## Thiol-sulfoxonium ylide photo-click reaction for bioconjugation

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# Table of Contents

<ul> <li>2. Supplementary material and information for organic chemistry</li></ul>
2.1. General information
2.2. Synthesis of sulfoxonium ylide12
2.3. General procedure A for the thiol-sulfoxonium ylide reaction
3. Supplementary material and information for peptide modification21
3.1. General information
3.2. General procedure B for the reaction of peptide
3.3. Characterization data for the reaction of peptide 723
3.4. Characterization data for the reaction of peptide 8
3.5. Characterization data for the reaction of peptide 9
3.6. Characterization data for the reaction of peptide 1041
3.7. Characterization data for the reaction of peptide 11
3.8. Characterization data for the reaction of peptide 1245
3.9. Characterization data for the reaction of peptide 13
3.10. Characterization data for the reaction of peptide 14
3.11. Characterization data for the reaction of peptide I-III
4. Mechanistic experiments
4.1. Luminescence screening
4.2. Stern-Volmer luminescence quenching studies
4.3. UV/vis absorption studies
4.4. Cyclic Voltammogram65
5. Supplementary material and information for protein modification
5.1. General information
5.2. General procedure C for the reaction of protein and proteome67
5.3. LC-MS analysis67
5.4. LC-MS/MS analysis67
6. Supplementary material and information for chemoproteomics analysis
6.1. Western blot
6.2. MS-based proteomics69
7. Reference
8. NMR spectra

## 1. Supplementary figures and tables





b) Catalyst free S-H insertion of aryl thiols

$$R_{2} \xrightarrow[]{} R_{1} + HS \xrightarrow[]{} R_{3} \xrightarrow[]{} Catalyst free \\ MeCN, 24 h \xrightarrow[]{} R_{3} \xrightarrow[]{} R_{3} \xrightarrow[]{} R_{1}$$

c) Acid-catalytic S-H insertion of aliphatic thiols

**Figure S1**. Recent reports of X-H insertion of sulfoxonium ylides. (**a**) Transition metal catalytic X-H insertion.<sup>1-6</sup> (**b**) Catalyst free S-H insertion of aryl thiols.<sup>7</sup> (**c**) Acid-catalytic S-H insertion of aliphatic thiols.<sup>7</sup>

Table S1. Screening of photocatalysts for thiol-sulfoxonium ylide reaction.

	Ac. N COOH	PhotoCat. (2%mol) Blue LED (450 nm) Ac. S + DMS water, rt, 20 min Ac. H	60
Entry	PhotoCat.	Quenching fraction F	Yield (%)
1	_	-	N.D.
2	$Ru(bpz)_3(PF_6)_2$	<5	<10
3	Rose Bengal (RB)	9	37
4	$Ir(dtbbpy)(ppy)_2(PF_6)$	13	38
5	MesAcrClO <sub>4</sub>	13	48
6	Riboflavin	26	63
7	Flavin mononucleotide (FMN)	33	81
8	Riboflavin tetrabutyrate (RFTB)	39	83
9	Riboflavin tetraacetate (RFTA)	43	86
10	Alloxazine-Bu	32	76 <sup>a</sup>

Conditions: **1a** (50 mM), **2** (25 mM) and photocat. (2% mol) under light irradiation (450 nm, 18 W) for 20 min in  $D_2O$  at rt with dimethyl sulfone as internal standard. <sup>a</sup> The 400 nm, 18 W light irradiation was used.



Figure S2. The chemical structure of the photocatalysts used in this work.



Figure S3. Luminescence spectra of the screening of the photocatalysts with sulfoxonium ylide 1a.



**Figure S4.** Kinetic studies of the reaction between **1a** and **2**. (**a**) Kinetic study of the S-H insertion of sulfoxonium ylide by <sup>1</sup>H NMR analysis. Blue, black, green and red arrows point to signals of sulfoxonium ylide **1a**, Ac-Cys-OH **2**, the product **2a** and leaving DMSO. Conditions: **1a** (50 mM), **2** (25 mM) and RFTA (2% mol) under light irradiation (450 nm, 18 W) in D<sub>2</sub>O at rt with dimethyl sulfone as internal standard. (**b**) <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) assignment of  $\alpha$ -carbonyl methylene of the purified product **2a** from H<sub>2</sub>O. (**c**) The yield versus time plot of the standard reaction. (**d**) Estimation of the second-order reaction constant ( $k_{obs} = 0.172 \text{ M}^{-1} \text{ s}^{-1}$ ) using the linearly-fitted region of the 1/[**2**] versus time plot. (**e**) The data was from **Figure S6b**. Conditions: **2** (25 mM) and RFTA (2% mol) with sulfoxonium ylide **1a** plot. The data was from **Figure S6b**. Conditions: **2** (25 mM) and RFTA (2% mol) with sulfoxonium ylide **1a** plot. State and the second of the standard reaction of sulfoxonium ylide **1a** plot. The data was from **Figure S6b**. Conditions: **2** (25 mM) and RFTA (2% mol) with sulfoxonium ylide **1a** (5, 10, 25, 50 and 100 mM) under light irradiation (450 nm, 18 W) in D<sub>2</sub>O at rt for 5 min with dimethyl sulfone as internal standard.



**Figure S5.** Low reactive and inactive sulfoxonium ylides for the visible-light-induced thiol-sulfoxonium ylide reaction. The trace amount of products were detected by LC-MS.

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		Ć	1a Ac-Cys-	соон в он, 2	RFTA (2%mol) lue LED (450 nm) Ac vater, rt, 5 min		+ DMSO	
	( <b>a</b> ) 50 ml	M sulfoxonium yli	de 1a			( <b>b</b> ) 25	mM Cys 2	
Entry	Cys concentration (mM)	5 min yield (%) for Cys	product concentration (mM)	Entry	Ylide concentration (mM)	5 min yield (%) for Cys	product concentration (mM)	5 min yield (%) for Ylide
1	50	31	15.5	1	100	73	18.3	18
2	25	62	15.6	2	50	63	15.8	32
3	10	89	8.9	3	25	29	7.3	29
4	5	90	4.5	4	10	12	3.0	30
5	2.5	92	2.3	5	5	6	1.5	30

Figure S6. The kinetic studies with various concentrations of Cys or sulfoxonium ylide.

**a**) The 5 min reaction efficiency of gradient change of Cys concentrations by fixing the concentration of sulfoxonium ylide **1a** (50 mM). For the low concentration of **2**, all of the reactions (entry 3-5) exhibited almost quantitative conversions. For the high concentration of **2**, entry 1 and 2 shown that the same amount of product **2a** (15.5 and 15.6 mM) was observed for 50 mM and 25 mM **2**, suggesting that the reaction rate remains the same as the Cys **2** concentration increases.

**b**) The 5 min reaction efficiency of gradient change of sulfoxonium ylide concentrations by fixing the concentration of Cys **2** (25 mM). Not as fast as the reactions with excessive **1a**, the excess of **2** led to around 30% conversions of **1a** (entry 4 and 5). Moreover, the conversions of equivalent and slightly excess **1a** (entry 2 and 3) were also around 30%, and the rate of product **2a** generation was linearly related to the concentration of starting concentration of **1a** (5-50 mM, also see **Figure S4e**), indicating that the activation rates of sulfoxonium ylide **1a** were almost the same under the fixed photocatalytic conditions.



**Figure S7**. UV/vis absorption spectra of the starting materials: RFTA, sulfoxonium ylide **1a** and Ac-Cys-OH **2**.



Figure S8. Stern-Volmer luminescence quenching analysis for RFTA with sulfoxonium ylide 1a and Ac-Cys-OH 2.



**Figure S9**. Mechanistic studies of the reaction between sulfoxonium ylide 1a and Ac-Cys-OH 2 by *in* situ <sup>1</sup>H NMR. A standard reaction was defined as: 1a (50 mM), 2 (25 mM) and RFTA (2%mol) under light irradiation (450 nm) for 20 min in  $D_2O$  (or other mentioned solvent) at rt. Green arrows point to

signals of the product **2a**. (**a**) Control experiments. Bottom to top traces: **1a** (50 mM) and RFTA (2%mol) under light irradiation (450 nm) at 0 min and 20 min in D<sub>2</sub>O at rt in the absence of **2**; **2** (25 mM) was added after 20 min irradiation; and the trace of standard reaction at 20 min. (**b**) The reactions in polar protic solvent. Bottom to top traces: a standard reaction was conducted in CD<sub>3</sub>OD at 0 min and 20 min; and the trace of standard reaction in nonpolar and polar aprotic solvent. Bottom to top traces: a standard reaction was conducted in DMSO- $d_6$  at 0 min and 20 min; a standard reaction was conducted in MeCN- $d_3$  at 0 min and 20 min; and **1a** (50 mM), *n*-PrSH (25 mM) and RFTA (2%mol) under light irradiation (450 nm) at 0 min and 20 min in D<sub>2</sub>O at rt. The red asterisk marks the peak of the active hydrogen atom of sulfoxonium ylide **1a**.



**Figure S10**. Reaction with peptides. (**a**) Optimization of the reaction between sulfoxonium ylide **1a** and model peptide **7**. Conditions: **1a** (10 mM), **7** (1 mM), photocat. (0.5 mM) and thiourea (10 mM) under light irradiation (450 nm) for 1 min at rt using PB buffer (pH 7.4 with 25% MeCN) as solvent. Conversion was determined by LC-MS.<sup>a</sup> The conversion in parenthesis refers to the reactions under nitrogen protection. (**b**) HPLC traces the reaction. The red asterisk marks the peak of oxidized by-product (disulfide). (**c**) Estimation of the conversion versus time plot. (**d**) Estimation of the conversion versus pH plot.

H <sub>2</sub> N-LAVCHMFPG-C		SH C H M F P G - C btide, 7 (1 mM)	PB buffer (pH 7.4, 25% MeCN) Bule LED (450 nM) 1 min, r.t.	I-LAVCHMFPG-COOH	
-	Entry	Additive	Additive loading (mM	I) Conversion (%)	
-	1		20	77	
	2		10	76	
	3	Thiourea	5	66	
	4		1	48	
	5		0.5	45	
	6	Phenylthiourea	10	55	
	7	Benzylthiourea	10	17	
	8	Urea	10	36	
	9	Phenylthiourea	10	51	

Table S2. Optimization of additives for peptide cysteine modification.

Conditions: **1a** (10 mM), **7** (1 mM), RFTA (0.5 mM) and additive (10 mM) under light irradiation (450 nm, 40 W) for 1 min at rt using PB buffer (pH 7.4, 25% MeCN) as solvent.



**Figure S11**. Scope of the peptide reaction without free cysteine residue. Conditions: sulfoxonium ylide 1 (10 mM), peptide **I-III** (1 mM), RFTA (0.5 mM) and thiourea (10 mM) under light irradiation (450 nm) for 1 min at rt in PBS buffer (pH 7.4, 25% MeCN was added to dissolve peptides) as solvent.

## 2. Supplementary material and information for organic chemistry

#### 2.1. General information

All chemical reagents are commercially available from *Energy Chemical* without purification. The reactions were monitored by TLC (silica gel-G). Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker 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 a Q\_Exactive\_Focus. Mass Spectrometry (MS) to screen the molecular weight of HPLC fractions were carried out on SHIMAZU LC-MS 8030 in positive ion mode.

