Supplementary Information

Incorporation of Viridicatin alkaloids-like scaffolds into DNA-encoded chemical libraries

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1. Materials and general methods

1.1 Materials

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Headpiece (**HP**, 5'-/5Phos/GAGTCA/iSp9/iUniAmM/i-Sp9/TGACTCCC-3', MW = 4937), Headpiece-primer (**HP-P**, 5'-/5Phos/ACCTTCGG TCGGGAGTCA/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 DMA, EtOH, 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. All reactions were performed in Axygen® 0.6 mL Snaplock Microcentrifuge Polypropylene Tube (Product Number: MCT-060-L-C) or Axygen® 200 µL 96-well Polypropylene PCR Microplate (Product Number: PCR-96M2-HS-C). For detailed technical information, the reader is directed to the homepage of Axygen: http://www.axygen.com/.

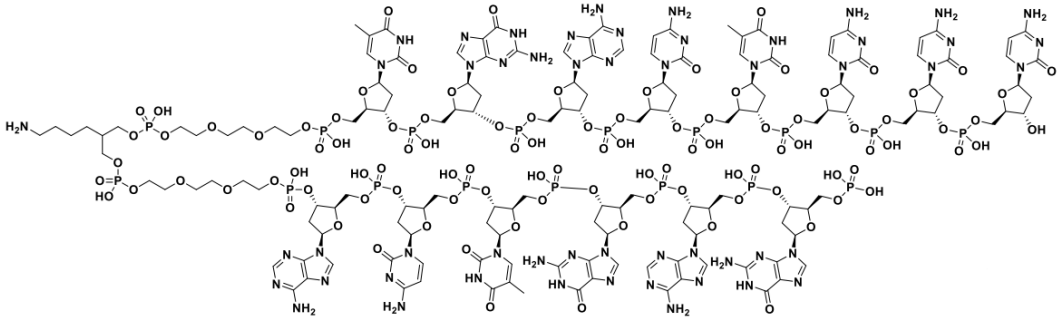


Figure S1. Structure of **HP**. (MW = 4937)

1.2 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 reaction analysis:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	18
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 reaction analysis:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	17
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 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.

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).

1.3 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 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 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

1.4 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 (2 μL, 2000 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.

1.5 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, 10 pmol of treated DNA samples was loaded on the gel, and the gel was run at 200 V

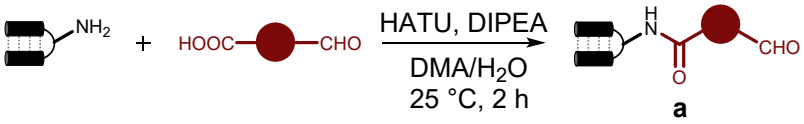
for 50 - 60 min. DNA fragments were visualized and analyzed by Bio-Rad Chemidoc^{TM} Image System (Bio-Rad, CA, USA).

1.6 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. Multiplicity abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet (dd = doublet of doublets), t = triplet, q = quartet, m = multiplet.

2. Experiments for on-DNA synthesis of Viridicatin

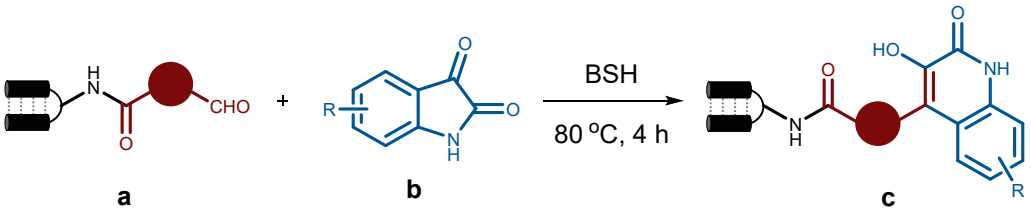
2.1 On-DNA amide coupling



a

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. Aldehyde-containing 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, 1 equiv). 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. After HPLC purification, the collected product was vacuum-dried overnight and redissolved in H_2O for subsequent experiments.

2.2 On-DNA Viridicatin synthesis



a b c

400 pmol DNA-conjugated amine was dissolved in sodium borate buffer 10 μL (250 mM, pH 11.5) , followed by addition of BSH (100 mM in EtOH, 8 μL, 2000 equiv) and 8 μL EtOH. The reaction mixture was vortexed, centrifuged, and proceed at 25 °C for 20 min. Then istain (500 mM in DMA, 4 μL, 5000 equiv) and 10 μL EtOH was added and the reaction was incubated at 80 °C for 4 h. After purification by ethanol precipitation, the reaction was analyzed by UPLC-MS. After HPLC purification, the collected product was vacuum-dried overnight and redissolved in H_2O for subsequent experiments.

3 General procedure for subsequent diversification

3.1 Ester bond formation

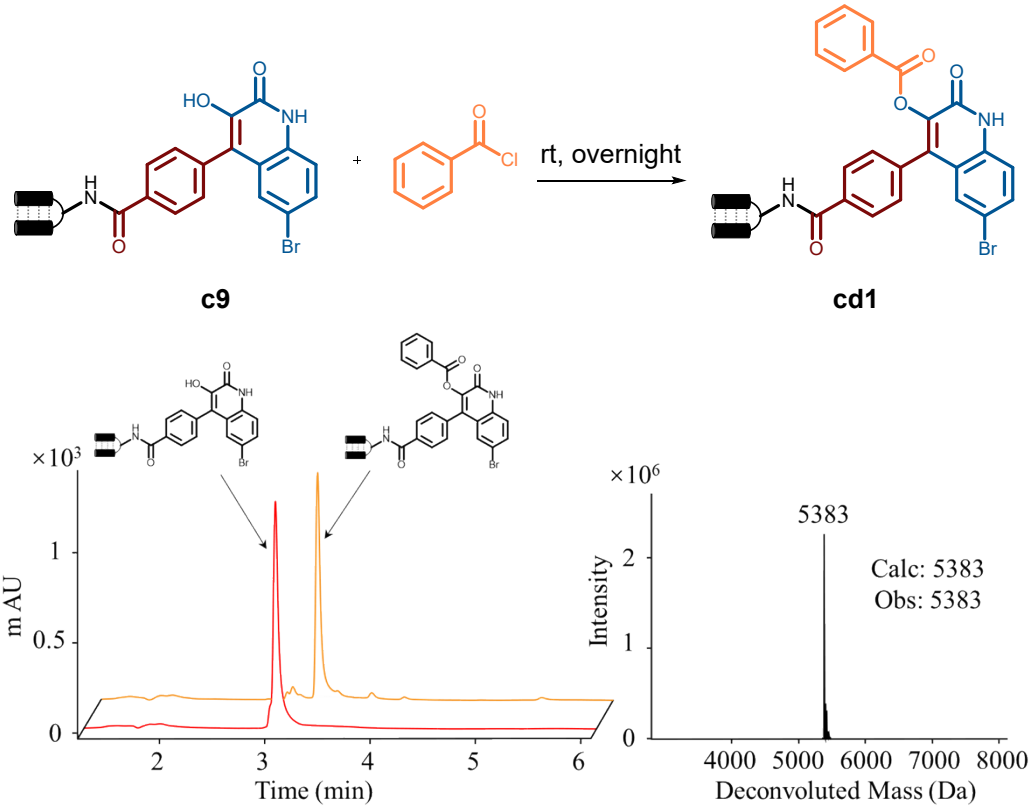
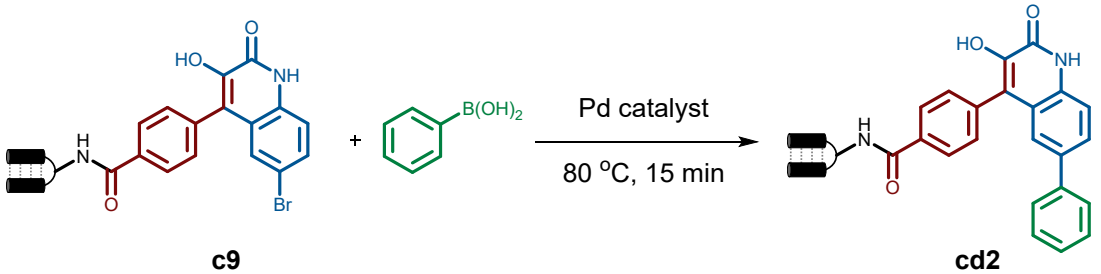


Figure S2. UPLC chromatogram and deconvoluted MS of **cd1**.

200 pmol **c9** was dissolved in sodium borate buffer (10 μL, 250 mM, pH 9.4), followed by addition of benzoyl chloride (200 nmol, 100 mM in EtOH, 2 μL, 1000 equiv). The reaction mixture was vortexed, centrifuged, and placed at 25 °C for overnight. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5383 Da; observed: 5383 Da.

3.2 C-C bond formation



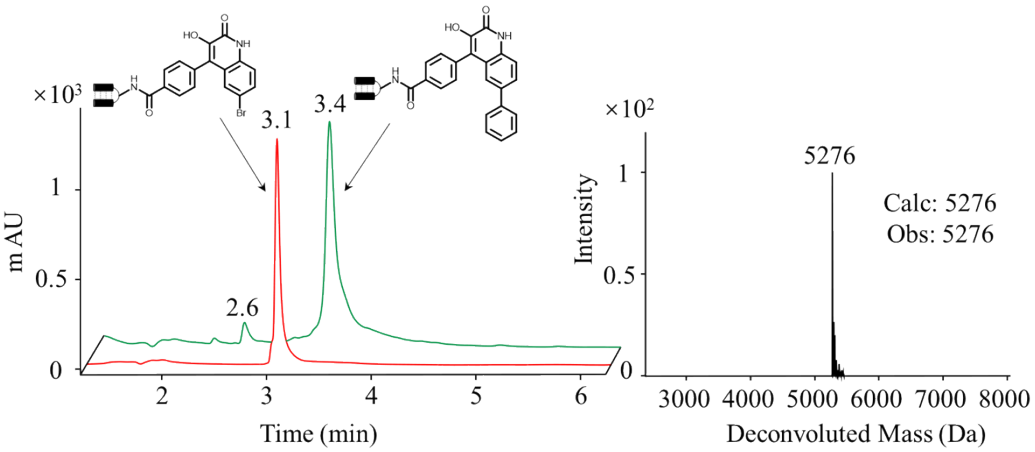
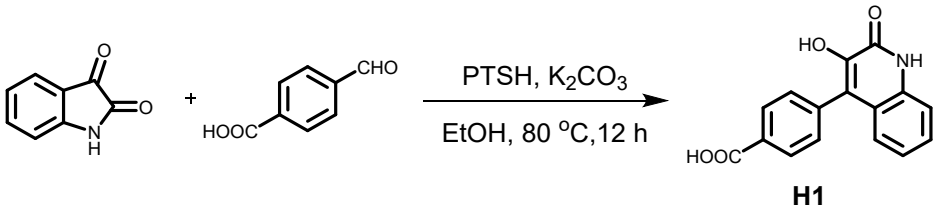


Figure S3. UPLC chromatogram and deconvoluted MS of **cd2**.

To the solution of **c9** (0.2 nmol, 2 μL, 100 μM in H_2O, 1 equiv) was added phenylboronic acid (1000 nmol, 5 μL, 200 mM in dioxane/H_2O (v/v = 1/1), 5000 equiv), CsOH (3600 nmol, 6 μL, 600 mM in H_2O, 18000 equiv), sSPhos-Pd-G2 (20 nmol, 2 μL, 10 mM in DMA, 100 equiv), and H_2O (5 μL). The reaction mixture was vortexed, centrifuged, and incubated at 80 °C for 15 min.[1, 2] The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5276 Da; observed: 5276 Da.

4. Structural validation

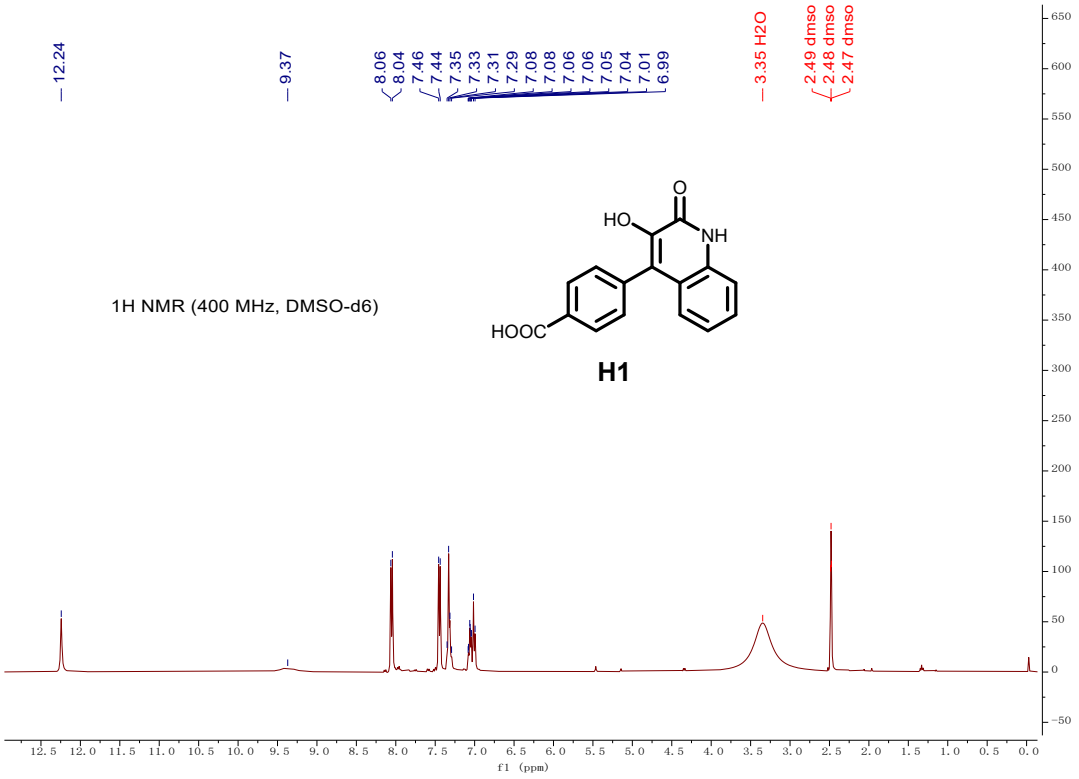
4.1 Off-DNA synthesis of Viridicatin



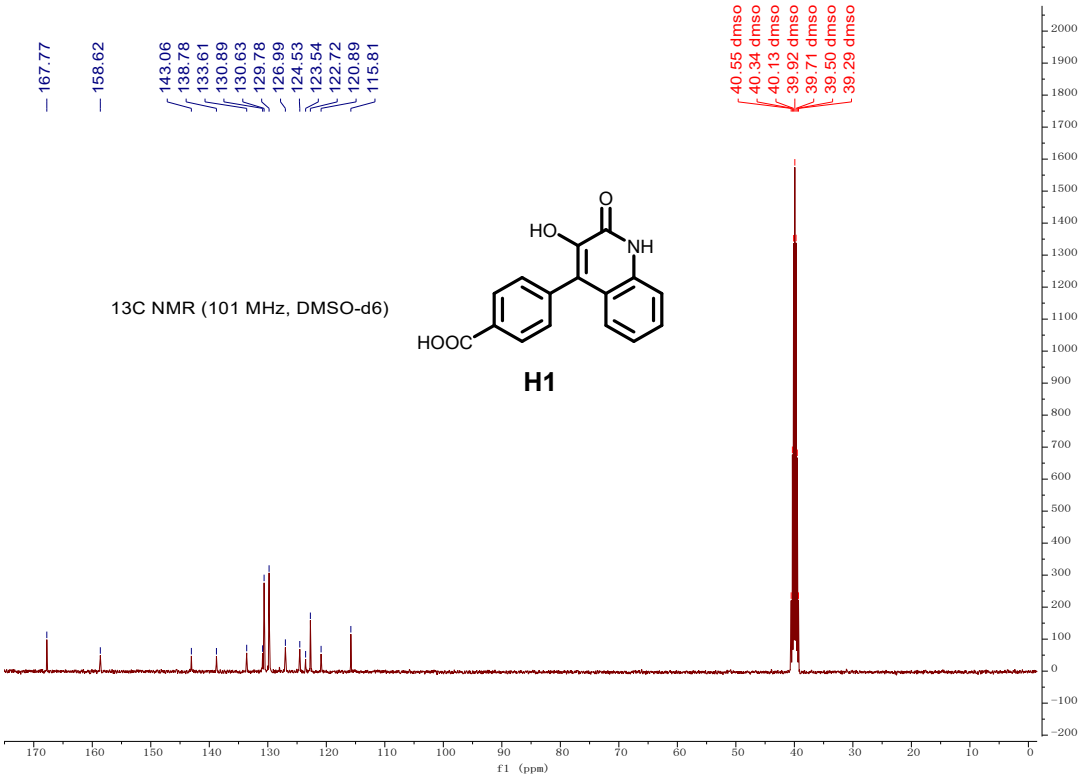
H1

4-(3-Hydroxy-2-oxo-1,2-dihydroquinolin-4-yl)benzoic acid[3]. A mixture of aldehydes (2 mmol) and *p*-toluenesulfonyl hydrazide (2 mmol) in ethanol was stirred at room temperature until complete consumption of starting materials (monitored by TLC). Then, isatins (2 mmol) and K_2CO_3 (8 mmol) was added to the reaction mixture and stirred at 80 °C for 8 h. After the reaction was finished, the solvent poured into water, then add concentrated hydrochloric acid to adjust the system pH to weak alkalinity and filtrated to afford a yellowish solid (363 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 9.37 (s, 1H), 8.05 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.32 (d, J = 6.7 Hz, 2H), 7.06 (td, J = 7.3, 6.2, 2.0 Hz, 1H), 7.00 (d, J = 7.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.8, 158.6, 143.1, 138.8, 133.6, 130.9, 130.6, 129.8, 127.0, 124.5, 123.5, 122.7, 120.9, 115.8. HRMS (ESI) calculated for $C_{16}H_{11}NO_4$ $[M + Na]^+$, 304.0586; observed, 304.0589.

^{1}H-NMR of **H1**

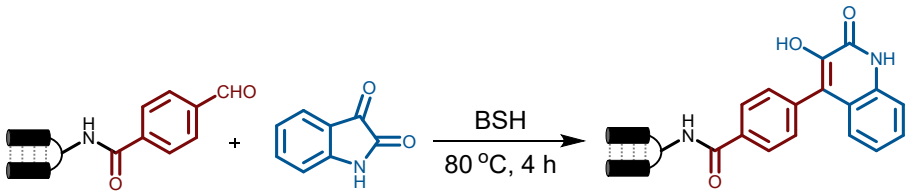


^{13}C-NMR of **H1**



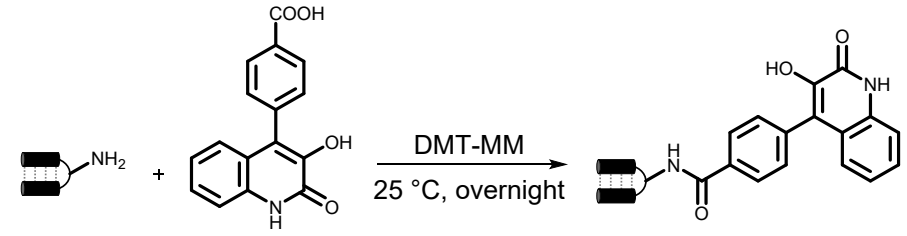
4.2 Co-injection experiment

Route A

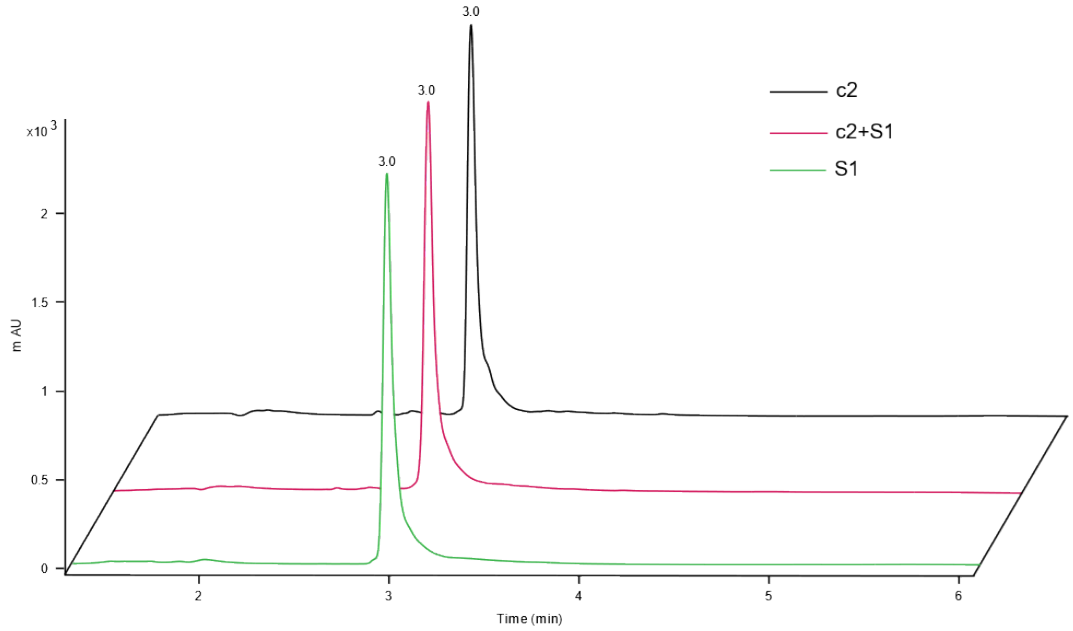


a1 b2 c2

Route B



HP H1 S1



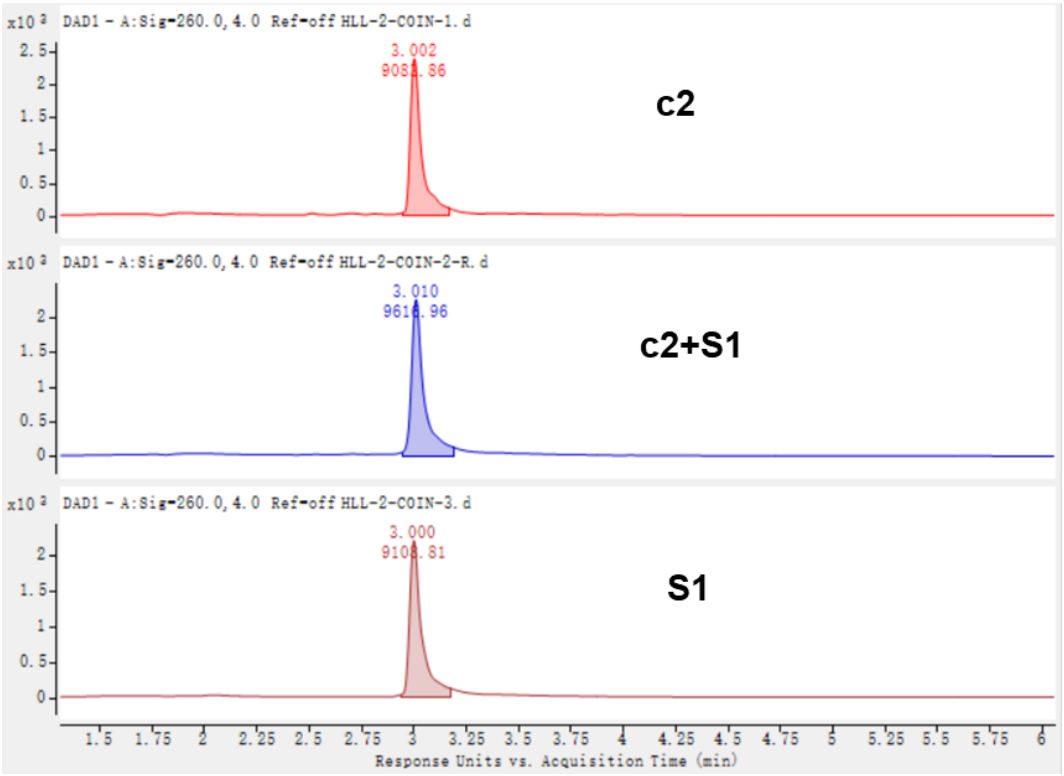
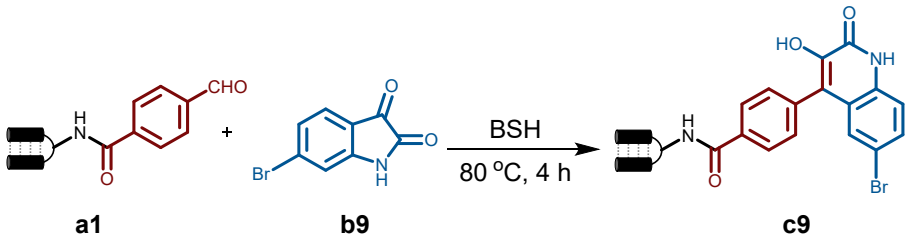


Figure S4. Co-injection experiment of **c2** and **S1** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (red curve) had the same retention time as the other two peaks (**c2** from route A, black curve; **S1** from route B, green curve).

5. Scale-up reaction



a1 **b9** **c9**

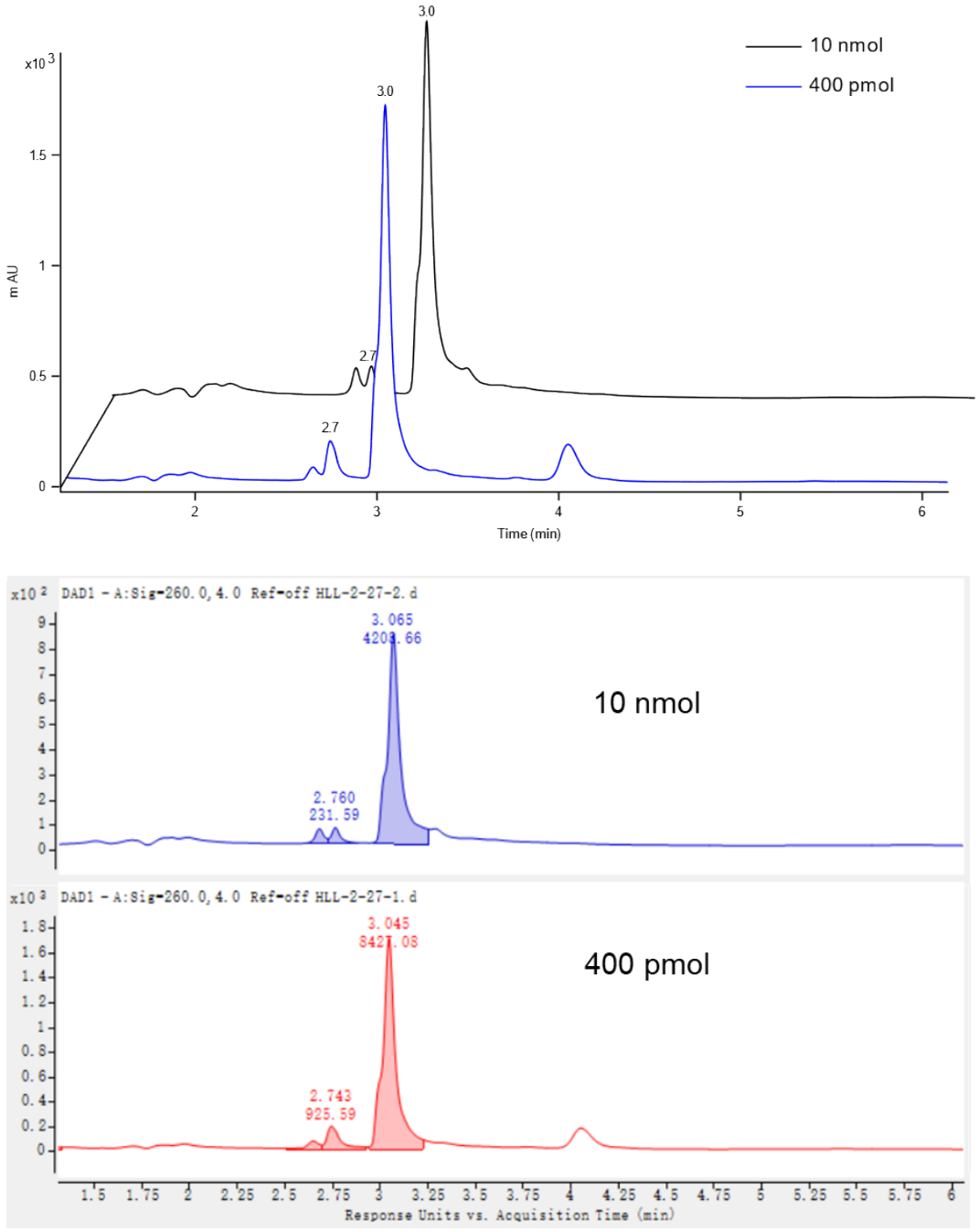


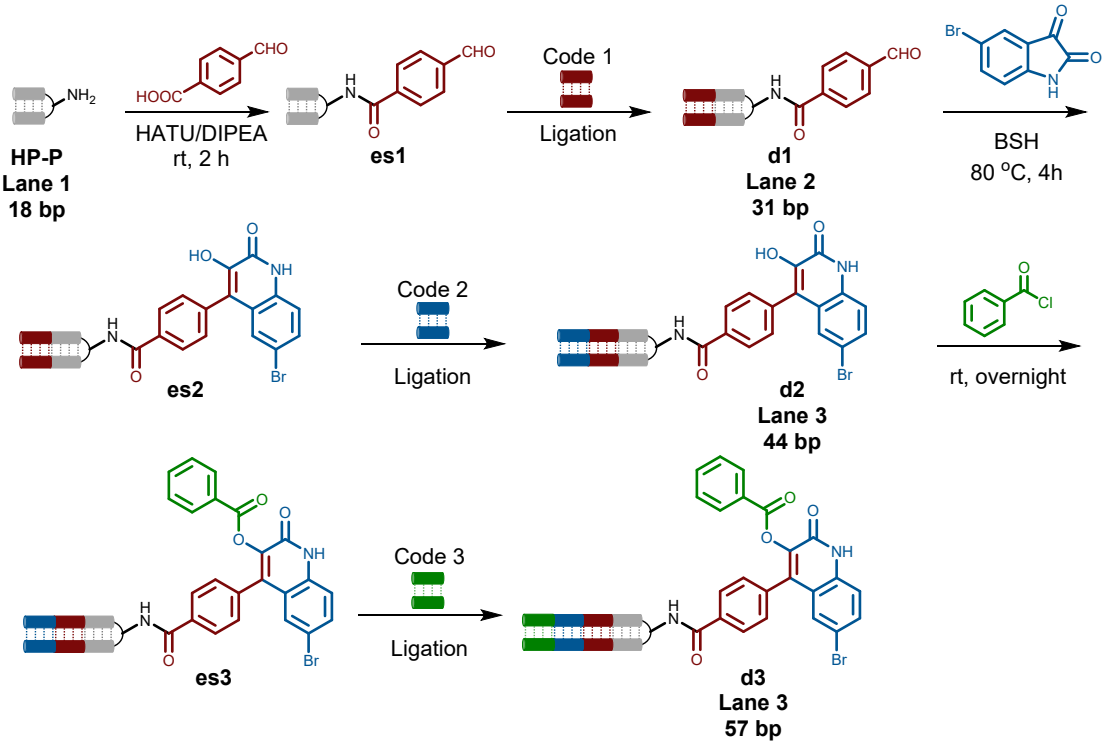
Figure S5. UPLC chromatogram of **c9** at 400 pmol and 10 nmol scales.

10 nmol DNA-conjugated amine was dissolved in sodium borate buffer 10 μL (250

mM, pH 11.5) , followed by addition of BSH (100 mM in EtOH, 8 μL,80 equiv) and 8 μL EtOH. The reaction mixture was vortexed, centrifuged, and proceed at 25 °C for 20 min. Then istain (500 mM in DMA, 4 μL, 200 equiv) and 10 μL EtOH was added and the reaction was incubated at 80 °C for 4 h. After purification by ethanol precipitation, the reaction was analyzed by UPLC-MS. After HPLC purification, the collected product was vacuum-dried overnight and redissolved in H_2O for subsequent experiments.

6. Enzymatic ligation and quantitative PCR (qPCR) testing

6.1 Enzymatic ligation



HP-P was initially coupled with 4-formylbenzoic acid via amide formation and tagged with Code 1 to afford **d1**. Then the product reacted with isatins (**b9**), followed by encoding with Code 2 to afford **d2**. After **d2** reacted with benzenecarbonyl chloride and encoded with Code 3 to afford **d3**. However, the ester hydrolysis by-product appeared after DNA ligation (**Figure S8**). Finally, **d1**, **d2** and **d3** are denatured by incubating at 95 °C in a dry bath for 10 min, and all the ligation product was isolated by ethanol precipitation. The resulting pellets were vacuum-dried and dissolved in nuclease-free water.

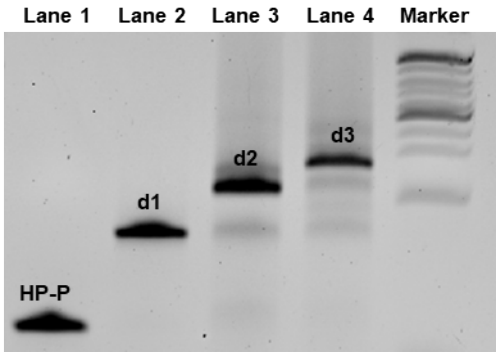


Figure S6. 20% denatured PAGE analysis of DEL-encoding compatibility. Lane 1, HP-P; lane 2, DNA conjugate **d1**; lane 3, DNA conjugate **d2**; lane 4, DNA conjugate

d3.

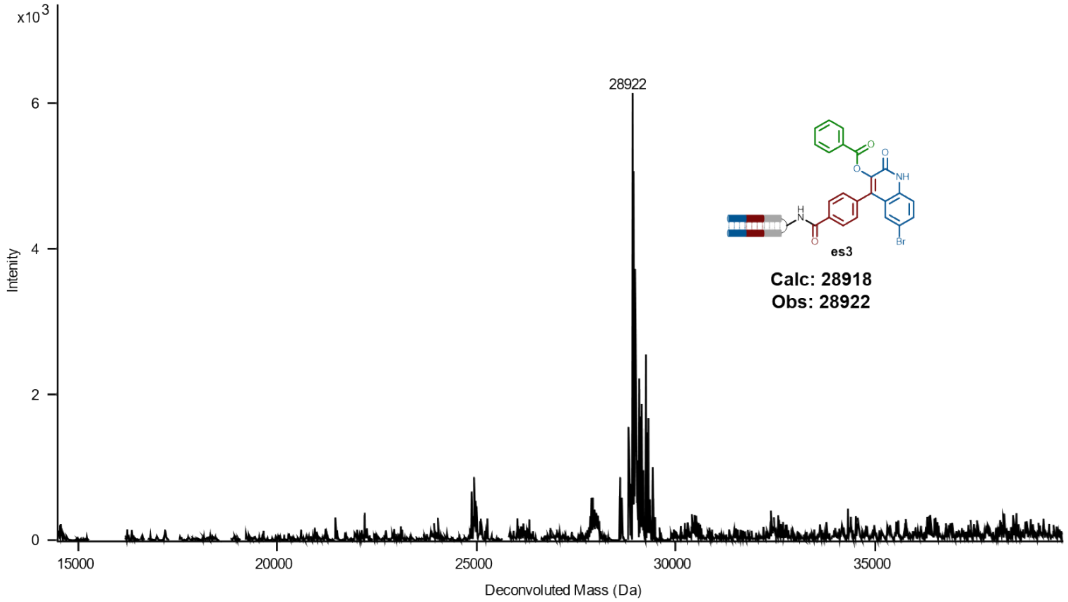


Figure S7. Deconvoluted MS of **es3**.

