Supporting information

Photoaffinity SAM analogues for the identification of SAM-binding proteins

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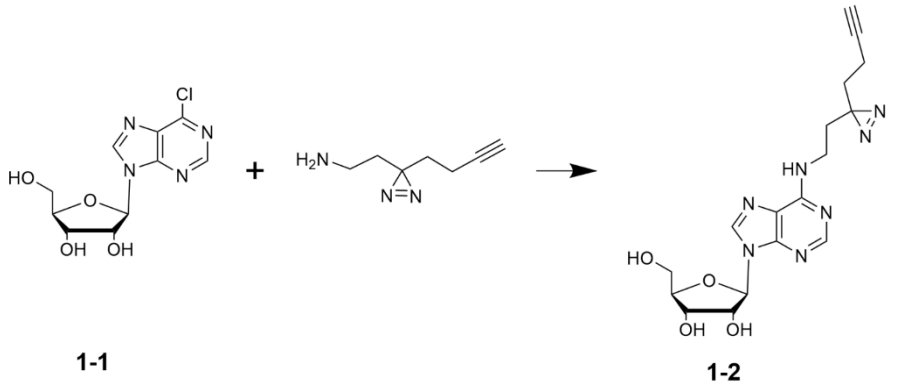
A. Reagents and Methods

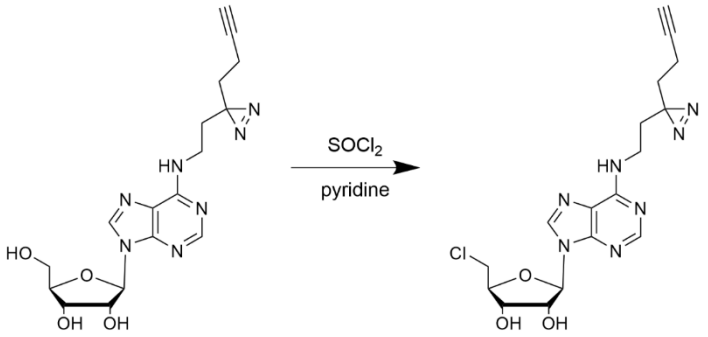
General methods. The reagents for organic synthesis were purchased from Sigma-Aldrich or Bidepharm. ^{1}H NMR was performed on JEOL 600 MHz and Bruker 800 MHz spectrometers. NMR data were analyzed via MestReNova. Analytic HPLC analysis was performed on a SHIMADZU C18 column (150 mm × 4.6 mm, 5 μm) monitored at 215 and 260 nm. Preparative HPLC purification was carried out using SHIMADZU C18 column (250 mm × 4.6 mm, 5 μm) monitoring at 215 and 260 nm. The solvents used for analytical HPLC analysis and preparative HPLC purification were water with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B). LCMS analysis was carried out on a Bruker miorOTOF-QII with a Phenomenex Luna C18 column (100 mm × 4.6 mm, 2.5 μm). The solvents for LCMS were water with 0.1% formic acid (solvent A) and acetonitrile (solvent B). Protein purification was performed in an anaerobic chamber (Coy Laboratory Products).

Synthesis of Probe 1



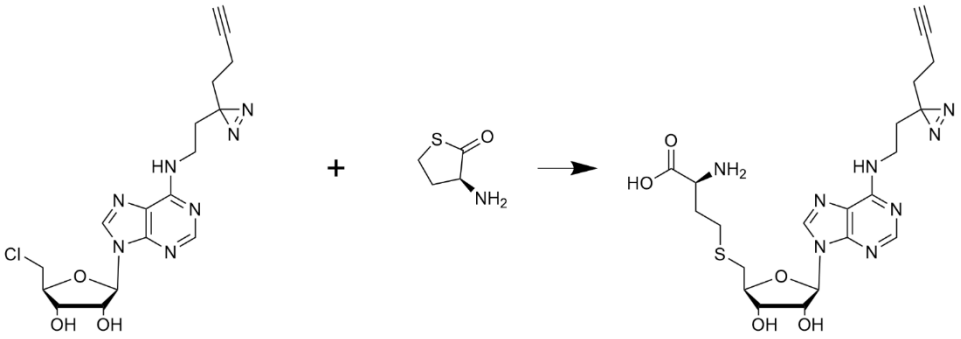
1-1 **1-2**

Triethylamine (30.3 mg, 0.3 mmol) and 3-(3-butyn-1-yl)-3H-diazirine-3-ethanamine (20 mg, 0.15 mmol) were added to a solution of **1-1** (30 mg, 0.10 mmol) in absolute alcohol (4 mL). The mixture was stirred at 80℃ for 5 h and then cooled to room temperature, after which the solvent was removed under vacuum. The diluted crude material was loaded onto preparative HPLC for purification. The product was eluted at 29 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **1-2** as a white powder. (19 mg, 49% yield). LCMS (ESI) calcd. for $C_{17}H_{21}N_7O_4$ $[M+H]^+$ 388.1728, obsd. 388.1699. ^{1}H NMR (800 MHz, D_2O) δ 8.50 (s, 1H), 8.42 (s, 1H), 6.15 (s, 1H), 4.77 (t, J = 5.0 Hz, 1H), 4.43 (t, J = 4.7 Hz, 1H), 4.27 (q, J = 3.8 Hz, 1H), 4.02 (s, 1H), 3.91 (dd, J = 12.8, 3.0 Hz, 1H), 3.84 (dd, J = 12.8, 4.2 Hz, 1H), 3.49 (s, 1H), 2.28 (t, J = 2.6 Hz, 1H), 2.05 – 1.93 (m, 4H), 1.74 (t, J = 7.1 Hz, 2H).



1-2 **1-3**

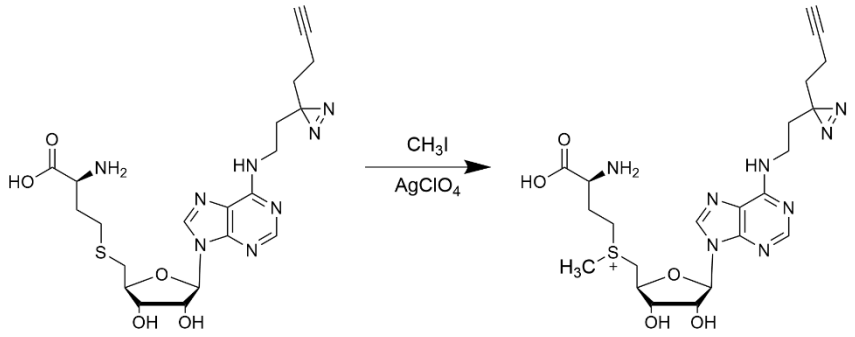
To a solution of **1-2** (9.7 mg, 0.025 mmol) in anhydrous CH_3CN (2 mL), anhydrous pyridine (4 μL, 0.05 mmol) was added while the whole was cooled in an ice bath. Then $SOCl_2$ (11 μL, 0.15 mmol) was slowly added to the stirred suspension. The mixture was stirred at 0 to 5°C for 4 h and was further stirred at room temperature overnight. The solvent was removed under vacuum, and the residue was redissolved in a mixture containing 10 mL of methanol, 1 mL of water and 2 mL of NH_4OH and stirred for 30 minutes at room temperature. The solvent was removed under vacuum. The diluted crude was loaded onto preparative HPLC for purification. The product was eluted at 31 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded compound **1-3** as a white powder. (6.2 mg, 61% yield). LCMS (ESI) calcd. for $C_{17}H_{20}ClN_7O_3$ $[M+H]^+$ 406.1389, obsd. 406.1338. ^1H NMR (800 MHz, D_2O) δ 8.50 (s, 1H), 8.41 (s, 1H), 6.18 (s, 1H), 4.86 (t, J = 5.2 Hz, 1H), 4.53 (t, J = 5.0 Hz, 1H), 4.46 (q, J = 4.6 Hz, 1H), 3.97 (dd, J = 12.4, 3.9 Hz, 1H), 3.93 (dd, J = 12.4, 4.8 Hz, 1H), 3.63 (s, 1H), 3.48 (s, 1H), 2.27 (d, J = 2.7 Hz, 1H), 2.05 – 1.93 (m, 4H), 1.74 (t, J = 7.1 Hz, 2H).



1-3 **1-4**

A solution of **1-3** (5 mg, 0.012 mmol) in DMSO (1 mL) was degassed by bubbling N_2 through it for 5 minutes before L-homocysteinethiolactone and HCl (2.8 mg, 0.018 mmol) were added. The mixture was heated to 65°C for 1 h. The mixture was then cooled to room temperature, treated with 4 N NaOH (28 μL, 0.11 mmol), and heated to 80°C for 0.5 h. The mixture was subsequently cooled to rt and treated with 10% TFA (0.5 mL). The resulting solution was loaded onto preparative HPLC for purification. The product was eluted at 28 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **1-4** as a white powder. (1.1 mg, 18% yield). LCMS (ESI) calcd. for $C_{21}H_{28}N_8O_5S$ $[M+H]^+$ 505.1976, obsd. 505.1908. ^1H NMR (800 MHz, D_2O) δ 8.39 (s, 1H), 8.33 (s, 1H), 6.10 (d, J = 5.1 Hz, 1H), 4.87 (s, 1H), 4.42 (t, J = 5.2 Hz, 1H), 4.33 (q, J = 5.3 Hz, 1H), 3.82 (t, J = 6.7 Hz, 1H), 3.66 – 3.51 (m, 2H), 3.01 (m, 2H), 2.68 (t, J = 7.5 Hz, 2H), 2.19 – 2.05 (m, 3H),

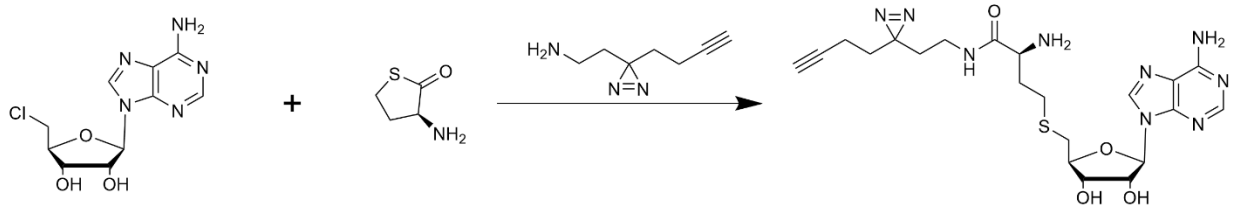
2.00 (td, J = 7.1, 2.8 Hz, 2H), 1.93 (t, J = 6.6 Hz, 2H), 1.71 (t, J = 7.1 Hz, 2H).



1-4 **1-5**

1-4 (2 mg, 0.004 mmol) was dissolved in acetic acid (200 μL) and formic acid (200 μL). Methyl iodide (2.7 μL, 0.044 mmol) was added at 0°C. This mixture was stirred for 20 min at 0°C. $AgClO_4$ (5.2 mg, 0.025 mmol) was dissolved in 50 μL of acetic acid and added to the former solution at 0°C. This mixture was stirred for 1 h at 0°C. The solvent was subsequently removed under vacuum. After being diluted with 2 mL of cold water, the mixture was stirred for 0.5 h and filtered to remove the precipitated AgI. The resulting solution was loaded onto preparative HPLC for purification. The product was eluted at 26 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **1-5** (probe 1) as a white powder (1.25 mg, 60% yield, 8:7 mixture of stereoisomers). LCMS (ESI) calcd. for $C_{22}H_{31}N_8O_5S^+$ $[M]^+$ 519.2133, obsd. 519.2162. ^{1}H NMR (800 MHz, D_2O) δ 8.42 (s, 1H), 8.39 (s, 1H), 6.16 (s, 1H), 4.87 (s, 1H), 4.62 – 4.55 (m, 2H), 3.99 – 3.93 (m, 1H), 3.94 – 3.80 (m, 2H), 3.70 – 3.52 (m, 2H), 3.51 – 3.42 (m, 2H), 3.00, 2.96 (s, s, 3H), 2.39 – 2.31 (m, 2H), 2.28 (d, J = 2.8 Hz, 1H), 2.05 – 1.92 (m, 4H), 1.74 (t, J = 7.2 Hz, 2H).