#### General method for preparative LC

Preparative LC were performed on a Shimadzu LC-6AD system equipped with Shimadzu Shim-pack GIST C18 column ( $20 \times 250$  mm, 5 µm; room temperature). Water (containing 0.1% TFA, A phase) and pure CH<sub>3</sub>CN (B phase) were used as solvents in linear gradient mixtures at a flow rate of 8 mL·min<sup>-1</sup>.

#### 2.2. Synthesis of sulfoxonium ylide

#### 2.2.1. Preparation of 4-nitrophenol ester

4-Nitrophenol esters **S3-S7** were prepared as intermediates for the synthesis of sulfoxonium ylides. The preparation of 4-nitrophenol ester was followed the reported procedure.<sup>8</sup>

**S3**, white powder, yield 82%. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.34 – 8.26 (m,

2H), 8.19 - 8.10 (m, 2H), 7.44 - 7.36 (m, 2H), 7.04 - 6.96 (m, 2H), 3.91 (s, 3H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  164.57, 164.06, 156.09, 145.40, 132.68, 125.36, 122.81, 120.85, 114.23, 55.75.

$$\mathbf{S4}$$
, white powder, yield 87%. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.34 – 8.26 (m, 2H).

8.26 – 8.17 (m, 2H), 7.45 – 7.37 (m, 2H), 7.26 – 7.15 (m, 2H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*) δ 167.91, 165.36, 163.40, 155.71, 145.60, 133.22, 133.13, 125.42, 124.96, 124.93, 122.75, 116.34, 116.12. <sup>19</sup>F NMR (376 MHz, Chloroform-*d*) δ -102.96.

 $\int_{S}^{N_{O_2}}$  S5, white powder, yield 75%. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.40 – 8.31 (m, 2H),

8.06 (dd, *J* = 3.8, 1.3 Hz, 1H), 7.77 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.25 (dd, *J* = 5.0, 3.8 Hz, 1H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*) δ 159.63, 155.36, 145.49, 135.60, 134.62, 131.74, 128.41, 125.35, 122.61.

**S6**, white powder, yield 90%. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.36 – 8.27 (m, 2H), 7.48 – 7.40 (m, 2H), 6.19 (s, 2H), 3.90 (s, 6H), 3.89 (s, 3H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  163.72, 159.57, 156.21, 145.28, 126.12, 125.22, 122.78, 115.76, 90.73, 56.18, 55.60.

S7, white powder, yield 85%. In NMR (300 MHz, Chloroform-*a*) 68.27 - 8.21 (in, 2H), 7.31 - 7.25 (m, 2H), 5.15 (t, J = 6.4 Hz, 1H), 3.51 (q, J = 6.2 Hz, 2H), 2.82 (t, J = 6.1 Hz, 2H), 1.43 (s, 9H).

#### 2.2.2. Preparation of intermediates for the synthesis of 11 and 1m



To a round-bottomed flask was added 2,2'-(ethane-1,2-diylbis(oxy))bis(ethan-1-ol) (3.0 g, 20 mmol) dissolved in 100 mL THF, and cooled in an ice bath. NaOH (1.6 g, 40 mmol) was added in one portion, and 3-bromoprop-1-yne (2.4 g, 20 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 50 mL H<sub>2</sub>O was added. The aqueous solution was neutralized with 1 M dilute HCl, and extracted with  $CH_2Cl_2$  (3×30 mL). The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crud 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-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-ol was dissolved with Tosyl chloride (TsCl, 3.8 g, 20 mmol) and Et<sub>3</sub>N (2.0g, 20 mmol) in 100 mL CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed to stir for 2 h. The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (5:1). S8, yellow oil, yield 75% for two steps. <sup>1</sup>H NMR (400 MHz, Chloroform-d) & 7.78 -7.70 (m, 2H), 7.34 - 7.27 (m, 2H), 4.13 (d, J = 2.4 Hz, 2H), 4.12 - 4.09 (m, 2H), 3.67 - 3.60 (m, 4H), 3.60 - 3.55 (m, 2H), 3.53 (s, 4H), 2.39 (s, 3H), 1.98 (s, 1H). <sup>13</sup>C NMR (101 MHz, Chloroform-d) δ 144.80, 132.93, 129.81, 127.92, 79.61, 74.60, 70.65, 70.48, 70.36, 69.26, 69.02, 68.61, 58.32, 21.59.

The intermediate **S8** (6.6g, 10 mmol), methyl 4-hydroxybenzoate (1.4 g, 10 mmol) and addition  $Et_3N$  (1.0 g, 10 mmol) were added in a round-bottomed flask with 100 mL  $CH_2Cl_2$  as solvent, and the reaction was stirred and continued for 16 h. After reaction completion, the solvent was removed, and then 50 mL H<sub>2</sub>O was added. The crud mixture was extracted with  $CH_2Cl_2$  (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 crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (5:1). **S9**, yellow oil, yield 84%. <sup>1</sup>H NMR (500 MHz, Chloroform-*d*)  $\delta$  7.99 – 7.93 (m, 2H), 6.94 – 6.88 (m, 2H), 4.20 – 4.14

(m, 4H), 3.86 (d, *J* = 4.3 Hz, 5H), 3.75 – 3.69 (m, 2H), 3.69 – 3.63 (m, 6H), 2.42 (t, *J* = 2.4 Hz, 1H).

To a round-bottomed flask was added intermediate **S9** (1.6 g, 5 mmol) dissolved in 50 mL methanol, and cooled in an ice bath. An aqueous solution of LiOH (50 mL, 1 M) was added dropwise. The solution was allowed to warm to room temperature and stirred for 1 h. After reaction completion, the organic solvent was removed, and the aqueous solution was neutralized with 1 M dilute HCl, and extracted with  $CH_2Cl_2$  (3×30 mL). The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under reduced pressure, and the crude intermediate was used directly for next step. Under an ice bath, to a round-bottomed flask was added the crude intermediate, 4-nitrophenol (0.83 g, 5 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 1.9 g, 10 mmol) and 4-dimethylaminopyridine (DMAP, 0.06 g, 10% mol) dissolved in 50 mL CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed to warm to room temperature and stir for 16 h. The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (5:1). **S10**, yellow oil, yield 71% for two steps. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.32 – 8.23 (m, 2H), 8.15 – 8.07 (m, 2H), 7.46 – 7.37 (m, 2H), 7.06 – 6.98 (m, 2H), 4.27 – 4.21 (m, 2H), 4.19 (d, *J* = 2.4 Hz, 2H), 3.94 – 3.87 (m, 2H), 3.78 – 3.66 (m, 8H), 2.52 (t, *J* = 2.4 Hz, 1H).



added Under bath, round-bottomed flask was the 4-(((tertan ice to а butoxycarbonyl)amino)methyl)benzoic acid (2.5 g, 10 mmol), 4-nitrophenol (1.7 g, 10 mmol), EDC (3.8 g, 20 mmol) and 4-dimethylaminopyridine (DMAP, 0.12 g, 10% mol) dissolved in 100 mL CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed to warm to room temperature and stir for 16 h. The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na2SO4. The crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (2:1). S11, white powder, yield 86%. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.32 – 8.24 (m, 2H), 8.16 – 8.09 (m, 2H), 7.45 – 7.35 (m, 4H), 5.18 (t, J = 6.3 Hz, 1H), 4.40 (d, J = 6.2 Hz, 2H), 1.45 (s, 9H). <sup>13</sup>C NMR (101 MHz, Chloroform-d)  $\delta$ 164.15, 155.86, 146.21, 145.50, 130.78, 127.56, 126.16, 125.38, 122.77, 115.68, 80.05, 44.42, 28.51.

To a round-bottomed flask was added intermediate **S11** (1.9 g, 5 mmol) dissolved in 100 mL of 1/1 mixture of trifluoroacetate (TFA) and CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed to stir for 6 h. After reaction completion, the organic solvent was removed under reduced pressure, and neutralized with dilute NaHCO<sub>3</sub>, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×30 mL). The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under reduced pressure, and the crude intermediate was used directly for next step. To a round-bottomed flask was added the crude intermediate, Biotin-NHS ester (1.7 g, 5 mmol) dissolved in 100 mL CH<sub>2</sub>Cl<sub>2</sub>. The solution was allowed to warm to room temperature and stir for 16 h. The organic phase was washed with saturated NaCl solution (2×20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crud product was purified by flash column chromatography using eluent solution CH<sub>2</sub>Cl<sub>2</sub>/MeOH (20:1). **S12**, yellow solid, yield 57% for

two steps. <sup>1</sup>H NMR (400 MHz, Methanol- $d_4$ )  $\delta$  8.00 – 7.83 (m, 4H), 7.59 – 7.51 (m, 4H), 4.51 (dd, J = 7.9, 4.8 Hz, 1H), 4.45 (s, 1H), 4.29 (dd, J = 7.9, 4.4 Hz, 1H), 4.20 (s, 2H), 3.38 (s, 2H), 3.21 (dt, J = 9.9, 5.3 Hz, 1H), 2.96 (dd, J = 12.8, 5.0 Hz, 1H), 2.73 (d, J = 12.7 Hz, 1H), 2.32 (t, J = 7.3 Hz, 2H), 1.78 – 1.56 (m, 4H), 1.45 (p, J = 7.8 Hz, 2H).

#### 2.2.3. Synthesis of sulfoxonium ylide

The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>7, 9</sup> Generally, the acyl chloride or 4-nitrophenol ester was used as substrates. The synthesis of **1a** was described as an example.

To a round-bottomed flask was added trimethylsulfoxonium iodide (6.6 g, 30 mmol) and potassium t-butoxide (*t*-BuOK, 6.7 g, 60 mmol) dissolved in 200 mL dry tetrahydrofuran (THF) under nitrogen protection, and heated in an oil bath. The solution was allowed to reflux and stirred for 3 h. After reaction completion, the mixture was cooled in an ice bath. To this cooled solution, a solution of benzoyl chloride (1.4 g, 10 mmol) in 20 mL dry THF was added dropwise. After the addition, the solution was allowed to warm to room temperature and stirred for additional 6 h. After the reaction completion, the organic phase was washed with saturated NaCl solution (2×50 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crud product was purified by flash column chromatography using eluent solution  $CH_2Cl_2/MeOH$  (20:1).

**1a**, white powder, yield 85%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>7</sup> The acyl chloride was used as substrates. <sup>1</sup>H NMR (500 MHz, Chloroform-*d*)  $\delta$  7.77 – 7.68 (m, 2H), 7.40 – 7.27 (m, 3H), 5.00 (s, 1H), 3.42 (s, 6H). <sup>13</sup>C NMR (126 MHz, Chloroform-*d*)  $\delta$  182.44, 138.98, 130.77, 128.21, 126.59, 69.30, 42.17. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>10</sub>H<sub>13</sub>O<sub>2</sub>S 197.0631, found 197.0632.

procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S5** was used as substrates. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  7.78 – 7.70 (m, 2H), 6.98 – 6.90 (m, 2H), 5.54 (s, 1H), 3.80 (s, 3H), 3.55 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO- $d_6$ )  $\delta$  180.42, 161.44, 132.50, 128.60, 113.67, 71.66, 55.71, 41.16. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>11</sub>H<sub>15</sub>O<sub>3</sub>S 227.0736, found 227.0732.

