Supplementary Information

Histidine-Specific bioconjugation via visible-light-promoted thioacetal activation

Chuan Wan,a Yuena Wang,a Chenshan Lian,b Qi Chang,b Yuhao An,b Jiean Chen,b Jinming Sun,a Zhanfeng Hou,b Dongyan Yang,c Xiaochun Guo,a Feng Yin,b Rui Wang,b and Zigang Li*a,b

a. State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen, 518055, P. R. China.
b. Pingshan translational medicine center, Shenzhen Bay Laboratory, Shenzhen, 518118, P. R. China.
c. College of Chemistry and Chemical Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou, 510225, P. R. China.
† These authors contributed equally to this work
* Address correspondence to yinfeng@szbl.ac.cn, wangrui@szbl.ac.cn, lizg.sz@pku.edu.cn, lizg@szbl.ac.cn.
# Table of Contents

1. Supplementary figures and discussions ....................................................................................... 3

2. Supplementary material and methods for biochemistry ........................................................... 17
   2.1 General procedure ................................................................................................................... 17
   2.2 Chemicals and Reagents ...................................................................................................... 17
   2.3 Cell Culture .......................................................................................................................... 17
   2.4 Gel fluorescence analysis of probe-labelled proteins .......................................................... 17
   2.5 Histidine-specific profiling using mass spectrometry .......................................................... 18
   2.6 LC-MS/MS Analysis .............................................................................................................. 18
   2.7 Data Processing ................................................................................................................... 18

3. Chemical procedures and compound characterizations ........................................................... 19
   3.1 General procedure A and characterization for the reaction between Boc-His-OMe and thioacetal 1 ................................................................................................................................ 19
   3.2 General procedure B for the synthesis of thioacetals and thioketals .................................. 23
   3.3 Synthesis of TA8 and TA9 ..................................................................................................... 27
   3.4 Synthesis of TA1 and TA10 ................................................................................................... 28
   3.5 Synthesis of TA2 and TA11 ................................................................................................... 29
   3.6 Synthesis of tert-butyl ((ethylthio)(4-methoxyphenyl)methyl)carbamate S8 ..................... 32
   3.7 Synthesis of intermediates .................................................................................................. 32

4. Supplementary material and methods for the reactions of peptide ......................................... 35
   4.1 General information ............................................................................................................. 35
   4.2 General procedure C for the reaction between peptide and thioacetal ............................. 35

5. References ..................................................................................................................................... 58

6. NMR spectra ............................................................................................................................... 59
1. Supplementary figures and discussions

**Scheme S1.** Examples of metal-approaches for His modification on protein. (a) Intracellular reactions promoted by bis(histidine) miniproteins stapled using Palladium(II) complexes\(^{[1]}\). (b) Histidine-directed arylation/alkenylation of backbone N–H bonds mediated by Copper(II)\(^{[2]}\).
Scheme S2. Examples for His modification on protein. (a) Histidine-selective labelling at the proximity of the active site of enzyme surface[3]. (b) Histidine modification using thiophosphorodichloridate reagents that mimic post-translational histidine phosphorylation[4]. (c) Peptide modification via radical-mediated chemoselective C–H alkylation of histidine using C4-alkyl-1,4-dihydropyridine (DHP) reagents under visible-light-promoted conditions[5]. (d) Site-selective protein conjugation at histidine using a bis-alkylation reagent, PEG(10kDa)-mono-sulfone[6].
Pummerer reaction is a well-studied organic transformation and generally requires stoichiometric amounts of strong electrophilic activators including acid anhydride, trimethylxonium, dimethylthiosulfonium fluoroborate (DMTSF), and TMSCI (Scheme S3a). Recently, thioacetals and intermediates of aldehyde-thiol condensation were well demonstrated as thionium precursor to undergo connective Pummerer-type reaction by previous literatures, where polar organic solvents, such as TFE, and catalytical Lewis acids, such as BF$_3$ OEt$_2$, Cu(OTf)$_2$ and phosphate, were required (Scheme S3b). Therefore, we initialed our investigation by the reaction between thioacetal and aromatic amino acids to screen a biocompatible method for Pummerer-type modification of proteins. All of the aromatic amino acids (His, Trp, Tyr and Phenylalanine, Phe) were reacted with thioacetal 1a under a polar condition (TFE solvent) with irradiation of Blue LED (10 W). The reaction of derivatives of Trp and His gave products with 89% and 71% yield (Scheme S4a and Scheme S4b), and evidences of NMR spectra validated their alkylation positions are C$_2$ for Trp and N$_3$ for His, respectively. In contrast, derivatives of Phe didn’t observe any product under this condition, and trace of product of Tyr and 1a was detected in a
more polar solvent 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (Scheme S4). These results proved the feasibility of the modification of aromatic amino acid by Pummerer-type reaction and encouraged us to develop more biocompatible conditions. In order to ensure the dissolution of thioacetal at a 20 mM concentration, MeCN/H$_2$O mixed solvent was investigated. In MeCN/20% H$_2$O solvent, the reaction between Boc-His-OMe and 1a exhibited a very inefficient manner. Hence, we then shifted our focus to photoredox-catalysts.

Scheme S4. Visible-light-promoted Pummerer-type reaction between thioacetal and derivatives of aromatic amino acids: (a) S$_2$Ar reaction of Boc-Trp-OMe with TFE solvent; (b) Nucleophilic reaction of Boc-His-OMe 2 with TFE solvent; SEAr reaction of (c) Boc-Phe-OMe and (d) Boc-Tyr-OMe with TFE solvent; (e) SEAr reaction of Boc-Tyr-OMe with HFIP solvent.
Table S1. Optimization of conditions$^a$

| Entry | Catalyst | Light | Conversion (%)$^b$ |
|-------|----------|-------|-------------------|
| 1     | -        | blue LED | <10 |
| 2     | -        | blue LED (12 h) | 36 |
| 3     | Cu(OTf)$_2$ | blue LED | 18 |
| 4     | Cu(MeCN)$_4$BF$_4$ | blue LED | 33 |
| 5     | [Ir(ppy)$_2$(dtbbpy)](PF$_6$) | blue LED | 62 (39)$^c$ |
| 6     | Ru(bpz)$_3$(PF$_6$)$_2$ | blue LED | 10 |
| 7     | Phenanthrene | blue LED | 47 |
| 8     | 3,6-Di(pyridin-2-yl)-1,2,4,5-tetrazine | blue LED | 44 |
| 9     | MesAcrClO$_4$ | blue LED | 79 (66)$^c$ |
| 10    | 1,2,4,5-Tetracyanobenzene | blue LED | 52 |
| 11    | Rhodamine B | blue LED | 32 |
| 12    | Riboflavin tetrabutyrate | blue LED | 85 |
| 13    | Rose Bengal | blue LED | 92 (84)$^c$ |
| 14    | Rose Bengal | dark | trace |
| 15    | Rose Bengal | white LED | 67 |
| 16    | Rose Bengal | green LED | 62 |
| 17    | Rose Bengal | red LED | 48 |

$^a$ Conditions: 20 mM thioacetal 1a (4 equiv.), 5 mM Boc-His-OMe (1 equiv.) and 10 mol% catalyst in MeCN/H$_2$O(4/1) under irradiation of LED light (10 W) or dark at 30 °C under air for 1 hour. $^b$ Conversions were determined by HPLC. $^c$ Isolated yields in the parentheses.
Figure S1. Chemical structures of photocatalysts used in this work.
Figure S2. Characterization of the reaction kinetics between thioacetal 1 and Boc-His-OMe: (a) Estimation of the yield versus time plot by LC-MS. (b) Determination of the second-order rate constant for the reaction. The observed rates calculated from different concentrations of 1 and Boc-His-OMe were plotted against the concentration of 1 to obtain the rate constant k from the slope of the plot. (c) The LC traces of the reaction.

Conditions: thioacetal 1a (4 equiv., 20 mM, 10 mM, 5 mM, 1 mM or 0.5 mM), Boc-His-OMe (1 equiv., 5 mM, 2.5 mM, 1.25 mM, 0.25 mM or 0.125 mM) and 10 mol% Rose Bengal (RB) in MeCN/H₂O(4/1) under irradiation of blue LED (10 W) at 30 °C under air for 10 min, 30 min, 1 h, 2 h, 4 h and 6 h.
Figure S3. (a) LC-MS analysis of the reaction between various amino acids and thioacetal 1a under optimized conditions. The red asterisk indicates trace by-products from RB and thioacetal. (b) LC-MS analysis of the reaction between additional Fmoc-protected amino acids and thioacetal 1a under optimized conditions. (c) The chemo-selectivity between aromatic and nucleophilic AAs by LC-MS analysis via a one-pot reaction. Top trace: the standard reaction of 1a and 2; bottom trace: a mixed reaction between 2, Boc-Trp-OMe, Boc-Phe-OMe, Boc-Tyr-OMe, Boc-Ser-OH, Boc-Lys-OH and 1a under optimized conditions. Aldehyde by-product refers to 4-methoxybenzaldehyde produced by hydrolysis of substrate 1a. (d) The comparison between the standard reaction of peptide 6 with 1a and 4-methoxybenzaldehyde.

Conditions: 20 mM thioacetal 1a (4 equiv.), 5 mM amino acid (1 equiv.) and 10 mol% catalyst in MeCN/H$_2$O(4/1) under irradiation of blue LED (10 W) at 30 °C under air for 1h.
Figure S4. LC-MS analysis of the reaction between Boc-Trp-OMe and thioacetal 1a. Conditions: 20 mM thioacetal 1a (4 equiv.), 5 mM Boc-Trp-OMe (1 equiv.) and 10 mol% RB in specified solvent under irradiation of blue LED (10 W) at 30 °C under air for 16 h.
Figure S5. LC-MS analysis of the reaction between Ac-Cys-OH and thioacetal 1a. Conditions: 20 mM thioacetal 1a (4 equiv.), 5 mM Ac-Cys-OH (1 equiv.) and 10 mol% catalyst or 5 mM Ac-Cys-OH (1 equiv.) in MeCN/H$_2$O(4/1) under irradiation of blue LED (10 W) at 30 °C under air for 1h.