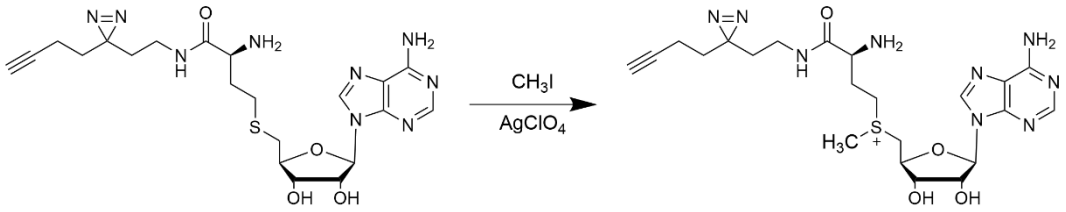
Synthesis of Probe 2



2-1 **2-2**

A solution of **2-1** (20 mg, 0.07 mmol) in DMSO (2.5 mL) was degassed by bubbling N_2 through it for 5 min before adding 3-(3-butyn-1-yl)-3H-diazirine-3-ethanamine (20.6 mg, 0.15 mmol), potassium carbonate (29 mg, 0.21 mmol), L-homocysteinethiolactone, and HCl (16 mg, 0.105 mmol). The mixture was heated to 65°C for 1 h. The reaction was then cooled to room temperature, treated with 4 N NaOH (275 μL, 1.1 mmol), and heated to 80°C for 0.5 h. The mixture was subsequently cooled to rt and treated with 10% TFA (1 mL). The resulting solution was loaded onto preparative HPLC for purification. The product was eluted at 27 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **2-2** as a white powder. (10.9 mg, 31% yield). LCMS (ESI) calcd. for $C_{21}H_{29}N_9O_4S$ $[M+H]^+$ 504.2136, obsd. 504.2102. ^{1}H NMR (800 MHz, D_2O) δ 8.46 (s, 1H), 8.38 (d, J = 1.5 Hz, 1H), 6.07 (d, J = 4.7 Hz, 1H), 4.80 – 4.75 (m, 1H), 4.37 (t, J =

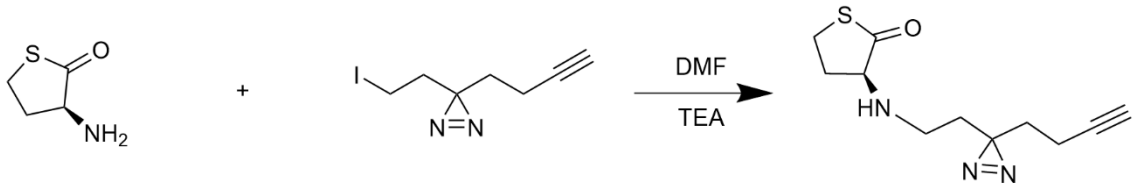
4.6 Hz, 1H), 4.31 – 4.23 (m, 1H), 4.00 – 3.90 (m, 1H), 3.14 – 2.97 (m, 2H), 2.98 – 2.84 (m, 2H), 2.71 – 2.58 (m, 2H), 2.28 (dq, J = 2.7, 1.3 Hz, 1H), 2.19 – 2.05 (m, 2H), 1.96 – 1.85 (m, 2H), 1.71 – 1.52 (m, 4H).



2-2 **2-3**

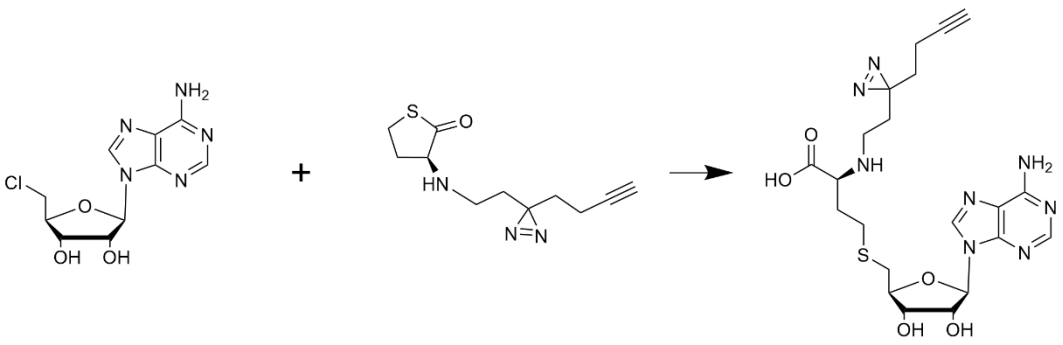
2-2 (10 mg, 0.02 mmol) was dissolved in acetic acid (200 μL) and formic acid (200 μL). Methyl iodide (14 μL, 0.22 mmol) was added at 0℃. This mixture was stirred for 20 min at 0℃. $AgClO_4$ (20 mg, 0.025 mmol) was dissolved in 50 μL of acetic acid and added to the former solution at 0℃. This mixture was stirred for 1 h at 0℃. The solvent was subsequently removed under vacuum. After being diluted with 2 mL of cold water, the mixture was stirred for 0.5 h and filtered to remove the precipitated AgI. The resulting solution was loaded onto preparative HPLC for purification. The product was eluted at 26 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **2-3** (probe 2) as a white powder. (7.4 mg, 71% yield, 5:4 mixture of stereoisomers). LCMS (ESI) calcd. for $C_{22}H_{32}N_8O_4S^+$ $[M]^+$ 518.2292, obsd. 518.2317. ^{1}H NMR (800 MHz, D_2O) δ 8.42 (s, 1H), 8.41 (s, 1H), 6.16 (d, J = 3.7 Hz, 1H), 4.88 (t, J = 4.9 Hz, 1H), 4.65 – 4.52 (m, 2H), 4.12 – 3.96 (m, 3H), 3.62 – 3.43 (m, 2H), 3.09 – 2.87 (m, 5H), 2.50 – 2.39 (m, 2H), 2.39 – 2.34 (m, 1H), 2.01 – 1.92 (m, 2H), 1.74 – 1.54 (m, 4H).

Synthesis of Probe 3



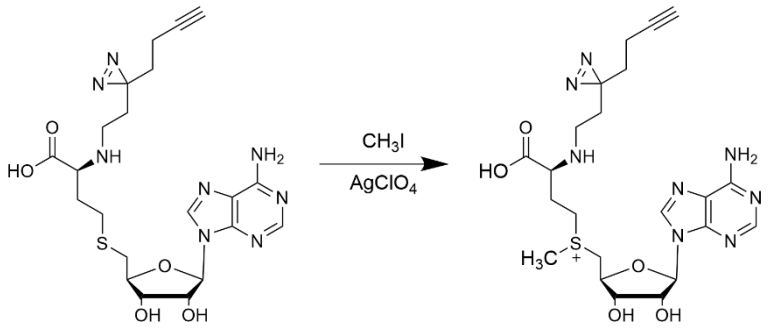
3-1 **3-2**

Triethylamine (30.3 mg, 0.3 mmol) was added to a solution of **3-1** (30.6 mg, 0.2 mmol) in DMF (2 mL). The mixture was stirred at 0℃ for 20 min. Then 3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3H-diazirine (74.4mg, 0.3 mmol) was slowly added to the stirred suspension. The mixture was stirred at room temperature overnight and treated with 10% TFA (200 μL). The diluted crude material was loaded onto preparative HPLC for purification. The product was eluted at 26 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **3-2** as a white powder. (23.2 mg, 49% yield). LCMS (ESI) calcd. for $C_{11}H_{15}N_3OS$ $[M+H]^+$ 238.1009, obsd. 238.0975. ^{1}H NMR (600 MHz, D_2O) δ 4.23 (dd, J = 13.1, 6.9 Hz, 1H), 3.39 – 3.34 (m, 2H), 3.14 – 2.95 (m, 2H), 2.76 – 2.71 (m, 1H), 2.29 (t, J = 2.6 Hz, 1H), 2.20 – 2.11 (m, 1H), 1.97 – 1.91 (m, 2H), 1.83 – 1.69 (m, 2H), 1.60 (t, J = 7.1 Hz, 2H).



3-2 **3-3**

A solution of 5'-chloro-5'-deoxyadenosine (16 mg, 0.056 mmol) in DMSO (2.5 mL) was degassed by bubbling N_2 through it for 5 min before adding **3-2** (19.9 mg, 0.084 mmol). The mixture was heated to 65℃ for 1 h. The reaction was then cooled to room temperature, treated with 4 N NaOH (275 µL, 1.1mmol), and heated to 80℃ for 0.5 h. The mixture was subsequently cooled to rt and treated with 10% TFA (1 mL). The resulting solution was loaded onto preparative HPLC for purification. The product was eluted at 28 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **3-3** as a white powder. (5.9 mg, 21% yield). LCMS (ESI) calcd. for $C_{21}H_{28}N_8O_5S$ $[M+H]^+$ 505.1976, obsd. 505.1859. ^{1}H NMR (800 MHz, D_2O) δ 8.46 (d, J = 5.5 Hz, 1H), 8.38 (s, 1H), 6.07 (d, J = 4.9 Hz, 1H), 4.79 (td, J = 5.1, 2.4 Hz, 1H), 4.37 (t, J = 5.1 Hz, 1H), 4.29 – 4.25 (m, 1H), 3.76 – 3.71 (m, 1H), 3.03 – 2.91 (m, 4H), 2.67 – 2.56 (m, 2H), 2.32 (q, J = 2.6 Hz, 1H), 2.07 (tt, J = 7.4, 4.2 Hz, 2H), 2.00 – 1.94 (m, 2H), 1.81 – 1.74 (m, 2H), 1.65 – 1.59 (m, 2H).



3-3 **3-4**

3-3 (5 mg, 0.01 mmol) was dissolved in acetic acid (200 µL) and formic acid (200 µL). Methyl iodide (6.9 uL, 0.11 mmol) was added at 0℃. This mixture was stirred for 20 min at 0℃. $AgClO_4$ (10.4 mg, 0.05 mmol) was dissolved in 50 µL of acetic acid and added to the former solution at 0℃. This mixture was stirred for 1 h at 0℃. The solvent was subsequently removed under vacuum. After being diluted with 2 mL of cold water, the mixture was stirred for 0.5 h and filtered to remove the precipitated AgI. The resulting solution was loaded onto preparative HPLC for purification. The product was eluted at 26 min with a flow rate of 10 mL/min with the following gradient: 0% solvent B for 10 min, then 0% to 60% solvent B over 60 min. Freeze drying of the HPLC fractions yielded **3-4** (probe 3) as a white powder. (2.7 mg, 52% yield, 9:8 mixture of stereoisomers). LCMS (ESI) calcd. for $C_{22}H_{31}N_8O_5S^+$ $[M]^+$ 519.2133, obsd. 519.2192. ^{1}H NMR (800 MHz, D_2O) δ 8.44 (s, 2H), 6.17 (d, J = 4.1 Hz, 1H), 4.88 – 4.82 (m, 1H), 4.64 – 4.54 (m, 2H), 4.05 – 3.90 (m, 2H), 3.70 – 3.40 (m, 3H), 3.02 – 2.95 (m, 5H), 2.41 – 2.33 (m, 2H), 2.32 – 2.24 (m,

1H), 2.08 – 2.01 (m, 2H), 1.88 – 1.81 (m, 2H), 1.74 – 1.65 (m, 2H).

