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Supporting Information

Laccase-Catalyzed Tyrosine Click with 1-Methyl-4-Arylurazole: Rapid Labeling on Protein Surfaces

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1. Supporting Data

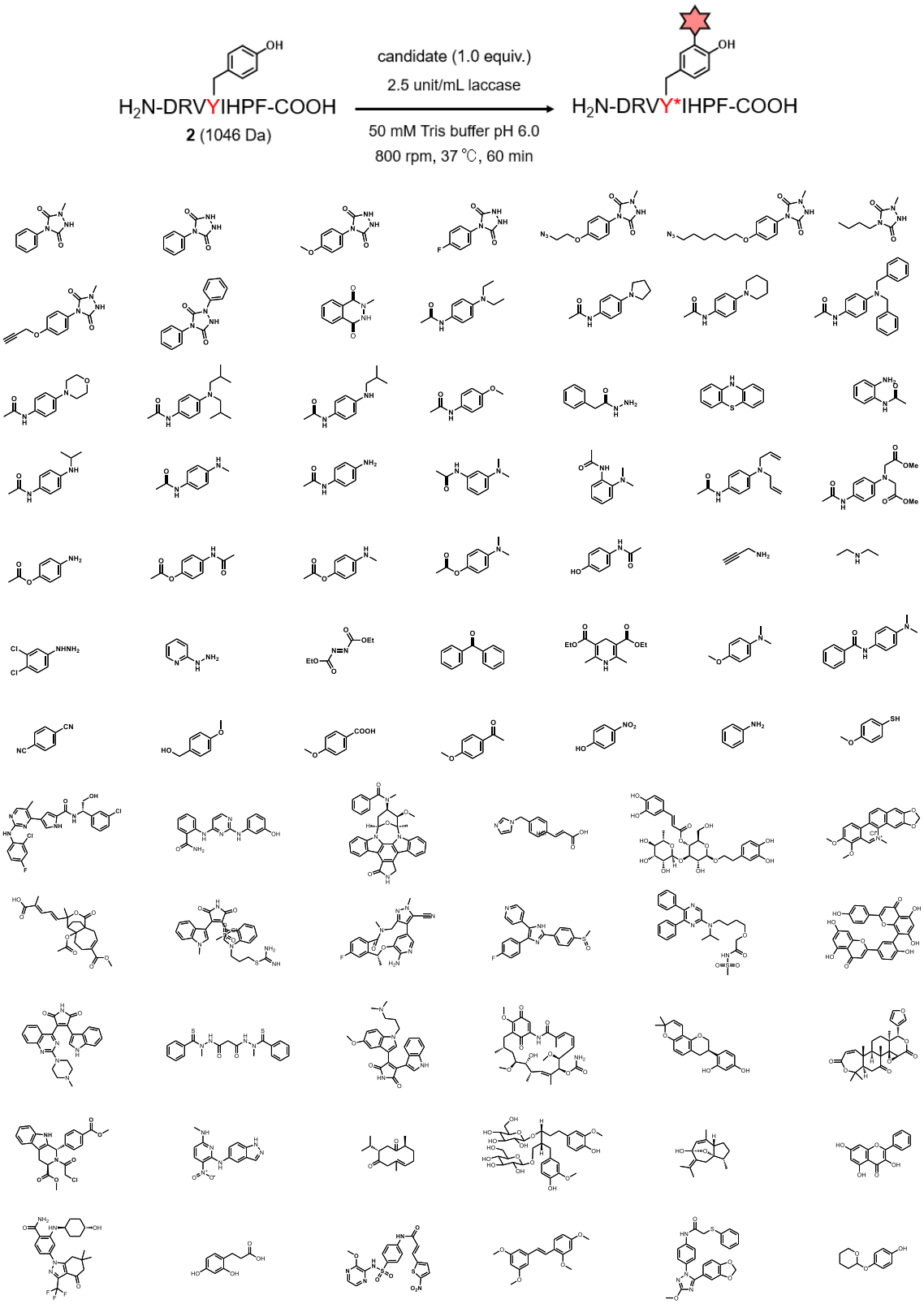


Figure S1. Compounds used for screening Tyr labeling reagents.

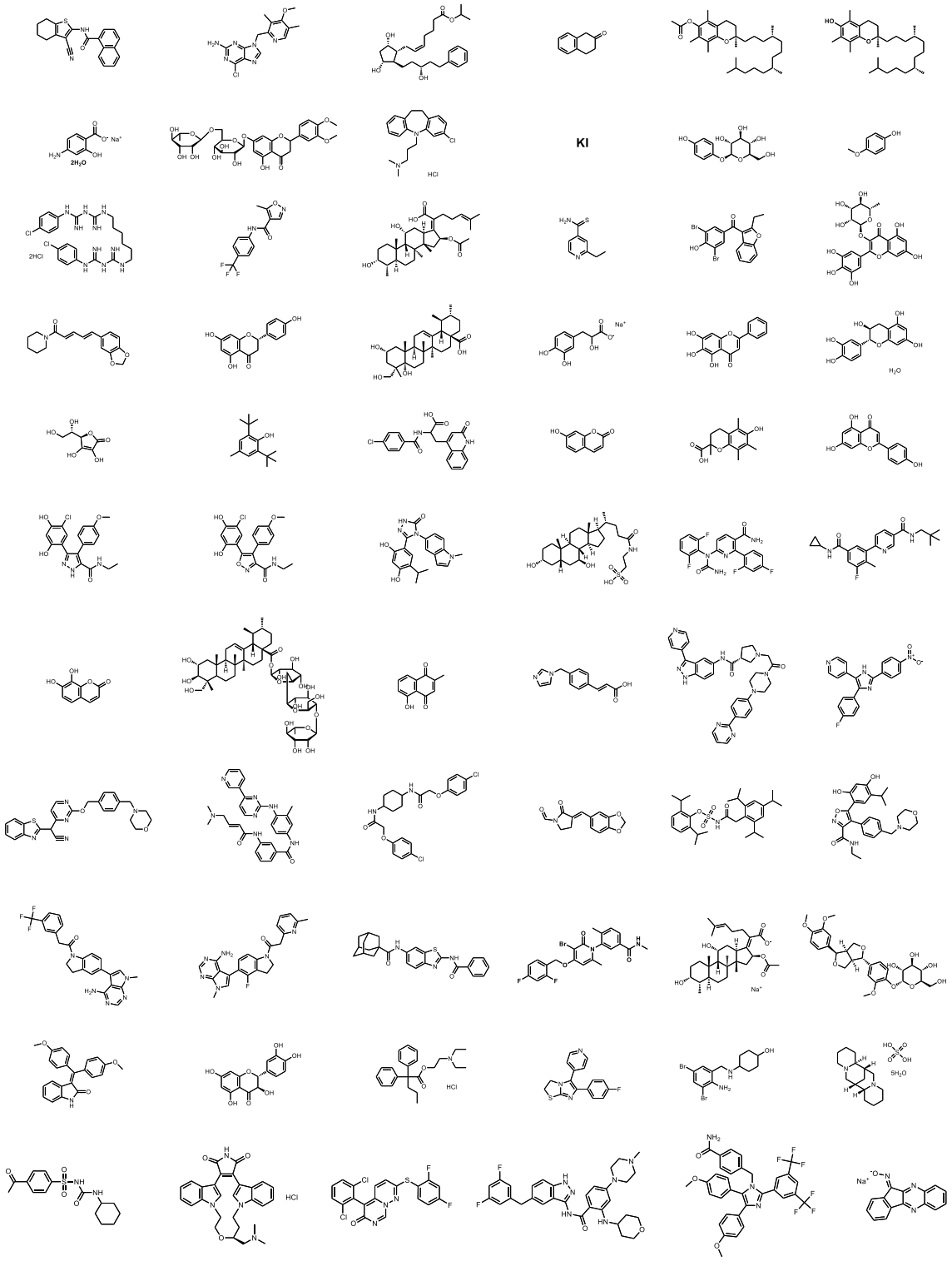


Figure S1. Compounds used for screening Tyr labeling reagents (continued).

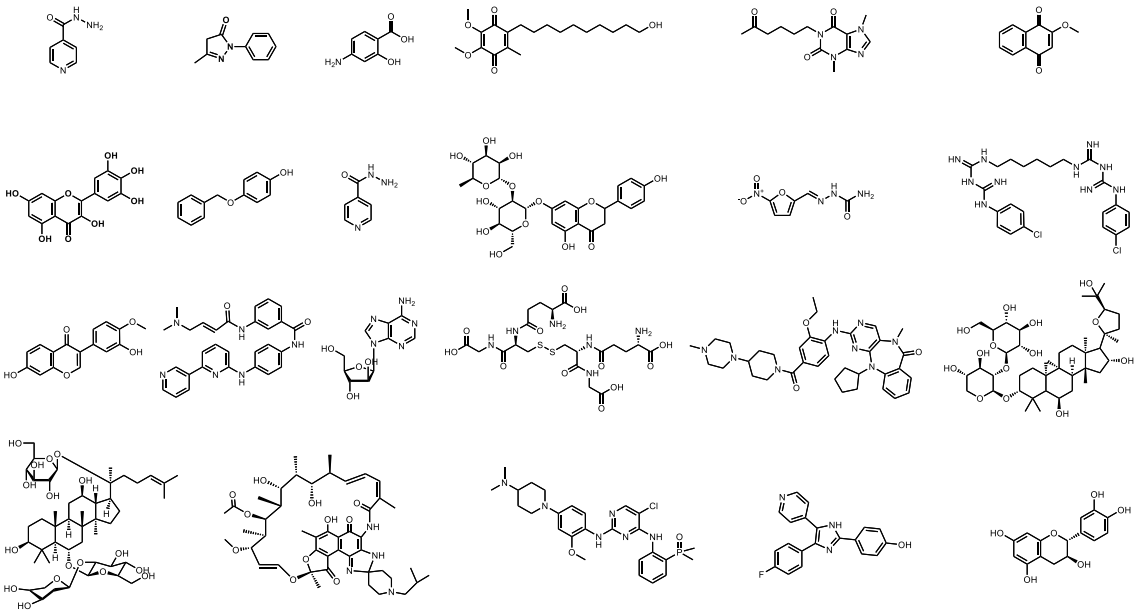


Figure S1. Compounds used for screening Tyr labeling reagents (continued).

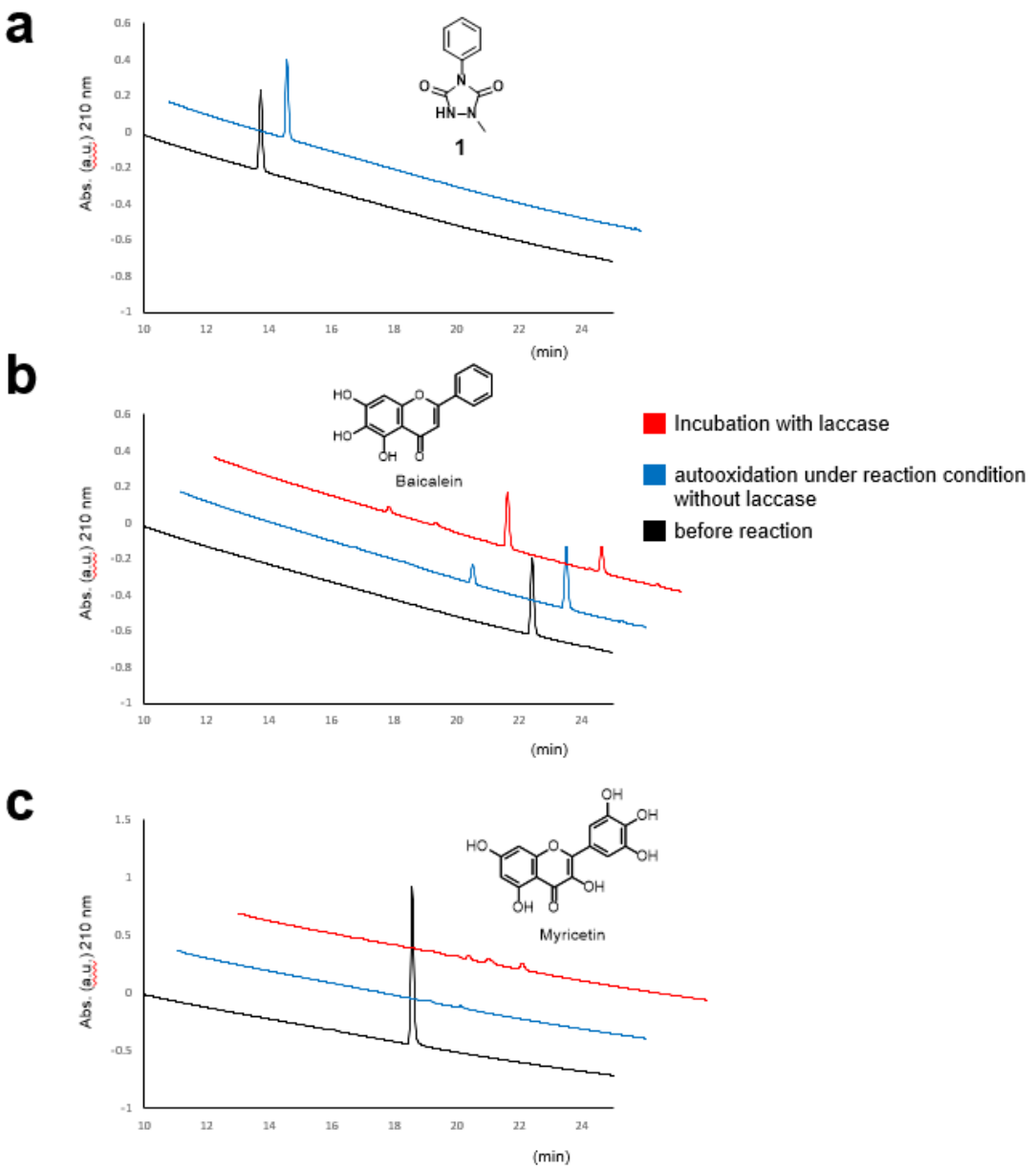


Figure S2. Autooxidation of MAUra and Catechols. (a) Autooxidation under the reaction conditions for MAUra. For oxidation by laccase, refer to Figure S7b. (b,c) Stability and reactivity of baicalein (b) and myricetin (c).

Baicalein and myricetin were selected as representative catechols from the screened compounds to evaluate their stability against autooxidation under the reaction conditions and their stability in the presence of laccase. Additionally, under the screening conditions, no MS peaks corresponding to reaction products between these catechols and angiotensin II were detected. In contrast, MAUra was found to be a stable compound and successfully labeled angiotensin II, as shown in Figure 2.

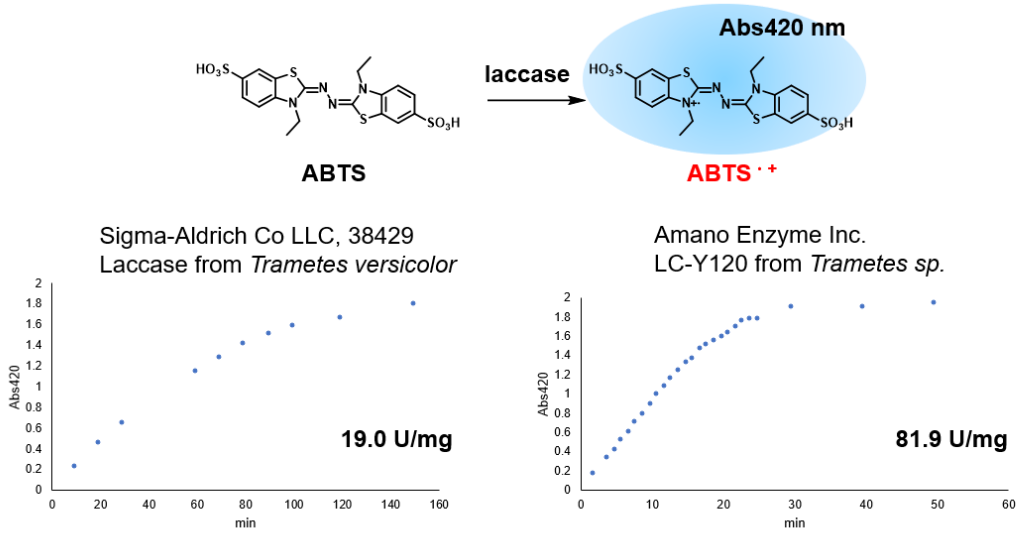


Figure S3. Measurement of laccase activity.

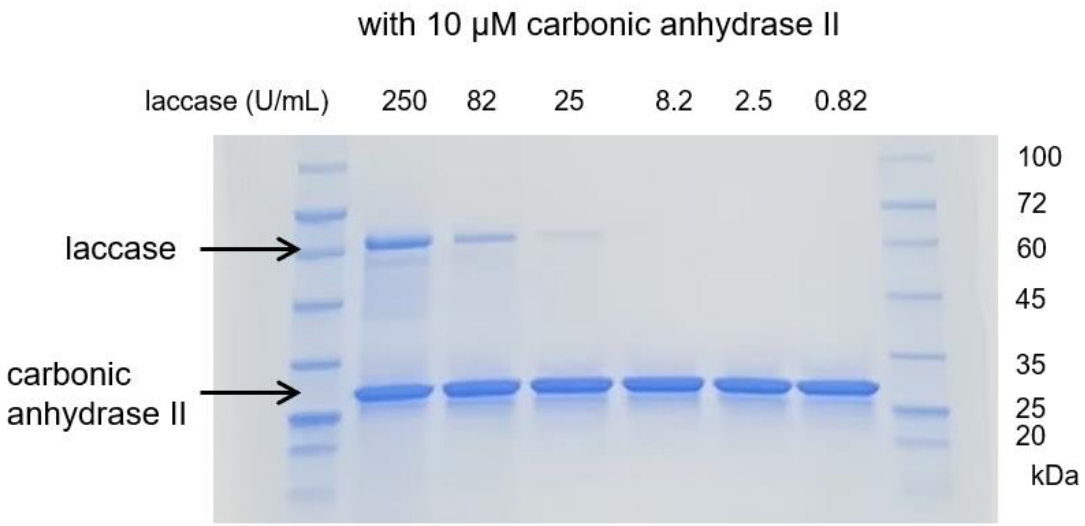


Figure S4. Protein concentration corresponding to the activity of Laccase.

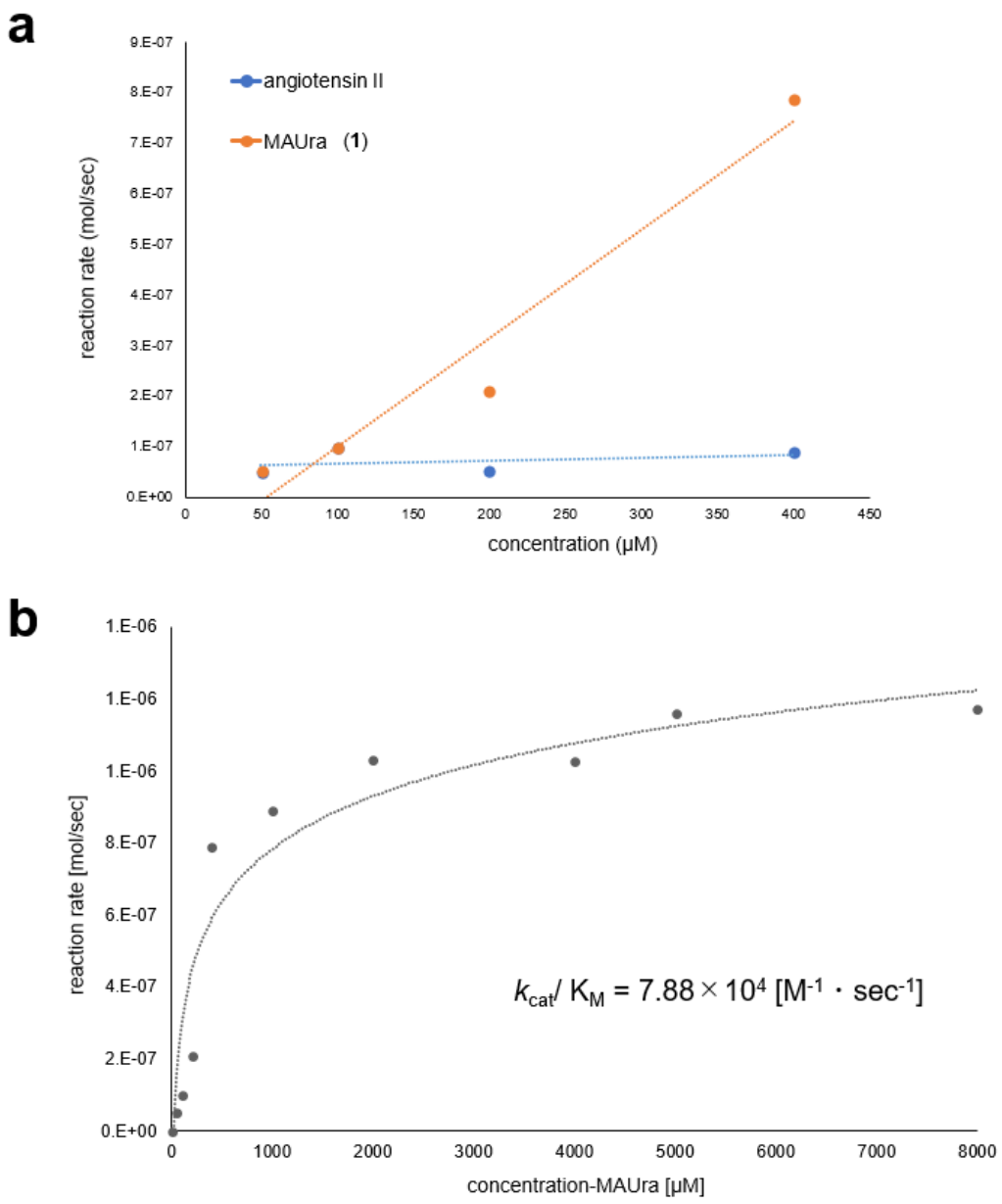


Figure S5. Substrate concentration dependence and reaction kinetics. (a) Relationship between substrate concentrations and the rate of the Tyr labeling reaction. (b) k_{cat}/K_m estimated from MAUra concentration and reaction rate (2.5 U/mL laccase = 13 nM).

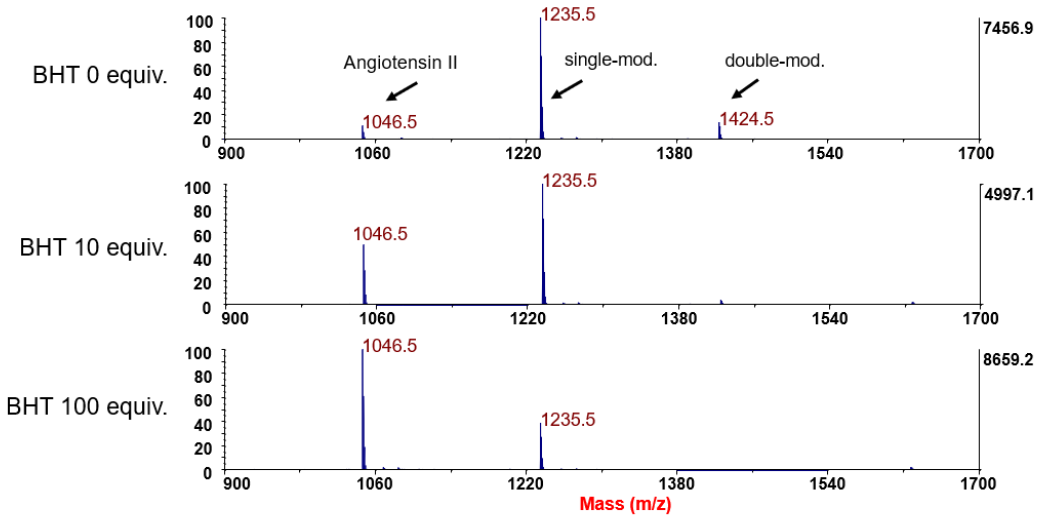


Figure S6. Reaction inhibition by radical scavenger BHT.

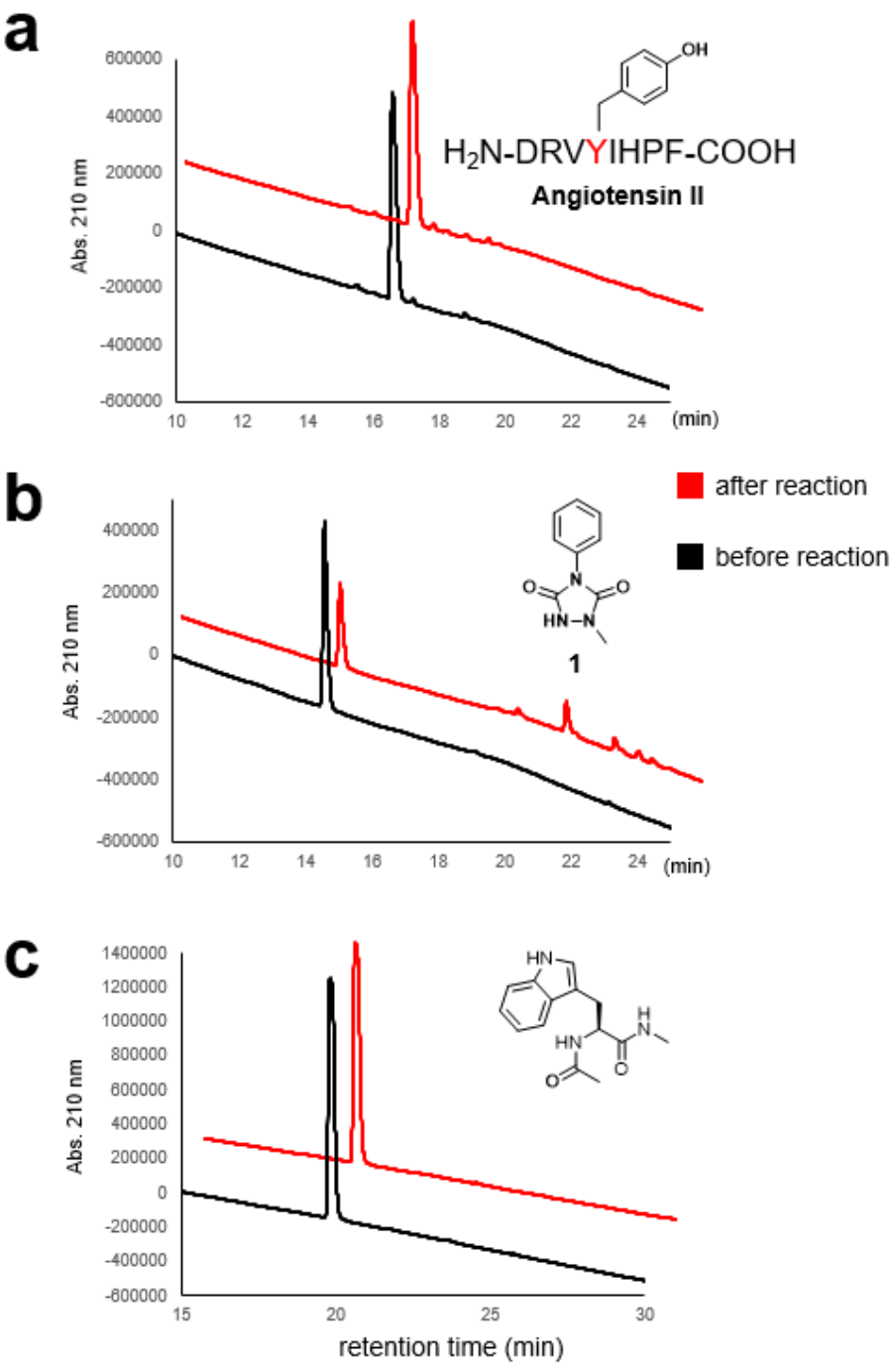


Figure S7. Reaction of each substrate with laccase. (a) Angiotensin II. (b) MAUra **1**. (c) Ac-Trp-NHMe.

Although tyrosine-containing peptides were not degraded by mixing with laccase, approximately 50% of MAUra was degraded after incubation for 60 min in the absence of tyrosine residues. These results suggest that laccase oxidatively activates MAUra. The incomplete degradation observed in the reaction between laccase and MAUra may reflect the reversible nature of the MAUra radical.

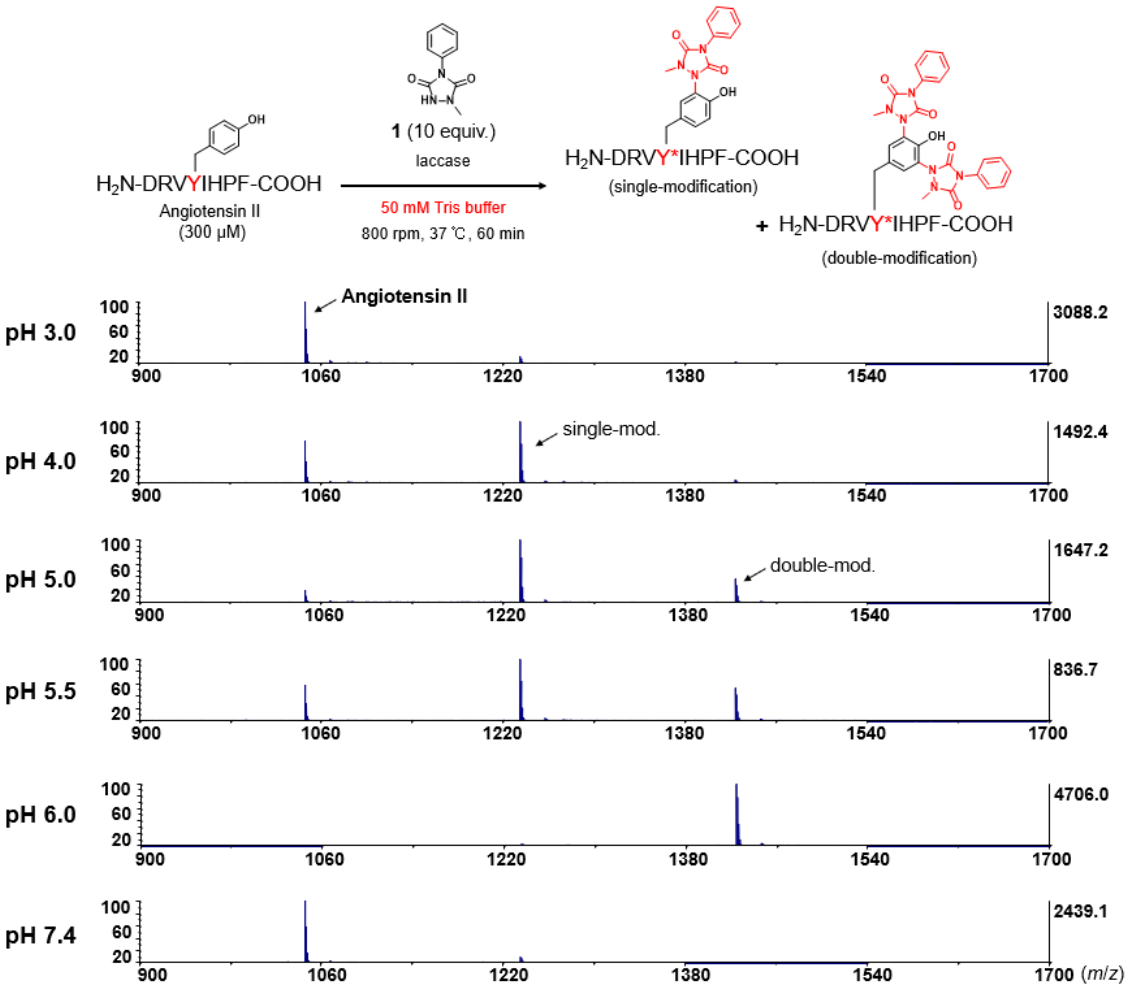


Figure S8. Effect of pH on laccase-catalyzed Tyr click.