Figure S6. Hydrolysis of Boc-His-OMe product. Conditions: 5 mM 1a (4 equiv.) in specified solvent at 37 °C shaker for 24 h. (a) In PBS buffer. (b) In aqueous solution with Brønsted acid.
Figure S7. (a) Other Thioacetal probes used in this work; (b) Chemical structures of unreactive thioacetals and thioketals.
Figure S8. (a) Stern–Volmer quenching studies between RB (10 μM) and thioacetal 1a (0, 1, 2, 5, 10, 20 mM, respectively). (b) Estimation of the $I_0/I$ versus concentration of 1a plot by luminescence spectra. $I_0$, the luminescence emission intensity of photocatalyst RB, $I$, the luminescence emission intensity of photocatalyst RB with specific concentration of 1a. (c) GC-MS analysis for the study of mechanism. Conditions: 10 mM thioacetal 1a (4 equiv.), 2.5 mM Boc-His-OMe (1 equiv.) and 10 mol% Rose Bengal (RB) in MeCN/H$_2$O(4/1) under irradiation of blue LED (10 W) at 30 °C under air for 1 h.
Figure S9. Selective Histidine labeling. (a) ESI-TOF Spectrum of unmodified MB and 1a modified MB (MB/1a 100 μM /2 mM, 5% Rose, pH 7.4, 37 °C for 0.5 h under blue light). (b) Labeling of MCF7 cell lysates with TA8 in the presence or absence of IAA. (c) Labeling of MCF7 cell lysates with TA8 in the presence or absence of NHS-Ace.
Figure S10. Histidine containing proteome quantification from Label-free TOP-ABPP (a) venny (b) PCA and (c) volcano analysis of TA8 labelled proteome in three experimental groups.
2. Supplementary material and methods for biochemistry

2.1 General procedure

All chemical reagents are commercially available without purification. The reactions were monitored by TLC (silica gel-G). Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker 300 MHz, 400 MHz or 500 MHz spectrometer using trimethylsilane (TMS) as internal standard under ambient temperature (20 °C). High-Resolution Mass Spectrometry (HRMS) were measured on Orbitrap Exploris 480.

2.2 Chemicals and Reagents

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated. Leuproprelin (peptide 4) was purchased from Sangon Biotech; Angiotensin II (peptide 5) and Melanotan I (peptide 6) were purchased from Wuxi Asiapeptide Biotechnology Co. Ltd.. The procedures for the preparation of peptide 7 and 8 were illustrated in Section 4.1. Bovine Serum Albumin (BSA) was purchased from Sangon Biotech. Myoglobin (MB) and Carbonic Anhydrase (CA) were purchased from Sigma-Aldrich. TAMRA-azide (named as “TAMRA-N₃” in this study), Biotin-dadps-azide (named as “DADPS biotin-N₃” in this study) and PC biotin-PEG₃-azide (named as “PC biotin-N₃” in this study) was purchased from BIOCONE (Chengdu, CHINA). Sequencing-grade trypsin and NeutrAvidin™ agarose were purchased from Thermo Scientific. All solutions were made with ultrapure Milli-Q water (Millipore, Bedford, MA). BSA, MB and CA were dissolved in PBS buffer, pH 7.4. The peptide and probes were dissolved in DMSO to a stock concentration of 100 mM.

2.3 Cell Culture

MCF-7 cells were maintained at 37 °C in a humidified atmosphere containing 5% carbon dioxide using Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 2 mM insulin and 10% fetal bovine serum (FBS). We separated cells once they grew to 80-90% confluence in a 1:2 ratio. After 12~14 hours, subculture would achieve to approximately 80% confluence on 100 mm polystyrene tissue culture plates. The cells should be in log phase growth and healthy. On ice, we discard old medium and wash cells twice with ice-cold PBS. Pre-cooled microcentrifuge tube resuspended pellet in 1 ml cold DPBS buffer. Cells were sonicated until pellet is disturbed then centrifuged at x12,000 rpm 30 min. The final supernatant protein was transferred to a new tube and quantified at 562 nm using BCA (Thermo Scientific).

2.4 Gel fluorescence analysis of probe-labelled proteins

For probe labelling assay, BSA or proteins extracted from MCF7 cells were incubated with probes at indicated concentration at 37 °C for 2 h with blue light. A freshly pre-mixed click chemistry reaction cocktail was then added (50 μM TAMRA-N₃, 100 μM TBTA, 1 mM TCEP, and 1 mM CuSO4) to the mixture above for another 1 h at RT. After reaction, the labelled proteins were precipitated by pre-chilled acetone (-20 °C) to remove excessive reagents. Precipitated proteins were subsequently collected by centrifugation (13000 rpm x 10 min at 4 °C) and dissolved in PBS containing 1.2% SDS and then boiled using 4x SDS-PAGE loading buffer at 95 °C for 10 min. The samples were analyzed by 12% SDS-PAGE gels (polyacrylamide gel electrophoresis) and imaged by Bio-Rad ChemiDoc MP. Then the gels were then stained with Coomassie staining (CBB) and
scanned. For competition assay, proteins were treated with IAA or sulfo-NHS acetate at indicated concentration as above processes.

2.5 Histidine-specific profiling using mass spectrometry

We had 3 groups of proteins named as “DMSO”, “10 μM TA8” and “80 μM TA-8”. Then biotin was ligated to the probe-labelled proteins in as groups with click chemistry reaction. After reaction, the labelled proteins were precipitated by pre-chilled acetone (-20 °C) to remove excessive reagents. Precipitated proteins were subsequently collected by centrifugation (13000 rpm x 10 min at 4 °C) and dissolved in PBS containing 1.2% SDS then diluted to 0.2% SDS. Upon incubated with NeutrAvidin™ agarose beads for 3 h at 29 °C to capture the biotin-labelled proteins, the beads were washed with cold PBS (twice) and cold water (twice). The captured proteins on beads were reduced by DTT, treated by IAA, and digested at 37 °C overnight by trypsin into peptides. The supernatant was collected by centrifugation (1,000 g, 1 min) and combined with 2 X 50 μl water after washing beads, stored at -80 °C then acidified to a final concentration of 2% (v/v) formic acid and desalted for LC-MS/MS analysis.

2.6 LC-MS/MS Analysis

After dry in the speed VAC, obtained samples were loaded onto a Thermo analytical column (75 μm i.d. × 25 cm) C18 column with an Easy-nLC 1200 chromatography pump coupled with Orbitrap Exploris™480. For each analysis, we reconstituted peptides in 15 μl 0.1% FA and loaded 4 μl onto the column for running. Peptides in each running were separated on a 120 min (5-40% ACN) gradient. Parameters are as follows in Full MS/ data dependent -MS2 TopN mode: mass analyzer over m/z range of 350−1500 with a mass resolution of 60000 (at m/z=200) in a data-dependent mode, 1.6 m/z isolation window. 20 most intense ions are selected for MS/MS analysis at a resolution of 15000 using collision mode of HCD. Because of different fragmentation, it is divided into the following types of modifications: TA2(△M=218.07654), TA2-2(△M=179.05306), TA2-3(△M=144.05751), TA6-5(△M=105.03404), TA2-Peg2-1(△M=350.15518), TA2-Peg2-2(△M=179.05306), TA2-Peg2-3(△M=276.13616).

2.7 Data Processing

Spectral data was searched against the Uniprot/Swiss-prot protein database using PD 2.4 and filtered to 1% FDR (false discovery rate) at the protein level. Default parameters used following exceptions: a minimum of 1 unique peptide was required for quantitation; peptide matching between runs was included and peptides containing oxidation (O), N-terminal acetylation (protein N-term), and carbamidomethyl (C) as variable modifications; only tryptic peptides with two missed cleavage sites were allowed; fragment mass tolerance was set to 0.02 Da for MS/MS fragment ions; mini and max peptide lengths were 6 and 144. GO enrichment was performed for cellular components of labelled proteins, and Uniprot accession numbers of identified reactive-histidine-containing proteins were subjected to LIMMA R package analysis.
3. Chemical procedures and compound characterizations

3.1 General procedure A and characterization for the reaction between Boc-His-OMe and thioacetal 1

![Chemical structure of thioacetal 1 and Boc-His-OMe](image)

To a colorless transparent glass vial charged with an appropriate magnetic stir bar was added thioacetal 1 (108 mg, 0.4 mmol, 4 equiv.), Boc-His-OMe 2 (26.9 mg, 0.1 mmol) and Rose Bengal (10.2 mg, 0.01 mmol 10 mol%), and 20 mL of MeCN/H₂O=4/1 was added as solvent. The vial was then sealed and placed on a magnetic stirrer about 2 cm away from a 10 W blue LED lamp for 1h. After reaction completion, the organic solvents were removed on a rotary evaporator, 10 mL of H₂O was added and the crud mixture was extracted with CH₂Cl₂ (3×10 mL). The organic phase was washed with saturated NaCl solution (2×5 mL) and dried over anhydrous Na₂SO₄. The crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (4:1). 3a (yellow oil, yield 84%): ¹H NMR (300 MHz, Chloroform-d) δ 7.73 – 7.61 (m, 1H), 7.21 – 7.09 (m, 2H), 6.96 (s, 1H), 6.86 – 6.80 (m, 2H), 6.13 (d, J = 1.6 Hz, 1H), 5.87 (t, J = 9.5 Hz, 1H), 4.53 (dt, J = 8.4, 4.8 Hz, 1H), 3.76 (s, 3H), 3.62 (s, 3H), 3.04 (td, J = 15.7, 15.3, 9.1 Hz, 2H), 2.50 – 2.43 (m, 1H), 2.34 (ddd, J = 12.6, 7.4, 2.1 Hz, 1H), 1.58 (ddt, J = 14.2, 7.3, 3.7 Hz, 2H), 1.40 (s, 9H), 0.95 (t, J = 7.4 Hz, 3H). ¹³C NMR (75 MHz, Chloroform-d) δ 172.49, 159.78, 155.53, 136.53, 138.10, 129.97, 127.65, 115.86, 114.14, 79.60, 64.01, 55.30, 53.63, 52.06, 33.92, 30.49, 28.30, 22.17, 13.41. HRMS: (ESI): [M+H]⁺ Calcd. m/z 464.22137, found m/z 464.22250, Error: 2.45ppm.