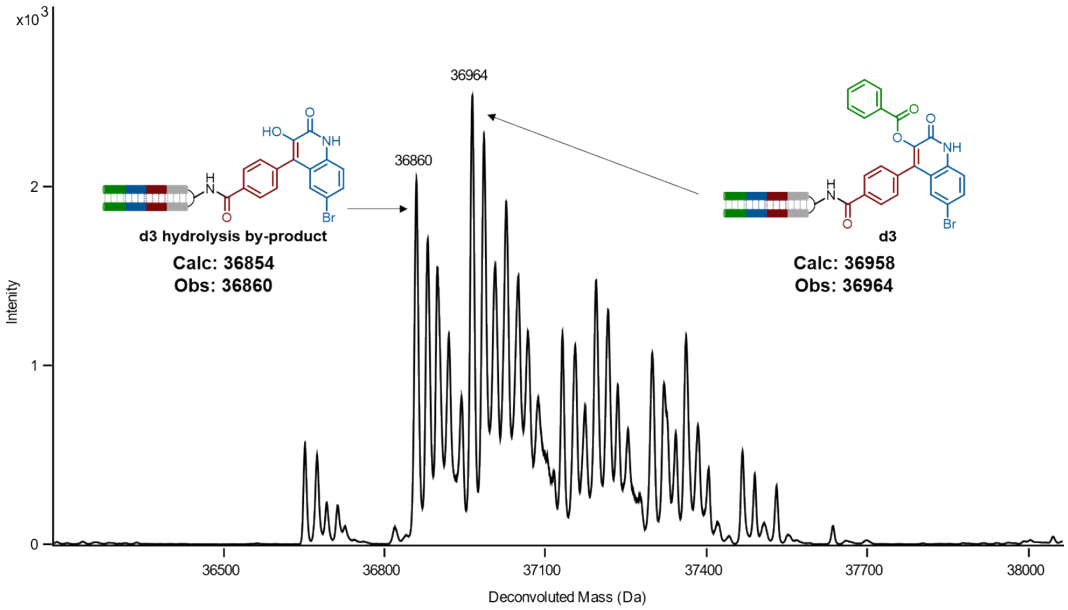


Figure S8. Deconvoluted MS of **d3**.

6.2 Quantitative PCR (qPCR) testing

The DNA damage was evaluated by applying q-PCR test. Ligations without any chemical reactions were used as a control experiment (**HP** to **Sample-1** and **HP-P-CHO** to **Sample-2**). Ligation experiments followed the general procedures described above. qPCR was performed on the BIO-RAD CFX96TM Real-Time System. The sample mixtures of 20 μL total volume contained the following: 1 μL ddH_2O, 4 μL primer mix (5 μM each, reverse and forward primer), 5 μL diluted DNA sample, and

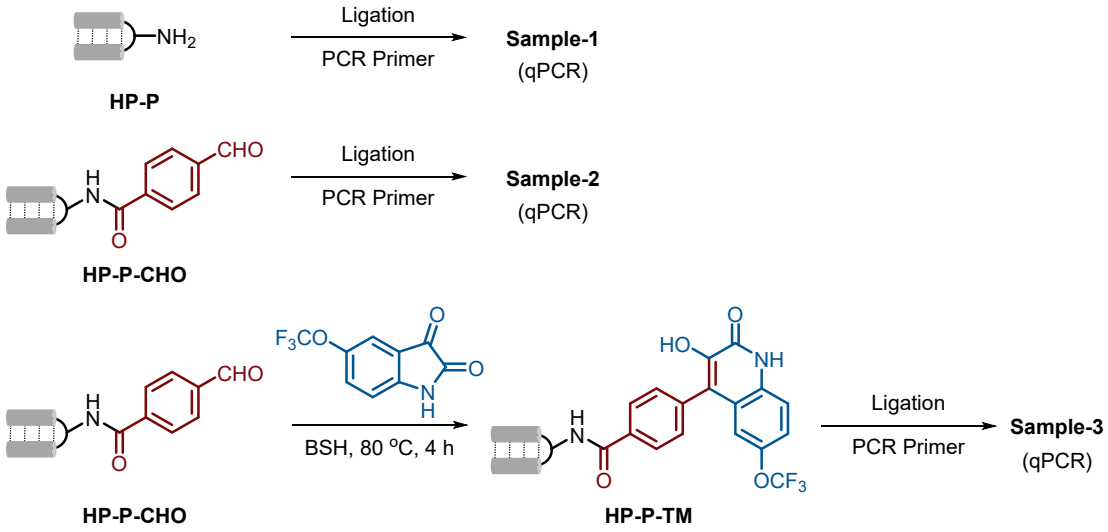
10 μL 2 × SP qPCR mix (BG0014). The PCR reaction was carried out with the following thermocycling program: 95 °C, 60 s; 40 cycles of (94 °C, 20 s; 94 °C, 10 s; 55 °C, 10 s). Every assay was repeated at least three times.

qPCR forward primer:

5'-GTTGGAAGCCAGCCCTCAGTGACAGAGAATATGTGTAGAGGCTCGGG TGCTCTG-3'

qPCR reverse primer:

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTCCCTCGCGCC ATCAGAC-3'



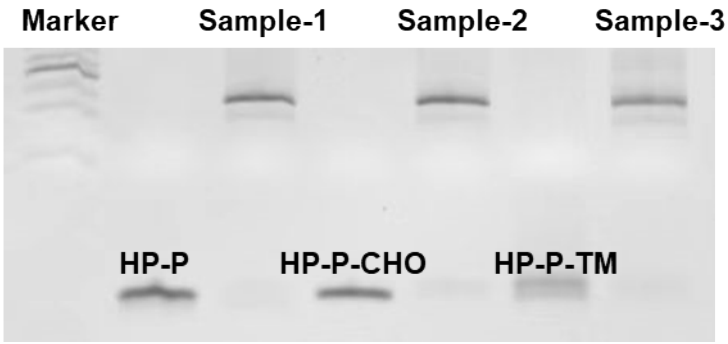


Figure S9. 20% denatured PAGE analysis of ligations.

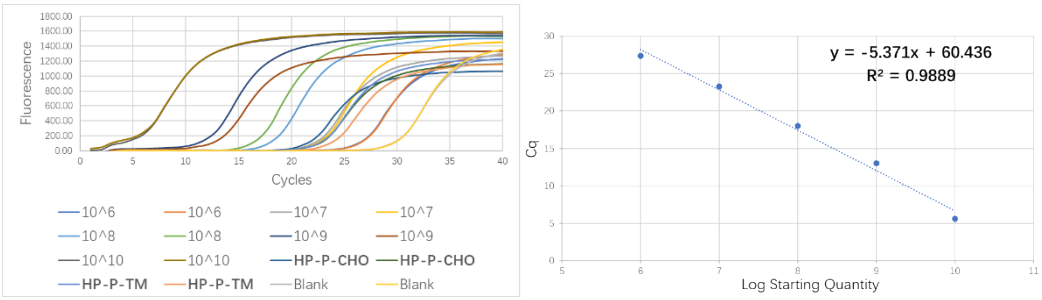


Figure S10. qPCR analysis of nucleic acid template and samples. Amplification curve and calibration curve of qPCR analysis. Starting from the same amount of **HP-P**, the recovery ratio of **HP-P-TM** to **HP-P** was 57%; the recovery ratio of **HP-P-CHO** to **HP-P** was 90%; the recovery ratio of **HP-P-TM** to **HP-P-CHO** was 63%.

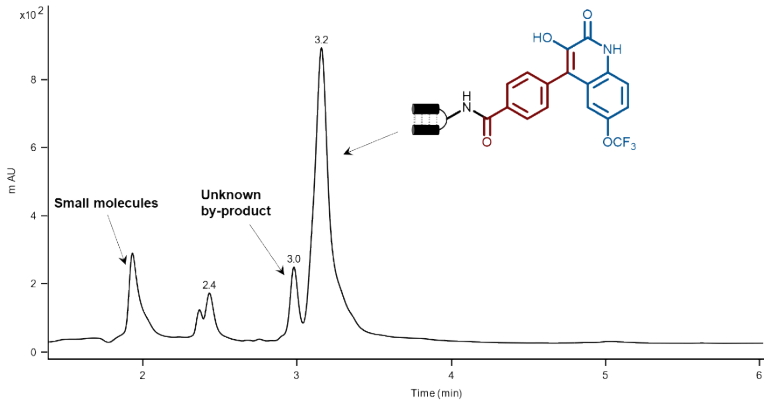
7. UPLC chromatogram and deconvoluted MS

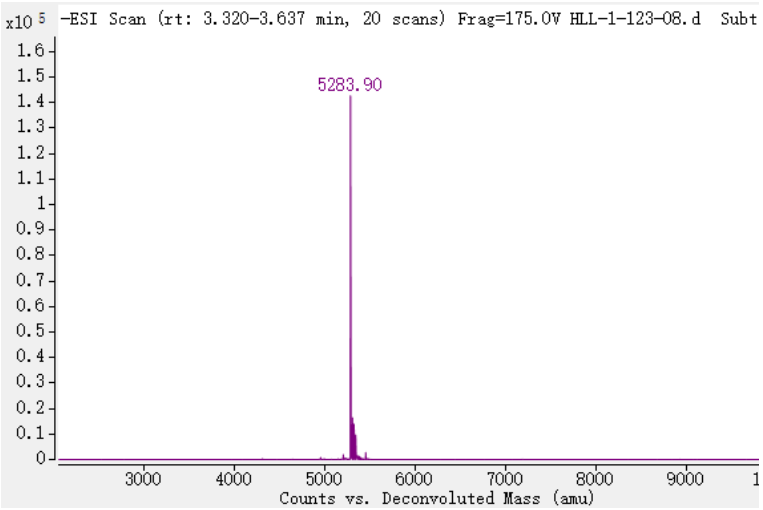
7.1 Substrate scope of isatins

UPLC chromatogram and deconvoluted MS of **c1**

Conversion: 80%

Calculated Mass: 5284 Da; Observed Mass: 5284 Da

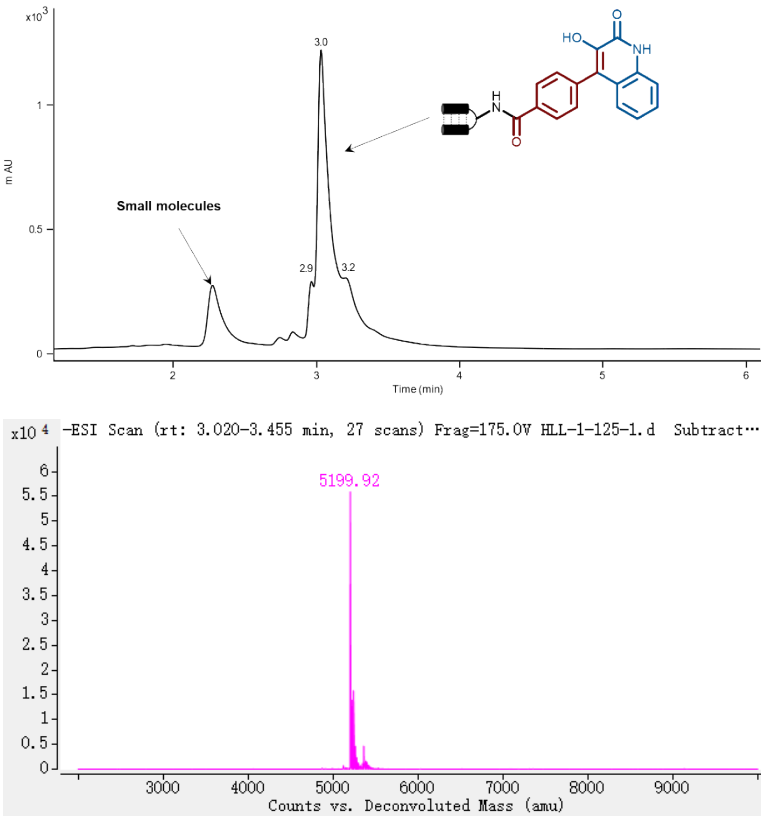




UPLC chromatogram and deconvoluted MS of **c2**

Conversion: 70%

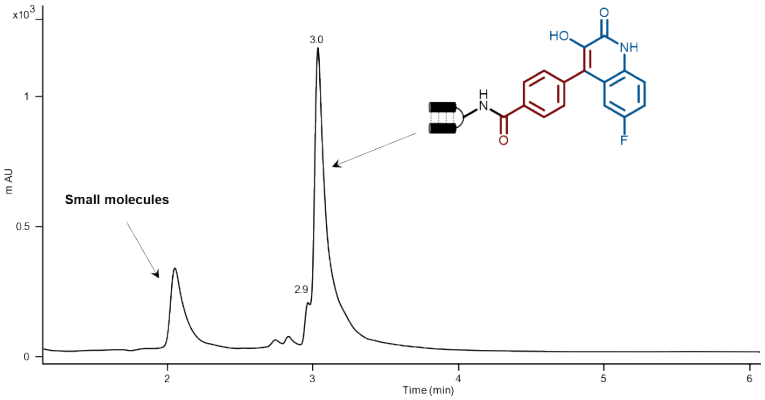
Calculated Mass: 5200 Da; Observed Mass: 5200 Da

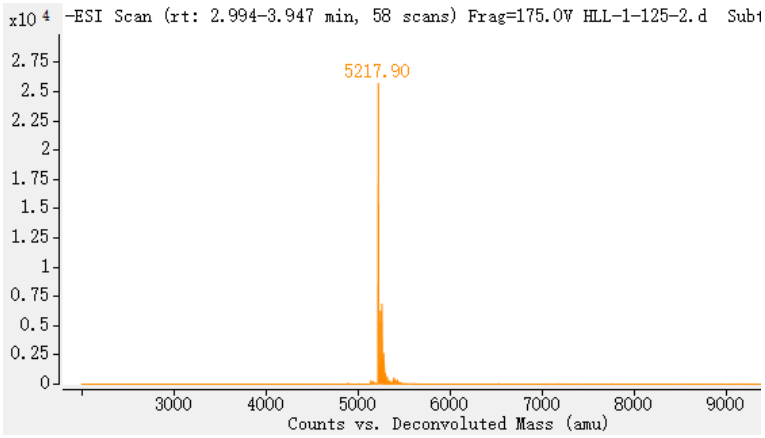


UPLC chromatogram and deconvoluted MS of **c3**

Conversion: >90%

Calculated Mass: 5218 Da; Observed Mass: 5218 Da

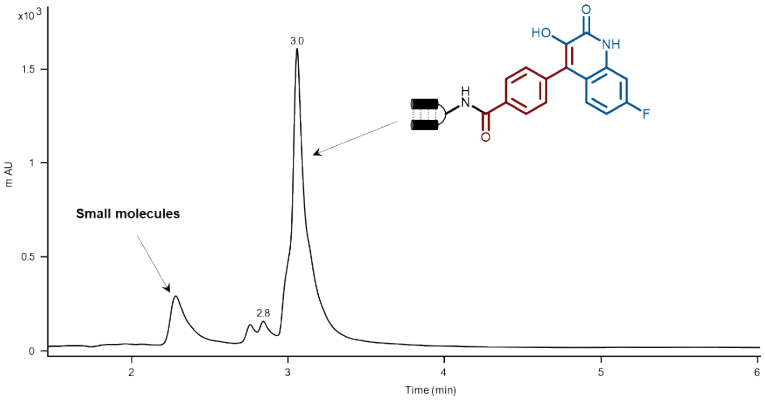


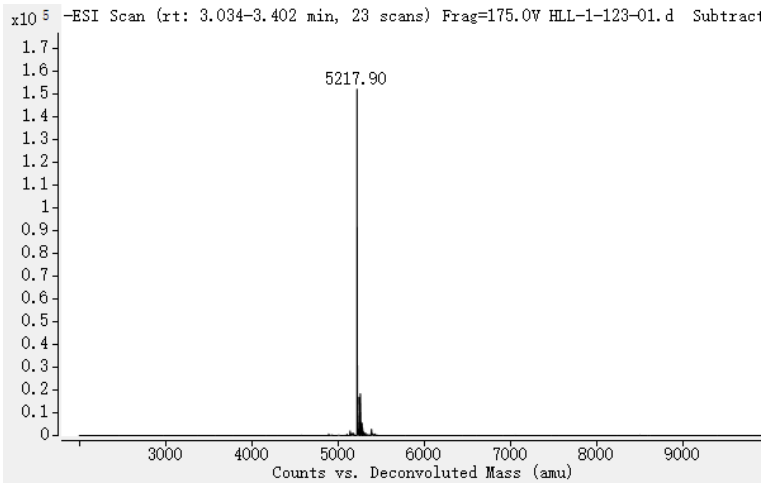


UPLC chromatogram and deconvoluted MS of **c4**

Conversion: >90%

Calculated Mass: 5218 Da; Observed Mass: 5218 Da

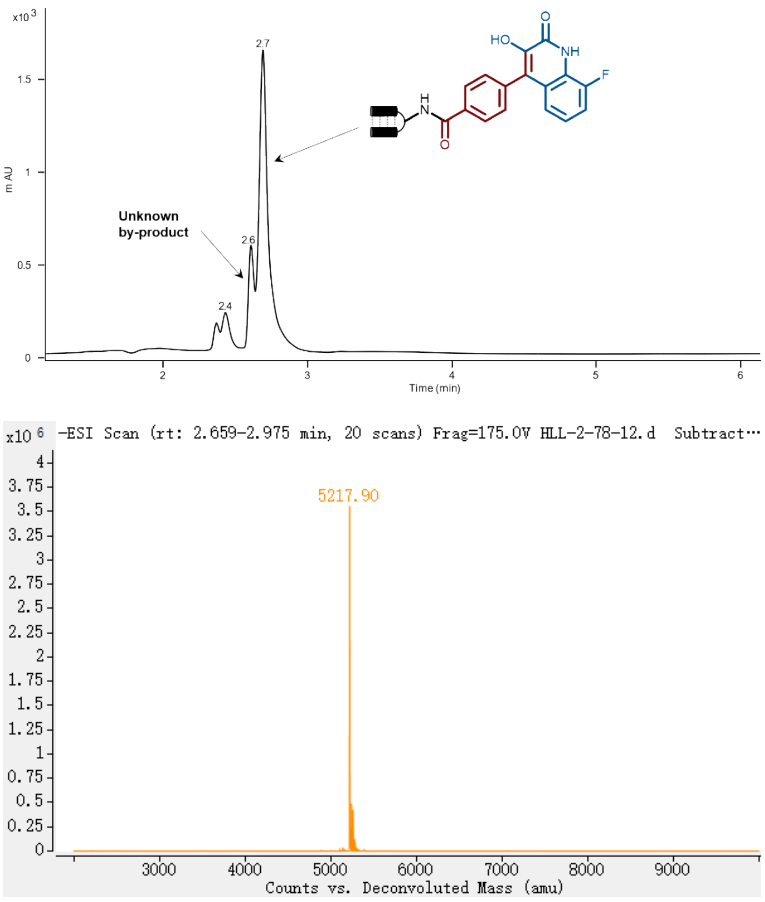




UPLC chromatogram and deconvoluted MS of **c5**

Conversion: 71%

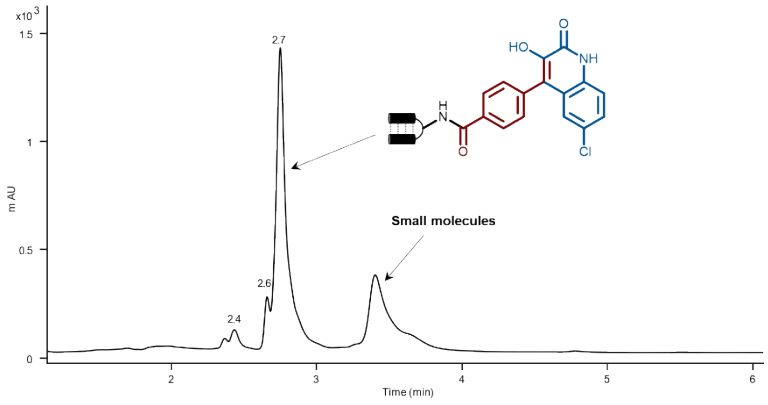
Calculated Mass: 5218 Da; Observed Mass: 5218 Da

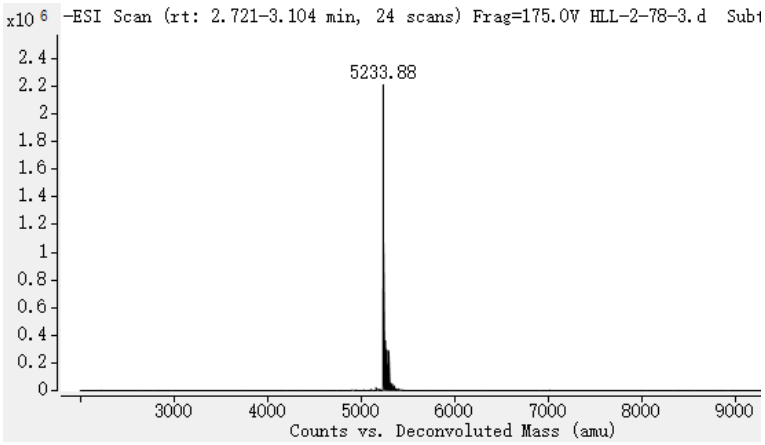


UPLC chromatogram and deconvoluted MS of **c6**

Conversion: 82%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

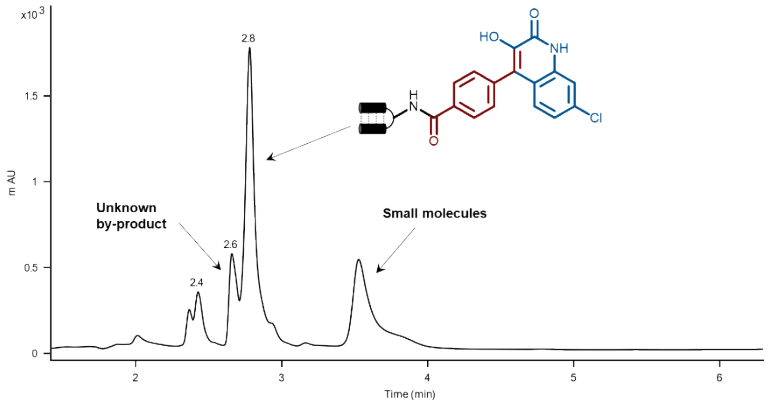


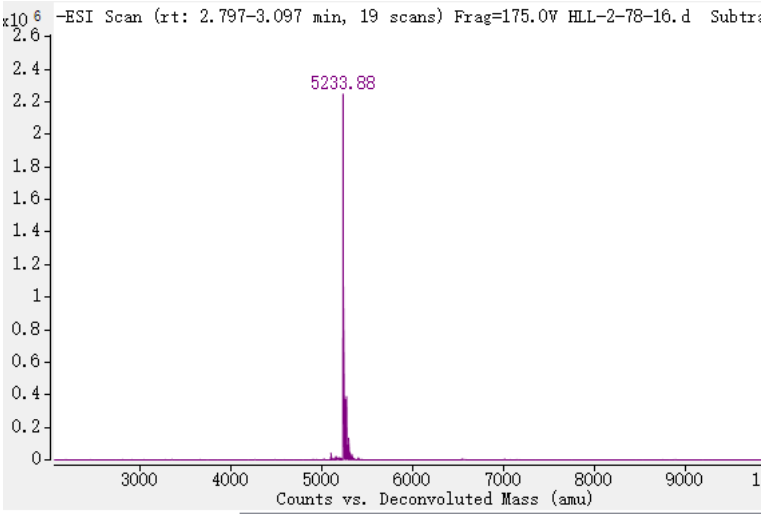


UPLC chromatogram and deconvoluted MS of **c7**

Conversion: 65%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

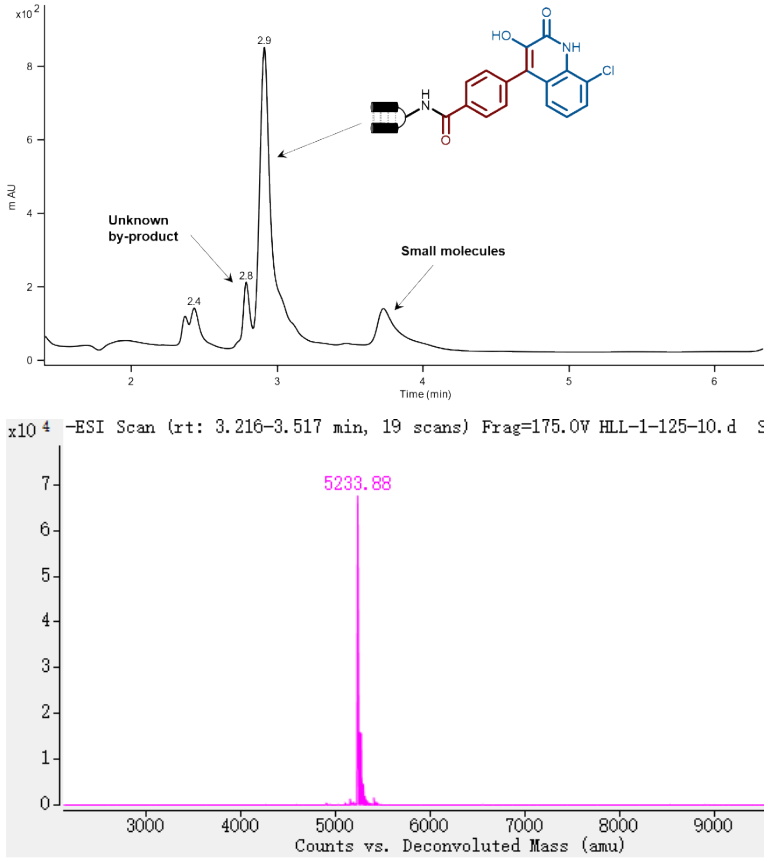




UPLC chromatogram and deconvoluted MS of **c8**

Conversion: 80%

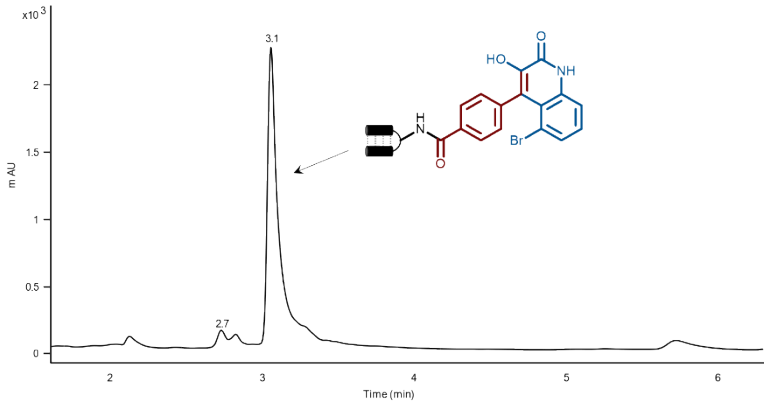
Calculated Mass: 5234 Da; Observed Mass: 5234 Da

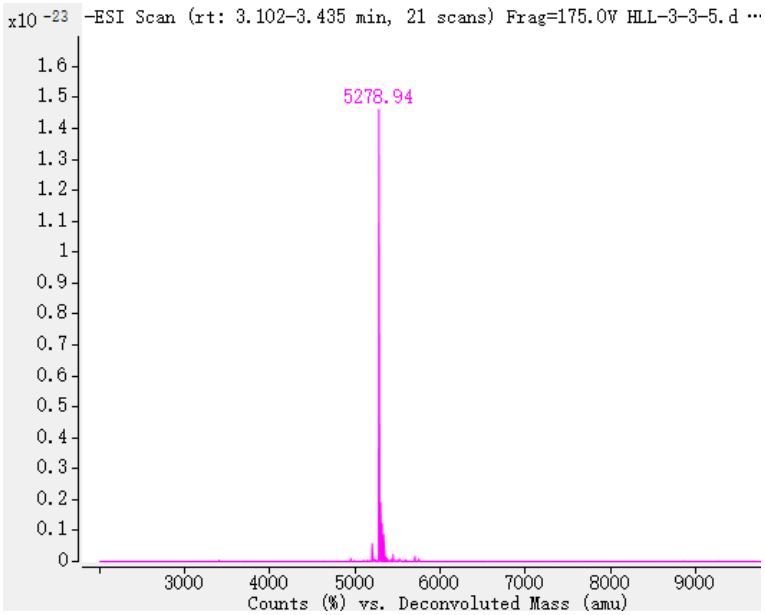


UPLC chromatogram and deconvoluted MS of **c9**

Conversion: >90%

Calculated Mass: 5279 Da; Observed Mass: 5279 Da

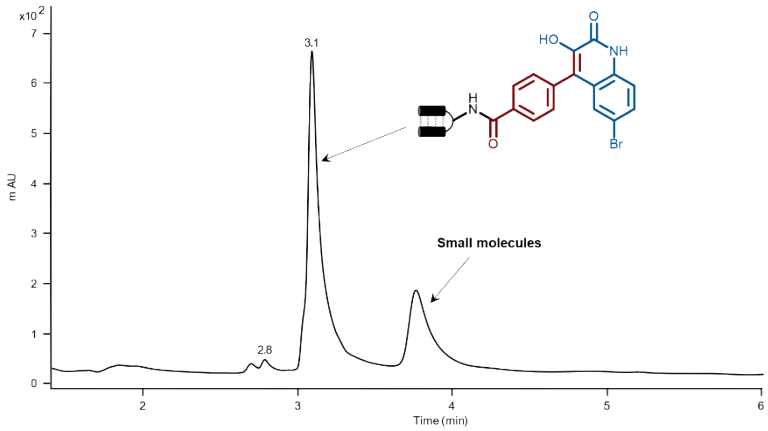


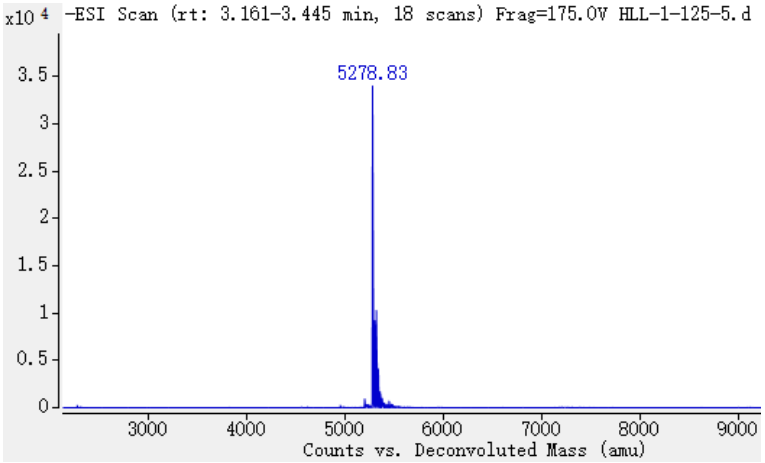


UPLC chromatogram and deconvoluted MS of **c10**

Conversion: >90%

Calculated Mass: 5279 Da; Observed Mass: 5279 Da

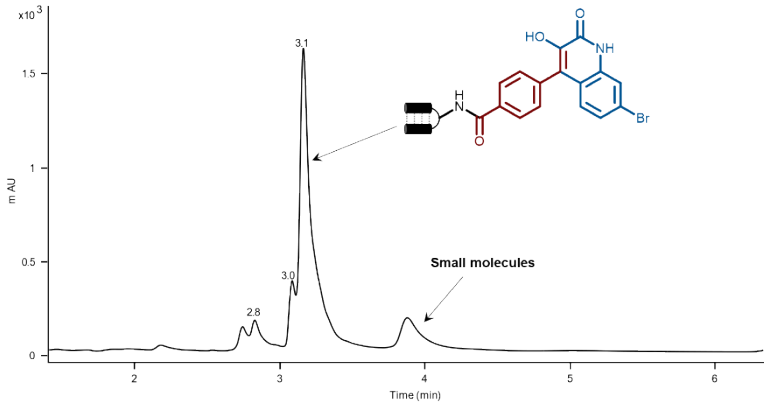


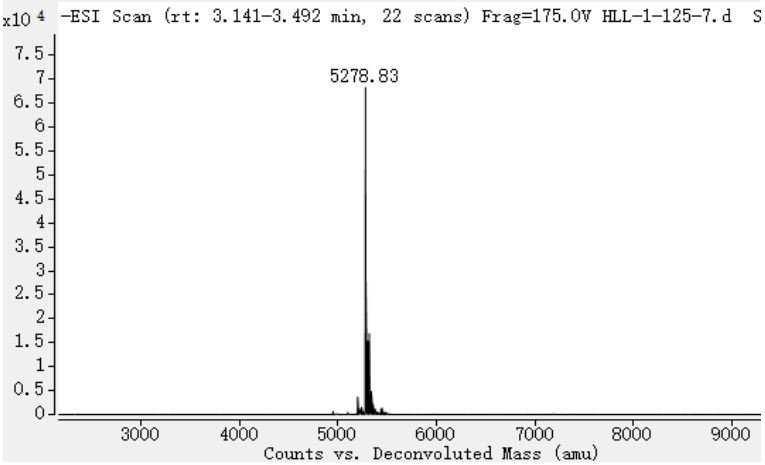


UPLC chromatogram and deconvoluted MS of **c11**

Conversion: 80%

Calculated Mass: 5279 Da; Observed Mass: 5279 Da

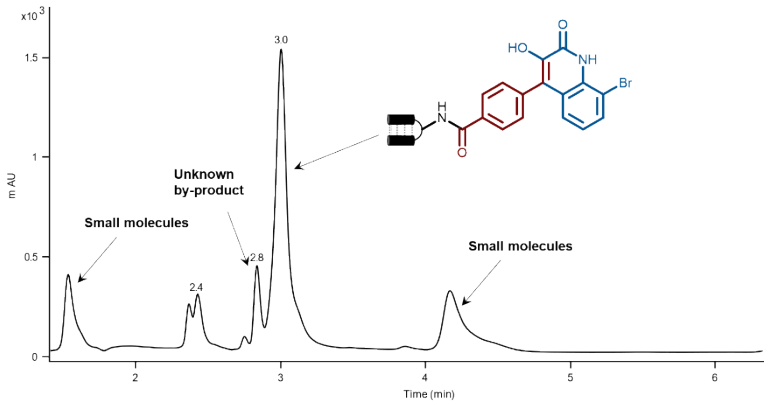


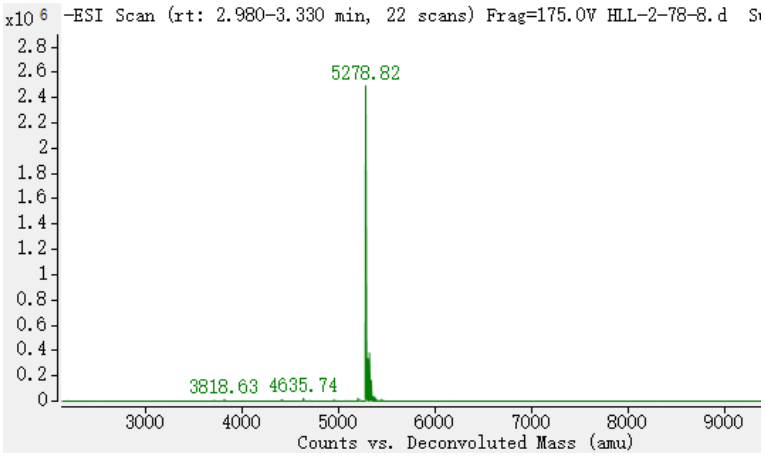


UPLC chromatogram and deconvoluted MS of **c12**

Conversion: 71%

Calculated Mass: 5279 Da; Observed Mass: 5279 Da

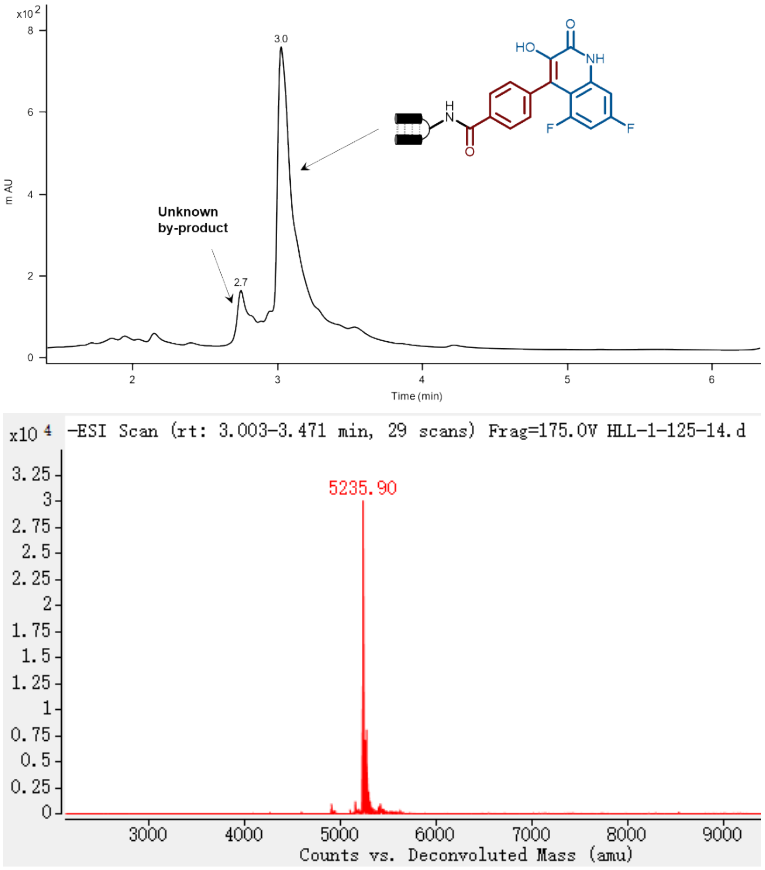




UPLC chromatogram and deconvoluted MS of **c13**

Conversion: 84%

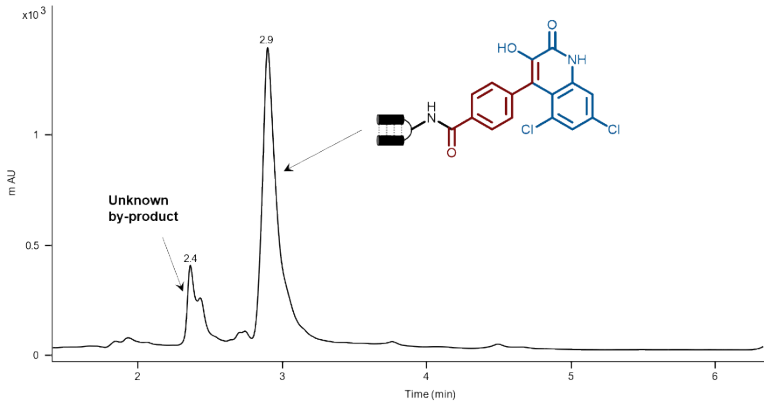
Calculated Mass: 5236 Da; Observed Mass: 5236 Da

