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Supporting Information

DNA-compatible one-pot synthesis of multi-substituted dihydrofuran via pyridinium ylide-mediated cyclization

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Table of Contents

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 Da), Headpiece-primer (**HP-P**, 5'/5Phos/ACCTTCGGTCGGGAGTCA/iSp9/iUniAmM/iSp9/TGACTCCCGACCGAAGGTTG-3') and code sequences were received from HitGen Inc. (Shuangliu District, Chengdu, China). All the DNA sequences were written in 5'- to 3'- orientation unless otherwise noted. Chemicals and reagents were purchased from several commercial suppliers including J&K Scientific, Bidepharm, Adamas, and Sigma-Aldrich, and were generally used from aliquots dissolved in DMA, ACN, H_2O 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. Watsons 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). For detailed technical information, the reader is directed to the homepage of Axygen: http://www.axygen.com.

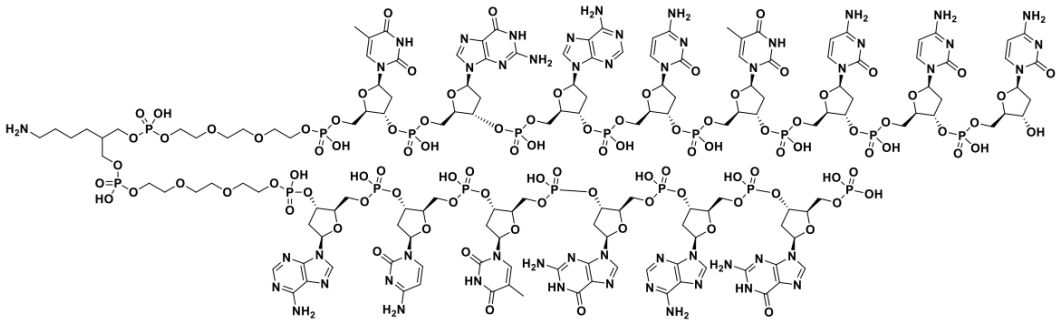


Figure S1. Structure of **HP** (MW = 4937).

1.2 General methods for DNA analysis

On-DNA reaction analysis (UHPLC-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 UHPLC. 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 UHPLC 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.

Table S1. Analytical method for **d1-d72**

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

Table S2. Analytical method for co-injection experiment

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
12	0.3	40
12.1	0.3	90
13	0.3	90
13.1	0.3	5
14	0.3	5

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

Table S3. Analytical method for 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

Time (min)	Flow (mL/min)	%B
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 conversions of on-DNA products were determined by UV absorbance (260 nm) peak area integration using the following equation: Conversion% = UV peak area (desired product)/UV peak area (total DNA recovered), while 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.[1]

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. The solution was maintained at -80 °C for 2 h and then 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.

Table S4. 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% MeCN

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 (20 μL), T4 DNA ligase (1.5 μL, 2000 units/μL) and nuclease-free water (to the total volume of 50 μL). The reaction was incubated at 20 °C for 2 h 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, 5 pmol of treated DNA samples was loaded on the gel, and the gel was run at 150 V for 50–60 min. DNA fragments were visualized and analyzed by Bio-Rad ChemidocTM Image System (Bio-Rad, CA, USA).

1.6 General information for off-DNA synthesis

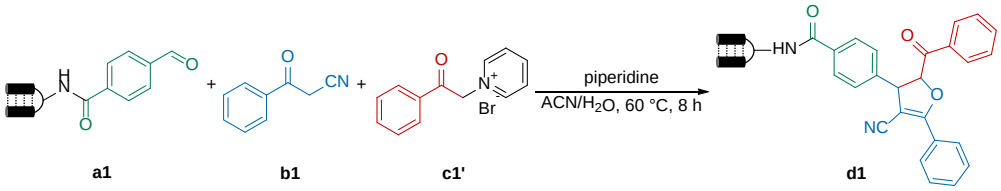
Off-DNA reactions were monitored by TLC. Analytical TLCs were performed with 0.25 mm silica gel HSGF254. The TLC plates were visualized by ultraviolet light. Flash chromatography was conducted on silica gel 60 (SiO_2, 100–200

mesh). Isolated compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS. Chemical shifts (δ) were recorded on an Agilent 400 MHz spectrometer reported in parts per million (ppm). [$CDCl_3$] (H δ = 2.50; C δ = 77.23) 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. High resolution mass spectra (HRMS) were acquired on a 7 Tesla SolariX FT-ICR MS (quadrupole, Bruker Daltonics, Bremen, Germany) with an ESI source or GCT Premier (waters, United States) with an EI source.

2. Condition optimization and general procedures for on-DNA synthesis of 2,3-dihydrofuran

2.1 Condition optimization

Table S5. Optimization of conditions for the on-DNA synthesis of 2,3-dihydrofuran

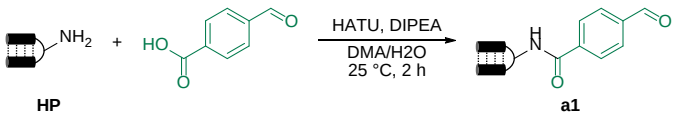


Entry	Base	Temperature	Time	Conversion
1	pyridine	60 °C	8 h	48%[a]
2	piperidine	60 °C	8 h	>95%
3	TEA	60 °C	8 h	86%
4	Cs_2CO_3	60 °C	8 h	0%[b]
5	KOH	60 °C	8 h	0%[b]
6	piperidine	40 °C	8 h	43%[b]
7	piperidine	25 °C	12 h	0%[c]

[a]DNA-conjugated aldehydes remained; [b]DNA-conjugated aldehydes remained and byproduct generated; [c]only byproduct generated.

Reaction conditions: To the solution of DNA conjugate **a1** (4 μL, 100 μM in H_2O, 400 pmol, 1 equiv.), were added 7 μL H_2O, 13 μL ACN, **b1** (2 μL, 100 mM in ACN, 200 nmol, 500 equiv.), base (2 μL, 200 mM in H_2O, 400 nmol, 1000 equiv.) and **c1'** (2 μL, 100 mM in H_2O, 200 nmol, 500 equiv.) sequentially. The reaction mixture was vortexed, centrifuged and incubated in the dry bath. Regioisomers were observed. Deconvoluted molecular mass: calculated: 5314 Da; observed: 5314 Da. Conversions were determined by UHPLC-MS.

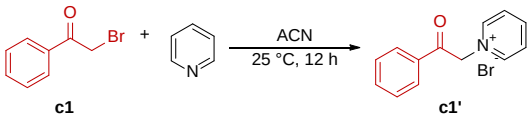
2.2 Preparation for DNA-conjugated aldehydes by amide coupling



HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. 4-Formylbenzoic 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 in the dry bath for 2 h. After purification by ethanol precipitation, the reaction was analyzed by UHPLC-MS. The collected product was vacuum-dried overnight and redissolved in H_2O for subsequent experiments.

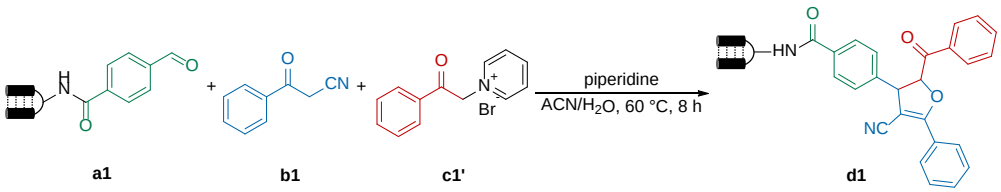
2.3 General procedure for formation of pyridinium ylide precursors



c1 **c1'**

The α-bromoketone **c1** (10 μL, 400 mM in ACN, 1 equiv.) was mixed with pyridine (10 μL, 400 mM in ACN, 1 equiv.) and proceed at 25 °C in the dry bath for 12 h. In most cases, a heavy precipitate formed. An equal volume of H_2O was added to dissolve the precipitate, yielding a solution for subsequent experiments. Unless otherwise noted, pyridinium ylide precursors described in the supporting information were performed under this standard condition. Note: a freshly prepared solution was required.

2.4 General procedure for on-DNA synthesis of 2,3-dihydrofuran scaffolds



a1 **b1** **c1'** **d1**

Standard Condition: To the solution of DNA conjugate **a1** (4 μL, 100 μM in H_2O, 400 pmol, 1 equiv.), were added 7 μL H_2O, 13 μL ACN, **b1** (2 μL, 100 mM in ACN, 200 nmol, 500 equiv.), piperidine (2 μL, 200 mM in H_2O, 400 nmol, 1000 equiv.), and **c1'** (2 μL, 100 mM in H_2O, 200 nmol, 500 equiv.) sequentially. The reaction mixture was vortexed, centrifuged and incubated at 60 °C in the dry bath for 8 h. The product was obtained by ethanol precipitation and

analyzed by UHPLC-MS immediately (Conversion: >90%). Regioisomers were observed. Deconvoluted molecular mass: calculated: 5314 Da; observed: 5314 Da. Unless otherwise noted, on-DNA synthesis of 2,3-dihydrofuran scaffolds described in the supporting information was performed under this standard condition.

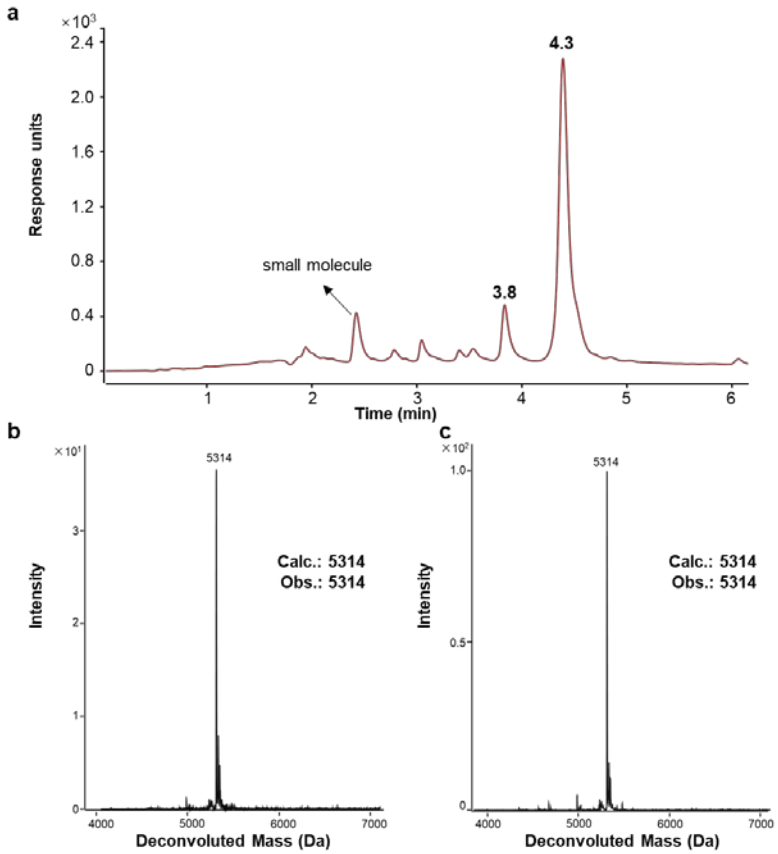
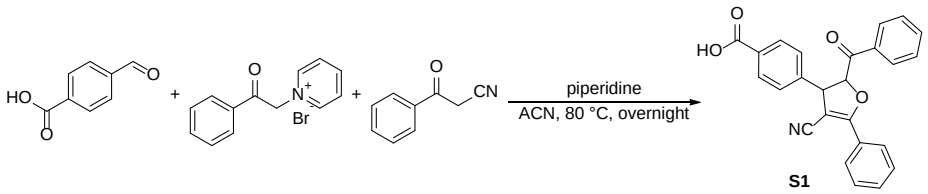


Figure S2. UHPLC chromatogram and deconvoluted MS of **d1**. **a**, UHPLC chromatogram of **d1**. **b**, Deconvoluted MS of the chromatographic peak eluting at 3.8 min. **c**, Deconvoluted MS of the chromatographic peak eluting at 4.3 min.

3. Structural validation

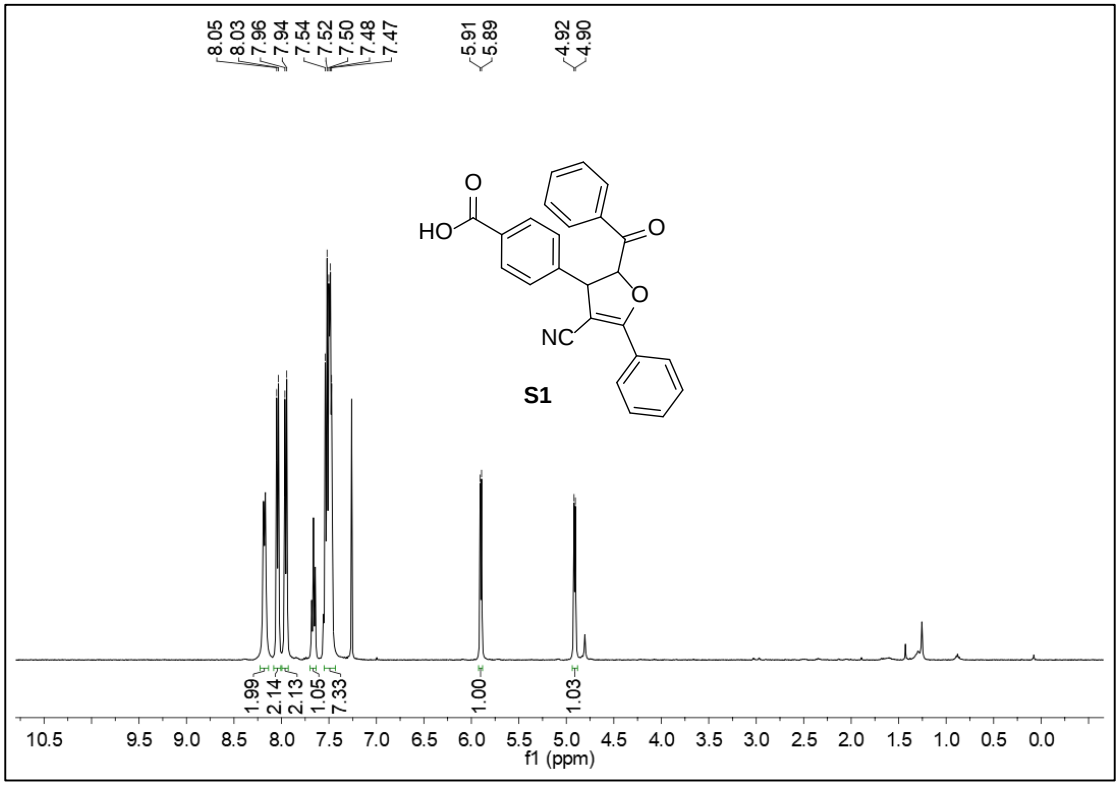
3.1 Off-DNA synthesis of 2,3-dihydrofuran S1



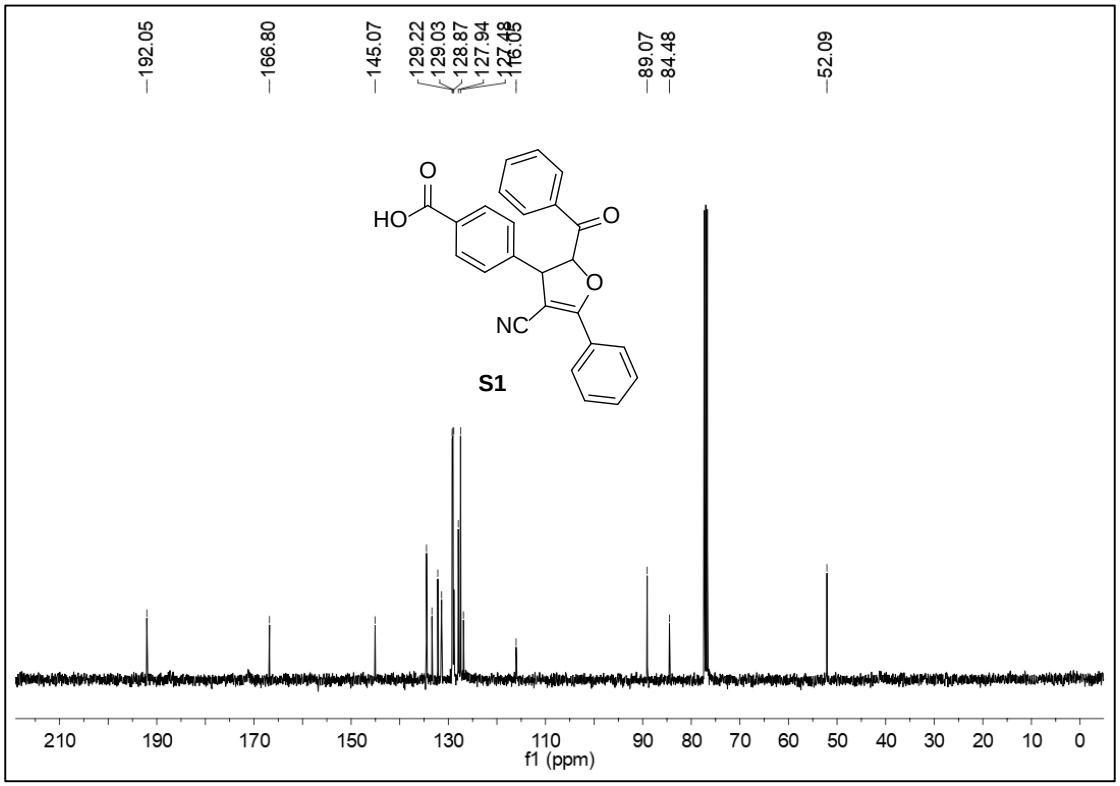
4-(2-benzoyl-4-cyano-5-phenyl-2,3-dihydrofuran-3-yl)benzoic acid (S1):

Authentic **S1** was prepared according to procedures reported in the literature.[2] A mixture of β-ketonitrile (1.0 mmol, 1.0 equiv.), pyridinium salt (1.0 mmol, 1.0 equiv.), aldehyde (1.0 mmol, 1.0 equiv.), and piperidine (1.2 mmol, 1.2 equiv.) in ACN (10 mL) was refluxed overnight. The reaction mixture was poured into 50 mL water. The product was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was distilled off under reduced pressure, and the residue was purified by flash column chromatography with EtOAc/n-hexane as an eluent to obtain the desired product **S1** as a faint yellow viscous oil (152 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 7.6 Hz, 2H), 8.04 (d, *J* = 7.3 Hz, 2H), 7.95 (d, *J* = 7.6 Hz, 2H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.55 – 7.43 (m, 7H), 5.90 (d, *J* = 5.7 Hz, 1H), 4.91 (d, *J* = 5.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.1, 166.8, 145.1, 134.5, 133.4, 132.2, 131.4, 129.2, 129.0, 128.9, 127.9, 127.5, 126.9, 116.1, 89.1, 84.5, 52.1. HRMS (TOF ESI): m/z: $[M + H]^+$ Calcd for $C_{25}H_{18}NO_4^+$: 396.1230; Found: 396.1234.

^{1}H NMR (400 MHz, $CDCl_3$) of **S1**



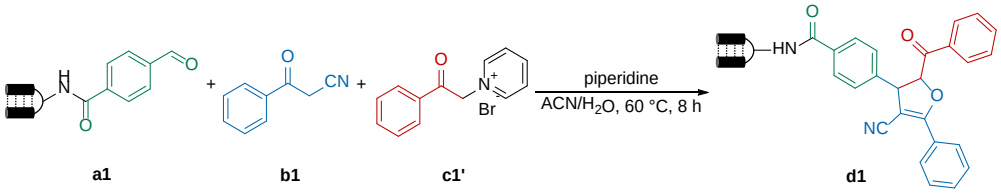
^{13}C NMR (101 MHz, $CDCl_3$) of **S1**



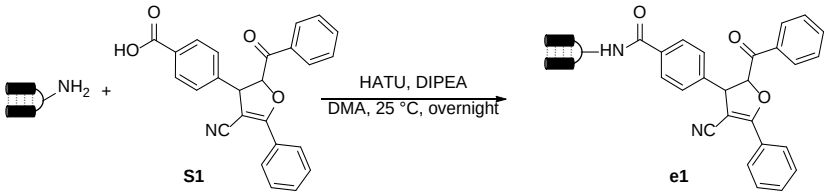
During the off-DNA synthesis of the corresponding small molecule, excellent regioselectivity was observed, and TLC analysis revealed predominantly a major product. Due to the significant dominance of the trans isomers, isolation proved difficult, hindering the acquisition of pure cis-isomers. We directly used this sample as the off-DNA synthesized small molecule standard for conjugation with DNA.

3.2 Co-injection experiment

Route A



Route B



DNA conjugate **d1** was prepared according to the general procedure for synthesis of 2,3-dihydrofuran. DNA conjugate **e1** was prepared according to the general procedure for amide coupling. DNA conjugates **d1**, **e1**, and their equimolar mixture (**d1**/**e1**), were analyzed by UHPLC-MS using the same analysis method. For all the LC chromatograms, two chromatographic peaks emerged, exhibiting distinct retention times but the identical molecular weight. The retention times of the peaks were consistent across all samples (Figure S3). This data validated the structure of the on-DNA generated product, revealing the presence of two regioisomers. Besides, in most of the substrates we investigated, there were two peaks indicating the presence of regioisomers, though the regioselectivity varies for different substrates.

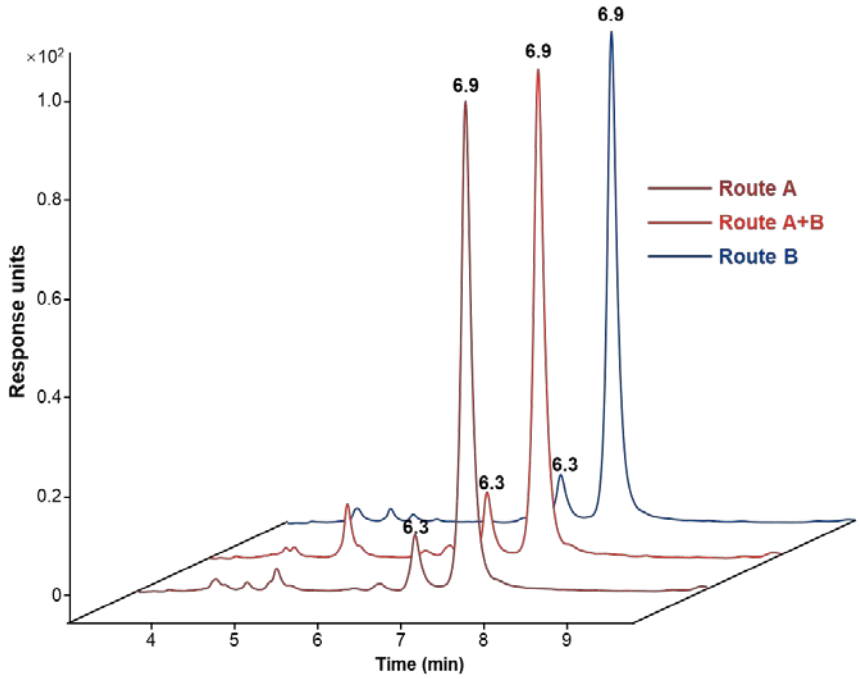
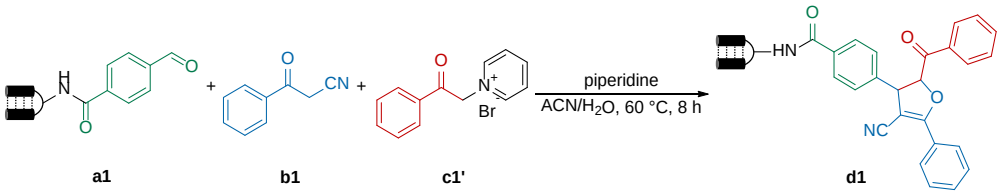


Figure S3. Co-injection experiment of **d1** and **e1** from two independent synthetic routes. HPLC chromatography showed that the two peaks from the co-injection (red curve) had the same retention time as those from **route A** and **route B**, respectively (**d1** from **route A**, brown curve; **e1** from **route B**, dark blue curve).

4. Scale-up reaction



To the solution of DNA-conjugated aldehyde **a1** (10 μL, 1 mM in H_2O, 10 nmol, 1 equiv.) were added 50 μL H_2O, 80 μL ACN, **b1** (20 μL, 100 mM in ACN, 2000 nmol, 200 equiv.), piperidine (20 μL, 200 mM in H_2O, 4000 nmol, 400 equiv.), and **c1'** (20 μL, 100 mM in H_2O, 2000 nmol, 200 equiv.) sequentially. The reaction mixture was vortexed, centrifuged, and incubated at 60 °C in the dry bath for 8 h. After purification by ethanol precipitation, the reaction was analyzed by UHPLC-MS.

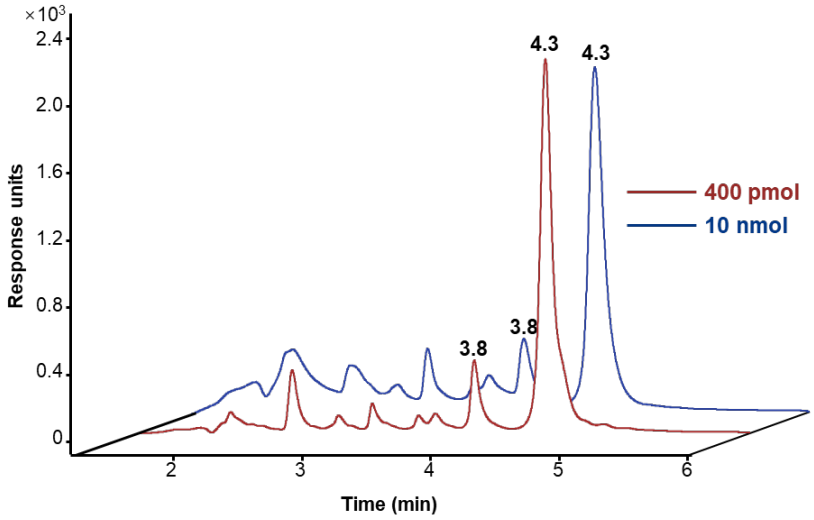
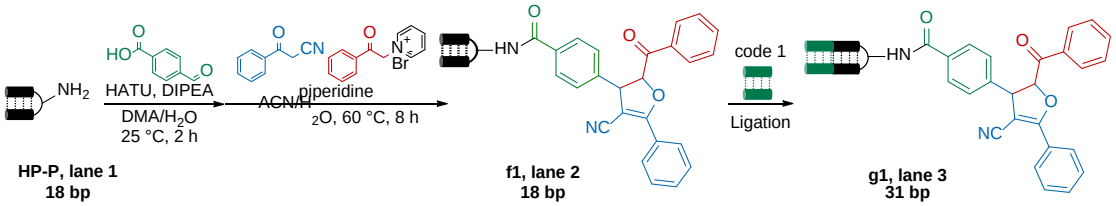


Figure S4. UHPLC chromatogram of **d1** at 400 pmol (brown curve) and 10 nmol (dark blue curve) scales.

5. Enzymatic ligation



HP-P was initially coupled with 4-formylbenzoic acid, then reacted with **b1** and **c1'** to afford **f1** (conversion: 87%), and then tagged with code 1 to afford **g1**. The reaction mixture was denatured by incubating at 95 °C in a dry bath for 10 min, and then all the ligation product was isolated by ethanol precipitation. The resulting pellets were vacuum-dried and dissolved in nuclease-free water.

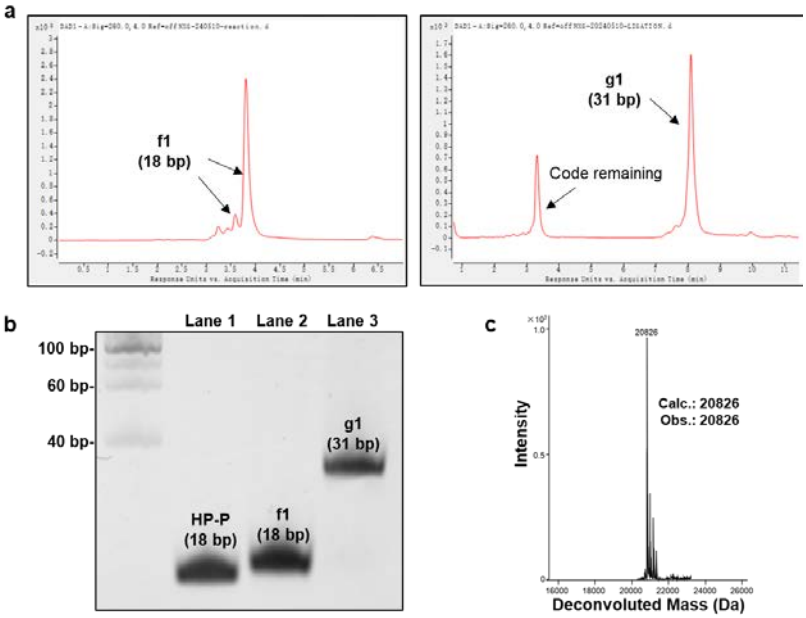
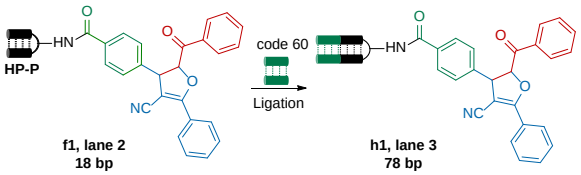


Figure S5. Enzymatic DNA ligation by T4 ligase. **a**, UHPLC chromatogram of **f1** and **g1**. **b**, 20% denaturing PAGE analysis of DEL-encoding compatibility. Lane 1, **HP-P**; Lane 2, DNA conjugate **f1**; Lane 3, DNA conjugate **g1**. **c**, Deconvoluted MS of **g1**.

6. General procedure for quantitative polymerase chain reaction (qPCR)



DNA damage was assessed by qPCR. **HP-P** (25 pmol) and DNA conjugate **f1** (25 pmol) were ligated with a 60 bp DNA tag (code 60) for 12 h to generate **HP-P-code 60** and **h1** in parallel. Ligation reactions were heat-inactivated at 95 °C for 10 min, diluted and subjected to qPCR without ethanol precipitation. (**HP-P** was quantified by its absorbance at 260 nm, while DNA conjugate **f1** was relatively quantified by comparing the densitometry of bands in gel electrophoresis.)

qPCR was performed on the BIO-RAD CFX96TM Real-Time System. Each 20 µL reaction contained: 1 µL ddH2O, 4 µL primer mix (10 µM each of reverse and forward primer), 5 µL diluted DNA sample, and 10 µL 2 × SP qPCR mix (BG0014). Thermal cycling conditions were: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 65 °C for 15 s. All Cycle Threshold (C_T) values represent the average of at least three replicates.

Code 60-T (5’-3’):

CTCCCTCGCGCCATCAGACGACCACGCAAGAGCTTTATGTACGAGCAAA GCCTGTGTTCA

Code 60-B (5’-3’):

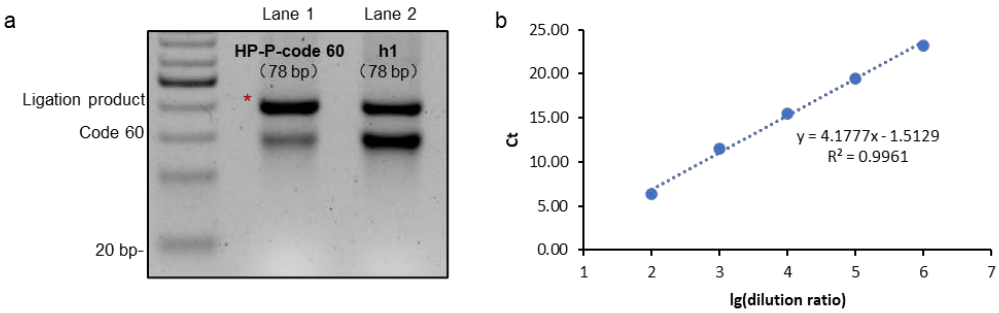
AACACAGGCTTTGCTCGTACATAAAGCTCTTGCGTGGTCGTCTGATGGCG CGAGGGAGGC

Forward Primer (5’-3’):

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGACTCCCGACCGAA GGTTG

Reverse Primer (5’-3’):

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTCCCTCGCGCCAT CAGAC

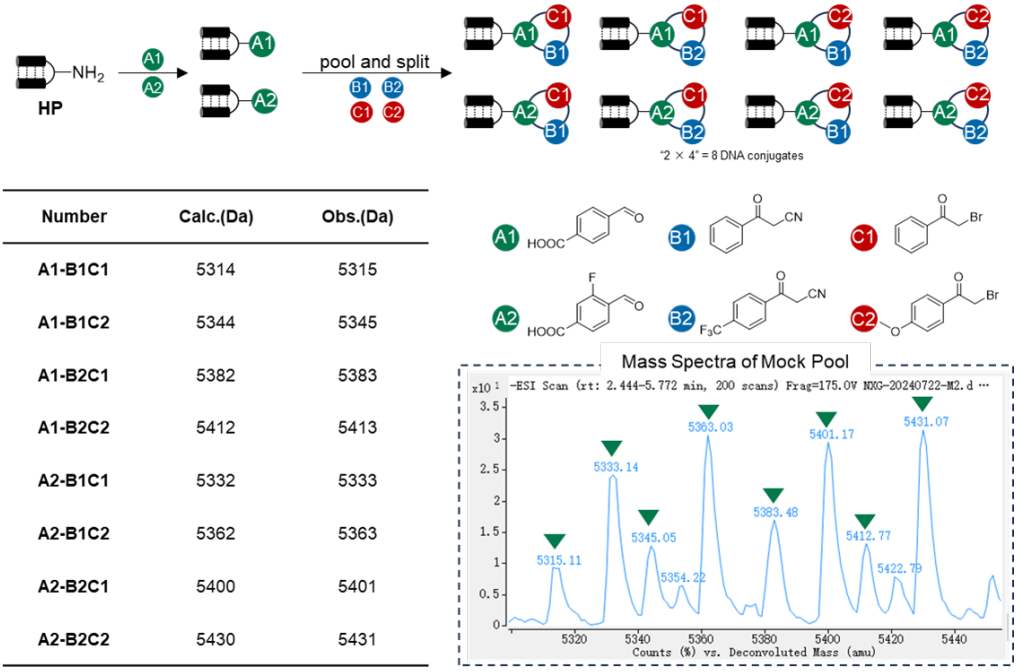


c

Sample	Average C_T	Amplification ratio
Control (HP-P-code 60)	15.27	100%
h1	14.73	129%

Figure S6. qPCR analysis of DNA damage. **a**, 20% denaturing PAGE analysis of ligation products. Lane 1, **HP-P-code 60**; Lane 2, DNA conjugate **h1**. **b**, Standard curve generated using serial dilutions of **HP-P-code 60** as a control for qPCR. **c**, the C_T values and DNA amplification ratios (the control and experimental group).

7. General procedure for DNA-encoded mock pool synthesis



Number	Calc.(Da)	Obs.(Da)
A1-B1C1	5314	5315
A1-B1C2	5344	5345
A1-B2C1	5382	5383
A1-B2C2	5412	5413
A2-B1C1	5332	5333
A2-B1C2	5362	5363
A2-B2C1	5400	5401
A2-B2C2	5430	5431

Figure S7. DNA-encoded mock pool synthesis

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. **A1** or **A2** (10 μL, 200 mM in DMA), HATU (10 μL, 400 mM in DMA) and DIPEA (10 μL, 400 mM in DMA) were mixed by vortex and allowed to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to **HP** solution (10 μL, 10 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 UHPLC-MS. The DNA-conjugated aldehydes were dissolved in nuclease-free water, quantified using a BioTek Epoch UV-Vis spectrometer, and then pooled at 2 nmol each to form the cycle 1 product. The pooled cycle 1 product was dissolved in 44 μL H_2O and divided into four tubes with 1 nmol mixture of DNA-conjugated aldehydes each**,** and separately reacted with a different combination of **B1**-**C1**, **B1**-**C2**, **B2**-**C1**, **B2**-**C2** under standard condition. After pool and purification by ethanol precipitation, the reaction mixture was analyzed by UHPLC-MS. As illustrated in **Figure S7**, we verified the two-round synthetic crude mixture by MS to find all eight DNA conjugates.

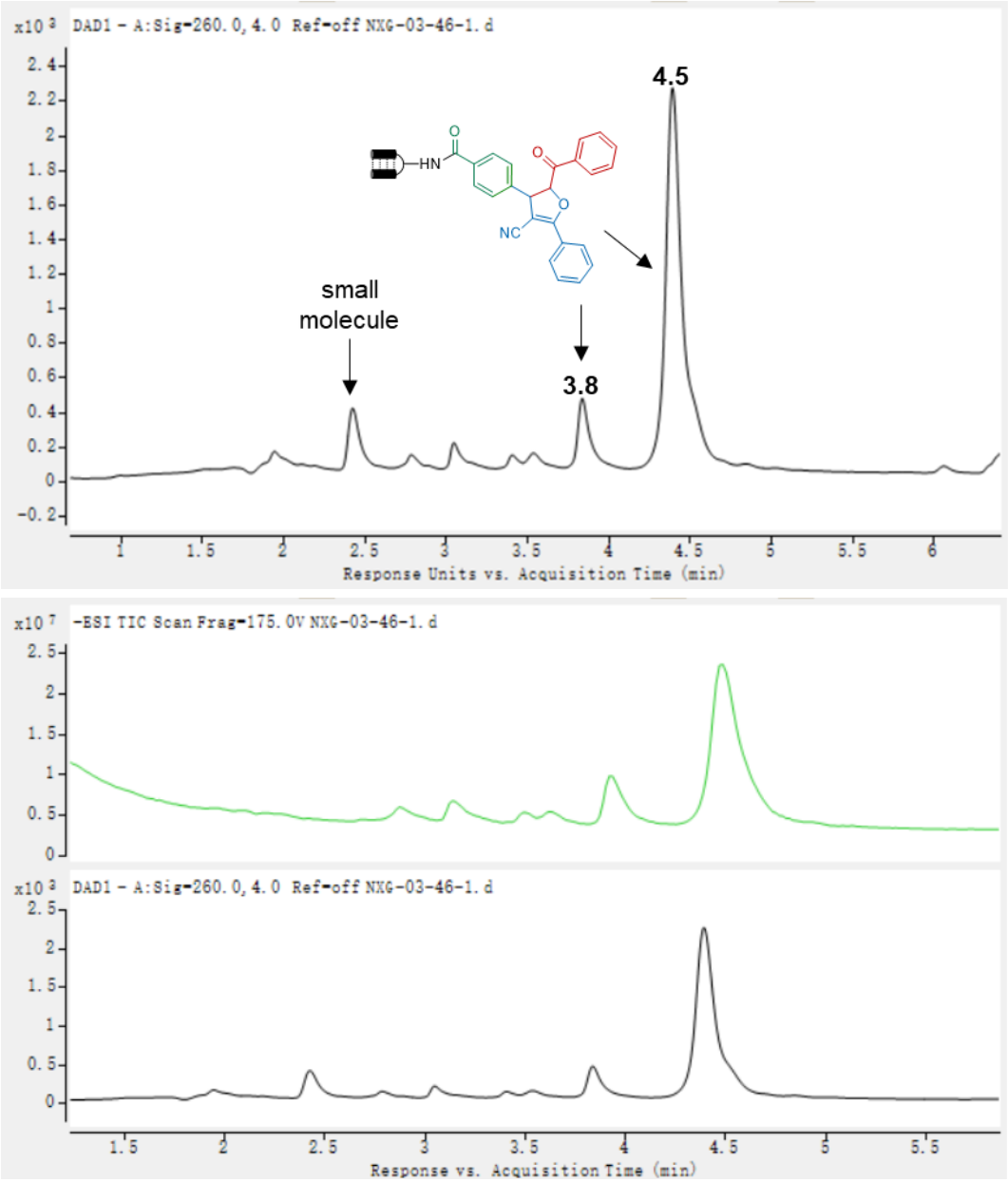
8. UHPLC chromatogram and deconvoluted MS

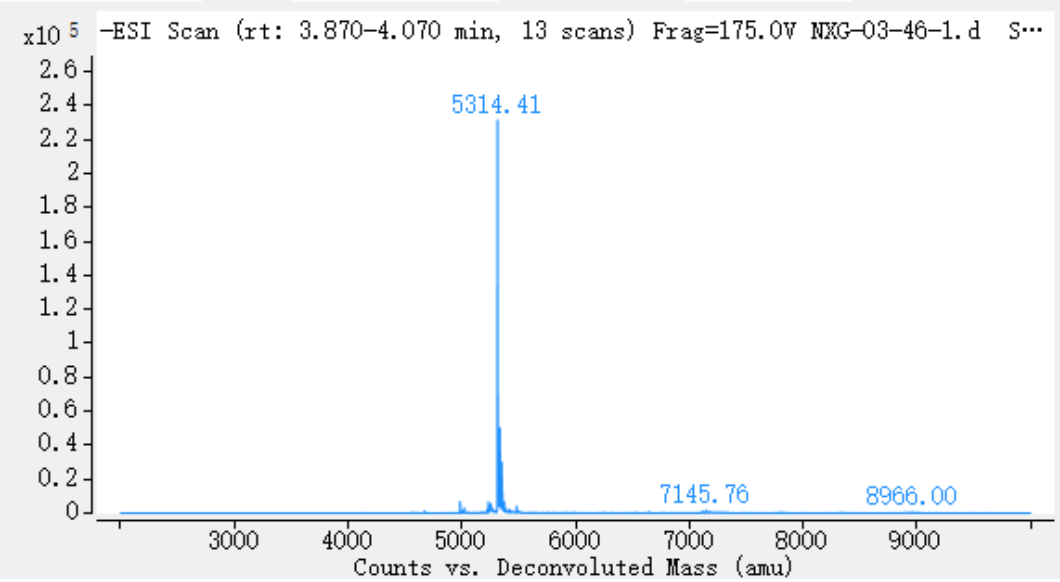
8.1 Scope of β-ketonitriles

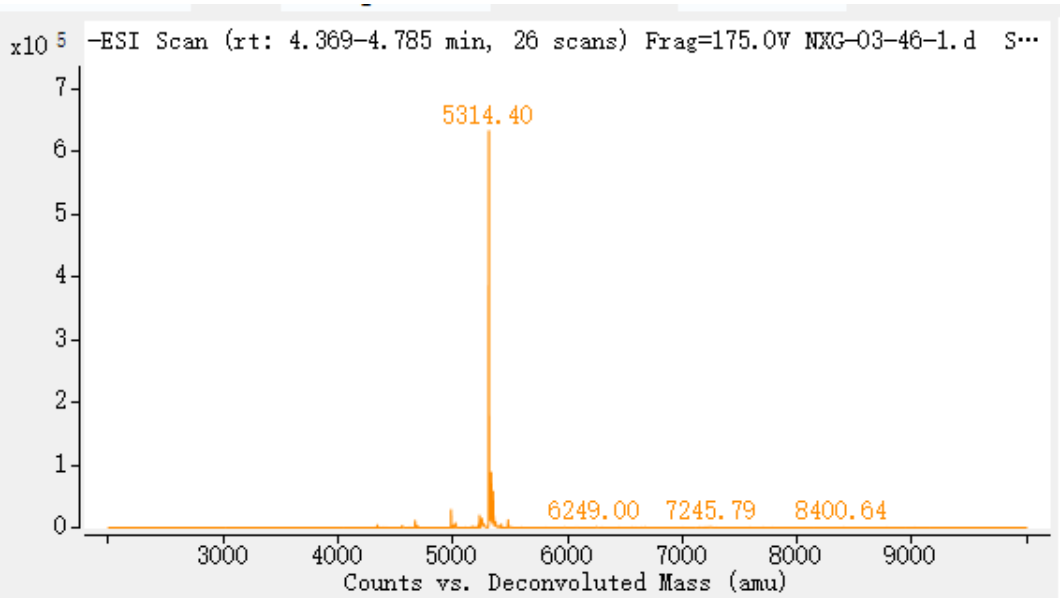
UHPLC chromatogram and deconvoluted MS of **d1**

Conversion: >90%

Calculated Mass: 5314 Da; Observed Mass: 5314 Da



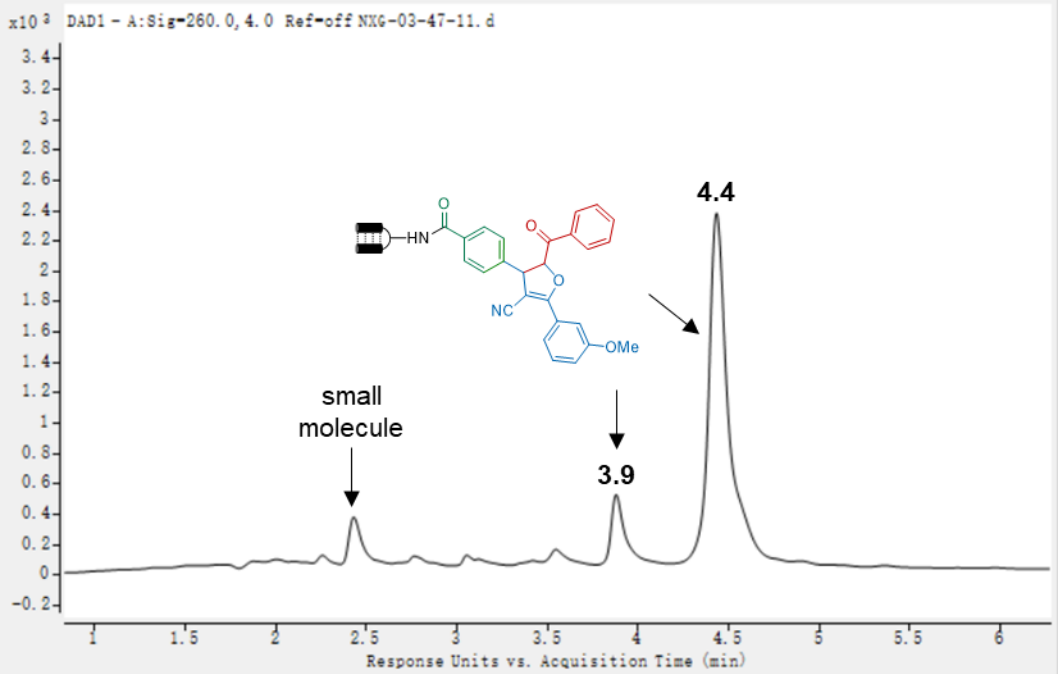

x10 5
-ESI Scan (rt: 3.870-4.070 min, 13 scans) Frag=175.0V NXG-03-46-1.d S…
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5314.41
7145.76
8966.00
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

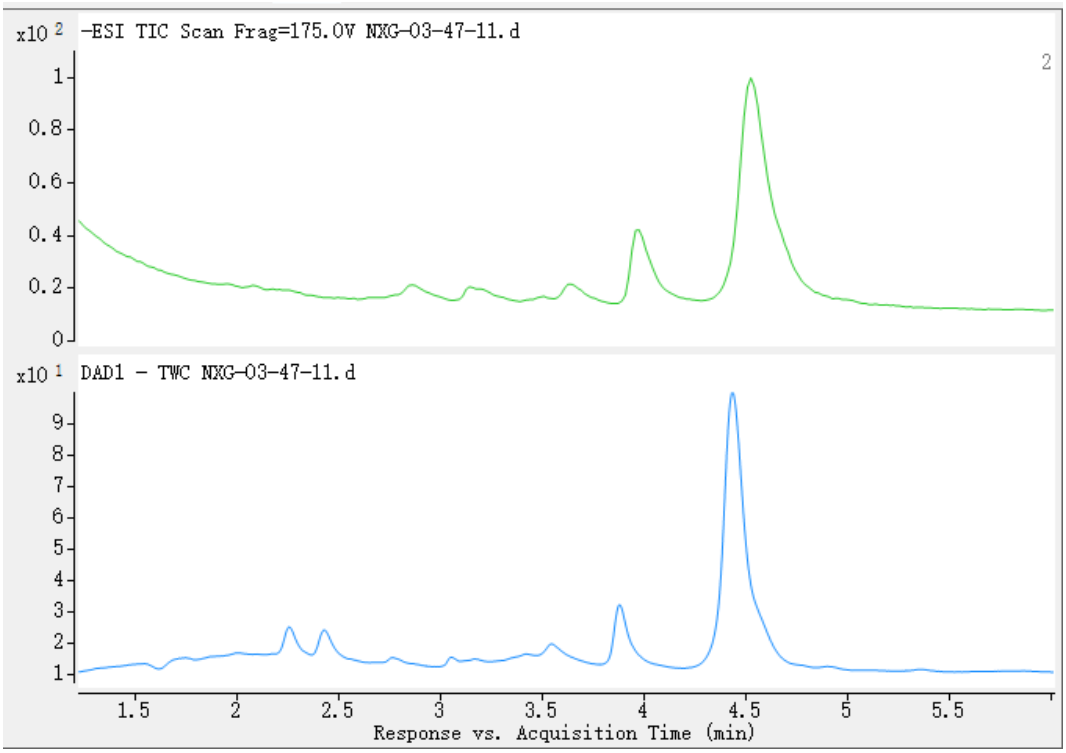

x10 5
-ESI Scan (rt: 4.369-4.785 min, 26 scans) Frag=175.0V NXG-03-46-1.d S…
7
6
5
4
3
2
1
0
5314.40
6249.00
7245.79
8400.64
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

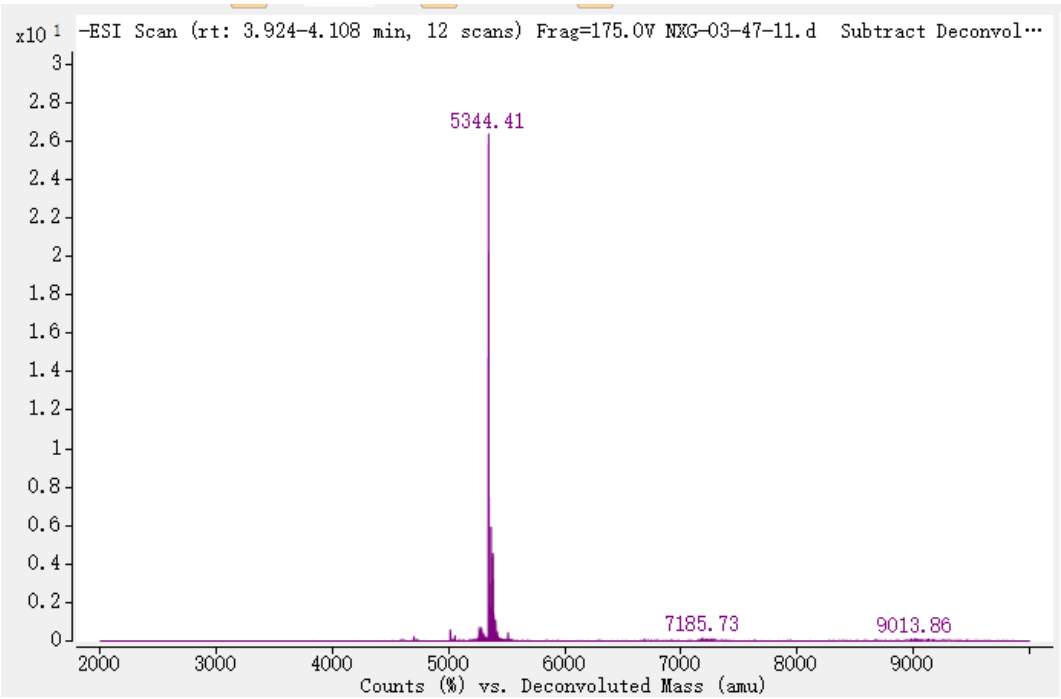
UHPLC chromatogram and deconvoluted MS of **d2**

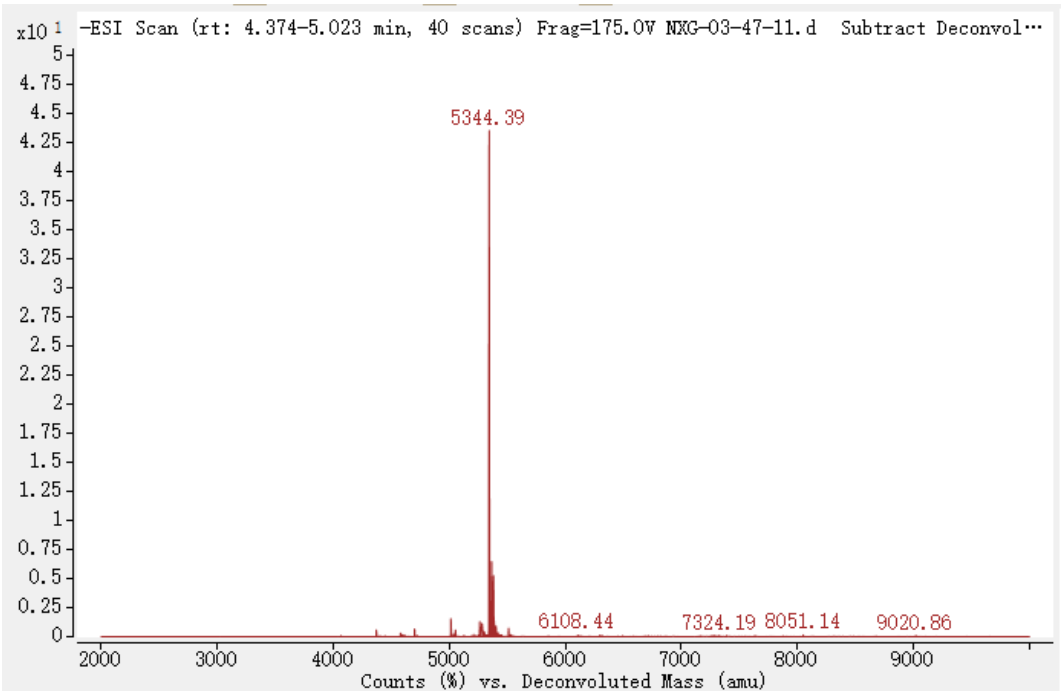
Conversion: >90%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da





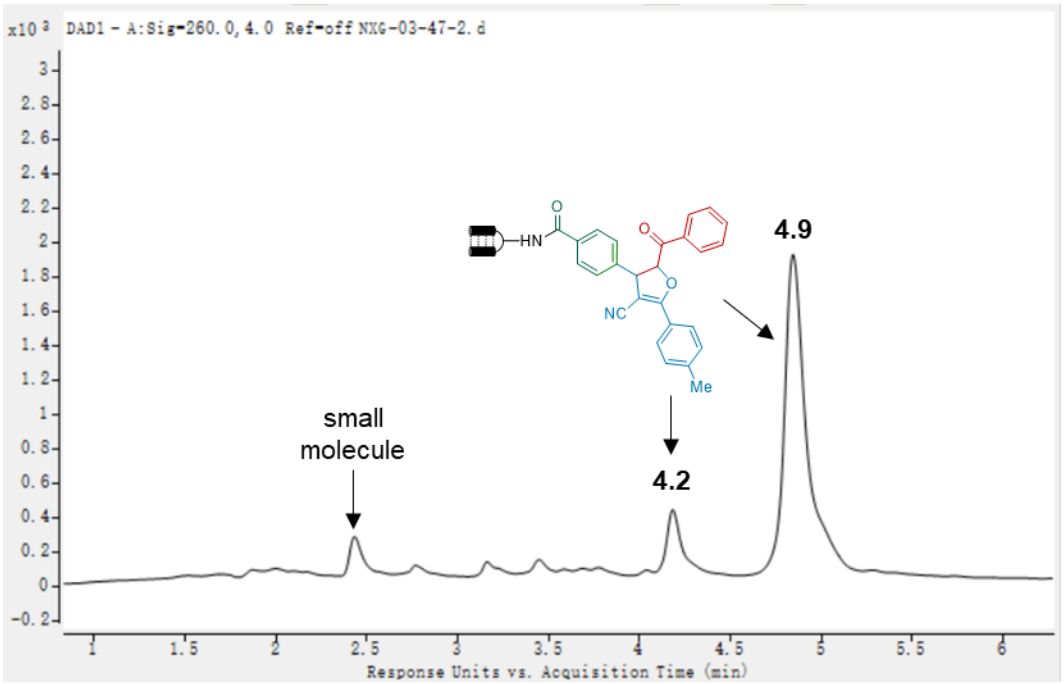

-ESI Scan (rt: 3.924-4.108 min, 12 scans) Frag=175.0V NXG-03-47-11.d Subtract Deconvol…
x10 1
5344.41
7185.73
9013.86
Counts (%) vs. Deconvoluted Mass (amu)

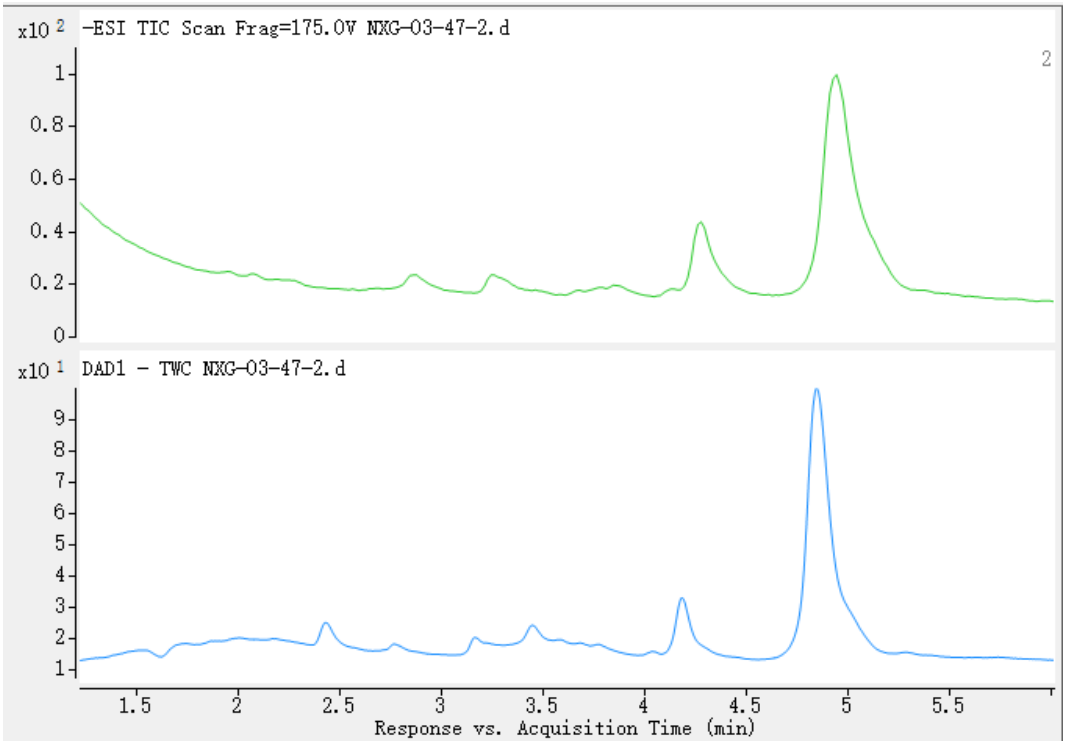

-ESI Scan (rt: 4.374-5.023 min, 40 scans) Frag=175.0V NXG-03-47-11.d Subtract Deconvol…
x10 1
5344.39
6108.44
7324.19
8051.14
9020.86
Counts (%) vs. Deconvoluted Mass (amu)

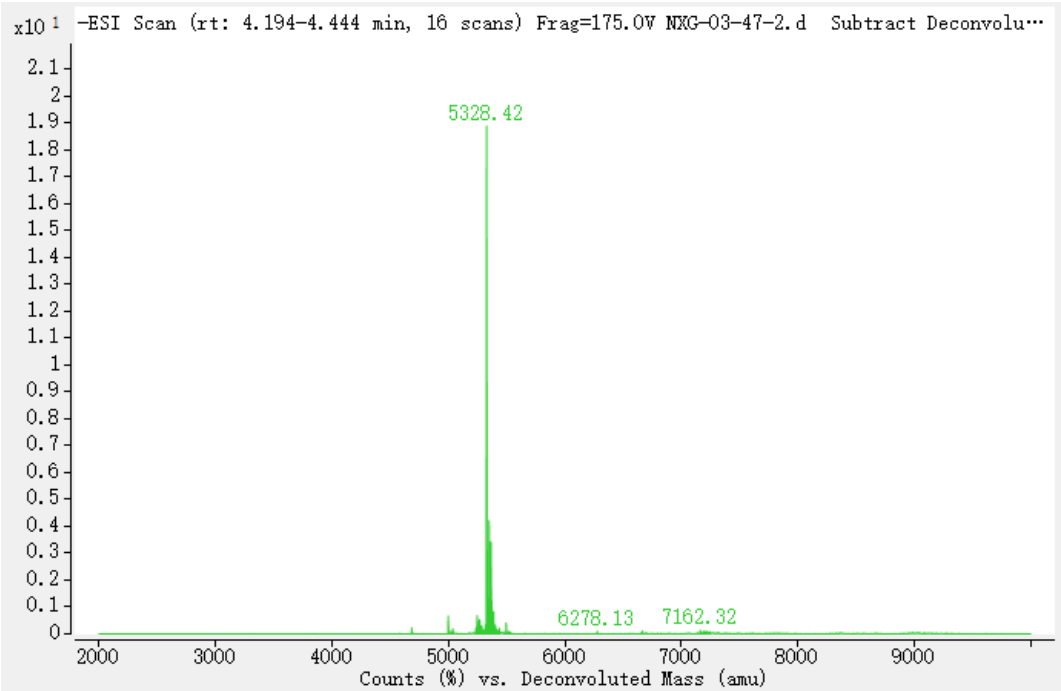
UHPLC chromatogram and deconvoluted MS of **d3**

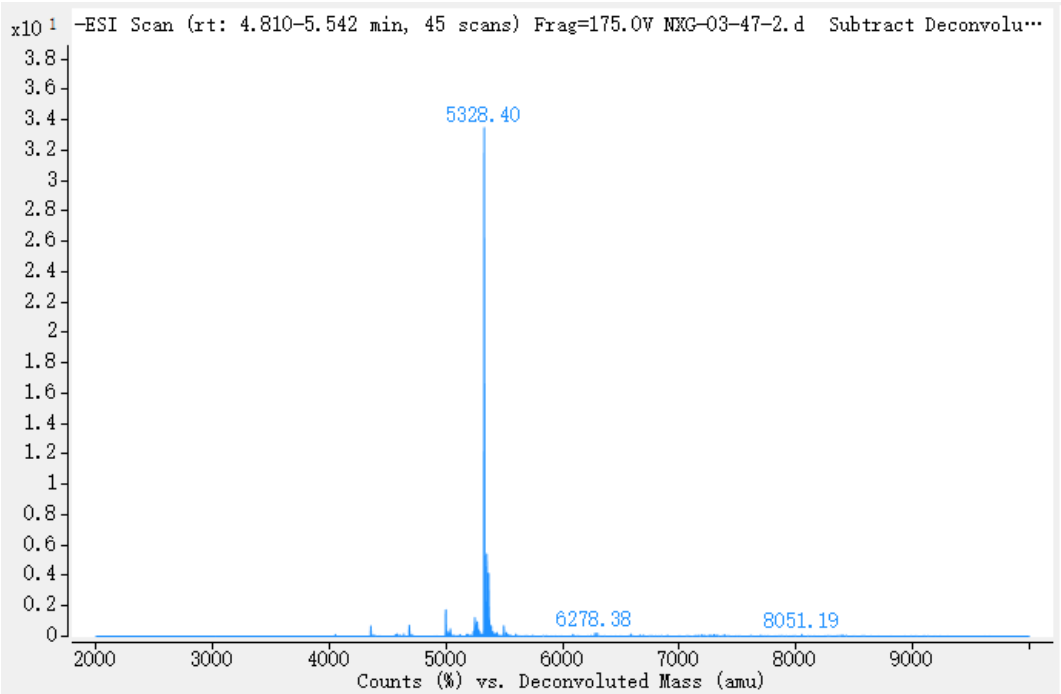
Conversion: >90%

Calculated Mass: 5328 Da; Observed Mass: 5328 Da





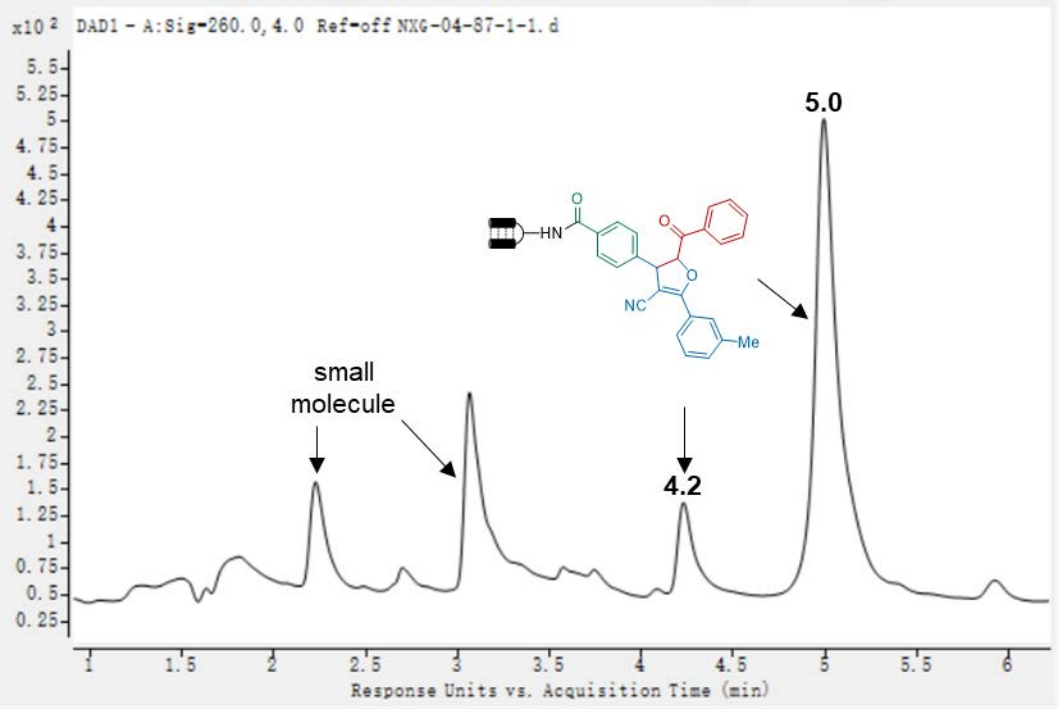

x10 1
-ESI Scan (rt: 4.194-4.444 min, 16 scans) Frag=175.0V NXG-03-47-2.d Subtract Deconvolu…
5328.42
6278.13
7162.32
Counts (%) vs. Deconvoluted Mass (amu)

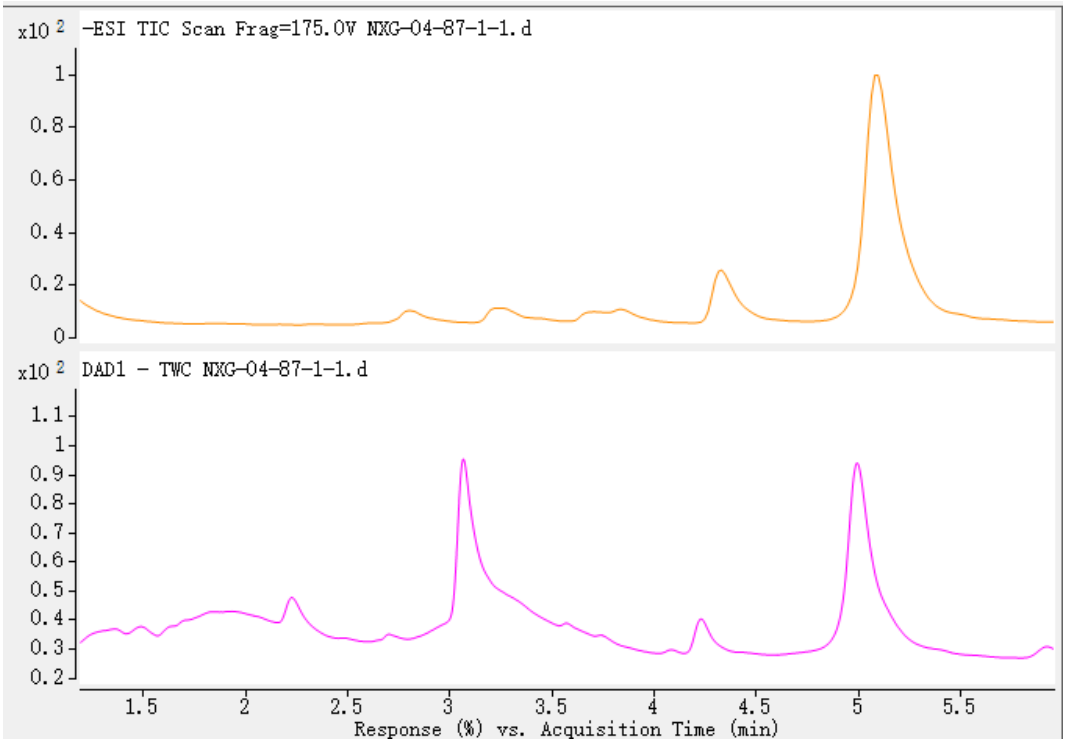

x10 1
-ESI Scan (rt: 4.810-5.542 min, 45 scans) Frag=175.0V NXG-03-47-2.d Subtract Deconvolu…
5328.40
6278.38
8051.19
Counts (%) vs. Deconvoluted Mass (amu)

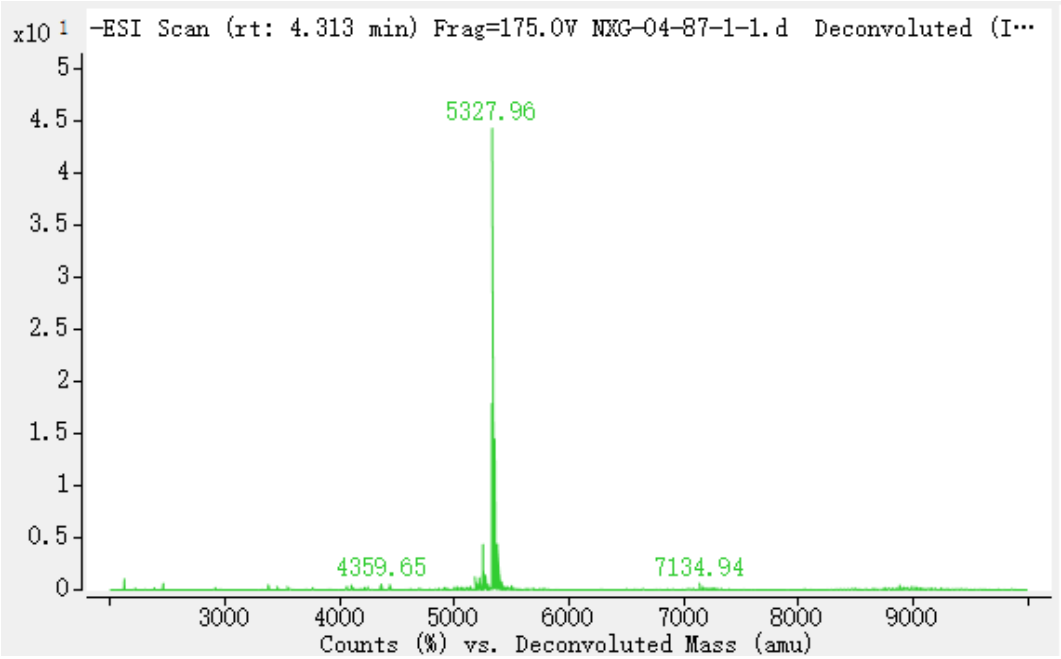
UHPLC chromatogram and deconvoluted MS of **d4**

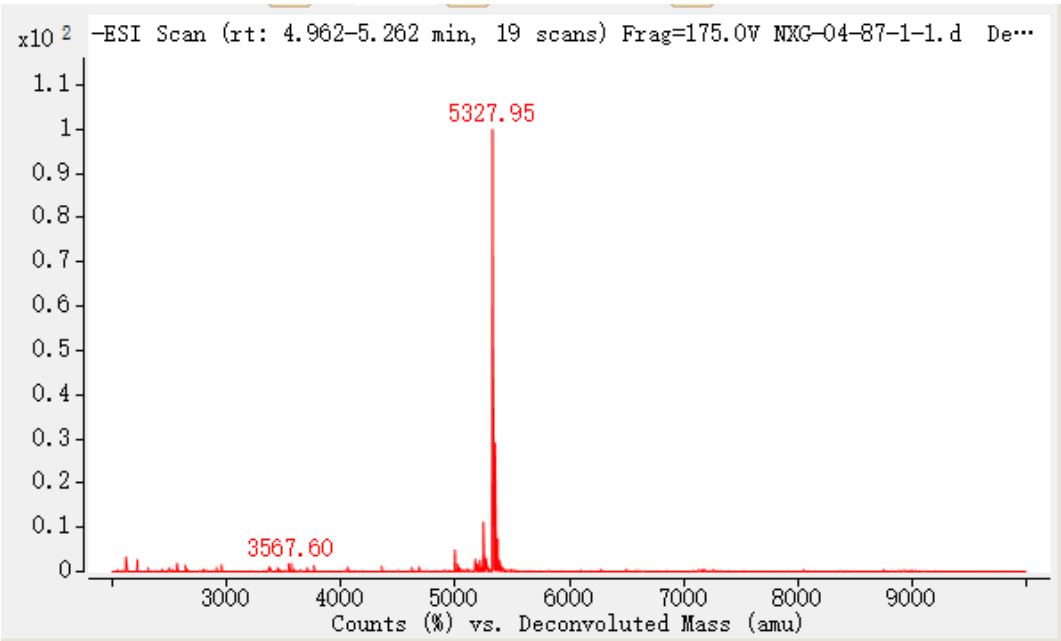
Conversion: >90%

Calculated Mass: 5328 Da; Observed Mass: 5328 Da





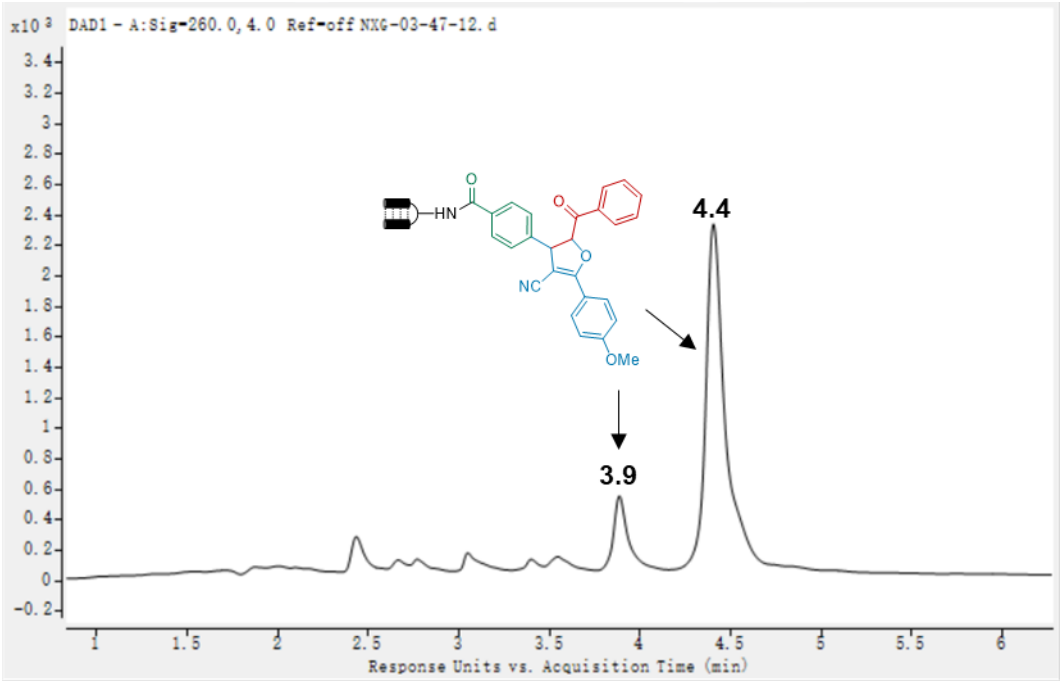

x10 1
-ESI Scan (rt: 4.313 min) Frag=175.0V NXG-04-87-1-1.d Deconvoluted (I…
5327.96
4359.65
7134.94
Counts (%) vs. Deconvoluted Mass (amu)

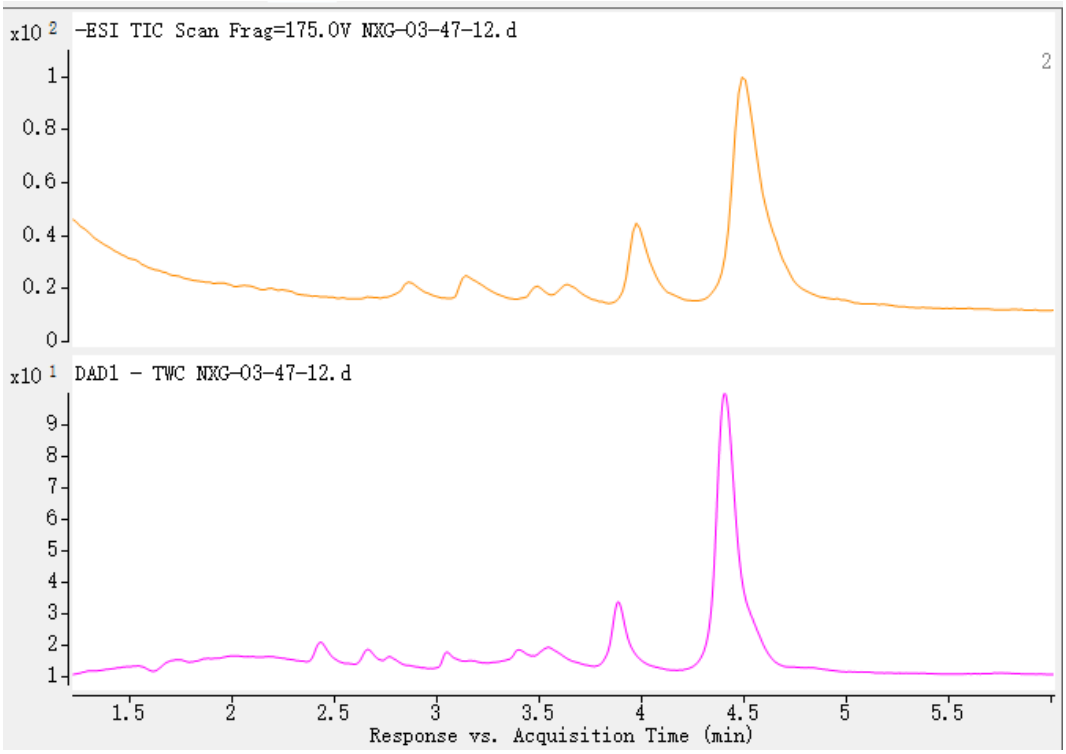

x10 2
-ESI Scan (rt: 4.962-5.262 min, 19 scans) Frag=175.0V NXG-04-87-1-1.d De…
5327.95
3567.60
Counts (%) vs. Deconvoluted Mass (amu)

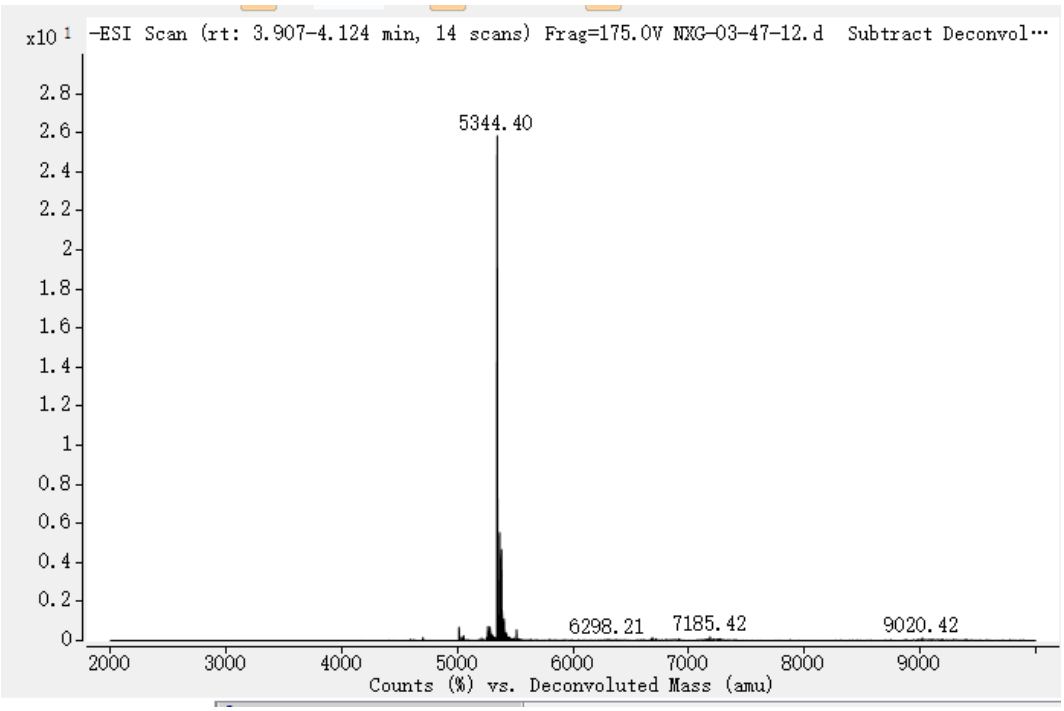
UHPLC chromatogram and deconvoluted MS of **d5**

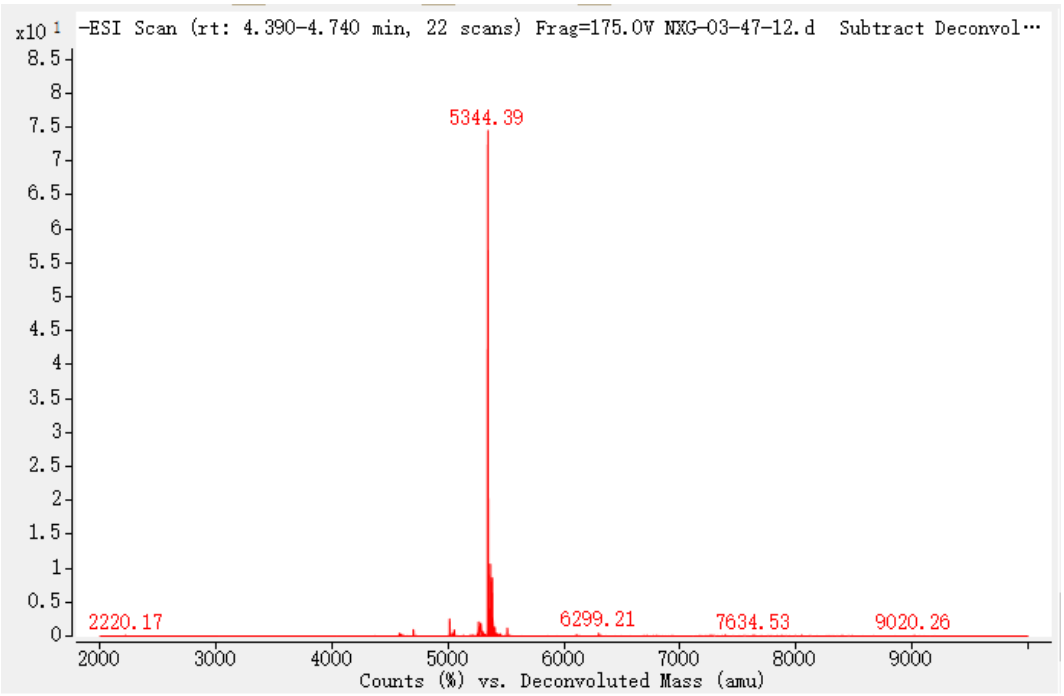
Conversion: >90%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da





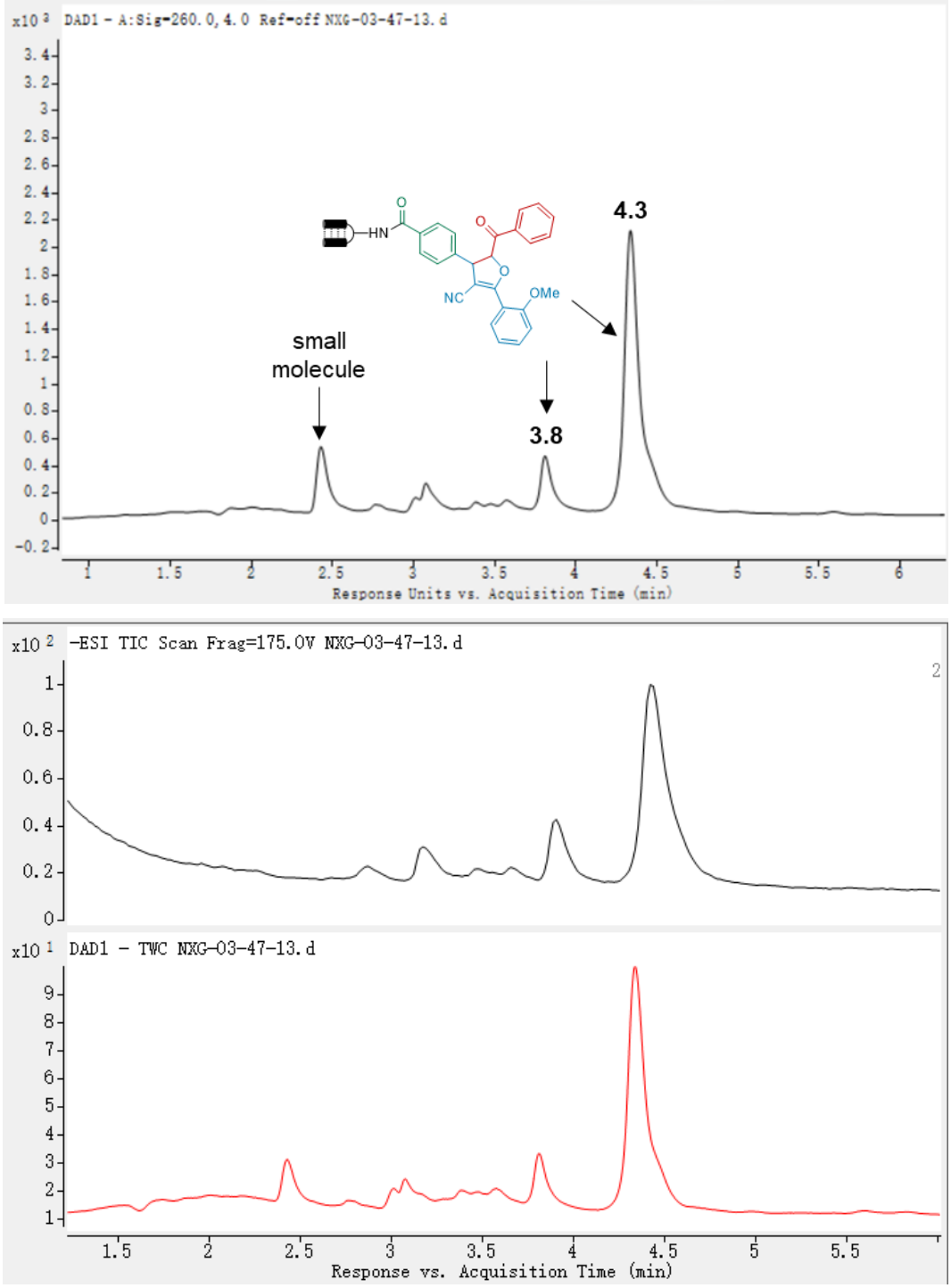

x10 1
-ESI Scan (rt: 3.907-4.124 min, 14 scans) Frag=175.0V NXG-03-47-12.d Subtract Deconvol…
5344.40
6298.21
7185.42
9020.42
Counts (%) vs. Deconvoluted Mass (amu)