Characterization of the product 3a

Characterization of the product 3a

In order to determine the reacted nitrogen atoms, we further performed three 2D NMR experiments (¹H-¹H COSY, ¹H-¹³C HSQC and ¹H-¹³C HMBC) for the product 3a. The corresponding full spectra of 3a have been added in the Section 6. NMR spectra. Based on the analysis of 2D spectra and ¹H, ¹³C NMR spectra, the detailed assignments of ¹H and ¹³C NMR signal have been summarized in the following table. To simplify the discussion, we numbered all carbon atoms in product 3a, and different colors were used to distinguish the three main parts of the structure, and the atom 4 is an important position for the determination of the structure.

| No. | ¹H NMR chemical shift | ¹³C NMR chemical shift |
|-----|-----------------------|------------------------|
| 1   |                       |                        |
| 2   |                       |                        |
| 3   |                       |                        |
| 4   |                       |                        |
| 5   |                       |                        |
| 6   |                       |                        |
| 7   |                       |                        |
| 8   |                       |                        |
| 9   |                       |                        |
| 10  |                       |                        |
| 11  |                       |                        |
| 12  |                       |                        |
| 13  |                       |                        |
| 14  |                       |                        |
| 15  |                       |                        |
| 16  |                       |                        |
| 17  |                       |                        |
| 18  |                       |                        |
| 19  |                       |                        |
| 20  |                       |                        |
After the confirmation of the $^1$H and $^{13}$C NMR signal, the signal of the $^1$H nuclear and remotely coupled $^{13}$C nuclear from the $^1$H-$^{13}$C HMBC ($^1$H detected heteronuclear multiple bond correlation) spectrum is important for the determination of the reacted position on imidazole. Two possible product structures and the $^1$H-$^{13}$C HMBC signal of the atom 4 ($^1$H δ 6.1, $^{13}$C δ 64.0) are shown in the following figure. As a remotely coupling NMR experiment, two different coupling signals would provide for the two possible structures. For the N3 reacted position, the atom 4 would couple with atom 10 ($^1$H δ 7.7, $^{13}$C δ 136.5) and 12 ($^1$H δ 6.9, $^{13}$C δ 115.8). For the N1 reacted position, the atom 4 would couple with atom 10 and 11 ($^{13}$C δ 138.1). Thus, we can determine its structure by the key signal from atom 11 or 12. Apparently, the coupling signal on {6.14, 115.77} ($^1$H4→$^{13}$C12), {6.14, 136.70} ($^1$H4→$^{13}$C10), {7.67, 64.01} ($^1$H10→$^{13}$C4) and {6.97, 64.01} ($^1$H12→$^{13}$C4) were found in the following HMBC spectrum. As a result, we confirmed that the reacted position on imidazole for this reaction is the N3 position.
Synthesis of 3b:

(yellow oil, yield 86%) ¹H NMR (400 MHz, Chloroform-d) δ 7.70 (dd, J = 13.0, 1.1 Hz, 1H), 7.10 – 6.96 (m, 3H), 6.85 – 6.74 (m, 2H), 6.12 (d, J = 3.4 Hz, 1H), 5.71 (t, J = 8.1 Hz, 1H), 4.54 (q, J = 5.3 Hz, 1H), 3.61 (d, J = 7.5 Hz, 3H), 3.15 – 2.98 (m, 2H), 2.53 – 2.32 (m, 2H), 1.58 (dt, J = 14.3, 7.3 Hz, 2H), 1.41 (s, 9H), 0.98 (t, J = 7.3 Hz, 3H). ¹³C NMR (101 MHz, Chloroform-d) δ 172.49, 157.74, 155.72, 136.46, 128.77, 127.84, 116.51, 116.06, 80.05, 64.60, 53.75, 52.34, 34.10, 30.64, 28.42, 22.33, 13.56. HRMS: (ESI): [M+H]⁺ Calcd. m/z 450.20572, found m/z 450.20605, Error: 0.75 ppm.

Synthesis of 3c:

(yellow oil, yield 79%) ¹H NMR (500 MHz, Chloroform-d) δ 7.67 (d, J = 3.9 Hz, 1H), 7.22 – 7.11 (m, 2H), 6.97 (s, 1H), 6.94 – 6.89 (m, 2H), 6.17 (d, J = 2.8 Hz, 1H), 5.87 (dd, J = 19.5, 8.4 Hz, 1H), 4.66 (d, J = 2.4 Hz, 2H), 4.55 (dt, J = 8.7, 5.0 Hz, 1H), 3.63 (s, 3H), 3.12 – 2.97 (m, 2H), 2.55 – 2.36 (m,
3H), 1.42 (d, J = 2.0 Hz, 9H), 1.24 – 1.23 (m, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) δ 157.79, 136.57, 130.89, 127.75, 115.87, 115.20, 79.66, 78.17, 75.86, 63.75, 55.87, 52.12, 30.59, 28.39, 26.04, 14.03. HRMS: (ESI): [M+H]$^+$ Calcd. m/z 474.20572, found m/z 474.20609, Error: 0.77 ppm.

Synthesis of 3d:

(yellow oil, yield 81%) $^1$H NMR (500 MHz, Chloroform-d) δ 7.66 (d, J = 6.9 Hz, 1H), 7.14 (dd, J = 8.6, 5.1 Hz, 2H), 6.95 (s, 1H), 6.90 (dd, J = 8.7, 1.7 Hz, 2H), 6.13 (d, J = 3.2 Hz, 1H), 5.86 (dd, J = 19.3, 8.3 Hz, 1H), 4.64 (d, J = 2.4 Hz, 2H), 4.53 (dt, J = 8.8, 5.2 Hz, 1H), 3.61 (s, 3H), 3.08 – 2.96 (m, 2H), 2.54 – 2.27 (m, 3H), 1.55 (dtt, J = 21.8, 11.5, 6.0 Hz, 2H), 1.39 (s, 9H), 0.94 (t, J = 7.3 Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) δ 172.47, 157.76, 155.58, 136.58, 130.98, 115.91, 115.17, 79.64, 78.17, 75.89, 64.03, 55.84, 53.70, 52.11, 33.97, 30.54, 28.37, 22.22, 13.46. HRMS: (ESI): [M+H]$^+$ Calcd. m/z 488.22137, found m/z 488.22284, Error: 3.01 ppm.

Synthesis of 3e:

(yellow oil, yield 62%) $^1$H NMR (500 MHz, Chloroform-d) δ 7.72 – 7.59 (m, 1H), 7.19 – 7.10 (m, 2H), 6.98 – 6.89 (m, 3H), 6.22 – 6.06 (m, 1H), 5.93 – 5.83 (m, 1H), 5.82 – 5.73 (m, 1H), 5.18 (d, J = 10.1 Hz, 1H), 5.12 – 5.03 (m, 2H), 4.66 (t, J = 2.4 Hz, 2H), 4.59 – 4.52 (m, 1H), 3.63 (t, J = 3.4 Hz, 3H), 3.10 (dd, J = 11.3, 7.1 Hz, 2H), 2.61 – 2.53 (m, 2H), 2.51 – 2.49 (m, 1H), 1.42 (s, 9H). $^{13}$C NMR (126 MHz, Chloroform-d) δ 155.60, 136.62, 134.26, 130.75, 127.77, 117.18, 115.84, 115.21, 79.66, 78.15, 75.89, 64.06, 55.86, 53.71, 52.14, 34.76, 30.69, 28.40. HRMS: (ESI): [M+H]$^+$ Calcd. m/z 486.20572, found m/z 486.20593, Error: 0.44 ppm.

Synthesis of 3f:
\[ \text{Boc-Trp-OMe-1a} \] (yellow oil, yield 89%) \textsuperscript{1}H NMR (300 MHz, Chloroform-\text{d}) \( \delta \) 8.93 (d, \( J = 34.6 \) Hz, 1H), 7.56 (d, \( J = 7.5 \) Hz, 1H), 7.50 – 7.30 (m, 3H), 7.22 – 7.05 (m, 2H), 6.94 – 6.76 (m, 2H), 5.54 (d, \( J = 2.2 \) Hz, 1H), 5.24 (dd, \( J = 11.7, 8.1 \) Hz, 1H), 4.66 (q, \( J = 6.0 \) Hz, 1H), 3.75 (s, 3H), 3.58 (s, 3H), 3.30 (dt, \( J = 13.7, 6.4 \) Hz, 2H), 2.42 (th, \( J = 20.1, 7.3, 6.4 \) Hz, 2H), 1.63 (dq, \( J = 14.3, 6.7, 4.8 \) Hz, 2H), 1.44 (d, \( J = 8.8 \) Hz, 9H), 0.99 (td, \( J = 7.3, 2.2 \) Hz, 3H). \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \( \delta \) 172.92, 158.93, 155.31, 135.56, 135.37, 131.62, 129.25, 128.45, 122.09, 119.66, 118.72, 114.18, 111.16, 107.63, 79.80, 55.20, 54.25, 52.27, 44.72, 34.43, 28.29, 27.34, 22.57, 13.58.

3.2 General procedure B for the synthesis of thioacetals and thioketals

\[
\begin{align*}
\text{R}_1\text{R}_2\text{O} & \quad \text{R}_3\text{SH} \\
\text{NBS} & \quad \text{R}_1\text{R}_2\text{S}S\text{R}_3
\end{align*}
\]

To a round-bottomed flask was charged with mercaptan (4 equiv.) and aldehydes/ketones (1 equiv.), and CH\textsubscript{2}Cl\textsubscript{2} was added to dissolve the starting materials. A catalytic amount of N-Bromosuccinimide (NBS, 5 mol\%) was added to the mixture. The solution was allowed to stir for 2 h at room temperature. After reaction completion, the organic phase was washed with saturated NaCl solution for two times and dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and concentrated on a rotary evaporator. The crude product was purified by flash column chromatography using eluent solution Hexane/EtOAc or CH\textsubscript{2}Cl\textsubscript{2}/MeOH.

Synthesis of 1 followed General Procedure B using 4-methoxybenzaldehyde and propane-1-thiol as substrates:
(colorless oil, yield 95%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.47 – 7.30 (m, 2H), 6.96 – 6.78 (m, 2H), 4.87 (s, 1H), 3.79 (s, 3H), 2.52 (qt, $J$ = 12.5, 7.3 Hz, 4H), 1.60 (dt, $J$ = 14.6, 7.3 Hz, 4H), 0.96 (t, $J$ = 7.3 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 159.05, 132.61, 128.83, 113.78, 55.24, 52.47, 34.29, 22.57, 13.55.