1c, white powder, yield 81%. The synthesis of sulfoxonium ylide was followed the similar

1b, white powder, yield 89%. The synthesis of sulfoxonium ylide was followed the similar

procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S6** was used as substrates. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.88 – 7.80 (m, 4H), 7.22 (t, *J* = 8.9 Hz, 4H), 5.63 (s, 2H), 3.58 (s, 11H), 2.52 (p, *J* = 1.9 Hz, 1H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  179.47, 165.15, 162.69, 136.44, 136.41, 129.32, 129.23, 115.41, 115.20, 72.72, 41.03. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  -111.12. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>10</sub>H<sub>12</sub>FO<sub>2</sub>S 215.0537, found 215.0533.

**1d**, yellow solid, yield 56%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>7</sup> The acyl chloride was used as substrates. <sup>1</sup>H NMR (400 MHz,

Chloroform-*d*)  $\delta$  7.80 – 7.71 (m, 2H), 6.99 – 6.91 (m, 2H), 4.91 (s, 1H), 4.71 (d, J = 2.4 Hz, 2H), 3.48 (s, 6H), 2.52 (t, J = 2.4 Hz, 1H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  181.79, 159.75, 132.58, 128.44, 114.44, 78.34, 75.95, 67.67, 55.94, 42.72. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>15</sub>O<sub>3</sub>S 251.0736, found 251.0738.

**1e**, yellow powder, yield 66%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>7</sup> The acyl chloride was used as substrates. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  7.74 (d, *J* = 8.6 Hz, 2H), 6.72 – 6.64 (m, 2H), 4.93 (s, 1H), 3.53 (s, 6H), 3.04 (s, 6H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  182.52, 152.31, 128.20, 126.43, 111.02, 66.39, 42.86, 40.26. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>18</sub>NO<sub>2</sub>S 240.1053, found 240.1052.

**1f**, white solid, yield 83%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S8** was used as substrates. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  6.07 (s, 2H), 4.52 (s, 1H), 3.78 (s, 3H), 3.75 (s, 6H), 3.48 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  182.30, 161.12, 157.99, 114.47, 90.73, 73.11, 56.01, 55.39, 42.69. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>19</sub>O<sub>5</sub>S 287.0948, found 287.0949.

**1g**, yellow solid, yield 71%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>7</sup> The acyl chloride was used as substrates. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.69 – 8.63 (m, 2H), 7.62 – 7.56 (m, 2H), 5.03 (s, 1H), 3.52 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  179.74, 150.39, 145.86, 120.63, 70.21, 42.36. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>9</sub>H<sub>12</sub>NO<sub>2</sub>S 198.0583, found 198.0584.

 $h_{s}^{7}$  **h**, yellow solid, yield 84%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S7** was used as substrates. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.63 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.48 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.09 (dd, *J* = 5.0, 3.7 Hz, 1H), 5.54 (s, 1H), 3.57 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO) δ 174.72, 147.57, 129.74, 128.24, 127.09, 71.85, 41.15. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>8</sub>H<sub>11</sub>O<sub>2</sub>S<sub>2</sub> 203.0195, found 203.0196.

<sup>Boc</sup>  $\beta$  **1i**, yellow powder, yield 72%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S9** was used as substrates. <sup>1</sup>H NMR (500 MHz, Chloroform-*d*)  $\delta$  5.24 (s, 1H), 4.43 (s, 1H), 3.37 (s, 6H), 3.32 (q, *J* = 6.1 Hz, 2H), 2.34 (t, *J* = 6.2 Hz, 2H), 1.39 (s, 9H). <sup>13</sup>C NMR (126 MHz, Chloroform-*d*)  $\delta$  189.17, 156.07, 79.01, 70.41, 42.22, 40.02, 37.33, 28.55. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>11</sub>H<sub>22</sub>NO<sub>4</sub>S 264.1264, found 264.1266.

11, yellow solid, yield 58%. The synthesis of sulfoxonium ylide was

followed the similar procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S12** was used as substrates. <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  7.75 – 7.67 (m, 2H), 6.91 – 6.82 (m, 2H), 4.90 (s, 1H), 4.16 (d, *J* = 2.4 Hz, 2H), 4.14 – 4.11 (m, 2H), 3.87 – 3.80 (m, 2H), 3.74 – 3.68 (m, 2H), 3.68 – 3.61 (m, 6H), 3.46 (s, 6H), 2.41 (t, *J* = 2.4 Hz, 1H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  181.93, 161.06, 131.82, 128.41, 114.11, 79.79, 74.75, 70.95, 70.76, 70.56, 69.77, 69.22, 67.70, 67.58, 58.52, 42.65. HRMS m/z (ESI- TOF):  $[M+H]^+$  calcd for  $C_{19}H_{27}O_6S$  383.1523, found 383.1517.



**1m**, yellow solid, yield 71%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>9</sup> The 4-nitrophenol ester **S14** was used as substrates. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.37 (t, J = 6.0 Hz, 1H), 7.74 – 7.66 (m, 2H), 7.28 – 7.21 (m, 2H), 6.41 (d, J = 26.1 Hz, 2H), 5.57 (s, 1H), 4.31 (d, J = 2.7 Hz, 1H), 4.13 (d, J = 5.4 Hz, 1H), 3.54 (s, 6H), 3.17 (d, J = 5.0 Hz, 2H), 3.09 (ddd, J = 8.6, 6.1, 4.3 Hz, 1H), 2.83 (dd, J = 12.4, 5.0 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.15 (t, J = 7.4 Hz, 2H), 1.67 – 1.45 (m, 4H), 1.39 – 1.27 (m, 2H). <sup>13</sup>C NMR (101 MHz, DMSO- $d_6$ )  $\delta$  180.69, 172.74, 163.34, 142.58, 138.58, 127.27, 126.96, 72.62, 61.65, 59.80, 56.04, 49.20, 42.32, 41.17, 35.75, 28.83, 28.63, 25.91. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>21</sub>H<sub>30</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 452.1672, found 452.1681.

J = 7.2 Hz, 2H), 3.42 (s, 6H), 1.19 (t, J = 7.1 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  166.35, 133.61, 132.53, 128.44, 127.07, 70.38, 59.00, 43.26, 14.81. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>17</sub>O<sub>3</sub>S 241.0893, found 241.0893.

S2, colorless solid, yield 89%. The synthesis of sulfoxonium ylide was followed the similar procedure of previous reports.<sup>10</sup> The Tosyl fluoride was used as substrates. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  7.77 – 7.69 (m, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.52 (s, 1H), 3.50 (s, 6H), 2.37 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO- $d_6$ )  $\delta$  145.83, 141.46, 129.59, 125.76, 62.11, 43.15, 21.38. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>10</sub>H<sub>15</sub>O<sub>3</sub>S<sub>2</sub> 247.0457, found 247.0460.

### 2.2.4. Synthesis of sulfoxonium ylide 1j and 1k

The methylation of **1g** and **1h** was followed a same procedure. The synthesis of **1j** was described as an example. To a round-bottomed flask was charged with **1g** (0.24 g, 1 mmol). The flask was sealed and refiled with nitrogen for 3 times.  $CH_2Cl_2$  was added by a syringe, and the flask was cooled in an ice bath. Methyl trifluoromethanesulfonate (MeOTf, 0.25 g, 1.5 mmol) was added dropwise. The reaction solution was allowed to stir for 1 h at room temperature, and white was precipitated. The product was filtered and washed with ether and  $CH_2Cl_2$ . The obtained powder was dried under vacuum, and the pure pyridinium salt **1j** was provided.

**1j**, white solid, yield 62%. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.05 – 7.94 (m, 4H), 5.78 (s, 1H), 3.63 (s, 9H), 3.60 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO- $d_6$ )  $\delta$  178.32, 148.76, 141.19, 128.27, 120.83, 73.92, 56.89, 40.98. <sup>19</sup>F NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -77.73. HRMS m/z (ESI-TOF): [M]<sup>+</sup> calcd for C<sub>13</sub>H<sub>20</sub>NO<sub>2</sub>S 254.1209, found 254.1208.

 $\frac{1}{100} \text{ k, yellow oil, yield 76\%. }^{1} \text{ H NMR (400 MHz, DMSO-}d_{6}) \delta 9.04 (d, J = 6.5 \text{ Hz, 2H}), 8.37 \\ - 8.30 (m, 2\text{H}), 6.14 (s, 1\text{H}), 4.36 (s, 3\text{H}), 3.66 (s, 6\text{H}). }^{13}\text{C NMR (101 MHz, DMSO-}d_{6}) \delta 173.23, 152.99, \\ 146.63, 124.52, 77.89, 48.12, 40.62. }^{19}\text{F NMR (376 MHz, DMSO-}d_{6}) \delta -77.74. \text{ HRMS m/z (ESI-TOF):} \\ \text{[M]}^{+} \text{ calcd for } C_{10}\text{H}_{14}\text{NO}_{2}\text{S 212.0740, found 212.0741.} \\ \end{array}$ 

#### 2.3. General procedure A for the thiol-sulfoxonium ylide reaction

To a glass flask was added sulfhydryl compound 2 (0.5 mmol), sulfoxonium ylide 1 (1 mmol), RFTA (2% mol) and 8 mL water. The flask was then sealed and equipped with magnetic bar. The reaction was stirred and irradiated with 450 nm LED light (18 W) for 60 min. The resulting solution was then purified directly *via* preparative HPLC after filtration. Desired distillates were identified by MS and lyophilized to obtain target products.

<sup>ACS</sup>  $\rightarrow$  <sup>COOH</sup> **2a**, white powder, yield 81%. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  12.82 (s, 1H), 8.25 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 7.4 Hz, 2H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 4.42 (td, *J* = 8.6, 5.0 Hz, 1H), 4.13 (d, *J* = 15.0 Hz, 1H), 4.02 (d, *J* = 15.1 Hz, 1H), 2.91 (dd, *J* = 13.6, 4.9 Hz, 1H), 2.73 (dd, *J* = 13.6, 8.8 Hz, 1H), 1.83 (s, 3H). <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  194.93, 172.27, 169.48, 135.37, 133.59, 128.89, 128.72, 51.62, 37.60, 33.07, 22.50. HRMS m/z (ESI-TOF): [M-H]<sup>-</sup> calcd for C<sub>13</sub>H<sub>14</sub>NO<sub>4</sub>S 280.0649, found 280.0643.

<sup>H</sup> <sup>II</sup> **2b**, white powder, yield 65%. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.28 (d, J = 8.1 Hz, 1H), 8.00 – 7.92 (m, 2H), 7.09 – 7.00 (m, 2H), 4.43 (td, J = 8.6, 4.9 Hz, 1H), 4.07 (d, J = 14.8 Hz, 1H), 3.96 (d, J = 14.8 Hz, 1H), 3.84 (s, 3H), 2.92 (dd, J = 13.6, 4.9 Hz, 1H), 2.74 (dd, J = 13.5, 8.9 Hz, 1H), 1.85 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO- $d_6$ )  $\delta$  193.44, 172.25, 169.46, 163.41, 131.06, 128.06, 114.02, 55.66, 51.51, 37.18, 32.96, 22.42. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>18</sub>NO<sub>5</sub>S 312.0900, found 312.0897.