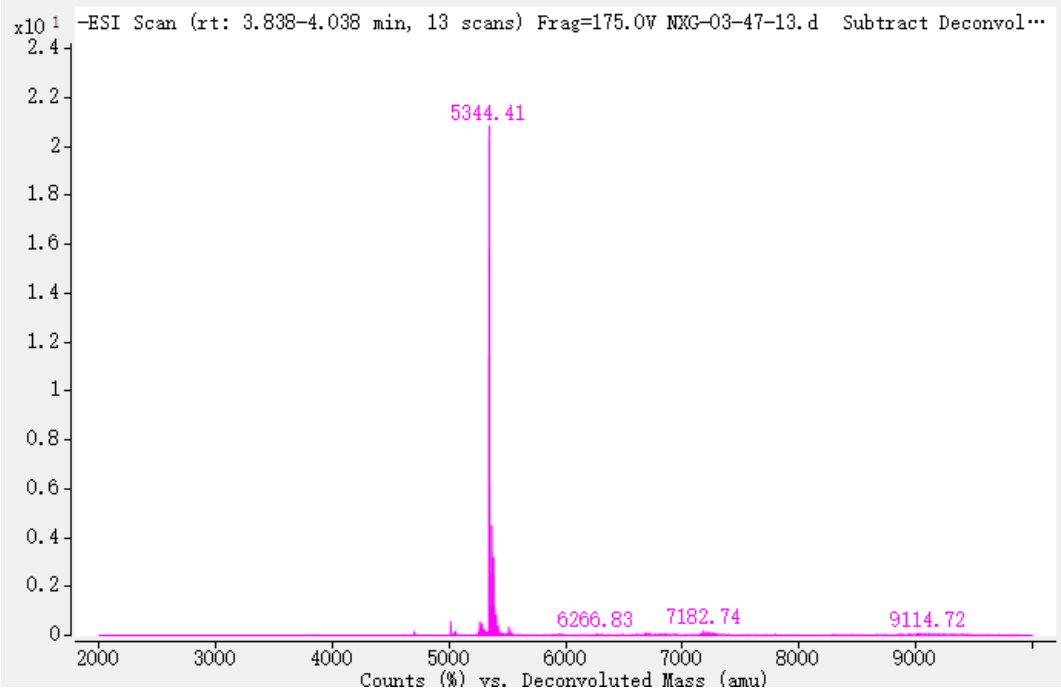

x10 1
-ESI Scan (rt: 4.390-4.740 min, 22 scans) Frag=175.0V NXG-03-47-12.d Subtract Deconvol…
5344.39
2220.17
6299.21
7634.53
9020.26
Counts (%) vs. Deconvoluted Mass (amu)

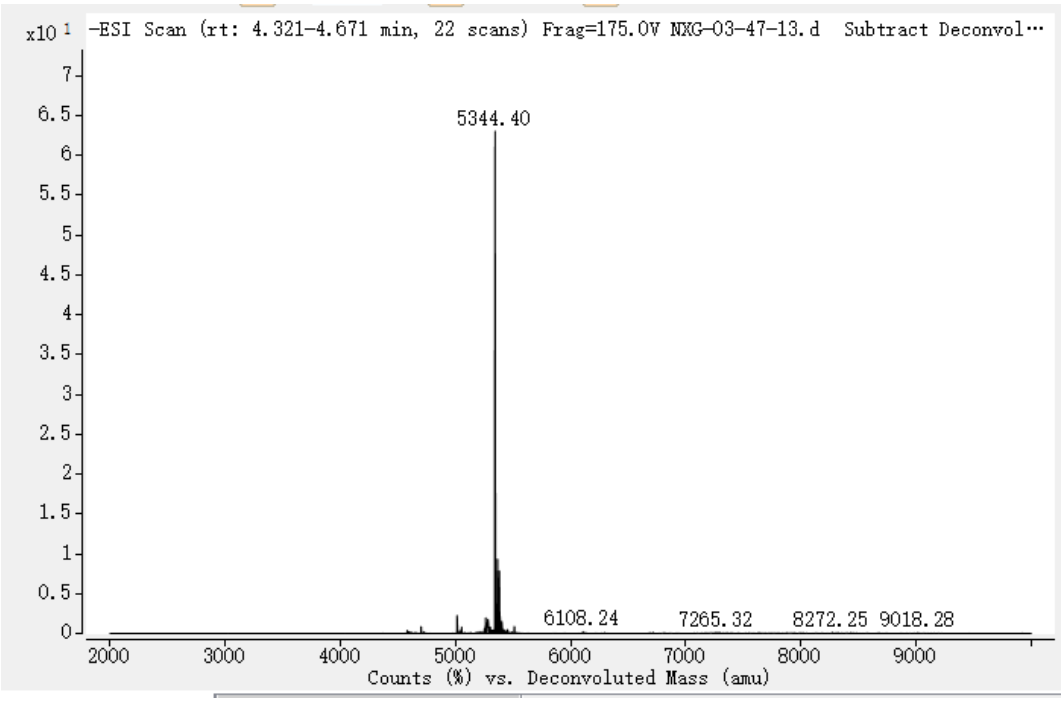
UHPLC chromatogram and deconvoluted MS of **d6**

Conversion: >90%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da



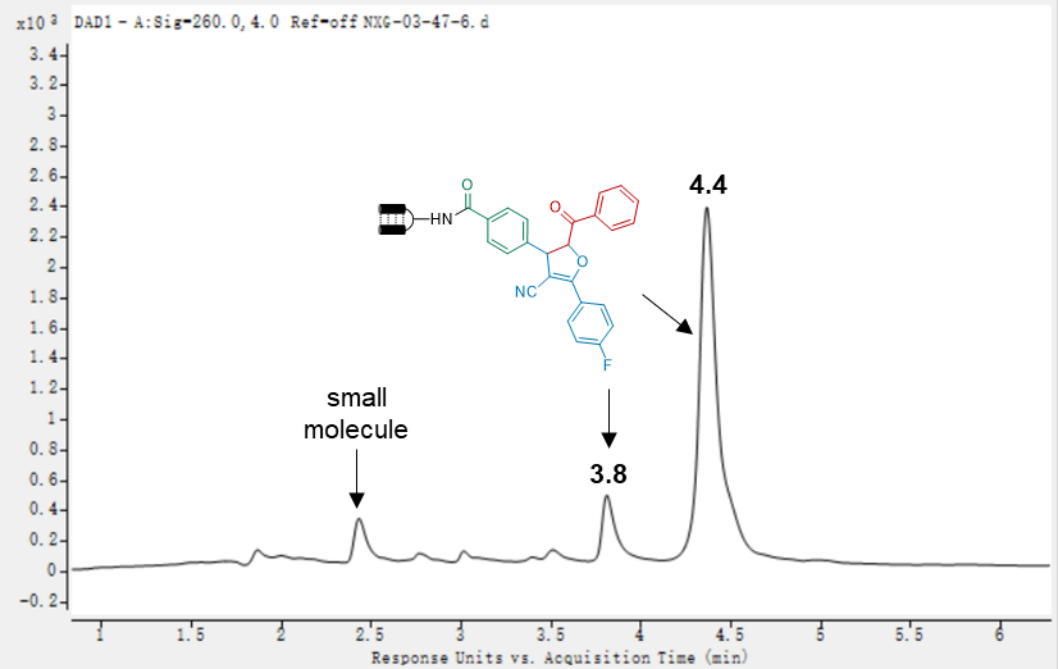

-ESI Scan (rt: 3.838-4.038 min, 13 scans) Frag=175.0V NXG-03-47-13.d Subtract Deconvol…
x10 1
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5344.41
6266.83
7182.74
9114.72
2000
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

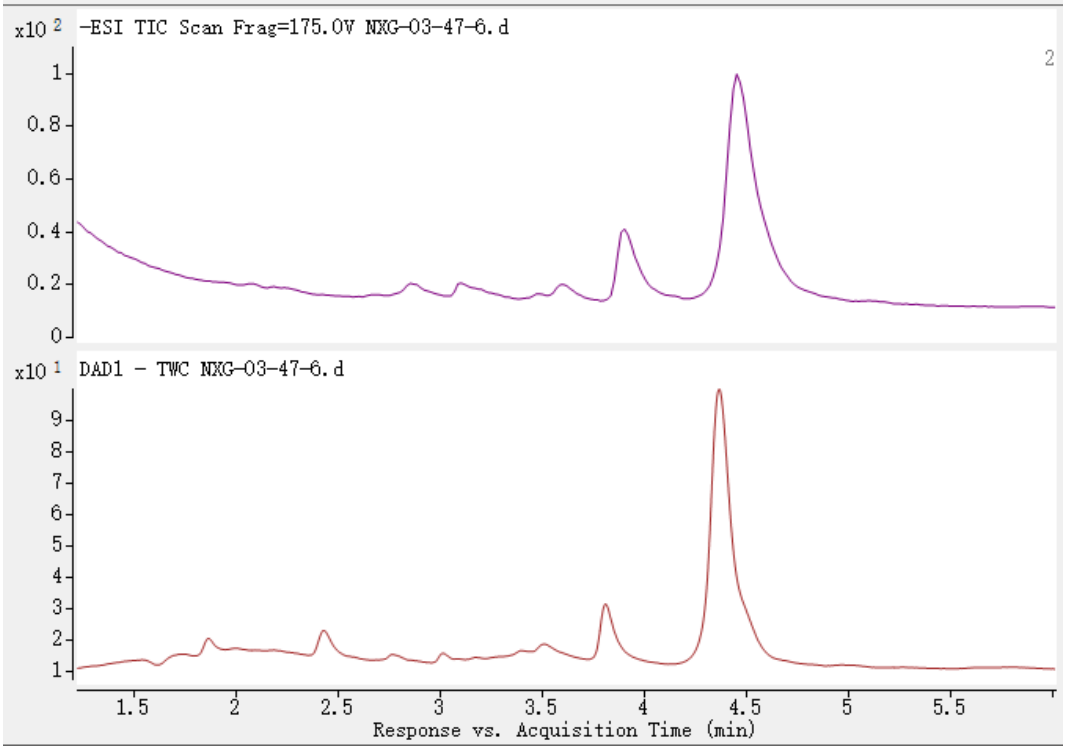

-ESI Scan (rt: 4.321-4.671 min, 22 scans) Frag=175.0V NXG-03-47-13.d Subtract Deconvol…
x10 1
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5344.40
6108.24
7265.32
8272.25
9018.28
2000
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

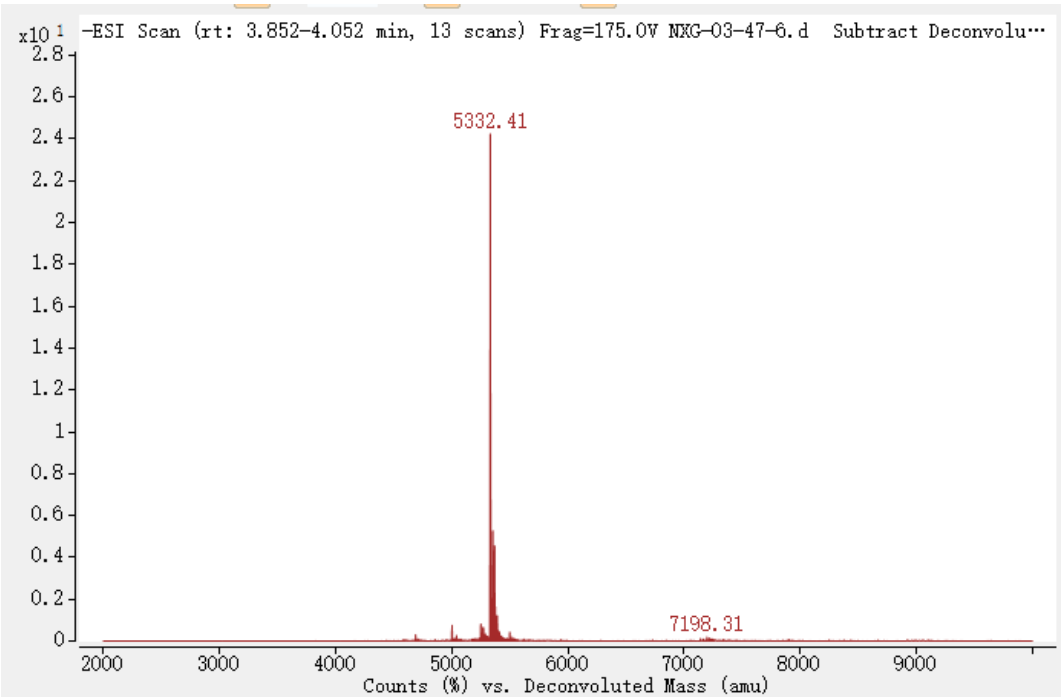
UHPLC chromatogram and deconvoluted MS of **d7**

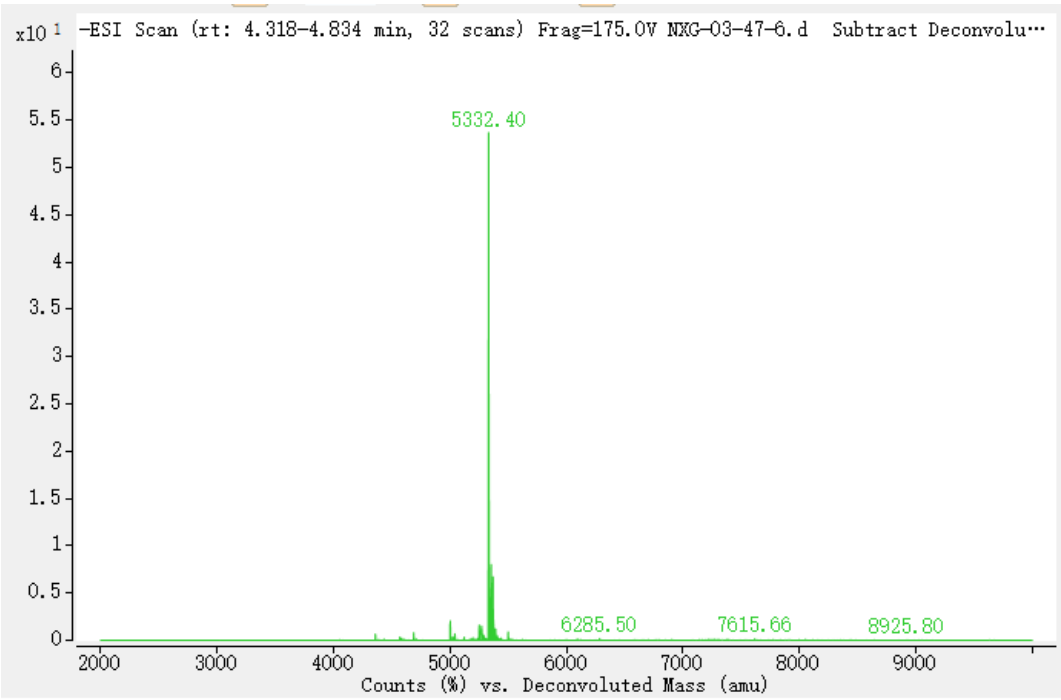
Conversion: >90%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da





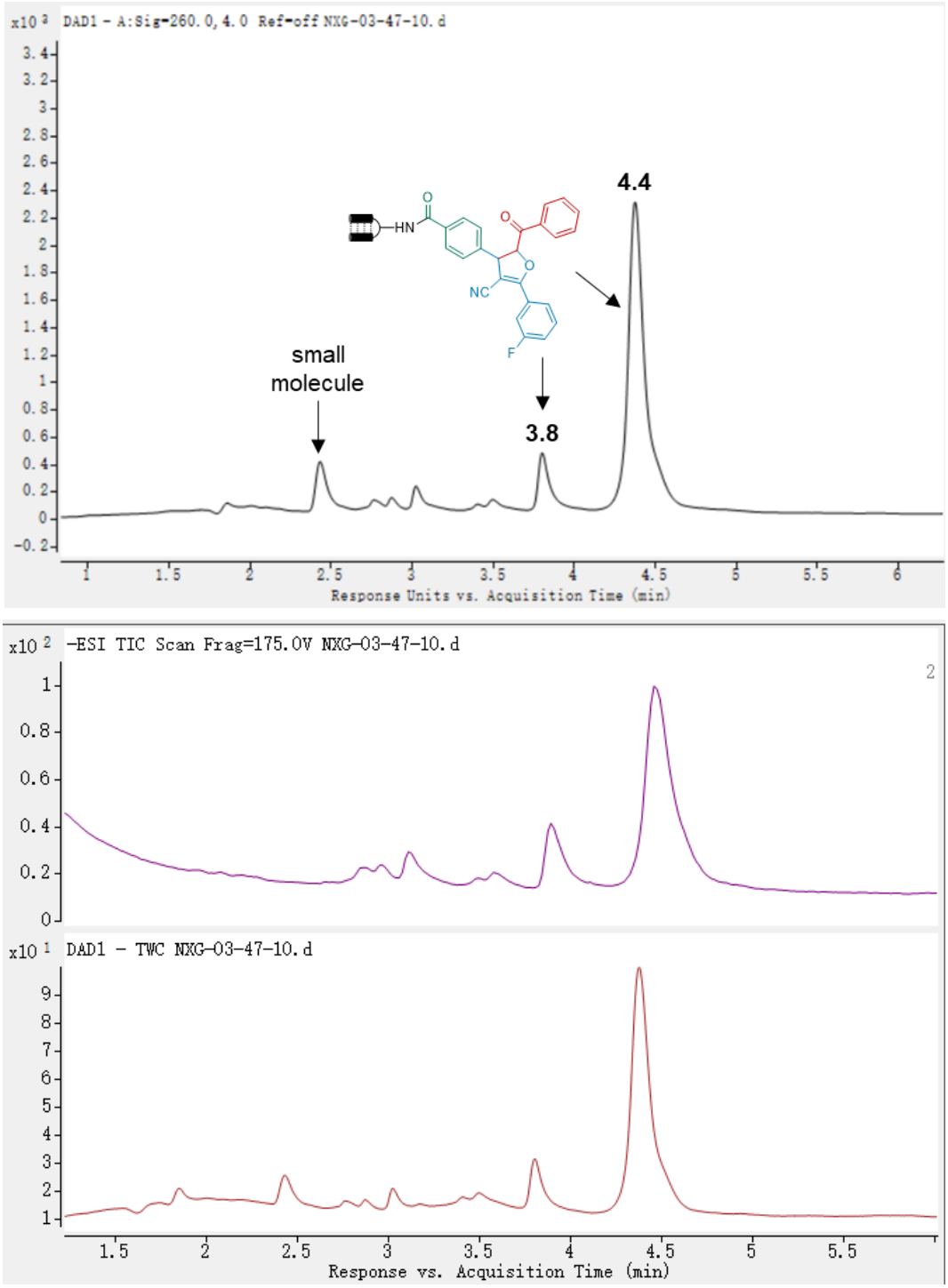

-ESI Scan (rt: 3.852-4.052 min, 13 scans) Frag=175.0V NXG-03-47-6.d Subtract Deconvolu…
x10 1
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5332.41
7198.31
2000
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

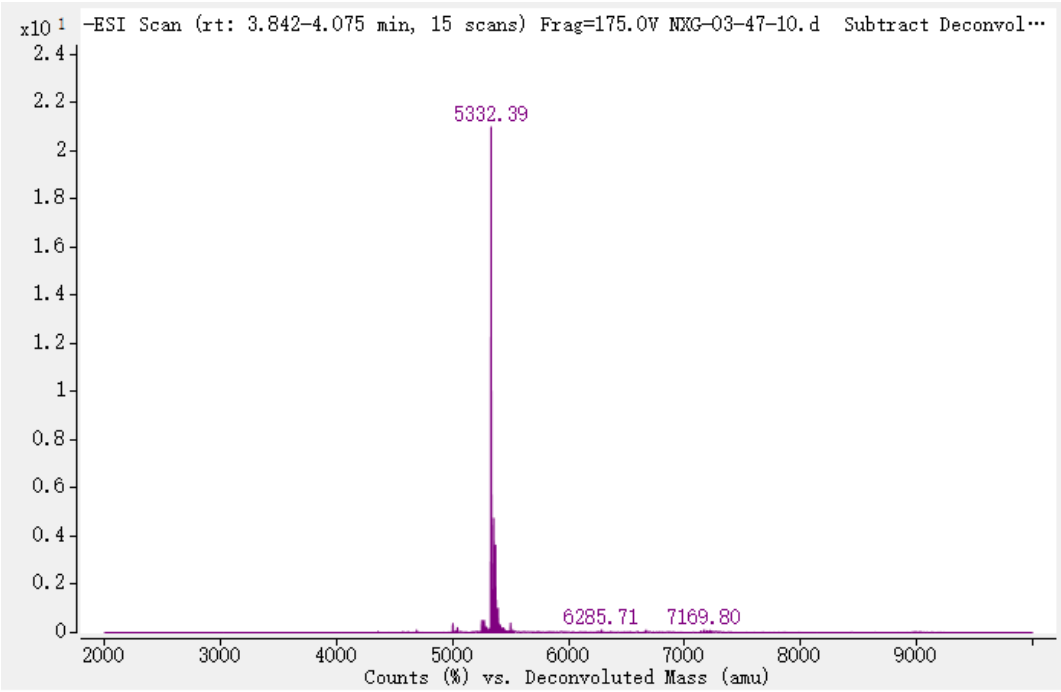

-ESI Scan (rt: 4.318-4.834 min, 32 scans) Frag=175.0V NXG-03-47-6.d Subtract Deconvolu…
x10 1
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5332.40
6285.50
7615.66
8925.80
2000
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

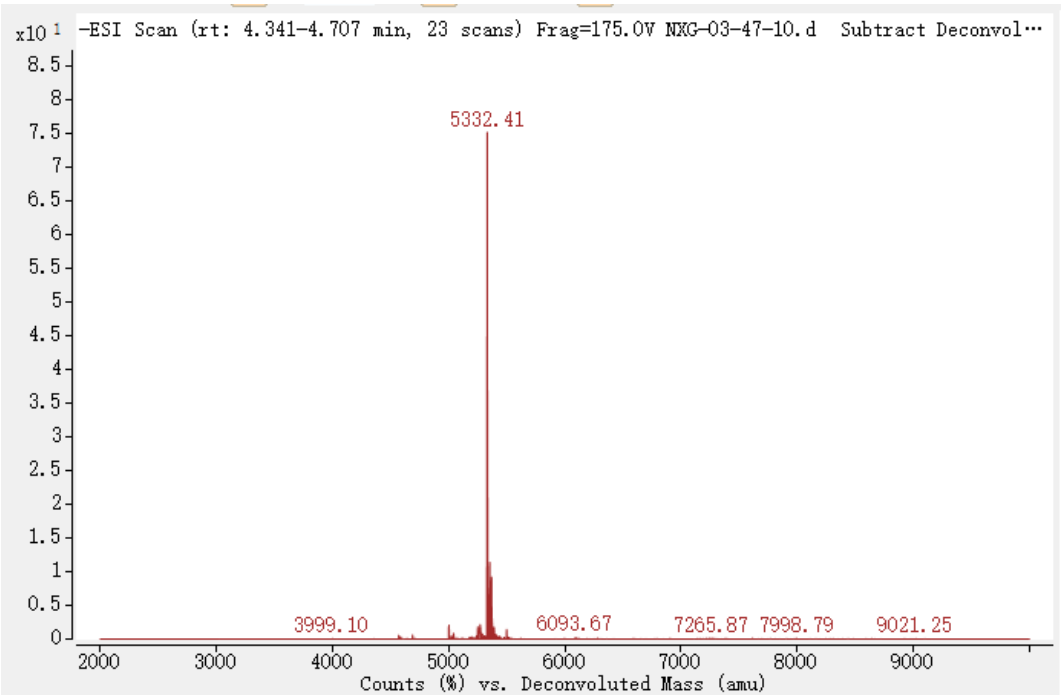
UHPLC chromatogram and deconvoluted MS of **d8**

Conversion: >90%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da



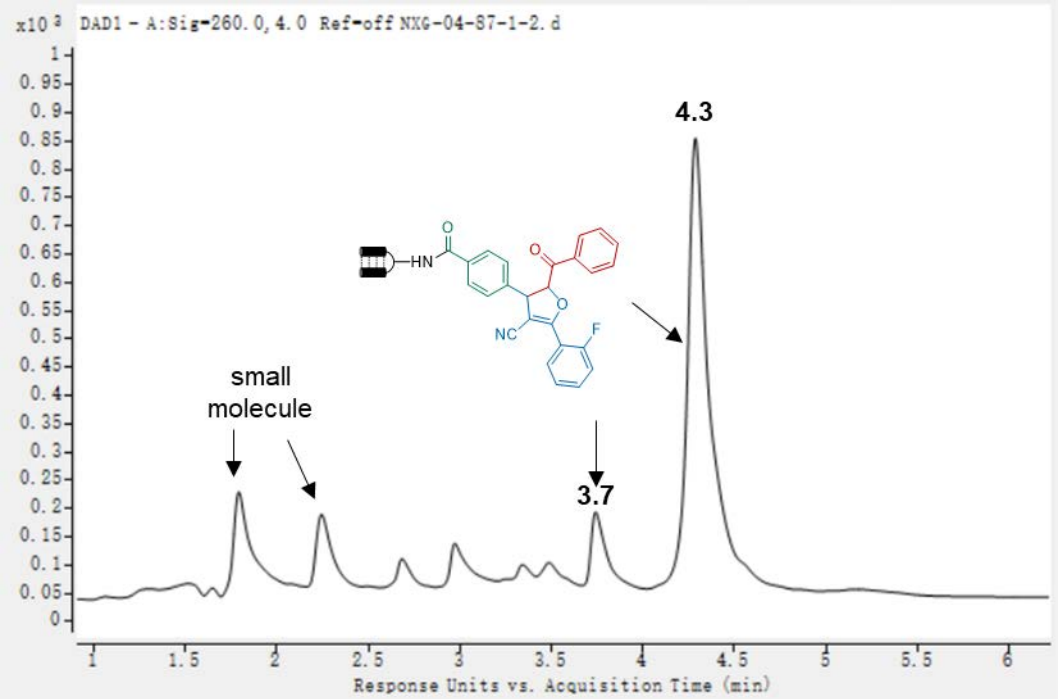

-ESI Scan (rt: 3.842-4.075 min, 15 scans) Frag=175.0V NXG-03-47-10.d Subtract Deconvol…
x10 1
5332.39
6285.71
7169.80
Counts (%) vs. Deconvoluted Mass (amu)

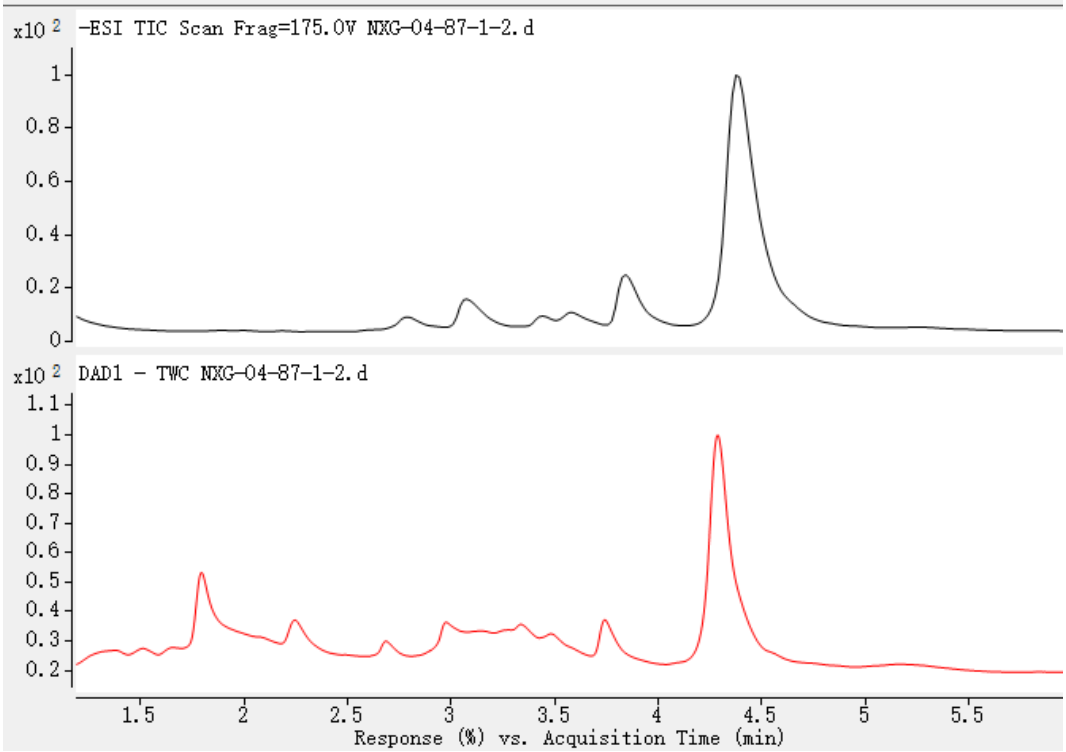

-ESI Scan (rt: 4.341-4.707 min, 23 scans) Frag=175.0V NXG-03-47-10.d Subtract Deconvol…
x10 1
5332.41
3999.10
6093.67
7265.87
7998.79
9021.25
Counts (%) vs. Deconvoluted Mass (amu)

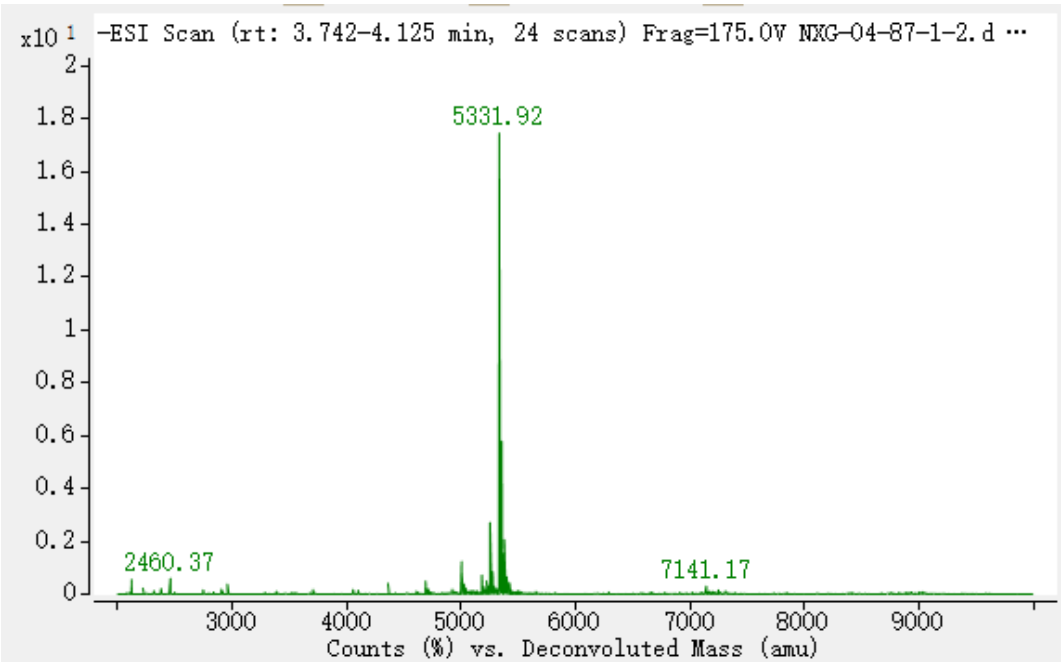
UHPLC chromatogram and deconvoluted MS of **d9**

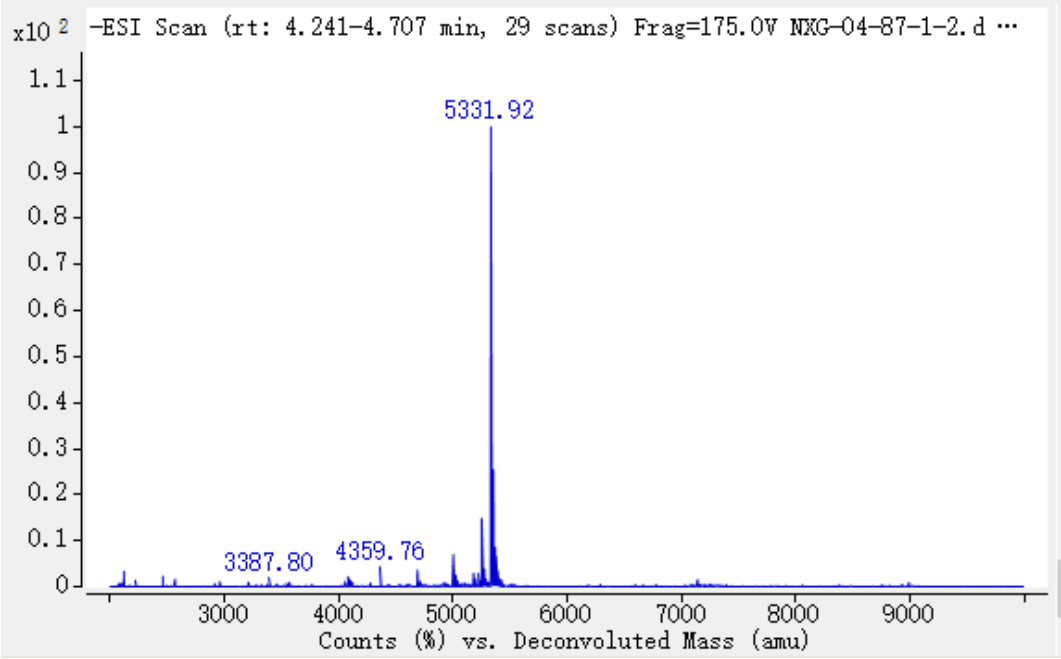
Conversion: 78%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da





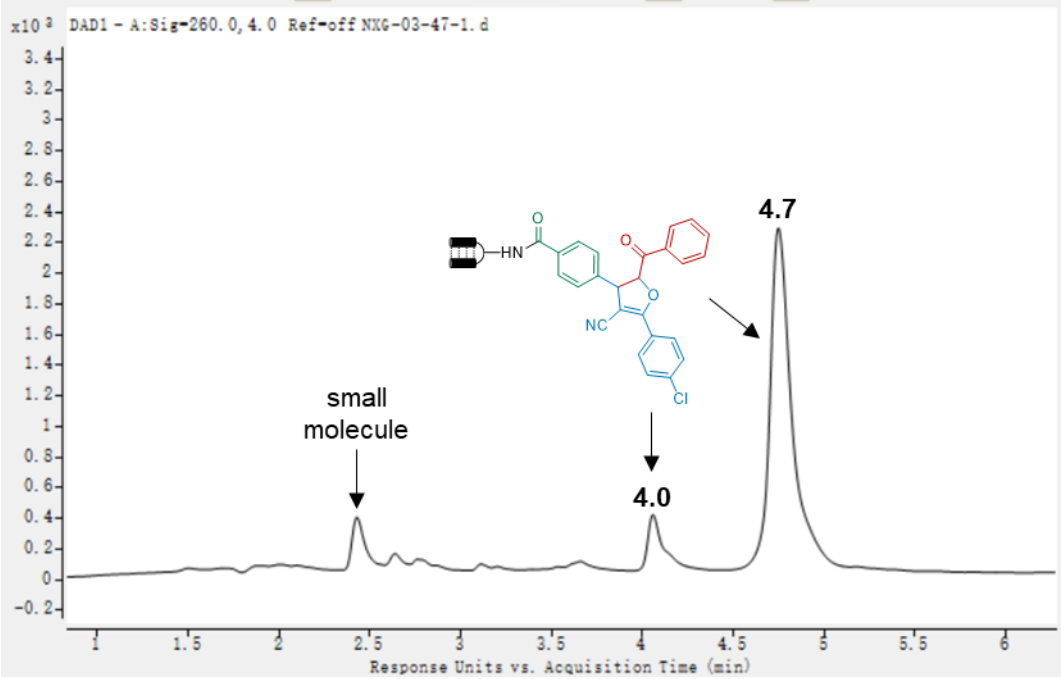

x10 1
-ESI Scan (rt: 3.742-4.125 min, 24 scans) Frag=175.0V NXG-04-87-1-2.d ...
5331.92
2460.37
7141.17
Counts (%) vs. Deconvoluted Mass (amu)

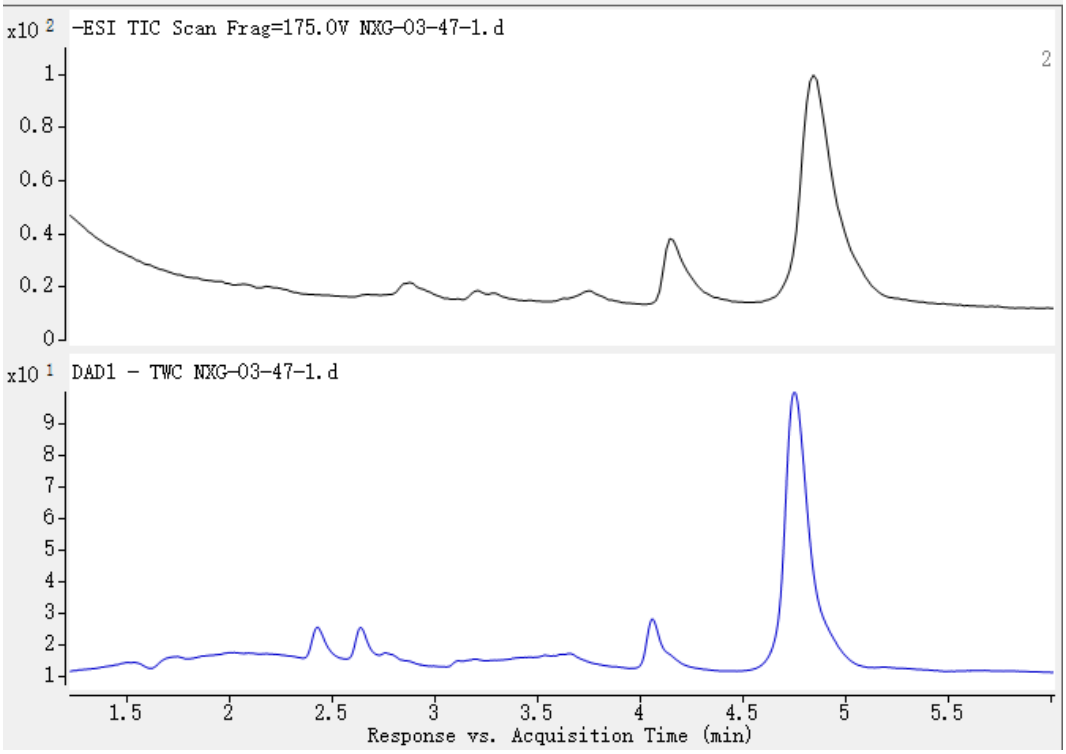

x10 2
-ESI Scan (rt: 4.241-4.707 min, 29 scans) Frag=175.0V NXG-04-87-1-2.d ...
5331.92
3387.80
4359.76
Counts (%) vs. Deconvoluted Mass (amu)

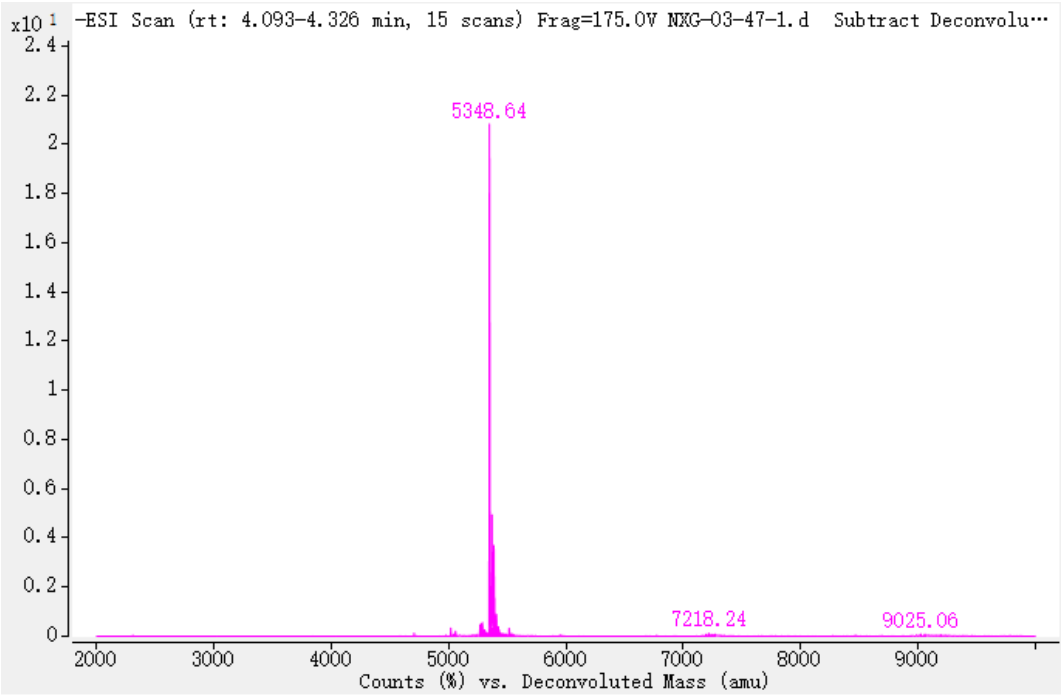
UHPLC chromatogram and deconvoluted MS of **d10**

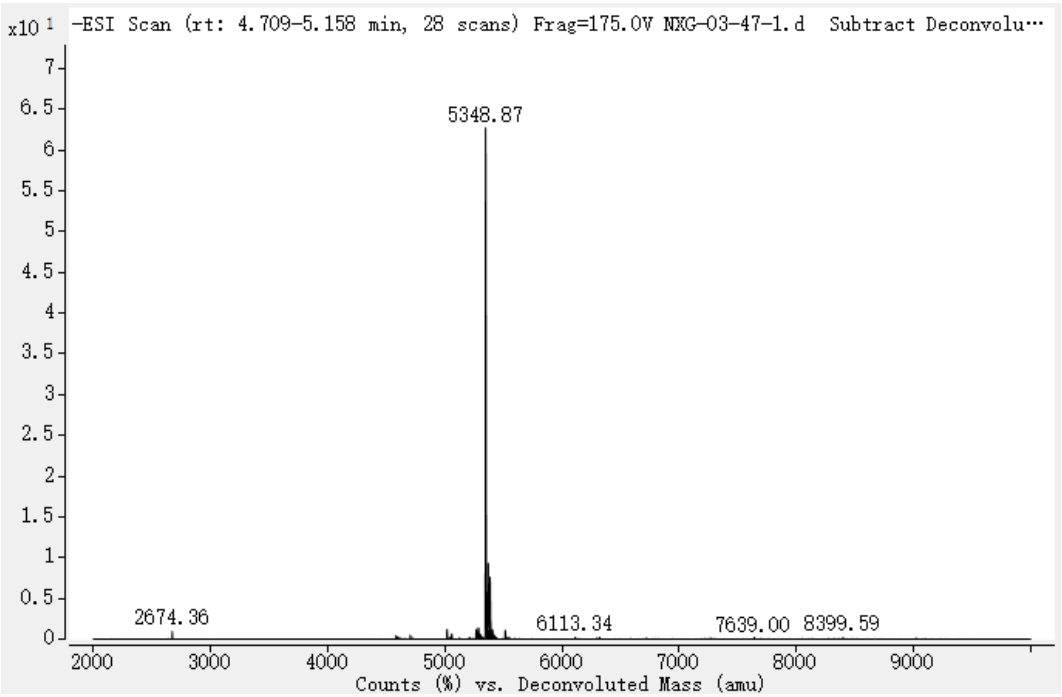
Conversion: >90%

Calculated Mass: 5348 Da; Observed Mass: 5348 Da





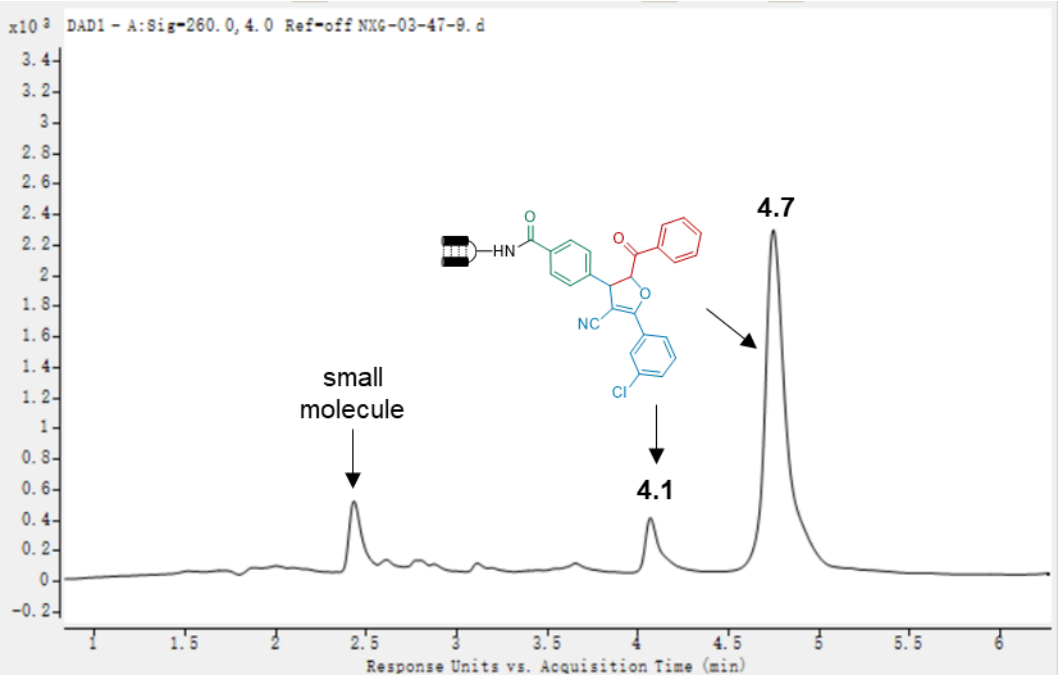

-ESI Scan (rt: 4.093-4.326 min, 15 scans) Frag=175.0V NXG-03-47-1.d Subtract Deconvolu…
x10 1
5348.64
7218.24
9025.06
Counts (%) vs. Deconvoluted Mass (amu)

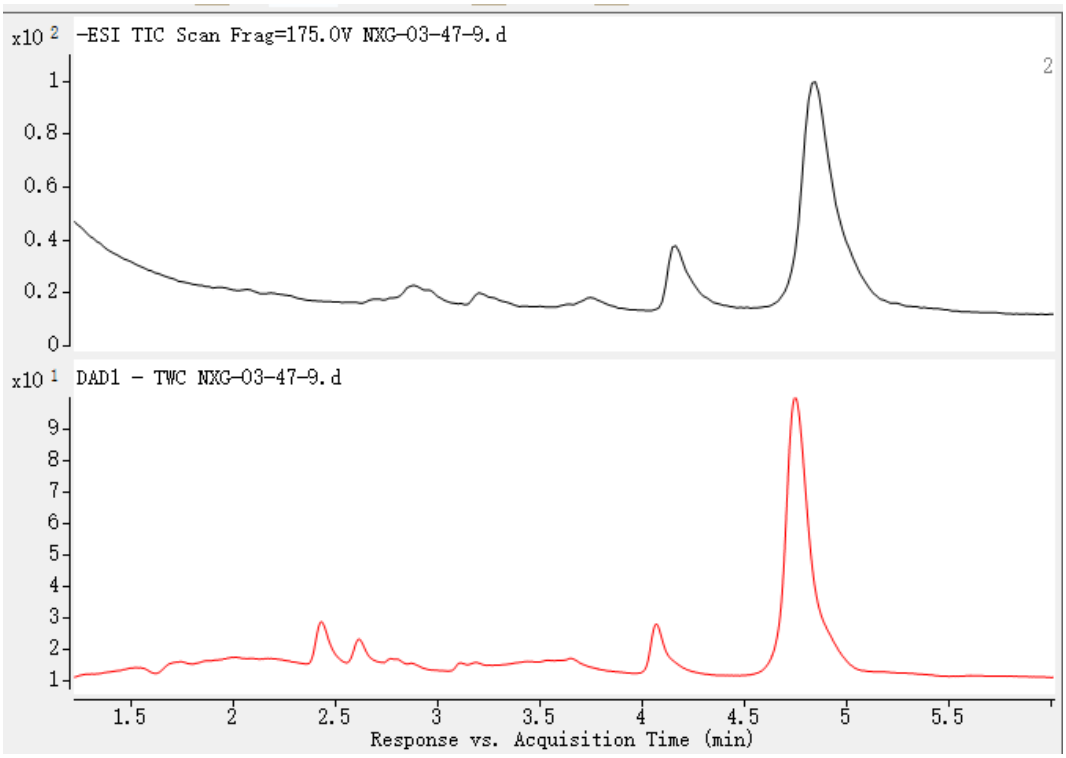

-ESI Scan (rt: 4.709-5.158 min, 28 scans) Frag=175.0V NXG-03-47-1.d Subtract Deconvolu…
x10 1
5348.87
2674.36
6113.34
7639.00
8399.59
Counts (%) vs. Deconvoluted Mass (amu)

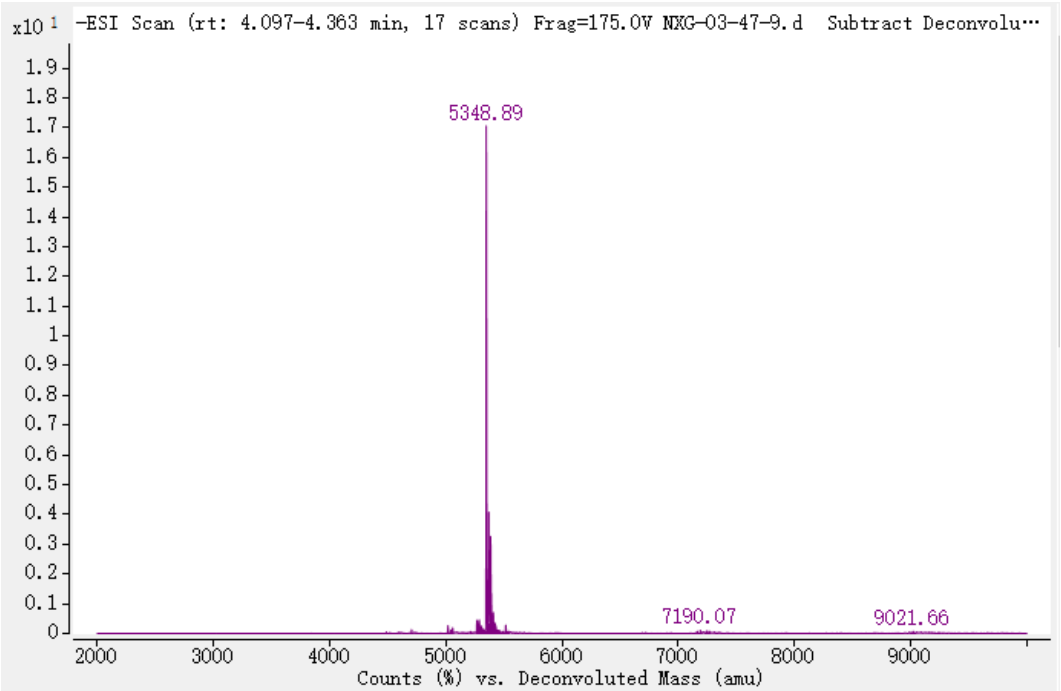
UHPLC chromatogram and deconvoluted MS of **d11**

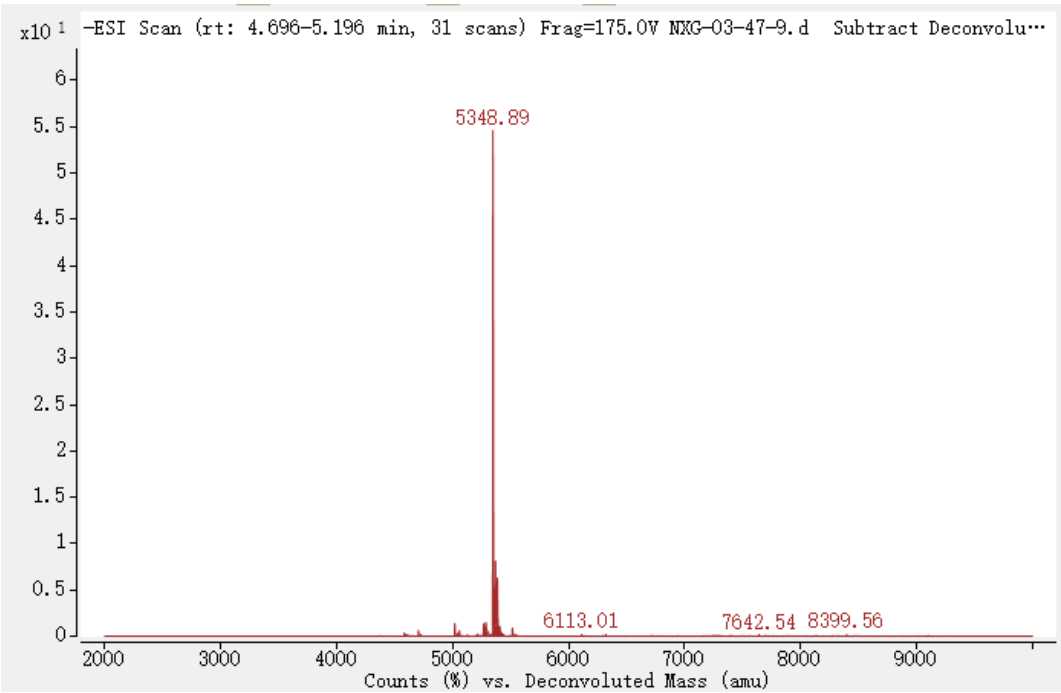
Conversion: >90%

Calculated Mass: 5348 Da; Observed Mass: 5348 Da





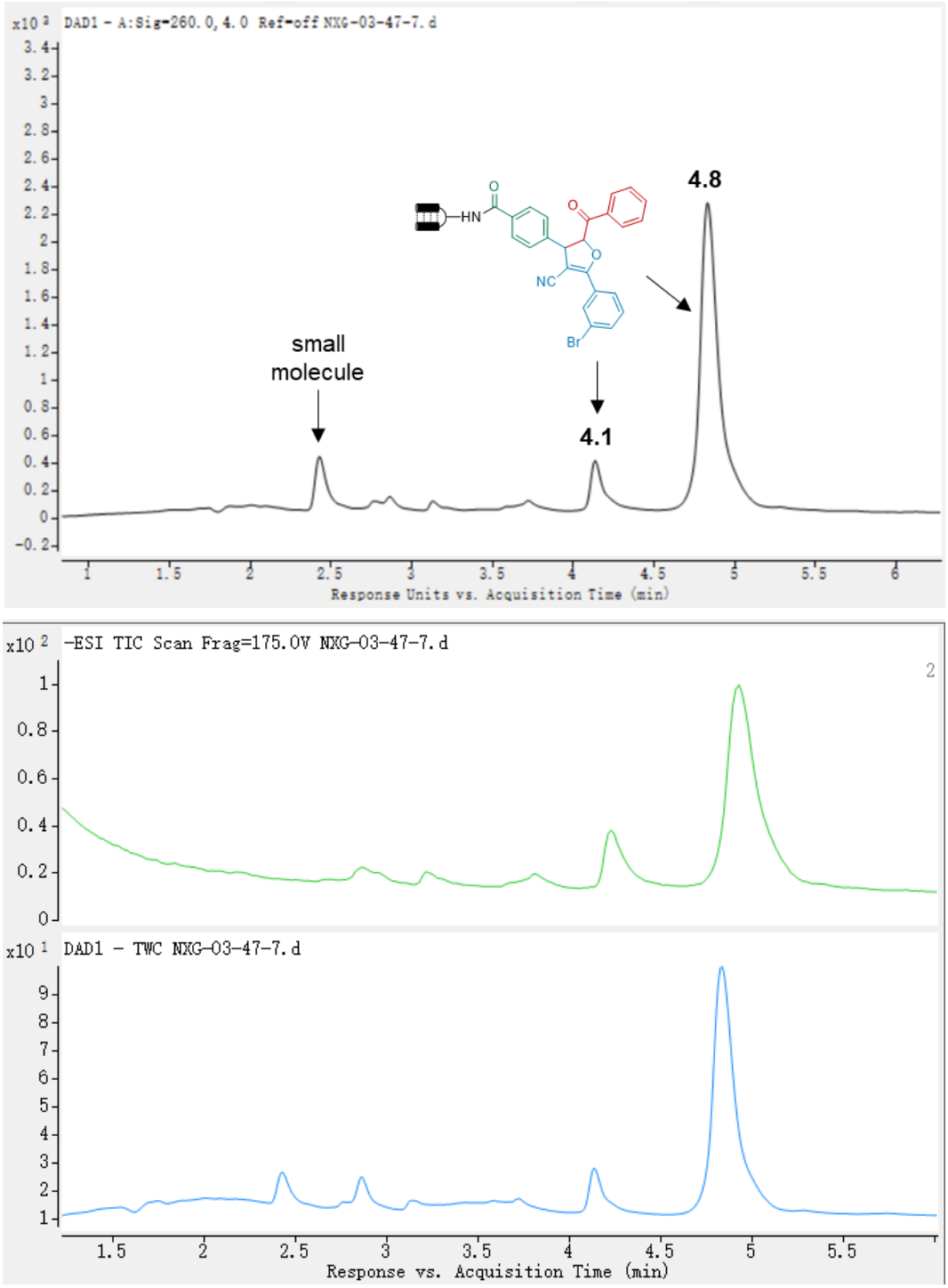

x10 1
-ESI Scan (rt: 4.097-4.363 min, 17 scans) Frag=175.0V NXG-03-47-9.d Subtract Deconvolu…
5348.89
7190.07
9021.66
Counts (%) vs. Deconvoluted Mass (amu)

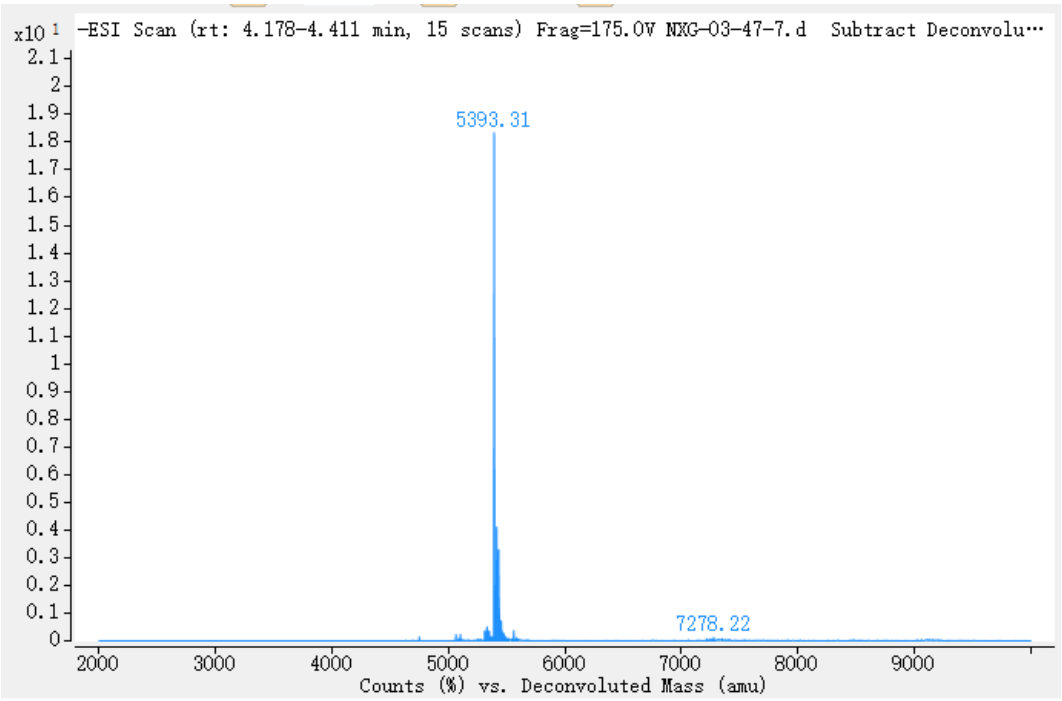

x10 1
-ESI Scan (rt: 4.696-5.196 min, 31 scans) Frag=175.0V NXG-03-47-9.d Subtract Deconvolu…
5348.89
6113.01
7642.54
8399.56
Counts (%) vs. Deconvoluted Mass (amu)

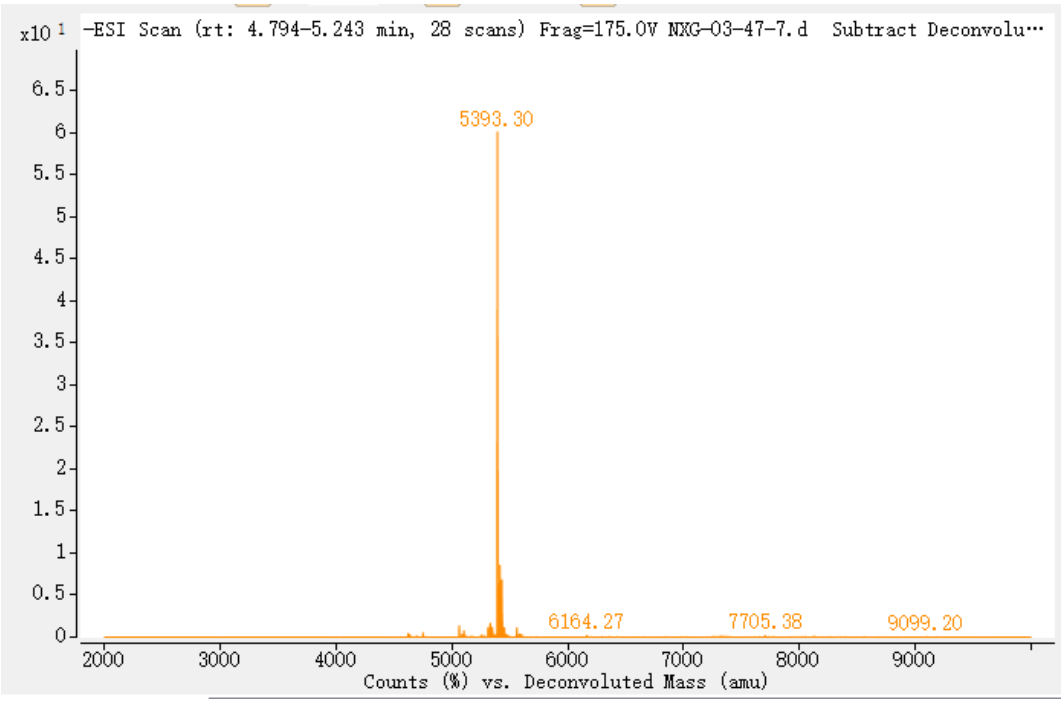
UHPLC chromatogram and deconvoluted MS of **d12**

Conversion: >90%

Calculated Mass: 5393 Da; Observed Mass: 5393 Da



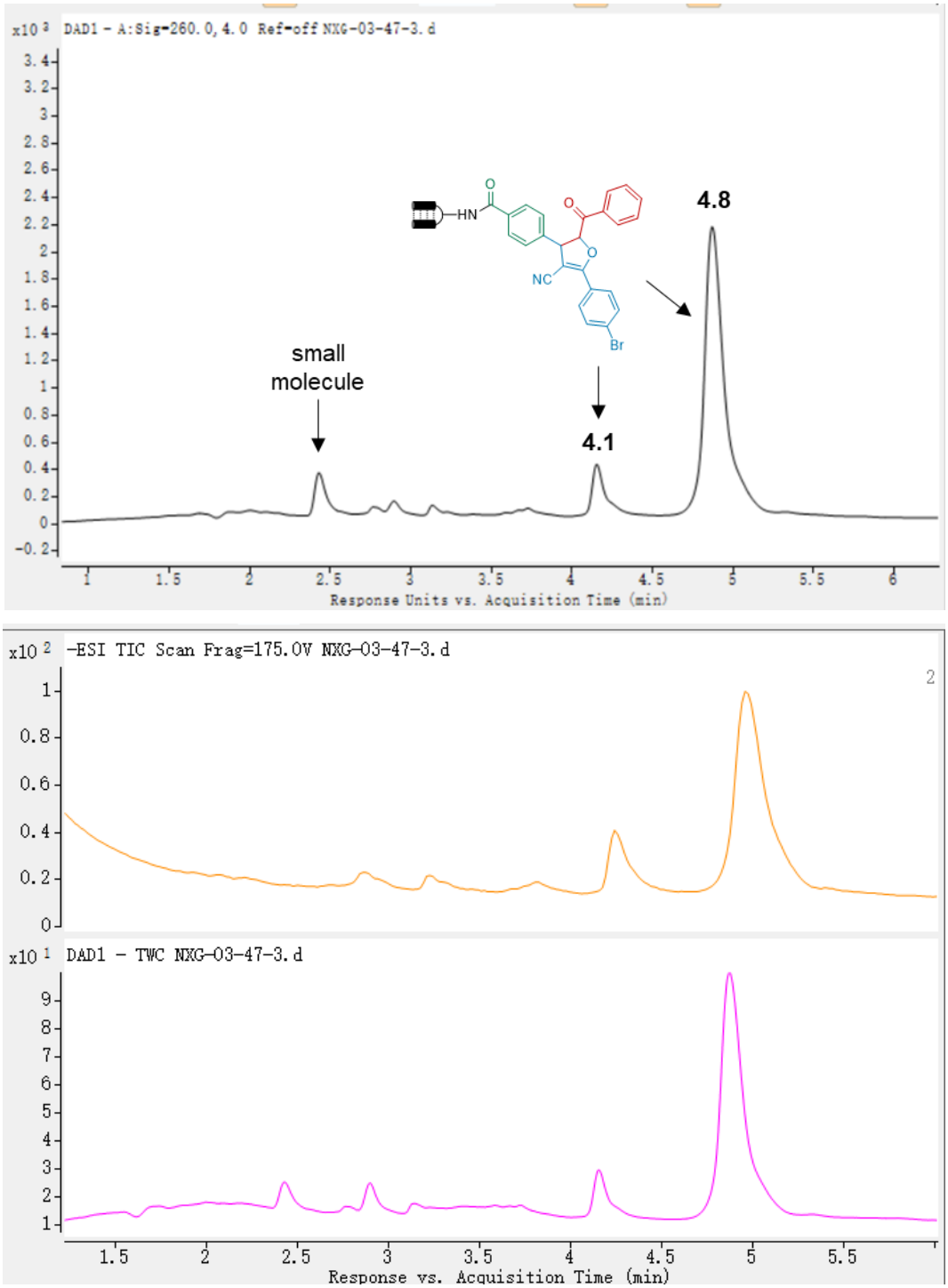

x10 1
-ESI Scan (rt: 4.178-4.411 min, 15 scans) Frag=175.0V NXG-03-47-7.d Subtract Deconvolu…
5393.31
7278.22
Counts (%) vs. Deconvoluted Mass (amu)

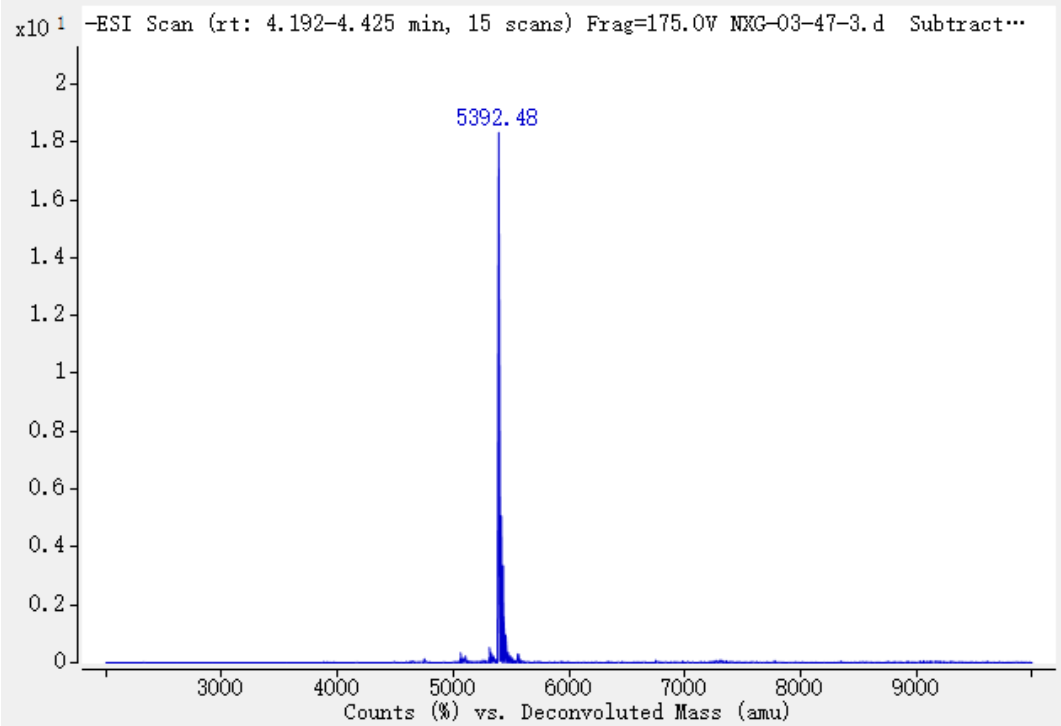

x10 1
-ESI Scan (rt: 4.794-5.243 min, 28 scans) Frag=175.0V NXG-03-47-7.d Subtract Deconvolu…
5393.30
6164.27
7705.38
9099.20
Counts (%) vs. Deconvoluted Mass (amu)

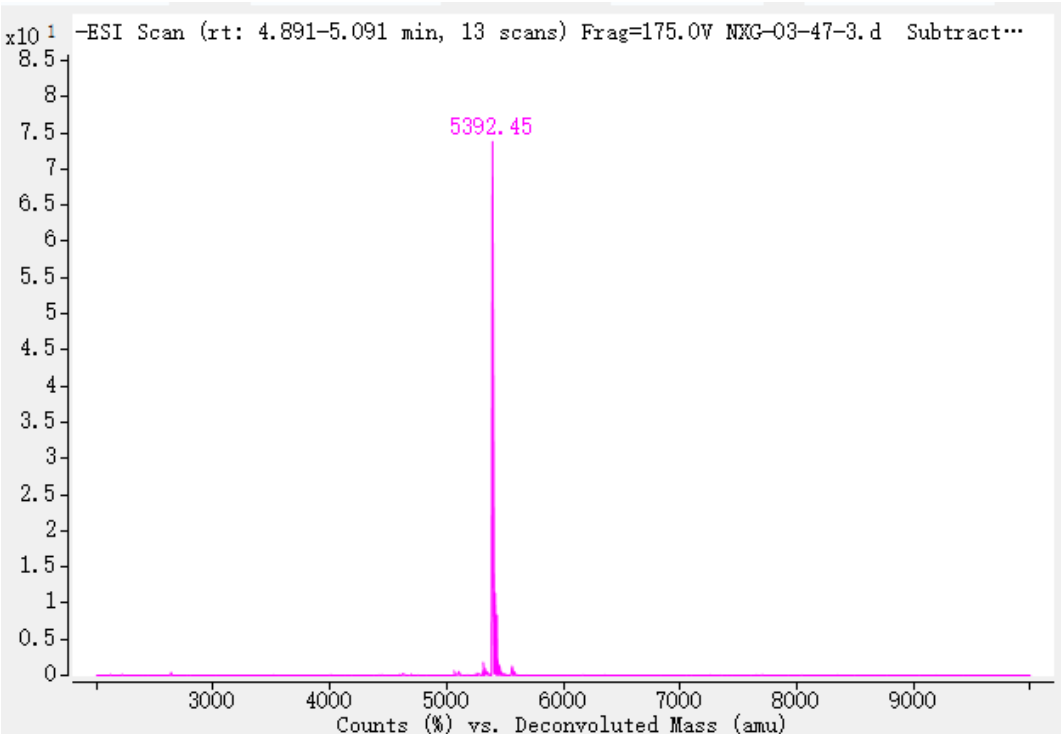
UHPLC chromatogram and deconvoluted MS of **d13**

Conversion: >90%

Calculated Mass: 5393 Da; Observed Mass: 5392 Da



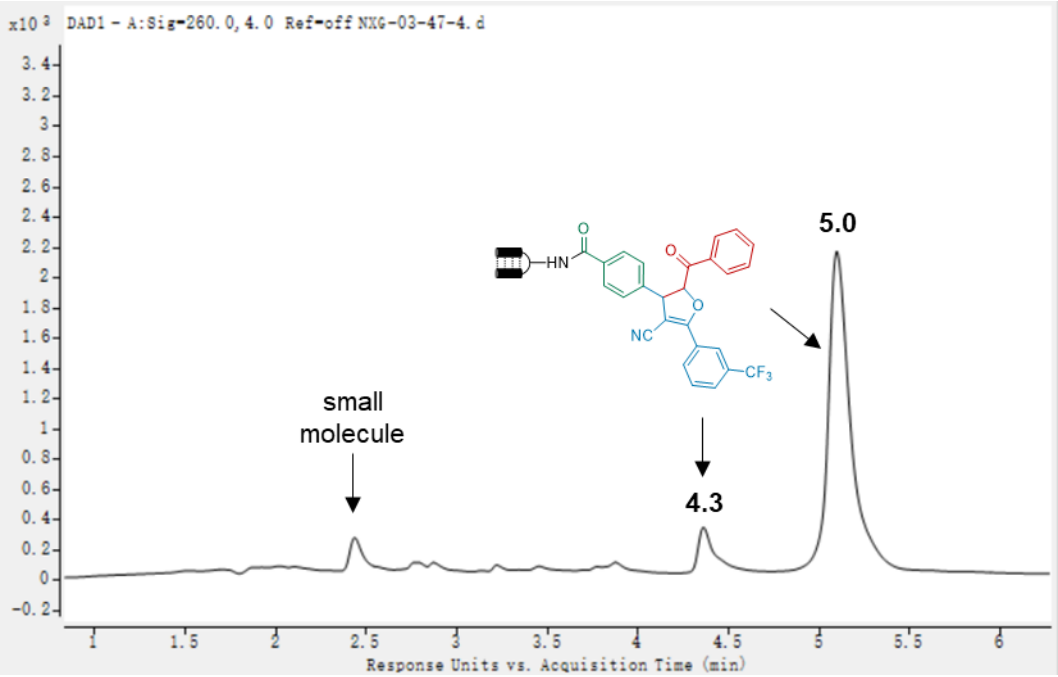

x10 1
-ESI Scan (rt: 4.192-4.425 min, 15 scans) Frag=175.0V NXG-03-47-3.d Subtract…
5392.48
Counts (%) vs. Deconvoluted Mass (amu)

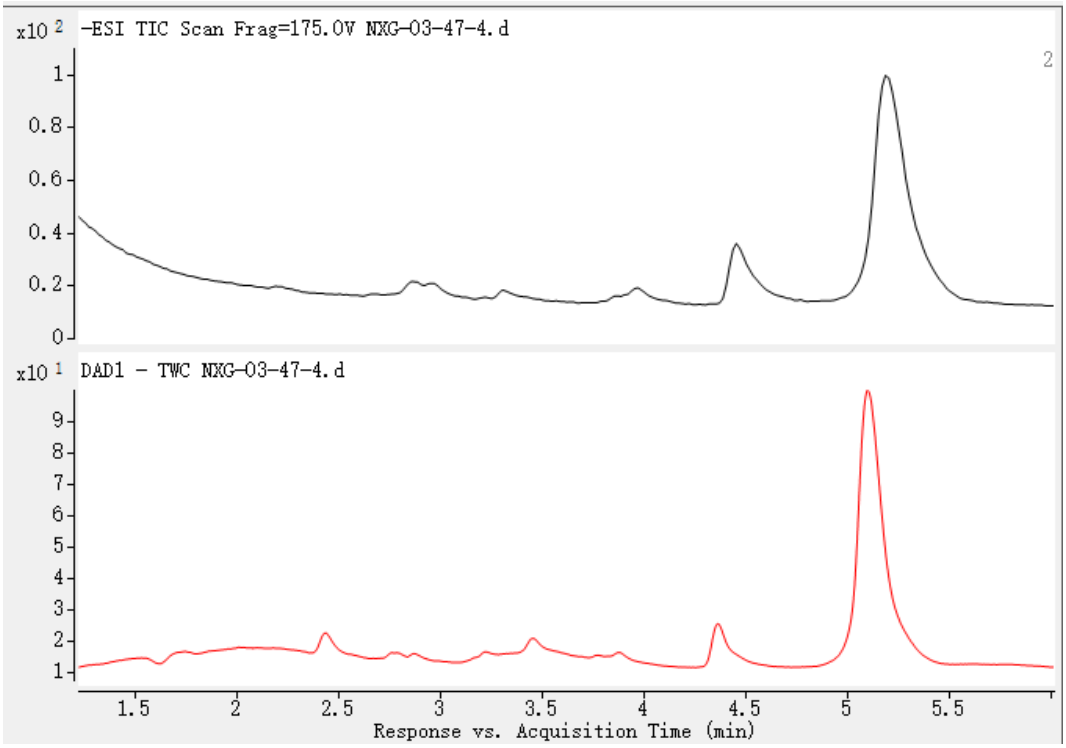

x10 1
-ESI Scan (rt: 4.891-5.091 min, 13 scans) Frag=175.0V NXG-03-47-3.d Subtract…
5392.45
Counts (%) vs. Deconvoluted Mass (amu)

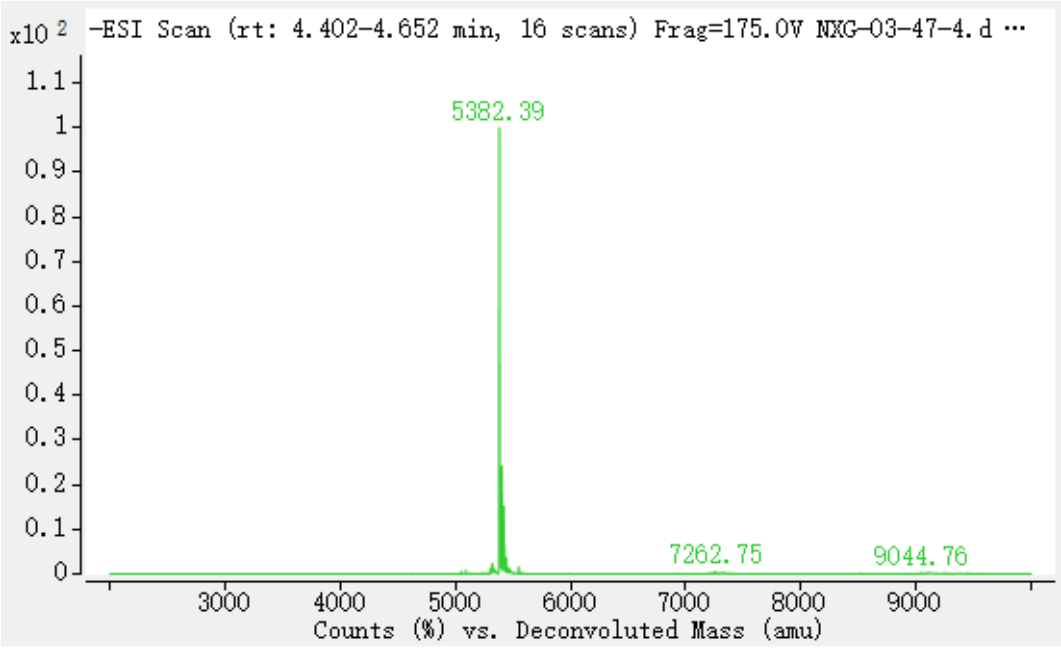
UHPLC chromatogram and deconvoluted MS of **d14**

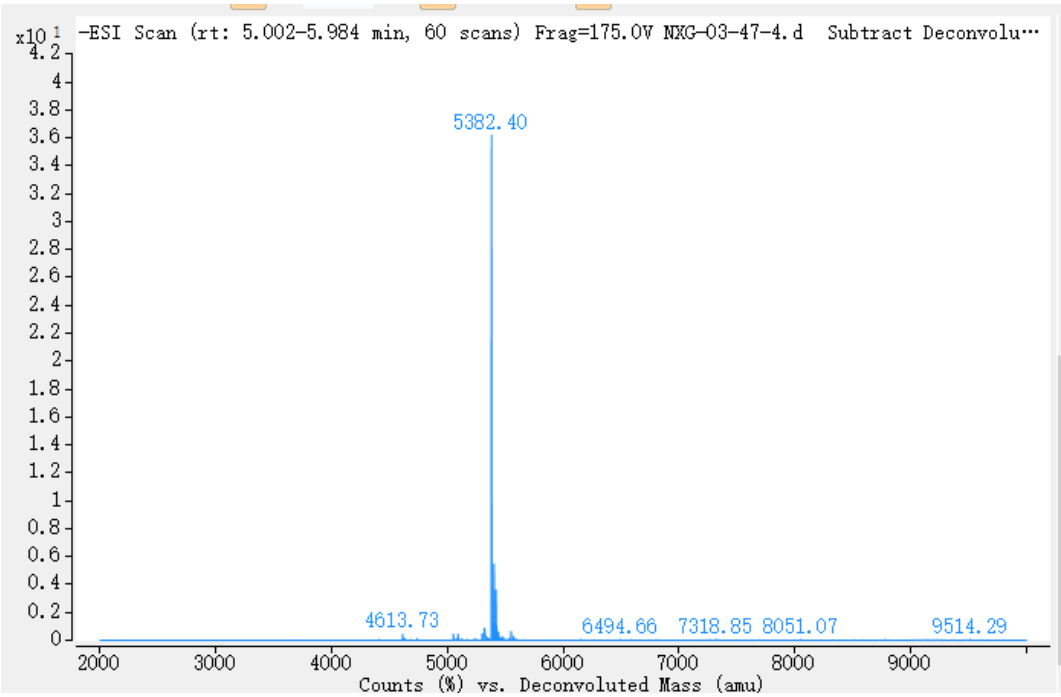
Conversion: >90%

Calculated Mass: 5382 Da; Observed Mass: 5382 Da





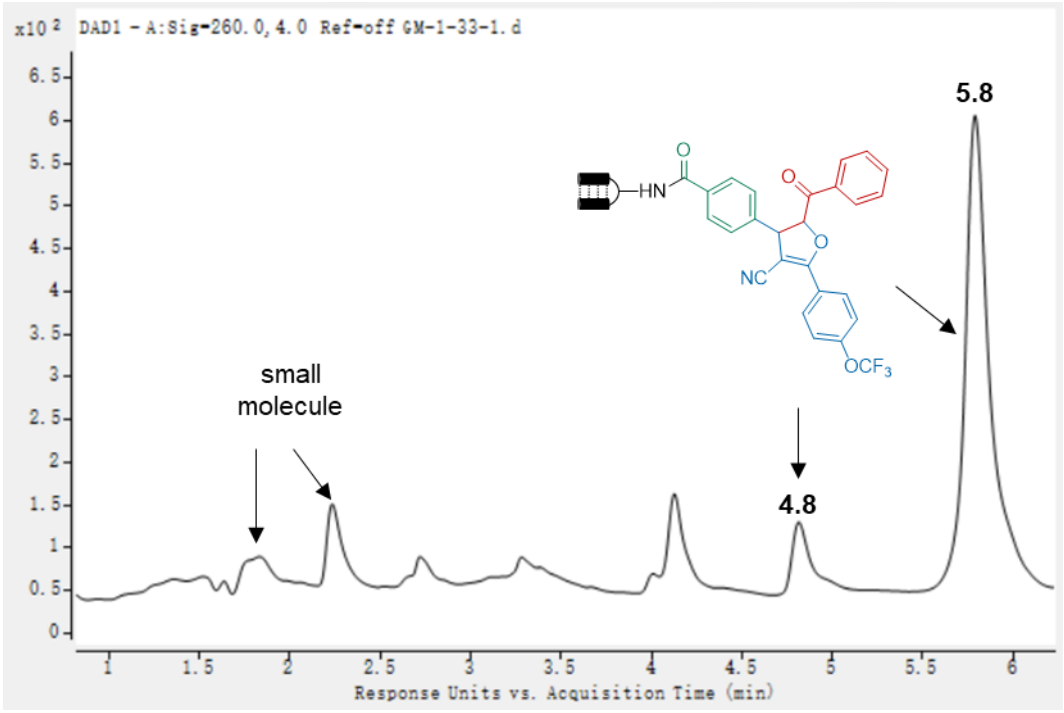

x10 2
-ESI Scan (rt: 4.402-4.652 min, 16 scans) Frag=175.0V NXG-03-47-4.d ...
5382.39
7262.75
9044.76
Counts (%) vs. Deconvoluted Mass (amu)