Synthesis of TA3 followed General Procedure B using 4-(prop-2-yn-1-yloxy)benzaldehyde S15 and ethanethiol as substrates:

(light yellow oil, yield 93%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.47 – 7.30 (m, 2H), 7.02 – 6.82 (m, 2H), 4.90 (s, 1H), 4.66 (d, $J$ = 2.3 Hz, 2H), 2.71 – 2.37 (m, 5H), 1.20 (t, $J$ = 7.4 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 157.05, 133.43, 128.85, 114.78, 78.49, 75.67, 55.84, 51.75, 26.20, 14.30.

Synthesis of TA4 followed General Procedure B using S15 and propane-1-thiol as substrates:

(light yellow oil, yield 91%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.35 (d, $J$ = 8.7 Hz, 2H), 6.93 – 6.83 (m, 2H), 4.84 (s, 1H), 4.61 (d, $J$ = 2.4 Hz, 2H), 2.60 – 2.36 (m, 5H), 1.53 (h, $J$ = 7.1 Hz, 4H), 0.91 (t, $J$ = 7.3 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 157.01, 133.59, 128.86, 114.72, 78.58, 75.76, 55.81, 52.48, 34.25, 22.55, 13.58.

Synthesis of TA5 followed General Procedure B using S15 and prop-2-ene-1-thiol as substrates:

(light yellow oil, yield 82%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.42 – 7.30 (m, 2H), 6.99 – 6.85 (m, 2H), 5.85 – 5.72 (m, 2H), 5.13 – 5.06 (m, 4H), 4.74 (s, 1H), 4.67 (d, $J$ = 2.4 Hz, 2H), 3.25 (dd, $J$ = 13.7, 7.1 Hz, 2H), 3.03 (dd, $J$ = 13.7, 7.2 Hz, 2H), 2.53 (dt, $J$ = 4.5, 2.2 Hz, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 157.13, 133.81, 132.77, 129.23, 117.57, 114.85, 78.46, 75.68, 55.84, 49.68, 35.24.
Synthesis of TA6 followed General Procedure B using S15 and 2-mercaptoethan-1-ol as substrates:

(white powder, yield 90%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.34 (d, $J = 8.7$ Hz, 2H), 6.89 (d, $J = 8.7$ Hz, 2H), 5.03 (s, 1H), 4.63 (d, $J = 2.3$ Hz, 2H), 3.66 (t, $J = 6.0$ Hz, 4H), 3.43 (s, 2H), 2.82 – 2.69 (m, 2H), 2.67 – 2.56 (m, 2H), 2.54 (t, $J = 2.3$ Hz, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 157.23, 132.92, 128.93, 114.97, 78.43, 75.98, 61.34, 55.88, 52.45, 35.10.

Synthesis of TA7 followed General Procedure B using 4-formyl-N-(prop-2-yn-1-yl)benzamide and propane-1-thiol as substrates. The preparation of 4-formyl-N-(prop-2-yn-1-yl)benzamide was followed the same procedure as described in previous literature.$^9$

(yellow oil, yield 88%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.75 (d, $J = 8.3$ Hz, 2H), 7.46 (d, $J = 8.2$ Hz, 2H), 6.91 (t, $J = 4.8$ Hz, 1H), 4.84 (s, 1H), 4.19 (dd, $J = 5.3, 2.5$ Hz, 2H), 2.55 – 2.33 (m, 4H), 2.23 (t, $J = 2.5$ Hz, 1H), 1.53 (h, $J = 7.2$ Hz, 4H), 0.90 (t, $J = 7.4$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.84, 144.64, 133.06, 127.91, 127.43, 79.59, 71.65, 52.57, 34.28, 29.67, 22.46, 13.47.

Synthesis of S1 followed General Procedure B using 4-methoxybenzaldehyde and ethane-1,2-dithiol as substrates.

(white powder, yield 94%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.56 – 7.35 (m, 2H), 6.97 – 6.73 (m, 2H), 5.63 (s, 1H), 3.79 (s, 3H), 3.50 (ddd, $J = 7.0, 5.9, 3.9$ Hz, 2H), 3.45 – 3.25 (m, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 159.36, 131.79, 129.15, 113.85, 56.04, 55.33, 40.22.

Synthesis of S2 followed General Procedure B using S15 and ethane-1,2-dithiol as substrates.
(white powder, yield 92%) \(^1\)H NMR (300 MHz, Chloroform-\(d\)) \(\delta\) 7.52 – 7.41 (m, 2H), 6.97 – 6.86 (m, 2H), 5.62 (s, 1H), 4.65 (d, \(J = 2.4\) Hz, 2H), 3.50 – 3.36 (m, 2H), 3.36 – 3.21 (m, 2H), 2.57 (t, \(J = 2.4\) Hz, 1H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 157.26, 133.03, 129.27, 114.84, 78.70, 75.96, 55.96, 55.88, 40.31.

Synthesis of \(\text{S3}\) followed General Procedure B using \(\text{S15}\) and propane-1,3-dithiol as substrates.

(yellow oil, yield 49%) \(^1\)H NMR (300 MHz, Chloroform-\(d\)) \(\delta\) 7.48 – 7.36 (m, 2H), 7.02 – 6.91 (m, 2H), 6.00 (s, 1H), 4.51 (ddd, \(J = 9.2, 6.4, 2.9\) Hz, 1H), 3.91 (td, \(J = 9.0, 6.2\) Hz, 1H), 3.34 – 3.09 (m, 2H), 2.53 (t, \(J = 2.4\) Hz, 1H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 157.80, 132.00, 128.96, 115.02, 78.43, 75.69, 71.79, 55.81, 34.08.

Synthesis of \(\text{S4}\) followed General Procedure B using \(\text{S15}\) and dropwise addition of 1 equivalent of 2-mercaptoethanol as substrates.
(white powder, yield 69%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.50 – 7.38 (m, 2H), 7.01 – 6.88 (m, 2H), 5.50 (s, 1H), 4.67 (d, $J = 2.4$ Hz, 2H), 3.70 – 3.55 (m, 1H), 3.20 – 2.99 (m, 3H), 2.51 (t, $J = 2.4$ Hz, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 157.36, 132.86, 128.52, 114.84, 78.43, 75.63, 72.92, 55.82, 52.75, 36.53.

Synthesis of S6 was followed General Procedure B using 1-(4-methoxyphenyl)ethan-1-one and propane-1-thiol as substrates.

\[
\begin{aligned}
&\text{O} \\
&\text{S6}
\end{aligned}
\]

(light yellow oil, yield 63%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.74 – 7.55 (m, 2H), 6.96 – 6.80 (m, 2H), 3.81 (s, 3H), 2.49 (t, $J = 7.4$ Hz, 4H), 2.02 (s, 3H), 1.54 (h, $J = 7.4$ Hz, 4H), 0.95 (t, $J = 7.4$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 158.47, 136.02, 128.24, 113.28, 59.81, 55.21, 32.90, 30.33, 22.37, 13.84.

Synthesis of S7 was followed General Procedure B using 1-(4-(prop-2-yn-1-yloxy)phenyl)ethan-1-one S16 and propane-1-thiol as substrates.

\[
\begin{aligned}
&\text{S7}
\end{aligned}
\]

(light yellow oil, yield 61%) $^1$H NMR (300 MHz, Chloroform-$d$) $\delta$ 7.78 – 7.49 (m, 2H), 7.05 – 6.79 (m, 2H), 4.67 (d, $J = 2.4$ Hz, 2H), 2.46 (t, $J = 7.4$ Hz, 4H), 2.18 (s, 1H), 1.98 (s, 3H), 1.51 (q, $J = 7.3$ Hz, 4H), 0.93 (t, $J = 7.4$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 156.46, 136.99, 128.26, 114.21, 78.49, 75.57, 59.77, 55.78, 32.88, 30.29, 22.33, 13.83.

### 3.3 Synthesis of TA8 and TA9

\[
\begin{aligned}
&\text{HO-}\text{O-}\text{O-}\text{O-}\text{OH} \\
&\text{Br} \quad \text{NaOH} \\
&\text{a) TsCl} \\
&\text{b) HO-}\text{CHO} \\
&\text{R'\text{-SH}} \\
&\text{NBS}
\end{aligned}
\]
To a round-bottomed flask was added 2,2’-(ethane-1,2-diylbis(oxy))bis(ethan-1-ol) (1.5 g, 10 mmol) dissolved in 50 mL THF, and cooled in an ice bath. NaOH (0.8 g, 20 mmol) was added in one portion, and 3-bromoprop-1-yne (1.18 g, 10 mmol) was added dropwise after 1h. The solution was allowed to warm to room temperature and stirred for 16 h. After reaction completion, the solvent was removed, and then 20 mL H<sub>2</sub>O was added. The aqueous solution was neutralized with 1 M dilute HCl, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×20 mL). The organic phase was washed with saturated NaCl solution (2×10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified by flash column chromatography using eluent solution Hexane/EtOAc (1:1). The purified intermediate was used directly for next step. The intermediate 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-ol was dissolved with 4-methylbenzenesulfonyl chloride (TsCl, 1.9 g, 10 mmol) and Et<sub>3</sub>N (1.01g , 10 mmol) in 50 mL CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed to stir for 2 h. 4-hydroxybenzaldehyde ( 1.22 g, 10 mmol) and addition Et<sub>3</sub>N (1.01g , 10 mmol) were added in one portion, and the reaction was continued for 16 h. After reaction completion, the solvent was removed, and then 50 mL H<sub>2</sub>O was added. The crude mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×20 mL). The organic phase was washed with saturated NaCl solution (2×10 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified by flash column chromatography using eluent solution Hexane/EtOAc (2:1).

**S9** (colorless oil, yield 64% for two steps): ¹H NMR (500 MHz, Chloroform-d) δ 9.83 (s, 1H), 7.83 – 7.73 (m, 2H), 7.04 – 6.92 (m, 2H), 4.17 (dd, J = 5.4, 4.3 Hz, 2H), 4.15 (d, J = 2.4 Hz, 2H), 3.84 (dd, J = 5.4, 4.1 Hz, 2H), 3.71 – 3.67 (m, 2H), 3.64 (tdd, J = 4.3, 2.7, 0.9 Hz, 6H), 2.40 (t, J = 2.4 Hz, 1H).

¹³C NMR (126 MHz, Chloroform-d) δ 190.82, 163.87, 131.96, 130.05, 114.91, 79.66, 74.64, 70.89, 70.66, 70.47, 69.48, 69.11, 67.79, 58.41.

