Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supplementary Information

Development of on-DNA vinyl sulfone synthesis for DNA-encoded chemical library

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1. Abbreviations

ACN: acetonitrile

DIPEA: *N*, *N*-diisopropylethylamine

DMA: *N*, *N*-dimethylacetamide

MeOH: methanol

DMT-MM: 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride

HATU: *O*-(7-aza-1-benzotriazolyl)-*N, N, N', N'*-tetramethyluroniumhexafluorophosphate

HFIP: 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol

HP: headpiece

HP-P: headpiece primer

HPLC: high performance liquid chromatography

MW: molecular weight

NMR: nuclear magnetic resonance

PAGE: polyacrylamide gel electrophoresis

TBE: tris-borate-EDTA

TEAA: triethylammonium acetate

TEA: trimethylamine

TLC: thin layer chromatography

TIC: total ion chromatogram

UPLC-MS: ultra performance liquid chromatography-mass spectrum

UV: ultraviolet

2. Materials and general methods

2.1. Materials

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Headpiece (**HP**, 5'-/5Phos/GAGTCA/iSp9/iUniAmM/iSp9 /TGACT CCC-3'), Headpiece-primer (**HP-P**, 5'-/5Phos/ACCTTCGGTCGGGAGTCA /iSp9/iUniAmM/iSp9/TGACTCCCGACCGAAGGTTG-3') and code sequences were received from HitGen Inc. (Shuangliu District, Chengdu, China). All the DNA sequences were written in 5'- to 3'- orientation unless otherwise noted. Chemicals and reagents were purchased from several commercial suppliers including J&K Scientific, Bidepharm, Adamas, and Sigma-Aldrich, and were generally used from aliquots dissolved in MeOH, DMA or other solvents, depending on solubility and optimized reaction conditions. T4 DNA ligase and 10× ligation buffer (500 mM Tris pH 7.5, 500 mM NaCl, 100 mM $MgCl_2$, 100 mM DTT and 25 mM ATP) were purchased from HitGen Inc.. All the buffer and aqueous solutions, NaCl (5 M), basic borate buffer (250 mM sodium borate/boric acid, pH 9.4), acetate buffer (3 M sodium acetate/acetic acid, pH 5.2) were prepared in-house. Cestbon water was used in all the reactions unless otherwise stated. All the gel images were captured by a Bio-Rad Chemidoc™ image system.

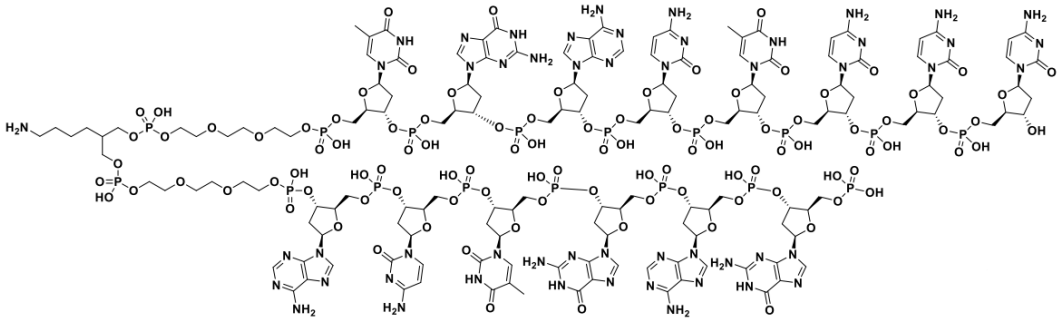


Figure S1. Structure of **HP** (5'/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3'), MW = 4937.

2.2. General methods for DNA analysis (UPLC-MS method)

Analysis of on-DNA reactions by UPLC-MS. The detection was performed by a high-resolution mass spectrometry-Agilent 6230 Time-of-Flight (TOF) mass spectrometer connected to an Agilent 1290 UPLC. After reaction, an aliquot of the reaction mixture was diluted with water to make the sample approximately 1 μM, and then 10~20 μL of the sample was injected into a reversed-phase UPLC column (Agilent, AdvanceBio Oligonucleotide, C18, 2.1×50 mm, 2.7 μm, maintained at 60 °C) at flow rate of 0.3 mL/min. The effluent was detected by UV absorbance at 260 nm and analysed on Agilent TOF (6230 B) in negative ion mode.

LCMS method of DNA reaction analysis:

Time (min)	Flow (mL/min)	%B
initial	0.3	5.0
1	0.3	15.0
2	0.3	25.0
5.5	0.3	30.0
6	0.3	90.0
6.5	0.3	90.0
7	0.3	5.0
8	0.3	5.0

Solvent A: 200 mM HFIP and 8 mM TEA in H_2O; Solvent B: MeOH

LCMS method of DNA ligation analysis:

Time (min)	Flow (mL/min)	%B
initial	0.3	3.0
1	0.3	12.0
2.5	0.3	18.0
4	0.3	20.0
6	0.3	22.0
9	0.3	30.0
10	0.3	85.0
11	0.3	85.0

12	0.3	3.0

Solvent A: 200 mM HFIP and 8 mM TEA in H_2O; Solvent B: MeOH

Conversion calculation. The conversion of on-DNA products was determined by UV absorbance (260 nm) peak area integration using the following equation: Conversion% = UV (desired products)/UV (total products), ignoring UV extinction coefficient difference for DNA species and assuming 100% DNA recovery. Any non-oligo material with UV absorbance at 260 nm was subtracted from the conversion calculation.[1]

Analysis of molecular mass. Observed m/z was calculated as m/z = [M – z]/z for the negative ion mode. Data visualization and integration were performed on BioConfirm 10.0 software (Agilent, v10.0).

2.3. General methods for DNA conjugates purification

General procedure for ethanol precipitation. To an on-DNA reaction mixture was added 10% (V/V) 5 M NaCl solution and 3 times volume of absolute cold ethanol. Alternatively, to a DNA ligation mixture was added 10% (V/V) 3 M acetate buffer (pH 5.2) and 3 times volume of absolute cold ethanol. After swirling and centrifuging, the solution was maintained at -80 °C for 2 h and then was centrifuged at 13500 rpm for 30 minutes at 4 °C by using Eppendorf 5424R centrifuge. The supernatant was discarded and the pellet was rinsed with 200 μL cold 75% ethanol. After centrifuging at 13500 rpm for 10 minutes at 4 °C, the supernatant was discarded again and the DNA pellet was dried by Speedvac (CV200, JM company, Beijing, China), which was equipped with cryotrap (JM86, JM company, Beijing, China). The recovered sample was dissolved in the appropriate solvent for subsequent experiments.

General method for HPLC purification. Preparative reversed-phase high-pressure liquid chromatography (RP-HPLC) for the DNA conjugate was performed on Waters 1575EF Series with the column (Eclipse-XDB C18, 5 μM, 9.4 × 250 mm) using eluent A (100 mM TEAA in H_2O) and eluent B (100 mM TEAA in 80% ACN) with gradient: 10% B (0 - 1 min), 10% to 30% B (1 - 11min), 30% to 100% B (11 - 11.1 min), 100% B (11.1 - 12 min), 100% to 10% B (12 - 12.1 min), 10% B (12.1 - 16 min). Fractions containing the product were combined and lyophilized overnight.

2.4. General procedure for DNA ligation

This reaction contained variably-derivatized **HP-P** starting material (10 nmol in H_2O, 1 equiv), code (12 nmol in H_2O, 1.2 equiv), 10× ligation buffer (4 μL), T4 DNA ligase (1 μL, 1000 units/μL) and nuclease-free water (to the total volume of 40 μL). The reaction mixture was incubated at 20 °C for overnight before performing gel analysis. The crude product was purified by ethanol precipitation and used for the next step of synthesis without further purification.

2.5. General procedure for polyacrylamide gel

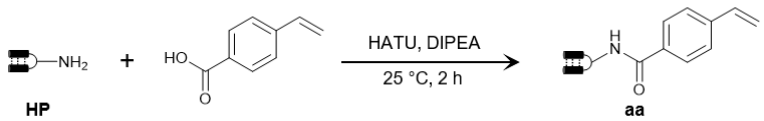
Ligation reaction was monitored by gel electrophoresis with 20% urea polyacrylamide gel in 1× TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) system referenced by a 20 bp DNA ladder (Takara, Japan). First, the DNA samples were denatured at 95 °C for 10 min and mixed with loading buffer. Then, 10 pmol of treated DNA samples was loaded on the gel, and the gel was run at 200 V for 50 - 60 min. DNA fragments were visualized and analyzed by Bio-Rad Chemidoc^{TM} Image System (Bio-Rad, CA, USA).

2.6. General methods for monitoring reaction and characterizing small molecules

Reactions were monitored by TLC and general staining reagents were used to analyze TLC intuitively. There were no unknown compounds, and known compounds were characterized by ^{1}H-NMR or identified by comparison of their physical and spectroscopic data with those reported in the literature. NMR spectrum was recorded on Agilent 400 MHz spectrometer using residual non-deuterated solvent ($CDCl_3$) as the internal standard. Multiplicity abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet (dd = doublet of doublets), t = triplet, q = quartet, m = multiplet. Unless otherwise noted, all deuterated solvents were purchased from Adamas.

3. General procedure for on-DNA vinyl sulfone formation

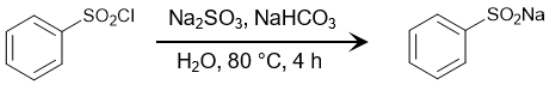
3.1. Preparation of DNA-conjugated alkenes by amide coupling



HP **aa**

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. 4-Vinylbenzoic acid (20 μL, 200 mM in DMA, 200 equiv), HATU (10 μL, 400 mM in DMA, 200 equiv), and DIPEA (10 μL, 400 mM in DMA, 200 equiv) were mixed by vortex and allowed to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to HP solution (40 μL, 20 nmol). The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 h. After ethanol precipitation, the reaction was analyzed by UPLC-MS. The separated and collected conjugates were vacuum-dried overnight, redissolved in H_2O for subsequent experiments. Deconvoluted molecular mass: calculated: 5067 Da; observed: 5067 Da.

3.2. Preparation of sodium sulfinates

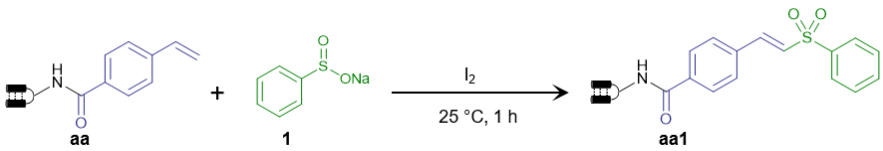


1

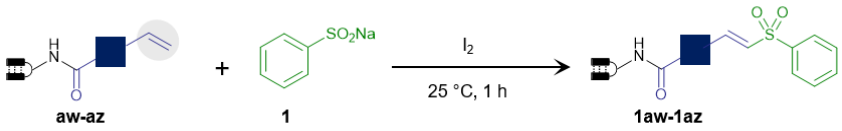
Benzenesulfonyl chloride (1 equiv) was added to a mixture of Na_2SO_3 (2 equiv) and $NaHCO_3$ (2 equiv) in water (1 M). The result mixture was stirred at 80 °C for 4 h. After cooling down to room temperature, the water was removed in vacuum, then ethanol was added. Then the precipitate was filtered off and the filtrate was concentrated in vacuum to obtain the final product. All the sulfinates are known compounds. They were identified by comparison of their physical and spectroscopic data with those reported in the literature.[2] Unless otherwise noted, sodium sulfinates described in the supplementary information were

synthesized under this standard condition.

3.3. On-DNA vinyl sulfone synthesis



Standard Condition: To the solution of DNA-conjugated **aa** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv) was added H_2O (8 μL), **1** (2 μL, 200 mM in H_2O, 400 nmol, 2000 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 2000 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: >90%). Deconvoluted molecular mass: calculated: 5207 Da; observed: 5207 Da. Unless otherwise noted, on-DNA vinyl sulfones described in the supplementary information were synthesized under this standard condition.



For DNA-conjugated aw-az: To the solution of DNA-conjugated (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv) was added H_2O (8 μL), **1** (2 μL, 200 mM in H_2O, 400 nmol, 2000 equiv), and iodine (2 μL, 50 mM in MeOH, 100 nmol, 500 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above

4. General procedure for subsequent diversification of vinyl sulfone

4.1. Amide coupling

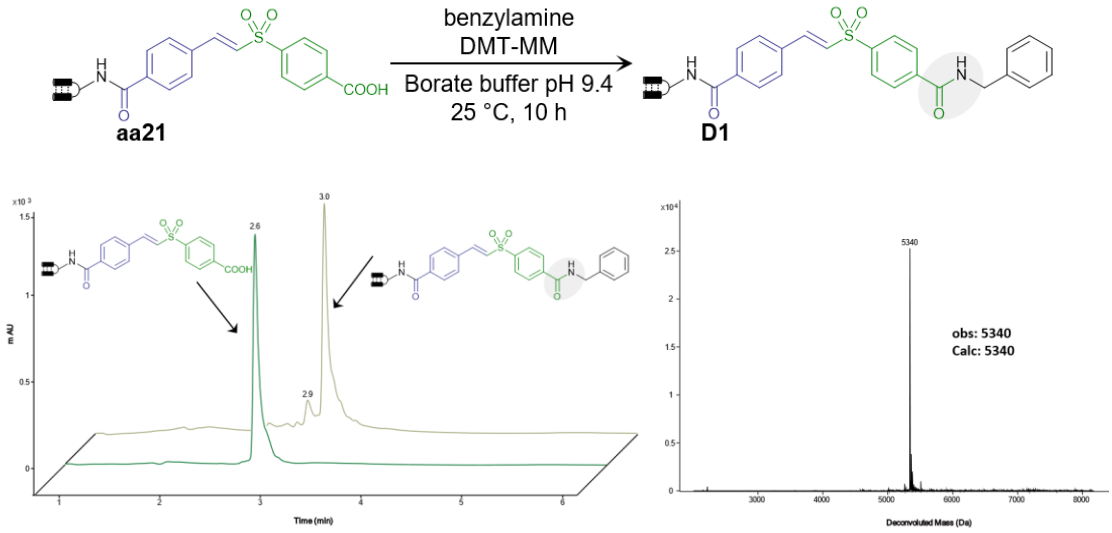
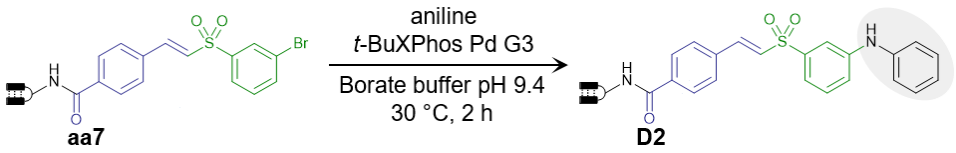


Figure S2. UPLC chromatogram and deconvoluted MS of **D1**.

To the solution of **aa21** (0.2 nmol, 1 μL, 200 μM in H_2O, 1 equiv) was added benzylamine (1500 nmol, 5 μL, 300 mM in ACN/H_2O, 1:1, 750 equiv), Borate buffer pH 9.4 (2250 nmol, 9 μL, 250 mM in H_2O, 11250 equiv), DMT-MM (4500 nmol, 5 μL, 900 mM in H_2O, 22500 equiv). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 10 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: 88%). Deconvoluted molecular mass: calculated: 5340 Da; observed: 5340 Da.

4.2. Buchwald−Hartwig amination



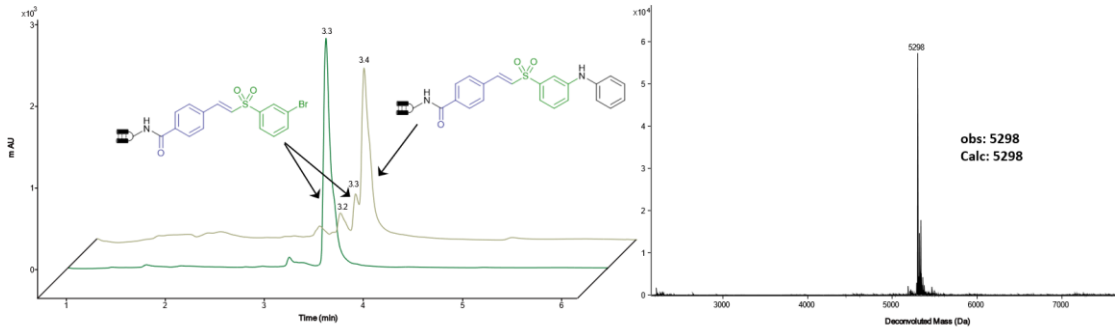
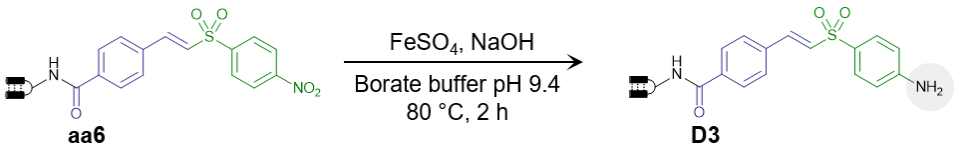


Figure S3. UPLC chromatogram and deconvoluted MS of **D2**.

To the solution of **aa7** (0.2 nmol, 2 μL, 100 μM in H_2O, 1 equiv) was added aniline (1600 nmol, 4 μL, 400 mM in DMA, 8000 equiv), Borate buffer pH 9.4 (375 nmol, 1.5 μL, 250 mM in H_2O, 1875 equiv), *t*-BuXPhos Pd G3 (150 nmol, 1.5 μL, 100 mM in DMA, 750 equiv), and H_2O (5 μL). The reaction mixture was vortexed, centrifuged, and incubated at 30 °C for 2 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: 70%). Deconvoluted molecular mass: calculated: 5298 Da; observed: 5298 Da.

4.3. Nitro reduction



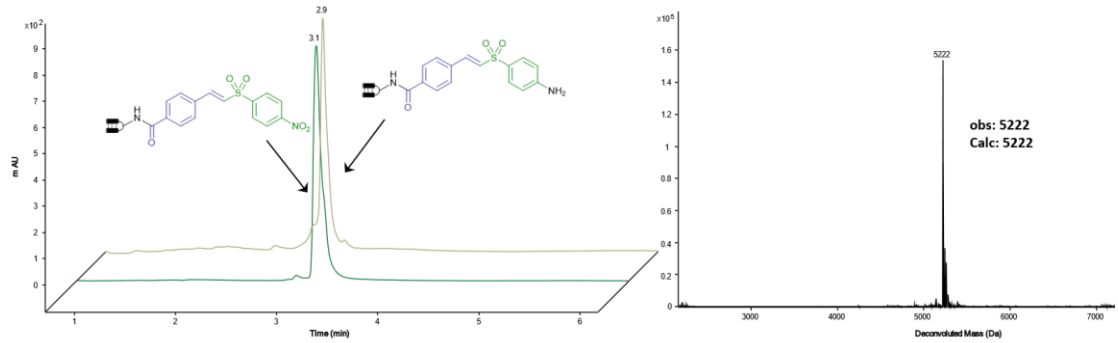


Figure S4. UPLC chromatogram and deconvoluted MS of **D3**.

To the solution of **aa6** (0.2 nmol, 1 equiv) in Borate buffer pH 9.4 (2500 nmol, 10 μL, 250 mM in H_2O, 12500 equiv), was added $FeSO_4$ (1000 nmol, 5 μL, 200 mM in H_2O, 5000 equiv), NaOH (3000 nmol, 3 μL, 1000 mM in H_2O, 15000

equiv). The reaction mixture was vortexed, centrifuged, and incubated at 80 °C for 2 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5222 Da; observed: 5222 Da.

4.4. Tetrazole formation

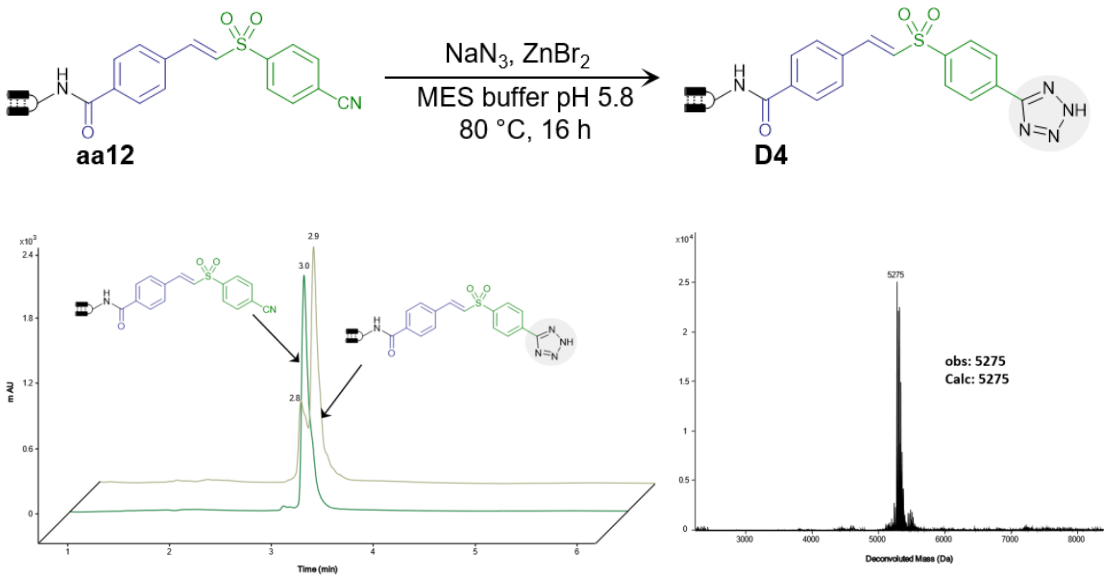
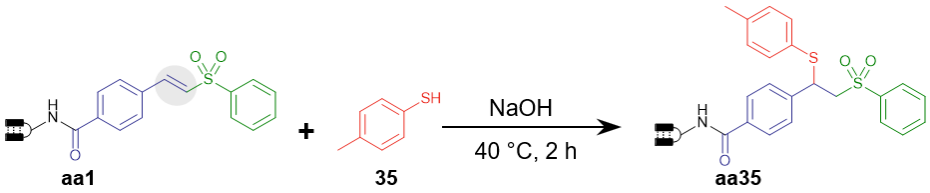


Figure S5. UPLC chromatogram and deconvoluted MS of **D4**.

To the solution of **aa12** (0.2 nmol, 10 μL, 20 μM in H_2O, 1 equiv) was added MES buffer pH 5.8 (2500 nmol, 10 μL, 250 mM in H_2O, 12500 equiv), 1,4-dioxane (18 μL), NaN_3(2000 nmol, 5 μL, 400 mM in H_2O, 10000 equiv), $ZnBr_2$ (500 nmol, 2 μL, 250 mM in H_2O, 2500 equiv). The reaction mixture was vortexed, centrifuged, and incubated at 80 °C for 16 h. After the reaction mixture was cooled down to room temperature, sodium cysteinate (1000 nmol, 5 μL, 200 mM in H_2O, 5000 equiv) was added and then heated at 80 °C for 15 min before being quenched by EtOH precipitation. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: 75%). Deconvoluted molecular mass: calculated: 5275 Da; observed: 5275 Da.

4.5. On-DNA thiol-Michael addition reaction of vinyl sulfone and subsequent sulfoxide formation

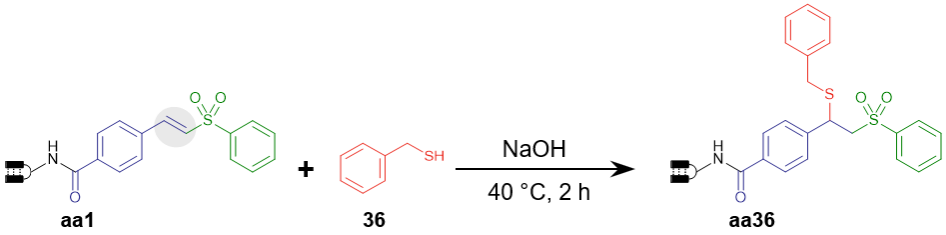
Scheme S1. Synthesis of **aa35**.



aa1 35 aa35

To the solution of DNA-conjugated **aa1** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv) was added H_2O (16 μL), NaOH (4 μL, 250 mM in H_2O, 1000 nmol, 5000 equiv), and **35** (8 μL, 200 mM in DMA, 1600 nmol, 8000 equiv). The mixture was vortexed, centrifuged, and placed at 40 °C for 2 h. The product was obtained by ethanol precipitation as described above (Conversion: 80%). Deconvoluted molecular mass: calculated: 5331 Da; observed: 5331 Da.

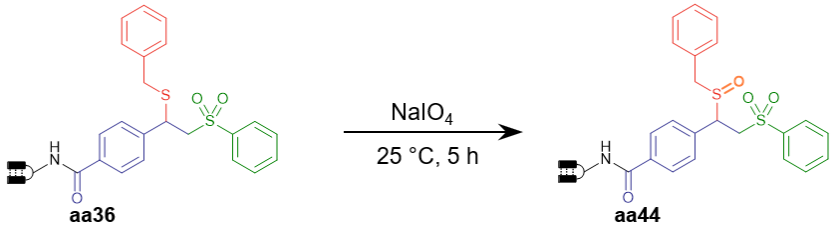
Scheme S2. Synthesis of **aa36**

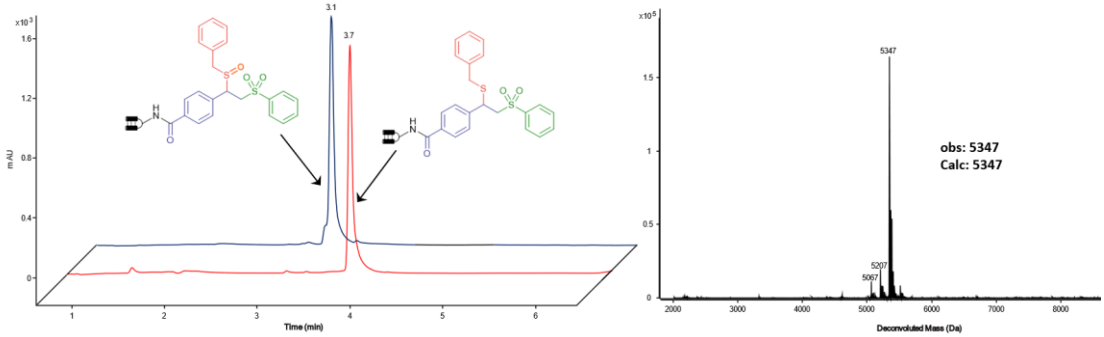


aa1 36 aa36

To the solution of DNA-conjugated **aa1** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv) was added DMA (16 μL), H_2O (6 μL), NaOH (2 μL, 250 mM in H_2O, 500 nmol, 2500 equiv), and **36** (4 μL, 200 mM in DMA, 800 nmol, 4000 equiv). The mixture was vortexed, centrifuged, and placed at 40 °C for 2 h. The product was obtained by ethanol precipitation as described above (Conversion: >90%). Deconvoluted molecular mass: calculated: 5331 Da; observed: 5331 Da. Unless otherwise noted, on-DNA thiol-Michael addition reaction described in the supplementary information were condducted under this standard condition.

Scheme S3. Synthesis of sulfoxide **aa44**.



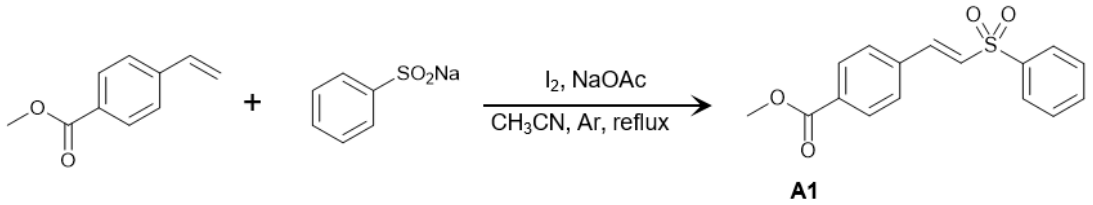


To the solution of **aa36** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv) was added H_2O (43 μL) and $NaIO_4$ (5 μL, 100 mM in H_2O, 500 nmol, 2500 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 5 h. The product was obtained by ethanol precipitation as described above (Conversion: >90%). Deconvoluted molecular mass: calculated: 5347 Da; observed: 5347 Da.

5. Structural confirmation

5.1. Off-DNA synthesis of authentic A1

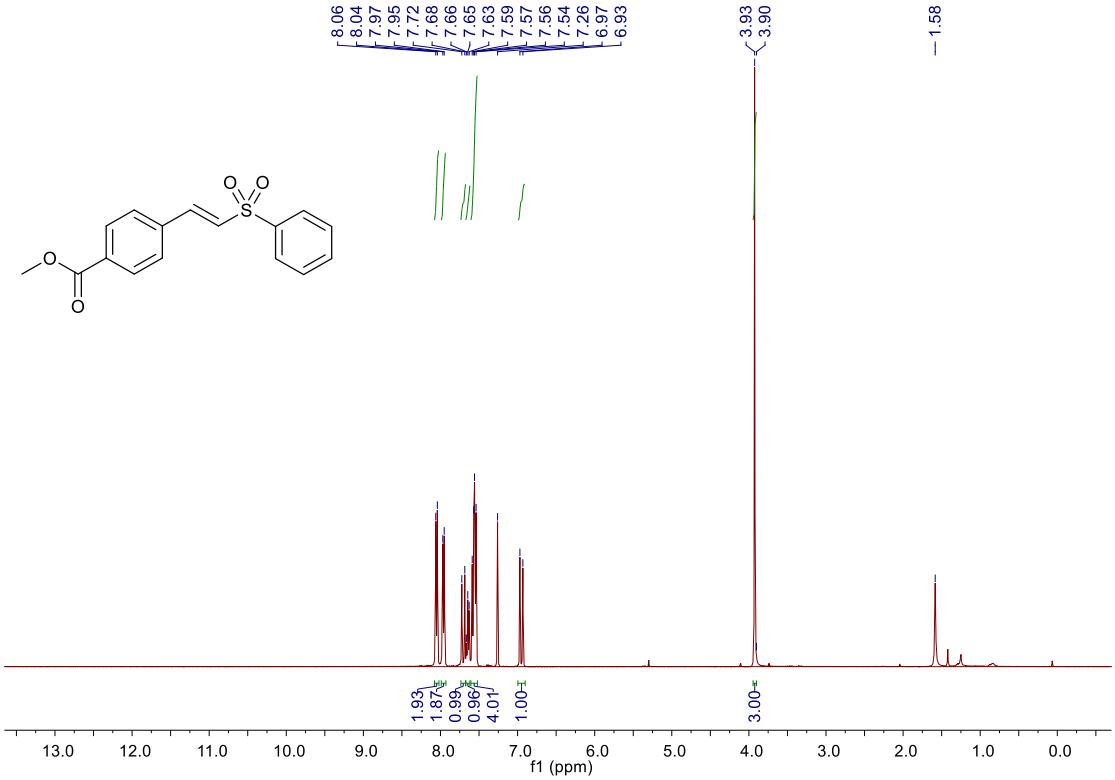
Scheme S4. Synthesis of authentic **A1**



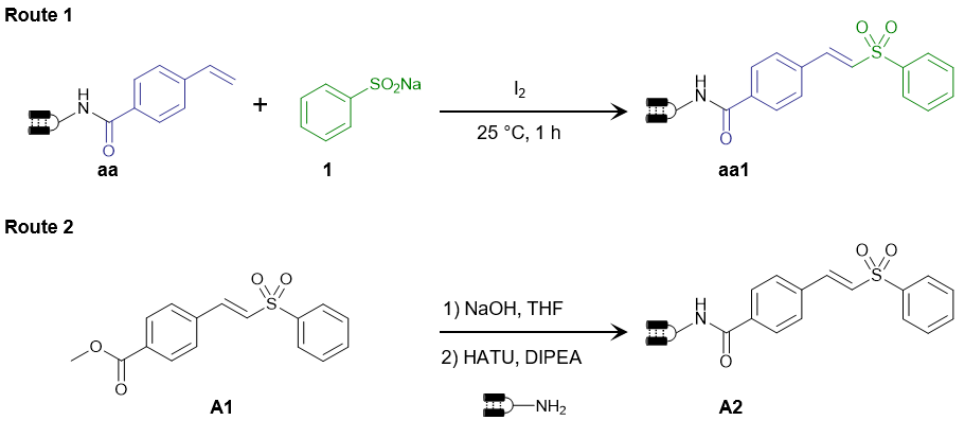
A1

Methyl (E)-4-(2-(phenylsulfonyl)vinyl)benzoate (**A1**).[3] To a suspension of benzenesulfinic acid sodium salt (147.6 mg, 0.9 mmol, 3 equiv) and NaOAc (36.9 mg, 0.45 mmol, 1.50 equiv) in MeCN (1.2 mL) was added styrene (50 mg, 0.3 mmol, 1 equiv) followed by iodine (114.3 mg, 0.45 mmol, 1.50 equiv). The mixture was heated to reflux for 3 h before being allowed to cool and the excess iodine was quenched with 10% aq. sodium thiosulfate. Sat. aq. $NaHCO_3$ was added and the product was extracted into EtOAc (3 x 20 mL). The organic phase was combined and dried with Na_2SO_4. The solvent was removed in vacuo to give a residue, which was purified by flash column chromatography on silica gel using petroleum ether/ethyl acetate (5:1) as eluent, affording **A1** as a white amorphous solid (66 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.3 Hz, 2H), 7.96 (d, *J* = 7.4 Hz, 2H), 7.70 (d, *J* = 15.4 Hz, 1H), 7.65 (t, *J* = 7.4 Hz, 1H), 7.56 (dd, *J* = 12.2, 8.0 Hz, 4H), 6.95 (d, *J* = 15.4 Hz, 1H), 3.93 (s, 3H).

^{1}H-NMR of **A1**


8.06
8.04
7.97
7.95
7.72
7.68
7.66
7.65
7.63
7.59
7.57
7.56
7.54
7.26
6.97
6.93
3.93
3.90
1.58
1.93
1.87
0.99
0.96
4.01
1.00
3.00
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

5.2. Co-injection experiment



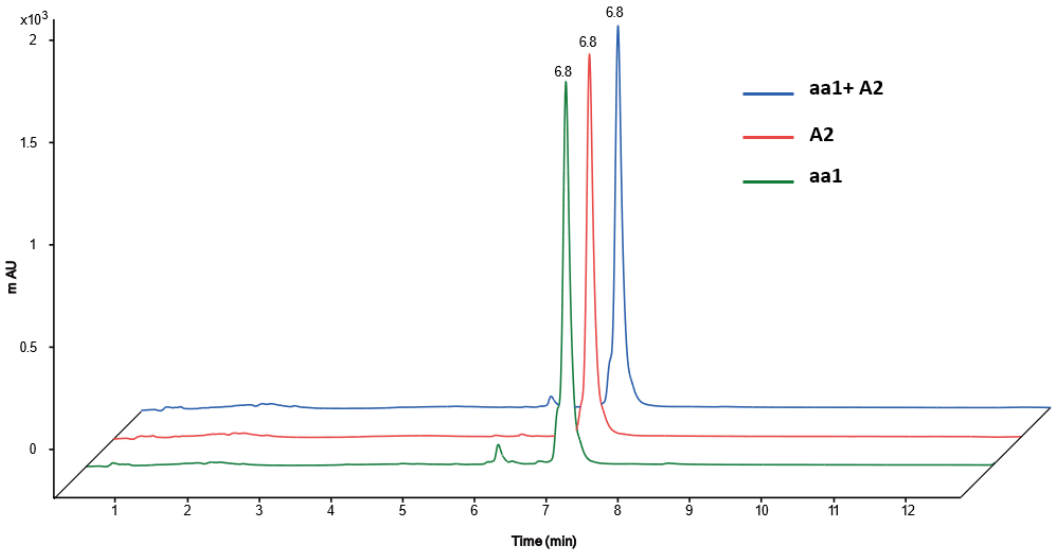
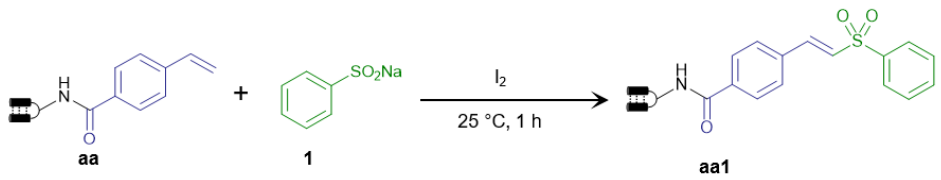


Figure S6. Co-injection experiment of **aa1** and **A2** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (curve in blue) had the same retention time as the other two peaks (**aa1** from route 1, curve in green; **A2** from route 2, curve in red)

6. Scale-up reaction

6.1. Scale-up reaction of aa1



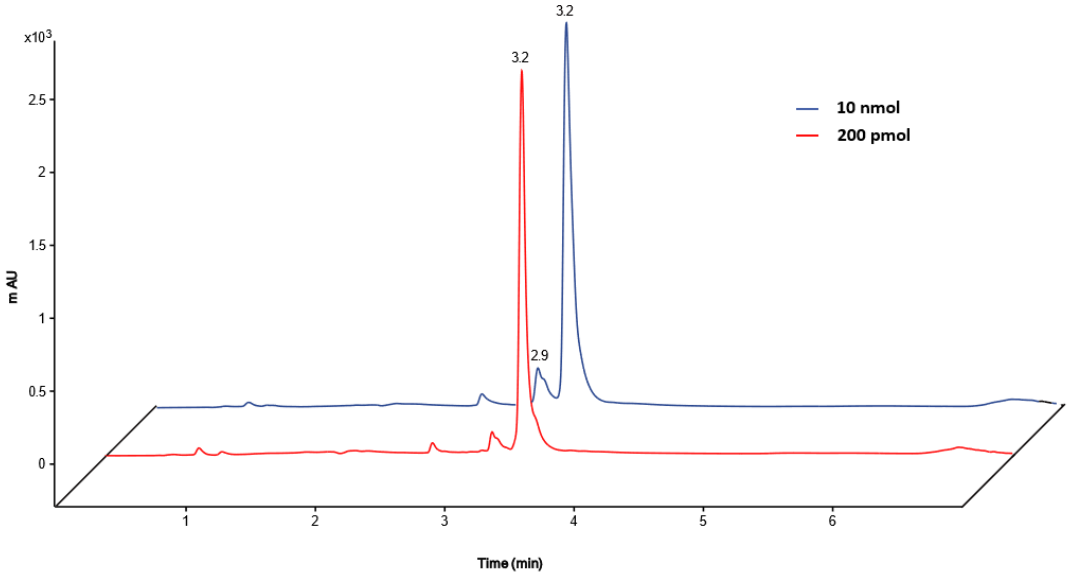
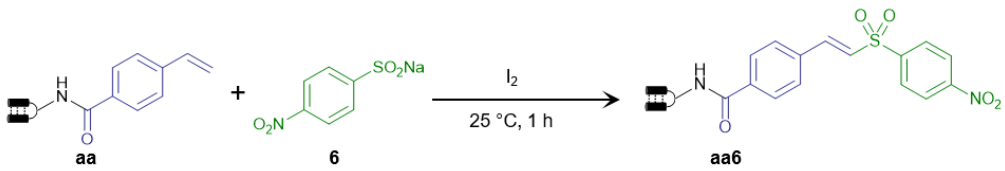


Figure S7. UPLC chromatogram of **aa1** at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated **aa** (2 μL, 5 mM in H_2O, 10 nmol, 1 equiv) was added H_2O (8 μL), **1** (2 μL, 200 mM in H_2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 88%). Deconvoluted molecular mass: calculated: 5207 Da; observed: 5207 Da.