Expression and anaerobic purification of *Ph*Dph2

*Ph*Dph2 was overexpressed in *E. coli* BL21(DE3) with pRARE2, and purified as previously described[1] but with the following modifications. The cells were cooled to 16°C before being induced at OD600 of 0.6 with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were incubated in a shaker at 16°C and 200 rpm for 18 h before being harvested. We used sonication (Scientz, China) to lyse the cells at 0°C for 17 min anaerobically (power 250 W, working for 4 sec with a 6 sec interval) (Figure S1)

Expression and anaerobic purification of ArsL

*Bg*ArsL was overexpressed in *E. coli* Rosetta (DE3), and purified as previously described[2] but with the following modifications. Purification of ArsL was performed in an anaerobic glovebox (Mikrouna, China). The cell pellet was suspended in 30 mL of lysis buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 10 mM $MgCl_2$, and 5 mM imidazole). Lysozyme (50 mg), DNase I (1 mg), and PMSF (1 mM) were added and incubated at 4°C for 0.5 h. The mixture was disrupted by sonication (Scientz, China) at 0°C for 17 min anaerobically (power 250 W, working for 4 sec with a 6 sec interval). The cell debris was removed by centrifugation (Thermo, Germany) at 14000g for 30 min. The supernatant was incubated for 45 min with 2 mL of Ni-NTA resin (Genscript) preequilibrated with lysis buffer. The Ni-NTA resin was loaded onto a polypropylene column and washed with 30 mL lysis buffer, followed by 50 mL of 30 mM imidazole in lysis buffer. ArsL was eluted using elution buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 10 mM $MgCl_2$, 300 mM imidazole). The elution fractions were concentrated to 1 mL and buffer-exchanged to desalting buffer (200 mM Tris-HCl pH 7.4, 150 mM NaCl, and 10% glycerol) using a 10-DG desalting column (GE Healthcare). The purified proteins were concentrated using Amicon Ultra15 centrifugal filter devices (Millipore). The protein concentration was determined by BCA protein auantification kit (Figure S1).

Expression and purification of AcnA and EDD84_07545

The codon-optimized gene fragments of AcnA and EDD84_07545 from *Burkholderia gladioli* were synthesized by GENE CREATE (Wuhan, China) and inserted into the vector pET28a. The plasmid was used to transform the *E. coli* BL21 (DE3) strain. The cells were grown in 1 liter of LB medium supplemented with 30 μg/mL kanamycin at 37°C and 200 rpm. When the OD600 reached 0.6, the cultures were cooled to 16°C. Protein expression was induced by 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were harvested after incubation at 16°C and 200 rpm for 18 h.

The cell pellet was suspended in 30 mL of lysis buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 10 mM $MgCl_2$, and 5 mM imidazole). Lysozyme (50 mg), DNase I (1 mg), and PMSF (1 mM) were added, and the mixture was incubated at 4°C for 0.5 h. The mixture was subsequently lysed with a cell disruptor (Scientz China). The cell debris was removed by centrifugation (Thermo, Germany) at 14000g for 30 min. The supernatant was incubated for 45 min with 2 mL of Ni-NTA resin (Genscript) preequilibrated with lysis buffer. The Ni-NTA resin was loaded onto a polypropylene column and washed with 30 mL of lysis buffer, followed by 50 mL of 30 mM imidazole in lysis buffer. The protein was eluted using elution buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 10 mM $MgCl_2$, 300 mM imidazole). The elution fractions were concentrated to 1 mL and buffer-exchanged to desalting buffer (200 mM Tris-HCl pH 7.4, 150 mM NaCl, and 10%

glycerol) using a 10-DG desalting column (GE Healthcare). The purified proteins were concentrated using Amicon Ultra15 centrifugal filter devices (Millipore). The protein concentration was determined by a BCA protein quantification kit (Figure S1).

Expression and purification of TPI1 and MET6

Yeast TPI1 and MET6 genes were amplified from yeast genomic DNA were extracted from BY4741 using Pierce Yeast DNA Extraction Kit and inserted into the vector pET28a. The plasmid was used to transform the *E. coli* Rosetta (DE3) strain. The cells were grown in 1 liter of LB medium with 30 μg/mL kanamycin and 20 μg/mL chloramphenicol at 37°C and 200 rpm. When the OD600 reached 0.6, the cultures were cooled to 16°C. Protein expression was induced by 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were harvested after incubation at 16°C and 200 rpm for 18 h. The cells were lysed and proteins were purified as described for AcnA.

Click chemistry

Rh-N_3 or Biotin-N_3 (final concentration of 500 μM), BTTAA (final concentration of 4 mM), $CuSO_4$ (final concentration of 2 mM), and sodium ascorbate (final concentration of 100 mM) were added to the samples in order, and the reaction mixture was incubated at room temperature for 1 h in the dark.

Labeling of *Ph*Dph2 with probes

The labeling experiment was performed as previously described[3]. Briefly, *Ph*Dph2 (10 μM) was incubated with 100 μM probes for 1 hour at 4°C. The samples were then UV-irradiated at 365 nm for 30 min on ice. For competition labeling, *Ph*Dph2 was incubated with 10 mM SAM for 1 h at 4°C prior to incubation with probes. Samples without UV-irradiation or probes were used as negative controls. After being subjected to click chemistry with Rh-N_3, the samples were mixed with loading buffer and separated by SDS-PAGE. The gel was subsequently fixed in destaining buffer (10% acetic acid, 40% methanol and 50% H_2O) for 1 h. A fluorescence image of the gel was recorded by a Typhoon imager, and an image of the protein gel with Coomassie blue staining was recorded with a digital camera.

The lysate of *Ph*Dph2-overexpressing cells (2 mg/mL) was incubated with 100 μM probes for 1 hour at 4°C. The samples were then UV-irradiated at 365 nm for 30 min on ice. For competition labeling, the lysate of *Ph*Dph2-overexpressing cells was incubated with 10 mM SAM for 1 hour at 4°C prior to incubation with probes. The negative control was labeled as previously described.

Labeling of cell lysates with probes

The cells were lysed in 1% NP-40 buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 1% protease inhibitor cocktail, 1% NP-40) with sonication. After centrifugation at 14,000 g for 30 min at 4°C, the supernatant was collected. The protein concentration was determined by a BCA protein quantification kit. The cell lysates (2 mg/mL) were incubated with 100 μM probes for 1 hour at 4°C and the labeling experiment was performed as described for *Ph*Dph2.

Pulldown experiments with *Burkholderia gladioli* and *Saccharomyces cerevisiae* via probes

For the pulldown experiments, as previously described,[4] the cell lysates (2 mg/mL) were incubated with or without 100 μM probes for 1 hour at 4°C. After the samples were UV-irradiated at 365 nm for 30 min on ice and subjected to click chemistry with biotin-N_3, the proteins were precipitated with methanol/chloroform/water (4:2:1) and resolubilized in 4% SDS buffer (4% SDS,

150 mM NaCl, 50 mM triethanolamine). The samples were subsequently diluted in PBS buffer to decrease the SDS concentration below 0.4%, and incubated with streptavidin beads for 1.5 hours at 4°C. The beads were pelleted by centrifugation at 5000 g for 5 min and subsequently washed (4×1 mL) with washing buffer (0.4% SDS in PBS). The beads were then washed (4×1 mL) with washing buffer (0.2% SDS in PBS), followed by three washes with 1 mL of PBS.

On-bead digestion

Streptavidin beads in 600 μL of 6 M urea in PBS were treated with 10 mM TCEP for 30 min at 37 °C with gentle rotation and then with 25 mM IAA for 30 min at 37 °C with gentle rotation. After the supernatant was discarded, the beads were washed with 1 mL of PBS and resuspended in 200 mL of 2 M urea in PBS, and then treated with 2 μg of trypsin in 200 μL of 2 M urea in PBS with 1 mM $CaCl_2$ for digestion overnight at 37°C. The digested peptides were enriched with C18 Ziptip for MS detection.

In-gel trypsin digestion

In-gel trypsin digestion was performed as previously described[5]. The gel pieces were treated with 200 μL of 100 mM ammonium bicarbonate: acetonitrile (1:1, vol/vol) for 60 min at 37°C. After the supernatant was discarded, and the gel pieces were washed with 100 μL acetonitrile and incubated with 25 mM DTT in 100 mM ammonium bicarbonate for 45 min at 55°C. After the supernatant was discarded, the gel pieces were washed with 100 μL acetonitrile and incubated with 55 mM iodoacetamide (IAA) in 100 mM ammonium bicarbonate for 30 min at room temperature in the dark. After the supernatant was discarded, the gel pieces were washed with 100 μL of acetonitrile and digested with 2 mg of trypsin in 100 μL of trypsin buffer (200 mM ammonium bicarbonate: CH_3CN: H_2O=1:1:8) at 30°C for 18 hours. Formic acid was added to the samples to a final concentration of 1.0% to stop the enzymatic reaction. The supernatant was collected, and the gel pieces were incubated with 30 μL of 50% acetonitrile with 1‰ formic acid for 45 min. The supernatant was collected, the gel pieces were incubated with 30 μL of 90% acetonitrile with 1‰ formic acid for 45 min. The supernatants were combined and dried under vacuum.

LC-MS/MS analysis for protein identification

The digestion products were separated via a 120 min gradient elution at a flow rate of 0.300 μL/min with a Thermo Scientific UltiMate™ 3000 HPLC system, which was directly interfaced with a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer. The analytical column was a custom-made fused silica capillary column (75 μm ID, 15 cm length) packed with C-18 resin (100 Å, 2 μm, Dr.Maisch, Ammerbuch, Germany). Mobile phase A consisted of 0.1% formic acid, and mobile phase B consisted of 80% acetonitrile and 0.1% formic acid. The Fusion mass spectrometer was operated in the data-dependent acquisition mode using Xcalibur software, and there was a single full-scan mass spectrum in the Orbitrap (350-1550 m/z, 120,000 resolution). The 20 most intense ions with charges ≥2 were fragmented with a normalized collision energy of 30.

The MS/MS spectra from each LC-MS/MS run were searched against the *Saccharomyces cerevisiae* and *Burkholderia gladioli* databases downloaded from UniProt and NCBI using Proteome Discoverer (version PD3.0, Thermo-Fisher Scientific, USA). The search criteria were set as follows: full tryptic specificity was required, two missed cleavage sites were allowed, oxidation (M) was set as a variable modification and carbamidomethyl (C) was set as a fixed modification. The precursor ion mass tolerances were set at 20 ppm for all MSs acquired in an Orbitrap mass analyzer, and the fragment ion mass tolerance was set at 0.02 Da for all MS2 spectra acquired. The

peptide false discovery rate (FDR) was calculated using percolator provided by PD. FDR was determined based on PSMs when searching against the reverse, decoy database. Peptides only assigned to a given protein group were considered unique. The false discovery rate (FDR) was also set to 0.01 for protein identification.

Data analysis

LC-MS/MS was performed as previously described[6] but with the following modifications. The total intensity sum of the peptides for each identified protein was calculated for both samples and the ‘‘Experimental’’ to ‘‘Control’’ ratios were determined. Proteins with Score Sequest HT >5, Coverage >5 and peptides >1 were retained. Next, proteins with a peptide intensity $Log_2(FC) > 4.7$ and p-value <0.02 were retained. Proteins with <1.6 total peptides in the ‘‘Experimental’’ sample compared with the ‘‘Control’’ sample were filtered. Finally, missing values from a normal distribution were replaced and the p-values resulted from the Welch t-test.

B. Supplementary Figures

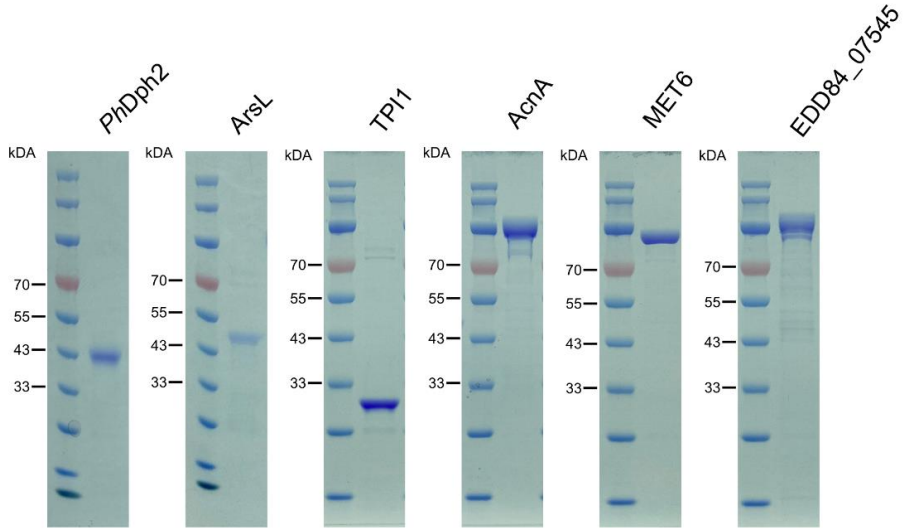


Figure S1. SDS−PAGE gels of the purified proteins.

