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Supplementary Information

DNA-compatible synthesis of enaminones via amination of allenic ketones

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1. Abbreviations

ACN: acetonitrile

DIPEA: *N*, *N*-diisopropylethylamine

DMA: *N*, *N*-dimethylacetamide

DMSO: dimethyl sulfoxide

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

HRMS: high-resolution mass spectrometry

MW: molecular weight

NMR: nuclear magnetic resonance

PAGE: polyacrylamide gel electrophoresis

TBE: tris-borate-EDTA

TEAA: triethylammonium acetate

TEA: trimethylamine

TLC: thin layer chromatography

UPLC-MS: ultra-performance liquid chromatography-mass spectrometry

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/TGACTCCC-3', MW = 4937), 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 allenic ketones[1,2] were purchased from several commercial suppliers including J&K Scientific, Bidepharm, Adamas, and Sigma-Aldrich, and were generally used from aliquots dissolved in DMA, EtOH, DMSO, ACN, 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.. Aqueous solutions, including NaCl (5 M), basic borate buffer (250 mM, sodium borate/boric acid, pH 9.4), and acetate buffer (3 M, sodium acetate/acetic acid, pH 5.2) were prepared in-house. Cestbon water was used in the reactions unless otherwise stated. All the gel images were captured by a Bio-Rad Chemidoc™ image system.

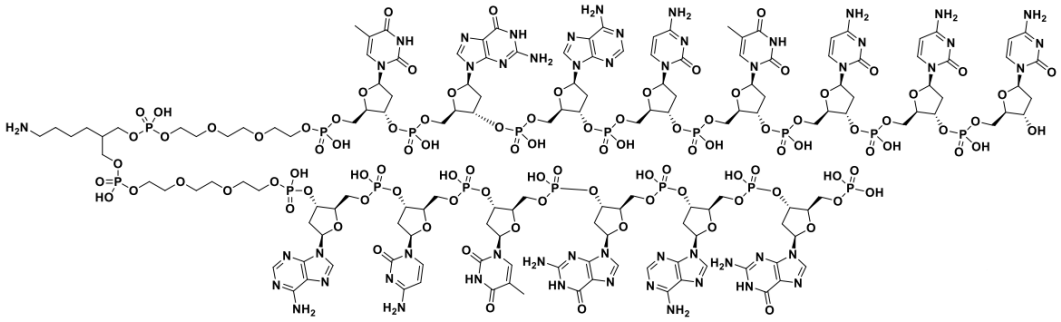


Figure S1. Structure of **HP**.

2.2 General methods for DNA analysis

On-DNA reaction analysis (UPLC-MS method). 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 the reaction, an aliquot of the reaction mixture was diluted with water to make the sample approximately 1 μM. 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 a flow rate of 0.3 mL/min. The effluent was detected by UV absorbance (260 nm) and analyzed on Agilent 6230 TOF in negative ion mode.

LCMS method of on-DNA synthesis of enaminones analysis:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
2	0.3	25
5.5	0.3	30
6	0.3	90
6.5	0.3	90
7	0.3	5
8	0.3	5

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

LCMS method of co-injection analysis:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
2	0.3	25
5.5	0.3	30
6	0.3	90
6.5	0.3	90
7	0.3	5
8	0.3	5

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

LCMS method of on-DNA synthesis of polyfunctionalized benzenes analysis:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	20
5.5	0.3	26
6	0.3	90
6.5	0.3	90
7	0.3	5
8	0.3	5

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
0	0.3	3
1	0.3	12
2.5	0.3	18
4	0.3	20
6	0.3	22
9	0.3	30
10	0.3	85
11	0.3	85
12	0.3	3

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

Conversion calculation. The conversion of on-DNA product was determined by UV absorbance (260 nm) peak area integration using the following equation: Conversion% = UV peak area (desired product)/UV peak area (total products), ignoring UV extinction coefficient difference for DNA species and assuming 100% DNA recovery. Any non-oligo material with UV absorbance (260 nm) was subtracted from the conversion calculation.[3]

Molecular mass analysis. 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

Ethanol precipitation. To an on-DNA reaction mixture was added 10% volume of NaCl solution (5 M) and 3 times volume of absolute cold ethanol. Alternatively, to a DNA ligation mixture was added 10% volume of acetate buffer (3 M, 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 Eppendorf 5425R 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 ddH_2O for subsequent experiments.

HPLC purification. Preparative reversed-phase high-performance 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). Fractions containing the product were combined and lyophilized overnight.

RP-HPLC method of purification:

Time (min)	Flow (mL/min)	%B
0	4	10
1	4	10
11	4	30
11.1	4	100
12	4	100
12.1	4	10
16	4	10

Solvent A: 100 mM TEAA in H_2O; Solvent B: 100 mM TEAA in 80% ACN

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 was incubated at 20 °C overnight before performing gel analysis. The crude product was purified by ethanol precipitation and used for the next step.

2.5 General procedure for polyacrylamide gel

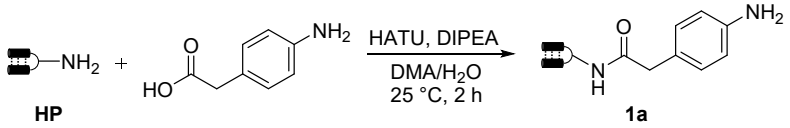
The ligation reaction was monitored by gel electrophoresis with 20% urea polyacrylamide gel in 1× TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) system referenced by a 20 bp DNA ladder (Takara, Japan). First, the DNA samples were denatured at 95 °C in a dry bath for 10 min and mixed with loading buffer. Then, 5 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™ Image System (Bio-Rad, CA, USA).

2.6 General information for off-DNA synthesis

Off-DNA reactions were monitored by TLC. Analytical TLCs were performed with 0.25 mm silica gel HSGF254. The TLC plates were visualized by ultraviolet light. Flash chromatography was conducted on silica gel 60 (SiO_2, 100–200 mesh). All the new compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS. The ^{1}H and ^{13}C NMR spectra were recorded on an Agilent 400 MHz spectrometer using the residual solvent resonance as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm). [D_6] DMSO (H δ = 2.50; C δ = 40.0) was used as solvents. Multiplicity abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet (dd = doublet of doublets), t = triplet, q = quartet, m = multiplet.

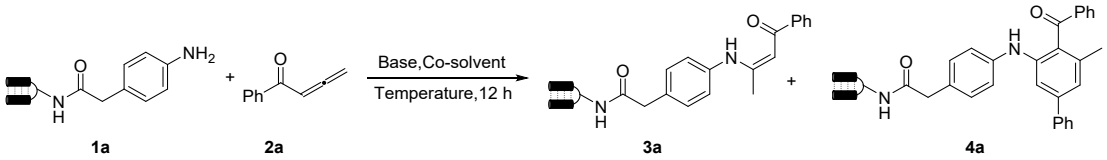
3. Experiments for on-DNA synthesis of enaminones

3.1 Preparation of DNA-Conjugated benzenamines by amide coupling

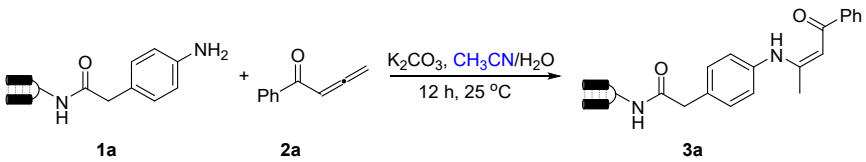


HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. 2-(4-aminophenyl)acetic acid (20 μL, 200 mM in DMA, 200 equiv.), HATU (10 μL, 400 mM in DMA, 200 equiv.), and DIPEA (10 μL, 400 mM in DMA, 200 equiv.) were mixed by vortex and allowed to pre-activate for 10 minutes at 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 purification by ethanol precipitation, the reaction was analyzed by UPLC-MS. The collected product was vacuum-dried overnight and redissolved in H_2O for subsequent experiments.

3.2 Optimization of the condition of enaminones synthesis using aryl amines



Reaction Condition: To the solution of DNA conjugate **1a** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv.) was added **base** (4 μL 200 mM in H_2O, 800 nmol, 4000 equiv.), **2a** (2 μL, 200 mM in corresponding solvent, 400 nmol, 2000 equiv.) and H_2O (4 μL) and Co-solvent (8 μL). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C, 40 °C or 60 °C for 12 h. The final products were obtained by ethanol precipitation and analyzed by UPLC-MS.



Standard Condition: To the solution of DNA conjugate **1a** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv.) was added K_2CO_3 (4 μL 200 mM in H_2O, 800 nmol,

4000 equiv.), **2a** (2 μL, 200 mM in ACN, 400 nmol, 2000 equiv.) and H_2O (4 μL) and ACN (8 μL). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 12 h. The final products were obtained by ethanol precipitation and analyzed by UPLC-MS. (Conversion: 87%). Deconvoluted molecular mass: calculated: 5214 Da; observed: 5214 Da.

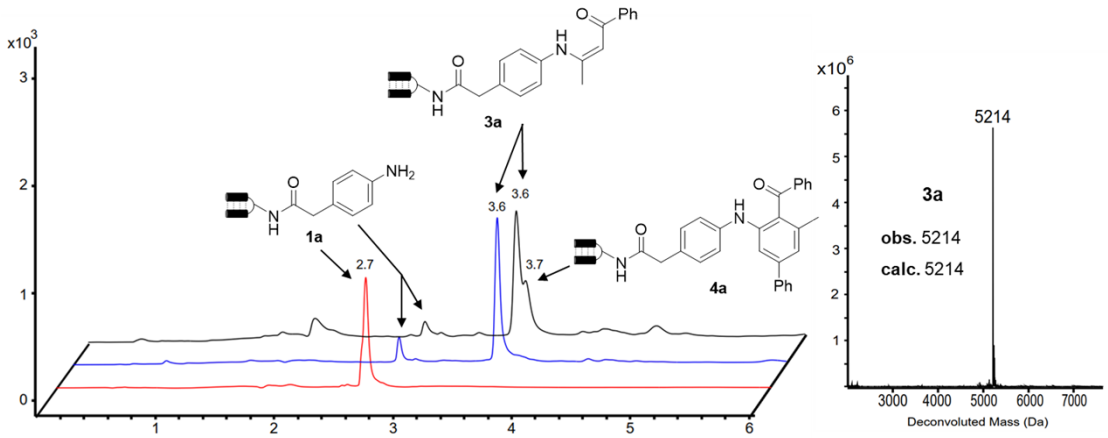
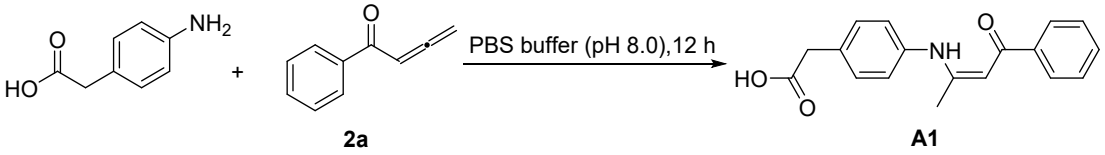


Figure S1. The UPLC chromatography and deconvoluted MS of the enaminone **3a**. (start materials **1a**, red curve; standard condition, blue curve; entry **4** in main text, black curve;).

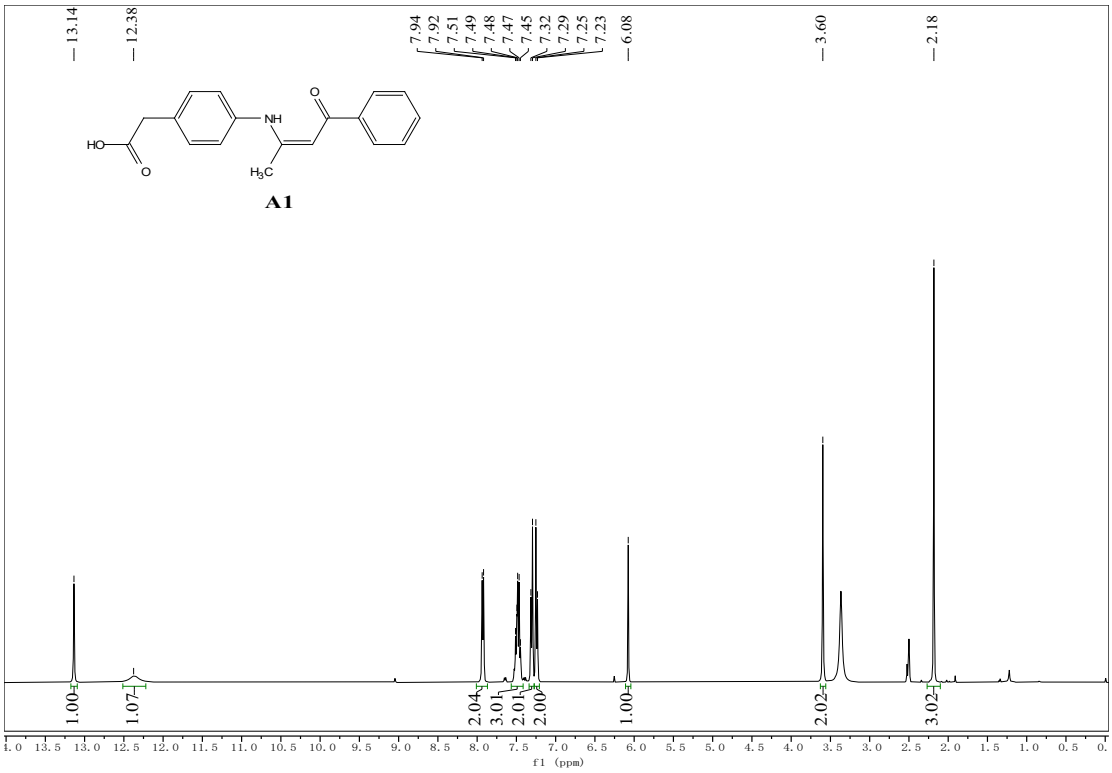
4. Structural confirmation

4.1 Off-DNA synthesis of authentic A1

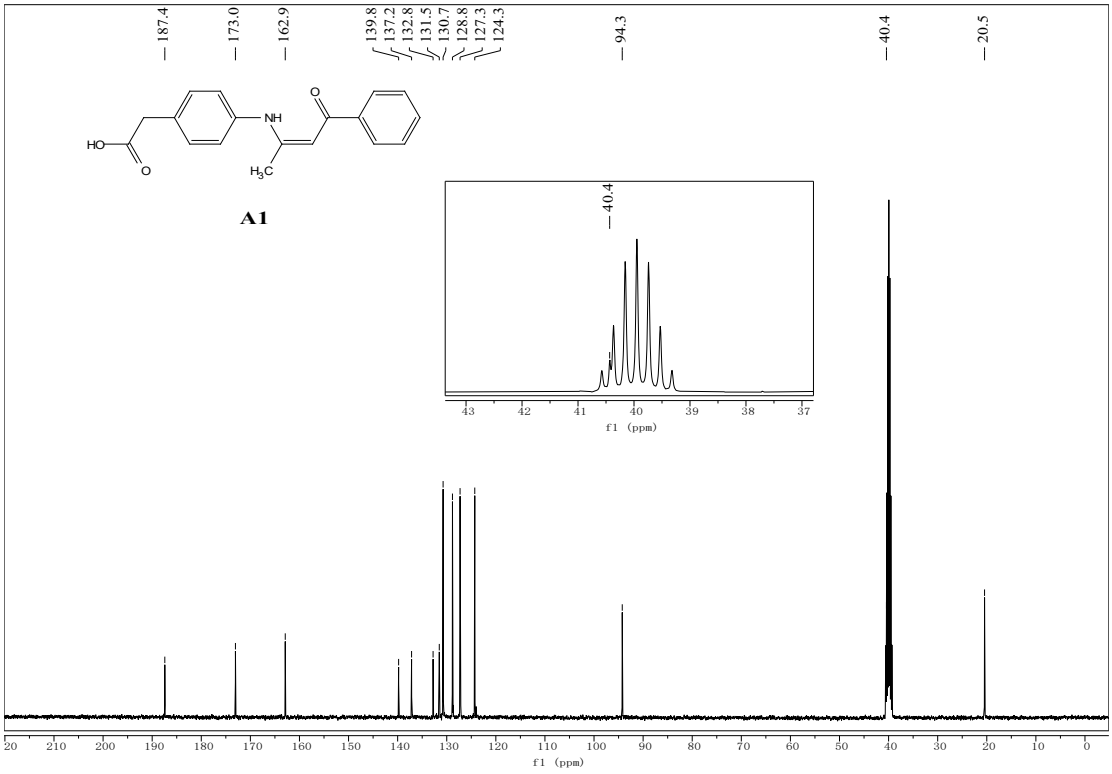


Authentic **A1** were prepared from the corresponding aldehydes according to slightly modified procedures reported in the literature.[4] a 15 mL vial was charged with allenic ketone **2a** (0.40 mmol, 1.0 equiv.), amine (0.80 mmol, 2.0 equiv.) and ACN: PBS buffer (pH 8.0) = 1:1 (6 mL) under ambient conditions. After the completion of the reaction (monitored by TLC, stirred for 5 h), the mixture was diluted with water (3 mL) and CH_2Cl_2 (3 mL)**,** The layers were separated and aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue so obtained was purified by flash column

chromatography with PE:EA (3:1) as an eluent to afford (Z)-2-(4-((4-oxo-4-phenylbut-2-en-2-yl)amino)phenyl)acetic acid (85 mg, 72%) as a brown solid. **^{1}H NMR (400 MHz, DMSO-d_6):** δ 13.14 (s, 1H), 12.38 (br.s, 1H), 7.93 (d, *J* = 7.1 Hz, 2H), 7.54 – 7.43 (m, 3H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 6.08 (s, 1H), 3.60 (s, 2H), 2.18 (s, 3H).**^{13}C NMR (101 MHz, DMSO-d_6)**: δ 187.4, 173.0, 162.9, 139.8, 137.2, 132.8, 131.5, 130.7, 128.8, 127.3, 124.3, 94.3, 40.4, 20.5. **HRMS (ESI, m/z):** calcd for $C_{18}H_{18}NO_3^+$ [M+H]$^+$: 296.1281; found: 296.1279.



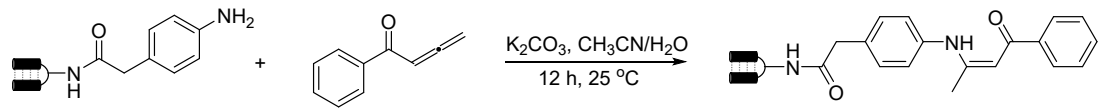
^{1}H NMR (400 MHz, DMSO-d_6) of A1



^{13}C NMR (101 MHz, DMSO-d_6) of A1

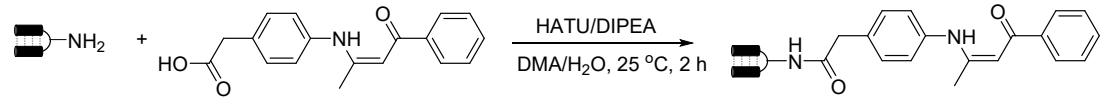
4.2 Co-Injection experiment

Route A



1a **2a** **3a**

Route B



HP **A1** **A2**

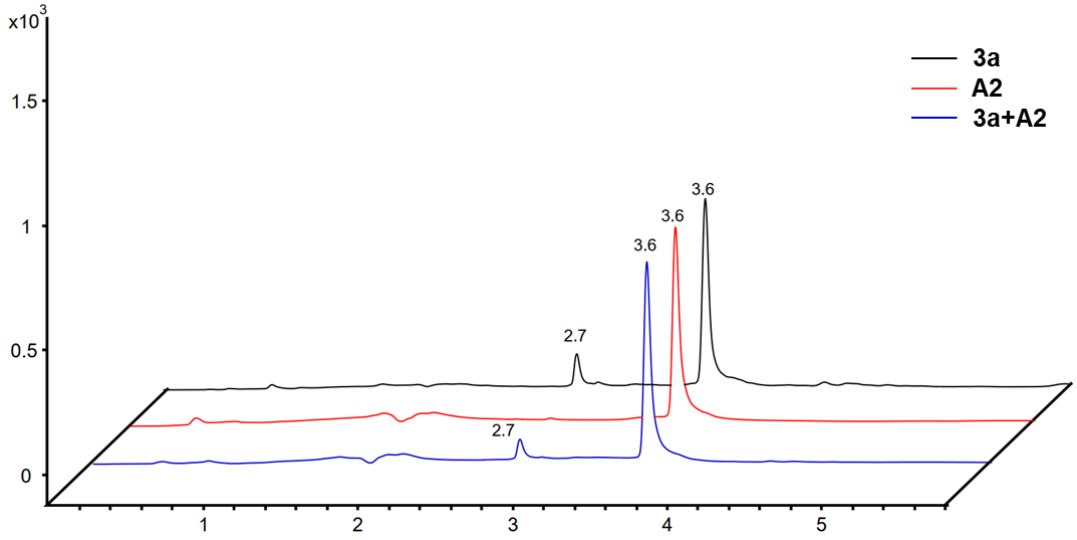
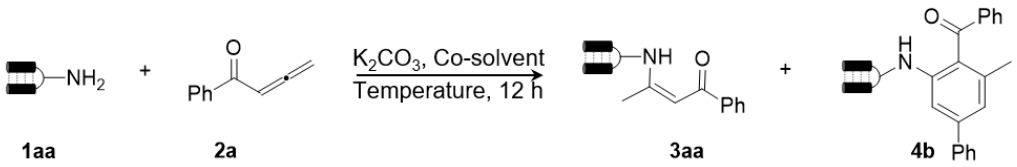


Figure S2. Co-injection experiment of **A2** and **3a** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (blue curve) had the same retention time as the other two peaks (**3a** from route A, black curve; **A2** from route B, red curve).

5. Optimization of the condition of enaminones synthesis using aliphatic amines



1aa **2a** **3aa** **4b**

Entry[a]	Co-solvent	Temperature(°C)	Yield[b](%)	
			3aa	4b
1	ACN/H_2O = 1:1	25	63%	25%
2	DMF/H_2O = 1:1	25	52%	0
3	DMA/H_2O = 1:1	25	70%	0
4	THF/H_2O = 1:1	25	10%	13%
5	EtOH/H_2O = 1:1	25	43%	20%
6	NMP/H_2O = 1:1	25	48%	24%
7	DMA/H_2O = 1:1	40	83%	5%
8	DMA/H_2O = 1:1	60	76%	8%
9	DMA/H_2O = 2:1	40	90%	0
10	DMA/H_2O = 3:1	40	81%	0

[a] Reaction conditions: HP **1aa** (2 μL, 100 μM in H_2O, 200 pmol, 1 equiv.), **2a** (2 μL, 200 mM in corresponding solvent, 400 nmol, 2000 equiv.), K_2CO_3 (4 μL 200

mM in H_2O, 800 nmol, 4000 equiv.), Total volume: 20 μL (water and organic solvent add in proportion). 12 h. [b] Conversions were determined by UPLC-MS.

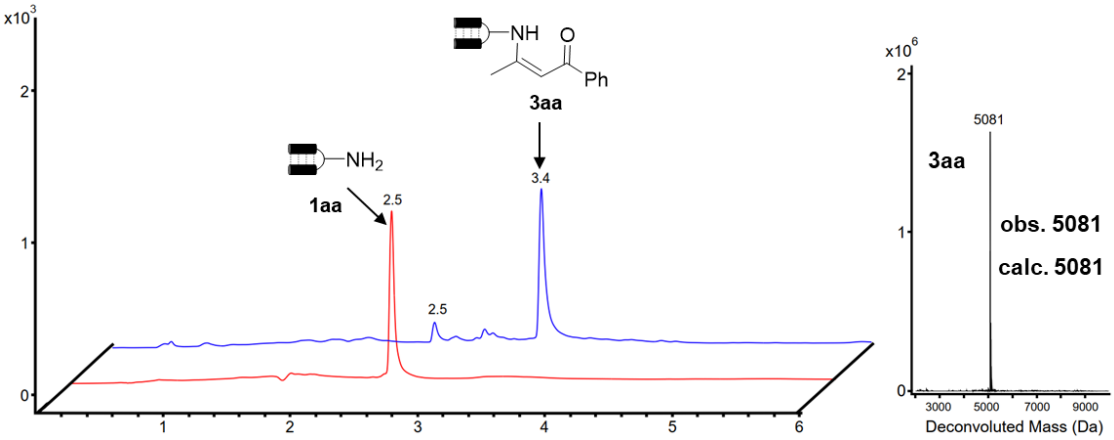


Figure S3. The UPLC chromatography and deconvoluted MS of the enaminone **3aa**. (**1aa**, red curve; standard condition (entry **9**), blue curve;).

6. Scale-up of the reaction

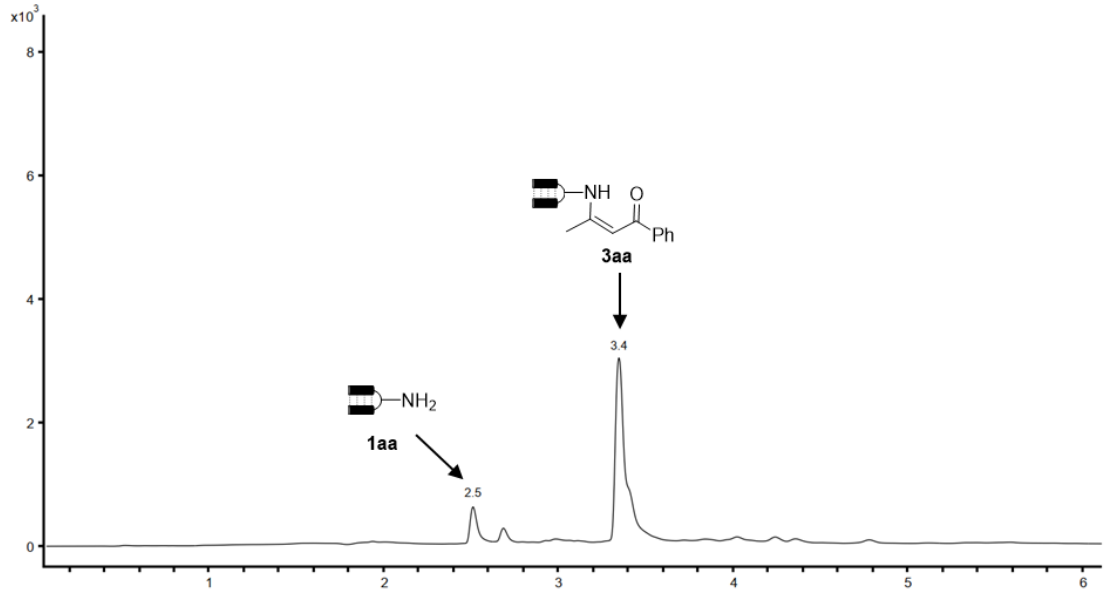


Figure S4. UPLC chromatogram of **3aa** 10 nmol scales.

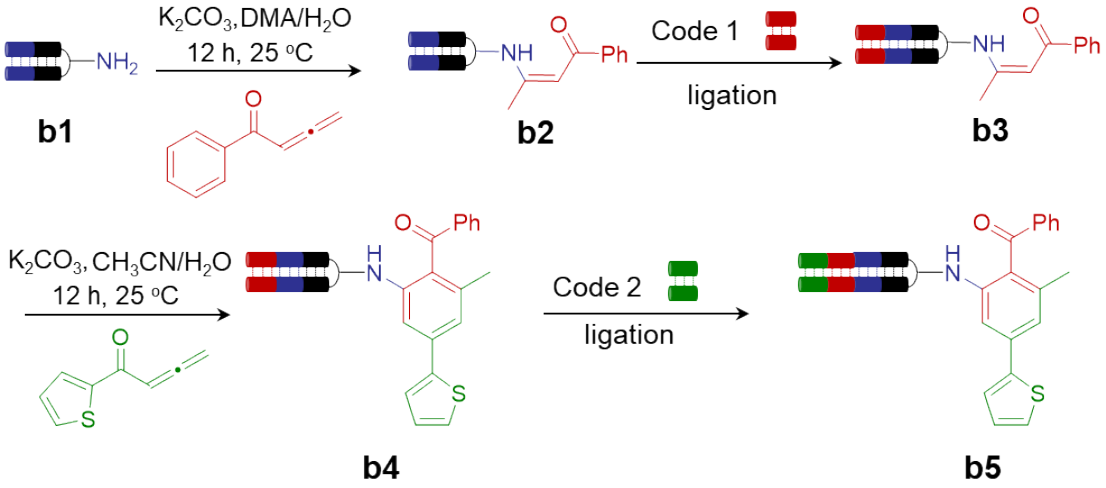
To the solution of **HP 1aa** (5 μL, 2 mM in H_2O, 10 nmol, 1 equiv.) was added K_2CO_3 (8 μL, 200 mM in H_2O, 1600 nmol, 160 equiv.), **2a** (4 μL, 200 mM in DMA, 800 nmol, 80 equiv.) and ACN (23 μL). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 12 h. The products were obtained by ethanol precipitation and analyzed by UPLC-MS. (Conversion: 82%). Deconvoluted molecular mass: calculated: 5081 Da; observed: 5081Da.

7. Procedure of on-DNA synthesis of polyfunctionalized benzenes from enaminone 3aa.

To the solution of DNA conjugate enaminone **3aa** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv.) was added K_2CO_3 (4 μL 200 mM in H_2O, 800 nmol, 4000 equiv.), corresponding allenic ketones (2 μL, 200 mM in ACN, 400 nmol, 2000 equiv.) and ACN (12 μL). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 12 h. The products were obtained by ethanol precipitation and repeated the above reaction procedure once. After purification by ethanol precipitation, the reaction was analyzed by UPLC-MS.

8. DNA barcode integrity test

8.1 Enzymatic ligation



Scheme S5. Protocol of three-dimensional DEL synthesis.

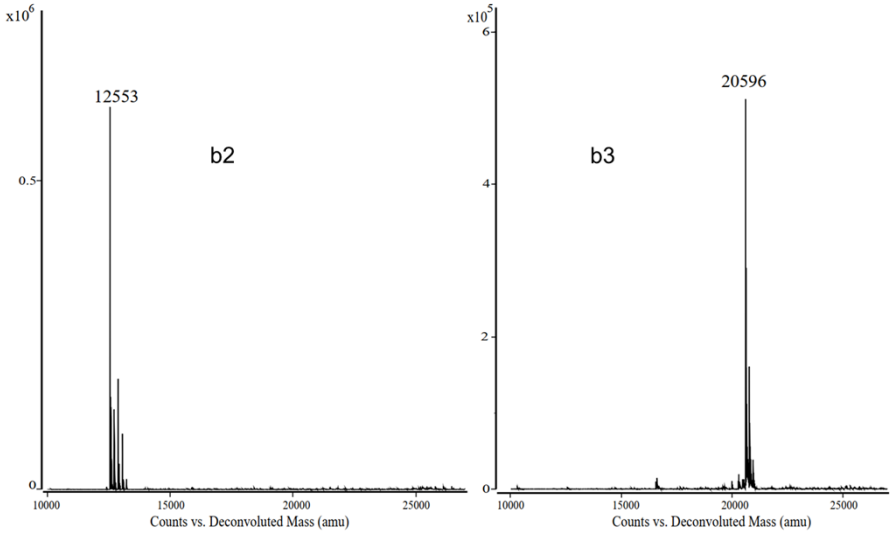
Step 1: The preparation of the enaminone **b2** from headpiece-primer (HP-P) **b1** (10 nmol) was carried out under standard DMA/H_2O condition and purified by HPLC (80% conversion. deconvoluted molecular mass: calculated: 12553 Da; observed: 12553 Da).

Step 2: To the solution of DNA conjugate **b2** (31.8 μL in water, 2 nmol) was added code 1 (2.2 μL, 1mM in H_2O, 1.1 equiv) and 10×ligation buffer (4 μL), mixed by vortex, then T4 DNA ligase (2 μL, 350 units/μL) was added and mixed gently. The reaction mixture was incubated at 20 °C for 16 h. the reaction

conversion was monitored by UPLC-MS analysis (The system cannot undergo denaturation for the instability of enaminones at high temperatures). Until the conversion was completed, the ligation product **b3** was isolated by ethanol precipitation. (Deconvoluted molecular mass: calculated: 20593 Da; observed: 20596 Da).

Step 3: To the solution of ligation product **b3** (200 pmol in 2 μL H_2O, 1 equiv) was added K_2CO_3 (4 μL 200 mM in H_2O, 800 nmol, 4000 equiv.), **2k** (2 μL, 200 mM in ACN, 400 nmol, 2000 equiv.) and ACN (12 μL). The mixture was vortexed, centrifuged, and placed at 25 °C for 12 h. The annulation product **b4** was obtained by ethanol precipitation as described above (>90% conversion. Deconvoluted molecular mass: calculated: 20725 Da; observed: 20728 Da).

Step 4: After the annulation reaction, product **b4** (10 μL, 50 pmol), code 2 (0.5 μL, 65 pmol, 1.3 equiv) and 4×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 **b5** was isolated by ethanol precipitation as described above. Deconvoluted molecular mass: calculated: 28746 Da; observed: 28751 Da.



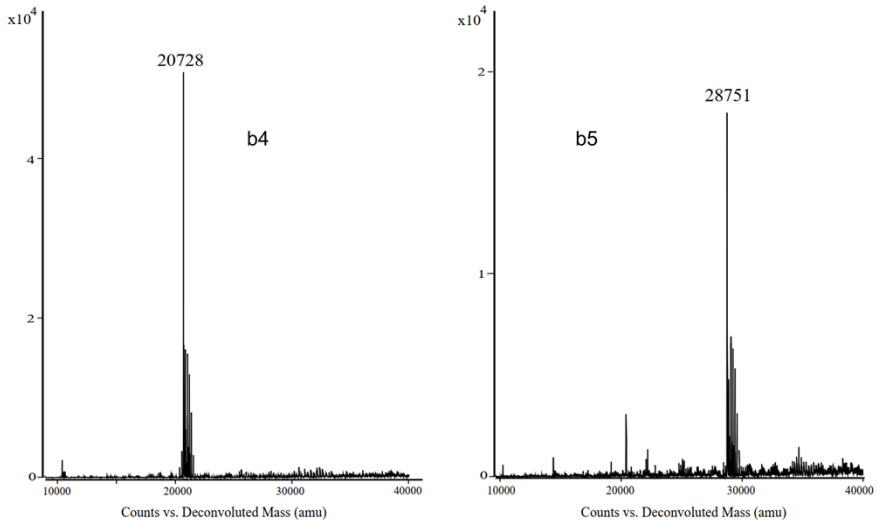
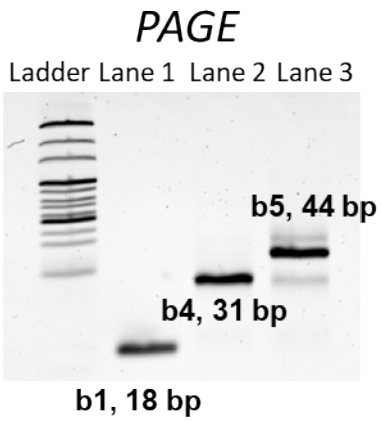


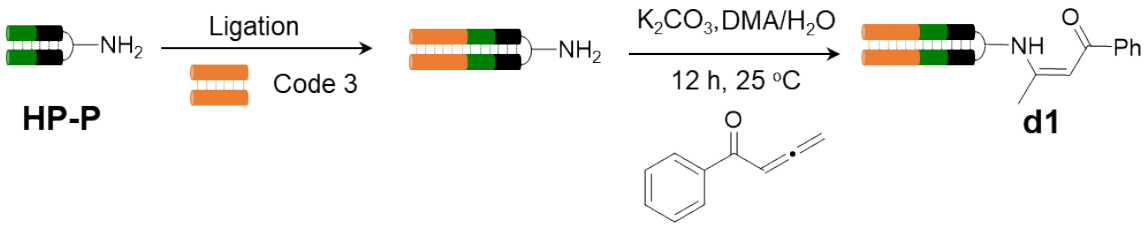
Figure S6. Deconvoluted MS of **b2**, **b3**, **b4** and **b5**



Scheme S7. 20% denatured PAGE analysis of **b4** and **b5**. Lane 1, DNA ladder; Lane 2, **HP-P** starting material; Lane 2, annulation product **b4**; lane 3, ligation product **b5**.