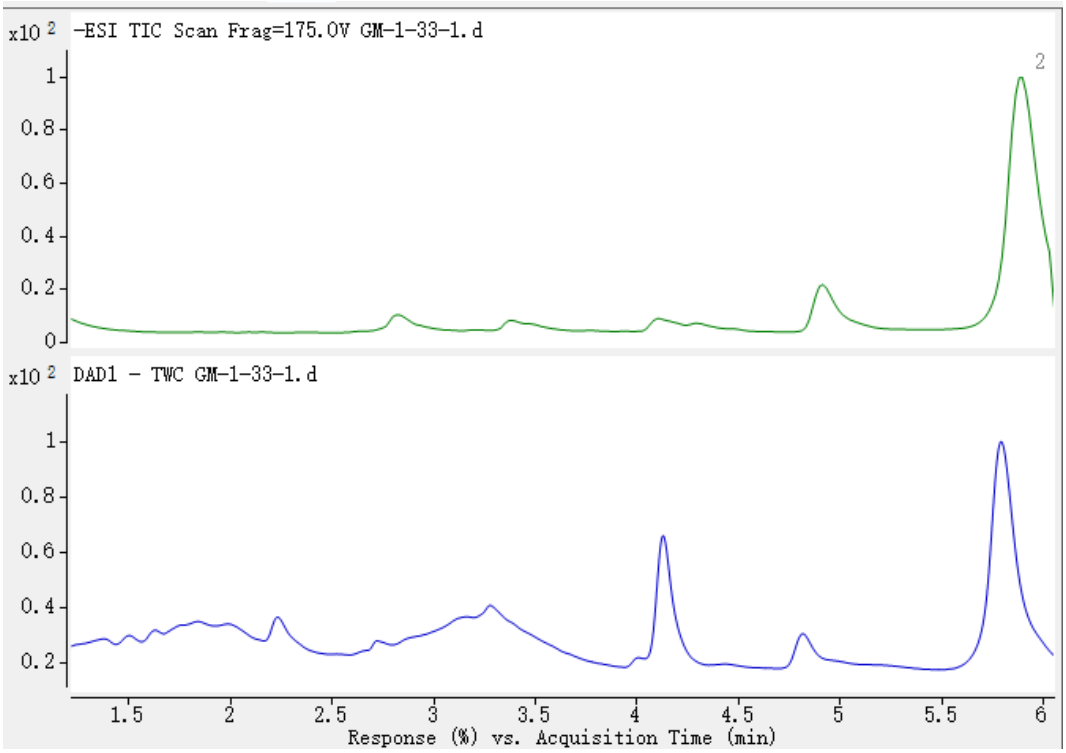

x10 1
-ESI Scan (rt: 5.002-5.984 min, 60 scans) Frag=175.0V NXG-03-47-4.d Subtract Deconvolu...
5382.40
4613.73
6494.66
7318.85
8051.07
9514.29
Counts (%) vs. Deconvoluted Mass (amu)

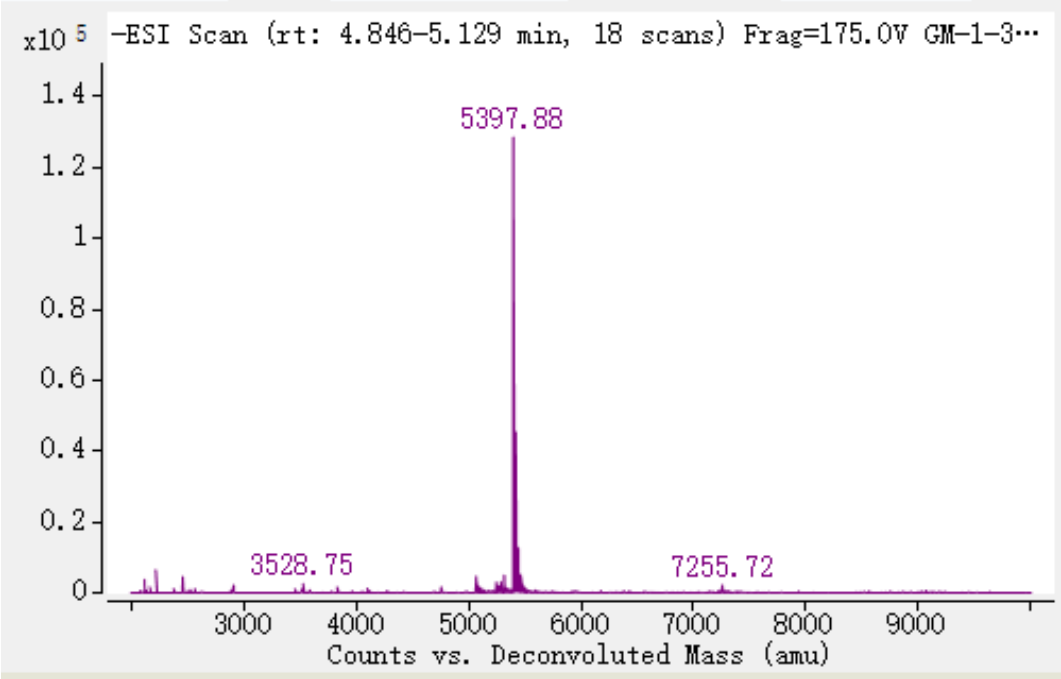
UHPLC chromatogram and deconvoluted MS of **d15**

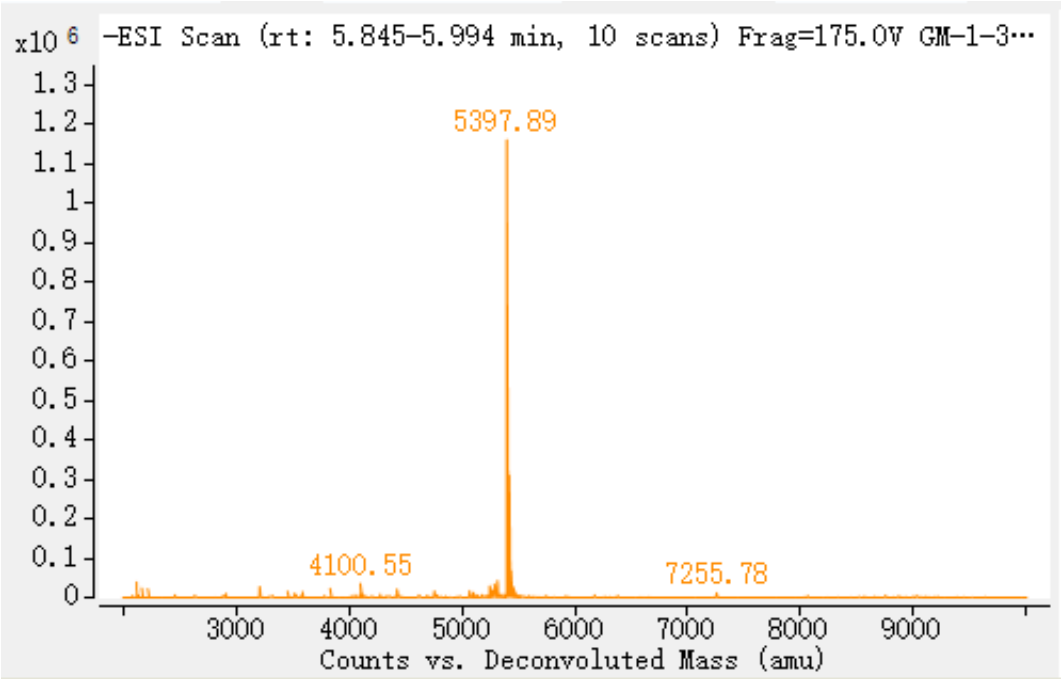
Conversion: 80%

Calculated Mass: 5398 Da; Observed Mass: 5398 Da





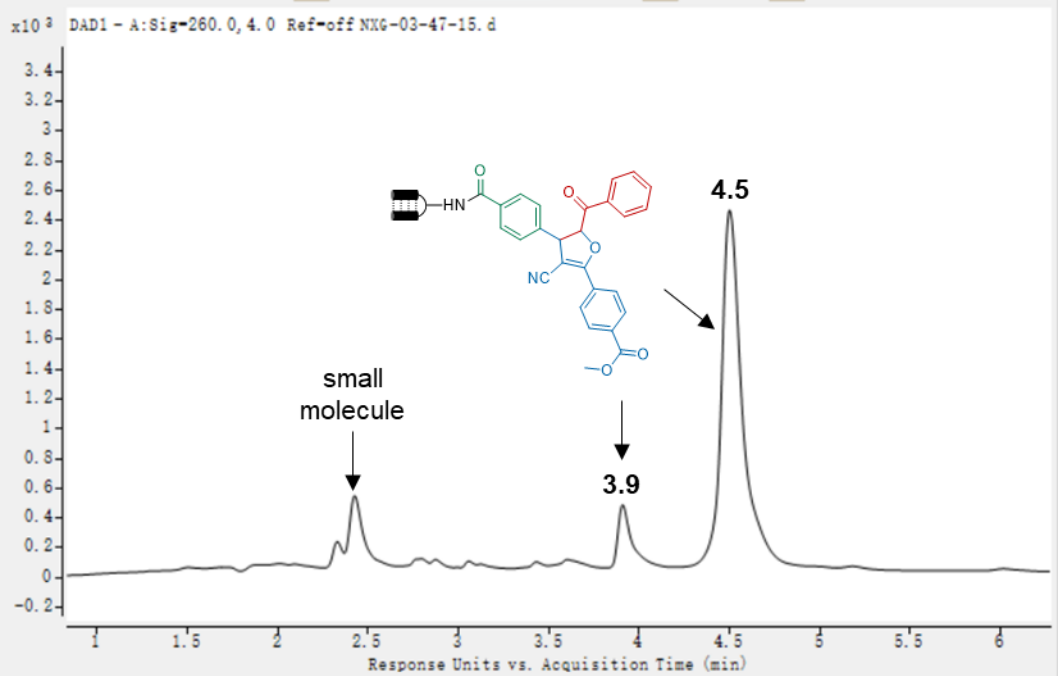

x10 5
-ESI Scan (rt: 4.846-5.129 min, 18 scans) Frag=175.0V GM-1-3…
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5397.88
3528.75
7255.72
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

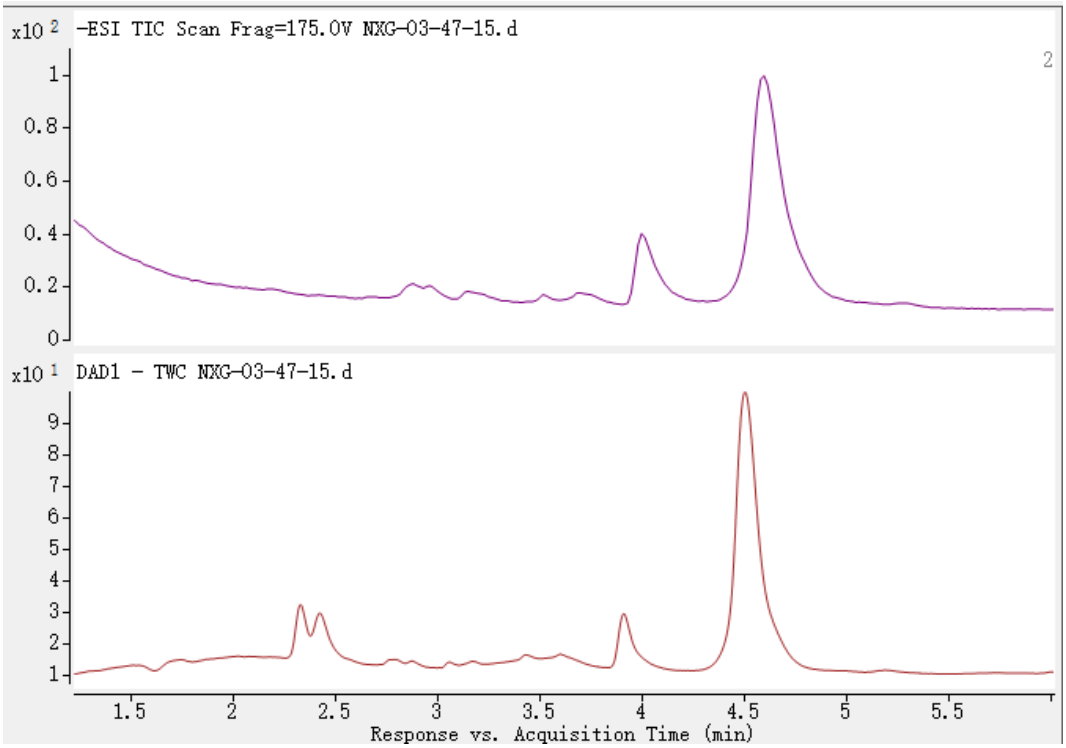

x10 6
-ESI Scan (rt: 5.845-5.994 min, 10 scans) Frag=175.0V GM-1-3…
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5397.89
4100.55
7255.78
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

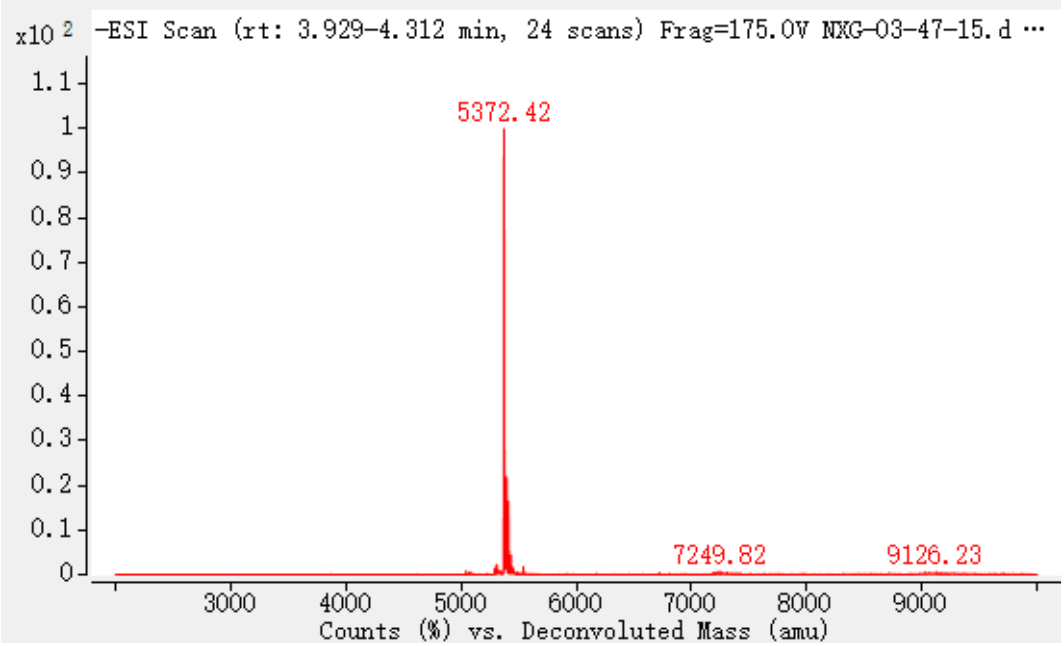
UHPLC chromatogram and deconvoluted MS of **d16**

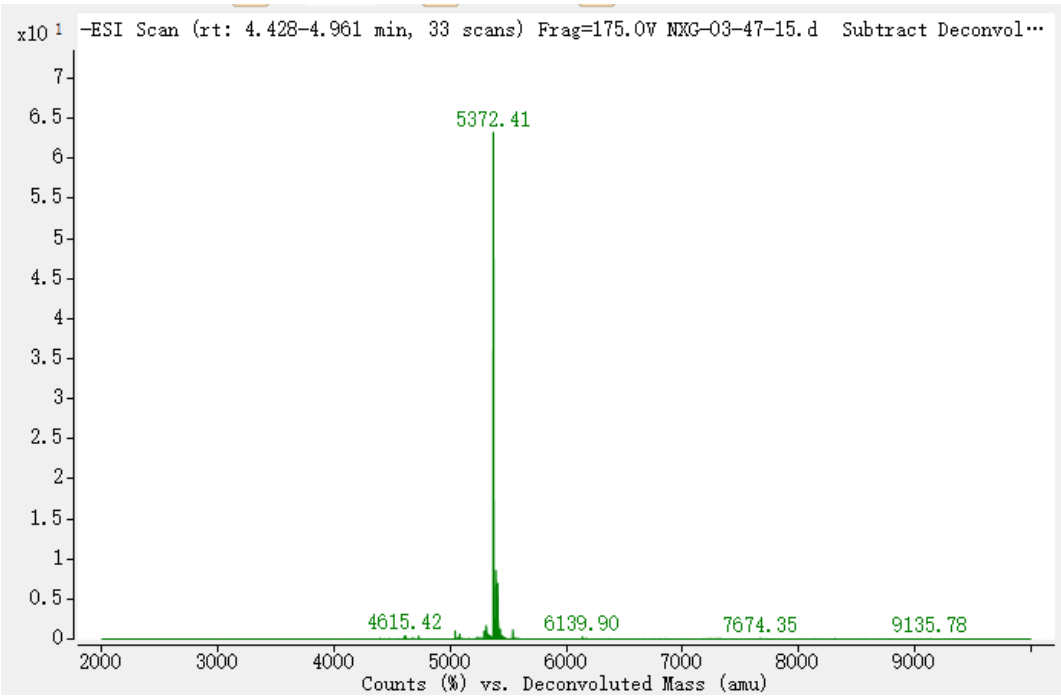
Conversion: >90%

Calculated Mass: 5372 Da; Observed Mass: 5372 Da





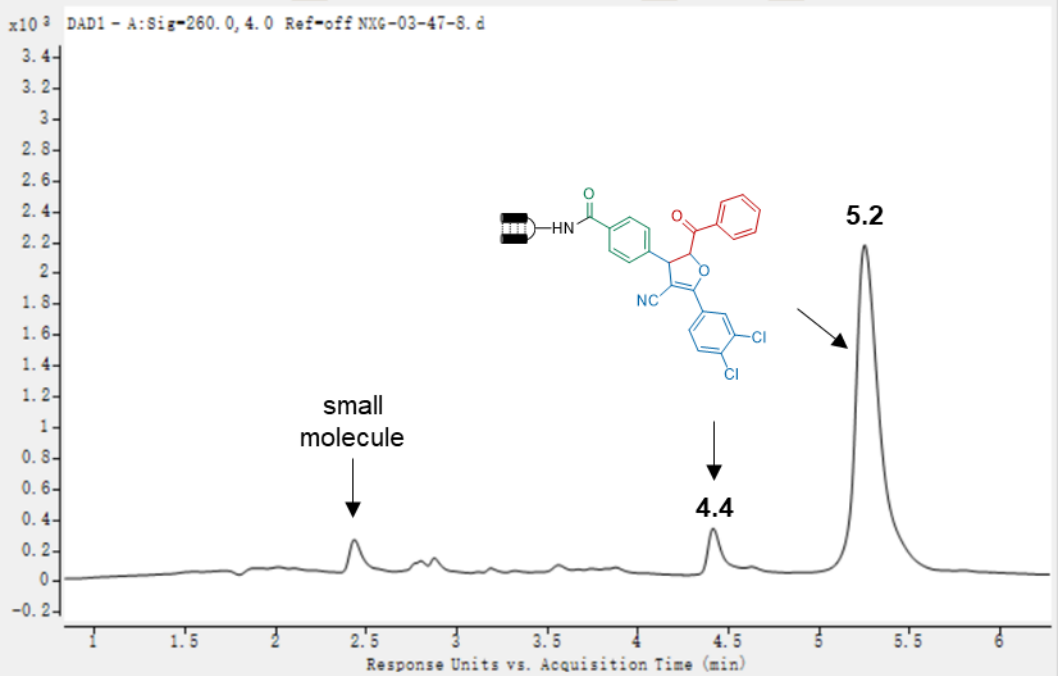

x10 2
-ESI Scan (rt: 3.929-4.312 min, 24 scans) Frag=175.0V NXG-03-47-15.d ...
5372.42
7249.82
9126.23
Counts (%) vs. Deconvoluted Mass (amu)

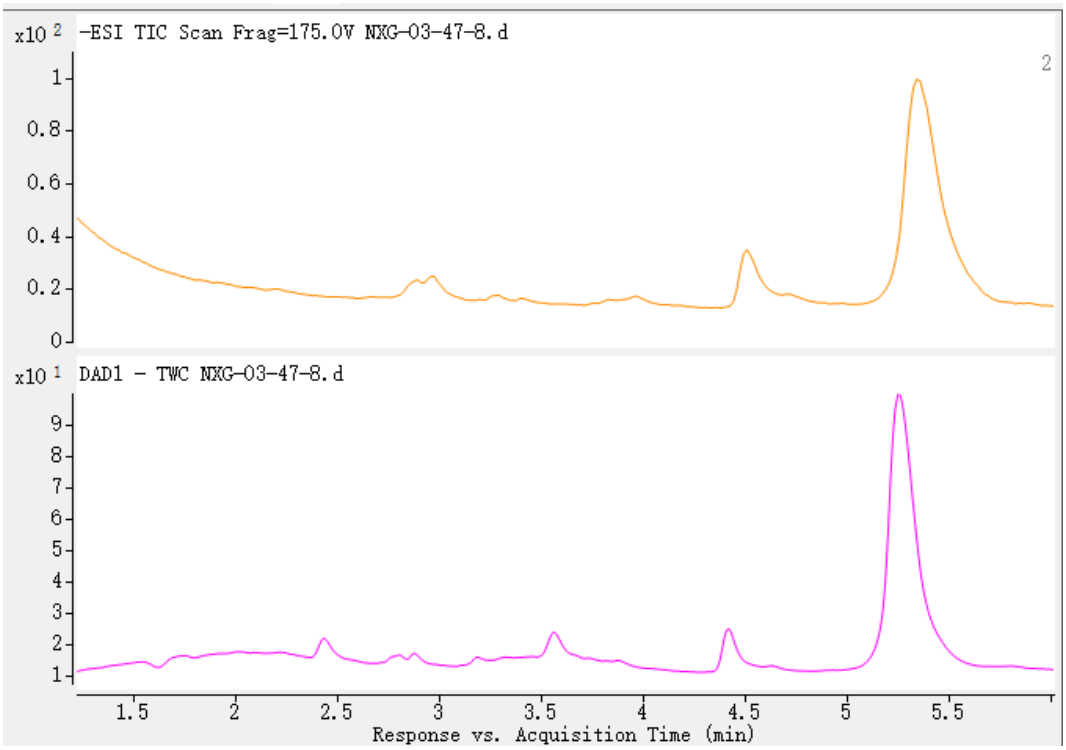

x10 1
-ESI Scan (rt: 4.428-4.961 min, 33 scans) Frag=175.0V NXG-03-47-15.d Subtract Deconvol...
5372.41
4615.42
6139.90
7674.35
9135.78
Counts (%) vs. Deconvoluted Mass (amu)

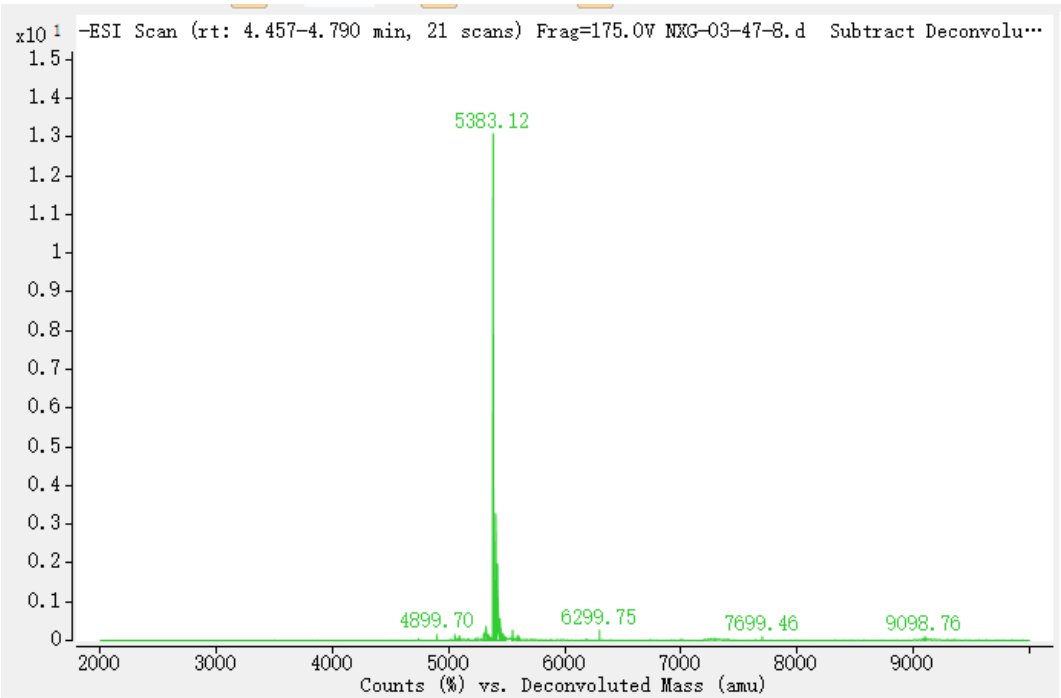
UHPLC chromatogram and deconvoluted MS of **d17**

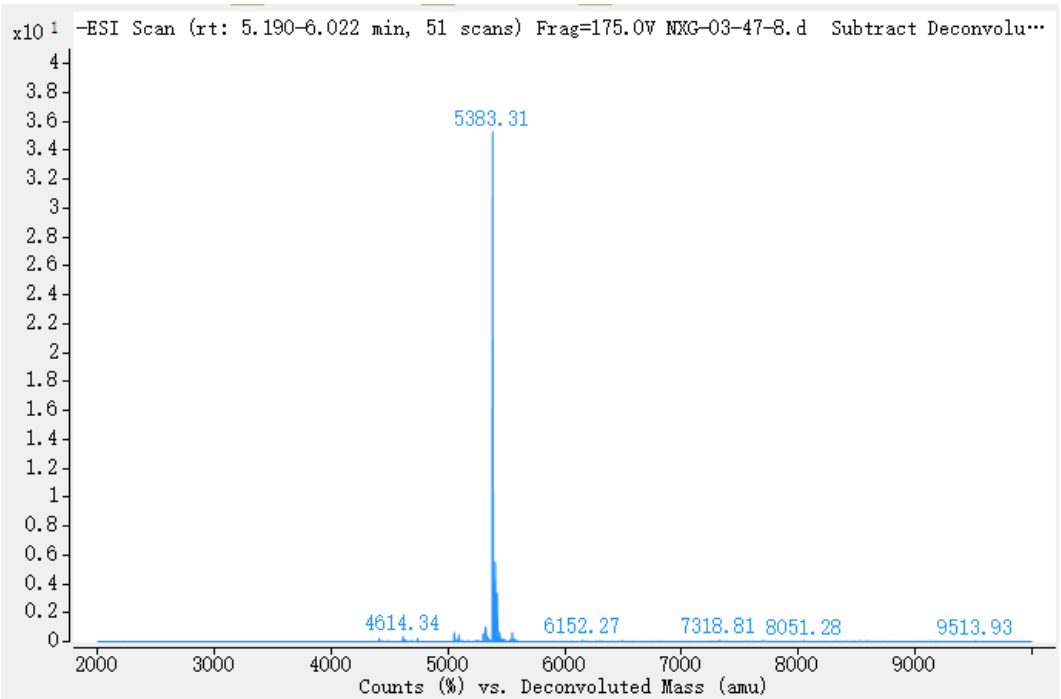
Conversion: >90%

Calculated Mass: 5383 Da; Observed Mass: 5383 Da





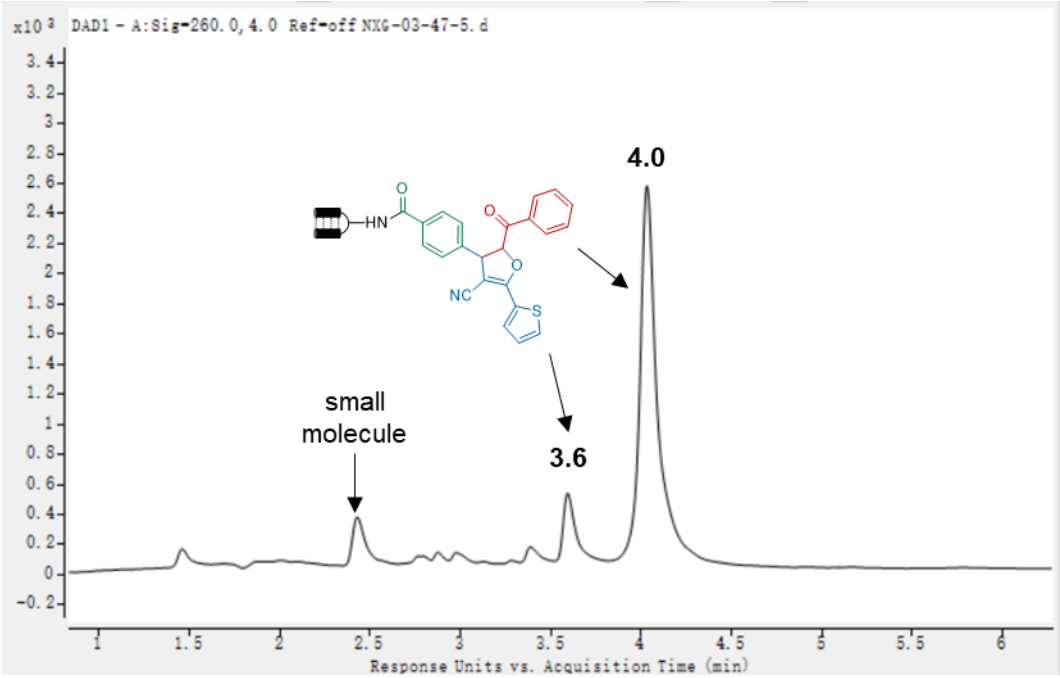

-ESI Scan (rt: 4.457-4.790 min, 21 scans) Frag=175.0V NXG-03-47-8.d Subtract Deconvolu…
x10 1
5383.12
4899.70
6299.75
7699.46
9098.76
Counts (%) vs. Deconvoluted Mass (amu)

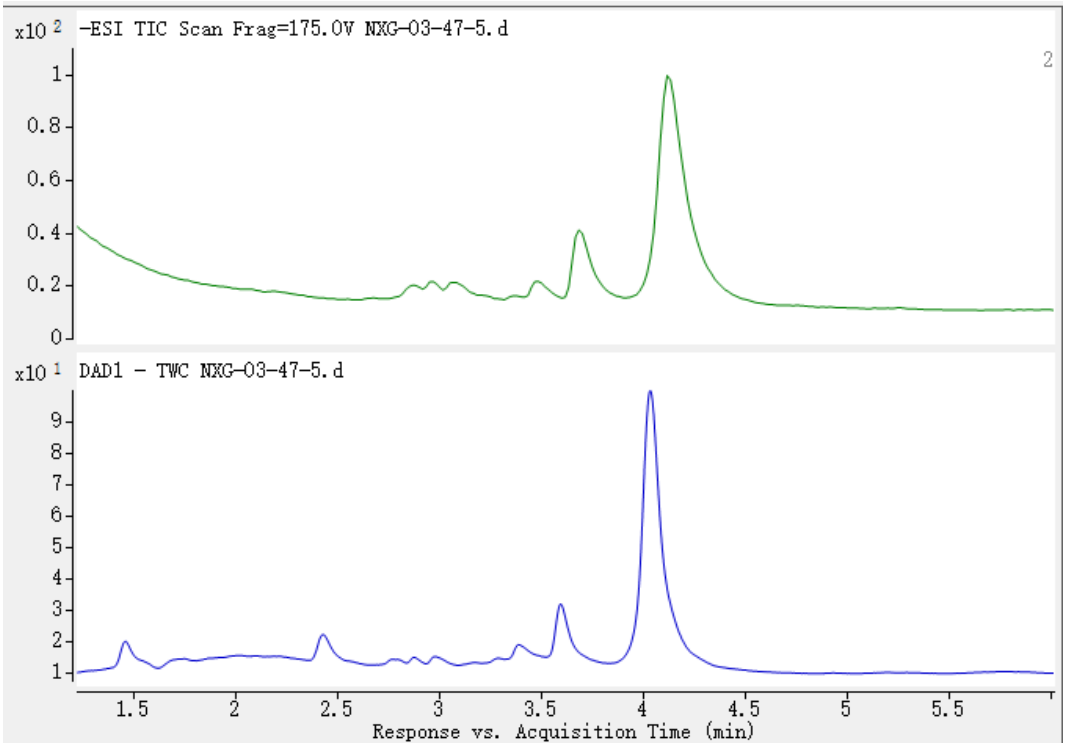

-ESI Scan (rt: 5.190-6.022 min, 51 scans) Frag=175.0V NXG-03-47-8.d Subtract Deconvolu…
x10 1
5383.31
4614.34
6152.27
7318.81
8051.28
9513.93
Counts (%) vs. Deconvoluted Mass (amu)

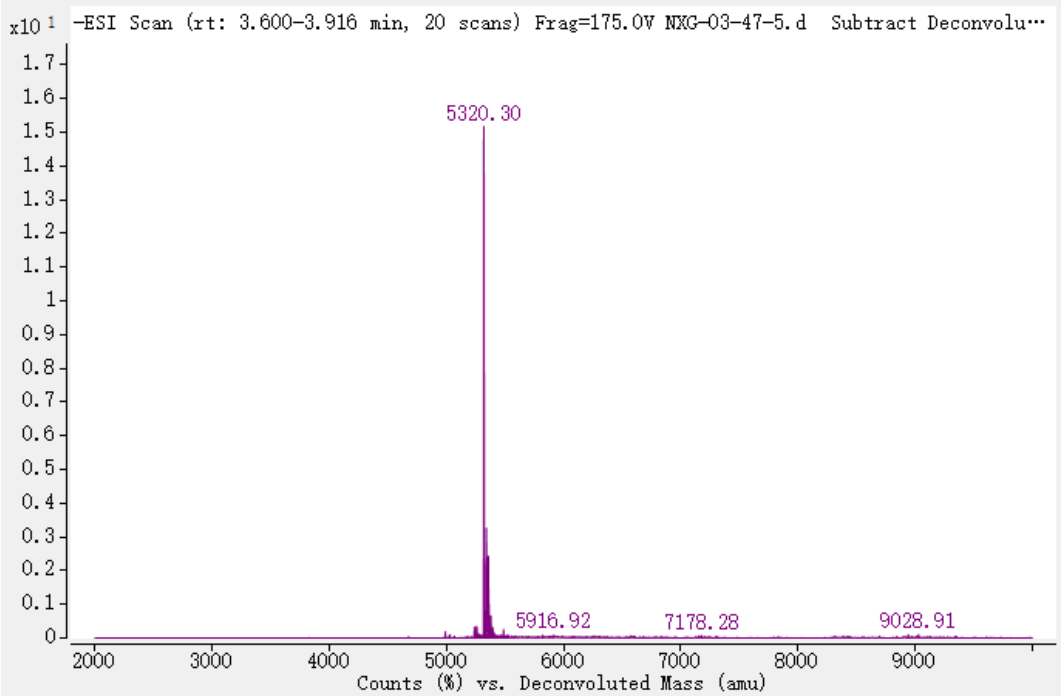
UHPLC chromatogram and deconvoluted MS of **d18**

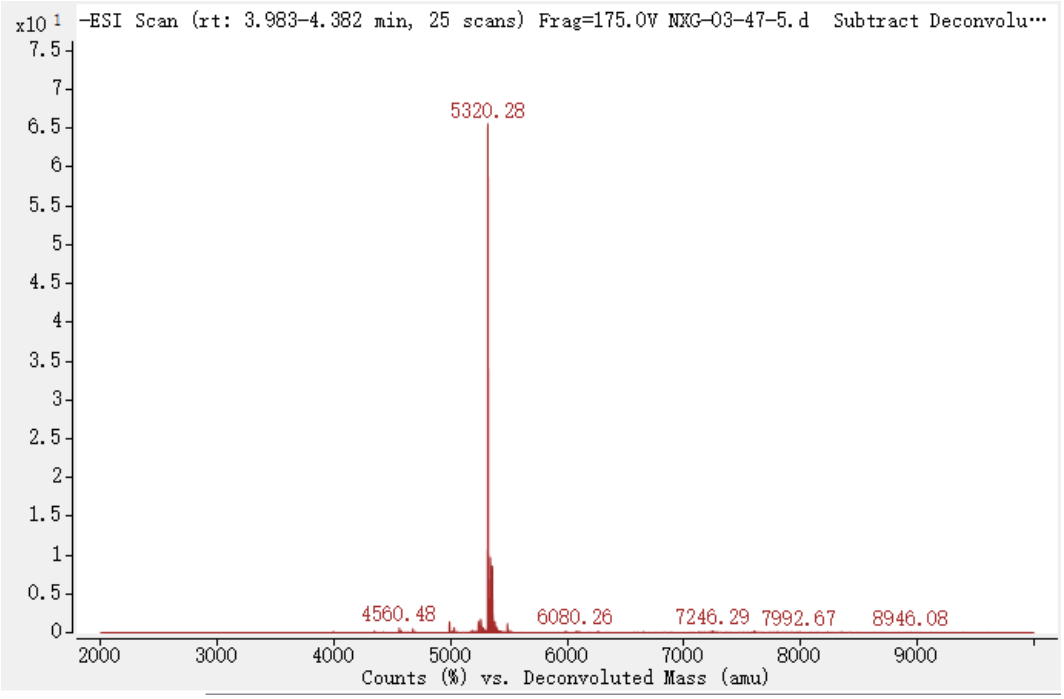
Conversion: >90%

Calculated Mass: 5320 Da; Observed Mass: 5320 Da





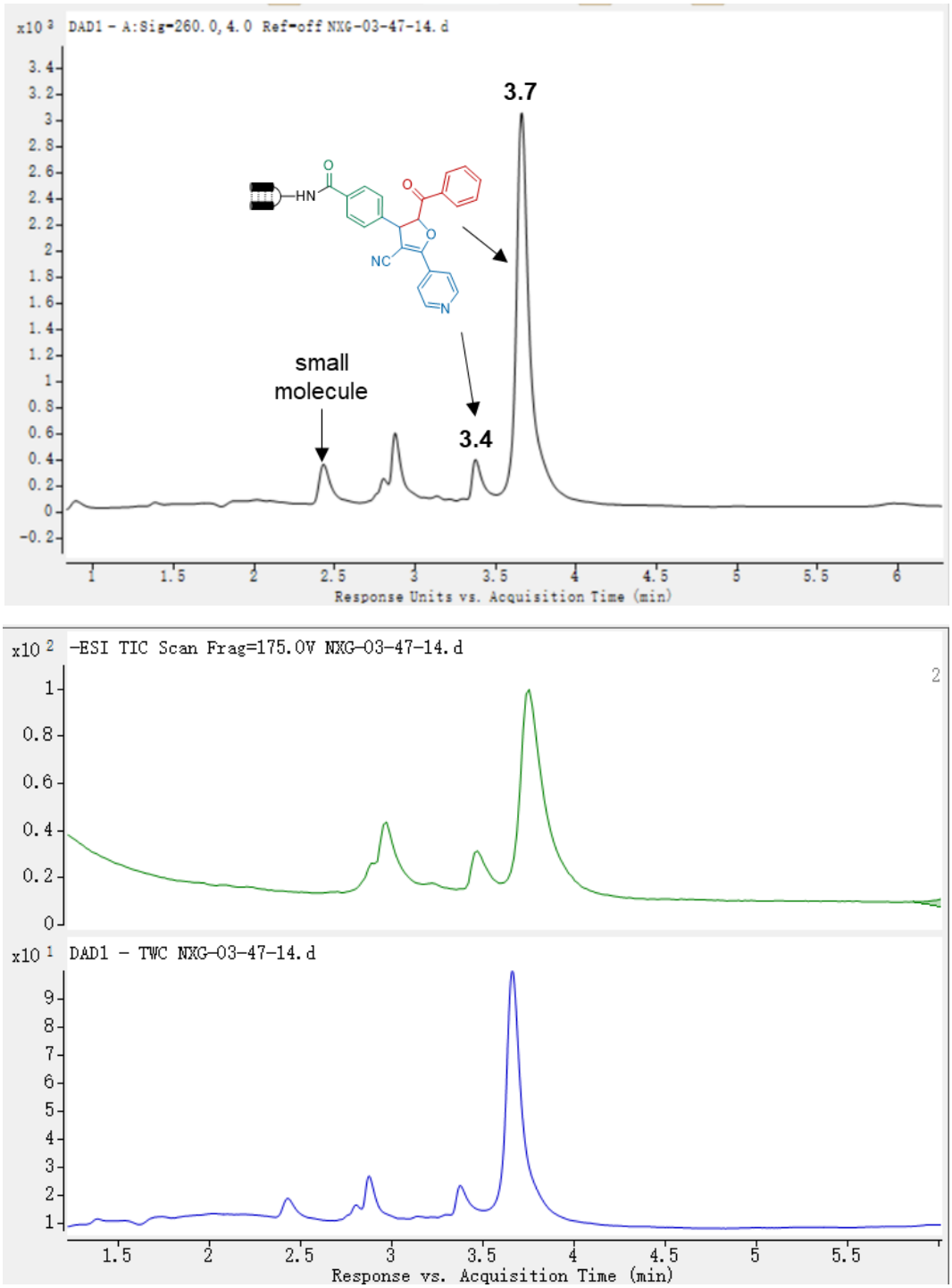

x10 1
-ESI Scan (rt: 3.600-3.916 min, 20 scans) Frag=175.0V NXG-03-47-5.d Subtract Deconvolu…
5320.30
5916.92
7178.28
9028.91
Counts (%) vs. Deconvoluted Mass (amu)

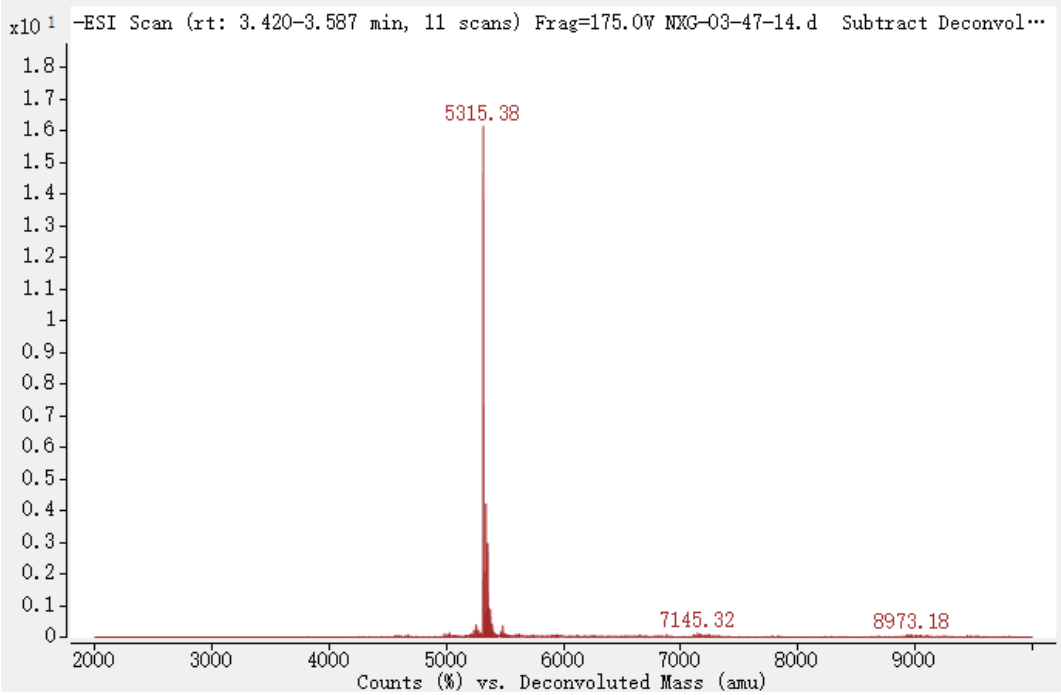

x10 1
-ESI Scan (rt: 3.983-4.382 min, 25 scans) Frag=175.0V NXG-03-47-5.d Subtract Deconvolu…
5320.28
4560.48
6080.26
7246.29
7992.67
8946.08
Counts (%) vs. Deconvoluted Mass (amu)

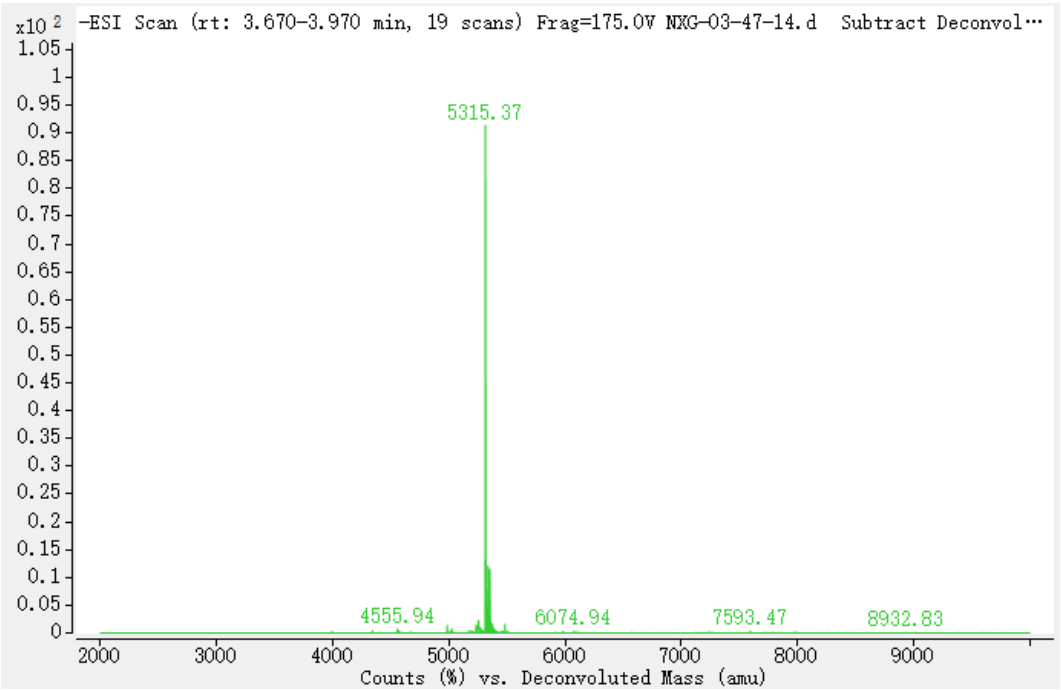
UHPLC chromatogram and deconvoluted MS of **d19**

Conversion: 85%

Calculated Mass: 5315 Da; Observed Mass: 5315 Da



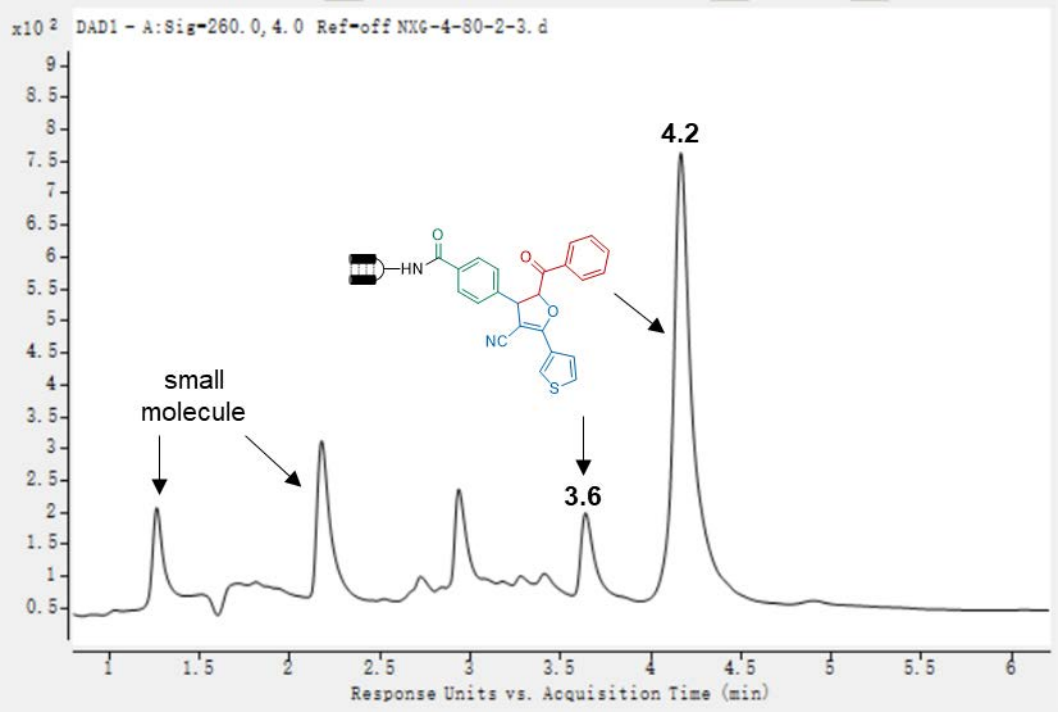

x10 1
-ESI Scan (rt: 3.420-3.587 min, 11 scans) Frag=175.0V NXG-03-47-14.d Subtract Deconvol…
5315.38
7145.32
8973.18
Counts (%) vs. Deconvoluted Mass (amu)

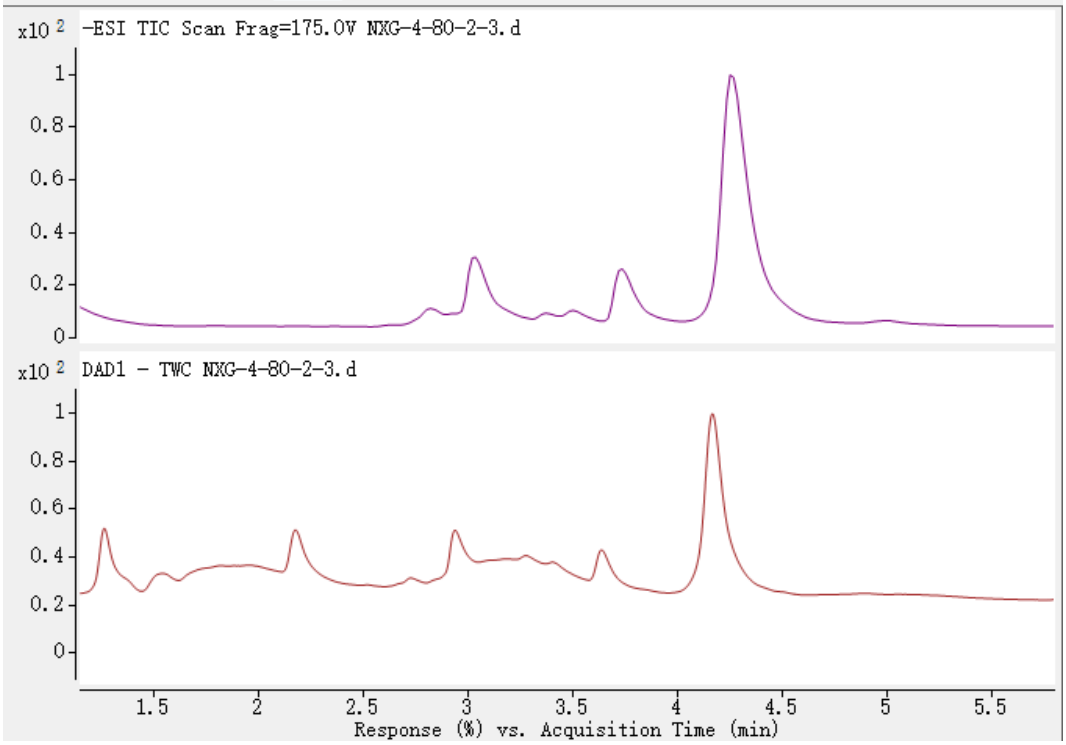

x10 2
-ESI Scan (rt: 3.670-3.970 min, 19 scans) Frag=175.0V NXG-03-47-14.d Subtract Deconvol…
5315.37
4555.94
6074.94
7593.47
8932.83
Counts (%) vs. Deconvoluted Mass (amu)

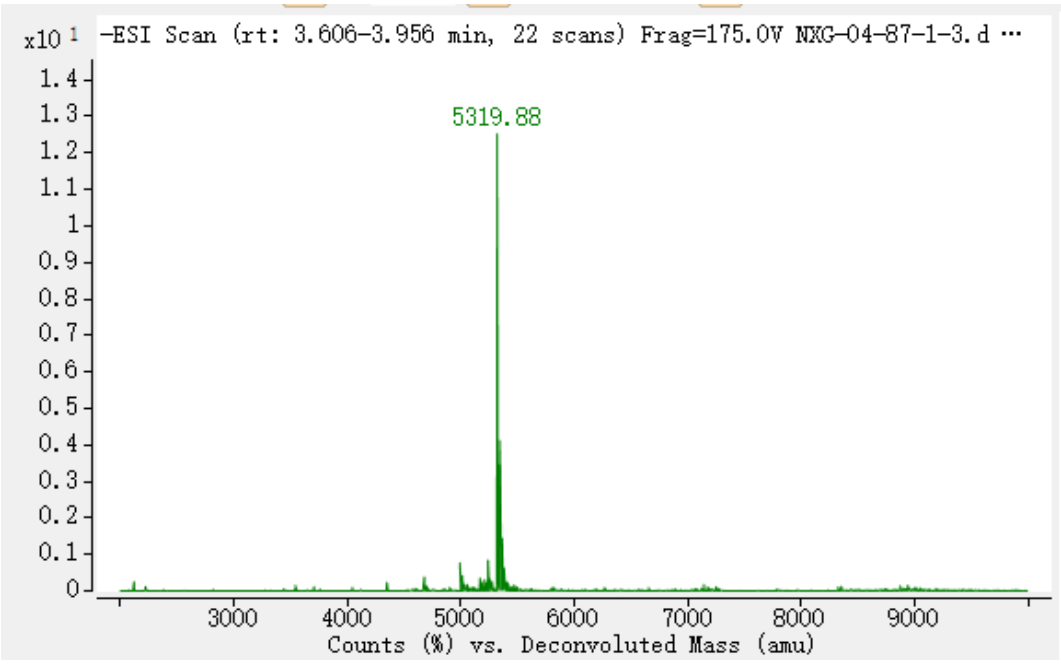
UHPLC chromatogram and deconvoluted MS of **d20**

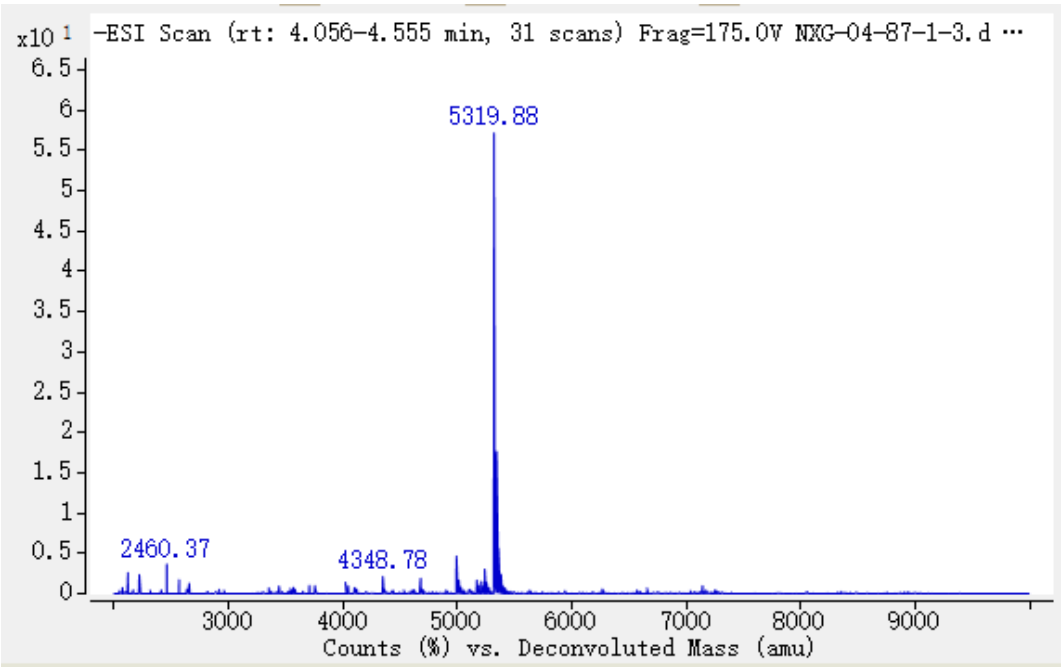
Conversion: 81%

Calculated Mass: 5320 Da; Observed Mass: 5320 Da






x10 1
-ESI Scan (rt: 3.606-3.956 min, 22 scans) Frag=175.0V NXG-04-87-1-3.d ...
5319.88
Counts (%) vs. Deconvoluted Mass (amu)

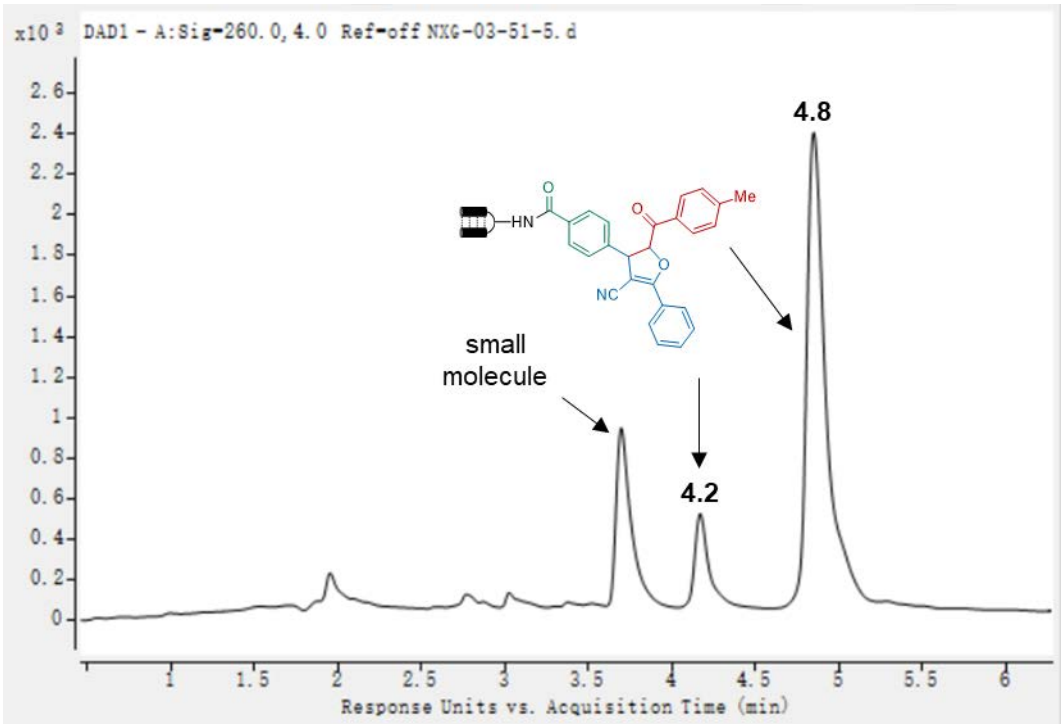

x10 1
-ESI Scan (rt: 4.056-4.555 min, 31 scans) Frag=175.0V NXG-04-87-1-3.d ...
5319.88
2460.37
4348.78
Counts (%) vs. Deconvoluted Mass (amu)

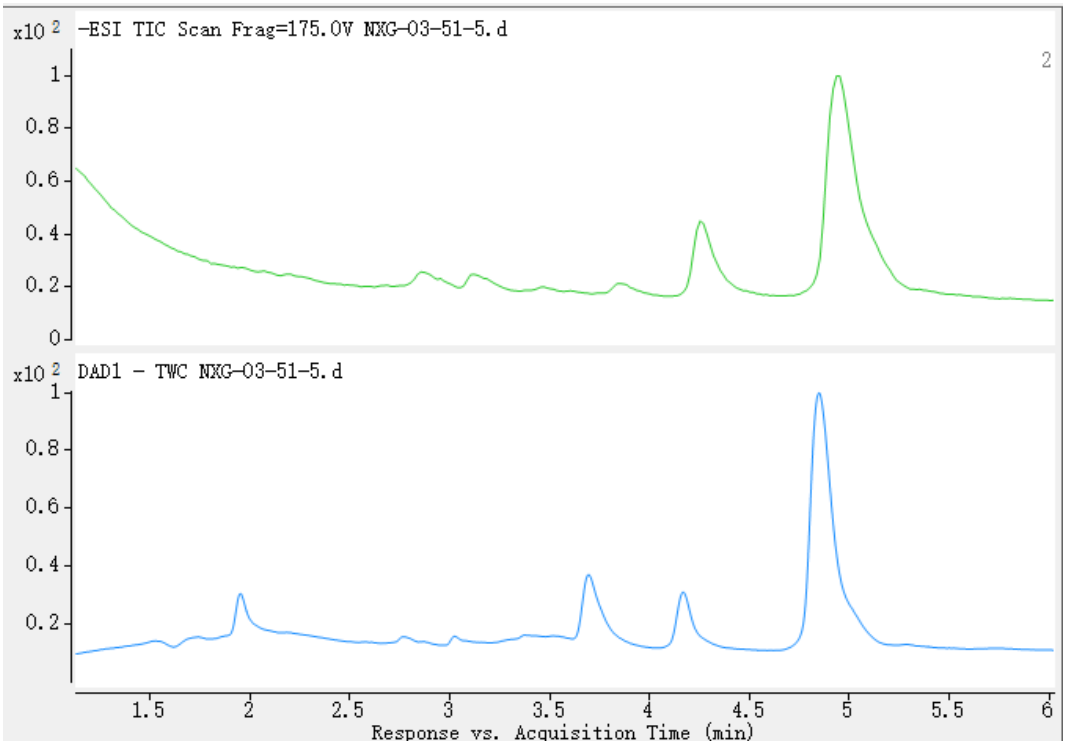
8.2 Scope of pyridinium ylide precursors

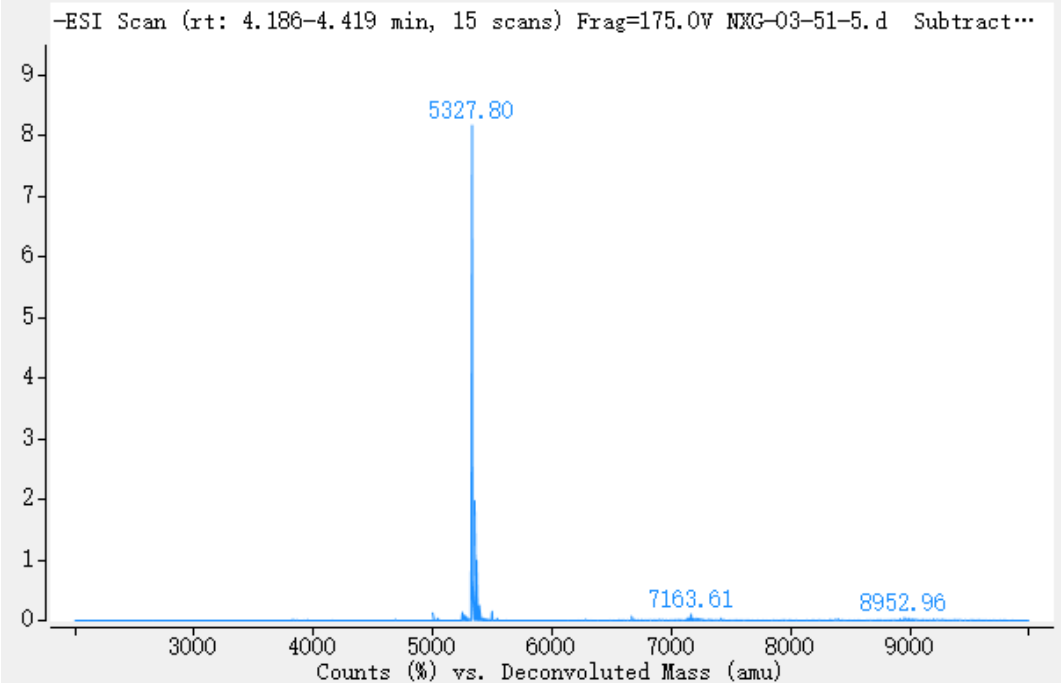
UHPLC chromatogram and deconvoluted MS of **d21**

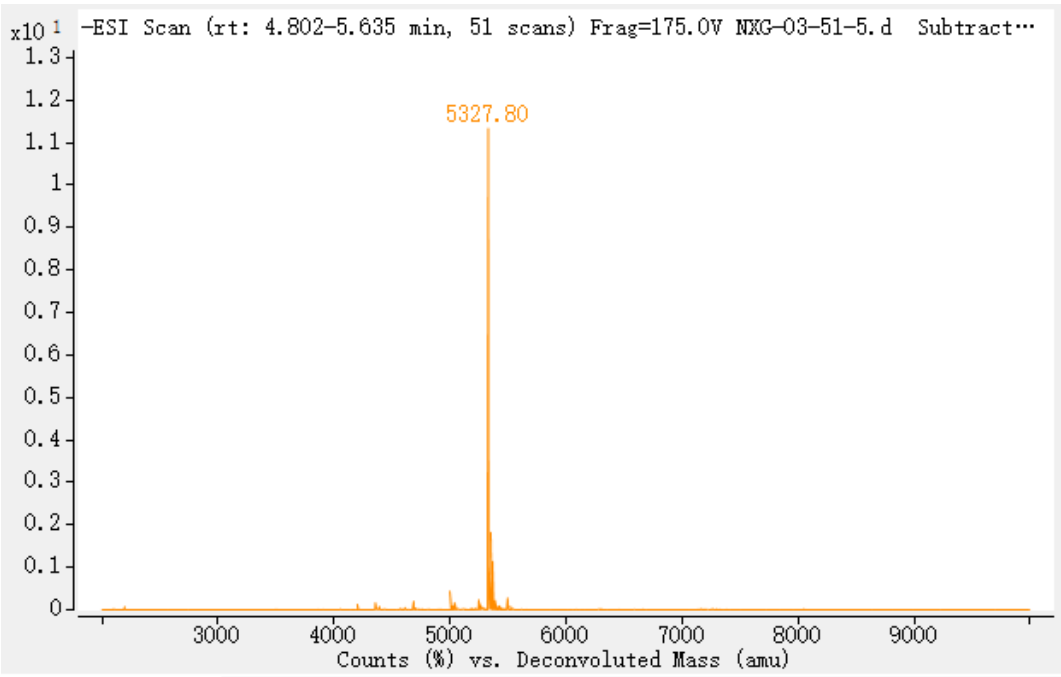
Conversion: >90%

Calculated Mass: 5328 Da; Observed Mass: 5328 Da





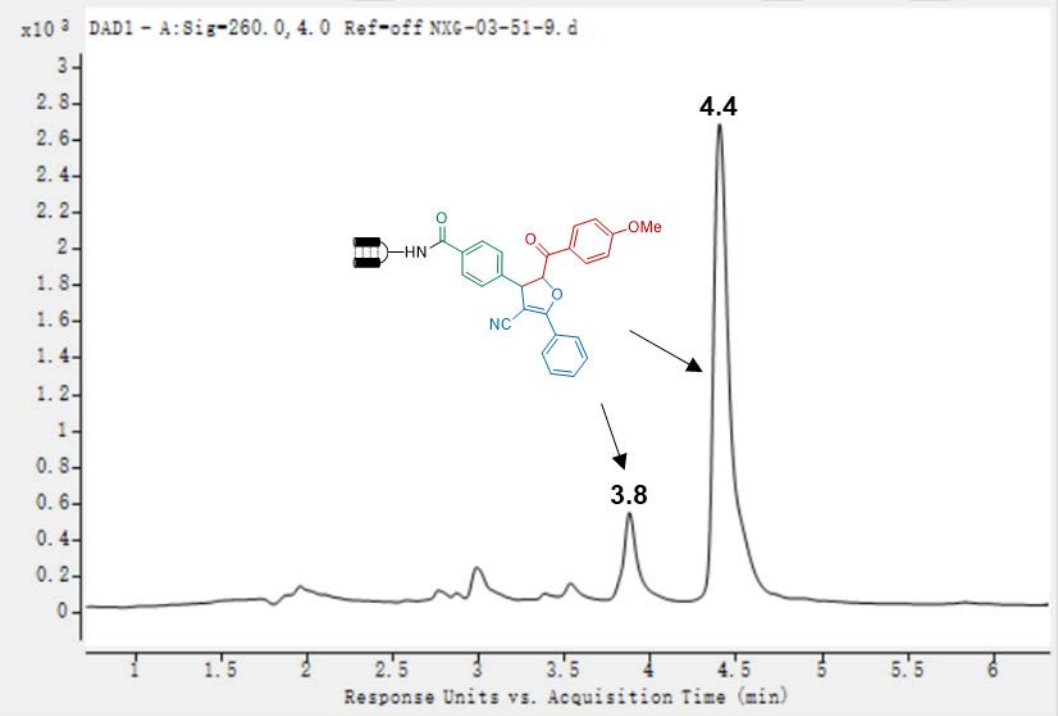

-ESI Scan (rt: 4.186-4.419 min, 15 scans) Frag=175.0V NXG-03-51-5.d Subtract…
5327.80
7163.61
8952.96
Counts (%) vs. Deconvoluted Mass (amu)

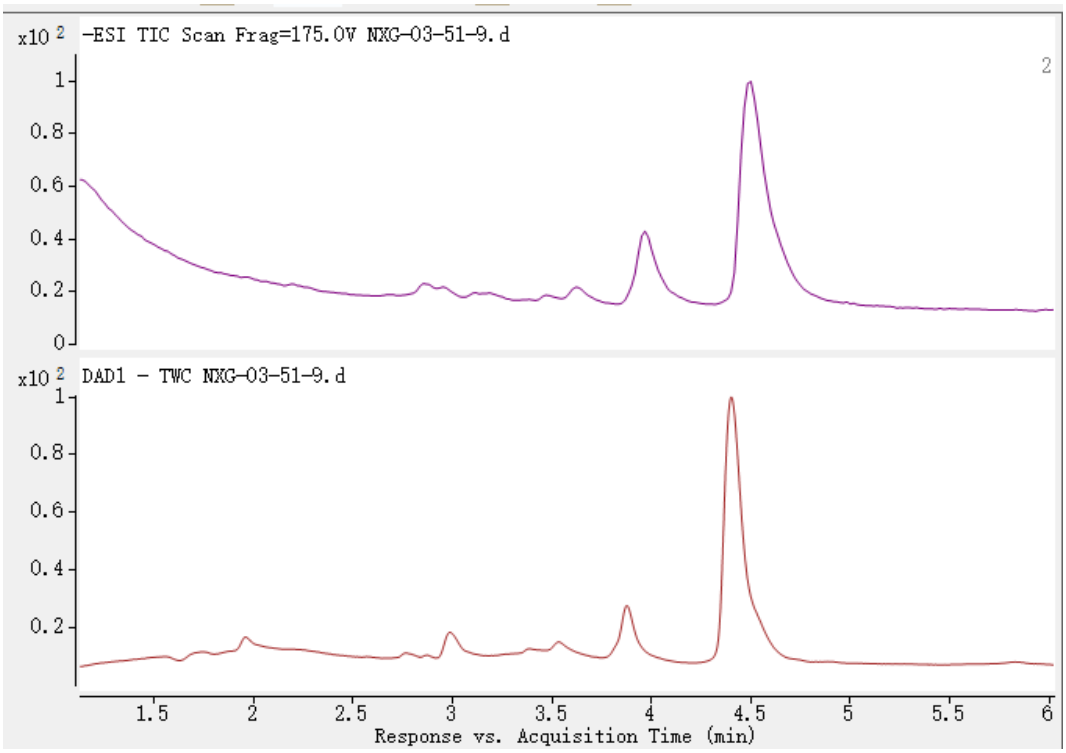

x10 1
-ESI Scan (rt: 4.802-5.635 min, 51 scans) Frag=175.0V NXG-03-51-5.d Subtract…
5327.80
Counts (%) vs. Deconvoluted Mass (amu)

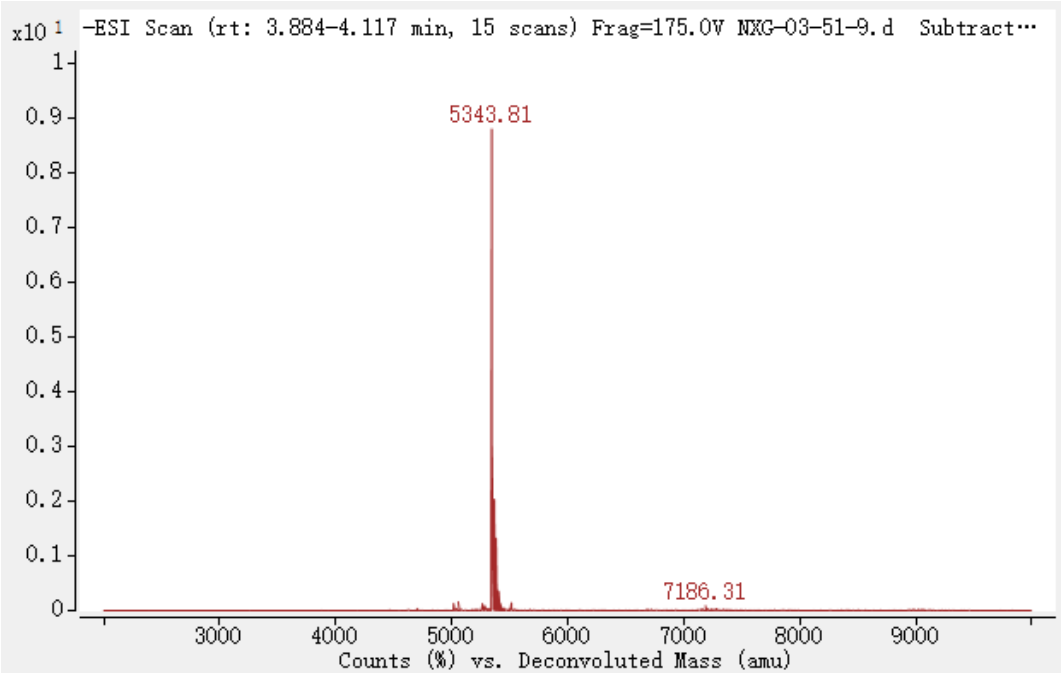
UHPLC chromatogram and deconvoluted MS of **d22**

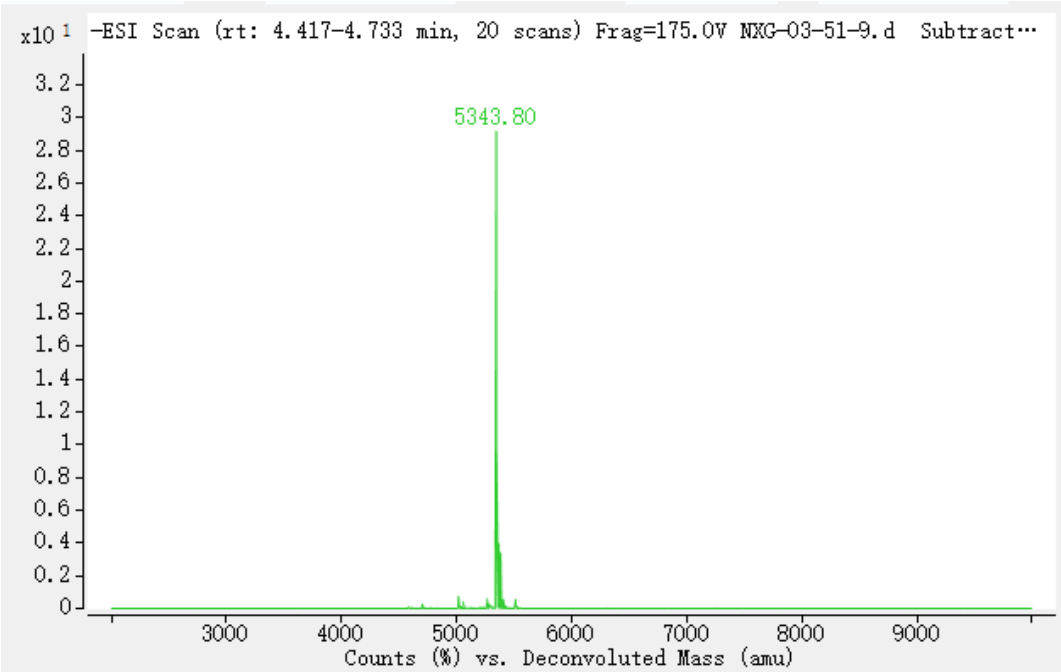
Conversion: >90%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da





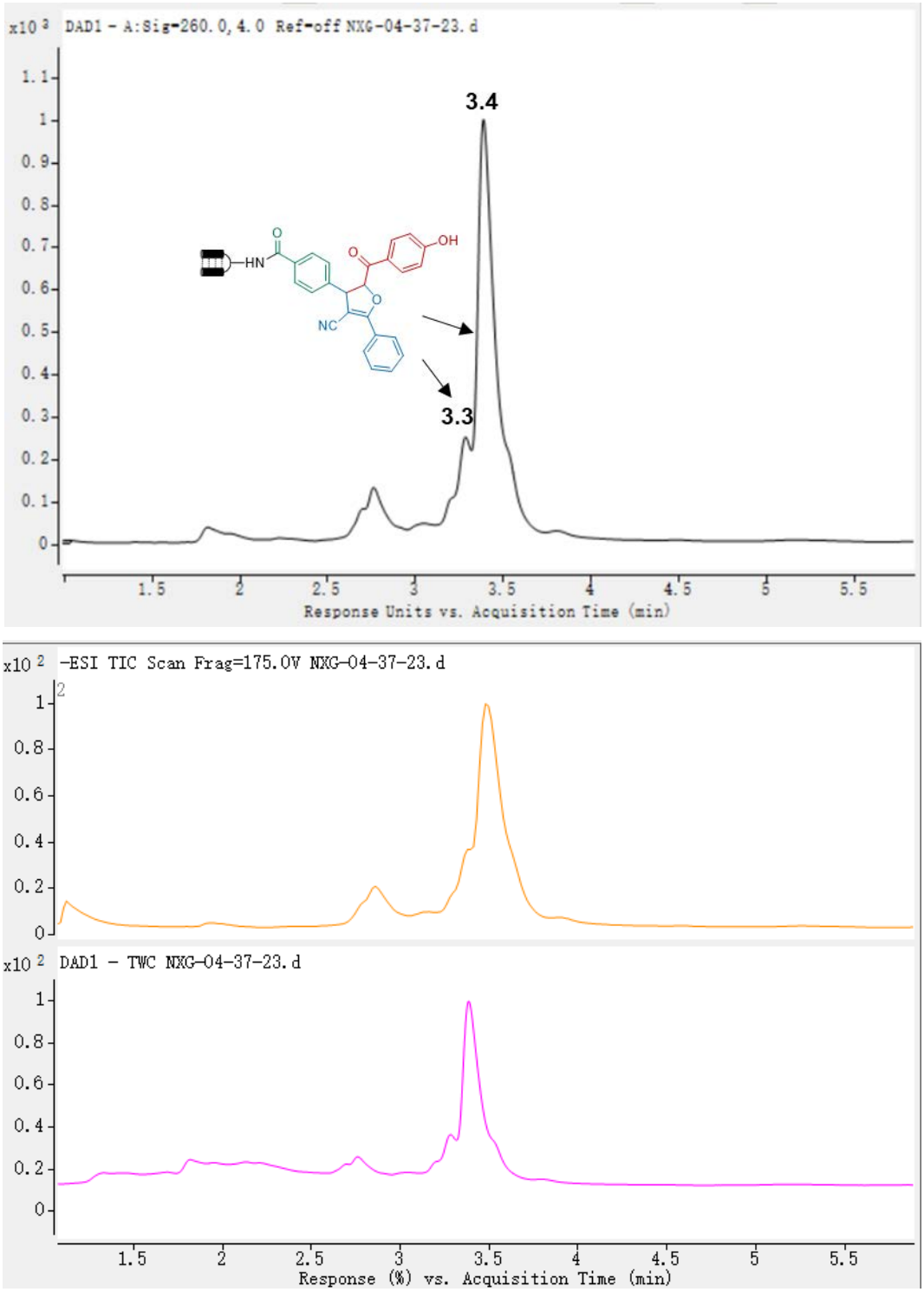

x10 1
-ESI Scan (rt: 3.884-4.117 min, 15 scans) Frag=175.0V NXG-03-51-9.d Subtract…
5343.81
7186.31
Counts (%) vs. Deconvoluted Mass (amu)

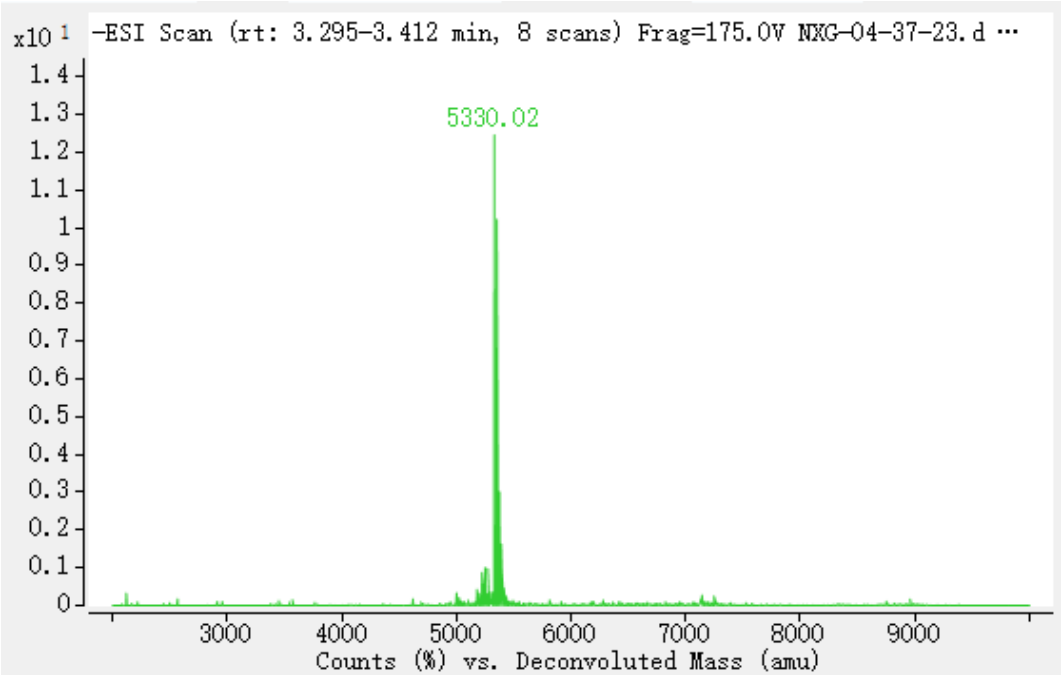

x10 1
-ESI Scan (rt: 4.417-4.733 min, 20 scans) Frag=175.0V NXG-03-51-9.d Subtract…
5343.80
Counts (%) vs. Deconvoluted Mass (amu)

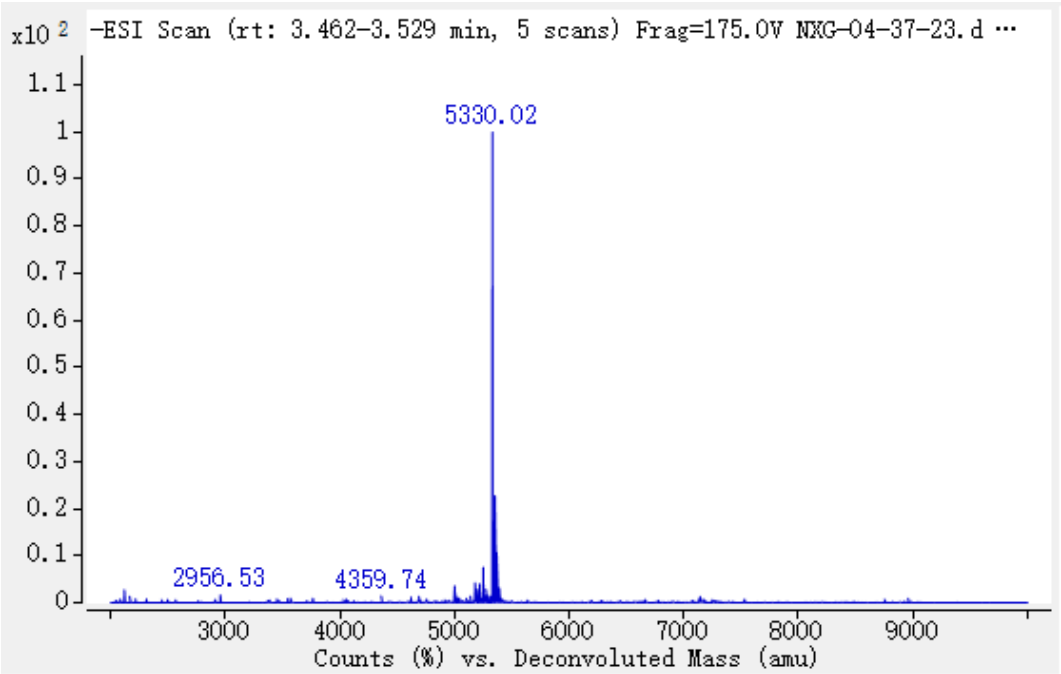
UHPLC chromatogram and deconvoluted MS of **d23**