**2c**, white powder, yield 82%. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.20 (d, J = 8.1 Hz, 1H), 8.04 - 7.95 (m, 2H), 7.35 - 7.24 (m, 2H), 4.36 (td, J = 8.6, 4.9 Hz, 1H), 4.06 (d, J = 14.9 Hz, 1H), 3.95 (d, J = 15.0 Hz, 1H), 2.84 (dd, J = 13.6, 4.9 Hz, 1H), 2.66 (dd, J = 13.6, 8.9 Hz, 1H), 1.77 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO- $d_6$ )  $\delta$  193.49, 172.20, 169.45, 166.46, 163.95, 131.95, 131.92, 131.76, 131.66, 115.96, 115.74, 51.45, 37.37, 32.90, 22.40. <sup>19</sup>F NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -74.10, -105.50, -105.52, -105.54. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>15</sub>FNO<sub>4</sub>S 300.0700, found 300.0702. <sup>AC</sup>  $H^{\circ}$  COOH **2d**, white powder, yield 72%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.28 (d, *J* = 8.1 Hz, 1H), 8.00 – 7.93 (m, 2H), 7.12 – 7.06 (m, 2H), 4.91 (d, *J* = 2.4 Hz, 2H), 4.43 (td, *J* = 8.5, 4.9 Hz, 1H), 4.07 (d, *J* = 14.8 Hz, 1H), 3.96 (d, *J* = 14.9 Hz, 1H), 3.63 – 3.62 (m, 1H), 2.96 – 2.86 (m, 1H), 2.78 – 2.68 (m, 1H), 1.85 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  193.69, 172.48, 169.77, 161.48, 131.20, 128.88, 115.08, 79.08, 79.00, 56.02, 51.74, 37.43, 33.19, 22.65. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>18</sub>NO<sub>5</sub>S 336.0900, found 336.0899.

<sup>H</sup> **C 2e**, white powder, yield 41%. <sup>1</sup>H NMR (400 MHz, Methanol- $d_4$ )  $\delta$  7.95 – 7.87 (m, 2H), 6.82 – 6.74 (m, 2H), 4.68 (dd, J = 8.6, 4.6 Hz, 1H), 4.00 – 3.86 (m, 2H), 3.11 (s, 7H), 2.92 (dd, J = 13.9, 8.6 Hz, 1H), 2.03 (s, 3H). <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  194.17, 172.36, 172.06, 154.09, 130.95, 122.81, 110.67, 51.94, 38.89, 36.54, 33.33, 21.05. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>15</sub>H<sub>21</sub>N<sub>2</sub>O<sub>4</sub>S 325.1217, found 325.1217.

<sup>H</sup>  $\overset{\parallel}{_{0}}$  **2g**, colorless oil, yield 79%. <sup>1</sup>H NMR (400 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  8.76 (s, 2H), 7.90 (s, 2H), 4.61 (dd, *J* = 8.4, 4.6 Hz, 1H), 4.10 – 3.90 (m, 2H), 3.02 (d, *J* = 9.4 Hz, 1H), 2.84 – 2.78 (m, 1H), 1.95 (s, 3H). <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  193.69, 172.02, 171.65, 149.94, 142.40, 122.42, 51.70, 32.98, 31.41, 21.05. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>15</sub>N<sub>2</sub>O<sub>4</sub>S 283.0747, found 283.0747.

<sup>Ac</sup> N<sup>+</sup> COOH **2h**, yellow powder, yield 74%. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.31 (d, *J* = 8.1 Hz, 1H), 8.07 – 7.99 (m, 2H), 7.27 (dd, *J* = 4.9, 3.8 Hz, 1H), 4.46 (td, *J* = 8.6, 4.9 Hz, 1H), 4.04 (d, *J* = 14.8 Hz, 1H), 3.97 (d, *J* = 14.8 Hz, 1H), 2.99 (dd, *J* = 13.6, 5.0 Hz, 1H), 2.80 (dd, *J* = 13.5, 8.9 Hz, 1H), 1.87 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  188.43, 172.23, 169.56, 142.36, 135.67, 134.44, 128.94, 51.57, 37.41, 33.22, 22.44. HRMS m/z (ESI-TOF): [M+H]<sup>+</sup> calcd for C<sub>11</sub>H<sub>14</sub>NO<sub>4</sub>S<sub>2</sub> 288.0359, found 288.0357.

<sup>1</sup>M<sub>2</sub> <sup>H</sup> <sup>b</sup> **3a**, white powder, yield 76%. <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ )  $\delta$  8.46 (d, J = 8.2 Hz, 1H), 7.97 (dd, J = 12.7, 6.4 Hz, 3H), 7.62 (t, J = 7.3 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 4.49 (q, J = 8.7 Hz, 1H), 4.11 (d, J = 15.1 Hz, 1H), 4.03 (d, J = 15.1 Hz, 1H), 3.50 (ddd, J = 40.2, 17.1, 4.9 Hz, 2H),

3.40 (t, J = 6.1 Hz, 1H), 2.92 (dd, J = 13.2, 4.3 Hz, 1H), 2.68 (dd, J = 13.2, 9.8 Hz, 1H), 2.54 (s, 2H), 2.33 (hept, J = 7.9 Hz, 2H), 1.96 (q, J = 6.8 Hz, 2H). <sup>13</sup>C NMR (126 MHz, DMSO- $d_6$ )  $\delta$  195.21, 172.48, 172.42, 171.72, 170.04, 159.36, 159.11, 158.86, 158.61, 135.48, 133.66, 128.96, 128.77, 120.83, 118.46, 116.08, 113.71, 53.62, 52.37, 40.43, 37.82, 33.84, 31.70, 27.07. HRMS m/z (ESI-TOF): [M-H]<sup>-</sup> calcd for C<sub>18</sub>H<sub>22</sub>N<sub>3</sub>O<sub>7</sub>S 424.1184, found 424.1187.

**4a**, yellow oil, yield 90%. The crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (15:1). <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.01 – 7.93 (m, 2H), 7.60 – 7.51 (m, 1H), 7.50 – 7.41 (m, 2H), 3.77 (s, 2H), 2.53 (td, J = 7.3, 0.8 Hz, 2H), 1.66 – 1.56 (m, 2H), 0.96 (td, J = 7.3, 0.8 Hz, 3H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  194.67, 135.28, 133.36, 128.85, 128.71, 37.07, 34.35, 22.34, 13.42. The NMR spectra are consistant with the reported literature.<sup>7</sup>

5a, yellow oil, yield 87%. The crud product was purified by flash column chromatography

using eluent solution Hexane/EtOAc (5:1). <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.01 – 7.93 (m, 2H), 7.64 – 7.54 (m, 1H), 7.52 – 7.43 (m, 2H), 3.89 (s, 2H), 3.77 (t, *J* = 5.9 Hz, 2H), 2.77 (t, *J* = 5.9 Hz, 2H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  195.27, 135.10, 133.72, 128.83, 60.72, 37.15, 35.37. The NMR spectra are consistant with the reported literature.<sup>11</sup>

**6a**, yellow oil, yield 91%. The crud product was purified by flash column chromatography using eluent solution Hexane/EtOAc (5:1). <sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.35 (dt, *J* = 4.6, 1.6 Hz, 2H), 7.96 (dt, *J* = 8.5, 1.3 Hz, 2H), 7.64 – 7.54 (m, 1H), 7.47 (td, *J* = 7.8, 1.6 Hz, 2H), 7.13 (dt, *J* = 4.6, 1.5 Hz, 2H), 4.40 (s, 2H). <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  192.93, 149.40, 147.56, 134.99, 134.09, 128.97, 128.68, 120.94, 38.04. HRMS m/z (ESI-TOF): [M-H]<sup>-</sup> calcd for C<sub>13</sub>H<sub>12</sub>NOS 230.0634, found 230.0637.

## 3. Supplementary material and information for peptide modification

#### 3.1. General information

All chemical reagents are commercially available from *Energy Chemical* without purification. Peptide 7, 12, 13, 14, Angiotensin II and Melanotan I were purchased from Wuxi Asiapeptide Biotechnology Co. Ltd.. The procedures for the preparation of peptide 8-11 were illustrated in the following section. High-Resolution Mass Spectrometry (HRMS) and MS/MS analysis were measured on a Q\_Exactive\_Focus. Mass Spectrometry (MS) to screen the molecular weight of HPLC fractions were carried out on SHIMAZU LC-MS 8030 in positive ion mode.

## **General procedure for SPPS**

Peptide 8-11 were synthesized on Rink Amide MBHA resin by Fmoc solid-phase peptide 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 N<sub>2</sub> 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.

## General method for estimating the conversions and LC/MS analysis

LC-MS conversions were estimated by UV absorption at 220 nm of the peak corresponding to the sulfoxonium ylide adducted product versus the sum of all peptide peaks (also checked by MS): % conversion =  $A_p/A_{all}$ .  $A_p$  is the peak area of sulfoxonium ylide-adducted products;  $A_{all}$  is the peak area of all peptide peaks. The remaining substrates, disulfide by-products, oxidized by-products (MS+16) and other peptidic by-products were calculated. Note that, some of the peaks possess MS signals around the peptide substrates, but we couldn't clearly assign the by-reactions. Thus, we termed these peaks as other peptidic by-products. All of the conversions were reported as an average of three trials. 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) or Inertsil ODS-SP C18 column (4.6 × 250 mm, 5 µm; room temperature). Water (containing 0.1% TFA, A phase) and pure CH<sub>3</sub>CN (B phase) were used as solvents in linear gradient mixtures at a flow rate of 1 mL·min<sup>-1</sup>.

#### General method for preparative LC

Preparative LC were performed on a Shimadzu LC-6AD system equipped with Shimadzu Shim-pack GIST C18 column ( $20 \times 250$  mm, 5 µm; room temperature). Water (containing 0.1% TFA, A phase) and pure CH<sub>3</sub>CN (B phase) were used as solvents in linear gradient mixtures at a flow rate of 8 mL·min<sup>-1</sup>.

#### 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/ $\mu$ l 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.

#### 3.2. General procedure B for the reaction of peptide

A 20 mM MeCN stock solution of RFTA was made up of 5.7 mg RFTA (0.01 mmol) and 0.5 mL MeCN, a 1 M water stock solution of thiourea was made up of 76 mg thiourea (1 mmol) and 1 mL deionized water, and a 100 mM water stock solution of sulfoxonium ylide was made up of specific sulfoxonium ylide (0.1 mmol) and 1 mL deionized water. These stock solutions were stored at room temperature away from light. To a 2 mL vial was added 200 uL specific peptide stock solution (1 mM, PB buffer, pH 7.4, 25% MeCN), 20  $\mu$ L specific sulfoxonium ylide (100 mM), 2  $\mu$ L thiourea (1 M) and 5  $\mu$ L RFTA (20 mM) stock solution. The vial was then caped and equipped with magnetic bar. The reaction was stirred and irradiated with 450 nm LED light (40 W) for 1 min, as shown in the following figure. The resulting solution was then analyzed directly *via* HPLC after filtration. Desired distillates were identified by MS and lyophilized to obtain target products.



## 3.3. Characterization data for the reaction of peptide 7



The reaction was followed **General procedure B** using peptide 7 (1 mM), sequence:  $NH_2$ -LAVCHMFPG-OH, and sulfoxonium ylide **1a-1m**. A stock solution of peptide 7 (1 mM) was made up by 5 mL PB buffer (pH 7.4, 25% MeCN) and 5 mg peptide 7.

## 3.3.1. Characterization of 7a



LC trace of the reaction of peptide 7 and 1a and purified product 7a. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Inertsil ODS-SP 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product 7a. Calculated Mass [M+H]<sup>+</sup>: 1092.50; [M+2H]<sup>2+</sup>: 546.75;

Mass Found (ESI+) [M+H]<sup>+</sup>: 1092.50; [M+2H]<sup>2+</sup>: 546.75.