The synthesis of TA8 and TA9 were followed General Procedure B using S9 and propane-1-thiol/2-mercaptoethan-1-ol as substrates.

**TA8** (colorless oil, yield 91%): ¹H NMR (500 MHz, Chloroform-d) δ 7.38 – 7.29 (m, 2H), 6.91 – 6.78 (m, 2H), 4.81 (s, 1H), 4.17 (d, J = 2.5 Hz, 2H), 4.11 – 4.07 (m, 2H), 3.88 – 3.76 (m, 2H), 3.70 (ddt, J = 6.5, 3.6, 2.0 Hz, 2H), 3.67 – 3.65 (m, 6H), 2.54 – 2.41 (m, 4H), 2.40 (d, J = 2.4 Hz, 1H), 1.54 (pd, J = 7.7, 1.0 Hz, 4H), 0.91 (t, J = 7.3 Hz, 6H).

¹³C NMR (126 MHz, Chloroform-d) δ 158.32, 158.0, 132.85, 132.85, 114.53, 79.69, 74.59, 70.85, 70.69, 70.48, 69.74, 69.13, 67.47, 58.43, 52.51, 34.33, 22.60, 13.58.

**TA9** (colorless oil, yield 83%): ¹H NMR (400 MHz, Chloroform-d) δ 7.34 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.04 (s, 1H), 4.22 – 4.13 (m, 2H), 4.08 (d, J = 4.4 Hz, 2H), 3.88 – 3.76 (m, 2H), 3.67 (q, J = 5.4 Hz, 10H), 3.19 (t, J = 38.8 Hz, 2H), 2.78 (dq, J = 12.0, 6.8, 5.7 Hz, 2H), 2.63 (dt, J = 13.1, 5.8 Hz, 2H), 2.46 (s, 1H).

¹³C NMR (101 MHz, Chloroform-d) δ 158.52, 132.26, 128.94, 114.72, 79.67, 74.84, 70.75, 70.60, 70.39, 69.67, 69.08, 67.47, 61.38, 58.40, 52.54, 35.21.

### 3.4 Synthesis of TA1 and TA10

![Chemical structure of TA1 and TA10](image)
4-Dimethylaminopyridine (DMAP, 48 mg, 0.4 mmol) was added to D-Biotin solution (488 mg, 2 mmol) in 100 mL CH₂Cl₂, followed by dicyclohexylcarbodiimide (DCC, 496 mg, 2.4 mmol). After stirring for 1 h, a solution of 4-hydroxybenzaldehyde (268 mg, 2.2 mmol) in CH₂Cl₂ (20 mL) was added and stirred at room temperature for 16 h. The crystals were filtered through celite and washed on the filter with CH₂Cl₂ (100 mL). The combined solution was washed successively with saturated Na₂CO₃(aq) and brine, then dried over Na₂SO₄. The solvent was then removed in vacuum to afford the crude product, which was purified by silica gel chromatography with CH₂Cl₂/CH₃OH (40:1) to furnish the product.

**S10** (white solid, yield 72%):

1H NMR (300 MHz, DMSO- d₆) δ 9.94 (s, 1H), 8.14 – 7.83 (m, 2H), 7.32 (d, J = 8.5 Hz, 2H), 4.32 (dd, J = 7.7, 4.6 Hz, 1H), 4.17 – 4.13 (m, 1H), 3.17 – 3.06 (m, 1H), 2.80 (dd, J = 12.5, 4.9 Hz, 1H), 2.64 – 2.54 (m, 3H), 1.69 – 1.58 (m, 3H), 1.52 – 1.36 (m, 3H).

13C NMR (75 MHz, DMSO) δ 192.66, 171.98, 163.50, 155.50, 134.19, 131.57, 123.18, 61.59, 59.75, 55.75, 40.22, 33.68, 28.34, 24.60.

The synthesis of **TA1** and **TA10** were followed General Procedure B using **S10** and propane-1-thiol/2-mercaptoethan-1-ol as substrates.

**TA1** (white solid, yield 86%):

1H NMR (300 MHz, Methanol- d₄) δ 7.52 – 7.42 (m, 2H), 7.10 – 7.01 (m, 2H), 4.98 (s, 1H), 4.48 (dd, J = 7.8, 4.3 Hz, 1H), 4.30 (dd, J = 7.9, 4.5 Hz, 1H), 3.27 – 3.15 (m, 1H), 3.03 – 2.76 (m, 2H), 2.64 – 2.43 (m, 6H), 1.77 (ddd, J = 15.3, 7.4, 4.6 Hz, 3H), 1.70 – 1.52 (m, 7H), 0.94 (t, J = 7.4 Hz, 6H).

13C NMR (75 MHz, MeOD) δ 180.18, 172.35, 164.68, 150.12, 138.71, 128.56, 121.31, 61.98, 60.21, 55.57, 51.91, 39.72, 33.93, 33.42, 29.21, 28.30, 24.46, 22.23, 12.46.

**TA10** (white solid, yield 81%):

1H NMR (300 MHz, Methanol- d₄) δ 7.51 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.6 Hz, 2H), 4.45 (dd, J = 7.7, 4.8 Hz, 1H), 4.27 (dd, J = 7.8, 4.4 Hz, 1H), 3.77 – 3.57 (m, 5H), 3.20 (dt, J = 9.5, 5.3 Hz, 1H), 2.90 (dd, J = 12.8, 4.8 Hz, 1H), 2.70 (ddt, J = 35.0, 14.5, 7.3 Hz, 7H), 1.81 – 1.45 (m, 6H).

13C NMR (75 MHz, MeOD) δ 172.45, 164.67, 150.26, 138.45, 128.74, 121.55, 62.01, 61.07, 60.23, 55.63, 52.36, 39.86, 34.37, 33.53, 28.37, 24.52.

**3.5 Synthesis of TA2 and TA11**
The synthesis of S11 was followed General Procedure B using 4-formylbenzoic acid and propane-1-thiol as substrates.

**S11** (white solid, yield 89%): \(^1\)H NMR (400 MHz, Chloroform-d) \(\delta\) 8.10 (d, \(J = 8.3\) Hz, 2H), 7.57 (d, \(J = 8.3\) Hz, 2H), 4.92 (s, 1H), 2.71 – 2.40 (m, 4H), 1.60 (h, \(J = 7.2\) Hz, 4H), 0.97 (t, \(J = 7.3\) Hz, 6H). \(^{13}\)C NMR (101 MHz, Chloroform-d) \(\delta\) 171.83, 147.12, 130.66, 128.77, 128.01, 52.90, 34.46, 22.64, 13.62.

DMAP (0.12 g, 1 mmol) was added to S11 (1.42 g, 5 mmol) in 150 mL CH\(_2\)Cl\(_2\), followed by 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 1.92 g, 10 mmol). After stirring for 10 min, 1-hydroxypyrrolidine-2,5-dione (NHS, 0.67 g, 5.5 mmol) was added and stirred at room temperature for 16 h. The solution was washed successively with brine for 3 times, then dried over Na\(_2\)SO\(_4\). The solvent was then removed in vacuum to afford the crude product, which was recrystal by Hexane/EtOAc to purify the product.
S12 (white solid, yield 82%): ¹H NMR (400 MHz, Chloroform-d) δ 8.01 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.3 Hz, 2H), 4.84 (s, 1H), 2.78 (s, 4H), 2.52 – 2.32 (m, 4H), 1.45 (hept, J = 6.8 Hz, 4H), 0.84 (t, J = 7.4 Hz, 6H). ¹³C NMR (101 MHz, Chloroform-d) δ 169.59, 161.57, 148.53, 130.87, 128.40, 124.38, 52.57, 34.35, 25.79, 22.55, 13.54.

To a round-bottomed flask was added 2,2'-(ethane-1,2-diylbis(oxy))bis(ethan-1-amine) (1.78 g, 12 mmol) and Et₃N (0.41 g, 4 mmol) dissolved in 100 mL MeCN. S12 (1.53 g, 4 mmol) in 20 mL MeCN solution was added dropwise. The solution was allowed to stir for 16 h. After reaction completion, the solvent was removed, and then 50 mL H₂O was added. The aqueous solution was extracted with CH₂Cl₂ (3×50 mL). The organic phase was washed with saturated NaCl solution (2×50 mL) and dried over anhydrous Na₂SO₄. The crude product was purified by flash column chromatography using eluent solution CH₂Cl₂/MeOH (30:1).

S13 (colorless oil, yield 61%): ¹H NMR (500 MHz, Chloroform-d) δ 7.76 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.18 (s, 1H), 4.84 (s, 1H), 3.68 – 3.53 (m, 8H), 3.46 (t, J = 5.1 Hz, 2H), 2.80 (t, J = 5.0 Hz, 2H), 2.57 – 2.38 (m, 4H), 2.17 (s, 2H), 1.59 – 1.46 (m, 4H), 0.90 (t, J = 7.3 Hz, 6H). ¹³C NMR (126 MHz, Chloroform-d) δ 169.17, 144.24, 133.99, 127.83, 127.43, 72.85, 70.27, 70.10, 69.89, 52.67, 41.49, 39.80, 34.34, 22.52, 13.52.

Synthesis of NHS-Biotin S14 was followed the same procedure as described by previous literature.[10]

S14 (white solid, yield 91%): ¹H NMR (300 MHz, DMSO-d₆) δ 6.42 (s, 1H), 6.36 (s, 1H), 4.34 – 4.24 (m, 1H), 4.19 – 4.07 (m, 1H), 3.13 – 3.05 (m, 1H), 2.89 – 2.80 (m, 1H), 2.79 (s, 4H), 2.70 (d, J = 3.1 Hz, 1H), 2.65 (t, J = 7.4 Hz, 2H), 1.73 – 1.44 (m, 4H), 1.44 – 1.30 (m, 2H).

¹³C NMR (75 MHz, DMSO) δ 170.73, 169.39, 163.15, 61.43, 59.61, 55.67, 30.42, 28.26, 28.02, 25.87, 25.65, 24.74.