8.2 Sanger sequencing

We carried out enzymatic ligations of a 60-mer oligo DNA code with control DNA headpiece-primer (**HP-P**) and subsequently obtained DNA conjugate enaminone **d1** through standard conditions.



Scheme S8. Preparation of sequencing substrate **d1**

All DNA samples were amplificated by two rounds of PCR steps to reach

181 bp length before sequencing. Following the manufacturer's guidelines, the DNA ligation sample was mixed with 2 μL of 10 μM primer PCR1-F and PCR1-R, 10 μL 2 × Es Taq MasterMix (Dye) to reach a 20 μL system finally. PCR cycles are as follows: 94 °C for 2 min, and then 30 cycles of 94 °C for 30 s (denaturing), 56 °C for 30 s (annealing), 72 °C for 30 s (extension). The products were analyzed with 2% agarose stained with ethidium bromide. After PCR1, PCR2 was executed as the same step by using PCR1 sample. DNA sequencing was sent to Sanger sequencing (Tsingke Biotechnology Co., Ltd.) by using primer PCR2-F.

Code 3 (60 nt):

5'-AAC ACA GGC TTT GCT CGT ACA TAA AGC TCT TGC GTG GTC GTC TGA TGG CGC GAG GGA GGC-3'

5'-CTC CCT CGC GCC ATC AGA CGA CCA CGC AAG AGC TTT ATG TAC GAG CAA AGC CTG TGT TCA-3'

PCR1-F: 5'-GTT GGA AGC CAG CCC TCA GTG ACA GAG AAT ATG TGT AGA GGC TCG GGT GCT CTG-3'

PCR1-R: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GCC TCC CTC GCG CCA TCA GAC-3'

PCR2-F: 5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCG TCT CGT GGG CTC GGA GAT G-3'

PCR2-R: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GCC TCC CTC GCG CCA TCA GAC-3'

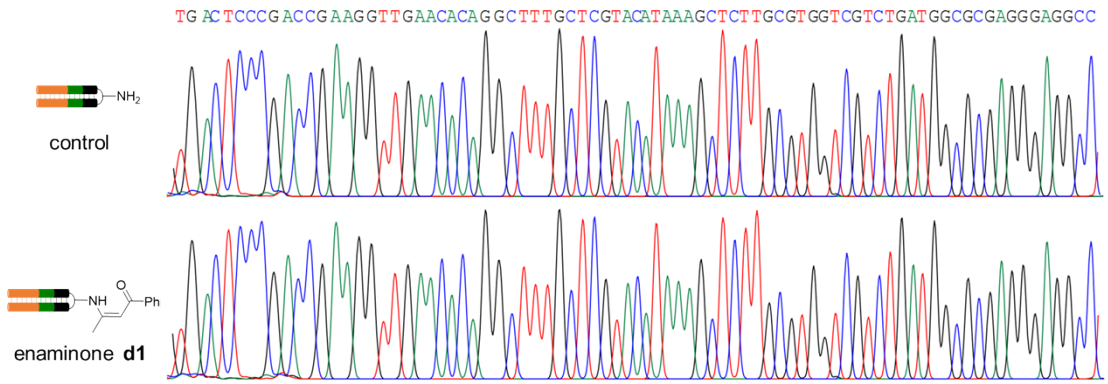
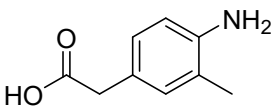
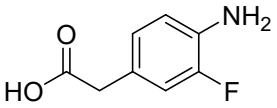
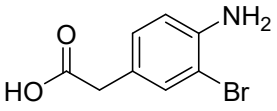
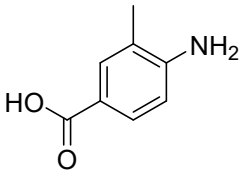
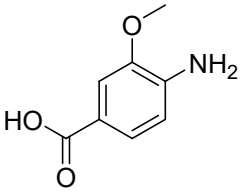
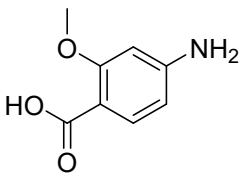


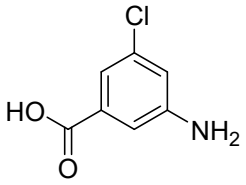
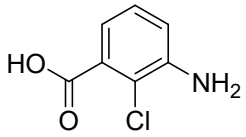
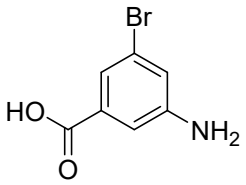
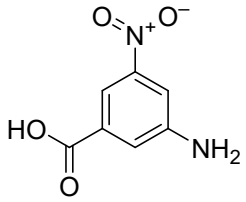
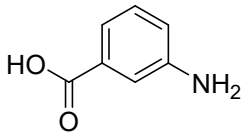
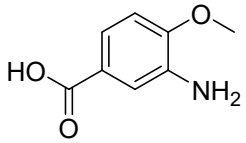
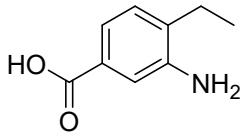
Figure S9. Sanger sequencing results of samples before and after chemical reactions

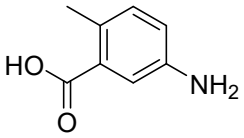
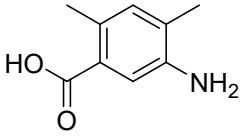
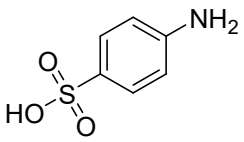
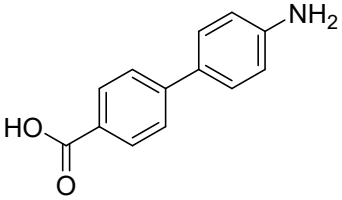
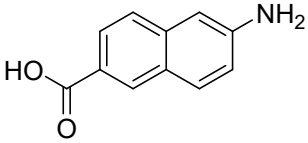
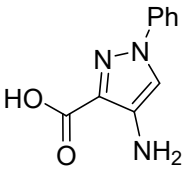
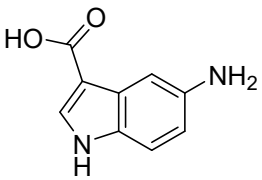
Sanger sequencing validated that no base pair damage was detected after the reaction was performed.

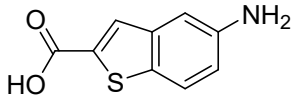
9. UPLC chromatogram and deconvoluted MS

9.1 Substrate scope of DNA-conjugated aryl amines 1b-1w

Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
1b		**3b**	5228	5228	85%
1c		**3c**	5232	5232	71%
1d		**3d**	5292	5293	70%
1e		**3e**	5214	5214	85%
1f		**3f**	5230	5230	76%
1g		**3g**	5230	5230	88%

1h		**3h**	5234	5234	74%
1i		**3i**	5234	5234	42%
1j		**3j**	5278	5279	81%
1k		**3k**	5245	5245	36%
1l		**3l**	5200	5200	>90%
1m		**3m**	5230	5230	>90%
1n		**3n**	5228	5228	>90%

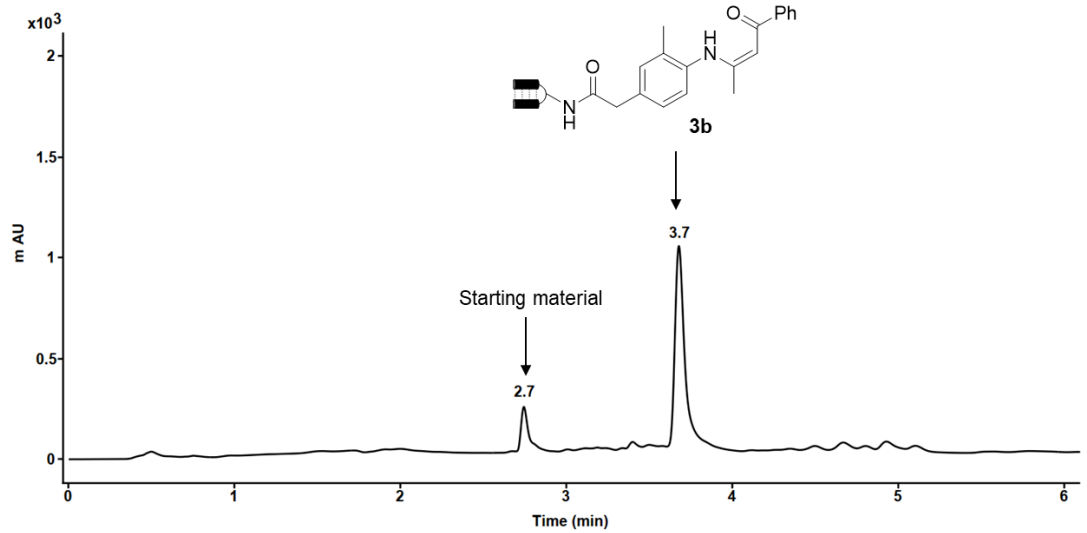
1o		**3o**	5214	5214	>90%
1p		**3p**	5228	5228	>90%
1q		**3q**	5236	5236	52%
1r		**3r**	5276	5276	>90%
1s		**3s**	5250	5250	86%
1t		**3t**	5266	5266	87%
1u		**3u**	5239	5239	87%