ESI Mass spectrum of oxidized by-product of 7 (disulfide). Calculated Mass [M+H]<sup>+</sup>: 1945.90; [M+2H]<sup>2+</sup>: 973.95; [M+3H]<sup>3+</sup>: 649.64; Mass Found (ESI+) [M+H]<sup>+</sup>: 1946.90; [M+2H]<sup>2+</sup>: 973.95; [M+3H]<sup>3+</sup>: 649.64.



De novo ms/ms analysis of purified product 7a.

## 3.3.2. Characterization of 7b



LC trace of the reaction of peptide 7 and 1b and purified product 7b. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7b**. Calculated Mass [M+H]<sup>+</sup>: 1122.52; [M+2H]<sup>2+</sup>: 561.77; Mass Found (ESI+) [M+H]<sup>+</sup>: 1122.52; [M+2H]<sup>2+</sup>: 561.77.



De novo ms/ms analysis of purified product 7b.

**<sup>3.3.3.</sup>** Characterization of 7c



LC trace of the reaction of peptide 7 and 1c and purified product 7c. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7c**. Calculated Mass [M+H]<sup>+</sup>: 1110.50; [M+2H]<sup>2+</sup>: 555.76; Mass Found (ESI+) [M+H]<sup>+</sup>: 1110.50; [M+2H]<sup>2+</sup>: 555.76.



De novo ms/ms analysis of purified product 7c.

## 3.3.4. Characterization of 7d



LC trace of the reaction of peptide 7 and 1d and purified product 7d. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7d**. Calculated Mass [M+H]<sup>+</sup>: 1146.52; [M+2H]<sup>2+</sup>: 573.76; Mass Found (ESI+) [M+H]<sup>+</sup>: 1146.52; [M+2H]<sup>2+</sup>: 573.76.



ESI Mass spectrum of by-product (7 MS+17). Calculated Mass [M+H]<sup>+</sup>: 991.45; Mass Found (ESI+) [M+H]<sup>+</sup>: 991.55.



De novo ms/ms analysis of purified product 7d.

## 3.3.5. Characterization of 7e



LC trace of the reaction of peptide 7 and 1e and purified product 7e. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7e**. Calculated Mass [M+H]<sup>+</sup>: 1135.56; [M+2H]<sup>2+</sup>: 568.28; Mass Found (ESI+) [M+H]<sup>+</sup>: 1135.56; [M+2H]<sup>2+</sup>: 568.28.



De novo ms/ms analysis of purified product 7e.

## 3.3.6. Characterization of 7f



LC trace of the reaction of peptide 7 and 1f and purified product 7f. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column).

## 3.3.7. Characterization of 7g





LC trace of the reaction of peptide 7 and 1g and purified product 7g. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7g**. Calculated Mass [M+H]<sup>+</sup>: 1093.50; [M+2H]<sup>2+</sup>: 547.25; Mass Found (ESI+) [M+H]<sup>+</sup>: 1093.50; [M+2H]<sup>2+</sup>: 547.25.



ESI Mass spectrum of by-product (7g MS+19). Calculated Mass [M+H]<sup>+</sup>: 1112.50; Mass Found (ESI+) [M+H]<sup>+</sup>: 1112.30.



De novo ms/ms analysis of purified product 7g.

## 3.3.8. Characterization of 7h



LC trace of the reaction of peptide 7 and 1h and purified product 7h. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7h**. Calculated Mass [M+H]<sup>+</sup>: 1098.46; [M+2H]<sup>2+</sup>: 549.73; Mass Found (ESI+) [M+H]<sup>+</sup>: 1098.46; [M+2H]<sup>2+</sup>: 549.73.



De novo ms/ms analysis of purified product 7h.

## 3.3.9. Characterization of 7i



LC trace of the reaction of peptide 7 and 1i and purified product 7i. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column).

## 3.3.10. Characterization of 7j

$$H_{2N} \xrightarrow{} H_{2N} \xrightarrow{$$



LC trace of the reaction of peptide 7 and 1j and purified product 7j. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Inertsil ODS-SP 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7j**. Calculated Mass [M]<sup>+</sup>: 1149.56; [M+H]<sup>2+</sup>: 575.28; Mass Found (ESI+) [M]<sup>+</sup>: 1149.56; [M+Na]<sup>2+</sup>: 586.28; [M+H]<sup>2+</sup>: 575.28.



ESI Mass spectrum of Oxidized **7j** (MS+16). Calculated Mass [M]<sup>+</sup>: 1165.56; [M+H]<sup>2+</sup>: 583.28; Mass Found (ESI+) [M+H]<sup>2+</sup>: 583.05.



De novo ms/ms analysis of purified product 7j.

## 3.3.11. Characterization of 7k



LC trace of the reaction of peptide 7 and 1k and purified product 7k. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Inertsil ODS-SP 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7k**. Calculated Mass [M]<sup>+</sup>: 1107.51; [M+H]<sup>2+</sup>: 554.26; Mass Found (ESI+) [M]<sup>+</sup>: 1107.51; [M+Na]<sup>2+</sup>: 565.25; [M+H]<sup>2+</sup>: 554.26.



De novo ms/ms analysis of purified product 7k

#### 3.3.12. Characterization of 7l



LC trace of the reaction of peptide 7 and 11 and purified product 71. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **71**. Calculated Mass [M+H]<sup>+</sup>: 1278.59; [M+2H]<sup>2+</sup>: 639.80; Mass Found (ESI+) [M+H]<sup>+</sup>: 1278.59; [M+2H]<sup>2+</sup>: 639.80.



ESI Mass spectrum of By-product (7 MS+7). Calculated Mass [M+H]<sup>+</sup>: 980.45; Mass Found (ESI+) [M+H]<sup>+</sup>: 980.80.



De novo ms/ms analysis of purified product 71.

3.3.13. Characterization of 7m




LC trace of the reaction of peptide 7 and 1m and purified product 7m. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **7m**. Calculated Mass [M+H]<sup>+</sup>: 1347.60; [M+2H]<sup>2+</sup>: 674.31; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 674.31; [M+H+Na]<sup>2+</sup>: 685.30; [M+2Na]<sup>2+</sup>: 696.29.



ESI Mass spectrum of Oxidized **7m** (MS+16). Calculated Mass [M+H]<sup>+</sup>: 1363.60; [M+2H]<sup>2+</sup>: 682.31; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 681.60.



De novo ms/ms analysis of purified product 7m.

#### 3.4. Characterization data for the reaction of peptide 8



The reaction was followed **General procedure B** using peptide **8** (1 mM), sequence: Ac-WDE<u>C</u>-NH<sub>2</sub>, and sulfoxonium ylide **1a**. A stock solution of peptide **8** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 1 mg peptide **8**.



LC trace of the reaction of peptide **8** and **1a** and purified product **8a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **8a**. Calculated Mass [M+H]<sup>+</sup>: 705.26; Mass Found (ESI+) [M+H]<sup>+</sup>: 705.26.





ESI Mass spectrum of oxidized by-product of **8** (disulfide). Calculated Mass [M+H]<sup>+</sup>: 1193.44; [M+2H]<sup>2+</sup>: 586.23; Mass Found (ESI+) [M+H]<sup>+</sup>: 1193.44; [M+2H]<sup>2+</sup>: 586.23.



De novo ms/ms analysis of purified product 8a.

#### 3.5. Characterization data for the reaction of peptide 9



The reaction was followed **General procedure B** using peptide **9** (1 mM), sequence: Ac-WDE<u>C</u>-NH<sub>2</sub>, and sulfoxonium ylide **1a**. A stock solution of peptide **9** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 1 mg peptide **9**.



LC trace of the reaction of peptide **9** and **1a** and purified product **9a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **9a**. Calculated Mass [M+H]<sup>+</sup>: 751.37; Mass Found (ESI+) [M+H]<sup>+</sup>: 751.37.



ESI Mass spectrum of by-product (9 MS+78). Calculated Mass [M+H]<sup>+</sup>: 711.32; Mass Found (ESI+) [M+H]<sup>+</sup>: 711.15.



ESI Mass spectrum of oxidized by-product of **9** (disulfide). Calculated Mass  $[M+H]^+$ : 1262.63;  $[M+2H]^{2+}$ : 632.32; Mass Found (ESI+)  $[M+2H]^{2+}$ : 632.32.



De novo ms/ms analysis of purified product 9a.

#### 3.6. Characterization data for the reaction of peptide 10



The reaction was followed **General procedure B** using peptide **10** (1 mM), sequence: Ac-WDE<u>C</u>-NH<sub>2</sub>, and sulfoxonium ylide **1a**. A stock solution of peptide **10** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 1 mg peptide **10**.



LC trace of the reaction of peptide **10** and **1a** and purified product **10a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **10a**. Calculated Mass [M+H]<sup>+</sup>: 711.24; Mass Found (ESI+) [M+H]<sup>+</sup>: 711.24.



ESI Mass spectrum of oxidized **10a** (MS+16). Calculated Mass [M+H]<sup>+</sup>: 727.24; Mass Found (ESI+) [M+H]<sup>+</sup>: 727.30.



ESI Mass spectrum of oxidized by-product of **10** (disulfide). Calculated Mass [M+H]<sup>+</sup>: 1183.38; [M+2H]<sup>2+</sup>: 592.20; Mass Found (ESI+) [M+H]<sup>+</sup>: 1183.38; [M+2H]<sup>2+</sup>: 592.20.



De novo ms/ms analysis of purified product 10a.

#### 3.7. Characterization data for the reaction of peptide 11



The reaction was followed **General procedure B** using peptide **11** (1 mM), sequence: Ac-WDE<u>C</u>-NH<sub>2</sub>, and sulfoxonium ylide **1a**. A stock solution of peptide **11** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 1 mg peptide **11**.



LC trace of the reaction of peptide **11** and **1a** and purified product **11a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **11a**. Calculated Mass [M+H]<sup>+</sup>: 709.28; Mass Found (ESI+) [M+H]<sup>+</sup>: 709.28.





ESI Mass spectrum of oxidized by-product of **11** (disulfide). Calculated Mass [M+H]<sup>+</sup>: 1179.45; [M+2H]<sup>2+</sup>: 590.23; Mass Found (ESI+) [M+H]<sup>+</sup>: 1179.45; [M+2H]<sup>2+</sup>: 590.23.



De novo ms/ms analysis of purified product 11a.

#### 3.8. Characterization data for the reaction of peptide 12



The reaction was followed **General procedure B** using peptide **12** (1 mM), sequence: NH<sub>2</sub>-SY<u>C</u>DEFNWQTRHKM-OH, and sulfoxonium ylide **1a**, **1l** and **1m**. A stock solution of peptide **12** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 3.7 mg peptide **12**.

#### 3.8.1. Characterization of 12a



LC trace of the reaction of peptide **12** and **1a** and purified product **12a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **12a**. Calculated Mass [M+2H]<sup>2+</sup>: 982.42; [M+3H]<sup>3+</sup>: 655.28; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 982.42; [M+3H]<sup>3+</sup>: 655.28.



ESI Mass spectrum of oxidized by-product of **12** (disulfide). Calculated Mass [M+H]<sup>+</sup>: 3686.54; [M+2H]<sup>2+</sup>: 1844.78; [M+3H]<sup>3+</sup>: 1230.19; [M+4H]<sup>4+</sup>: 922.89; Mass Found (ESI+) Calculated Mass [M+H]<sup>+</sup>: 3686.54; [M+2H]<sup>2+</sup>: 1844.78; [M+3H]<sup>3+</sup>: 1230.19; [M+4H]<sup>4+</sup>: 922.89.