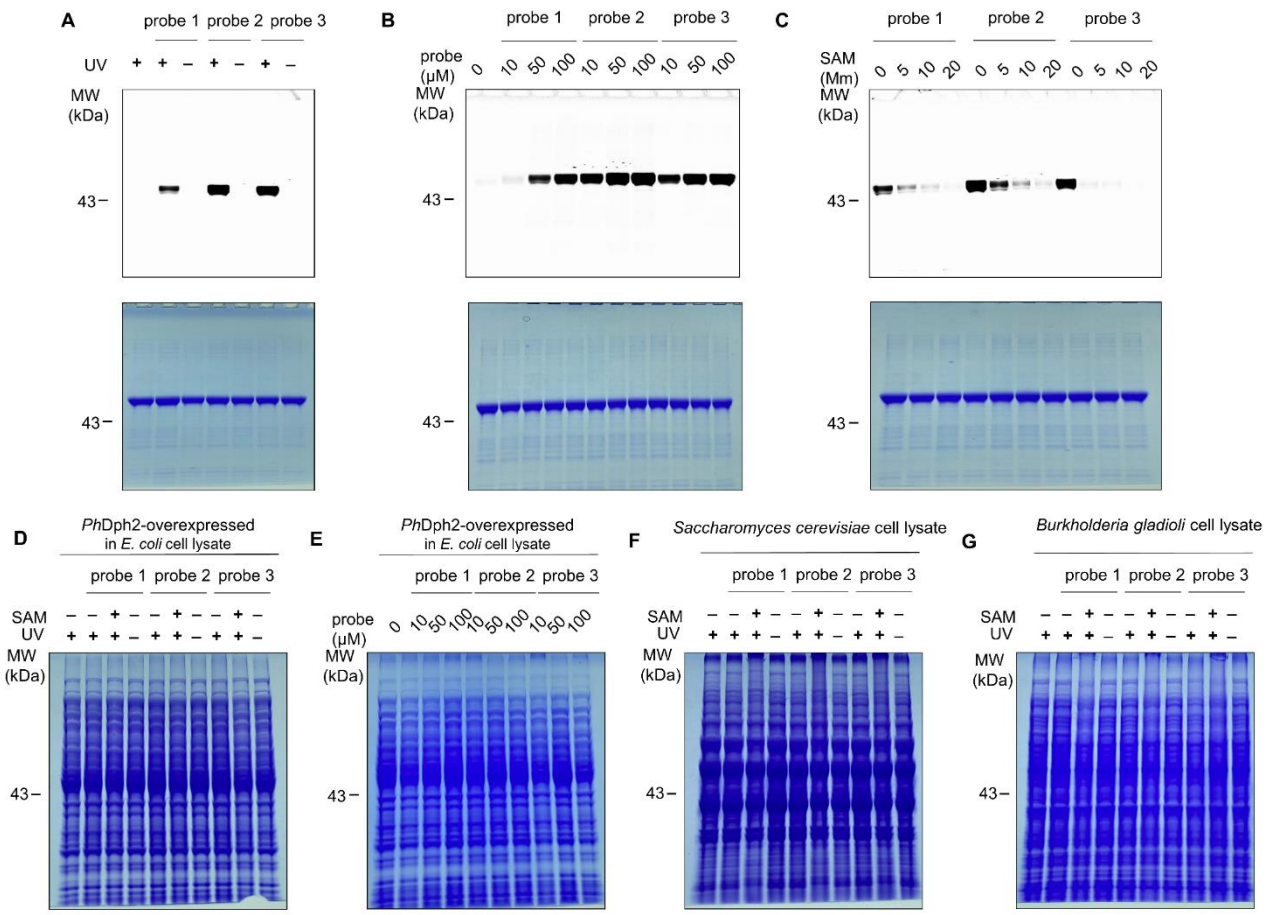


Figure S2. Full image of the in-gel fluorescence analysis (Figures 2-4). (A) In-gel fluorescence analysis of *Ph*Dph2 treated with probes 1–3. (B) Fluorescence in-gel analysis of *Ph*Dph2 with increased concentrations of probes 1–3. (C) Different concentrations of SAM were used as the competitors. (D-E) Coomassie blue staining of the *Ph*Dph2-overexpressed *E. coli* cell lysates and increasing concentrations of probes 1−3. Coomassie blue stain of the *Saccharomyces cerevisiae* (F) and *Burkholderia gladioli* (G) cell lysates treated with probes 1−3.

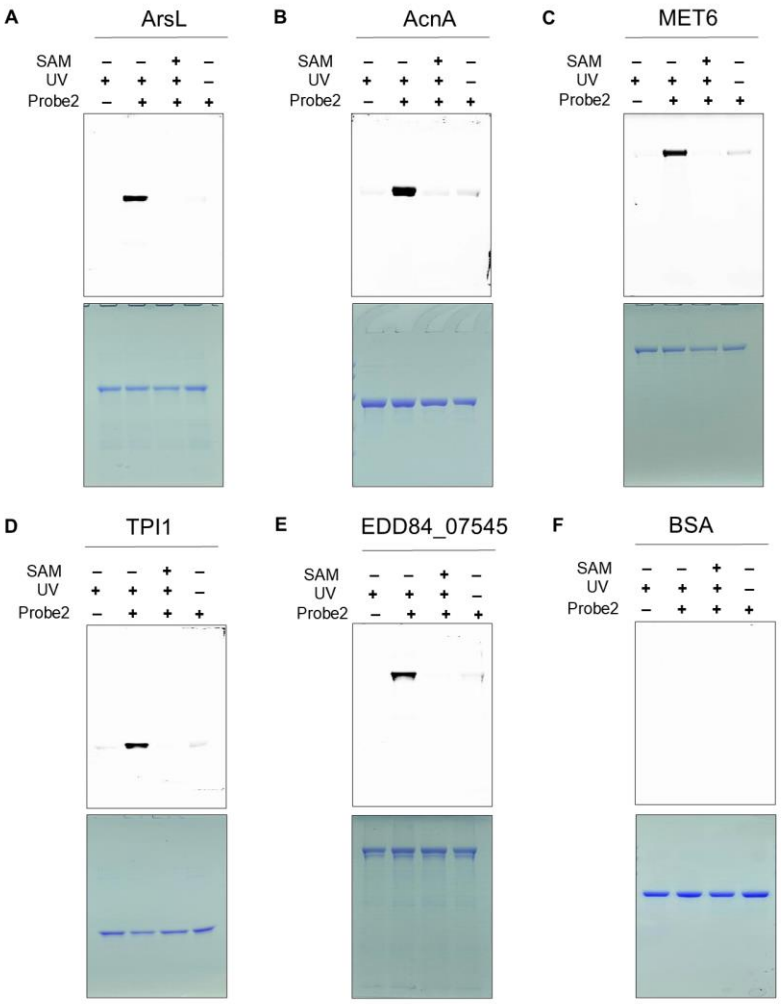


Figure S3. Full image of the in-gel fluorescence analysis (Figure 6). Verification of five SAM-binding proteins (A-E) and BSA (F) by fluorescence in-gel analysis.

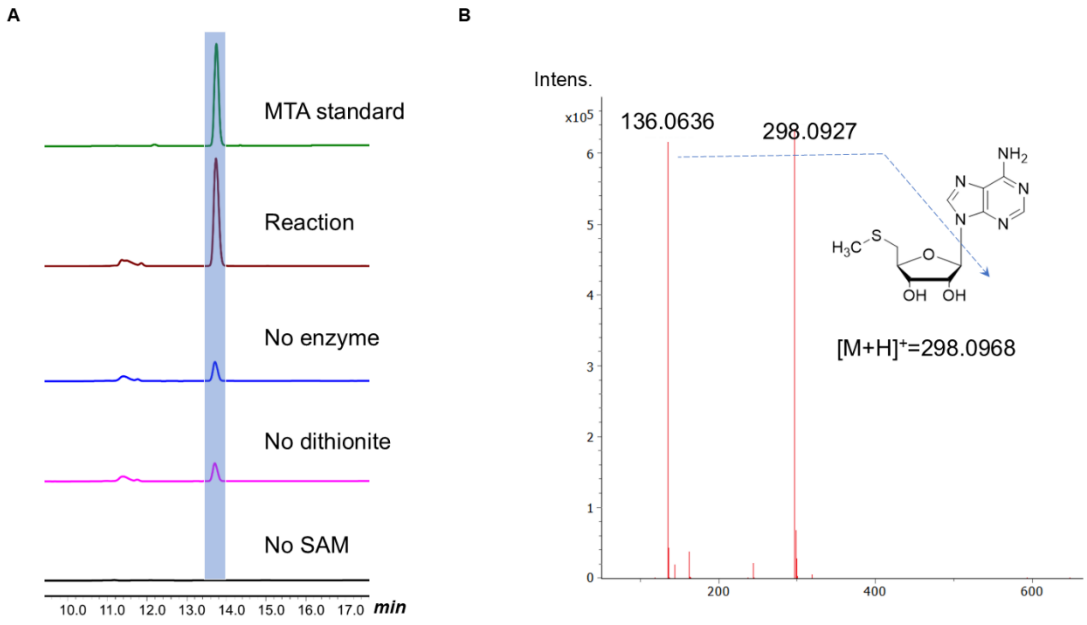


Figure S4. Enzymatic assay of the SAM cleavage reaction of ArsL. (A)HPLC traces of the ArsL reactions. (B) High resolution MS of the isolated MTA peak in the reaction.

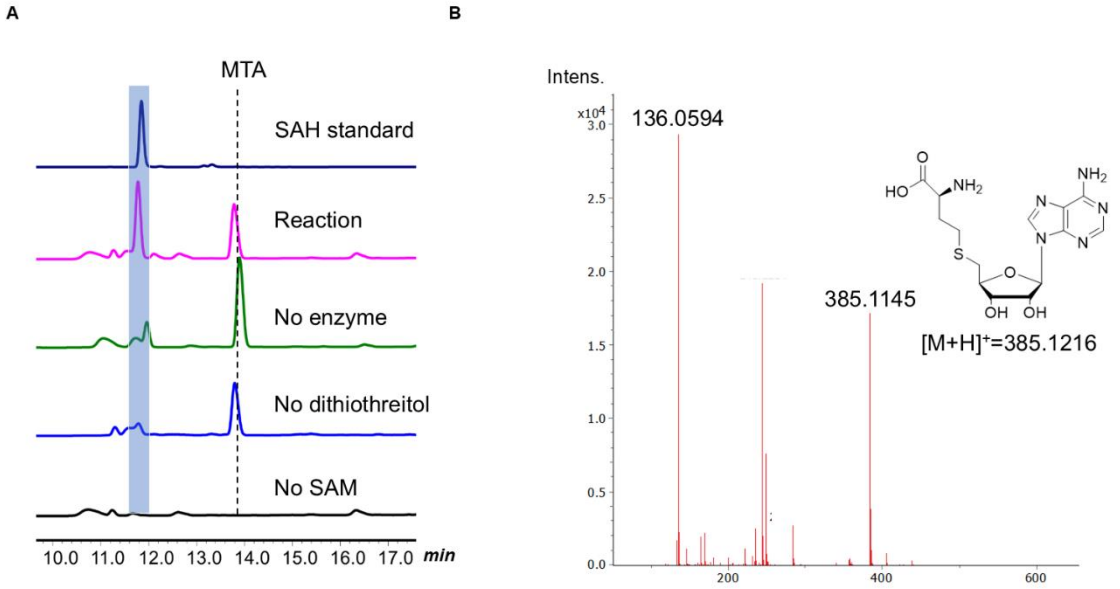


Figure S5. Enzymatic assay of the SAM cleavage reaction of EDD84_07545. (A)HPLC traces of the EDD84_07545 reactions. The peak of 13.8 minutes was decomposed MTA. (B) High resolution MS of the isolated SAH peak in the reaction.