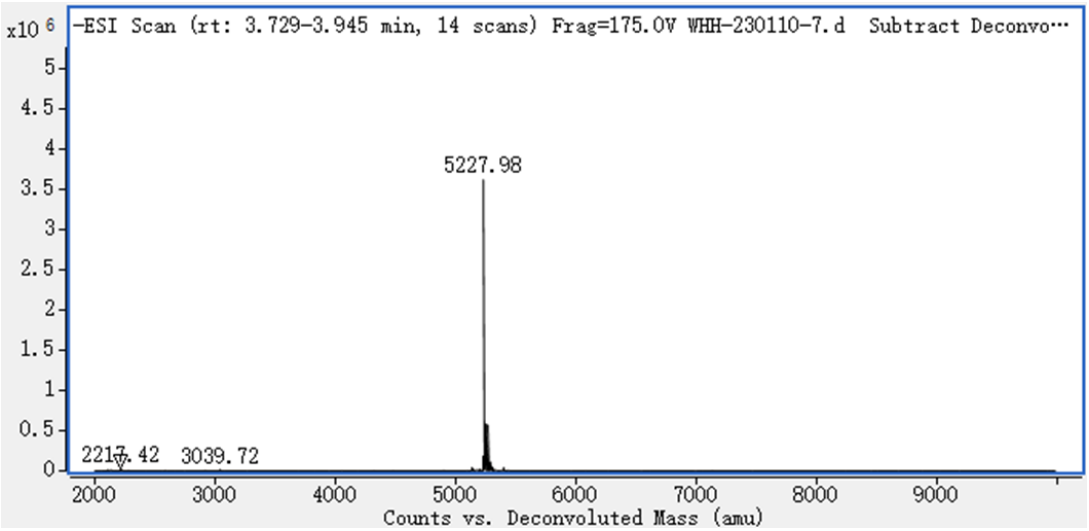
1v		**3v**	5256	5256	>90%

UPLC chromatogram and deconvoluted MS of **3b**

Conversion: 85%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

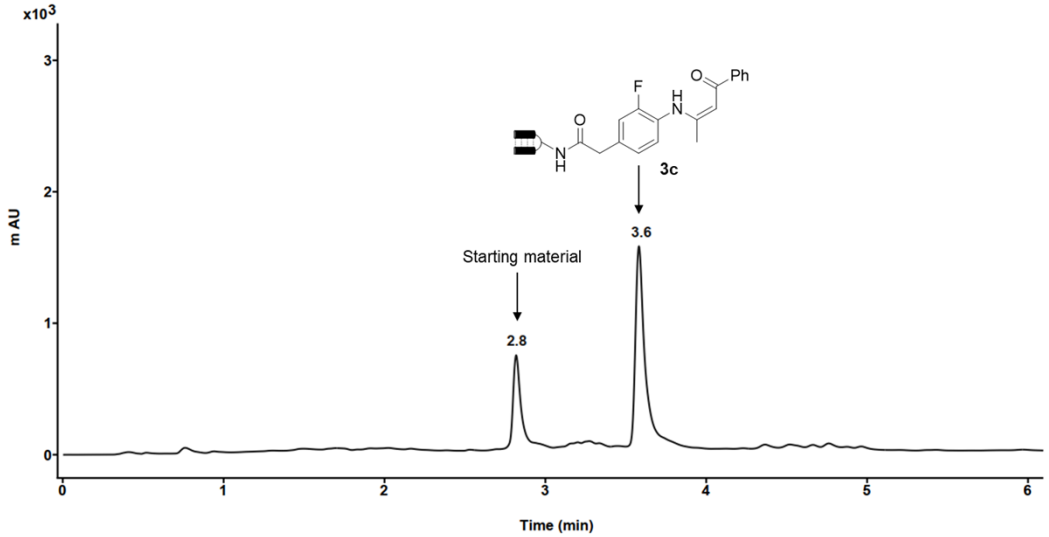


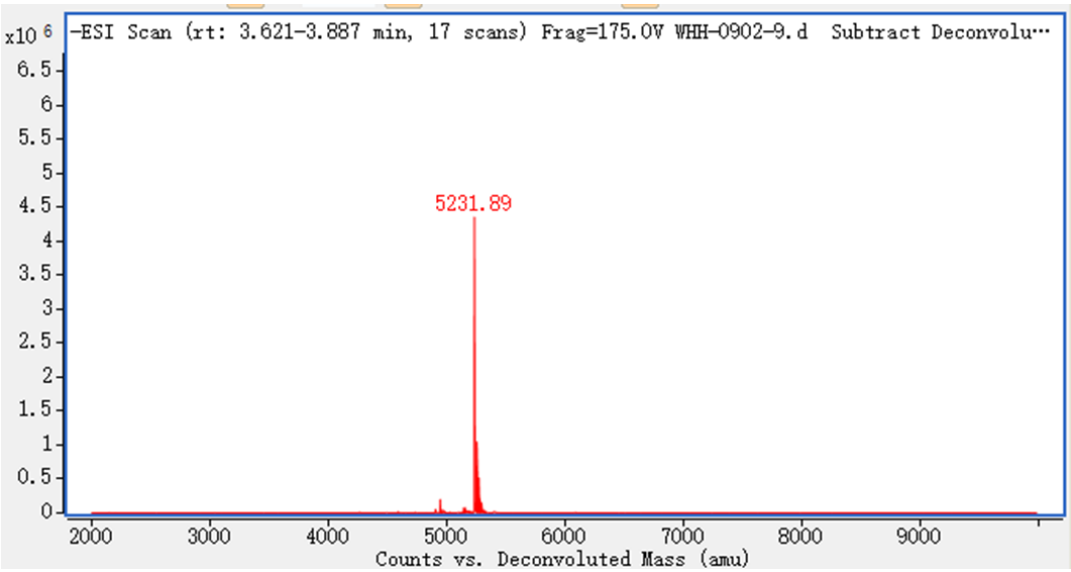


UPLC chromatogram and deconvoluted MS of **3c**

Conversion: 71%

Calculated Mass: 5232 Da; Observed Mass: 5232 Da

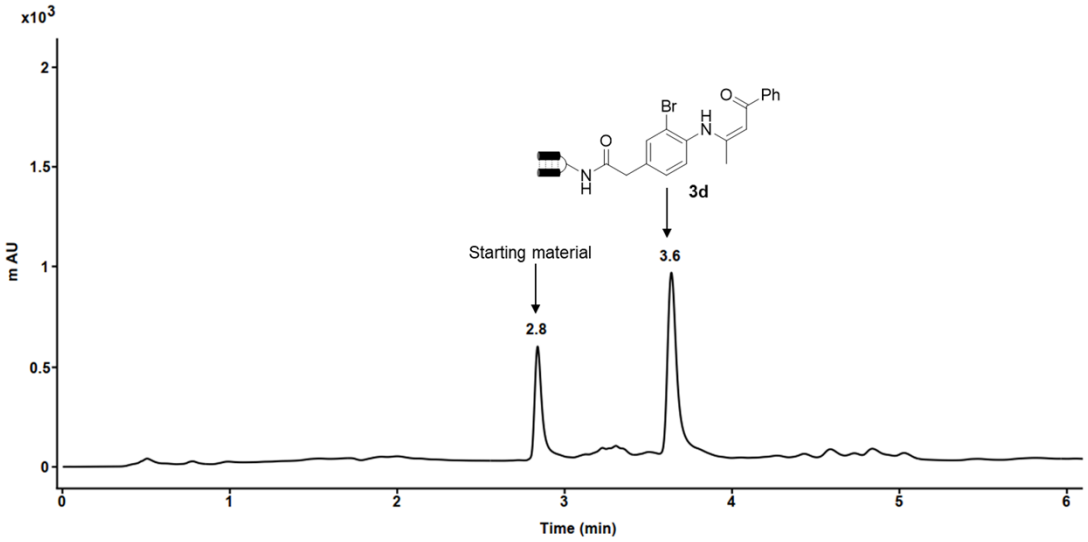


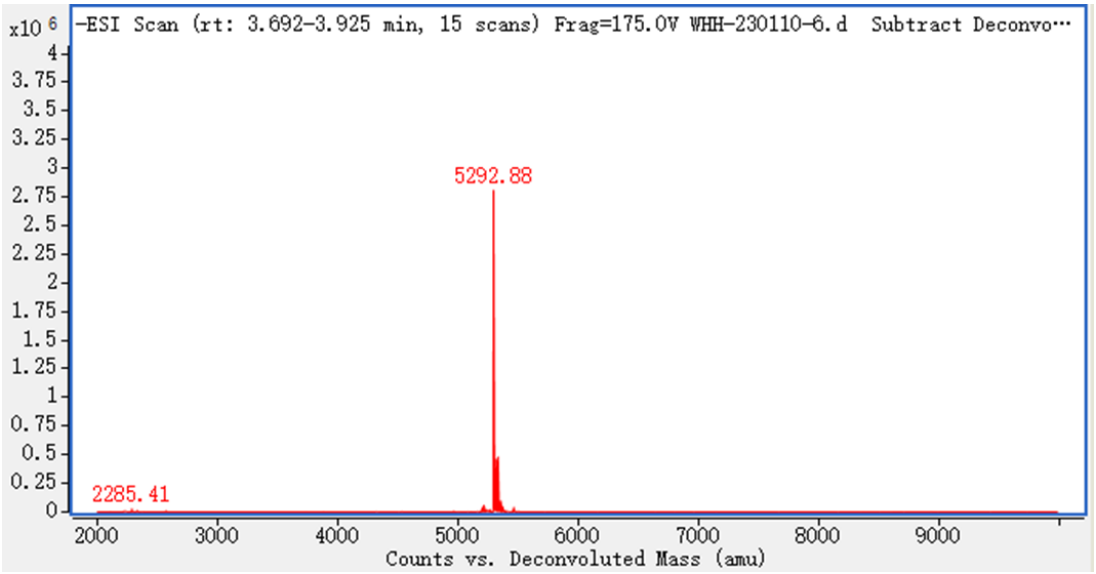


UPLC chromatogram and deconvoluted MS of **3d**

Conversion: 70%

Calculated Mass: 5292 Da; Observed Mass: 5293 Da

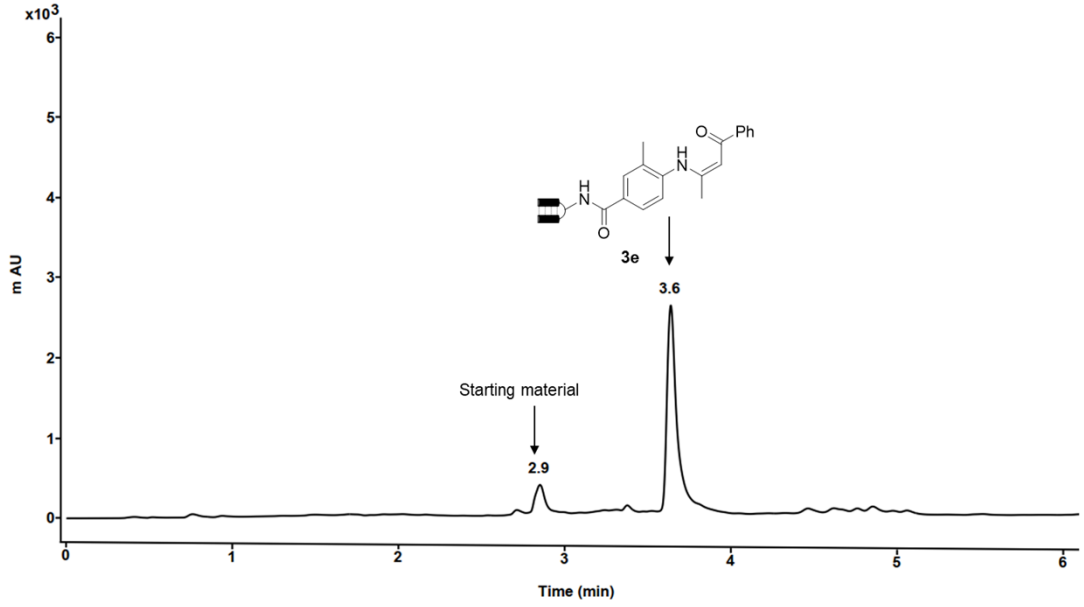


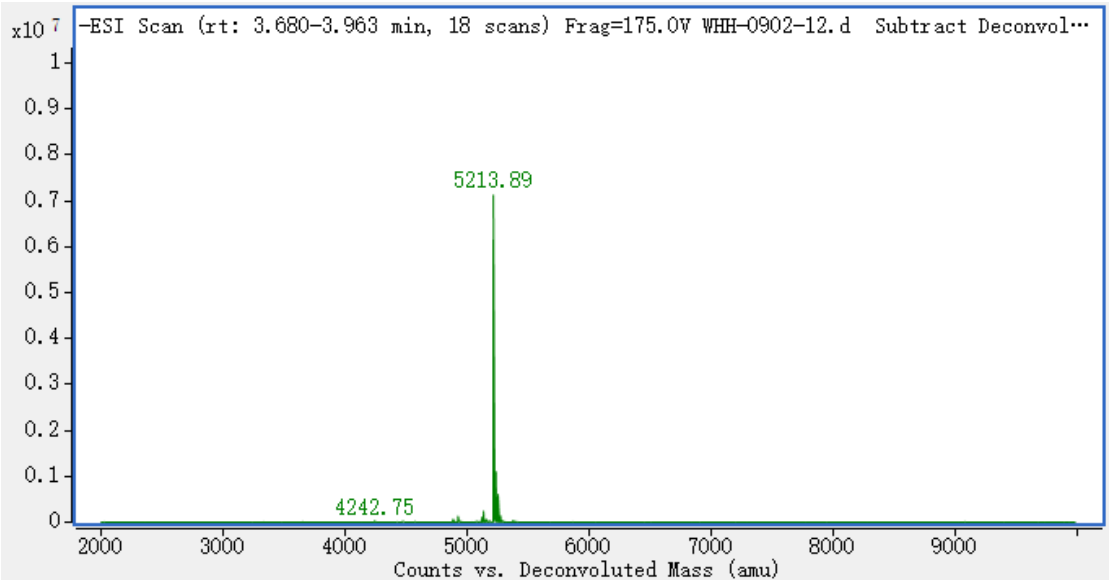


UPLC chromatogram and deconvoluted MS of **3e**

Conversion: 85%

Calculated Mass: 5214 Da; Observed Mass: 5214 Da

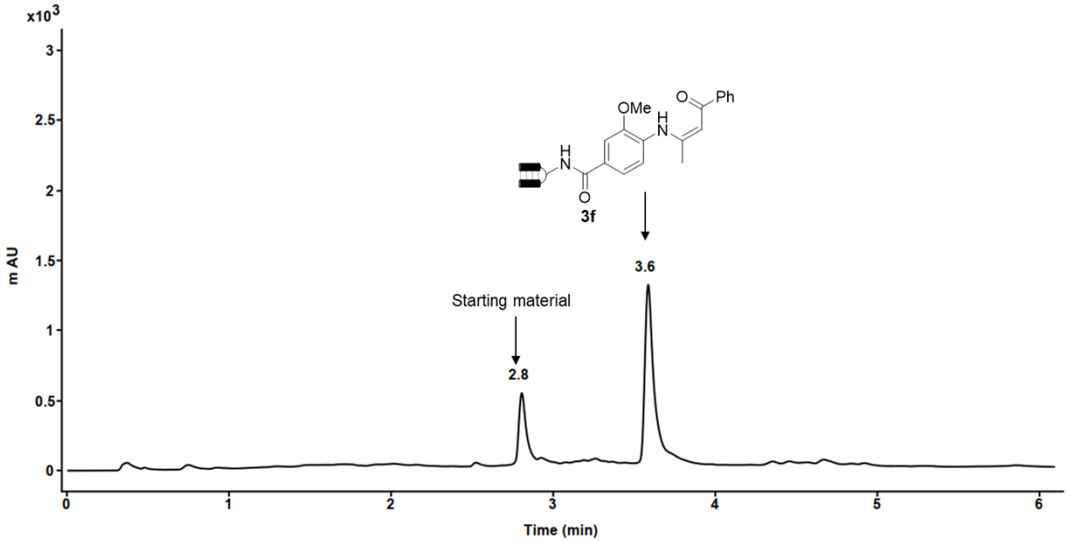


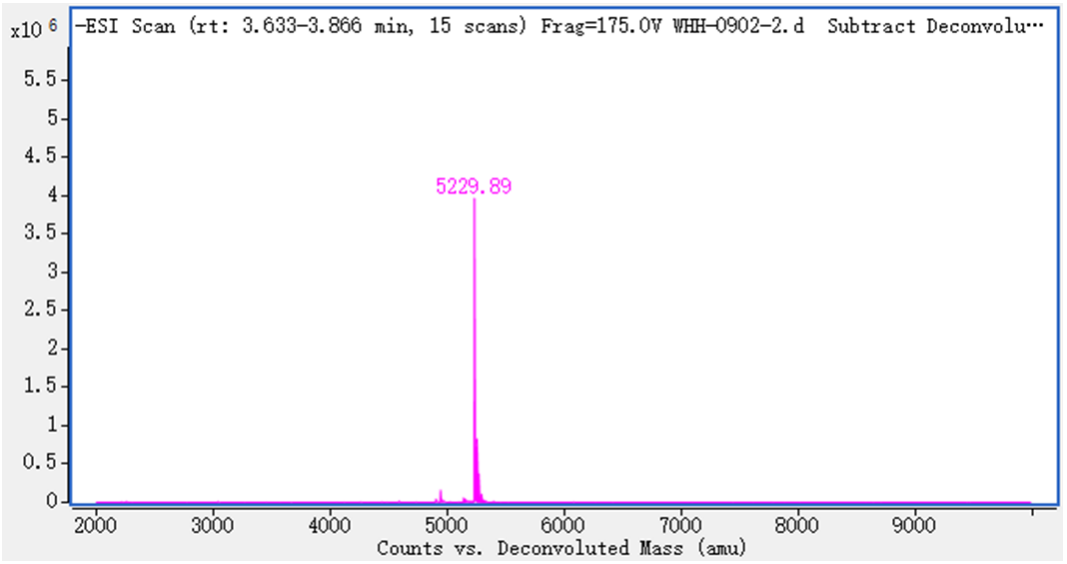


UPLC chromatogram and deconvoluted MS of **3f**

Conversion: 76%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

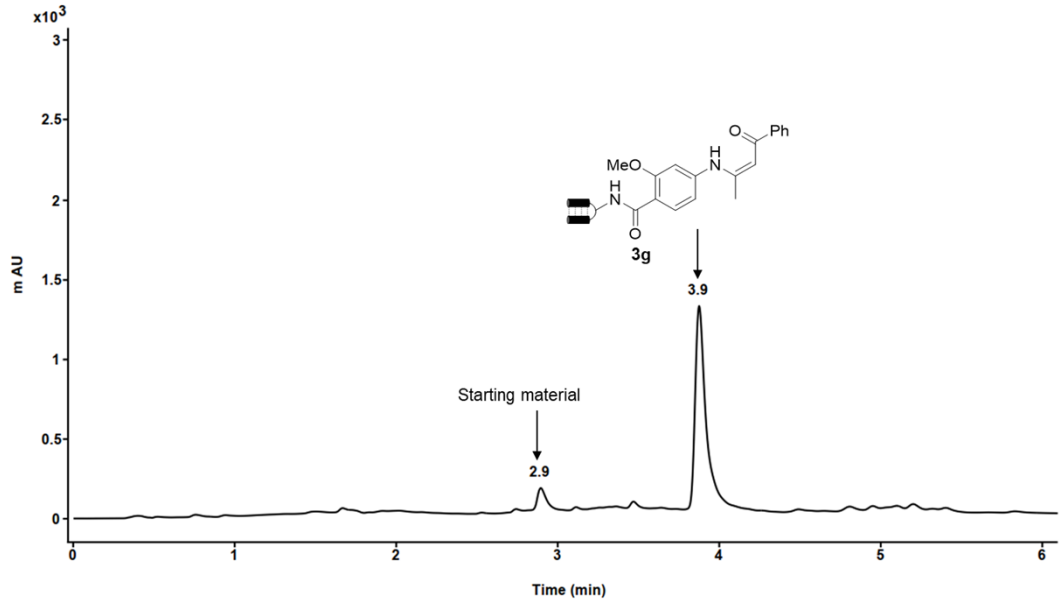


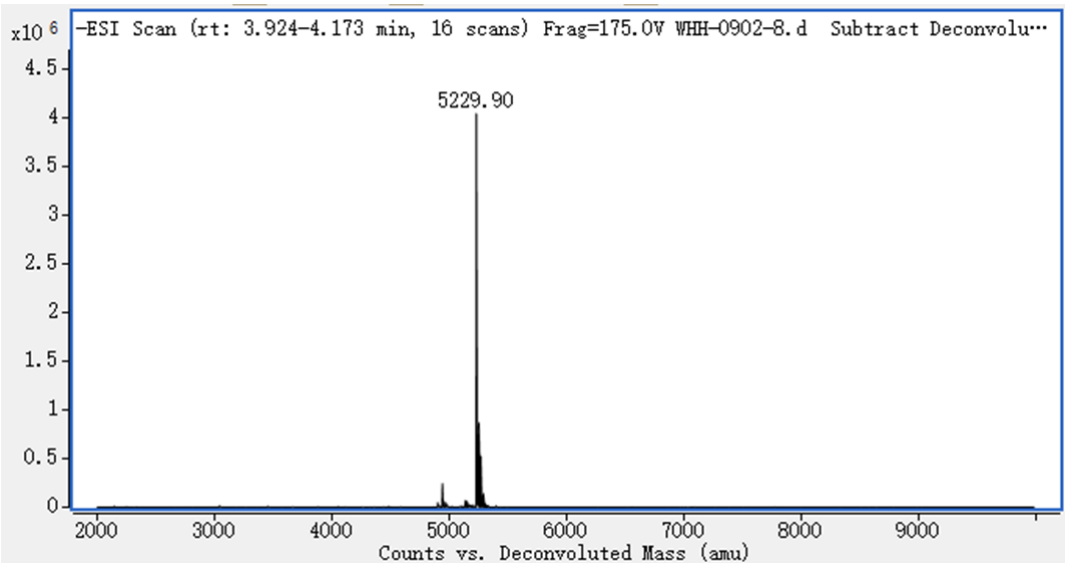


UPLC chromatogram and deconvoluted MS of **3g**

Conversion: 88%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

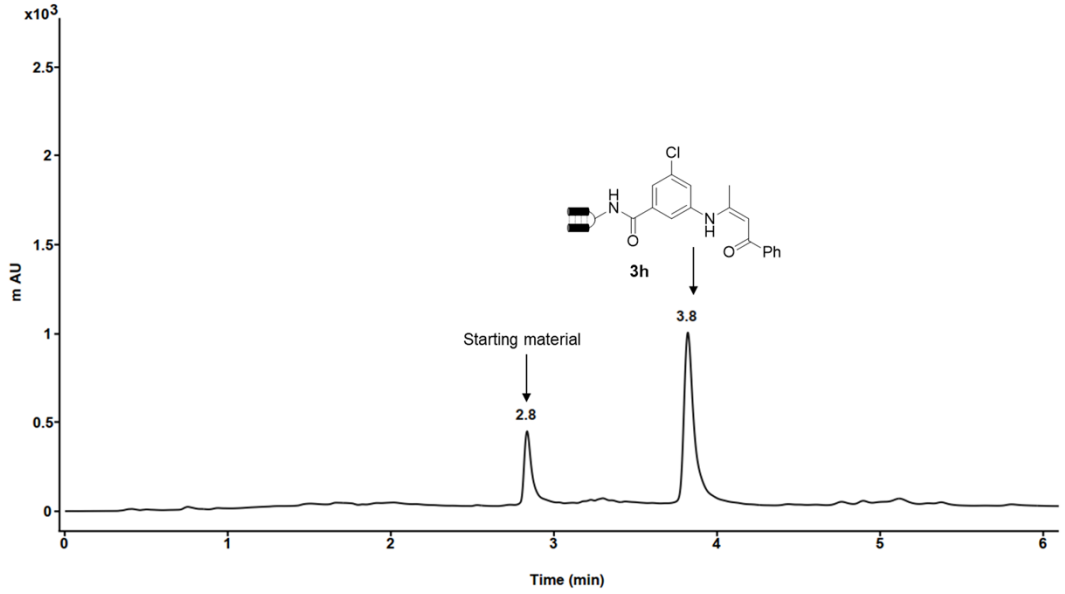


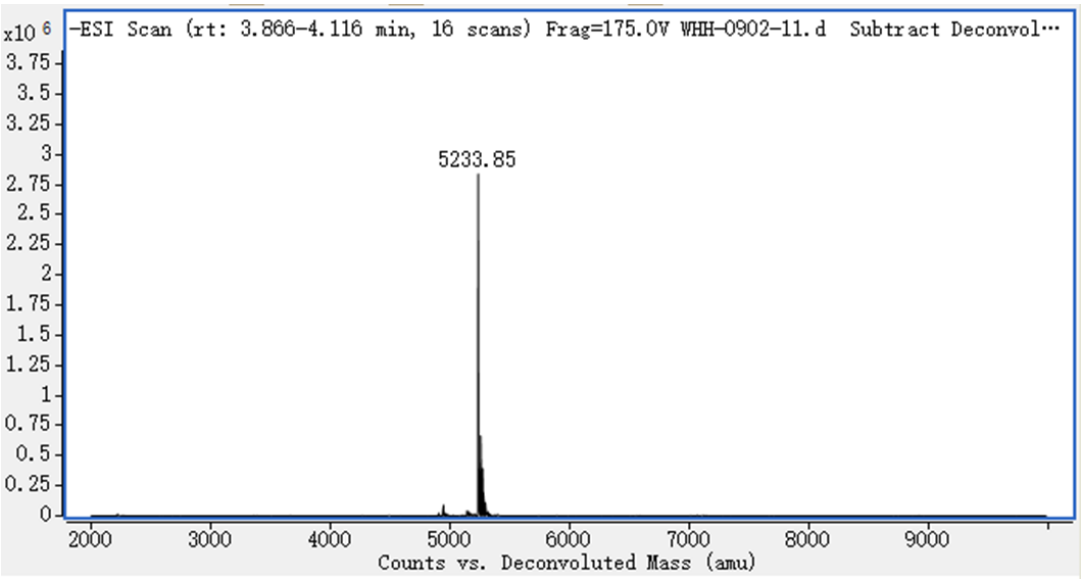


UPLC chromatogram and deconvoluted MS of **3h**

Conversion: 74%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

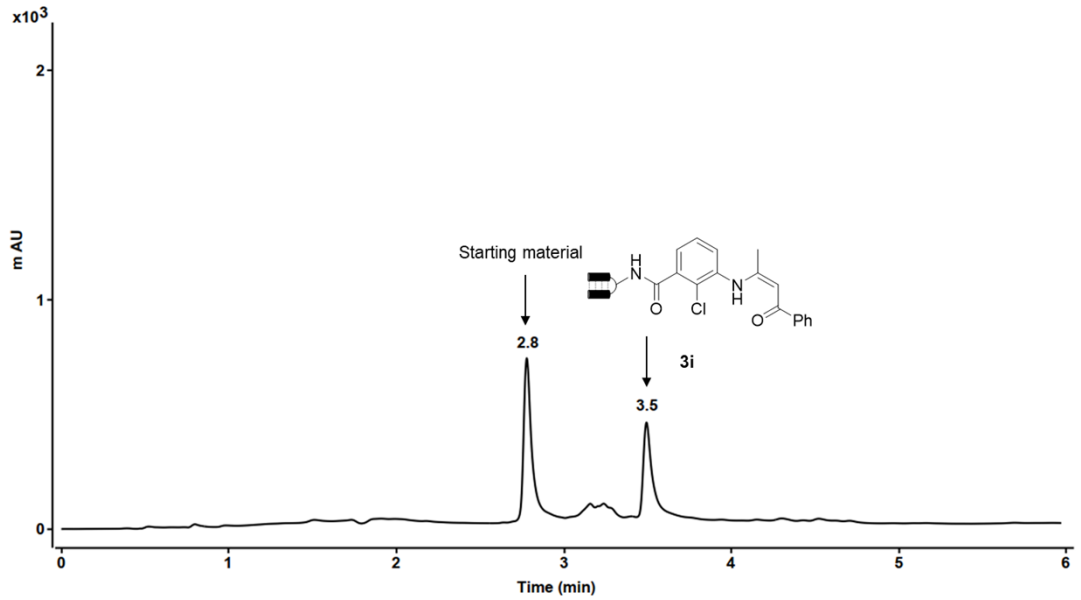


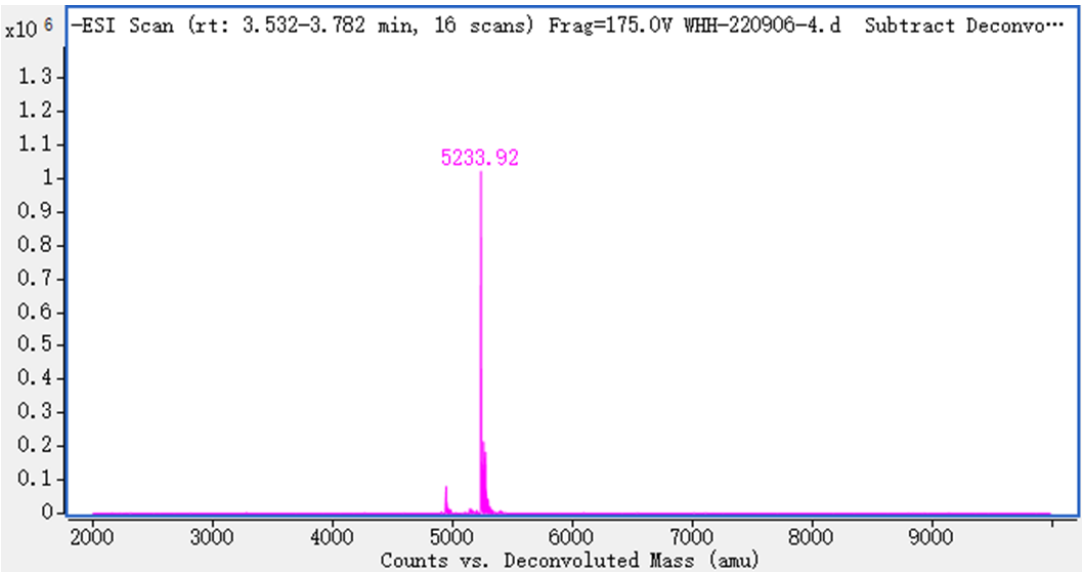


UPLC chromatogram and deconvoluted MS of **3i**

Conversion: 42%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

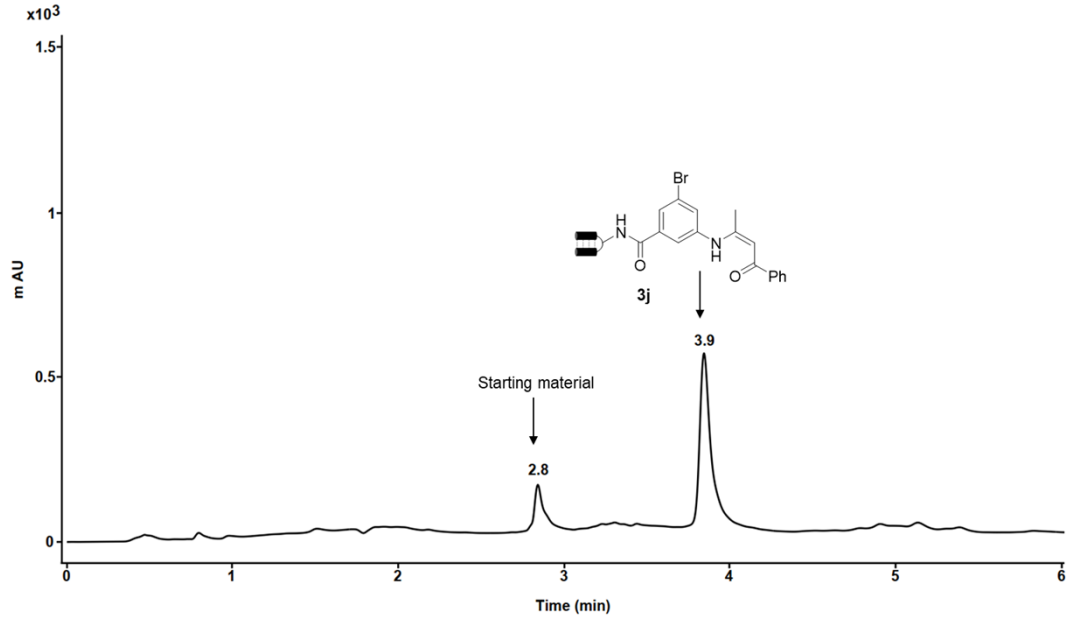


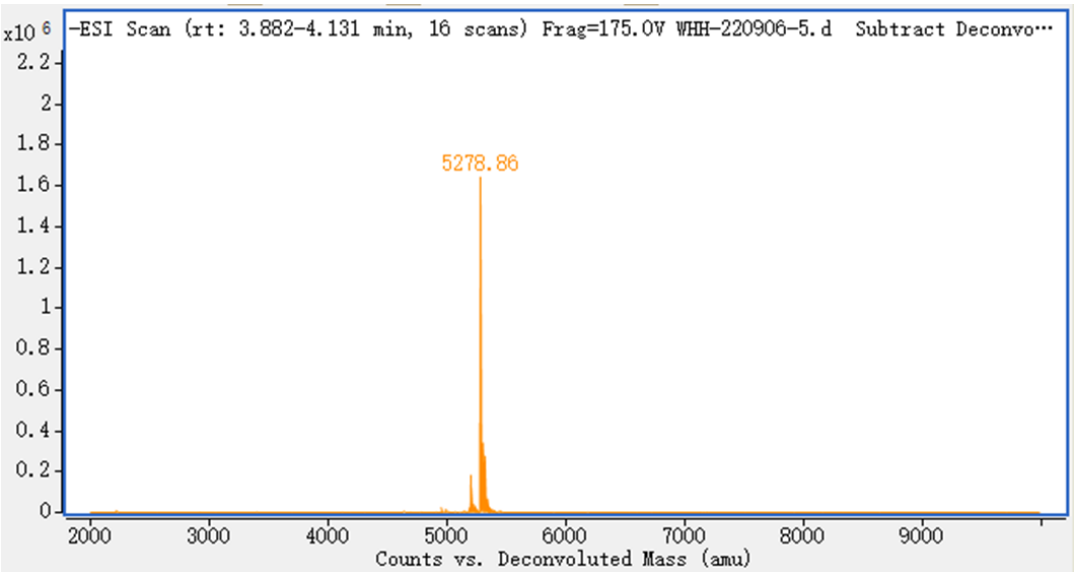


UPLC chromatogram and deconvoluted MS of **3j**

Conversion: 81%

Calculated Mass: 5278 Da; Observed Mass: 5279 Da

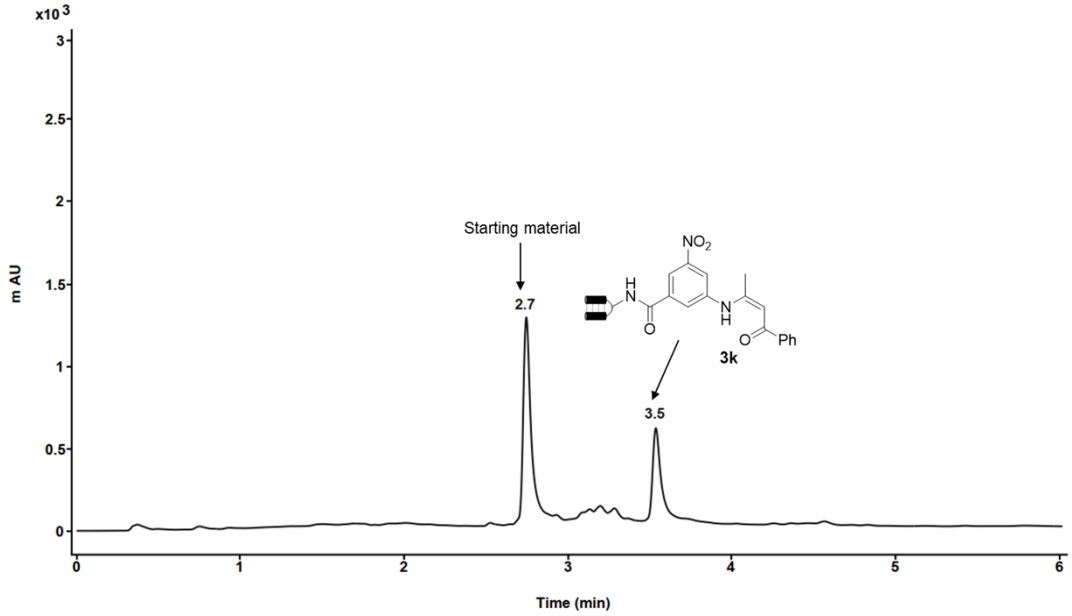


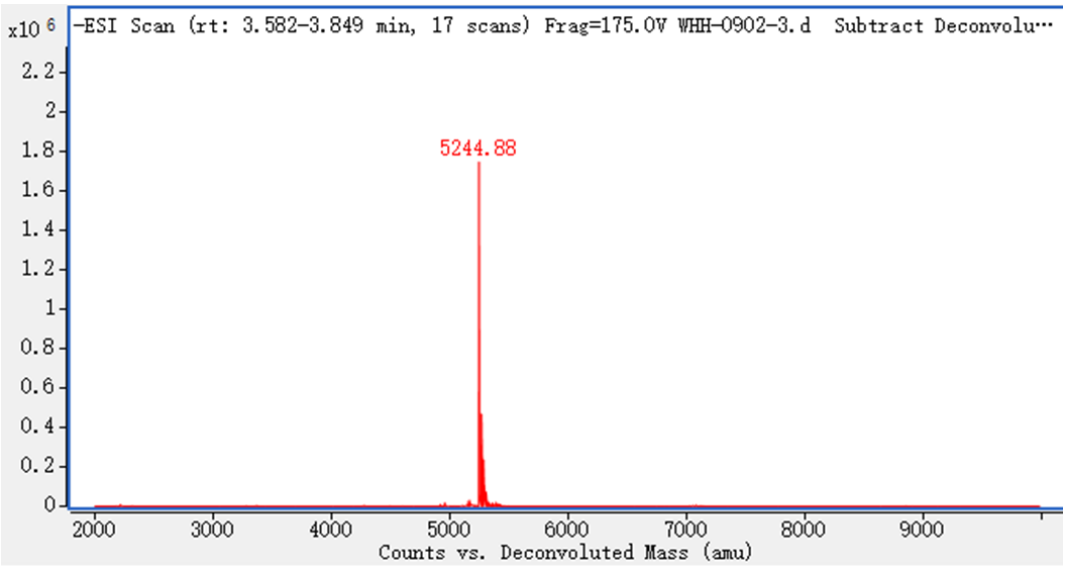


UPLC chromatogram and deconvoluted MS of **3k**

Conversion: 36%

Calculated Mass: 5244 Da; Observed Mass: 5244 Da

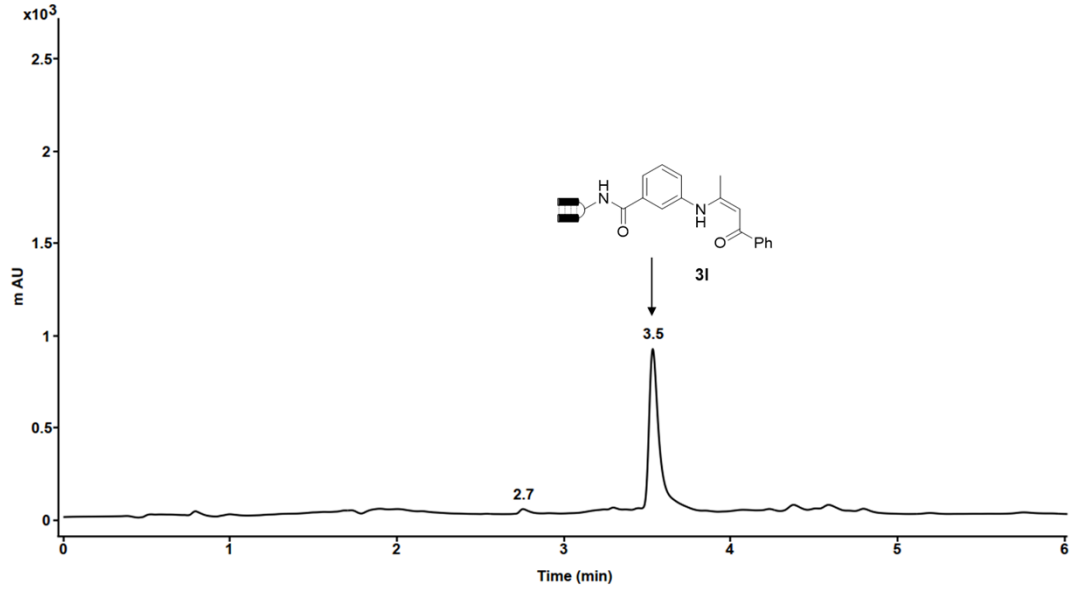


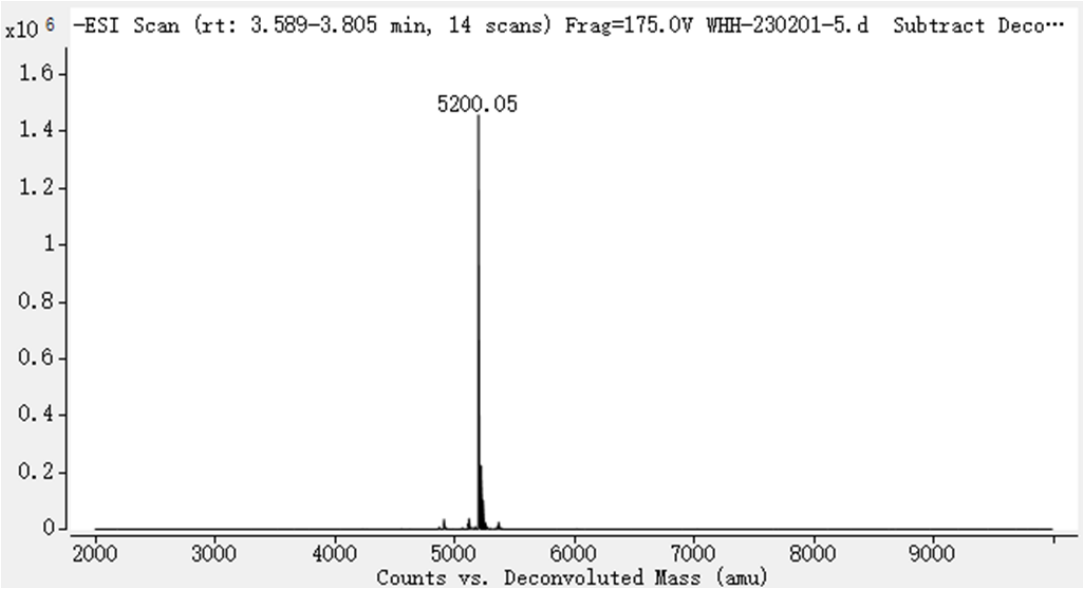


UPLC chromatogram and deconvoluted MS of **3l**

Conversion: >90%

Calculated Mass: 5200 Da; Observed Mass: 5200 Da

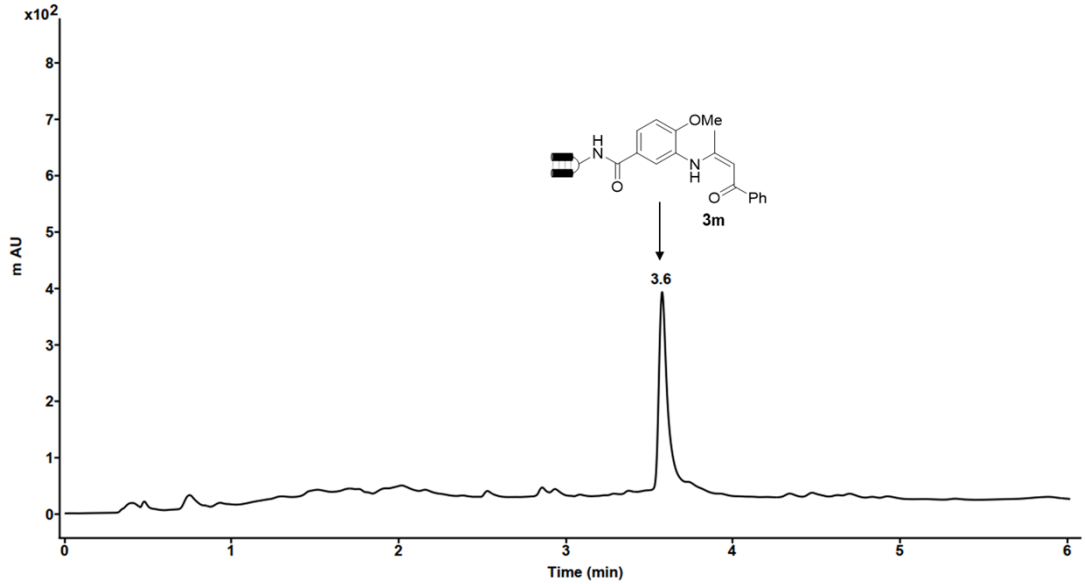


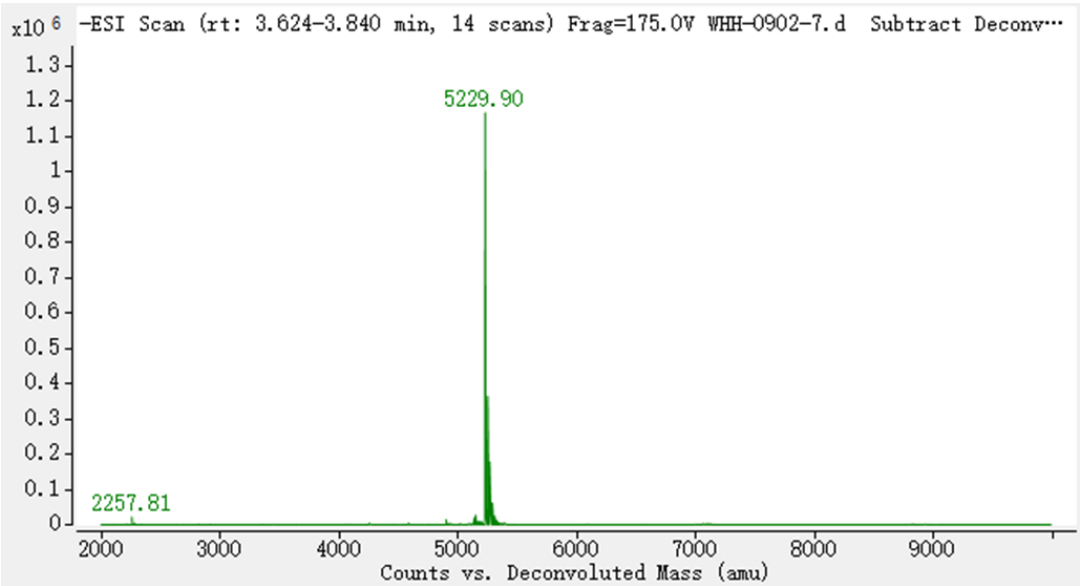


UPLC chromatogram and deconvoluted MS of **3m**

Conversion: >90%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

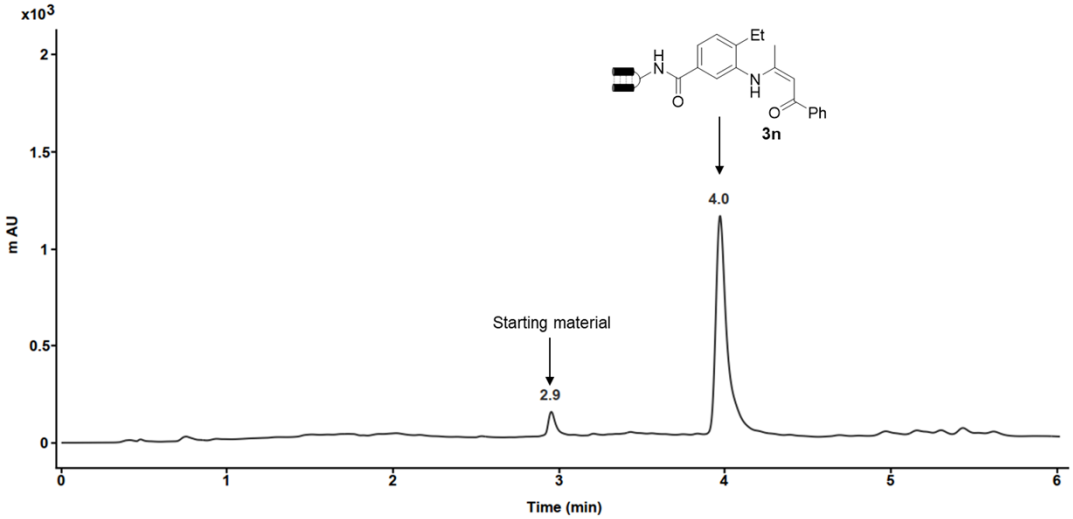


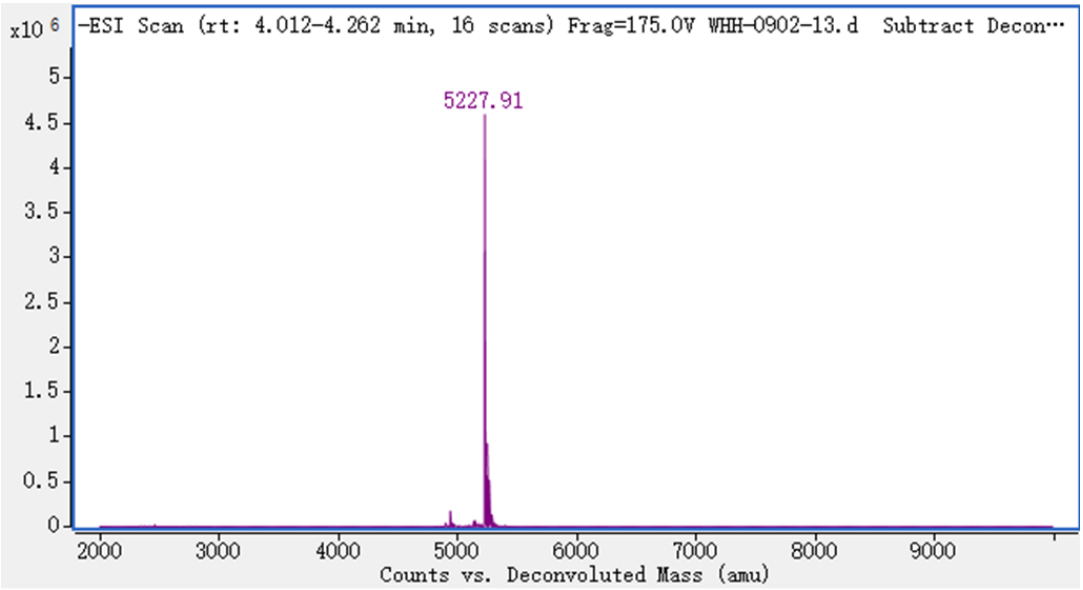


UPLC chromatogram and deconvoluted MS of **3n**

Conversion: >90%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

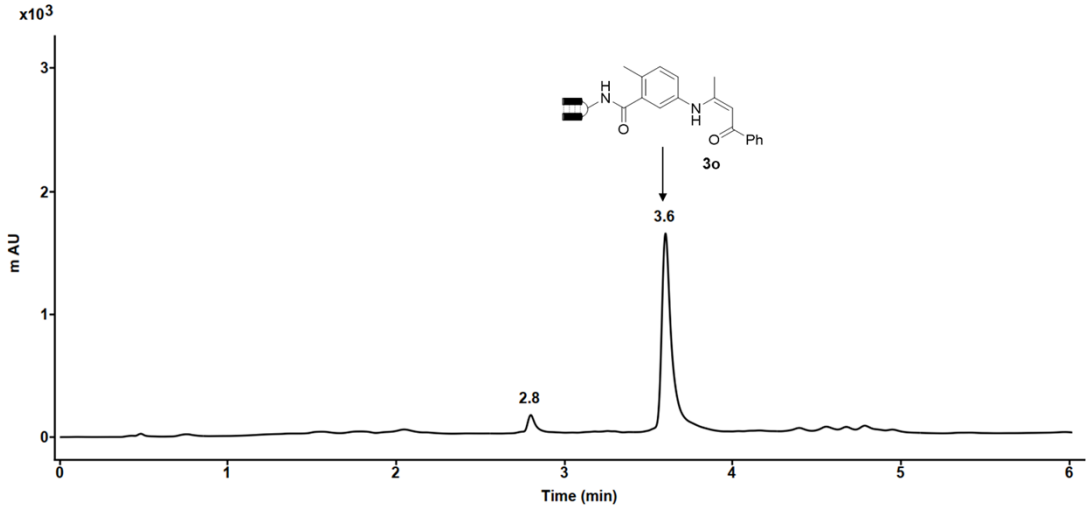


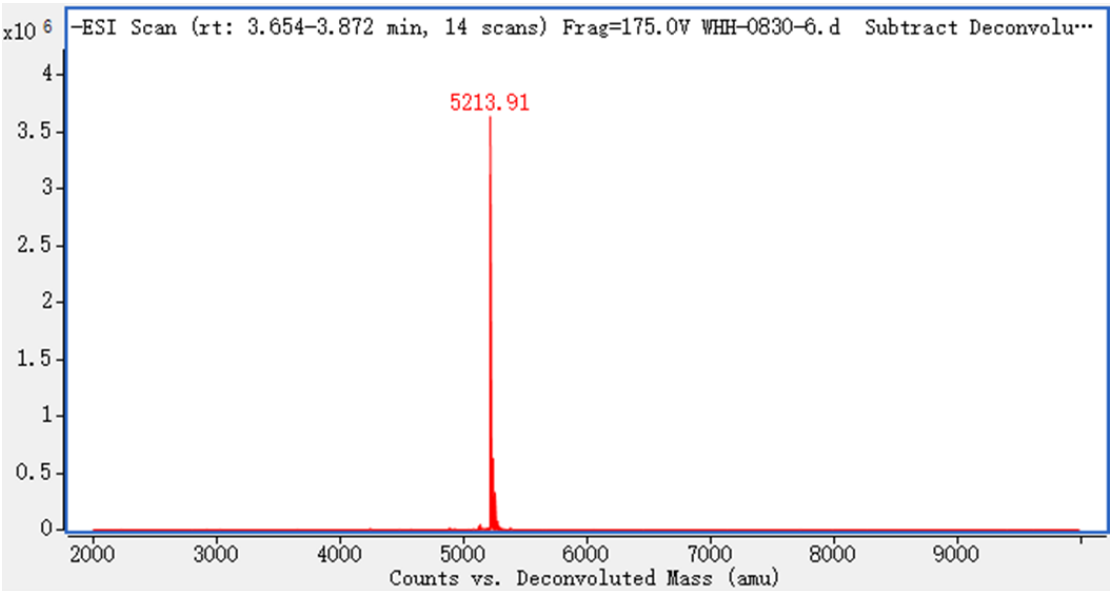


UPLC chromatogram and deconvoluted MS of **3o**

