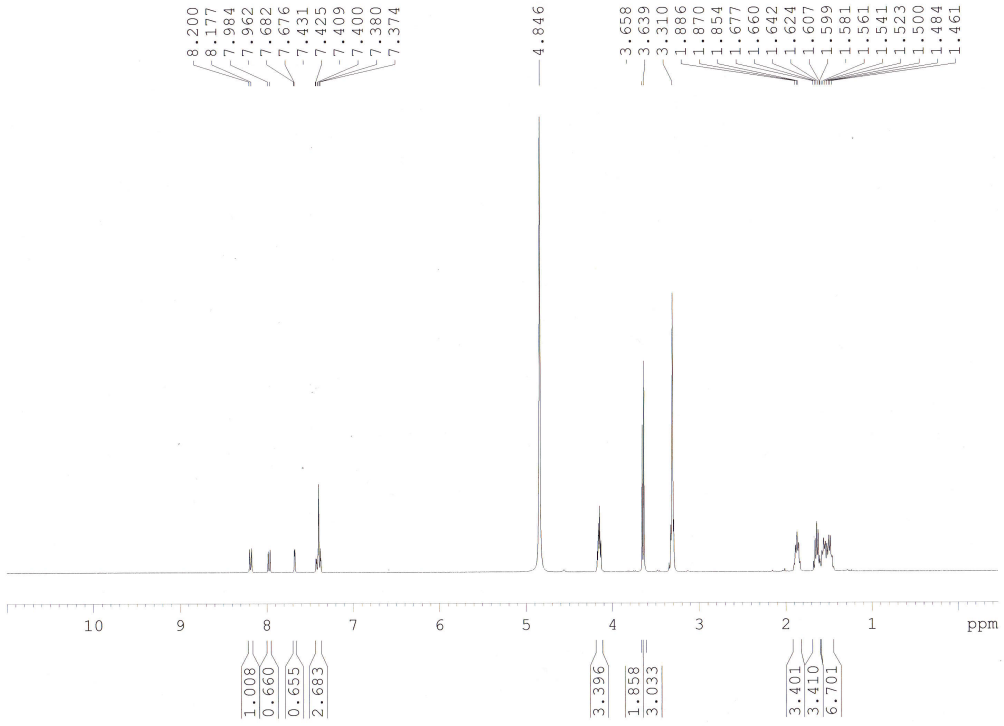
Biotin-PEG2-maleimide (Cosmo Bio) (final conc. 200 μM) was added to modified BSA and incubated at room temperature for 1 hr. Excess modification reagent was removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000×g, 4 min). For the detection of biotinylated proteins, separated protein with SDS-PAGE was transferred to a PVDF membrane (GE Healthcare) using Trans-Blot® Turbo (Bio Rad) (1.3 A 25 V per 1 mini gel, 7 min). The membrane was blocked with Immuno Block® (DS Pharma) and treated with horseradish peroxidase (HRP)-conjugated streptavidin (SAv-HRP, Sigma-Aldrich), and the blot was treated with ECL kit (GE Healthcare). The chemiluminescence images were obtained with Fusion Solo 4S (Vilber Lourmat). The signal intensities were quantified by ImageJ software.

3. ^{1}H-NMR data for reported compounds, 2, 5, 6, 8-10

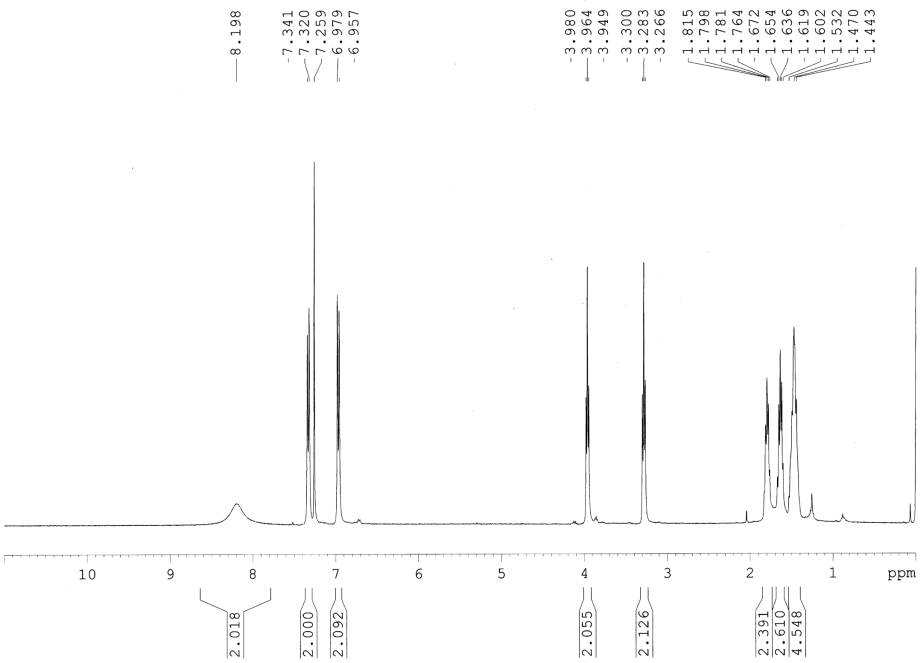
^{1}H-NMR spectrum of compound **2** (400 MHz; DMSO-d_6) [1)]