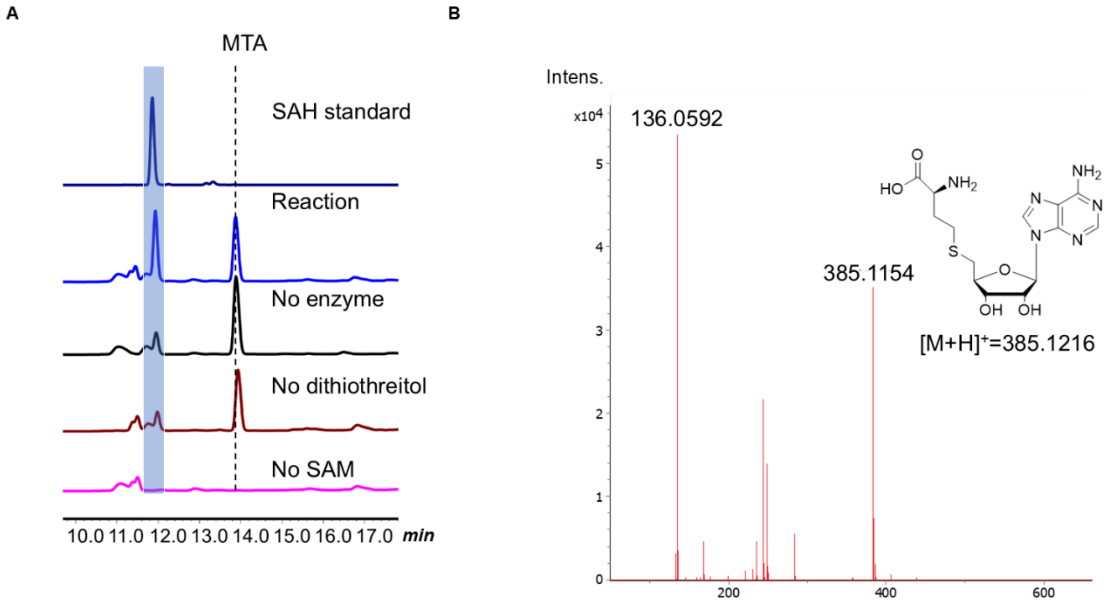


Figure S6. Enzymatic assay of the SAM cleavage reaction of AcnA. (A)HPLC traces of the AcnA reactions. The peak of 13.8 minutes was decomposed MTA. (B) High resolution MS of the isolated SAH peak in the reaction.

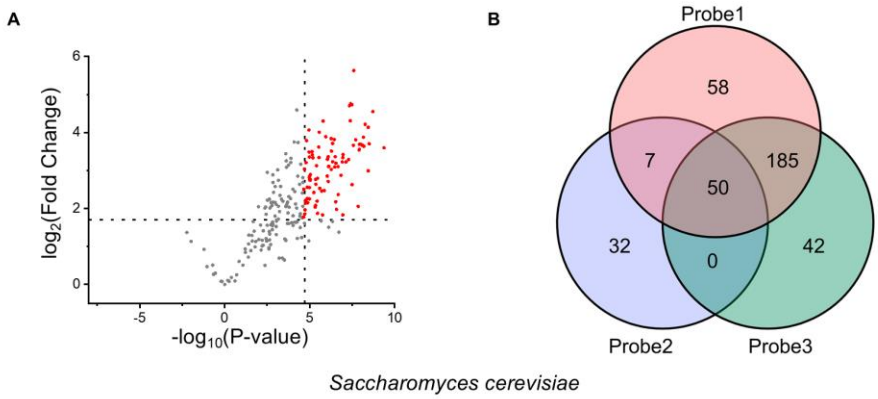


Figure S7. (A) Volcano plots showing the quantification results of enriched proteins with 100 μM probe 2 compared with the control (no probe) of *S. cerevisiae*. The ratios of Log2(FC) ＞4.7 and p-value <0.02 were considered high-confidence Proteins in all three independent replicates. (B) Venn diagram showing the overlap between the sets of experiments for proteins in *S. cerevisiae* labeled with probes 1-3.

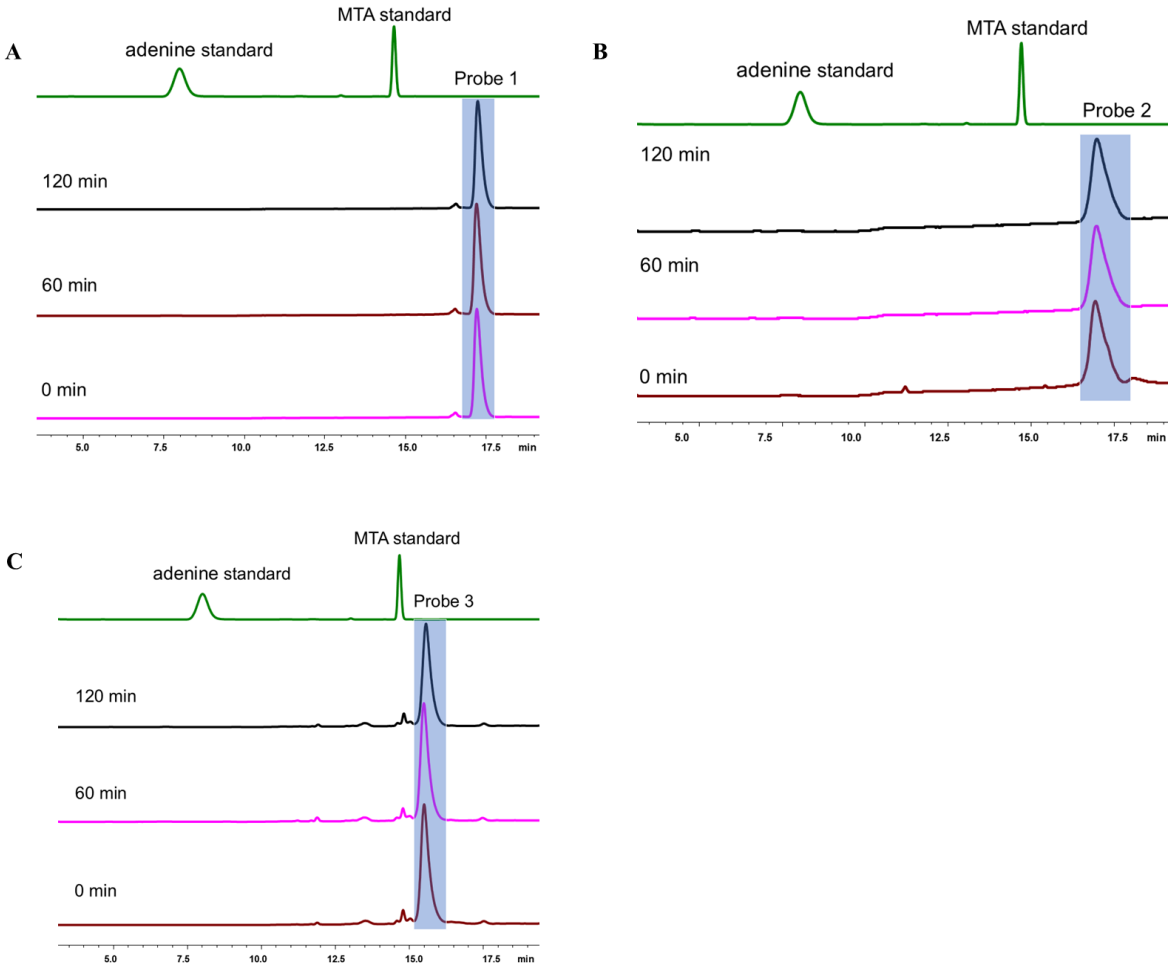


Figure S8. Stability test of probes. (A) Stability test of Probe 1 at pH 7.4. 99% of Probe 1 remained at 120 minutes. (B) Stability test of Probe 2 at pH 7.4. 99% of Probe 1 remained at 120 minutes. (C) Stability test of Probe 2 at pH 7.4. 99% of Probe 3 remained at 120 minutes.