Conversion: >90%

Calculated Mass: 5214 Da; Observed Mass: 5214 Da

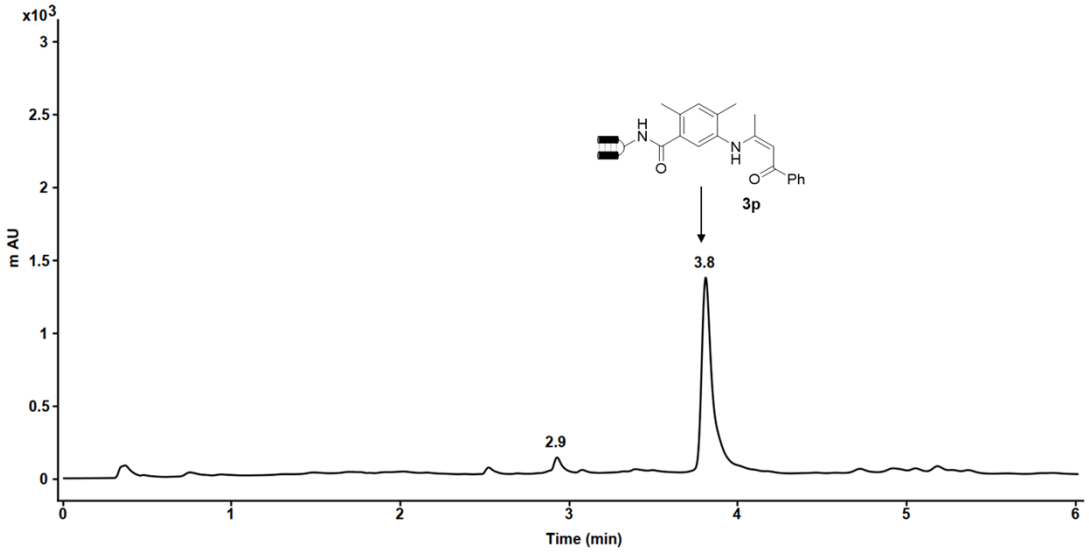


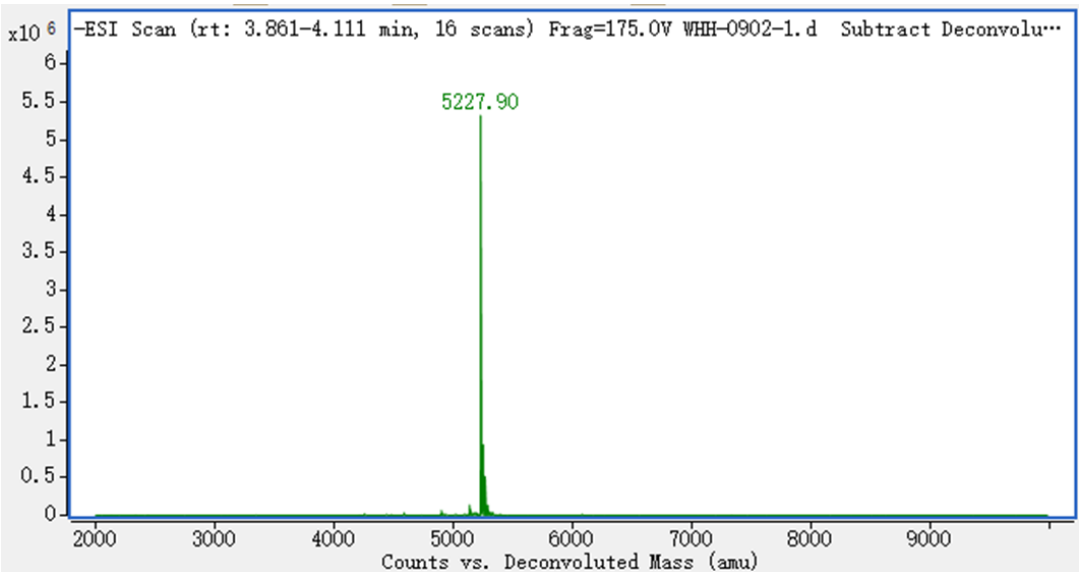


UPLC chromatogram and deconvoluted MS of **3p**

Conversion: >90%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

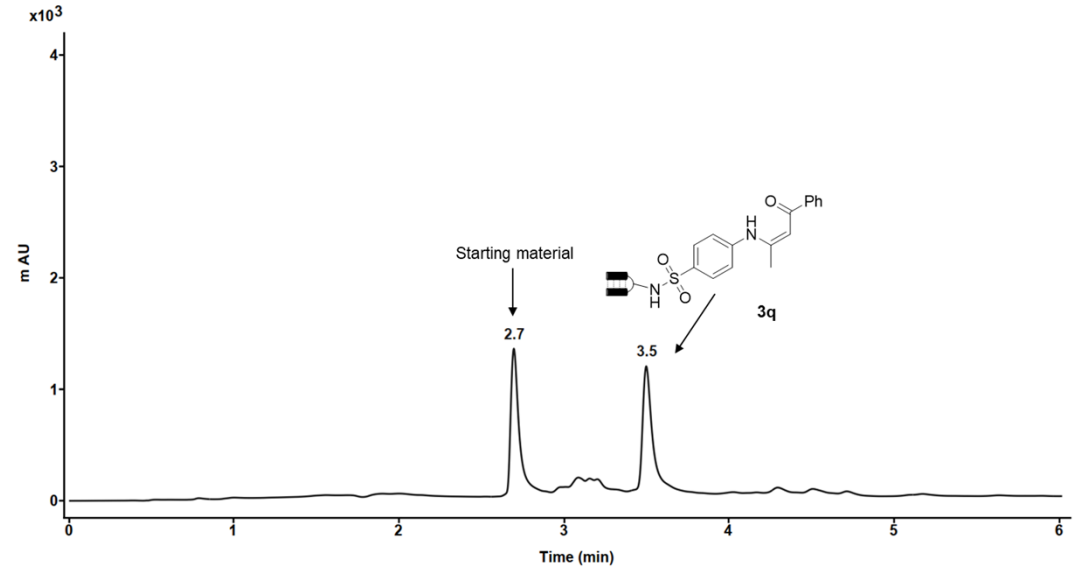


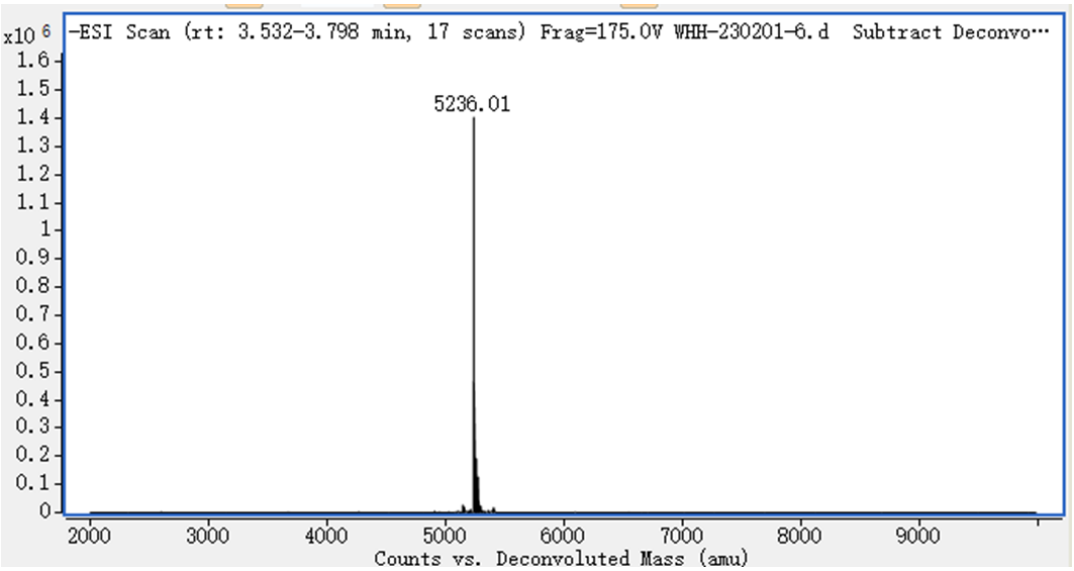


UPLC chromatogram and deconvoluted MS of **3q**

Conversion: 52%

Calculated Mass: 5236 Da; Observed Mass: 5236 Da

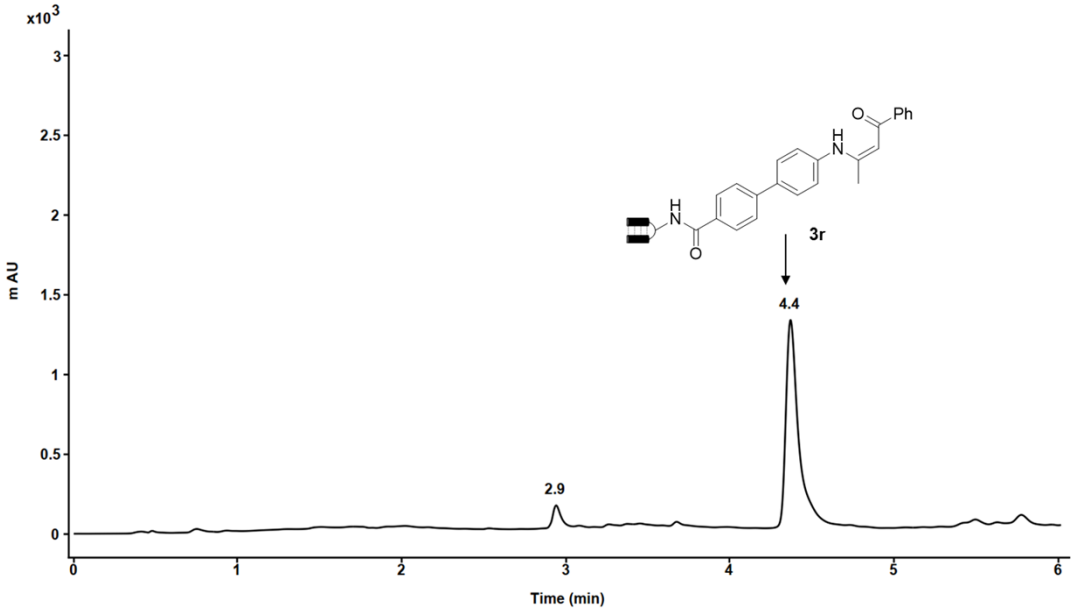


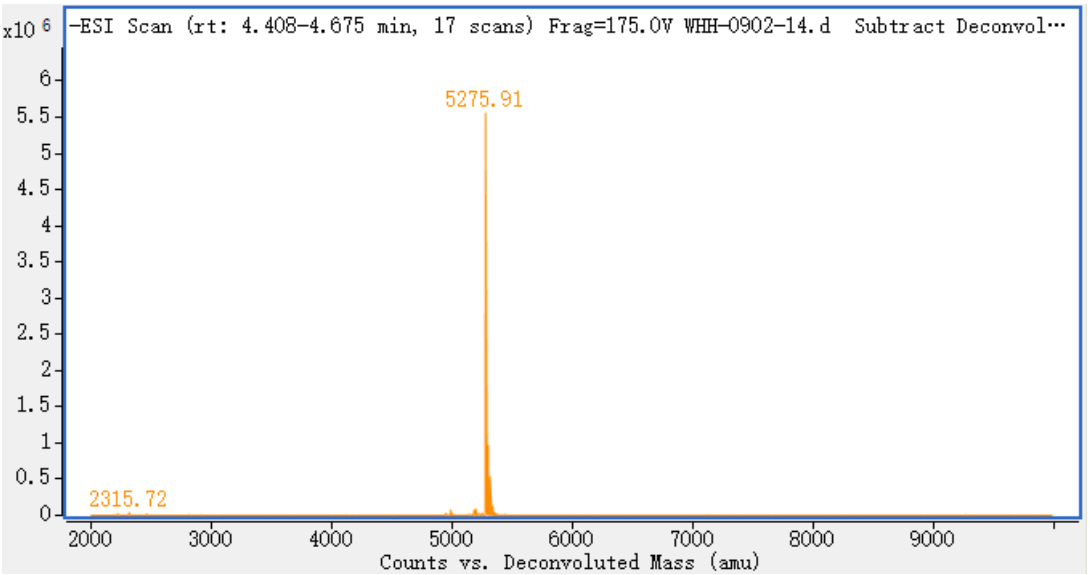


UPLC chromatogram and deconvoluted MS of **3r**

Conversion: >90%

Calculated Mass: 5276 Da; Observed Mass: 5276 Da

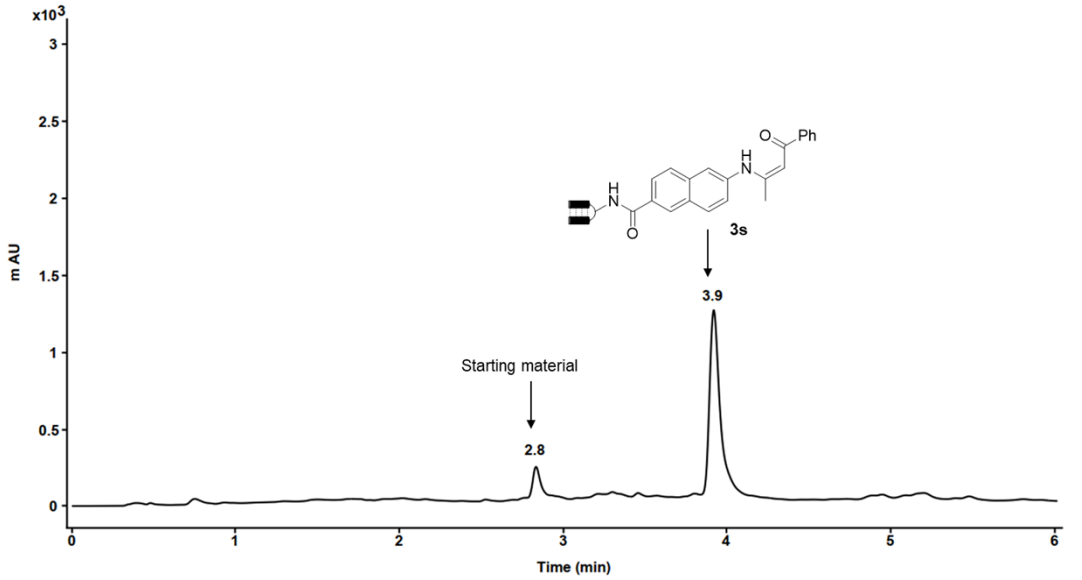


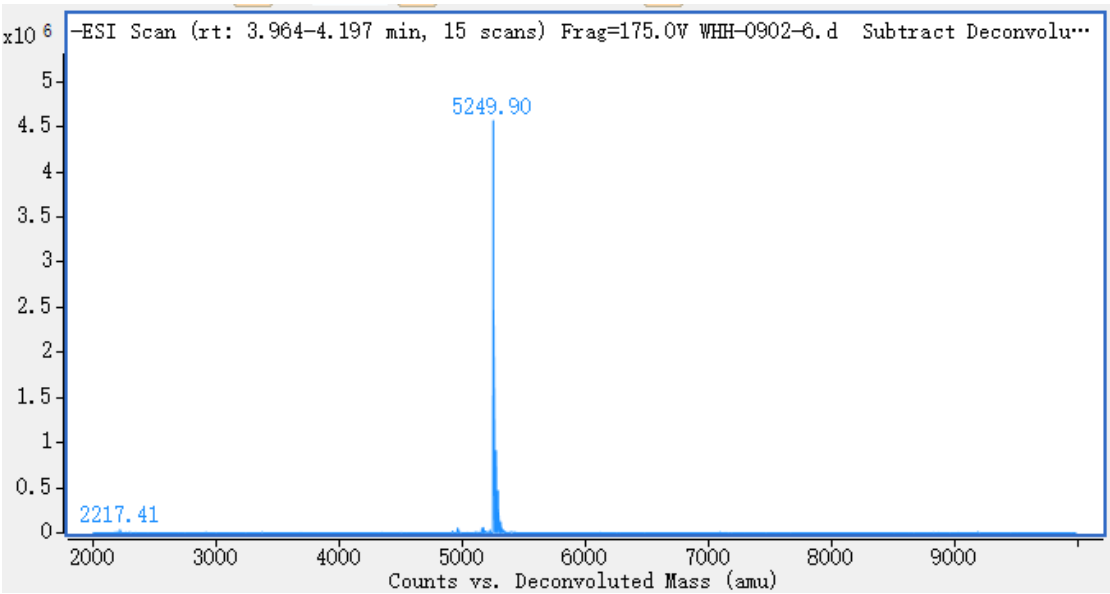


UPLC chromatogram and deconvoluted MS of **3s**

Conversion: 86%

Calculated Mass: 5250 Da; Observed Mass: 5250 Da

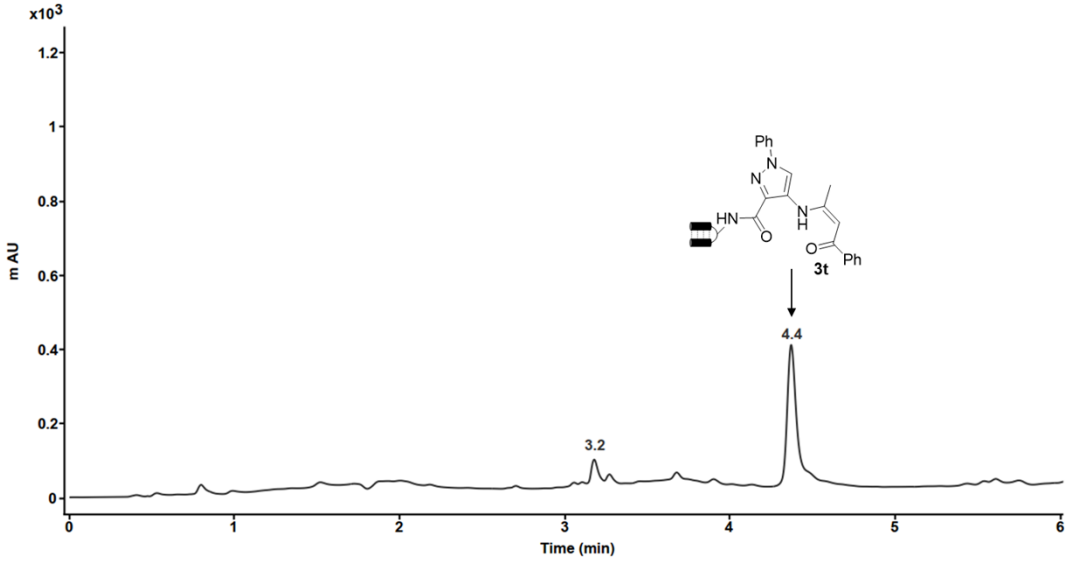


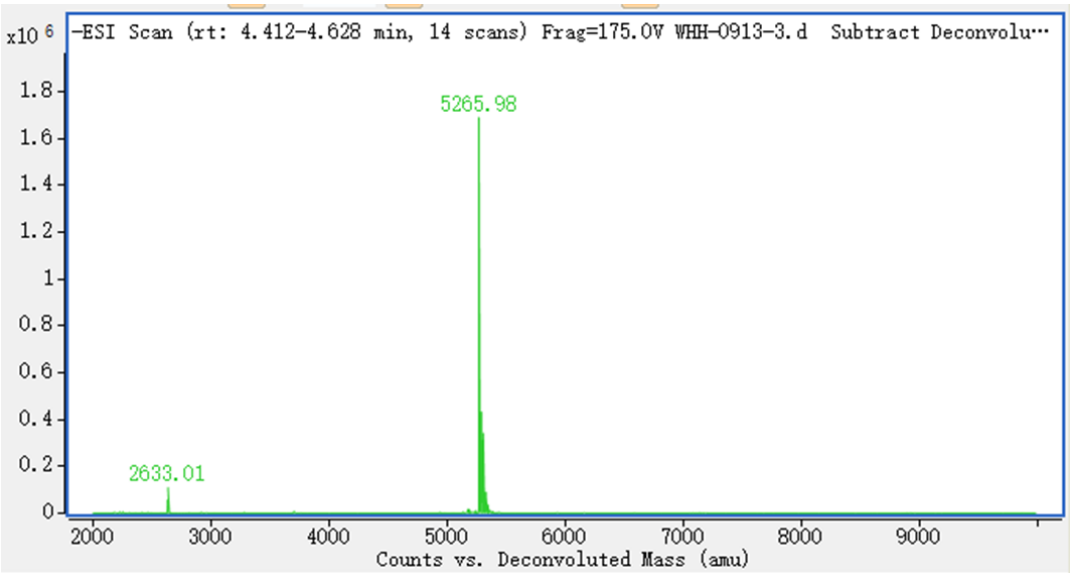


UPLC chromatogram and deconvoluted MS of **3t**

Conversion: 87%

Calculated Mass: 5266 Da; Observed Mass: 5266 Da

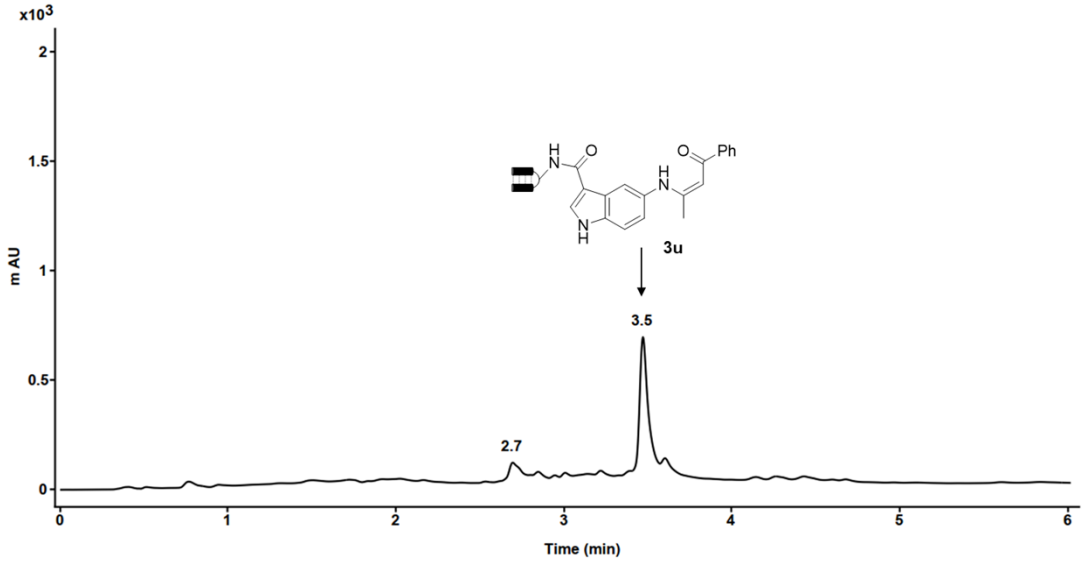


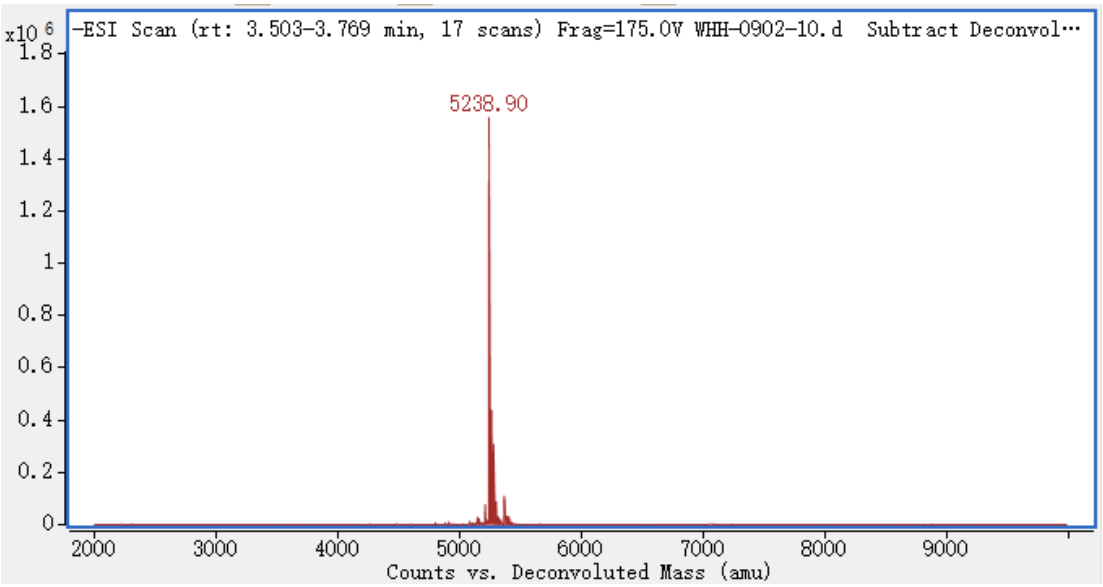


UPLC chromatogram and deconvoluted MS of **3u**

Conversion: 87%

Calculated Mass: 5239 Da; Observed Mass: 5239 Da

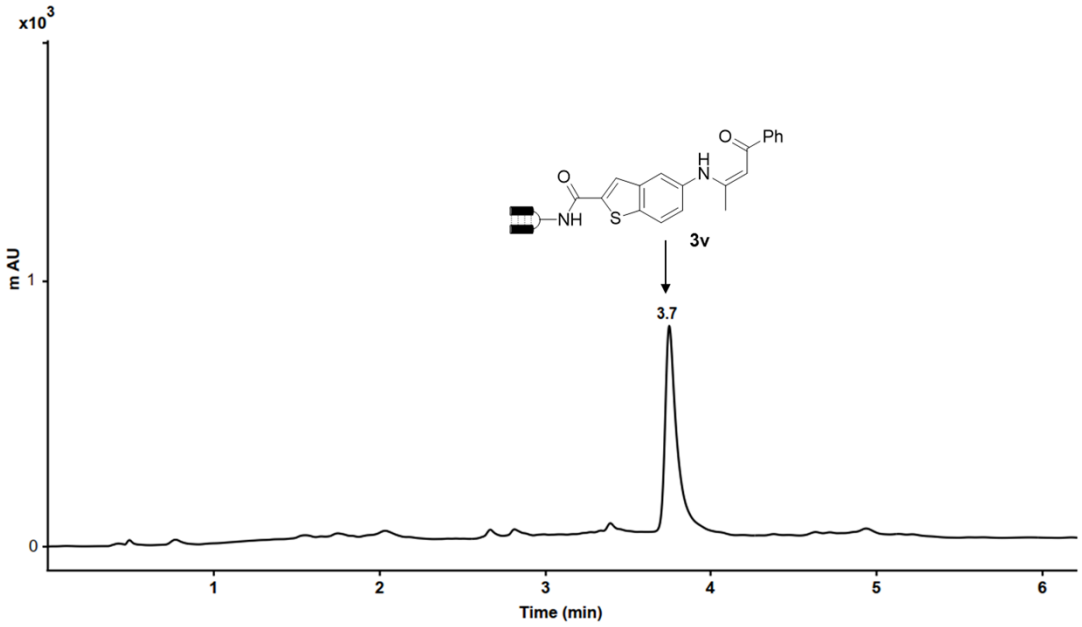


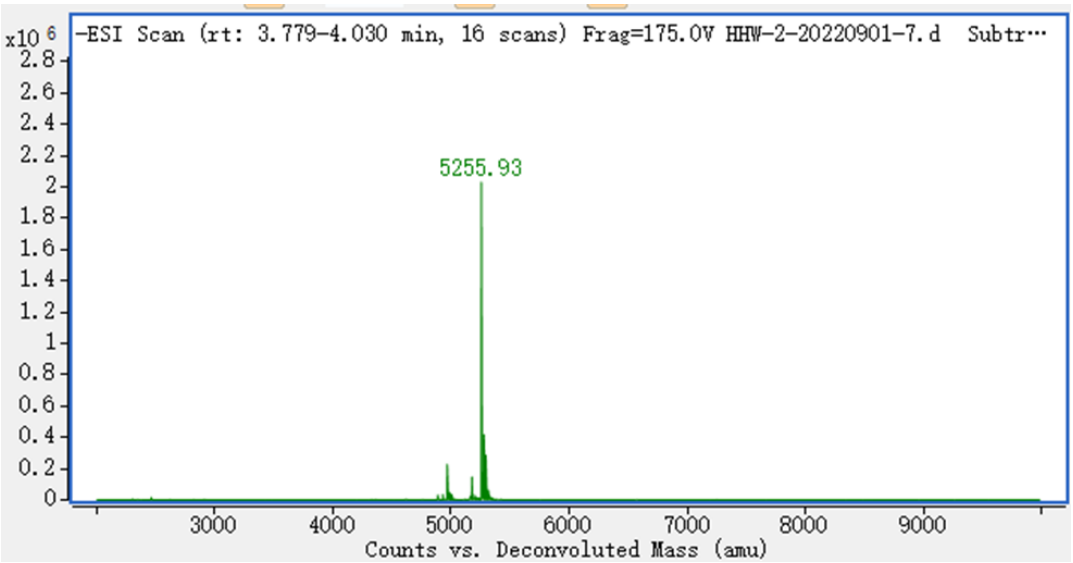


UPLC chromatogram and deconvoluted MS of **3v**

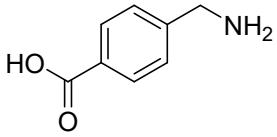
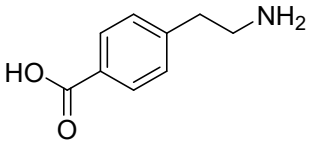
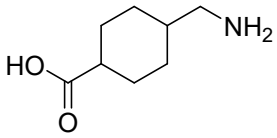
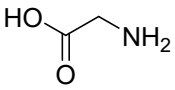
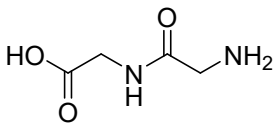
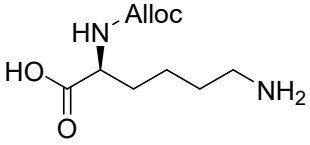
Conversion: >90%

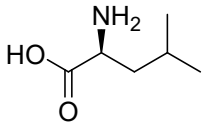
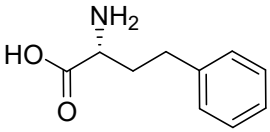
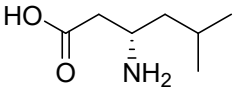
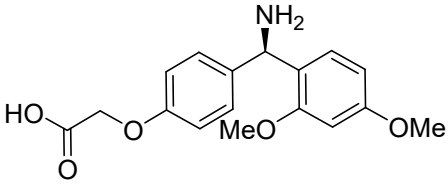
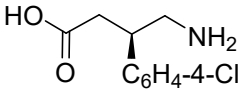
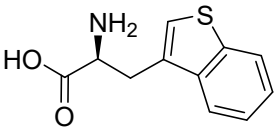
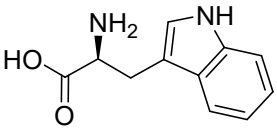
Calculated Mass: 5256 Da; Observed Mass: 5256 Da

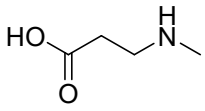
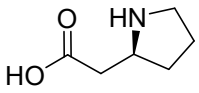




9.2 Substrate scope of DNA-conjugated aliphatic amines 1ab-1ap

Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
1ab		**3ab**	5214	5214	>90%
1ac		**3ac**	5228	5228	>90%
1ad		**3ad**	5220	5220	>90%
1ae		**3ae**	5138	5138	70%
1af		**3af**	5195	5195	81%
1ag		**3ag**	5293	5293	>90%

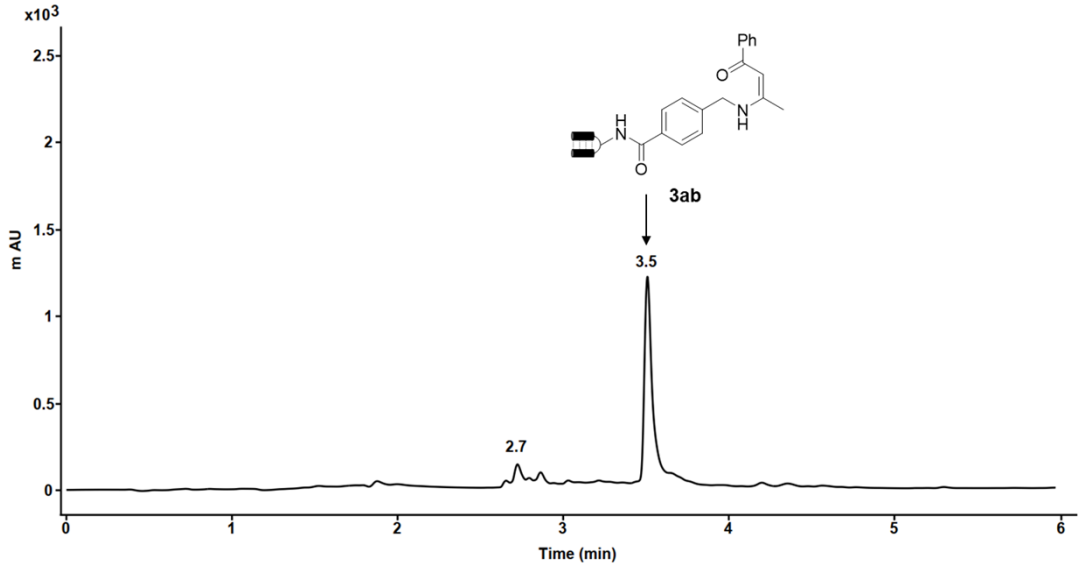
1ah		**3ah**	5194	5194	69%
1ai		**3ai**	5242	5242	76%
1aj		**3aj**	5208	5208	>90%
1ak		**3ak**	5380	5380	>90%
1al		**3al**	5276	5276	>90%
1am		**3am**	5284	5284	88%
1an		**3an**	5266	5267	74%

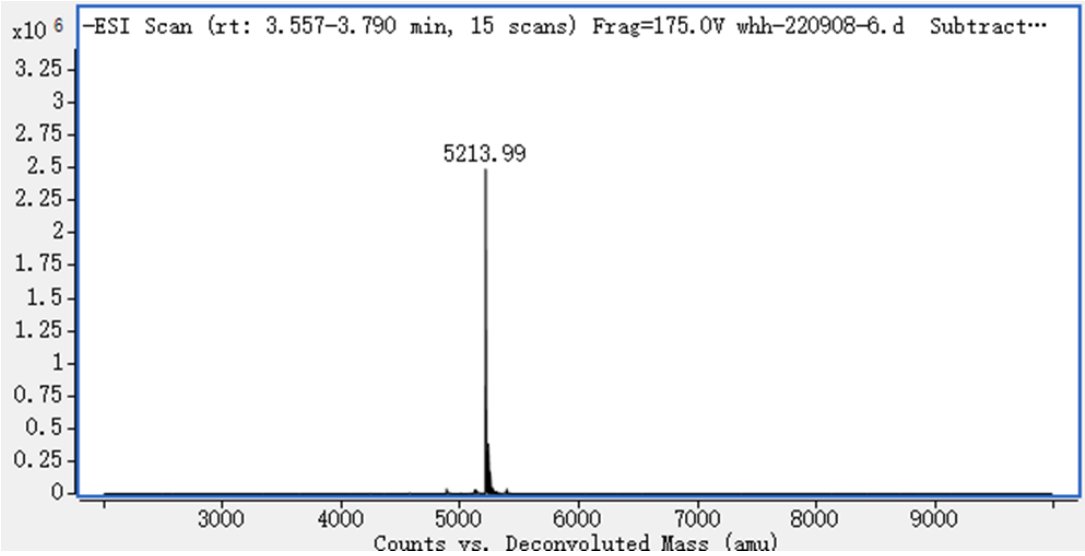
1ao		**3ao**	5166	5166	56%
1ap		**3ap**	5192	5192	67%