6.2. Scale-up reaction of aa6



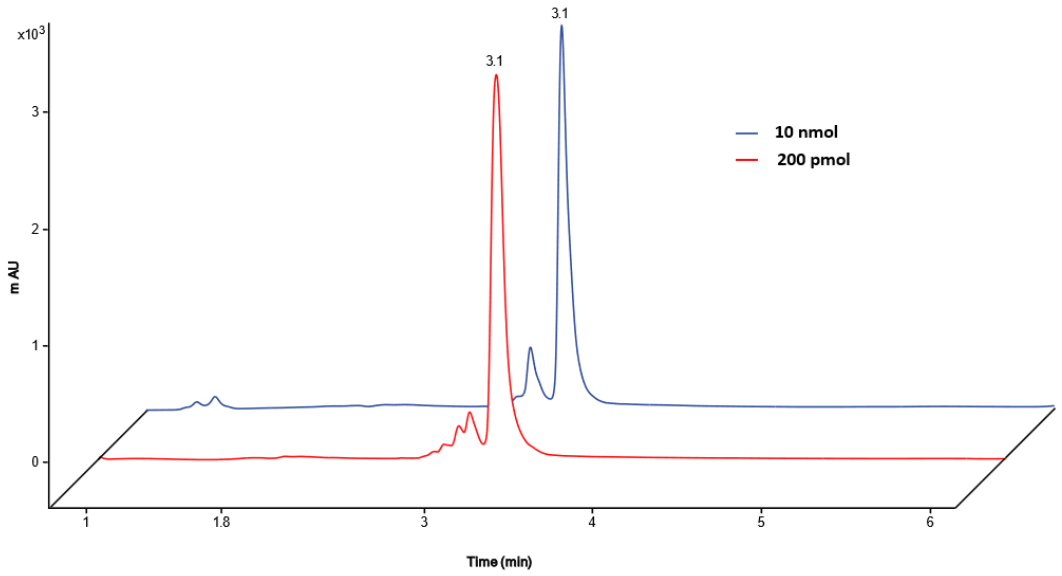
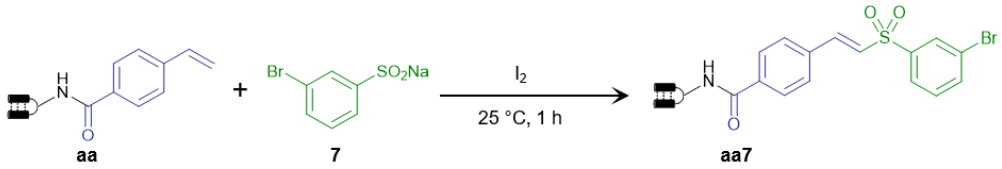


Figure S8. UPLC chromatogram of **aa6** at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated **aa** (2 μL, 5 mM in H_2O, 10 nmol, 1 equiv) was added H_2O (8 μL), **6** (2 μL, 200 mM in H_2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 86%). Deconvoluted molecular mass: calculated: 5252 Da; observed: 5252 Da.

6.3. Scale-up Reaction of aa7



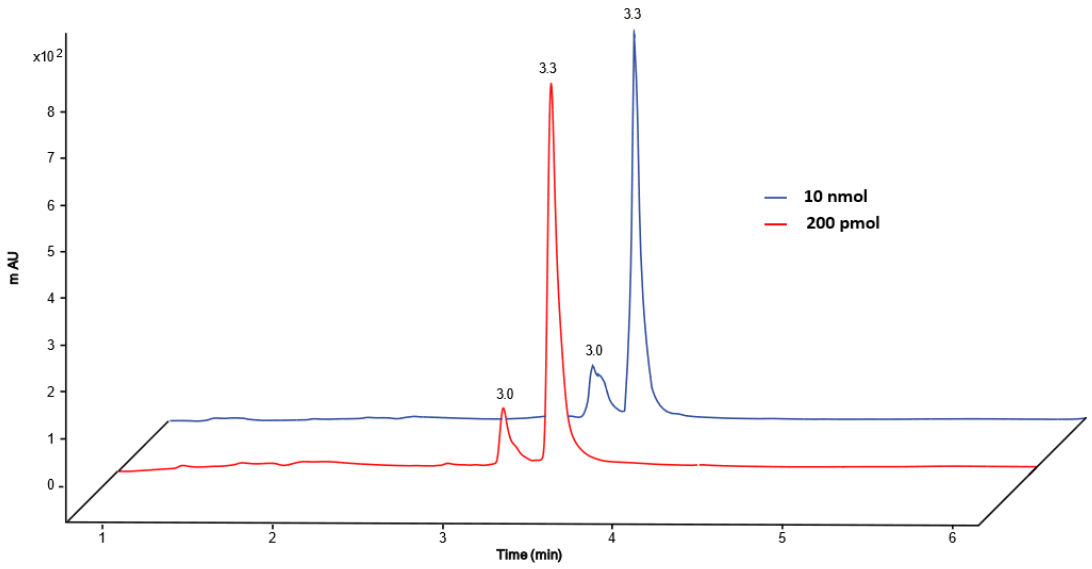
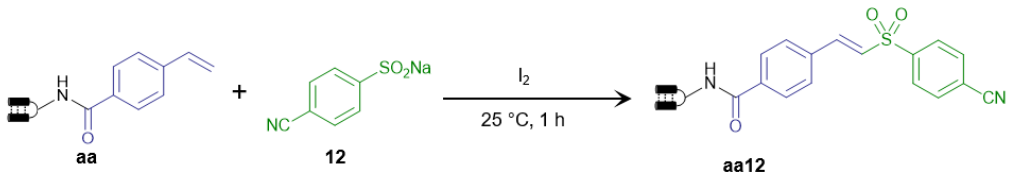


Figure S9. UPLC chromatogram of **aa7** at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated **aa** (2 μL, 5 mM in H_2O, 10 nmol, 1 equiv) was added H_2O (8 μL), **7** (2 μL, 200 mM in H_2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 81%). Deconvoluted molecular mass: calculated: 5286 Da; observed: 5286 Da.

6.4. Scale-up reaction of aa12



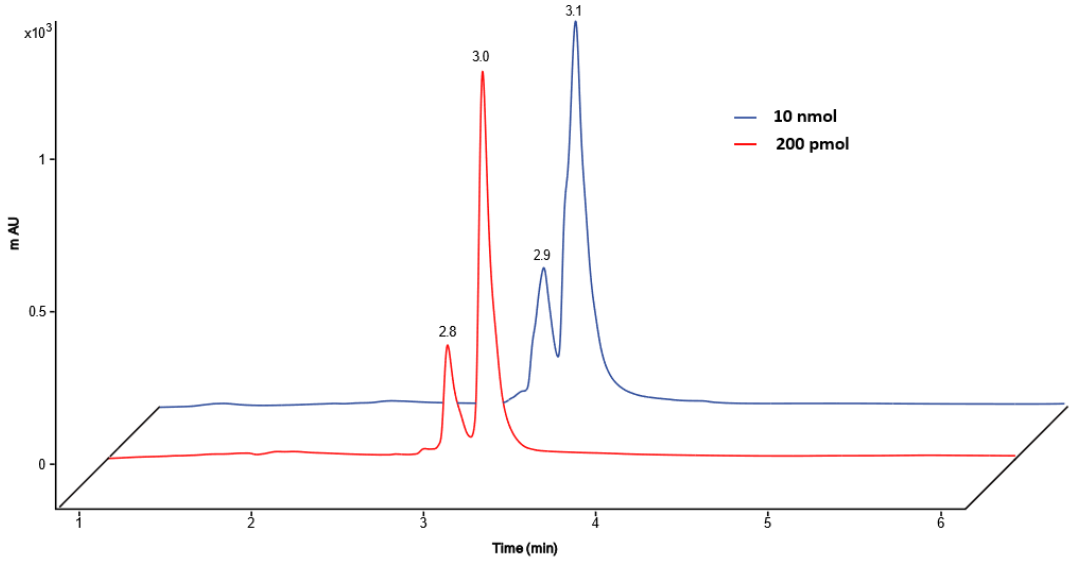
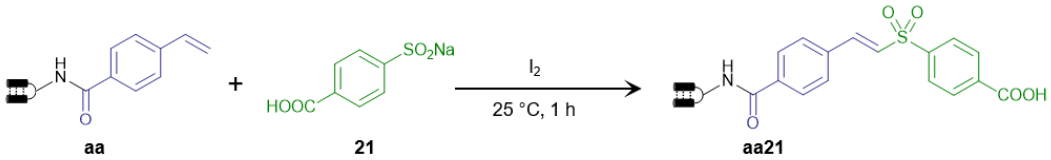


Figure S10. UPLC chromatogram of **aa12** at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated **aa** (2 μL, 5 mM in H_2O, 10 nmol, 1 equiv) was added H_2O (8 μL), **12** (2 μL, 200 mM in H_2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 77%). Deconvoluted molecular mass: calculated: 5232 Da; observed: 5232 Da.

6.5. Scale-up reaction of aa21



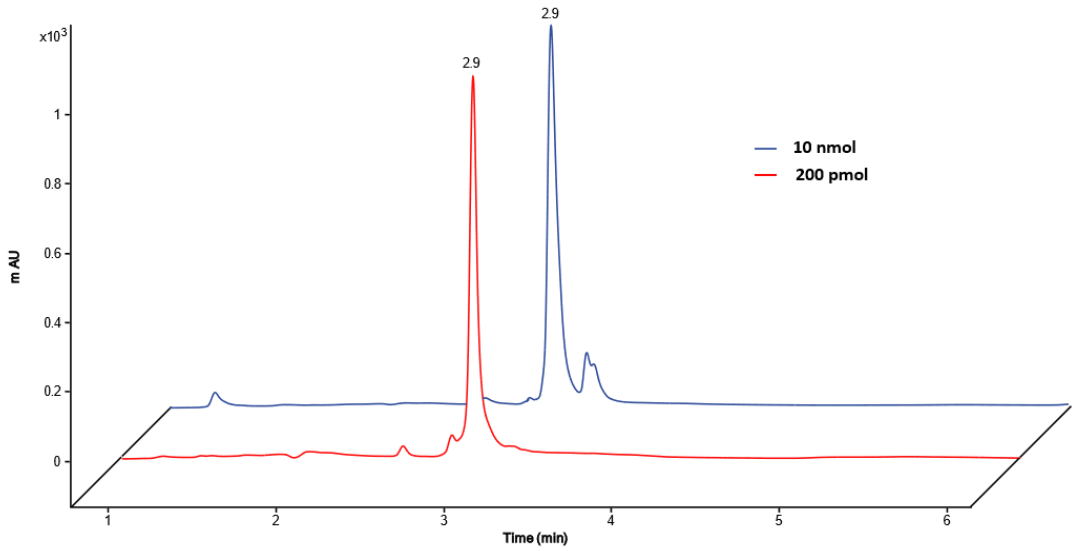
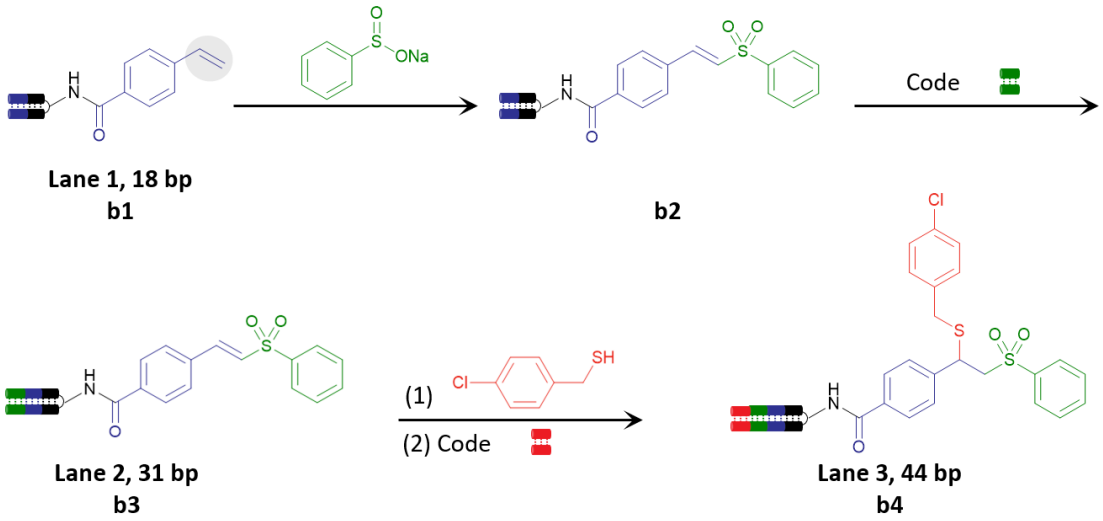


Figure S11. UPLC chromatogram of **aa21** at 200 pmol and 10 nmol scale, respectively.

To the solution of DNA-conjugated **aa** (2 μL, 5 mM in H_2O, 10 nmol, 1 equiv) was added H_2O (8 μL), **21** (2 μL, 200 mM in H_2O, 400 nmol, 40 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 40 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 83%). Deconvoluted molecular mass: calculated: 5251 Da; observed: 5251 Da.

7. Enzymatic ligation

Scheme S5. Protocol of two-cycle DEL synthetic route and enzymatic ligation.



Headpiece-primer (**HP-P**, 20 nmol**)** was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. 4-Vinylbenzoic acid (10 μL, 200 mM in DMA, 100 equiv), HATU (5 μL, 400 mM in DMA, 100 equiv), and DIPEA (5 μL, 400 mM in DMA, 100 equiv) were mixed by vortex and allowed to pre-activated for 10 minutes at 25 °C, and then the mixture was transferred to **HP-P** solution in a 0.6 mL tube and mixed. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 hours. After ethanol precipitation, the resulting pellet was vacuum-dried and dissolved in nuclease-free water (200 μL). The reaction was analyzed by UPLC-MS.

To the solution of DNA conjugate **b1** (2 μL, 2.5 mM in H_2O, 5 nmol, 1 equiv) was added H_2O (8 μL), **1** (2 μL, 200 mM in H_2O, 400 nmol, 80 equiv), and iodine (2 μL, 200 mM in MeOH, 400 nmol, 80 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 1 h. The product was obtained by ethanol precipitation as described above (Conversion: 90%). Deconvoluted molecular mass: calculated: 12679 Da; observed: 12679 Da.

DNA conjugate **b2** (16 μL, 6 nmol), code (6 μL, 7.2 nmol, 1.2 equiv) and 2× ligation buffer (25 μL) were added into 0.6 mL tube, mixed by vortex, then T4 DNA ligase (3 μL, 350 units/μL) was added and mixed gently. The reaction

mixture was incubated at 20 °C for 16 h. Before ligation confirmation by UPLC-MS analysis, the reaction system was denatured by incubating at 95 °C for 10 min, and the ligation product was isolated by ethanol precipitation as described above. Deconvoluted molecular mass: calculated: 20719 Da; observed: 20720 Da.

To the solution of DNA conjugate **b3** (2 μL, 0.5 mM in H_2O, 1 nmol, 1 equiv) was added H_2O (6 μL) and DMA (16 μL), NaOH (2 μL, 250 mM in H_2O, 500 nmol, 500 equiv), and 4-Chlorobenzylmercaptan (4 μL, 200 mM in H_2O, 800 nmol, 800 equiv). The mixture was vortexed, centrifuged, and placed at 40 °C for 2 h. The product was obtained by ethanol precipitation as described above (>90% conversion). Deconvoluted molecular mass: calculated: 20878 Da; observed: 20879 Da. After the reaction, product (10 μL, 50 pmol), code (0.5 μL, 65 pmol, 1.3 equiv) and 2× ligation buffer (10 μL) was added into 0.6 mL tube, mixed by vortex, then T4 DNA ligase (0.2 μL, 350 units/μL) was added and mixed gently. The reaction mixture was incubated at 20 °C for 16 h. Before ligation confirmation by UPLC-MS analysis, the reaction system was denatured by incubating at 95 °C for 10 min, and the ligation product was isolated by ethanol precipitation as described above. Deconvoluted molecular mass: calculated: 28900 Da; observed: 28983 Da ($28900+2Na^+ + K^+$).

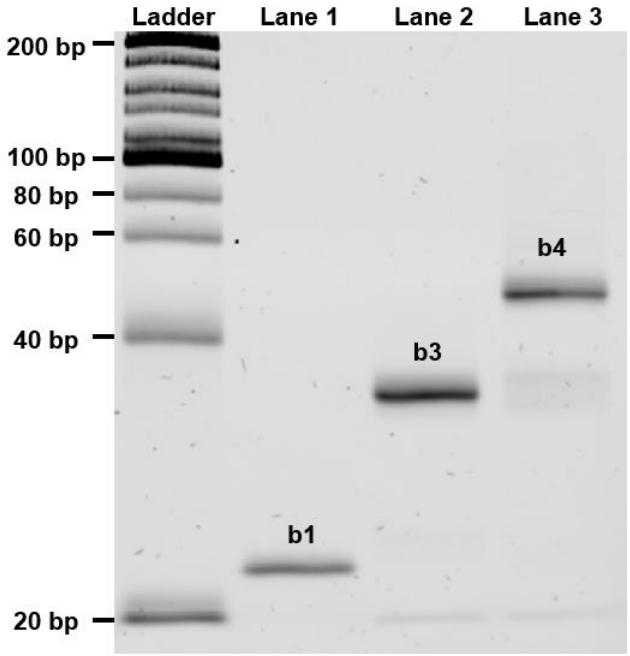


Figure S12. 20% denatured PAGE analysis of DEL compatibility of on-DNA vinyl sulfone and subsequent thiol-Michael addition reaction.

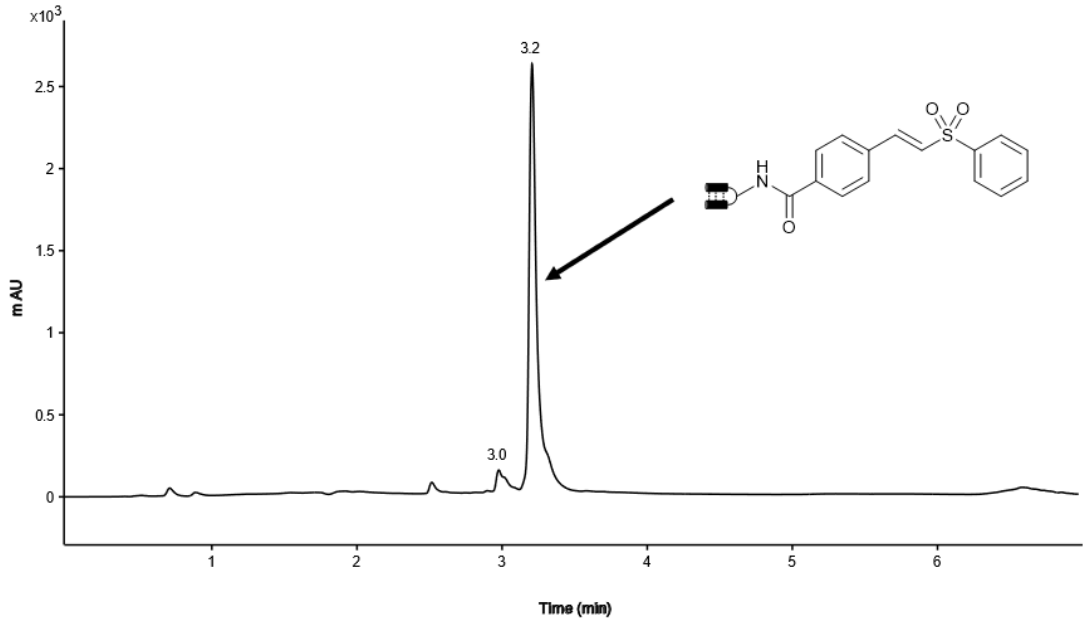
8. UPLC chromatogram and deconvoluted MS

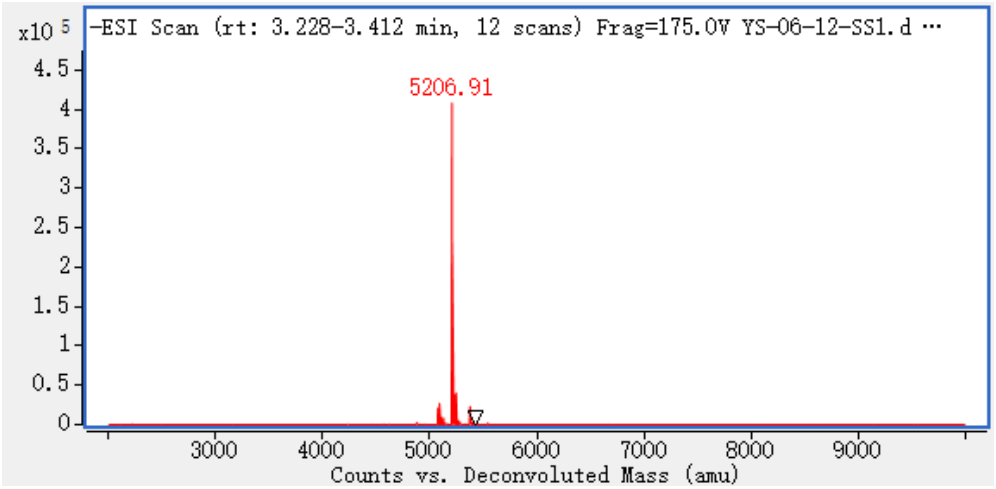
8.1. Substrate scope of sodium sulfinates