ESI Mass spectrum of oxidized **12** (MS+16). Calculated Mass [M-H]<sup>-</sup>: 1858.78; [M-2H]<sup>2-</sup>: 928.89; Mass Found (ESI+) Calculated Mass [M-2H]<sup>2-</sup>: 928.80.



De novo ms/ms analysis of purified product 12a.

The MS/MS searching on other nucleophilic residues of product 12a

The MS/MS searching on K site:

2+ y 13 y 12	y4 y3
SYCDEF	N WQTRHKM
ha	bs bs b11 b13

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	469.1388	598.1813	745.2498	859.2927	1045.3720	1173.4306	1274.4783	1430.5794	1567.6383	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	299.5943	373.1285	430.1500	523.1896	587.2189	637.7428	715.7933	784.3228	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1365.6521	1218.5837	1104.5407	918.4614	790.4029	689.3552	533.2541	396.1952	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	683.3297	609.7955	552.7740	459.7344	395.7051	345.1812	267.1307	198.6012	75.5328

The MS/MS searching on R site:

### 

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	469.1388	598.1813	745.2498	859.2927	1045.3720	1173.4306	1274.4783	1548.6212	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	299.5943	373.1285	430.1500	523.1896	587.2189	637.7428	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1365.6521	1218.5837	1104.5407	918.4614	790.4029	689.3552	415.2122	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	683.3297	609.7955	552.7740	459.7344	395.7051	345.1812	208.1097	139.5803	75.5328

The MS/MS searching on H site:

# 

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	469.1388	598.1813	745.2498	859.2927	1045.3720	1173.4306	1274.4783	1430.5794	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	299.5943	373.1285	430.1500	523.1896	587.2189	637.7428	715.7933	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1365.6521	1218.5837	1104.5407	918.4614	790.4029	689.3552	533.2541	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	683.3297	609.7955	552.7740	459.7344	395.7051	345.1812	267.1307	139.5803	75.5328

The MS/MS searching on Y site:

## $\begin{array}{c} 2 + & y^{13} & y^{11} & y^{10} & y^{18} & y^{18} & y^{12} & y^{12} & y^{12} & y^{12} & y^{12} \\ S & Y & C & D & F & N & W & Q & T & R & H & K \\ _{D3} & _{D4} & _{D5} & _{D6} & _{D7} & _{D6} & & _{D11} & _{D12} & _{D13} \end{array}$

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	369.1445	472.1537	587.1806	716.2232	863.2916	977.3345	1163.4139	1291.4724	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	44.5233	185.0759	236.5805	294.0939	358.6152	432.1494	489.1709	582.2106	646.2399	696.7637	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1594.6889	1491.6798	1376.6528	1247.6102	1100.5418	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	938.4007	797.8481	746.3435	688.8300	624.3088	550.7745	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

The MS/MS searching on S site:

## $\begin{array}{c} \begin{array}{c} 2+ \\ S \end{array} Y \hspace{0.5cm} C \hspace{0.5cm} \int \hspace{-.5cm} D \hspace{0.5cm} E \hspace{0.5cm} F \hspace{0.5cm} N \hspace{0.5cm} N \hspace{0.5cm} W \hspace{0.5cm} V \hspace{0.5cm} Y \hspace{0.5cm}$

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	206.0812	369.1445	472.1537	587.1806	716.2232	863.2916	977.3345	1163.4139	1291.4724	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	103.5442	185.0759	236.5805	294.0939	358.6152	432.1494	489.1709	582.2106	646.2399	696.7637	774.8143	843.3437	907.3912	*
y+	*	1757.7523	1594.6889	1491.6798	1376.6528	1247.6102	1100.5418	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	879.3798	797.8481	746.3435	688.8300	624.3088	550.7745	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

The MS/MS searching on T site:

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	469.1388	598.1813	745.2498	859.2927	1045.3720	1173.4306	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	299.5943	373.1285	430.1500	523.1896	587.2189	696.7637	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1365.6521	1218.5837	1104.5407	918.4614	790.4029	571.3133	415.2122	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	683.3297	609.7955	552.7740	459.7344	395.7051	286.1603	208.1097	139.5803	75.5328

The MS/MS searching on D site:

## 

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	587.1806	716.2232	863.2916	977.3345	1163.4139	1291.4724	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	294.0939	358.6152	432.1494	489.1709	582.2106	646.2399	696.7637	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1376.6528	1247.6102	1100.5418	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	688.8300	624.3088	550.7745	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

The MS/MS searching on E site:

#### 2+ S Y C D E F N W Q T R H K M b2 b5 b6 b7 b8 b11 b12 b13

#	S(1/*)	V(2/13)	C(3/12)	D(4/11)	F(5/10)	F(6/9)	N(7/8)	W(8/7)	0(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
	5(17)	1(2/13)	C(0/12)	0(4/11)	2(0)10)	1(0/0)	11(170)	(0//)	4(0)0)	1(10/5)	14(11)-1)	11(12/0)	14(10/2)	
b+	88.0393	251.1026	354.1118	469.1388	716.2232	863.2916	977.3345	1163.4139	1291.4724	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	358.6152	432.1494	489.1709	582.2106	646.2399	696.7637	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1247.6102	1100.5418	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	624.3088	550.7745	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

The MS/MS searching on Q site:

#### 2+ S Y C D E F N W Q T R H K M b2 b3 b3 b3 b11 b12 b13

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	469.1388	598.1813	745.2498	859.2927	1045.3720	1291.4724	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	299.5943	373.1285	430.1500	523.1896	646.2399	696.7637	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1365.6521	1218.5837	1104.5407	918.4614	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	683.3297	609.7955	552.7740	459.7344	336.6841	286.1603	208.1097	139.5803	75.5328

The MS/MS searching on N site:

#### 2+ y15 y12 S Y C D E F N W Q T R H K M b2 b2 b7 b8 b11 b12 b13

#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	88.0393	251.1026	354.1118	469.1388	598.1813	745.2498	977.3345	1163.4139	1291.4724	1392.5201	1548.6212	1685.6801	1813.7751	*
b++	44.5233	126.0550	177.5595	235.0730	299.5943	373.1285	489.1709	582.2106	646.2399	696.7637	774.8143	843.3437	907.3912	*
y+	*	1875.7941	1712.7308	1609.7216	1494.6947	1365.6521	1218.5837	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	938.4007	856.8690	805.3644	747.8510	683.3297	609.7955	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

In order to further confirm the potential functionalization on other nucleophilic residues, we have used the pLabel (v2.4) software<sup>12</sup> to automatically match the b-/y- ion fragments of products under the default parameters with a TOL (0.5 Da), the matching mass tolerance of fragment ions. As shown above, the analysis on the C site exhibited abundant MS fragments and the key fragment **b3** and **y12** were found. On the contrary, the analysis on the other nucleophilic sites exhibited a small number of fragments. Especially, the reliable match for site-adjacent key fragments were absent for these residues. For example, even though a few key fragments, **y3** (689.4) and **y4** (267.1), for K and H site were coincidently found, but the fragments between C3 and W8 were completely absent in the searching. Thus, the results demonstrated that the cysteine site is the only possible adducted position in product **12a**.



LC trace of the reaction of peptide **12** and **11** and purified product **121**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column).

#### 3.8.3. Characterization of 12m



LC trace of the reaction of peptide **12** and **1m** and purified product **12m**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **12m**. Calculated Mass [M+H]<sup>+</sup>: 2217.93; [M+2H]<sup>2+</sup>: 1109.97; [M+3H]<sup>3+</sup>: 740.31; Mass Found (ESI+) [M+H+Na]<sup>2+</sup>: 1120.46; [M+2H]<sup>2+</sup>: 1109.97; [M+3H]<sup>3+</sup>: 740.31.



ESI Mass spectrum of oxidized **12** (MS+16). Calculated Mass [M-H]<sup>-</sup>: 1858.78; [M-2H]<sup>2-</sup>: 928.89; Mass Found (ESI+) Calculated Mass [M-2H]<sup>2-</sup>: 929.00.



ESI Mass spectrum of oxidized **12m** (MS+16). Calculated Mass [M+H]<sup>+</sup>: 2233.92; [M+2H]<sup>2+</sup>: 1117.46; [M+3H]<sup>3+</sup>: 745.31; Mass Found (ESI+) Calculated Mass [M+3H]<sup>3+</sup>: 746.15.



De novo ms/ms analysis of purified product 12m.

#### 3.9. Characterization data for the reaction of peptide 13



The reaction was followed **General procedure B** using peptide **13** (1 mM), sequence: NH2-LITQLMPFGCLLE, and sulfoxonium ylide **1a**, **1l** and **1m**. A stock solution of peptide **13** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 2.9 mg peptide **13**.

#### 3.9.1. Characterization of 13a



LC trace of the reaction of peptide **13** and **1a** and purified product **13a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **13a**. Calculated Mass [M+H]<sup>+</sup>: 1581.81; [M+2H]<sup>2+</sup>: 791.41; Mass Found (ESI+) [M+H]<sup>+</sup>: 1581.81; [M+2H]<sup>2+</sup>: 791.41.



ESI Mass spectrum of oxidized by-product of **13** (disulfide). Calculated Mass [M+H]<sup>+</sup>: 2924.50; [M+2H]<sup>2+</sup>: 1463.75; [M+3H]<sup>3+</sup>: 975.84; Mass Found (ESI+) [M+H]<sup>+</sup>: 2924.50; [M+2H]<sup>2+</sup>: 1463.75; [M+3H]<sup>3+</sup>: 975.84.



De novo ms/ms analysis of purified product 13a.