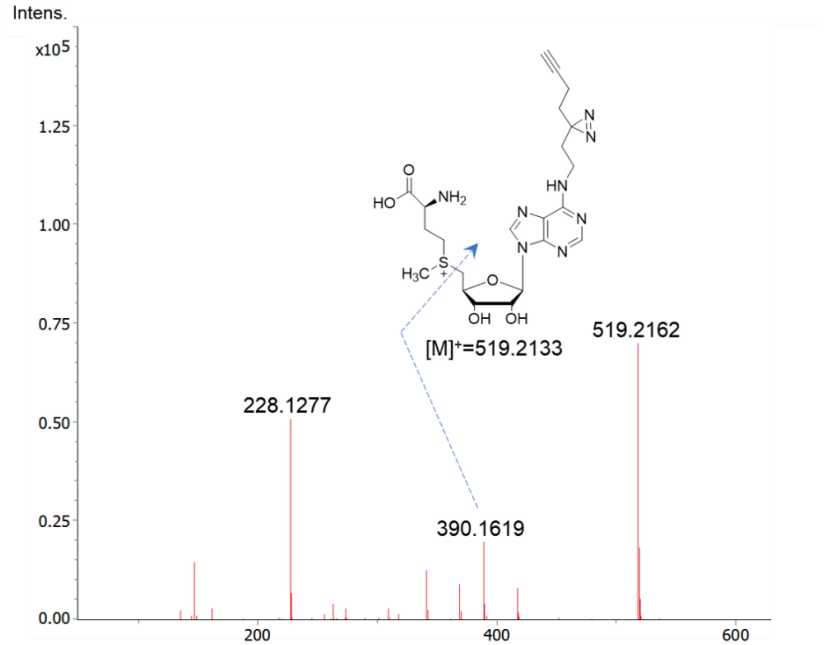


Figure S9. LC-MS analysis of compound **1-5**

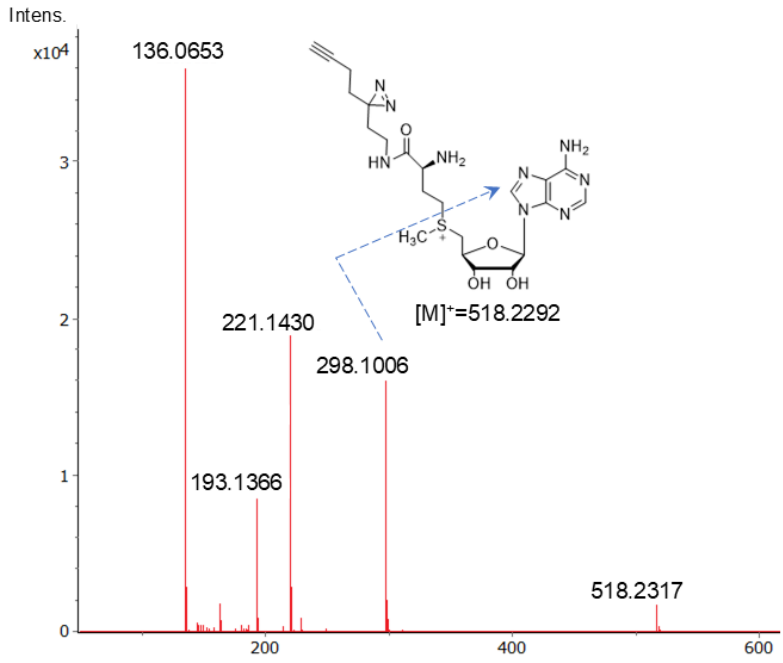


Figure S10. LC-MS analysis of compound **2-3**

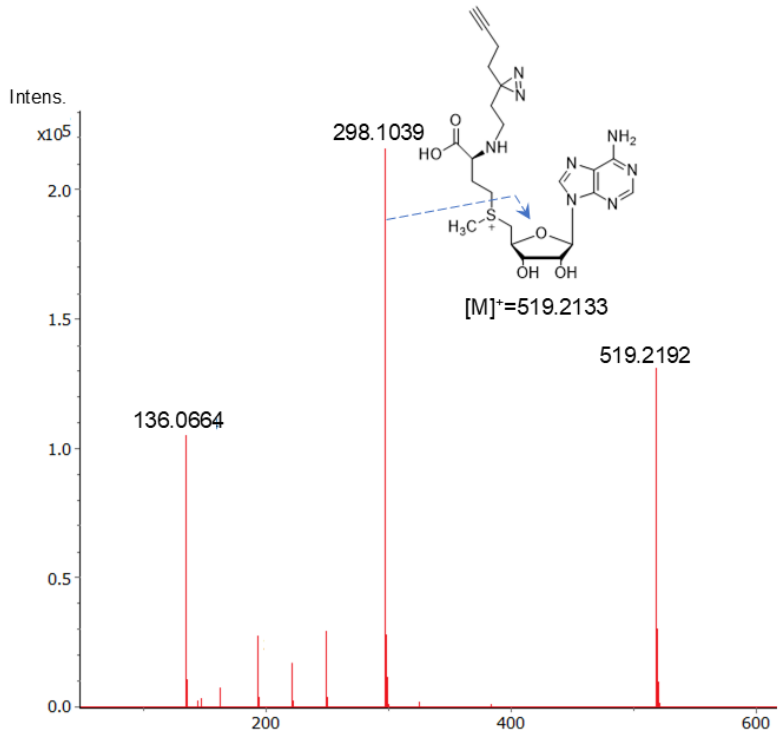


Figure S11. LC-MS analysis of compound **3-4**

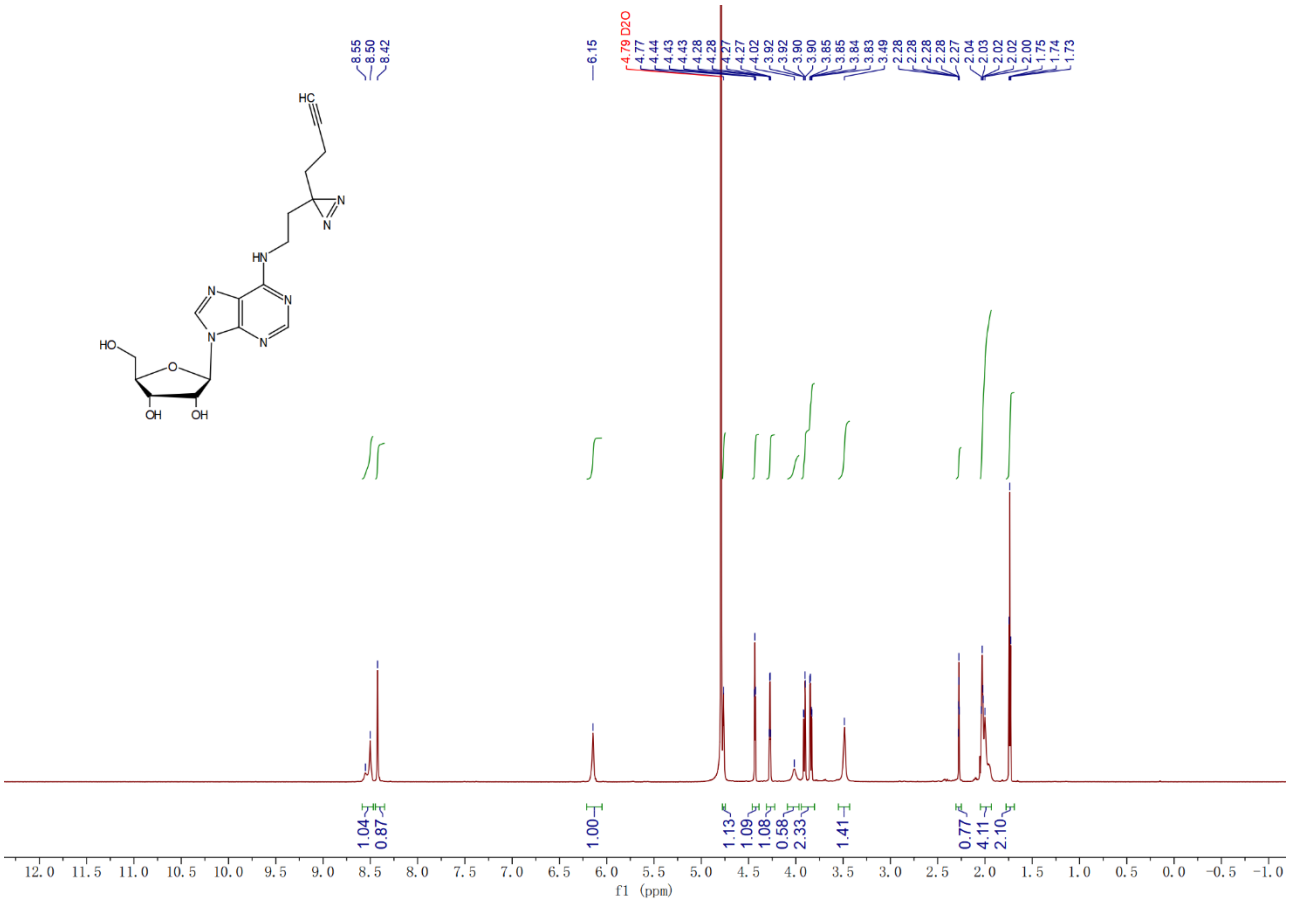


Figure S12. ^{1}H-NMR spectrum of compound **1-2**.

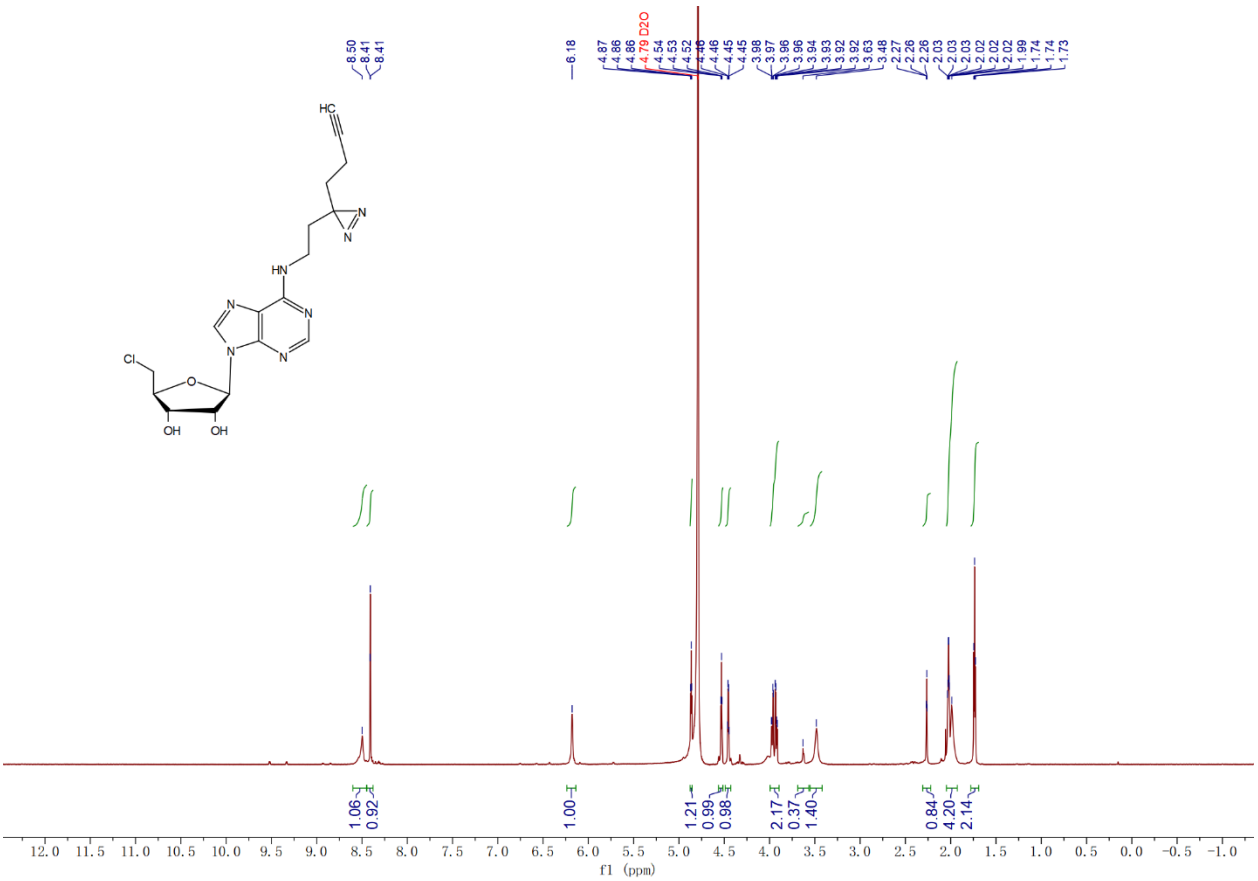


Figure S13. ^{1}H-NMR spectrum of compound **1-3**.

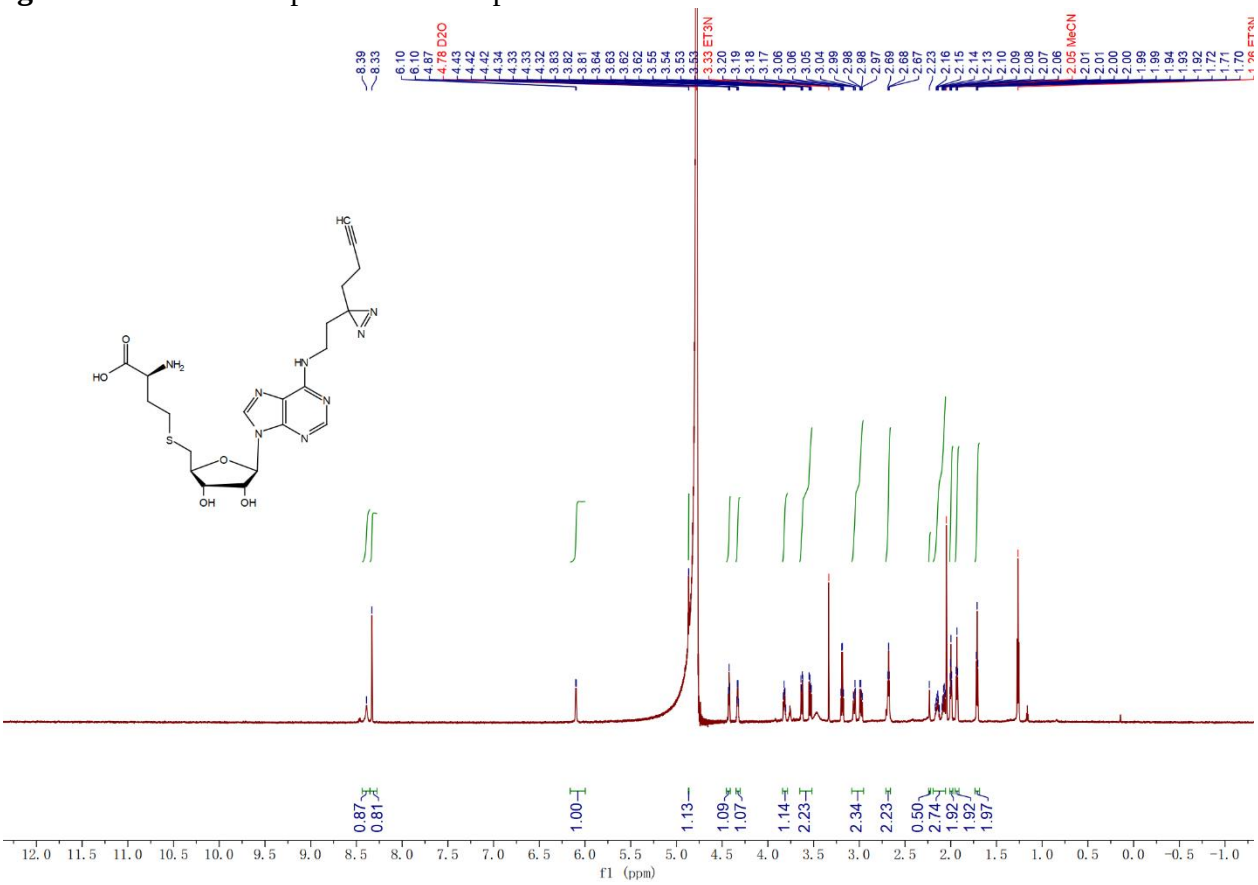


Figure S14. ^{1}H-NMR spectrum of compound **1-4**.