UPLC chromatogram and deconvoluted MS of **aa1**

Conversion: >90%

Calculated Mass: 5207 Da; Observed Mass: 5207 Da

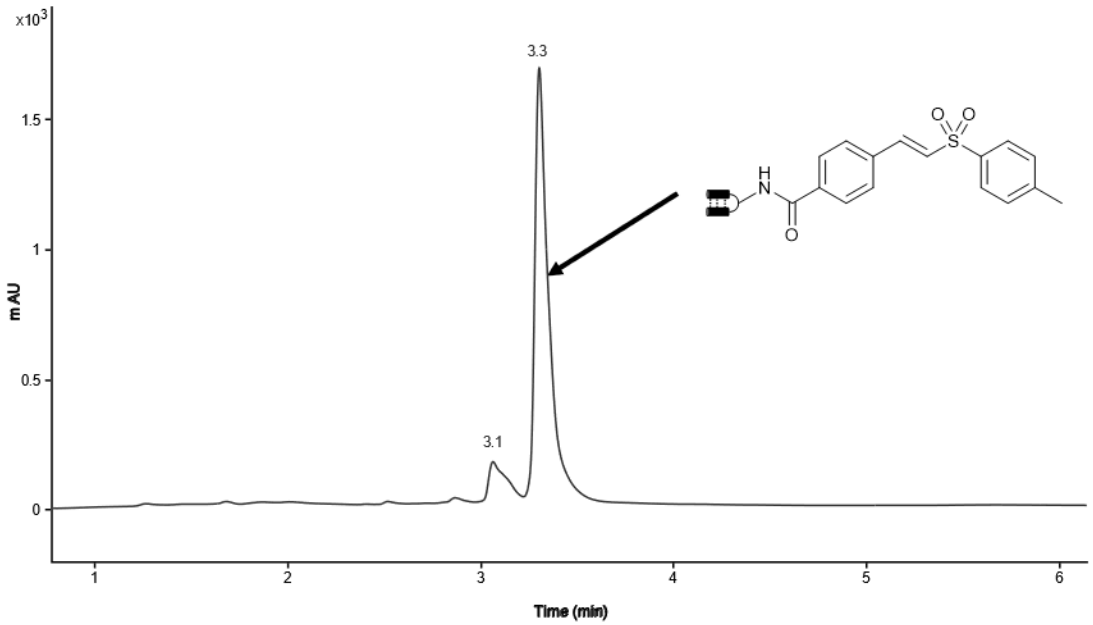


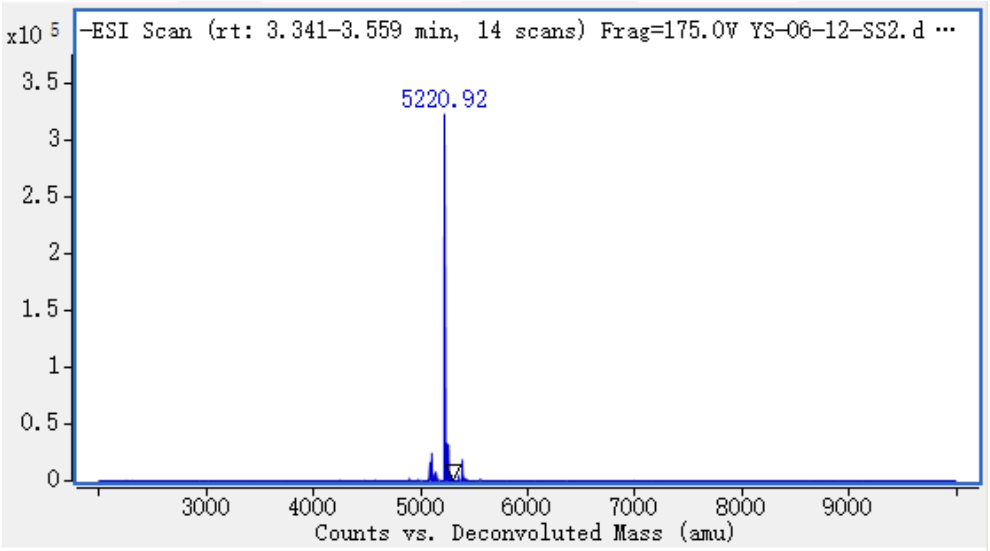


UPLC chromatogram and deconvoluted MS of **aa2**

Conversion: 87%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da

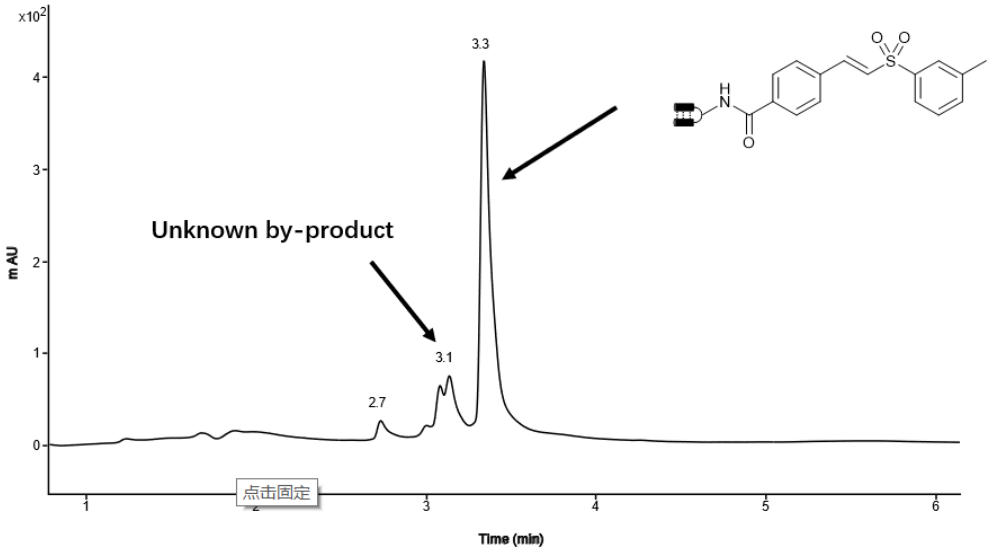


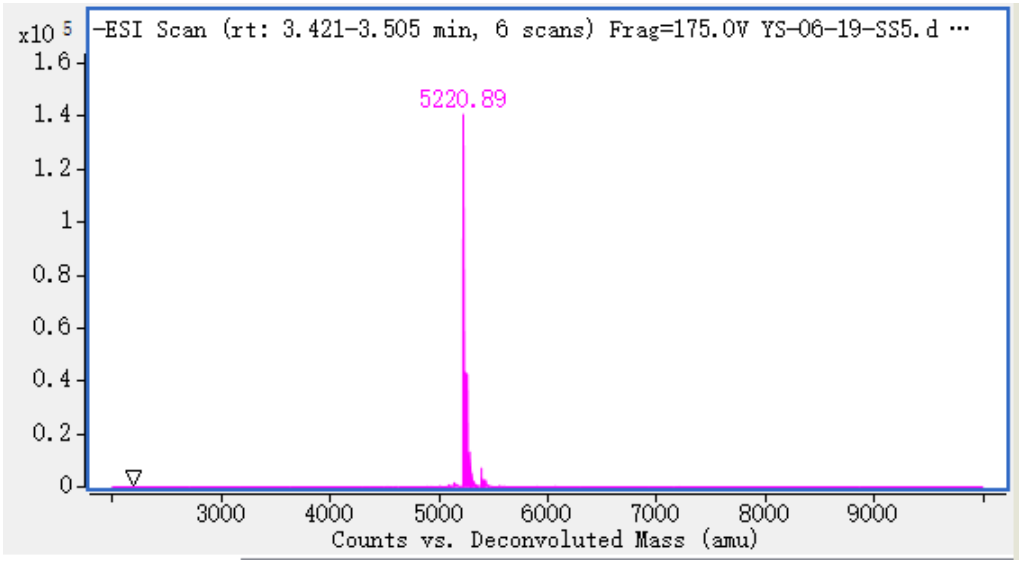


UPLC chromatogram and deconvoluted MS of **aa3**

Conversion: 82%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da

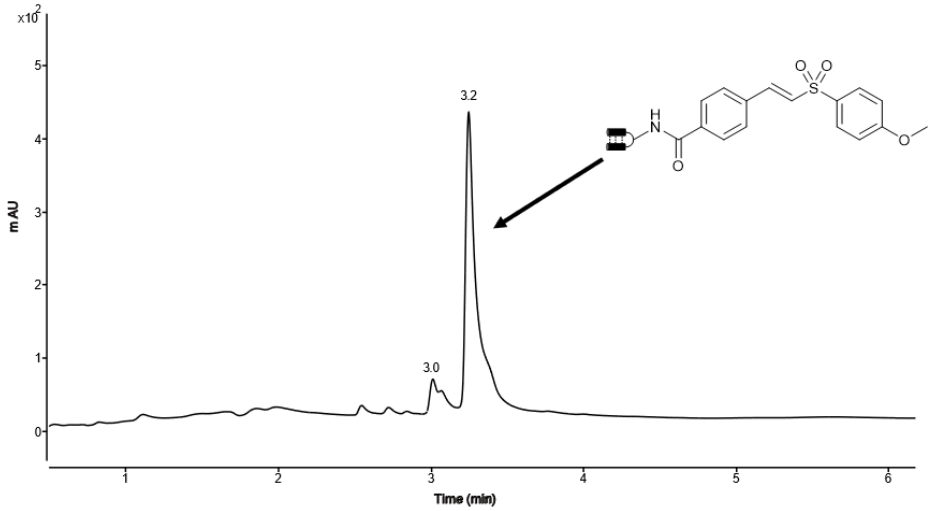


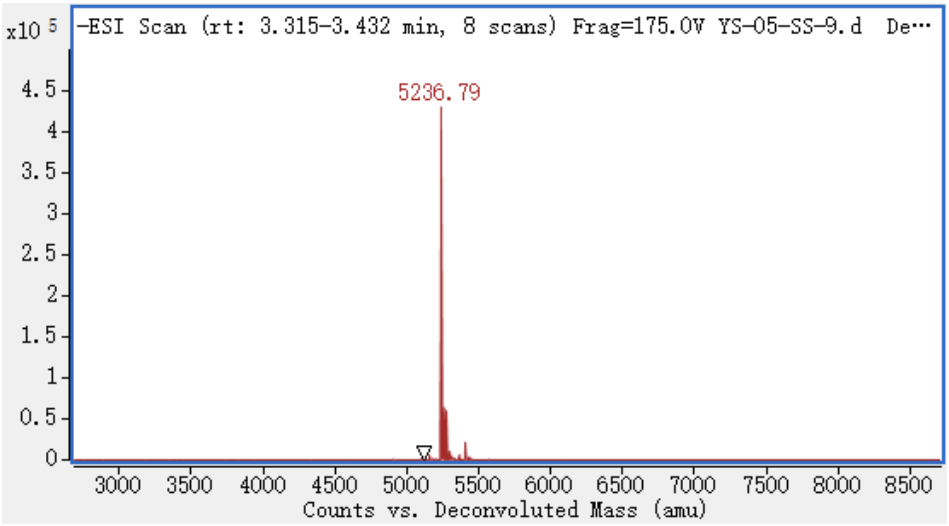


UPLC chromatogram and deconvoluted MS of **aa4**

Conversion: 86%

Calculated Mass: 5237 Da; Observed Mass: 5237 Da

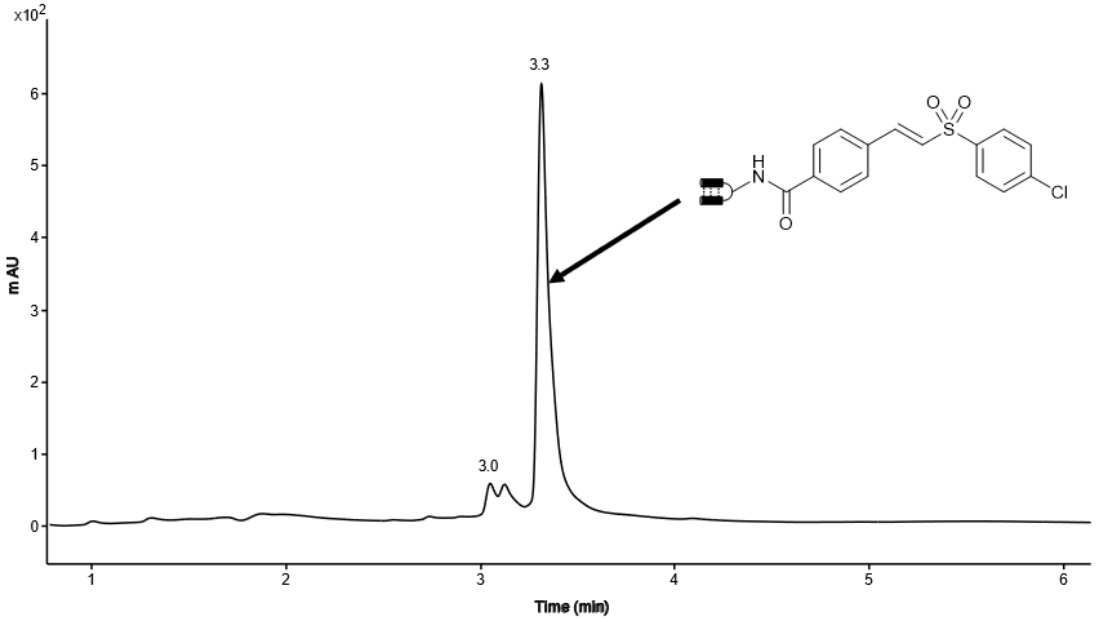


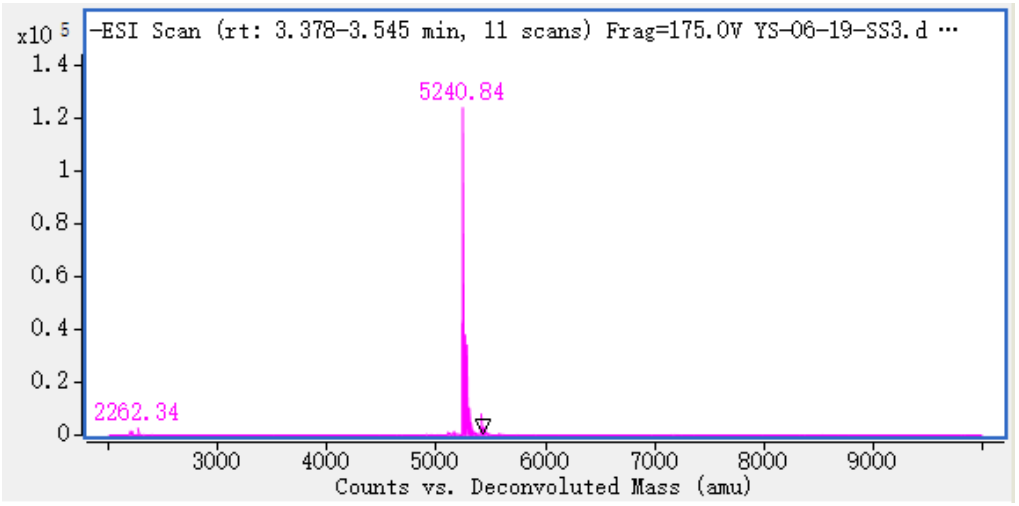


UPLC chromatogram and deconvoluted MS of **aa5**

Conversion: >90%

Calculated Mass: 5241 Da; Observed Mass: 5241 Da

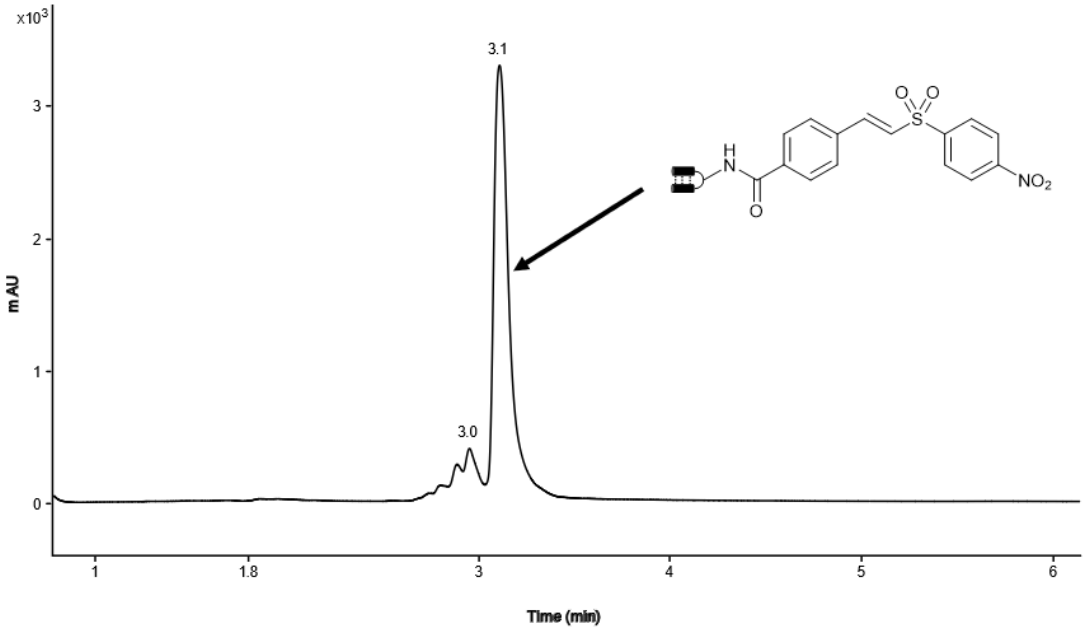


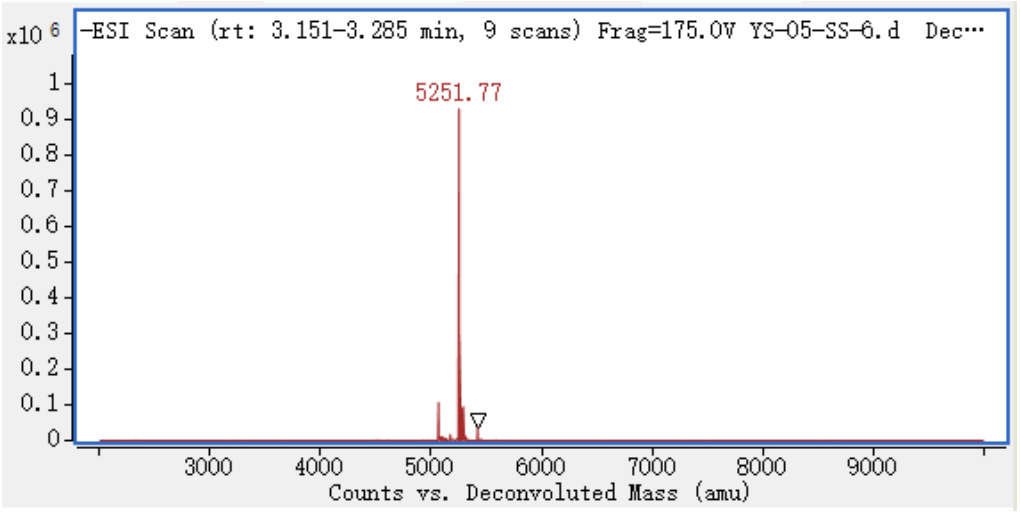


UPLC chromatogram and deconvoluted MS of **aa6**

Conversion: 80%

Calculated Mass: 5252 Da; Observed Mass: 5252 Da

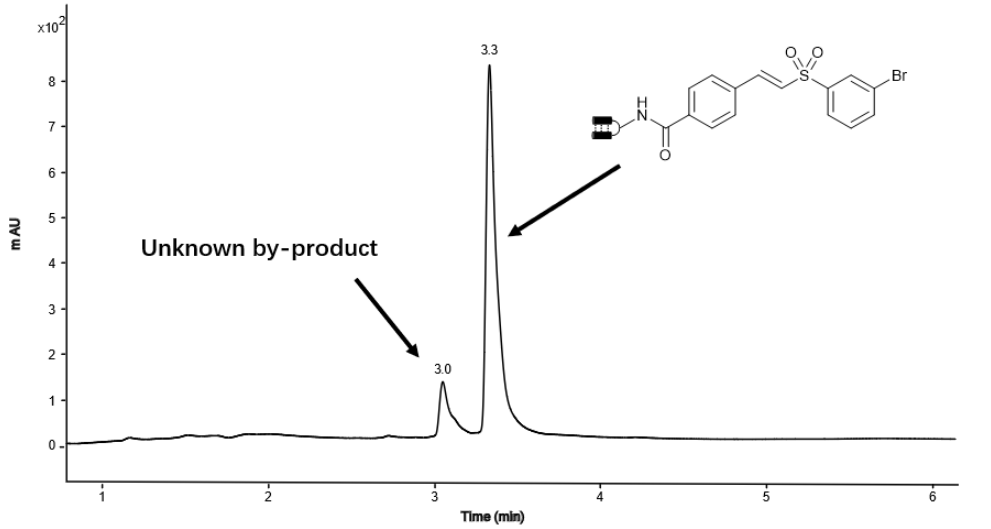


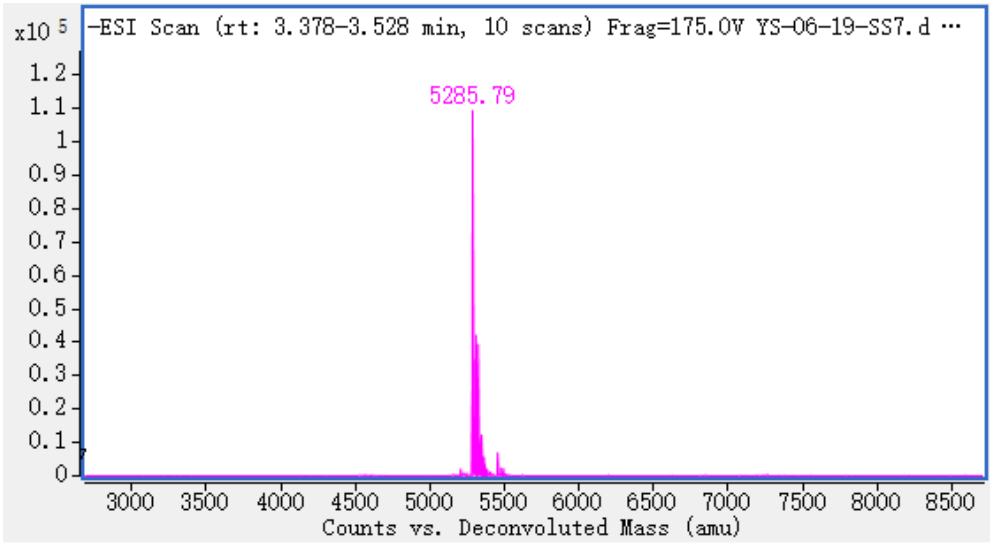


UPLC chromatogram and deconvoluted MS of **aa7**

Conversion: 80%

Calculated Mass: 5286 Da; Observed Mass: 5286 Da

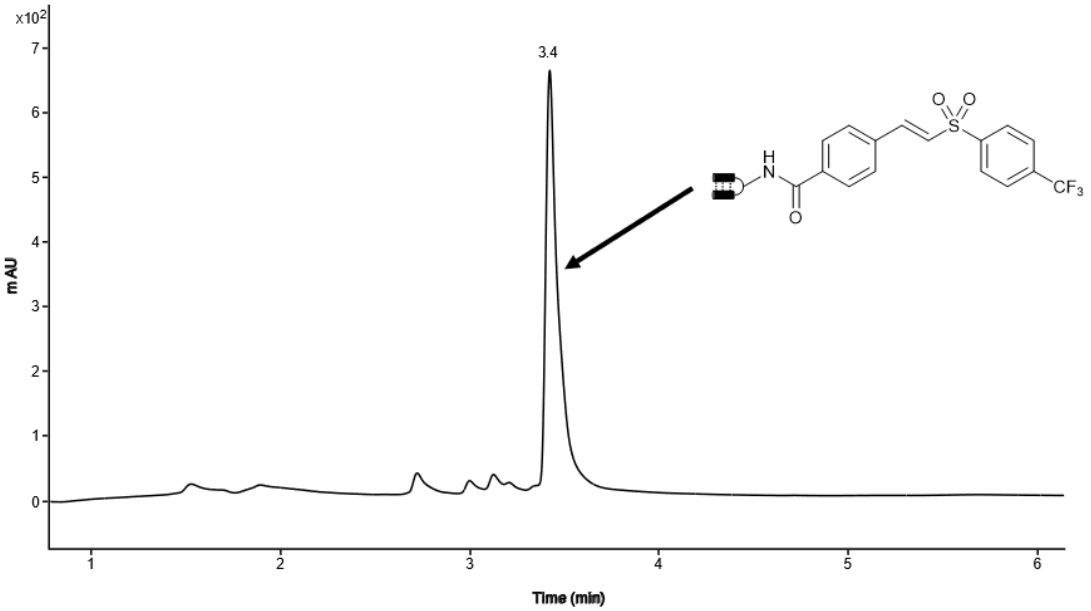


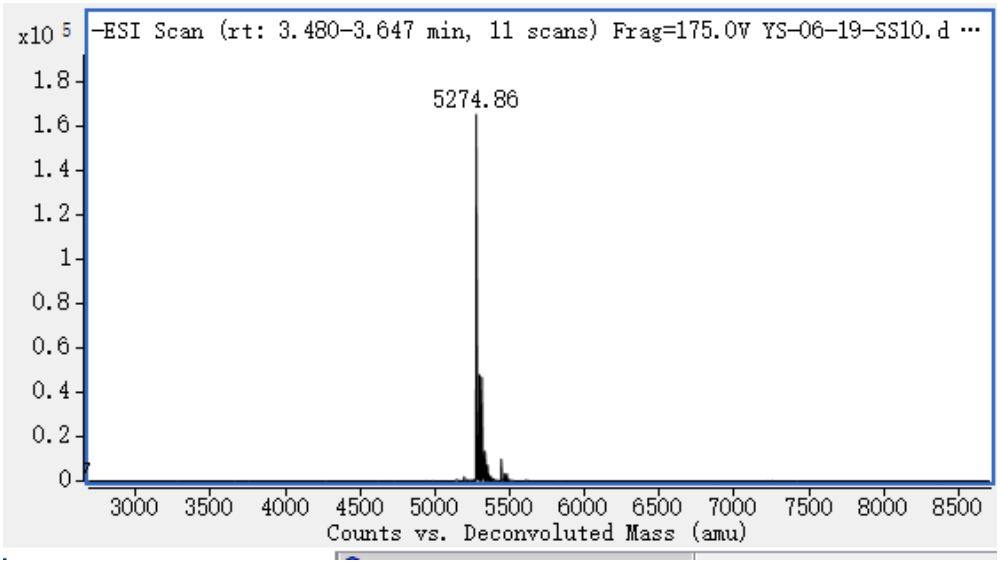


UPLC chromatogram and deconvoluted MS of **aa8**

Conversion: >90%

Calculated Mass: 5275 Da; Observed Mass: 5275 Da

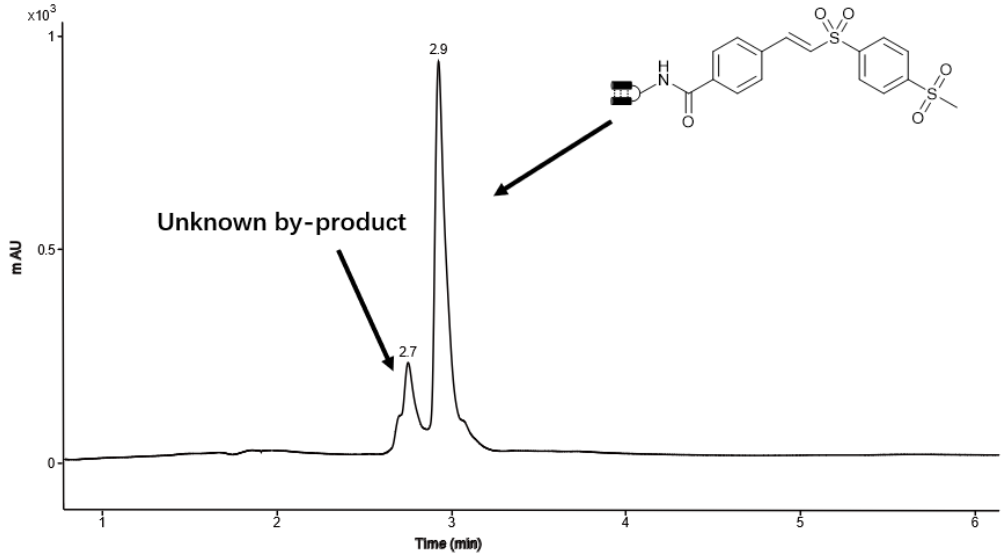


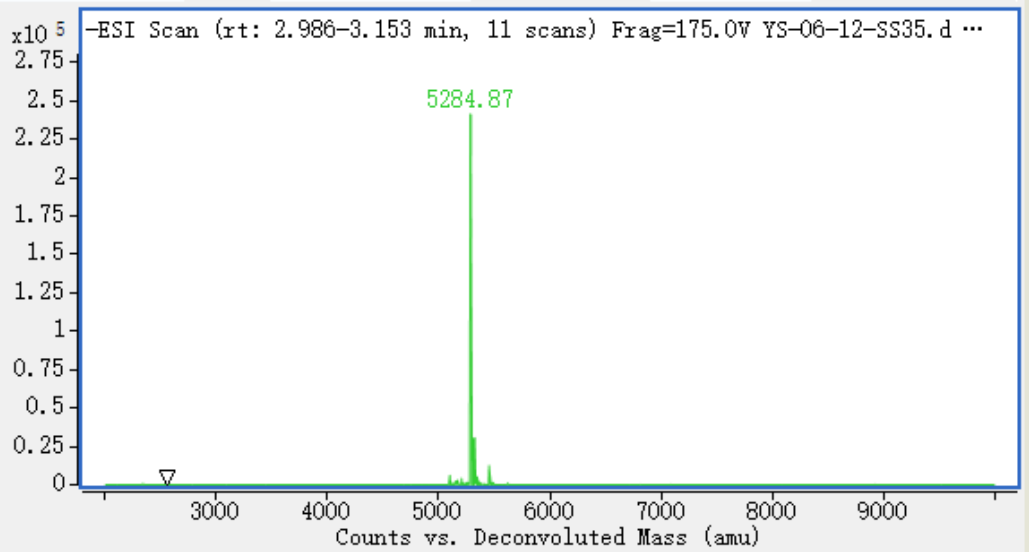


UPLC chromatogram and deconvoluted MS of **aa9**

Conversion: 78%

Calculated Mass: 5285 Da; Observed Mass: 5285 Da

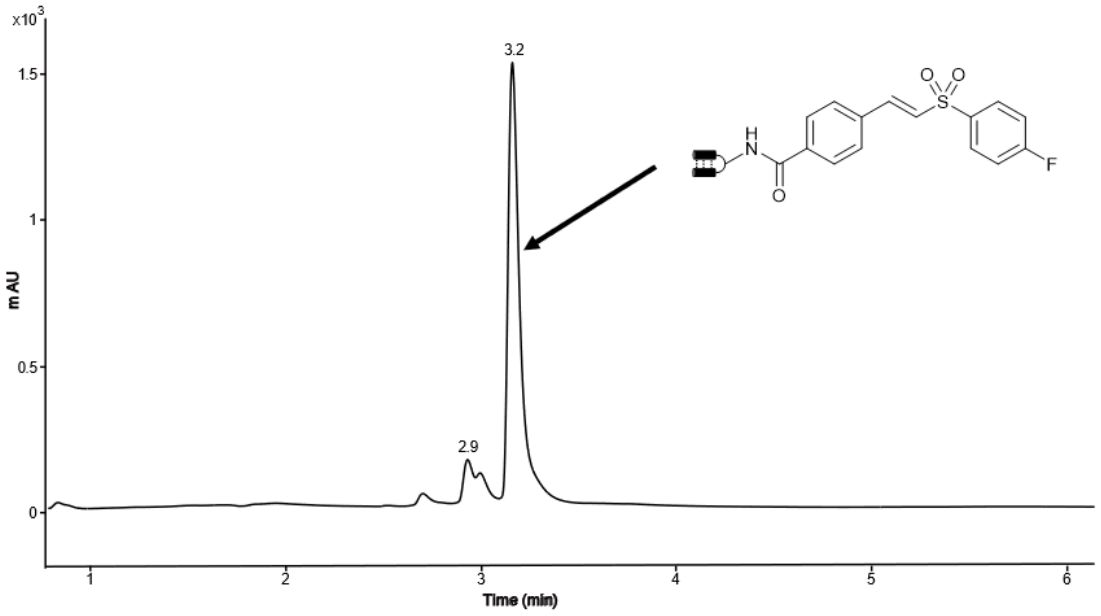


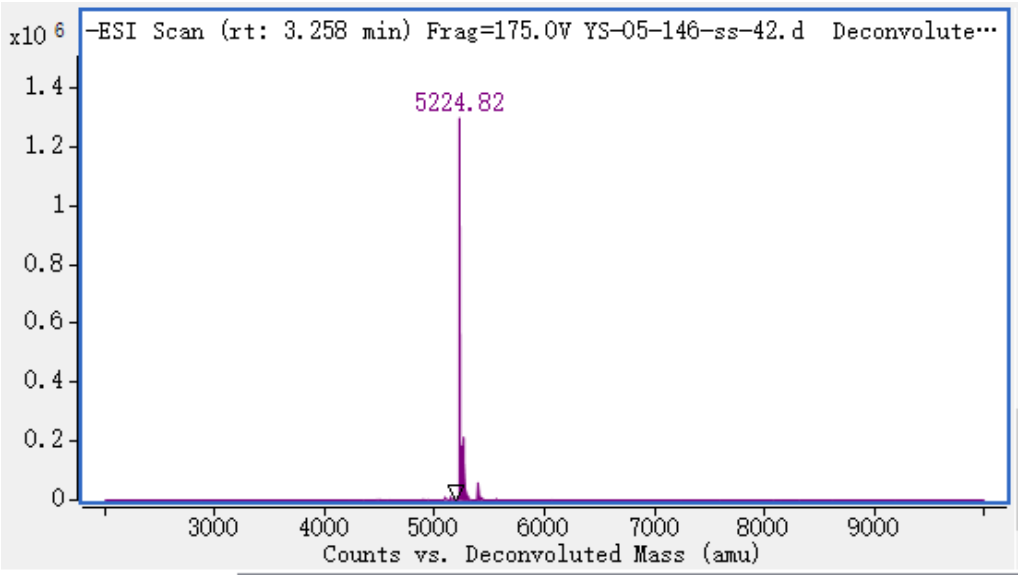


UPLC chromatogram and deconvoluted MS of **aa10**

Conversion: 83%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da

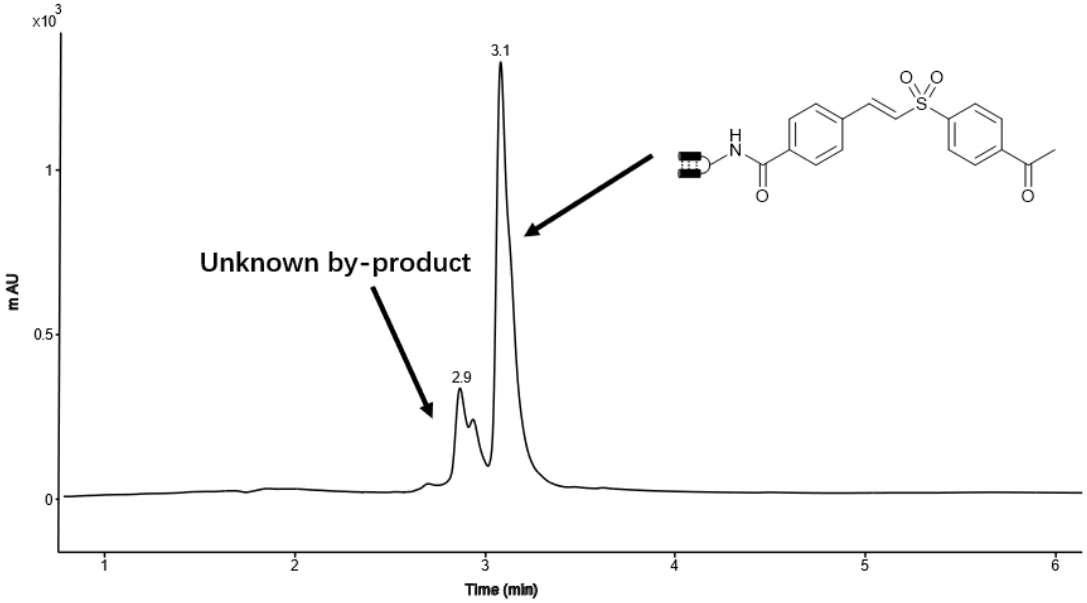


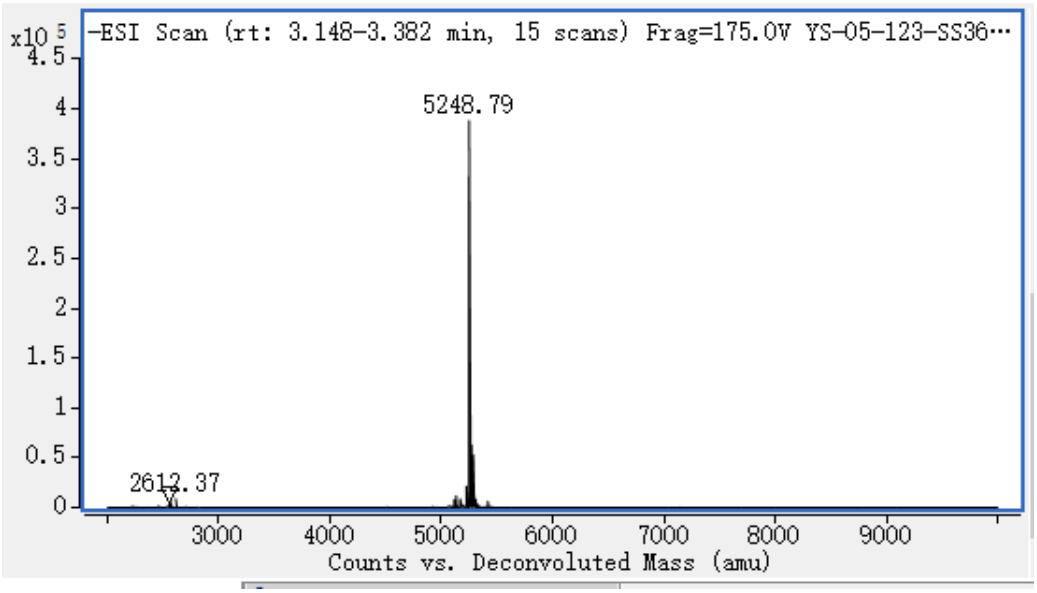


UPLC chromatogram and deconvoluted MS of **aa11**

Conversion: 86%

Calculated Mass: 5248 Da; Observed Mass: 5248 Da

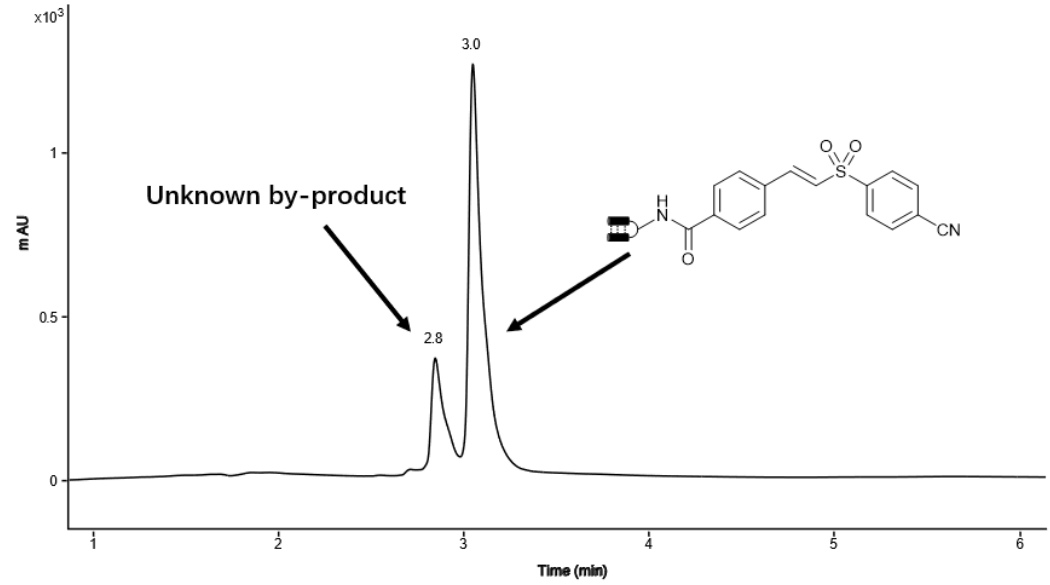


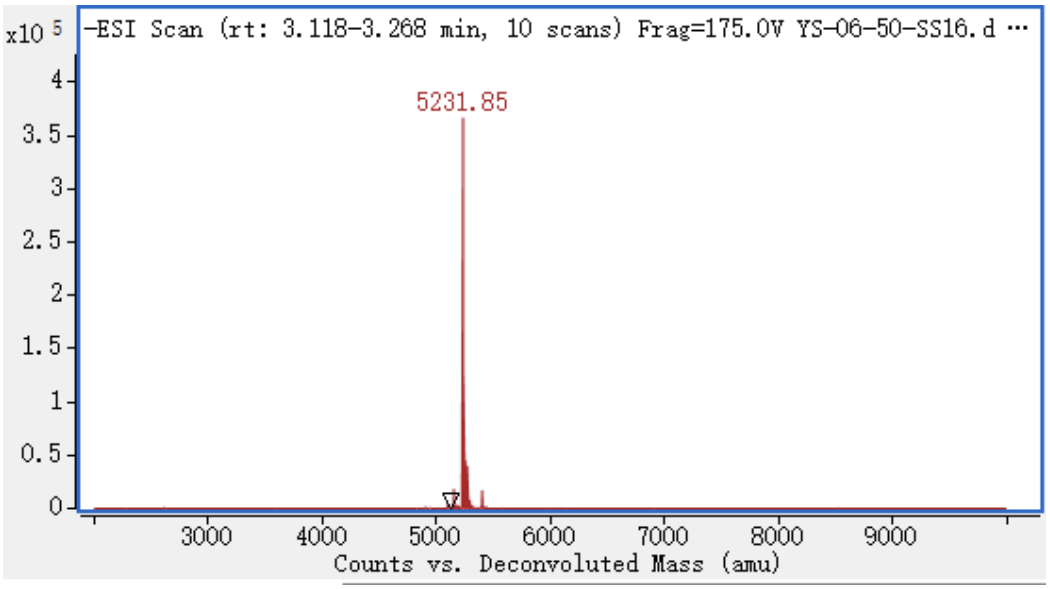


UPLC chromatogram and deconvoluted MS of **aa12**

Conversion: 75%