Conversion: 86%

Calculated Mass: 5330 Da; Observed Mass: 5330 Da



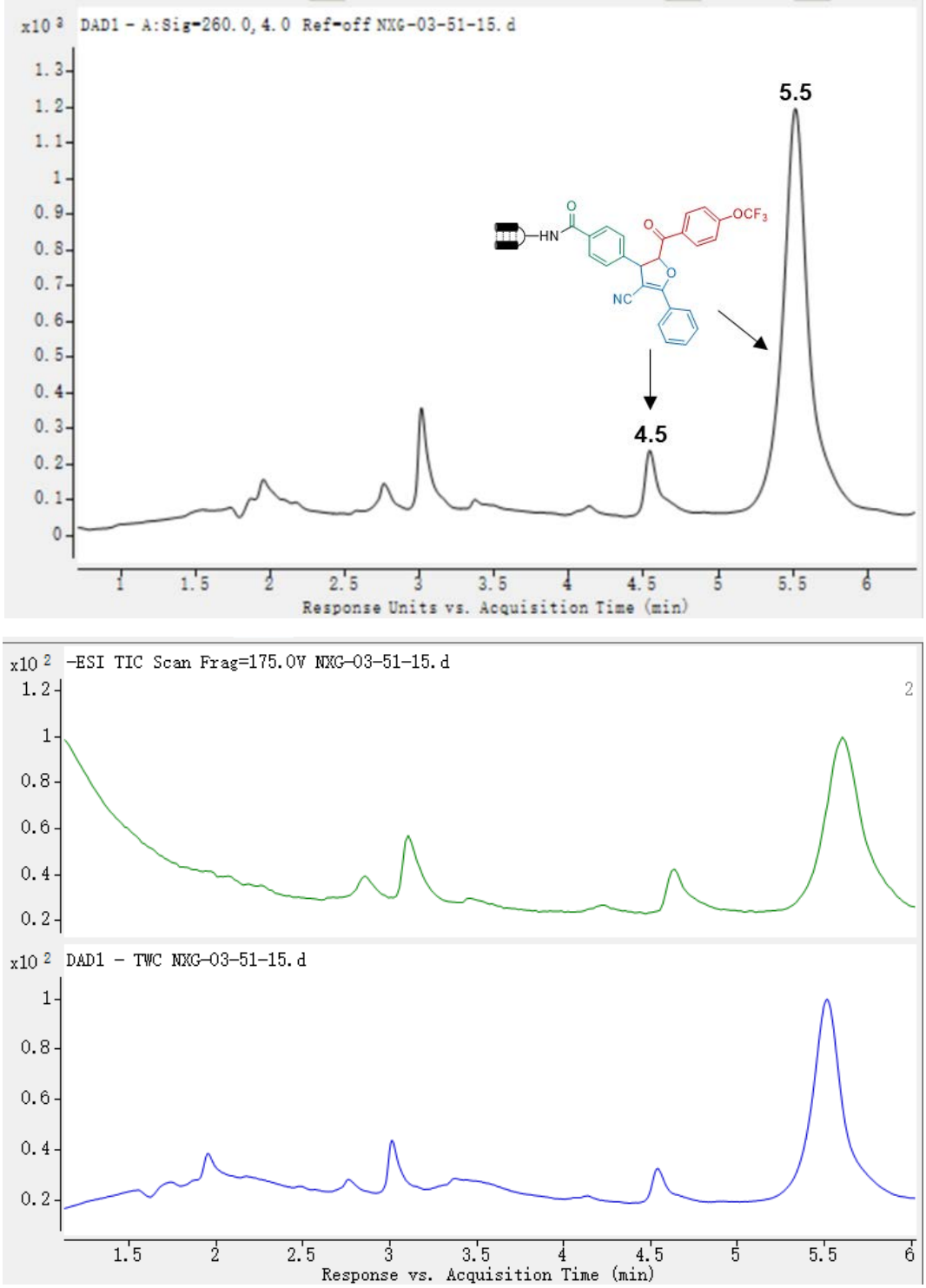

x10 1
-ESI Scan (rt: 3.295-3.412 min, 8 scans) Frag=175.0V NXG-04-37-23.d ...
5330.02
Counts (%) vs. Deconvoluted Mass (amu)

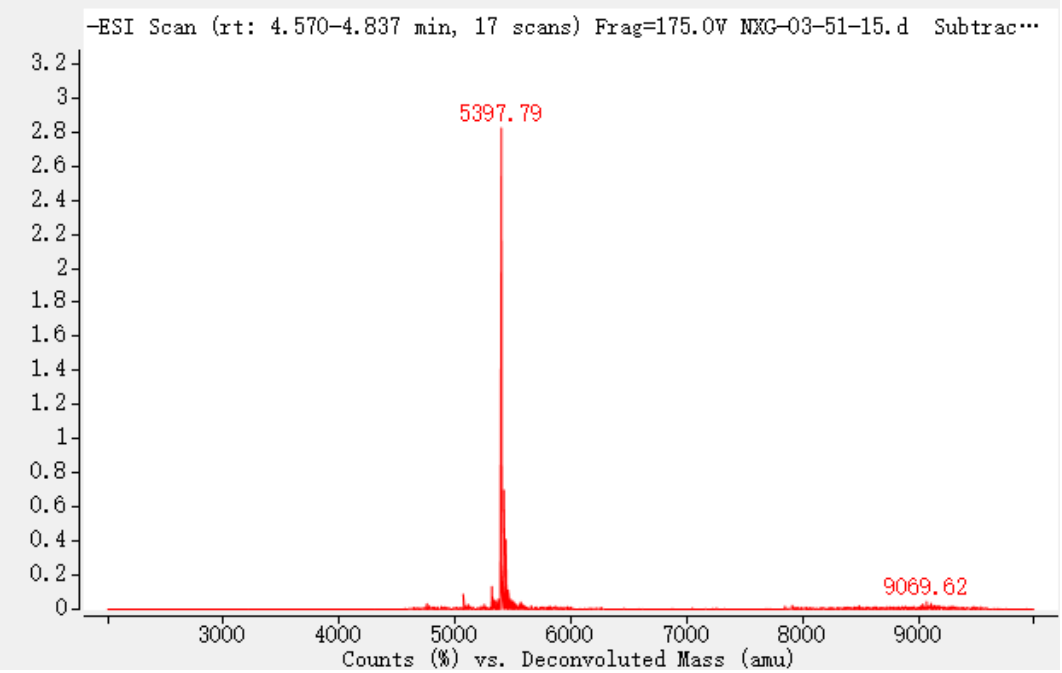

x10 2
-ESI Scan (rt: 3.462-3.529 min, 5 scans) Frag=175.0V NXG-04-37-23.d ...
5330.02
2956.53
4359.74
Counts (%) vs. Deconvoluted Mass (amu)

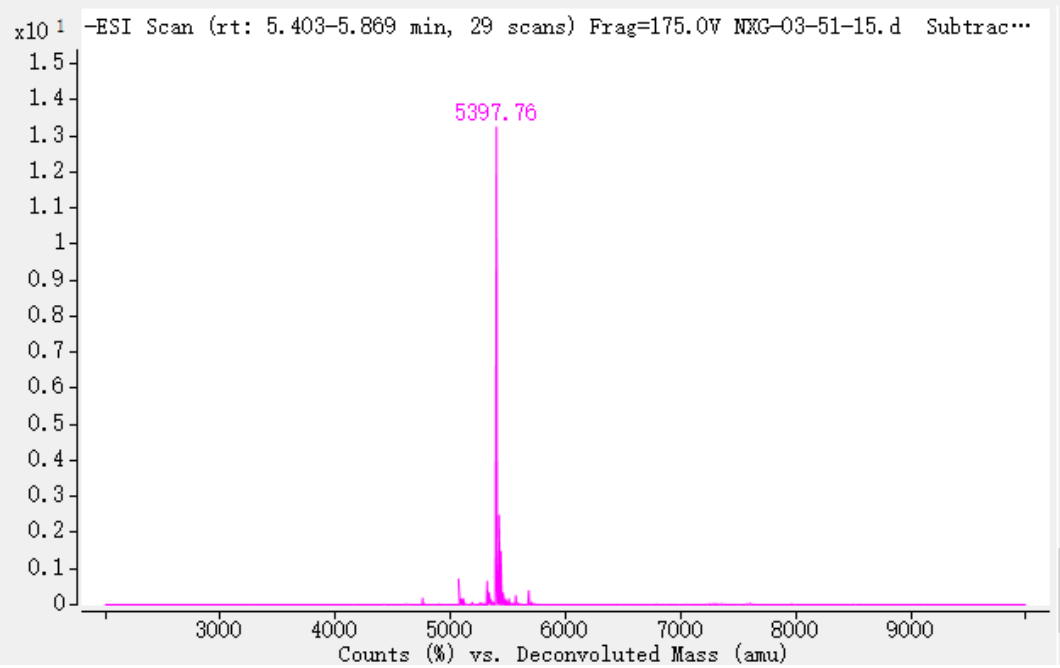
UHPLC chromatogram and deconvoluted MS of **d24**

Conversion: 87%

Calculated Mass: 5398 Da; Observed Mass: 5398 Da



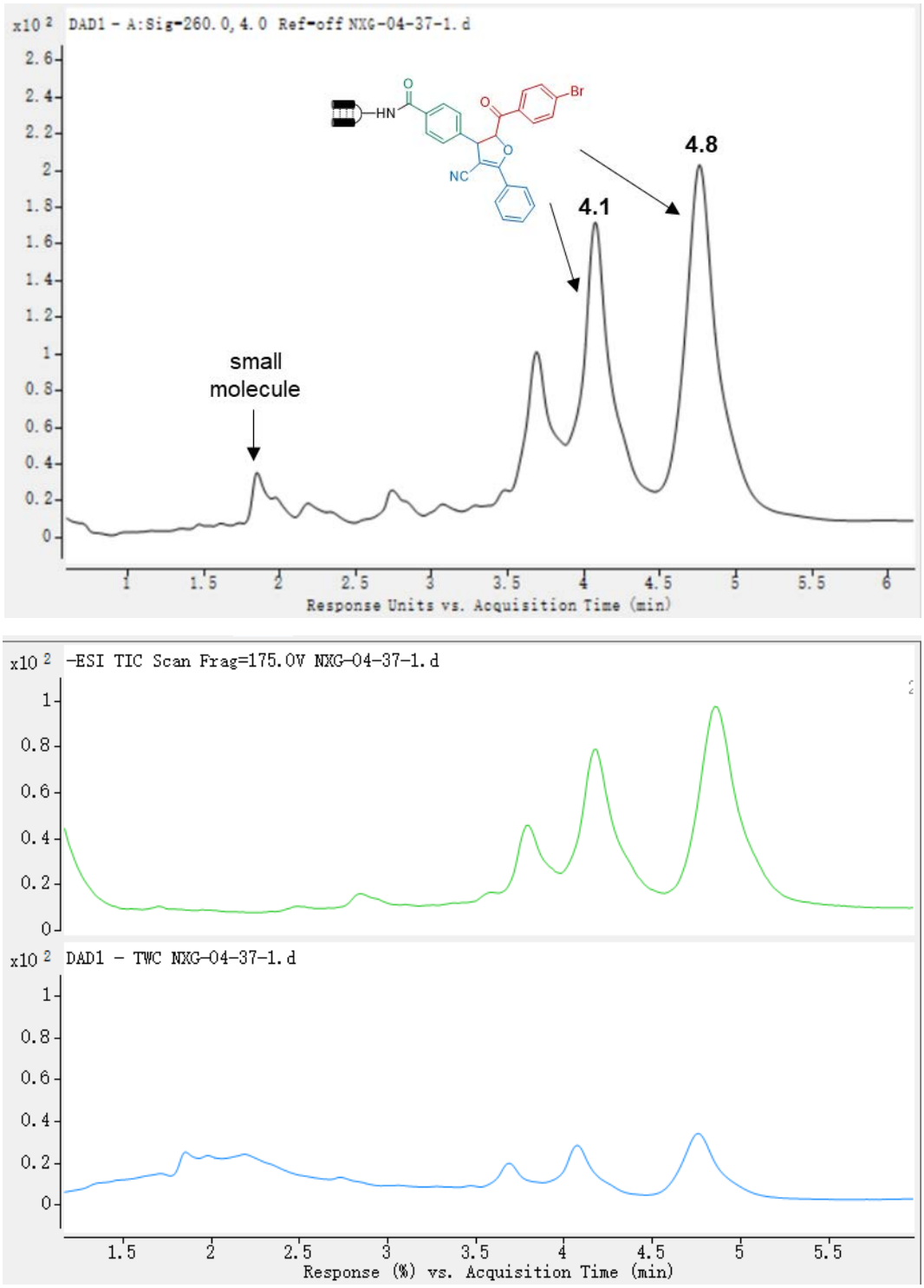

-ESI Scan (rt: 4.570-4.837 min, 17 scans) Frag=175.0V NXG-03-51-15.d Subtrac…
5397.79
9069.62
Counts (%) vs. Deconvoluted Mass (amu)

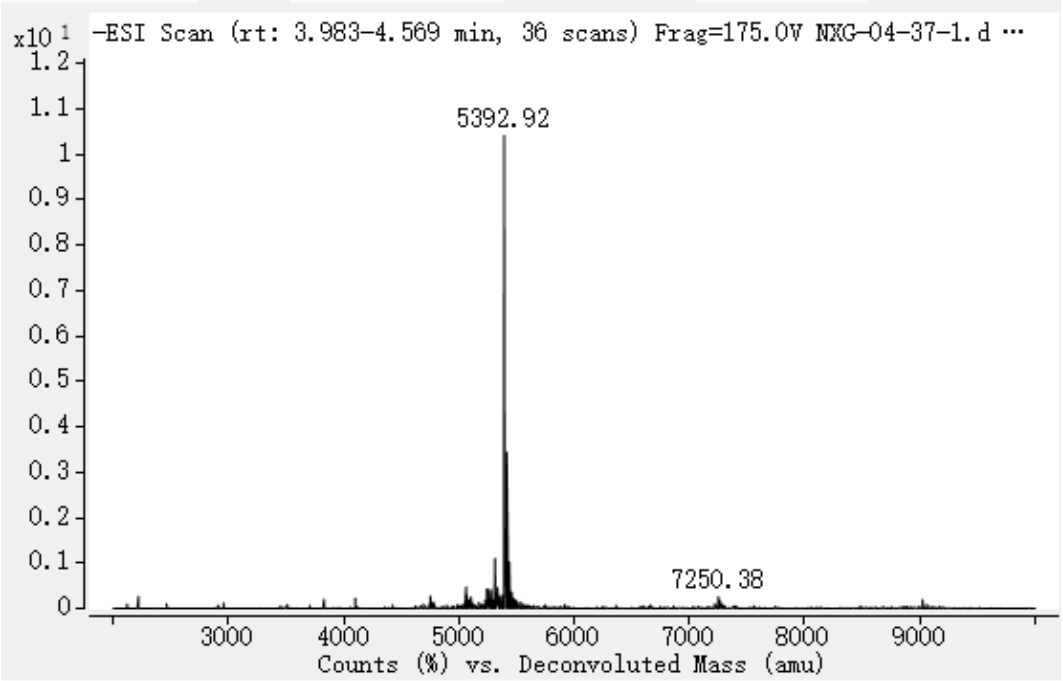

x10 1
-ESI Scan (rt: 5.403-5.869 min, 29 scans) Frag=175.0V NXG-03-51-15.d Subtrac…
5397.76
Counts (%) vs. Deconvoluted Mass (amu)

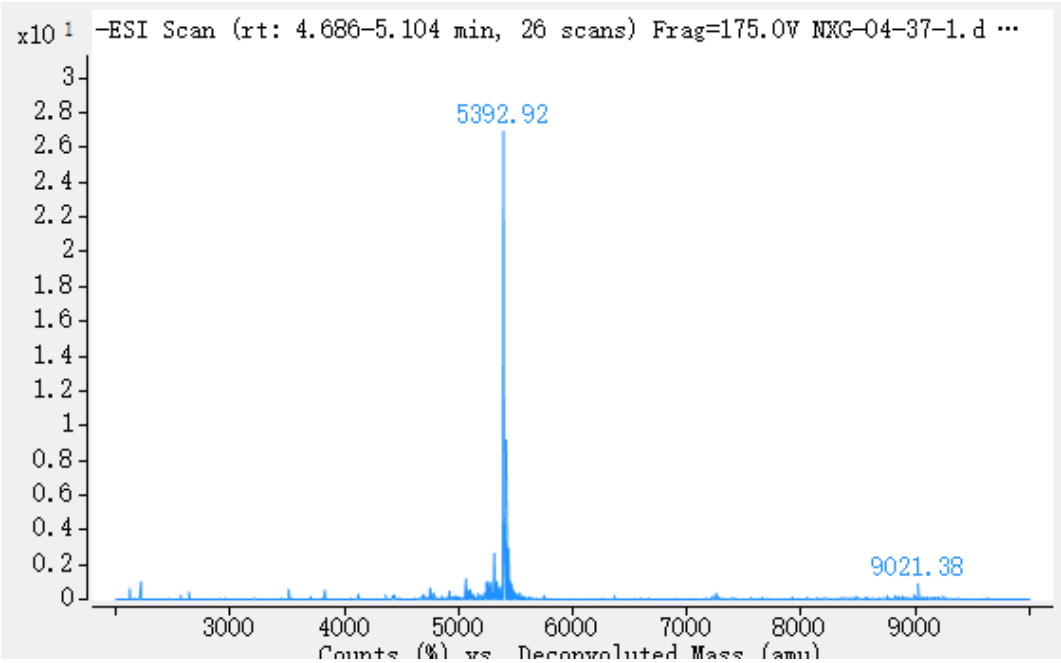
UHPLC chromatogram and deconvoluted MS of **d25**

Conversion: 79%

Calculated Mass: 5393 Da; Observed Mass: 5393 Da



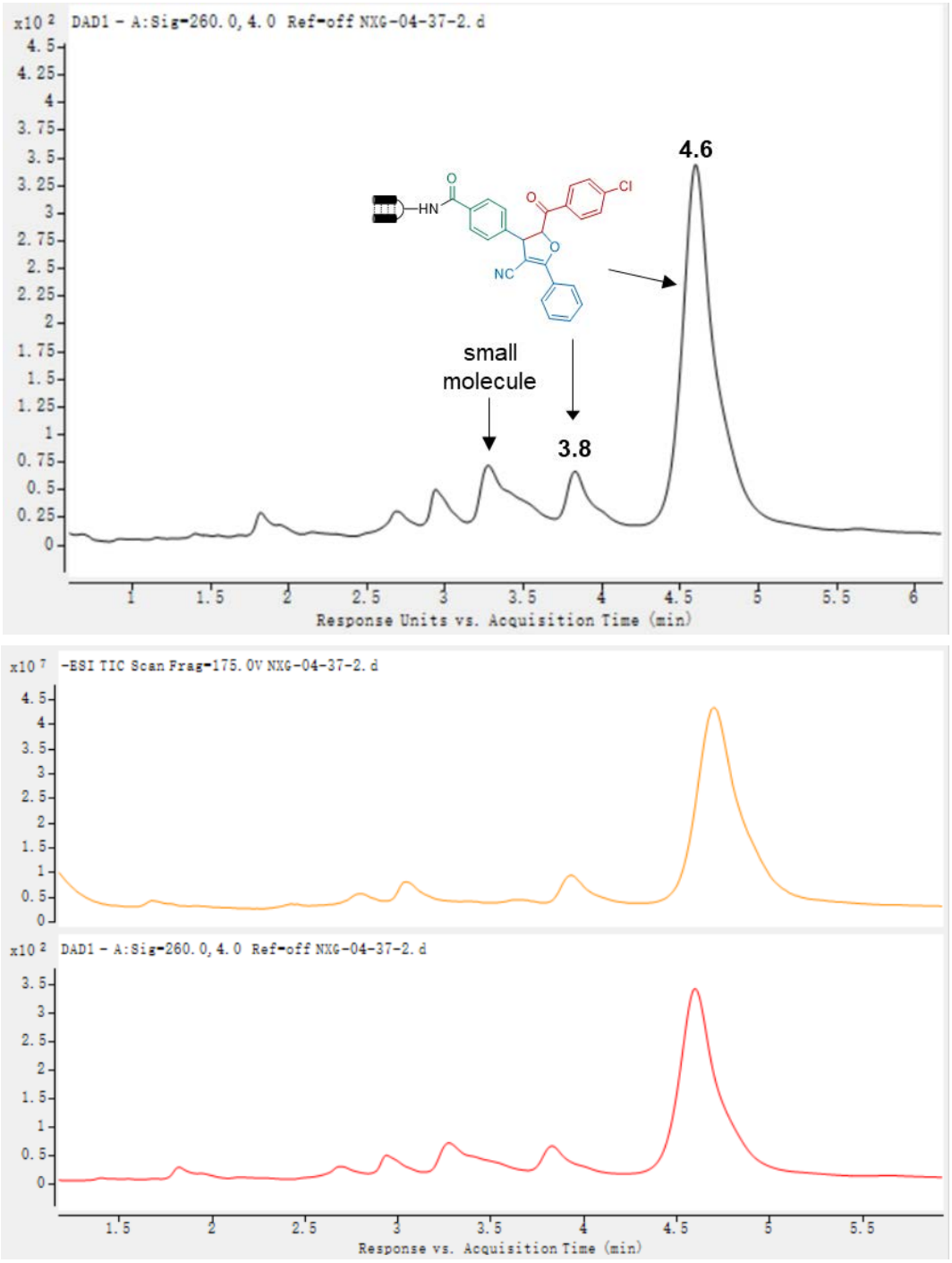

x10 1
-ESI Scan (rt: 3.983-4.569 min, 36 scans) Frag=175.0V NXG-04-37-1.d ...
5392.92
7250.38
Counts (%) vs. Deconvoluted Mass (amu)

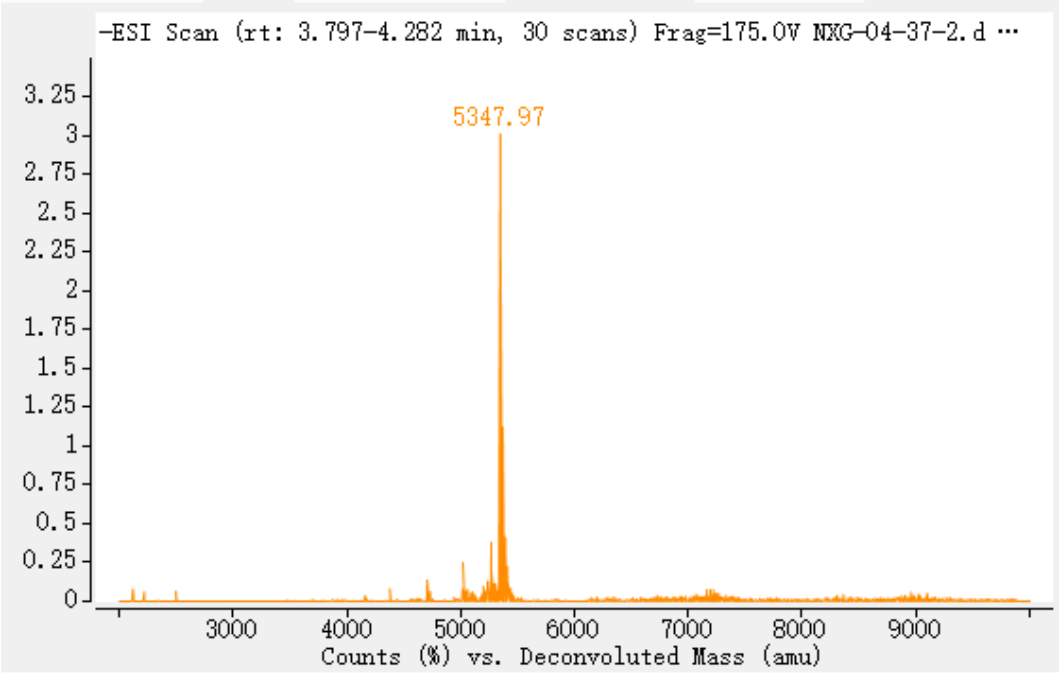

x10 1
-ESI Scan (rt: 4.686-5.104 min, 26 scans) Frag=175.0V NXG-04-37-1.d ...
5392.92
9021.38
Counts (%) vs. Deconvoluted Mass (amu)

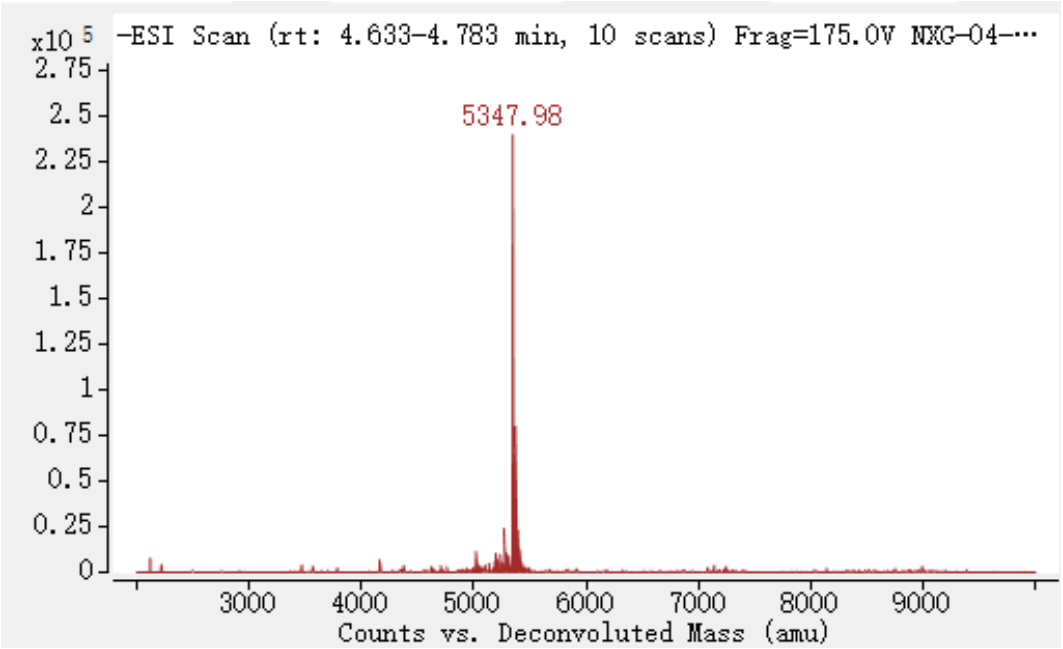
UHPLC chromatogram and deconvoluted MS of **d26**

Conversion: 89%

Calculated Mass: 5348 Da; Observed Mass: 5348 Da



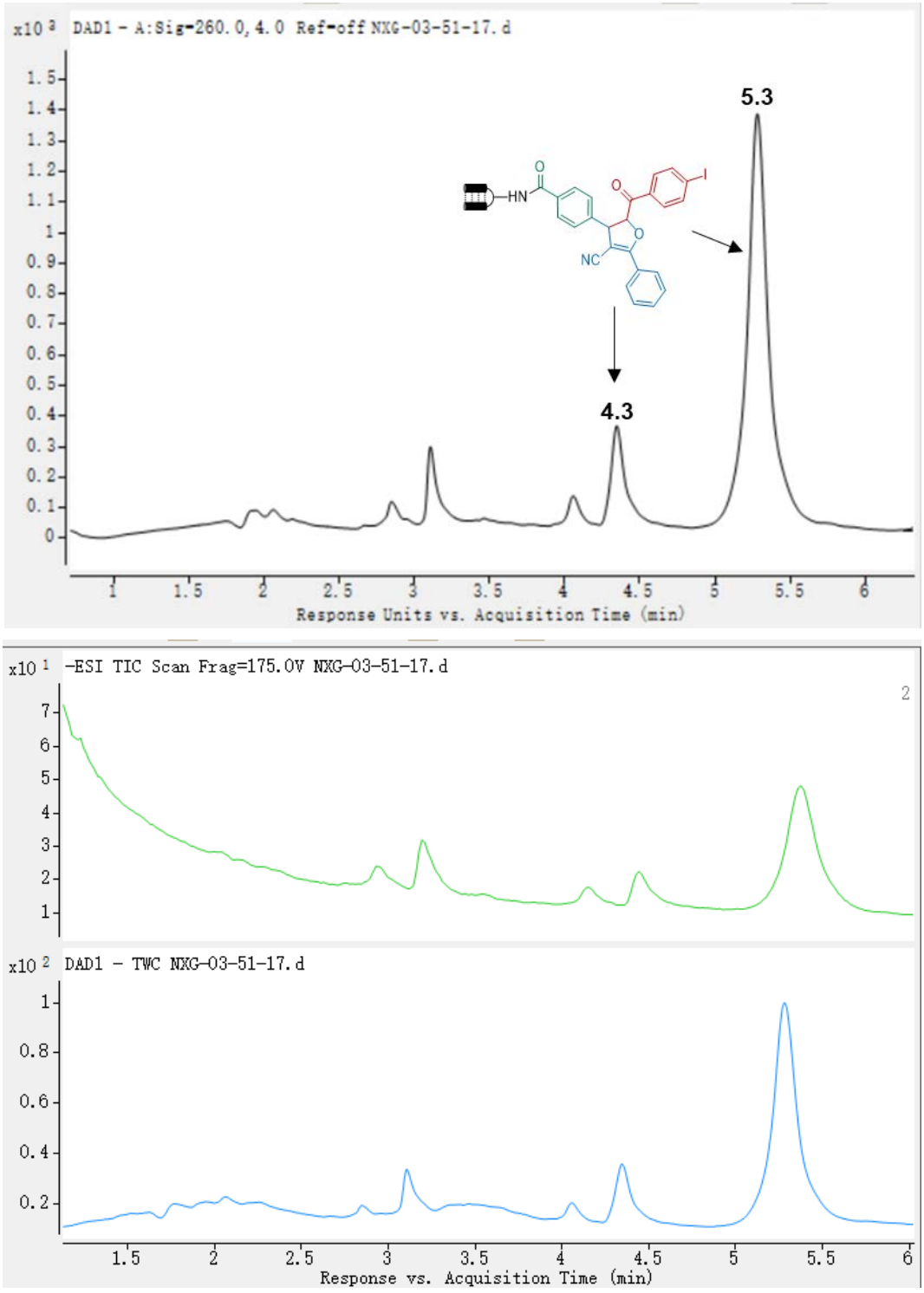

-ESI Scan (rt: 3.797-4.282 min, 30 scans) Frag=175.0V NXG-04-37-2.d ···
5347.97
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

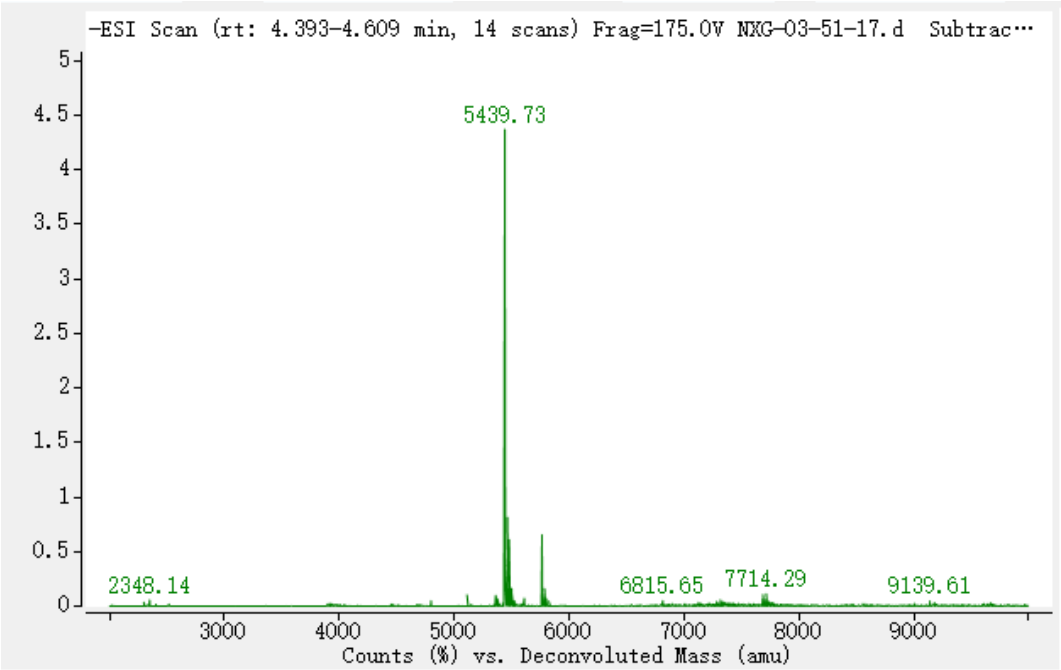

x10 5
-ESI Scan (rt: 4.633-4.783 min, 10 scans) Frag=175.0V NXG-04-···
5347.98
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

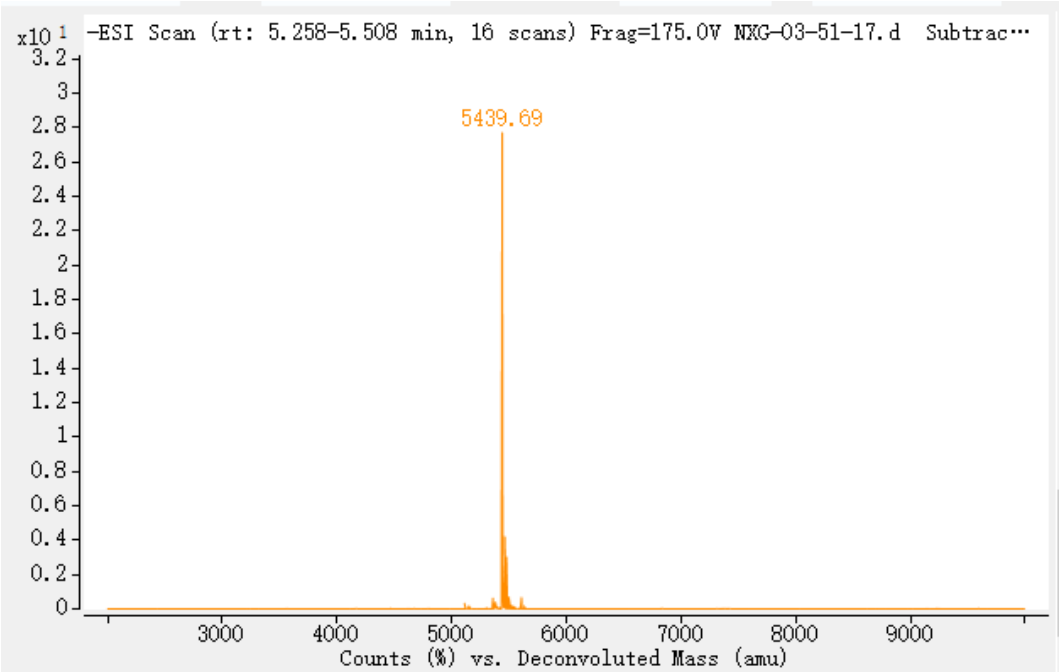
UHPLC chromatogram and deconvoluted MS of **d27**

Conversion: 85%

Calculated Mass: 5440 Da; Observed Mass: 5440 Da



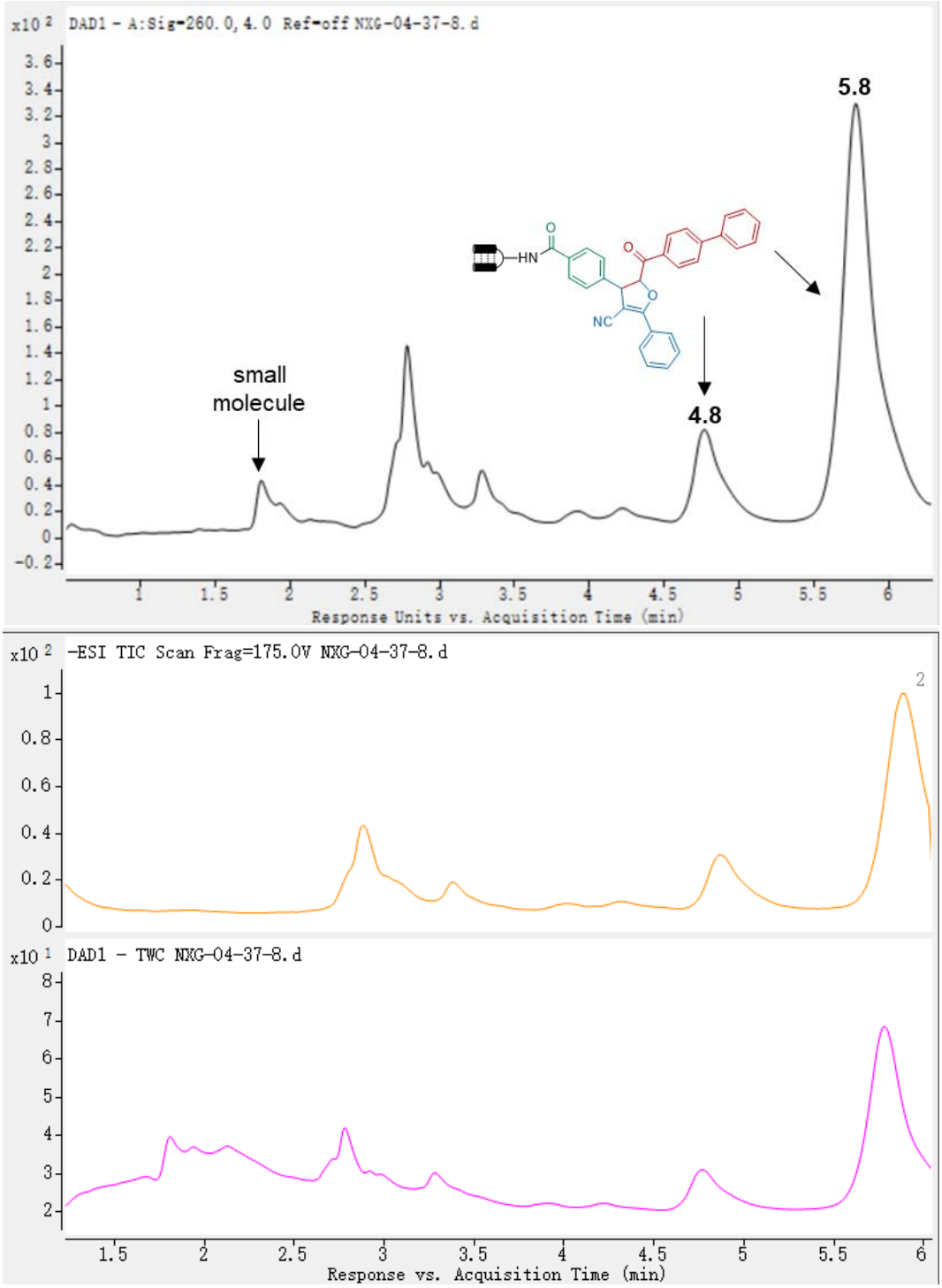

-ESI Scan (rt: 4.393-4.609 min, 14 scans) Frag=175.0V NXG-03-51-17.d Subtrac…
5439.73
2348.14
6815.65
7714.29
9139.61
Counts (%) vs. Deconvoluted Mass (amu)

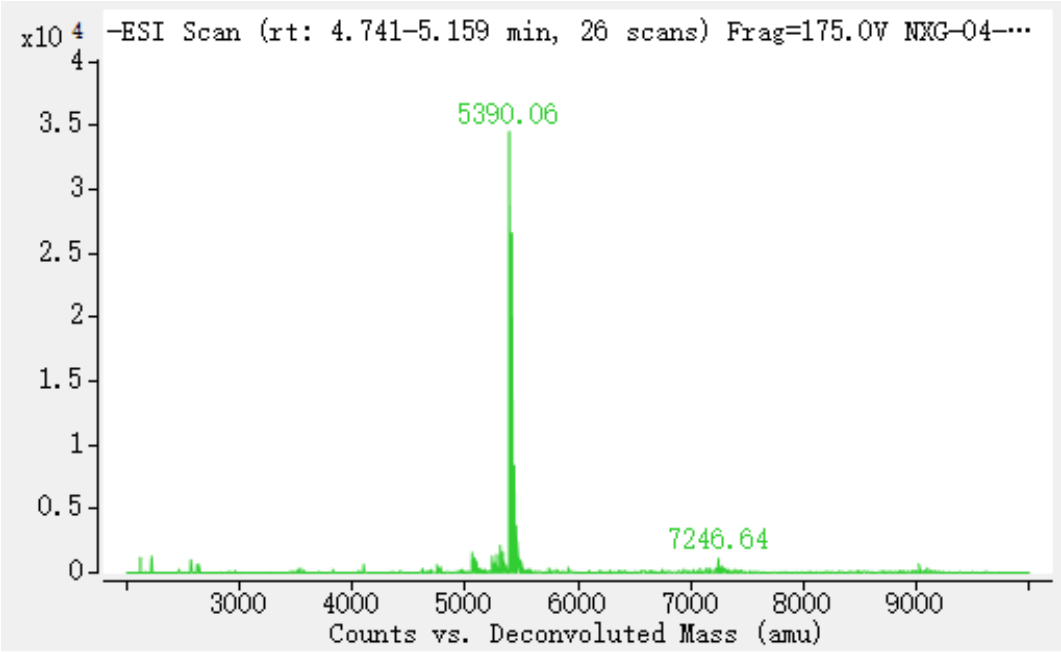

x10 1
-ESI Scan (rt: 5.258-5.508 min, 16 scans) Frag=175.0V NXG-03-51-17.d Subtrac…
5439.69
Counts (%) vs. Deconvoluted Mass (amu)

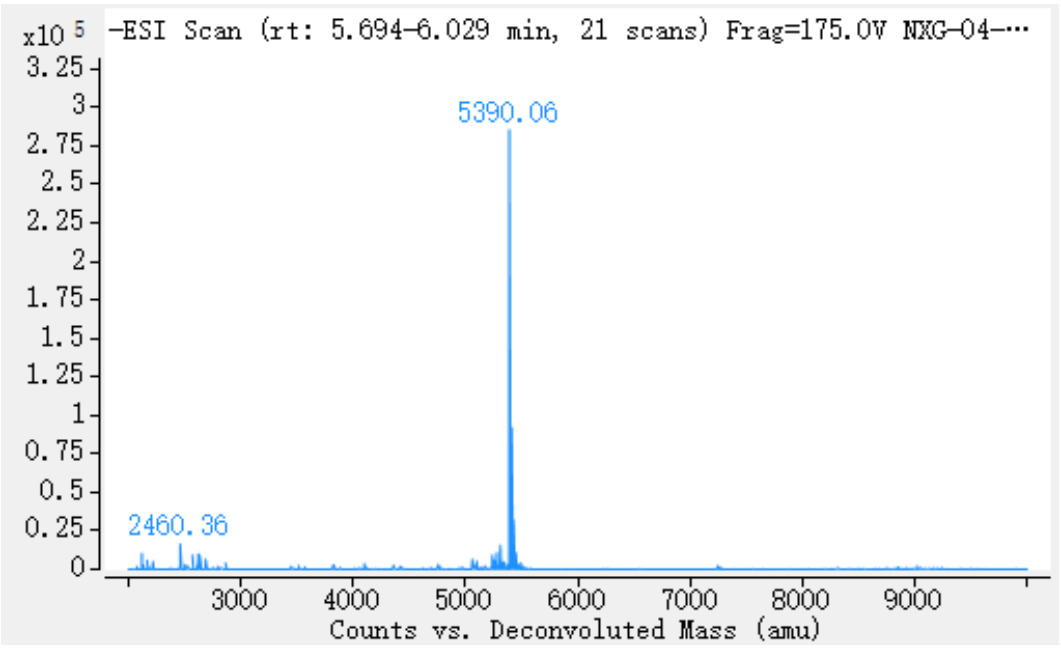
UHPLC chromatogram and deconvoluted MS of **d28**

Conversion: 72%

Calculated Mass: 5390 Da; Observed Mass: 5390 Da



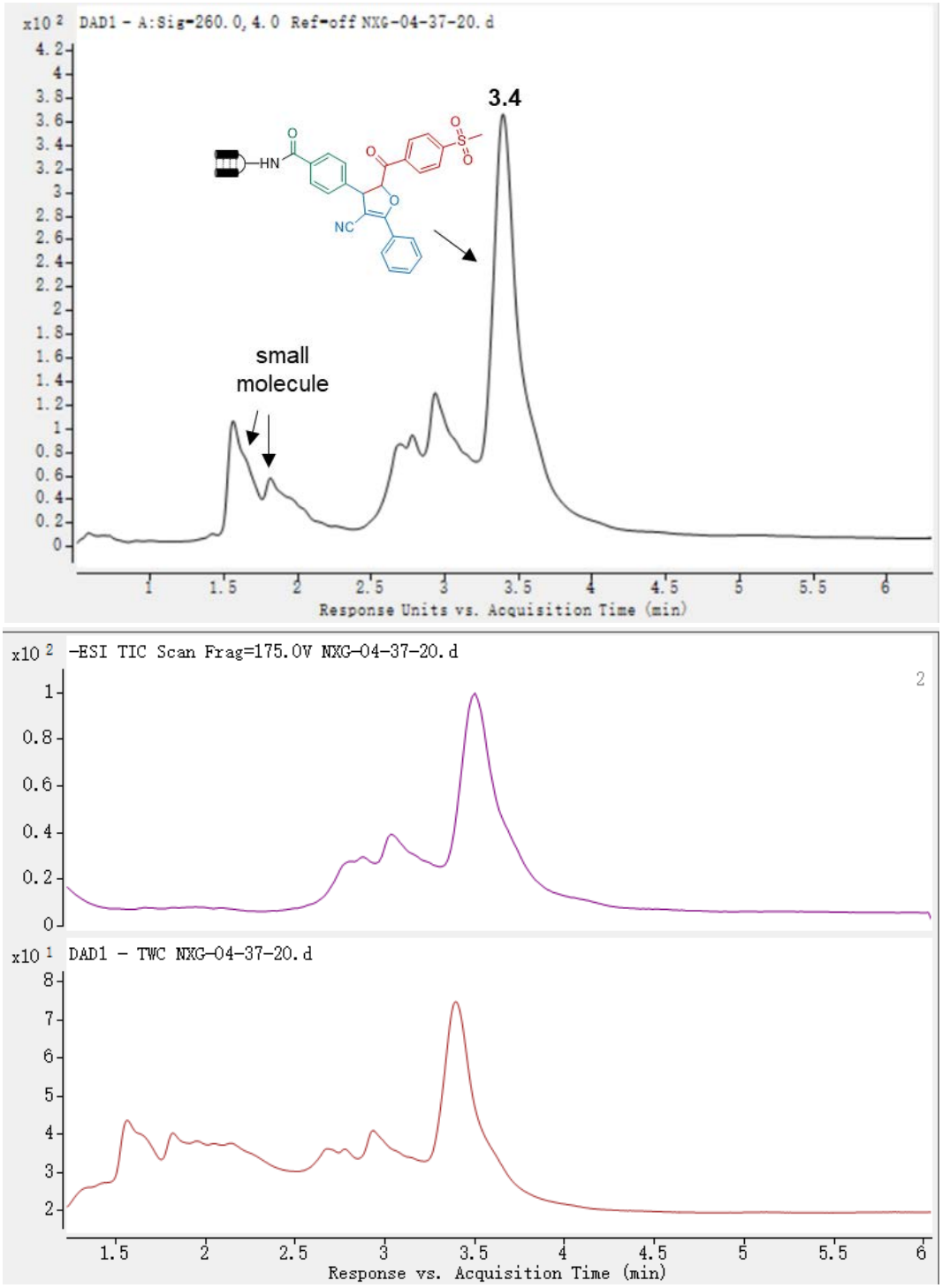

-ESI Scan (rt: 4.741-5.159 min, 26 scans) Frag=175.0V NXG-04-…
x10 4
4
3.5
3
2.5
2
1.5
1
0.5
0
5390.06
7246.64
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

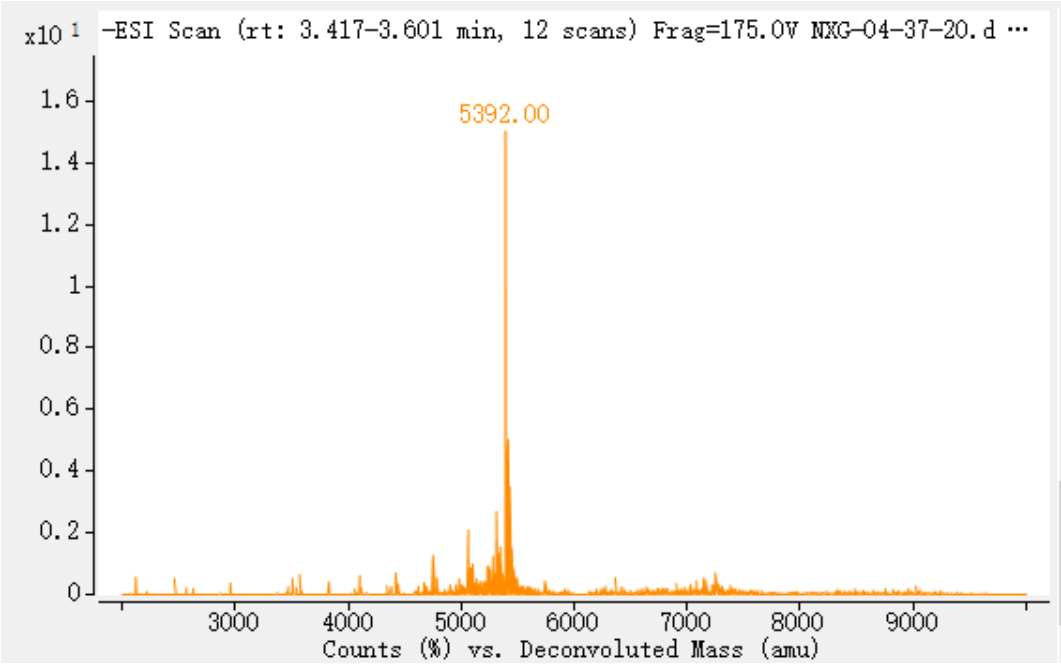

-ESI Scan (rt: 5.694-6.029 min, 21 scans) Frag=175.0V NXG-04-…
x10 5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
5390.06
2460.36
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

UHPLC chromatogram and deconvoluted MS of **d29**

Conversion: 68%

Calculated Mass: 5392 Da; Observed Mass: 5392 Da

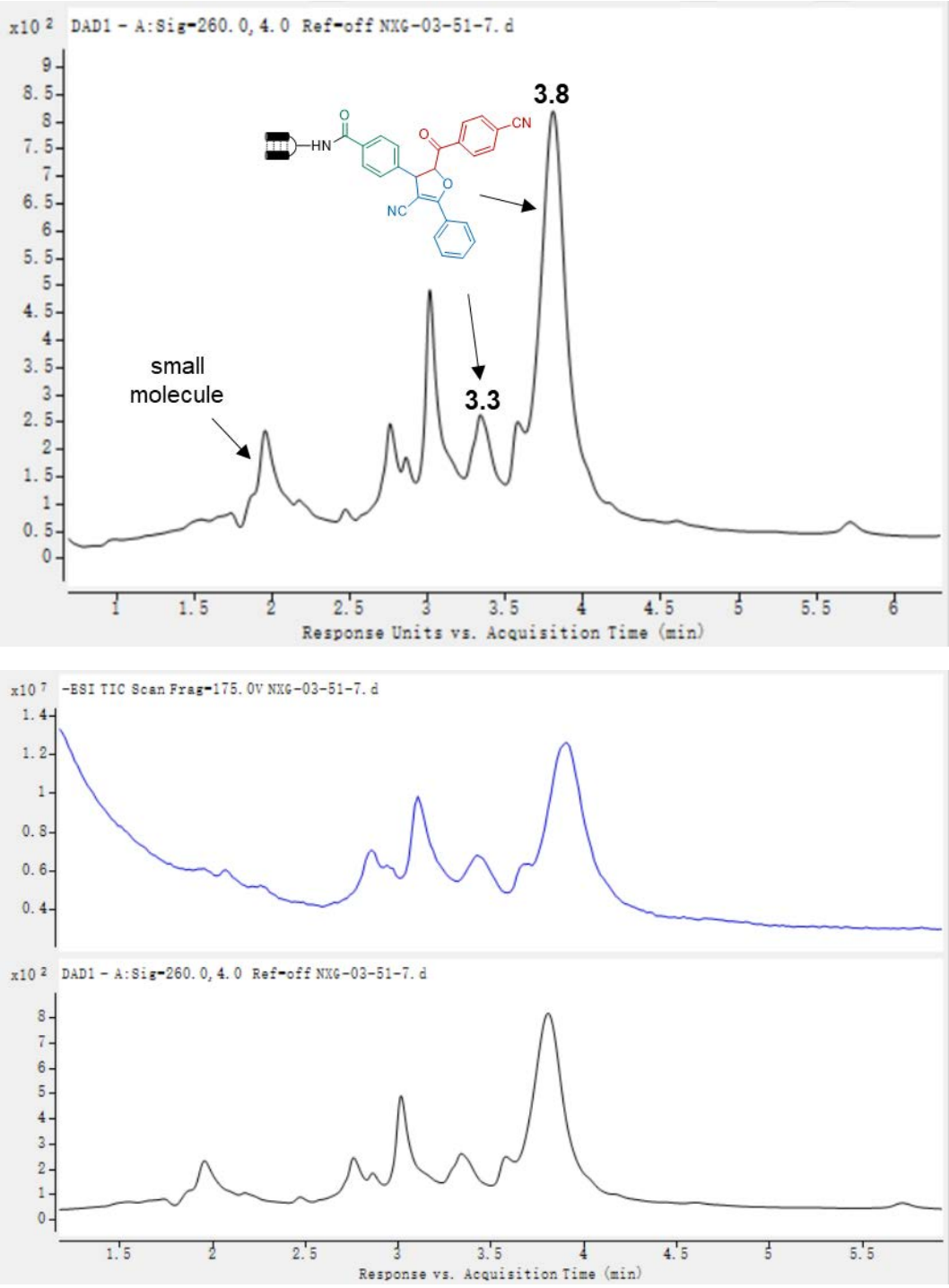


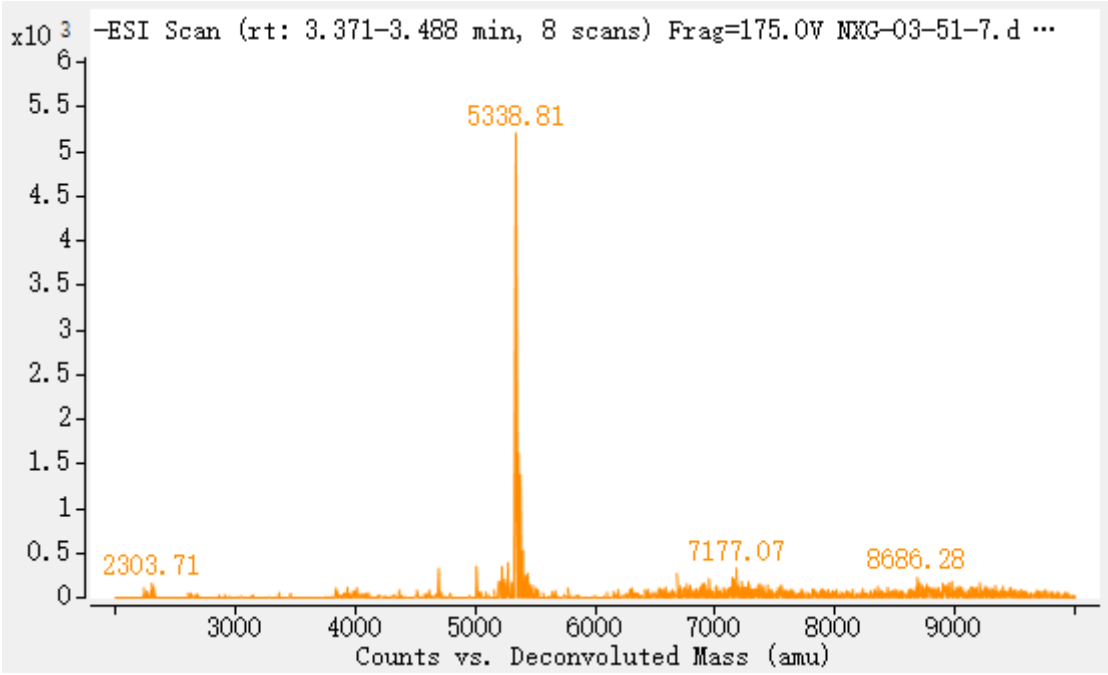

x10 1
-ESI Scan (rt: 3.417-3.601 min, 12 scans) Frag=175.0V NXG-04-37-20.d ...
5392.00
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

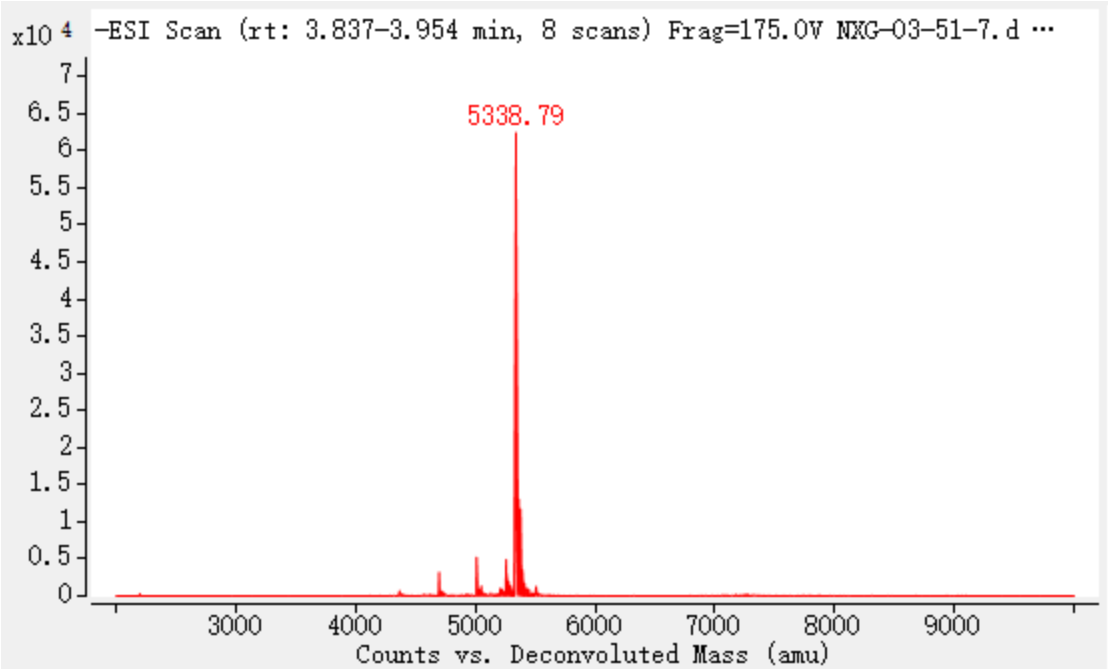
UHPLC chromatogram and deconvoluted MS of **d30**

Conversion: 69%

Calculated Mass: 5339 Da; Observed Mass: 5339 Da



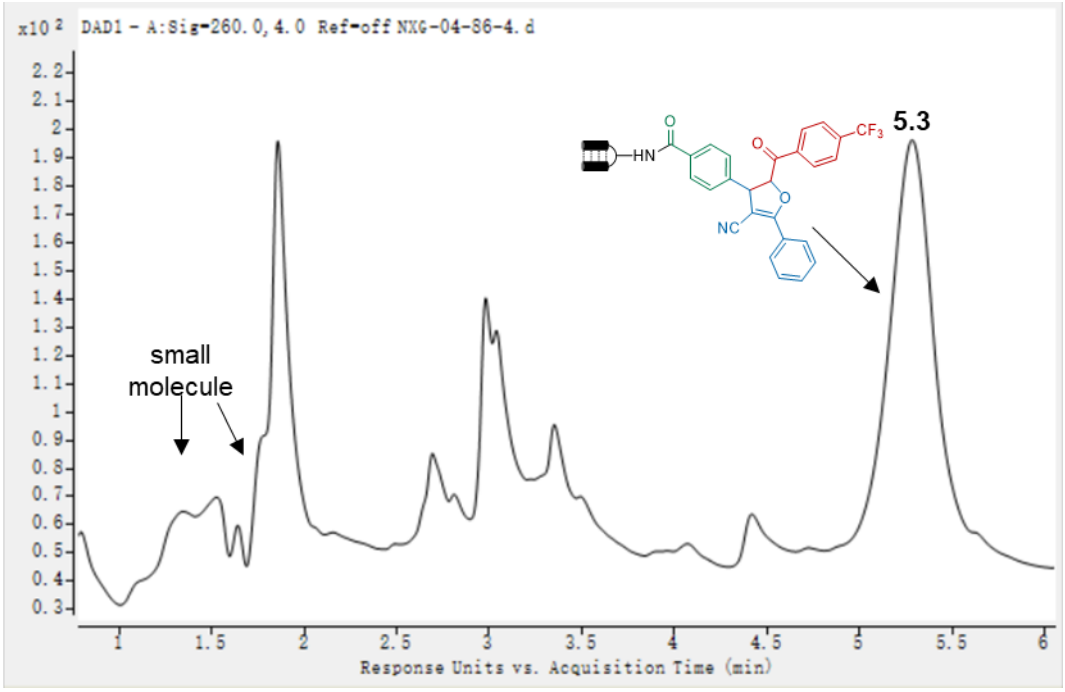

x10 3
-ESI Scan (rt: 3.371-3.488 min, 8 scans) Frag=175.0V NXG-03-51-7.d ...
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5338.81
2303.71
7177.07
8686.28
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

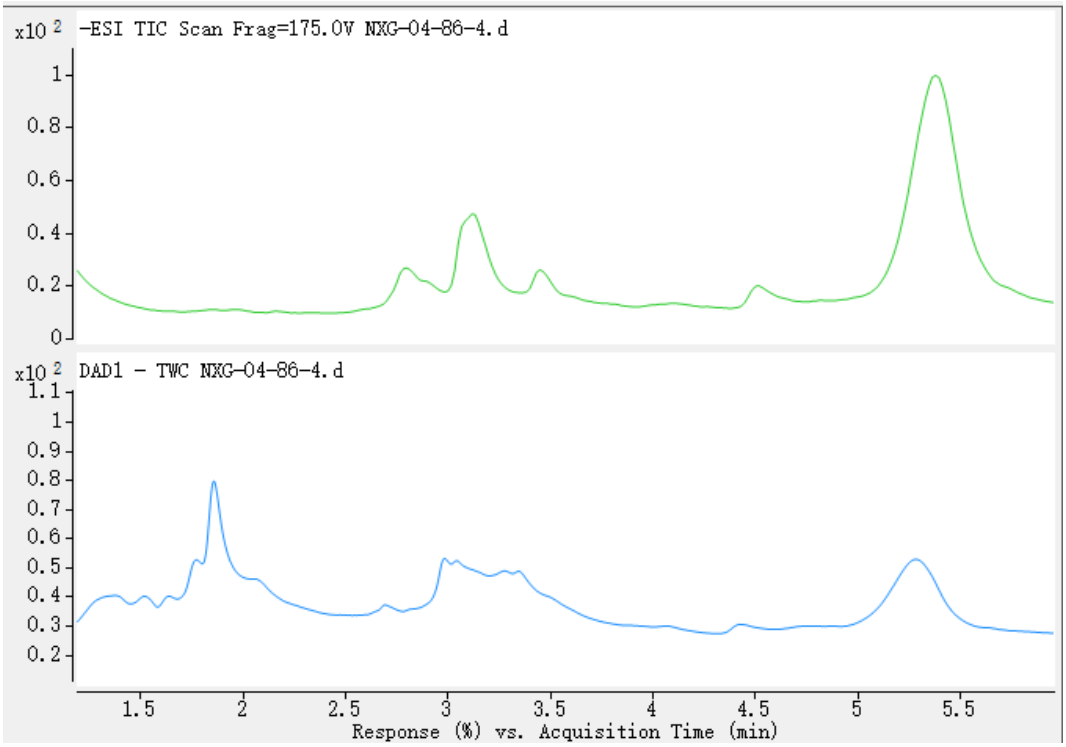

x10 4
-ESI Scan (rt: 3.837-3.954 min, 8 scans) Frag=175.0V NXG-03-51-7.d ...
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5338.79
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

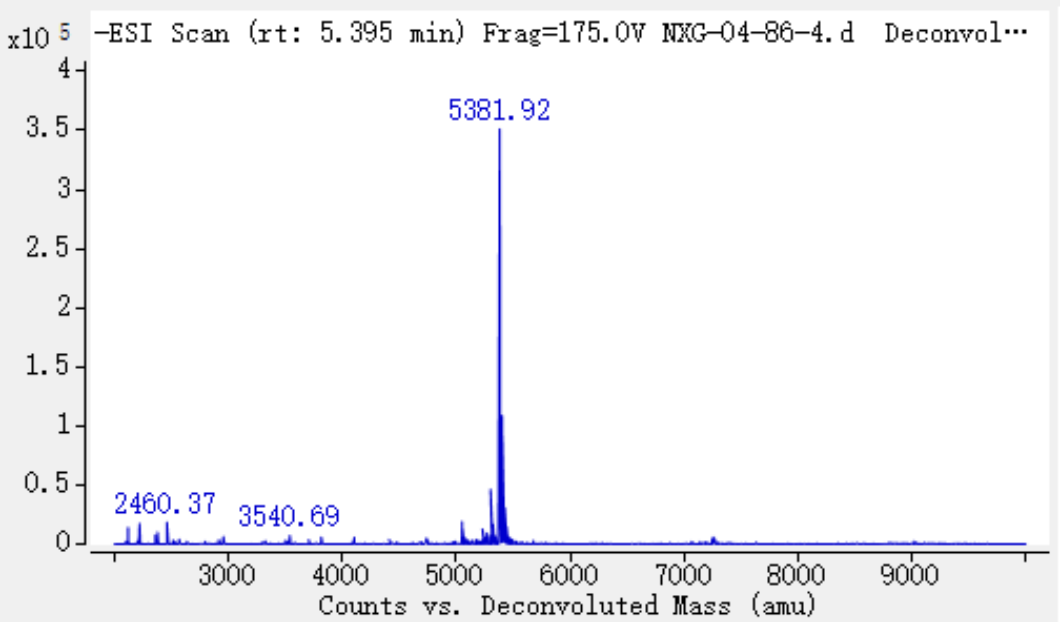
UHPLC chromatogram and deconvoluted MS of **d31**

Conversion: 57%

Calculated Mass: 5382 Da; Observed Mass: 5382 Da



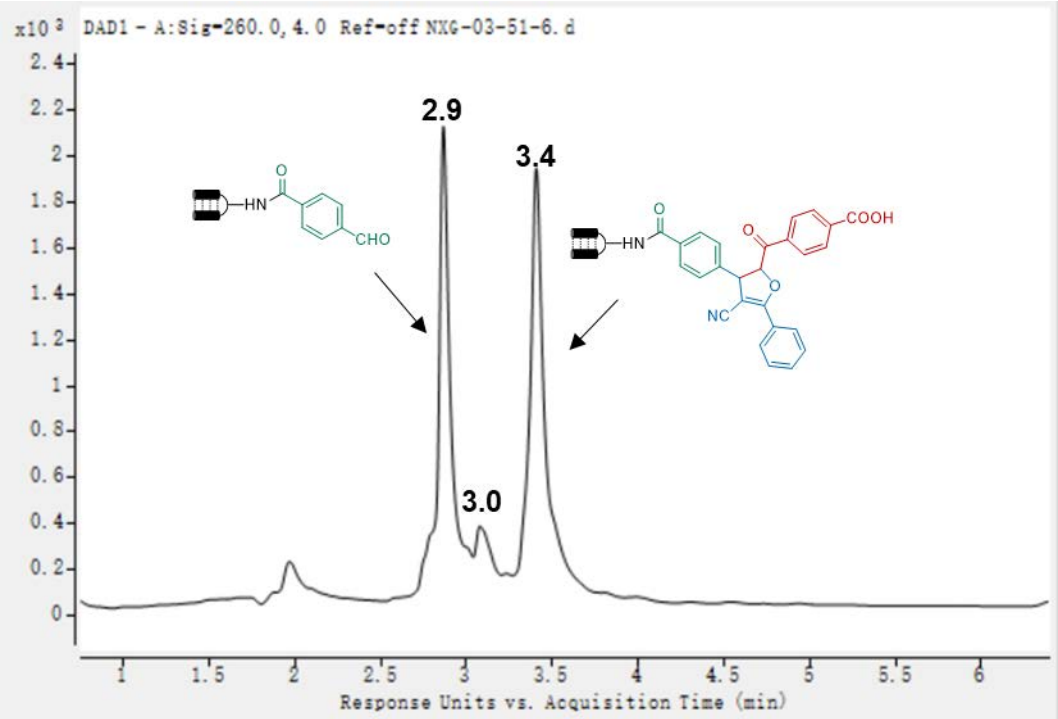


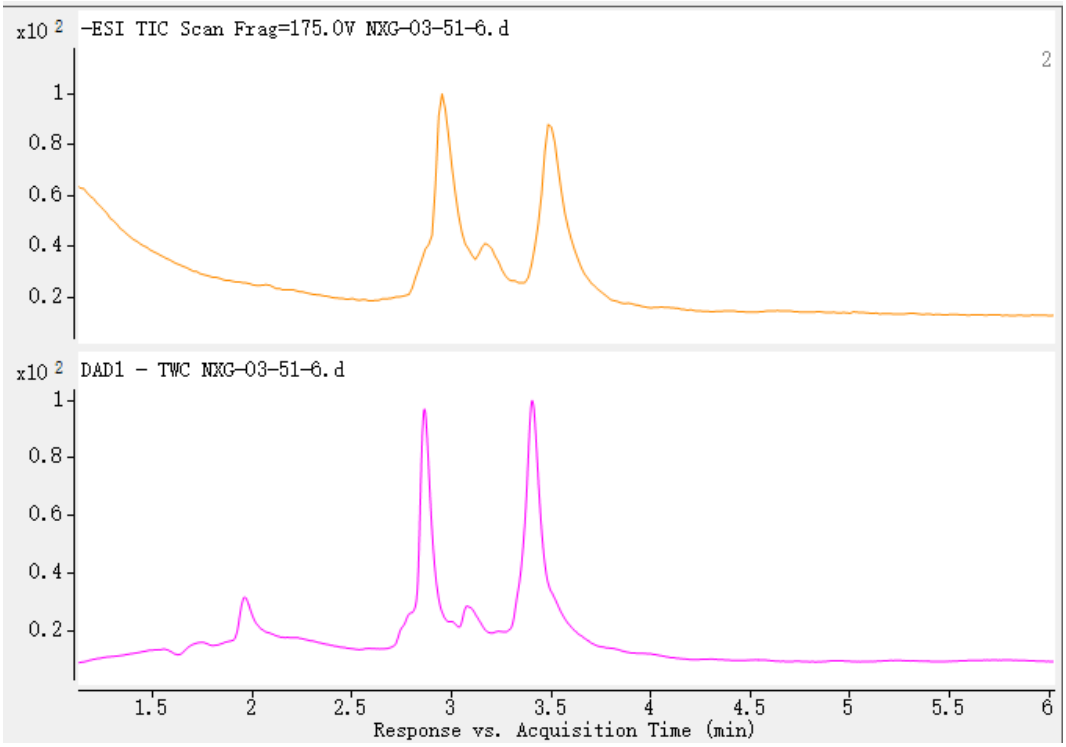

x10 5
-ESI Scan (rt: 5.395 min) Frag=175.0V NXG-04-86-4.d Deconvol…
4
3.5
3
2.5
2
1.5
1
0.5
0
5381.92
2460.37
3540.69
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

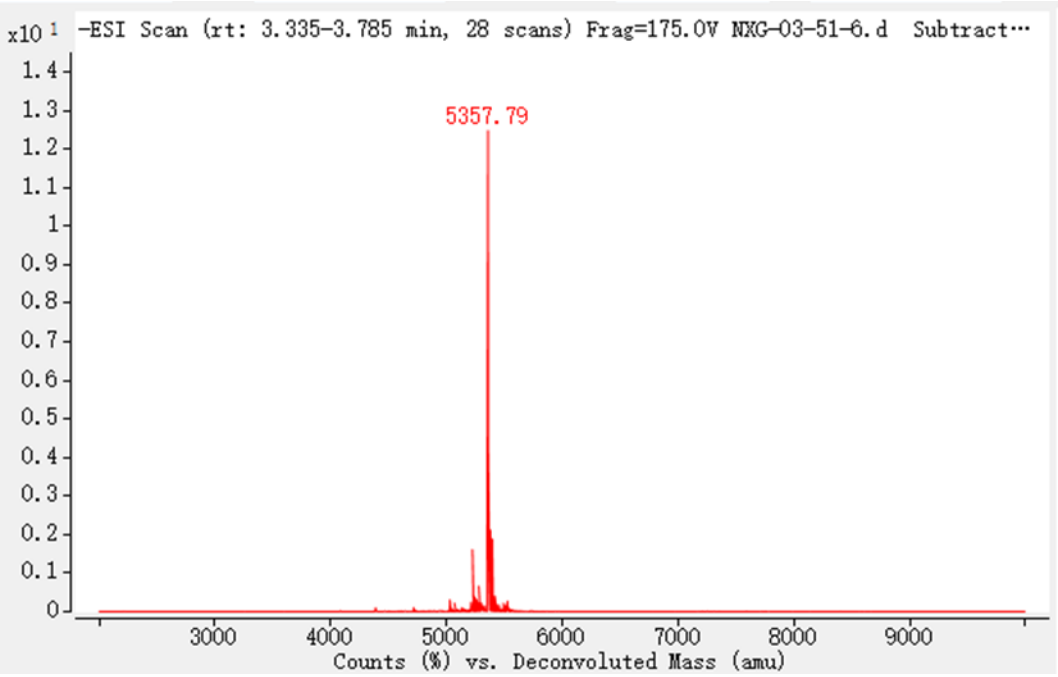
UHPLC chromatogram and deconvoluted MS of **d32**

Conversion: 48%

Calculated Mass: 5358 Da; Observed Mass: 5358 Da



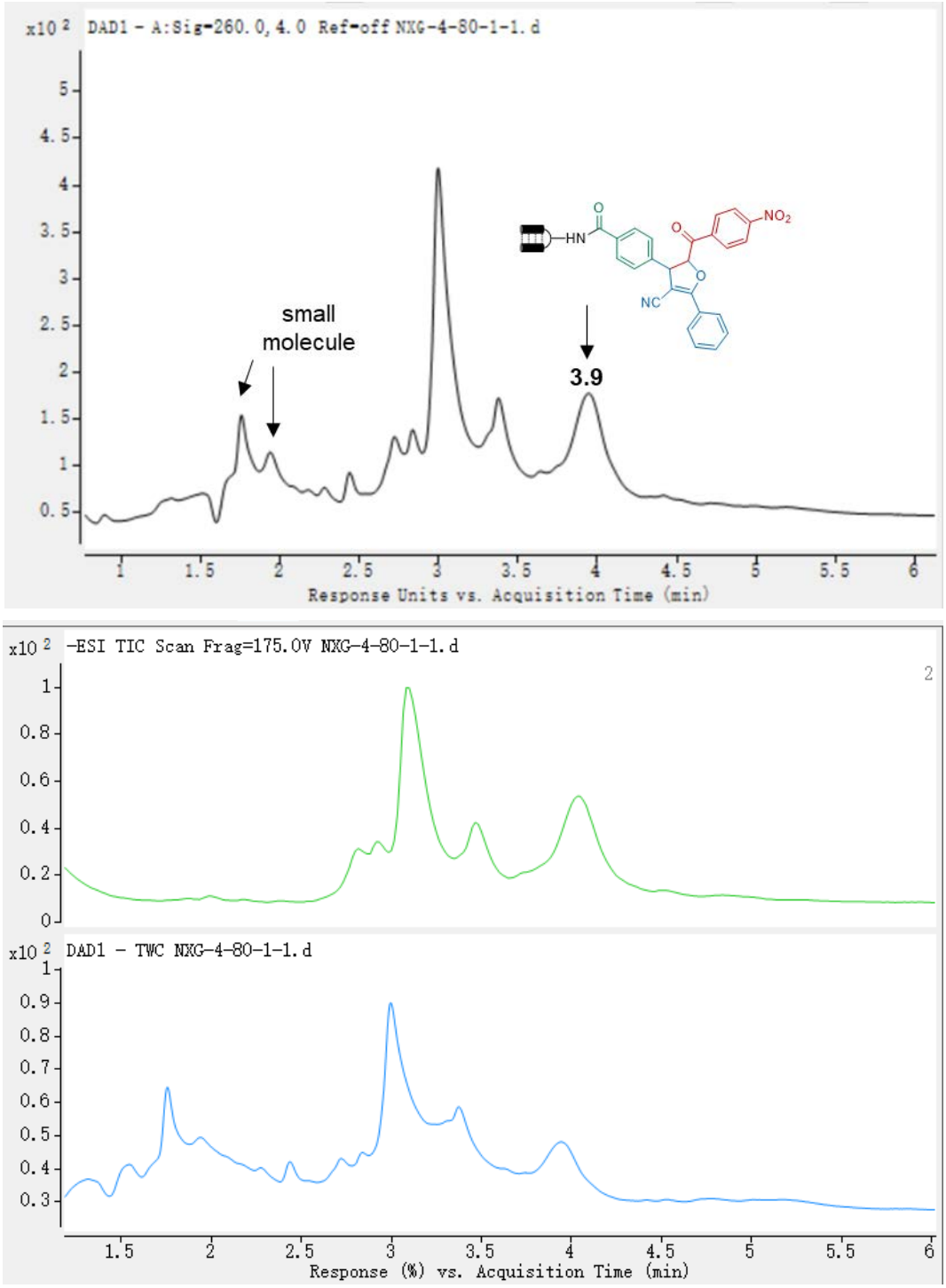


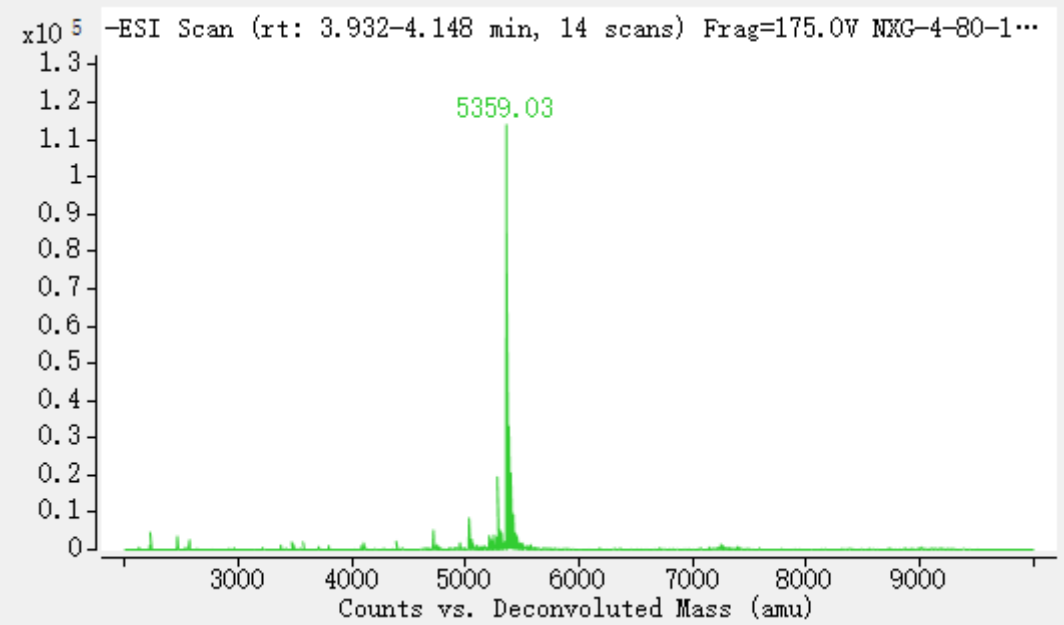

x10 1
-ESI Scan (rt: 3.335-3.785 min, 28 scans) Frag=175.0V NXG-03-51-6.d Subtract…
5357.79
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

UHPLC chromatogram and deconvoluted MS of **d33**

Conversion: 27%

Calculated Mass: 5359 Da; Observed Mass: 5359 Da

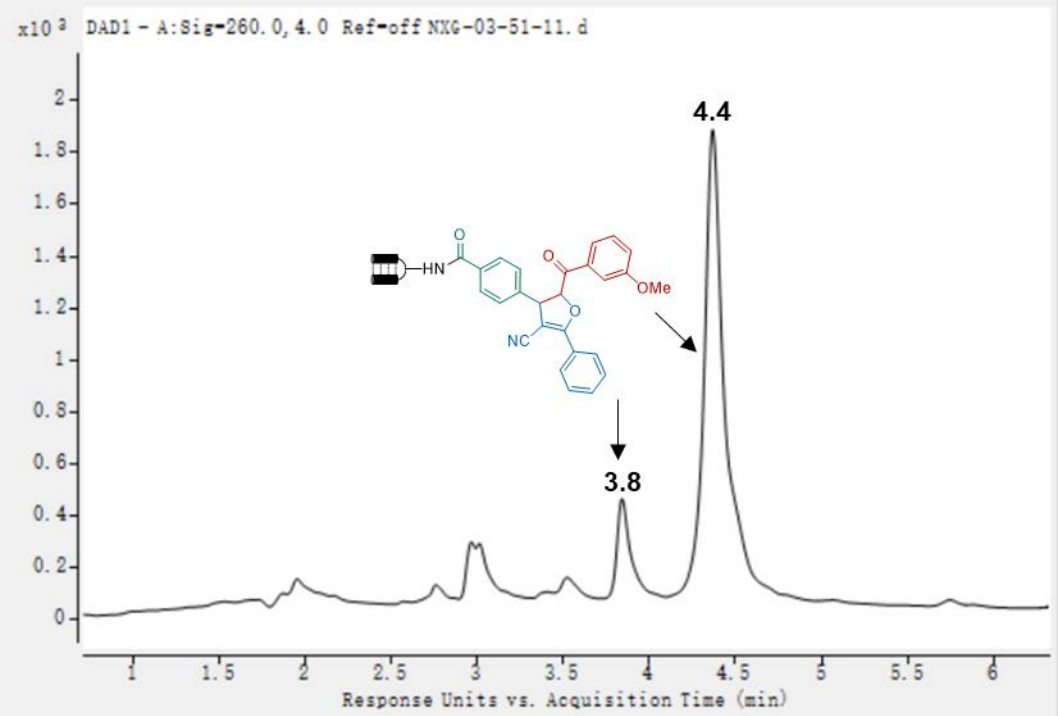


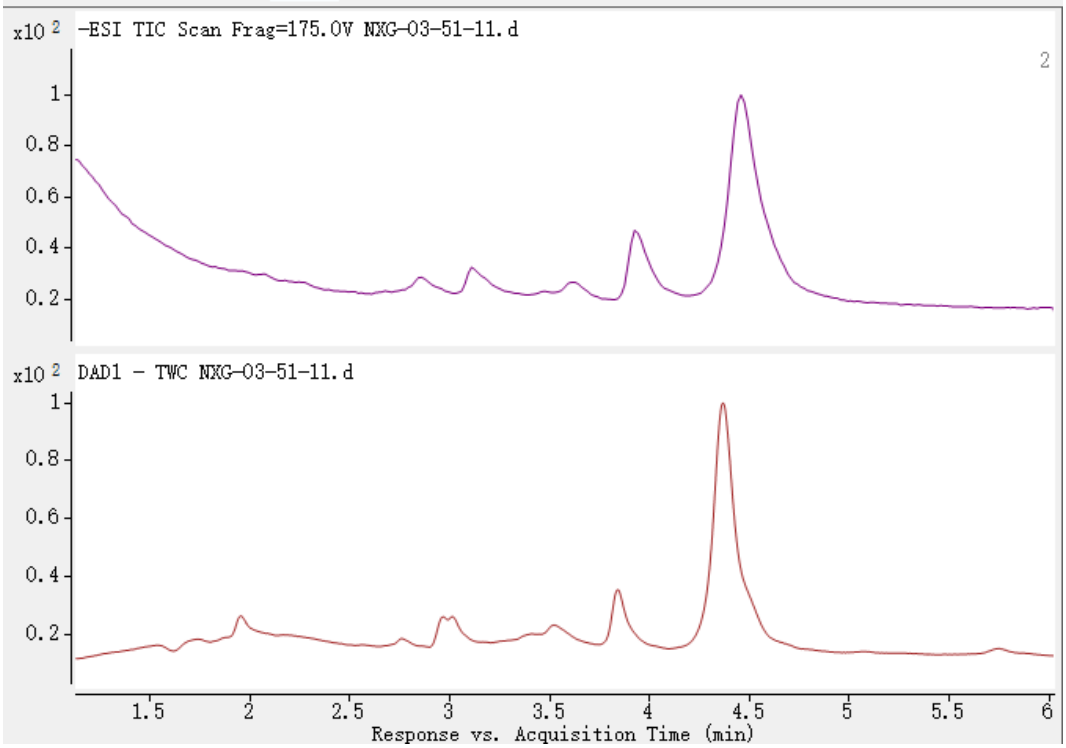

x10 5
-ESI Scan (rt: 3.932-4.148 min, 14 scans) Frag=175.0V NXG-4-80-1…
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5359.03
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

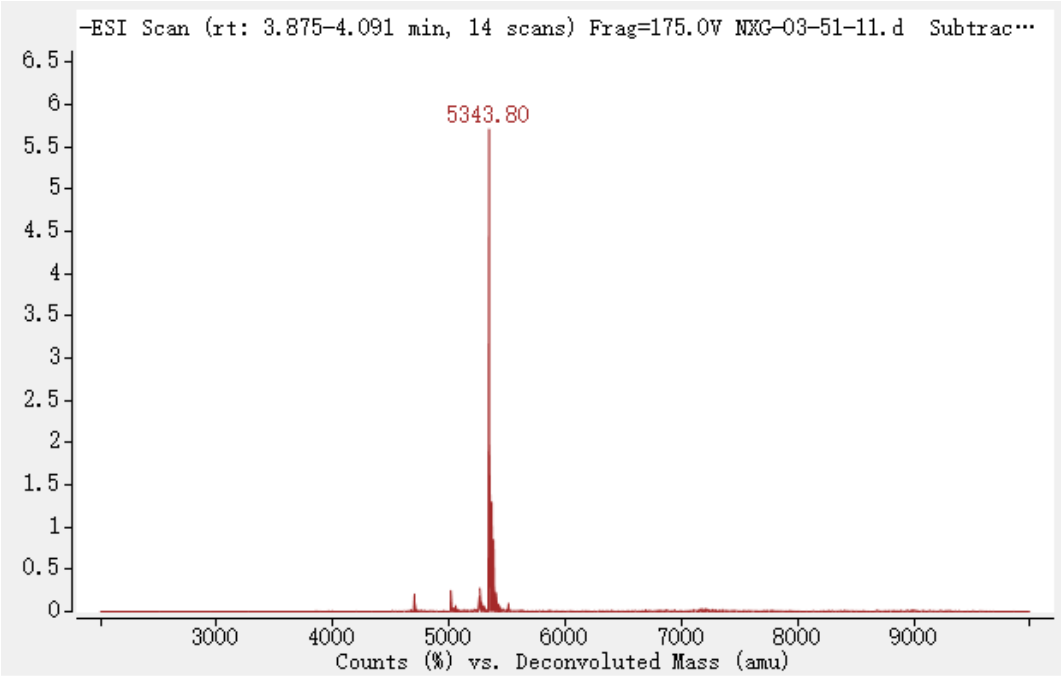
UHPLC chromatogram and deconvoluted MS of **d34**