To a round-bottomed flask was added S13 (0.41 g, 1 mmol), NHS-Biotin S14 (0.34 g, 1 mmol) and Et₃N (0.1 g, 1 mmol) dissolved in 50 mL MeCN. The solution was allowed to stir for 16 h. After reaction completion, the solvent was removed, and then 50 mL H₂O was added. The aqueous solution was extracted with CH₂Cl₂ (3×50 mL). The organic phase was washed with saturated NaCl solution (2×50 mL) and dried over anhydrous Na₂SO₄. The crude product was purified by flash column chromatography using eluent solution CH₂Cl₂/MeOH (40:1).

TA2 (colorless oil, yield 89%): ¹H NMR (500 MHz, Chloroform-d) δ 7.77 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 4.8 Hz, 1H), 6.81 (t, J = 5.1 Hz, 1H), 6.72 (s, 1H), 5.99 (s, 1H), 4.84 (s, 1H), 4.47 – 4.38 (m, 1H), 4.28 – 4.18 (m, 1H), 3.68 – 3.53 (m, 8H), 3.49 (dt, J = 10.9, 5.3 Hz, 2H), 3.36 (dtt, J = 14.3, 8.8, 4.1 Hz, 2H), 3.07 (q, J = 7.3 Hz, 1H), 2.82 (dd, J = 12.8, 4.7 Hz, 1H), 2.72 – 2.61 (m, 2H), 2.55 – 2.40 (m, 4H), 2.15 (t, J = 7.6 Hz, 2H), 1.72 – 1.48 (m, 8H), 1.36 (p, J = 7.3 Hz, 2H), 0.91 (t, J = 7.3 Hz, 6H). ¹³C NMR (126 MHz, Chloroform-d) δ 173.63, 167.27, 164.47, 144.37, 133.82, 127.86, 127.47, 70.17, 70.05, 69.89, 61.87, 60.31, 55.73, 52.72, 40.48, 39.89, 39.12, 35.95, 34.38, 28.29, 28.08, 25.64, 25.49, 22.54, 13.54. HRMS: (ESI): [M+H]⁺ Calcd. m/z 641.28596, found m/z 641.28638, Error: 0.65 ppm.

DMAP (0.048 g, 0.4 mmol) was added to Rhodamine B (0.96 g, 2 mmol) in 150 mL CH₂Cl₂, followed by 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.77 g, 4 mmol). After
stirring for 10 min, 1-hydroxyypyrrrolidine-2,5-dione (NHS, 0.27 g, 2.2 mmol) was added and stirred at room temperature for 16 h. The solution was washed successively with brine for 3 times, then dried over Na₂SO₄. The solvent was then removed in vacuum to afford the crude product, which was directly used for next step.

To a round-bottomed flask was added S13 (0.41 g, 1 mmol), the crude intermediate NHS-Rhodamine B and Et₃N (0.1 g, 1 mmol) dissolved in 50 mL MeCN. The solution was allowed to stir for 16 h. After reaction completion, the solvent was removed, and then 50 mL H₂O was added. The aqueous solution was extracted with CH₂Cl₂ (3×50 mL). The organic phase was washed with saturated NaCl solution (2×50 mL) and dried over anhydrous Na₂SO₄. The crude product was purified by flash column chromatography using eluent solution CH₂Cl₂/MeOH (40:1).

TA11 (red oil, yield 51% for two steps): ¹H NMR (500 MHz, Chloroform-d) δ 7.88 – 7.83 (m, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.42 – 7.38 (m, 2H), 7.23 (t, J = 4.9 Hz, 1H), 7.06 – 7.01 (m, 1H), 6.49 – 6.39 (m, 4H), 6.29 (dd, J = 8.9, 2.4 Hz, 2H), 4.84 (s, 1H), 3.57 (p, J = 4.7 Hz, 4H), 3.46 (dd, J = 5.5, 3.5 Hz, 2H), 3.37 – 3.27 (m, 12H), 3.13 (t, J = 7.0 Hz, 2H), 2.52 – 2.42 (m, 4H), 1.55 – 1.50 (m, 4H), 1.12 (t, J = 7.1 Hz, 12H), 0.92 – 0.89 (m, 6H). ¹³C NMR (126 MHz, Chloroform-d) δ 168.44, 167.11, 153.61, 153.18, 148.05, 144.07, 133.98, 132.59, 130.83, 128.99, 128.20, 127.93, 127.86, 127.51, 127.35, 123.80, 122.83, 108.85, 98.96, 70.33, 69.99, 67.93, 64.85, 52.70, 45.08, 39.91, 39.44, 34.31, 22.54, 13.53, 12.44. HRMS: (ESI): [M]+ Calcd. m/z 839.42344, found m/z 839.42407, Error: 0.75 ppm.

3.6 Synthesis of tert-butyl ((ethylthio)(4-methoxyphenyl)methyl)carbamate S8

S8 white solid, yield 62% ¹H NMR (500 MHz, Chloroform-d) δ 7.30 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 8.7 Hz, 2H), 5.95 (d, J = 9.5 Hz, 1H), 5.26 (s, 1H), 3.75 (s, 3H), 2.77 – 2.47 (m, 2H), 1.42 (s, 9H), 1.29 (t, J = 7.3 Hz, 3H). ¹³C NMR (126 MHz, Chloroform-d) δ 159.27, 154.69, 132.15, 127.55, 127.30, 122.83, 108.85, 98.96, 70.33, 69.99, 67.93, 64.85, 52.70, 45.08, 39.91, 39.44, 34.31, 22.54, 13.53, 12.44. HRMS: (ESI) [M]+ Calcd. m/z 839.42344, found m/z 839.42407, Error: 0.75 ppm.

3.7 Synthesis of intermediates

Synthesis of intermediate 4-(prop-2-yn-1-yloxy)benzaldehyde S15

4-hydroxybenzaldehyde (12.2g, 100 mmol) and 3-bromoprop-1-yne (11.8g, 100 mmol) were dissolved in 300 mL EtOH. Anhydrous K₂CO₃ (13.8g, 100 mmol) was added in one portion. The mixture was allowed to heated to reflux in oil bath for 6 h. After reaction completion, the solvent
was removed, and then 500 mL H₂O was added. The aqueous solution was extracted with EtOAc (3×100 mL). The organic phase was washed with 0.1 M HCl (2×100 mL) and saturated NaCl solution (2×100 mL) and dried over anhydrous Na₂SO₄. The solvent was then removed in vacuum to afford the crude product, which was recrystallized by Hexane/EtOAc to purify the product.

S15 (white solid, yield 87%): ^1^H NMR (300 MHz, Chloroform-d) δ 9.84 (s, 1H), 7.95 – 7.67 (m, 2H), 7.16 – 6.94 (m, 2H), 4.73 (d, J = 2.4 Hz, 2H), 2.57 (t, J = 2.4 Hz, 1H). ^1^C NMR (75 MHz, CDCl₃) δ 190.85, 162.34, 131.87, 130.48, 115.14, 77.55, 76.44, 55.91.

Synthesis of intermediate 1-(4-(prop-2-yn-1-yloxy)phenyl)ethan-1-one S16

![Chemical structure of S16](image)

The preparation of S16 was followed the same procedure of the synthesis of S15 using 1-(4-hydroxyphenyl)ethan-1-one and 3-bromoprop-1-yne as substrates.

S16 (white solid, yield 88%): ^1^H NMR (300 MHz, Chloroform-d) δ 8.00 – 7.82 (m, 2H), 7.06 – 6.90 (m, 2H), 4.73 (d, J = 2.4 Hz, 2H), 2.55 (t, J = 2.4 Hz, 1H), 2.53 (s, 3H). ^1^C NMR (75 MHz, CDCl₃) δ 196.80, 161.25, 130.95, 130.49, 114.53, 77.73, 76.19, 55.80, 26.34.

Synthesis of thioacetal 4-(bis(propylthio)methyl)phenol S17

![Chemical structure of S17](image)

Synthesis of S17 was followed General Procedure B using 4-hydroxybenzaldehyde and propane-1-thiol as substrates. (colorless oil, yield 93%) ^1^H NMR (400 MHz, Chloroform-d) δ 7.36 – 7.24 (m, 2H), 6.84 – 6.74 (m, 2H), 4.86 (s, 1H), 2.61 – 2.42 (m, 4H), 1.57 (h, J = 7.2 Hz, 4H), 0.94 (t, J = 7.3 Hz, 6H). ^1^C NMR (101 MHz, Chloroform-d) δ 155.41, 132.58, 129.19, 115.61, 52.63, 34.48, 22.68, 13.68.

Synthesis of S17 was followed General Procedure B using pentanal and propane-1-thiol as substrates.

![Chemical structure of S18](image)

(colorless oil, yield 94%) ^1^H NMR (400 MHz, Chloroform-d) δ 3.73 (t, J = 7.0 Hz, 1H), 2.70 – 2.49 (m, 4H), 1.82 – 1.74 (m, 2H), 1.61 (qd, J = 7.4, 3.2 Hz, 4H), 1.55 – 1.46 (m, 2H), 1.35 – 1.29 (m, 2H),
1.00 (t, $J = 7.3$ Hz, 6H), 0.91 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 52.07, 36.04, 32.23, 29.81, 22.94, 22.40, 14.07, 13.78.
4. Supplementary material and methods for the reactions of peptide

4.1 General information

All chemical reagents are commercially available without purification. **High-Resolution Mass Spectrometry (HRMS) and MS/MS Spectrometry** were measured on a *Q* _Exactive_ _Focus_.

**General procedure for SPPS**: Peptide 7 and 8 were synthesized on Rink Amide MBHA resin (peptide 7) or Fmoc-Wang resin (peptide 8) by Fmoc solid-phase synthesis (SPPS). Rink-amide resin was pre-swelled with DCM for 30min, filtered, the Fmoc (9-fluorenylmethyloxycarbonyl) group was removed with 50% (vol/vol) morpholine for 30min*2; the resin was sequentially washed with DCM and DMF for three times. Fmoc-protected amino acids (2.0 equiv.) and HATU (2.0 equiv.) were dissolved in DMF, followed by DIPEA (3.0 equiv.). The mixture was pre-activated for 1min and added to the resin for 1 h with N₂ bubbling, repeated once. The resin was washed sequentially with DCM, DMF for three times, then dried under a stream of nitrogen for next step. For cleavage of resin, the final resin was treated with TFA/TIS/water (95:2.5:2.5) at room temperature for 3 h and concentrated under a stream of nitrogen. The crude peptides were precipitated and washed with cold hexane/diethyl ether (1:2, v/v) at 4°C, redissolved in 50% acetonitrile in water. Crude peptides were purified by preparative HPLC.