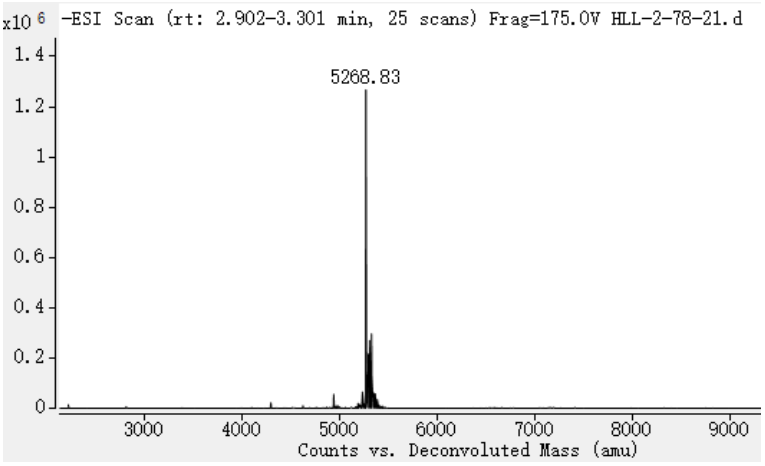


UPLC chromatogram and deconvoluted MS of **c14**

Conversion: 80%

Calculated Mass: 5269 Da; Observed Mass: 5269 Da

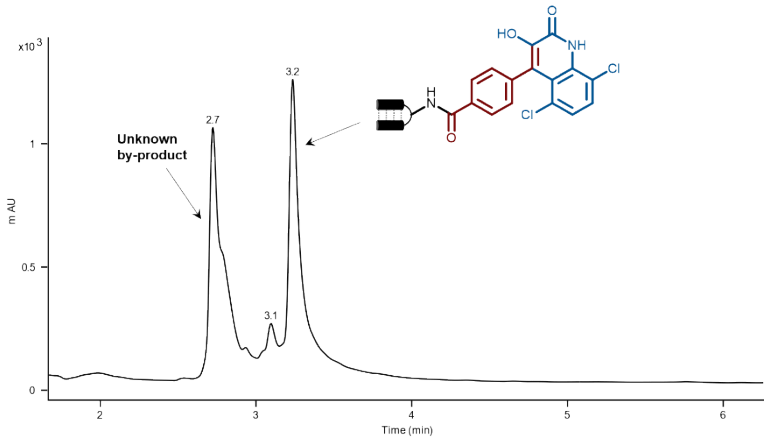


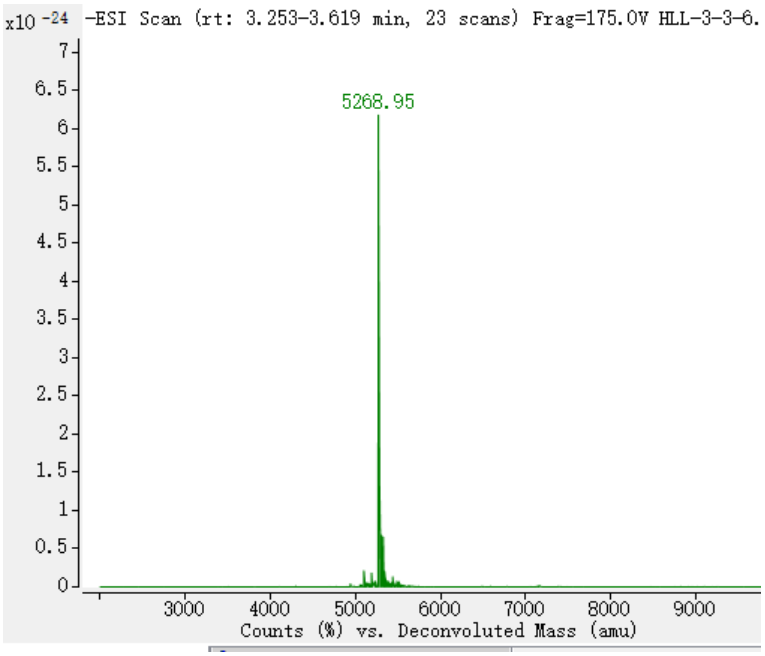


UPLC chromatogram and deconvoluted MS of **c15**

Conversion: 46%

Calculated Mass: 5269 Da; Observed Mass: 5269 Da

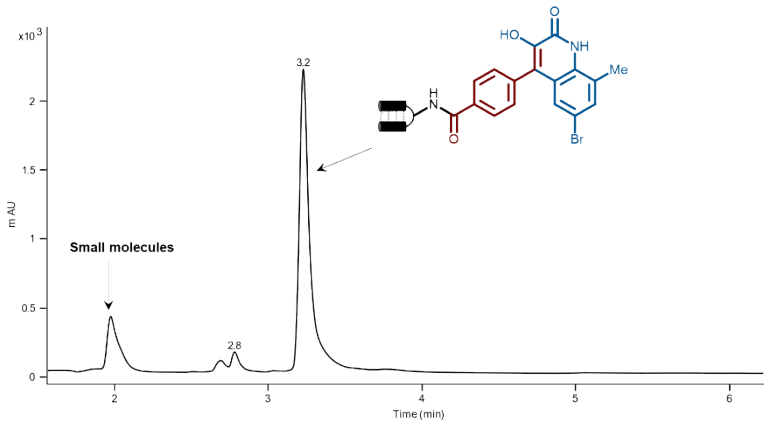


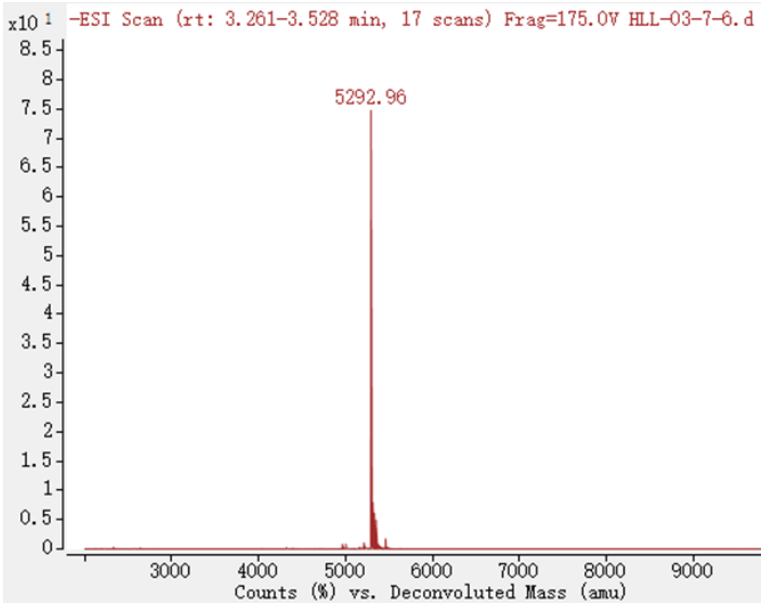


UPLC chromatogram and deconvoluted MS of **c16**

Conversion: >90%

Calculated Mass: 5293 Da; Observed Mass: 5293 Da

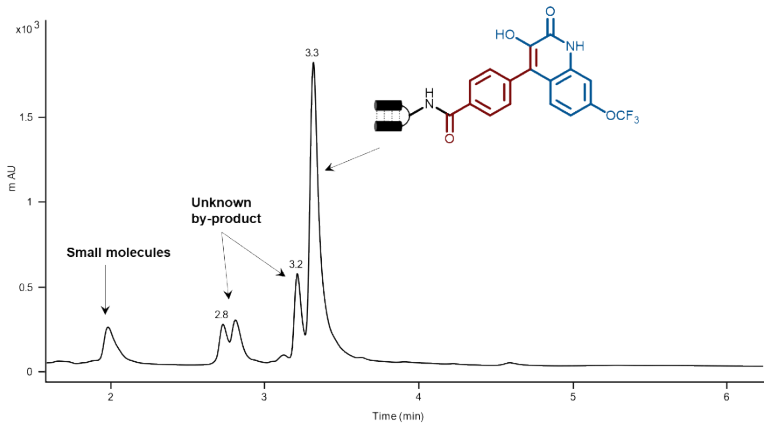


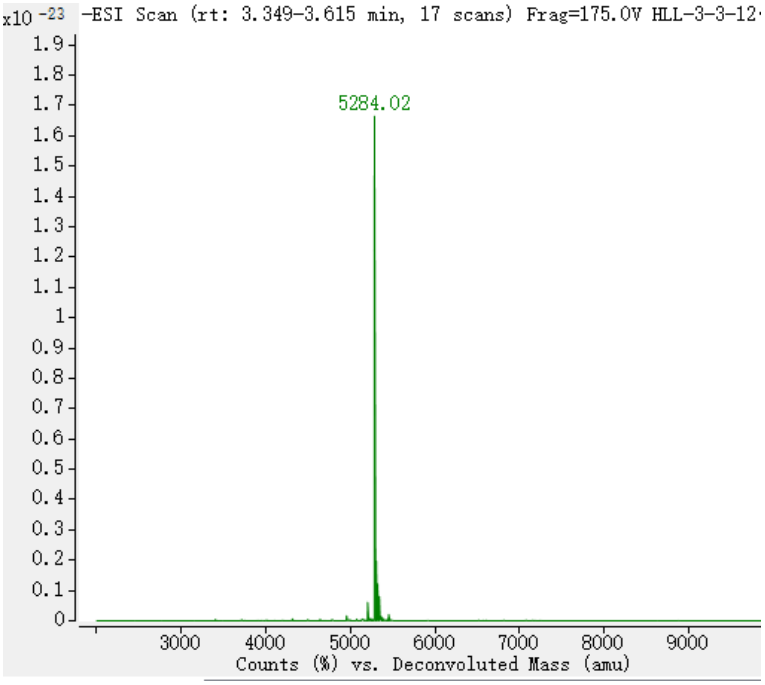


UPLC chromatogram and deconvoluted MS of **c17**

Conversion: 68%

Calculated Mass: 5284 Da; Observed Mass: 5284 Da

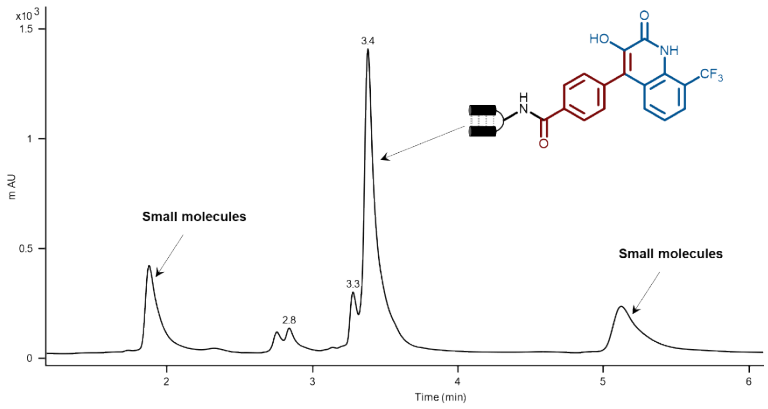


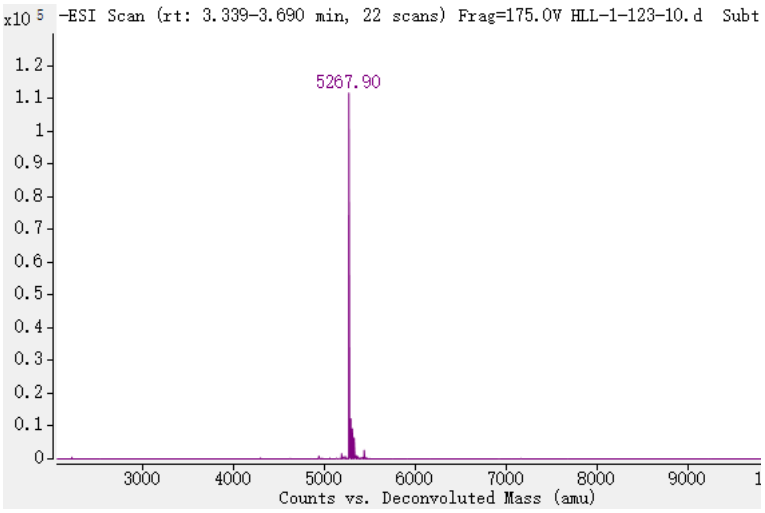


UPLC chromatogram and deconvoluted MS of **c18**

Conversion: 81%

Calculated Mass: 5268 Da; Observed Mass: 5268 Da

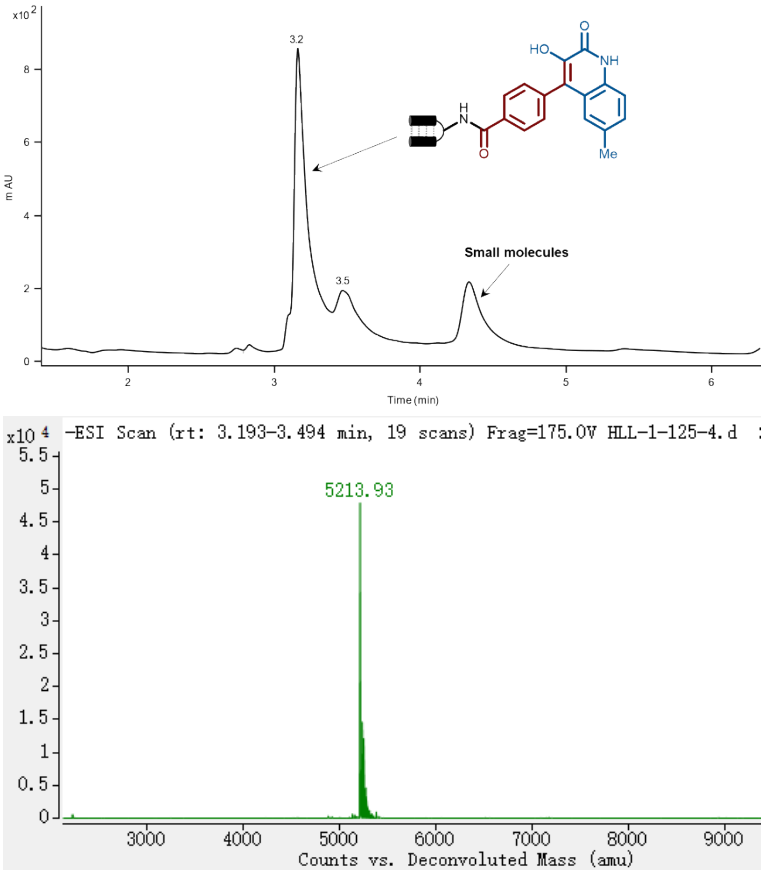




UPLC chromatogram and deconvoluted MS of **c19**

Conversion: 70%

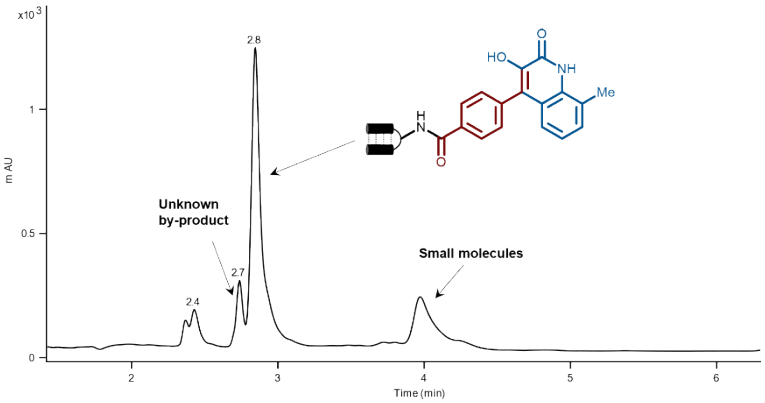
Calculated Mass: 5214 Da; Observed Mass: 5214 Da

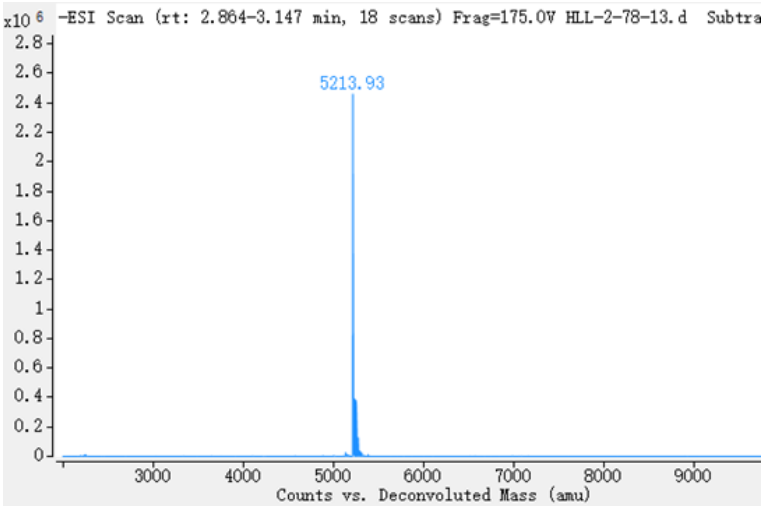


UPLC chromatogram and deconvoluted MS of **c20**

Conversion: 74%

Calculated Mass: 5214 Da; Observed Mass: 5214 Da

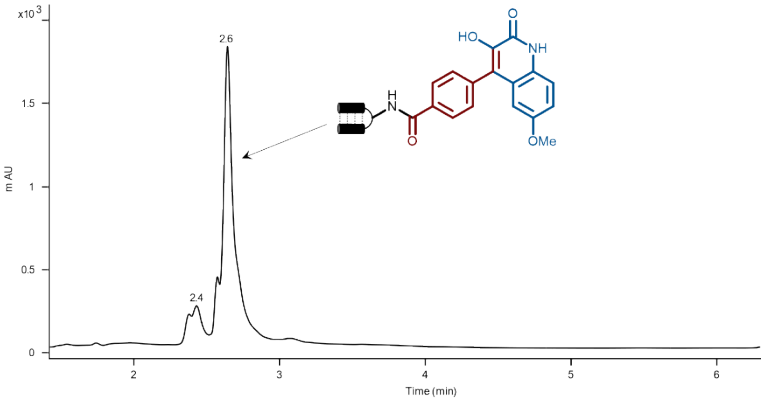


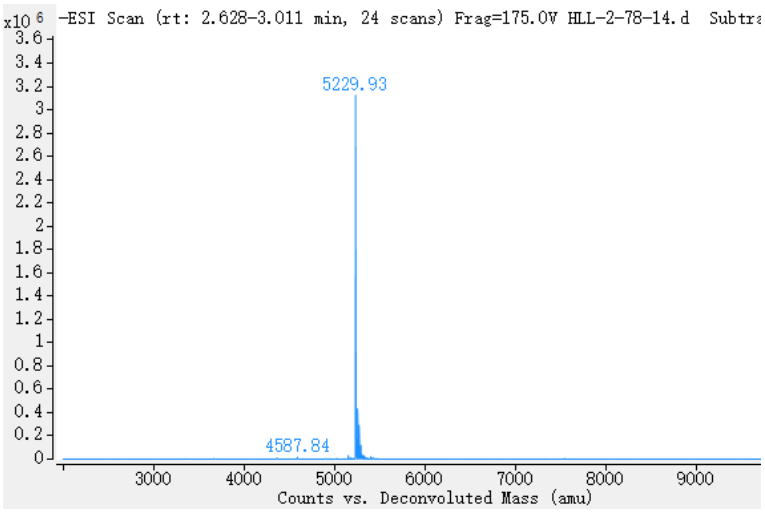


UPLC chromatogram and deconvoluted MS of **c21**

Conversion: 75%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

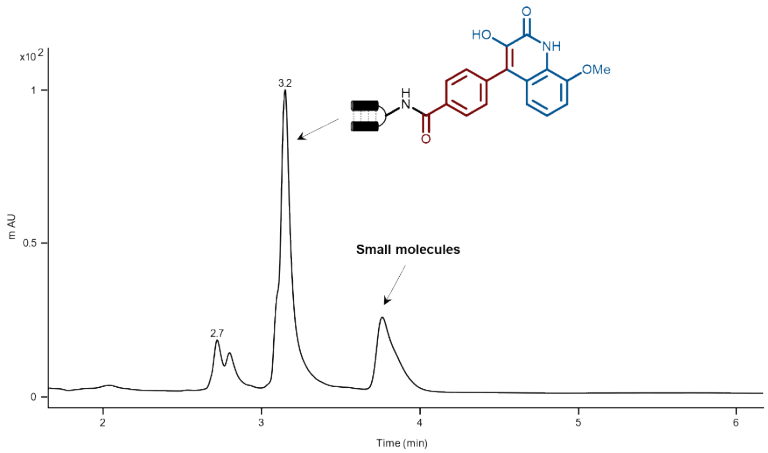


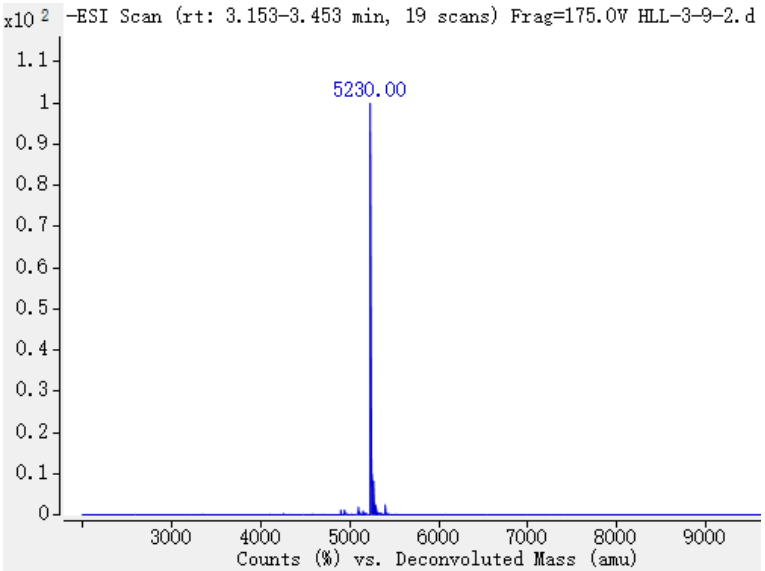


UPLC chromatogram and deconvoluted MS of **c22**

Conversion: 81%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

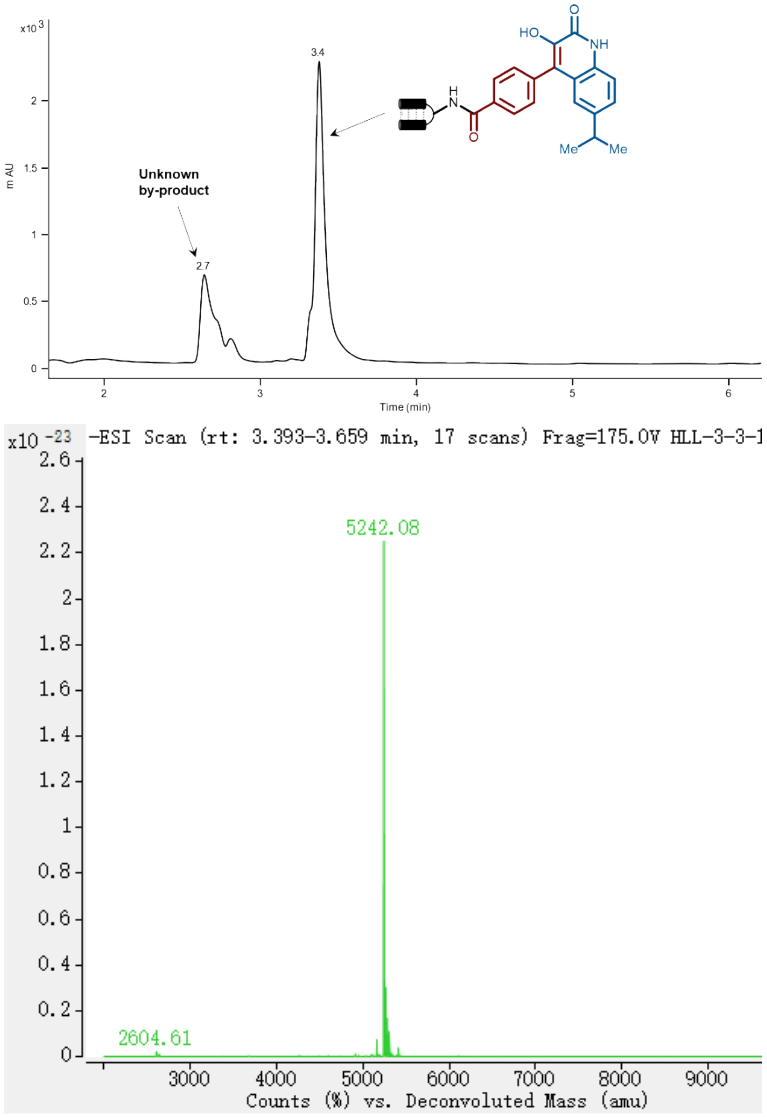




UPLC chromatogram and deconvoluted MS of **c23**

Conversion: 70%

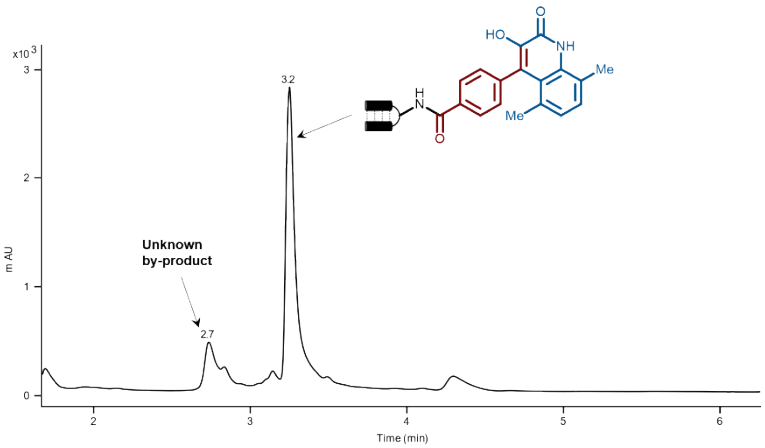
Calculated Mass: 5342 Da; Observed Mass: 5342 Da