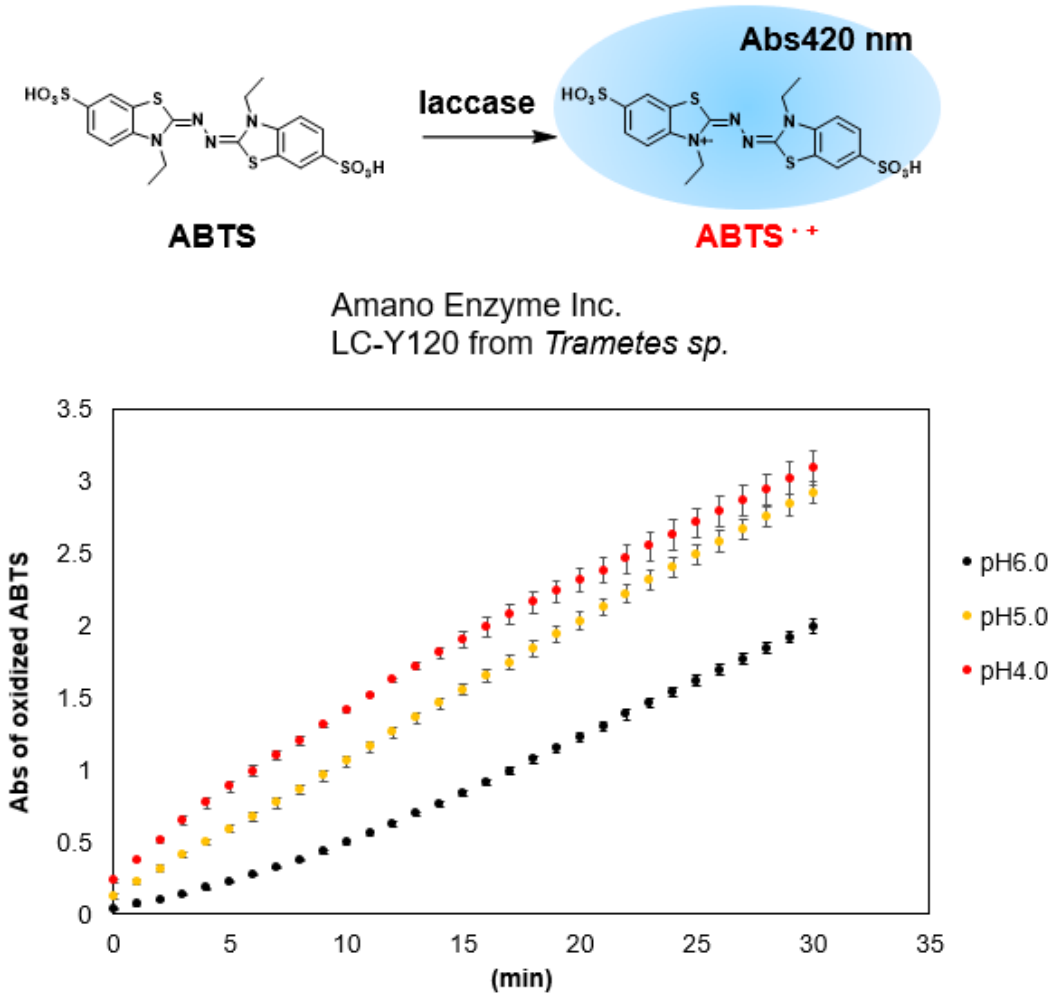


Figure S9. pH dependence of the oxidation of ABTS by laccase.

Although the activity of laccase is more pronounced at low pH, the ratio of the anionic form of MAUra present was also an important factor, with pH 6.0 being the most efficient reaction condition.

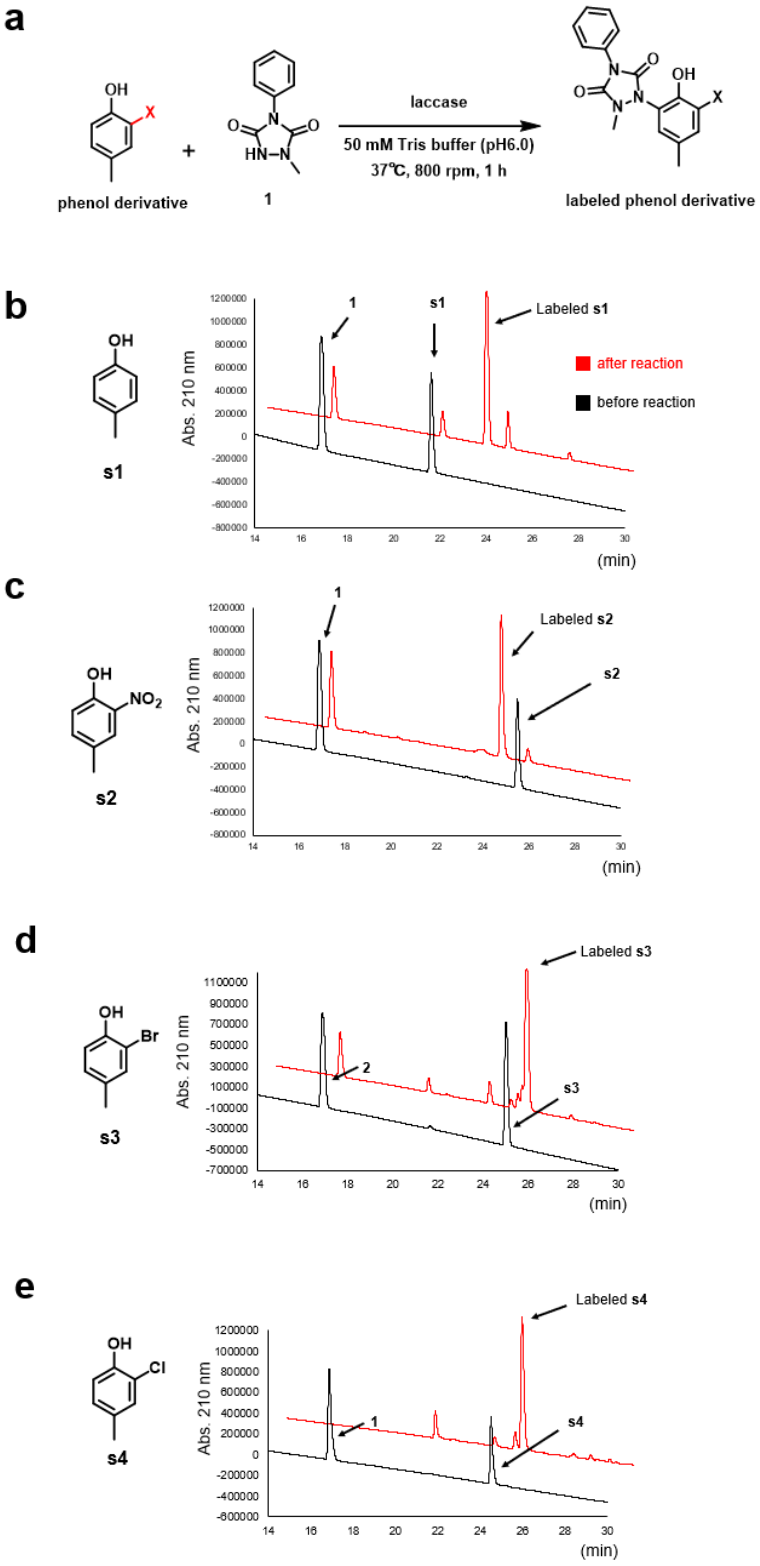


Figure S10. Labeling reaction with compound **1** to ortho-substituted phenols.

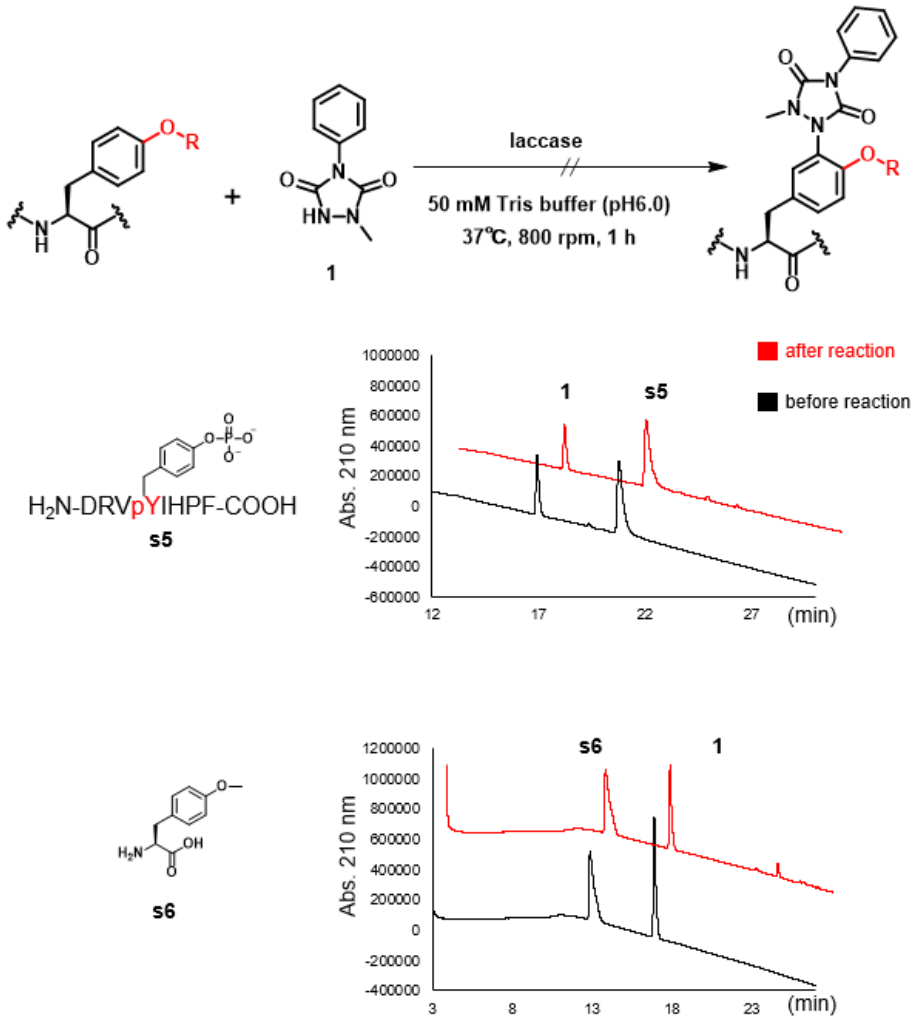


Figure S11. Labeling reaction with compound **1** to phosphotyrosine and *O*-methyl tyrosine.

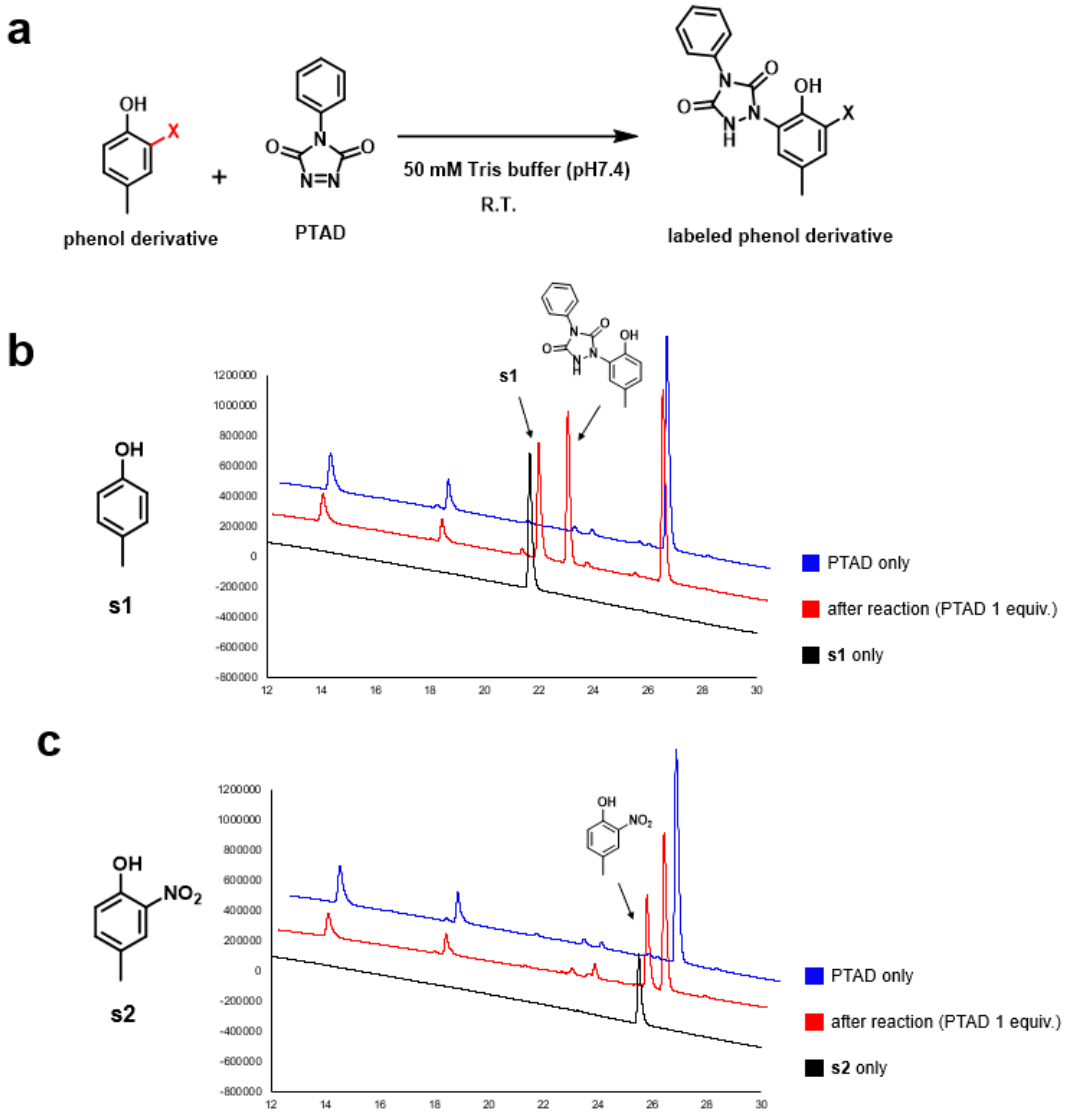


Figure S12. PTAD labels *para*-cresol but not 2-nitro-*para*-cresol.

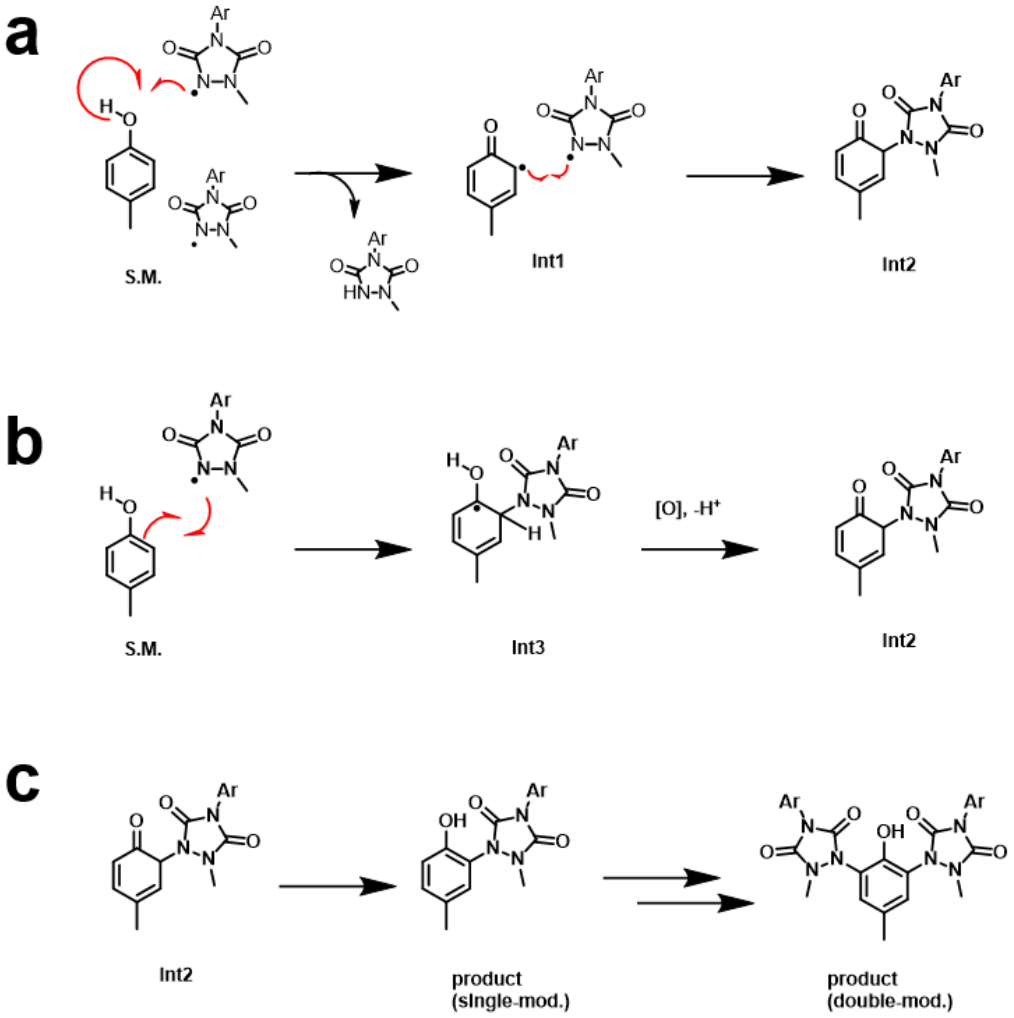


Figure S13. Proposed reaction mechanism. There are two possible mechanisms for the formation of the bond between MAUra radicals and Tyr, shown in (a) and (b). (a) The mechanism where the MAUra radical abstracts one electron from tyrosine, generating a tyrosyl radical. The tyrosyl radical then forms a bond with another MAUra radical molecule. (b) The MAUra radical forms a bond with a tyrosine residue, and the resulting radical species (Int 3) undergoes further single-electron oxidation. The electron acceptor is assumed to be either laccase or a MAUra radical. (c) Mechanism from Int 2: Tautomerization leads to the formation of a single-modified product, which can further undergo a second labeling step, resulting in double-modification.

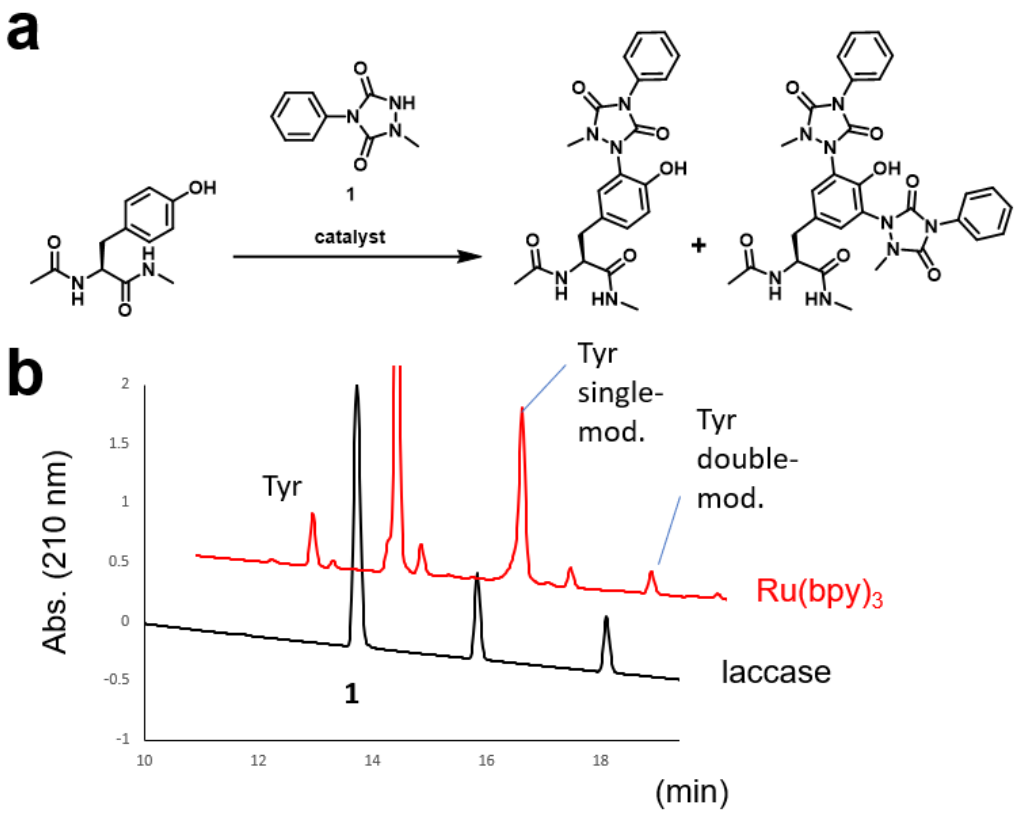


Figure S14. Comparison of conventional methods and confirmation of double-modification product. (a) Reaction scheme for labeling of Ac-Tyr-NHMe. (b) Comparison of the product of laccase method with the method previously reported to cause double-modification.[1] Reaction conditions: 100 μ M Ac-Tyr-NHMe, 100 μM $Ru(bpy)_3Cl_2$, 10 mM MES pH 6.0 containing 20% DMSO, 1 mM **1**, LED 455 nm 5 min (Ru). 100 μM Ac-Tyr-NHMe, 8.2 U/mL laccase, 1 mM **1**, 50 mM Tris buffer pH 6.0, 800 rpm 20 min (laccase).

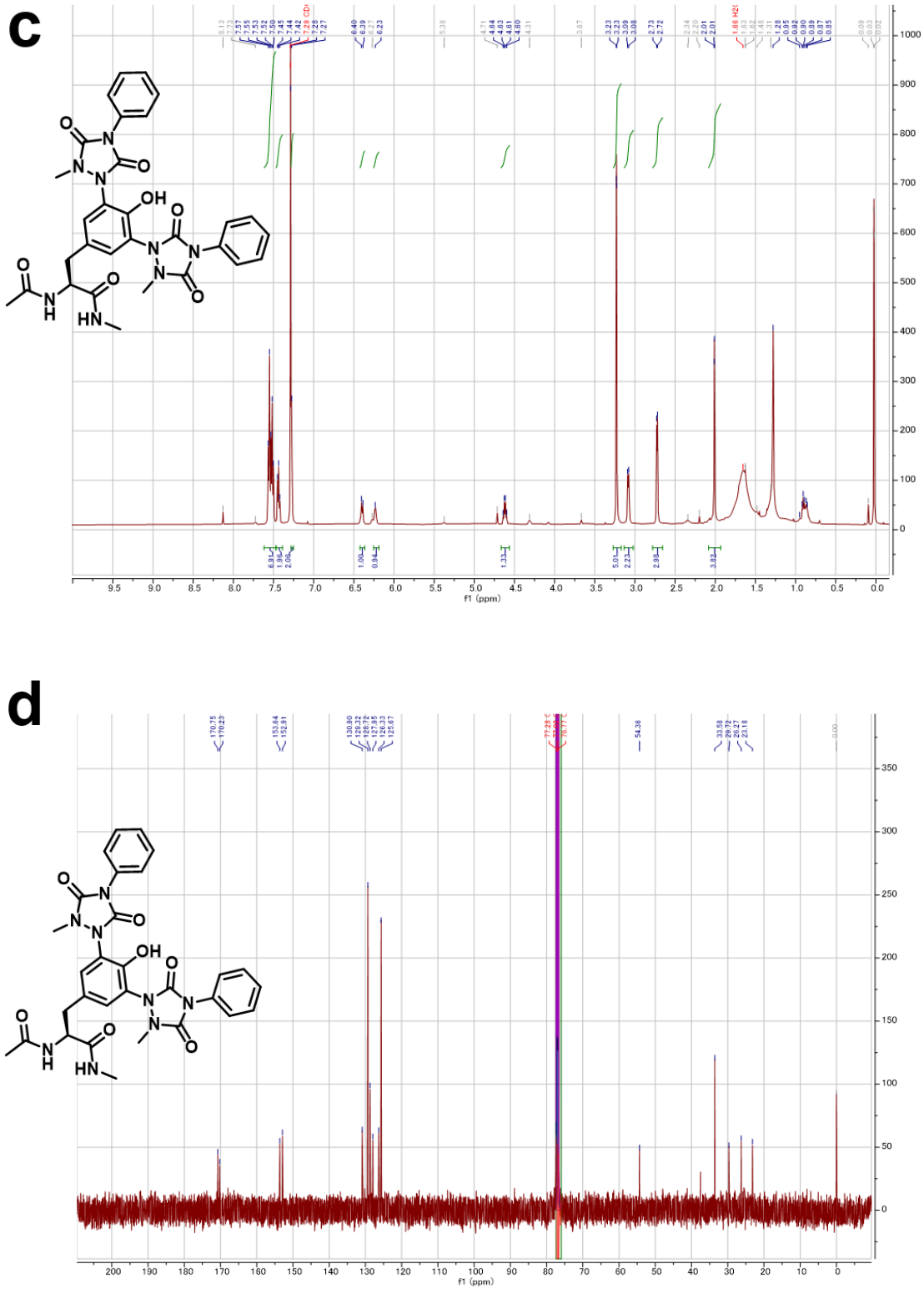
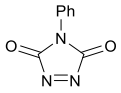
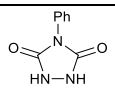
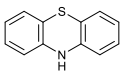
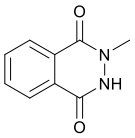
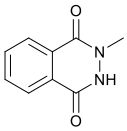
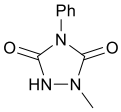


Figure S14(continued). (c) ^{1}H-NMR of double-modification product. (d) ^{13}C-NMR of double-modification product.

Table S1. Comparison of the current method and previously reported Tyr labeling methods.

Entry	Method	Labeling reagent	Reaction conditions	Conversion (%)	Side reaction	detail
1	PTAD[2]	 **s7** (10 equiv.)	5 min	70%	NH_2 modification by byproduct	Figure S15
2	electrochemistry[3]	 **s8**(10 equiv.)	400 mV vs Ag/AgCl 60 min	80%	NH_2 modification by byproduct	Figure S16
3	electrochemistry[4]	 **s9** (10 equiv.)	900 mV vs Ag/AgCl 50% CH_3CN 30 min	>95%	oxidation	Figure S17
4	hemin[5]	 **7** (1.0 equiv.)	hemin (10 μM) H_2O_2 (10 equiv.) 60 min	85%	N.D.	Figure S18
5	HRP[6]	 **7** (1.0 equiv.)	HRP (45 nM) H_2O_2 (1.0 equiv.) 60 min	70%	N.D.	Figure S19
6	laccase	 **1** (1.0 equiv.)	laccase (2.5 U/mL) shaking, 37° C 60 min	>95%	N.D.	Figure 1
7			laccase (82 U/mL) shaking, 37° C 5 min	>95%	N.D.	-

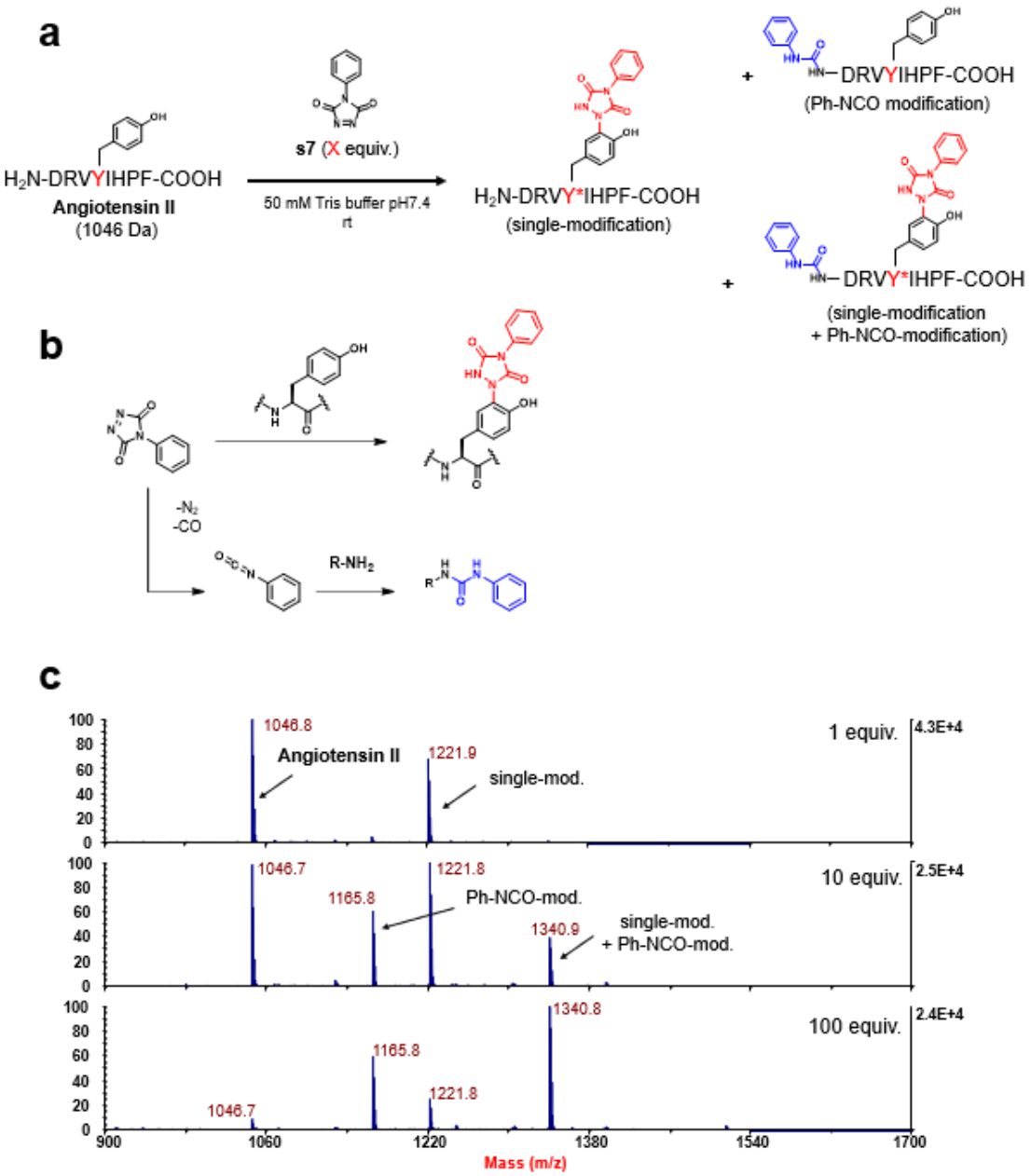


Figure S15. Excessive addition of PTAD promotes side reactions with amino groups and does not induce double modification.