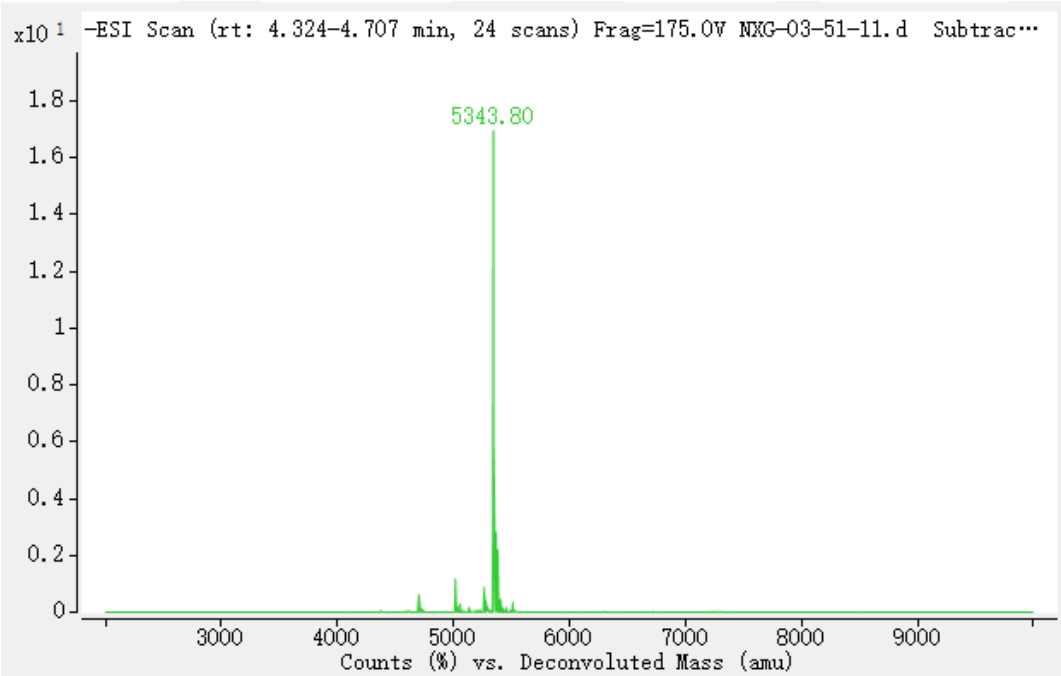
Conversion: 83%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da





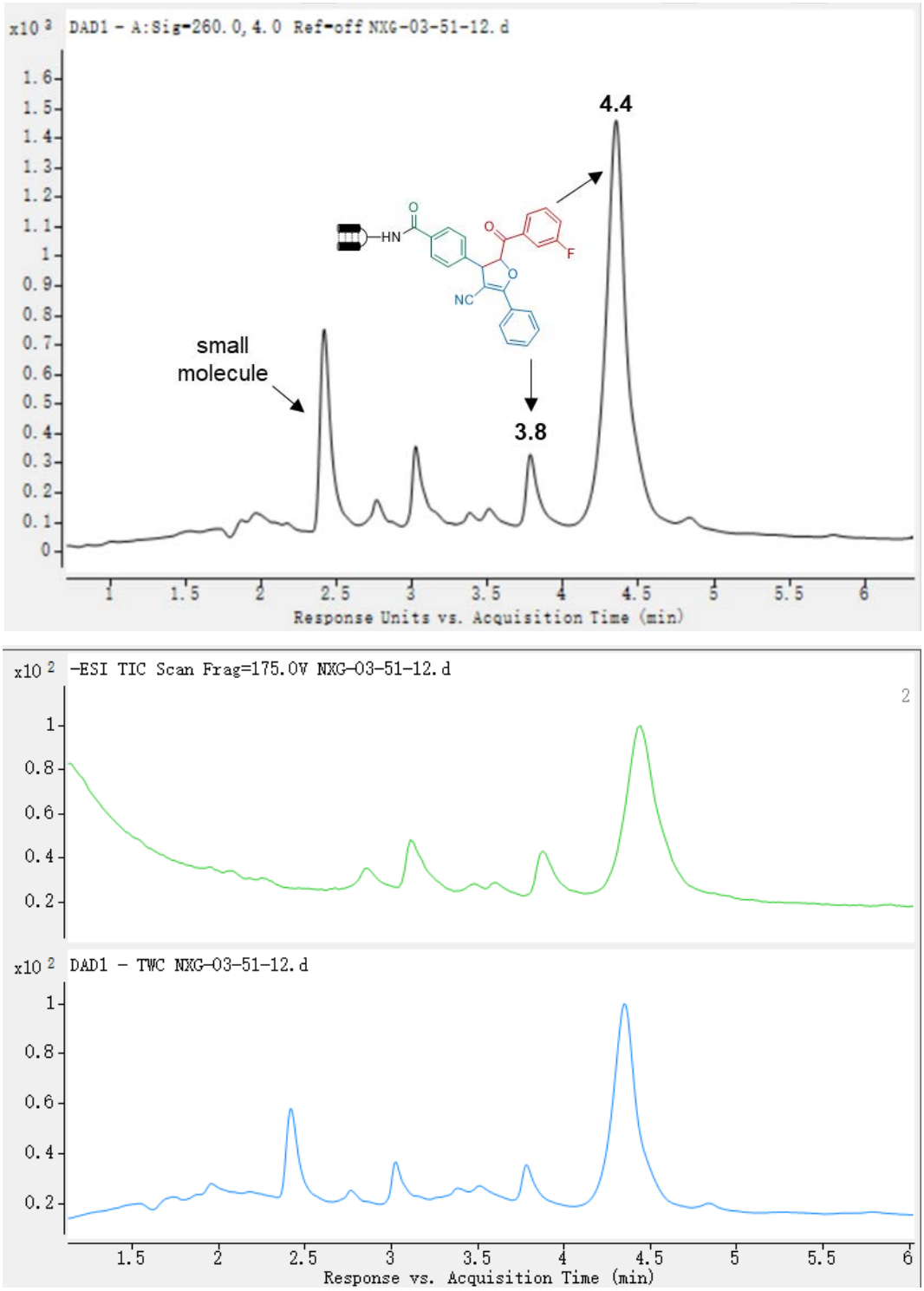

-ESI Scan (rt: 3.875-4.091 min, 14 scans) Frag=175.0V NXG-03-51-11.d Subtrac…
5343.80
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

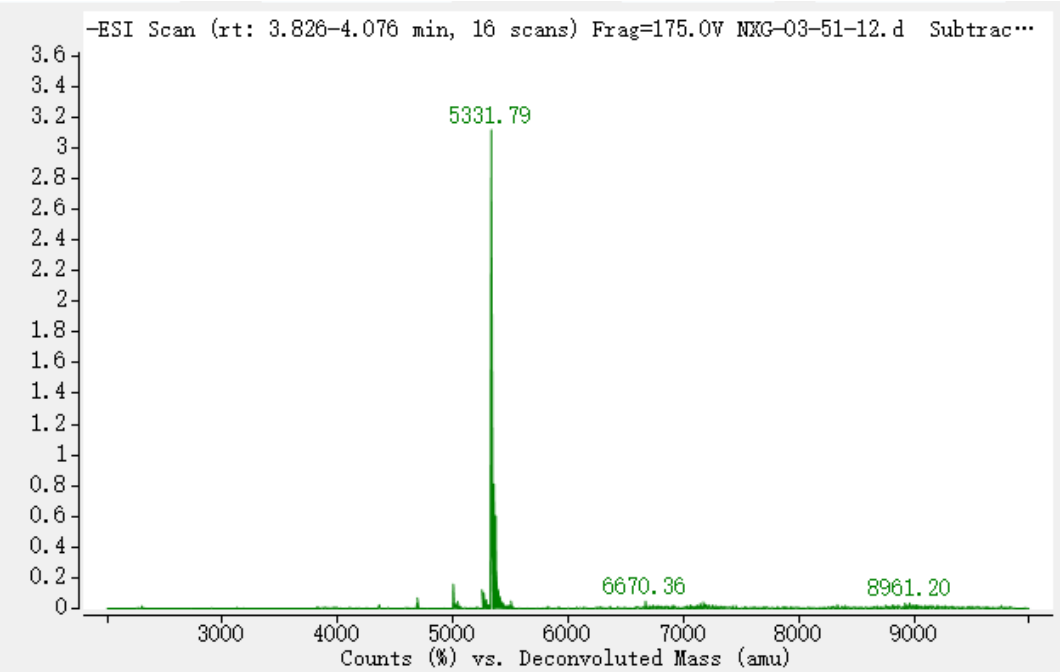

x10 1
-ESI Scan (rt: 4.324-4.707 min, 24 scans) Frag=175.0V NXG-03-51-11.d Subtrac…
5343.80
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

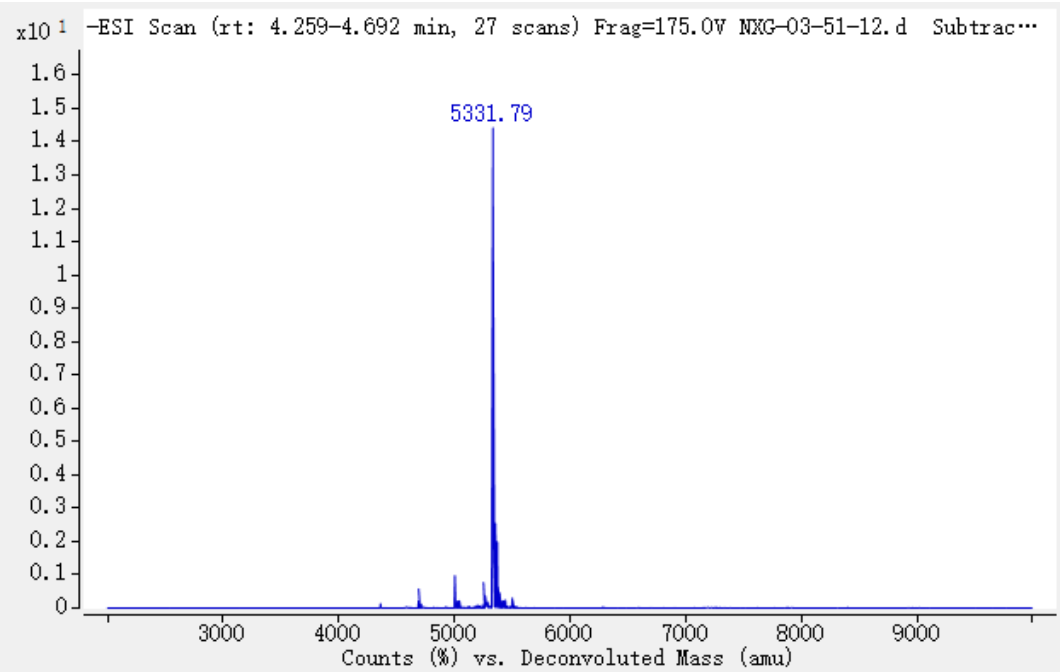
UHPLC chromatogram and deconvoluted MS of **d35**

Conversion: 78%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da



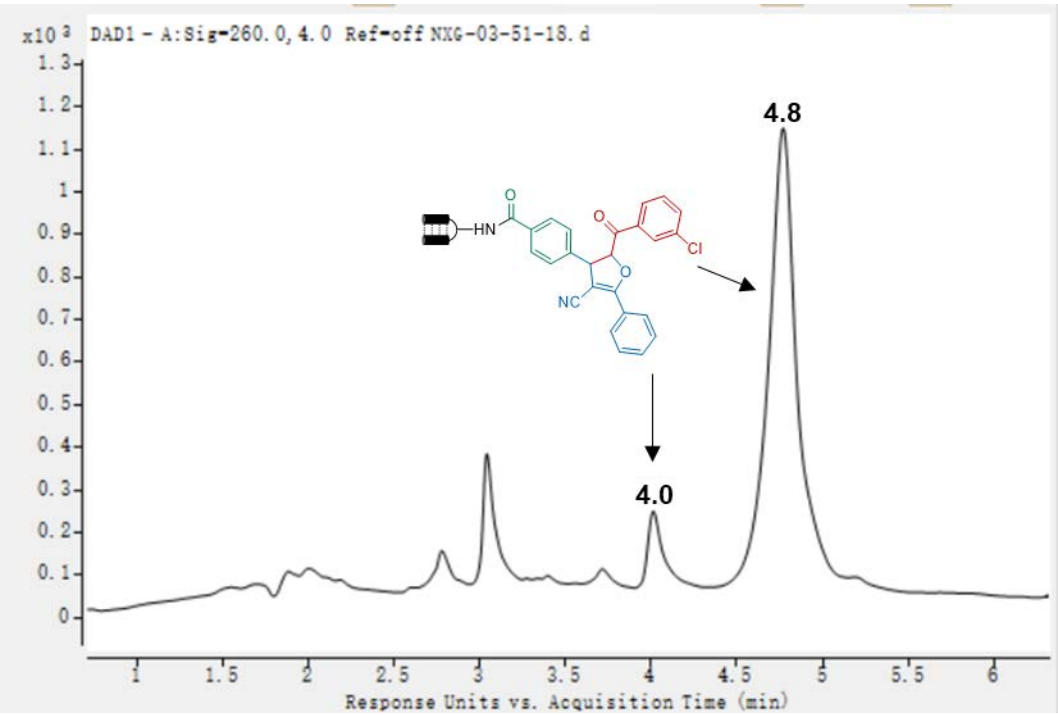

-ESI Scan (rt: 3.826-4.076 min, 16 scans) Frag=175.0V NXG-03-51-12.d Subtrac…
5331.79
6670.36
8961.20
Counts (%) vs. Deconvoluted Mass (amu)

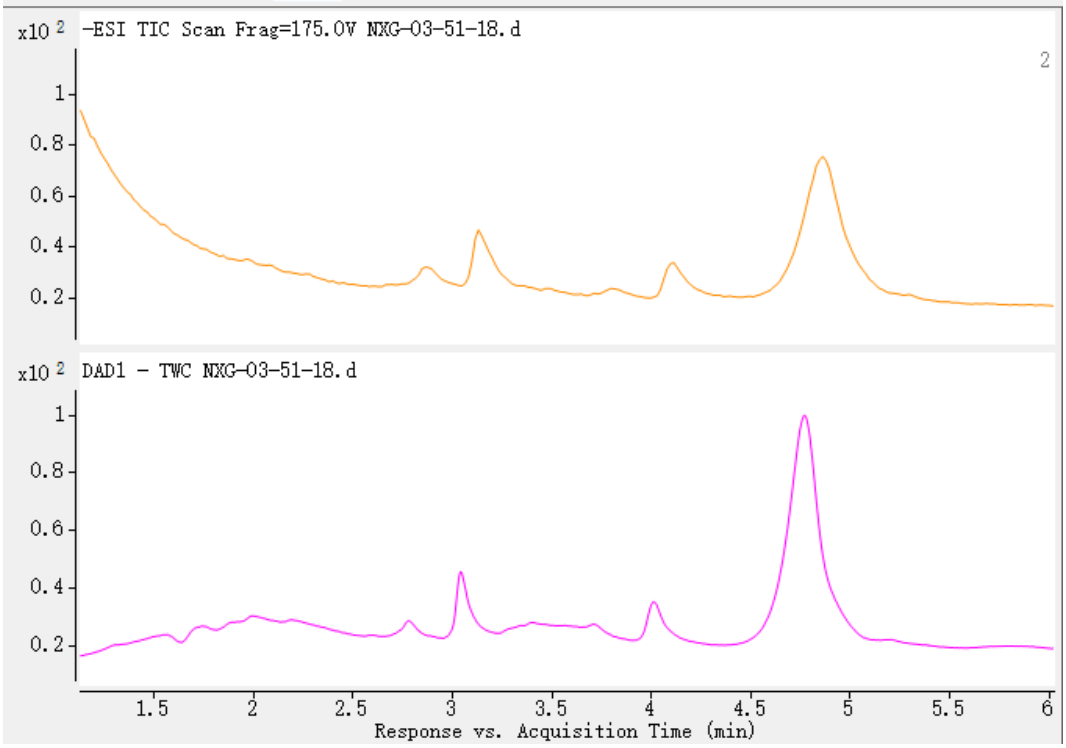

x10 1
-ESI Scan (rt: 4.259-4.692 min, 27 scans) Frag=175.0V NXG-03-51-12.d Subtrac…
5331.79
Counts (%) vs. Deconvoluted Mass (amu)

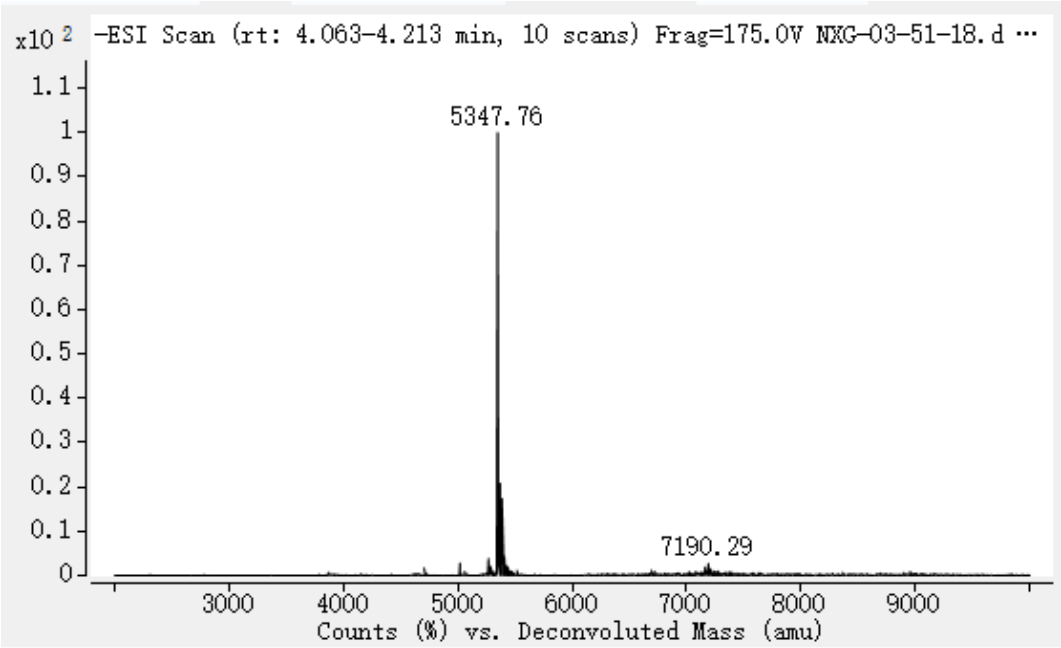
UHPLC chromatogram and deconvoluted MS of **d36**

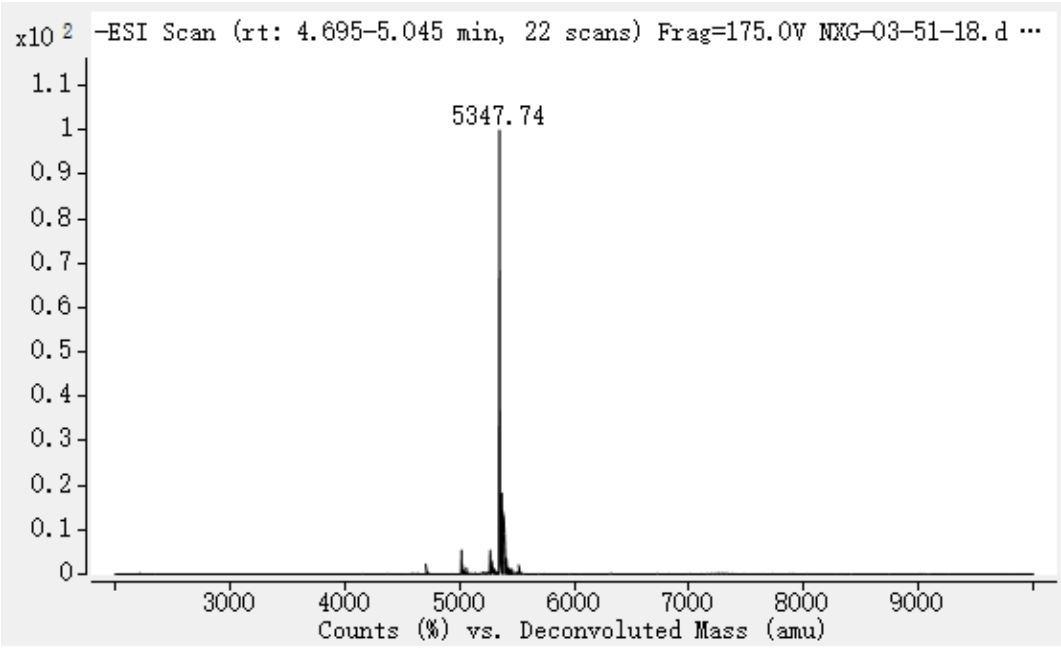
Conversion: 87%

Calculated Mass: 5348 Da; Observed Mass: 5348 Da





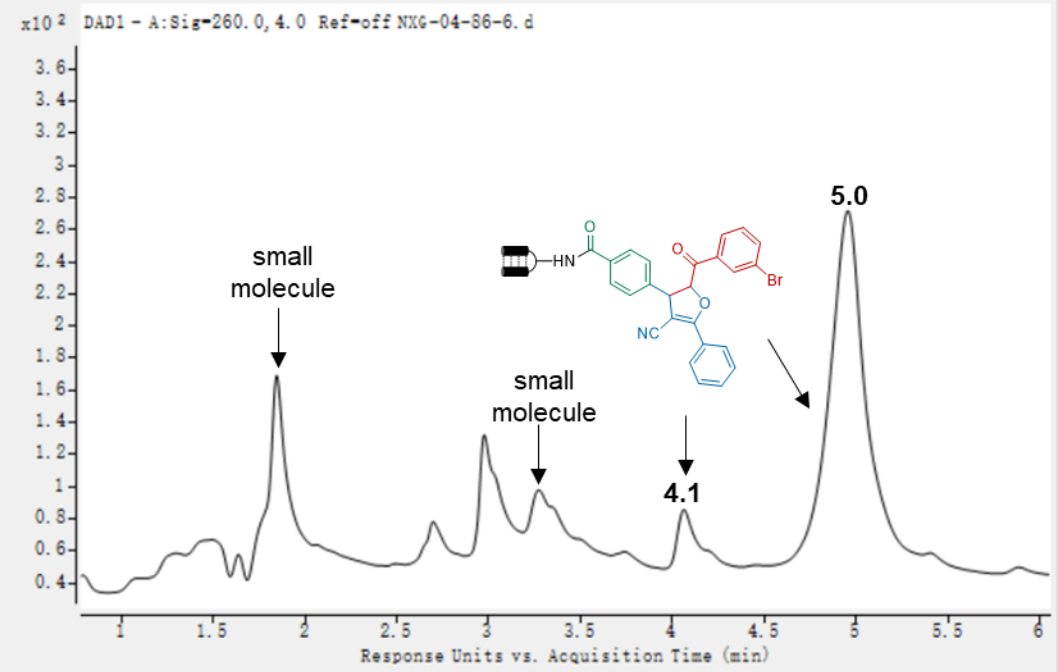

x10 2
-ESI Scan (rt: 4.063-4.213 min, 10 scans) Frag=175.0V NXG-03-51-18.d ...
5347.76
7190.29
Counts (%) vs. Deconvoluted Mass (amu)

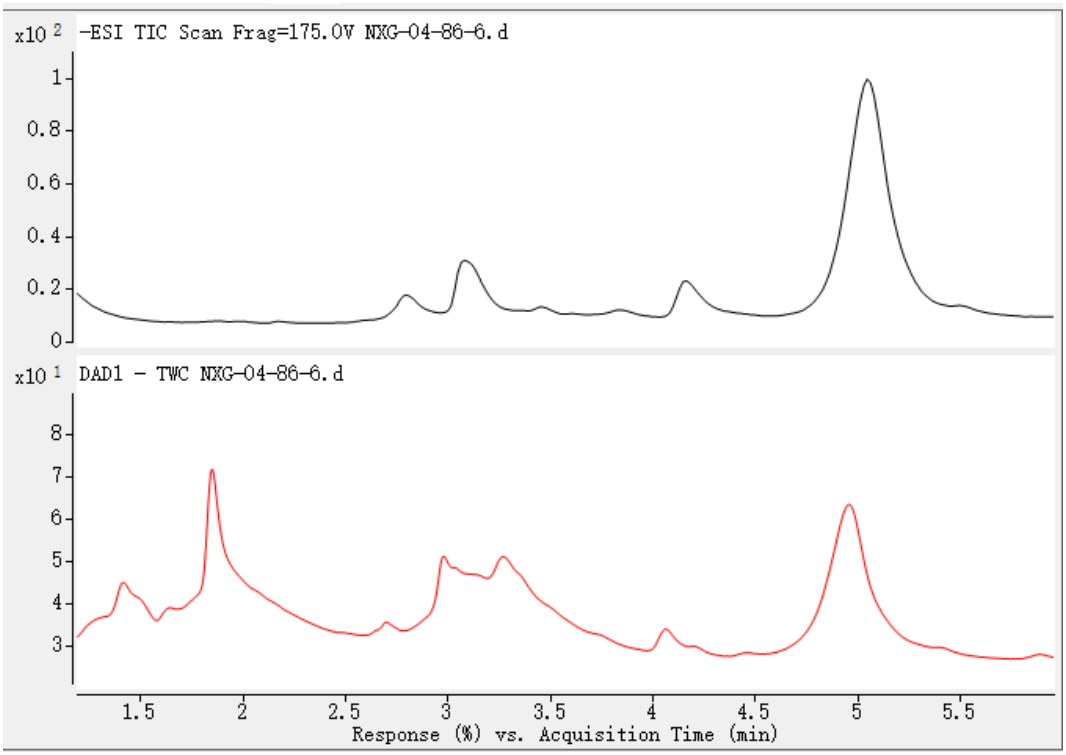

x10 2
-ESI Scan (rt: 4.695-5.045 min, 22 scans) Frag=175.0V NXG-03-51-18.d ...
5347.74
Counts (%) vs. Deconvoluted Mass (amu)

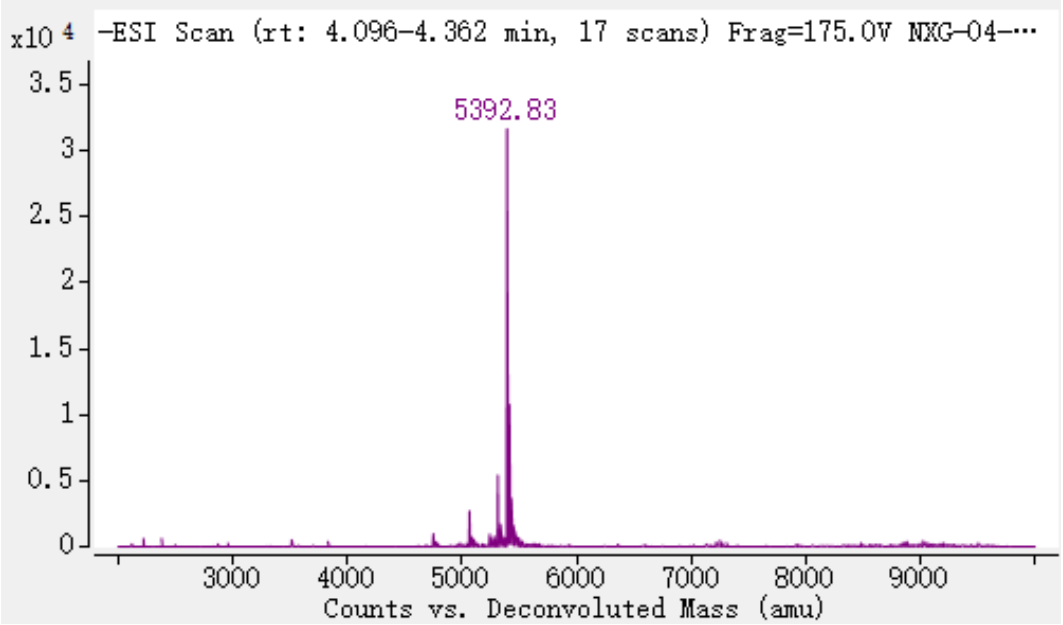
UHPLC chromatogram and deconvoluted MS of **d37**

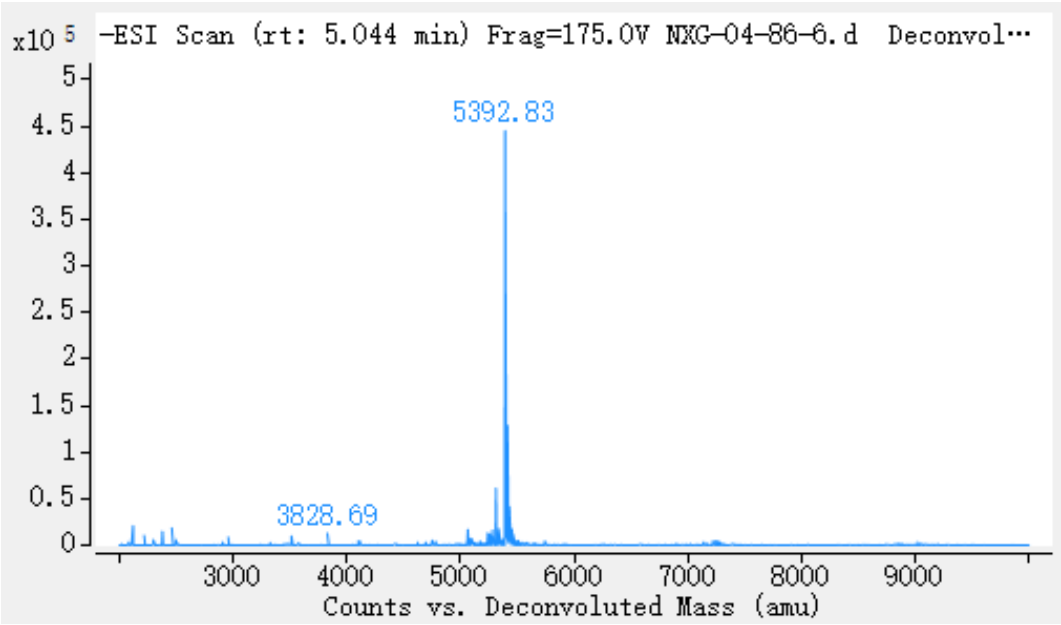
Conversion: 76%

Calculated Mass: 5393Da; Observed Mass: 5393 Da





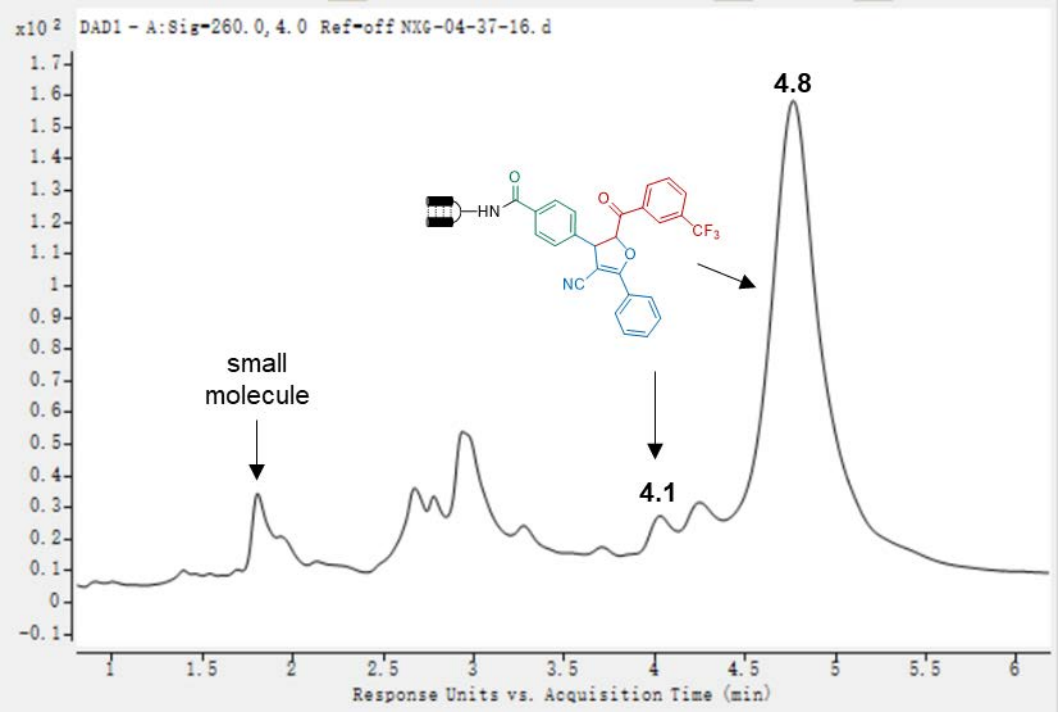

x10 4
-ESI Scan (rt: 4.096-4.362 min, 17 scans) Frag=175.0V NXG-04-…
5392.83
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

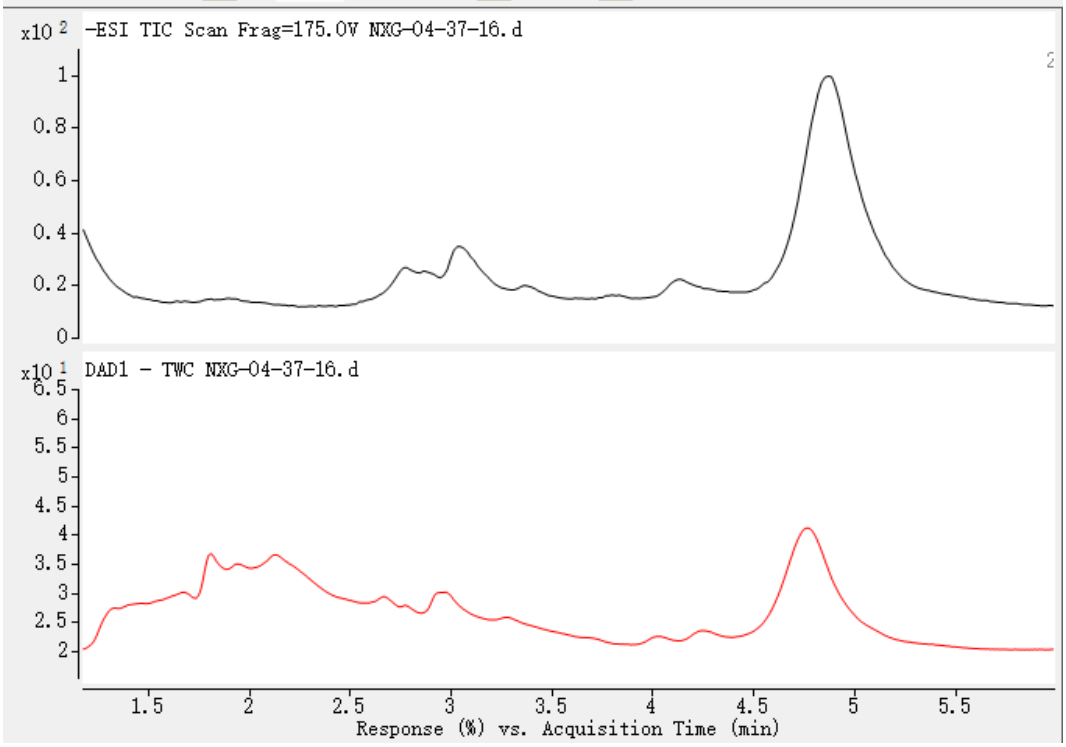

x10 5
-ESI Scan (rt: 5.044 min) Frag=175.0V NXG-04-86-6.d Deconvol…
5392.83
3828.69
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

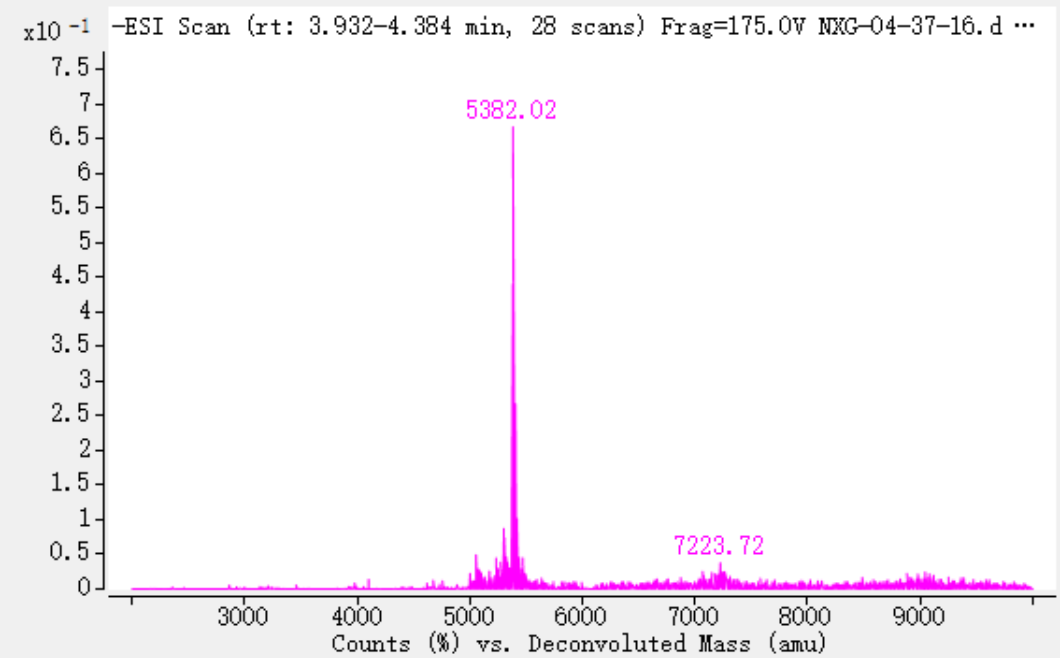
UHPLC chromatogram and deconvoluted MS of **d38**

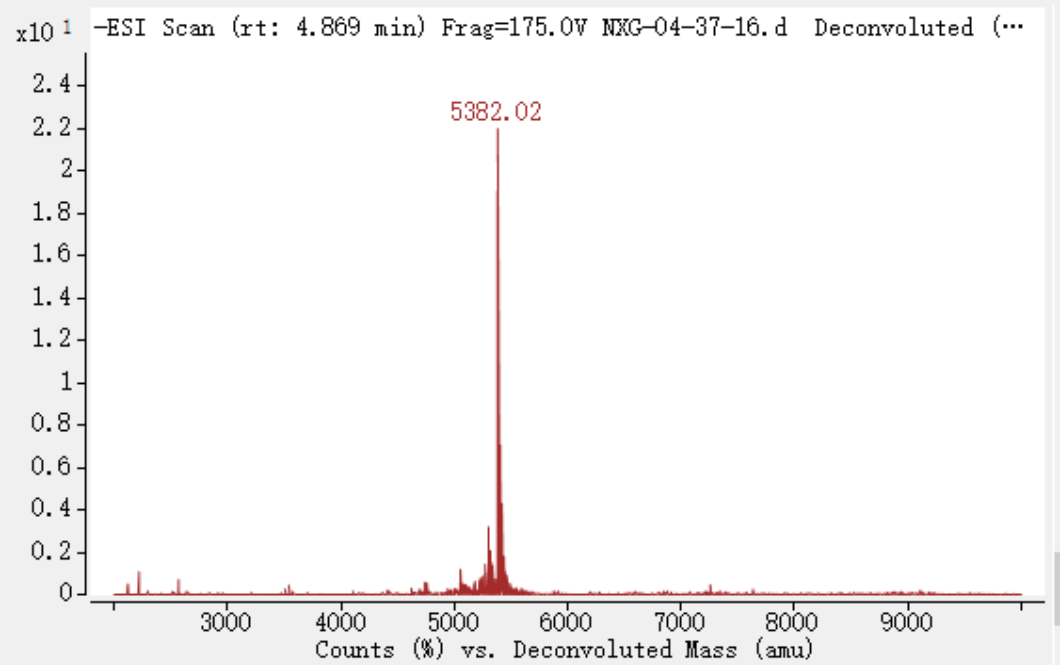
Conversion: 75%

Calculated Mass: 5382 Da; Observed Mass: 5382 Da





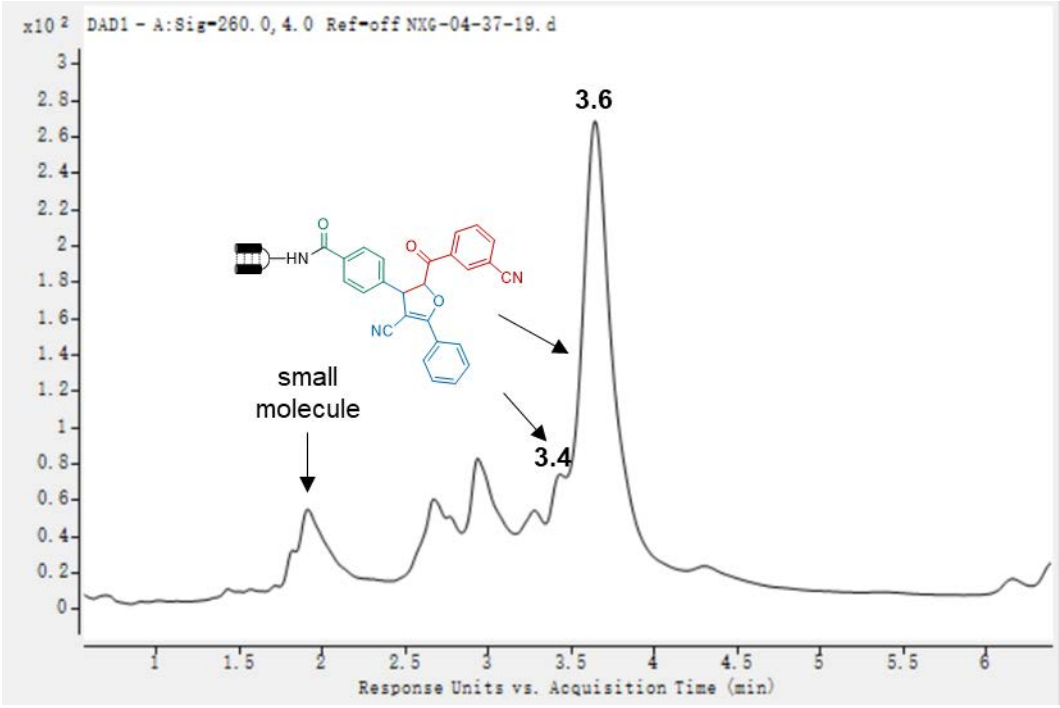

x10 -1
-ESI Scan (rt: 3.932-4.384 min, 28 scans) Frag=175.0V NXG-04-37-16.d ...
5382.02
7223.72
Counts (%) vs. Deconvoluted Mass (amu)

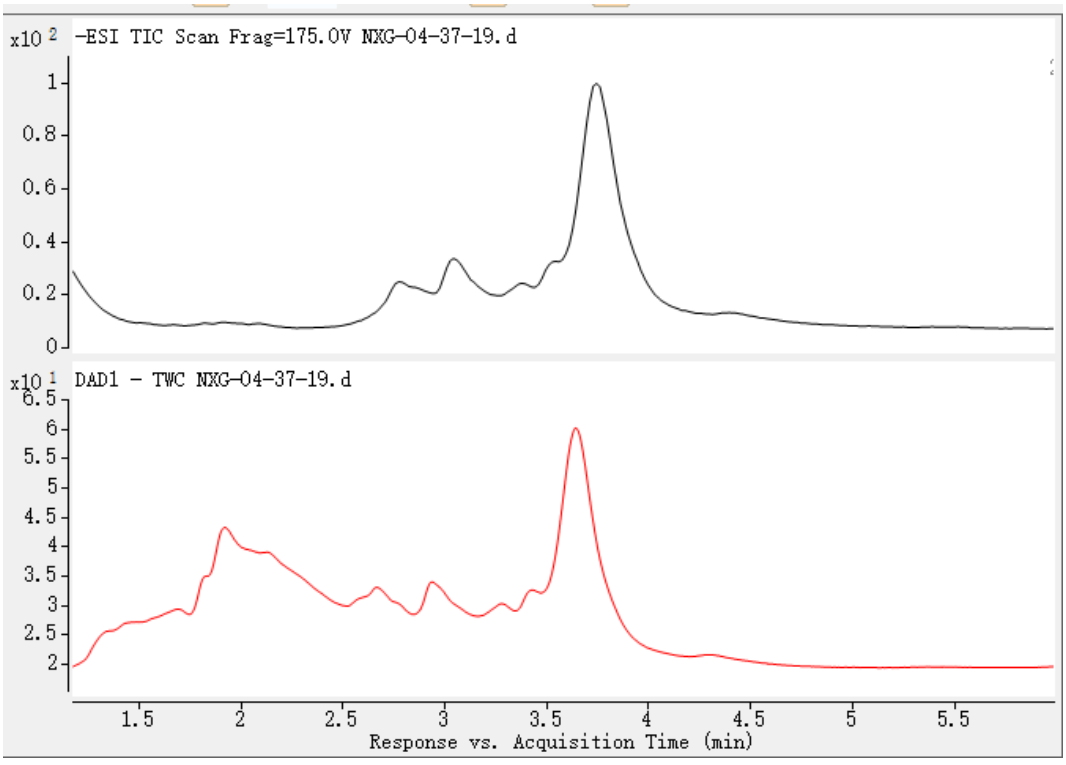

x10 1
-ESI Scan (rt: 4.869 min) Frag=175.0V NXG-04-37-16.d Deconvoluted (...
5382.02
Counts (%) vs. Deconvoluted Mass (amu)

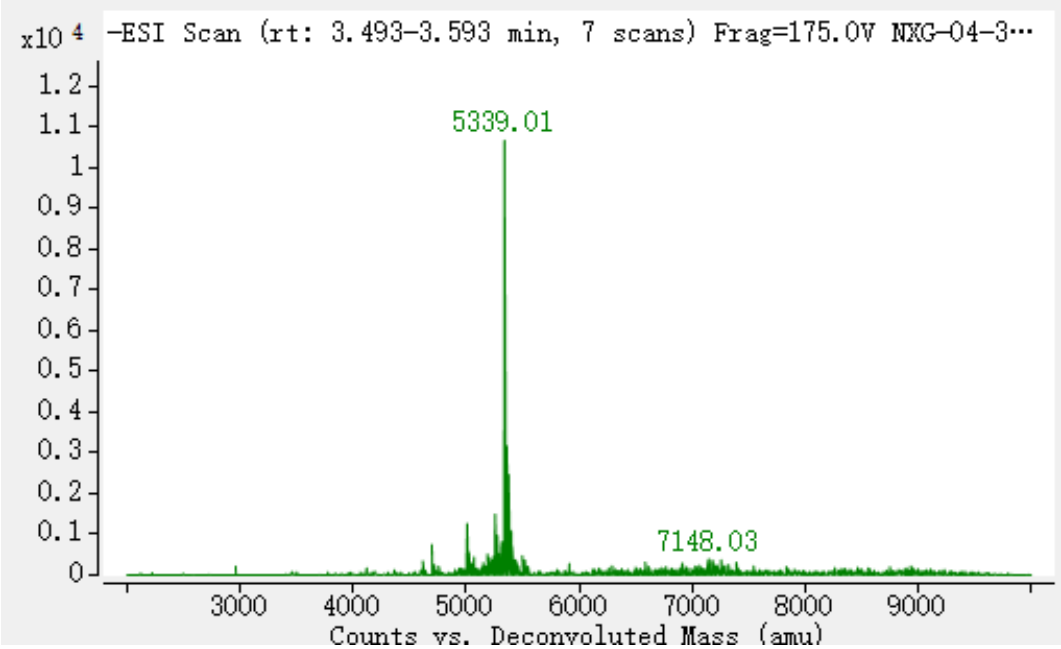
UHPLC chromatogram and deconvoluted MS of **d39**

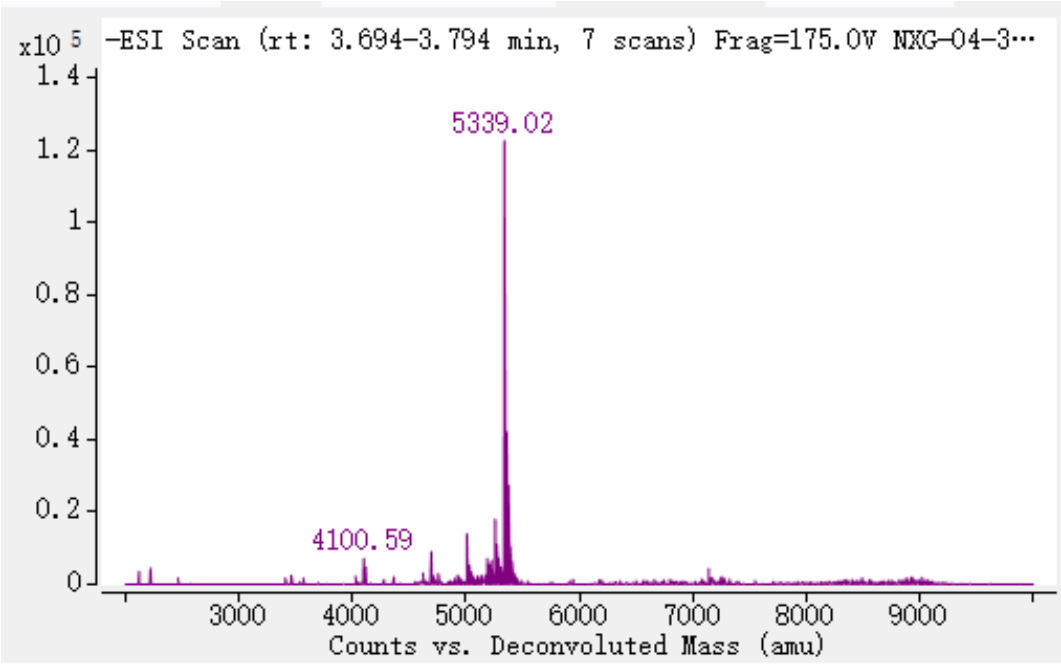
Conversion: 63%

Calculated Mass: 5339 Da; Observed Mass: 5339 Da





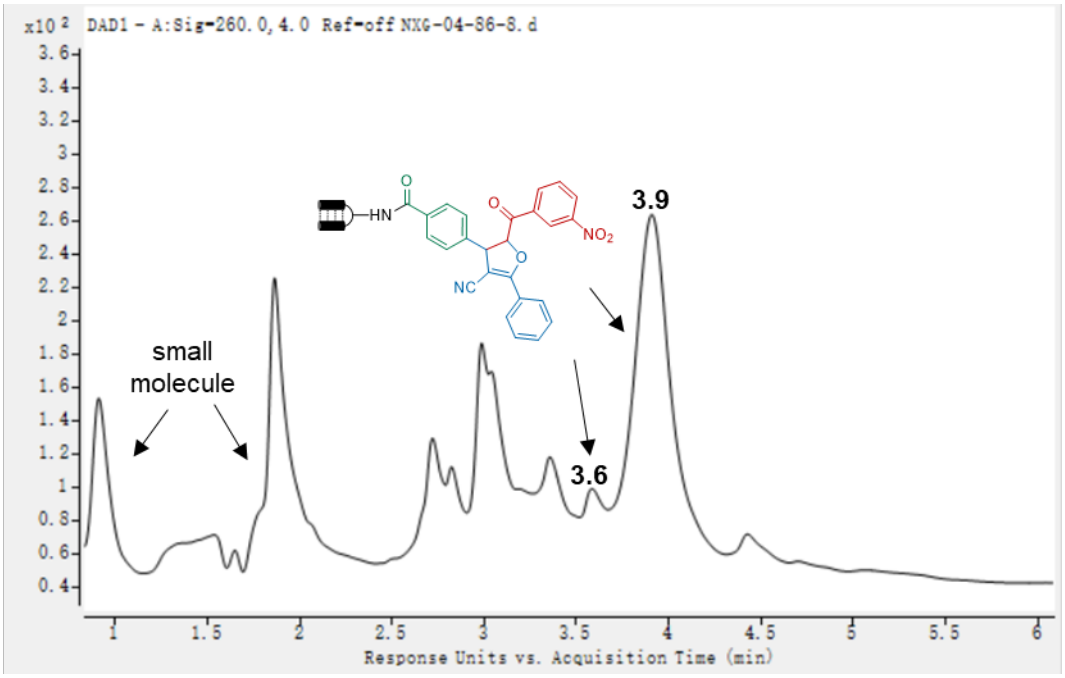

x10 4
-ESI Scan (rt: 3.493-3.593 min, 7 scans) Frag=175.0V NXG-04-3…
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5339.01
7148.03
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

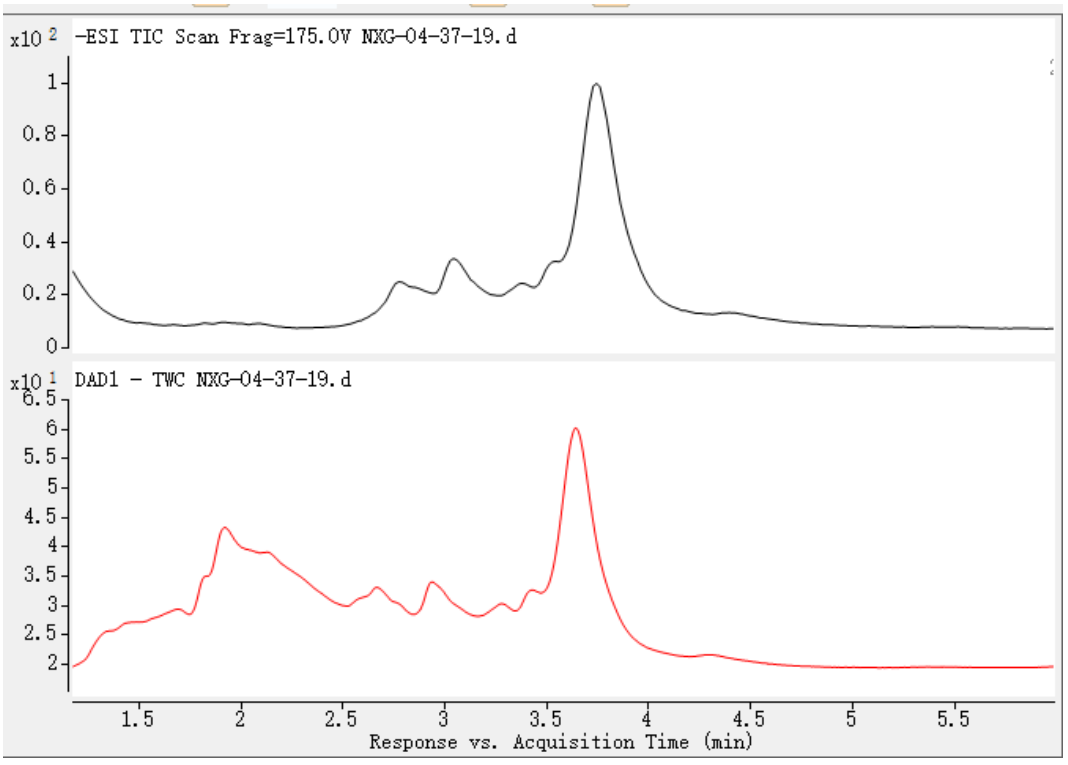

x10 5
-ESI Scan (rt: 3.694-3.794 min, 7 scans) Frag=175.0V NXG-04-3…
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5339.02
4100.59
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

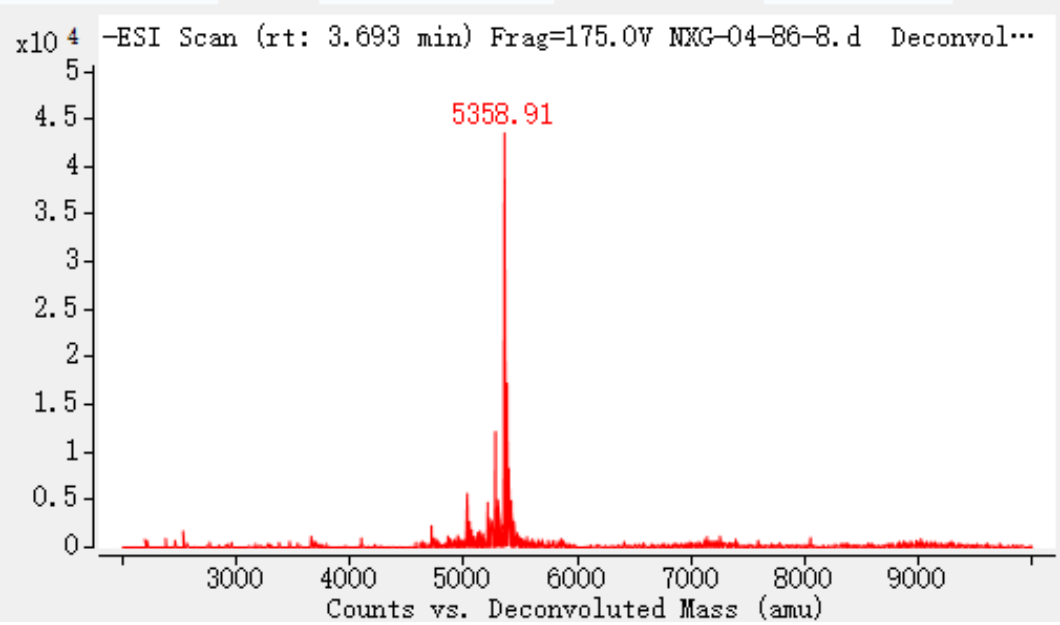
UHPLC chromatogram and deconvoluted MS of **d40**

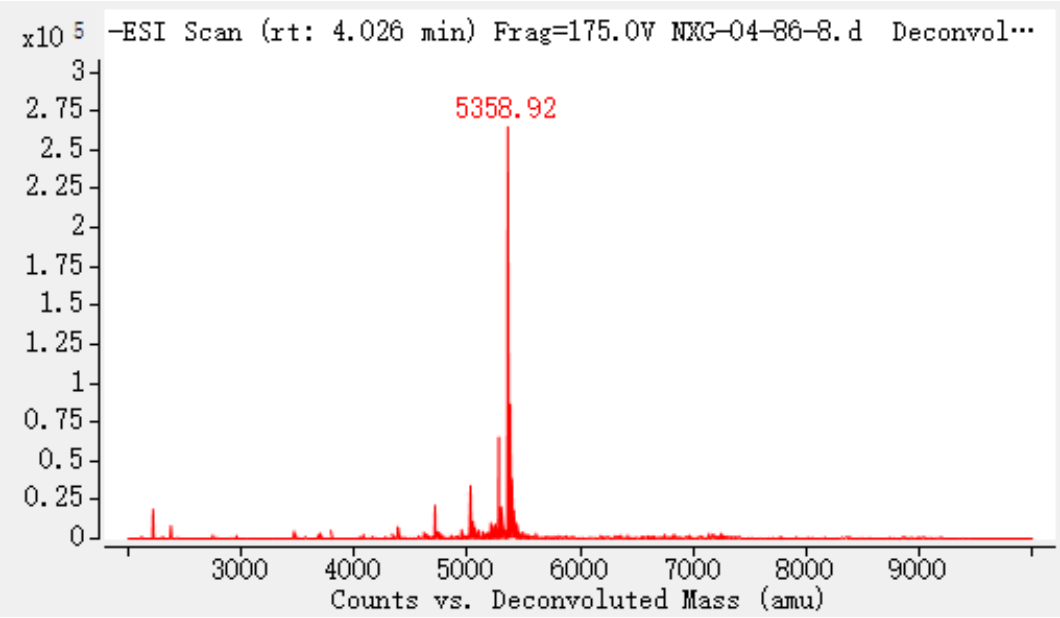
Conversion: 54%

Calculated Mass: 5359 Da; Observed Mass: 5359 Da





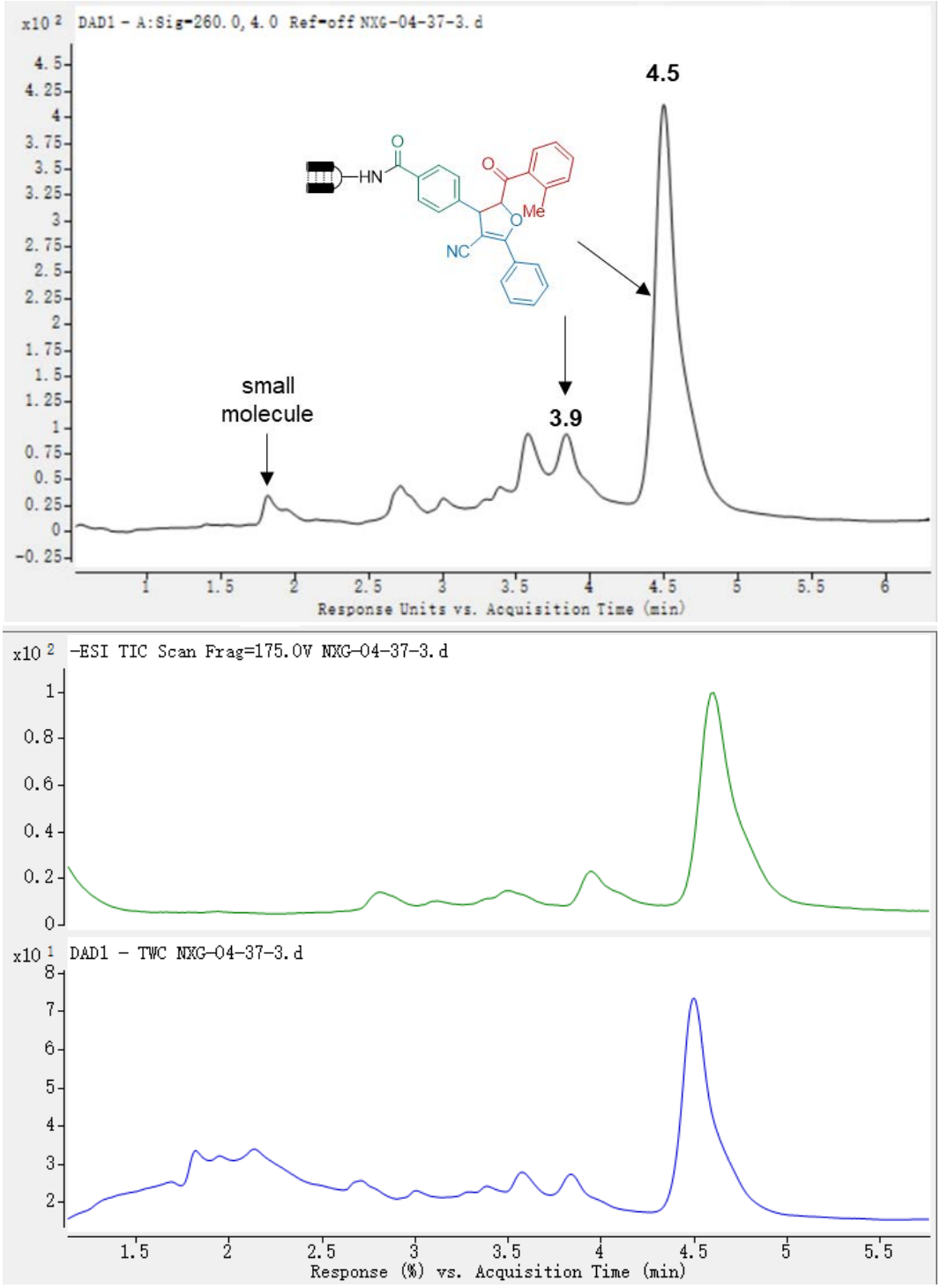

x10 4
-ESI Scan (rt: 3.693 min) Frag=175.0V NXG-04-86-8.d Deconvol…
5358.91
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

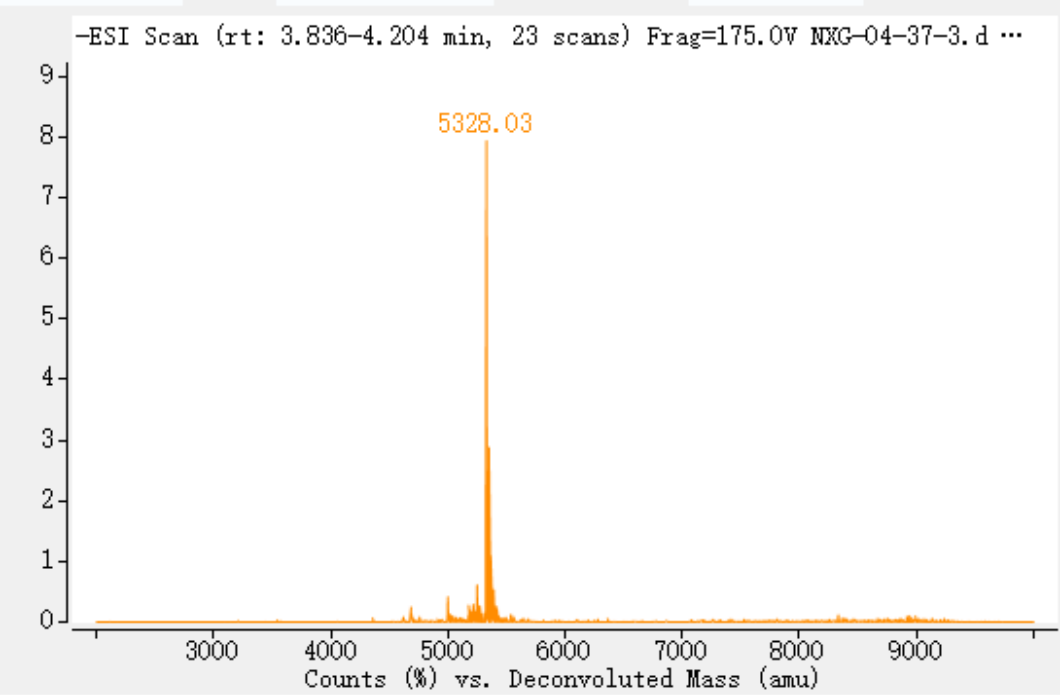

x10 5
-ESI Scan (rt: 4.026 min) Frag=175.0V NXG-04-86-8.d Deconvol…
5358.92
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

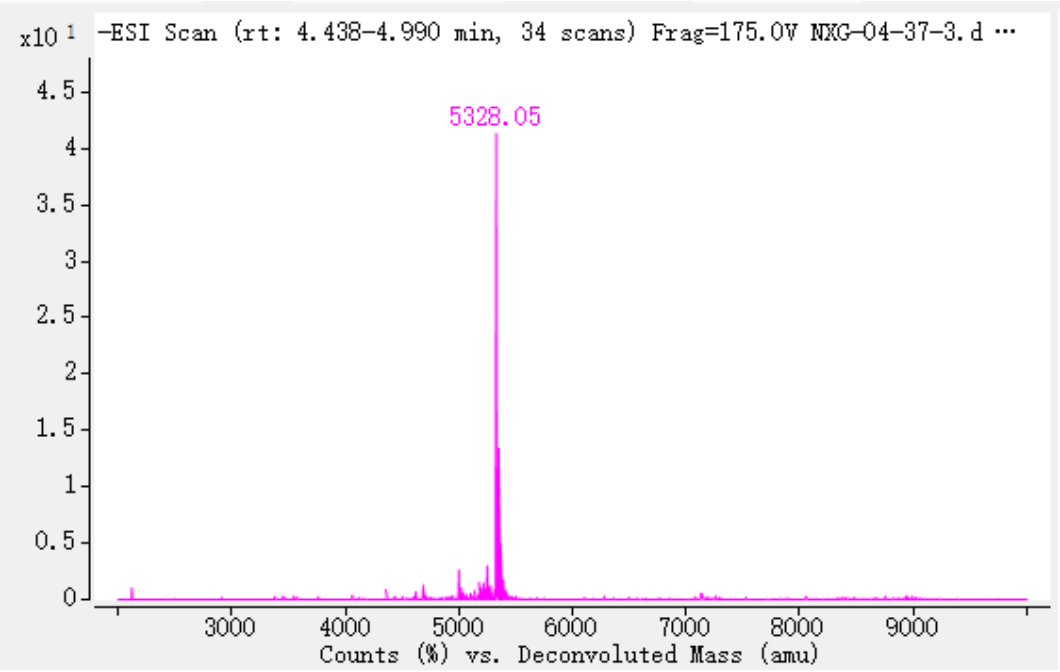
UHPLC chromatogram and deconvoluted MS of **d41**

Conversion: 79%

Calculated Mass: 5328 Da; Observed Mass: 5328 Da



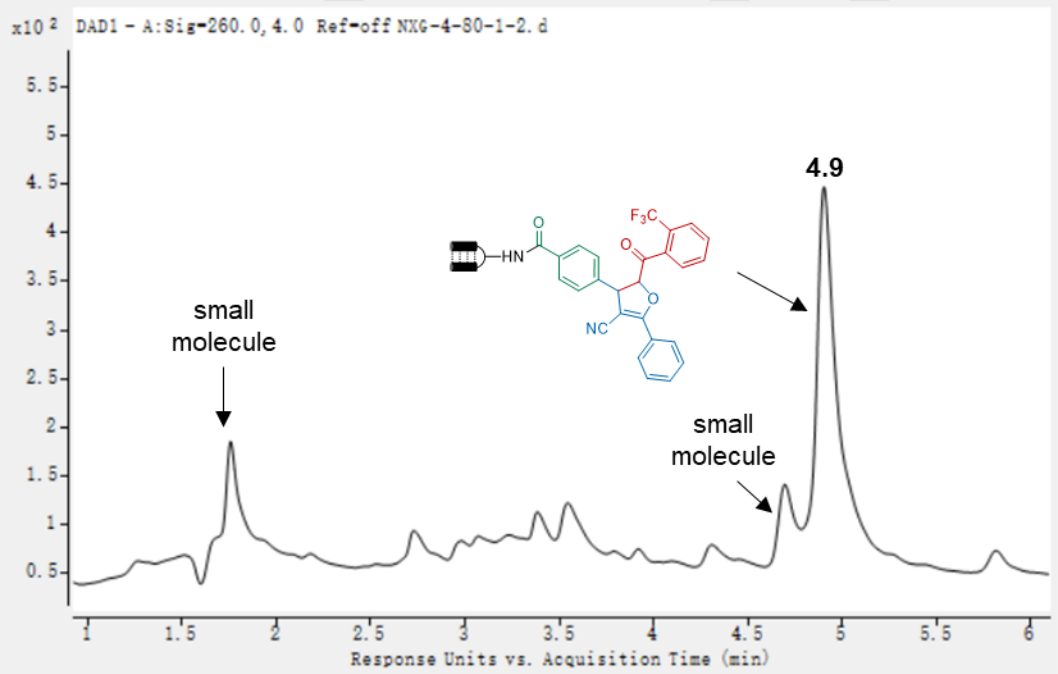

-ESI Scan (rt: 3.836-4.204 min, 23 scans) Frag=175.0V NXG-04-37-3.d ...
5328.03
9
8
7
6
5
4
3
2
1
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

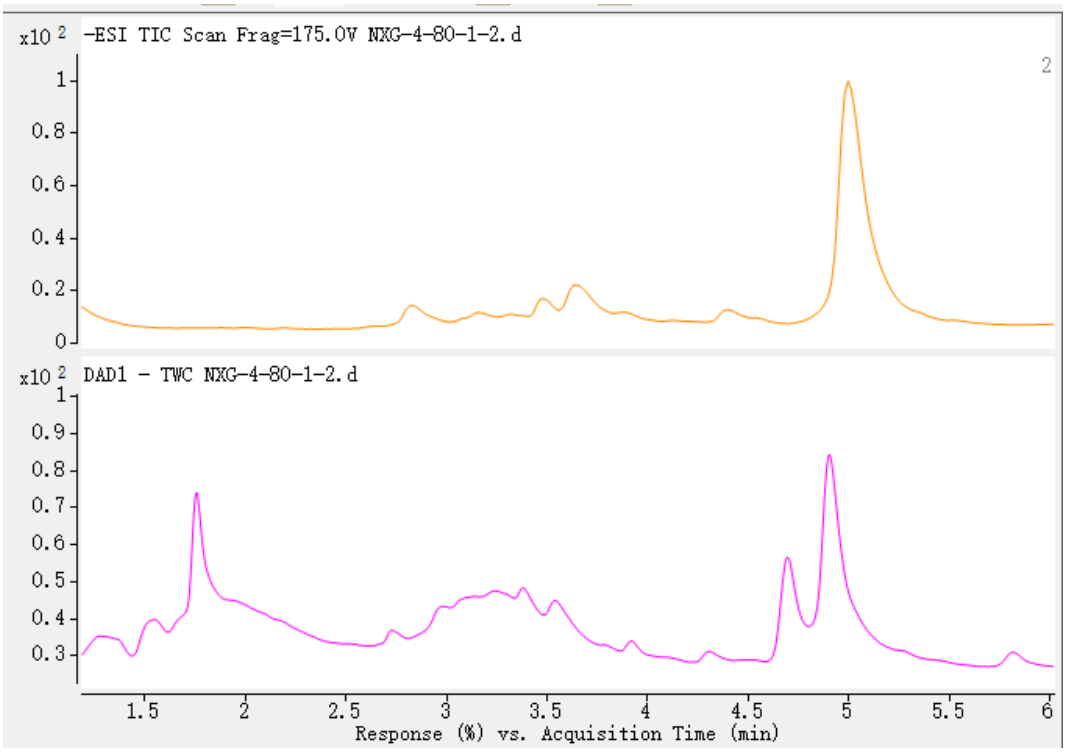

x10 1
-ESI Scan (rt: 4.438-4.990 min, 34 scans) Frag=175.0V NXG-04-37-3.d ...
5328.05
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

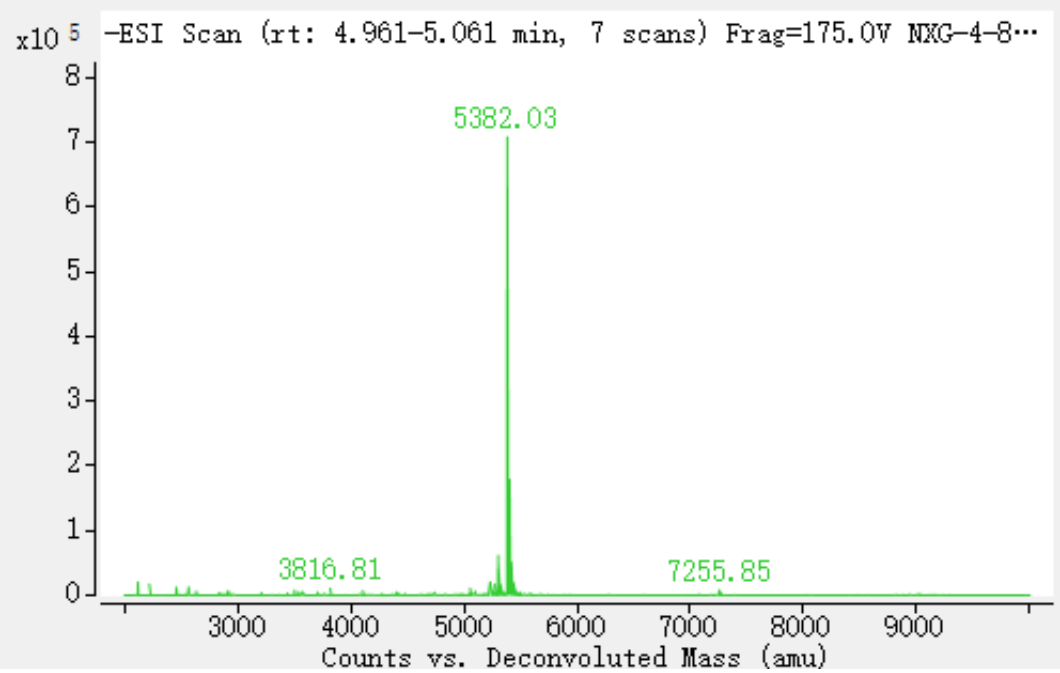
UHPLC chromatogram and deconvoluted MS of **d42**

Conversion: 80%

Calculated Mass: 5382 Da; Observed Mass: 5382 Da



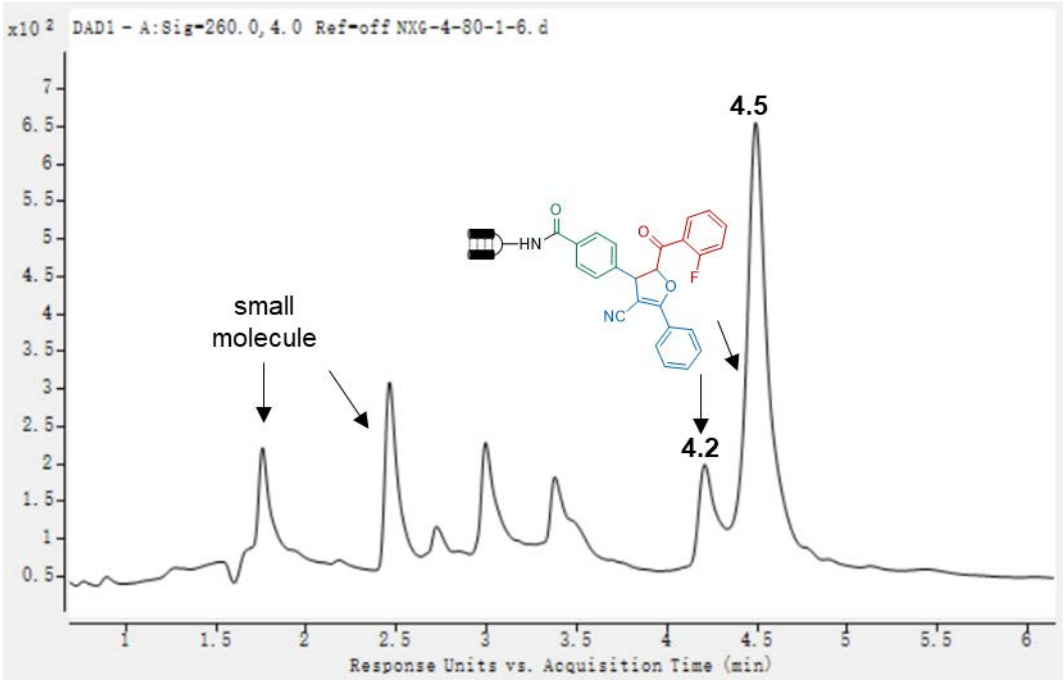


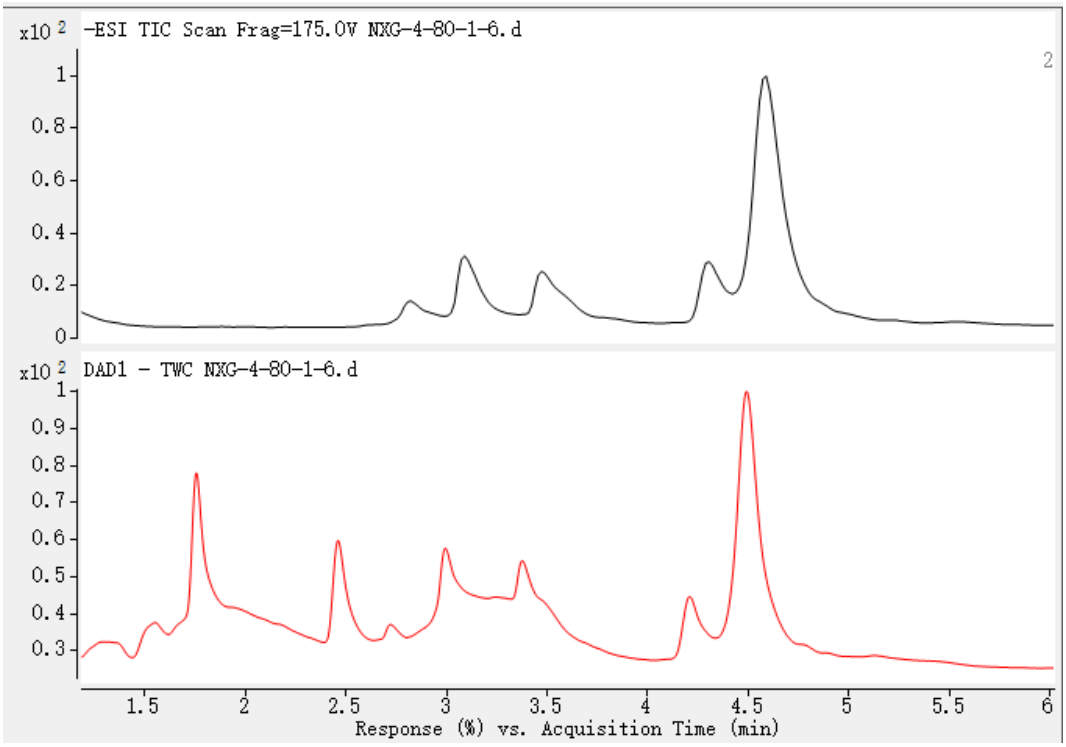

x10 5
-ESI Scan (rt: 4.961-5.061 min, 7 scans) Frag=175.0V NXG-4-8…
8
7
6
5
4
3
2
1
0
5382.03
3816.81
7255.85
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

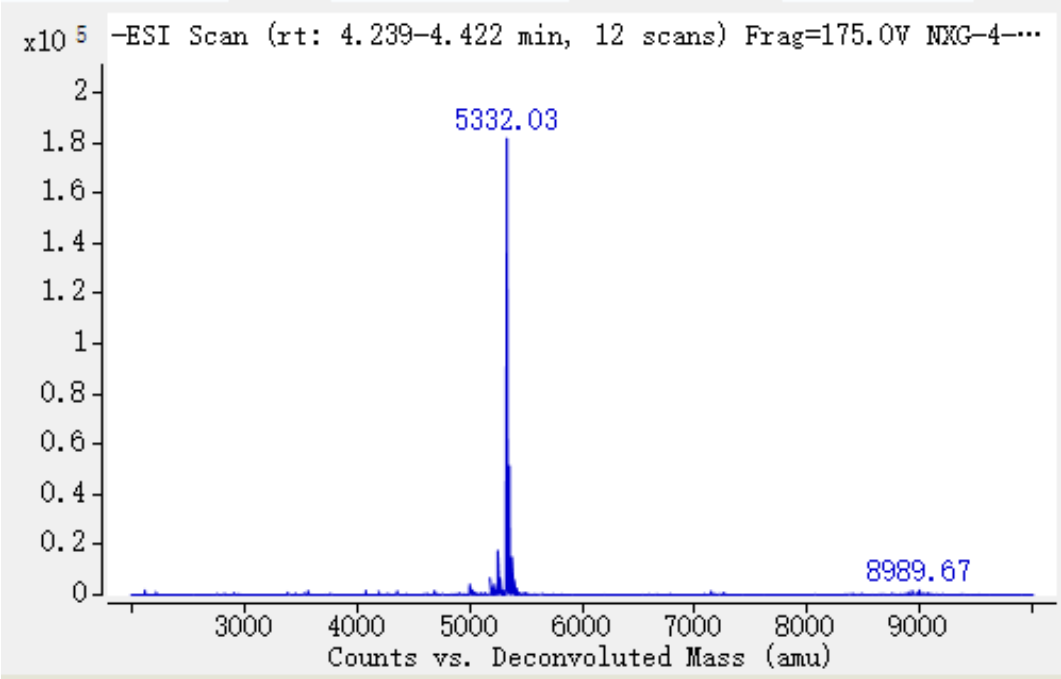
UHPLC chromatogram and deconvoluted MS of **d43**

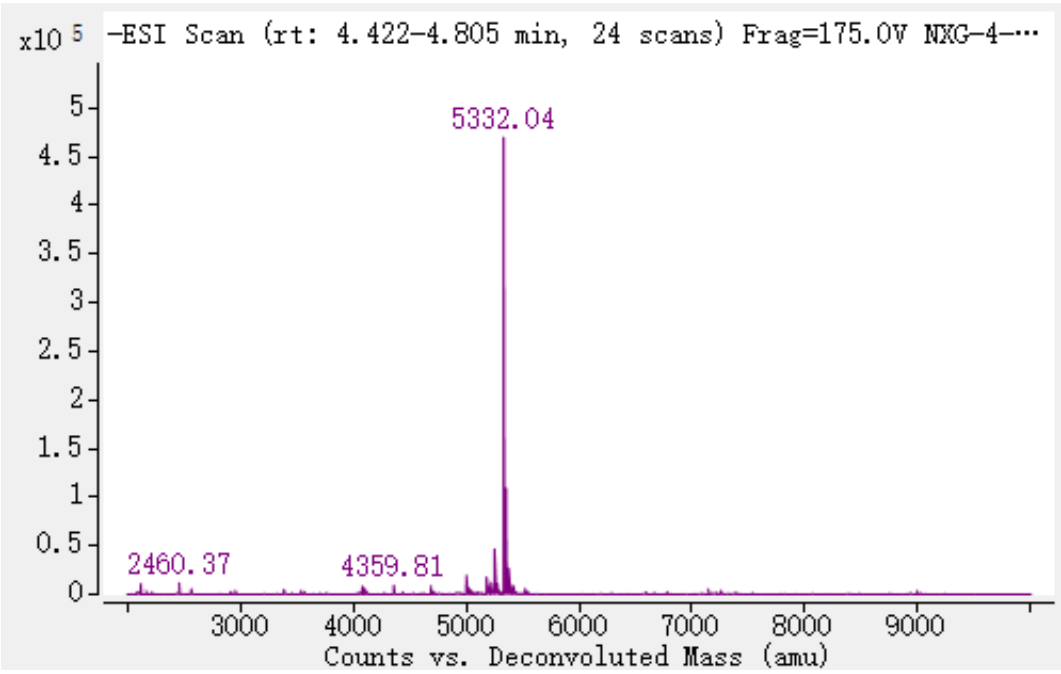
Conversion: 70%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da