**Acetamidation of peptide 8**: A solution of un-protected peptide 8 (5.0 mg, 2 mM, PBS buffer 7.4 with 50% MeCN) was added iodoacetamide (IAM, 20 mM), and the resulted mixture was shaken in an incubator shaker (37 °C) for 1 hours. The crude reaction mixture was directly purified by preparative HPLC after filtration. 4.0 mg (78%) white powder was obtained as target product.

4.2 General procedure C for the reaction between peptide and thioacetal

A 10 mM MeCN/H₂O (1/1) stock solution of RB and a 200 mM MeCN stock solution of thioacetal was made up. These stock solutions were stored at room temperature away from light. To a 2 mL vial was added 100 µL solution of specific peptide (1 mM, in MeCN/H₂O=4/1) solvent and 2 µL RB (10 mM), 10 µL specific thioacetal (200 mM) stock solution. The vial was then caped and equipped with magnetic bar. The reaction was set in a reaction chamber equipped with magnetic stirrer, 450 nm LED lamp (10 W) and exhaust fan to maintain the reaction temperature at about 30 °C for 1 hour, as shown in the following figure. The resulting solution was then analyzed with an internal standard (dibenzyl sulfoxide, 0.2 mM) via HPLC-MS after filtration. The distillates of target products were collected and further analyzed by HRMS and MS/MS spectrometry. Desired distillates of preparative LC were identified by MS and lyophilized to obtain target products, and analyzed by NMR. Liquid nitrogen was used to quickly freeze distillates and lyophilize them as quickly as possible using a lyophilizer.
LC-MS yields were estimated by UV absorption at 220 nm of the peak corresponding to the thioacetal adducted product versus the internal standard (dibenzyl sulfoxide): % yield = \( \frac{A_p}{A_{st}} \times k \). \( A_p \) is the peak area of thioacetal-adducted products; \( A_{st} \) is the peak area of the internal standard; \( k \) is the quantity coefficient between specific peptide and standard. The quantity coefficients were measured by the correlation of a gradient concentration. The isolated yield of 4a was obtained by a 10 mg level reaction, and isolated by a preparative LC. Note that the lyophilization of desired distillates need to be quick due to the potential hydrolyzation of thioacetal adducted products in weak acidic solution.

**General method for LC/MS analysis**

Analytical LC-MS were performed on a Shimadzu LC-MS 8030 system equipped with Kromasil 100-5-C18 column (4.6 × 250 mm, 5 µm; room temperature). Water (containing 0.1% TFA, A phase) and pure CH\(_3\)CN (B phase) were used as solvents in linear gradient mixtures at a flow rate of 1 mL·min\(^{-1}\).

**General method for preparative LC**

Preparative LC were performed on a Shimadzu LC-6AD system equipped with Shimadzu Shim-pack GIST C18 column (20 × 250 mm, 5 µm; room temperature). Water (containing 0.1% TFA) and pure CH\(_3\)CN were used as solvents in linear gradient mixtures at a flow rate of 8 mL·min\(^{-1}\). Due to the potential hydrolyzation of thioacetal addeduct products in weak acidic solution (Figure S6), we tried a mobile phase of pure water to separate the products, and found that 0.1% TFA in water is essential. Thus, we tried quick freezing samples using liquid nitrogen and lyophilizing.

**General method for MS/MS analysis**

The fragmentation of modally modified peptides was investigated in positive electrospray ionization mode, loaded onto a Thermo Q Exactive Focus Orbitrap LC-MS/MS system. The protonated molecule was generated by spraying a 0.5 ng/µl solution in 20:80 water:methanol + 0.1% formic acid (FA) with a flow rate of 0.28 mL/min. Parameters are as follows in Full MS/ data dependent-MS2 TopN mode: mass analyzer over \( m/z \) range of 145–2175 with a mass resolution of 70,000 (at \( m/z = 200 \)) in a data-dependent mode. MS/MS spectra were obtained using collision energy values at 25% normalized activation energy with a HCD (High Energy Collision Dissociation) mode.
The reaction was followed General Procedure C using peptide 4 (100 μL 1 mM solution, sequence: NH-PyrHWSYLLR-NHEt) and thioacetal 1a, 1c, 1d and 1e. A stock solution of peptide 4 (1mM) was made up by 2 mL solvent (MeCN/H₂O = 4/1) and 2.4 mg peptide 4.

Before the reaction, a quantity relationship of matter between peptide 4 and the internal standard (dibenzyl sulfoxide) was established by a gradient concentration. The volume of the reaction mixture was adjusted to 500 μL with MeCN/H₂O (1/1), and then 2 μL dibenzyl sulfoxide stock solution (50 mM in MeCN/H₂O = 1/1) was added. The resulted solution was filtrated and analyzed with LC-MS.

A 10 mg level reaction between 1a and peptide 4 was followed the similar procedure. 10.0 mg of peptide 4 was dissolved in the solvent (5 mL MeCN/H₂O = 4/1) in a 20 mL vial. 45 mg thioacetal 1a and 1.1 mg RB was added to the mixture. The vial was then caped and equipped with magnetic bar. The reaction was set in a reaction chamber equipped with magnetic stirrer, 450 nm LED lamp (10 W) and exhaust fan to maintain the reaction temperature at about 30 °C for 1 hour. The resulting solution was then purified via a preparative LC after filtration. (10-80% B phase over 20 min, 8 mL·min⁻¹ flow rate, 0.01% TFA, λ = 220 nm, Shimadzu Shim-pack GIST C18 20 × 250 mm, 5 µm column). Desired distillates of preparative LC were identified by MS and lyophilized to obtain target products, and analyzed by NMR. Liquid nitrogen was used to quickly freeze distillates and lyophilize them as quickly as possible using a lyophilizer. 6.8 mg (59%) white powder was obtained as target product.

Characterization data of 4a

HPLC traces of the quantity relationship of matter, crud reaction and purified 4a. (10-80% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.
ESI Mass spectrum of purified product 4a. Calculated Mass [M+H]^+: 1403.75; Mass Found (ESI+) [M+H]^+: 1403.75.

MS/MS analysis of 4a
$^1$H NMR (400 MHz, MeOD) analysis of 4a. Blue arrows point to signals of adducted thioacetal.

A control spectrum of $^1$H NMR (400 MHz, MeOD) analysis of Leuprorelin
1H-1H COSY NMR spectrum of 4a
Characterization data of 4b

HPLC traces of the quantity relationship of matter, crud reaction and purified 4b. (10-80% B phase over 25 min, 1 mL·min\(^{-1}\) flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

ESI Mass spectrum of purified product 4b. Calculated Mass \([\text{M+H}]^+\): 1413.71; Mass Found (ESI+) \([\text{M+H}]^+\): 1413.71.
| # | b   | b-H₂O | b-NH₃ | b(2+) | Seq | y   | y-H₂O | y-NH₃ | y(2+) | #   |
|---|-----|-------|-------|-------|-----|-----|-------|-------|-------|-----|
| 1 | 112.04 | 94.03 | 95.01 | 56.52 | pE  | 1351.69 | 1333.68 | 1334.66 | 676.345 | 9   |
| 2 | 391.14 | 373.13 | 374.11 | 196.07 | [H+143.05] | 1240.66 | 1222.65 | 1223.63 | 620.83 | 8   |
| 3 | 577.22 | 559.21 | 560.19 | 289.11 | W   | 961.56 | 943.55 | 944.53 | 481.28 | 7   |
| 4 | 664.25 | 646.24 | 647.22 | 332.625 | S   | 775.48 | 757.47 | 758.45 | 388.24 | 6   |
| 5 | 827.31 | 809.3 | 810.28 | 414.155 | Y   | 688.45 | 670.44 | 671.42 | 344.725 | 5   |
| 6 | 940.40 | 922.39 | 923.37 | 470.7 | L   | 525.39 | 507.38 | 508.36 | 263.195 | 4   |
| 7 | 1053.48 | 1035.47 | 1036.45 | 527.24 | L   | 412.30 | 394.29 | 395.27 | 206.65 | 3   |
| 8 | 1209.58 | 1191.57 | 1192.55 | 605.29 | R   | 299.22 | 281.21 | 282.19 | 150.11 | 2   |
| 9 | 1306.64 | 1288.63 | 1289.61 | 653.82 | P   | 143.12 | 125.11 | 126.09 | 72.06 | 1   |

**MS/MS analysis of 4b**
Characterization data of 4c

HPLC traces of the quantity relationship of matter, crud reaction and purified 4c. (10-80% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

ESI Mass spectrum of purified product 4c. Calculated Mass [M+H]⁺: 1425.71; Mass Found (ESI+) [M+H]⁺: 1425.71.
MS/MS analysis of 4c
Characterization data of 4d

HPLC traces of the quantity relationship of matter, crude reaction and purified 4d. (10-80% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

ESI Mass spectrum of purified product 4d. Calculated Mass [M+H]⁺: 1429.71; Mass Found (ESI+) [M+H]⁺: 1429.71.
MS/MS analysis of 4d
The reaction was followed General Procedure C using peptide 5 (100 µL 1 mM solution, sequence: NH₂-DRVYHPF-OH) and thioacetal 1a. A stock solution of peptide 5 (1mM) was made up by 2 mL solvent (MeCN/H₂O = 4/1) and 2.2 mg peptide 5.

Before the reaction, a quantity relationship of matter between peptide 5 and the internal standard (dibenzyl sulfoxide) was established by a gradient concentration. The volume of the reaction mixture was adjusted to 500 µL with MeCN/H₂O (1/1), and then 2 µL dibenzyl sulfoxide stock solution (50 mM in MeCN/H₂O = 1/1) was added. The resulted solution was filtrated and analyzed with LC-MS.

Characterization data of 5

HPLC traces of the quantity relationship of matter, crude reaction and purified 5. (10-80% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.
ESI Mass spectrum (positive charge) of purified product 5. Calculated Mass \([M+H]^+: 1240.63; \) Mass Found (ESI+) \([M+H]^+: 1240.63, [M+Na]^+: 1262.61. 

ESI Mass spectrum (negative charge) of purified product 5. Calculated Mass \([M-H]: 1238.61; \) Mass Found (ESI+) \([M-H]: 1238.61, [M+CF_3COO]: 1352.60. 