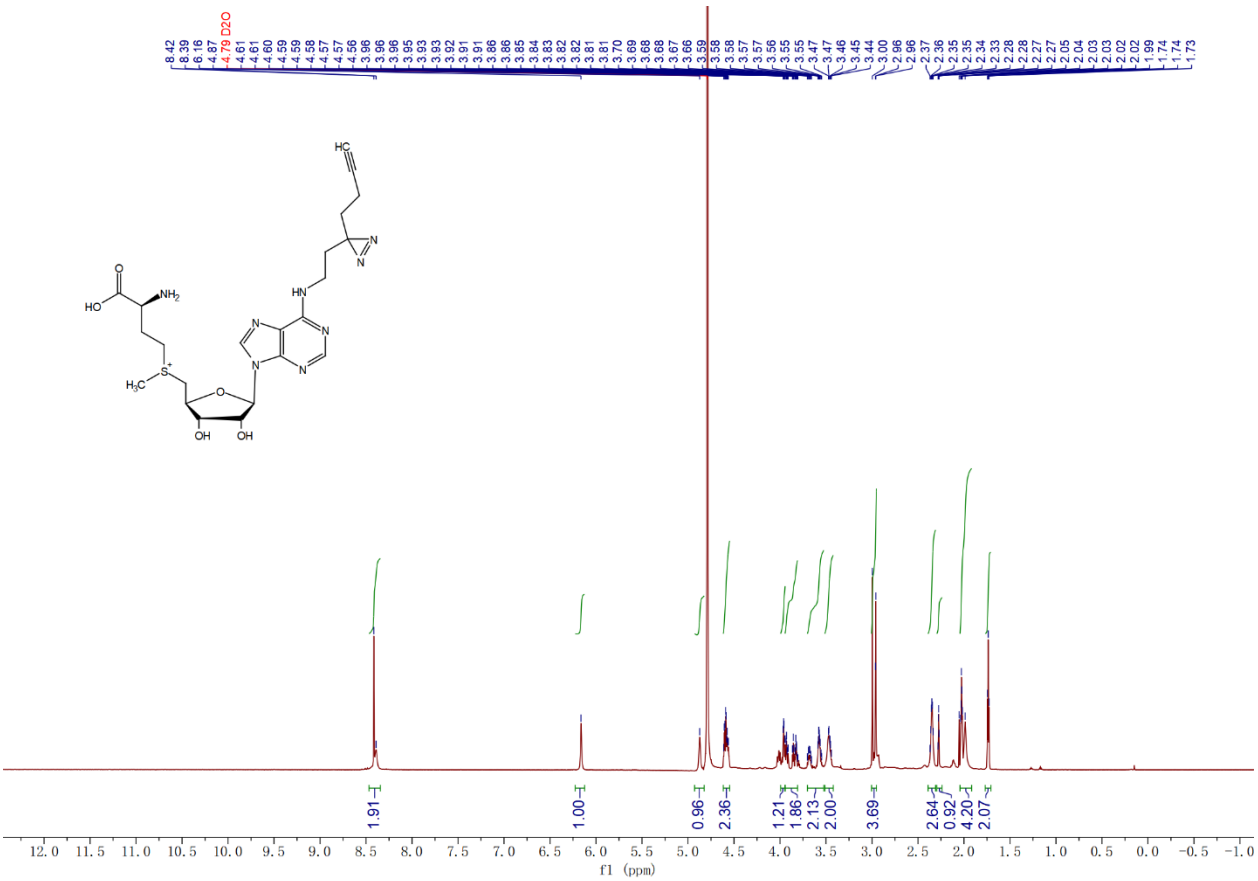


Figure S15. [1]H-NMR spectrum of compound **1-5**.

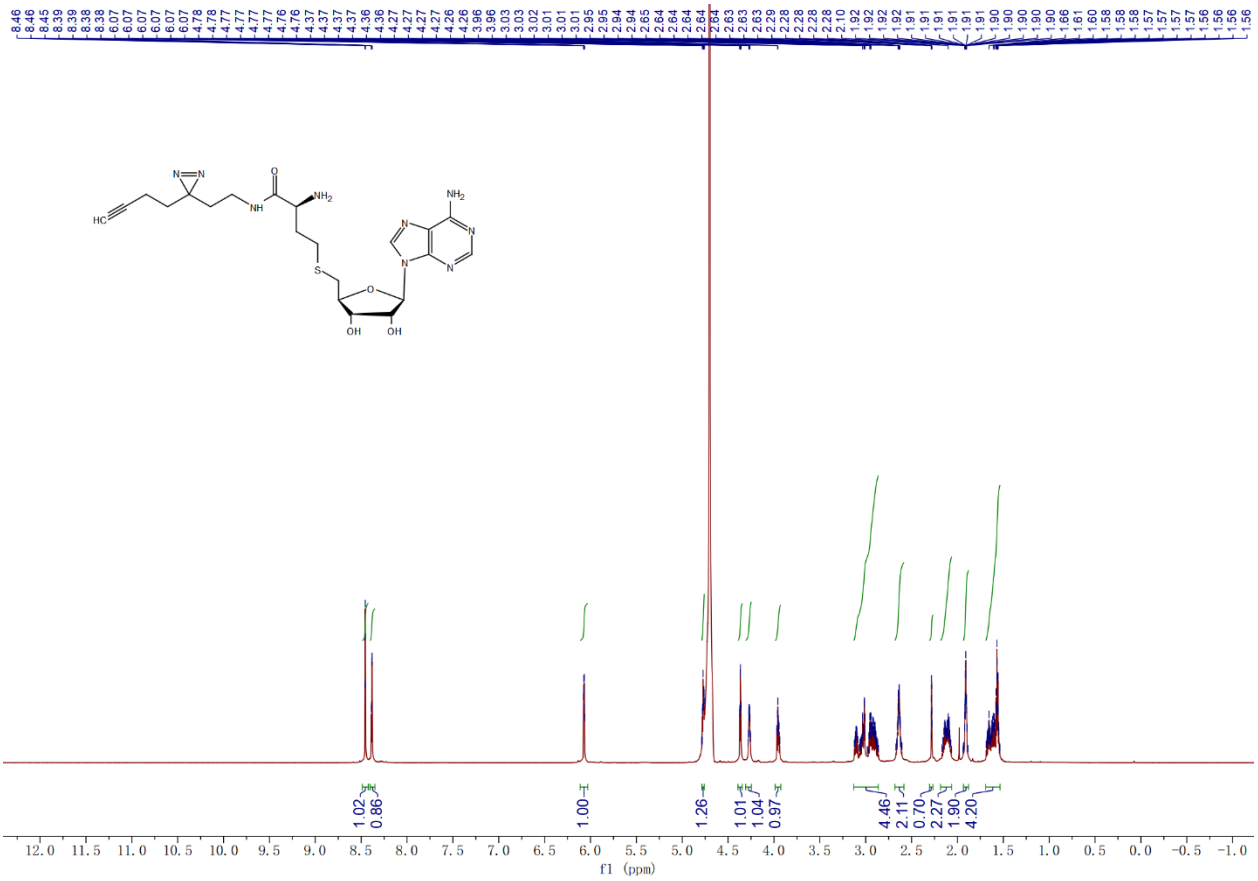


Figure S16. [1]H-NMR spectrum of compound **2-2**.