UPLC chromatogram and deconvoluted MS of **3ab**

Conversion: >90%

Calculated Mass: 5214 Da; Observed Mass: 5214 Da

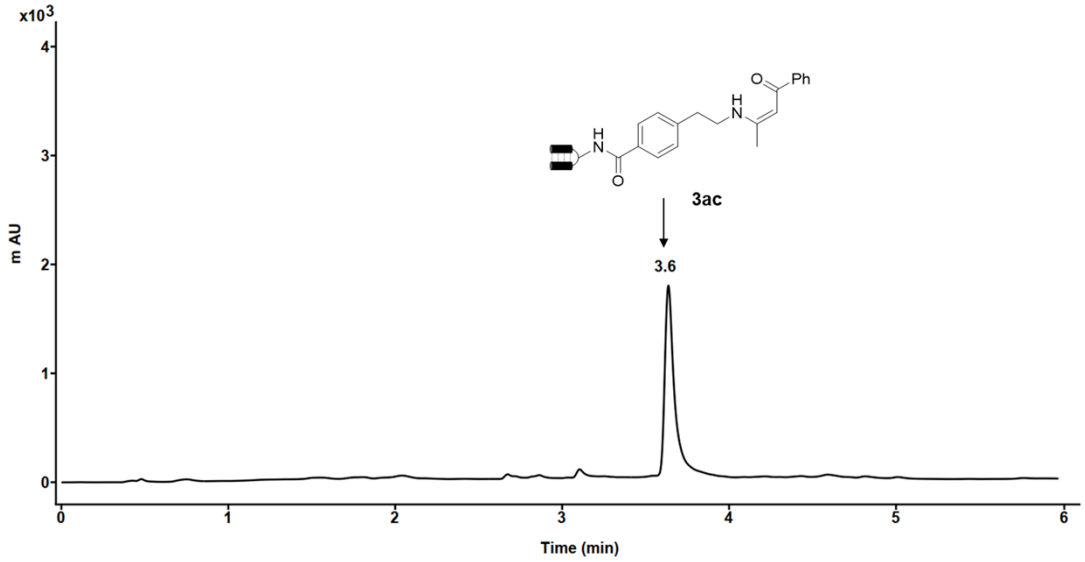


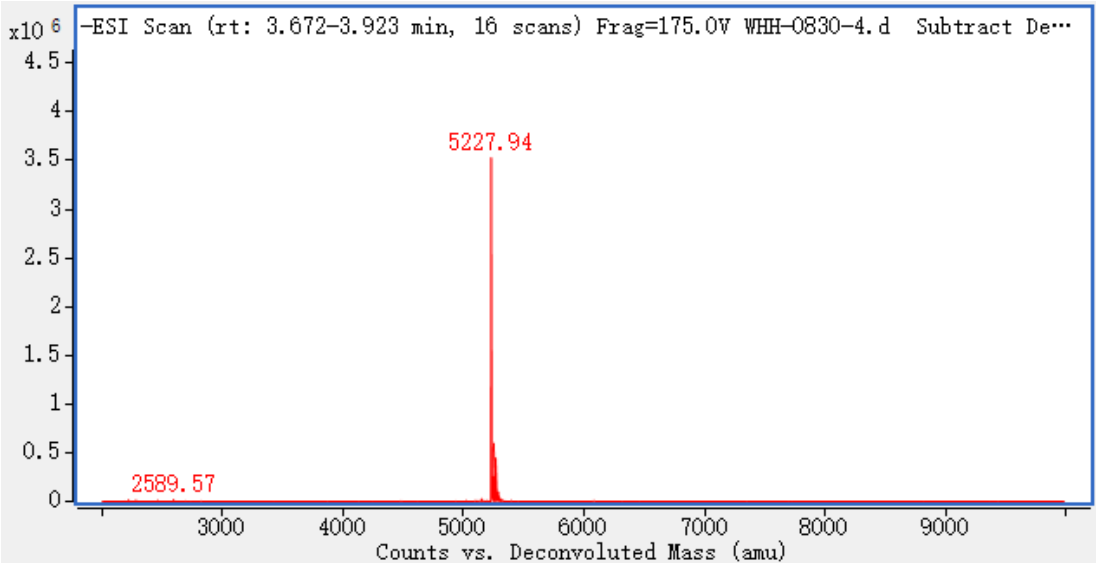


UPLC chromatogram and deconvoluted MS of **3ac**

Conversion: >90%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

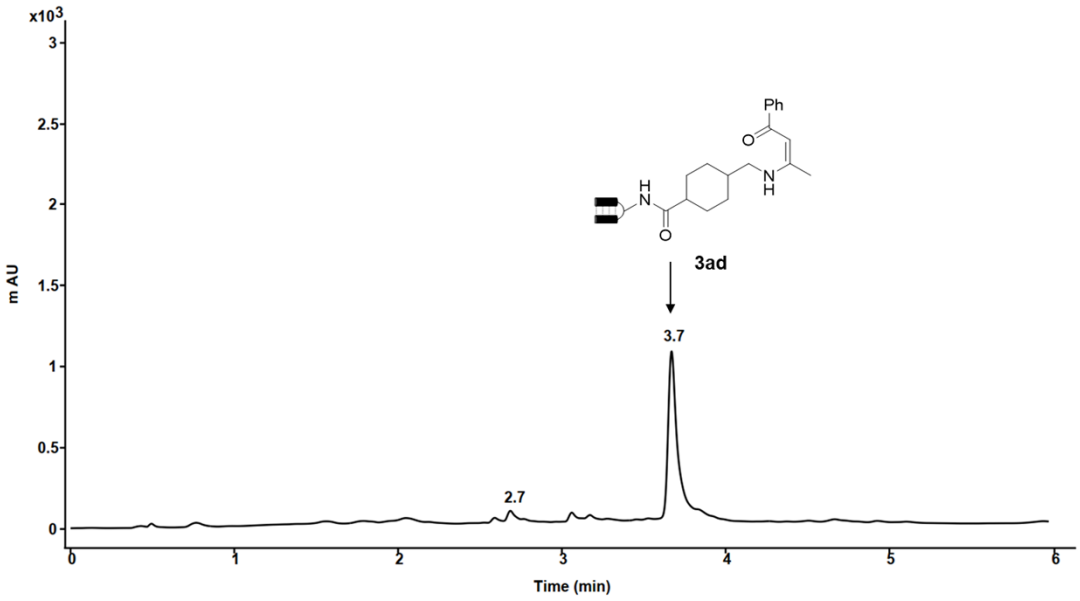


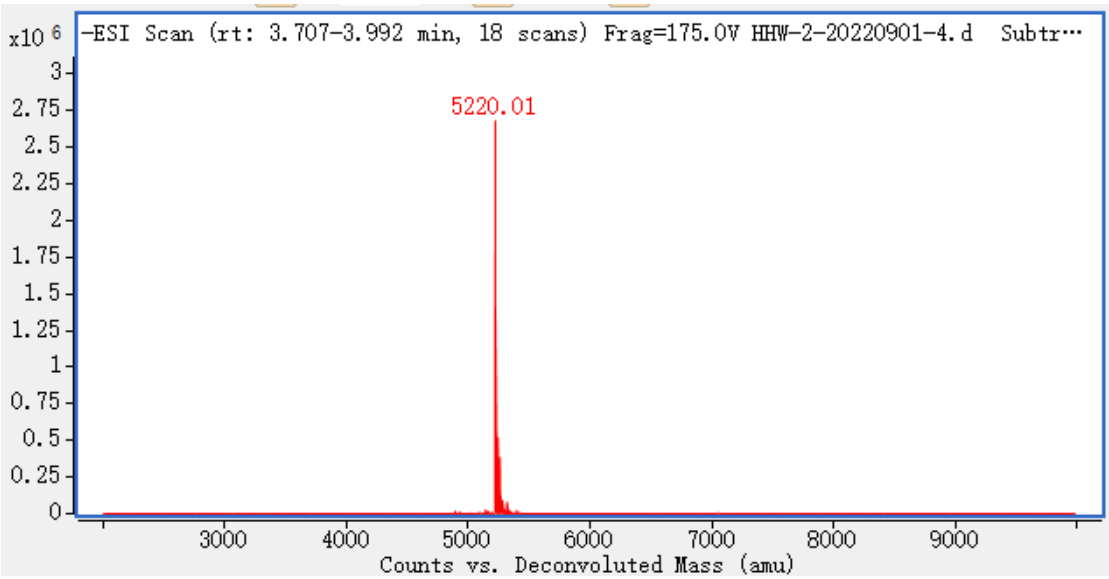


UPLC chromatogram and deconvoluted MS of **3ad**

Conversion: >90%

Calculated Mass: 5220 Da; Observed Mass: 5220 Da

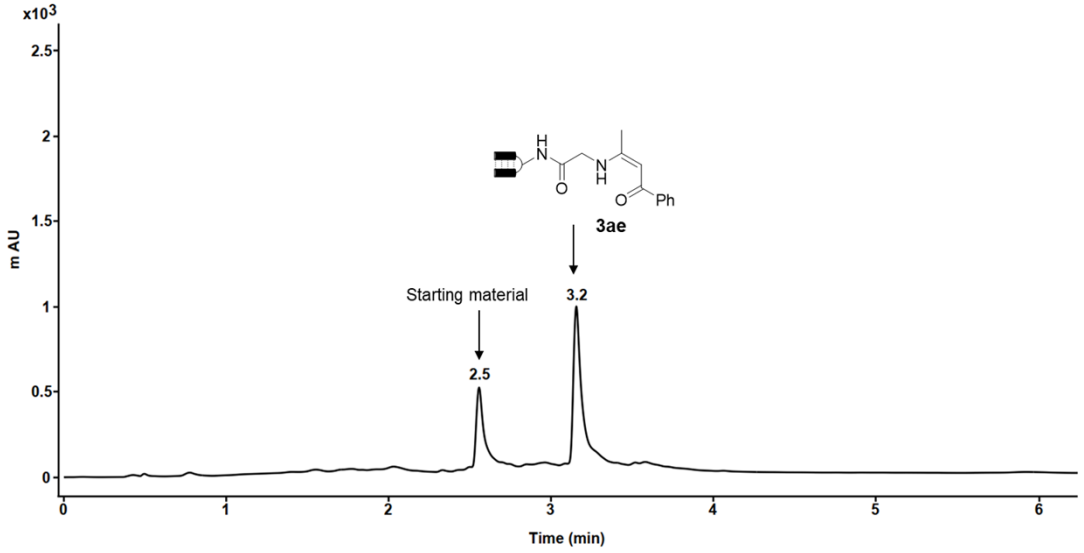


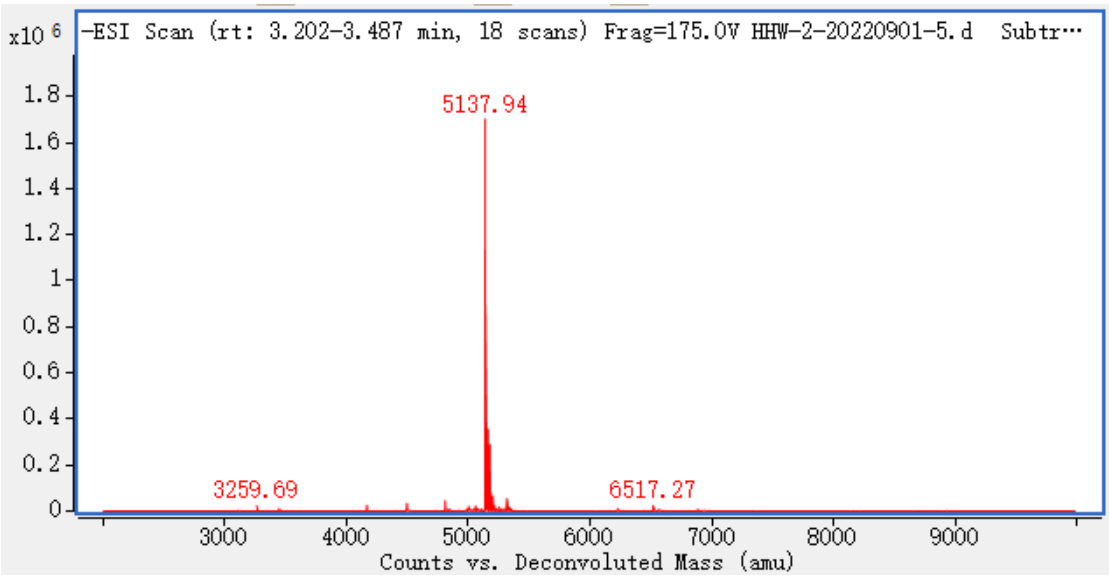


UPLC chromatogram and deconvoluted MS of **3ae**

Conversion: 70%

Calculated Mass: 5138 Da; Observed Mass: 5138 Da

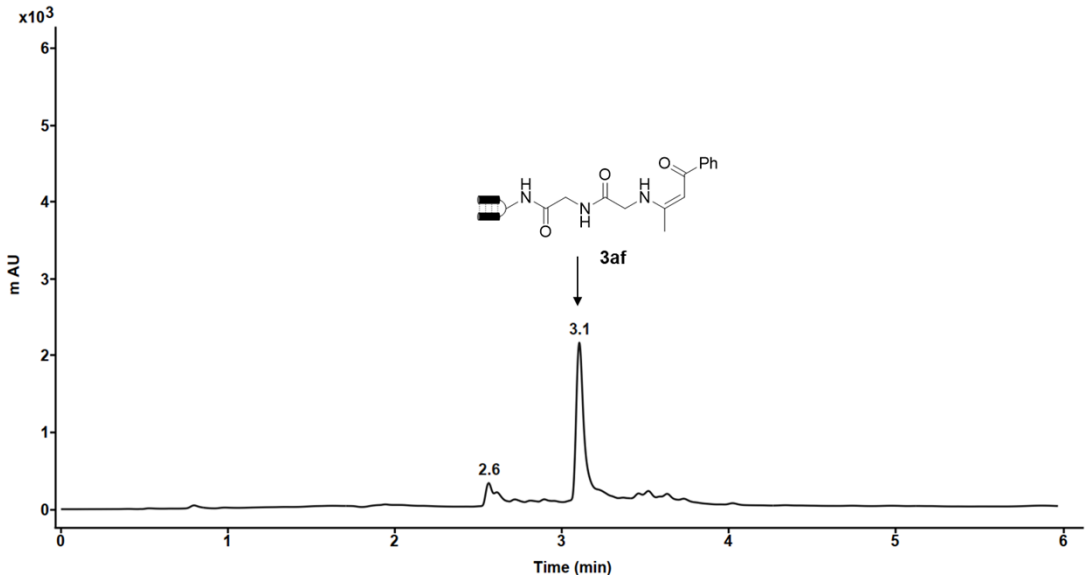


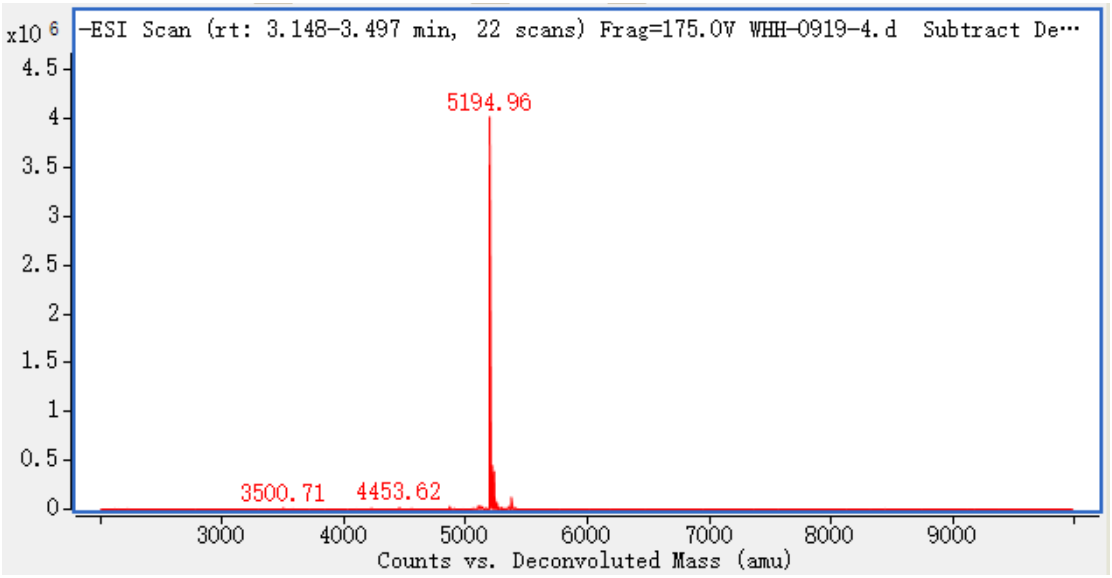


UPLC chromatogram and deconvoluted MS of **3af**

Conversion: 81%

Calculated Mass: 5195 Da; Observed Mass: 5195 Da

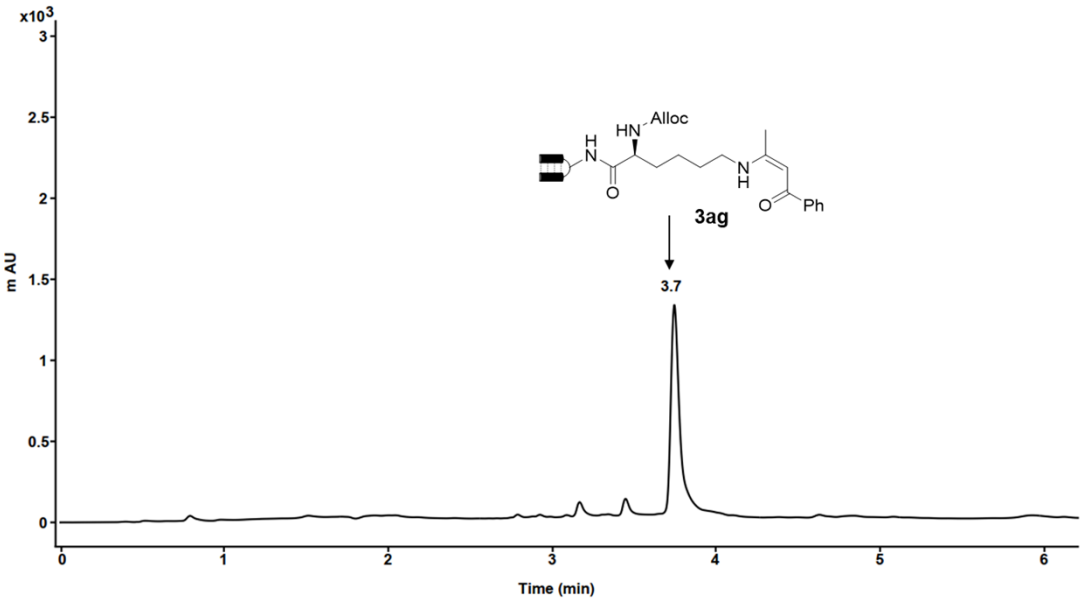


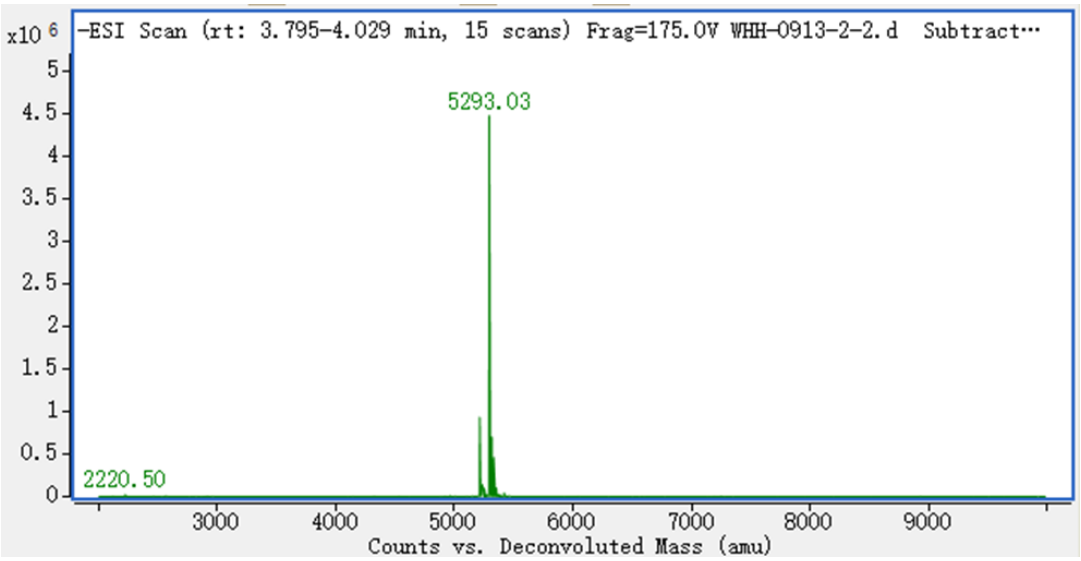


UPLC chromatogram and deconvoluted MS of **3ag**

Conversion: >90%

Calculated Mass: 5293 Da; Observed Mass: 5293 Da

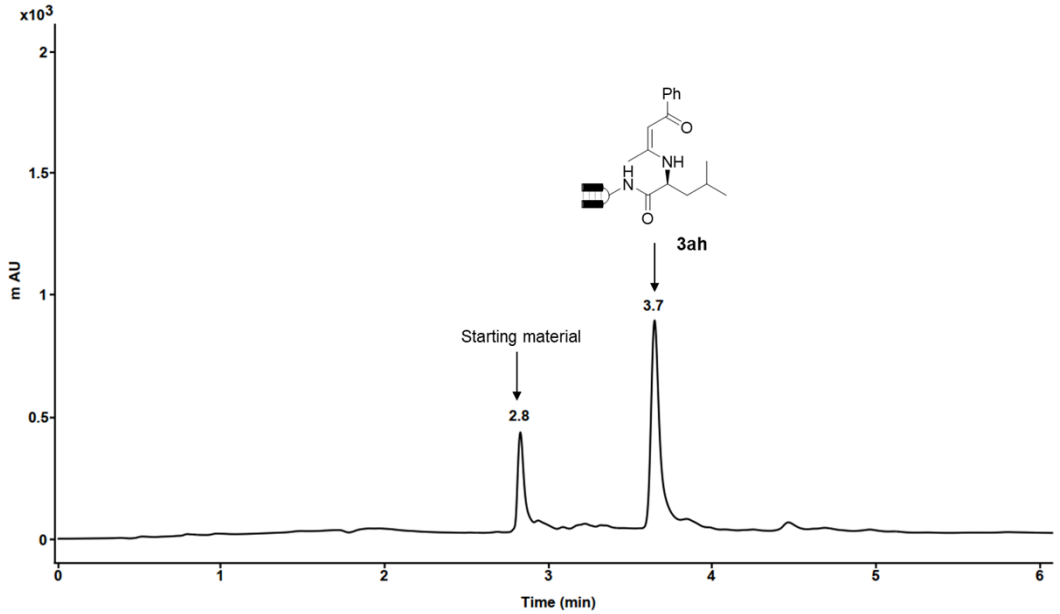


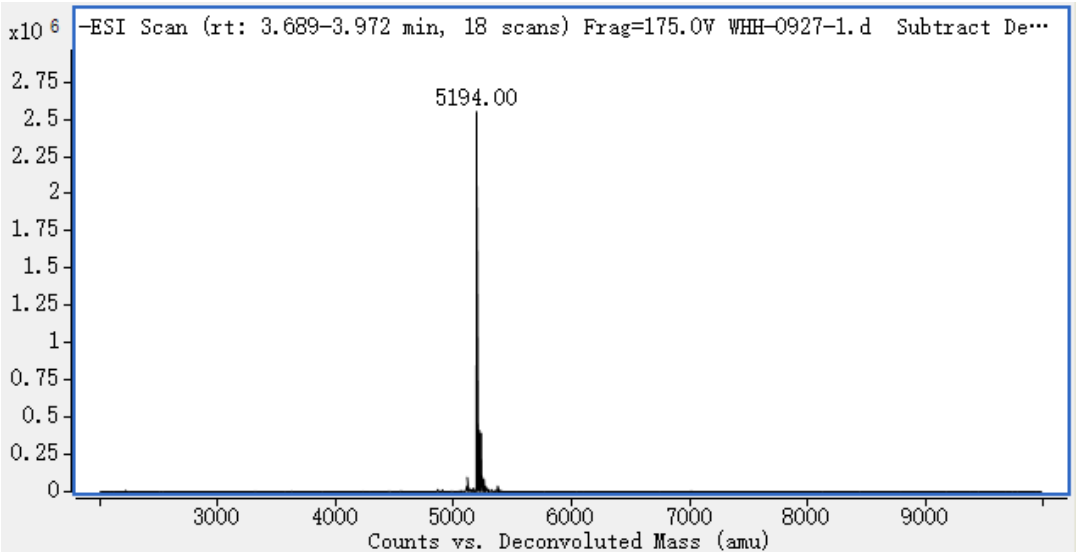


UPLC chromatogram and deconvoluted MS of **3ah**

Conversion: 69%

Calculated Mass: 5194 Da; Observed Mass: 5194 Da

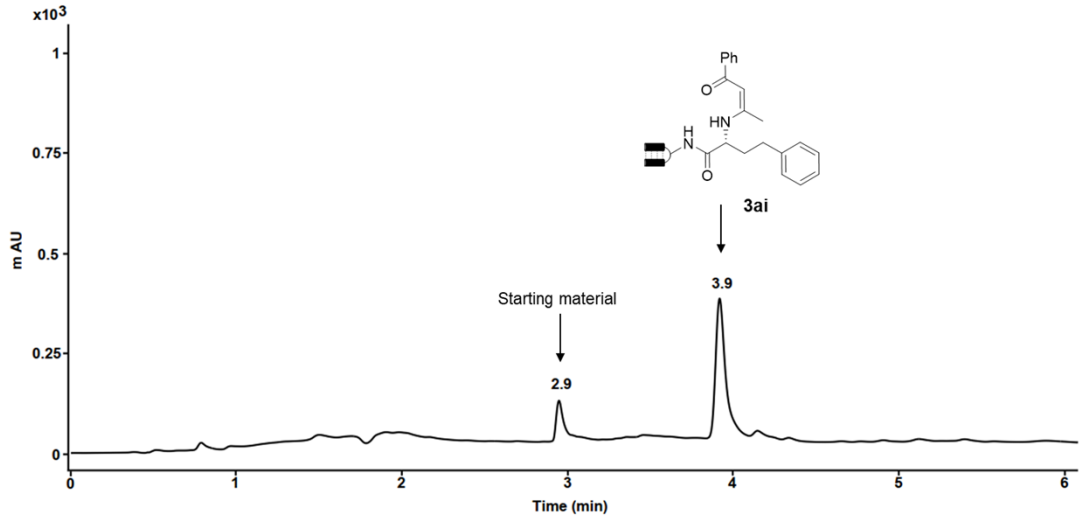


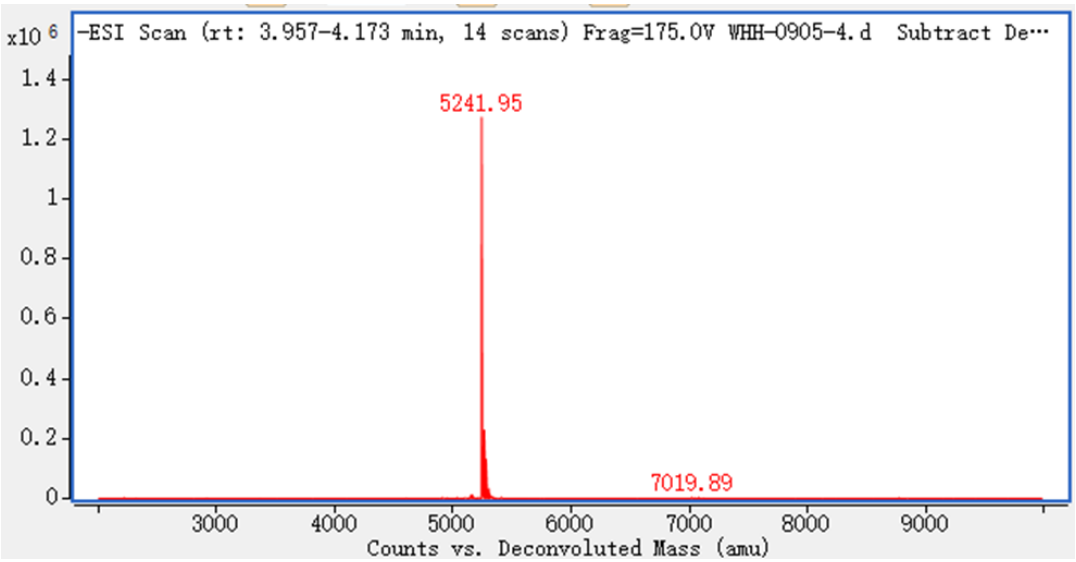


UPLC chromatogram and deconvoluted MS of **3ai**

Conversion: 76%

Calculated Mass: 5242 Da; Observed Mass: 5242 Da

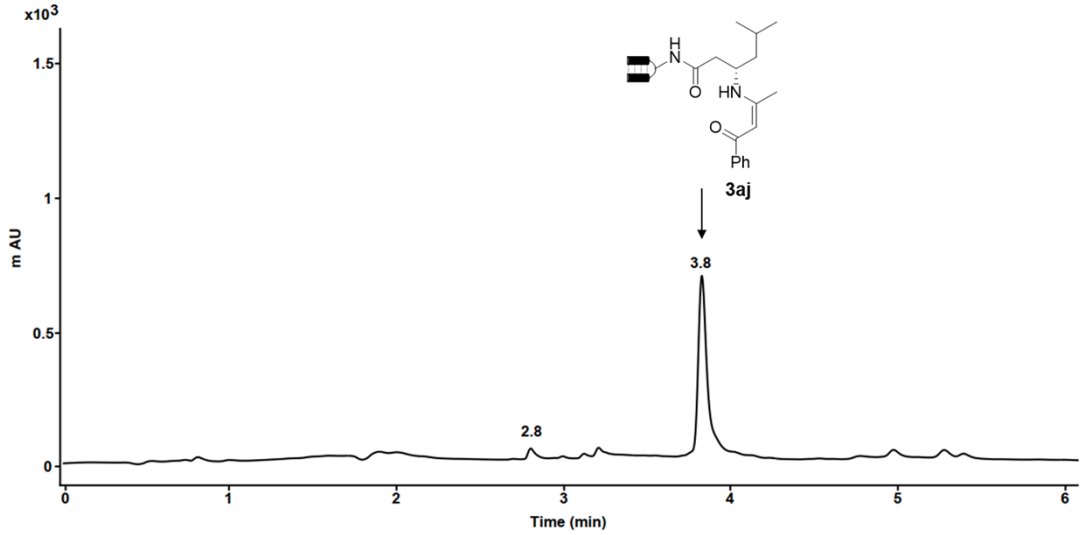


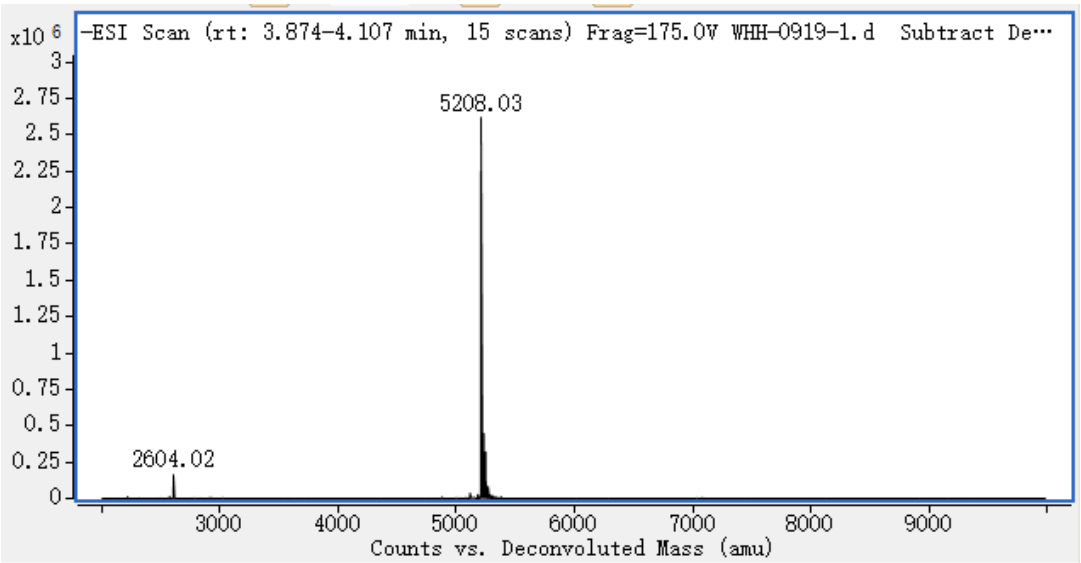


UPLC chromatogram and deconvoluted MS of **3aj**

Conversion: >90%

Calculated Mass: 5208 Da; Observed Mass: 5208 Da

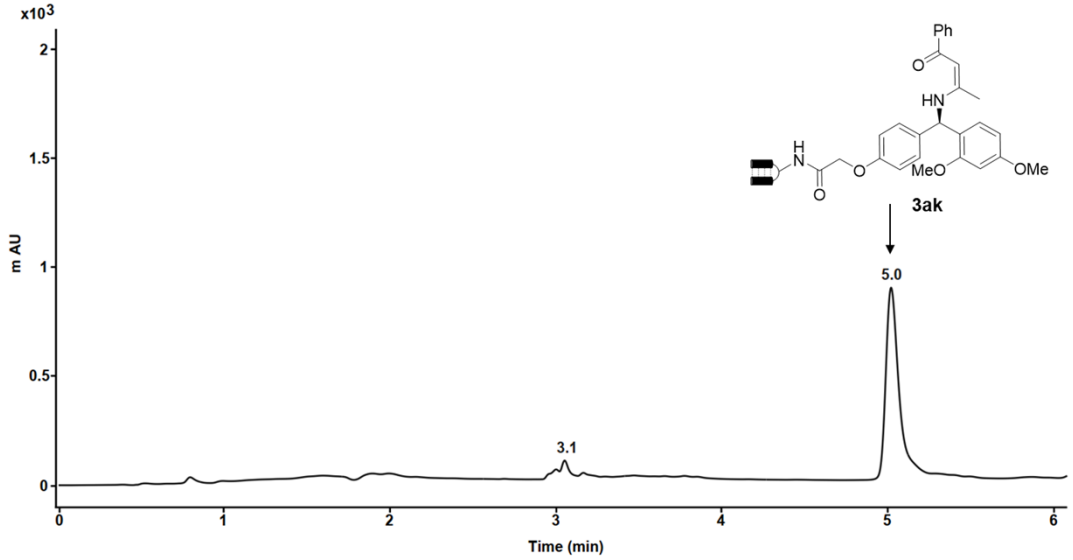


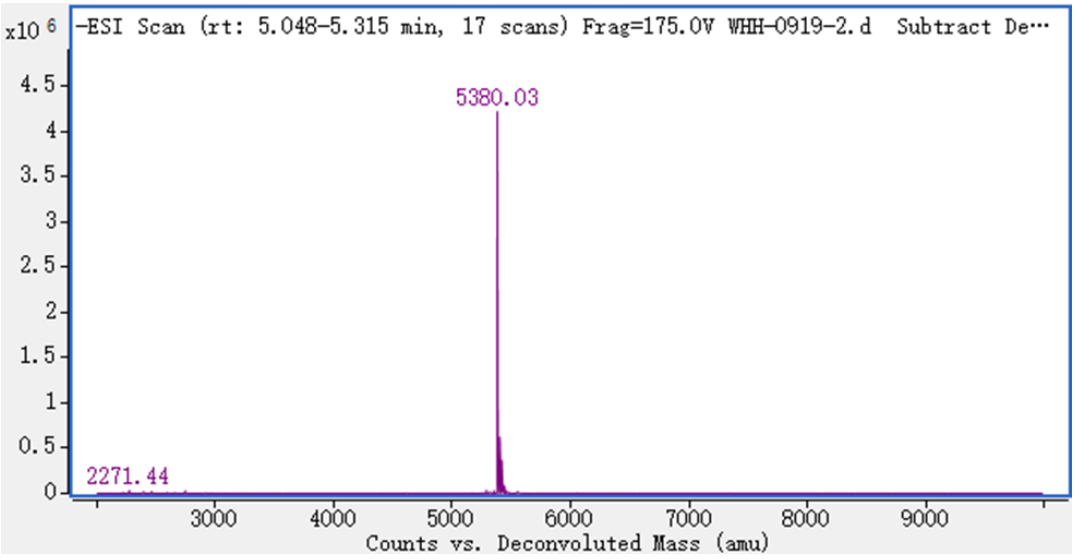


UPLC chromatogram and deconvoluted MS of **3ak**

Conversion: >90%

Calculated Mass: 5380 Da; Observed Mass: 5380 Da

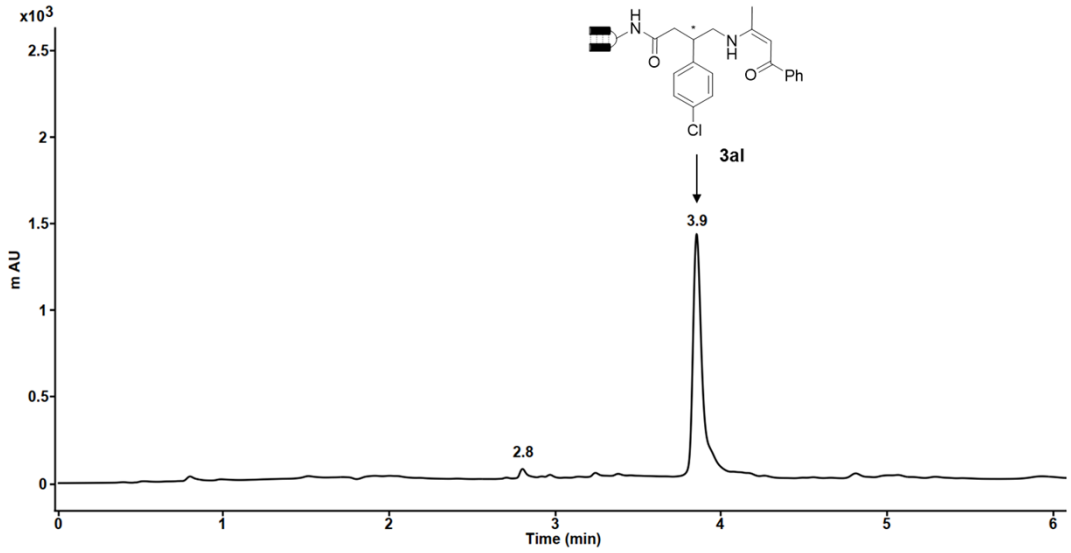


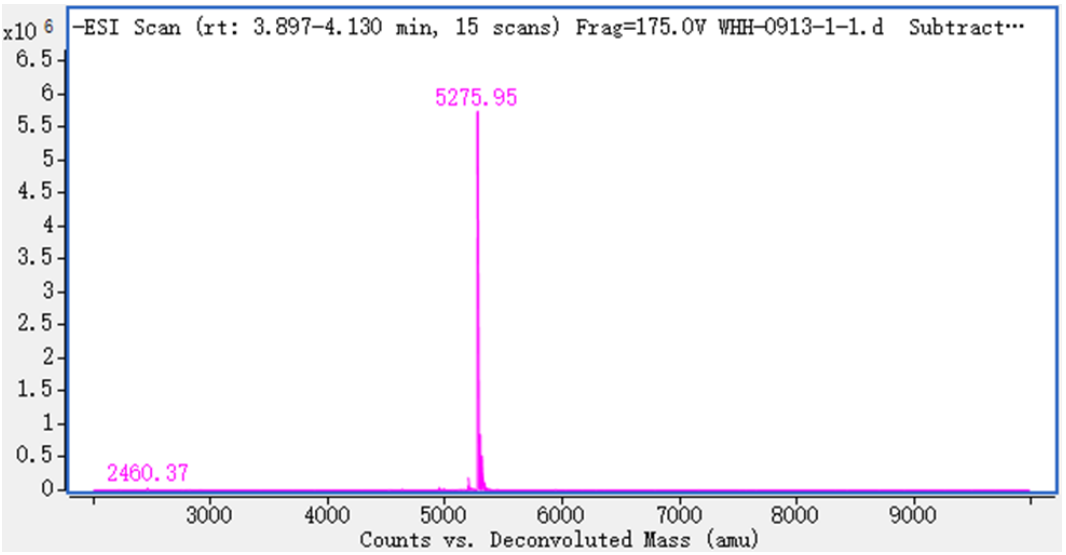


UPLC chromatogram and deconvoluted MS of **3al**

Conversion: >90%