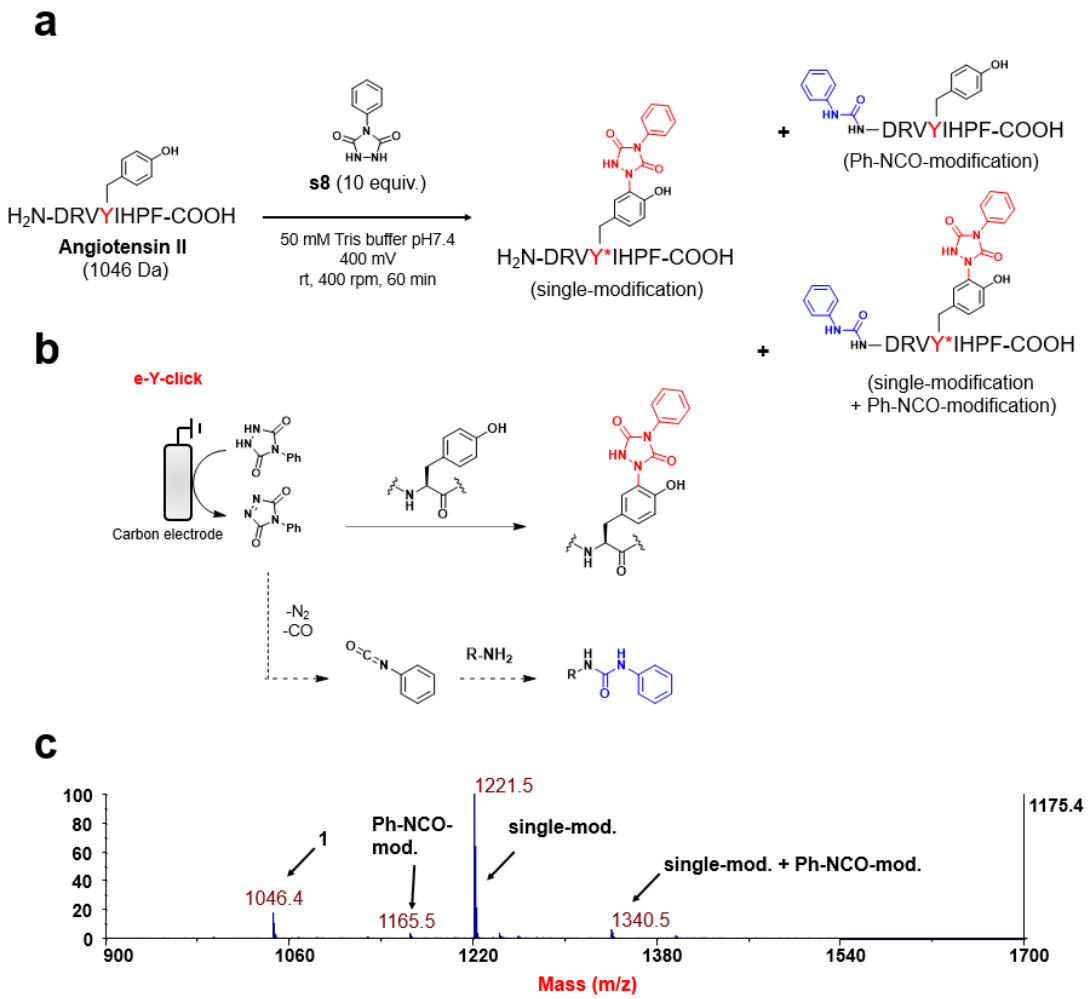


Figure S16. Tyrosine labeling using electrochemical generation of PTAD. (a) Scheme of tyrosine labeling. (b) Generation of PTAD on electrodes. (c) Result of MALDI-TOF MS.

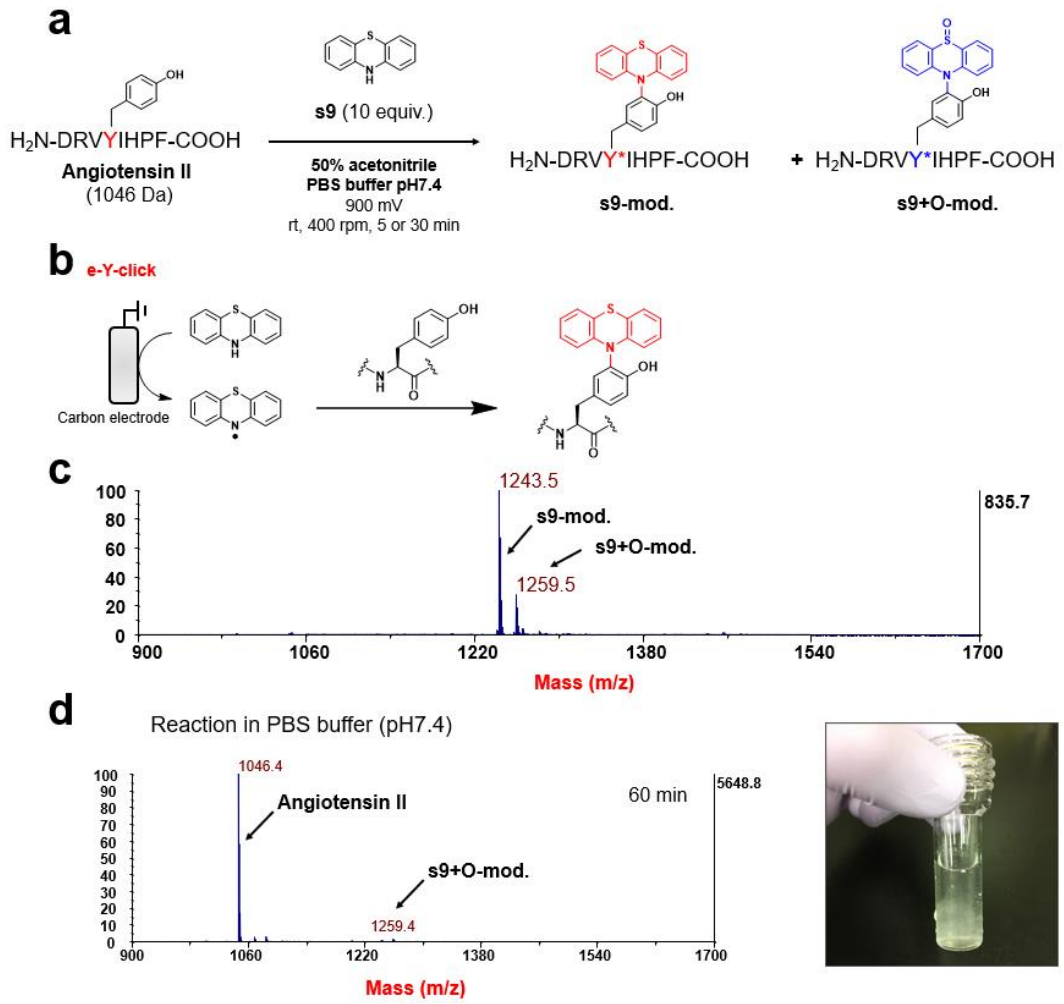


Figure S17. Tyrosine labeling using the electrochemical activation of phenothiazine. (a) Scheme of tyrosine labeling. (b) Generation of phenothiazine radical on electrodes. (c) The result of MALDI-TOF MS. (d) Reaction efficiency when PBS buffer is used instead of 50% acetonitrile buffer.

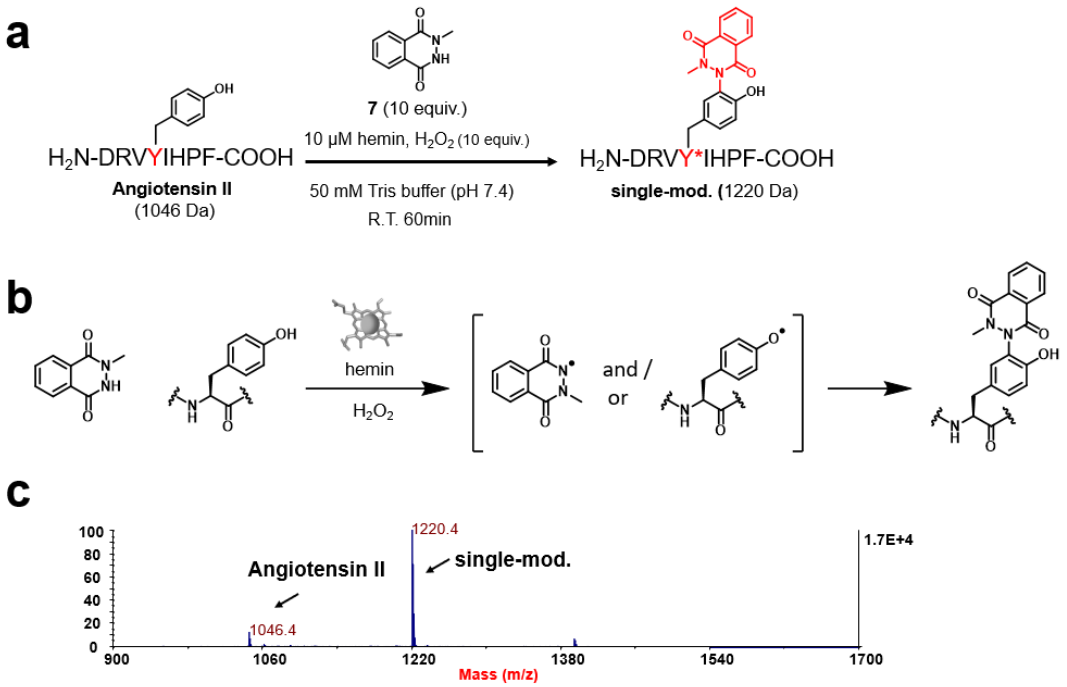


Figure S18. Tyrosine labeling using hemin and compound **7**. (a) Scheme of tyrosine labeling. (b) Estimated reaction mechanism. (c) The result of MALDI-TOF MS.

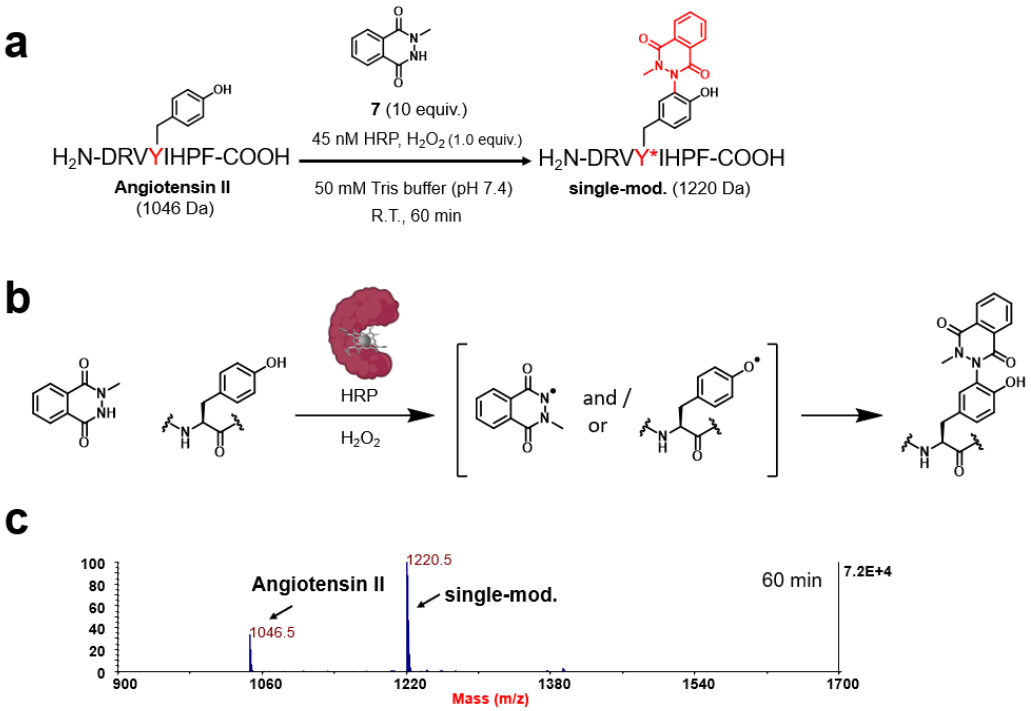


Figure S19. Tyrosine labeling using HRP and compound **7**. (a) Scheme of tyrosine labeling. (b) Estimated reaction mechanism. (c) The result of MALDI-TOF MS.

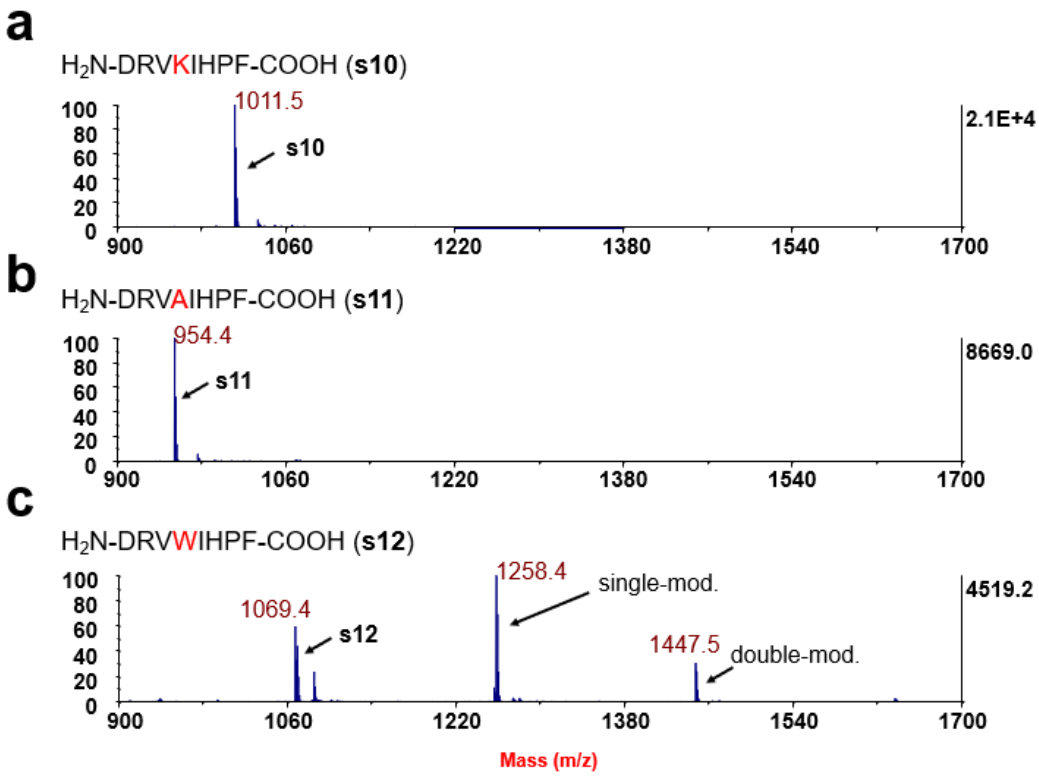


Figure S20. Reactivity to amino acid residues other than tyrosine. (a) Angiotensin II Y4K peptide. (b) Angiotensin Y4A peptide. (c) Angiotensin II Y4W peptide.

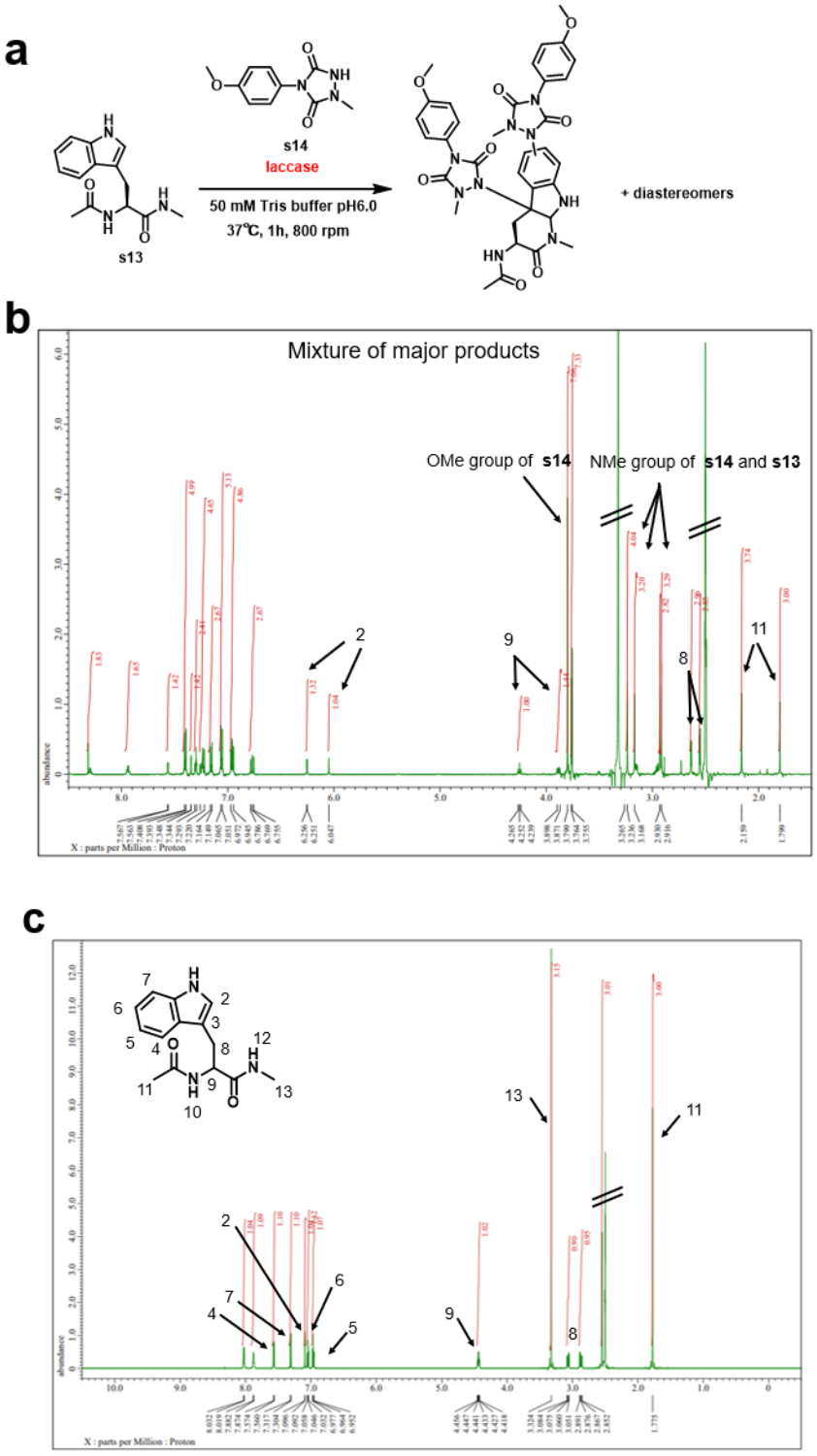


Figure S21. NMR analysis of modified Trp derivative. (a) Reaction scheme. (b) NMR spectrum of labeling product. (c) NMR spectrum of starting material.

This NMR result is consistent with papers reported on the binding mode of Trp and urazole.[7]

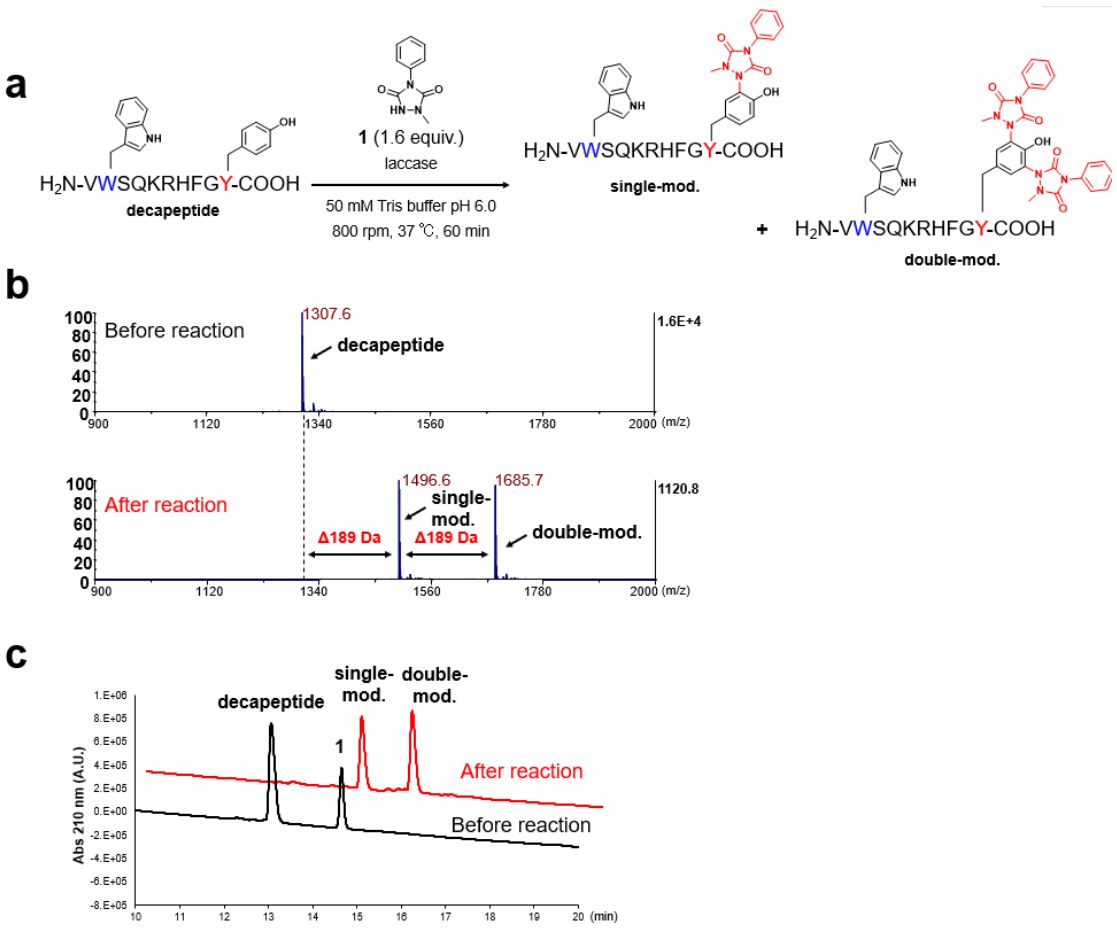


Figure S22. Verification of selectivity for tyrosine click. (a) Reaction scheme. (b) MALDI-TOF MS analysis before and after the reaction. (c) HPLC analysis before and after the reaction.

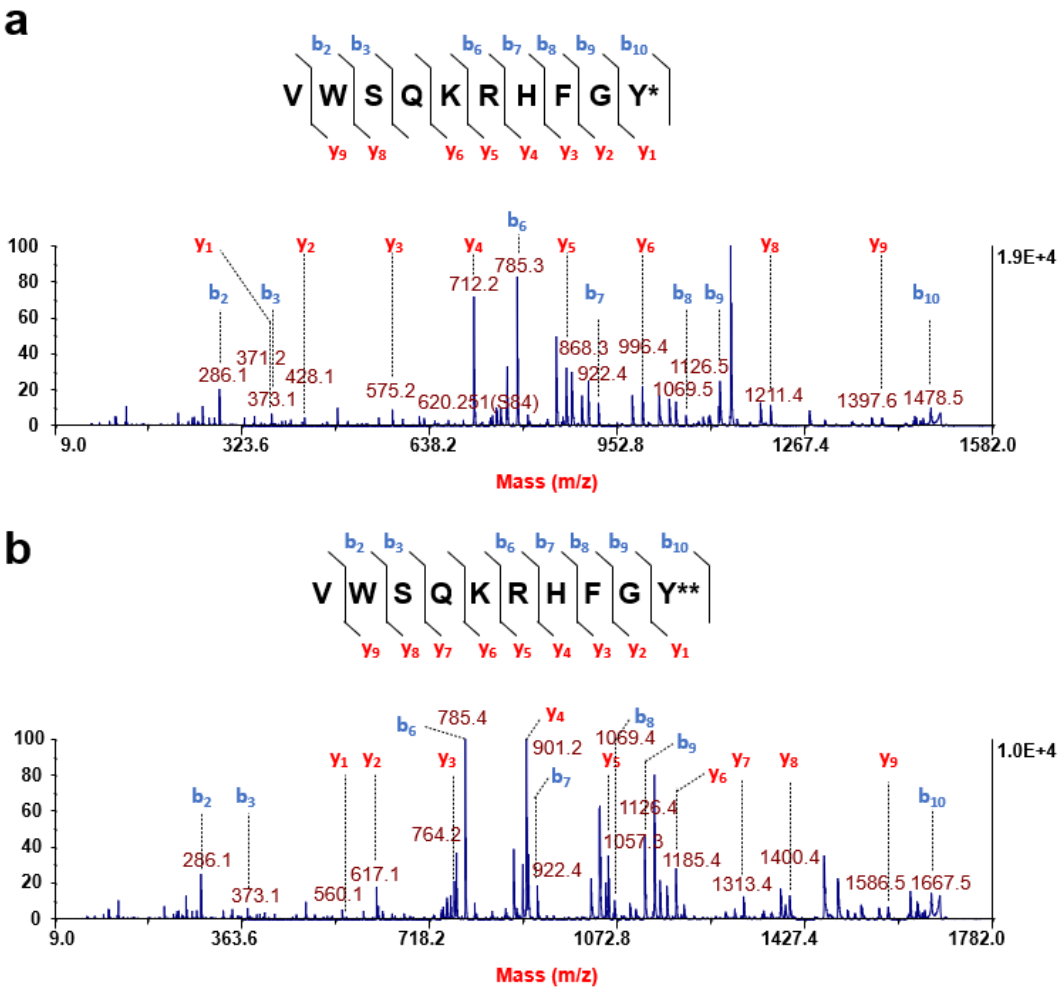


Figure S23. MS/MS analysis of the products in Figure S22. (a) MS/MS analysis of the single modified decapeptide. (b) MS/MS analysis of the double-modified decapeptide.

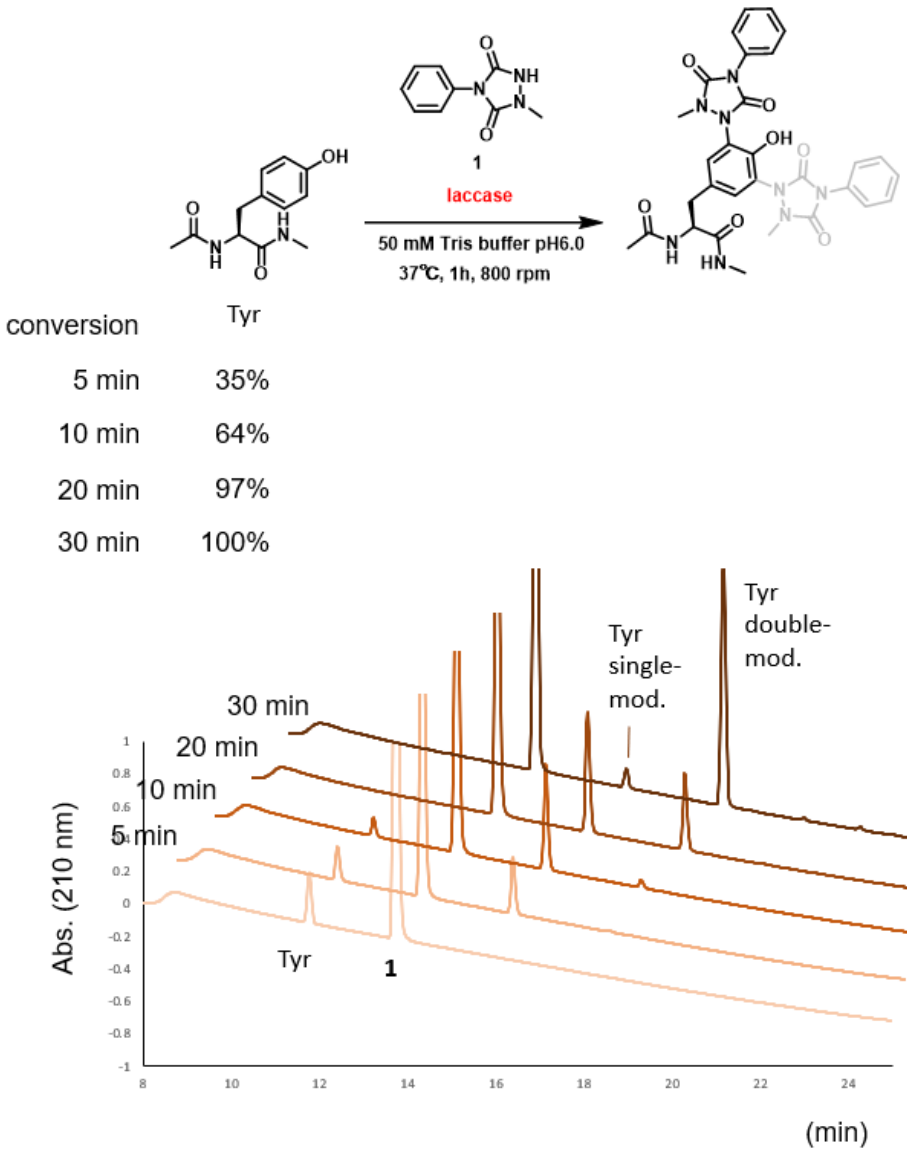


Figure S24. Kinetic analysis of the Ac-Tyr-NHMe labeling reaction.

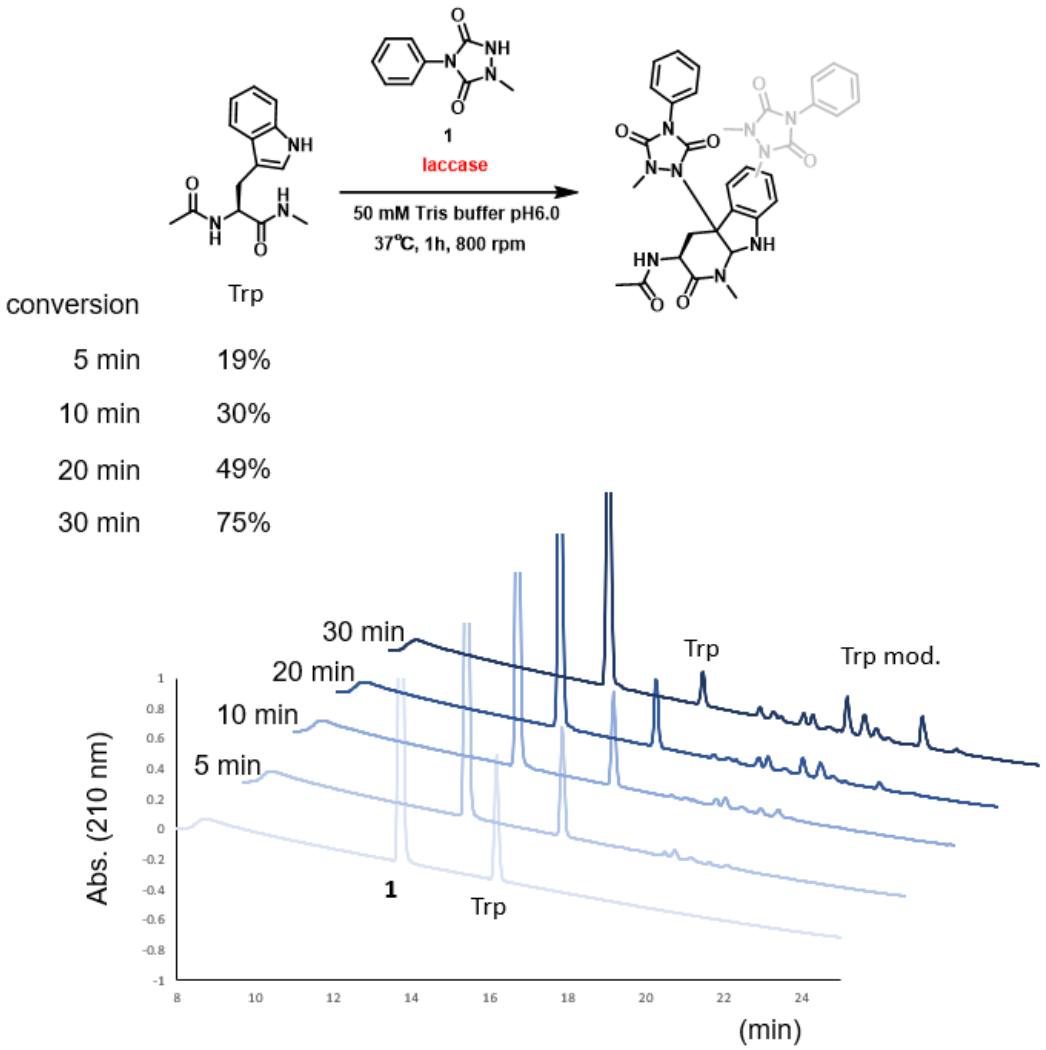
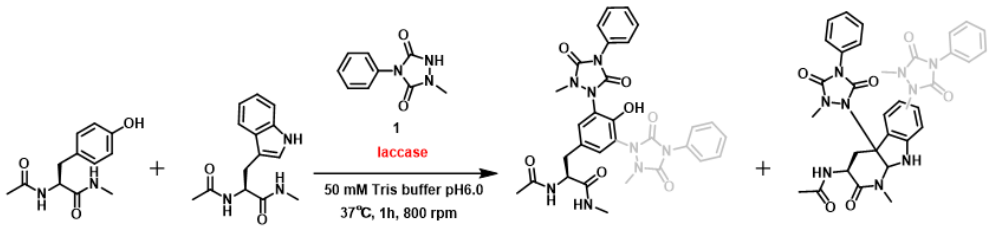


Figure S25. Kinetic analysis of the Ac-Trp-NHMe labeling reaction.



conversion	Tyr	Trp
5 min	31%	4%
10 min	57%	6%
20 min	93%	11%
30 min	97%	26%

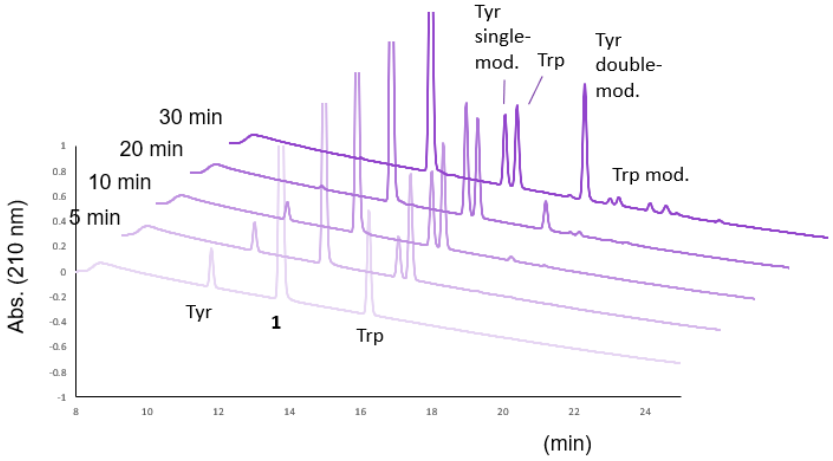


Figure S26. Kinetic analysis of labeling reactions for 1:1 mixtures of Ac-Tyr-NHMe and Ac-Trp-NHMe.