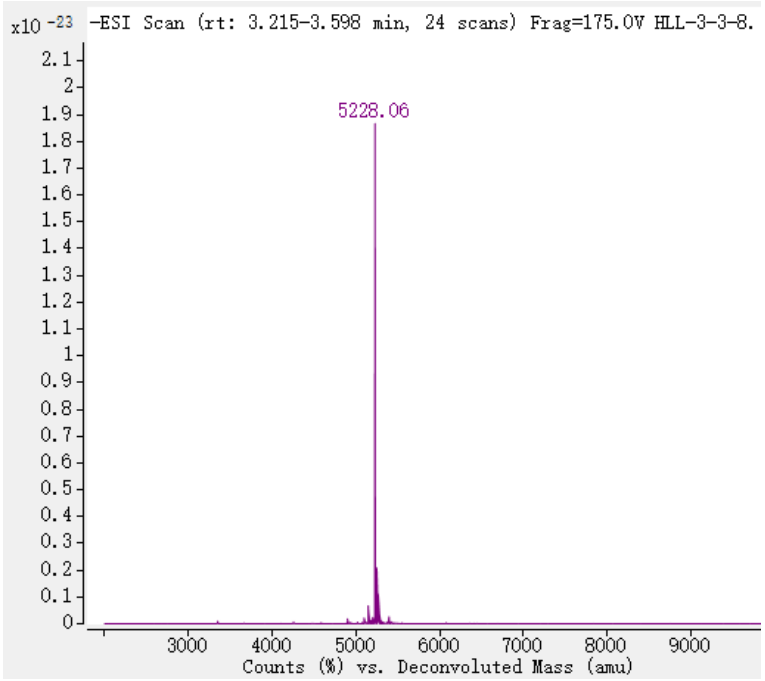


UPLC chromatogram and deconvoluted MS of **c24**

Conversion: 81%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

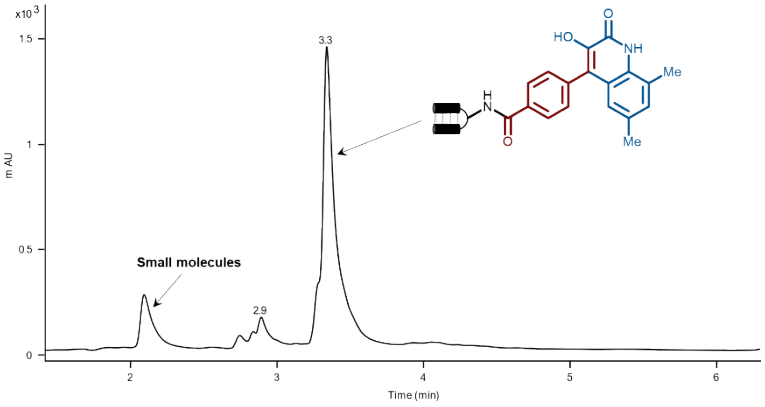


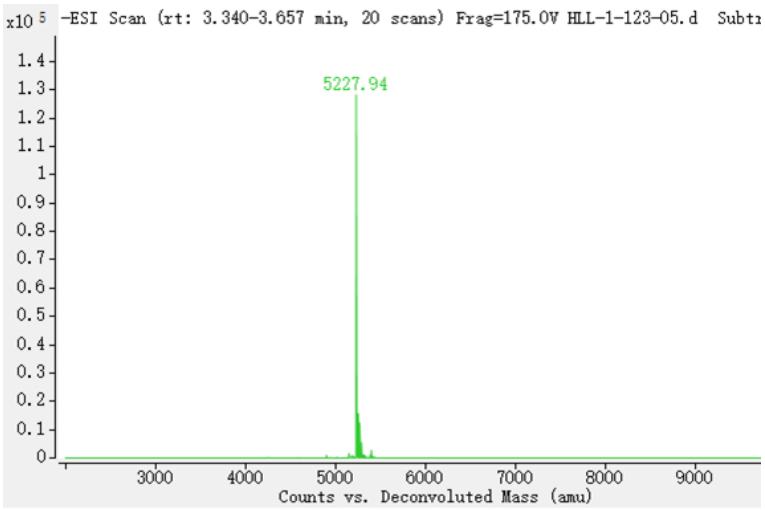


UPLC chromatogram and deconvoluted MS of **c25**

Conversion: 82%

Calculated Mass: 5228 Da; Observed Mass: 5228 Da

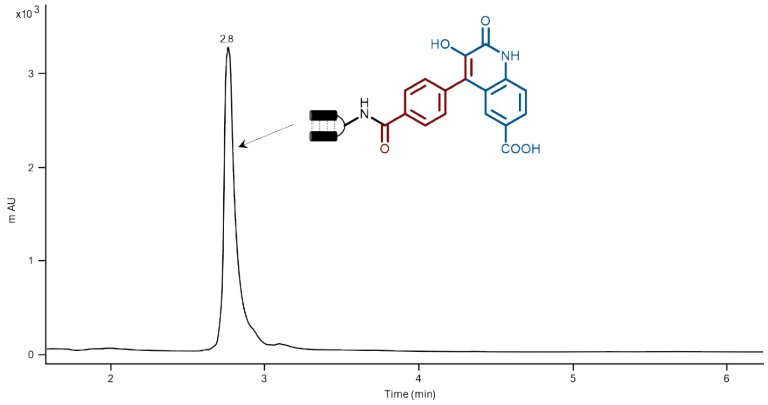


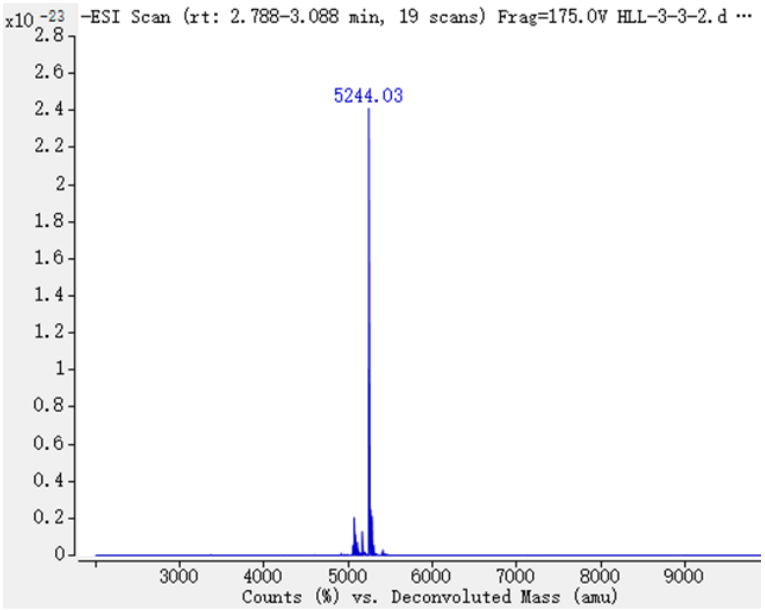


UPLC chromatogram and deconvoluted MS of **c26**

Conversion: >90%

Calculated Mass: 5244 Da; Observed Mass: 5244 Da

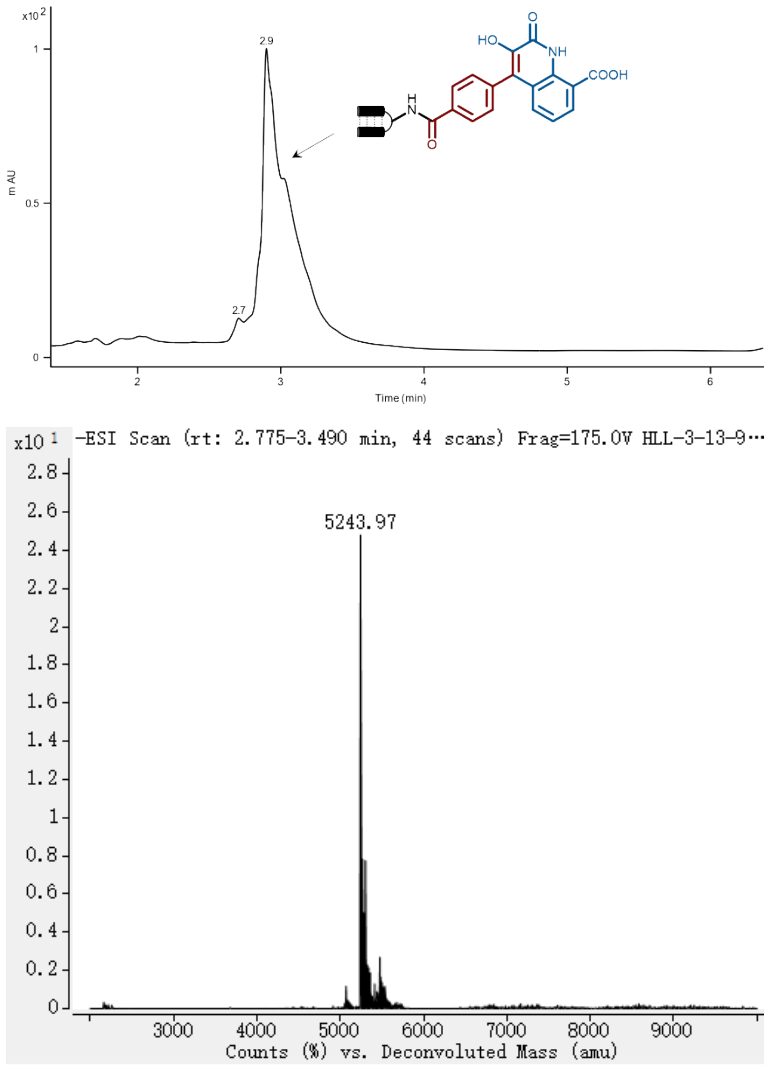




UPLC chromatogram and deconvoluted MS of **c27**

Conversion: %

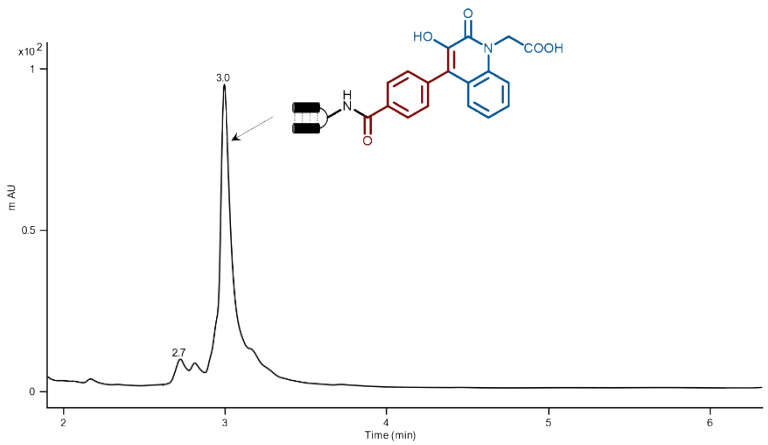
Calculated Mass: 5244 Da; Observed Mass: 5244 Da

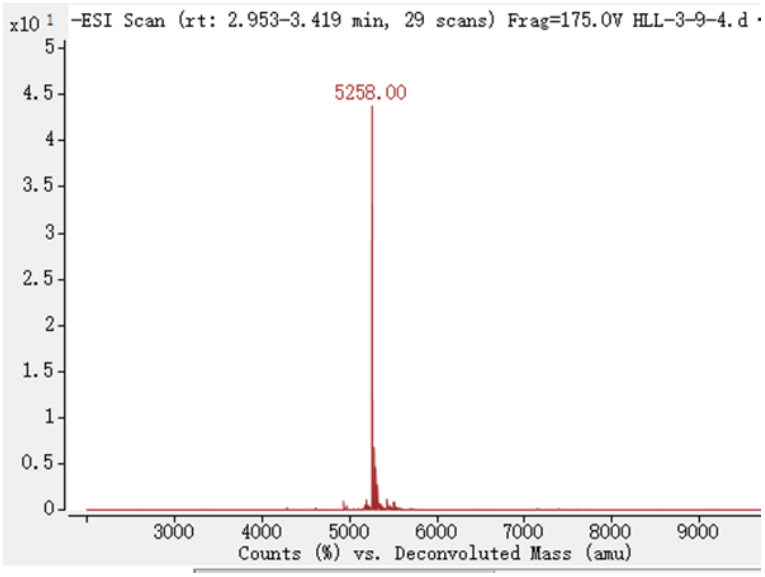


UPLC chromatogram and deconvoluted MS of **c28**

Conversion: 89%

Calculated Mass: 5258 Da; Observed Mass: 5258 Da

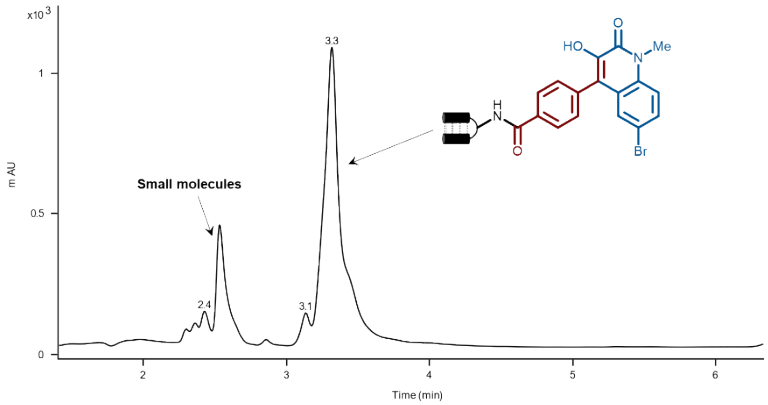


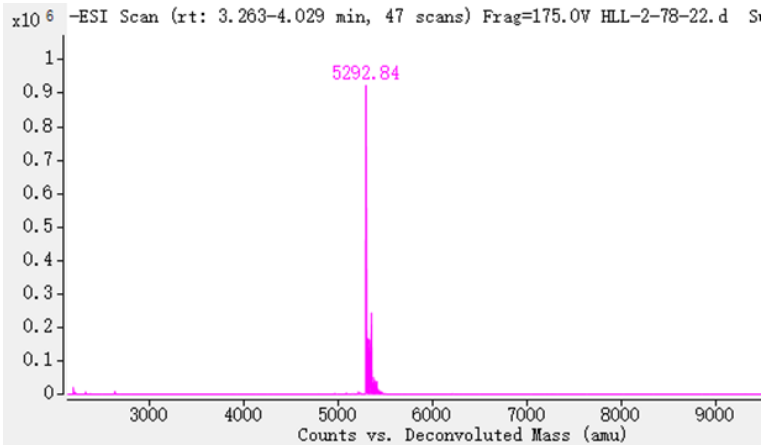


UPLC chromatogram and deconvoluted MS of **c29**

Conversion: 80%

Calculated Mass: 5293 Da; Observed Mass: 5293 Da

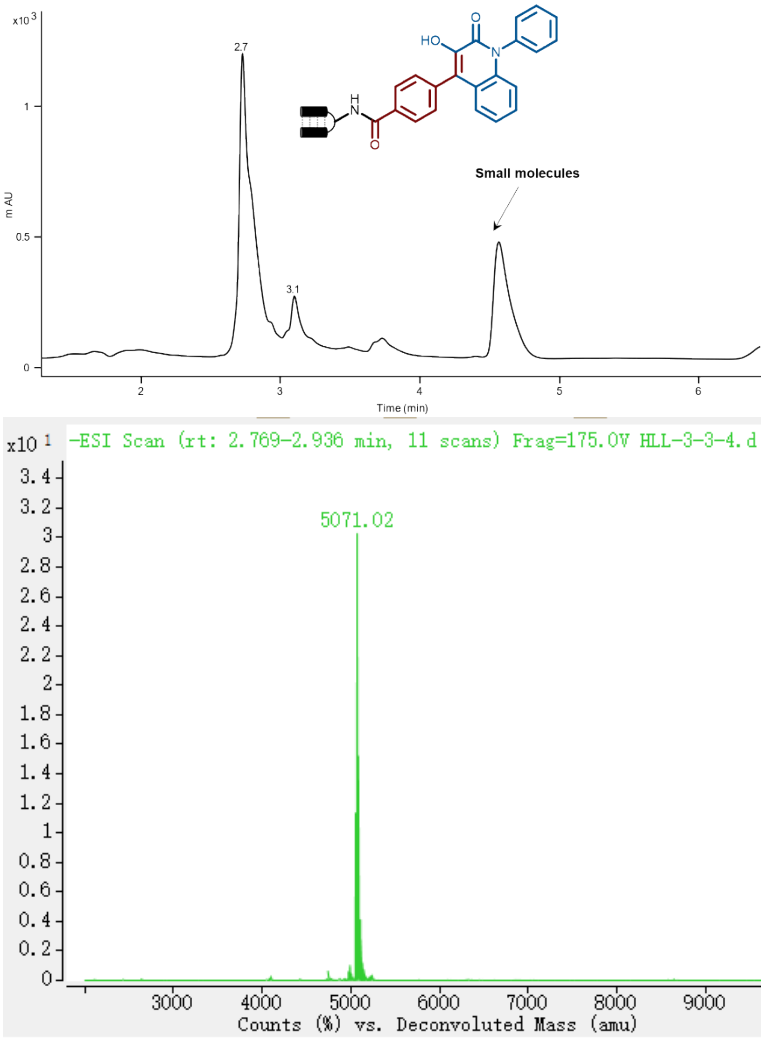




UPLC chromatogram and deconvoluted MS of **c30**

Conversion: 0%

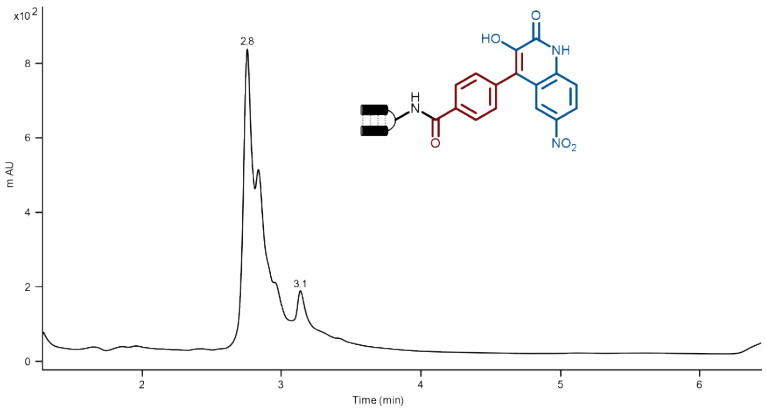
Calculated Mass: 5276 Da; Observed Mass: 5071 Da

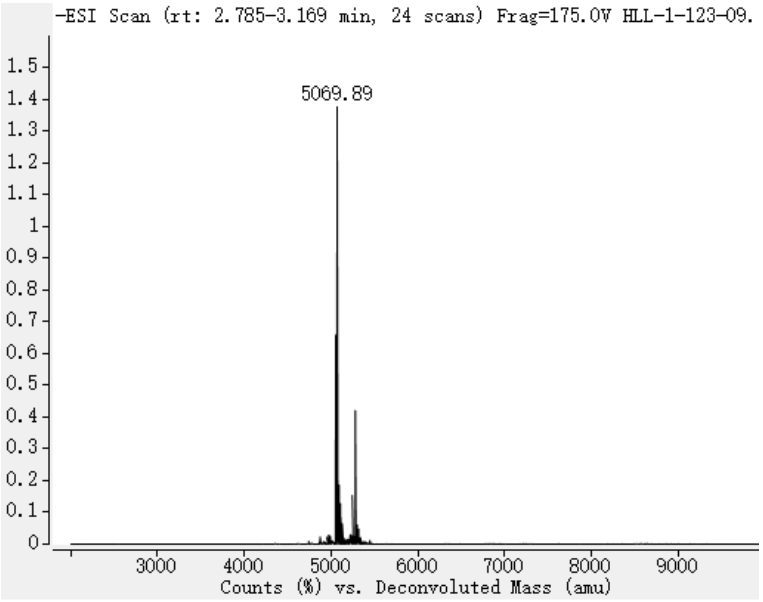


UPLC chromatogram and deconvoluted MS of **c31**

Conversion: 0%

Calculated Mass: 5245 Da; Observed Mass: 5069 Da



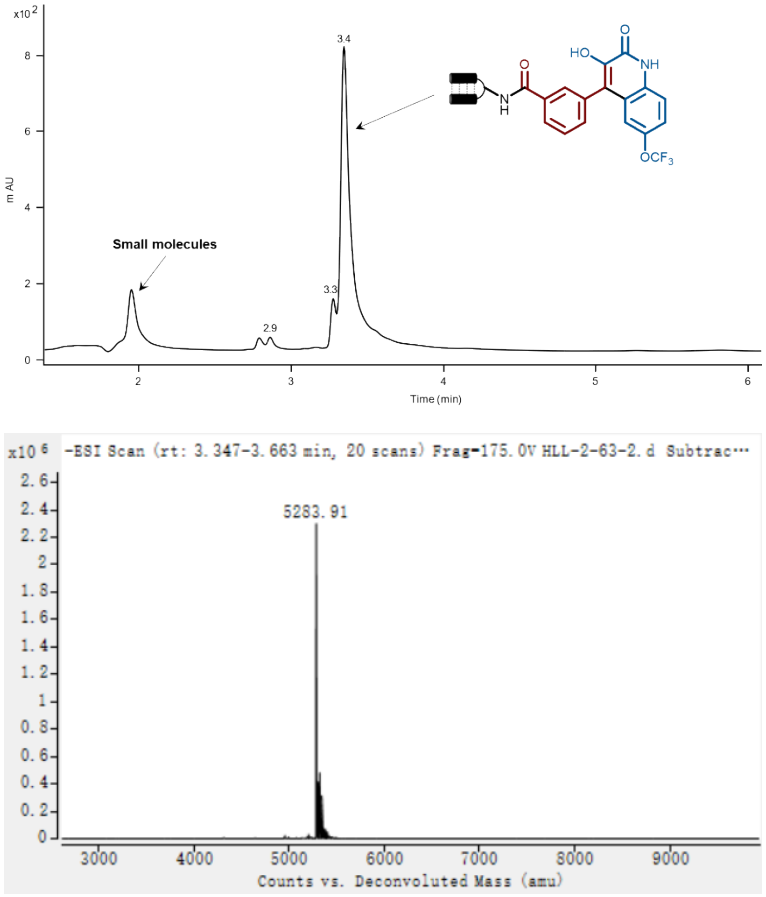


7.2 Substrate scope of DNA-tagged aldehydes

UPLC chromatogram and deconvoluted MS of **c32**

Conversion: >90%

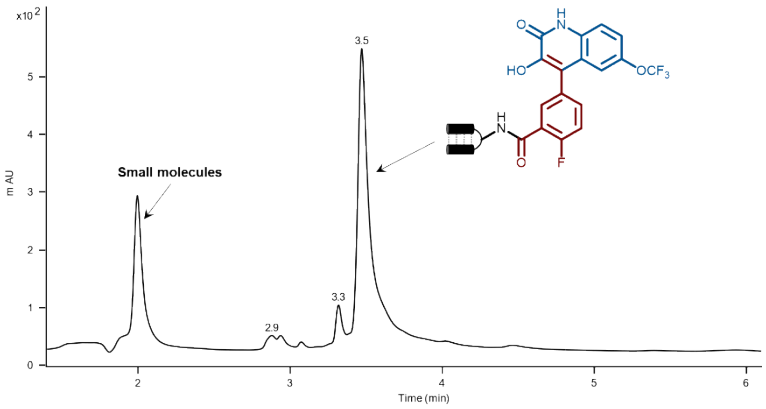
Calculated Mass: 5284 Da; Observed Mass: 5284 Da

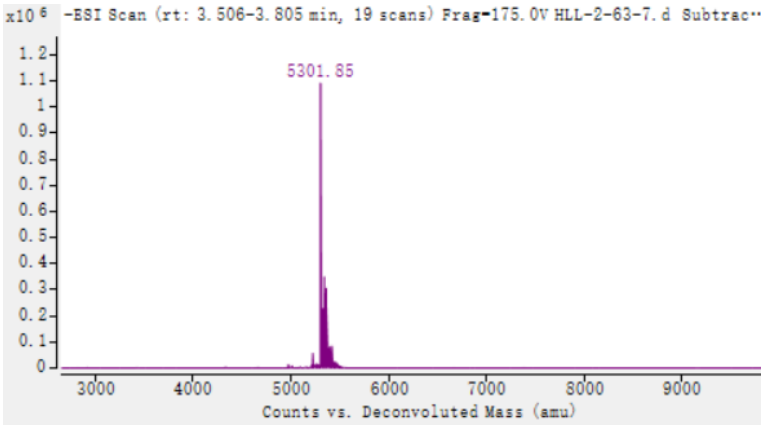


UPLC chromatogram and deconvoluted MS of **c33**

Conversion: >90%

Calculated Mass: 5302 Da; Observed Mass: 5302 Da

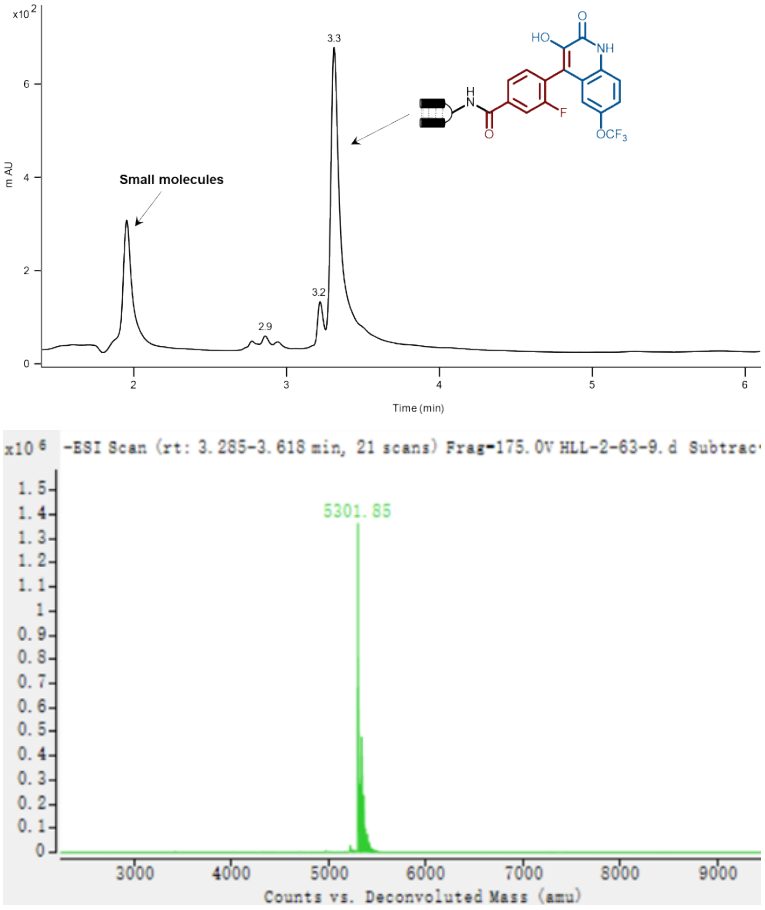




UPLC chromatogram and deconvoluted MS of **c34**

Conversion: 88%

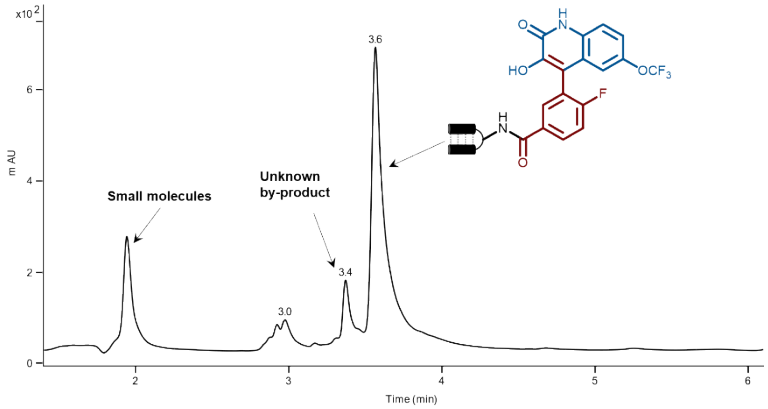
Calculated Mass: 5302 Da; Observed Mass: 5302 Da



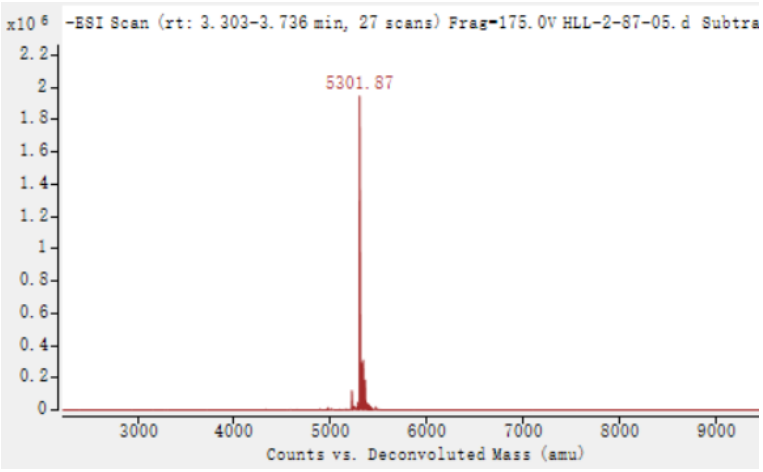
UPLC chromatogram and deconvoluted MS of **c35**

Conversion: 84%

Calculated Mass: 5302 Da; Observed Mass: 5302 Da



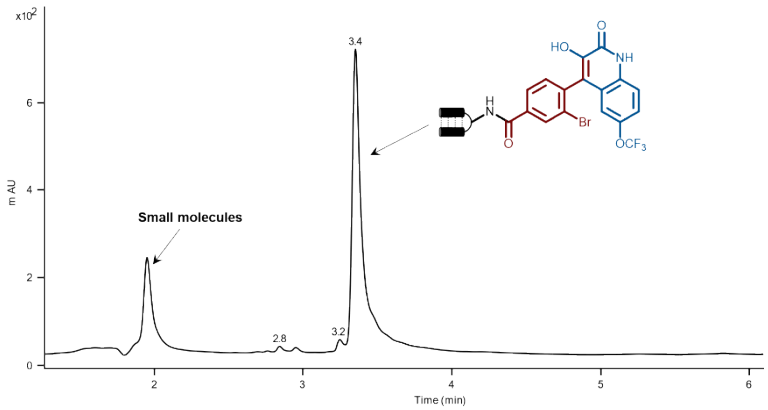
10

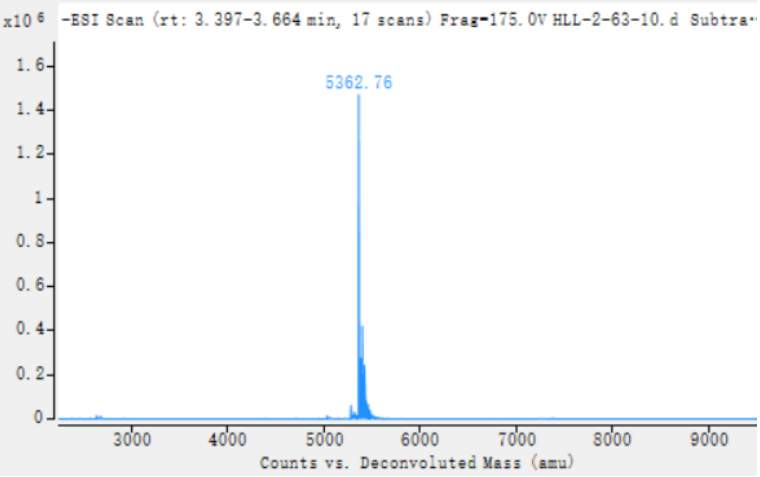


UPLC chromatogram and deconvoluted MS of **c36**

Conversion: >90%

Calculated Mass: 5363 Da; Observed Mass: 5363 Da

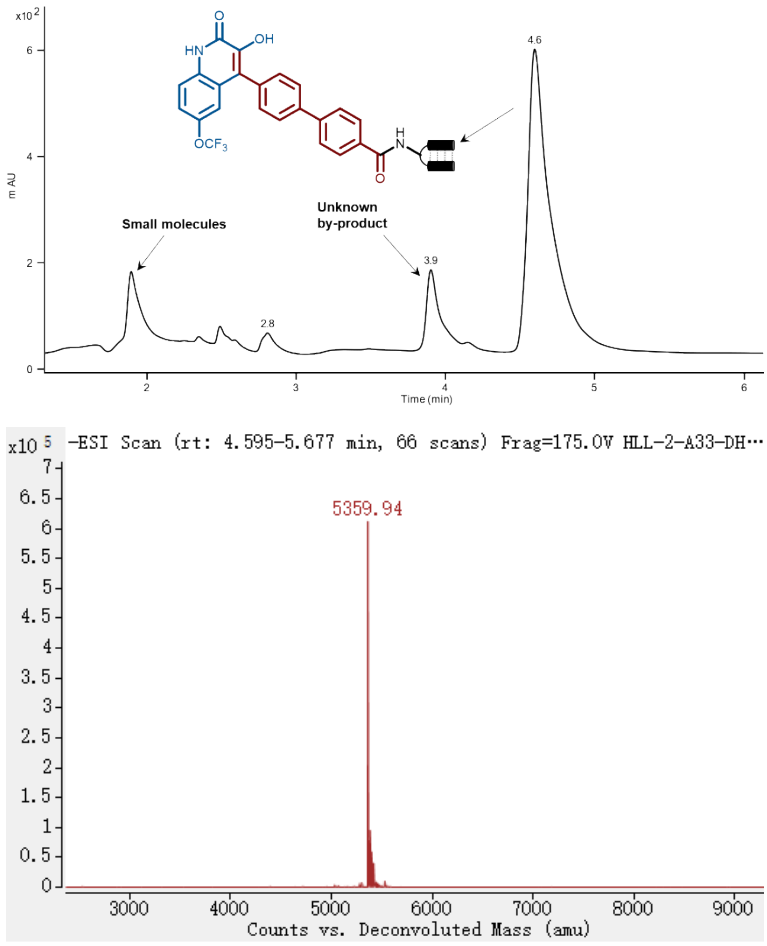




UPLC chromatogram and deconvoluted MS of **c37**

Conversion: 78%

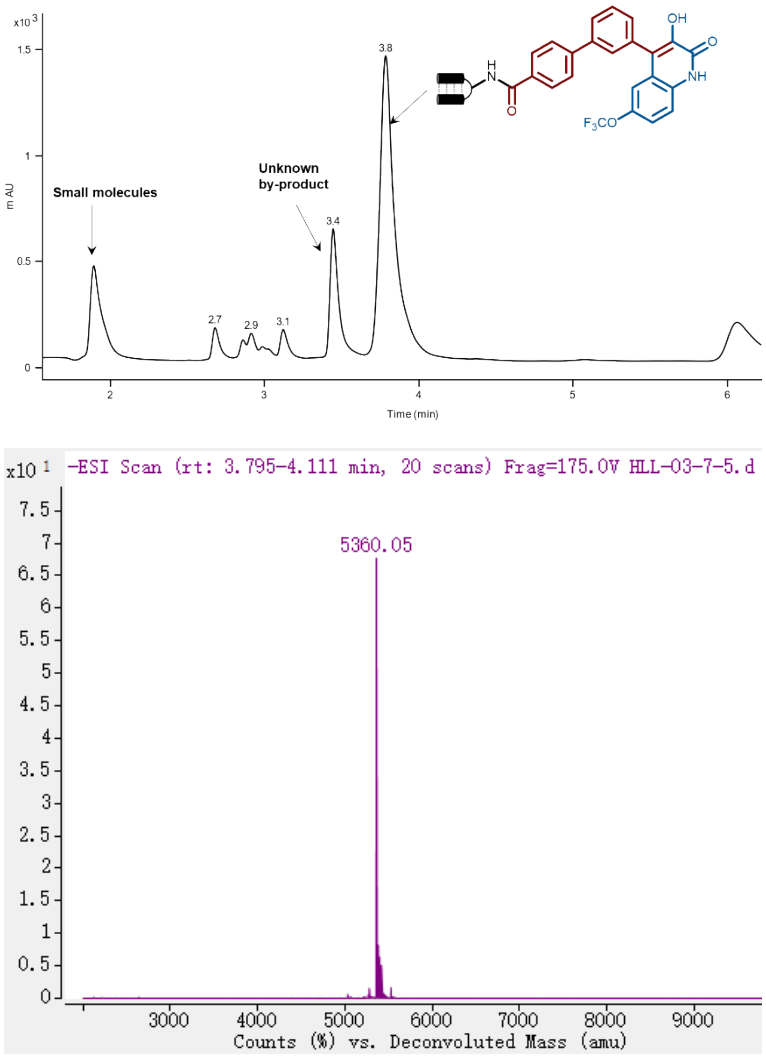
Calculated Mass: 5360 Da; Observed Mass: 5360 Da



UPLC chromatogram and deconvoluted MS of **c38**

Conversion: 73%

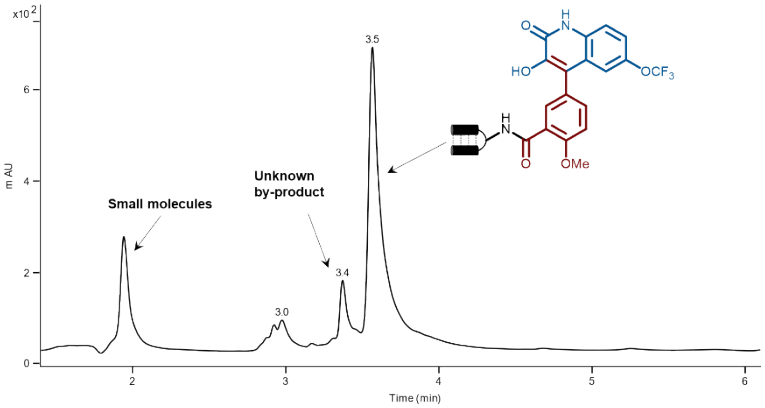
Calculated Mass: 5360 Da; Observed Mass: 5360 Da