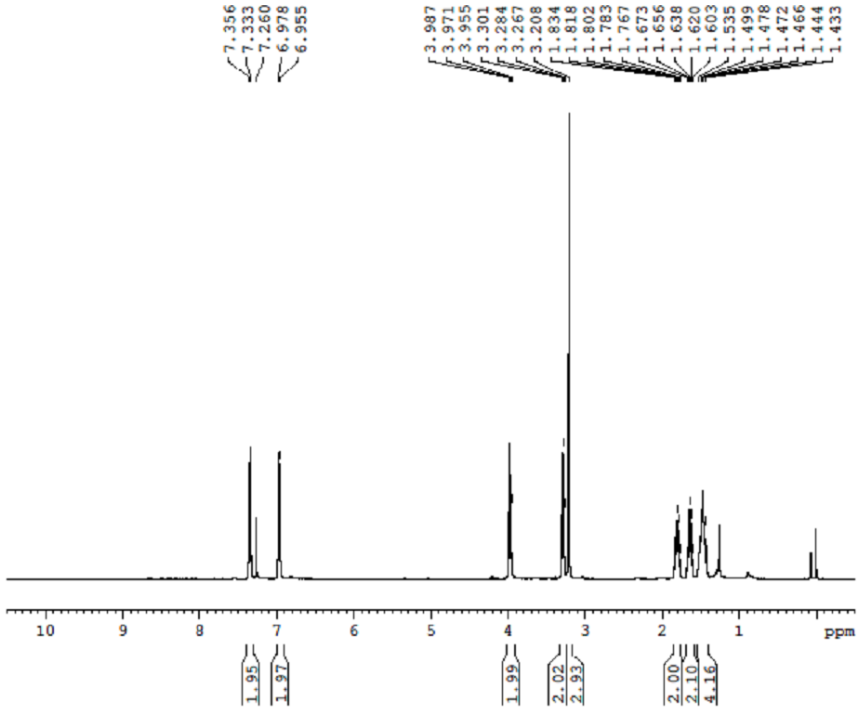
^{1}H-NMR spectrum of compound **5** (isomer mixture, 400 MHz; CD_3OD) [1)]



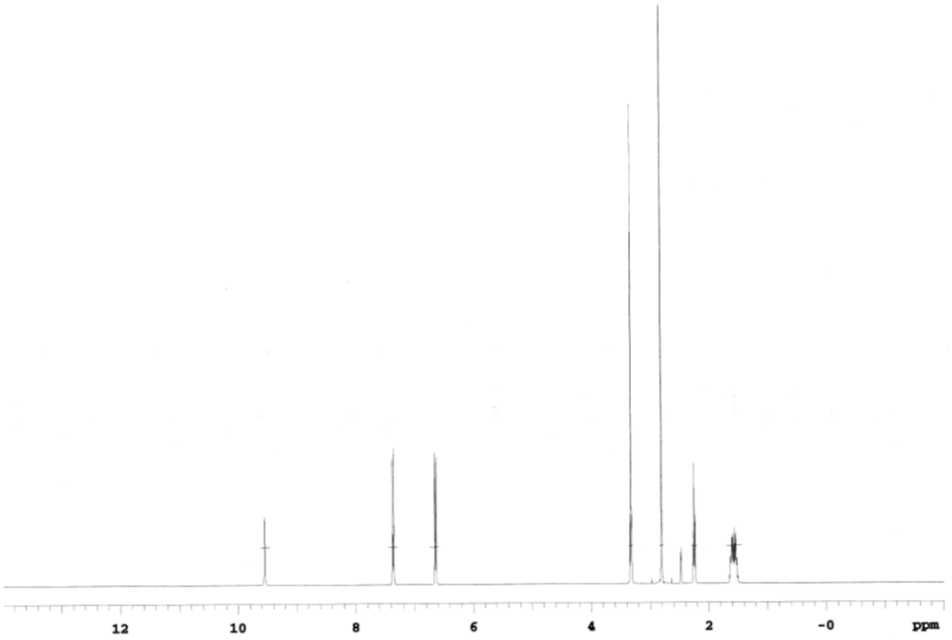
^{1}H-NMR spectrum of compound **6** (400 MHz; $CDCl_3$) [1)]