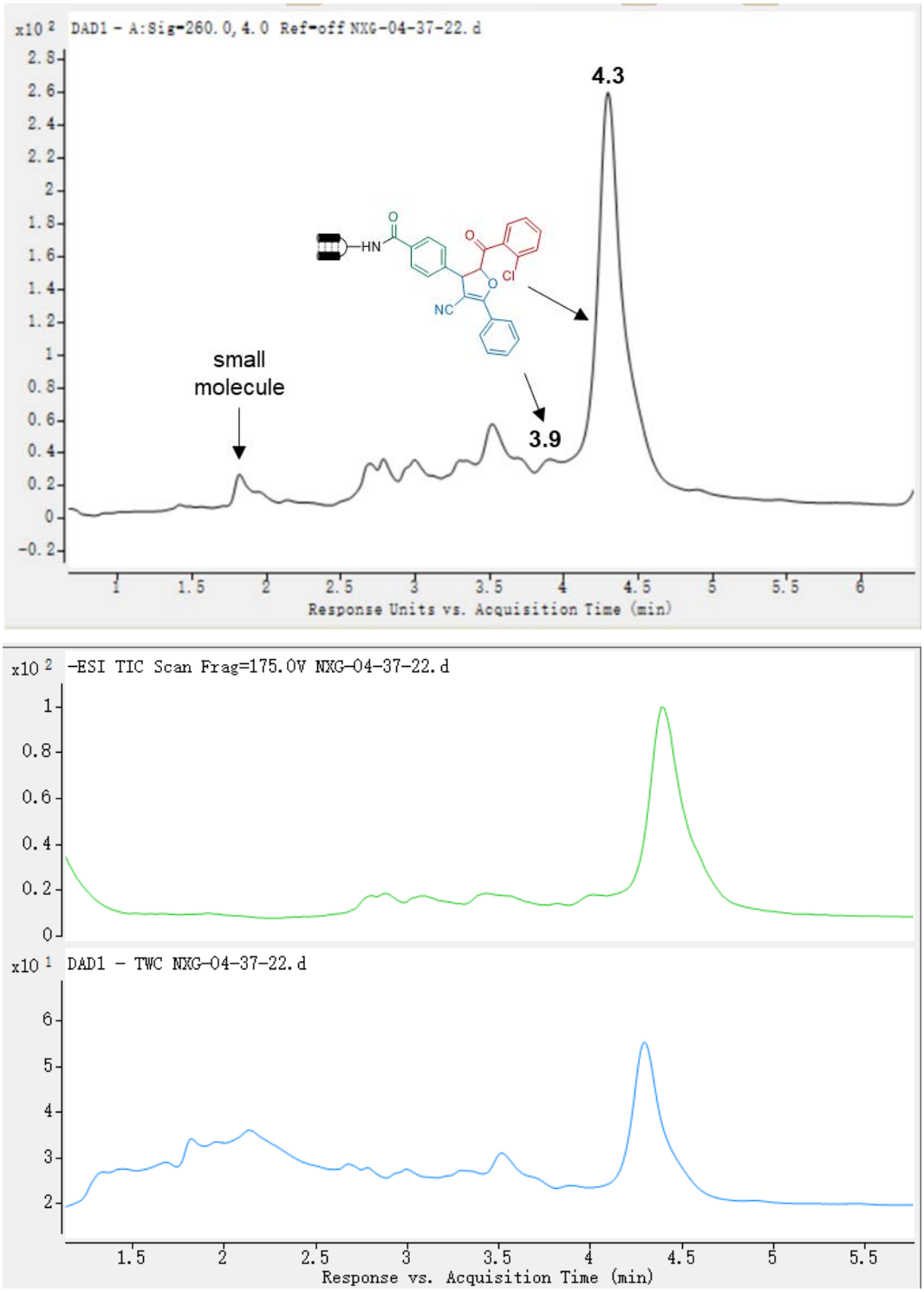

x10 5
-ESI Scan (rt: 4.239-4.422 min, 12 scans) Frag=175.0V NXG-4-…
5332.03
8989.67
Counts vs. Deconvoluted Mass (amu)

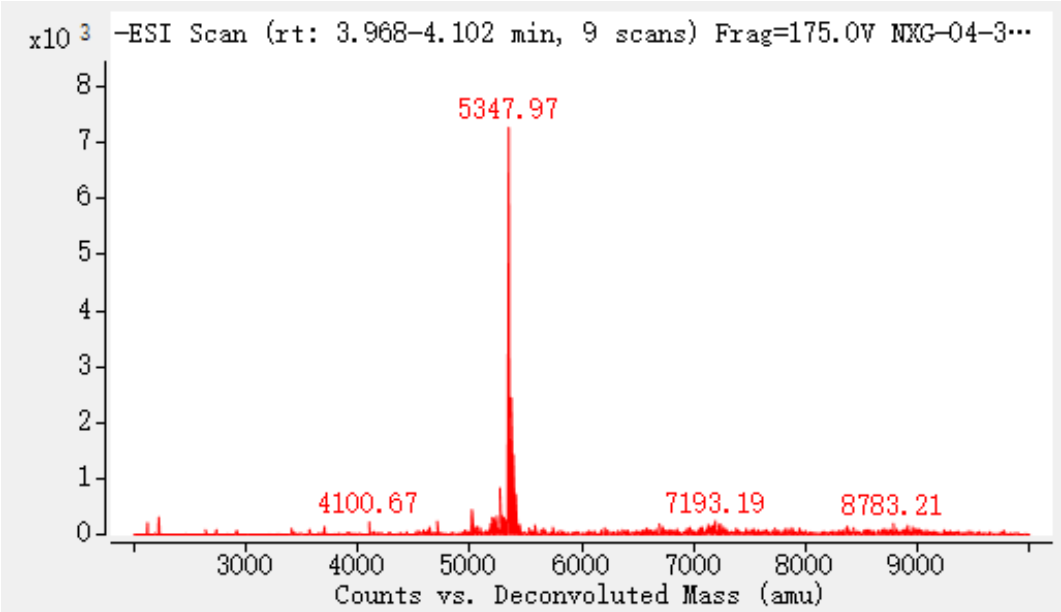

x10 5
-ESI Scan (rt: 4.422-4.805 min, 24 scans) Frag=175.0V NXG-4-…
5332.04
2460.37
4359.81
Counts vs. Deconvoluted Mass (amu)

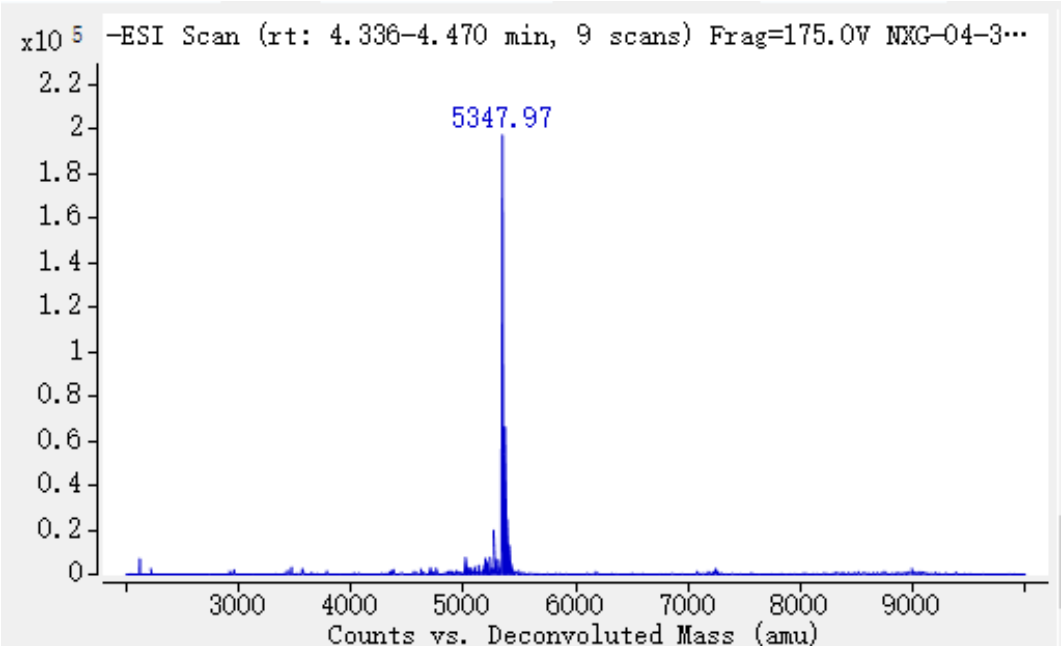
UHPLC chromatogram and deconvoluted MS of **d44**

Conversion: 65%

Calculated Mass: 5348 Da; Observed Mass: 5348 Da



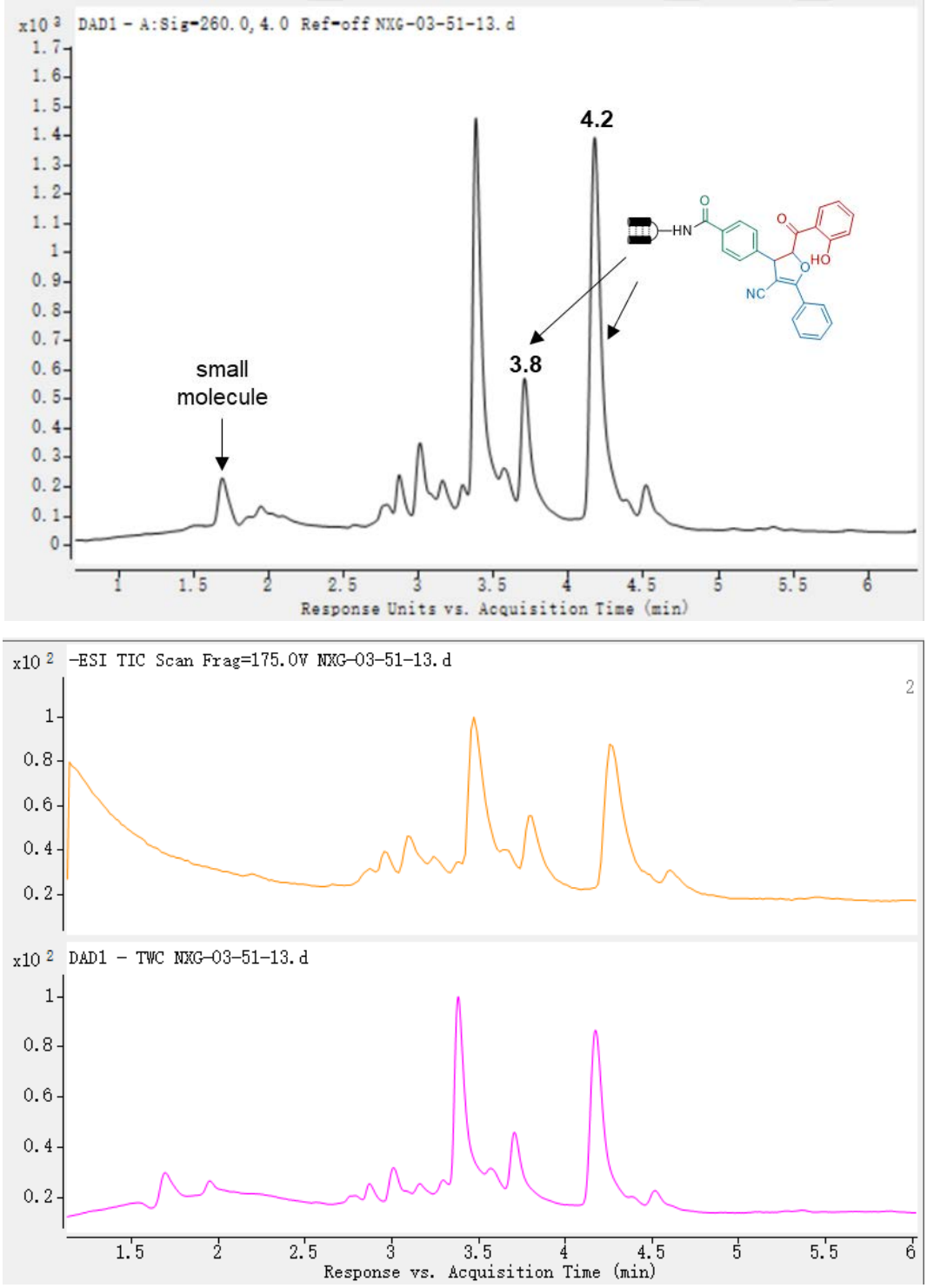

x10 3
-ESI Scan (rt: 3.968-4.102 min, 9 scans) Frag=175.0V NXG-04-3…
5347.97
4100.67
7193.19
8783.21
Counts vs. Deconvoluted Mass (amu)

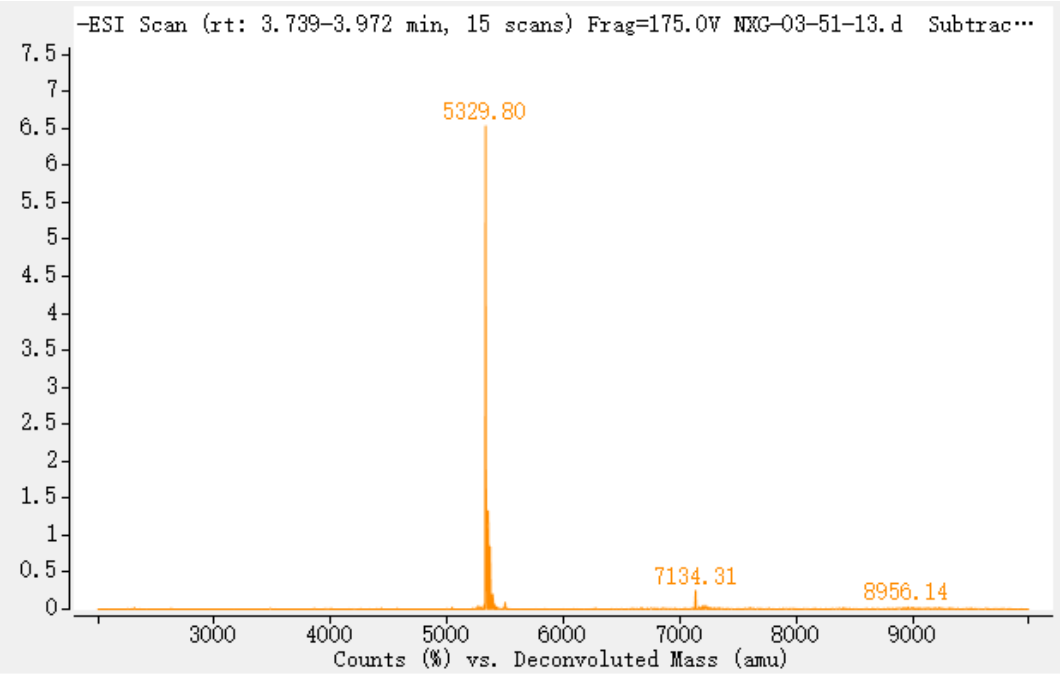

x10 5
-ESI Scan (rt: 4.336-4.470 min, 9 scans) Frag=175.0V NXG-04-3…
5347.97
Counts vs. Deconvoluted Mass (amu)

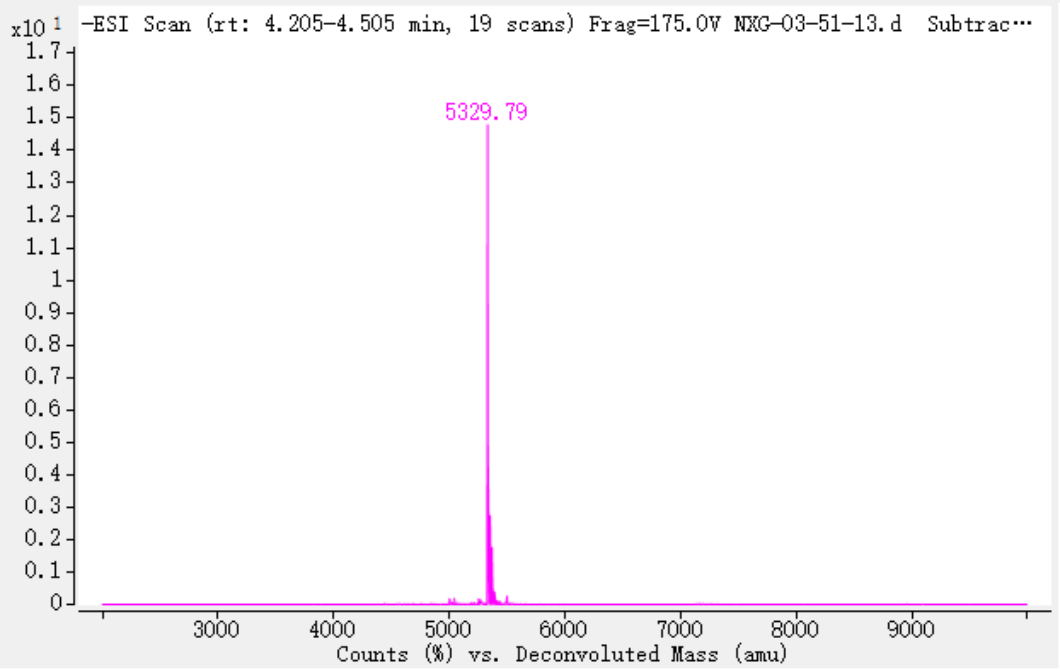
UHPLC chromatogram and deconvoluted MS of **d45**

Conversion: 41%

Calculated Mass: 5330 Da; Observed Mass: 5330 Da



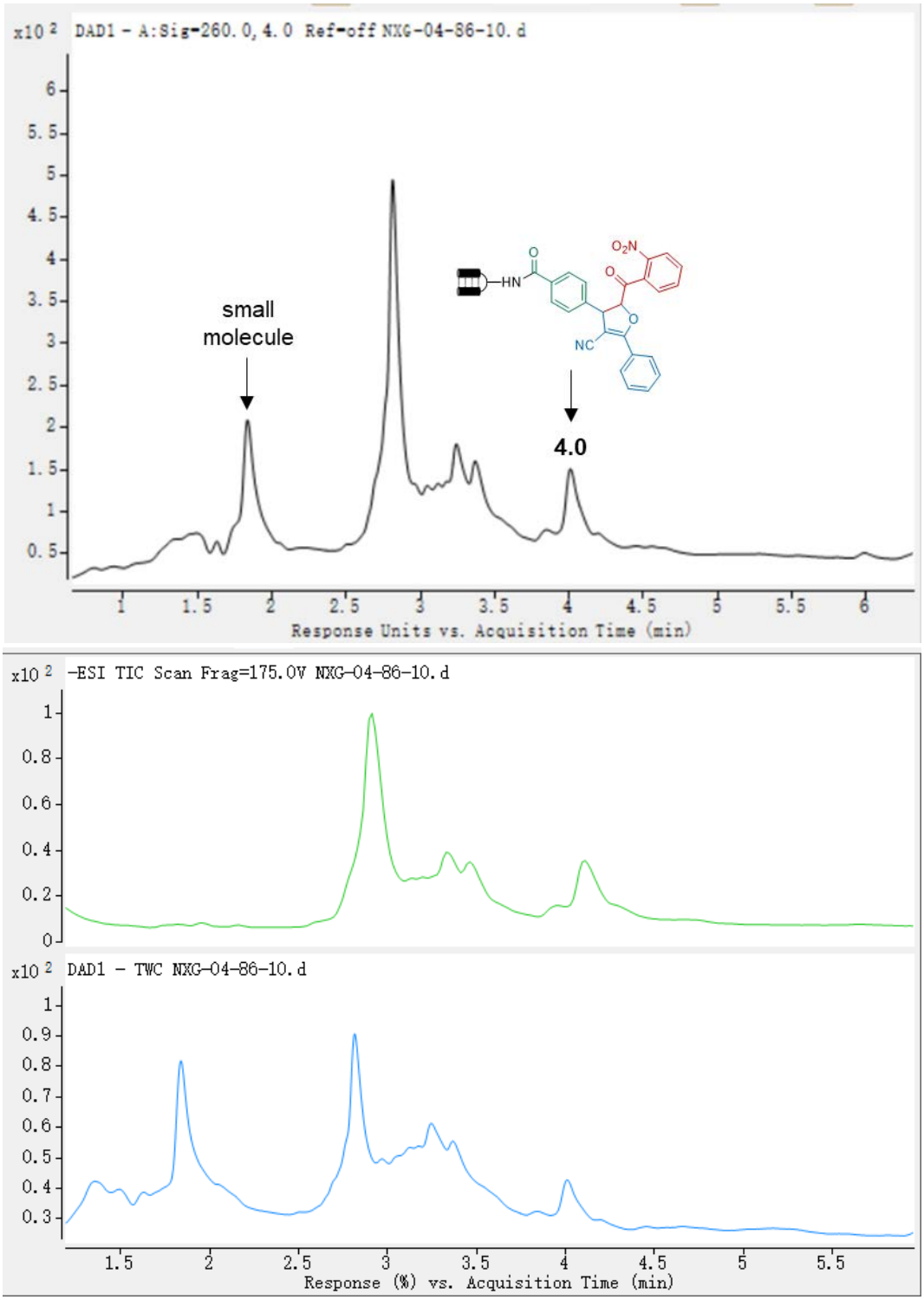

-ESI Scan (rt: 3.739-3.972 min, 15 scans) Frag=175.0V NXG-03-51-13.d Subtrac…
5329.80
7134.31
8956.14
Counts (%) vs. Deconvoluted Mass (amu)

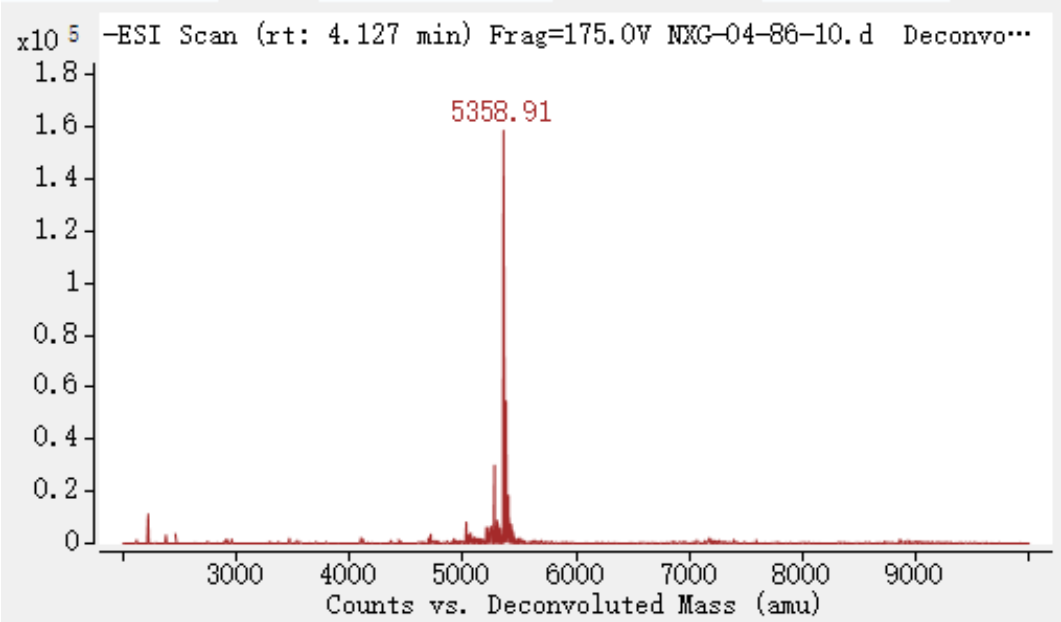

x10 1
-ESI Scan (rt: 4.205-4.505 min, 19 scans) Frag=175.0V NXG-03-51-13.d Subtrac…
5329.79
Counts (%) vs. Deconvoluted Mass (amu)

UHPLC chromatogram and deconvoluted MS of **d46**

Conversion: 11%

Calculated Mass: 5359 Da; Observed Mass: 5359 Da

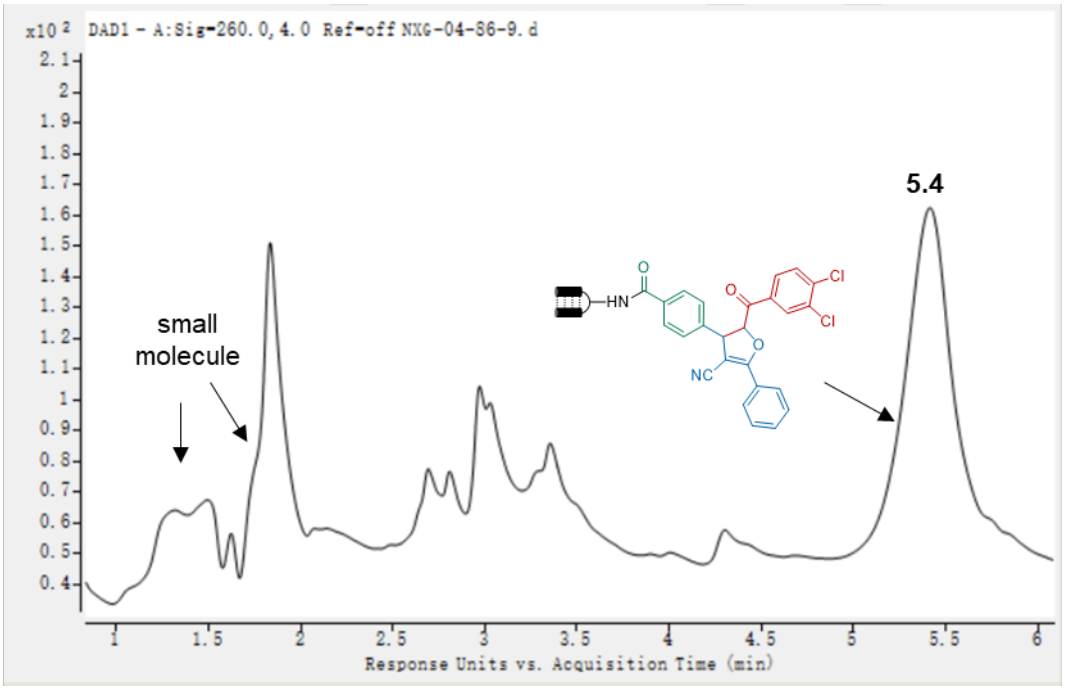


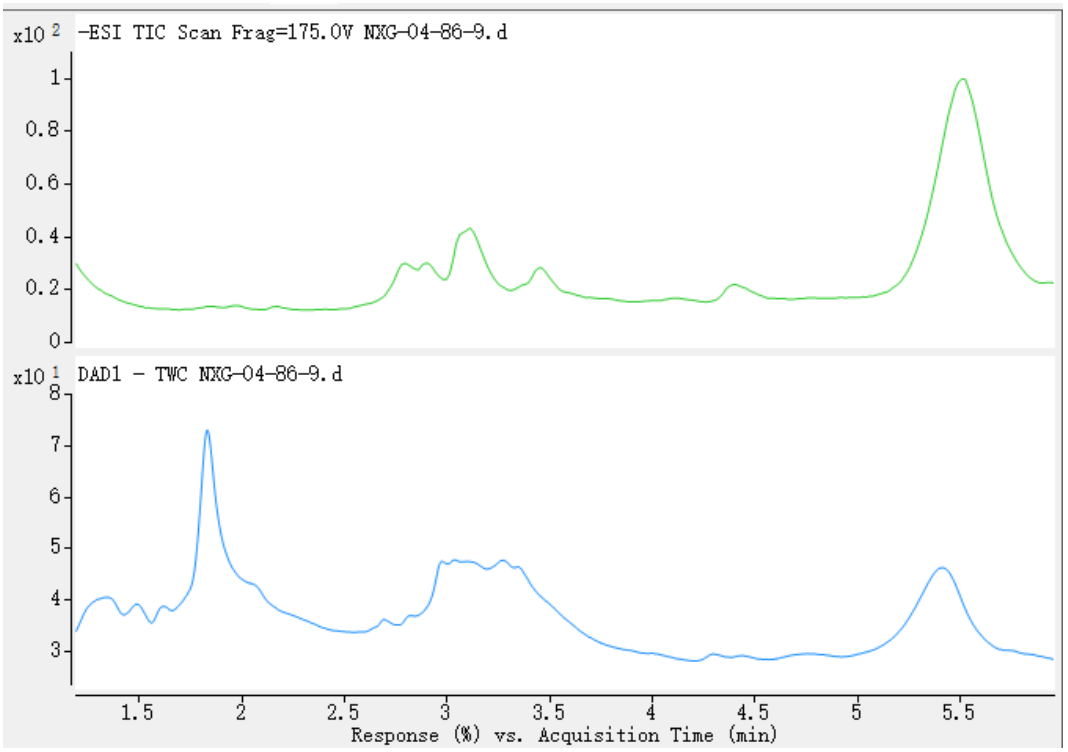

x10 5
-ESI Scan (rt: 4.127 min) Frag=175.0V NXG-04-86-10.d Deconvo…
5358.91
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

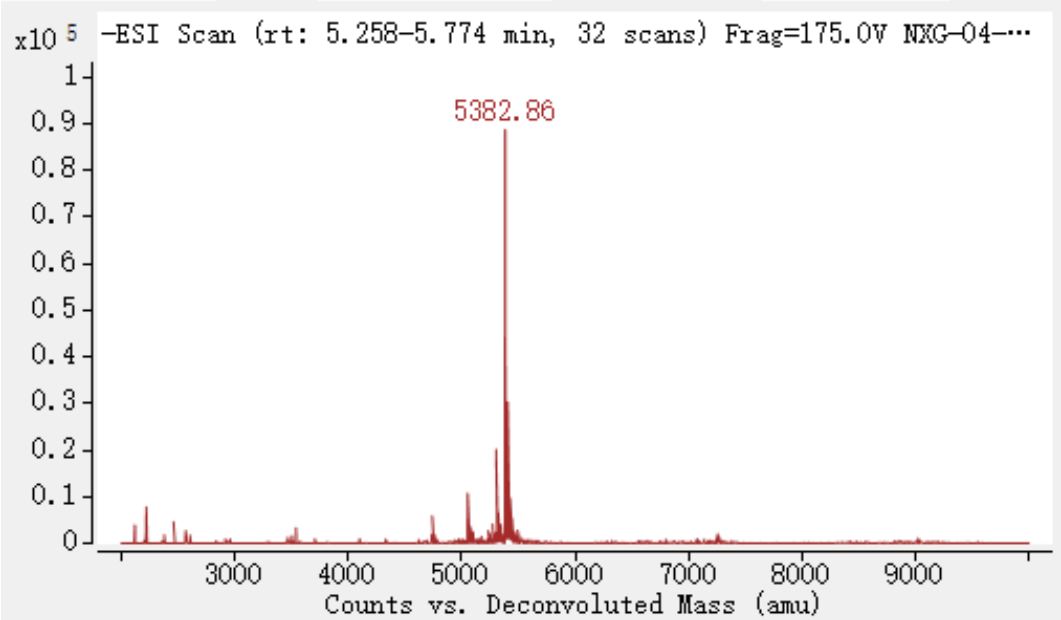
UHPLC chromatogram and deconvoluted MS of **d47**

Conversion: 58%

Calculated Mass: 5383 Da; Observed Mass: 5383 Da



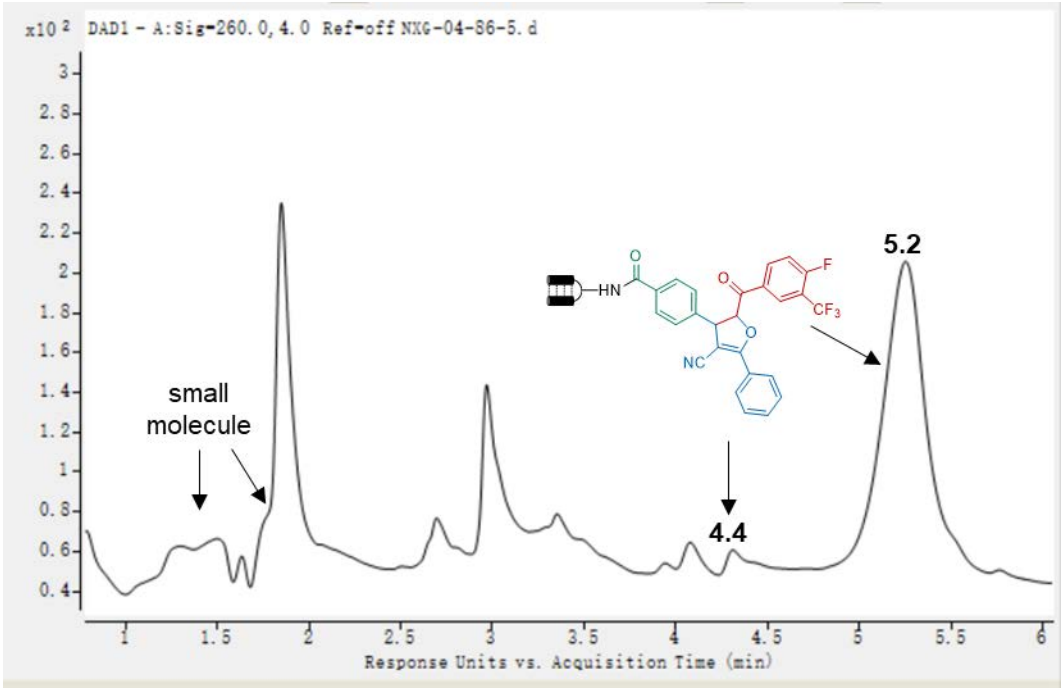


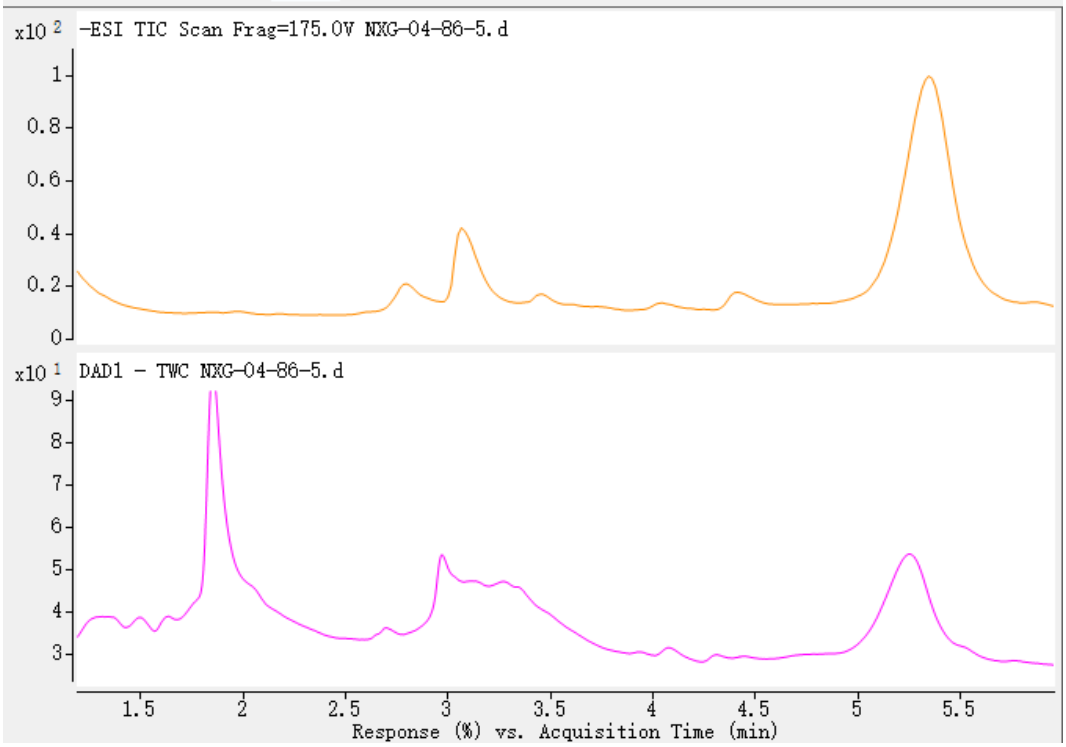

x10 5
-ESI Scan (rt: 5.258-5.774 min, 32 scans) Frag=175.0V NXG-04-…
5382.86
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

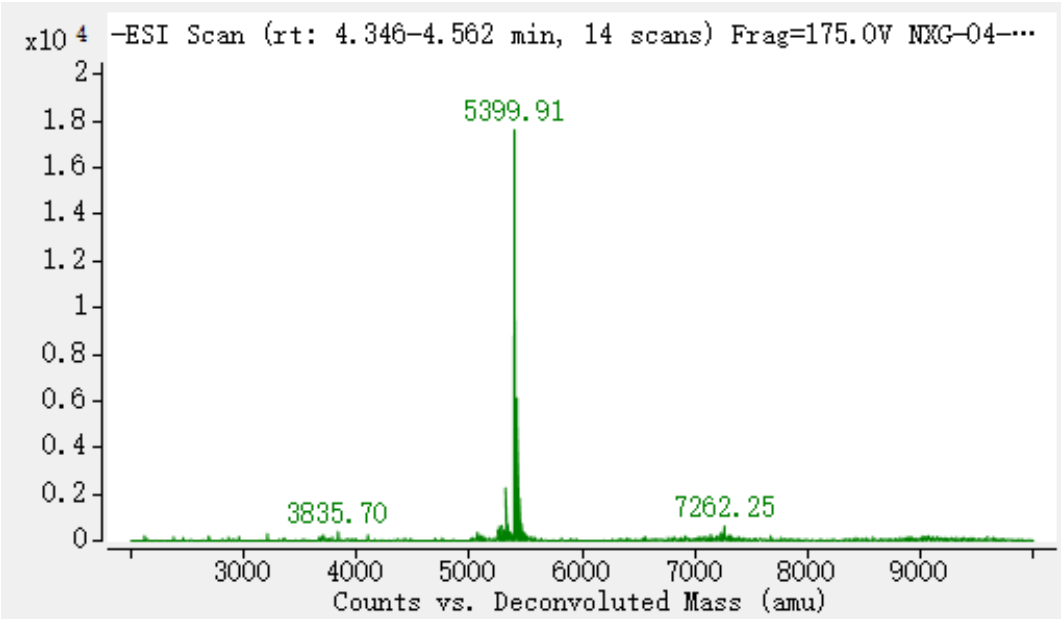
UHPLC chromatogram and deconvoluted MS of **d48**

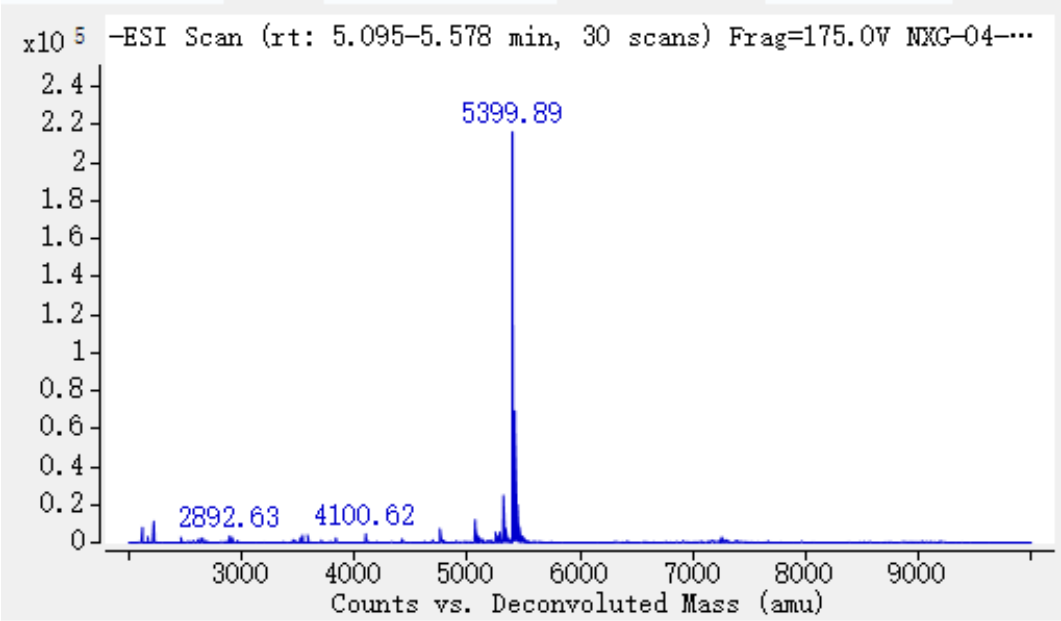
Conversion: 65%

Calculated Mass: 5400 Da; Observed Mass: 5400 Da





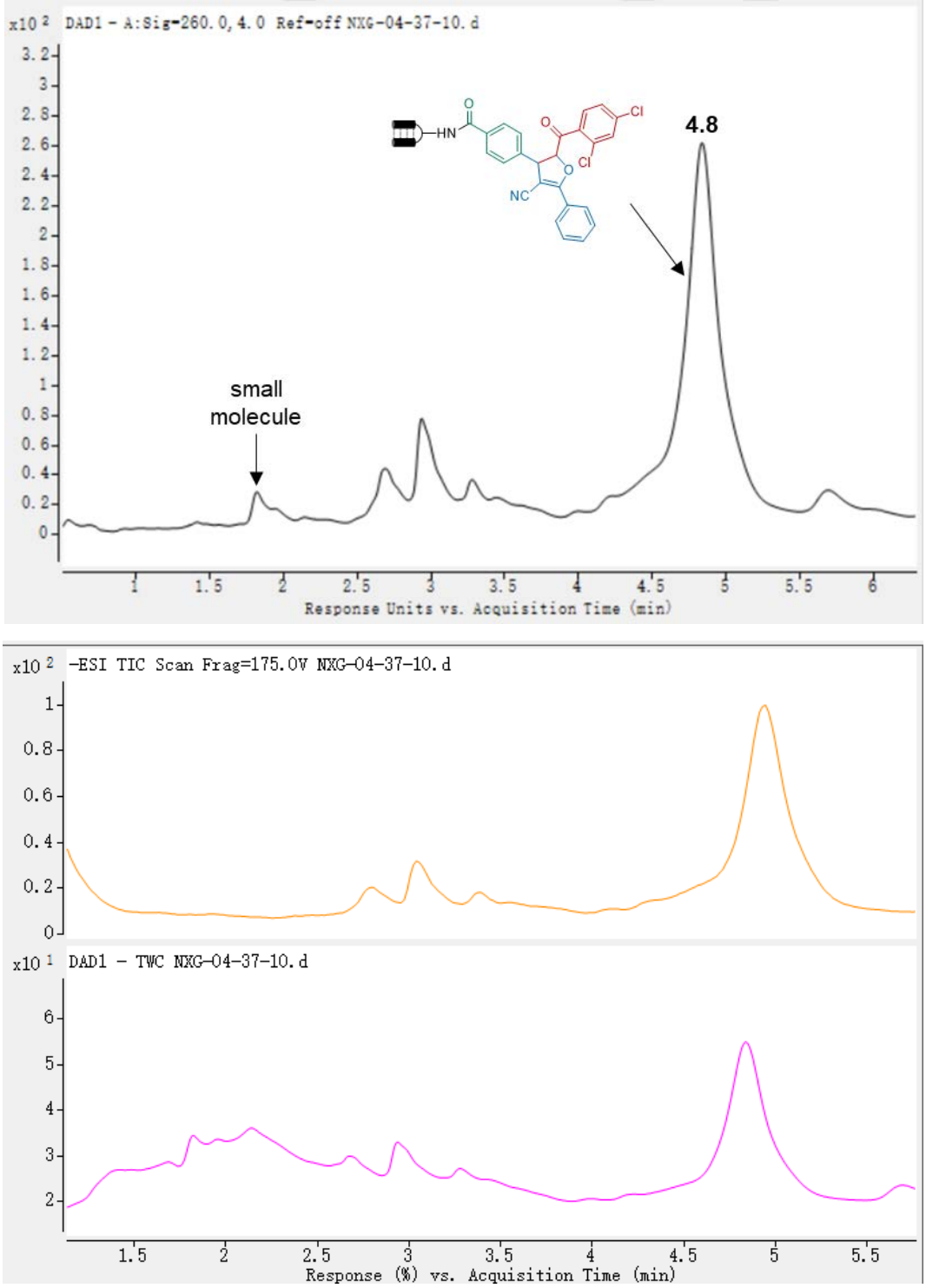

x10 4
-ESI Scan (rt: 4.346-4.562 min, 14 scans) Frag=175.0V NXG-04-…
5399.91
3835.70
7262.25
Counts vs. Deconvoluted Mass (amu)

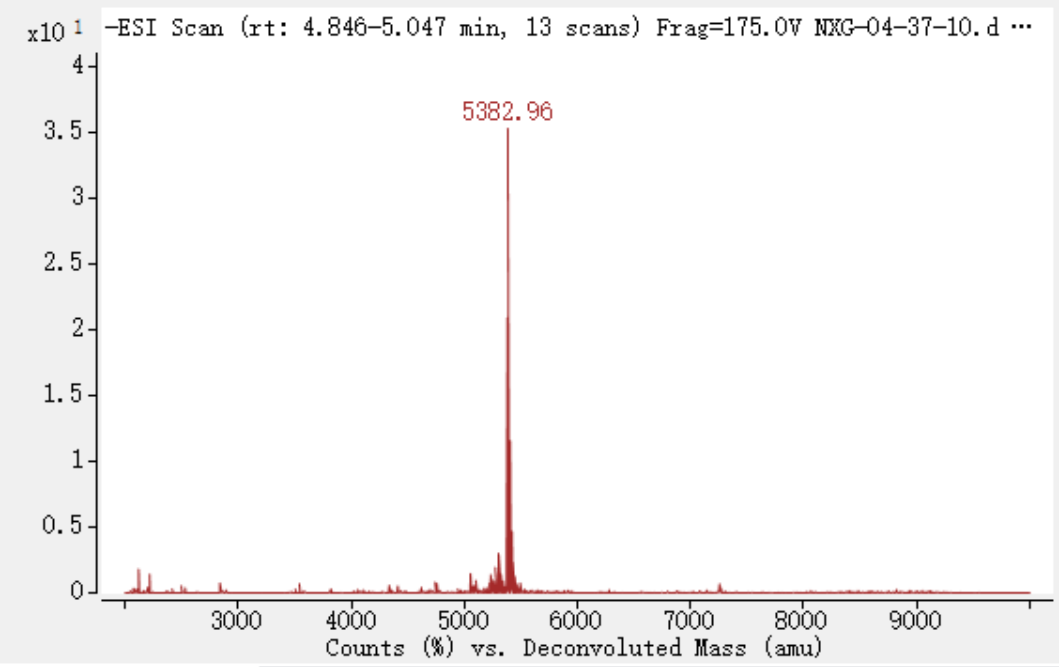

x10 5
-ESI Scan (rt: 5.095-5.578 min, 30 scans) Frag=175.0V NXG-04-…
5399.89
2892.63
4100.62
Counts vs. Deconvoluted Mass (amu)

UHPLC chromatogram and deconvoluted MS of **d49**

Conversion: 78%

Calculated Mass: 5383 Da; Observed Mass: 5383 Da

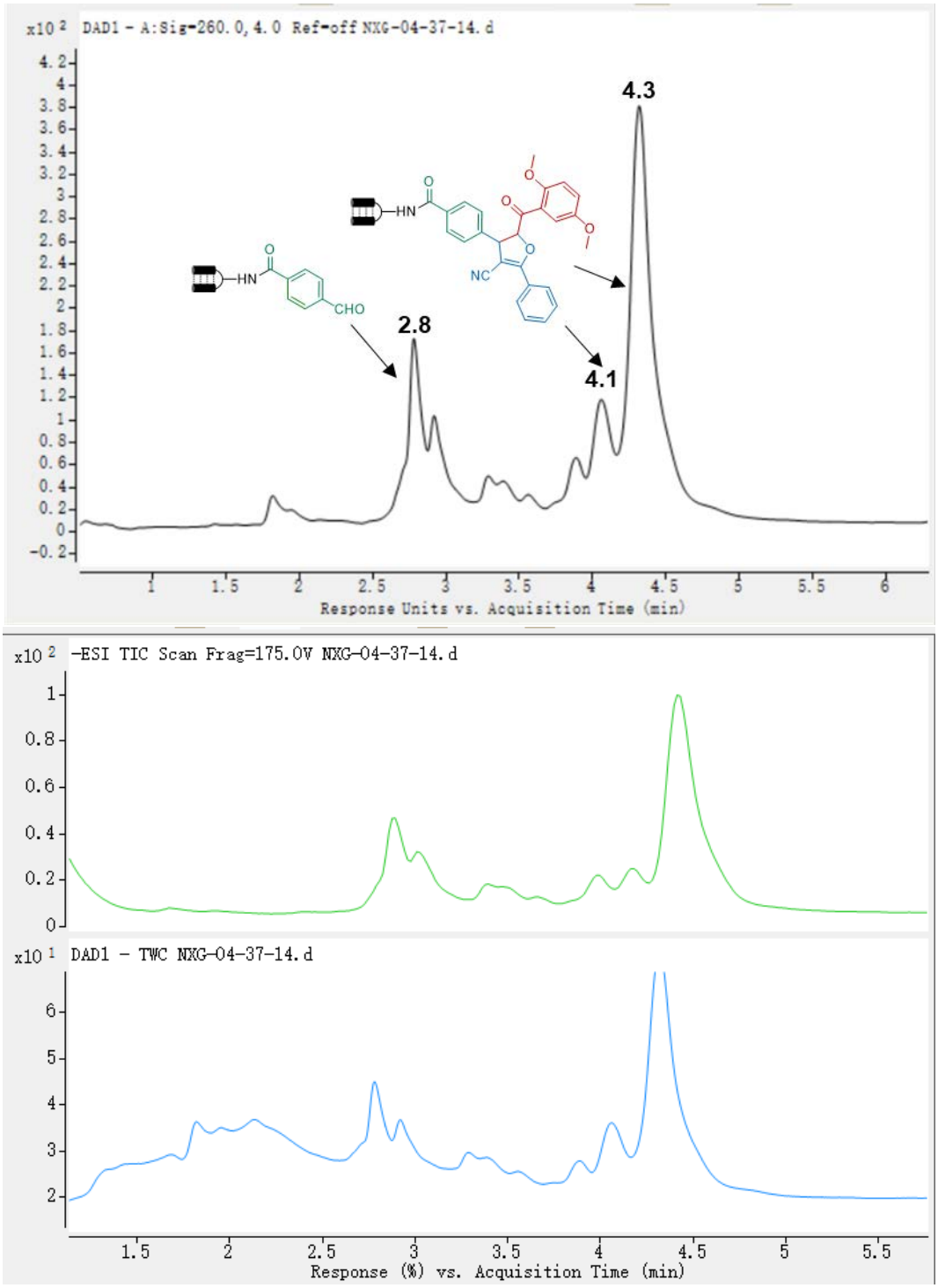


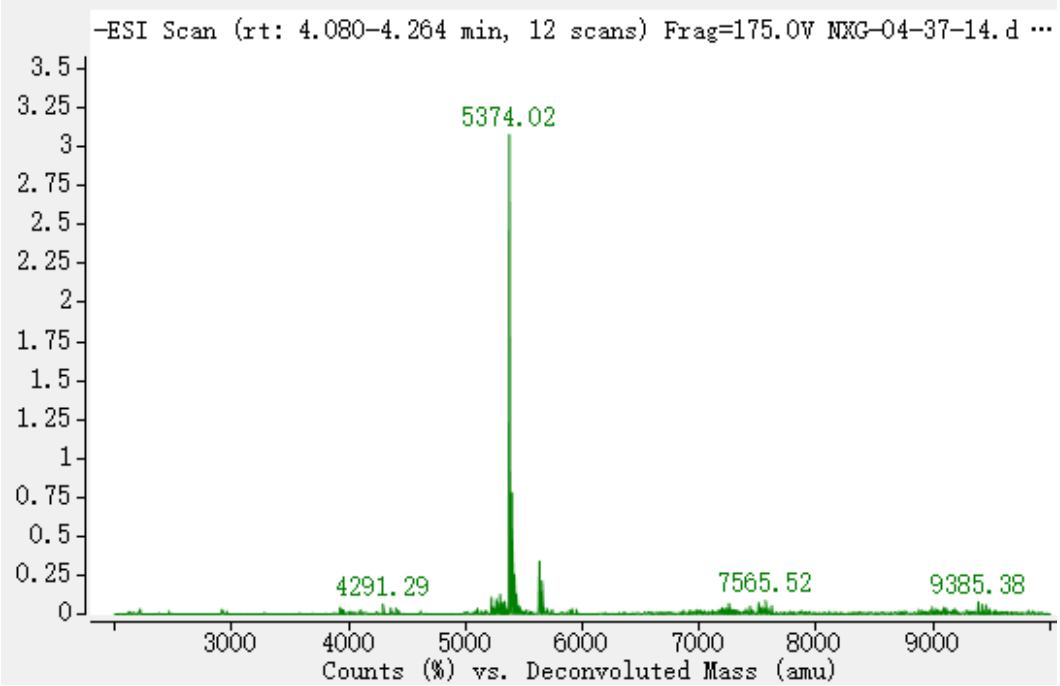

x10 1
-ESI Scan (rt: 4.846-5.047 min, 13 scans) Frag=175.0V NXG-04-37-10.d ...
5382.96
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

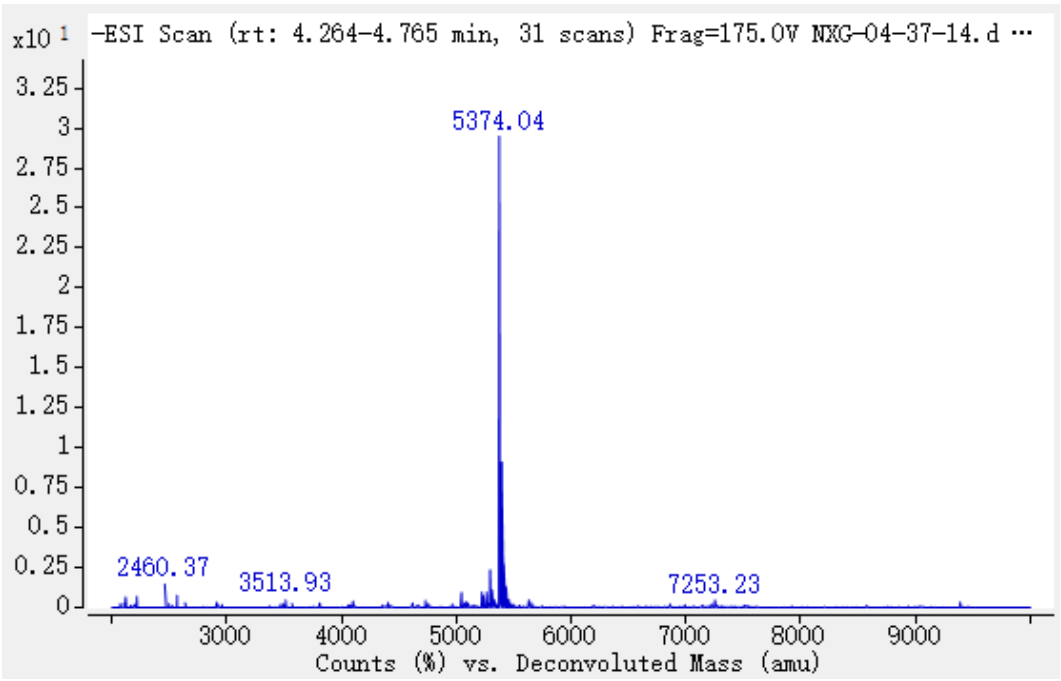
UHPLC chromatogram and deconvoluted MS of **d50**

Conversion: 65%

Calculated Mass: 5374 Da; Observed Mass: 5374 Da



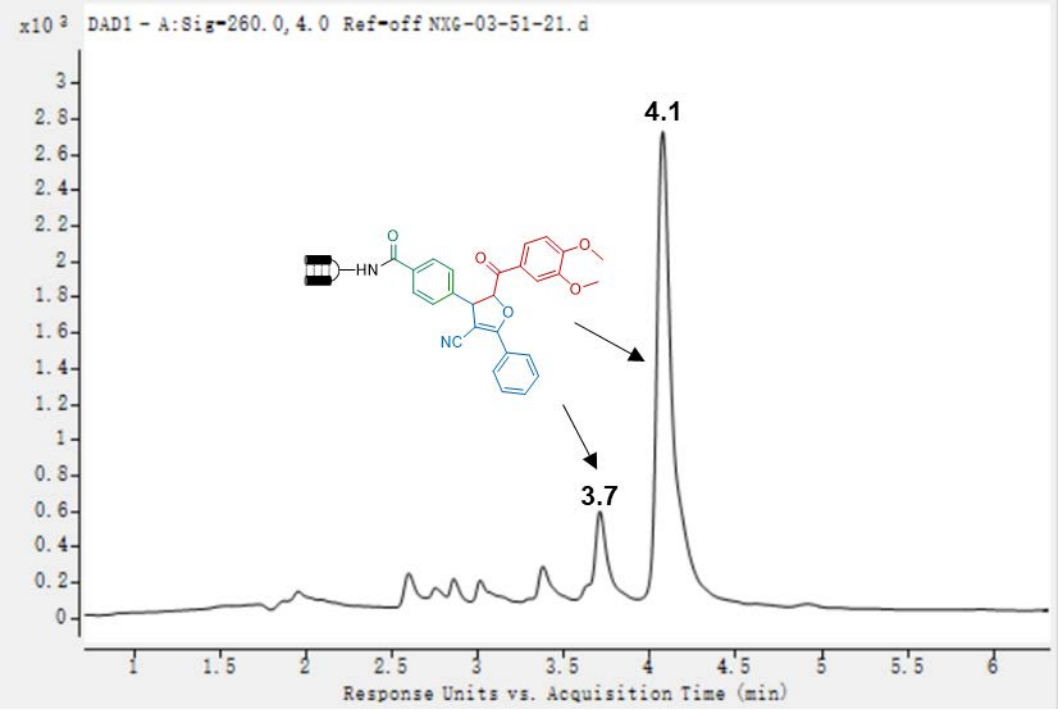

-ESI Scan (rt: 4.080-4.264 min, 12 scans) Frag=175.0V NXG-04-37-14.d ...
5374.02
4291.29
7565.52
9385.38
Counts (%) vs. Deconvoluted Mass (amu)

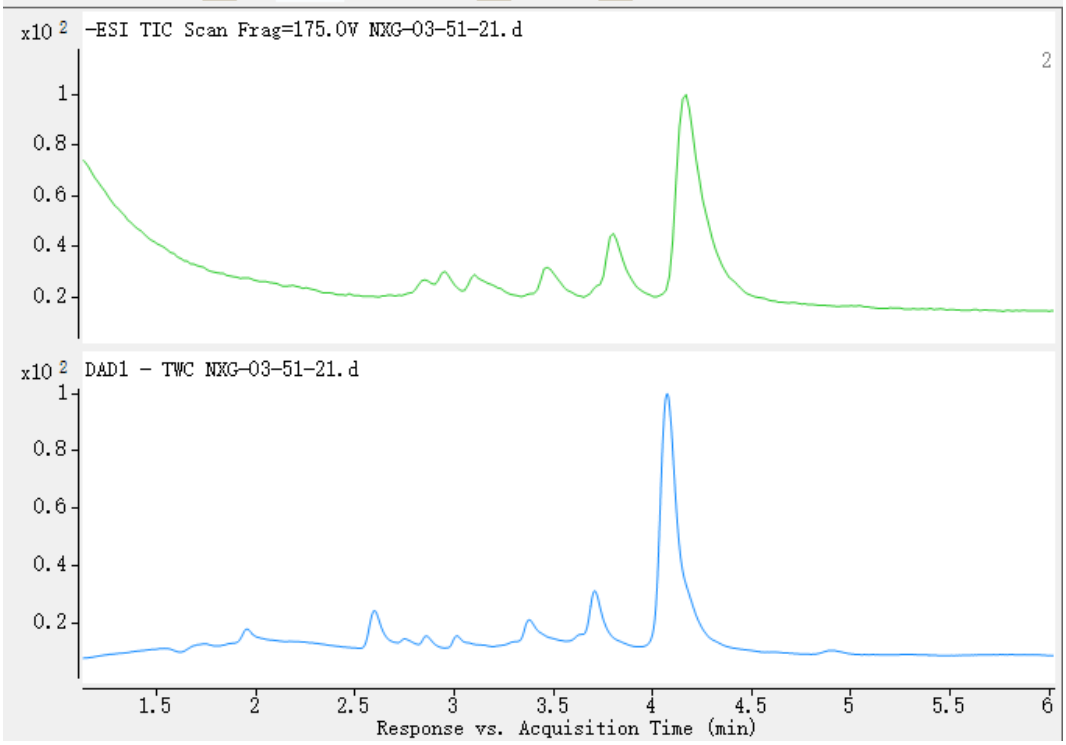

x10 1
-ESI Scan (rt: 4.264-4.765 min, 31 scans) Frag=175.0V NXG-04-37-14.d ...
5374.04
2460.37
3513.93
7253.23
Counts (%) vs. Deconvoluted Mass (amu)

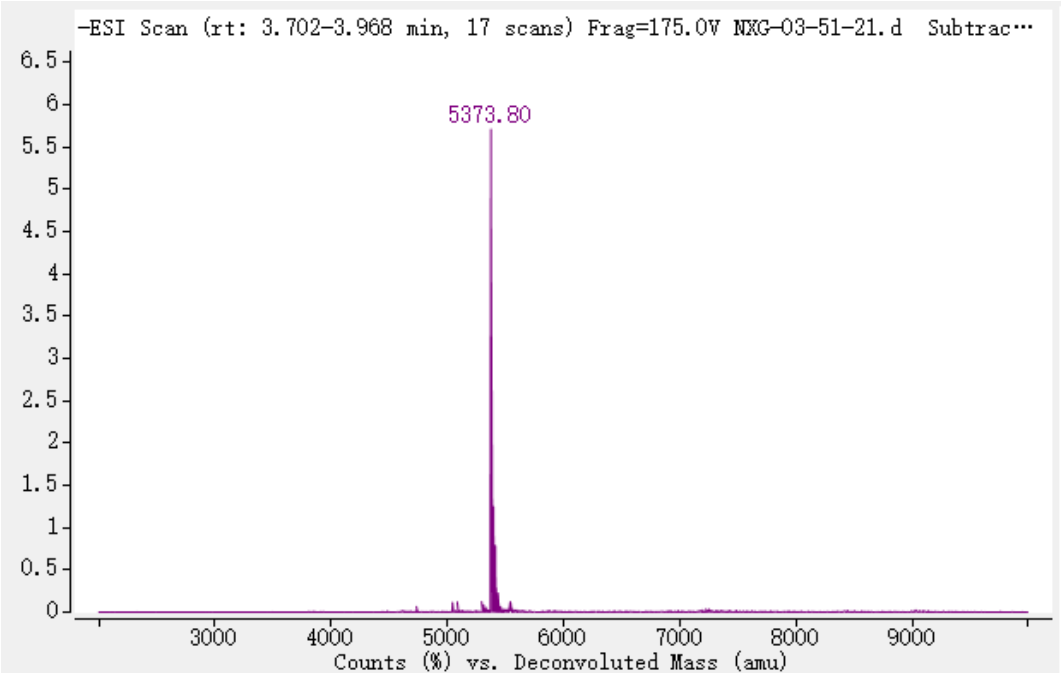
UHPLC chromatogram and deconvoluted MS of **d51**

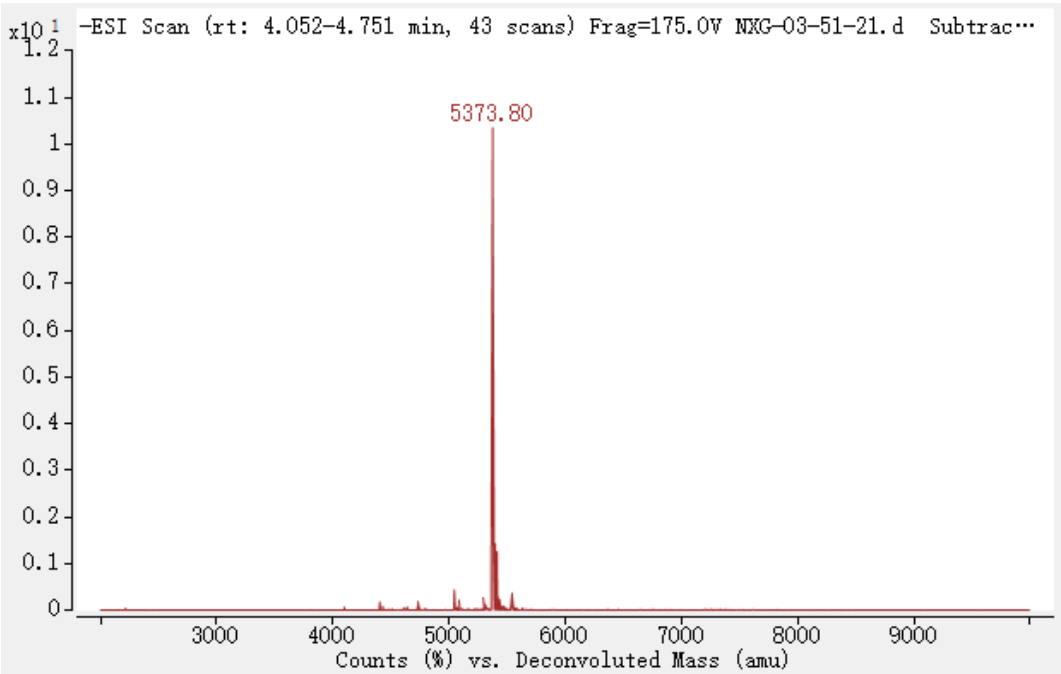
Conversion: 85%

Calculated Mass: 5374 Da; Observed Mass: 5374 Da





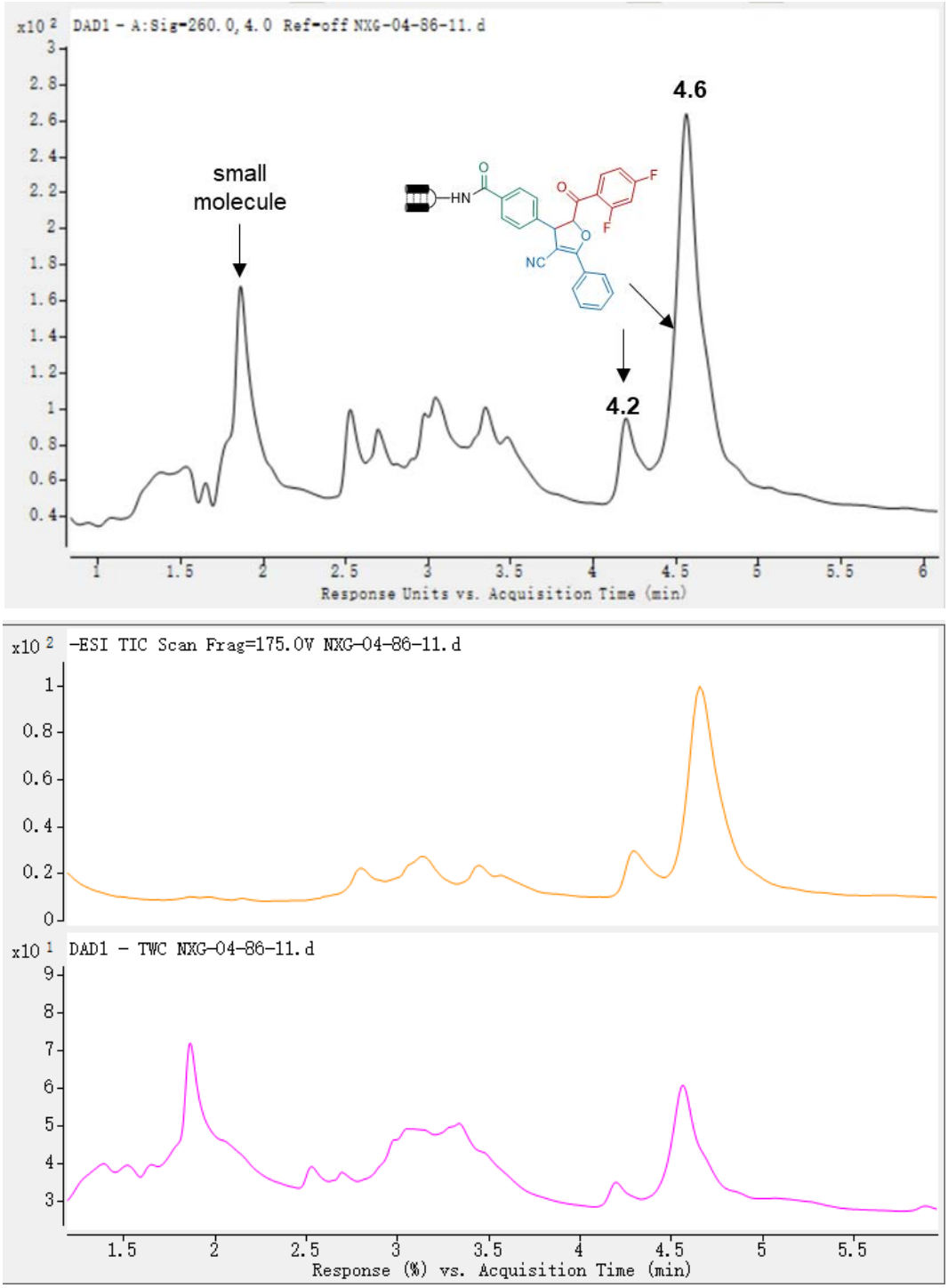

-ESI Scan (rt: 3.702-3.968 min, 17 scans) Frag=175.0V NXG-03-51-21.d Subtrac…
5373.80
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

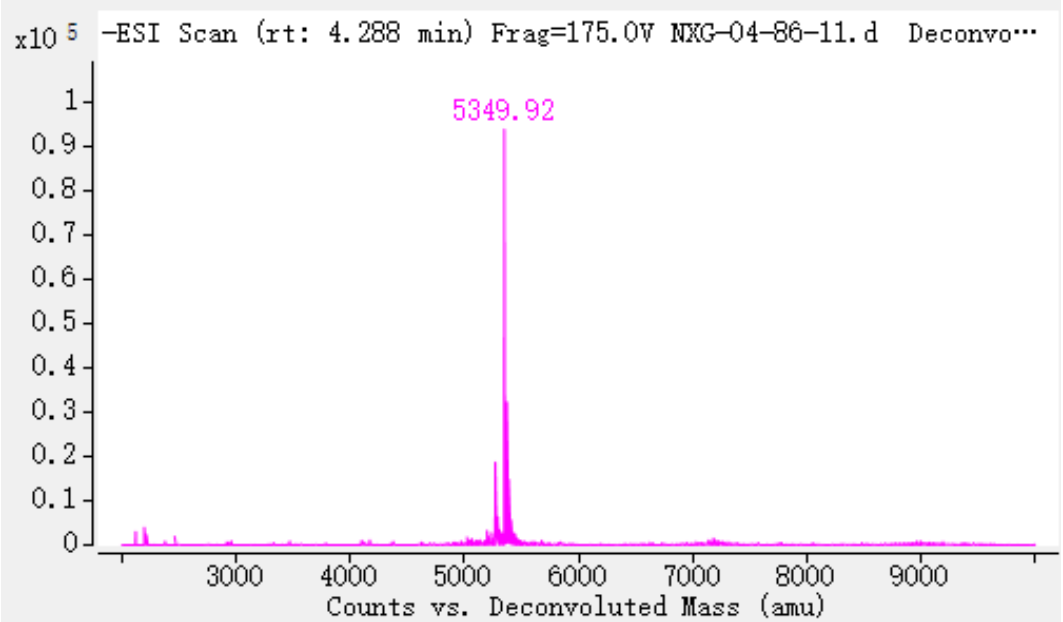

x10 1
-ESI Scan (rt: 4.052-4.751 min, 43 scans) Frag=175.0V NXG-03-51-21.d Subtrac…
5373.80
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

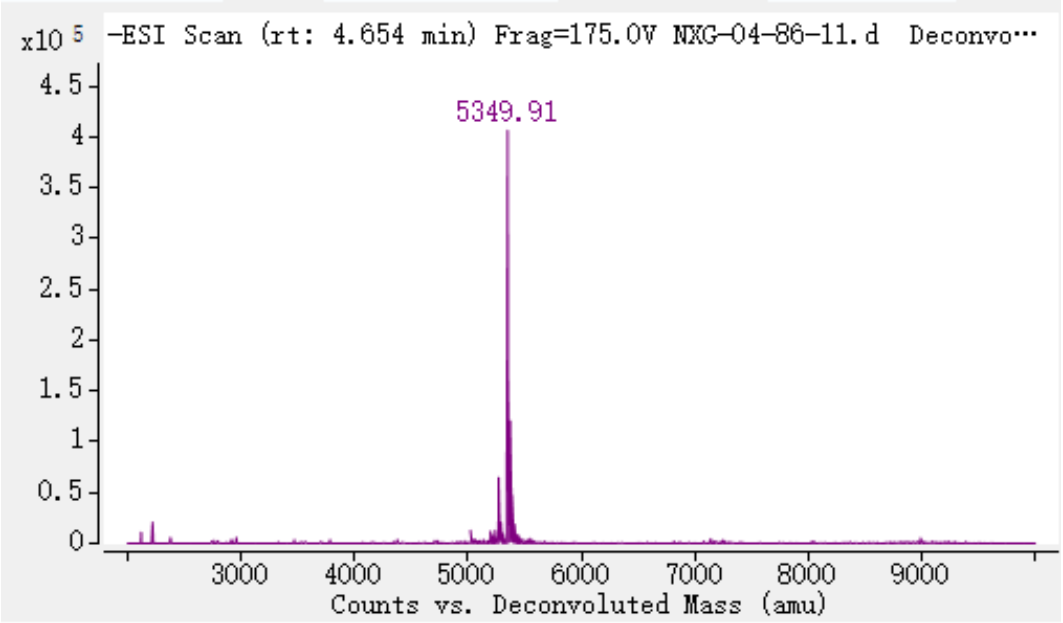
UHPLC chromatogram and deconvoluted MS of **d52**

Conversion: 55%

Calculated Mass: 5350 Da; Observed Mass: 5350 Da



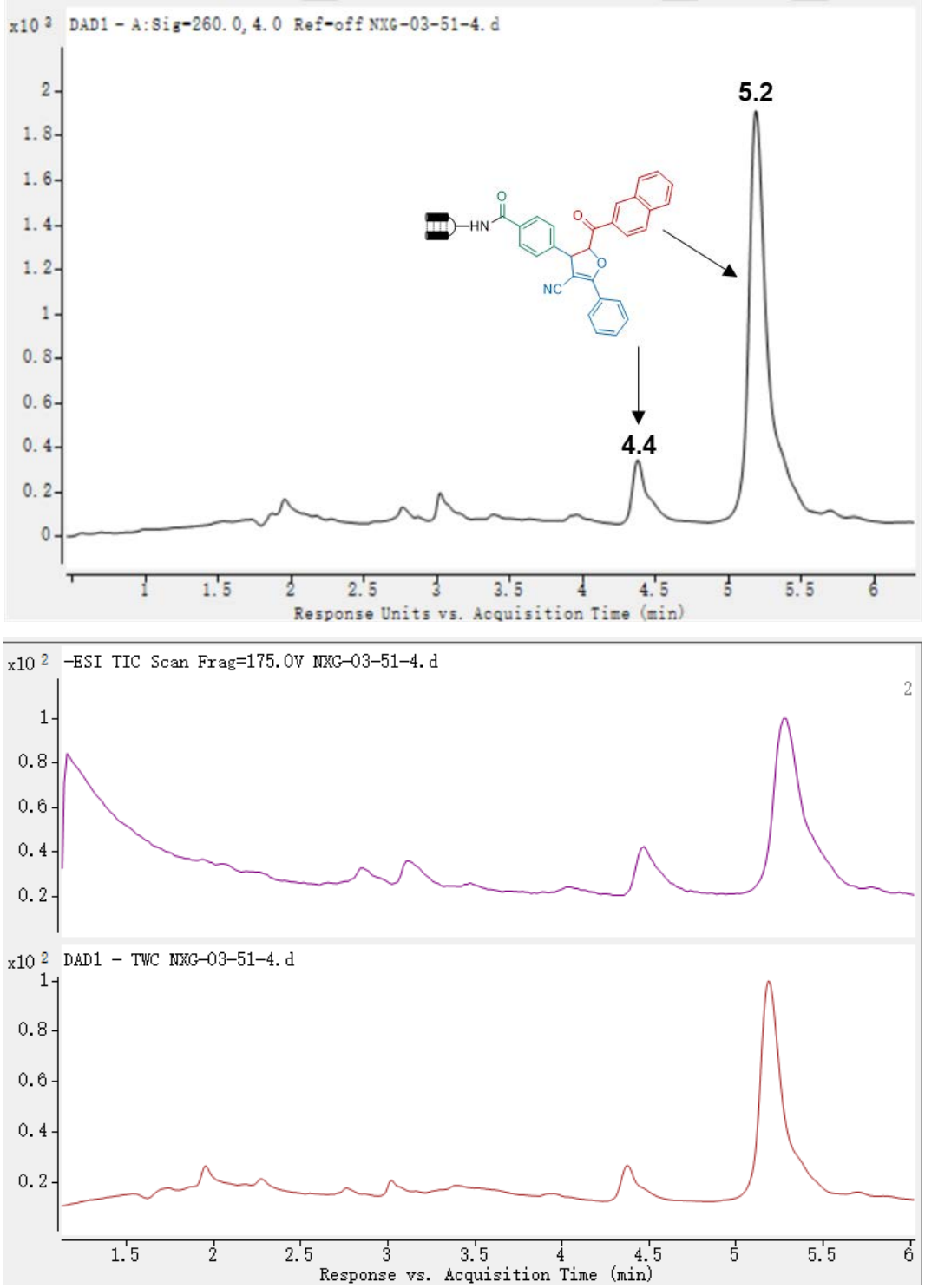

x10 5
-ESI Scan (rt: 4.288 min) Frag=175.0V NXG-04-86-11.d Deconvo…
5349.92
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

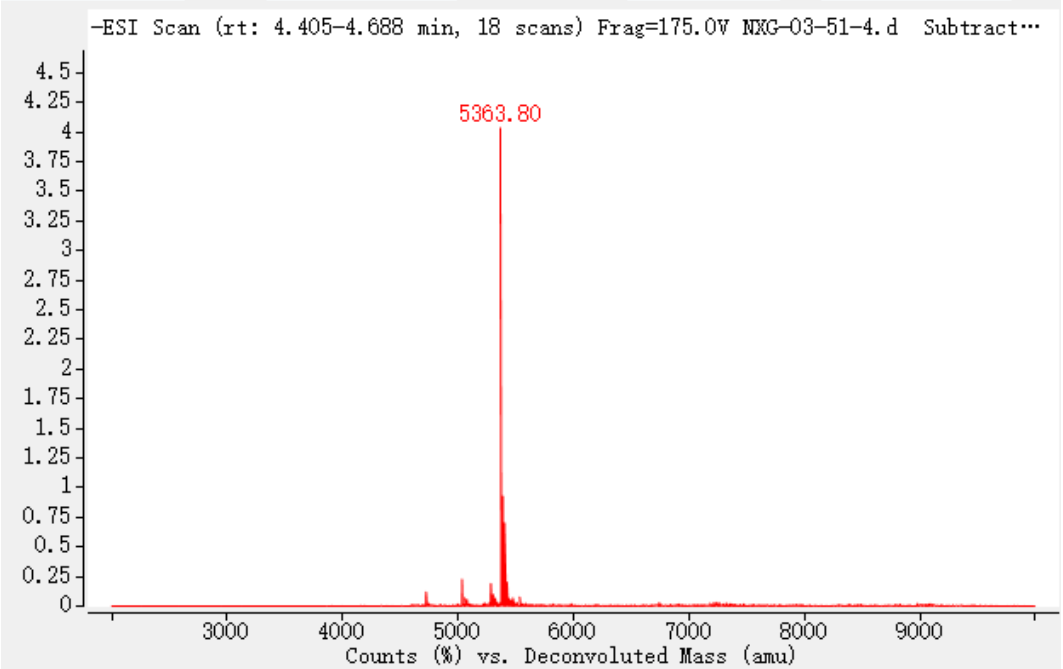

x10 5
-ESI Scan (rt: 4.654 min) Frag=175.0V NXG-04-86-11.d Deconvo…
5349.91
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

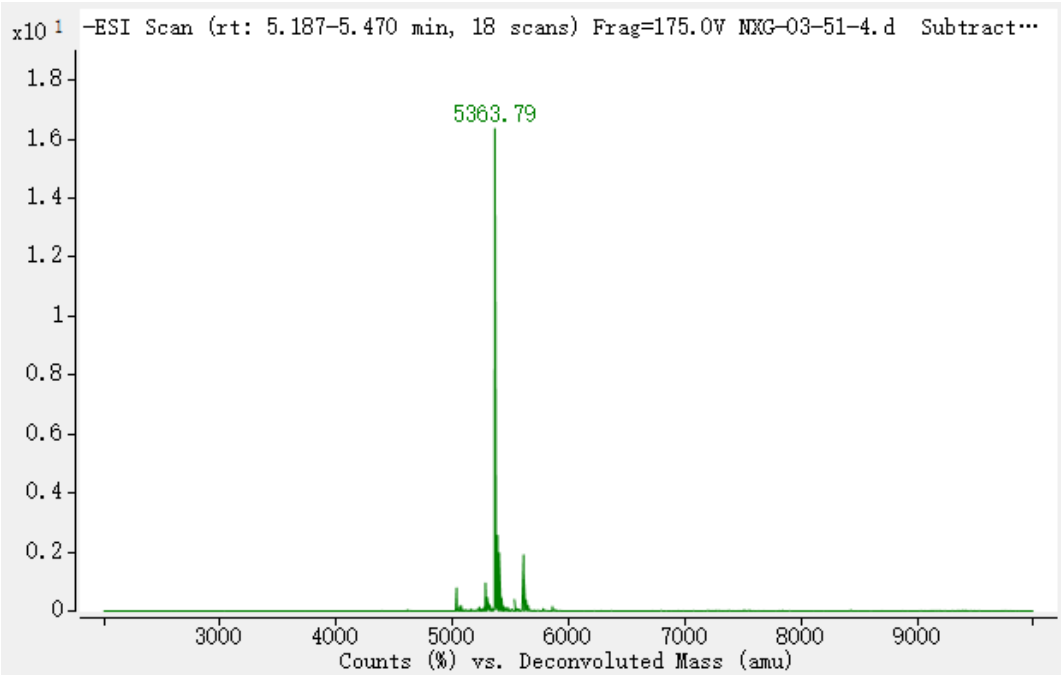
UHPLC chromatogram and deconvoluted MS of **d53**

Conversion: >90%

Calculated Mass: 5364 Da; Observed Mass: 5364 Da



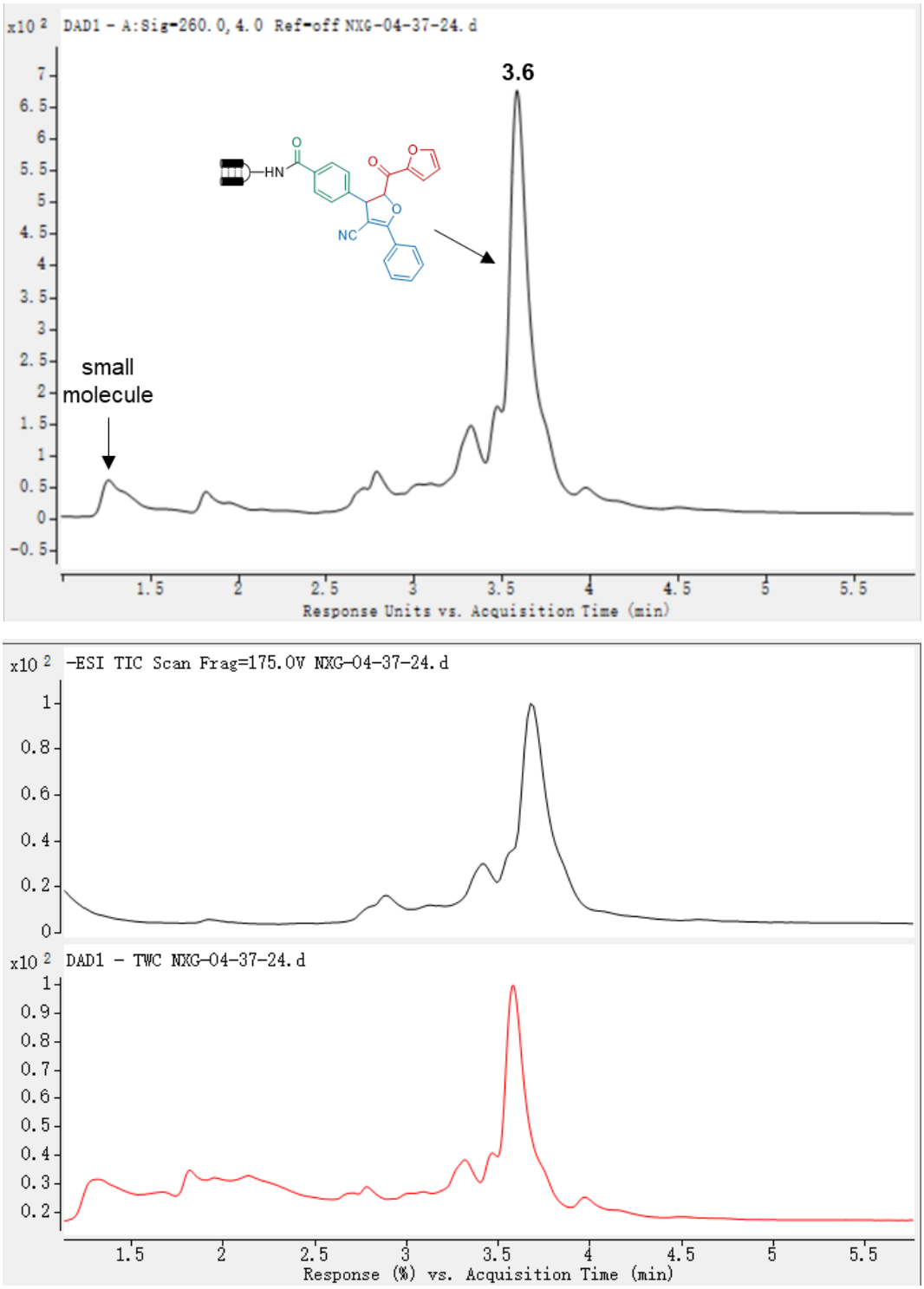

-ESI Scan (rt: 4.405-4.688 min, 18 scans) Frag=175.0V NXG-03-51-4.d Subtract…
5363.80
Counts (%) vs. Deconvoluted Mass (amu)