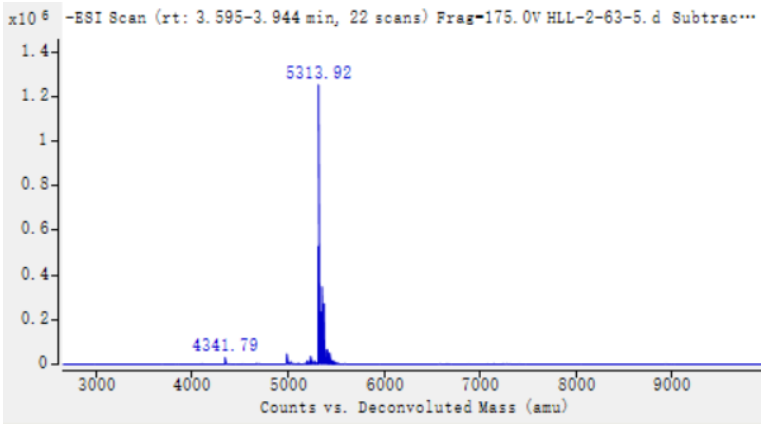


UPLC chromatogram and deconvoluted MS of **c39**

Conversion: 78%

Calculated Mass: 5314 Da; Observed Mass: 5314 Da

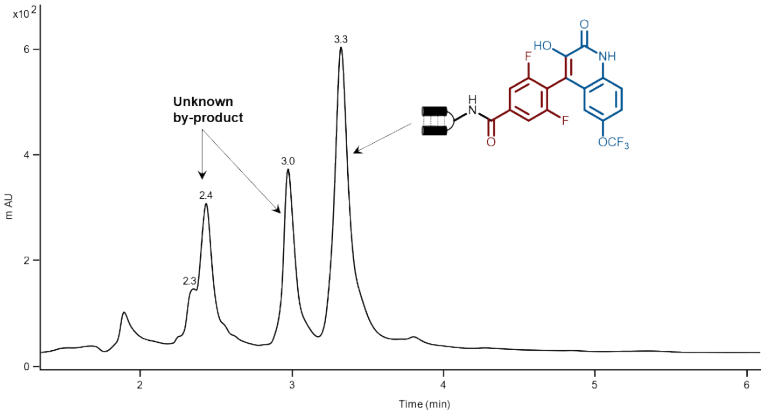


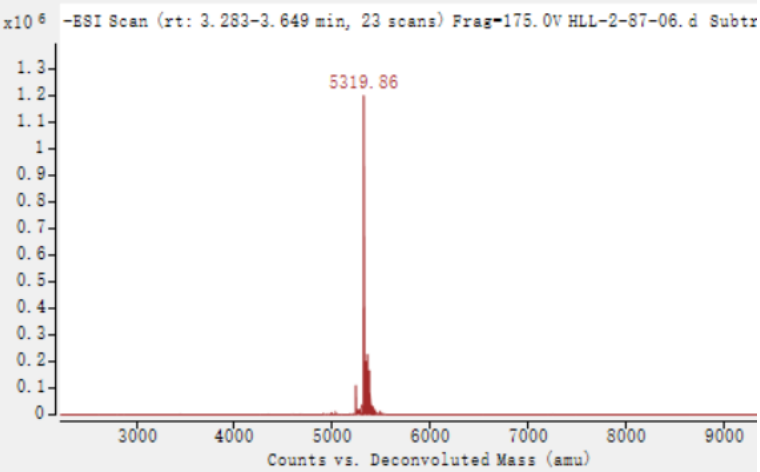


UPLC chromatogram and deconvoluted MS of **c40**

Conversion: 45%

Calculated Mass: 5320 Da; Observed Mass: 5320 Da

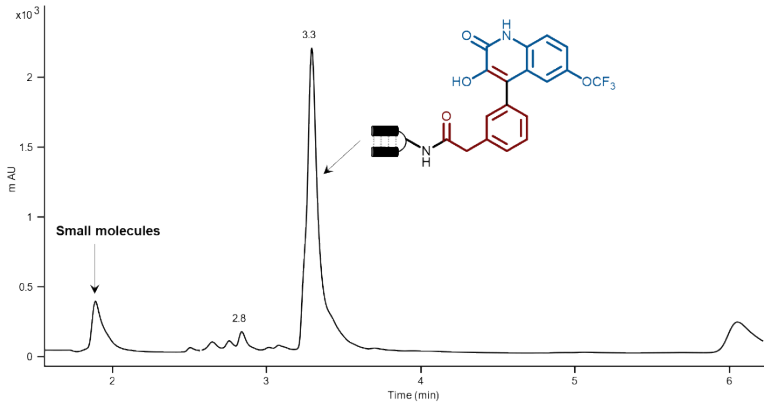


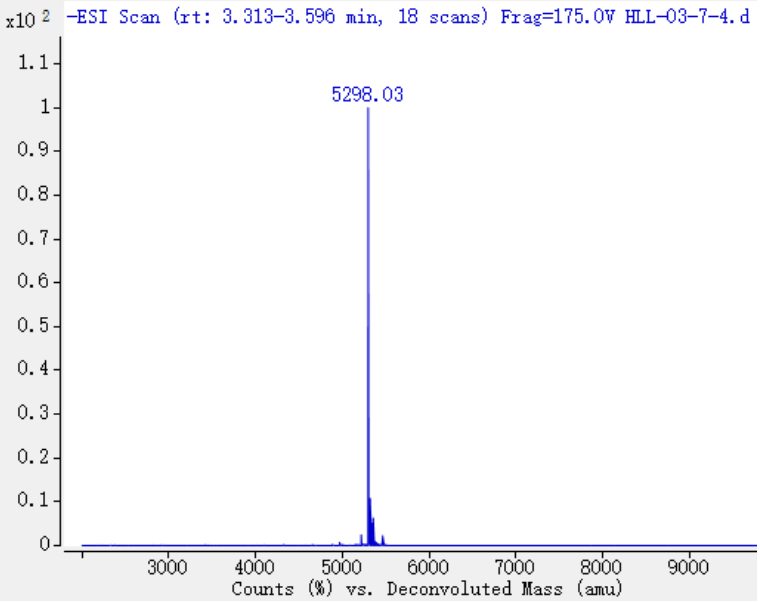


UPLC chromatogram and deconvoluted MS of **c41**

Conversion: >90%

Calculated Mass: 5298 Da; Observed Mass: 5298 Da

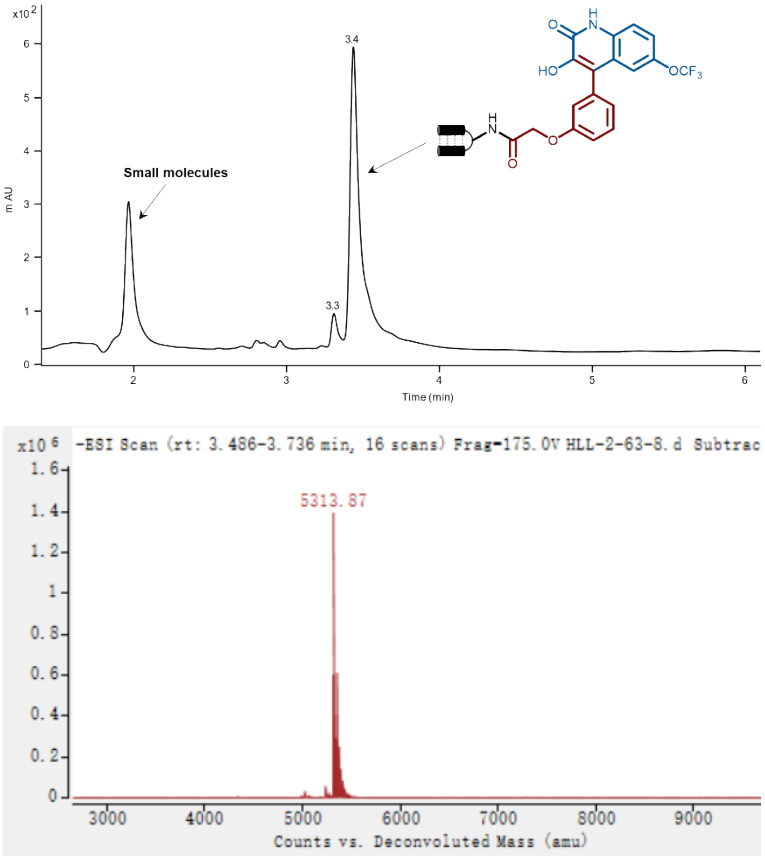




UPLC chromatogram and deconvoluted MS of **c42**

Conversion: >90%

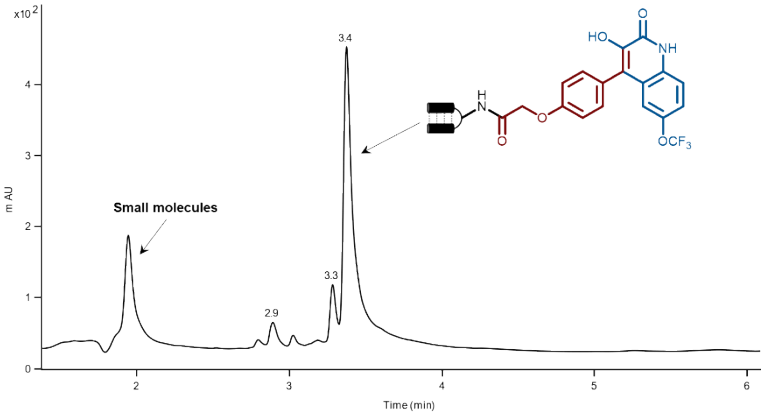
Calculated Mass: 5314 Da; Observed Mass: 5314 Da

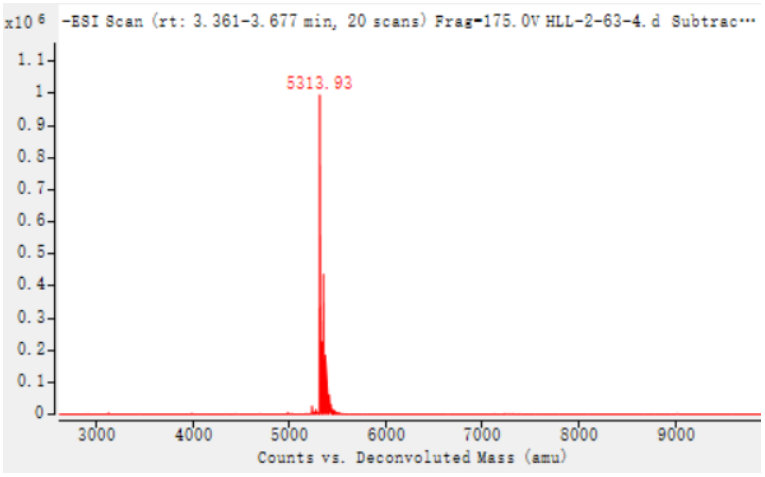


UPLC chromatogram and deconvoluted MS of **c43**

Conversion: 81%

Calculated Mass: 5314 Da; Observed Mass: 5314 Da

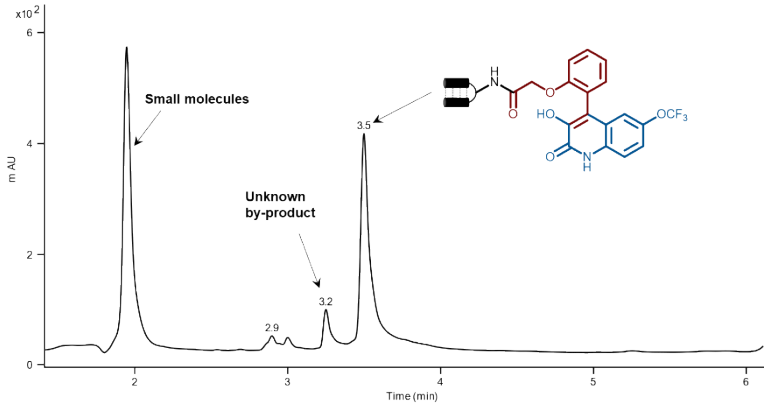


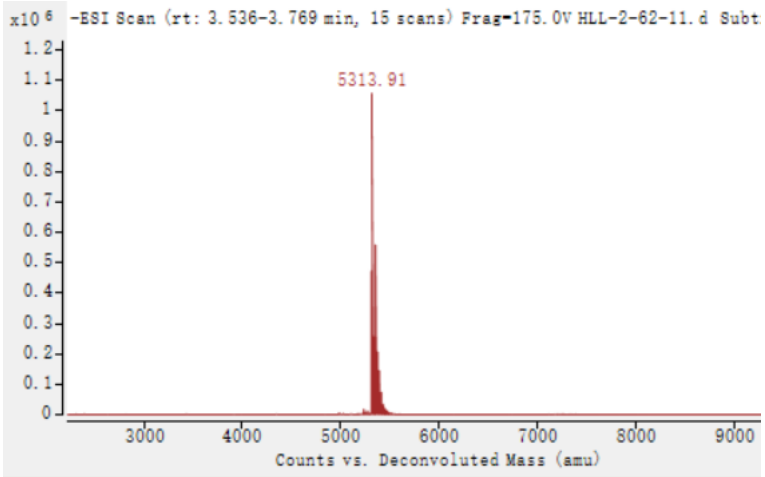


UPLC chromatogram and deconvoluted MS of **c44**

Conversion: 79%

Calculated Mass: 5314 Da; Observed Mass: 5314 Da

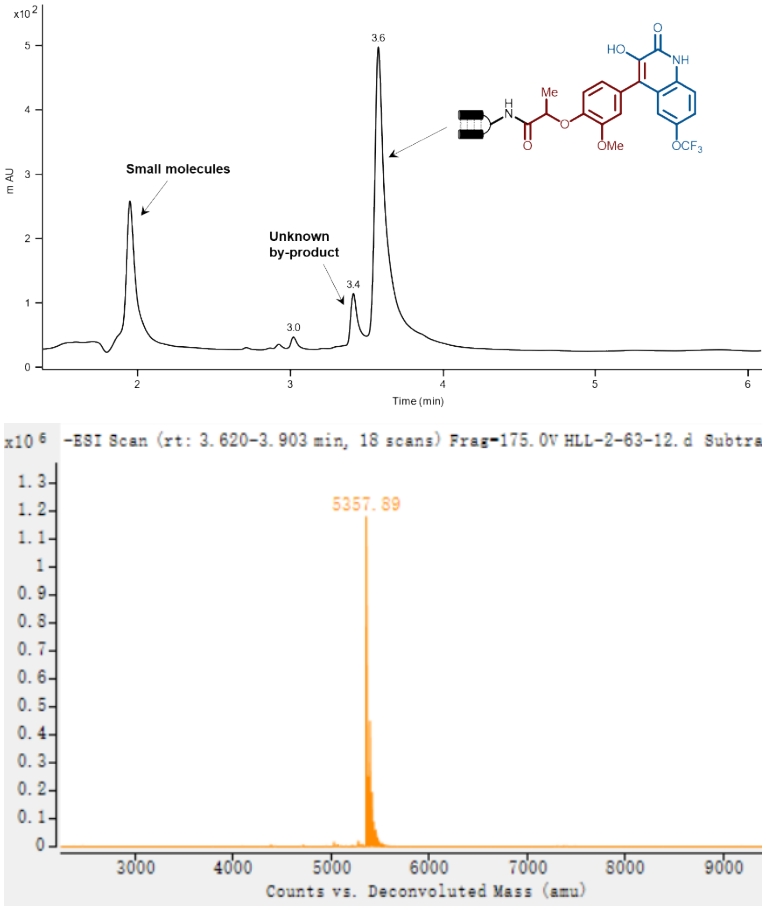




UPLC chromatogram and deconvoluted MS of **c45**

Conversion: 84%

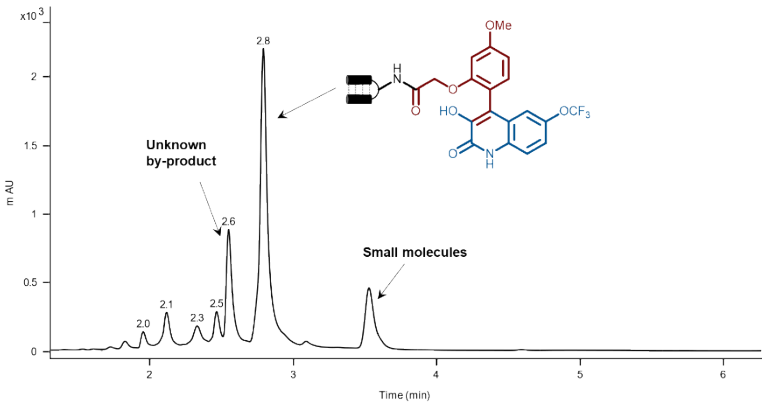
Calculated Mass: 5358 Da; Observed Mass: 5358 Da

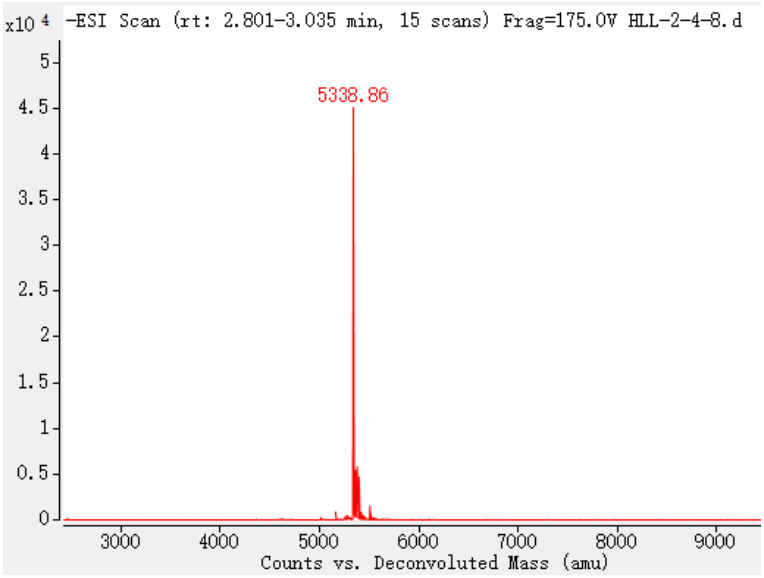


UPLC chromatogram and deconvoluted MS of **c46**

Conversion: 65%

Calculated Mass: 5339 Da; Observed Mass: 5339 Da

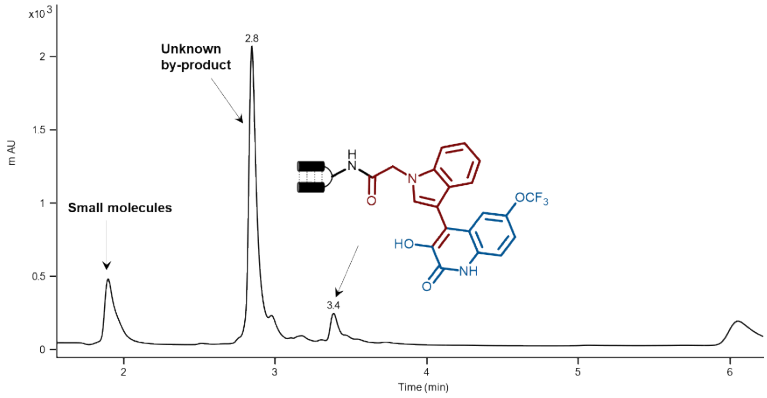


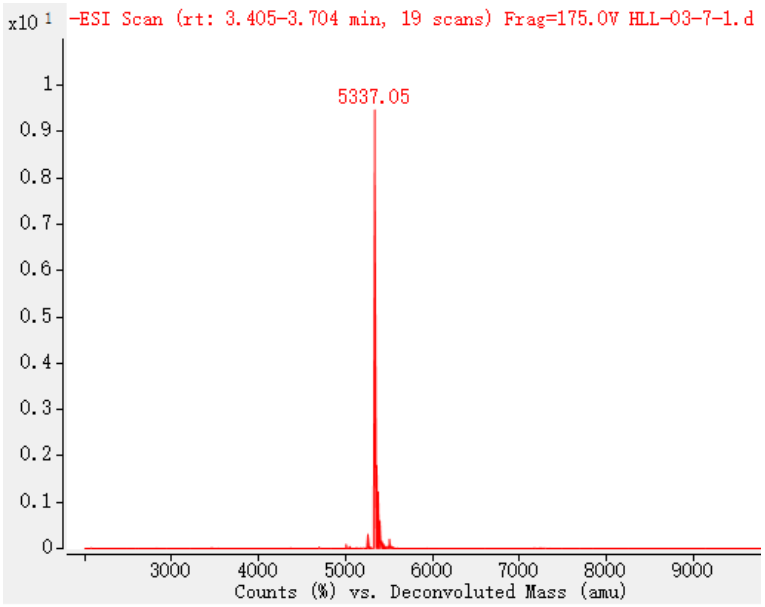


UPLC chromatogram and deconvoluted MS of **c47**

Conversion: 14%

Calculated Mass: 5337 Da; Observed Mass: 5337 Da

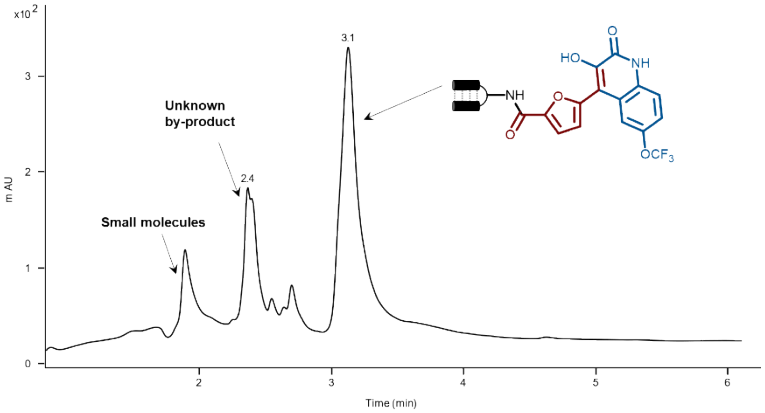


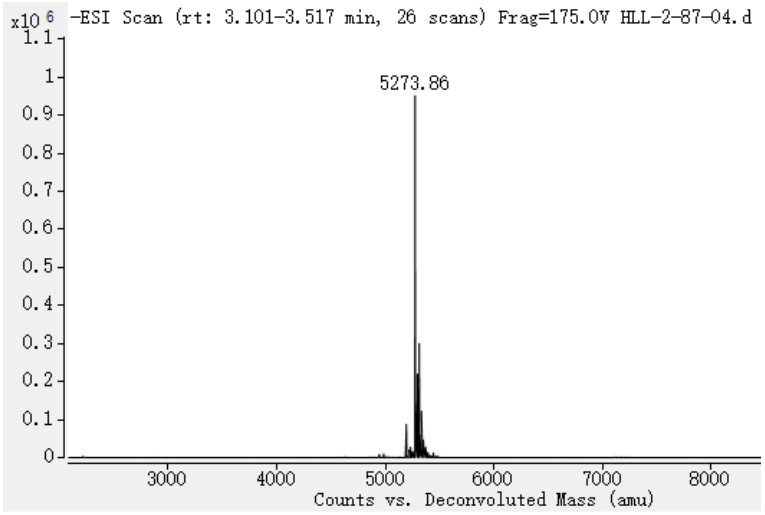


UPLC chromatogram and deconvoluted MS of **c48**

Conversion: 64%

Calculated Mass: 5274 Da; Observed Mass: 5274 Da

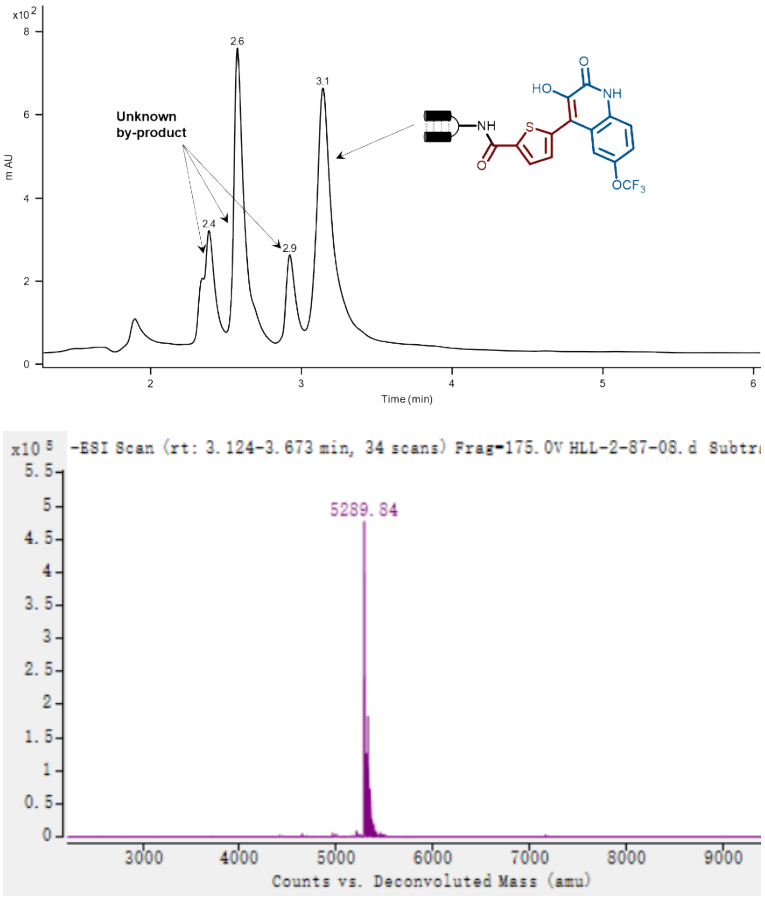




UPLC chromatogram and deconvoluted MS of **c49**

Conversion: 47%

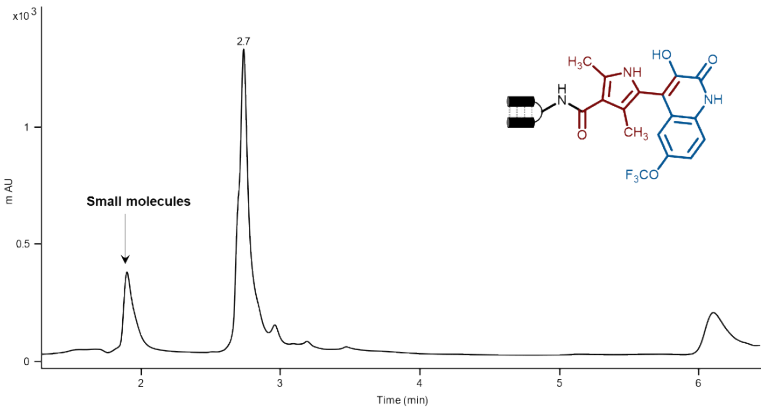
Calculated Mass: 5290 Da; Observed Mass: 5290 Da

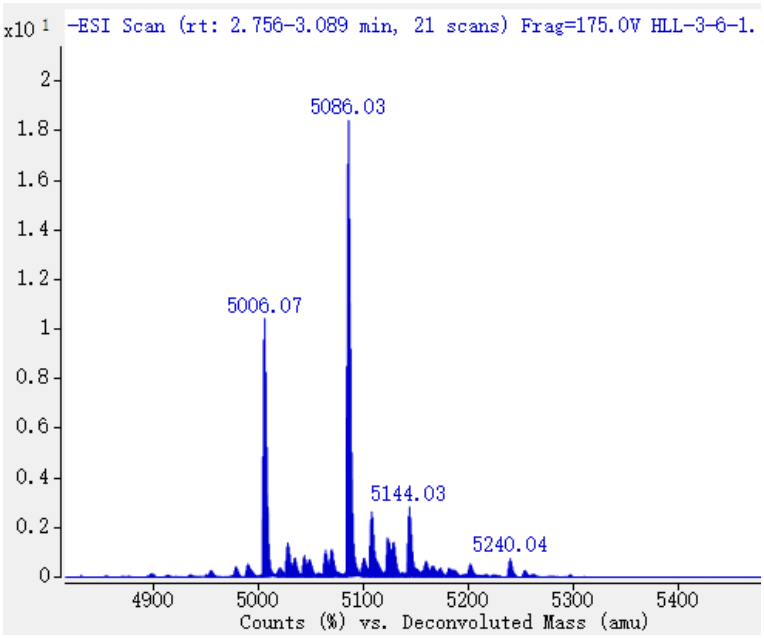


UPLC chromatogram and deconvoluted MS of **c50**

Conversion: 0%

Calculated Mass: 5301 Da; Observed Mass: 5086 Da

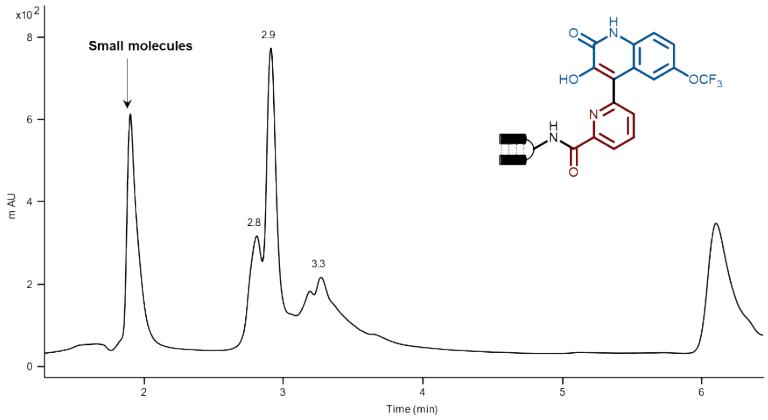


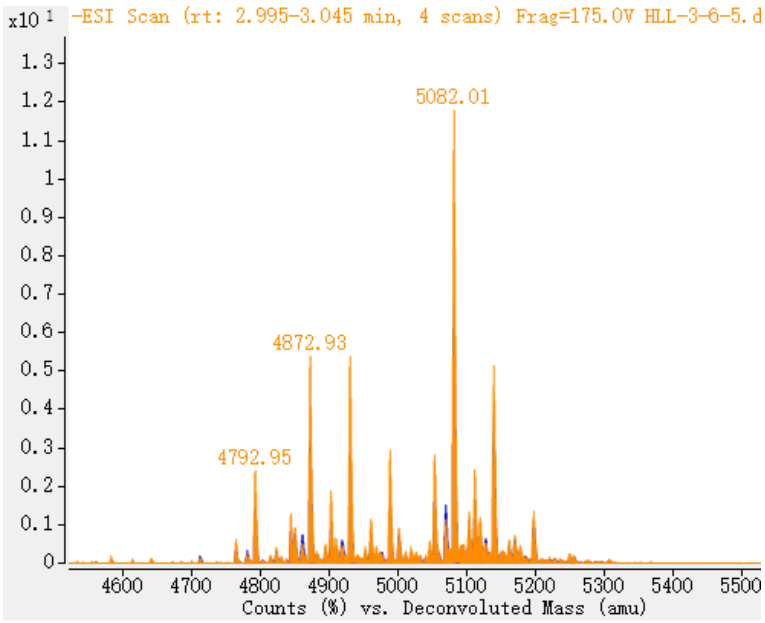


UPLC chromatogram and deconvoluted MS of **c51**

Conversion: 0%

Calculated Mass: 5285 Da; Observed Mass: 5082 Da



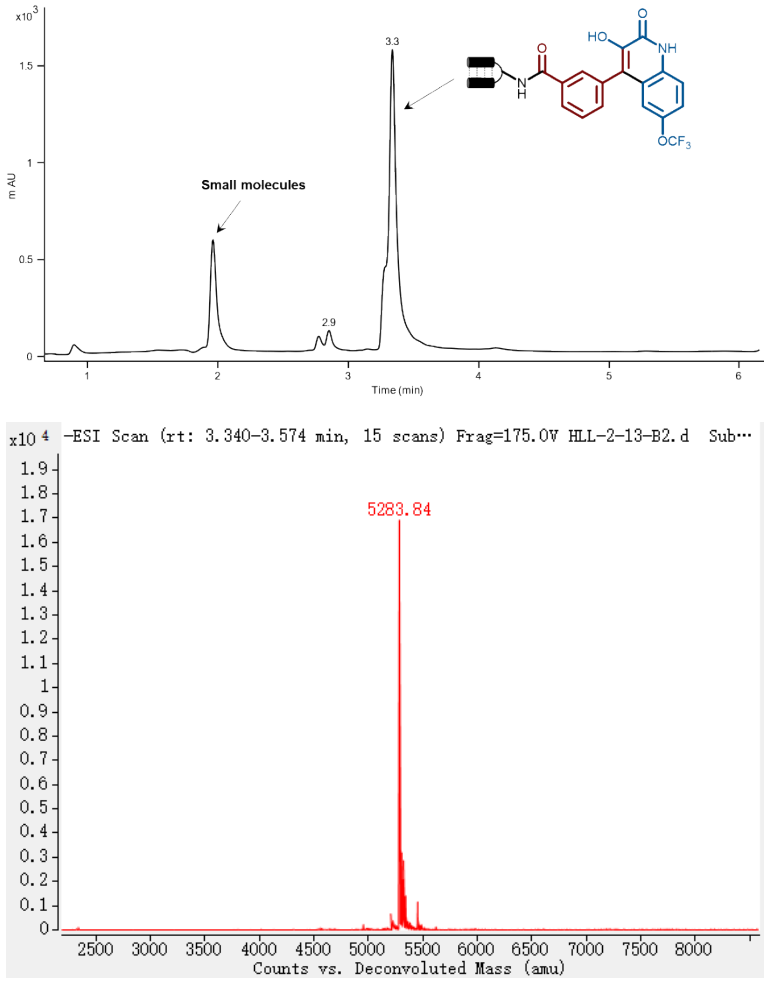

x10 1
-ESI Scan (rt: 2.995-3.045 min, 4 scans) Frag=175.0V HLL-3-6-5.d
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5082.01
4872.93
4792.95
4600
4700
4800
4900
5000
5100
5200
5300
5400
5500
Counts (%) vs. Deconvoluted Mass (amu)

7.3 Cross substrate scope of DNA-tagged aldehydes and isatins

UPLC chromatogram and deconvoluted MS of combination of **a2** and **b1**

Conversion: 82%

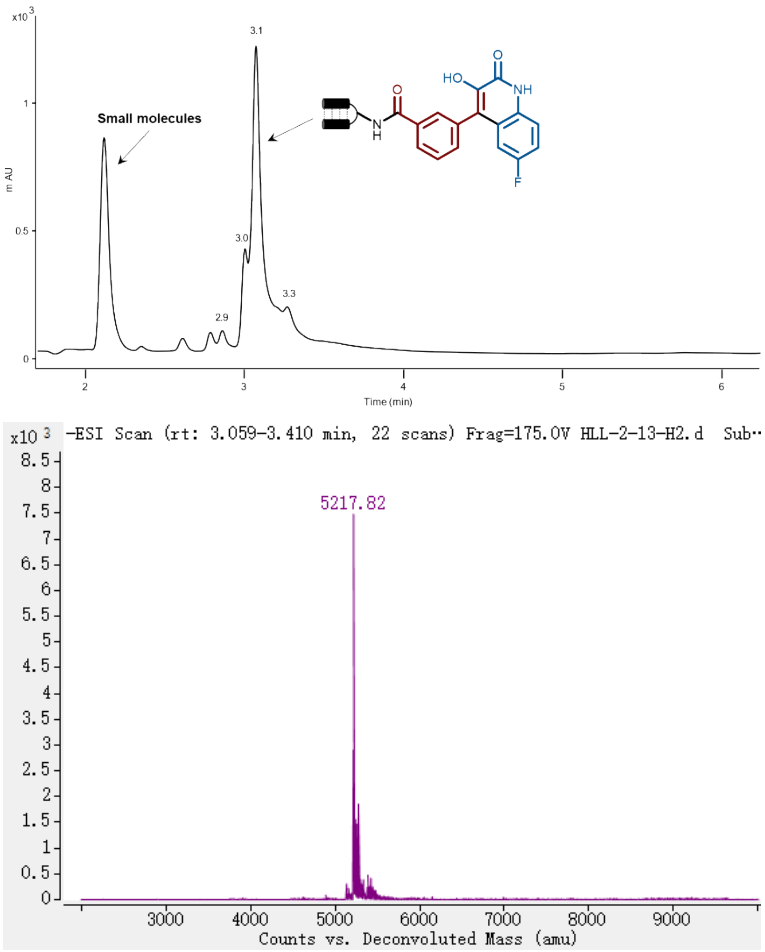
Calculated Mass: 5284 Da; Observed Mass: 5284 Da