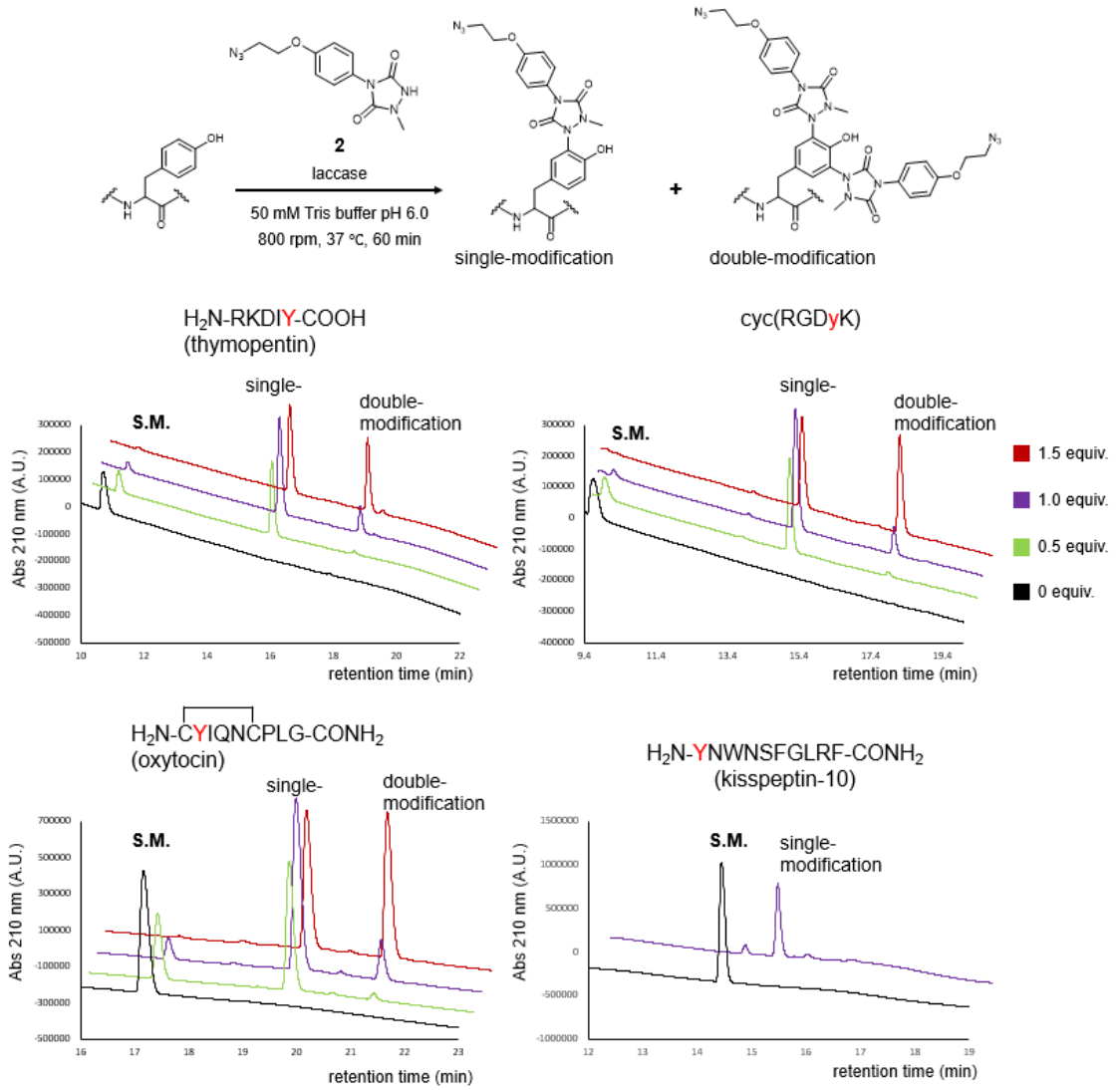


Figure S27. Laccase-catalyzed tyrosine click using peptides as substrates. Thymopentin, cycRGDyK, and oxytocin were labeled at a peptide concentration of 300 μM, 25 U/mL laccase, and kisspeptin-10 was labeled at a peptide concentration of 500 μM, 42 U/mL laccase.

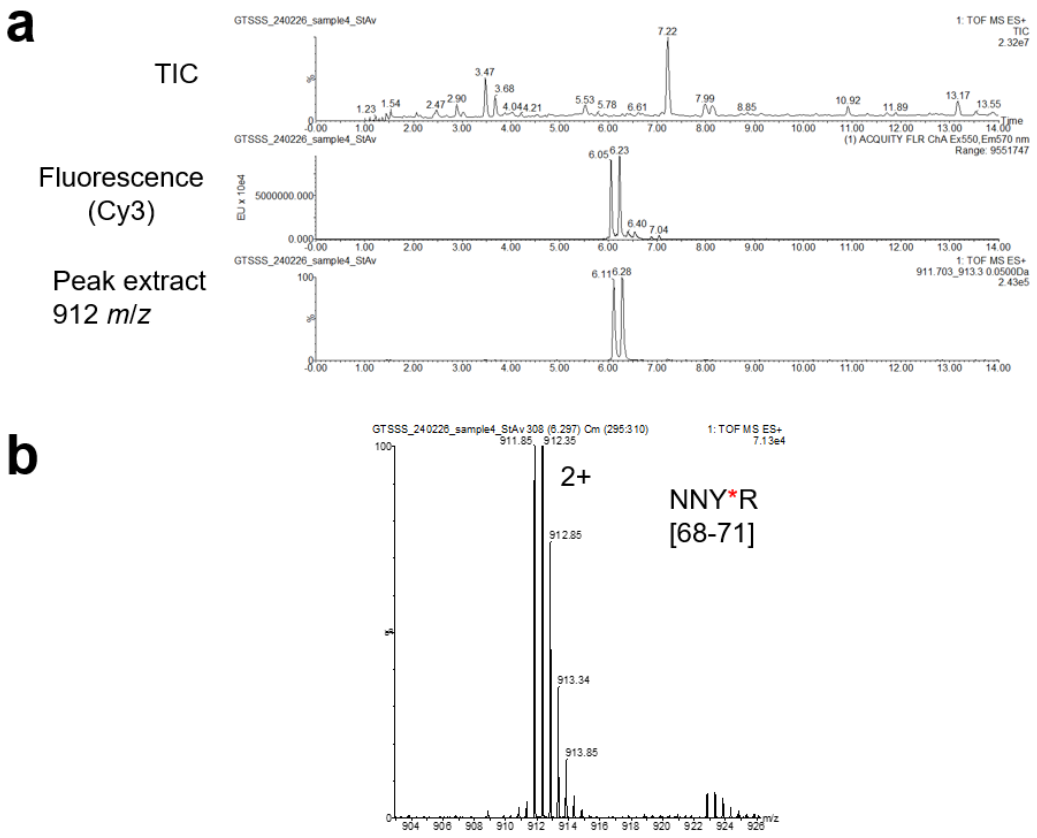


Figure S28. LC-MS of tryptic digests of Cy3-labeled streptavidin. (a) Result of LC-MS. (b) MS analysis of peaks at retention times corresponding to Cy3-fluorescent peaks, indicating the presence of Y70-labeled peptides.

The proteins were labeled with 3.0 equivalents of compound **2** and laccase (8.2 U/mL) at 37 °C, 800 rpm for 1 h. The labeling site was then Cy3-labeled with 10 equivalents of DBCO-Cy3, and the peptide fragments obtained by trypsin digestion in gel were analyzed using LC-MS, as shown in Figures S28–S32.

The appearance of two peaks for one modified fragment was most likely due to isomerism resulting from the click reaction between DBCO and the azide group. This was also suggested by the appearance of two peaks when the reaction products of compound **2** and DBCO-Cy3 were analyzed by HPLC under the same conditions (See Figure S33).

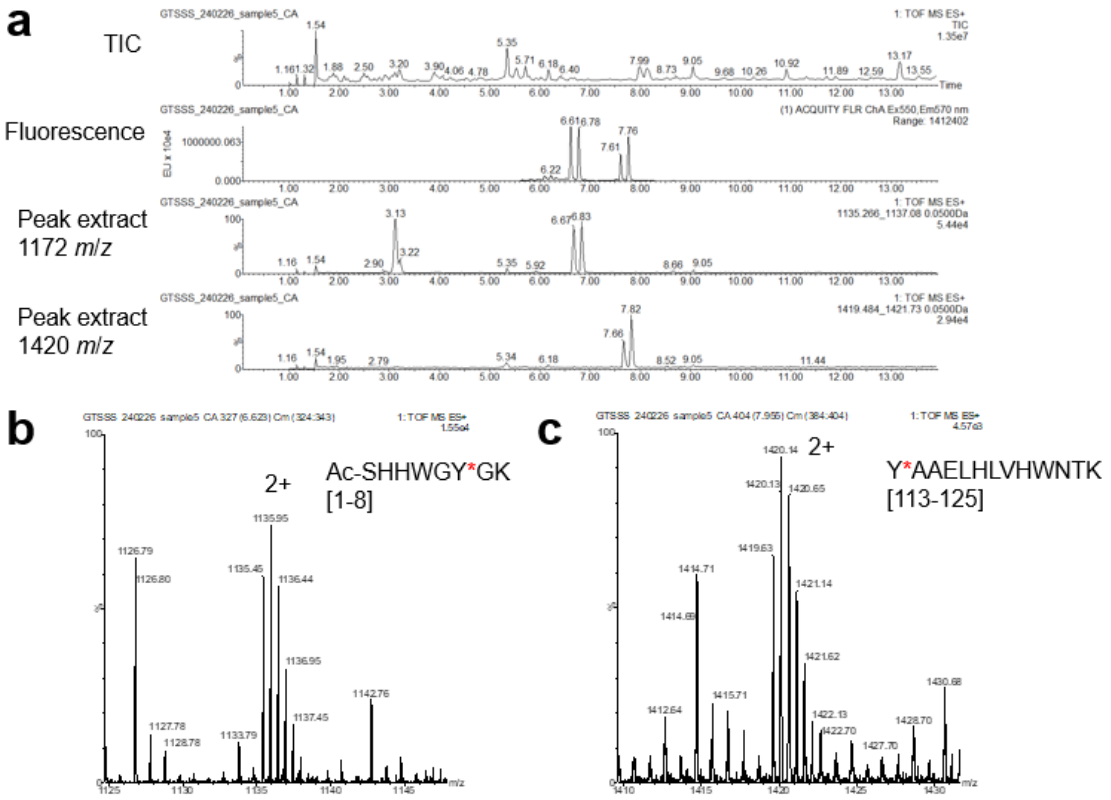


Figure S29. LC-MS of tryptic digests of Cy3-labeled carbonic anhydrase II. (a) The result of LC-MS. (b,c) MS analysis of peaks at retention times corresponding to Cy3-fluorescent peaks, indicating the presence of Y6- and Y113-labeled peptides.

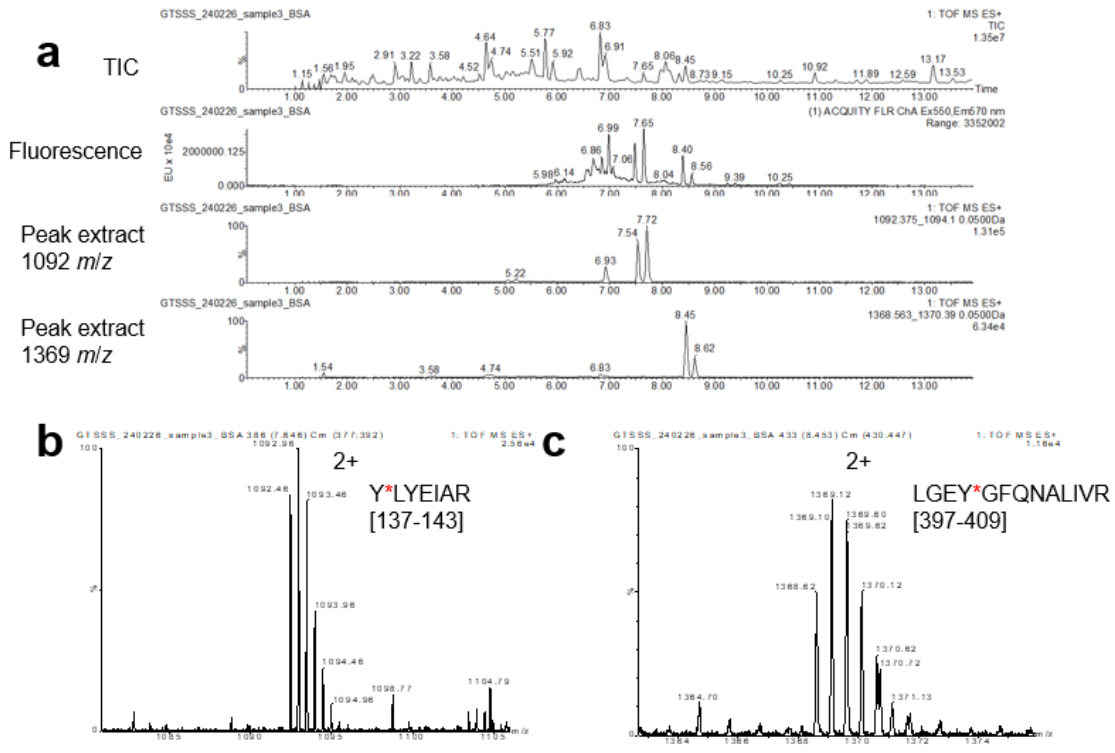


Figure S30. LC-MS of tryptic digests of Cy3-labeled bovine serum albumin. (a) Result of LC-MS. (b,c) MS analysis of peaks at retention times corresponding to Cy3-fluorescent peaks, indicating the presence of Y137- and Y400-labeled peptides.

Peptide fragments with minor labeling sites detected earlier than the retention time of 7.5 min could not be identified.

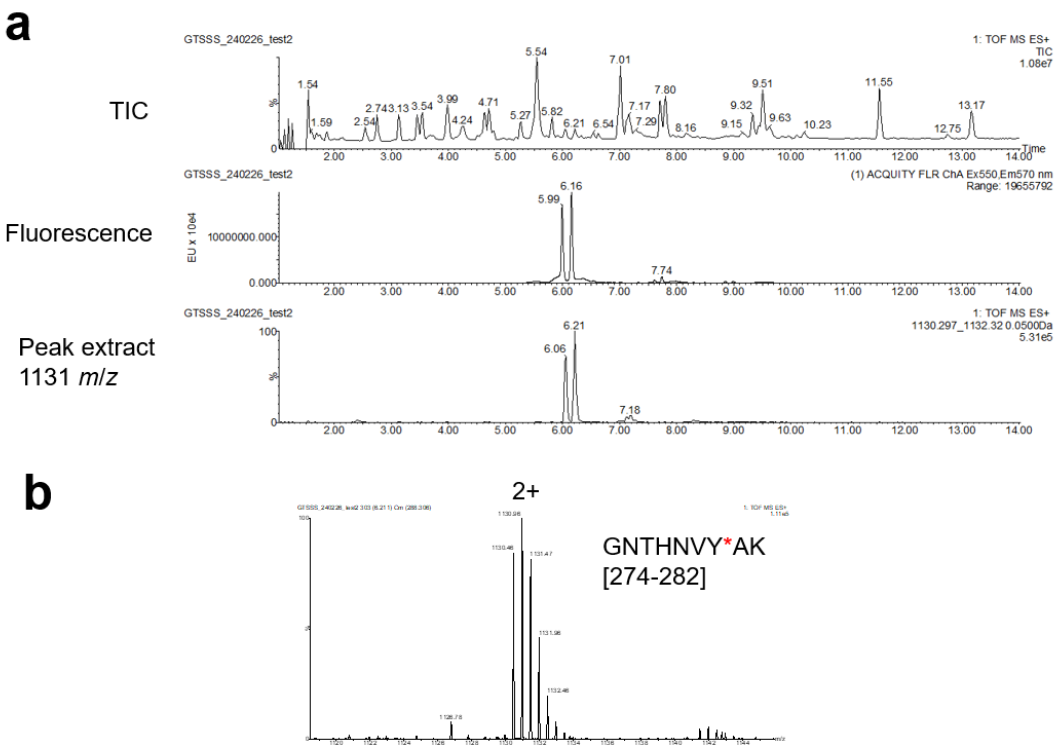


Figure S31. LC-MS of tryptic digests of Cy3-labeled glucose oxidase. (a) Result of LC-MS. (b) MS analysis of peaks at retention times corresponding to Cy3-fluorescent peaks, indicating the presence of Y280-labeled peptides.

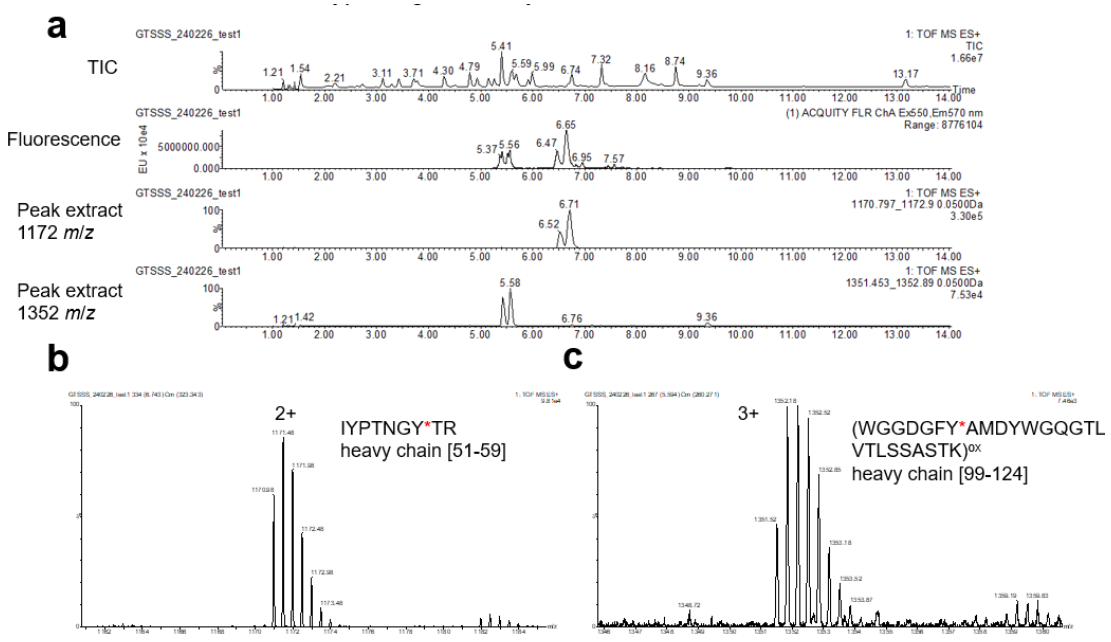


Figure S32. LC-MS of tryptic digests of Cy3-labeled trastuzumab. (a) Result of LC-MS. (b,c) MS analysis of peaks at retention times corresponding to Cy3-fluorescent peaks, indicating the presence of Y57 (heavy chain)- and Y105 (heavy chain)-labeled peptides.

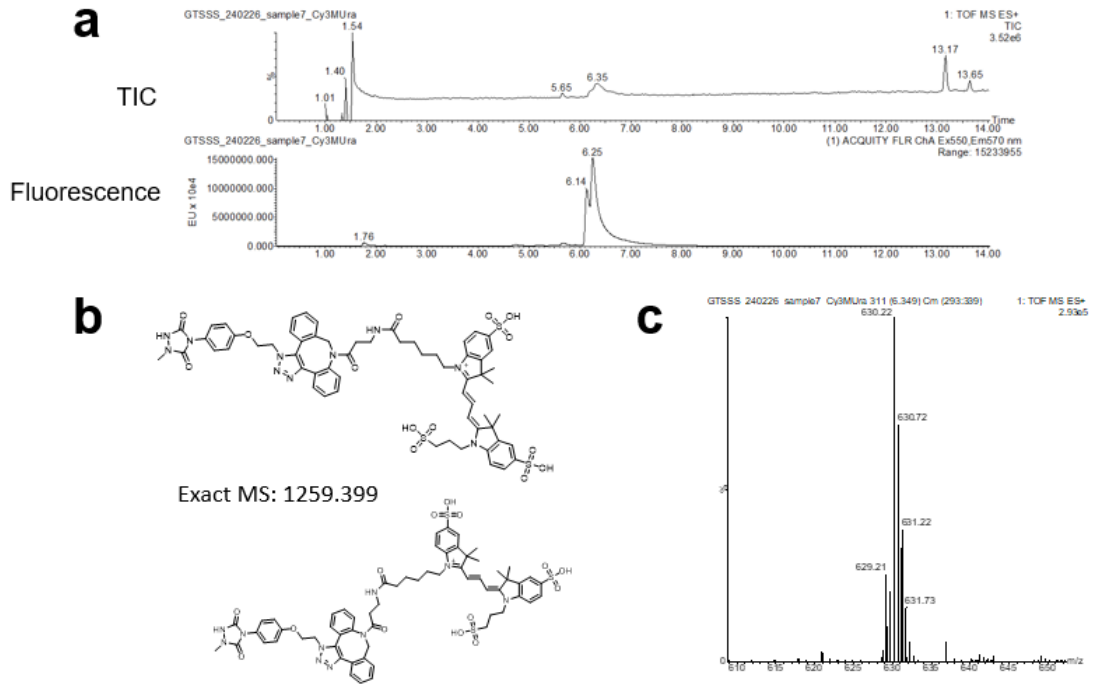


Figure S33. Control experiment for the reaction of compound **2** with DBCO-Cy3, suggesting that the two trend peaks originate from diastereomers produced during the triazole formation step. (a) Result of LC-MS. (b) Structures assumed as diastereomers. (c) MS analysis of peaks at retention times corresponding to Cy3-fluorescent peaks, indicating the presence of a compound in which **2** and DBCO-Cy3 are bound.

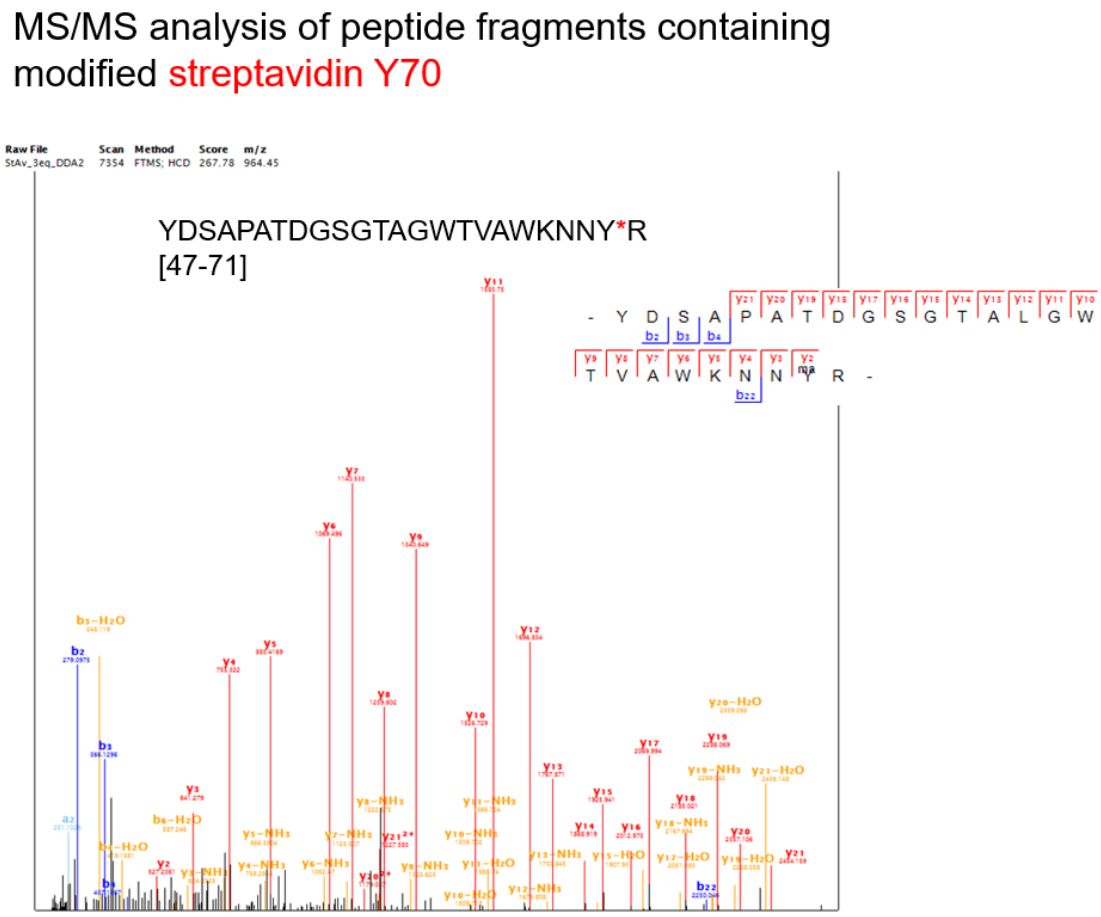


Figure S34. MS/MS analysis of peptide fragments containing modified streptavidin Y70.

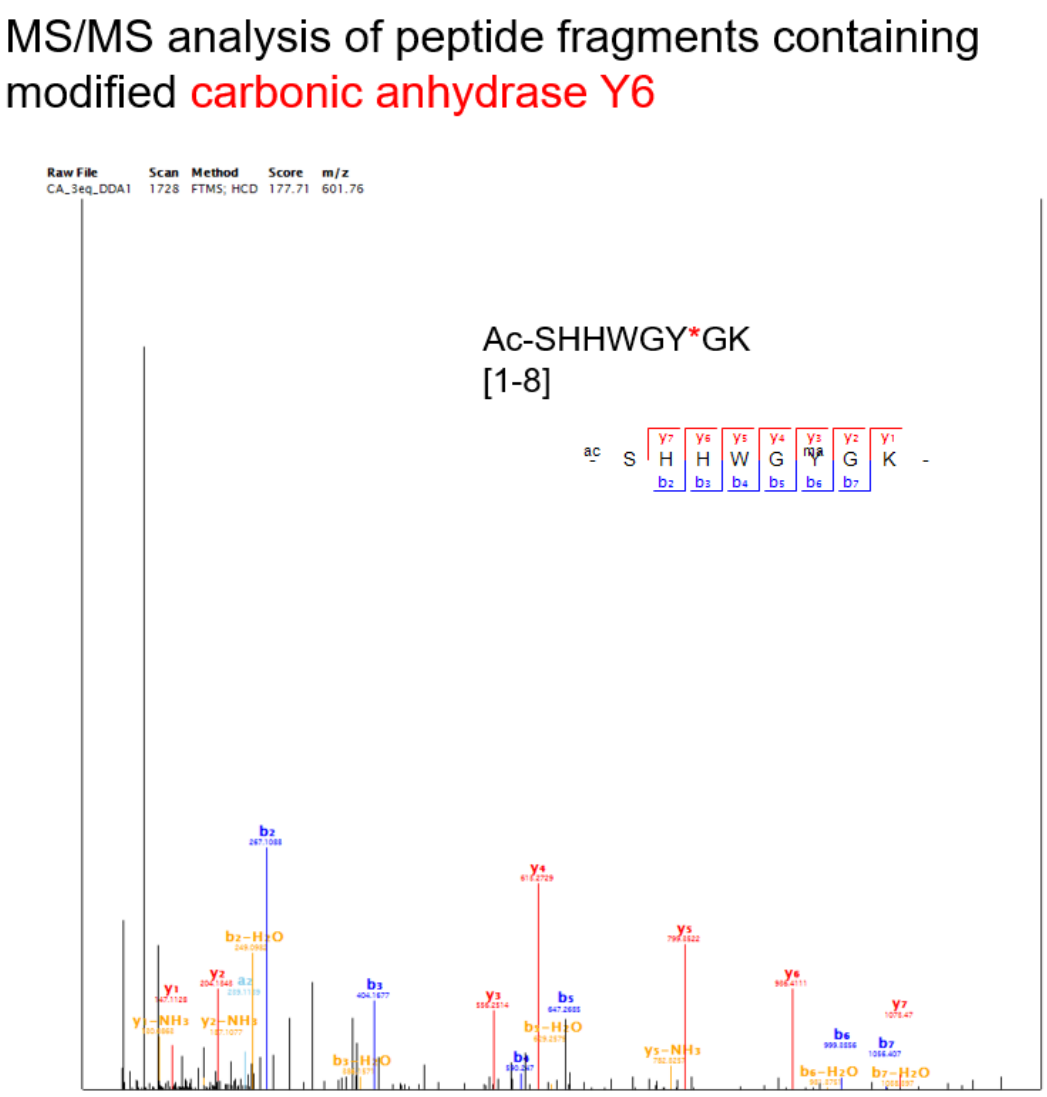


Figure S35. MS/MS analysis of peptide fragments containing modified carbonic anhydrase Y6.

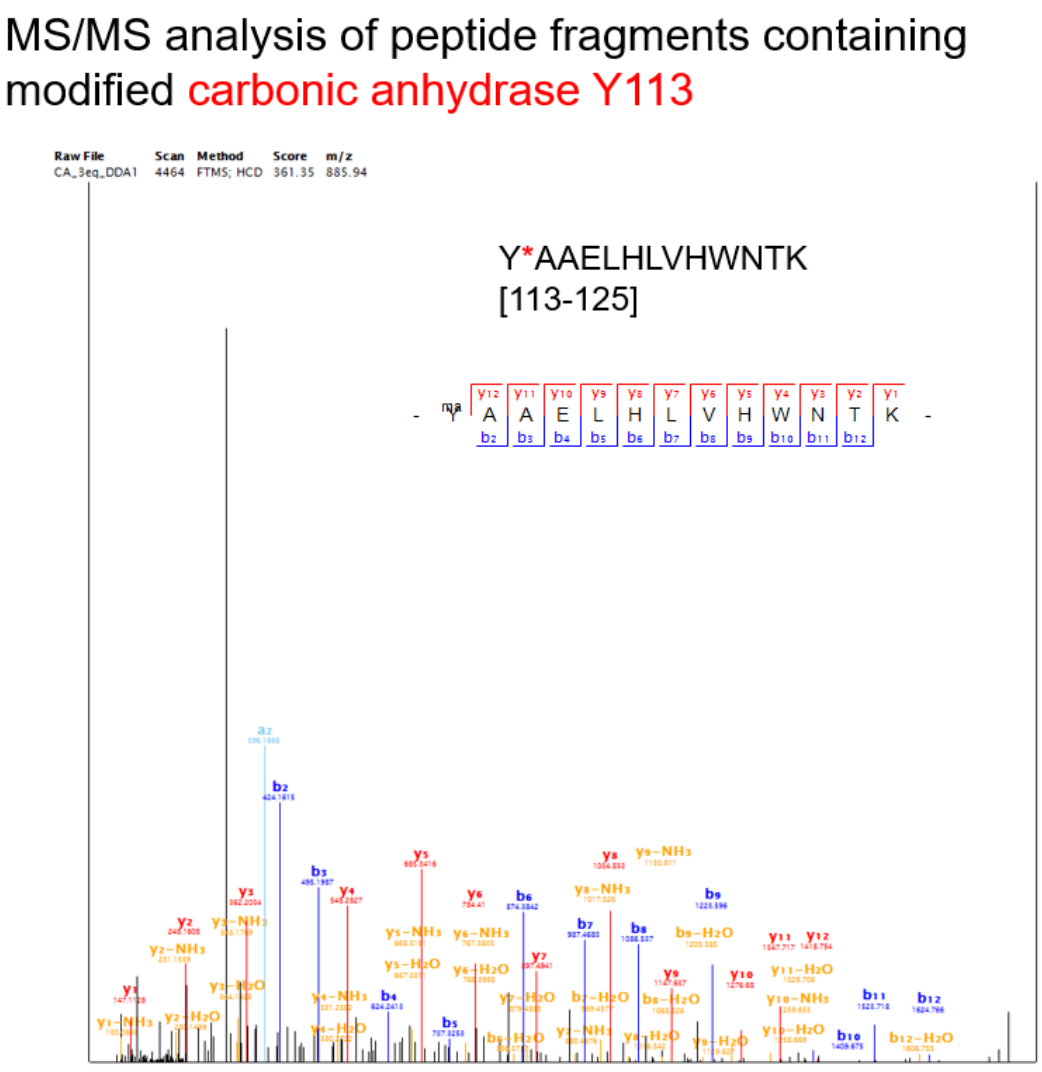


Figure S36. MS/MS analysis of peptide fragments containing modified carbonic anhydrase Y113.