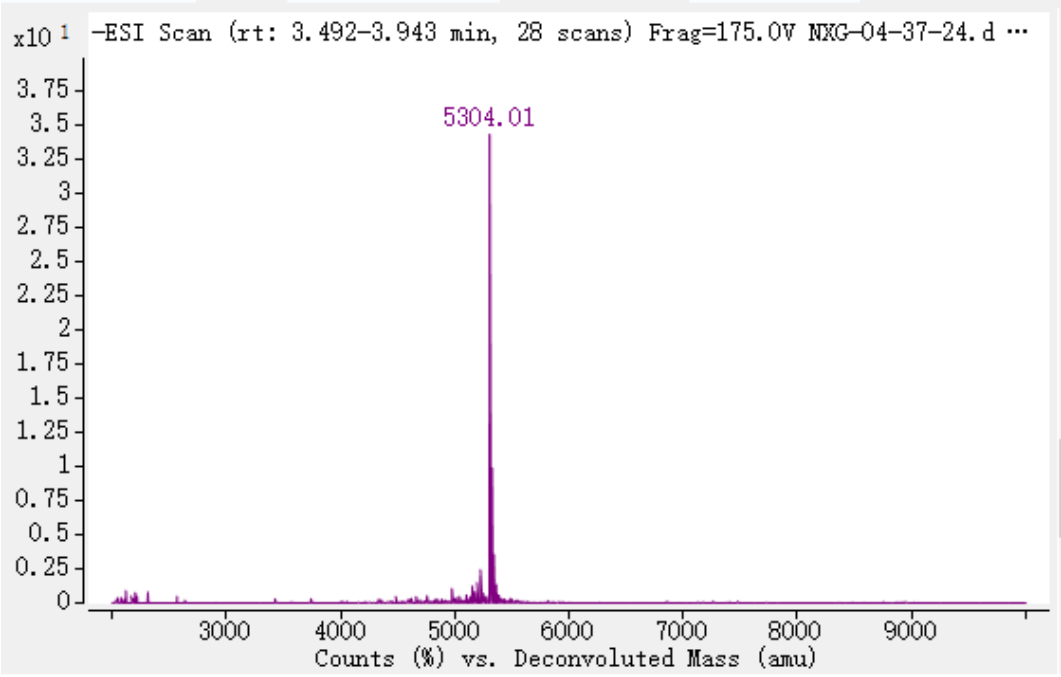

x10 1
-ESI Scan (rt: 5.187-5.470 min, 18 scans) Frag=175.0V NXG-03-51-4.d Subtract…
5363.79
Counts (%) vs. Deconvoluted Mass (amu)

UHPLC chromatogram and deconvoluted MS of **d54**

Conversion: 70%

Calculated Mass: 5304 Da; Observed Mass: 5304 Da



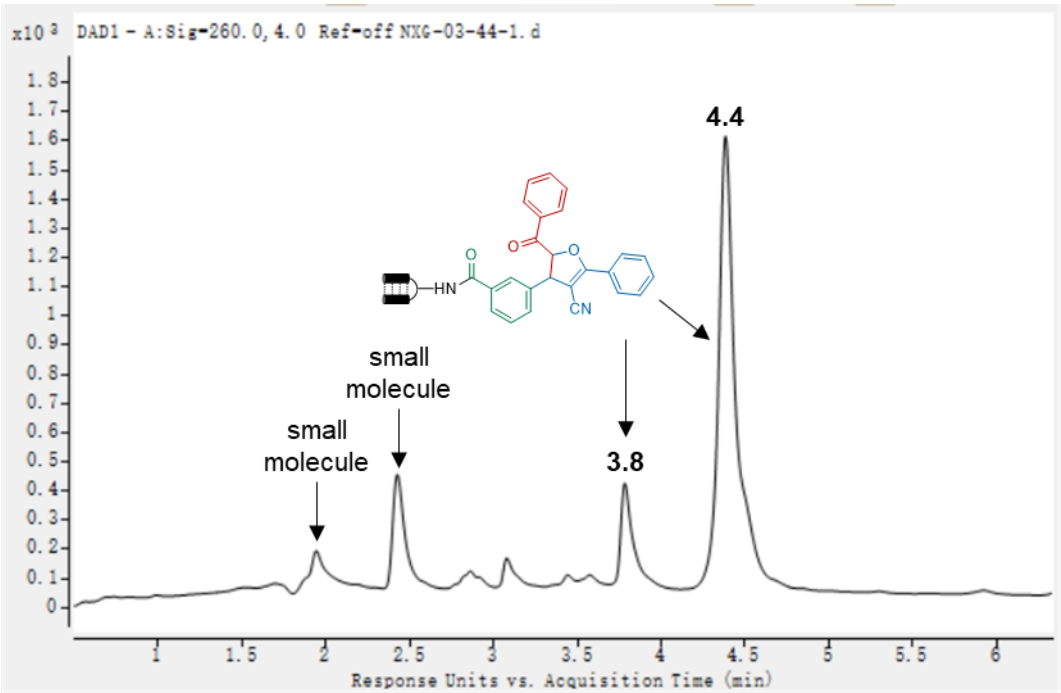

x10 1
-ESI Scan (rt: 3.492-3.943 min, 28 scans) Frag=175.0V NXG-04-37-24.d ...
5304.01
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

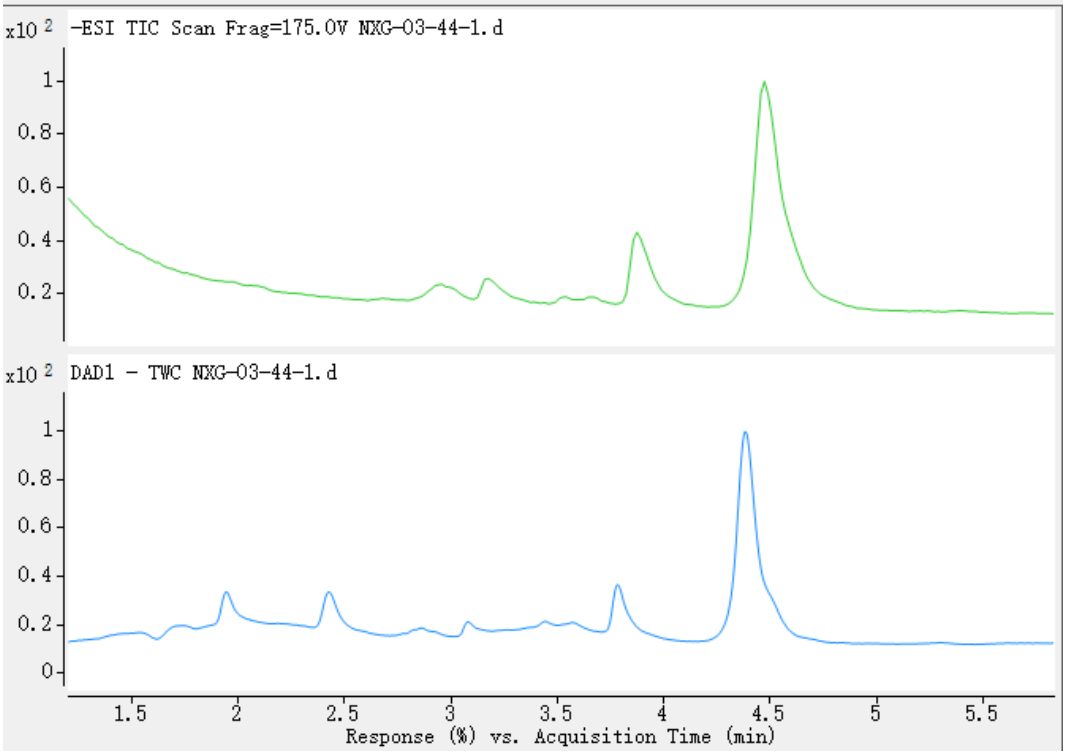
8.3 Substrate scope of DNA-conjugated aldehydes

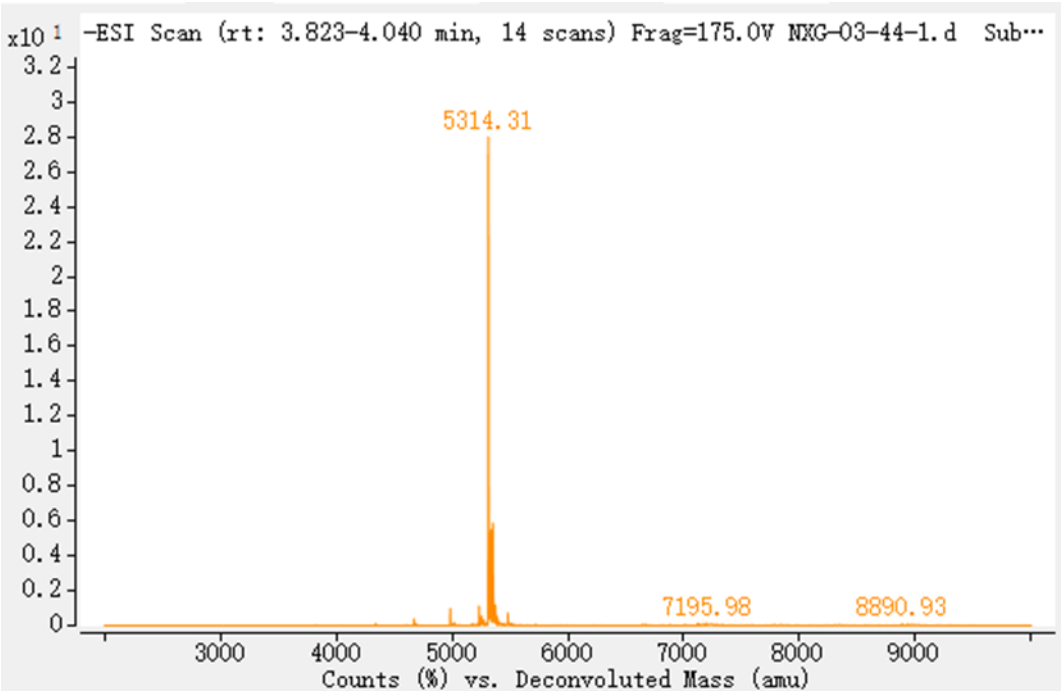
UHPLC chromatogram and deconvoluted MS of **d55**

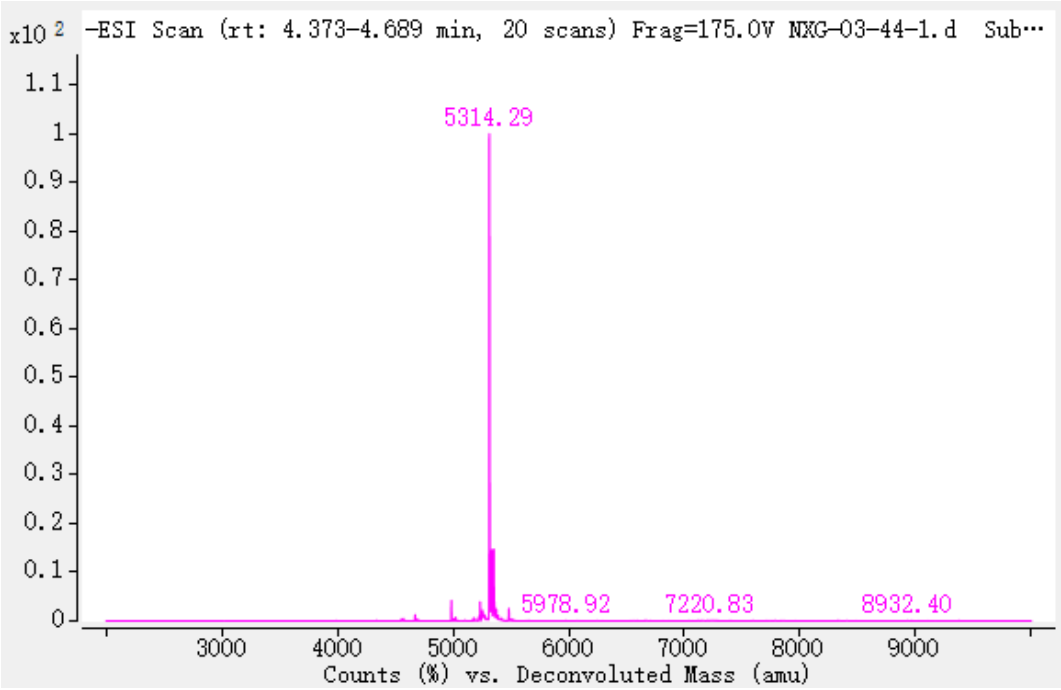
Conversion: >90%

Calculated Mass: 5314 Da; Observed Mass: 5314 Da





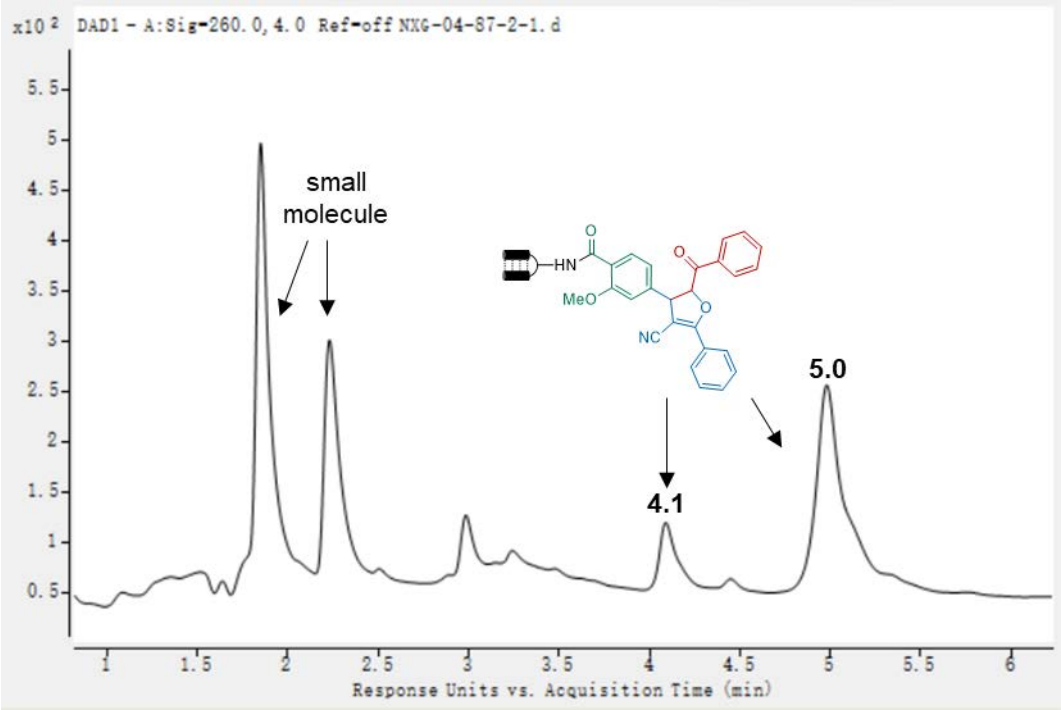

x10 1
-ESI Scan (rt: 3.823-4.040 min, 14 scans) Frag=175.0V NXG-03-44-1.d Sub…
5314.31
7195.98
8890.93
Counts (%) vs. Deconvoluted Mass (amu)

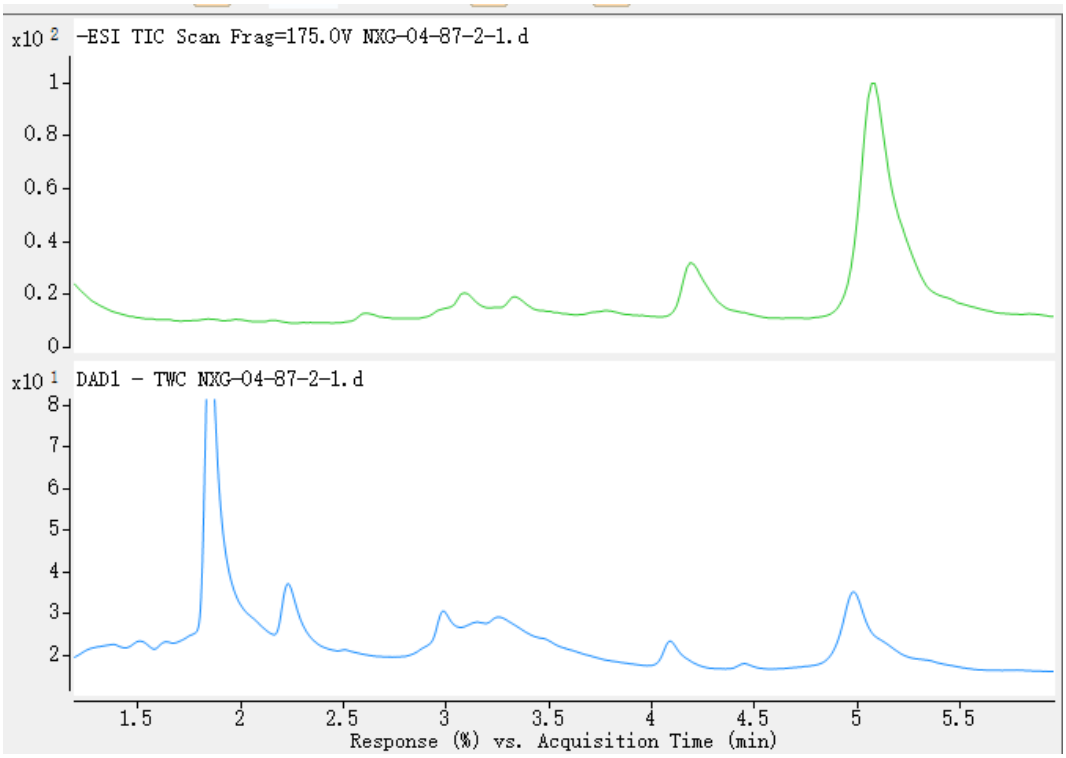

x10 2
-ESI Scan (rt: 4.373-4.689 min, 20 scans) Frag=175.0V NXG-03-44-1.d Sub…
5314.29
5978.92
7220.83
8932.40
Counts (%) vs. Deconvoluted Mass (amu)

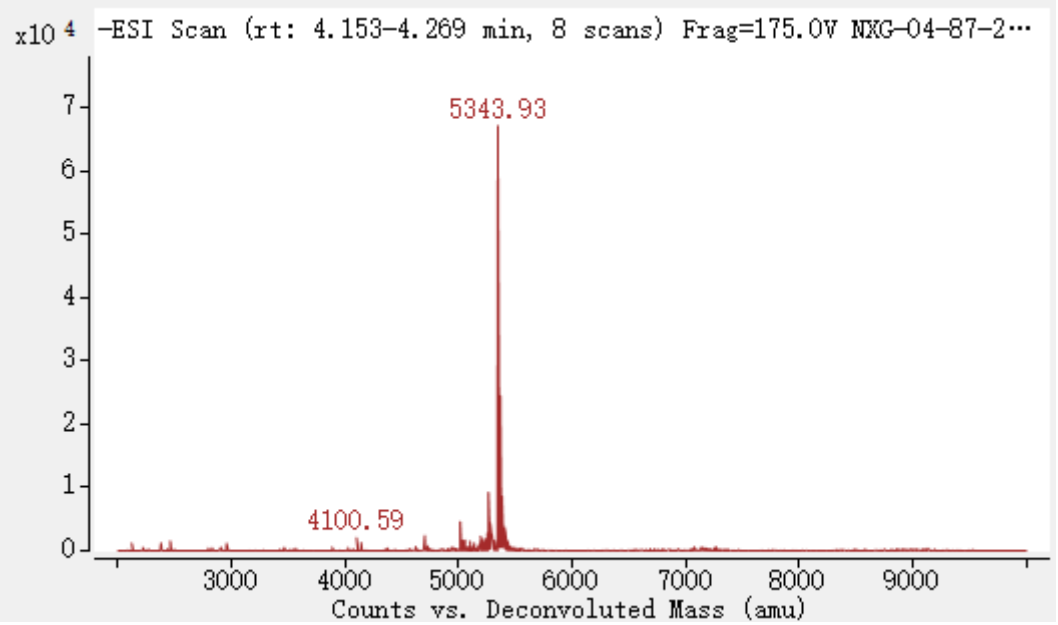
UHPLC chromatogram and deconvoluted MS of **d56**

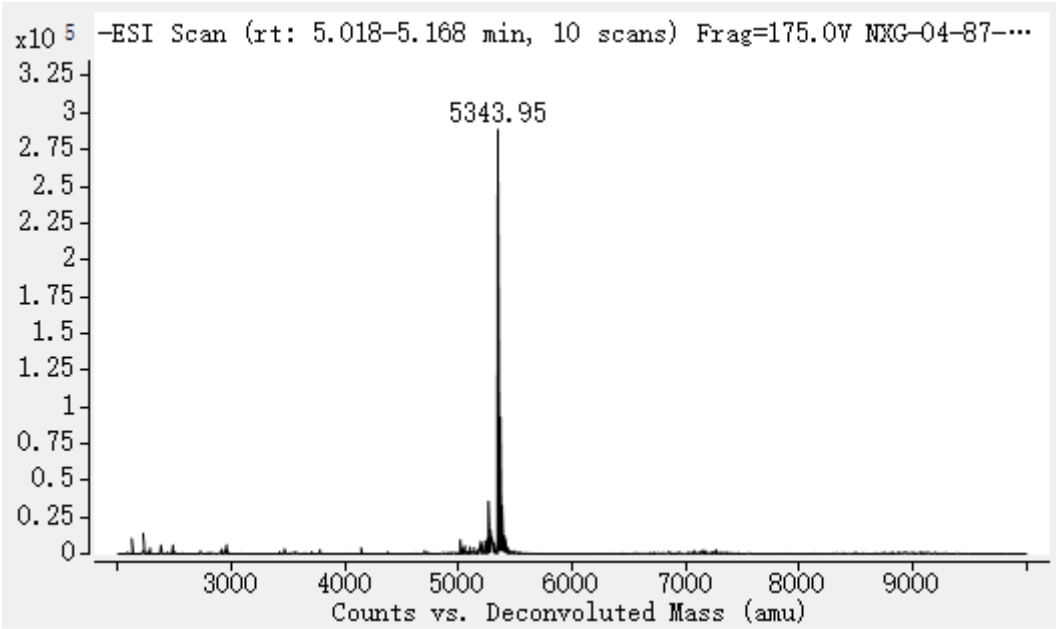
Conversion: 68%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da





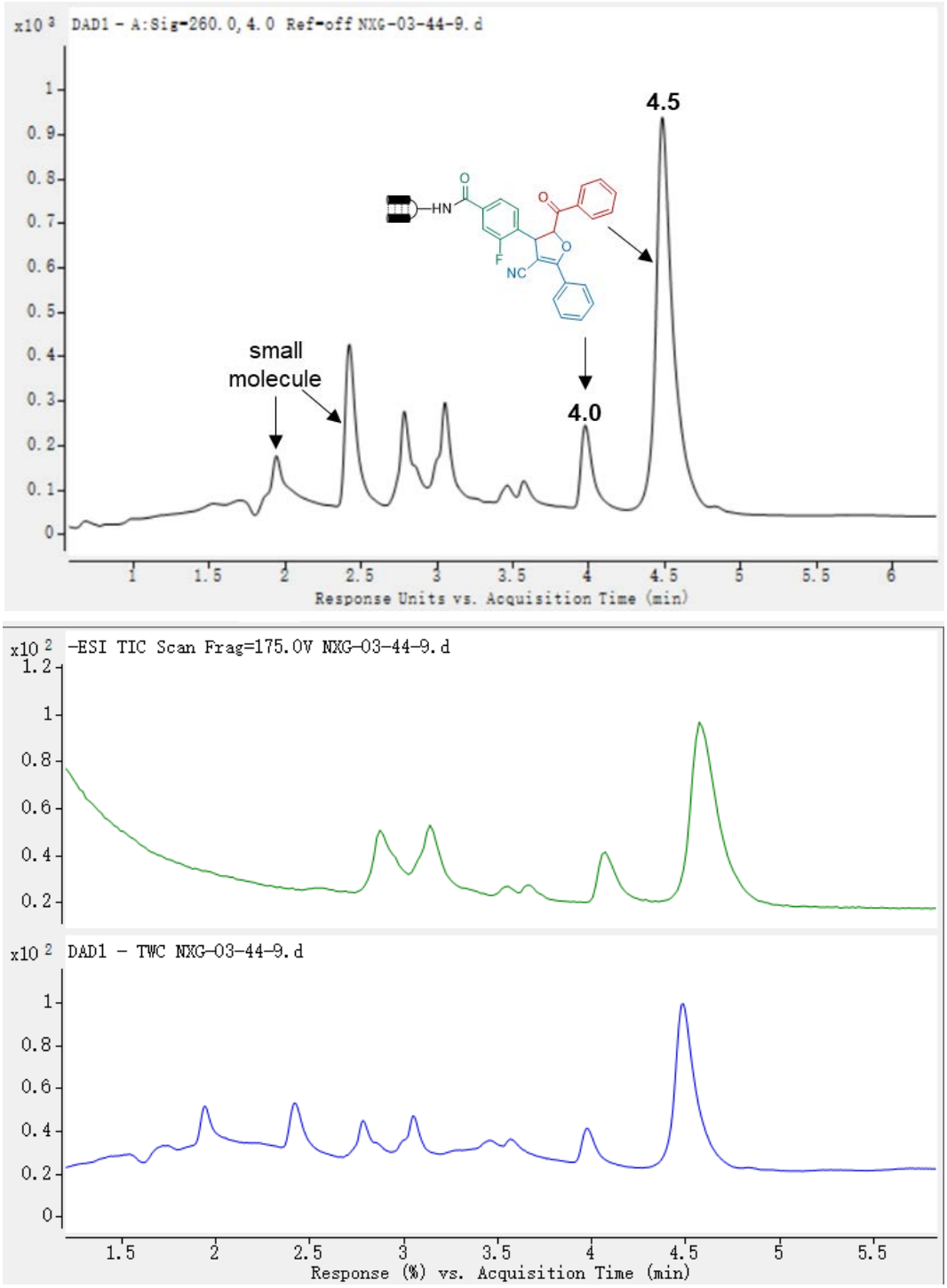

x10 4
-ESI Scan (rt: 4.153-4.269 min, 8 scans) Frag=175.0V NXG-04-87-2…
7
6
5
4
3
2
1
0
5343.93
4100.59
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

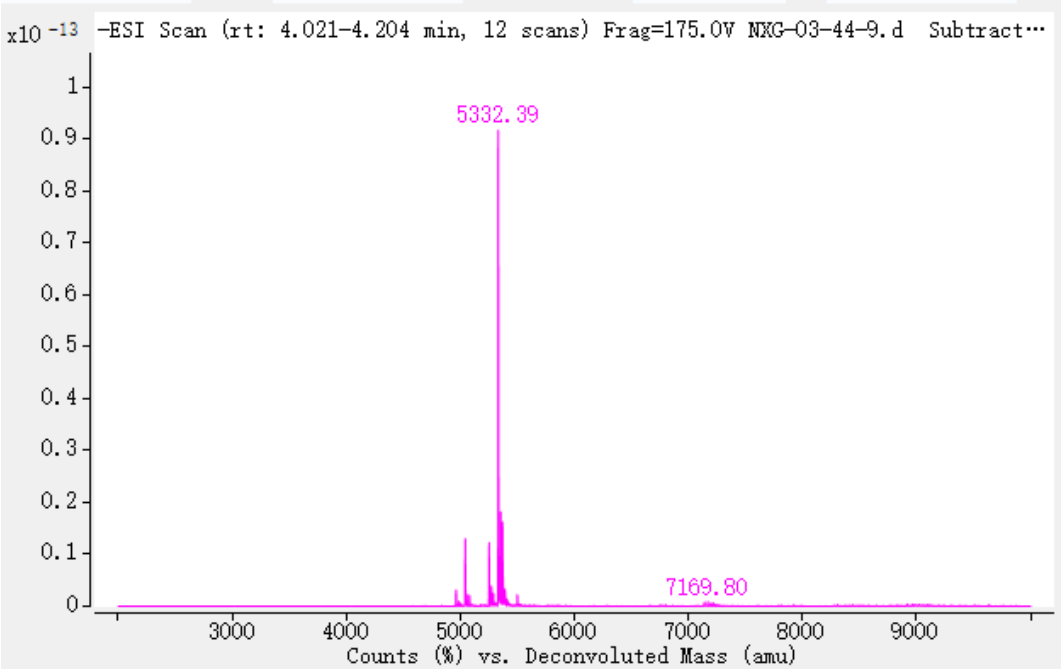

x10 5
-ESI Scan (rt: 5.018-5.168 min, 10 scans) Frag=175.0V NXG-04-87-…
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
5343.95
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

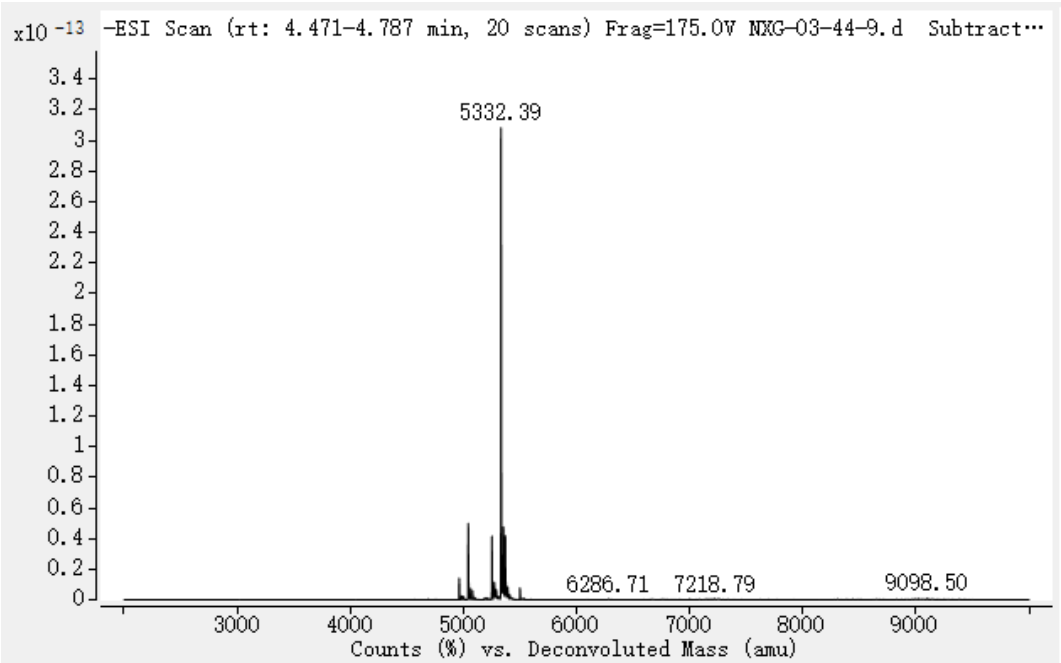
UHPLC chromatogram and deconvoluted MS of **d57**

Conversion: 65%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da



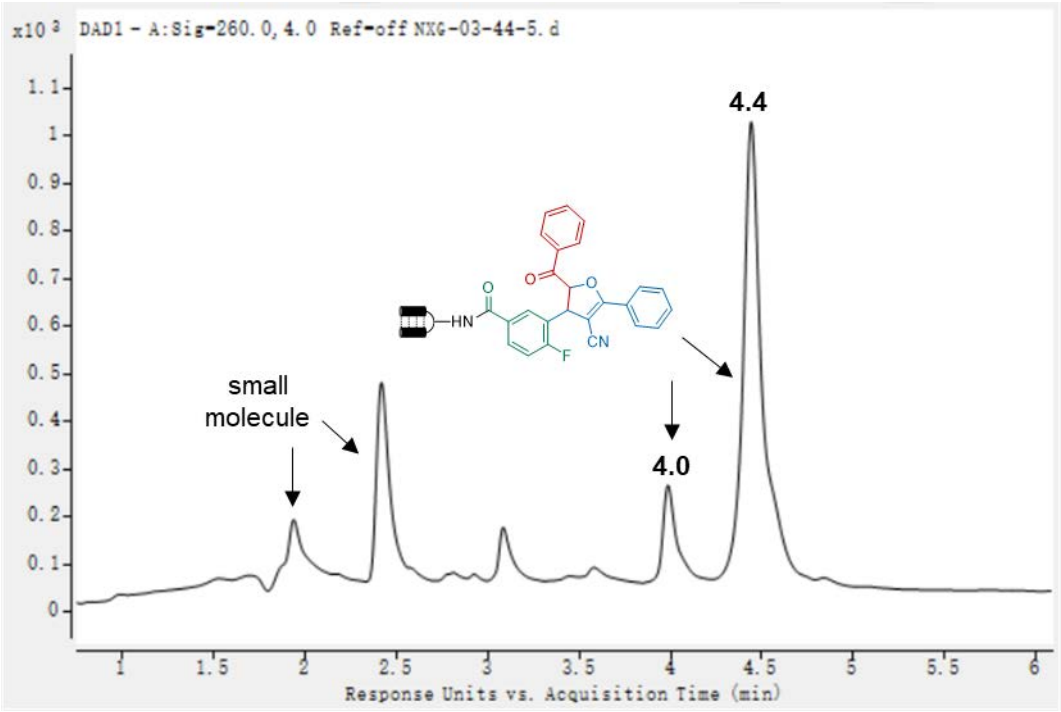

x10 -13
-ESI Scan (rt: 4.021-4.204 min, 12 scans) Frag=175.0V NXG-03-44-9.d Subtract…
5332.39
7169.80
Counts (%) vs. Deconvoluted Mass (amu)

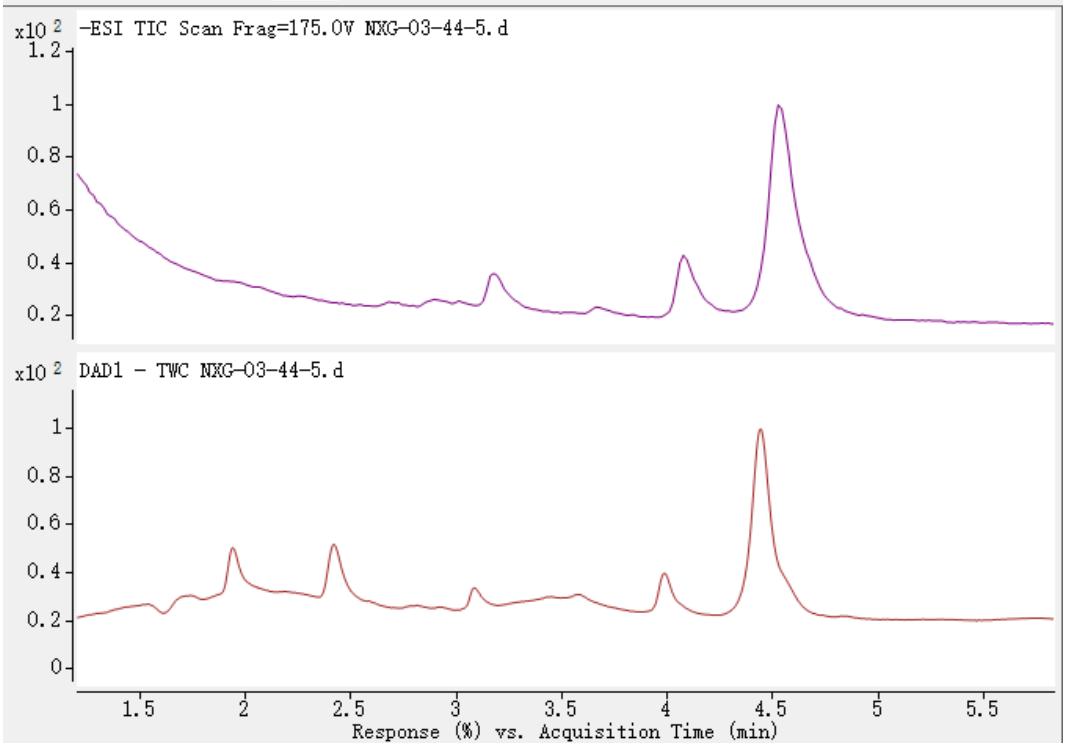

x10 -13
-ESI Scan (rt: 4.471-4.787 min, 20 scans) Frag=175.0V NXG-03-44-9.d Subtract…
5332.39
6286.71
7218.79
9098.50
Counts (%) vs. Deconvoluted Mass (amu)

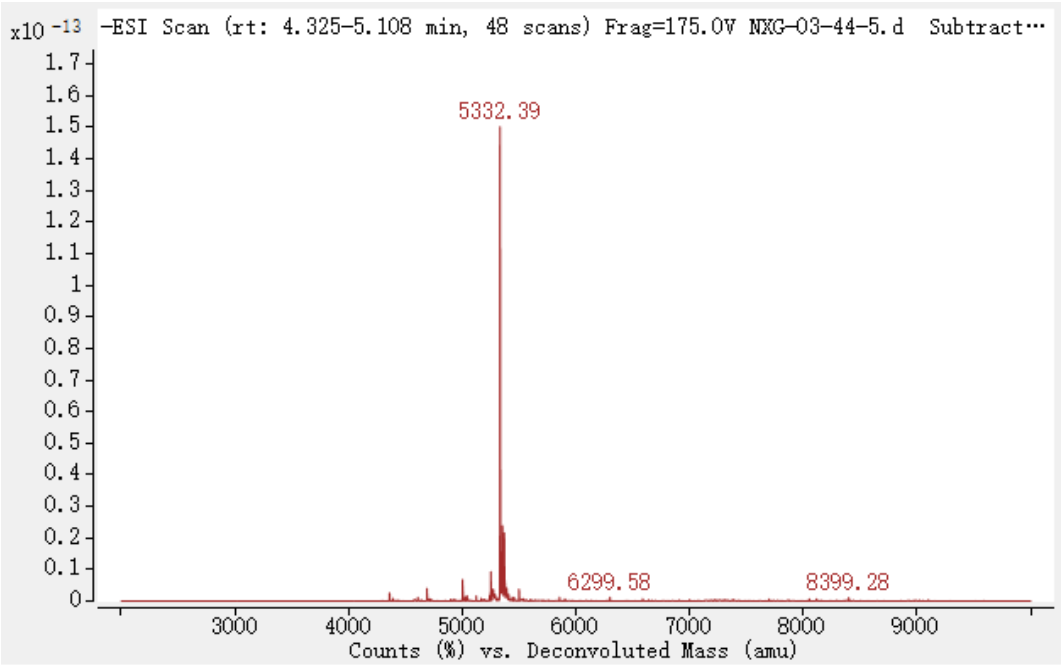
UHPLC chromatogram and deconvoluted MS of **d58**

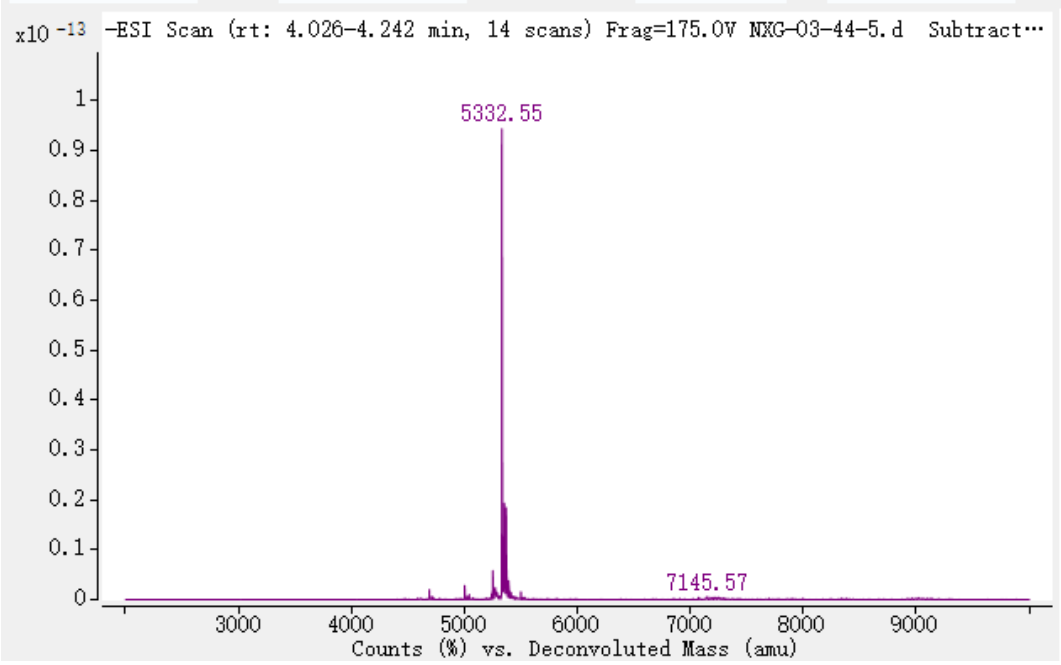
Conversion: >90%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da





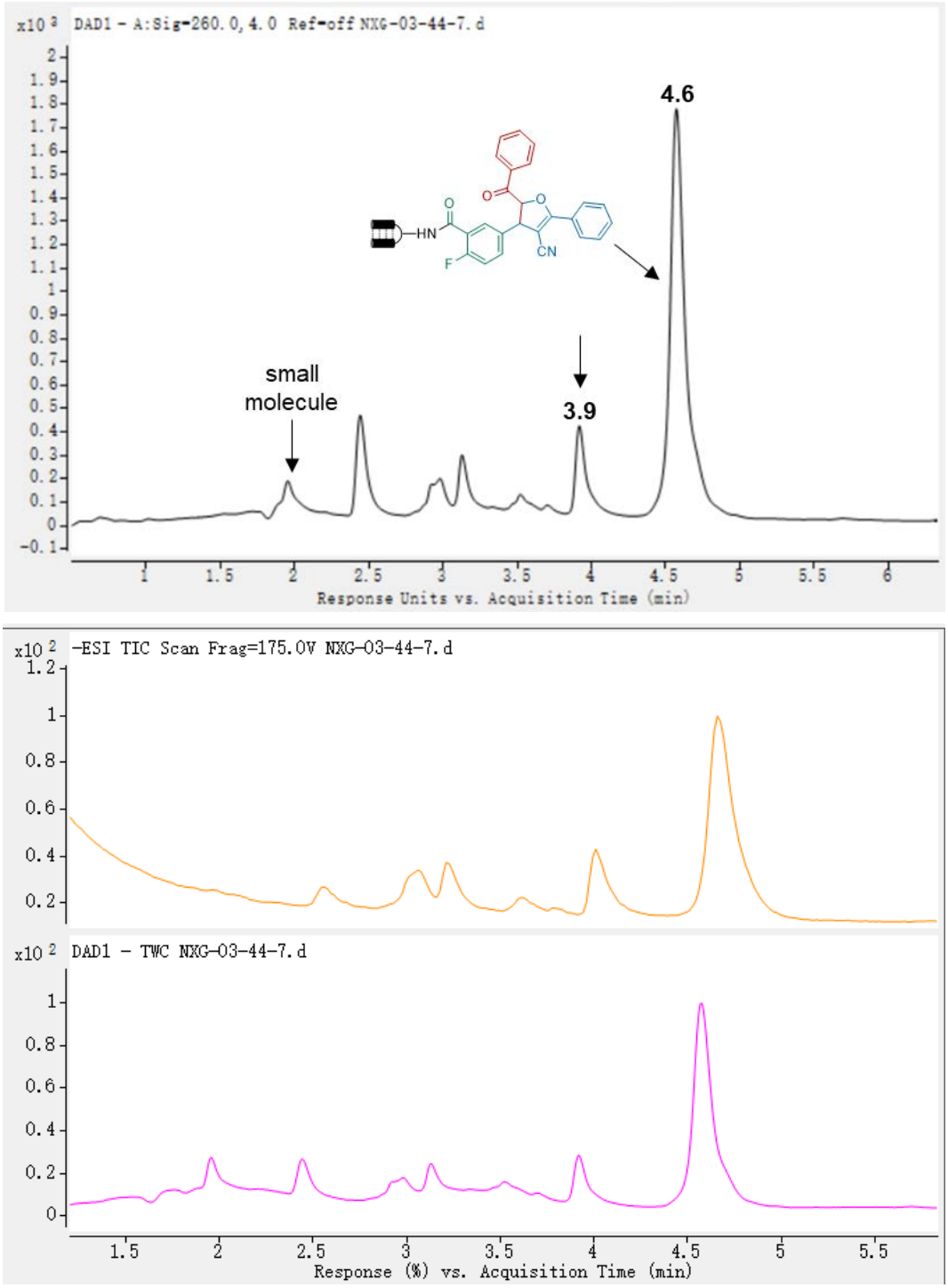

x10 -13
-ESI Scan (rt: 4.325-5.108 min, 48 scans) Frag=175.0V NXG-03-44-5.d Subtract…
5332.39
6299.58
8399.28
Counts (%) vs. Deconvoluted Mass (amu)

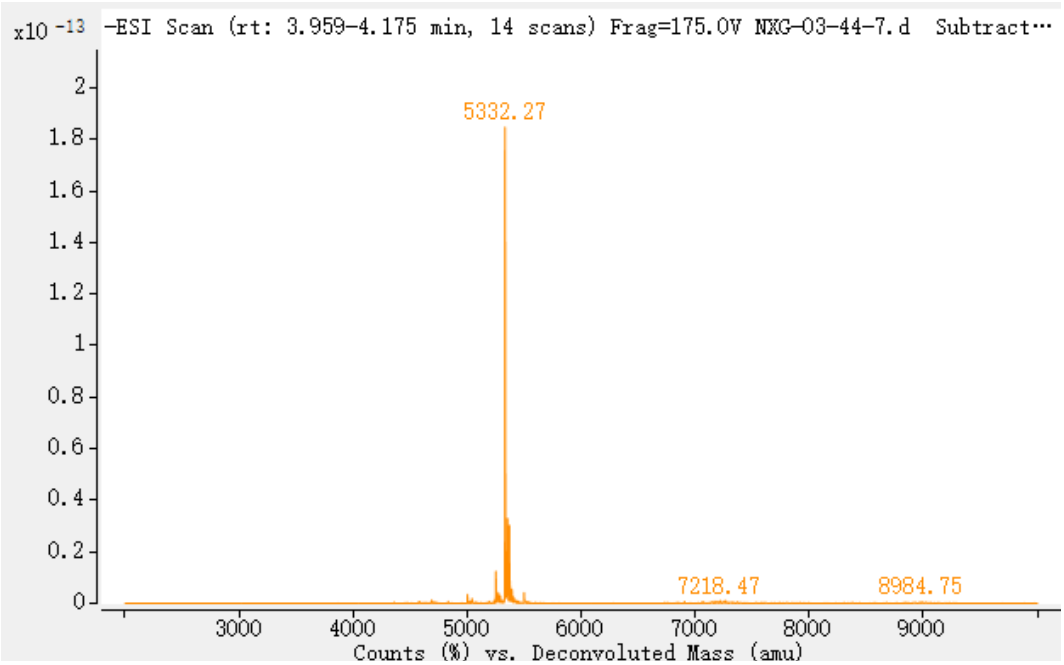

x10 -13
-ESI Scan (rt: 4.026-4.242 min, 14 scans) Frag=175.0V NXG-03-44-5.d Subtract…
5332.55
7145.57
Counts (%) vs. Deconvoluted Mass (amu)

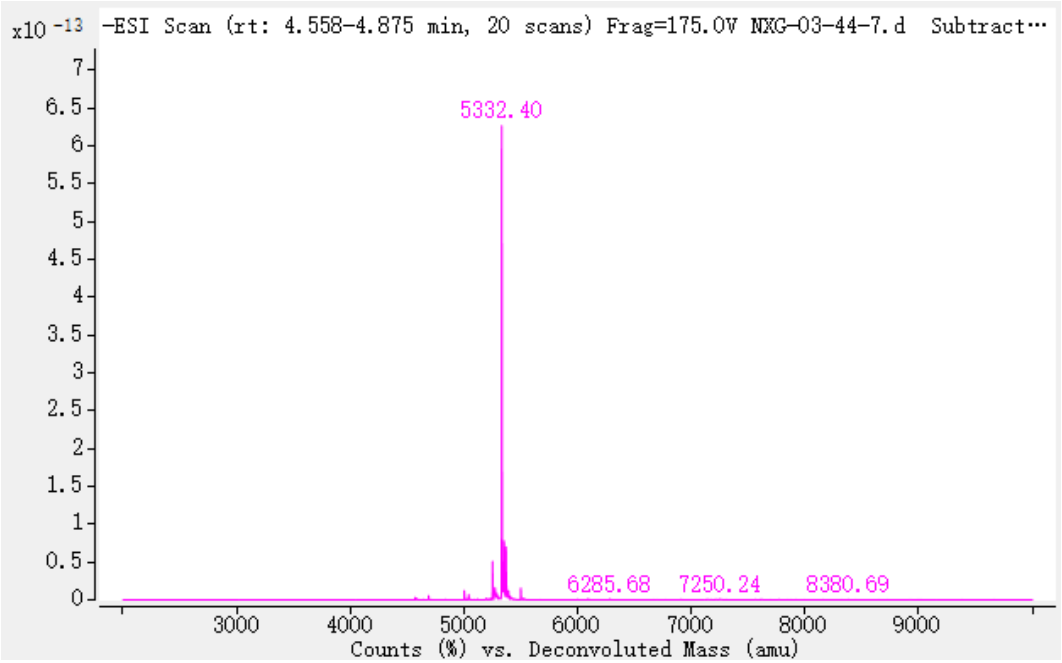
UHPLC chromatogram and deconvoluted MS of **d59**

Conversion: 75%

Calculated Mass: 5332 Da; Observed Mass: 5332 Da



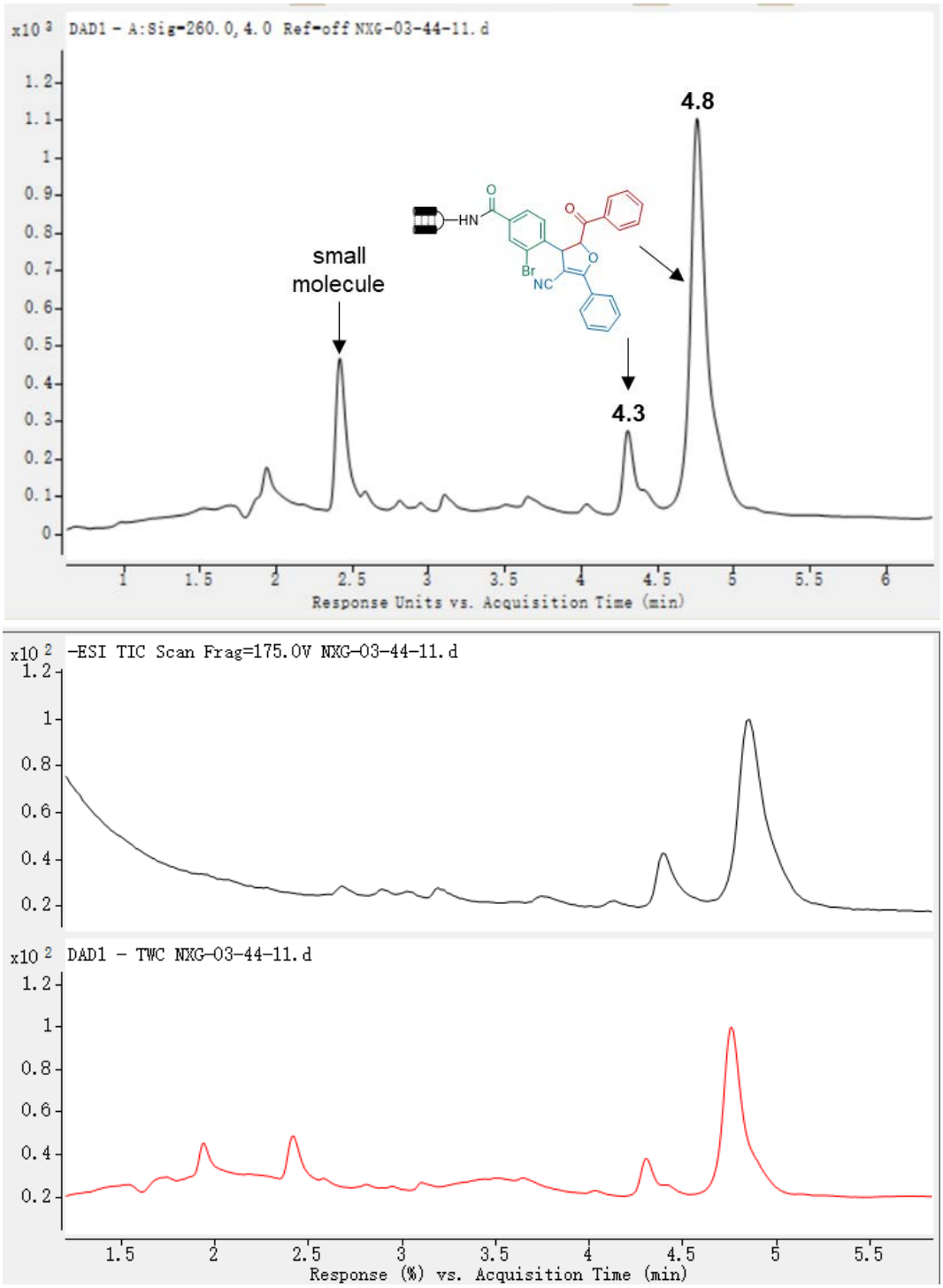

x10 -13
-ESI Scan (rt: 3.959-4.175 min, 14 scans) Frag=175.0V NXG-03-44-7.d Subtract…
5332.27
7218.47
8984.75
Counts (%) vs. Deconvoluted Mass (amu)

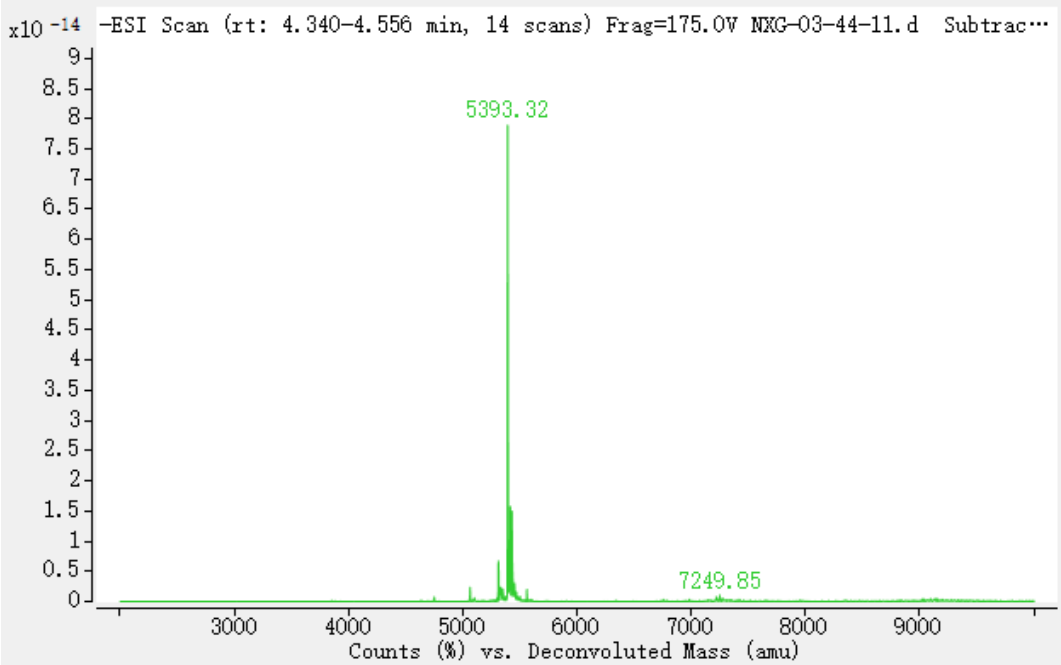

x10 -13
-ESI Scan (rt: 4.558-4.875 min, 20 scans) Frag=175.0V NXG-03-44-7.d Subtract…
5332.40
6285.68
7250.24
8380.69
Counts (%) vs. Deconvoluted Mass (amu)

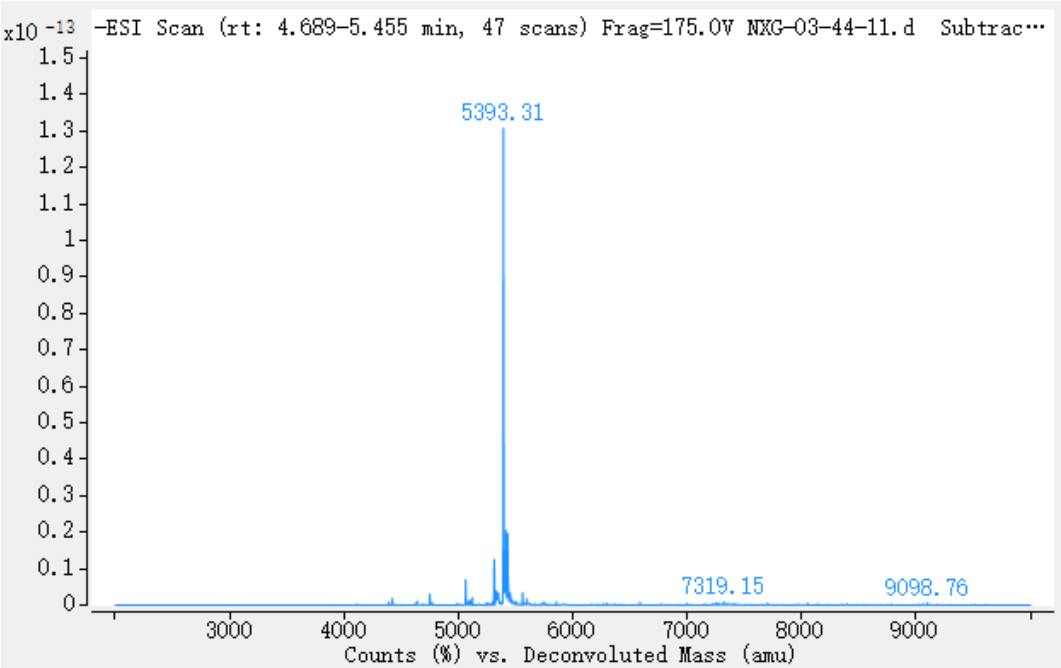
UHPLC chromatogram and deconvoluted MS of **d60**

Conversion: 89%

Calculated Mass: 5393 Da; Observed Mass: 5393 Da



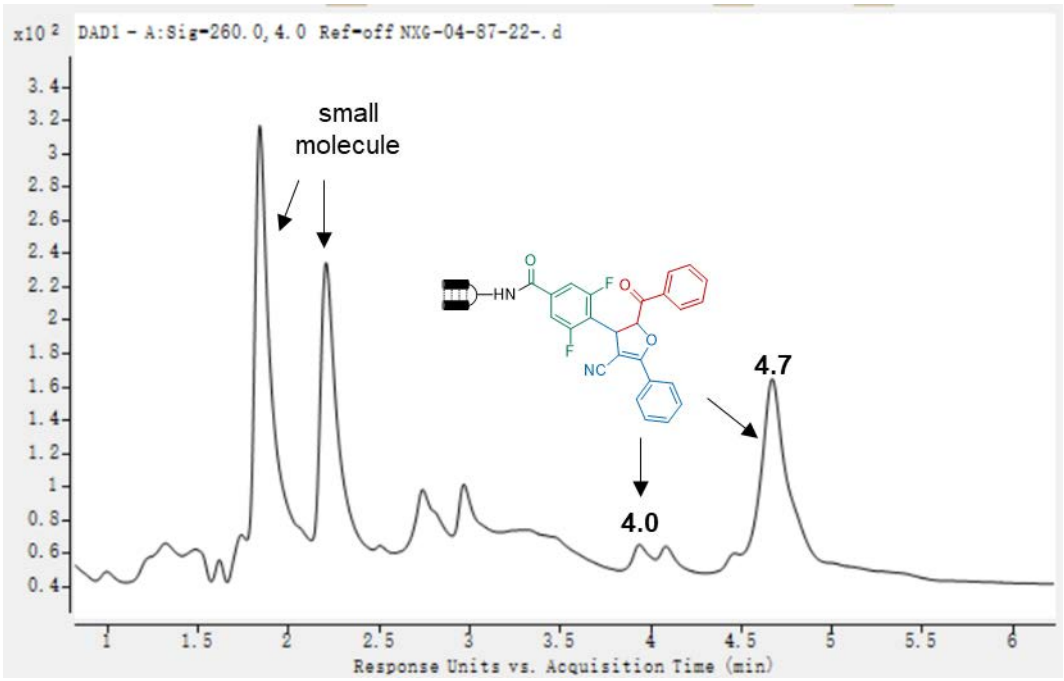

x10 -14
-ESI Scan (rt: 4.340-4.556 min, 14 scans) Frag=175.0V NXG-03-44-11.d Subtrac…
5393.32
7249.85
Counts (%) vs. Deconvoluted Mass (amu)

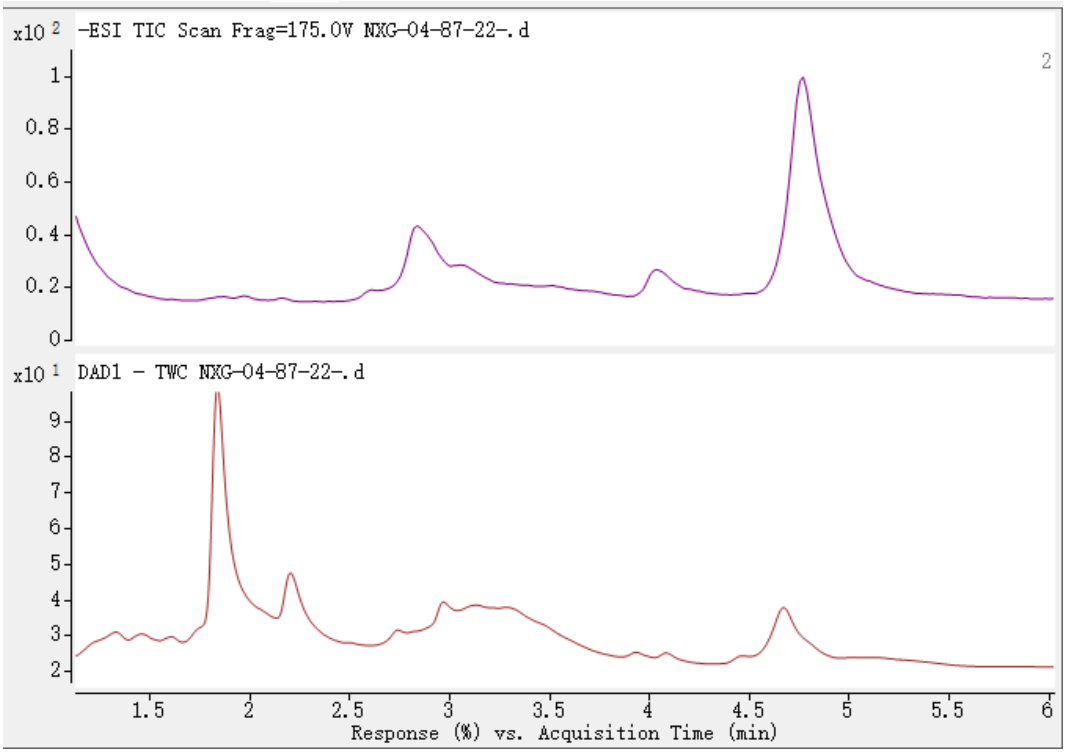

x10 -13
-ESI Scan (rt: 4.689-5.455 min, 47 scans) Frag=175.0V NXG-03-44-11.d Subtrac…
5393.31
7319.15
9098.76
Counts (%) vs. Deconvoluted Mass (amu)

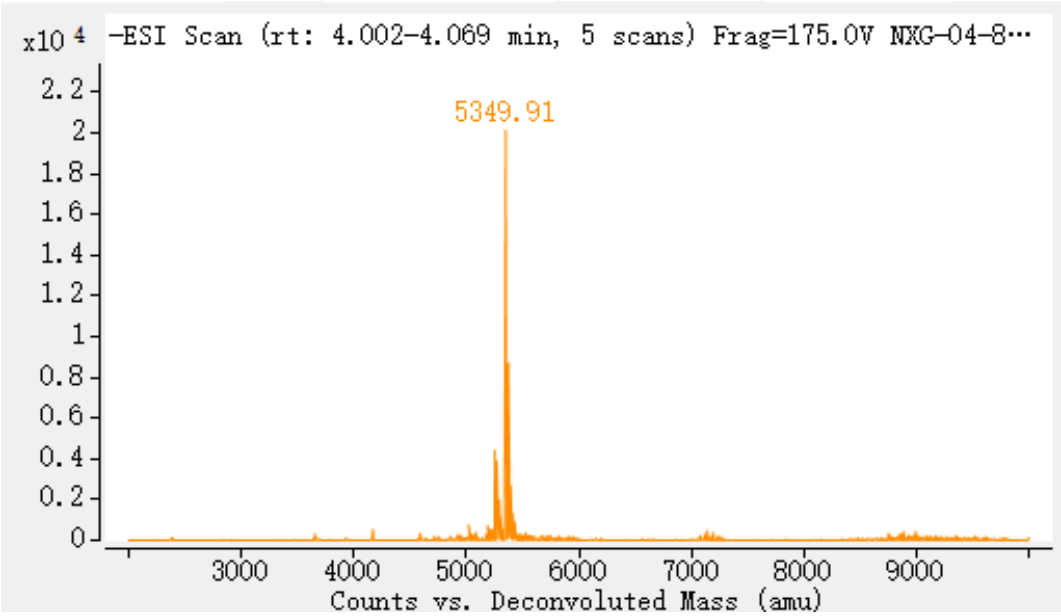
UHPLC chromatogram and deconvoluted MS of **d61**

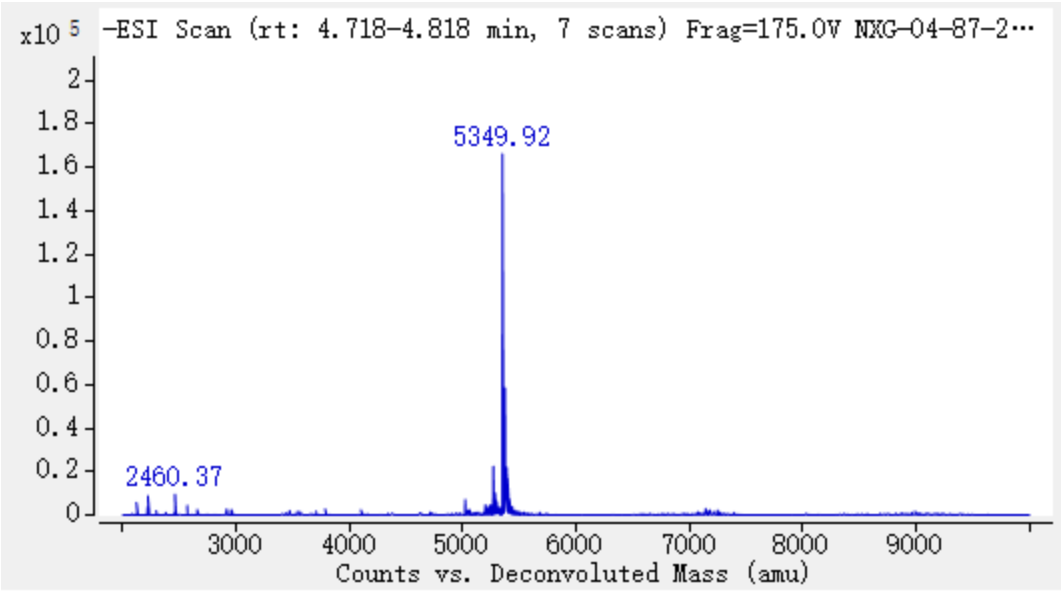
Conversion: 66%

Calculated Mass: 5350 Da; Observed Mass: 5350 Da





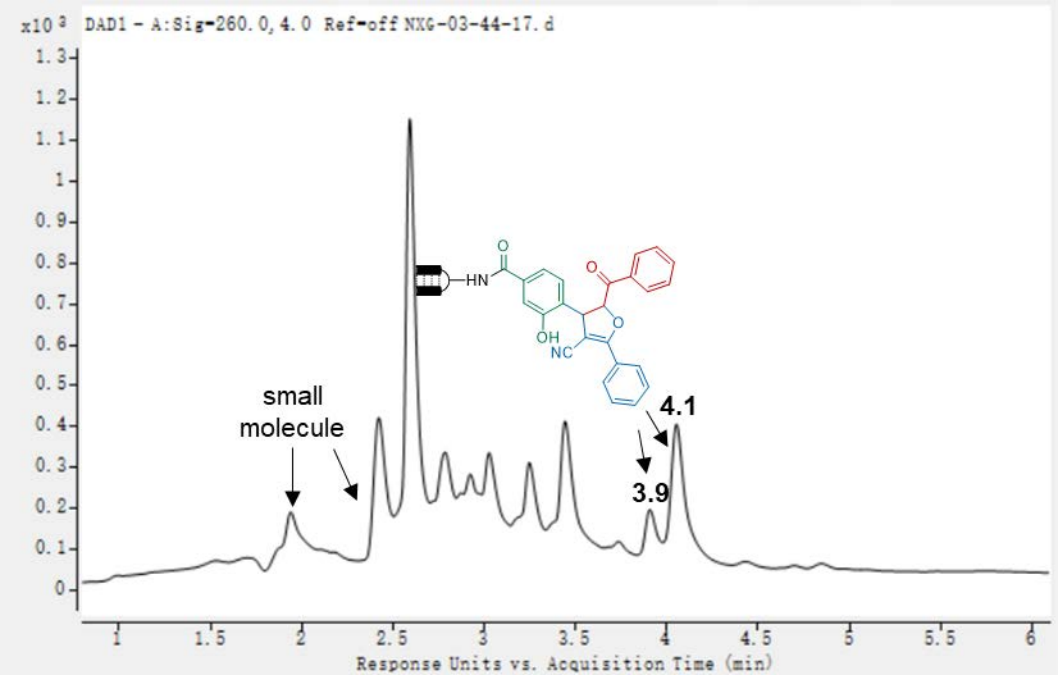

x10 4
-ESI Scan (rt: 4.002-4.069 min, 5 scans) Frag=175.0V NXG-04-8…
5349.91
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

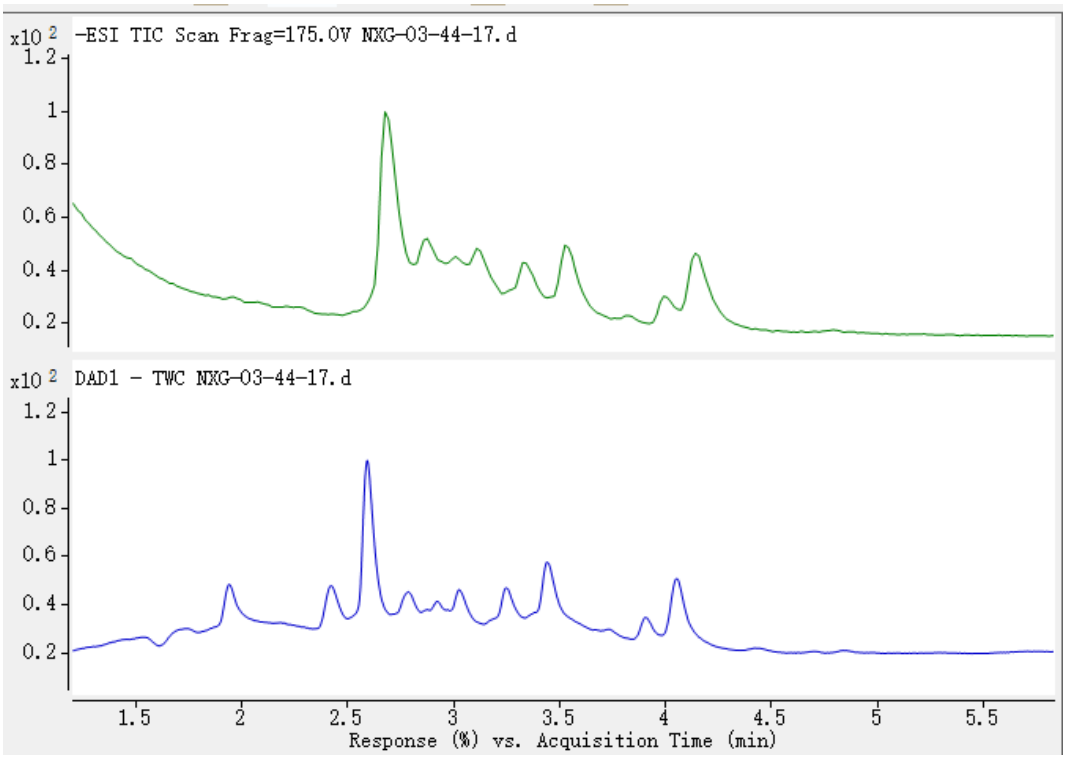

x10 5
-ESI Scan (rt: 4.718-4.818 min, 7 scans) Frag=175.0V NXG-04-87-2…
5349.92
2460.37
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

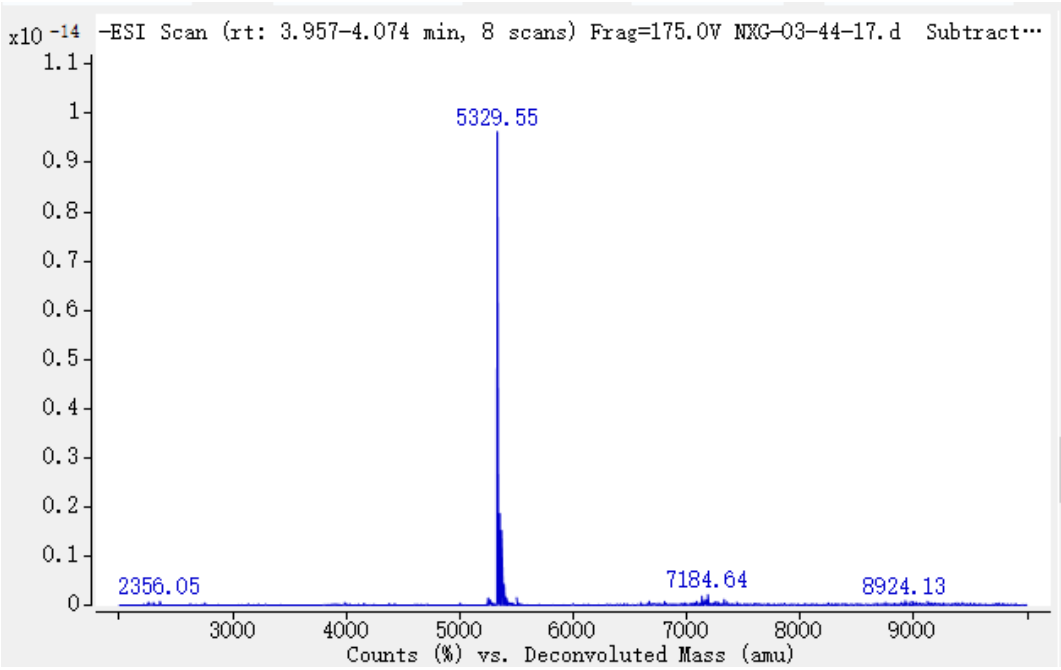
UHPLC chromatogram and deconvoluted MS of **d62**

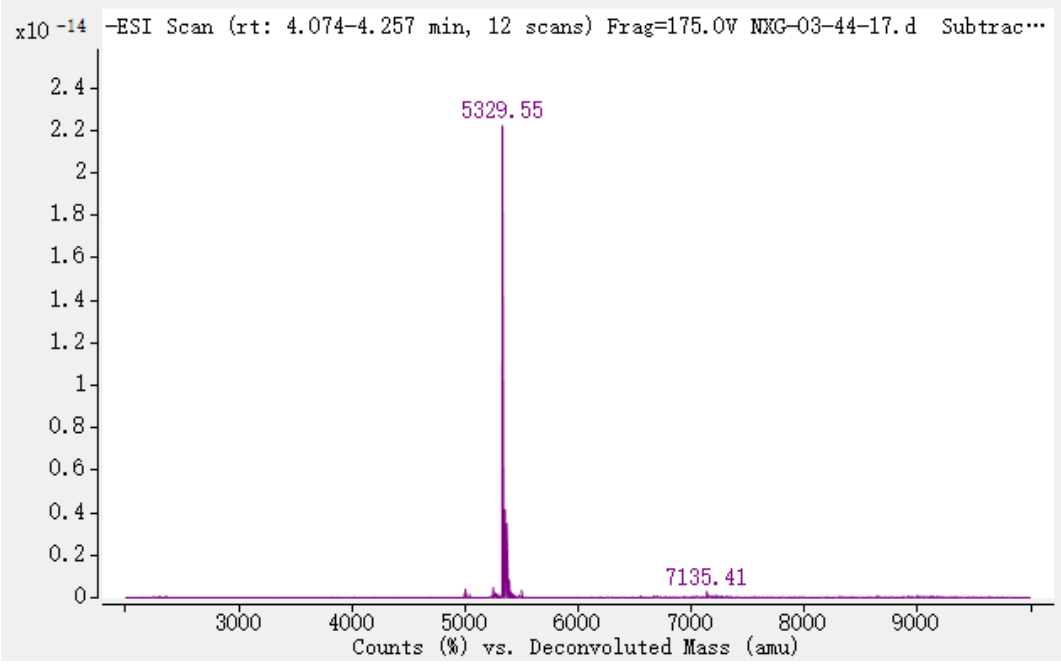
Conversion: 14%

Calculated Mass: 5330 Da; Observed Mass: 5330 Da





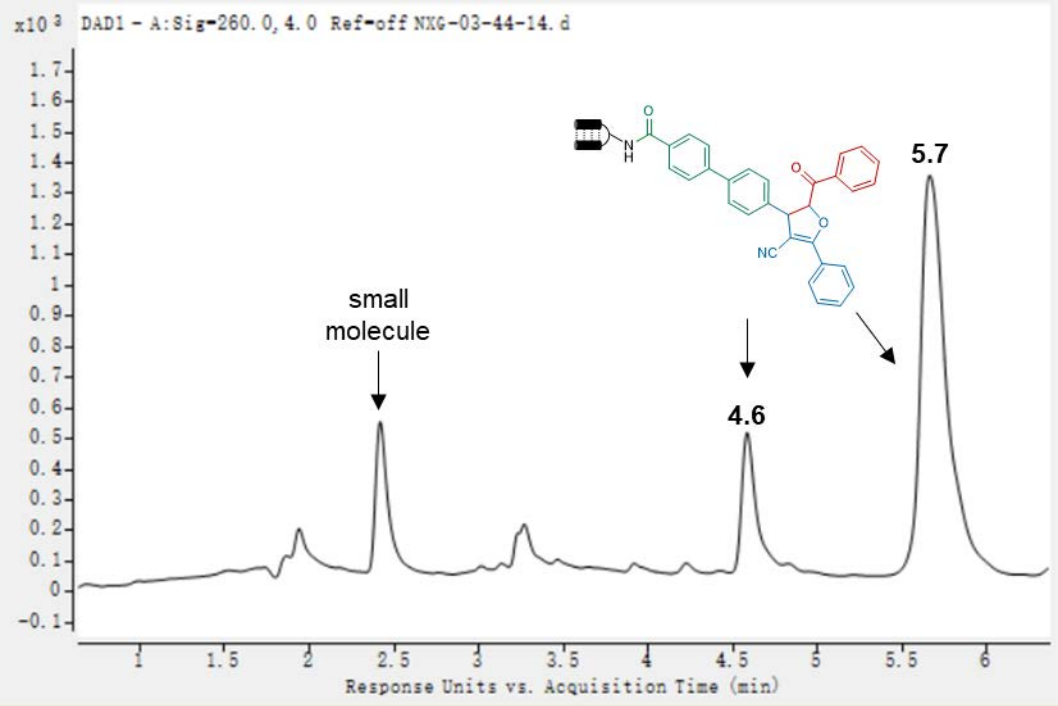

x10 -14
-ESI Scan (rt: 3.957-4.074 min, 8 scans) Frag=175.0V NXG-03-44-17.d Subtract…
5329.55
2356.05
7184.64
8924.13
Counts (%) vs. Deconvoluted Mass (amu)

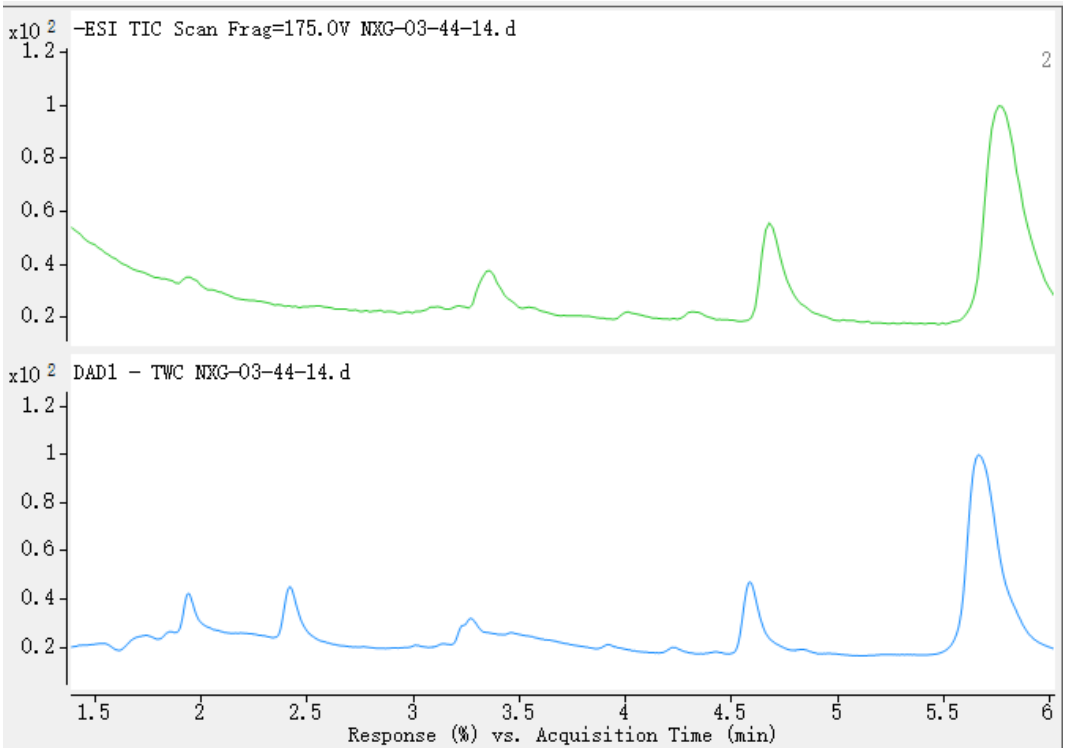

x10 -14
-ESI Scan (rt: 4.074-4.257 min, 12 scans) Frag=175.0V NXG-03-44-17.d Subtrac…
5329.55
7135.41
Counts (%) vs. Deconvoluted Mass (amu)

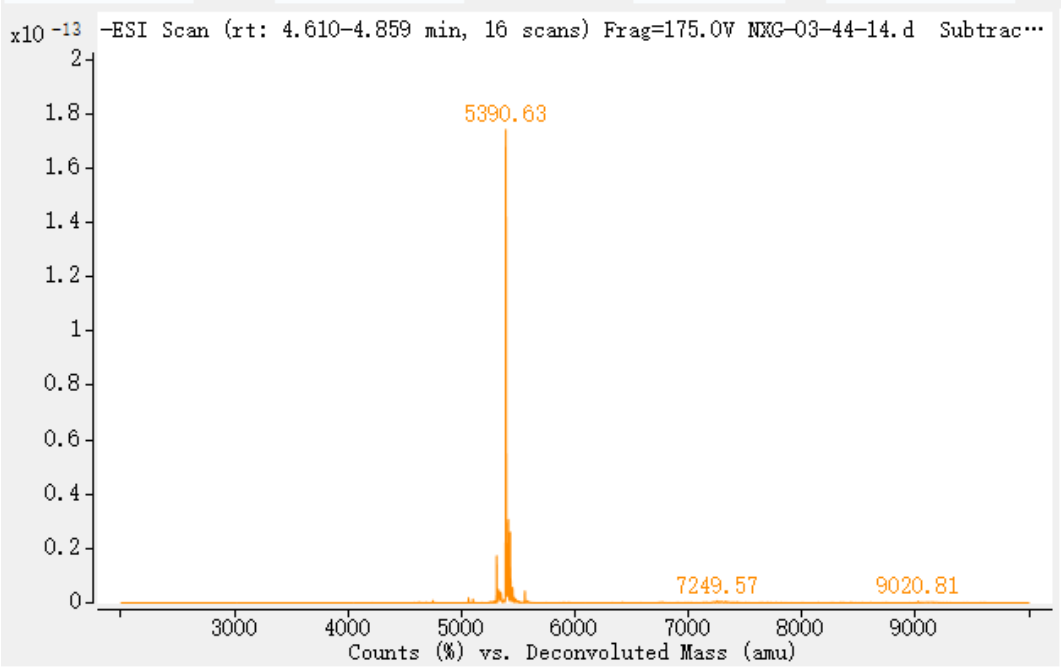
UHPLC chromatogram and deconvoluted MS of **d63**