UPLC chromatogram and deconvoluted MS of combination of **a2** and **b3**

Conversion: 60%

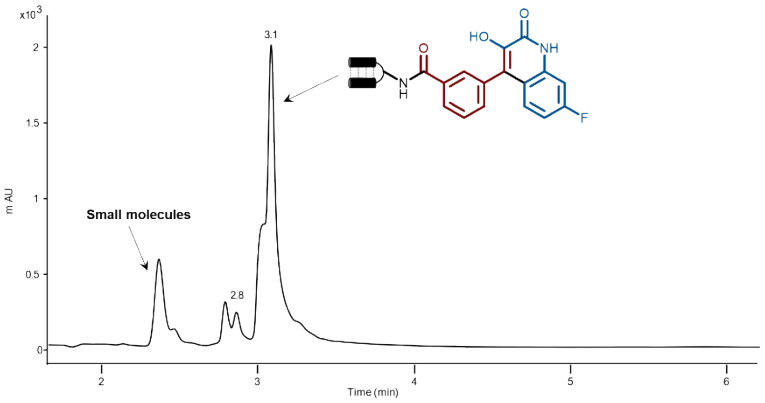
Calculated Mass: 5218 Da; Observed Mass: 5218 Da

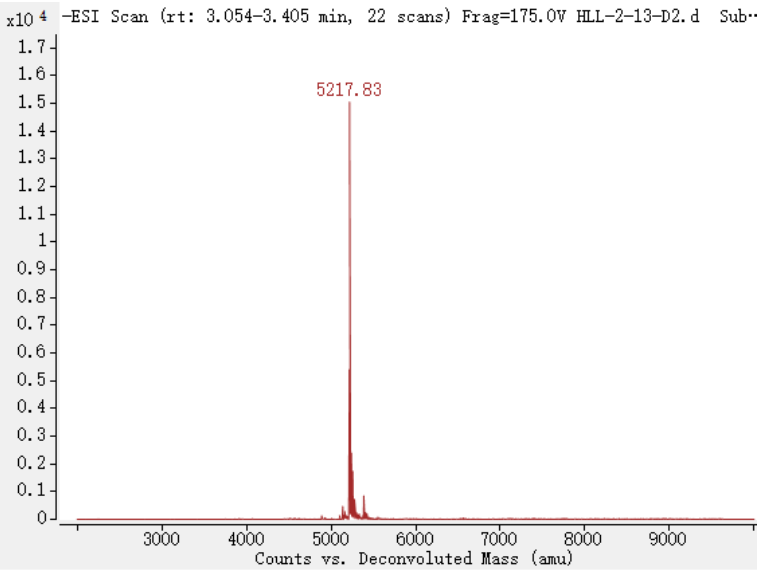


UPLC chromatogram and deconvoluted MS of combination of **a2** and **b4**

Conversion: 67%

Calculated Mass: 5218 Da; Observed Mass: 5218 Da

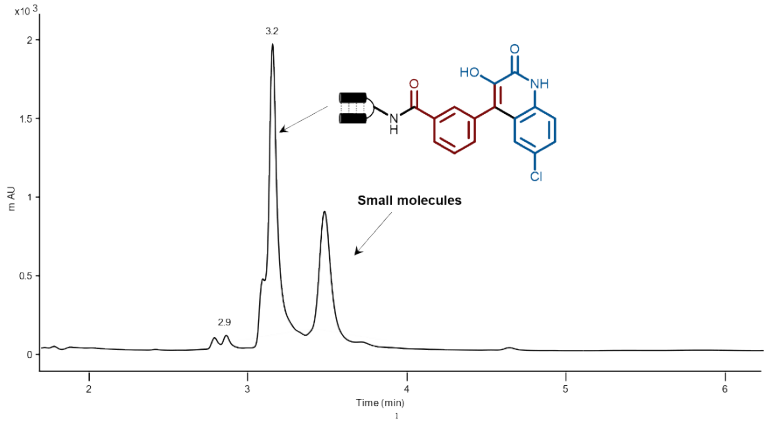


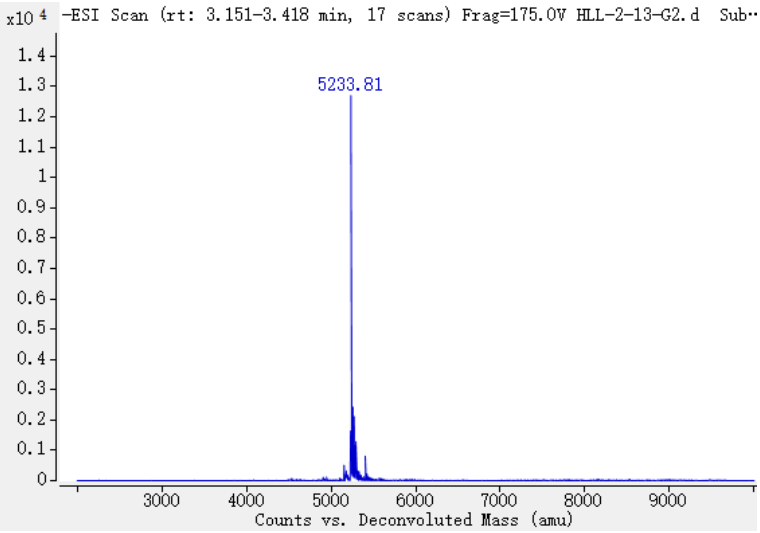


UPLC chromatogram and deconvoluted MS of combination of **a2** and **b6**

Conversion: 84%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

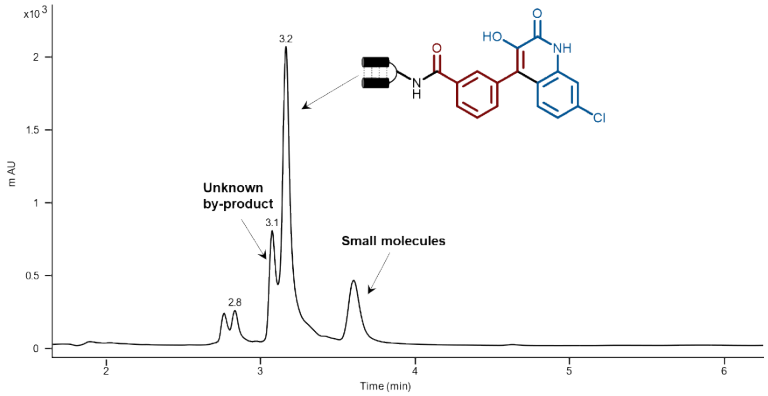


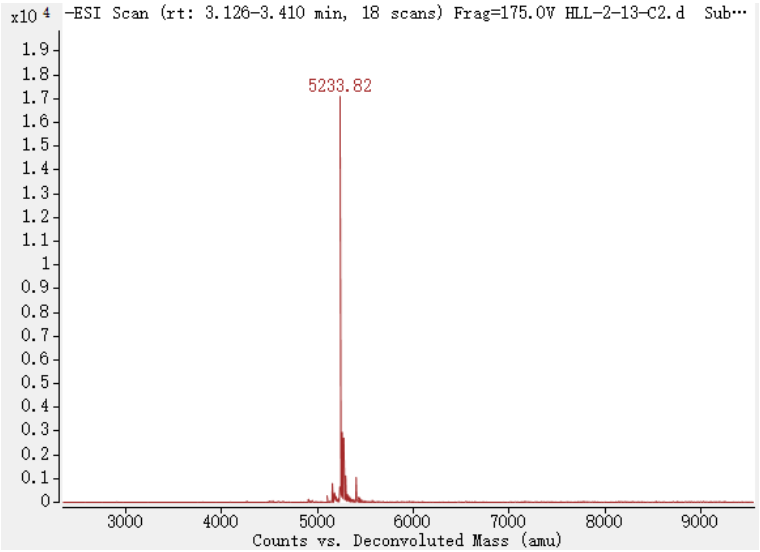


UPLC chromatogram and deconvoluted MS of combination of **a2** and **b7**

Conversion: 68%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

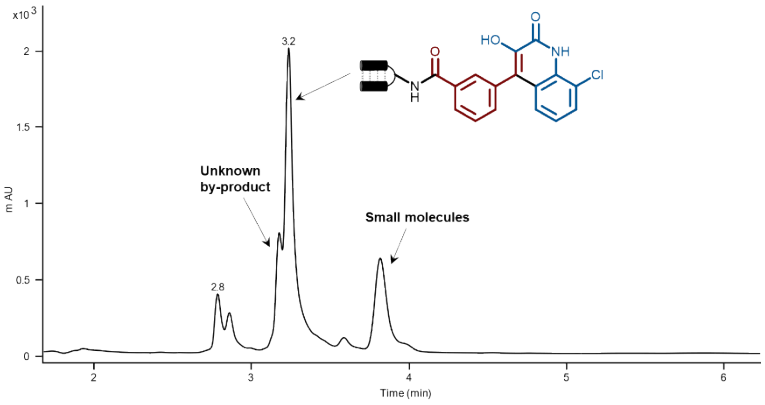


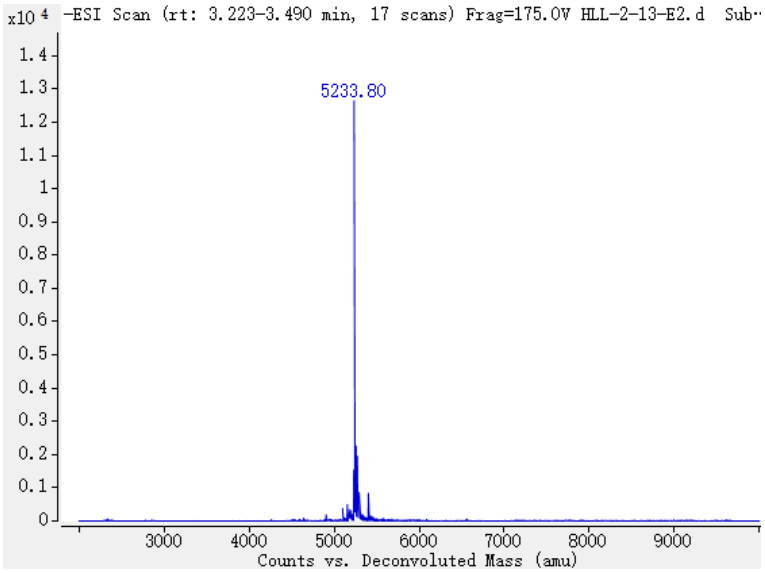


UPLC chromatogram and deconvoluted MS of combination of **a2** and **b8**

Conversion: 65%

Calculated Mass: 5234 Da; Observed Mass: 5234 Da

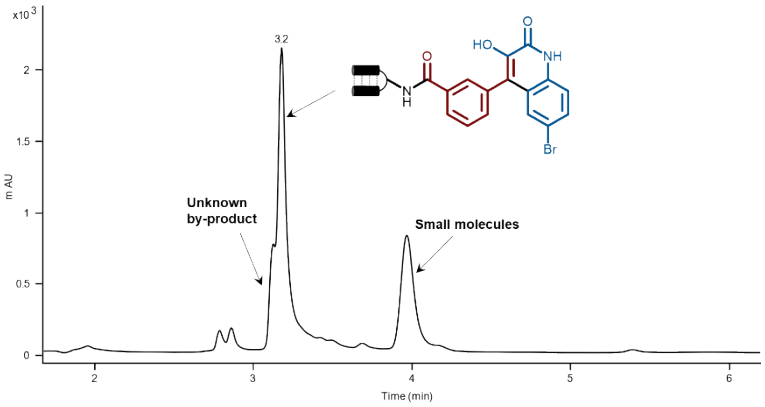


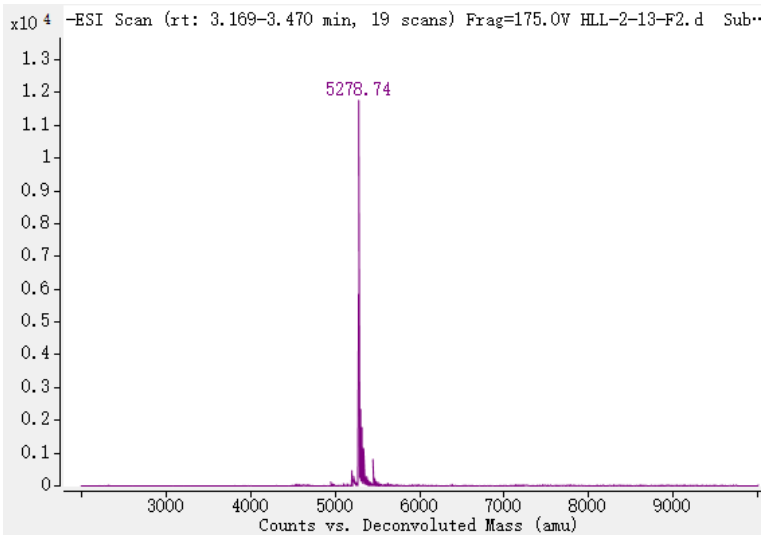


UPLC chromatogram and deconvoluted MS of combination of **a2** and **b10**

Conversion: 75%

Calculated Mass: 5279 Da; Observed Mass: 5279 Da

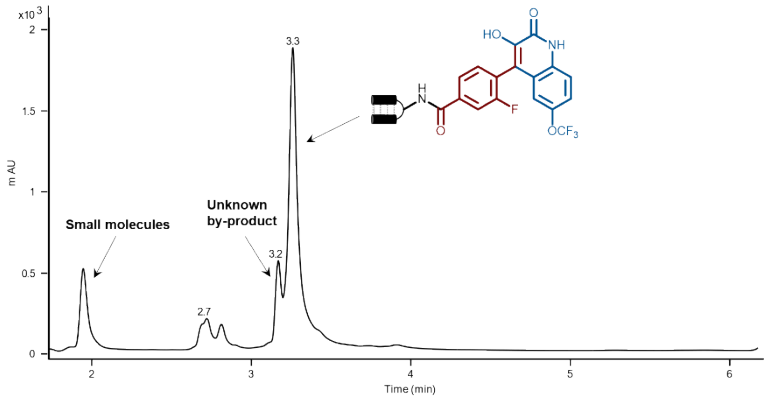


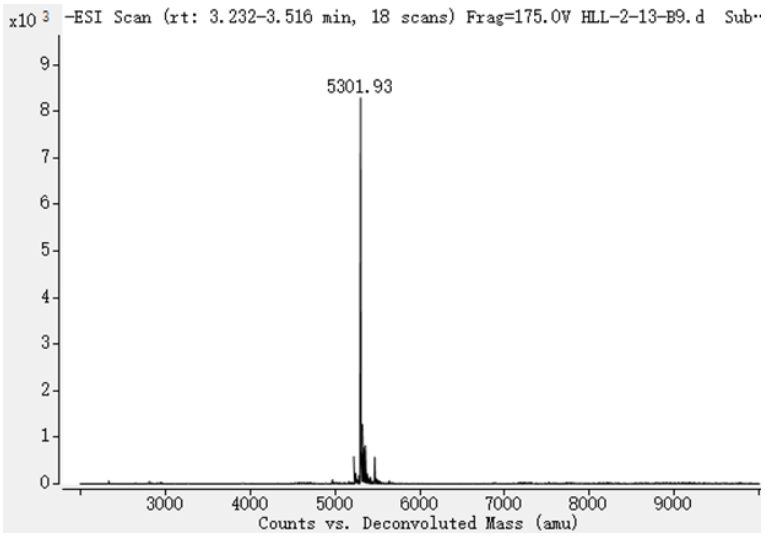


UPLC chromatogram and deconvoluted MS of combination of **a4** and **b1**

Conversion: 71%

Calculated Mass: 5302 Da; Observed Mass: 5302 Da

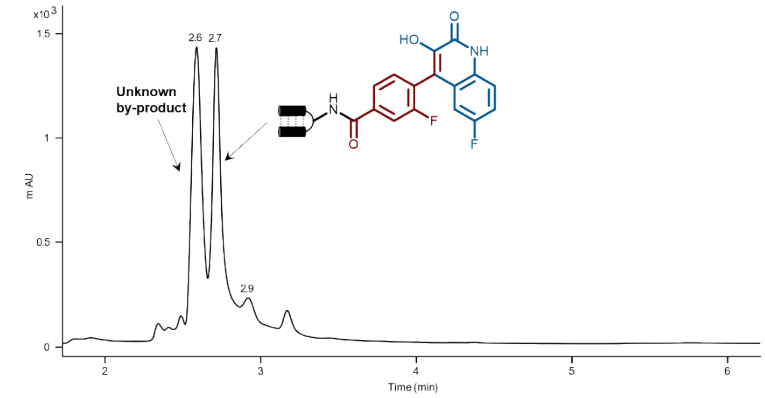


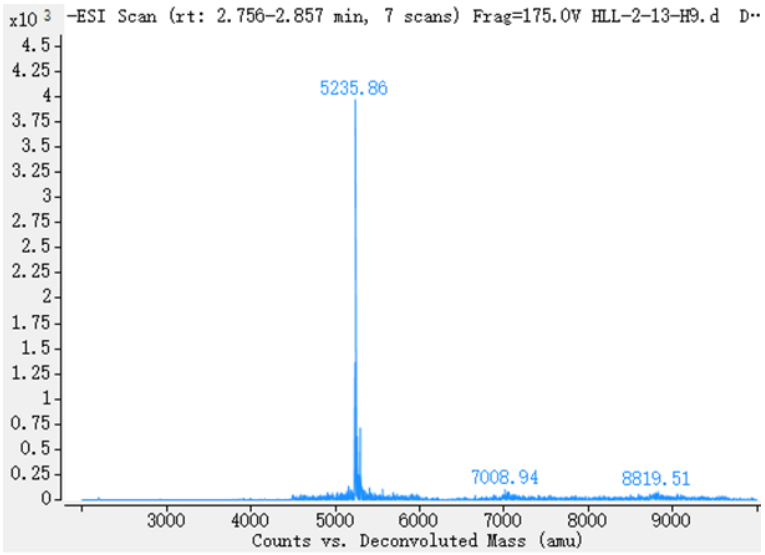


UPLC chromatogram and deconvoluted MS of combination of **a4** and **b3**

Conversion: 40%

Calculated Mass: 5236 Da; Observed Mass: 5236 Da

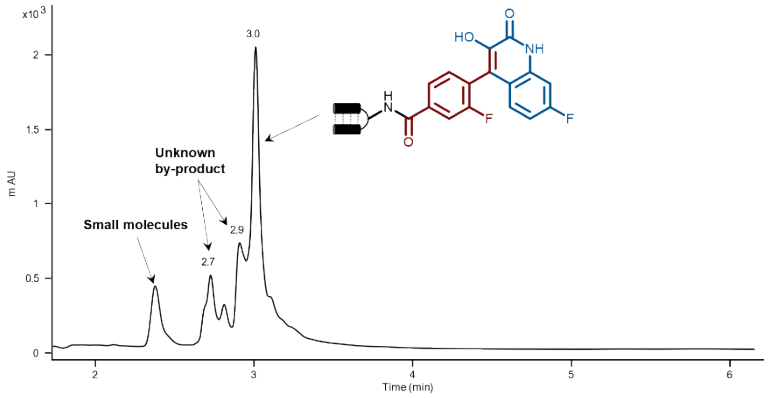


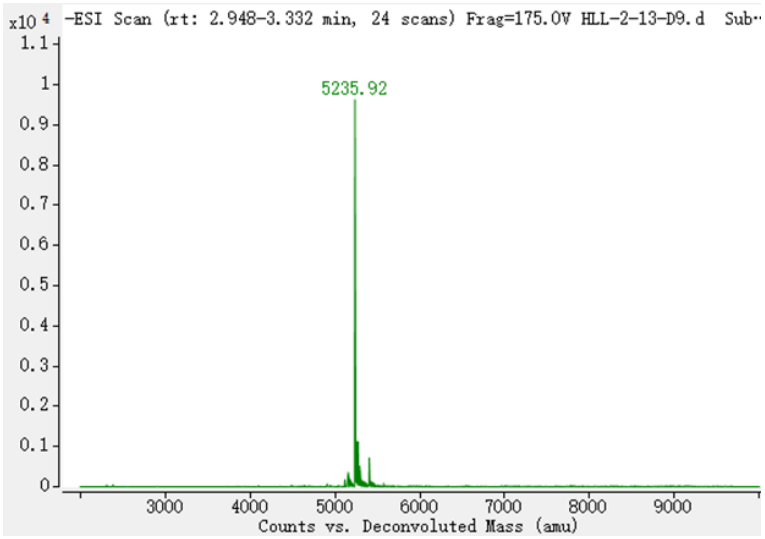


UPLC chromatogram and deconvoluted MS of combination of **a4** and **b4**

Conversion: 63%

Calculated Mass: 5236 Da; Observed Mass: 5236 Da

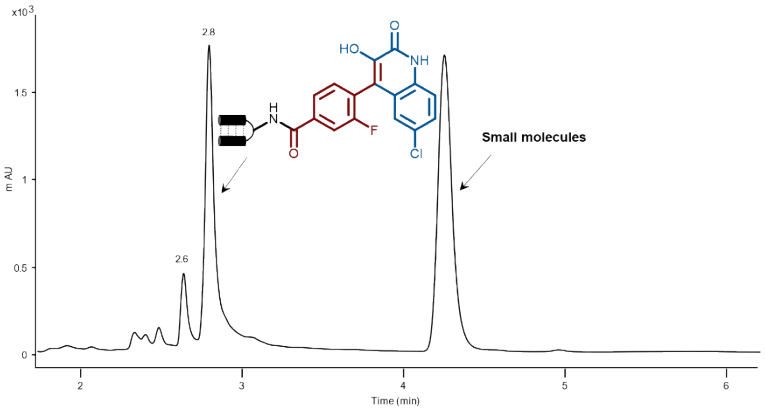


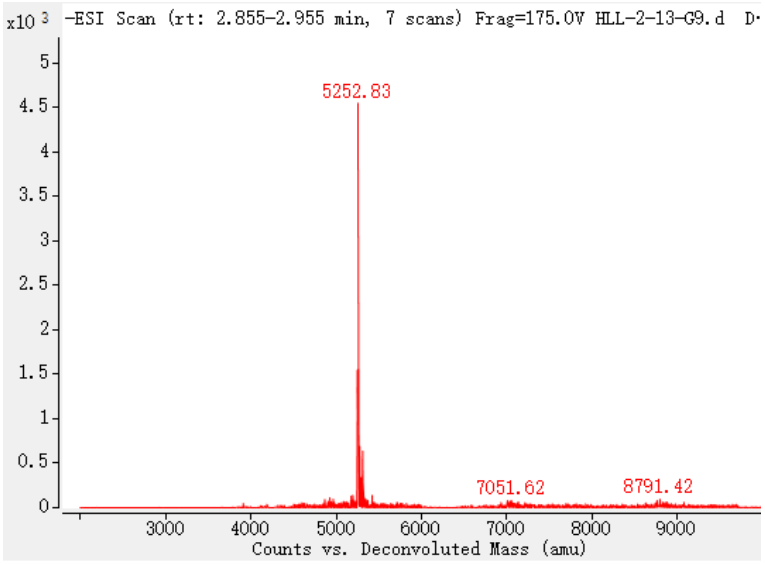


UPLC chromatogram and deconvoluted MS of combination of **a4** and **b6**

Conversion: 89%

Calculated Mass: 5253 Da; Observed Mass: 5253 Da

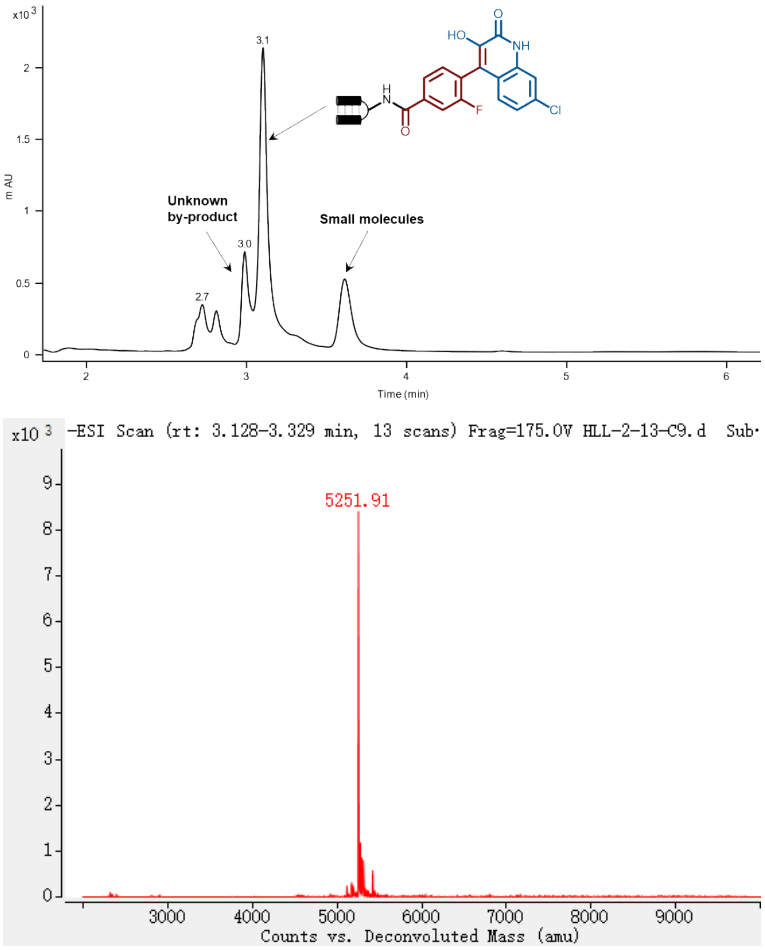




UPLC chromatogram and deconvoluted MS of combination of **a4** and **b7**

Conversion: 63%

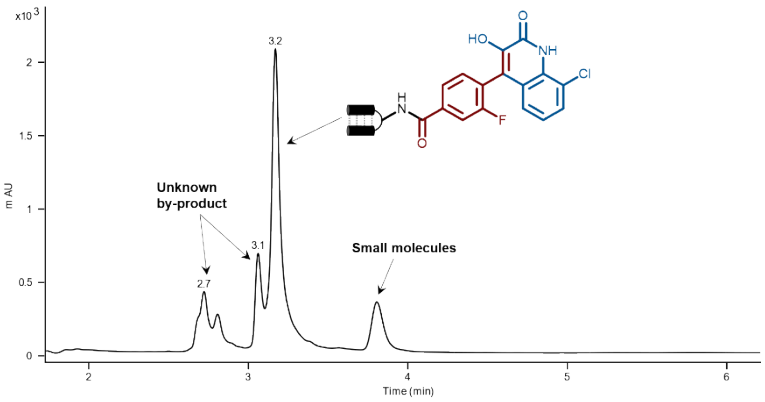
Calculated Mass: 5253 Da; Observed Mass: 5252 Da