MS/MS analysis of peptide fragments containing modified bovine serum albumin Y137

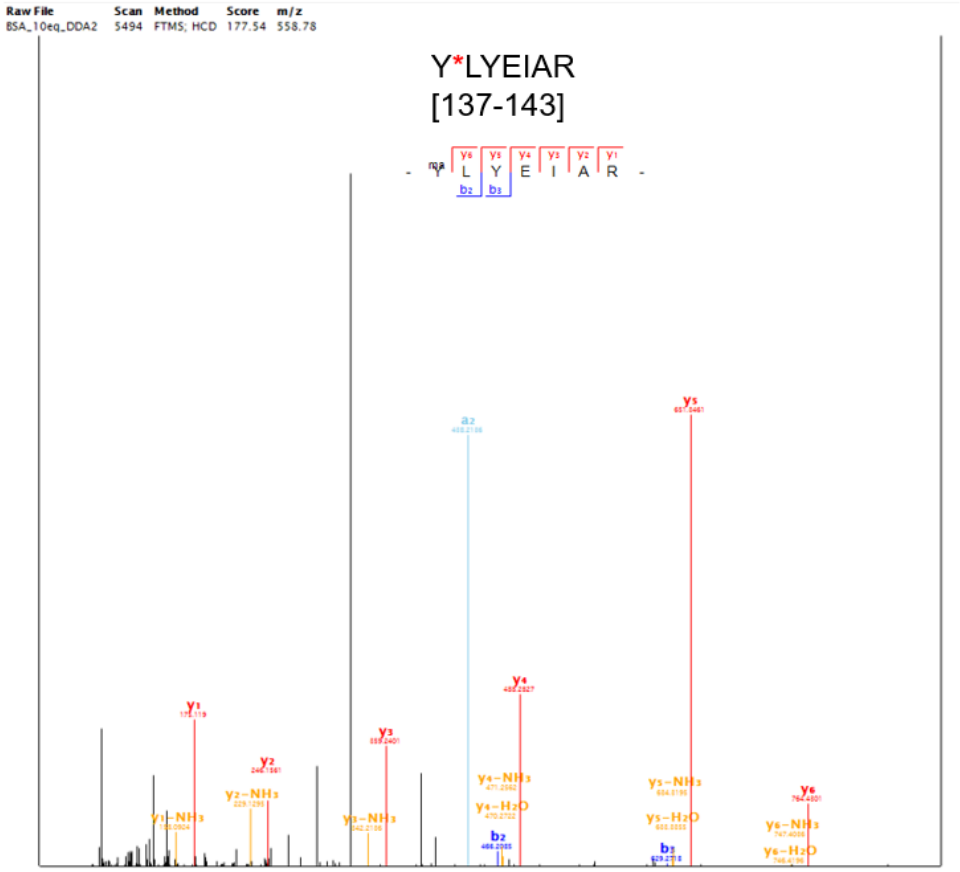


Figure S37. MS/MS analysis of peptide fragments containing modified bovine serum albumin Y137.

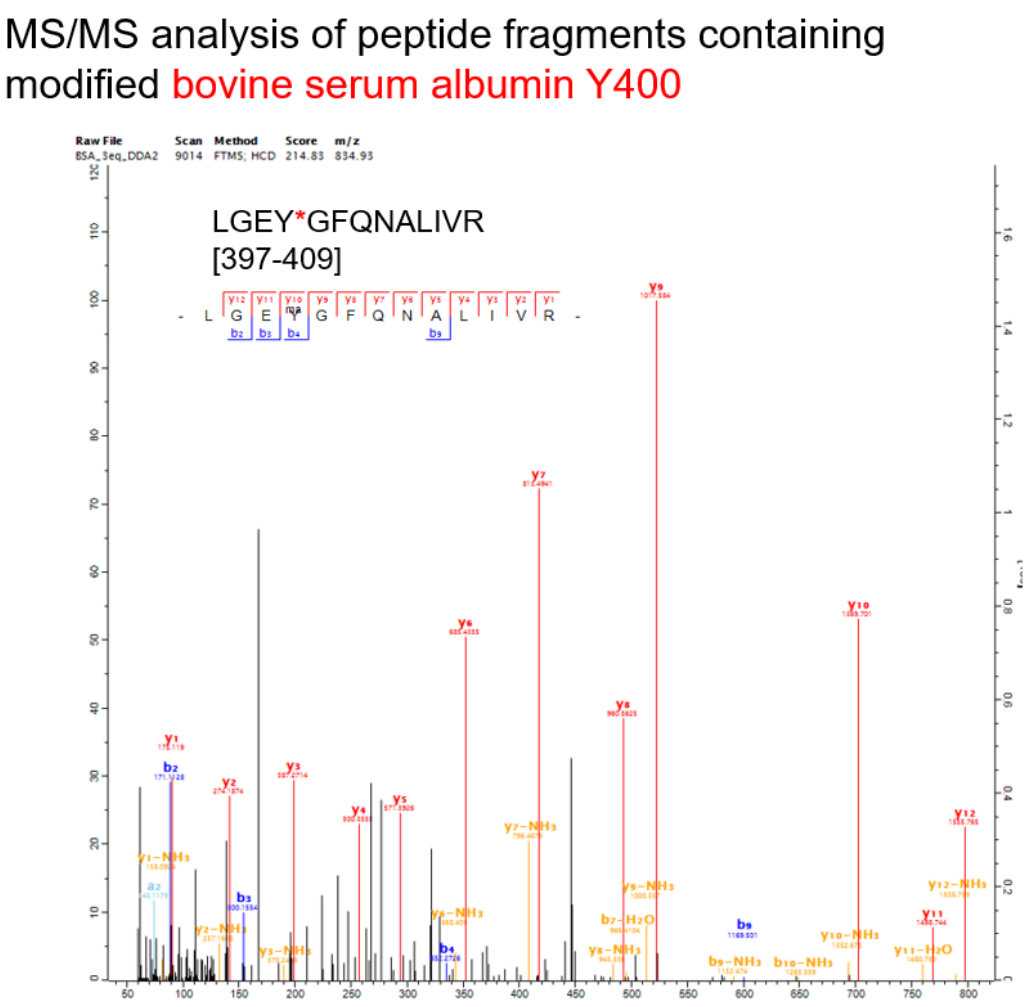


Figure S38. MS/MS analysis of peptide fragments containing modified bovine serum albumin Y400.

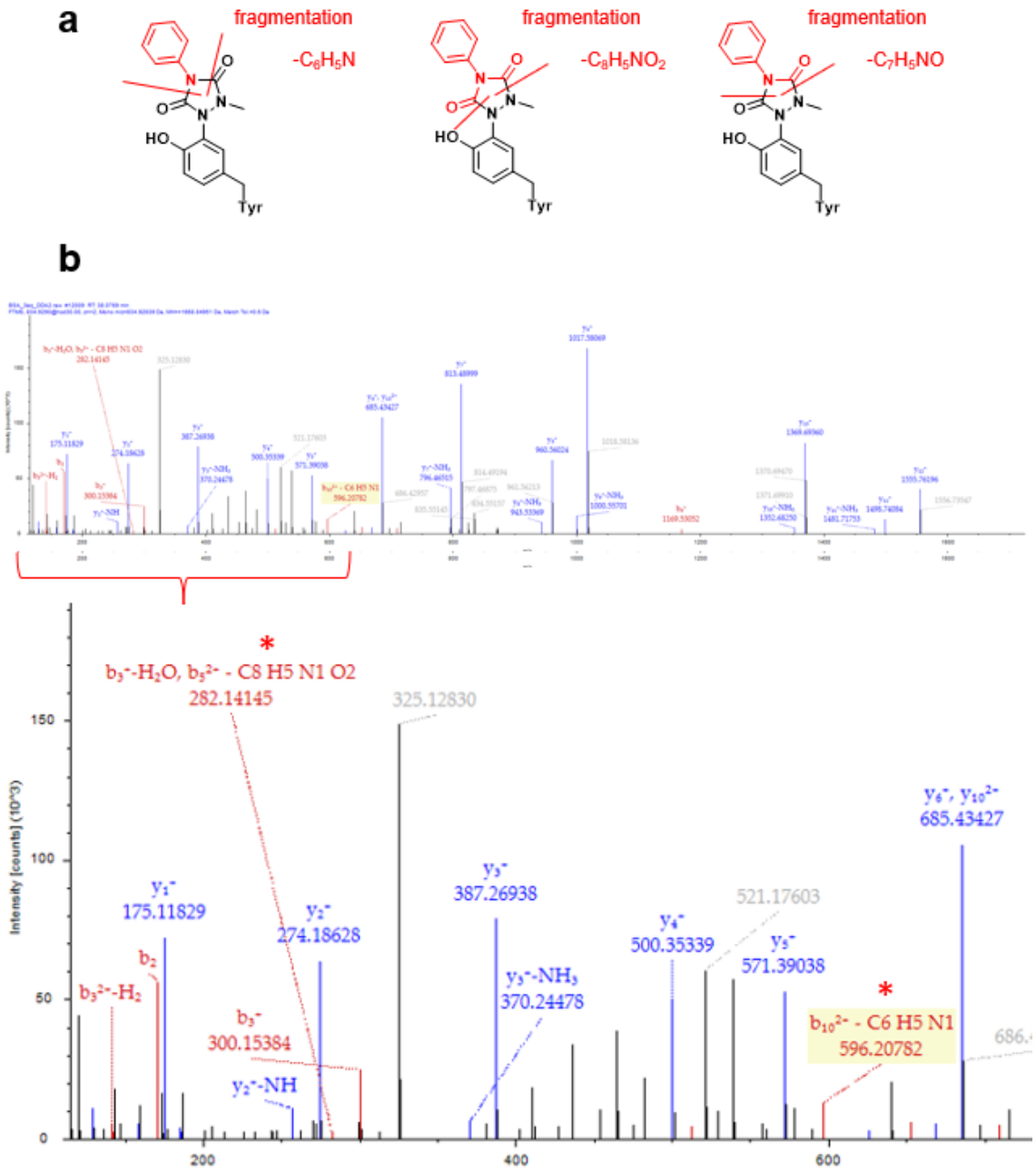


Figure S39. Analaysis considering fragmentation within MAUra structure. (a) Possible patterns of fragmentation due to neutral losses. (b) MS/MS analysis of peptide fragments containing modified bovine serum albumin Y400 considering the fragmentation.

It was suggested that these fragmentation are minor fragmentations.

MS/MS analysis of peptide fragments containing modified glucose oxidase Y280

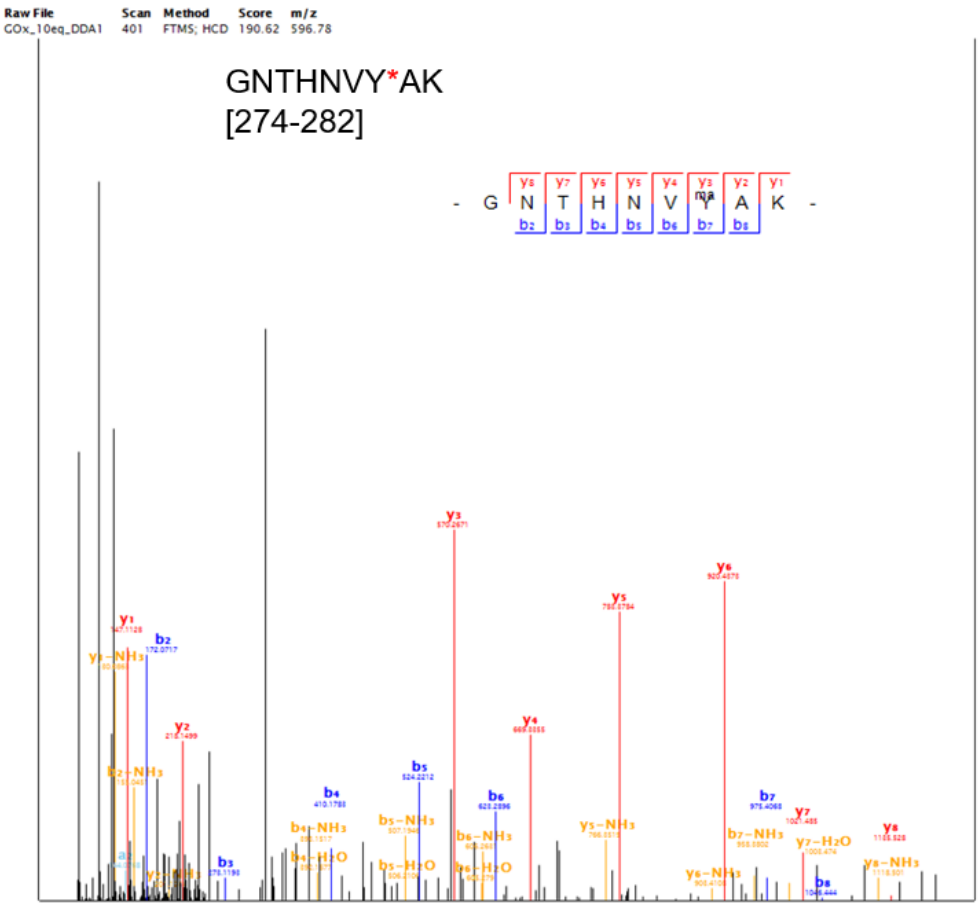


Figure S40. MS/MS analysis of peptide fragments containing modified glucose oxidase Y280.

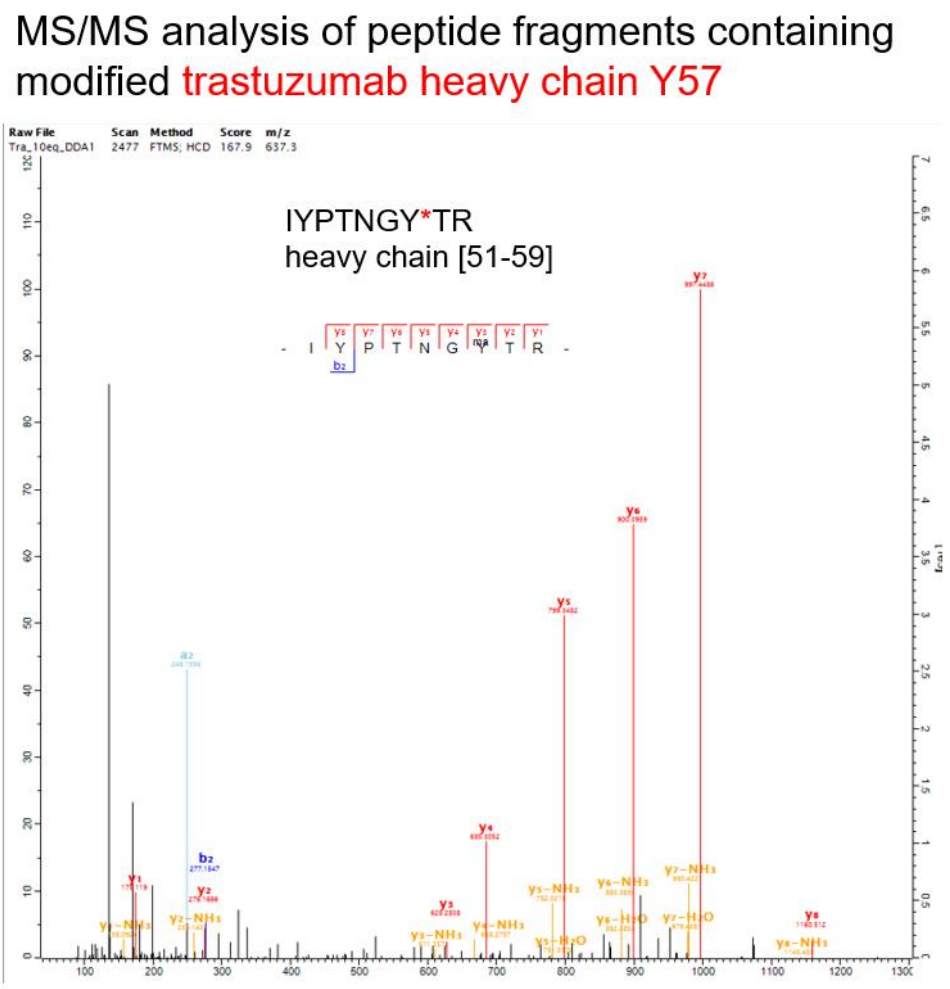


Figure S41. MS/MS analysis of peptide fragments containing modified trastuzumab heavy chain Y57.

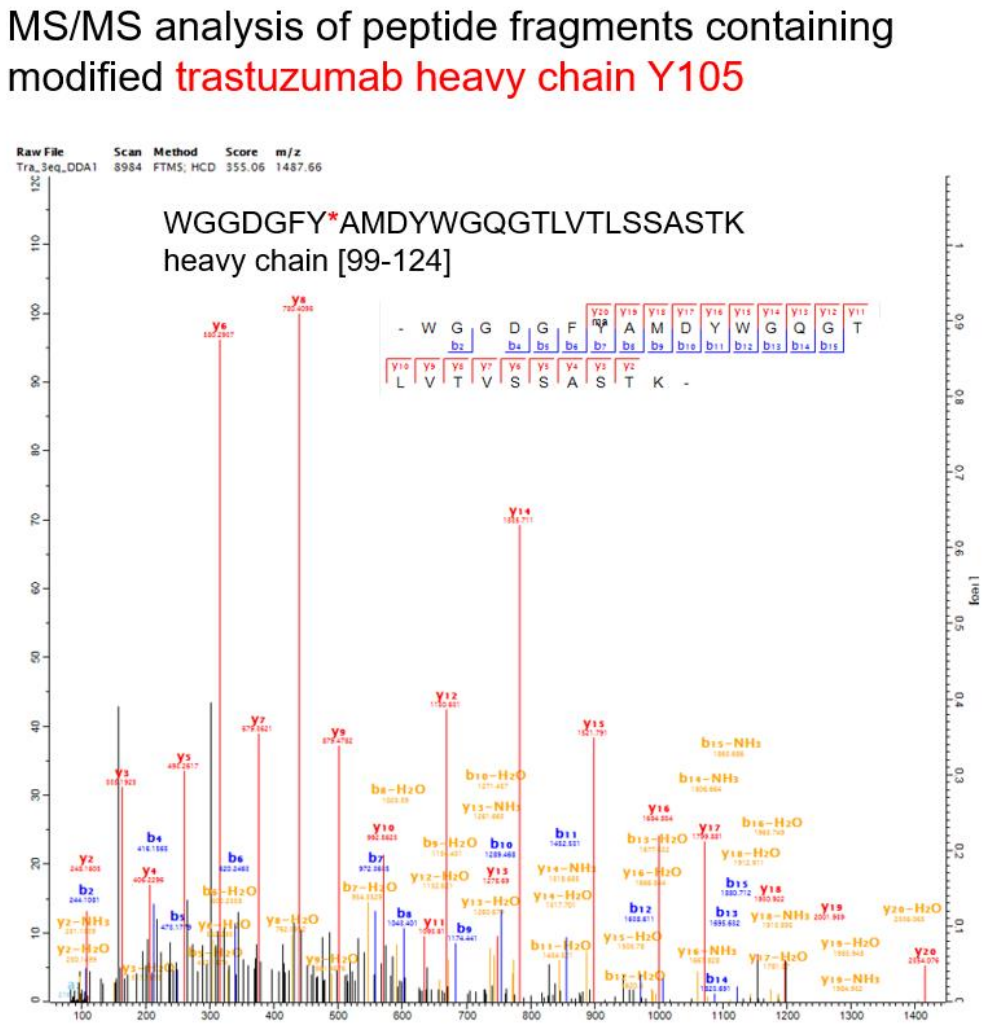


Figure S42. MS/MS analysis of peptide fragments containing modified trastuzumab heavy chain Y105.

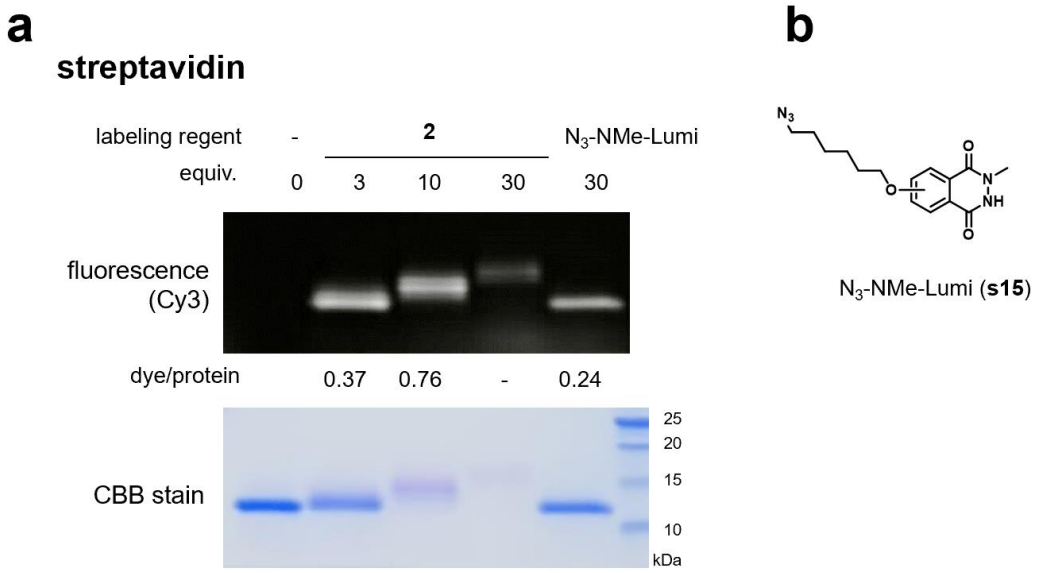


Figure S43. Effect of equivalent amount of labeling reagent on streptavidin labeling. (a) Fluorescence of gel after SDS-PAGE and CBB staining image. (b) Structure of **s15**.

Refer to "Quantification of dye labeled on protein" section in chapter 2-5 for how to evaluate dye/protein.

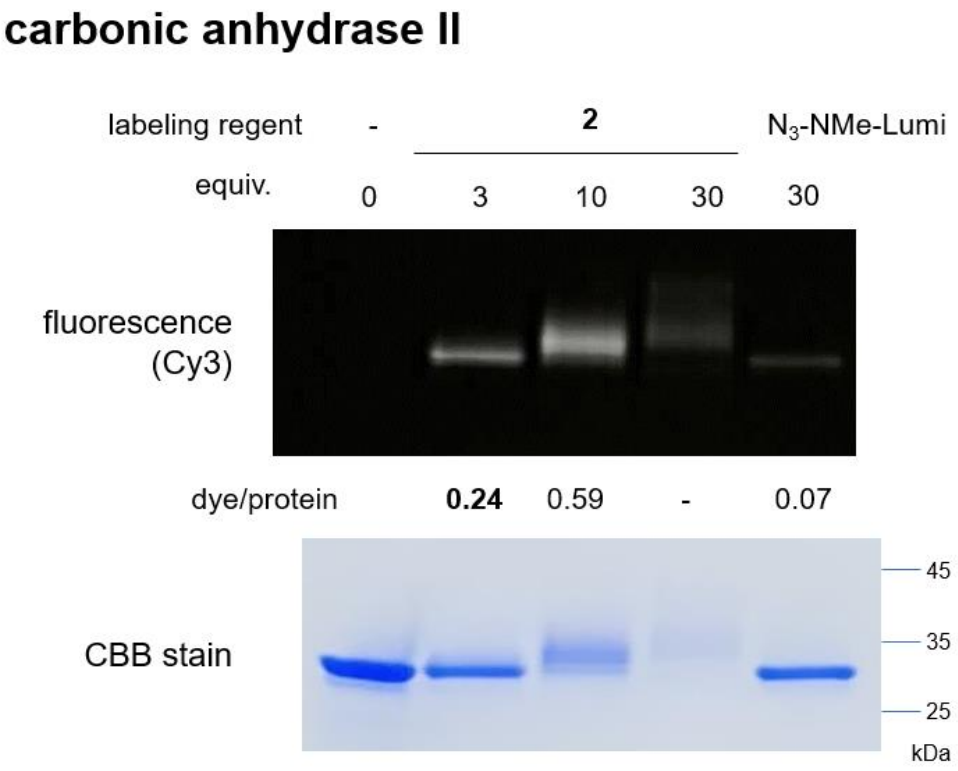


Figure S44. Effect of equivalent amount of labeling reagent on carbonic anhydrase II labeling.

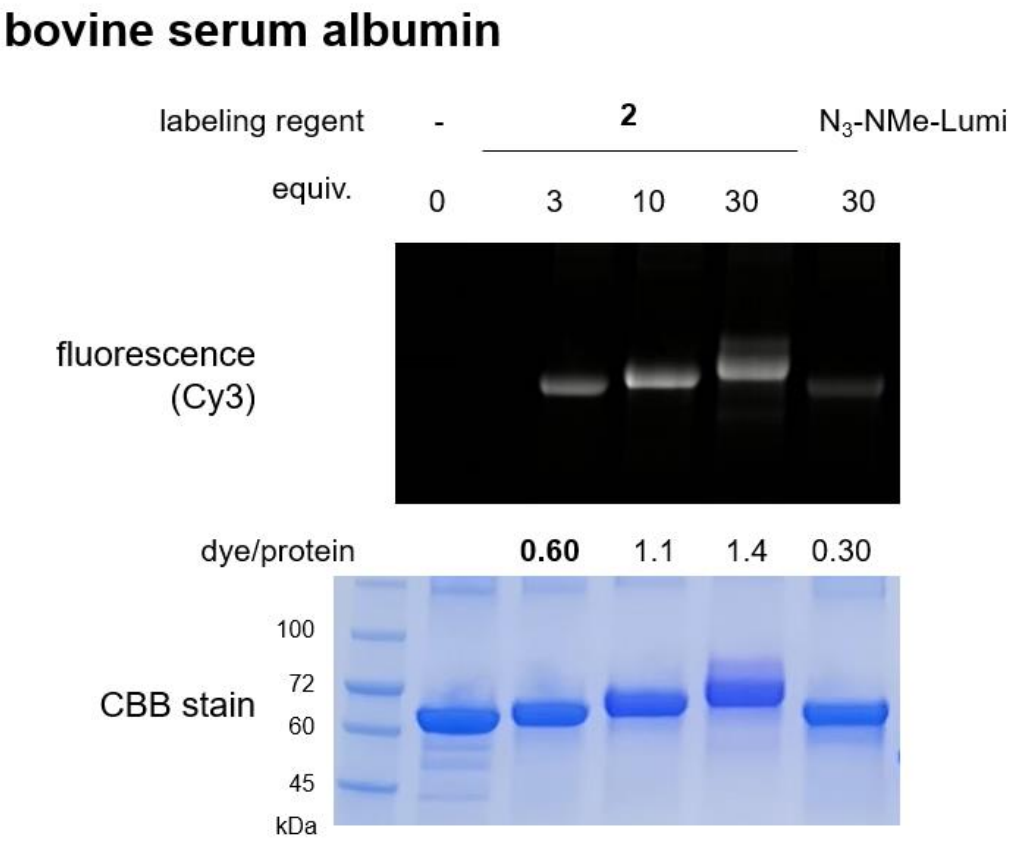


Figure S45. Effect of equivalent amount of labeling reagent on bovine serum albumin labeling.

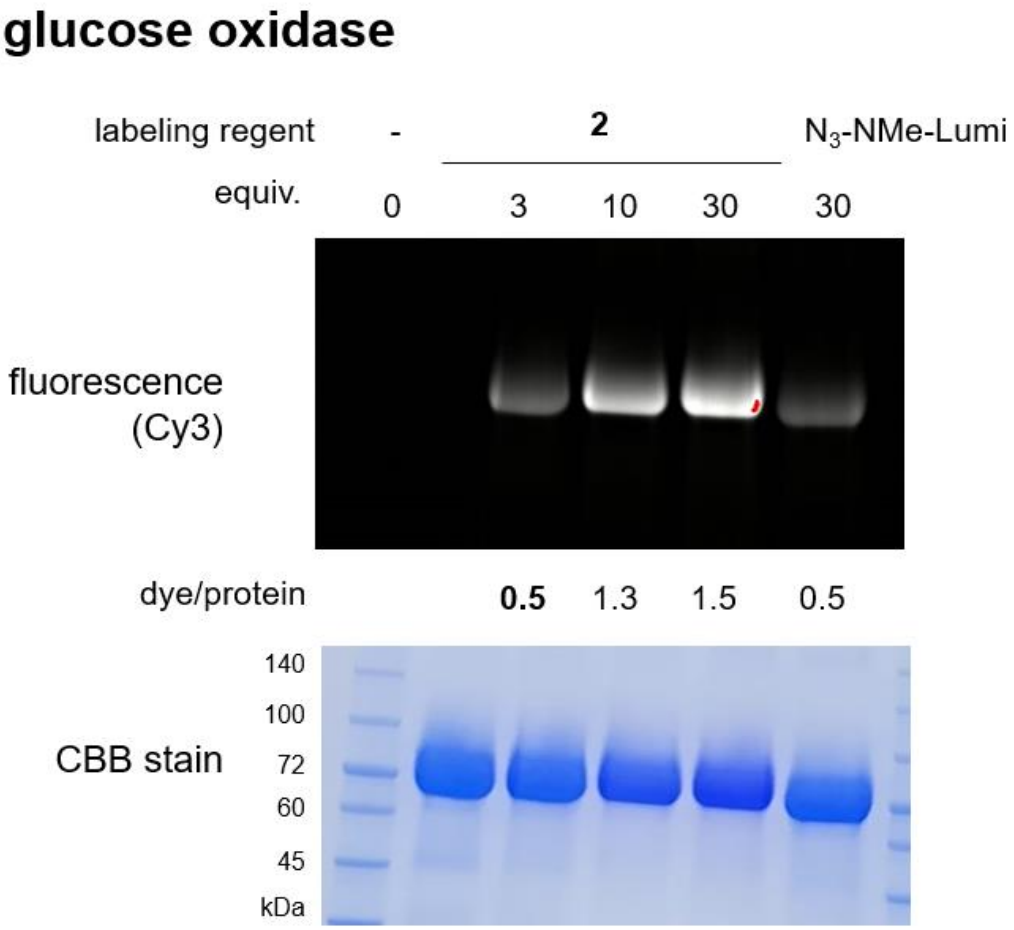


Figure S46. Effect of equivalent amount of labeling reagent on glucose oxidase labeling.