Calculated Mass: 5276 Da; Observed Mass: 5276 Da

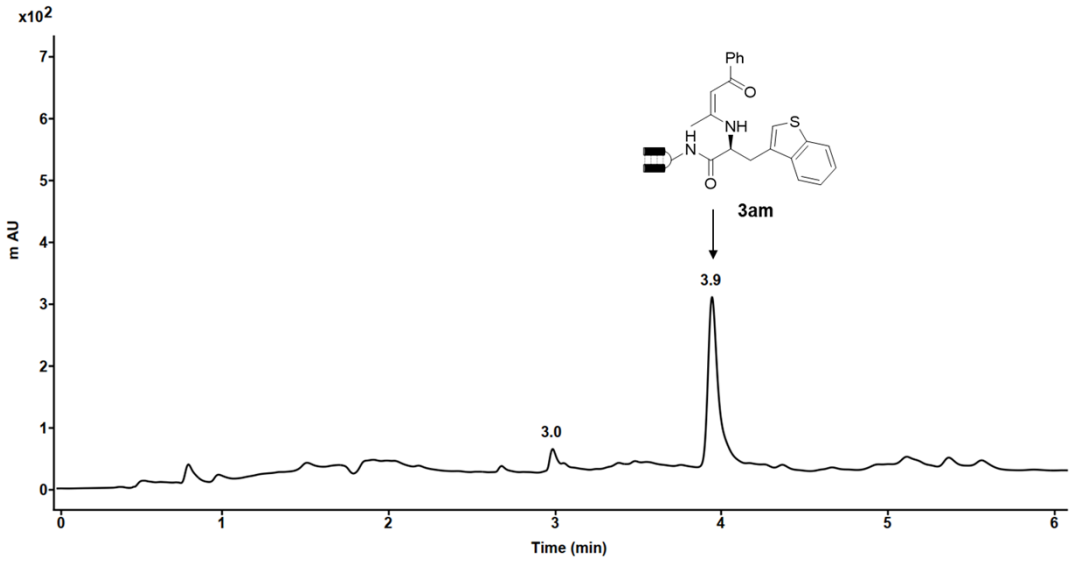


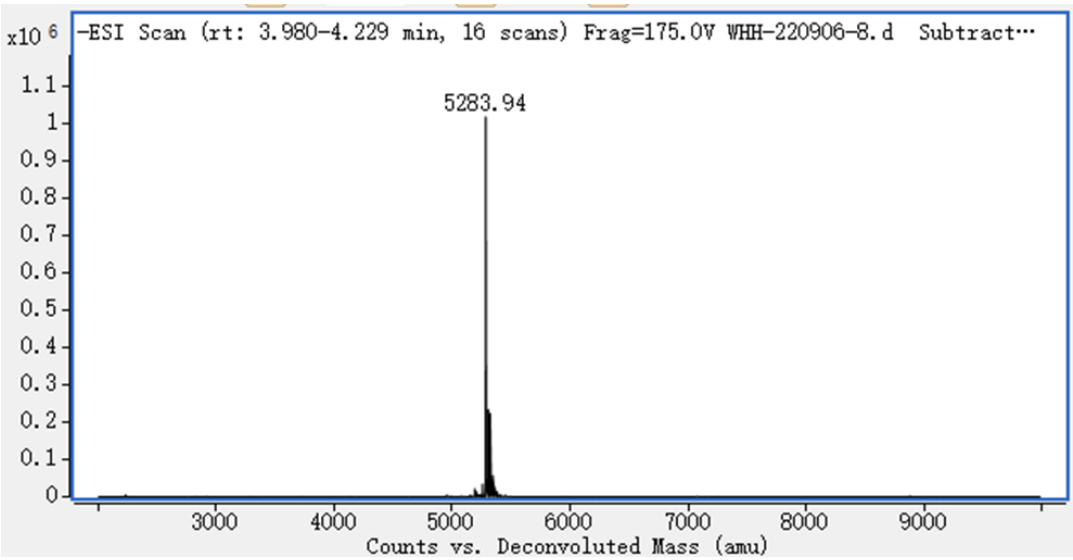


UPLC chromatogram and deconvoluted MS of **3am**

Conversion: 88%

Calculated Mass: 5284 Da; Observed Mass: 5284 Da

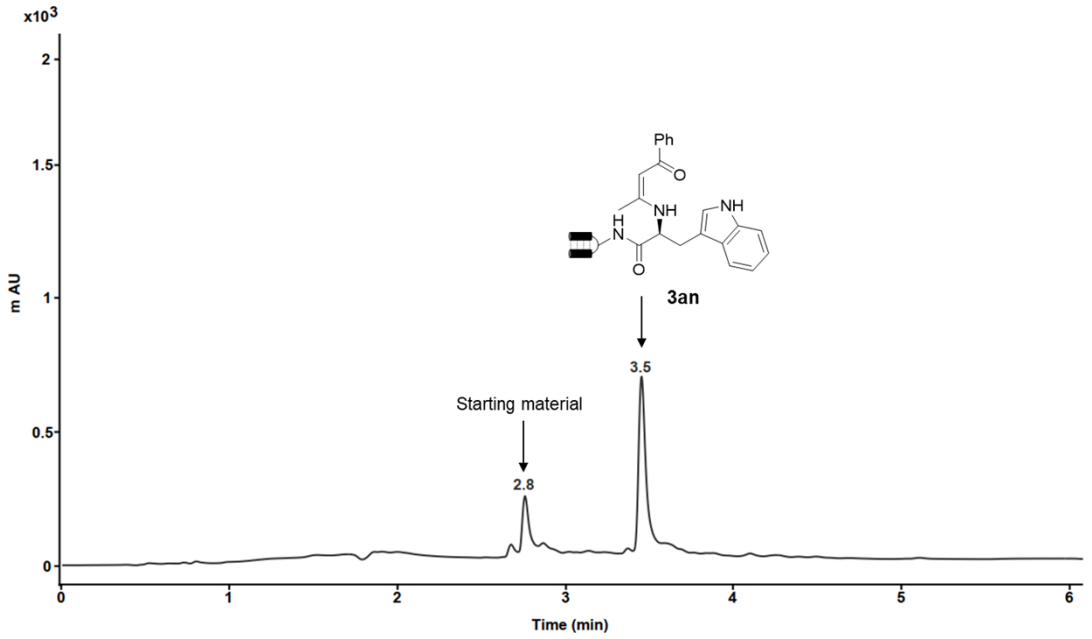


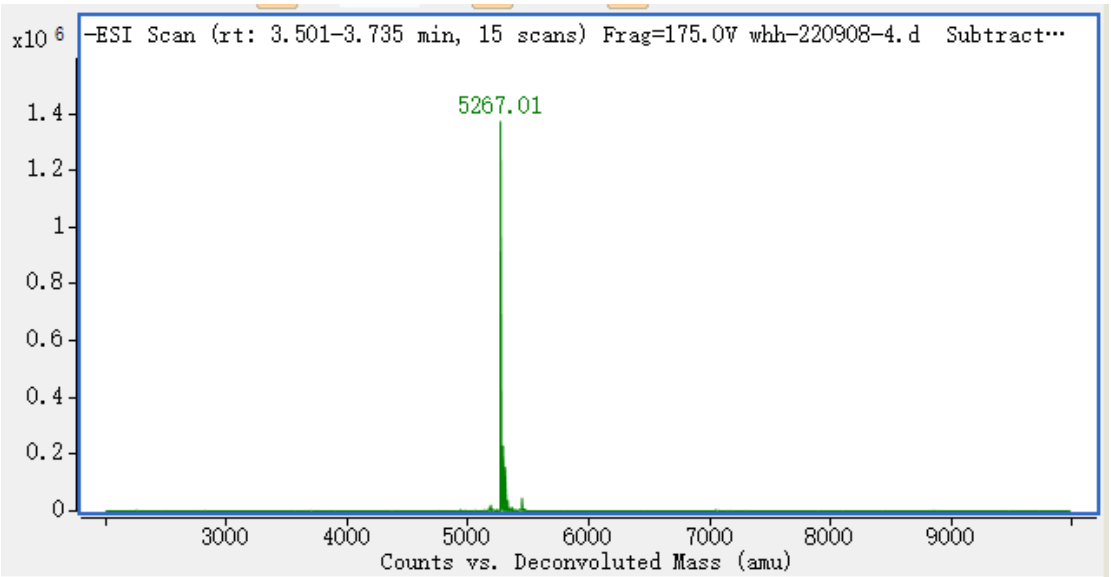


UPLC chromatogram and deconvoluted MS of **3an**

Conversion: 74%

Calculated Mass: 5267 Da; Observed Mass: 5267 Da

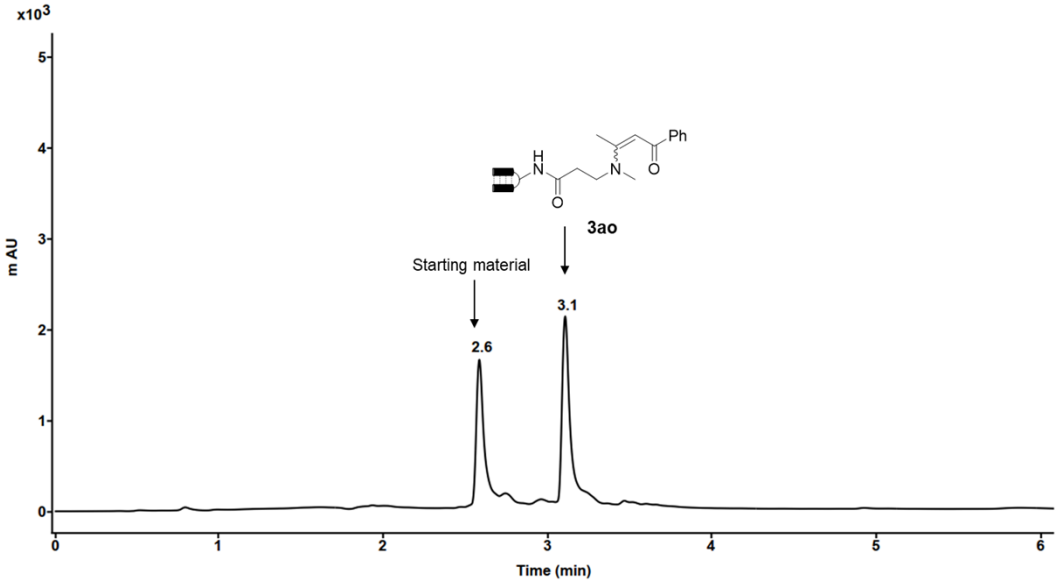


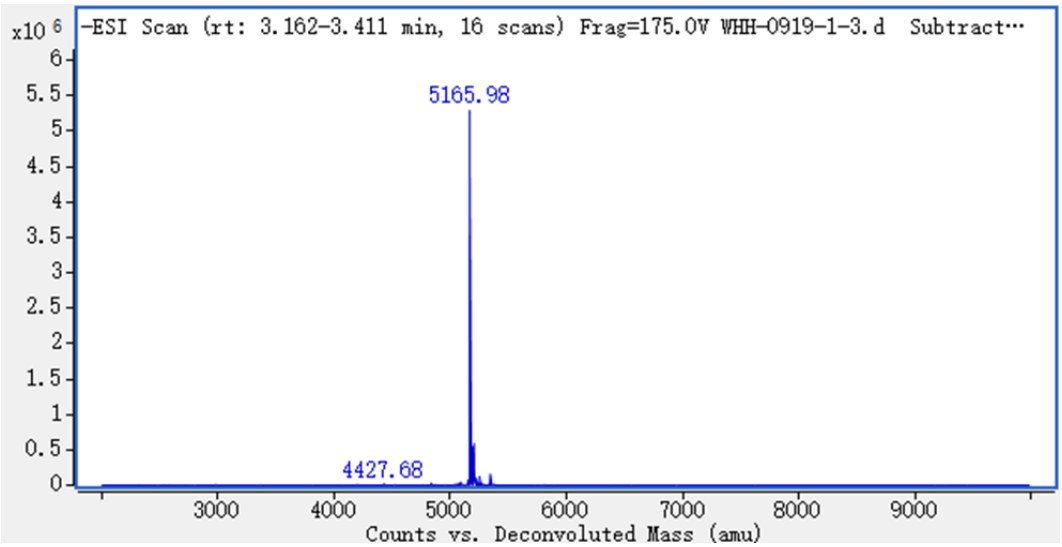


UPLC chromatogram and deconvoluted MS of **3ao**

Conversion: 56%

Calculated Mass: 5166 Da; Observed Mass: 5166 Da

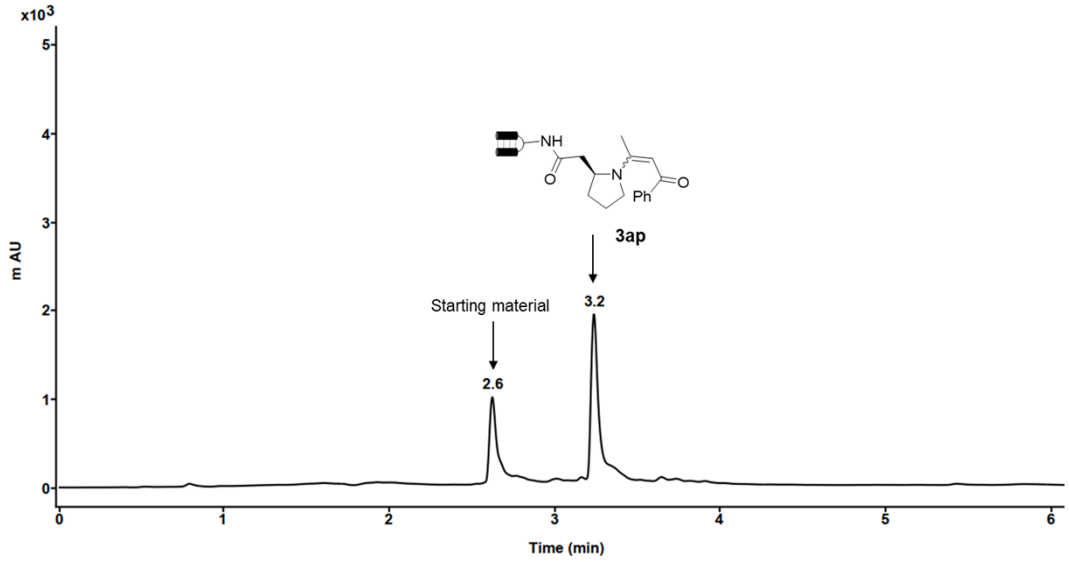


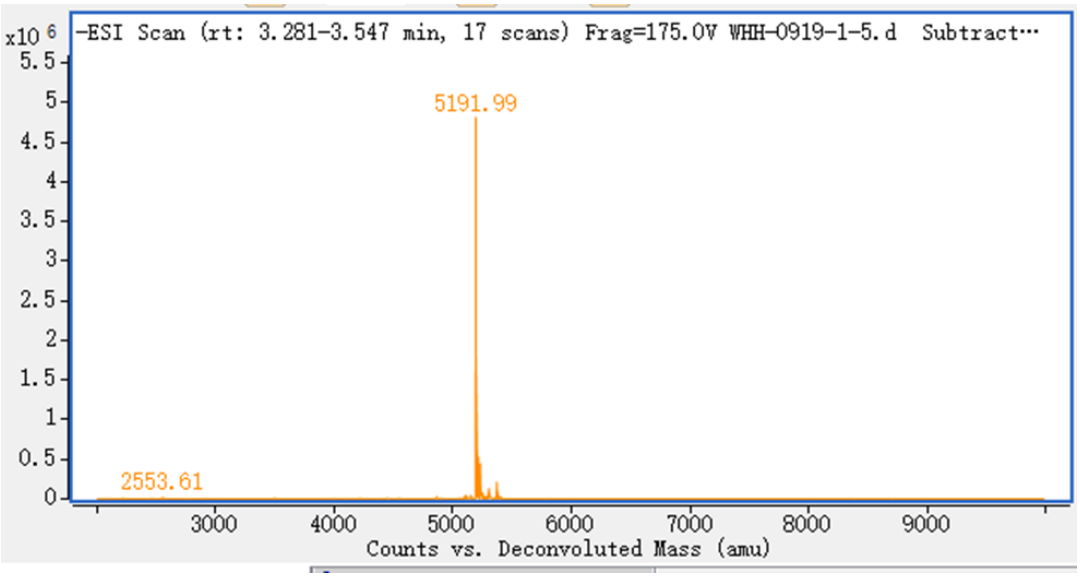


UPLC chromatogram and deconvoluted MS of **3ap**

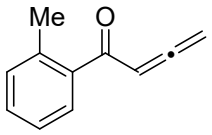
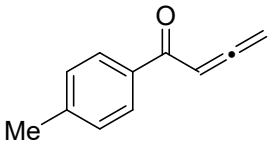
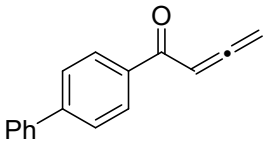
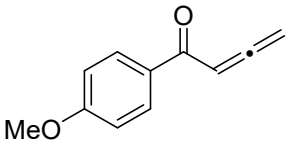
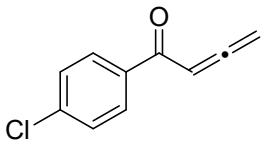
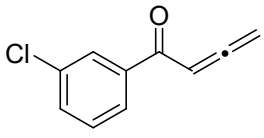
Conversion: 67%

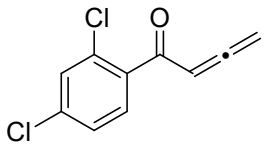
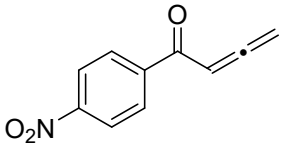
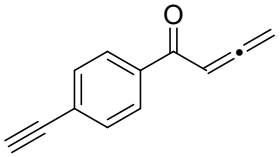
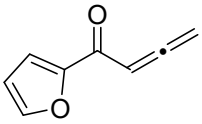
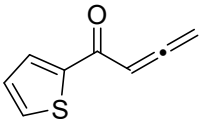
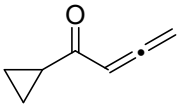
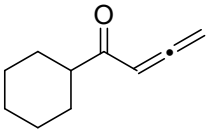
Calculated Mass: 5192 Da; Observed Mass: 5192 Da

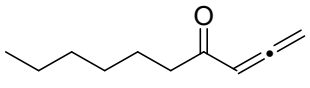
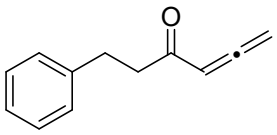
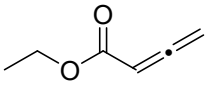




9.3 Substrate scope of allenic ketones 2b-2p

Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
2b		**3bb**	5228	5228	>90%
2b		**3bc**	5228	5228	>90%
2c		**3bd**	5290	5290	>90%
2d		**3be**	5244	5244	68%
2e		**3bf**	5248	5248	>90%
2f		**3bg**	5248	5248	>90%

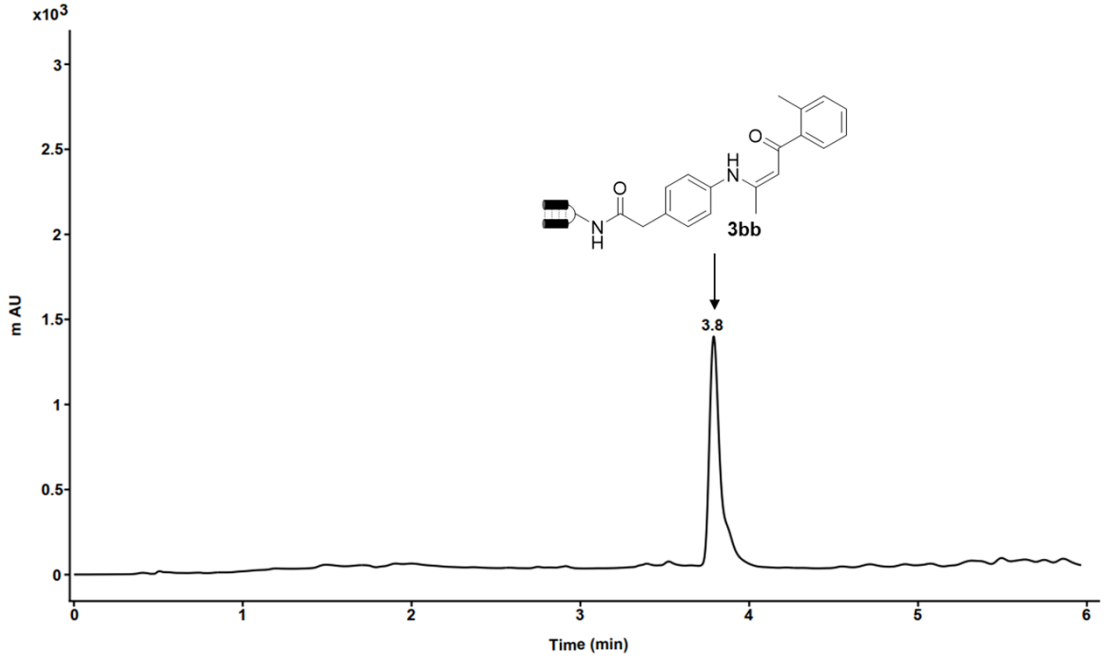
2g		**3bh**	5282	5283	>90%
2h		**3bi**	5259	5259	42%
2i		**3bj**	5238	5238	>90%
2j		**3bk**	5204	5204	>90%
2k		**3bl**	5220	5220	83%
2l		**3bm**	5178	5178	>90%
2m		**3bn**	5220	5220	87%

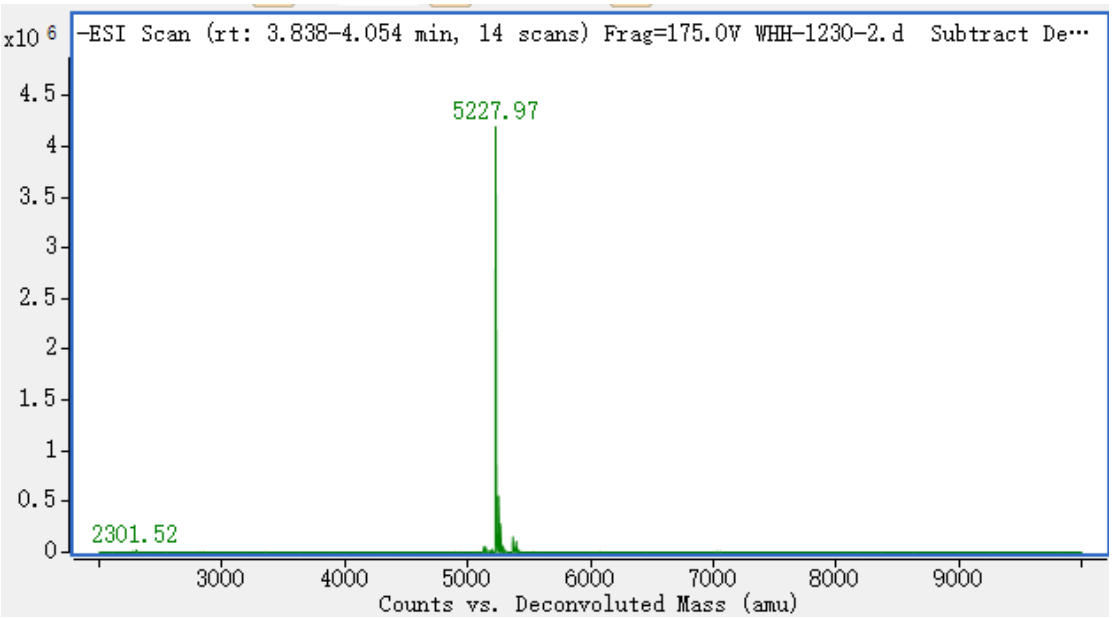
2n		**3bo**	5222	5222	>90%
2o		**3bp**	5242	5242	>90%
2p		**3bq**	5182		0%