MS/MS (negative charge) analysis of 5
The reaction was followed General Procedure C using peptide 6 (100 µL 1 mM solution, sequence: Ac-SYSNleEHfRWGKPV-NH₂) and thioacetal 1a. A stock solution of peptide 6 (1mM) was made up by 1 mL solvent (MeCN/H₂O = 4/1) and 1.7 mg peptide 6.

Before the reaction, a quantity relationship of matter between peptide 6 and the internal standard (dibenzyl sulfoxide) was established by a gradient concentration. The volume of the reaction mixture was adjusted to 500 µL with MeCN/H₂O (1/1), and then 2 µL dibenzyl sulfoxide stock solution (50 mM in MeCN/H₂O = 1/1) was added. The resulted solution was filtrated and analyzed with LC-MS.

Characterization data of 6

HPLC traces of the quantity relationship of matter, crude reaction and purified 6. (10-70% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.
ESI Mass spectrum of purified product 6. Calculated Mass [M+2H]^{2+}: 921.47; Mass Found (ESI+) [M+2H]^{2+}: 921.47.

MS/MS analysis of 6
The reaction was followed General Procedure C using peptide 7 (100 μL 1 mM solution, sequence: NH₂-RRMEH₂RMEW-NH₂) and thioacetal 1a. A stock solution of peptide 7 (1 mM) was made up by 1 mL solvent (MeCN/H₂O = 4/1) and 1.3 mg peptide 7.

Before the reaction, a quantity relationship of matter between peptide 7 and the internal standard (dibenzyl sulfoxide) was established by a gradient concentration. The volume of the reaction mixture was adjusted to 500 μL with MeCN/H₂O (1/1), and then 2 μL dibenzyl sulfoxide stock solution (50 mM in MeCN/H₂O = 1/1) was added. The resulted solution was filtrated and analyzed with LC-MS.

Characterization data of 7

HPLC traces of the quantity relationship of matter, crude reaction and purified 7. (10-80% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product. The red asterisks indicate oxidative products with both oxidized Met and modified His.
ESI Mass spectrum of purified product 7. Calculated Mass [M+H]^+: 1523.72, [M+2H]^2+: 762.36; Mass Found (ESI+) [M+H]^+: 1523.72, [M+H+Na]^2+: 773.35, [M+2H]^2+: 762.36.

MS/MS analysis of 7

ESI Mass spectrum of purified product 7 with both mono-oxidized Met and modified His. Calculated Mass [M+H]^+: 1539.71; Mass Found (ESI+) [M+H]^+: 1539.71.
MS/MS analysis of 7 with both mono-oxidized Met and modified His
ESI Mass spectrum of purified product 7 with both di-oxidized Met and modified His. Calculated Mass [M+H]^+: 1556.71, [M+2H]^2+: 778.36; Mass Found (ESI+) [M+H]^+: 1556.71, [M+2H]^2+: 778.36.

MS/MS analysis of 7 with both di-oxidized Met and modified His
The reaction was followed General Procedure C using peptide 8 (100 µL 1 mM solution, sequence: NH₂-SYCFDEFNWQTRHKM-NH₂) and thioacetal 1a. A stock solution of peptide 7 (1 mM) was made up by 1 mL solvent (MeCN/H₂O = 4/1) and 1.9 mg peptide 8.

Before the reaction, a quantity relationship of matter between peptide 7 and the internal standard (dibenzy sulfoxide) was established by a gradient concentration. The volume of the reaction mixture was adjusted to 500 µL with MeCN/H₂O (1/1), and then 2 µL dibenzyl sulfoxide stock solution (50 mM in MeCN/H₂O = 1/1) was added. The resulted solution was filtrated and analyzed with LC-MS.

Characterization data of 8

HPLC traces of the quantity relationship of matter, crude reaction and purified 8. (10-80% B phase over 25 min, 1 mL·min⁻¹ flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product. The red asterisks indicate oxidative products with both oxidized Met and modified His.
ESI Mass spectrum of purified product 8. Calculated Mass [{M+2H}]^{2+}: 1048.94; Mass Found (ESI+) [{M+2H}]^{2+}: 1048.94, [{M+H+Na}]^{2+}: 1059.93.

MS/MS analysis of 8

ESI Mass spectrum of purified product 8 with both mono-oxidized Met and modified His. Calculated Mass [{M+2H}]^{2+}: 1056.94; Mass Found (ESI+) [{M+2H}]^{2+}: 1056.94, [{M+H+Na}]^{2+}: 1067.93.
MS/MS analysis of 8 with both mono-oxidized Met and modified His
5. References

[1] S. Learte-Aymami, C. Vidal, A. Gutierrez-Gonzalez, J. L. Mascarenas, *Angew. Chem. Int. Ed. Engl.* **2020**, *59*, 9149-9154.

[2] J. Ohata, M. B. Minus, M. E. Abernathy, Z. T. Ball, *J Am Chem Soc* **2016**, *138*, 7472-7475.

[3] Y. Takaoka, H. Tsutsumi, N. Kasagi, E. Nakata, I. Hamachi, *J. Am. Chem. Soc.* **2006**, *128*, 3273-3280.

[4] S. Jia, D. He, C. J. Chang, *J. Am. Chem. Soc.* **2019**, *141*, 7294-7301.

[5] X. Chen, F. Ye, X. Luo, X. Liu, J. Zhao, S. Wang, Q. Zhou, G. Chen, P. Wang, *J. Am. Chem. Soc.* **2019**.

[6] K. Peciak, E. Laurine, R. Tommasi, J. W. Choi, S. Brocchini, *Chem. Sci.* **2019**, *10*, 427-439.

[7] D. Kaiser, I. Klose, R. Oost, J. Neuhaus, N. Maulide, *Chem. Rev.* **2019**, *119*, 8701-8780.

[8] a) R. Parnes, H. Reiss, D. Pappo, *J. Org. Chem.* **2018**, *83*, 723-732; b) R. Parnes, D. Pappo, *Org. Lett.* **2015**, *17*, 2924-2927; c) R. Parnes, S. Narute, D. Pappo, *Org. Lett.* **2014**, *16*, 5922-5925.

[9] C. S. McKay, M. G. Finn, *Angew Chem Int Ed Engl* **2016**, *55*, 12643-12649.

[10] K. Susumu, H. T. Uyeda, I. L. Medintz, T. Pons, J. B. Delehanty, H. Mattoussi, *J Am Chem Soc* **2007**, *129*, 13987-13996.

[11] N. George, M. Bekkaye, G. Masson, J. Zhu, *European Journal of Organic Chemistry* **2011**, *2011*, 3695-3699.
6. NMR spectra

$^1$H NMR spectrum of 1a

$^{13}$C NMR spectrum of 1a
$^{1}H$ NMR spectrum of Boc-Trp-OMe-1a

$^{13}C$ NMR spectrum of Boc-Trp-OMe-1a
$^1$H-$^1$H COSY NMR spectrum of Boc-Trp-OMe-1a

$^1$H-$^{13}$C HSQC NMR spectrum of Boc-Trp-OMe-1a
$^1$H-$^1$C HMBC NMR spectrum of Boc-Trp-OMe-1a

$^1$H NMR spectrum of 3a
$^{13}$C NMR spectrum of 3a

$^1$H-$^1$H COSY NMR spectrum of 3a
$^1$H-$^{13}$C HSQC NMR spectrum of 3a

$^1$H-$^{13}$C HMBC NMR spectrum of 3a
$^1$H NMR spectrum of 3b

$^{13}$C NMR spectrum of 3b
$^{1}H$ NMR spectrum of $3c$

$^{13}C$ NMR spectrum of $3c$
$^{1}H$ NMR spectrum of 3d

$^{13}C$ NMR spectrum of 3d
$^1$H NMR spectrum of 3e

$^{13}$C NMR spectrum of 3e
$^1$H NMR spectrum of 3f

$^{13}$C NMR spectrum of 3f
$^1$H NMR spectrum of TA3

$^{13}$C NMR spectrum of TA3
$^1$H NMR spectrum of TA4

$^{13}$C NMR spectrum of TA4
$^1$H NMR spectrum of TA5

$^{13}$C NMR spectrum of TA5
$^1$H NMR spectrum of TA6

$^{13}$C NMR spectrum of TA6
$\text{H NMR spectrum of TA7}$

$\text{^{13}C NMR spectrum of TA7}$
$^1$H NMR spectrum of TA8

$^{13}$C NMR spectrum of TA8
$^1$H NMR spectrum of TA9

$^{13}$C NMR spectrum of TA9
$^1$H NMR spectrum of TA1

$^{13}$C NMR spectrum of TA1
$^1$H NMR spectrum of TA10

$^{13}$C NMR spectrum of TA10
$^1$H NMR spectrum of TA11

$^{13}$C NMR spectrum of TA11
$^{1}H$ NMR spectrum of $S1$

$^{13}C$ NMR spectrum of $S1$
$^1$H NMR spectrum of $S_2$

$^{13}$C NMR spectrum of $S_2$
$^1$H NMR spectrum of S3

$^{13}$C NMR spectrum of S3
$^1$H NMR spectrum of S4

$^{13}$C NMR spectrum of S4
$^1$H NMR spectrum of S5

$^{13}$C NMR spectrum of S5
$^1$H NMR spectrum of S6

$^{13}$C NMR spectrum of S6
$^1$H NMR spectrum of S7

$^{13}$C NMR spectrum of S7
$^1$H NMR spectrum of S8

$^{13}$C NMR spectrum of S8
\(^1\text{H} \text{NMR spectrum of S9}\)

\(^{13}\text{C} \text{NMR spectrum of S9}\)
$^1$H NMR spectrum of S10

$^{13}$C NMR spectrum of S10
$^1$H NMR spectrum of S11

$^{13}$C NMR spectrum of S11
$^1$H NMR spectrum of S12

$^{13}$C NMR spectrum of S12
$^1$H NMR spectrum of S13

$^{13}$C NMR spectrum of S13
$^{1}H$ NMR spectrum of S14

$^{13}C$ NMR spectrum of S14
$^1$H NMR spectrum of S15

$^{13}$C NMR spectrum of S15
$^1$H NMR spectrum of S16

$^{13}$C NMR spectrum of S16
$^1$H NMR spectrum of S17

$^{13}$C NMR spectrum of S17
$^1$H NMR spectrum of S18

$^{13}$C NMR spectrum of S18