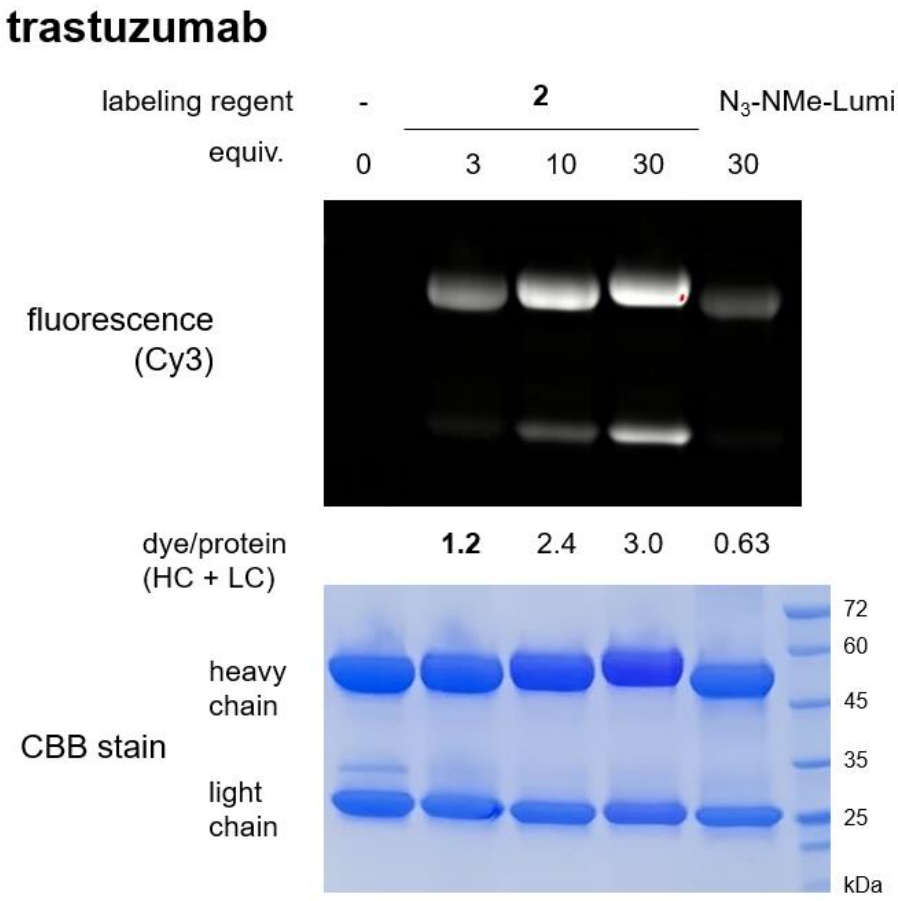


Figure S47. Effect of equivalent amount of labeling reagent on trastuzumab labeling.

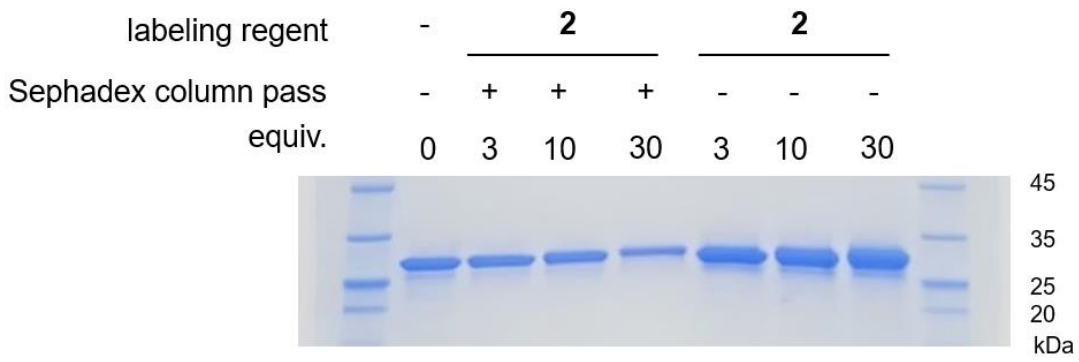


Figure S48. Trapping of Protein Aggregates by Sephadex Column

The decrease in protein recovery observed under conditions using 30 equivalents of modifying agent for streptavidin and carbonic anhydrase II is likely due to increased hydrophobicity on the protein surface, caused by excessive attachment of the modifying agent to low molecular weight proteins, leading to protein aggregation. This phenomenon is also observed when unreacted modifying agents are removed using a Sephadex column, without performing DBCO-Cy3 labeling after compound **2** modification. A comparison using CBB staining with and without column treatment revealed that aggregated proteins are not able to pass through the column for removing small molecules, resulting in decreased protein recovery.

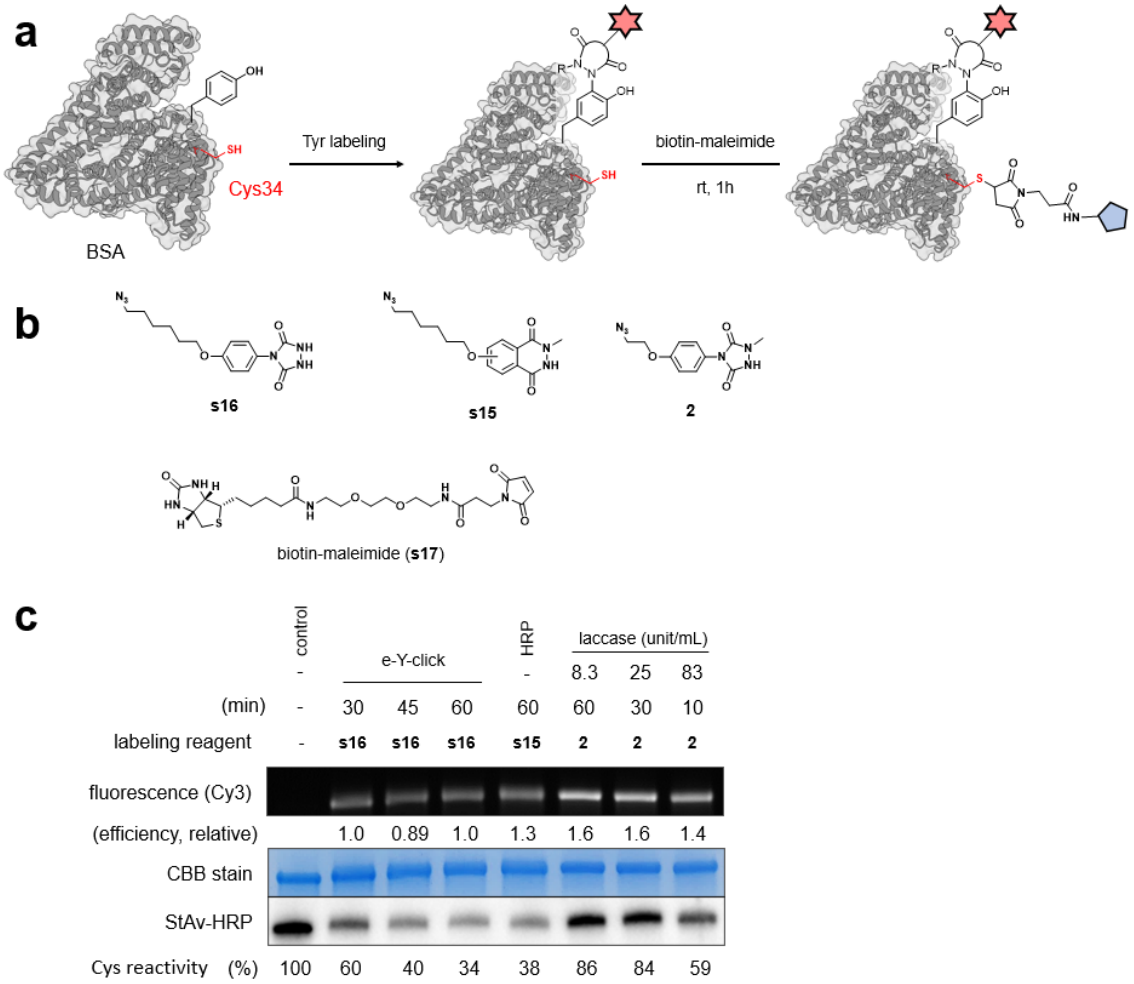


Figure S49. Comparison of laccase-catalyzed tyrosine click with electrochemical and HRP-based methods. (a) Scheme of tyrosine labeling and Cys34 labeling. (b) Structures of labeling reagent. (c) Efficiencies of tyrosine labeling and Cys34 reactivity after tyrosine labeling reactions.

Free cysteine residues (Cys) on proteins are sensitive to oxidative damage, and oxidation of free Cys is one of the major oxidative side reactions. The Cys residues remaining after Tyr labeling with Cy3 were labeled with biotin-maleimide. If Cys is damaged, the thiol on the Cys residue is converted to sulfinic acid, sulfonic acid, or other oxidized forms., and thus loses its nucleophilicity and cannot be labeled with maleimide. These results suggest that the Cys residues of BSA are oxidatively damaged even under eY-click conditions, in which the reaction can be controlled at low voltages. In contrast, the current method using laccase caused less oxidative damage to Cys than the conventional method.

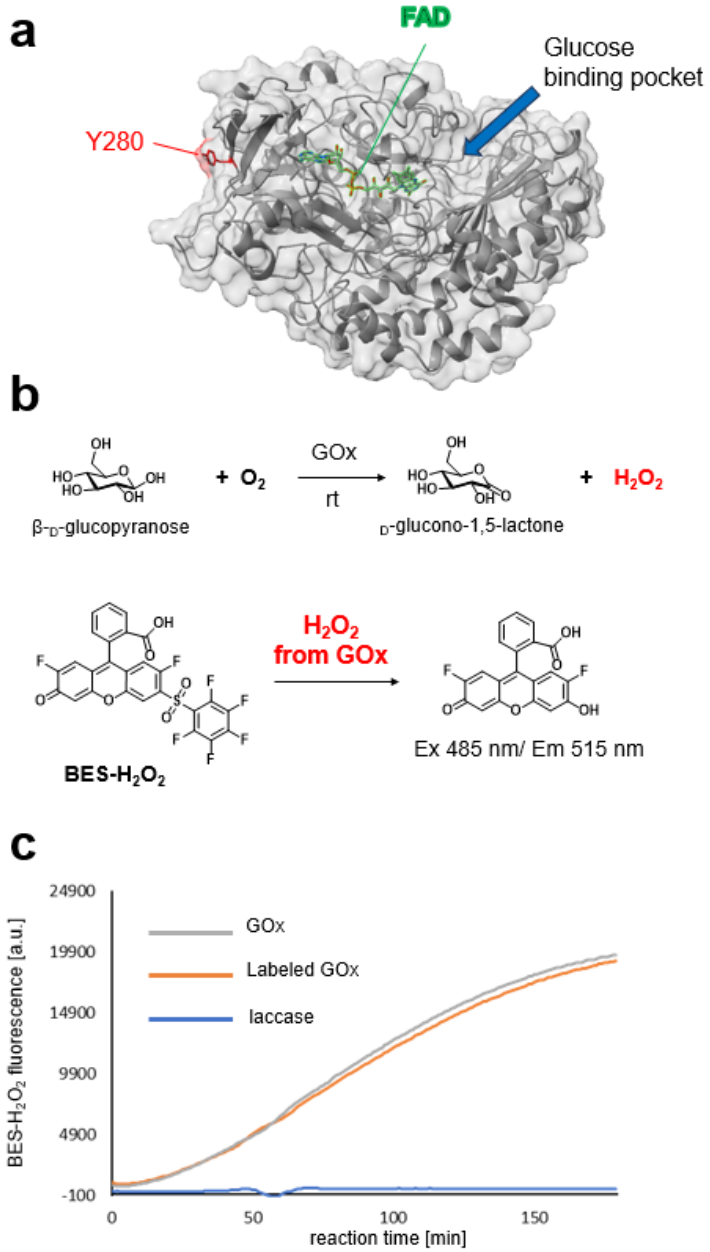


Figure S50. Activity of GOx after tyrosine labeling. (a) Structure of GOx (PDB ID: 1CF3), labeling site, and enzymatic active site. (b) Scheme of H_2O_2 generation during glucose metabolism by GOx and detection of H_2O_2 using H_2O_2-selective fluorogenic chemical probe BES-H_2O_2. (c) Change in fluorescence over time.

Notably, a single Tyr can be labeled with high selectivity, considering that 28 Tyr are present in GOx. Conveniently, Y280 is located on the opposite side of the glucose binding pocket, making it a labeling site that is unlikely to impair GOx activity. When the H_2O_2 production activity of GOx labeled with compound **2** was measured upon the addition of glucose, the Gox activity was hardly impaired

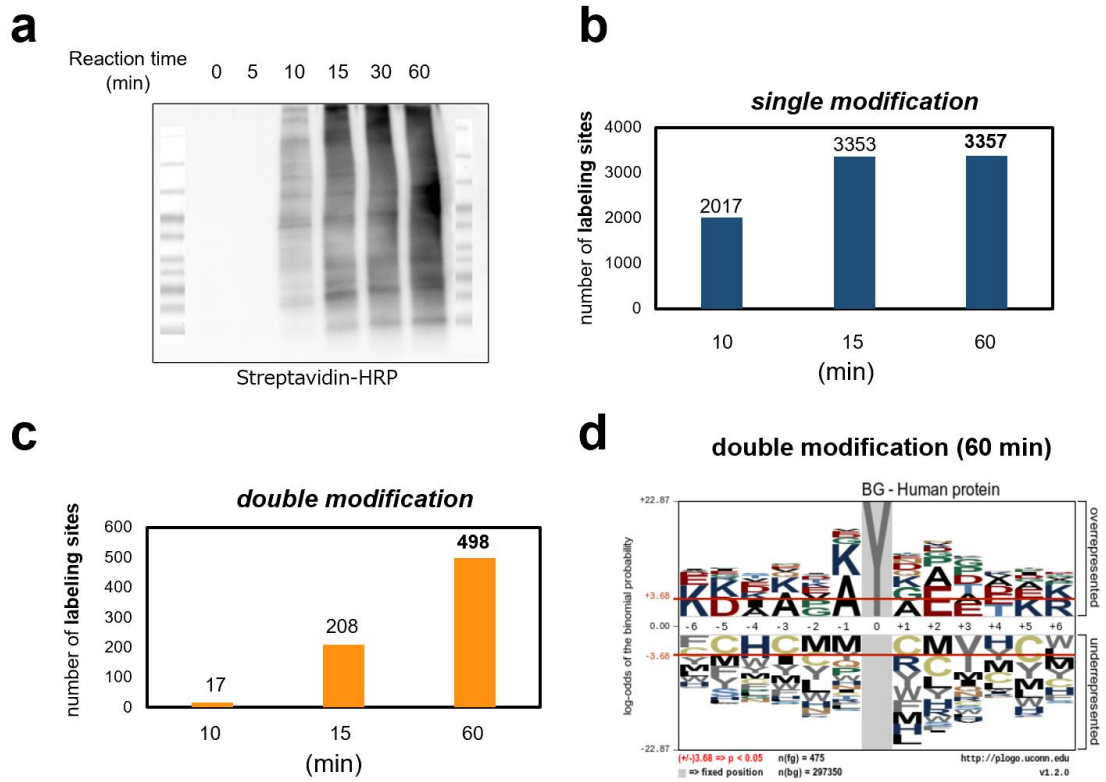


Figure S51. Analysis of Tyr residues labeled by MAUra-DTB **8**. (a) Quantitative change in labeling efficiency over reaction time detected by western blotting with streptavidin-HRP. (b,c) Change of single- and double-modification sites in reaction time. (d) Motif analysis of double-modification sites.

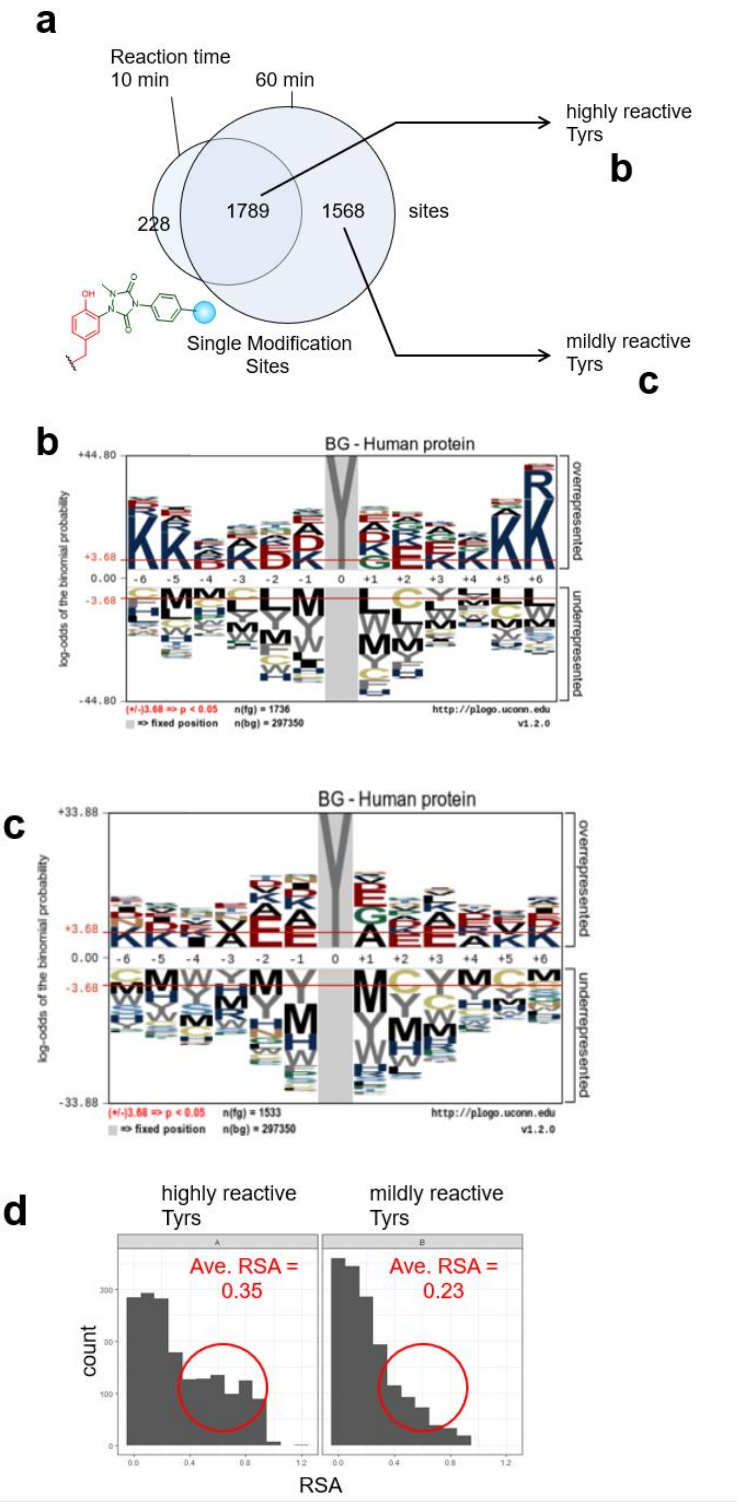


Figure S52. Comparison between highly reactive and mildly reactive Tyrs. (a) Definition of highly reactive and mildly reactive Tyrs. (b, c) motif analysis of highly (b) and mildly (c) reactive Tyrs. (d) Comparison of the distribution of RSA values.

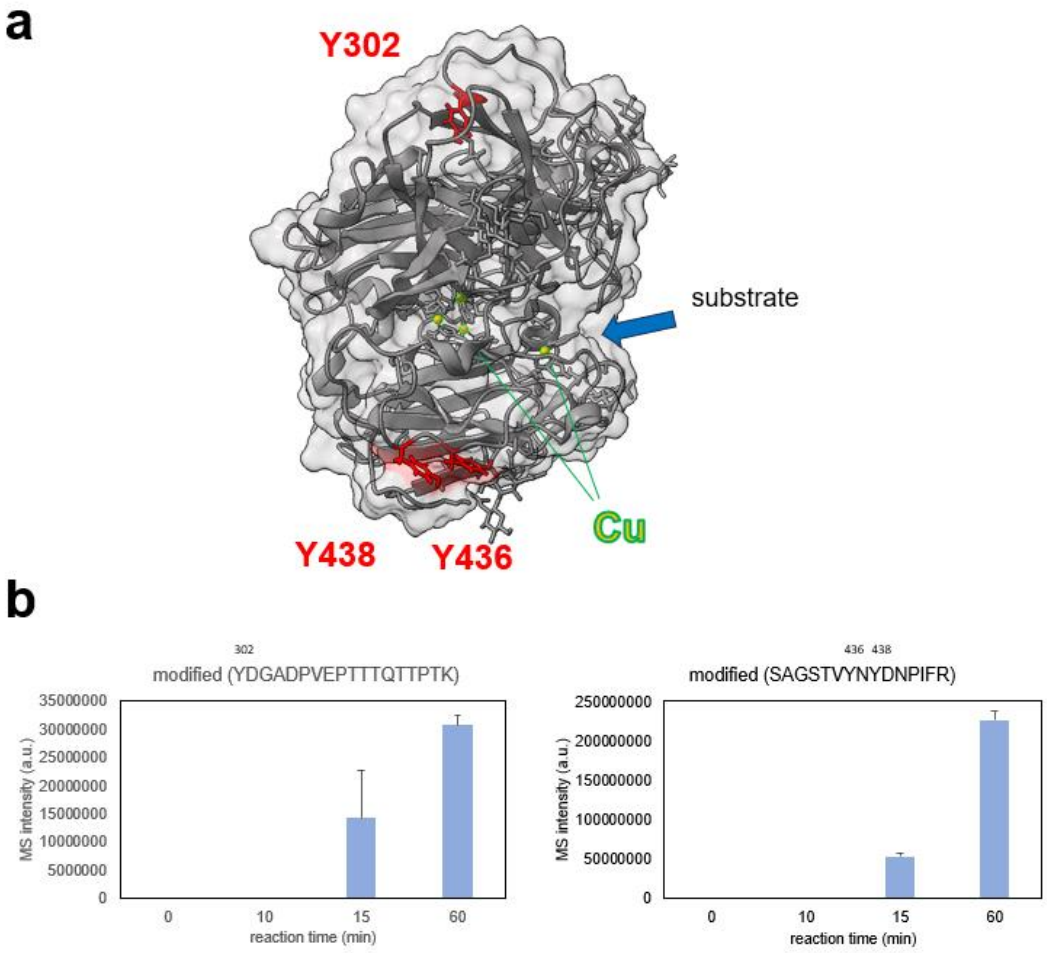


Figure S53. Self-labeling in laccase. (a) Structure of laccase (PDB ID: 3PXL), labeling sites, and substrate binding site. (b) MS1 intensity of peptides containing modified Y302 at each reaction time. (c) MS1 intensity of peptides containing modified Y436 or Y438 (single-modification) at each reaction time.

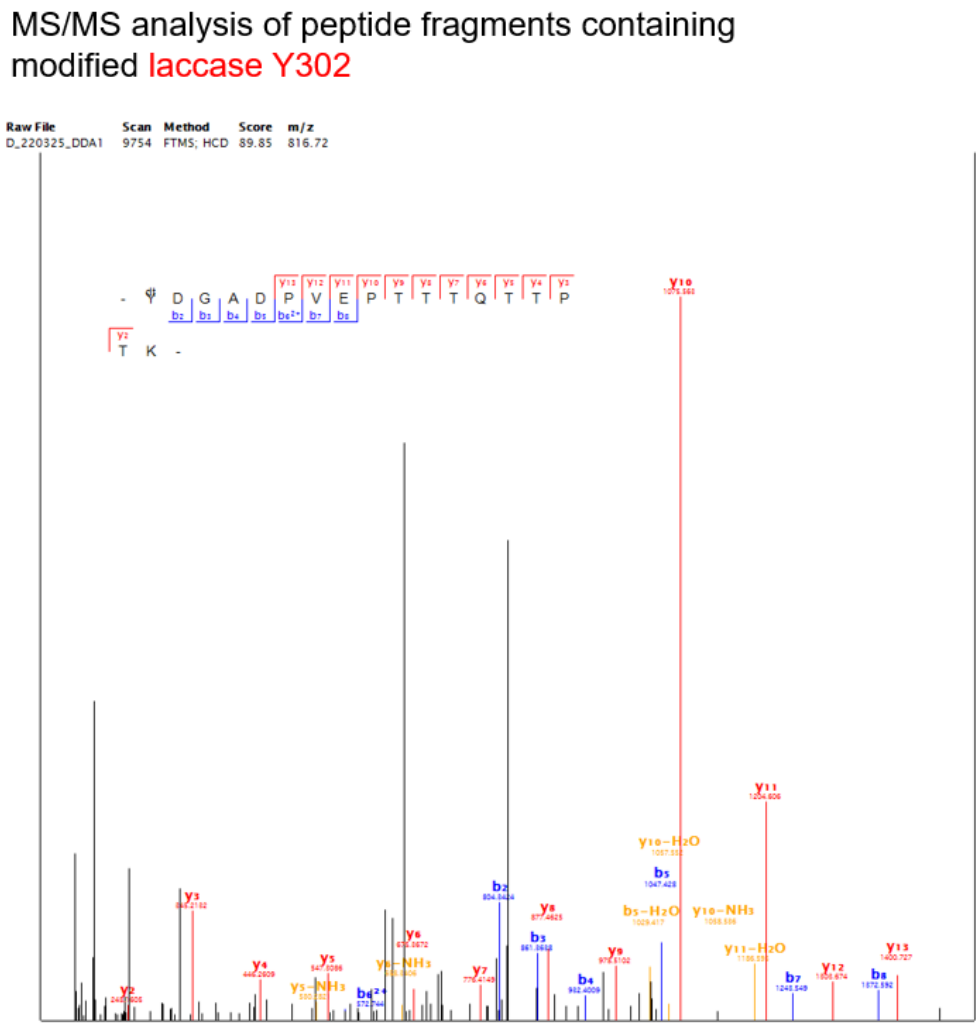


Figure S54. MS/MS analysis of peptide fragments containing modified laccase Y302

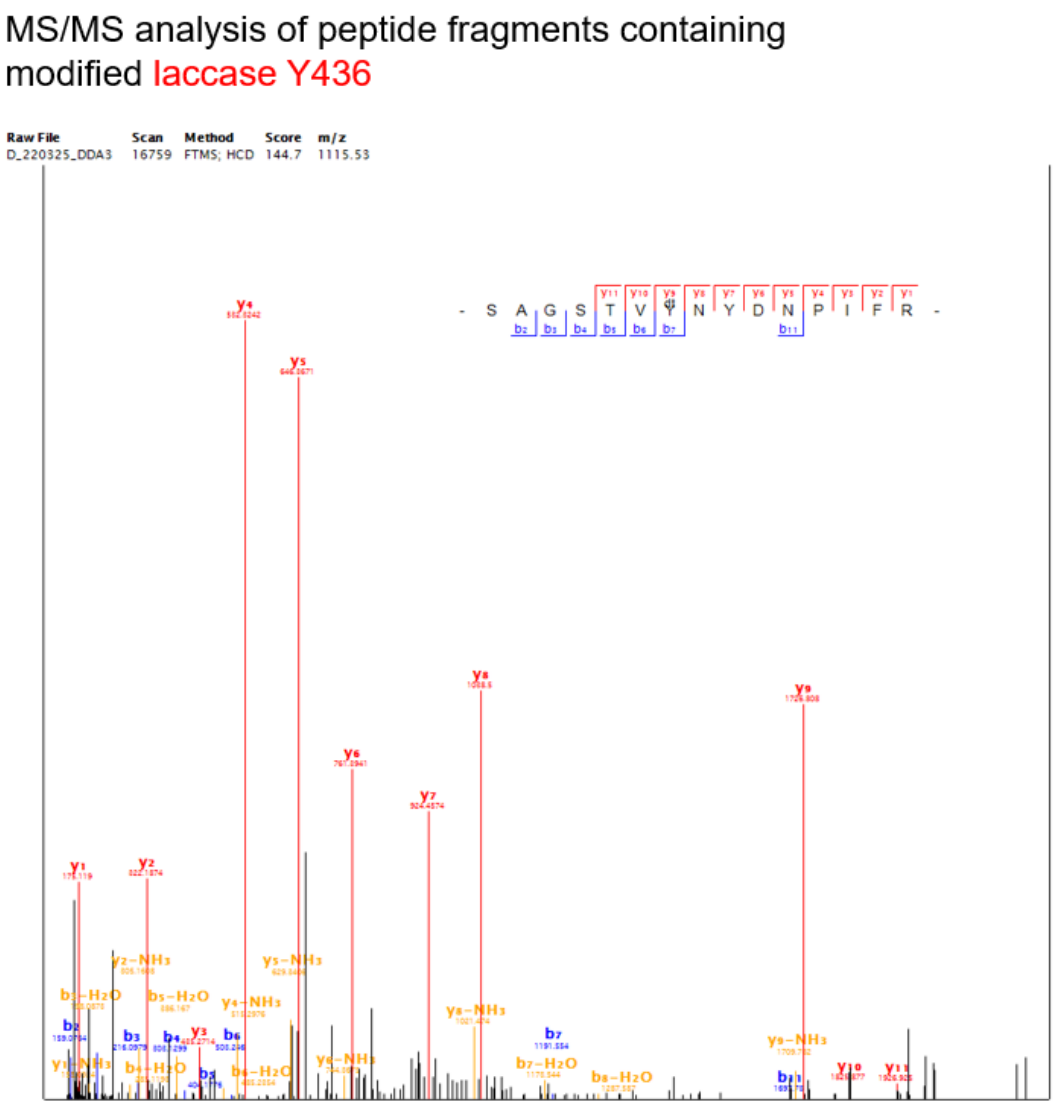


Figure S55. MS/MS analysis of peptide fragments containing modified laccase Y436.

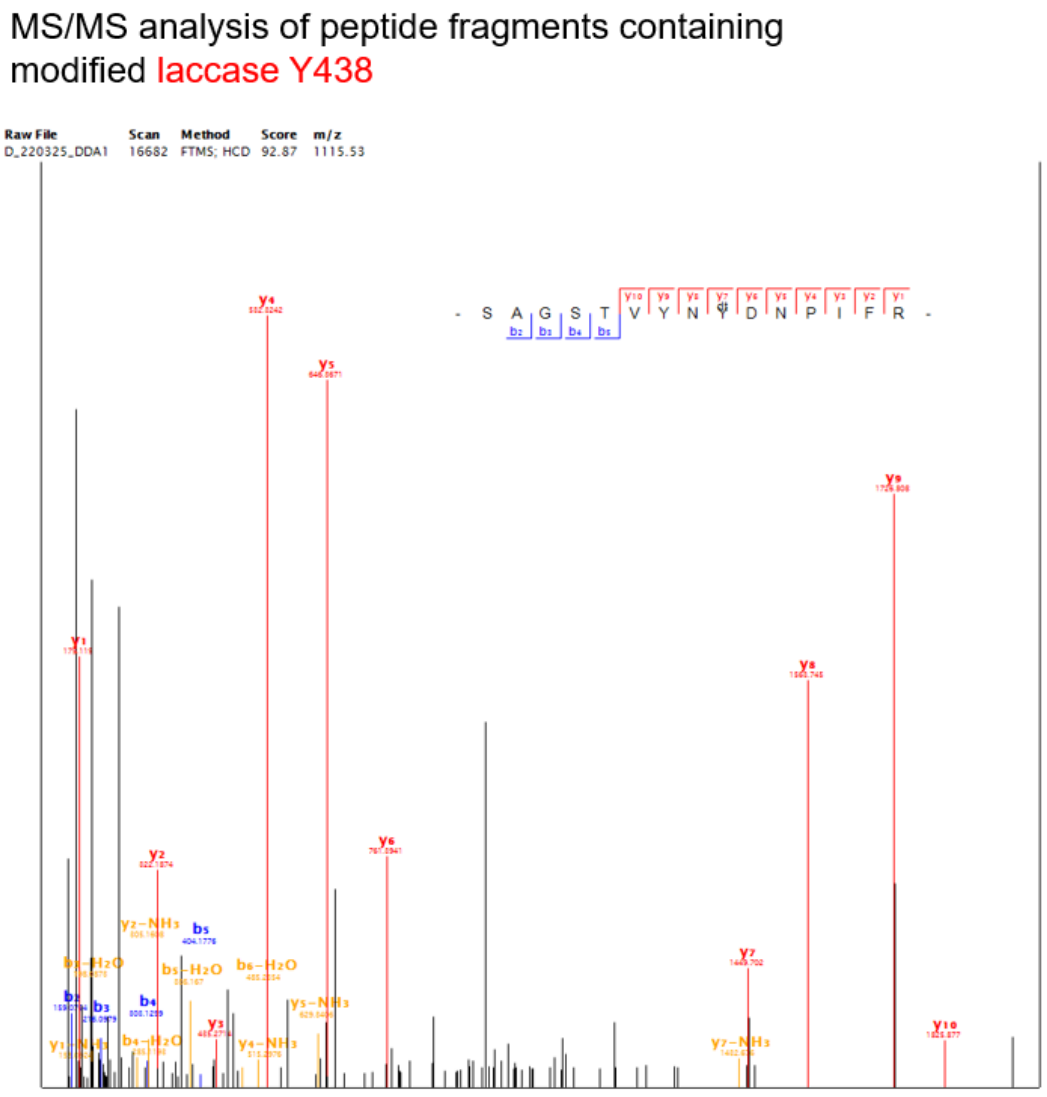


Figure S56. MS/MS analysis of peptide fragments containing modified laccase Y438.