#### The MS/MS searching on other nucleophilic residues of product 13a

The MS/MS searching on T site:

#	L(1/*)	I(2/12)	T(3/11)	Q(4/10)	L(5/9)	M(6/8)	P(7/7)	F(8/6)	G(9/5)	C(10/4)	L(11/3)	L(12/2)	D(*/1)
b+	114.0913	227.1754	446.2649	574.3235	687.4076	818.4481	915.5008	1062.5692	1119.5907	1222.5999	1335.6840	1448.7680	*
b++	57.5493	114.0913	223.6361	287.6654	344.2074	409.7277	458.2541	531.7883	560.2990	611.8036	668.3456	724.8876	*
y+	*	1468.7215	1355.6374	1136.5479	1008.4893	895.4052	764.3647	667.3120	520.2436	463.2221	360.2129	247.1288	134.0448
y++	*	734.8644	678.3223	568.7776	504.7483	448.2062	382.6860	334.1596	260.6254	232.1147	180.6101	124.0681	67.5260

The MS/MS searching on Q site:

#	L(1/*)	I(2/12)	T(3/11)	Q(4/10)	L(5/9)	M(6/8)	P(7/7)	F(8/6)	G(9/5)	C(10/4)	L(11/3)	L(12/2)	D(*/1)
b+	114.0913	227.1754	328.2231	574.3235	687.4076	818.4481	915.5008	1062.5692	1119.5907	1222.5999	1335.6840	1448.7680	*
b++	57.5493	114.0913	164.6152	287.6654	344.2074	409.7277	458.2541	531.7883	560.2990	611.8036	668.3456	724.8876	*
y+	*	1468.7215	1355.6374	1254.5897	1008.4893	895.4052	764.3647	667.3120	520.2436	463.2221	360.2129	247.1288	134.0448
y++	*	734.8644	678.3223	627.7985	504.7483	448.2062	382.6860	334.1596	260.6254	232.1147	180.6101	124.0681	67.5260

The MS/MS searching on D site:

#	L(1/*)	I(2/12)	T(3/11)	Q(4/10)	L(5/9)	M(6/8)	P(7/7)	F(8/6)	G(9/5)	C(10/4)	L(11/3)	L(12/2)	D(*/1)
b+	114.0913	227.1754	328.2231	456.2817	569.3657	700.4062	797.4590	944.5274	1001.5488	1104.5580	1217.6421	1330.7262	*
b++	57.5493	114.0913	164.6152	228.6445	285.1865	350.7067	399.2331	472.7673	501.2781	552.7827	609.3247	665.8667	*
y+	*	1468.7215	1355.6374	1254.5897	1126.5311	1013.4471	882.4066	785.3538	638.2854	581.2640	478.2548	365.1707	252.0866
y++	*	734.8644	678.3223	627.7985	563.7692	507.2272	441.7069	393.1805	319.6463	291.1356	239.6310	183.0890	126.5470

In order to further confirm the potential functionalization on other nucleophilic residues, we have used the pLabel (v2.4) software<sup>12</sup> to automatically match the b-/y- ion fragments of products under the default parameters with a TOL (0.5 Da), the matching mass tolerance of fragment ions. As shown above, the analysis on the C site exhibited abundant MS fragments and the key fragment **b10** and **y5** were found. On the contrary, the analysis on the other nucleophilic sites exhibited a small number of fragments. Especially, the reliable match for site-adjacent key fragments were absent for these residues. Thus, the results demonstrated that the cysteine site is the only possible adducted position in product **13a**.

#### 3.9.2. Characterization of 131





LC trace of the reaction of peptide **13** and **11** and purified product **131**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **13I**. Calculated Mass [M+H]<sup>+</sup>: 1768.93; [M+2H]<sup>2+</sup>: 884.97; Mass Found (ESI+) [M+H]<sup>+</sup>: 1768.93; [M+2H]<sup>2+</sup>: 884.97.



ESI Mass spectrum of by-product (**13** MS-102). Calculated Mass [M+H]<sup>+</sup>: 1361.76; [M+2H]<sup>2+</sup>: 681.38; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 681.35.



ESI Mass spectrum of by-product (**13** MS+190). Calculated Mass [M+H]<sup>+</sup>: 1653.76; [M+2H]<sup>2+</sup>: 827.38; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 827.60



De novo ms/ms analysis of purified product 131.

#### 3.9.3. Characterization of 13m



LC trace of the reaction of peptide **13** and **1m** and purified product **13m**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **13m**. Calculated Mass [M+H]<sup>+</sup>: 1837.91; [M+2H]<sup>2+</sup>: 919.46; Mass Found (ESI+) [M+H]<sup>+</sup>: 1837.91; [M+2H]<sup>2+</sup>: 919.46.



De novo ms/ms analysis of purified product 13m.

#### 3.10. Characterization data for the reaction of peptide 14



The reaction was followed **General procedure B** using peptide **14** (1 mM), sequence: NH2-VHLLE<u>C</u>AWLEIGLV-OH, and sulfoxonium ylide **1a**, **1l** and **1m**. A stock solution of peptide **14** (1 mM) was made up by 2 mL PB buffer (pH 7.4, 25% MeCN) and 3.2 mg peptide **14**.

#### 3.10.1. Characterization of 14a



LC trace of the reaction of peptide **14** and **1a** and purified product **14a**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **14a**. Calculated Mass [M+H]<sup>+</sup>: 1712.91; [M+2H]<sup>2+</sup>: 857.46; Mass Found (ESI+) [M+H]<sup>+</sup>: 1712.91; [M+2H]<sup>2+</sup>: 857.46.



ESI Mass spectrum of the remaining **14**. Calculated Mass [M+H]<sup>+</sup>: 1594.87; [M+2H]<sup>2+</sup>: 797.94; Mass Found (ESI+) [M+H]<sup>+</sup>: 1594.87; [M+2H]<sup>2+</sup>: 797.94.



De novo ms/ms analysis of purified product 14a.

#### The MS/MS searching on other nucleophilic residues of product 14a

The MS/MS searching on T site:

$$\begin{array}{c} 2 + & y_6 \hspace{0.1 cm} y_5 \hspace{0.1 cm} y_5 \hspace{0.1 cm} y_4 \hspace{0.1 cm} y_2 \hspace{0.1 cm} y_2 \hspace{0.1 cm} y_1 \\ V \hspace{0.1 cm} H \hspace{0.1 cm} L \hspace{0.1 cm} L \hspace{0.1 cm} E \hspace{0.1 cm} C \hspace{0.1 cm} | \hspace{0.1 cm} A \hspace{0.1 cm} | \hspace{0.1 cm} W \hspace{0.1 cm} | \hspace{0.1 cm} L \hspace{0.1 cm} E \hspace{0.1 cm} | \hspace{0.1 cm} I \hspace{0.1 cm} | \hspace{0.1 cm} G \hspace{0.1 cm} | \hspace{0.1 cm} L \hspace{0.1 cm} | \hspace{0.1 cm} V \\ b_6 \hspace{0.1 cm} b_7 \hspace{0.1 cm} b_8 \hspace{0.1 cm} b_9 \hspace{0.1 cm} b_{10} \hspace{0.1 cm} b_{11} \hspace{0.1 cm} b_{12} \end{array}$$

-						1	-							
#	V(1/*)	H(2/13)	L(3/12)	L(4/11)	E(5/10)	C(6/9)	A(7/8)	W(8/7)	L(9/6)	E(10/5)	I(11/4)	G(12/3)	L(13/2)	V(*/1)
b+	100.0757	355.1765	468.2605	581.3446	710.3872	813.3964	884.4335	1070.5128	1183.5969	1312.6394	1425.7235	1482.7450	1595.8290	*
b++	50.5415	178.0919	234.6339	291.1759	355.6972	407.2018	442.7204	535.7600	592.3021	656.8234	713.3654	741.8761	798.4181	*
y+	*	1613.8396	1358.7388	1245.6548	1132.5707	1003.5281	900.5189	829.4818	643.4025	530.3184	401.2758	288.1918	231.1703	118.0863
y++	*	807.4234	679.8730	623.3310	566.7890	502.2677	450.7631	415.2445	322.2049	265.6629	201.1416	144.5995	116.0888	59.5468

The MS/MS searching on E5 site:

#	V(1/*)	H(2/13)	L(3/12)	L(4/11)	E(5/10)	C(6/9)	A(7/8)	W(8/7)	L(9/6)	E(10/5)	I(11/4)	G(12/3)	L(13/2)	V(*/1)
b+	100.0757	237.1346	350.2187	463.3027	710.3872	813.3964	884.4335	1070.5128	1183.5969	1312.6394	1425.7235	1482.7450	1595.8290	*
b++	50.5415	119.0709	175.6130	232.1550	355.6972	407.2018	442.7204	535.7600	592.3021	656.8234	713.3654	741.8761	798.4181	*
y+	*	1613.8396	1476.7807	1363.6966	1250.6125	1003.5281	900.5189	829.4818	643.4025	530.3184	401.2758	288.1918	231.1703	118.0863
y++	*	807.4234	738.8940	682.3519	625.8099	502.2677	450.7631	415.2445	322.2049	265.6629	201.1416	144.5995	116.0888	59.5468

The MS/MS searching on E10 site:

#	V(1/*)	H(2/13)	L(3/12)	L(4/11)	E(5/10)	C(6/9)	A(7/8)	W(8/7)	L(9/6)	E(10/5)	I(11/4)	G(12/3)	L(13/2)	V(*/1)
b+	100.0757	237.1346	350.2187	463.3027	592.3453	695.3545	766.3916	952.4709	1065.5550	1312.6394	1425.7235	1482.7450	1595.8290	*
b++	50.5415	119.0709	175.6130	232.1550	296.6763	348.1809	383.6994	476.7391	533.2811	656.8234	713.3654	741.8761	798.4181	*
y+	*	1613.8396	1476.7807	1363.6966	1250.6125	1121.5700	1018.5608	947.5237	761.4443	648.3603	401.2758	288.1918	231.1703	118.0863
y++	*	807.4234	738.8940	682.3519	625.8099	561.2886	509.7840	474.2655	381.2258	324.6838	201.1416	144.5995	116.0888	59.5468

In order to further confirm the potential functionalization on other nucleophilic residues, we have used the pLabel (v2.4) software<sup>12</sup> to automatically match the b-/y- ion fragments of products under the default parameters with a TOL (0.5 Da), the matching mass tolerance of fragment ions. As shown above, the analysis on the C site exhibited abundant MS fragments and the key fragment **b6** were found. On the contrary, the analysis on the other nucleophilic sites exhibited a small number of fragments. Especially, the reliable match for site-adjacent key fragments were absent for these residues. For example, even though the key fragments, **y5** (648.4), for E site were coincidently found, but the fragments between C6 and E10 were completely absent in the searching. Thus, the results demonstrated that the cysteine site is the only possible adducted position in product **14a**.

3.10.2. Characterization of 14l





LC trace of the reaction of peptide **14** and **11** and purified product **141**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **14I**. Calculated Mass [M+H]<sup>+</sup>: 1899.00; [M+2H]<sup>2+</sup>: 950.50; Mass Found (ESI+) [M+H]<sup>+</sup>: 1899.00; [M+2H]<sup>2+</sup>: 950.50.



ESI Mass spectrum of by-product (14 MS+21). Calculated Mass [M+H]<sup>+</sup>: 1615.86; [M+2H]<sup>2+</sup>: 808.43; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 808.60.



ESI Mass spectrum of by-product (**14** MS+107). Calculated Mass [M+H]<sup>+</sup>: 1701.86; [M+2H]<sup>2+</sup>: 851.43; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 851.40.



De novo ms/ms analysis of purified product 14l.

#### 3.10.3. Characterization of 14m



LC trace of the reaction of peptide **14** and **1m** and purified product **14m**. (10-80% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The table refer to the normalized integration of the peptide peaks.



ESI Mass spectrum of purified product **14m**. Calculated Mass [M+H]<sup>+</sup>: 1968.01; [M+2H]<sup>2+</sup>: 985.01; Mass Found (ESI+) [M+H]<sup>+</sup>: 1968.01; [M+2H]<sup>2+</sup>: 985.01.



ESI Mass spectrum of by-product (**14** MS+107). Calculated Mass [M+H]<sup>+</sup>: 1701.86; [M+2H]<sup>2+</sup>: 851.43; Mass Found (ESI+) [M+2H]<sup>2+</sup>: 851.20.



De novo ms/ms analysis of purified product 14m.

#### 3.11. Characterization data for the reaction of peptide I-III

#### 3.11.1. The reaction peptide I



The reaction was followed **General procedure B** using peptide I (1 mM), sequence:  $NH_2$ -SYC(IAM)DEFNWQTRHKM-OH, and sulfoxonium ylide **1a**. A stock solution of peptide I (1 mM) was made up by 1 mL PB buffer (pH 7.4, 25% MeCN) and 1.9 mg peptide I.



LC trace of the reaction of peptide I and 1a. (10-70% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Inertsil ODS-SP 4.6 × 250 mm, 5 µm column).