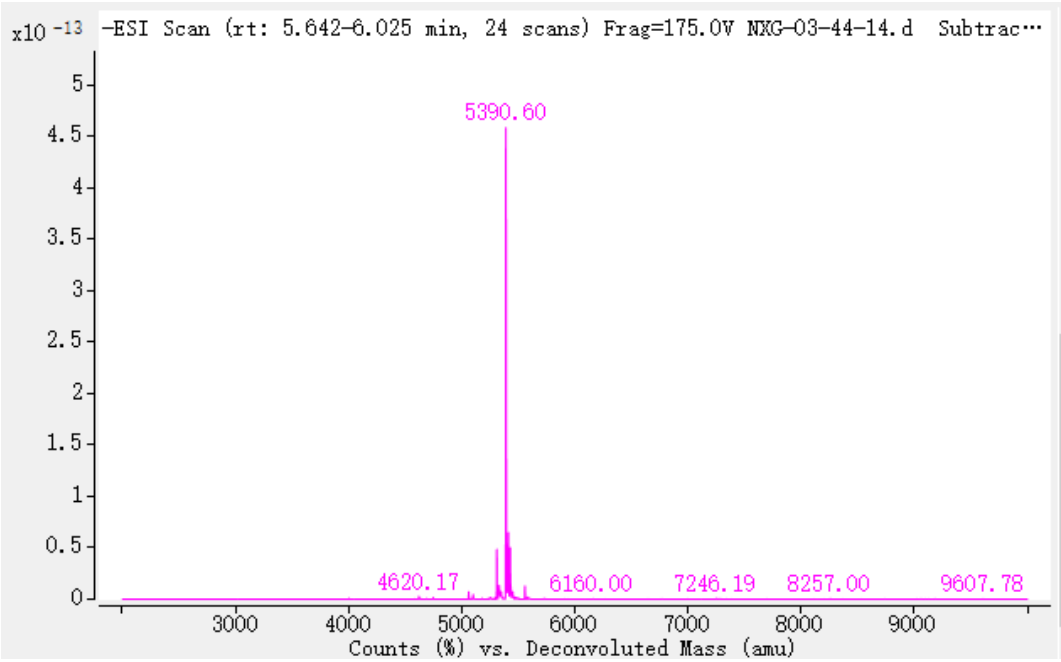
Conversion: 90%

Calculated Mass: 5390 Da; Observed Mass: 5390 Da





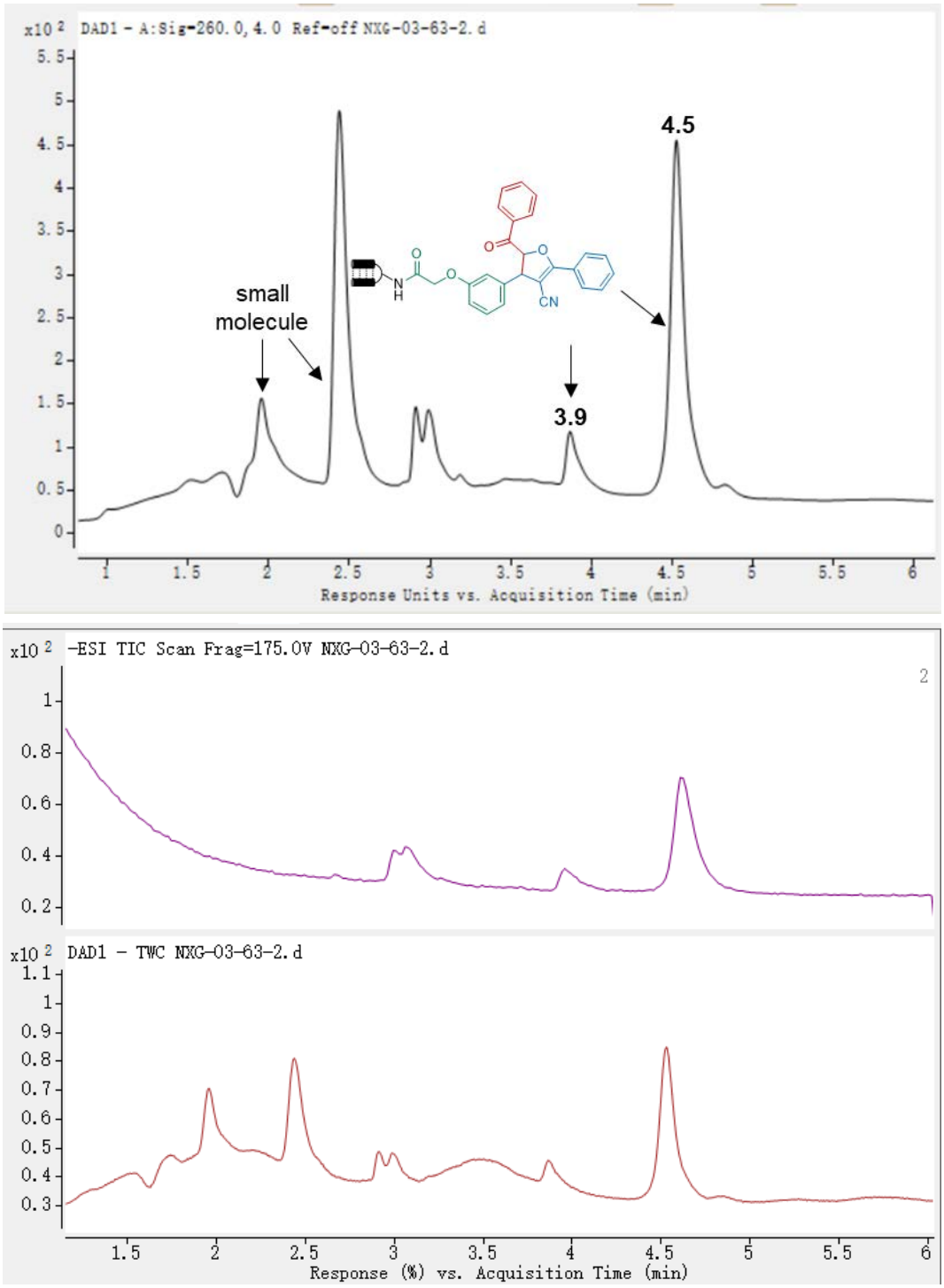

x10 -13
-ESI Scan (rt: 4.610-4.859 min, 16 scans) Frag=175.0V NXG-03-44-14.d Subtrac…
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5390.63
7249.57
9020.81
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

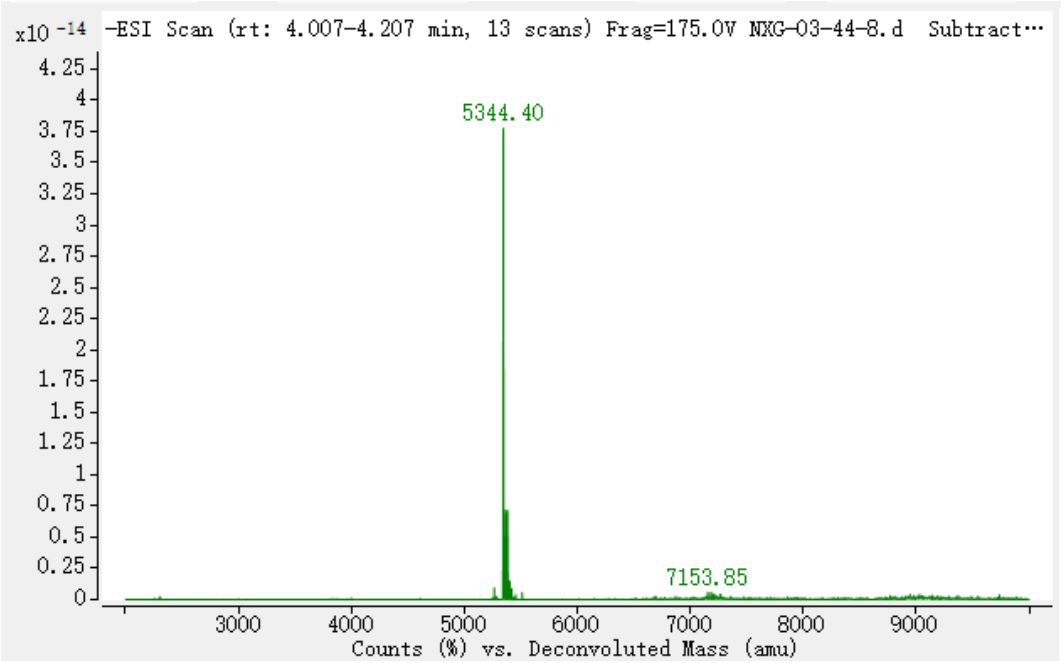

x10 -13
-ESI Scan (rt: 5.642-6.025 min, 24 scans) Frag=175.0V NXG-03-44-14.d Subtrac…
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5390.60
4620.17
6160.00
7246.19
8257.00
9607.78
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

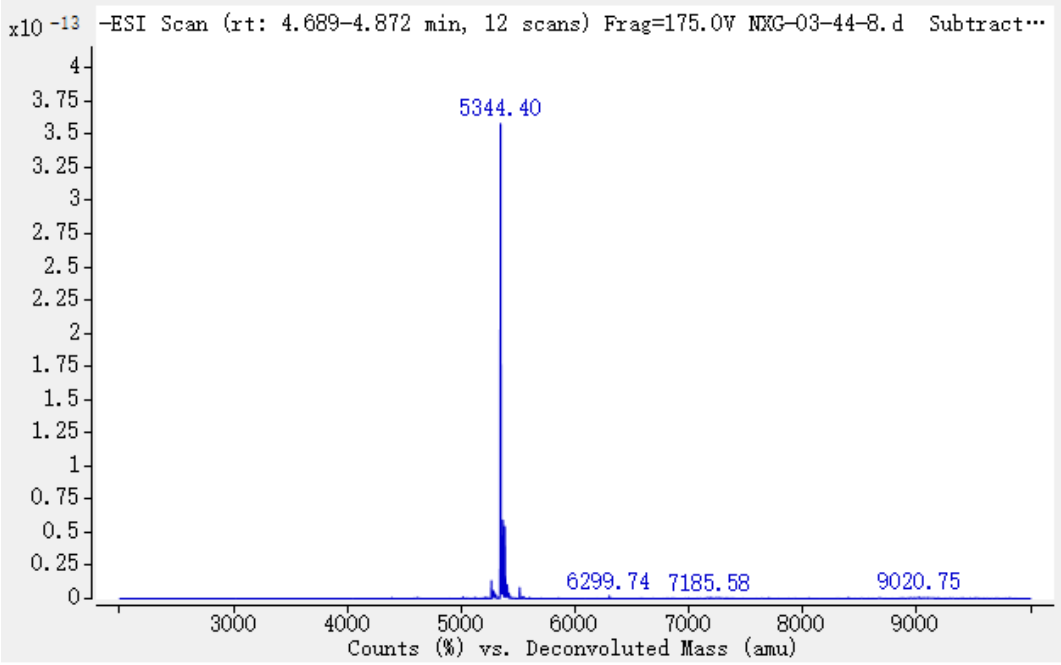
UHPLC chromatogram and deconvoluted MS of **d64**

Conversion: 81%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da



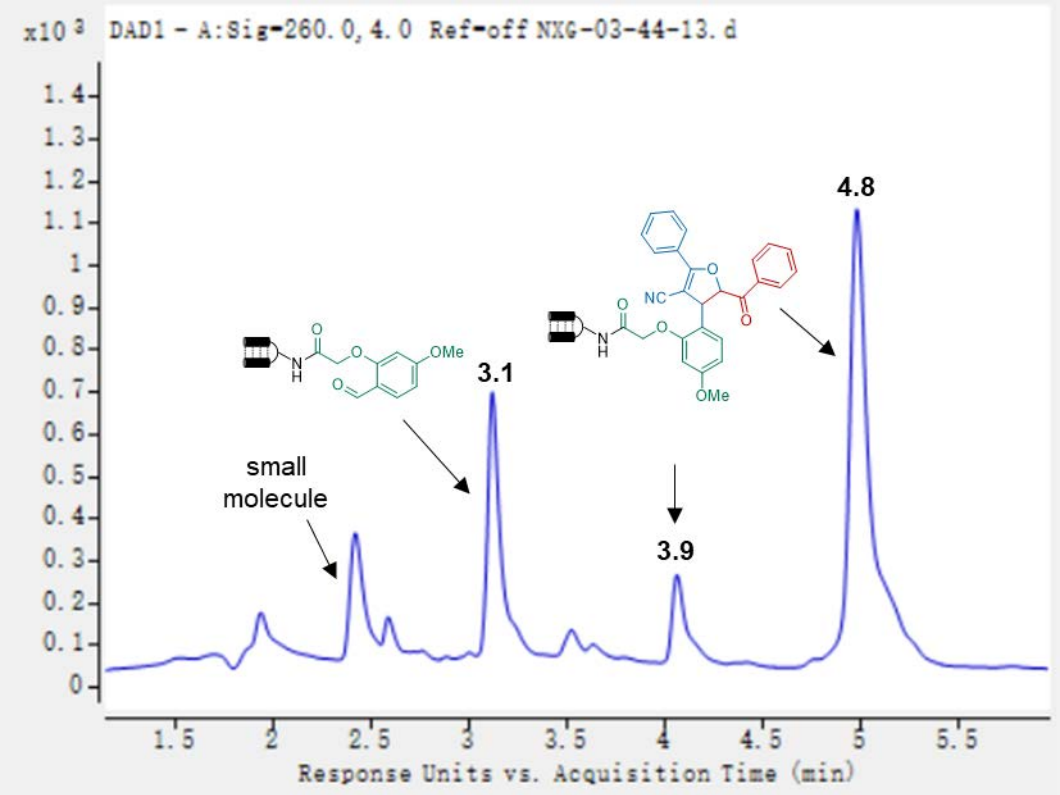

x10 -14
-ESI Scan (rt: 4.007-4.207 min, 13 scans) Frag=175.0V NXG-03-44-8.d Subtract…
4.25
4
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
5344.40
7153.85
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

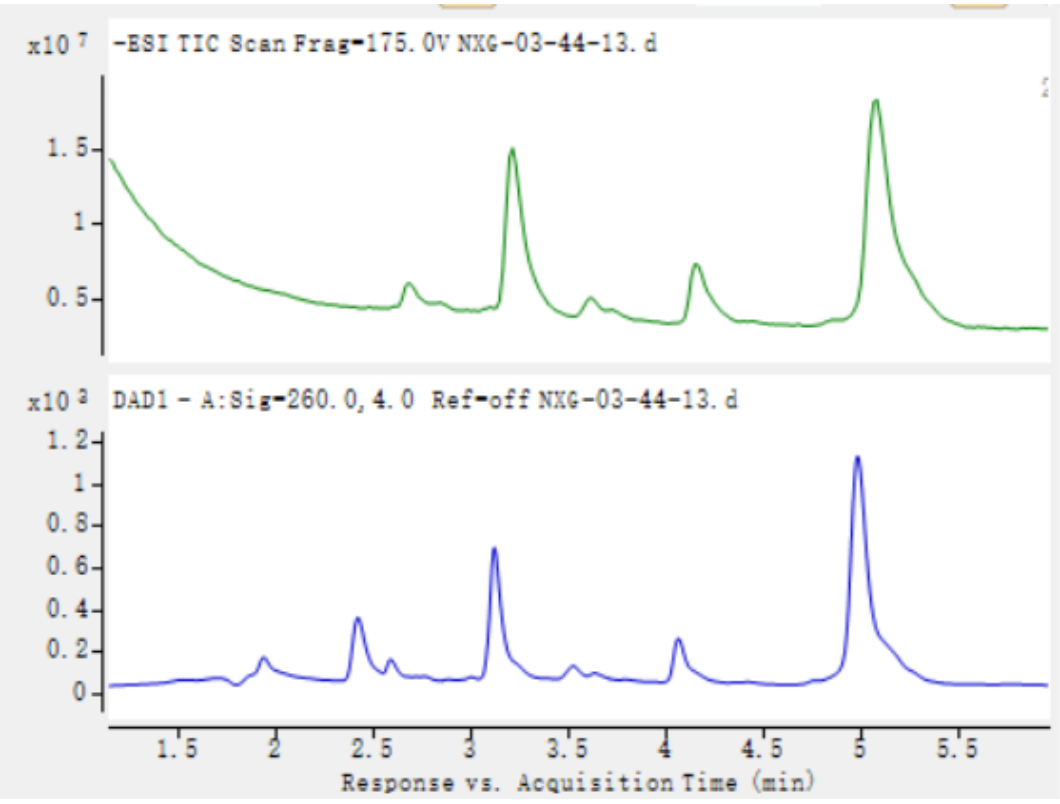

x10 -13
-ESI Scan (rt: 4.689-4.872 min, 12 scans) Frag=175.0V NXG-03-44-8.d Subtract…
4
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
5344.40
6299.74
7185.58
9020.75
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

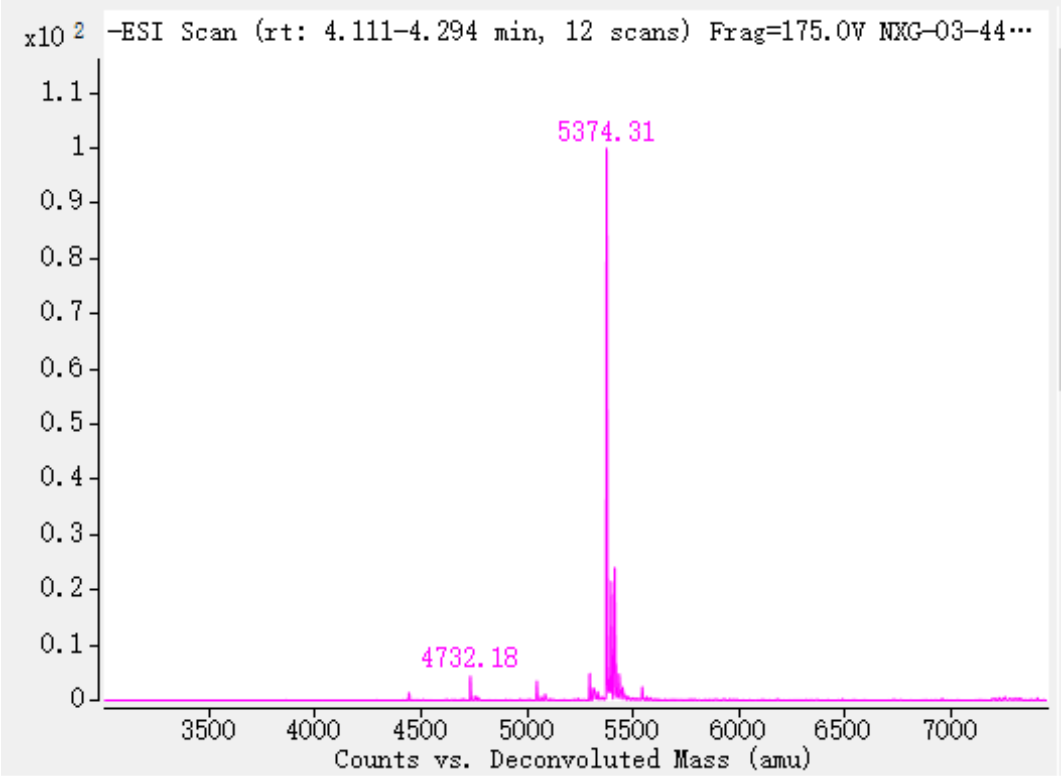
UHPLC chromatogram and deconvoluted MS of **d65**

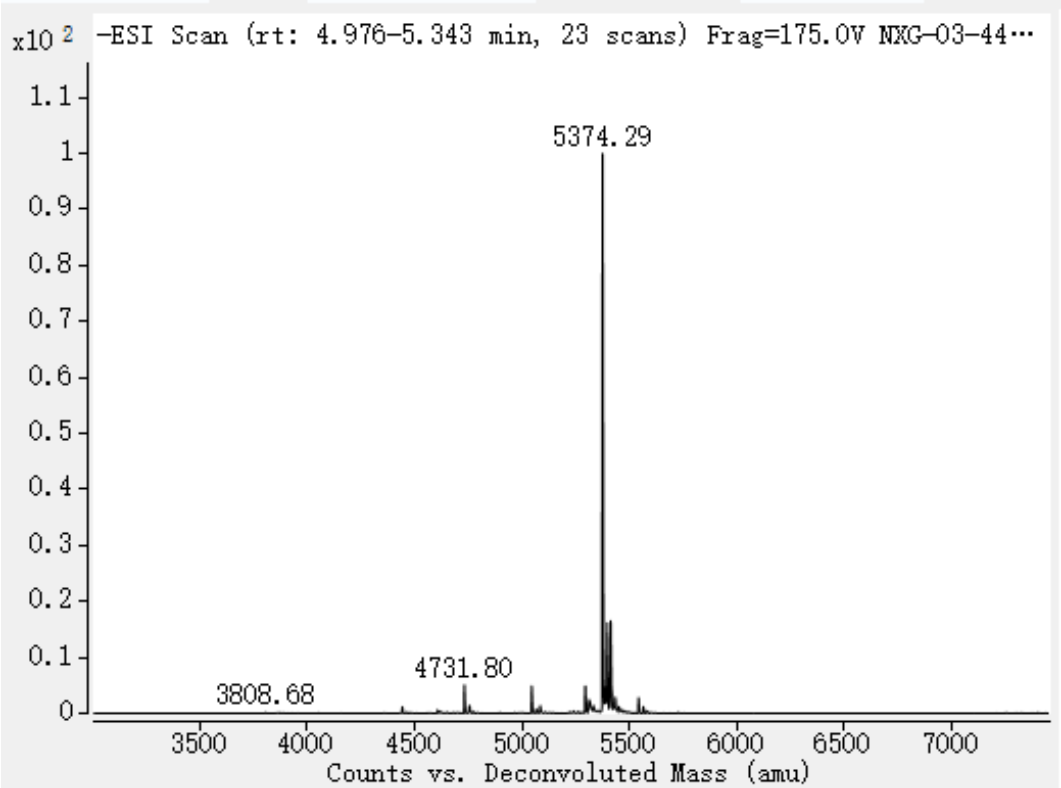
Conversion: 65%

Calculated Mass: 5374 Da; Observed Mass: 5374 Da





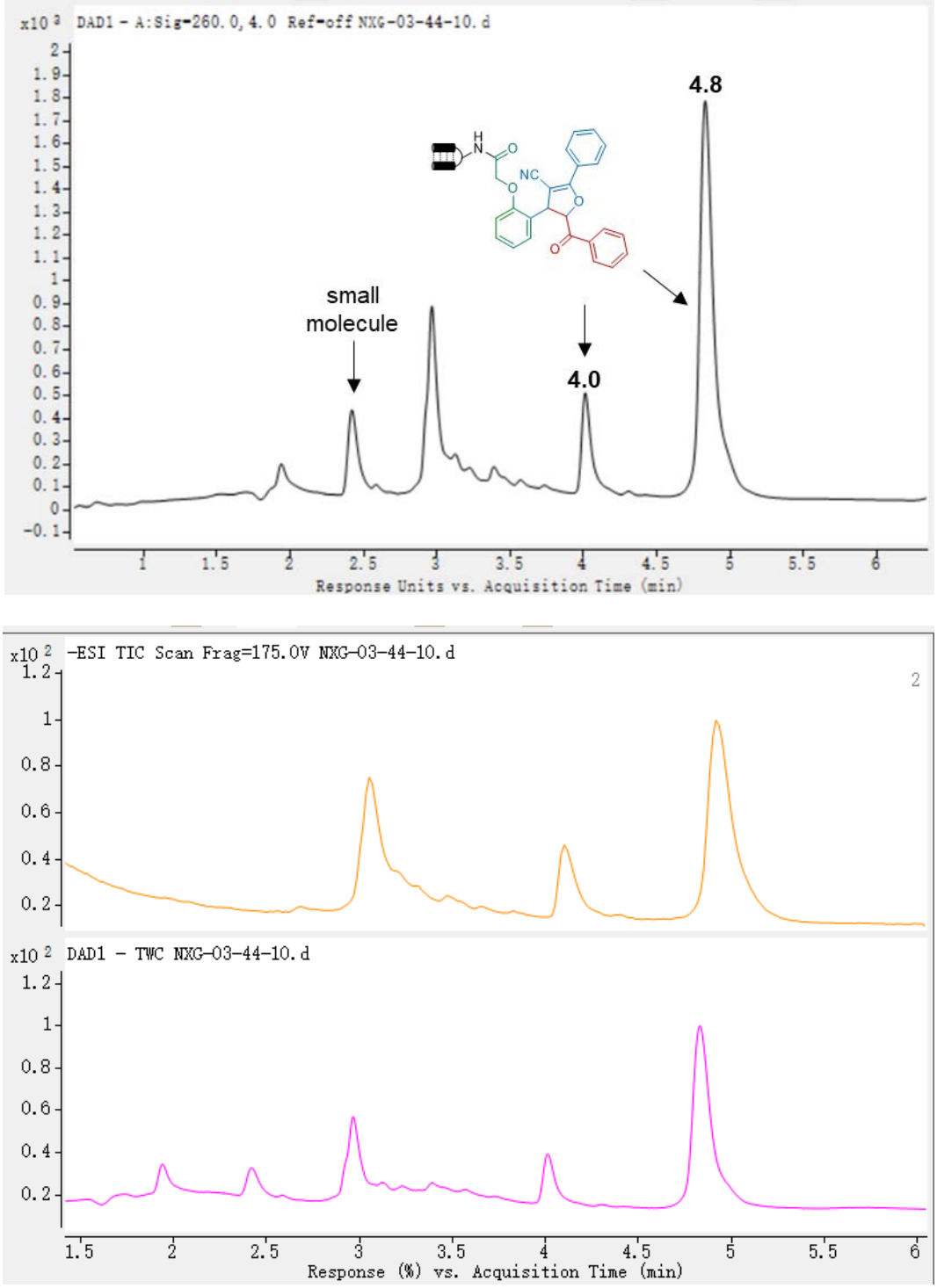

x10 2
-ESI Scan (rt: 4.111-4.294 min, 12 scans) Frag=175.0V NXG-03-44…
5374.31
4732.18
Counts vs. Deconvoluted Mass (amu)

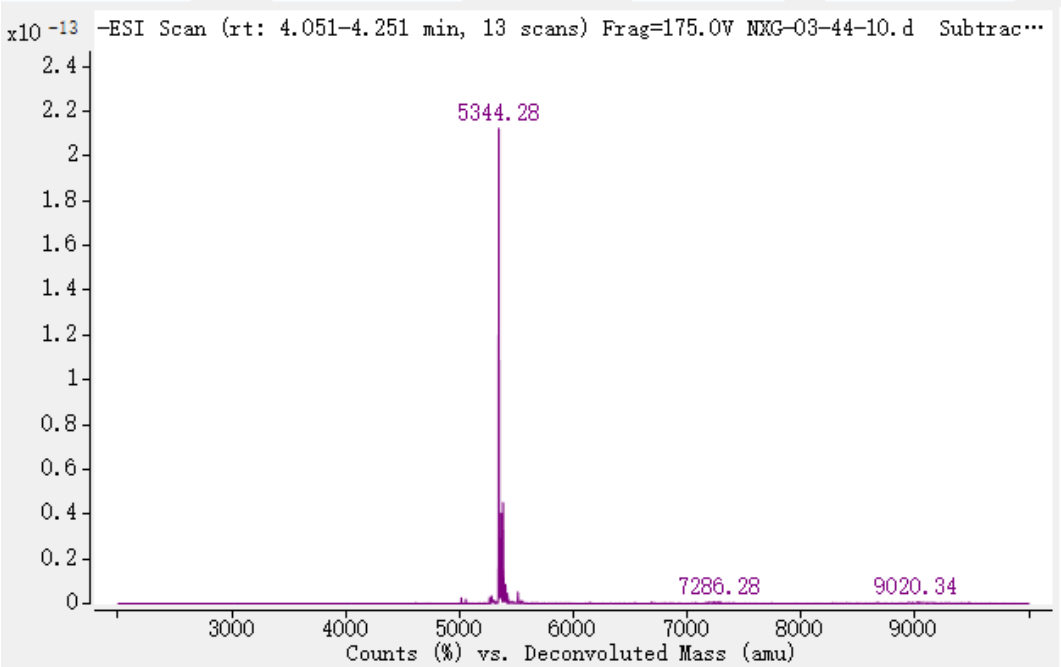

x10 2
-ESI Scan (rt: 4.976-5.343 min, 23 scans) Frag=175.0V NXG-03-44…
5374.29
4731.80
3808.68
Counts vs. Deconvoluted Mass (amu)

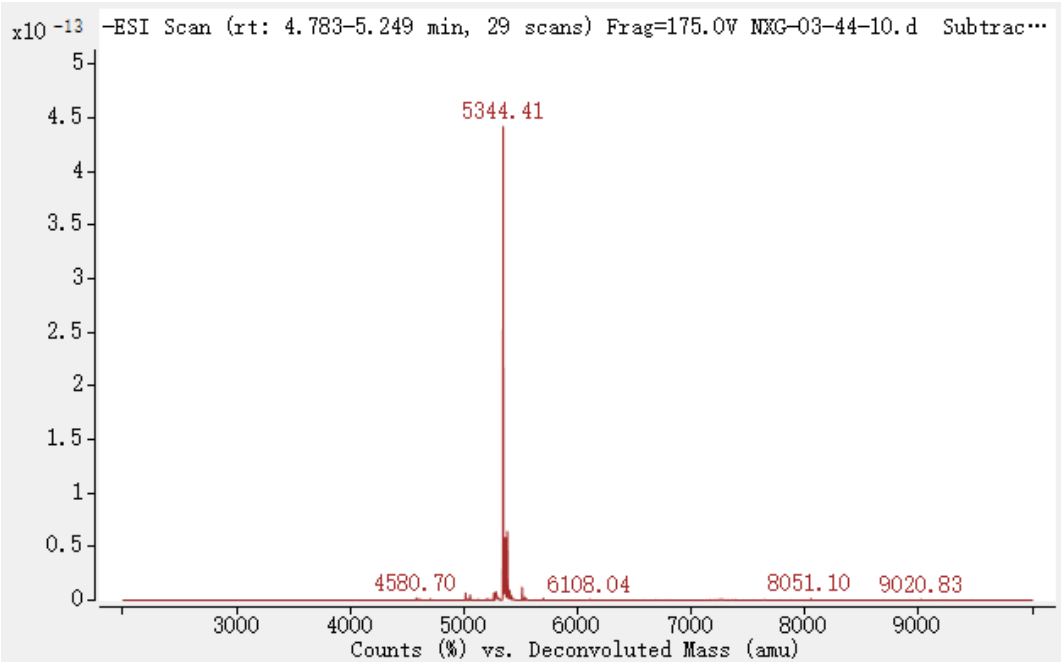
UHPLC chromatogram and deconvoluted MS of **d66**

Conversion: 62%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da



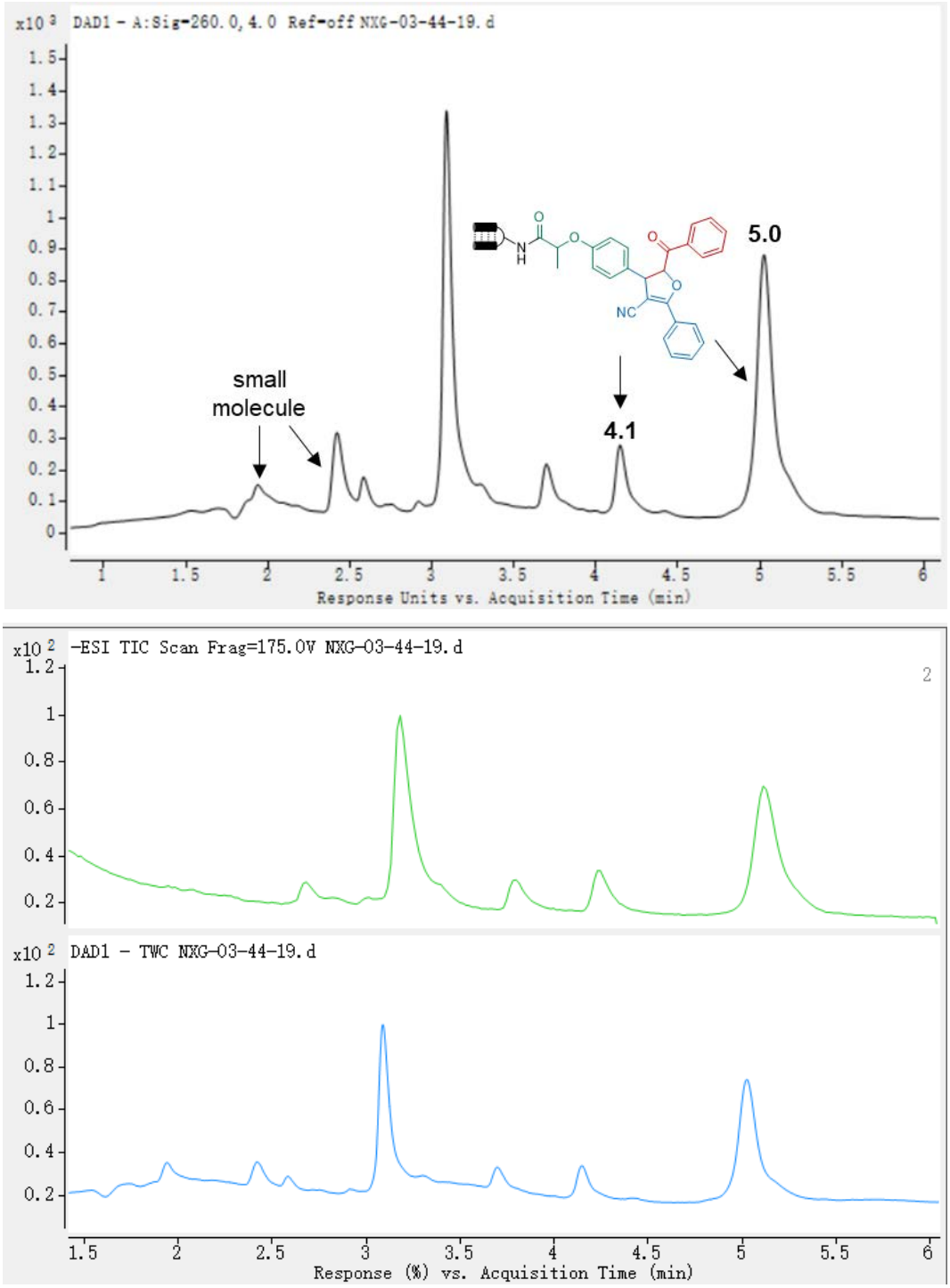

x10 -13
-ESI Scan (rt: 4.051-4.251 min, 13 scans) Frag=175.0V NXG-03-44-10.d Subtrac…
5344.28
7286.28
9020.34
Counts (%) vs. Deconvoluted Mass (amu)

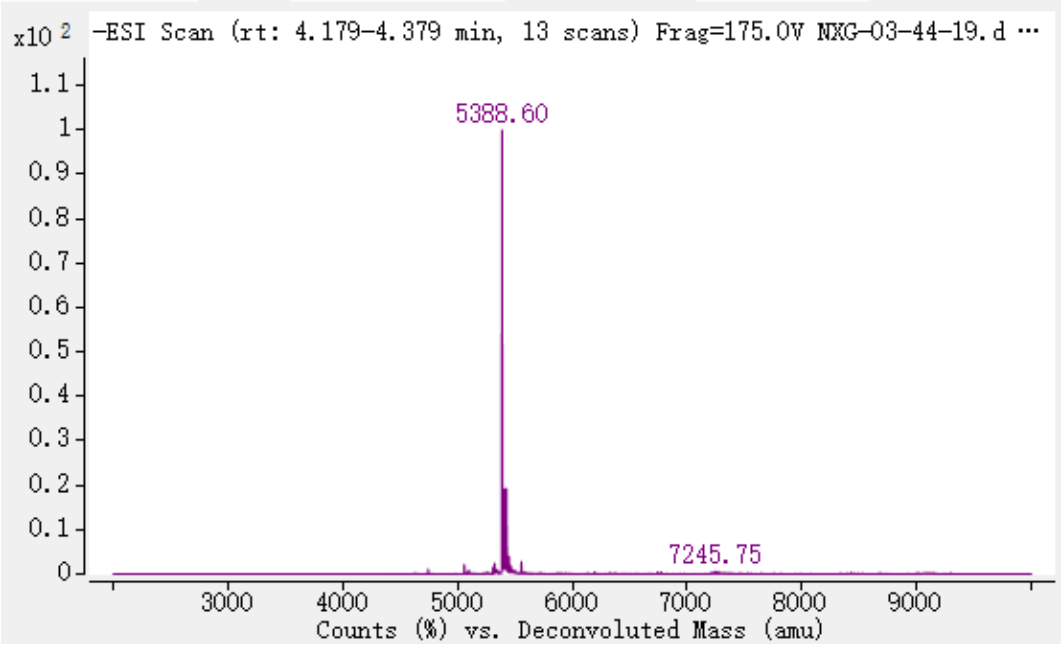

x10 -13
-ESI Scan (rt: 4.783-5.249 min, 29 scans) Frag=175.0V NXG-03-44-10.d Subtrac…
5344.41
4580.70
6108.04
8051.10
9020.83
Counts (%) vs. Deconvoluted Mass (amu)

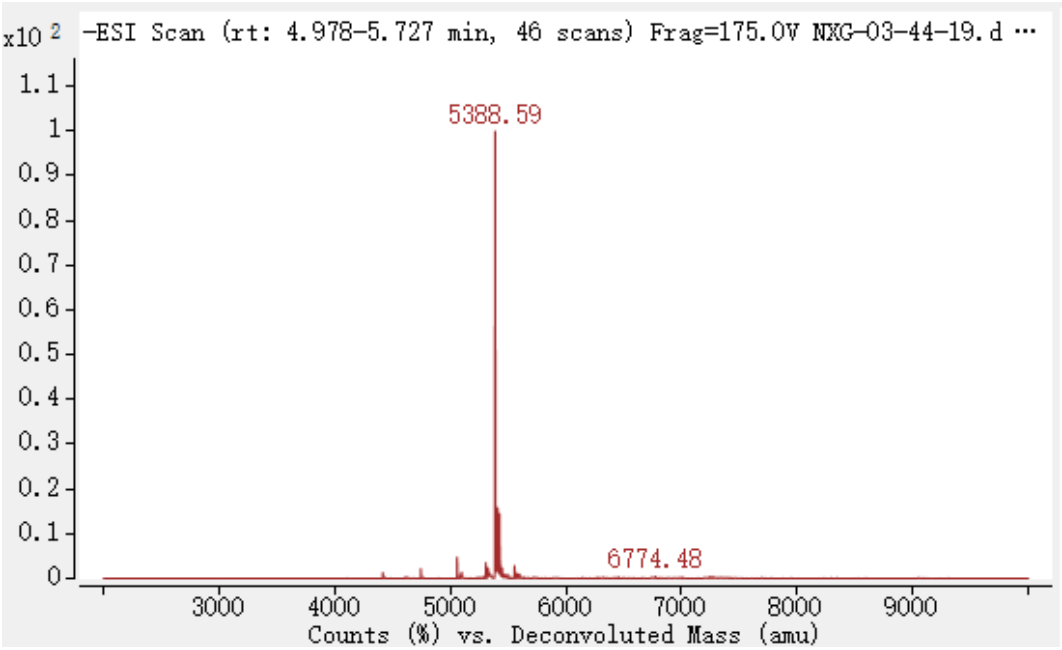
UHPLC chromatogram and deconvoluted MS of **d67**

Conversion: 55%

Calculated Mass: 5388 Da; Observed Mass: 5388 Da



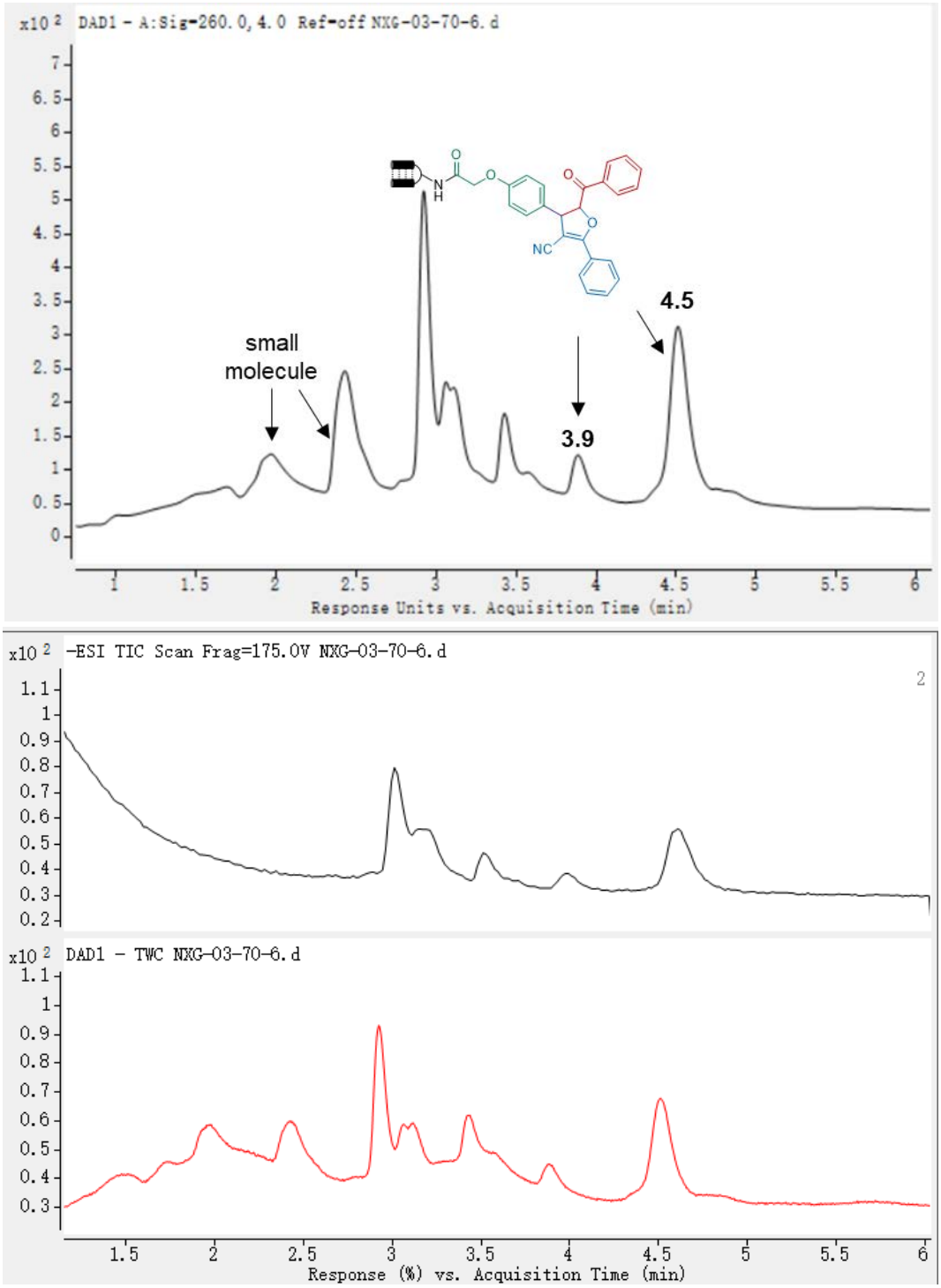

x10 2
-ESI Scan (rt: 4.179-4.379 min, 13 scans) Frag=175.0V NXG-03-44-19.d ...
5388.60
7245.75
Counts (%) vs. Deconvoluted Mass (amu)

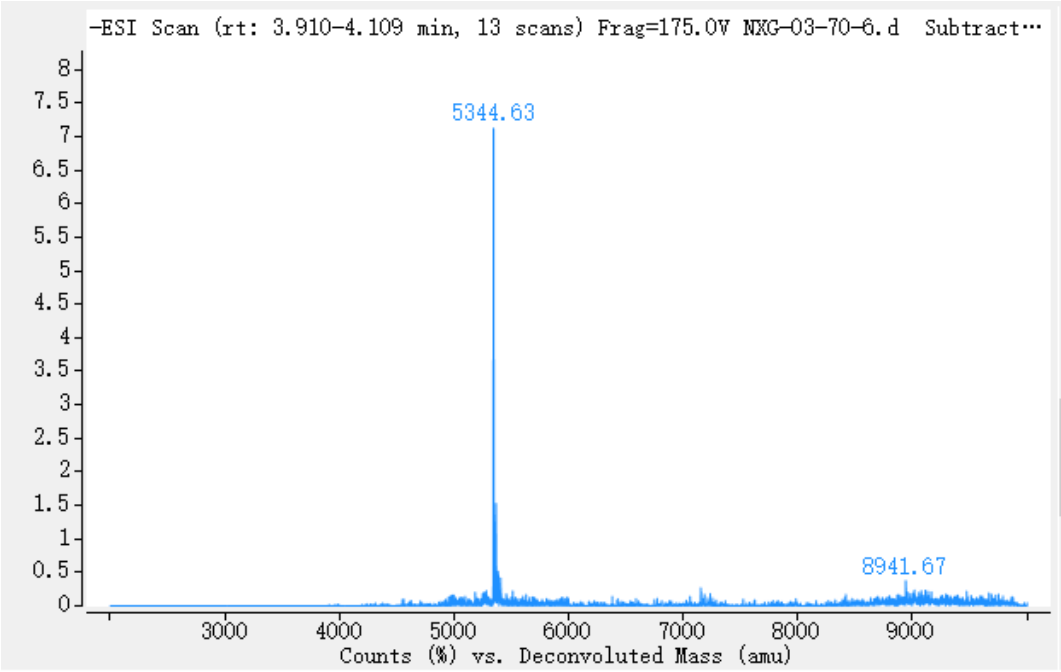

x10 2
-ESI Scan (rt: 4.978-5.727 min, 46 scans) Frag=175.0V NXG-03-44-19.d ...
5388.59
6774.48
Counts (%) vs. Deconvoluted Mass (amu)

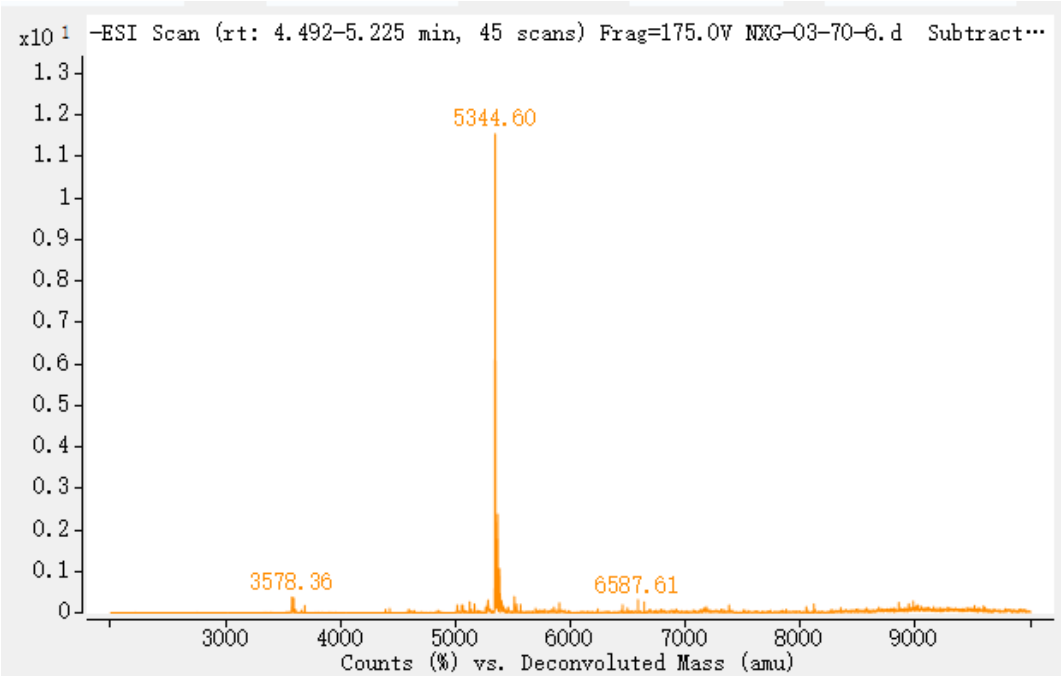
UHPLC chromatogram and deconvoluted MS of **d68**

Conversion: 35%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da



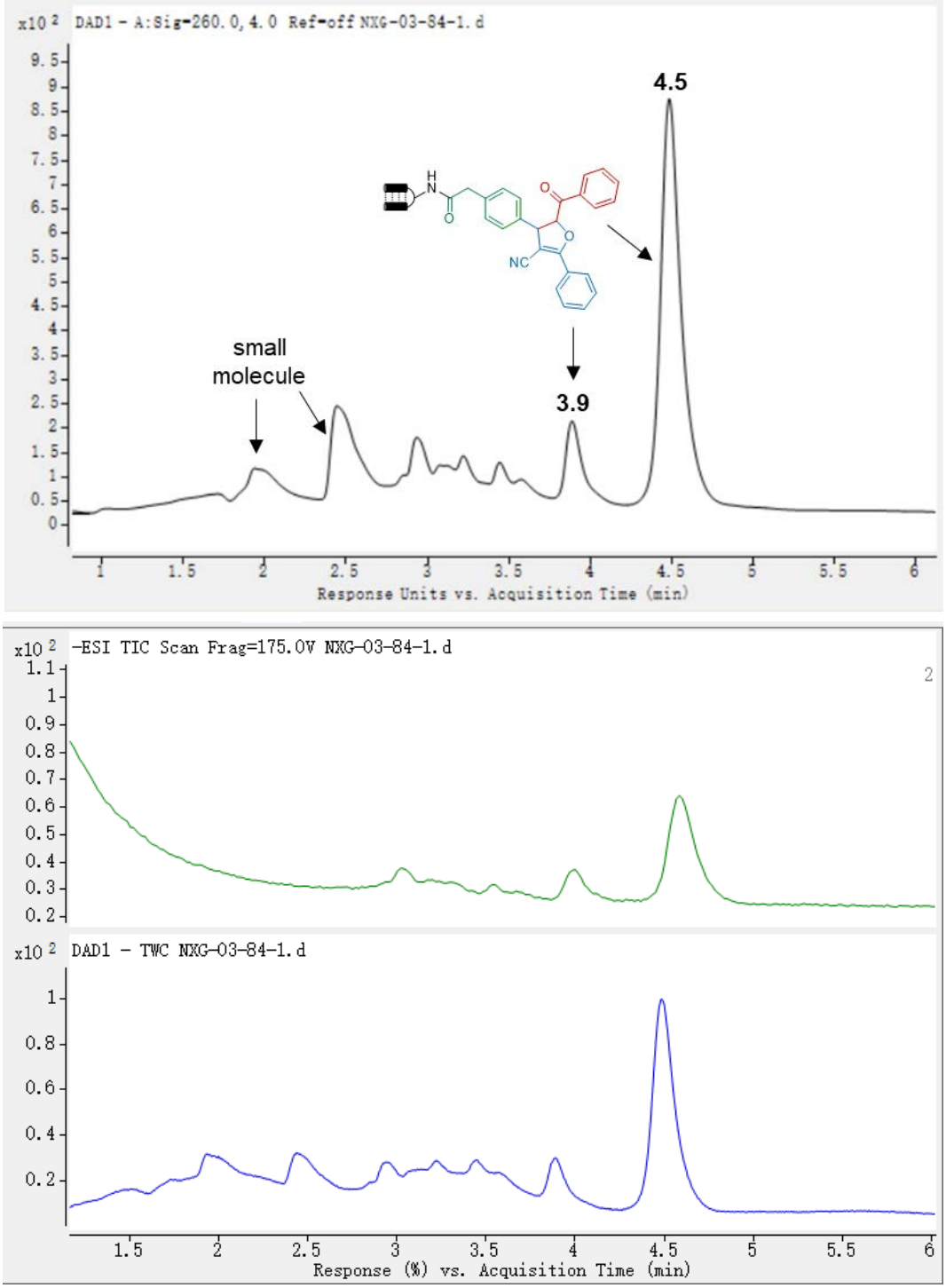

-ESI Scan (rt: 3.910-4.109 min, 13 scans) Frag=175.0V NXG-03-70-6.d Subtract…
5344.63
8941.67
Counts (%) vs. Deconvoluted Mass (amu)

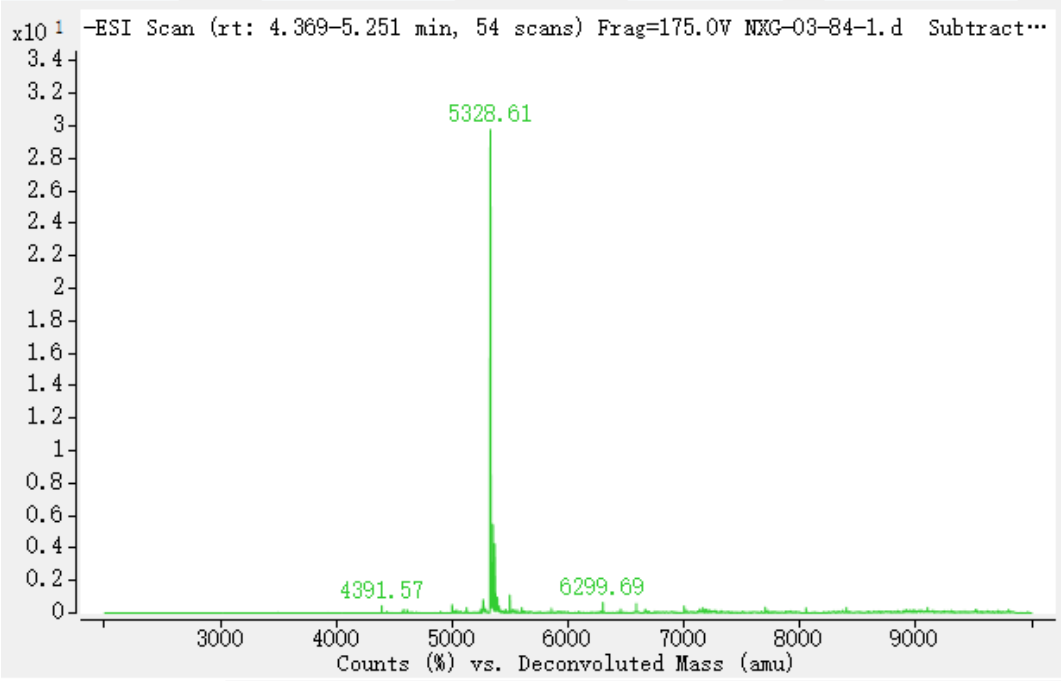

x10 1
-ESI Scan (rt: 4.492-5.225 min, 45 scans) Frag=175.0V NXG-03-70-6.d Subtract…
5344.60
3578.36
6587.61
Counts (%) vs. Deconvoluted Mass (amu)

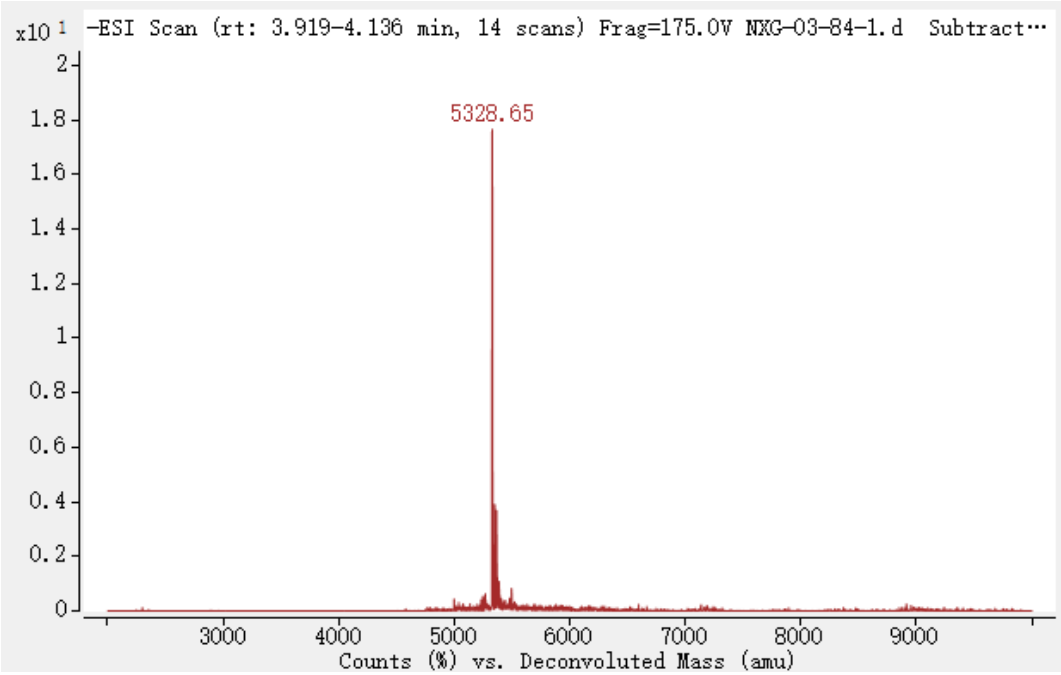
UHPLC chromatogram and deconvoluted MS of **d69**

Conversion: 87%

Calculated Mass: 5328 Da; Observed Mass: 5328 Da



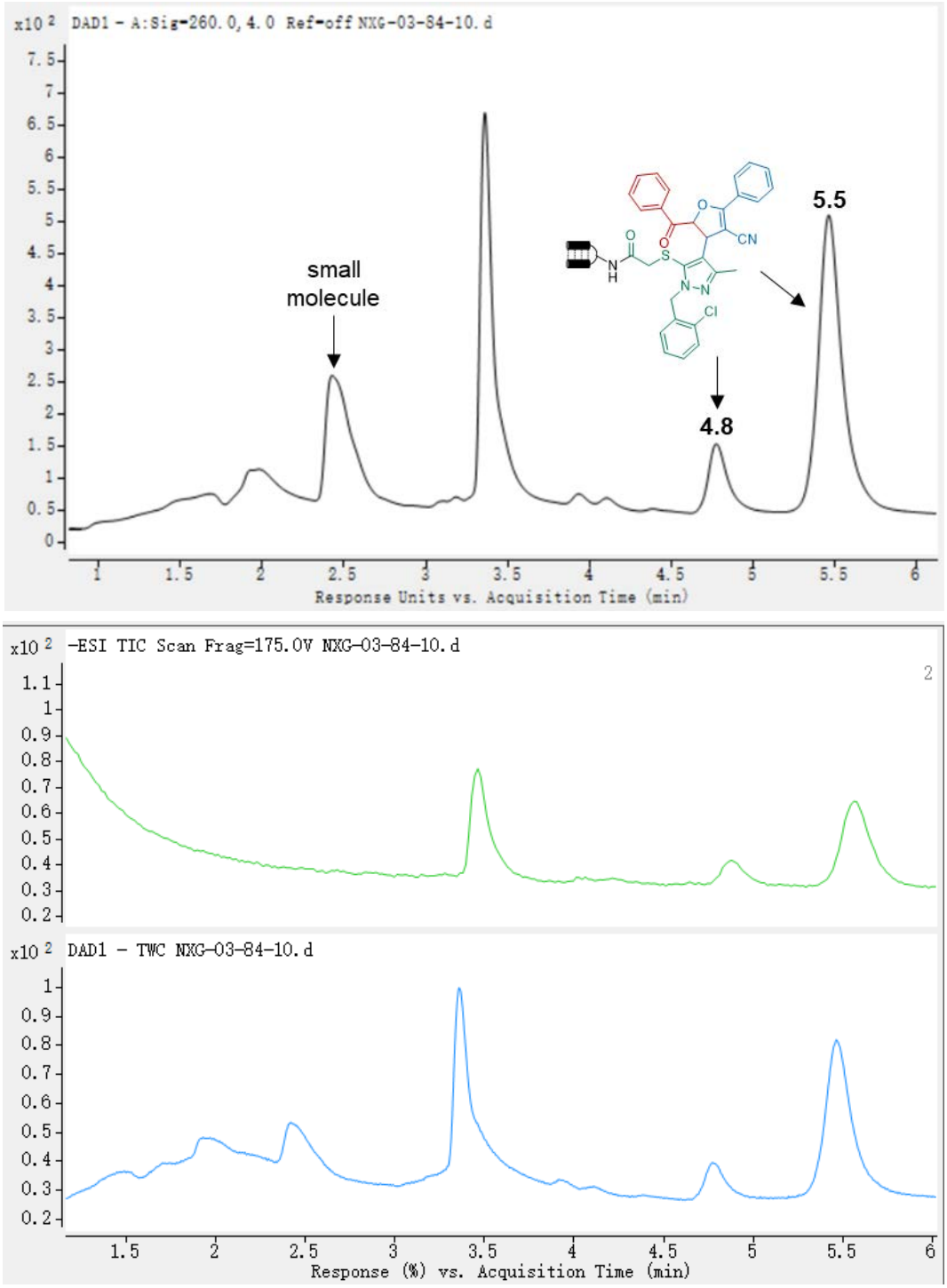

x10 1
-ESI Scan (rt: 4.369-5.251 min, 54 scans) Frag=175.0V NXG-03-84-1.d Subtract…
5328.61
4391.57
6299.69
Counts (%) vs. Deconvoluted Mass (amu)

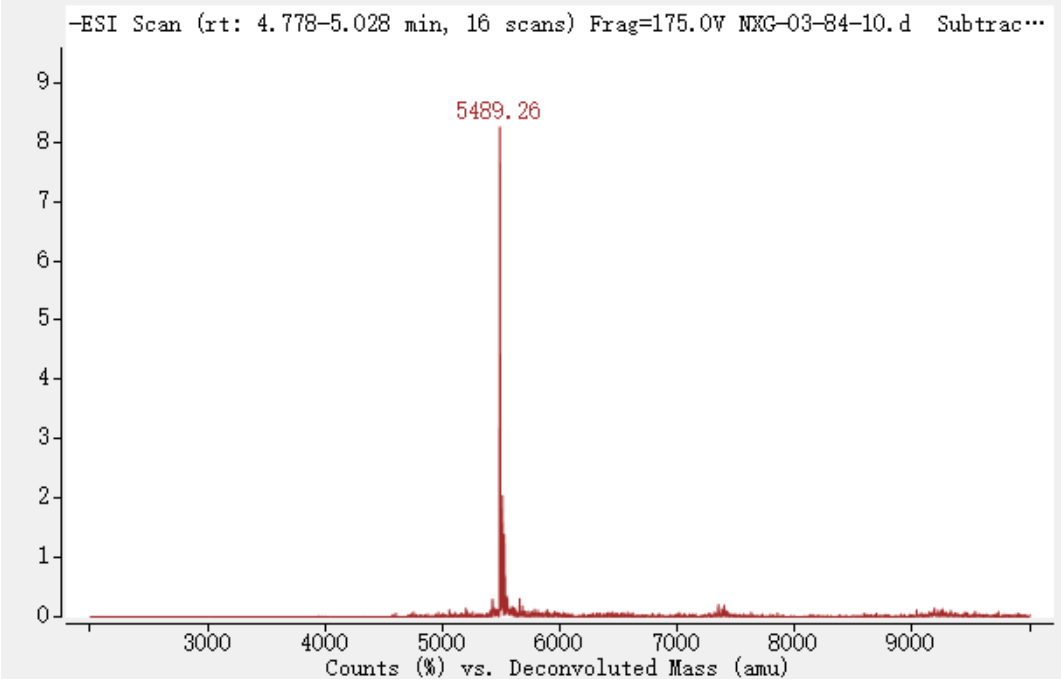

x10 1
-ESI Scan (rt: 3.919-4.136 min, 14 scans) Frag=175.0V NXG-03-84-1.d Subtract…
5328.65
Counts (%) vs. Deconvoluted Mass (amu)

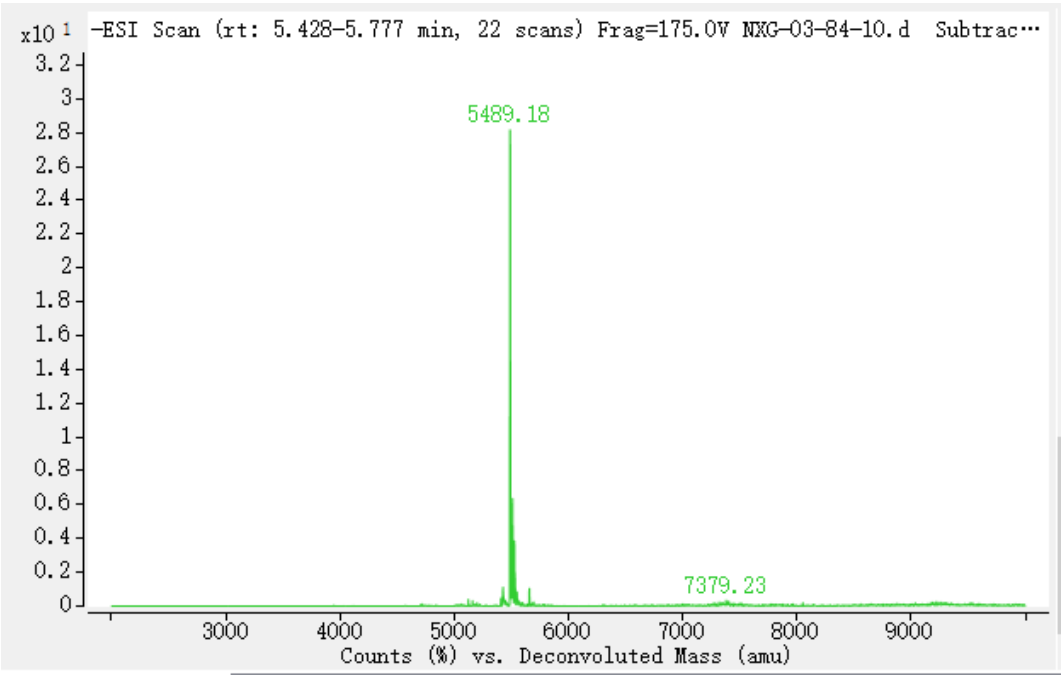
UHPLC chromatogram and deconvoluted MS of **d70**

Conversion: 61%

Calculated Mass: 5489 Da; Observed Mass: 5489 Da



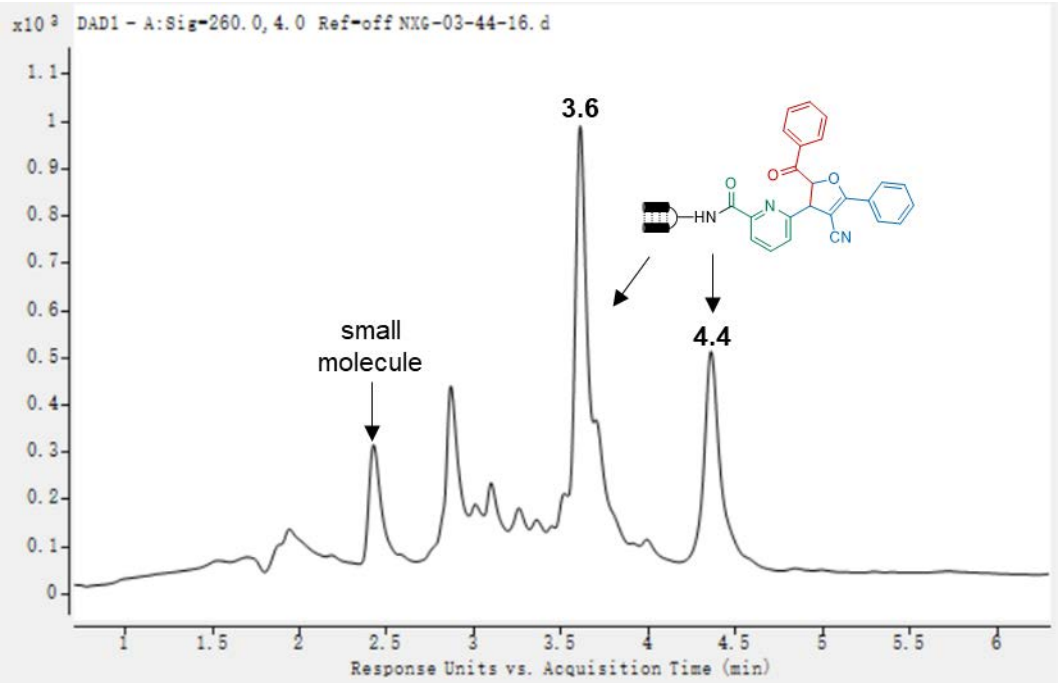

-ESI Scan (rt: 4.778-5.028 min, 16 scans) Frag=175.0V NXG-03-84-10.d Subtrac…
5489.26
Counts (%) vs. Deconvoluted Mass (amu)

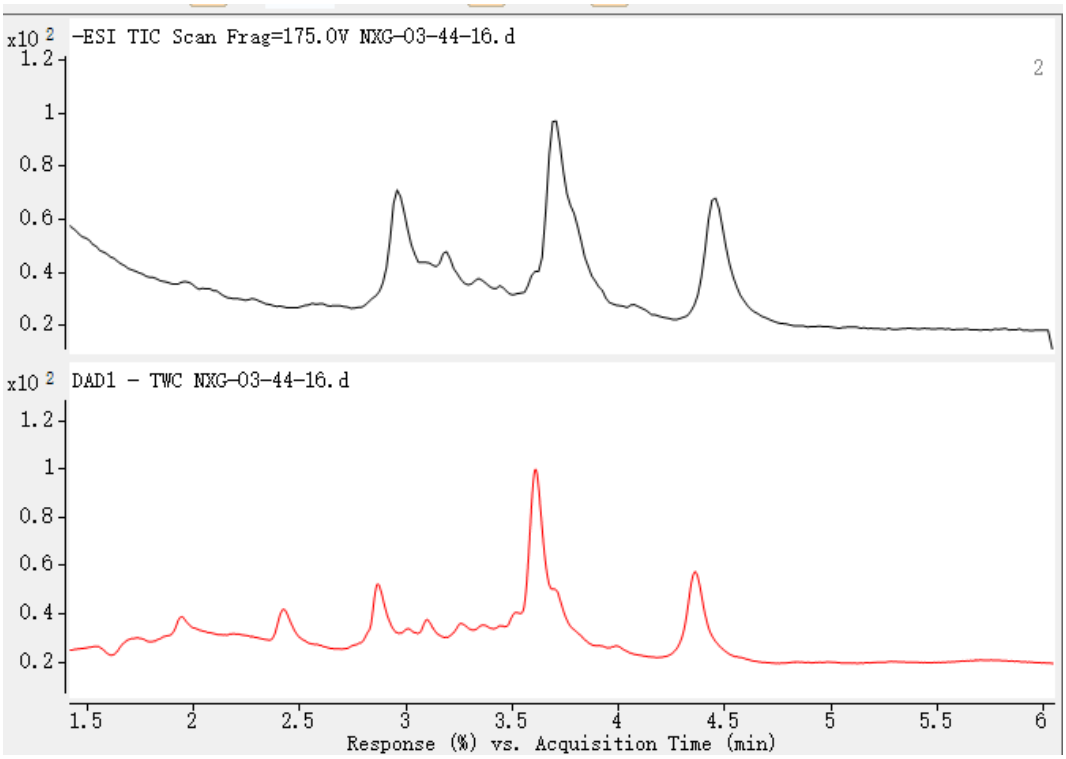

x10 1
-ESI Scan (rt: 5.428-5.777 min, 22 scans) Frag=175.0V NXG-03-84-10.d Subtrac…
5489.18
7379.23
Counts (%) vs. Deconvoluted Mass (amu)

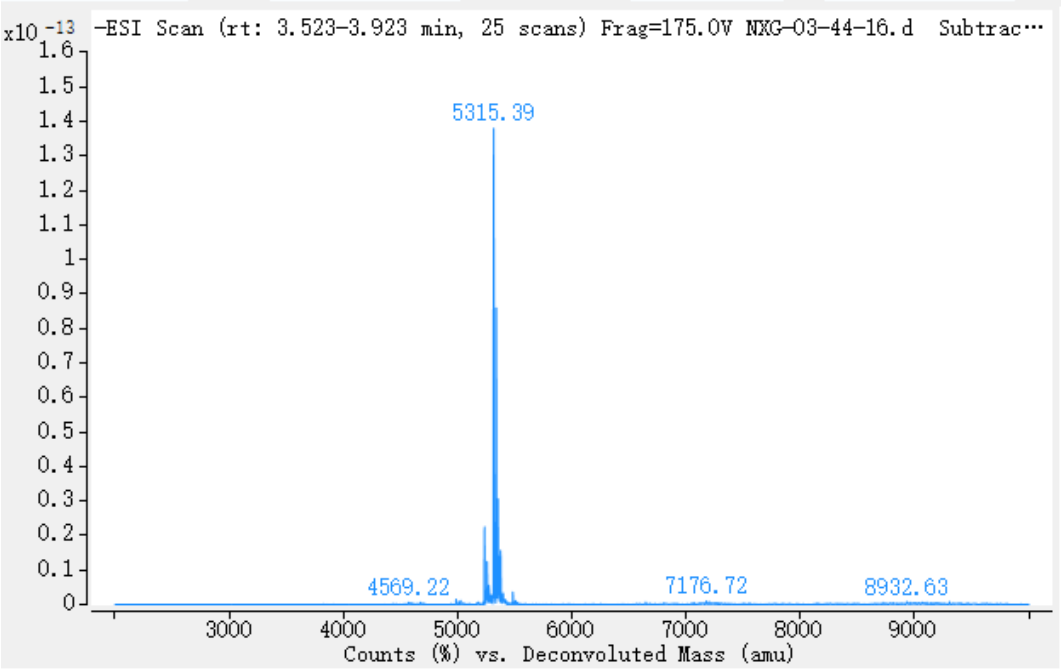
UHPLC chromatogram and deconvoluted MS of **d71**

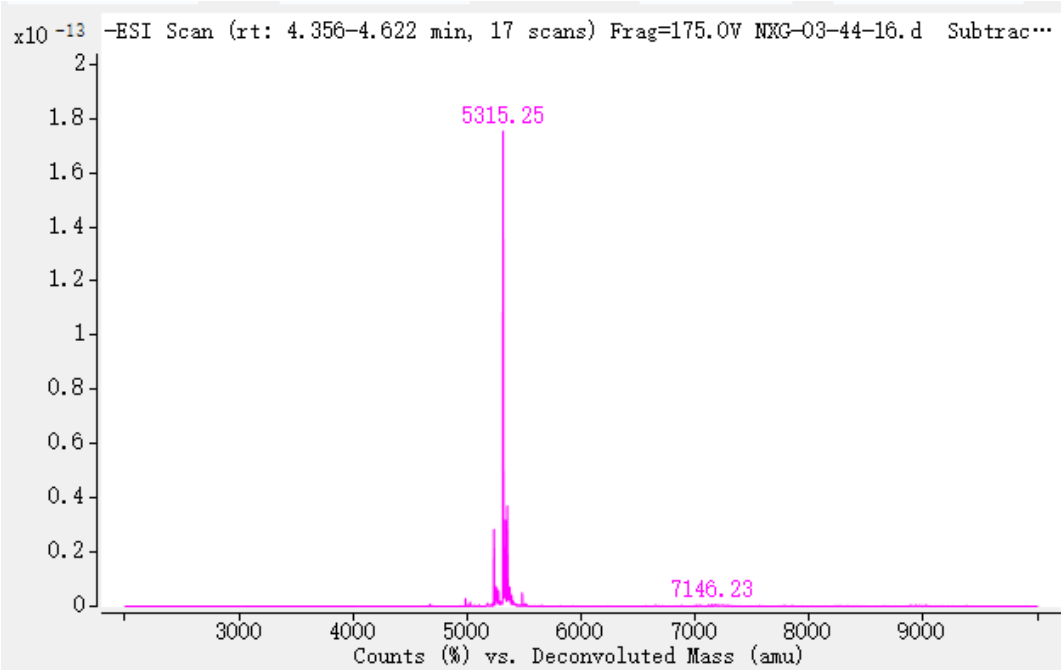
Conversion: 46%

Calculated Mass: 5315 Da; Observed Mass: 5315 Da





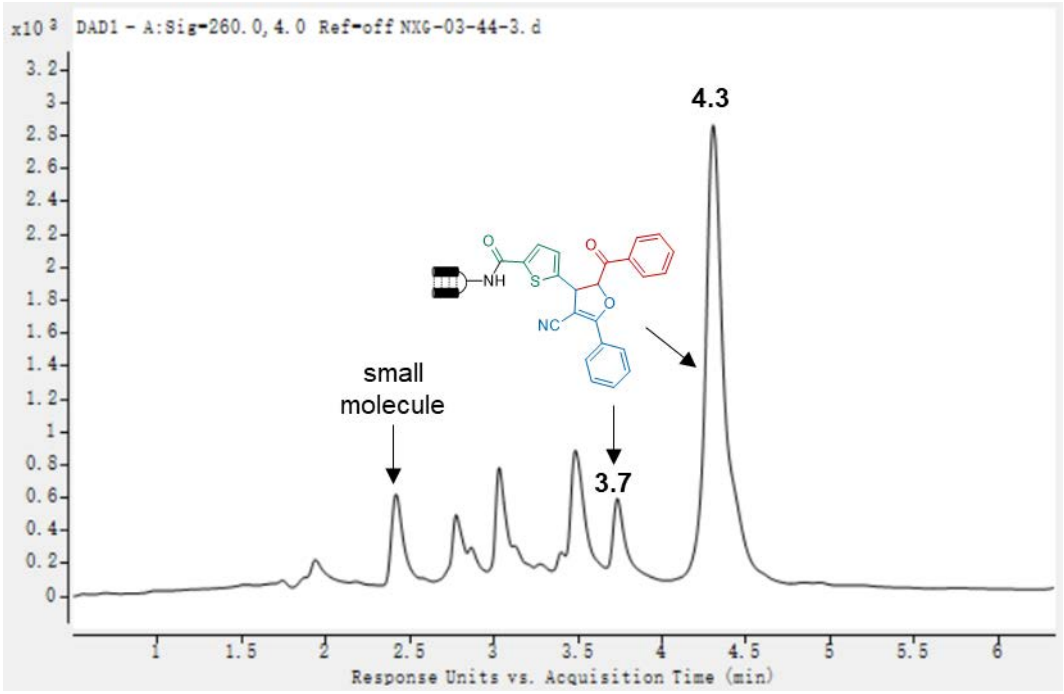

x10 -13
-ESI Scan (rt: 3.523-3.923 min, 25 scans) Frag=175.0V NXG-03-44-16.d Subtrac…
5315.39
4569.22
7176.72
8932.63
Counts (%) vs. Deconvoluted Mass (amu)

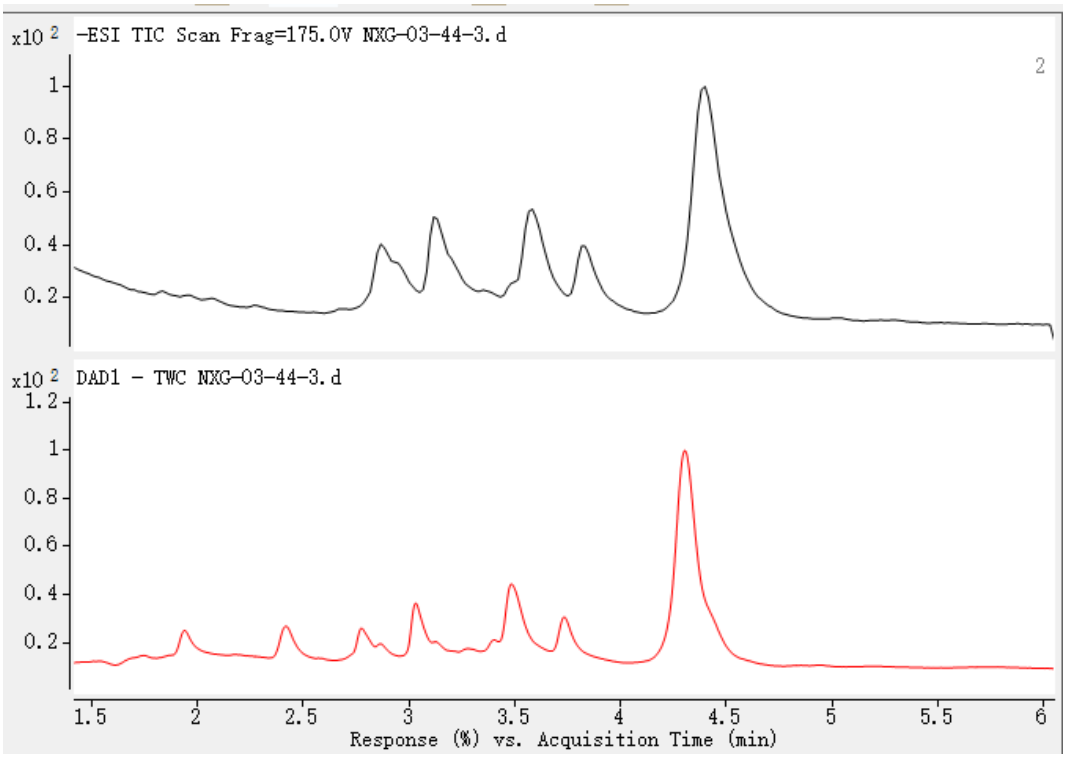

x10 -13
-ESI Scan (rt: 4.356-4.622 min, 17 scans) Frag=175.0V NXG-03-44-16.d Subtrac…
5315.25
7146.23
Counts (%) vs. Deconvoluted Mass (amu)

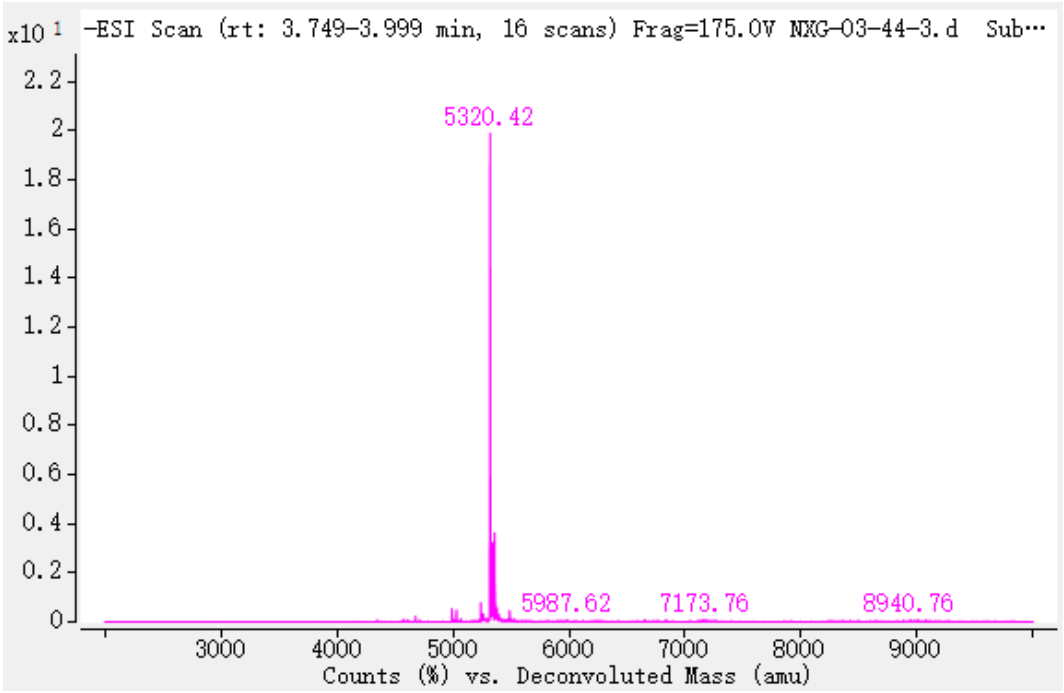
UHPLC chromatogram and deconvoluted MS of **d72**

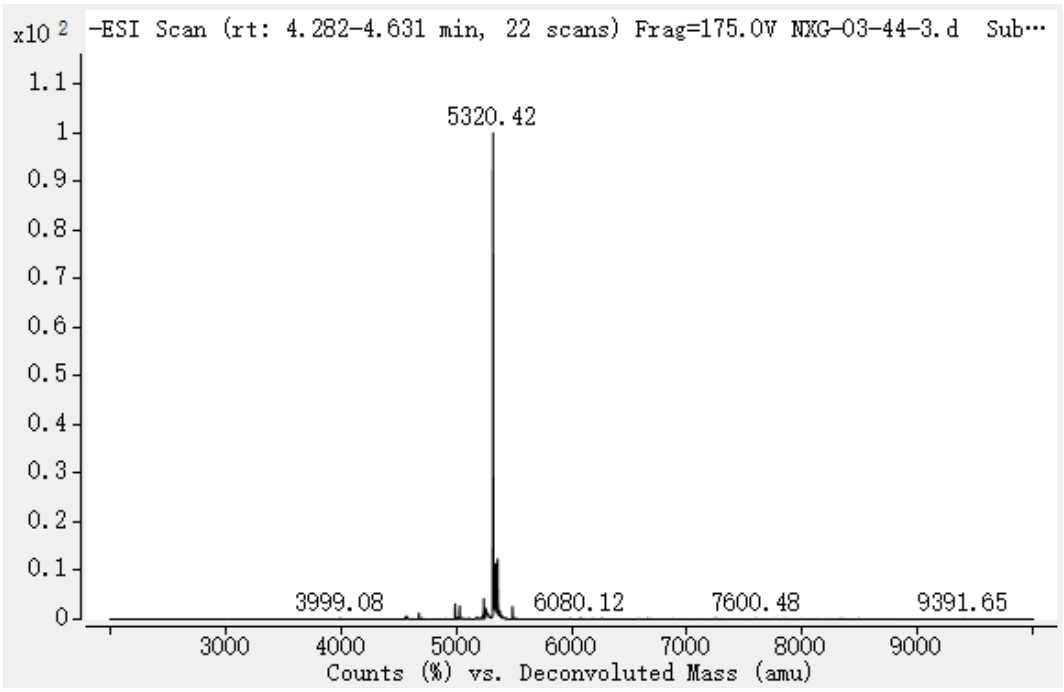
Conversion: 62%

Calculated Mass: 5320 Da; Observed Mass: 5320 Da






x10 1
-ESI Scan (rt: 3.749-3.999 min, 16 scans) Frag=175.0V NXG-03-44-3.d Sub…
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5320.42
5987.62
7173.76
8940.76
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)


x10 2
-ESI Scan (rt: 4.282-4.631 min, 22 scans) Frag=175.0V NXG-03-44-3.d Sub…
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5320.42
3999.08
6080.12
7600.48
9391.65
3000
4000
5000
6000
7000
8000
9000
Counts (%) vs. Deconvoluted Mass (amu)

9. References

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2. M. R. Demidov, V. A. Osyanin, D. V. Osipov and Y. N. Klimochkin, *J. Org. Chem.*, 2021, **86**, 7460-7476.