Calculated Mass: 5232 Da; Observed Mass: 5232 Da

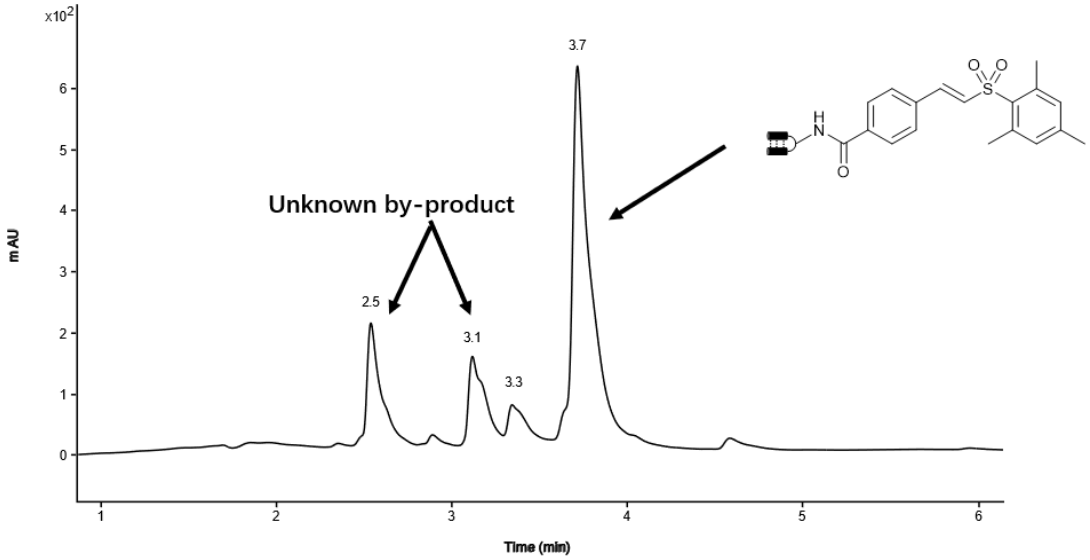


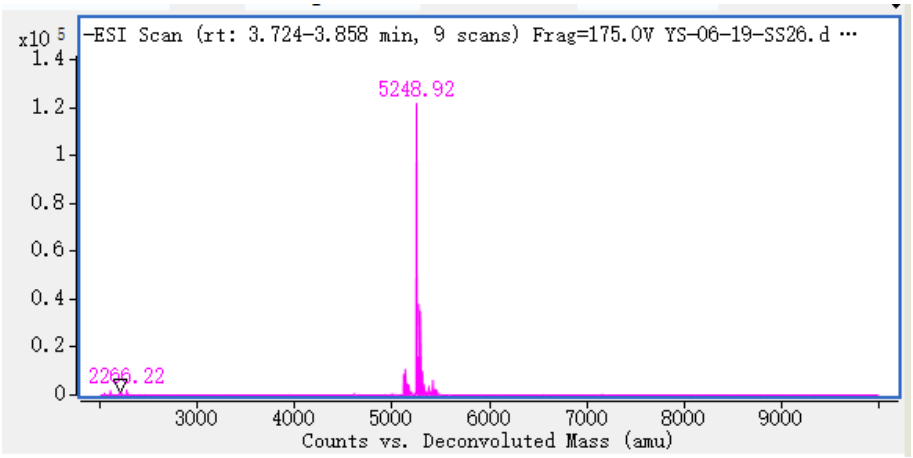


UPLC chromatogram and deconvoluted MS of **aa13**

Conversion: 57%

Calculated Mass: 5249 Da; Observed Mass: 5249 Da

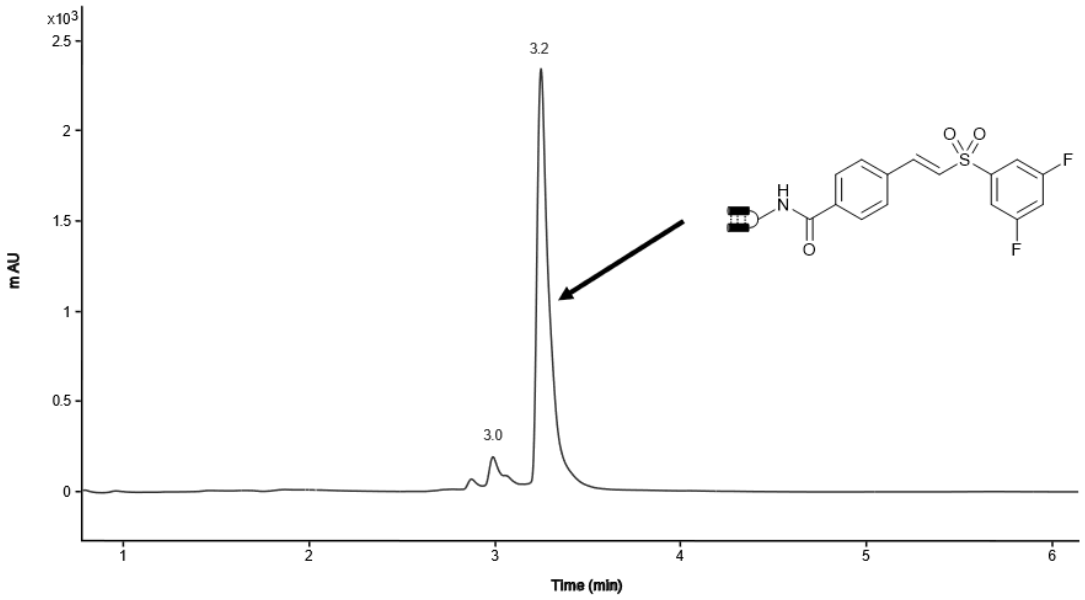


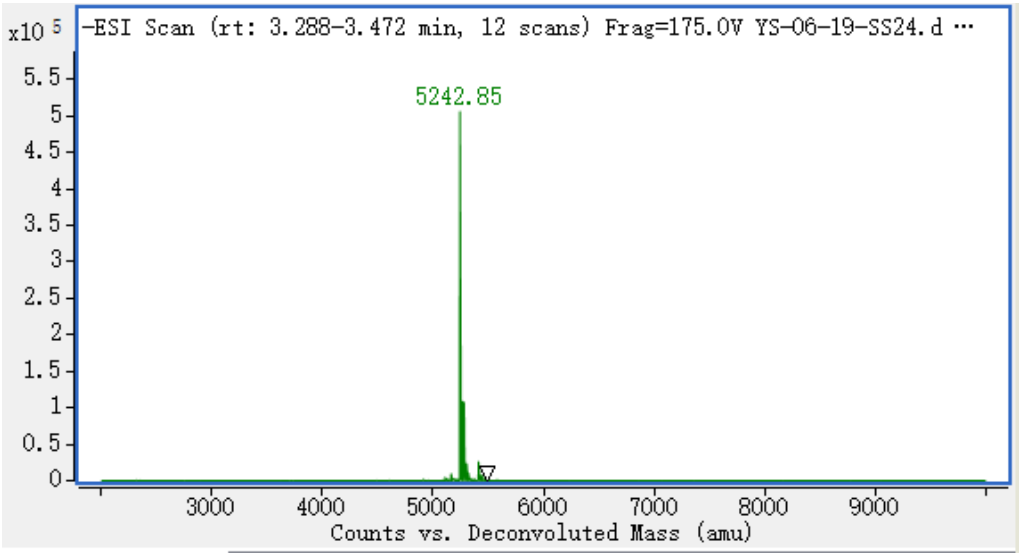


UPLC chromatogram and deconvoluted MS of **aa14**

Conversion: >90%

Calculated Mass: 5243 Da; Observed Mass: 5243 Da

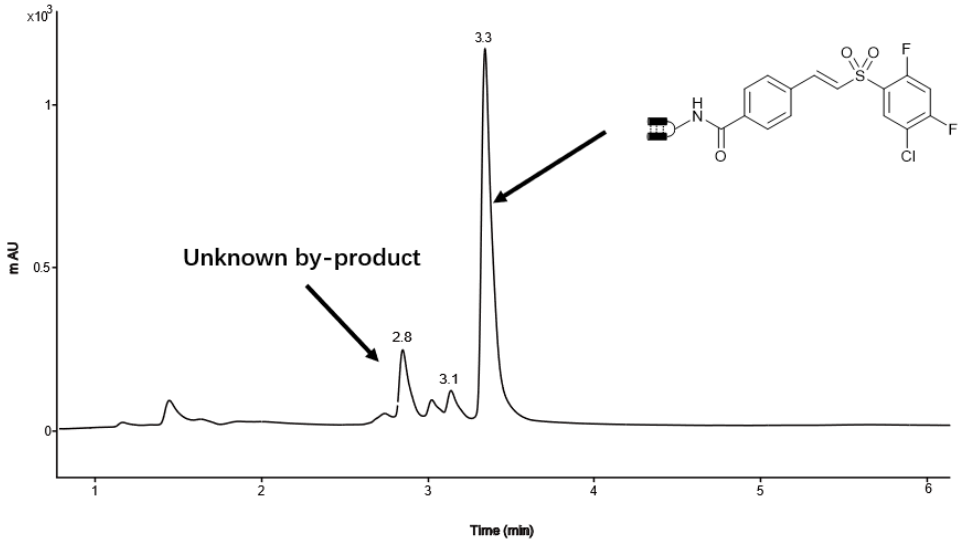


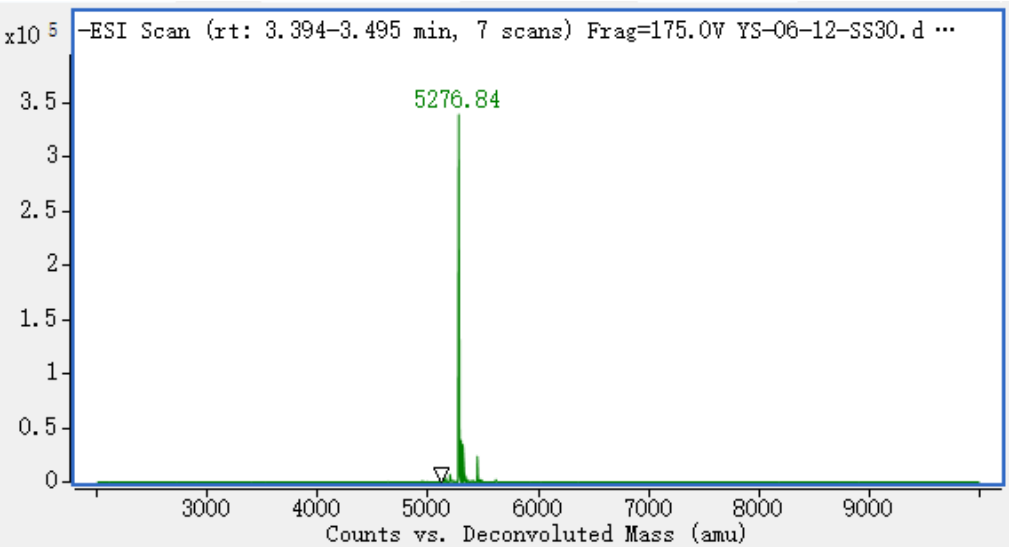


UPLC chromatogram and deconvoluted MS of **aa15**

Conversion: 76%

Calculated Mass: 5277 Da; Observed Mass: 5277 Da

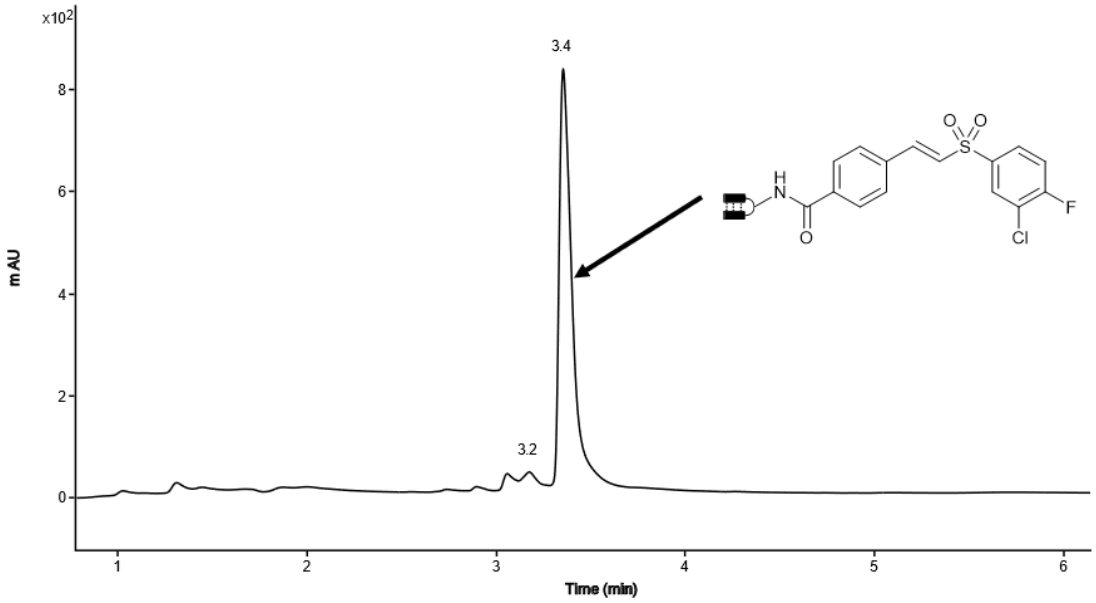


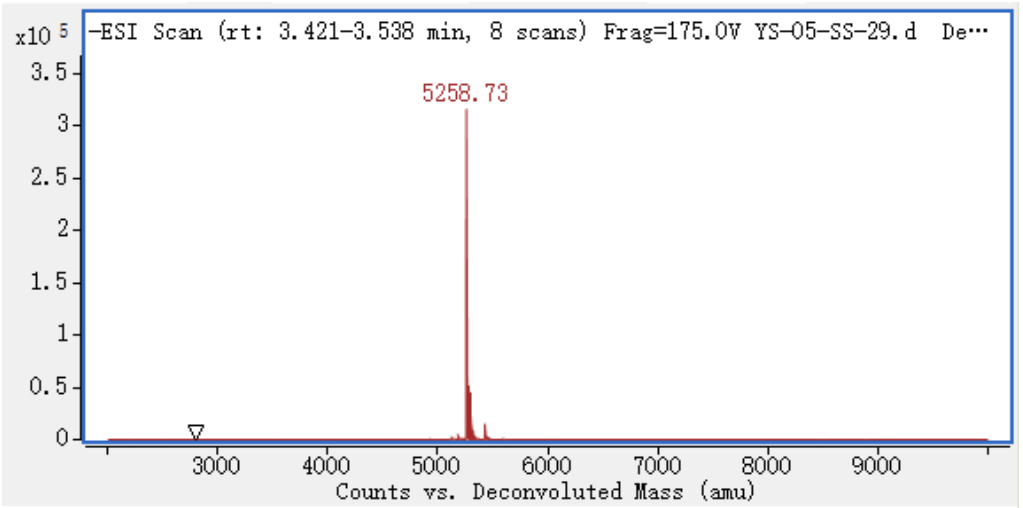


UPLC chromatogram and deconvoluted MS of **aa16**

Conversion: >90%

Calculated Mass: 5259 Da; Observed Mass: 5259 Da

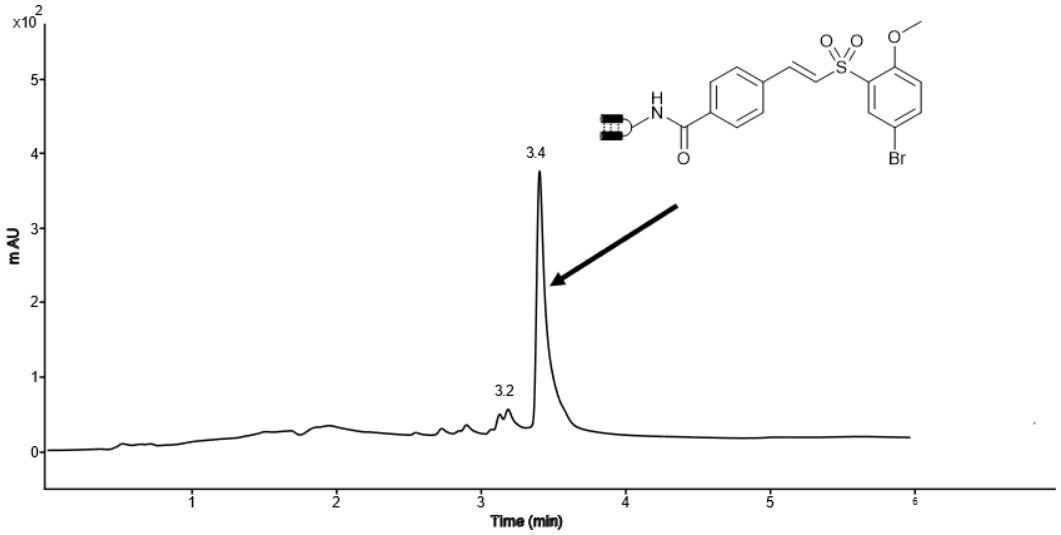


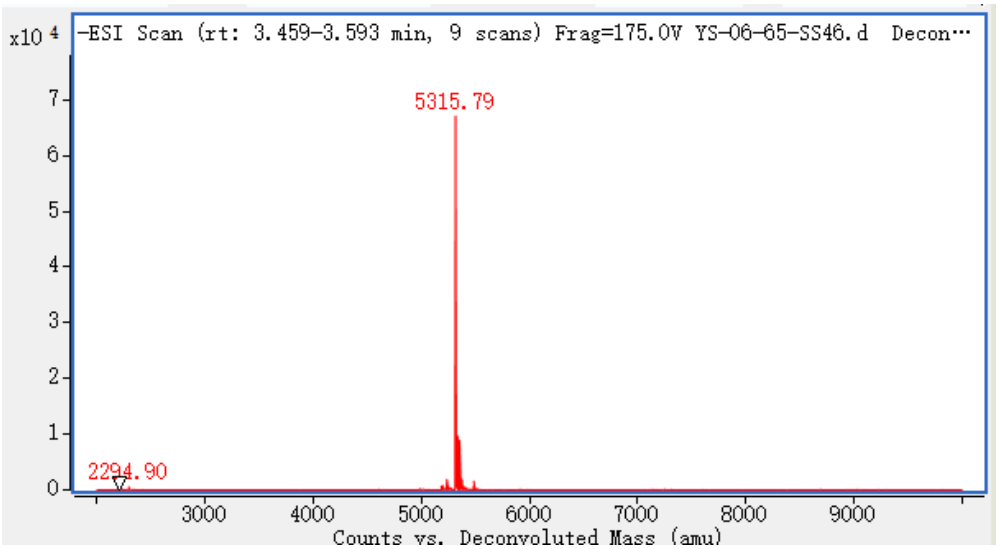


UPLC chromatogram and deconvoluted MS of **aa17**

Conversion: 82%

Calculated Mass: 5316 Da; Observed Mass: 5316 Da

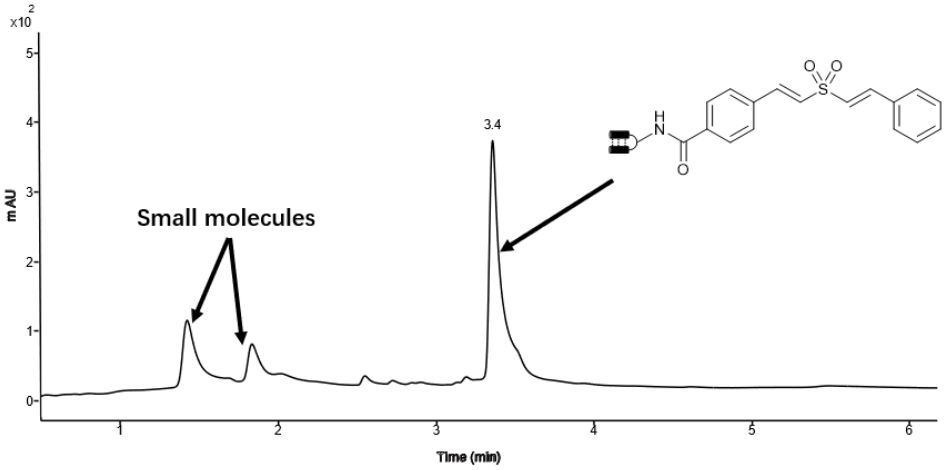


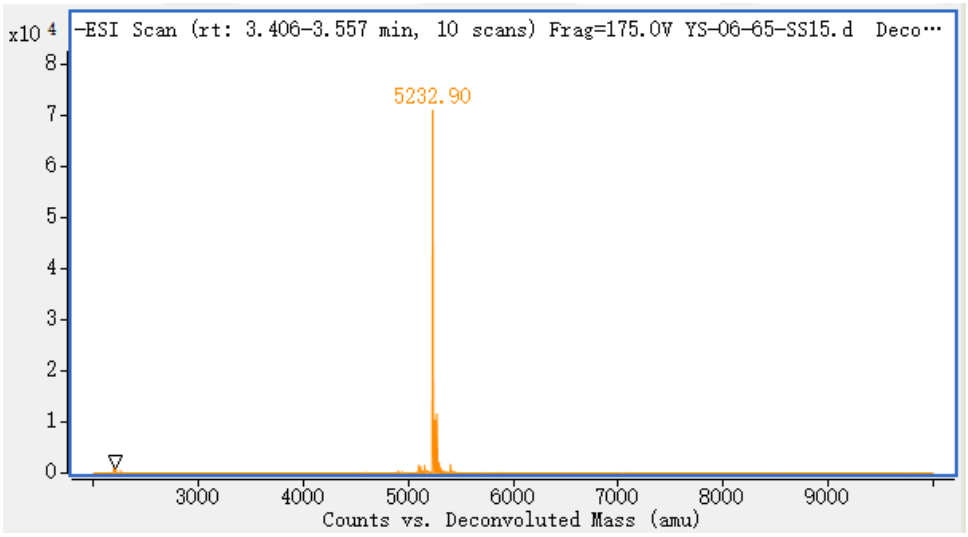


UPLC chromatogram and deconvoluted MS of **aa18**

Conversion: >90%

Calculated Mass: 5233 Da; Observed Mass: 5233 Da

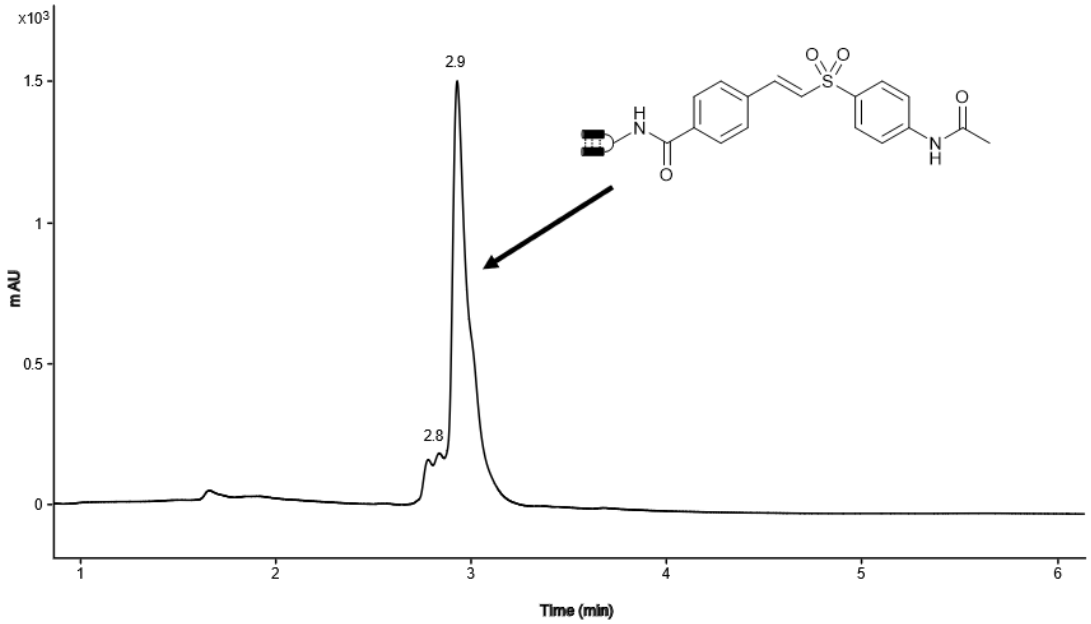


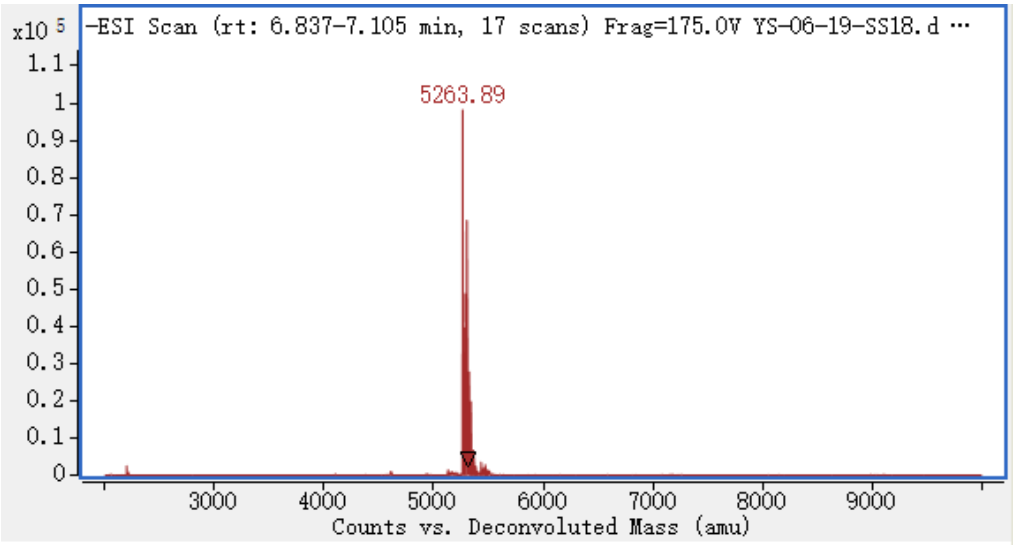


UPLC chromatogram and deconvoluted MS of **aa19**

Conversion: 82%

Calculated Mass: 5264 Da; Observed Mass: 5264 Da

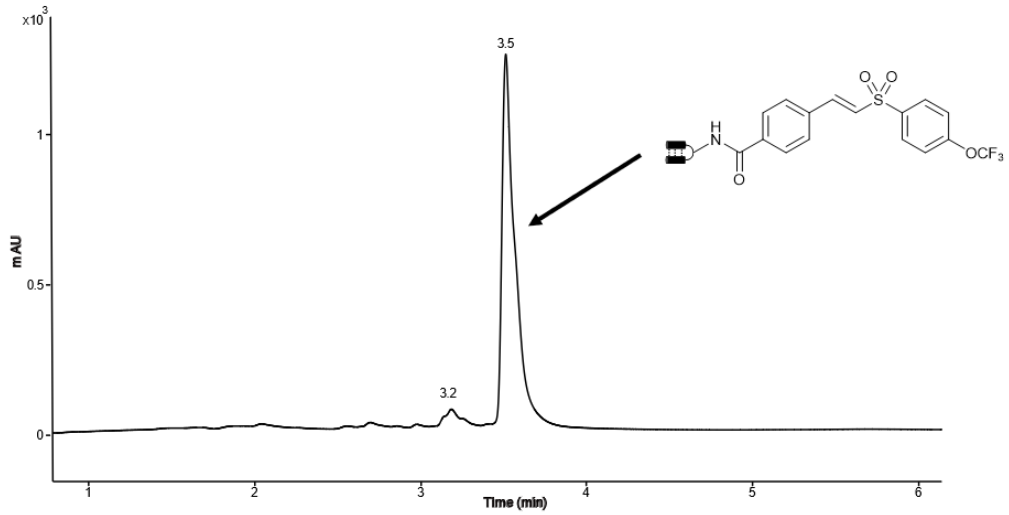


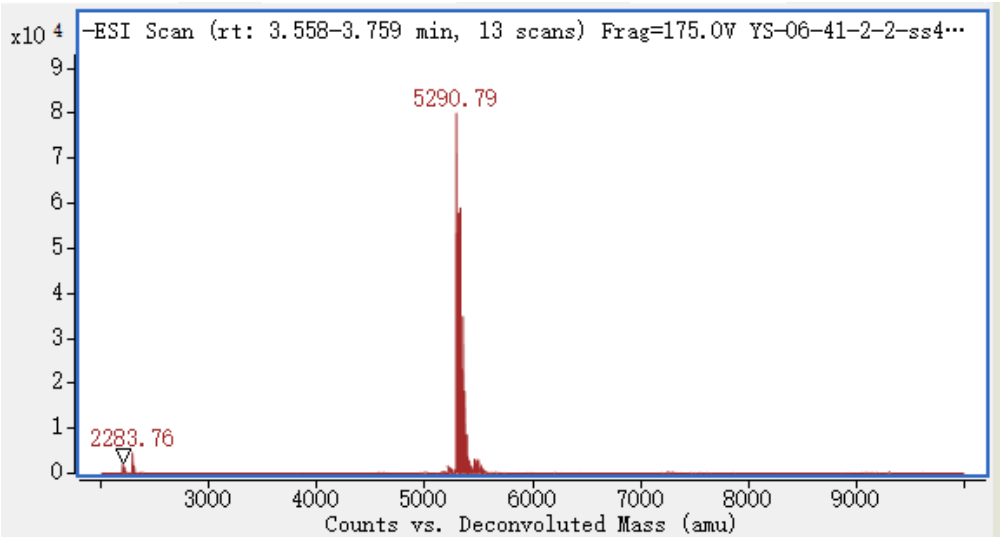


UPLC chromatogram and deconvoluted MS of **aa20**

Conversion: >90%

Calculated Mass: 5291 Da; Observed Mass: 5291 Da

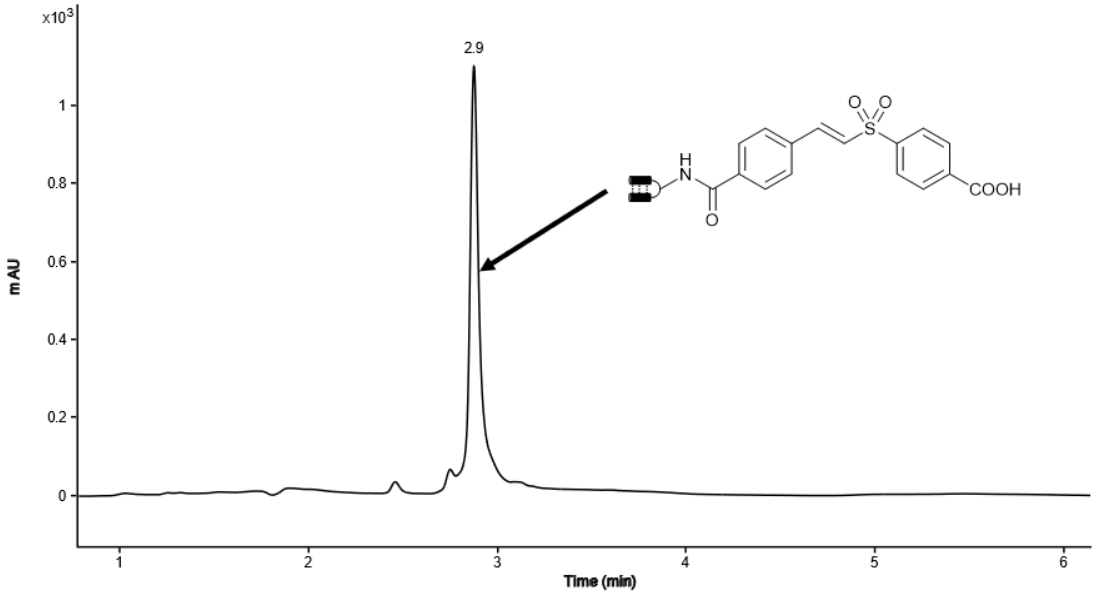


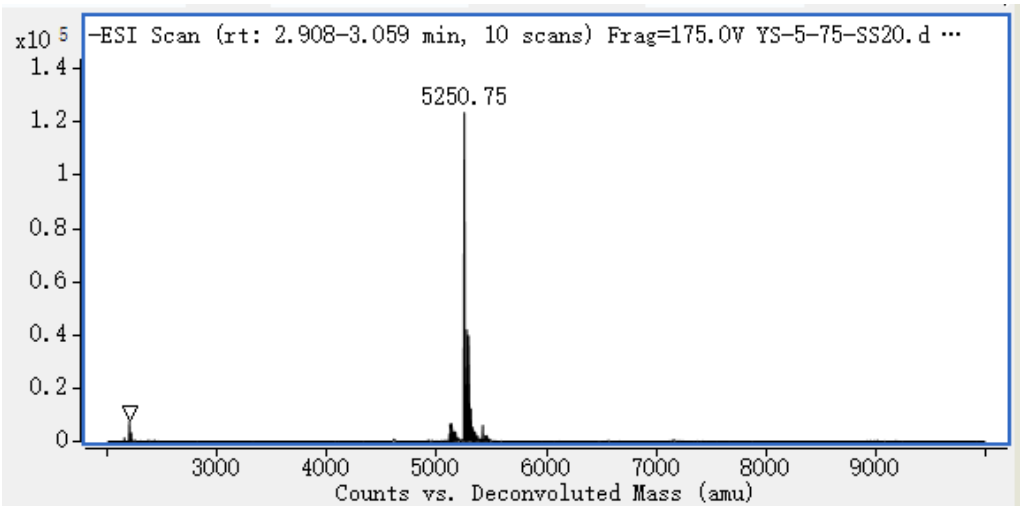


UPLC chromatogram and deconvoluted MS of **aa21**

Conversion: >90%

Calculated Mass: 5251 Da; Observed Mass: 5251 Da

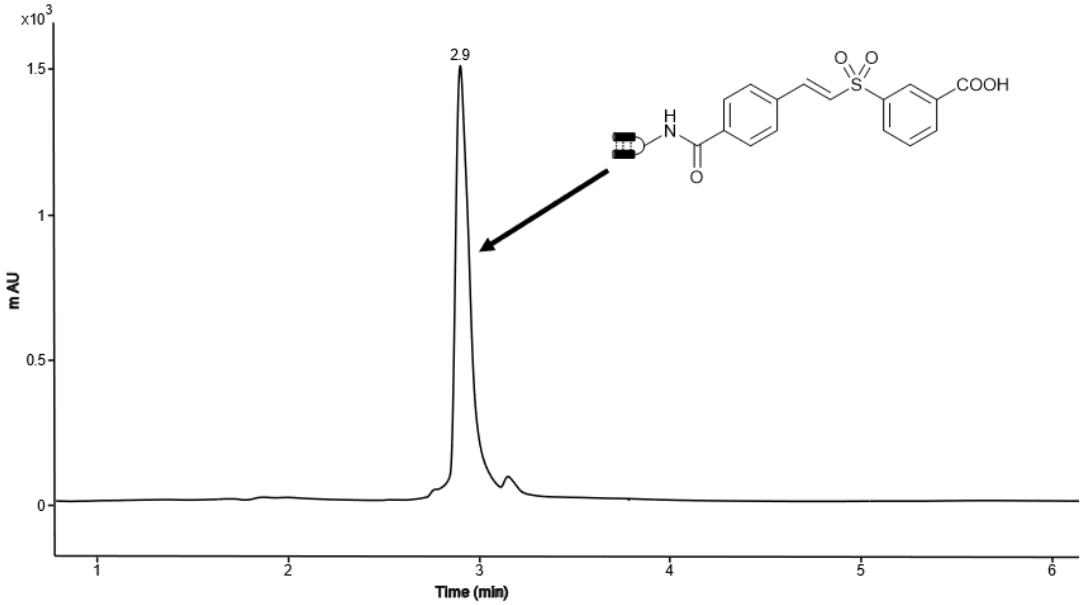


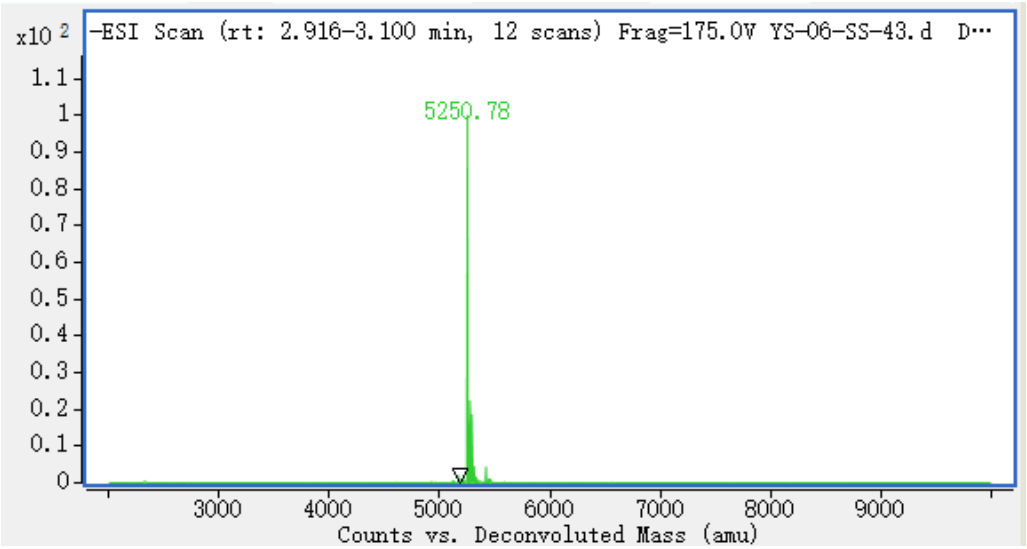


UPLC chromatogram and deconvoluted MS of **aa22**

Conversion: >90%

Calculated Mass: 5251 Da; Observed Mass: 5251 Da

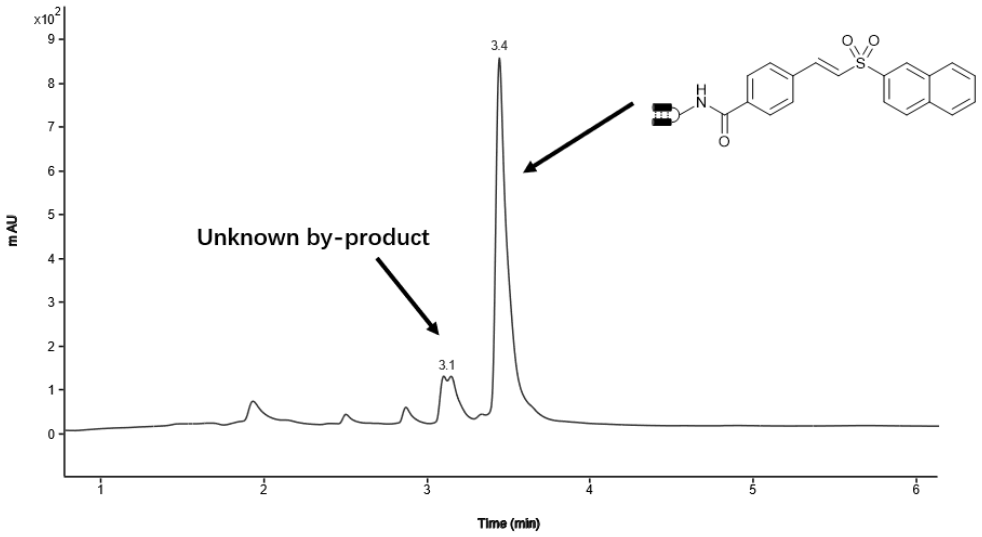


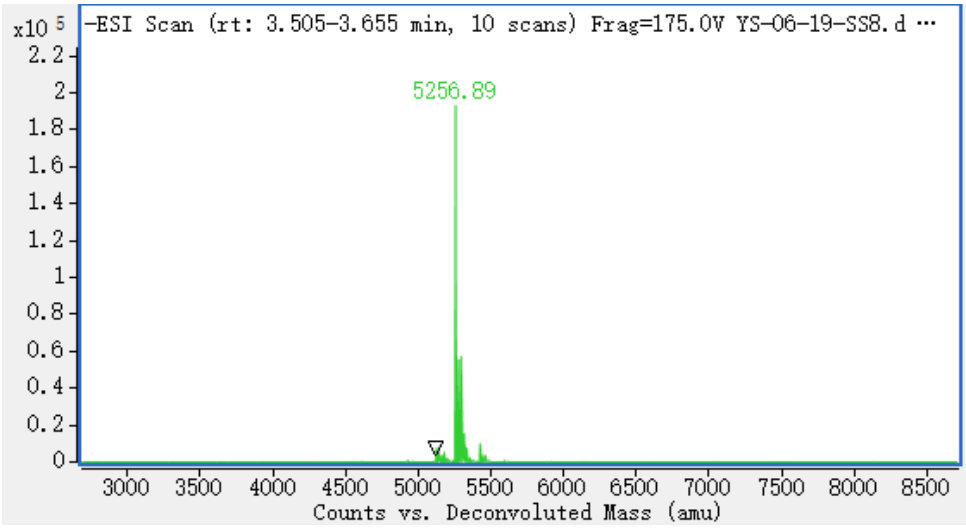


UPLC chromatogram and deconvoluted MS of **aa23**

Conversion: >90%

Calculated Mass: 5257 Da; Observed Mass: 5257 Da

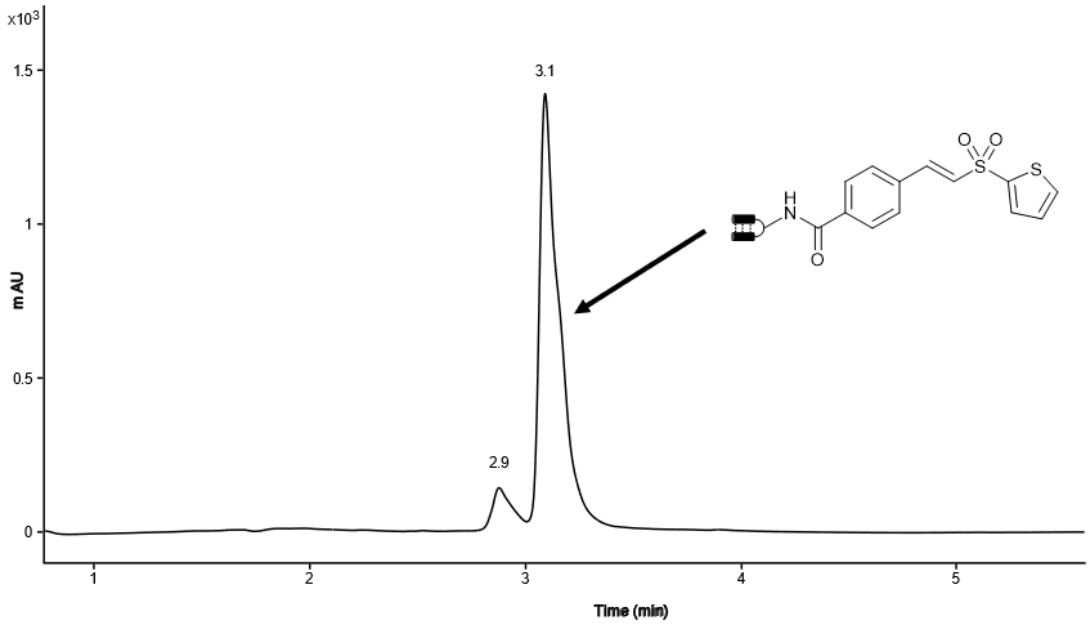