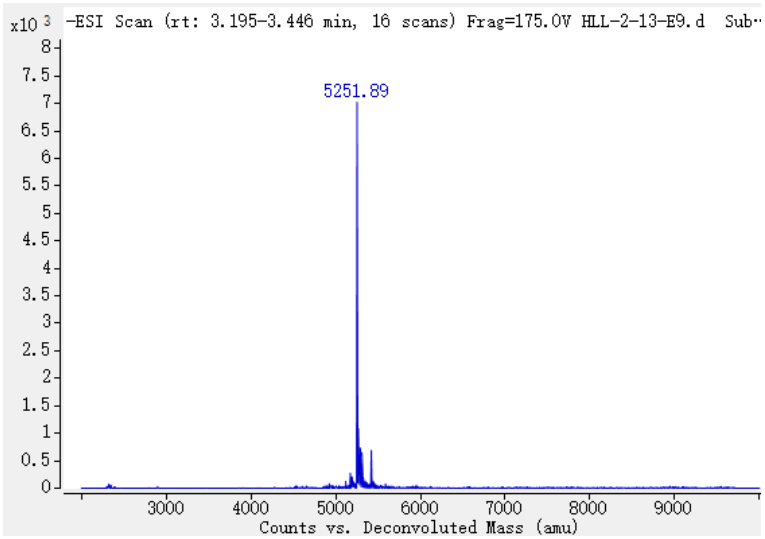


UPLC chromatogram and deconvoluted MS of combination of **a4** and **b8**

Conversion: 63%

Calculated Mass: 5253 Da; Observed Mass: 5252 Da

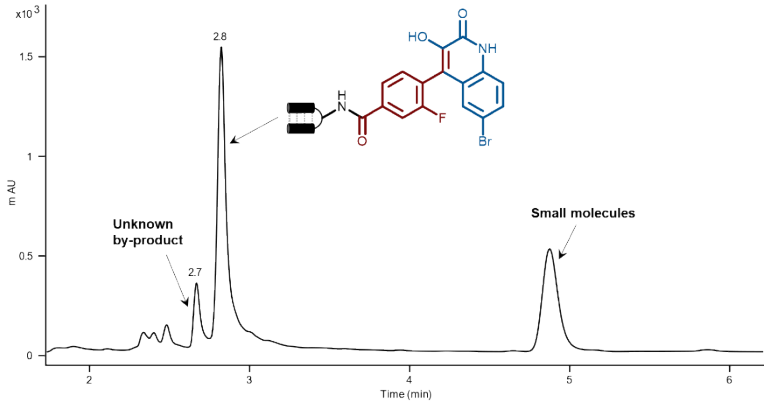


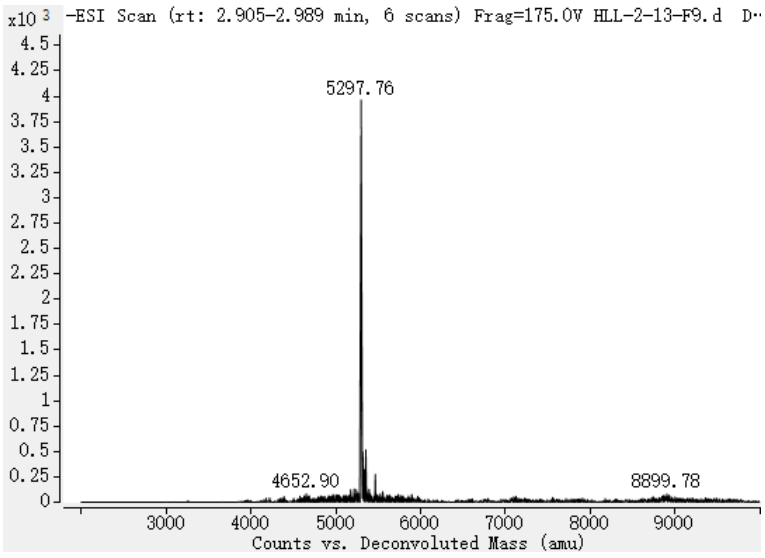


UPLC chromatogram and deconvoluted MS of combination of **a4** and **b10**

Conversion: 76%

Calculated Mass: 5297 Da; Observed Mass: 5298 Da

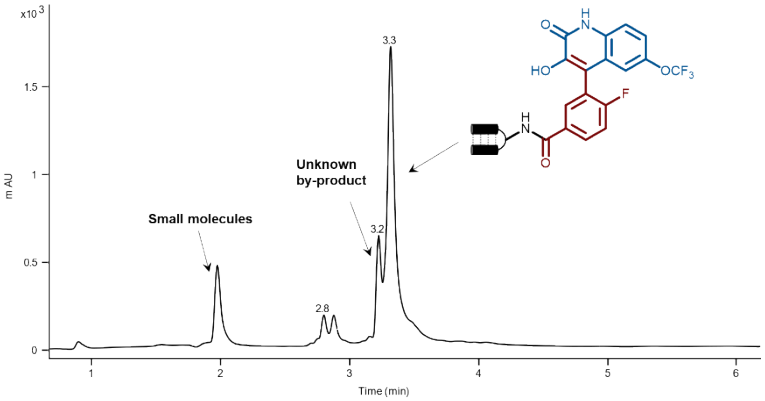


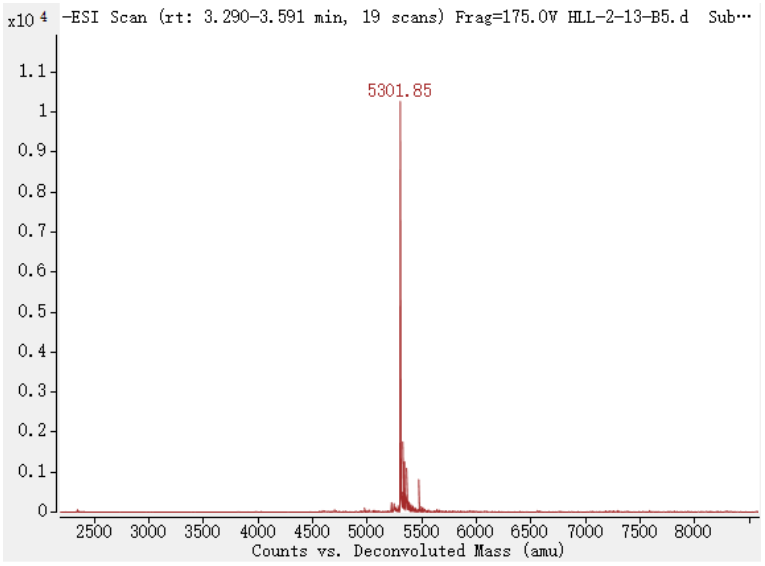


UPLC chromatogram and deconvoluted MS of combination of **a5** and **b1**

Conversion: 72%

Calculated Mass: 5302 Da; Observed Mass: 5302 Da

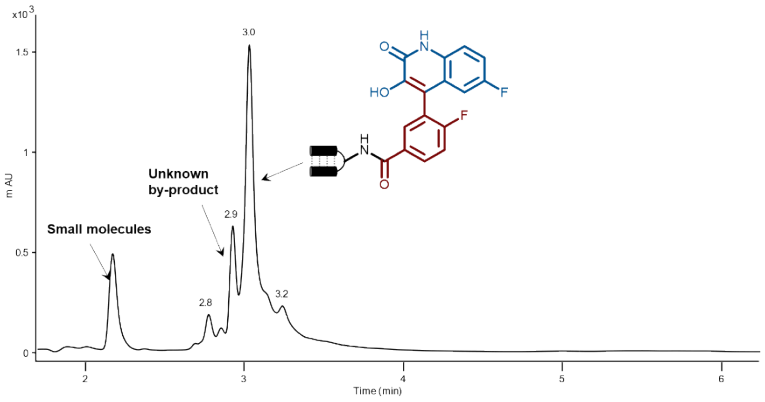


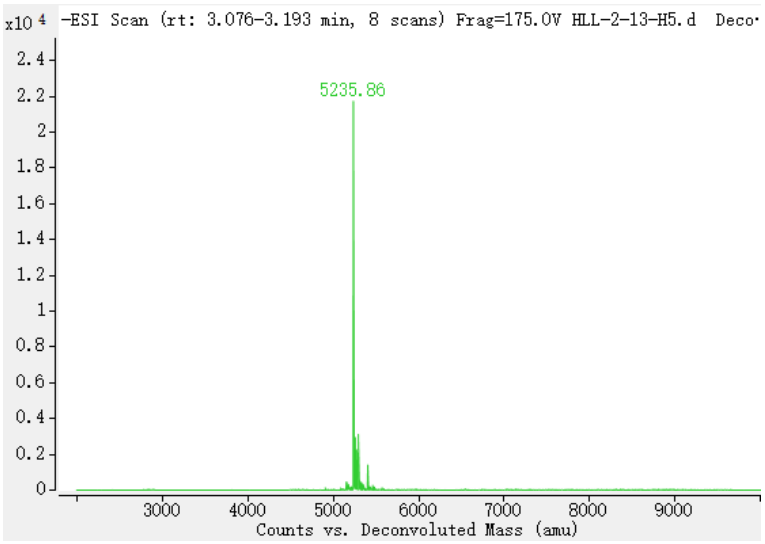


UPLC chromatogram and deconvoluted MS of combination of **a5** and **b3**

Conversion: 62%

Calculated Mass: 5236 Da; Observed Mass: 5236 Da

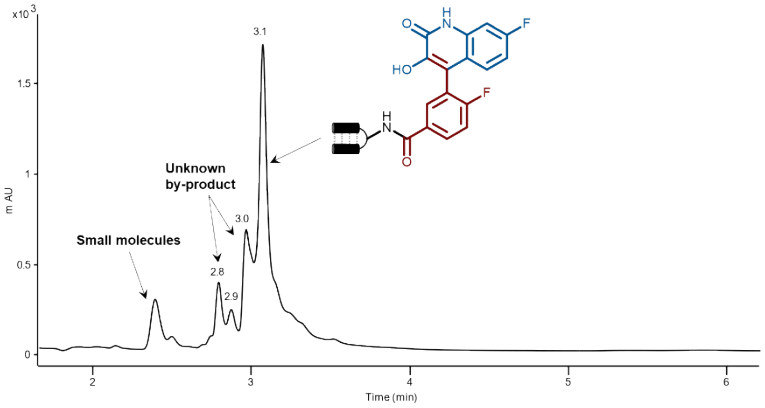


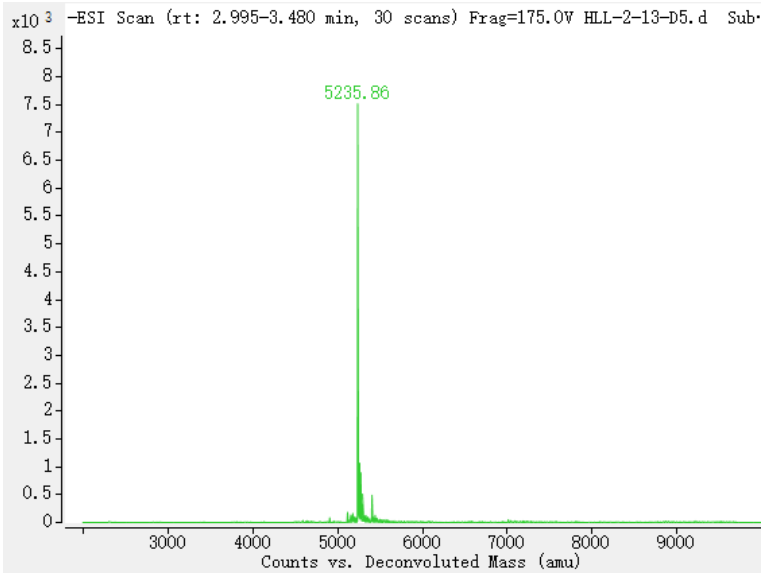


UPLC chromatogram and deconvoluted MS of combination of **a5** and **b4**

Conversion: 66%

Calculated Mass: 5236 Da; Observed Mass: 5236 Da

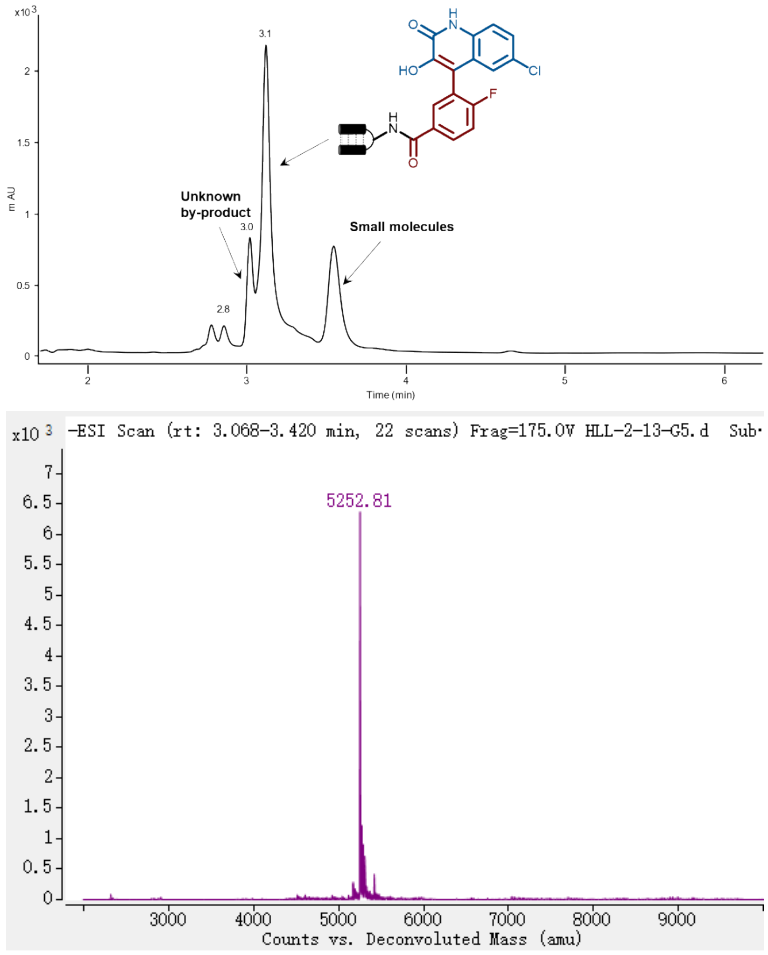




UPLC chromatogram and deconvoluted MS of combination of **a5** and **b6**

Conversion: 72%

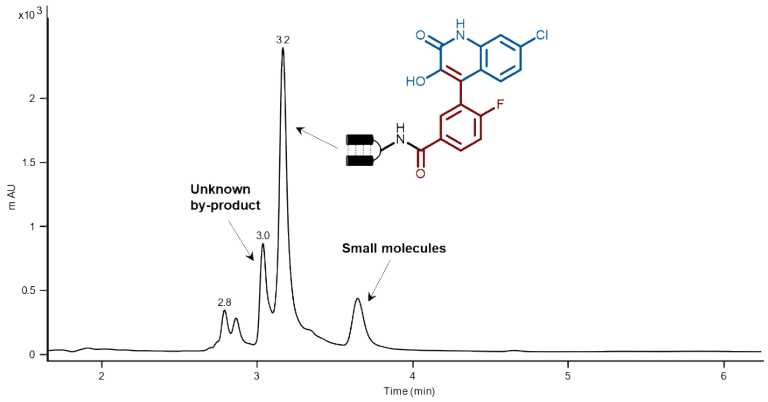
Calculated Mass: 5253 Da; Observed Mass: 5253 Da

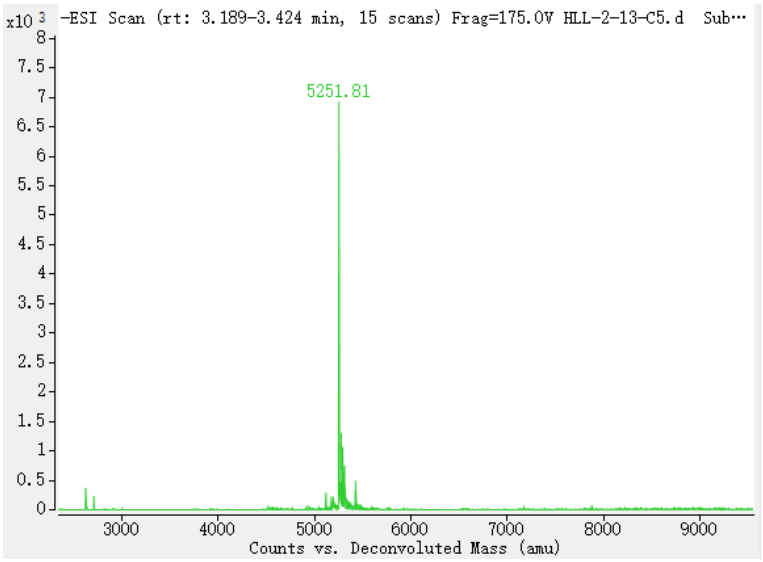


UPLC chromatogram and deconvoluted MS of combination of **a5** and **b7**

Conversion: 66%

Calculated Mass: 5253 Da; Observed Mass: 5252 Da

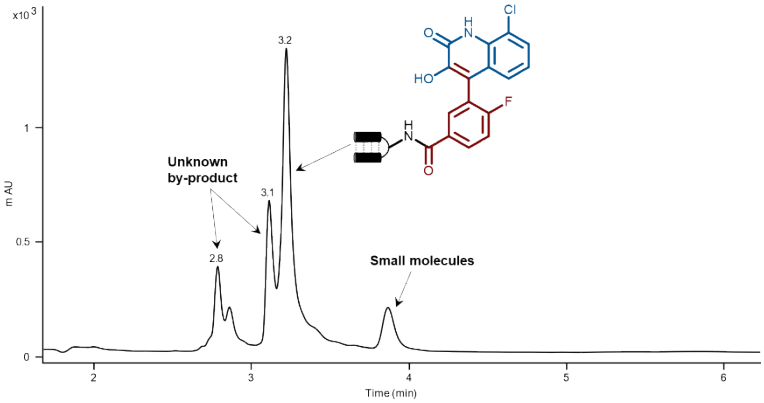


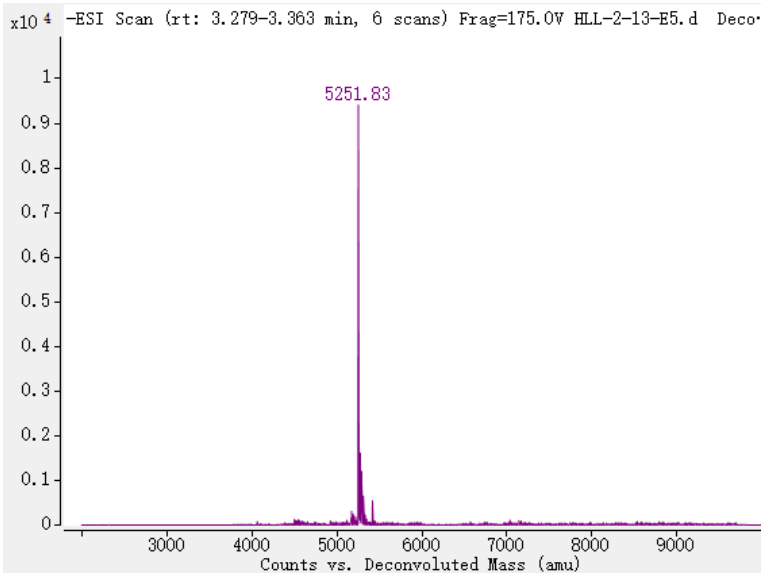


UPLC chromatogram and deconvoluted MS of combination of **a5** and **b8**

Conversion: 60%

Calculated Mass: 5253 Da; Observed Mass: 5252 Da

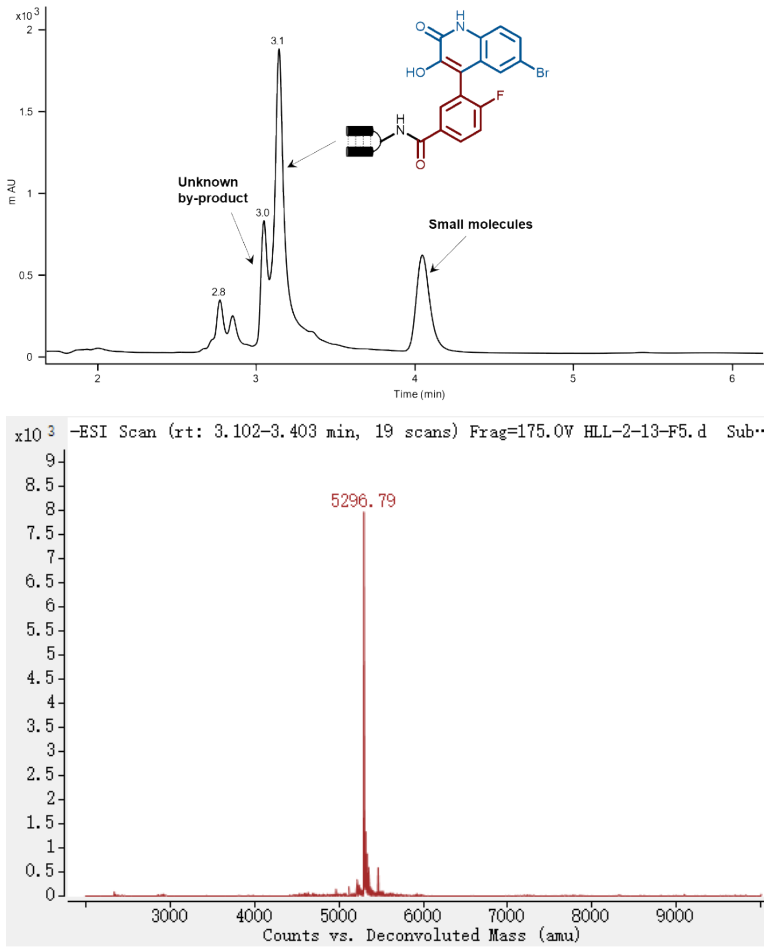




UPLC chromatogram and deconvoluted MS of combination of **a5** and **b10**

Conversion: 66%

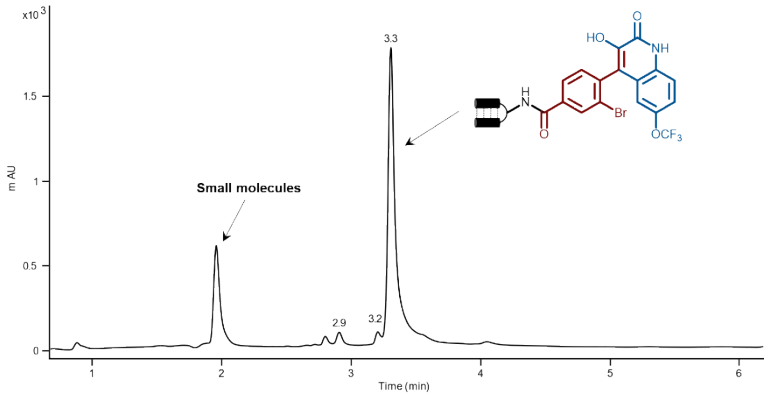
Calculated Mass: 5297 Da; Observed Mass: 5297 Da

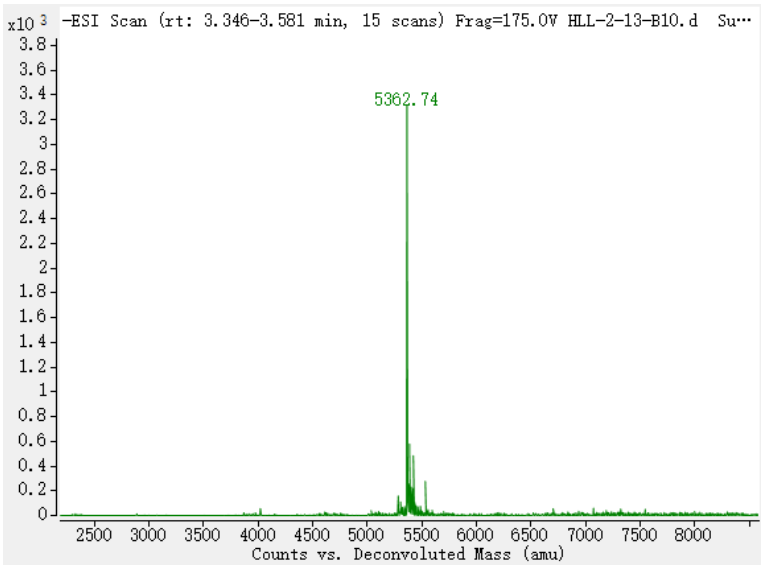


UPLC chromatogram and deconvoluted MS of combination of **a6** and **b1**

Conversion: 88%

Calculated Mass: 5363 Da; Observed Mass: 5363 Da

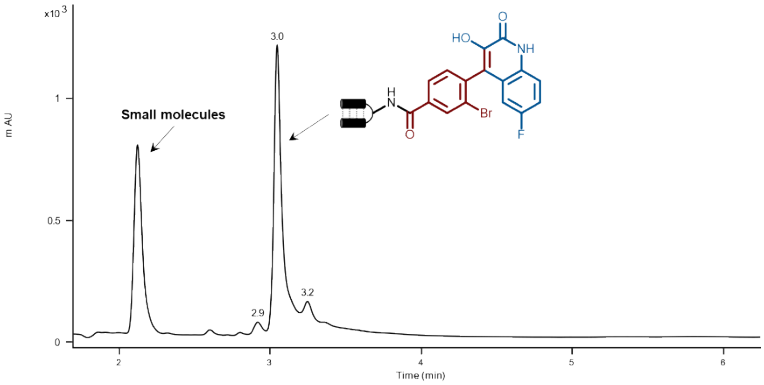


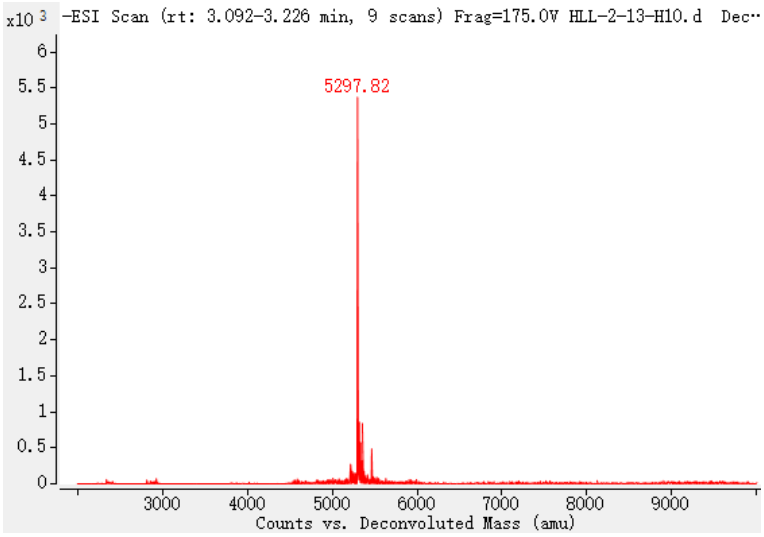


UPLC chromatogram and deconvoluted MS of combination of **a6** and **b3**

Conversion: 71%

Calculated Mass: 5297 Da; Observed Mass: 5298 Da

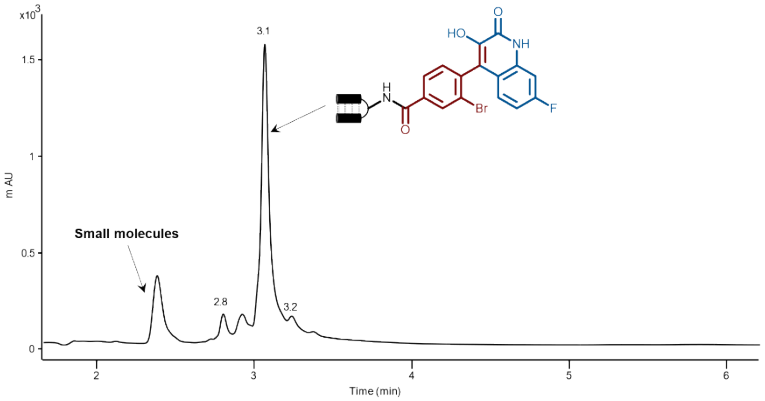


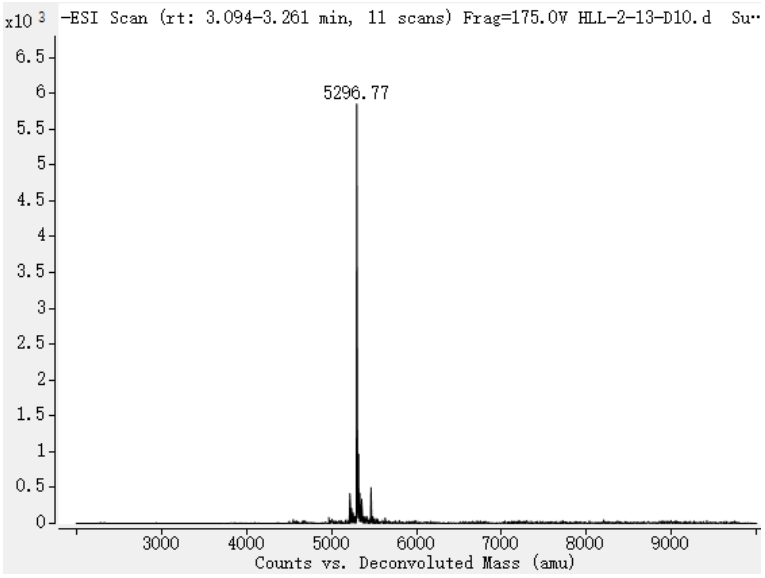


UPLC chromatogram and deconvoluted MS of combination of **a6** and **b4**

Conversion: 73%

Calculated Mass: 5297 Da; Observed Mass: 5297 Da

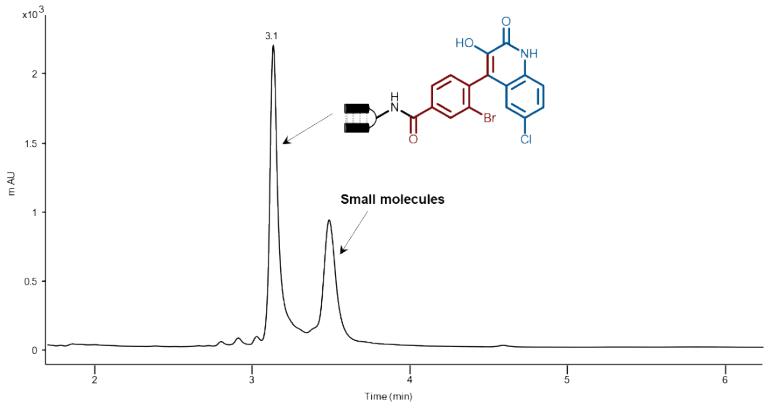


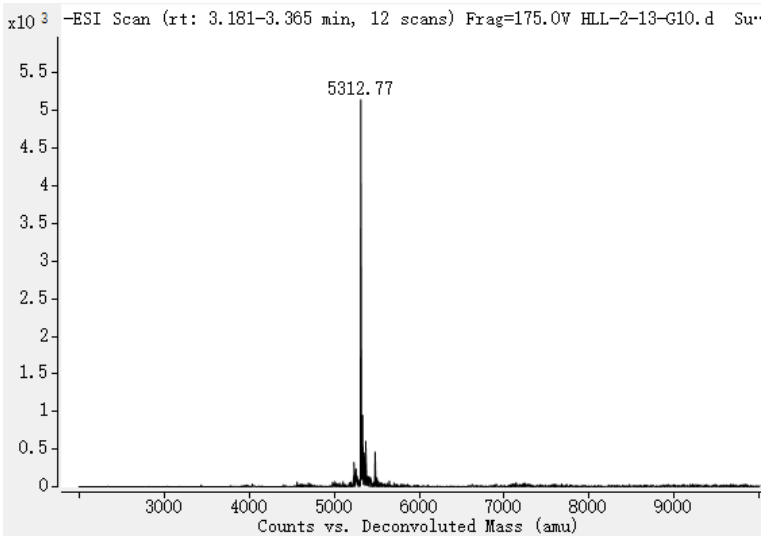


UPLC chromatogram and deconvoluted MS of combination of **a6** and **b6**

Conversion: >90%

Calculated Mass: 5314 Da; Observed Mass: 5313 Da

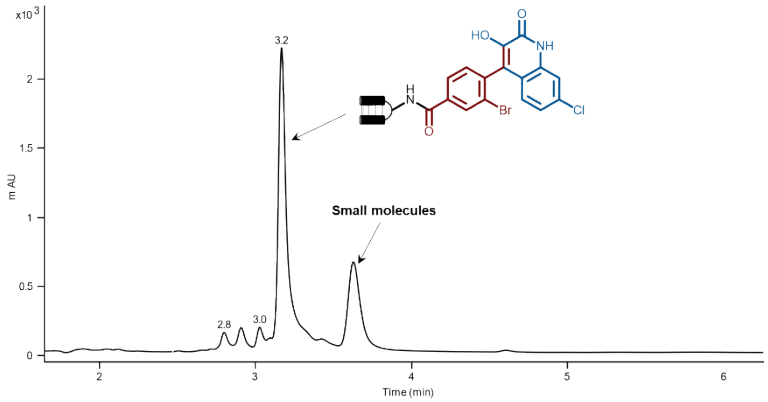


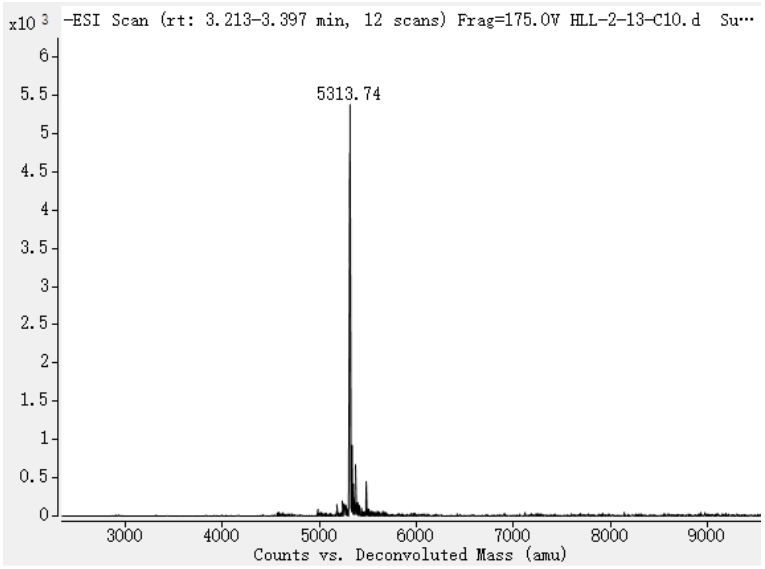


UPLC chromatogram and deconvoluted MS of combination of **a6** and **b7**

Conversion: 80%

Calculated Mass: 5314 Da; Observed Mass: 5314 Da

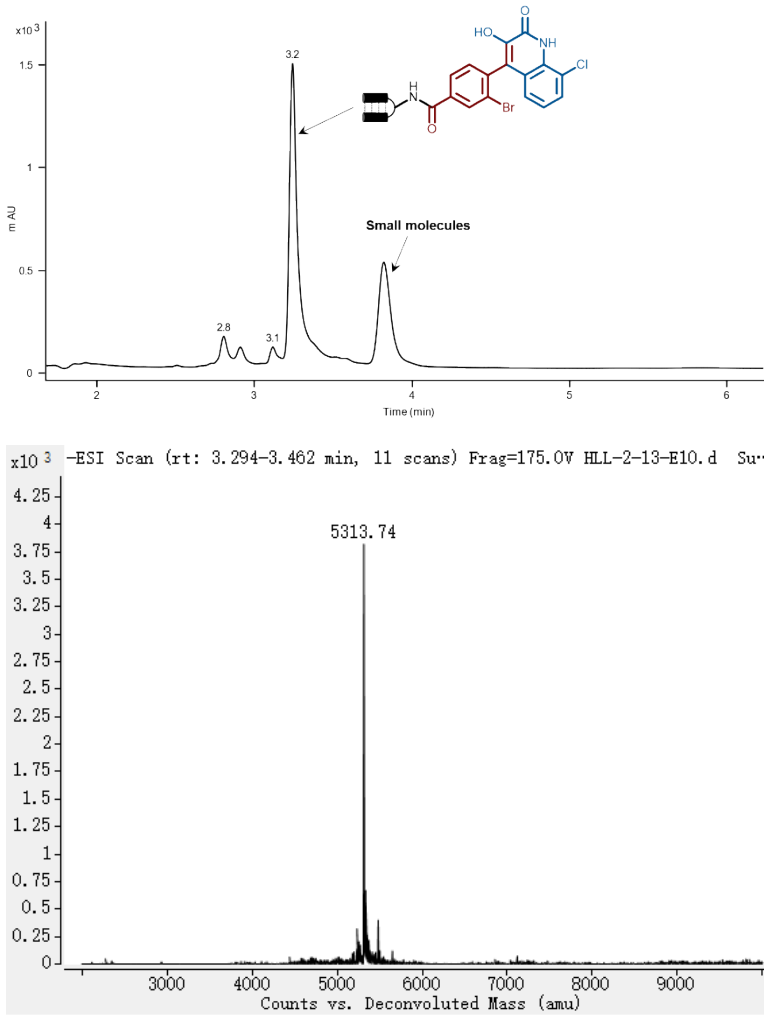




UPLC chromatogram and deconvoluted MS of combination of **a6** and **b8**

Conversion: 80%

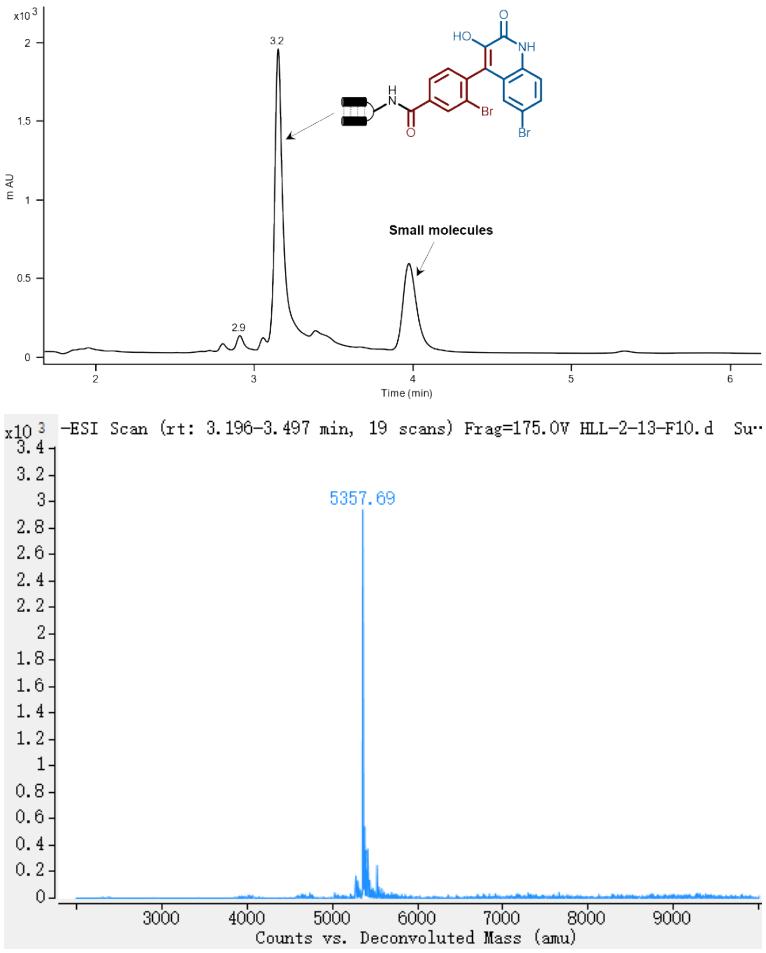
Calculated Mass: 5314 Da; Observed Mass: 5314 Da



UPLC chromatogram and deconvoluted MS of combination of **a6** and **b10**

Conversion: 79%

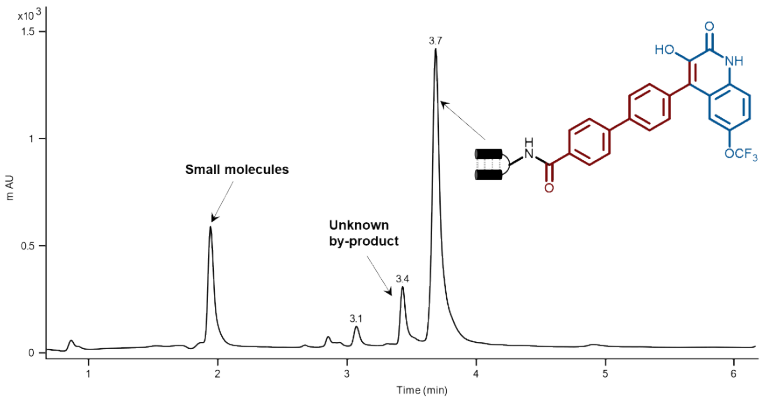
Calculated Mass: 5358 Da; Observed Mass: 5358 Da