UPLC chromatogram and deconvoluted MS of **3bb**

Conversion: >90%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

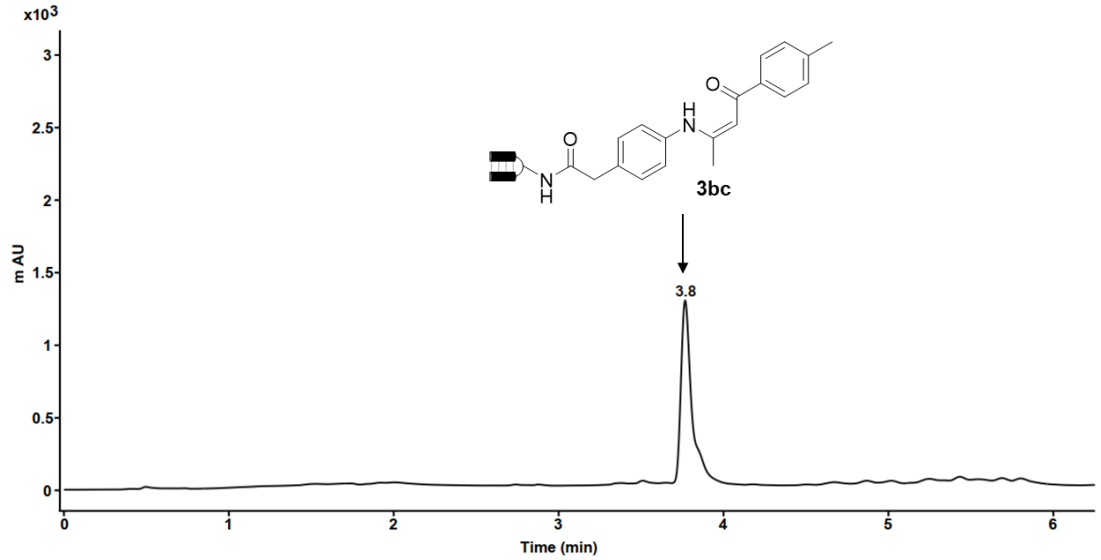


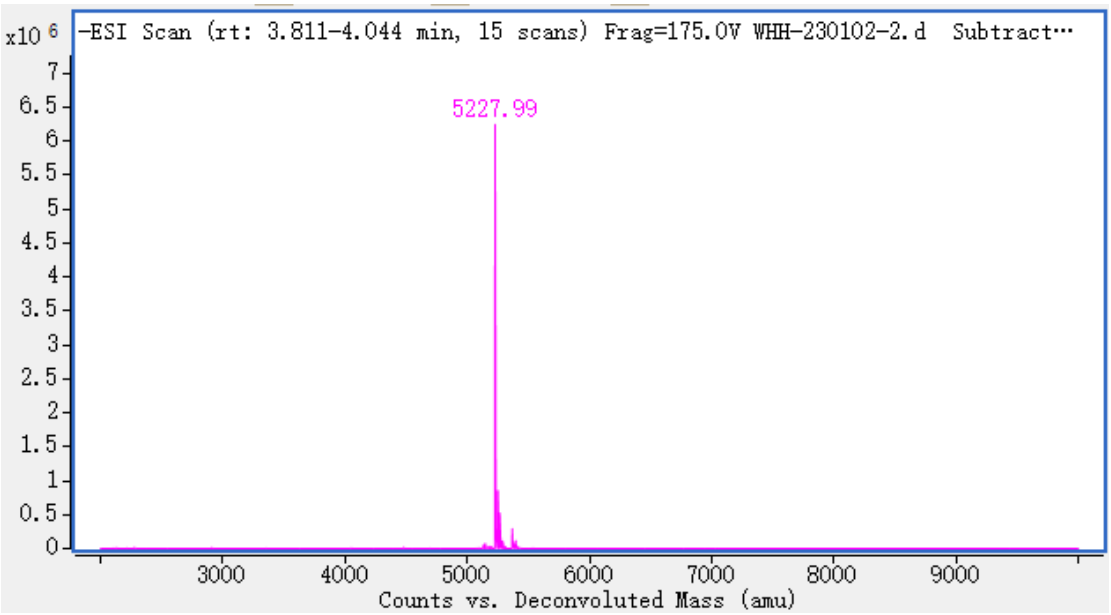


UPLC chromatogram and deconvoluted MS of **3bc**

Conversion: >90%

Calculated Mass: 5228 Da; Observed Mass: 5228Da

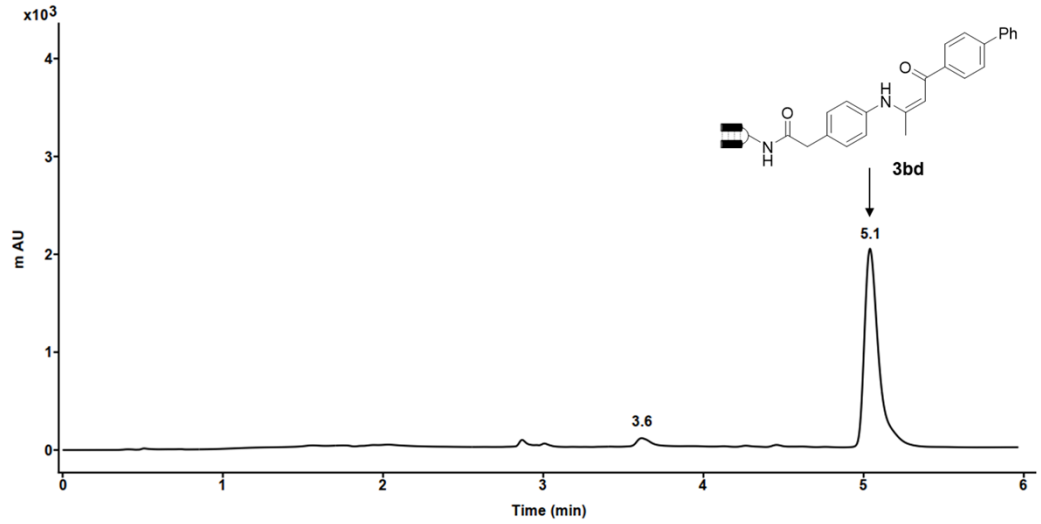


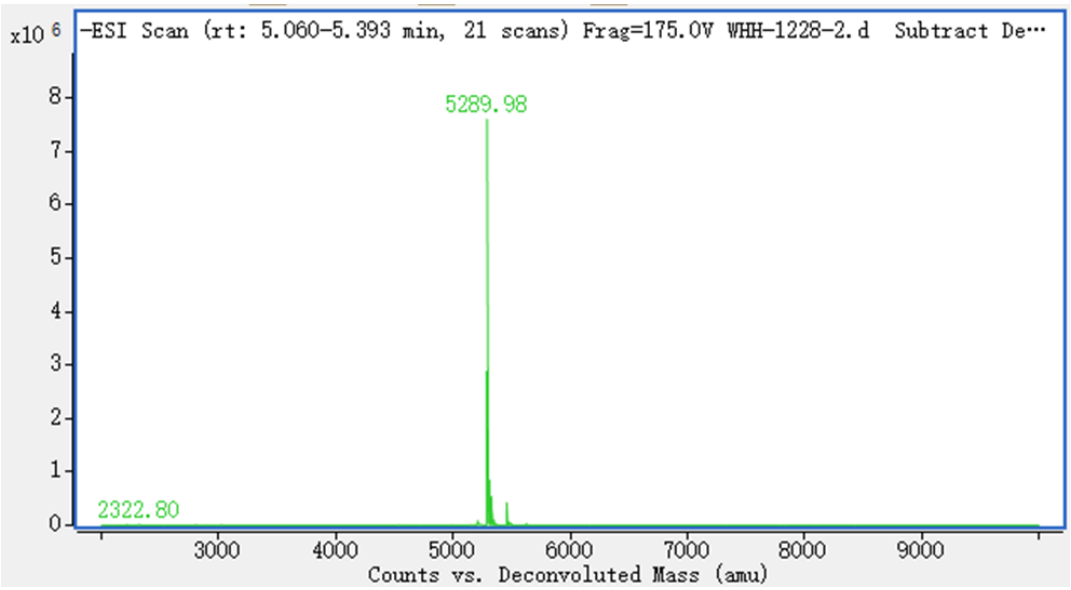


UPLC chromatogram and deconvoluted MS of **3bd**

Conversion: >90%

Calculated Mass: 5290 Da; Observed Mass: 5290 Da

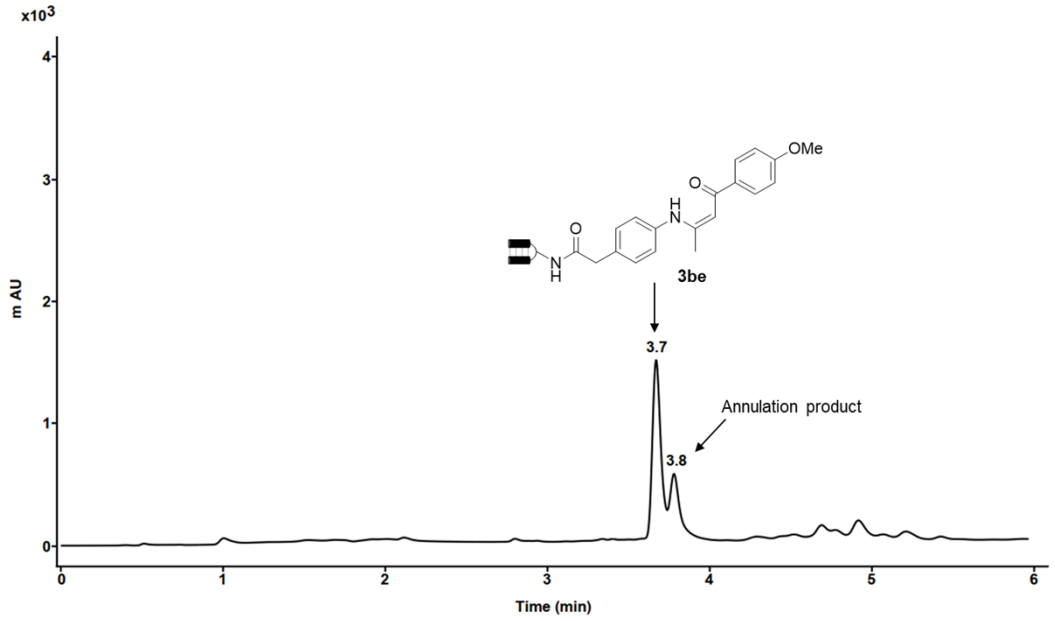


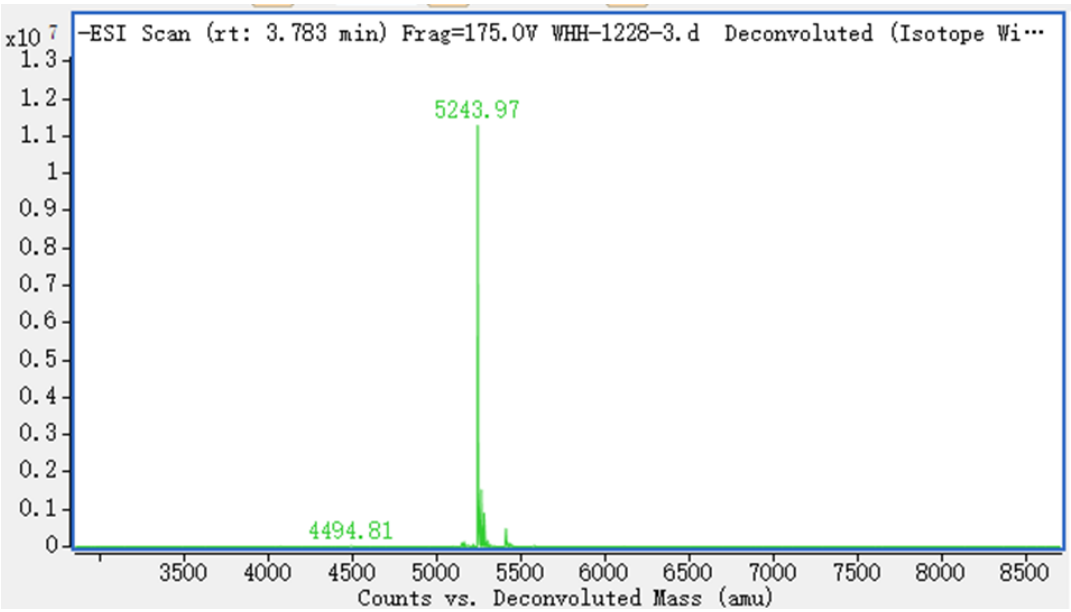


UPLC chromatogram and deconvoluted MS of **3be**

Conversion: 68%

Calculated Mass: 5244 Da; Observed Mass: 5244 Da

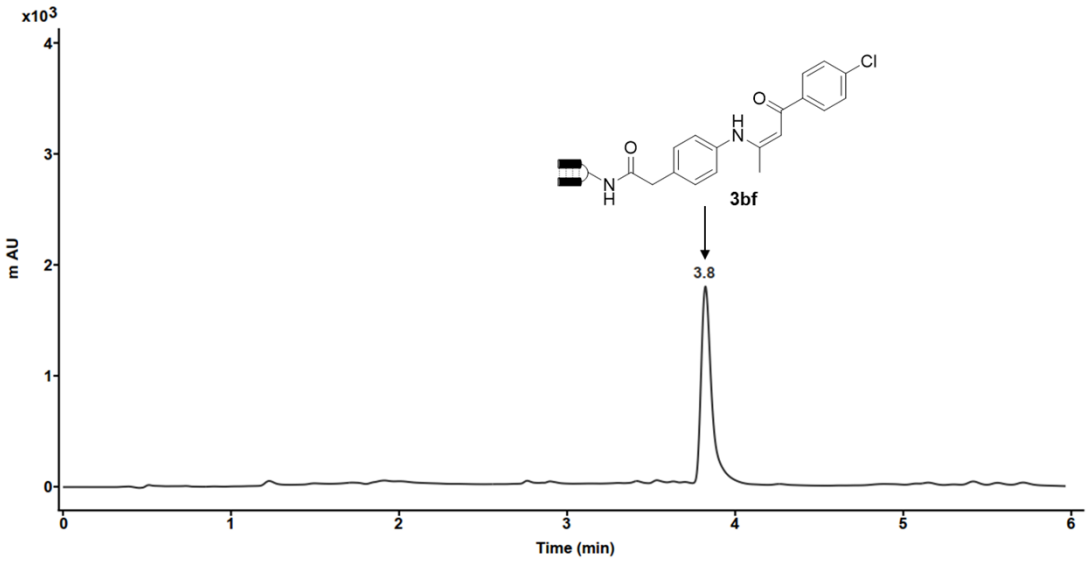


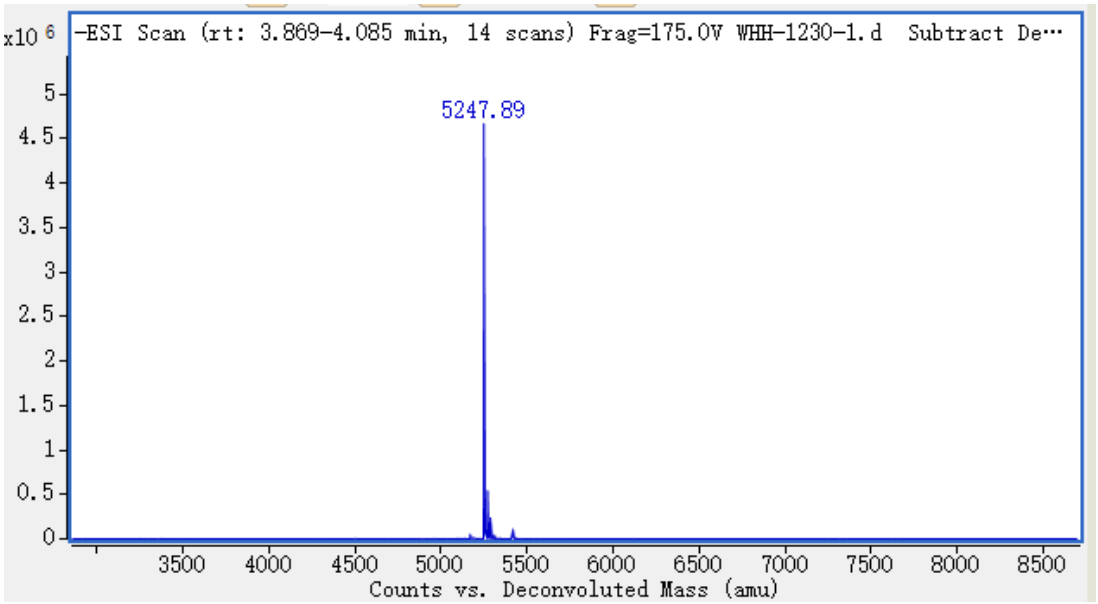


UPLC chromatogram and deconvoluted MS of **3bf**

Conversion: >90%

Calculated Mass: 5248 Da; Observed Mass: 5248 Da

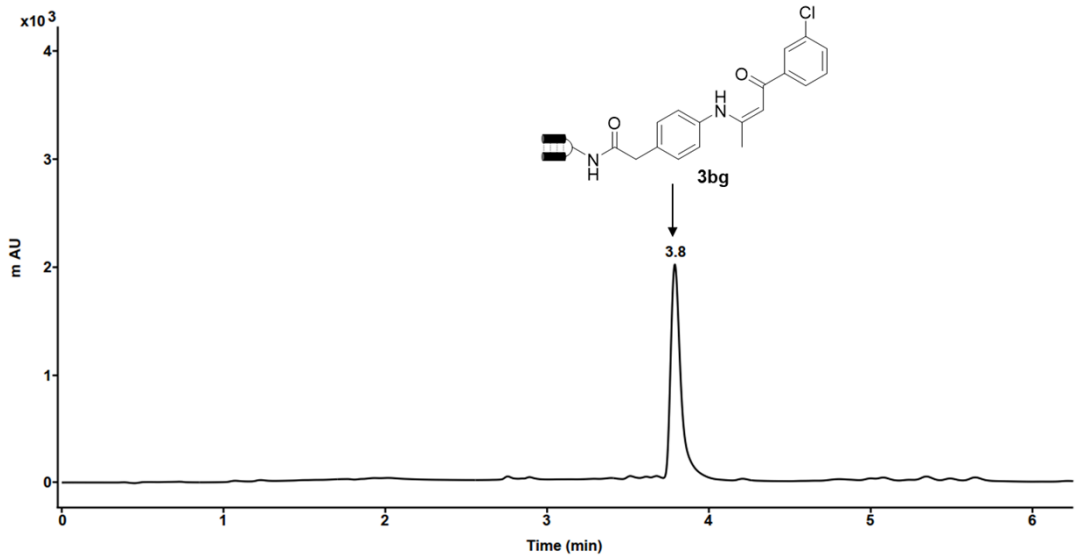


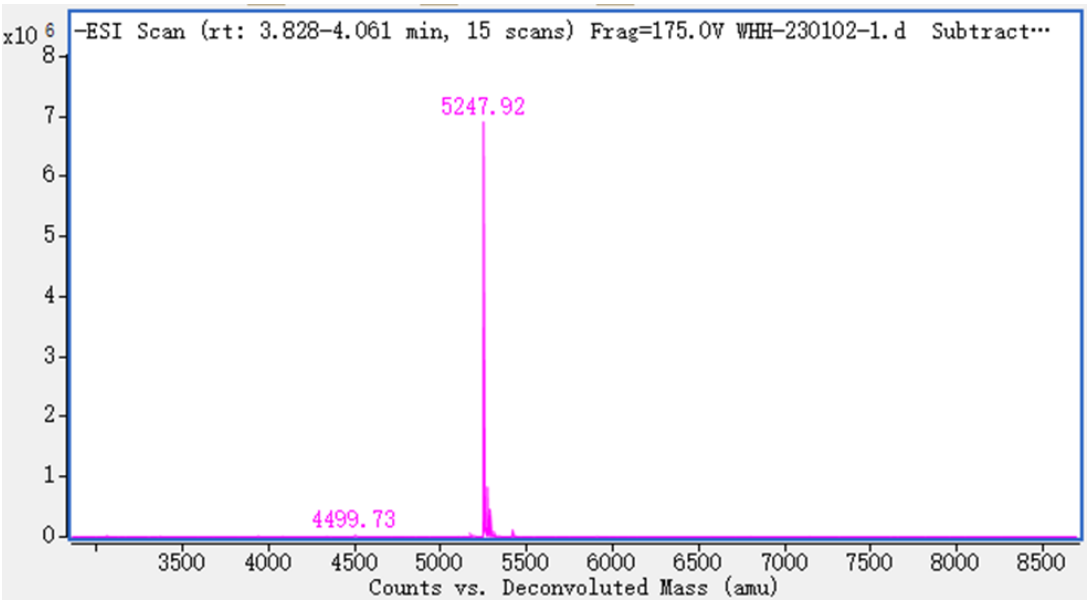


UPLC chromatogram and deconvoluted MS of **3bg**

Conversion: >90%

Calculated Mass: 5248 Da; Observed Mass: 5248 Da

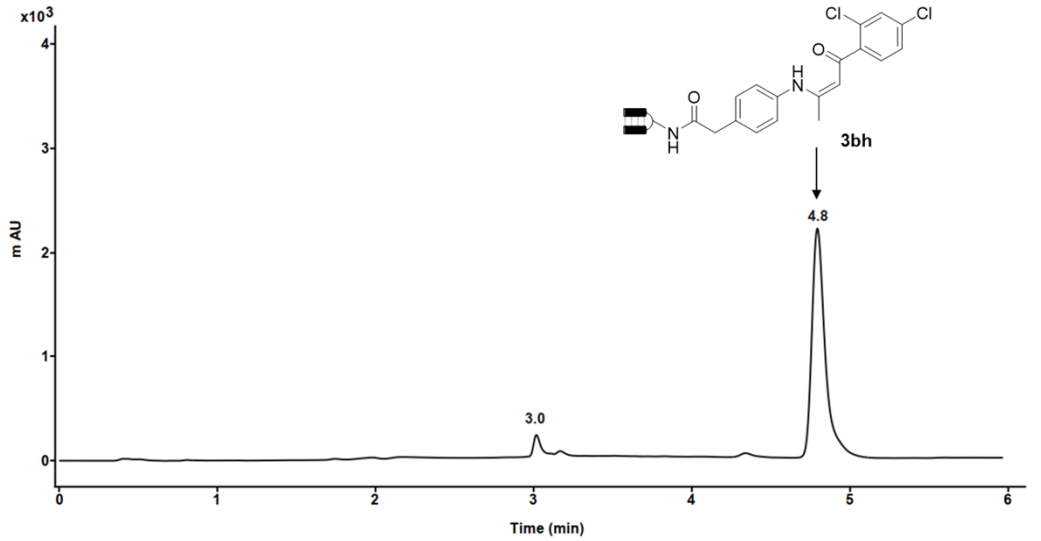


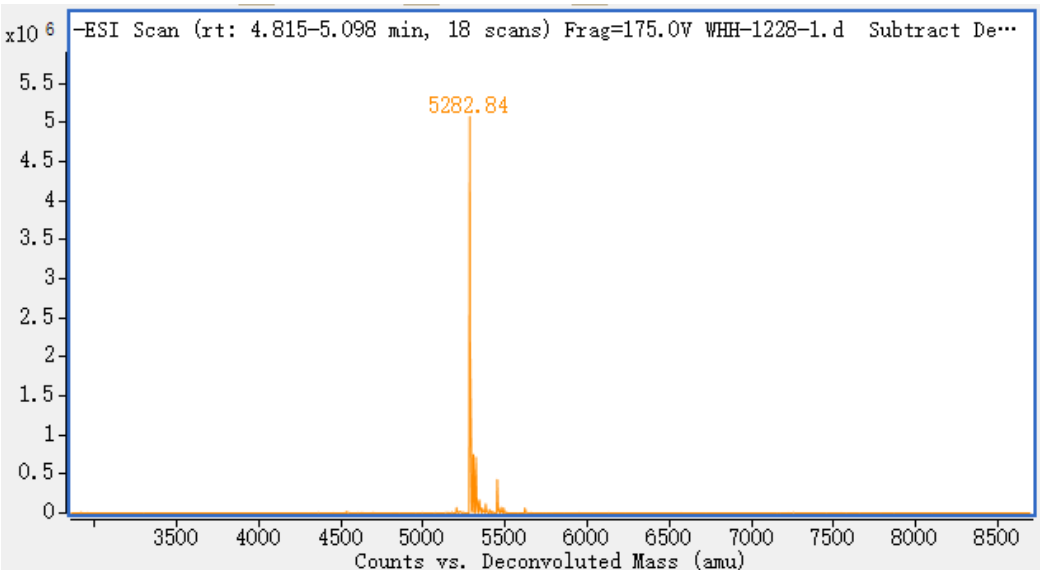


UPLC chromatogram and deconvoluted MS of **3bh**

Conversion: >90%

Calculated Mass: 5282 Da; Observed Mass: 5283 Da

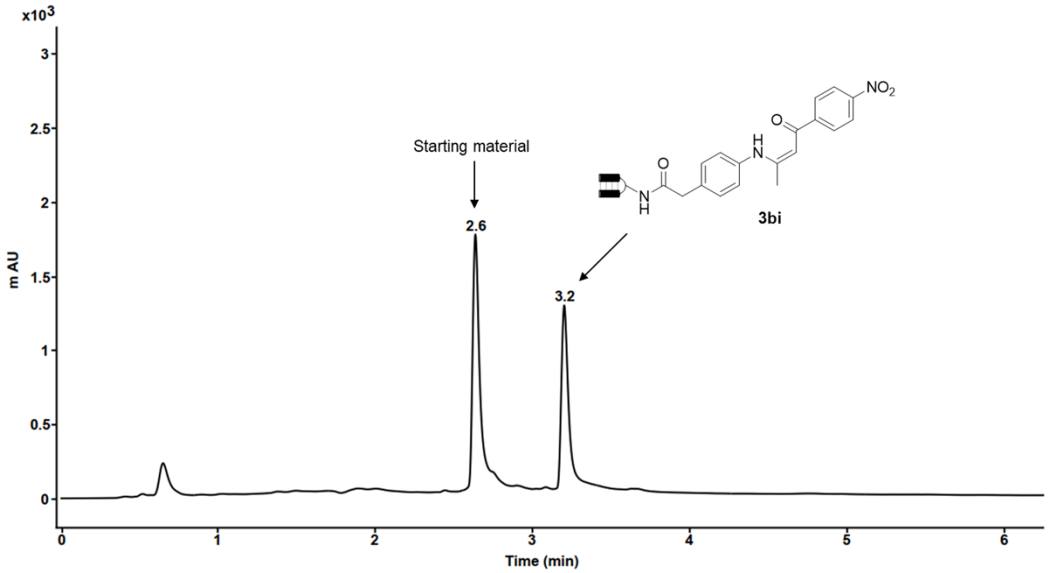


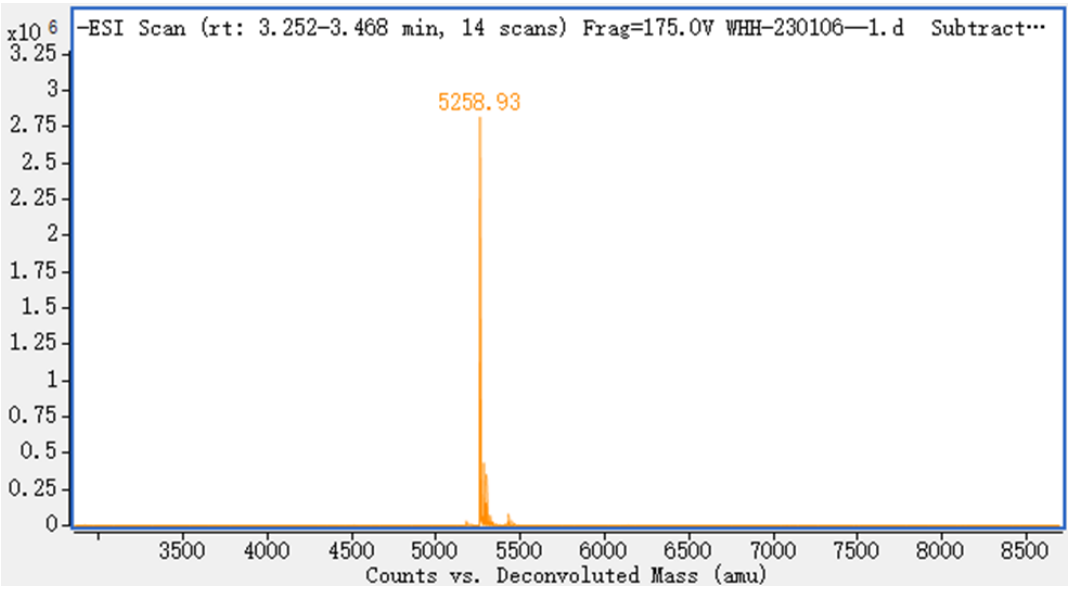


UPLC chromatogram and deconvoluted MS of **3bi**

Conversion: 42%

Calculated Mass: 5259 Da; Observed Mass: 5259 Da

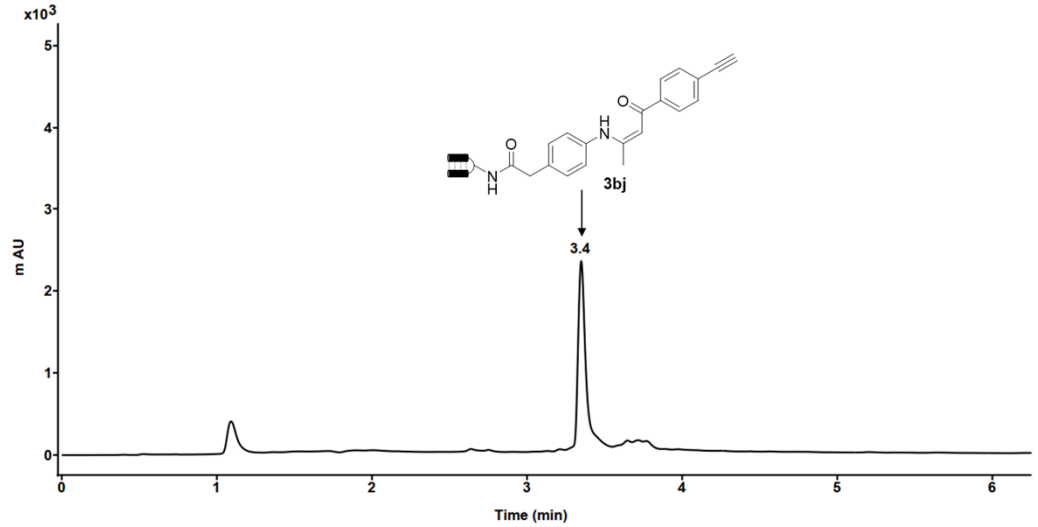


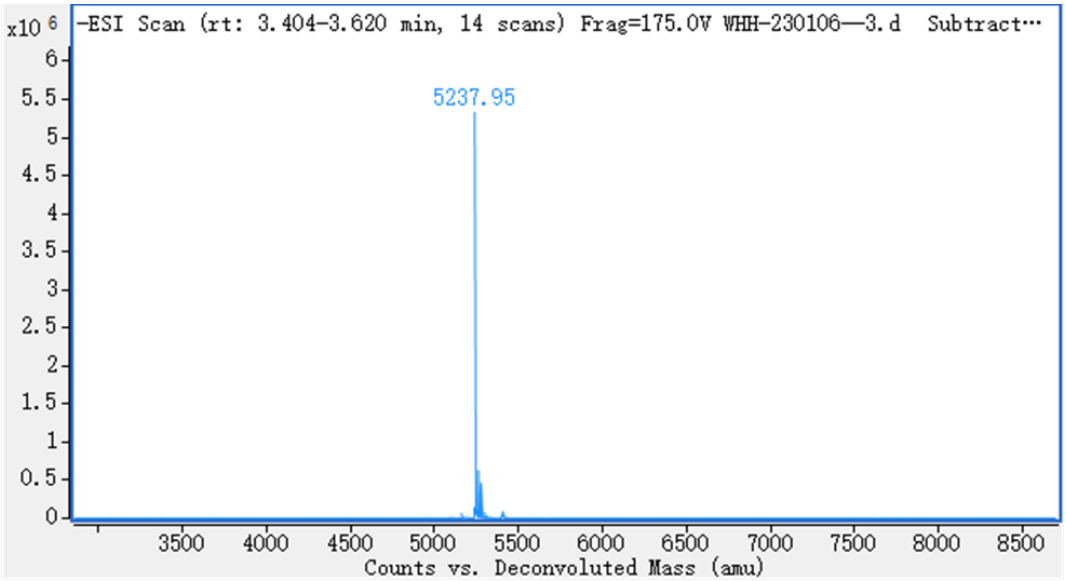


UPLC chromatogram and deconvoluted MS of **3bj**

Conversion: >90%

Calculated Mass: 5238 Da; Observed Mass: 5238 Da

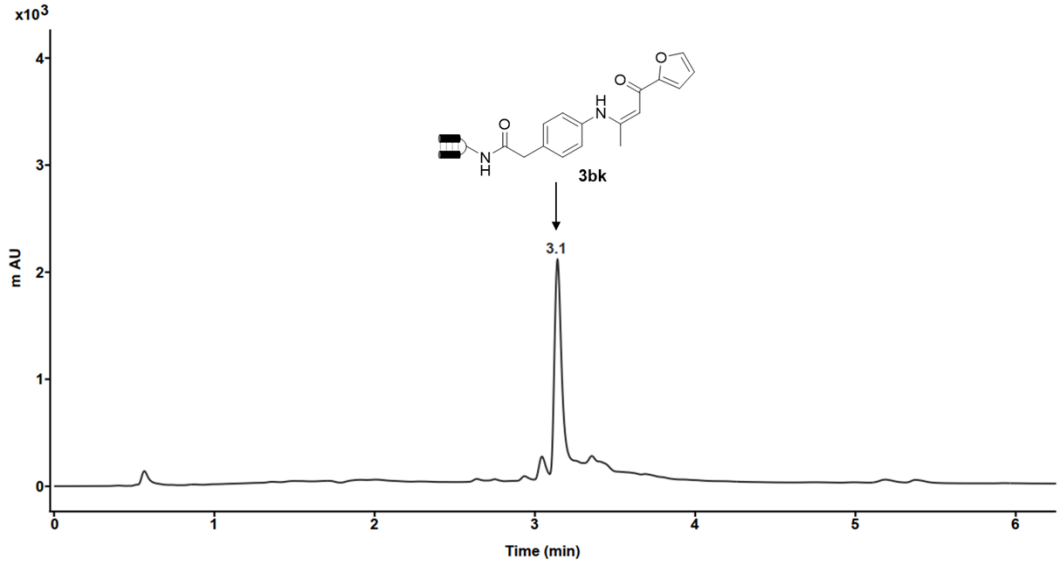


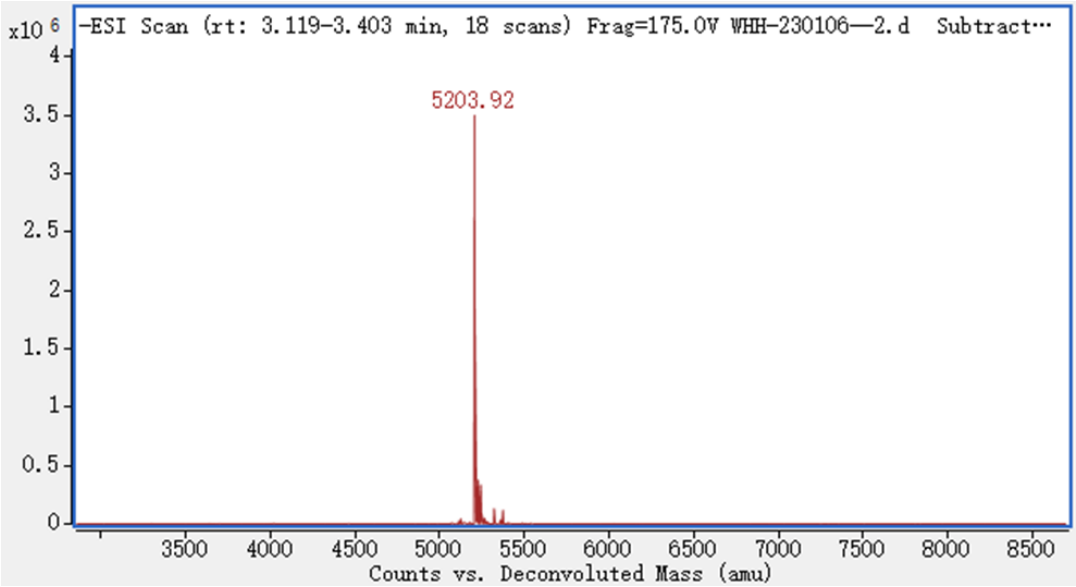


UPLC chromatogram and deconvoluted MS of **3bk**

Conversion: >90%

Calculated Mass: 5204 Da; Observed Mass: 5204 Da

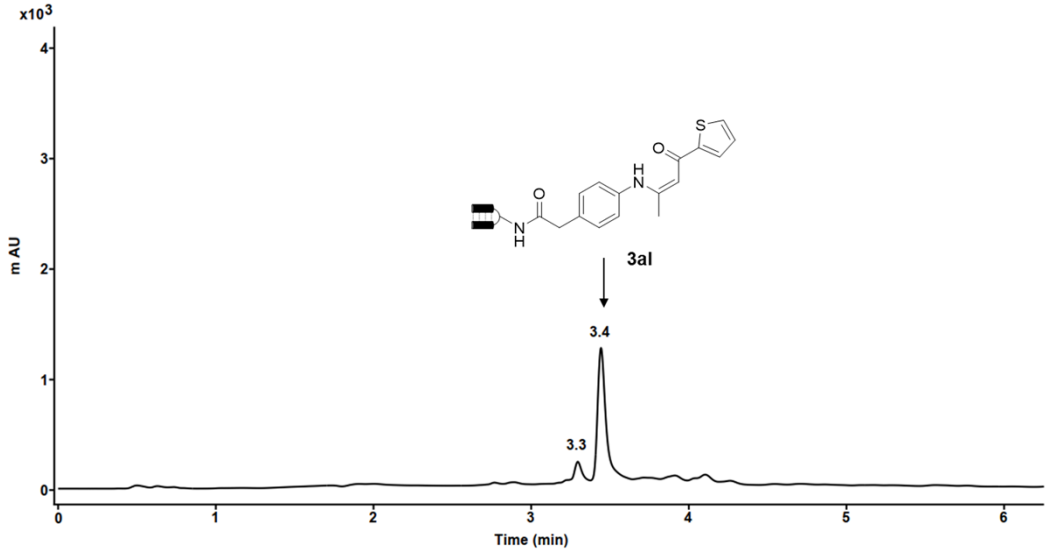


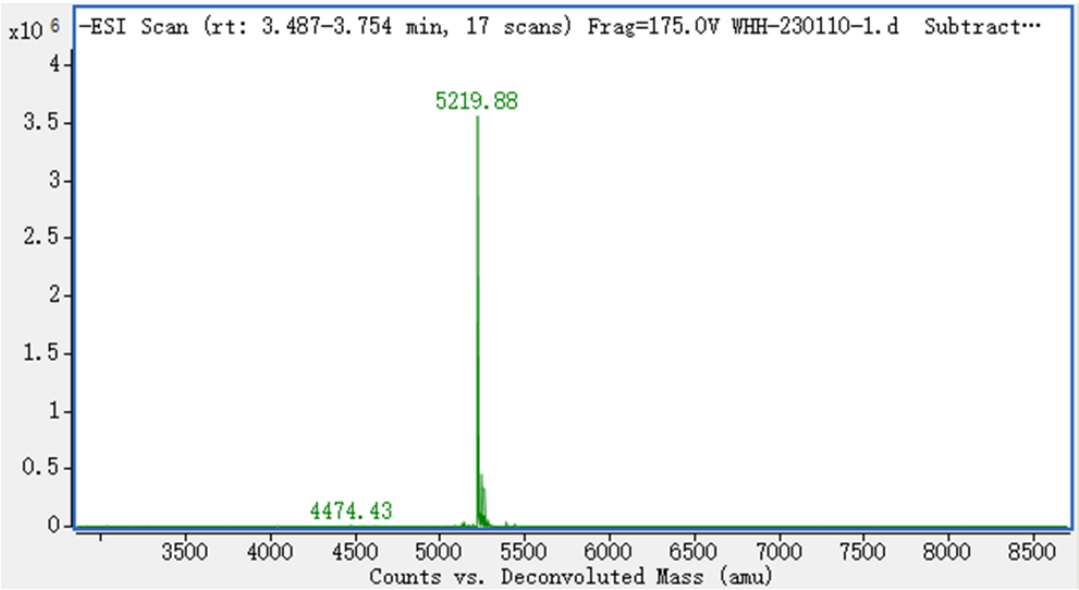


UPLC chromatogram and deconvoluted MS of **3bl**

Conversion: 83%

Calculated Mass: 5220 Da; Observed Mass: 5220 Da

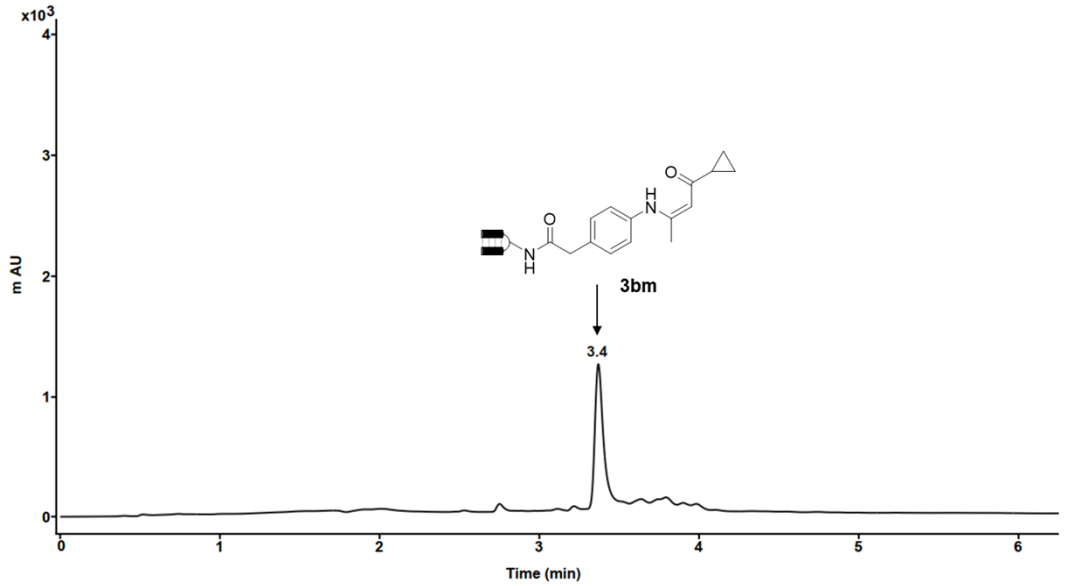


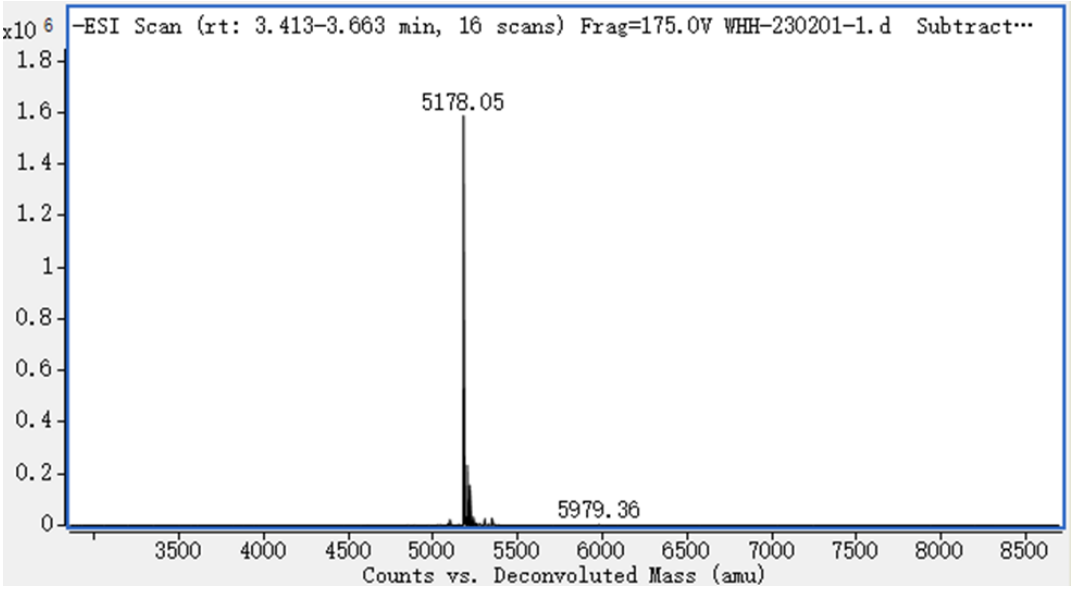


UPLC chromatogram and deconvoluted MS of **3bm**

Conversion: >90%

Calculated Mass: 5178 Da; Observed Mass: 5178 Da

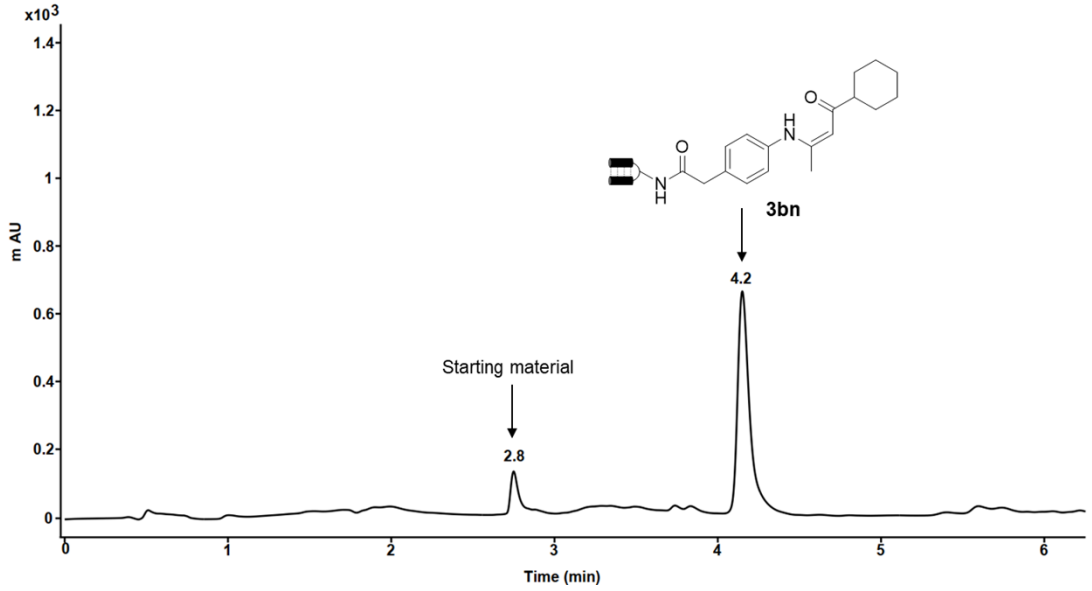


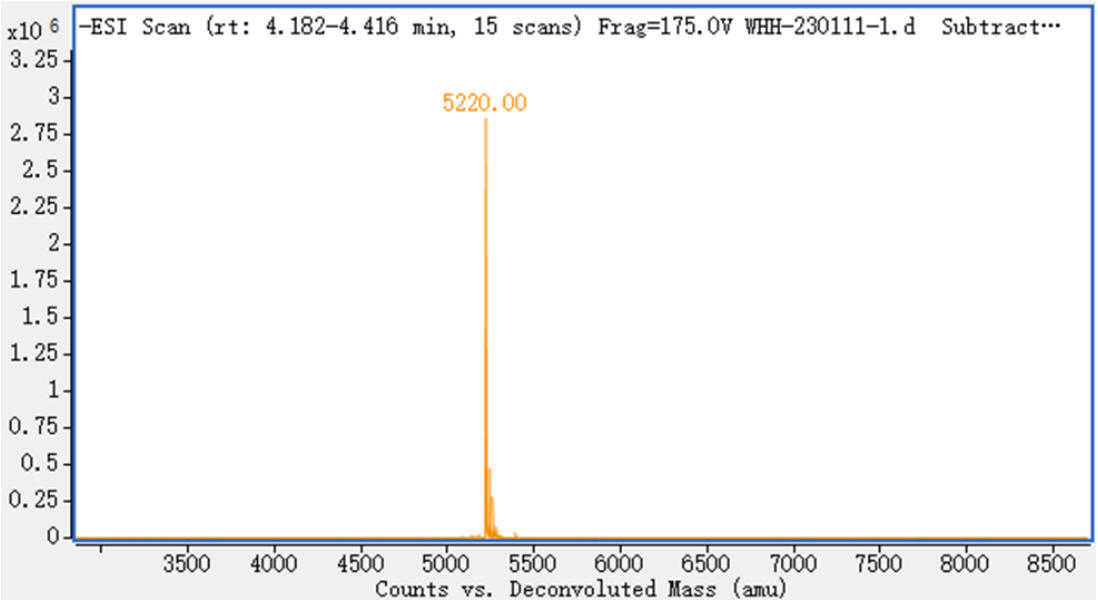


UPLC chromatogram and deconvoluted MS of **3bn**

Conversion: 87%

Calculated Mass: 5220 Da; Observed Mass: 5220 Da

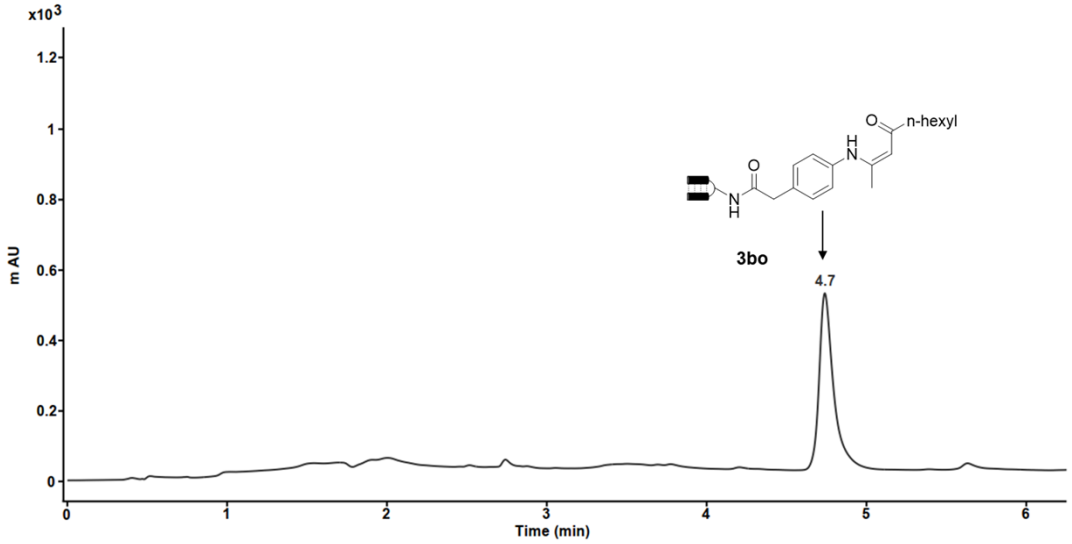