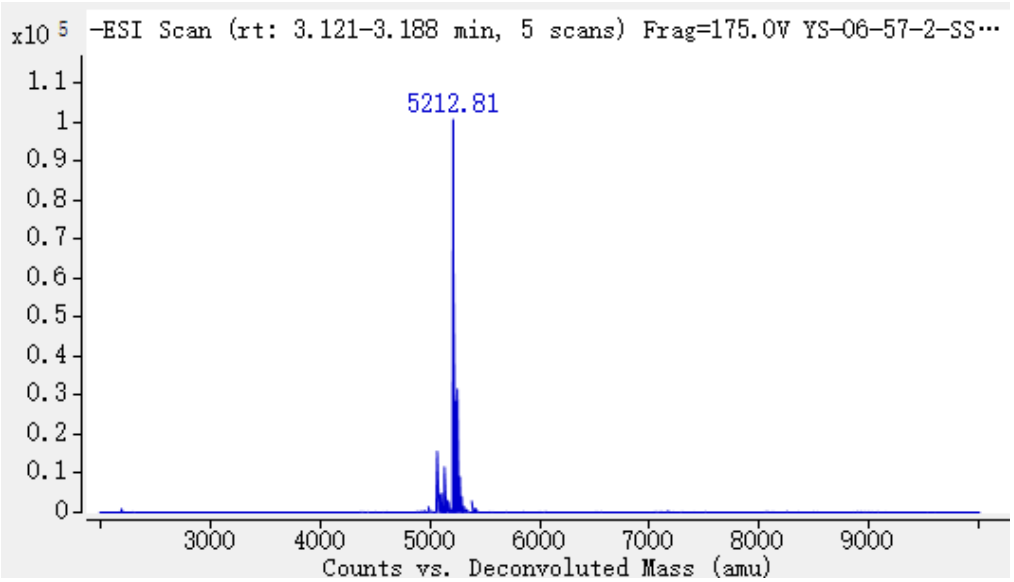


UPLC chromatogram and deconvoluted MS of **aa24**

Conversion: 80%

Calculated Mass: 5213 Da; Observed Mass: 5213 Da

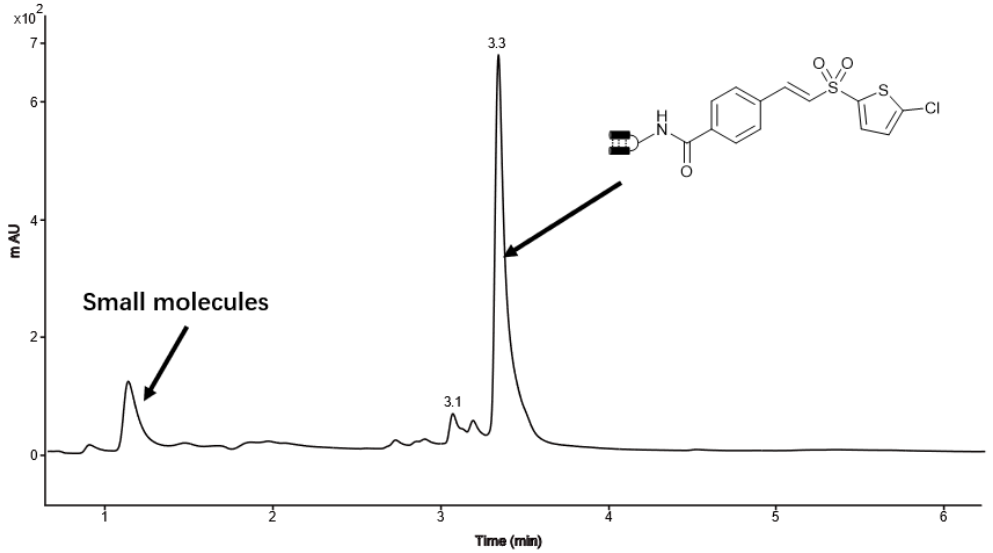


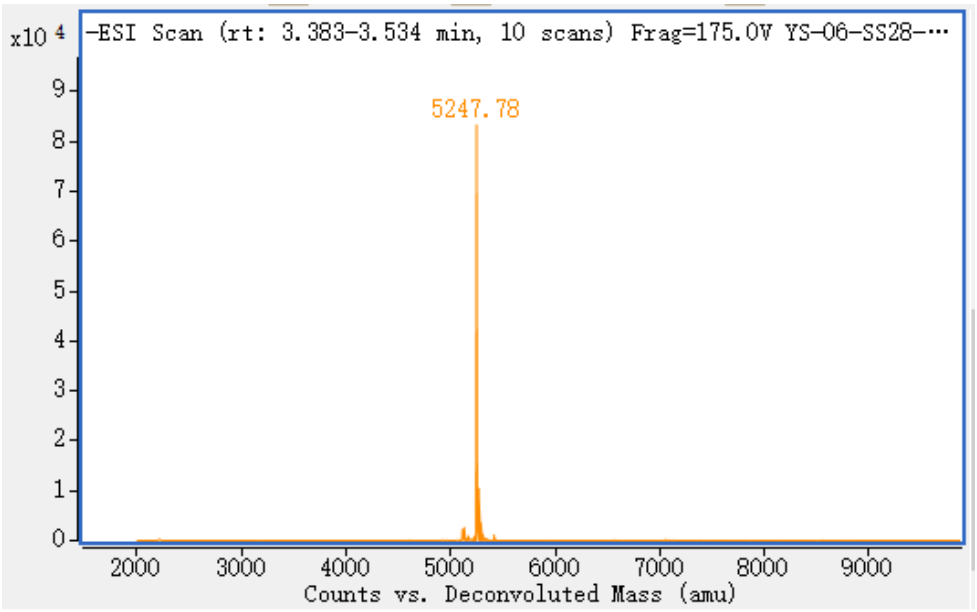


UPLC chromatogram and deconvoluted MS of **aa25**

Conversion: 85%

Calculated Mass: 5248 Da; Observed Mass: 5248 Da

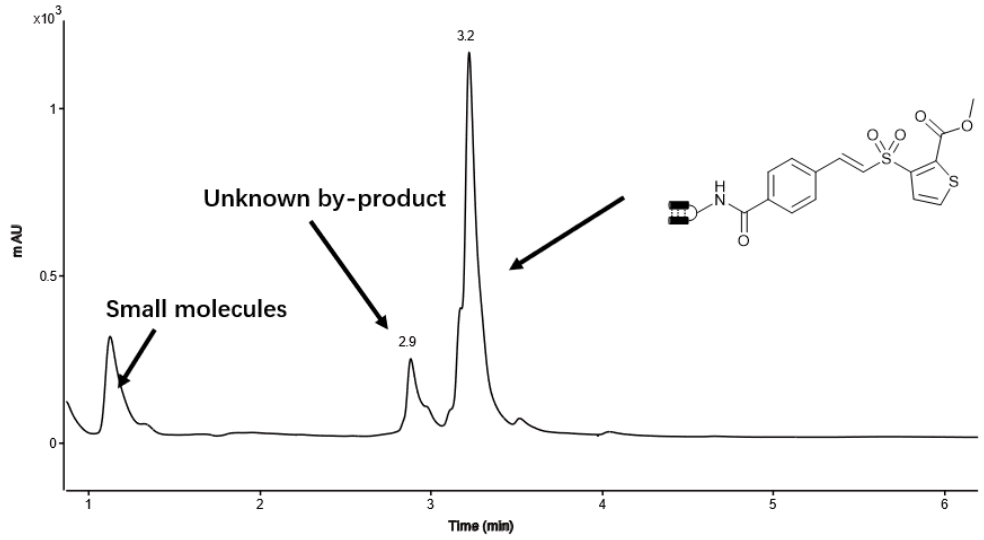


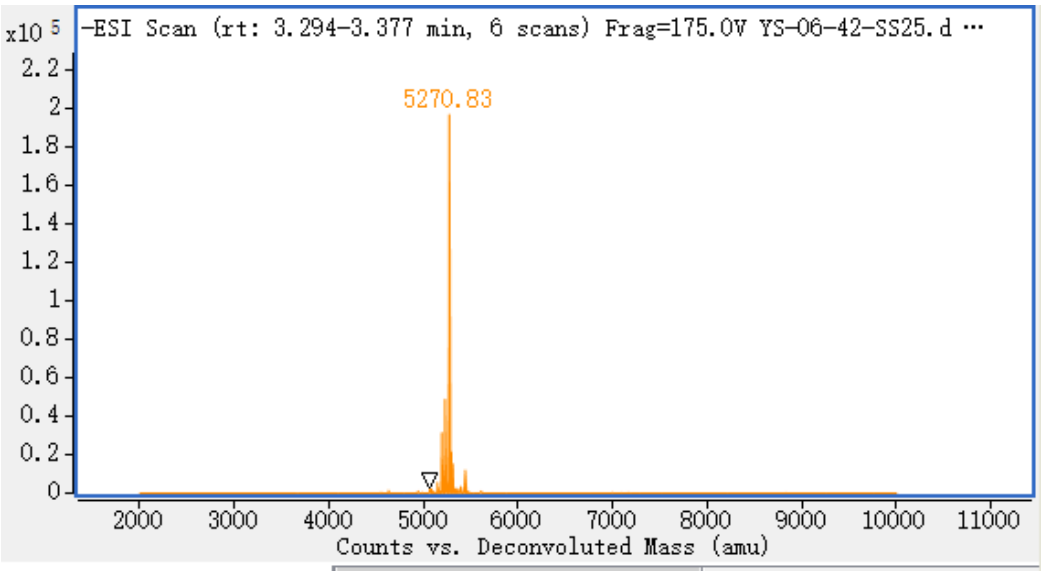


UPLC chromatogram and deconvoluted MS of **aa26**

Conversion: 61%

Calculated Mass: 5271 Da; Observed Mass: 5271 Da

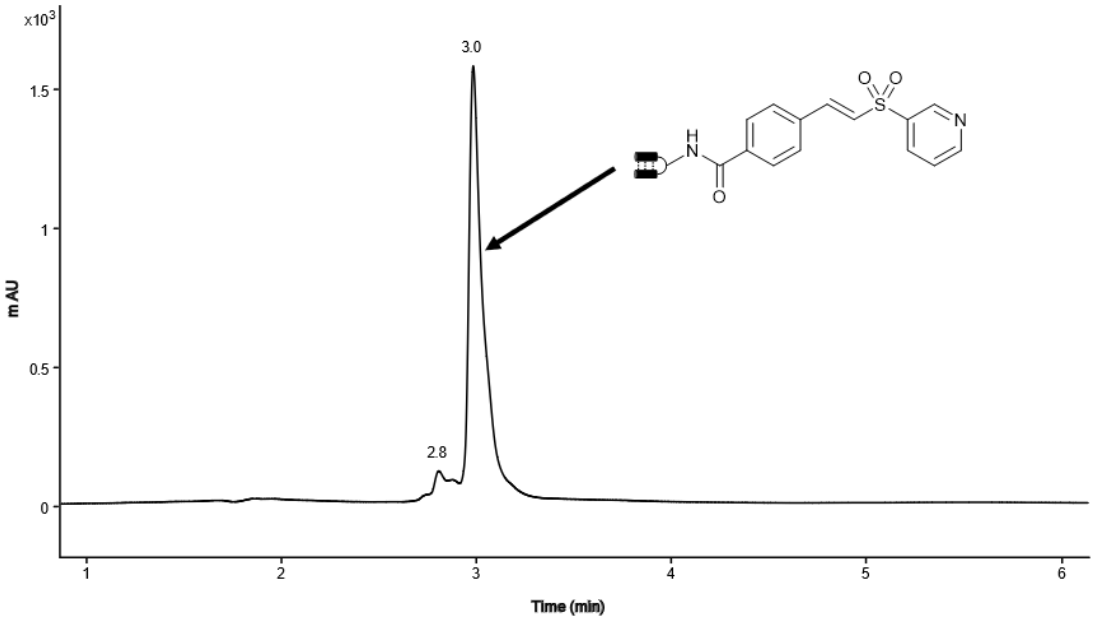


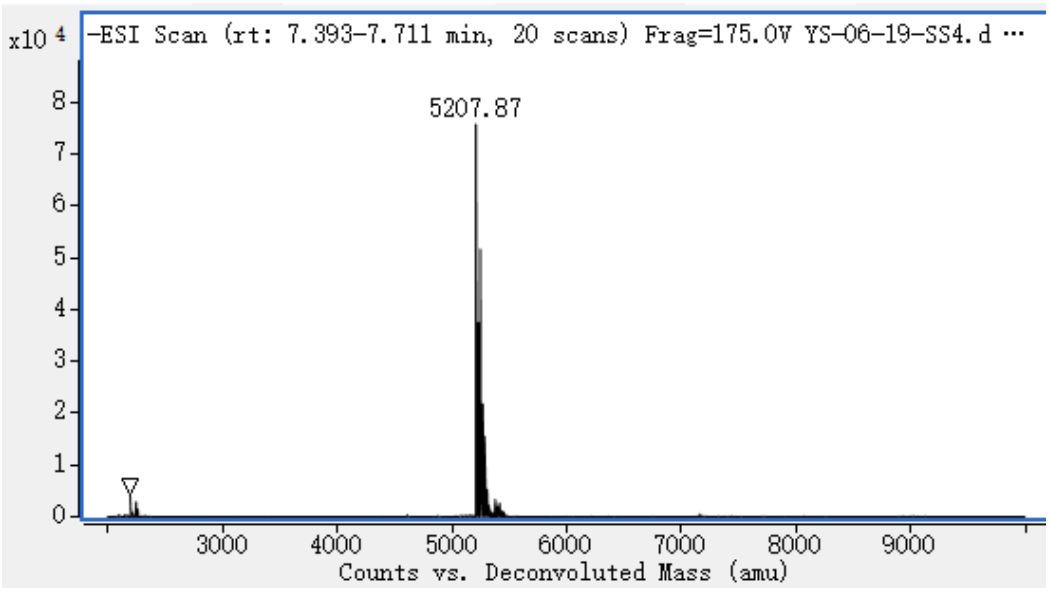


UPLC chromatogram and deconvoluted MS of **aa27**

Conversion: 90%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da

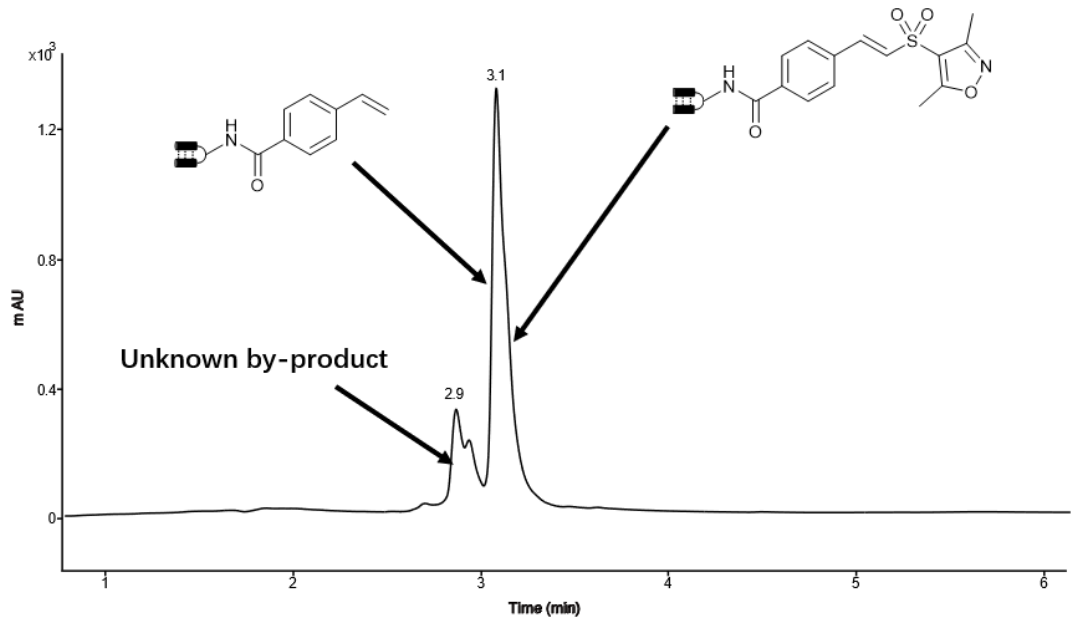


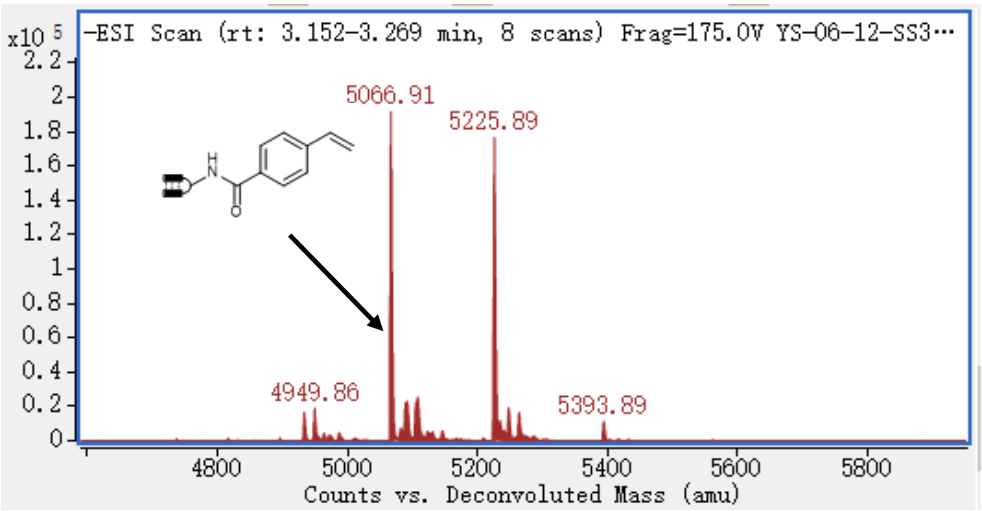


UPLC chromatogram and deconvoluted MS of **aa28**

Conversion: 38%

Calculated Mass: 5226 Da; Observed Mass: 5226 Da

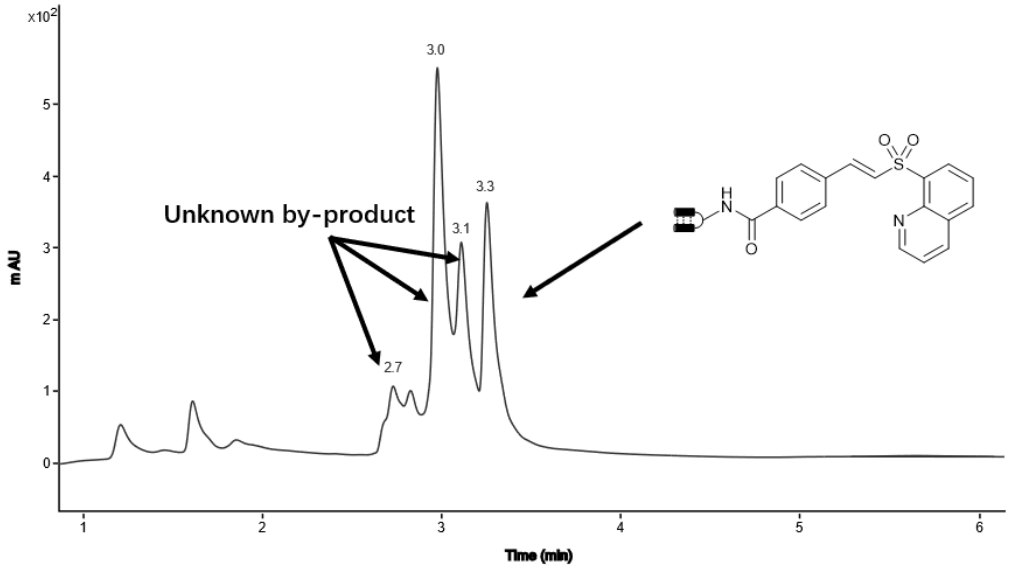


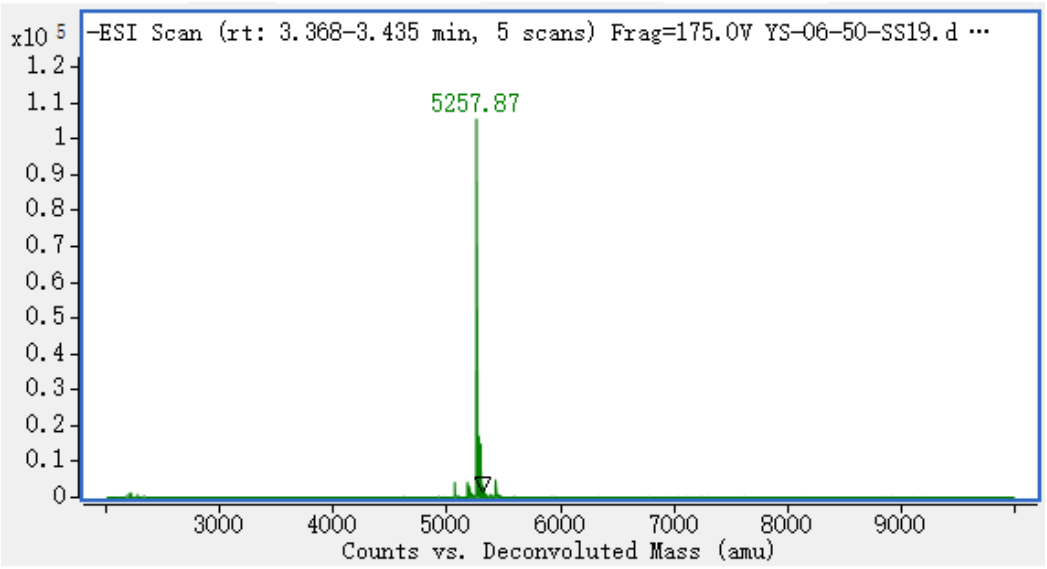


UPLC chromatogram and deconvoluted MS of **aa29**

Conversion: 28%

Calculated Mass: 5258 Da; Observed Mass: 5258 Da

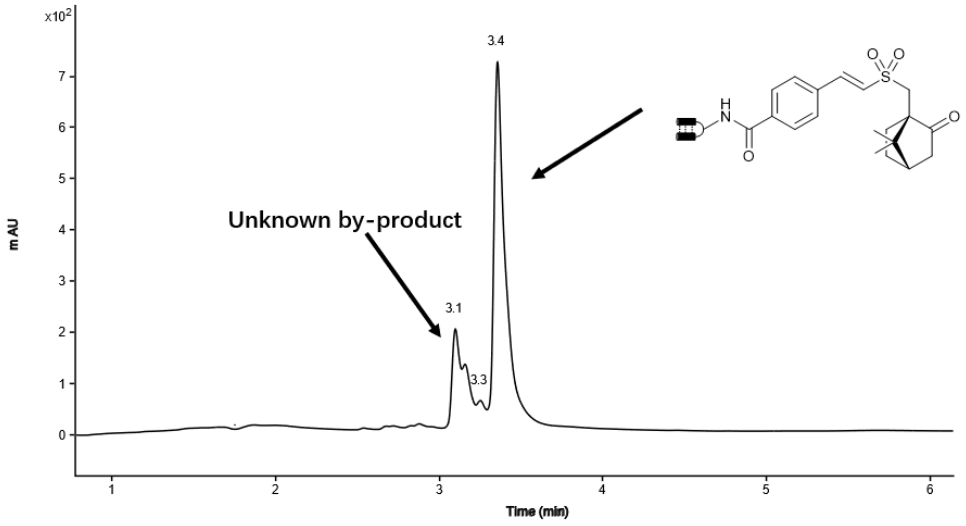


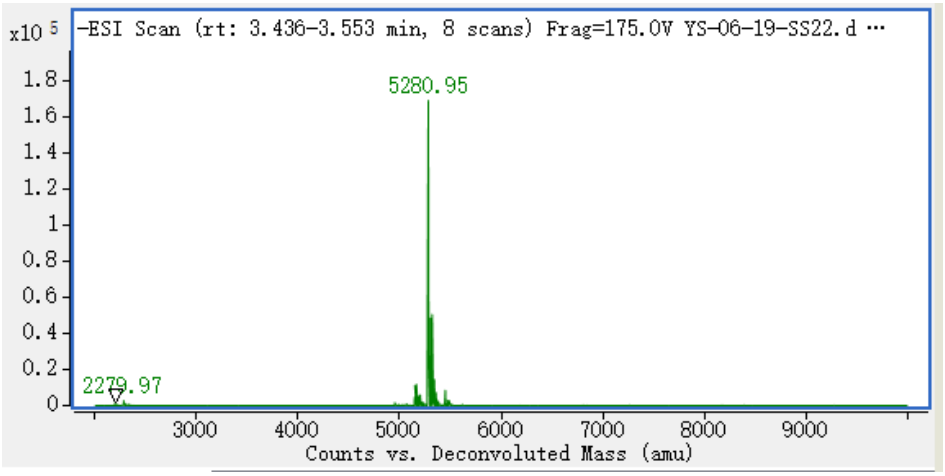


UPLC chromatogram and deconvoluted MS of **aa30**

Conversion: 75%

Calculated Mass: 5280 Da; Observed Mass: 5281 Da

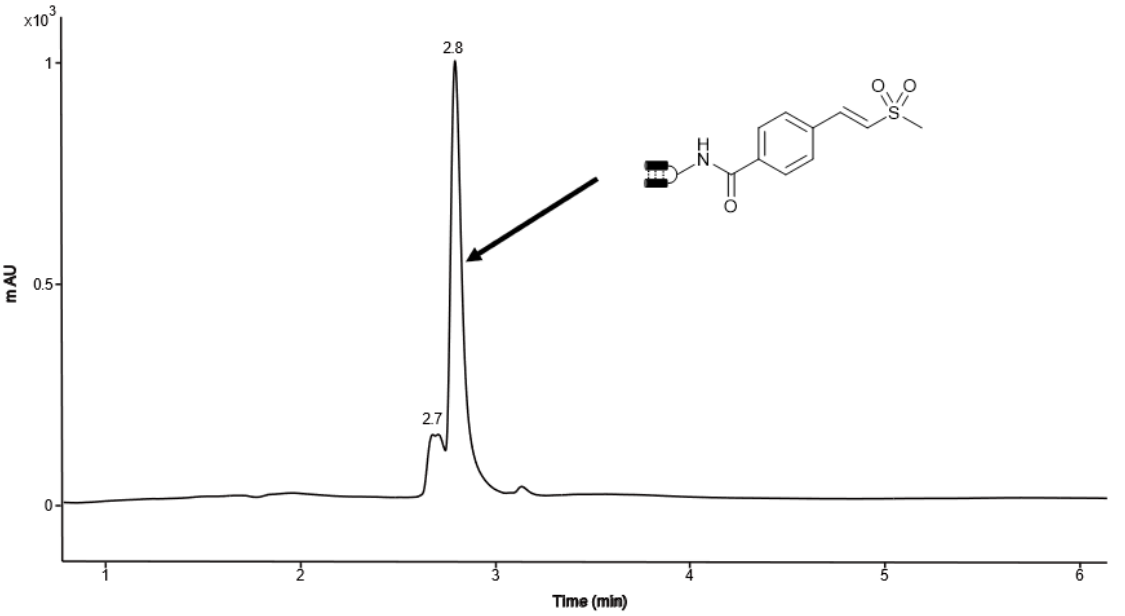


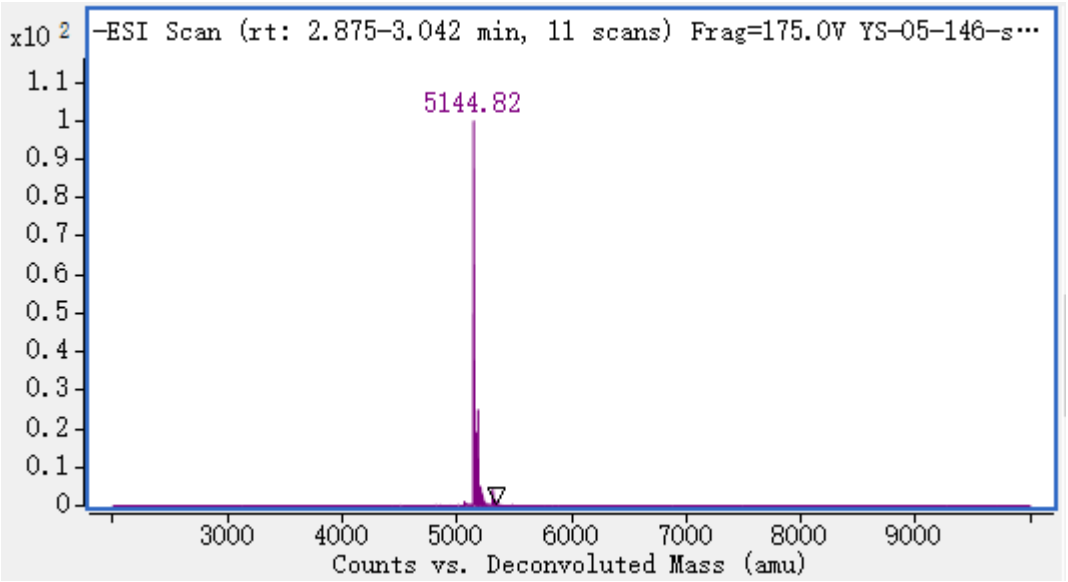


UPLC chromatogram and deconvoluted MS of **aa31**

Conversion: 85%

Calculated Mass: 5145 Da; Observed Mass: 5145 Da

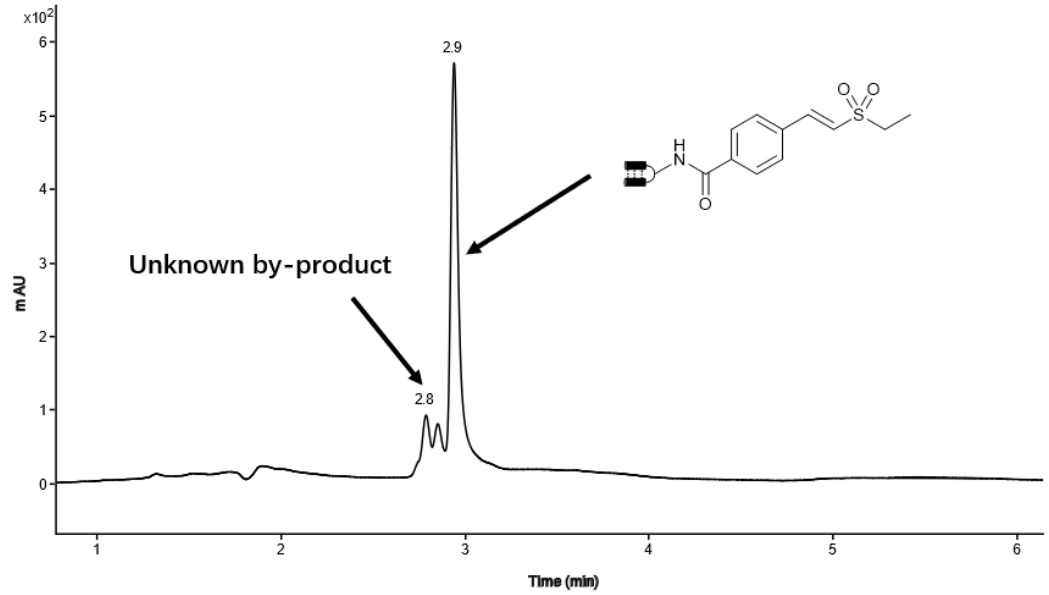


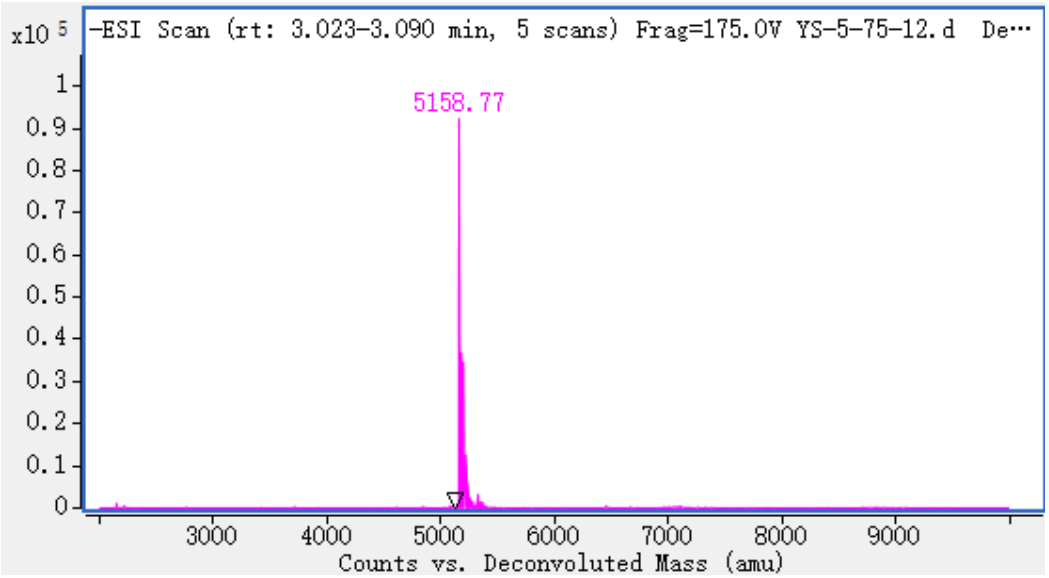


UPLC chromatogram and deconvoluted MS of **aa32**

Conversion: 70%

Calculated Mass: 5159 Da; Observed Mass: 5159 Da

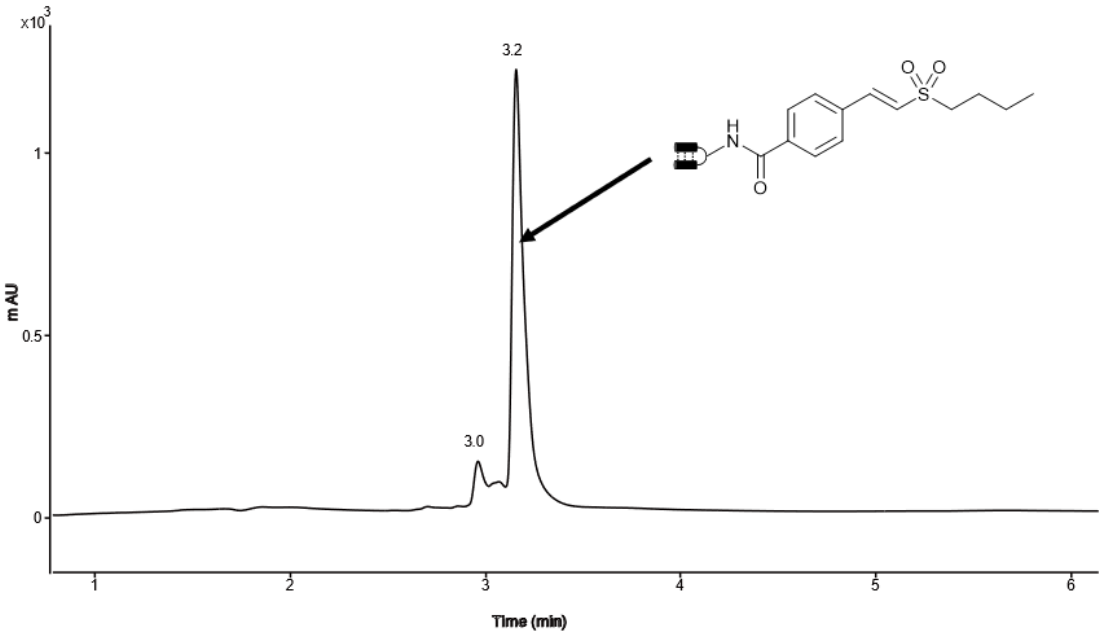


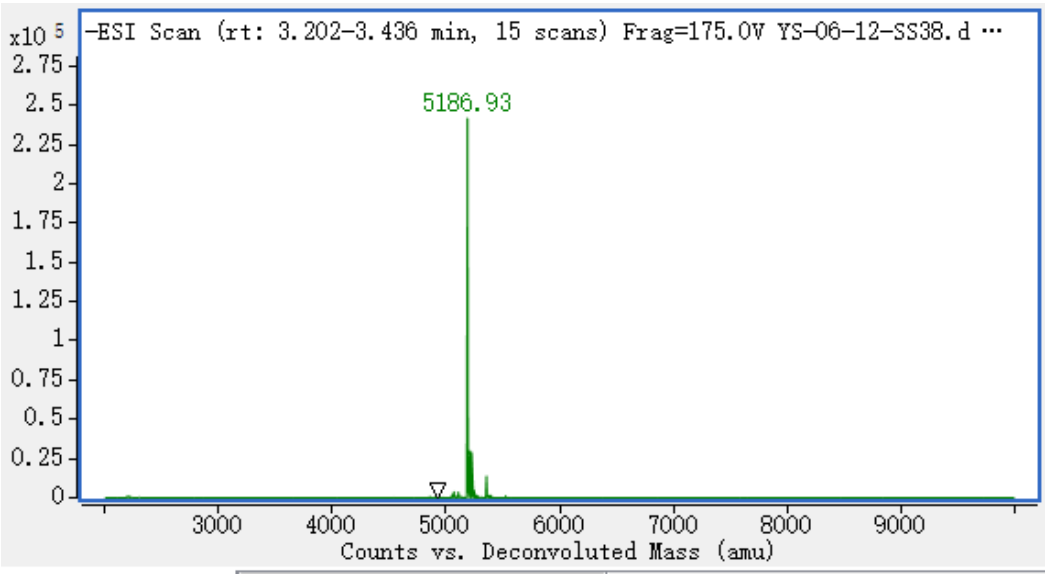


UPLC chromatogram and deconvoluted MS of **aa33**

Conversion: 80%

Calculated Mass: 5187 Da; Observed Mass: 5187 Da

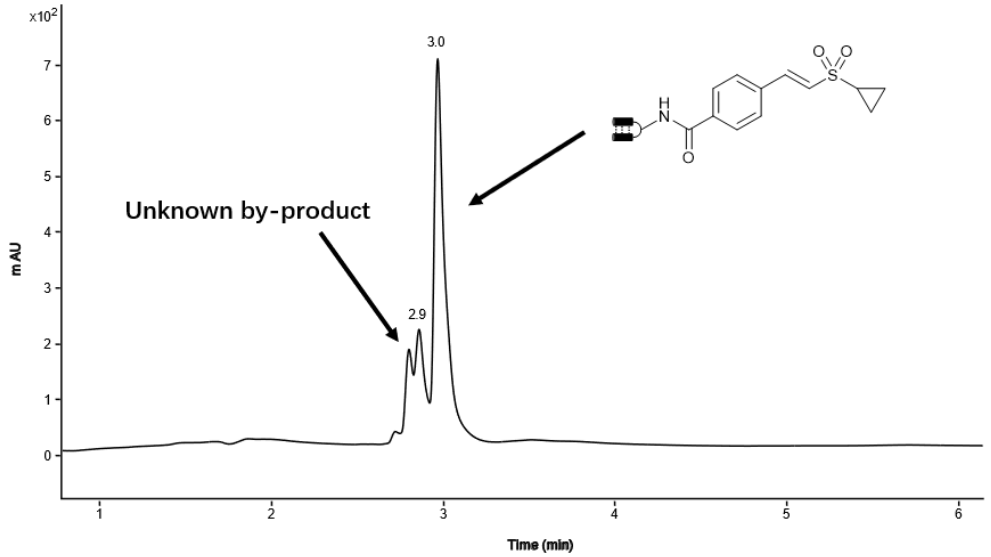


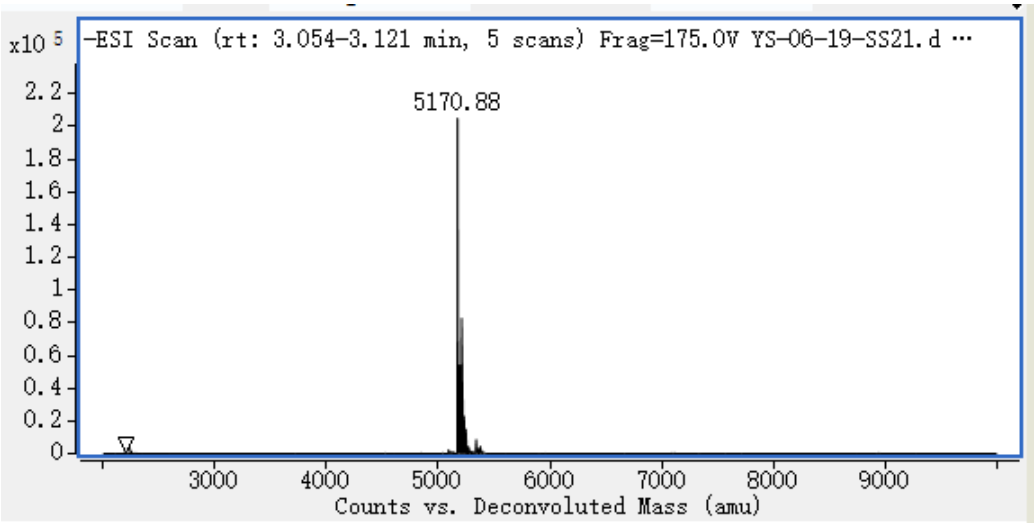


UPLC chromatogram and deconvoluted MS of **aa34**

Conversion: 72%

Calculated Mass: 5171 Da; Observed Mass: 5171 Da



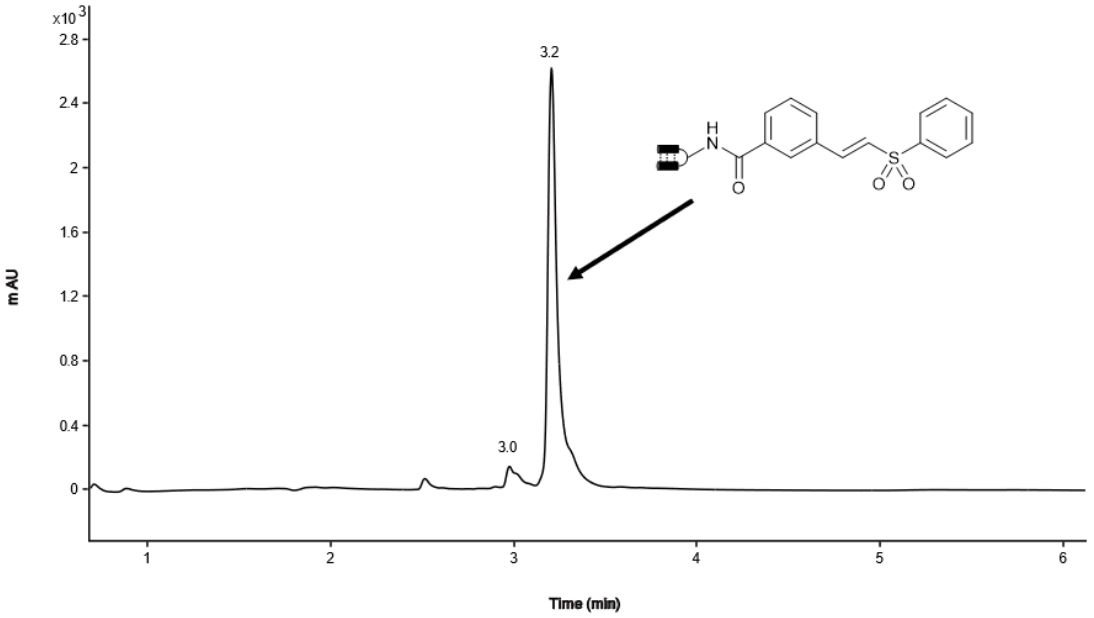


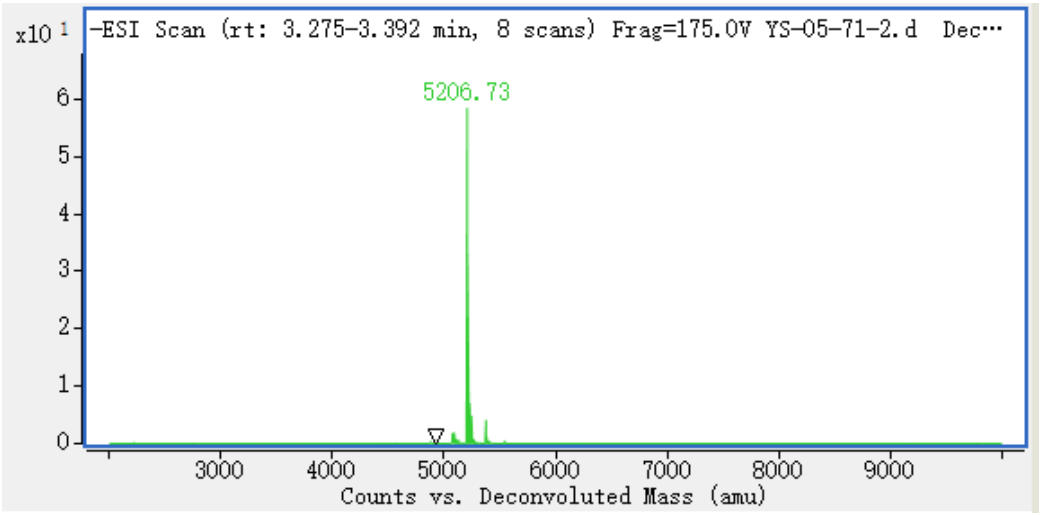
8.2. Substrate Scope of DNA Conjugated Alkenes