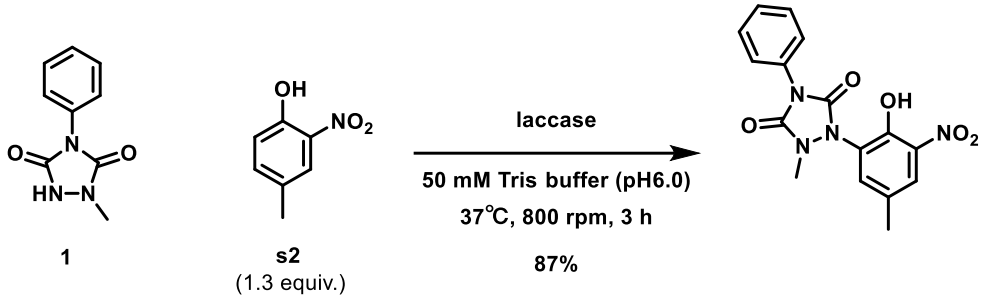
2. Experimental section

2-1. General

Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL ECA-600 (600 MHz ^{1}H, 150 MHz ^{13}C) instrument in the indicated solvents. Chemical shifts are reported in units, parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in CD_3OD (3.31 ppm for ^{1}H, 49.00 ppm for ^{13}C), CD_3CN (1.94 ppm for ^{1}H, 1.32 and 118.26 ppm for ^{13}C) or DMSO-d_6 (2.50 ppm for ^{1}H, 39.52 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; and, *J* coupling constant in hertz. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-TOF-MS instrument (micro TOF II). Analytical thin layer chromatography (TLC) was performed using a silica gel 60 GF254 glass plate (Merck). Silica gel (Fuji Silysia, CHROMATOREX PSQ 60B, 50-200 μm) was used for column chromatography. MALDI-TOF MS was performed using Bruker UltrafleXtreme or ABSCIEX TOF/TOFTM 5800. Analytical HPLC was carried out on JASCO PU-4580 HPLC Pump, JASCO LG-4580 Quaternary Gradient Unit, and JASCO DG-4580 Degassing Unit with a JASCO MD-2018 Plus Photodiode Array Detector, JASCO CO-4060 Column Oven, JASCO As-455 HPLC Autosampler, and JASCO LC-NetII/ADC Interface Box using a C18 reverse phase column (Inertsil ODS-4, 150 × 4.6 mm, 5 μm (GL Science Inc.)). The HPLC conditions were as follows: mobile phase A was 0.1% formic acid (FA) in H_2O and mobile phase B was 0.1% FA in CH_3OH. The absorbance was recorded using a Shimadzu UV-1200 instrument. Commercial reagents were purchased from Tokyo Chemical Industry Co., Ltd., Sigma-Aldrich Co. LLC, FUJIFILM Wako Pure Chemical Corporation, Nacalai Tesque, Thermo Fisher Scientific, Roche, or Cosmo Bio Co., Ltd. and used directly without further purification. Biotin-PEG2-maleimide was purchased from Cosmo Bio. Phenothiazine (**10**) was purchased from the Tokyo Chemical Industry. Hydrogen peroxide solution, *p*-nitrophenyl acetate, and *p*-cresol (**s1**) were purchased from Nacalai Tesque. Hemin, $(BimH)_3$, DBCO-Cy3, PTAD, desthiobiotin, and propargylamine were purchased from Sigma-Aldrich. BES-H_2O_2 was purchased from Wako Pure Chemical Corporation. Angiotensin II were purchased from Sigma-Aldrich. Oxytocin and kisspeptin-10 were purchased from PEPTIDE INSTITUTE, Inc.. Thymopentin and cycRGDyK were purchased from GL Biochem, Ltd. Laccase was provided by Amano Enzymes, Inc. Laccase from *Trametes versicolor* was purchased from Sigma-Aldrich. Bovine serum albumin (BSA), carbonic anhydrase (CAII), glucose oxidase (GOx), streptavidin, and horseradish peroxidase (HRP) were purchased from Sigma-Aldrich. Trastuzumab (Herceptin®) was purchased from Chugai Pharmaceutical. NeutrAvidin beads (Sera-Mag™ SpeedBeads NeutrAvidin-coated Magnetic Particles) were purchased from Cytiva. RIPA buffer was purchased from Nacalai. Sephadex G-25 gel was purchased from GE Healthcare. A HiPPR detergent removal resin column kit was purchased from Thermo Fisher Scientific. The C18 pipette tips were purchased from Nikkyo Technos Co., Ltd.

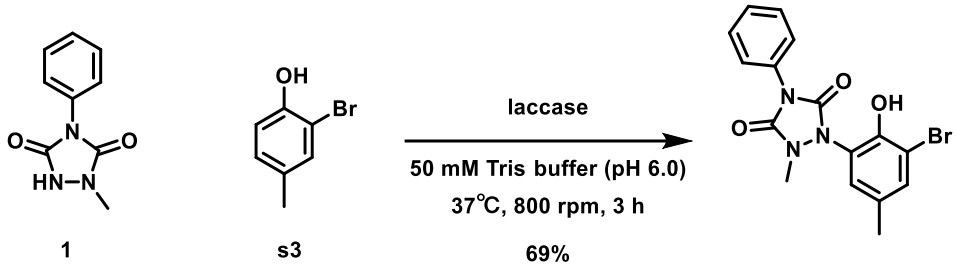
2-2. Chemical synthesis

2-Nitro-*p*-cresol labeling using laccase



2-nitro-*p*-cresol **s2** (33.0 mg, 0.215 mmol), **1** (31.2 mg, 0.163 mmol), and laccase (Amano Enzyme Inc., 7.0 mg) were dissolved in 30 mL 50 mM Tris buffer (pH 6.0). Subsequently, 10 mL of the mixture was stirred open to air at 37 °C for 3 h in a 25 mL tube. This reaction was repeated three times to react with all of the mixture (10 mL × 3 times). After the reaction, the mixture was extracted with $CHCl_3$ and concentrated under reduced pressure. The crude was purified by silica gel chromatography (Isorela One) to yield the product (48.5 mg, 87%) as a yellow amorphous solid. ^{1}H-NMR (600 MHz, CD_3CN) δ_H (ppm) 8.10 (s, 1H), 7.72 (s, 1H), 7.57-7.50 (m, 4H), 7.49-7.45 (m, 1H), 3.07 (s, 3H), 2.40 (s, 3H); ^{13}C-NMR (600 MHz, CD_3CN) δ_C (ppm) 154.5, 154.1, 151.1, 140.5, 135.9, 132.8, 131.0, 130.1, 129.5, 127.4, 127.4, 126.7, 33.2, 20.1; HRMS (ESI-TOF): *m/z* calced. for $C_{16}H_{13}N_4O_5$ $[M-H]^-$: 341.0880, found 341.0899.

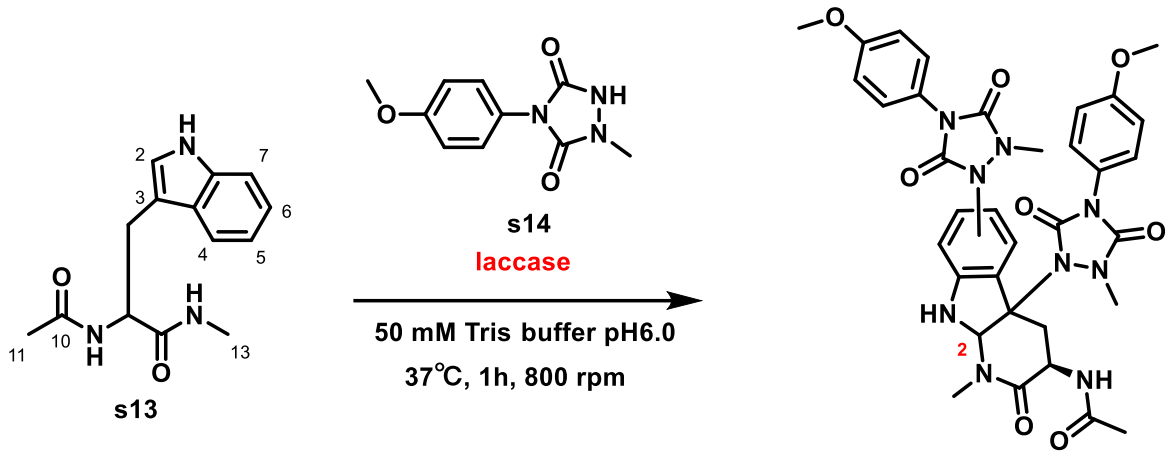
2-Bromo-*p*-cresol labeling using laccase



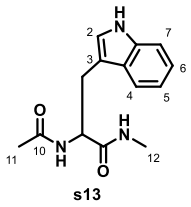
2-bromo-*p*-cresol **s3** (34.0 mg, 0.180 mmol), **1** (34.1 mg, 0.178 mmol), and laccase (Amano Enzyme Inc., 7.4 mg) were dissolved in 30.0 mL of 50 mM Tris buffer (pH 6.0). 10.0 mL of the mixture was stirred open to air at 37 °C for 3 h in a 25 mL tube. This reaction was repeated three times for all mixtures. After the reaction, the mixture was extracted with ethyl acetate and concentrated under reduced pressure. The crude was purified by silica gel chromatography (Isorela One) to yield the product (46.1 mg, 69%) as a white amorphous solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ_H (ppm) 7.58-7.54 (m, 2H), 7.52-7.48 (m, 2H), 7.43-7.39 (m, 2H), 7.06 (d, 1H, J = 1.8 Hz), 3.20 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ_C (ppm) 154.0, 152.7, 146.2, 133.7, 132.1, 131.1, 129.4, 128.7, 125.7,

125.5, 124.1, 113.3, 33.8, 20.5; HRMS (ESI-TOF): *m/z* calced. for $C_{16}H_{13}N_3O_3Br$ $[M-H]^-$: 374.0135; found: 374.0144.

Ac-Trp-NHMe labeling using laccase

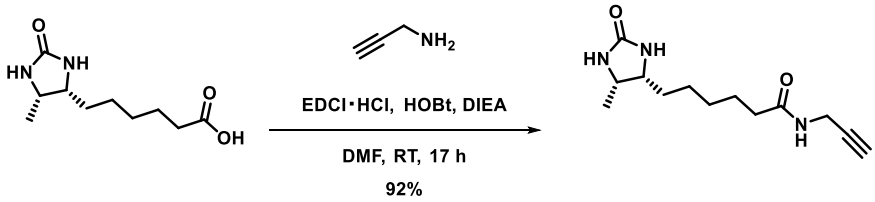


Ac-Trp-NHMe **s13** (14.4 mg, 55.4 µmol, 1.0 equiv.) and compound **s14** (12.3 mg, 55.4 mmol, 2.0 equiv.) were added to 10 mL of 50 mM Tris pH6.0 in a reaction vessel to adjust the concentration of each compound to 5.5 mM. Laccase powder was added to the reaction mixture to adjust the concentration of laccase to 0.3 mg/mL and the reaction mixture was stirred at room temperature for 1 h. An identical reaction mixture was prepared and the two reaction solutions were combined. The reaction mixture was then concentrated *in vacuo*. The residue was further purified using flash chromatography (chloroform: methanol). The fraction containing the target molecule was purified by preparative HPLC using an H_2O-MeOH gradient system. A fraction containing the target molecule was lyophilized to obtain a solid (3.1 mg, 4 % yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ_H 8.32-8.25 (m, 1H), 7.99-7,90 (m, 1H), 7.57 (d, J = 2.4 Hz, 1H), 7.40 (d, J = 9.0 Hz, 4H, Ar from compound **s11**), 7.35 (d, J = 2.4 Hz, 1H), 7.34-7.29 (m, 2H), 7.28-7.22 (m, 4H, Ar from compound **s11**), 7.16 (d, J = 9.0 Hz, 2H), 7.06 (d, J = 8.4 Hz, 4H, Ar from compound **s11**), 6.98-6.94 (m, 4H, Ar from compound **s11**), 6.79-6.75 (m, 2H), 6.25 (s, 1H, position 2), 6.05 (s, 1H, position 2), 4.25 (t, J = 7.8 Hz, 1H, position 9), 3.90-3.87 (m, 1H, position 9), 3.80 (s, 6H, OMe from compound **s11**), 3.77-3.75 (m, 6H, OMe from compound **s11)**, 3.27-3.24 (m, 3H, NMe), 3.17 (s, 3H, NMe), 2.93 (s, 3H, NMe), 2.92 (s, 3H, NMe), 2.66-2.60 (m, 2H, position 8), 2.57-2.53 (m, 2H, position 8), 2.16 (s, 3H, position11), 1.80 (s, 3H, position11) (See Figure S21). Yellow amorphous.

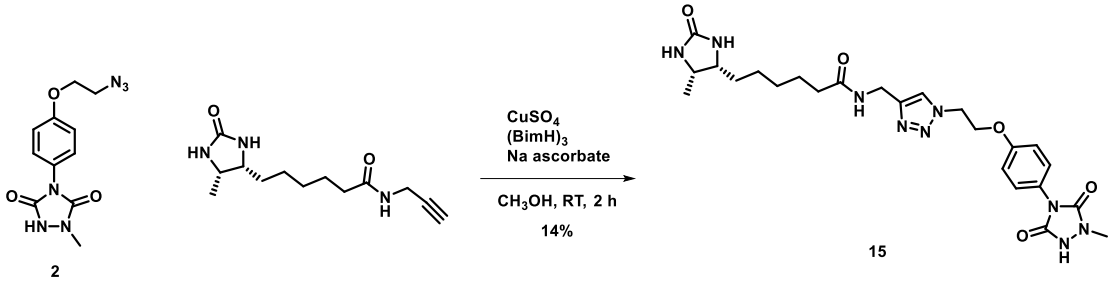


s13

(Reference): Ac-Trp-NHMe **s10**. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H 8.02 (d, J = 7.8 Hz, 1H, NH), 7.89-7.86 (m, 1H, NH), 7.57 (d, J = 8.4 Hz, 1H, position 4), 7.31 (d, J = 7.8 Hz, 1H, position 7), 7.09 (d, J = 2.4 Hz, 1H, position 2), 7.05 (t, J = 7.2 Hz, 1H, position 6), 6.96 (t, J = 7.2 Hz, 1H, position 5), 4.46-4.41 (m, 1H, position 9), 3.32 (s, 3H), 3.07 (dd, J = 14.4 Hz, 5.4Hz, 1H, position 8), 2.87 (dd, J = 14.4, 9.0 Hz, 1H, position 8), 2.55 (s, 3H, position 12), 1.78 (s, 3H, position 11). White solid.



Synthesis of alkyne-conjugated desthiobiotin. Desthiobiotin (304.4 mg, 1.42 mmol), HOBt · H_2O (223.4 mg, 1.46 mmol), and EDCI · HCl (526.3 mg, 2.96 mmol) were added to a solution of propargylamine (140 μL, 2.13 mmol) in DMF (5.0 mL) at room temperature. After stirring at room temperature for 17 h, the reaction mixture was concentrated under vacuum. The residue was purified using HPLC (5–100% CH_3OH/H_2O) to obtain the target compound as a white solid (329.3 mg, 92%). ^{1}H NMR (600 MHz, CD_3OD) δ_H (ppm) 3.94 (d, J = 2.4 Hz, 2H), 3.81 (m, 1H), 3.70 (m, 1H), 2.58 (s, 1H), 2.21 (t, J = 7.8 Hz, 2H), 1.63 (m, 2H), 1.52–1.27 (m, 6H), 1.10 (d, J = 6.6 Hz, 2H); ^{13}C NMR (600 MHz, CD_3OD) δ_C (ppm) 175.7, 166.2, 80.7, 72.1, 57.3, 52.7, 36.6, 20.7, 30.1, 29.3, 27.1, 26.6, 15.6; HRMS (ESI-TOF): *m/z* calced. for $C_{13}H_{21}N_3O_2Na$ $[M+Na]^+$: 274.1526, found 274.1554.



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Synthesis of MAUra-DTB 8. Alkyne-conjugated desthiobiotin (51.7 mg, 206 μmol) and $(BimH)_3$ (17.0 mg, 41.7 μmol) were added to a solution of compound **2** (56.8 mg, 206 μmol) in CH_3OH (2.0 mL). An aqueous solution of CuSO4 (9.6 mg, 60.0 μmol, 300 μL in H_2O) and Na ascorbate (1.020 g) were added to the solution. After ultrasonication for 5 min and stirring for 2 h at room temperature, the reaction mixture was concentrated under vacuum. The residue was purified using PTLC ($CHCl_3$:CH_3OH = 4:1) and HPLC (40% CH_3OH/H_2O) to yield the target compound **8** as a brown amorphous (14.6 mg, 14%) solid. ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm) 7.95 (s, 1H), 7.33 (d, 2H,

J= 8.4 Hz), 7.01 (d, 2H, *J*= 7.8 Hz), 4.81 (m, 2H), 3.79 (m, 1H), 3.66 (m, 1H), 3.23 (s, 1H), 2.21 (t, 2H, *J*= 7.2 Hz), 1.62 (m, 2H), 1.47-1.25 (m, 6H), 1.08 (d, 3H, *J*= 6.6 Hz); ^{13}C-NMR (600 MHz, DMSO-d_6) δ_C (ppm) 172.1, 162.9, 155.7, 145.2, 127.9, 126.8, 126.2, 123.2, 114.7, 113.9, 66.5, 55.4, 54.5, 50.6, 49.9, 49.0, 35.1, 34.1, 29.5, 28.4, 25.1, 15.5; HRMS (ESI-TOF): *m/z* calced. for $C_{24}H_{32}N_9O_5$ [M-H]$^-$: 526.2549; found: 526.2521.

2-3. Cell lysate preparation

Cell culture

A431 cells were incubated in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS). Cells were incubated in a cell incubator with 5% CO_2 at 37 °C.

Preparation of cell lysate

A431 cells (2.0×10^7 cells) were washed with phosphate-buffered saline (PBS) three times, and EDTA solution (1 mL, 0.5 mM) was added. After 5 min of incubation at 37 °C, the suspension was added to PBS, and the supernatant was removed after centrifugation (1400 rpm, 4 ℃, 5 min). The cells (2.0×10^7 cells) were washed three times with PBS, and 1.0 mL of 1× RIPA buffer (50 mM Tris-HCl buffer; pH = 7.6, 150 mM NaCl, 1% Nonidet P 40 substitute, 0.5% sodium deoxycholate, protease inhibitor cocktail) was added. After incubation on ice for 15 min, the sample was vortexed for 10 s and centrifuged (10000 × g, 4 °C, 10 min) to yield the A431 cell lysate as the supernatant. The concentration of the cell lysate was determined using the BCA protein assay.

2-4. Labeling

Peptide labeling using laccase

A solution of the labeling reagent was added to a solution of the peptide (50 mM Tris or phosphate buffer, pH 3.0-8.0). Laccase (Amano Enzyme Inc. or Sigma-Aldrich; final concentration 2.5-82 U/mL) was added to the mixture and mixed, and the mixture was stirred at 800 rpm (using a Thermo Fisher Scientificshaker incubator) and 37 ℃. The reaction was quenched by the addition of aqueous TFA. (final concentration 0.1%). The peaks of the modified peptides were detected using MALDI-TOF and HPLC.

***p*-Cresol derivative labeling using laccase (analysis scale)**

A solution of compound **1** (final concentration 1 mM) was added to a solution of *p*-cresol derivative (final concentration 1 mM) in 50 mM Tris buffer (pH 6.0). Laccase was added to the mixture and mixed, the mixture was stirred at 800 rpm (using a Thermo Fisher Scientific shaker incubator) and

37 ℃. The reaction was quenched by the addition of aqueous TFA (final concentration 0.1%). The reaction mixture was diluted 2.9 times with 0.1% TFA and the mixture was analyzed by HPLC.

Labeling of Ac-Tyr-NHMe and Ac-Trp-NHMe

A solution of compound **1** (final 1 mM) was added to a solution of Ac-Tyr-NHMe and/or Ac-Trp-NHMe (100 μM, in 50 mM Tris buffer, pH 6.0). Laccase (Amano Enzyme Inc. or Sigma-Aldrich; final concentration 8.2 U/mL) was added to the mixture and mixed, and the mixture was stirred at 800 rpm (using a Thermo Fisher Scientificshaker incubator) and 37 ℃. The reaction was quenched by the addition of aqueous TFA. (final concentration 0.1%). The peaks of the modified peptides were detected using HPLC (Arc HPLC system, Waters).

Peptide labeling using PTAD

A solution of PTAD **s7** was added to a solution of peptide (final concentration 100 μM in 50 mM Tris buffer, pH 7.4) and reacted for 5 min. The reaction was quenched by the addition of aqueous TFA (final concentration 0.1%). Peaks of the modified peptides were detected using MALDI-TOF MS.

Peptide labeling using e-Y-click[3]

Voltage was applied to a mixture of angiotensin II (100 μM) and 4-phenylurazole **s8** (1 mM) in 50 mM Tris buffer (pH 7.4) using the experimental device (400 mV vs Ag/AgCl, room temperature, 5–60 min, stirring speed: 400 rpm, graphite). Peaks of the modified peptides were detected using MALDI-TOF MS.

Peptide labeling using phenothiazine[4]

Voltage was applied to a mixture of angiotensin II (100 μM) and phenothiazine **s9** (1 mM) in 50% CH_3CN in PBS buffer (pH 7.4) or PBS buffer using the experimental device shown (900 mV vs Ag/AgCl, room temperature, 5–60 min, stirring speed: 400 rpm, graphite plate anode, nickel plate cathode, under N_2). Peaks of the modified peptides were detected using MALDI-TOF MS.

Peptide labeling using hemin[5]

N-Me luminol **7** (from a 100 mM stock solution in DMSO; final concentration, 100 μM) and hemin (from 1 mM freshly dissolved in DMSO, final concentration 500 nM or 10 μM) were added to a solution of angiotensin II (final concentration 100 μM) in 50 mMTris buffer (pH 7.4). H_2O_2 (final concentration: 1 mM) was added to the mixture and allowed to react at room temperature for 0-60 min. Peaks of the modified peptides were detected using MALDI-TOF MS.

Peptide labeling using HRP[6]

N-Me luminol **7** (from a 100 mM stock solution in DMSO; final concentration 100 uM) and HRP (final concentration 45 nM) were added to a solution of angiotensin II (final concentration 100 μM) in 50 mMTris buffer (pH 7.4). H_2O_2 (final concentration, 100 or 300 μM) was added to the mixture and reacted at room temperature for 0-60 min. Peaks of the modified peptides were detected using MALDI-TOF MS analysis.

Protein labeling using laccase

A solution of labeling reagent was added to a solution of protein (final concentration 10 μM in 50 mM Tris buffer (pH 6.0)). Laccase (Amano Enzyme Inc.) was added to the mixture and mixed, and the mixture was stirred at 800 rpm (using a Thermo Fisher Scientific shaker incubator) and 37 °C. For proteins labeled with azide-conjugated labeling reagents, labeling reagents were removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000 × g, 4 min) after the reaction. A solution of DBCO-Cy3 (final concentration 30 or 100 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) or a Zeba Spin Desalting Column (Thermo Fisher Scientific, 7 K MWCO, 0.5 mL) (1500 × g, 2 min).

Protein labeling using HRP[6,8]

A solution of azide-conjugated *N*-Me luminol **s15** (final concentration 300 μM) was added to a solution of protein (final concentration 10 μM in 50 mM Tris buffer (pH 7.4)). HRP (final 45 nM) and H_2O_2 (25 μM) were added to the mixture and mixed, and the mixture was incubated at 37 °C for 1 h. After 1 h, the 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 concentration 100 μM) was added to the filtrate, and the mixture was incubated at 37 °C for 30 min. After 30 min, the excess DBCO reagent was removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000 × g, 4 min). SDS-PAGE was performed using the same amount of protein in each well.

E-Y-click of BSA

Voltage was applied to a mixture of BSA (10 μM) and **s16** (300 μM) in 50 mM Tris buffer (pH 7.4) using the experimental device (400 mV vs Ag/AgCl, room temperature, 30–60 min, stirring speed: 400 rpm). The 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 concentration 100 μM) was added to the filtrate and the mixture was incubated at 37 °C for 30 min. After 30 min, the excess DBCO reagent was removed using a Sephadex G-25 gel (GE Healthcare) filtration column (2000 × g, 4 min).

2-5. Analysis

MALDI-TOF MS analysis of labeled peptides

For experiments on peptide labeling reactions, the reaction mixture was diluted 10 times with 0.1% TFA and was mixed with CHCA solution (5.0 mg/mL solution in acetonitrile: 0.1% TFA aq.= 0.5 μL: 0.5 μL), and the mixture was placed on MALDI-TOF plate and dried at room temperature. Modified protein peaks were detected using MALDI-TOF MS (ABSCIEX TOF/TOF^{TM} 5800).

HPLC analysis

Labeled peptides or substrates oxidized by laccase (Amano Enzyme Inc.) were analyzed using HPLC (210 nm absorbance of HPLC peaks). The following micropump (1 mL/min) gradient method was used: Mobile phase A: 0.1% aqueous FA, mobile phase B: 100% CH_3OH. 0−5 min: 5% B, 5−27 min: 5−100% B, 27−32 min: 100% B

Laccase activity assay

Laccase activity was evaluated from the oxidation of ABTS. The absorbance at 420 nm of solutions of laccase (final concentration 0.01 mg/mL) and ABTS (final concentration 100 μM) in 100 mM sodium acetate buffer (pH 4.5) was measured at room temperature.

Quantification of dye labeled on protein

After the procedure described in the "Protein labeling using laccase" section, the amount of Cy3 bound to the recovered protein was quantified using a NanoDrop One (Thermo Fisher Scientific). The following values were used for the Cy3 quantification settings: Coefficient (L/mol·cm): 150,000, Wavelength (nm): 550, 260 nm correction: 0.04, 280 nm correction: 0.05.

Estimation of free cysteine oxidation of BSA[9]

Biotin-PEG2-maleimide (Cosmo Bio; final concentration 200 μM) was added to modified BSA and incubated at room temperature for 1 h. The 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, protein bands separated with SDS-PAGE were transferred to a PVDF membrane (GE Healthcare) using TransBlot® 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 (StAv-HRP, Sigma-Aldrich). The blot was treated with an ECL kit (GE Healthcare). Chemiluminescent images were obtained using Fusion Solo 4S (Vilber Lourmat). The signal intensities were quantified using Image J.

Oxidation of substrate using laccase

A solution of the substrate was added to a laccase solution (Amano Enzyme, Inc.) in 50 mM Tris buffer (pH 6.0). The substrate was mixed, and the mixture was stirred at 800 rpm (using a Thermo Fisher Scientific shaker incubator) and 37 ℃. The reaction was quenched by the addition of aqueous TFA (final concentration 0.1%). The reaction mixture was diluted 2.9 times with 0.1% aqueous TFA and analyzed by HPLC.

Enzymatic digestion of labeled proteins

Sample preparation for fluorescence detector-conjugated LC-MS and nanoLC-MS measurements for labeling site identification was performed using the iST kit (PREOMICS), following the manufacturer's protocol. The approximate amount of peptide after digestion was quantified using the A205 mode of a NanoDrop One (Thermo Fisher Scientific).

Fluorescence detector-coupled LC-MS measurement of Cy3-labeled peptide fragments

The peptide fragments obtained by enzymatic digestion of the labeled proteins were analyzed by LC-MS. The labeled peptide fragments were detected using LC-MS (ACQUITY UPLC, Xevo G2-XS QTof) to detect fluorescence (Ex/Em = 550/570 nm for Cy3 detection) using an AQUITY UPLC® BEH130 C18 1.7 µm 2.1 × 150 mm column. The micropump (0.4 mL/min) gradient method was used as follows: mobile phase A: 0.1% aqueous TFA; mobile phase B: 100% acetonitrile. 0−0.5 min: 15% B (0.31 mL/min), 0.5−15.5 min: 15−60% B, 15.5−16 min: 60−95% B, 16−18 min: 95% B, 18−18.5 min: 10% B. The MS data was analyzed by Masslynx v4.1.

NanoLC-MS/MS analysis for the identification of labeling site

NanoLC-MS/MS analysis was performed using an LC-nano-ESI-MS composed of a quadrupole-Orbitrap hybrid mass spectrometer (Q-Exactive; Thermo Fisher Scientific) equipped with a nanospray ion source and a nano HPLC system (Easy-nLC 1000; Thermo Fisher Scientific). The trap column used for the nano HPLC was a 2 cm × 75 µm capillary column packed with 3 µm C18-silica particles (Thermo Fisher Scientific) and the separation column was a 12.5 cm × 75 µm capillary column packed with 3 µm C18-silica particles (Nikkyo Technos Co., Ltd., Japan). The flow rate of the nano-HPLC system was 300 nL/min. The separation was conducted using a 10−40% linear acetonitrile gradient for 30 min in the presence of 0.1% formic acid. The nanoLC-MS/MS data were acquired in a data-dependent acquisition mode controlled by Xcalibur 4.0 (Thermo Fisher Scientific). The settings of data-dependent acquisition were as follows: the resolution was 70,000 for a full MS scan and 17,500 for MS2 scan; the AGC target was 3.0E6 for a full MS scan and 5.0E5 for MS2 scan; the maximum IT was 60 ms for both a full MS scan and MS2 scan; the full MS scan range was 310−1,500 m/z; and the top 10 signals were selected for MS2 scan per one full MS scan. MS/MS spectra were searched against the respective amino acid sequences using MaxQuant (freeware) with the default settings.[10]

The FASTA file corresponding to the protein sequences was used. For labeling, the oxidation (+O) of His, Met, and Tyr residues, acetylation (+C_2H_2O) at the N-terminus, and the adduct of MAUra (+$C_9H_7N_3O_2$; +189.054 Da) for Tyr residues were set as possible modifications.

Comparison of GOx activity before and after labeling

A solution of BES-H_2O_2 (final concentration 1 μM), GOx (final concentration 10 nM), and glucose (final concentration 90 mM, 50 mM Tris buffer pH 7.4) was added to 96-well-plates (100 μL/well) and incubated for 3 h at 25 ℃. Gox was labeled with 3.0 equiv. Compound **2** was used to label GOx. A time-dependent increase in fluorescence intensity was detected using a plate reader (Infinite 200 Pro, λex = 485 nm ± 20 nm/ λem = 530 nm ± 25 nm) (See Figure S50).