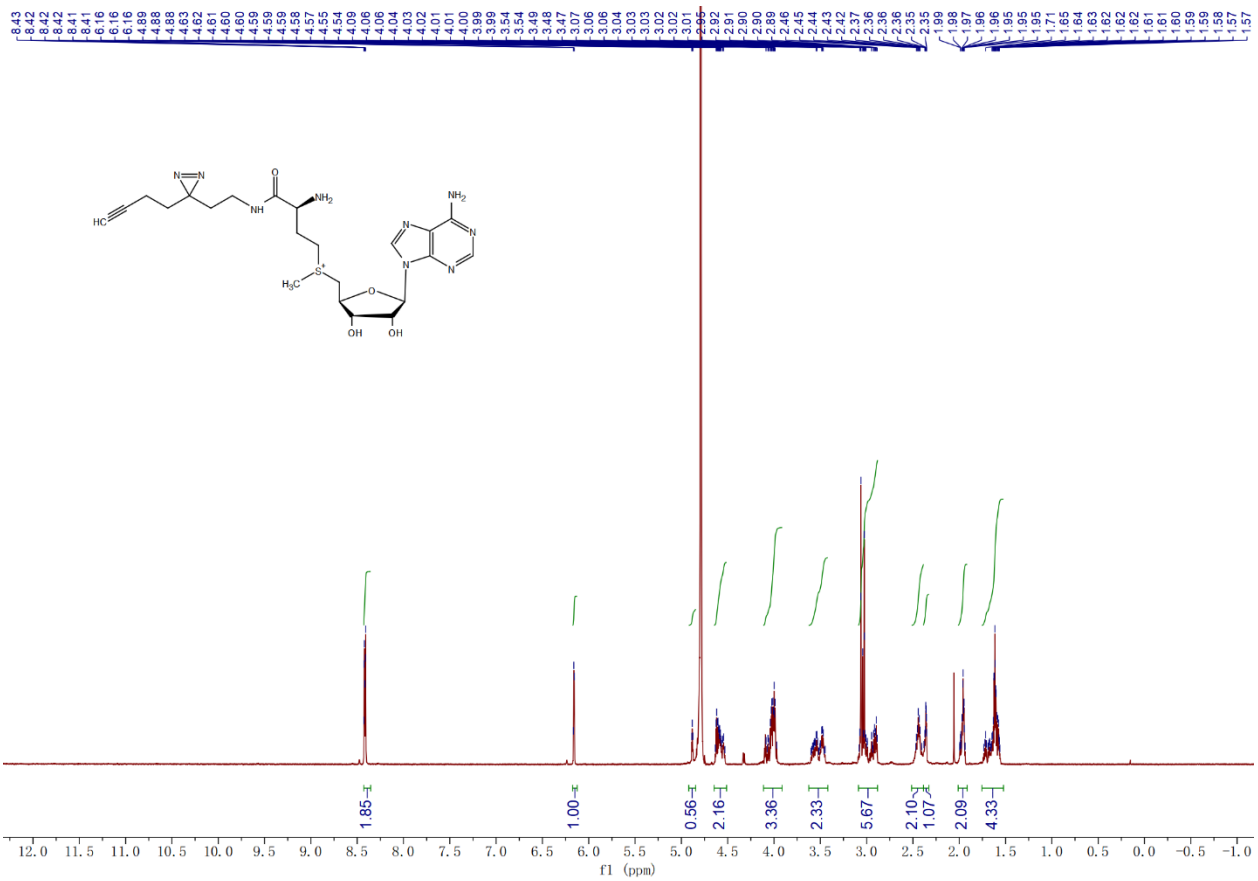


Figure S17. ^{1}H-NMR spectrum of compound **2-3**

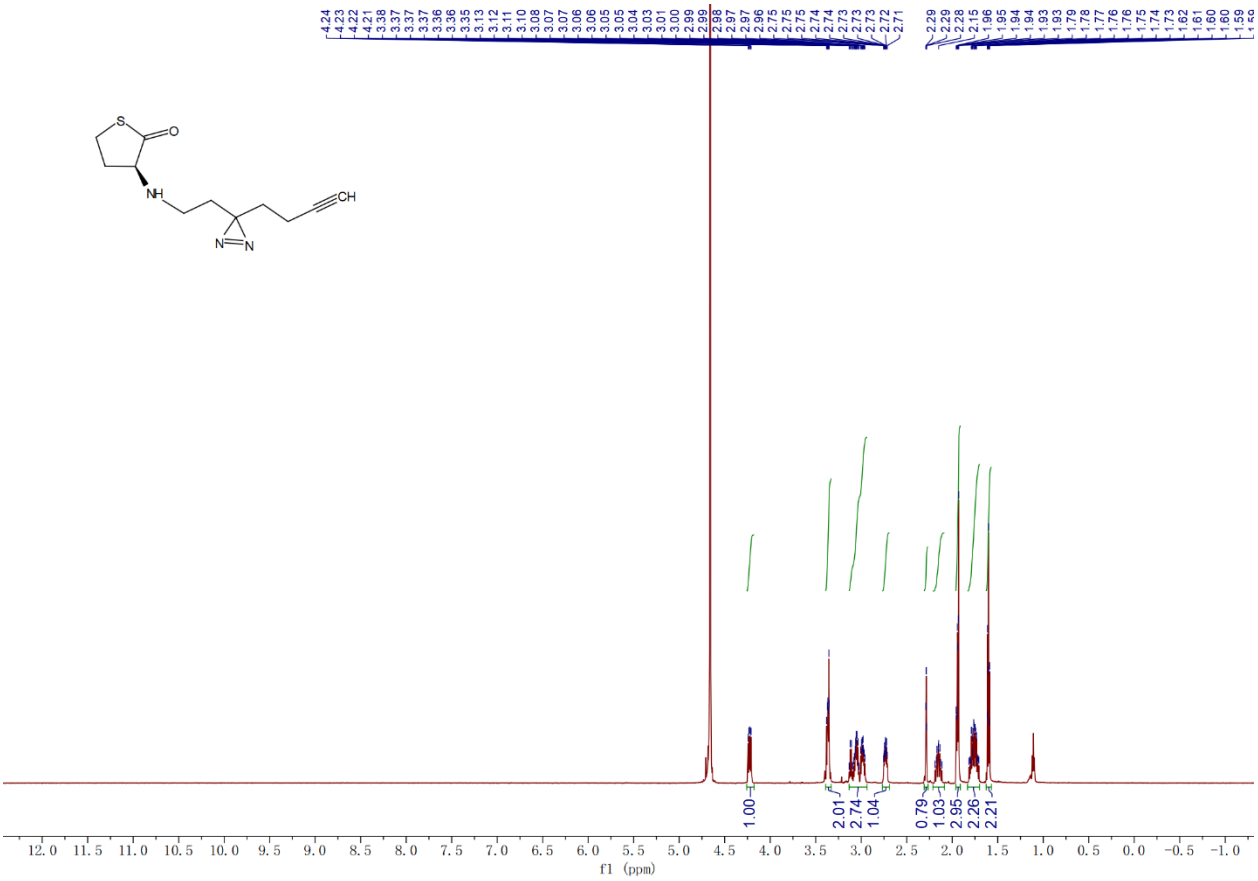


Figure S18. ^{1}H-NMR spectrum of compound **3-2**

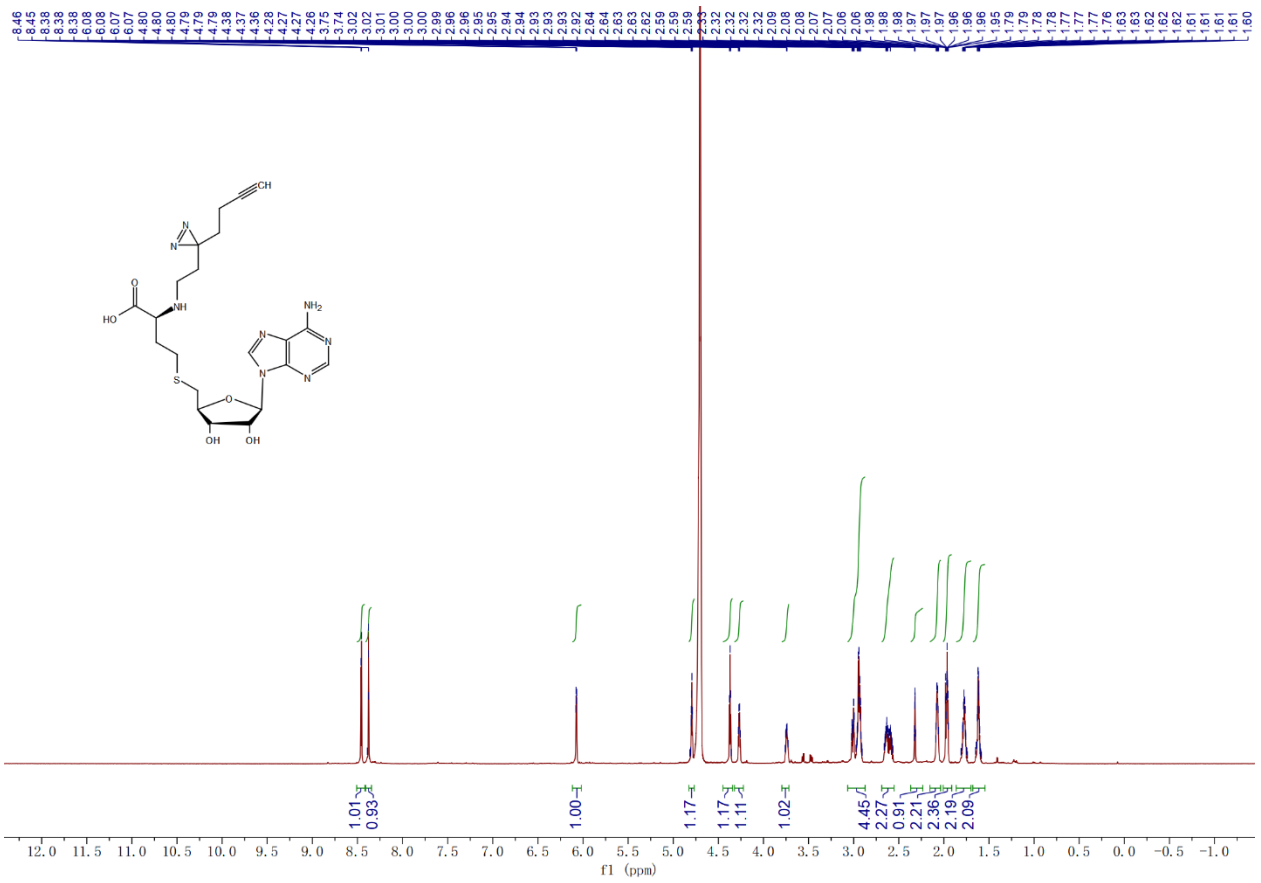


Figure S19. ^{1}H-NMR spectrum of compound **3-3**

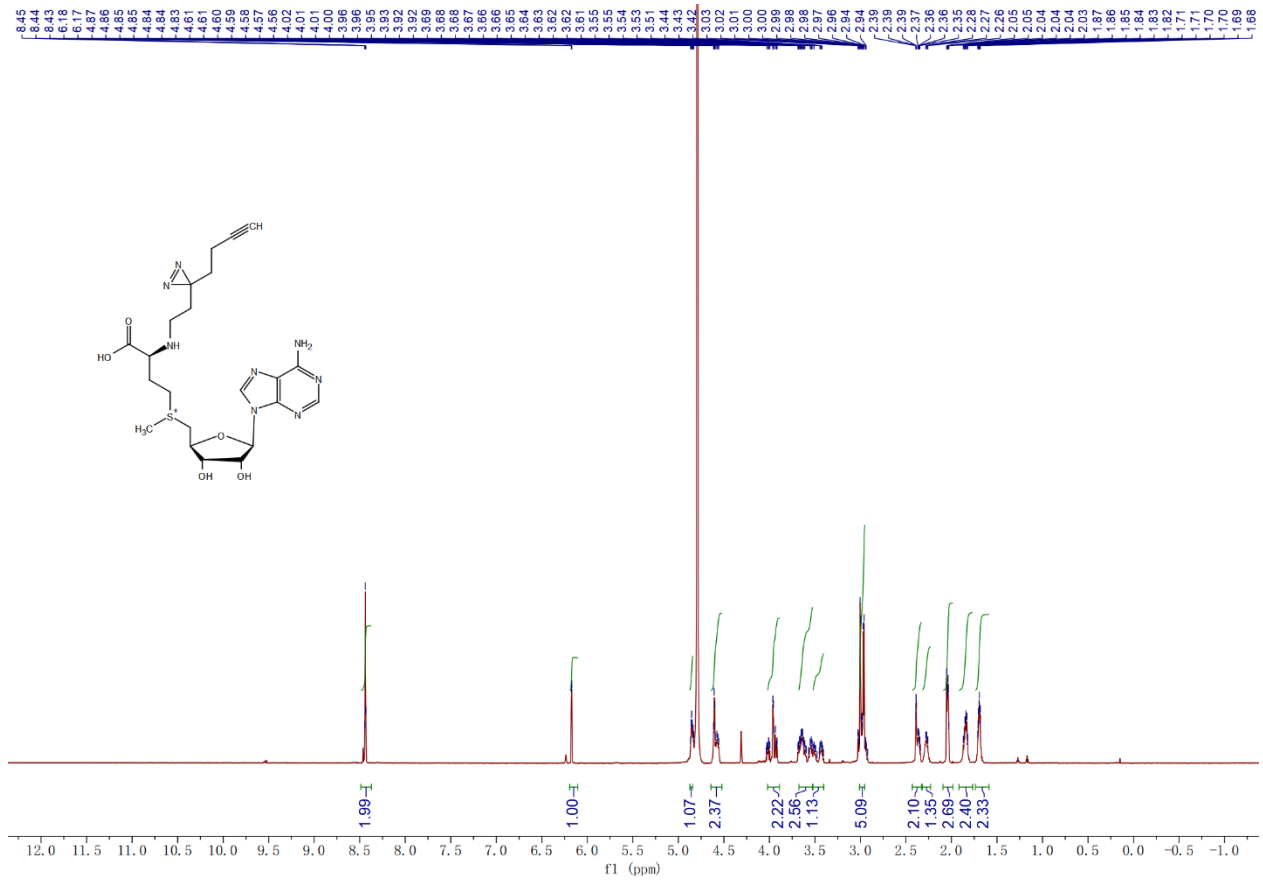


Figure S20. ^{1}H-NMR spectrum of compound **3-4**

Table S1. Overview of five proteins in figure 6

Rank	Uniprot/RefSeq/ GenBank Accession	Gene/ORF name/Locus_tag	Organism	Description
1	P00942	TPI1	Saccharomyces cerevisiae S288C	Triosephosphate isomerase
2	P05694	MET6	Saccharomyces cerevisiae S288C	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
3	WP_219608243.1	ArsL	Burkholderia gladioli	arsinothricin biosynthesis radical SAM protein ArsL
4	KKJ03637.1	AcnA	Burkholderia gladioli	aconitate hydratase
5	AYQ87247.1	EDD84_07545	Burkholderia gladioli	ribonucleoside-diphosphate reductase subunit alpha

Table S2. The proteins identified by probe 2 from *E. coli* cell lysate

Rank	Protein description	Coverage [%]	Peptides count	PSMs count	Unique peptides count
1	diphthamide biosynthesis enzyme Dph2 [Pyrococcus horikoshii OT3]	85	54	1295	49
2	Chain A, Structure of Adenylosuccinate Synthetase from E. Coli Complexed with Hadacidin, Gdp, 6-Phosphoryl-Imp, And Mg^{2+}	58	18	38	18
3	phosphopentomutase [Escherichia coli B7A]	67	18	39	17
4	aminopeptidase B [Escherichia coli HS]	58	17	35	17
5	PREDICTED: keratin 25D isoform 4 [Pan troglodytes]	26	17	37	16
6	epidermal cytokeratin 2 [Homo sapiens]	48	20	32	16
7	Chain A, Crystal Structure Of *E.Coli* Histidyl-Trna Synthetase Complexed With A Histidyl-Adenylate Analogue	51	16	28	16
8	protein S1	37	16	21	16
9	Chain A, Structure of Wild Type *E. Coli* Fabf (Kasii)	75	17	62	15

C. References

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