UPLC chromatogram and deconvoluted MS of **1ab**

Conversion: >90%

Calculated Mass: 5207 Da; Observed Mass: 5207 Da

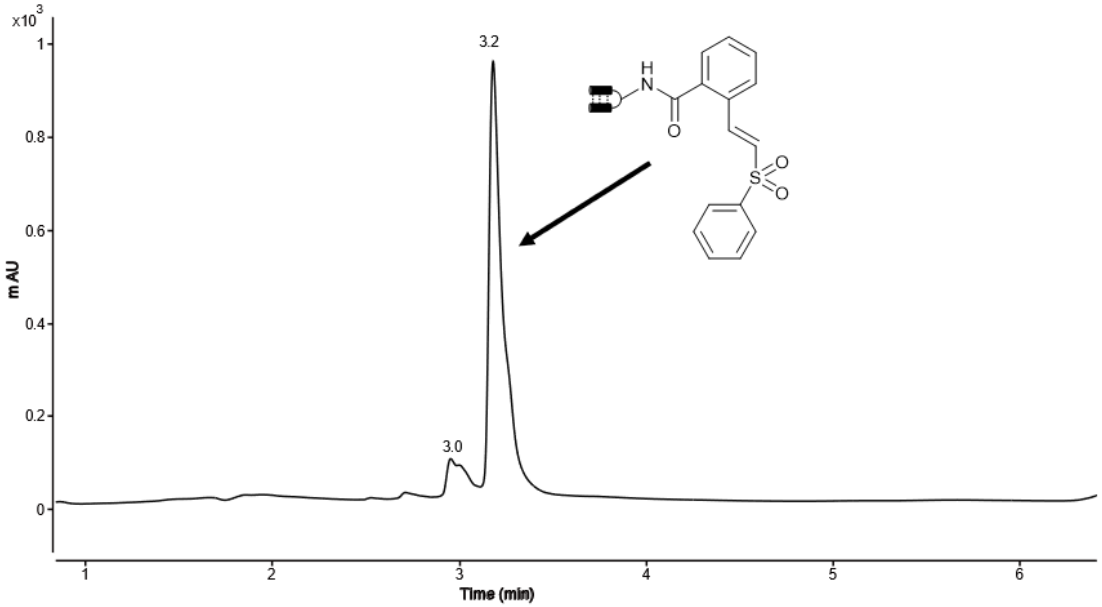


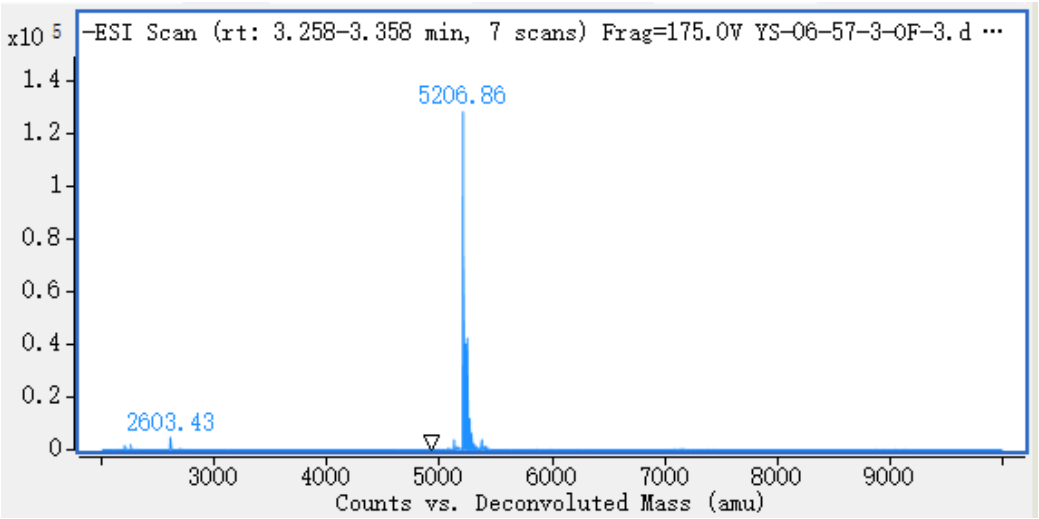


UPLC chromatogram and deconvoluted MS of **1ac**

Conversion: 90%

Calculated Mass: 5207 Da; Observed Mass: 5207 Da

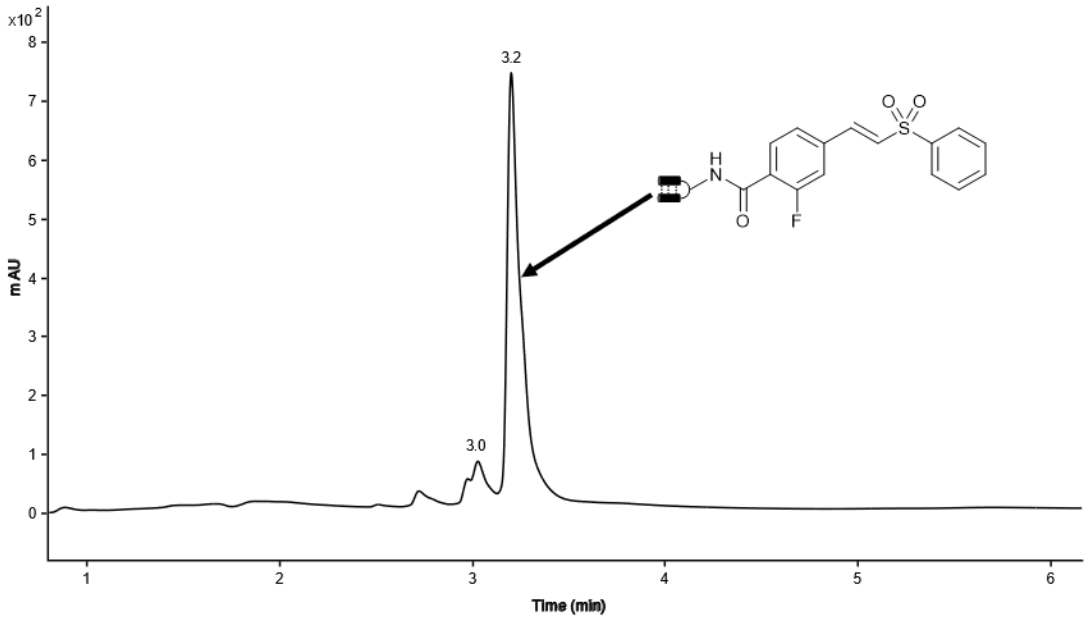


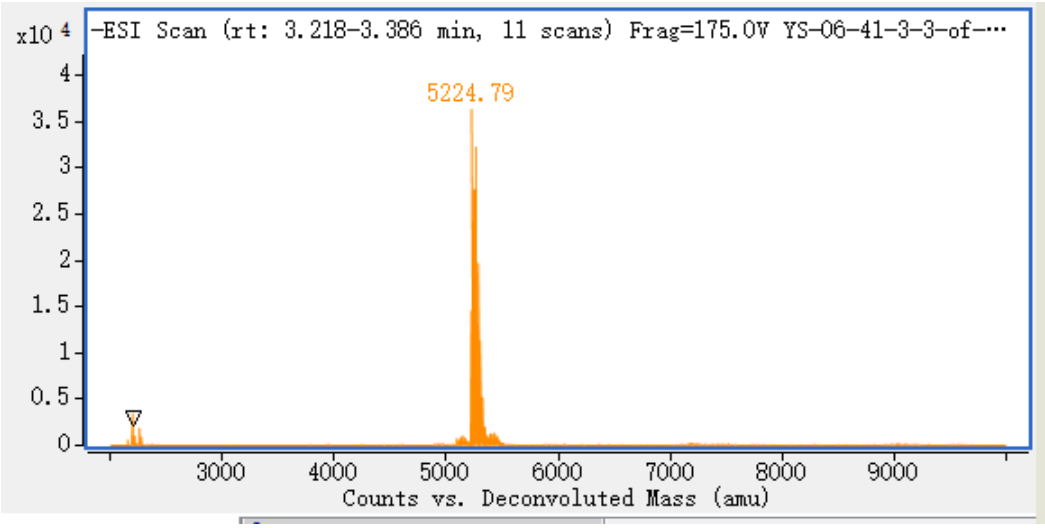


UPLC chromatogram and deconvoluted MS of **1ad**

Conversion: 88%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da

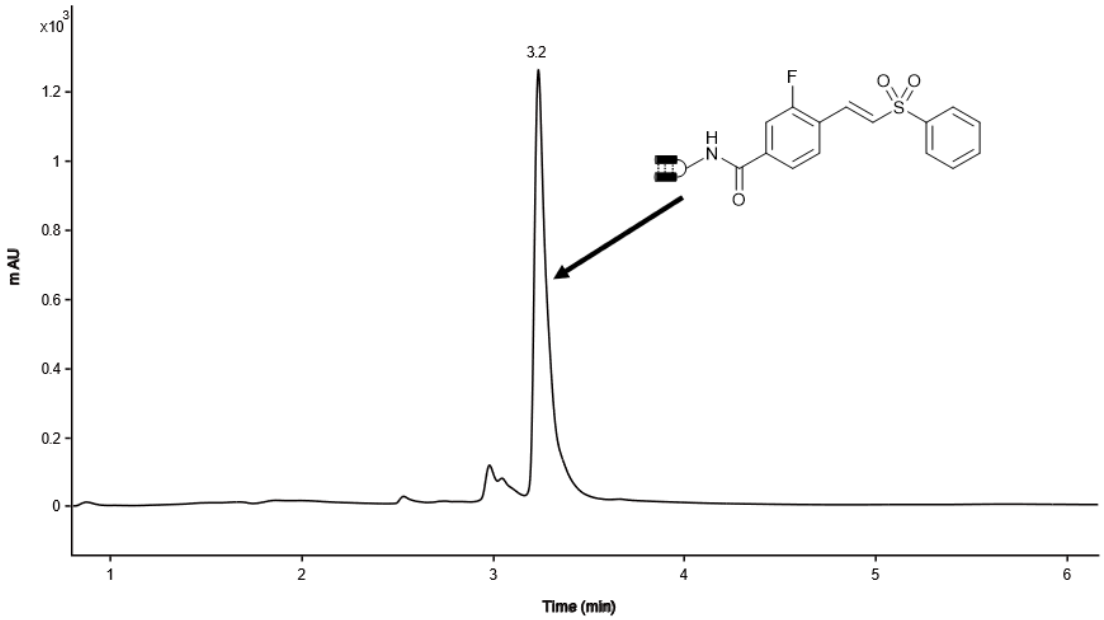


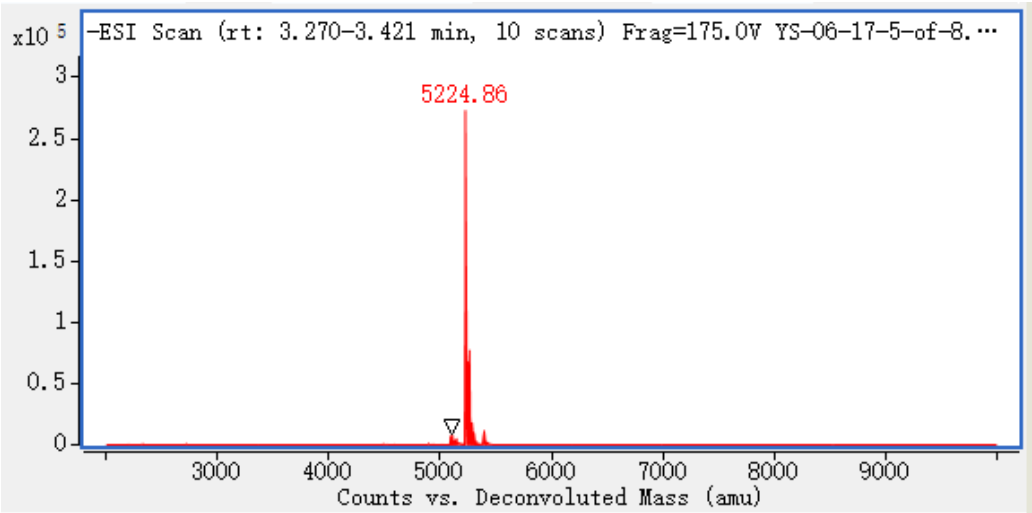


UPLC chromatogram and deconvoluted MS of **1ae**

Conversion: 90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da

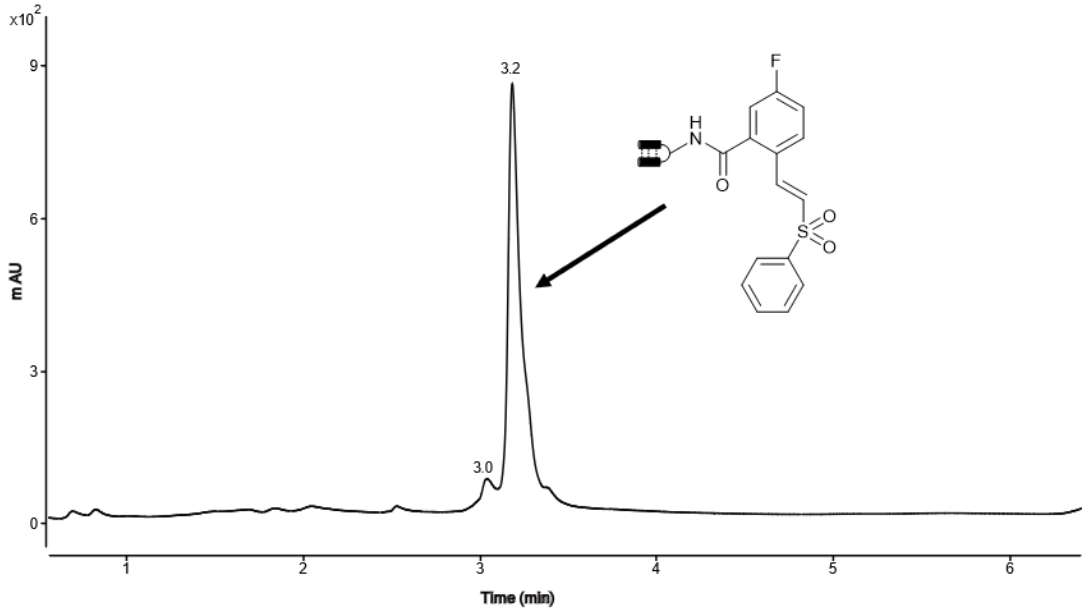


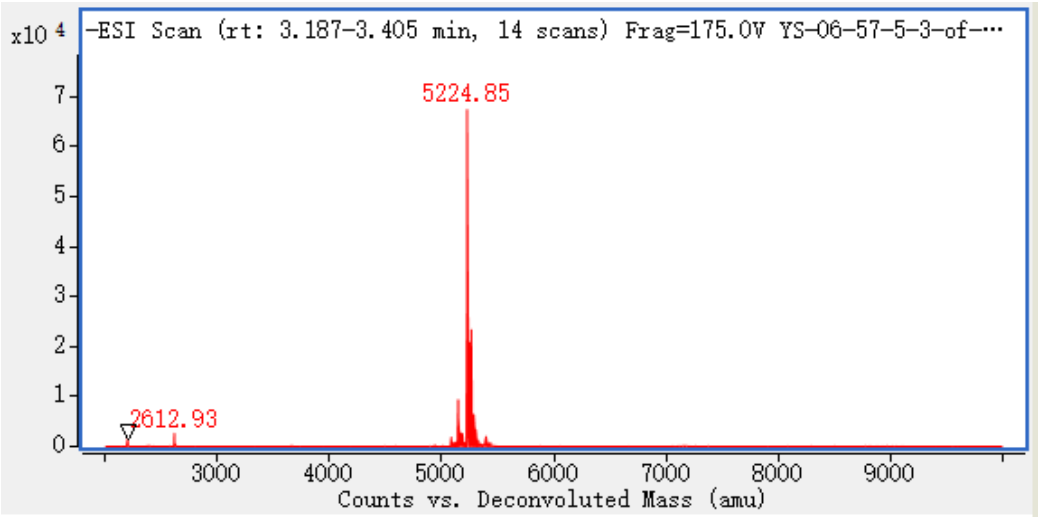


UPLC chromatogram and deconvoluted MS of **1af**

Conversion: >90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da

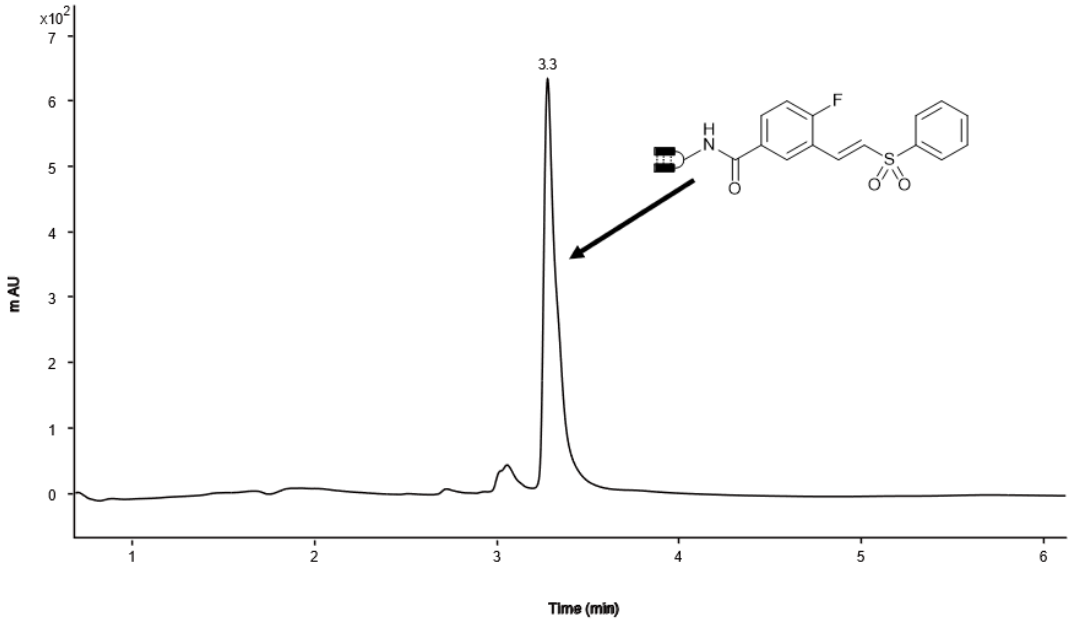


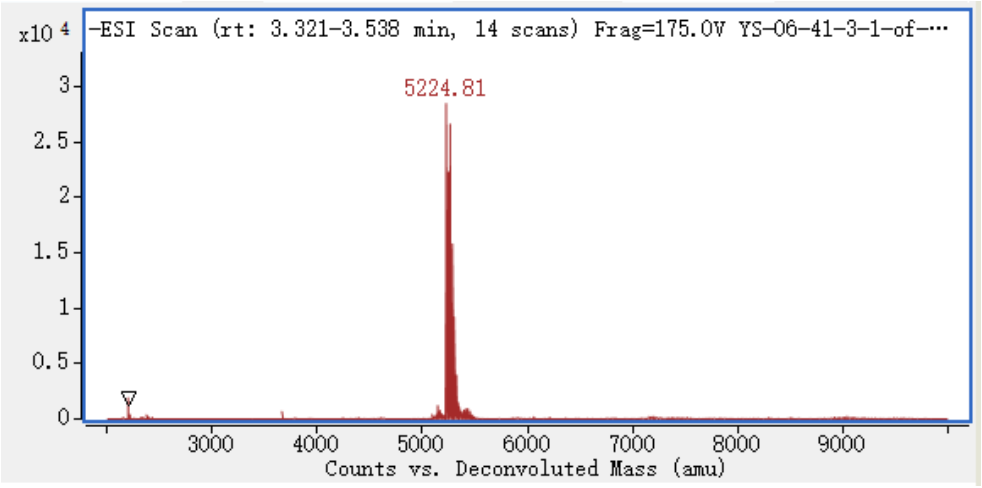


UPLC chromatogram and deconvoluted MS of **1ag**

Conversion: 90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da

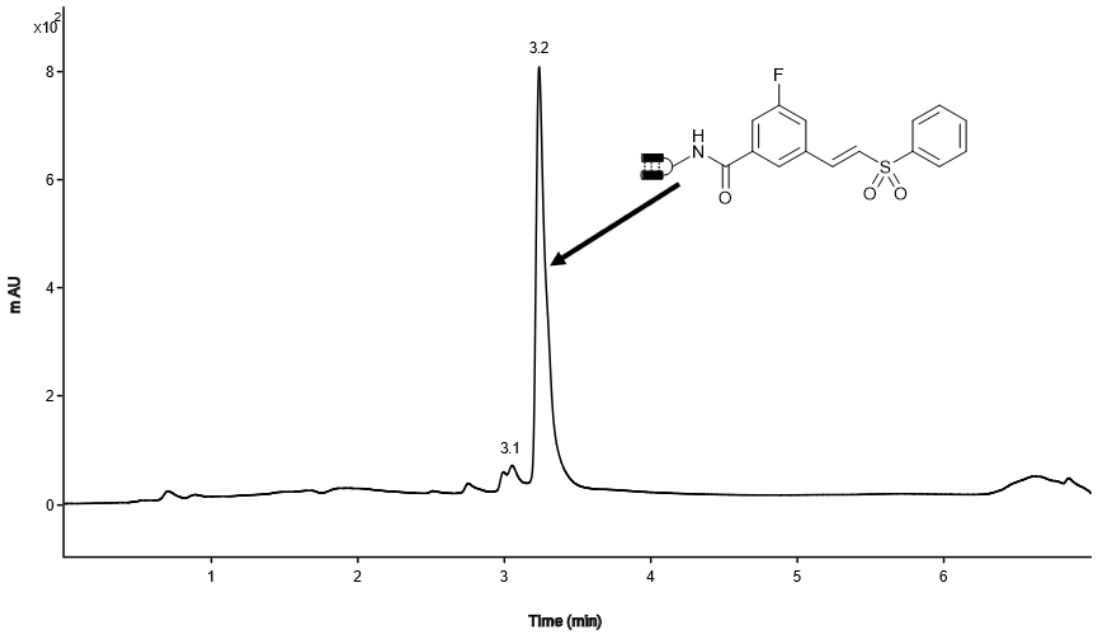


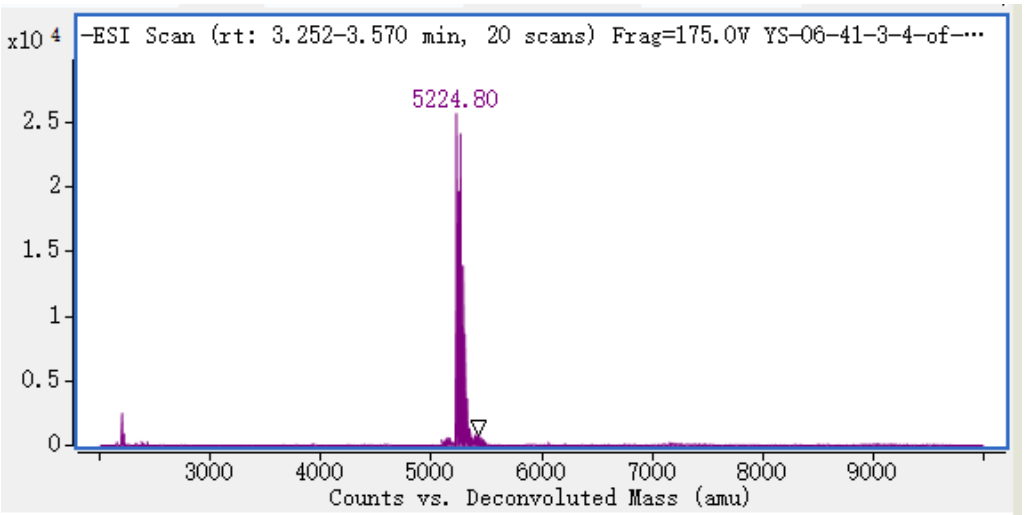


UPLC chromatogram and deconvoluted MS of **1ah**

Conversion: 90%

Calculated Mass: 5225 Da; Observed Mass: 5225 Da

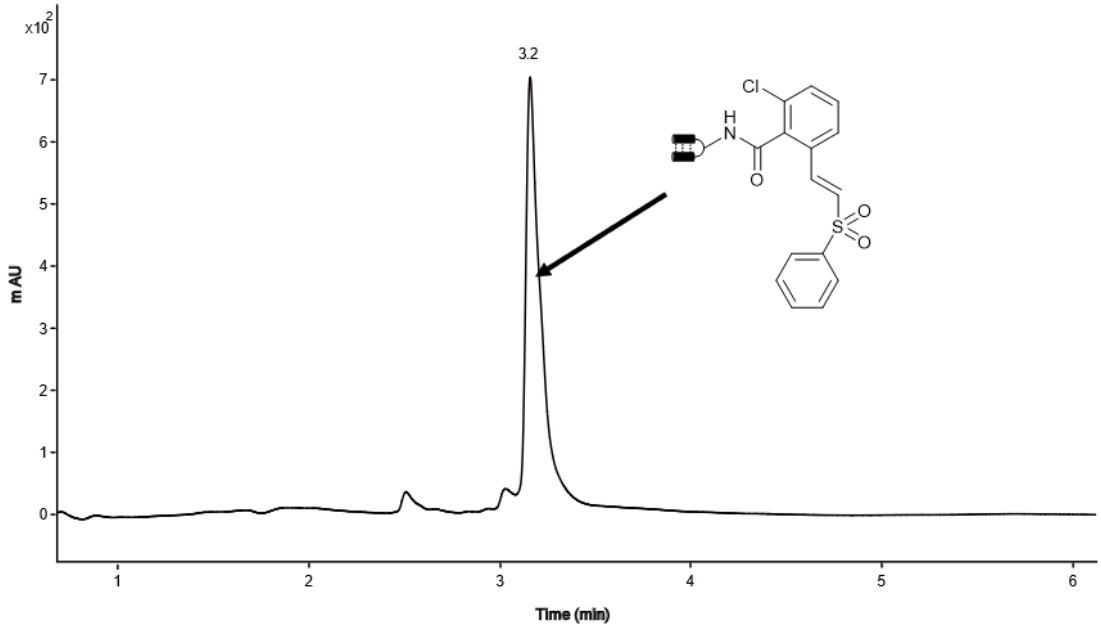


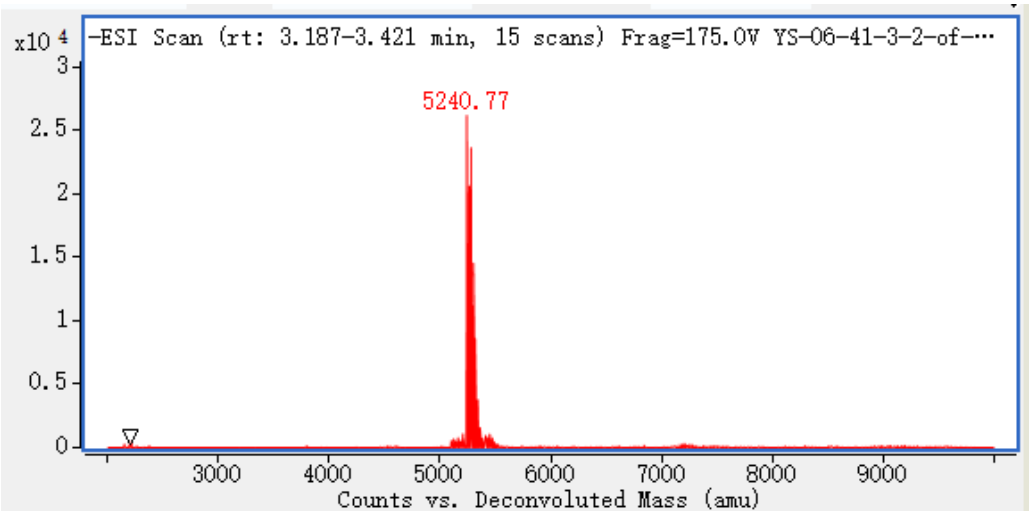


UPLC chromatogram and deconvoluted MS of **1ai**

Conversion: >90%

Calculated Mass: 5241 Da; Observed Mass: 5241 Da

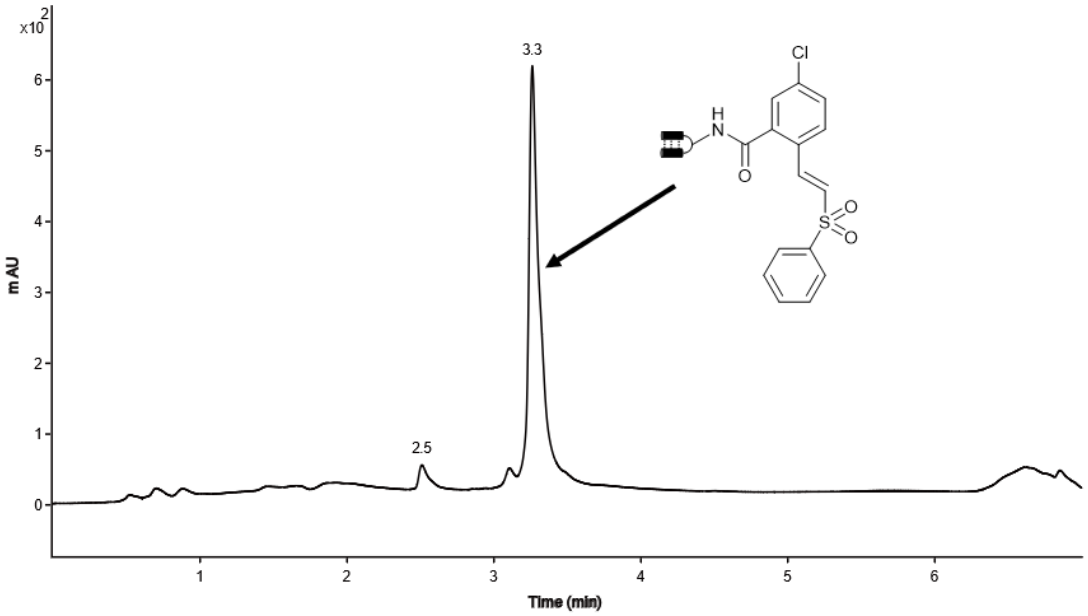


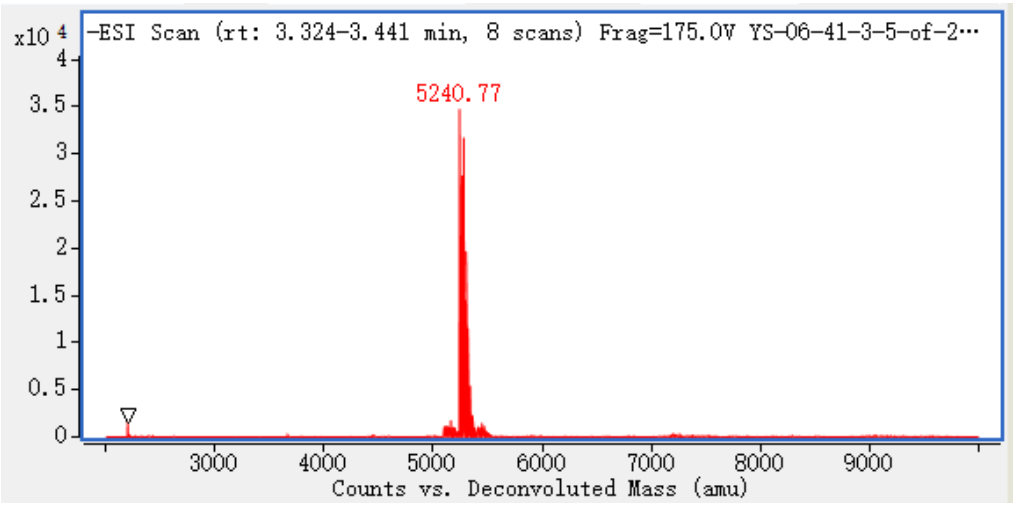


UPLC chromatogram and deconvoluted MS of **1aj**

Conversion: >90%

Calculated Mass: 5241 Da; Observed Mass: 5241 Da

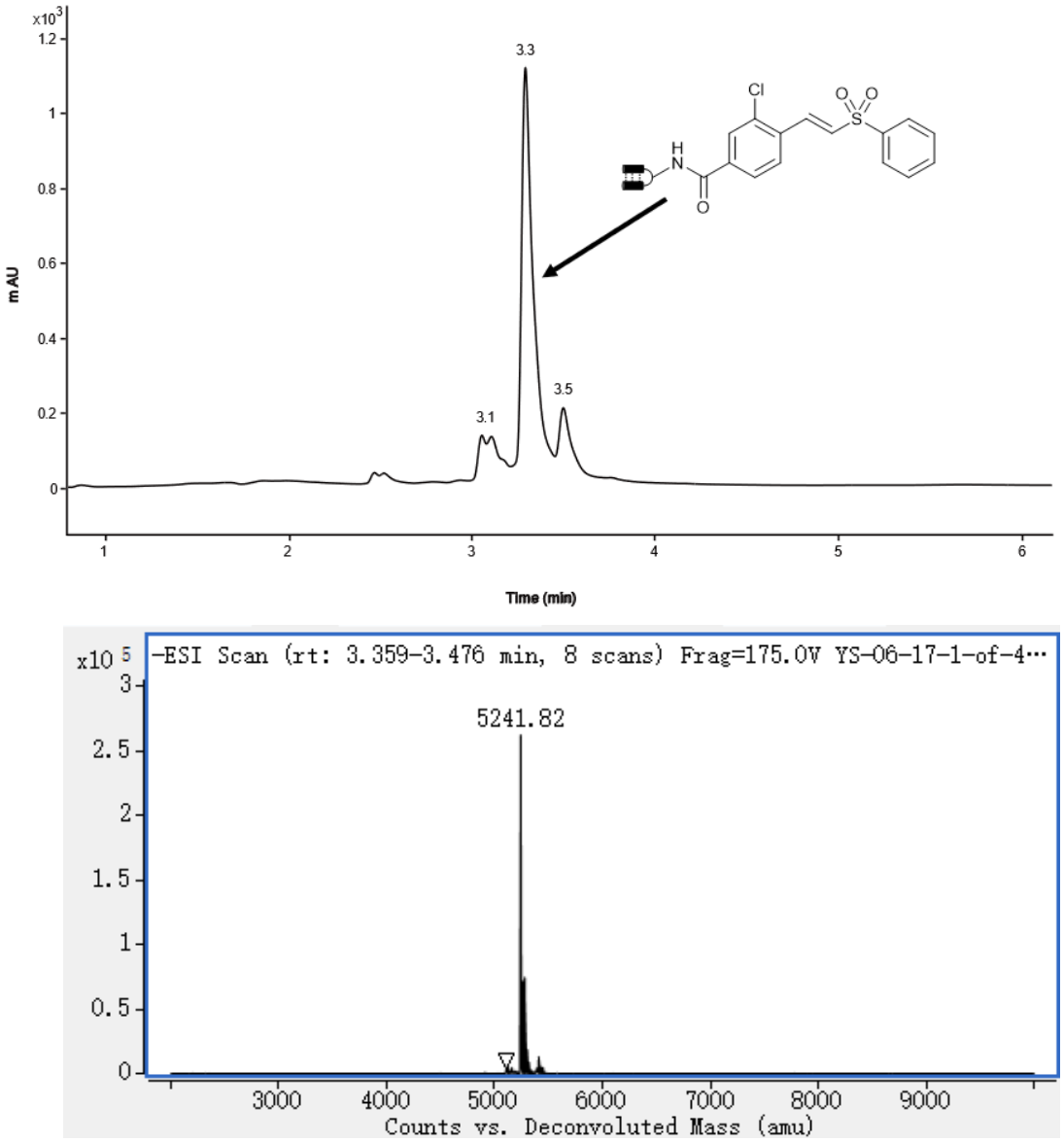




UPLC chromatogram and deconvoluted MS of **1ak**

Conversion: 72%

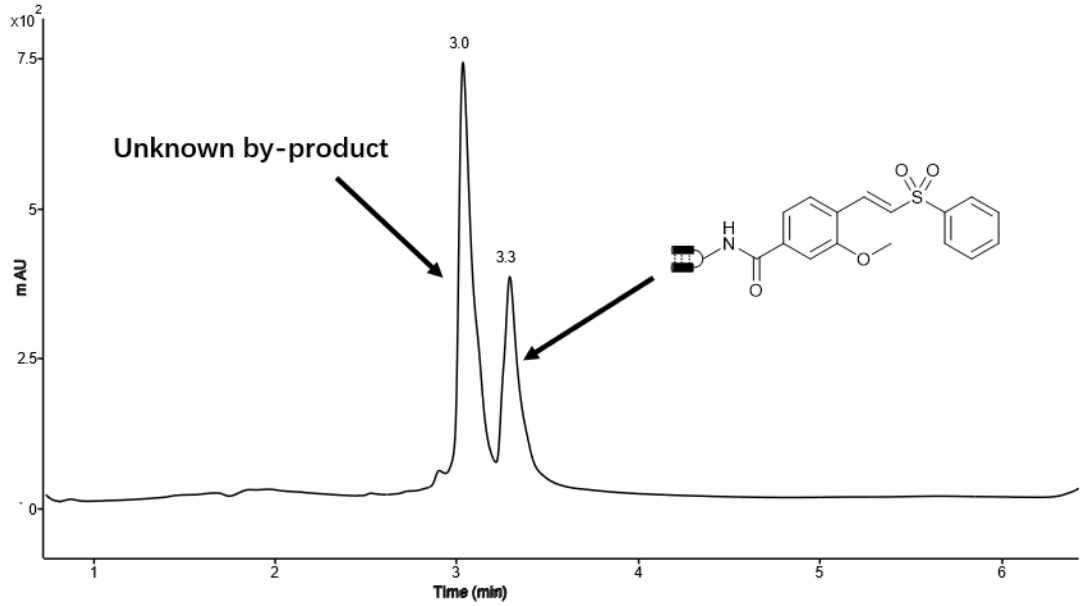
Calculated Mass: 5242 Da; Observed Mass: 5242 Da