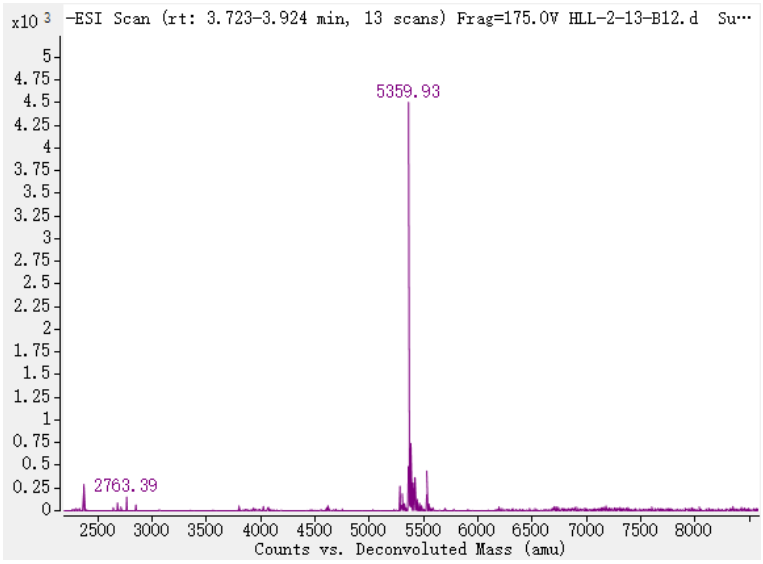


UPLC chromatogram and deconvoluted MS of combination of **a7** and **b1**

Conversion: 82%

Calculated Mass: 5360 Da; Observed Mass: 5360 Da

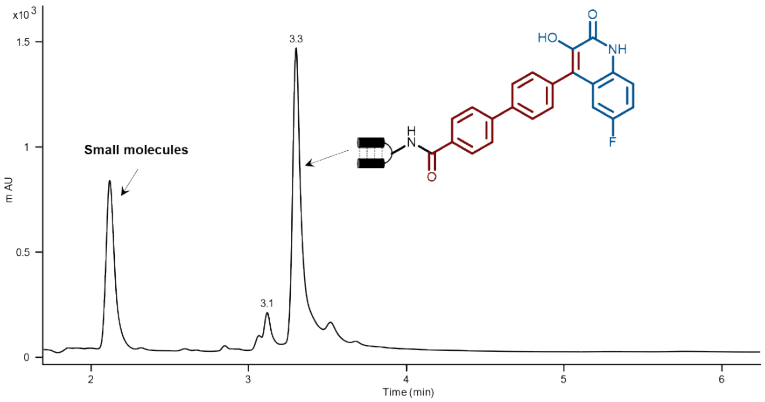


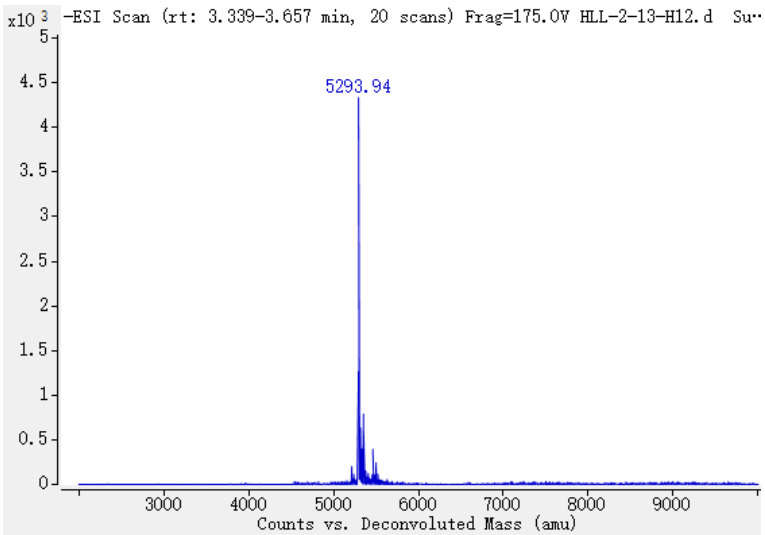


UPLC chromatogram and deconvoluted MS of combination of **a7** and **b3**

Conversion: 79%

Calculated Mass: 5294 Da; Observed Mass: 5294 Da

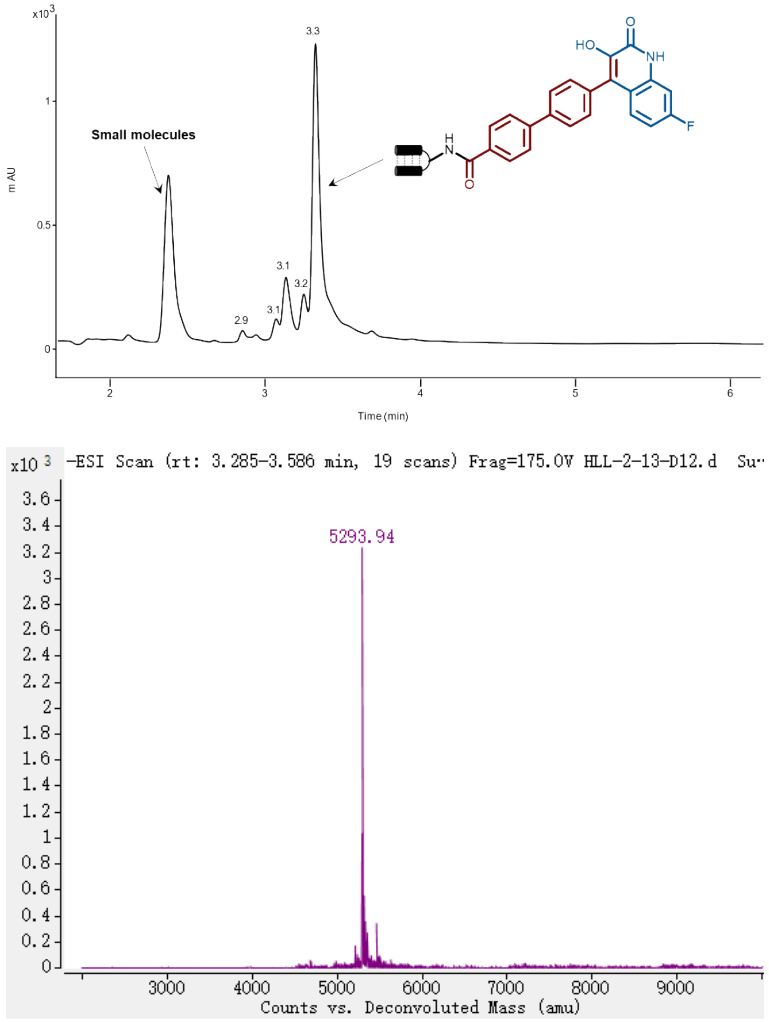




UPLC chromatogram and deconvoluted MS of combination of **a7** and **b4**

Conversion: 73%

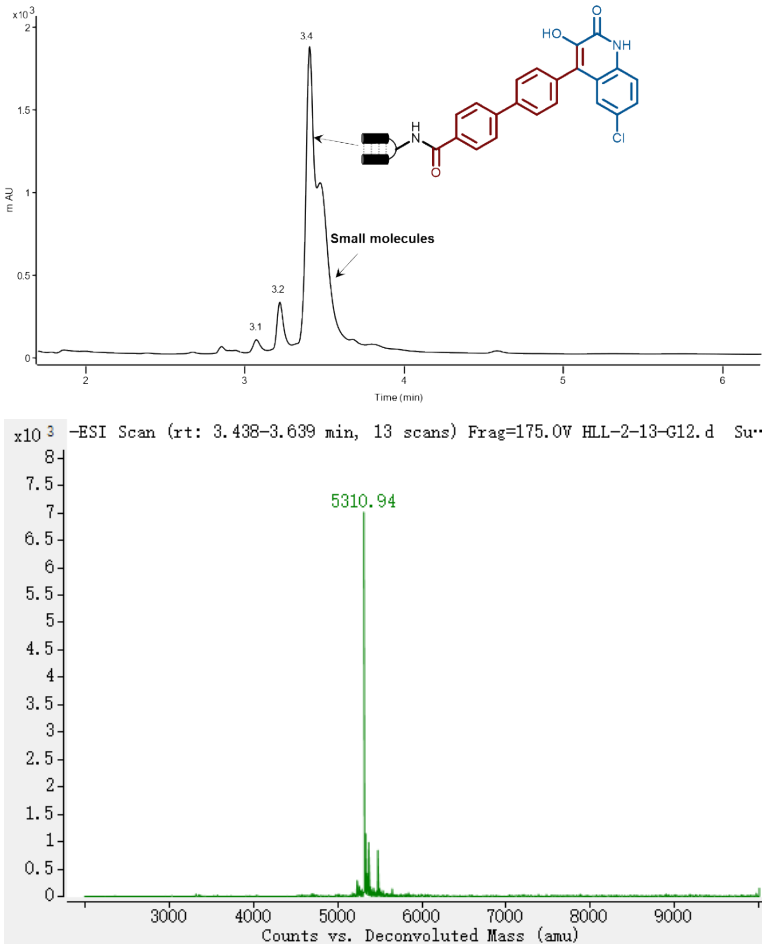
Calculated Mass: 5294 Da; Observed Mass: 5294 Da



UPLC chromatogram and deconvoluted MS of combination of **a7** and **b6**

Conversion: 38%

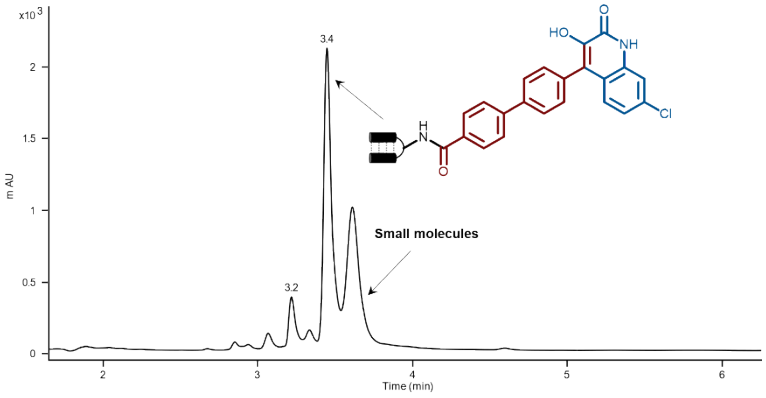
Calculated Mass: 5311 Da; Observed Mass: 5311 Da

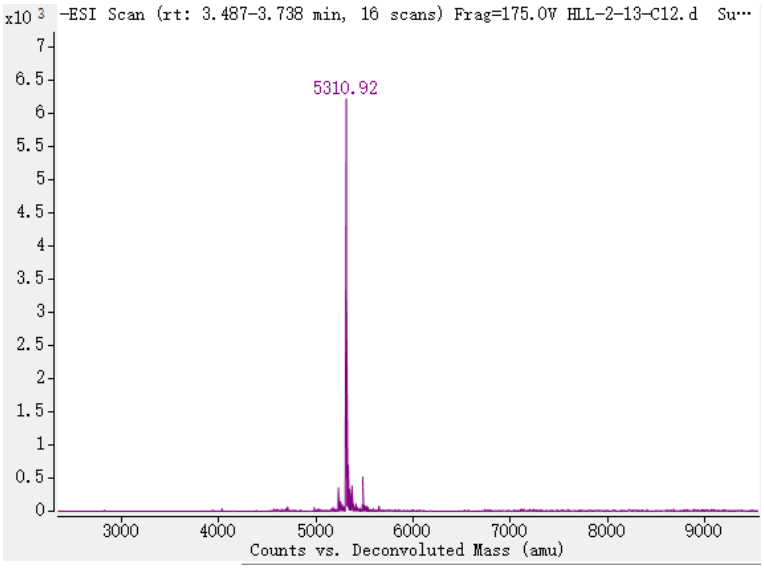


UPLC chromatogram and deconvoluted MS of combination of **a7** and **b7**

Conversion: 78%

Calculated Mass: 5311 Da; Observed Mass: 5311 Da

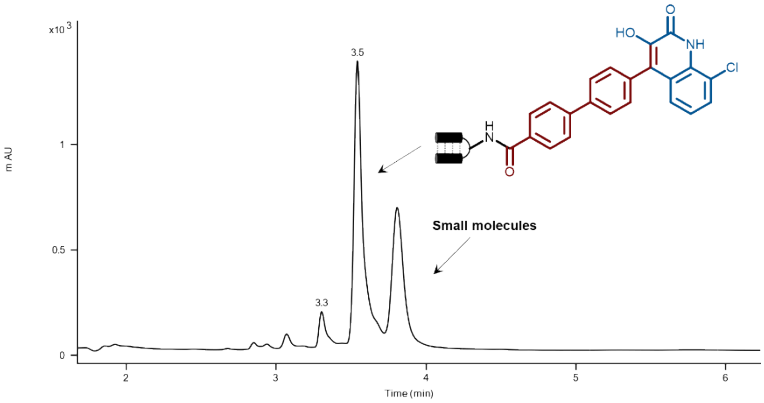


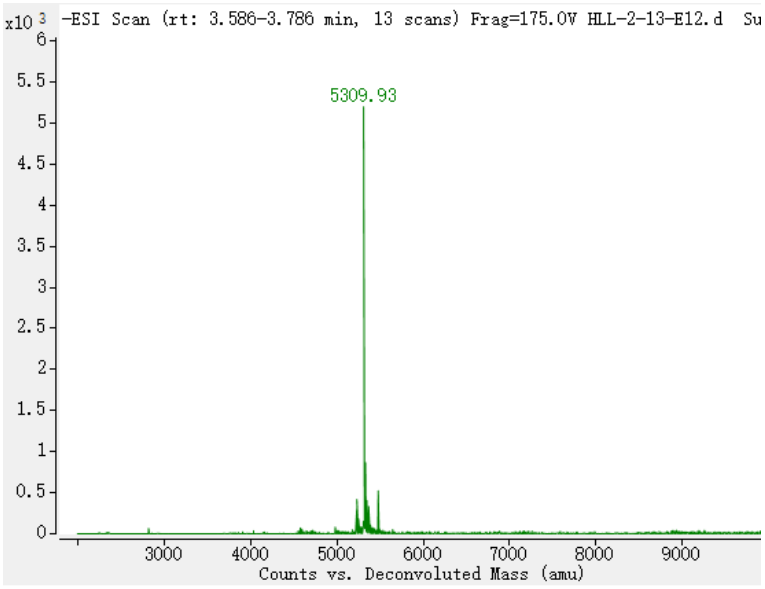


UPLC chromatogram and deconvoluted MS of combination of **a7** and **b8**

Conversion: 82%

Calculated Mass: 5311 Da; Observed Mass: 5310 Da

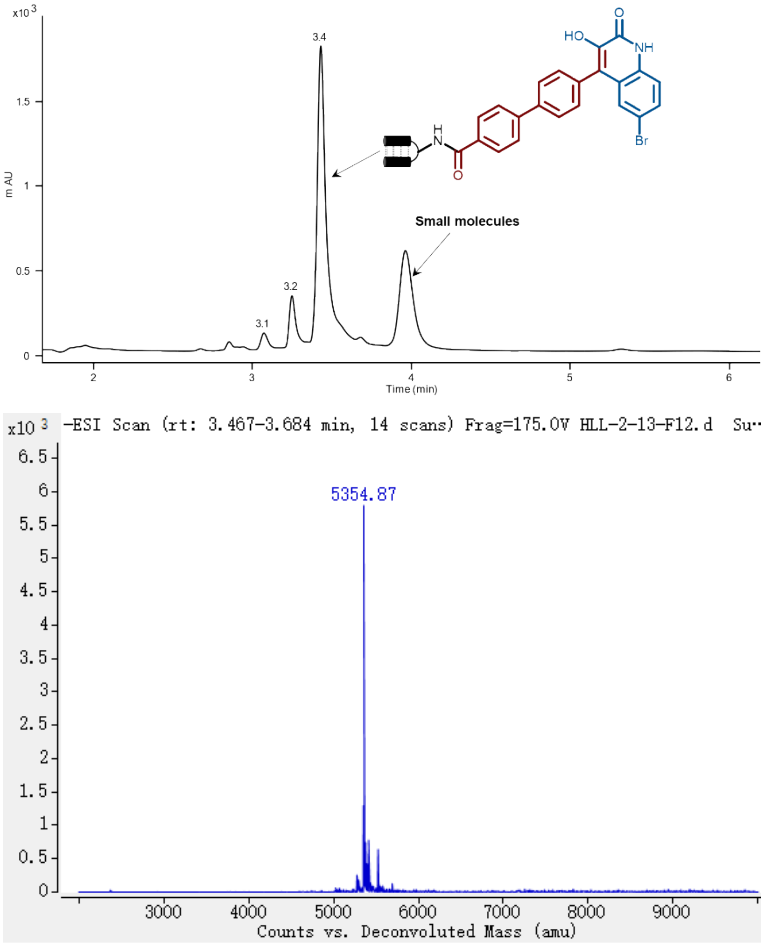




UPLC chromatogram and deconvoluted MS of combination of **a7** and **b10**

Conversion: 83%

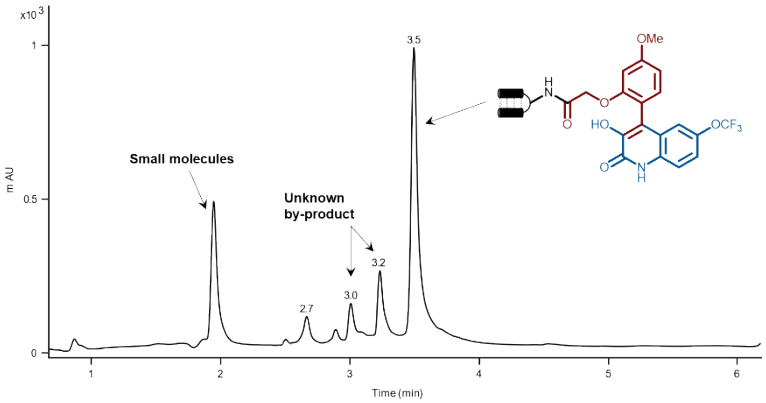
Calculated Mass: 5355 Da; Observed Mass: 5355 Da

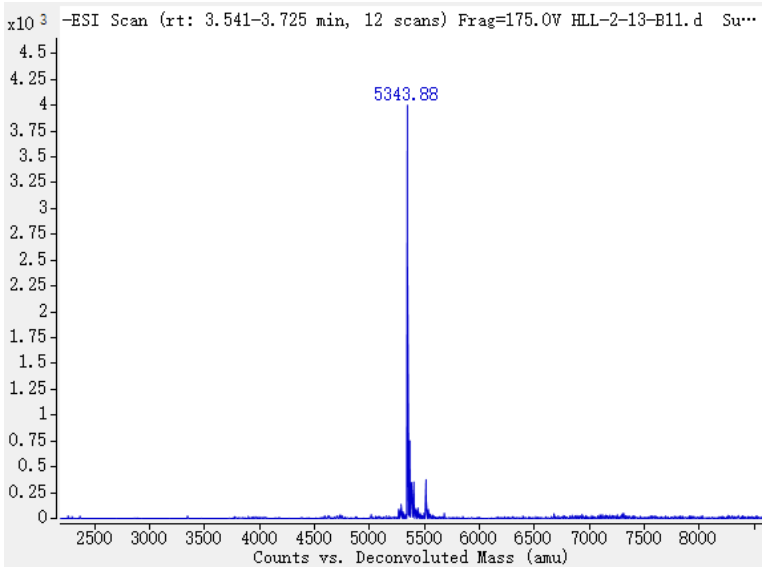


UPLC chromatogram and deconvoluted MS of combination of **a16** and **b1**

Conversion: 65%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da

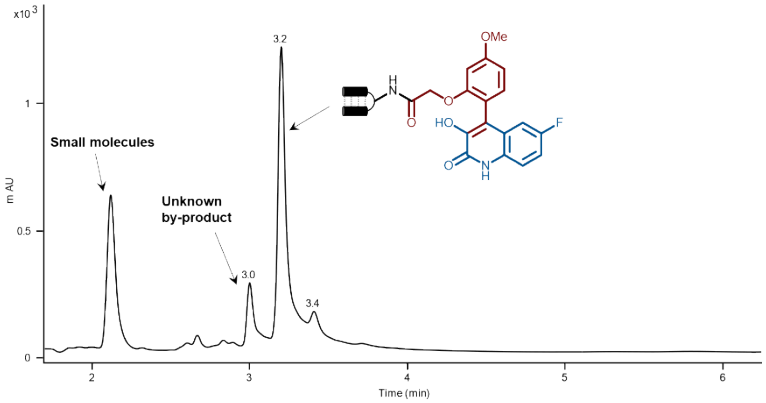


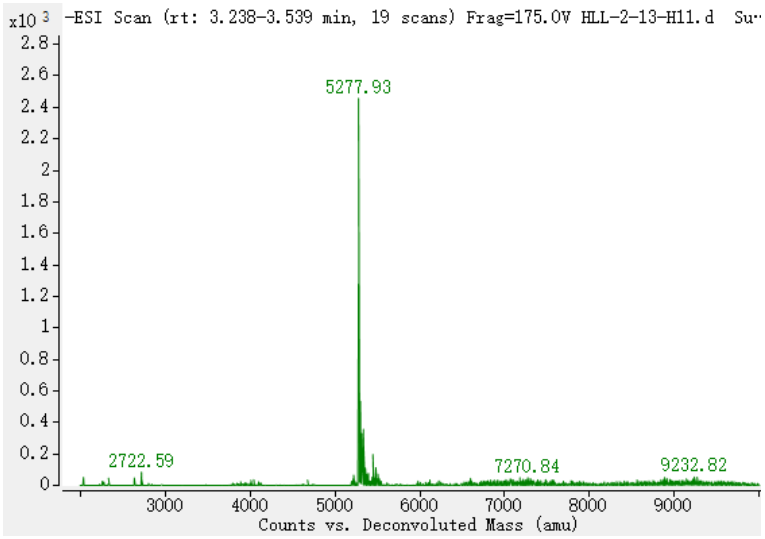


UPLC chromatogram and deconvoluted MS of combination of **a16** and **b3**

Conversion: 65%

Calculated Mass: 5278 Da; Observed Mass: 5278 Da

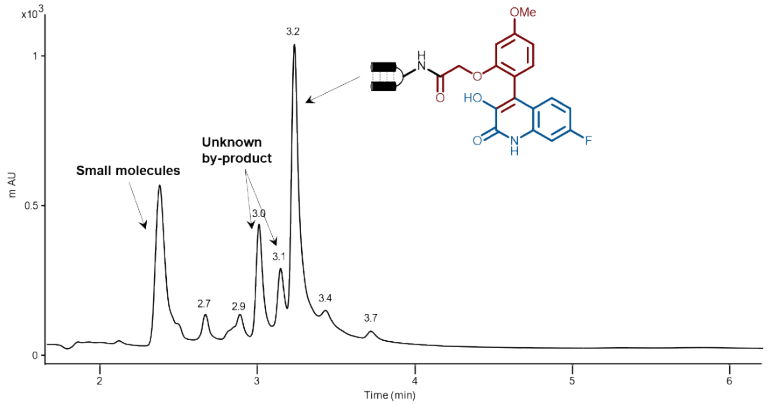


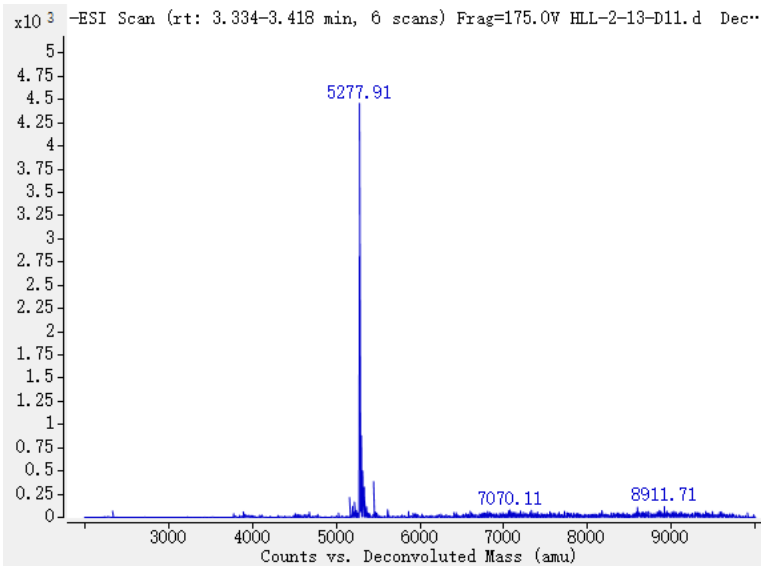


UPLC chromatogram and deconvoluted MS of combination of **a16** and **b4**

Conversion: 49%

Calculated Mass: 5278 Da; Observed Mass: 5278 Da

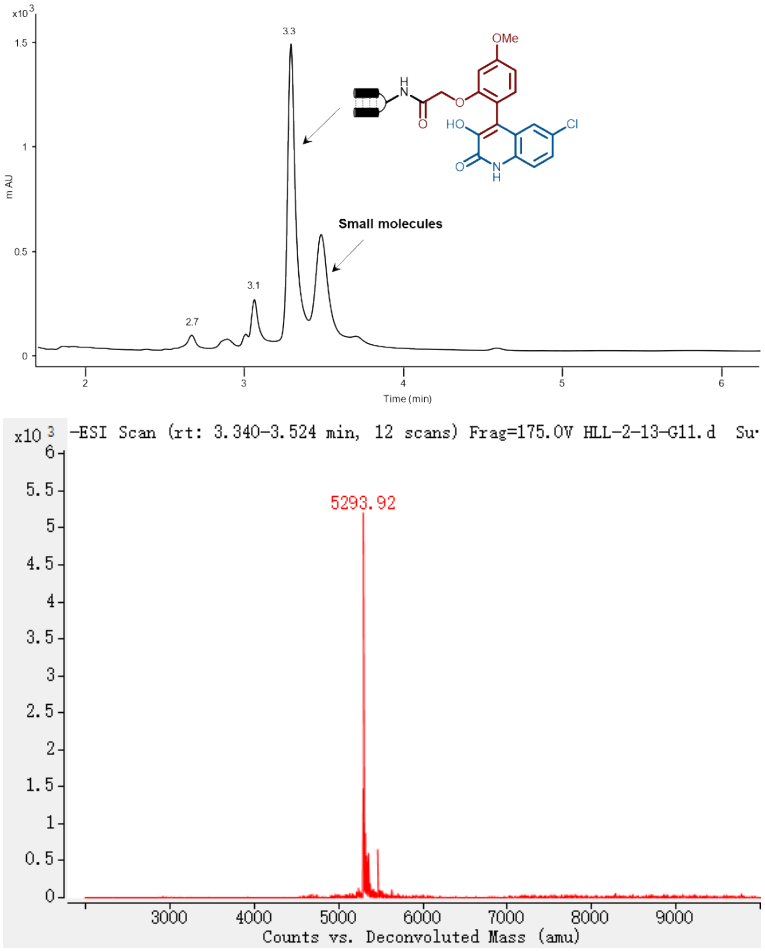




UPLC chromatogram and deconvoluted MS of combination of **a16** and **b6**

Conversion: 78%

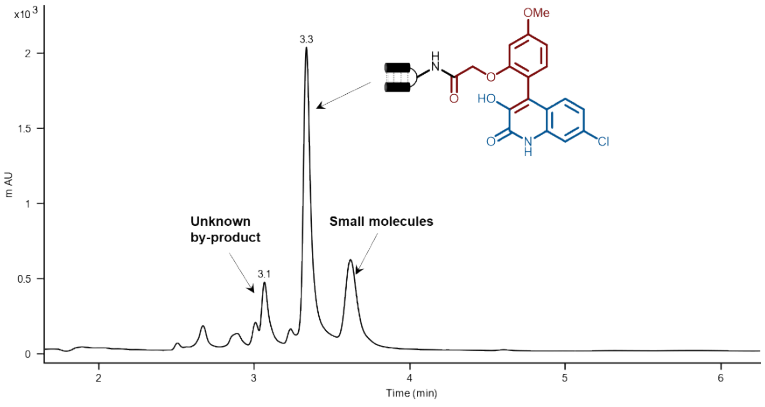
Calculated Mass: 5295 Da; Observed Mass: 5294 Da

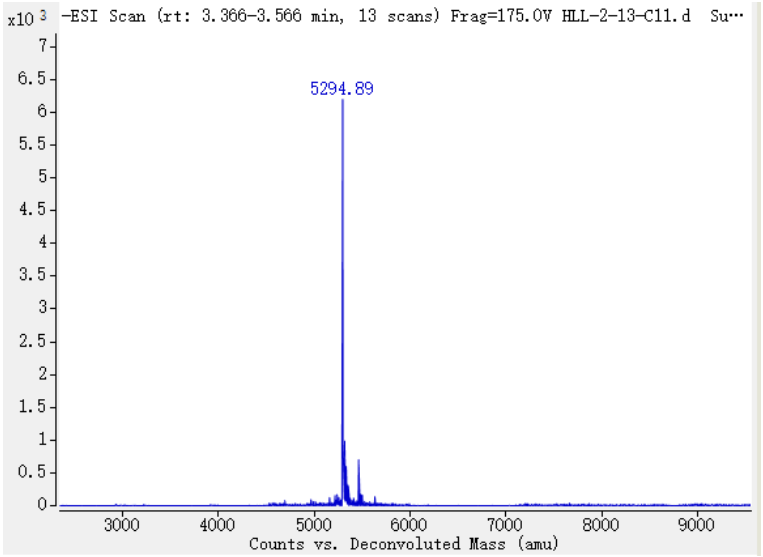


UPLC chromatogram and deconvoluted MS of combination of **a16** and **b7**

Conversion: 66%

Calculated Mass: 5295 Da; Observed Mass: 5295 Da

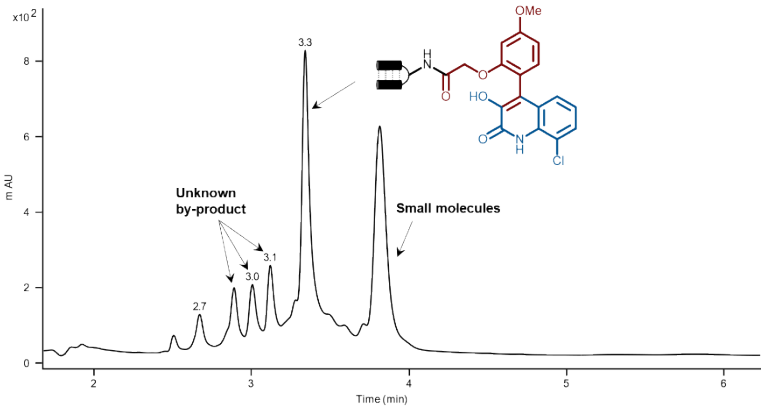


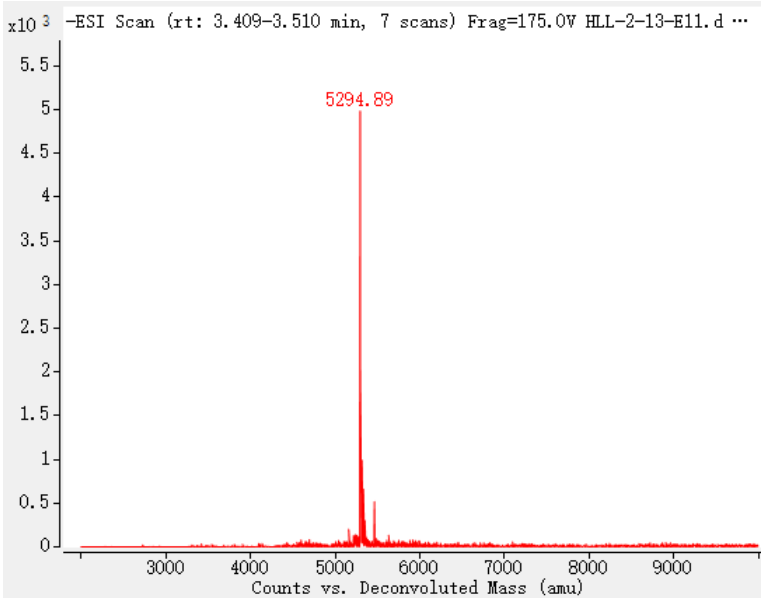


UPLC chromatogram and deconvoluted MS of combination of **a16** and **b8**

Conversion: 52%

Calculated Mass: 5295 Da; Observed Mass: 5295 Da

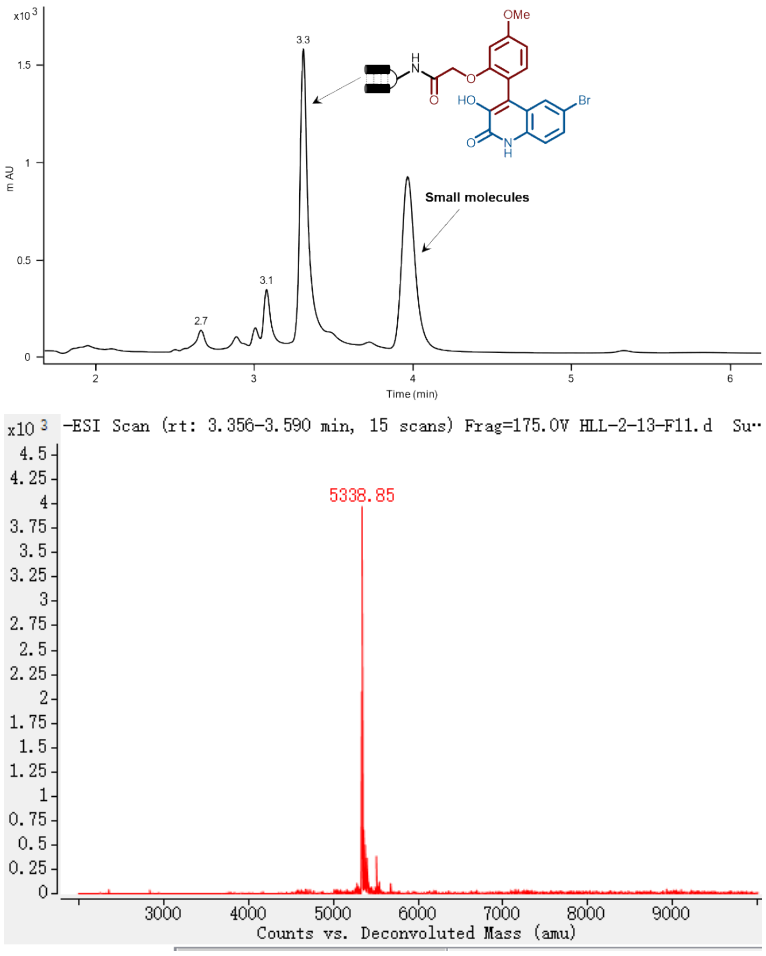




UPLC chromatogram and deconvoluted MS of combination of **a16** and **b10**

Conversion: 71%

Calculated Mass: 5339 Da; Observed Mass: 5339 Da



8. References

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2. X. Fang, Y. Wang, P. He, H. Liao, G. Zhang, Y. Li and Y. Li, *Org. Lett.*, 2022, **24**, 3291-3296.
3. Y. Tangella, K. L. Manasa, N. H. Krishna, B. Sridhar, A. Kamal and B. Nagendra Babu, *Org. Lett.*, 2018, **20**, 3639-3642.