Preparation of nanoLC-MS/MS sample from labeled cell lysate

A solution of MAUra-DTB **15** (final concentration 500 μM) was added to a solution of A431 cell lysate (final concentration 1 mg/mL in 50 mM Tris buffer (pH 6.0)). Laccase (Amano Enzyme Inc., final concentration 82 U/mL) was added to the mixture and mixed, and the mixture was stirred at 800 rpm (using a Thermo Fisher Scientific shaker incubator) and 37 °C. Chloroform/methanol precipitation of the labeled cell lysate was performed to remove the labeling reagent. Resulted proteins were dissolved by the addition of PTS (12 mM SDC, 12 mM SLS in 100 mM Tris-HCl (pH 9.0)) and heated at 95 ℃ for 5 min (final concentration of protein 1 mg/mL). This solution was supplemented with DTT (from a 100 mM solution in 100 mM NH_4HCO_3, final concentration 10 mM) and incubated at 37 °C for 30 min. Subsequently, 2-iodoacetamide (from 1 M solution in 50 mM NH_4HCO_3, final concentration of 50 mM) was added and the solution was incubated at 37 °C for 30 min. The resulting solution was diluted five-fold with 100 mM NH_4HCO_3. Lys-C solution (final 10 ng/μL) was added to the solution and incubated at room temperature for 3 h. After incubation, trypsin solution (final 20 ng/μL) was added to the solution, incubated overnight at 37 ℃, and quenched by aqueous TFA solution (final concentration 0.1%). The mixture was extracted with 500 μL of ethyl acetate and TFA (final concentration 0.5%) to remove SDC and SLS. The water layer was collected by configuration (15700 × g for 2 min at room temperature) and concentrated under reduced pressure. The residue was rehydrated with TBS and NeutrAvidin beads (Cytiva) (0.5 mg) were added and shaken at room temperature for 60 min. The beads were then washed three times with TBS. For the elution of desthiobiotin labeled peptide, beads were heated at 95 ℃ for 5 min. The supernatant was collected by magnetic separation. After collecting the supernatant, detergents in the solution were removed using a HiPPR Detergent Removal Resin Column Kit (Thermo Scientific) and desalted using C18 pipette tips (Nikkyo Technos Co., Ltd.). The desalted solution was then subjected to LC-MS/MS.

NanoLC-MS/MS analysis for comprehensive detection of labeled peptide from cell lysate

NanoLC-MS/MS analysis was performed using an LC-nano-ESI-MS composed of a quadrupole-Orbitrap hybrid mass spectrometer (Q-Exactive; Thermo Fisher Scientific) equipped with a nanospray ion source and a nano HPLC system (Easy-nLC 1000; Thermo Fisher Scientific). The trap column used for the nano HPLC was a 2 cm × 75 μm capillary column packed with 3 μm C18-silica particles (Thermo Fisher Scientific) and the separation column was a 12.5 cm × 75 μm capillary column packed with 3 μm C18-silica particles (Nikkyo Technos Co., Ltd., Japan). The flow rate of the nano-HPLC system was 300 nL/min. The separation was conducted using a 10−40% linear acetonitrile gradient for 70 min in the presence of 0.1% formic acid. The nanoLC-MS/MS data were acquired in a data-dependent acquisition mode controlled by Xcalibur 4.0 (Thermo Fisher Scientific). The settings of data-dependent acquisition were as follows: the resolution was 70,000 for a full MS scan and 17,500 for MS2 scan; the AGC target was 3.0E6 for a full MS scan and 5.0E5 for MS2 scan; the maximum IT was 60 ms for both a full MS scan and MS2 scan; the full MS scan range was 310−1,500 m/z; and the top 10 signals were selected for MS2 scan per one full MS scan. MS/MS spectra were searched against the human proteome (downloaded from UniProt database on Aug. 11th, 2021) with the sequences of 298 contaminant proteins preset in the software, using the Sequest HT algorithm within Proteome Discoverer 2.4 (Thermo Fisher Scientific). For the modification settings, oxidation (+ 15.995 Da) for His, Met, and Trp residues and $C_{24}H_{30}N_9O_5$ (+ 524.552 Da) for His, Trp, and Tyr residues were set as dynamic modifications and carbamidomethylation (+ 57.021 Da) for the Cys residue was set as a static modification.

Motif analysis of labeled sites

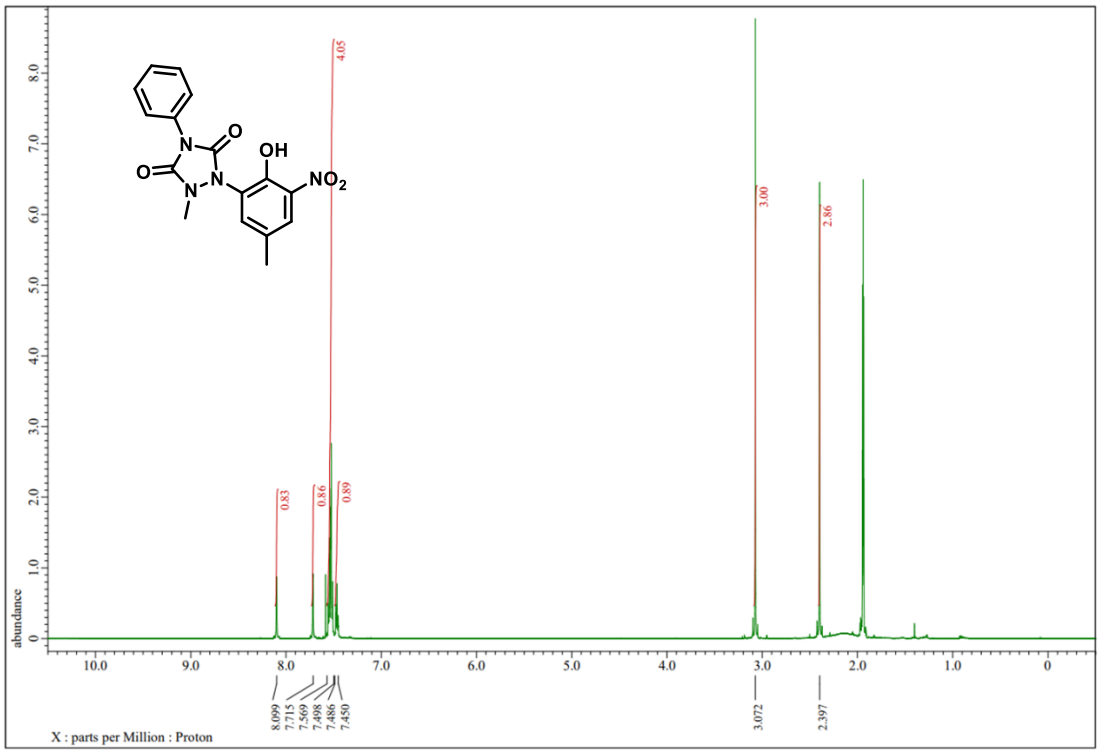
Using the labeled protein residue data and human protein sequence information obtained from UniProt (downloaded on July 22nd, 2022), a list of amino acid residues (-6 to +6) surrounding the labeled sites was generated using the R software. A motif analysis diagram was created from this list using pLogo (https://plogo.uconn.edu/) with human protein as the background.

Calculation of relative accessible surface area

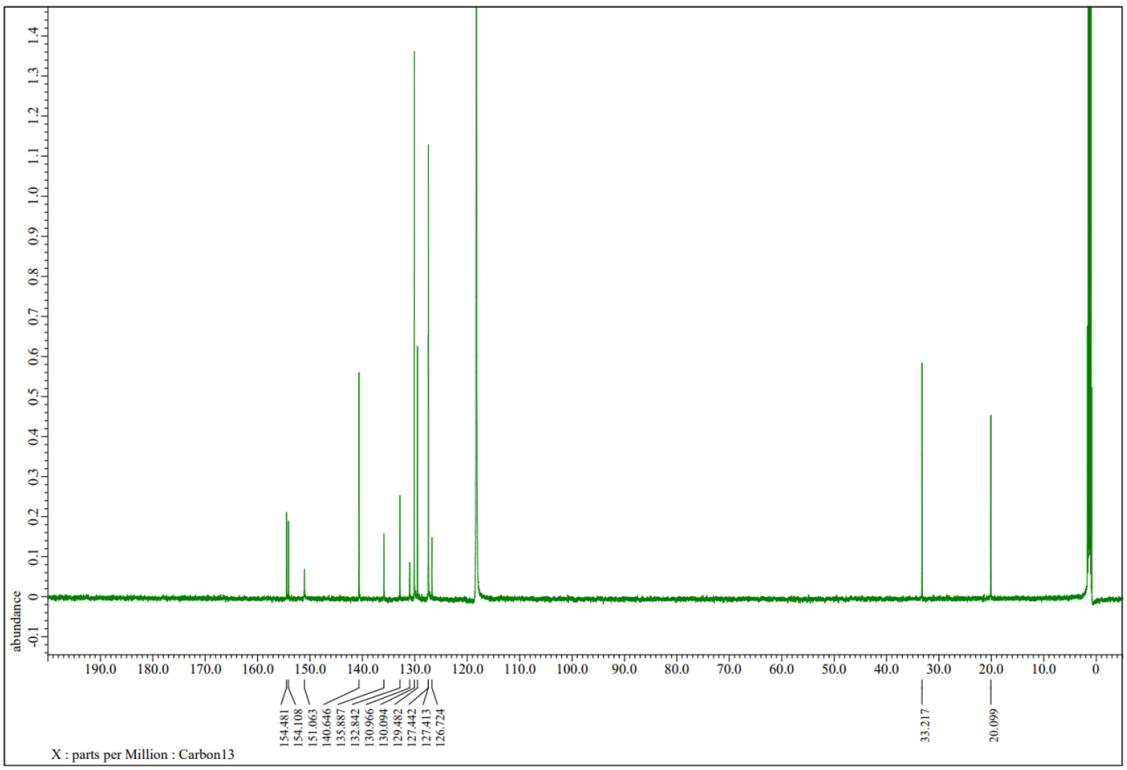
All human predicted structures were downloaded from the AlphaFold Structure Database (structure version 1). The accessible surface area (ASA) calculation of Tyr residues on predicted structures was performed using DSSP. The maximum accessible surface area for a Tyr residue (255.0) used for converting absolute ASA to relative ASA was taken from ref. 11.

3. ^{1}H- and ^{13}C-NMR spectrum data

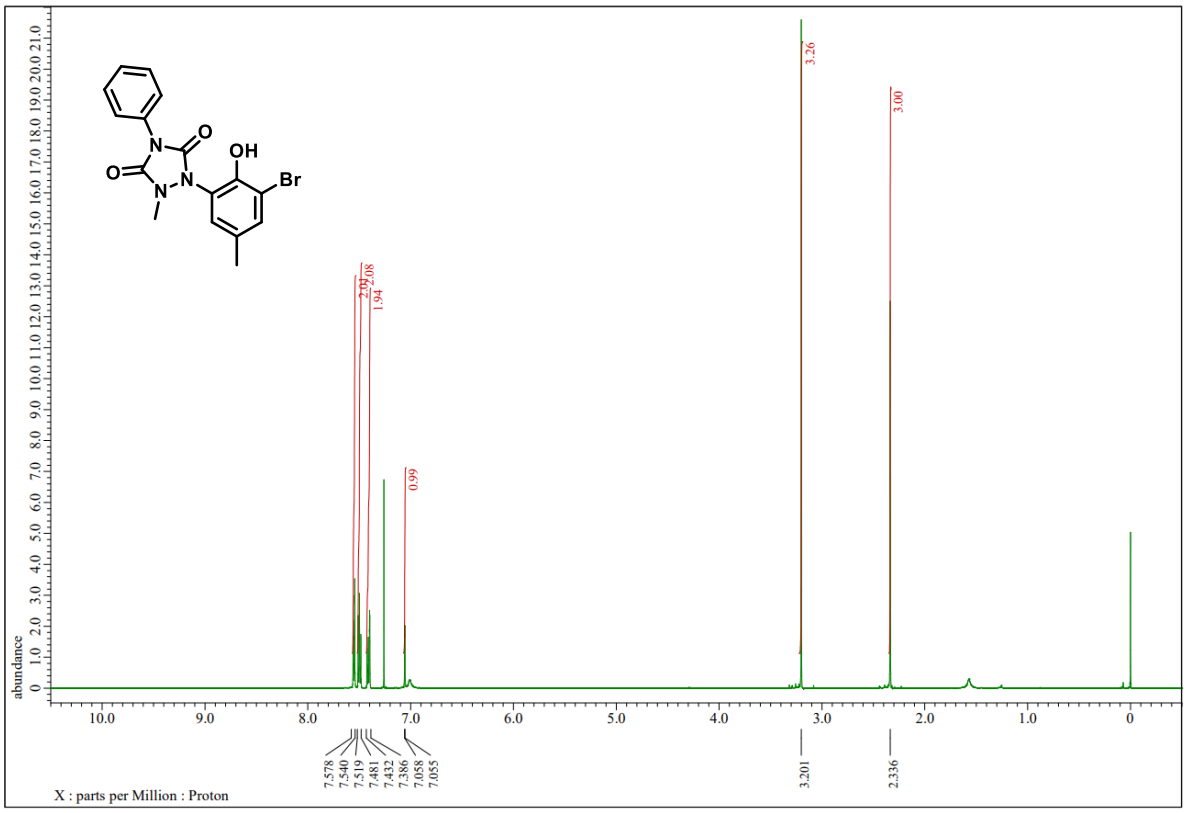
^{1}H NMR (600 MHz, CD_3CN)



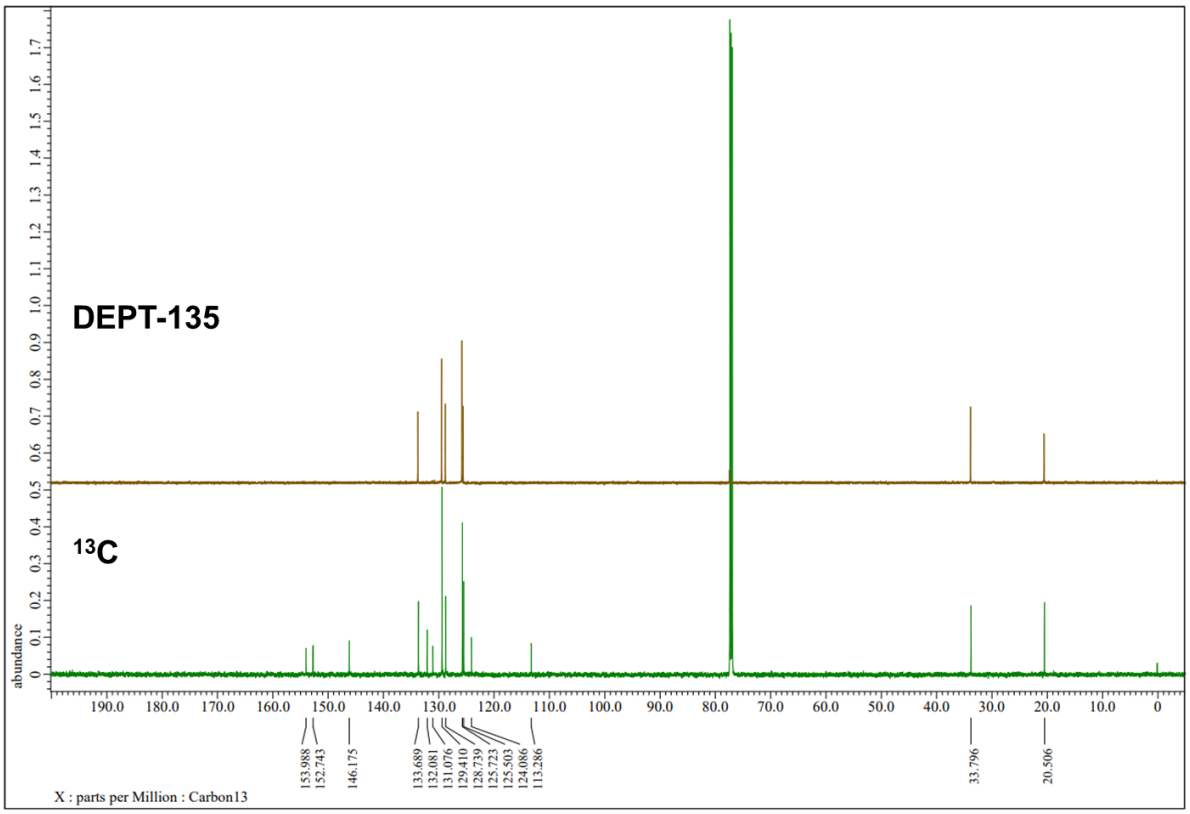
^{13}C NMR (150 MHz, CD_3CN)



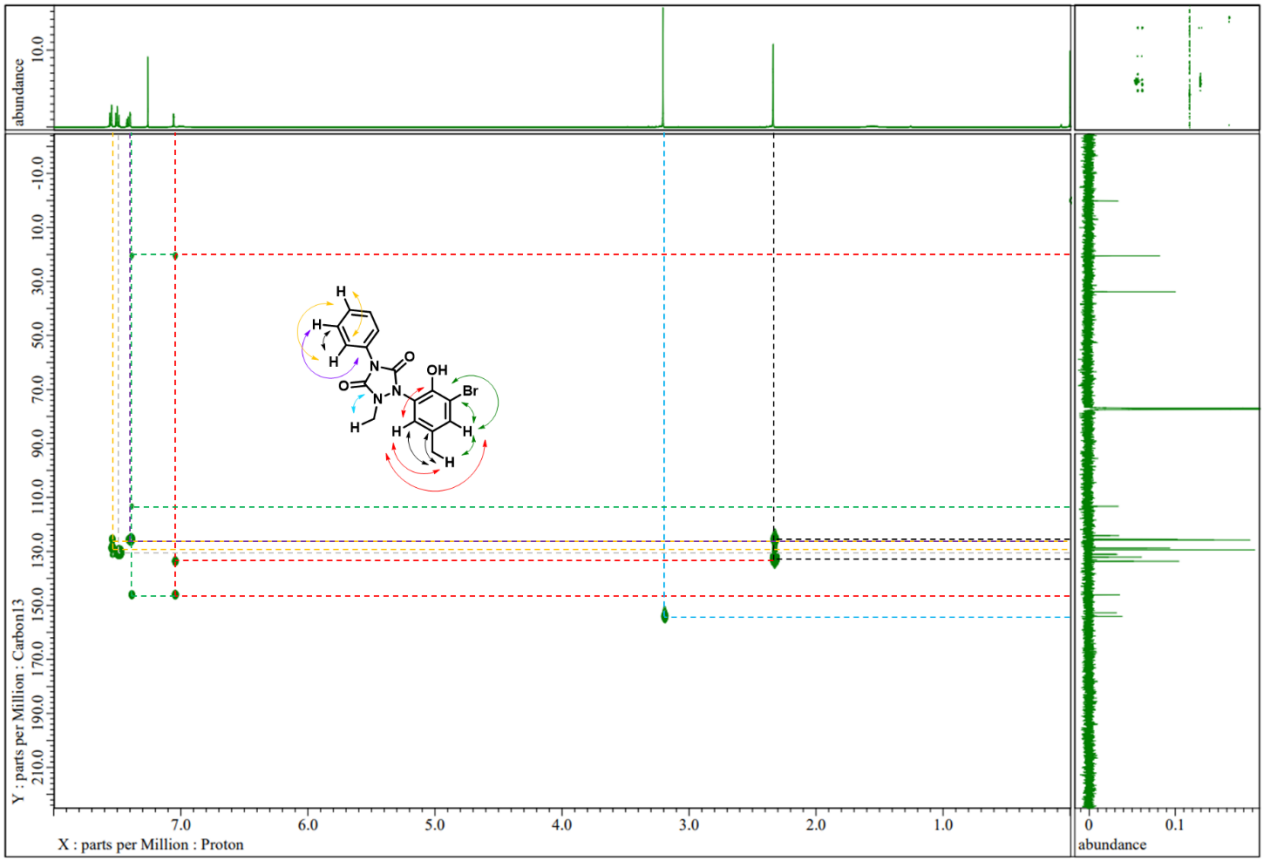
^{1}H NMR (600 MHz, $CDCl_3$)



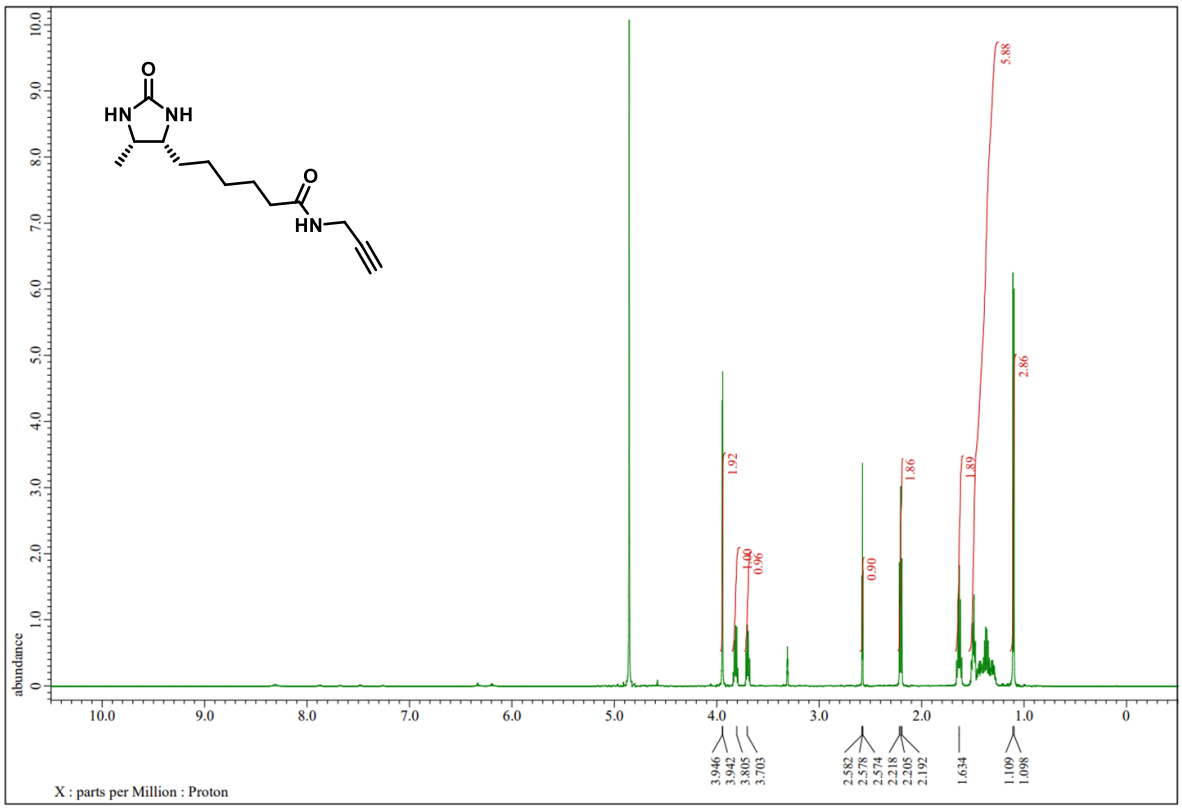
^{13}C NMR (150 MHz, $CDCl_3$)



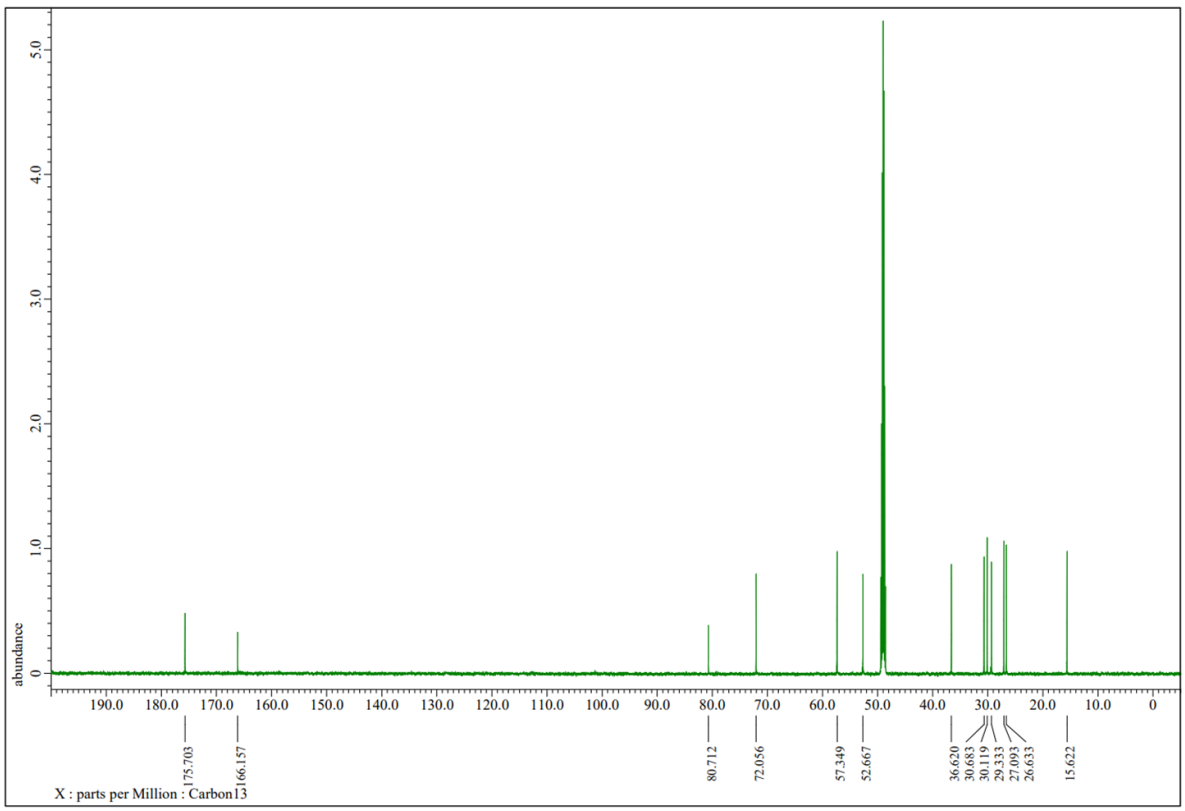
HMBC (600 MHz, $CDCl_3$)



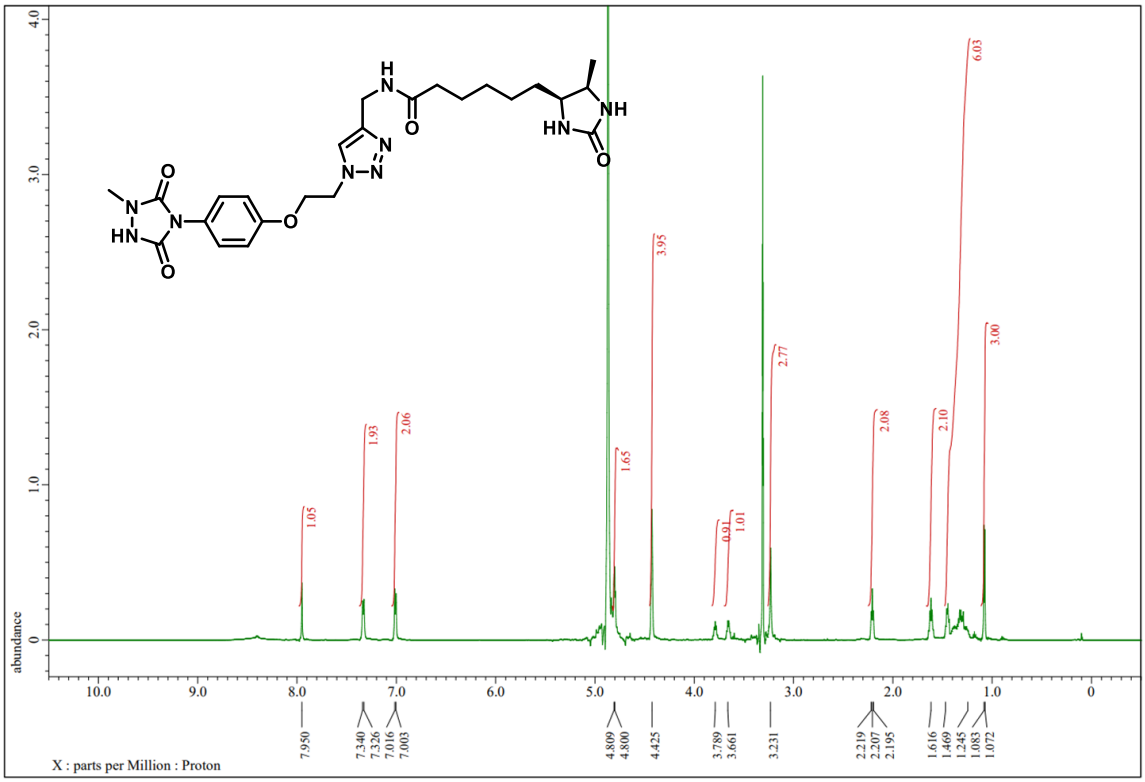
^{1}H NMR (600 MHz, CD_3OD)



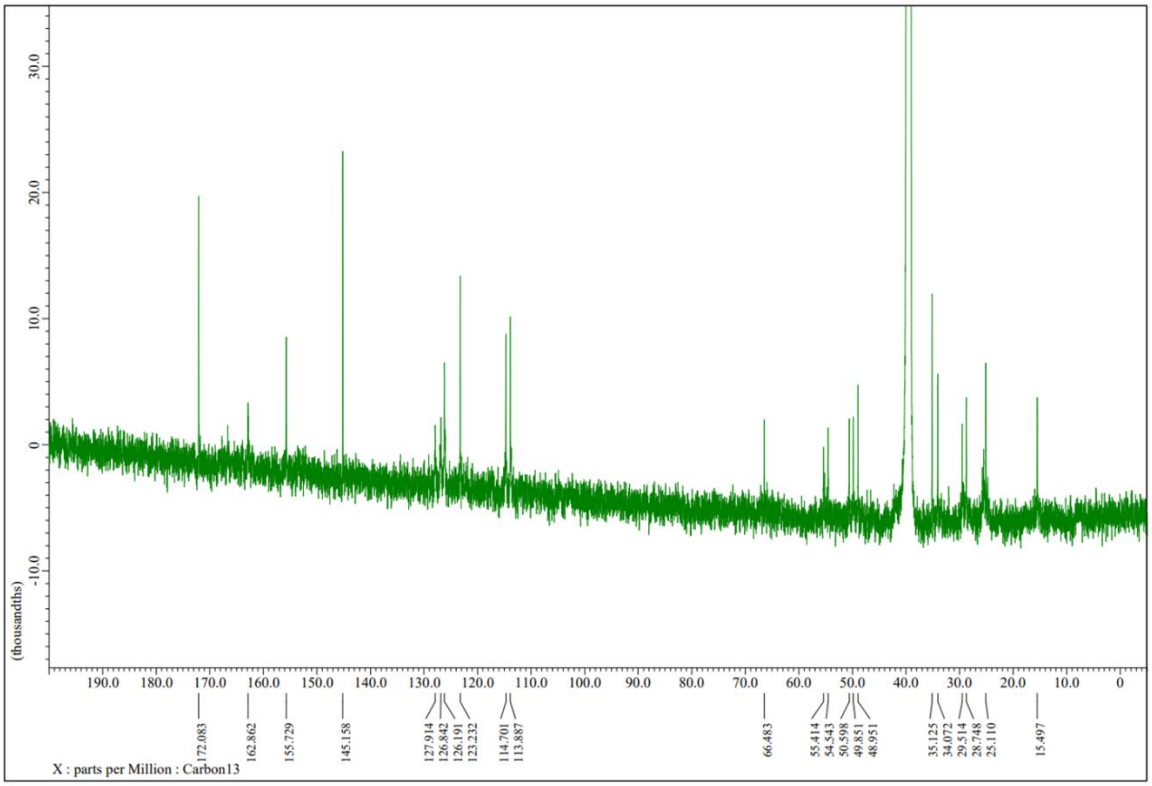
^{13}C NMR (150 MHz, CD_3OD)



^{1}H NMR (600 MHz, CD_3OD)



^{13}C NMR (150 MHz, DMSO-d_6)



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5. Acknowledgements

This study was partially supported by research grants from the JST FOREST Program (grant number: JPMJFR2005 to S. S.), Grant-in-Aid for Scientific Research (B) (23H02099 to S. S.), Naito Foundation (to S. S.), Grant-in-Aid for Transformative Research Areas (A), Biophysical Chemistry for Material Symbiosis (21H05503 to S. S.), and Scientific Research (A) (22H00436 M. I.). We thank the Cell Biology Center Research Core Facility at Tokyo Tech for the Q-Exactive mass spectrometry measurements, generous support from the FRIS CoRE, which is a shared research environment. This study was partially supported by the Research Support Project for Life Science and Drug Discovery (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from AMED under Grant Number JP23ama121019. We thank Dr. Izumi Sakamoto, Dr. Ryohei Uematsu, Naoko Hashimoto (GlyTech, Inc.) for their help in LC-MS experiment for the labeling site analysis.