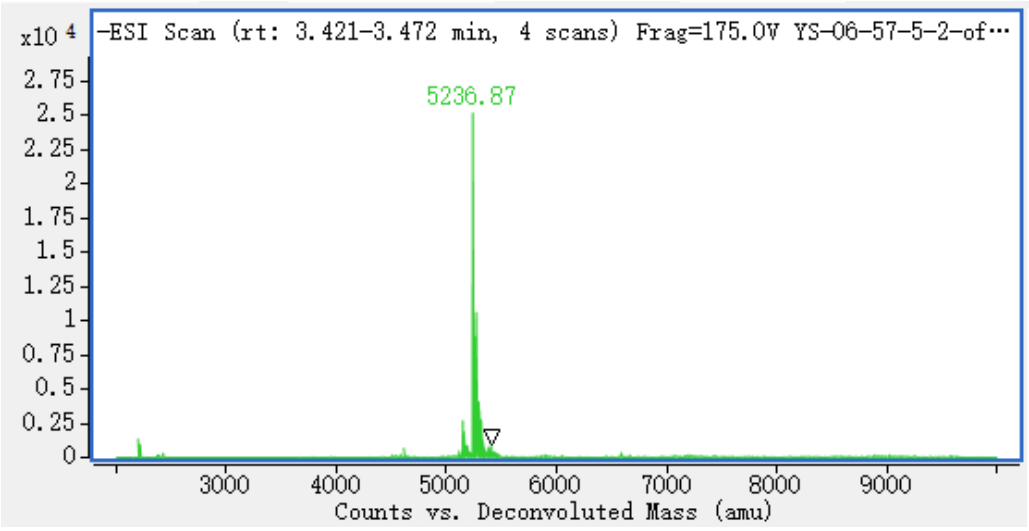


UPLC chromatogram and deconvoluted MS of **1al**

Conversion: 25%

Calculated Mass: 5237 Da; Observed Mass: 5237 Da

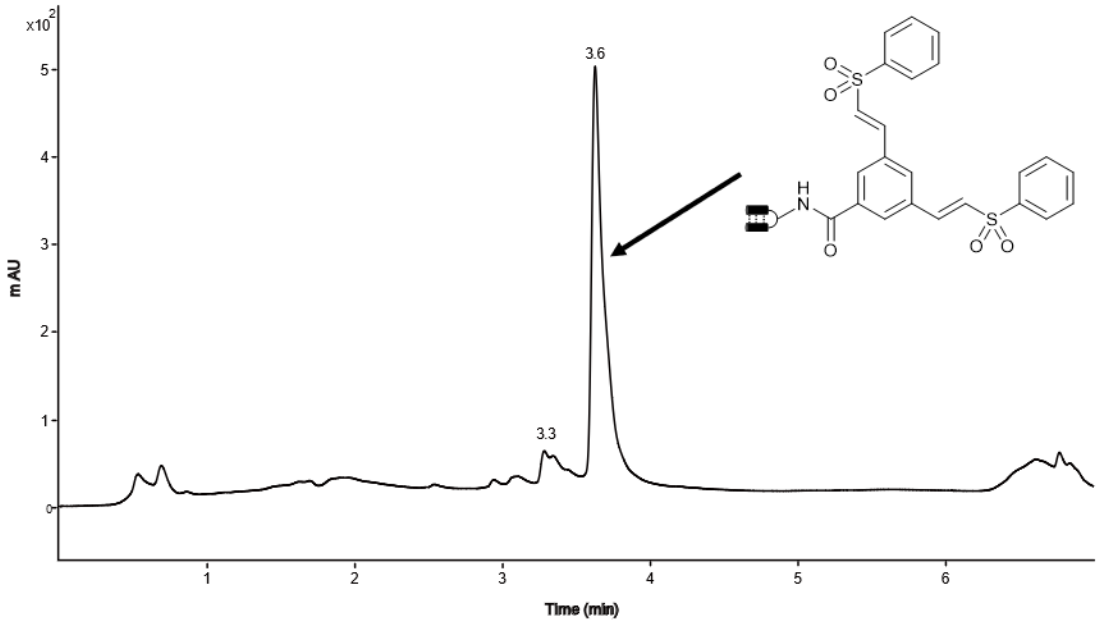


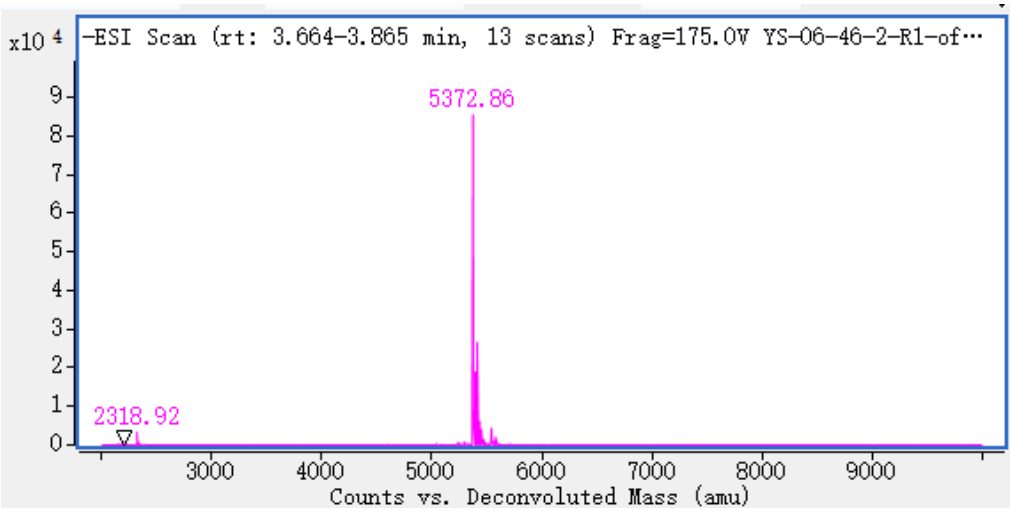


UPLC chromatogram and deconvoluted MS of **1am**

Conversion: 90%

Calculated Mass: 5373 Da; Observed Mass: 5373 Da

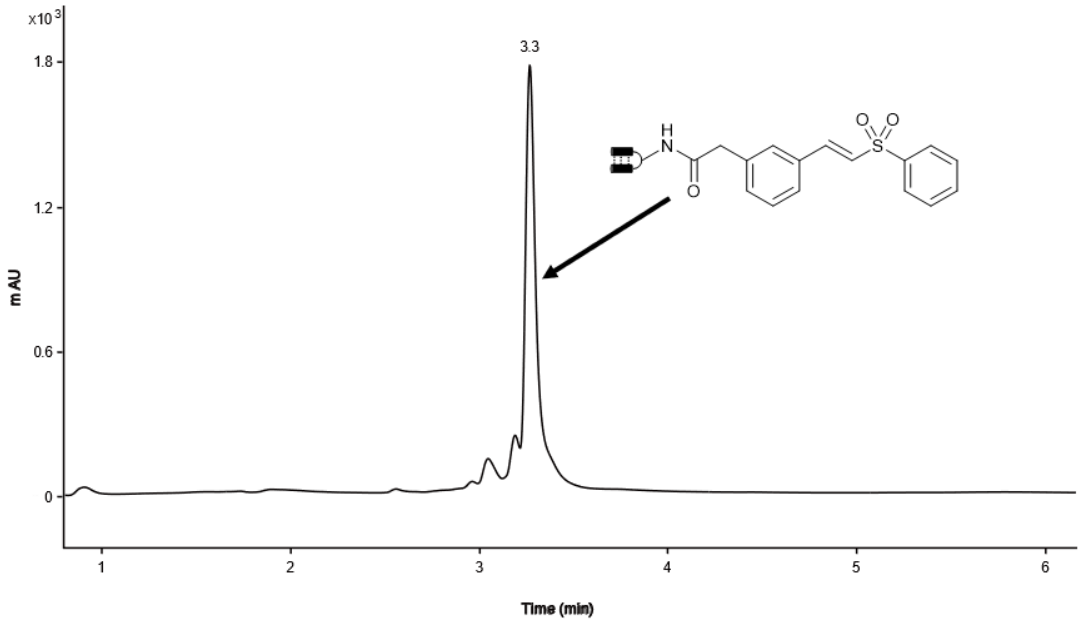


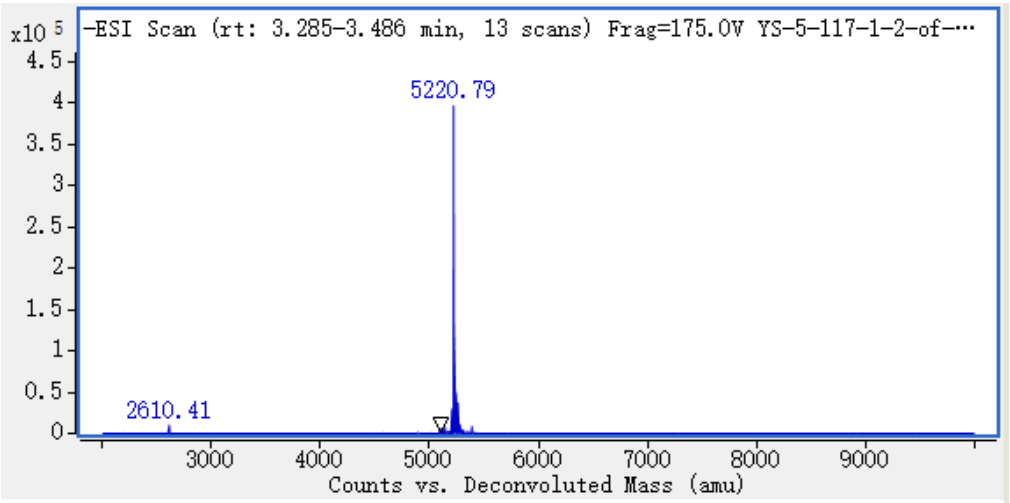


UPLC chromatogram and deconvoluted MS of **1an**

Conversion: 81%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da

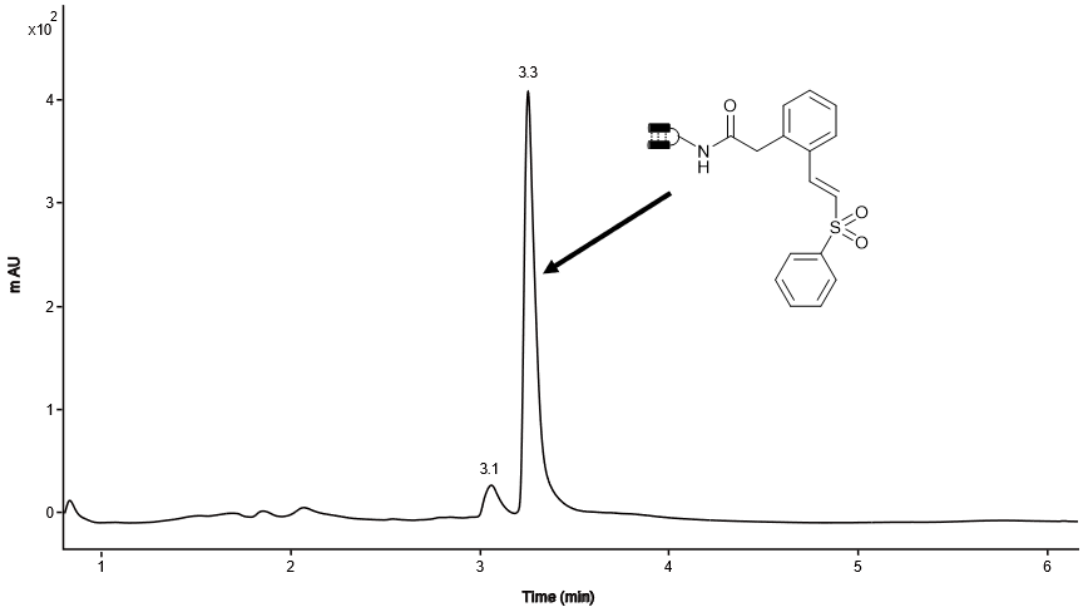


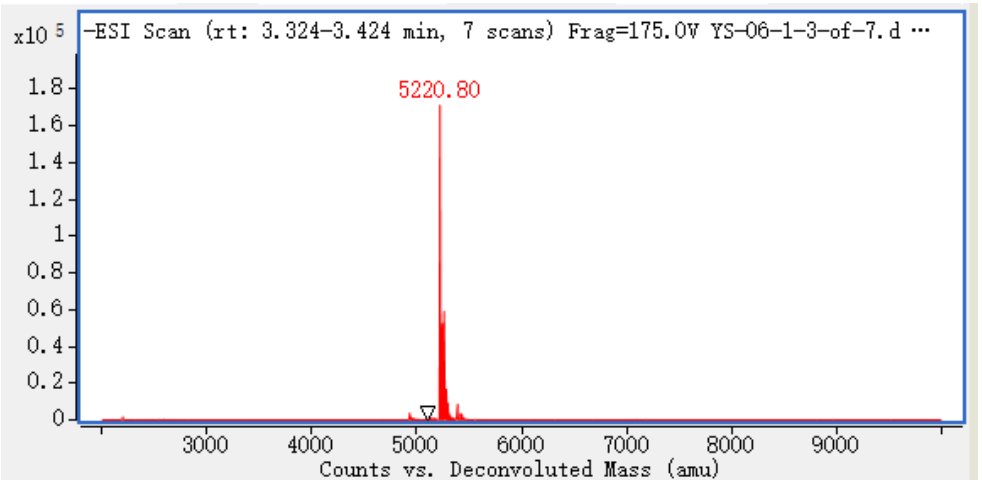


UPLC chromatogram and deconvoluted MS of **1ao**

Conversion: >90%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da

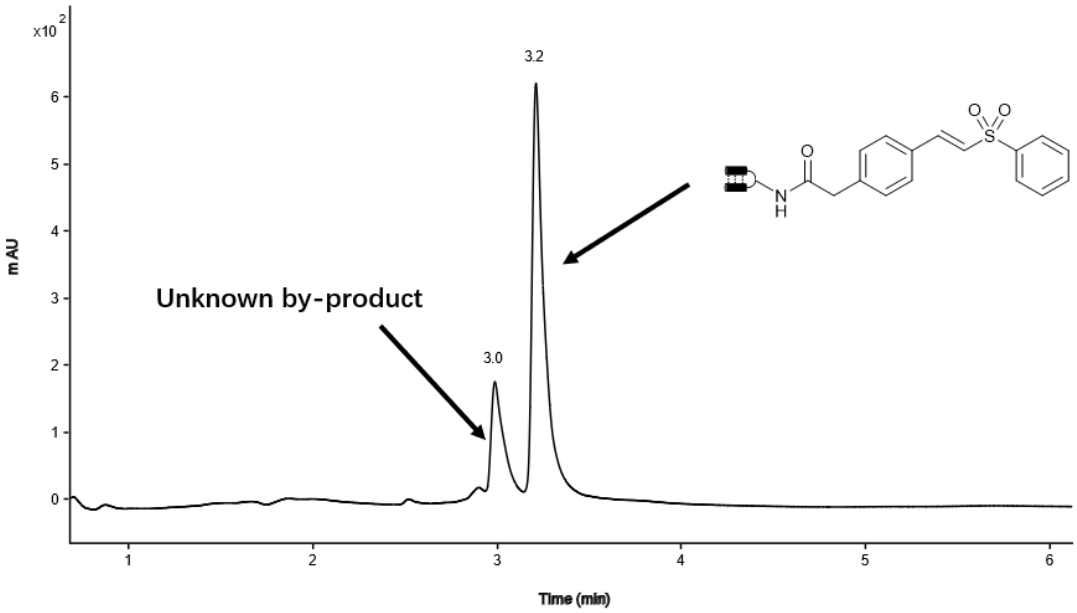


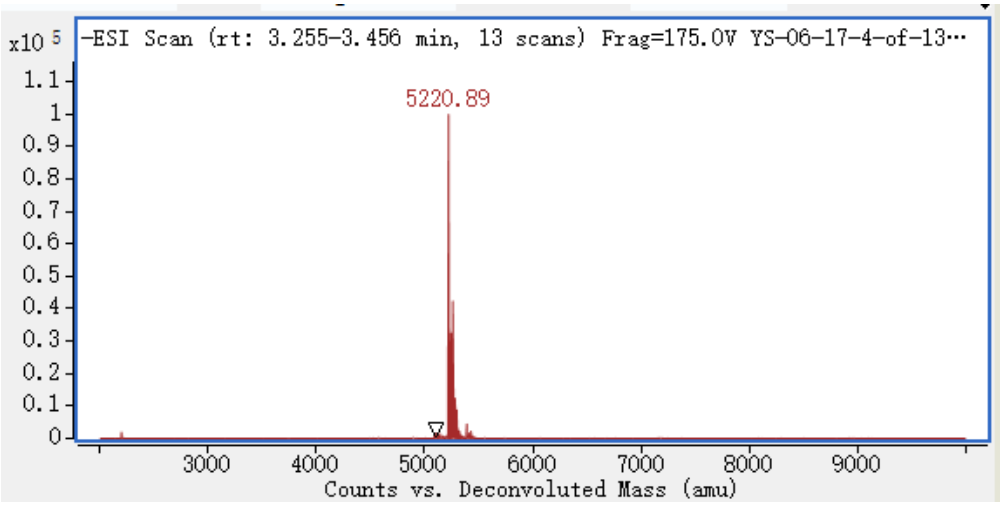


UPLC chromatogram and deconvoluted MS of **1ap**

Conversion: 76%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da

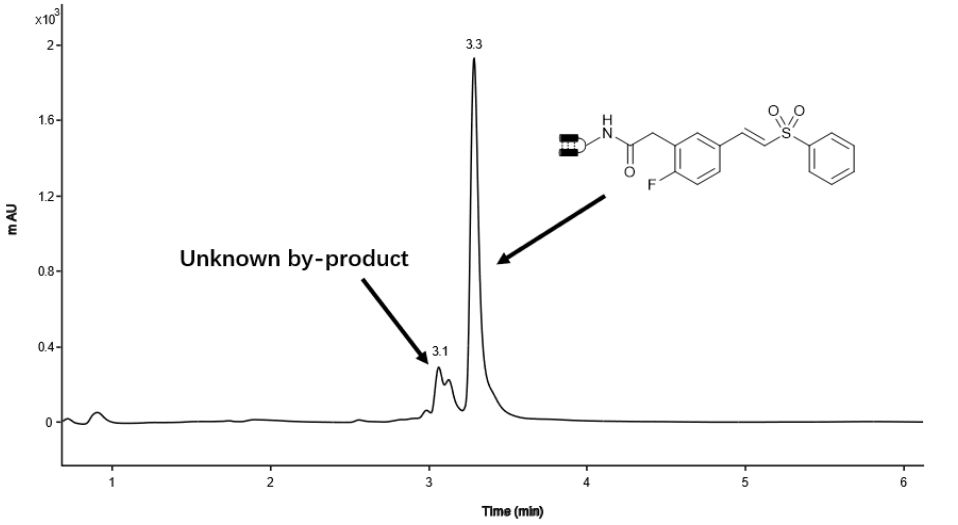


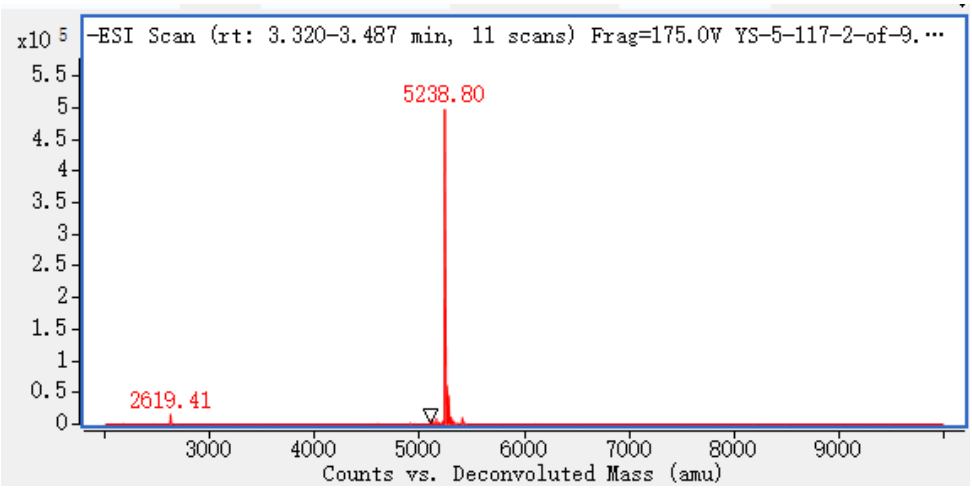


UPLC chromatogram and deconvoluted MS of **1aq**

Conversion: 81%

Calculated Mass: 5239 Da; Observed Mass: 5239 Da

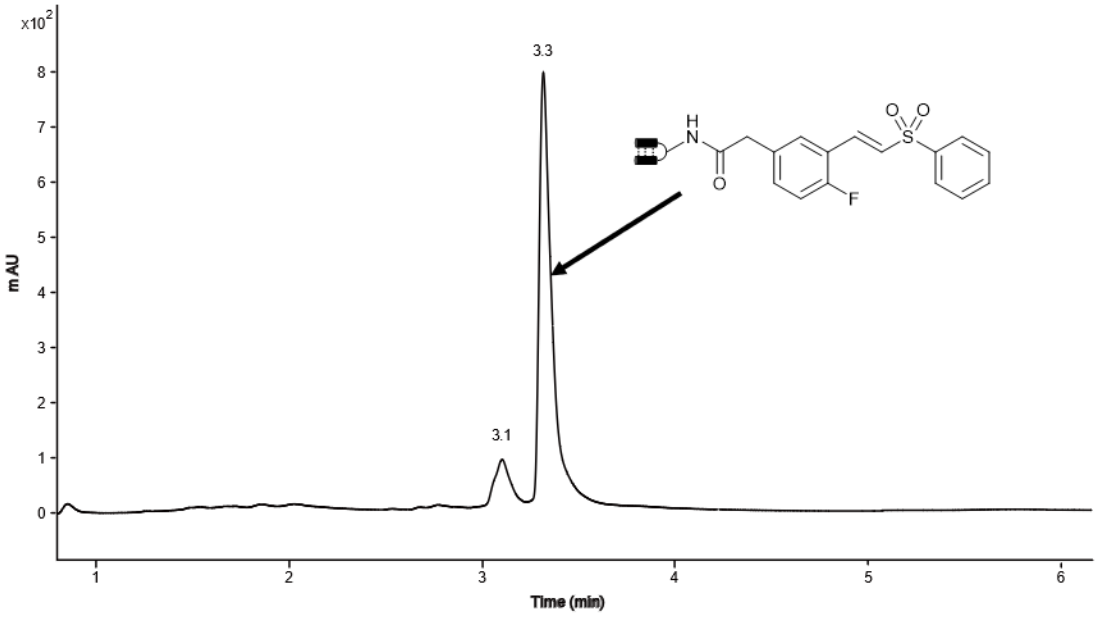


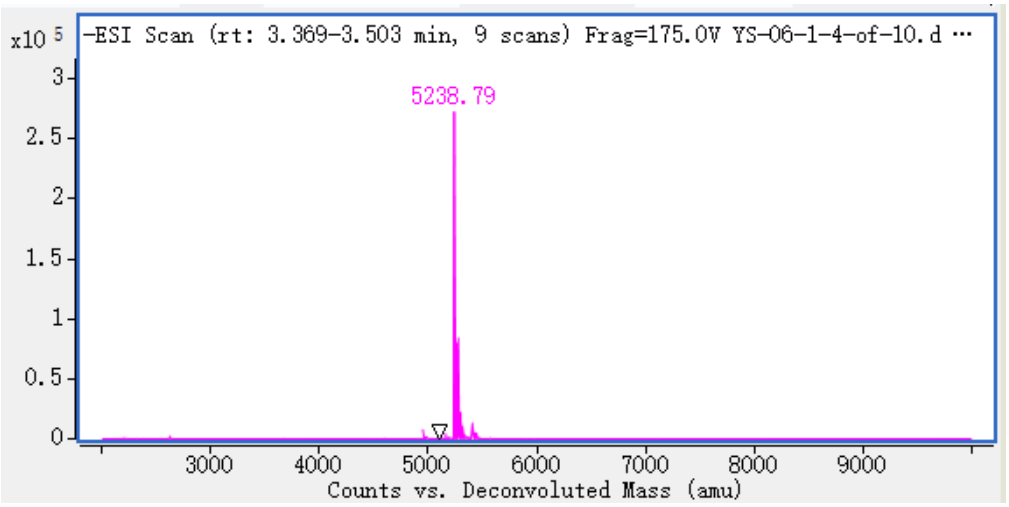


UPLC chromatogram and deconvoluted MS of **1ar**

Conversion: 87%

Calculated Mass: 5239 Da; Observed Mass: 5239 Da

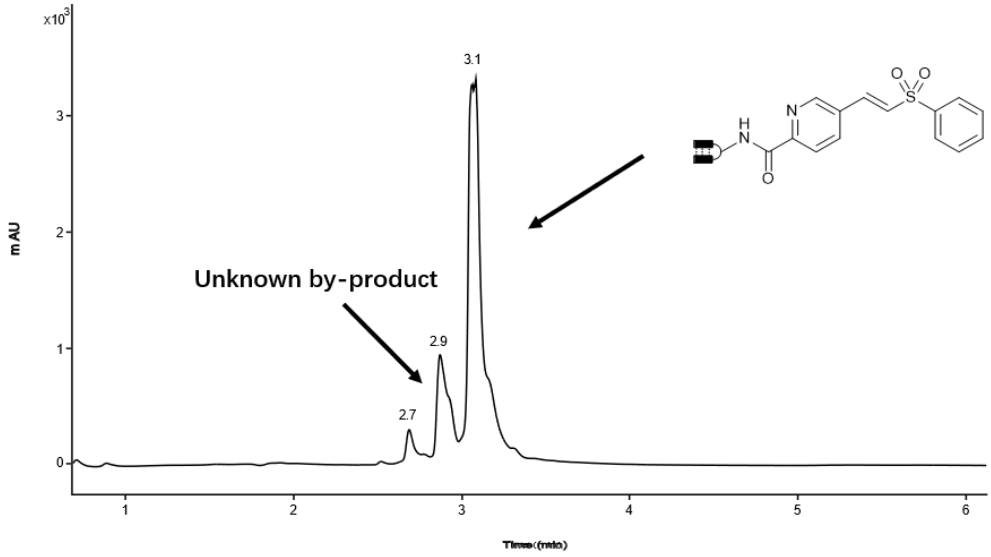


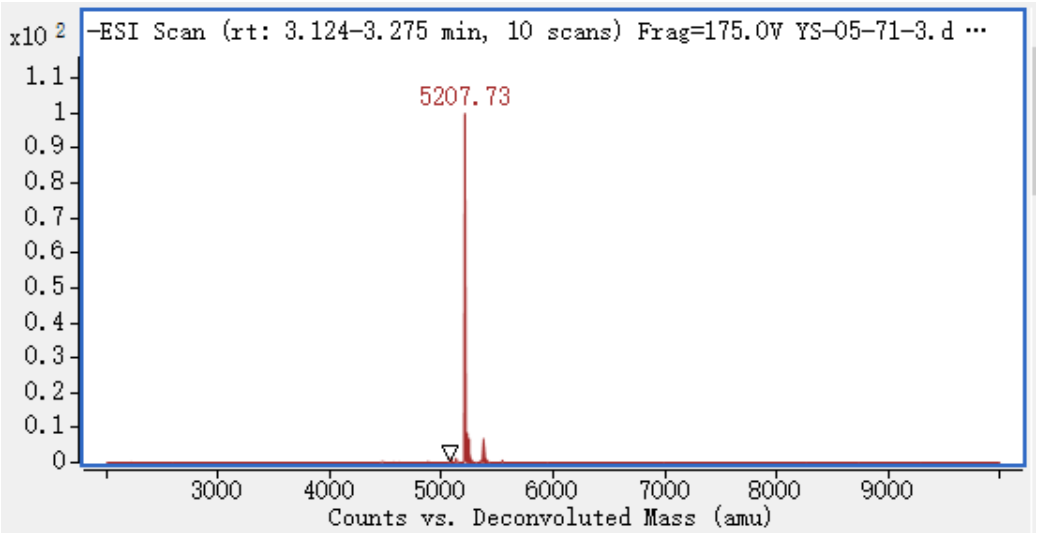


UPLC chromatogram and deconvoluted MS of **1as**

Conversion: 70%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da

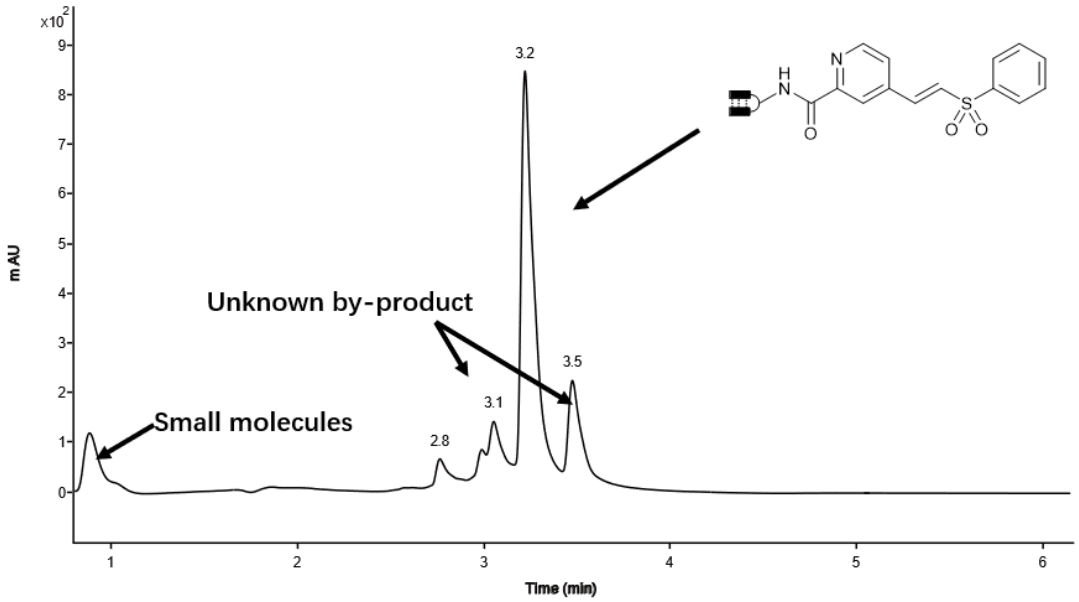


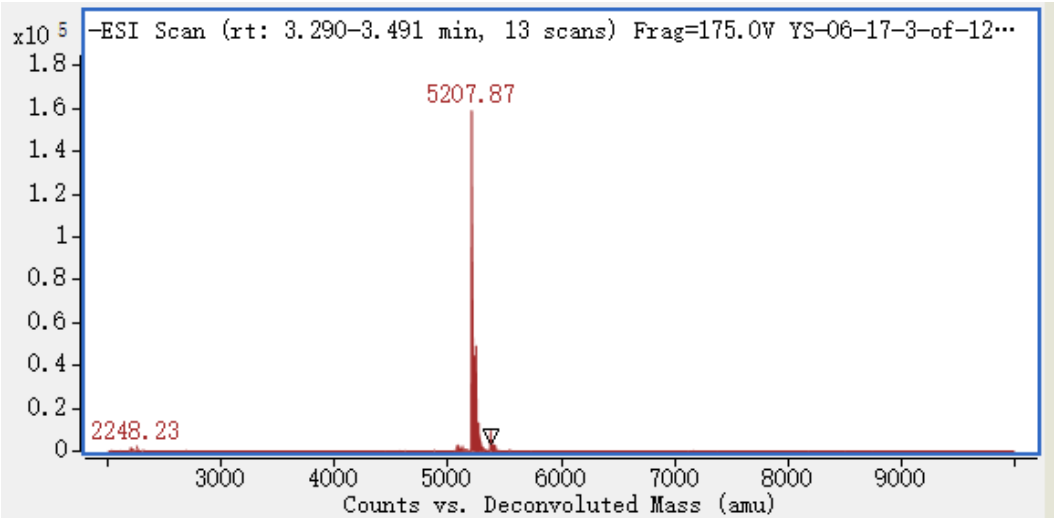


UPLC chromatogram and deconvoluted MS of **1at**

Conversion: 63%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da

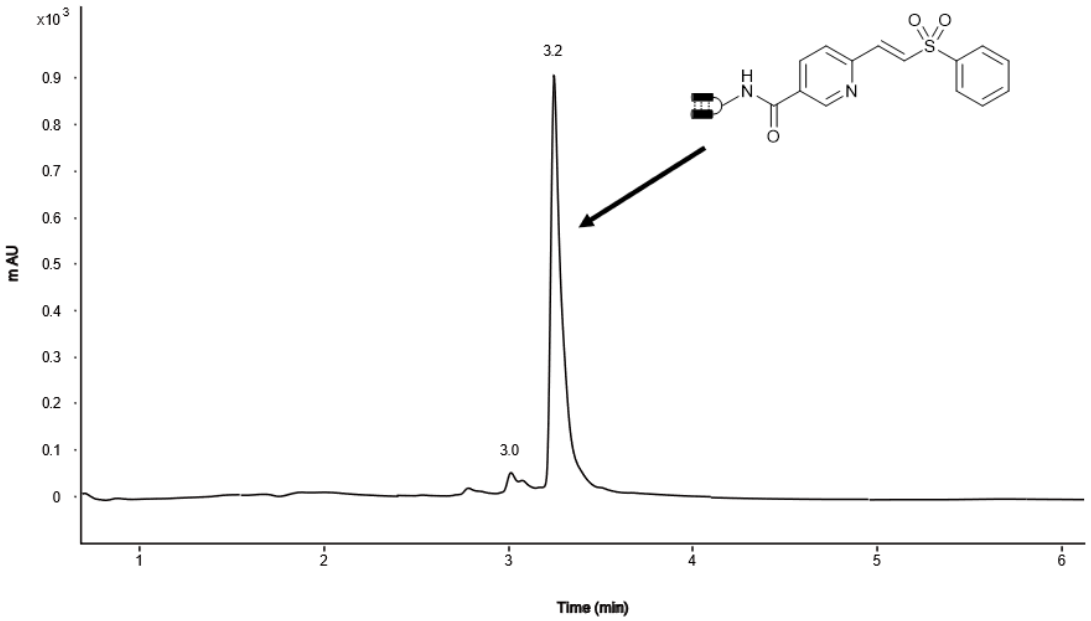


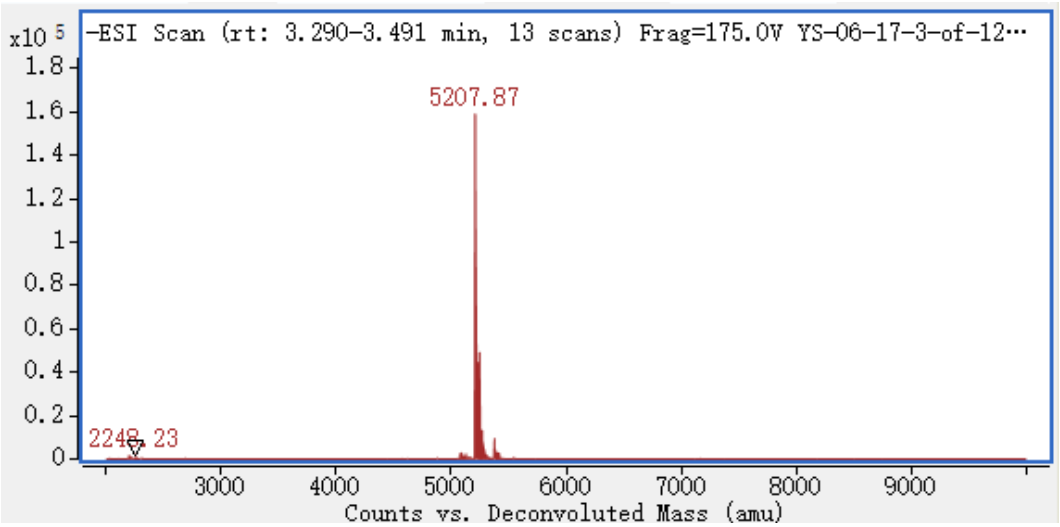


UPLC chromatogram and deconvoluted MS of **1au**

Conversion: >90%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da

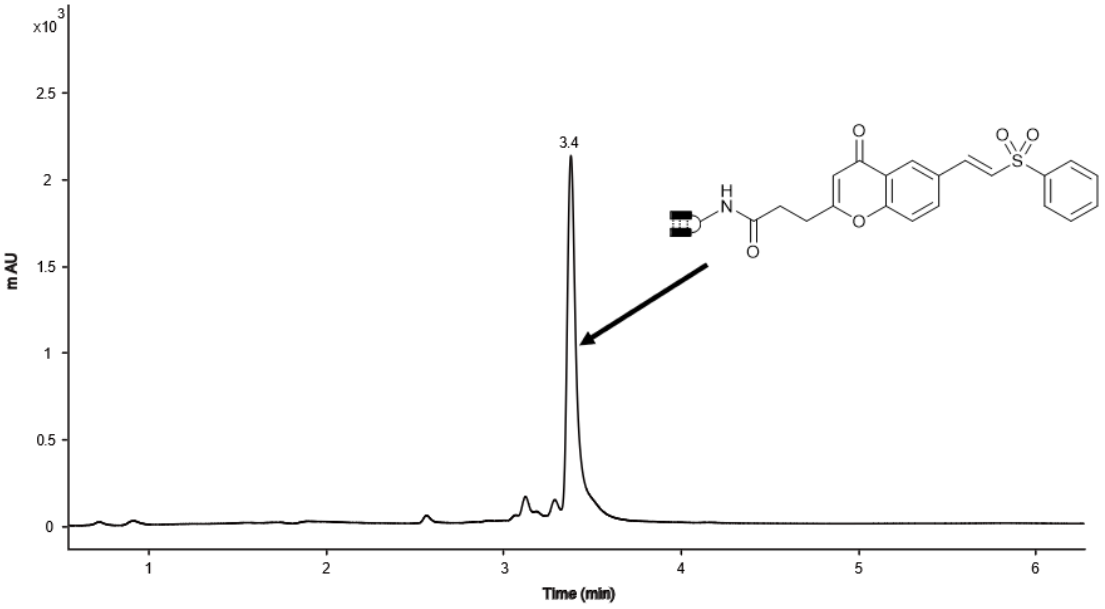


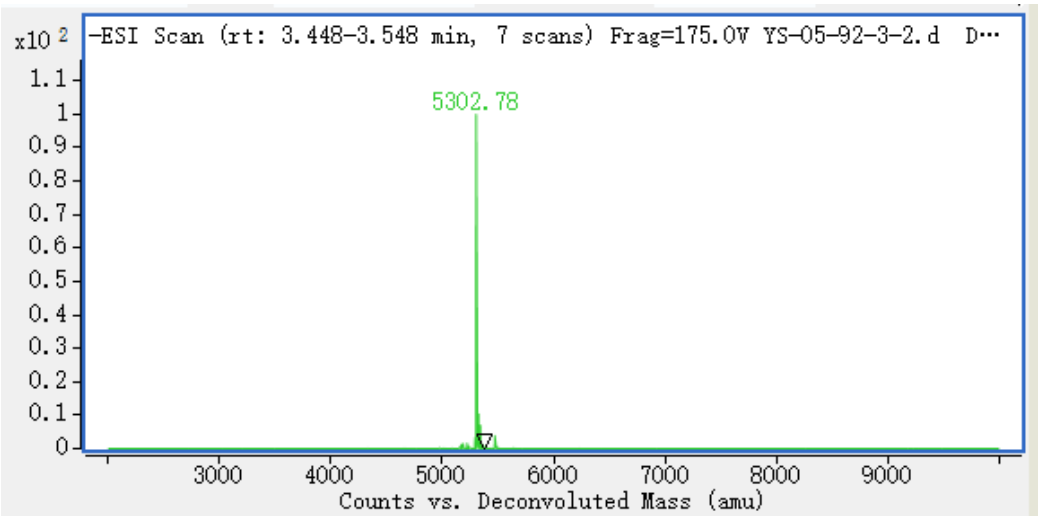


UPLC chromatogram and deconvoluted MS of **1av**

Conversion: >90%

Calculated Mass: 5303 Da; Observed Mass: 5303 Da

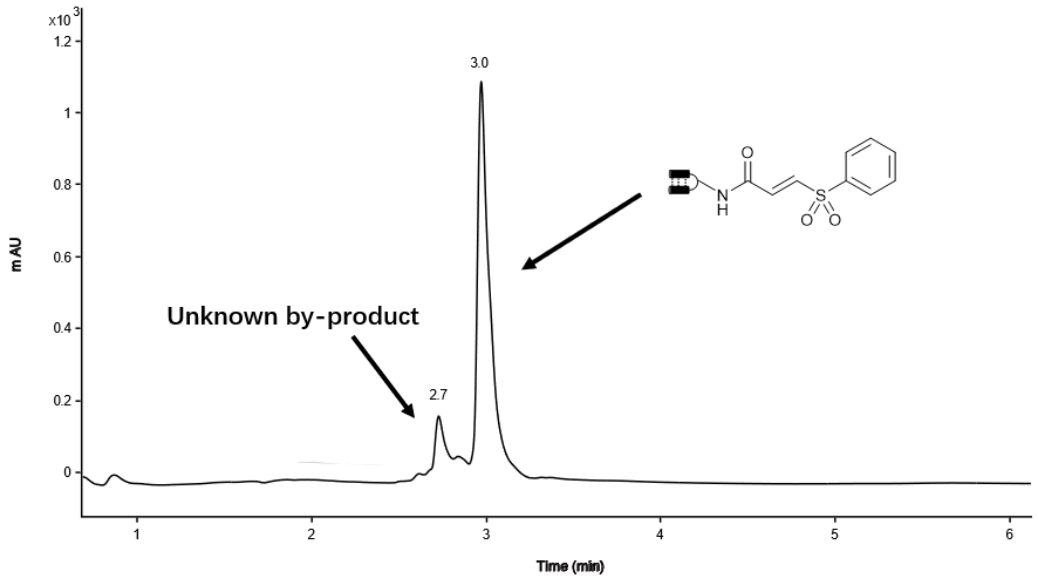


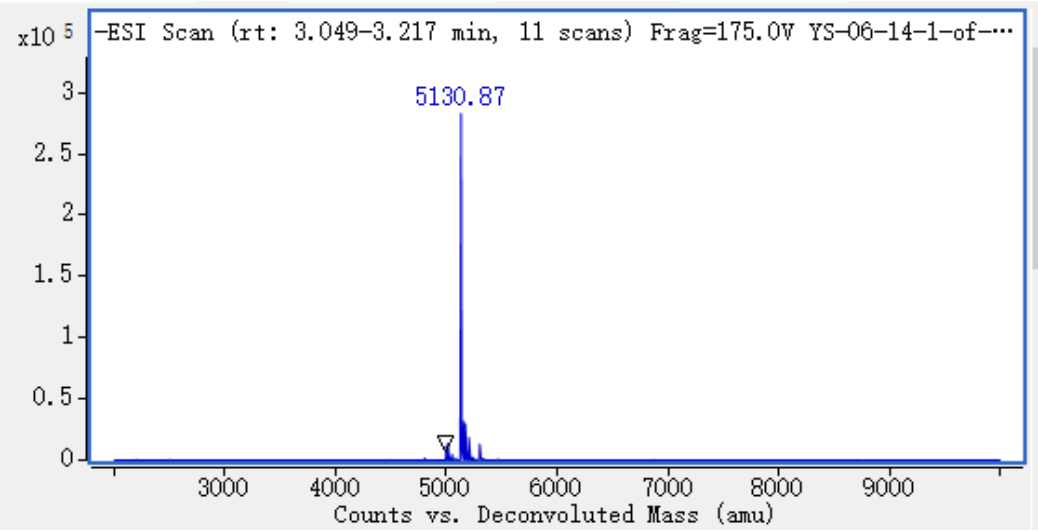


UPLC chromatogram and deconvoluted MS of **1aw**

Conversion: 82%

Calculated Mass: 5131 Da; Observed Mass: 5131 Da

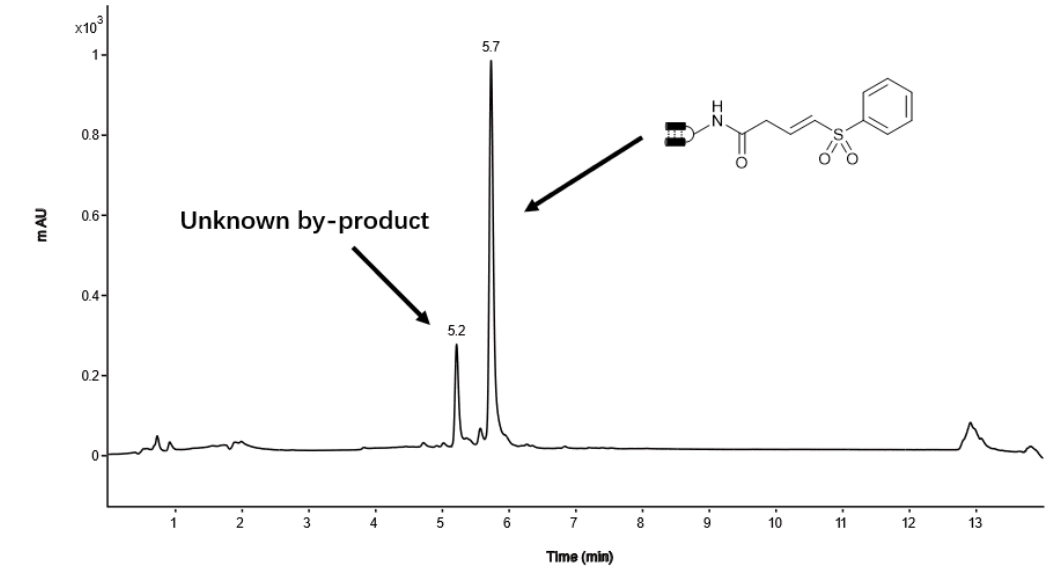


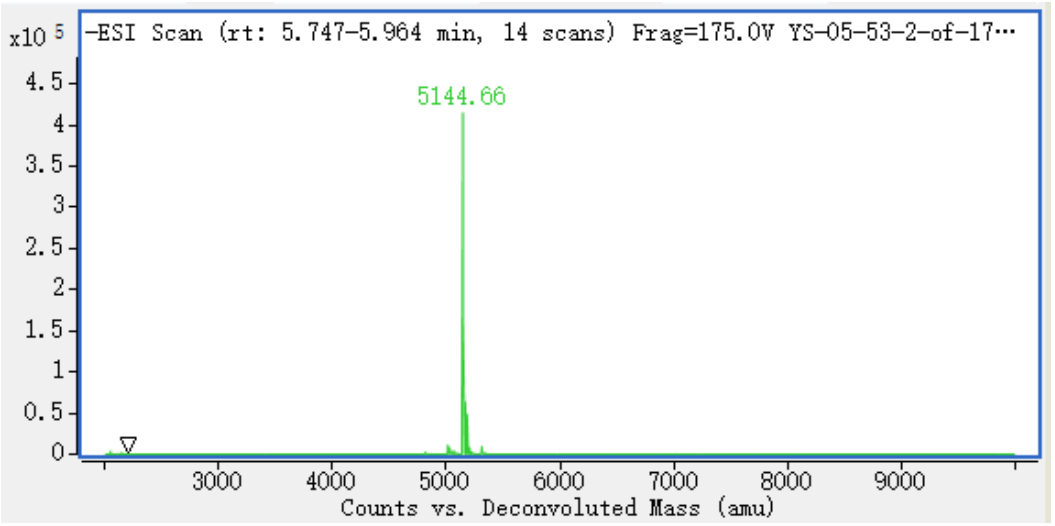