ESI Mass spectrum of remaining peptide I. Calculated Mass [M+H]<sup>+</sup>: 1901.80; [M+2H]<sup>2+</sup>: 951.41; Mass Found (ESI+) [M+H]<sup>+</sup>: 1902.81; [M+2H]<sup>2+</sup>: 951.91.

#### 3.11.2. The reaction peptide II



The reaction was followed **General procedure B** using peptide **II** (1 mM), sequence: NH<sub>2</sub>-DRVYIHPF-OH, and sulfoxonium ylide **1a**. A stock solution of peptide **II** (1 mM) was made up by 1 mL PB buffer (pH 7.4, 25% MeCN) and 1.1 mg peptide **II**.



LC trace of the reaction of peptide I and 1a. (10-70% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Inertsil ODS-SP 4.6 × 250 mm, 5 µm column).



ESI Mass spectrum of remaining peptide I. Calculated Mass [M+H]<sup>+</sup>: 1901.80; [M+2H]<sup>2+</sup>: 951.41; Mass Found (ESI+) [M+H]<sup>+</sup>: 1902.81; [M+2H]<sup>2+</sup>: 951.91.

#### 3.11.3. The reaction peptide III



The reaction was followed **General procedure B** using peptide **III** (1 mM), sequence: Ac-SYSNleEHfRWKPV-NH<sub>2</sub>, and sulfoxonium ylide **1a**. A stock solution of peptide **III** (1 mM) was made up by 1 mL PB buffer (pH 7.4, 25% MeCN) and 1.6 mg peptide **III**.



LC trace of the reaction of peptide I and 1a. (10-70% B phase over 25 min, 1 mL·min<sup>-1</sup> flow rate, 0.1% TFA,  $\lambda = 220$  nm, Inertsil ODS-SP 4.6 × 250 mm, 5 µm column).



ESI Mass spectrum of remaining peptide I. Calculated Mass [M+H]<sup>+</sup>: 1901.80; [M+2H]<sup>2+</sup>: 951.41; Mass Found (ESI+) [M+H]<sup>+</sup>: 1902.81; [M+2H]<sup>2+</sup>: 951.91.

#### 4. Mechanistic experiments

#### 4.1. Luminescence screening

The luminescence screening was performed by following a similar procedure of previous report<sup>13</sup>. In order to consistent with the reaction conditions, PB buffer (pH 7.4, 25% MeCN) was used as the solvent for luminescence experiments. A photocatalyst concentration of 20  $\mu$ M was used throughout the experiments along with substrate concentrations of 20 mM, which equates to 1000 equivalents of each potential quencher relative to the photocatalyst. The luminescence emission spectrum of each sample excited at 420 nm was measured twice and an average was taken. The emission intensity (*I*) at a predefined wavelength was noted and compared with that of the photocatalyst in isolation (*I*<sub>0</sub>). The amount of decrease in the emission intensity was then quantified as a "quenching percentage" (*F*) defined by the following formula:

$$F(\%) = 100 \left(1 - \frac{I}{I_0}\right)\%$$

Equation 1

The structure of the photocatalysts employed in this study are shown in **Figure S2**. The results were listed in Table **S1** and Figure **S10** to compare with the reaction conversions of **1a** and peptide **7**. The luminescence spectra were summarized in **Figure S3**.

#### 4.2. Stern-Volmer luminescence quenching studies

Stern-Volmer luminescence quenching studies were carried out using a 20  $\mu$ M solution of RFTA and variable concentrations of sulfoxonium ylide **1a** and Ac-Cys-OH **2** in PB buffer (pH 7.4, 25% MeCN). Two independent duplications were performed for two substrates. The solutions were irradiated at 420 nm and the luminescence was measured at 530 nm ( $I_0$  = emission intensity of the photocatalyst in isolation at the specified wavelength; I = observed intensity as a function of the quencher concentration). The luminescence spectra and were summarized in **Figure S7**.

#### 4.3. UV/vis absorption studies

UV/vis absorption spectra of the starting materials were observed in PB buffer (pH 7.4, 25% MeCN): RFTA (20 uM), sulfoxonium ylide **1a** (20 mM) and Ac-Cys-OH **2** (20 mM). The photocatalyst RFTA presented a maximum absorption at 450 nm in visible light region, and the substrates **1a** and **2** didn't show any absorption in visible light region as shown in **Figure S8**. Therefore, the photocatalysts were the only absorbing species within the reaction mixture.

### 4.4. Cyclic Voltammogram

Cycle voltammetry (CV) measurements were carried out using an electrochemical workstation (CHI600E). Insets show the reference of  $Fc/Fc^+$ 

1a HOMO Init E (V) = 0 High E (V) = 1.7 Low E (V) = 0 Init P/N = P Scan Rate (V/s) = 0.1 Segment = 2 Sample Interval (V) = 0.001 Quiet Time (sec) = 2



### RFTA HOMO

Init E (V) = 0 High E (V) = 2.6 Low E (V) = 0 Init P/N = P Scan Rate (V/s) = 0.1 Segment = 2 Sample Interval (V) = 0.001 Quiet Time (sec) = 2



#### 5. Supplementary material and information for protein modification

#### 5.1. General information

All chemical reagents are commercially available from *Energy Chemical* without purification. **High-Resolution Mass Spectrometry (HRMS)** and **MS/MS** analysis were measured on a Q\_Exactive\_Focus.

#### 5.2. General procedure C for the reaction of protein and proteome

A 20 mM MeCN stock solution of RFTA was made up of 5.7 mg RFTA (0.01 mmol) and 0.5 mL MeCN, a 1 M water stock solution of thiourea was made up of 76 mg thiourea (1 mmol) and 1 mL deionized water, and a 100 mM water stock solution of sulfoxonium ylide was made up of specific sulfoxonium ylide (0.1 mmol) and 1 mL deionized water. These stock solutions were stored at room temperature away from light. To a 2 mL vial was added 200 uL specific protein (50  $\mu$ M) or cell lysate (2 mg/mL) PB buffer (pH 7.4) solution and 0.4  $\mu$ L RFTA (20 mM), 10  $\mu$ L specific sulfoxonium ylide (100 mM) and 2  $\mu$ L thiourea (1 M) stock solution. The vial was then caped and equipped with magnetic bar. The sealed vial was degassed and refilled with nitrogen. The reaction was stirred and irradiated with 450 nm LED light (40 W) for 10 s, as shown in the following figure. The resulting solution was then subjected for the analyzation of ESI-TOF MS, MS/MS as well as Western-blotting.



#### 5.3. LC-MS analysis

Ultrafiltration centrifuge tubes are wetting before use by MilliQ water. The 50  $\mu$ L resulting solution for the reaction of BSA was added to ultrafiltration centrifuge tubes for the analyzation of ESI-TOF MS, respectively. The tubes were washing three times with 200  $\mu$ L MilliQ water to remove residue small molecule weight substrates by centrifugation with 5000 rpm. The protein concentration was determined by Nanodrop and waiting for ESI-TOF MS analysis.

#### 5.4. LC-MS/MS analysis

The 50  $\mu$ L resulting solution for BSA was added to 1.5 ml tube and 5  $\mu$ L 5× loading buffer was added to this mixture following by in-gel SDS-PAGE scanning. The gel was stained by coomassie blue and destained by decolorant. The protein lane was cut in to pieces, washed by ddH<sub>2</sub>O and decolorized. Cuticle was subjected to reduction and alkylation with 10 mM DTT at 37 °C for 30

min and 10 mM IAA in the dark at room temperature for 30 min. The mixture was washed with 100 $\mu$ L ddH<sub>2</sub>O, dehydrated by 50% acetonitrile, 100% acetonitrile and immersed with NH<sub>4</sub>HCO<sub>3</sub> solution containing 1  $\mu$ g trypsin overnight 17 hours. The supernatant was collected by centrifugation (1400 g, 3 min) and combined with 2 × 50  $\mu$ L extraction liquid after incubating for 30 min, add 5% (v/v) formic acid to stop digest, stored at -80 °C and desalted for LC-MS/MS analysis.

### 6. Supplementary material and information for chemoproteomics analysis

#### 6.1. Western blot

The probe **1m** incubated with BSA, Hela and MCF7 cell lysate under the above modified reaction condition, respectively, samples without **1m** as control. Loading buffer was added to these samples following by in-gel SDS-PAGE (10% acrylamide, Marker: MIKX<sup>TM</sup> Tris-Glycine 4-20% DB245 10-245 kD) and electrophoresis transfer. The nitrocellulose filter membrane was blocked by 5% milk for 1 hour and incubated with anti-biotin antibody (rabbit) for 3 hours at room temperature. The nitrocellulose filter membrane was scanned with e-BLOT by chemiluminescence.

#### 6.2. MS-based proteomics

The harvested cells were lysed by sonication (1s work, 1s break,10 pulses, 50% duty cycle, output setting = 3). The soluble (supernatant) and membrane (pellet) was separated by centrifugation (12000rpm, 10 min × 2 times, 4 °C). Protein concentration was determined by BCA assay. Each samples contained 2 mg proteomes and was treated with biotinylated sulfoxonium ylide (5 mM) in vitro for 10 seconds 450 nm light irradiation, then cold methanol (volume ratio = 1:3) was added to the above solution and appeared precipitation in a short time. The precipitation protein was separated by centrifugation at 4 °C, 14,000 rpm for 10 min and wash twice by 300 µL methanol. The precipitation followed by solubilized in 300  $\mu$ L PBS containing 1.2% SDS by sonication then dilute to 0.2% SDS in PBS. A volume of 50 µL of streptavidin-agarose beads slurry was added to the solution and incubated for 3 hours at 29 °C. After incubation, the beads were pelleted by centrifugation and washed (3  $\times$  1 mL cold PBS and 3  $\times$  1 mL cold water) and resuspended in PBS containing 6 M urea. Subsequently, 10 mM DTT was added to the beads containing solution and incubated at 37 °C for 30 min and 20 mM IAM was added and reacted at 37 °C for 30 min in dark. The bead mixture was diluted with PBS containing 2 M urea and incubated with 2 µg trypsin overnight. The system was acidified to a final concentration of 2% (v/v) formic acid and the beads were further washed (3  $\times$  0.6 mL cold PBS and 3  $\times$  1 mL cold water) to remove the unmodified peptides. The beads were then incubated with 60 µL washing buffer (80% acetonitrile, 0.1% formic acid in water) for 10min at 25 °C and for 10min at 72 °C, respectively, to release probe-modified peptides. Combined the washing buffer dried for LC-MS/MS analysis.
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## 8. NMR spectra



<sup>13</sup>C NMR spectrum of **1a** 























<sup>19</sup>F NMR spectrum of **1**j



S85



<sup>19</sup>F NMR spectrum of **1k** 









<sup>1</sup>H NMR spectrum of S2

























<sup>1</sup>H-<sup>13</sup>C HSQC NMR spectrum of 2a















<sup>1</sup>H-<sup>13</sup>C HSQC NMR spectrum of **2c** 







<sup>1</sup>H-<sup>1</sup>H COSY NMR spectrum of **2d** 



<sup>1</sup>H-<sup>13</sup>C HMBC NMR spectrum of **2d**




<sup>1</sup>H-<sup>13</sup>C HSQC NMR spectrum of **2e** 





S112













<sup>1</sup>H-<sup>13</sup>C HMBC NMR spectrum of **2h** 