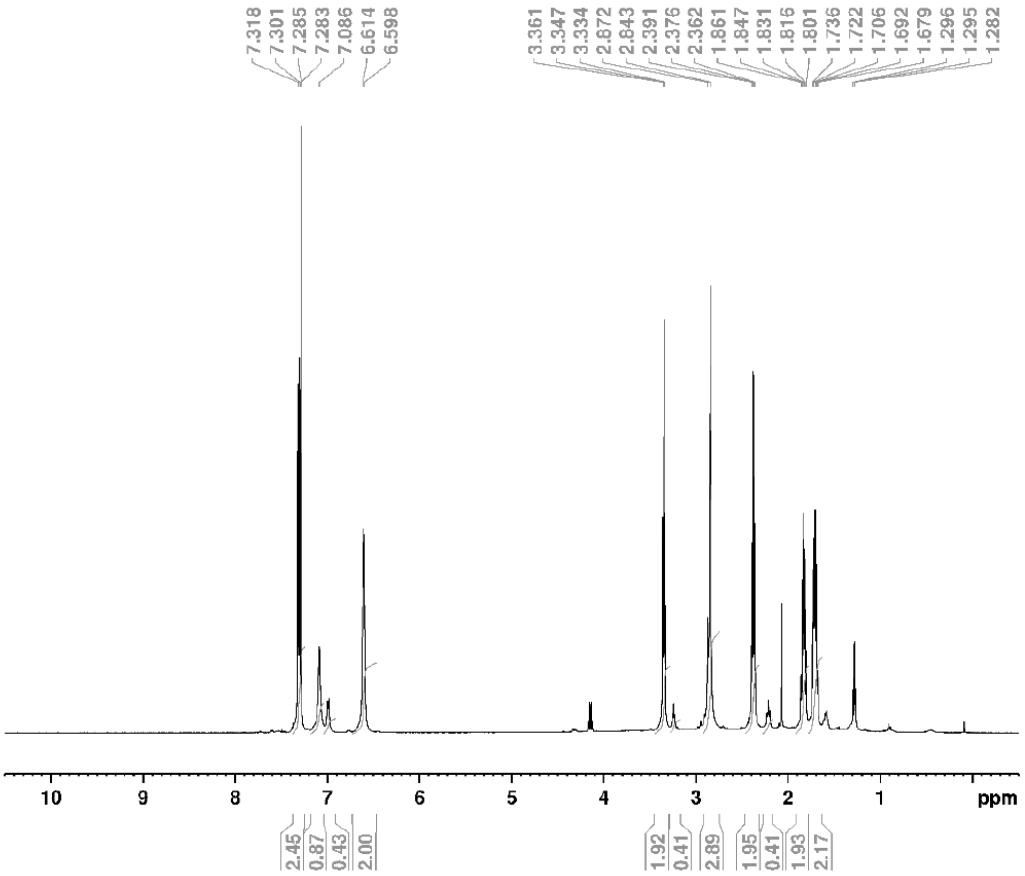
^{1}H-NMR spectrum of compound **8** (400 MHz; $CDCl_3$) [2)]



^{1}H-NMR spectrum of compound **9** (400 MHz; DMSO-d_6) [3)]



^{1}H-NMR spectrum of compound **10** (500 MHz; $CDCl_3$) [4)]



4. References

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2 S. Sato, K. Hatano, M. Tsushima and H. Nakamura, *Chem. Commun.*, 2018, **54**, 5871–5874.

3 S. Sato and H. Nakamura, *Angew. Chemie - Int. Ed.*, 2013, **52**, 8681–8684.

4 S. Sato, M. Yoshida, K. Hatano, M. Matsumura and H. Nakamura, *Bioorganic Med. Chem.*, 2019, **27**, 1110–1118.