UPLC chromatogram and deconvoluted MS of **1ax**

Conversion: 80%

Calculated Mass: 5145 Da; Observed Mass: 5145 Da

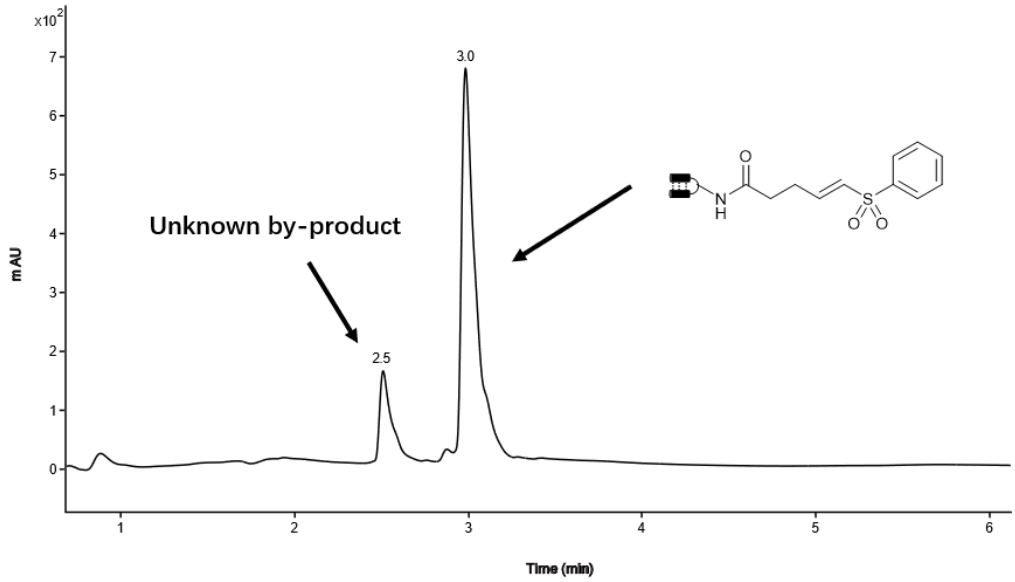


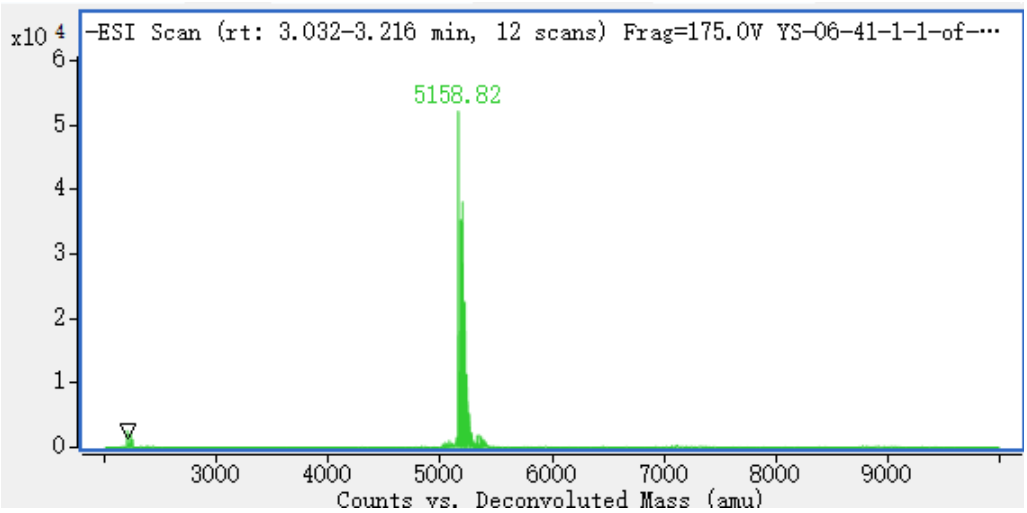


UPLC chromatogram and deconvoluted MS of **1ay**

Conversion: 82%

Calculated Mass: 5159 Da; Observed Mass: 5159 Da

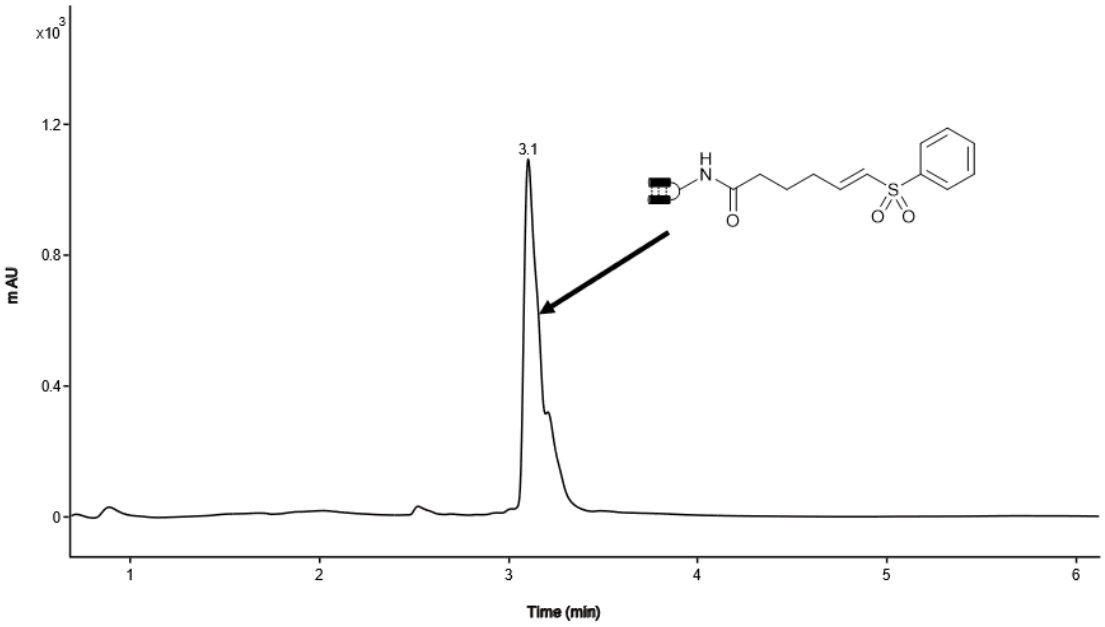


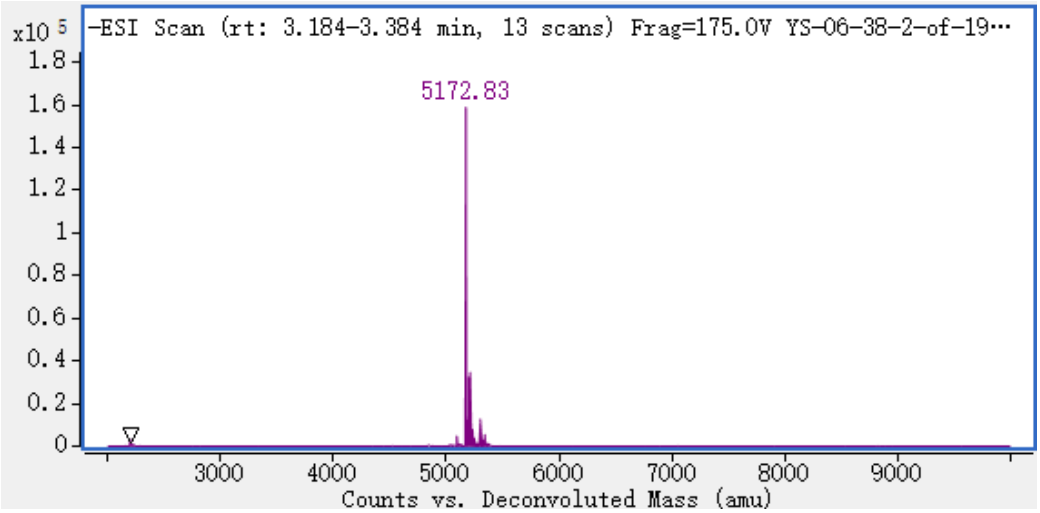


UPLC chromatogram and deconvoluted MS of **1az**

Conversion: 90%

Calculated Mass: 5173 Da; Observed Mass: 5173 Da



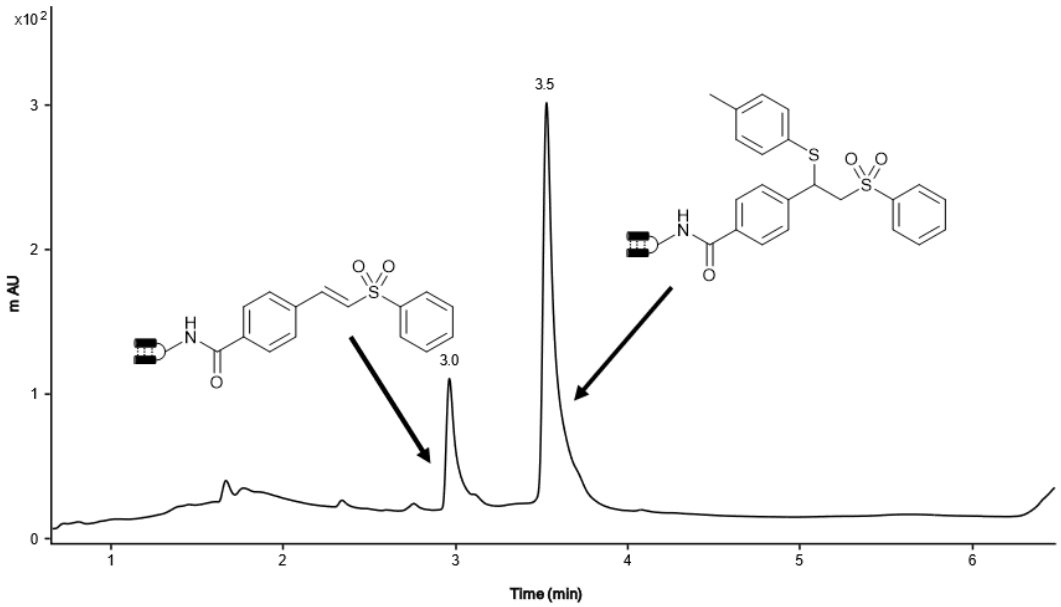


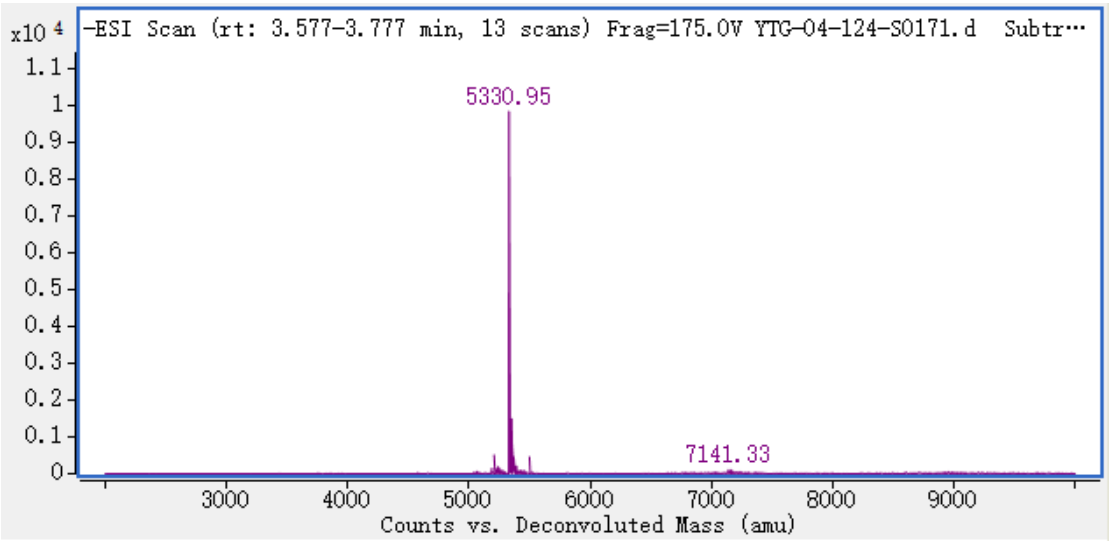
8.3. Substrate Scope of Thiols

UPLC chromatogram and deconvoluted MS of **aa35**

Conversion: 80%

Calculated Mass: 5331 Da; Observed Mass: 5331 Da

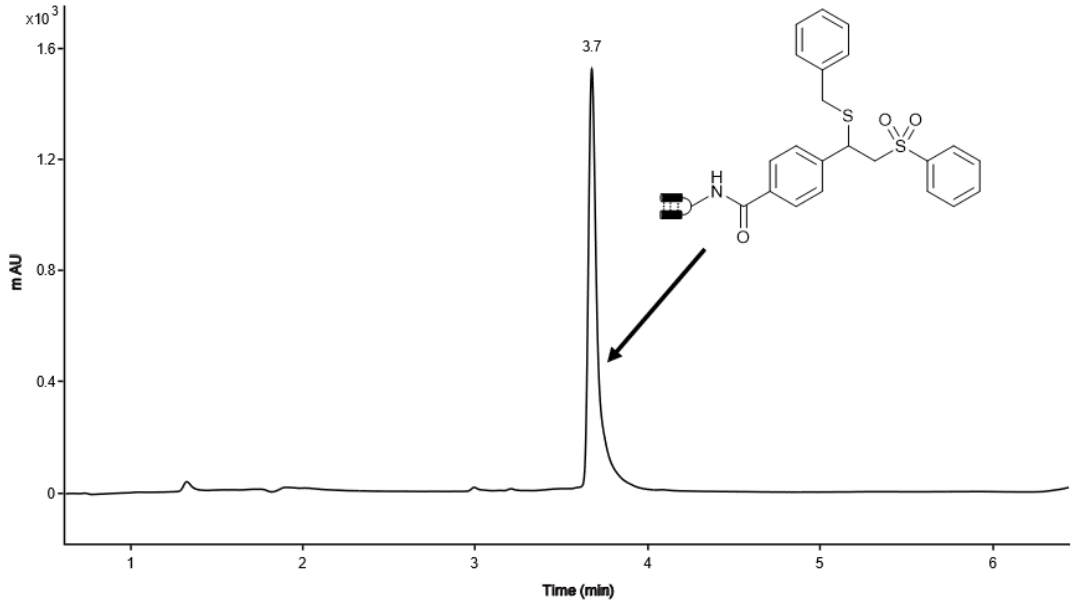


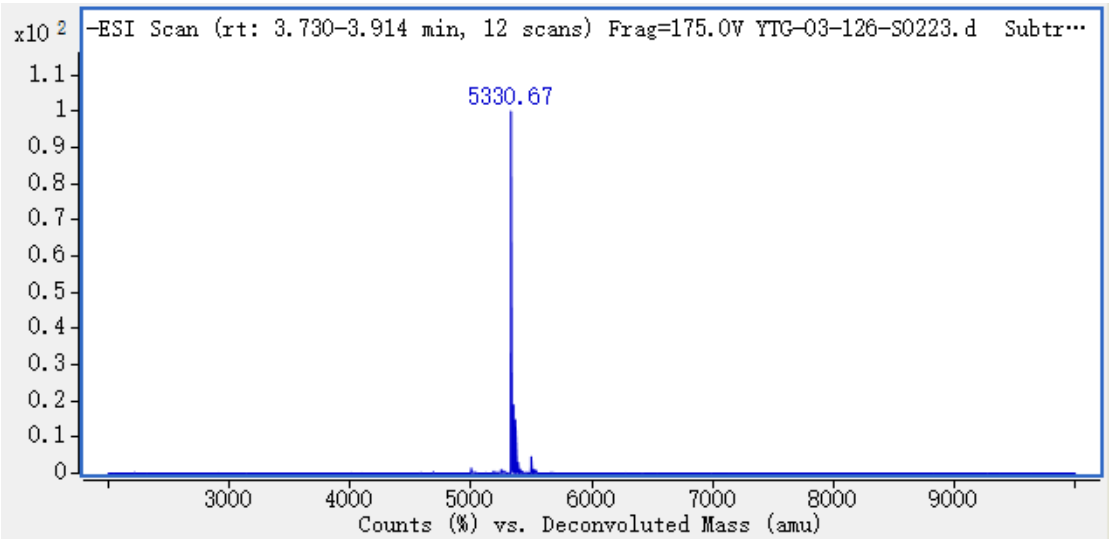


UPLC chromatogram and deconvoluted MS of **aa36**

Conversion: >90%

Calculated Mass: 5331 Da; Observed Mass: 5331 Da

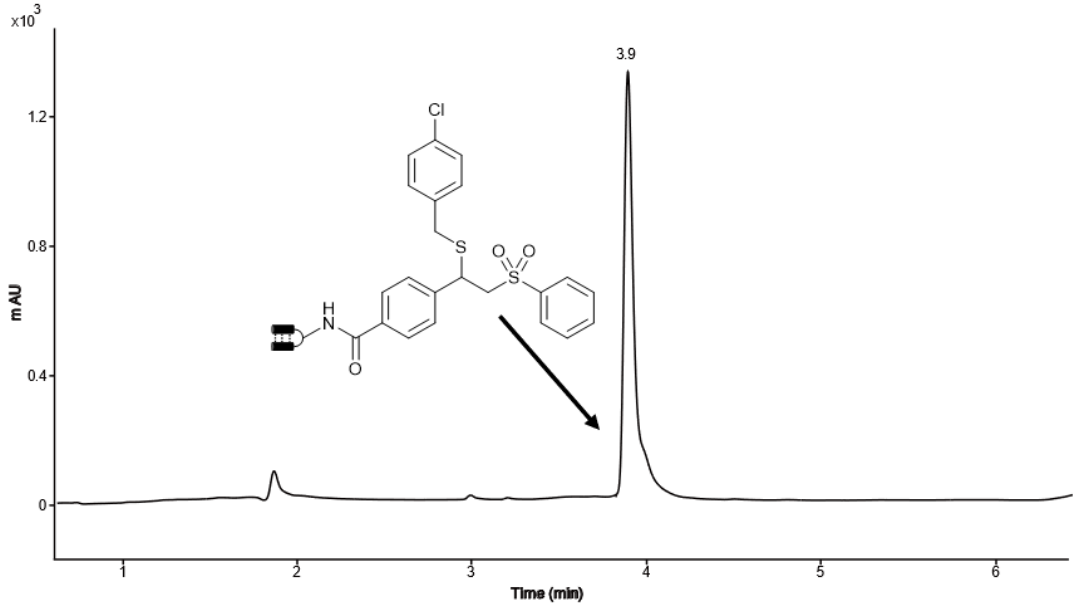


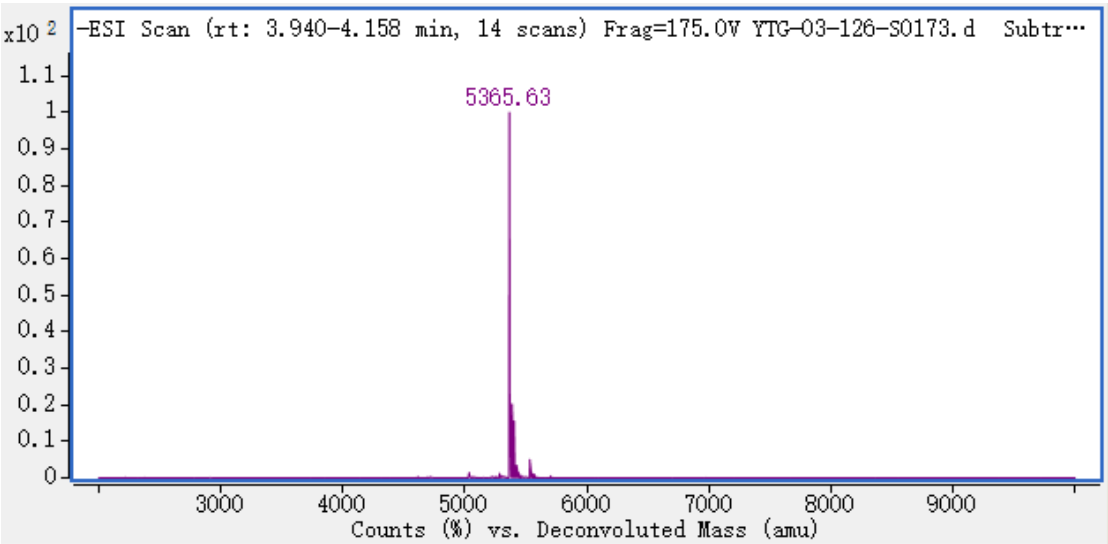


UPLC chromatogram and deconvoluted MS of **aa37**

Conversion: >90%

Calculated Mass: 5366 Da; Observed Mass: 5366 Da

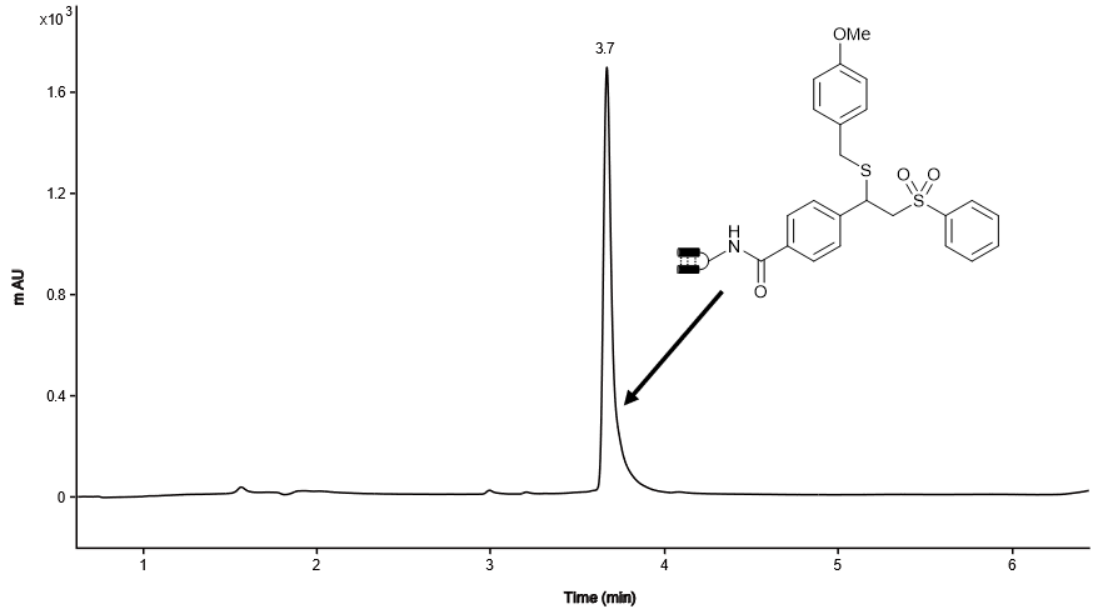


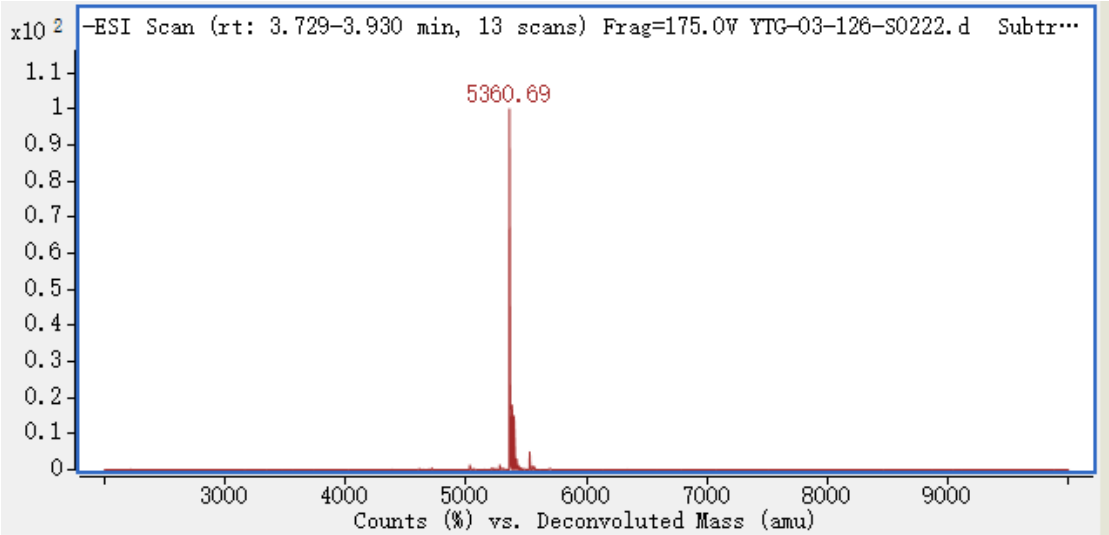


UPLC chromatogram and deconvoluted MS of **aa38**

Conversion: >90%

Calculated Mass: 5361 Da; Observed Mass: 5361 Da

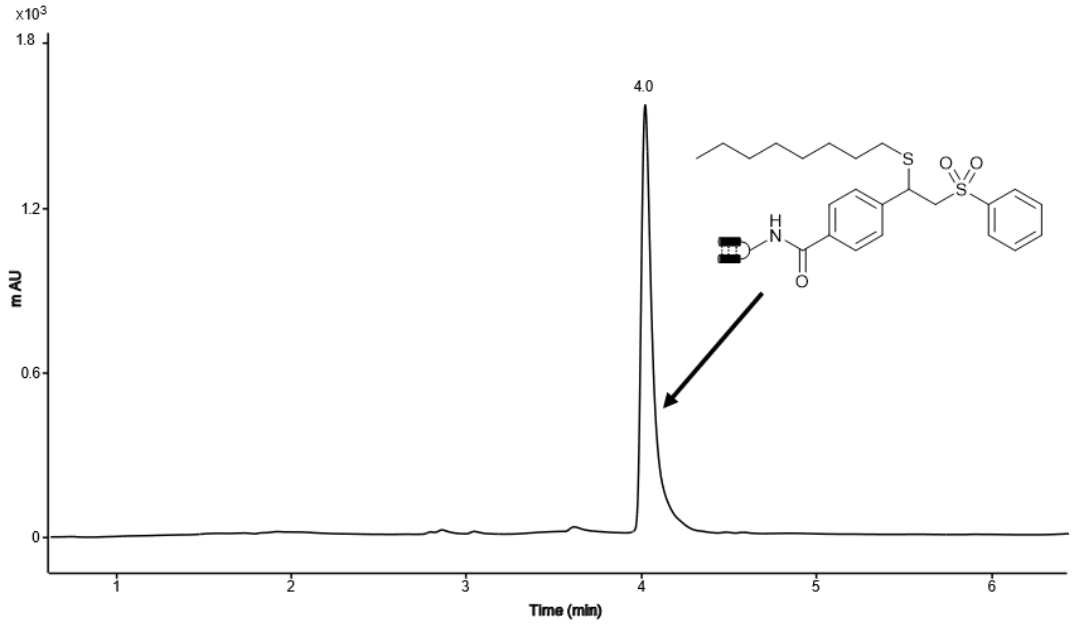


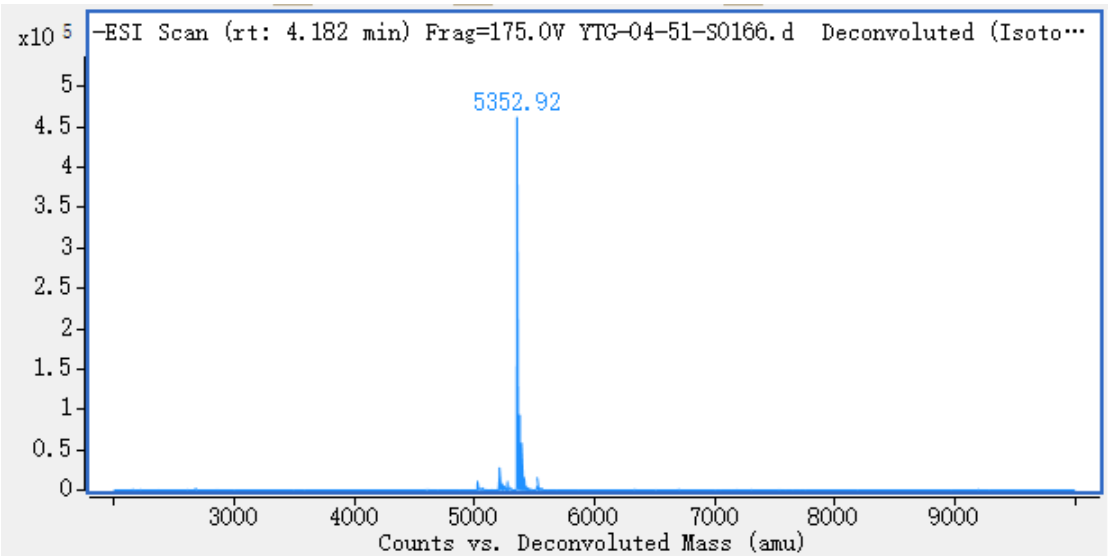


UPLC chromatogram and deconvoluted MS of **aa39**

Conversion: >90%

Calculated Mass: 5353 Da; Observed Mass: 5353 Da

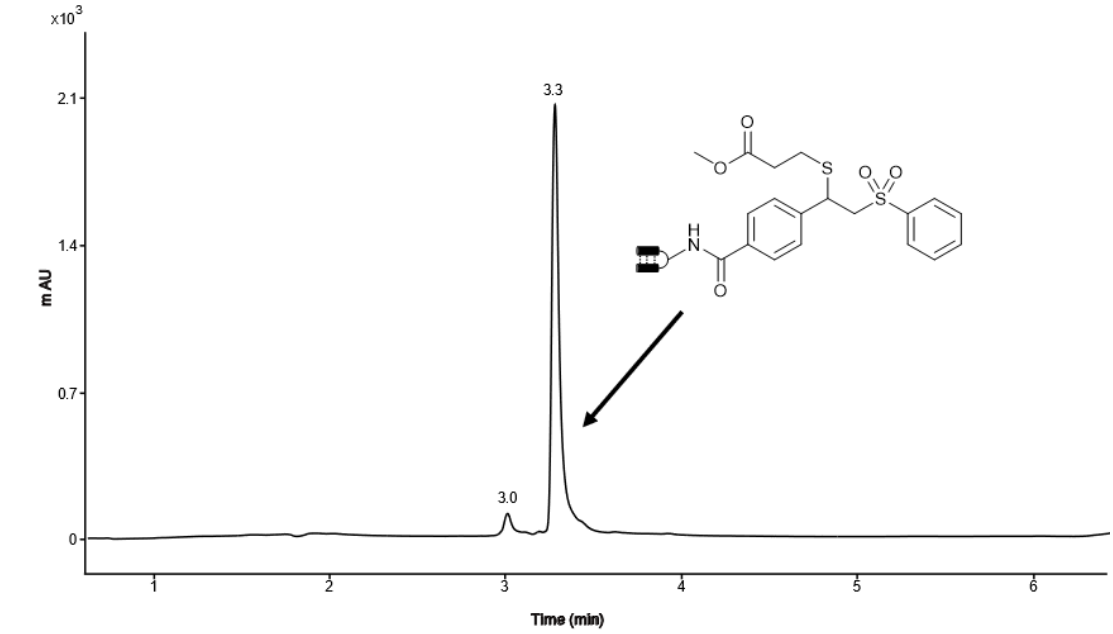


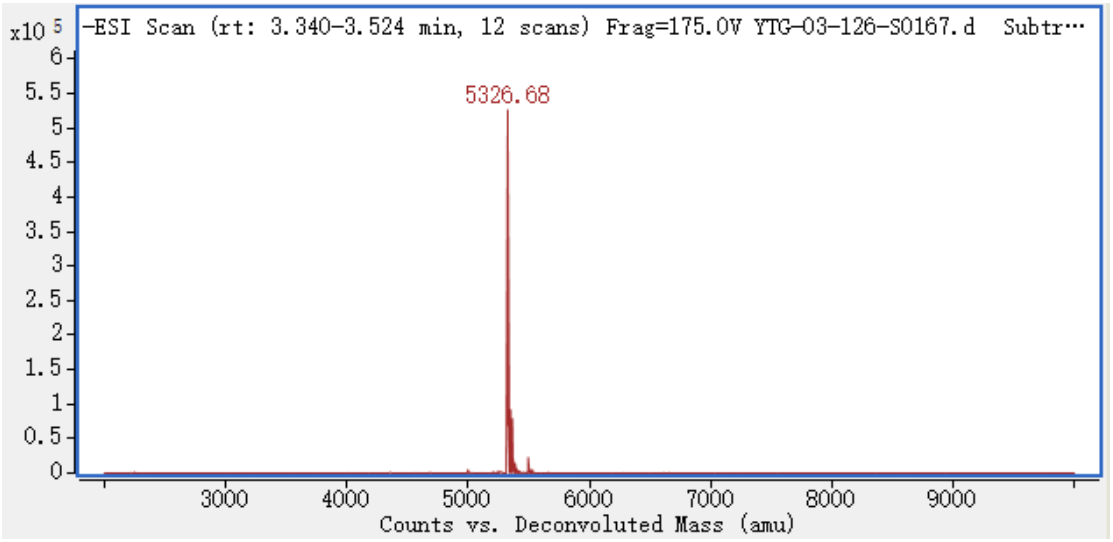


UPLC chromatogram and deconvoluted MS of **aa40**

Conversion: >90%

Calculated Mass: 5327 Da; Observed Mass: 5327 Da

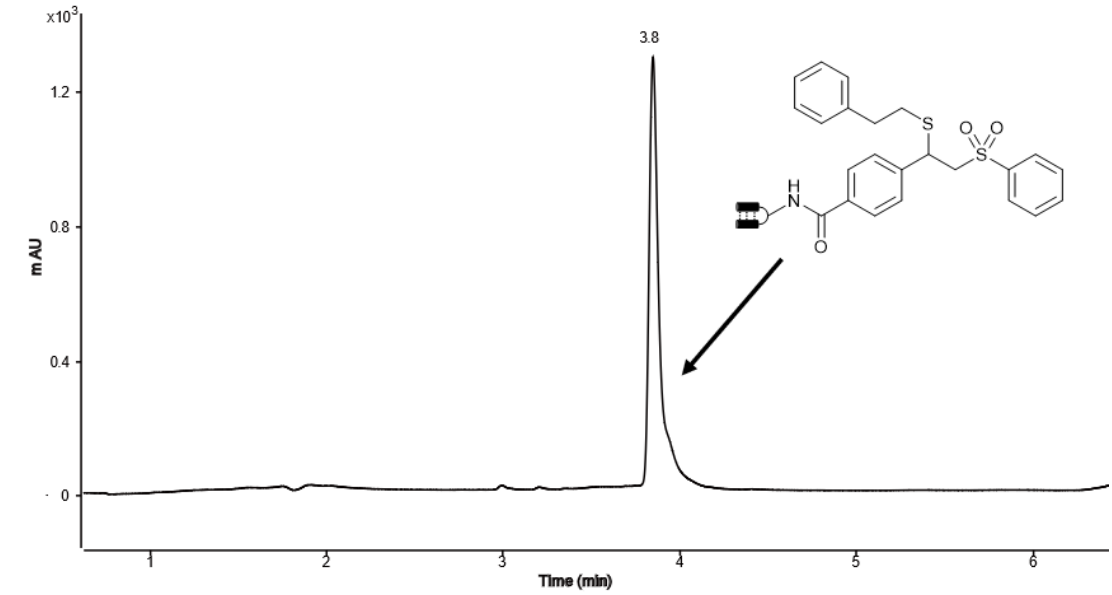


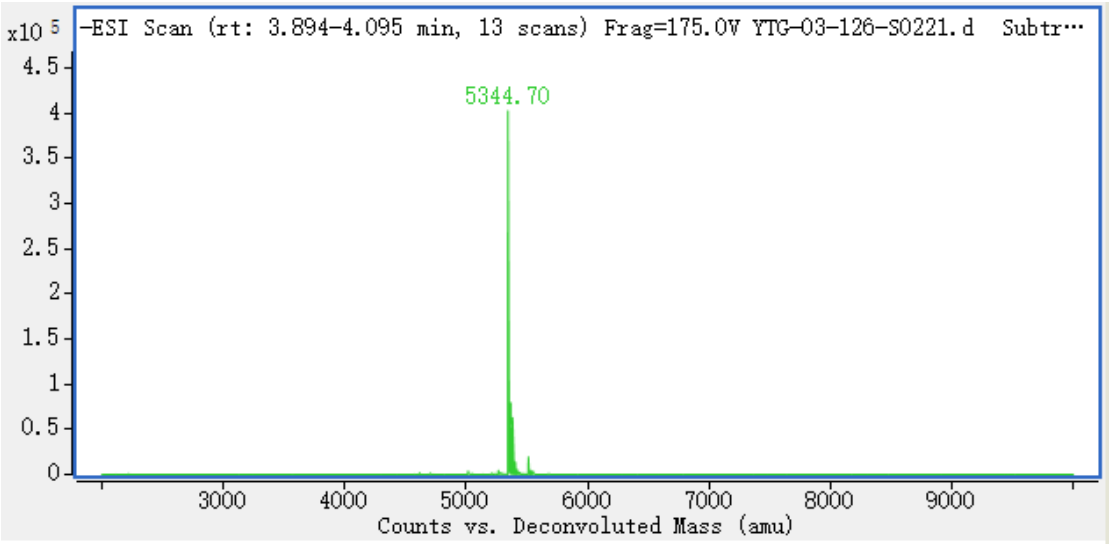


UPLC chromatogram and deconvoluted MS of **aa41**

Conversion: >90%

Calculated Mass: 5345 Da; Observed Mass: 5345 Da

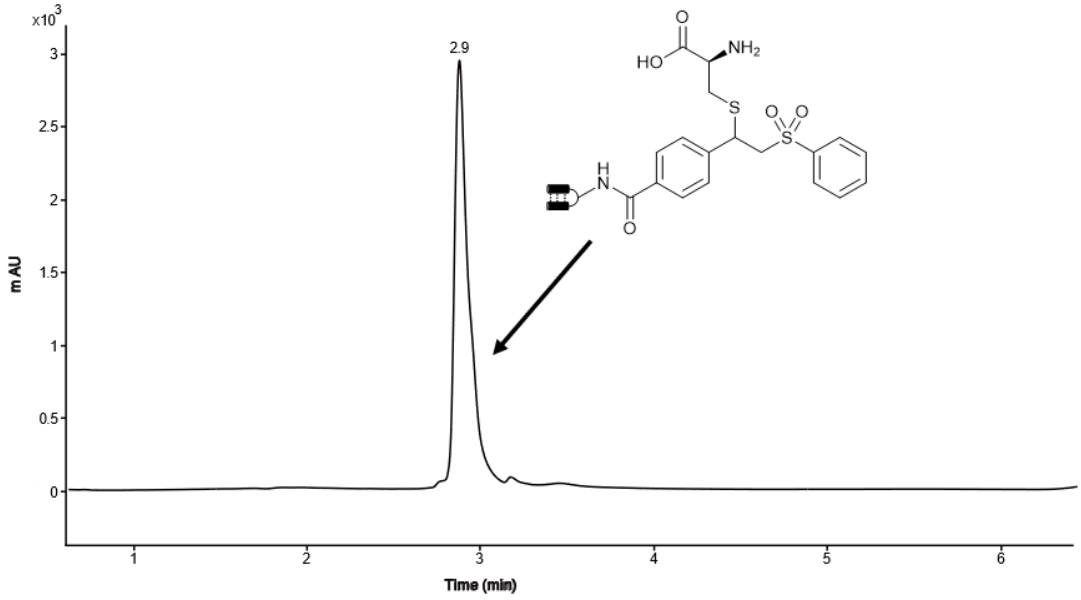


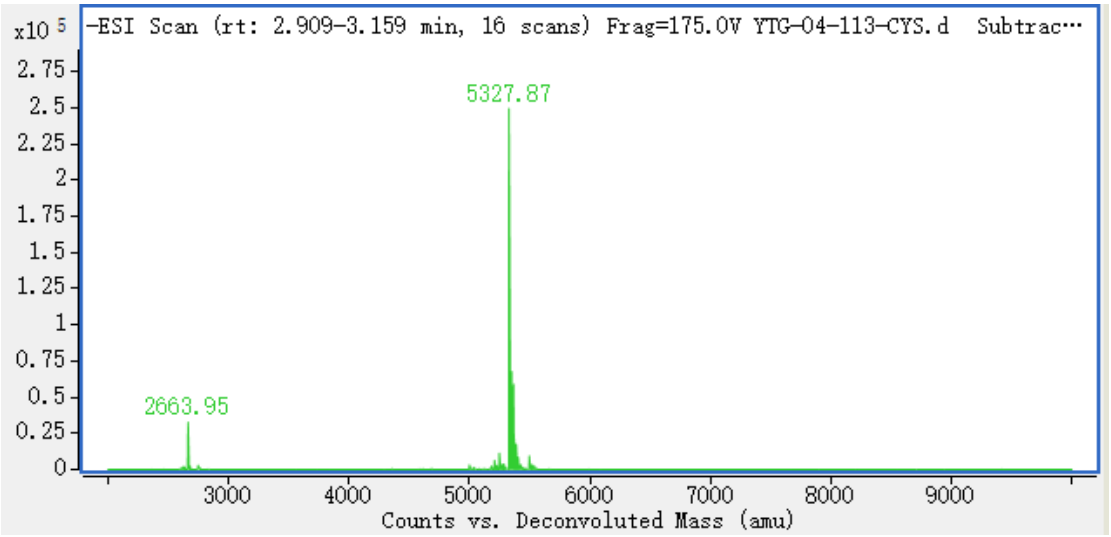


UPLC chromatogram and deconvoluted MS of **aa42**

Conversion: >90%

Calculated Mass: 5328 Da; Observed Mass: 5328 Da

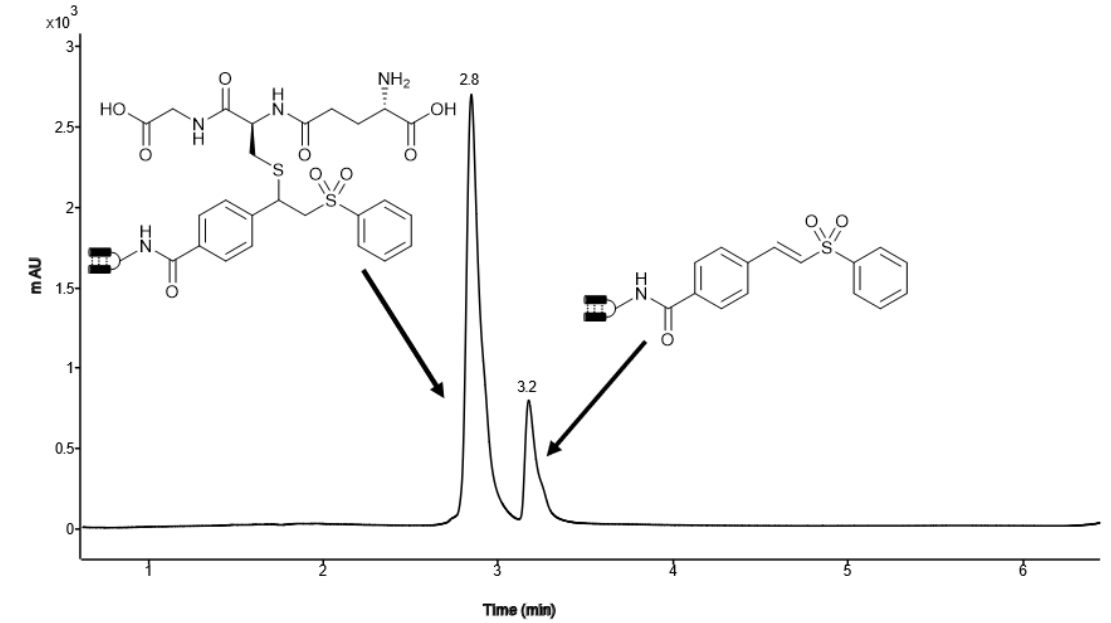


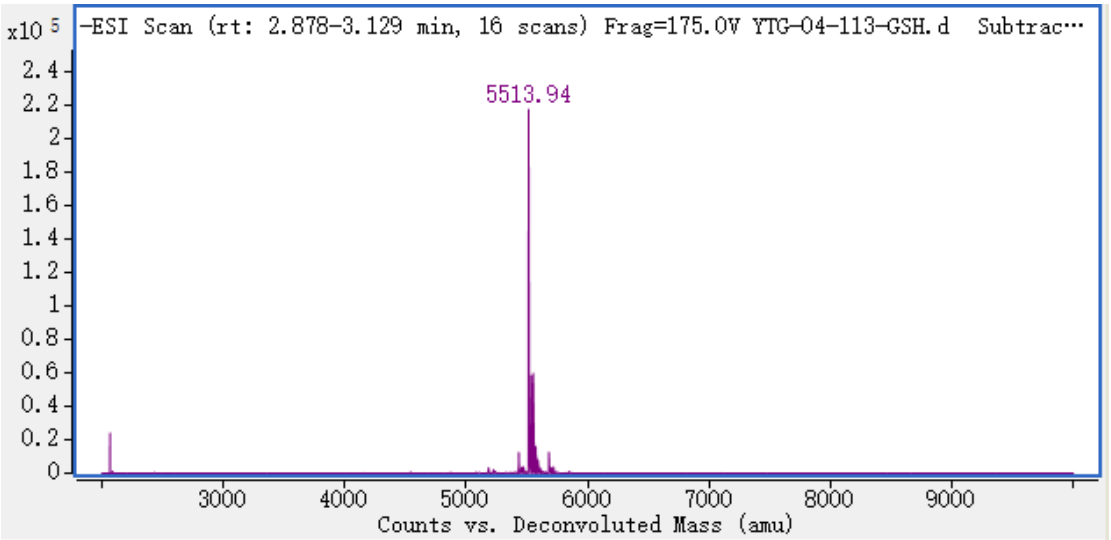


UPLC chromatogram and deconvoluted MS of **aa43**

Conversion: 80%

Calculated Mass: 5514 Da; Observed Mass: 5514 Da





9. References

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