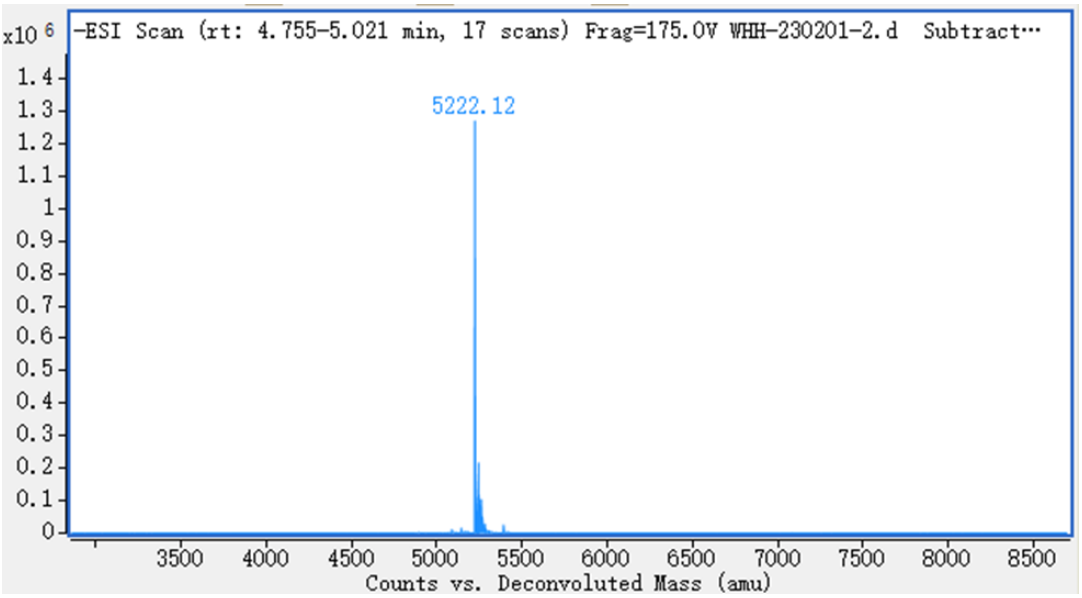


UPLC chromatogram and deconvoluted MS of **3bo**

Conversion: >90%

Calculated Mass: 5222 Da; Observed Mass: 5222 Da

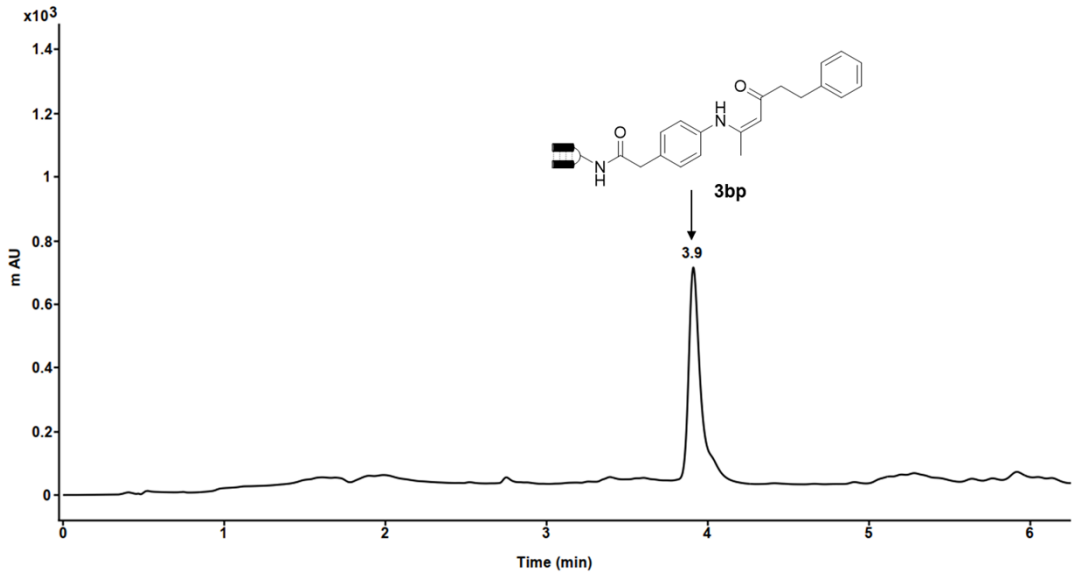


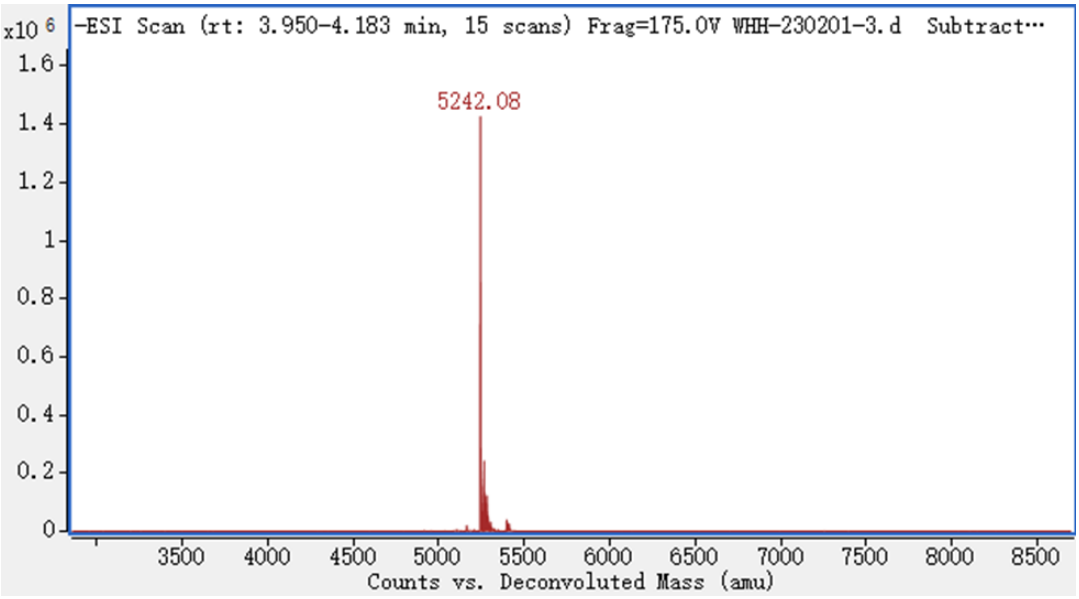


UPLC chromatogram and deconvoluted MS of **3bp**

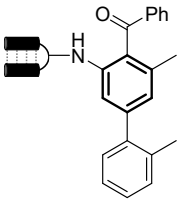
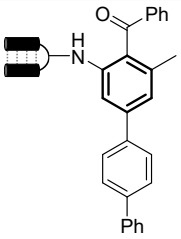
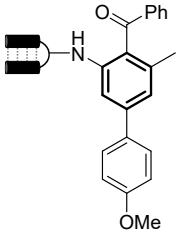
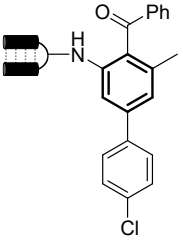
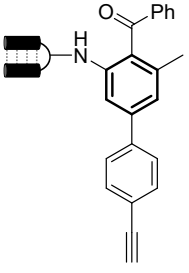
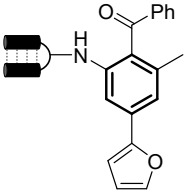
Conversion: >90%

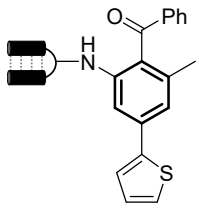
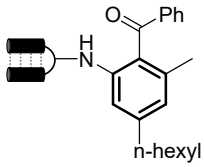
Calculated Mass: 5242 Da; Observed Mass: 5242 Da





9.4 Further assembly of polyfunctionalized benzenes using representative allenic ketones 2b, 2d, 2e, 2f, 2j, 2k, 2l, 2o.

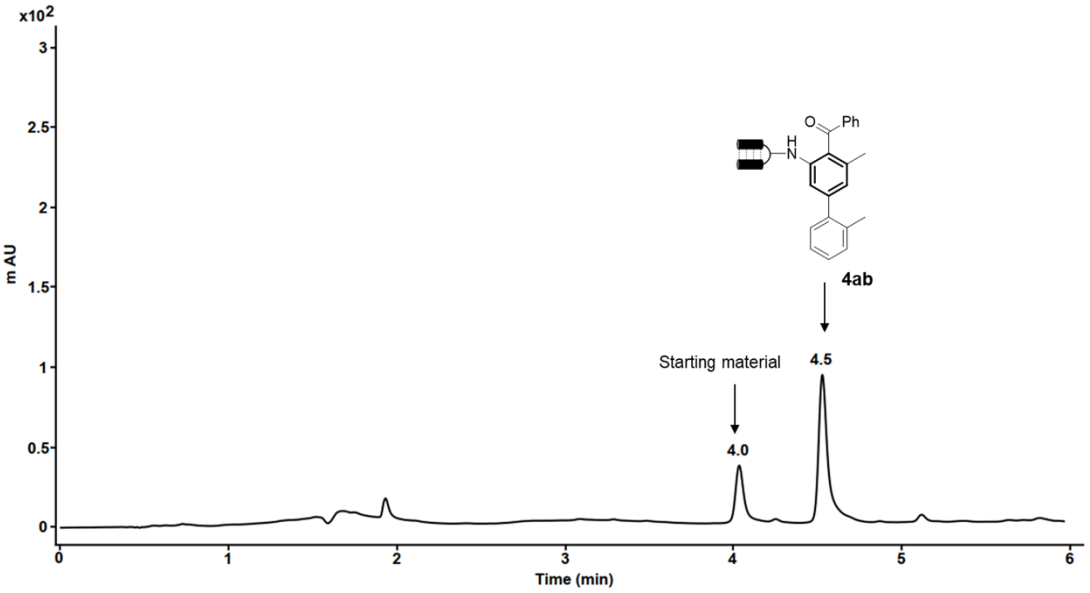
Compound	Product Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
2b		**4ab**	5221	5221	73%
2c		**4ac**	5283	5283	89%
2d		**4ad**	5237	5237	>90%
2e		**4ae**	5242	5242	86%
2i		**4af**	5231	5231	85%
2j		**4ag**	5197	5197	>90%

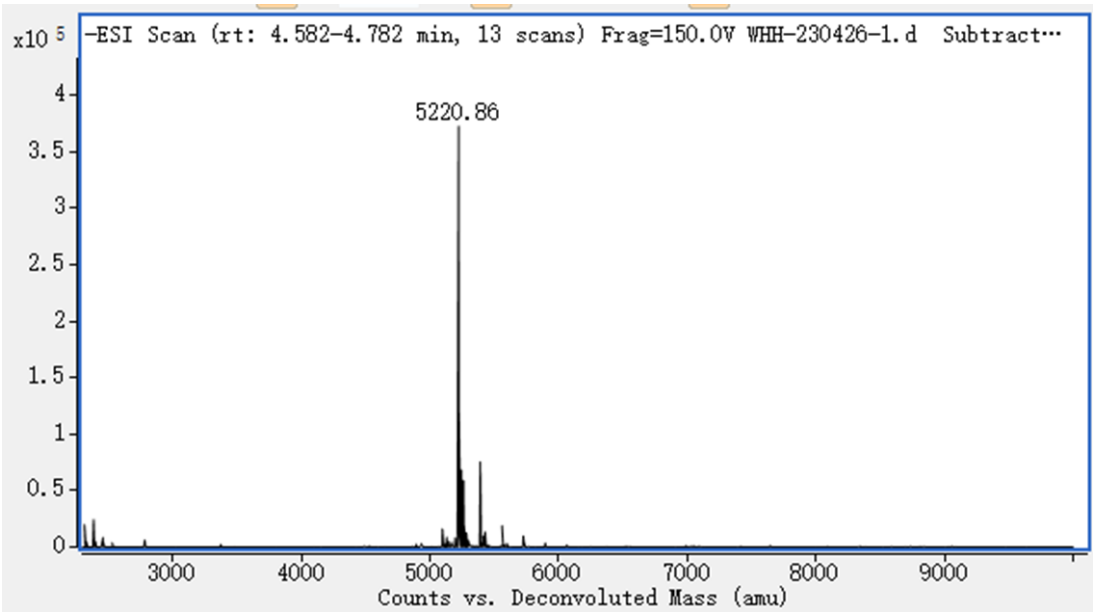
2k		**4ah**	5213	5213	>90%
2n		**4ai**	5215		0%

UPLC chromatogram and deconvoluted MS of **4ab**

Conversion: 73%

Calculated Mass: 5221 Da; Observed Mass: 5221 Da

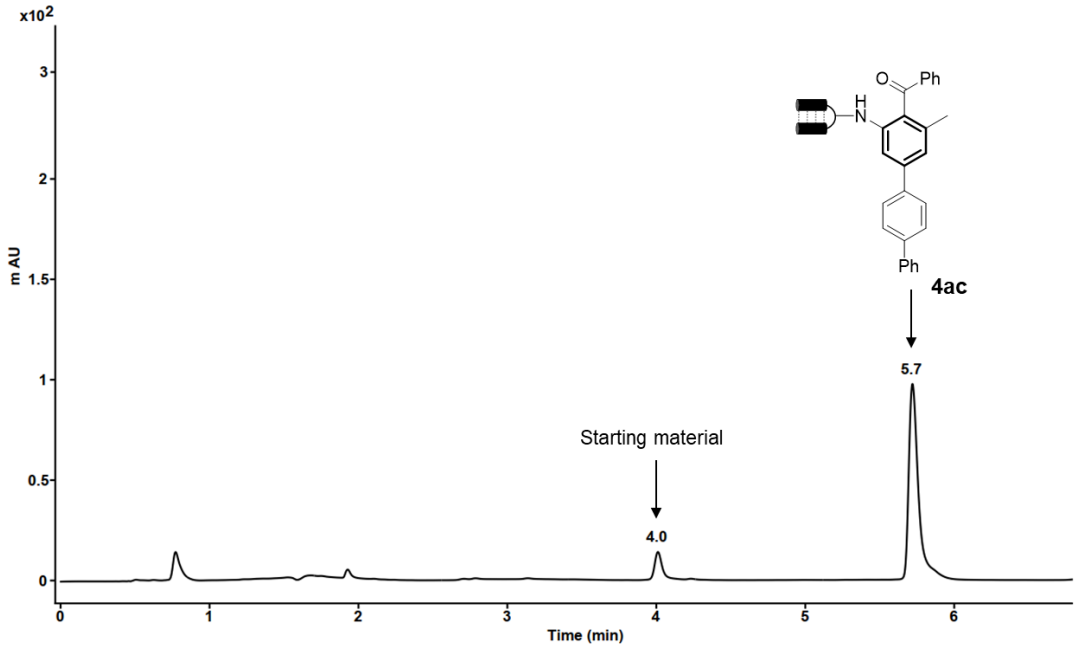


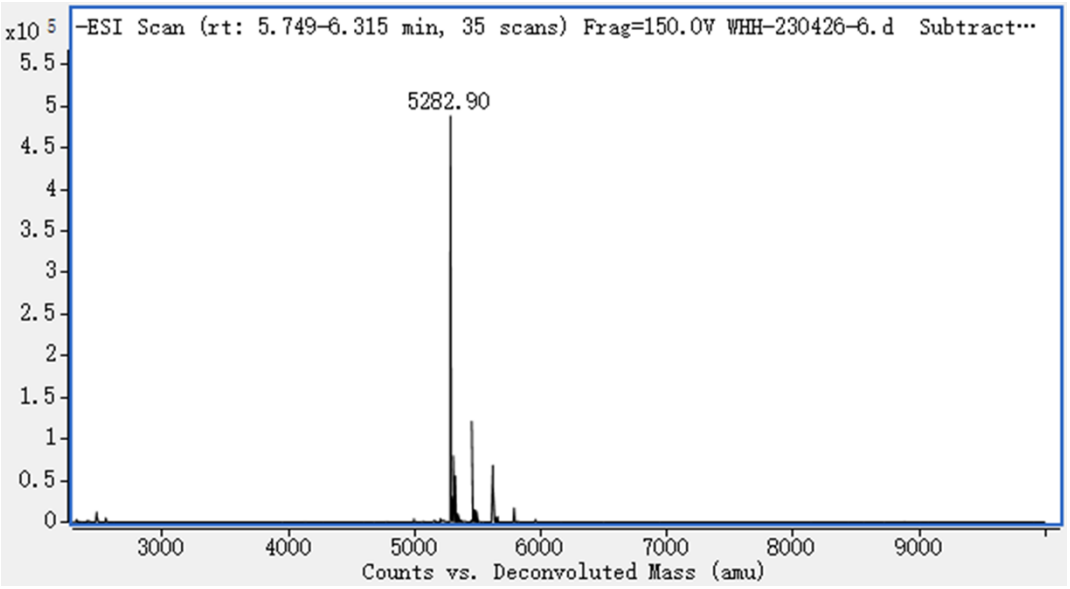


UPLC chromatogram and deconvoluted MS of **4ac**

Conversion: 89%

Calculated Mass: 5283 Da; Observed Mass: 5283 Da

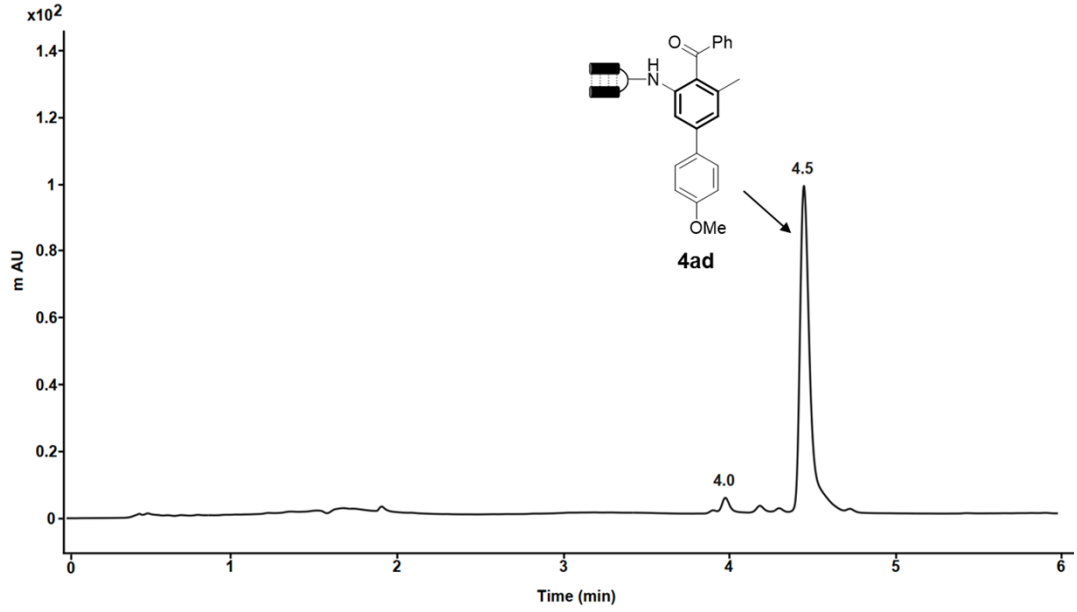


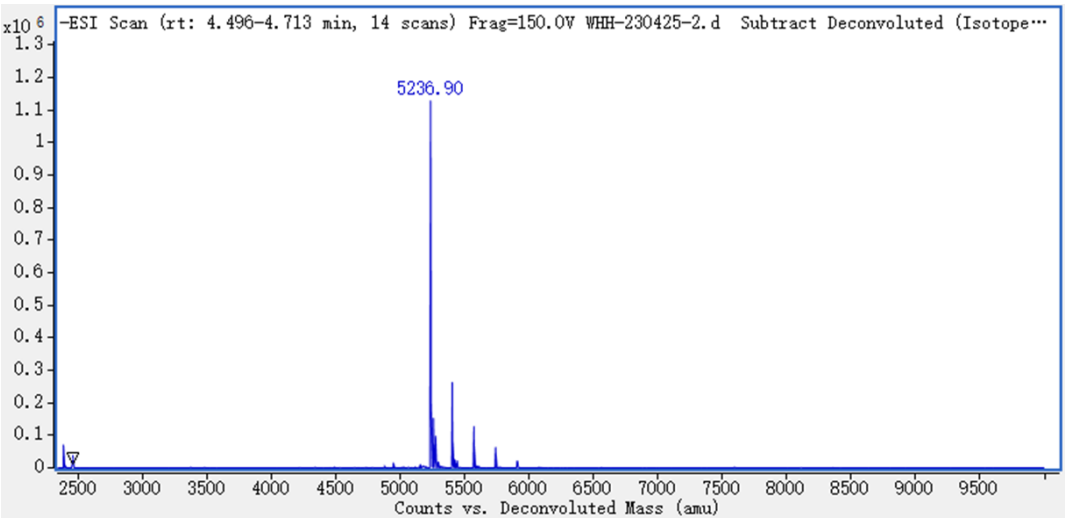


UPLC chromatogram and deconvoluted MS of **4ad**

Conversion: >90%

Calculated Mass: 5237 Da; Observed Mass: 5237 Da

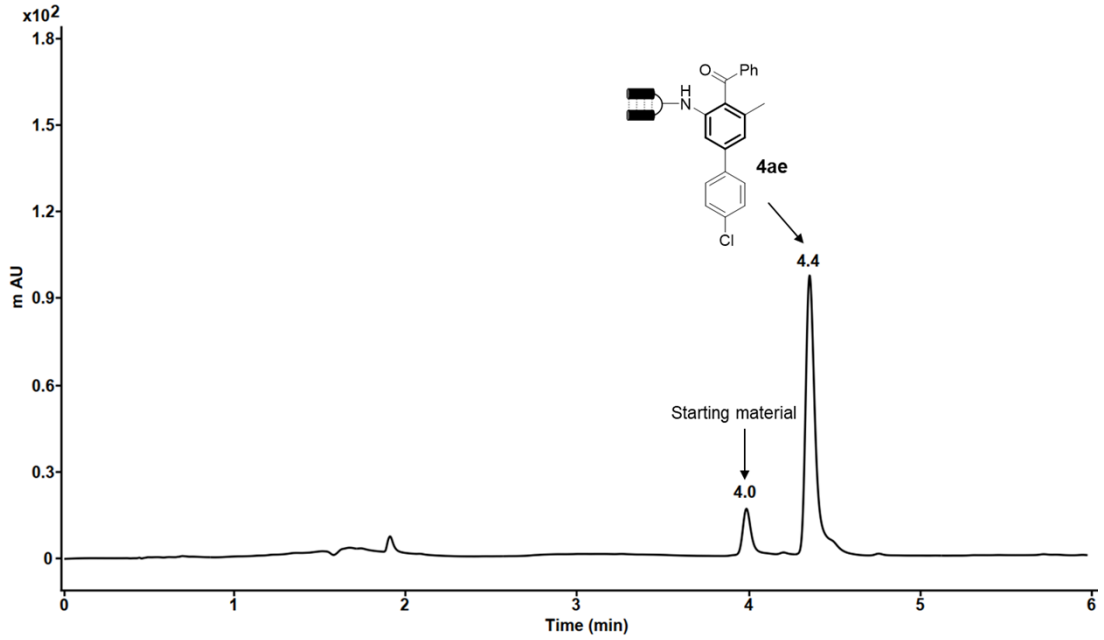


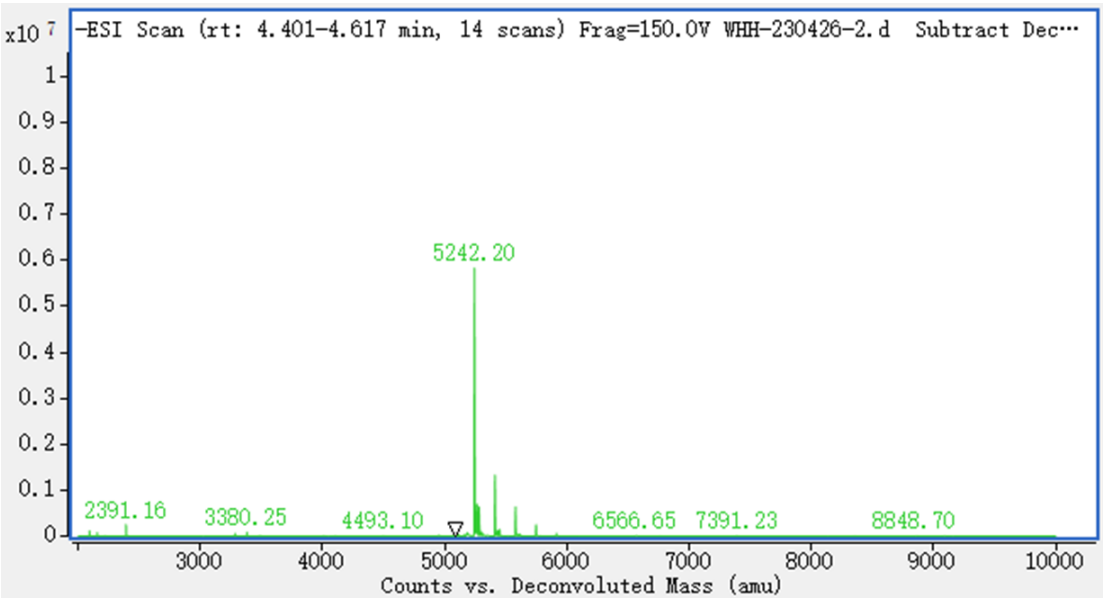


UPLC chromatogram and deconvoluted MS of **4ae**

Conversion: 86%

Calculated Mass: 5242 Da; Observed Mass: 5242 Da

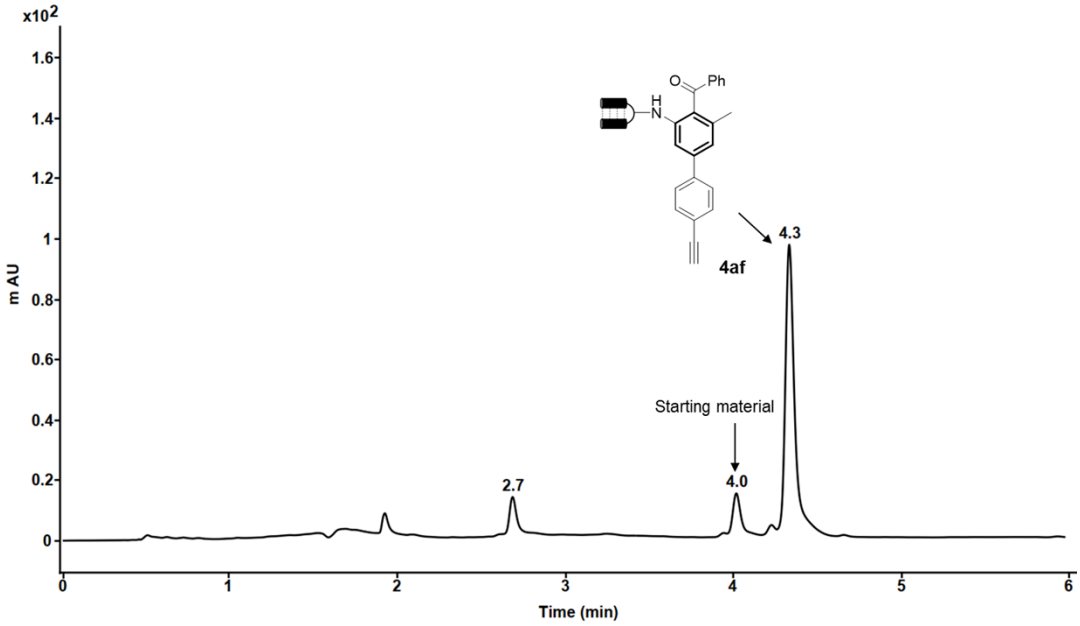


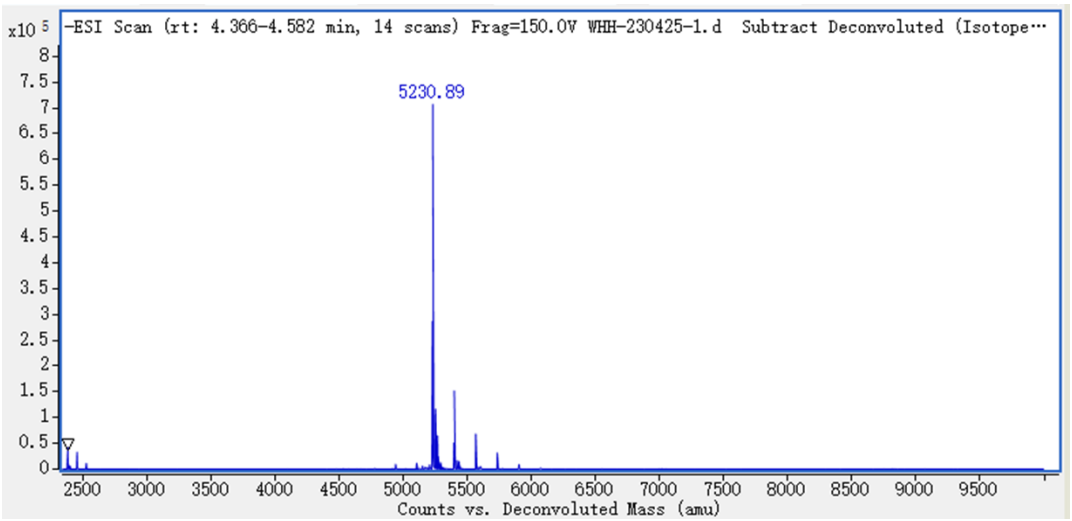


UPLC chromatogram and deconvoluted MS of **4af**

Conversion: 85%

Calculated Mass: 5231 Da; Observed Mass: 5231 Da

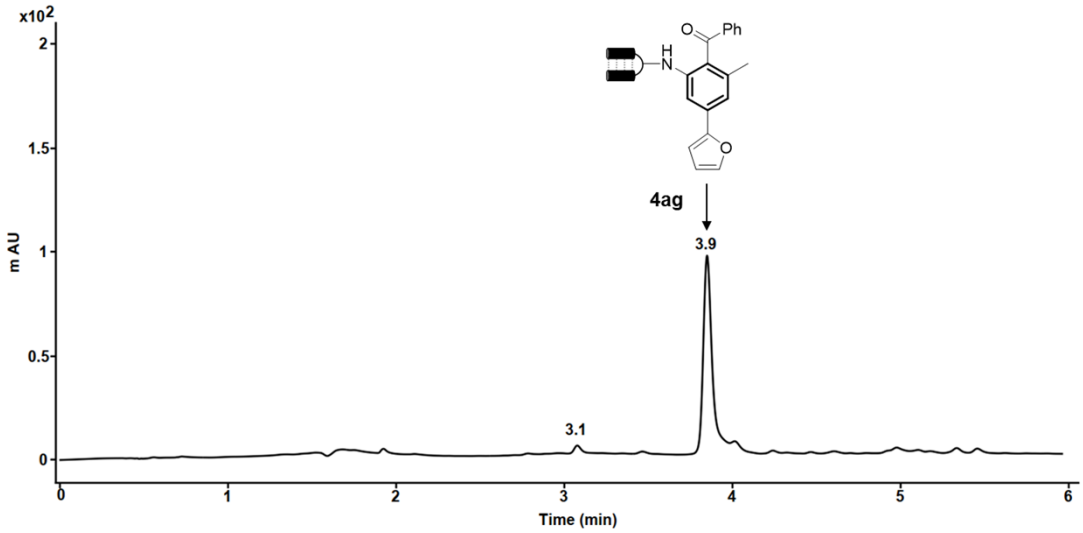


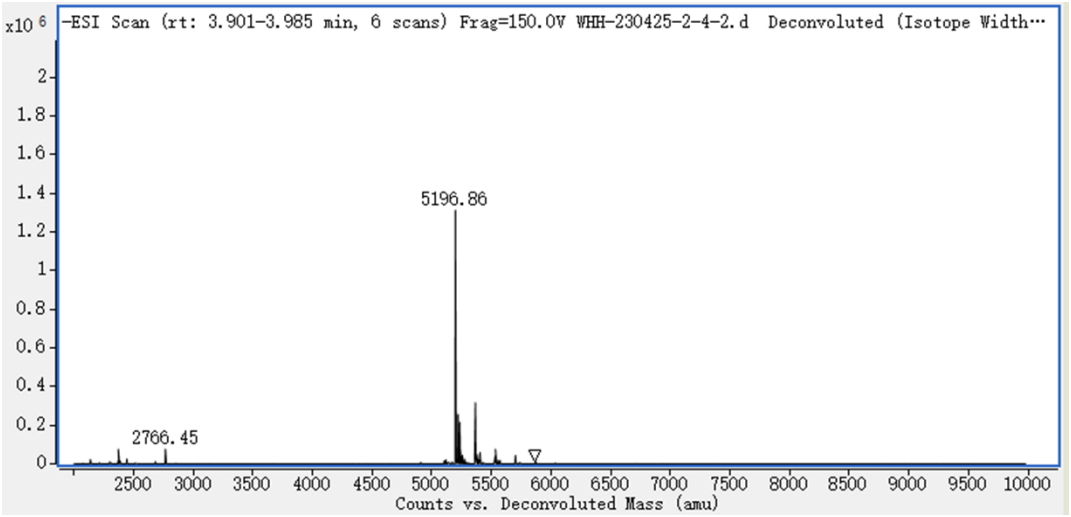


UPLC chromatogram and deconvoluted MS of **4ag**

Conversion: >90%

Calculated Mass: 5197 Da; Observed Mass: 5197 Da

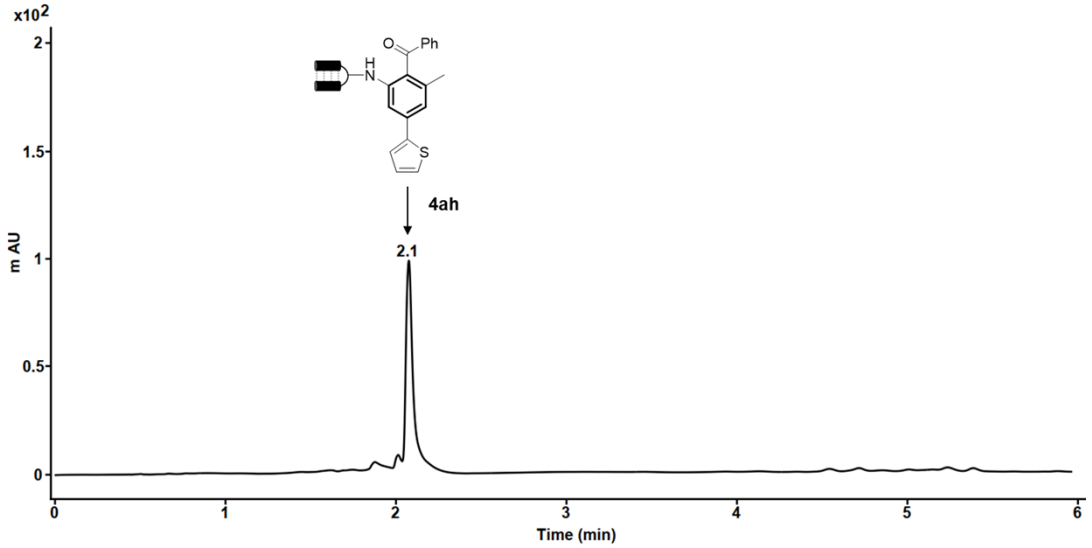


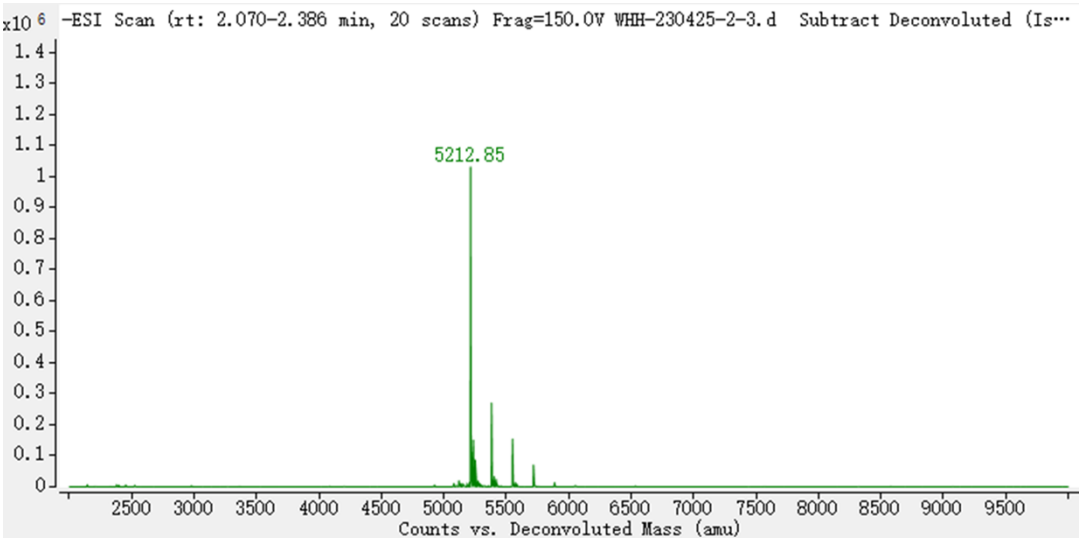


UPLC chromatogram and deconvoluted MS of **4ah**

Conversion: >90%

Calculated Mass: 5213 Da; Observed Mass: 5213 Da





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